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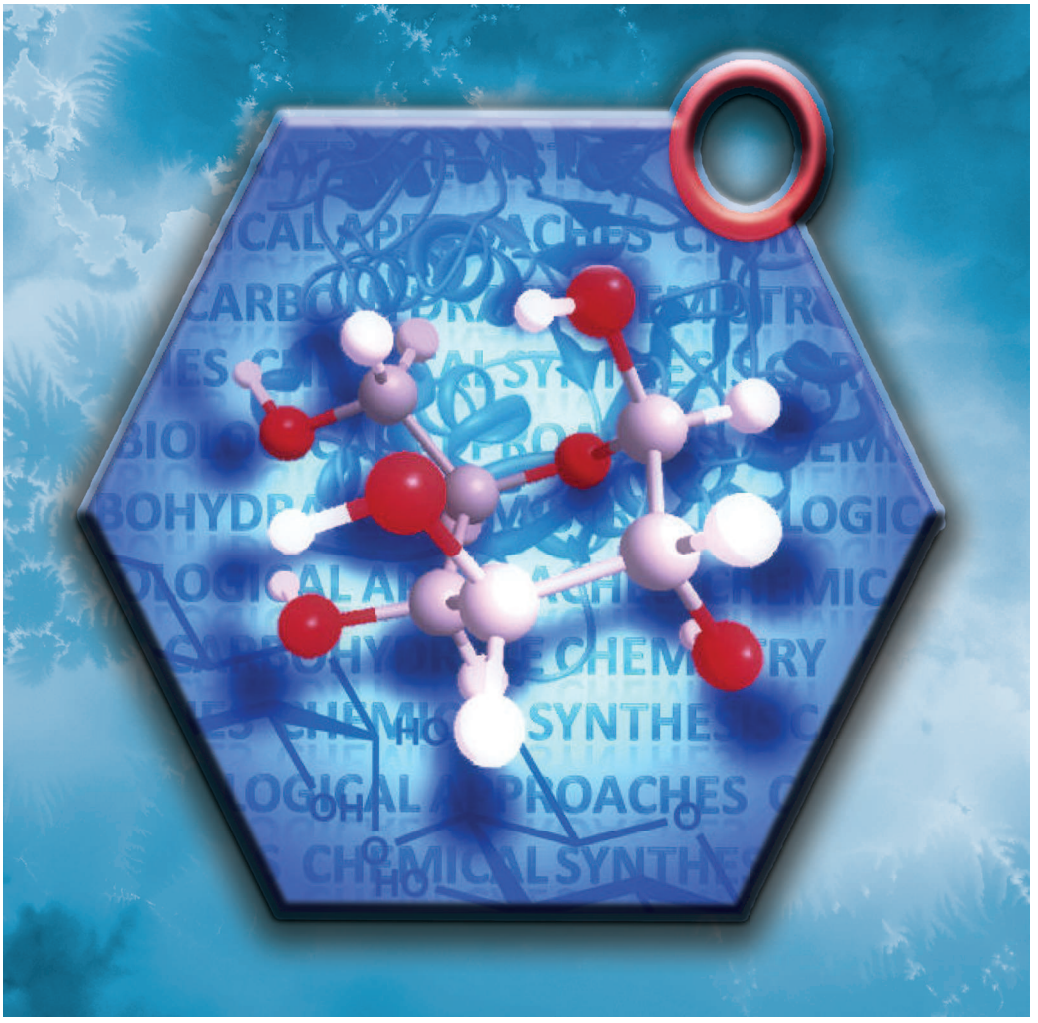
Edited by A Pilar Rauter and T K Lindhorst

Carbohydrate Chemistry

Chemical and Biological Approaches

Volume 35



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Carbohydrate Chemistry
Chemical and Biological Approaches

Volume 35

A Specialist Periodical Report

Carbohydrate Chemistry

Chemical and Biological Approaches
Volume 35

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Preface

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The *Specialist Periodical Reports* volumes on Carbohydrate Chemistry, published by the Royal Society of Chemistry, have become very well known and recognized in recent decades as an extremely useful book series for researchers in the field of carbohydrate chemistry. These volumes provide review coverage of all publications relevant to the chemistry of mono- and oligosaccharides in a given year, and each volume of the series has brought together references to all published work in given areas of the subject and served as a comprehensive database for the active research chemist. Prof. Robin Ferrier, the previous scientific editor, gave an outstanding contribution to the success of this series up to his retirement in 2002, when volume 34 was published. This series has lapsed since then, but the Royal Society of Chemistry is now re-initiating the publication of *Specialist Periodical Reports* volumes on Carbohydrate Chemistry, with a new philosophy for the series. Considering the easy access that researchers now have to published work in their area of interest, this series aims to bring together the latest developments in topical highlights and critical reviews, covering the related subjects from chemistry to biology in the last five years. This new approach will be of great advantage to any researcher who wishes to find, in a single book, chemical and biological science related to the topic that has been selected for that particular issue of the series, thus avoiding time-consuming literature searches involving journals specialized in chemistry, biology or other related areas.

The first volume of this re-invigorated series is volume 35, which covers firstly various aspects of the chemistry at the anomeric center, starting with a critical revision of the enthalpic, conformational and also kinetic anomeric effects in pyranosides. Methodologies for 1,2-*cis*-*O*- and 1,2-*cis*-*C*-glycosylation, stereoselective synthesis of biologically relevant glycosyl phosphates and synthetic approaches for C-1 glycals, which are currently used for *C*-glycosylation, are also discussed. Nitro sugars and transformations affecting their anomeric center leading to a diversity of molecular structures are reviewed as well. In addition, the importance of the chemistry at C-1 for the preparation of carbohydrate scaffolds involving bicyclic lactones or thionocarbamates is highlighted. Furthermore, it is shown how the production of fine chemicals and of bioactive molecules containing sugars and porphyrines can take advantage of the chemistry at the anomeric center.

Blocking the anomeric reactivity allows one not only to inhibit enzymes, which interfere with biological functions, but also to mimic glycosides by stable compounds of pharmacological relevance. Hence, modifications of the anomeric centre of carbohydrates are also reviewed, as well as compounds of that type which have found application as drugs.

Glycosidic bond formation by enzymes is particularly attractive for the preparation of oligosaccharides, avoiding tedious protection, activation, and deprotection strategies. Thus, a chapter of this volume is dedicated to the enzymatic synthesis of oligosaccharides by glycosidases, also using modified substrates and including glycosidase mutants.

Carbohydrate conformation plays a key role for molecular recognition processes, and their determination contributes to the understanding of many biological processes. The latest NMR methodological developments for conformation determination are discussed here, and representative examples are given.

The final chapter is dedicated to the state-of-the-art methods for determining lectin affinity and specificity for oligosaccharides. These play a key role in many biological processes relevant to cell communication and disease states, and are involved in cancer development and metastasis, inflammation and host-pathogen recognition.

Overall, this volume, covering various approaches for syntheses, NMR conformational studies and methods for determining lectin–carbohydrate interactions essential for recognition processes, demonstrates the interdisciplinary character of modern carbohydrate research, together with the importance of combining chemical and biological approaches to achieve innovation in glycomics. It is our hope, that with this new *Specialist Periodical Reports* volume on Carbohydrate Chemistry, we have assembled a collection of contributions that capture the thriving nature of carbohydate research.

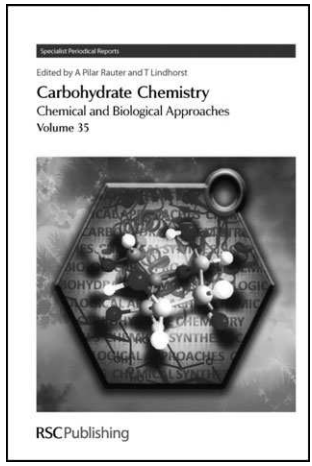
Last not least, we wish to thank the authors of this volume for their commitment in writing their contributions within such a short period of time, allowing its publication in time for presentation at Eurocarb XV in Vienna!

The scientific editors,
Amelia Pilar Rauter and Thisbe K. Lindhorst

The next volume

Volume 36 will be dedicated to carbohydrate-based vaccines and to the utilization of the azide functionality in chemical and biological areas of the glycosciences. There are recent reports on interesting applications of azides in this area, and as starting materials for a variety of important bioactive carbohydrates. Hence, the synthesis, reactivity and application of azides in glycomics will also be included in this volume.

Some promising contributions have already been confirmed, and we thus can already look forward to the next volume!



Cover

Tetrahydropyran-enclosed ball-and-stick depiction of a glucose molecule, and (in the background) part of an α-glycosyl-(1 → 4)-D-glucose oligosaccharide and a glycosidase, all representative of the topics covered in *Carbohydrate Chemistry – Chemical and Biological Approaches*.
Cover prepared by R. G. dos Santos.

Preface 7
Amelia Pilar Rauter and Thisbe K. Lindhorst

Anomeric effects in pyranosides and related acetals 13
Sandrine Gerber-Lemaire and Pierre Vogel
Introduction 13
The enthalpic anomeric effect 13
The conformational anomeric effect 16
The kinetic anomeric effect 24
Conclusion 29

Stereoselective synthesis of 1,2-*cis*-glycosylic linkages 33
Ana T. Carmona, Antonio J. Moreno-Vargas and Inmaculada Robina
Introduction 33
Stability of 1,2-*cis*-glycosylic linkages 35
Factors influencing anomeric control in 1,2-*cis*-glycosylations 36
Methods for the preparation of 1,2-*cis*-*O*-glycosides 37
Methods for the preparation of 1,2-*cis*-*C*-glycosyl compounds 48

Synthesis of anomeric phosphates of aldoses and 2-ulosonic acids 71
Alla Zamyatina and Paul Kosma
Introduction 71
Instalment of glycosyl phosphate linkage by glycosylation 72
Synthesis of glycosyl phosphates by exposure of hemiacetals to activated derivatives of phosphoric acid 81
Approaches to glycosyl phosphates based on P(III)-intermediates 85
Synthesis of anomeric phosphates of aldulosonic acids 93
Conclusions 94

Glycosidic bicyclic lactones as new carbohydrate scaffolds 99
Yves Queneau, Stéphane Chambert, Sylvie Moebs, Arkadiusz Listkowski and Rouba Cheaib
Carbohydrate-based bicyclic lactones: an introduction 99
Carboxymethyl glycoside lactones (CMGLs): synthesis 108
Uses of CMGLs towards mono- and difunctional systems 114
Conclusion 121

Thionocarbamates on carbohydrate scaffolds—from synthesis to bioactivity 127
Ana Catarina Simão, Jolanta Rousseau, Sandrina Silva, Amelia Pilar Rauter, Arnaud Tatibouët and Patrick Rollin
Introduction 127
Synthesis of thionocarbamates on carbohydrate scaffolds 128
Reactivity of thionocarbamates on carbohydrate scaffolds 147
Bioactivity 160
Conclusions 165

Recent advances in nitro sugar chemistry 173
Raquel G. Soengas, Juan C. Estévez, Amalia M. Estevez, Fernando Fernández and Ramón J. Estévez
Introduction 173
Preparation of nitro sugars 173
Reactivity of nitro sugars 180

Porphyrinyl-type sugar derivatives: synthesis and biological applications 199

José A. S. Cavaleiro, Maria A. F. Faustino and João P. C. Tomé

Introduction 199
Synthesis of porphyrinyl-type sugar derivatives 200
Biological applications 223
Concluding remarks 227

Sugars as chiral synthons in the preparation of fine chemicals 232

Sławomir Jarosz, Marta Magdycz and Bartosz Lewandowski

Introduction 232
From simple sugars to cyclic (non-sugar) derivatives: transfer of chirality 232
Modified carbohydrates 243
Transformation of disaccharides 252
Conclusion 255

Blocking the anomeric reactivity, how and why 259

Francesco Nicotra, Laura Cipolla, Barbara La Ferla and Ana Catarina Araújo

Introduction 259
Replacement of the anomeric oxygen 260
Replacement of the endocyclic oxygen 275
Other modifications 283
Conclusions 284

Recent strategies for the preparation of C-1 glycals 289

Ana M. Gómez and J. Cristóbal López

C-1 Glycals – an introduction 289
From cyclic precursors 289
From acyclic precursors 302
Conclusions 306

Glycosidases in synthesis 310

Lenka Weignerová, Pavla Bojarová and Vladimír Křen

Introduction 310
Glycosidase as a promising tool for synthesis 310
Regioselectivity of glycosidases 316
Substrate specificity 317
Glycosidases in industry 322
Enzyme engineering—mutant glycosidases 323
Conclusion 325

Recent advances on the application of NMR methods to study the conformation and recognition properties of carbohydrates 333

Ana Ardá, F. Javier Cañada, Jesús Jiménez-Barbero, João P. Ribeiro and Maria Morando

Introduction 333
The access to new NMR parameters and methodological developments 333
Applications: Saccharides in solution 337
Applications: The interaction of saccharides with other natural and synthetic molecules 343

Specificity and affinity studies in lectin/carbohydrate interactions 356

Ondrej Sulak, Emilie Lameignère, Michaela Wimmerova and Anne Imberty

Introduction 356
Hemagglutination + ELLA 356
Glyco chips: new tools for screening lectin specificity 357
Fluorescence and fluorescence-based thermal shift assay 359
NMR 360
Frontal chromatography 361
Quartz crystal microbalance 362
Surface plasmon resonance 363
Isothermal titration calorimetry 365
Molecular modelling 366
Concluding remarks 367

Anomeric effects in pyranosides and related acetals

Sandrine Gerber-Lemaire and Pierre Vogel*

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The anomeric effect, originally defined as the preference of an electronegative substituent at the anomeric position of pyranosides to stand in an axial rather than an equatorial position, results from the combination of multiple steric, stereoelectronic and medium interactions. A more generalized definition of the enthalpic anomeric effect refers to the *gem*-dioxy stabilizing effect which makes acetals more stable than their 1,*n*-dialkoxyalkane isomers ($n > 1$). This effect is far more important than the difference in stability between conformers of acetals as those found in axial and equatorial pyranoses and pyranosides. This review gives an up-dated view of enthalpic, conformational and also kinetic anomeric effects in pyranosides. The latter manifest themselves during heterolytical processes occuring at the anomeric centers of pyranosides and analogous compounds.

1 Introduction

Known for alkyl pyranosides since 1905 (*e.g.*: alkyl α-D-glucopyranosides being more stable than their β-anomers)[1] and rediscovered in 1955 by Edward,[2] and by Lemieux and Chü in 1958,[3] the conformational anomeric effect designs the contrasteric effect observed in acetals which renders the more sterically encumbered *gauche*/*gauche* conformers more stable than their *anti*/*gauche* and *anti*/*anti* conformers. Following the early interpretation of Lemieux based on electrostatic interactions, several explanations have been advanced to account for the origin of the conformational anomeric effect. This review presents the different models that provide insights in the understanding of anomeric effects.

2 The enthalpic anomeric effect

Acetals are more stable than their 1,*n*-dialkoxyalkane isomers ($n > 1$). Examples (Table 1) are given by comparing the standard heats of formation (gas phase)[4] of 1,2-diethoxyethane ($\Delta H_f^\circ = -98.06 \pm 0.25$ kcal/mol) with that of 1,1-diethoxyethane ($\Delta H_f^\circ = -108.4 \pm 0.74$ kcal/mol), of 1,3-diethoxypropane ($\Delta H_f^\circ = -104.3$ kcal/mol) with that of 2,2-diethoxypropane ($\Delta H_f^\circ = -121.1$ kcal/mol), or of 1,4-dioxane ($\Delta H_f^\circ = -75.4$ kcal/mol) with that of 1,3-dioxane ($\Delta H_f^\circ = -80.9$ kcal/mol).

The *gem*-dioxy substitution stabilizing effect is called the ''enthalpic anomeric effect''.[5] If one takes alkanes as reference compounds, one finds

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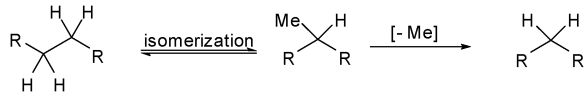
Table 1 Standard heats of formation [ΔH°_f (gas) in kcal/mol] of selected compounds. Evidence of the stabilizing *gem*-dioxy-substituent effects (= enthalpic anomeric effect)[a]



R = Et	−39.94 ± 0.019	[−1.1]	−41.02 ± 0.23	[+6]	−35.08 ± 0.14
R = Me	−30.03 ± 0.16	[−2]	−32.07 ± 0.15	[+7]	−25.02 ± 0.12
R = Cl	−31.5 ± 0.84	[0][c]	−31.5 ± 0.3	[+8.7]	−22.8 ± 0.6
R = CN	+50.11 ± 0.21	[+6.5][c]	+56.6 (est)[b]	[+7]	+63.64 ± 0.24
R = OMe	−81.9 ± 0.2	[−11.2][d]	−93.15 ± 0.20	[+10][e]	−83.21 ± 0.19
R = Oet	−98.06 ± 0.2	[−10.4][d]	−108.41 ± 0.74	[+10][e]	−98.73 ± 0.2

[a] Taken from NIST Chemistry Webbook. [b] Estimated from $CH_2(CN)_2$ adding −7 kcal/mol (exchange of a CH_2 group by a CHMe group). [c] Enthalpy difference indicates a destabilization effect for *gem*-disubstitution with respect to alkanes. [d] Enthalpy difference shows a stabilization effect for *gem*-dialkoxy-disubstitution with respect to alkanes. [e] Enthalpy difference shows increases of enthalpic anomeric effect for tertiary acetals compared to secondary acetals.

that exchange of a CH_2–CH_2 group by a CH(Me) group (isomerization) corresponds to a decrease of heat of formation of *ca.* −2 kcal/mol (for systems not affected by significant changes in gauche interactions). In the case of *n*, *n* + 1-dialkoxyalkanes isomerizing into *n*,*n*-dialkoxyalkanes, the change associated with the exchange of a RO–CH_2–CH_2–OR group with a $(RO)_2CH(Me)$ group amounts to −10 to −11 kcal/mol, which is about −8 to −9 kcal/mol more negative than for alkanes. This difference is a stabilization effect associated with *gem*-dialkoxy-disubstitution. Interestingly in the case of dichlorides and dicarbonitriles, *gem*-disubstitution leads to destabilizations of *ca.* +2 and +8 kcal/mol, respectively (Table 1). The stabilizing *gem*-dioxy substitution effect is not always the same. It depends on the nature of the acetal. Beckhaus *et al.* proposed the following correction $\Delta\Delta H_{anom}$ for the anomeric effect ($\Delta\Delta H_{anom} = [\Delta H_{dioxy} - \Delta H_{oxy}] - [\Delta H_{oxy} - \Delta H_{alkyl}]$) (Fig. 1) to be applied to the additivity rule which allows estimation of heats of formation.[6]

Methyl substitution of *n*-alkanes introduces a change in the standard heats of formation [ΔH°_f (gas)] of −7 kcal/mol [exchange of a CH_2 group for a CH(Me) group]. In the case of CH_3CH_2OMe, becoming $(CH_3)_2CHOMe$, $\Delta\Delta H^\circ_f$ = −8.5 kcal/mol and for 1,3-dioxane, methyl substitution at C(2) is accompanied with a more negative change of −11.6 kcal/mol. Even more dramatic are the 4-methyl and 2-methyl substituent effects on the standard heats of formation of 1,3-dioxane that reach −9.6 and −14 kcal/mol, respectively (Fig. 2).[4]

A strong stabilizing geminal disubstitution effect is also observed for difluoro disubstituted hydrocarbons (*e.g.*: 2MeF ⇄ CH_2F_2 + CH_4,

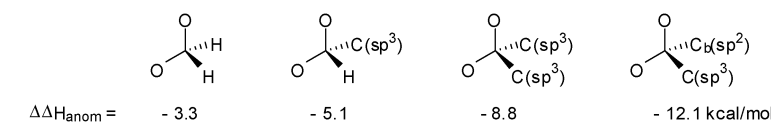


Fig. 1 Corrections for the anomeric effect.

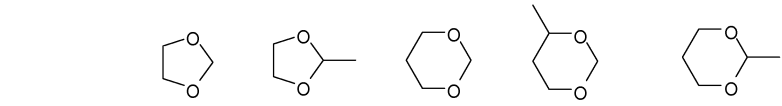


Fig. 2 Methyl substituent effects on cyclic acetals.

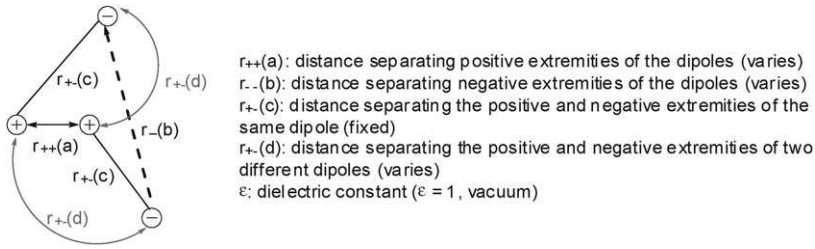


Fig. 3 Modelisation of enthalpic anomeric effect.

$\Delta H^\circ_r = -12.3$ kcal/mol; $2MeOMe \rightleftarrows (MeO)_2CH_2 + CH_4$, $\Delta H^\circ_r = -13$ kcal/mol) while dibromo and dichloro derivatives (*e.g.*: $2MeCl \rightleftarrows CH_2Cl_2 + CH_4$, $\Delta H^\circ_r = -0.2$ kcal/mol) do not benefit from this effect. The origin of this enthalpic anomeric effect is essentially an electronic one. Considering the two oxy or two fluoro substituents as two dipoles, electrostatic theory predicts the existence of families of geometries leading to a stabilization ($E_{el} < 0$).[7] This phenomenon can be modeled by the relationship given in Fig. 3 for an ensemble of two positive charges q_+ and two negative charges q_-. The attractive interactions between negative and positive extremities of two different dipoles overcome the repulsing interactions resulting from the juxtaposition of two positive extremities of the two dipoles and from the repulsion between the two negative extremities of the dipoles.

$$E_{el} = \left[\frac{1}{r_{++}(a)} + \frac{1}{r_{--}(b)} - \frac{1}{r_{+-}(c)} - \frac{1}{r_{+-}(d)}\right] \frac{|q_+||q_-|}{4\pi\varepsilon}$$

Balance between repulsive and attractive electrostatic effects depends on charges q_+,q_- (electronegativity differences of the C–X bonds) or the nature of the dipole (type of bonds) and their orientation. In the case of acetals, one needs to consider an ensemble of not only two dipoles but of, at least, four (Fig. 4).

As we shall see, the conformational anomeric effects can be assigned to stability difference between the various conformers that this ensemble of

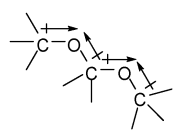


Fig. 4 Acetals relative stability depends on at least four dipoles.

dipoles can adopt. As a general rule, the latter enthalpy differences are much smaller than the enthalpic anomeric effects (*gem*-dioxysubstitution effects) defined above.

3 The conformational anomeric effect

The conformational anomeric effects design the contrasteric effects observed in acetals which render the more sterically encumbered *gauche*/*gauche* conformers more stable than their *anti*/*gauche* and *anti*/*anti* conformers. Such effects were first evidenced by Jungins in 1905 and rediscovered by Edward in 1955 and by Lemieux and Chiu in 1958. They observed the higher stability of alkyl α-D-glucopyranosides in comparison with their β-anomers (Fig. 5).[8]

Following these first discoveries, it was soon established that the conformational anomeric effect is not restricted to carbohydrates but applies to fragments of type R–X–A–Y where X stands for an atom bearing lone pairs, A represents an atom of intermediate electronegativity (*i.e.* C, P, S) and Y is an atom of higher electronegativity than A (*i.e.* N, O, halogens). These systems display a preference for the substituent on the anomeric carbon to adopt a synclinal (*gauche*) position rather than a anti-periplanar (*anti*) position (Fig. 6).[9]

Consequences of the conformational anomeric effect are largely expressed in monosaccharides and their derivatives. One recognizes the conformational *endo*-anomeric effect for pyranosides with a polar X group at C(1) (contrasteric electronic stabilization effect: Fig. 7A) and conformational *exo*-anomeric effect for glycosides (acetals) in which the alkyl group of the exocyclic moiety is synclinal (Fig. 7B, C).

3.1 Conformational endo-anomeric effect in hexoses

In many monosaccharides the proportion of the α-anomer in aqueous solution is higher than the expected ratio only based on steric inteactions. In particular, while the predicted ratio in aqueous solution at 25 °C based on the A value for axial *vs*. equatorial cyclohexanol is 11/89, the observed ratio for D-glucose is 36/64 for α-pyranose/β-pyranose.[10] Based on

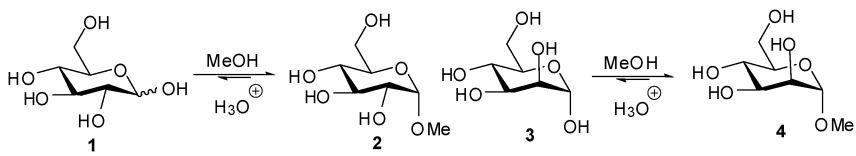


Fig. 5 Glycosylation of D-glucose and D-mannose according to Fischer.

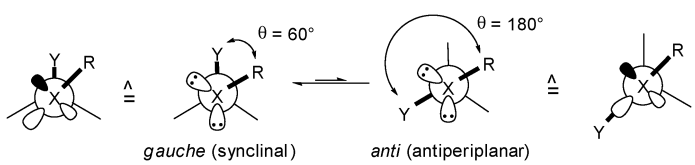


Fig. 6 Generalized conformational anomeric effect.

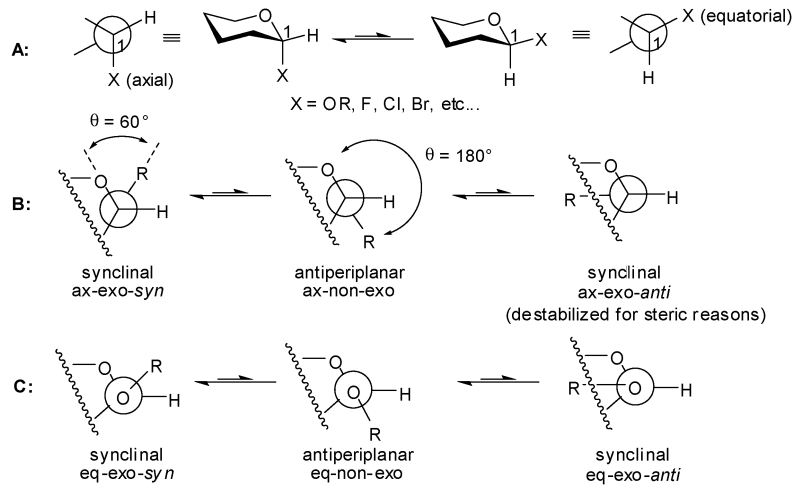
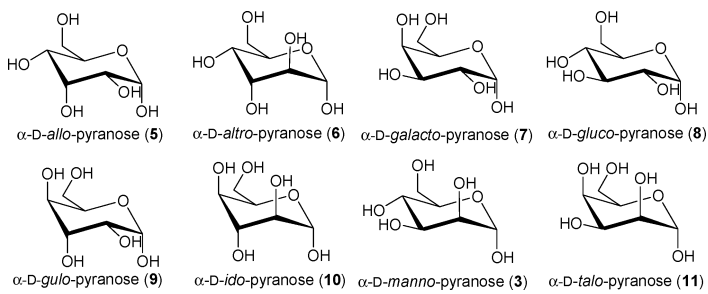


Fig. 7 (A) Conformational endo-anomeric effect; (B) conformational exo-anomeric effect in axial *O*-pyranosides (X = OR); (C) conformational exo-anomeric effect in equatorial *O*-pyranosides (X = OR).

quantitations by ^{13}C-NMR, Serianni *et al.*[11] disclosed the ratios of the different forms of aldohexoses in water (Table 2).

The authors also evidenced an increase of the percentages of α-*talo*-pyranose and α-*talo*furanose upon ^{2}H substitution at C(1) concomitantly with a decrease of the percentages of β-conformers. This observed shift on the talopyranose anomeric equilibrium was attributed to the preference of the shorter C–^{2}H bond (relative to the C–^{1}H bond) to assume an equatorial orientation.

Table 2 Percentage of cyclic and acyclic forms of D-aldopyranoses in water at 30 °C



Monosaccharide	α-*p*	β-*p*	α-*f*	β-*f*	Hydrate	Aldehyde
D-*allo* (**5**)	14.6	77.1	3.0	5.3	0.0063	0.0032
D-*altro* (**6**)	26.8	41.0	8.6	13.4	0.079	0.014
D-*galacto* (**7**)	31.2	62.7	2.3	3.7	0.046	0.006
D-*gluco* (**8**)	37.6	62	0.1	0.28	0.0059	0.004
D-*gulo* (**9**)	12.2	83.7	0.94	3.04	0.077	0.005
D-*ido* (**10**)	33.7	37.3	12.14	16.12	0.7	0.094
D-*manno* (**3**)	66.23	32.85	0.64	0.24	0.022	0.0044
D-*talo* (**11**)	42.1	28.7	17.9	11.1	0.052	0.029

α-*p*: α-pyranose, β-*p*: β-pyranose, α-*f*: α-furanose, β-*f*: β-furanose.

3.2 Theories for conformational anomeric effects

The first interpretation of the conformational anomeric effect, given by Edward,[2] invoked more favorable electrostatic interactions in the axial anomers than in the equatorial anomers of carbohydrates (Fig. 8).

For instance, in structure **12-e**, the C–X and C–O dipole moments are additive, leading to a destabilization of the molecule by increasing the energy. In structure **12-a**, offset of the C–X and C–O dipole moments minimizes electrostatic interactions, thus leading to a more stable conformation. This electrostatic model was supported by the observed increase of the percentage of the equatorial conformation of 2-methoxy tetrahydropyran (**14**) when moving from a non-polar to a polar solvent (Table 3).[12] In this model, the polar groups are not polarizable and lead to dipole/dipole (hard/hard) interactions.

The decrease of the anomeric effect in polar solvents was also supported by quantum mechanics calculations.[13] Nevertheless further studies on the anomeric effect demonstrated the limitations of the electrostatic model. In particular, Juaristi *et al.*[14] demonstrated that, at low temperature, the dependence of conformational equilibria of 2-carbomethoxy-1,3-dithiane upon solvent shows an opposite trend to the stronger anomeric effect in less polar media observed at 25 °C (Table 4).

Similar observations have been reported by Pinto[15] for the conformational equilibrium in 2-[(4-methoxyphenyl)seleno]-1,3-dithiane (Fig. 9). Although, at 273 and 300 K, the proportion of the more polar equatorial isomer **17** increases as the dielectric constant of the medium increases, no apparent correlation exists at low temperature.

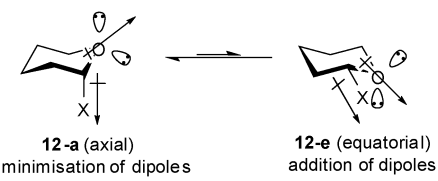
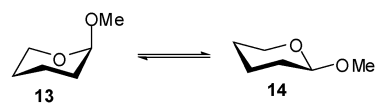


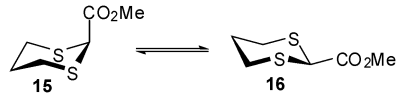
Fig. 8 Interpretation of the conformational endo-anomeric effect according to Edwards (hard/hard interactions).

Table 3 Conformation dependence on solvent polarity according to Lemieux



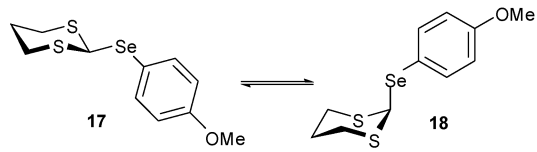
Solvent	Dielectric constant	Ratio **13**/**14**
CCl_4	2.2	83/17
C_6H_6	2.3	82/18
CS_2	2.6	80/20
$CHCl_3$	4.7	71/29
$(CH_3)_2C(O)$	20.7	72/28
CH_3OH	32.6	69/31
CH_3CN	37.5	68/32
H_2O	78.5	52/48

Table 4 Conformation dependence on solvent polarity according to Juaristi for 2-carbomethoxy-1,3-dithiane



Solvent	Dielectric constant	ΔG° (kcal/mol)
CD_2Cl_2	8.9	0.83[a]
$(CD_3)_2C(O)$	20.7	0.92[b]
CD_3OD	32.6	1.13[b]

[a] $T = -100$ °C; [b] $T = -90$ °C.



solvent (ε)	K at 180K	K at 185K	K at 273K	K at 300K
PhMe (2.4)	9.9	9.1	2.42	2.10
CH_2Cl_2 (4.7)	5.4	4.8	2.01	1.87
$(CH_3)_2CO$ (4.7)	8.1	6.8	1.68	1.34

Fig. 9 Equilibrium data for conformational equilibrium in 2-[(4-methoxyphenyl)seleno]-1,3-dithiane according to Pinto.

The rationalization of the conformational anomeric effect solely based on electrostatic interactions fails to account for these solvent effects. Another interpretation based on bond polarizability in 1,1-dialkoxyalkyl systems calls electronic transfer from a non bonding electron pair of one oxygen atom to the empty σ^*_{C-O} orbital from the other alkoxy substituent (Fig. 10).[16]

In this model, one considers the acetals to be composed of polarizable dipolar moieties that can be stabilized by electron transfer from an electron-rich moiety (non-bonding electron on oxygen; low ionisation energy) to adjacent polar and polarizable moieties (high electron affinity). A strong overlap between n(O) and σ^*_{C-O} orbitals optimizes this electronic transfer. As these orbitals are not spherical, n(O)/σ^*_{C-O} overlap depends on

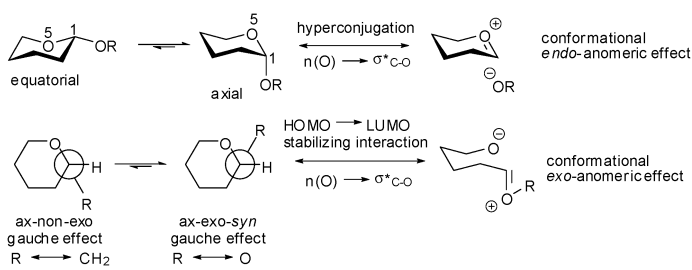


Fig. 10 Stereoelectronic interpretation of the conformational endo- and exo-anomeric effects [n(O) → σ^*_{C-O} hyperconjugation].

conformation (torsional angle θ, Fig. 6 and 7). For alkyl pyranosides, the electronic transfer $n(O) \rightarrow \sigma^*_{C-O}$ involving the intracyclic oxygen atom with the C(1)–OR bond is more favorable in axial conformers (conformational endo-anomeric effect). This theory predicts also that rotamers in which the R group and the O(5) oxygen atom are gauche should be favored over rotamers presenting the R group and the intracyclic O(5) oxygen atom in antiperiplanar relationship (Fig. 10). Manifestation of this exo-anomeric effect is given by a clear difference in C–O bond lengths.[17] In equatorial pyranosides (synclinal, eq-exo-*syn*, Fig. 7C), the intracyclic C–O bond is longer than the exocyclic C–O bond.[18]

At that stage, a correct understanding of the non-bonding electron pairs of oxygen (and other heteroatoms involved in conformational anomeric effect) is of crucial importance.[19] In many instances, a sp^3-hybridized oxygen atom has been used to describe the properties of acetals (with two sp^3 orbitals occupied each by two electrons pairs: rabbit ears). Nevertheless, electron distribution about oxygen atom in H_2O,[20] ethers and related compounds are better represented by using a sp^2-hybridized oxygen atom.[21] In sp^2 hybridized oxygen atom, the $sp^2(O)$ orbital is a sub-HOMO equivalent to the combination $1/\sqrt{2}\ sp^3(O) + 1/\sqrt{2}\ sp^3(O)$ and the HOMO (localized on the ethereal oxygen atom) is equivalent to $1/\sqrt{2}\ sp^3(O) - 1/\sqrt{2}\ sp^3(O)$. As the latter is higher in energy (lower ionization energy) than the former localized orbital (higher ionization energy), it contributes more efficiently to any electron transfer, for instance into a hyperconjugating C–X bond. Consequently, we prefer to use sp^2-hybridized rather than sp^3-hybridized oxygen atoms to describe the non bonding electron pairs involved in conformational anomeric effect. The σ conjugation[22] of the type $2p(O') \rightarrow \sigma^*_{C-O''}$ (negative hyperconjugation)[23] also intervene on the stability difference of 1,7-dioxaspiro[5.5]undecanes (6,6-spiroketals) (Fig. 11).[24] In these systems, the contrasteric effect renders the more sterically encumbered *gauche*/*gauche* conformers (**19**) more stable than their *anti*/*gauche* (**20**) and *anti*/*anti* (**21**) conformers. Noteworthy, the model depicted in Fig. 11 for the conformational anomeric effect which intends to overlap between 2p(O) (HOMO) and σ^*_{C-O} (LUMO) orbitals is in agreement with the dihedral (torsional) angle θ measured in 6,6-spiroketals.[25] In fact, the analysis of the crystal structures of [6,6]-spiroketal derivatives recorded in the Cambridge Structural Database revealed that the dihedral angle θ (Fig. 7) is larger than 60° in most cases {mean value: 62° for 84 [6,6]-(*gauche*/*gauche*)-spiroketal structures}.

3.3 Conformational anomeric effects in pyranosides

In the case of 2-hydroxytetrahydropyran, the axial conformer **22** is calculated to be more stable than its equatorial conformer **23** in vacuum (Fig. 12). Solvent effects change the equilibrium constant and the equatorial form **23** is favored in aqueous solution, in agreement with data. The magnitude of the conformational endo-anomeric effect in **22** is estimated to 2.0 kcal/mol (gas phase; stereoelectronic effects overwhelming the steric

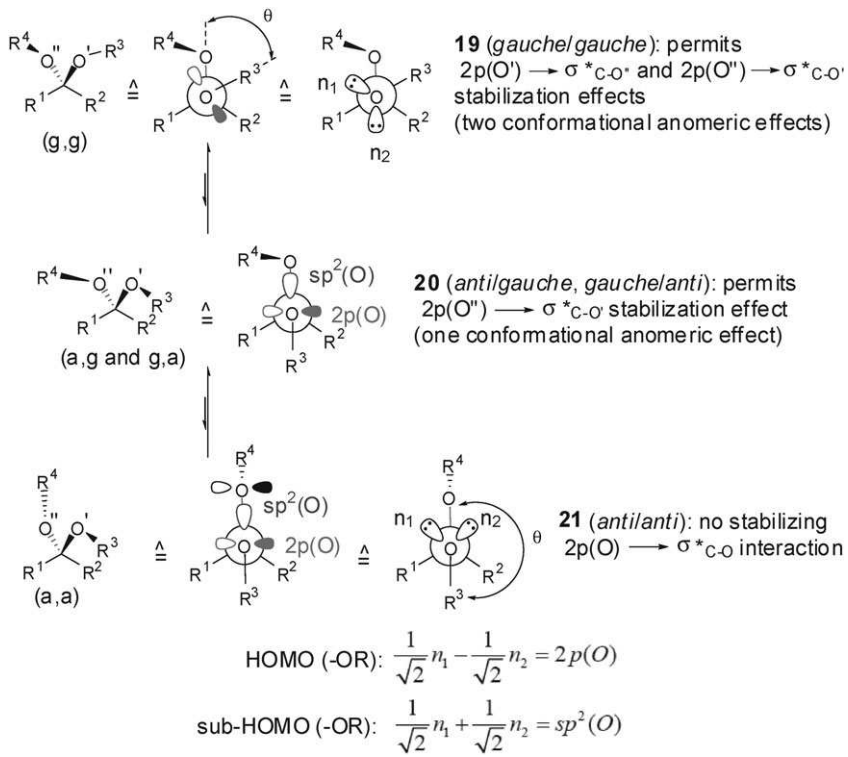


Fig. 11 Possible conformations of hemiacetals and acetals.

gauche effect, as given by the comparison of the free enthalpy difference between axial/equatorial 2-hydroxytetrahydropyran and cyclohexanol).

The hydroxyl group in **22** and **23** exhibits conformational exo-anomeric effect of 2.3 (**22s** ⇆ **22a**) and 2.9 (**23s** ⇆ **23a**) kcal/mol.[26] Similar computational and experimental effects are found for 2-methoxytetrahydropyrans (**13** ⇆ **14**).[27]

As conformational anomeric effects represent only a fraction (−1 to −3 kcal/mol) of the global enthalpic anomeric effect or *gem*-dioxy stabilizing effect (−6 to −17 kcal/mol), additional factors have to be taken into account. Depending on substitution, steric factors can affect the relative stability of acetal conformers. Dubois *et al.*[28] have demonstrated that in furanose–pyranose derivatives a bulky substituent at the furanose

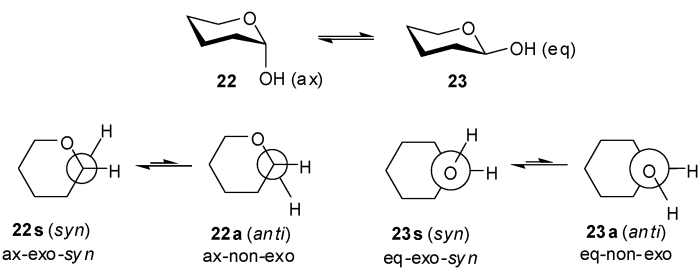


Fig. 12 Conformational equilibria for 2-hydroxytetrahydropyran.

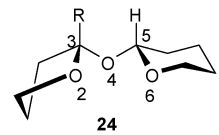


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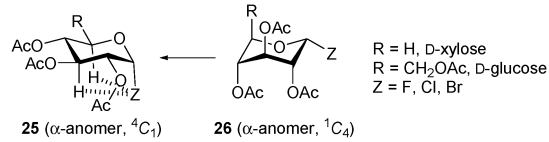
Fig. 13 Relative *syn* position of the two rings in furanose-pyranose structures bearing bulky R substituents.

anomeric center induces an axial conformation of the pyranose ring (Fig. 13, generic structure **24**). This phenomenon is accompanied by an alternation between short and long C–O bonds [short O(2)–C(3) and O(4)–C(5) bonds, long C(3)–O(4) and C(5)–O(6) bonds] which illustrates a dominant endo-anomeric effect for the furanose ring and a dominant exo-anomeric effect for the pyranose ring. Both rings lie in *syn* relationship on the same side of the C(3)–O(4)–C(5) plane.

Furthermore, it has been demonstrated in acetals of phenyl alkyl ketones that the presence of the aromatic moiety at the anomeric carbon induces a further stabilization resulting in very strong anomeric stabilization.[29] This phenomenon is reminiscent of the stronger methyl substitution effects discussed above for 1,3-dioxolane and 1,3-dioxane (see also Table 1) than for alkyl ethers and alkanes.

Difference in stability between *gauche* and *anti* conformers is also solvent dependent. *Ab initio* molecular dynamics simulations of glucose in water demonstrated a similar average number of hydrogen bonds for both anomers, which is in line with the small free energy difference between the two structures (0.35 kcal/mol).[30] However, while water molecules flow freely around the anomeric oxygen of the most abundant β-anomer [with equatorial C(1)–OH], they bind more tightly and in a more ordered manner to the α-anomer [with axial C(1)–OH]. Through the analysis of the conformational equilibria for D-xylose and D-glucose in aqueous solution, Brady *et al.*[31] proposed that the observed anomeric equilibria result from a competition of internal and solvation terms of opposite signs. Increased hydrogen bonding around the anomeric hydroxyl group is observed for the β-anomers which present a greater hydrophilic surface area. The authors evidenced that in solution there are significant intrasolute and solute solvent terms of opposite signs which stabilize the α-anomer [with axial C(1)–OH] and the β-anomer [with equatorial C(1)–OH], respectively. On their side, Nishio *et al.*[32] suggested that the origin of the anomeric effect in monosaccharides and analogues residues, at least in part, in the stabilization induced by hydrogen bonds between the electronegative atom at the anomeric position and axial C–H groups on the ring (Fig. 14). In D-*xylo* and D-*gluco* pyranosyl halides, the authors assume that the five-membered CH/Z (Z = F, Cl, Br) hydrogen bonds, which can only occur in the $^4C_1$ conformers, is responsible for the marked preference for axial orientation of the Z substituent at C(1) ($^4C_1$-conformer of α-anomer **25** with axial Z). This hypothesis is supported by the non-bonded distance between the Z substituent and the axial C–H groups which is shorter than the van der Waals distance.

Recently, quantum calculations and topological analysis using the Quantum Theory of Atoms in Molecules (QTAIM) provide an explanation



25 (α-anomer, $^4C_1$) **26** (α-anomer, $^1C_4$)

Fig. 14 Axial preference of pyranosyl halides for D-*xylo* and D-*gluco* configurations.

of the conformational anomeric effect for acetals that is not in line with the $n(O) \rightarrow \sigma^*_{C-O}$ hyperconjugation model. The conformational energy variations are accompanied by an electron population redistribution that implies the CH_2 group in $MeOCH_2OMe$, and by extension the CR^1R^2 group in other acetals. The stabilization of the *gauche* conformers of $(MeO)_2CH_2$ is accompanied by a progressive reduction of the electron population of the hydrogens of the central CH_2 group as the number of their gauche interactions to lone pairs rises. The electron population removed from these atoms during the conformational change is gained in the *gauche* conformers by the oxygen atoms, which results in more negative energy.[33] The electrostatic effects (hard interactions) thus overwhelm the polarizability effect (hyperconjugation: soft interactions).

3.4 Conformation of *O*- and *C*-disaccharides

Kishi and co-workers have proposed that *O*-glycosides and *C*-glycosyl compounds share the same conformational characteristics in solution.[34] This is the case, for instance, for the *C*-disaccharide **27** (Fig. 15).[35] It adopts a major conformation similar to that proposed for methyl 3-*O*-(α-D-galactopyranosyl)-α-D-mannopyranoside (**28**).

Thus the major *syn* conformer of **27** implies a zig–zag arrangement of the $\sigma_{C(1')-C(2)}$ and $\sigma_{C(3)-CH2}$ bonds, what is expected for all carbon chain. This suggests that the stereoelectronic effect [hyperconjugation $n(O) \rightarrow \sigma^*_{C-O}$] invoked to explain conformational exo-anomeric effect in pyranosides might not be the dominating factor, a steric factor favors already the *syn* conformers. β,β-Trehalose and its *C*-disaccharide adopt the same major conformation in solution.[36] Molecular mechanics calculations suggested, however, that α,β- and β,β-*C*-trehaloses exhibit a larger number of low energy conformers and a larger area of a map energy than the corresponding *O*-disaccharides. The preferred conformations of the axial *C*-glycosyl bond are in agreement with the conformational exo-anomeric effect. Equatorial *C*-glycosyl bonds are rather flexible, influenced by the polarity of the medium and the formation of inter-residue hydrogen

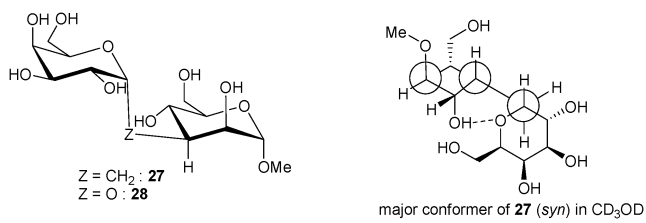


Fig. 15 Conformation of *C*-disaccharide **27**.

bonds.[37] In the case of *C*-disaccharide β-C-Gal(1→3)-β-Glc-OMe, it was shown by molecular mechanics and NMR spectroscopy that the *C*-disaccharide populates two distinctive conformational families in solution, the normal *syn* (exo-anomeric) conformation, which is the predominant conformation of the parent *O*-glycoside, and the *anti*-conformation, which has not been detected for the *O*-disaccharide.[38]

In the case of *C*-lactose bound to peanut agglutin, its conformation is identical to the conformation of its parent *O*-lactose bound to the same protein. This has led to the claim that the conformational similarity between *O*-glycosides and *C*-glycosyl compounds is a general phenomenon.[39] In other studies, Jimenez-Barbero and co-workers have reported that similar conformations for *O*-glycosides and *C*-glycosyl analogues do not persist at least for *C*- and *O*-lactose β(1→4)-glycosidic linkage[40] and for *C*- and *O*-mannobiose [α(1→2)-glycosidic linkage].[41] Regarding the use of *C*-disaccharides as *O*-disaccharide isosteres, it appears that, due to the low energy difference among conformers, conformations different from the major one existing in water solution may be bound by biopolymers without major energy conflict.[42]

4 The kinetic anomeric effect

The stereoelectronic interpretation of the conformational anomeric effects in alkyl pyranosides (Fig. 10) implies that the intra-ring O(5) oxygen assists lengthening and weakening of the C(1)–OR, which then becomes more prone to cleavage. When the HOMO 2p(O) of O(5) is antiperiplanar or nearly antiperiplanar to the axial C(1)–OR bond, the latter is activated for cleavage. This model sets the basis of the so-called antiperiplanar lone pair effect.[43] For instance, hydrolysis of 9-oxabicyclo[3.3.1]non-1-yl-2,4-dinitrophenolate **29** is about 10^{13} times slower than that of 2-methyltetrahydropyran-2-yl-2,4-dinitrophenolate **30** (Fig. 16). Both acetals undergo S_N1 solvolysis generating oxycarbenium ion intermediates **31** and **32**, respectively. In the case of the bicyclic cation **31**, the rigid geometry prohibits 2p(O) ↔ 2p(C^+) interaction that is possible in the pyranosyl cation **32**. Therefore, there is a stereoelectronic constraint in **29** that prohibits the α-oxy-substituent to stabilize the forming carbenium ion intermediate.[43,44]

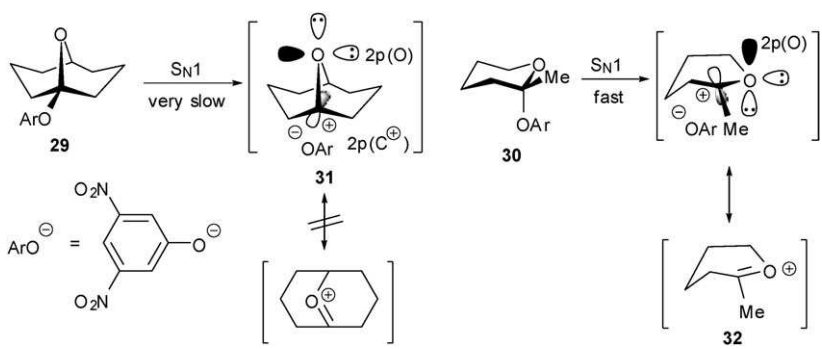


Fig. 16 Hydrolysis of 2,4-dinitrophenolates.

This effect amounts to $\Delta\Delta G^{\#} = RT\ln 10^{13} = \cong 17.7$ kcal/mol at 25 °C in the cases of the hydrolyses of **29** and **30**.

Therefore any flexible acetal will undergo conformational changes to permit 2p(O) ↔ 2p(C^+) stabilizing interaction to intervene in the transition state of its heterolysis. This is also true for pyranosides for which the free energy difference between chair, boat and sofa conformers rarely surpasses 10 kcal/mol.

It has also been demonstrated that synperiplanar oxygen lone pairs can play a similar role in influencing reaction rates.[45] In the early 1980's, Kirby[44] observed that the spontaneous hydrolysis of aryl acetal **34**, with equatorial leaving group OAr, was three times faster than the hydrolysis of its isomer **33**, with an axial leaving group OAr. The latter bears a lone pair on the ring oxygen antiperiplanar to the bond of the leaving group (Fig. 17). Kirby suggested reaction of the equatorial isomer **34** by way of a non-ground state conformation **36**, presenting a chair-boat conformation, in which a lone pair of the intracyclic oxygen moiety is antiperiplanar to the C–OAr bond.

In their study of Brønsted acid induced cleveage of α [with axial C(1)–OMe] and β [with equatorial C(1)–OMe] glycopyranosides, Fraser-Reid *et al.*[46] demonstrated that the β/α rate ratios for hydrolysis of methyl pyranosides (Table 5) can be explained by the different intermediates and transition-state structures through which proceed the heterolysis of α and β isomers (Fig. 18).

Correlations between the O(5)–C(1) and C(1)–O(1) bond lengths in both axial and equatorial isomers demonstrated that n(O)–σ*(C–O) interactions stabilizing the S_N1 transition states are already manifesting themselves in

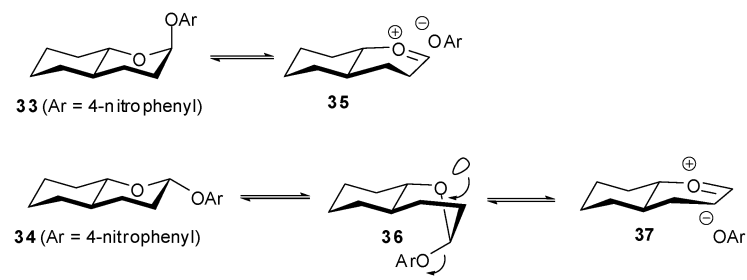


Fig. 17 Spontaneous hydrolysis of axial and equatorial acetals.

Table 5 β/α rate ratios for hydrolysis of methyl pyranosides

Pyranoside	β/α rate ratio
Glucose	1.9
2-Deoxyglucose	2.5
Mannose	2.4
Galactose	1.8
Xylose	2.0
Rhammose	2.3
Arabinose	1.5

Conditions: HCl 0.5 M, 75 °C.

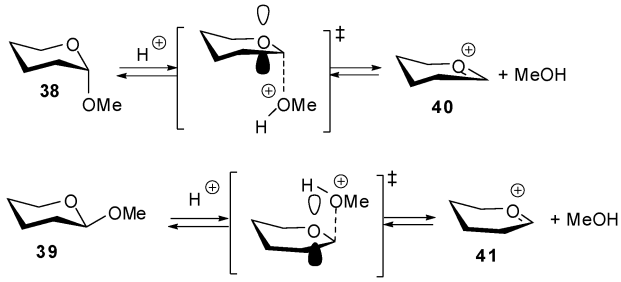
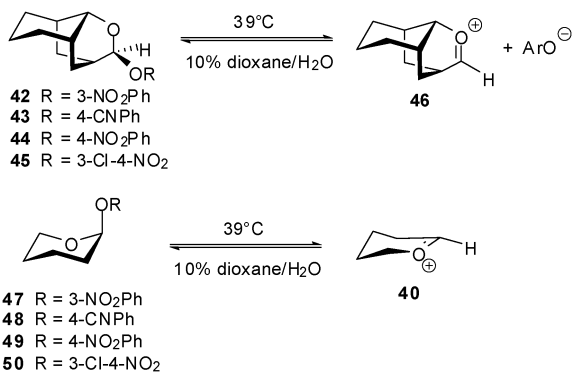


Fig. 18 Acidic hydrolysis of 2-methoxytetrahydropyrans according to Fraser-Reid.[46]

the ground state of these acetals. For methoxytetrahydropyran models **38** and **39**, the protonated axial anomer proceed through a half-chair transition state, thus leading to a half-chair oxycarbenium ion (**40**), while the protonated equatorial anomer **39** progresses through a *endo*-sofa transition state, thus leading to a sofa oxocarbenium ion (**41**).

Stereoelectronic effects on the reactivity of fixed tetrahydropyranyl acetals **42–45** have also been reported.[47] Rates of spontaneous hydrolysis at 39 °C were measured and compared with the rates of hydrolysis of the corresponding axial tetrahydropyranyl acetals **47–50** (Table 6). The spontaneous hydrolyses of acetals **42–45** are somewhat faster than for the axial tetrahydropyranyl acetals **47–50**, with a greater difference for better leaving groups. X-ray analysis revealed that the C–OAr bonds are shorter and the C–O intracyclic bonds are longer in tricyclic acetals **42–45** than in the corresponding tetrahydropyranyl acetals **47–50**. Considering the torsional strain which is generated in the transition state from chairs **47–50** to half-chair **40** (which is not generated in the pathway from **42–45** to **46**), the above observations suggest that the n(O)–σ*(C–O) donation from the

Table 6 Relative rates of hydrolysis for tetrahydropyranyl acetals



Leaving group ArO^-	k (s^{-1})	Relative rate
3-NO_2Ph	**42**: 2.85×10^{-5}	**47**/**42**: 1/2.7
4-CNPh	**43**: 1.23×10^{-4}	**48**/**43**: 1/3
4-NO_2Ph	**44**: 1.32×10^{-3}	**49**/**44**: 1/3.6
3-Cl-4-NO_2	**45**: 4.25×10^{-3}	—

Conditions: 10% dioxane/water, 39.2 °C.

synperiplanar lone pairs of the ring oxygen in tricyclic acetals **42–45** is weaker than from the antiperiplanar lone pairs in **47–50**. This cannot be a definitive conclusion as the kinetic selectivities observed are all very small. Differential solvation effects might also explain the results.

While acetal cleavage depends on n(O)–σ*(C–O) overlap between the lone pairs electrons on the donor oxygen and the σ* antibonding orbital of the C–OR bond to the leaving group, kinetic anomeric effects have been proposed to account for the diastereoselectivity observed in the addition of nucleophiles to glycosyl derivatives and analogues. For instance, Vasella *et al.*[48] suggested that kinetic anomeric effects are responsible for the high diastereoselectivities of the additions of lithium dialkyl phosphite to *N*-glycosylnitrones (Fig. 19).

The role of the kinetic anomeric effect was demonstrated by the difference in diastereoselectivities observed for the addition of $LiPO_3Me_2$ to *N*-glycosylnitrone **51** and to the deoxy analogue **52**. In THF, the difference in diastereoselectivities corresponds to a value of 1.1 kcal/mol while in CH_2Cl_2 it gives a value of 0.6 kcal/mol. Moreover the deoxy derivative **52** undergoes a slower reaction than its parent glycosylnitrone **51**.

Another relevant example is found with the addition of 3-aminopropan-1-ol to 5-bromo-5-deoxy-D-xylose. The formation of the least stable stereomer **58** is 20 times as fast as that of **59** (at equilibrium [59]/[58] = 7.3).[49] This kinetic selectivity was interpreted in terms of transition structures **60** and **61** which imply *N*-alkylation of a tetrahydrooxazine intermediate as the discriminating step. The faster formation of the least stable product **58** arises from transition state **60** in which *N*-alkylation corresponds to an axial attack of the oxazine intermediate. This is easier than equatorial attack in transition state **61** (Fig. 20).

Pikho and co-workers[50] found that nonanomeric [6,5]-spiroketals (having a pyranoside moiety with an equatorial C–O acetal bond) can be formed under conditions of kinetic control. For instance, **62** undergoes acid catalyzed spiroacetalization giving the "anomeric" (most stable) acetal **63**

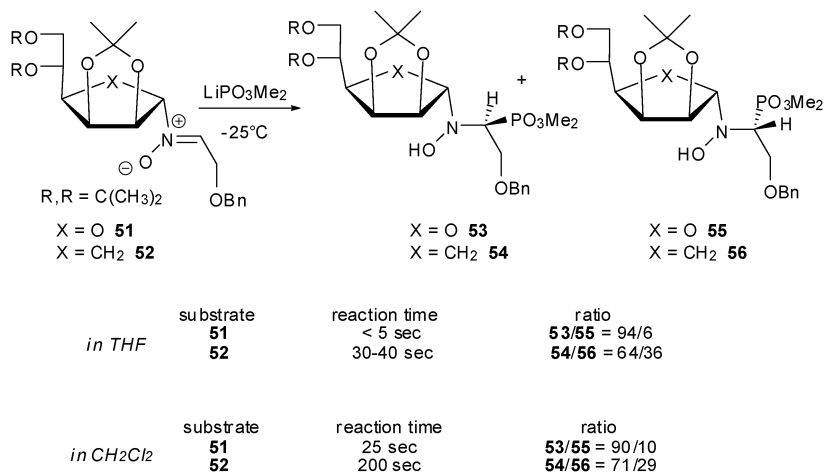


Fig. 19 Addition of lithium dialkyl phosphate to *N*-glycosylnitrones and analogues.

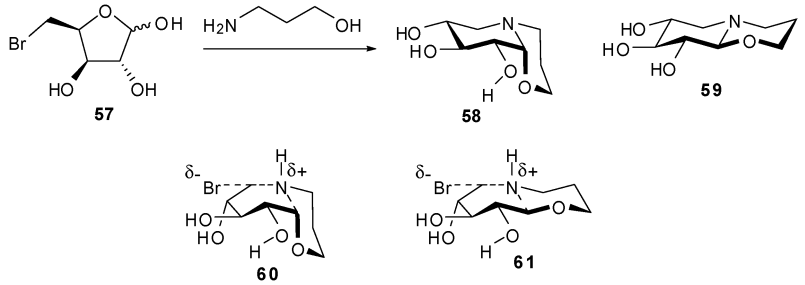


Fig. 20 *N*-Alkylation of tetrahydro-1,3-oxazines according to Berges.[49]

(*gauche*, *gauche*) when treated with CF_3COOH in THF/H_2O at 25 °C. Using a weaker acid such as $ClCH_2COOH$, the less stable, ''nonanomeric'' isomeric spiroketal **64** (*gauche*, *anti*) forms competitively with **63**. These results have been interpreted in terms of formation of oxycarbenium ion intermediates that can undergo cyclization according to the two diastereomeric transition states **65** and **66** (Fig. 21). Pseudo-axial attack (**65**) leads to the ''anomeric'' spiroketal **63**, whereas pseudo-equatorial attack (**66**) generates the ''nonanomeric'' isomer **64**. Under stronger acidic conditions **64** isomerizes into **63**. These interesting results show that the stability difference between the anomeric (**63**) and nonanomeric acetal **64** does not manifest itself in the transition states **65** and **66**. The latter must be seen as early transition state toward product formation. Considering the reverse heterolysis **63** → **65** and **64** → **66**, the stabilizing $2p(O) \leftrightarrow \sigma^*_{(C(1)–O(5'))}$ interaction in **63**, which is suppressed in **64**, does not make transition state **65** more stable than **66**, what is reminiscent to the observation that synperiplanar as well as antiperiplanar oxygen lone pairs can play similar role in influencing heterolytical reaction rates of acetals and derivatives.[45] It is therefore not surprising that the glycosylation of alcohols under acidic conditions see their α *vs.* β stereoselectivity under kinetically controlled

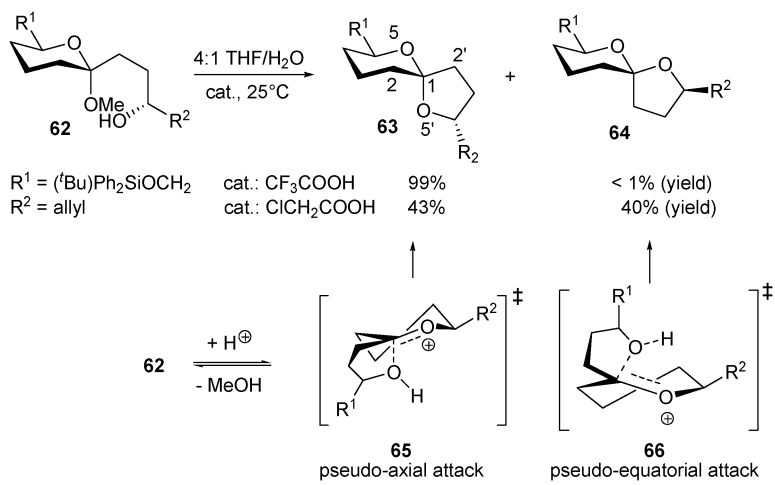


Fig. 21 Direct kinetic formation of ''nonanomeric'' [6.5]-spiroketals in aqueous media.

conditions strongly influenced by the nature of the glycosyl donor (relative configuration of the leaving group and of the other substituents) and of the glycosyl acceptor, by the nature of the promotor, the medium and temperature.[51]

5 Conclusion

In pyranosides and related acetals, *gem*-dialkoxy disubstitution affects the thermodynamic (10–12 kcal/mol of stabilization with respect to *n*,*n* + 1-dialkoxyalkane isomers) and dynamic properties of these compounds. The stability differences (conformational endo- and exo-anomeric effects) between various staggered conformers of acetals (contrasteric stabilization effect of 2–3 kcal/mol) represent only a fraction of the enthalpic anomeric effect. Thus, the least stable *anti*,*anti* conformers, in which hyperconjugative $n(O) \leftrightarrow \sigma^*_{C-O}$ stabilizing effect is not permitted, benefit of 6–10 kcal/mol of stabilization with respect to their isomeric *n*,*n* + 1-dialkoxyalkanes. Thus, the dipole/dipole (hard/hard) stabilizing interactions play a more important role than $n(O) \leftrightarrow \sigma^*_{C-O}$ (soft/soft: polarisability effect) in pyranosides and acetals. It is not a surprise that the relative stability of pyranosides and acetal conformers depend very much on the nature of the substituents (introducing other dipole/dipole interaction, hydrogen bridging, *etc*.) and the medium. Although it is demonstrated that $2p(O) \leftrightarrow 2p(C^+)$ interaction strongly stabilizes oxycarbenium intermediates and thus affects the rate of the heterolytical reactions at the anomeric center of pyranosides (and furanosides), no simple rule can be given for the α *vs*. β-stereoselectivity under kinetically controlled conditions. The rate of heterolytical dissociation at the anomeric center and of nucleophilic quenching of the intermediate glycosyl cation intermediates does not depend only on the axial/equatorial position of the leaving groups, but on several other factors such as the nature of the leaving group (formation of tight, solvent-separated, free ion pairs), the relative configuration and nature of the substituents of the glycosyl donor and glycosyl acceptor as well as on the medium, concentration and temperature.

Acknowledgements

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Stereoselective synthesis of 1,2-*cis*-glycosylic linkages

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Stereoselective methods for the preparation of 1,2-*cis*-*O*-glycosides and 1,2-*cis*-*C*-glycosyl compounds are presented.

1. Introduction

The stereoselective formation of glycosylic linkages is one of the most important goals in oligosaccharide chemistry because of the crucial role of the glycoside structure in the biological processes involving oligosaccharides.[1] The α/β-stereoselectivity in glycosylation reactions is affected by the steric and stereoelectronic effects around the anomeric center which depend on the conformation of the glycosyl donor substrates. Although high levels of stereoselectivity can be controlled with judicious choice of reaction conditions, such as, solvent, temperature and promoter as well as the protecting and leaving group pattern on the glycosyl donor, complete control of the stereochemistry of the anomeric center during the glycosylation process is a difficult task.

The anomeric linkages can be classified according to the relative and absolute configuration at C-1 and C-2 (Fig. 1). 1,2-*cis*-Glycosylic linkages

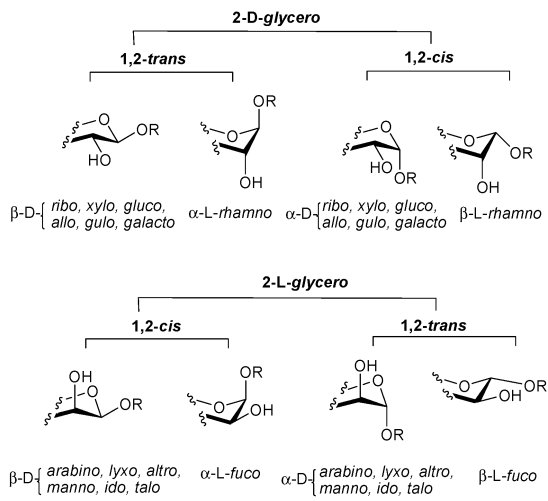

Fig. 1 Different types of glycosidic linkages.

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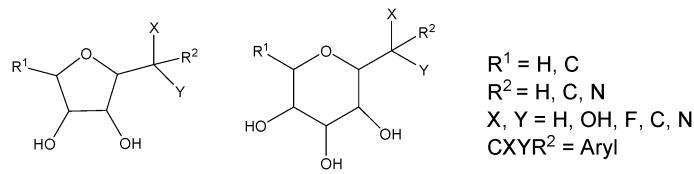


Fig. 2 Different types of *C*-glycosyl compounds.

are present in α-(ribo-, xylo-, allo-, gluco-, gulo- and galacto)pyranosides for 2-D-*glycero* series, and in β-(arabino-, lyxo-, altro-, manno-, ido- and talo)pyranosides for 2-L-*glycero* series. 1,2-*trans*-Glycosylic linkages are found in β-(ribo-, xylo-, allo-, gluco-, gulo- and galacto)pyranosides for 2-D-*glycero* series, and in α-(arabino-, lyxo-, altro-, manno-, ido- and talo)pyranosides for 2-L-*glycero* series. 1,2-*cis* Glycosyl residues are found in α-L-fuco- and β-L-rhamnopyranosides while 1,2-*trans* glycosyl residues are found in β-L-fuco- and α-L-rhamnopyranosides.

Closely related to the above compounds, *C*-glycosyl compounds which are *O*-glycoside analogues with a methylene or substituted methylene group (CH_2, CHX, CXY) replacing the *exo*-oxygen atom of the glycosylic bond, are also an important class of compounds[2] (Fig. 2). *C*-glycosyl compounds show improved chemical and biochemical stability compared to their corresponding *O*-glycosides[3] and hence, serve as carbohydrate analogues resistant to metabolic processes and have potential therapeutic value. *C*-glycosyl compounds found in nature as well as synthetic analogues present quite often interesting biological properties[4] or are useful drugs.[5]

The important role of *O*-glycosides and *C*-glycosyl compounds in biology and biomedicine has been a major incentive for devising new processes for their chemical and enzymatic syntheses. Numerous glycosylation methods for the synthesis of *O*-glycosylic linkages have been reported.[6] The last two or three decades have witnessed dramatic improvements in the methods available for the synthesis of complex oligosacharides,[7] and also a wide range of methodologies have been developed for the synthesis of their *C*-glycosyl counterparts.[8]

While the formation of 1,2-*trans*-glycosides can be easily controlled by the intramolecular neighbouring group participation of acyl groups at C-2,[9,10] the formation of 1,2-*cis*-glycosides usually requires donors with a non-assisting functionality at C-2. Despite the favorable stereoelectronic preference, this usually leads to a mixture of anomers that requires time-consuming purification protocols, reduced yields and limits one-pot multistep glycosylations.

1,2-c*is*-*O*-Glycosylic linkages and their mimetics 1,2-*cis*-*C*-glycosylic linkages are present in many naturally occurring compounds of biological, pharmaceutical and industrial interest. The introduction of these types of linkages requires special glycosylation strategies and tuning of the reaction parameters. Enormous efforts have been devoted to the search for new strategies of stereoselective synthesis of 1,2-*cis* glycosylic linkages.

The aim of this chapter is to describe the methods currently available for the stereoselective synthesis of compounds having 1,2-*cis*-*O*-glycosylic or 1,2-*cis*-*C*-glycosylic linkages.[11]

2. Stability of 1,2-*cis*-glycosylic linkages

When an electron-withdrawing substituent different from OH, is axial at C-1 (α-anomer for D-sugars in $^4C_1$ and for L-sugars in $_1C^4$ conformation), there is a stabilization *via* hyperconjugation owing to the periplanar orientation of both non-bonding orbital of the endocyclic oxygen electron lone pair and the anti-bonding orbital of the axially substituted compound (endo-anomeric effect).[12] The interaction of the non-bonding exocyclic oxygen electron lone pair with the anti-bonding orbital in an axially substituted compound can also contribute to the stabilization of the molecule (exo-anomeric effect).[13] However, in the equatorial conformations, only the exo-anomeric effect is possible. It has been determined that the endo-anomeric effect is stronger than the exo-anomeric effect in axial conformations. Dipole stabilizations and electrostatic repulsions[14] as well as solvent effect have been also taken into consideration to explain the anomeric effect.[12,15] 1,2-*cis*-β-D- or -β-L-Glycosides bearing an equatorial electron-withdrawing group at C-1 are thermodynamically less stable than the corresponding axial anomers due to the lack of the endo-anomeric effect. Additionally, those belonging to 2-L-*glycero* series having an axial group at C-2 (*arabino*, *lyxo*, *altro*, *manno*, *ido*, *talo*) are also destabilized by means of steric repulsion by the axial C-2 substituent (Fig. 3).

In summary, for *O*-glycosides, α-anomers (α-D and α-L) are thermodynamically more stable than the β-anomers (β-D and β-L) due to the anomeric effect. Therefore 1,2-*cis*-α-D- or α-L-glycosides are preferentially obtained, in general. On the other hand, the lower stability of glycosides such as β-D-arabinosides and β-D-mannosides requires special methods for their preparation, thus promoting the development of new synthetic methodologies.

The substitution of an oxygen atom by a methylene group modifies both the structural parameters and the electronic properties.[16] Hence, the exo-anomeric effect due to the presence of the acetal function is no longer seen in *C*-glycosyl compounds and consequently, the associated variation in the steric interactions between the residues is not seen either. Kishi and co-workers have proposed that *O*-glycosides and *C*-glycosyl compounds share the same conformational characteristics in the free state.[17] However, it has been claimed that this is not a general phenomenon and different conformational behavior of *C*-glycosyl compounds and their *O*-linked counterparts have been described.[18] Intuitively, it seems plausible that,

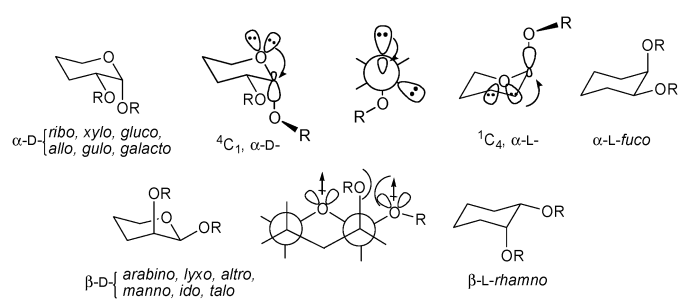


Fig. 3 Endo-anomeric effect.

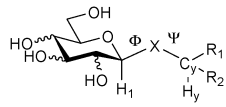
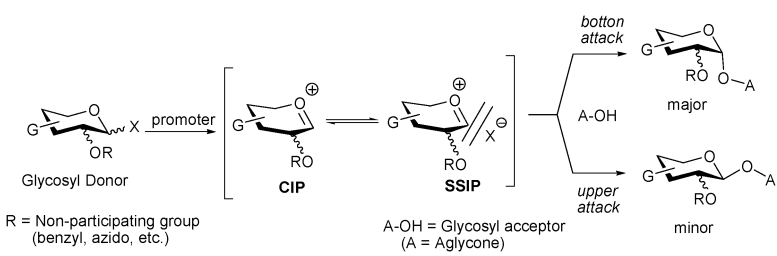


Fig. 4 Definition of Φ and Ψ angles in *C*-glycosyl compounds.

because of the longer interglycosylic distances, the rotameric equilibrium around Ψ angles of *C*-glycosyl compounds should involve smaller energy barriers than those around Ψ angles of *O*-glycosides. In the absence of stereoelectronic effects, the spatial orientation of the OH-group at the 2-position of the pyranose ring should strongly influence the conformational equilibrium. The importance of steric effects around Φ angle could be obtained through systematic comparison of differently configurated *C*-glycosyl compounds. Studies along this topic have been reported[19] (Fig. 4).

3. Factors influencing anomeric control in 1,2-*cis*-glycosylations

An *O*-glycosylation reaction is a nucleophilic displacement of a leaving group attached to the anomeric carbon of a sugar moiety by an alcohol ROH, or by the OH group of another partially protected sugar moiety. Mechanistic considerations indicate that this nucleophilic reaction in most cases often follows a S_N1-type mechanism,[20] and therefore, low stereoselectivity could, in principle, be expected. The major requirement to control the stereochemical outcome of 1,2-*cis*-glycosylations is the use of glycosyl donors bearing a non-participating substituent at C-2. The choice of the combination leaving group/promoter/solvent plays a crucial role for the anomeric stereocontrol of a glycosylation, especially when a non-participating group is at C-2 position, because they influence the equilibrium between the proposed intermediates of the reaction: covalent donors, contact ion pair (CIP), or solvent separated ion pair (SSIP). Similar considerations can be extended to the formation of *C*-glycosyl compounds with a carbon nucleophile (Scheme 1). Structural elements of the reactants and a careful choice of reaction conditions give good 1,2-*cis* stereoselectivities. For instance, Mukaiyama coupling conditions ($SnCl_2$, $AgClO_4$) with galactosyl fluorides that proceeded predominantly with α-stereoselectivity, have been used by Ogawa and co-workers in the synthesis of an α-(1→4)-dodecagalacturonic acid.[21]



Scheme 1 Glycosylation mechanisms.

The participation of solvents plays an important role. The use of non-polar solvents, favours the glycosylation reaction to follow a S_N2-like

pathway. Under S_N1-type conditions, the participation of solvents can markedly improve the stereoselectivity in glycosylation of donors with non-assisting functionalities. In diethyl ether or THF as solvents and using strong acid promoters, the solvent participates forming equatorial oxonium cations due to the reverse anomeric effect,[12,22] which favours thermodynamically stable α-glycosides. Recently, catalytic ($HClO_4$) stereoselective glycosylations with glycosyl diphenyl phosphates in dioxane/Et_2O (1:1) afforded glycosides in good yields and good to excellent 1,2-*cis*-α-selectivities.[23] The presence of acetonitrile as a co-solvent can favour a β-D (or β-L) or α-D (or α-L) selectivity by the so-called ''Nitrile Effect''. Acetonitrile, as polar solvent, favours a S_N1 mechanism solvating the oxonium cation with preference at the α-face and forms the kinetically controlled α-nitrilium-nitrile complex. This complex can render the β-anomer by nucleophilic substitution by an alcohol. On the other hand, the more thermodynamically stable β-nitrilium-nitrile complex due to the reverse anomeric effect, favours the α-anomer after reaction with the alcohol.[24] Isolation of Ritter-type products reinforce the formation of the initial nitrilium-nitrile complex.[25] Recently,[26] the nitrile effect was used to increase the β-selectivity of a number of L-rhamnopyranosylations[27] and the α-selectivity for the synthesis of 1-adamantanyl thiosialoside.[28] High-throughput optimization of the solvent system was performed in the construction of three continuous 1,2-*cis*-α-glucosidic linkages, resulting in mixtures of $CHCl_3$ and ethereal solvents having a synergetic effect in enhancing the 1,2-*cis*-selectivity.[29]

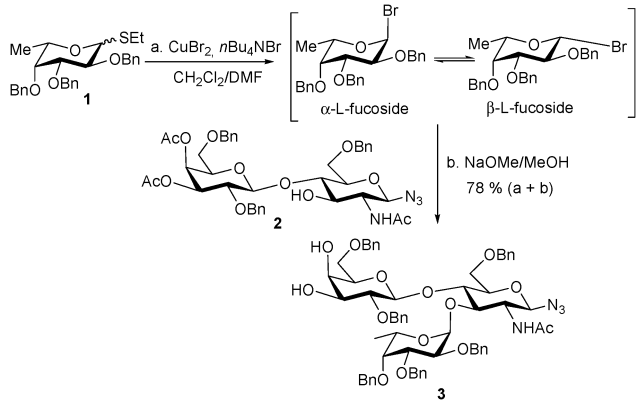
However, in spite of the fact that good results were obtained by an appropriate choice of reactants and of the reaction conditions, a great deal of attention has been devoted during recent years to the search for successful comprehensive methods for 1,2-*cis*-glycosylations. They are discussed in the next sections.

4. Methods for the preparation of 1,2-*cis*-*O*-glycosides

4.1 *In situ* anomerization procedure

Lemieux first introduced this method[30] which is based on the equilibrium established between an α-glycopyranosyl halide and the more reactive β-glycosyl halide catalysed by halide ions derived from tetra-alkylammonium halides. The reaction proceeds with inversion of a highly reactive β-halide with the alcohol component *via* nucleophilic substitution. This reaction is thought to proceed through several intermediates and requires very reactive glycosyl halides (armed) and long reaction times, in particular when the original tetra-alkyl ammonium bromides are used as catalysts. The use of other liophilic promoters such as $HgBr_2$,[31] $AgClO_4$ and AgTfO[32] makes it possible to carry out the reaction with even less reactive halides. However, the stereoselective outcome of the glycosylation is very dependent not only on the reactivity of the catalyst, but also on the reactivity of both the halide and the acceptor. Careful adjustment of the reactivity of the two different components is essential in order to obtain satisfactory results. This procedure has proven to be very useful for derivatives with D-*gluco*, D-*galacto* and L-*fuco* configuration.[12,33] Kunz and co-workers have recently used the *in situ* anomerization conditions in the synthesis of Lewis X azide **3**

by reaction of thioglycoside **1** with lactosamine derivative **2** in the presence of tetrabutylammonium bromide (Scheme 2).[34]



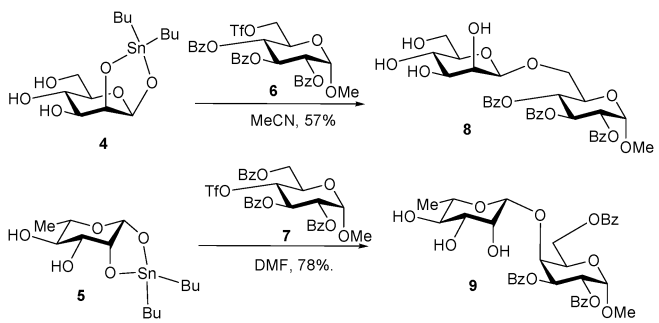
Scheme 2 Synthesis of Le^x azide **3** by *in situ* anomerization.

4.2 Glycosylation by heterogeneous catalysis

The Koenigs-Knorr method in the presence of an insoluble silver salt proceeds mainly with inversion of configuration. Silver silicate and silver-silicate-aluminate have often been used as the heterogeneous catalyst. This procedure has been traditionally used for the synthesis of β-mannosides and has been recently reviewed.[35] However, it only works well with very reactive halides and sufficiently reactive alcohol components.

4.3 Glycosylation through locked anomeric configuration

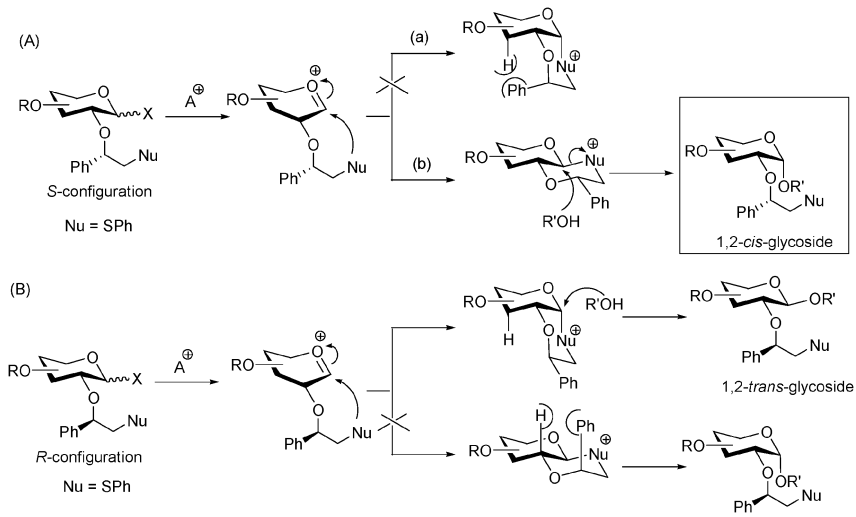
This method implies the use of 1,2-stannylene acetals as glycosyl donors (Scheme 3). The reaction of *cis*-1,2-stannylene acetals of D-mannose and L-rhamnose (**4** and **5**, respectively) with primary and secondary trifluoromethanesulfonates derived from sugars **6** and **7**, affords 1,2-*cis*-linked disaccharides such as β-D-mannopyranoside **8** and β-L-rhamnopyranoside **9**. In the case of secondary triflates, the new glycosylic linkage is formed with complete inversion of the configuration in the electrophile.[36] Nicolaou and co-workers[37] have used stannylene chemistry to couple two sugar moieties through both anomeric centres.



Scheme 3 Synthesis of β-D-mannosides and β-L-rhammnosides by glycosylation *via* locked anomeric configuration.

4.4 Neighbouring group participation

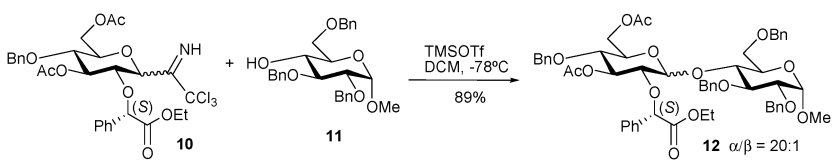
The formation of 1,2-*cis*-glycosides can be also controlled by the participation of certain groups (Scheme 4). Thus, Boons and co-workers[38] have reported the use of (1*S*)-phenyl-2-(phenylsulfanyl)ethyl moiety at C-2 of a glycosyl donor, as a participating neighbouring group that leads stereoselectively to the formation of α-glycosides. The participating group gives a quasi-stable anomeric sulfonium ion formed as a *trans*-decalin ring system due to steric and electronic factors. The alternate *cis*-fused system will place the phenyl substituent in an axial position inducing unfavourable steric interactions (Scheme 4(A), pathway a). Subsequent displacement of the equatorial anomeric sulfonium ion by a sugar alcohol leads to the formation of the 1,2-*cis*-glycoside (Scheme 4(A), pathway b).



Scheme 4 (A) Neighbouring group participation by an (*S*)-auxiliary at C-2 leading to 1,2-*cis*-glycosides. (B) Neighbouring group participation by an (*R*)-auxiliary at C-2 leading to 1,2-trans-glycosides.

Alternatively, the use of an auxiliary with an (*R*)-stereochemistry at the phenyl-2-(phenylsulfanyl)ethyl moiety will lead to the formation of a 1,2-*trans*-glycoside because in this case the *trans*-decalin system will exert unfavourable steric interactions. The proposed mechanism has been supported by computational studies.[39]

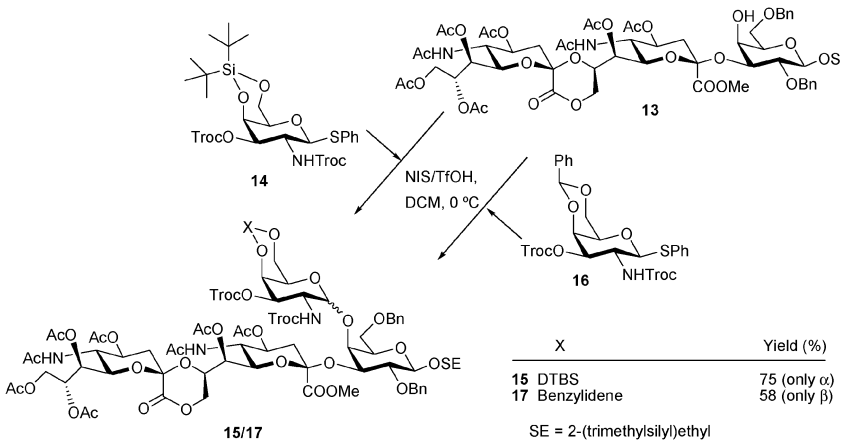
As an example, glycosylation of trichloroacetimidate **10** with acceptor **11** using catalytic amounts of TMSOTf in DCM at −78 °C gave disaccharide **12** in 89% yield and good α-stereoselectivity (Scheme 5).



Scheme 5 Stereoselective 1,2-*cis*-glycosylations with chiral auxiliaries at C-2 of glycopyranosyl donors.

4.5 Locking donor conformation with bulky protecting groups

The introduction of a cyclic di-*tert*-butylsilylene (DTBS) protecting group at 4-OH and 6-OH in a *galacto* type glycosyl donor, has been used as donor for successful 1,2-*cis*-galactosylation reactions despite the presence of C-2 participating functionalities.[40] Thus, reaction of complex acceptor **13** with 4,6-*O*-di-*tert*-butylsilylene (DTBS)-protected phenylthio-galactoside **14** gave mainly the 1,2-*cis*-glycoside **15**, whereas using 4,6-di-*O*-benzylidene protected phenylthio-galactoside **16**, the 1,2-*trans*-glycoside **17** was mainly obtained due to the neighbouring group participation of NHTroc group[41] (Scheme 6). Other related examples were recently described.[42] The method is also applicable to the formation of GalN-Ser/Thr linkages,[43] found in natural oligosaccharides such as globo and isoglobo glycolipids (Gal-α-(1→4)-Gal sequence) and mucin-type glycoproteins.



Scheme 6 Di-*tert*-butylsilylene-directed α-galactosylation.

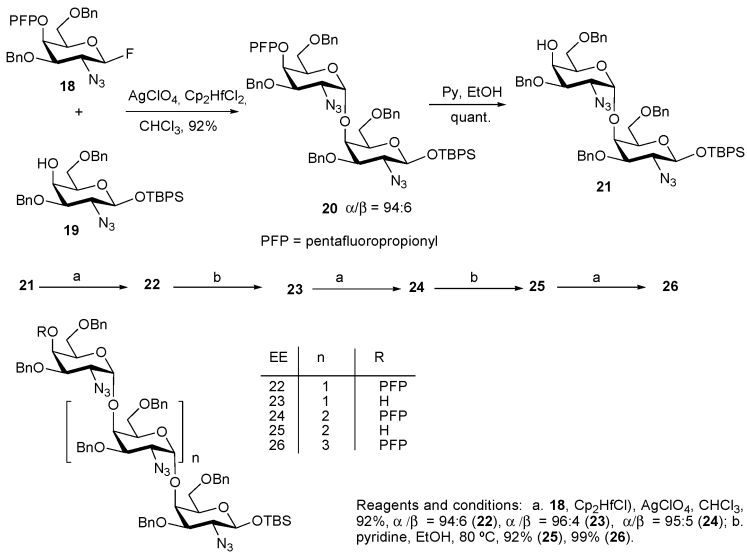
1,2-*cis*-Selective glycosylations influenced by bulky protecting groups were also carried out with furanose moieties. Thus, stereoselective β-(1,2-*cis*)-selective arabinofuranosylation was achieved using the 3,5-*O*-di-*tert*-butylsilylene[44] or 3,5-*O*-di-isopropylsilylene (TIDPS) protected arabinofuranosyl donors. The latter was used in the synthesis of an hepta-arabinofuranoside fragment of a Mycobacterial arabinan.[45]

Manabe and Ito have reported[46] the use of *N*-benzyl-2,3-*trans*-oxazolidinones as selective glycosyl donors for the synthesis of 1,2-*cis* glycosylic linkages.

4.6 Polar driving stereodirection for 1,2-*cis*-galactosylation by strong electron-withdrawing groups

Protection of the OH-4 on a Gal*p*N donor promotes the stereoselective construction of the α-(1→4)-Gal*p*NAc linkages. This strategy was applied to the synthesis of the hexasaccharide α-Gal*p*NAc-(1→4)-α-Gal*p*NAc-(1→4)-[β-Glc*p*-(1→3)]-α-Gal*p*NAc(1→4)-α-Gal*p*NAc-(1→4)-Gal*p*NAc

by coupling 2-azido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-pentafluoropropionyl(PFP)-β-galactopyranosyl fluoride **18** and acceptor **19** (Scheme 7).[47] The 1,2-*cis* selectivity of 4-PFP-galactoyranosyl donors has been explained by electrostatic considerations. An α-glycosylation will neutralize the strong dipole moment caused by the PFP group in the axial orientation, thus enhancing the α-selectivity. A stepwise synthesis starting from disaccharide **21** led to the linear pentasaccharide **26**.

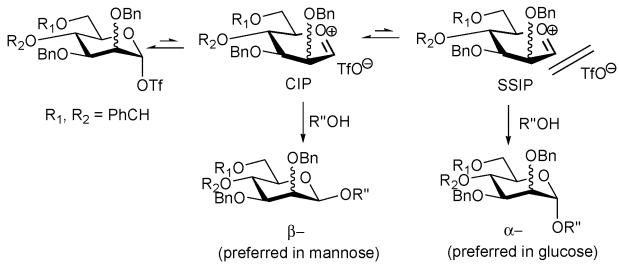


Scheme 7 Synthesis of a linear pentasaccharide.

4.7 *Via* the transient formation of glycopyranosyl triflates

Crich and co-workers have developed a methodology for the stereoselective formation of hindered glycosides such as β-mannopyranosides[48] and α-glucopyranosides from thioglycosides having either non-participating or participating protecting groups at C-2. Thus, activation at −78 °C with PhSOTf/2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) or *S*-(4-methoxyphenyl)-benzenethio-sulfinate/Tf_2O/DTBMP, or 1-benzenesulfinylpiperidine (BSP)/Tf_2O cleanly leads to the formation of glycosyl triflates. Subsequent coupling with a range of secondary and tertiary glycosyl acceptors, gives high β-selectivity with an α-mannosyl triflate while the corresponding α-glucosyl triflate is α-selective.[49] This was supported by parallel observations from other groups using sulfoxides as triflate precursors.[50] The reaction takes place through the intermediacy of a covalent glycosyl triflate, which has the α-configuration in both *manno* and *gluco* series. The stereoselective construction of 1,2-*cis* glycosylic linkages depends on the equilibrium between the intermediates of the reaction: covalent donors, contact ion pair (CIP), or solvent separated ion pair (SSIP) (Scheme 8).[51] Factors destabilizing the oxycarbenium ion result in a diminished concentration of

the SSIP and in an increased β-selectivity. Variations on the O2–C2–C3–O3 torsional interactions as the covalent triflates collapse to the ion pairs, are responsible for the glucose/mannose stereoselectivity shift. In mannose, the formation of the oxycarbenium ion is more endothermic because the torsion angle is compressed as the ion is formed, whereas in glucose it is relaxed and so, the formation of the glucosyl oxycarbenium ion is less endothermic than that of its mannosyl counterpart. This leads to a higher concentration of solvent separated ion pairs in glucose than in mannose. β-Selectivity results from the attack on the covalent triflate or the associated CIP, whereas α-selectivity arises from attack on the SSIP.[52]



Scheme 8 Glycosylation through transient glycosyl triflates.

The benzylidene acetal exerts its influence through a combination of torsional factors that come into play as the covalent triflate chair conformer flattens to the oxycarbenium ion[53] and to an electronic effect, associated with locking C5–C6 in the *tg* conformation (Fig. 5) by maintaining the C5–C6 bond in the most electron-withdrawing *tg* conformer. In the *tg* conformer, the C6–O6 bond acts as a dipole with the negative terminus directed away from the electron-deficient center of the oxycarbenium ion, thereby destabilizing this key glycosyl transfer intermediate, whereas in the *gg* and *gt* conformers, this dipole is gauche to the developing positive charge, making the oxycarbenium ion more stable.[54] This reversal of selectivity has been the subject of computational investigations.[55]

Substituents at C-2 and C-3 on the *gluco* and *manno* framework influence markedly the 1,2-*cis*-stereoselectivity. Substitution of a benzyloxy group at C-2 or C-3 by a fluorine atom, on series of 4,6-*O*-benzylidenegluco- and mannopyranosyl donors decreases the selectivity, thus reinforcing the influence of the O2–C2–C3–O3 torsion angle.[56] Significant improvement in the β-selectivity of many mannosylation reactions was achieved with the introduction on the 4,6-*O*-benzylidenemannose framework of a benzyloxy group at C-3 and little bulked and modest disarmed propargyl ethers at C-2.[57] In particular, the introduction of 4-trifluoromethylbenzenepropargyl ethers at C-2 gives extremely β-selective glycosylations. This protecting

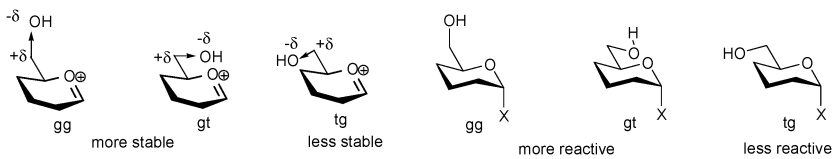
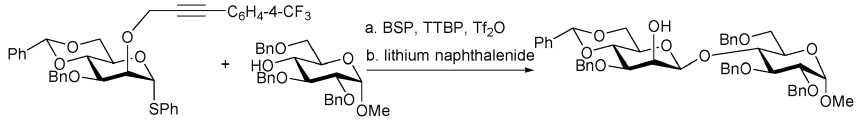


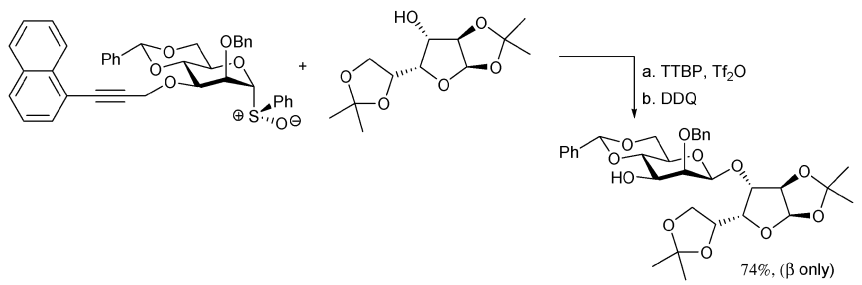
Fig. 5



Scheme 9 β-Selective glycosylations with a 4-trifluoromethyl-benzenepropargyl ether at C-2.

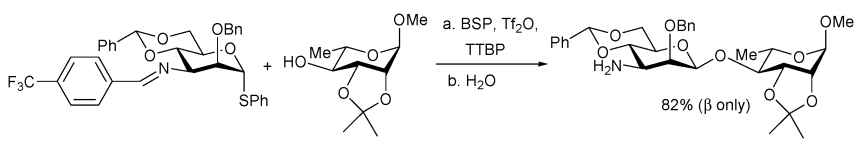
group offers the possibility of orthogonal cleavage in a single step with lithium naphthalenide (Scheme 9).[58]

Similarly, β-L-rhamnopyranosides are readily formed with good selectivity by means of thioglycoside donors protected with a 2-*O*-sulfonate and 4-*O*-benzoyl esters and activated with the combination of 1-benzenesulfinyl piperidine and triflic anhydride in the presence of 2,4,6-tri-*tert*-butyl-pyrimidine (TTBP).[59] The introduction of a naphthylpropargyl ether group at C-3 of a 4,6-*O*-benzylidene-2-*O*-benzyl-mannosyl donor in conjunction with the sulfoxide glycosylation method, afforded extremely β-selective coupling reactions and the possibility of orthogonal cleavage in a single step with DDQ (Scheme 10).[60]



Scheme 10 β-Selective glycosylations with a naphthylpropargyl ether at C-3.

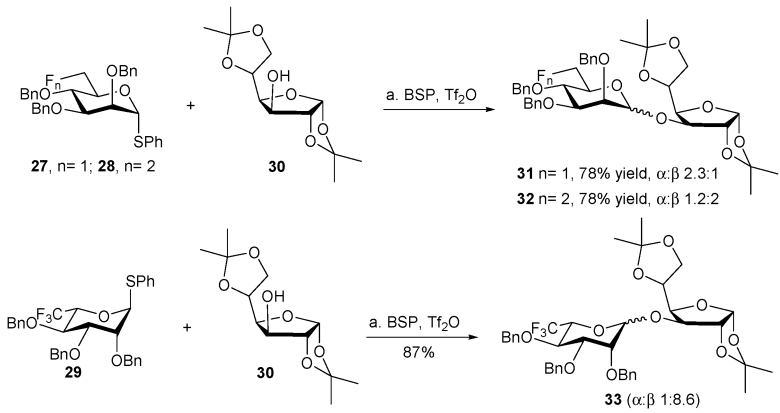
In the 3-amino-3-deoxyglucopyranoside and 3-amino-3-deoxymanno-pyranoside series, the introduction of phthalimido and *p*-trifluoromethyl-benzylidene imine, respectively, as nitrogen protecting groups and using the BSP glycosylation method, afforded glycosylic linkages with complete 1,2-*cis*-selectivity (Scheme 11).[61]



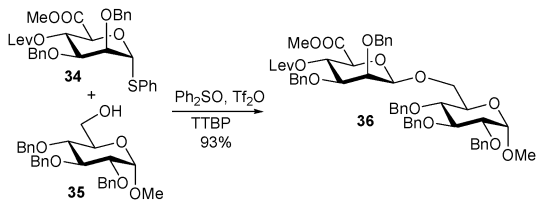
Scheme 11

The electron-withdrawing capability of the disarming substituent at the 6-position also controls the stereochemical outcome of the Crich glycosylation method. Thus, 6-mono- and difluoro substituted *S*-phenyl 2,3,4-tri-*O*-benzyl-D-mannopyranosides **27** and **28** and the trifluoro analogue of *S*-phenyl

2,3,4-tri-*O*-benzyl-α-L-rhamnopyranoside **29** react with 1,2-5,6-di-*O*-isopropylidene-α-D-glucofuranose **30** to give disaccharides **31**–**33** in good yield (Scheme 12). The β/α ratio of the disaccharide products increases, going from nonfluorinated substrates to monofluoro donor **27**, to difluoro donor **28**, and further to trifluoro donor **29**. The β-selectivity of rhamnopyranoside derivative **33** is higher than that of the mannopyranosyl donors due to the higher electronegativity of the trifluoro methyl group and obviously due to steric factors.[62] The C5-carboxylate group of 1-thiomannuronate ester donors also leads to the predominant formation of the 1,2-*cis* products. Thus, glycosylations with disarmed 1-thiomannuronate **34** in the presence of Ph_2SO/Tf_2O take place with high β-selectivity[63] (Scheme 13).



Scheme 12



Scheme 13 Glycosylation reaction of a mannuronate ester donor.

Mechanistically, it has been recently postulated that the C5-carboxylate ester prefers to occupy an axial position in the oxycarbenium intermediate, thereby favoring the formation of the $^3H_4$ half-chair over the $^4H_3$ conformer. Nucleophilic attack on the $^3H_4$ half-chair intermediate occurs in a β-fashion, providing the 1,2-*cis*-mannuronates with excellent stereoselectivity (Fig. 6).[64] The stereocontrol of mannuronate esters is independent of the type of donor employed and agrees well with this postulate.[65]

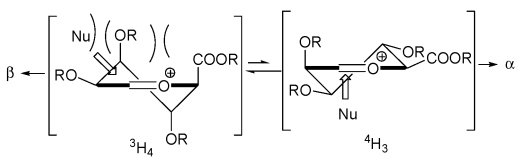
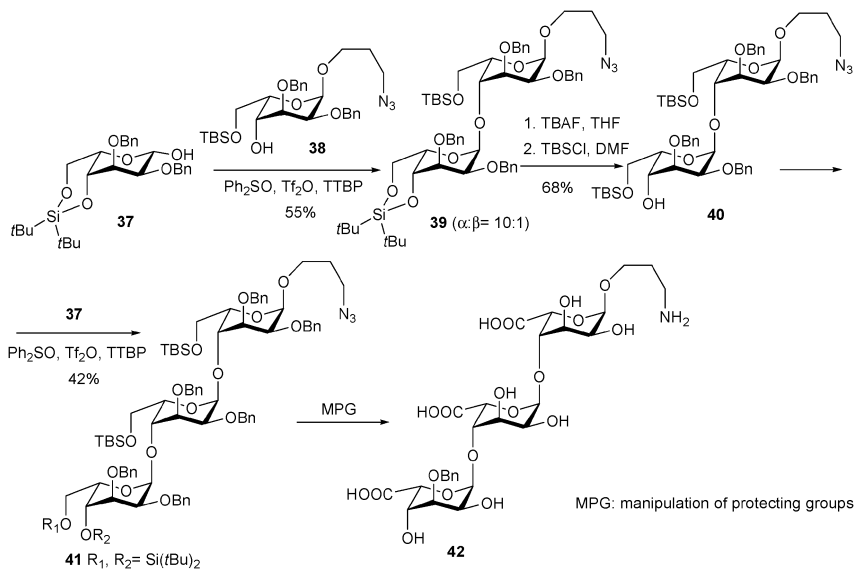


Fig. 6 Mannuronate ester oxycarbenium ion conformers.

The potential of the mannuronate ester donors in the formation of the β-mannosidic linkage has been demonstrated with the construction of a mannuronic acid alginate pentamer using a convergent orthogonal glycosylation.[64]

Codée and Marel[66] have described the synthesis of a guluronic acid trimer that forms part of L-guluronic acid alginates. The synthesis starts from di-*O*-*tert*-butylsilylene gulose **37**, that is coupled in the presence of Ph_2SO/Tf_2O to gulose **38** providing disaccharide **39** in 55% yield and good α-stereoselectivity (Scheme 14). Liberation of the hydroxy group at C-4′ furnishes disaccharide acceptor **40** which was elongated by subsequent glycosylation with **37** furnishing trimer **41**, obtained as a single diastereomer in a moderate 42% yield. Further protective group manipulations gives triacid **42** in excelent yield. It was believed that the high 1,2-*cis* selectivity came from the conformational preferences of the intermediate solvent-separated oxycarbenium ion which adopts the most favourable $^3H_4$ conformation.



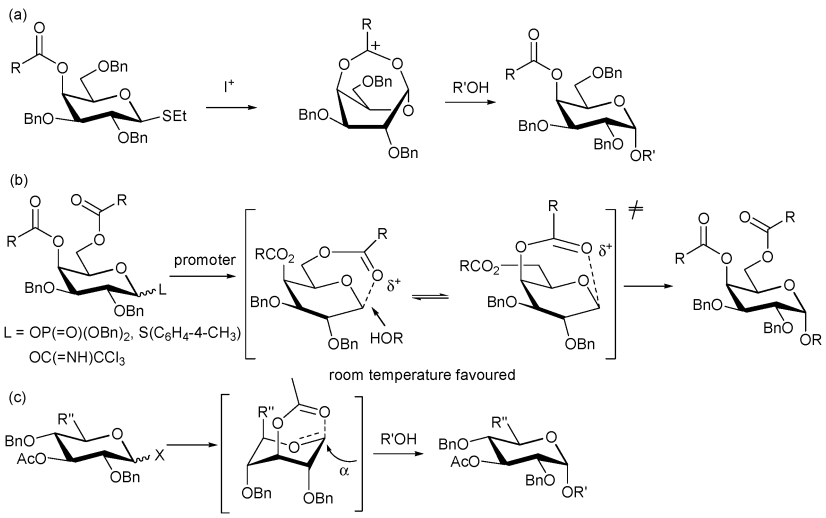
Scheme 14

The Crich method has been also applied for the direct stereocontrolled synthesis with 4,6-*O*-benzylidene acetal derivatives of D- and L-*glycero*-β-D-manno-heptopyranosides and was applied to a fully stereocontrolled block synthesis of tetra- and hexasaccharides β-D-*glycero*-D-*manno*-Hep*p*-(1→4)-[α-L-Rha*p*-(1→3)-β-D-*glycero*-D-*manno*-Hep*p*-(1→4)]$_n$-α-L-Rha*p*-OMe (n = 1, 2) corresponding to the repeating units of the glycan from the surface-layer glycoprotein from *Bacillus thermoaerophilus*.[67]

4.8 By assistance of remote substituents

The stereoselectivity of 1,2-*cis* glycosylations is improved by anchimeric assistance of remote substituents at C-3, C-4 or C-6 in the glycosyl donors.[68]

An enhanced α-selectivity over the perbenzylated glycosyl donor was observed in the reaction of different galactosyl donors having acyl groups at C-4,[69] C-6,[70] C-3[71] and C-4/C-6,[72] with a variety of acceptors under appropriate activation conditions (Scheme 15). In all cases the reaction takes place through an incipient oxycarbenium ion which is stabilized by the electron-donating substituents. The more stable oxycarbenium ion produces the α-anomer. The anchimeric participation of a remote 3-*O*-acyl substituent was also reported for the glycosylation with 2-deoxy-*ribo*-hexopyranosyl donors[73] and with 3-*O*-benzoyl-2,4-di-*O*-benzyl-fucosyl bromide, which proceeded with high α-stereoselectivity.[74] Other remote substituent effects were observed with allyloxy groups in the preparation of orthogonally protected chitosan oligosaccharides.[75]



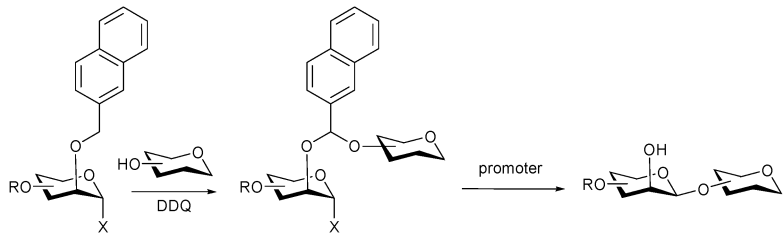
Scheme 15 Stereoselective glycosylations by (a) a remote C-4 *O*-acyl group, (b) remote C-4, C-6 *O*-acyl groups, (c) a remote C-3 *O*-acyl group.

4.9 Intramolecular aglycon delivery approach (IAD)

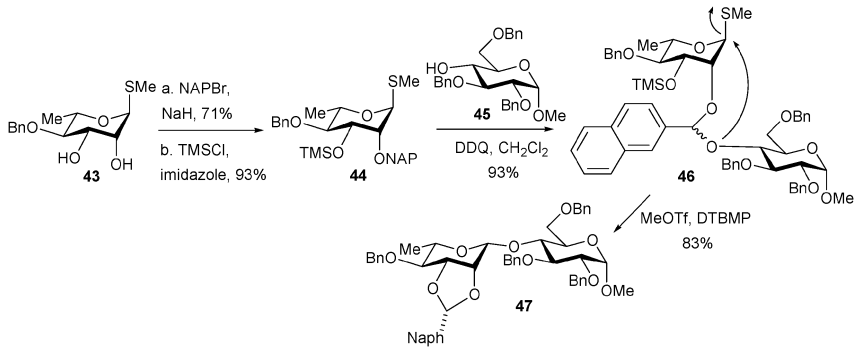
In this approach, the glycosyl donor and the glycosyl acceptor are linked by the 2-OH of the donor and the free OH of the acceptor. It is one of the most predictable and reliable methods for achieving 1,2-*cis* stereocontrol. Acetals, mixed *p*-methoxybenzylacetals and silicon tethering have been widely used as well as iodonium mediated tethering acetals derived from vinyl, allyl and allenyl ethers. These methodologies have been revised.[6,76]

Ito and co-workers[77] have recently reported the use of naphthylmethyl (NAP) ether mediated IAD (Scheme 16) for the construction of several 1,2-*cis* linkages (β-mannopyranosides, β-arabinofuranosides and α-gluco-pyranosides) of the non-reducing terminal structure of the tetrasaccharide $Glc_3Man_9GlcNAc_2$, a common precursor of all *N*-linked glycans. They also demonstrate the versatility of this approach with the stereoselective synthesis of the hindered β-L-rhamnopyranosides.[78] Rhamnosyl donor 2-*O*-NAP thioglycoside **44** was prepared from thiorhamnoside **43** after regioselective alkylation and subsequent protection. Reaction of **44** with

glucosyl acceptor **45** in the presence of DDQ as oxidant, gave the corresponding mixed acetal **46** in good yield. Subsequent IAD using MeOTf as promoter afforded disaccharide **47** as a single isomer (Scheme 17).

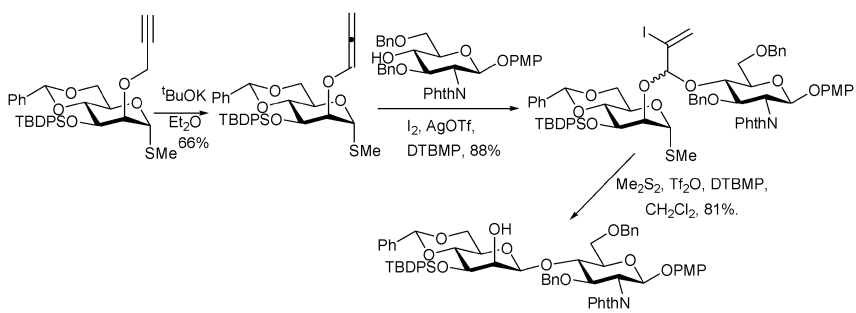


Scheme 16 Intramolecular aglycon delivery approach using NAP ether protected glycosyl donors.



Scheme 17 Synthesis of β-L-rhamnopyranosides.

Fairbanks and co-workers[79] have also recently described the use of propargyl mediated IAD for the synthesis of the key Manβ(1→4)GlcNAc linkage of *N*-glycan oligosaccharides. Isomerisation of a 2-*O*-progargyl group of *manno* thioglycoside donors to an allene is followed by iodonium ion mediated mixed acetal formation with the 4-OH of protected GlcNAc acceptors. Subsequent intramolecular glycosylation occurs with complete control of anomeric stereochemistry. The methodology represents a considerable improvement over the allyl IAD approach in terms of efficiency of the intramolecular glycosylation step for disarmed monosaccharide donors (Scheme 18).



Scheme 18 Intramolecular aglycon delivery approach using allene protected glycosyl donors.

5. Methods for the preparation of 1,2-*cis*-*C*-glycosyl compounds

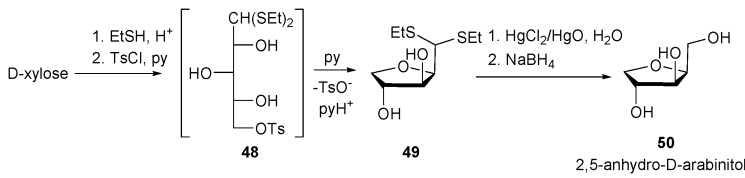
A wide range of methodologies has been developed for the synthesis of *C*-glycosyl compounds using sugars as starting materials and "*de novo*" procedures.[80,81] These methods are classified according to the type of reaction to create the anomeric C–C bond. We are presenting here only those related with the preparation of 1,2-*cis*-*C*-glycosyl compounds.

5.1 Acid-catalyzed dehydration

Dehydration of pentitols, hexitols, 1-amino-1-deoxypentitols and 1-amino-1-deoxyhexitols by heating in strong acid media, affords anhydropentitols presenting in some cases 1,2-*cis*-*C*-glycosylic linkages.[82]

5.2 1,5-Elimination of sulfonic esters on anomeric acetal derivatives

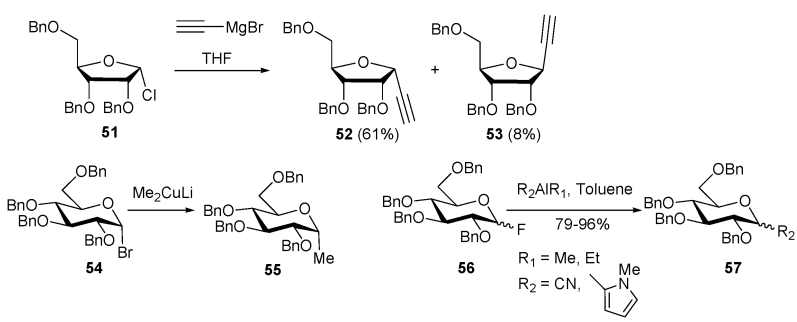
The dithioacetal of D-xylose generates a primary tosylate **48** that can undergo a 1,5-elimination under basic conditions giving the corresponding 2,5-anhydropentose dithioacetal **49**. Hydrolysis of the dithioacetal and $NaBH_4$ reduction furnishes the corresponding 2,5-anhydropentitol **50**[83] (Scheme 19).



Scheme 19

5.3 Nucleophilic displacement with *C*-nucleophiles

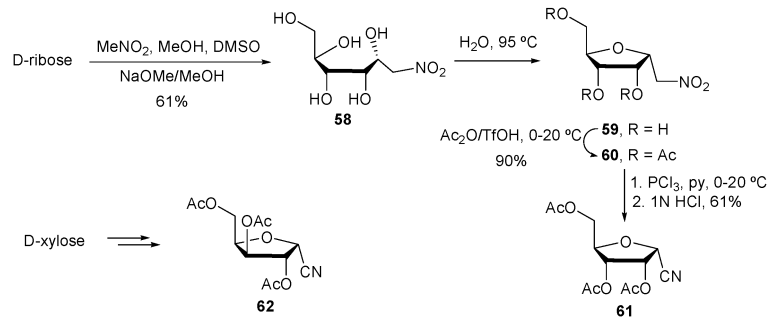
The reaction of α-D-ribofuranosyl chloride **51** with HC≡CMgBr is α-selective giving a separable mixture of **52** and **53** isolated in 61 and 8% yield, respectively. Other organometallic reagents[84] have also been used, namely lithium dimethyl cuprates,[85] in the preparation of methyl α-*C*-glucopyranosyl compound **55** from 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl bromide **54**. Reactions of glycosyl fluorides **56** and organoaluminium reagents such as Me_2AlCN,[86] or aluminated heterocycles,[87] also proceeded with retention of configuration at the anomeric center giving good α-stereoselectivities (Scheme 20).



Scheme 20

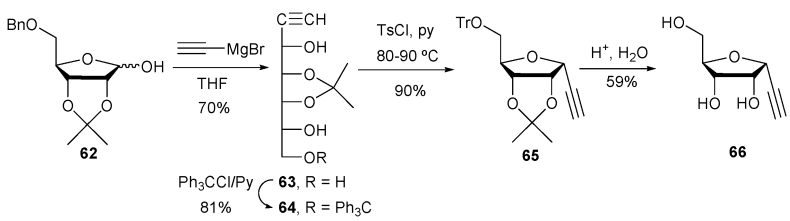
5.4 Nucleophilic addition of *C*-nucleophiles to sugar hemiacetals

5.4.1 Nitronate addition followed by conversion to cyanides. Köll and co-workers[88] have proposed a route to 1,2-*cis*-furanosyl cyanides (**61** and **62**) based on the nitromethanation of D-ribose and D-xylose, followed by dehydration and peracetylation of the 2,5-anhydro-1-deoxy-1-nitropentitol **58**. Reaction with PCl_3 in pyridine and subsequent acid treatment gives 3,4,6-tri-*O*-acetyl-α-D-altrononitrile (**61a**) and 3,4,6-tri-*O*-acetyl-α-D-gulononitrile (**61b**) (Scheme 21).



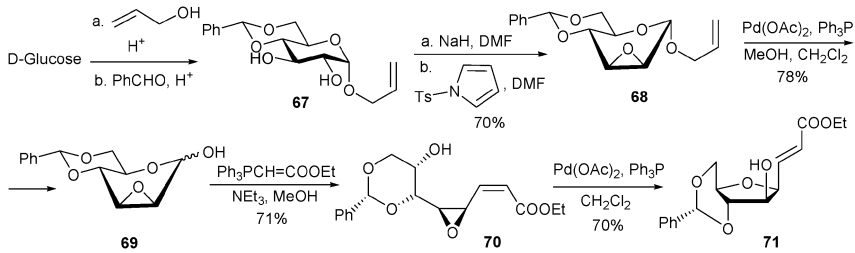
Scheme 21

5.4.2 Adition of Grignard reagents. Reaction of 2,3-*O*-isopropylidene-D-ribose (**62**) with ethynyl magnesium bromide gives 1,2-dideoxy-4,5-*O*-isopropylidene-D-*allo*-hept-1-ynitol (**63**), which is then converted into its 7-*O*-trityl ether (**64**). Treatment of **64** with TsCl/pyridine provides 2,3-*O*-isopropylidene-5-*O*-trityl-α-D-ribofuranosylethyne (**65**) which upon deprotection affords derivative **66** (Scheme 22).[89]



Scheme 22

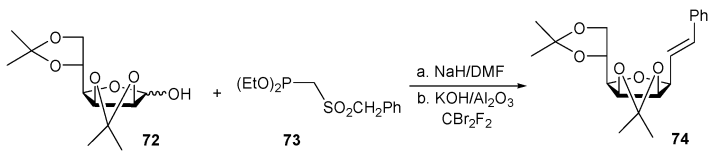
5.4.3 Olefination of aldose derivatives. A stereoselective route to 1,2-*cis*-*C*-furanosyl compounds was reported[90] starting from D-glucose. Fischer allylation and semi-protection as 4,6-*O*-benzylidene acetal gives diol **67** which is converted into epoxide **68** by double deprotonation, followed by selective tosylation at O-2 and spontaneous displacement of the resulting tosylate.[91] Removal of the allyl group with $Pd(PPh_3)_4$ affords hemiacetal **69**, which is converted to the (*Z*)-alkene **70** by (*Z*)-selective stabilized Wittig reaction with $Ph_3P{=}CHCOOEt$ in MeOH in the presence of Et_3N (*Z*/*E* 4:1). Reaction of the allyl epoxide moiety in **70** with Pd(0) catalyst provides the corresponding π-allyl intermediates, which can be trapped by



Scheme 23

the internal hydroxyl group to form 1,2-*cis*-β-*C*-furanoside **71** in 70% yield (Scheme 23).

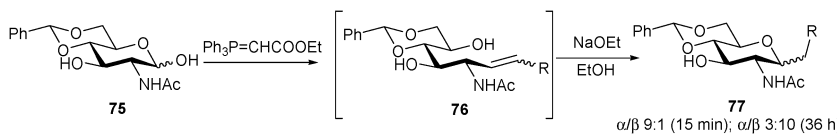
Similarly, starting from 2,3:5,6-di-*O*-isopropylidene-D-mannofuranose **72**, a one-pot procedure that implies the synthesis of glycosyl sulfones by Horner-Wadsworth-Emmons olefination with phosphonate **73**, followed by subsequent Michael addition and Ramberg-Bäcklund reaction[92] gives compound **74** in 78% yield[93] (Scheme 24).



Scheme 24

Suitably semi-protected pyranoses can react with soft carbon nucleophiles generating mixtures of alditols that can undergo elimination of water and intramolecular addition of the δ-hydroxy group to the intermediate alkenes.[94,95]

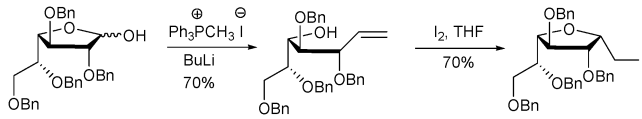
Other anions such as nitronate anion,[96] or the conjugate base of β-ketoesters,[97] give similar results. Usually, the 1,2-*cis*-*C*-glycosyl compound is formed under kinetic conditions. Thus, in the case of the reaction of the GlcNAc derivative **75** with the Wittig reagent $Ph_3P{=}CHCOOEt$, the α-*C*-pyranosyl compound **77**α is the major product under conditions of kinetic control. Under basic conditions, **77**α is slowly epimerized into the more stable β-*C*-pyranosyl derivative **77**β (Scheme 25).



Scheme 25 *C*-Pyranosyl compounds by reaction of the sugar-aldehyde intermediates equilibrating with the pyranoses.

Russo and co-workers[98] have reported a high stereoselective method for the preparation of 1,2-*cis*-*C*-glycosyl compounds based on the iodocyclization of some hept- and hexenitols obtained by Wittig reaction of sugar

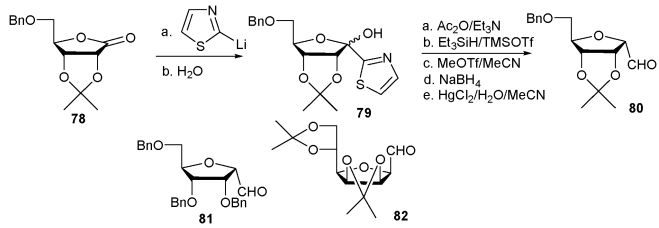
hemiacetals. Thus, reaction of perbenzylated *gluco*, *manno*, *galacto* and *ribo* hemiacetals with methyltriphenylphosphonium iodide and BuLi followed by treatment with iodine in THF afforded the 1,2-*cis*-iodomethylglycosyl compounds with complete or high stereoselectivity (Scheme 26).



Scheme 26 1,2-*cis*-*C*-Furanosyl compounds generated by iodocyclization of heptenitols.

5.5 Organometallic addition to aldonolactones

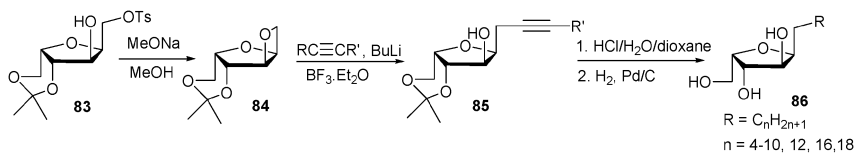
Dondoni and Schermann[99] have used the addition of 2-lithiothiazole to γ-pentonolactones followed by reduction with Et_3SiH or $(^iPr)_3SiH$/Lewis acid for the synthesis of the sugar aldehyde **80** (Scheme 27). The same procedure was applied to the preparation of **81** and **82**. This strategy applied to 1,5-glucoaldonolactones gave a mixture of α- and β-*C*-glucopyranosyl aldehydes.



Scheme 27 Dondoni's synthesis of carbaldehyde **80**.

5.6 Addition to 1,3:2,5-dianhydrohexitol derivatives

Reaction of dianhydro alditol **84** with lithium acetylides in the presence of $BF_3 \cdot Et_2O$ followed by acidic work-up and catalytic hydrogenation affords 1,2-*cis*-*C*-furanosyl compounds **86**[100] (Scheme 28).

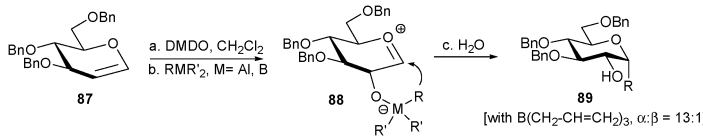


Scheme 28 Köll's synthesis of 1-C-*n*-alkyl-2,5-anhydro-1-deoxy-L-iditols.

5.7 Use of glycal epoxides

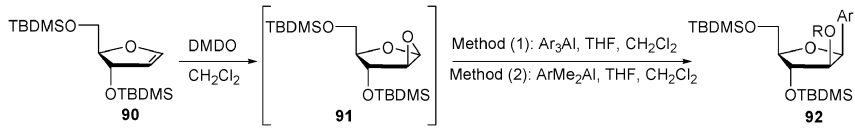
$AlMe_3$, $Me_2Al{-}{\equiv}{-}SiMe_3$, $Me_2AlCH{=}CH_2$, $Al(CH{=}CH_2)_3$, $AlPh_3$, $Al(\alpha\text{-furyl})_3$ and $Al(CH_2CH{=}CH_2)_2$ as well as $B(CH_2{-}CH{=}CH_2)_3$ have also been used as nucleophiles with glycal-epoxides. In these cases, the carbon nucleophiles are delivered *cis* with respect to the 2-hydroxy group of

the *C*-glycosyl compound. This is explained by the formation of cationic intermediates of type **88** resulting from the heterolytic opening of the epoxide, and subsequent intramolecular delivery of the carbon nucleophile to the anomeric center giving **89** after aqueous work-up[101] (Scheme 29).



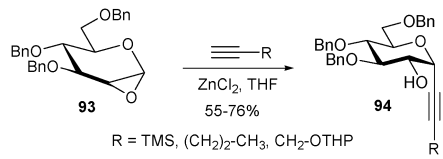
Scheme 29 One-pot synthesis of α-*C*-glycosyl compounds from glycals using organoaluminium and organoboron reagents.

Seitz and co-workers[102] have used Ar_3Al and $ArMe_2Al$ for the *cis*-selective opening of 1,2-anhydroarabinose **90**, obtaining compound **92** in moderate to good yield (Scheme 30).



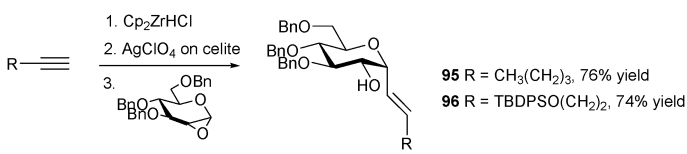
Scheme 30

Starting from 3,4,6-tri-*O*-benzyl-D-glucal epoxide **93** (Scheme 31), α-*C*-glycosyl compounds **94** are obtained in good stereoselectivity by treatment with a lithium acetylide and zinc chloride.[103]



Scheme 31

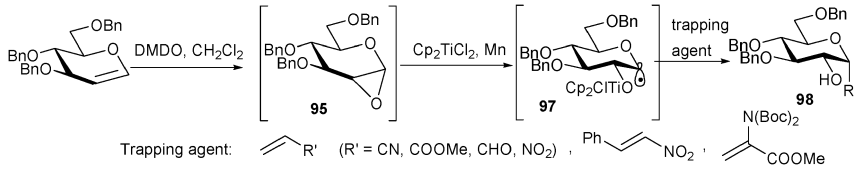
In addition, Wipf and co-workers[104] have used silver(I)-catalyzed addition of zirconocenes to 3,4,6-tri-*O*-benzyl-D-glucal epoxide **93** for the stereoselective synthesis of α-*C*-glucosyl compounds **95** and **96** following a similar mechanism as in the reaction with organoaluminium and organoboron reagents (Scheme 32).



Scheme 32

The same procedure was also applied to the synthesis of α-*C*-galactosyl compounds. Similarly, reductive ring opening of 1,2-anhydro sugar **93** with titanocene(III) chloride produces an anomeric radical **97** that can be trapped

with a variety of agents. The reaction stereospecifically affords α-glycosides **98** and produces a free *C*-2 hydroxyl group allowing for further elaboration (Scheme 33).[105]

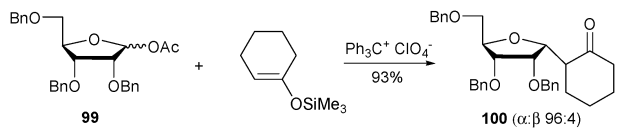


Scheme 33

5.8 Electrophilic *C*-glycosylation

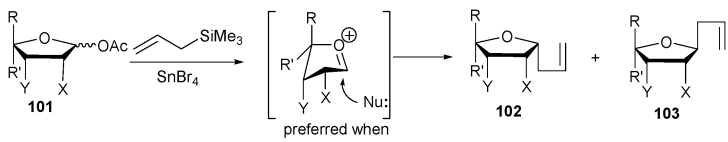
Suitably protected glycosyl halides or acetates, upon Lewis-acid promoted S_N1 heterolysis, generate glycosyl cation intermediates that can react with electron-rich arenes, heteroarenes, Me_3SiCN, enoxysilanes, enamines, allyl silanes and stannanes, acetylenyl silanes and stannanes affording *C*-glycosyl compounds.

5.8.1 Glycosyl acetates. Reaction of 1-*O*-acetyl-2,3,5-tri-*O*-benzyl-D-ribofuranose (**99**) with various enoxysilanes in the presence of $TrClO_4$ afforded 1,2-*cis*-*C*-glycosyl compounds with high stereoselectivity and yields higher than 90% (Scheme 34).[106]



Scheme 34

Lewis-acid promoted *C*-allylations of furanose acetates **101** with allyl-(trimethyl)silane are α-selective and the selectivity is governed by the alkoxy group at C-3. The lowest energy conformers bear the 3-alkoxy group in a pseudoaxial orientation (Scheme 35). To a lesser extent, the 2-substituent

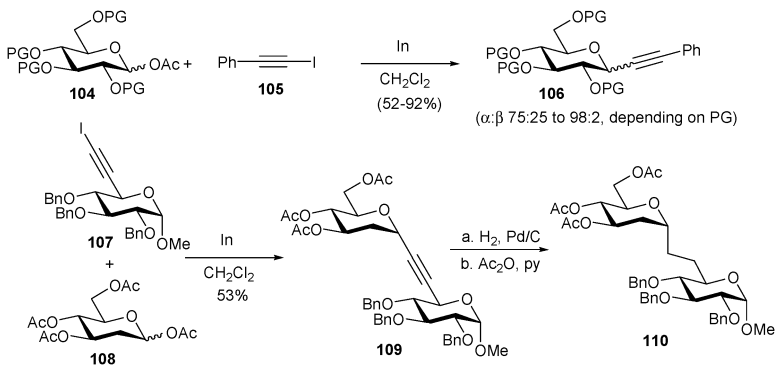


R	R'	Y	X	yield	α:β
CH_2OBn	H	OBn	OBn	93%	>99:1
CH_2OBn	H	OBn	H	94%	>99:1
CH_2OBn	H	OBn	F	81%	>99:1
CH_2OBn	H	Me	H	93%	93:7
H	H	OBn	H	93%	5:95
H	H	$OSiMe_2$(t-Bu)	H	90%	94:6
H	H	OBn	H	93%	94:6

Scheme 35

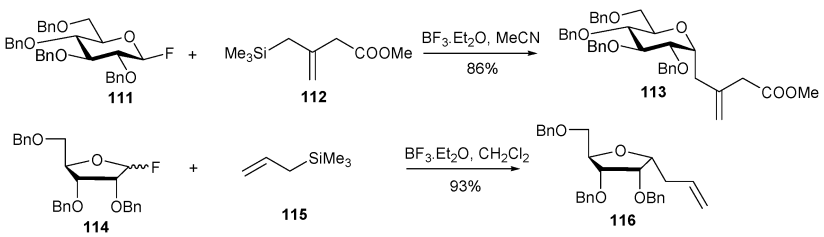
prefers to occupy a pseudoequatorial position, a preference which is accommodated by the ribose configuration but not by the arabinose configuration. The alkyl group at C-4 exerts no influence on the stereoselectivity of the allylation reaction.[107]

Anomeric acetates **104** have been also used as electrophiles toward alkynylindium reagents **105** under Barbier conditions. Good yields and 1,2-*cis*-stereoselectivities were observed during the reaction (Scheme 36). The alkynylation was applied to the synthesis of an α-(1→6)-*C*-disaccharide (**110**) analogue of methyl isomaltoside.[108]



Scheme 36

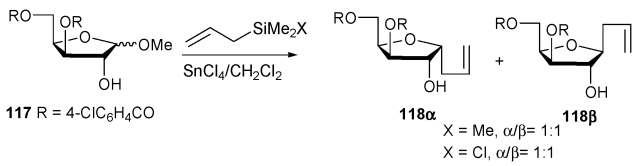
5.8.2 Glycosyl halides. One of the best methods for the preparation of *C*-pyranosyl and *C*-furanosyl compounds (**113** and **116**) is the Sakurai's allylation with allylsilanes.[109] Reaction of allylsilanes with methyl pyranosides and pyranosyl chlorides in the presence of a catalytic amount of Me_3SiOTf or Me_3SiI produces the corresponding pyranosylprop-3-enes.[110] Glycopyranosyl 1,3-diylphosphates are useful glycosyl donors in *C*-glycosylation with Me_3SiCN and allyltrimethylsilane.[111] The α- *vs.* β-selectivity depends on the structure of the allylsilanes.[112] Reaction of $Me_3SiCH_2C(=CH_2)CH_2CO_2Me$, with 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl fluoride (**111**) gives α-*C*-pyranosyl compound **113** in 86% yield (Scheme 37). When the reaction was carried out with 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl fluoride, the corresponding α-*C*-galactopyranosyl compound was obtained in high yield (90%).



Scheme 37

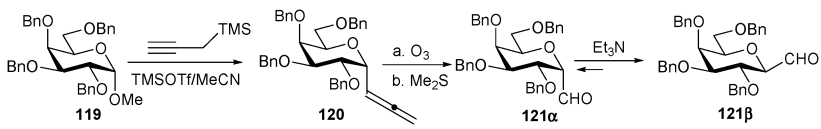
The reaction is also applicable to five-membered ring scaffolds. Thus, D-ribonofuranosyl fluoride **114** reacts with allyl(trimethyl)silane to give α-*C*-ribofuranosyl compound **116** with high stereoselectivity.[113]

5.8.3 Methyl pyranosides. With $CH_2{=}CH{-}CH_2SiMe_3$ in CH_2Cl_2, the $SnCl_4$-promoted allylation of **117** gives a 1:1 mixture of the two possible *C*-furanosyl compounds **118**. In contrast, with the chlorodimethylsilane analogue $CH_2{=}CH{-}CH_2{-}SiMe_2Cl$, a 5:1 mixture of **118**α and **118**β is obtained. In the second reaction, the improved α-stereoselectivity is attributed to an intermediate tethering or coordination of the allyl reagent with the 2-hydroxy group of the furanoside, thus favoring delivery of the nucleophile to the α-face of the anomeric center[114] (Scheme 38).



Scheme 38

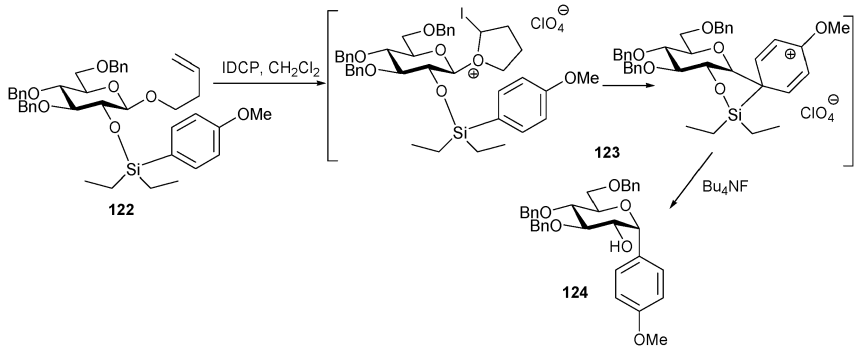
When methyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranoside **119** is treated with propargyltrimethylsilane and Me_3SiOTf in MeCN, the *C*-glycosyl allene **120** is obtained. Its ozonolysis provides the α-*C*-glycosyl aldehyde **121**α. Epimerization of **121**α with 10% Et_3N in isopropanol/CH_2Cl_2 gives the β-*C*-glycosyl aldehyde **121**β[115] (Scheme 39).



Scheme 39

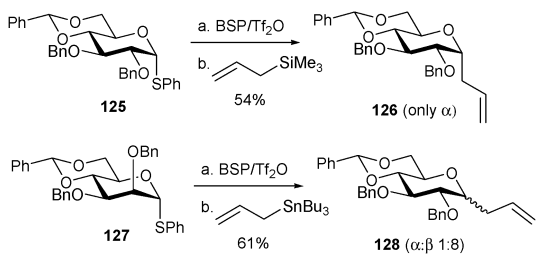
The reaction of suitable protected *p*-nitrobenzoyl-α-D-glucopyranose with (*E*)-penta-2,4-dienyltrimethylsilane or allyltrimethylsilane in $BF_3.Et_2O$ in acetonitrile afforded stereoselectively (*E*)-5-(tetra-*O*-benzyl-α-D-glucopyranosyl)-penta-1,3-diene and 3-(tetra-*O*-benzyl-α-D-glucopyranosyl)prop-1-ene in 45% and 60% yield, respectively.[116]

5.8.4 Use of aryl pyranosides. Fries-type rearrangement. An useful approach to the synthesis of aryl *C*-glycosyl aromatic compounds is the Fries-type rearrangement of aryl *O*-glycosides induced by Lewis acids. Although in most cases 1,2-*trans*-glycosyl compounds are formed, an intramolecular version promoted by iodonium dicollidine perchlorate (IDCP) has been described by Rousseau and Martin[117] leading to 1,2-*cis*-derivatives as depicted in Scheme 40 for the synthesis of **124**.



Scheme 40

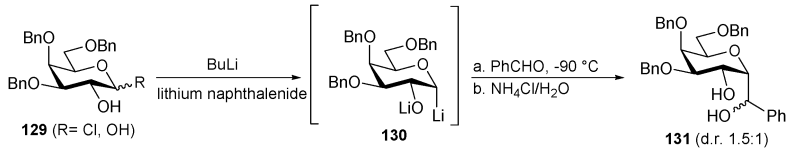
5.8.5 Use of glycosyl triflates, Crich's method. Crich and co-workers[118] have used 1-benzenesulfinyl/triflic anhydride activation of 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-thiohexopyranosides (**125** and **127**), in the reaction with allylsilanes and stannanes, and with silyl enol ethers to give *C*-glycosyl compounds following a selectivity pattern parallel to that of *O*-glycosides. With *gluco* configuration the α-anomers (*e.g.*: **126**) are formed exclusively, whereas the mannose series provides the β-isomers (*e.g.*: **128**) with high selectively (Scheme 41).



Scheme 41

5.9 Nucleophilic *C*-glycosylation

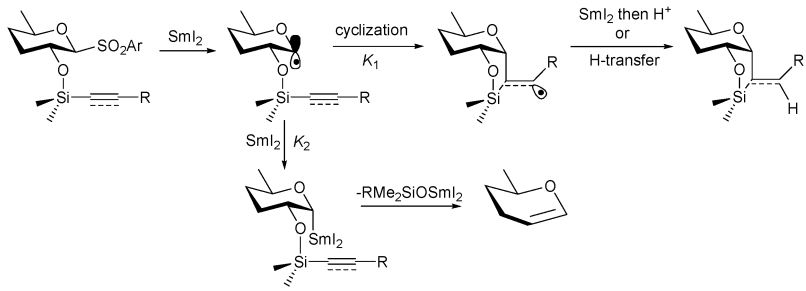
5.9.1 Use of pyranosyllithium reagents. Sugar-derived anomeric nucleophiles have been obtained by halogen/metal exchange of fully protected pyranosyl halides with polar organometallic species such as BuLi and Grignard reagents. If the pyranosyl halide is not a 2-deoxy derivative, quick β-elimination of a ROM entity occurs. The formation of the glycal can be avoided if the 2-substituent is an alcoholate. Glycosyllithium species are configurationally stable at −78 °C and react with aldehydes with *syn* diastereoselectivity.[119] This method has been also applied by Kessler and co-workers for a stereoselective synthesis of α-*C*-galactopyranosyl compound **131** as is outlined in Scheme 42.[120]



129 (R= Cl, OH) **130** **131** (d.r. 1.5:1)

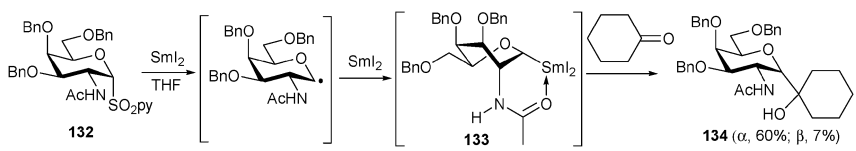
Scheme 42

5.9.2 Use of pyranosylsamarium diiodides. In 1994, Beau and co-workers[121] developed a mild and rapid stereoselective synthesis of 1,2-*cis*-*C*-glycosyl compounds *via* the reductive samariation of glycosyl 2-pyridyl sulfones bearing a silicon-tethered unsaturated group at the C2–OH position. This reaction proceeds through a glycosyl radical followed by a 5-*exo* cyclization. The procedure has been successfully applied using glycosyl 2-pyridyl sulfones to the formation of α-*C*-gluco and α-*C*-mannopyranosyl compounds (Scheme 43).



Scheme 43

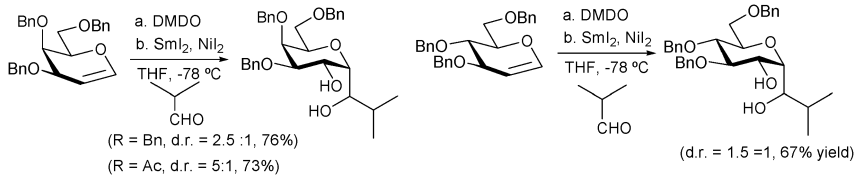
Skrydstrup, Beau and co-workers have applied the reductive samariation of glycosyl 2-pyridyl sulfones in the presence of a carbonyl substrate to galactosamine **132** obtaining good results for the synthesis of α-*C*-galactosamine derivatives. (*e.g.*: **134**). With carbonyl substrates, α:β stereoselectivities range from 20:1 to 5:1. The results are interpreted in terms of α-oriented anomeric glycosyl samarium(III) intermediate (**133**) that is stabilized through chelation of the metal by the 2-acetamido group[122] (Scheme 44). The method has been applied to the synthesis of 1,2-*cis*-*C*-linked-oligosaccharides.[123]



132 **133** **134** (α, 60%; β, 7%)

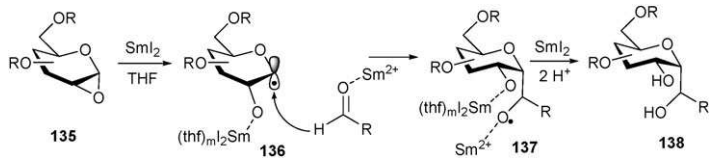
Scheme 44

Chiara and Sesmilo[124] found that suitably protected glycal epoxides (1,2-anhydropyranoses) can be coupled with aldehydes *via* Barbier reaction induced by SmI_2 containing a catalytic amount of NiI_2 (1 mol%) at −78 °C in THF. α-*C*-Glycosyl compounds are predominantly or exclusively obtained with aldehydes independently of the protecting groups or configuration of the starting glycal (Scheme 45).



Scheme 45

A possible mechanistic pathway is illustrated in Scheme 46 for anhydro sugars **135**.

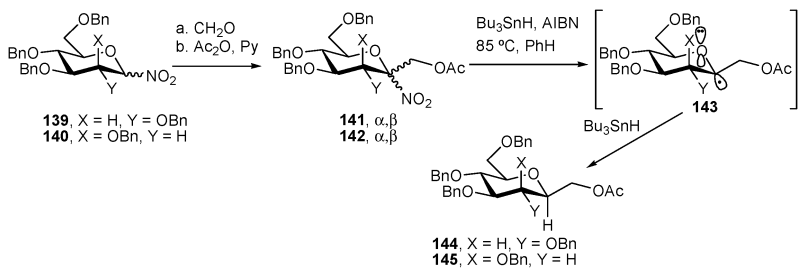


Scheme 46

Single electron transfer (SET) from SmI_2 to the epoxide group of **135** leads regioselectively to an α-anomeric radical intermediate in the form of a solvated samarium(III) alkoxide **136**, that after intermolecular radical addition to an activated carbonyl group and subsequent (or concomitant) kinetic trapping of the generated alkoxy radical produces the *C*-glycosyl derivative **138** after hydrolysis. Interactions between the solvated samarium(III) alkoxide at the C-2 atom in **136** and the incoming complexed carbonyl moiety along the kinetically favored axial trajectory of attack are desestabilizing in the case of ketones, thus explaining a preferential 1,2-*trans* approach in these compounds.

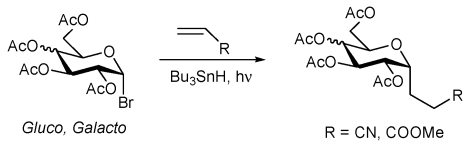
5.10 Radical *C*-glycosylation

5.10.1 Radical reduction of 1-nitro-*C*-glycosyl compounds. In 1983, Vasella and co-workers[125] reported a stereoselective method for the synthesis of α-*C*-mannopyranosyl compounds by reduction of 1-nitro-*C*-mannopyranosyl derivatives with Bu_3SnH in the presence of α,α′-azoisobutyronitrile (AIBN) radical initiator. These reactions involve the formation of anomeric centered radicals. Thus, in the case of D-*manno* configuration as in **140**, the 1,2-*cis*-*C*-pyranosyl compound **145** was obtained in 84% yield. The intermediate pyranosyl radicals **143** prefer α-attack by the tin hydride. Thus for D-glucopyranosyl derivatives, the corresponding 1,2-*trans*-*C*-pyranosyl compound **144** is obtained preferentially (Scheme 47).



Scheme 47

5.10.2 Intermolecular radical *C*-glycosylation. Reaction of peracetylated α-D-pyranosyl bromides[126] and β-D-pyranosyl selenoglycosides[127] with acryl derivatives in the presence of Bu_3SnH and with irradiation produce the corresponding α-D-*C*-glycosyl compounds (Scheme 48). The method is useful for the synthesis of 1,2-*cis*-*C*-glycosyl derivatives with *gluco* and *galacto* configuration.



Scheme 48

These reactions are believed to involve pyranosyl radical intermediates **146** and **147**[128] (Fig. 7).

Pyranosyl radicals B_{25} have substantial co-planar arrangement with the C(2)–O bond and the singly occupied orbital at C(1). The α-face selectivity for the reaction of radical acceptor alkenes is thus controlled by conformational and steric factors. The conformational bias in the pyranosyl radicals is influenced by stereoelectronic factors [interaction of the singly occupied HOMO at C(1) with the neighboring functions]. Radical acceptor alkenes such as fumarodinitrile, methacrylonitrile,[129] dimethyl maleate, methyl acrylate, dimethyl acetylenedicarboxylate, butyl vinyl ether and *N*-ethyl maleimide[130] can also be used in the radical *C*-glycosylation in the presence of Bu_3SnH. The method is not applicable for the synthesis of β-*manno*-*C*-pyranosyl compounds because the corresponding pyranosyl radical in a preferred $^4C_1$ conformation for the same reasons, favours the formation of 1,2-*trans*-*C*-glycosyl derivatives. The method has been also applied to prepare α-*C*-glycosyl derivatives of *N*-acyl-D-galactosamine,[131] D-glucosamine and D-galactosamine structures.[132]

Praly and co-workers[133] have developed procedures for the preparation of α-D-*C*-glycosyl derivatives that use a catalytic amount of Bu_3SnCl in the presence of $NaBH_4$ or Bu_4NBH_3CN in excess as source of hydrogen. Thus, photo-induced radical glycosylation of acrylonitrile or diethyl vinylphosphonate with **148** gives α-*C*-glucopyranosyl compounds **149** and **150** in 79 and 70% yield, respectively (Scheme 49).

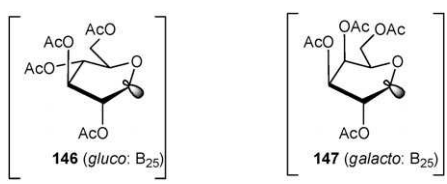
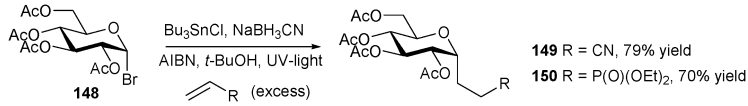
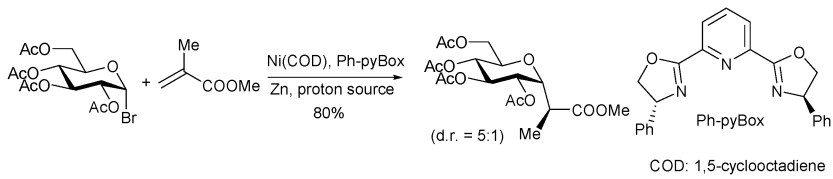


Fig. 7



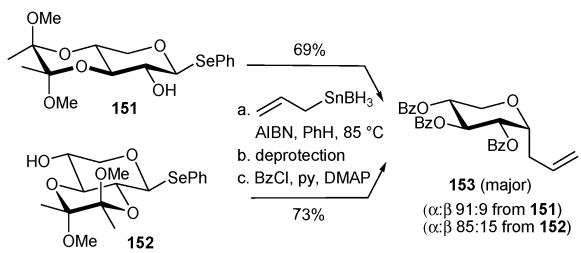
Scheme 49

Instead of Bu_3SnH, titanocene(III) chloride and zirconocene(III) chloride can be employed as effective and mild reagents for the conversion of glycosyl halides to *C*-glycosyl compounds (or glycals in the absence of radical acceptors).[134] A Ni-catalyzed mild method for α-*C*-glycosylation has been also reported.[135] Stereoselective coupling with 2-substituted acrylates was made possible through the use of pyBox ligand, Zn as the terminal reductant, 2,4-dimethyl-3-pentanol as proton source and dimethylacetamide (DMA) as solvent (Scheme 50).



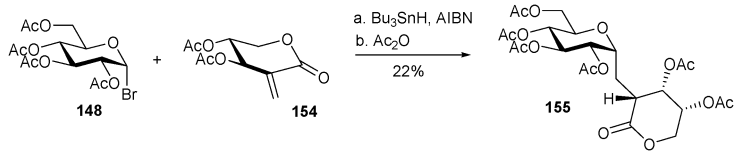
Scheme 50

Alternatively, pyranosyl radicals can be generated through the reduction of 3,4,6-tri-*O*-benzyl glucal epoxide with Cp_2TiCl_2 and manganese metal.[136] With the conformationally restricted 1-phenylseleno-D-xylose derivatives **151** and **152** ($^4C_1$ conformation) their reaction with $Bu_3SnCH_2–CH{=}CH_2$ in the presence of AIBN (Scheme 51) affords the corresponding α-*C*-pyranosyl derivatives (**153**) preferentially.[137]



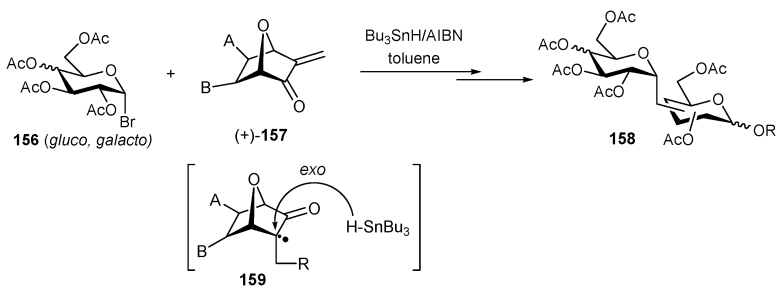
Scheme 51

The first examples of *C*-disaccharides obtained applying radical *C*-glycosylation of sugar-derived exocyclic enones was reported by Giese and Witzel.[138] The reaction of glucosyl bromide **148** and enone **154** afforded a mixture of stereoisomeric *C*- glucopyranosyl compounds from which pure **155** was obtained in 22% yield by precipitation (Scheme 52).



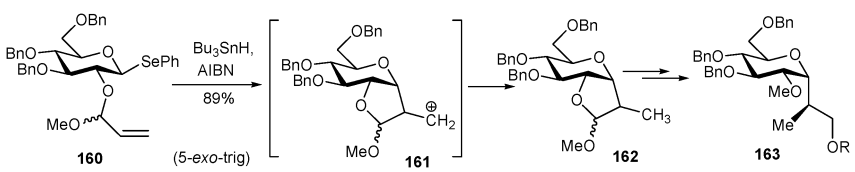
Scheme 52

α(1→2)-*C*-Linked, α(1→3)-, α(1→4)-, and α(1→5)-*C*-linked disaccharides have been obtained through addition of 2,3,4,6-tetra-*O*-acetylglycopyranosyl radical **156** to 3-methylidene-7-oxabicyclo[2.2.1]heptan-2-one derivatives **157** (Scheme 53).[139] In these cases, the intermediate radicals of type **159** resulting from the addition of glycopyranosyl radicals, react with Bu_3SnH exclusively from their *exo* faces for steric reasons. The method has been applied to the synthesis of the *C*-disaccharides containing α-*C*-(1→3)-D-fucopyranosyl residue and *N*-acetyl galactosamine (which is an inhibitor of human α-1,3-fucosyltransferase VI), α-*C*-(1→3)-D-mannopyranosyl residue and *N*-acetyltalosamine[140] and α-*C*-(1→3)-L-fucopyranosyl residue and *N*-acetylgalactosamine.[141]



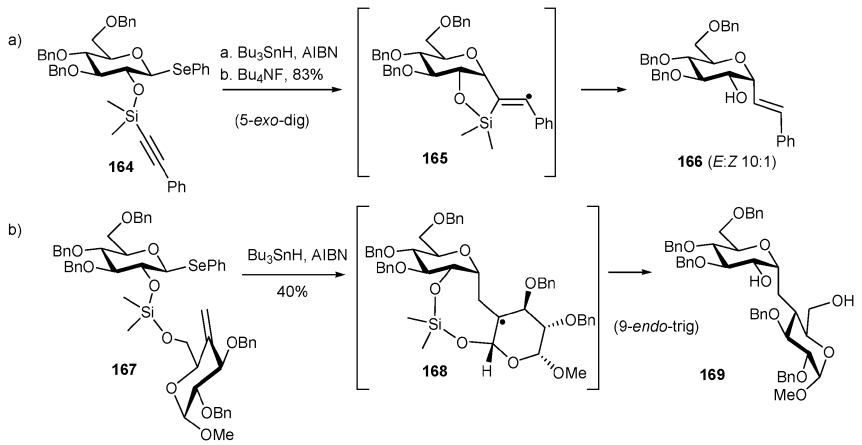
Scheme 53

5.10.3 Intramolecular radical *C*-glycosylation. A radical intramolecular delivery approach for the synthesis of 1,2-*cis*-glycosyl compounds has been carried out using alkene or alkyne acetal HO–C(2) groups based on tin hydride-5-*exo*-radical cyclization because of the geometric requirement for the *cis*-ring fusion. Thus, radical cyclization of acetal **160** gives **162** that can be modified to liberate the linking hydroxyl group furnishing α-*C*-glycosyl compound **163** (Scheme 54).



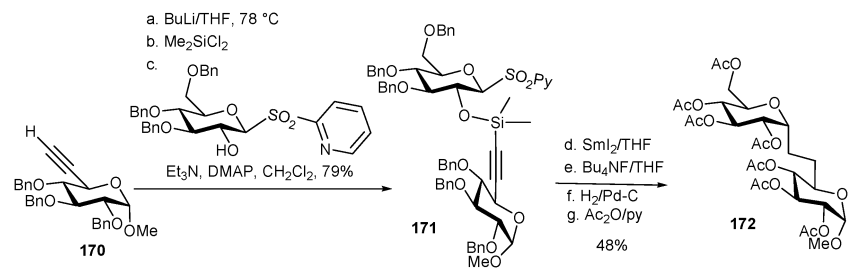
Scheme 54

This strategy was later refined by Stork and co-workers[142] who used silicon (*e.g.*: **164**) as the tethering atom [Scheme 55a)]. The latter strategy was adapted by Sinay and co-workers[143] for the synthesis of *C*-disaccharides (*e.g.*: **169**) by employing 8- and 9-*endo* radical cyclizations with the readily prepared silaketal connectors [Scheme 55b)].



Scheme 55

Skrydstrup, Beau and co-workers[122] have adapted Stork's method to the SmI_2-reduction of glycosyl pyridyl sulfones bearing a silicon-tethered unsaturated group at HO–C(2). An example is shown with the synthesis of methyl α-*C*-*iso*-maltoside **172** from alkyne **170** *via* the 5-*exo*-dig radical cyclization of **171** (Scheme 56).[144]



Scheme 56

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Synthesis of anomeric phosphates of aldoses and 2-ulosonic acids

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This review summarizes recent approaches towards the selective formation of anomeric aldose and aldulosonic acid phosphates of biological relevance, in particular as precursors for the synthesis of nucleotide-activated sugars.

1. Introduction

Glycosyl 1-phosphates play a vital role in many life processes. They are principal intermediates in the biosynthesis of nucleotide-activated sugars (NDP-sugars) which function as nucleotide donor substrates for glycosyltransferases involved in the assembly of oligosaccharide chains of glycoproteins and glycolipids. Glycosyl phosphates are also important constituents of larger biomolecules and represent the key intermediates in the metabolism of sugars and their transformation into nucleotides. Due to the growing interest in a profound investigation of biosynthetic processes, the availability of glycosyl phosphates and the respective NDP-sugars has become a subject of intensive research. The most effective methods for the synthesis of sugar nucleotides require initial preparation of glycosyl 1-phosphates, which are generally quite expensive and rarely commercially available.

Approaches to the synthesis of glycosyl phosphate diesters as constituents of glycopolymers of the outer membrane of bacteria, yeasts and protozoa and the latest achievements in the field have been profoundly reviewed and will not be covered in this chapter.[1,2]

Glycosyl phosphates and phosphites have been recognised as promising glycosyl donors for chemical glycosidation reactions in the last decade and a variety of synthetic methods towards introduction of anomeric phosphates and phosphites as leaving groups has been developed. The latest progress in this field has been recently summarised.[3] Since stereospecificity of the arising glycosidic linkage depends only marginally on the configuration of the anomeric carbon with the leaving phosphate or phosphite, limited attention has been paid to the stereocontrolled instalment of the glycosyl phosphite/phosphate functionality. The fact that most glycosyltransferases are capable of processing only a single anomeric form of the corresponding NDP-sugars emphasizes the importance of generating stereopure substrates as biochemical probes and, consequently, the significance of synthetic approaches towards preparation of anomerically pure glycosyl phosphates

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as precursors. The synthesis of glycosyl phosphates is a demanding task owing to the lability of the anomeric phosphate intermediates, whereas the most challenging aspect is the control of the anomeric configuration. This ~10 year update concentrates on the application of both long-existing and well established methodologies and some recently elaborated approaches to glycosyl phosphates on selected examples. In addition, the scope of transformations will be outlined which can be performed after introduction of the anomeric phosphate moiety without altering this labile functionality in terms of possible nucleophilic displacement, hydrolytic decomposition and anomerisation reactions.

Existing approaches to chemical synthesis of glycosyl 1-phosphates can be roughly divided into two main categories wherein the sugar, activated at the anomeric position, acts as an electrophilic component undergoing a nucleophilic displacement reaction by a phosphate anion, or, conversely, wherein the anomeric hydroxyl group acts as nucleophilic constituent participating in the reaction with an activated phosphate (*e.g.* a protected phosphoric acid anhydride) or phosphite (*e.g.* a phosphoramidite), respectively. The first group of methods involves the use of selected, well-established glycosylation reactions employing phosphate glycosyl acceptors and electrophilic glycosyl donors such as glycosyl halides, nitrates, trichloroacetimidates, orthoesters, activated 1-thio- and pentenyl derivatives as well as glycals.[4] The second category of approaches, wherein the carbohydrate functions as the nucleophilic component, implies either activation of the anomeric hydroxyl group by 1-*O*-lithiation followed by reaction with protected phosphoric acid anhydride (*e.g.* tetrabenzyl pyrophosphate) or coupling of the hemiacetal to activated phosphate (*e.g.* phosphoryl chlorides in the presence of base) or phosphite derivatives (*e.g.* phosphoramidites, imidazolylphosphites), respectively.

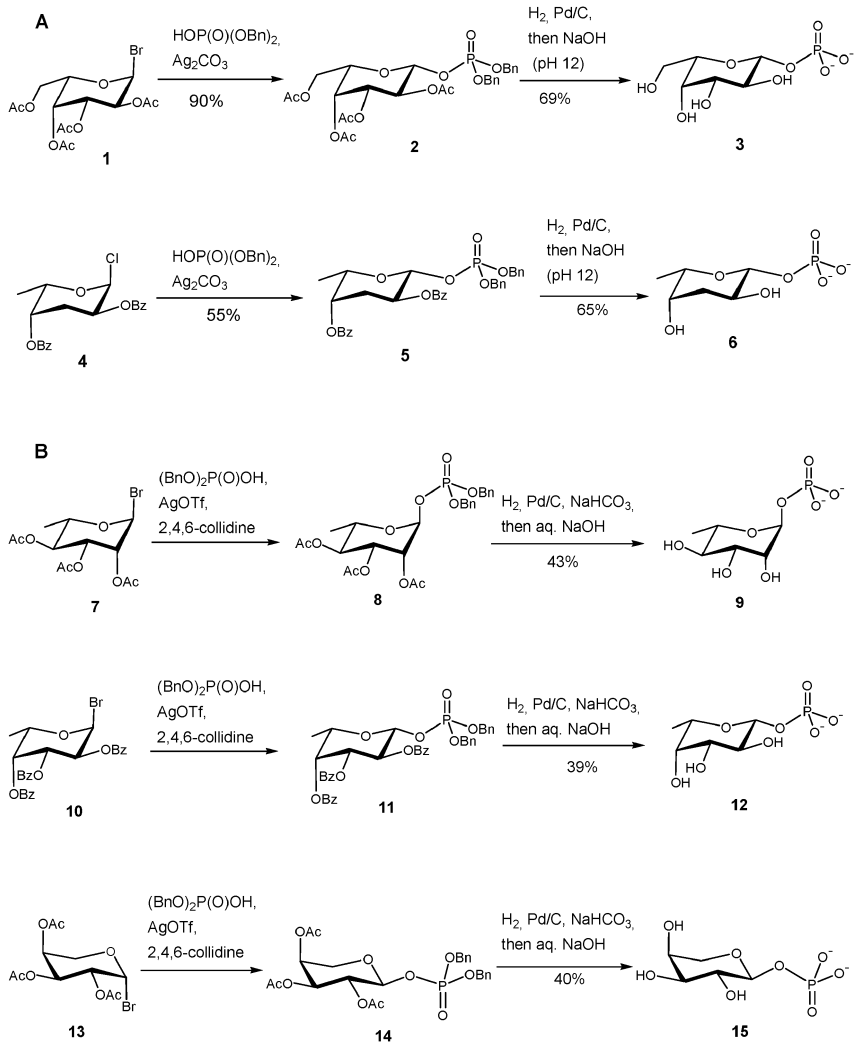
2. Instalment of glycosyl phosphate linkage by glycosylation

2.1 Glycosyl halides as glycosyl donors in the synthesis of anomeric phosphates

The typical and widely used procedure for the activation of the anomeric centre involves the initial preparation of acylated glycosyl halides, which are then subjected to S_N2-like displacement by a phosphate anion in the presence of a silver-containing promoter and a base.

For the synthesis of β-L-galactose-containing GDP derivatives as mimetics of GDP β-L-fucose, several analogues of β-L-galactose 1-phosphate were prepared by using the corresponding α-halogenides as starting material.[5] Reaction of the bromide **1** with dibenzyl phosphate in the presence of silver carbonate provided exclusively the β-anomeric phosphotriester **2** in 90% yield (Scheme 1, A). Since phosphorylation of the α-bromide of 3,6-dideoxy derivative of L-galactose resulted in a 1:5 α/β anomeric mixture, the donor had to be exchanged to the more stable chloride **4**, which provided a higher β-selectivity and better overall yield of the protected phosphate **5** in the subsequent phosphorylation step.[5] Final deblocking was achieved by hydrogenolysis of the benzyl ester groups

followed by basic hydrolysis (pH 12) of the benzoate or acetate groups allowing formation of the anomeric monophosphates **3** and **6**, respectively.



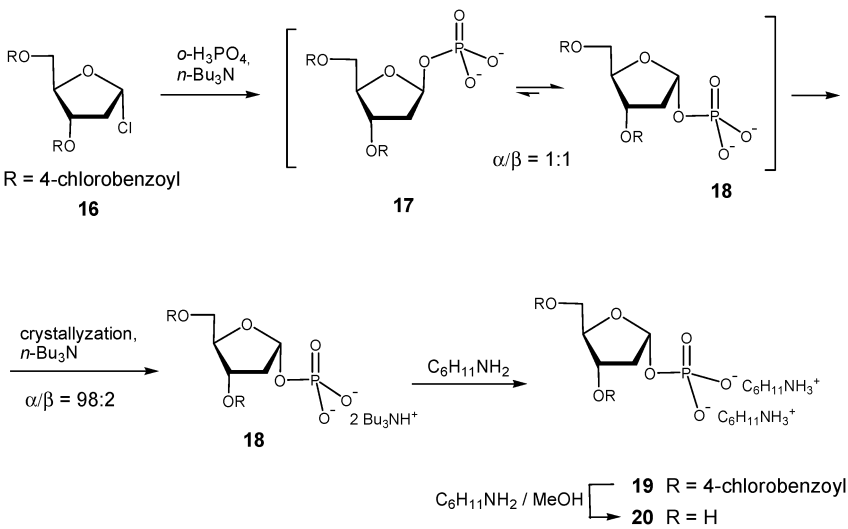
Scheme 1

Stereochemically pure glycosyl phosphates of α-L-rhamnose, β-L-fucose and α-L-arabinose were prepared in sufficient yields from the corresponding acylated bromides by silver triflate-catalysed reaction with dibenzyl phosphate in the presence of 2,4,6-collidine.[6] Glycosyl bromides **7**, **10** and **13** were obtained by standard procedures and subjected, without isolation, to silver triflate-promoted nucleophilic displacement reaction with dibenzyl phosphate, followed by hydrogenolysis to afford α-L-rhamnopyranosyl phosphate **9**, β-L-fucopyranosyl phosphate **12** and α-L-arabinopyranosyl phosphate **15**, respectively, in ~40% yield over 3 steps (Scheme 1, B). Due to neighbouring group participation, the sterical outcome was governed by bottom-face attack at the anomeric centre resulting in 1,2-*trans* stereochemistry.

2.2 Employment of glycosyl halides for generation of glycosyl phosphates in 2-deoxyaldoses

2-Deoxy sugars are both essential components of natural and synthetic antibiotics and, as constituents of nucleotide-activated sugars, important biochemical probes for investigating glycosyltransferase-catalysed glycosylations. Due to the absence of a 2-hydroxy group, the anomeric phosphates of 2-deoxy-aldoses are much more labile to hydrolytic cleavage compared to 2-hydroxy sugars, which renders their chemical synthesis quite difficult.

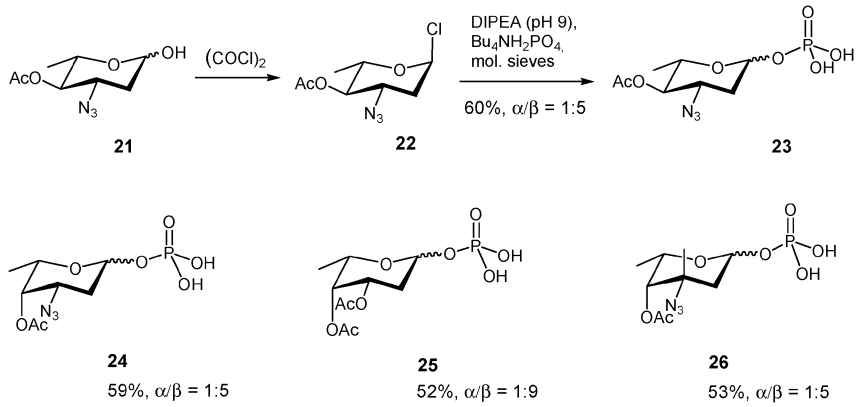
2-Deoxy-α-D-ribosyl-1-phosphate **20**, a key substrate in the preparation of 2′-deoxynucleosides, was stereoselectively prepared by crystallization-induced asymmetric transformation in the presence of an excess of *ortho*-phosphoric acid and tri(*n*-butyl)amine under strictly anhydrous conditions (Scheme 2).[7] Initial S_N2 displacement of Cl in α-glycosyl chloride **16** by phosphoric acid resulted in a 1:1 α/β anomeric mixture of **17** and **18** due to the rapid anomerisation of the α-chloride in polar solvents. Under acidic conditions, in the presence of an excess of H_3PO_4, an equilibration between the α and β anomers gradually changed in favour of the thermodynamically more stable α-counterpart. By selective crystallization of the mono tri(*n*-butyl)ammonium salt of the α-phosphate from the mixture, the equilibrium could be shifted towards the desired α-D-ribosyl phosphate **18** (α/β = 98.5:1.5), which was isolated as bis-cyclohexylammonium salt **19** and deprotected to furnish compound **20**.



Scheme 2

Since glycosyltransferases transferring 2-deoxy-L-sugars only accept β-configured NDP-sugar substrates, the approaches towards generation of the thermodynamically disfavoured and somewhat unstable 2-deoxy-β-glycosyl phosphates are of importance. Reducing phosphates of β-L-acosamine **23**, β-L-daunosamine **24**, 2-deoxy-β-L-fucose **25** and β-L-vancosamine **26**, which

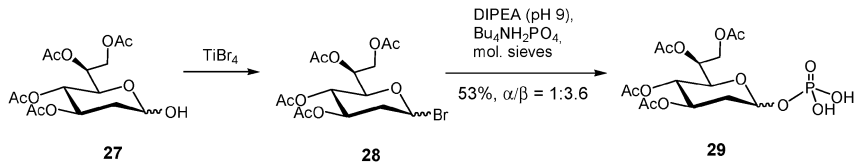
are essential constituents of most anthracycline antitumour antibiotics and the glycopeptide antibiotic vancomycin, respectively, were prepared as precursors of TDP-activated sugars (Scheme 3).[8,9] The synthetic route involved the initial preparation of acetylated glycosyl halides, which were then reacted with tetrabutylammonium dihydrogen phosphate (TBAP) in the key phosphorylation step. Shifting the reaction mechanism from S_N1 to S_N2-like displacement of α-halides by increasing the stability of the α-configured anomeric leaving group (Cl > Br > I) of the donor and an exchange of the solvent from dichloromethane to acetonitrile (nitrile effect) to favour the β-face nucleophilic attack by the phosphate (TBAP) substantially improved the β-selectivity. Thus, hemiacetal **21** was transformed into α-glycosyl chloride **22** which, in turn, was subjected to S_N2-like displacement by phosphate anion to furnish the anomeric phosphate **23** in good yield and high β-selectivity (Scheme 3).



Scheme 3

The intrinsically unstable ADP L-*glycero*-β-D-*manno*-heptopyranose, which serves as the substrate of bacterial heptosyl transferases, easily decomposes to the 1,2-cyclic phosphate and AMP due to intramolecular attack of the anomeric phosphate onto the axially disposed hydroxyl group at position 2.[10] An analogue of the ADP heptose, ADP 2-deoxy-β-L-*galacto*-heptopyranose has been synthesised from the corresponding 2-deoxy-heptosyl phosphate.[11] The synthesis comprised preparation of the acetylated anomeric bromide **28** from the corresponding hemiacetal **27**, followed by coupling with TBAP in the presence of DIPEA in acetonitrile, which resulted in an inseparable 1:3.6 α/β anomeric mixture **29** (Scheme 4). Attempts to improve the stereoselectivity by initial preparation of the β-enriched anomeric phosphotriesters, which are usually readily separable *via* column chromatography, failed due to the inherent instability of β-glycosidic phosphotriesters of the 2-deoxypyranoses.[11]

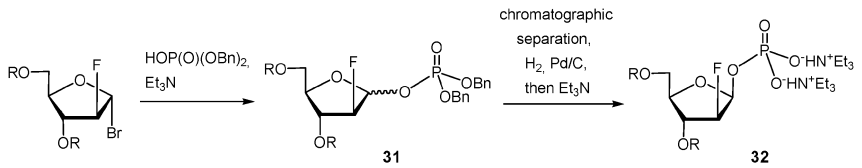
Since 2-deoxy-2-fluoro derivatives of aldopyranoses and furanoses are far more stable towards hydrolytic conditions than their 2-deoxy counterparts, the synthesis of the former entails primary preparation of the anomeric phosphotriesters **31**, which are chromatographically separated into individual diastereomers and subsequently deprotected on phosphorus



27 28 29

Scheme 4

(Scheme 5). Thus, the progenitor **32** of the 2-deoxy-2-fluoro analogue of polyprenyl β-D-arabinosyl phosphate was synthesised by the reaction of aldofuranosyl bromide **30** with dibenzyl phosphate in the presence of triethylamine which afforded predominantly the β-configured dibenzyl phosphotriester **31**, readily separable as individual diastereomer by conventional silica gel chromatography. Hydrogenolysis of the phosphate benzyl protecting groups provided β-phosphate **32**.[12]



30 31 32

R = Bz — 82%, α/β =1:6.4 for R = Bz

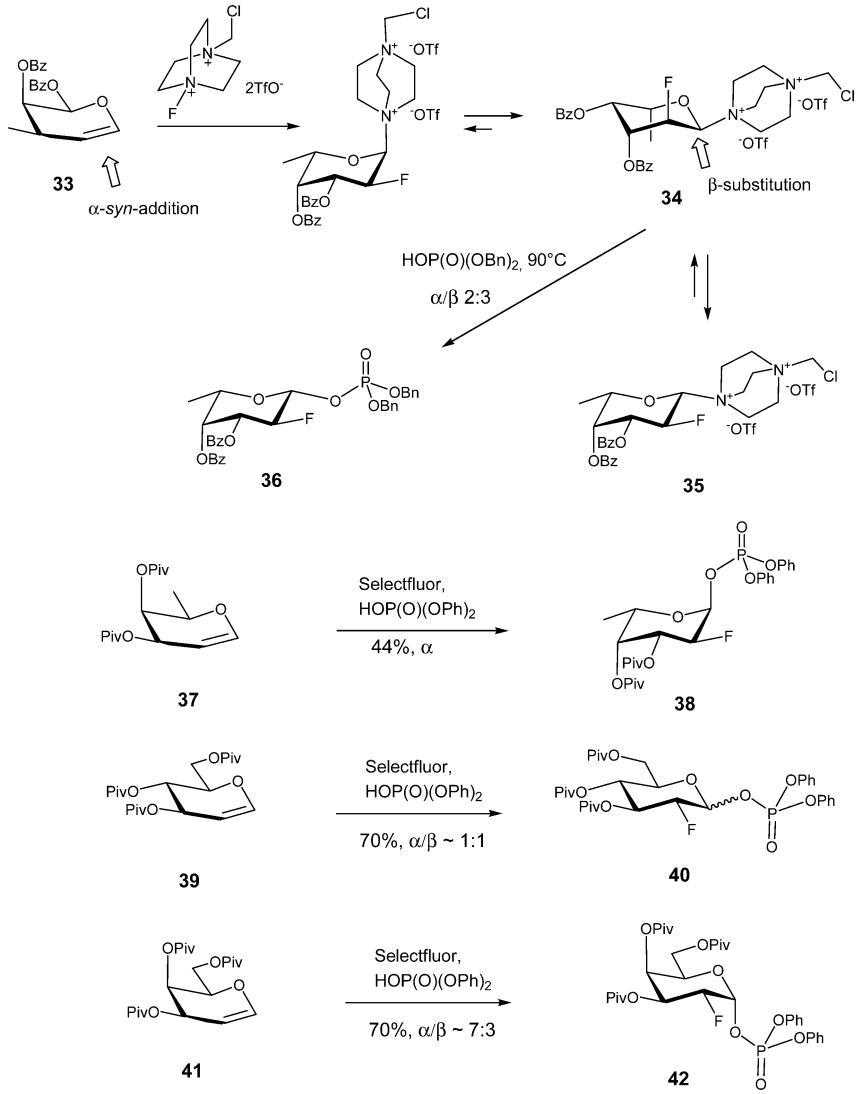
R = *t*-Bu(CH$_3$)$_2$Si — 67%, α/β =1:5 for R = *t*-Bu(CH$_3$)$_2$Si

Scheme 5

2.3 2-Deoxy-2-fluoro-aldopyranosyl phosphates by stereoselective fluorophosphorylation of glycals

The synthesis of a series of biologically important 2-deoxy-2-fluoro glycosyl phosphates in the fucose-, glucose- and galactose-series has been achieved by two-step one-pot electrophilic fluorination—nucleophilic addition reaction with Selectfluor® on glycals.[13] Selectfluor® is initially reacted with glycals to afford regio- and stereoselective fluorination at position 2, followed by nucleophilic addition of phosphoric acid diester at the anomeric centre to furnish the corresponding glycosyl phosphates. Formation of the equatorially oriented fluorination product was rationalised on the basis of initial formation of reactive *syn*-adduct **34** in $^4C_1$ conformation which slowly epimerized into thermodynamically more stable **35**, that should favour higher α-selectivity of the following nucleophilic attack (Scheme 6). Nucleophilic addition of the phosphate was suggested to proceed *via* competitive S_N1 and S_N2 mechanisms, whereas increasing steric hindrance of the nucleophile favours an α-selectivity of the process.[13] Thus, one-pot addition of dibenzyl phosphate at elevated temperature furnished 2-fluoro fucosyl phosphate **36** with prevalence of the β-anomer. Starting from pivaloylated glycal **37** by employment of diphenyl phosphate as nucleophile, the stereospecificity was clearly shifted towards the α-phosphate **38**, which was isolated as a single product due to *in situ* decomposition of the unstable

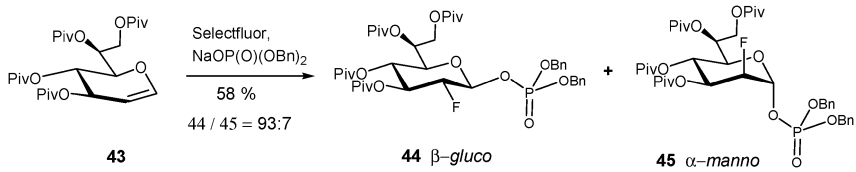
β-fucosyl phosphate. Reaction of tripivaloyl glucal **39** and galactal **41** with Selectfluor® and diphenyl phosphate resulted in a 1:1 anomeric mixture of 2-fluoro-glucosyl 1-phosphate **40** but preferred formation of the α-configured galactosyl 1-phosphate **42**.



Scheme 6

Recently, the synthesis of the 2-fluoro analogue of ADP heptose has been accomplished utilizing the glycal **43** as educt.[14] Reaction of **43** with Selectfluor® and sodium dibenzyl phosphate proceeded in a diastereoselective fashion to give the β-anomeric phosphotriester **44** as major isomer with concomitant formation of α-*manno* configured product **45**. Employment of more sterically demanding *tert*-butyldimethylsilyl protecting groups improved β-*gluco*-selectivity and afforded the corresponding 2-fluoro 1-phosphate in 51% yield with excellent β-selectivity (α/β = 6:94,

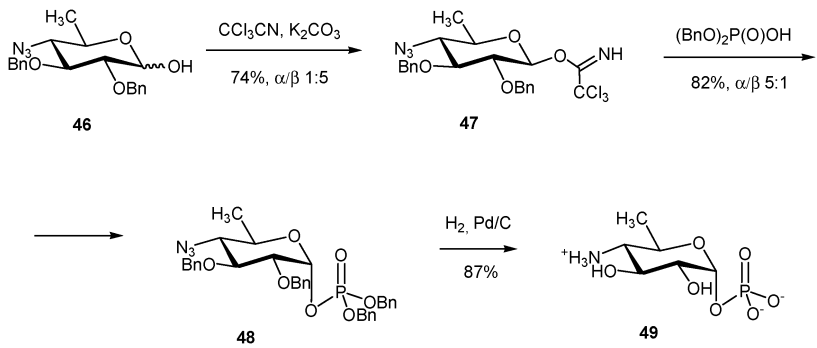
gluco-product). The selectivity invoked for the formation of the β-*gluco*-product was explained by *syn*-addition of Selectfluor® from the α-side followed by nucleophilic displacement with the phosphate anion to furnish the product with inverted anomeric configuration (Scheme 7).[14]



Scheme 7

2.4 Trichloroacetimidates as glycosyl donors for generation of anomeric phosphates

Use of the β-enriched trichloroacetimidate donor **47** (containing a nonparticipating group at C-2) in phosphate–anion-mediated glycosylation resulted, as expected, in a mixture of anomeric phosphotriesters **48** (α/β = 5:1) in good yield (Scheme 8).[15] Debenzylation with simultaneous reduction of the 4-azide group conducted at atmospheric pressure in aq. dioxane at pH 6 did not encounter any problems despite the possibility that the formed amino group could poison the catalyst and prevent a full conversion. The obtained 4-amino-4,6-dideoxy-α-D-glucosyl phosphate **49** was further transformed into the TDP-derivative as biosynthetic precursor of acarbose.

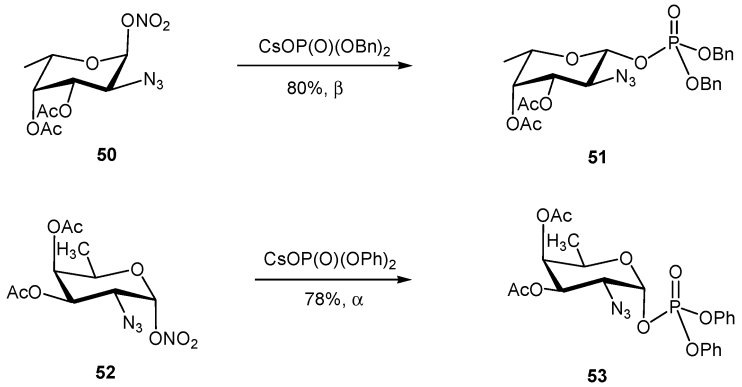


Scheme 8

2.5 Glycosyl nitrates as glycosyl donors

The propensity of the cesium salts of week acids to accelerate S_N2 type displacement reactions was exploited for the stereodirected conversion of glycosyl nitrates into anomeric phosphotriesters by exposure of phosphoric acid diesters to cesium salts (Scheme 9).[16] Thus, glycosyl nitrate **50**, obtained by azidonitration of 3,4-di-*O*-acetyl-L-fucal, was treated with

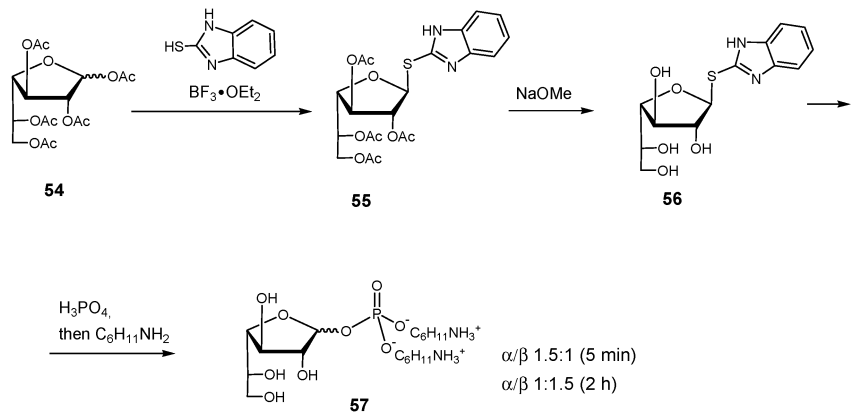
cesium dibenzyl phosphate under mild conditions to furnish the β-phosphate **51**. On the other side, when cesium diphenyl phosphate was used for the reaction with α-D-glycosyl nitrate **52**, the α-phosphate **53** was isolated as a single product. The nature of the phosphate protecting groups was found to significantly modify the anomeric distribution of the phosphorylated products: by employment of diphenyl phosphate α-selectivity was observed, whereas glycosylation of dibenzyl phosphate favoured β-selectivity.[16]



Scheme 9

2.6 Thioimidoyl derivatives as glycosyl donors

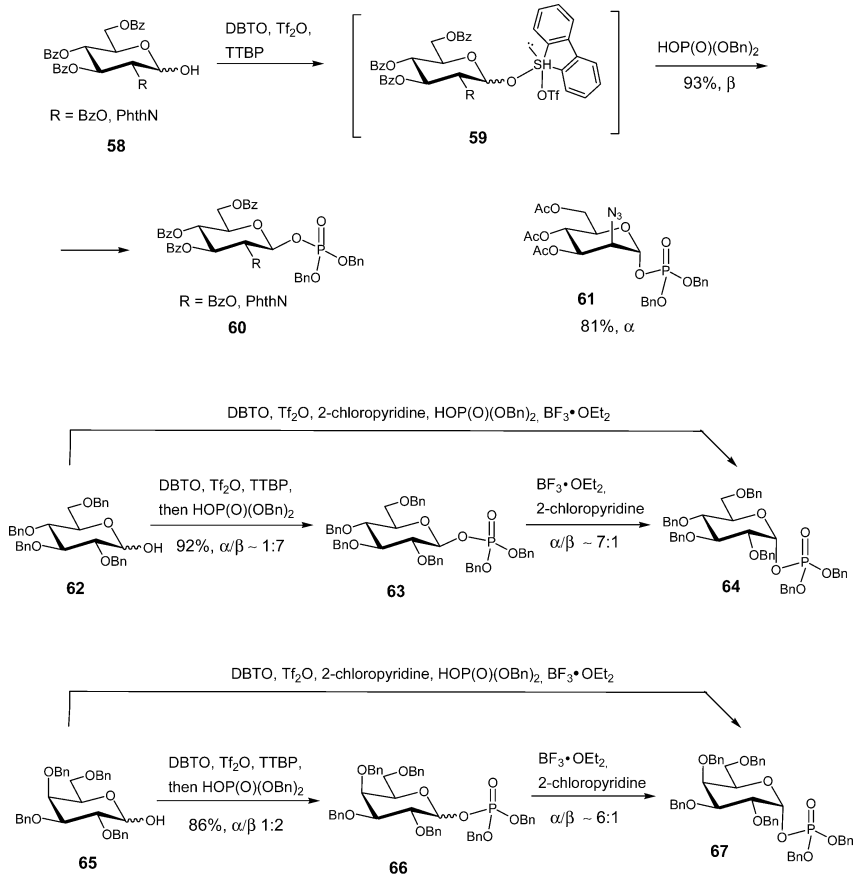
A nonstereospecific instalment of the glycosidic phosphate in hexofuranoses has been achieved by employment of unprotected thioimidoyl derivatives.[17,18] Based on the concept of remote activation of thioglycosides, harbouring a protonated aromatic aglycon, by dry phosphoric acid, a series of inseparable anomeric mixtures of hexafuranosyl phosphates of galactose, mannose, glucose and fucose was synthesised. A typical reaction sequence included initial conversion of the peracetylated galactofuranose **54** into β-thiofuranoside **55** by $BF_3 \cdot OEt_2$—catalysed reaction with 2-mercaptobenzimidazole (Scheme 10).[17] Subsequent deacetylation afforded unprotected thioglycosyl donor **56**, which was subjected to condensation with dry phosphoric acid to provide phosphate **57** as anomeric mixture. It is worthy to note that the desired α-product resulting from S_N2-type reaction was formed in excess (α/β = 1.5:1) within the first few minutes and subsequently anomerised in favour of the sterically less hindered β-counterpart (α/β = 1:1.5) after 2 h in the presence of phosphoric acid. Since the anomeric effect in furanoses is equal in the stabilisation of both α- and β-anomers, the steric interactions in the transition state should be taken into consideration. By limiting reaction times and careful adjustment of purification protocols, D-gluco-, D-manno- and D-fucofuranosyl phosphates were obtained as α/β mixtures slightly enriched in α-anomer, in 35%, 50% and 80% overall yields, respectively, starting from the corresponding peracetates.[18]



Scheme 10

2.7 Glycosyl phosphates by dehydrative glycosylation

An interesting approach towards the formation of glycosyl 1-phosphates by dehydrative glycosylation of nucleophilic dialkyl phosphate acceptors with



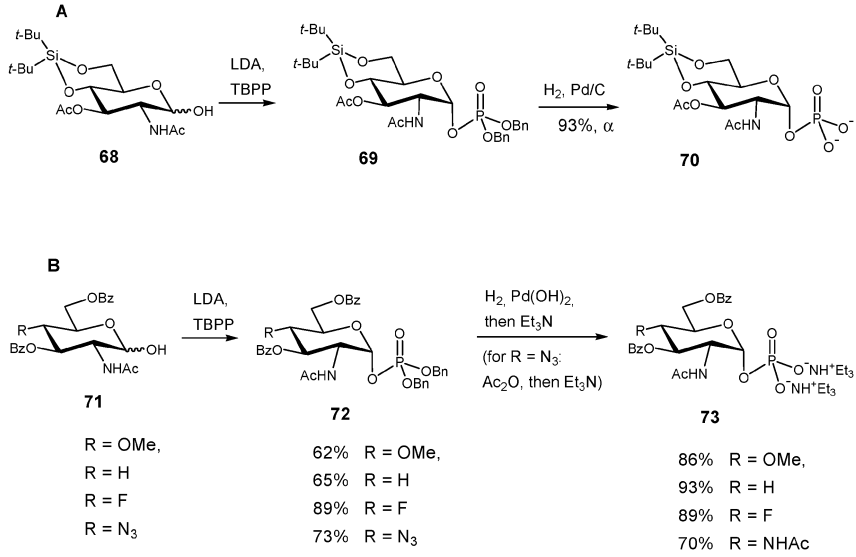
Scheme 11

hemiacetal donors catalysed by activated sulfonium reagents has been developed and applied to the preparation of protected anomeric phosphates of gluco-, galacto- and mannopyranoses.[19] In the standard phosphorylation procedure, the selectively protected hemiacetals **58**, **62** and **65** were activated, in the presence of an excess of the acid scavenger 2,4,6-tri-*tert*-butylpyridine (TTBP), by dibenzothiophene-5-oxide (DBTO) and triflic anhydride to likely generate *in situ* the anomeric oxosulfonium (or sulfurane) species **59**, acting as electrophile (Scheme 11).[19,20] Further treatment, in a one-pot reaction, with dibenzyl phosphate acceptor furnished the corresponding glycosyl phosphotriester **60**. The stereochemical outcome was strongly dependent on the type of protecting group and configuration at C-2. Accordingly, exclusively 1,2-*trans* anomeric phosphates were formed giving 2-*O*-acyl and 2-*N*-phthalyl substituted *gluco*-products **60** and 2-azido-substituted *manno*-derivative **61**, respectively, whereas introduction of a nonparticipating protecting group at C-2 led to diminished β-selectivity ($\alpha/\beta \approx 1{:}7$ for perbenzylated *gluco*-compound **63** and $\alpha/\beta \approx 1{:}2$ for galactopyranosyl phosphate **66**). An anomerisation of the 1:7 α/β anomeric mixture **63** with $BF_3 \cdot OEt_2$ in the presence of 2-chloropyridine eventually led to the predominant formation of the α-phosphate **64** ($\alpha/\beta \approx 7{:}1$). Similar Lewis acid–catalysed anomerisation in favour of the α-phosphate was also observed for galactosyl phosphate **67**. Combination of both phosphorylation and anomerisation steps in a single one-pot reaction (**62** → **64** and **65** → **67**) ensured high yields of glycosyl phosphates and remarkable α-selectivity in *gluco*- and *galacto*-series.

3. Synthesis of glycosyl phosphates by exposure of hemiacetals to activated derivatives of phosphoric acid

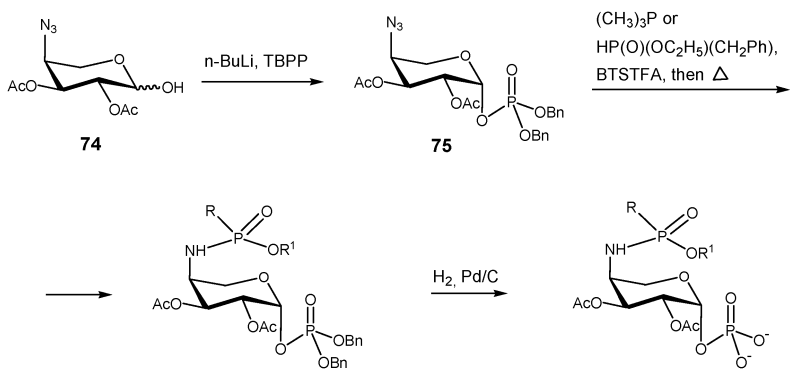
3.1 Synthesis of glycosyl phosphates by activation of the anomeric hydroxyl group

Activation of the hemiacetal hydroxy group by 1-*O*-lithiation followed by treatment with tetrabenzyl pyrophosphate still remains one of the most frequently used procedures for the stereoselective instalment of α-configured anomeric phosphates in divergently protected 2-deoxy-2-acylamino hexopyranoses and derivatives.[20–24] Accordingly, hemiacetal **68** having 4,6-*O*-cyclic silyl protection was treated with tetrabenzyl pyrophosphate (TBPP) in the presence of lithium diisopropylamide (LDA) which afforded solely α-configured phosphotriester **69** in nearly quantitative yield (Scheme 12, A).[21] In the absence of a 4,6-cyclic protecting group, the yields of phosphorylation varied between 90% for a 4,6-di-*O*-benzoyl-masked GlcNAc derivative[22] and 60–65% for 4-deoxy- and 4-*O*-methyl protected phosphotriesters **72** (Scheme 12, B).[23] Interestingly, the outcome was substantially improved (up to 90%) in the case of α-glycosyl phosphates of 4-deoxy-4-fluoro and 4-deoxy-4-azido functionalised GlcNAc-pyranoses **72**.[23] Deblocking of the phosphotriesters by convenient hydrogenation provided corresponding glycosyl phosphates **73**. The 1-*O*-lithiation/TBPP approach was successfully employed for α-phosphorylation of the anomeric hydroxyl group of peracetylated chitobiose,[24] peracetylated 4-deoxy-D-*lyxo*-hexose and D-lyxose.[25]



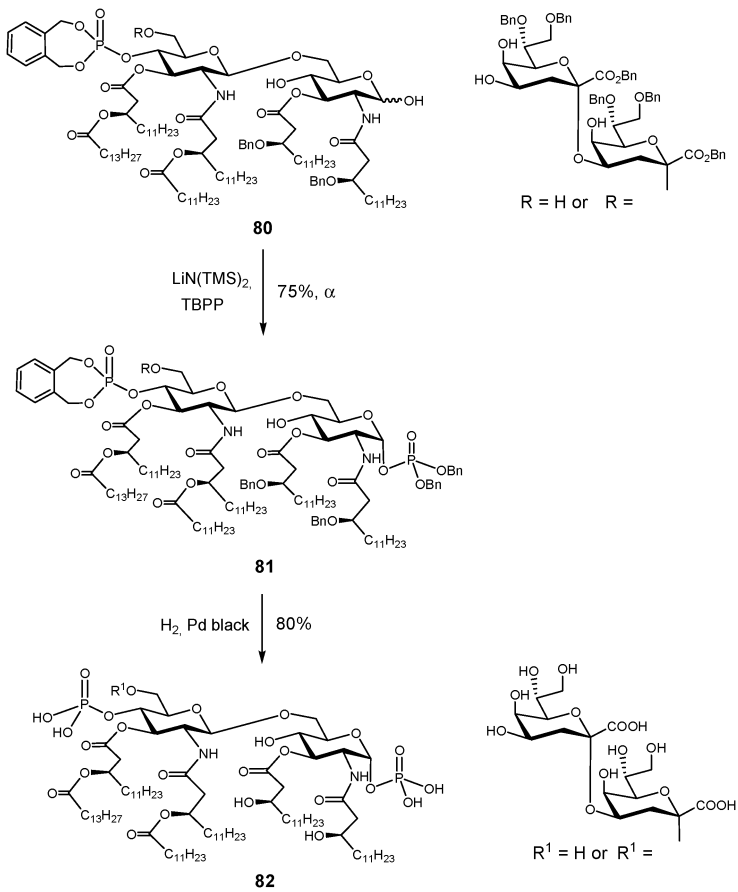
Scheme 12

In the pentafuranose series the 1-*O*-lithiation approach was also utilized for stereoselective anomeric phosphorylation of 4-amino-4-deoxy-L-arabinose.[26] Accordingly, hemiacetal **74** was reacted with butyl lithium and tetrabenzyl pyrophosphate giving rise to β-L-configured phosphotriester **75** in good stereochemical purity (Scheme 13). In an effort to produce inhibitors of bacterial polymyxin resistance mechanisms, the azido functionality of **75** was derivatized to phosphoramidate **76** by a modified Staudinger reaction and to phosphonamidate **77** by reaction with ethyl benzyl *H*-phosphinate in the presence of bis(trimethylsilyl)trifluoroacetamide (BTSTFA) followed by thermal rearrangement, respectively. Hydrogenolysis of the benzyl phosphate esters gave free phosphates **78** and **79** which were further subjected to the coupling with UMP-morpholidate.[26]



Scheme 13

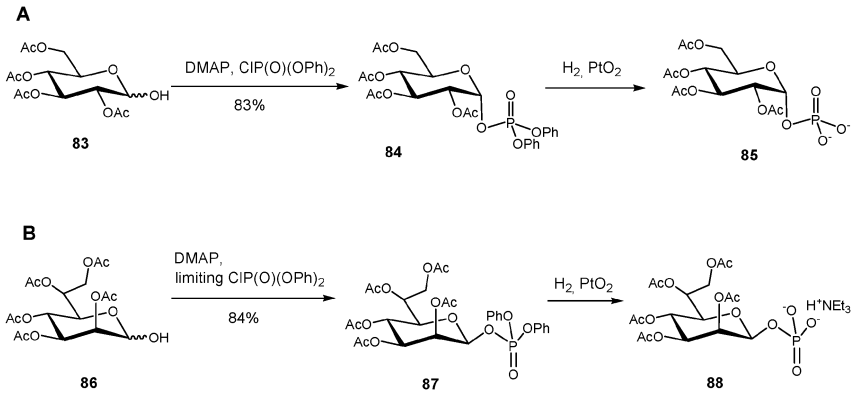
The phosphoryl group at the 1-position of synthetically prepared lipid A is normally introduced by 1-*O*-lithiation followed by phosphorylation with either tetrabenzyl pyrophosphate[27–30] or dibenzyl phosphoryl chloride.[31] Thus, anomeric phosphate was installed in the *E. coli* lipid A progenitor **81** by the reaction of hemiacetal **80** with tetrabenzyl pyrophosphate in the presence of lithium bis(trimethylsilyl)amide $LiN(TMS)_2$ at −78 °C (Scheme 14).[27,29] This procedure provided rather high yields and remarkable α-stereoselectivity. The cleavage of benzyl protecting groups from anomeric phosphotriester **81** was achieved in the final deprotection step by hydrogenolysis on Pd-black which afforded bis-phosphoryl lipid A **82** (Scheme 14). Similarly, R*e*-LPS partial structure from *H. pylori*,[28] lipid A from *C. trachomatis*[32] and *P. gingivalis*[33] were synthesised by this highly efficient approach. Application of *n*-butyl lithium in complex with dibenzyl phosphoryl chloride for the introduction of the reducing phosphate in the synthesis of lipid A from *P. gingivalis* provided exclusively the α-configured anomeric phosphotriester derivative.[31]



Scheme 14

3.2 Synthesis of glycosyl phosphates by reaction of hemiacetals with activated phosphoric acid derivatives

An approach comprising direct acylation of the anomeric hydroxyl group with diphenyl phosphoryl chloride in the presence of DMAP for stereoselective synthesis of glycosyl phosphates in gluco-, galacto- and manno-series was introduced by Sabesan and Neira in 1992.[34] This easily reproducible and effective methodology was then successfully used by many groups to prepare a wide range of pyranosyl phosphates with excellent stereochemical control. Accordingly, the 1,2-*cis* glycosyl phosphate of peracetylated $^{13}C_6$-glucose **85** was efficiently synthesised from the corresponding hemiacetal **83** by initial treatment with DMAP to ensure enrichment with the thermodynamically more stable α-anomer followed by reaction with diphenyl phosphorylchloride to provide phosphotriester **84**, which was further hydrogenolytically deprotected to afford **85** (Scheme 15, A).[35]



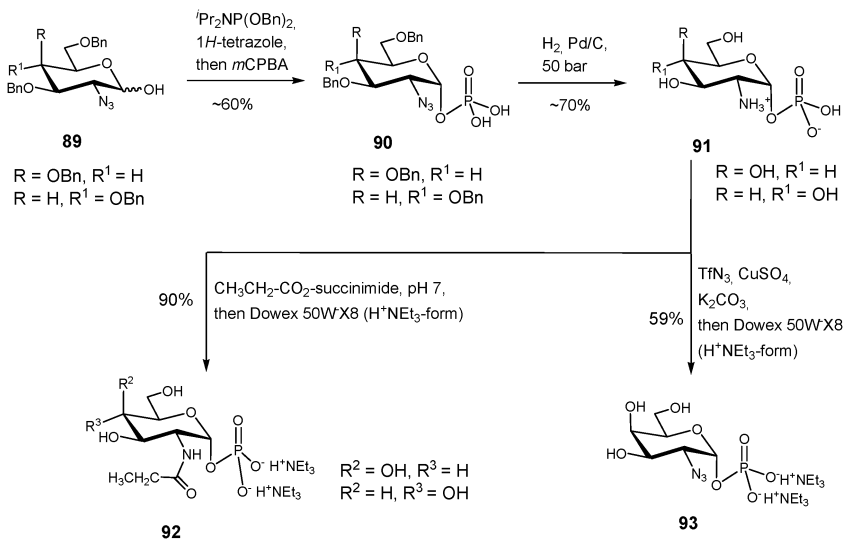
Scheme 15

The diastereoselective synthesis of β-mannosyl phosphates through nucleophilic attack on the phosphorus atom of a less stable but more reactive equatorial hydroxyl group is a further task which can be attempted under conditions where equilibration of the hemiacetal forms is possible. In essence, the problem is solved by limiting an excess of the electrophilic phosphate component under conditions promoting accelerated equilibration of the axial and equatorial hemiacetals. The higher nucleophilicity of equatorially oriented 1-OH group was thus exploited in the stereoselective synthesis of β-anomeric *glycero*-D-*manno*-heptosyl phosphate (Scheme 15, B).[10] Reaction of peracetylated hemiacetal **86** with a limited amount of diphenyl phosphoryl chloride in the presence of a large excess of DMAP afforded β-glycosyl phosphotriester **87** in 84% isolated yield along with a minor amount of α-anomer (α/β = 1:9). The same methodology was applied to the synthesis of a peracetylated mannosyl phosphate, which was achieved with a clear predominance of the β-anomer (α/β = 1:4).[36]

4. Approaches to glycosyl phosphates based on P(III)-intermediates

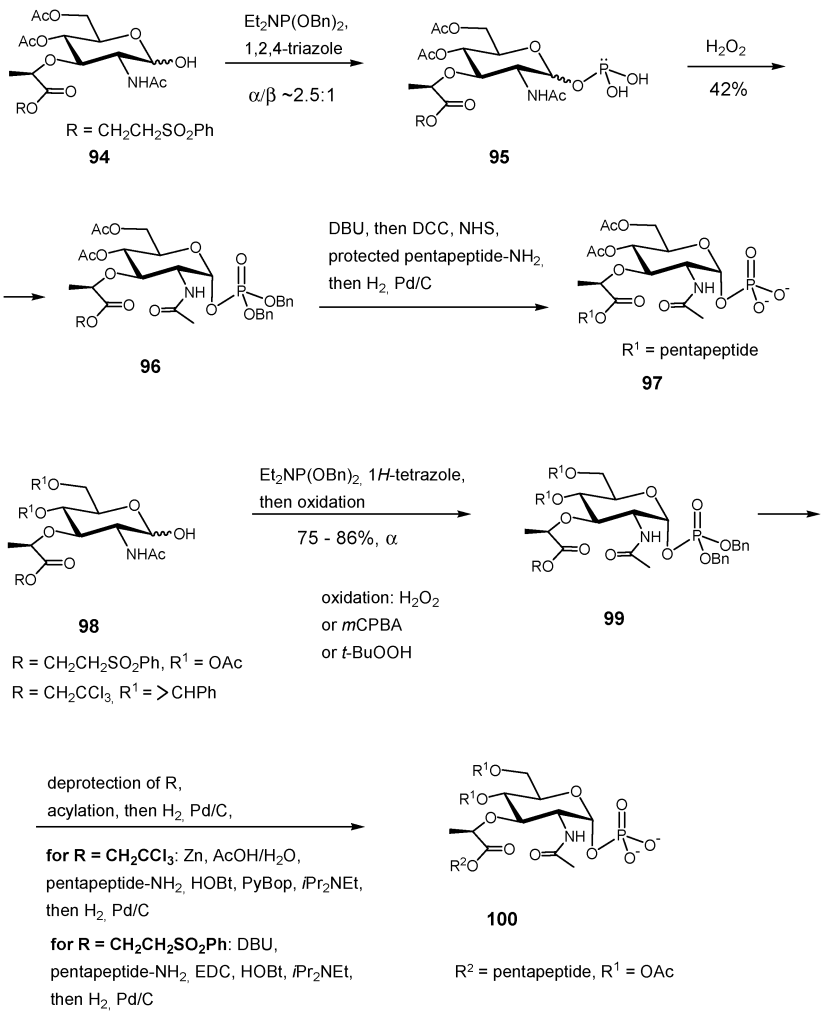
4.1 Phosphoramidite approach in the synthesis of glycosyl phosphates

The generality of the phosphoramidite approach, known for its mildness and simplicity of handling, has been widely demonstrated in the synthesis of a variety of glycosyl phosphites and their ensuing conversion to phosphates. *N*-substituted unnatural analogues of UDP-galacto- and UDP-glucosamine have been suggested as alternative substrates for GalNAc-[37] and GlcNAc-transferases, respectively,[38] and were synthesised using the corresponding 2-acylamino- and 2-azido-protected glycosyl phosphates **92** and **93**, respectively, as progenitors (Scheme 16). To this end, the phosphoramidite methodology was successfully employed for the instalment of the anomeric phosphate into the azido-functionalised hemiacetal **89**. After oxidation of the intermediate phosphite triester with *meta*-chloroperbenzoic acid (*m*CPBA), the resulting dibenzyl-protected α-phosphate **90** was isolated after chromatography in ~60% yield. The β-configured product has not been detected, though the possibility of the initial formation of some amount of β-phosphite, which could have been destroyed by oxidation and contact with silica gel, could not be excluded. Simultaneous deprotection and reduction of the azide functionality by hydrogenation under 50 bar with an excess of Pd/C catalyst was carried out in a homogeneous-phase solvent mixture of ethyl acetate–methanol–water to account for low solubility and amphipathic properties of the inner salt **91** and needed 36 h for completion. Mild and high-yield *N*-acylation of the amino–phosphate **91** with *N*-propionyloxysuccinimide at neutral pH in THF-water gave acylamino-derivative **92** as a building block for further coupling to UMP. To furnish the 2-azido analogue of UDP-GalNAc, the amino group of **91** was subjected to copper(II)-catalysed diazo transfer reaction with triflic azide and potassium carbonate in a mixture of protic solvents. The α-anomeric 2-azido phosphate **93** was isolated as the triethylammonium salt.



Scheme 16

The convenience and efficacy of the phosphoramidite approach was profoundly investigated and convincingly demonstrated in the synthesis of UDP-*N*-acetylmuramyl-pentapeptide (Park Nucleotide), a biosynthetic precursor in the peptidoglycan biosynthetic cascade.[39–42] The anomeric hydroxyl group of the lactol **94** was reacted with dibenzyl *N,N*-diethyl-phosphoramidite in the presence of 1,2,4-triazole which afforded the mixture of anomeric phosphites **95** with a clear bias towards the α-anomer (α/β 2.5:1) (Scheme 17).[39] Oxidation with hydrogen peroxide and chromatographic isolation provided the α-phosphate **96** in 42% overall yield. The more labile 1,2-*trans*-configured counterpart was destroyed upon oxidation and contact with silica. Treatment with DBU followed by DCC-promoted acylation and, finally, hydrogenolytic debenzylation furnished glycosyl phosphate **97** which was further used as a building block for the synthesis of Park Nucleotide. A substantial improvement in terms of both anomeric selectivity and yield was introduced by increasing the acidity of the catalyst



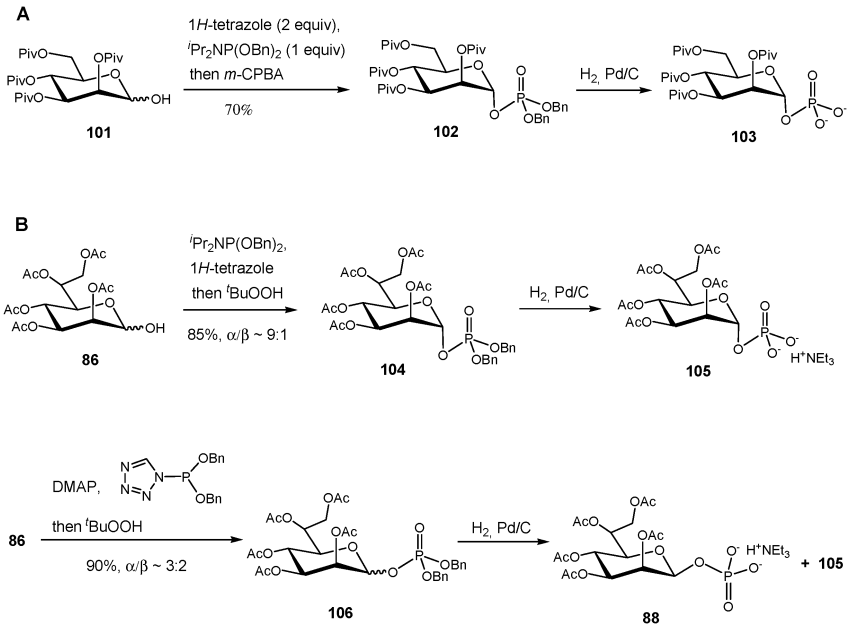
Scheme 17

by exchanging 1,2,4-triazole to 1*H*-tetrazole.[40–42] Thus, the hemiacetal **98** was treated with dibenzyl *N*,*N*-diethylphosphoramidite in the presence of 1*H*-tetrazole. The resulting phosphite triester was immediately oxidised by either *tert*-butylhydroperoxide (*t*BuOOH) or *meta*-chloroperbenzoic acid which afforded exclusively α-phosphate **99** in 75–86% yield depending on the protecting groups of the sugar moiety and oxidizing agent (Scheme 17). Since the *in situ* activation of phosphoramidite is the rate-limiting step of phosphitylation, the employment of 1*H*-tetrazole (pK_a = 4.9) instead of triazole (pK_a = 10.0) leads to accelerated activation and formation of phosphorotetrazolidite. The latter being more reactive then phosphoro-imidazolidite, is instantly consumed in the next step by the thermodynamically more stable α-hemiacetal prior to its possible anomerisation into more reactive β-counterpart, thereby improving the sterical outcome. The glycosyl phosphotriester **99** was stable under mild basic (DBU) and acidic (Zn/AcOH) conditions which were applied for the liberation of the carboxyl group, which was further activated and coupled to the free amino group of the protected pentapeptide. Hydrogenolytic debenzylation furnished anomeric monophosphate **100** as progenitor of UDP-*N*-acetylmuramyl-pentapeptide.

Although addition of activated phosphoramidite to hemiacetals of *manno*-pyranoses under thermodynamic control has been reported to deliver exclusively α-phosphates in some cases,[43] anomeric mixtures with preponderance of α-anomer have been reported in other examples.[10,44] Since formation of phosphorotetrazolidite is a rate-limiting step of the process, initial activation of phosphoramidite followed by addition of nucleophilic hemiacetal should accelerate condensation and favour the formation of the thermodynamic α-product. Indeed, reaction of hemiacetal **101** with dibenzyl phosphorotetrazolidite assured exclusive α-selectivity of the resulting glycosyl phosphate **102**.[43] The accumulation in the reaction mixture of mildly acidic 1*H*-tetrazole, which is liberated upon reaction of tetrazolidite with hydroxylic component, could also favour predominant formation of the α-phosphate (Scheme 18, A). Conventional hydrogenolysis afforded the α-mannosyl phosphate **103**.

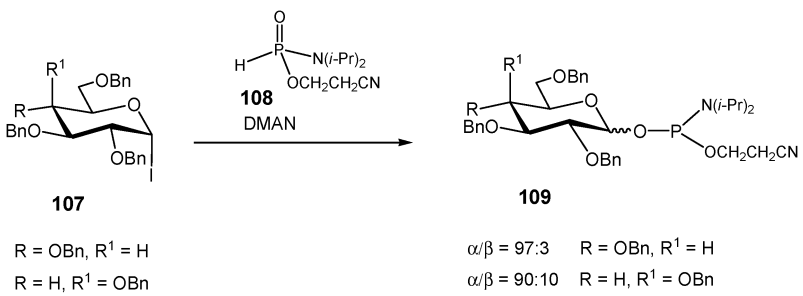
In the commonly used phosphoramidite procedure the phosphoramidite reagent undergoes activation by 1*H*-tetrazole in the presence of a nucleophilic component, which, in turn, is then rapidly consumed by the phosphoro-tetrazolidite formed *in situ*. Starting from heptose **86**, this treatment resulted in formation of the α-glycosyl phosphate **104** along with a minor amount of β-phosphate (α/β = 9:1) (Scheme 18, B). Shifting the equilibrium from the thermodynamically preferred formation of α-phosphite towards kinetically controlled generation of β-product could be achieved by limiting the amount of dibenzyl phosphorotetrazolidite in the reaction and using an excess of DMAP.[10] In this way, the glycosyl phosphotriester **106** could be enriched with the β-anomer (α/β = 3:2). The diastereomers were readily separated to furnish, after hydrogenation, pure β- and α-heptosyl phosphates **88** and **105**, respectively, which were further converted into ADP-activated derivatives, respectively (Scheme 18, B).

An innovative approach towards glycosyl phosphoramidites, which can be subsequently converted into the corresponding phosphite triesters,



Scheme 18

entails glycosylation of H-phosphonamidate derivative **108** with glycosyl iodide **107** in the presence of 1,8-bis(dimethylamino)naphthalene (DMAN). The resulting *N*,*N*-diisopropylamino cyanoethyl phosphoramidites **109** were formed with remarkable stereospecificity in favour of the α-anomers (Scheme 19).[45]

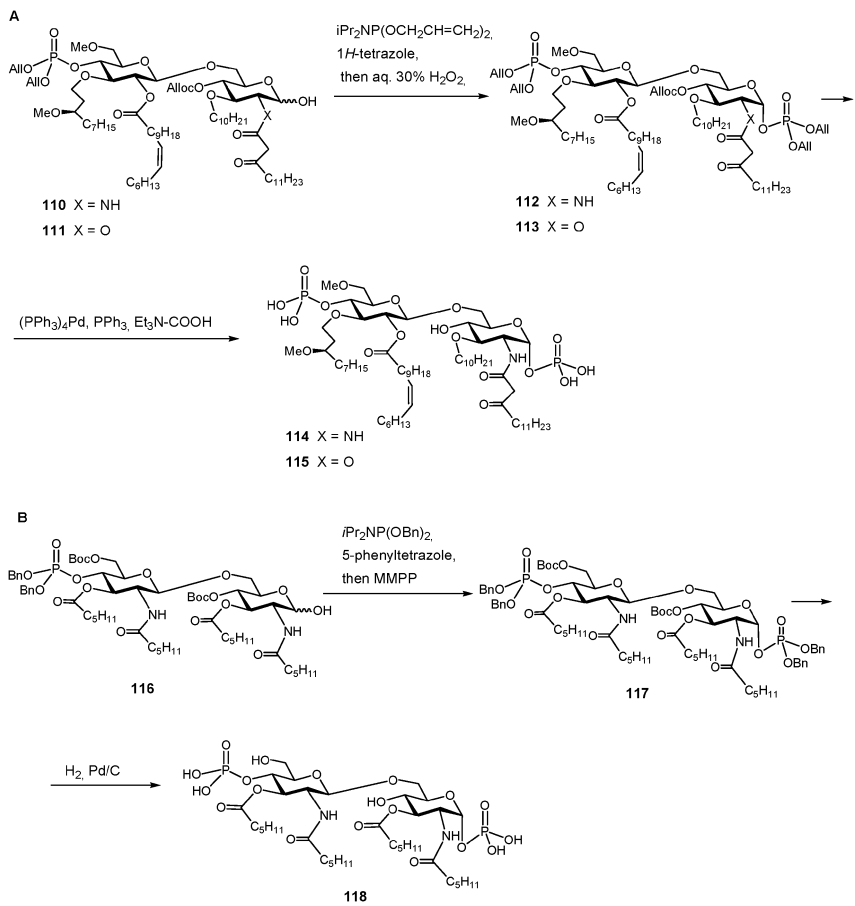


Scheme 19

4.2 Phosphoramidite approach for the instalment of α-anomeric phosphate in lipid A synthesis

The mild conditions offered by the approach of phosphitylation of the anomeric hemiacetals and subsequent oxidation of phosphites to phosphates suggest a very attractive alternative to the 1-*O*-lithiation method used in lipid A synthesis considering the complexity of the substrates. Indeed, an application of the phosphoramidite methodology was reported to be effective for the stereoselective instalment of the α-anomeric phosphate

moiety in lipid A derivatives. Accordingly, the hemiacetal of a fully protected lipid A (compound E5564) precursor **110** was subjected to reaction with 2 equivalents of *N*,*N*-diisopropyl diallylphosphoramidite in the presence of an excess of 1*H*-tetrazole, which gave, after oxidation with 30% aq. H_2O_2, phosphotriester **112** as α-anomer in 58% yield (Scheme 20, A).[46] Similarly, the glucose—analogue of the most potent LPS antagonist E5564 **113** was prepared as exclusive α-anomer in 79% yield from the corresponding hemiacetal **111**.[47] The replacement of 2-*O*-acyl or 2-*N*-acyl functionalities to a 2-*O*-alkyl chain dramatically decreased the stereoselectivity in phosphoramidite-based phosphorylation of the anomeric centre giving rise to an inseparable anomeric mixture (α/β = 4:5).[48] Cleavage of the allyl protecting group from phosphotriesters **112** and **113**, respectively was achieved in the final deprotection step by treatment with tetrakis(triphenylphosphine)-palladium(0) ($(PPh_3)_4Pd$), triphenylphosphine (Ph_3P) and triethylamine—formic acid to furnish E5564—analogues **114** and **115** in high yields. Interestingly, the authors report on the failure to perform the same deprotection procedure on non-natural α-(1 → 6)-connected disaccharide α-Glc*p*-(1 → 6)-α-Glc*p*N-(1 → P) due to formation of numerous by-products.[46,48]



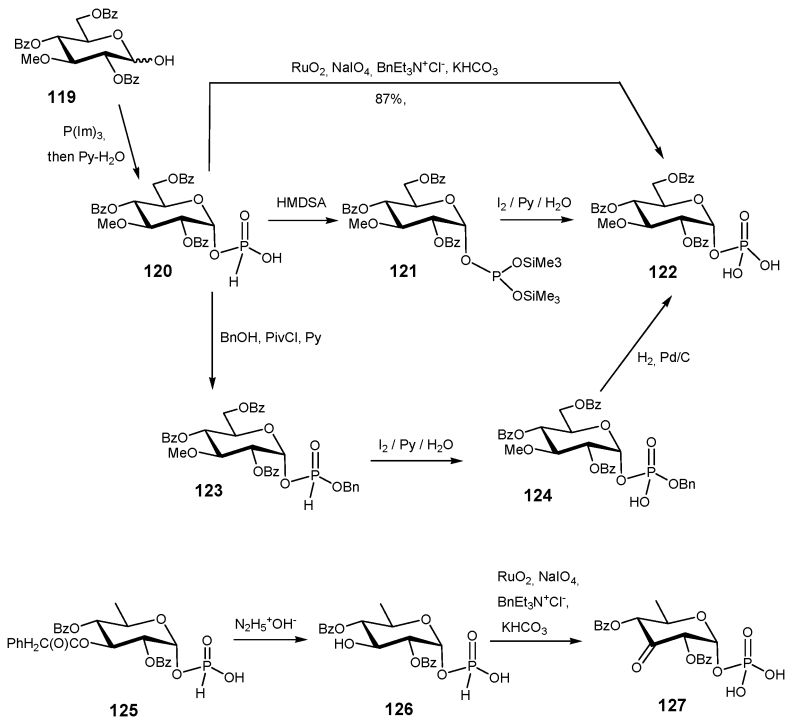
Scheme 20

The phosphoramidite approach was also successfully applied for the anomeric phosphorylation in the synthesis of short-chain analogues of tetraacylated lipid A (lipid A 406).[49] Thus, the hemiacetal **116** was reacted with equal molar amounts of *N*,*N*-diisopropyl dibenzylphosphoramidite and 5-phenyltetrazole and was then subsequently oxidised with magnesium monoperoxyphthalate (MMPP) which afforded the α-configured phosphotriester **117** as a single product in 76% yield (Scheme 20, B). The outstanding α-selectivity of the 1-OH phosphitylation using the phosphoramidite approach in the preparation of *H. pylori* lipid A was rationalised on the basis of extreme instability of the anomeric β-phosphate.[50] The stereochemical outcome of the anomeric phosphitylation of *N*-acylglucosamine derivatives in the lipid A synthesis seems to be dependent not only on the nature (acyl, β-hydroxyacyl or alkyl) of the 2-*N*-substituent, but also on the length of the 2-*N*-fatty acid chain, since an application of the phosphoramidite approach for the introduction of the glycosyl phosphate in *Chlamydia* lipid A having C-20 residues, resulted in a 3:2 α/β mixture of phosphotriesters.[32]

4.3 Application of H-phosphonate approach in the preparation of glycosyl 1-phosphates

The H-phosphonate approach entails phosphitylation of a hydroxylic component with an activated unprotected derivative of phosphonic acid to furnish H-phosphonate (a stable tetra-coordinate form of the unprotected monophosphite) followed by oxidation on phosphorus. The H-phosphonate approach is not generally used for the synthesis of monophosphates due to the difficulty of oxidation of the H-phosphonate monoesters and the necessity either to render the phosphorus atom of H-phosphonate into the three-coordinate form by silylation (to form disilylphosphite) or to introduce a protecting group (to form H-phosphonate diester) prior to oxidation. Nevertheless, in rare cases, the H-phosphonate procedure turns out to be a method of choice if, for instance, the simultaneous oxidation of functional groups other than H-phosphonate is required.[51]

Glycosyl H-phosphonates are usually prepared by the reaction of a hemiacetal with phosphorotriimidazolide (prepared *in situ* from phosphorus trichloride and excess of imidazole) as shown for **119** to give diimidazolyl phosphite which is then hydrolyzed by treatment with aqueous pyridine to α-glycosyl phosphite (H-phosphonate) **120** (Scheme 21). Oxidation of H-phosphonate is known to be very sluggish and inefficient. Hence compound **120** was first converted to a silyl phosphite **121** and to H-phosphonate diester **123**, which were then oxidized by iodine in aqueous pyridine to provide glycosyl phosphate **122** or phosphodiester **124**, respectively, in moderate overall yields. As an alternative to this two-step procedure, a direct oxidation approach of anomeric H-phosphonates with ruthenium tetraoxide—sodium periodate reagent in a two-phase bicarbonate buffer system was developed (Scheme 21, **120** → **122**).[51] RuO_4-oxidation of the H-phosphonate **120** was complete within 1 h after addition of 2 equivalents of $NaIO_4$ to furnish phosphate **122** in nearly 90% yield. The procedure was also employed for simultaneous oxidation of a hydroxyl group



Scheme 21

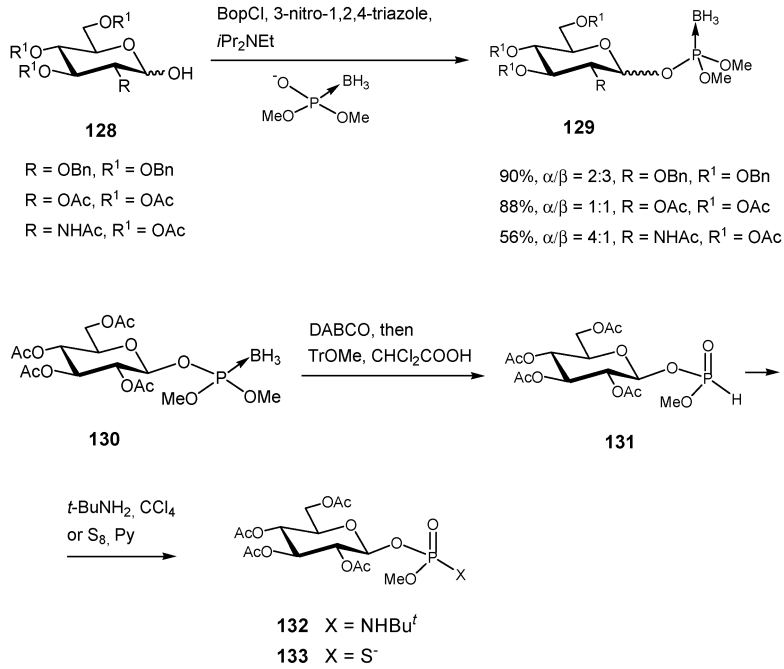
and the phosphite functionality in 6-deoxy-α-glucosyl-H-phosphonate **126**, generated from **125**, to produce 3-keto-6-deoxy-α-glucosyl phosphate **127**, which was further transformed into the corresponding TDP-derivative.

4.4. Isosteric analogues of glycosyl phosphates: phosphorothioates, boranophosphates, phosphoroamidates

The phosphite triester methodology offers, among other advantages, the possibility of transformation of intermediate phosphite triester into isosteric P-substituted phosphate analogues such as phosphorothioates and boranophosphates in which one of the nonbridging oxygen atoms is replaced by a sulphur or BH_3 group, respectively. Largely due to their stability towards enzymatic degradation, phosphorothioates and isoelectronic boranophosphates are the most common types of phosphate modification in oligonucleotides and are currently finding extended application as glycosyl phosphate modifications. P-substitution might be either introduced into the previously stereospecifically installed glycosyl phosphite functionality by direct oxidation with elemental sulphur or reaction with boronating reagents,[52,53] or by means of condensation of the hemiacetal with a specially prepared boranophosphorylating reagent.

According to the latter approach, reducing sugars **128** were reacted with triethylammonium dimethyl boranophosphate in the presence of bis(2-oxo-3-oxazolidinyl)phosphinic chloride as condensing reagent, 3-nitro-1,2,4-triazole as nucleophilic catalyst and *N,N*-diisopropyl-*N*-ethylamine to provide boranophosphate triester derivatives **129** as anomeric

mixture (Scheme 22).[54] Since boranophosphotriesters are remarkably more stable than their phosphate triester counterparts, the anomers were readily separable by silica gel chromatography without any noticeable decomposition. Partial deprotection on phosphorus of the β-boranophosphate **130** by treatment with 1,4-diazabicyclo-[2.2.2]-octane and subsequent treatment with tritylium-cation, generated *in situ* from TrOMe and dichloroacetic acid, afforded H-phosphonate **131**, a versatile precursor to a variety of P-substituted phosphate derivatives. Thus, compound **131** was converted



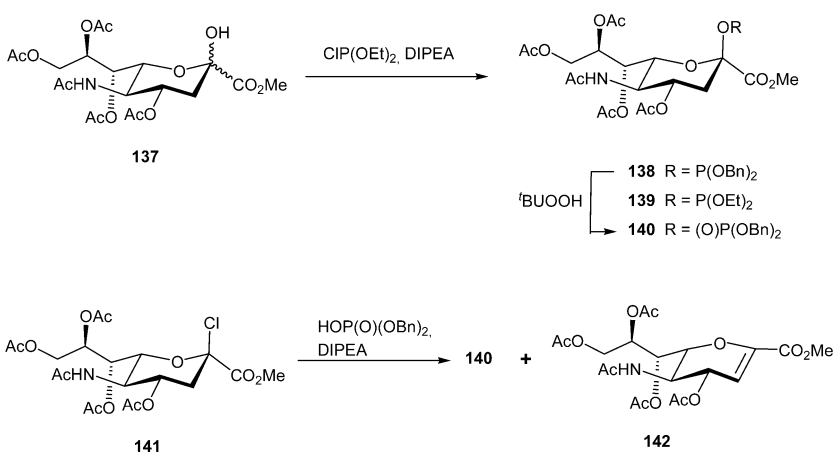
Scheme 22

into glycosyl phosphoramidate and phosphorothioate derivatives **132** and **133**, respectively. Boranophosphates were also obtained by glycosylation of dimethoxy H-phosphonate with corresponding glycosyl iodides **134** in the presence of 1,8-bis(dimethylamino)naphthalene (DMAN) to furnish glycosyl phosphites **135** which were further subjected to boronation with $BH_3 \cdot THF$ to give boranophosphate triesters **136** (Scheme 22).[45]

5. Synthesis of anomeric phosphates of aldulosonic acids

Aldulosonic acids are involved in many important areas of glycobiology and glycomedicine, such as *N*-acetylneuraminic acid (Neu5Ac), 3-deoxy-D-*glycero*-D-*galacto*-non-2-ulosonic acid (Kdn) and 3-deoxy-D-*manno*-oct-2-ulosonic acid (Kdo).

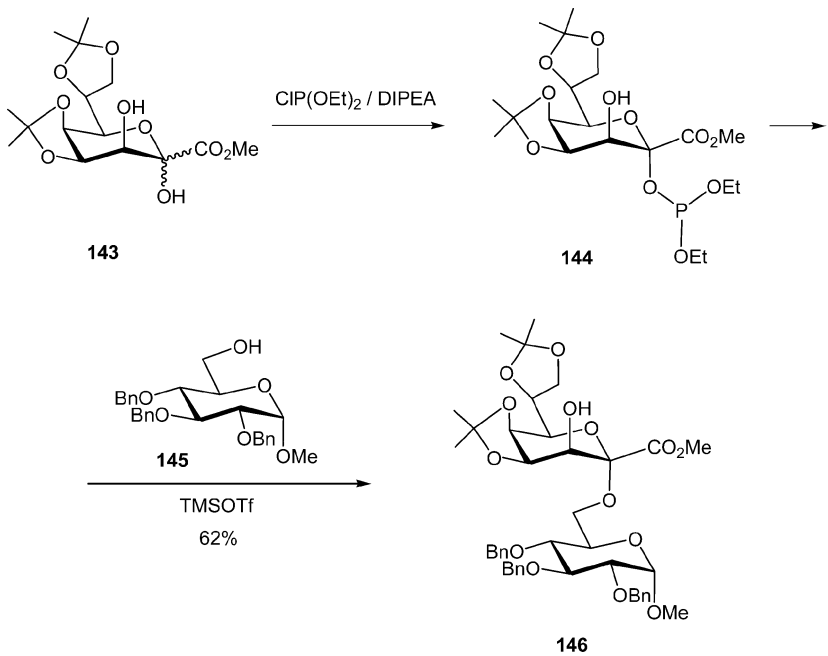
Anomeric phosphates of sialic acids have been investigated mainly in the context of developing suitable glycosyl donors as well as precursors to generate the corresponding CMP activated sugar nucleotides. The use of sialyl phosphates and phosphites has enlarged the synthetic repertoire to efficiently synthesize sialyl glycosides and has been covered by several in-depth reviews in the past few years.[3,55,56] Two approaches towards sialyl phosphites have first been reported by the groups of Schmidt and Wong, respectively, based on the reaction of the hemiketal hydroxy group of **137** either *via* 1*H*-tetrazole promoted conversion using *N*,*N*-diethyl dibenzyl-phosphoramidite or with dialkyl phosphochloridite in the presence of Hünig base providing the β-anomeric phosphites **138** and **139** in good to high yields, respectively (Scheme 23).[57–60] Oxidation of the phosphite **138** in the presence of *tert*-butylhydroperoxide furnished the corresponding phosphotriester derivative **140** in high yield. The phosphite triester **139** may serve as an efficient glycosyl donor to be coupled to nucleoside phosphomonoester derivatives as demonstrated in the synthesis of CMP neuraminic acid.[61] The anomeric phosphotriester derivative **140** has also been prepared by glycosylation of dibenzyl phosphate with the Neu5Ac chloride derivative **141**. Good yields were obtained when using Hünig-base as the counterion thereby minimizing the formation of the glycal ester



Scheme 23

byproduct **142** (Scheme 23).[62] Due to the deactivating influence of the carboxylic group in aldulosonic acid halides, however, the more frequently used methods rely on the reaction of active phosphite reagents with the anomeric hydroxy group.

Synthesis of anomeric Kdo phosphates and phosphites has received much less attention. This fact is partly due to the inherent lability of CMP-Kdo which has a half-life time of 34 min in contrast to CMP Neu5Ac which is a stable compound.[63] The hydrolytic lability of CMP Kdo resides in the axial orientation of the 5-OH group and CMP Kdo has so far only been synthesized using the enzymatic activity of the CMP-Kdo synthetase (CKS) which converts the β-pyranose tautomer of Kdo in the presence of CTP into the corresponding sugar nucleotide derivative. A 3-hydroxy-derivative of Kdo, termed Ko, has been detected in several bacterial lipopolysaccharides. The 4,5;7,8-diisopropylidene protected D-*glycero*-D-*talo*-oct-2-ulosonic derivative **143** was transformed into the corresponding anomeric phosphite triester derivative **144** by reaction of the hemiketal with diethyl phosphochloridite in the presence of Hünig-base in 91% yield (Scheme 24).[64] The compound was used as a Ko glycosyl donor in a TMSOTf promoted coupling reaction with methyl 2,3,4-tri-*O*-benzyl glucopyranoside **145** as acceptor to give the α-(2→6)-linked disaccharide **146** in 62% yield. The chemistry and potential glycosyl donor properties of Kdo phosphite and phosphate derivatives still remain to be explored in more detail.



Scheme 24

6. Conclusions

The first group of approaches to glycosyl phosphates, wherein the carbohydrate acts as electrophilic component, entails common glycosylation methods and

has been shown to be effective in the introduction of the glycosyl phosphate moiety with varying degrees of anomeric selectivity. These approaches require the initial preparation of the appropriate glycosyl donor, which, due to intrinsic hydrolytic lability, may readily decompose leading to the loss of valuable carbohydrate material. By contrast, the second group of methods to anomeric phosphates is based on an activation of commercially available phosphate or phosphite reagents, which is usually taken in excess to warrant the completeness of transformation and to minimize the loss of the carbohydrate hemiacetal constituent.

Being a good leaving group, the glycosyl phosphate functionality is usually introduced in the late stages of multi-step syntheses to minimize the number of following chemical transformations and to reduce the risk of its performance as glycosyl donor.

To make an appropriate choice of the procedure for the instalment of anomeric phosphate the following criteria are of considerable importance: (1) the costs and difficulty of the synthesis of the carbohydrate component; (2) the chemical properties of the sugar constituent (*e.g.* 2-deoxy-, 2-acylamino-2-deoxy-sugar, *etc.*) and the applicability of protecting groups (*e.g.* instalment of participating or nonparticipating group at C-2), (3) the necessity of stereoselective introduction of the anomeric phosphate (*e.g.* the possibility of using an anomeric mixture in biological applications); (4) the option of chromatographic separation of the mixture of anomeric phosphates (if formed) and the stability of the desired anomer; (5) the complexity of the carbohydrate constituent (functional groups) and consequent requirements to the mildness of phosphorylation procedure.

Abbreviations

ADP	adenosine diphosphate
All	allyl
AMP	adenosine monophosphate
BopCl	bis(2-oxo-3-oxazolidinyl)phosphinic chloride
BTSTFA	bis(trimethylsilyl)trifluoroacetamide
*m*CPBA	*meta*-chloroperbenzoic acid
CKS	CMP-Kdo synthetase
CMP	cytidine monophosphate
CTP	cytidine triphosphate
DABCO	1,4-diazabicyclo[2.2.2]octane
DBTO	dibenzothiophene-5-oxide
DBU	1,8-diazabicyclo-[5.4.0]undec-7-ene
DCC	dicyclohexylcarbodiimide
DIPEA	*N*,*N*-diisopropylethylamine
DMAN	1,8-bis(dimethylamino)naphthalene
DMAP	dimethylaminopyridine
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
GDP	guanidine diphosphate
Im	imidazole
HMDSA	hexamethyldisilazane
HOBt	hydroxybenzotriazole
DMAP	4-*N*,*N*-dimethylaminopyridine
LDA	lithium diisopropylamide
LPS	lipopolysaccharide

MMPP	magnesium monoperoxyphthalate
NDP	nucleoside diphosphate
NHS	n-hydroxysuccinimide
Piv	pivaloyl
PyBop	benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluoro-phosphate
Selectfluor®	1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(triflate)
TBAP	tetrabutylammonium phosphate
TBPP	tetrabenzyl pyrophosphate
TBS	*tert*butyldimethylsilyl
*t*BuOOH	*tert*-butylhydroperoxide
TDP	thymidine diphosphate
TMS	trimethylsilyl
Tr	trityl
TTBP	tri-*tert*butyl-pyridine
UDP	uridine diphosphate
UMP	uridine monophosphate

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Glycosidic bicyclic lactones as new carbohydrate scaffolds

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1. Carbohydrate-based bicyclic lactones: an introduction

Although less intensively studied than aldonolactones,[1] several examples of bicyclic carbohydrate-based lactones, either sugar-linked or sugar-fused lactones, have been reported in the literature.[2] Among the latter derivatives, new bicyclic systems involving the anomeric position and OH-2, namely carboxymethyl glycoside lactones (CMGLs), have been synthesised in our group and found to behave as efficient mono- or disaccharide delivery synthons.[3] Structures of this type, which involve the anomeric center in the lactone ring, can be referred to as ''glycosidic lactones''. This chapter will give an overview on the preparation and uses of such lactones for the synthesis of various *pseudo* glucoconjugates (carbohydrate aminoacid hybrids, pseudo-disaccharides, pseudoglycolipids). Before, as an introduction, a brief review of the literature will first aim at highlighting other known (glycosidic or not) bicyclic sugar-fused lactones. Some have been targeted as interesting final compounds: the carbohydrate moiety then may help elaborating a chiral structure with a suitable topology or a significant biological profile may be enhanced by the lactone. The presence of the reactive heterocycle has also been exploited from a synthetic point of view.

Sugar lactones, like the readily available D-glucono-1,5-lactone, have been widely used as useful building blocks to elaborate various glycoconjugates often relying on the opening of the monocyclic compound.[4] Owing to their structural diversity and their ability to display a number of substituents in a sterically defined manner, carbohydrates have emerged as attractive featuring groups for different architectures, from sugar amino acids[5] to glycoproteins[6] or dendrimers.[7] Some are used in glycobiology[8] where the pyranose or furanose form is essential for recognition. The polycyclic combination of a saccharidic structure with a lactone allows maintaining the carbohydrate ring feature after the ring-opening by a nucleophile. This makes thus possible to easily connect a carbohydrate scaffold to another molecular block.[9]

In some sugar-based lactones, the carbohydrate moiety can serve as a chiral template. For example, the synthesis and characterisation of a novel chiral bicyclic caprolactone **1** was reported in 2003 in six steps from 1,2,5,6-di-*O*-isopropylidene-α-D-glucofuranose.[10] The introduction of

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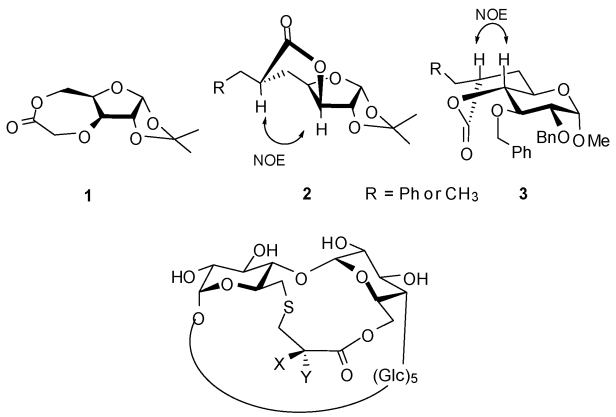
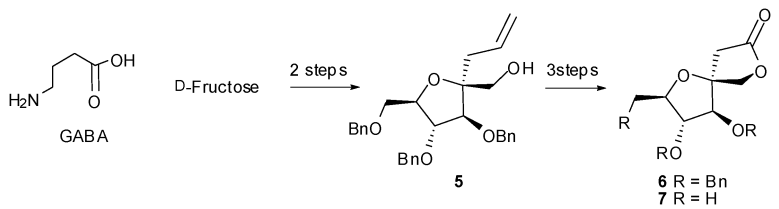


Fig. 1

ethylbromoacetate allows the further elaboration of the seven-membered ring. Initially targeted as a monomer, it was never engaged in ring-opening polymerisation. Alves *et al.*[11] also gave an example of the "off-template" diastereoselective induction by the carbohydrate skeleton in the synthesis of δ-lactones **2** or **3** by 6-exo-trig radical carbocyclisation. The stereochemical outcome proved to be dependent not only on the nature of carbohydrate but also on the pyranose or the furanose form (Fig. 1).

The modification of cyclodextrins (CDs) reported recently[12] is another example of structures associating a lactone functionality and the chiral structure of carbohydrates. The introduction of the appropriate enantiomer of *N*-dansyl cysteine on the primary side of the macrocycle led to an intramolecular condensation to give the lactones **4** in a vector-selective manner *i.e.* controlling topology (Fig. 1). Both functional CDs adopting different conformations exhibit distinct photophysical behaviours (the fluorescence of the dansyl moiety being environmentally sensitive) and unequal binding affinity to sodium adamantanecarboxylate.

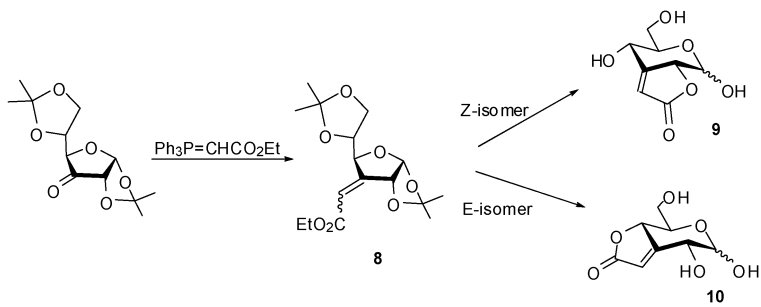
Given the biological relevance of γ-butyrolactones in therapeutic candidates, Araújo *et al.*[13] suggested to evaluate sugar-fused γ-butyrolactones and lactams as new potential GABA receptor ligands. A *C*-fructoside scaffold, rich in stereochemistry with a rigid skeleton, was used to elaborate the pharmacophore feature through a *spiro* junction between the anomeric position and the hydroxyl group at C-1 of fructose. A *C*-allylation of the fully benzylated D-fructose furnished a key intermediate **5** converted in 3 steps into the spirolactone **6**, then **7** after hydrogenolysis (Scheme 1).



Scheme 1

Compounds **6** and **7** (and other lactam analogs) are able to displace titriated muscinol, a selective agonist, in receptor binding studies at $GABA_A$ receptors using rat brain membrane preparations. Interestingly, the sugar moiety does not interfere with binding and appropriate substitution could so be used to modulate the pharmacokinetic properties or lipophilicity.

Sugar-fused lactones, abundant in both naturally occurring and synthetic products are also relevant as molecular targets with significant biological profile. The formation of intramolecular lactones is known for example to occur in glycolipids like gangliosides containing sialic acid residues. Sialoglycoconjugates are indeed very keen to intramolecular esterification due to the proximity of the anomeric carboxylic acid to neighbouring hydroxyl groups. These polycyclic lactones are suggested to be more immunogenic and tumor-specific than their native open form and are as such potential targets as cancer vaccines.[14] The particular role of sugars containing α,β-unsaturated carbonyl systems as scaffolds in carbohydrate chemistry and as potential bioactive compounds was recently reviewed by the Rauter and co-workers.[2] This group brought a major contribution to the field and some of these butenolides were recently synthesized in a stereocontrolled fashion.[15] A stereoselective Wittig reaction on readily available furanos-3-uloses (from hexoses see Scheme 3, but also performed from pentoses) was the key step to get (*E*) and (*Z*)-α,β-unsaturated esters **8** (about *Z*/*E* 5/1). An acidic hydrolysis cleaved the labile protecting groups and induced both lactonisation and isomerisation into the 2,3 and 3,4-pyranose-fused butenolides **9** and **10** in α,β anomeric mixtures. The antimicrobial activities against six pathogenic bacteria were evaluated for some of them but a pyranoid α,β-unsaturated δ-lactone proved to be the most active against the tested pathogenic species (Scheme 2).

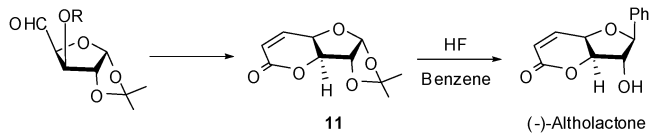


Scheme 2

A similar approach had been described for the synthesis of the mycotoxin patulin (2,3-pyranose-fused unsaturated lactone) from a 2-arabinopyranos-2-uloside derivative,[16] and more recently, for the synthesis of a potent germination stimulant and some analogous compounds from D-xylose or commercially available D-glucuronic acid γ-lactone.[17]

A furanose-fused α,β-unsaturated δ-lactone was also prepared from ester **11** as a key chiral intermediate for the synthesis of the enantiomer of (+)altholactone, a natural product with cytotoxic and antitumor activities.[18] A Reformatsky reaction with ethylbromoacetate or a Wittig

reaction with a triphenylphosphorane reagent[19] on α-D-xylo-pentodialdofuranose derivative were both used to introduce the lateral carboxylic chain for intramolecular lactonisation (Scheme 3).



Scheme 3

Like in gangliosides, lactones might be found in some bacterial capsular polysaccharides containing 1-carboxyethylsubstituents. But their identification remains problematic due to the conditions of isolation and preparation of analytic samples. To facilitate their detection by NMR, and in order to determine if the formation or hydrolysis of lactones occurred during analytical procedures, synthetic model substances, 2,3- and/or 3,4-lactones based on gluco-**12**, manno-**13**, and galactopyranosides **14** were prepared and characterized by NMR spectroscopy (Fig. 2).[20] The relative lactonisation rates in acetic acid-d_4 and hydrolysis rates in buffered D_2O were evaluated.

Similarly, a newly discovered sialic acid **15** has been suggested to play a significant role in glycoproteins but only by indirect evidence as its hydrolysis is suspected to occur upon isolation. Allevi *et al.*[21] have thus very recently described the transformation of *N*-acetylneuraminic acid (NeuAc) **16** into its 1,7-lactone **15**. Different classes of sialyl lactones had already been prepared as the equatorial or axial configuration of the carboxyl group gives either the 1,4-(**17**), 1,7-(**18**) or the spirolactone (**19**) (Scheme 4).[14,22] A bulky and easily removable acylating agent (CbzCl) activated the carboxyl group for the chemoselective 1,7-ring closure and was selectively introduced at the anomeric secondary hydroxyl group. A simple hydrogenolysis of the Cbz group affords quantitatively **15**, stable under crystalline form at 4 °C. This synthesis allowed to confirm the instability in water and in acidic conditions and pointed out the possible formation of the lactone during the acylation of various sialic acids, the analysis of which must be carefully set up. Besides the potential biological activities of such compounds, the presence of a reactive heterocycle directly connected to a carbohydrate appeared as really attractive and has been successfully exploited. In 1997, during their research for new NeuAc analogs, Gervay's group suggested sialyllactones as synthetic intermediates to provide sugar aminoacids by reaction with nucleophilic amines. 1,4- and spirolactones were thus

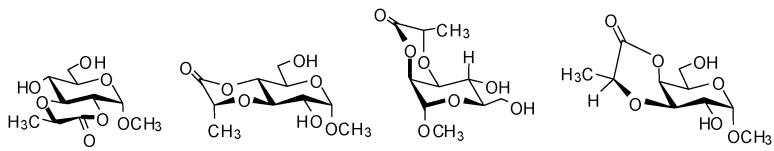
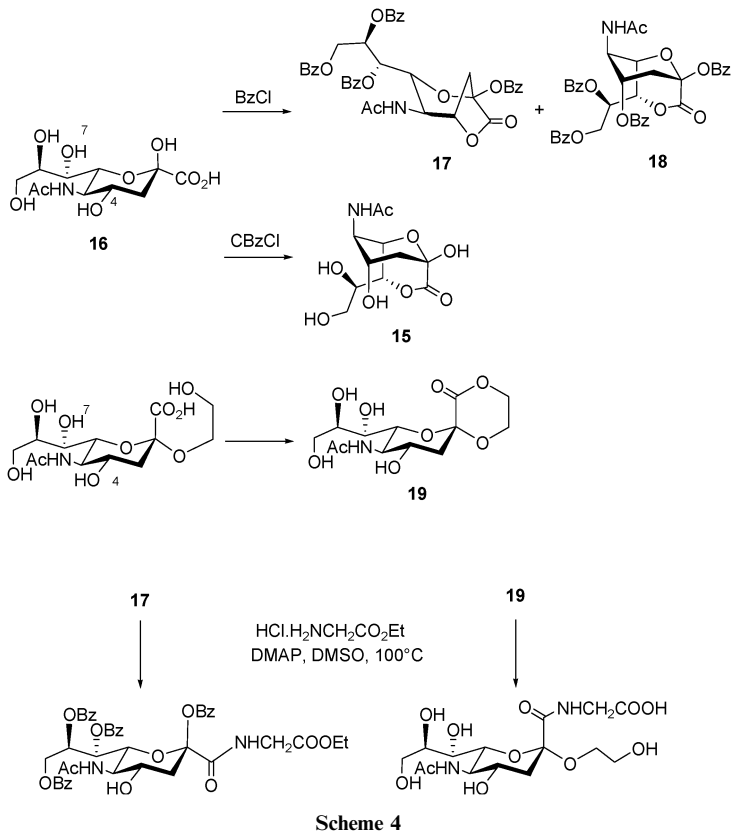


Fig. 2

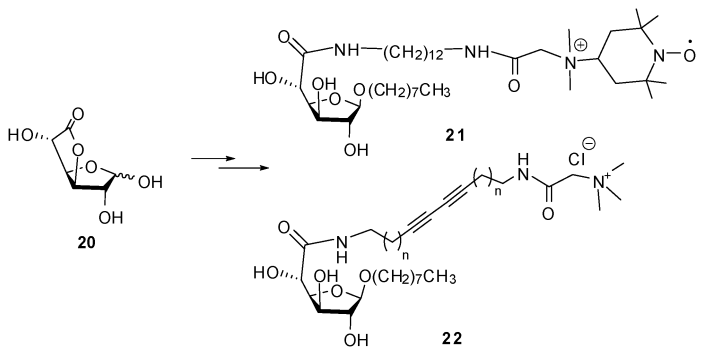


Scheme 4

submitted to ring opening reactions with glycine ethyl ester hydrochloride salt.[22] Even after long reaction times, the yields remained however modest probably due to steric congestion. In 1994, Camarasa *et coll.*[23] had already mentioned the possibility to prepare 2′-C and 3′-C-branched-chain nucleosides by radical cyclisation from γ-butyrolactones of nucleosides albeit in low yields.

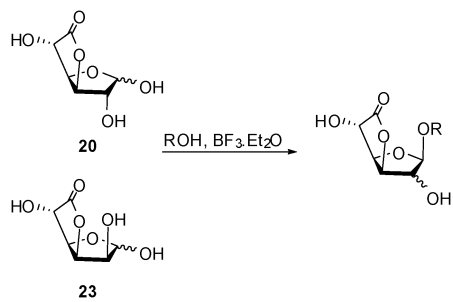
More recently, D-glucofuranurono-6,3-lactones **20** (as well as D-*manno* and *galacto* derivatives) found their application as readily available precursors for the preparation of novel unsymmetrical bolaamphiphiles.[24,25] A correlation is indeed sought between the structure of the bipolar molecule and the one of the supramolecular aggregates they can formed and which may be used to develop new advanced materials. To the neutral glycosidic polar head, with a more or less long alkyl chain in the anomeric position, various diamines had been added *via* lactone opening. Selective monoacylation allowed further functionalisation of the free amine either by introduction of a spin-active nitroxide permitting ESR studies (**21**)[25] or of a cationic glycine betaine by peptide ligation (**22**) (Scheme 5). The variation in the nature and length of oligomethylene bridging chain[24,26] linking both polar heads led to differences in the observed self-assembled morphologies.

Such lactones had already been exploited in Rennes by Ferrières *et al.* for the stereoselective formation of alkyl furanosides from the readily available D-glucuronic acid **20**.[27] Similarly, the analogous



Scheme 5

D-mannofuranurono-6,3-lactone, **23**, obtained by acidic hydrolysis of alginic acid, was used in glycosidation reactions with alcohols in the presence of boron trifluoride-diethyl etherate.[28] The β-D-mannofuranoside derivatives were thus obtained with good β-anomer selectivity without using a tethering glycosylation strategy (Scheme 6). A preferential coordination between the boron trifluoride promoter and the α-anomer induced by the lactone ring could explain the anomeric selectivity, opposite to the one previously obtained with Dowex 50W-X4 resin.[29]

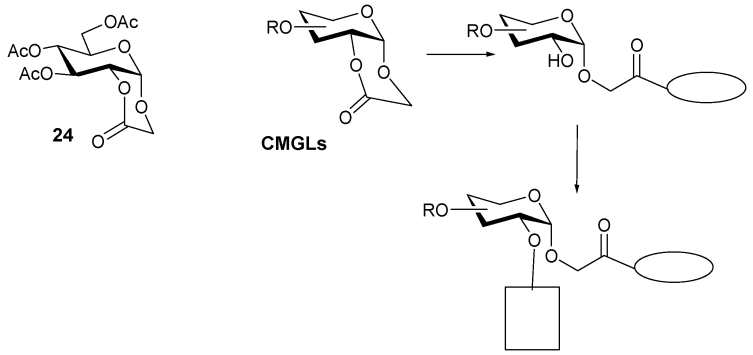


Scheme 6

Besides a chiral remote induction for glycosidation, other glycosidic sugar-fused lactones have been also directly used as saccharidic delivery synthons.

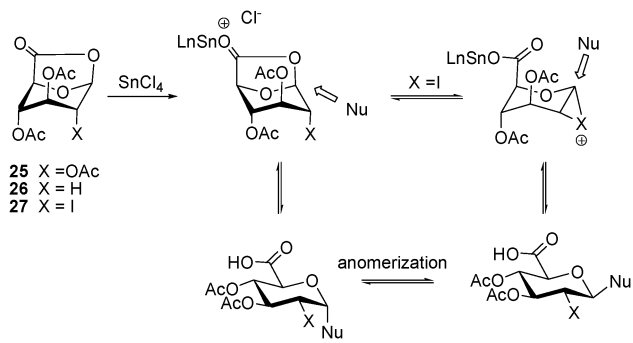
In 2000, we described for the first time the preparation and the use of carboxymethyl α-D-glucoside lactone **24**.[3a] Since then, we have developed a full methodology for appending sugars onto various molecular blocks (aminoacids, aminodeoxysuagrs, steroids, porphyrins) by reaction of lactone **24** and analogous lactones (CMGLs) based on many different sugars (Scheme 7).[3] The nucleophilic opening of the lactone occurs without affecting the configuration of the anomeric center. The chemodifferentiation is done by the release of a free 2-OH position available for further functionalisation. The preparation and applications of these pseudo-glycosyl donors, which is the main purpose of this chapter, are developed in the sections 2 and 3.

When the anomeric hydroxyl group is engaged in the ester function, Lewis-acid anomeric activation of these glycosyl donors triggers the



Scheme 7

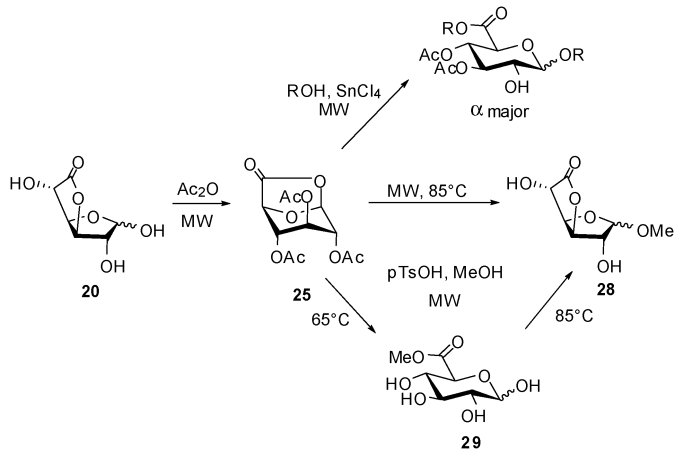
opening of the lactone. 1,6-Lactones are for example also available from the previously mentioned uronic acids and stereoselective glycosylations by nucleophilic opening at the anomeric position have been performed specially by Murphy's group (Scheme 8). Using tin(IV) chloride as promoter, the glycosyl donors, the lactone **25** and the 2-deoxy parent lactone **26** gave α-anomers in a highly stereoselective manner while 2-deoxy-2-iodo derivative **27** led to β-anomers.[30]



Scheme 8

cis-1,2-Glycosides were thus obtained from **25** (despite the neighbouring acetate) and **26** with β-silyloxy azido acids or with silylether glycosyl acceptors. A SN_2 process was first believed to explain the diastereoselectivity when iodide better anchimeric assistance could force the attack of the nucleophile from the upper face. A more extensive study of possible mechanistic pathways proved that epimerisation of kinetically formed β-anomer took place and was dependent on the temperature, the nature of the acceptor and catalyzed by the released carboxylic acid.[31] Rat *et al.*[32] were interested in the benefit of microwave irradiation in solvent-free conditions both for the synthesis of **25** and glucuronic acid lactone derivative **28** from **20** (Scheme 9). Lactone **28** was obtained as an anomeric mixture of methyl glycosides from D-glucuronic acid or from **25** in the presence of *p*TsOH and methanol. At lower temperature (65 °C *vs.* 85 °C) **25** was instead converted into the methyl glucopyranuronate. Besides the acceleration, under $SnCl_4$

catalysis, a concomitant esterification of the released carboxylic acid was observed as well as an increased stereoselectivity due to an anomerisation favored by microwave heating.



Scheme 9

The three 1,6-lactones **25**, **26**, **27** provide rapid access to glucuronate synthons. According to Hoffmann,[33] such lactones can be regarded as tethered anomeric acetates acting as intramolecular leaving groups and releasing a free carboxylic group incorporated in the glycosidic framework. A chemodifferentiation is realised between positions 1 and 6 of the sugar and the presence of the carboxylic acid both facilitates the purification and may stabilise the deoxy compound towards acid. After reaction of the released free carboxylic group with diamines, diene **29** or diyne **30** could be obtained from **25** and were subsequently involved in intramolecular ring-closing metathesis to form glycophanes **31** (Fig. 3) (novel hybrids of sugars and cyclophanes believed to be of interest in bioactive molecule development and in biomimetic, supramolecular and materials chemistry). The nature of the amide (secondary or tertiary) turned out to be critical

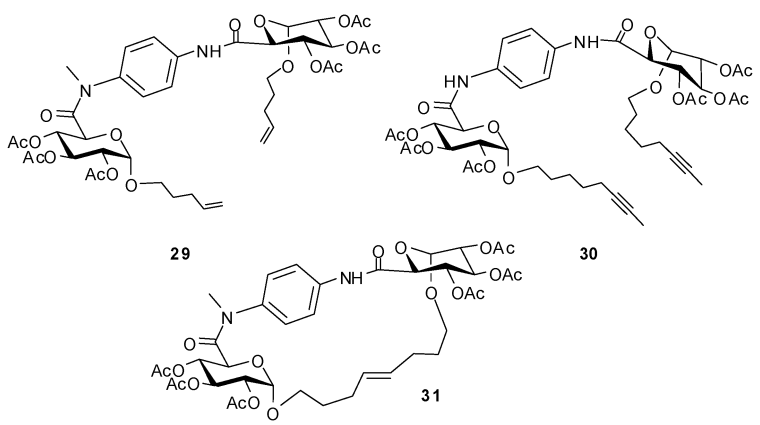
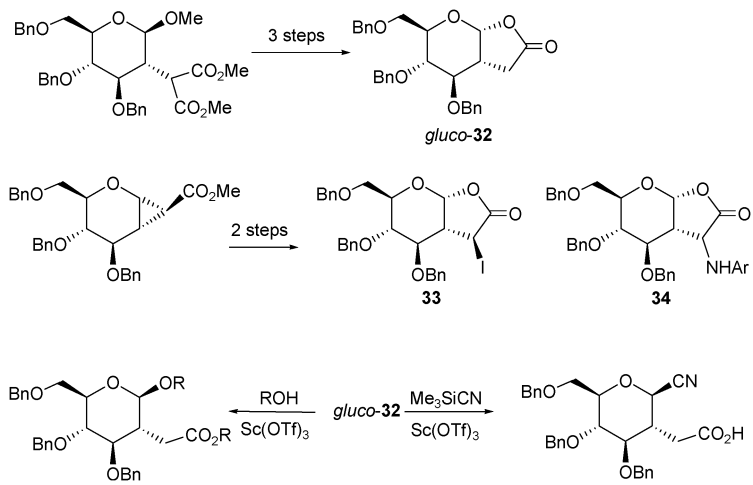


Fig. 3

for the geometric arrangement of the sugar moieties and so for the solubility in water.[34]

Very recently, Linker and co-workers[35] disclosed their convenient synthesis of new unsubstituted carbohydrate 1,2-lactones like **32** (gluco configuration). Two recent reports had described structures with substituted lactone ring **33** and **34**[36] (Scheme 10), rare examples of carbohydrate 1,2-lactones being found in the literature, except higher oxydized analogues or more complex structures.[37] The synthesis in three steps from the corresponding glycals, by addition of dimethyl malonate, mild saponification followed by direct cyclisation after acid-catalysed thermolysis, was realized from hexoses (glucose, galactose), pentoses (xylose, arabinose) and disaccharides (maltose, lactose) into carbohydrate-fused γ-lactones.



Scheme 10

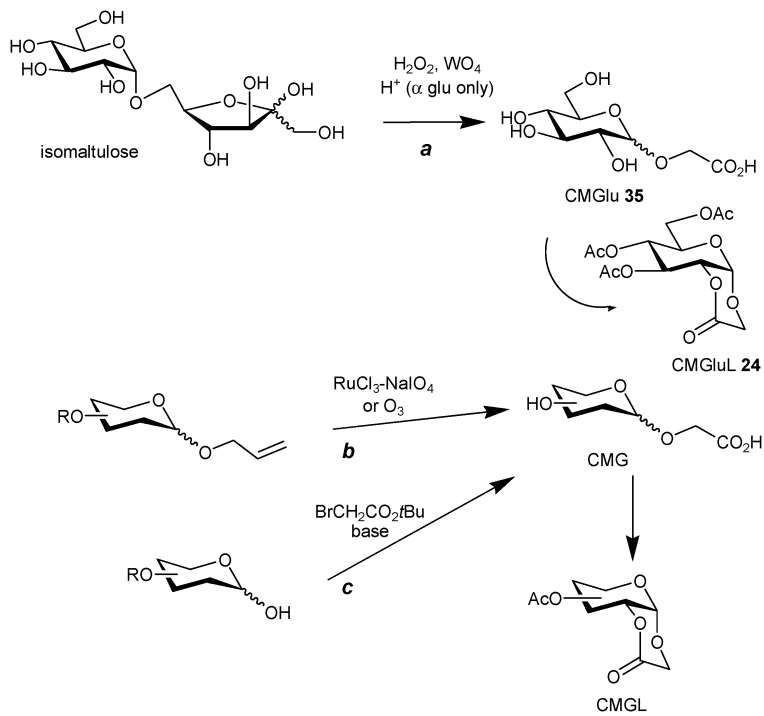
Stereoselective reactions at the anomeric center with nucleophiles were developed to provide C-2 branched saccharides. Ring-opening products were obtained from the *gluco*-configured lactone **32** after addition of oxygen, nitrogen, sulphur or even hydride nucleophiles in the presence of $Sc(OTf)_3$. The released free carboxylic acid was most often directly esterified under these conditions. Carbohydrates acceptors proved to be also suitable nucleophiles although modest yields for the unesterified disaccharides were obtained. β-Selectivity was observed, albeit depending on nucleophiles and reaction times, and an epimerisation into the α-anomer also occurred. The introduction of carbon susbstituents was also validated by reaction with silylated nucleophiles (Me_3SiCN, $Me_3Siallyl$ . . .) or electron-rich arenes, providing stereoselectively interesting β-*C*-glycosul compounds. Applications in total synthesis are suggested by straightforward transformations of these functionalised intermediates.

The following sections will now focus on the synthesis and the uses of carboxymethyl glycoside lactones which we developed in our group.

2. Carboxymethyl glycoside lactones (CMGLs): synthesis

Our work on the carboxymethyl glycoside lactones (CMGLs) originated from our interest in the chemistry of unprotected sugars.[38] Looking for new carboxylated species obtained from available disaccharides,[39] we studied the oxidation of isomaltulose (6-α-D-glucopyranosyl-D-fructofuranose) and found that carboxymethyl tri-*O*-acetyl-α-D-glucopyranoside (CMG, **35**) could be obtained in a straightforward manner compared to previous syntheses.[3a] Fischer glycosylation of glycolic acid by glucose in the presence of hydrochloric acid, described by Petersson *et al*., is also a very straightforward method (despite its low 6% yield), but it leads to a 70:30 α/β mixture.[40] In the process of its characterisation by acetylation, the 2-*O*-lactone (CMGluL, **24**) was identified, and was found to be easily opened by reaction with nucleophilic species leading to neoglucoconjugates.[3b,41–43]

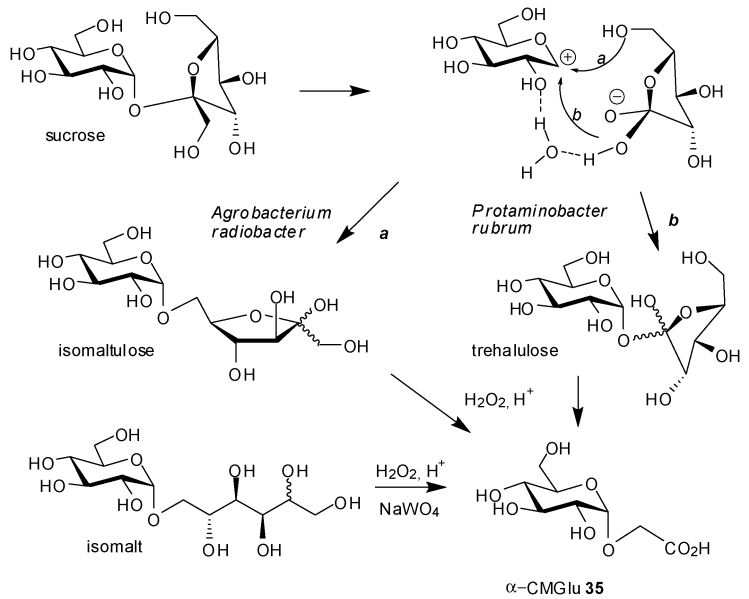
Therefore, taking into account the potentialities of such lactones as carbohydrate delivery synthons (*vide infra*), several routes leading to carboxymethyl glycosides (and thus subsequently to the lactones) were investigated, in order to get as many structural variations as possible for widening the scope of their use in synthesis. In addition to the isomaltulose oxidation method (route a), the oxidation of allyl glycosides (route b), and the anomeric alkylation with *tert*-butylbromoacetate (route c) were studied (Scheme 11). These three methods are detailed in the following sections.



Scheme 11

2.1 Oxidation of isomaltulose and related sugars: synthesis of CMGlu and CMGL

Disaccharides such as isomaltulose (6-α-D-glucopyranosyl-D-fructofuranose), obtained in one step from sucrose by bioconversion (Scheme 12),[44] Isomalt®, the hydrogenated derivative of isomaltulose and trehalulose (1-α-D-glucopyranosyl-D-fructopyranose) have been shown to be interesting substrates for chemistry.[45]

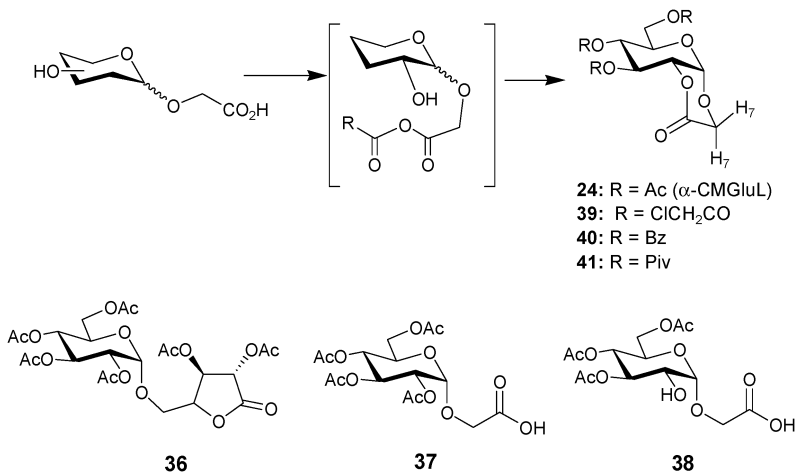


Scheme 12

Oxidation of isomaltulose by various methods has been reported. For example, using air oxygen under basic conditions, isomaltulose provides glucosyl-α-D-arabinonates, whereas more carboxylated derivatives are formed under platinum-catalysed oxidation conditions.[46] Our efforts were directed towards the use of aqueous hydrogen peroxide, easily available and generating no by-products.[47] The oxidation of carbohydrates by hydrogen peroxide under acidic or basic conditions can be more or less efficient depending on the presence of free hemiacetals at anomeric position, as well as the presence of additives such as metal salts. Degradative oxidation-decarboxylation reaction can often be observed, leading to short acids as final products. Under acidic conditions, hydrogen peroxide oxidation of isomaltulose led to α-CMGlucoside **35**. The addition of sodium tungstate is known to promote the oxidative cleavage of glycols *via* peroxotungstate species,[48] for example leading to erythronic acid-terminated oligoglucosides from starch or maltodextrins.[49] In the case of isomaltulose, it allowed slightly higher yields and permitted to work at increased pH. However, the yields were not significantly better. When isomalt was used as starting material, the presence of tungstate salts proved to be indispensable.[3d] A typical procedure consists in heating isomaltulose with excess hydrogen

peroxide at pH 2 at 85–90 °C for several hours. Under basic conditions,[50] formation of α-CMG was much slower. CMGlu could be obtained on a 5–10 g scale in *ca.* 35% yield.

It is at the stage of the characterisation that we identified the lactone **24** (α-CMGluL, Scheme 13), for which ^{1}H and ^{13}C one- and two-dimensional NMR spectroscopic analyses gave clear evidences of the bicyclic structure. Notably, HMBC C–H correlations were observed between H-7a,b and C-1 and between H-7a,b and C═O. It is supposed that a mixed anhydride intermediate is formed first, followed by cyclisation reaction with OH-2. Such a glucoside lactone had never been fully characterised, although a similar structure was suggested as an intermediate in the case of a β-lactoside.[51] A comparable system has also been reported in a recent patent among the intermediates towards a lipid-A analog.[52] A few side products were identified and provide clues on the outcome of the oxidation. For example, the presence of lactone **36** (Scheme 13) can be explained by incomplete oxidation when the reaction was not carefully maintained at 90 °C. Also, glucose pentaacetate can be present when the pH is not well controlled and carboxylic acid forms of CMG, either acetylated at OH-2 or not (**37** and **38**), reflect the opening of the lactone during the reaction or at the work-up stages. Three different acetylating systems (acetic anhydride/pyridine, acetic anhydride/DMF/Et_3N, acetic anhydride/sodium acetate) were used with very similar results. An important aspect is the possible opening of the lactone at the work-up or purification stage, which have to be performed relatively rapidly, after careful removal of remaining base before column chromatography.[3d] Three other lactones **39–41** were obtained by treatment of CMG with chloroacetyl chloride, pivaloyl anhydride or benzoyl chloride.



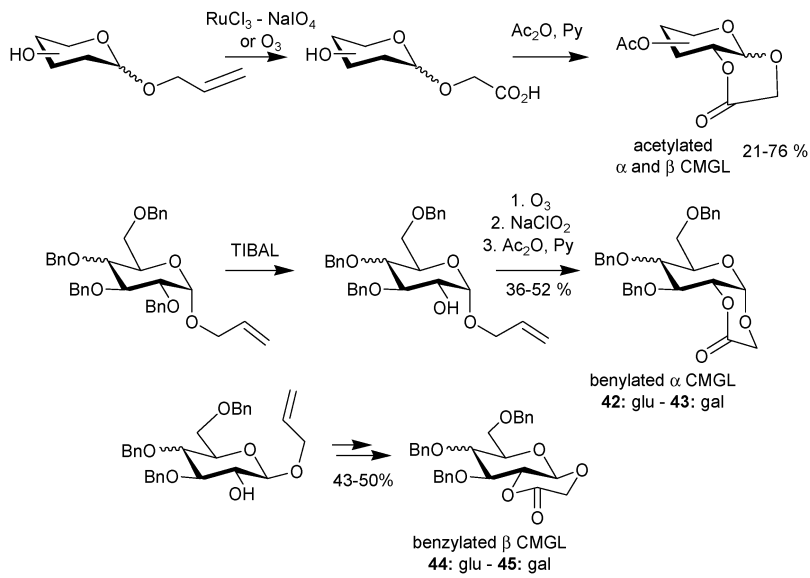
36 **37** **38**

Scheme 13

2.2 The allyl glycoside route

Allyl glycosides can be obtained either by Fischer or Koenigs-Knorr type glycosylations. They can be oxidized by the $RuCl_3$–$NaIO_4$ system,[53]

developed by Sharpless and co-workers,[54] by bishydroxylation followed by glycolic cleavage,[55] or by ozonolysis.[56] Both acetylated and benzylated lactones derived from glucose and galactose were obtained by this route. Oxidation of the double bond of glucose and galactose allyl glycosides with ruthenium trichloride led to peracetylated carboxymethyl glycosides,[3c] which were transformed into the corresponding lactones by a deacetylation-reacetylation sequence. A more straightforward process to the same lactones is the direct oxidation of allyl glycosides by ozonolysis, followed by reaction with $NaClO_2$ and subsequent lactonisation under acetylation conditions, without intermediate purification. When this last sequence is applied to the mixture of anomers, the mixture of α and β lactones (eventually difficult to separate) is obtained in 38 to 58% overall yields (Scheme 14). The possibility to get lactones with benzyl protecting groups was then studied. Such lactones would allow easier further manipulations on their adducts. The proper allyl α-glycosides (glc and gal) were obtained by triisobutylaluminium (TIBAL) de-*O*-alkylation, a method developed by Sinaÿ and co-workers which selectively deprotects the 2 position of α-glucosides or galactosides.[57] Then lactones **42** and **43** were synthesized by ozonolysis, oxidation of the resulting aldehydes with $NaClO_2$ and, finally, lactonisation under acetylation conditions. The β allyl glycosides prepared by the orthoester strategy, led to the corresponding β lactones **44** and **45**. It should be noted that benzylated lactones exhibited lower stability often spontaneously opened by traces of water when stored. Unlike, most acetylated lactones were found to be stable.



Scheme 14

2.3 The anomeric alkylation *via tert*-butylbromoacetate route

An alternative sequence consists in connecting the sugar to a carboxymethyl residue instead of an allyl one. Fischer and Helferich reported the synthesis

of ethoxycarbonylmethyl β-D-glucopyranoside and its subsequent saponification to the carboxylic function in 1911.[58] Since then, many examples of glycolic acid esters glycosylation were described, using various precursors (bromide, fluoride, trichloroacetimidate).[51,59] An acid-catalysed reaction with glycolic acid ethyl ester was described in the presence of a perbenzylated substrate,[55b] and the use of the silver(I) salt of glycolic acid was reported.[60] In place of a glycolic residue, bisdimethylacetal of glycolylaldehyde was also used, with a subsequent oxidation step by $NaClO_2$.[61] Our approach was to combine reaction of α-halogenoacetic acid derivatives with alcohols or amines and the anomeric alkylation strategy.[3e]

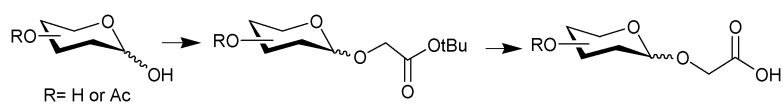
Anomeric alkylation has been extensively studied by Schmidt and co-workers,[62] and the main alkylating agents used were dialkyl sulphates, benzyl bromide, allyl bromide[63] or various *O*-triflates, allowing the synthesis of disaccharides.[64] The stereochemical outcome of anomeric alkylation is known to depend on many parameters such as the base, the solvent and its effect on solubility and concentration, the temperature, chelation effects, presence of additives, and nature of the electrophilic species.[62–65]

First, alkylation with *tert*-butyl bromoacetate of acetylated sugars, having only available the anomeric hemiacetal hydroxyl group, was studied (Table 1 and Scheme 15). Such substrates are easily obtained from the peracetylated derivatives by selective anomeric deprotection of the readily available corresponding peracetylated sugars.[66] For example, reaction of tetra-acetyl glucose with *tert*-butyl bromoacetate occurred readily under mild conditions (room temperature, DMF, K_2CO_3). The two isomers were easily separated at this stage, with a 6.1:1 selectivity in favour of the α-anomer. A series of mono- and disaccharides were subjected to the same procedure leading to

Table 1 Anomeric alkylation of OH-1 of acetylated or unprotected sugars with *tert*-butyl bromoacetate

Sugar	Yield of anomeric alkylation from acetylated sugars having OH-1 free (%)[a]	α:β	Yield of anomeric alkylation from unprotected sugars (%)[b]	α:β
Glucose	88	6.1:1	50	1:2.8
Mannose	91	32.3:1	55	1:1
Galactose	94	8.1:1	65 (furanoside)	>19:1
Fucose	74	4.8:1	n.d.	n.d.
N-Ac-Glucosamine	85	6.7:1	77	2.8:1
Lactose	68	3.3:1	57	1:2
Maltose	88	4.9:1	49	1:1.8
Cellobiose	85	3.3:1	n.d.	n.d.

[a] DMF, K_2CO_3 (2.5–5 equiv.), *tert*-butyl bromoacetate (2 equiv.), r.t. [b] NaH, DMF, *tert*-butyl bromoacetate (1 equiv.), r.t.



Scheme 15

the corresponding glycosides, all with significant selectivity for α anomers. Although full deprotection is necessary for the further transformation to the lactones, deprotection of only the *tert*-butyl ester can be achieved leading to the acetylated carboxylic acid glycosides, which are also useful synthons for grafting sugars. From totally unprotected sugars and in the presence of sodium hydride, monoalkylated products at the anomeric position were obtained in fair yields. Glucose, mannose, *N*-acetyl glucosamine, maltose and lactose gave the desired pyranoside products, whereas galactose led to a mixture in which the α-furanoside was the major product. Such a behaviour of galactose has already been reported.[63] In terms of selectivity at the anomeric position, a higher proportion of the β anomer (α:β from 1:1 to 1:2) was obtained from the unprotected sugars using NaH (except for galactose and *N*-acetyl glucosamine).

The two sets of conditions (partially acetylated sugars, K_2CO_3, or unprotected sugars, NaH) leading to different proportions of α or β anomers, allowed to prepare a variety of bicyclic carboxymethyl glycosides. Full deprotection of the various glycosides obtained by anomeric alkylation (1 M aq. NaOH in MeOH with possible intermediate TFA *tert*-butyl esters cleavage) led to the carboxymethyl glycosides, which were acetylated by treatment with acetic anhydride in pyridine (Fig. 4).

A large series of lactones was thus prepared, with variations in the sugar type, the anomeric configuration, mono- or disaccharides, and benzyl protected lactones. The potential of these lactones is illustrated in the

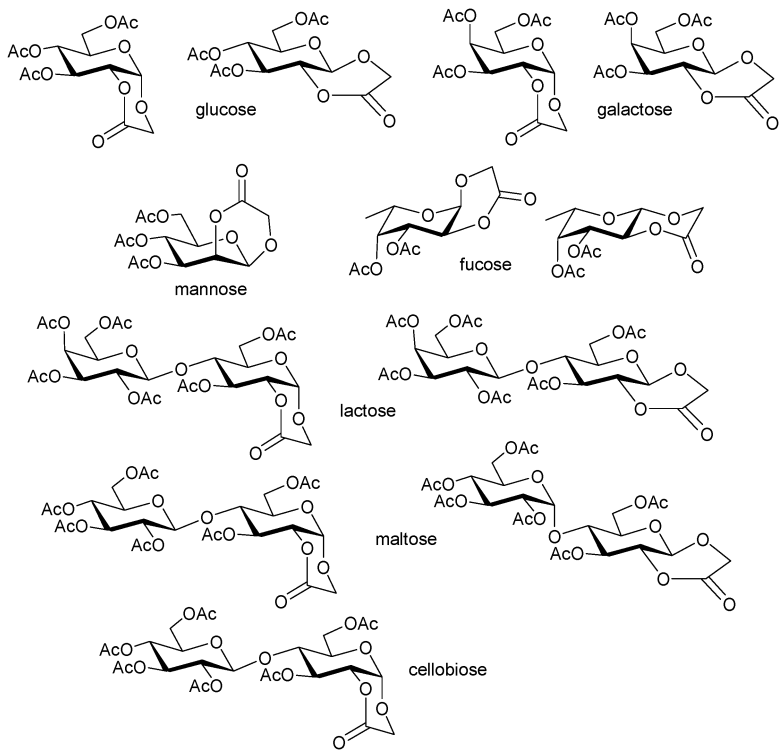


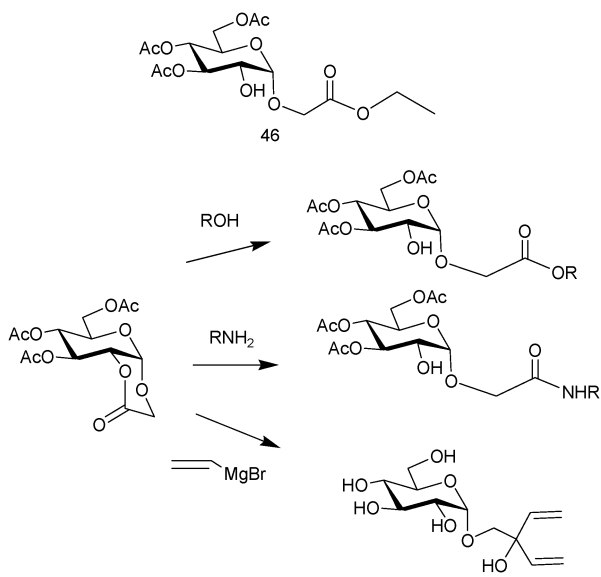
Fig. 4

following sections, which describe the formation of their adducts by reaction with various nuclephilic species by simple addition. The free OH at position 2 could be functionalised giving access to more elaborated products. The intermediate carboxymethyl glycosides, obtained by removal of the *tert*-butyl ester with TFA, are interesting synthons as well, used as connecting system in many applications.[51,53c,d,55a,b,56b,59b,g,67] In this respect, the anomeric alkylation route explored by our group proved to be general, convenient and straightforward.

3. Uses of CMGLs towards mono- and difunctional systems

3.1 General aspects

The ability of these lactones to be readily opened by nucleophilic species was first detected by the presence of small amounts of CMG ethyl ester **46**, having a free OH at position 2, in the mother liquors of a recrystallisation of the lactone in ethanol. Building on this observation, we then extensively studied the lactone opening process in the case of alcohols and amines. For alcohols, either base or acid catalysis could promote the reaction (without any catalyst, the process was very slow). However, the difficulty to remove selectively the acetyl groups without cleaving the newly formed ester make the reaction of little synthetic interest if unprotected adducts are targeted. The reaction of the lactones with amines, leading to the corresponding amides, provided a much wider scope to these synthons as illustrated by the following sections. The case of a carbon nucleophile was also rapidly explored but in its presence, the ester protecting groups proved to be not enough stable though clues for the formation of carbon-carbon bonds were obtained (Scheme 16).



Scheme 16

3.2 Monofunctional pseudo-conjugates from CMGLs

3.2.1 Glycoaminoacids. Glycopeptides are an important class of biomolecules and therefore, many methods for the synthesis of mimetics have been reported.[9d,68,69] It was thus interesting to evaluate the CMGLs as precursors of new glycoaminoacid hybrids.[3b] Hydrochloride salts of aminoacids protected as esters were used. The reaction proved to be very general and a series of adducts were obtained (Fig. 5). Typical changes in the proton NMR spectra for the CMG conjugates compared to the lactone were observed, notably H-2, H-3 and the two H-7 of the carboxymethyl linkage. The presence of a new stereogenic center in the aminoacid moiety was a concern as the basic conditions for the coupling reaction (necessary for the neutralisation of the hydrochloride and liberation of the free amine) could possibly provoke an epimerisation. The final deprotection of the acetyl groups to the free sugar in basic conditions could also be problematic. The example of aspartic acid dimethyl ester was used to check this, both the L- or the D-amino acids being easily available: in the two diastereoisomers formed, specific AB system patterns at the side chain CH_2 were observed by NMR spectroscopy. It was thus cleary verified that both compounds are different and pure after the coupling step. For the deacetylation step, different methods were evaluated. Hydrazine in either methanol or methanol-dichloromethane, known to respect amino acid chirality[70] could be used without any epimerisation. However, methyl ester protecting groups are not compatible with the hydrazine/methanol method and lead to hydrazides (which are actually also interesting compounds). The MeONa–MeOH system led to 1:1 mixtures of epimers. The system which

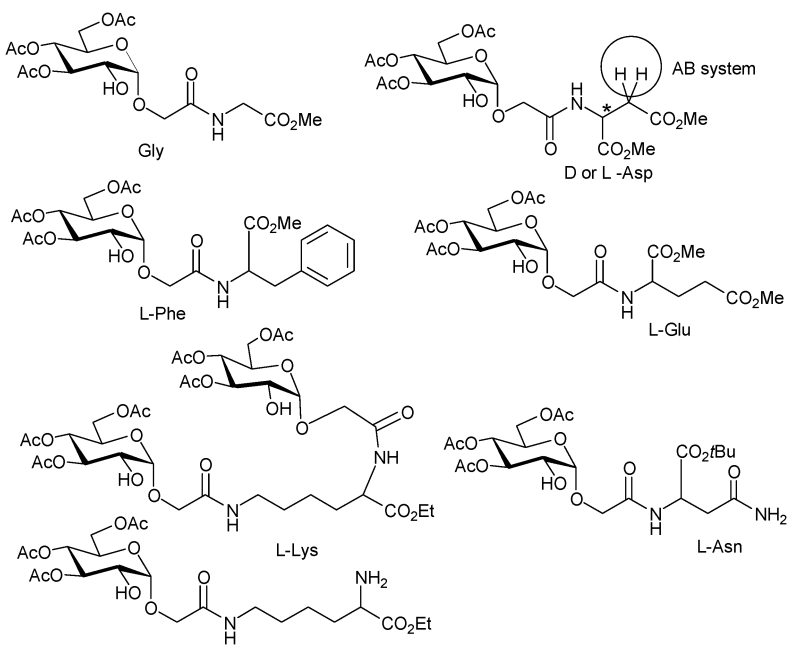
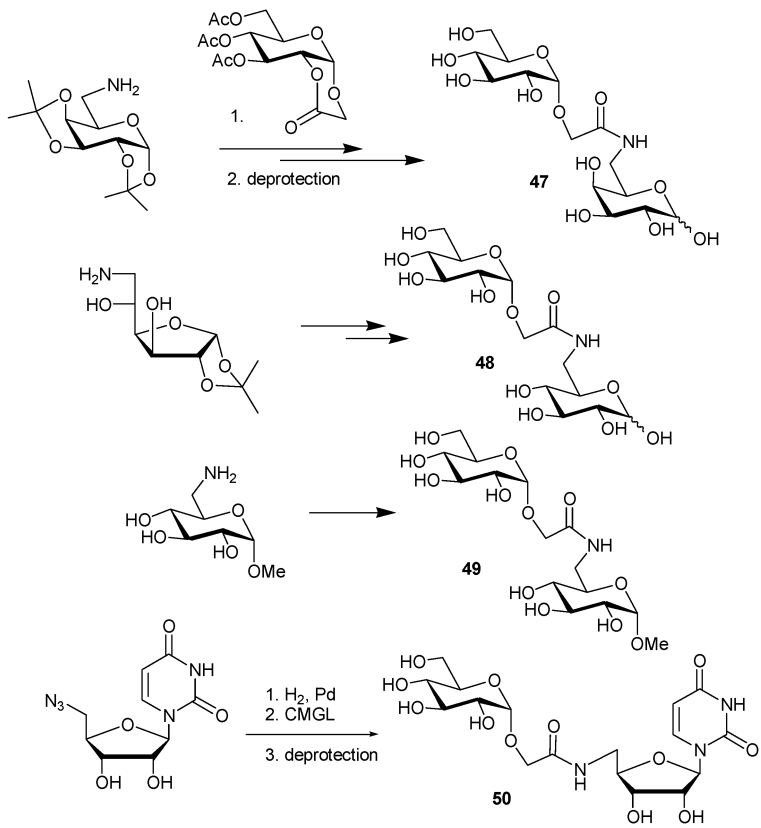


Fig. 5

was considered as the most general one was acetyl chloride in methanol, which liberates small quantities of HCl in the medium. When lysine was used, which possesses an amino group on the side chain, a monoamide and a diamide were obtained, the major compound being the *N*,*N*′-bis(glycosyl) diacetamide. The monoamide has still the side chain amino group available for incorporation into a peptide sequence. The alternative lysine monoamide, functionalised at the side chain nitrogen atom, could be prepared from benzyloxycarbonyl *N*-protected lysine methyl ester.

3.2.2 Pseudo-oligosaccharides and nucleotide sugars. Oligosaccharides and nucleotides are also key biomolecules. Amide-linked saccharidic structures have been proposed as analogs[69,71] and some new compounds could be prepared from CMGLs (Scheme 17).[41] Several pseudo-disaccharides **47–49** were prepared by reaction of lactone **24** with aminodeoxy sugars. In this reaction the carboxymethyl inter-glycosidic linkage can nearly be considered, in size, as one extra carbohydrate monomer, making these equivalent to trisaccharide mimetics (similar oligosaccharides with a four- to six carbon connection were reported in the literature, some of them being competitive inhibitors of the hydrolysis of *p*-nitrophenyl α-maltotriose by porcine alpha-amylase).[72]



Scheme 17

Unlike alcohols, free amines are reactive enough for the opening of the lactone function without catalysis. Therefore, in the case of aminodeoxy sugars, competitive reaction of alcohol functions, which leads to undesired *N*-acetylation of the starting aminosugar by intermolecular *O*-to-*N* acetyl exchange, was nearly not observed. The best conditions were found with THF as solvent and a very small excess of CMGL, or DMF when aminosugars with unprotected OH groups were used. Removal of acetyl groups was achieved under Zemplén conditions, though in the case of compounds with both isopropylidene and acetyl groups, a 0.5 M HCl solution at 50 °C could remove all protecting groups, without competitive acidic cleavage of the glycosidic bond. An example of sugar/nucleoside adduct **50** (Scheme 17), a possible analog of glucosyltransferases substrate UDP-Glc, was also prepared from 5′-deoxy-5′-azidouridine.

3.2.3 Pseudo-glycolipids. The interest for glycolipids is connected with their occurrence in biological systems, as well as their physicochemical properties, the two viewpoints being sometimes correlated. For example in membranes, lipid rafts are sub-domains which contain liquid-ordered phases.[73]

In the past years, we have been involved in the synthesis and the study of the physicochemical properties (surface activity and thermotropic liquid-crystalline behaviour) of synthetic glycolipids, notably in the context of the use of simple and available sugars as starting materials.[74,75] Therefore the potential of CMGLs in this field was also explored, looking more precisely at the thermotropic behaviour of amides **51–56** derived from aliphatic amines with chain length ranging from 6 to 16 carbon atoms, and **57–58** from aminodeoxysteroids (Fig. 6).[42] Bolaform systems **59** were also

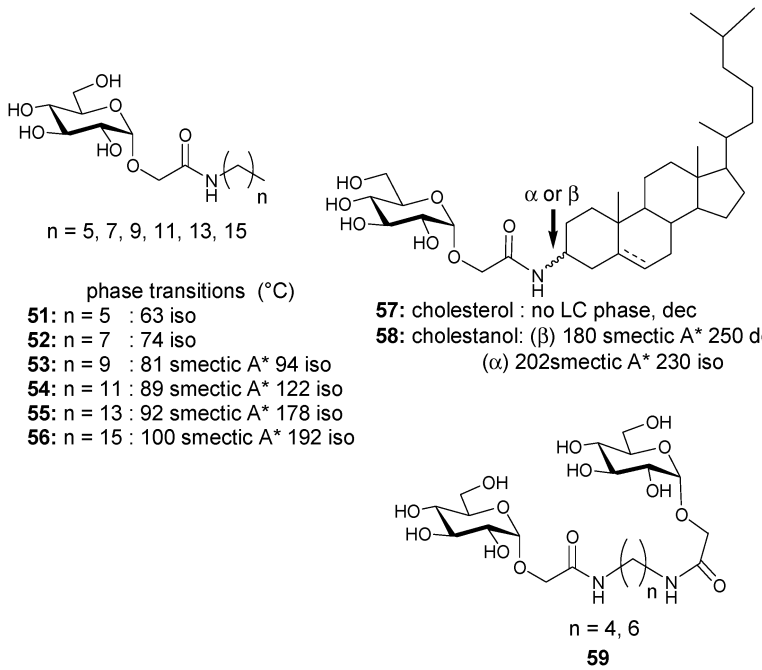


Fig. 6

obtained from diamines. A minimum chain length of 10 carbon atoms was found to be necessary for exhibiting liquid crystal phases (lamellar). The amide function and the α-glucosidic bond are very stable and allow several heating and cooling cycles for microscope observations and DSC analysis experiments, unlike the other sucrose derivatives (esters or hydroxyalkyl ethers) previously investigated.[75]

From 3-aminocholesterols (α and β) and cholestanol, four pseudo-glycosteroids were obtained by reaction with CMGL in THF. The amphiphilic glycosteroids were obtained after removal of acetyl groups. In this series, only saturated steroid amides **58** (derived from aminodeoxycholestanol) were found to exhibit a liquid crystalline phase, though at high temperature and within a limited range, a rapid decomposition occurred probably due to unsufficient flexibility in the structure. Recently, more flexible systems have been prepared by intercalating a spacer between the sugar and the steroid moieties, which have exhibited much wider ranges of liquid crystalline phases (unpublished results).

3.2.4 Glycosylated porphyrins and miscellaneous products. A few other examples of functional glyco-compounds were prepared, persuing the idea that attaching a carbohydrate moiety can provide increased polarity and water solubility to another molecular construct. Many types of compounds have been prepared in this regard (Fig. 7). For example, polymerisable

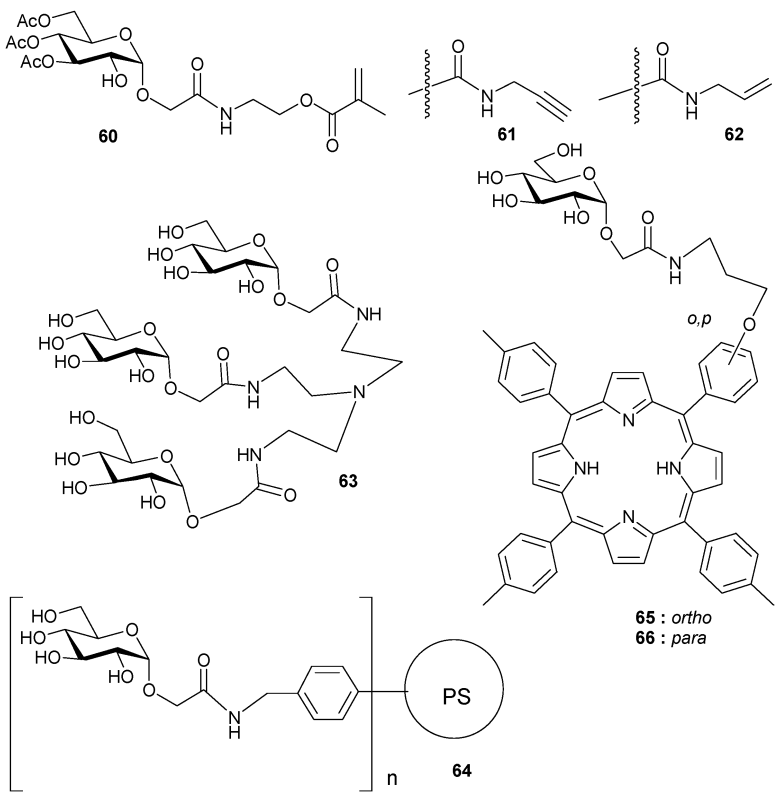


Fig. 7

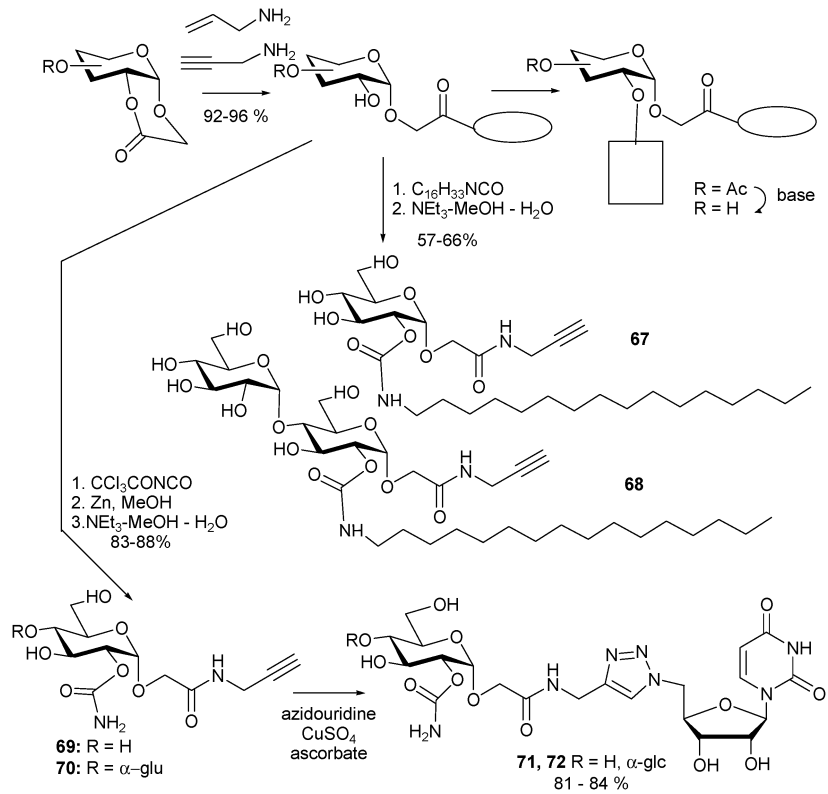
molecules could be prepared, such as the acrylic derivative **60** and the alkyne **61** which can undergo click type cycloaddition with azides, or alkene **62** which can be used in many reaction sequences, including cross metathesis. Such compounds have proven their interest towards new carbohydrate containing polymers, and the results will be reported in due time. Multivalent species were also obtained such as the triamide **63**, and reaction with an amino-resin (**64**) could be used for removing excess CMGL from the reaction mixture by filtration.

Other conjugates for which it is interesting to bring better solubility and eventually improve life-time, are glycoporphyrins. Such products have demonstrated their interest as photosensitisers for cancer photochemotherapy,[76] and the sugar moieties are known to modulate their amphiphilicity and some membrane interactions,[77] and in some cases, to increase their plasmatic life-time.[78] Cancer cell surface targeting could also be possible by binding to specific membrane receptors.[79] The glucosylated porphyrins **65** and **66** were thus prepared by reaction of CMGL (Fig. 7) with aminopropylated monohydroxyphenyltritolylporphyrins.[43] A decay of fluorescence was observed for these porphyrins in H_2O/THF (8/2) compared to THF, suggesting the formation of aggregates.[80] The photoactivity of the glucosylated porphyrins was clearly improved compared to the non glycosylated ones. *In vitro* photocytotoxicity (K562 chronic leukaemia cell line) evaluation showed that the *ortho* porphyrin **65** was significantly more active than the *para* one **66**, although less active than Photofrin®. Early necrotic death more than secondary necrosis was induced, probably due to the induction of apoptosis.

3.3 1,2-Difunctional pseudo-conjugates from CMGLs

The synthetic potential of the CMGL synthons is not limited to their ability to be easily opened in the presence of nucleophilic species. Indeed, subsequent functionalisation at position 2 provides 1,2-bisfunctionalised carbohydrates in an easy, general and competitive manner (Scheme 18).[3e] This offers an alternative to other methods, notably glycosylation reactions[81] using intermediates such as 1,2-isopropylidene acetals,[82] 1,2-orthoesters,[83] 1,2-*O*-stannylene acetals,[84] glycals and 1,2-anhydrosugars,[85] and to selective de-*O*-benzylation of position-2 with TIBAL, DIBAL-H[57] or Lewis acid catalysts,[86] or to the one-pot access to 3-*O*-benzyl-4,6-*O*-benzylidene glucosides by tandem catalysis recently reported.[87] The 1,2-lactones recently reported by Linker and co-workers[35] are also synthons which provide 1,2-bisfunctionalised carbohydrate derivatives having 2-C-2 deoxy substituents. The CMGL approach is not a way to make glycosides, but a method for grafting sugars in which the anomeric configuration has been fixed at the synthon level instead of resulting from selectivity control.

Functionalisation at OH-2 thus led to a series of 1,2-bisfunctionalised platforms prepared from two model lactones, one monosaccharidic (α-gluco) and one disaccharidic (α-malto). Allylamine and propargylamine were used as model functional appendages due to the wide scope of their possible subsequent chemistry. Their addition on the lactones occurs in high yield and very mild conditions (room temperature, THF, no catalyst,



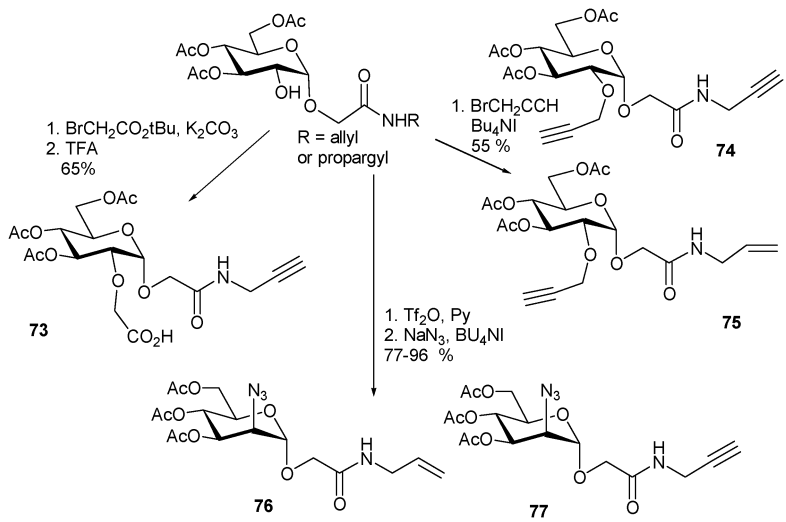
Scheme 18

82–96%, Scheme 18). Three reactions at OH-2 were investigated, namely carbamatations by reaction with isocyanates, etherifications by reaction with alkyl bromides, and substitution by an azide after intermediate triflate formation.

Hexadecyl isocyanates react with the opened lactones and the obtained carbamates were directly deacetylated leading to the deprotected 1,2-bisfunctionalised systems **67** and **68** in very good overall yield. Reaction with trichloroacetylisocyanate was also performed and, after a rapid silica gel chromatography, the resulting trichloroacetyl carbamate was treated with zinc in methanol leading to $OCONH_2$ carbamate residue, following a procedure used for preparing moenomycin-type inhibitors.[88] The OH-2 to $OCONH_2$ sequence and the final deprotection using NEt_3/H_2O/MeOH led to compounds **69** and **70** in very good overall yields. These compounds were further transformed by Cu(I)-mediated Huisgen cycloaddition with 5′-azido-5′-deoxyuridine, leading to new triazole conjugates in very good yield, which are potential analogs of glycosyltransferase substrates.

Introduction of an ester (Scheme 19) was achieved by reaction of the free OH-2 with *tert*-butyl bromoacetate in DMF in the presence of K_2CO_3 in 72% yield. From which the corresponding free acid **73** was obtained in quantitative yield. Propargylation of OH-2 was also performed by reaction with propargyl bromide in the presence of NaH which led to diynes **74** and

75 in moderate yields. Finally, azido-alkenes or alkynes were prepared by subsequent triflate formation and sodium azide substitution in very good overall yields, leading to the bifunctional 2-deoxy-2-azido *manno* derivatives **76** and **77**. The ability of such compounds to be used as AB monomers is currently under investigation.



Scheme 19

4. Conclusion

Carboxymethyl glycoside lactones are easily prepared by diverse routes and react readily with nucleophilic species. Their reaction with amines, a simple addition which does not require any intermediate activation, is very general, and many examples of new pseudo-glycoconjugates prepared by this method are described. Moreover, 1,2-bisfunctionalized carbohydrate systems are efficiently constructed by reaction of the free OH on position 2 obtained after the opening of the lactone ring. Applications at the frontier of materials and biology have been envisaged and further studies in these fields will be reported in the near future.

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Thionocarbamates on carbohydrate scaffolds—from synthesis to bioactivity

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2-Thioxotetrahydro-1,3-*O*,*N*-heterocycles are commonly named cyclic thiocarbamates or thionocarbamates. The structural association of five- and six-membered thionocarbamates with diverse carbohydrate scaffolds has shown a promising potential in modern organic synthesis and in the preparation of biomolecules mimics. The principal pathways for their synthesis, the recent and most important developments of their chemical transformations as well as some examples of their biological activities will be considered in the following discussion.

1. Introduction—interest of thionocarbamates connected on carbohydrate scaffolds

2-Thioxotetrahydro-1,3-*O*,*N*-heterocycles, which are commonly known as cyclic thiocarbamates or thionocarbamates, have been the subject of extensive studies because of their important biological activity.[1] The thionocarbamate function is very useful for the design of more complex heterocycles, which are attractive from both chemical and biological points of view.[2] In the light of these facts, the interest of connecting five- or six-membered ring thionocarbamates on carbohydrate scaffolds was stirred up. Noteworthy are the saccharidic 1,3-oxazolidine-2-thiones (OZT) and 1,3-oxazine-2-thiones through the broad variety of their applications. In fact, such structural arrangements have given birth to analogues of natural compounds such as pseudo *C*- and *N*-nucleosides,[3–5] spironucleosides[6,7] or spiro-*C*-glycosyl compounds.[8,9] Sugar-derived OZT have been reported to behave as inhibitors of D-fructose transporter protein GLUT5.[10] From a synthetic point of view, the five-membered thionocarbamate system has gained further importance as a chiral auxiliary due to its broad applicability in chiral induction, as this heterocycle is structurally related to Evans' oxazolidinones.[11,12] In this context, saccharidic OZTs have been used in inverse hetero-Diels–Alder reactions.[13] Thionocarbamates on carbohydrate scaffolds are have promising structural features for biologically active compounds such as modified nucleoside derivatives[14–21] as well as bicyclic *N*-thiocarbonyl iminosugars as potential glycosidase inhibitors.[22,23] Regarding synthetic applications, sugar-derived OZTs have been recently

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used in copper promoted palladium-catalyzed Suzuki and Stille cross-coupling reactions with a range of organoboryl and organostannyl reagents[24,25] as well as in desulfurative Sonogashira cross-coupling reactions with alkynes.[26]

This review summarizes the most relevant methods for the preparation of thionocarbamates on carbohydrate scaffolds, the recent and most important developments of their chemical transformations and some examples concerning their biological activities.

2. Synthesis of thionocarbamates on carbohydrate scaffolds

From a structural point view, the most explored thionocarbamates in carbohydrate chemistry are five-membered 1,3-oxazolidine-2-thiones.[27–29] With respect to the synthesis of aromatic analogues of OZTs-1,3 oxazoline-2-thiones (OXTs), only two acyclic D-fructose-derived OXTs were recently reported by Rollin and co-workers.[30]

From a structural point of view, the carbohydrate template can have either furan or pyran rings although in some cases open chain structures can be formed. A large variety of aldopentoses (*e.g.* D- and L-arabinose, D-ribose, D-xylose), aldohexoses (*e.g.* D-glucose, D-mannose, D-galactose) as well as ketohexoses (*e.g.* D-fructose, L-sorbose) can be used as scaffolds.

Thionocarbamates and carbohydrate scaffolds may be connected to give either fused, anchored or spiro compounds (Fig. 1).

Considering the preparative aspects, the main synthetic pathway is the reaction of a β-aminoalcohol with a thiocarbonyl-source under basic conditions. The different approaches are summarized in Scheme 1.[27–29]

The second- and oldest-method for the preparation of thionocarbamates on carbohydrate scaffolds consists of the condensation of thiocyanic acid, generated *in situ* from potassium thiocyanate and a protic acid, with an anomeric-free sugar to form the thermodynamically more stable five-membered ring OZT as the major compound (Scheme 2).[27,28]

Some examples of thionocarbamates syntheses on carbohydrate scaffolds from their isothiocyanate precursors were reviewed by Ortiz-Mellet[27,28] and more recently by Fernandez-Bolanos.[29] The present review will consider the

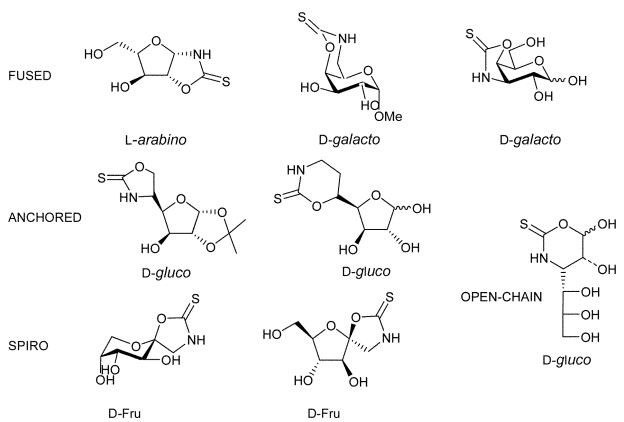
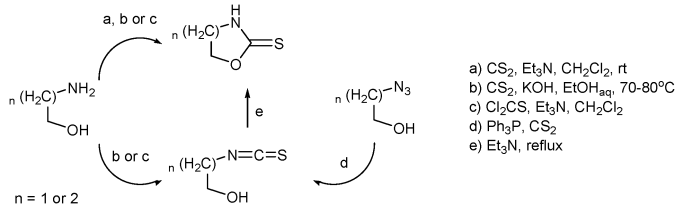
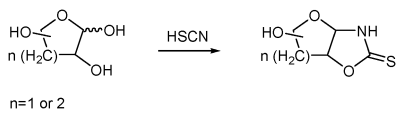


Fig. 1 Different possibilities of thionocarbamate connection on carbohydrate scaffolds.



Scheme 1



Scheme 2

synthetic possibilities for connecting a thionocarbamate moiety to carbohydrate scaffolds and discuss the reactivity of such hybrid structures.

2.1 Condensation reaction with a thiocarbonyl source-CS_2 or $CSCl_2$

In carbohydrate chemistry, the most described method for the preparation of saccharidic thionocarbamates involves preliminary introduction of the amine function on a partially or non-protected saccharidic template. The condensation of amino sugars with carbon disulfide or thiophosgene leads to cyclization in 1,3-oxazolidine- or 1,3-oxazine-2-thiones. This reaction involves the formation of an intermediate isothiocyanate, which reacts further with a β- or γ-located hydroxyl group. The viability and facility of this process depends on the saccharidic ring size and the inherent strain. Some major rules can be put into light from the cases studied:[30]

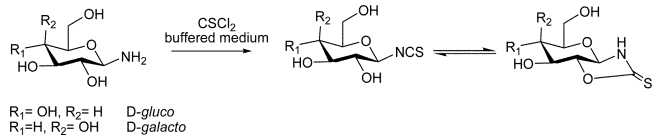
(1) the formation of five-membered thionocarbamates is favoured against their six- membered counterparts;

(2) furan systems are more prone to afford bicyclic derivatives than their pyran tautomers;

(3) the relative arrangement of both reacting groups is highly important: *trans*-diaxially-arranged isothiocyanate and hydroxyl groups in a confomationally rigid pyran ring do not cyclize to give a saccharidic thionocarbamate.[31]

This methodology applied to aldohexoses differing in amine position on saccharidic template (anomeric position, C-6, C-2, C-3 or C-5) will be discussed.

2.1.1 Glycosylamines. Aiming at the synthesis of *O*-unprotected glycosylthioureas from anomeric isothiocyanates in aldohexose series (D-*gluco*, D-*galacto* and D-*manno*), Fuentes and co-workers[32] have reported a crucial result: in *β*-D-*gluco* and *β*-D-*galacto* series, when the glycopyranosylamines condense with thiophosgene in buffered medium, an equilibrium between the anomeric isothiocyanate and the corresponding OZT is established, thus showing the possibility of building up a *trans*-fused system between an OZT and a pyran ring (Scheme 3).



Scheme 3

In contrast, when β-D-mannopyranosylamine reacts with thiophosgene, only the *cis* bicyclic thionocarbamate is formed and the transient isothiocyanate cannot be detected (Scheme 4). The different behaviour of the *cis* and *trans* hydrindane-type systems can be explained by the strain in the ring fusion for a *trans* species.

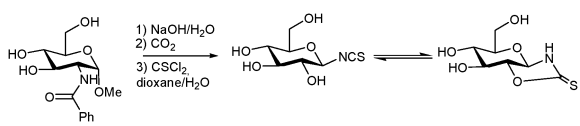
Scheme 4

The above results agree with the fact that bicyclic compounds can be formed either in a 5 + 5 or 6 + 5 ring fusion, but in both cases the *cis* isomers are more stable than the *trans* isomers.[33]

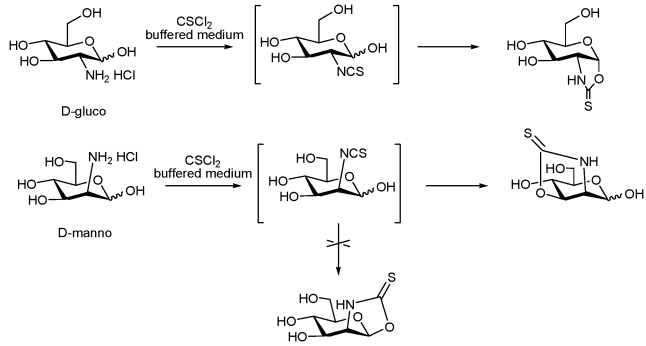
2.1.2 Saccharidic templates bearing an amino group in C-2, C-6, C-3, C-5. Generally, an amine located on non-anomeric position reacts similarly: through the condensation reaction with carbon disulfide or thiophosgene, the preliminary formed isothiocyanate leads to spontaneous or base-induced cyclization into 1,3-oxazolidine- or 1,3-oxazine-2-thiones, as previously mentioned. Several extensive studies are reported about the selective introduction of thionocarbamate moieties on carbohydrate scaffolds.[3,22,23,32*a*,34–38]

2-Amino-2-deoxy aldoses. The behaviour of *O*-unprotected sugars is exemplified in D-*gluco* series: after basic hydrolysis of the starting 2-benzamidoglycoside followed by buffering the medium with carbon dioxide and treatment with thiophosgene, an intermediate isothiocyanate was obtained.[32*a*] However, NMR revealed a temperature-dependent equilibrium of this isothiocyanate with a *trans*-fused OZT (Scheme 5).

Synthesis of *cis*-fused OZT starting from 2-amino-2-deoxy-D-glucose and D-mannose hydrochlorides in conditions similar to those applied for glycosylamines was recently published (Scheme 6).[36] In contrast with data previously reported for *trans*-fused thiocarbamate,[32] the isomeric isothiocyanates were not detected.



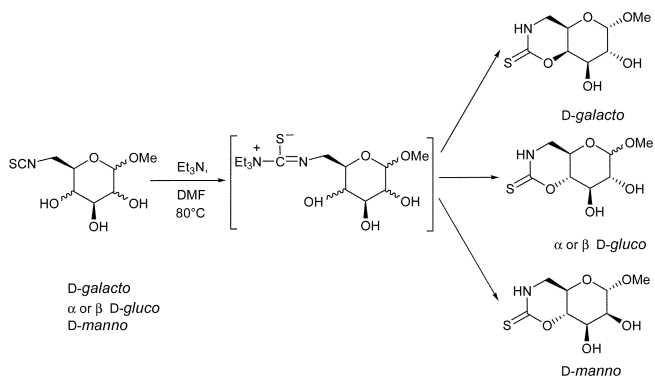
Scheme 5



Scheme 6

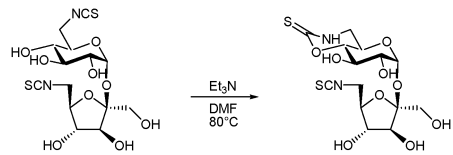
It is worth mentioning that in D-*manno* series, the 2,3-*cis*-fused OZT is formed exclusively, with the 1,2-*cis*-regioisomer remaining undetected. This can be explained by a weaker nucleophilicity of the hydroxyl group in the anomeric position.

6-Amino-6-deoxy aldoses. Following the same process in D-*gluco*, D-*galacto* and D-*manno* series, Ortiz Mellet and co-workers have extensively studied the utilization of 6-deoxy-6-isothiocyanates as precursors of fused tetrahydro-1,3-oxazine-2-thiones. The cyclization reaction was performed under two distinct conditions: (1) the anomeric position of aldoses was blocked as a methyl pyranoside; (2) the aldoses remained in the form of a hemiacetal.[3,34] Reaction of methyl 6-amino-6-deoxyaldopyranosides with thiophosgene led to the corresponding 6-isothiocyanates. The subsequent intramolecular cyclisation to afford the fused oxazine-2-thione required triethylamine catalysis. It was postulated that the nucleophilic attack of the γ-located hydroxyl on C-4 might be activated by formation of a zwitterionic complex between the isothiocyanate and Et_3N (Scheme 7). The same reaction was observed for 6,6′-dideoxy-6,6′-diisothiocyanato-α,α'-sucrose (Scheme 8).[37]



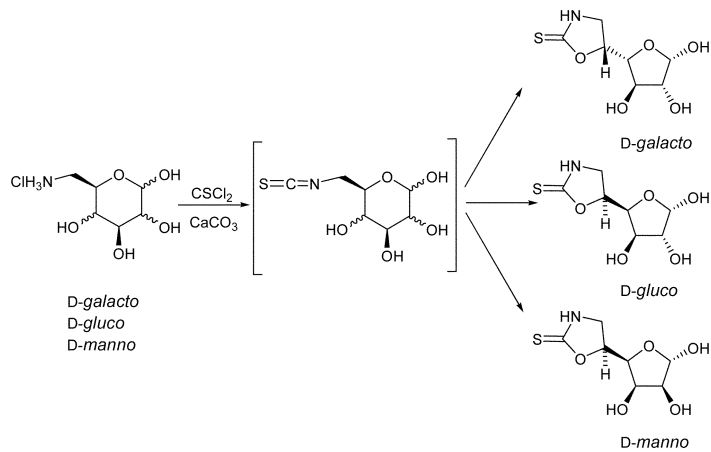
Scheme 7

Nevertheless, in the case of saccharides with a free hemiacetal group, the reactivity was shown to be different. Acid-catalyzed full deprotection of



Scheme 8

6-deoxy-1,2:3,4-di-*O*-isopropylidene-6-isothiocyanato-α-D-galactopyranose delivers the hemiacetal, which *via* opening-closing of the pyran ring rearranges towards a more favoured furan structure by involvement of the β-hydroxyl group. Other OZTs anchored on furan forms were similarly obtained from *O*-deprotected 6-amino-6-deoxyhexoses (Scheme 9).

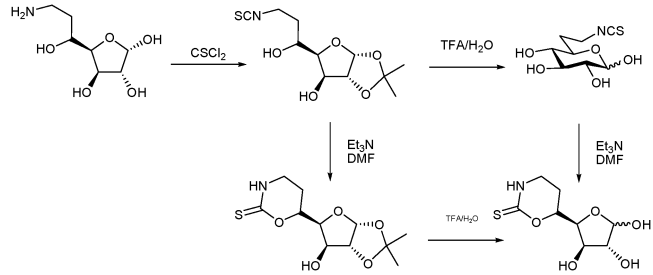


Scheme 9

It should be noted that this reaction occurs with total regioselectivity: only one five- membered ring thionocarbamate linked to the furanose moiety was formed.

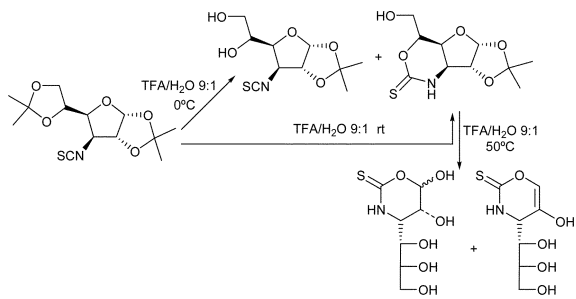
7-Amino-6,7-dideoxy-α-heptopyranosides in D-*gluco*, D-*galacto* and D-*manno* series were obtained *via* homologation reaction of 6-deoxy-6-iodoglycopyranosides in three steps.[38] The key reaction in this synthesis is the nucleophilic displacement of the iodide by cyanide anion. After reduction of the nitrile, the amine obtained undergoes condensation with thiophosgene to generate the corresponding isothiocyanate. Acid-catalyzed removal of the 1,2-*O*-isopropylidene acetal moiety led to the *O*-unprotected sugar isothiocyanate, which proved stable in the absence of base. Treatment of this isothiocyanate with triethylamine in DMF at 80 °C afforded the anomeric mixture of furanose-anchored 1,3-oxazine-2-thiones. The same thionocarbamate could also be obtained through (1) base-induced intramolecular annelation of the isothiocyanate and (2) deprotection of the acetal under acidic conditions (Scheme 10).

3-Amino-3-deoxy aldoses. Another central study has been carried out by Fuentes and co-workers on the introduction of an isothiocyanate at C-3 of aldohexoses and its reaction with sugar secondary hydroxyl groups.[35] From



Scheme 10

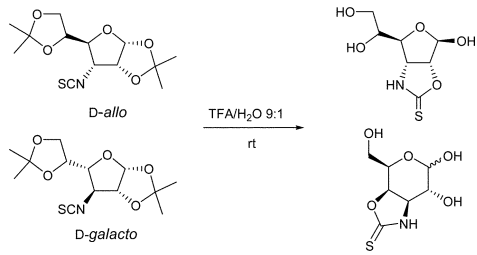
commercially available starting materials, they synthesized 3-deoxy-1,2:5,6-di-*O*-isopropylidene-3-isothiocyanato-α-D-hexofuranoses from D-*gluco*, D-*allo* and D-*galacto* series. As the isothiocyanato function is compatible with the acidic conditions used for acetal deprotection, they investigated the reactivity of those 3-isothiocyanates in two ways, either with selectively 5,6-*O*-deprotected or with fully deprotected species. Treatment of 1,2:5,6–di-*O*-isopropylidene-3-isothiocyanato-α-D-glucofuranose with 90% aqueous TFA at low temperature resulted in the formation of a mixture of the isothiocyanate and the tetrahydro-1,3-oxazine-2-thione. At room temperature, the tetrahydro-1,3-oxazine-2-thione was produced quantitatively (Scheme 11).



Scheme 11

Furthermore, no intramolecular cyclization occurred when D-allofuranosyl and D-galactofuranosyl isothiocyanates were selectively *O*-deprotected. The hydrolysis produced the corresponding isothiocyanates devoid of 5,6-protection, which remained reluctant to cyclization, even under triethylamine catalysis. Their different configuration can explain those results: in the *gluco* series, both the isothiocyanate and the C-4 appendage are in *cis* relationship, while it is *trans* in the other two series (Scheme 12). Contrary to a pyran frame, the furanose ring prevents formation of an oxazine ring when both reactive ends are *trans* configurated.

Complete *O*-deprotection of the hexofuran-3-yl isothiocyanates led to three types of compounds, depending on the stereochemical relationship of the isothiocyanato group and the reactive OH. Deprotection of D-*allo* and D-*galacto* derivatives is a good illustration of the influence of a *cis*-relationship between those two groups on the geometry of the molecule. The 2,3-*cis* relationship in D-*allo* series induced the formation of a 2,3-OZT imposing a

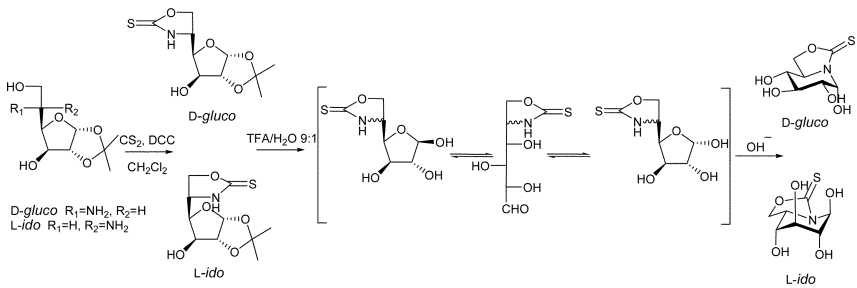


Scheme 12

furanose ring, while the 3,4-*cis* relationship in D-*galacto* series led to a 3,4-OZT which forced the carbohydrate into a pyranose form.

In contrast, the absence of any *cis* relation in D-*gluco* series forced the molecule to generate more stable tetrahydro- and dihydrooxazinethione-type moieties (Scheme 11).

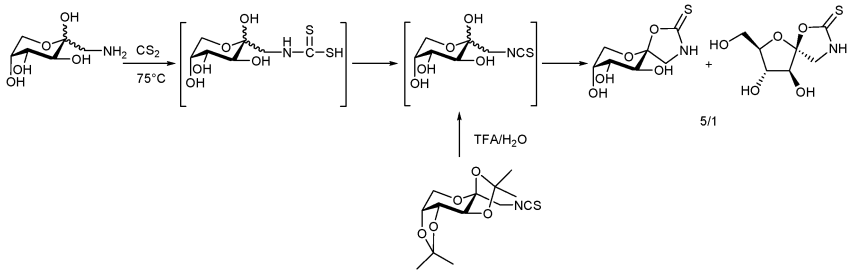
5-Amino-5-deoxy aldoses. Similarly to the precedent approach, a regioselective access to C-5 anchored OZTs was developed from 5-amino-5-deoxy-1,2-*O*-isopropylidene-α-D-gluco- and α-L-idofuranose through condensation with carbon disulfide (Scheme 13):[22,23]



Scheme 13

Most differently from the C-3 analogues, acid-catalyzed hydrolysis of the 1,2-acetal did not result in a rearrangement of the formed OZTs, and mixtures of α, β furanoses were obtained. Stereocontrolled intramolecular cyclization induced by reaction of these mixtures with a base leads to indolizidine-type bicyclic compounds. These derivatives are structurally related to the imino sugars—glycosidase inhibitors family. The reaction proceeds *via* nucleophilic attack of the nitrogen on the masked aldehyde group. It should be noted that NMR analysis shows that for each sugar a single stereoisomer is formed, with *R*- (for the D-*gluco* derivative) or *S*-configuration (for the L-*ido* derivative) at the pseudoanomeric center.

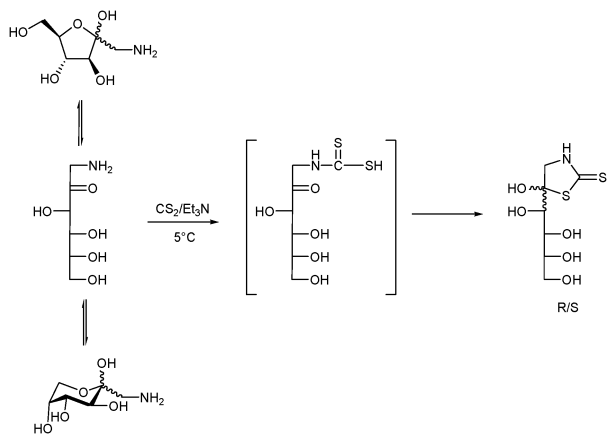
1-Amino-1-deoxy ketoses. In the case of ketohexoses, the formation of thionocarbamates is more complex than for aldohexoses. The condensation of 1-amino-1-deoxy-D-fructose with carbon disulfide *via* intermediate isothiocyanate led to *β*-pyran and *β*-furan forms connected to spiro-OZTs (Scheme 14).[6]



Scheme 14

The mixture of spiro OZTs can be obtained by full deprotection of 1-deoxy-2,3:4,5-di-*O*-isopropylidene-1-isothiocyanato-D-fructopyranose under acidic conditions (Scheme 14).[7] A longer reaction time or higher temperature may cause further isomerisation to an open-chain dithiocarbamate derivative.

Condensation of 1-amino-1-deoxy-D-fructose with carbon disulfide in the presence of excess triethylamine at 5 °C, leading to a (5*R*)/(5*S*)-diastereoisomeric mixture of open-chain 1,3-thiazolidine-2-thiones, was reported in 1975 by Jochims (Scheme 15).[39]



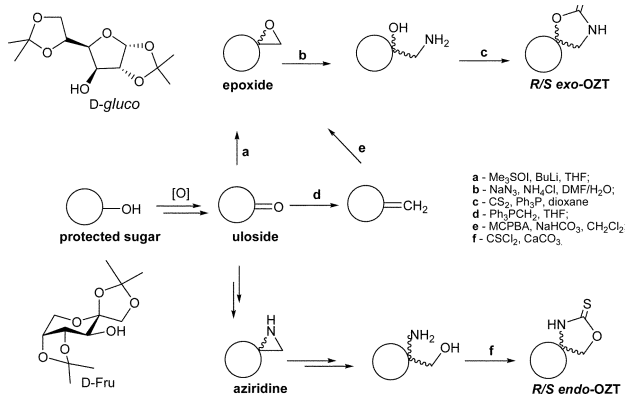
Scheme 15

The results presented in Schemes 14 and 15 can be explained by the formation of the corresponding dithiocarbamate, whose cyclization depends on the reaction conditions. Thus, excess triethylamine prompts spontaneous cyclization of the dithiocarbamate through implication of the carbonyl group of D-fructose. However the formation of spiro OZTs requires more severe conditions in the absence of base.[40] In that case, regression of the dithiocarbamate towards the corresponding isothiocyanate can be envisaged, followed by attack of the D-fructose anomeric hydroxyl group to afford two epimeric spiro-OZTs. The hypothesis is confirmed by the hydrolysis of 1-deoxy-2,3:4,5-di-*O*-isopropylidene-1-isothiocyanato-D-fructose (Scheme 14), which results in spontaneous cyclization to afford the same spiro-OZT derivatives.

The results previously shown for sucrose emphasize that selectivity of thionocarbamate formation is dependent on the configuration and structure of the sugar. Thus the *trans*-decalin-type system is easily formed on the glucopyran form, whereas the isothiocyanate linked to the fructofuran moiety remains inactive even if the presence of triethylamine in DMF at 80 °C (Scheme 8).[37]

2.1.3 Epoxide or aziridine pathway to saccharidic thionocarbamates. The original approach *via* the formation of epoxides and aziridines to elaborate spiro-OZTs at C-3 position of D-glucose and D-fructose was reported recently by Rollin and co-workers.[41]

The purpose of this study was to explore the introduction of an OZT moiety onto the specific C-3 site of both 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose and 1,2:4,5-di-*O*-isopropylidene-β-D-fructopyranose, taking advantage of the well-defined frame of both carbohydrate structures to generate all possible OZT-isomers. These spiroheterocyclic structures could be constructed according to a simplified sequence based on a key stereoselective approach from uloses *via* epoxides or aziridines (Scheme 16).



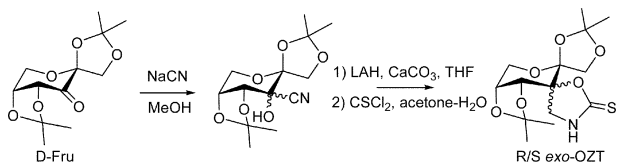
Scheme 16

The *exo* spiro-OZTs could be produced through a standard reaction sequence (Scheme 16): a 3-keto sugar, readily obtained by oxidation of a protected sugar, was converted into the epoxide, leading to both possible epimers. Completion of the sequence involved regioselective nitrogen introduction to afford the amino-alcohol and subsequent cyclisation to the *exo*spiro-OZT. Epoxidation through the sulfonium ylide approach gave moderate yields of the corresponding epoxides. The alternative pathway involves two steps: (1) Wittig methylenation; (2) stereoselective *m*-CPBA epoxidation, and allows better yields and improved stereoselectivity.

In conclusion, the epoxide approach could be performed in a stereoselective manner depending on the conditions used. On both templates, the (*3S*)-epoxides were obtained with very good stereoselectivity, whereas their epimeric counterparts proved more difficult to synthesize. Only the D-Fru (*3R*)-epoxide could be obtained in reasonable yield and good

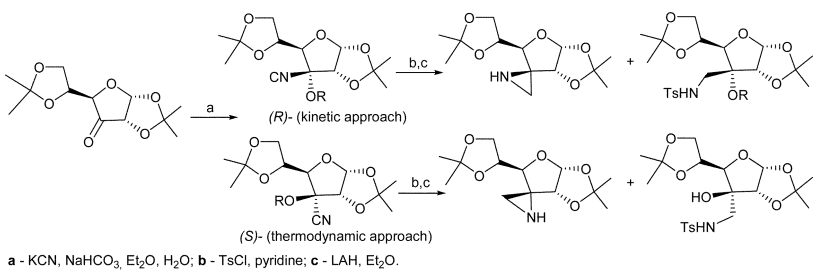
stereoselectivity. The above epoxides were then regioselectively cleaved by sodium azide under protic conditions to afford the corresponding azido-alcohols in excellent yields (83% to 96%). Direct cyclization to the corresponding OZT was performed following a one-pot protocol, involving a modified Staudinger condensation and spontaneous cyclization of the intermediate hydroxylated isothiocyanate.

A different approach involving cyanohydrin formation from the 3-keto sugar was also explored in the D-Fru series (Scheme 17). A mixture of epimeric cyanohydrins was quantitatively formed by reaction with sodium cyanide in methanol, albeit without stereoselectivity. Chromatographic separation of (*R*)- and (*S*)-isomers was straightforward and the former epimer was selected to exemplify the two-step transformation into an OZT. Reduction of this nitrile by lithium aluminum hydride led to the corresponding aminoalcohol, which was further condensed with thiophosgene to afford the (3*R*)-spiro-OZT in *ca.* 30% overall yield. Despite its shorter pathway, the cyanohydrin route to the OZT was not exploited further, mainly because of the disappointing yields in the last two steps.



Scheme 17

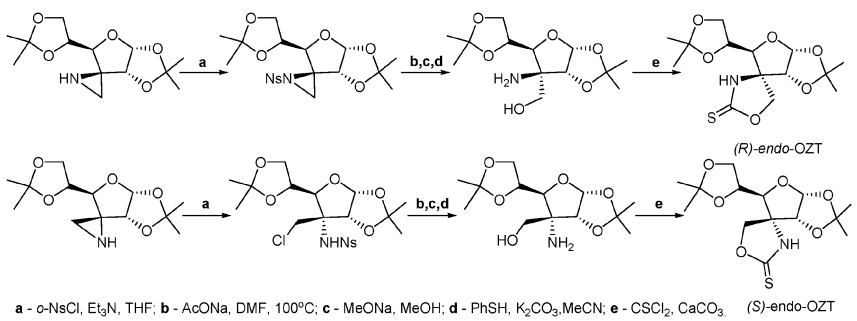
The *endo*-spiro-OZT could be prepared through a reaction sequence similar to that applied for the *exo*-epimer, with spiro-aziridine intermediates replacing the key spiro-epoxides (Scheme 18). Cyanohydrin formation from ketones was tried under kinetic or thermodynamic conditions, and only reaction with the D-*gluco* derived keto sugar offered efficient stereoselectivity, while no selectivity was observed for reaction with the keto sugar obtained from protected D-fructose. The (*R*)-cyanohydrin was prepared in excellent yield under kinetic conditions (KCN, $NaHCO_3$, 0 °C, 10 min); a modified thermodynamic procedure was applied to produce the (*S*)-epimer in 85% yield (Scheme 18).



Scheme 18

The different steric hindrance of hydroxyl groups in (*3R*)- and (*3S*)-cyanohydrins was revealed by activation of α-hydroxynitriles through *O*-tosylation to afford the (*R*)-tosylate in 85% yield and the (*S*)-tosylate in

only 66% yield. Lithium aluminum hydride reduction of the cyano group into the amino group caused spontaneous intramolecular cyclization. A highly selective reaction occurred with the (*3R*)-cyanohydrin, to furnish the aziridine in 90% yield. The side-reaction of tosyl migration to form the sulfonamide was limited to 5% yield. In contrast, applying identical conditions to the (*3S*)-cyanohydrin afforded the corresponding aziridine in moderate yield (53%), together with its (*3R*)-epimer (3%) and a 32% yield of sulfonamide was observed. As expected, direct acetolysis of aziridines failed: the ring-opening of a spiro-aziridine often requires strong activation *via* *N*-sulfonylation (Scheme 19). The *N*-nosylated (*3R*)-epimer was therefore prepared in 60% yield, subsequently undergoing acetolysis in moderate yield (58%). When applying the same *N*-sulfonylation conditions to the (*3S*)-aziridine, a different behavior was observed: *N*-nosylation was followed by chloride ion counter-attack, affording a 68% yield of 3-chloromethyl sulfonamide, which could finally be converted into the acetate in nearly quantitative yield. The final sequence involved methanolysis and standard thiolate-induced *N*-desulfonylation to afford precursor epimeric β-amino alcohols. Conversion of the latter into the target OZTs was performed by condensing thiophosgene under basic conditions to yield the desired (*R*)- and (*S*)-*endo*-1,3-oxazolidine-2-thiones in 78 and 70% yield, respectively (Scheme 19).

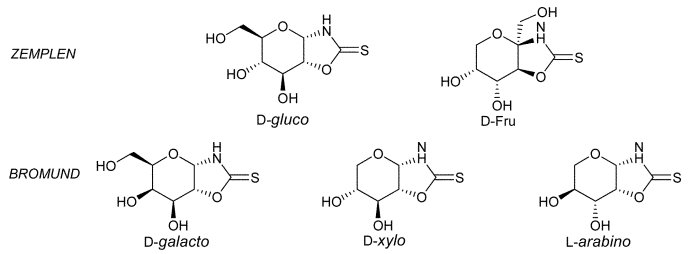


Scheme 19

In conclusion, the formation of spiro-aziridine only showed efficient selectivity with furan from derivatives. Following a three-step sequence, the (*R*)- and (*S*)- spiro-aziridines were prepared in 73% and 30% yield, respectively. Further steps using a *N-ortho*-nosyl activation/protection system afforded the corresponding *endo*-spiro-OZTs in overall 20% and 41%, respectively.

2.2 Reactions with thiocyanic acid

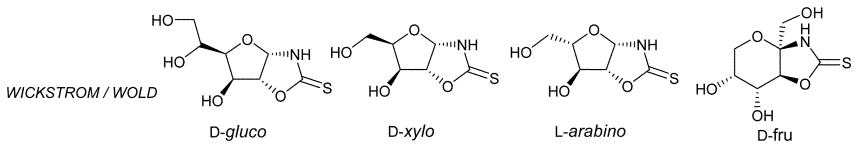
2.2.1 OZTs from aldoses and ketoses. The second and the oldest-as well as the less studied—method is based on the condensation of *O*-unprotected sugars with thiocyanic acid, generated *in situ* from potassium thiocyanate and a protic acid. The reaction involves the free anomeric position and a γ- or δ-hydroxyl group able to promote intramolecular cyclization of a transient open-chain isothiocyanate, to form the thermodynamically most stable OZT. The first results obtained by Zemplén in D-*gluco* and D-Fru series reported the formation of OZTs fused to pyran backbones (Scheme 20).[42]



Scheme 20

Zemplén's work was revisited by Bromund *et al.*[43] by exploring the reaction in diverse aldose series (D-*galacto*, D-*xylo*, L-*arabino*). Similar bicyclic fused structures of OZT on pyran backbones were proposed (Scheme 20).

The structural determination of those bicyclic compounds remained under discussion for some time, until Wickstrom *et al.*[44] could (1) confirm the formation of fused bicyclic OZT-sugars and, more important (2) ascertain the furan forms for aldoses and the pyran form for D-fructose (Scheme 21). Those results were later confirmed by Jochims *et al.* through the first NMR analysis performed on OZTs.[45]



Scheme 21

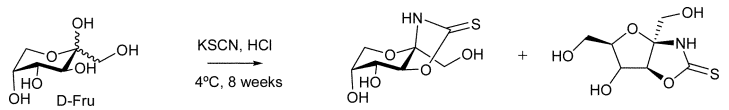
In 1975, Ranganathan used the OZT derived from D-arabinofuranose as a precursor to purine nucleosides.[14,15] The D-xylofuran-fused OZT was reported later by Imbach *et al.*[16,17] for the same purpose of synthesizing α- and β-D-xylofuranosyl nucleosides.

In conclusion, the work developed so far has shown the possibility to condense aldoses with thiocyanic acid to produce bicyclic furanthiono-carbamates in good yields.[10,20,21]

Ketoses should react under a similar scheme. Indeed they do but an important problem in the chemistry of ketoses consists on the lack of selectivity due to: (1) the complexity of their tautomeric equilibria and (2) their tendency to form tertiary oxocarbenium ions under acidic conditions. Thus, mixtures of open-chain, cyclic and dehydrated products are frequently obtained.[7] The discussion about OZT structures obtained from D-fructose as proposed by Zemplén, Wickstrom and more recently by Grouiller *et al.* continues today. In fact, the first authors claimed the fusion of OZT on a pyran form of D-fructose, while Grouiller suggested the formation of a mixture of fused OZTs with β-pyran (major) and β-furan (minor) forms (Scheme 22).[18]

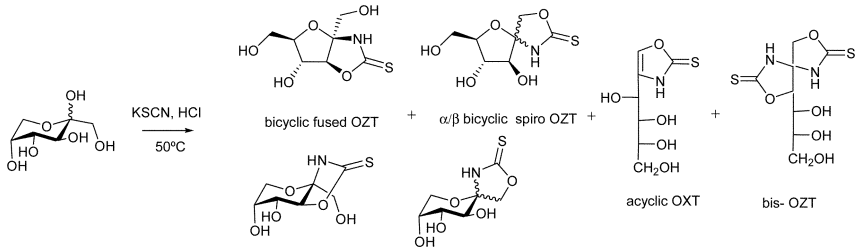
Similarly to the synthesis of spiro-OZTs in D-Fru series (Scheme 14),[6] the reaction mechanism probably proceeds *via* an intermediate isothiocyanate which undergoes nucleophilic attack of the C-3 hydroxyl group.

In order to learn more about how aldoses and ketohexoses behave when confronted to thiocyanic acid, Girniene *et al.* have developed a synthetic



Scheme 22

procedure leading directly to fused OZTs from *O*-unprotected or partially protected sugars.[10] Slightly modifying the conditions applied by Bromund on non-protected aldoses (D- and L-arabinoses, D-xylose, D-ribose), bicyclic OZT-sugar systems were prepared with good to excellent yields (70–97%) and the geometries of the sugar ring were completely defined. A furan form was obtained, as confirmed by ^{1}H and ^{13}C NMR spectra, and the anomeric configuration was controlled by the location of the hydroxyl group in C-2 position. In the case of ketohexoses, condensations are not so simple. In fact, by reacting those with HSCN, one can expect the formation of up to 9 different thionocarbamates: fused and spiro bicycles on pyran or furan skeletons, as well as acyclic OXTs and bis-OZTs (Scheme 23).

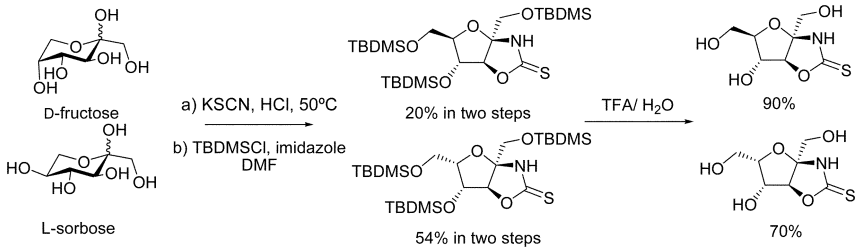


Scheme 23

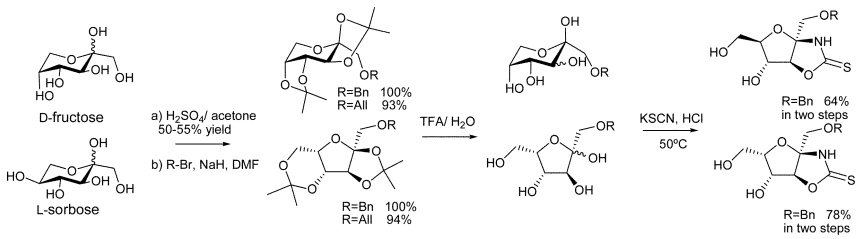
The above structures have been postulated from the synthesis of 1,3-oxazolidin-2-one (OZO) analogues studied by Lichtenthaler *et al.*[46] The reaction of D-fructose with potassium cyanate, can deliver four OZO structures-one furan-fused structure and three spiro derivatives. The reaction of D-fructose and L-sorbose with thiocyanic acid in aqueous solution was examined by Tatibouët *et al.*[9] as expected, the complex mixture of products obtained did not allow an easy separation by column chromatography. According to Grouiller,[18] the crude material was per-*O*-silylated (TBDMS), in order to facilitate isolation of the fused bicyclic thionocarbamates.! For both ketose series, the same type of furan-fused thionocarbamate was isolated. Acid-catalyzed deprotection returned naked OZTs in good yields (Scheme 24).[10]

With a view to increasing the selectivity in OZT formation (*i.e.* reducing the number of products shown in Scheme 23), selective hydroxyl protections were performed. Protection of the C-1 alcohol would induce limitation to fused structures (Scheme 25),[10] whereas 3-*O*-protection would only allow formation of *spiro*-derivatives (Scheme 26).[47]

Blocking the C-1 OH of D-fructose and L-sorbose (Scheme 25) was effected in excellent yields through regioselective isopropylidene acetalation of the free ketoses, followed by etherification (benzylation or allylation) of the remaining primary alcohol. Acid-catalyzed hydrolysis of the isopropylidene groups and condensation with HSCN efficiently produced a sole fused bicyclic OZT.



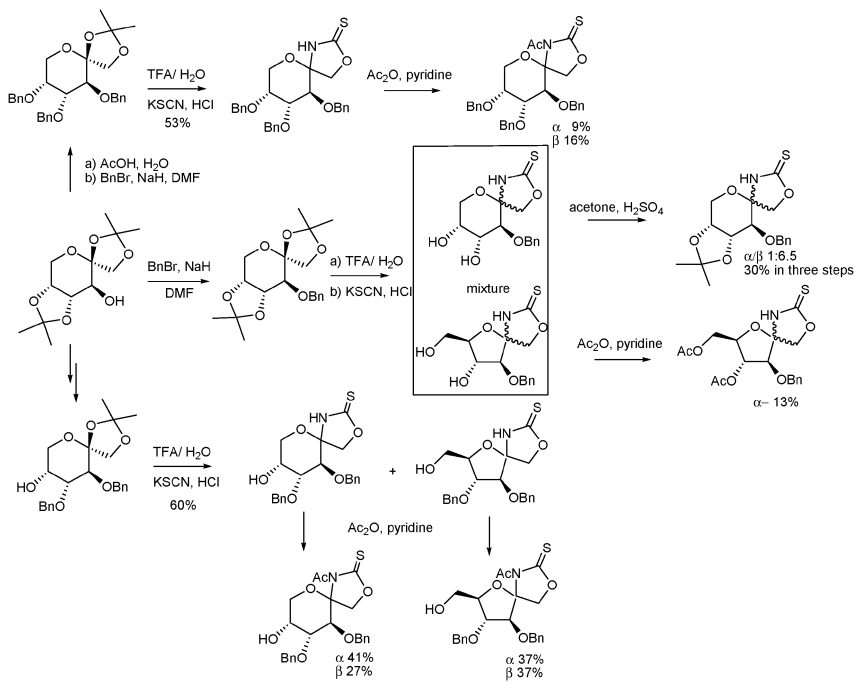
Scheme 24



Scheme 25

The synthesis of these bicyclic systems was achieved in reasonable yields (30–35% in D-Fru series and 34–43% in L-Sor series).

In the continuation of this work, a broad range of OZTs was prepared from selectively protected derivatives of D-fructose and L-sorbose (Scheme 26).[47]



Scheme 26

A 3-*O*-benzylated D-fructose derivative was produced from 1,2:4,5-di-*O*-isopropylidene-β-D-fructopyranose under standard conditions (Scheme 26). Transient 3-*O*-benzylated D-fructose resulting from acidic hydrolysis was reacted with thiocyanic acid without intermediate purification. This sequence afforded a mixture of isomeric spiro-OZT on pyran and furan templates, which was difficult to separate, even if standard acetylation was applied.

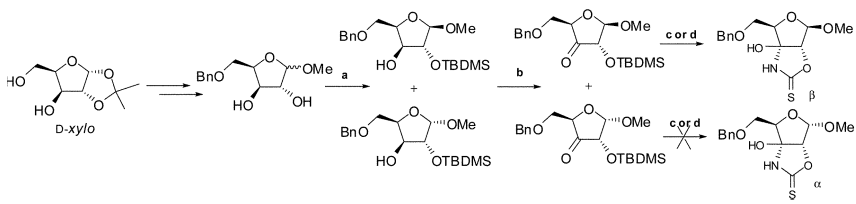
The 3,4,5-tri-*O*-benzyl D-fructopyran derivative should lead to the spiro-pyran forms. Selective deprotection of the 4,5-*O*-isopropylidene, followed of perbenzylation and condensation with thiocyanic acid gave the expected spiro-pyran OZT in reasonable yield (53%), but standard acetylation was not very efficient for the separation of the two epimers (Scheme 26).

The 3,4-di-*O*-benzylated D-fructopyran derivative (Scheme 26) would limit the possibilities for spiro-furan and -pyran derivatives. Application of the usual two-step process afforded with a 60% overall yield a mixture of OZTs in which the spiro-furan forms predominated (2:1 ratio) over the spiro-pyran forms. After column chromatography separation, acetylation allowed isolation of each epimer either α-furan (37%) and β-furan (35%) forms as well as α-pyran (41%) and β-pyran (27%) forms.

In conclusion, the present study describes the first selective formation of OZTs on ketohexose templates with a characterization of five out of the seven possible structures (Scheme 23).

2.2.2 OZTs from keto sugars. An original approach for the synthesis of OZTs from carbohydrate-based α-hydroxyketones was recently published by Silva *et al.*[48a] who investigated the reactivity of carbohydrate-based α-hydroxyketone in the presence of thiocyanic acid.

Starting from a partially protected D-xylose, the sequence of protection-deprotection reactions was performed. The highly regioselective *O*-silylation, followed by oxidation at C-3 afforded the corresponding α- and β-keto sugars. Condensation of both anomers with thiocyanic acid following a slightly modified procedure only gave access to the β-furanoside OZT. The formation of the corresponding α-anomer was not observed (Scheme 27).

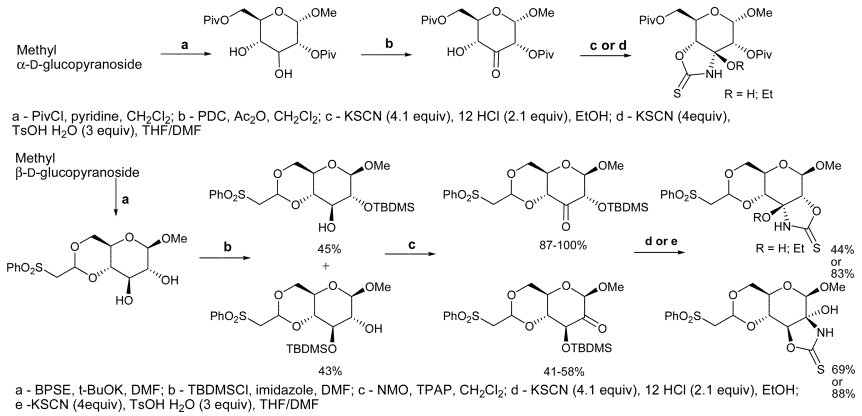


a - TBDMSCl, imidazole, DMF; b - PDC, Ac2O, CH2Cl2; c - KSCN (4.1 equiv), 12 HCl (2.1 equiv), EtOH; d - KSCN (4equiv), TsOH H_2O (3equiv), THF/DMF

Scheme 27

In order to extend this approach to pyran rings, the methyl α- and β-D-glucopyranosides were tested. Two ways were selected for generating accessible ketones.

The first involved a chemoselective 2-step sequence: (1) selective *O*-pivaloylation of OH-6 and OH-2 2) selective oxidation at C-3 to afford the target ketone (Scheme 28). When the condensation with HSCN was carried out in refluxing ethanol, a good overall transformation yield of 83% was obtained: two OZTs were formed in 22% (R = H) and 61% (R = Et) yield, respectively. Performing the reaction in a mixture of aprotic solvents (THF/DMF) afforded the target OZT (R + H) in 79% yield (Scheme 28).

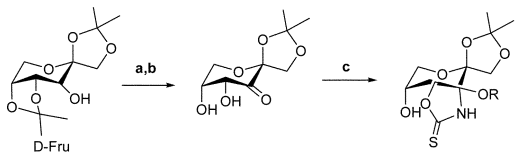


Scheme 28

The second approach involved initial 4,6-*O*-protection of the glucopyran template by a PSE acetal-an atypical acid-resistant protective group (Scheme 28). Regioselective oxidation under previously described conditions was unsuccessful, therefore masking either C-2 or C-3 hydroxyl groups was undertaken. Non-regioselective *O*-silylation led to a separable mixture of isomers. Subsequent oxidation of both compounds was performed using the TPAP-NMO system, which proved more efficient than PDC to afford the corresponding keto sugars. Condensation with thiocyanic acid in standard conditions (KSCN, 12 M HCl, EtOH) led to the formation of isomeric OZTs in 44% and 69% yield, respectively. When employing the THF/DMF-TsOH system, the corresponding hydroxy-OZTs were obtained in 83% and 88% yield, respectively. Applying the same sequence in the α-series failed to produce the target OZTs.

The approach was carried out on a ketohexo backbone bearing acid-sensitive ketal groups (Scheme 29). 1,2:4,5-Di-*O*-isopropylidene-*β*-D-fructopyranose readily underwent PDC oxidation of the 3-OH, followed by selective acid-catalyzed hydrolysis of the 4,5-ketal to afford a partially protected ketone in 94% overall yield. For the subsequent HSCN condensation, adapted acidic conditions had to be established to avoid 1,2-isopropylidene cleavage under thermal conditions and the target OZT (R = H) could be isolated in 60% yield. When performed in ethanol, the condensation afforded the acetalic counterpart (R = Et) albeit in lower yield.

Some features were put into light: (a) condensation of thiocyanic acid on carbohydrate-based α-hydroxyketones favors the formation of a fused OZT over an OXT, whereas the opposite is observed on acyclic systems;[48b] taking the different geometries into account, this is indicative of a thermodynamic

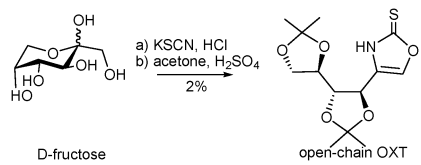


a - PDC, Ac_2O, CH_2Cl_2; **b** - AcOH, H_2O; **c** - KSCN (4equiv), TsOH H_2O (3equiv), THF/DMF

Scheme 29

effect in favor of the OZT formation; (b) the position and orientation of the hydroxyl group involved is critical with regard to the stereochemistry of the OZT formed; (c) the anomeric configuration has a decisive influence on the formation of the OZT between positions 2 and 3 on the carbohydrate backbone: with the keto sugars obtained from α-D-xylofuranoside and α-D-glucopyranoside, which share the same 1,2-*cis* relationship, no reaction occurred, while on both β-anomers, effective condensations took place. Such behaviour might appear as a consequence of two possible phenomena: (a) the approach of HSCN, which should take place on the ring face congested by the masked α-hydroxyl, might be blocked because of steric or electronic repulsion (b) unfavorable repulsive effects in the case of a 1,2-*cis* relationship to the anomeric oxygen (Fig. 1) might bring an additional limitation to the construction of a fused OZT. In summary, we have reported for the first time the installation on carbohydrate templates of hemiaminals of the 4-hydroxy-OZT type.

2.2.3 Open-chain OXT from a ketose. Recently Leconte *et al*. reported the formation of an open-chain oxazoline-2-thione (OXT) in low yield from D-fructose based on hydroxyketone chemistry (Scheme 30).[30]



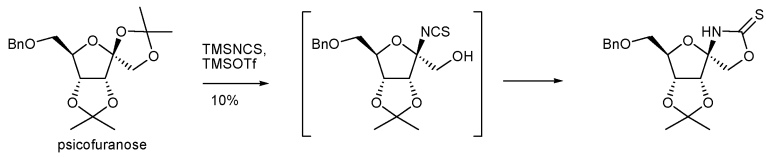
Scheme 30

The target OXT was accessible even when the starting ketone was masked by a ketal function and moreover, water was shown to be a well-adapted solvent.

2.3 Non-conventional methods

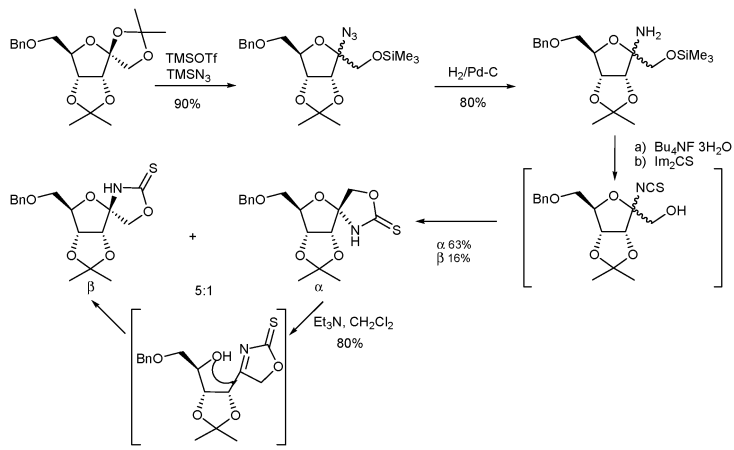
An interesting approach for the synthesis of spiro-OZTs and oxazine-2-thiones in D-psicofuran and D-fructopyran series involved as a key-step the reaction with trimethylsilyl isothiocyanate, azide or cyanide.[8] The reaction mechanism is similar to the one described above—after nucleophilic attack on the anomeric position, the formed isothiocyanate undergoes spontaneous cyclization to afford the corresponding spiro-thionocarbamate.

However, direct introduction of isothiocyanate on the psicofuran backbone proved to be less efficient (Scheme 31).



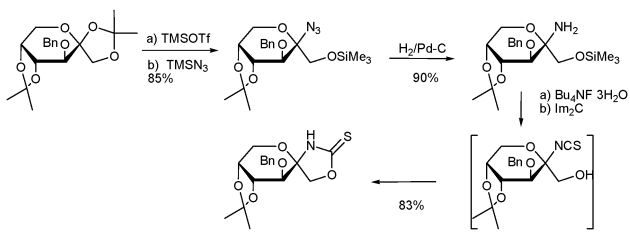
Scheme 31

In contrast, the synthetic pathway using trimethylsilyl triflate and trimethylsilyl, azide followed by hydrogenation, de-*O*-silylation, condensation with thiocarbonyl diimidazole and spontaneous cyclization of an intermediate isothiocyanate, afforded a β-anomer in good yield (Scheme 32).



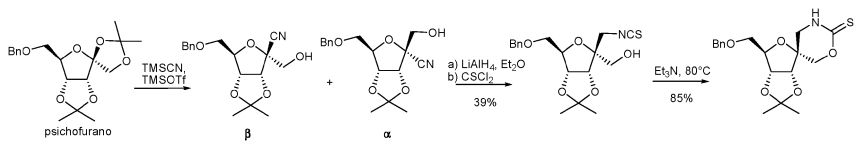
Scheme 32

The spiro-OZT on a pyran ring of D-fructose was prepared in a similar manner (Scheme 33). In this case, the obtained yield over three steps was 75% and the stereoselectivity at the anomeric position was better (β:α 9:1).

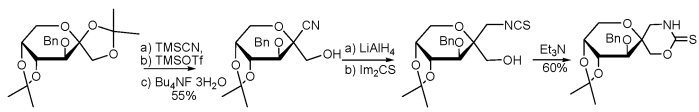


Scheme 33

The synthesis of 1,3-oxazine-2-thiones from D-psicofuranose (Scheme 34) and D-fructopyranose (Scheme 35) was performed using the following procedure: introduction of cyano group at the anomeric position led to a



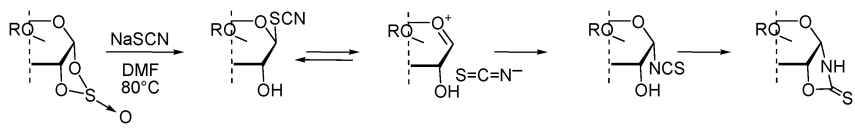
Scheme 34



Scheme 35

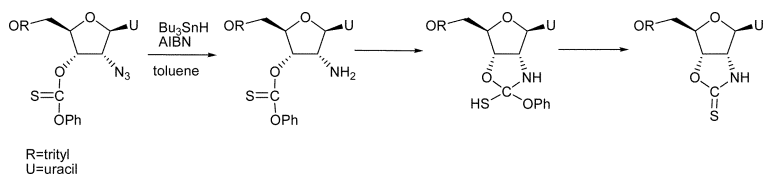
mixture of α- and β-nitriles, which were reduced into the corresponding amines; after condensation with thiophosgene, the mixture of isothiocyanates was formed. Final base-induced cyclisation produced the target thionocarbamates in good yield.

Uzan *et al*. have reported an alternative route to 1,2-fused thionocarbamates from 1,2-carbohydrate sulfites.[49] The reaction proceeded *via* formation of a β-configured thiocyanate, which further epimerizes to the α-anomer, ready to undergo cyclisation into the fused OZT (Scheme 36).



Scheme 36

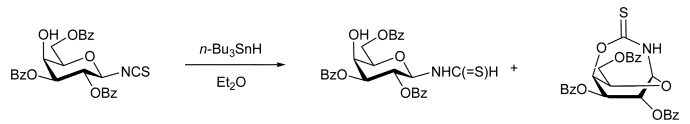
A fused OZT was obtained in the course of the reduction of vicinal azido-thiocarbonates *via* formation of the intermediate amine, which attacks the thiocarbonyl group (Scheme 37). The condensation reaction proved faster than the deoxygenation process for the synthesis of 2′-amino-2′,3′-dideoxyuridine[50] or methyl 3-amino-4,6-*O*-benzylidene-3-deoxy-2-*O*-phenoxythiocarbonyl-α-L-talopyranoside.[51]



Scheme 37

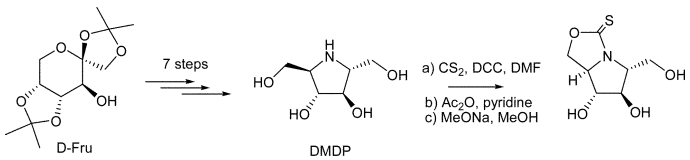
Reduction of a selectively protected *β*-D-galactopyranosyl isothiocyanate, having a free OH group at C-4, gave the corresponding glycosyl thioformamide, and a seven-membered ring thionocarbamate was also formed, albeit in very low yield (Scheme 38).[52]

One last approach to thionocarbamates on carbohydrate scaffolds was developed in D-Fru series: the intermediate imino sugar (*2R*,*3R*,*4R*,*5R*)-2,5-bis(hydroxymethyl)-3,4-dihydroxypyrrolidine (DMDP) obtained in



Scheme 38

seven steps from 1,2:4,5-di-*O*-isopropylidene-*β*-D-fructopyranose was further converted by carbon disulfide/ DCC into an indolizine-type thionocarbamate fused from the nitrogen side (Scheme 39).[53]



Scheme 39

The condensation of DMDP with carbon disulfide/dicyclohexylcarbodiimide (DCC) afforded the indolizine derivative—potentially biological active thionocarbamate on carbohydrate scaffold, fused from the nitrogen side.

3. Reactivity of thionocarbamates on carbohydrate scaffolds (*N*- and *S*-reactivity)

The simplest OZTs or those anchored on saccharidic backbones offer two remarkable reactive sites: nitrogen and sulfur. The reactivity of both sites was extensively investigated and used to explore original reactions in carbohydrate chemistry by Rollin *et al.*[2k,13,54,55] (Fig. 2)

The differences in reactivity between the nitrogen and the sulfur atom in OZTs reveal that most reactions can be interpreted with reference to Pearson's HSAB theory.[56] In the case of 1,3-oxazolidine-2-thiones, one may consider the nitrogen atom as a «harder» basic center than the «softer» sulfur atom.

Among chiral auxiliaries, 1,3-oxazolidine-2-thiones (OZTs) have attracted much interest for their various applications in different synthetic transformations.[2b] Such simple structures, directly related to far better known chiral oxazolidinones,[11,12,57] have been explored in asymmetric Diels-Alder reactions and asymmetric alkylations, but mainly in condensation of their *N*-acyl derivatives with aldehydes. Chiral OZTs have shown interesting characteristics in anti-selective aldol reactions[58] or combined asymmetric addition.

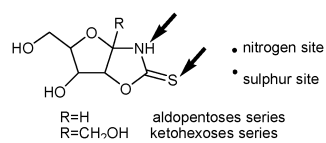


Fig. 2

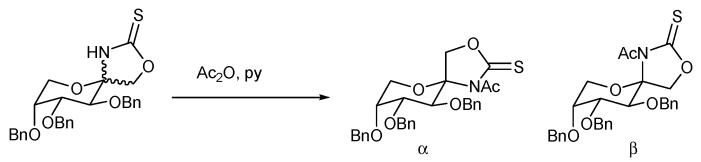
3.1. *N*-Reactivity

In order to study the reactivity of the nitrogen atom in saccharidic OZT moieties Rollin and co-workers[13,55] explored some standard reactions: *N*-acylation, *N*-sulfonylation, *N*-vinylsulfonylation by Michael addition, reductive elimination, conjugated addition and cycloaddition.

N-functionalised OZTs being enantiopure compounds can be seen as important tools in asymmetric synthesis.

3.1.1 *N*-Acylation. Acyl chlorides or carboxylic anhydrides, which generally have a «hard» electrophilic character, can selectively convert OZTs into *N*-acylated derivatives.[59]

A well-spread example is the acetylation, which is commonly used as a protective reaction but can also be applied as a post-functionalization in order to separate isomers[47] (Scheme 40). In some cases, however, the separation of *N*-acetyl OZTs can be tedious, due to their relative instability.

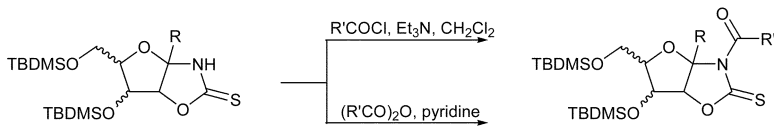


Scheme 40

Methods for the *N*-acylation of similar heterocycles, such as 'simple' thiazolidinethiones, have been reported since 1977, namely acyl chlorides in miscellaneous conditions,[58h] or carboxylic acids under DCC-activation.[60,61] However the easiest and most effective method involves acyl chlorides or carboxylic anhydrides in the presence of an amine.[47] Applying that procedure on carbohydrate scaffolds Rollin and co-workers[62] reported the synthesis of diverse *N*-acylated OZTs. The reactions were performed with good yields and the *N*-selective acylation was ascertained by NMR—namely the thiocarbonyl ^{13}C chemical shift (Scheme 41). Thanks to the dual nature of the carbanion drifting in the reaction,[59b,60] no competitive formation of the thioester, as mentioned by Plusquellec *et al.* in the case of benzothiazole, was observed.

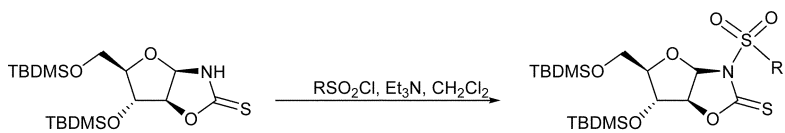
Comparing the results given in Scheme 41 with those reported for 'simple' OZTs, it can be concluded that *N*-acylation of OZTs fused on saccharidic backbones is not affected by the saccharidic structure, the increase in steric hindrance (aldopentose to ketohexose) or the protection of C-1 position (OBn, OTBDMS).

3.1.2. *N*-Sulfonylation. The *N*-sulfonylation reaction (Scheme 42) was performed using different sulfonyl chlorides and some enantiopure sulfonamido derivatives were synthesized.[54] The *N*-selectivity of sulfonylation was ascertained by ^{13}C NMR.[62]



R	Sugar series	R'	Yield
H	D-*arabino*	CH_3	80%
		OtBu	100%
		Ph	72%
		CH=CHPh	93%
CH_2OBn	D-Fru	OtBu	100%
		CH=CHPh	90%
	L-Sor	OtBu	98%
		CH=CHPh	80%
$CH_2OTBDMS$	D-Fru	CH=CHPh	87%
	L-Sor	CH=CHPh	85%

Scheme 41



R	Yield
CH_3	72%
4-$CH_3C_6H_4$	70%

Scheme 42

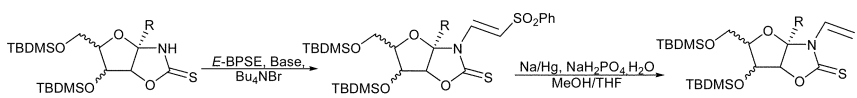
3.1.3 *N*-Vinylsulfonylation by Michael addition and reductive elimination. The literature is relatively scarce regarding general methods for the synthesis of *O*-[63], *S*-[64] and *N*-vinylsulfones.[65] Rollin, De Lucchi and co-workers have developed an easy way for *O*- and *S*-vinylsulfonylation on saccharidic backbones, using electrophilic 1,2-bis(phenylsulfonyl)ethylene (BPSE).[66–68] BPSE is recognised as a very useful reagent in organic synthesis thanks to the sulfonyl electroattractor dual system which strongly activates the unsaturation. For this property, its application as a highly reactive dienophile was tested in Diels-Alders reactions,[69] cycloadditions[66,70] but also as a good Michael acceptor with an extension toward asymmetric reactions.[71] BPSE can undergo two successive nucleophilic additions. A first addition leads to a heterovinylsulfone-type mono-adduct, which may then undergo a second attack to form a phenylsulfonylethylidene (PSE) acetal. This is a new protective device in carbohydrate chemistry,[72] with promising properties owing especially to its remarkable resistance against acidic media.[72–74] Depending on the type of nucleophile and its Brønsted acidity,

the basic conditions used can be different. The first addition is stereo-conservative, which means that the configuration of the heterovinylsulfone formed depends on the configuration (*E*) or (*Z*) of the BPSE used: retention of configuration is indeed resulting from a Michael addition-elimination.[75]

The specific reactivity of sulfones has been used namely to conduct highly efficient reductive desulfonylations: in that case, a monoelectronic transfer can selectively afford the corresponding vinyl derivative. The literature reports several reduction methods, including lithium naphtalenide, samarium diiodide, magnesium or sodium/mercury amalgam in a buffered medium.[76] Although the use of mercury is not the most advantageous in terms of toxicity, both the amalgam preparation and handling are easy. In particular for the final product purification, a simple filtration is usually sufficient. Sodium amalgam can also be replaced by magnesium in methanol, however, a loss of efficiency is observed.[77]

We report here some literature examples 13, 55 of *N*-vinylsulfonylation and reduction to *N*-vinyl derivatives. Reactions were applied to different types-fused, anchored, spiro-OZTs on carbohydrate scaffolds and the examples shown indicate that the location of the OZT moiety with respect to the anomeric carbon can sometimes influence the results obtained.

(a) At the anomeric carbon. The *N*-vinylsulfonylation was applied successfully to some fused OZT in an aldopentose and ketohexoses series. A combination of diisopropylethylamine and the phase transfer agent Bu_4NBr in DMF (especially in the case of hindered compounds) allowed the formation of *N*-vinylsulfonyl compounds in good yields. This amine (Hunig base) makes the process of mono-addition quite efficient. Concerning ketohexose derivatives with the hydroxyl group in position 1 protected with a bulky group, no reactivity was observed due to steric hindrance.[55] Reductive desulfonylation conditions applied to the *N*-vinylsulfonyl OZTs proceeded well (Scheme 43).[55]



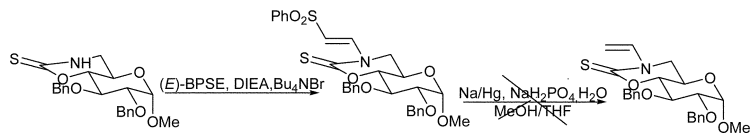
R	Sugar series	*N*-vinylsulfone.		*N*-vinyl OZT
H	D-*arabino*	Et_3N	91%	76%
CH_2OBn	D-Fru	DIEA	70%	98%
	L-Sor	DIEA	72%	96%
$CH_2OTBDMS$	D-Fruc	DIEA	no reaction	-
	L-Sor	DIEA	no reaction	-

Scheme 43

Fused-OZTs

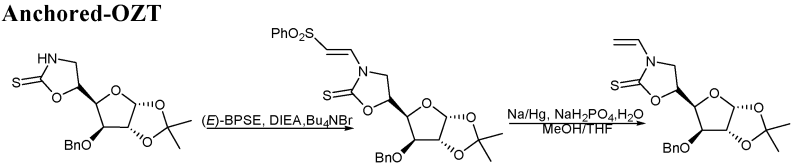
(b) Apart from the anomeric carbon. *N*-vinylsulfonylation and reductive elimination on OZT moieties away from the anomeric carbon were performed by Rollin and co-workers. using the conditions mentioned in

3.1.3.a. However the yields were generally found superior than those for OZTs at the anomeric carbon, the reason for that being a better accessibility to the nitrogen site. The results of the sodium amalgam reduction to *N*-vinyl derivatives were good for OZTs (Schemes 45–47); however, one fused 1,3-oxazine-2-thione did not undergo successful desulfonylation (Scheme 44), degradation being mostly observed in the reductive process. This unexpected result suggested the instability of *N*-vinyl-1,3-oxazine-2-thiones, which may be due to the tensions induced in the 6-membered ring.[55]



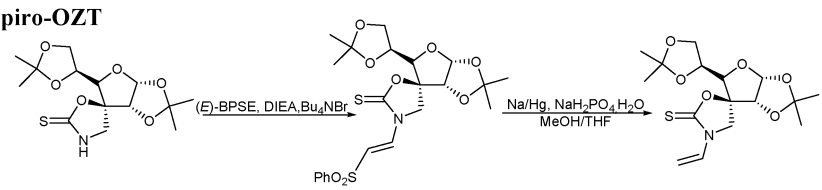
Scheme 44

Anchored-OZT

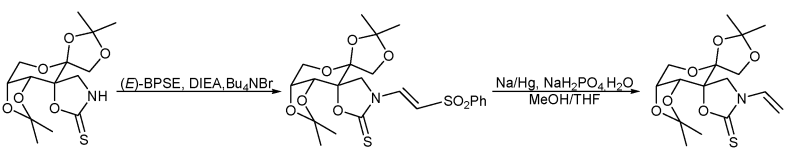


Scheme 45

Spiro-OZT



Scheme 46



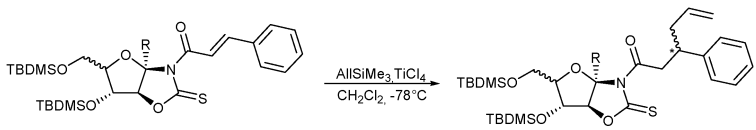
Scheme 47

3.1.4 ***N*-Functionalized OZTs.** 2-Thioxo-1,3-*O*,*N*-heterocycles form an important class of heterocyclic compounds with varied chemical, biological and pharmaceutical applications. In particular, optically active OZTs have been largely applied as chiral auxiliaries in a wide range of synthetic transformations (asymmetric aldolisations, stereoselective alkylations, *etc* . . .). Several research teams have applied that methodology for the synthesis of biologically-relevant natural compounds (antibiotics, carbanucleosides, *etc* . . .).[2,78]

3.1.5 Addition reactions

3.1.5.1 Conjugate addition. In general, the use of chiral auxiliaries able to promote chirality transfer with a predictable stereochemistry on newly generated stereocenters is recognized as indispensable in asymmetric synthesis.[79]

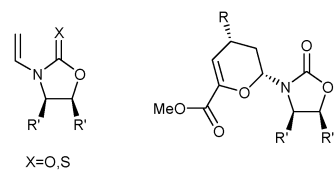
As mentioned before, chiral OZTs have proven to be very efficient auxiliaries in a large number of stereoselective reactions. Rollin and co-workers. have studied diastereoselective conjugate addition reactions catalyzed by Lewis acids on chiral OZTs, foreseeing OZTs on saccharidic scaffolds to be bulky enough to allow good facial selectivity in the allyltrimethylsilane attack. The results are shown in Scheme 48. Conjugate addition on the *N*-cinnamoyl D-*arabino*-OZT took place with reasonable yield but without any stereoselectivity. With a view to increase steric congestion in the reactive zone, D-Fru and L-Sor OZT derivatives were synthesized. However, the diversely 1-*O*-protected acceptors tested only gave disappointing results, both in terms of chemical yield and stereoselectivity. the presence of an additional appendage only slightly improved the diastereoisomeric ratio.



R	Sugar series	Yield (%)(d.r.)
H	D-*arabino*	68 (1:1)
CH_2OBn	D-Fru	75 (0.6:1)
	L-Sor	42 (0.7:1)
$CH_2OTBDMS$	D-Fru	no reaction
	L-Sor	

Scheme 48

3.1.5.2 Cycloaddition. Recently *N*-vinyloxazolidinones were presented as new potential chiral dienophiles in inverse hetero-Diels-Alder reactions to activated 1-oxabutadienes, leading with high diastereocontrol to hetero-adducts under $Eu(fod)_3$-catalyzed conditions (Scheme 49).[17]

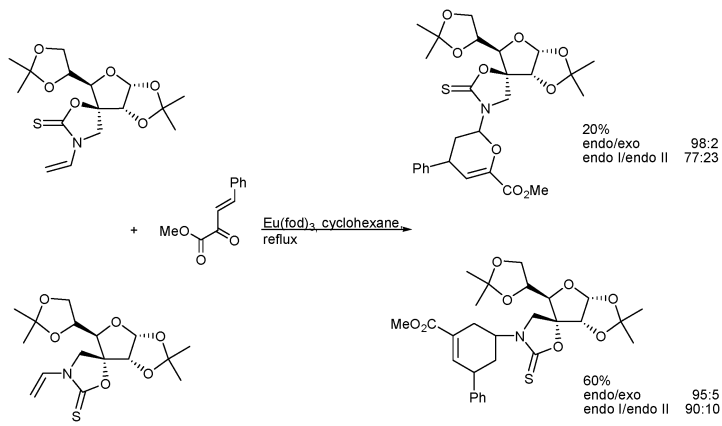


Scheme 49

Specifically functionalized OZTs are helpful synthons[10,21] which are emerging as new chiral auxiliaries with high potential in chirality transfer.[56,80] The efficient Michael addition-reductive elimination process to produce

carbohydrate-derived *N*-vinyl OZTs (3.1.4.) opens the way to their application in stereoselective cycloadditions.[13]

Heterocycloaddition of a glucofuran-based spiro-OZT bearing an *N*-vinyl moiety with benzylidenepyruvic acid methyl ester, under the standard conditions established by Dujardin for *N*-vinyloxazolidinones, proceeded in low yield, albeit with a high *endo*-selectivity, while facial selectivity remained modest (77:23), when compared with previous values. However its C-3 epimeric spiro-OZT gave a surprisingly good yield of the desired heterocycloadduct with a high selectivity for the *endo*-diastereoisomer in addition to a very good facial selectivity (90:10) (Scheme 50).



Scheme 50

When comparing these results with those previously obtained using carbohydrate-based vinyl ethers as chiral dienophiles, this improved facial diastereoselectivity to heterodienes under similar conditions is noteworthy.[81] The efficient chiral transfer in the second example might mostly be attributed to the specific architecture of the 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose moiety.Those findings open the way to develop well-defined spiro-carbohydrate templates towards improved auxiliaries for chirality transfer in a wide range of syntheses.

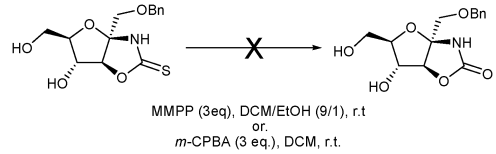
3.2 *S*-Reactivity

3.2.1 Conversion to carbamates. Cyclic carbamates derived from sugars and related compounds have repeatedly been encountered, the first being an inosamine derivative, obtained by Curtius degradation of dihydroshikimic acid.[82,83]

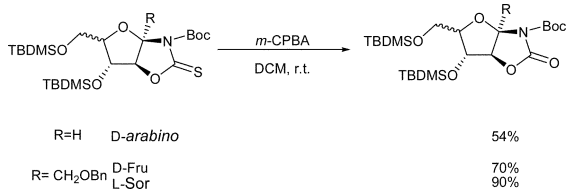
The conversion of thiocarbonyl compounds into carbonyl compounds, long attracted the interest of organic chemists and a variety of methods allowing this conversion have been developed, recurring to diverse organic and inorganic reagents.[84]

With respect to OZTs anchored on carbohydrate scaffolds, the direct replacement of the sulfur by oxygen showed to be tricky (Scheme 51).

However, good yields of 1,3-oxazolidin-2-ones were obtained through *m*-CPBA oxidation of *N*- and *O*-protected OZTs (Scheme 52).[10]



Scheme 51

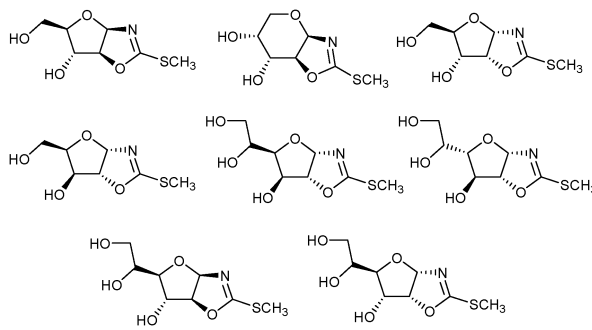


Scheme 52

This transformation opens a new access to enantiomerically pure 1,3-oxazolidin-2-ones, a class of compounds which has been extensively studied either in medicinal chemistry[85–87] or in stereocontrolled synthesis.[11,12]

3.2.2 *S*-Alkylation. The nucleophilic character of the C═S bond in thionocarbamates was explored with alkylation reactions. Normally and in agreement with Pearson's theory,[56,88,89] the R–X reagents behave as soft electrophiles, providing preferential high-yielding *S*-alkylation.

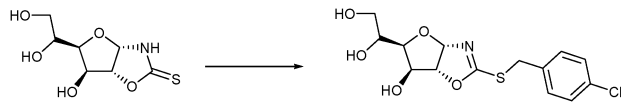
Coxon and co-workers[90] have studied the reaction of naked glyco-OZTs with methyl iodide in the presence of sodium methoxide in methanol and the corresponding 2-methylsulfanyl-glyco-oxazolines were obtained in good yields in many aldose series (Scheme 53).



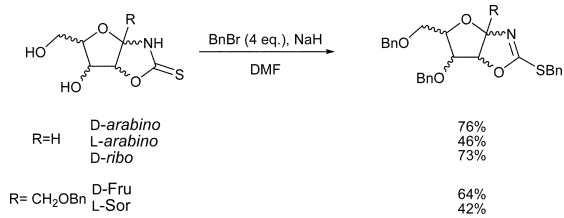
Scheme 53

Similarly, Tóth and co-workers converted a D-glucofurano-derived OZT into the corresponding 2-*p*-chlorobenzylsufanyloxazoline in 77% yield (Scheme 54).[91]

Girniene *et al.*[21] have applied classic benzylation conditions to naked carbohydrate-fused OZTs to produce per-*O*,*S*-benzylated derivatives (Scheme 55).



Scheme 54



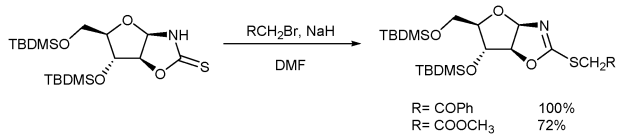
Scheme 55

However, the direct peralkylation method produces quite variable yields: this fact was previously mentioned and explained by Fujita *et al.*[92] who observed that, in the presence of excess BnBr or under heating, the *S*-benzylated derivatives can undergo rearrangement into *N*-benzylated isomers.

In order to increase the S-benzylation yields, Girniene *et al.* have successfully opted for a previous protection of the hydroxyl groups (Scheme 56).

Scheme 56

In addition, it was shown that preliminary protection of the hydroxyl groups allowed the formation of diverse S-alkylated products in very good yield (Scheme 57).



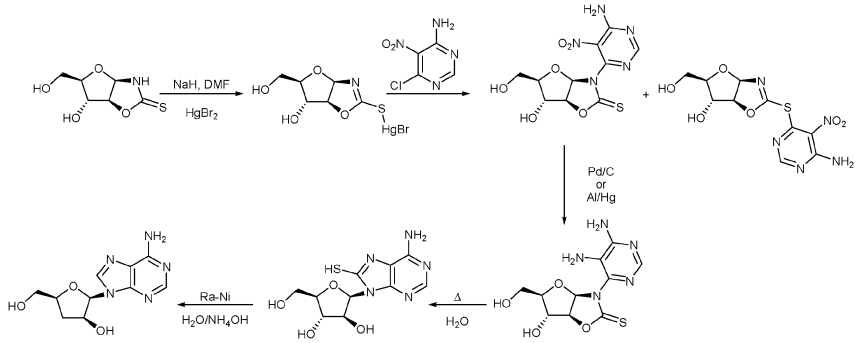
Scheme 57

3.2.2.1 Access to modified nucleosides. Base-modified nucleosides and nucleotides are very important for their biological properties. They can be found as antiviral agents (HBH, VZV, AIDS),[93–95] in the study of DNA degradation,[96,97] as fluorescent agents and as chemical probes of DNA structure.[98–101] The access to nucleosides can be achieved by different methods:

• Use of glycosylation in order to effect connection between the base and the carbohydrate structure—which can be tricky due to anomeric stereoselectivity problems;[102,103]

• Construction of the nucleic base *via* a multi-step process, centered on the monosaccharide structure.[104]

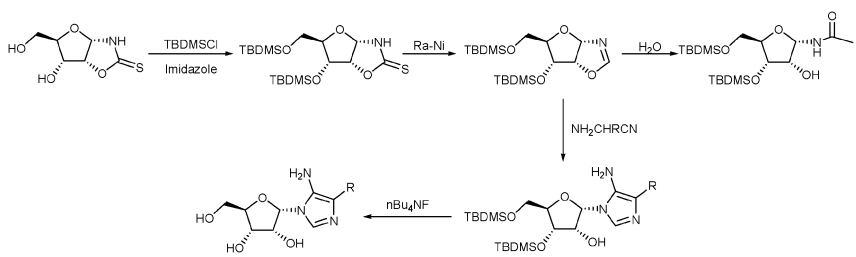
The use of thionocarbamates in the synthesis of nucleoside analogues was explored for the first time by Ranganathan, who described a five-step synthesis of 9-β-D-arabinofuranosyladenine, starting from a D-*arabino*-anchored OZT[14] (Scheme 58).



Scheme 58

Despite of the originality of the procedure, the second condensation step with the pyrimidine base gave poor yields, especially due to the formation of both regio-isomers.

Some years later, Imbach *et al.*[16] prepared base-modified α-nucleosides, starting from a D-*ribo*-anchored OZT (Scheme 59).

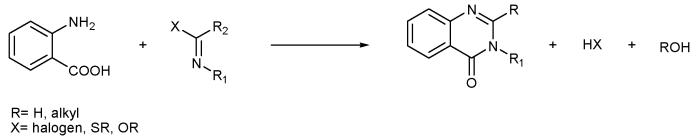


Scheme 59

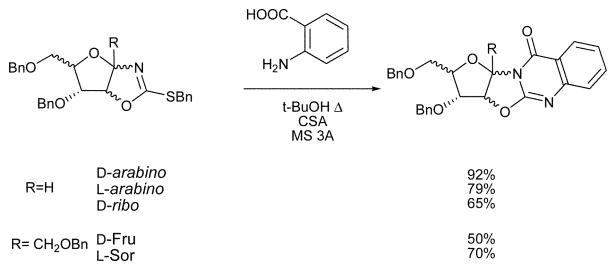
The same synthetic scheme was applied to synthesize nucleoside analogues with natural or modified base, starting from α- and β-D-*xylo*-[17] and β-D-*arabino*-derivatives.[105] Grouiller also used the procedure with β-D-fructose to prepare nucleoside derivatives.[18] However, the fragility of the oxazoline intermediate and low yields obtained limited the methodology.

In 2004, Girniene *et al.* used OZTs as starting structures to develop a convergent preparation of fused quinazolinone-sugars and quinazoline-dione nucleosides. Based on well-documented cyclocondensation reactions of anthranilic acids[106–110] (Scheme 60), the strategy was focused on the condensation of carbohydrate-connected 2-alkylsulfanyloxazolines with diverse anthranilic acids.[21]

Application of the condensation process to per-*O*-benzylated OZTs led to new homochiral quinazolinone derivatives in satisfactory yields (Scheme 61).

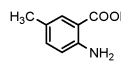
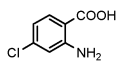
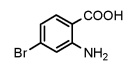
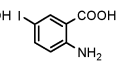
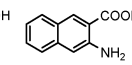


Scheme 60



Scheme 61

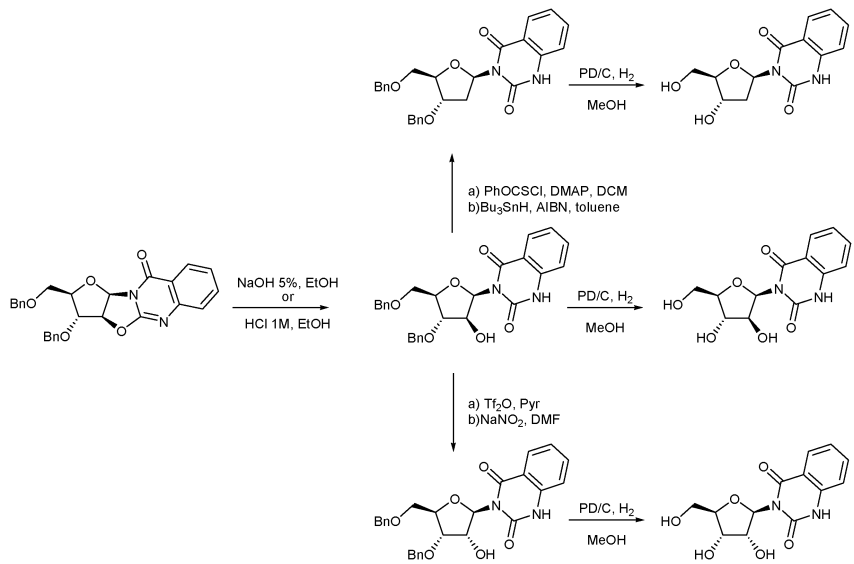
Diversely substituted anthranilic acids were condensed with per-*O*-benzylated D-*arabino*-alkylsulfanyloxazoline as a model: the results showed that the presence of an electron-withdrawing group hampered the reaction to some extent.

Amino acids					
Condensation yield (%)	89	86	94	78	65

Having in hand an efficient and short process for the formation of 2,2′-anhydronucleosides, Girniene *et al*. explored the ability of the newly formed heterocycles to generate the corresponding nucleosides and deoxynucleosides, investigating the ring-cleavage of quinazolinones on a D-*arabino* model. Reacting the previously prepared D-*arabino* condensed derivative under basic or acidic conditions led to the quinazoline 2,4-dione, thus allowing preparation of both the nucleoside analogue and its C-2 epimer. Through a Barton-McCombie process, the 2-deoxynucleoside was also synthesized (Scheme 62).

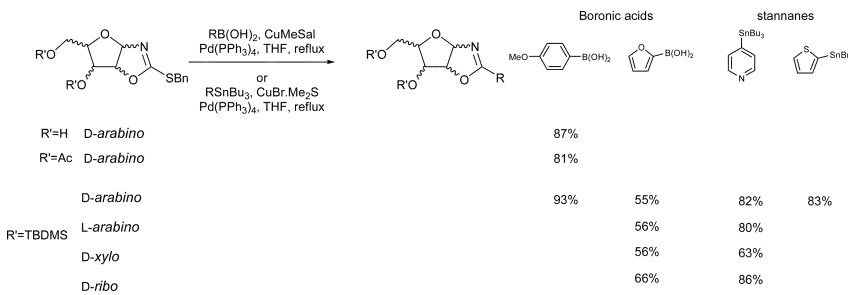
3.2.2.2 Palladium-catalyzed coupling reactions. In the last few decades, palladium compounds have been used as catalysts to develop new synthetic transformations, mainly for carbon-carbon and carbon-heteroatom coupling reactions (as Suzuki, Stille, Sonogashira, *etc* . . .) in generally mild reaction conditions. The high functional group tolerance and broad availability of starting materials have contributed to the growing success of many palladium cross-coupling reactions as one of the major tools for the construction of complex molecules.[111–117]

Recently, with a view to overcome the difficulty on the preparation of aryl or alkenyl halides or sulfonates, thioamides and their *S*-alkyl derivatives have been proposed as a new class of electrophilic partners. This palladium cross-coupling methodology was developed by Liebeskind and mostly applied to heteroaromatic templates.[118–121]



Scheme 62

Applying the coupling conditions established by Guillaumet *et al.*,[119] Leconte *et al.* have developed the coupling reaction of carbohydrate-connected benzylsulfanyl OZTs with boronic acids and stannanes in good yields (Scheme 63).[122]



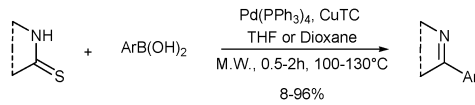
		Boronic acids		stannanes	
R'=H	D-*arabino*	87%			
R'=Ac	D-*arabino*	81%			
R'=TBDMS	D-*arabino*	93%	55%	82%	83%
	L-*arabino*		56%	80%	
	D-*xylo*		56%	63%	
	D-*ribo*		66%	86%	

Scheme 63

The results given above clearly demonstrate the good leaving group ability of the readily introduced benzylsulfanyl moiety in copper-promoted, Pd-catalyzed cross-coupling reactions. Moreover, the use of non-protected polyhydroxylated substrates has proven to be compatible with the cross-coupling conditions.

Kappe has recently published a modified desulfurative cross-coupling method, using a direct reaction on thioamides under microwave assistance.[123,124] It was shown that, in contrast to cross-coupling alkylsulfanyl-*N*-heteroaromatics and boronic acids, 2 to 3 equivalents of CuTc cofactor were needed to achieve high yielding conversions (Scheme 64).

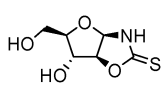
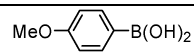
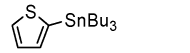
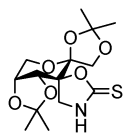
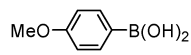
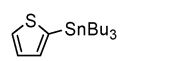
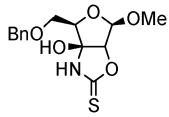
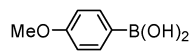
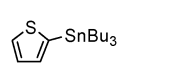
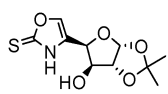
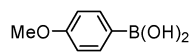
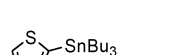
Rollin *et al.*[25] have applied those direct coupling conditions in a Suzuki-type process-using *p*-methoxyphenyl boronic acid and CuTc-to three carbohydrate-based OZTs but the coupling reaction only resulted in moderate yields (42%–47%). Using the same thionocarbamates, a direct Stille



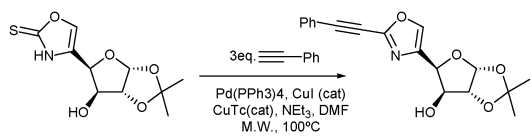
Scheme 64

reaction, using 2-tributylstannylthiophene and CuBr in excess was performed, albeit with disappointing yields. The preceding thionocarbamates were therefore converted into the respective 2-benzylsulfanyloxazolines, which were further submitted to Suzuki and Stille coupling conditions. On all three OZT substrates, the Suzuki and Stille couplings took place with dramatically improved yields: the two-step procedure thus appeared a good alternative to the direct cross-coupling process. In sharp contrast, the direct cross-coupling protocol in Suzuki and Stille reactions looked far superior when applied to the aromatic parent OXT structure[90,125] (Table 1).

Table 1 Suzuki/Stille coupling reaction yield (direct coupling *vs.* two-step procedure)

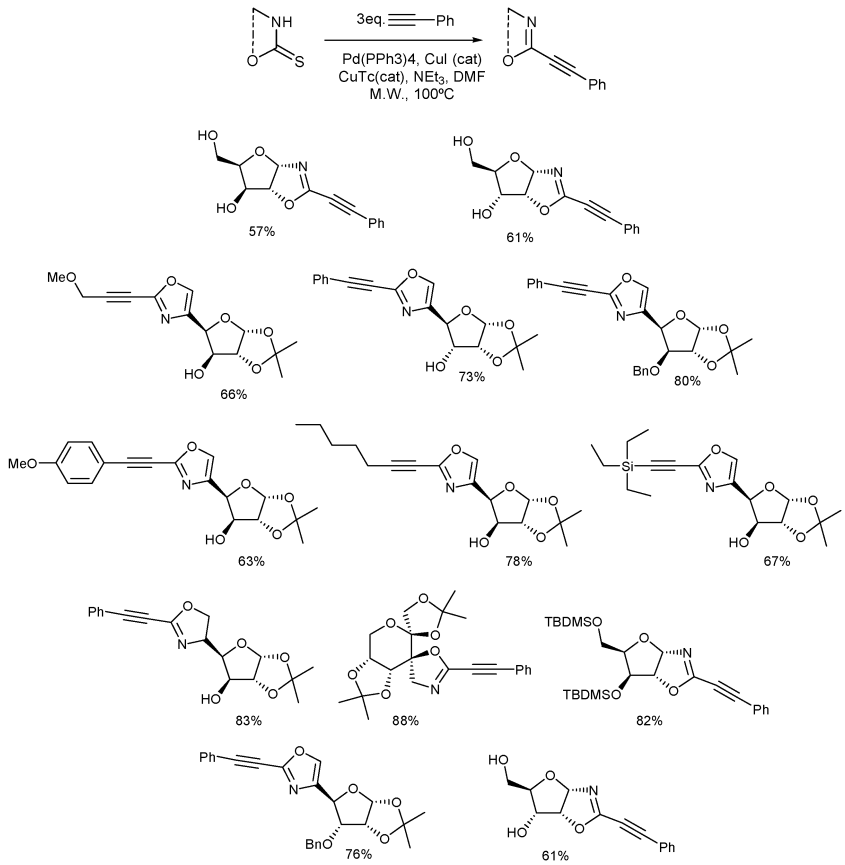
Substrate	Coupling reagent	Direct coupling yield (%)	Two-step procedure yield (%)
		47	76
		72	77
		45	83
		75	80
		42	80
		27	67
		86	—
		86	—

In addition, Rollin *et al.*[26] have also developed a new modified Sonogashira cross-coupling reaction of OZTs, in which copper(I) is used in catalytic amount, allowing the formation of C–C bonds to produce alkynyloxazoles (Scheme 65).



Scheme 65

Generalization of the above results to different alkynes and different carbohydrate-based thionocarbamates showed the flexibility of the method for C–C bond formation on either the aromatic OXTs or non aromatic OZTs in building up a small library of analogues (Scheme 66).



Scheme 66

4. Bioactivity

The synthesis of 2-thioxo-1,3-*O*,*N*-heterocycles connected to glycopyran and glycofuran scaffolds involves the formation of fused, anchored or spiro structures. The synthesis of such compounds is an attractive challenge from a chemical point of view but also from the growing interest concerning their biological properties.

Those structures correspond to analogues of natural compounds such as pseudo *C*- and *N*-nucleosides,[3,4] spironucleosides[7] or even spiro *C*-glycosyl compounds.[8] Furthermore, OZT saccharidic derivatives are potentially useful as chiral intermediates and synthons in aza sugars synthesis (glycosidase inhibitors),[22,23] or as nucleoside precursors.[14–18,105] In addition, the formation of OZTs at the anomeric or other positions on carbohydrates was exploited to mimic the ketohexose conformations in inhibiting fructose transporter GLUT5.[10,17,18,20,21]

4.1 GLUT5

The chemistry of D-fructose has not been much developed when compared to D-glucose, even though the ketose is one of the most abundant simple sugars in nature and is therefore a significant component of human dietary sugar intake.[126] Unlike the case of D-glucose, which is absorbed in the intestine by the transporter sodium-dependent D-glucose SGLT 1,[127] the consumption of D-fructose in mammals is carried out *via* a sodium-independent specialised transporter, known as GLUT5.[128]

Understanding the sugars assimilation at cellular level allows clearly comprehension of installation mechanisms of type 2 diabetes (diabetes non-insulin dependent). As a result, the comprehension of the structure and function of this transmembrane protein is indeed crucial.[129] This protein belongs to a family of transporters (glucose transporters, GLUTs), which by the recent sequencing of human genome has been extended from five to thirteen isoforms, and classified into three subfamilies: (1) GLUT 1-4 are D-glucose specific, (2) GLUT 5, 9 and 11 seem to be D-fructose specific (3) GLUT 6, 8, 10 and 12, the specificity of which remains to be clarified.

Thus, specific inhibitors of this protein (and other proteins) are reagents of choice to develop chemical probes.[130] In addition to an imperfectly understood transport role for GLUT5 and the new isoforms, these proteins are distributed in some specific tissues. GLUT5 is present in the intestinal tissues,[131] kidney[128*b*] and spermatozoa but substantial levels are also present in adipose and muscle tissue.[128*a*,132] The tissue expression levels of the GLUT5 transporter are altered in development and are responsive to alterations in D-fructose in the diet.[133] Furthermore, levels of GLUT5 transporter in intestine are increased in experimental diabetes and type 2 diabetes.[131*d*,134] Their physiological implications must be determined. As a result construction of probes selective for each isoform is an important goal.

The interaction analysis between the D-fructose and its transporter is complicated due to its ability to exist in solution in four different tautomeric forms -α,β-pyranose and α,β-furanose. Despite its large-scale accessibility and elucidation of its structure over a century ago,[135] the chemistry of fructose[126] makes also the preparation of simple derivatives in tautomeric fixed structures difficult, particularly in furanoid forms.

In a previous work, using D-fructose pyran- and furan- forms as inhibitors of D-fructose transport in CHO (Chinese Hamsters Ovary)-GLUT5 cells, Rollin, Holman and co-workers established that both ring forms were tolerated. The approach used was to block each hydroxyl function with allylic ether: it was concluded that two sites, O-2 (pyranose and furanose) and O-6 (furanose) could be modified and addressed a visualization of vital interactions with the protein. These interactions were considered to occur because the D-fructofuranose form is relatively symmetrical: for that reason, the binding site can arise either in anomeric center side or on the other side of the molecule. Hence D-fructopyranose appears to present to GLUT5 transporter by hydroxyl 3, 4, 5 recognition (Fig. 3).

With the aim to explore this model of interaction, Rollin and co-workers have developed a method using the OZTs moieties to block the D-fructose structure and analogues. The OZT structures can be regarded as analogues of D-fructose

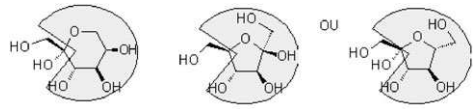


Fig. 3

in pentose series (derived from D- and L-arabinose, D-ribose and D-xylose), as well as in ketohexose series (derived from D-fructose and L-sorbose). The interest of these molecules is to establish a blocked furan structure through the presence of the OZT cycle, which will be presented to the protein.

In order to understand the partern of recognition, the authors decided to compare the influence of various factors on the recognition process: (1) the type of osidic scaffold (aldopentoses or ketohexoses), (2) the group (benzyl, allyl) at O-1 position in ketohexoses, (3) the stereochemistry of C-5, (4) comparison of the pentose series. The aim of the study was therefore to develop analogues of D-fructose in which each position was substituted and thereby determine the importance of each of hydroxyl groups in the binding and interaction with GLUT5. This necessitated an investigation of the positions around the D-fructose structure that could accommodate bulky groups and, as described here, factors that could be used to increase the affinity of D-fructose analogues for its transporters.

The *in vitro* bioassays allowed to determine the inhibition constant of D-fructose transport by the CHO cells. This measure is carried out by competition with radioactive D-fructose. The study put in evidence that pentose-OZT derivatives are not recognized by the protein transporter. Only the ketohexose-OZT derivatives expressed some inhibition of GLUT5. These inhibition constants showed to be much effective with L-Sor derivatives than with D-Fru derivatives and even better than D-fructose itself (K_i = 15.5 mM) (Table 2).

Table 2 Association constant of D-fructose transport

OZT derivatives	Association constant K_i [mM]
D-fructose	15.5 ± 2.9
D-*arabino*-OZT	104.5 ± 21.3
L-*arabino*-OZT	122.8 ± 27.6
D-*xylo*-OZT	106.2 ± 27.1
D-*ribo*-OZT	109.9 ± 24.3
D-Fru-Bn-OZT	32.6 ± 3.6
D-Fru-All-OZT	20.8 ± 3.8
D-Fru-OZT	24.9 ± 3.2
L-Sor-Bn-OZT	12.4 ± 1.5
L-Sor-All-OZT	14.5 ± 2.1
L-Sor-OZT	17.4 ± 6.7

These blocked structures gave a picture of the interaction of D-fructose with the protein. It is assumed that a ketohexose C-1 additional arm (absent in pentose series), is required in order to achieve some inhibition, but a certain freedom of substitution is possible (OBn, OAll or OH). Comparison of D-Fru- and L-Sor-OZTs showed that substituted derivatives are better

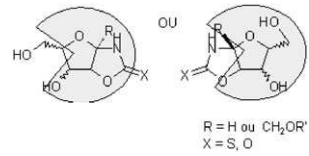


Fig. 4

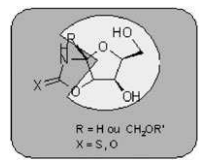


Fig. 5

inhibitors than unsubstituted. It means that bulky groups can occupy a position out of the binding site (C1) and so recognition is performed by the other 'face' of the molecule.

The L-sorbofurano configuration is more efficient to perform GLUT5 inhibition which means that this configuration certainly allows a better recognition by GLUT5.

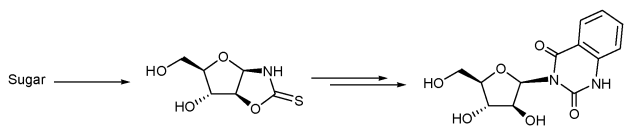
The L-Sor-OZT derivatives mimic the D-fructofuranose interaction with GLUT5 using a recognition face by carbons C-4 and C-6. This conclusion confirms the model initially proposed (Fig. 4 and 5).

4.2 Nucleosides

Over the past years, synthetic base-modified nucleosides and nucleotides have displayed important impact in diverse fields. Their biological properties have found application as antiviral tools against hepatitis virus (HBV), herpes virus (VZV) and human immunodeficiency (HIV).[94,95,136] Many of those compounds exhibit antiproliferative, antibiotic and antifungal activities and some have been used as probes for DNA damages[96,97,137] as well as in the anti-sense approach and DNA-probe technology with fluorescence properties.[99]

It is believed that introducing diversity either into the carbohydrate or the heterocyclic moieties of nucleosides (*e.g.* methylated bases) leads to gifted molecules with therapeutical potential in RNA duplex,[138] or in the bacteria world as new antibiotics.[98,101,102,139]

The approach developed involving a sugar-derived OZT paves the way to novel type 'artificial nucleosides' as presented in the example below (Scheme 67).[20]



Scheme 67

On the other hand, Kifli and co-workers[139] have synthesised original nucleosides with modifications in the sugar unit, which are antiviral and anticancer agent analogues.[140] As presented in Fig. 6, the nucleoside with a

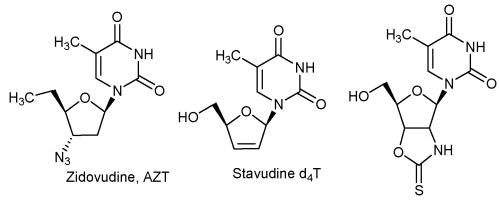


Fig. 6

fused-OZT moiety could be regarded as a Zidovudine (AZT) or Stavudine (d4T) analogue.

In general OZT moieties in carbohydrates scaffolds are very important structures due to the wide range of biological and pharmaceutical applications. But many further applications still remain to be developed.

4.3 Imino sugars

Imino sugars (carbohydrate mimic with an *N*-atom replacing the endocyclic oxygen) represent an important class of glycosyl hydrolase inhibitors. Thanks to their inhibitory potential, they are promising probes for structure and function studies of enzymatic catalysis (glycoconjugate function studies) as well as useful for targeting and turnover.[141] Furthermore these compounds also find applications as chemotherapeutic agents for the treatment of cancer,[142] diabetes[143] and inflammation or viral replication.[144] Actually there are many imino sugars available but the problem still resides in lack of specificity.

The compounds shown in Fig. 7 show good inhibitory properties towards various α- and β-glycosidases,[53,145] but a better enzyme specificity is detected for castanospermine. Taking this into account, Garcia-Fernandez and co-workers[23,146] have synthesised castanospermine analogues starting from OZTs anchored on carbohydrate scaffolds (Scheme 68) and other imino sugar analogues have also been synthesised *via* an OZT moiety (Scheme 69).

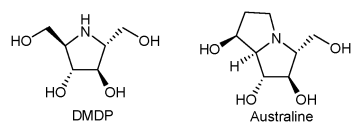
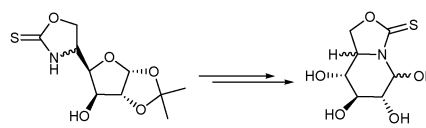
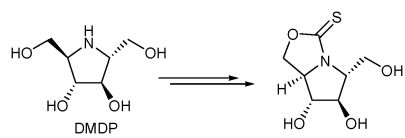


Fig. 7



Scheme 68



Scheme 69

5. Conclusions

In summary, it was shown that a thionocarbamate function can be connected on carbohydrate scaffolds according to three different possibilities: fused, anchored and spiro. Each linkage can be selectively obtained depending on the synthetic pathway and/or the carbohydrate backbone.

Among chiral auxiliaries, 1,3-oxazolidine-2-thiones (OZTs) have attracted important interest thanks to there various applications in different synthetic transformations. These simple structures, directly related to the well-documented Evans' oxazolidinones, have been explored in asymmetric Diels-Alder reactions and asymmetric alkylations (*N*-enoyl derivatives), but mainly in condensation of their *N*-acyl derivatives on aldehydes. Those have shown interesting characteristics in anti-selective aldol reactions or combined asymmetric addition. Normally, the use of chiral auxiliaries which can accomplish chirality transfer with a predictable stereochemistry on new generated stereogenic centers, are indispensable in asymmetric synthesis. The use of OZTs as chiral copula has proven efficient and especially useful for a large number of stereoselective reactions. In addition, OZT heterocycles are helpful synthons that can be specifically functionalized.

Carbohydrate-based OZTs can easily undergo regioselective S-alkylation: For example, S-benzylation allowed sulfur activation and appeared as particularly useful in condensations with 1,2-aminoaromatic acids to produce nucleoside precursors and in the exploitation of Suzuki and Stille Pd cross-coupling reactions. For both Suzuki and Stille modified reactions, the success of the direct Pd catalysed, Cu(I)-mediated carbon-carbon cross-coupling depends on the aromatic/non aromatic nature of the ring. The development and generalization of a new modified Sonogashira cross coupling reaction, in which copper(I) is used in catalytic amount, allowed the formation of alkynyl C–C bonds, using for the first time thionocarbamates as electrophiles.

There are many attractive applications for OZTs anchored on carbohydrate scaffolds, not only as tools in synthesis but also as promising biological entities. These molecules can be used as intermediates in the original synthesis of 'artificial' nucleosides, imino sugar analogues or in the study of the fructose transporter, GLUT5, aiming at a deeper knowledge of biological mechanisms involved in a variety of diseases.

Acknowledgements

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Recent advances in nitro sugar chemistry

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This chapter reports the most relevant recent contributions in nitro sugar chemistry. It includes the following aspects: preparation of nitro sugars, formation of carbon–carbon bonds and transformation of the nitro group into other functionalities.

1. Introduction

Nitro sugars are valuable compounds and synthetic intermediates of growing chemical interest that have been known for more than forty years. Thus, the first revision on nitro sugar chemistry was published in 1969[1] and the advances in this field during the seventies and the early eighties were reviewed in 1986.[2]

Initially, nitro sugars were mainly used for the preparation of the corresponding amino sugars, due to their biological importance. Later on, some biologically active nitro sugars were reported,[3] and it was discovered that the nitro group mimics the carboxyl group in several biological systems.[4]

In the last twenty years, nitro sugars became powerful chemical tools on account of their usefulness for the construction of carbon–carbon bonds prior to the transformation of the nitro group into a variety of other chemical functionalities. As a result, a diverse range of funcionalized carbohydrates and other derivatives as carbasugars, cyclitols and heterocycles have been prepared.

This chapter includes the most relevant recent contributions to the preparation and synthetic applications in nitro sugar chemistry.

2. Preparation of nitro sugars

The importance of the chemistry and biochemistry of nitro sugars has sparkled a great interest in their preparation. Their synthesis was reviewed by Wade and Giuliano in 1990[5] and since then many synthetic efforts have been made to efficiently prepare several kinds of nitro sugars.

Nitro sugars are mainly prepared by oxidation, nucleophilic substitution and aldol- or Michael-type condensations.

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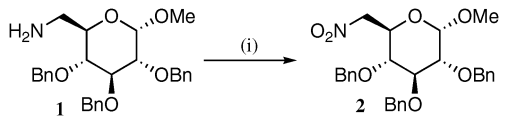
2.1 Oxidation of other nitrogenated sugar derivatives

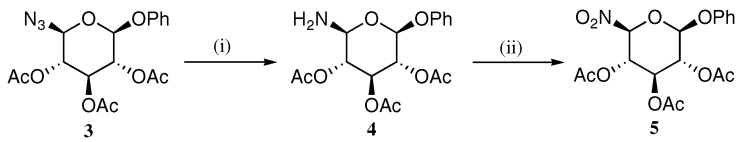
Oxidation is a general method for the preparation of nitro sugars from other sugar derivatives, as amino or azido sugars and sugar oximes.

Amino sugars can usually be oxidized to the corresponding nitro sugars using *m*-chloroperbenzoic acid (MCPBA).[6] Thus, Kobertz *et al*. synthesized nitro sugar **2** from amino sugar **1** by treatment with MCPBA in 1,2-dichloroethane under reflux (Scheme 1).[7]



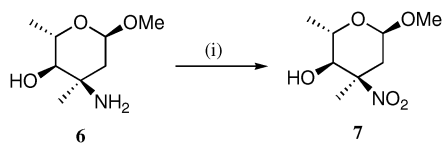
Scheme 1 *Conditions*: (i) MCPBA, $ClCH_2CH_2Cl$, reflux.

Although this is a common method for the preparation of nitro sugars, several other oxidizing agents have been employed. Thus, in 1998 Giuliano *et al*. reported the oxidation of amino to nitro groups in methyl-branched amino sugars by treatment with excess dimethyldioxirane (DMDO).[8] This procedure was used in 2002 by Rye *et al*. for the conversion of azido sugar **3** into nitro sugar **5** *via* the corresponding amine **4** (Scheme 2).[9]



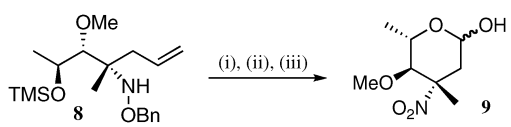
Scheme 2 *Conditions*: (i) PtO_2, H_2 (ii) DMDO, acetone.

Giuliano *et al*. have also reported a procedure for the oxidation of tertiary amino sugars to the corresponding nitro sugars, using ozone and OXONE as oxidizing agents (Scheme 3).[10]



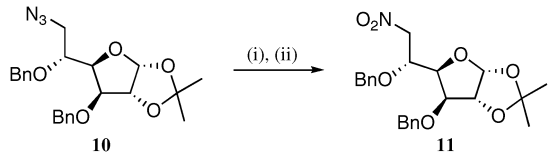
Scheme 3 *Conditions*: (i) O_3, OXONE, $HNaCO_3$, acetone.

Nitro sugars can also be prepared by oxidation of hydroxylamines. This strategy was used in 1999 by Nicolau *et al*. for the preparation of nitro sugar **9**, a component of the natural occurring antibiotic everninomicin,[11] that was obtained by ozonolysis of compound **8** followed by sequential treatment of the resulting compound with TFA and triphenylphosphine (Scheme 4).



Scheme 4 *Conditions*: (i) O_3, iC_8H_8/CCl_4, −78 °C (ii) TFA, −78 to 25 °C (iii) Ph_3P, −78 to 25 °C.

Azido sugars can be easily obtained by S_N2 reaction of triflates, mesylates or tosylates with sodium azide.[12] The conversion of azido sugars into nitro sugars is then another useful procedure for the preparation of nitro sugars. This procedure, due to Corey *et al*., requires the treatment of an azide with triphenylphosphine followed by ozonolysis of the resulting phosphorous imine, in order to afford the nitro sugar.[13] This approach was recently used for the preparation of the D-glucose derived nitro sugar **11**, a valuable intermediate for the preparation of cyclopentane based β-amino acids (Scheme 5).[14]

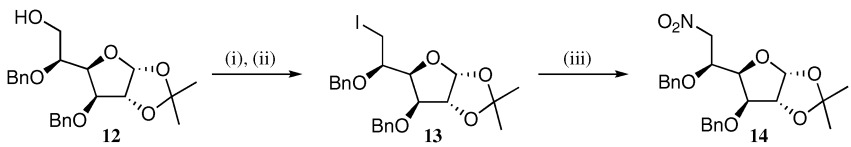


10 **11**

Scheme 5 *Conditions*: (i) PPh_3, CH_2Cl_2 (ii) O_3, CH_2Cl_2.

2.2 Nitrite displacement of iodosugars

A nitro group can alternatively be introduced in a sugar by nucleophilic displacement of iodide by a nitrite ion. This procedure was recently used in the preparation of the L-idose derived nitro sugar **14**, an intermediate in the synthesis of polyhydroxylated cispentacin analogues (Scheme 6).[15]



12 **13** **14**

Scheme 6 *Conditions*: (i) ClTs, Py (ii) NaI, acetone, reflux (iii) $NaNO_2$, phloroglucinol, DMSO.

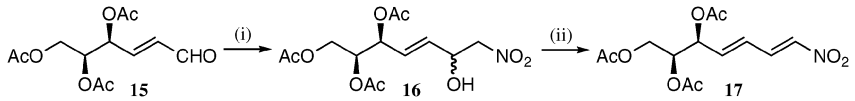
This approach to nitro sugars is limited to primary iodo sugars and gives poor results when the carbon bearing the iodo substituent is sterically hindered.

2.3 Nitroaldol (Henry) reaction of nitro alkanes with sugar aldehydes

The base-catalyzed reaction of nitroalkanes and sugar-based aldehydes (the Henry reaction) is one of the most common procedures for the lengthening of the carbon skeleton of a carbohydrate.[16] The mild reaction conditions required for the formation of C–C bonds by this method are usually compatible with most of the protective groups and masked functionalities involved in multistep synthesis from sugars.[17]

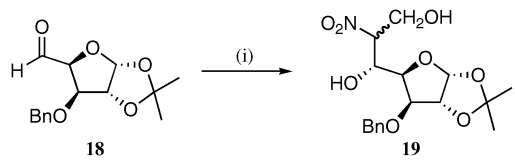
Henry reactions of nitro sugars can be promoted by catalytic amounts of mild bases and it has widely extended the use of triethylamine. A recent example involves the condensation of sugar derived α,β-unsaturated aldehyde **15** with nitromethane in the presence of triethylamine, to give

nitroaldol **16**.[18] Acetylation and subsequent elimination afforded the conjugated nitroalkene **17** (Scheme 7).



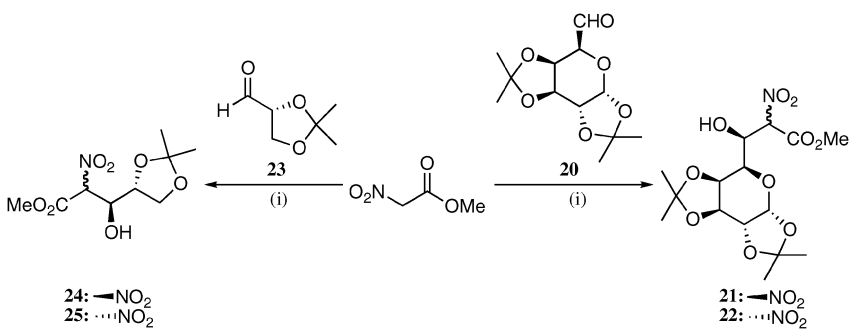
Scheme 7 *Conditions*: (i) CH_3NO_2, Et_3N (ii) Ac_2O, Py.

Kambe and Yasuda[19] discovered the effectiveness of the fluoride as a Henry reaction catalyst. Since then, the use of diverse fluorides is a common method to catalyze nitro aldol condensations, since they provide mild conditions that are particularly convenient for sensitive products as carbohydrates. The most common fluorides are potassium fluoride[20] and tetrabutyl ammonium fluoride.[21] A recent example relates to the TBAF catalyzed addition of nitroethanol to the D-glucose derivative **18**, to give nitro sugars **19** (Scheme 8).[22]



Scheme 8 *Conditions*: (i) $NO_2CH_2CH_2OH$, TBAF, THF.

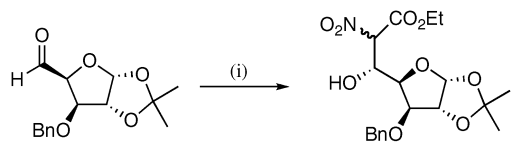
The discovery of the hetereogeneous phase catalyzed nitroaldol condensations found immediate applications in carbohydrate chemistry, due to the very mild conditions required, which are usually compatible either with acid and base sensitive protecting groups. These heterogeneous phase conditions were first introduced by Hanessian *et al.*[23] for the Henry reaction of carbohydrates and used in 1995 by Gómez-Guillén *et al.*[24] in their studies on the condensation of methyl nitroacetate with several sugar aldehydes, catalyzed by silica gel. Thus, the Henry reaction of methyl nitroacetate with aldehyde **20** afforded isomers **21** and **22**. On the other side, the nitro aldol condensation of methyl nitroacetate and 2,3-*O*-isopropyliden-D-glyceraldehyde (**23**) generated the 2-epimeric-3*S*-nitroalcohols **24** and **25** (Scheme 9).



Scheme 9 *Conditions*: (i) SiO_2.

The classical Henry reaction conditions (base catalyzed addition) have some drawbacks: sometimes the nitro alcohols are obtained in low yields and diastereoselectivities are not always high. To improve these results, a number of modifications were introduced. One of them is the Seebach's silyl nitronate method,[25] that involves a reaction between an aldehyde with a silyl nitronate prepared by metalation of the corresponding nitro alkane with LDA, followed by reaction of the resulting nitronate with *tert*-butyldimethylsilyl chloride.[26]

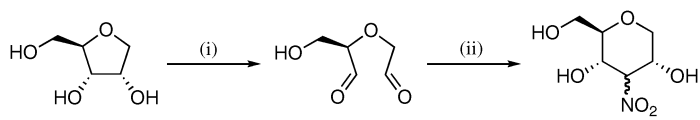
However, this multistep procedure is experimentally complex. A simpler variation described in 1991[27] consists of the reaction of an aldehyde and a nitro compound in the presence of triethylamine, TBAF and *tert*-butyldimethylsilyl chloride. Under these conditions, nitro sugars are obtained in good yieds and higher diastereoselectivities than those afforded by the standard conditions. This procedure was used in several synthesis of 2-nitro-2-deoxyaldoses, as for the condensation of 1,1-diethoxy-2-nitroethane and 1,2:3,4-di-*O*-isopropylidene-α-D-galacto-hexodialdo-1,5-piranose.[28] More recently, it was applied to the addition of ethyl nitroacetate to the D-glucose derived aldehyde **18**, to give nitro sugar derivatives **26**, key precursors of polysubstituted cyclohexane α-amino acids (Scheme 10).[29]



18 **26**

Scheme 10 *Conditions*: (i) $NO_2CH_2CO_2Et$, TBAF, Et_3N, TBDMSCl, THF.

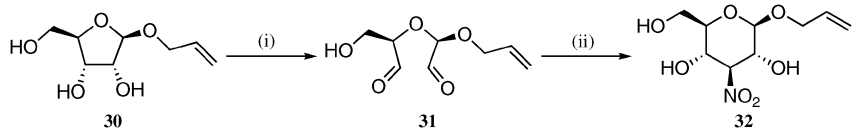
Reaction of nitromethane and monosaccharide-derived dialdehydes is a useful tool that has been broadly used for the preparation of nitro and amino sugars, and carbocycles.[30] Dialdehydes can easily be obtained by oxidative cleavage of conveniently protected monosaccharides with sodium periodate. Their subsequent Henry reaction with a nitroalkene, commonly nitromethane, usually gives isomeric mixtures that require the isolation of the major isomer.[31] Thus, treatment of the D-ribose derivative **27** with sodium periodate gave dialdehyde **28**, which was subjected to a Henry reaction with nitromethane, to afford nitrosugar **29** as an epimeric mixture (Scheme 11).[32]



27 **28** **29**

Scheme 11 *Conditions*: (i) $NaIO_4$ (ii) CH_3NO_2, Et_3N.

More recently, a similar transformation was used for the preparation of the nitro sugar derivative **32**, a precursor of a carbohydrate mimic of 2-deoxystreptamine (Scheme 12).[33]



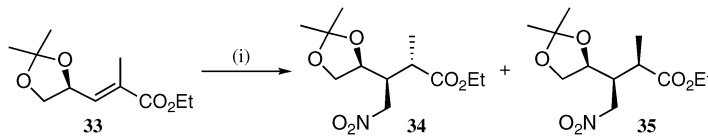
30 31 32

Scheme 12 *Conditions*: (i) $NaIO_4$, H_2O (ii) CH_3NO_2, NaOMe.

2.4 Michael addition of nitro alkanes to α,β-unsaturated carbonyl sugar derivatives

The Michael addition of nitroalkanes to sugar-based α,β-unsaturated carbonyl compounds is another powerful method for the preparation of nitro sugar derivatives.

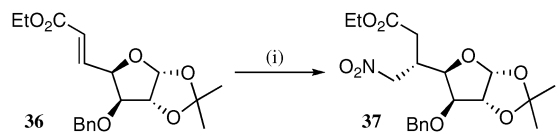
After their preliminary studies on the conjugate addition of nitromethane to sugar-derived enoates,[34] Costa *et al.* reported more exhaustive studies that include the addition of nitromethane to enoate **33** in the presence of TBAF, to give a mixture of the two adducts **34** and **35** (Scheme 13).[35]



33 34 35

Scheme 13 *Conditions*: (i) CH_3NO_2, TBAF, THF.

In 2008, Chakraborty *el al.* reported that Michael addition of nitromethane to the sugar olefinic derivative **36** provided the sugar-based β-nitro acid ester **37** (Scheme 14), which was used as starting material for the preparation of a novel type of cyclohexyl amines.[36]

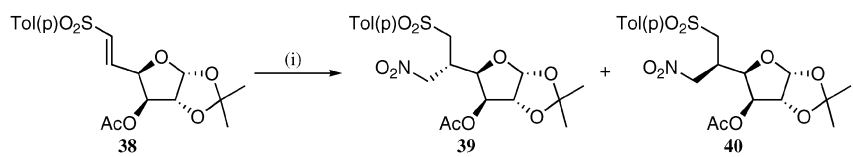


36 37

Scheme 14 *Conditions*: (i) CH_3NO_2, K_2CO_3, EtOH.

Michael addition of nitromethane to D-glucose based vinyl sulfone **38** to afford nitro sugars **39** and **40** was also recently described (Scheme 15).[37]

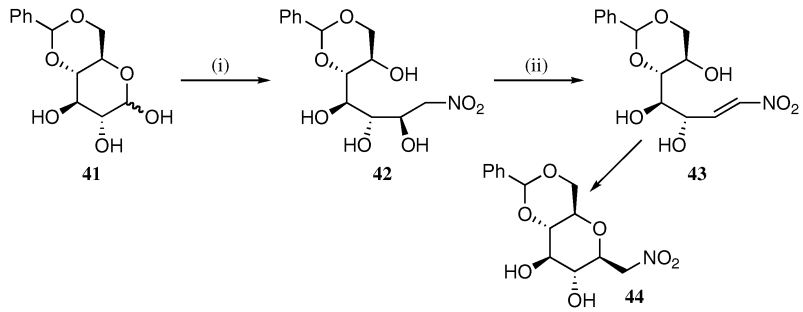
The old condensation[38] of 4,6-*O*-benzylidene-D-glucose with nitromethane in the presence of sodium methoxide to give D-heptitol **42** and cyclic nitromethyl β-D-glucopiranose (**44**, 5% yield) has recently been revisited.



38 39 40

Scheme 15 *Conditions*: (i) CH_3NO_2, NaH, 1,4-dioxane.

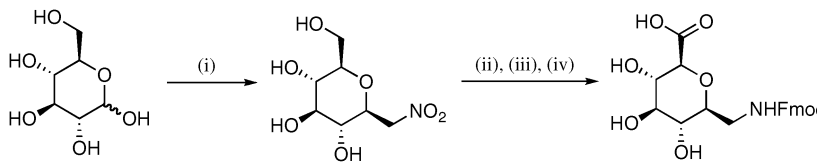
New conditions (aprotic solvents and highly active bifunctional catalysts 2-hydroxypyridine and DBU/molecular sieves) were found, that allowed a 70% yield of **44** to be obtained in a single step (Scheme 16).[39]



Scheme 16 *Conditions*: (i) CH_3NO_2, 2-hydroxypyridine, DBU/molecular sieves (ii) $-H_2O$.

This sequence involves a Henry reaction of **41** with nitromethane followed by a dehydration of the resulting acyclic D-heptitol derivative **42** and a Michael intramolecular addition of the resulting nitroolefin **43**.

A similar protocol was used by Prof. Gervay-Hage in a recent synthesis of uronic acids (Scheme 17).[40]



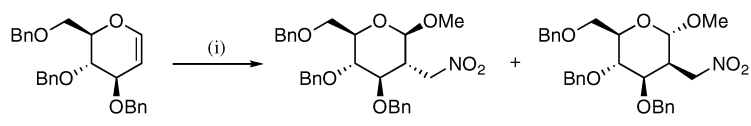
Scheme 17 *Conditions*: (i) CH_3NO_2, NaOMe, MeOH (ii) H_2, Pd/C, MeOH (iii) FmocCl, 10% Na_2CO_3 (vi) TEMPO, NaOCl.

Treatment of D-glucose with nitromethane and sodium methoxide afforded nitrosugar **46** which, on reduction of the nitro group, protection of the resulting amine with Fmoc-Cl and TEMPO oxidation afforded **47** in a three-step sequence.

2.5 Radical mediated methods

Although the generation of nitroalkyl radicals by oxidative transition-metal-mediated reactions is known for many years, their application in carbohydrate chemistry was not investigated until recently.[41]

The first addition of nitroalkanes to glycals was recently described. It involved a radical mediated addition of nitromethane to tri-*O*-benzyl-D-glucal (**48**) in the presence of CAN and KOH and methanol as solvent, to give the diastereomeric mixture **49** + **50** (Scheme 18).[42]

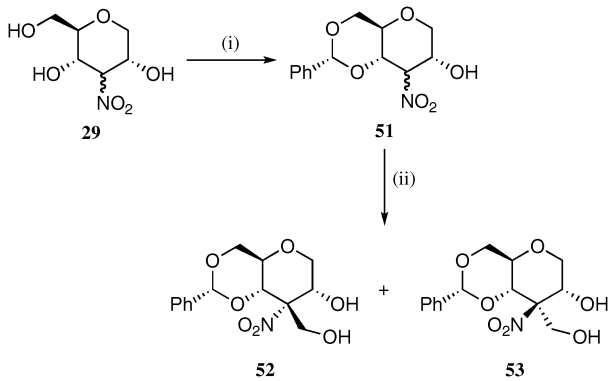


Scheme 18 *Conditions*: (i) CH_3NO_2, KOH, CAN, MeOH, 0 °C.

3. Reactivity of nitro sugars

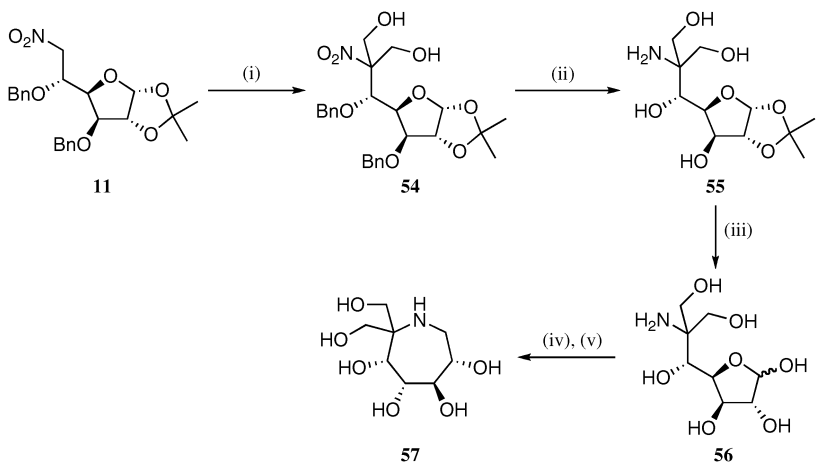
3.1 Henry reaction

Nitroaldol reactions between nitro sugars and aldehydes have been used for the preparation of branched-chain nitro sugar derivatives. Thus, Hossain *et al*. have prepared *C*-branched nitro hexitols **52** and **53** by a synthteic route starting from nitro sugar **29**, which was prepared by Henry reaction as depicted in Scheme 11 (page 5). It involves a protection with benzaldehyde followed by a nitroaldol reaction of the resulting epimeric mixture **51** with formaldehyde, catalyzed by tetramethyl guanidine. The resulting compounds **52** and **53** were used as the starting materials for the synthesis of various modified anhydrohexitol nucleosides (Scheme 19).[43]



Scheme 19 *Conditions*: (i) PhCHO, $ZnCl_2$ (ii) HCHO, TMG.

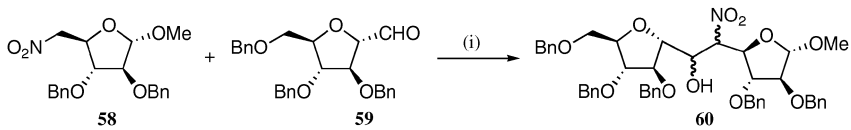
Henry reaction of nitro sugar **11** with formaldehyde allowed the introduction of two hydroxymethyl groups at the carbon bearing the nitro group, and hence opened a specific route for the preparation of branched-chain imino sugar **57** and analogues (Scheme 20).[44]



Scheme 20 *Conditions*: (i) $(HCHO)_n$, TBAF, THF (ii) NH_4HCO_2, Pd black, MeOH, 50 °C (iii) TFA/H_2O (iv) $NaHCO_3$, THF, 40 °C (v) $NaCNBH_3$, AcOH, MeOH.

Thus, treatment of **11** with paraformaldehyde and TBAF in THF provided the novel branched-chain nitro sugar **54**. Reduction of its nitro group to the amino group of **55** followed by deprotection of its anomeric position, resulted in the amine **56**, that finally led to compound **57**, the first reported branched-chain seven-membered imino sugar, after a synthetic sequence consisting of a reductive amination.

The Henry reaction of nitro sugars and sugar aldehydes is a powerful tool for the formation of *C*-glycosydic bonds. A recent example is the preparation of disaccharide precursor **60** stated in Scheme 21.[45] It involves a Henry reaction of nitro sugar **58** and aldehyde **59** in acetonitrile in the presence of catalytic potassium fluoride, to give nitro alcohol **60**, a precursor of the corresponding *C*-disaccharide.



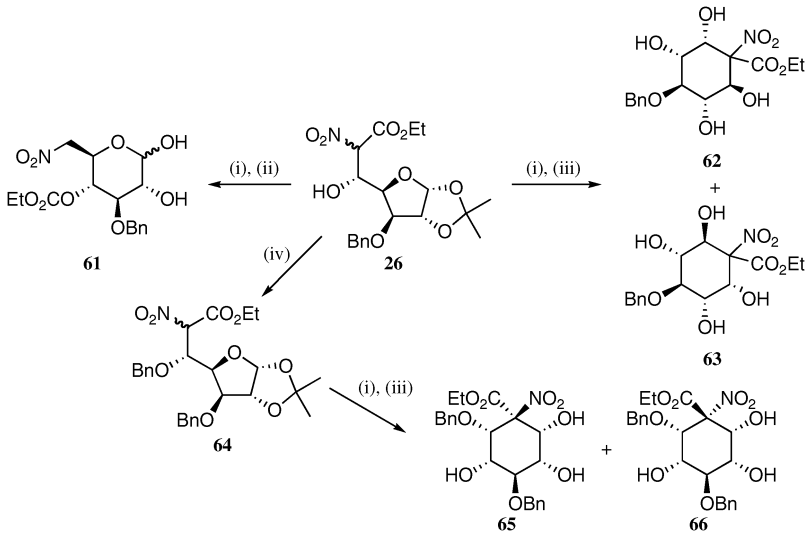
Scheme 21 *Conditions*: (i) KF, 18-crown-6, MeCN.

Intramolecular nitroaldol reactions are a useful choice for the conversion of sugars into polyhydroxylated nitro cyclopentanes, nitro cyclohexanes and their derivatives.[46] Baer *et al*. in the course of their studies on the cyclization of 6-deoxy-6-nitrohexoses under kinetic and thermodynamic control,[47] established the reaction pathway involved in the formation of nitroinositols mediated by intramolecular Henry reactions. Firstly, a nitronate is formed and then, under thermodynamic control conditions, an epimerization occurs before cyclization. But, under kinetic controlled conditions, the cyclization occurs first.[48]

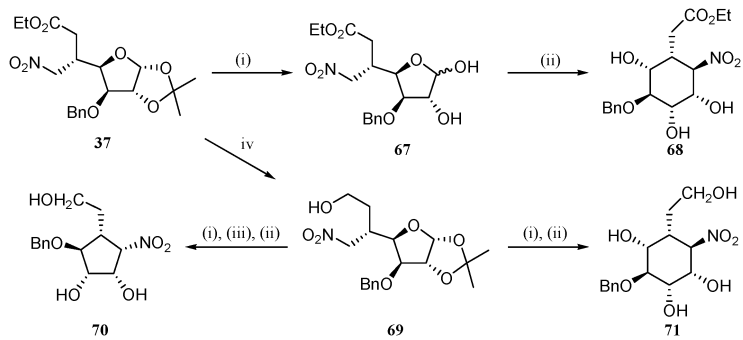
Intramolecular Henry reactions of nitro sugars are usually diastereoselective; sometimes a single isomer results from an epimeric mixture, on account of the reversivility of the Henry reaction, which allows equilibration through open chain intermediates.[49] A recent example involving the D-glucose derived nitro acid ester **26** is shown in Scheme 22.

Removal of the acetonide protecting group of **26** by treatment with a 1:1 TFA/water solution, gave an unstable mixture which was directly subjected to Henry reaction conditions. The outcome of this reaction indicated that the results are strongly influenced by the base used in the reaction. Thus, when TBAF was the base, surprisingly, nitro sugar derivative **61** was obtained, as a result of a C→O migration of the carbethoxyethyl group.[50] But when 2% aqueous sodium bicarbonate was the base, a racemic mixture of ethyl α-nitrocyclohexane carboxylates **62** and **63** resulted. Moreover, when the hydroxy group at C-5 position of compound **26** was protected as a benzyloxy to prevent racemization, the intramolecular nitroaldol reaction now produced a 2:1 separable mixture of epimers **65** and **66**.[51]

Another recent example supporting the influence of the nature of the base in intramolecular Henry reactions in nitro sugars is depicted in Scheme 23. The intramolecular nitroaldol cyclization of compound **67** using alkoxides, hydroxides or carbonates gave unsatisfactory results, but when this compound and DABCO (3 eq.) were refluxed in benzene, surprisingly, only



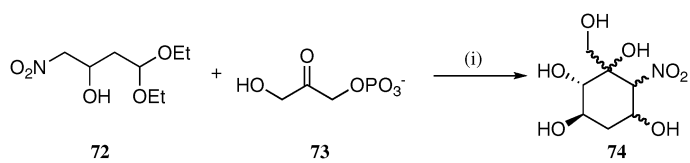
Scheme 22 *Conditions*: (i) TFA/H_2O (ii) TBAF, THF (iii) 2% aq. $HNaCO_3$ (iv) 2,2,2-benzyltrichloroacetimidate, TfOH, C_6H_{12}, CH_2Cl_2.



Scheme 23 *Conditions*: (i) TFA/H_2O (ii) DABCO, benzene, reflux (iii) $NaIO_4$ (iv) LAH, THF.

diastereomer **68** was obtained in high yield. This remarkable diastereoselectivity was also observed in the cyclization of the hemiacetal derived from **69** leading to the six-membered carbocycle **71** or the five-membered carbocycle **70**, obtained when oxidative cleavage of C1–C2 bond occurred prior to the treatment with DABCO.[52]

Recently, a chemoenzimatic catalized Henry reaction has been reported by El Blidi *et al.*[53] Nitroaldol cyclization between the masked 3-hydroxy-4-nitrobutyraldehyde **72** and dihydroxyacetone phosphate (DHAP) **73**, catalyzed by fructose-1,6-biphosphate aldolase (RAMA), afforded the nitrocyclohexane **74** (Scheme 24).



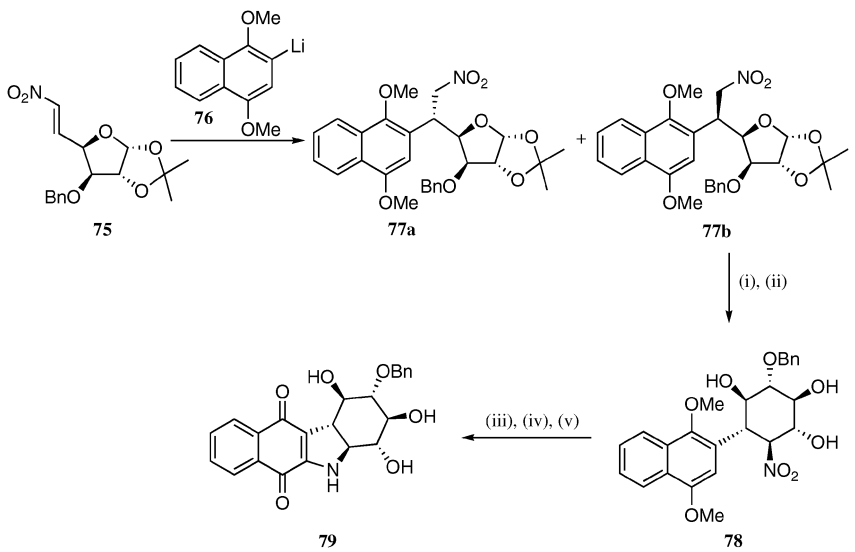
Scheme 24 *Conditions*: (i) fructose-1,6-biphosphate aldolase.

3.2 Michael reaction

Michael additions to sugar nitro olefins have received considerable less attention than the Henry reaction involving nitro sugars. Early examples of Michael addition include the synthesis[54] of licoricidin by Paulsen in 1982, the addition of phosphorous nucleophiles to a D-glucose based nitro olefin[55] by Yamashita in 1987 and the synthesis of the natural antibiotic polyoxin published by Barret in 1990.[56]

Usually, the diastereoselectivity in Michael additions is the one predicted by the Felkin-Anh model.[57] However, it was discovered that in the case of the addition of highly hindered nucleophiles, as potassium phthalimide and succinimide, the major product has the opposite configuration to the one predicted by this model, because of the presence of steric hindrance interactions.[58]

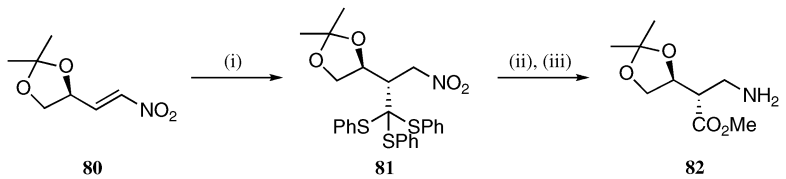
Michael addition of 1,4-napthoquinones[59] to sugar nitro olefins was shown to proceed in excellent yield and high diastereoselectivity,[60] as examplified by the recently reported synthesis of polyhydroxylated hexahydrobenzo[*b*]carbazoldione **79** (Scheme 25).[61] It involves a Michael addition of the lithium salt of the masked naphthoquinone **76** to the nitroolefine **75**, to give a 5:1 epimeric mixture of nitrocompounds **77a** and **77b**. Removal of the acetonide of **77b** followed by intramolecular nitroaldol cyclization gave only nitro cyclohexane **78**, which was transformed into tetrahydroxyhexahydrobenzo[*b*]carbazoldione **79**, according to the reaction sequence stated in Scheme 25.[62]



Scheme 25 *Conditions*: (i) Dioxane/TFA/H_2O (3:1:2), 50 °C (ii) 2% aq. $NaHCO_3$ (iii) H_2, Ni-Raney, MeOH (iv) CAN, CH_3CN, H_2O (v) TFA, 60 °C.

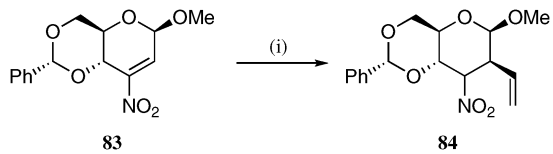
Addition of the lithium salt of tris(triphenylthio)methane to the D-glyceraldehyde-derived nitroolefin **80** resulted in a stereoselective Michael addition leading to adduct **81**, which was then converted into the novel

β-amino acid ester **82** (Scheme 26).[63] This protocol constitutes a new promising approach to the synthesis of β-amino acids.



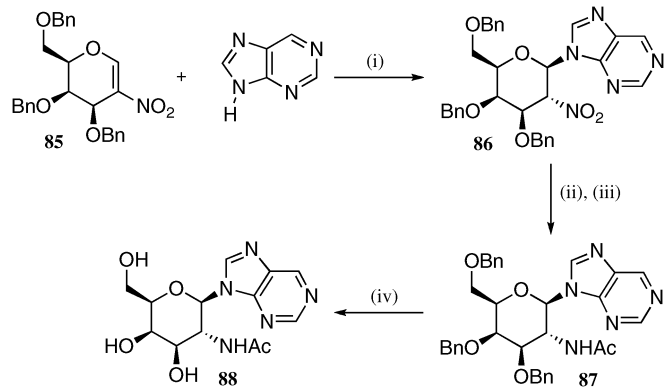
Scheme 26 *Conditions*: (i) $CH(SPh)_3$, *n*-BuLi, THF, −78 °C; (ii) HgO, $BF_3 \cdot OEt_2$, $MeOH/H_2O/THF$; (iii) H_2, Pd/C, AcOEt.

Another recent example of a Michael addition of organometallic reagents to sugar nitroolefins consists of the addition of vinyl magnesium bromide to nitroolefin **83**, that afforded nitro sugar **84** (Scheme 27).[64]



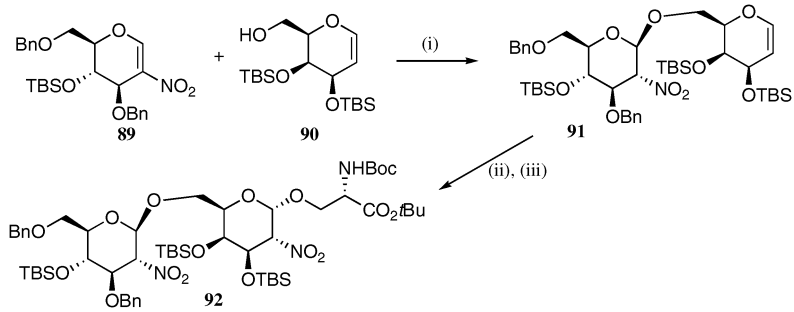
Scheme 27 *Conditions*: (i) $CH_2{=}CHMgBr$, THF $MeOH/H_2O/THF$; (iii) H_2, Pd/C, AcOEt.

2-Nitroglycals[65] are excellent Michael-type acceptors,[66] where *O*-, *N*-, *S*-, *C*- and *P*-nucleophiles can be used as donors.[67] An application of this kind of reaction was reported by Schmidt *et al*. for a new synthesis of 2-deoxy-2-nitro-D-galactose nucleoside **86** and of *N*-acetyl-D-galactosamine nucleoside **88**, based on addition reactions to 3,4,6-tri-*O*-benzyl-2-nitro-D-galactal **85** (Scheme 28).[68]



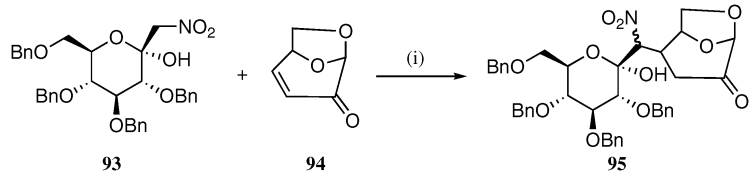
Scheme 28 *Conditions*: (i) DBU, THF; (ii) Raney Nickel, H_2, EtOH; (iii) Ac_2O, Py; (iv) $Pd(OH)_2/C$, H_2, THF.

The 2-nitroglycal chemistry has also recently been used for the synthesis of mucin core structures and derivatives (Scheme 29).[69]



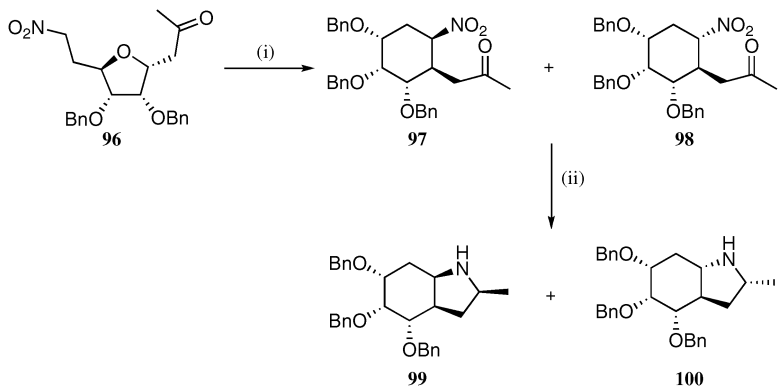
Scheme 29 *Conditions*: (i) KO*t*Bu; (ii) Ac_2O, HNO_3, Et_3N, CH_2Cl_2; (iii) Boc-L-Ser-O*t*Bu, KO*t*Bu.

A salient example regarding a Michael addition of nitronates to α,β-unsaturated carbonyl compounds is the stereoselective addition of glucosyl nitromethane **93** to levoglucosenone (**94**) in the presence of tetramethylguanidine (TMG) (Scheme 30).[70]



Scheme 30 *Conditions*: (i) TMG, MeCN.

More recently, an efficient transformation of 6-nitro-*C*-glycofuranosyl derivative **96** into indoline derivatives **99** and **100** has been reported. Treatment of **96** with a base (4% NaOMe or 1% K_2CO_3 in MeOH) at room temperature produced nitro cyclohexanols **97** and **98**, *via* β-elimination followed by an intramolecular Michael addition (Scheme 31).[71]



Scheme 31 *Conditions*: (i) 4% NaOMe or 1% K_2CO_3; (ii) Zn, AcOH.

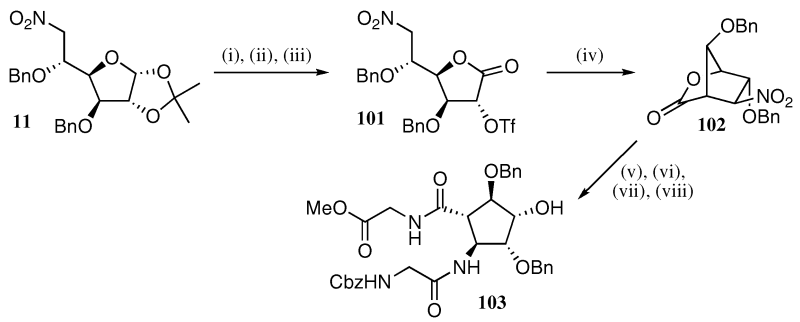
3.3 Alkylation of nitronates

Alkylation of nitronates is a complex chemical reaction. Due to the ambident character of the nitronate anion, nitronates can be alkylated either at the carbon or at the oxygen atoms. The mechanism of this reaction

has been studied extensively[72] and the factors influencing the *C*- or *O*-alkylation of ambident anions has been reviewed by le Noble.[73]

In general, nitronates undergo *O*-alkylation. However, some procedures have been developed that overcome the preference for *O*-alkylation in favour of *C*-alkylation. For example, the double deprotonated nitronates introduced by Seebach[74] can be *C*-alkylated and this strategy was used for the synthesis of nitro furanosyl and pyranosyl compounds.[75]

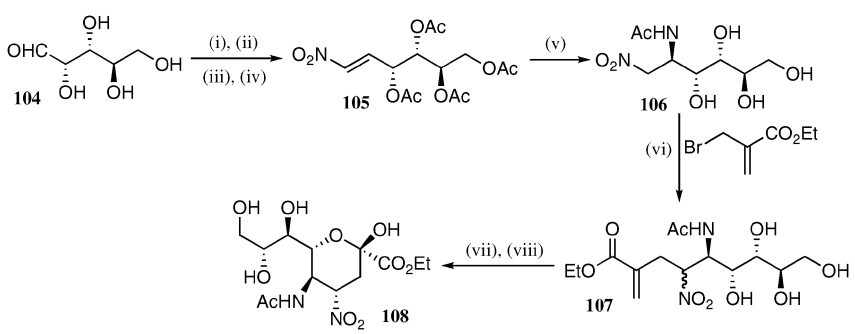
C-Alkylation has also been observed in intramolecular reactions where the *O*-alkylation is not favoured.[76] This property has been recently exploited in a stereocontrolled intramolecular cyclization of nitronate of nitro sugar **101** (Scheme 32), that provided the novel bicyclic nitrolactone **102**, a synthetic precursor of tripeptide **103**, that includes the first reported polyhydroxylated cyclopentane β-amino acid.[77]



Scheme 32 *Conditions*: (i) TFA/H_2O; (ii) Br_2, $BaCO_3$, dioxane/H_2O; (iii) TfO_2, Py, CH_2Cl_2; (iv) TBAF, THF; (v) H_2, Raney Ni, MeOH; (vi) $ClCO_2Et$, Cbz-Gly, Et_3N, DMF, MeOH; (vii) $NH_2NH_2xH_2O$, MeOH; (viii) *t*-$BuNO_2$, HCl, Gly-OMe, Et_3N, DMF, dioxane.

A sligth modification of this synthetic methodology allowed the development of a novel route to 4-amino-5-(hydroxymethyl)cyclopentane-1,2,3-triols, some of them display significant glycosidase inhibition properties. In addition, a similar approach starting from L-idose allowed the first polyhydroxylated cispentacine to be prepared.[78]

In another recent example, the D-*arabino*-tetraacetoxy-1-nitrohex-1-ene **105** was obtained in three steps (Scheme 33). The reaction sequence began



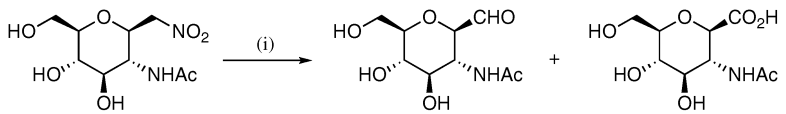
Scheme 33 *Conditions*: (i) CH_3NO_2, NaOMe/MeOH; (ii) Amberlite H^+; (iii) Ac_2O, cat. H_2SO_4; (iv) $NaHCO_3$ (s), benzene, reflux; (v) sat. NH_3/MeOH, 0 °C to rt; (vi) NaOMe/MeOH; (vii) O_3, CH_2Cl_2, MeOH, –78 °C; (viii) DMS, –78 °C to rt, fractional crystallization.

with a base promoted Henry reaction of D-arabinose with nitromethane, after which peracetylation followed by elimination of OAc gave nitrohexene **105**. The subsequent Michael addition of NH_3 to the double bond of **105** provided the acetamidonitromannitol **106**, which upon treatment with ethyl α-(bromomethyl)acrylate in NaOMe/MeOH, gave the coupled enoate ester **107**-(*R*,*S*), as a result of the *C*-alkylation of the nitronate of **106**. This compound was finally converted into ethyl 4-deoxy-4-nitrosialate **108**.[79]

3.4 Conversion of the nitro group in other functionalities

3.4.1 The Nef reaction. The Nef reaction consists of the conversion of a nitro group into a carbonyl group.

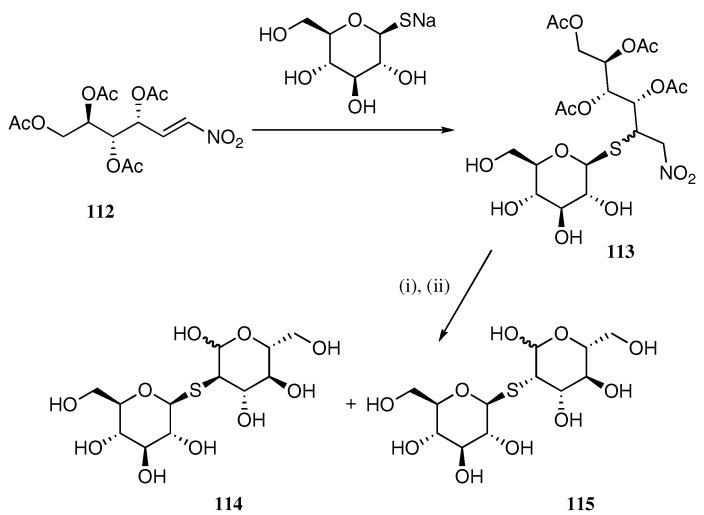
The addition of nitromethane to the carbonyl function of a sugar followed by the conversion of the nitro into a carbonyl group is a classical strategy for the elongation of a sugar carbon chain.[80] In a recent application, nitromethylation of *N*-acetyl-D-glucosamine afforded the corresponding nitromethyl derivative **109**, that when subjected to a Nef reaction provided *C*-glycosyl derivatives **110** and **111** (Scheme 34).[81]



109 **110** **111**

Scheme 34 *Conditions*: (i) NaOH, H_2O, O_3.

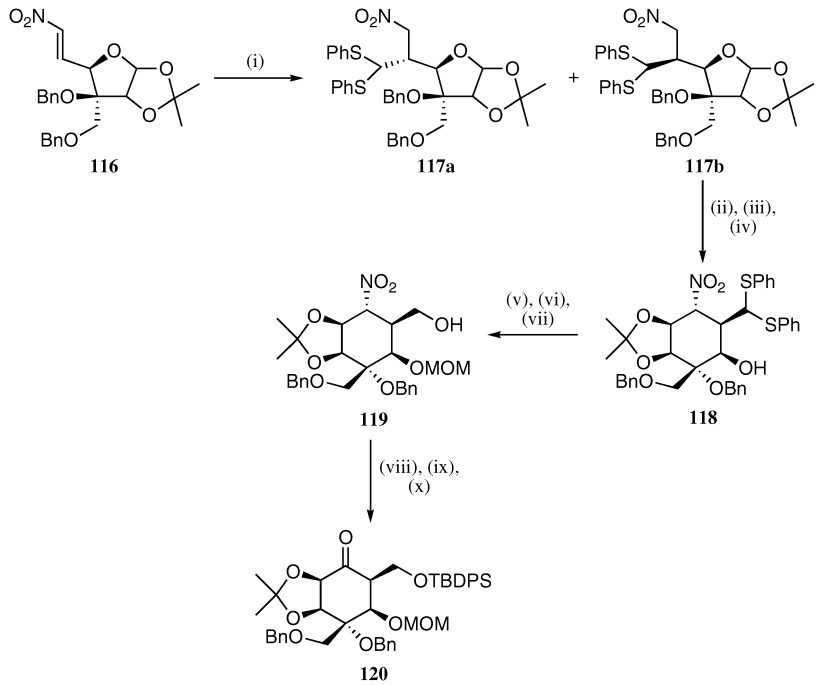
A Michael addition-Nef reaction sequence was used in a synthesis of disaccharides. Addition of the sodium salt of 1-thio-D-glucose to sugar nitroolefin **112** gave a mixture of isomers **113**. The subsequent deacetylation, followed by a Nef reaction, afforded the *S*-disaccharides **114** and **115** (Scheme 35).[82]



114 **115**

Scheme 35 *Conditions*: (i) AcCl, MeOH; (ii) H_2SO_4, NaOH, $Ba_2(CO)_3$.

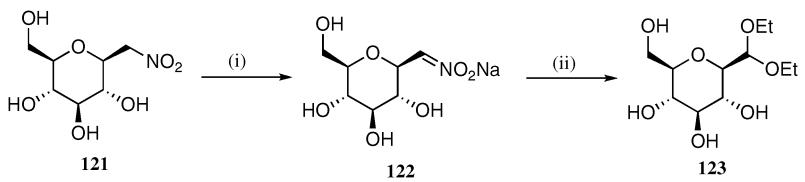
Recently, a synthesis of tetrodotoxin from D-glucose was described (Scheme 36). After a Michael addition of the lithium salt of bis(phenylthio)-methane to the nitroolefin **116**, the major component (**117b**) of the resulting epimeric mixture **117a** + **117b** was subjected to a reaction sequence that involved an intramolecular nitroaldol reaction, to give the complex nitro cyclohexane derivative **118**.



Scheme 36 *Conditions*: (i) $CH_2(SPh)_2$, *n*-BuLi, THF; (ii) 80% AcOH; (iii) $NaHCO_3$, $MeOH/H_2O$; (iv) DMP, PTSA; (v) $CH_2(OMe)_2$, P_2O_5, CH_2Cl_2; (vi) NBS, $MeCN/H_2O$; (vii) $NaBH_4$; (viii) TBDPSCl, Im, CH_2Cl_2; (ix) O_3, *t*-BuOK, toluene; (x) Zn, AcOH.

Finally, a Nef reaction allowed the generation of the carbonyl group of compound **119**.[83]

An extension of the Nef reaction of *C*-glycosylnitromethanes has recently been reported (Scheme 37).[84]



Scheme 37 *Conditions*: (i) NaOMe, MeOH; (ii) HCl, EtOH.

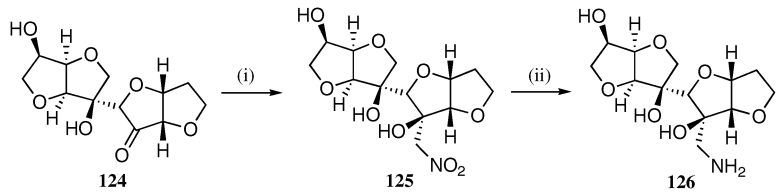
Protonated *aci*-nitro forms of *C*-glycosylnitromethanes that are resistant to the Nef reaction in aqueous acidic media undergo a modified Nef reaction in acidified ethanol. These reaction conditions allowed the

C-glycosylmethanal diethyl acetal of β-D-gluco configuration **123** to be obtained in moderate yield.[85]

3.4.2 Reduction of the nitro group

Nitro sugars have been broadly used as precursors of the corresponding amino sugars, most of them naturally occurring and biologically relevant compounds. The standard procedure involves a catalytic hydrogenation and several catalysts have been used for this purpose.

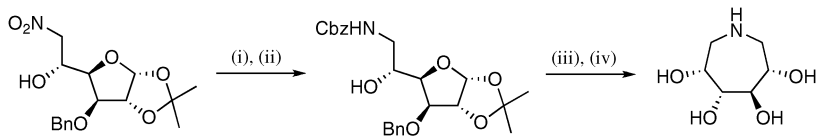
For example, the amino derivative of the higher carbon sugar **126** (a compound with promising biological activities) was recently prepared by Henry reaction of nitromethane and the higher carbon keto sugar **124**, followed by reduction of the nitro group to the amino group under catalytic hydrogenation using Pd/C as the catalyst (Scheme 38).[86]



124 125 126

Scheme 38 *Conditions*: (i) CH_3NO_2, Et_3N; (ii) H_2, Pd/C, MeOH.

Raney Nickel was also used as the catalyst for the reduction of nitro sugars to amino sugars by hydrogenation.[87] Under these conditions, nitro sugar **127** was reduced to amino sugar derivative **128**, without removal of the benzyl protecting group. Compound **128** was finally converted into azepane **129** (Scheme 39).[88]

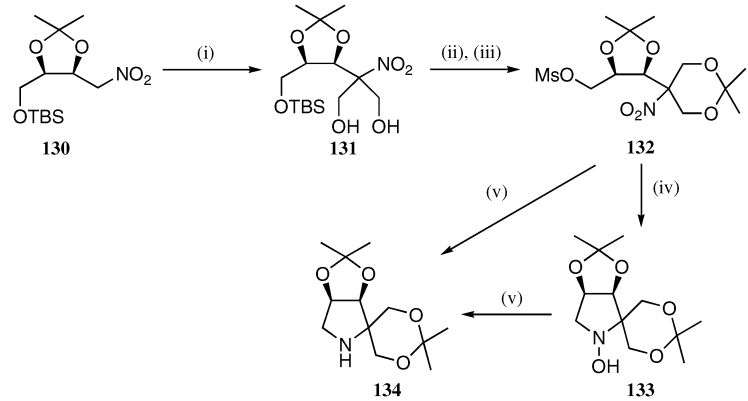


127 128 129

Scheme 39 *Conditions*: (i) H_2, Raney Ni; (ii) ClCbz, $NaHCO_3$; (iii) TFA/H_2O; (iv) H_2, Pd/C.

For the reduction of the nitro group, ammonium formate can be used instead of hydrogen.[89] The reaction is fast and the work-up requires a simple filtration, so this procedure has been widely used for the conversion of nitro to amino groups. A recent example deals with the synthesis of the new branched-chain imino sugar **134**. It starts with a double Henry reaction of nitro sugar **130** that allowed compound **132** to be obtained *via* compound **131**. Reduction of the nitro group of **132** to amino was followed by a concomitant cyclization leading to imino sugar derivatives **133** or **134** (Scheme 40).

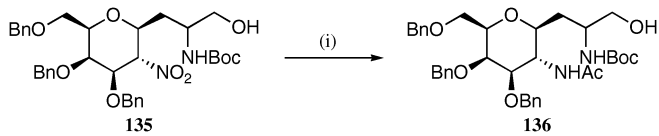
When the hydrogenation was carried out with palladium black and ammonium formate in methanol at room temperature, the compound isolated was the hydroxylamine **133**. Further hydrogenation of this



Scheme 40 *Conditions*: (i) $(HCHO)_n$, AcONa, THF; (ii) TBAF, THF; (iii) ClMs, Py; (iv) $COONH_4$, Pd black, r.t.; (v) $COONH_4$, Pd black, 50 °C.

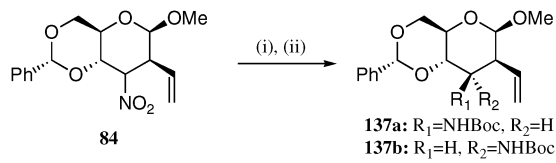
compound with palladium black and ammonium formate in methanol at 50 °C provided the corresponding imino sugar **134**. Under these conditions, nitro sugar **132** was directly converted to **134**.[90]

Nitro groups in nitro sugars can also be reduced using Zn/HCl, as in the case of the reduction nitro sugar **135** to amino sugar **136** (Scheme 41).[91]



Scheme 41 *Conditions*: (i) Zn/HCl, THF-AcOH.

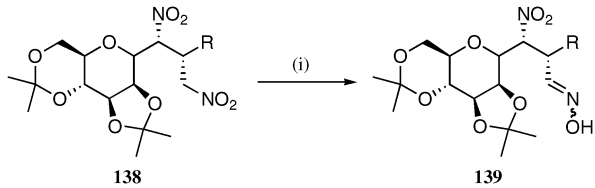
Recently, nitro sugar **84**, obtained by Michael addition of vinyl magnesiane to a sugar nitroolefin (Scheme 27, page 12), was reacted with lithium aluminium hydride and the resulting free amine was protected as NHBoc, to give a mixture of epimers **137a** and **137b** (Scheme 42).[92]



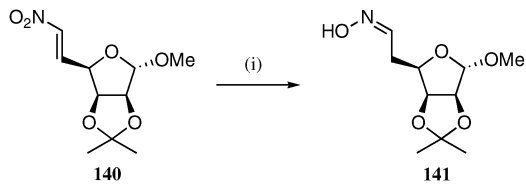
Scheme 42 *Conditions*: (i) $LiAlH_4$, THF; (ii) Boc_2O, Et_3N.

A primary nitromethyl group can be reduced to the corresponding oxime by a radical reaction with tin.[93] The reaction is remarkably selective for primary nitro groups over secondary groups (Scheme 43).[94]

In 2000, Koos described a procedure for the reduction of the nitroolefin **140** to 5-deoxy sugar oxime **141** using tin(II) chloride as a reducing agent (Scheme 44).[95]



Scheme 43 *Conditions*: (i) HBu_3Sn, ABCN.

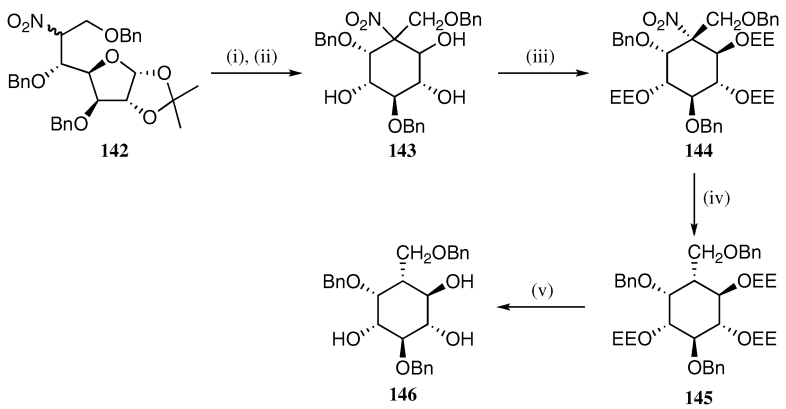


Scheme 44 *Conditions*: (i) $SnCl_2 \cdot 2H_2O$, AcOEt.

3.5 Substitution and elimination of the nitro group

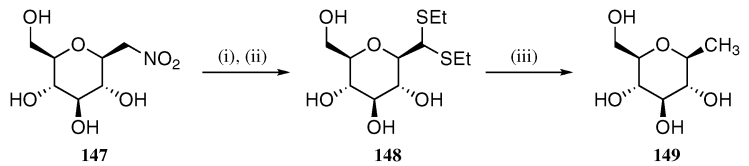
The nitro group can be displaced by electron transfer reactions ($S_{RN}1$)[96] or nucleophilic substitution processes.[97] Thus, some electron transfer reactions of 1-deoxy nitro sugars have been used in several synthesis of biologically important carbohydrates in the past.[98] But the most important radical substitution reaction of the nitro group in carbohydrate chemistry is by far the replacement of the nitro group by an hydrogen.[99] Typical examples include the synthesis of carbasugars from nitro sugars by intramolecular Henry reaction followed by removal of the nitro group.[100]

In a recent application of this strategy, nitrocyclohexane **143** (prepared from nitrosugar **142** by intramolecular Henry reaction) was subjected to a radical denitration by $HSnBu_3$, after protection of the hydroxyl groups to avoid side reactions. Inositol **146** was selectively obtained in good yield, once the hydroxyl protecting groups were removed (Scheme 45).[101]



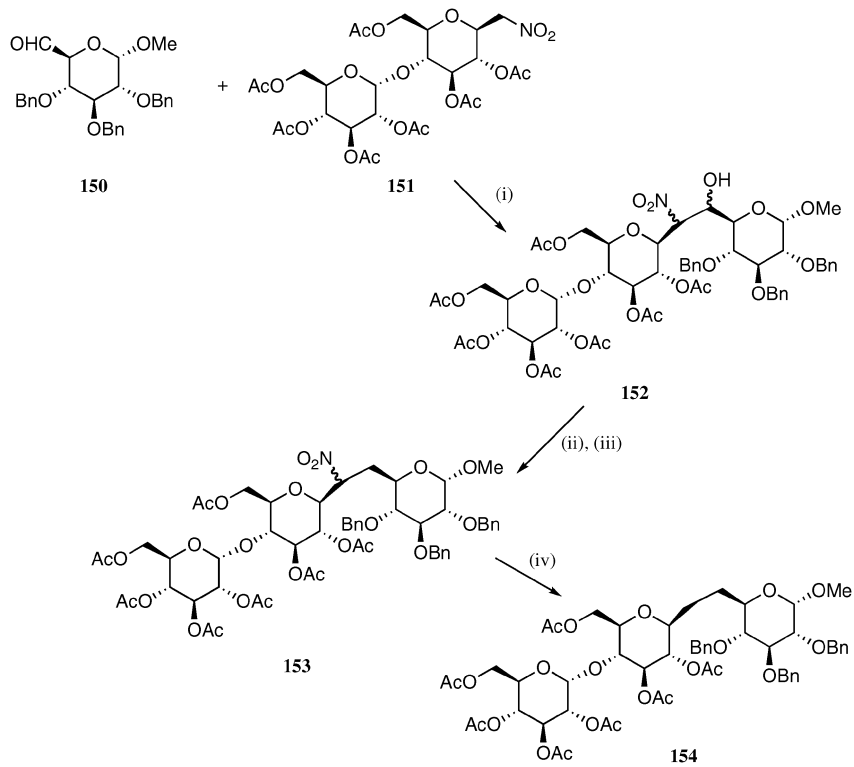
Scheme 45 *Conditions*: (i) TFA/H_2O; (ii) 2% aq. $HNaCO_3$, MeOH; (iii) $CH_3CH_2OCH{=}CH_2$, PPTS; (iv) $HSnBu_3$, AIBN, toluene; (v) PPTS, EtOH, 50 °C.

Under these conditions, tertiary nitro compounds and also nitro groups in an allyl or benzyl position or in a vicinal position to a keto or ester groups are readily denitrated. Nevertheless, inactivated secondary nitroalkyl groups and primary nitro groups are much more resistant to the direct replacement by hydrogen and a large excess of $HSnBu_3$ in boiling toluene is required.[102] An interesting solution to this problems has been recently reported (Scheme 46).[103] It involves an acid-catalyzed solvolysis of a *C*-glycopyranosylmethyl-hydrogen nitronate group with ethanethiol affording *C*-glycopyranoylmethanal diethyl dithioacetal, followed by a desulfuration with freshly activated Raney Nickel.



Scheme 46 *Conditions*: (i) NaOMe, MeOH; (ii) HCl, EtSH; (iii) Raney-Ni, MeOH.

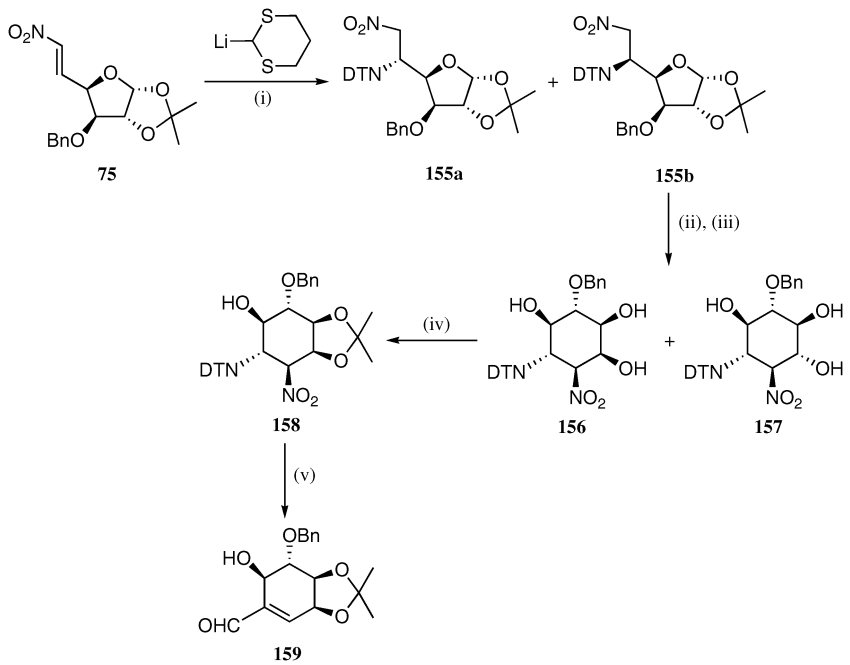
Henry reaction of 1-deoxy-1-nitroaldoses followed by denitration is a valuable strategy to obtain *C*-glycosyl compounds[104] and this procedure was used for the preparation of several *C*-polysaccharides.



Scheme 47 *Conditions*: (i) KF, 18-crown-6, MeCN; (ii) Ac_2O, Py; (iii) $NaBH_4$, EtOH; (iv) $HSnBu_3$, AIBN, toluene, reflux.

For example, the *O*,*C*-trisaccharide **153** was obtained by a Henry reaction of nitro disaccharide **151** and sugar aldehyde **150** followed by the dehydration of β-nitro alcohol **152** and reduction of the resulting nitroolefin, to give nitro sugar **153**. Finally, a radical elimination of the nitro group afforded the target **154** (Scheme 47).[105]

On the other hand, an E_{1CB} elimination of a nitro group in nitro sugars has recently been reported as part of a novel synthesis of rancinamicyns.[106] Thus, Michael addition of the lithium salt of 1,3-dithiane to sugar nitroolefin **75** afforded the separable mixture of isomers **155a** and **155b** (Scheme 48). The major isomer **155b** was submitted to a two step sequence including an intramolecular Henry reaction, to give the mixture of carbocycles **156** and **157**, which reacted with dimethoxypropane and PTSA to produce compound **158** selectively. Finally, treatment of **158** with MeI and aqueous sodium hydrogen carbonate provided compound **159** as the result of the liberation of the masked carbonyl group followed by a kinetically and thermodynamically favoured $E_{1C}B$ elimination of the nitro group.

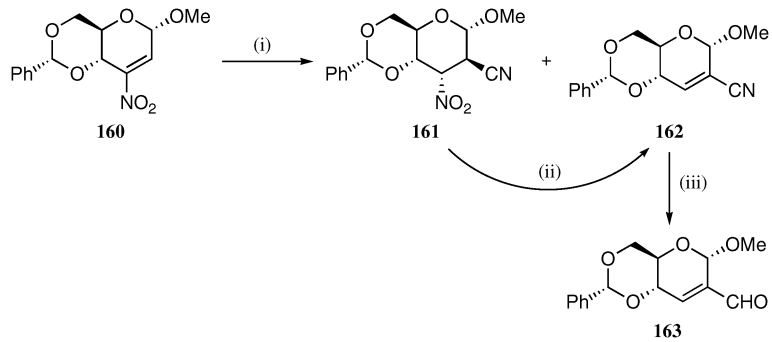


Scheme 48 *Conditions*: (i) THF, −78 °C; (ii) 75% AcOH, reflux; (iii) 2% aq. $NaHCO_3$, MeOH; (iv) $(CH_3)_2C(OMe)_2$, *p*-TsOH, acetone; (v) MeI, aq. $NaHCO_3$, MeCN, 35 °C.

A similar $E_{1C}B$ elimination of a nitro group in nitro sugars was involved in a recently reported synthesis of methyl 4,6-*O*-benzylidene-2,3-dideoxy-2-*C*-formyl-α-D-*erythro*-hex-2-enopyranoside **163** (Scheme 49).[107]

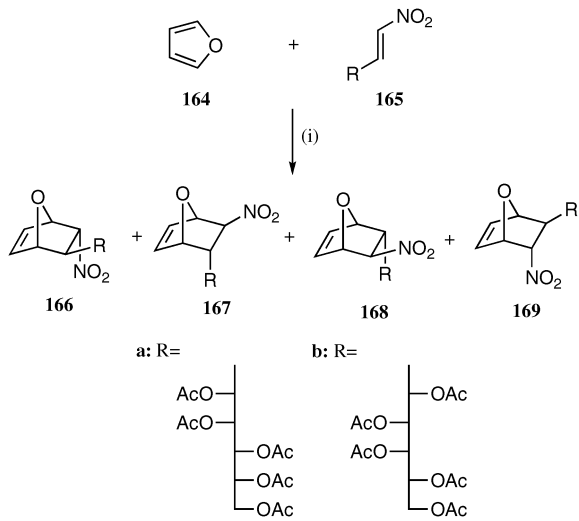
3.6 Cycloaddition reactions of nitro sugars

Nitroalkenes are relatively good dienophiles in Diels-Alder cycloadditions. Thus, the easily available sugar-derived chiral nitroalkenes D-*manno* and

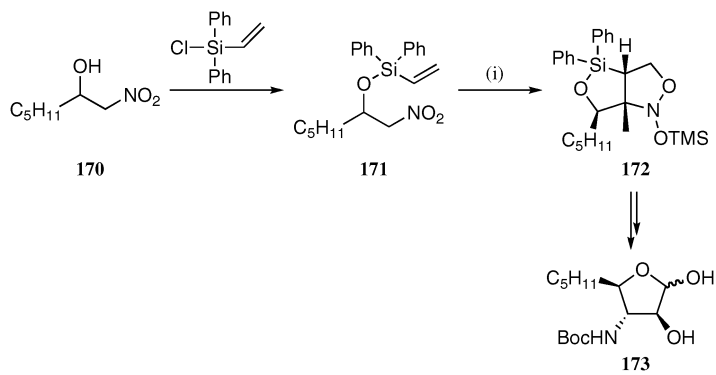


Scheme 49 *Conditions*: (i) KCN, H_2O, Amberlite, CH_3CN; (ii) Et_3N, acetone/H_2O; (iii) DIBAL-H, CH_2Cl_2.

D-*galacto*-3,4,5,6,7-penta-*O*-acetyl-1,2-dideoxy-1-nitrohept-1-enitols **165a** and **165b** reacted with furan under high pressure to afford the bicyclic adducts **166–169** (Scheme 50).[108]



Scheme 50 *Conditions*: (i) 13 Kbar, CH_2Cl_2.



Scheme 51 *Conditions*: (i) ClTMS, Et_3N.

Intramolecular cyclizations of silyl nitronates were also used in the preparation of aminosugars. In 2003 Kudoh *et al.* reported the stereoselective conversion of 2-nitroalkanols by silyl nitronate generation followed by an intramolecular nitronate-olefin [3 + 2] cycloaddition reaction (Scheme 51).[88]

Acknowledgements

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Porphyrinyl-type sugar derivatives: synthesis and biological applications

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This report deals with the synthesis and biological evaluations concerning photodynamic therapy properties of porphyrinyl- and dihydroporphyrinyl-type sugar derivatives. The chosen conjugates have *O*- and *S*-glycosidic moieties or ether and ester functions.

1. Introduction

Carbohydrates are abundant compounds in Nature appearing as monomers, oligomers or polymers and as components of many other natural molecules. They play vital roles to living organisms. Human beings eat them in direct or indirect ways, and use their polymeric forms for many purposes in everyday life. Also glycoconjugates are present in proteins, cells and in entire organisms. It is known that carbohydrates are involved in many cellular processes (*e.g.*, cell–cell recognition, cellular transport).[1–3]

Porphyrin-type derivatives also play in Nature's vital functions. They are involved in our respiration processes, in photosynthesis and in drug detoxification. Apart from such biological significance of porphyrins a great deal of work has been carried out in recent decades on the possible applications for such macrocycles. Applications on the medical side are already known; the promising ones are centred on the action against cancer and microorganisms. Photodynamic therapy (PDT) of cancer cells and treatment of age-related macular degeneration are two therapeutics already taking place in several countries with approved formulations (Photofrin® and Visudyne®) on the markets.[4,5]

Sugars can be used in diagnostic tests, vaccines and other therapeutics. Certain glycoconjugates can then be considered as potential new drugs. Particularly in glycoporphyrin derivatives the sugar groups will rule the amphiphilicity of the conjugate and also can act at specific interactions taking place at the cellular membrane area. Certainly the sugar moiety will bring a higher plasmatic life and allow cancer cell targeting due to membrane receptor bindings. In such ways sugar-porphyrin derivatives might become compounds of great medical significance; synthetic work leading to such compounds and evaluation of their biological properties have been targets in recent years for several research groups.[6a,b]

This review deals with the synthesis of porphyrinyl-type sugar conjugates developed and biologically accessed, and considers the involvement of macrocycles of the porphyrin and chlorin (dihydroporphyrin) types, with *O*- and *S*-glycosides or ether and ester functions.

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2. Synthesis of porphyrinyl-type sugar derivatives

The biological significance of glycoporphyrins, their scarce natural occurrence and their potential applications have made the availability feature of such compounds a scientific target for several research groups. Taking advantage of established knowledge about synthesis or chemical modifications of a porphyrin[7–9] and also about the linkage of sugar species to other compounds, many porphyrinylsugar derivatives have been prepared and their biological activities evaluated. Several synthetic approaches were considered and natural and synthetic porphyrin derivatives were linked to sugar units by amide or ester, ether or thioether and amine functionalities.[6,10] To allow easy purification of the sugar-porphyrin derivatives, the sugar hydroxyl functions are usually protected by suitable groups during the synthetic procedure which can be cleaved at a later stage.

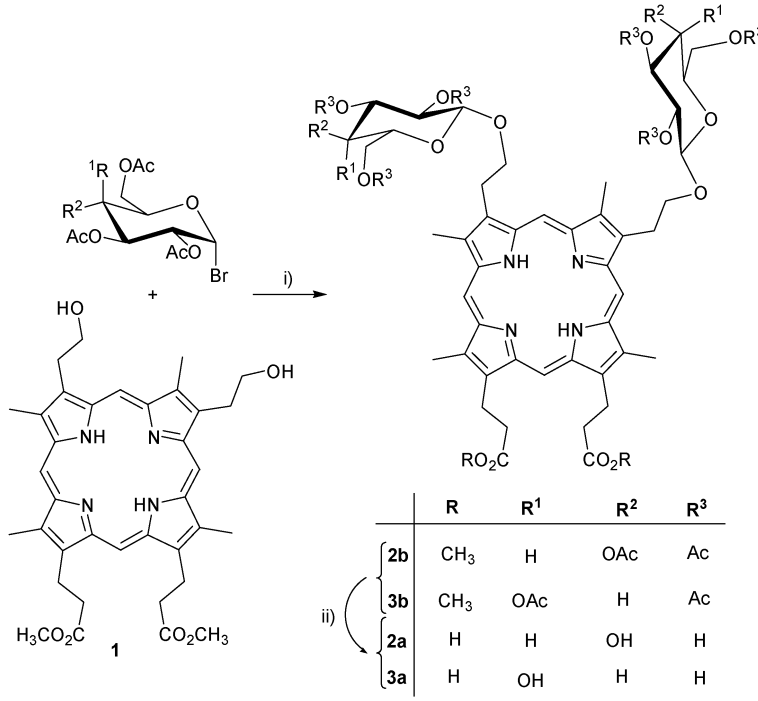
2.1 *O*-Glycosidic porphyrins and other conjugates

Throughout the eighties, the ability of porphyrins to be accumulated in tumour tissues and, in particular, the good PDT results achieved by the hematoporphyrin derivative HpD, a porphyrin mixture which can be obtained from heme, has transformed the search for new chemically pure photosensitisers into a primary goal for the scientific community. In addition to its chemical purity, the photosensitiser would also have to be soluble in physiologic media and easily excreted from the body after therapy. Within this context, Franck *et al.*[11] presented in 1989 the first water soluble, diglycoside derivatives **2** and **3** (Scheme 1). These derivatives are structurally related to natural occurring protoporphyrin-IX. The *O*-glycosylation of the derivative **1** with the 2,3,4,6-tetra-*O*-acetyl-1-bromo-α-D-glucopyranose or 2,3,4,6-tetra-*O*-acetyl-1-bromo-α-D-galactopyranose was carried out in nonpolar solvents with solid, surface-active silver catalyst. The corresponding deprotected diglucoside **2a** and digalactoside **3a** were obtained by alkaline treatment of the acetylated derivatives **2b** and **3b**, respectively. The main advantage of compounds **2** and **3**, when compared with HpD or its patented formulation Photofrin®, a purified fraction of HpD, is that they are chemically and stereoisomerically pure.

Recently Cavaleiro *et al.* described an easy synthetic approach to glycoporphyrins from zinc(II) protoporphyrin-IX dimethyl ester **4** and *O*-allyl carbohydrate acetonides **5A–E** (D-ribose (A), D-galactose (B), D-glucose (C), and two isomeric derivatives (D) and (E) of D-fructose) by cross-metathesis (Scheme 2).[12] Two equivalents of each carbohydrate and the Grubbs catalyst were used, giving the carbohydrate derivatives **6** in a range of 74% to 93% yields.

Using the same methodology but now using Zn(II) vinylporphyrin **7** and equimolar amounts of the allylic acetonides new β-substituted glycoporphyrins **8** were obtained (Scheme 2).[12]

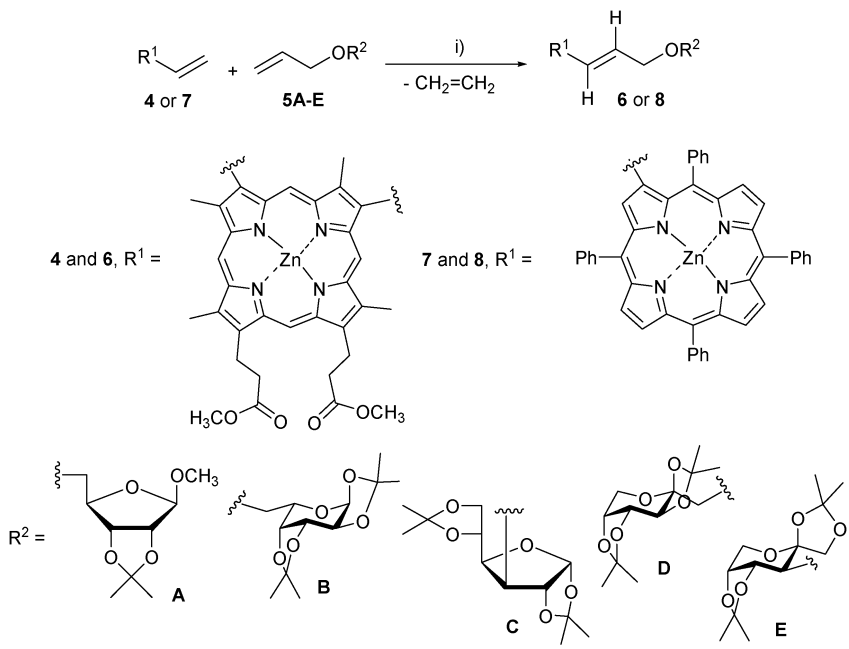
The direct attachment of adequately substituted *meso*-arylporphyrin to the saccharide units has been another synthetic strategy. *Meso*-tetraarylporphyrins were used as templates to append, directly or by a spacer, several sugar units at the *ortho*, *meta* or *para* positions of the phenyl ring (Fig. 1).



	R	R^1	R^2	R^3
2b	CH_3	H	OAc	Ac
3b	CH_3	OAc	H	Ac
2a	H	H	OH	H
3a	H	OH	H	H

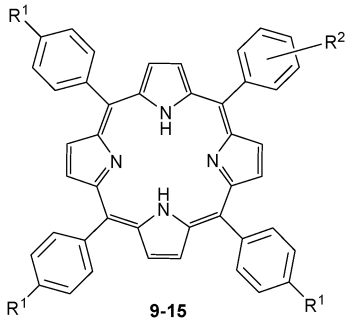
i)Ag_2CO_3, Na_2SO_4, CH_2Cl_2, 20 ºC, 1-7 d, Argon; ii) KOH, MeOH, reflux, 24 h

Scheme 1



i) Grubbs' second-generation catalyst (25 mol%), CH_2Cl_2, reflux

Scheme 2



9-15

	R^1	R^2
9(*p*)	H	OH
10(*p*)	H	*β*-OGlc
11(*o*,*p*)	CH_3	OH
12(*o*,*p*)	CH_3	$O(CH_2)_3OH$
13(*o*,*p*)	CH_3	$O(CH_2)_3(\alpha)$-OGlc
14(*o*,*p*)	CH_3	$O(CH_2)_3NH_2$
15(*o*,*p*)	CH_3	$O(CH_2)_3NHC(O)CH_2(\alpha)OGlc$

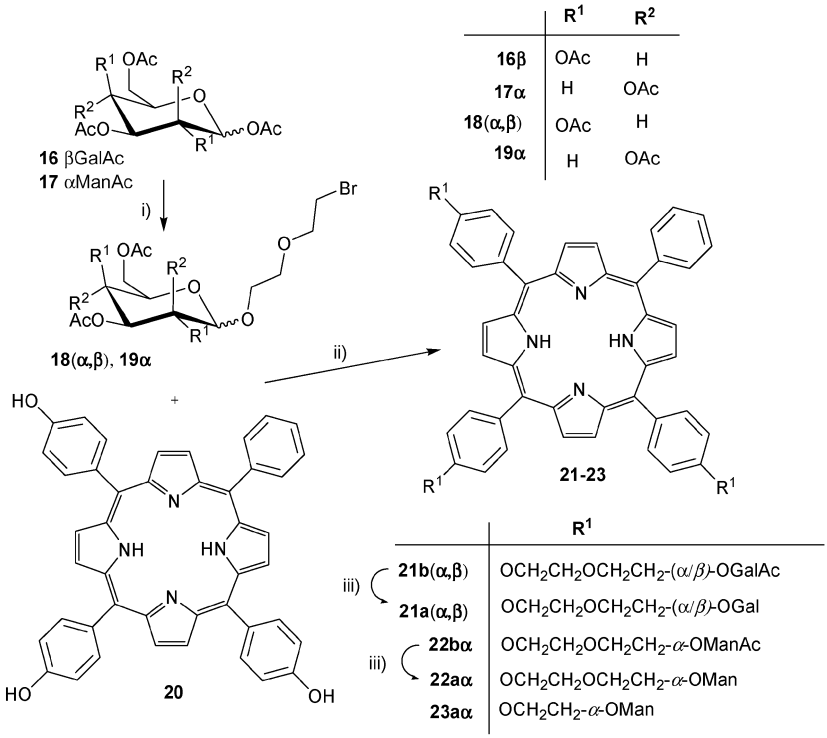
Fig. 1

Using such strategy, the glycoporphyrins **10**, **13** and **15** have been synthesised.[6,13,14] In the case of derivative **10**, the tetrapyrrolic macrocycle is directly linked to the glucose unit by a beta glycosidic linkage in the *para* position of the phenyl ring, but derivatives **13** and **15** have a spacer between the glucose unit and the porphyrinic moieties. For these derivatives, the linkage to the macrocycle is accomplished by *ortho* and *para* positions. Their preparation involves 3,4,6-tri-*O*-acetylcarboxymethyl-α-D-glucopyranoside-2-*O*-lactone as the carbohydrate moiety supplier. The reaction occurs between an excess of the lactone and the primary amine of porphyrin **14**(*o*,*p*) in very good yield (95%). The porphyrin derivatives **12**(*o*,*p*) and **14**(*o*,*p*) containing the spacer arms were obtained through an easy procedure from derivative **11**(*o*,*p*).[6,14]

Maillard has also prepared a series of glycoconjugated porphyrins and evaluated their PDT activity against human retinobastoma cells.[15] The ethylene glycol-linked glycoconjugated porphyrins **21a–23a** were prepared by following the synthetic procedures described in Scheme 3. The *O*-glycosylated porphyrins were obtained by the reaction of porphyrin **20** with the bromo-substituted glycosides **18(α,β)** and **19α**, followed by the *O*-deprotection step of derivatives **21b** and **22b**. The requisite bromo-substituted glycosides **18(α,β)** and **19α** were prepared by the reaction of *β*-galactopyranose **16** and α-mannopyranose **17** penta-acetate respectively, with 2-(2-bromoethoxy)ethanol in the presence of BF_3-etherate.[15] They have also prepared the derivative **23α** containing the monoethylene glycol linker.[16]

The condensation of glycosylated benzaldehydes with pyrrole has been another approach to prepare porphyrinylsugar derivatives.[14,17–24]

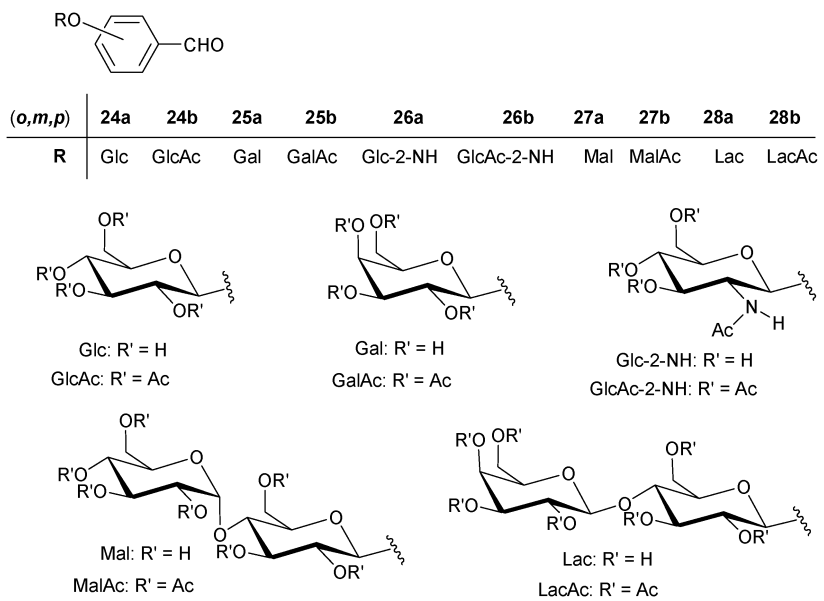
Firstly, it is necessary to prepare the glycosylaldehydes. Glycosylbenzaldehydes, such as those containing tetra-acetyl-glucosyl, -galactosyl, -glucosamino, -maltosyl and -lactosyl moieties **24–28** shown in Fig. 2 were prepared according to the method described by Halazy *et al.* For instance, the *para*-glucosylated benzaldehyde **24b** can be prepared in 30% yield by coupling α-bromo-tetra-acetylglucopyranose **30** with the 4-hydroxybenzaldehyde. The α-bromoglucopyranose derivative can be obtained by treating the penta-acetylglucopyranose **29** with HBr/acetic acid (Scheme 4).[25]



	R^1	R^2
16β	OAc	H
17α	H	OAc
18(α,β)	OAc	H
19α	H	OAc

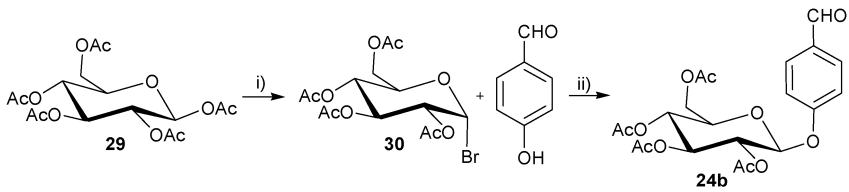
i) 2-(2-bromoethoxy)ethanol, $BF_3{\cdot}OEt_2$, dry CH_2Cl_2, 0 ºC to rt, 4h; ii) K_2CO_3, dry DMF, 60 °C, 15h; iii) $NaOCH_3$, CH_3OH

Scheme 3



(*o,m,p*)	24a	24b	25a	25b	26a	26b	27a	27b	28a	28b
R	Glc	GlcAc	Gal	GalAc	Glc-2-NH	GlcAc-2-NH	Mal	MalAc	Lac	LacAc

Fig. 2

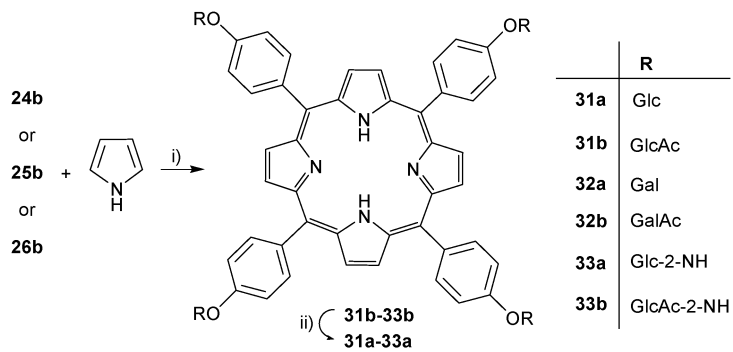


i) HBr, AcOH, rt, 3h; ii) NaOH (aq), Bu_4NBr, CH_2Cl_2, rt, 3 days

Scheme 4

Condensation of glycosylated benzaldehydes with pyrrole can take place in boiling acetic acid[26] or under Lindsey conditions[27] to afford glycosylated porphyrins. According to Lindsey's method, the porphyrinogens, intermediate tetrapyrrolic species, are obtained in dichloromethane at room temperature with a high-diluted solution of aldehydes and pyrrole, in the presence of $BF_3 \cdot OEt_2$ (a Lewis catalyst). Then, the porphyrinogens are oxidized *in situ* by treatment of the reaction mixture with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) or with another high potential quinone derivative.[28]

The condensation of the 4-(tetra-acetyl-β-D-glucosyloxybenzaldehyde) **24b**(*p*) or 4-(tetra-acetyl-β-D-galactosyloxybenzaldehyde **25b**(*p*) or 4-(2-*N*-acetyl-3,4,6-tri-acetyl-β-D-glucosyloxybenzaldehyde) **26b**(*p*) with pyrrole gave the corresponding *meso*-tetrakis(4-glycosylphenyl)porphyrins **31b–33b** (Scheme 5).[19,22,29]



i) (a) $BF_3{\cdot}OEt_2$, CH_2Cl_2; (b) *p*-chloranil, Δ; iii) $NaOCH_3$, CH_3OH, rt

Scheme 5

A similar approach was used to prepare the octaglucosylated porphyrin derivative **34a** obtained quantitatively after treatment of **34b** with sodium methoxide and dry methanol (Fig. 3). The aldehyde used in the preparation of octaglucosylated derivative **34b** was obtained in 90% yield by condensation of 3,5-dihydroxybenzaldehyde with acetobromoglucose **30** in the presence of Ag_2O.[22] The condensation of pyrrole and the 2,5-bis(tetra-acetylglucopyranosyloxy)benzaldehyde occurred under Lindsey conditions and yielded porphyrinylsugar derivative **34b** in 32%.[22]

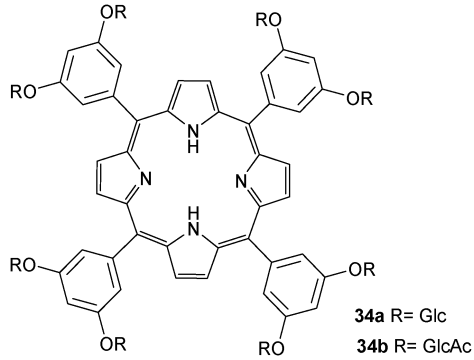


Fig. 3

The target of photodynamic action within the cell can vary between the cell membrane,[30] mitochondria[31] and nucleus[32,33] and can be dependent on the structure of photosensitisers.[34] A high number of new photosensitisers has been designed in order to achieve better tissue selectivity and higher efficiency.[34–41] The photophysical and the biological properties of the photosensitisers can be ruled by the nature and the number of sugar substituents. Furthermore, the presence of hydrophobic substituent groups in the *meso* positions, such as phenyl, pentafluorophenyl and alkyl groups, could increase the interaction of those molecules with the lipidic parts of cell membranes, whereas the glycosyl moieties could become functional components involved in cell recognition.[42,43]

In order to prepare asymmetric *meso*-porphyrinylsugar derivatives (mono-, di- and tri-glycosylarylporphyrins), pyrrole was condensed with a mixture of glycosylated and nonglycosylated aldehydes.[17,21,24,44–46] Several porphyrinylsugar derivatives were prepared by this synthetic methodology. The saccharide unit can be directly linked to the porphyrin macrocycle **35–50** or separated from the phenyl group by a spacer, such as in the case of derivative **51–53** shown below (Fig. 4).

Several examples of coupling peptides to porphyrin derivatives have been reported in the literature and the important biological phenomena and transport processes in which these molecules are involved have been exploited for PDT.[47–50]

Krausz *et al*. have synthesised two series of amino acid porphyrinylsugar derivatives (Fig. 5). One of them involves the coupling of adequate glycoporphyrin derivatives, prepared by pyrrole/aldehyde condensation methodology, with 9-fluorenylmethoxycarbonyl-L-alanine (Fmoc-L-alanine) to give the tri-, di-, and mono-alanine glycoporphyrin derivatives **54–57** after the deprotection step; the other series (**58**) involves a glucosylamino acid moiety instead of the alanine in their preparation.[21,44]

A specific peptide motif linked to the chemotherapeutic molecule can enhance its efficacy, reduce their toxicity and also improve tumour cell targeting.[45,51,52] Therefore, a range of glycoporphyrins **59** and **60** bearing the Arginine-Glycine-Aspartate (RGD motif) moiety were synthesised (Fig. 6).

	R	R'
35B	2-NO_2Ph-	4-O-β-GlcPh-
36	4-NO_2Ph-	4-O-β-GlcPh-
37A	4-NO_2Ph-	2-O-β-GlcPh-
38B	2-NH_2Ph-	4-O-β-GlcPh-
39	4-NH_2Ph-	4-O-β-GlcPh-
40A	4-NH_2Ph-	2-O-β-GlcPh-
41B	2-NH-Alanine-Ph-	4-O-β-GlcPh-
42	4-NH-Alanine-Ph	4-O-β-GlcPh-
43A	4-NH-Alanine-Ph	2-O-β-GlcPh-
44B	4-OHPh-	4-O-β-GlcPh-
45B	4-$CH_2CH{=}CH_2$Ph-	4-O-β-GlcPh-
46B	2-$O(CH_2)_3CO$-RGD-Ph-	4-O-β-GlcPh-
47B	2-$O(CH_2)_3CO_2H$-Ph-	4-O-β-GlcPh-
48B	4-$O(CH_2)_3CO_2H$-Ph-	4-O-β-GlcPh-
49B	Ph-	4-O-β-GlcPh-
50B	C_6F_5-	4-O-β-GlcPh-
51	4-CH_3Ph-	4-O-$(CH_2)_3O$-β-GlcPh-
52A	4-CH_3Ph-	2,4-NH-serine-O-β-GlcPh-
53A	4-CH_3Ph-	2,4-NHC(O)CH(NH_2)CH_2-O-β-GlcPh-

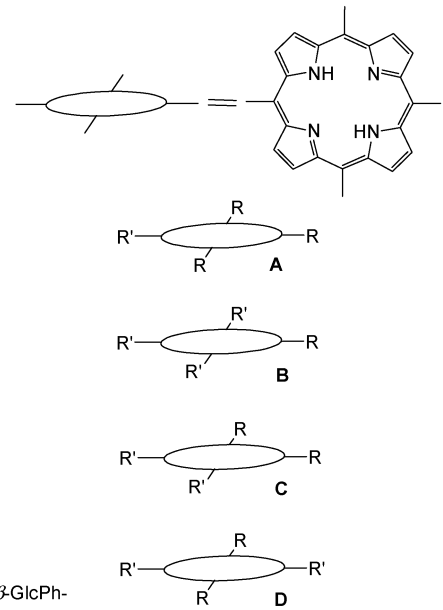
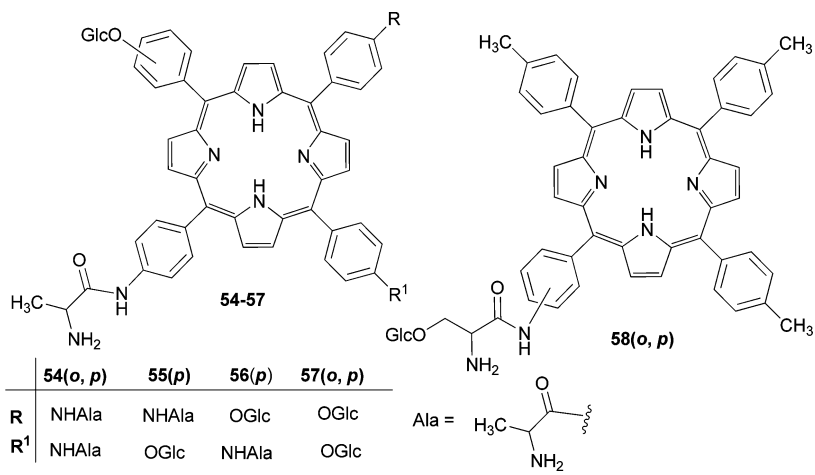


Fig. 4



	54(*o*, *p*)	55(*p*)	56(*p*)	57(*o*, *p*)
R	NHAla	NHAla	OGlc	OGlc
R^1	NHAla	OGlc	NHAla	OGlc

Fig. 5

Porphyrin dimers were also considered as photosensitisers and evaluated for PDT cancer treatment.[53,54] The corresponding preparation involves porphyrinic precursors which can be prepared by following the Lindsey methodology.[27,28] Thus, once those precursors have been prepared, several *O*-glycosylporphyrin dimers were synthesised.[55–58]

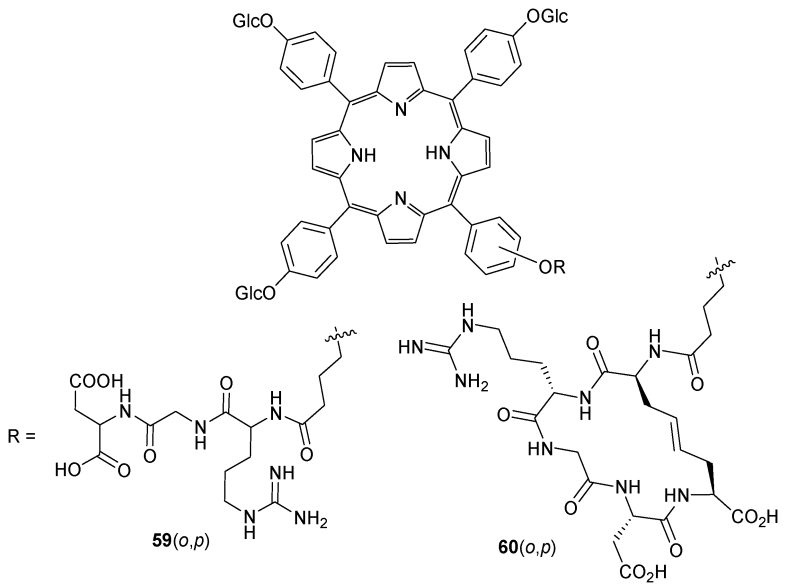


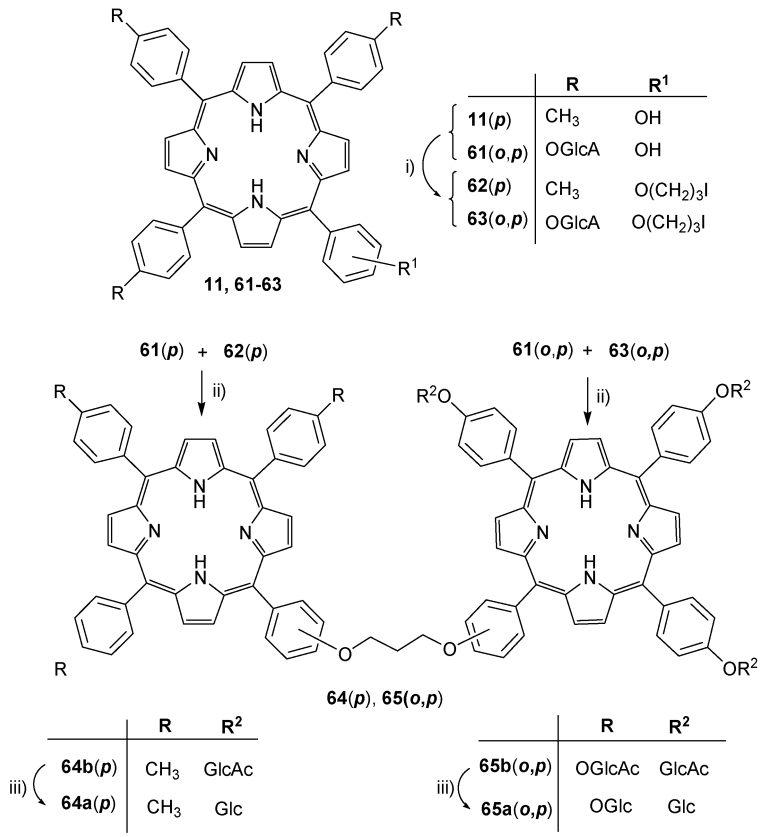
Fig. 6

To obtain the *O*-glycosylporphyrin dimers with ether linkage **64a**(*p*) and **65a**(*o*,*p*) the synthetic procedure described in the Scheme 6 has been followed.[56]

Cationic glycosyl-bisporphyrins **66** and **67**[57] and a neutral porphyrin dimer **68** containing a pentapeptide moiety with RGD sequence as a spacer have also been prepared (Fig. 7).[58]

Polymeric *O*-glycoconjugates were also prepared[59–64] and recently their biological properties have been evaluated, particularly their antimicrobial activities.[62–64] The goal was to join in a bioactive material the photosensitizing properties of the porphyrins with the ability of the cellulose after esterification by fatty acids, allowing the formation of plastic materials.[65,66] Some authors have reported photobactericidal plastic films based on esterified cellulose and porphyrin derivatives.[62–64] To prepare these esterified plastic films, lauric acid, protoporphyrin-IX and 5-(4-hydroxyphenyl)-10,15,20-tristolylporphyrin have been used. Chloroacetyl chloride and 5-(4-pyridyl)-10,15,20-tristolylporphyrin were also used in the cellulose functionalisation. The porphyrin-appended cellulose ester polymers **69–71** (Fig. 8) were obtained by 'one pot, two-steps' esterification reactions. Firstly, cellulose was dissolved in DMA/LiCl solvent system and the porphyrin derivative was grafted onto cellulose by reaction with TsCl in the presence of pyridine. Thus, esterification of porphyrinic-cellulose intermediates was carried out in the same condition with lauric acid.[62] Compound 5-(4-hydroxyphenyl)-10,15,20-tristolylporpohyrin **72** was converted into derivatives **73** and **74** (Scheme 7) and then grafted into the cellulose polymer using either the 4- or 11-carbon spacer arms.[63]

The synthesis of the polymeric cationic glycoconjugate **80** was carried out accordingly the methodology depicted in Scheme 8. Cellulose **77** dissolved in DMA/LiCl was reacted with chloroacetyl chloride leading to halogenated



i) $I(CH_2)_3I$, K_2CO_3, DMF, 6 h; ii) K_2CO_3, DMF, 24 h; iii) NaOMe, $MeOH/CH_2Cl_2$ (8:2).

Scheme 6

plastic film **78**. The plastic film **78** synthesised was dissolved in DMF and then reacted with the porphyrin **79** at room temperature and the porphyrinic plastics were isolated by precipitation with chloroform.[64] Different porphyrin content of porphyrinic plastic film was obtained by variation of the stoichiometry of porphyrin per anhydroglucose unit.[62–64]

2.2 *O*-Glycosyl chlorin derivatives

Several research groups probably inspired by both the attractive features of PDT and the commercialization of Photofrin® and Visudyne®, have put considerable effort on the development and study of the so-called second generation photosensitisers.

The development of PDT agents that absorb light above 630 nm (where tissues are more transparent) is desirable because it would allow a greater depth of penetration and potentially greater destruction of malignant tissues. With such targets in mind, a second generation of photosensitisers, related to chlorins and related compounds have been developed and evaluated as putative PDT agents in recent years. In particular, chlorins containing aromatic groups fused to porphyrin macrocycles (benzochlorins, naphthochlorins, and pyridochlorins) have attracted particular attention.[1,69,70]

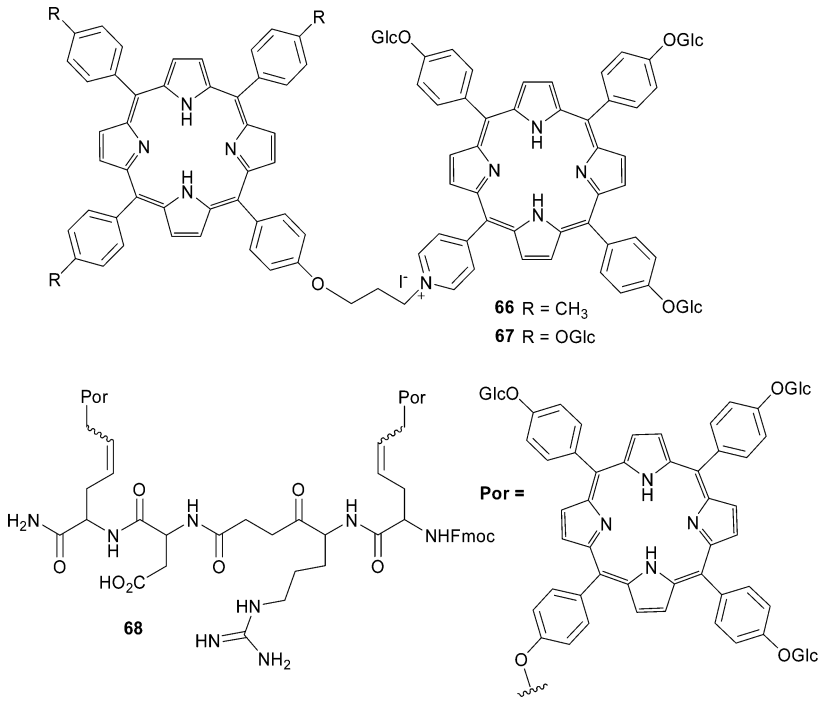


Fig. 7

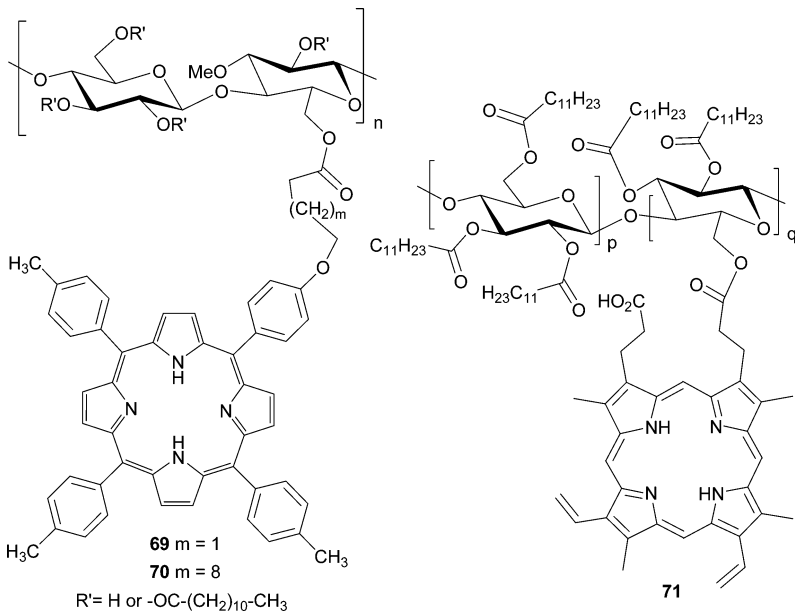
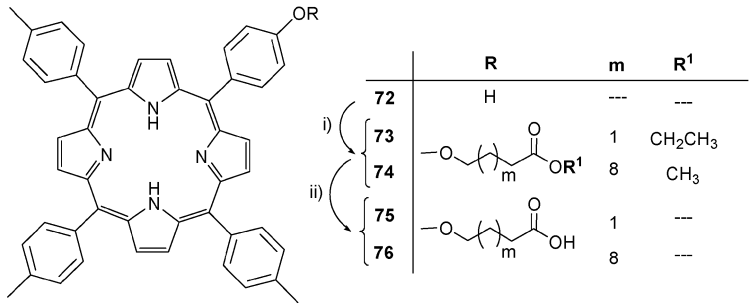


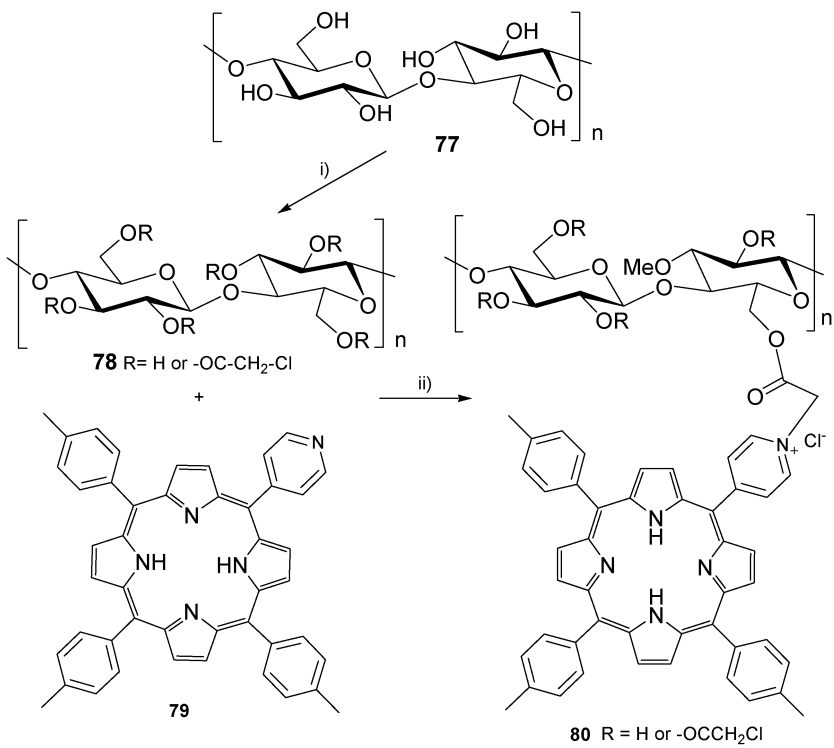
Fig. 8

Different studies performed with purpurinimides, a reduced macrocycle that showed a long wavelength absorbance, by Pandey and co-workers[67,71]



i) Br-$(CH_2)_3$-$CO_2CH_2CH_3$ (10 equiv) or Br-$(CH_2)_{10}$-CO_2CH_3 (10 equiv), K_2CO_3 (20 equiv), DMF, rt.
ii) KOH/CH_3CH_2OH (1 mol.dm^{-3}), DMF, 100 °C

Scheme 7

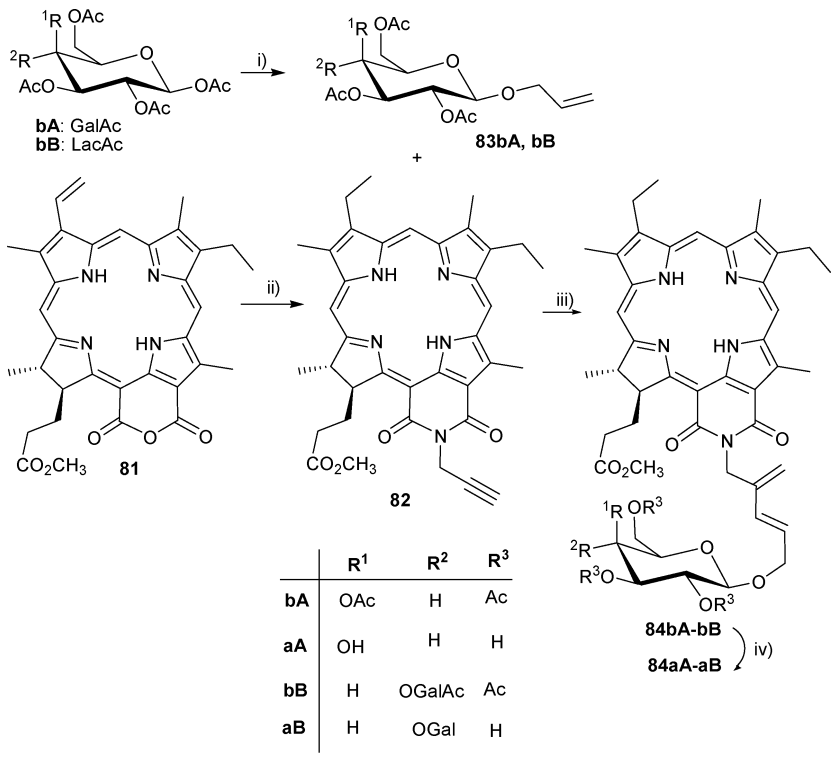


i) $ClCH_2COCl$, DMA/LiCl, rt, 2 h; ii) DMF anhydrous, 55 °C, 24 h

Scheme 8

have demonstrated that the purpurinimide system provides an "ideal" system to investigate structure-activity relationships. Several purpurin-18-*N*-alkylimides were studied so as to evaluate them, namely in relation to the influence of their lipophilicity and photodynamic activity.[71] They have also extended these studies to the galectin-specific photosensitisers based on purpurinimide core,[67] having subsequently introduced the sugar units at various positions of the molecule.[68] Several glycosylated-purpurinimide derivatives were prepared, and the sugar units were joined to the macrocycle by a diene, an amide or ethylene linkers. The purpurin-18-methyl ester **81**

was obtained from methylpheophorbide-a which had been isolated from *Spirulina pacifica*. From the reaction of purpurin **81** with propargylamine results the corresponding propargylimide derivative **82** in good yield. The galactosyl-purpurinimide **84** with a diene linker was prepared by the enyne cross metathesis which occurs between a purpurin-18-*N*-propagylimide-17-propionic ester derivative **82**, the alkyne component, and the allylgalactose penta-acetate **83**, the alkene component, in the presence of Grubbs ruthenium catalyst (Scheme 9).[67,72] The acetylated allyl β-D-galactopyranoside **83** was prepared from galactopyranose penta-acetate by BF_3-etherate induced glycosylation.[73] Treatments of the galactopyranosyl-purpurinimide **84b** with sodium methoxide in methanol/dichloromethane led to the expected conjugate **84a**.[67]



	R^1	R^2	R^3
bA	OAc	H	Ac
aA	OH	H	H
bB	H	OGalAc	Ac
aB	H	OGal	H

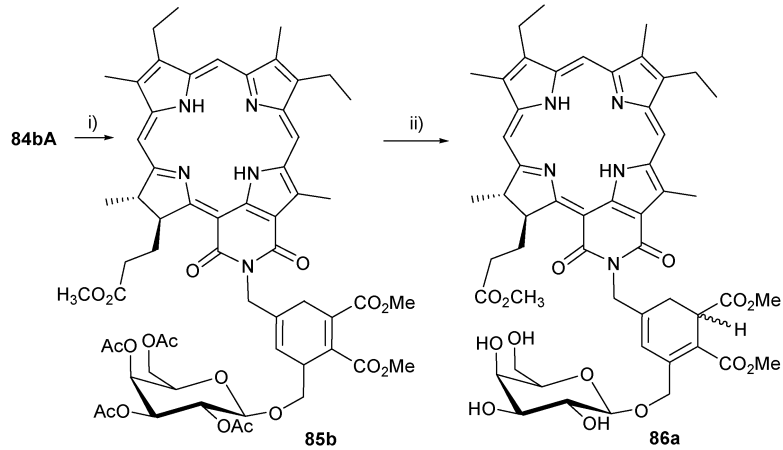
i) NH_2CH_2CCH in benzene, reflux, 12 h; ii) $BF_3{\cdot}OEt_2$, allyl alcohol in CH_2Cl_2, 0 °C to rt;
iii) Grubbs' catalyst, CH_2Cl_2, Argon, 48 h; iv) NaOMe/MeOH/CH_2Cl_2.

Scheme 9

The Diels-Alder cycloaddition of galactopyranosyl-chlorin conjugate **84bA** with dimethyl acetylenedicarboxylate, in refluxing toluene, afforded the corresponding Diels-Alder adduct **85b** (Scheme 10). Cleavage of the acetyl groups in **85b** gave rise to the required glycosylated chlorin derivative **86a** as the major product in 44% yield.[67]

Based on biological results, a series of carbohydrate-photosensitiser conjugates in position 3 was prepared and biologically evaluated.[68]

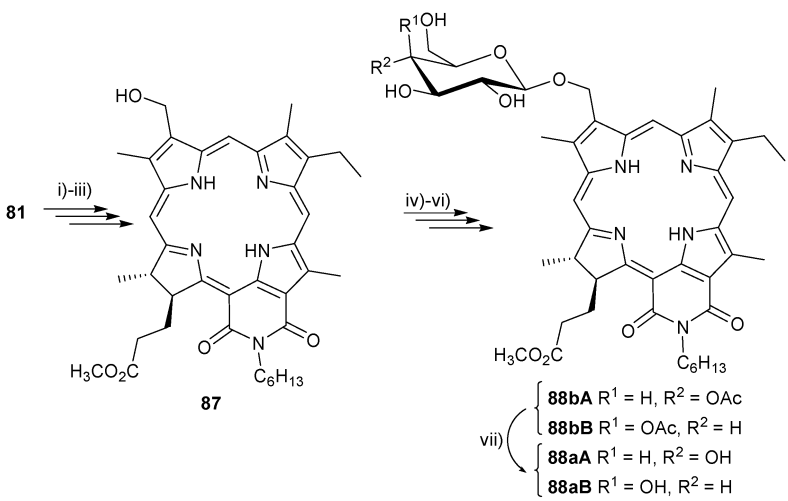
The glycosidic conjugates **88** were prepared by reaction of 3-hydroxymethylpurpurin-18-*N*-hexylimide-17-propionic ester **87** with the



i) dimethyl acetylenedicarboxylate, toluene, reflux, 3 h, Argon; ii) NaOMe/MeOH, 1 h.

Scheme 10

penta-acetate glucose or penta-acetate galactose derivatives in the presence of boron trifluoride diethyl etherate to produce a protected sugar-purpurinimide conjugates **88b** which, upon deacetylation with sodium methoxide, gave the compounds **88a**. The conversion of purpurin-18-methyl ester **81** into **87** was achieved after the sequential treatment of **81** with 1-hexylamine and with catalytic amount of methanolic KOH and diazomethane. The vinyl group present in position 3 was oxidised with osmium tetraoxide/sodium periodate into the corresponding formyl group which, on reacting with sodium borohydride, afforded the compound **87** (Scheme 11).[68]

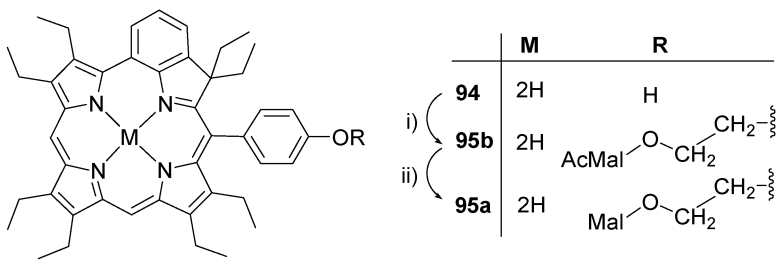


i) $C_6H_{13}NH_2$; ii) KOH/CH_3OH; iii) CH_2N_2; iv) OsO_4, $NaIO_4$; v) $NaBH_4$; vi) $Glc(OAc)_5$ or $Gal(OAc)_5$, $BF_3.Et_2O$, vii) NaOMe/MeOH

Scheme 11

Since the purpurinimide suggested to be an "ideal" system to investigate structure-PDT activity relationships, several lactose-photosensitiser conjugates were also prepared and their PDT activity evaluated in RIF cells.[68] The lactosylderivative **84aB** with a diene linker was prepared by enyne cross metathesis (Scheme 9) and the biological evaluation revealed that a lactose chlorin conjugate with a diene linker was more effective *in vitro* when compared to the non glycosylated precursor.[67] Based on these results, Pandey *et al*. prepared lactose-purpurinimide conjugates with the lactose moiety in different positions (3, 8 and 12) and with the lactose unit linked to the macrocycle *via* an amide or ethylene bond.[67]

To achieve these lactose-purpurinimide conjugates **89–93**, in the macrocycle positions 3, 8, 12, the purpurin-18-methyl ester **81** had to undergo several chemical transformations to obtain the desired hydroxymethyl group.[68] Such alcohol derivatives were reacted with lactose octaacetate in the presence of boron trifluoride diethyl etherate to induce the acetylated lactose-purpurinimide derivative which, after deacetylation reaction conditions, yielded the desired compounds in excellent amounts (Scheme 12).



i) 1-bromoethoxy-*per*-acetyl-maltose, K_2CO_3, DMF, 60 °C, ii) NaOMe/MeOH

Scheme 12

The derivative **90** was obtained by condensation of the purpurin-18-*N*-hexylimide-17-propionic acid with aminolactose heptaacetate in the presence of benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) followed by the deacetylation procedure. The lactose-photosensitiser conjugate linked by an ethylene moiety was also prepared by following a similar approach. The purpurin-18-methyl ester **81** was converted into *N*-(3-iodobenzyl)*meso*-purpurin-18-*N*-hexylimide-17-propionic ester by hydrogenation over Pd/C followed by reaction with 3-iodo-benzylamine. Afterwards, the propargyllactose heptaacetate reacted with *N*-(3-iodobenzyl)*meso*-purpurin-18-*N*-hexylimide-17-propionic ester in the presence of tris(dibenzylidieneacetone)dipalladium(0) (Pd_2-dba_3) which, after deacetylation conditions, afforded the derivative **89** (Fig. 9).[68]

Momenteau *et al*. described the preparation of amphiphilic glyco-conjugated meso-monoarylbenzochlorins from meso-monoarylporphyrins.[74] The reaction of metal free benzochlorin **94** with 1-bromoethoxy-per-acetyl-maltose, in DMF and in the presence of potassium carbonate, gives compound **95b** in 95% yield (Scheme 12). After deacetylation of **95b** maltosylchlorin **95a** was obtained quantitatively.

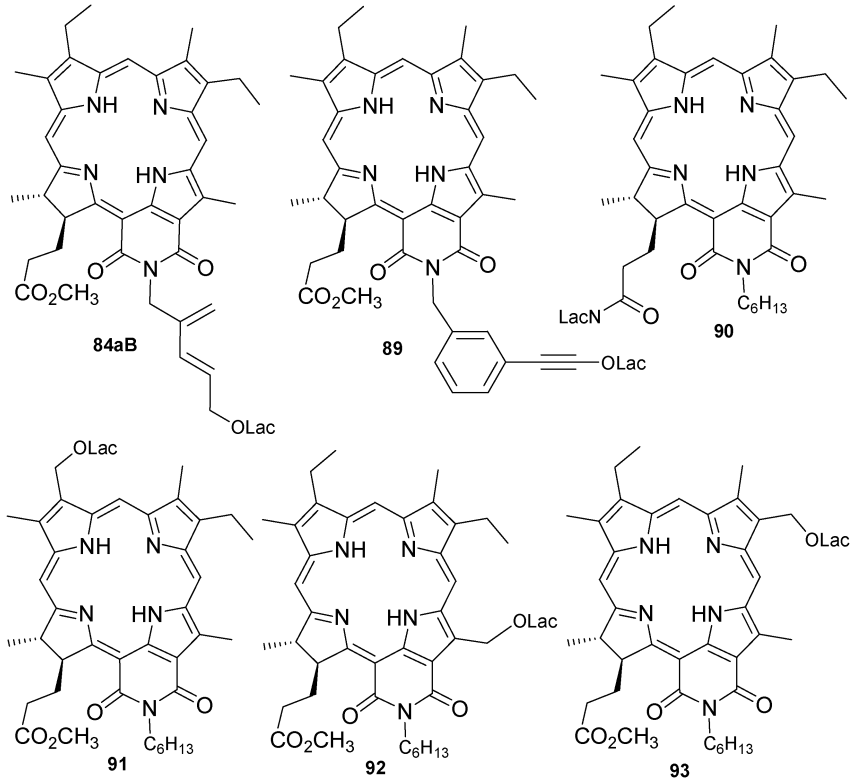
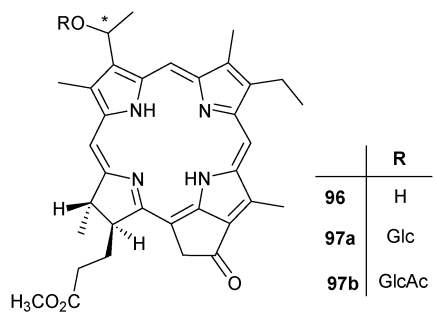


Fig. 9

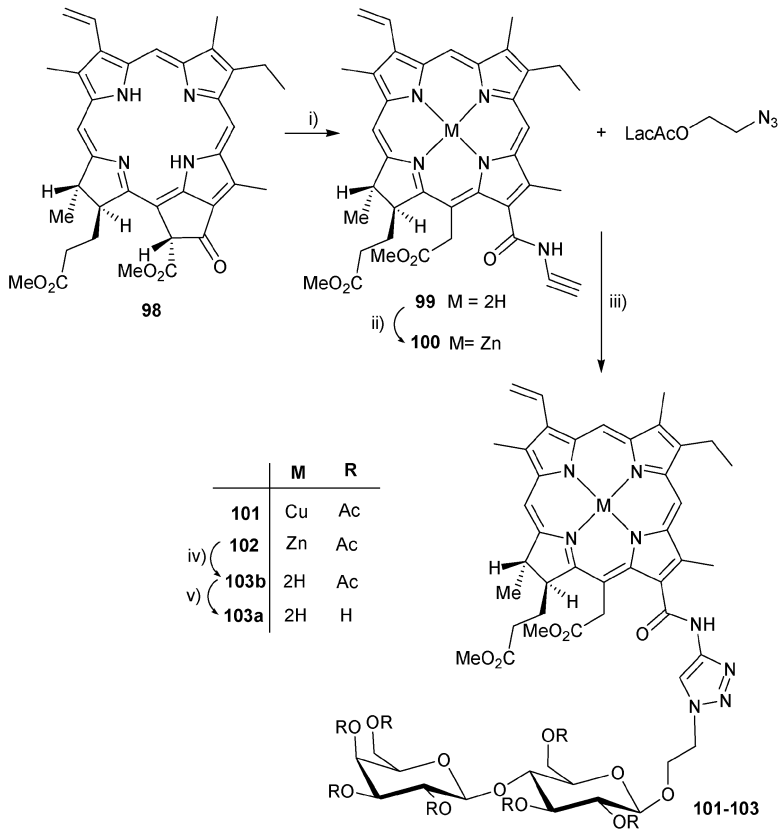
Furthermore some *O*- and *S*-glycosylated pyropheophorbide-*a* derivatives have been prepared.[75] The *O*-glycosylated pyropheophorbide derivative **97** was obtained by carbohydrate per-acetate glycosylation acid catalyzed method of derivative **96** (Fig. 10). The reagent proportions and the reaction times have been shown to rule the reaction yield. The best results were found when the reagents ratio was 2:1:2 (glycoside: pyropheophorbide:Lewis acid). Under these conditions the reaction yield was 36% with 83% of anomeric purity. The glycosylated derivatives **97** are diastereomeric mixtures.



96, **97β/α** (83:17)

Fig. 10

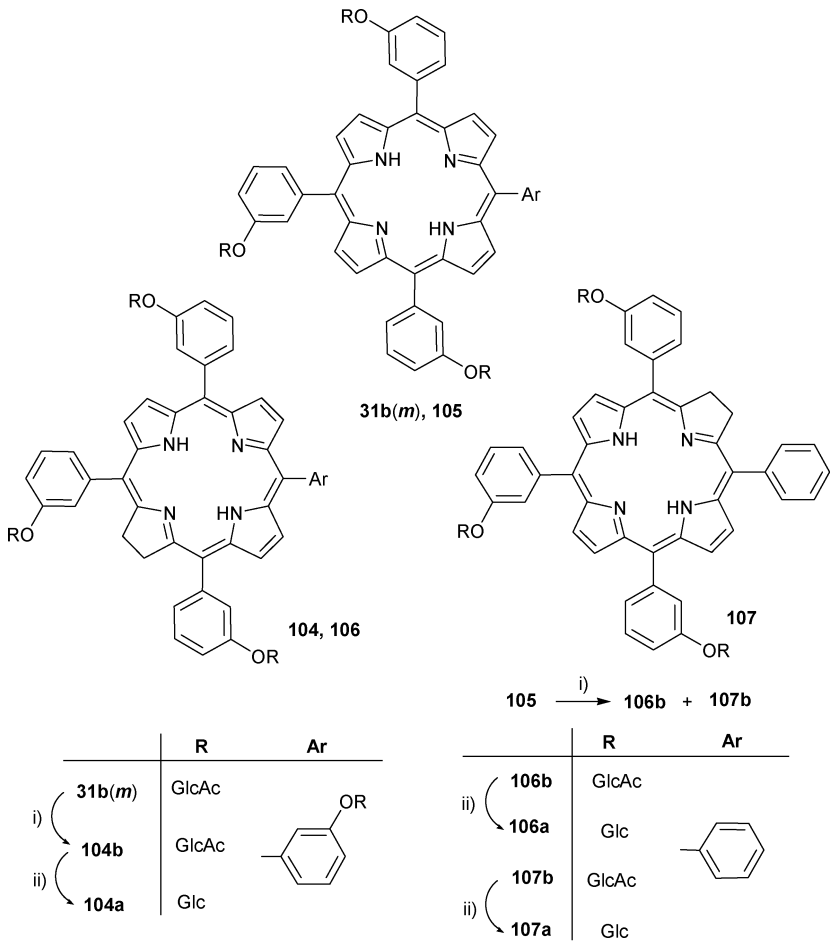
More recently the synthesis of a chlorin e_6 β-D-lactose conjugate **101** was carried out by 1,3-dipolar cycloaddition of a sugar azide to a propargyl derivative of chlorin e_6 **99**, derived from methylpheophorbide-a **98** (Scheme 13). The cycloaddition was carried out in the presence of a catalytic amount of copper(I) iodide and the yield of the target products were around 80%. Using amide Zn complex **100** allowed the demetallation in a weakly acidic medium and the hydrolysis of the carbohydrate acetyl groups to get the metal-free water-soluble glycochlorin **103a**.[76]



i) propargylamine, CH_2Cl_2; ii) $Zn(MeCOO)_2$, $CHCl_3$/MeOH; iii) CuI, MeCN, Et_3N; iv) HCl; v) NaOMe/MeOH

Scheme 13

Blais *et al*. prepared tri- and tetra-meta-glucosylated chlorin derivatives.[77] The chlorin derivatives **104**, **106** and **107** were prepared from the corresponding glucoconjugate porphyrin derivatives **31b(m)** and **105**,[19] by the diimide reduction procedure[78] to achieve the reduced macrocycles (Scheme 14). The gluco-unprotected derivatives **104a**, **106a** and **107a** were obtained in quantitative yield by treatment of chlorins **104b**, **106b** and **107b** with sodium methoxide in methanol. Rreduction of the tetra-glucoconjugated porphyrin **31b(m)** gave rise to compound **104b** in 89% yield, the



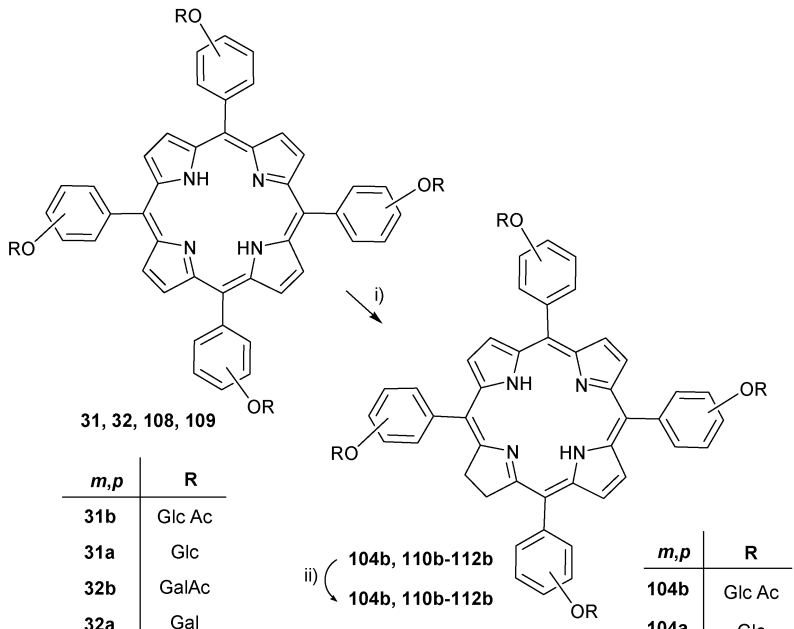
i) a) *p*-toluenesulfonylhydrazide, K_2CO_3, dry pyridine, 100 °C, argon, b) ethyl acetate, *o*-chloranil; ii) NaOMe/MeOH.

Scheme 14

triglucosylated porphyrin **105** when submitted to the same reduction process gave, in 72% yield, a mixure of the two unseparable isomeric chlorins **106b** and **107b** in a 1:1 ratio.

Yano *et al*. following the same methodology have prepared several *meta* and *para* tetra-glycoconjugate chlorin derivatives.[79] Starting with *meta* and *para* tetra-β-glycopyranosylconjugate porphyrins **31b**, **32b**, **108b** and **109b**, the chlorin derivatives **104b**, **110–112b** have been obtained after treatment with *p*-toluenesulfonylhydrazide (Scheme 15).[78] The corresponding gluco-unprotected derivatives were prepared after treatment with sodium methoxide.

More recently, Cavaleiro *et al*. described a new method to prepare novel glyco-dihydro- and diglycol-tetrahydroporphyrins (chlorins **116**, **117**, bacteriochlorins **119** and isobacteriochlorins **118**), through CuCl-catalyzed cyclopropanation reactions of α-diazoacetates derived from di-acetonides of several glycosides **113bA-bD** with meso-tetrakis(pentafluorophenyl)-porphyrinatozinc(II) **115** (Scheme 16).[10]



m,p	R
31b	Glc Ac
31a	Glc
32b	GalAc
32a	Gal
108b	XylAc
108a	Xyl
109b	AraAc
109a	Ara

m,p	R
104b	Glc Ac
104a	Glc
110b	GalAc
110a	Gal
111b	XylAc
111a	Xyl
112b	AraAc
112a	Ara

i) a) *p*-toluenesulfonylhydrazide, K_2CO_3, dry pyridine, 100 °C, N_2; b) benzene, *o*-chloranil, rt; ii) NaOMe, MeOH/$CHCl_3$.

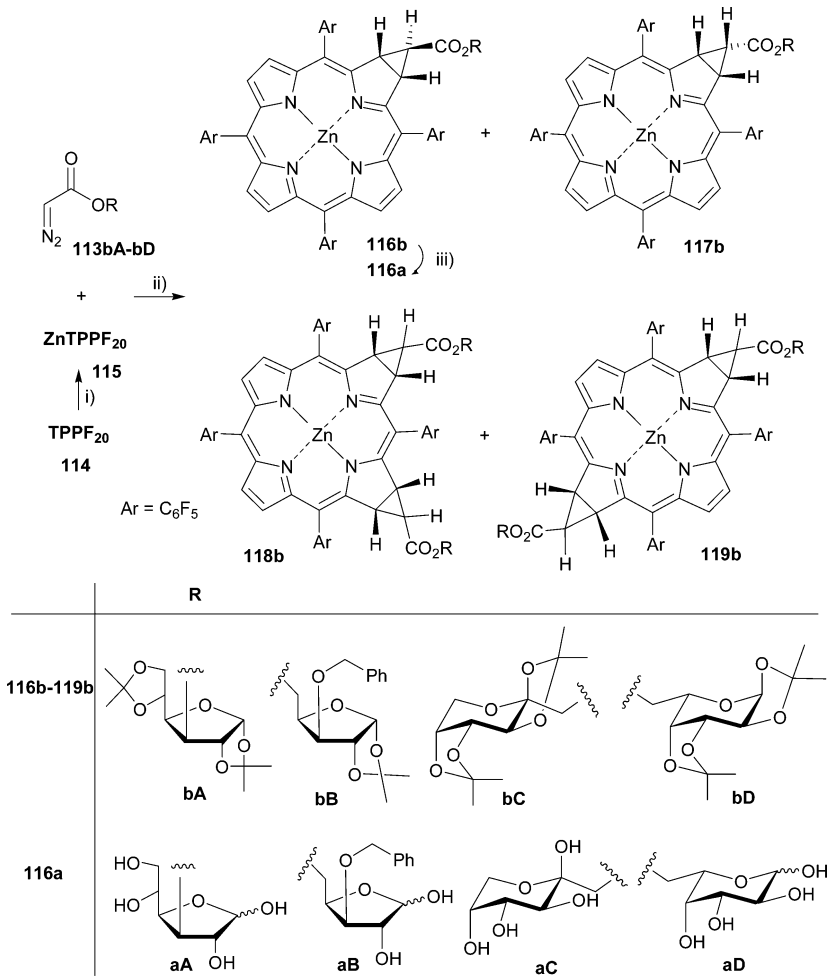
Scheme 15

2.3 *S*-Glycosyl porphyrins

Porphyrin *S*-glycosides can be considered to be good mimics of the *O*-glycoside derivatives with enhanced stability toward enzymatic hydrolysis.[80]

Two series of *S*-glycosylated porphyrins have also been designed: the derivatives from protoporphyrin-IX derivatization and the *meso*-aryl substituted porphyrins.[81–83] The most comum *S*-glycosidic moieties on these glycoporphyrins are derived from thioglucose, thiomannose and thiogalactose.

The thioglycosidic analogues **121–122** (**A–C**) have been prepared according to the general route shown in Scheme 17. Each one of the thioglycosides 2,3,4,6-tetra-*O*-acetyl-1*S*-acetyl-1-thio-β-D-galactopyranose **A**, 2,3,4,6-tetra-*O*-acetyl-1*S*-acetyl-1-thio-β-D-glucopyranose **B** and 2,3,4,6-tetra-*O*-acetyl-1*S*-acetyl-1-thio-β-D-mannopyranose **C**, was added to 2,4-bis-(2-bromoethyl)deuteroporphyrin dimethyl ester **120**, in a mixture of DMF and diethylamine, giving rise to compounds **121b** (**A–C**) in good yields (Scheme 17). The sugar acetate protecting groups can be cleaved by treatment with sodium methoxide in methanol, giving rise to compounds



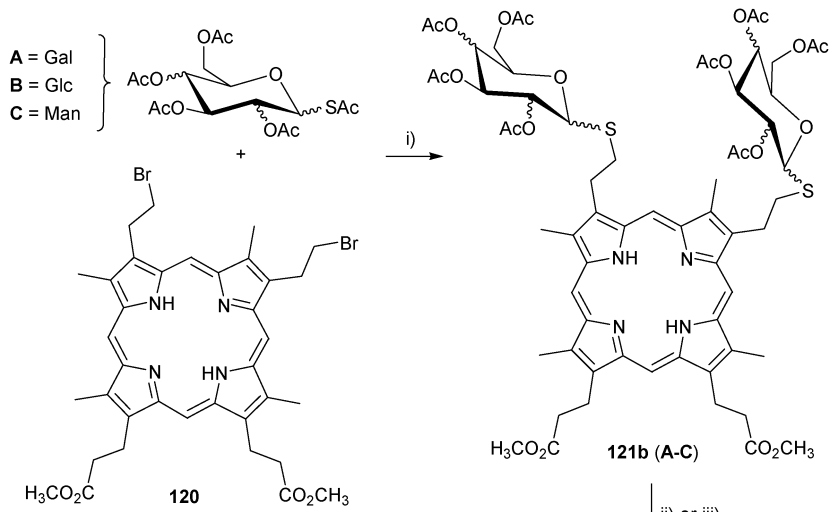
i) $Zn(OAc)_2 \cdot 2H_2O$, $CHCl_3$/MeOH (2:1), reflux; ii) CuCl (2 mol%), CH_2Cl_2, 40 °C; iii) THF/H_2O (9:1), rt, 30 min.

Scheme 16

121a (**A–C**); the acetyl groups and the methyl ester ones have been cleaved with KOH/MeOH, giving rise to the water soluble derivatives **122a** (**A–C**).[81]

The same thiosugar derivatives were condensed with the monobrominated porphyrin **123** in dry DMF, giving rise to the expected compounds **124b** in good yields and from these, by cleavage of the acetyl groups, the desired compounds **124a** have been obtained (Scheme 18).[81]

Other thioglycosylated *meso*-tetraarylporphyrins, the thiosugar-diethylene glycol porphyrin derivatives **126** and **127** (Scheme 19), were described by Maillard *et al.*[15] Condensation of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranose (**B**) or 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranose (**C**) with the 5,10,15-tris(*p*-bromoetoxyetoxyphenyl)-20-phenylporphyrin **125**, gave rise to derivatives **126b** and **127b**. These by deacetylation gave compounds **126a** and **127a** quantitatively.[15]



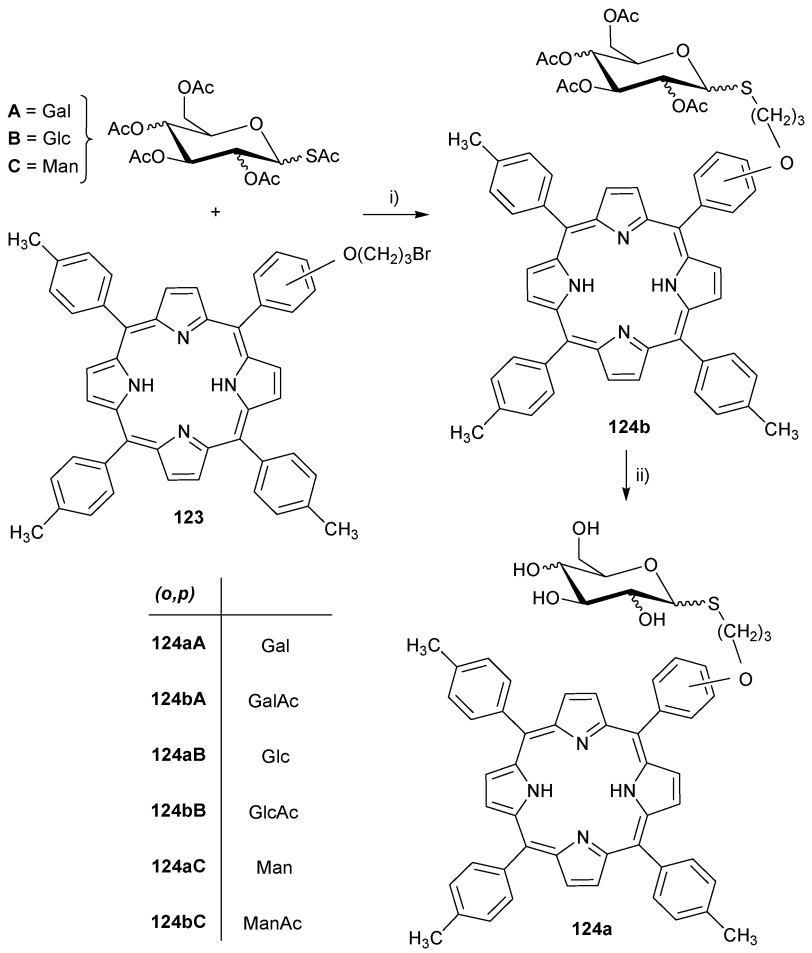
	Sugar unit	R
121aA	Gal	CH_3
121bA	GalAc	--
121aB	Glc	CH_3
121bB	GlcAc	--
121aC	Man	CH_3
121bC	ManAc	--
122aA	Gal	H
122aB	Glc	H
122aC	Man	H

i) DMF, Et_2NH, N_2, rt; ii) NaOMe, MeOH; iii) KOH, CH_2Cl_2/MeOH, reflux

Scheme 17

As already described the *p*-fluoro atom of the pentafluorophenyl groups of the *meso*-tetrakis(pentafluorophenyl)porphyrin (**$TPPF_{20}$**) can be substituted by nucleophiles, such as sulphur derivatives.[82] The synthetic route leading to several thioglycosylated compounds was based on that methodology. Tetrakis(thioglucosyl) **128b** and tetrakis(thiogalactosyl) **129a** derivatives of **$TPPF_{20}$** have been synthesised in high yields. The overall yield for compound **128a** after the three steps is 88% and the yield for **129a** is 92%.[80] The synthesis of both families can be accomplished using either the protected sugar (i) followed by deprotection (iii) or directly by using the unprotected sugar (ii) (Scheme 20).

Since glycosylated derivatives of this porphyrin core have been shown to be effective photosensitisers, inducing necrosis and/or apoptosis in several cancer cell lines, Drain's group used **$TPPF_{20}$** as a core platform to efficiently generate a variety of solution-phase combinatorial libraries.[83] This

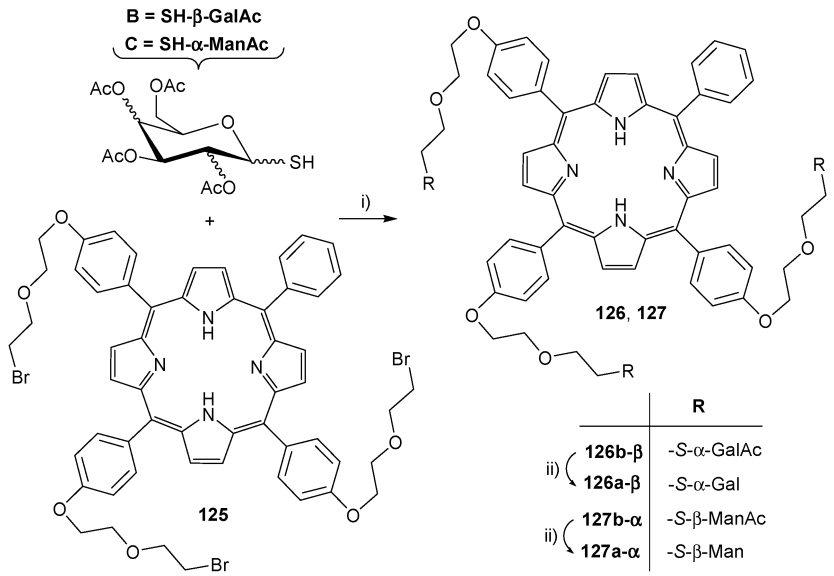


(o,p)	
124aA	Gal
124bA	GalAc
124aB	Glc
124bB	GlcAc
124aC	Man
124bC	ManAc

i) Et_2NH, dry DMF, N_2, rt; ii) NaOMe, CH_2Cl_2/MeOH

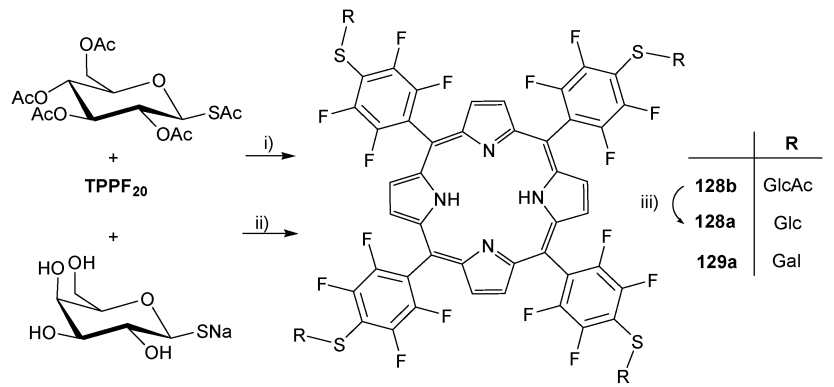
Scheme 18

approach allows a simple one step reaction that can lead to many porphyrinoids with diverse peripheral substituents. Several motifs known to be taken up by cancer cells and/or to provide amphiphilicity were used to modulate the photophysical and chemical properties of the macrocycle core (Fig. 11). Three different libraries with 21, 55 and 666 members were prepared from the addition of different thiol motifs and characterized by mass spectrometry. For example, the combinatorial library (L1) with 21-member porphyrins were prepared by reacting one equiv. of $TPPF_{20}$ with 1.4 equiv. of each one of the three thiols (per-*O*-acetyl-glucosyl- and per-*O*-acetyl-xylosylthioacetate, and 4-mercaptopyridine; in a 1:4.2 ratio of $TPPF_{20}$ to thiol) in DMF, in presence of DEA for 24 h at r.t. A statistical mixture of 21 compounds were formed (Fig. 11). The selections of the winning compounds from the libraries were performed using human breast cancer cells (MDA-MB-231). The selected porphyrins by the cancer cells, compounds with high affinities and/or uptake into the cells, were then



i) K_2CO_3, dry acetone, 20 ºC, 15 h; ii) NaOMe, MeOH, rt.

Scheme 19



i) Et_2NH, DMF, rt; ii) DMF, rt, overnight; iii) NaOMe, CH_2Cl_2/MeOH.

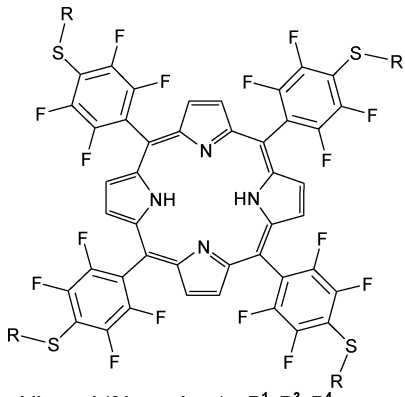
Scheme 20

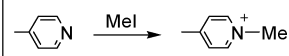
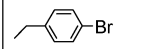
assayed by MALDI-MS. This study indicated that both glycosylation and amphipathicity are key properties for a winning photosensitiser.

Reaction between 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose and **130**, in DMSO at room temperature, gave porphyrin derivative **131** in 72% yield (Scheme 21).[82] The alkylation of **131** with a range of alkyl iodides gave the cationic products **132b–135b**, as their iodide salts.[82] Finally, after deprotection of the sugar residues using sodium methoxide in methanol, compounds **132a–135a** have been obtained.

2.4 *S*-Glycosyl chlorins

For the preparation of *S*-glycosylated pyropheophorbide derivatives **137–140** (Fig. 12), chlorin **136** was gradually added to a suspension of the



	Thiol motifs*
R^1	Glc
R^2	Glc(2-NHAc)
R^3	Xyl
R^4	
R^5	
R^6	$-(CH_2)_4CH_3$
R^7	$-(CH_2)_5CH_3$
R^8	$-(CH_2)_7CH_3$
R^9	F

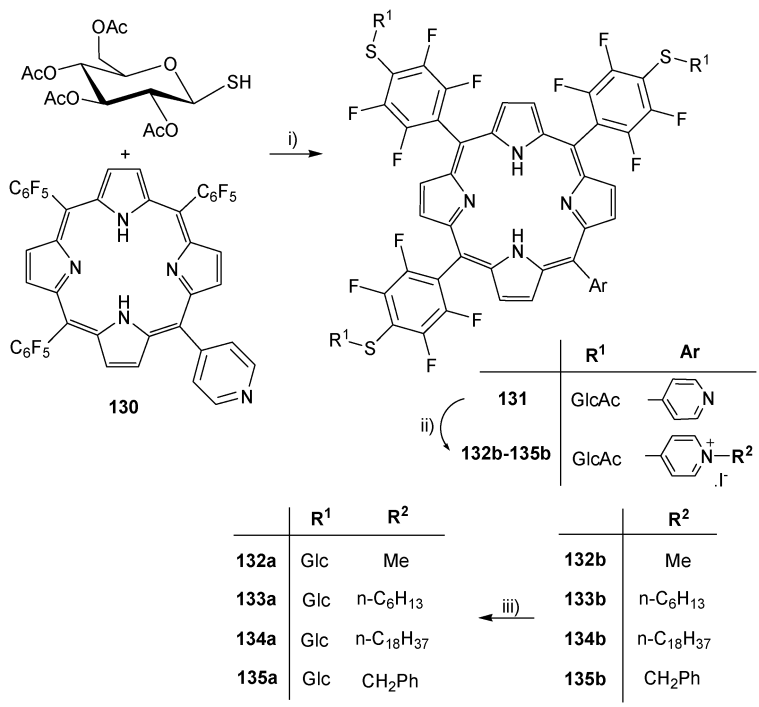
Library 1 (21-members) = R^1, R^3, R^4

Library 2 (55-members) = R^1, R^2-R^4

Library 3 (666-members) = R^1, R^3-R^9

* The sugars were used as acetate derivatives which were subsequently deprotected.

Fig. 11



	R^1	Ar
131	GlcAc	
132b-135b	GlcAc	

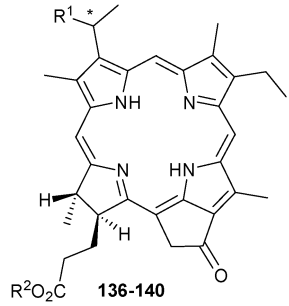
	R^1	R^2
132a	Glc	Me
133a	Glc	n-C_6H_{13}
134a	Glc	n-$C_{18}H_{37}$
135a	Glc	CH_2Ph

	R^2
132b	Me
133b	n-C_6H_{13}
134b	n-$C_{18}H_{37}$
135b	CH_2Ph

i) DMF, rt, 16 h; ii) R'-I, DMF, rt, 16 h; iii) NaOMe, MeOH, rt, 1 h.

Scheme 21

thioglycosyl derivatives, sodium β-D-glucopyranosylmercaptide and sodium β-D-galactopyranosylmercaptide.[75] The thioglucoside **137** was obtained in 54% yield, and the corresponding thiogalactoside **138** was obtained in 44% yield. In order to compare the photophysical and biological properties of all the pyropheophorbide sugar derivatives the methyl esters **139** and **140** were



	R^1	R^2
136	Br	H
137	-*S*-Glc	H
138	-*S*-Gal	H
139	-*S*-Glc	CH_3
140	-*S*-Gal	CH_3

Fig. 12

also prepared by treatment the corresponding acids with 3% sulfuric acid in methanol. The initial results obtained with all these amphiphilic conjugates **137–140** showed a tendency to aggregate in aqueous and aqueous/ethanolic solutions.

3. Biological applications

Porphyrins have demonstrated to be promising photosensitisers to be used in PDT of cancer and in other diseases. The wider applications of PDT require chemically pure photosensitisers which should be readily accessible and selective to the target, good singlet oxygen generators, with a short washout period and being soluble in physiological media. If many porphyrinic macrocycles could fulfil these features, the sugar units could confer amphiphilicity and water solubility to the macrocycle, and also allowing cancer cell targeting due to membrane receptor bindings.

The first porphyrinylsugar derivatives prepared by Frank and co-workers, the bis(D-glucosyl)isohematoporphyrin **2a** and bis(D-galactosyl)isohematoporphyrin **3a**, exhibit an unusual water solubility for a porphyrin derivative and also maintain the photophysical properties of their precursor **1**, namely singlet oxygen production.[11]

The *in vitro* and *in vivo* activity of the reduced purpurinylsugar derivatives was also evaluated in RIF cells and in C3H mice bearing RIF tumours, respectively.[67,68] Since cancer cells express galectins, in particular galectin-1 and galectin-3, the carbohydrate-recognizing proteins, to investigate the effect of the lactose moiety in the photosensitising activity, a series of purpurinimide-lactose conjugates **84aB**, **89–93** were considered. The glucosyl and galactosyl sugar conjugates **88**, **84a**, **86a** have also been evaluated. Among these analogues, the conjugate containing the lactose moiety at position 3 showed the best efficacy in comparison with the other related compounds in *in vitro* studies performed with RIF cells. In fact, among the positional isomers **91–93**, compound **91** with the lactose moiety in position 3 was 4–5 times more potent than the isomers at positions 8 and 12. When the different lactose-photosensitiser linkers among the lactose-purpurinimide conjugates **84aB** and **89–91** were compared, it was the latter which showed the best efficacy. All the carbohydrate-purpurinimide conjugates, including the monomeric conjugates **88aA** and **88aB** (galactose and glucose), exhibited almost similar efficacies in RIF cells under the same

experimental conditions (LD_{50} = 0.21 μM, 1 J/cm^2 light dose) and were almost 5-fold more effective than their nonconjugate purpurinimide **81** (LD_{50} = 1.0 μM, 1 J/cm^2 light dose).[68] However, the *in vivo* PDT activity in C3H mice bearing with RIF tumour of all derivatives shows that compound **91** produces the best long-term activity when compared with the other conjugates. Interestingly, the monomeric derivatives **88aA** and **88aB** (glucose and galactose), under similar treatment conditions, were ineffective, despite the best uptake pattern. Also the *in vivo* PDT studies in C3H mice bearing with RIF tumour the galactosylpurpurinimide **86a** and the lactosylpupurimimide derivative **84aB**, under similar treatment conditions, gave 50% tumour response at day 30.[67] The modelling studies suggest that the six membered ring of the linker diminishes the flexibility of the spacer and mimics the glucose unit of βGal(1-4)-Glc.[67] Although the global reasons for this behaviour are still unclear, molecular modelling studies about the interaction of lactose-photosensitiser conjugates with Human Galectin-1 (Gal-1) and Galectin-3 (Gal-3), β-galactoside recognizing proteins (widely studied due to their differential expression in tumour cells), have shown that the conjugation of purpurinimide with carbohydrate moieties gives high specificity for Gal-3 and the different conjugates showed differences in binding affinities.[68]

Derivative **10(p)** was evaluated against HSV-1 and HSV-2. The results obtained have shown that the presence of a sugar moiety confers higher activity when compared with the porphyrinic precursor **9(p)**.[13]

The photocytotoxicity of compounds **15** against K562 human chronic myelogenous leukemia cells has been evaluated in comparison with Photofrin®. Although the *ortho* derivative **15** showed to be more active than the *para* analogue, it is less active than Photofrin® at the same concentration. Even with the structural bases of this biological discrepancy being presently unknown, the importance of the sugar unit in the macrocycle periphery in improving the photoactivity of porphyrin derivatives is clear. Irrelevant ($\sim$15%) cell death was observed when the nonglucosylated compound **9(b)** was evaluated under the same conditions.[6]

Since the retinoblastoma is the most common malignant intraocular tumour in children and keeping in mind the severe side effects related to the therapeutic modality currently in use (carboplatin chemotherapy), PDT has been considered as an alternative treatment.[15] A series of diethylene glycol-linked *O*- and *S*-galacto/mannoconjugated porphyrins have been explored *in vitro* on a human retinoblastoma cell line (Y79). The photoefficiency of the glycosylated **21a(α,β)**, **22aα**, **126** and **127** was compared with that of the parent diethylene glycol porphyrin **20** and the corresponding monoethylene glycol-linked mannosylated porphyrin **23aα**.[15]

The biological evaluation has demonstrated that the porphyrinylsugar derivatives photoactivity is dependent on the nature of the linker and the nature and anomeric configuration of the glycoside residue.[15] The derivative **22a**α with the diethylene glycol linker showed a higher cellular uptake than the derivative **23aα** which presents mono-ethylene glycol as a linker between the sugar unit and the tetrapyrrolic macrocycle. Furthermore, the porphyrinylsugar derivatives **21a**β, **22a**α, **126a**β, **23aα** have shown a higher cellular uptake than the nonglycosylated derivative **20**. In addition, differences in

the cellular uptake between the derivatives **21a**α and **21a**β have also been found, according to which the beta anomer presented a higher uptake than the alfa analogue. This study also revealed that the cellular internalisation of the porpyrinylsugar derivatives may occur *via* an active mechanism involving a cellular transporter or receptor. It is important to point out that tumoral cell membranes can be overexpressed in such sugar receptors. This fact is supported by the inhibition effects on cellular uptake observed when the cells were pre-incubated with glucose or glycosylated albumin before incubation with the sugar photosensitiser conjugate.[15] The preliminary *in vitro* PDT treatment of Y79 cells has demonstrated that mannosylporphyrin **22a**α and galactosyl derivative **21a**α exhibit a particularly high photoactivity, although they had shown a significantly different cellular uptake.

The sugar-dependent photocytotoxic property of tetra and octaglycosylated tetraphenylporphyrins was evaluated against the HeLa cell line.[22] Although the symmetric tetra-glycosylated porphyrin derivatives **31–33** are similar to octa-glycosylated derivative **34** in terms of singlet oxygen production, the *in vitro* photocytotoxic results of the tetra-glycosylated derivatives **31–33** have shown that the latter are more efficient in killing HeLa cells than the octa-derivative **34**. Mikata attributes this lack of efficiency of **34a** to its bulky spherical structure.[22]

The photodynamic effect on red blood cells of the *para*-tetraglucosylporphyrin **31a** and its analogous with the sugar unit in *orto* position has also been analysed. Considering the possibility of the photosensitiser causing hemolysis, this study became crucial.[29] The two symmetric derivatives have proved to be ineffective in these cells. However, the asymmetric derivatives **49B** and **50B** are phototoxic and changes in hematological parameters were observed.[29]

The asymmetric derivatives were prepared in order to establish the structure-activity relationship. Indeed, the porphyrinylsugar derivatives are known to have good solubility in water and the glycosyl moieties should be functional components for cell recognition. However, the selectivity of these compounds could also be due to the hydrophilic, lipophilic balance. Krausz and co-workers have been preparing nitro and amino glycosylated porphyrins and testing them *in vitro* for their antibacterial photoactivity against *E. coli* and *S. aureus*.[23] More than thirty glycosylated compounds (protected and unprotected) were tested against two bacterial strains (Gram positive and Gram negative). From the nitroderivatives evaluated, only the two nitro glycosylated porphyrins **36B** and **36C** induced growth inhibition of Gram positive bacteria.[17,23] In respect to the amino derivatives, the photosensitisers carrying simultaneously glucosyl and amino groups displayed again a photobactericidal effect against Gram-positive bacteria. The derivative **39D** having glucosyl moieties in diagonally opposed phenyl rings is the only one that exhibited no activity. However, compound **46B** bearing three *O*-*β*-D-glucosyl units and one RGD group expressed a greater photobiocidal activity than the related compound whose glucosyl units were substituted by methyl groups.[17]

The glycosylated derivatives **51A–D** were tested against the yeast *Saccharomyces cerevisiae* and displayed different photoactivities.[24] In this case, the most photoactive compounds were the mono-glycosylated **51A** and

the bis-glycosylated **51C** derivatives. The least photoactive compound was the derivative **51B**. These differences in photoactivity could be attributed to the amphiphilic character of the porphyrinylsugar derivatives. The derivative **51A** and **51C** showed a clear separation between the hydrophobic an hydrophilic poles, in contrast to the derivatives **51B** and **51D**.[24]

The series of amino acid porphyrinylsugar derivatives **54–58** was tested against K562 human chronic myelogenous leukaemia cells and its *in vitro* photocytotoxic effect was compared to hematoporphyrin. Although the *in vitro* photoactivity of the amino acid porphyrinylsugar derivatives **55–57** induced limited immediate cell death when compared to the immediate effect of hematoporphyrin, they led to a large percentage of cell death after 24 h postirradiation incubation in the dark, provided that the incubation was longer than 60 min.[44] The compounds **54** and **58** bearing only one sugar unit have shown a lower effect, therefore confirming the importance of the number and nature of amino acids and glucose present in the macrocycle.[44] The glycoporphyrins **59–60** bearing the RGD motif were also evaluated against the K562 human chronic myelogenous leukemia cells.[45,46] These compounds were shown to be active against the K562 cell line.[45] The same research group has also tested the bis-porphyrinyl derivatives **64–68** against the same cell line; the results obtained have shown that derivatives with sugar moieties in both macrocycles **65a**, **67** display a very low activity which could be due to the high hydrophilicity of those molecules.[57] On the other hand, dimers **64a** and **66**, which are amphiphilic molecules, were shown to be more active against the K562 cell line.[57] Thus, dimer **64a** was the most active one.

Therefore, the efficacy of the photoactivity is once again influenced by the hydrophilic/lipophilic character of the compounds.[57,58]

Considering that severe health hazards and diseases that can be induced by the adhesion and proliferation of bacteria on the surface of numerous materials, bioactive materials incorporated in the surfaces will be required. The cellulose-functionalised with porphyrin **69–71** and **80** were shown to kill gram positive and gram negative bacteria upon irradiation with visible light. Such materials could be used in industrial, household and medical environments, and more generaly in areas that would benefit from permanent and efficient surface disinfection.[62–64]

Blais *et al*. prepared tri- and tetra-meta-glucosylated chlorin derivatives.[77] The aim was to assess how the sugar units linked to chlorin derivatives affect the photoactivity, cell internalization and subcellular localization in HT29 human adenocarcinoma cells. This was compared with the action due to meso-tetrakis(*m*-hydroxyphenyl)chlorin, a compound being formulated as photosensitiser (Foscan®) for palliative treatment of head and neck cancers.

The presence of the sugar moieties in the chlorin structures brought a change in the octanol-water partition coefficient. Also the biological evaluation carried out with these compounds revealed that the tetra-glucoconjugated chlorin **104a** was poorly internalized and weakly photoactive but the asymmetrical triglucosylated chlorins **106a** and **107a** exhibit a high *in vitro* photoactivity and a preferential mitochondrial affinity. In spite of the lower levels of cell uptake of **106a** and **107a** compared to Foscan®,

the phototoxicity of the triglucosylated compounds was higher. The cellular uptake and the cells localization seem to be correlated with the amphiphilicity of the compounds. These results have encouraged the evaluation of the photodynamic activity of the glucosylated chlorins *in vivo* and to study their metabolism *in vivo* or in cell based assays. The *in vivo* metabolism is an important issue when considering the use of glycoconjugated compounds in PDT treatment, since cleavage of the glycoside bond by glycosidases will result in modifications of amphiphilic properties, biodistribution, blood clearance and drug-cell interaction.[84] There is indication that the glycosidase activity is higher in certain tissues especially in tumoral tissues when compared to normal ones. Glycosidase promoted metabolism of glycoconjugated porphyrins and chlorins can thus occur after administration *in vivo* of these compounds. However, a certain specificity and stability can be achieved. In fact, studies carried out on the *in vitro* metabolism of compound **106a** and **107a** have demonstrated that the three sugar moieties in chlorins **106a** and **107a** undergo sequential hydrolysis and also there is dehydrogenation of the macrocycles to the corresponding porphyrins.[84]

All the deacetylated glycopyranosyl conjugated derivatives studied were biologically evaluated *in vitro* with HeLa cells and the results compared with those due to tetrasulfonated porphryrin (TPPS4).[79,85] The glycochlorins **110a**(*m*,*p*)-**112a**(*m*,*p*) showed higher uptake by HeLa cells than TPPS4 and cellular uptake similar or greater than the corresponding porphyrins **32a**(*m*,*p*), **108a**(*m*,*p*) and **109a**(*m*,*p*).

Thioglycosylated derivatives has been shown to resist to the enzymatic hydrolysis of the β-thioglucosydic bonds by β-glucosidases.

The new derivatives **121a** (**A–C**) and **122a** (**A–C**) were evaluated for their photocytotoxic activities in comparison with Photofrin®, the commercial anti-cancer hematoporphyrin derivative formulation. Compounds **121a** (**A–C**) have revealed to be more PDT efficient than Photofrin®, but surprisingly compounds **122a** (**A–C**) have shown to be inactive.

The thioglycosylated meso-tetraarylporphyrins **124**(*o*,*p*) (**A–C**) photocytotoxicities were evaluated against K562 chronic leukaemia cell lines.[81]

All these derivatives showed significant photocytotoxic activity, with a phototoxic effect lower than the one observed with the commercial formulation Photofrin®, when the dead cells were counted immediately after irradiation.

The tetrakis(thioglycosyl) **128a** and **129a** derivatives can act as potent photosensitisers in their potential medicinal applications, this being due to their important photophysical properties.[80]

4. Concluding remarks

It is well established that sugars and porphyrin macrocycles play vital roles in living organisms. Several medicinal applications are also known for such groups of compounds. Facing the potentialities demonstrated by porphyrin derivatives against cancer and microorganisms, even those which are antibiotic resistant species, it can be expected that in the near future big developments will appear concerning the appearance of new drugs. Having

in mind the present knowledge about chemistry and biological properties of porphyrins and sugars, it is expected that new sugar-porphyrin conjugates will come and will play a major role for Man's welfare.

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Sugars as chiral synthons in the preparation of fine chemicals

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1. Introduction

The material reviewed in this paper covers *only* the last five years, with emphasis on the most recent results. It will not be comprehensive and will deal only with such transformations of simple sugars (and to some extent disaccharides), which lead to interesting optically pure compounds of (at least potential) biological interest, or to novel monosaccharides with the skeleton substantially different from the parent sugar. No simple classical sugar chemistry such as: selective protection-deprotection, or standard transformations and application of properly blocked sugars as chiral auxiliaries will be reported. Also, no application of simple sugars for the preparation of achiral materials (such as *e.g.* hydroxymethylene furfural[1]) or aromatic derivatives with sugar-like substituents[2] will be discussed.

This material will be divided into several main topics describing the methodology of the synthesis of important classes of compounds, which will be illustrated by selected examples.

2. From simple sugars to cyclic (non-sugar) derivatives: transfer of chirality

One of the most important classes of sugar mimetics are, undoubtedly, imino sugars/aza sugars. The first report on their biological activity as glycosidase inhibitors goes back to 1970's. Since then, these polyvalent molecules were introduced to the clinical applications; several iminosugars are now available on the pharmaceutical market. The growing interest in the application of such compounds in the clinical use resulted (especially in the last decade) in high activity for development of new (even more efficient than the existing ones) synthetic methods. An excellent review on the synthesis and therapeutic applications of imino sugars was recently presented.[3]

Other class of polyhydroxylated derivatives acting as sugar mimics consists of carbasugars *i.e.* sugars in which the ring-oxygen atom is replaced by the carbon atom. Their synthesis can be initiated either from achiral (or racemic) derivatives (this approach will not be dealt with) or more conveniently from simple sugars (chirons). Syntheses of these important compounds were comprehensively reviewed recently.[4]

Synthesis of β-lactam antibiotics from sugars (either as chiral auxiliary or chiron), describing the general methodology developed in 1990s by Chmielewski and based on [2 + 2] cycloaddition of isothiocyanides to sugar olefins, was also comprehensively reviewed.[5]

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2.1 Aza sugars and imino sugars

The synthesis of these important compounds is very well explored. They can be now prepared almost routinely by a number of methods including the RCM cyclization of the properly activated sugar diolefins, 1,3-dipolar cycloaddition of nitrones and olefins (recent review ref. 6) or other types of cyclizations.

2.1.1 The 1,3-dipolar cycloaddition. The interesting approach to aza-disaccharides in which the aza-part is linked to 'normal' sugar was proposed by Martin.[7] The sugar nitrone **1** reacted with 5-*O*-allyl-galactofuranoside to afford adduct **2** (Fig. 1).

A simple synthesis of a 7-membered aza sugar from the unsaturated aldehyde **4** was proposed by Moutel.[8] The same aldehyde **4** was used in the synthesis of modified aza sugars such as **5** (Fig. 2).[9]

An interesting approach to polyhydroxylated 7-membered branched aza sugars (*e.g.* **8**) was proposed by Estevez.[10] Nitro sugar **6** underwent the

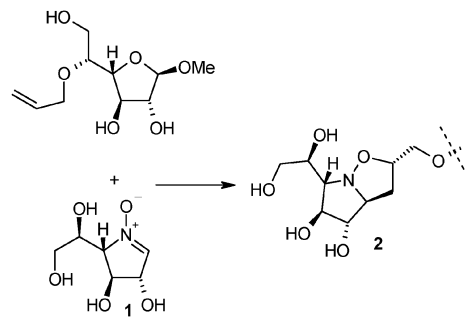


Fig. 1 Synthesis of aza-disaccharides *via* 1,3-dipolar cycloaddition.

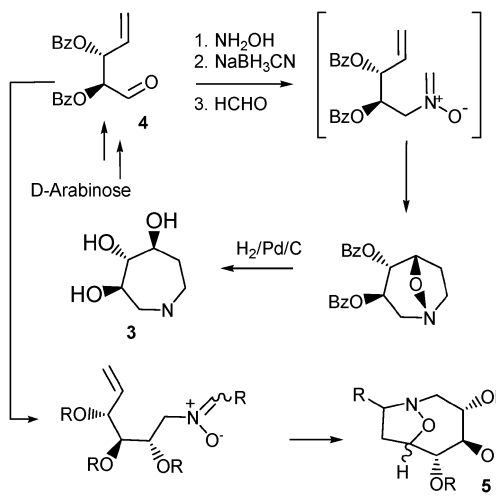


Fig. 2 Synthesis of 7-membered aza sugar derivatives.

Henry reaction with formaldehyde to afford **7**, which was converted into the target derivative **8** (Fig. 3).

Synthesis of bicyclic aza sugars from D-xylose was reported. A key step involved reaction of the nitrone **9** with ethyl acrylate providing intermediate **10**, which was finally converted into aza sugars (Fig. 4).[11]

The 1,3-dipolar cycloaddition of nitrone **12** (derived from D-glucose) was also used for the preparation of bicyclic aza sugars of type **14** (route **a** Fig. 5).[12] Another classical approach (route **b**) allowed to prepare the analogs (*e.g.* **17**).[13,14]

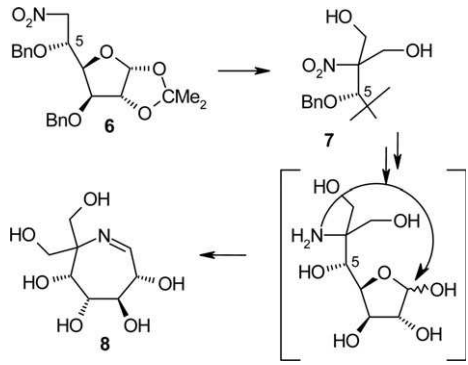


Fig. 3 Approach towards branched aza sugars.

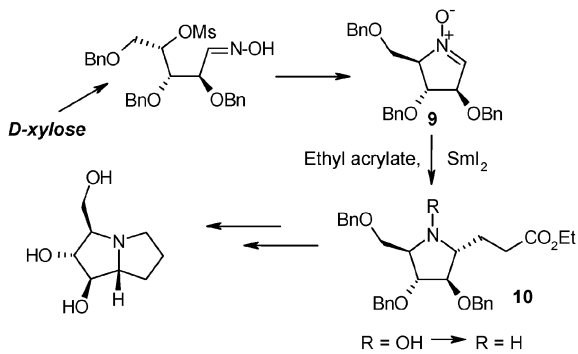


Fig. 4 Bicyclic azasugars obtained from D-xylose.

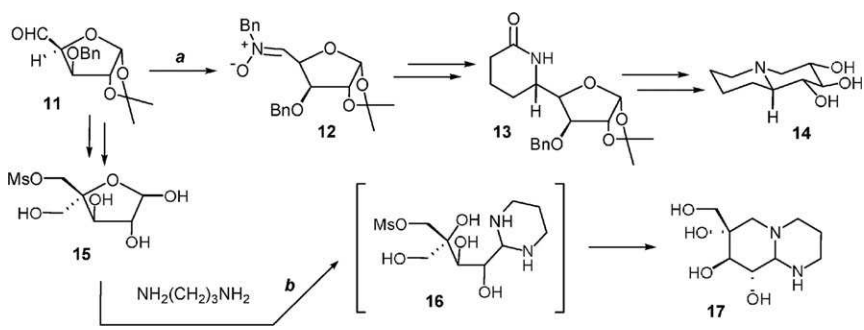
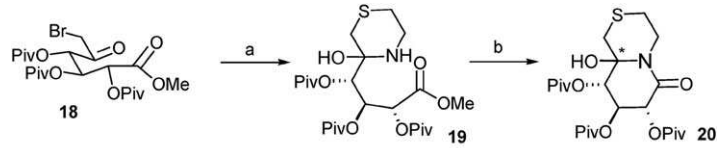


Fig. 5 Dipolar cycloaddition route towards bicyclic aza sugars.



(a) $HS(CH_2)_2NH_2$, CH_3OH; (b) $NaCNBH_3$, CH_3OH then Na_2CO_3, $CHCl_3$.

Fig. 6 Approach to fused bicyclic aza sugar derivatives.

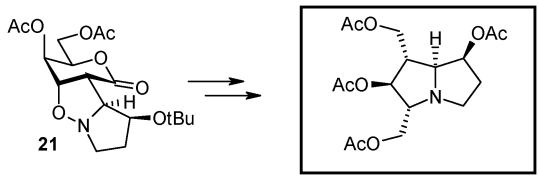


Fig. 7 1,3-Dipolar cycloaddition as key step in the synthesis of 1-homoaustraline.

Interesting structural variation of target aza sugars was initiated from compound **18**, readily available in a few standard steps from methyl glucoside (Fig. 6).[15]

The 1,3-dipolar cycloaddition of a five-membered cyclic nitrone derived from malic acid and unsaturated D-*threo*-hexonolactone led to a single adduct **21**, which was transformed into 1-homoaustraline *via* a sequence of well defined reactions (Fig. 7).[16] Synthesis of similar derivatives was presented recently.[17]

2.1.2 RCM approach to aza sugars. RCM approach to aza sugars, exemplified in Fig. 8, is used as standard method.[18]

N-allylamine **23** (obtained by standard transformations of 6-iodoglucoside **22**) underwent cyclization into the monocycle **24**. However, when the amine was *in situ* protected as a Boc derivative it could be subjected to the RCM process. The products were further converted into the bicyclic aza sugar **26**.[19] A similar approach to eight-membered ring aza sugars was recently reported (Fig. 9).[20]

The bicyclic aza sugar **28** was prepared by Laventine[21] using RCM as the key step (Fig. 10).

Preparation of polyhydroxylated 6-oxanortropane glycomimetics (*e.g.* **32**), structurally related to the glycosidase inhibitor family of the calystegines, was reported. The synthetic strategy involves the furanose/piperidine

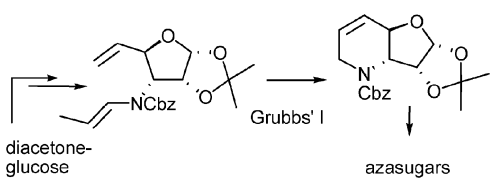


Fig. 8 Synthesis of aza sugars *via* RCM strategy.

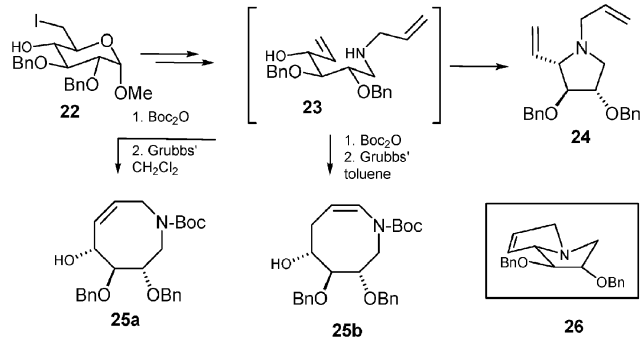


Fig. 9 Application of RCM in the synthesis of eight-membered ring aza sugars.

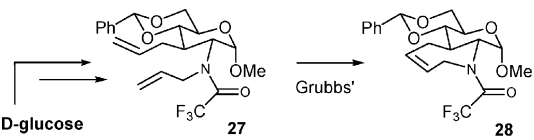


Fig. 10 Approach towards bicyclic derivatives of aza sugars.

rearrangement of 5-deoxy-5-ureido-L-idose precursors, followed by intramolecular glycosylation involving the primary hydroxyl group (Fig. 11).[22]

Another approach, based on mercuration of unsaturated sugar **34** was proposed by Zhang (Fig. 12).[23]

The first examples of the eight-membered iminoalditols were obtained from 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose by ring-closing metathesis. The (2*R*,3*R*,4*R*,5*S*)-2-hydroxymethyl-azocane-3,4,5-triol, with D-*gluco* configuration at the C2–C5, is a weak inhibitor of glycosidases.[24] The RCM reaction using Grubbs' II catalyst proceeded smoothly to give the epimeric mixture of azocanes **38** in 73% yield (Fig. 13).

Several, optically pure imino sugars have been synthesised by Fleet[25] from simple mono-saccharides in a short, high yield synthetic sequences. The novelty of the presented approach involved an application of benzhydryl protecting group which allowed to avoid epimerisation at C-2 (Fig. 14).

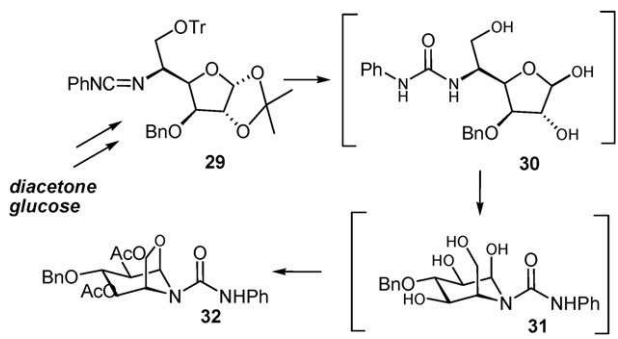


Fig. 11 Furanose/piperidine rearrangement in the synthesis of 6-oxanortropane glycomimetics.

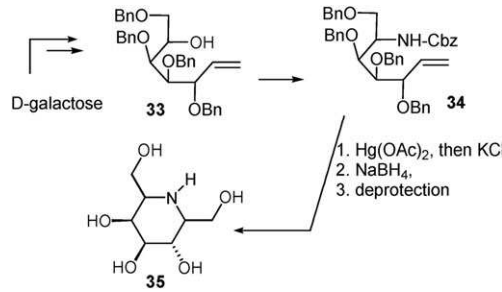
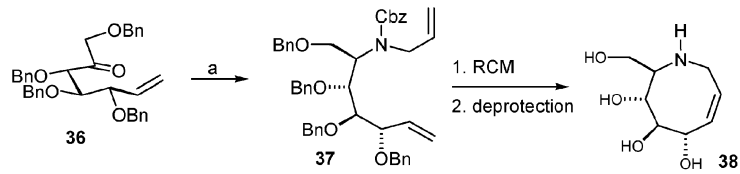


Fig. 12 Synthesis of a simple aza sugar based on mercuration of its unsaturated precursor.



a. (i) allylamine, AcOH, $NaBH_3CN$, MS 3A, MeOH, (ii) CBzCl.

Fig. 13 Example of eight-membered ring iminoalditol obtained *via* RCM.

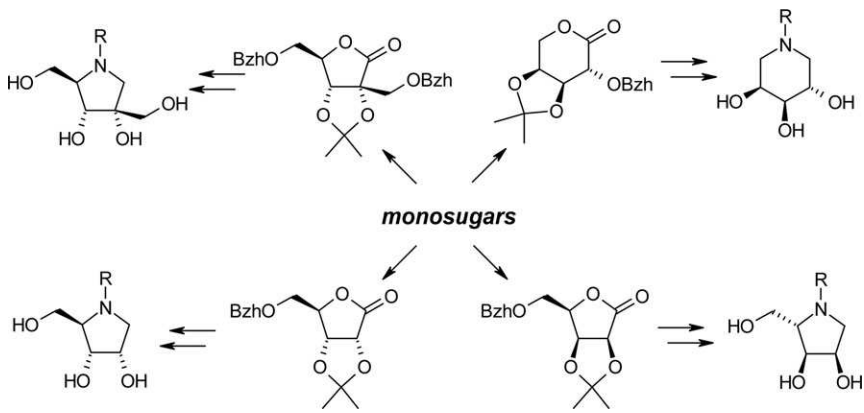


Fig. 14 Application of benzhydryl protecting group for the efficient synthesis of imino sugars.

2.2 Carbasugars

The first convenient synthetic method leading to such derivatives was reported by Ferrier in 1979 and since then the so-called Ferrier-II rearrangement became the major method used for their preparation.[26] Modification was proposed by Sinaÿ, who applied the reagent triisobutylaluminum (TIBAL) to induce the rearrangement.[27] Another useful general method to prepare carbasugars (with various size of the ring) consists of metathetic cyclization of sugar derived acyclic diolefins (Fig. 15).[28]

Besides these general methods a number of others such as 1,3-dipolar cycloaddition, aldol condensation *etc*. are applied to prepare carbasugars.

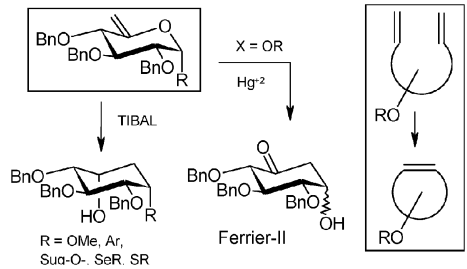


Fig. 15 Major synthetic strategies to carbasugars.

2.2.1 Monocyclic carbasugars. The 1,3-dipolar cycloaddition was used in the synthesis of polyhydroxylated compound with the seven-membered ring (Fig. 16).[29]

Similar approach to polyhydroxylated (amino) hexitols was proposed by Chakraborty (Fig. 17).[30]

The same group proposed also a general route to carbocycles of type **49**.[31] Starting from D-glucose, the precursor **46** was prepared in a few standard steps; it could be used either for the synthesis of imino sugars or carbocycles as shown in Fig. 18.

The key transformation in the synthesis of carbanucleosides, reported by Horvath, was based on the Ferrier-II rearrangement and led to the important derivative: ara-cyclohexenyl-A (Fig. 19).[32]

Approach to such compounds was also accomplished by the 1,3-dipolar cycloaddition of the proper precursor **53** (Fig. 20).[33]

Another method used for the preparation of carbanucleosides was based on the RCM cyclization of simple sugar derivatives as shown in Fig. 21.[34]

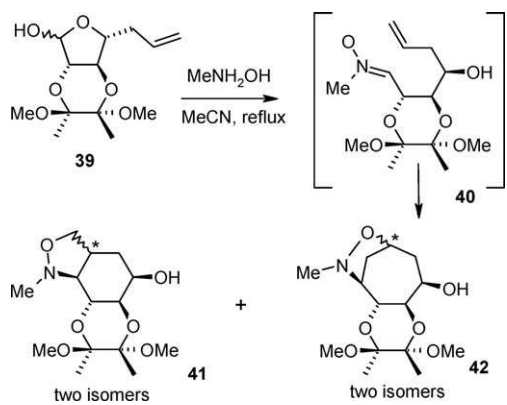


Fig. 16 Application of cycloaddition for the synthesis of 7-membered ring carbasugar derivatives.

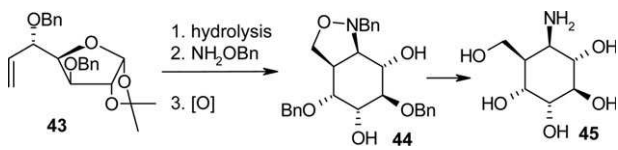


Fig. 17 Synthesis of polyhydroxylated hexitols.

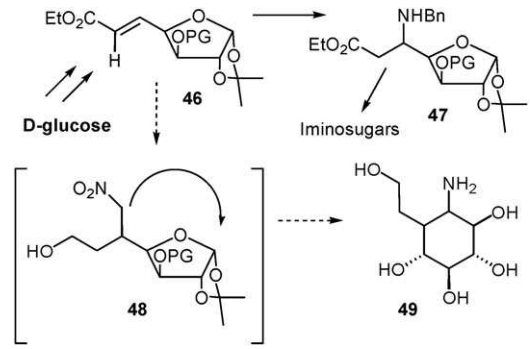


Fig. 18 Imino sugars and carbocycles obtained from D-glucose.

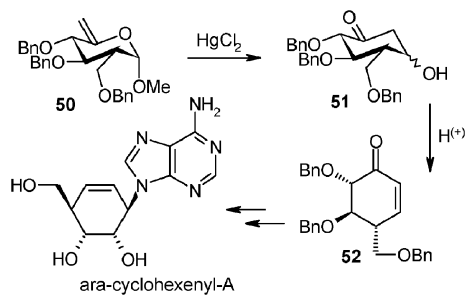


Fig. 19 Synthesis of carbanucleosides based on the Ferrier-II rearrangement.

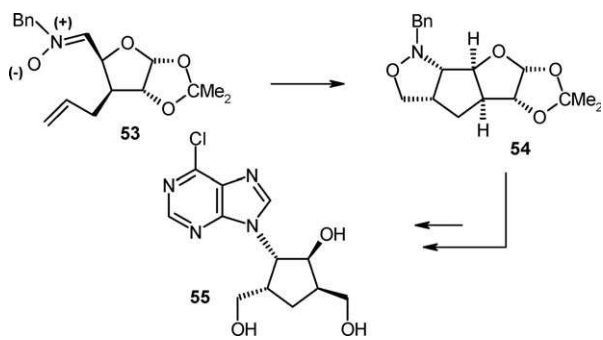


Fig. 20 Carbanucleosides *via* the 1,3-dipolar cycloaddition.

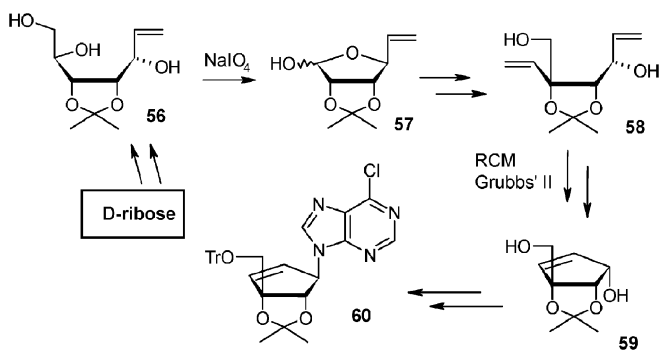


Fig. 21 Route towards carba-nucleosides based on RCM.

A [4.3.0]bicyclic nucleoside precursor, containing an unsaturated hydroxylated 3′,4′-*trans* linkage, has been efficiently synthesized from diacetone-D-glucose. The key-step involved the ring-closing metathesis (RCM) of **62** with the second generation Grubbs' catalyst (Fig. 22).[35]

An interesting approach to both enantiomers of biologically active cyclophellitol, based on the latent symmetry concept, from the same common starting material D-xylose was proposed by Kireev *et. al*. Functionalization of this monosaccharide at the C-1 provided compound **64**, while similar processes initiated from the '*end*' (C5) afforded the **ent-64** (Fig. 23).[36] Proper functionalization of these intermediates led to both enantiomers of cyclophellitol.

Another approach to (+)-cyclophellitol, proposed by Madsen, was based on the RCM cyclization of the sugar diolefin **68** (Fig. 24).[37]

A general method for the preparation of carbasugars with various ring size consists of the metathesis of properly prepared sugar diolefins.

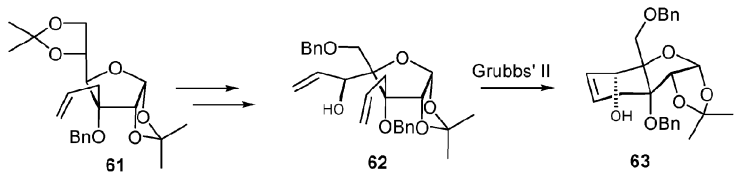


Fig. 22 Key step towards unsaturated carbanucleoside involving RCM.

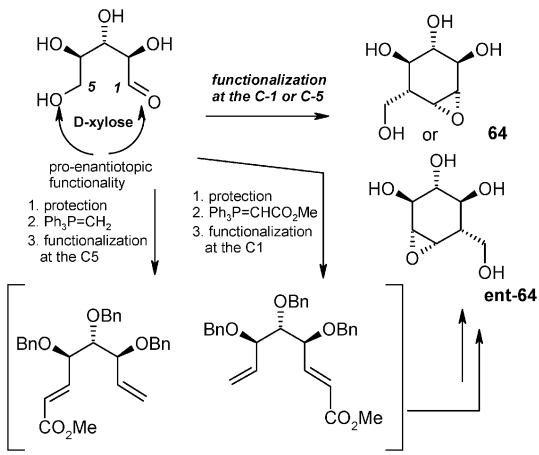


Fig. 23 Approach towards both enantiomers of cyclophellitol from D-xylose.

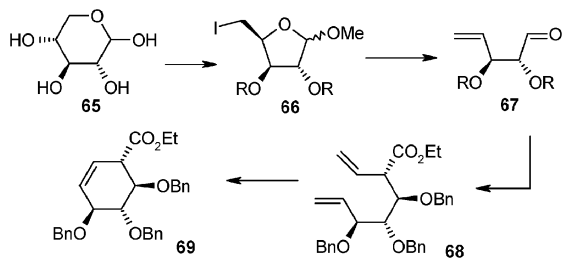


Fig. 24 (+)-Cyclophellitol obtained by RCM strategy.

Preparation of penta-substituted C8-glycomimetics (*e.g.* **72**) and their glycosidase inhibitory activity was recently studied (Fig. 25).[38]

The popular methodology for the construction of carbocyclic ring consists of the intramolecular aldol condensation of properly activated monosugars. This may be exemplified by transformation of **73** into the dicarbonyl derivative **74** which, upon treatment with base (or acid), provided the desired carbocycle (Fig. 26).[39] Other differently substituted derivatives were also prepared by this approach.[40]

Synthesis of the potential herbicide 5-fluoro-(+)-MK7607 was recently described, starting from the properly protected monosaccharide, which was transformed into the intermediate difluorocarbasugar **79**[41] (Fig. 27).

An interesting 6-*exo*-dig radical cyclization leading to carbasugars was reported by Lopez (Fig. 28).[42]

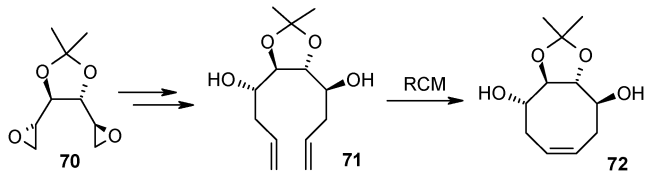


Fig. 25 Glycosidase inhibitor obtained by RCM.

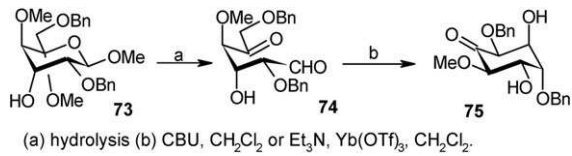


Fig. 26 Carbasugar derivative obtained by intramolecular aldol condensation.

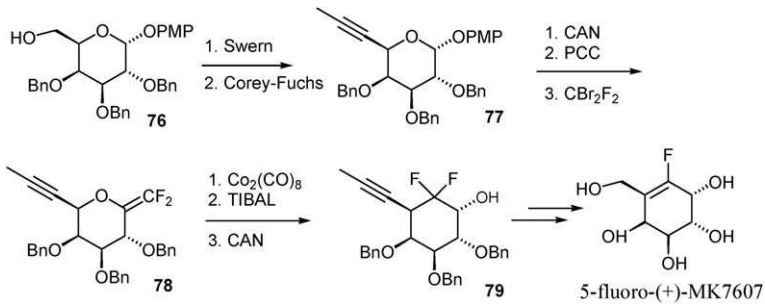


Fig. 27 Synthesis of the difluorocarbasugar **79**, a precursor of 5-fluoro-(+)-MK7607, from a properly protected monosugar.

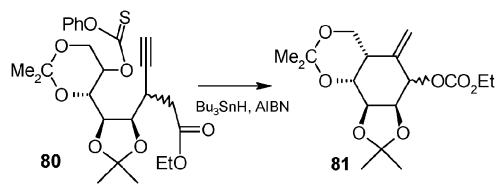


Fig. 28 Application of radical reaction in the synthesis of carbasugar derivatives.

2.2.2 Bicyclic derivatives. Polyhydroxylated carbo-bicyclic derivatives may be regarded as carbasugars with the rigid structure resulting from the presence of the additional carbocyclic ring. The most convenient way for construction of the bicyclic skeleton consists of the Diels-Alder reaction of properly functionalized trienes (intramolecular version) or dienes and olefins (intermolecular).

An interesting approach to carbobicyclic polyhydroxylated compounds was proposed by Jarosz.[43] It was based on a Lewis acid transformation of sugar allyltins **82** into dieno-aldehydes **83** with the *E*-geometry across the internal double bond exclusively. The Wittig-type reaction of **83** afforded a triene, which underwent cyclization under high pressure, providing derivatives of bicyclo[4.3.0]nonane **84**. Alternatively, dienoaldehyde **83** was converted into the phosphonate **85**, which afforded the bicyclo[4.4.0]-decene derivatives (**86**) upon reaction with an aldehyde and simultaneous cyclization of the resulting triene.[43] (Fig. 29).

Transformation of such derivatives into fully hydroxylated compounds was performed by rather standard methods.[44]

The Diels-Alder reaction between the diene **87** and the olefin **88** afforded the adduct **89** in good yield (Fig. 30).[45]

Pursuing the synthesis of *angelmicin B*, Mootoo prepared the tricyclic intermediate fragment **93** from D-xylose derivative **90** (Fig. 31).[46]

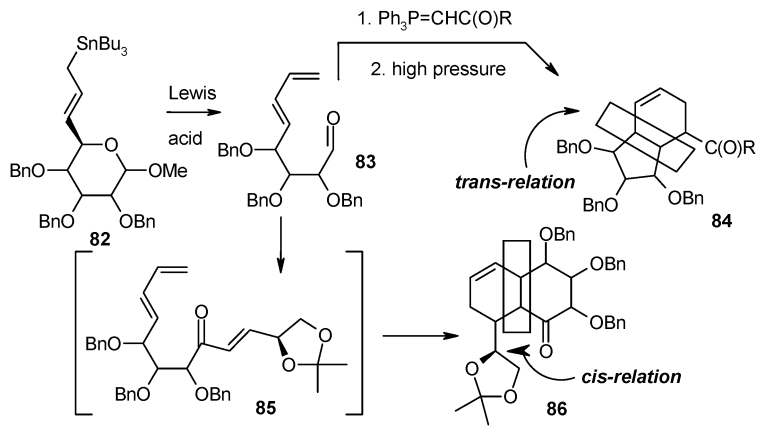


Fig. 29 Synthetic route towards polyhydroxylated bicycles.

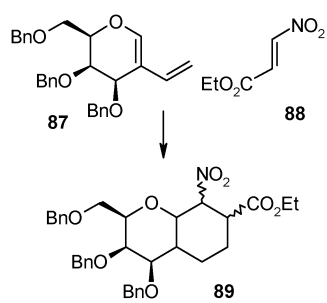


Fig. 30 Diels-Alder cycloaddition producing a bicyclic monosugar derivative.

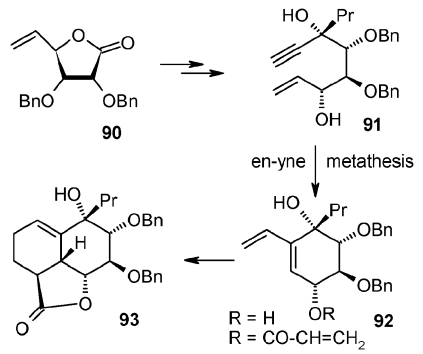


Fig. 31 Synthesis of a tricyclic framework using ene-yne metathesis and Diels-Alder reaction.

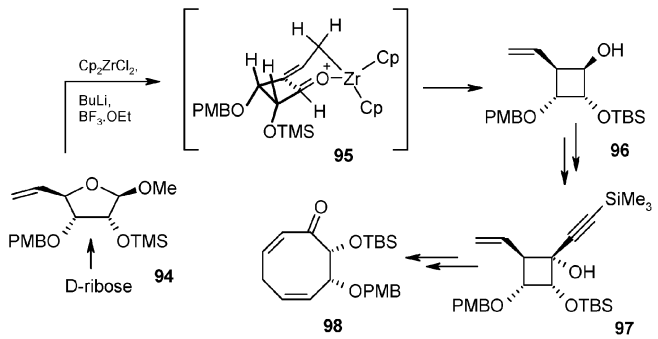


Fig. 32 Synthetic route from D-ribose towards cyclooctane derivative.

An approach to derivatives of polyhydroxylated cyclooctane from D-ribose was proposed by Paquette.[47] The crucial step involved a ring scission induced with zirconocene (Fig. 32).

3. Modified carbohydrates

This chapter deals with the preparation of rare sugars, including so-called higher carbon sugars (with more than 10 carbon atoms in the chain) and 'normal-sized' carbohydrates with highly modified structure (with exception of aza sugars and carbasugars already mentioned in 2.1 and 2.2). Unusual transformations of monosaccharides will also be briefly discussed. No common standard reactions of simple sugars will be reported.

3.1 Higher carbon sugars

Sugar allyltins (mentioned already in 2.2.3.) were used for the preparation of higher carbon sugars. This may be illustrated by reaction of the furanoside **99** with di-*O*-isopropylidene-D-arabinose performed under high pressure, which provided compound **100** (Fig. 33).[43,48]

Another approach to higher carbon sugars is based on the Wittig-type condensation between two properly activated sugar sub-units. The

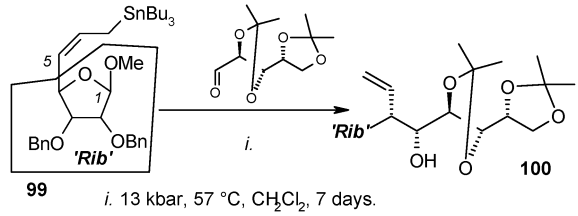


Fig. 33 Synthesis of higher carbon sugars.

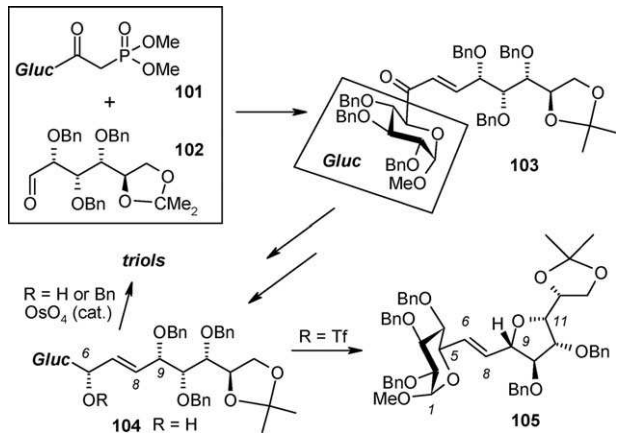


Fig. 34 Approach to higher carbon sugars *via* the Wittig-type reaction; Unusual rearrangement of the enone induced by triflate leaving group.

phosphonate **101** reacted with the open-chain D-*gluco* aldehyde **102** to afford the enone **103**. Reduction of the carbonyl group provided allyl alcohol **104** (+ diastereoisomer), which was oxidized (OsO_4) to a triol (Fig. 34). The interesting S_N2' rearrangement (with the participation of the benzyloxy group) was observed upon treatment of the alcohol with triflic anhydride.[49]

Several polyhydroxylated natural products with the α,β-unsaturated lactone ring (*e.g.* anamarine) display significant pharmacological activity. A new useful approach to such compounds was presented recently (Fig. 35).[50]

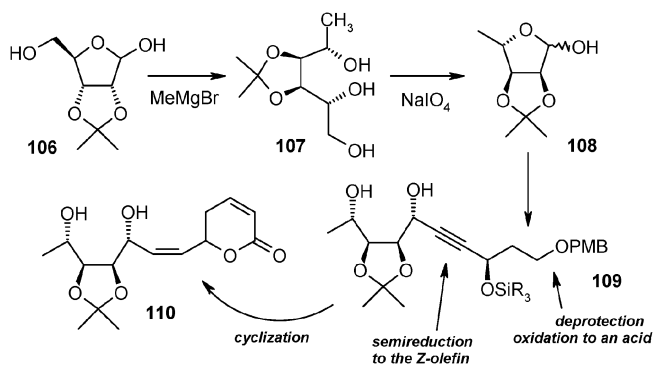


Fig. 35 Unsaturated sugar lactones obtained from monosaccharides.

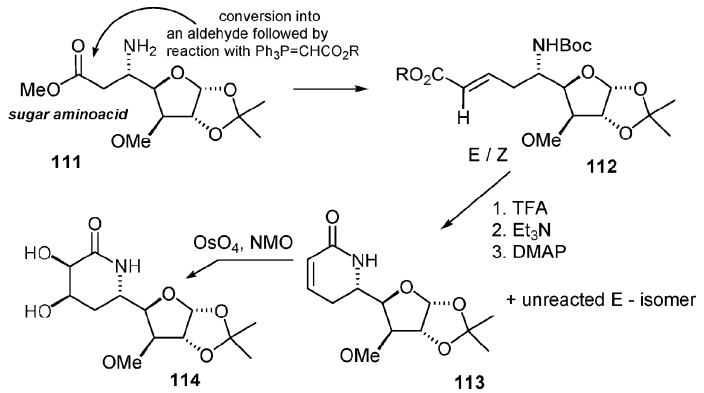


Fig. 36 Aza disaccharides from sugar aminoacids.

Stereoselective synthesis of higher aza disaccharides from *C*-linked carbo-β-aminoacids was proposed by Indian scientists (Fig. 36).[51]

Similar derivatives (formally being *C*-disaccharides with the 'normal' and aza sugar rings) were prepared by Vogel (Fig. 37).[52]

An elegant synthesis leading to the α,β-unsaturated polyhydroxylated lactones possessing an antifungal activity was proposed by Ramana (Fig. 38).[53]

The 1,3-dipolar cycloaddition between the sugar nitrone and sugar olefins was proposed[54] and an example is shown in Fig. 39.[54c]

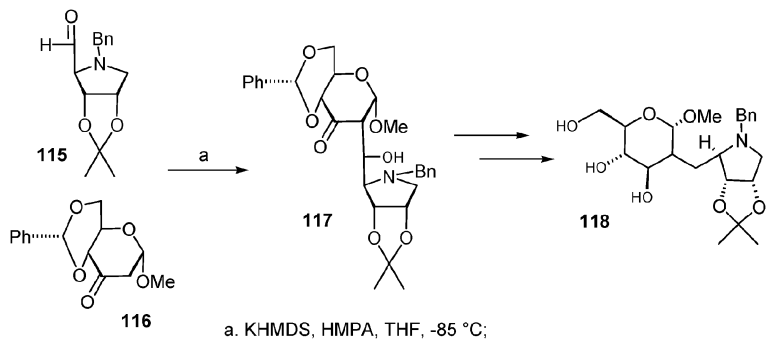


Fig. 37 Vogel's approach towards aza *C*-disaccharides.

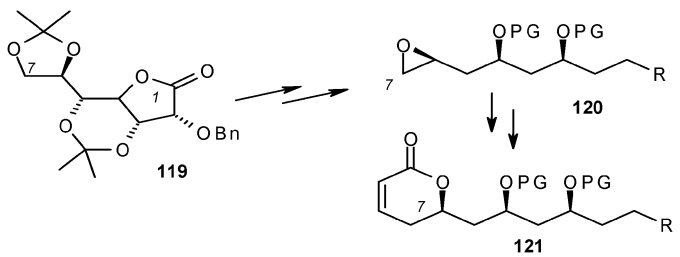


Fig. 38 Synthesis of antifungal polyhydroxylated lactones.

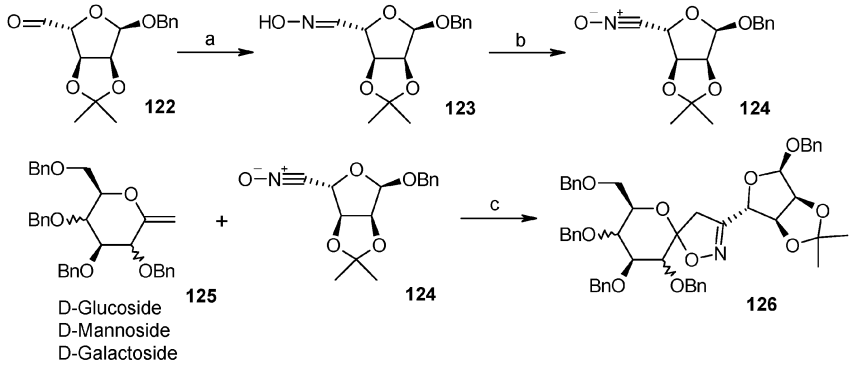


Fig. 39 Dipolar cycloaddition methodology in the synthesis of disaccharide derivatives.

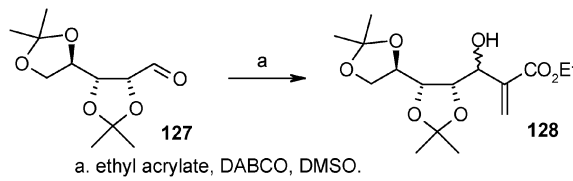


Fig. 40 Baylis-Hillman reaction as a key step in the synthesis of heptoses.

The synthesis shown in Fig. 40 provided access to heptoses,[55] but according to our definition, this is not a 'higher sugar' synthesis. However, the approach to such derivative was based on the Baylis-Hillman reaction of acyclic sugar-derived aldehydes, a reaction not commonly applied in sugar chemistry and worth to mention in this review.

3.2 'Normal-sized' sugars with highly modified structure

An elegant route to glycophostones (*e.g.* **131**)—cyclic phosphonate analogs of biologically relevant sugars was proposed by Hanessian.[56] Similar derivative **132** from diacetono-mannose was prepared by Cristau (Fig. 41).[57]

The convenient approach to analogs of the naturally occurring glucosidase inhibitor salacinol, was presented by Pinto.[58] In a number of papers he described the synthesis of such derivatives. The general motif is shown in Fig. 42.

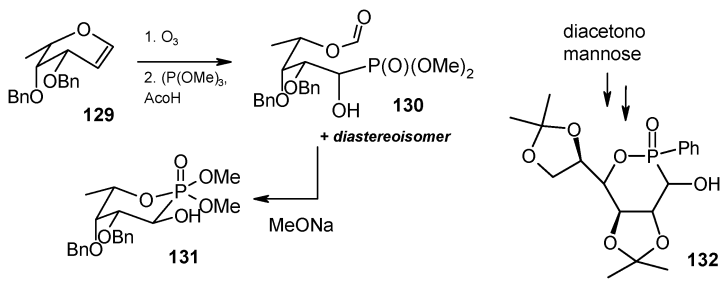


Fig. 41 P-analogs of monosugars.

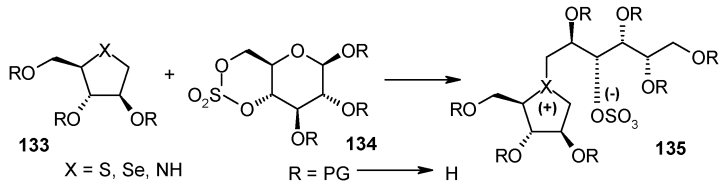


Fig. 42 Approach to the synthesis of salacinol analogs.

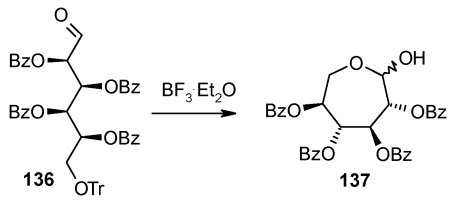


Fig. 43 Septanose derivatives obtained from hexose.

An elegant route to derivatives of septanose from the open-chain hexoses has been reported (Fig. 43).[59] The protected septanose **137** was converted in few standard steps into free septanosides.

Rauter's group exploited the synthesis of sugar derived bicyclic butenolides (*e.g.* **140**, Fig. 44),[60] which possess cytotoxic and antitumor activities. The key structural feature of such compounds consists of the presence of the α,β-unsaturated lactone, which allows them to act as Michael acceptors for the addition of enzymes' nucleophiles.

Another interesting example of the application of sugars in the synthesis of bioactive compounds was presented by Nicotra's group.[61] In this approach fructose was used as the starting material for the preparation of a series of GABA ligands, having a considerable therapeutic potential (Fig. 45).

The intramolecular cyclization of the open chain glucose derivative **143** leading to substituted tetrahydrofuran **146** was observed by Cumpstey (Fig. 46).[62]

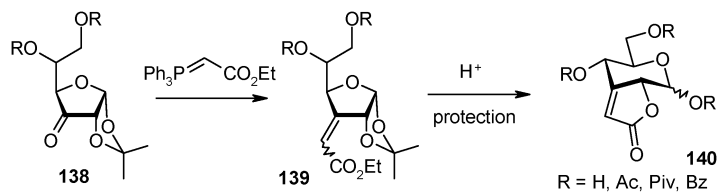


Fig. 44 Cytotoxic and antitumor agents obtained from monosugars.

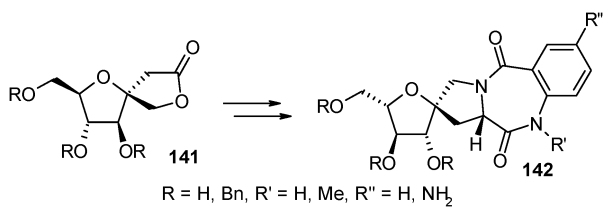


Fig. 45 GABA ligands obtained from fructose.

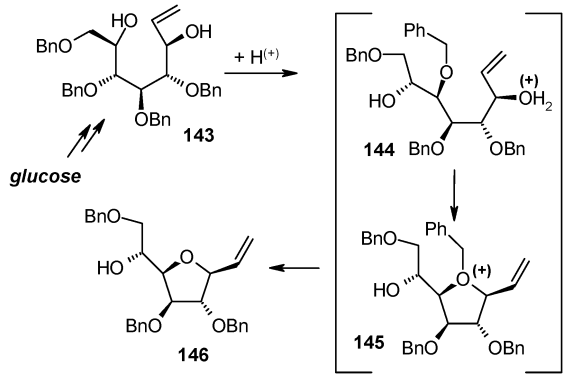


Fig. 46 Acid induced cyclization towards THF derivative.

Thioaldoses exhibit remarkable biological activities, such as inhibition of glycosidase. For example, 5-thio-D-glucose inhibits D-glucose transport across membranes and also the release of insulin. Treatment of **149** with DIC/HOBt as coupling reagents gave, after cyclisation, 4,5-*O*-isopropylidene 6-thio-D-galactono-1,6-lactone (Fig. 47).[63]

The radical approach to natural product xylobovide starting from D-glucose was reported (Fig. 48).[64]

The synthesis of 1,2,3-triazole-fused tetracyclic compounds *via* intramolecular 'click chemsitry' approach was presented (Fig. 49).[65]

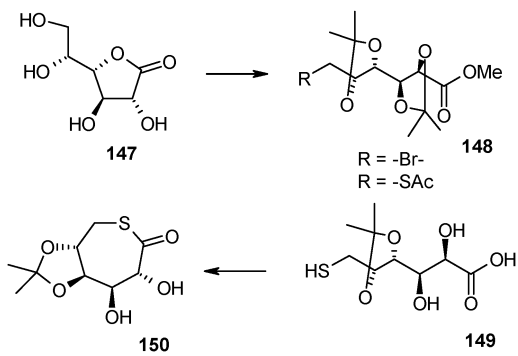


Fig. 47 Preparation of thioaldose derivative.

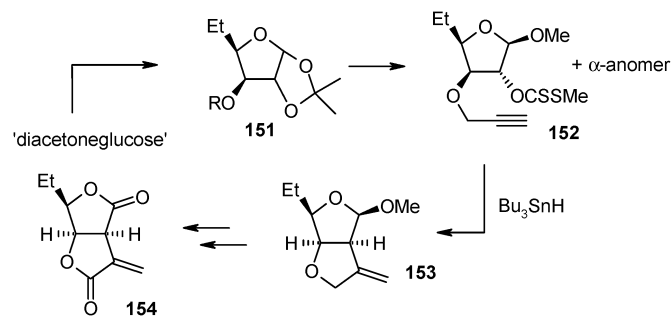


Fig. 48 Synthesis of xylobovide *via* a radical 5-exo-dig cyclisation.

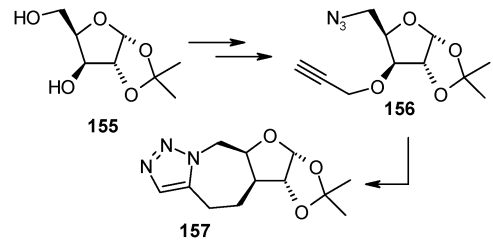
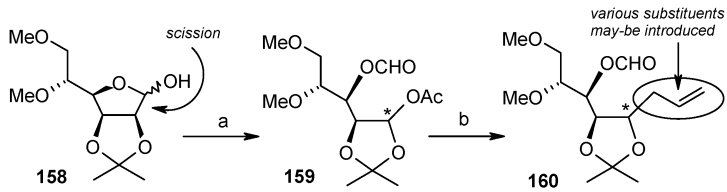


Fig. 49 'Click' chemistry approach to tetracyclic monosugar derivatives.



(a) $PhI(OAc)_2$, I_2, CH_2Cl_2, hv; (b) CH_2=$CHCH_2TMS$, BF_3OEt_2, CH_2Cl_2.

Fig. 50 Novel methodology for preparation of highly functionalised polyhydroxylated compounds.

An interesting ring scission involving breaking of the C1–C2-bond induced by $PhI(OAc)_2$ was observed by Spanish scientists;[66] an example is shown in Fig. 50. Compound **159**, obtained after the scission is usually a mixture of stereoisomers differing in configuration at the newly created stereogenic center (original C-2), with one strongly predominating (Fig. 50).

Tributyltin radical mediated cyclization of the glucose derived exo-methylene furanose derivatives led to highly functionalized *cis*-fused bicyclic ethers. The product could subsequently be transformed into optically active tricyclic nucleoside analogue or oxepine derivative (Fig. 51).[67]

Synthesis of a sugar derived allene and its intramolecular silver mediated etherification followed by ring closing metathesis has been explored for building the tricyclic framework of eunicin (Fig. 52).[68]

Novel highly functionalized dipeptide isosters were synthesized *via* diastereoselective alkyl-arylation protocol of a glucose-derived (R)-*tert*-butanesulfinylimine. One of these novel sugar amino acid derivatives, a D-Ala-Ser/Thr isostere, was applied in a peptide synthesis protocol to afford a cyclic tetramer (Fig. 53).[69]

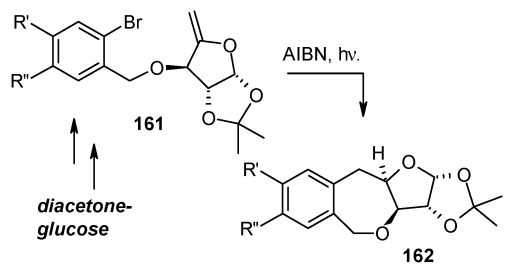


Fig. 51 Radical approach towards highly functionalised fused bicyclic ethers.

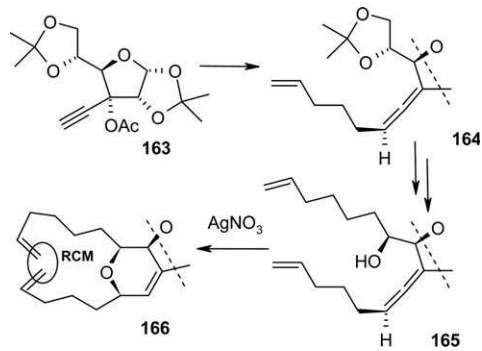


Fig. 52 Silver catalysed cyclisation in the synthesis of tricyclic skeleton of eunicin.

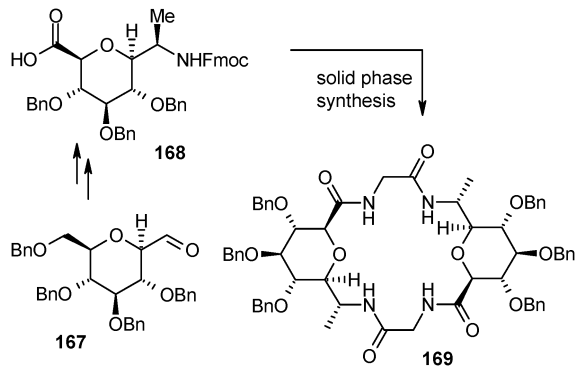


Fig. 53 Synthesis of macrocyclic sugar aminoacids.

An interesting entry to macrocyclic sugar-receptors was proposed and other compounds of this type were also prepared (Fig. 54).[70]

An efficient convergent approach has been developed for the construction of novel, nonnatural polysubstituted carbohydrate-based macrolides. A key step in the synthesis consisted of formation of a macrocyclic ring *via* a ring-closing metathesis (Fig. 55). The obtained macrolide analogs have been screened for biological activity against Gram-positive and Gram-negative bacteria, yeasts, and molds.[71]

Fleet reported the synthesis of a new class of carbopeptoid-cyclodextrin, cyclo[(6-amino-6-deoxy-D-galactonic acid)$_4$] with a 28-membered ring in which

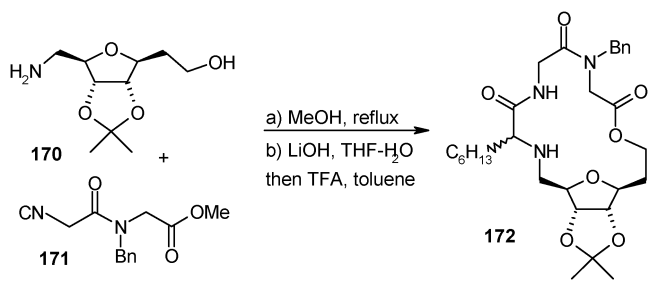


Fig. 54 Sugar-based macrocyclic receptors.

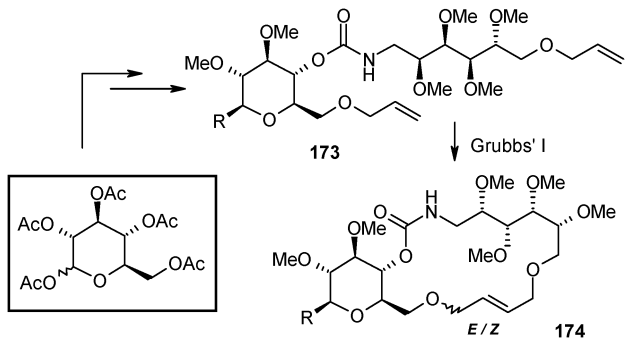


Fig. 55 Synthesis of macrolide analogues from sugars.

the key step consisted of cyclisation of a protected linear fully hydroxylated tetramer obtained in several steps from D-galactonolactone (Fig. 56).[72]

Stereoselective intramolecular oxymercuration of carbohydrate derivatives was proposed as the key reaction for efficient preparation of mono- and dihydroxylated unsymmetrical bis-tetrahydrofuran skeletons present in naturally occurring biologically active acetogenins. The *trans*- and *syn*-selective intramolecular oxymercurations were explored in an enantioselective synthesis of the bis-tetrahydrofuran skeleton of mucoxin (Fig. 57).[73]

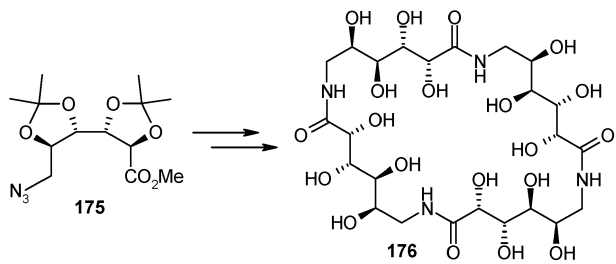


Fig. 56 New class of carbopeptoid-cyclodextrins.

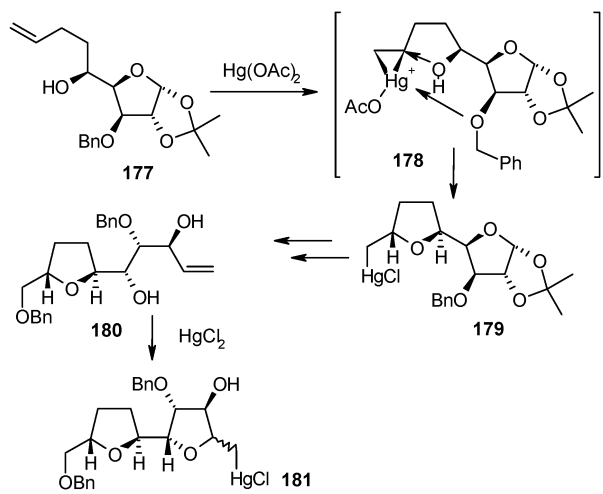


Fig. 57 Synthesis of bioactive bis-tetrahydrofurans *via* oxy-mercuratio.

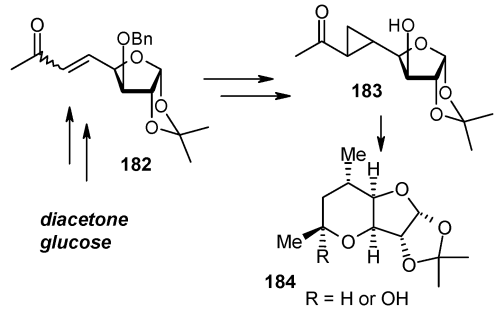


Fig. 58 Approach to the polyketide acid unit of nagahamide A.

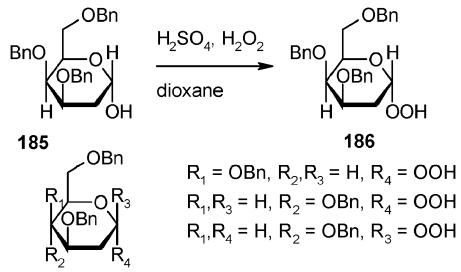


Fig. 59 Anomeric hydroperoxides from 2-deoxy-sugars.

This approach was also used to prepare the C10–C34 fragment of asimitrin.[74]

A carbohydrate based approach to the synthesis of polyketide acid unit present in *nagahamide A* has been reported. Reductive transformation of **183** followed by cyclization allowed to prepare **184** (Fig. 58).[75]

Anomeric hydroperoxides are readily prepared by treatment of 2-deoxy sugars with H_2O_2 in the presence of acid (Fig. 59). They are used as reagents for enantioselective epoxidation of α,β-unsaturated olefins (*e.g.* chalcone); in the presence of sodium hydroxide, the epoxidations showed exceptionally high asymmetric induction.[76]

4. Transformation of disaccharides

Application of disaccharides to the synthesis of fine chemicals is restricted mostly to sucrose and lactose.

Sucrose is the most abundant disaccharide isolated from natural sources. All aspects of its chemistry were recently comprehensively reviewed.[77] In this paper the new applications of sucrose as synthons in the preparation of fine chemicals will be reported.

The easily available hexa-*O*-benzylsucrose (**187**) was used as a starting material for the preparation of macrocyclic receptors. Several derivatives of type **188** with various cavities and different number of nitrogen and oxygen atoms were prepared (Fig. 60).[78] The azacrown macrocycles showed remarkable enantioselectivity towards α-phenylethylammonium cations. For example diazacrown derivative **188** (Y = O; X = NBn; n = 2) did

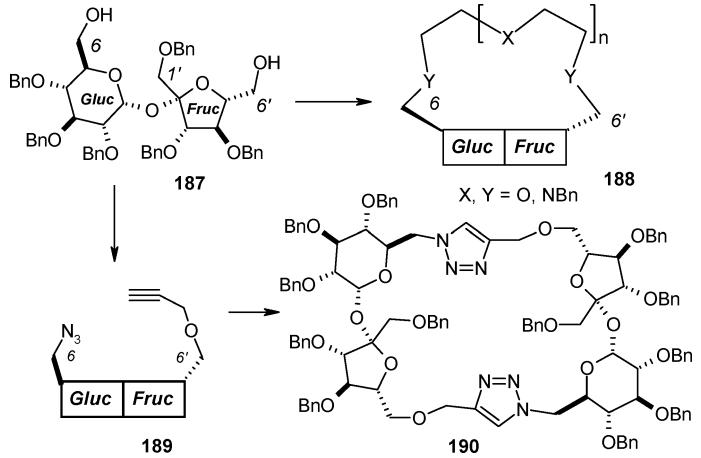


Fig. 60 Synthesis of various types of macrocycles from sucrose.

not form any complex with the *R*-amine while with the *S*-isomer the stability constant was $K_a = 945\ M^{-1}$.[79]

The partially protected sucrose with the free terminal positions was also applied to the synthesis of macrocycles with higher symmetry using either metathesis[80] or click-chemistry approach (exemplified in Fig. 60).[81]

The convenient method to obtain sucrose based polymers was proposed by Barros's group.[82] The preparation of a monomer is depicted in Fig. 61. Selective protection of the 6′-OH (fructose part) followed by benzylation of the remaining seven hydroxyl groups and regeneration of the 6′-OH afforded the monoalcohol. Reaction of this derivative with crotoyl chloride (and others) provided the monomer **191** ready for polymerization (Fig. 61).

An approach to modified disaccharides (utilising the 'click' chemistry methodology) was proposed by Nilsson (Fig. 62).[83]

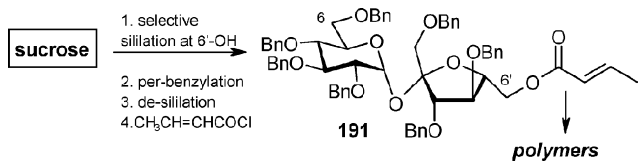


Fig. 61 Preparation of sucrose based monomers.

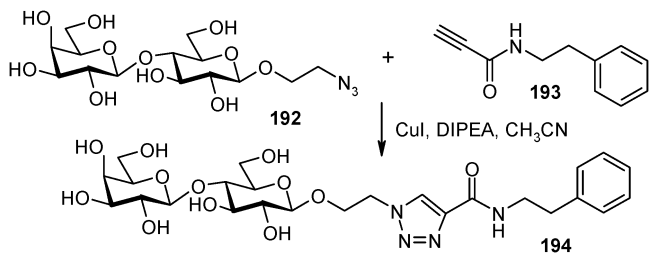


Fig. 62 Modification of disaccharides by the 'click' cycloaddition protocol.

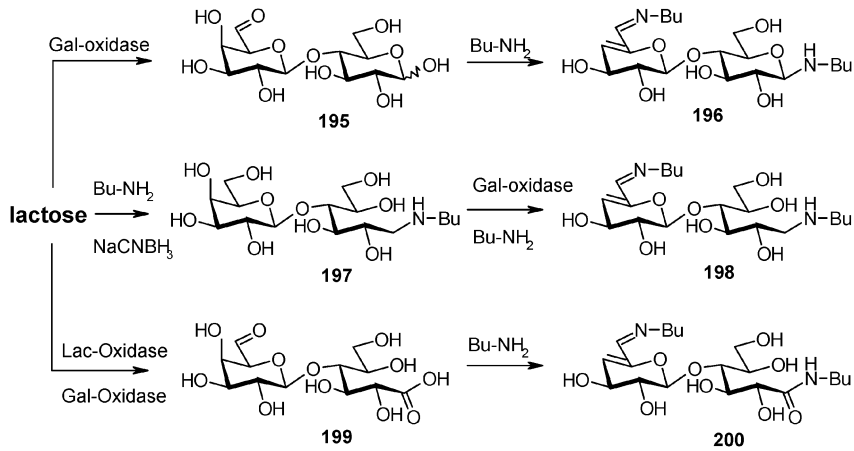


Fig. 63 Enzymatic modification of lactose.

A number of modified lactoses were prepared by enzymatic approach (Fig. 63).[84]

An interesting approach to unusual glycosides (with relatively large carbocyclic aglycon) from lactose was proposed by Thiem's group.[85] They postulated a conversion of the glucose part into the cyclooctanone skeleton (Fig. 64). Similar approach to the glucose derivative provided non-substituted cyclooctane.[86]

The group of Stütz developed a convenient methodology of the transformation of maltose into 1-deoxynojirimycin derivative (Fig. 65).[87]

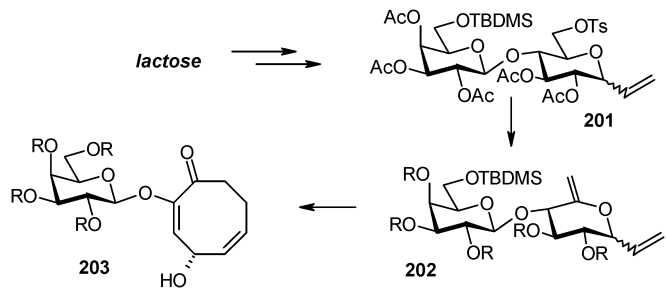
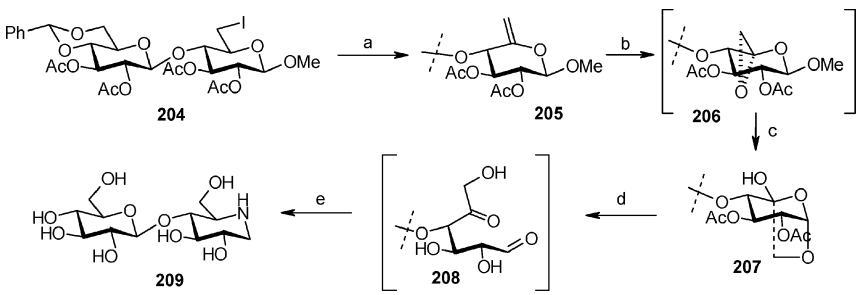


Fig. 64 Conversion of lactose to monosugar derivative of cyclooctanone.



a. AgF, Py; b. MCPBA, c. silica gel, d. MeONa, MeOH then Amberlite IR 120 (H^+); e. H_2, $Pd(OH)_2/C$, NH_4OH.

Fig. 65 Synthesis of 1-deoxynojirimycin derivative from maltose.

5. Conclusion

Application of simple sugars as starting materials for the preparation of optically pure complex products represents nowadays the classic methodology of organic synthesis; it is covered by many comprehensive reviews, cited here only in references.[88] These references provide the precise 'state-of-art' in the synthesis of aza sugars, carbasugars and other derivatives.

The material discussed in our paper, not being comprehensive, is focused mostly on the application of these methodologies in practical synthesis. It provides a general overview of the synthetic methodologies for such important compounds and is illustrated by selected and recent examples from the original literature.

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Blocking the anomeric reactivity, how and why

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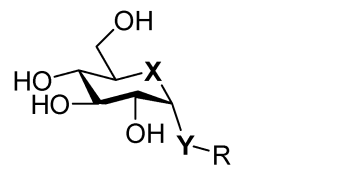
1. Introduction

The peculiarity of monosaccharides is the capacity, once cyclised in their hemiacetalic or hemiketalic form, to link anther sugar or an aglycon generating more complex molecules. Nature exploits this behaviour generating a wide variety of polysaccharides and glycoconjugates implicated in a series of biological processes of physiological relevance. The dynamicity of generation and cleavage of the glycosidic bound is guaranteed by two classes of enzymes, the glycosyltransferases which generate the glycosidic bound and the glycosidases that cleave it. This dynamicity is very important for the metabolism of carbohydrates and for the modification of biomolecules such as the glycosylation of proteins, which changes their conformation and consequently the biological function. Blocking the anomeric reactivity allows not only to inhibit the enzymes interfering with those biological functions, but also to generate stable mimics of glycosides of pharmacological relevance. In analogy with the case of peptides, in which peptidomimetics stable to proteases are generated for pharmaceutical purposes, glycomimetics stable to glycosidases but also to acidic cleavage are promising potential drugs.

Different strategies have been developed in order to block the anomeric reactivity of the glycosidic linkage, all based on the substitution of the acetal function with a more stable function. In order to do that, one of the two acetal oxygen atoms, or both, must be replaced. Fig. 1 schematically shows the possible modifications of the anomeric centre generating glycomimetics.



X=O, Y=C, C-glycosides
X=O, Y=S, S-glycosides
X=O, Y=N, N-Glycosides
X=O, Y=P, P-glycosides
X=C, Y=O or C, carbasugars
X=N, Y=C, iminosugars
X=S, Y=O
X=P, Y=O

Fig. 1 Glycomimetics obtained by modification of the anomeric functionality.

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2. Replacement of the anomeric oxygen

The anomeric oxygen can be replaced by a hydrogen atom or a carbon atom, generating ethers in which the anomeric reactivity is completely blocked, or by a nitrogen or sulphur atom which modifies the reactivity with respect to the parent *O*-glycoside. It is worthy of note that all these type of compounds are generated in leaving organisms. Peculiar is the case of replacement of the anomeric oxygen with a phosphor atom which has been proposed to generate not isosteric analogues of natural phosphates.

2.1 Replacement of the anomeric oxygen with a hydrogen atom or with a double bound

The replacement of the anomeric oxygen with a hydrogen atom blocks the anomeric reactivity generating ethers, defined anhydroalditols.

Anhydroalditols can be easily generated by Lewis acid catalysed reduction of the corresponding aldoses or glycosides with triethylsilane (Fig. 2). The reaction requires protection of the hydroxyl groups, which can be performed temporarily by preliminary treatment with bis(trimethylsily1)-trifluoroacetamide (BSTFA).[1]

The limit of this modification lies on the impossibility to mime the anomeric substituent, which makes these compounds not really useful as carbohydrate mimics.

Much more interesting, in terms of applications, is the case in which the anomeric oxygen is replaced by a double bound. This is the case of Zanamivir® (Fig. 3)[2] an anti-influenza drug approved by the US Food

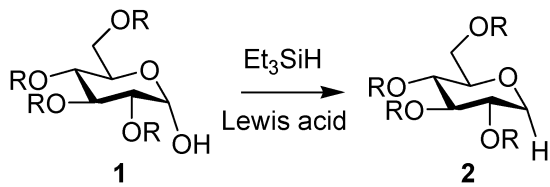
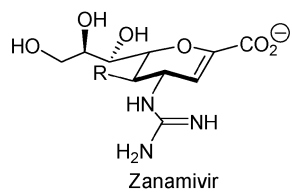


Fig. 2 Reduction of a protected aldose **1** to glycitol **2**.



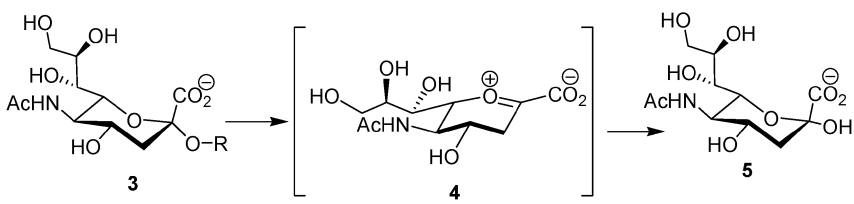


Fig. 3 Zanamivir®, an antiviral drug which inhibits viral sialidases and below the mechanism of hydrolysis of sialosides with the transition state **4** mimed by Zanamivir®.

and Drug Administration in 1999. The sp^2 hybridization of the anomeric carbon atom mimes the transition state for the cleavage of the glycosidic linkage, making these compounds inhibitors of glycosidases. In the case of Zanamivir®, the protection against influenza viruses lies on the fact that the viral infection requires, as first action, the displacement of the sialic acids **3** terminating the glycoconjugates protecting the cell wall. This displacement is performed by viral sialidases that are inhibited by Zanamivir®.

In this context it is interesting to note that also glyconolactones, in which the anomeric carbon is hybridized sp^2, are week glycosidase inhibitors.[3]

2.2 Replacement of the anomeric oxygen with a carbon atom: *C*-glycosyl compounds

The replacement of the anomeric oxygen with a carbon atom generates a class of stable glycomimetics defined *C*-glycosyl compounds. The proper choice of configuration and nature of substituent linked through a carbon atom to the "anomeric" centre allows the generation of stable mimics of glycosides.

Bioactive *C*-glycosyl compounds are known since long time, *C*-nucleosides in particular, such as pirazomycin or showdomycin (Fig. 4), isolated from natural sources, showed a variety of interesting biological properties such as antitumor, antiviral or antibacterial activities.[4]

C-Glycosyl aromatic compounds such as undamycinone B present DNA binding properties and cytotoxicity, therefore being promising antitumor agents,[5] whereas the UDP-galactose mimic displaces time-dependent inactivation of UDP-galactopyranose mutase (Fig. 5).[6]

The *C*-glycosyl analogue of the immunostimulant galactosilceramide KRN7000 (Fig. 6) resulted 1000 times more protective than the *O*-glycoside in mouse against malaria infection, and 100 times more effective against lung melanoma.[7]

The synthesis of *C*-glycosyl compounds has been widely reviewed,[8] and the first examples goes back to 1945–1950, when Hurd reacted glycosyl halides with Grignard reagents.[9] A great variety of synthetic methods have been developed, and the details can be found in the reviews reported in the references. Here we report just a general scheme showing the different synthetic strategies and some recent results of biological interest (Fig. 7).

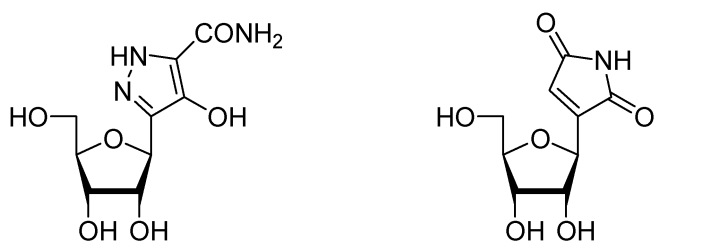


Fig. 4 Examples of *C*-nucleosides biologically active.

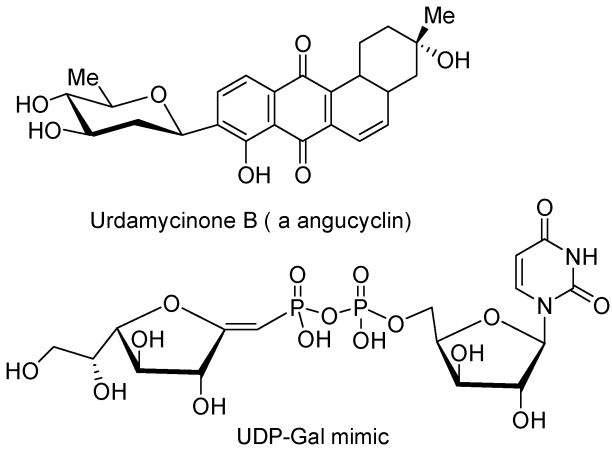


Fig. 5 Examples of bioactive *C*-glycosyl compounds.

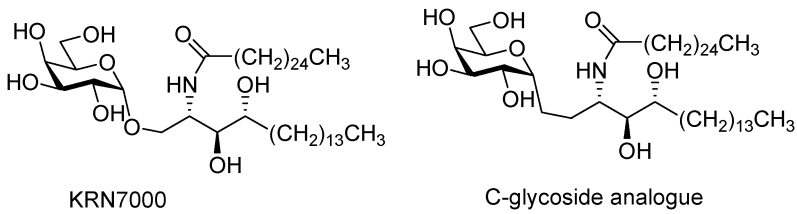


Fig. 6 The immunostimulant galactosilceramide KRN7000 and the *C*-glycosyl compound analogue which resulted more potent.

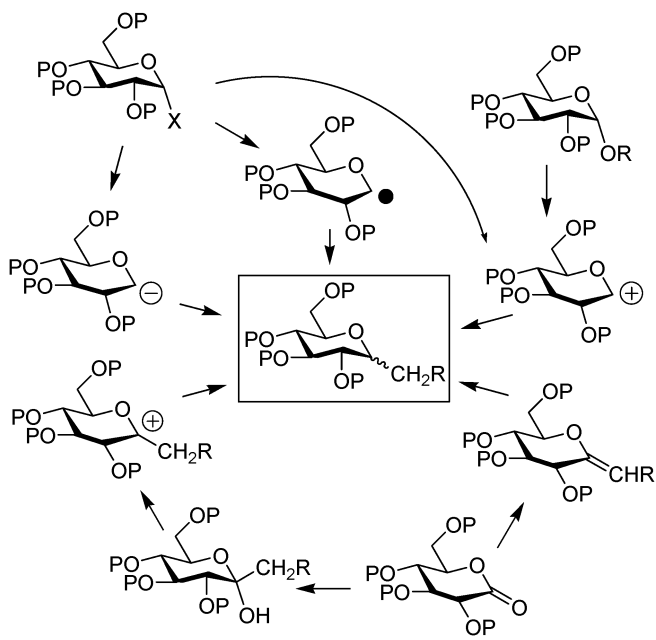


Fig. 7 General scheme for *C*-glycosyl compounds synthesis.

The nature of the anomeric centre is electrophilic, and the electrophilicity can be enhanced converting the anomeric OH into a better leaving group such as a halide or a trichloroimidate. Therefore the anomeric centre can be attached by a carbon nucleophile. In Fig. 4 the electrophilicity of the anomeric centre is represented by a carbocation, but the *C*-glycosylation process can occur also *via* a S_N2 mechanism or *via* an ion-pair, depending on the nature of the nucleophile. Stabilized carbanions, such as malonates,[10] nitromethane[11] and cyanide anions,[12] and organometallic reagents such as Grignard reagents, displace glycosyl halides generating *C*-glycosyl compounds. Alternatively a Lewis acids will generate an anomeric carbocation that can react with a proper carbon–carbon double bound, such as allyltrimethylsilane,[13] vinylsilanes,[14] propargylsilanes,[15] silylenol ethers,[16] enamines,[17] and also simple alkenes[18] and aromatic compounds (Fig. 8).[19]

Also glyconolactones can be exploited to generate *C*-glycosyl compounds. In this case the carbon atom is inserted at the anomeric centre by addition of organolitium reagents generating the corresponding lactol that can be reduced to the corresponding *C*-glycosyl compound by Lewis acid catalysed reaction with triethylsilane.[13*b*]

The control of the stereochemistry of the *C*-glycosylation reaction is particularly problematic and at the same time is an interesting task. It depends not only on the type of *C*-glycosylation procedure, but also on the nature of the substrate. It is not possible to define general roles, but in many cases it is possible to generate stereoselectively α or β-*C*-glycosyl compounds. This is the case of the *C*-glucopyranosyl compound **10**, generated stereoselectively by Lewis acid catalysed reaction of a glucopyranoside with allyltrimethylsilane, whereas the corresponding β-isomer can be generated by reaction of gluconolactone with allylmagnesium bromide

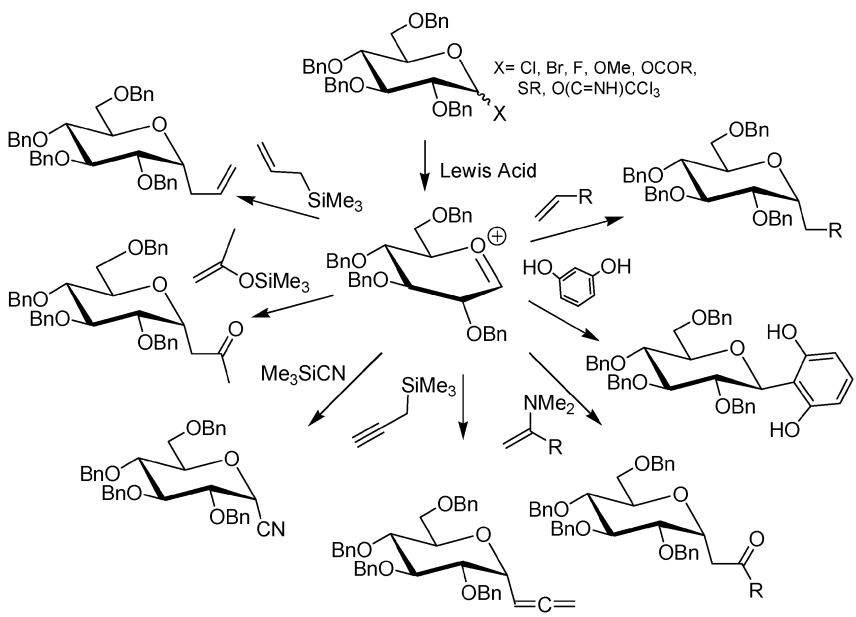


Fig. 8

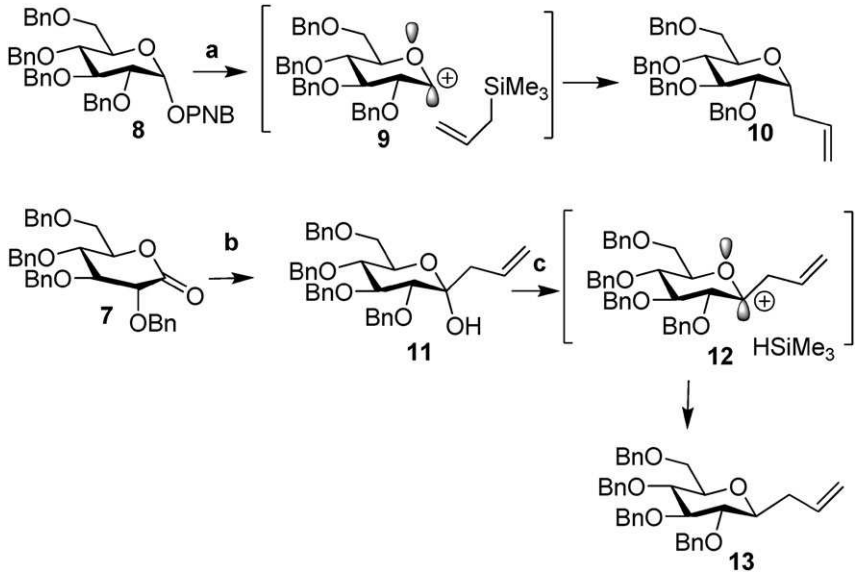


Fig. 9 Stereoselective synthesis of *C*-glucopyranosides. Reagents and conditions: (a) BF_3OEt_2, allyltrimethylsilane; (b) AllylMgBr; (c) BF_3OEt_2, Et_3SiH. (PNB = *p*-nitrobenzoate).

and reduction of the obtained lactol **11** with triethylsilane and a Lewis acid (Fig. 9). The stereocontrol of both processes lies on the anomeric effect that favours the attack of the nucleophile on the anomeric carbocation free orbital oriented at 180° with respect to the axial lon pair of the ring oxygen, that is from the α face.

Examples have also been reported in which glyconolactones are olefinated generating exoenitols (Fig. 10) for example by methylenation (Tebbe reaction),[20] difluoromethylenation,[21] and dichloromethylenation,[22] the Wittig olefination.[23]

Aldoses can be converted into *C*-glycosyl compounds also exploiting the reaction of the carbonyl group which a stabilised Wittig reagent or Wittig-Horner reagent, (Fig. 11a)[24] or with 1,3-dicarbonyl compounds (Fig. 11b).[25] Interestingly these reactions have been performed also with unprotected aldoses. Alternatively, $Ph_3P{=}CH_2$[26] as been used, as the only non stabilized

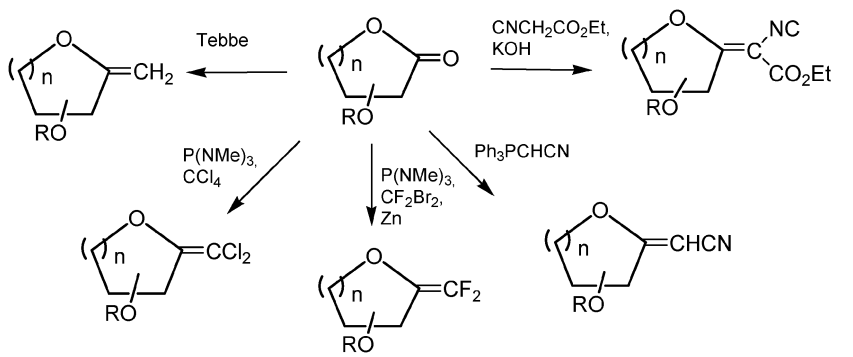


Fig. 10 Methods of olefination of glyconolactones.

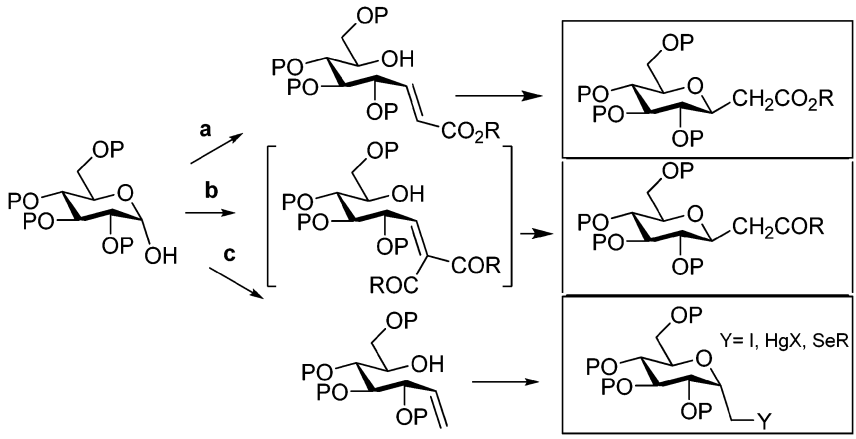


Fig. 11 Generation of *C*-glycosyl compounds by Wittig reaction.

Wittig reagent. The reaction produces open chain enitols which can generate *C*-glycosyl compounds by cyclization. In the cases in which an electron withdrawing group is conjugated to the double bound, the cyclization occurs even spontaneously (like in procedure b) *via* Michael reaction, otherwise the double bound must be activated by an electrophile such as mercuric salts,[26] iodine,[27] NIS or epoxydation.[24a]

As far as the stereochemistry concerns, when the cyclization occurs through a Michael reaction, the thermodynamic product is generally obtained, but some exceptions have been observed such as that of *N*-acetyl glucosamine protected as 4,6-benzylidene, the reaction of which with [(ethoxycarbonyl)methylene]triphenylphosphorane afforded the α-*C*-glycosyl compound stereoselectively.[28]

When the cyclization occurs by activation of the carbon–carbon double bound, the anomeric configuration of the product depends on the stereochemistry of attack of the electrophile to the double bound. The attack preferably occurs from the less hindered face of the most stable conformation, that in which the allylic hydroxyl group lies on the same plane of the double bond.

This is well exemplified by the example reported in Fig. 12, in which tetrabenzyl glucose **14** is methylated with metylenetriphenylphosphorane and the obtained hexoenitol **15** treated with $Hg(OAc)_2$.[26] The attack occurs stereoselectively from the *si* face generating a mercuronium ion which is attached by the hydroxyl group forming the *C*-glycosyl compound **17**. Alternatively the reactivity of the anomeric centre can be inverted generating glycosylanions or organometallics.[29,30]

The main problem of these protocols is β-elimination which occurs if a leaving group such as an alkoxy is present in the adjacent position. The elimination is prevented if the anomeric anion is strongly stabilized, for example by a nitro group,[31] or in presence of a bad leaving group, such as the anions $–O^-$[32] or $–NAc^-$ (Fig. 13).[33]

Anomeric metallations can be performed from glycosyl halides or from glycals, converting them into glycosyl-$SnBu_3$[34] or glycosyl-$Mn(CO)_5$[35] or even into glycosyl-SO_2Ph[36] or glycosyl-SO_2Py, which are all converted

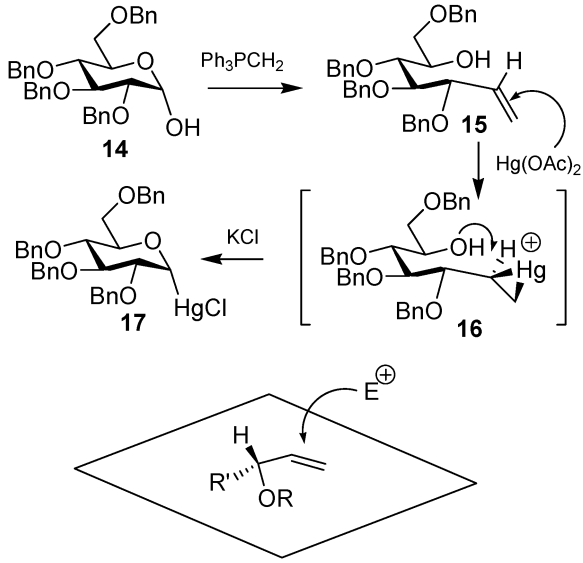


Fig. 12

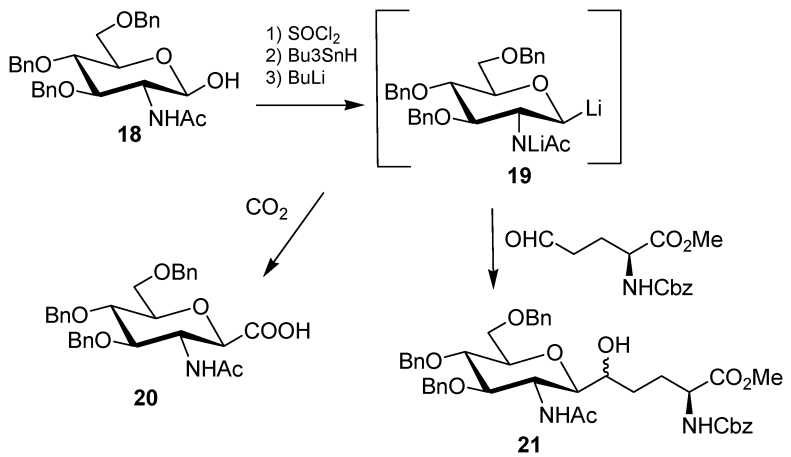


Fig. 13 Example of anomeric metallation, avoiding β-elimination, in the generation of *C*-glycosyl compounds.

in situ into the more reactive organolithium reagent. Glycosyl-SO_2Py in particular has been used to generate *in situ* with samarium diiodide a nucleophilic anomeric carbon.[37] This is probably the most efficient method to generate *C*-glycosyl compounds, including *C*-disaccharides (Fig. 14) exploiting a *C*-glycosyl nucleophilic intermediate.

Finally, the anomeric centre of a sugar can be converted into a radical.[38] This conversion has been performed since 1982[39] treating glycosyl halides with trialkylstannanes. These radicals were trapped with methyl acrylate[40] or acrylonitrile.[41] The glycosyl radical can be generated by irradiation or with radical promoters such as AIBN, starting from glycosyl halides, phenylselenides, methylthiocarbonates or *p*-anisyltellurides. Allystannanes, allylthioethers and variety of carbon–carbon double bonds activated with

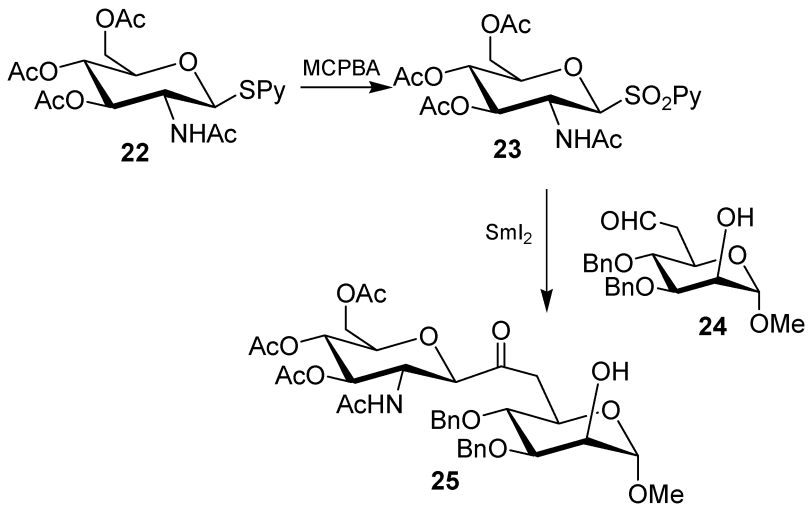


Fig. 14 Synthesis of a *C*-disaccharide exploiting the SmI_2 catalyzed reaction of a glycosyl pyridylsulphone.

an electron withdrawing group have been used as radical scavengers, including a properly functionalised sugar in order to generate a *C*-disaccharide (Fig. 15).[42]

The stereochemistry of the reaction of glycosyl radicals is strongly influenced by the anomeric effect. Glucopyranosides and manno-pyranosides afford stereoselectively the α-*C*-glycosyl compounds whereas in furanosidic structures the stereochemistry is not always predictable.

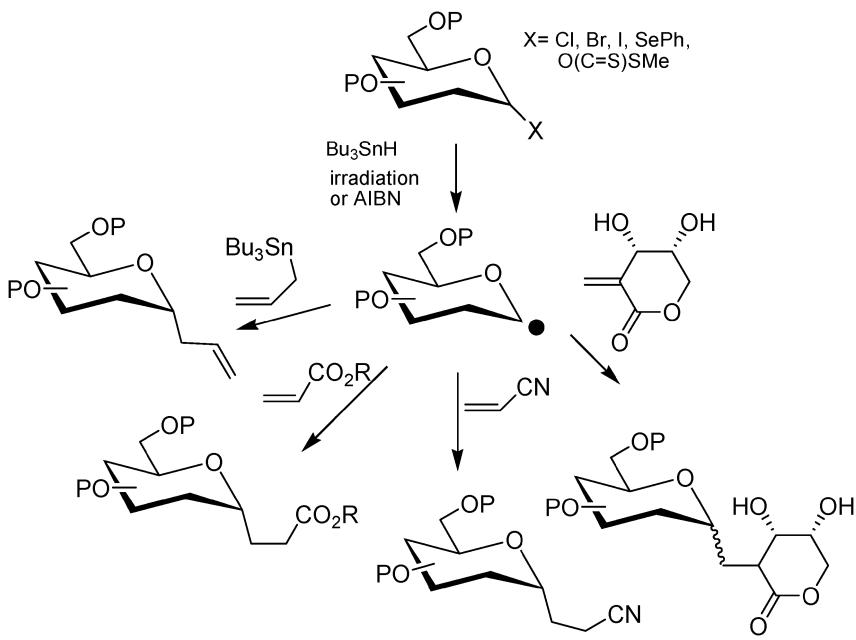


Fig. 15 General scheme of *C*-glycosylation *via* a glycosyl radical.

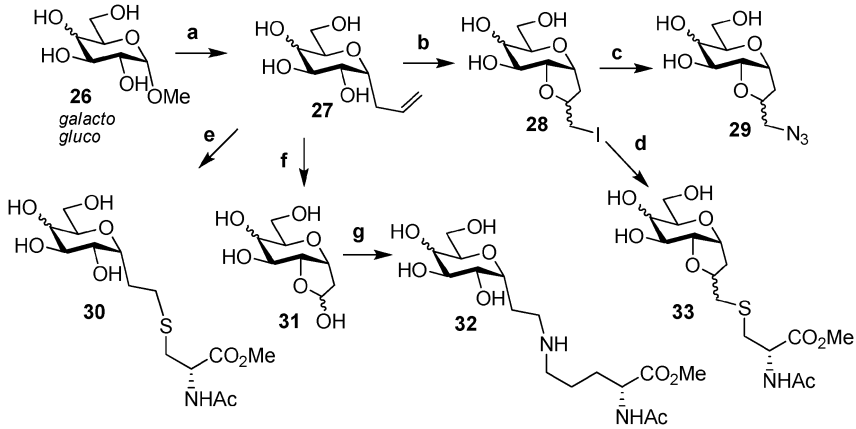


Fig. 16 Manipulation of deprotected *C*-glucopyranosides. Reagents and conditions: (a) i. BTSFA, CH_3CN, reflux; ii. allyltrimethylsilane, TMSOTf, 0 °C-room temp., 12 h; (b) NIS, H_2O, 2 h; (c) Bu_4NN_3, H_2O, 80 °C, 72 h; (d) Cs_2CO_3, Cys-NAc-OMe, MeOH-DMF 2:1; (e) hν, *N*-acetyl L-cysteine methyl ester, MeOH/H_2O; (f) i. O_3, ii. Me_2S; (g) *N*-acetyl L-lysine methyl ester, MeOH, AcOH, $NaBH_3CN$.

The allylation *via* allyltrimethylsilane is becoming one of the most efficient *C*-glycosylation procedures due to the simplicity of the experimental reaction condition and the stereoselectivity of the process. Interestingly, this allylation can be performed also on deprotected sugars, provided that an *in situ* silylation is performed.[1] The allylic appendage was differently manipulated, also in the absence of protecting groups, in order to afford for example *C*-glycosyl compounds linked to amino acids (Fig. 16),[43] and the primary hydroxyl group was selectively oxidised with TEMPO in order to generate the glycuronic acid derivatives.

2.3 Replacement of the anomeric oxygen with a nitrogen atom: *N*-glycosides

The replacement of the anomeric oxygen with nitrogen generates aminals that are generally not very stable. Their stability can be increased when the electron pair of the nitrogen is delocalised by resonance. This is the case of natural *N*-glycosides such as ribonucleosides or *N*-acetyl-glucosamine linked to asparagine (GlcNAc-Asn) in *N*-linked glycoproteins (Fig. 17).

The fact that an aldose reacts chemoselectively with amino derivatives such as hydroxylamines or hydrazones generating oximes or hydrazones,

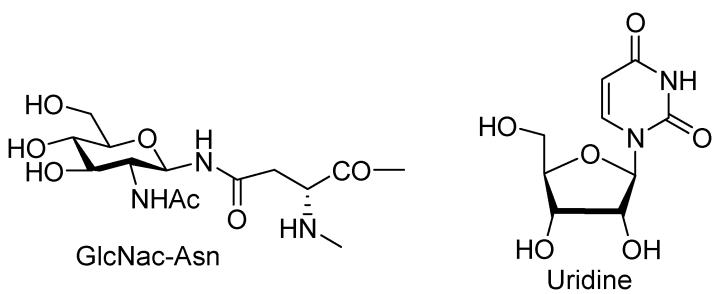


Fig. 17 Naturally occurring *N*-glycosides.

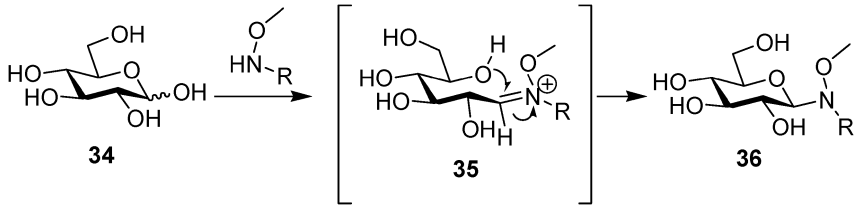


Fig. 18

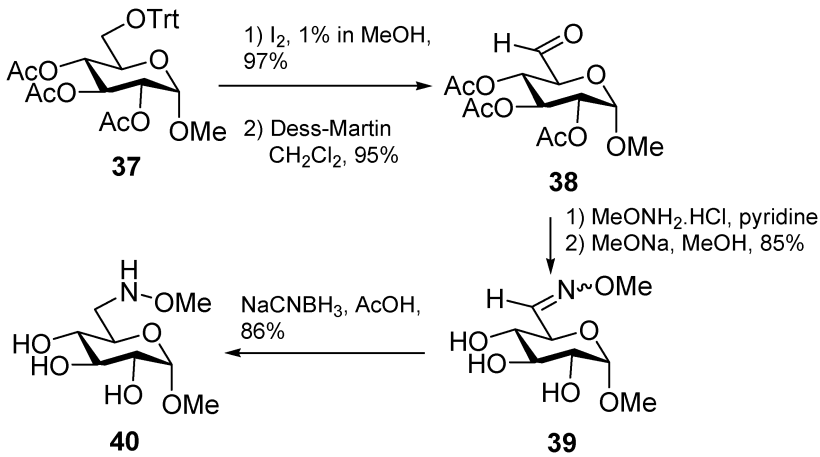


Fig. 19 Synthesis of methyl 6-desoxy-6-methoxyamino-α-D-glucopyranoside.

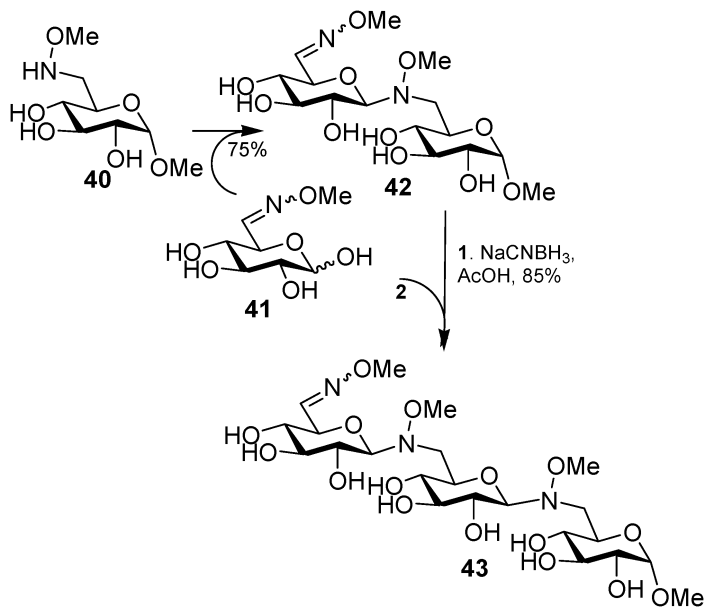


Fig. 20 Iterative synthesis of pseudoloigosaccharides using 6-desoxy-6-methoxyamino-α-D-glucopyranose.

has stimulated the interest on the possibility to find a method that allow to link unprotected carbohydrate moieties to other biomolecules. However this process generally affords imino derivatives in which the cyclic nature of the sugar is lost. To overcome this disadvantage, *N*-substituted alcoxyamines have been used, so that the iminium ion that is generated easily undergoes the expected cyclization (Fig. 18).[44]

The methoxyamino function can be inserted in a sugar exploiting the procedure reported in Fig. 19, or eventually exploiting TEMPO for the oxidation in order to avoid protection–deprotection steps. Pseudo-disaccharides, trisaccharides and so on can be generated chemoselectively in a iterative way adopting the protocol reported in Fig. 20.[45] Methoxyamino-glycosides are stable to glycosidases and are indefinitely stable at pH 2.

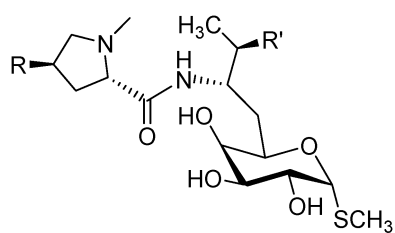
2.4 Replacement of the anomeric oxygen with a sulphur atom: *S*-glycosides

S-Glycosides are known since long time, several thioglycosides presenting interesting biological activities have been isolated from many different organisms and are treated in another chapter of this book.

Lyncomicin A and B (Fig. 21) are thioglycoside-based antibiotics produced by *Streptomycies lincolnensis*.[46] They consist of an aminooctose moiety, the α-methylthiolincosaminide, linked by an amidic bond to a *N*-propilhygric acid (lincomycin A) or an ethylhygric acid (lincomycin B) unit.

Lincomycins bacteriostatic activity is based on the inhibition of the bacterial proteic synthesis. Lincomycin A in particular have been largely employed in clinical therapy of infections caused by micoplasmas, Gram-positive bacteria, and cocci but it presents a lot of side effects overcome by 7-clindamycin (Fig. 21), a semi-synthetic drug with larger spectrum of action and major antibacterial activity.

Simple *S*-glycosides, in particular thiophenyl glycosides, have found application in glycosylation reaction due to the fact that the thioether substituent can be selectively converted into a good leaving group by treatment with a thiophilic reagent. In particular thioglycosides have been successfully employed in sequential glycosylation.[47] The versatility of thioglycosides allowed the development of chemoselective, orthogonal and iterative glycosylation strategies recently reviewed.[48] Thiophenyl glycosides can be easily synthesised from anomeric acetates by reaction with thiophenol catalysed with BF_3OEt_2[49] or with $InCl_3$ and $TiCl_4$ as co-activator.[50]



Lincomycin A R=Pr, R'=OH
Lincomycin B R=Et, R'=OH
Clindamycin R=Pr, R'=Cl

Fig. 21 Lincomycin A and B, and clindamycin, thioglycosides with antibiotic activity.

The interest in *S*-glycosides lies not only on the synthetic potentiality, but also on the fact that *S*-glycosides are generally stable to glycosidases. A variety of analogues of disaccharides in which the two sugars are linked through a sulphur atom have been synthesized for this purpose.[51] Furthermore, *S*-glycosides can be generated from glycosylthiols **44** exploiting chemoselective reactions, which is very useful for neoglycosylation of biomolecules such as proteins.

Different procedures have been developed to generate thiodisaccharides, based on two strategies: (a) generation of a glycosylthiol **44** and reaction with an electrophilic centre on the second sugar **45**, (b) replacement of an –OH of the sugar with an –SH and reaction with a glycosyl donor **46** (Fig. 22).

An example of synthesis of a thiodisaccharide exploiting procedure (a) is reported in Fig. 23. Saponification of glycopyranosyl thioacetate **48** generated the thio-anion **49** which displaced a triflate in position 4 of galacto-derivative **50**, generating the thiodisaccharide **51**.[52]

Another example in which the reaction of a glycosylthiol **52** displaces a triflate on a second sugar **53**, generating a thiodisaccharide **54** is reported in Fig. 24 for the synthesis of a mimetic of glycosaminoglycan chondroitin (**55**).[53]

This compound was designed as potential inhibitor of chondroitin AC lyase from *Flavobacterium heparinum*, useful for the structural analysis of the enzyme active site.

Instead of a triflate, the electrophile on the glycosyl acceptor can be an α,β-unsaturated carbonyl group. This is the case reported in Fig. 25, in which a stereoselective Michael addition of the 1-thiosugar **56** to the α,β-conjugated system of levoglucosenone **57**, generated after deprotection a couple of L-fucopyranosyl-4-thiodisaccharides **61** and **62** presenting inhibitory activity on α-L-fucosidase.[54]

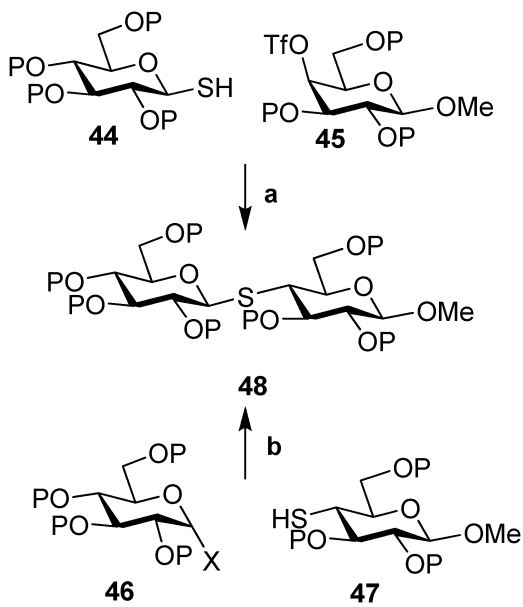


Fig. 22 Two different strategies to generate thiodisaccharides.

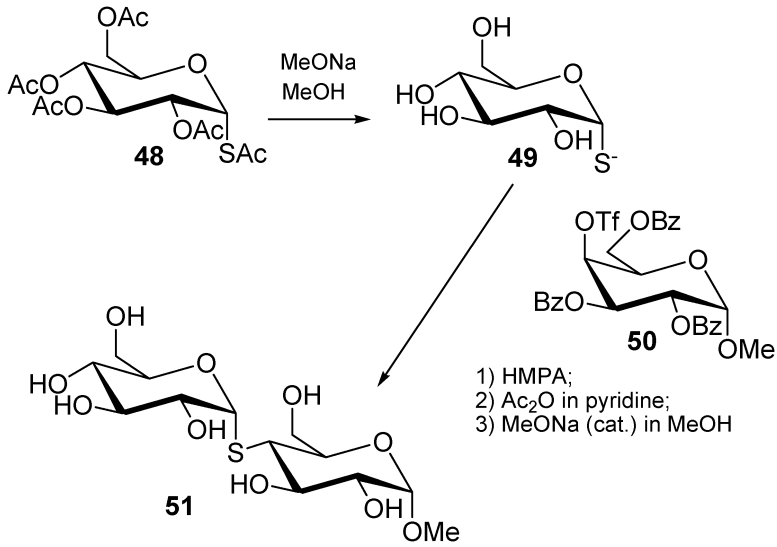


Fig. 23

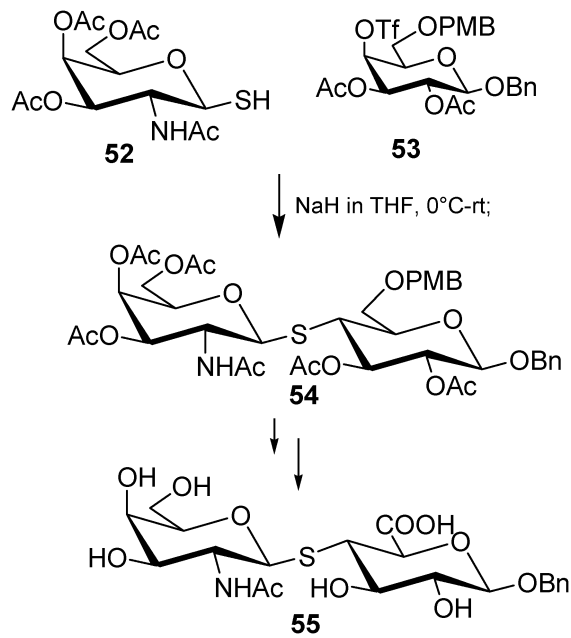


Fig. 24

Glycosylthiols have been used also to neo-gycosylate peptides and proteins, taking advantage in particular on the chemoselectivity of the –S–S– bound formation.

A cysteine residue was incorporated into the protein backbone in a specific position and activated to thioglycosylation by treatment with phenylselenenyl bromide. The electrophilic character of the sulphur atom in the resulting S–Se bond makes possible its nucleophilic substitution by 1-thio mono- or oligosaccharides (Fig. 26).[55]

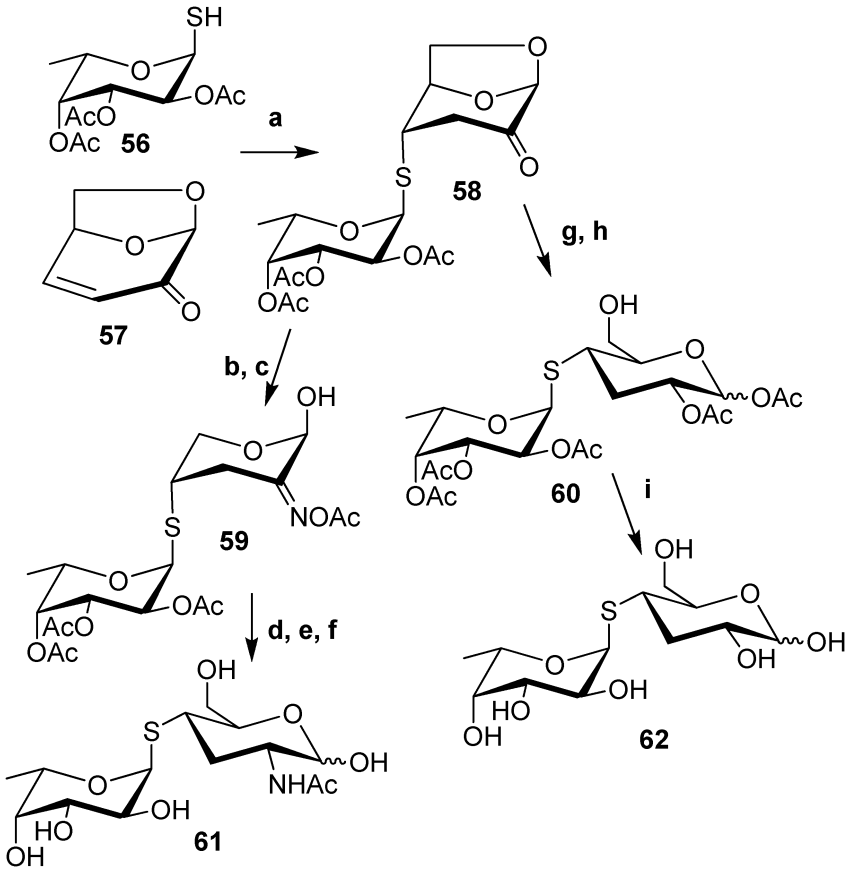


Fig. 25 Synthesis of *S*-disaccharides inhibitors of α-L-fucosidases *via* Michael addition. Reagents and conditions: (a) Et_3N, benzene; (b) NH_2OH:HCl/EtOH/pyridine; (c) Ac_2O, pyridine; (d) 9-BBN/H_2O_2/NaOH; (e) BF_3OEt/Ac_2O; (f) MeOH/H_2O/Et_3N; (g) L-selectride; (h) BF_3OEt/Ac_2O; (i) MeOH/H_2O/Et_3N.

The second strategy (b) for the synthesis of thiodisaccharides requires the replacement of an –OH of the sugar with an –SH (**64**) and then the reaction of this function with a glycosyl donor (**63**). An interesting example of the

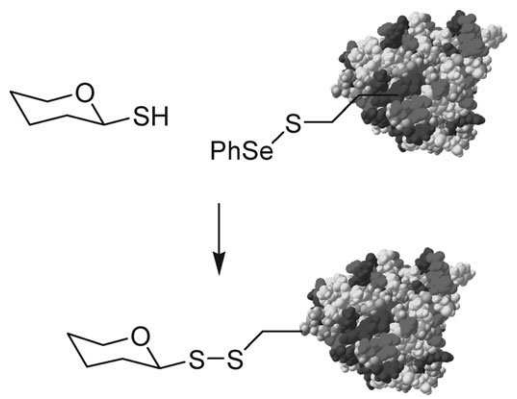


Fig. 26 Protein glycosylation *via* Glyco-SeS strategy.

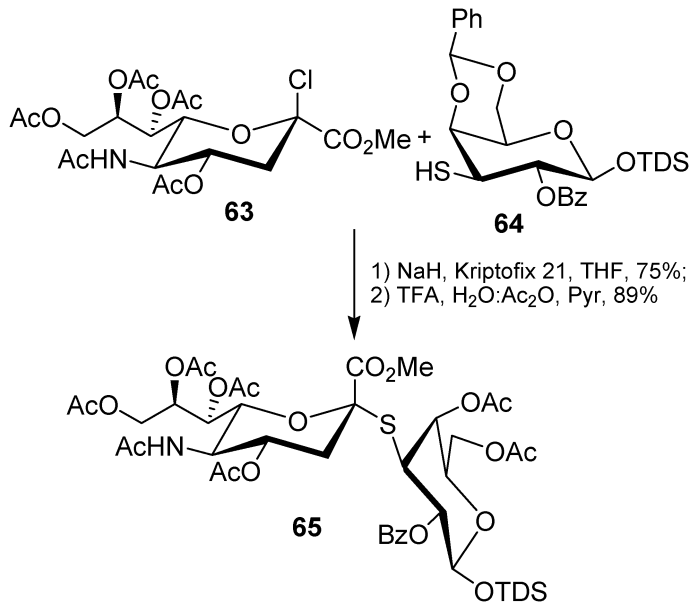


Fig. 27 Synthesis of Neu5Acα(2→3)-*S*-Gal *via* an S_N2 reaction employing a thio-acceptor.

use of this strategy is reported in Fig. 27, for the synthesis of the Neu5Acα(2→3)-*S*-Gal **65**.[56]

Interestingly, this approach has been used also enzimatically, exploiting thioglycoligases. The alanine acid/base mutants of two retaining β-glycosidases, the β-glucosidase from *Agrobacterium sp*. Abg E171A (Fig. 28) and the β-mannosidase from *Cellulomonas fini* Man2 E429A have been used to synthesize 1,4-β-*S*-disaccharides such as **68**.[57]

A double mutant (acid/base and nucleophile) Abg E71A E358G was developed[58] which efficiently catalyzes thioglycoside formation from a glycosyl fluoridine donor (**69**) with inverted anomeric stereochemistry. This represents a significant improvement, as glycosyl fluoride donors are much more accessible (Fig. 29).

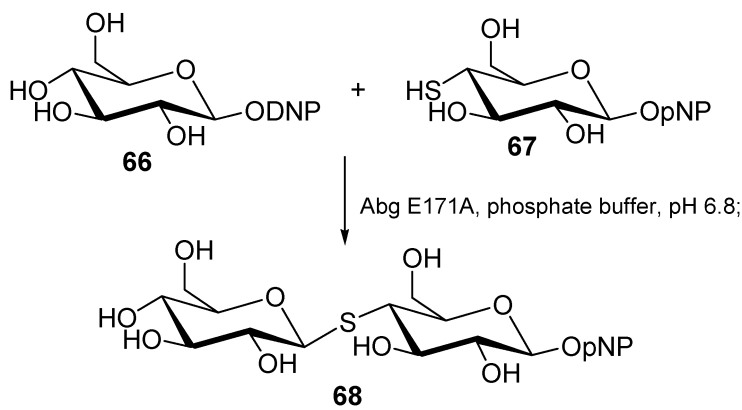


Fig. 28 Enzymatic synthesis of a thio-disaccharide.

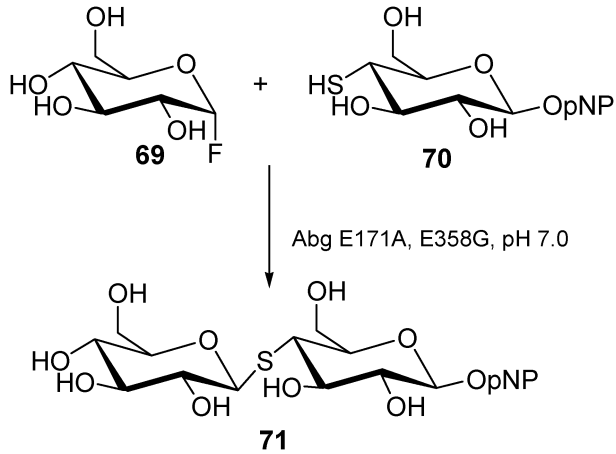


Fig. 29 Enzymatic synthesis of 1,4-thiosaccharides catalyzed by the double mutant Abg E71A E358G.

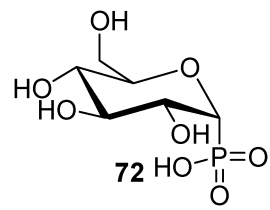


Fig. 30 Glucopyranosyl phosphonate, a mimic of glucose 1-phosphate.

2.5 Replacement of the anomeric oxygen with a phosphor atom

The replacement of the anomeric oxygen with a phosphor atom has been reported for the generation of non-isosteric analogues of glycosyl phosphates (Fig. 30).[59] This class of compounds however has not found applications.

3. Replacement of the endocyclic oxygen

The replacement of the endocyclic oxygen of the sugar with a different element is another approach exploited to modify the anomeric reactivity. The carbon atom is the most obvious element to be used, the carbocycle so generated (cyclitol) completely loses the anomeric reactivity. The chemistry of cyclitols will not be described in this chapter as it is treated in another chapter of the same book.

Another interesting class of compounds is that in which the endocyclic oxygen is replaced by nitrogen.

3.1 Replacement of the endocyclic oxygen with a nitrogen: imino sugars

The replacement of the endocyclic oxygen of the sugar with a nitrogen atom generates a class of compounds defined imino sugars.

Since the discovery in 1966 of nojirimycin (Fig. 31),[60] an antibiotic produced by several *Streptomyces* and found to be a 5-amino-5-deoxy-D-glucopyranose, many efforts have been devoted to study such compounds.[61]

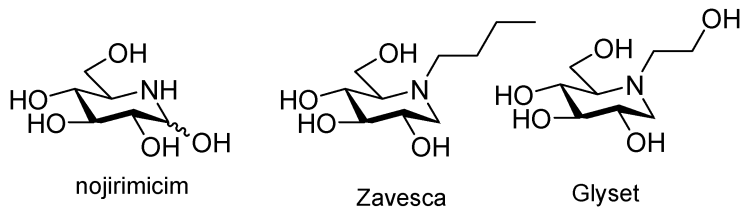


Fig. 31 Examples of imino sugars: nojirimicin, an antibiotic produces by several Streptomyces, Zavesca® and Glyset, drug on the market.

As a matter of facts, in recent years, imino sugars have been investigated as antiviral,[62] antitumor[63] agents. Zavesca® (*N*-butyldoexynojirimicin) is on the market for treatment of type 1 Gaucher disease,[64] a lysosomal storage disorder, and Glyset® (or miglitol, *N*(2-hydroxyethyl)-doexynojirimicin for treatment of non insulin dependent diabetes (Fig. 30)

The bioactivity of imino sugars is due to the fact that they inhibit glycosidases. Once protonated, the imino sugar mime the oxonium ion intermediate of the reaction catalysed by those enzymes (Fig. 32), and therefore binds the active site.

Recently, imino sugars have found application as active site specific chaperones (ASSC) for the treatment of lysosomial storage disorders.[65] An ASSC is a small molecule that bind the catalytic domain of an enzyme inducing the regeneration of the active conformation of misfolded proteins.

In other words, beside the possibility to inhibit an active enzyme there is the possibility to use very low concentrations of an imino sugars to activate an inactive (misfolded) enzyme.

A great variety of synthetic strategies have been reported to synthesize imino sugars, taking also in account that the structural variety is very big, including bicyclic structures and oligosaccharide mimics. Here we try to outline the general strategy and describe few recent and interesting examples

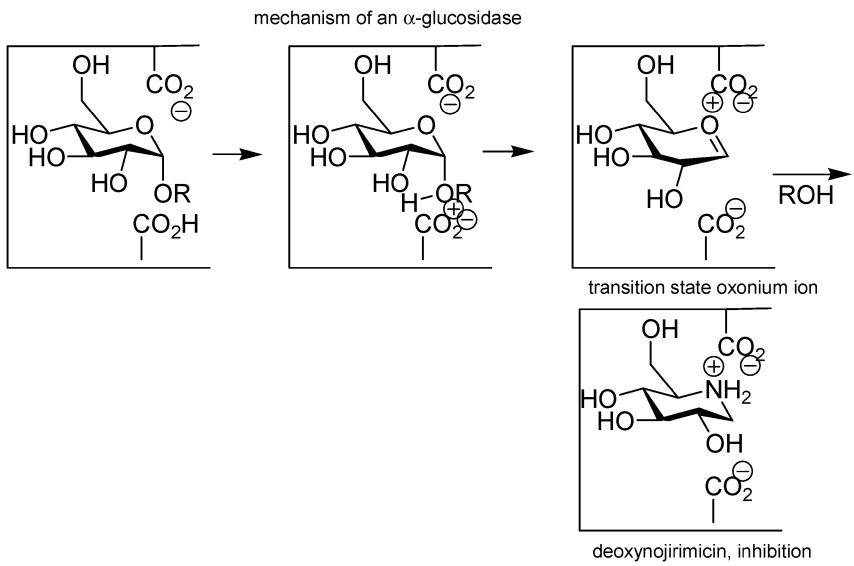


Fig. 32 Mechanism of α-glucosidase and rationale of the inhibition by deoxynojirimicin.

of synthesis of imino sugars, a description of the multifarious approaches can be found in the references.

The synthesis of an imino sugars can start from the parent sugar, in order to take advantage of the chirality and functionalisation of the part of the molecule not involved in the transformation. In this case the conversion requires (a) introduction of nitrogen, (b) cyclization. A feature of imino sugars must be taken into consideration: in order to be stable they must lack of the anomeric oxygen.

Suppose for simplicity to refer to nojirimicin as target, in a retrosynthetic scheme the nitrogen ($-NH_2$ or $-N_3$) can be inserted alternatively in position 1 or in position 5, whereas the other position must be electrophilic (represented as synthon with a carbocation) (Fig. 33).

The synthetic equivalents of the carbocations can be, of course a carbonyl group or a carbon with a leaving group –X, an epoxide or an activated double bound.

And how to insert the nitrogen in the open chain synthetic intermediates (one more retrosynthetic step)? With the same approach, insertion of an amine or an azide on an electrophilic carbon. Which means that in principle the nitrogen can be inserted at the same time on both positions. The example reported in Fig. 34 is an application of this approach.

An easy example of insertion of the nitrogen at the anomeric position and subsequent cyclization is reported in Fig. 35.[66] Tetrabenzyl-D-glucono-lactone **76** reacts with ammonia generating the amide **77** and therefore liberating the hydroxyl grout at C-5. By Swern oxidation of this hydroxyl group, the ketone **78** is formed which undergoes the nucleophilic attack from the $-NH_2$ of the amide. By treatment with $NaBH_3CN$ reduction of the so formed imide occurs, generating the imino sugar lactam **79**.

Many syntheses of imino sugars have been performed inserting the nitrogen in the sugar chain and then exploiting it for the subsequent cyclization at the anomeric centre. Fig. 36 describes one of the numerous examples of synthesis of imino sugars following this strategy.[67]

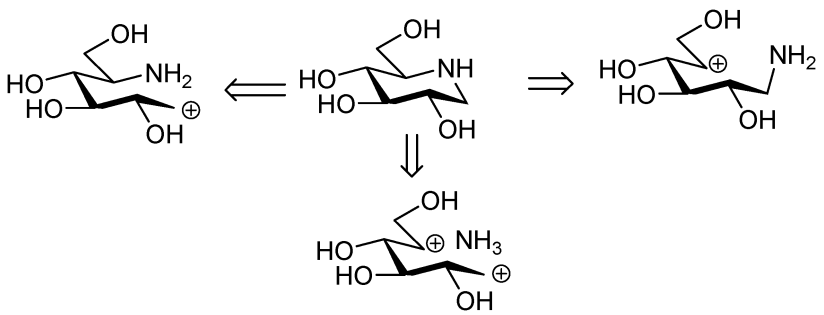


Fig. 33 General retrosynthetic scheme for imino sugars synthesis.

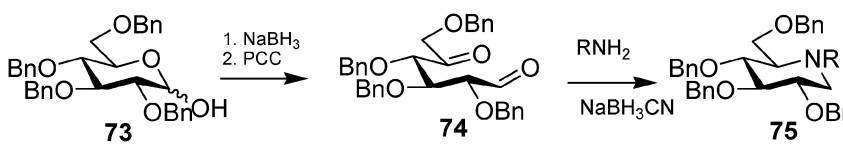


Fig. 34 Example of synthesis of an imino sugars by contemporaneous insertion of the nitrogen on the two carbons involved in the piperidine cycle.

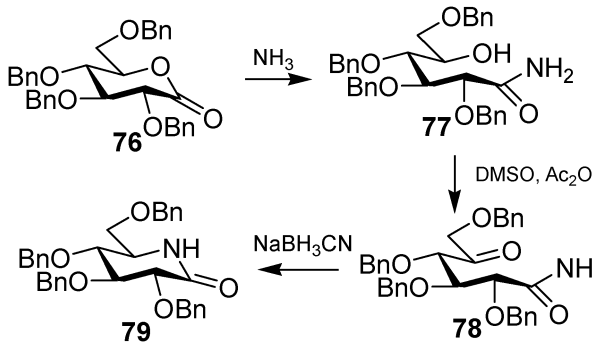


Fig. 35 Example of synthesis of an imino sugars by insertion of the nitrogen at the anomeric centre and subsequent cyclization.

The synthesis of imino sugars has been performed from non-carbohydrate starting materials. For example, a substituted pyridine has been used for an asymmetric synthesis of deoxynojirimicin (Fig. 37).[68]

Another example, in which the piperidine cycle is generated *de novo*, exploits a hetero Diels-Alder cycloaddition of 1-*p*-tolylsulfinyl-1,3-pentadiene **91** with benzylnitrosoformate, that generates an oxazine **92** with complete regioselectivity and π-facial diastereoselectivity.[69] Osmilation of the double bond inserts stereoselectively two hydroxyl groups on the oxazine skeleton, protection and catalytic hydrogenation finally afforded the enantiomerically pure imino sugars **94** (Fig. 38).

Imino sugars have been obtained in an elegant way exploiting aldolases. The idea is simple, aldolases such as fructose 1,6-diphosphate aldolase catalyse an aldol condensation between dihydroxyacetone 1-phosphate and different aldehydes.[70] Performing this reaction with an aldehyde properly functionalyded with an azido group (**94**), an azidiketone (**95**) is formed that upon reduction generates the imino sugars by intramolecular reductive amination (Fig. 39). The procedure was brilliantly improved using non phosphorylated dihydroxyacetone and performing the process in one pot.[70a]

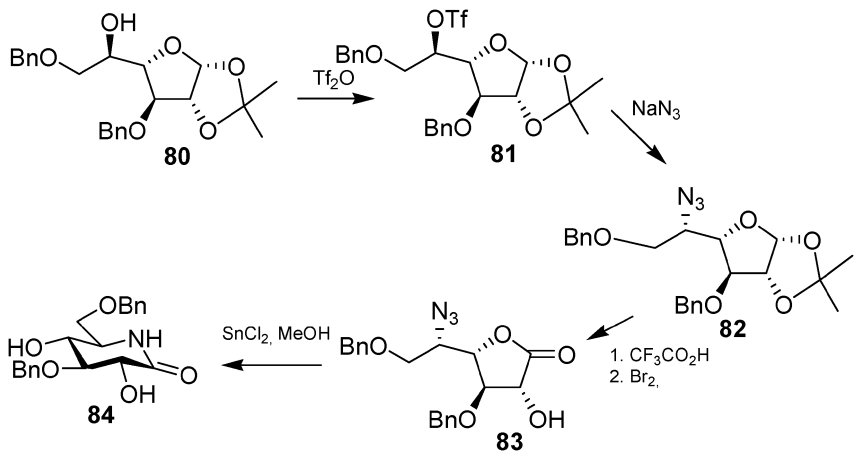


Fig. 36 Example of synthesis of an imino sugars by insertion of the nitrogen on the sugar chain and subsequent cyclization at the anomeric centre.

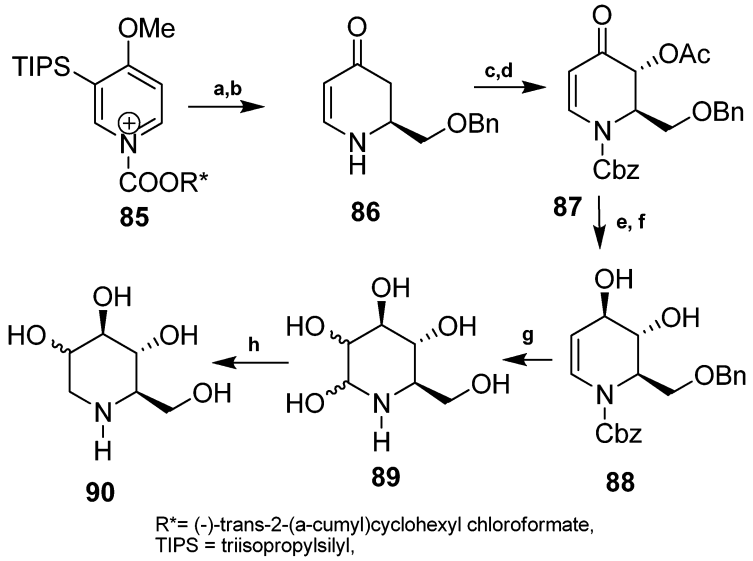


Fig. 37 Example of synthesis of an imino sugars starting from an achiral non-carbohydrate precursor. Reagents and conditions: (a) $BnOCH_2$(2-thienyl)$CuCnLi_2$, THF, −78 °C; 10% HCl; (b) NaOMe, MeOH, reflux; HCl, *i*-PrOH; (c) *n*-BuLi, PhOCOCl, THF, −78 °C; (d) $Pb(OAc)_4$, toluene, reflux; (e) HCl, EtOH; (f) $Me_4NBH(OAc)_3$, acetone, AcOH; (g) OsO_4, NMO; (h) $Pd(OH)_2$, H_2.

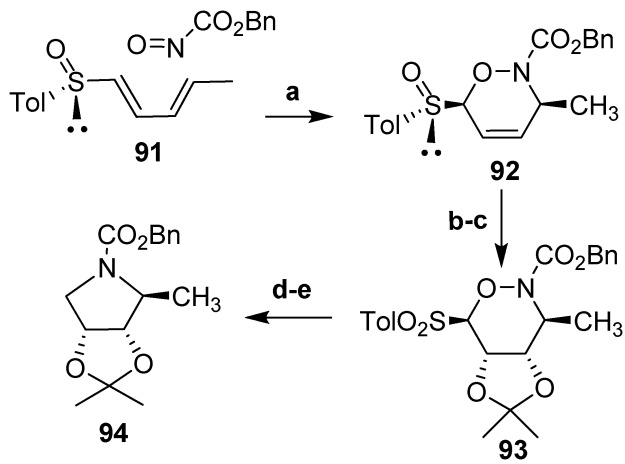


Fig. 38 Synthesis of imino sugars *via* Diels-Alder cycloaddition on sulfinyl pentadiene. Reagents and conditions: (a) CH_2Cl_2, −78 °C to 0 °C; (b) OsO_4, NMO; (c) DMP, PTSA; (d) Pd/C, H_2; (e) $ClCO_2Bn$.

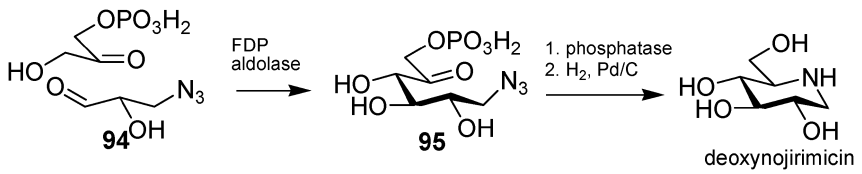


Fig. 39 Chemoenzymatic synthesis of imino sugars exploiting fructose 1,6-diphosppate (FDP) aldolase.

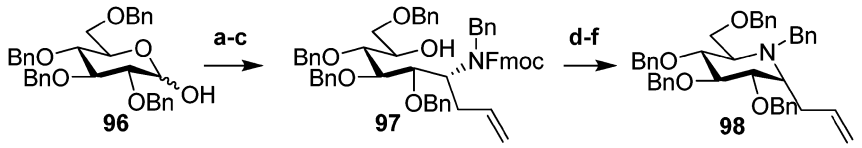


Fig. 40 Synthesis of α-*C*-glycosyl derivative of nojirimycin and its analogues. Reagents and conditions: (a) $BnNH_2$, PTSA; (b) $CH_2{=}CHCH_2MgBr$, Et_2O; (c) FmocCl, Na_2CO_3; (d) PCC; (e) piperidine, DMF; (f) $NaHB(OAc)_3$, AcOH, Na_2SO_4, 1,2-dichloroethane, −35 °C.

As already outlined, in general imino sugars lack the "anomeric" oxygen, because it would make them unstable. In order to introduce "anomeric" substituents, which is important to increase the selectivity of inhibition, carbon chains have been therefore introduced, and these molecules have been also named imino-*C*-glycosyl compounds. Different approaches have been developed to generate such kind of compounds, with α or β anomeric configuration and with different functionalities at the anomeric appendage in order to allow the generation of imino-disaccharides, imino-glyco-conjugates, imino-glycodendrimers and so on.

The insertion of a functionalised carbon chain, and at the same time of the amine, can be performed at the anomeric centre of the substrate as describe in Fig. 40.[71] Starting from 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose **96**, the amino group was introduced by reaction with benzylamine, followed by treatment of the obtained hemiaminal with allylmagnesium bromide. The obtained acyclic aminoalcohol **97** was subsequently cyclised *via* oxidation of the free hydroxyl group and reductive amination, to generate the protected α-*C*-glycosyl derivative of nojirimycin **98**. The allylic appendage allows a variety of chemical manipulations.

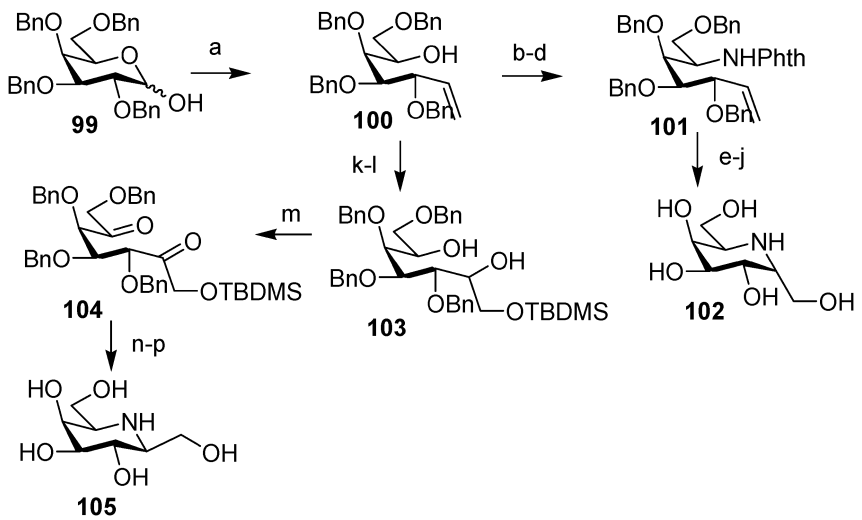


Fig. 41 Synthesis of *C*-glycosyl compounds of galactonojirimycins. Reagents and conditions: (a) $Ph_3P{=}CH_2$, PhMe; (b) $pO_2NC_6H_4CO_2H$, Ph_3P, DEAD; (c) MeONa; (d) Phthalimide, Ph_3P, DEAD; (e) N_2H_4, MeOH; (f) BnOCOCl; (g) $(CF_3CO_2)_2Hg$; (h) I_2, THF; (i) H_2, Pd/C; (j) KOH, MeOH; (k) OsO_4, NMO; (l) *t*$BuMe_2SiCl$; (m) oxalyl chloride, DMSO, −60 °C then Et_3N; (n) NH_4HCO_2, $NaBH_3CN$; (o) AcOH–H_2O; (p) Me_3SiI then H_2O.

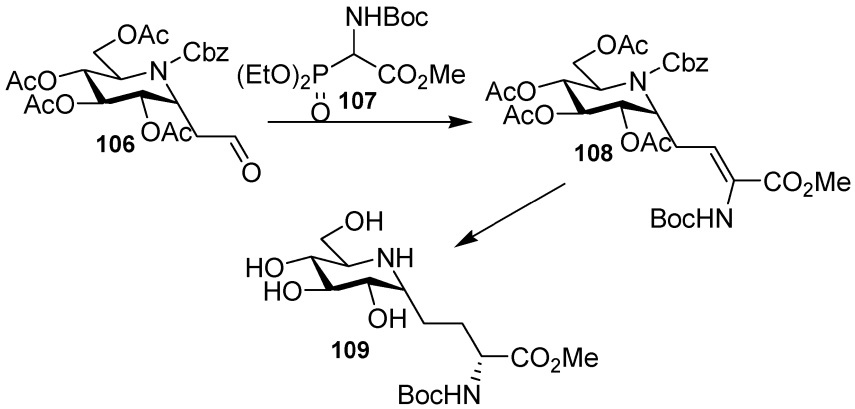


Fig. 42 Synthesis of nojirimycinyl *C*-(L)-serine.

An alternative approach, in which the anomeric centre of the sugar is reacted with a carbon nucleophile, but the amino group is subsequently inserted at the other side or in both sides at the same time, is reported in Fig. 41 for the synthesis of α- and β-homogalactonojirimycin.[72] Reaction of 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranose with methylenetriphenylphosphorane generated an enitol on which the amino group was inserted as phtalimide by a double inversion process of the 5-OH, under Mitsunobu's conditions.

The "anomeric" appendage of imino sugars has been exploited to generate mimics of glycoconjugates. An example is reported in Fig. 42 for the synthesis of nojirimycinyl *C*-(L)-serine **109**,[73] in which the ozonolysis of the allylic appendage generates the aldehydes **106**, on which the aminoacidic moiety was inserted by Wittig-Horner reaction.

Deacetylation and catalytic hydrogenation with Pearlman's catalyst, in the absence of any asymmetric catalyst, afforded the serine conjugate **109** in a 6:1 ratio in favor of the desired (L)-isomer.

In order to improve the selectivity of imino sugars as glycosidase inhibitors, imino-*C*-disaccharides have been synthesized with the expectation that they could fit much better to enzymes with more that one recognition site.[74] In order to generate such compounds, the imino sugar has been attached to a second sugar unit at different positions, and exploiting a variety C–C forming reactions. In the example of Fig. 43, an

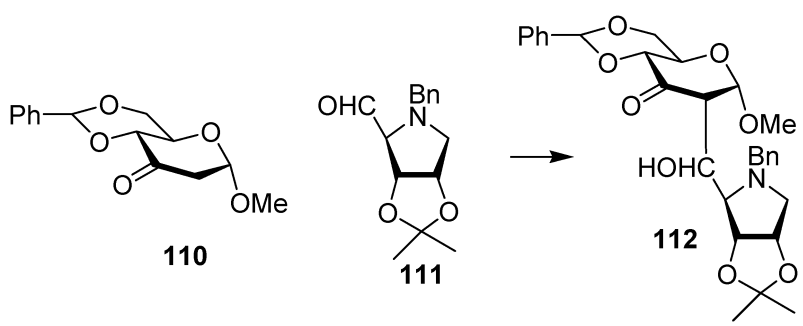


Fig. 43

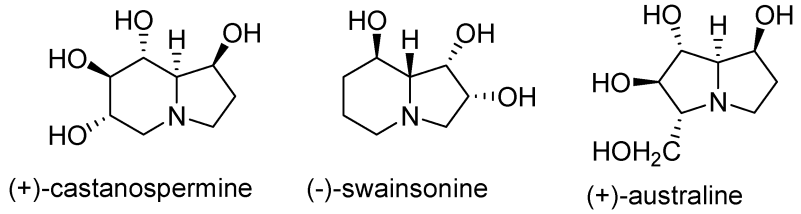


Fig. 44 Examples of naturally occurring bicyclic imino sugars.

aldol condensation between the imino sugars aldehydes **111** and sugar–ketone **110** is exploited.[75]

A variety of naturally occurring imino sugars presenting interesting biological activity, such as castanospermine, swainsonine or australine are bicyclic compounds (Fig. 44).

As a matter of fact, the second cycle increases the conformational rigidity of the molecule sometime increasing the activity and selectivity of the inhibitor. In the case of castanospermine for example, it has been postulated that the increase of inhibitory activity on α-glucosidase with respect to nojirimicin, is due to the orientation of the hydroxyl group at C-6 (referred to glucose) that in the case of castanospermine is restricted (Fig. 45).[76]

Among the many syntheses of caspanospermine, worthy of note is that reported in Fig. 46, in which the insertion of the nitrogen and the formation of both cycles occurs one-pot by reductive amination of a tri-carbonyl precursor **116** generated from aldehydes **113**.[77]

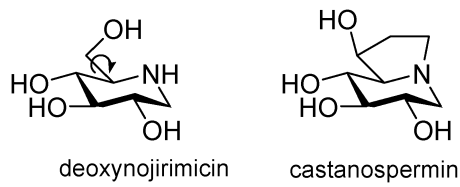


Fig. 45 Restricted conformation of castanospermine with respect to deoxynojirimicin.

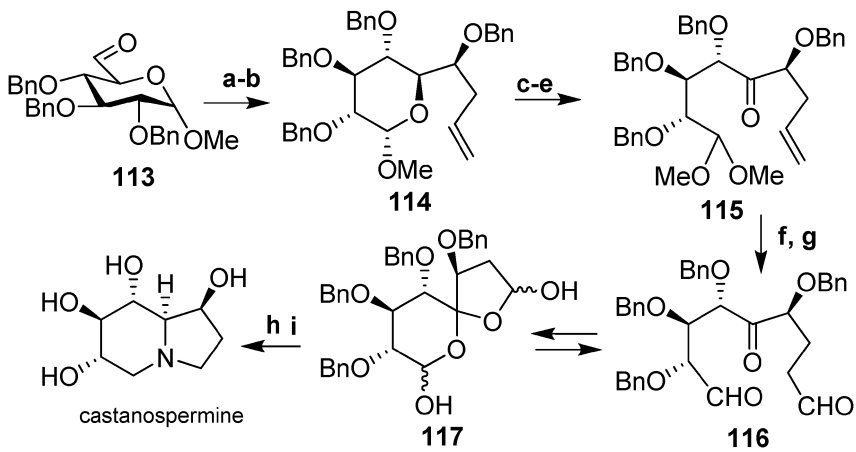


Fig. 46 Reagents and conditions: (a) allyl bromide, Sn, CH_3CN/H_2O, ultrasound; (b) BnBr, NaH, DMF; (c) IDCP, CH_2Cl_2/MeOH; (d) Zn, 95% EtOH, △; (e) DMSO/Ac_2O; (f) O_3, CH_2Cl_2, −78 °C, then Ph_3P; (g) 9 MHCl, THF; (h) NH_4HCO_2, $NaCNBH_3$, MeOH; (i) 10% Pd–C, MeOH/HCOOH.

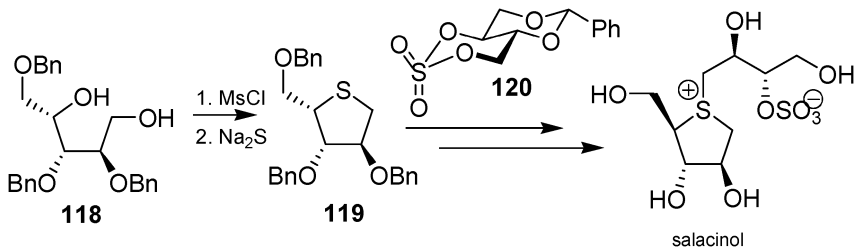


Fig. 47

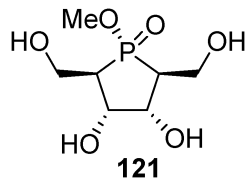


Fig. 48

3.2 Replacement of the endocyclic oxygen with a sulphur atom

Salacinol (Fig. 47) and some similar glycidic compounds in which a sulphur atom substitutes the endocyclic oxygen, has been isolated from the roots, stems, and leaves of Salacia reticulata, Salacia oblonga, and Salacia chinensis, which have traditionally been used for the treatment of diabetes in Sri Lanka, India, and Thailand. The antidiabetic activity of these compounds can be attributed to their capacity to inhibit α-glucosidases.[78] The mechanism of action is substantially the same of imino sugars, the positively charged sulphonium ion mimicking the oxonium ion transition state of glycosidases.

The chemistry and biological activity of salacinol and related zwitterionic glycosidase inhibitors has been recently reviewed.[79] From the synthetic point of view, the thio-sugar is generated from the proper sugar precursor, such as **118** in Fig. 46 for the synthesis of salacinol, performing a double nucleophilic substitution with Na_2S.

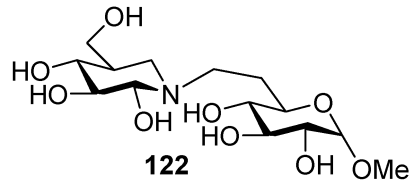
Instead of the sulfur atom, also Selenium was introduced, exploiting the same chemistry.[79]

3.3 Replacement of the endocyclic oxygen with a phosphono group

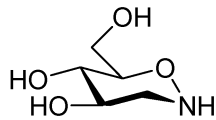
The endocyclic oxygen of carbohydrates has been replaced also with a phosphono group (Fig. 48).[80] However these compounds have not found applications.

4. Other modifications

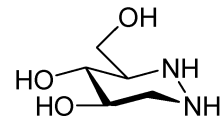
In order to modify the anomeric reactivity, also the anomeric carbon as been replaced with a different atom. The most studied case is that of the so defined 1-azasugars, in which a nitrogen is located at the anomeric position.[81] Such compounds, like imino sugars to which are strictly related, are able to inhibit glycosidases. Interestingly, the presence of the trivalent



122



123



124

Fig. 49

nitrogen at the anomeric position, allows to link easily an anomeric substituent, an example in which a second sugar unit is linked *via* a carbon bridge is reported in Fig. 49.[82]

In most cases, 1-azasugars present a piperidine or pyrrolidine skeleton, in other words a methylene group is positioned in place of the endocyclic oxygen, like in the case of compound **122**. In other cases the endocyclic oxygen is maintained, like in the case of compound **123**, or is replaced by a nitrogen (**124**).

Finally, the anomeric carbon has bees replaced by a phosphono group[83] or a sulphur, sulfoxi or sulphono group.[84]

5. Conclusions

In conclusion, the anomeric centre of carbohydrates has been modifies in a great variety of ways, with the main intent to generate stable analogies of glycosides or mimics of the transition state of glycosidases. The common feature, or at least potentiality, of these compounds is to inhibit carbohydrate processing enzymes, and therefore to interfere in the physiological of pathological processes in which they are implicated. Some of these compounds, such as Zanamivir, Zavesca or Miglitol, have found application as drugs against influenza, Gaucher disease or diabetes, but it is expected that the applications will be extended in the next future to other pathologies, in particular cancer and bacterial and viral infections.

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Recent strategies for the preparation of C-1 glycals

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C-1 Glycals, a relevant type of unsaturated branched sugar, are currently used in synthetic approaches towards *C*-glycosyl compounds and natural products. In this chapter we present a brief overview of the synthetic strategies employed for their preparation, which have been classified according to the type of starting material employed. Further modifications carried out on the C-1 glycals fall beyond the scope of this review and will not be discussed.

1. C-1 Glycals – an introduction

The term glycal is used to define sugar derivatives having a double bond between C-1 and C-2. Accordingly, C-1 glycals are $\Delta^{1,2}$ unsaturated carbohydrate derivatives with a carbon substituent at the anomeric position. These compounds are versatile synthetic intermediates, owing to the variety of transformations associated with their enol ether functionality, and have found ample use in the preparation of C-glycosyl compounds,[1] carbohydrate mimics,[2] and natural products.[3]

The preparation of C-1 glycals has been largely addressed by synthetic modifications on cyclic carbohydrate derivatives, although strategies that rely on ring forming reactions from acyclic derivatives have recently emerged (Fig. 1).

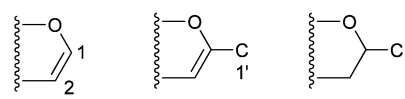

Fig. 1

2. From cyclic precursors

Among the approaches to C-1 glycals from cyclic carbohydrate precursors, those based on the formation of the C-1–C-1′ bond on a glycal have been by far the more exploited. Other methods based on the formation of the anomeric C–C bond followed by the installation of the $\Delta^{1,2}$ unsaturation have also been described.

2.1 Synthesis from glycals

The relatively acidic nature of the vinylic anomeric hydrogen in glycals is at the origin of most of the protocols for C-1–C-1′ bond formation in C-1

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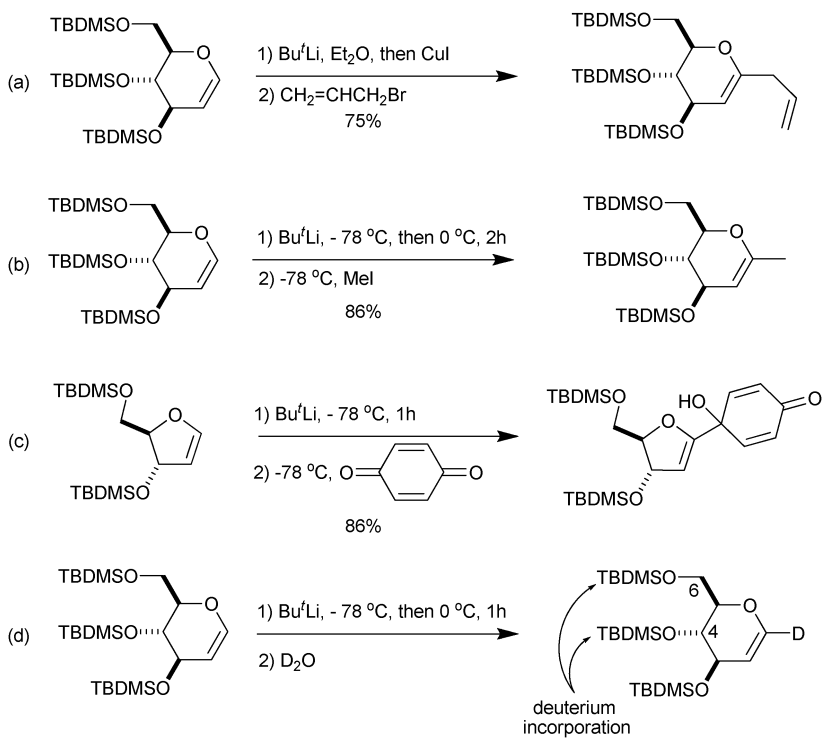
glycals. Thus, deprotonated glycals have been incorporated in synthetic strategies to C-1 glycals, either by their reaction with carbon electrophiles or by stannylation combined to palladium-catalyzed coupling reactions.

2.1.1 Methods based on the deprotonation of glycals. Independent studies from three research groups[4–6] demonstrated that pyranoid glycals could be deprotonated with strong bases (*tert*-butyl lithium,[4,5] or butyl lithium–potassium *tert*-butoxide[6]) and that the ensuing (lithiated) species[7] were able to react with various electrophiles.

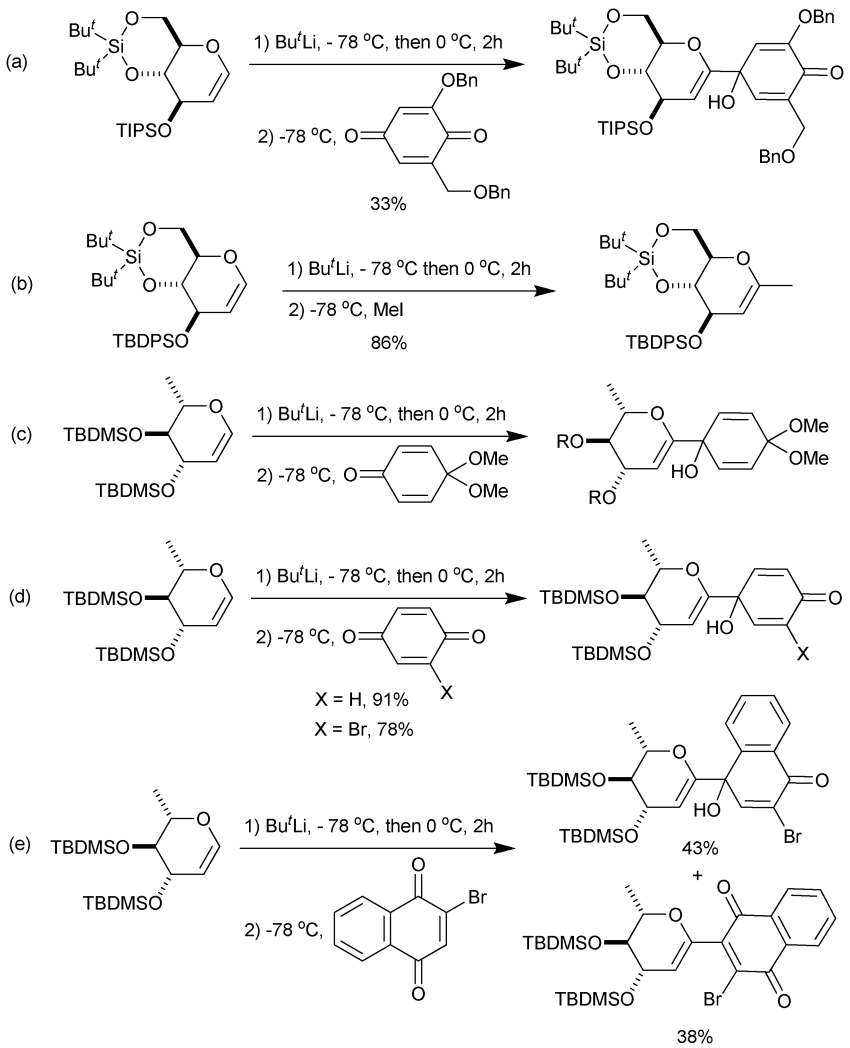
Accordingly, the direct lithiation of glycals followed by reaction with carbon electrophiles provides direct access to the corresponding C-1 glycals (Scheme 1a[5],b[4]). This chemistry has also been applied to furanoid glycals (Scheme 1c).[8] However, this transformation suffers from certain limitations associated with the competing deprotonation of the oxygen protecting groups in the glycal. In this context, benzyl protecting groups can not be employed,[4] and *tert*-butyldimethylsilyl (TBDMS) protecting groups experience lithiation α to silicon on the C6 TBDMSO methyl groups in addition (and in competition) to vinylic deprotonation (Scheme 1d).[9–11]



Scheme 1 Glycal lithiation-alkylation sequence.

This caveat could be circumvented with the use of triisopropylsilyl (TIPS) protecting groups (Scheme 2a,b),[12–16] or when 6-deoxy glycals (devoid of the C-6 oxygen substituent) are used as substrates (Scheme 2c,d,e).[13,17,18]

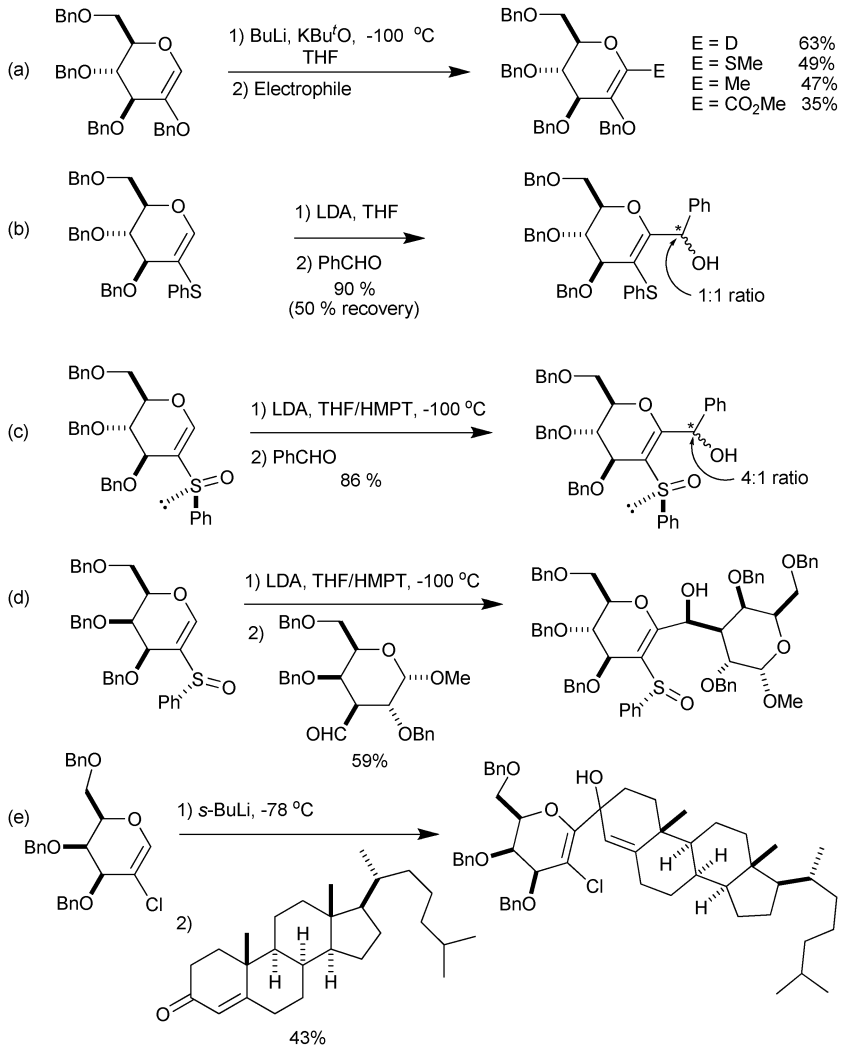
This strategy has been extensively used by Parker's group in the synthesis of *C*-glycosyl aromatic antibiotics (Scheme 2a,c,d,e) by transformation of the easily accessible quinol C-1 glycals into aryl C-1 glycals, and thence to *C*-glycosyl compounds.[19]



Scheme 2 Formation of C-1 glycals from lithiated glycols.

A different approach to facilitating direct C1-lithiation of glycals was devised by Schmidt's group by incorporating appropriate heteroatom substituents at C-2. Activation of the C-1 position, in C-2 activated glycals, was rationalized by inductive effects and stabilization of the resulting lithiated species *via* complexation. This approach is compatible with the use of *O*-benzyl protected glycals. A 2-*O*-benzyl glycal was successfully deprotonated by Schlosser's base (*n*-BuLi, KO^{t}Bu) and trapped with several electrophiles (Scheme 3a).[20] Sulfur substituents at C-2 gave improved results, thus 2-(phenylthio)-glycals could be cleanly lithiated with LDA

(Scheme 3b).[20] However, best results were observed when 2-(phenylsulfinyl) glycals[21] were used, and these were considered the substrates of choice by Schmidt's group in their strategies to C-1 glycals.[22] In the case of 2-(phenylsulfinyl) glycals both sulfoxide diastereomers can be lithiated, varying the degrees of diastereoselectivity observed in the coupling products (Scheme 3c).[23] When carbohydrate containing aldehydes were used as electrophiles, C-glycals could be produced as single diastereoisomers (Scheme 3d).[24]

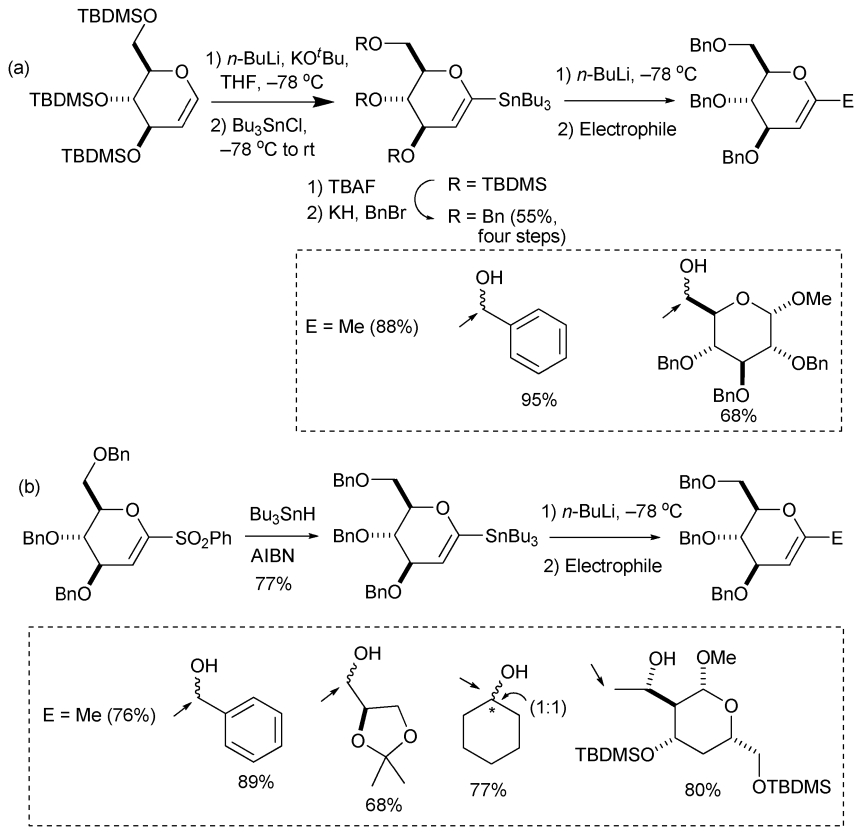


Scheme 3 Access to C-1 glycals by C-1 lithiation of C-2 activated glycols.

More recently, reports by Quayle and co-workers, have shown that benzylated 2-chloroglucal derivatives can be lithiated, with *sec*-butyllithium in THF at −78 °C, and the ensuing lithiated species trapped with a variety of carbon electrophiles that include enolizable carbonyl derivatives.[25] 4-Cholesten-2-one underwent 1,2-addition to yield a 5:1 mixture

(α:β facial selectivity) of chloro adducts (Scheme 3e). Similar chemistry has also been aplied to *O*-methyl protected 2-fluoro glucal.[26]

A widely extended alternative approach to the generation of C-1 lithiated glycals is based on the tin–lithium exchange of 1-tributylstannyl derivatives.[27] Thus, whereas direct deprotonation of a glycal requires a strong base and higher temperatures, tin–lithium exchange on 1-tributylstannyl glycals can be effected under milder conditions by treatment with *n*-BuLi at −78 °C. Hanessian first reported this protocol on a silylated glucal (Scheme 4a).[6] Thus, stannylation (*n*-BuLi, KO^{t}Bu) followed by protecting group replacement (TBDMS → Bn) and tin–lithium exchange (*n*-BuLi, −78 °C) led to a C-1 lithiated benzyl glucal that was able to react with methyl iodide, and a hexose-derived aldehyde (Scheme 4a).[6] 1-Lithiated glycals also react with epoxides that had been activated by boron trifluoride etherate (Scheme 4b).[28]



Scheme 4 Tin–lithium exchange, on 1-stannyl glycals, followed by reaction with carbon electrophiles.

The advantage of this protocol over the direct lithiation method is that the lithiated glycal was produced under less demanding conditions. However, the first lithiation step is not avoided and thence the acidity related restrictions to protecting groups will still apply. In this context, Beau devised a route to 1-stannyl glycals based on a radical-mediated

addition-elimination process from 1-phenylsulfonyl glycals. Under this conditions the, above mentioned, protecting group requirements no longer apply (Scheme 4b).[4] Two additional synthetic routes to 1-tributylstannyl glycals have been devised by Kocienski's group: (i) Ni(0)-catalysed coupling of C-1 phenylsulfonyl glycals with tributylmagnesium bromide,[29] and (ii) lithiation of C-1 phenylsulfinyl glycals followed by quenching with tributylstannyl chloride.[30]

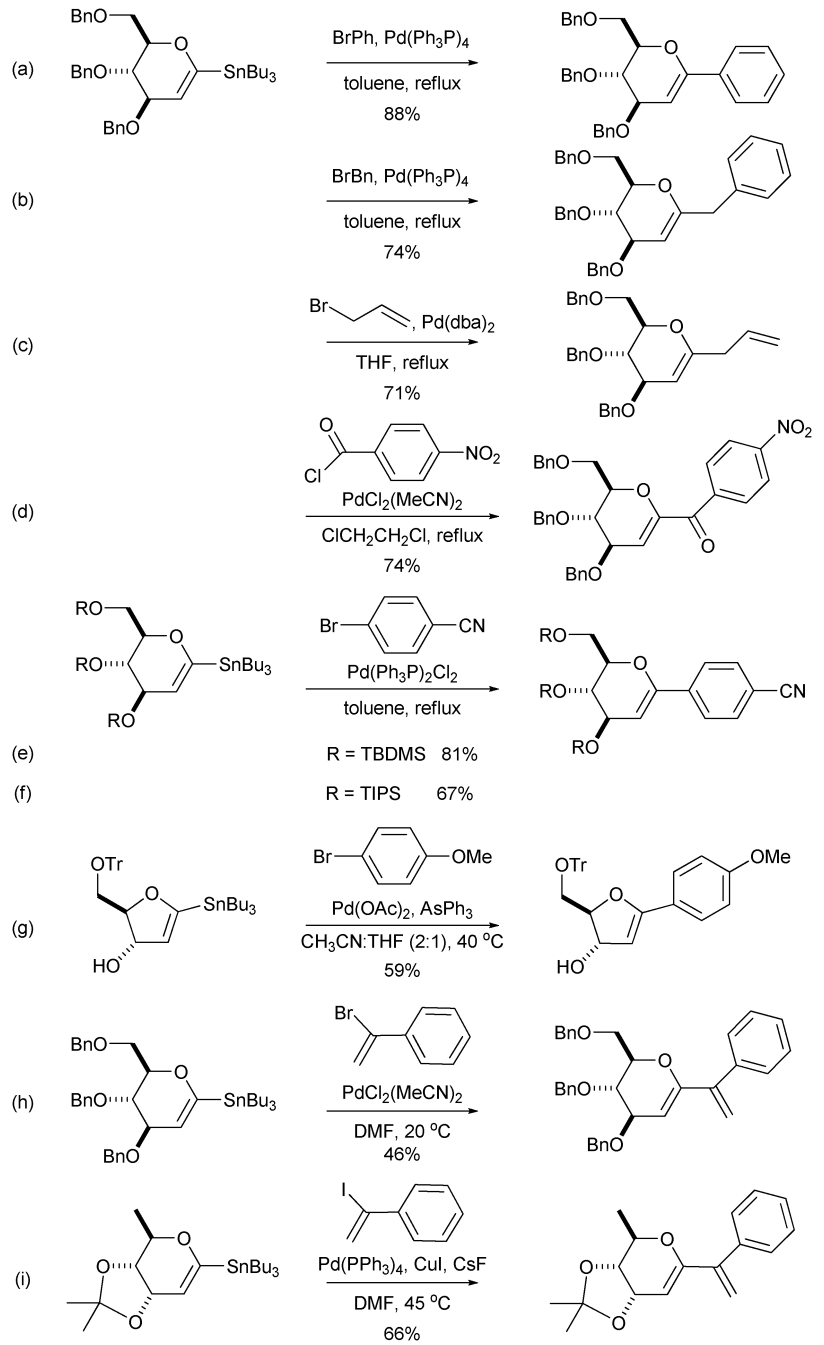
Several research groups have applied this strategy for the preparation of C-1 glycals.[31]

2.1.2 Methods based on palladium catalysed cross-coupling reactions. The relevance of C-1 stannylated glycals in the preparation of C-1 glycals not only stems from their ability to generate C-1 lithiated species but, more interestingly, from their capacity to participate in palladium-catalyzed coupling reactions with organic halides, a process independently reported by the groups of Beau[32] and Friesen.[33]

This coupling reaction often requires a careful choice of catalyst and solvent. Thus, Beau's group reported the use of tetrakis(triphenylphosphine)-palladium(0) $Pd(PPh_3)_4$ in refluxing toluene for the coupling of aryl and benzyl bromides with a benzylated C-1 stannyl glucal (Scheme 5a,b).[32] $Pd_2(dba)_3$ in the presence of triphenylphosphine was used for the coupling with allyl bromide (Scheme 5c), and $PdCl_2(MeCN)_2$ was used for the acylation of the above mentioned glucal (Scheme 5d).[32] Friesen's group reported the use of $(PPh_3)_2PdCl_2$ for the coupling reaction with a variety of *o*- and *p*- substituted bromobenzenes (Scheme 5e,f).[33,34] Furanoid glycals also undergo palladium mediated coupling with iodoarenes (Scheme 5g).[35] Vinyl halides underwent cross-coupling reactions with C-1 stannyl glycals under the agency of $Pd(MeCN)_2Cl_2$ in DMF (Scheme 5h),[36] or with palladium in the presence of copper (I) salts and fluoride ion [$Pd(PPh_3)_4$, CuI, CsF] in DMF (Scheme 5i).[37]

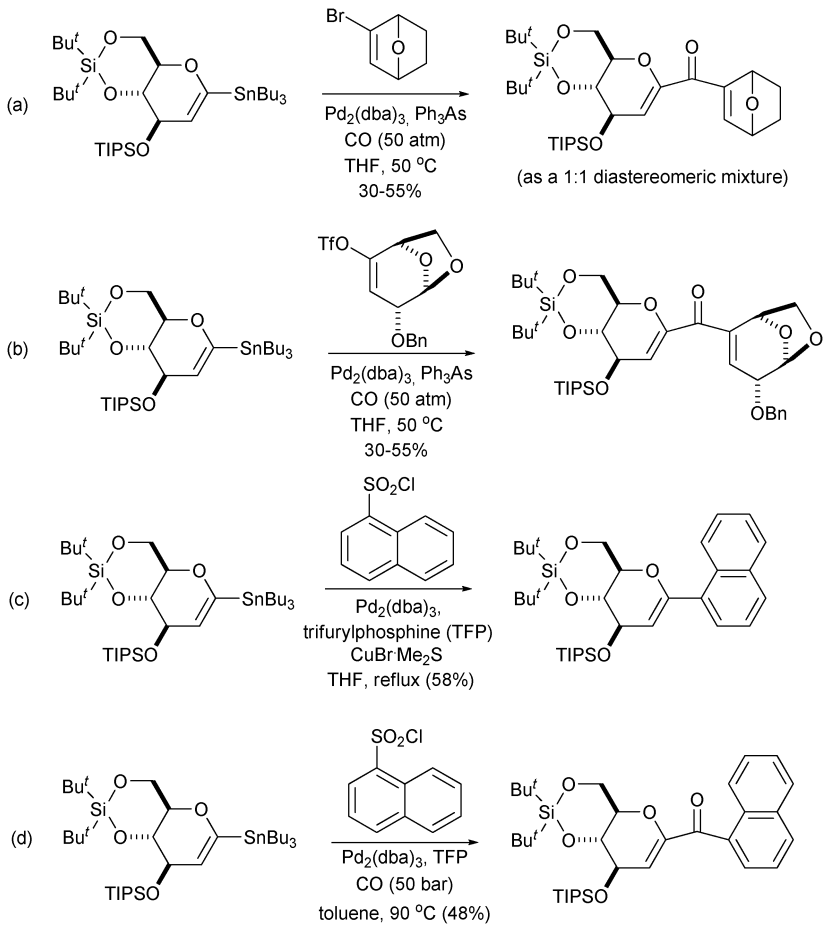
Recent contributions from Vogel's group have shown that, under CO atmosphere and in the presence of $Pd_2(dba)_3$ and Ph_3As, 1-stannyl glycals can be carbonylated and coupled to organic halides (Scheme 6a),[38] or vinyl triflates (Scheme 6b),[39] in carbonylative Stille cross-coupling processes.[40] Also of interest is the palladium catalyzed cross-coupling reaction of 1-tributylstannyl glycals with arenesulfonyl chlorides.[41] This process takes place with concomitant desulfitation (Scheme 6c), and, when carried out in the presence of CO, also results in carbonylative Stille cross-coupling processes (Scheme 6d). However, these carbonylative Stille couplings are highly influenced by steric factors. Thus, 1-stannyl triisopropylsilyl glucal failed to give any coupling product with the vinyl bromide shown in Scheme 6a,[38] and the desulfitative carbonylative Stille cross-coupling of 1-naphthalenesulfonyl chloride was unsuccessful when a bulkier tin galactal derivative was used instead of the glucal derivative shown in Scheme 6d.[41]

Palladium catalyzed cross-coupling reactions of 1-substituted glycals have not only been limited to tributylstannyl derivatives. In fact, the versatility of this approach is significantly enhanced by the fact that C-1 zinc-, indium-, or iodine-substituted glycals (easily accesible from glycals, see Scheme 7)



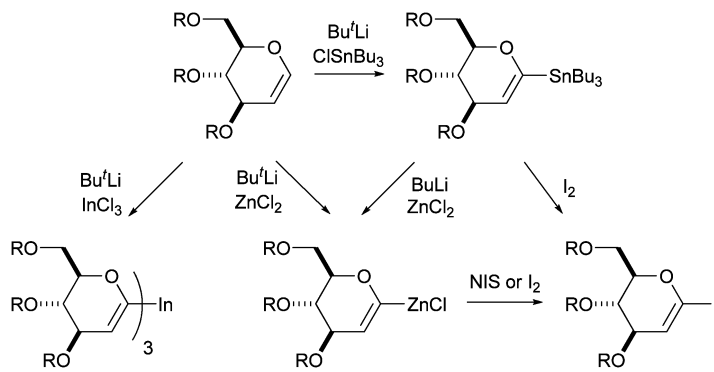
Scheme 5 Palladium-catalyzed coupling reactions of C-1 stannylated glycals with organic halides.

can also be harnessed to cross-coupling reactions. Lithiation of glycals followed by trapping of the resulting anion with indium trichloride or zinc dichloride provides facile access to 1-indium or zinc derivatives, respectively (Scheme 7). The latter have also been prepared from 1-tributylstannyl glycals, which also serve as starting materials in the preparation of 1-iodo



Scheme 6 Carbonylative Stille cross-coupling of vinyl halides, triflates, and sulfonyl chlorides.

glycals by treatment with iodine. A direct, one-pot, route to 1-iodo glycals *via* zincglycals has also been reported (Scheme 7).[34b]



Scheme 7

These variety of glycal derivatives is of considerable synthetic value, and have been exploited by several research groups. In a report by Quayle and

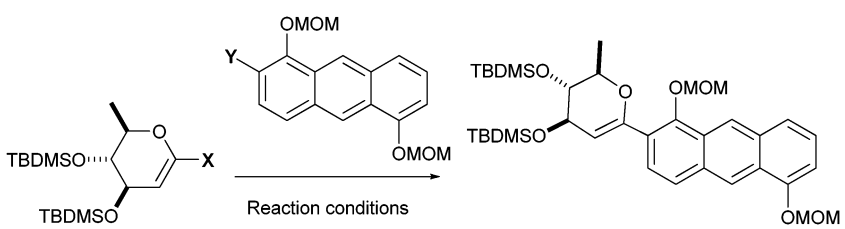
co-workers, the Stille cross-coupling reaction of a stannylated glycal with an alkenyl iodide was accompanied by loss of stereochemistry, however the related Negishi cross-coupling with a chlorozinc glycal [$Pd(PPh_3)_4$ or $Pd_2(dba)_3] \cdot CHCl_3$, $As(Ph)_3$, rt] furnished the sought diene without erosion of double bond stereochemistry.[42] Ousmer *et al.* observed that whereas homocoupling was the major observed process in the Stille cross-coupling of a C-1 tributylstannyl glucal, the corresponding Negishi coupling [$Pd_2(dba)_3$] $CHCl_3$, P(*o*-tolyl)$_3$, THF, rt] of a related zincated glucal led to the coupled product in 90% yield.[43] Holzapfel and Portwig described the Negishi coupling [$Pd(PPh_3)_2Cl_2$, THF, reflux] of 2-phenylsulphonyl bromozinc glucals with aryl halides for the preparation of C-1 aryl glucals.[44]

The use of an organoindium glucal in palladium catalyzed [$Pd(PPh_3)_2$] cross-coupling reactions with aryl iodides gave moderate yields (40–60%) of C-1 aryl glucals.[45]

Tius and co-workers illustrated the possibilities of differently functionalized glycals in their route to vineomycinone B2 methyl ester (Table 1).[46] They tested differently substituted glycals, in coupling reactions with various aromatic derivatives (Table 1), and found that the Negishi coupling of a chlorozinc glycal with a iodoarene was the method of choice for the preparation of the sought aryl C-1 glycal (Table 1, entry 4).

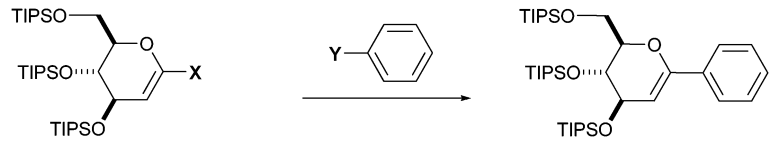
1-Iodo glycals are also useful substrates in the preparation of C-1 aryl and alkyl glycals. Friesen and Loo described the preparation and use of 1-iodo-3,4,6-tri-*O*-(triisopropylsilyl)-D-glucal in palladium-catalyzed couplings of metalated aromatic compounds (Table 2, entries 2–5).[12] They noticed that changing from TBDMS to TIPS protecting groups in Stille coupling reactions of C-1 stannylated glycals (compare in Scheme 5, 5e *versus* 5f) was a major and disadvantageous modification. On this basis, they reasoned that a reversal in the sense of the coupling reaction, making the bulkier carbohydrate moiety the more reactive organic halide partner, could be beneficial. In fact, they found that the coupling of metalated aromatics, including $ArB(OH)_2$ (Table 2, entry 3), $ArB(OMe)_2$ (Table 2, entry 4), and ArZnCl (Table 2, entry 5), with 1-iodo glucal takes place in good yields and, that the glycal dimer, the major by-product in the "normal" Stille coupling, was not formed.

Table 1 Tius' exploratory work on route to vineomycinone B2 methyl ester



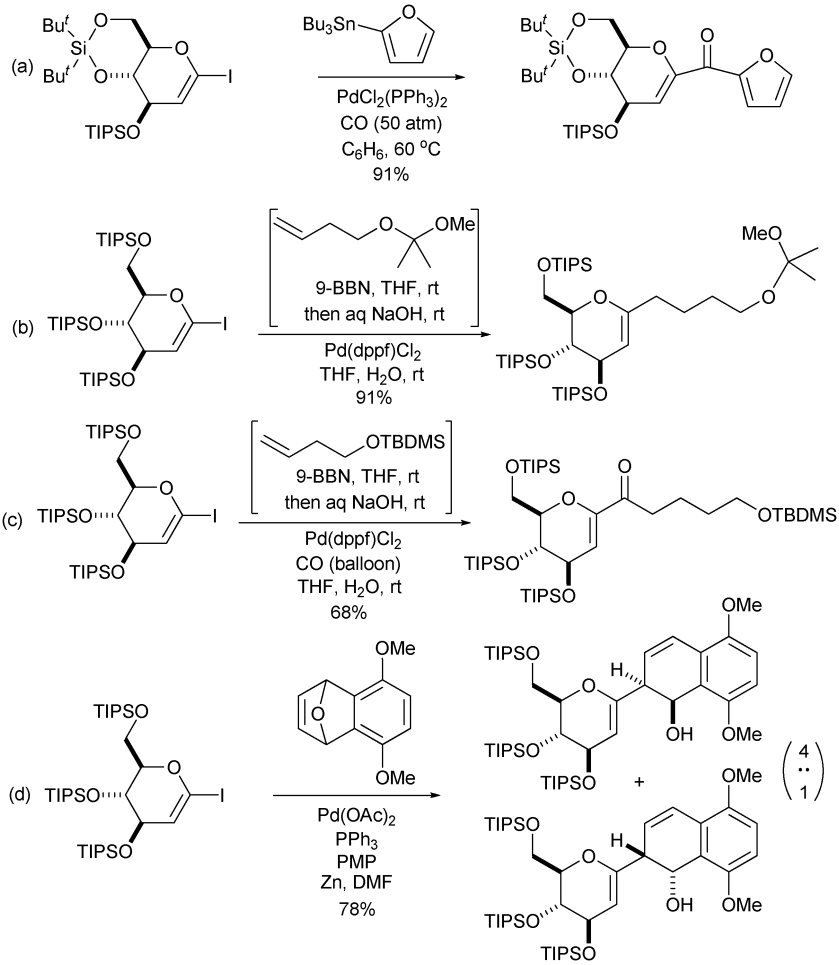
Entry	X	Y	Reaction conditions	Yield
1	I	ZnCl	$Ni(dppp)Cl_2$; ether, THF, rt	32%
2	I	$SnBu_3$	$Pd(CH_3CN)_2Cl_2$, PPh_3; $CHCl_3$; 80°C	0%
3	$SnBu_3$	I	$Pd(CH_3CN)_2Cl_2$, DMF; 80 °C	31%
4	ZnCl	I	$Pd(PPh_3)_2Cl_2$ + DIBAL; THF; rt	78%

Table 2 Transition metal-catalyzed coupling of different 1-substituted glycals



Entry	X	Y	Reaction conditions	Yield
1	$SnBu_3$	Br	$Pd(PPh_3)_4$, THF, reflux, 15h	70%
2	I	$SnBu_3$	$Pd(PPh_3)_2$, Cl_2, THf, reflux, 24h	20%
3	I	$B(OH)_2$	$Pd(PPh_3)_2$, Cl_2, THF-aq Na_2CO_3/75 °C/1.5 h	81%
4	I	$B(OMe)_2$	$Pd(PPh_3)_2$, Cl_2, THF-aq Na_2CO_3/rt/15 h	90%
5	I	ZnCl	$Pd(PPh_3)_2$, Cl_2, THF/rt/30 min	90%

Several other research groups have also made use of 1-iodo glycals in their syntheses of C-1 alkyl and aryl glycals (Scheme 8). Vogel and co-workers reported the use of 1-iodo glycals in carbonylative Stille couplings with

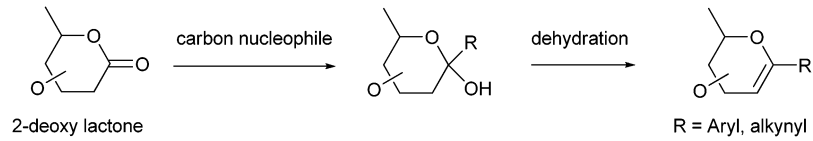


Scheme 8

tributylstannyl partners (Scheme 8a),[38] and Potuzak and Tan showed the utility of the *B*-alkyl Suzuki–Miyaura coupling, and that of its carbonylative version for accessing C-1 alkyl and C-1 acyl glycals, respectively, from a variety of terminal olefins (Scheme 8b,c).[47] Martin and co-workers reported the palladium-catalyzed ring opening of oxabenzonorbornadienes in the presence of 1-iodo glycals to furnish dihydronaphtols (Scheme 8d) that were readily oxidized to C-1 (2-naphthyl) glycals.[48]

2.2 Synthesis from other cyclic derivatives

2.2.1 Synthesis from carbohydrate derived 2-deoxy lactones. Routes to C-1 glycals from 2-deoxy sugar pyranolactones have been described. The general strategy is outlined in Scheme 9.

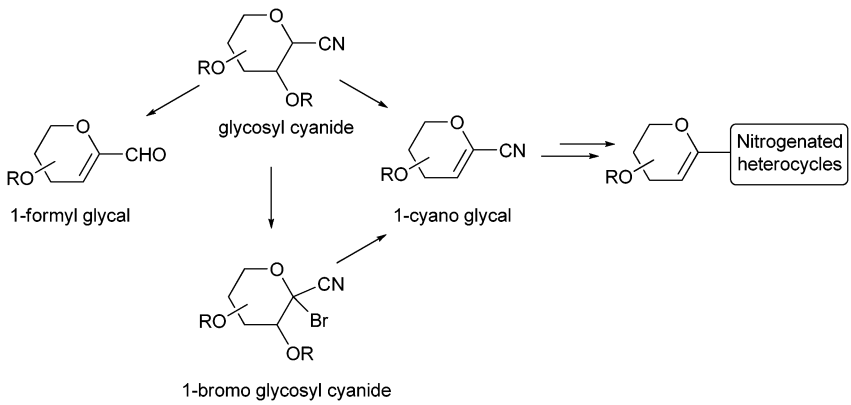


Scheme 9 Routes to C-1 glycals from 2-deoxy lactones.

Sulikowski and co-workers reported the addition of aryl magnesium bromides to 2-deoxy lactones followed by dehydration with Martin's sulfurane ($Ph_2S[OC(CF_3)_2Ph]_2$)[49] as a route to C-1 aryl glycals.[50] The preparation of a C-1 alkynyl glycal based on an analogous addition of an alkynyl lithium, followed by $POCl_3$/pyridine mediated dehydration has also been described.[51] More recently, a general method for the one-pot the preparation of C-1 aryl glycals has appeared, it entails addition of aryllithium reagents to 2-deoxysugar lactones followed by dehydration of the intermediate hemiketals with a mixture of pyridine, DMAP, and TFAA, at −78 °C.[52] This method provides access to C-1 aryl glycals (glucal, rhamnal) in good yields (75–92% yield), although galactose derivatives gave low yields of the corresponding glycals.

2.2.2 Methods based on β-elimination from *C*-glycosyl derivatives. Glycosyl cyanides have been thoroughly used as starting materials in the preparation of 1-formyl, and 1-cyano-glycals, mainly through base-induced elimination procedures (Scheme 10).

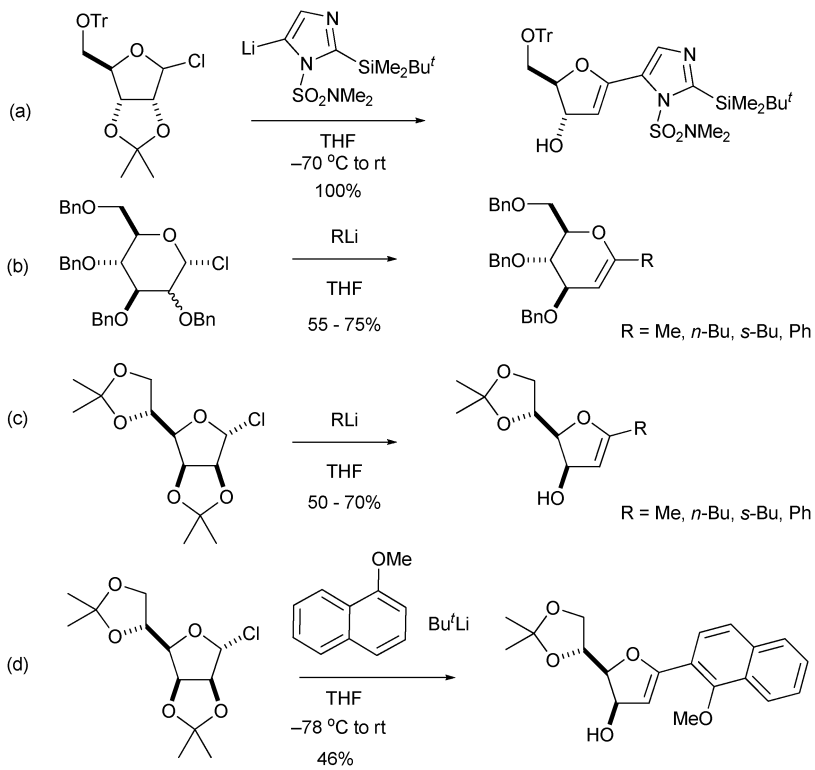
Dettinger *et al*. described in 1979 the synthesis of a 1-formyl galactal derivative and evaluated its potential as a galactosidase inhibitor.[53] Schmidt and co-workers also employed 1-formyl galactal derivatives in the preparation of potential galactosyl transferase inhibitors.[2d,f] Routes to 1-cyano glycals have been mainly explored and described by Somsak's group, either from 1-bromo glycosyl cyanides (zinc dust/NEt_3 or pyridine)[54] or from glycosyl cyanides (DBU).[55] These compounds have been transformed into nitrogenated heterocycles owing to their resemblance to nucleosides,[55,56] or as potential inhibitors in the shikimate pathway.[57,58] 1-Cyano galactal has also been used in the preparation of heptulosonic acid derivatives and analogues.[59] On the other hand, Withers evaluated 1-cyano glucal as a



Scheme 10 Synthetic routes to 1-formyl and 1-cyano glycals from glycosyl cyanides.

potential glycosidase inhibitor.[2g] Finally, glycosyl acetylenes bearing ether protecting groups have been transformed into C-1 alkynyl glycals upon treatment with *n*BuLi.[60]

2.2.3 Synthesis from glycosyl chlorides. Glycosyl chlorides bearing isopropylidene acetal or ether (including benzyl) protecting groups react with alkyl or aryl lithium reagents to give C-1 alkyl or aryl glycals (Scheme 11).[61,62] The reaction has been applied to furanosyl chlorides



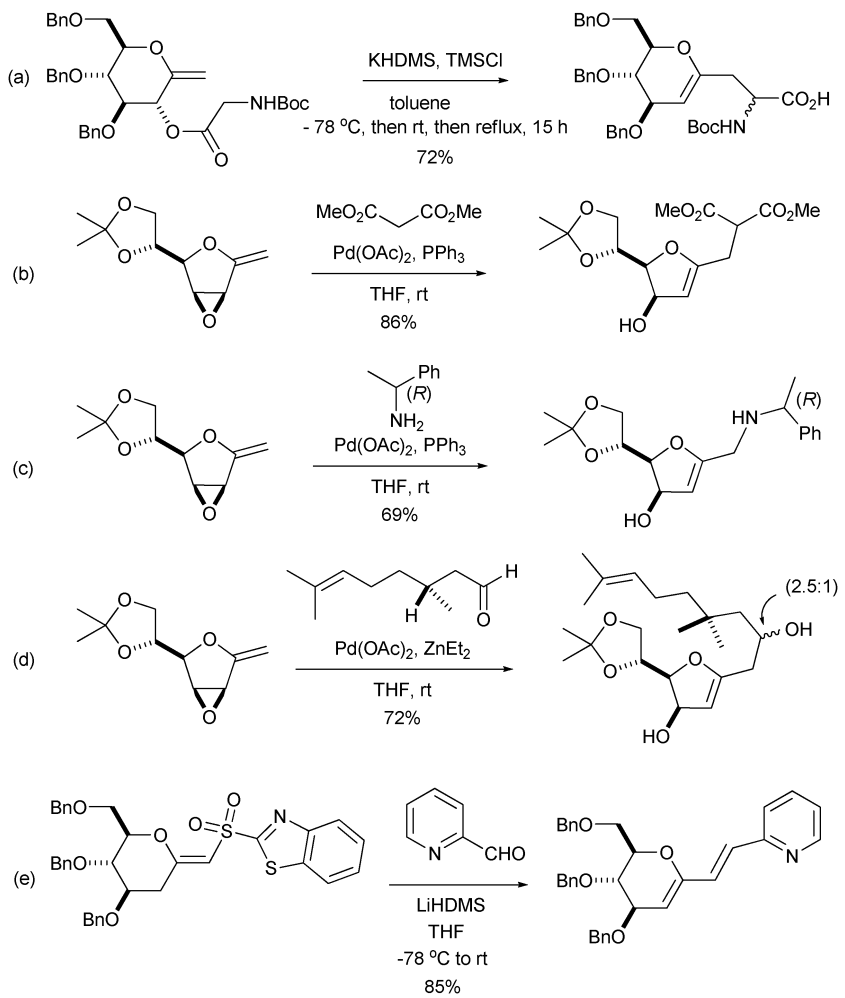
Scheme 11 Reaction of glycosyl chlorides with organolithium reagents.

and 5-lithio imidazoles (Scheme 11a),[61] and to pyranosyl and furanosyl chlorides (Scheme 11b, c and d),[62] with commercially available organolithium reagents. The stereochemistry of the substituent at C-2 seems to be irrelevant to the outcome of the reaction (Scheme 11b), and aryl lithium derivatives generated by directed *ortho*-metalation,[63] could be also used in the preparation of C-glycals (Scheme 11d).[64] Lithium phenyl acetylide, however failed to give the expected C-alkynyl glycal.

2.2.4 Synthesis from exo-glycals. Several routes to C-1 glycals from 1-*exo*-methylene pyranoses and furanoses have been described recently.

A Claisen–Ireland rearrangement has been applied to 1-exo-methylene pyranoses bearing enolizable ester functionalities at C-2 (Scheme 12a).[65]

1-*exo*-Methylene-2,3-anhydro furanoses have been transformed into functionalized C-1 glycals by palladium [$Pd(PPh_3)_4$] catalyzed nucleophilic addition (Scheme 12b,c).[66] Reaction of the same substrates with $Pd(PPh_3)_4$



Scheme 12 Synthesis of C-1 glycals from *exo*-glycals.

in the presence of diethyl zinc resulted in umpoled species able to react with electrophiles (Scheme 12d).[67]

Conjugated C-1 glycals have also been prepared from *C*-glycosylidene vinyl sulfones by means of a modified Julia olefination (Scheme 12e).[68]

3. From acyclic precursors

The use of acyclic sugar-derived precursors provides a complementary approach to that shown in Section 2, from cyclic carbohydrate derivatives. Among the strategies to C-1 glycals from acyclic carbohydrate precursors, two protocols that differ in the C–C bond formed have been developed, one in which the formation the C-1–C-2 double bond is formed by way of ring-closing metathesis (RCM) and other that involves the construction of the C2–C3 bond in the glycal ring throuhg an oxocarbenium ion–enol ether cyclization.

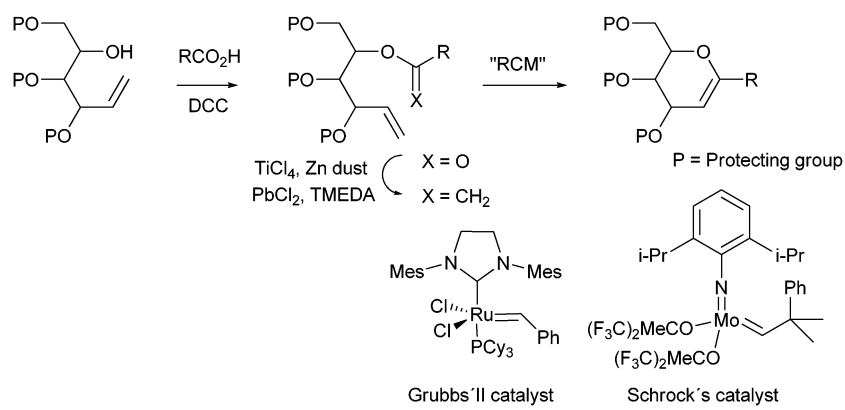
3.1 Ring-closing metathesis

Postema and co-workers have developed a general synthetic sequence to C-1 glycals based on RCM that is outlined in Scheme 13.[69]

The strategy begins with the dehydrative coupling of a suitable carbohydrate-based alcohol with an aproppriate acid. Methylenation of the ensuing ester using the Takai procedure[70] is then followed by RCM to give C-1 glycals. In their early work, they used Schrock's molybdenum as the catalyst for RCM but in more recent work the RCM key step has been accomplished by exposure of the enol-ethers to Grubbs' second-generation ruthenium catalyst.

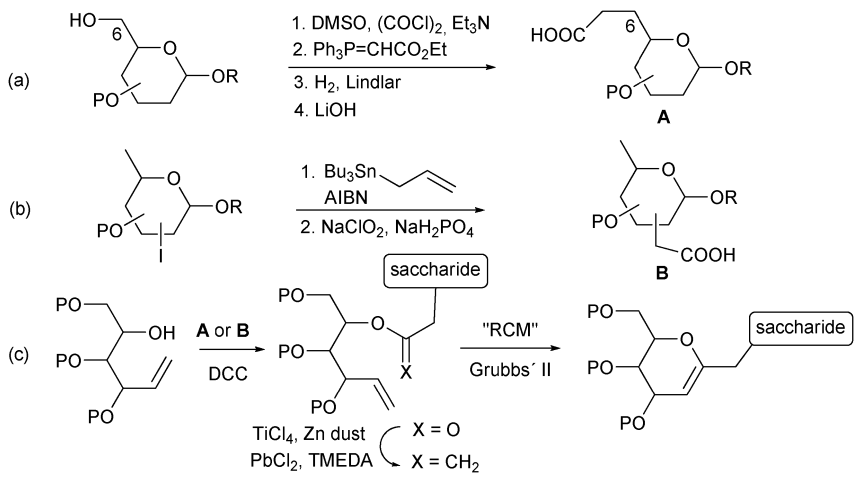
The convergence of the approach is clear and the generality of the method relies only on the availability of the required carboxylic acid.

The method was first applied to the preparation of a number of simple alkyl and aryl C-1 glycals,[71] but since then the scope of this approach have been extended with the preparation of a small library of significant C-1 glycals including steroidal, and glycerolipidic residues endowed with potential biological activity.[72]



Scheme 13 Ring-closing metathesis approach for the synthesis of C1-glycals.

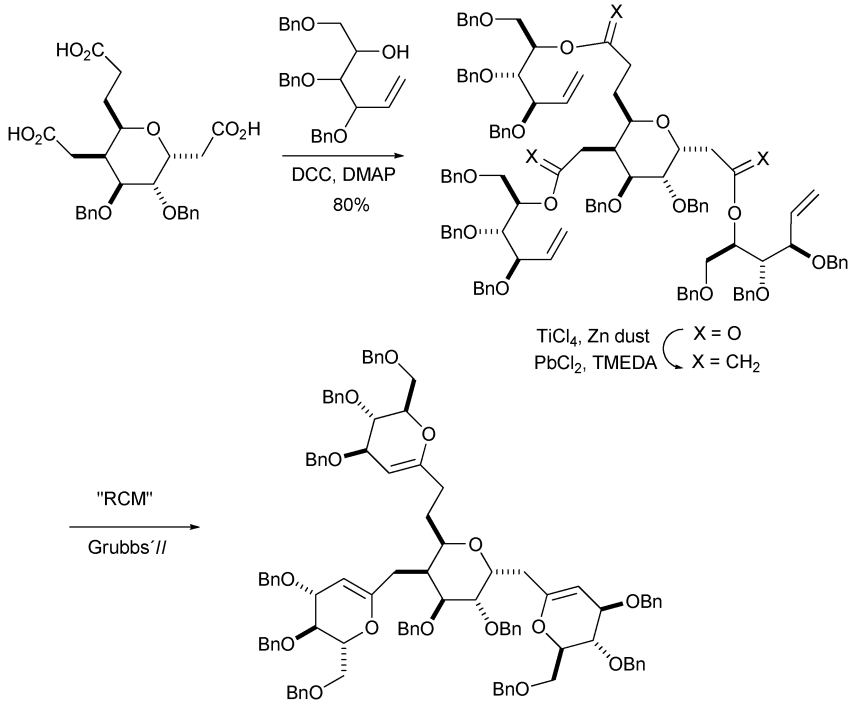
This protocol permits a facile entry to C-saccharide glycals when the carboxylic acid functionality is attached to a pre-existing pyranose ring. This chemistry is illustrated in Scheme 14. The carboxylic acid moiety can be installed, by chain extension, at any position of the saccharide partner. Thus, it can be incorporated at C-6, through a sequence involving: oxidation, Wittig reaction, hydrogenation and saponification (Scheme 14a),[73] or at any other position in the saccharide ring by free radical allylation followed by oxidative cleavage of the ensuing double bond (Scheme 14b).[74] Ester formation, mediated by DCC, methylenation, and RCM proved to be routine to produce a collection of C-disaccharide glycals (Scheme 14c).



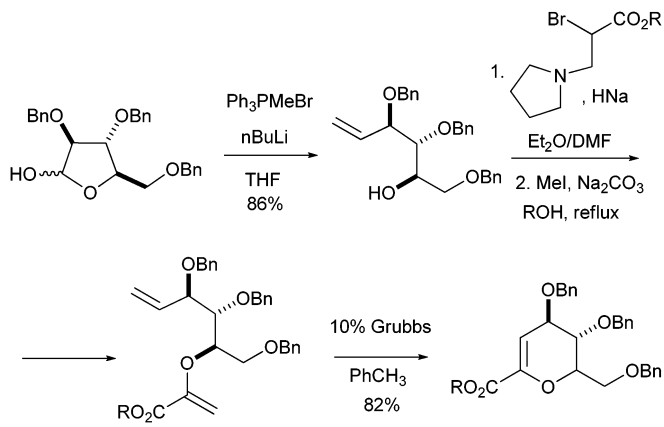
Scheme 14 Ring-closing metathesis approach to C-saccharide glycols.

In addition, this route was shown to be useful for the synthesis of bis-C-1 glycal[75] or tris-C-1 glycal saccharides[76] through a double or triple esterification, methylenation, and enol ether-olefin RCM cyclization. It is remarkable that the reaction sequence worked well even when both groups to be cyclized are on the same side of the pyranose ring (no other cyclized by-products were observed) (Scheme 15). Scheme 15 illustrates the reaction of branched pyranose triester which was methylenated to generate the RCM-precursor. Subsequent exposure of the resulting tris-enol ether to 50 mol% of Grubbs' second generation catalyst provided the desired tris-C-glycal. This compound was subsequently transformed into a β-C-tetrasaccharide by hydroboration (44% yield over three steps).[76]

In related work, Rutjes and co-workers reported a four-step protocol from conveniently protected sugar derivatives to substituted C-1 glycals (Scheme 16).[77] The key step in their synthetic route included RCM of highly functionalized α-alkoxyacrylates. The potential of these glycal derivatives was illustrated by their conversion to natural products such as DAH (3-deoxy-D-arabino-2-heptulosonic acid) or KDO (3-deoxy-D-manno-2-ulosonic acid).



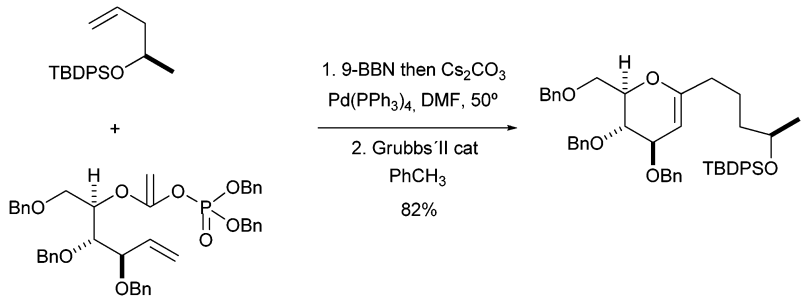
Scheme 15 Triple ring-closing metathesis of a tris-C-glycal.



Scheme 16 Ring-closing metathesis of carbohydrate-derived α-alkoxyacrylates.

Recent contributions from Sasaki's group have exploited a Suzuki–Miyaura coupling/RCM sequence for the convergent synthesis of a variety of C1-glycals and using a small set of readily available precursors. Thus, as illustrated in Scheme 17, alkylboranes (prepared by hydroboration of the appropriate olefin), are *in situ* coupled with sugar-derived enol phosphates. Subsequent RCM with Grubbs' second generation catalyst, then afforded desired C-1 glycals in good overall yields. It is noteworthy that the intermolecular Suzuki–Miyaura coupling predominates over the intramolecular Heck cyclization of the enolphosphate. The C-1 glycals prepared

in this manner have been successfully applied to the synthesis of spiroketals, including cytotoxic metabolites.[78]

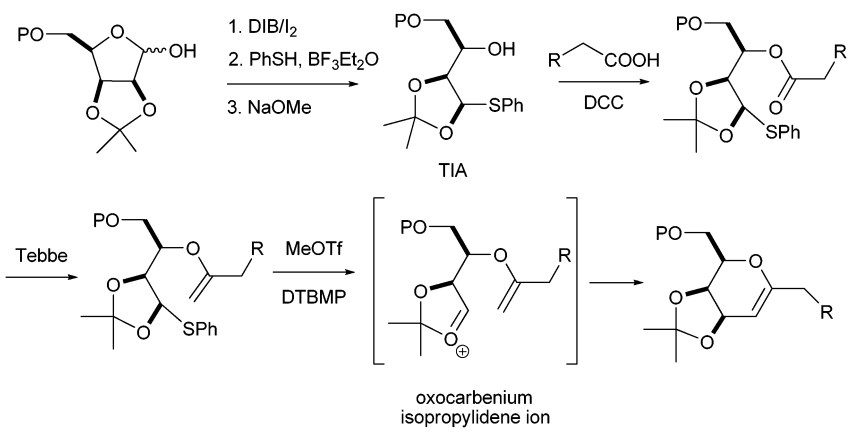


Scheme 17 Synthesis of C-glycals based on Suzuki–Miyaura coupling/RCM sequence.

3.2 Oxocarbenium ion–enol ether cyclization

Mootoo and co-workers disclosed a procedure for the preparation of C-1 substituted galactals based on the intramolecular capture of an oxocarbenium ion by an enol ether residue.[79] In their approach, the key intermediate 1-thio-1,2-*O*-isopropylidene acetals (TIA) are activated with methyl triflate to generate the crucial annulating synthon (1,2-*O*-isopropylidenated oxocarbenium ion).

The overall strategy is highly convergent and it entails the assembly of 1-thio-1,2-isopropylidene alcohol, obtained in three straigthtforward operations from D-lyxose, with a carboxylic acid (Scheme 18). Then, olefination of the ensuing ester followed by activation of the thioacetal, with methyl triflate in the presence of 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP), leads to the C-1 substituted glycals in high yield and as single stereo- and regioisomer. The protocol have been applied to different types of carboxylic acids, thus providing a convergent entry to C-galactals with aglycon substituents of varying complexities.[80]



Scheme 18 Synthesis of C-glycals based on oxocarbenium ion-enol ether cyclization.

4. Conclusions

This chapter has examined the many strategies reported for the preparation of C-1 glycals. Presently, most of the strategies rely in the modification of cyclic, unsaturated, carbohydrate derivatives, and within them glycals have been the favorite starting materials. Several routes to C-1 glycals from 1-*exo*-methylene derivatives have also appeared. More recently, strategies based on acyclic derivatives, that use mainly ring-closing metathesis, have become popular and are currently showing their versatility.

From the existing literature reports, methods can be found for the preparation of almost any type of C-1 glycal. However, the increasing relevance of these compounds appears to guarantee a continued effort in the development of synthetic strategies towards C-1 glycals.

Abbreviations

AIBN	2,2-azobisisobutyronitrile
9-BBN	9-borabicyclo[3,3,1]nonane
Bn	benzyl
DCC	dicyclohexylcarbodiimide
DIB	diacetoxy iodobenzene
DIBAL	diisobutylaluminum hydride
DMF	dimethylformamide
DMAP	4-*N*,*N*-dimethylaminopyridine
DTBMP	2,6-di-*tert*-butyl-4-methylpyridine
h	hour
HMPT	hexamethylphosphorous triamide
KHDMS	potassium hexamethyldisilazane
KO^{t}Bu	potassium tertbutoxide
LiHDMS	lithium hexamethyldisilazane
PMP	1,2,2,6,6-pentamethylpiperidine
rt	room temperature
TBDMS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
TIA	1-thio-1,2-*O*-isopropylidene acetals
TIPS	triisopropylsilyl
TMEDA	tetramethylethylenediamine

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Glycosidases in synthesis

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1. Introduction

In recent decades, there has been a boom in carbohydrate chemistry, evoked by the increasing need for new carbohydrate materials and by the dynamic development of glycomics. Oligosaccharides can be prepared by classical organic chemistry methods,[1] however, the tedious protection, activation, and deprotection strategies lead to intolerably low yields and consumption of material and time.[2,3] In this respect, enzymatic synthesis, ideally stereo- and regiospecific, comes in play. An efficient and elegant glycosidic bond formation can be accomplished by two enzyme groups—glycosyltransferases (EC 2.4.1.-)[4–6] and glycosidases (EC 3.2.1.-). The classification and properties of these enzymes and their application in oligosaccharide synthesis have been described in a number of detailed reviews.[7–11] In this chapter, we will focus on glycosidases, their substrate specificity, and their application in oligosaccharide synthesis, including the latest trends: use of modified substrates and mutant glycosidases.

2. Glycosidase as a promising tool for synthesis

2.1 Classification

Glycosidases (*O*-glycoside hydrolases; EC 3.2.1.-) are *in vivo* determined to the cleavage of oligo- and polysaccharides by glycosyl transfer to water. They are able to form the glycosidic linkage under 'unnatural' conditions, where a carbohydrate hydroxyl moiety acts as a more efficient nucleophile than water. Such conditions can be achieved by a variety of strategies including reduction of water activity and use of glycosyl donors activated by a good leaving group. Nowadays, the sub-subclass of glycosidases encompasses 149 valid entries in IUBMB enzyme nomenclature system (http://www.chem.qmul.ac.uk/iubmb/enzyme/EC3/cont3aa.html, Jan 15, 2009). Enzyme classification into families reflecting the amino acid sequence similarities was introduced, which is now the base of the frequently updated CAZy database (http://www.cazy.org).[12] CAZy also proposes 'supraclass' division based on the protein fold, which is better conserved than their sequences. Thus, some of the almost 100 families are grouped in over ten 'clans'.[12]

2.2 How do glycosidases work?

Traditionally, the glycosidases used for synthetic purposes are exoglycosidases, which transfer only the non-reducing terminal monosaccharide unit

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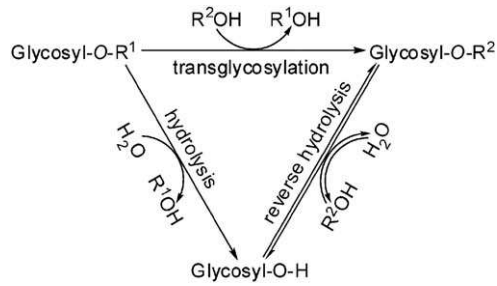
of substrates. They operate both in the transglycosylation and the reverse hydrolysis modes (Scheme 1).

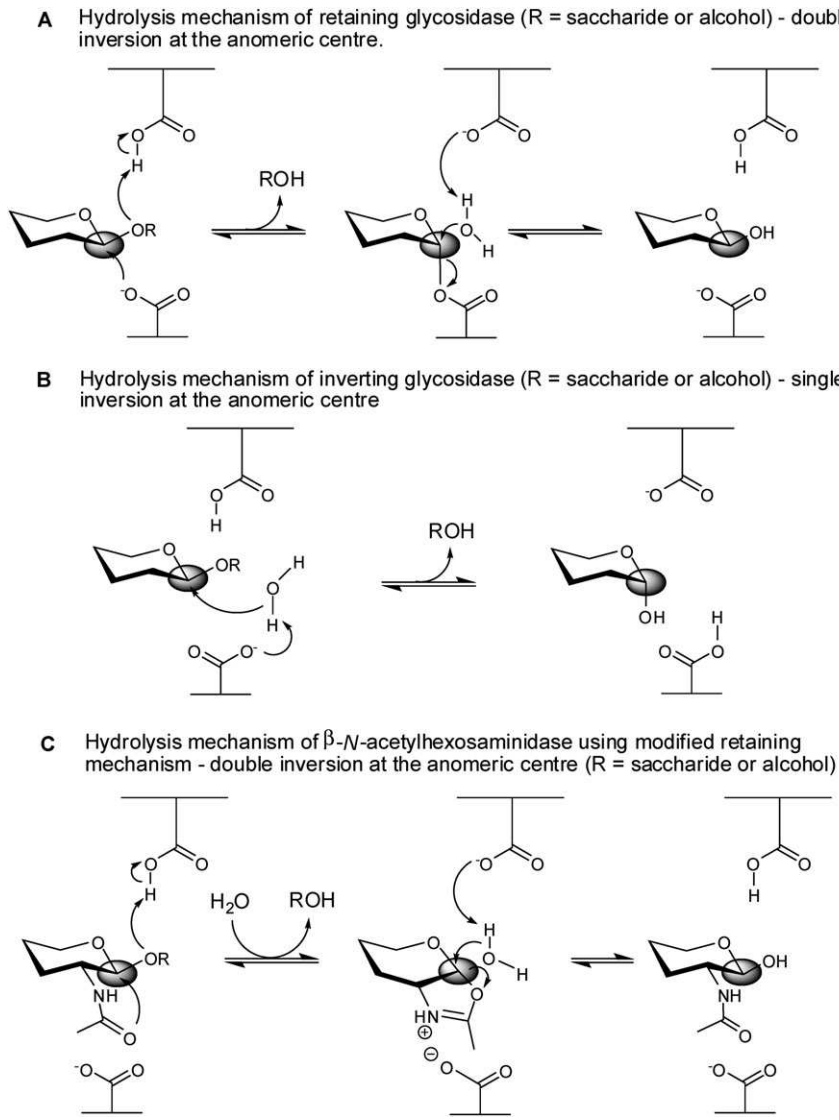


Scheme 1 Reactions catalyzed by glycosidases.

Glycosidases synthesize the glycosidic bond in two ways, depending on the substrate structure and the composition of the reaction medium. In the thermodynamically controlled or equilibrium process, a free monosaccharide is combined with a nucleophile under the exclusion of a water molecule. This process, commonly referred to as 'reverse hydrolysis' (Scheme 1), is in its nature a condensation reaction. The equilibrium constant strongly favors hydrolysis over glycoside formation. A shift towards product formation can be reached by decreasing the water activity by high reactant concentrations (*e.g.*, 80–90% w/w total sugar concentration), addition of salts, by removing the product from the reaction mixture (*e.g.*, on an active carbon column) or by reaction medium engineering, *e.g.*, using organic solvents or microwave field.[10] Generally, increased reaction temperature (50–60 °C) is necessary to bring the reaction to equilibrium on a reasonable time scale. Reaction times are days or even weeks and yields do not exceed 15%. Although not as widely used as the kinetically controlled transglycosylation, this approach resulted in several noteworthy products, *e.g.*, non-reducing sugars[13] and thioglycosides.[14] Reverse hydrolysis is a widely used method for glycosylation of (mainly primary) alcohols.

The kinetically controlled reaction design—transglycosylation (Scheme 1)—employs an activated glycoside. For this design, the choice of a leaving group, as well as the water activity reduction,[15,16] are crucial. The product can accumulate at much higher concentrations than in the equilibrium distribution and, as a result, the reaction gives considerably higher yields—generally in the range of 20–40%. Water acts as a competing nucleophile and causes parasitic hydrolysis of the reactant. Most glycosidase-catalyzed reactions are performed in this way.

The glycosidase mechanism on the molecular level has been extensively studied.[17,18] Its detailed knowledge is a prerequisite for manipulation of glycosidase activities *via* protein engineering or design of new inhibitors. The glycosidic bond hydrolysis can result in the net inversion of configuration at the anomeric carbon (inverting enzymes) or *vice versa* (retaining enzymes). Both ways involve similar oxocarbenium-ion-like transition states (Scheme 2). Retaining glycosidases (Scheme 2A), which often have transglycosylating abilities, hydrolyze *via* a double-displacement



Scheme 2 Hydrolysis mechanisms of retaining (A) and inverting (B) glycosidase and β-*N*-acetylhexosaminidase, which uses a modified retaining mechanism (C).

mechanism. The catalytic machinery involves two catalytic carboxylates: an acid/base and a nucleophile. In the first step (glycosylation), the former carboxylate provides an acid-catalyzed leaving group departure simultaneously with a nucleophilic attack by the other residue to form the glycosyl-enzyme intermediate. In the second step (deglycosylation), the acid/base carboxylate acts as a general base to activate the incoming nucleophile (water or another acceptor), which hydrolyzes the glycosyl-enzyme intermediate yielding a new glycosidic linkage. Inverting glycosidases (Scheme 2B) act by a single-step, acid/base catalyzed mechanism: the leaving group is directly displaced by the nucleophilic water molecule. β-*N*-Acetylhexosaminidases (Scheme 2C) utilize a double-displacement mechanism, in which the

nucleophile is not donated by the enzyme but by the 2-acetamido group of the substrate itself, forming an oxazoline intermediate (Scheme 2).

Classical examples of retaining glycosidases are β-galactosidase, β-fructofuranosidase, and hen lysozyme; representatives of inverting glycosidases include α-L-rhamnosidase, trehalase, and β-amylase. Inverting glycosidases do not catalyze transglycosylation reactions—they just work in the reverse hydrolysis mode.[19]

2.3 Properties of glycosidases

Glycosidases are readily available from natural sources like seeds, microorganisms or fungal cultures, as well as higher organisms (typically plant seeds, mollusks, *etc.*). Commercial crude enzyme preparations constitute another worthy source. Availability, stability and easy handling are the main advantages of glycosidases over glycosyltransferases. Glycosidases are absolutely stereoselective, with the exception of glycosyl fluoride hydrolysis, where the enzyme is able to cleave 'wrong' fluoride anomers.[20] However, even in this case the cleavage consistently yields one anomer. The glycosidase stereoselectivity has practical applications, *e.g.*, for separating mixtures of chemically prepared α/β glycosides.[21] The enantioselectivity of glycosidases towards the aglycon moiety can result in significant enantiomeric enrichment of racemic mixtures. Glycosidases are generally able of chiral discrimination; however, mostly with a rather average enantiomeric excess ($<65\%$).[22,23]

Glycosidases are rather undemanding in the choice of substrates, which greatly broadens their applications compared to glycosyltransferases. However, they also suffer from several significant drawbacks, such as low regioselectivity and yields. Thus, the choice of an appropriate attitude and an enzyme type remains strongly dependent on the specific application.

2.4 Diversity of glycosidases

2.4.1 Mannosidases. α-Mannosidases are successfully applied in reverse hydrolysis yielding *manno*-oligosaccharides. The reactions mostly afford mixtures of regioisomers;[24] however, several α-mannosidases exhibit a relatively high selectivity, like α-mannosidase from *Aspergillus phoenicis* with (α1-2) or (α1-6) activity depending on the cultivation medium[25] and the recombinant α-mannosidase from *Penicillium citrinum* with (α1-2) activity.[26] Maitin *et al.*[26] used these selective enzymes to purify the (1–3) regioisomer from the complex mixture after the almond α-mannosidase-catalyzed reaction.

β-Mannosidic linkage, particularly β-D-Man*p*- and β-D-Man*p*NAc-(1 → 4)-D-Glc*p*NAc, is one of the most difficult bonds to synthesize chemically and only few methods can achieve complete anomeric stereoselectivity.[3,27] Successful β-mannosylations of various acceptors were accomplished using retaining glycosidases, however, generally with modest yields.[28–31] Moreover, the need of activated β-mannopyranosides as donors leads into a vicious circle. A prospective solution is offered by mutant β-mannosidases, *e.g.*, β-mannosynthase from *Cellulomonas fimi*[32] or β-mannansynthase from

Cellovibrio japonicus.[33] They ensure high yields and employ easily available α-mannosyl fluorides.

2.4.2 Fucosidases. α-L-Fucosidases (EC 3.2.1.51), the only members of glycoside hydrolase family 29, are involved in many biological processes like inflammation, growth regulation, receptor interactions, and antigenicity. Their substrate specificity vitally depends on the enzyme source (bacteria, moulds, mollusks, plants, and mammals). High yields were reached in regioselective synthesis of (α1-3)-fucosylated *N*-acetyllactosamine and lactose (51% and 34%, respectively) by α-L-fucosidase from *Alcaligenes sp*.[34] The high selectivity for synthesizing the (α1-3)-linkage was also demonstrated by other microbial α-L-fucosidases from *Penicillium multicolor*,[35,36] the latter showing decent activity in DMSO, DMF, and dioxane.[35]

β-D-Fucosidases (EC 3.2.1.38) are only scarcely studied in the literature. This activity was introduced to *Escherichia coli* β-galactosidase (lac Z) by genetic manipulation.[37]

2.4.3 α-L-Rhamnosidases. The use of α-L-rhamnosidases (EC 3.2.1.40) in synthetic reactions has been rather exceptional so far, also due to their inverting character. However, their hydrolytic potential has important industrial applications. Several enzymes have been isolated from commercial 'hesperidinase' and 'naringinase' enzyme preparations (*e.g*., from *Aspergillus niger* and *Penicillium decumbens*),[38–40] microbial[41–44] and fungal sources,[45–49] and prepared by cloning and recombinant expression.[50] Martearena *et al.* published the first study on α-L-rhamnosylation of aliphatic alcohols by reverse hydrolysis using L-rhamnose. He also studied the use of various β-rhamnosides as glycosyl donors,[19] but transglycosylation does not proceed with inverting glycosidases. As a result, activated substrates are always first hydrolyzed and the released rhamnose is used in the reverse hydrolysis mode (Křen and Pišvejcová, unpublished results). Recently synthesis of rutinosides and rutinose by reverse hydrolysis catalyzed by fungal α-L-rhamnosidases were published.[51]

2.4.4 Galactosidases. α-D-Galactosidases (EC 3.2.1.22) are known to be potentially useful in industrial applications, mainly in the sugar industry, where they improve the crystallization of sucrose *via* the hydrolytic cleavage of raffinose, they can enhance the bleaching effect in the pulp and paper industry.[52] α-D-Galactosidases are also widely used for the removal of α-gal residues from polysaccharides. As a result, the removal of raffinose, stachyose and leguminous polysaccharides from seeds or soymilk has a positive impact on the acceptance of soy-based foods, because these sugars cause intestinal discomfort and flatulence.[53] α-D-Galactosidase was applied in the pre-treatment of animal feed to hydrolyze nonmetabolizable sugars, thereby increasing their nutritional value.[54] The increasing interest in these enzymes in biomedicine is due to their therapeutic applications, for example in the treatment of Fabry′s disease,[55–59] the conversion of a B type blood cell to an O type cell[60–62] or their potential use in xenotransplantation by removing α-gal type immunogenic epitopes. The introduction of an α-gal

residue into various structures can alter the physiological activities of the new compound, *e.g.* attaching an α-gal epitope.[63] More examples of α-D-galactosidase applications has been recently reviewed by us.[11]

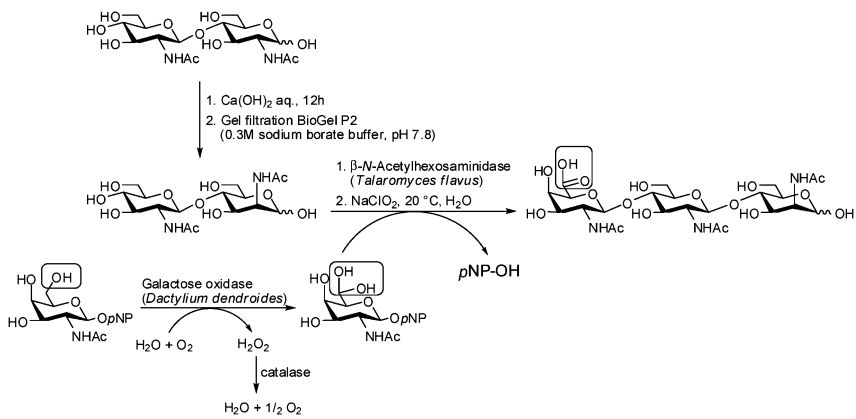
2.4.5 β-*N*-Acetylhexosaminidases. β-*N*-Acetylhexosaminidases (EC 3.2.1.52) catalyze the hydrolysis of terminal β-D-Glc*p*NAc and β-D-Gal*p*NAc residues in nature. β-*N*-Acetylhexosaminidases are widely distributed in plants, animals, fungi, and microorganisms. They belong among the most active lysosomal glycosidases and they are fundamental for chitin degradation. In human organism, β-*N*-acetylhexosaminidase activity and isoenzyme pattern are clinically important markers of various disorders, especially in nephrology, urology, and pediatry, notably also of Tay-Sachs and Sandhoff diseases.[64–66] Their industrial applications[67] comprise structural characterization of the glycosylation pattern of glycolipids and glycoproteins, synthesis of glyco-structures by transglycosylation, and design of antifungal agents in medicine and agriculture.

β-*N*-Acetylhexosaminidases have excellent ability for wide synthetic applications with natural and also modified substrates. A series of successful enzymatic syntheses of different hexosamine oligosaccharides in reverse[68,69] or transgylcosylation mode were described.[70–85]

2.5 Glycosidases in multi-enzyme reactions

The synthesis of complex carbohydrate structures can preferably be accomplished using several enzymes in one-pot or sequential mode. Crude intermediate products may be either directly processed by the follow-up enzyme, or a fast and simple purification step may be included like desalting, concentrating, *etc*. This approach saves both time and costs, however, it requires a relatively high specificity, regioselectivity and yields in all the steps, which somehow limits the choice of glycosidases available.

A typical multi-enzyme reaction, presented in diverse variations, comprises the combined use of a glycosidase and a glycosyltransferase. If they are applied in one pot, the glycosidase product is directly processed by



Scheme 3 Synthesis of β-D-Gal*p*NAcA-(1 → 4)-D-Glc*p*NAc by coupled use of galactose oxidase from *Dactylium dendroides* and β-*N*-acetylhexosaminidase from *Talaromyces flavus*, followed by *in situ* chemical oxidation.[76]

a selective transferase and thus, its secondary hydrolysis is avoided. Suzuki *et al.*[86] combined β-galactosidase from *Bacillus circulans* and α-2,3-sialyltransferase from rat liver with chemical modification to obtain a mucin-type tetrasaccharide linked to protected L-serine.

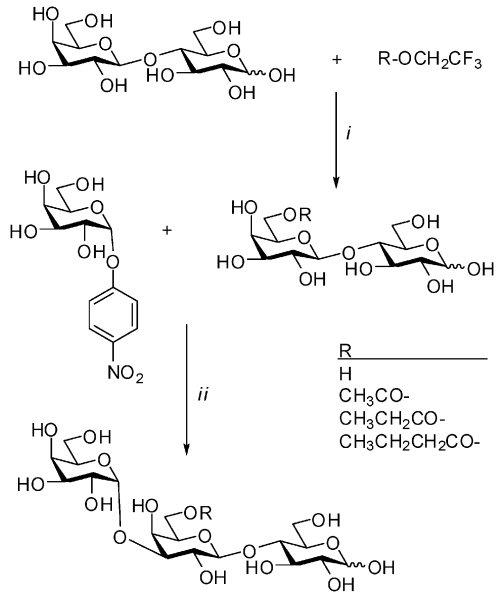
Another useful enzyme combination is the tandem: galactose oxidase—glycosidase. An example is the successive use of galactose oxidase from *Dactylium dendroides* and β-*N*-acetylhexosaminidase from *Talaromyces flavus*, followed by *in situ* chemical oxidation, which afforded an immunoactive disaccharide of β-D-Gal*p*NAcA-(1 → 4)-D-Glc*p*NAc[76] (Scheme 3).

3. Regioselectivity of glycosidases

Contrary to glycosyltransferases, glycosidases exhibit a rather poor regioselectivity. If more than one acceptor hydroxyl is present, the transglycosylation reaction mostly results in complex mixtures, difficult to separate. In general, the primary hydroxyl group reacts preferentially to the secondary ones, yielding (1-6)-linked products. The regioselectivity of the transfer reaction is time dependent—when the transglycosylation reaches its thermodynamic equilibrium, the predominating regioisomer, which is the least willingly hydrolyzed one. By the rational selection of glycosidase from various sources, it is possible to prepare various regioisomers. Both the structure of the aglycon and the configuration of the glycosidic linkage can influence the regioselectivity of the product formed. The regioselectivity of a glycosidase-catalyzed reaction is also influenced by the reaction environment—cosolvents[13] or ionic liquids.[87,88] Most glycosidases have a higher affinity to glycosides with hydrophobic aglycons than to free sugars. Glycosidases catalyze the synthesis of anomerically pure alkyl glycosides in one step with a broad specificity for the alcohol acceptor (saccharide, alkyl or aryl). Not only alcoholic acceptors (saccharides, alcohols, hydroxy amino acids, nucleosides, ergot alkaloids) but also non-alcoholic ones (oximes, thiols) can be glycosylated.[89]

Glycosidase catalysis has also resulted in several surprising products throughout its existence, non-reducing disaccharides being among the most important ones.[13,70,90] They are commonly formed by enzymes specific for non-reducing substrates, however, their formation by other exoglycosidases, naturally operating at the non-reducing end of an oligosaccharide chain and leaving the reducing end intact, is a rarity. We described a transglycosylation with β-*N*-acetylhexosaminidase from *Aspergillus oryzae* yielding the non-reducing β-D-Glc*p*NAc-(1 ↔ 1)-β-D-Man*p*.[13] Similarly, β-*N*-acetylhexosaminidases from *Aspergillus flavofurcatis*, *Aspergillus oryzae* and *Aspergillus tamarii* transferred the β-D-Glc*p*NAc moiety onto D-galactose and lactose yielding non-reducing di- and trisaccharides.[70] Another example is the synthesis of 6′-sulfo-β-D-Gal*p*-(1 ↔ 1)-α-D-Glc*p* by β-galactosidase from *Bacillus circulans*.[90]

In some cases, a changed or enhanced regioselectivity can be accomplished by partially protected acceptors, especially those with the C-6 position blocked. From the most trivial point of view, this approach limits the choice of the available acceptor hydroxyls.[75,91,92] The C-6 modification of the acceptor may also considerably change the enzyme affinity (Scheme 4).[92]



Scheme 4 6′-*O*-Acyl-lactose (acetyl, propionyl, butyryl) derivatives prepared *via* the selective enzymatic acylation of lactose by the protease subtilisin were used as acceptors for enzymatic transglycosylations catalyzed by α-D-galactosidase from *Talaromyces flavus*, forming *iso*-globotriose α-Gal(1 → 3)-β-Gal-(1 → 4).[92] (*i*) Protease N, pyridine, 37 °C; (*ii*) α-galactosidase from *Talaromyces flavus*.

Efficient selective protection of C-6 hydroxyls by acylation can be achieved by lipases[91,92] or proteases like subtilisin.[75,92,93]

Some authors exemplified different regioselectivity on glycosylations of various *O*- and thio-β-D-glycopyranosides (phenyl, benzyl, phenylethyl, phenylthio, benzylthio, *etc*.), including a *C*-glycosyl compound.[29]

4. Substrate specificity

4.1 Specificity towards glycosyl acceptors

The specificity of glycosidases towards glycosyl acceptors is very broad. The structure of the acceptor molecule influences both the yield and the regioselectivity of glycosylation.

The reaction mixture is often complicated by condensation of monosaccharidic molecules yielding unwanted homodisaccharides. This problem is also reduced by the 'minimum water approach' (water activity of *ca.* 0.7–0.8). The organic solvent at low concentration probably deactivates the enzyme due to structural changes whereas high solvent concentrations with the necessary minimum water cause fixation of the enzyme structure in its active conformation. Unfortunately, glycosidases are rather instable in low-water media. This is a big drawback compared to highly solvent-resistant lipases.[94]

A very large group of common acceptors are linear, branched or aromatic alcohols. The glycosides formed are widely applicable as, *e.g.*, non-ionic detergents. The alcohol is mostly used as an organic cosolvent and the water

activity substantially influences the reaction yield. Rantwijk *et al.*[89] gives a detailed overview of glycosylations with various alcohols. Tertiary alcohols were considered as inert to glycosylation for a long time and, however, they can also be glycosylated, as demonstrated by α-galactosidase from *Talaromyces flavus* with *tert*-butyl alcohol,[95] or by cassava β-glucosidase with 2-methylbutan-2-ol and 2-methylpentan-2-ol.[96]

The enzymatic glycosylation of hydroxy amino acids, especially of L-serine, has recently attracted considerable attention, as the products are building blocks for the glycoprotein synthesis. The synthesis of α-D-Gal*p*NAc-(1 → *O*)-L-serine on a gram scale was performed by Ajisaka *et al.*[97] However, it is true that glycosidases are generally rather unwilling to glycosylate free underivatized biogenic amino acids. One of the possible explanations is that these structures are recognized as naturally occurring and, therefore, protected against pathological glycosylation.

Formation of thioglycosides (with sulfur replacing the glycosidic oxygen atom) by *O*-glycosidases is rather rare. There are just a few examples of glycosylation of simple thiols, however, no enzymatic syntheses of oligosaccharides with thioglycosidic linkages by wild-type enzymes. Meulenbeld *et al.*[14] used almond β-glucosidase for reverse hydrolysis with 1-thiopropane, 2-thiopropane and furfuryl thiol. β-Galactosidases from *Aspergillus oryzae* and *Penicillium multicolor* catalyzed the condensation of galactose and 2-mercaptoethanol yielding 2-hydroxyethyl 1-thio-β-D-galactopyranoside.[98] Several β-fructofuranosidases (the best one from *Candida utilis*) catalyzed the transfructosylation of 2-mercaptoethanol.[99] Stick and Stubbs[100] tested a set of thioacceptors for a transglycosylation catalyzed by β-glucosidase from *Agrobacterium sp.*, however, with no success. A reliable approach leading to thioglycosides represent thioglycoligases, the acid/base mutants of retaining glycosidases.[101] They require strong nucleophiles like thiosugars as acceptors that do not need general base catalysis, contrary to most wild-type enzymes. The general intolerance of glycosidases towards thioacceptors is probably due to the fact that the thiol is ionized to thiolate in the active site and this form is repulsed by the acid/base catalytic carboxylate, so that no reaction can proceed. Only when this residue is mutated to the uncharged alanine, the thiolate can bind and react in the active site.

4.2 Specificity towards glycosyl donors

4.2.1 Donors substituted at C-1—natural and synthetic substrates. The kinetically controlled transglycosylation reactions require the presence of a glycosyl donor, suitably activated by a leaving group at its anomeric position. A good glycosyl donor generally has two main features: it binds strongly to the enzymatic active site and enables a fast formation of the glycosyl-enzyme intermediate. The high affinity of enzyme to the glycosyl donor (*i.e.*, low K_m) and a fast reaction (*i.e.*, high k_{cat}) minimize the risk of product hydrolysis. The efficacy of a certain enzyme-donor system is commonly expressed as k_{cat}/K_m.

The structure of the leaving group is a decisive factor for donor properties. Natural substrates for glycosidases are polysaccharidic chains and the corresponding disaccharides are still used in transglycosylation reactions as

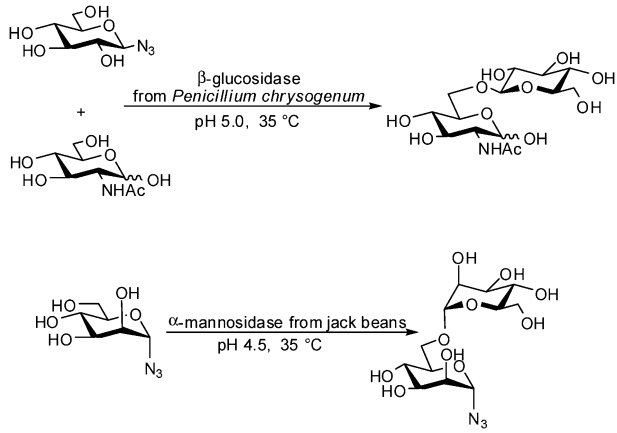
they are easily available and cheap (*e.g.*, lactose for β-galactosidases, *N*,*N*′-diacetylchitobiose for β-*N*-acetylhexosaminidases, sucrose for α-glucosidases, *etc.*) and sometimes lead to better results than the synthetic donors.[102] Obviously, the type of the neighboring monosaccharide unit and its linkage also greatly influence the enzyme performance—a demonstrative example is a comparison of *N*,*N*′-diacetylchitobiose and of its analogue β-D-Glc*p*NAc-(1 → 4)-D-Man*p*NAc as substrates for β-*N*-acetylhexosaminidase from *Aspergillus oryzae*.[103] The latter disaccharide was not accepted as substrate, which was nicely explained using a molecular model of both compounds docked in the enzymatic active site. However, the activation by a saccharide leaving group often leads to low yields due to insufficient activation and side hydrolytic and autocondensation reactions.

A relatively high donor concentration is essential for effective transfer with a low risk of side hydrolysis. The hydrophobic leaving groups, though providing more efficient substrate cleavage, often cause solubility problems. Organic co-solvents can overcome the problem, however, their use is not universal. Nitrophenyl glycosides tend to form autocondensation products in transglycosylation reactions (serving both as donor and acceptor). Therefore, new effective and highly soluble glycosyl donors are still sought after: 3-nitro- and 5-nitro-2-pyridyl glycosides,[71] and also other donors than *O*-glycosides.

In recent years, especially in connection with mutant glycosidases, a new class of glycosyl donors has been brought to public notice—glycosyl fluorides. Their use is not universal but they are successfully applied both as mechanistic probes and in syntheses. For details, see a recent review by Williams and Withers.[104] The main advantages of fluorine as a leaving group is its small size (causing minimum steric hindrance in the active site), its ready detection by ^{19}F NMR spectroscopy even directly in the active site, and its electron-withdrawing potential, which facilitates its easy departure during cleavage. Glycosyl fluorides are readily synthesized in both anomeric forms[105] and their stability is acceptable (weeks), though β-D-anomers are to be prepared prior to use. Their efficiency as donors is comparable to the best aryl glycosides known. Glycosyl fluorides, probably due to their reactivity and the extremely small size of fluorine, exhibit a noteworthy feature—especially inverting glycosidases are able to accept glycosyl fluoride anomers with the 'wrong' configuration and hydrolyze them. Several transglycosylation reactions have been described with glycosyl fluorides as donors.[106] An interesting modification represents ice reaction medium.[15]

A novel alternative to aryl glycosides and glycosyl fluorides are glycosyl azides. They combine some advantages of the above glycosyl donors, *e.g.*, the small size of a leaving group, strong nucleophilic character and delocalized π-electron density. Additionally, they are exceptionally stable and perfectly water-soluble. We used these donors in transglycosylation reactions with β-*N*-acetylhexosaminidases, where the corresponding fluorides are unstable.[73] Glycosyl azides are less effective donors than the corresponding *p*-nitrophenyl glycosides (considering k_{cat}, K_m), however, they do not inhibit the enzyme at higher concentrations and afford better transglycosylation yields, probably due to the reduction of water activity by high donor concentration in the reaction mixture. The synthetic

applications of glycosyl azides are not limited to β-*N*-acetylhexosaminidase-assisted catalysis—good results have also been obtained with β-galactosidases and β-glucosidases (Scheme 5).[107]



Scheme 5 Tranglycosylation reactions with glycosyl azides as glycosyl donors.[107]

Several other compounds appeared in the literature as donors for glycosidases, however, their use was restricted to individual applications and their broader utility is to be demonstrated yet. *N*-Acetyllactosamine[108] and *N*,*N*′-diacetylchitobiose[109] were used as donors for glycosylation by chitinase from *Bacillus sp*. (endoglycanase responsible for chitin hydrolysis). D-Glycals were employed in the synthesis of 2-deoxy-D-glycopyranosides by β-glucosidase from *Agrobacterium sp*.[29] Another noteworthy example is 1-*O*-acetyl-β-D-galactopyranose, which showed *ca*. 30-fold higher k_{cat}/K_m ratio than *p*-nitrophenyl β-D-galactopyranoside in the hydrolysis by *Penicillium sp*. β-galactosidase and was also used as a donor in transgalactosylations.[110] Use of thioglycosides as glycosyl donors with wild-type glycosidases has not been described yet, though Meulenbeld and Hartmans showed their hydrolysis.[111]

4.2.2 Donors substituted at the primary hydroxyl. MacManus *et al*.[112] presented the hydrolysis and transglycosylation of nine C-6 modified *p*-nitrophenyl glycosides by β-galactosidases from barley and from *Helix pomatia* stomach juice ('snail acetone powder', SNAP). The primary hydroxyl was substituted, *e.g*., by methyl, carbene, carbyne, and fluorine. The transglycosylation reactions yielded selectively (β1-4) or (β1-6) isomers, depending on the enzyme source (SNAP or barley, respectively). Hušáková *et al*.[75] performed selective transglycosylations with 6-*O*-acetylated glycosyl donors and acceptors catalyzed by β-*N*-acetylhexosaminidase from *Penicillim brasilianum*.

Transglycosylations with modified glycosyl donors offer the possibility of introducing a reactive substituent to a complex molecule, which can be further modified (*e.g*., reduced, oxidized or conjugated to other compounds

such as amines or hydrazides). We synthesized a novel disaccharide of β-D-Gal*p*NAcA-(1→4)-D-Glc*p*NAc from the C-6 aldehyde by transglycosylation and subsequent chemical oxidation.[76] Due to the presence of a carboxyl moiety, this molecule is a powerful ligand of activation receptors of human and rat natural killer cells, with potential applications in cancer therapy.[113] The β-*N*-acetylhexosaminidase employed accepted neither the uronic acid nor its methylester as substrates, which shows the limitations of C-6 modified substrates.

Another use of *p*-nitrophenyl β-D-*galacto*-hexodialdo-1,5-pyranoside on β-galactosidase from *Bacillus circulans* was shown by Weingarten and Thiem.[114] Apart from the aldehyde, eight other modified donors were tested, four of them being transferred (including β-D-fucopyranoside and α-L-arabinopyranoside). The same enzyme and β-galactosidase from *Escherichia coli* were used earlier with 4-methylumbelliferyl 6-sulfo-β-D-galactopyranoside as a donor yielding sulfated disaccharides.[90]

4.2.3 Donors substituted at the secondary hydroxyl. A high tolerance towards C-4 configuration is a typical feature of glycosidases from family 20 – β-*N*-acetylhexosaminidases. They hydrolyze and transfer both β-D-Glc*p*NAc and β-D-Gal*p*NAc structures, though with different affinities. The *gluco*-structures are generally more willingly accepted but some enzymes exhibit a clear preference for β-D-Gal*p*NAc structures, as shown by Weignerová *et al.*[72] It was shown that the addition of inorganic salts like $MgSO_4$ and cultivation conditions of the producing organism influence this ratio considerably. An unexpectedly important influence has also the aglycon moiety—we observed practically no hydrolysis of 2-acetamido-2-deoxy-β-D-galactopyranosyl azide by β-*N*-acetylhexosaminidases, in contrast to *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-galactopyranoside.[73] Molecular modelling—docking of the substrates into the active site of a β-*N*-acetylhexosaminidase has revealed sterical reasons for this phenomena.[73]

The tolerance of glycosidases towards C-4 hydroxyl modifications is not restricted to β-*N*-acetylhexosaminidases. A similar phenomenon was observed by glucosidases—they do not tolerate only galactopyranosides as substrates, but, more surprisingly, also D-fucopyranosides (6-deoxy-D-galactopyranosides), the latter with even higher affinity than the former, and D-xylopyranosides.[96] On the other hand, the cleavage of D-mannopyranosides (C-2 epimers) is mostly negligible.

Enzymatic recognition of glycosyl donors modified at a secondary hydroxyl was described for α-glucosidase from *Aspergillus niger* using 2-deoxy- and 3-deoxy-analogues of *p*-nitrophenyl α-D-glucopyranoside as donors.[115] Nishimura *et al.*[116] tested the hydrolysis of *p*-nitrophenyl β-D-glucopyranosides *O*-methylated at the C-2, C-3, C-4 and C-6 positions by six fungal and plant β-glucosidases. 6-*O*-Methyl was tolerated best (2–3 orders higher hydrolysis rate).

A very important group of secondary hydroxyl modifications represent fluoroglycosides.[104] When they interact with a retaining glycosidase, the highly electronegative fluorine on the carbohydrate ring causes the destabilization of both transition states and the reaction proceeds at a

strongly reduced rate, whereas a good leaving group enables trapping of the fluoroglycosyl-enzyme intermediate. As a result, fluoroglycosides are good mechanism-based inhibitors, useful for specific labeling and identification of the catalytic nucleophile.[16,117] 5-Fluoro-D-glycosyl fluorides work well even with α-glycosidases[118] and the substitution at C-5 enables to use this type of inhibitors even for β-*N*-acetylhexosaminidases,[119] as the C-2 is not blocked.

A wide range of modifications offers the C-2 amino group of hexosaminides. The acetamido group is a crucial structural feature for accepting the substrate by β-*N*-acetylhexosaminidases and they are quite sensitive towards its modifications. Our recent study[74] disclosed several β-*N*-acetylhexosaminidases (especially from *Penicillium oxalicum* and *Aspergillus oryzae*) with broad substrate specificity towards four *p*-nitrophenyl 2-acylamido-2-deoxy-β-D-glucopyranosides. The ability of these enzymes to cleave *N*-acyl modified substrates and to perform transglycosylations was demonstrated. The β-*N*-acetylhexosaminidases tolerated certain steric changes at C-2 (shorter or longer acyl groups, a hydroxyl instead of a hydrogen) but did not accept highly electronegative acyl groups, *e.g.*, trifluoroacetyl, nor the free amino group.

5. Glycosidases in industry

Glycosidases found their first applications in the food industry, mostly in relation to their hydrolytic activity. Their use in synthesis emerged only later. One of the first commercial products containing glycosidases was Takadiastase®, a mixture of amylolytic and proteolytic enzymes from *Aspergillus oryzae*, used as a digestive aid. β-Galactosidase is used for the hydrolysis of lactose in milk, which makes milk digestible even for lactose-intolerant people.[120] α-L-Rhamnosidases are applied for debittering of citrus juices[121,122] as well as for the enhancement of wine and fruit juice aroma.[123]

For the synthetic use of glycosidases in industry, there are two essential parameters: space-time yield (*i.e.*, the mass of product formed per volume of the reactor and time consumed) and product/waste ratio (*i.e.*, the amount of non-recyclable waste per kilogram product). From these viewpoints, the enzymatic transglycosylation is evidently far more attractive than the chemical synthesis (typically, the Königs-Knorr reaction).[89] Another fundamental parameter is the price and availability of reactants, mainly of the glycoside donor. The most common sugar donors available in bulk quantities (starch, cellulose, glucose, sucrose, lactose, and fructose) dictate the types of industrially suitable catalysts to be α- and β-glucosidases, β-galactosidase, β-fructofuranosidase, cyclomaltodextrin glucanotransferase (for α-glucosylation) and α- and β-amylases. Although many other glycosyl donors are more effective, they will hardly find applications in the large-scale production, possibly with the exception of some high-added-value special chemicals. De Rode *et al.*[124] recently reviewed perspectives for the industrial enzymatic production of glycosides, including reactor set-up and downstream processing.

Industrial applications of glycosides are predetermined by their aglycon. The condensation of a monosaccharide with a long-chain linear alcohol

results in efficient non-ionic surfactants and emulsifiers, used mainly in detergent and cosmetic industry.[125] Glycosides of unsaturated linear alcohols like terpenes have antimicrobial activity and glycosides of peptides and steroids are used as antibiotics, antitumor, and cardiotonic drugs.[63] Probably the only detergent and tenside produced by a glycosidase on a large-scale is hexyl β-D-glucopyranoside, synthesized from glucose by almond β-glucosidase in the reverse hydrolysis mode.[126] Downstream processing and purification of this compound by extraction with adsorption on alumina[126] were described in detail.

When the prebiotic activities of numerous oligosaccharides were discovered in the fifties and sixties, glycosidases found a range of applications in the production of nutraceutics. The industrial application of glycosidases and transglycosidases is widespread mainly in Japan. Especially fructo-, isomalto-, and galactoligosaccharides are used in bioindustry as health promoting 'functional sweeteners' due to their low energetic value, anti-caries and bifidogenic effects. Isomaltooligosaccharides, used as prebiotics, are prepared on the industrial scale by thermophylic cyclomaltodextrin glucanotransferase from *Bacillus stearothermophilus* in Hayashibara Biochemical Laboratories (Okayama, Japan) and in many others.[127]

Fructooligosaccharides (FOS) are (β2-1) fructose oligomers bound to glucose at the non-reducing end industrially produced from sucrose by 'fructosyltransferases' (sucrose 1-fructosyl transferases; EC 2.4.1.9).[128]

The nutritional and medical importance of ascorbate is well known. Besides this, it is also used as, *e.g.*, a skin-whitening agent in cosmetics in Japan due to its *in vivo* inhibitory effect on melanin synthesis.[129]

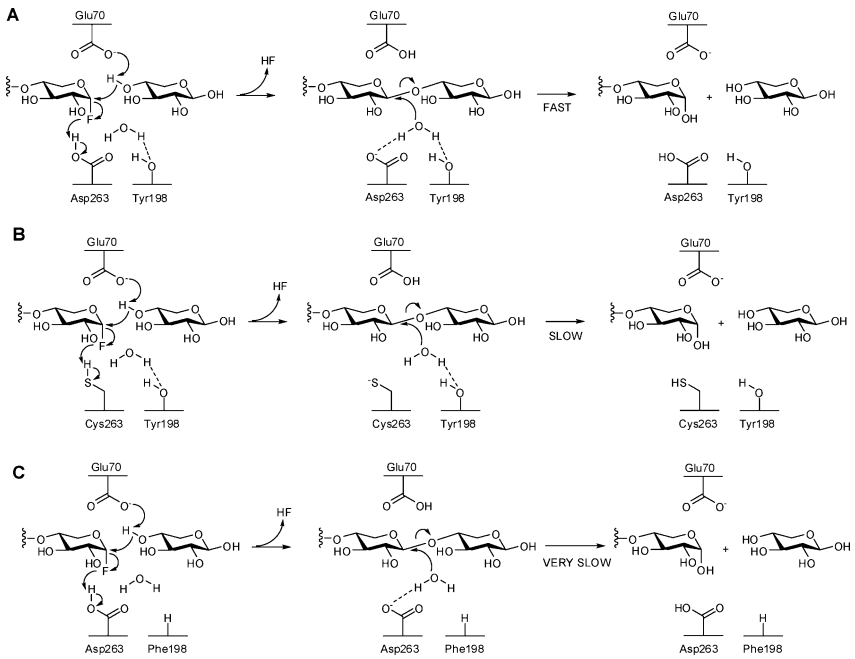
6. Enzyme engineering—mutant glycosidases

Since their introduction in 1998,[130,131] glycosynthases have caused a revolution in the high-yield enzymatic synthesis of carbohydrates and been the topic of numerous reviews.[132,133] The mutation of the active nucleophile to a non-nucleophile, such as alanine, renders a glycosidase hydrolytically inactive but the resulting mutant—glycosynthase—can transfer an activated sugar donor, such as a glycosyl fluoride, onto a suitable acceptor substrate. Since then, a number of other glycosynthases have been generated; for example the glycosynthase derived from *Thermus thermophilus* β-glycosidase, which can selectively synthesize the β1,3 glycosidic linkages in good yields,[134] or the xylosynthase derived from *Agrobacterium* sp. β-glucosidase.[135] Other xylosynthases followed, which were based on *endo*-1,4-β-xylanase from *Cellulomonas fimi*[136] and β-xylosidase from *Geobacillus stearothermophilus*.[137] Glycosynthases have proved their potential in a number of applications in the preparation of difficult-to-obtain glycosylated structures, such as therapeutically valuable glycosphingolipids,[138] and, very recently, glycosylated flavonoids.[139]

In 2006, Withers' group presented the first glycosynthase that was able to transfer glucuronyl residues.[140] Interestingly, the alanine mutant (Glu383Ala) was the most efficient in the transfer of both glucuronyl and galacturonyl moieties, which is in contrast to previous observations with other glycosynthases, where generally the serine mutants exhibited superior activity.[141]

Another glucuronyl synthase, Glu504Gly from *E. coli*, was applied in the β-glucuronylation of a range of alcohols.[142]

All previously described glycosynthases were derived from retaining glycosidases. However, Honda and Kitaoka have reported the first glycosynthase derived from an inverting enzyme: the reducing end xylose-releasing exo-oligoxylanase from *Bacillus halodurans*. First, they mutated the general-base residue[143] and later on the active-site tyrosine residue[144] (Scheme 6). This goes against the traditional belief that inverting glycosidases cannot be used in synthesis.



Scheme 6 Reaction mechanism of wild-type inverting xylanase from *Bacillus halodurans* (A) and two derived glycosynthases, Asp263Cys,[143] (B), and Tyr198Phe,[144] (C).

The wild type enzyme (A) has a high F^- releasing activity and the nucleophilic water molecule, involved in instant product hydrolysis, is stabilized in the active site by hydrogen bonding to the general base Asp263 and to Tyr198. By mutating the general base Asp263 to Cys (B) the hydrolytic activity is considerably diminished, but so is the F^- releasing activity. With the single mutation of the water-stabilizing Tyr298, the F^- releasing activity is slightly increased compared to the wild-type, whereas the hydrolyzing activity is drastically reduced. Thus, Tyr198Phe is a better inverting glycosynthase than any mutant of the base residue, such as Asp263Cys.

New advances have been accomplished in the development of thioglycoligases—retaining glycosidases in which the acid/base catalytic residue is substituted by another amino acid, mainly Ala and Gln.[101] These mutant enzymes use activated donor glycosides such as dinitrophenyl glycosides with thiol acceptors. Müllegger *et al.*[145] designed an improved mutant

thioglycoligase (Glu170Gln) from *Agrobacterium* sp. by saturation mutagenesis, which can use donor sugars with a relatively poor leaving group such as β-D-glucopyranosyl azide. Several other thioglycoligases have been generated, *e.g.* from *Thermotoga maritima* β-glucuronidase[140] or *Xanthomonas manihotis* β-galactosidase.[146] The latter was recently applied in the synthesis of a small library of thioglycosides screened as potential chaperones of unstable lysosomal glycosidases such as human lysosomal β-galactosidase hLyBga, responsible for the catabolism of gangliosides.[147] Thioglycoligase from *Agrobacterium* sp. was successfully employed in the thioglycosylation of a model glycoprotein, thus opening up new possibilities of *in vitro* generation of glycoproteins.[148] Removal of both the catalytic nucleophile and the catalytic acid/base residue from a glycosidase creates a double-mutant thioglycosynthase, which requires both an α-glycosyl fluoride and a thiol acceptor.[149] Thioglycoligases and thioglycosynthases represent the only reliable enzymatic pathway so far that yields thioglycosides, notably, only with a β-anomeric configuration. Though some natural glycosidases, such as *O*-GlcNAcase (β-*N*-acetylglucosaminidase),[150] were shown to reasonably cleave the thioglycosidic linkage, no syntheses of thiooligosaccharides by wild-type enzymes, apart from β-glycosylations of simple thiols,[11] have been reported to date—and no α-glycosylations whatsoever. Interestingly, *Thermotoga maritima* 6-phospho-β-glucosidase, a member of glycosyl hydrolase family 4 (see: http://afmb.cnrs-mrs.fr/CAZY/), was shown to efficiently cleave non-activated thioglycosides with kinetic parameters comparable to *O*-linked analogues.[151] This behaviour indicates that the catalytic mechanism involves anionic transition states (redox-elimination-addition mechanism), similar to, *e.g.*, *S*-adenosyl homocysteine hydrolase, and not the classical general-acid catalysis employed in all other glycosidases. Thus, we may speculate that glycosidases from glycosyl hydrolase family 4 can potentially be an alternative to mutant glycosidases in the synthesis of specific glycosidase inhibitors. Recently, Vocadlo and Davies[152] reported an updated revision on glycosidase mechanisms.

Besides (thio)glycosynthases and thioglycoligases, several other mutant glycosidases were produced with abolished hydrolytic activity in favour of synthesis: transsialidase from *Trypanosoma rangeli*[37] or β-transglycosidase from *Thermus thermophilus*.[153] A new β-fucosidase activity was introduced into *E. coli* β-galactosidase by genetic manipulation.[37]

7. Conclusion

This chapter aimed to demonstrate the key aspects of glycosidase-catalyzed reactions mainly focusing on the synthetic applications. The approach to enzymatic synthesis has crucially changed since its beginnings more than a hundred years ago—from the first surprising findings of unexpected spots on a paper chromatogram of saccharide hydrolysis to sophisticated reactions in ionic liquids, in ice or in biphasic systems with coated or immobilized glycosidases. The latest trend, which exploits the newest knowledge of genetic engineering, introduced mutants with astonishing properties that could not have evolved during the natural evolution and that become tractable tools in intended applications.

Synthesis by glycosidases is not a panacea for all the problems encountered in carbohydrate chemistry. However, the sometimes-heard arguments condemning glycosidases or enzymatic synthesis in general in favor of the 'reliable' chemical approach or highlighting glycosyltransferases over glycosidases are at least useless if not narrow-minded. Each of the methods has its advantages and the art of a good synthetic chemist is to design the best way to achieve the set goal. Importantly, synthesis by glycosidases should not be a mere '*l'art pour l'art*' technique and a proof of principle—they should be applied in order to solve real synthetic problems that cannot be overcome by other means. Hopefully, the simplicity, cheapness and adaptability of glycosidase-catalyzed reactions will find many more synthetic applications in the future.

Glossary

Endo-glycosidase an enzyme that cleaves internal linkages in a glycosidic chain, releasing an oligosaccharidic residue, for instance removing the entire intact oligosaccharide portion from a glycoprotein.

Exo-glycosidase an enzyme that cleaves a single glycosidic residue at the non-reducing end of an oligosaccharide chain.

Glycosynthase a retaining glycosidase, in which at the active site the catalytic nucleophile (Asp or Glu) is replaced by a non-nucleophilic residue (typically Ala, Ser or Gly). This mutated enzyme loses its hydrolytic activity and can catalyze tranglycosylations with suitable activated donors, such as glycosyl fluorides with inverted anomeric configuration, in a virtually quantitative yield. Very recently, this definition has been extended to inverting glycosidases and hexosaminidases.

Retaining/Inverting glycosidase a retaining glycosidase releases products from hydrolysis and transglycosylation that have the same configuration at the anomeric carbon as the original glycoside substrate. In contrast, an inverting glycosidase affords products that have opposite configuration to the processed glycoside. Both types of glycosidases differ in their mechanism.

Reverse hydrolysis a thermodynamically controlled equilibrium process, in which a free monosaccharide reacts with a nucleophile under exclusion of a water molecule and hence chemically, can be considered a condensation reaction.

Substrate engineering a glycosylation utilizing carbohydrate substrates that carry various functional groups and/or modifications in the molecule. In this way, structurally modified carbohydrate products can be produced, as well as regioselectivity and yield of the reaction influenced.

Thioglycoligase a retaining glycosidase with a substitution of the acid-base catalytic carboxylate at the catalytic site by a non-nucleophilic residue. This enzyme is able to catalyze high yielding glycosylations of nucleophilic thiosugars, such as pyranose acceptors carrying a thiol at C3, C4 or C6, using glycosyl donors with reactive leaving groups.

Thioglycosynthase a double mutant of a retaining glycosidase, in which both the catalytic nucleophile and the catalytic acid–base residue at the active site have been substituted by non-nucleophilic residues. It

can catalyze transglycosylations with glycosyl fluorides of inverted anomeric configuration (similarly to a glycosynthase) and thiosugar acceptors (similarly to a thioglycoligase).

Transglycosylation
: a kinetically controlled reaction, in which a glycosidase (typically, a retaining *exo*-glycosidase) transfers a glycosidic residue from an activated glycoside donor to an acceptor while retaining anomeric configuration.

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Recent advances on the application of NMR methods to study the conformation and recognition properties of carbohydrates

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1. Introduction

This report gathers selected and recent examples of studies of the conformation, structure, dynamics and binding features of saccharides and analogues. It has not been our intention to be exhaustive, since many groups are working in this field either on a regular basis or making frequent contributions. It has been our aim to present different examples of the application of NMR methods and protocols for analysing saccharide conformation.

We will start by presenting new advances from the NMR methodological viewpoint to later describe some key examples of applications of NMR for sugar conformation determination, by discriminating between the free and bound state and also from natural and synthetic sugars (or mimetics thereof).

2. The access to new NMR parameters and methodological developments

Some of the advances on the application of NMR parameters to extract structural information have involved detailed analysis of coupling constants. As leading examples, Serianni *et al*. have followed their previous contributions by measuring long range $^4J_{C1,H6}$ and $^4J_{C3,H6}$ in aldohexopyranosyl rings, and their dependency on the geometry of the C1-O5-C5-H6*R*/*S* and C3-C4-C5-H6*R*/*S* coupling pathways, respectively. For the α and β anomers of D-glucopyranosides, different rotameric distributions about the hydroxymethyl groups give rise to significant different values of $^4J_{C,H}$. This information can be useful for establishing, along with other coupling constants information, hydroxymethyl group conformation in oligosaccharides containing 1,6-glycosidic linkages.[1]

A different study was focused on the short range coupling constants within the exocyclic hydroxymethyl group.[2] In this case, coupling constants for the α and β anomers of D-Glc*p* and D-Gal*p* involving the CH_2OH group ($^1J_{C1,H1}$, $^2J_{C5,H6R}$, $^2J_{C5,H6S}$, $^2J_{C6,H5}$, $^3J_{C4,H6R}$, $^3J_{C4,H6S}$, $^3J_{H5,H6R}$, $^3J_{H5,H6S}$ and $^2J_{H6R,H6S}$) were calculated by DFT methods as a function of ω, θ and the glycosidic φ torsion angles, and new Karplus-type equations were reported. Experimental values were obtained and compared to those predicted by the derived Karplus relationships. Also, a new theoretical treatment for $^3J_{CXCH}$ with X = O, C, S was proposed, allowing to extend

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the use of these conformational constraints to the study of thio- and *C*-glycosyl linked oligosaccharide mimetics.

$^{2}J_{CCH}$ geometrical dependence has also been deeply studied for aldopyranosides.[3] These couplings have a primary dependence on the C–O torsion involving the carbon that bears the coupled proton, and a secondary dependence on the C–O torsion involving the coupled carbon. New parameterized Karplus-like equations were described for the quantitative treatment of $^{2}J_{C1,H2}$ and $^{2}J_{C2,H1}$, being the latter of particular interest for assessing preferred conformations about the glycosidic torsion angle (C2–C1–H1).

Correlations between the $J_{C,C}$ couplings and the aldopyranosyl ring configuration and conformation have also been deduced.[4] Previous correlations involving C1 and C6 were confirmed, extended and/or modified, and new structural correlations involving C1–C5, through single and dual pathways, specifically for $^{3}J_{C3,C5}$, $^{3+3}J_{C1,C4}$, $^{3+3}J_{C2,C5}$ were proposed.

The coupling constants involving the exchangeable hydroxyl protons, $^{3}J_{HCOH}$, $^{3}J_{CCOH}$ and $^{2}J_{COH}$, have been examined in aldopyranosides.[5] A generalized Karplus-like equation was proposed for $^{3}J_{HCOH}$, involving the non-anomeric OH groups. Separate equations are required for $^{3}J_{H1,OH1}$ couplings, since they are influenced by the additional effect of internal electronegative substituents, which causes a phase shifting of the Karplus curve. This fact is due partly to the non equivalent values of the *gauche* couplings. $^{3}J_{CCOH}$ showed the expected dependence on the C–C–O–H torsion angle, but a secondary contribution played by the orientation of substituents on the coupled carbon was also detected; separate equations were derived for couplings pathways containing an in-plane OH/OR or C substituents, and for those lacking this arrangement. Once more, different equations were needed to treat $^{3}J_{C2,COH}$, depending on the relative configuration at C1 and C2. Some values of $^{3}J_{CCOH}$ and $^{3}J_{HCOH}$ were found to be inconsistent with a free rotational model about their constituents bonds, suggesting a bias favoring specific C–O rotamers. This was the case of the H3′–C3′–O3′ torsion in methyl β-lactoside (for the non-reducing end), showing a preferred *gauche* conformation for this torsion, deduced from the values obtained for $^{3}J_{C4',O3'H}$ and $^{3}J_{H3',O3'H}$, a prerequisite for the previously proposed O3–O5′ hydrogen bond.

The coupling constant involving the exchangeable amide proton in *N*-acetylated amino sugars, $^{3}J_{HN,H2}$, was the target of an investigation with the aim to gain insights into the conformational properties of these biologically relevant sugars.[6] DFT methods were used to predict $^{3}J_{HN,H2}$ coupling constants in Glc*p*NAc and Gal*p*NAc, and the predicted values were used to derive empirical Karplus equations. However, a poor agreement was observed when experimental values were compared to those calculated by the Karplus equations for both α and β anomers of D-Glc*p*NAc. In principle, this fact is due to intramolecular effects (hydrogen bonds) that introduce a bias in the conformational population. Inclusion of the explicit solvent in the calculation was needed to maintain a realistic structure of the sugars during the DFT calculations. However, the still existing disagreement was also attributed to a slight overestimation of the coupling constants by the employed DFT methods.

Other NMR parameters, which are also of paramount importance for conformational analysis, involving the interchangeable protons, were subjected to investigation. The temperature coefficients of the amide protons in different *N*-acetyl glucosamine oligosaccharides were obtained.[7] The small variation of their values across the different environments, despite the differences observed in the amide group chemical shifts and $^{3}J_{H,H}$ coupling constants, suggests its limited use as a probe for inter- and intramolecular interactions. Amide nitrogen temperature coefficients, on the other hand, were also measured, showing to be more sensitive towards environmental structural variations.

The ^{1}H chemical shifts, coupling constants, temperature coefficients, exchange rates and inter-residual ROEs of the hydroxyl protons of various synthetic type II trisaccharides, analogues of β-D-Gal*p*-(1 → 4)-β-D-Glc*p*NAc-(1 → 2)-α-D-Man-(1 → O)$(CH_2)_7CH_3$, were reported and interpreted,[8] assisted with molecular dynamic simulations, to deduce key information on the conformational behavior of these important molecules in aqueous solution.

The employment of NMR-active isotopes permits to access experimental parameters which are intrinsically difficult to measure, unless a significant concentration of the sugar is present in the NMR tube. For instance, aqueous solutions of *N*-acetylneuraminic acid, labeled with ^{13}C at C1, C2, and/or C3, were analyzed to detect and quantify the various chemical species present in equilibrium at different pHs. In fact, in addition to the expected α and β pyranose forms, acyclic keto, keto hydrate and enol forms were identified on the basis of ^{13}C NMR spectroscopic data. Besides, DFT methods were employed to predict the effect of enol and hydrate structure on the coupling constant values $J_{C,H}$ and $J_{C,C}$ involving C2 and C3, finding that $^{2}J_{C2,H3}$ can be safely used to differentiate the *cis* and *trans* isomers of the enol forms.[9]

During the last decade, many examples and investigations have focused on the use of NMR parameters that may access to conformational features of saccharides in a NOE-independent manner. Among, these, residual dipolar couplings (RDC) have been specially targeted. A wise strategy, which may be applicable to the family of *N*-acetylated naturally occurring oligosaccharides implies the introduction of isotopically labelled acetyl groups. From the measured RDC values, a certain amount of orientational information can be extracted, sufficient to select reasonable conformation representations of the oligosaccharide. This method was applied to *O*-butyl-*N*,*N*′-di-*N*-acetylchitobiose, whose *N*-acetyl groups were labelled at both methyl and carbonyl carbons, by removing native acetyls and replacing them with a ^{13}C enriched version. The assignment of the two acetyl groups was based on a novel combination of MS and NMR. Finally, two possible conformations of the *O*-butyldisaccharide anchored to a lipid-bilayer like surface were selected to be consistent with the RDC and novel CSA offset data.[10]

These novel methodologies have also been applied to access to conformational information in the bound state. For a ligand-receptor complex in solution, averaged RDC are measured for the ligand atom pairs, which depend on the ligand molar ratio of the sugar when free and when

complexed with the receptor. Opposite to trNOESY or STD, both based on cross-relaxation effects, where the bound-state contributions to the average observed cross-relaxation (that scales up with the size of the complex) dominates, RDC are characterized by the absence of heavy weighting of the bound state data in the measured averaged RDC. Thus, if the molar fraction of the bound ligand is small, the RDCs from the bound state can be difficult to extract from the measured coupling. Two different approaches have been proposed with the aim of enhancing the alignment of the protein, what will consequently increase the magnitude of the alignment of the complex and thus, the magnitude of the RDCs of the ligand in the bound state. One way of doing that was to modify the protein by introduction of a polyhistidine-tag, which specifically associates with nickel-chelating lipids that were introduced into the bicelle-like liquid crystal media used for the sample alignment in RDC measurements. This methodology was validated by studying galectin-3 complexed to lactose.[11]

A different method implied the introduction of a hydrophobic alkyl chain to the protein, in such a way that it specifically associates with the hydrophobic interior of the lipid-bilayer-like aligned medium. In this manner, the study of the geometry of the sugar in the bound state can be inferred, rather than its orientation relative to the protein.[12]

Glycosyltransferases are key enzymes for carbohydrate processing. In this context,[13] *N*-acetylglucosaminyltransferase V (GnT-V) has been investigated to obtain structural information on its active site through the measurement of the conformation of natural ligands and their synthetic analogues. In this case, paramagnetic relaxation enhancement experiments, using a spin-labelled ligand analogue, were employed to characterize the relative orientation of two bound ligands. By this approach, minor effects on the site of the acceptor ligand in the presence of unpaired electron on the sugar donor were detected.

Paramagnetic-based NMR constraints have also been employed to analyse the interaction of galectin-3 with lactose.[14] A lanthanide-binding peptide showing minimal flexibility with respect to the protein was integrated into the *C*-terminus of the galectin-3-carbohydrate-binding domain. Pseudo-contact shifts (PCS) and field-induced residual dipolar couplings (RDC), permitted to infer the location and conformation of the natural ligand bound to the protein receptor. Under these conditions, RDCs and PCS reflect only bound-state properties of the ligand and can be safely analysed.

When analysing large molecules, the broadening of the NMR signals, caused by the fast transverse relaxation (T_2) is a current problem in the studies of large molecules, obviously including glycoproteins. Due to the small magnetic moment of the ^{13}C nucleus, the employment of carbon-detected instead of proton-detection experiments can partially or globally overcome this problem. This strategy has been wisely used by Yamaguschi *et al.*[15] in their study of ^{13}C labelled glycans attached to the Fc portion of immunoglobulin G (IgG).

New STD-based NMR sequences have been proposed with success to deal with mixtures of similar compounds, being one or some of them isotopically labelled,[16] or to discriminate ligand from receptor resonances,

by using one of them selectively labelled.[17] Sugar-receptor proteins are useful systems to test these new sequences, due to the usually favourable kinetic features of the binding processes.

A new NMR method for the determination of the anomeric configuration in mono- and disaccharides has been described.[18] The protocol is based on the different cross-correlated relaxation between proton chemical shift anisotropy (CSA) and dipolar relaxation for the α and β anomers of sugars. Only the α-anomers show the presence of CSA (H1 or H1′)-proton dipole (H1–H2 or H1′–H2′) in the longitudinal relaxation of the anomeric protons. The method is of special interest for cases in which vicinal coupling constants between H1 and H2 in both anomers α and β are similar and small, such as D-mannose, and the non-ambiguous description of the anomeric configuration needs additional measurements.

With regard to new pulse sequences, the increase of sensitivities provided by cryoprobes has also permitted the measurement of important parameters, basically inaccessible in the past. As leading example, taking as starting point the, in principle, rather insensitive INADEQUATE experiment, a novel method for the simultaneous measurement of one bond and long range scalar or residual dipolar coupling constants has been proposed.[19] It has been applied to a variety of conformational studies, to diastereomeric and enantiomeric ratio determinations in small molecules, and to structural research on aligned media. For instance, it has been applied for the measurement of ${}^{n}D_{C,C}$ in methyl β-D-xylopyranoside, and to obtain the ${}^{n}J_{C,C}$ coupling constants across the glycosidic linkage in methyl β-lactoside.

3. Applications: Saccharides in solution

3.1 Oligosaccharides

From the biological viewpoint, glycosaminoglycans (GAGS) are one of the most relevant oligo- and polysaccharides. The access to new NMR hardware and software, as well as the employment of new synthetic and molecular biology protocols have permitted important advances in the comprehension of their structural and conformational properties, as well as deeply study different examples of their interaction with receptors.

One key NMR-based study has focused on the evaluation of the dynamic properties of heparin-like hexasaccharides.[20] The analysis of T_1, T_2 and NOE ^{13}C-NMR data of biologically active synthetic compounds has shown that the sulphation pattern strongly influences the internal dynamics, and supports the importance of the GAGs flexibility on the selectivity of the interaction with fibroblast growth factors.

The binding of cations to heparin derivatives was also investigated by NOE and T_2 relaxation analysis.[21,22] The detailed effect of the presence of different cations was analysed, by differentiating the sulphation pattern of the several GAGs under analysis. NMR along with other techniques allowed concluding that the sequence alone does not define the conformation and flexibility for this class of molecules. Indeed, the associated cations must also be considered. Interestingly, the presence of particular cations

was capable of grossly altering the studied biological activity, even to the extent of converting former biologically inactive saccharides into active ones.

Further studies on the molecular recognition of GAGs by proteins have been analysed by Guerrini *et al.*, using different conjugate systems.[23,24] Previous results had shown that the anti-thrombotic activity of low molecular weight heparins (LMWH) was largely associated with the antithrombin binding pentasaccharide sequence AGA*IA ($GlcN_{NAc/NS,6S}$-GlcA-$GlcN_{NS,3,6S}$-$IdoUA_{2S}$-$GlcN_{NS,6S}$).[25,26] Now, the new NMR study, conducted along with fluorescence titrations and docking experiments on different disaccharide extensions on both sides of the AGA*IA sequence suggested that, to a lesser extent, additional contacts involving reducing and non-reducing extension of the AGA*IA sequence had indeed a contribution to the binding. Such results could be taken into account for the design of new and more potent LMWHs.

Contrasting this report, other studies have focused on the opposite effect—the inhibition of the effect of heparin-like molecules. The work conducted by Wang and Rabenstein[27] led to two synthetic peptides—analogues of the heparin-binding domain of heparin/heparin-sulphate-interacting protein—that showed properties as proteolytically stable therapeutic agents for the neutralization of the anticoagulant activity of heparin.

The conformational behaviour in solution of a dermatan-derived tetrasaccharide has been explored by means of NMR spectroscopy, especially by NOE-based conformational analysis. RDCs were also measured for the tetrasaccharide in a phage solution and interpreted in combination with restrained MD simulations. The RDC-derived data substantially confirmed the validity of the conformer distribution resulting from the NOE-derived simulations, but allowed an improved definition of the conformational behaviour of the oligosaccharides in solution, which show a moderate flexibility at the central glycosidic linkage. Differences in the shapes of the different species with the IdoA in skew and in chair conformations and in the distribution of the sulphate groups were also highlighted.[28]

Other recent NMR investigations of the interaction between heparin polymers and different protein receptors have focused on the heparin binding to full-length Tau systems.[29] In this particular example, the strength and location of the interactions was investigated, as well as the structural consequences derived from this important molecular interaction.

Probably, one of the most valuable advances in this field has dealt with the first chemoenzymatic synthesis of the stable isotope-enriched heparin from a uniformly double labelled ^{13}C, ^{15}N *N*-acetylheparosan from *E. coli* K5. Heteronuclear, multidimensional nuclear magnetic resonance spectroscopy was employed to analyze the chemical composition and solution conformation of *N*-acetylheparosan, the precursors, and heparin. Isotopic enrichment was found to provide well-resolved ^{13}C spectra with the high sensitivity required for conformational studies of these biomolecules. Stable isotope-labelled heparin was indistinguishable from heparin derived from animal tissues and might be employed as a novel tool for studying the interaction of heparin with different receptors.[30]

Other oligosaccharides have also been studied in different contexts. For instance, the substrate specificity of four different recombinant sialyltransferases (rST3Gal III, hST3Gal IV, hST6Gal I and hSTGal II) toward a synthetic β-D-Gal*p*-(1–4)-β-D-Glc*p*NAc-(1–2)-α-D-Man*p*-(1-O)$(CH_2)_7CH_3$ oligosaccharide and different analogues have been studied.[8] NMR experiments, in a mixture solvent of 85% H_2O and 15 % of deuterated acetone at low temperature, were acquired to detect hydroxyl and amide exchangeable protons. These provided additional structural information, apart from the usually overlapped ring proton signals. Under these conditions, the exchange rate with water is lowered, and chemical shifts, chemical shift differences, temperature coefficients, vicinal coupling constants, rates of exchange with water, and ROEs contacts were measured. From a practical viewpoint, the NMR assignment employed the scalar connectivities between OH groups and ring/exocyclic protons in standard 2D-experiments. As frequently found nowadays, the experimental data were supported by MD simulations in gas phase and, more important, in explicit solvent, to study the presence of intramolecular hydrogen bonding networks and the influence of solvation on the conformational and dynamic behaviour.

3.2 Glycopeptides

In the last years, the study of the conformation of glycopeptides and peptidoglycans has also been a topic of major development. As for other saccharides, the major difficulties to study this class of compounds by NMR consist in the assignment of the carbon-bound protons, due to the relative little dispersion of their chemical shift leading to overlapping signals; the lack of many inter-residual distance constrains from NOE-type experiments in D_2O, or even H_2O, and the intrinsically high flexibility, leading to the coexistence of several conformations in solution. Nevertheless, one of the advantages of using NMR techniques for elucidating conformation and dynamics of these biomolecules consists in the possibility to work under (almost) physiological conditions. For instance, Dziadek *et al.*[31] have studied the effect of *O*-glycosylation on the conformational properties of a designed peptide, representing the full length tandem repeat sequence of the human mucin MUC1 and its analogues. By the measurement of chemical shift deviation, temperature coefficients, coupling constants and ROESY-derived cross peaks, and by combining these experimental data with molecular mechanics and dynamics calculations, they proposed a valuable structural model of the immunogenetically relevant parts of the MUC1 peptide core.

Combining 2D-NOESY and 2D-ROESY NMR experiments with molecular modelling protocols, Kuhn and Kunz[32] have been able to study the saccharide-induced peptide conformational behaviour of the recognition region of LI-Cadherin. The detailed conformational analysis of this key biomolecule not only proves that the saccharide side chain exerts a marked influence on the conformation of the peptide chain, but also that the size and type of the saccharide indeed strongly affects the conformation of the main chain.

Different behaviour has been observed for key torsion angles of the glycosidic linkage of D-Gal*p*NAc-Ser and D-Gal*p*NAc-Thr motifs. The observed variations, which depend on the chemical nature of the substitution, allow the carbohydrate moiety to adopt completely different orientations in either case. In addition, the combination of NMR methods with MD simulations indicated different hydration patterns for both molecules and the existence of long lived water molecules. For instance, for the mucin-type simplest model, namely, the glycopeptide α-*O*-D-Gal*p*NAc-Ser diamide, the study revealed that intramolecular hydrogen bonds between sugar and peptide residues are very weak and, as a consequence, not strong enough to maintain the well-defined conformation of this type of molecule. In fact, the observed conformation of this model glycopeptide can be satisfactorily explained by the presence of water pockets/bridges between the sugar and the peptide moieties.[33] Additionally, DFT calculations reveal that not only the bridging water molecules, but also the surrounding water molecules in the first hydration shell are essential to keep the existing conformation. The fact that the water pockets found in α-*O*-D-Gal*p*NAc-Thr differ from those obtained for its serine analogue could be related to the different capability that the two model glycopeptides have to structure the surrounding water.[34]

Other studies by Chen *et al.*[35] have focused on how *O*-GlcNAcylation and *O*-phosphorylation can induce relevant differences in the structural and functional behaviour of the *N*-terminus of Murine Estrigen Receptor b (*m*ER-b). By using NOE data, chemical shift perturbations of NH protons and molecular dynamics simulations, subtle differences in the secondary structure of the characterized peptides were identified.

NMR studies on a synthetic 2 kDa peptidoglycan fragment of the bacterial cell wall have been carried out by Meroueh *et al.*,[36] achieving the complete ^{1}H, ^{13}C and ^{15}N resonance assignment. The NOE interactions obtained for this repeating unit allowed proposing a model for 3D of the bacterial cell wall, which was used in a subsequent investigation[37] to evaluate the interaction between the bacterial peptidoglycan cell wall and the peptidoglycan recognition protein (PGRPs).

3.3 Polysaccharides

NMR is also of paramount importance to identify structural features of polysaccharides, including primary and secondary structure (if any) information, as well as dynamic features. A few examples will be given herein, since a large number of polysaccharides structures has been analysed by NMR in these last few years. A typical procedure used by many research groups[38–44] employ standard NMR methods (based on the assignment of all, or most, of the ^{1}H and ^{13}C resonance signals, by combination of homonuclear COSY, TOCSY, NOESY, and heteronuclear ^{1}H–^{13}C HSQC and HMBC experiments) and chemical analyses to decode the repeating unit of the polysaccharides obtained from different natural organisms, including bacteria and fungi.

For instance, in a tour de force analysis, Florea *et al.*[45] were able to determine the complete structure of 59 *O*-glycans (ranging in size from

disaccharides to tetradecasaccharides) from a jelly coat present in Amphibia eggs, through a combination of 2D ^{1}H–^{1}H and ^{1}H–^{13}C NMR spectra, and ESI-MS/MS analysis. Also Becker *et al*. employed a combination of NMR with chemical analysis and additional biophysical techniques to deduce the structure of 2-sulfated, 3-linked α-L-galactan and α-L-fucans.[46] It was indicated that the extremely different conformations of the two polysaccharides could help to explain their distinct anticoagulant properties.

Knowing that nitric oxide and hyaluronic acid both play an important role in the wound healing process, Di Meo *et al*. chose to combine the properties of both entities and synthesized new NO-donors based on hyaluronic acid derivatives exhibiting a controlled NO-release under physiological conditions (*in vitro* tests). These derivatives were fully characterized by ^{1}H and ^{13}C NMR spectroscopy and their NO release monitored by means of UV spectrophotometric measurements.[47]

The description of hyaluronan conformation in solution has been scrutinized by Almond and Blundell in several elegant and recent studies.[48,49] In particular, ^{15}N-enriched oligosaccharides were investigated by employing very high-field NMR experiments and molecular dynamics simulations. The results obtained by amide proton chemical shift analysis, 2D and 3D correlation experiments, and $^{3}J_{HH}$ coupling constant measurements acquired under different conditions, were in agreement with the hydrodynamic and simulation data presented in the literature,[50,51] where it was suggested that the hyaluronan molecules were stiffened by a rapidly interchanging network of transient hydrogen bonds at the local level and did not significantly associated at a global level.

3.4 Glycomimetics

NMR has also been of crucial importance to derive key information on the emerging development of new carbohydrate mimetic structures.

For many years, the conformational behavior of *C*-glycosyl compounds has been studied. The initial design, which aimed to imitate natural *O*-glycosides, but with enhanced stability towards chemical and biological degradation, has been conceptually modified in several occasions, trying to get molecular probes which may help to understand key molecular recognition events. Several molecules of this family have been studied by employing J_{HH}, and in some cases $J_{19F\text{-}1H}$, values, combined with NOE NMR data, assisted by molecular mechanics and dynamics calculations. Besides, in some cases, their configuration was also determined by NMR. This has been the case of the two epimers of 3-carboxymethyl-*O*-D-Gal*p*-(1 → 1)-α-D-Man*p*-fluoro-*C*-glycoside at the *C*-pseudoglycoside stereogenic centre. The conformational results for these *C*-disaccharides, synthesized as potential analogues of the sialyl Lewis tetrasaccharide to bind P-selectin, revealed that the conformational equilibrium of both *R* and *S* epimers, can be described by significant populations of *non natural* conformers (*exo*-Gal/non*exo*-Man for *R*, and probably non-*exo*-Gal/*exo*-Man and/or *anti*-Gal/*exo*-Man for the *S* one), unlike its *O*-glycoside analogue. The existence of non-exoanomeric conformations is basically negligible for natural saccharides. Still, they were shown to interact with P-selectin with

similar activity as the parent *O*-disaccharide.[52] When the CHF interglycosidic bridge was replaced by a CF_2 group expecting to introduce conformational restrictions into the intersaccharide torsions, the non-*exo*-Gal/*exo*-Man conformer was found to be populated to a major extent, with a minor contribution of the conformer that corresponds to the ground state in the natural *O*-glycoside.[53] Also, drastically different conformational preferences between the *O*-glycoside and the *C*-glycosyl analogue were found, following the same strategy, for two *C*-disaccharide analogues of the sialyl-Tn antigen (with and without a hydroxyl group at the pseudo-glycosidic bridge atom).[54] Moreover, for β-*C*-D-Gal*p*-(1 → 4)-β-D-Glc*p*NAc-OMe, the *C*-glycosyl analogue of *N*-acetyllactosamine, three or even four different conformational families were found, being the major one the so-called *anti*-ψ, in contrast to the corresponding *O*-disaccharide, for which this conformer is just scarcely populated. Interestingly, selective T_1 measurements and STD NMR experiments demonstrated that still this analogue binds to viscumin (mistletoe lectin) and human galectin-1, while trNOESY showed that among the four existing conformations in the free state in water solution, just one of them is bound to *h*Gal-1. These findings evidence the existence of a conformational selection process correlated with the molecular recognition event.[55] The same research group studied the conformational behavior of the mammalian sulfoglycolipid sulfatide, which is involved in immunological phenomena by binding to CD-1 proteins and stimulating T-cells, and compared it to that of its *C*-glycosyl analogue, revealing similar conformational populations, but different dynamic behavior basing on the NOE intensities at different mixing times.[56] As additional example, emphasizing the rather distinct conformational behavior of *O*- and *C*-glycosyl compounds, the conformational distribution around the glycosylic torsion angles of β-*C*-D-Gal*p*-(1 → 3)-β-D-Glc*p*-OMe populates two distinctive conformational families, the *syn*-Ψ, which predominates in the *O*-analogue, and the *anti*-Ψ geometry, which has not been described for the natural saccharide.[57]

The conformational behaviour of hemicarbasucrose, a close congener of sucrose in which the endocyclic oxygen atom of the glucose moiety is replaced by a methylene group, has been studied by a combination of molecular mechanics and NMR spectroscopy (*J* and NOE data). It was shown that this carbadisaccharide populates two distinct conformational families in solution, one of them not detected for the *O*-disaccharide. Interestingly, in contrast to other carbasugars, it was found that hemicarbasucrose is less flexible than its natural congener.[58]

The conformational properties of a glyconucleoside containing an *S*-glycosidic linkage have been also studied and compared to those of the corresponding *O*-disaccharide. A *J* and NOE data examination allowed a full pseudorotational analysis, showing a switch in the conformational preferences between the two molecules, the natural and the mimetic one.[59]

A different strategy for the development of carbohydrate mimics has focused on the replacement of the non-pharmacophoric parts of active oligosaccharides by conformationally stable cyclic diols. Thus, looking for an analogue of the α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 6)-α-D-Man*p*-mannotrioside, one of the terminal arms of the $Man_9GlcNAc_2$

oligosaccharide, a pseudo-trisaccharide in which the central mannose was replaced with a carbocyclic diol, was studied by NOE interactions and $^{3}J_{H,H}$, in combination with molecular modelling.[60] The conformation resembled that of the parent molecule.

Other carbohydrate scaffolds with synthetic interest have also been investigated by NMR. For instance, the factors driving the conformational preferences of methyl azido-trideoxy-hex-5-enopyranosides have been discussed based on the analysis of the ^{1}H–^{1}H coupling constants which afford conformational information.[61]

Highly-functionalized difluorinated cyclooctenone templates, sugar analogues used for stereoselective oxidation reactions, have been synthesized and their dynamic properties evaluated, by using variable temperature ^{19}F NMR. In this manner, ΔG for the conformational exchange was calculated, which showed a rather restricted fluxional behavior. Also, $^{3}J_{H,F}$ and NOESY and ROESY led to the topological characterization of the exchange process, and allowed to propose a conformational exchange with pseudorotation at room temperature, which detailed structure calculations were not able to fully reproduce.[62] As additional example, a standard conformational analysis based on ^{3}J coupling constants and NOE data has also been applied to triazolo-carbohydrate mimetics.[63]

4. Applications: The interaction of saccharides with other natural and synthetic molecules

In the last years, a number of studies of the conformational features of the interaction between proteins and saccharides have been reported, providing new details, at atomic resolution, on these key processes. NMR experiments have been employed to analyse the interaction of sugars with different biomolecules, including proteins (lectins, antibodies and enzymes), nucleic acids and even other saccharides. Synthetic receptors, also dubbed artificial lectins have been employed to model sugar interactions. Finally, a few cases of the use of cyclodextrins as receptors will be mentioned, as examples of sugars being receptors of organic molecules, instead of being ligands.

In principle, there are different ways to characterize the complexes: On one hand, in favourable cases, it is possible to measure the NMR parameters of the receptor in the free and bound states, *i.e.*, differences in chemical shift and NOE-contacts, before and after the formation of the complex. On the other hand, it is usually more feasible to observe and detect changes in the ligand NMR signals before and after the binding process. In this latter case, the use of STD (saturation transfer difference) and TR-NOE (transferred NOE) experiments is of paramount importance.

4.1 Carbohydrate-protein interactions

GAGs are implicated in many binding processes. For instance, by using NMR, Herbert *et al.*[64] were able to demonstrate the key role of a single residue (His 402), when analysing the interaction between the factor H and chemically defined GAGs.

The combination of X-ray with NMR data for the CD44-hyaluronan (HA) complex,[65] allowed identifying two conformational forms of the

binding site. NMR experiments in solution revealed some unexpected features of these fundamental carbohydrate-protein interactions and provided key information on the mechanism for regulating CD44-HA binding.

A key example of the interaction of GAGs with receptor proteins by using NMR, has been the 3D structural study of the complex formed by the acidic fibroblast growth factor (FGF-1) and a specifically designed synthetic heparin hexasaccharide.[66] The use of this well defined synthetic heparin analogue, which did not induce any dimerization of the growth factor, allowed to perform a detailed NMR structural analysis of the heparin-FGF interaction, overcoming the limitations of NMR to deal with the high molecular mass and heterogeneity of the FGF-1 oligomers formed in the presence of natural heparin fragments. Previously, the bound conformation of the saccharide in the FGF1 binding site had been described using half-filtered NMR experiments, with a labelled protein and non labelled sugar.[67]

The molecular recognition of septanose carbohydrates has been investigated in depth by using concanavalin A[68] as a model lectin. Complex formation was analysed by STD experiments and showed the first direct evidence of binding, by a natural protein, for this class of ring-expanded carbohydrate molecules.

The design of carbohydrate ligands for DC-SIGN has been a topic of high interest during last years, because of the key role played by this C-type lectin in immunity and infection processes. In this field, the interactions of DC-SIGN with one 1,2-mannobioside mimic[69] has been investigated by NMR and docking experiments and the antiviral activity has also been measured. STD and TR-NOESY experiments showed that the mannobioside mimic is a good alternative as DC-SIGN ligand with a higher enzymatic stability than the corresponding disaccharide. In a second report, the binding modes of mannosyl trisaccharide ligands[70] were also evaluated using the same protocol. The data showed potential variations in the binding modes of the targets, as consequence of the existence of multiple binding possibilities.

The conformational and dynamic behaviour of oligomannosides[71] has also been investigated and their recognition by banana lectin has been evaluated by STD NMR methods and docking procedures, providing a preliminary view of their putative interaction mode.

Using a combining approach of mass spectrometry, NMR spectroscopy and molecular modelling, the interactions between the pentasaccharide[72] chain of ganglioside GM1 with two lectins, the galectin 1 and cholera toxin have been investigated. In this process the interaction is reached through a limitation of the flexibility in the oligosaccharide ligand. The possibility to select an energetically favoured glycan conformation for binding seems to be common. The advantage of this process is the reduction of the entropic penalty in the receptor-ligand interaction process, as compared with the entropic cost in case that a flexible ligand had to be fixed in a single bound-state conformation.

The mechanism by which furanosyl-containing saccharides bind to proteins has also received attention. For instance, the interaction of D-arabinofuranosyl glycans[73] with the CS-35 antibody, which is widely used in the characterization of these polysaccharides has been addressed

using a combination of chemical synthesis, mass spectrometry, titration microcalorimetry and NMR spectroscopy.

To enhance the knowledge on molecular recognition of lectins, hybrid glycopeptide mimetics with β-lactam scaffolds have been designed[74] and synthesised. The advantages of this so-called alternative "shape-modulating linker" design were demonstrated by combining NMR and docking approaches, and employing a fucose-binding lectin as model.

In order to assess the importance of carbohydrate-aromatic interactions for the molecular recognition of neutral sugar by proteins, three different AcAMP2-like peptides with different aromatic residues at the key binding site were prepared.[75] Their molecular recognition features towards the chitin-derived trisaccharide were deduced by NMR spectroscopy and molecular dynamics calculations. The thermodynamics of the binding processes with the artificial peptides were characterized by ^{1}H NMR and ^{19}F NMR spectroscopy, highlighting that the chemical nature of the aromatic residues strongly influenced the binding affinity. Within this context, different simple models[76,77] of the interaction between sugars and aromatic rings have been studied by NMR and different molecular mechanics, *ab initio*, and DFT calculations to predict the chemical features and structural requirements for an optimal interaction of glycans with aromatic moieties.

It is evident that the detailed knowledge of carbohydrate binding modules (CBM) is vital for the overall understanding of carbohydrate-active enzymes. In this context, the first structures of the CBM families 21 and 35, derived from *Rhizopus oryzae* glucoamylase and *Cellvibrio japonicus* β-1,4-mannanase Man5C, respectively have been deduced by using NMR.[78,79] The NMR structure of both free- and carbohydrate-bound protein was determined using standard 3D NMR experiments. The results of such structural investigations allowed deducing the fine details of the inter-family variations of CBMs by which these proteins recognize distinct carbohydrates.

Other enzyme-substrate or inhibitor interaction studies[80–82] have been addressed, using a combination of STD and trNOE NMR experiments, in order to collect details on the substrate bound conformation (ligand perspective). In other cases, the availability of a labelled protein receptor[83] have permitted to follow the induced chemical shift variations of the protein resonances upon ligand addition to the NMR tube by HSQC methods (protein perspective).

The mechanisms of bacterial resistance due to enzymatic inactivation of aminoglycoside antibiotics has been studied in different systems[84,85] In the streptomycin-*Bacillus subtilis* ANT(6) system, the structural and conformational information (tr-NOE/tr-ROE experiments) allowed the conclusively characterization of the antibiotic in both free- and bound-state, and it was stated that the molecular recognition event involved a conformational selection process. From the enzyme perspective, surprisingly, although highly specific for streptomycin, ANT(6) was still able to recognize different aminoglycosides in a non-specific manner. Several key aspects of aminoglycoside recognition by the resistance enzyme ANT(4′) have also been evaluated. From a methodological perspective, this analysis provided

evidence that a careful protocol involving the proper design of trNOE experiments with specifically targeted receptors and mutants can be employed to characterize the existence of simultaneous binding events of the ligand to different regions of a given protein receptor. From the molecular recognition point of view, it was shown that ANT(4′) displays a wide tolerance towards the conformational variation of the drug.

Following with bacterial infections and anti-adhesion strategies,[86–88] NMR based structural studies have been conducted on *O*-polysaccharide chains in these past few years,[38–40,42,43] in order to elucidate the molecular basis and the role of the lipopolysaccharides in these pathologies.

Landström *et al.*[89] have reported on the conformational preferences of an octasaccharide derived from the serogroup D1 lipopolysaccharide and its binding to the phage P22 tailspike protein (part of the bacteriophage's tail machinery). Docking studies successfully identified the binding region on the protein surface, whereas the analysis of 2D ^{1}H, ^{1}H-T-ROESY and transferred NOESY NMR experiments indicated that the bound octasaccharide had a similar conformation to that observed in solution.

The interaction between melittin (a 26 *a.a.* peptide that exhibits potent anti-microbial activity)[90–92] and lipopolysaccharides (the major constituent of the outer membrane of the Gram-negative bacteria) has been studied by NMR. It was demonstrated that the *C*-terminus of melittin adopts a helical structure in the complex with LPS, while the *N*-terminus appears in an extended conformation. STD experiments permitted to identify those residues of melittin in close proximity with LPS, which appeared to be located at the *C*-terminus and thus, engaged in the formation of helical structure.

Also conformational and interaction events related to viral infection processes have been studied by NMR. Rademacher *et al.*[73] presented a detailed NMR study that determined which sugar residues are involved in the binding with a calcivirus [namely, rabbit hemorrhagic disease virus (RHDV)]. Calciviruses are unusual because they dock to sugar residues on the surfaces of cells to be invaded rather than to proteins.[73] In this work, NMR experiments allowed to determine which parts of different histo-blood antigens are involved, directly or indirectly, in binding to the protein surface of virus-like particles (VLPs). These particles were expressed from the structural proteins of RHDV, and had the same shape, size and antigens as natural virus particles, but contained no nucleic acids. By systematic screening of several antigen fragments, the authors found that only those containing L-fucose were recognized by the VLPs. This crucial information could open the door for the rational design of compounds that obstruct the entry of viruses into cells.

4.2 Carbohydrate–carbohydrate and carbohydrate–nucleic acid interactions

Carbohydrates not only interact with protein receptors, but also with other carbohydrates and nucleic acids. Recently, gold nanoparticles (GNPs) have been prepared as new multivalent tools that mimic carbohydrate presentation on the cell surface. Using this tool, a weak calcium-mediated carbohydrate–carbohydrate interaction has been detected using NMR, employing

the *microciona prolifera* sponge trisaccharide as interacting system. DOSY and trNOESY NMR methods, assisted by MD simulations in explicit water permitted to provide a 3D model of the interaction.[93] In the case of nucleic acids, a series of complexes of sugar-oligoamides[94] with long tracts of calf thymus DNA (ct-DNA) have been studied by both TR-NOESY and STD experiments. The combination of the experimental data allowed the determination of their binding modes in the groove of ct-DNA, indicating that different sugars interact differently with the DNA, possibly due to the different amphiphilic character of their surface.

Also on the nucleic acid field, it has been shown[95] that the distribution pattern of $NH_2/NH_3^+/OH$ groups in natural aminoglycosides could indirectly influence their RNA binding properties and therefore their antibiotic functions. Indeed, the number and location of these polar groups is essential to modulate the conformation and dynamics of the glycoside with the concomitant implication for the RNA recognition process.

4.3 Interactions of carbohydrates with artificial receptors

The development of artificial receptors for carbohydrates has received increasing attention in recent years, being NMR one of the main tools to study the binding process. Very different chemical architectures have been described in order to mimic the molecular recognition process carried out by the carbohydrate binding proteins in Nature. They share however, common features: on one hand, the occurrence of groups capable of participating in hydrogen bonds and, on the other hand, the presence of aromatic moieties being able to form CH-π stacking interactions.

Thus, Roelens *et al*. have reported different affinities and selectivities of benzene tripodal based receptors in organic solvents following a well described titration protocol.[96] A cage structure built by linking two tripodal benzene units through amine and pyrrol groups showed complete selectivity for β-D-Glc*p* towards α-D-Glc*p*, and the ability to discriminate β monosaccharides of the *gluco* series from both the α and β anomers of the *galacto* and *manno* families.[97] Based on the same benzene tripodal scaffold, two opened structures containing pyrrolic and imino or amino groups were compared.[98] NMR titrations showed that they are able to significantly bind octyl glycosides of α- and β-D-Glc*p*, Gal*p*, Man*p* and Glc*p*NAc, preferentially recognizing the β-D-Glc*p* glycoside among them. When the same receptor was modified by including acetal groups linked to the pyrrol ring, high affinity for β-D-Man*p* was achieved.[99] If the same benzene platform bears three catechol units instead of the amino and pyrrol groups, multi equilibrium systems with several monosaccharides were detected, and selectivity for the α-mannoside towards β-glucoside was found.[100]

A similar strategy has been applied by Mazik and coworkers. The receptor is usually titrated with increasing amounts of the sugar, to evidence binding, to extract the values of the binding constants and to get the stoichiometry of the complexes formed. Reverse titrations and extraction experiments can also be performed. The structures of the receptors described are based on different scaffolds: the above mentioned hexasubstituted tripodal benzene moiety and 3,3′,5,5′-tetrasubstituted

diphenylmethane and biphenyl units, to which different chemical functional groups are added in different combinations, being able to form both neutral and ionic H bonds. For instance, aminopyridine, aminopyrimidine,[101] carboxylate,[102,103] hydroxy phenyl acetamide, crown ethers,[104] neutral and protonated trypthophan,[105] protonated 2-amino-1,8-naphtyridine,[106] oxime,[107,108] phenantroline,[109] indole and imidazole,[110] and hydroxymethyl groups[111] have been employed. Exploiting the same aromatic tris-amine template, Schmuck *et al.*[112] described a tris-cationic receptor capable of binding anionic carbohydrates such as uronic acids as well as the phosphates of glucose, mannose and galactose.

The 3,3′,5,5′-tetrasubtituted biphenyl unit constitutes also the floor and the roof of the receptor reported by the group of A. Davis. These scaffolds are linked by polar spacers, giving rise to a tricyclic cage arrangement. A variant of this structure that contains twelve carboxylate groups was shown to be able to bind carbohydrates in water with low affinities but with significant selectivities for mono- and disaccharides with all-equatorial stereochemistry. Hence, NMR titrations along with NOESY experiments led the authors to propose a structural model in which the monosaccharide sits into the hollow of the macrocyclic structure, with the equatorial hydroxyl groups interacting with the polar pillars and the axial ring protons facing the aromatic moieties. In addition, the binding constants were obtained by integration of the NMR signal of the complex against that of an internal standard.[113] When the same receptor was studied towards *O*-β-D-Glc*p*NAc, a strikingly stronger binding was detected, and higher selectivities were found.[114] A similar structure, but with a *meta*-terphenyl structure as a roof and floor, proved to recognize cellobiose towards other disaccharides with good affinities (similar to those of some lectins) and with exceptional selectivities.[115]

Quinoline and pyridine rings have also been employed as proton acceptors using different scaffold cores. Palde and coworkers[116] proposed a cyclohexane ring to which the above mentioned moieties are linked. They reported two hosts for the recognition of octyl monosaccharides in protic solvents (methanol) with interesting properties: while one of them displayed ability to act as a turn-on fluorescence sensor, the other one exhibited the highest affinity achieved so far for non covalent recognition under the described conditions. ^{1}H NMR titrations were applied for qualitative binding analysis, but binding constants were also compiled by using other analytical techniques. A structural model of the complex formed was suggested from the analysis of the obtained NOE data. On the other hand, Lu and coworkers[117] used naphthyridine as core structure. In this case, upon addition of the receptor to a solution of octyl pyranosides, the hydroxyl protons of the sugars experienced a downfield shift, which was attributed to their participation in hydrogen bonds with the receptor.

A different kind of host consisting of a peptide-based bicyclic structure has been described.[118] In this case, the chemical shifts changes were followed by HSQC spectra in deuterated acetic acid and in water, when titrated with cellobiose. In any case, a low but measurable binding affinity constant was found.

A 1,3,5-tris(hydroxyalkylphenyl)benzene unit has been used as a template for the development of various symmetrical hosts. Their possibilities to form stable hydrogen bonds and therefore, their abilities to recognize glycosides have been studied by titration NMR with different octyl glycosides. In most of the cases, the existence of association processes was evidenced.[119,120]

The so-called foldamers are unnatural oligomers that are able to adopt a well defined secondary structure through intermolecular non covalent forces. Some of them have been reported to be able to recognize saccharides. By means of ^{1}H NMR titrations, binding of two aromatic oligoamide foldamers to β-D-ribose, α-L-Glc*p*, β-L-Glc*p* and β-maltose with high affinity, but poor selectivity, was demonstrated.[121] A series of chiral aromatic hydrazide oligomers, in which the chirality was introduced by two *R* or *S*-proline units at the terminal backbones, also displayed differential recognition features towards α-L-Glc*p* and α-D-Glc*p* alkyl glycosides.

Some oligomers were observed to adopt a different fold from that on the free state upon complexation with saccharides. Goto *et al.*[122] have reported on the properties of an oligoresorcinol nonamer, which forms a double helix in water. This molecule, in the presence of isomaltooligosacharides, suffers a transformation process giving rise to a preferred-helical-heteroduplex, as suggested on the basis of circular dichroism and absorption spectra. The binding affinity was found to be highly affected by the chain length and the glycosidic linkage patterns of the oligosaccharide, showing selectivity for α-1,6-linkages. The complex with the glucose polymer, $Dex_{20,}$ was studied by NMR. ^{1}H chemical shift perturbations and NOE data were used to propose a possible structure for the 1:1 heteroduplex. Further evidence of the complex formation was obtained by DOSY experiments. Abe *et al.*[123] have also described co-oligomers of various lenghts involving (1*H*)-pyridinone and 4-alkoxypyridine rings that undergo supramolecular transformation upon saccharide recognition in chloroform. In this case, the ^{1}H NMR analysis permitted to deduce that the self-associated dimer changed into a single strand helical complex upon sugar binding.

4.4 Cyclodextrins

Carbohydrates not only act as ligands, but they can also provide scaffolds for molecular recognition processes. It is well known that cyclodextrins (CDs) are able to form an inclusion complex with specific guest molecules. In the last years, NMR experiments combined with other techniques have been used to highlight different recognition events.

The effect of modification of one residue with a different sugar in the rim has been studied by Hakkarainen *et al.*,[124] by substituting one α-D-Glc*p* unit for an altropyranose (Alt) residue, containing axial hydroxyl groups. It was observed that the presence of the altropyranose unit changed the rigidity of the macrocycle and enhanced the ring capacity to adapt itself to the shape of the guest. By analysing chemical shifts, temperature coefficients and vicinal coupling constants of the hydroxyl protons in the absence and presence of adamantane-1-carboxylic acid, the authors concluded that the introduction

of the new unit modifies the conformation of the novel CD, affects the hydrogen bond network and its behaviour in the complex formation.

A complete NMR approach has been employed to evaluate the complexation process of catechin A with β-CD and synthetic analogues.[125] The analysis of the variation of the proton chemical shifts indicated the formation of a 1:1 stoichiometric complex. 2D-ROESY provided detailed spatial information of the complex while the binding constants were obtained by using diffusion-order spectroscopy (DOSY) techniques.

The modification of the physical properties of some molecules as consequence of complexation with CDs is well known. Kongo *et al.*[126] studied the TB-β-CD/porphyrine complex and observed a drastic change in the photochemical and photophysical properties of porphyrine. The conformational analysis by NMR revealed strong NOE interactions between the ligand and the internal part of the CD, in agreement with a deep insertion of the porphyrine analogue into the CD's cavity. The NOE data provided structural information to propose the 3D model of 1:2 inclusion complexes.

There are several cases that have addressed molecular recognition of chiral drugs by modified CDs[127] as well as inclusion complexes of cationic, anionic and neutral organic compounds[128] in order to understand the role of hydrophobic and electrostatic interactions between the functional groups on host and guest.

NMR can be applied to demonstrate the existence of complexes with different stoichiometry, above the 1:1 ratio. The induction of supramolecular self-assembly of β-CDs as carriers of the antimicrobial agent chlorexidine has been investigated,[129] indicating that a high order complex is formed, with the consequently higher bioavailability of the guest molecule. In this case, the NMR T_1 and ROESY data were combined with ESIMS and ITC data. Using a similar approach,[130] the interaction between β-CD with pyromellitic diimide derivatives (PMDIs) has been described. These molecules lie just outside of the narrow rim of β-CD, due to their hydrophilic nature. However, modifying the β-CD by including a bulk substituent permitted the PMDIs to form inclusion complexes.

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Specificity and affinity studies in lectin/carbohydrate interactions

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1. Introduction

Lectins are ubiquitous carbohydrate-binding proteins, characterized by their specificity recognition towards complex oligosaccharides and their non-immune origin.[1,2] Their ability to distinguish oligosaccharides is central to many biological processes involving cell communication. They also play a role in many diseases being involved in cancer development and metastasis, inflammation and host-pathogen recognition. If recently characterized bacterial lectins display higher affinity for their target with K_d for monosaccharides in the micromolar range,[3] lectin-carbohydrate interactions are more often characterized by rather low affinity, which is generally compensated by the presence of several binding sites, resulting in avidity or velcro effect. The development of fast and effective methods for determining lectin affinity and specificity has been hampered by the technical problem of obtaining sufficient amount of pure and well characterized oligosaccharides. Nevertheless, a range of approaches have become available; they will be described in the present chapter.

2. Hemagglutination + ELLA

Lectins are often referred to as hemagglutinins because of their capacity to bind to red cells glycans and to reticulate them through multivalent interactions. Hemagglutination is therefore a simple, sensitive, and quasi-quantitative assay for detecting lectin activity. Inhibition of this process by monosaccharides and oligosaccharides is also routinely used as a screen for lectin specificity. The galactose binding (PA-IL) and the fucose binding (PA-IIL) proteins from the opportunistic bacterium *Pseudomonas aeruginosa* are soluble lectins that are likely to be involved in adhesion to host tissue and in biofilm formation.[3,4] Crude soluble protein extract obtained from *Pseudomonas aeruginosa* precipited papainized erythrocytes.[5] Hemagglutination test not only allowed for defining the monosaccharide specificity of each lectin but demonstrated that human milk, and not cow milk can block the binding of PA-IIL to fucosylated target.[5] Hemagglutination assays can also yield additional information on the binding process. For example, the lectin purified from shrimp *Fenneropenaeus chinensis* plasma showed a strong affinity for human A/B/O erythrocytes (RBC), mouse RBC and chicken RBC. The hemagglutinating (HA) activity of the lectin was

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reversibly sensitive to EDTA and dependent to Ca^{2+}, establishing the probable role of this divalent cation in sugar binding.[6]

The enzyme-linked lectin assay (ELLA) approach is based on modifications of the enzyme-linked immunosorbent (ELISA) test. This method permits to study the lectin specificity by determining the ability of a soluble compound to inhibit interaction between lectin and a reference ligand.[7] One of the binding partners is coated on the bottom of a microtiter plate and the labelled other binding molecule will adhere to the absorbed molecule. A library of soluble compounds can be tested for their ability to inhibit the binding. Determination of inhibitory potencies provides an estimate of the ligands affinity. Molecules can be labelled by different methods including fluorophore, biotin or conjugated antibody.

ELLA test provides a rapid method for evaluating lectin binding properties of soluble natural or synthetic lectin ligands in solution. For example, series of mono- to trivalent *N*-acetylglucosamine (GlcNAc) derivatives have been examined for their ability to inhibit binding of wheat germ agglutinin (WGA) to carbohydrate-coated microtiter plates. It was shown that the presentation of GlcNAc residues on the microtiter plates either as part of a glycoprotein or as a covalently immobilized monosaccharide derivative strongly influences results of the assay.[8] ELLA tests are also used for screening of glyco-derived compounds that may be active as anti-adhesive compounds by inhibiting the binding of bacterial lectins or adhesins to host glycoconjugates.[9] In the last two years, a large number of glycomimetics and glycodendrimers have been tested for their capacity to compete for the binding of the *Pseusomonas aeruginosa* PA-IIL to fucose. One strategy was to produce glycomimetics presenting terminal αFuc1-4GlcNAc disaccharide.[10] The other strategy used oligomers presenting two or three fucose residues,[11] or glycodendrimers built on either pseudo-oligonucleotidic or polypeptide scaffolds.[12,13]

New developments tend to associate ELLA with other methods in order to provide high-throughput screening. Recently, a simple ELLA assay coupled to a high-performance anion-exchange chromatography (HPAEC) has been used to analyze transferrin sialylation directly from serum.[14] In another study, a high-throughput screen based on the ELLA tests was developed to detect the synthesis of natural and non-natural gangliosides by cell lysates using the binding specificity of cholera toxin B-subunit for the oligosaccharide moiety of the ganglioside GM1. This screen, which is highly sensitive and can be used with crude cell extracts, would be useful in assaying both glycolipid biosynthesis and glycolipid hydrolysis.[15]

3. Glyco chips: new tools for screening lectin specificity

The development of microarray technologies that have been extensively used as high-throughput analytic tools for genomics, transcriptomics and proteomics research opened the route for carbohydrate microarrays. The advantages of carbohydrate microarray technology over other screening methods are multifold. Small amounts of carbohydrate compounds are sufficient for analyzing simultaneously a large number of glycan–protein interactions. Also, the dense presentation of oligosaccharides on the surface

results in multivalent presentation that mimics glycoconjugates at cell surface. The recent years have seen increasing availability of glycan arrays (glycochips) that were developed in different laboratories.[16–18] Each format differs in the type of glycans and the manner in which they are displayed. Some use noncovalent attachment to plastic or nitrocellulose membranes, and others use covalent attachment to plastic, gold, or glass. Glycan arrays based on neoglycolipids have been successful for screening lectins specificity and new approaches were also developed in this area for ligation of the ligands.[19] The Consortium for Functional Glycomics (www.functionalglycomics.org) proposes two different technics. The ELISA-based microplate array consists of a library of biotinylated synthetic and naturally occurring oligosaccharides attached by a spacer arm to streptavidin-coated microtiter plates (more than 240 glycans). The printed glycan microarray is the new format with a higher number of ligands (442 glycans) with amino linkers printed onto NHS-activated glass microscope slides.[20] The most recent development will implement bacterial polysaccharide glycan arrays that will be useful tools in host-pathogen interaction and immunology.[21]

The use of glycan array technology resulted in a very large amount of data in the last few years and only a selection will be highlighted here. In several cases, a high affinity ligand could be defined for lectins that were only characterized by their low affinity binding to monosaccharides. For example, codakine, the Fuc/Man binding C-type lectin from white clams demonstrated a very high specificity for biantennary *N*-glycans of the complex type (Fig. 1).[22]

Glycan array allows for the definition of fine differences in specificity among related lectins giving clues about the basis for differences in their biological activities. For example comparison of a set of human galectins demonstrated that Gal-1, Gal-2, and Gal-3 all bind *N*-acetyllactosamine but

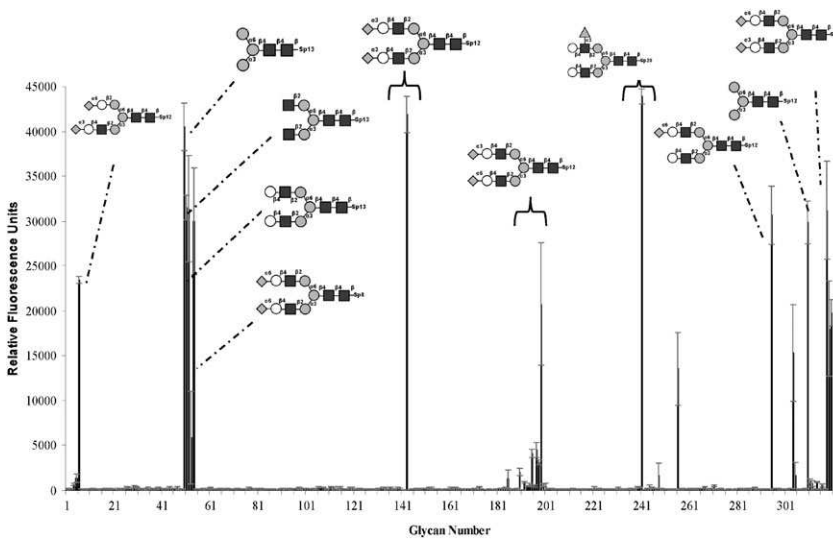

Fig. 1 Glycan array analysis of codakine as measured by fluorescence intensity (glycan array v3.0 at Consortium for Functional Glycomics). Purified codakine from white clams was purified with Alexa Fluor® 488 Protein Labeling Kit (Molecular Probes™, Invitrogen) and tested on glycan array v3.0 at Consortium for Functional Glycomics.

differ significantly in their recognition of *N*- and *O*-glycans.[23] Such side-by-side comparison of binding data is of first importance when it is correlated with the host and tissue specificity of pathogens. The most documented case is related to the risk of a new pandemic influenza that could be caused by human-adapted avian virus presenting hemagglutinin (HA) able to bind to human epithelia. Glycan microarray analysis of the hemagglutinins from modern and pandemic influenza viruses reveals different receptor specificities. Avian influenza virus bind to αNeuAc2-3Gal termini usually found in the intestinal tract and lower region of the respiratory tract whereas human-adapted virus recognize sialosides with α2-6 linkage located in upper region of the respiratory tract.[24] Recent work demonstrated that some mutations in hemagglutinin H5 have already occurred in some avian influenza strains that should be considered as "high-risk" because of their propensity to adapt to human receptors.[25]

Comparison of related pathogens is also of interest. For example PA-IIL from *P. aeruginosa* and BclA from *B. cenocepacia* are related lectins from opportunistic bacteria. Glycan array data indicate that although the lectins are closely related, BclA binds only to oligomannose glycans, whereas PA-IIL has a preference for fucosylated oligosaccharides.[10,26] For PA-IIL, the stronger binding is observed for αFuc1-4GlcNAc containing oligosaccharides and indeed Lewis a (Le^a) trisaccharide αFuc1-4(βGal1-3)GlcNAc is the best natural ligand for PA-IIL with a dissociation constant of 210 nM.[27]

4. Fluorescence and fluorescence-based thermal shift assay

Fluorescence methods in glycosciences are well recognized as they represent a powerful tool for studying protein-carbohydrate interactions.[28] Classical methods are oriented to follow direct changes in fluorescence of the protein or its ligand during interaction, or to measure changes in polarization or anisotropy. In the first case, it is expected that intrinsic protein fluorescence is changed during the ligand binding as fluorescence of the naturally occurred tryptophans in the molecule is influenced by their microenvironment.[29] Fluorescence-polarization experiments are based on the fact that a fluorescent molecule undergoes less depolarization when bound to a receptor than when unbound. This approach has been extensively used for screening library of fluorescein-labeled saccharides or derivatives toward different galectins.[30] Mono- or diamido-thiodigalactoside derivatives were demonstrated to be efficient inhibitors against all galectins, with galectin-3 displaying best Kd (<50 nM).[31]

The fluorescence-based thermal shift assay is a relatively new method, useful for identification of stabilizing effects after protein–ligand or generally macromolecule-solution additives interactions. The method takes the advantage of an environmentally sensitive fluorescence dye to monitor protein thermal unfolding. When hydrophobic fluorescence dye is added to the solution with properly folded protein, it is exposed to an aqueous environment and its fluorescence signal is quenched. As the temperature rises, the hydrophobic dye preferentially binds to the exposed hydrophobic core region of the unfolding protein and leads to a sharp decrease in quenching of fluoroprobe. Therefore, detected fluorescence emission can

be studied as a function of temperature. This thermal unfolding is an irreversible process with a sharp transition state defined as the thermal transition midpoint (T_m), when 50% of the biomolecules are unfolded. The fluorescence-based thermal shift assay can be performed in a commercially available real-time PCR instrument, where thermal melting curves can be screened rapidly in high-throughput mode on 96 samples plates with very low quantities of the sample in a range of several micrograms per well. The method represents an efficient technique to search for optimal buffers and stabilizing conditions. The ligand-binding affinity can be assessed from the shift of the unfolding temperature obtained in the presence of ligand.[32,33] Its current development for screening for ligand will for sure be applicable soon to lectin/carbohydrate interactions.

5. NMR

Several techniques of nuclear magnetic resonance can bring information about the interaction between lectins and oligosaccharides.[34] Titration can be classically followed by measurement of chemical shift differences and/or lines broadening but more sophisticated methods emerged for the determination of the bound conformation of the oligosaccharide and for the identification of the binding epitope. These ligand-based approaches, namely transferred nuclear Overhauser experiments (trNOEs)[35] and saturation transfer difference (STD),[36] focus on the observation of the ligand and do not require assignment of the protein moiety. TrNOEs have been mostly used to determine the conformation of oligosaccharide in lectin binding site, such as blood group A trisaccharide with *Dolichos biflorus* lectin.[37] STD experiments have broader ranges of application since they can be used for screening libraries of putative oligosaccharides in order to identify the ones with binding activities.[38] They are also used for identifying the part of the oligosaccharide that is directly contacting the protein binding site.

In the recent years, the combination of TrNOEs and STD experiments often associated with molecular modelling, appeared as a powerful tool for characterizing lectin/oligosaccharide interactions. For example several lectins that bind to HIV by interacting with its gp120 oligosaccharide part were investigated. DC-SIGN, which is present at the surface of immature dendritic cells, use several binding modes for interaction with oligomannosides.[39,40] On the opposite, the cyanobacterial lectin cyanovirin-N binds only to nonreducing terminal mannose of αMan1-2Man moiety.[41]

Since STD experiments do not require the assignment of the protein moiety, they can be run on large proteins and even on cells and viruses. Indeed promising data were obtained for the interaction between living cells tranfected with DC-SIGN and *S. cerevisiae* mannan.[42] Recently, virus-like particles (VLP) that are non-infectious protein architectures mimicking viruses, were tested by STDs. It was demonstrated that rabbit hemorrhagic disease virus binding to human blood group oligosaccharide is mediated by fucose.[43] STD experiments on avian influenza H5-containing virus-like particles demonstrated clear preference for αNeuAc2-3Gal containing oligosaccharides over αNeuAc2-6Gal containing ones.[44] NMR methods

appear therefore promising as the initial step in screening processes for oligosaccharide specificity towards capside proteins, but also membrane-bound receptors or bacterial adhesins.

6. Frontal chromatography

Frontal affinity chromatography (FAC) is a very sensitive quantitative method for determination of dissociation constants that has been developed in the 1970s.[45] FAC can be used for the analysis of relatively weak interactions, and is thus very suitable for analysis of sugar-protein interactions.[46] First generation of FAC systems used conventional open-type columns and therefore required relatively large amount of sample. Recent systems have been greatly improved by utilizing HPLC automated machines equipped with capsule-type miniature columns in line with fluorescence detectors (FAC-FD).[47] Miniature columns filled with lectin have been developed[48] to be used with fluorescent-labelled oligosaccharides[49] injected at a constant flow rate and concentration *via* a relatively large sample loop. With such approach, it was possible to quantitatively analyze the interactions between 13 galectins including 16 CRDs originating from mammals, chick, nematode, sponge, and mushroom, with 41 pyridylaminated (PA) oligosaccharides. The calculated equillibrium constants K_d resulted in conclusions how galectins have evolved their fine and dynamic sugar-binding specificities.[50] Recently developed system for automated FAC[47] enables up to one hundred analyses per day. Systematic data on lectin/carbohydrate specificity may be accumulated rapidly and lectin specificity can now be discussed in terms of K_d in a more systematic manner than ever.[51] Several different modes of analysis are also possible by interfacing it to, *i.e.*, a mass spectrometer.[52]

FAC can be effectively used for screening large number of ligands. Two GlcNAc-binding lectins, *Griffonia simplicifolia* lectin-II (GSL-II) and a novel fungal lectin from *Boletopsis leucomelas* (BLL) were investigated with their detailed oligosaccharide specificity using 146 glycans. As a result, it was demonstrated that both GSL-II and BLL recognize completely and partially agalactosylated complex glycans while their specificities are markedly different.[53] This approach was also used for a systematic comparison of specificity between *Ricinus communis* agglutinin I (RCA120) and *Erythrina* lectins (Fig. 2) with the use of more than one hundred pyridylaminated oligosaccharides demonstrating that the potentially toxic RCA120 can be replaced by other lectins in binding studies.[54] Recently, the systematic structure-function relationships of the extended mannose-binding-type Jacalin-related lectin (mJRL) subfamily was studied using 103 pyridylaminated glycans. Notably, the result of cluster analysis of the amino acid sequences clearly corresponded to the specificity classification found by affinity studies.[55]

Galectins consist of a family of related proteins with fine differences in oligosaccharides specificity, related to many different biological roles. Two studies based on FAC have been performed on galectins from the nematode *Caenorhabditis elegans*. Site-directed mutagenesis of the *N*-terminal lectin domain of galectin LEC-1 was performed to identify the amino acid residues important for carbohydrate recognition.[56] Later on, a detailed

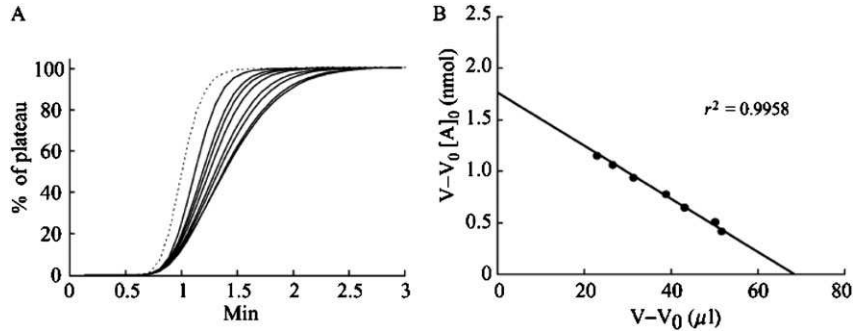

Fig. 2 Determination of B_t values (amount of functional immobilized ligand in the column) for the immobilized *Erythrina cristagalli* agglutinin. *p*-Nitrophenyl, (*p*NP)-lactose, diluted to various concentrations (8 to 50 μM), was used for concentration-dependence analysis. (A) The solid and dotted lines demonstrate elution profiles of *p*NP-lactose and control sugar (*p*NP-mannose), respectively. (B) Woolf-Hofstee-type plot was made by using $V\text{-}V_0$ values. Adapted from 47 with permission.

structural analysis of the *N*-glycans of *Caenorhabditis elegans* recognized by *C. elegans* galectin LEC-6 has been reported.[57] It confirmed that all pyridylaminated sugars, having affinity for LEC-6, contain a Gal-Fuc disaccharide unit, and that this unit is bound to the innermost GlcNAc residue of the *N*-glycan chain. During the last two years, additional characterization of galectins using FAC have investigated their diverse carbohydrate-binding properties as in the case of mammalian galectin 9.[58,59]

7. Quartz crystal microbalance

The Quartz Crystal Microbalance (QCM) is an ultra-sensitive mass sensor, capable of measuring mass changes in the nanogram range. QCM measures interaction using the property that the frequency change of a quartz crystal resonator is a linear function of the mass change per area. After the description of the first quartz crystal microbalance,[60] application to biological samples became possible when suitable oscillator circuits for operating in liquids were developed.[61]

In 2005 Pei and co-workers developed a quartz crystal microbalance biosensor system for real-time detection of lectin–carbohydrate interactions.[62] Yeast mannan was immobilised on polystyrene-coated quartz crystals and tested by interactions with Concanavalin A (ConA) and the results were in good agreement with a related enzyme-labelled lectin assay (ELLA) protocol. Further development included the generation of glycosurface on the gold surface of QCM through self-assembled strategy by the use of alkanethiol functional groups.[63] The resulting biosensor was used to detect the binding ability of ConA. Recently, mannose-stabilized gold nanoparticles were used as signal amplifier, yielding higher sensitivity.[64]

Comparison of two analytical approaches, atomic force microscopy (AFM) and quartz crystal microbalance, for studying the binding of Con A to glycosylated carboxypeptidase, demonstrated that both could determine the quantitative parameters characterizing the interaction.[65] Quantitative analyses of the interaction of Calreticulin (CRT), which is a soluble molecular chaperone of the endoplasmic reticulum, with various

disaccharides, including fluorine-substituted analogues were investigated using QCM.[66] In combination with saturation transfer differentiation NMR (STD NMR) the interaction mode between CRT and saccharides was studied emphasizing the importance of the glucose α1-3 linkage and of the 2- and 3-OH for the specificity.

Combining carbohydrate and lectin recognition events with an appropriate QCM transducer can yield sensor devices for on-line screening and detection of bacteria in food, water, clinical and biodefense areas. The binding of bacterial lipopolysaccharides to the lectin (Con A) attached to the surface of quartz crystal modified with mannose self-assembled monolayer (SAM) was used for detecting *E. coli* within a detection limit of a few hundred bacterial cells.[67] An alternative is to attach the lectin on a gold-plated quartz crystal surface *via* avidin-biotin binding.[68] Moreover, fast, sensitive and selective flow-injection using novel lectin-based quartz crystal microbalance biosensor assay were developed for the detection of pathogenic enterobacteria.[69] The proposed biosensor was able to carry out not only the label-free assay identification of studied pathogens (*H. pylori*, *C. jejuni*), which can serve as a promising alternative to the traditional bacteriological analysis for clinical diagnostics, but also performed the rapid and sensitive quantitative assay. The simple regeneration procedure using glycine made it possible to use the same sensor several times without losing the lectins activity.

Other applications of QCM biosensors include real-time monitoring of the agglutination process of human hepatic normal cells (L-02) and hepatic cancer cells (Bel7402) on QCM electrode in the absence and presence of ConA and wheat germ agglutinin.[70] This work demonstrated that the QCM measurement technique based on cell agglutination can be used for discriminating hepatic normal cells from hepatic cancer cells.

8. Surface plasmon resonance

Surface plasmon resonance (SPR) is a charge-density oscillation that may occur at the metal–dielectric interface. Photons of polarized light can interact with the free electrons of the metal layer, inducing a wave-like oscillation of the free electrons that leads to reduction of the reflected light intensity. The resonance angle at which the maximum loss of the reflected light intensity occurs is dependent on the optical characteristics of the system. It changes when molecules adsorb on the metal surface and the change is directly dependent on the total accumulated mass.

The main advantage of SPR biosensors is their ability to characterize binding reactions in real-time without labeling. Biosensor experiments involve an immobilization of one reaction partner on a biochip surface and monitoring its interactions with a second component passing over the surface free in solution. During the last decade, SPR became an established method for measuring biomolecular interactions since it represents a flexible and powerful tool to describe kinetics and/or steady-state equilibrium of a variety of molecular interactions. The experimental design data analysis methods are evolving along with widespread applications in ligand fishing, epitope mapping and interaction study of any biological system from

proteins, oligonucleotides, saccharides and lipids to small molecules or phage, viral particles and cells. A tandem integration of SPR instruments with mass spectrometers provides a unique analytical tool for functional proteomics and identification of binding partner.[71,72]

For lectin/carbohydrate interactions, either the protein or the glycan can be attached to the sensor surface, letting the counterpart passing along. In most published data, a modified glycosurface is prepared *via* different approaches and signal corresponding to the protein binding is detected. For example, novel sensing interface was prepared using chemoenzymatically synthesized biotinylated sialyldisaccharides.[73] This study showed a structure-dependent variation of sialoside-lectin binding for different lectins and different carbohydrate-immobilized surfaces. High stability of the surface is promising for high-throughput screening of weak biological interactions. Similarly, a series of 6′-sialyllactose-BSA conjugates were attached to chips to test the quality of influenza vaccine egg-derived and cell culture-derived lots.[74]

The high sensitivity and reproducibility of currently used instruments also allows to directly monitor the binding of small ligands, *i.e.* disaccharides or monosaccharides, to immobilized lectins.[75–77] Fig. 3 illustrates the binding of small glycans to BclA lectin from *Burkholderia cenocepacia*.[26]

High-throughput quantitative SPR analysis is an ultimate goal and challenge for the future. A typical example of a high-throughput SPR sensor is the SPR imaging (SPRi). SPRi systems measure changes in the intensity of light at fixed angle and wavelength and work in an array format so they are able to analyze higher number of samples in much shorter time. The binding preference of human Siglec7 to α2-8-linked disialic acid

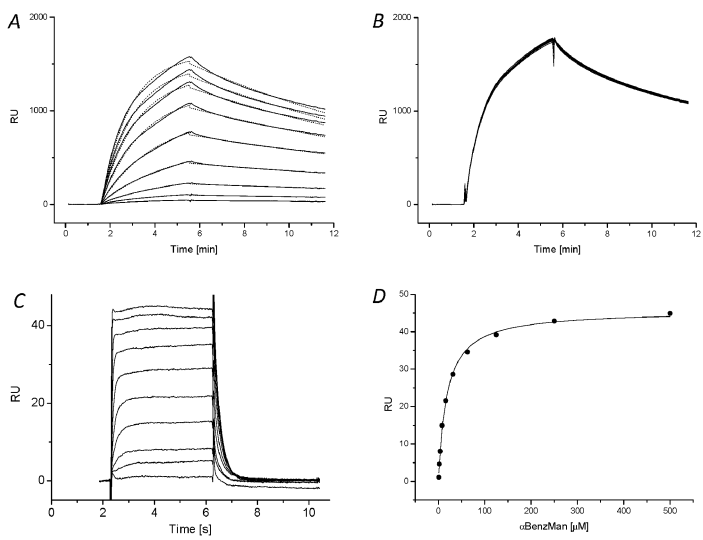

Fig. 3 SPR sensorgrams. Upper: Binding curves of 20 μg/mL of BclA to immobilized PAA-mannose in the presence of (A) 1.95 μM-0.25 mM α-benzyl-mannoside (the best ligand from tested monosaccharides) and (B) 0.95 mM-25 mM D-galactose (non-binder). Bottom: SPR sensorgrams for D-mannose binding to immobilized BclA. (C) Equilibrium steady state curves for D-mannose varying from 1 to 500 μM. (D) The corresponding binding curve derived from steady-state equilibrium values.

structures were determined on the carbohydrate microarray of 40 sialylated and unsialylated glycans.[78] Direct measurement of glycoproteins dotted on a gold surface at volumes down to nanolitres demonstrated sensitivity and suitability of SPRi for fast biomolecular screening.[79]

9. Isothermal titration calorimetry

Isothermal titration calorimetry (ITC) directly quantifies the heat effects accompanying association between molecular entities during a series of injections of ligand solution into binding partner solution. The binding isotherm obtained is then used for linear least-squares analysis in order to obtain the stoichiometry of binding, the change in enthalpy (ΔH) and the association constant. Heat release or uptake is a universal property of interaction and reaction, so the experiments can be performed in buffer, with not labelling or immobilizing of the molecules of interest. ITC is very well adapted to protein-carbohydrate interaction,[80] mainly because the large number of hydrogen bonds generally involved results in a strongly exothermic binding, giving rise to easily detectable heat peaks. Successful extraction of thermodynamic parameters from calorimetric data is easier when the system displays micromolar affinity. Nevertheless, low affinity systems, such as often observed in lectin-carbohydrate interactions, can be studied using ITC with appropriate strategies.[81]

In glycobiology, ITC is mainly used to complement specificity study and/or structural data by measuring the affinity of diverse carbohydrate ligands to lectins. It therefore complements glycan arrays and crystallography. For example, the C-type lectin from white clam, codakine, displays classical low affinity binding (K_d 0.3 mM) for mannose. The use of glycochips demonstrated its fine specificity for complex-type *N*-glycans and ITC could confirm that the biantennary nonasaccharide is a very high affinity ligand with Kd of 432 nM.[22] Similar approach was used to define the behavior of a fungal galectine-like protein that displays unusual affinity for chitooligosaccharides.[82]

Protein/carbohydrate interactions are classically described as low affinity ones, ranging from millimolar for monosaccharidic ligand to micromolar for oligosaccharide. Analysis of the thermodynamic contribution revealed that the poor free energy of binding ΔG-and hence weak affinity constant-is related to an entropy barrier.[80] In general, the enthalpy term is large and negative, but the entropy contribution counterbalances this effect ($\Delta G = \Delta H - T\Delta S$). This dogma is mostly true for plant and animal lectins, but the recent characterization of several bacterial lectins demonstrated different behavior. The thermodynamic behavior of a family of calcium-depent lectins present in opportunistic bacteria such as *Pseudomonas aeruginosa* (PA-IIL),[27,83] *Chromobacterium violaceum* (CV-IIL),[84] *Rastonia solanacearum* (RS-IIL)[85] and *Burkholderia cenocepacia* (BclA)[26] exhibited high affinity for monosaccharides.

As displayed in Fig. 4, for this family of proteins, the entropy of binding is either favorable or weakly unfavorable, while the enthalpy of binding is negative, resulting in micromolar affinity. The only exception is a PA-IIL ligand consisting of two fucose residues separated by a flexible linker that

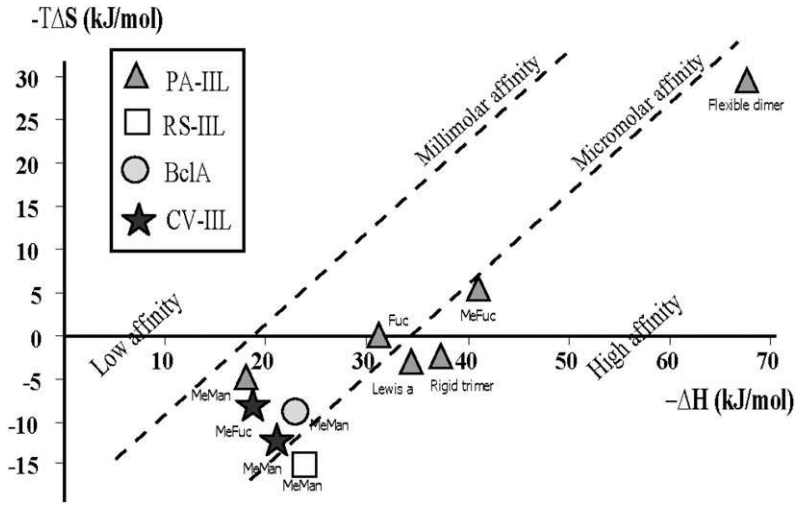

Fig. 4 Enthalpy-entropy plot obtained by ITC measurement on a family of related microbial lectins interacting with natural and synthetic carbohydrates.

generates a strong entropy barrier upond binding.[11] This example illustrates how ITC data can be used for optimizing the design of active compounds.

Recent developments are pushing towards elucidating complex binding phenomenon between clustered carbohydrate epitopes and multivalent lectins. Ligand valency often results in aggregation, and the kinetic of the process may complicate the ITC measurements.[86] For polymers presenting multiple carbohydrate epitopes, such as mucins, it has been proposed that the enhanced affinity observed for these interactions are due to internal diffusion of lectin molecules from epitope to epitope in these multivalent ligands before dissociation.[87,88]

10. Molecular modelling

Molecular modelling can be used to complete experimental studies on protein-carbohydrate interactions and this approach is an efficient tools for rationalizing the observed specificity. While a large variety of programs are available for modelling proteins, modelling glycans requires to select appropriate computational tools in order to take into account the conformational behavior of the glycosidic linkages. Some widely used force-field have been modified or extended in order to include energy parameters suitable for carbohydrates and such recent additions are available for CHARMM,[89] GROMOS[90] and AMBER[91] force-fields. This latter parameterization, named GLYCAM06, is widely used since it allows for simulation of oligosaccharides, glycoprotein and protein-oligosaccharides complexes.

For predicting the carbohydrate orientation in binding sites, flexible docking methods have to be used in order to account for the possible orientations of pendent groups (*i.e.* hydrogen bond network directed by hydroxyl/hydroxymethyl group orientations) and also for the conformational behaviour of the glycosidic linkage of oligosaccharides. The

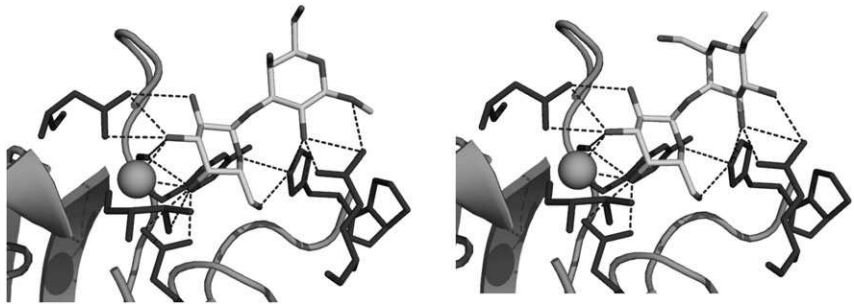

Fig. 5 Docking prediction for the binding of αGal1-4Gal (left) and αGal1-3Gal (right) in the binding site of PA-IL from *P. aeruginosa*. The calcium ion is represented by a sphere, the hydrogen bonds by dotted lines and the contact to calcium by fine lines.

AutoDock program[92] has been successfully used for the modelling of lectin/glycan interaction. Recent developments include the correct treatment of calcium ions for C-type lectins and other calcium dependent lectins.[93,94] The approach from Nurisso and coll. has been applied for predicting the binding of oligomannosides in langerin,[95] a human lectin involved in the recognition of gp120 from HIV. It was also used for comparing the binding modes of two disaccharides, αGal1-3Gal and αGal1-4Gal in the binding site of PA-IL from *Pseudomonas aeruginosa*.[96] Both disaccharides can establish a strong network of hydrogen bonds with the PA-IL binding sites, albeit with very different geometries (Fig. 5).

The ultimate goal in theoretical structural biology is the prediction of the affinity for a given lectin-carbohydrate interaction. Two types of approach could be used for calculating the free energy of binding, both requiring extensive molecular dynamics simulations, preferably in explicit water environment.[97] The direct free energy difference (ΔG) calculations require the initial and final states of the system to be evaluated in independent molecular dynamics calculation, whereas the thermodynamics integration requires a slow perturbation of the system from the initial to final states. Direct ΔG calculations were applied successfully on ConA interacting with oligosaccharides.[98] This model lectin was also used to investigate the role of hydration in the binding event using both free energy calculations[99] and perturbation methods.[100]

11. Concluding remarks

This review demonstrates that the analysis of lectin/glycan interactions is a very active field that benefits from important recent development in miniaturization of experiments and developments of high-throughput techniques. Mastering the clustering effect due to multivalency is still a challenge since there is no simple method for analyzing a complex behavior that includes competition between binding events and aggregation. Studying the physical forces driving protein/carbohydrate interaction is a part of the efforts for progressing in both the fundamental basis of this recognition and signalling events and the possible development of glycocompounds with therapeutical interest.

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