Marco Brito-Arias

Synthesis and Characterization of Glycosides

Second Edition

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To Carmina Daniela BR and Iryna K

Preface

There is no doubt that glycoside chemistry continues to be a dynamic and exciting field of organic chemistry. Within sugar chemistry, glycosides are of special interest not only because of the challenges represented by their synthesis and structural characterization, but also due to their important biochemical relevance, and hence their applications in a number of essential disciplines, such as pharmaceuticals, food, and biotechnology.

Important biomolecules such as DNA and RNA, or cofactors such as ATP and NAD are some of the natural glycosidic structures that play key roles at a biochemical level. Also, a considerable number and variety of natural and synthetic glycosides are being extensively used as antibiotics, antiviral, and antineoplastic agents.

There are also a significant number of chromophoric glycosides being used in molecular biology as substrates for detection of enzymatic activity of gene markers.

Solid-phase oligosaccharide synthesis despite the great progress recently reported by different groups continues to be a challenging task considering the diversity and complexity of glycosides, especially those present in cellular membranes. However, based on the satisfactory evolution of this approach, there is confidence that many complex molecules will be prepared just in the same way that solid-phase chemistry is currently used to prepare oligopeptides and oligonucleotides.

The aim of this book is to provide methods and strategies for the formation of glycosides, illustrated by the synthesis of important biologically active glycosides, and also to present an overview of the basic tools needed for the characterization of glycosides through NMR spectroscopy, X-ray diffraction, and mass spectrometry.

From the overwhelming number of excellent articles related to glycoside chemistry, it has not been an easy task to select those that are biologically important, and perhaps most importantly serve as didactic models for understanding more about the process of glycoside bond formation.

The book should also serve as a helpful guide to those professionals interested in sugar chemistry, especially regarding the design of synthetic routes, by evaluating suitable protecting and leaving groups, and the best reaction conditions needed for the preparation of glycosides.

la Laguna Ticomán cp., Mexico Marco Brito-Arias

Preface for Second Edition

The second edition is designed to serve as a textbook on glycoside chemistry with the main goal to provide updated information about the methods considered classical or of primary significance as well as novel variations or new methods for achieving glycosylation processes. This applies to glycosyl donors, promoters or activators, and protecting groups that have been currently reported as more efficient or with significance for preparing active substances of glycosidic nature with important implications in pharmaceutical, food, environmental, and biotechnological related disciplines. The second edition provides updated information on chemical shifts, and coupling constant data for complete structure assignment of glucopyranoses and pyranosyl disaccharides, as well as the main fragmentation pattern observed in mass spectrometry. I hope this new edition will expand its usefulness to those professionals involved in glycoside chemistry and will provide support in design of suitable methodologies in a novel or more efficient way. Finally the author would be grateful for receiving any comment intended to improve the quality of the material included.

Acknowledgments

The author would like to thank COFAA and SIP-IPN for their financial support.

Contents

Chapter 1
Glycosides, Synthesis and Characterization

1.1 Introduction

Monosaccharides are generally defined as aldoses and ketoses connected to a polyhydroxylated skeleton [1]. In an aqueous solution, monosaccharides are subject to internal nucleophilic addition to form cyclic hemiacetal structures. When addition occurs between -OH at C(4) or -OH at C(5), and the carbonyl group, a five- or a six-member ring is formed called a furanose or a pyranose respectively. It is also known that an equilibrium exists between the open and the cyclic form, being displaced to the latter by more than 90 %. Therefore, in aqueous solution, it is more accurate to consider that most sugars are present as cyclic molecules and behave chemically as hemiacetals.

The Haworth structure is a useful way to represent sugars. However, as it is known that for any six-membered rings a nonplanar conformation is assumed. The conformation exclusively preferred is called chair and the two possible conformations are $^4C_1$ and $_4C^1$. The first conformation is used for the D enantiomeric form and the second for the L form (Scheme 1.1).

On a chair conformation type $^4C_1$, an α anomeric hydroxyl group is positioned in the axial orientation while a β hydroxyl lies equatorial (Scheme 1.2).

As a result of this reversible ring formation process, a diastereomer mixture of anomers α and β is produced as indicated in Table 1.1 for some of the most common monosaccharides [1, 2].

The pioneering work in 1890 by Fischer [3] allowed him to determine the relative configuration and the synthesis of the most known aldohexoses. Based on the assumption that in D-glyceraldehyde, the hydroxyl group is placed to the right, he proposed correctly the structure of tetroses, pentoses, and aldohexoses (Scheme 1.3). The relative configuration of D-glyceraldehyde was later confirmed by X-ray diffraction by Bijvoet in 1951. Consequently, all the resulting biologically active distereoisomeric aldoses derived from D-glyceraldehyde conserve

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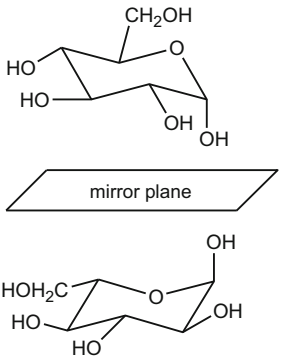


Scheme 1.1 α-D-glucopyranose-$^4C_1$ and α-L-glucopyranose-$^1C_4$

always the secondary alcohol next to the primary one to the right side in the Fischer projection. Ketoses with 3–6 carbons are naturally produced from 1,3-dihydroxyacetone, according to the tree shown in Scheme 1.4.

1.2 Reactions of Monosaccharides

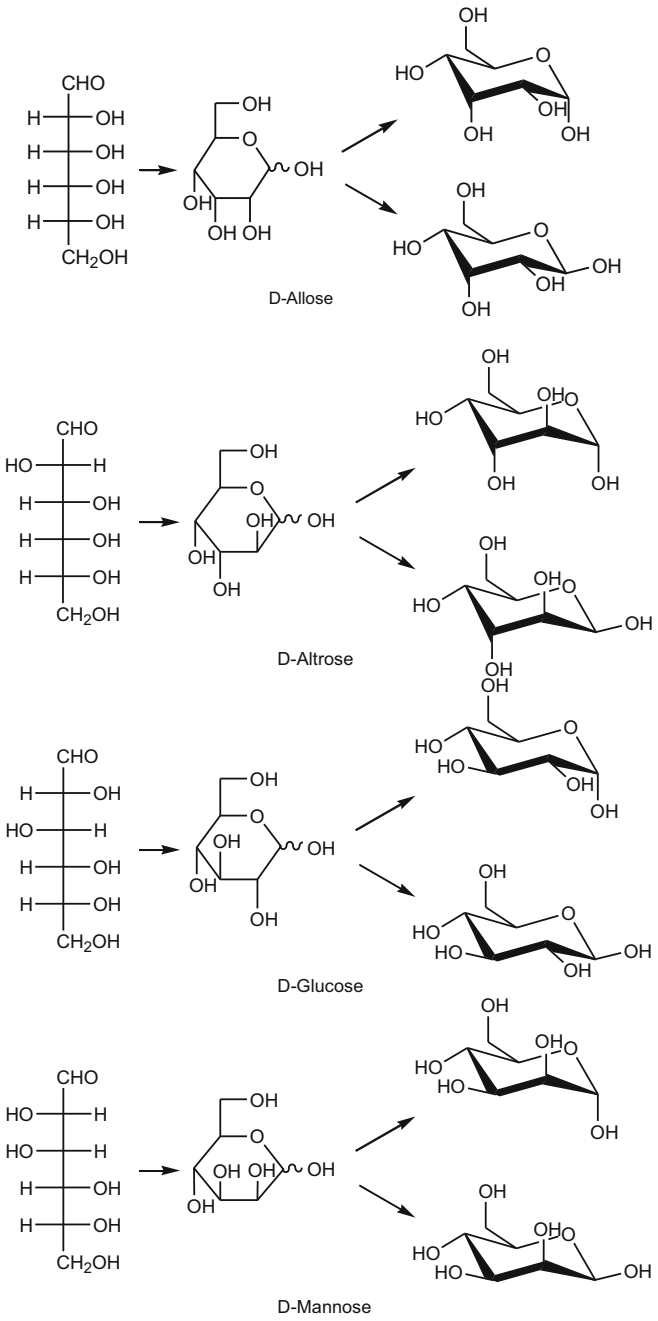
Carbohydrates own their reactivity to the hemiacetalic center and to the hydroxyl groups, with the primary group being more reactive than the secondary group. Aldoses and ketoses are susceptible to nucleophilic addition and the latter is less reactive due to steric hindrance. The cyclic forms are adopted when the hydroxyl group positioned at C-5 verifies an intramolecular nucleophilic addition to the carbonyl group producing an anomeric mixture of pyranosides (Scheme 1.5).

1.3 Chemical Modifications

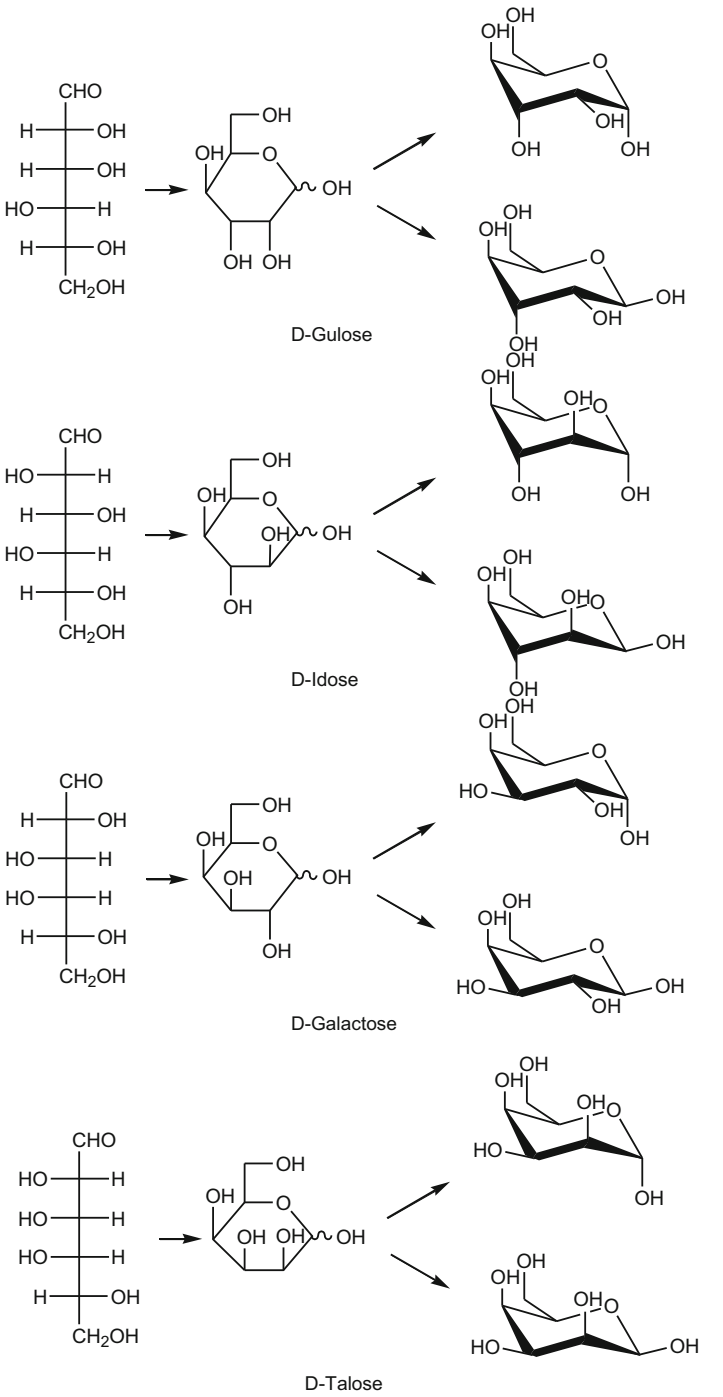
The classical reactions on monosaccharides are used initially for identification or sugars or to distinguish between aldoses and ketoses. They are also very useful for preparing key intermediates in the construction of glycosides. Some of the common reactions used to identify monosaccharides are:

1.3.1 Oxidations

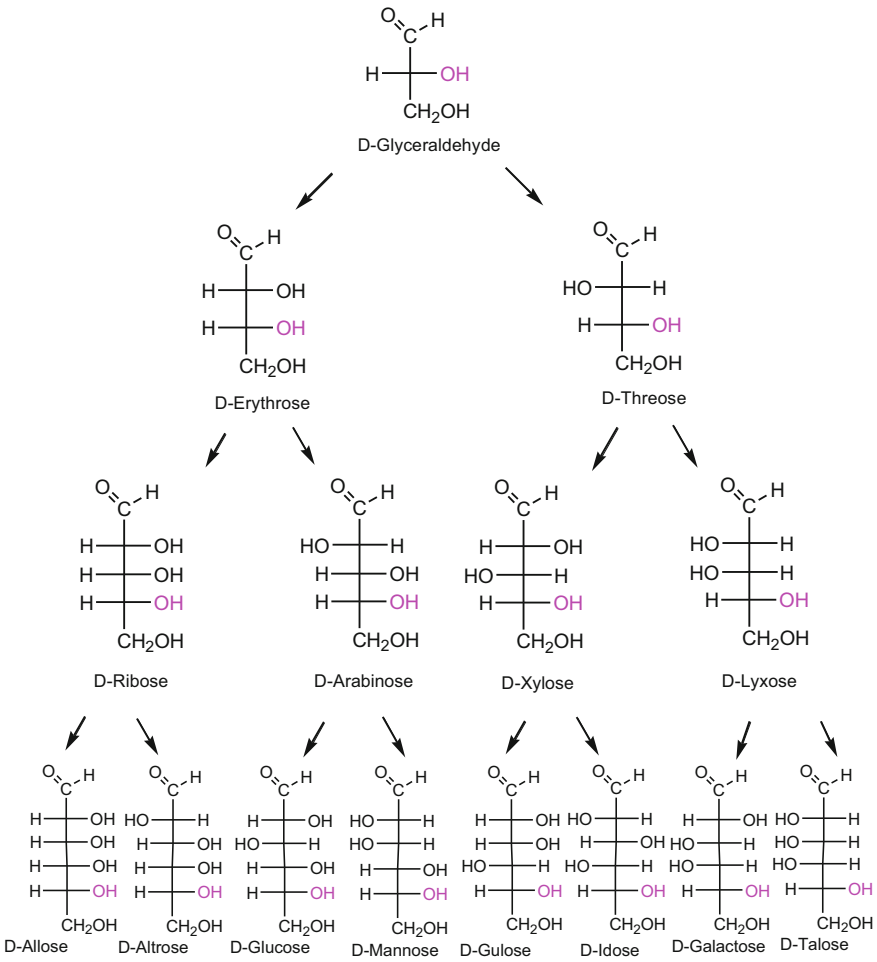
The oxidation of non-protected aldoses may result in carboxylic acid formation depending on the reaction conditions. Thus, with aqueous bromine a monocarboxylic acid (aldonic acid) is formed, whereas with nitric acid a dicarboxylic acid is favored (aldaric acid) (Scheme 1.6).



Scheme 1.2 Fischer projections, Haworth structures, and $^4C_1$ chair conformation of D-aldohexoses



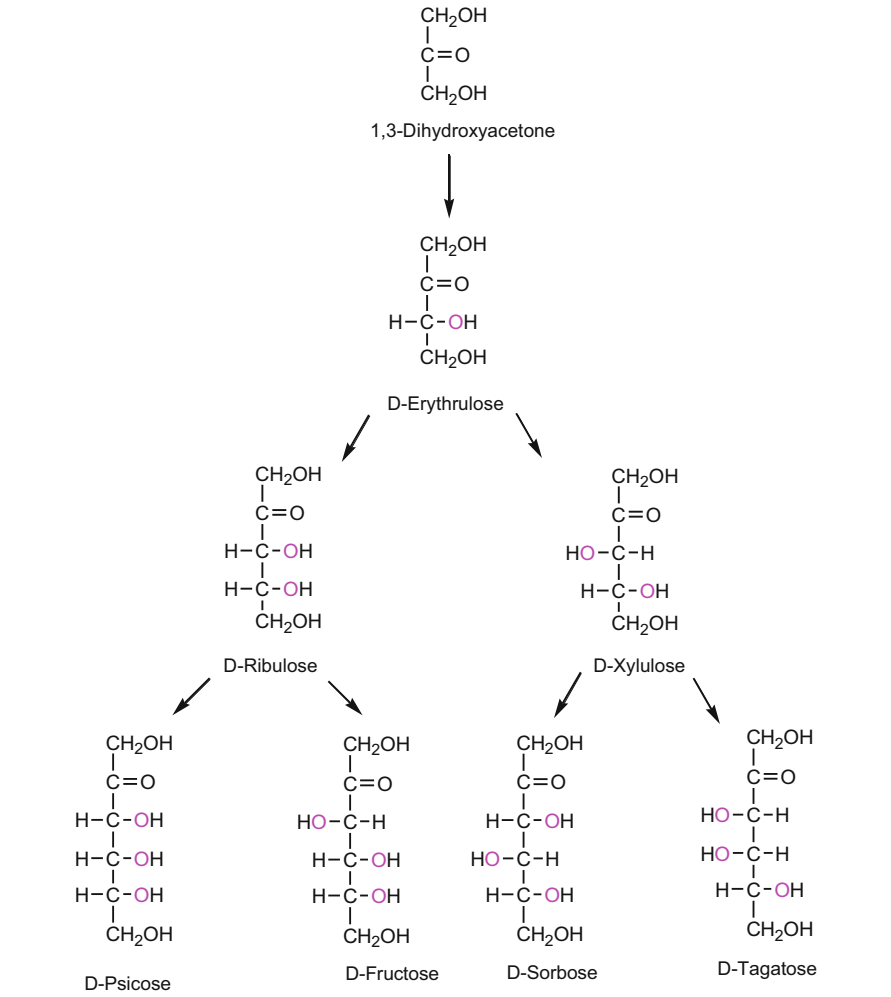
Scheme 1.3 Fischer projections of D-aldoses



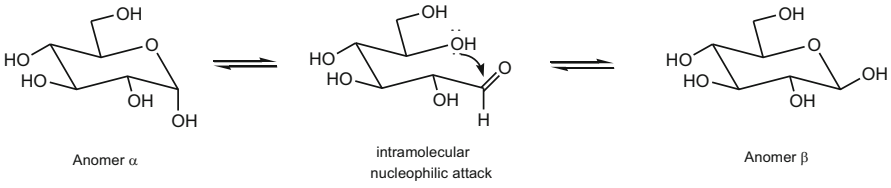
Scheme 1.3 (continued)

Table 1.1 Distribution of α β of some D-monosaccharides in solution at 31 °C

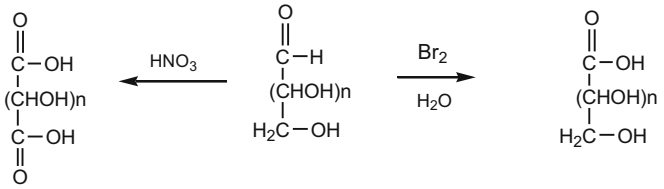
	% Pyranose		% Furanose	
Carbohydrate	α	β	α	β
Glucose	38	62	0.1	<0.2
Galactose	30	64	3	4
Mannose	65.5	34.5	0.6	0.3
Rhamnose	65.5	34.5	0.6	0.3
Fructose	2.5	65.0	6.5	25
Ribose	21.5	58.5	6.4	13.5
Xylose	36.5	63.0	0.3	0.3



Scheme 1.4 Fischer projections of the 2-ketoses

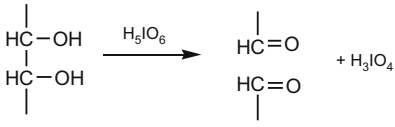


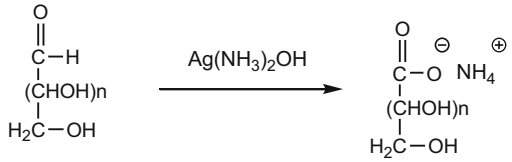
Scheme 1.5 Pyranose ring formation



Scheme 1.6 Oxidative aldose transformation into monocarboxylic and dicarboxylic acids

Scheme 1.7 Oxidative cleavage of diol by periodic acid





Scheme 1.8 Tollens reaction

1.3.2 Periodate Oxidation

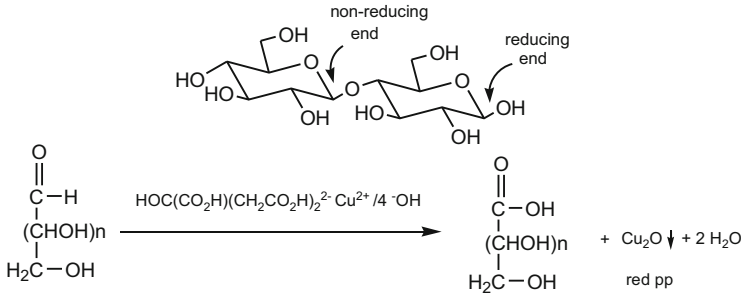
Periodic acid is an strong oxidizing agent and is capable of breaking 1,2-cis diols to generate carbonyl fragments after cleavage of the C–C bond (Scheme 1.7).

1.3.3 Tollens Reaction

This classical reaction is very useful for aldose identification and consists in the oxidation of the aldehyde function with a moderate oxidative agent (a silver ammonium salt) to form the glucuronide ammonium salt and metallic silver which produce the silver mirror effect (Scheme 1.8).

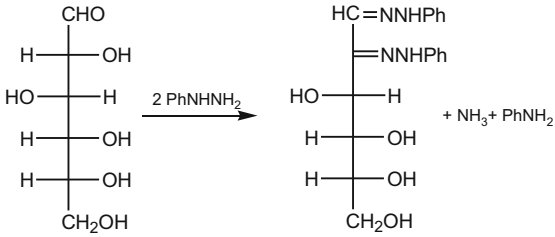
1.3.4 Benedict and Fehling Test

The test consist in the use of a copper citrate (Benedict reagent) or copper tartrate complex (Fehling reagent), which upon treatment with the sugar under study produces the glucuronide ion along with copper (I) oxide which is detected as a brick-red precipitate (Scheme 1.9).



Scheme 1.9 Benedict and Fehling test

Scheme 1.10 Osazone formation



Based on Tollens, Benedict, or Fehling test, sugars are classified into reducing when positive or non-reducing sugars if negative. Reducing sugars are hemiacetals in equilibrium with small amounts of open forms. Under basic conditions, aldoses and ketoses are positive for Tollens and/or Benedict/Fehling test as result of an aldose–ketose equilibrium via enediol intermediates.

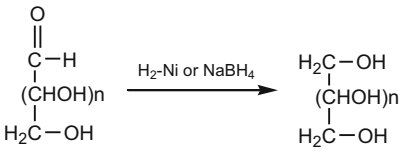
1.3.5 Nucleophilic Addition

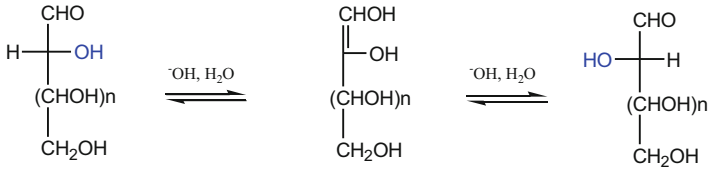
Aldoses and ketoses may react with a variety of nucleophiles, giving rise to addition/elimination products such as osazones and oximes, or addition products such as reduced derivatives when reacted with hydrides.

The reaction that allowed E. Fischer to determine the structure of common aldoses is the osazone formation and consisted in the reaction between hydrazine and aldoses (Scheme 1.10) to yield crystalline derivatives that can be identified through their melting point values.

The carbonyl group can be reduced by hydrogenation or hydride addition to produce corresponding alditols (Scheme 1.11). These reduced sugars are present in various fruits such as cherries, pears, and apples and are used as sugar substitutes for diabetics.

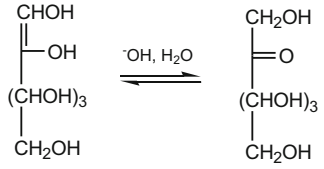
Scheme 1.11 Carbonyl reduction for the preparation of sorbitols





Scheme 1.12 Enediol rearrangement

Scheme 1.13 Enediol rearrangement



1.3.6 Enediol Rearrangement

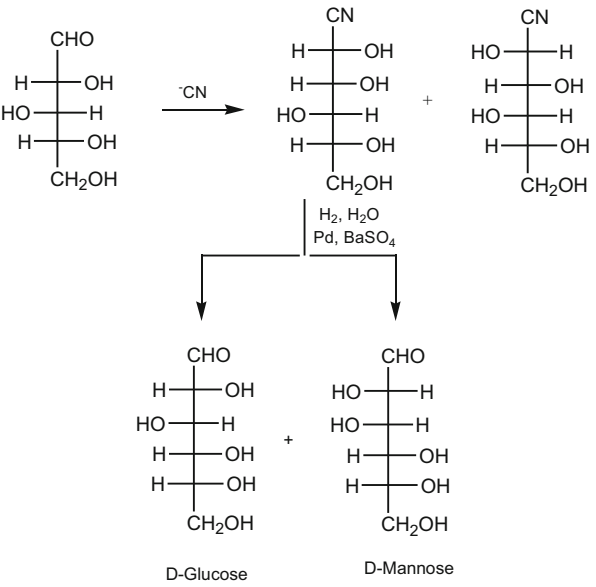
This transformation occurs in a basic medium and allows the conversion of epimers, defined as isomeric forms that differ in the position of the hydroxyl group at C-2. In this way it is possible to transform glucose to mannose through the enediol intermediate and vice versa (Scheme 1.12).

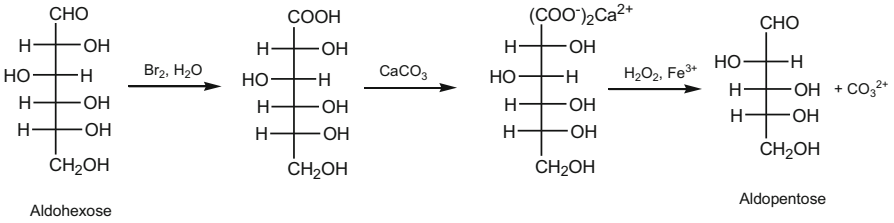
Another important isomerization process through the enediol rearrangement is the interconversion of glucose and fructose. Thus, the enolization proceeds by migration of proton at position 2, to carbon at 1 (Scheme 1.13).

1.3.7 Kiliani–Fischer Synthesis

This sequence was used to increase the number of carbons in a sugar. The reaction involves cyanohydrin formation by nucleophilic addition of cyanide to the aldehyde. The diastereoisomeric mixture of cyanohydrins obtained is partially reduced to produce the epimeric forms (Scheme 1.14).

Scheme 1.14 Kiliani–Fischer synthesis





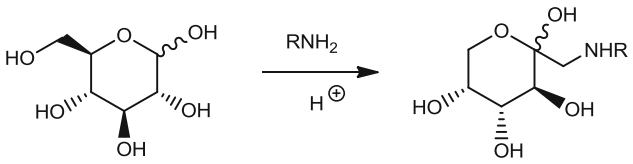
Scheme 1.15 Ruff degradation

1.3.8 Ruff Degradation

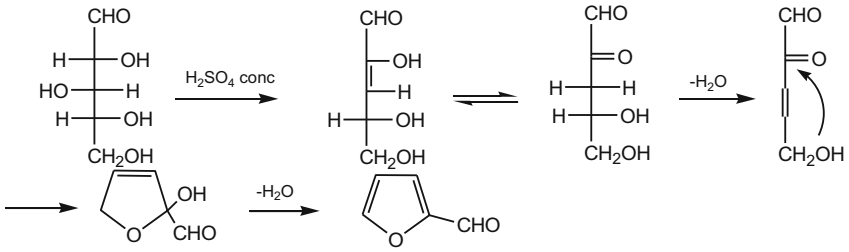
The process of reducing the monosaccharide skeleton in one carbon is known as Ruff degradation and consists in the oxidation of the aldehyde to the carboxylic acid through the use of calcium salt and subsequent peroxide treatment in the presence of ferric salts to produce the aldose reduced in one carbon (Scheme 1.15).

1.3.9 Amadori Rearrangement

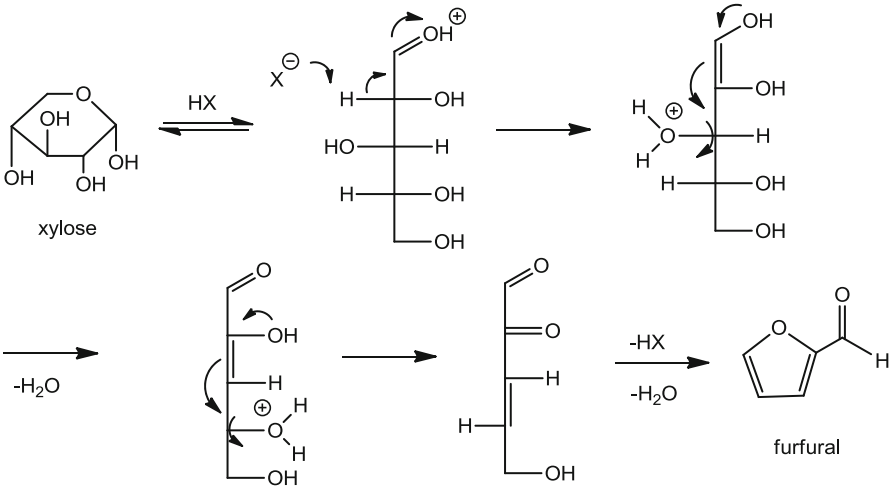
This reaction occurs between an unprotected aldose such as D-glucose and suitable amines, producing 1-amino-1-deoxy ketoses as a mixture of anomers. When the amino group comes from an amino acid the reaction is known as the Maillard reaction, which is an important modification in food science (Scheme 1.16) [4].



Scheme 1.16 Amadori rearrangement



Scheme 1.17 Conversion of pentoses to furfural



Scheme 1.18 Conversion of xylose to furfural

1.3.10 Conversion to Furfural Derivatives

Pentoses subjected to high acid concentrations can be transformed to furfural in quantitative yields. The sequence involves a tautomeric keto-enol equilibrium, dehydration, and intramolecular nucleophilic addition of the primary alcohol to the aldehyde to generate furfural (Scheme 1.17).

The main pentose source used for preparing furfural is xylose which under acidic medium is subjected series of dehydrations, enolization and intramolecular cyclization as shown in Scheme 1.18. Some of the conditions reported for preparing furfural are described in Table 1.2.

Table 1.2 Reaction conditions for preparation of furfural

Sugar source	Catalyst	Reference
Xylose	Solid acid/ZrO_2–Al_2O_3	[5]
Xylose	Atmospheric pressure by dilute sulfuric	[6]
Xylose	Halides in dilute aqueous acidic	[7]
Xylose	Vanadyl pyrophosphate	[8]
Xylose	Formic acid	[9]
Pentosan	Acid hydrolysis	[10]

Table 1.3 Reaction conditions for the preparation of hydroxymethylfurfural

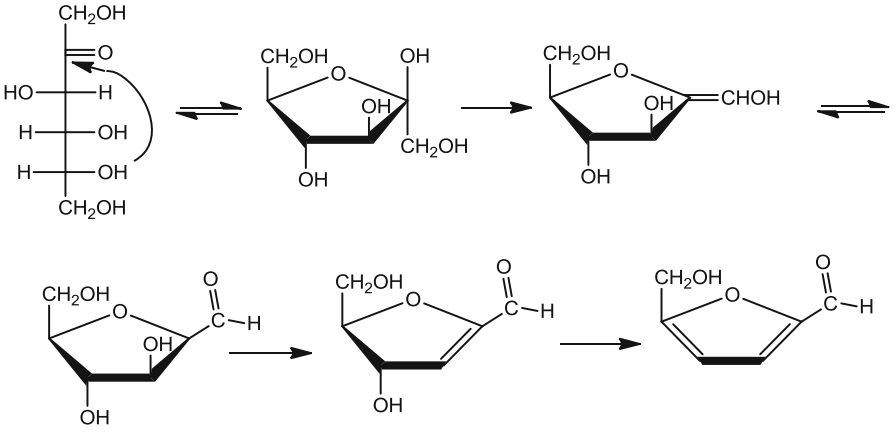
Sugar source	Catalyst	Reference
Starch-rich acorn biomass	Chromium halides	[13]
Rice straw	Single-phase and biphasic systems	[14]
High fructose	Ionic liquids	[15]
Fructose	Inorganic salt in alcohol	[16]
Fructose and sucrose	Protic ionic liquids	[17]
Fructose or glucose	Imidazolium ionic liquids with and without a catalyst	[18]
Alditols and ketohexoses	Polymer-mediated cyclodehydration	[19]
Fructose	Acidic resin-catalyzed	[20]
Glucose	Co-catalysts and solvents	[21]
Fructose	Phosphorous pentoxide in ionic liquid	[22]
Cellulose	Zinc chloride, MW	[23]
Sucrose	Ammonium halides	[24]
Fructose	Mesoporous SBA-15-SO_3H in ionic liquid BmimCl	[25]
Glucose	$SnCl_4$-tetrabutyl ammonium bromide	[26]

1.3.11 Preparation of 5-Hydroxymethylfurfural (HMF)

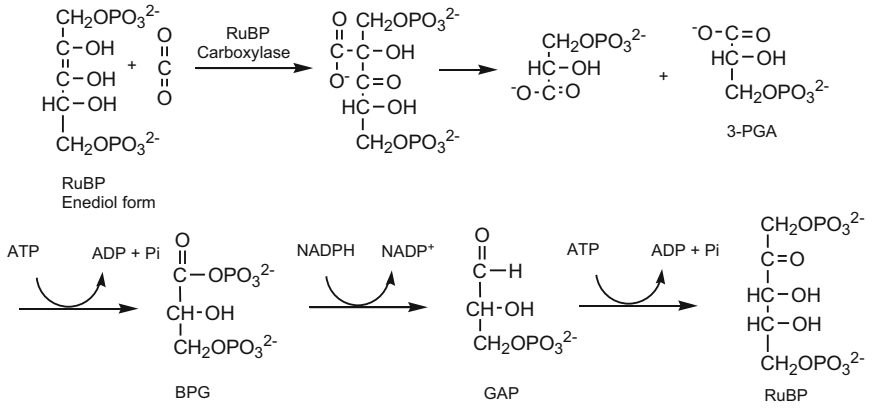
This valuable derivative is subjected to intensive studies since it can be used in the preparation of pharmaceuticals, liquid fuels, plastics, and other fine chemicals. The common sugar source is fructose and glucose, although starch, cellulose, and sucrose have been examined as a natural source for the preparation of HMF (Table 1.3) [11, 12]. The mechanism involves enol formation after the first dehydration, and two further dehydrations to furnish the furan ring (Scheme 1.19).

1.4 Biosynthesis of Sugars

Synthesis of carbohydrates in plants occurs through a mechanism of carbon dioxide fixation, and was understood through the use of long-lived radioactive isotope of carbon ^{14}C. After considerable investigations it was found that the initial CO_2 acceptor



Scheme 1.19 Conversion of xylose to furfural



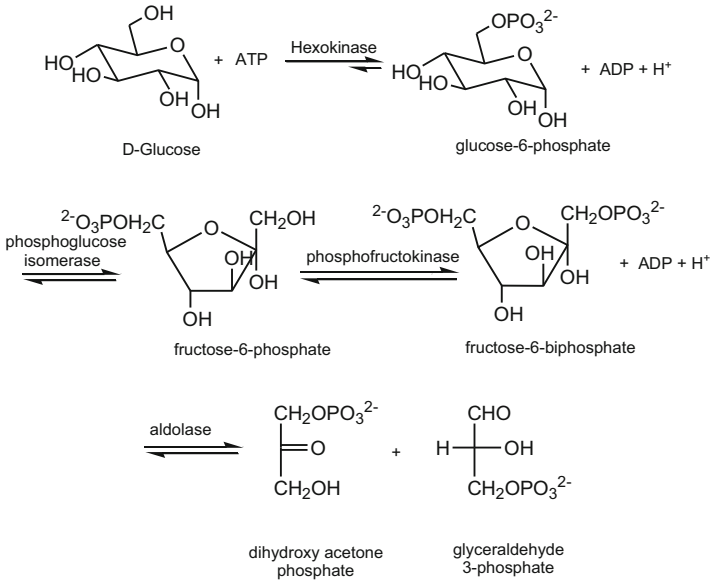
Scheme 1.20 Carbohydrate synthesis from CO_2 fixation

was the five-carbon compound ribulose 1,5-bis-phosphate (RuBP) which after incorporation of carbon dioxide produces a six-carbon molecule. The resulting molecule is fragmented into two molecules of 3-phosphoglycerate (PGA) that is one of the intermediates of glycolysis. This transformations takes place in the chloroplast by a large multisubunit enzyme, ribulose bisphosphate carboxylase "Rubisco." The following reaction sequence is cyclic and constitutes what is called the Calvin cycle which consists in formation glyceraldehyde 3-phosphate (G3P), and regeneration of RuBP. The overall process requires six CO_2 molecules fixed, 12 molecules of G3P produced which rearrange to regenerate six molecules of the five-carbon CO_2 acceptor RuBP (Scheme 1.20).

1.4.1 Sugars as Energy Sources

Metabolically the main monosaccharide useful for the production of energy is glucose. During glycolysis process glucose is enzymatically transformed and degraded to pyruvate which is membrane permeable and further introduced into the Krebs cycle.

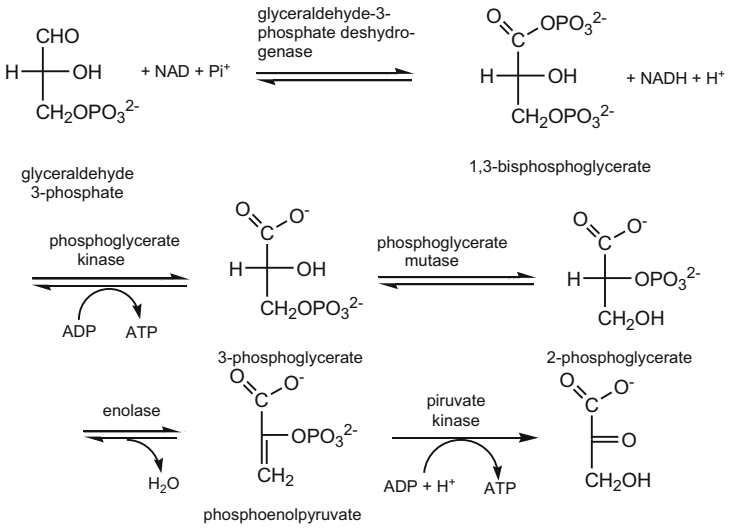
Carbohydrates are responsible of several biological events mainly related with the storage and production of energy, as metabolic intermediates and signal molecules. They are also constitutive structural units of essential biomolecules such as polysaccharides (starch, glycogen, cellulose), glycoproteins, glycolipids, and nucleotides. The process by which glucose is used as an energy source, to produce ATP and pyruvate is known as glycolysis and consists in a series of events represented in Scheme 1.21



The second cycle of glycolysis is divided into four steps.

1.5 Synthesis of Carbohydrates

The chemical synthesis of carbohydrates can be accomplished by chemical, enzymatic, or combined approach (chemoenzymatic). Their preparation by either of the mentioned methods has received considerable attention especially because they can be used as starting materials for the synthesis of biologically active carbohydrate derivatives known as mimetics or the synthesis of complex molecules such as oligosaccharides or glycopeptides.



Scheme 1.21 Glycolysis pathway

1.5.1 Chemical Synthesis

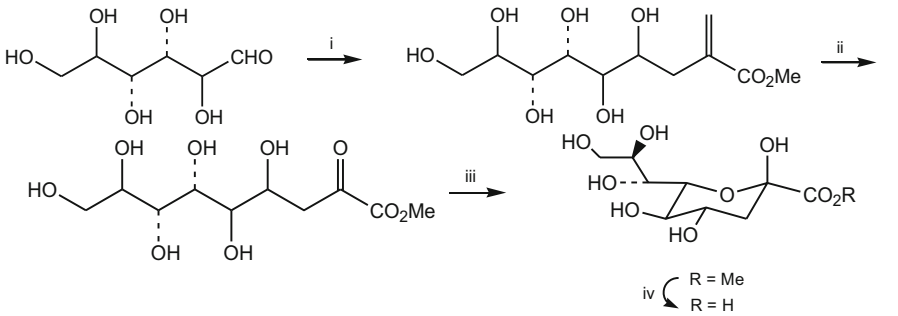
Access to potentially useful sugars or congeners can be obtained from natural sugars such as arabinose and mannose [27]. Thus, convenient routes have been implemented for the preparation of KDN from D-mannose [28], 3-deoxy-D-manno-2-octulosonic acid (KDO) from 2,3:4,5-di-*O*-isopropylidene-D-arabinose [29], D-*glycero*-D-galacto-heptose from D-arabinose [30], and KDN from D-mannose [31] (Scheme 1.22).

Different approximations for the preparation of monosaccharides from other sources have been reported. One method consists in the asymmetric synthesis of D-galactose via an iterative *syn*-glycolate aldol strategy. The general method is shown in Scheme 1.23 [32].

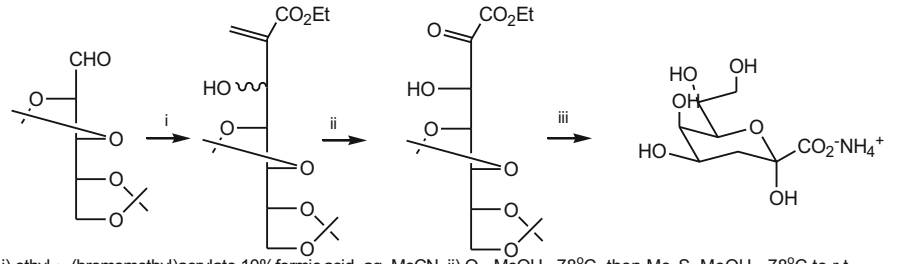
A promising and simple concept based on a two-step reaction sequence for preparing monosaccharides via the enantioselective organocatalytic direct aldol reaction of α-oxyaldehydes is recently described. The summarized sequence is illustrated in Scheme 1.24 [33].

An interesting strategy for preparing KDO and 2-deoxy-KDO from 2,3-*O*-isopropylidene-D-glyceraldehyde was reported, based on a hetero Diels–Alder reaction, followed by pyranoside ring formation. Diol formation and double inversion at C-4 and C-5 produced the target molecules (Scheme 1.25) [34].

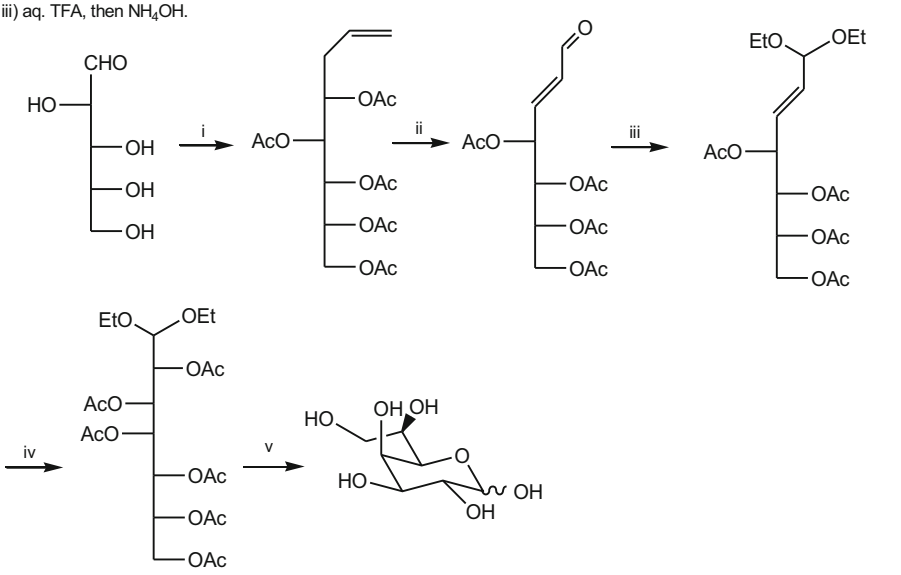
C-methylheptoses were suitable prepared from nonracemic butenolide as starting material. Asymmetric conjugate addition provided protected lactone which by methylation provided α-methyl lactone. Each of them under DIBALH treatment, produced C-methylheptoses (Scheme 1.26) [35].



i) methyl 2-(bromomethyl)acrylate, H_2O. ii) O_3, MeOH, -78°C, then Na_2SO_3. iii) spontaneous ciclization. iv) KOH, MeOH.

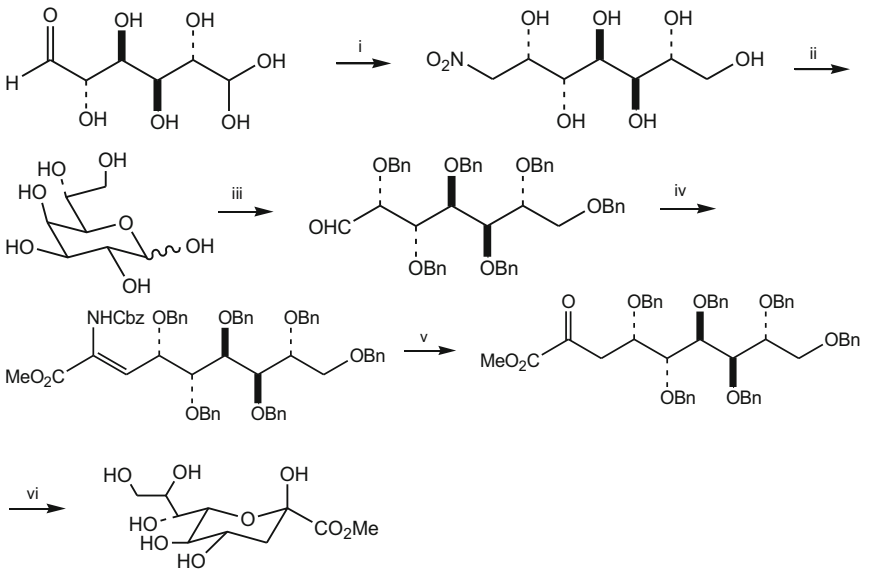


i) ethyl α-(bromomethyl)acrylate,10%formic acid, aq. MeCN. ii) O_3, MeOH, -78°C, then Me_2S, MeOH, -78°C to r.t.. iii) aq. TFA, then NH_4OH.



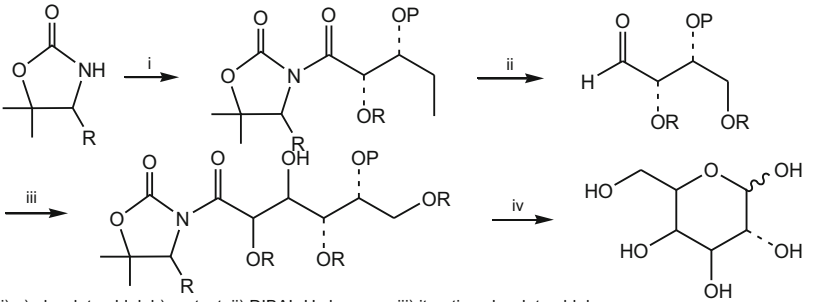
i) allyl bromide, ultrasonication, then Ac_2O, Py, DMAP. ii) OsO_4, KIO_4, then TBAF. iii) H_3O^+, $(HC(OEt)_3$. iv) OsO_4, NMO, then Ac_2O, Py, DMAP. v) NaOMe, MeOH, then H_3O^+.

Scheme 1.22 Chemical synthesis of sugar congeners from natural sugars



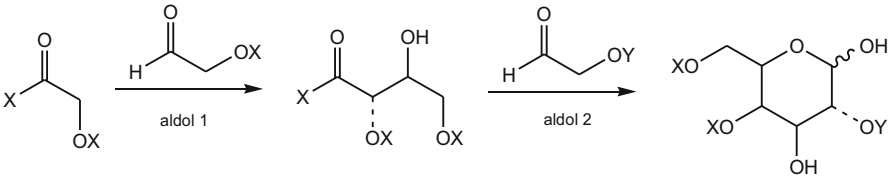
i) $MeNO_2$, DBU. ii) Nef oxidation iii) EtSH, HCl, then NaH, BnBr, DMF, then MeI, Na_2CO_3. iv) $(Et)_2P(O)CH(NHCBz)CO_2Me$, NaH, CH_2Cl_2. v) H_2, Pd-C. vi) H_2, $Pd(OH)_2$, then Dowex H^+, MeOH.

Scheme 1.22 (continued)

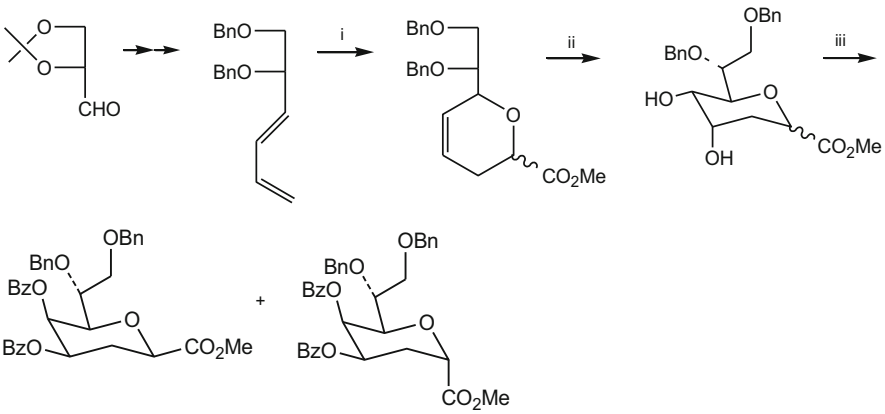


i) a) glycolate aldol. b) protect. ii) DIBAL-H cleavage. iii) iterative glycolate aldol. iv) cleavage and deprotection.

Scheme 1.23 Asymmetric synthesis of D-galactose

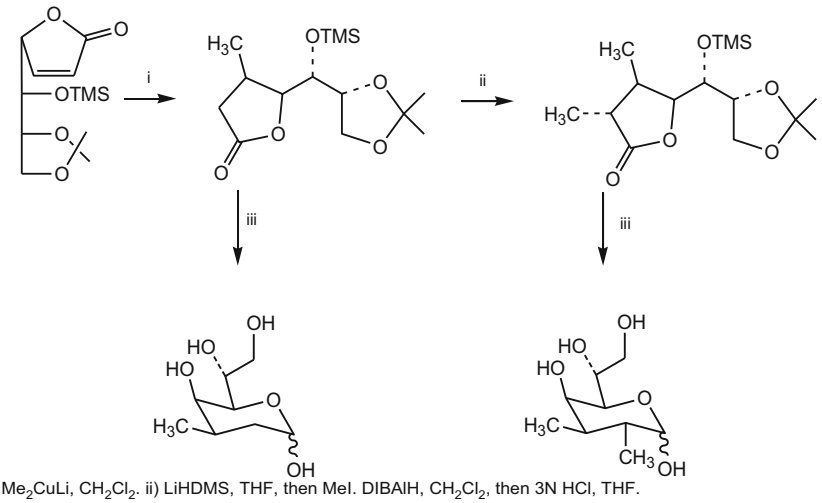


Scheme 1.24 Two-step carbohydrate synthesis



i) $HCOCO_2Bu$, 130°C, then MeOH, TsOH. ii) OsO_4, NMO. iii) Tf_2O, Py, then $PhCO_2NBu_4$.

Scheme 1.25 Synthesis of protected KDO and 2-deoxy-KDO

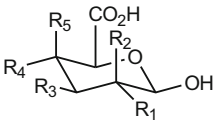


i) Me_2CuLi, CH_2Cl_2. ii) LiHDMS, THF, then MeI. DIBAlH, CH_2Cl_2, then 3N HCl, THF.

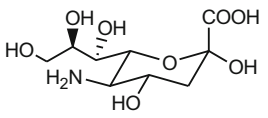
Scheme 1.26 Synthesis of methylheptoses

Naturally occurring sugar amino acids are another class of interesting modified carbohydrates found as structural components in nucleoside antibiotics. Most of them consist of *N*- and *O*-acyl derivatives of neuraminic acids, while others are found in the form of ipso-hydantoin furanosides (Scheme 1.27) [36].

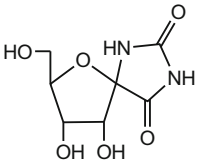
Some of these sugars amino acids have been synthesized via azide furanosides [37, 38], as it was the case for β-sugar amino acids shown in Scheme 1.28 [36].



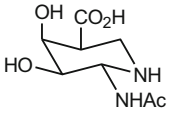
	R^1	R^2	R^3	R^4	R^5
glucosaminuronic acid	NH_2	H	OH	OH	H
galactosaminuronic acid	NH_2	H	OH	H	OH
monnosaminuronic acid	H	NH_2	OH	OH	H
4-amino-4-deoxy-glucuronic acid	OH	H	OH	NH_2	H



Neuraminic acid

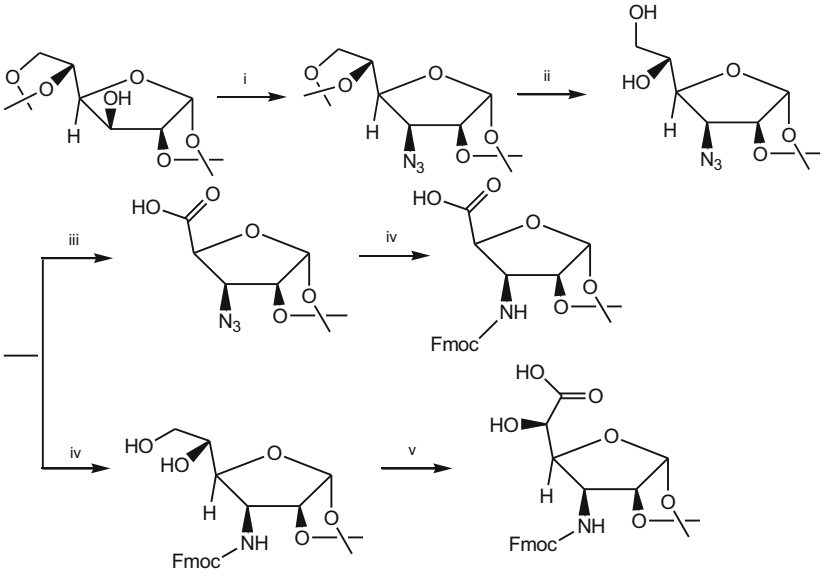


Hydantocidin



Siastatin B

Scheme 1.27 Naturally occurring sugar amino acids



i) a) Tf_2O, Py. b) NaN_3, Bu_4NCl (cat.), 69%. ii) 77% AcOH, quant. iii) a) $NaIO_4$. b) $KMnO_4$, 50% AcOH, 90%. iv) H_2, Pd/C, FmocCl, $NaHCO_3$. v) NaOCl, TEMPO (cat.), KBr, $NaHCO_3$, Bu_4NCl, 62%.

Scheme 1.28 Synthesis of protected sugar amino acids

1.5.2 C-*glycosyl Amino Acids*

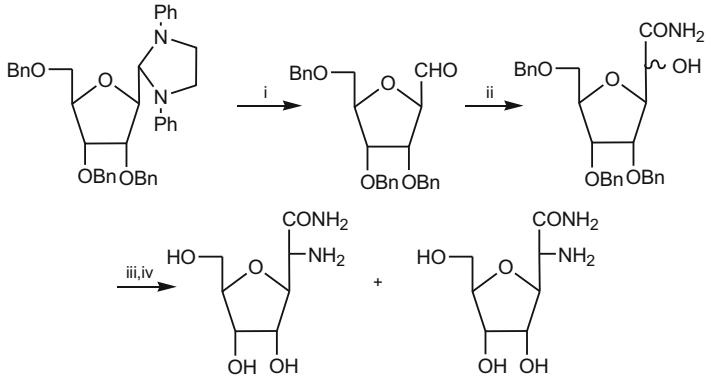
It has been mentioned that natural glycopeptides are classified into *O*-glycopeptides when the sugar residue establishes an *O*-glycosyl linkage with l-serine or l-threonine and *N*-glycopeptides if the linkage is with asparagine. There has been an increasing interest for preparing unnatural *C*-glyco amino acids as a potential building block in the assembly of modified glycopeptides that may serve in preparing therapeutically useful mimetics, displaying higher resistance to hydrolytic enzymes and also superior properties of the natural ones.

A recent review describes methods for the preparation of C-glycosyl glycines, alanines, serines, asparagines, tyrosines, and tryptophans [39].

For instance the synthesis of ribofuranosyl glycine was described under Strecker conditions, starting from 2-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)-1,3-diphenylimidazolidine which was after hydrolysis tosylated and reacted with cyanide and peroxide to give the α-hydroxy amide as a racemic mixture. The anomers were separated as *O*-mesyl derivatives which were transformed to azides and further reduced to the corresponding ribofuranosyl glycines (Scheme 1.29) [40].

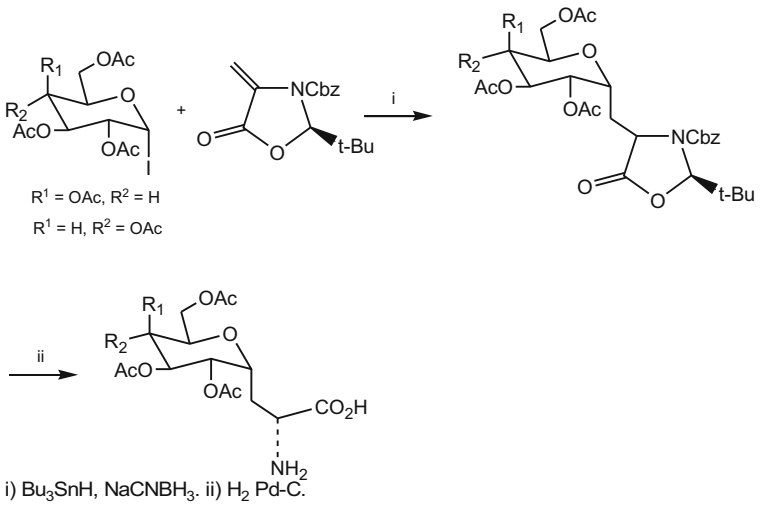
A method reported for the preparation of *C*-glycosyl alanines involves the use of (R)-methyleneoxazolidinone which was linked to the peracetylated iodosugars under promoted radical additions. The α-linked *C*-glycoside was subjected to hydrogenolysis to give α-D-galactosyl D-alanine and the α-D-glucosyl isomer (Scheme 1.30) [41].

C-analogs of glycosyl serines have been prepared by a number of methods and among them Strecker, Wittig, and Sharpless asymmetric aminohydroxylation reactions [42]. One of them describes their synthesis via coupling of anomeric pyridyl sulfone with an electrophiles center under samarium catalysis. The resulting C-glycosylation proceeds with α-selectivity (3.3:1). Final deprotection produced the C-glycosyl serine analog in good yield (Scheme 1.31) [43].

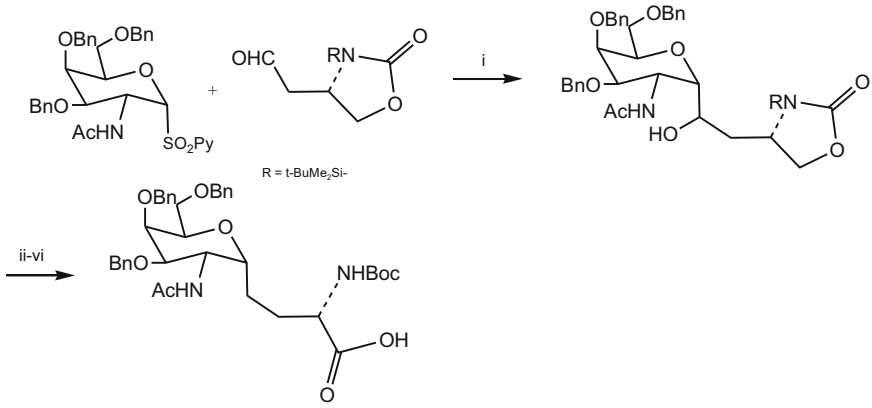


i) TsOH. ii) a) NaCN, K_2CO_3, H_2O. b) H_2O_2. 91%. iii) a) MsCl. b) LiN_3. iv) a) aq. HCl. b) H_2, Pd/C.

Scheme 1.29 Synthesis of anomeric ribofuranosyl glycines

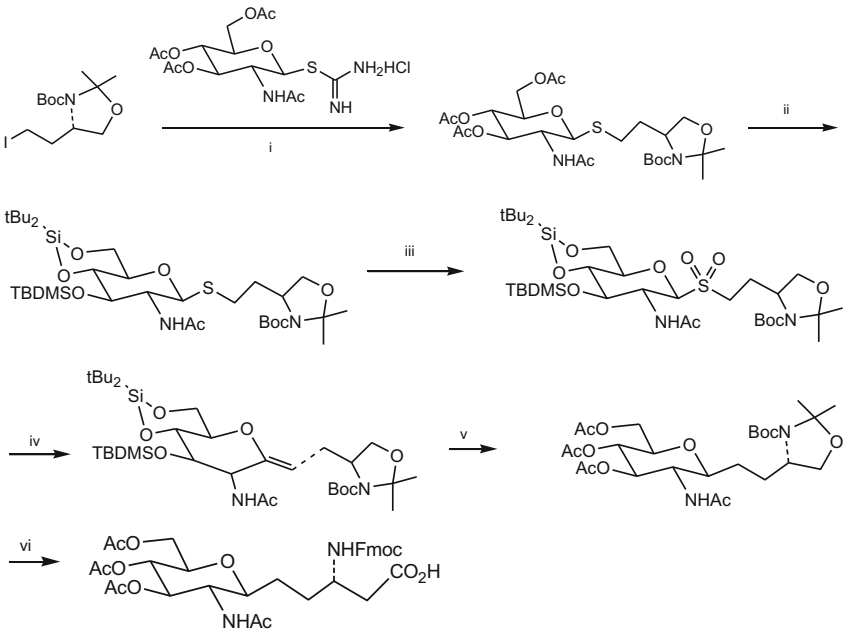


Scheme 1.30 Synthesis of α-D-galactosyl D-alanine and the α-D-glucosyl isomers



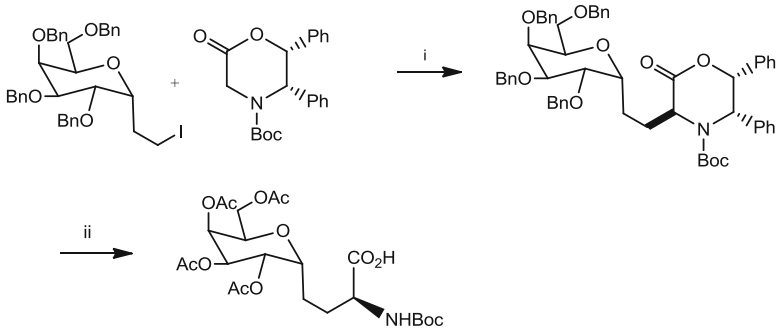
Scheme 1.31 Synthesis of C-glycosyl serine analog

More recently, the stereoselective synthesis of a *C*-glycoside analog N-fmoc-serine β-*N*-acetylglucosaminide has been described employing the Ramberg–Bäcklund (RB) rearrangement. This procedure involves the coupling reaction between isothiourea and protected iodide to produce thioglycoside in good yield. Oxidation to the sulfone was followed by the RB conditions KOH/Al_2O_3 in tBuOH/$(CBrF_2)_2$ at 50 °C providing the exoglycal derivative. The final step which involves hydrogenolysis, deprotection, and oxidation provided the desired C-glycosyl analog (Scheme 1.32) [44].



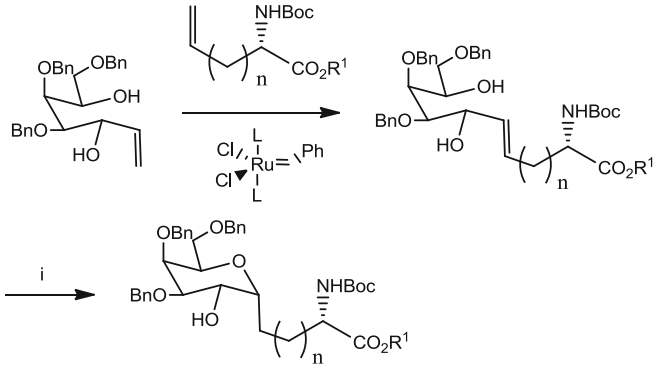
i) K_2CO_3, $Na_2S_2O_5$/acetone-H_2O, 98%. ii) a) Et_3N/MeOH-H_2O. b) $tBu_2Si(OTf)_2$, 2,6-lutidine/DMF, 88%. iii) mCPBA, Na_2HPO_4/CH_2Cl_2, 77%. iv) KOH/Al_2O_3, $CBrF_2CBrF_2$/tBuOH, 50ºC, 38%.
v) a) H_2, $Pd(OH)_2$/EtOAc, 78%. b) TBAF/THF. c) Ac_2O/Py, 68% (two steps). vi) a) TFA/$CHCl_3$.
b) FmocCl/*i*PrNEt/CH_2Cl_2-MeOH, 69% (two steps). c) Jones oxidation/acetone, 77%.

Scheme 1.32 Synthesis of C-glycoside serine analog by Ramberg–Bäcklund rearrangement



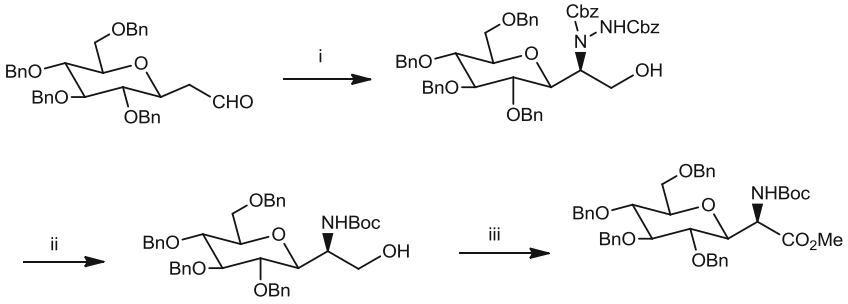
i) LiHMDS, THF, HMPA. ii) a) Li, NH_3 b) CH_2N_2. c) Ac_2O, Py

Scheme 1.33 Synthesis of C-glycosyl amino acids via oxazinone intermediate



i) a) $Hg(OTFA)_2$, aq KCl. b) BEt_3, $NaBH_4$

Scheme 1.34 Synthesis of C-glycosyl amino acids via cross-metathesis/cyclization strategy



i) L-proline, DBAD, CH_3CN, 0°C. ii) $NaBH_4$, CH_3CN, 0°C. ii) a) H_2, Ni-Raney MeOH-HOAc, rt. b) Boc_2O-$NaHCO_3$, dioxane, rt. iii) Jones, acetone 0°C. CH_2N_2, Et_2O, 0°C

Scheme 1.35 Synthesis of C-glycosyl amino acids from C-glycosylalkyl aldehydes

Alternatively C-glycosyl amino acids can be prepared by coupling reaction between α-Gal iodide and oxazinone under basic medium to provide the C-glycoside heterocycle which was finally deprotected to provide the C-linked D-glucopyranosyl and D-galactopyranosyl L-serines in 70 % yield (Scheme 1.33) [45].

Also the successful cross-metathesis/cyclization strategy has been implemented for preparing C-glycosyl amino acids, by using gluco-heptenitol with partner, allyl glycine in the presence of Grubbs catalyst (Scheme 1.34) [46].

A protocol based on α-amination of C-glycosylalkyl aldehydes leading to axially and equatorially linked C-glycosyl α-amino acids (glycines, alanines, and CH_2-serine isosteres) with either S or R is introduced via hydrazino alcohol intermediates which are subjected to hydrogenolysis and Jones oxidation to provide the desired C-glycosyl amino acids (Scheme 1.35) [47].

1.5.3 Enzymatic Synthesis

The enzymatic synthesis of monosaccharides and carbohydrates mimetics by enzyme catalysts is performed mainly by a group of lyases known as aldolases. This enzymes effects the conversion of hexoses from their three-carbon components via an aldol condensation [48]. There are over 30 aldolases identified and isolated, and classified into two types depending on the mechanism involved: Aldolase type 1 and type 2 which is Zn-dependent. The general reaction that they catalyze is the stereospecific addition of a ketone donor to an aldehyde acceptor (Scheme 1.36).

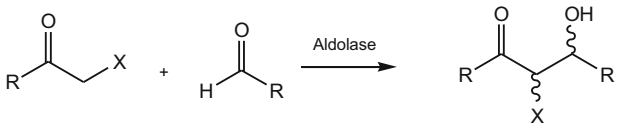
The aldolases used for synthetic purposes are classified into five groups depending on the ketone donor and the products formed:

Dihydroxyacetone phosphate (DHAP) aldolase
Pyruvate aldolase
2-deoxyribose 5-phosphate aldolase
Glycine aldolase.
Other aldolases

Examples of each of them are indicated in figure:

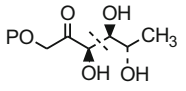
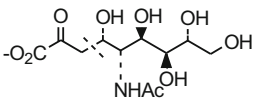
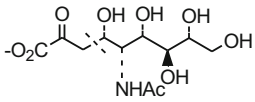
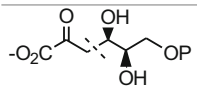
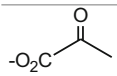
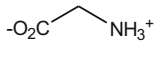
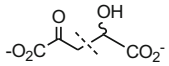
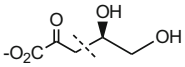
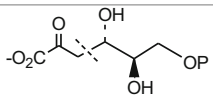
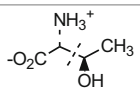
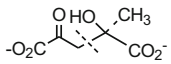
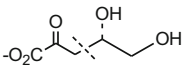
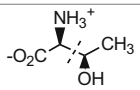
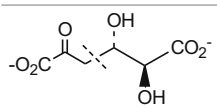
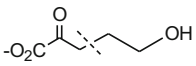
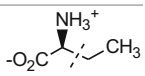
Aldolases have been also very useful for the preparation of a variety of common and uncommon monosaccharides. Fructose-1,6-diphosphate (FDP) aldolase effects the conversion of dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (G3P) to D-fructose-1,6-diphosphate (FDP). Table 1.4 summarizes the natural substrates, de donors and the products obtained through this reaction. Broken lines indicate the bond formed or broken [49].

DHAP aldolases catalyze the reversible asymmetrical aldol condensation of DHAP to L-lactaldehyde or D-glyceraldehyde 3-phosphate (G3P). There are four types of DHAP aldolases which are classified based on the condensation product formed: D-fructose 1,6-diphosphate (D-FDP) aldolase, which condenses DHP with G3P; D-tagatose 1,6-diphosphate (TDP) which utilizes the same substrates; fuculose 1-phosphate, catalyzing the condensation reaction between DHAP and L-lactaldehyde to produce L-fucolose 1-phosphate; and L-rhamnulose 1-phosphate aldolase which recognizes the same substrates to produce L-rhamnulose 1-phosphate (Scheme 1.37) [49].



Scheme 1.36 General scheme of enzymatic-mediated aldol condensation

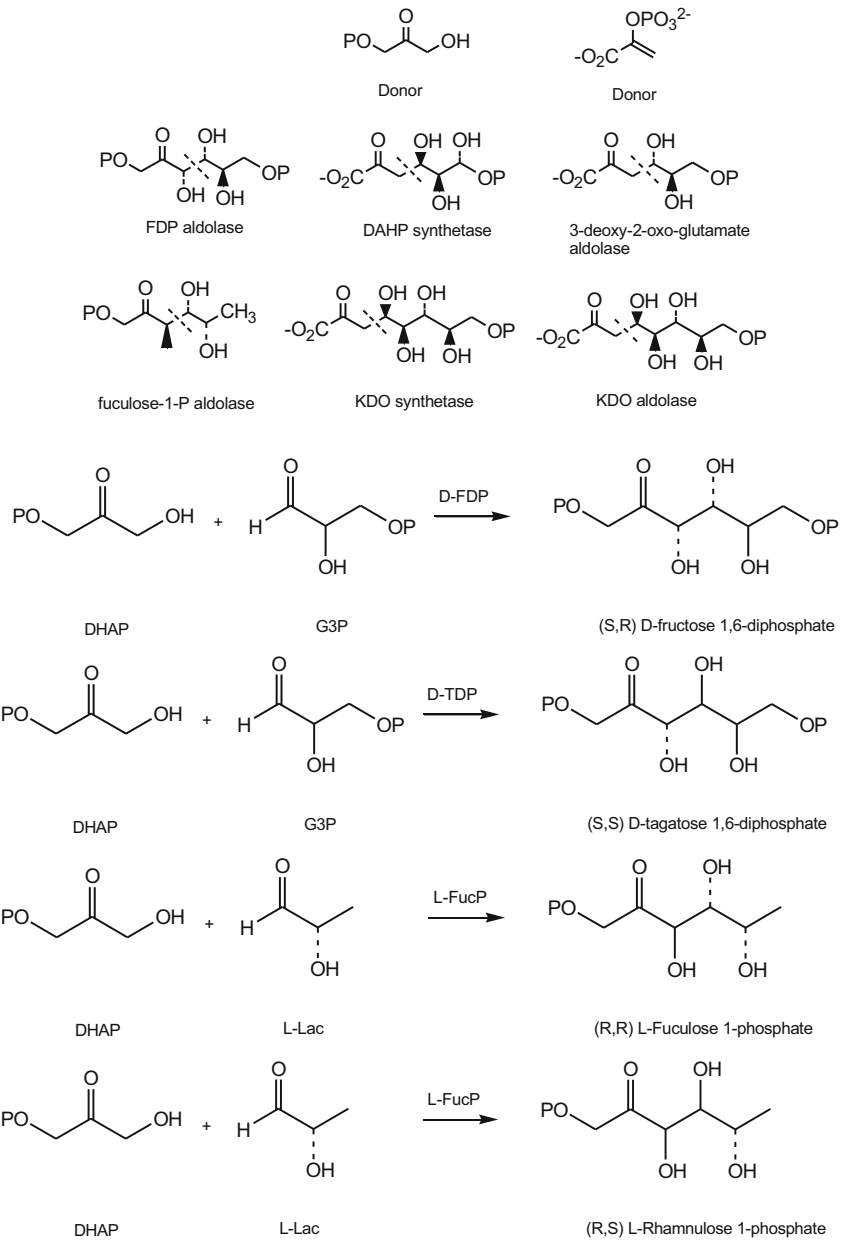
Table 1.4 Natural substrates for aldolases

 rhamnulose-1-Paldolase	 sialic acid synthetase	 sialic acid aldolase	
 3-deoxy-2-oxo-6-P-galactonate aldolase			
 Donor		 Donor	 Donor
 4-hydroxy-2-oxo-glutarate aldolase	 3-deoxy-2-oxo-L-arabinoate aldolase	 2-deoxyribose-5-P-aldolase	 D-Thr aldolase
 4-hydroxy-4-methyl-2-oxoglutarate aldolase	 3-deoxy-2-oxo-D-pentanoate aldolase		 L-Thr aldolase
 3-deoxy-2-oxo-D-glucarate aldolase	 hydroxybutyrate aldolase		 Ser-hydroxymethyl transferase

Likewise, DHAP-dependent aldolases are involved in the incorporation of dihydroxyacetone phosphate (DHAP) on pentose and hexose phosphate introducing consequently three carbons and two chiral centers (Scheme 1.38) [50].

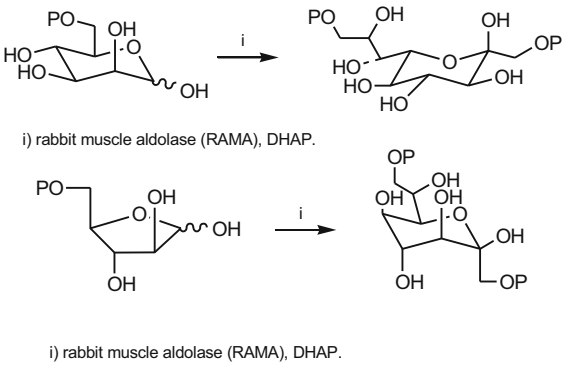
Another enzymatic aldol type reaction takes place on *N*-acetylneuraminic acid also known as sialic acid which after a reversible aldol reaction of *N*-acetyl-D-mannosamine and pyruvate produces *N*-acetyl-5-amino-3,5-dideoxy-D-glycero-D--galacto-2-nonulosonic acid (NeuAc) (Scheme 1.39) [51].

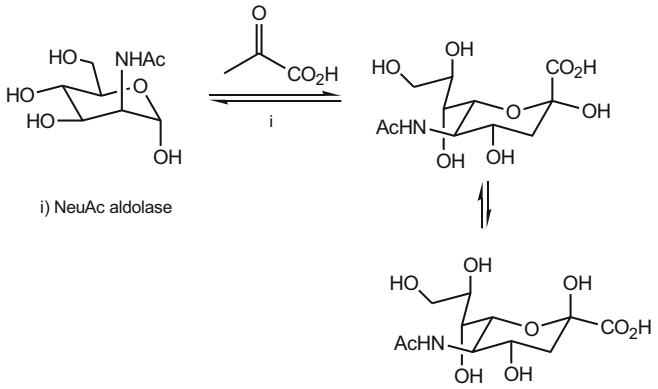
Ketoses can be transformed to aldoses through the use of isomerases [52]. In this way glucose derivatives can be obtained from fructose as shown in Scheme 1.40 [53].



Scheme 1.37 DHAP-dependent aldolases

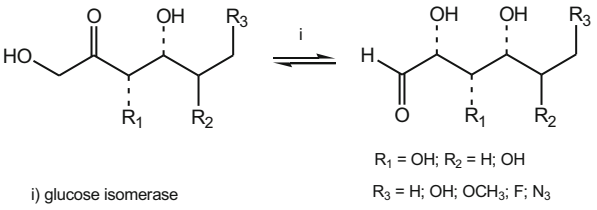
Scheme 1.38 Enzymatic preparation of pentose and hexose phosphate





Scheme 1.39 Enzymatic preparation of sialic acid analogs

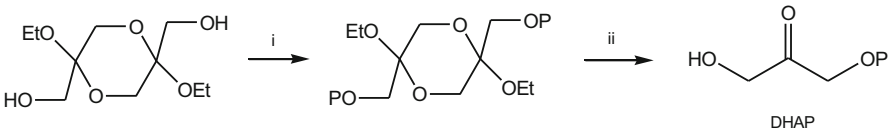
Scheme 1.40 Enzymatic isomerization of fructose to glucose derivatives



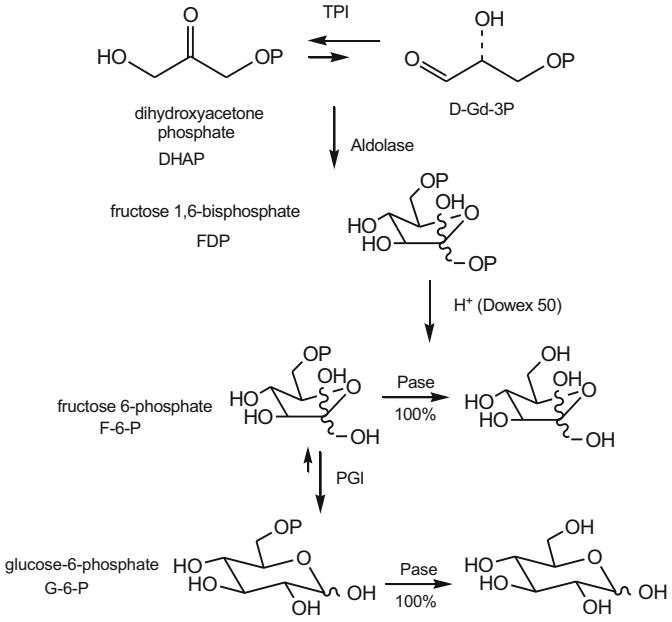
1.5.4 *Chemoenzymatic Synthesis*

The chemoenzymatic approach is a combination of the chemical and the enzymatic methodologies and intends to explode the versatility and availability of the chemical reagents with the high stereoselectivity and regioselectivity of the enzymes when they act as catalysts.

For instance the enzymatic synthesis of dihydroxyacetone phosphate (DHAP) is too expensive on large scale, and therefore the combined approach becomes the best choice. The reported procedure consist in the phosphorylation of dihydroxyacetone dimer with $(PhO)_2POCl$ followed by hydrolysis of the dimer to generate dihydroxyacetone phosphate in 61 % yield [54]. The chemically prepared DHAP is then used as an important material for the synthesis of natural monosaccharides and carbohydrates mimetics (Scheme 1.41).



i) a) $(PhO)_2POCl$. b) H_2/PtO_2. ii) H_2O, 65°C.



Scheme 1.41 Chemoenzymatic preparation of glucose

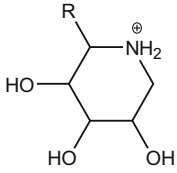
1.6 Synthesis of Carbohydrates Mimetics

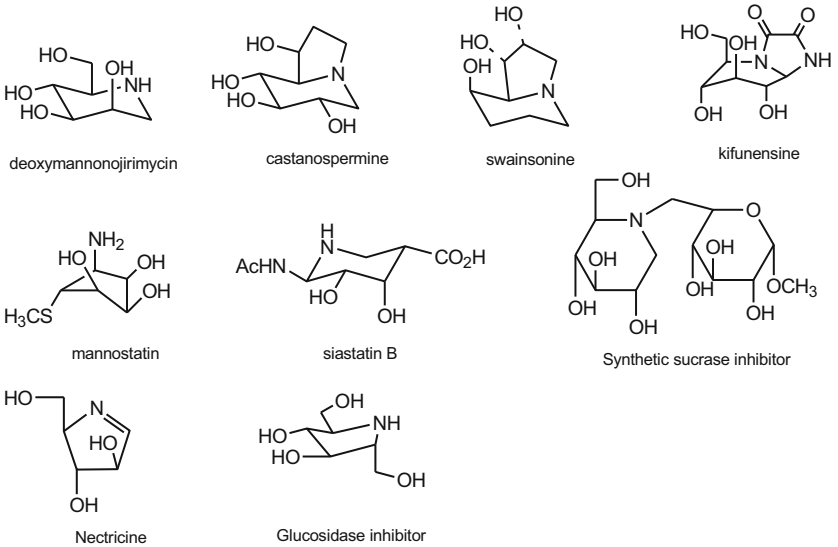
1.6.1 Iminosugars

This class of isosteric sugars also recognized as aza sugars has been the subject of intense study because their significant activity as α-glycosidase inhibitor, which is a promising strategy in the treatment against diabetes mellitus type II and other glycosidase associated disorders. It is believed that the mechanism for glycosidase inhibition and to some extend for glycosyltransferases involves the binding of the aza sugars to the active site by charge–charge and hydrogen bond interactions [55]. A significant variety and diversity of naturally occurring and synthetic aza sugars with glycosidase and glycosyltransferase inhibition activity have been reported [56–59]. The common feature of these derivatives is the replacement by chemical or enzymatic methods of the cyclic oxygen by a nitrogen atom. The representative example is known as deoxynojirimycin (Scheme 1.42) which has shown strong inhibition against a variety of α-glycosidases.

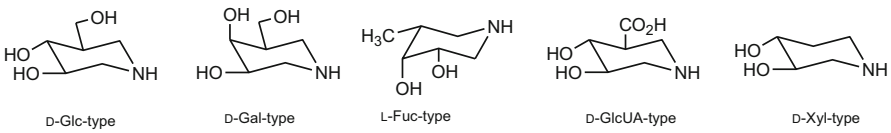
Representative examples of natural and synthetic aminoglycosides implicated in inflammation, metastasis, and blocking infection processes are depicted in Scheme 1.43.

Scheme 1.42 Iminosugar deoxynojirimycin

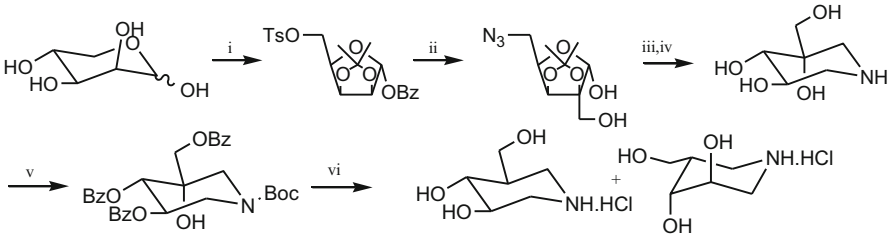




Scheme 1.43 Representative aminosaccharides



Scheme 1.44 Series of 1-*N*-iminosugars



Scheme 1.45 Synthesis of 1-*N* iminosugars

A series of 1-*N*-iminosugars including D-glucose-type, D-galactose-type, L-fucose-type, D-glucuronic acid-type, and D-xylose-type was synthesized and evaluated as glycosidase inhibitors (Scheme 1.44).

A general procedure for the preparation of 1-*N*-iminosugars consisted in the azido substitution of a 5-tosyl-1-*O*-benzoate, followed by aldol reaction, Pearlman hydrogenation, and cyclization (Scheme 1.45) [60].

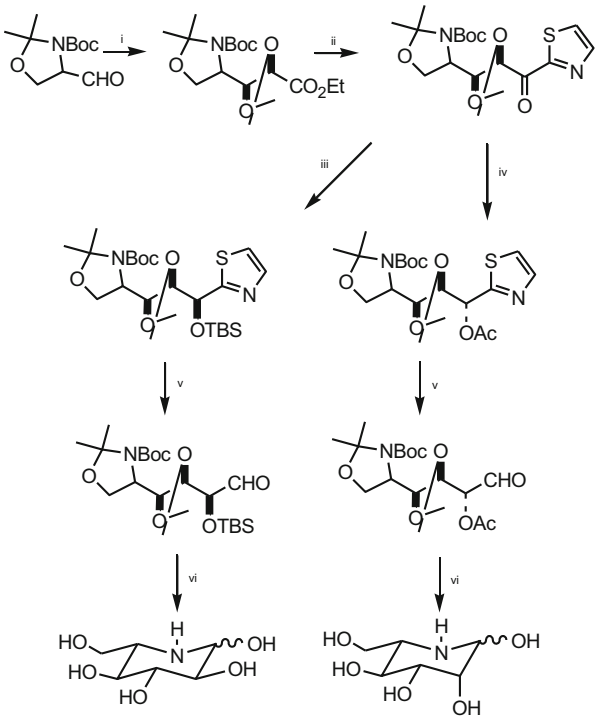
Another chemical approach described for the preparation of iminosugars consisted in the use of protected L-serine which was subjected to Wittig elongation, diol formation, and 2-lithiothiazole treatment, to produce a common thiazole derivative. This intermediate under the appropriate conditions will give rise to L-(−)-nojirimycin or L-(−)-mannonojirimycin (Scheme 1.46) [61].

Chemoenzymatic preparation of glycosidase inhibitors deoxynojirimycin and deoxymannojirimycin was described by using RAMA-aldolase for the aldol condensation and hydrogenolysis for azide reduction and ring formation (Scheme 1.47) [62].

Significant achievements have been made for the synthesis of aza sugars based on aldolase reactions particularly fructose-1,6-diphosphate [30], 2-deoxyribose-5-phosphate [63], fuculose-1-phosphate [64], sialic acid aldolase, and Pd/C-mediated reductive amination (Scheme 1.48).

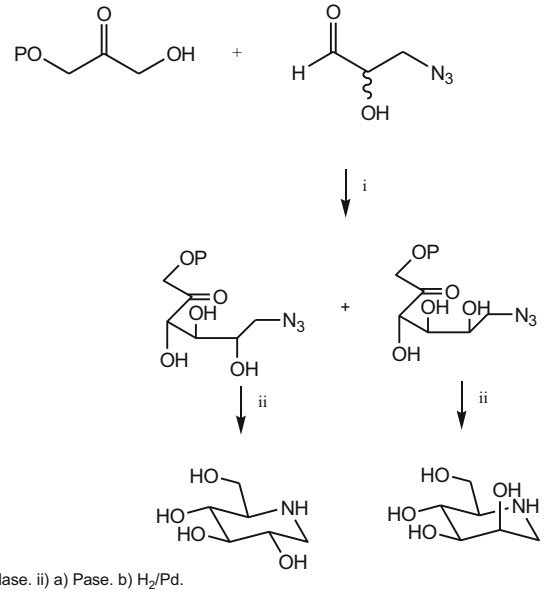
1.6.2 Amino Sugars

Amino sugars are another class of naturally and non-naturally sugars which might be considered distinct from the previous class in that the nitrogen is exocyclic. Their significance is clearly seen in a family of aminoglycoside antibiotics such as



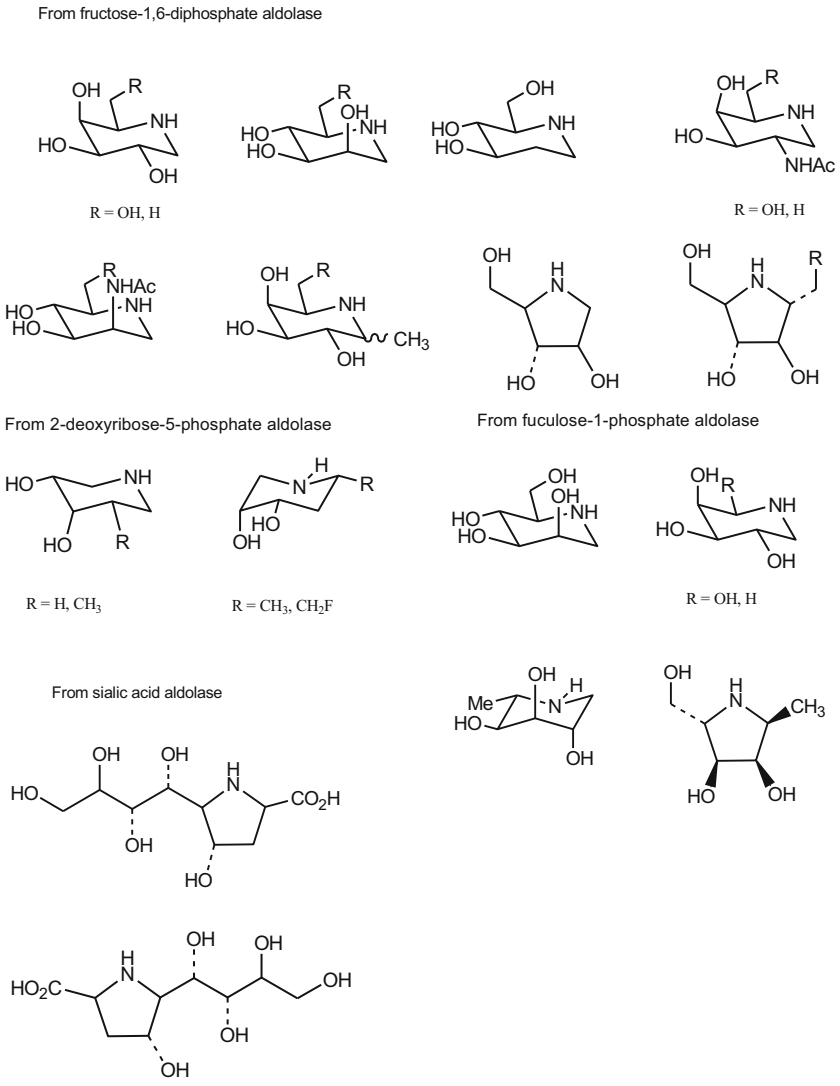
i) a) Ph_3PCHCO_2Et, b) OsO_4, NMO, c) DMP, TsOH. ii) 2-lithiothiazole, Et_2O iii) a) $NaBH_4$. b) TBSCl, imidazole iv) a) Red-Al, toluene, b) Ac_2O, Py, DMAP. v) a) MeI, MeCN. b) $NaBH_4$ c) $HgCl_2$, MeCN, H_2O. vi) TFA, H_2O.

Scheme 1.46 Chemical synthesis of L-(−)-nojirimycin and L-(−)mannonojirimycin



i) FDP aldolase. ii) a) Pase. b) H_2/Pd.

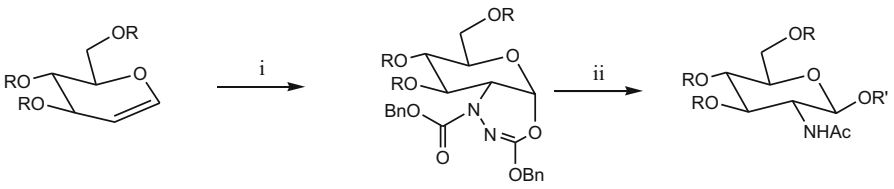
Scheme 1.47 Chemoenzymatic synthesis of iminosugar



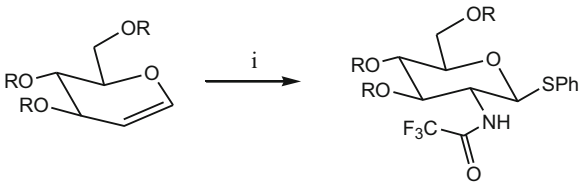
Scheme 1.48 Aza sugars prepared by aldolase reactions

neomycin, kanamycin which are widely used against both gram-positive and gram-negative bacteria. Although there is no unified protocol for the synthesis of amino sugars, they have been roughly classified into (a) non-azido (Scheme 1.49) and (b) azido approaches (Scheme 1.50) [65].

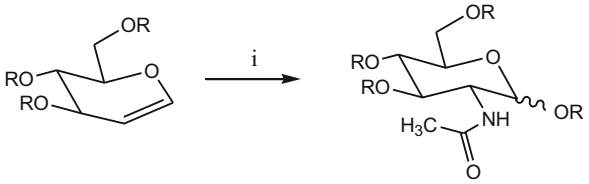
(a) The non-azido methodologies usually involves the introduction of an amino group at C-2, and glycals are usually the starting materials.
(b) The azido approach is a more common procedure for amino introduction on sugars due to its relative stability, good solubility in organic media, and easy



i) $BnO_2C-N{=}N-CO_2Bn$, hv. ii) a) R'OH, Lewis acid b) Raney Ni. 3) Ac_2O, Py
ref. [66]



i) (saltmen)Mn(N)$(CF_3CO)_2O$. ii) PhSH/BF_3-OEt_2



i) a) thianthrene -5-oxide, $AcNHSiMe_3$, Tf_2O, Et_2NPh. b) Amberlyst -15, HOR.

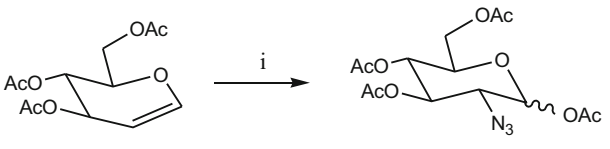
Scheme 1.49 Non-azido methods for the preparation of aminosaccharides [66–68]

conversion to amines through catalytic hydrogenolysis. Some of the methods reported involve the use of glycals, or protected saccharides containing free primary or secondary alcohols.

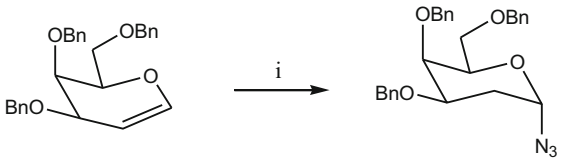
Epimerization of hydroxyl groups can be achieved by following an oxidation–reduction sequence in which a secondary alcohol is converted into a keto group, followed by stereoselective hydride reduction and nucleophilic substitution. It has been observed that epimerization by following the Mitsunobu protocol has not been satisfactory due to steric hindrance of the secondary hydroxyl groups on the pyranose ring [65].

1.6.3 Thiopyranoside Monosaccharides

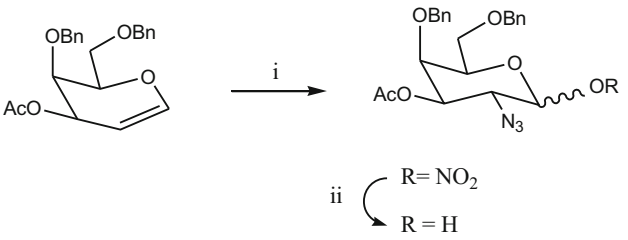
Thiosugars are another class of interesting carbohydrate mimetics. The synthesis of these derivatives can be achieved by using aldolases RAMA for the aldol condensation reaction. The following reaction sequence was used successfully for the preparation of deoxygluco, manno, galacto, and altropyranosides (Scheme 1.51) [75].



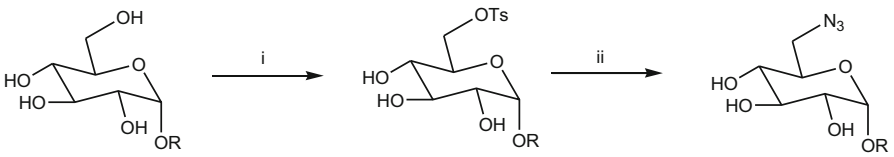
i) ClN_3, CH_2Cl_2 $(PhCO_2)_2$



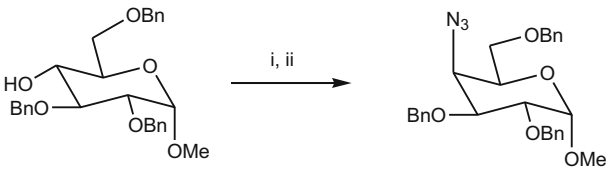
i) Me_3SiN_3, Me_3SiONO_2



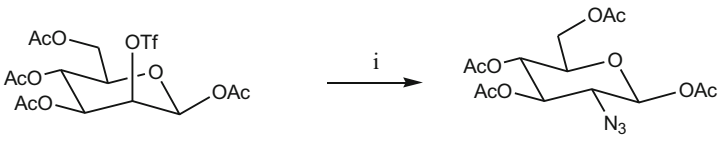
i) CAN, NaN_3, CH_3CN, -15°C, 45%. PhSH, DIEA, 91%.



i) TsCl, Py, r.t. ii) NaN_3, DMF

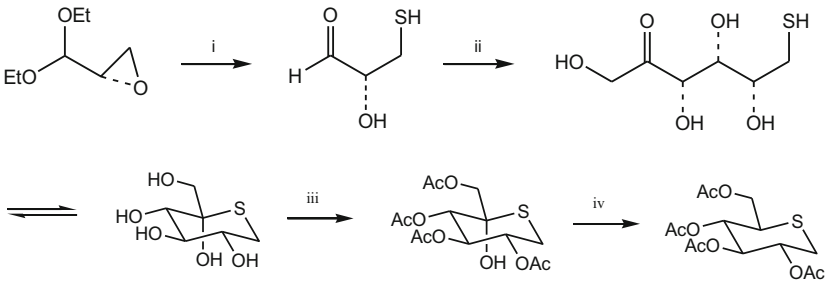


i) Tf_2O, Py/ CH_2Cl_2. ii) NaN_3, DMF



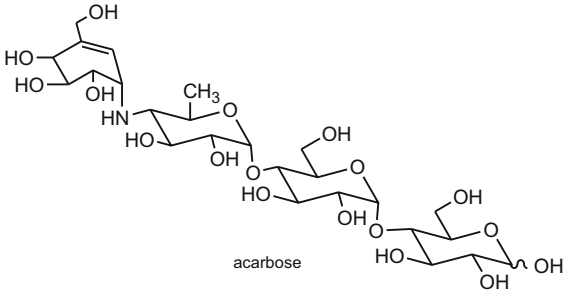
i) NaN_3, DMF

Scheme 1.50 Azido methods for the preparation of aminosaccharides [1, 69–74]



i) a) AcSK, AcSH. b) HCl. ii) a) DHAP FDP A. b) Pase. iii) Ac_2O, Py. iv) Et_3SiH, $BF_3.OEt_2$.

Scheme 1.51 Synthesis of thiomonosaccharides



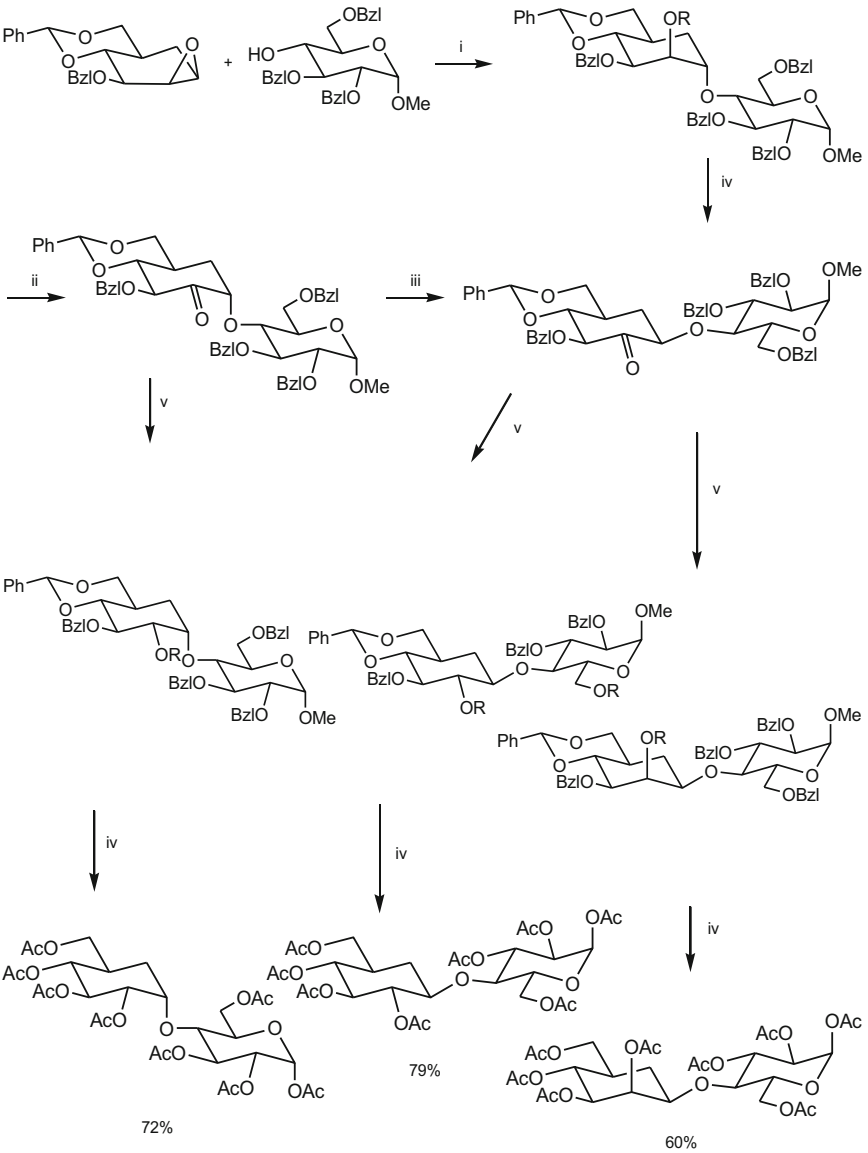
Scheme 1.52 Pseudotetrasaccharide acarbose

Another strategy for the synthesis of thiosugars involves the replacement of one of the oxygen atoms at the anomeric carbon of the glycoside by a sulfur atom leading to two distinctly different thiosugars, namely a 5-thioglycoside and a 1-thioglycoside [76].

1.6.4 Carbapyranoside-Saccharides

More recently this type of sugar mimics have received increasing attention since some of them present α-glucosidase activity and therefore considered for therapies for non-insulin dependent diabetes mellitus. Also they have been found to be active as agricultural antibiotics, and because of their recognition by glycosidases and glycosyltransferases as substrates and stability against enzymatic degradation, they have been used also to study oligosaccharide-chain biosynthesis [77, 78, 181]. The pseudotetrasaccharide Acarbose (Scheme 1.52) has been the first α-glucosidase inhibitor to be explored in humans as an antidiabetic agent along with the amino sugar 1-deoxynojirimycin Miglitol.

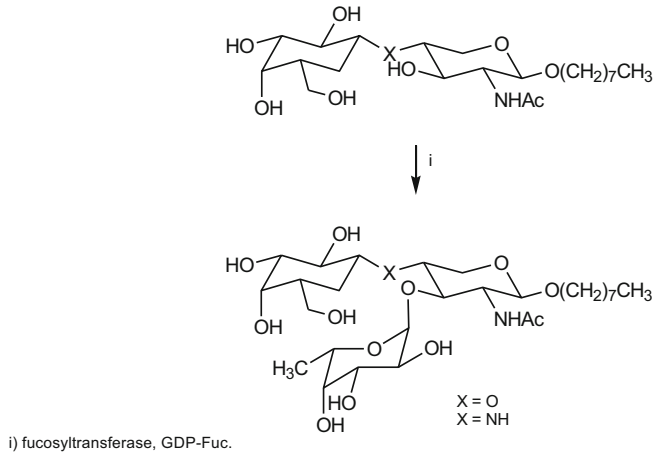
The chemical synthesis of carbamaltose, carbacellobiose, and related carbadisaccharides of biological interest according to the pathway indicated in Scheme 1.53 has been described [79].



i) DMF, NaH, 15-crown-5 ether, 50°C, 60% ii) DMSO, Ac_2O, r.t., 72% iii) DBU, PhCH3, 70°C, 56%. iv) a) $FeCl_3$, Ac_2O, -20°C b) H_2, Pd/C, EtOH. c) Ac_2O, Py. v) $NaBH_4$, CH_2Cl_2/MeOH, O°C.

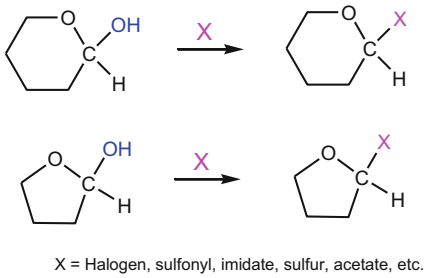
Scheme 1.53 Synthesis of carbapyranoside-disaccharides

Pseudo-*N*-acetyllactosaminides were found to be acceptors substrates for human-milk α-(1 → 3/4)-fucosyltransferase. A small scale reaction of the mentioned pseudodisaccharides with GDF-fucose resulted in conversion to pseudotrisaccharides (Scheme 1.54) [77].



Scheme 1.54 Chemoenzymatic synthesis of pseudotrisaccharides

Scheme 1.55 Anomeric carbon and activation to a good leaving group

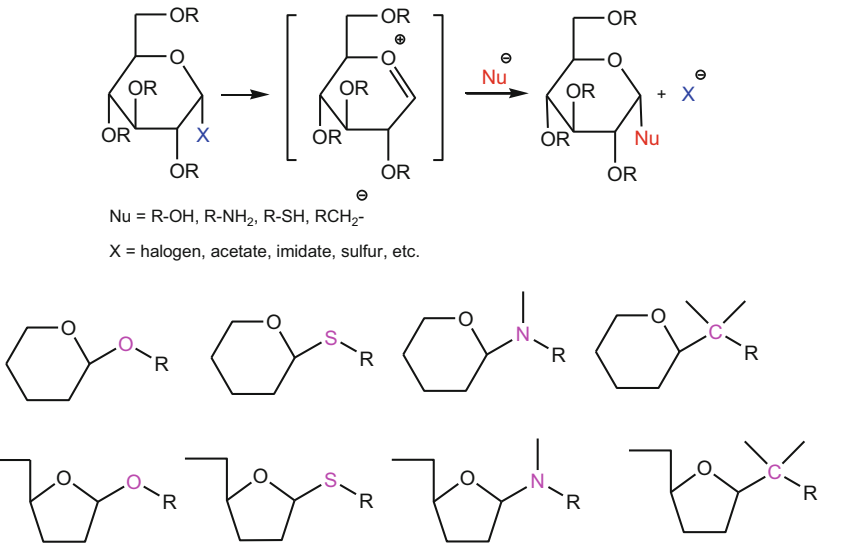


1.7 Glycoside Reactivity

The reactivity for the anomeric carbon C(1) is the typical for acetals and therefore the nucleophilic addition may occur. On the other hand, the other hydroxyl groups behave typically for alcohols. For coupling reaction with sugars the anomeric carbon is involved to produce a glycosidic bond, and usually must be activated with a good leaving group in order to form a new linkage (Scheme 1.55).

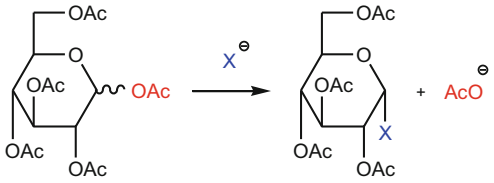
A glycoside is formed when the anomeric carbon of a sugar is connected through an heteroatom (except with *C*-glycosides) with an aliphatic or aromatic fragment known as aglycon.

The glycosidic bond is formed when a nucleophile (alcohol, amine, thiol or carbanion) substitutes the hydroxyl group at the anomeric position, which has been previously substituted by a good leaving group. Therefore when the nucleophiles are an alcohol, amine or carbanion, *O*-, *N*-, or *C*-glycosides are generated as result, as can be observed in Scheme 1.56.



Scheme 1.56 Nucleophile displacement on the anomeric carbon and general types of glycosides

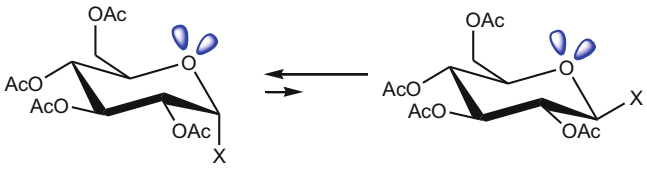
Scheme 1.57 Stereoselectivity of halides at the anomeric position



1.8 The Leaving Groups

As mentioned above, the anomeric hydroxyl group can be replaced under suitable conditions with a good leaving group. Initially, the use of halogens such as fluorine, chlorine, and bromine is the strategy of choice, and particularly the latter since it presents the best balance between reactivity and stability and this is why it has been extensively used for preparing glycosides. However, halides are in most cases labile and undergo decomposition. Consequently a number of other leaving groups have been designed for glycoside chemistry, and among them, imidates, sulfur, sulfonates, silyl groups, phosphates, and acetates are equally important alternatives. The use of iodide has been restricted due to its low reactivity and fluoride although limitedly has been more used for preparation of some α-glycosides [80, 81]. It has been found that in the absence of selective conditions, a leaving group can be found as a mixture of anomers, as in the case of the acetates. However, some others such as bromide and imidate can be introduced preferentially at the α-position (Scheme 1.57).

A well accepted hypothesis that explains the α-stereoselective preference assumed by the leaving group (halogens and imidate) is based on the anomeric



Scheme 1.58 Anomeric effect on halogens

effect, consisting in the electronic effect produced by the ring oxygen which gives rise to a repulsive effect between one of the oxygen lone pairs and the leaving group, forcing the latter to assume such a position [82] (Scheme 1.58).

1.9 Glycosyl Donors

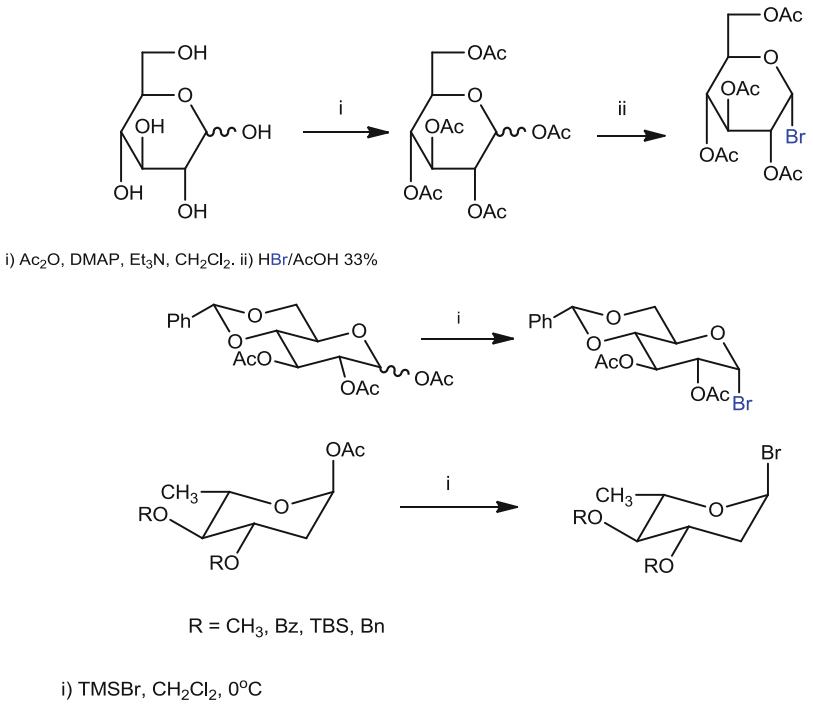
This term is used to define a glycosidic moiety that contains a leaving group at the anomeric position. When a glycosyl donor is reacted in the presence of a catalyst (also known as promoter) with a free alcohol called glycosyl acceptor, it will produce an *O*-glycosidic linkage. The first glycosyl donors developed and used specifically for glycoside formation were the glycosyl halides. As mentioned above, glycosyl bromide and chloride are the most widely used halides, and are the glycosyl donors used for the preparation of *O*-glycosides according to the methods reported by Michael, Koenigs–Knorr, and Helferich (see *O*-glycoside formation); however, iodide and fluorine glycosyl donors are gaining increased attention in the synthesis of *O*-glycosides.

1.9.1 Glycosyl Halides

2,3,4,6-tetraacetyl-α-D-glucopyranosyl bromide also known as acetobromoglucose is one of the most extensively used sugar intermediates for preparing glycosides derived from glucose [83]. The preparation involves the initial peracetylation of glucose with acetic anhydride in the presence of a catalyst, commonly pyridine, triethylamine, and dimethylaminopyridine, or sodium acetate and zinc chloride, in dichloromethane as solvent.

The resulting 1,2,3,4,6-pentaacetyl-α,β-D-glucopyranoside (as a mixture of anomers) is treated with a 33 % solution of HBr-acetic acid in dichloromethane at 5 °C during 12 h. The final product is obtained after crystallization from isopropyl ether to yield acetobromoglucose as a white solid. For sugar containing acid sensitive groups such as benzylidine, bromotrimethylsilane (TMS-Br) is used as an alternative (Scheme 1.59) [84, 85].

The ^{1}H NMR spectrum of acetobromoglucose shows signals for each of the ring protons, as well as for the primary alcohol and acetates. The well-defined spectrum



Scheme 1.59 Standard conditions for preparation of acetobromoglucose

allows the net identification of each proton, starting from the anomeric proton at δ 6.60 shifted downfield due to the presence of the halogen, with coupling constant of 4 Hz indicating an equatorial–axial interaction with H-2. Diaxial interactions are evident as triplets for H-3 and H-4, and axial–equatorial as double of double for H-2 (Scheme 1.60).

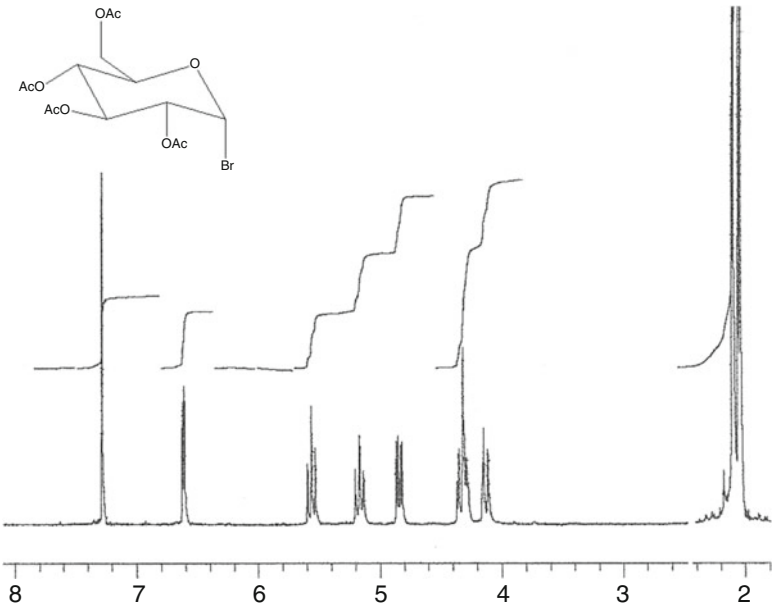
In the case of chlorine this can be suitable prepared by treatment of peracetylated saccharide with thionyl chloride in tin (IV) chloride at room temperature or boron chloride at 0 °C (Scheme 1.61) [86, 87].

Other conditions reported with sugars bearing sensitive groups such as azide group employs trimethylsilyl chloride, phosgene in DMF, or titanium(IV) chloride (Scheme 1.62) [84, 87, 88].

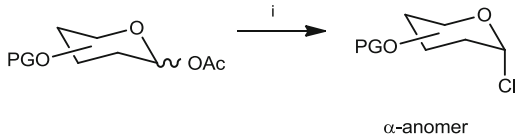
Another possibility used in the synthesis of branched sugars transform peracetylated nitro azide disaccharides with tetraethyl ammonium chloride at room temperature (Scheme 1.63) [89].

Iodine glycosyl donors once considered unstable glycosyl donors are having increasing participation as glycosyl donors, as it can be observed in studies for either armed or disarmed approaches. The common methods for preparing glycosyl iodides consist in the reaction peracetylated saccharide with hydrogen iodide in acetic acid, iodo trimethylsilane (TMSI) in toluene, and hexamethyldisilane (HMDS) with molecular iodine (Scheme 1.64) [90–92]

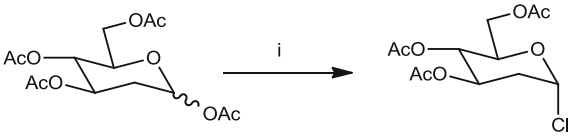
Likewise interconversion of glycosyl bromide to iodide can be accomplished by treatment with sodium iodide in acetone (Scheme 1.65).



Scheme 1.60 ^{1}H NMR of acetobromoglucose



i) $SOCl_2$ (2 eq)/$SnCl_4$ (1 eq), CH_2Cl_2, rt..



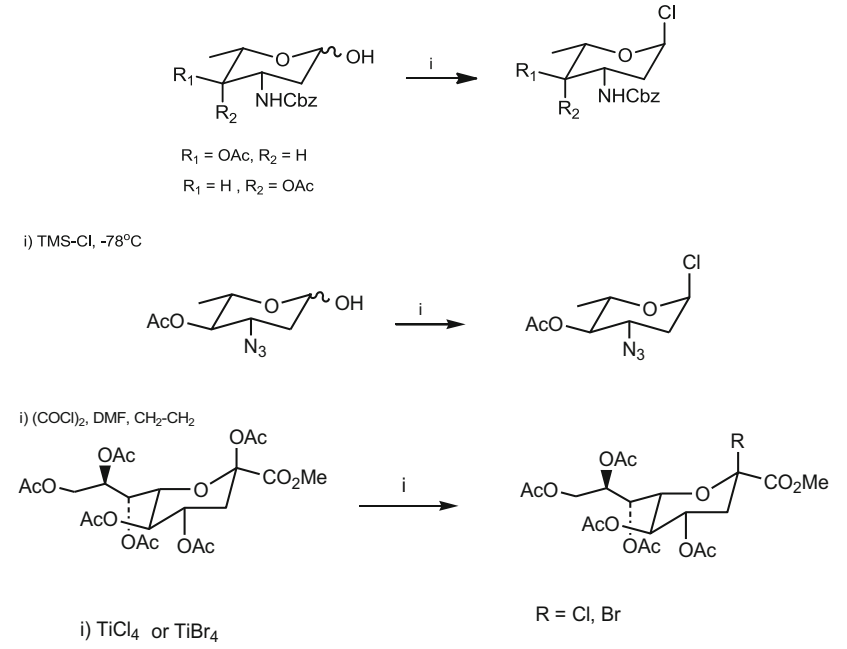
i) BCl_3, CH_2Cl_2, 0°C, 30 min

Scheme 1.61 Synthesis of α-glycosyl chloride from peracetylated sugars

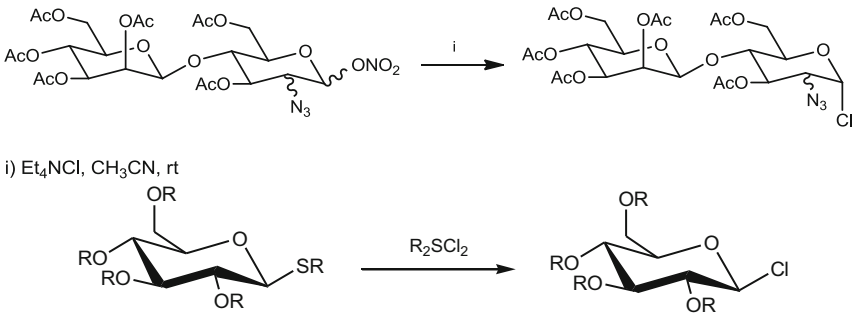
Also protected pivaloate glucuronide α-iodide donors can be suitably prepared by using hexamethyldisilane–I_2 mixture which generates Me_3SiI in situ in high yield (Scheme 1.66) [93].

On the other hand, protected per-*O*-TBS-β-D-galactofuranose was submitted to iodination under TMSI to furnish the corresponding galactofuranosyl iodide with 1,2-trans selectivity, and user further as glycosyl donor in the preparation of S- and C-galactofuranosides (Scheme 1.67) [94].

Glycosyl fluorides are used as glycosyl donors in the synthesis of various glycosides, and also are useful substrates for glycoside hydrolases and glycosyltransferases [96].

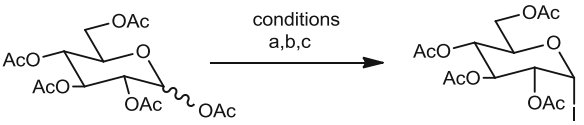


Scheme 1.62 Preparation of sensitive glycosyl chlorides

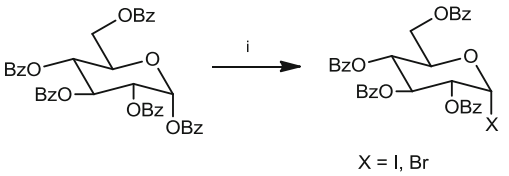


Scheme 1.63 Preparation of glycosyl chloride from nitro and sulfur glycosyl donors

They are considered thermally and chemically stable in relation to other glycosyl halides and also allows purification prior to the moisture-sensitive glycosylation reactions. Typical protocols for preparing glycosyl fluorides involves the conversion of glycosyl chlorides or bromides with fluorine salts such as AgF, $AgBF_4$, or ZnF_2 [97]. Another approach involves the use of diethylaminosulfurtrifluoride (DAST), however DAST-promoted fluorination of thioglycoside requires higher reaction temperatures, suggesting that the electrophilicity of the DAST-derived reactive species is rather low [98]. Additionally other fluorinated reagents such as XtalFluor [97] and HF-pyridine [99] have been proposed as useful alternative glycosyl donors for the preparation of glycosyl fluorides (Scheme 1.68).

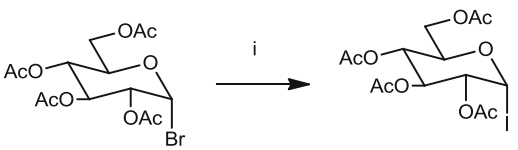


conditions a) HI/AcOH.
conditions b) TMSI, toluene
conditions c) HMDS, I_2.



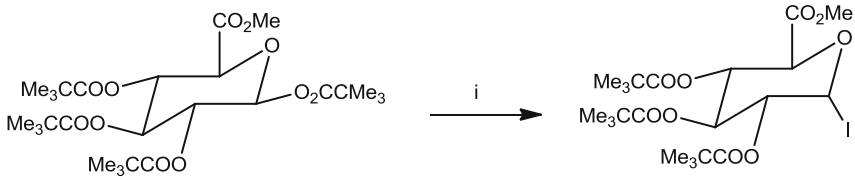
i) Me_3SiX ZnX_2 (cat), CH_2Cl_2, rt..

Scheme 1.64 Preparation of glycosyl iodides from peracetylated sugars [95]



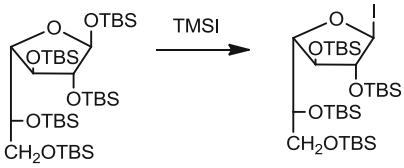
i) NaI, acetone, 76%

Scheme 1.65 Preparation of glycosyl iodides from acetobromopyranosides



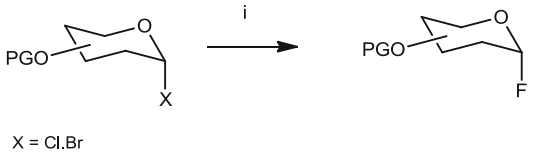
i) HMDS-I_2 or Me_3SiI 90%.

Scheme 1.66 Preparation of α-glucuronopyranoside iodides from pivaloate glucuronide



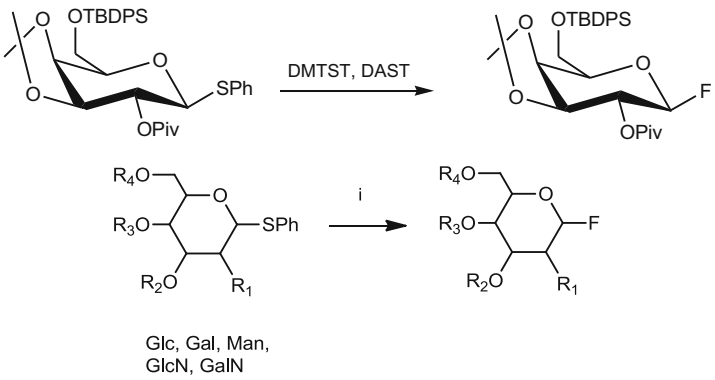
i) Me_3SiX ZnX_2 (cat), CH_2Cl_2, rt..

Scheme 1.67 Preparation of furanosyl and iodide as a glycosyl donors



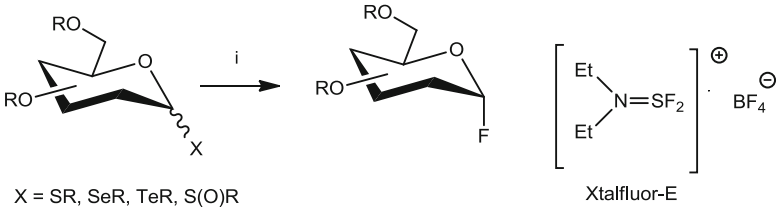
X = Cl.Br

i) AgF or $AgBF_4$ or ZnF_2



Glc, Gal, Man,
GlcN, GalN

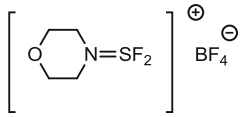
i) DAST (+ DMTST)



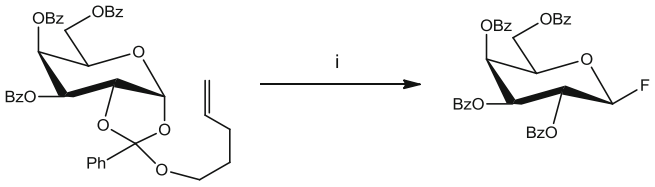
X = SR, SeR, TeR, S(O)R

Xtalfluor-E

i) Xtalfluor-E or M, CH_2Cl_2



Xtalfluor-M

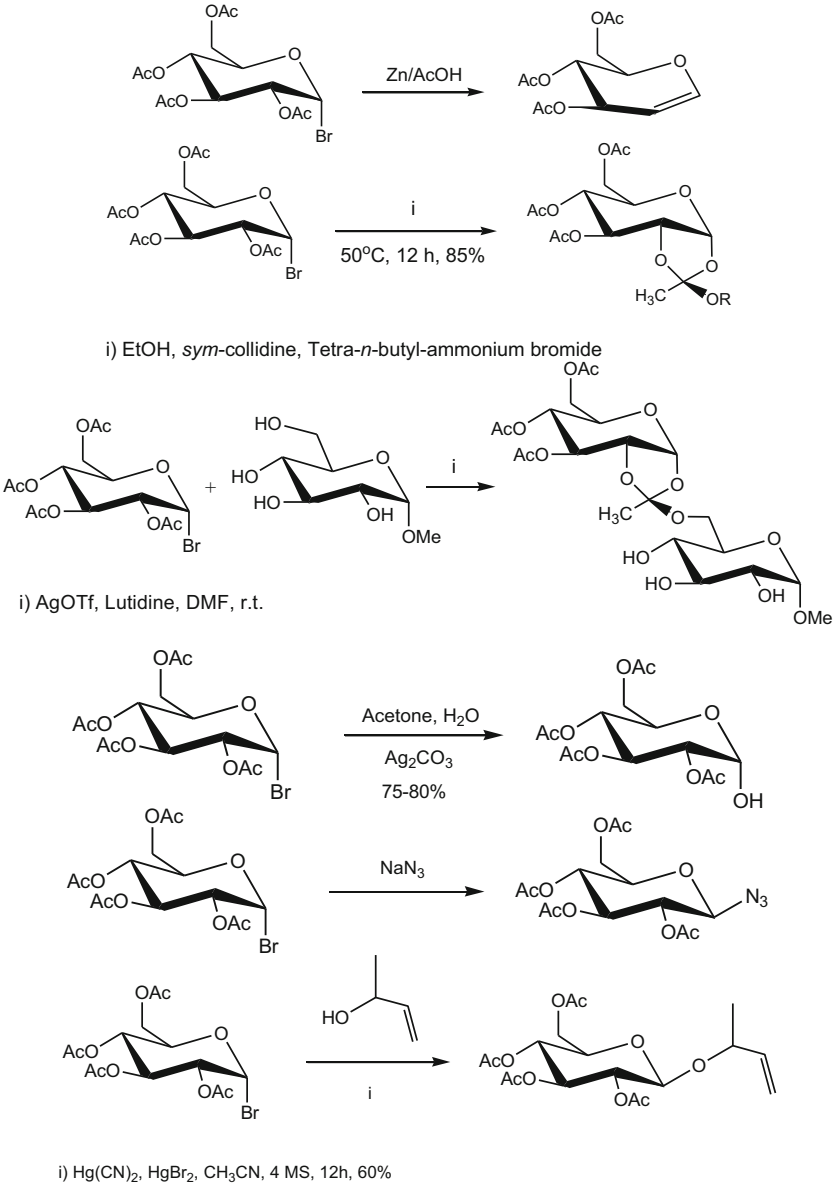


i) HF-Py, CH_2Cl_2, -40°C

Scheme 1.68 Methods for preparing glycosyl fluorides

1.9.2 Glycosyl Donor Interconversion

Besides their extensive use in the preparation of glycosides, glycosyl bromide can also be useful for conversion to other suitable glycosyl donors (Scheme 1.69), such as glycals [100, 182, 183], orthoesters [101, 184], and thiols [102]. Also, the glycosyl halides



Scheme 1.69 Some glycosyl donors obtained from acetobromoglucose

can be transformed to glycosyl imidate through the anomeric hydroxyl formation [103], or to amines via a reaction with azide salt and hydrogenolysis [78, 181].

Glycosyl acetates are also important glycosyl donors and can be used directly under the fusion strategy for the preparation of *O*- and *N*-glycosides. The fusion method consists in the reaction between the glycosyl acetate as glycosyl donor and the glycosyl acceptor in the presence of a Lewis acid as a promoter to generate the corresponding glycoside. Likewise, acetates can also be suitable precursors for the preparation of glycosyl donors such as halides, thiols [104], and imidates, the latter by a two-step process. The first step involves the removal of the anomeric acetate with a base; hydrazine, benzylamine, ammonia, and piperidine are the most preferred.

The resulting hydroxyl group is obtained as a mixture of anomers, and is subsequently used for the preparation of the glycosyl imidate (see Imidate Method). Another use of glycosyl acetates, is the transformation into anomeric amines, through the introduction of the azide group with trimethylsilyl azide under a Lewis acid catalyst, and further hydrogenolysis [71]. This reaction is useful for the preparation of some glycopeptides. Likewise 2-thiophenyl glycosides of Neu5Ac are suitably obtained by treatment of 2-*O*-acetyl, 2-chloro, or 2-chloro Neu5Ac glycosyl donors with PhSH in the presence of NIS/TfOH as promoter system (Scheme 1.70). Other activated agents for preparing S-alkyl and S-aryl glycosyl donors are methyl trifluoromethanesulfonate (MeOTf), dimethyl(methylthio)sulfonium trifluoromethanesulfonate (DMTST), iodo dicollidine perchlorate (IDCP), and phenyl selenyl trifluoromethanesulfonate (PhSeOTf) [105].

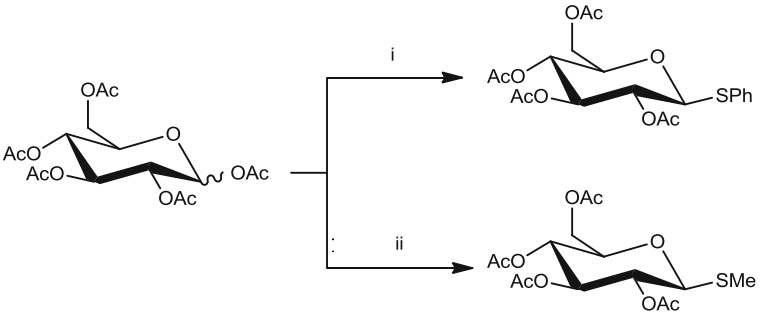
Thioglycosyl donors (e.g., -Sme, -SEt, -STol) may also be obtained from per-*O*-acetylated glycopyranosyl iodides with 1.2 M equiv. of the respective thiols with complete anomeric selectivity and in very good yield [91].

Thioglycosides are stable glycosyl donors widely used for the preparation of glycosides. The usual conditions for achieving this goal are the glycosyl acceptor and *N*-iodosuccinimide (NIS), or NIS-TfOH as promoter. Thioglycosides are also important starting material for the preparation of other glycosyl donors such as acetates, fluorine [104], chlorine [111], sulfoxides [112], or anomeric alcohols (Scheme 1.71).

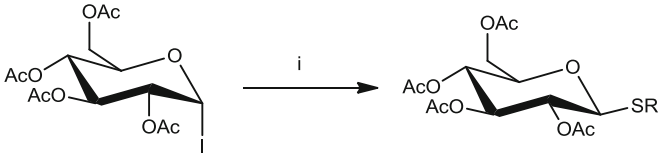
Glycals are becoming potentially useful glycosyl donors, and an increasing number of simple and complex glycosides have been reported. For this purpose the glycal is usually transformed to the oxirane. and immediately coupled with the glycosyl acceptor in the presence of a Lewis acid (see The Glycal Method). Moreover, glycals are also suitable intermediates for the preparation of a variety of glycosyl donors (Scheme 1.72) such as phosphates and thiophosphates [113], deoxysugars [114], Diels–Alder adducts [115], allyl glycosyl donors [116], and imidates [117].

1.10 Protecting Groups

An important additional requirement for achieving glycosidic coupling reactions, besides the fact that a good leaving group should be present, is the appropriate use of protecting groups. Their function is to shield those groups (particularly

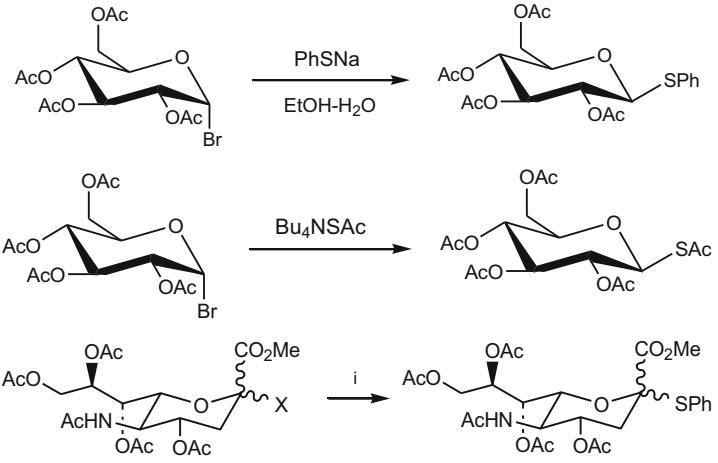


i) PhSH (1.1 eq.), $SnCl_4$ (0.7 eq.), CH_2Cl_2, 0°C, 4h, 82%. ii) MeSH (excess), $SnCl_4$ (0.7 eq.), CH_2Cl_2, -20°C, 3h, 85%.



R = Me, Et, Tol

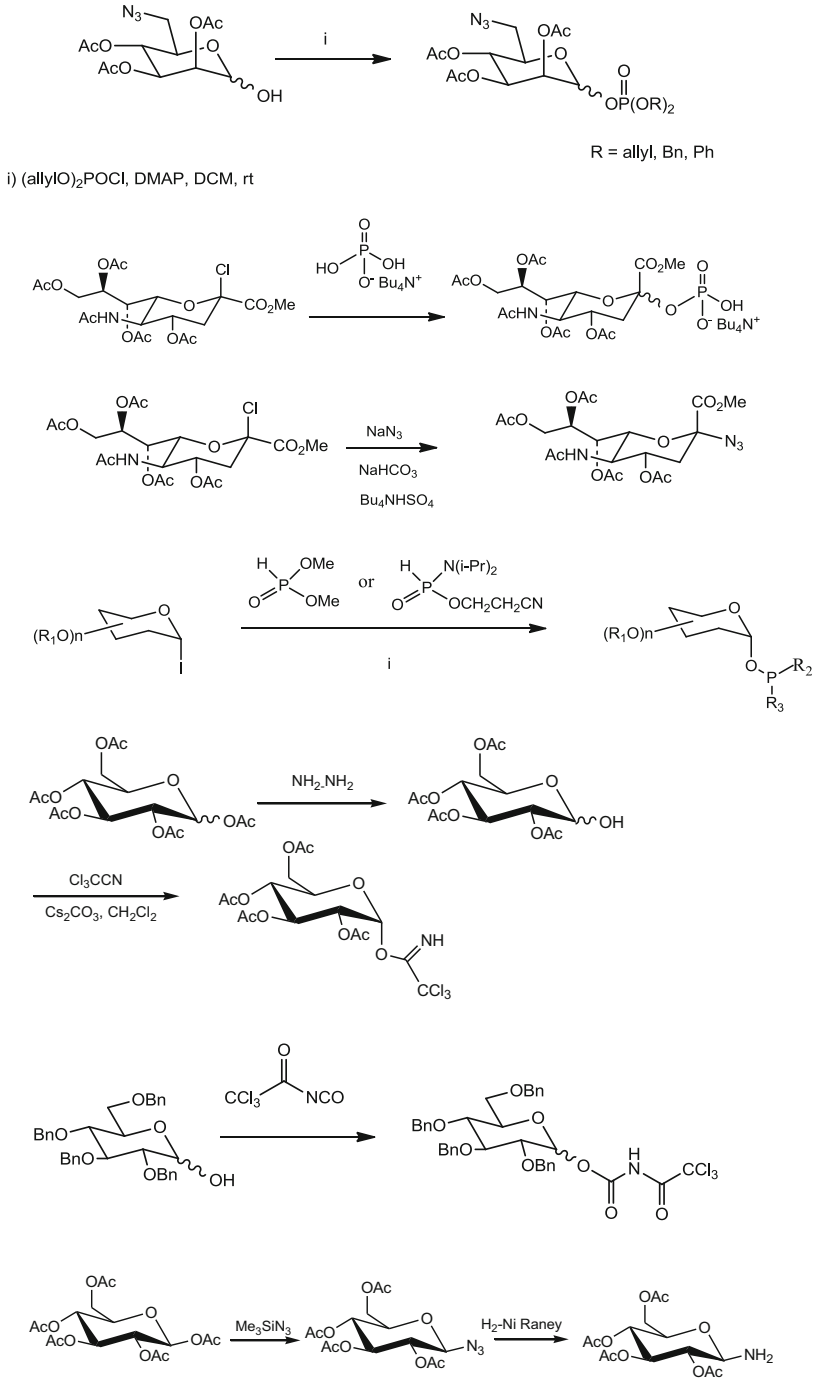
i) RSH 1.2 eq or MeSSMe for R = Me.



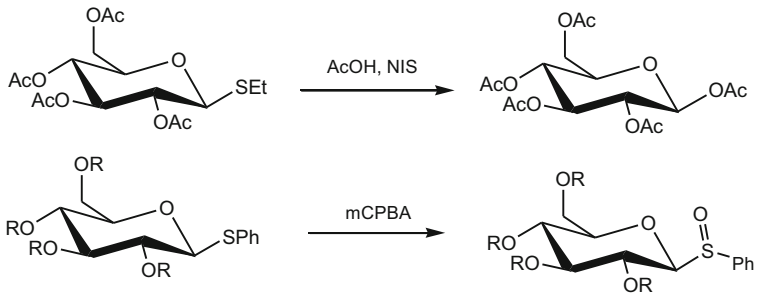
X = OAc, Cl, F.

i) PhSH, NIS/TfOH.

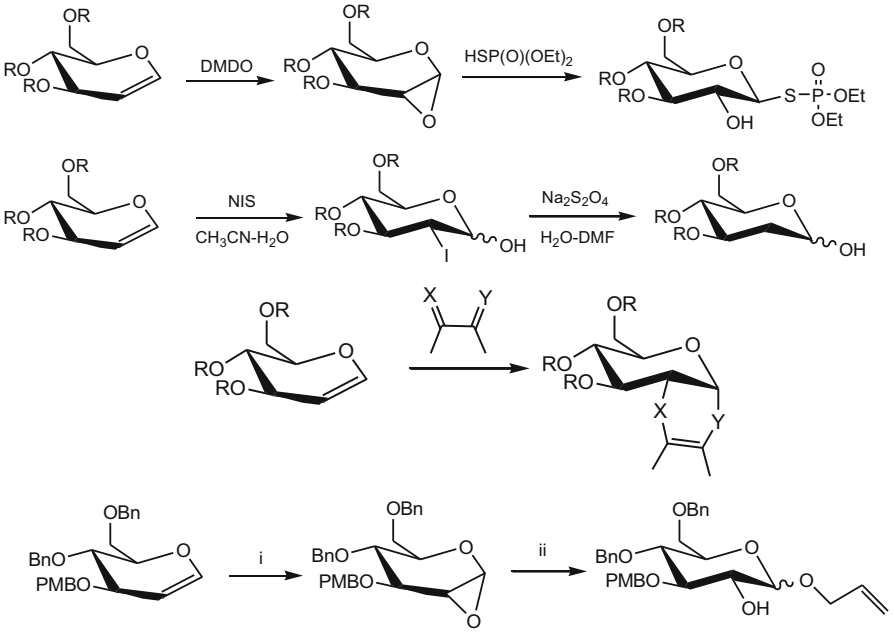
Scheme 1.70 Miscellaneous approaches for the preparation of glycosyl donors [106–110]



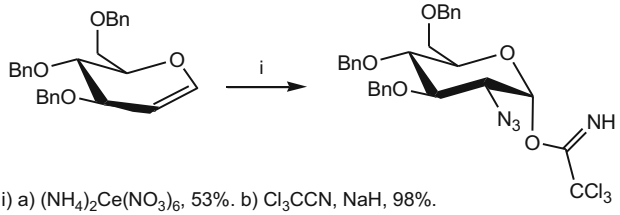
Scheme 1.70 (continued)



Scheme 1.71 Modifications of thio glycosyl donors

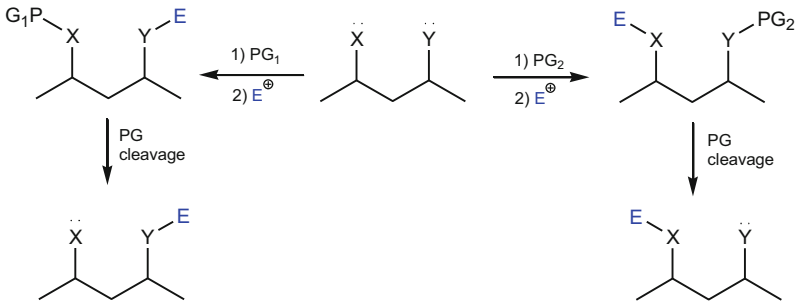


i) DMDO-acetone. ii) ally alcohol, r.t., 16h, 79%.



i) a) $(NH_4)_2Ce(NO_3)_6$, 53%. b) Cl_3CCN, NaH, 98%.

Scheme 1.72 Preparation of glycosyl donors and precursors from glycals



Scheme 1.73 Schematic representation of protecting group applicability

heteroatoms) that are wanted to keep unaltered during the coupling reaction and then release them under mild conditions that do not affect the glycosidic bond (Scheme 1.73).

A significant number of protecting groups [118] have been used and combined for pursuing the synthesis of complex natural products including glycosides.

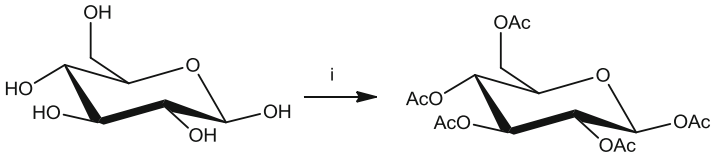
Due to its acetal character, the glycosidic bond is hydrolyzed under acidic conditions, and is significantly more resistant to base, hydride reduction, or hydrogenolysis.

The use of ethers such as methyl ether (-O-CH_3), methoxymethyl ether (-O-CH_2OCH_3, MOM), 2-methoxyethoxymethyl ether (-O-$CH_2OCH_2CH_2OCH_3$, MEM), and tetrahydropyranyl ether (-O-2-c-C_5H_9O, THP) have been widely used for protection of alcohols. However, in glycoside synthesis attention has to be paid since deprotection is carried out under acidic conditions, which might be hazardous for the glycosidic bond. Silyl derivatives are also another important choice for protection of hydroxyl groups [105]. Some of the most accepted silyl derivatives for carbohydrate hydroxyl protection are *tert*-butyl dimethylsilyl (TBDMS), triisopropylsilyl (TIPS), *tert*-butyl diphenylsilyl (TBDPS), and triethylsilyl (TES) ethers. Quantitative cleavage is usually achieved upon treatment with tetrabutylammonium fluoride (TBAF) or HF/pyridine.

The conventional protecting groups for the preparation of glycosides are the affordable acetates, benzoates, and benzyl protecting groups since they can be removed under basic and later neutral conditions, the best conditions for preserving the glycosidic bond. The standard conditions for either installing and removing the most common protecting group described are:

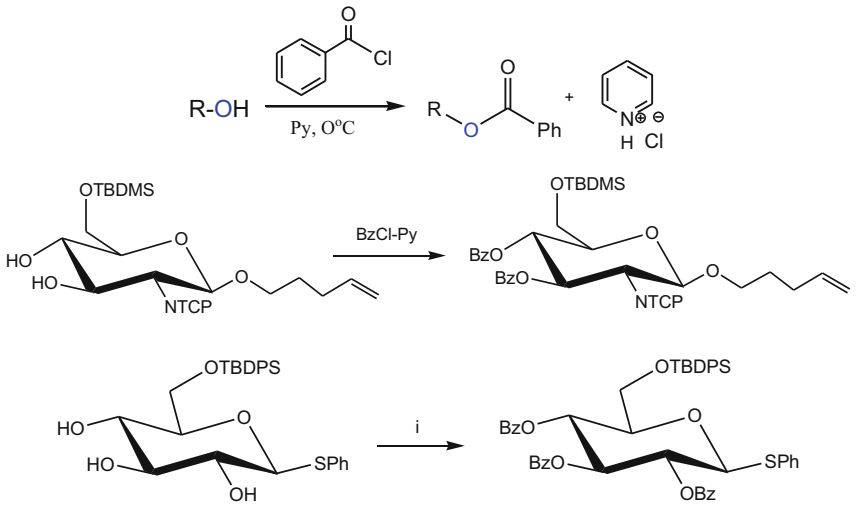
Acetate (**Ac**-). The standard procedure involves the use of acetic anhydride in the presence of pyridine or triethylamine as acid scavenger, and 4-(dimethylamino) pyridine (DMAP) that improves the rate of reaction. The cleavage of acetates proceeds smoothly with NaOMe solution also known as Zemplen conditions. Acetates are stable at pH from 1 to 8 and can be cleaved with lithium aluminum hydride (Scheme 1.74) [91].

Benzoyl (**Bz**-): This protecting group is more stable to hydrolysis than acetates and may resist a pH up to 10. The conditions for protection of alcohols are shown in Scheme 1.75 and involves the use of benzoyl chloride in pyridine or



conditions A: Ac_2O, CH_2Cl_2, Et_3N, DMAP, r.t.
B: Ac_2O, CH_2Cl_2, AcONa
C: Ac_2O, I_2 cat.*

Scheme 1.74 Standard protocol for the preparation of peracetylated sugars



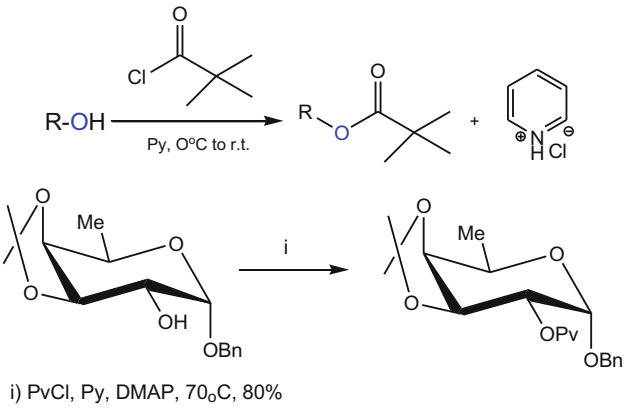
i) PhCOCl (4.0 eq.), Et_3N (8.0 eq.), 4-DMAP (0.2 eq), THF, 50°C, 15h, 92%.

Scheme 1.75 General procedure for the benzoylation of sugars

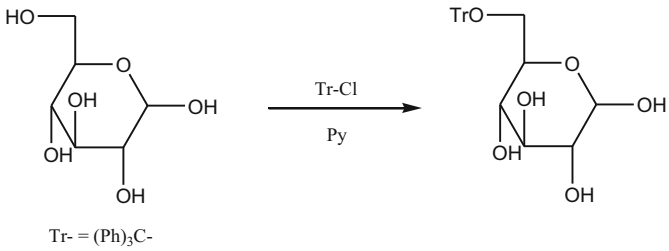
triethylamine [119, 185]. It is stable to hydrogenolysis and borohydrides but not to lithium aluminum hydride. The cleavage is usually achieved in 1 % NaOMe-MeOH solution.

Pivaloyl (**Pv**-): This protecting group, which is also known as trimethylacetyl chloride, is used for protection of primary and secondary alcohols in yield. An example of the use of this group is the protection of the hydroxyl group at position 2 of fucose derivative [120]. The standard conditions for protection are pivaloyl chloride in pyridine or DMAP and the cleavage is performed with $Bu_4N^{+-}OH$ at 20 °C (Scheme 1.76).

Trityl (**Tr**-): This bulky protecting group is selective for primary alcohols (Scheme 1.77). The protecting reaction proceeds in pyridine or DMAP-DMF [121]. The cleavage can be performed under neutral conditions with 1 % iodide in methanol, or weakly acidic in formic acid–ether solution.

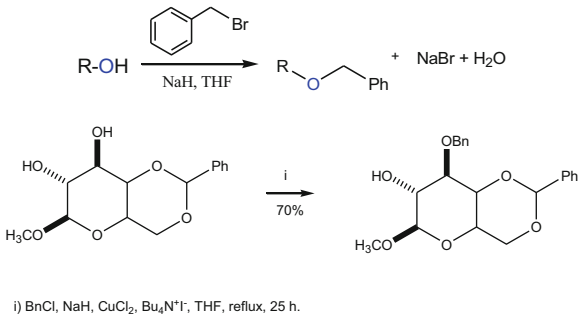


Scheme 1.76 Conditions and reagents for protection of alcohols with pivaloyl group



Scheme 1.77 Protection of primary alcohol with trityl protecting group

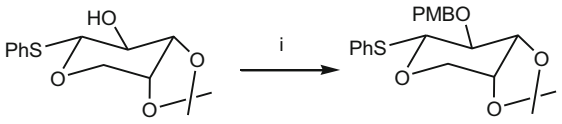
Scheme 1.78 General conditions for benzylation of carbohydrates



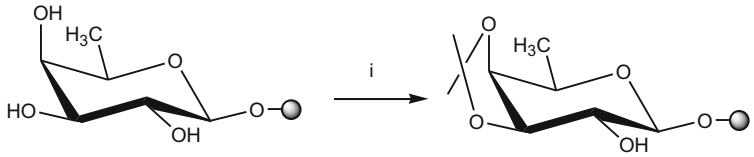
Benzyl (**Bn**-): This protecting group when attached with alcohol generates an ether (Scheme 1.78). However, unlike common ethers, this can be cleaved under neutral condition by hydrogenolysis. The usual conditions for attachment are NaH, THF, and benzyl bromide or chloride [122]. The conditions for removing this group are hydrogen, Pd/C 10 % or $Pd(OH)_2$/C 10 % in ethanol or ethyl acetate.

***p*-Methoxybenzyl** (**PMB**-): This benzyl derivative is installed by reacting the free alcohol with PMB-Cl under NaH, DMF conditions at 0 °C [123]. An example of its applications can be seen in the protection at the second position of acetonide

Scheme 1.79 General conditions for benzylation of carbohydrates with PMB

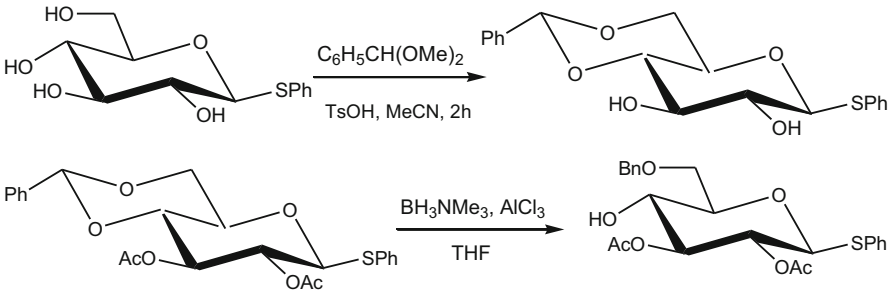


i) NaH, DMF, 0°C, 30 min., then 1.2 equiv. PMB-Cl, 2 h, 93%.



i) $(CH_3)_2C(OCH_3)_2$, camphorsulfonic acid, DMF, r.t., 3 h

Scheme 1.80 Acetonide formation for protection of diols



Scheme 1.81 Protection of 4 and 6 hydroxyl groups with benzylidene group and partial removal

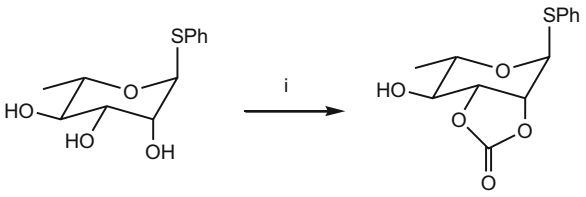
thioglycoside shown in Scheme 1.79. Deprotection is carried out under neutral conditions with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), in CH_2Cl_2 and H_2O (20:1), 1 h, at 25 °C, in a 91 % deprotection yield.

Acetonide (**$(CH_3)_2C(O)_2$-**): This protecting group is useful for protection of *cis* diols (Scheme 1.80) and the conditions are acetone, 2,2-dimethoxypropane, and *p*-toluenesulfonic acid or camphorsulfonic acid as catalyst [124]. Acetonides are usually stable at a pH between 4 and 12, and the regeneration of the diol can be achieved by treatment with aqueous acid.

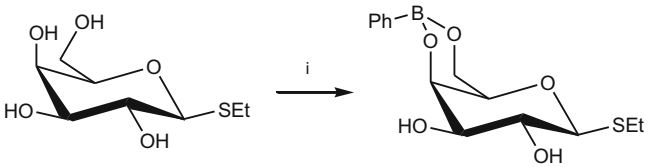
Benzylidene (**$PhCH(O)_2$-**): This classical protecting group is usually selected for protection of position 6 and 4, allowing the remaining positions to be modified. The benzylidene is attached under mild conditions and are useful for either –OH (4) in axial or equatorial positions (Scheme 1.81). Deprotection can be effected under different conditions, such as acid conditions, hydrogenolysis, and hydrides such as BH_3NMe_3, $AlCl_3$, THF, 60 °C, 1 h [125].

Carbonate (**$O{=}C(O)_2$-**): This group is suitable for protection of *cis* diols, and it has been used in the synthesis of complex oligosaccharides and also in solid-phase

Scheme 1.82 Standard conditions for protection of diols with carbonate protecting group

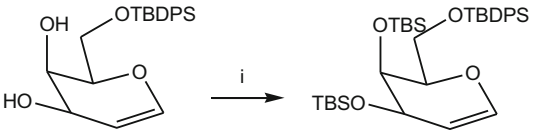


i) $(Cl_2)C=O$, Py, 0°C, 1h, 69%.

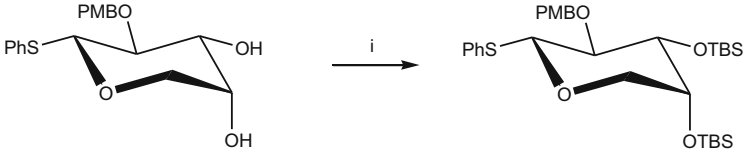


i) $PhB(OH)_2$.

Scheme 1.83 Protection of 4,6-OH groups with boronate



i) TBSOTf, Py.



i) TBSOTf (4.0 eq.), Et_3N (10.0 eq.), CH_2Cl_2, 0°C, 2h, 97%.

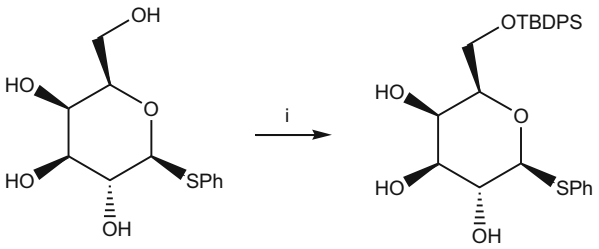
Scheme 1.84 Protection of secondary alcohols with TBS protecting group

oligosaccharide synthesis. The reagents and conditions used for protection are phosgene in pyridine at 0 °C during 1 h (Scheme 1.82), and the yield reported is around 70 % [126]

Boronate (**$PhB(O)_2$-**): This group has been proposed in solid-phase oligosaccharide synthesis [127] for simultaneous protection of 4,6-OH groups (Scheme 1.83). Deprotection is achieved with IRA-743 resin [128].

***Tert*-butyldimethylsilyl** (**TBS**): More recently introduced for protection of primary and secondary alcohols with reported yield protection around 90 % (Scheme 1.84).

Scheme 1.85 Protection of primary alcohols with TBDPS protecting group

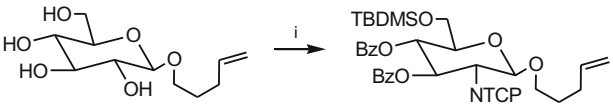


i) *t*-$BuPh_2SiCl$, imidazole, DMF, 100%.

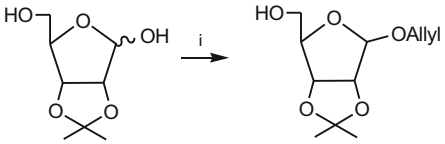
The standard conditions are *tert*-butyldimethylsilyltriflate in pyridine [129], and deprotection is usually achieved with butyl ammonium fluoride (Bu_4NF) in THF.

***tert*-butyldiphenylsilyl (TBDPS-)**: This protecting group is specific for primary alcohols and the yields reported are quantitatives (Scheme 1.85). This bulky silylated group has been used for the assembly of oligosaccharide libraries and has been compatible with the use of other highly selective groups [130]. The standard protection conditions are TBDPS-Cl, imidazole, DMF, or THF. Deprotection is achieved with hydrogen fluoride-pyridine or TBAF, cat. AcOH, THF, and a yield of 87 %.

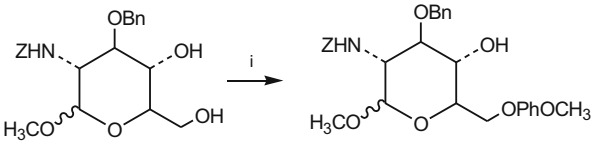
1.11 Selective Protections (Scheme 1.86)



i) TBDMSCl/Py. ii) BzCl/Py.

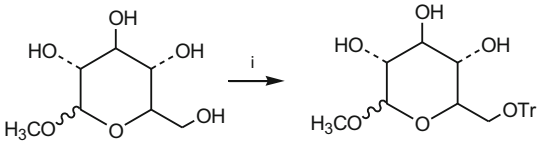


i) CH_2=$CHCH_2OCO_2Et$, $Pd_2(dba)_3$, THF, 65°C, 4h, 70%.

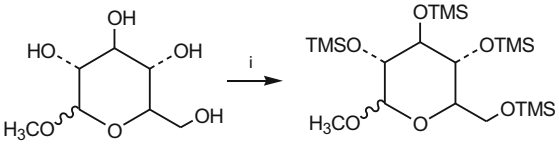


Z = benzyloxycarbonyl

i) 4-$CH_3OC_6H_5OH$, THF, DEAD, Ph_3P, 80°C, 82%.

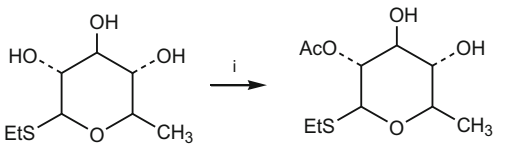


i) Ph_3CCl, DMAP, DMF, 25°C, 12h, 88%.

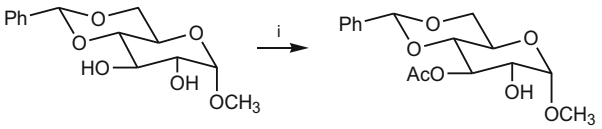


i) Me_3SiCl, Et_3N, THF, 25°C, 8h, 90%.

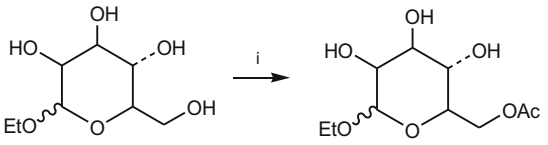
Scheme 1.86 Miscellaneous selective protections [119, 123, 125, 129, 131–156]



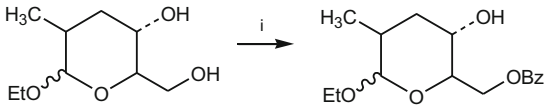
i) $MeC(OCH_3)_3$, TsOH, DMF, 96%.



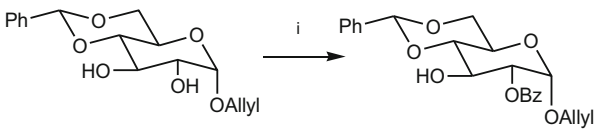
i) Lipase AK, vinyl acetate, 92%



i) Pancreatin, vinyl acetate, THF, TEA 95%.

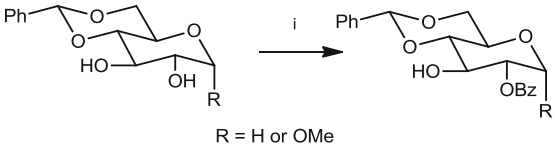


i) Ph_3P, DIAD, $PhCO_2H$, THF, 84%.



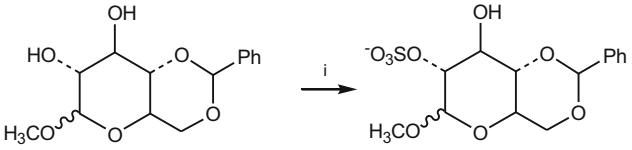
i) BzOBt, 92%

Scheme 1.86 (continued)

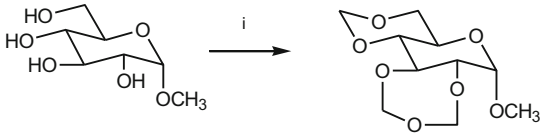


R = H or OMe

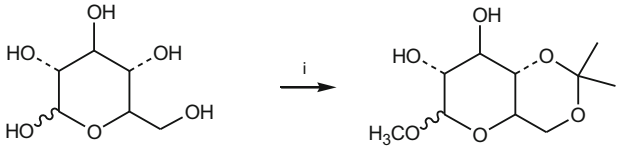
i) PPh_3, DEAD, BzOH/THF



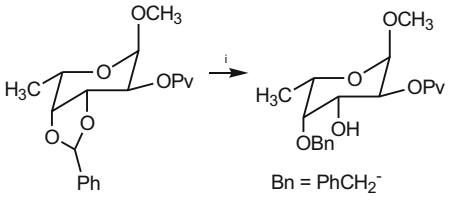
i) Pyr SO_3, Pyr, 69%



i) DMSO, $POCl_3$, 85%.



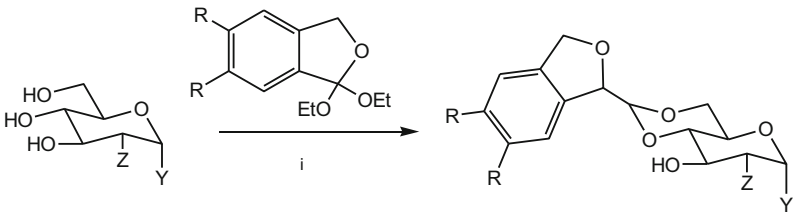
i) $CH_2=C(CH_3)OCH_3$, DMF, TsOH. 0°C, 95%



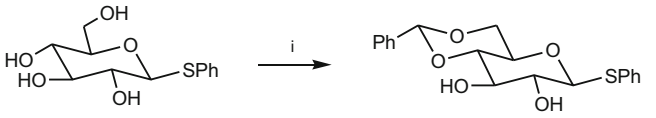
Bn = $PhCH_2^-$

i) NBS, CCl_4, 75%.

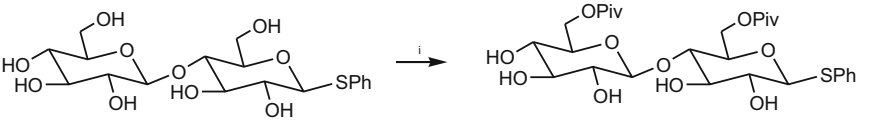
Scheme 1.86 (continued)



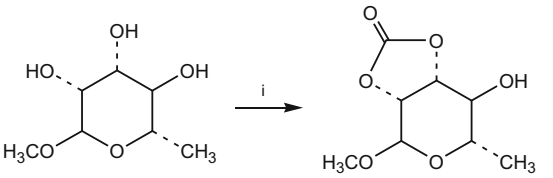
i) PPTS, ACN. 90%



i) $C_6H_5CH(OMe)_2$, TsOH, MeCN, 2h.

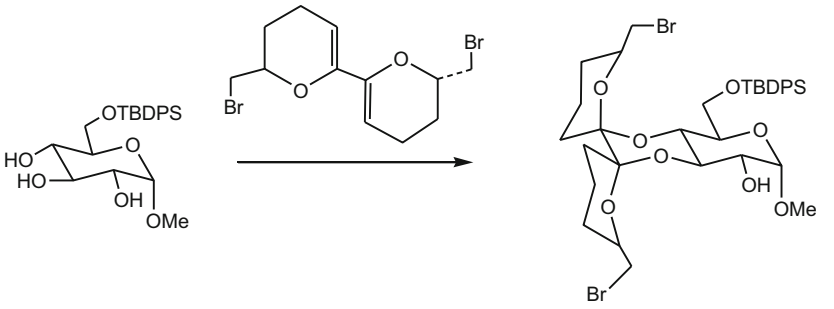


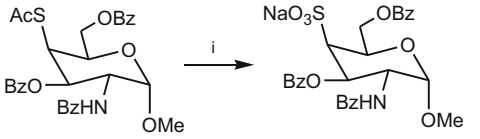
i) Pivaloyl chloride, Py, 91%.



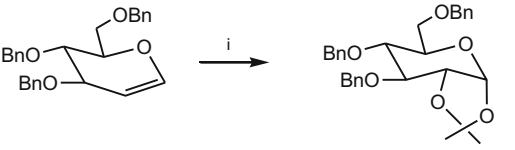
i) Cl_3CCOCl, Py, rt, 80%

Scheme 1.86 (continued)

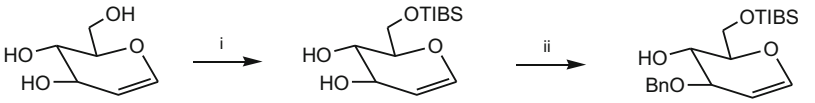




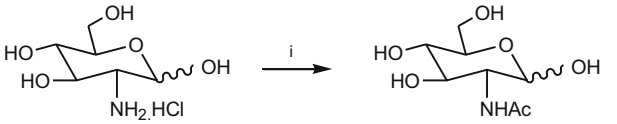
i) aq. H_2O_2 (33%), AcOH, NaOAc, 80°C, 8h.



i) DMDO, $ZnCl_2$, acetone.

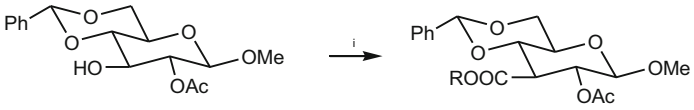


i) TIBS-Cl, Imidazole. ii) Bu_2SnO, BnBr, TBAI, 67%

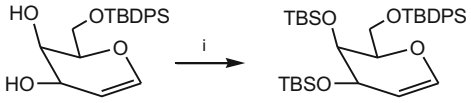


i) a) 1eq. NaOMe/MeOH. b) 1.2 eq. Ac_2O, 12h, 91%.

Scheme 1.86 (continued)



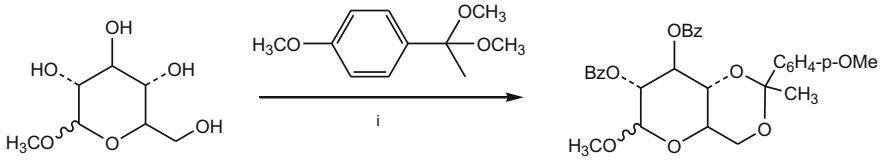
i) TMEDA, $ClCO_2R$, CH_2Cl_2, 0°C.



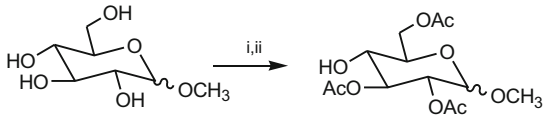
TBDPS = tert-butyldiphenylsilyl

TBS = tert-butyl dimethylsilyl

i) TBSOTf, Py.

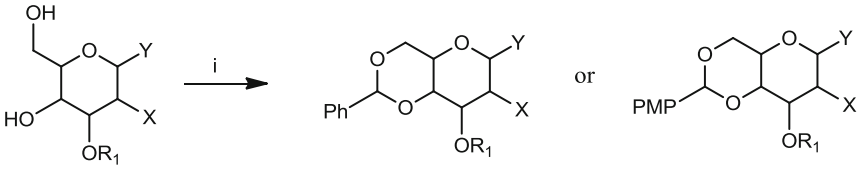


i) pyridinium p-toluensulfonate, 82-100%.



i) $(Bn_3Sn)_2O$, PhMe. ii) AcCl, r.t.

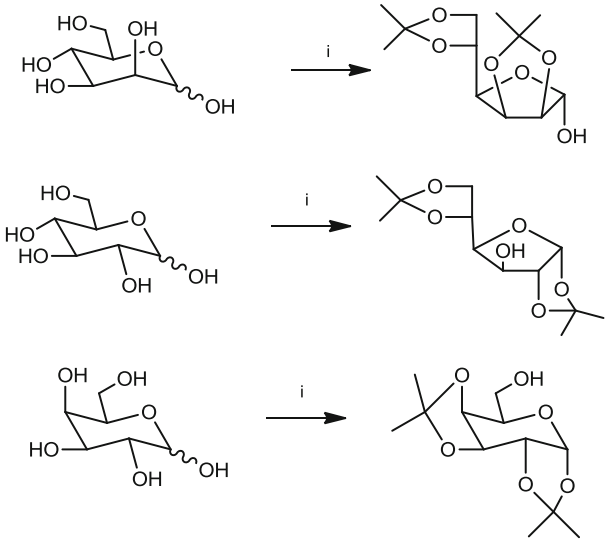
Scheme 1.86 (continued)



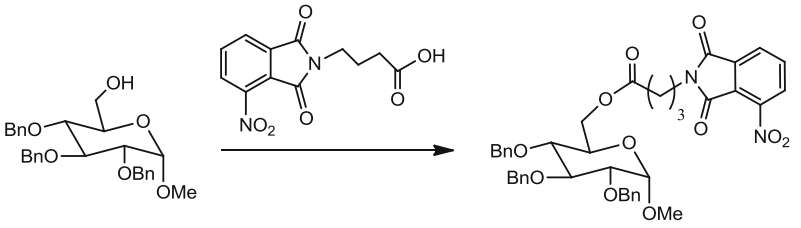
1. For monosaccharides
$FeCl_3$ (cat), $PhCH(OMe)_2$
or $PMPCH(OMe)_2$, CH_3CN

2. For disaccharides
$FeCl_3$ (1.2 equiv), CH_3CN, $PhCH(OMe)_2$
4A MS

R_1 = H/Ac/Bz/Bn; X= OH/OAc/OBz/OBn/Nphth
Y= SR_2/OR_2



i) tetrabutylammonium tribromide (TBATB), dry acetone, rt.



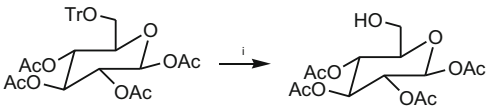
i) DCC, DMAP, CH_2Cl_2.

Scheme 1.86 (continued)

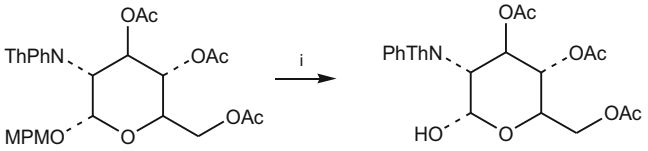
1.12 Selective Deprotections (Table 1.5, Scheme 1.87)

Table 1.5 Summary of common protecting and cleavage conditions

Protecting group	Protection conditions	Cleavage conditions
Acetyl (Ac-)	Ac_2O, Et_3N, DMAP, CH_2Cl_2	NaOMe-MeOH
Benzoyl (Bz-)	Bz-Cl, Py	NaOMe-MeOH
Pivaloyl (Pv-)	Pv-Cl, Py, DMAP	Bu_4NOH
Trityl (Tr-)	Tr-Cl, DMAP, DMF	1 % I_2-MeOH
Benzyl (Bn-)	Bn-Br, NaH, THF	H_2-$Pd(OH)_2$-EtOH
p-Methoxybenzyl (PMB-)	PMB-Cl, NaH, THF	DDQ, CH_2Cl_2-H_2O
Acetonide ($((CH_3)_2C(O)_2$-)	$(CH_3)_2CO$, 2,2-DMP, *p*-TsOH	AcOH-H_2O
Benzylidene ($PhCH(O)_2$-)	$PhCH(OCH_3)_2$, *p*-TsOH, CH_3CN	AcOH-H_2O, or H_2-$Pd(OH)_2$
tert-butyldimethylsilyl (TBS-)	TBS-OTf-Py	Bu_4NF-THF
tert-butyldiphenylsilyl (TBDPS-)	TBDPS-Cl-imidazole, DMF	HF-Py

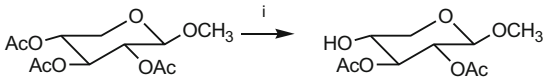


i) 1% I_2, MeOH.

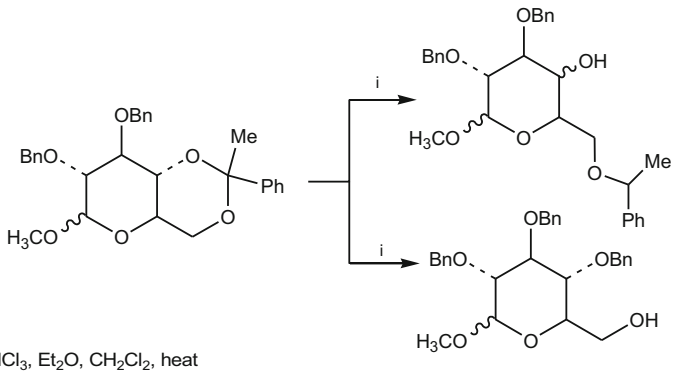


PhTh = phthalimido.
MPM = p-Methoxybenzyl ether p-$MeOC_6H_4CH_2OR$

i) CAN or NBS, CH_2Cl_2, H_2O.

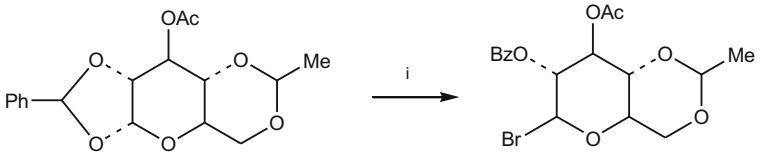


i) Lipase PS, n-pentyl-OH, 93%

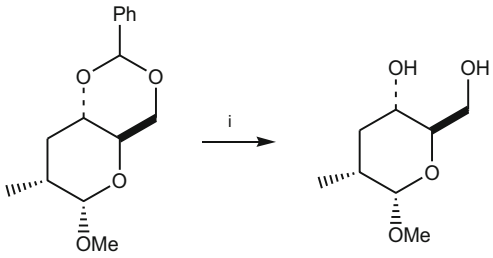


i) $LiAlH_4$, $AlCl_3$, Et_2O, CH_2Cl_2, heat

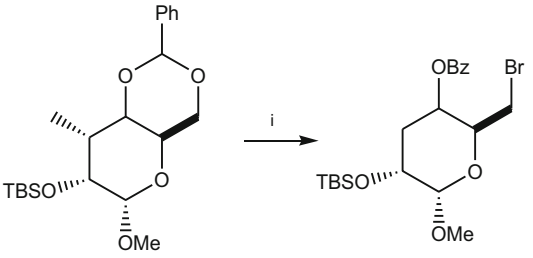
Scheme 1.87 Miscellaneous selective deprotections [120, 137, 152, 156–180]



i) $BrCCl_3$, CCl_4, hv, 30 min. 100%

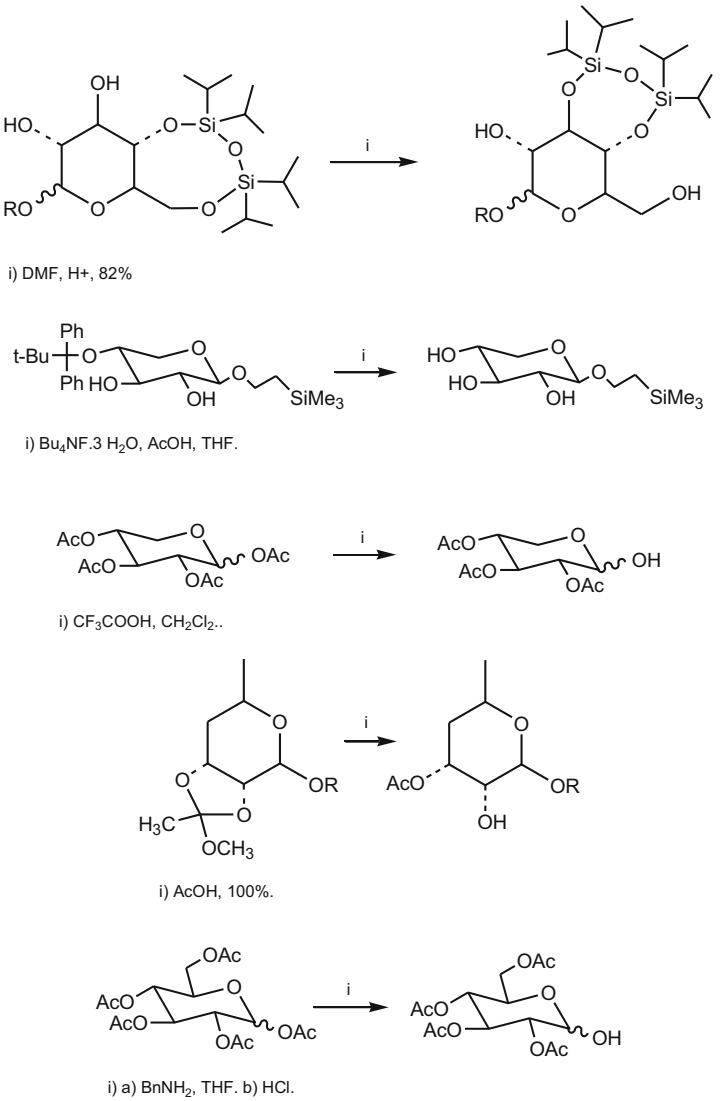


i) H_2, $Pd(OH)_2$, EtOH, 92%

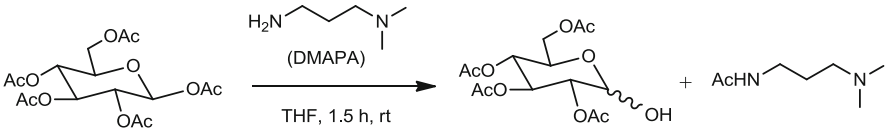


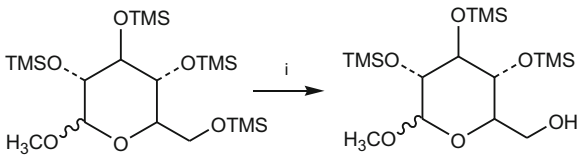
i) NBS, $BaCO_3$, CCl_4, Δ

Scheme 1.87 (continued)

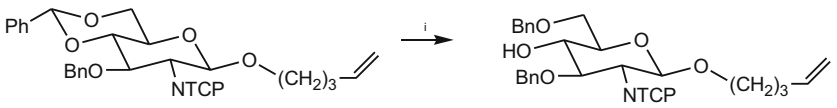


Scheme 1.87 (continued)

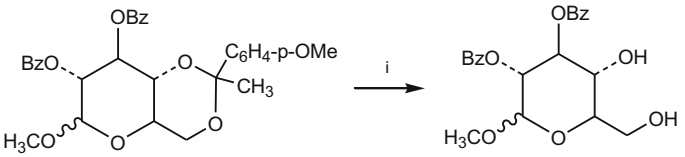




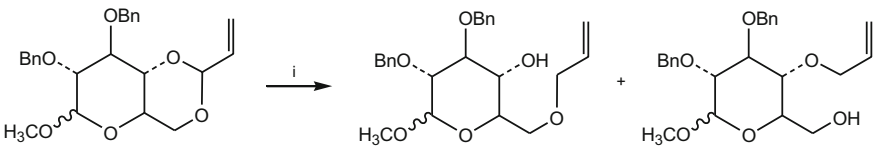
i) K_2CO_3, MeOH, 0°C, 45 min, 100%



i) a) $NaBH_3CN$. b) HCl, THF, Et_2O



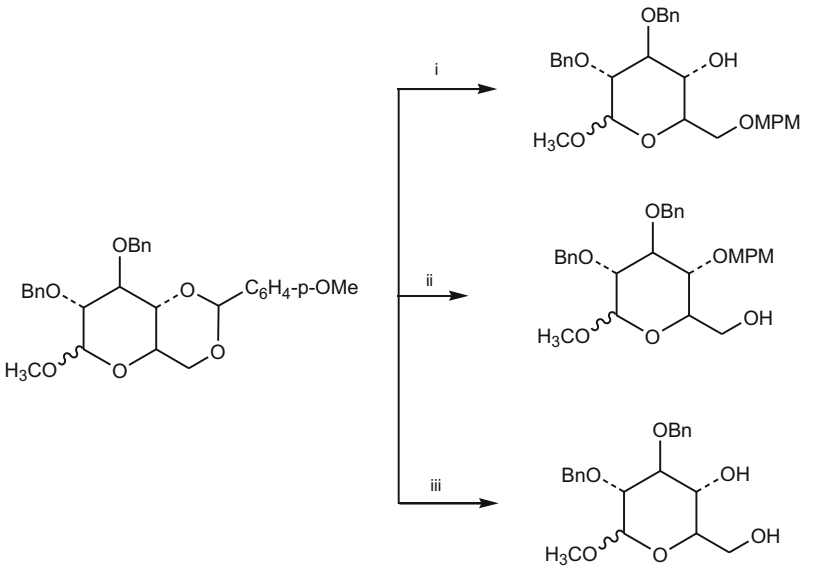
i) a) $SnCl_4$, CH_2Cl_2, -78°C. b) Bu_4NOH, 90%.



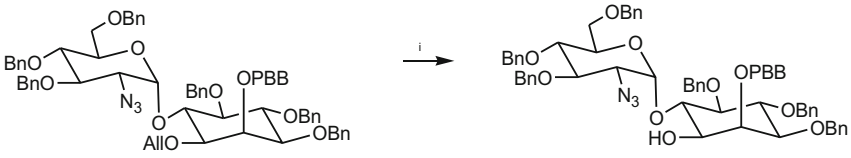
79%

i) a) $NaBH_3CN$. b) HCl, THF.

Scheme 1.87 (continued)

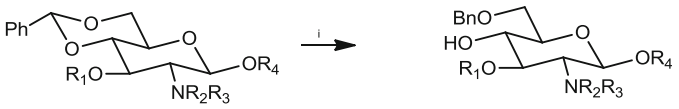


i) $NaBH_3CN$. b) TFA, DMF. ii) a) $NaBH_3CN$. b) TMSCl, CH_3CN. iii) CAN, CH_3CN-H_2O (9:1), 95%.



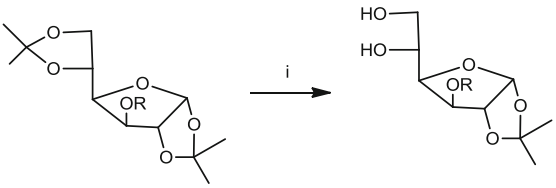
PBB = p-bromobenzyl

i) $Pd(OAc)_2$, (o-biphenyl)$P(^tBu)_2$, PhN(H)Me, NaO^tBu, 80°C. ii) $SnCl_4$, 84%.

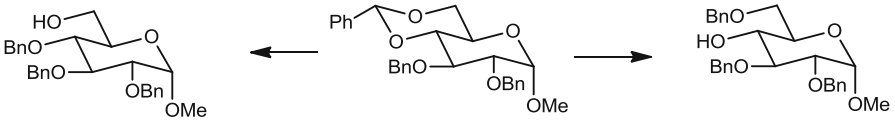


i) MsOH, $NaBH_3CN$, THF

Scheme 1.87 (continued)

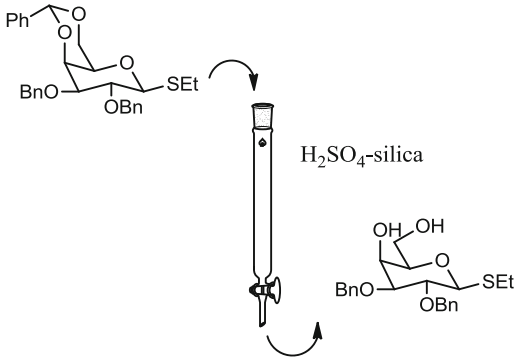


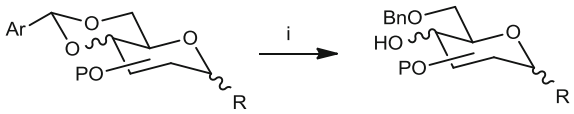
i) MeOH, Solid acid, H_2O, rt



$LiAlH_4$, $AlCl_3$, CH_2Cl_2, Et_2O or
$BH_3.SMe_2$, $AlCl_3$, THF or
BH_3-THF, rt*

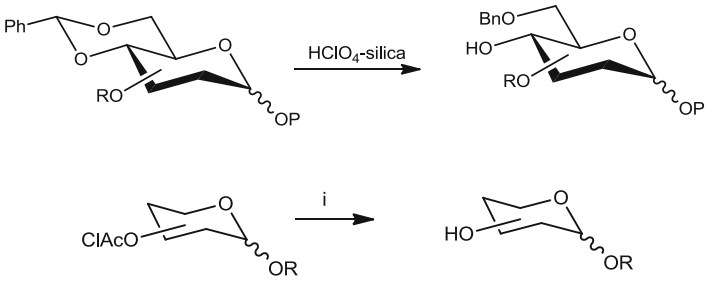
$BH_3.NMe_3$, $AlCl_3$, THF or
$BH_3.NMe_3$, $BF_3.OEt_2$, THF or
$BH_3.SMe_2$, $AlCl_3$, THF or
TfOH, EtSiH, -78°C*





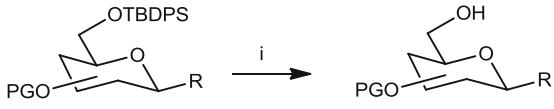
$NaBH_3CN$, I_2, CH_3CN, rt

Scheme 1.87 (continued)



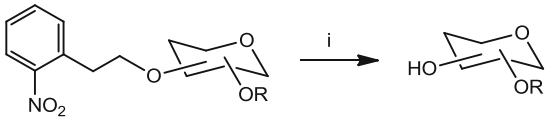
TBAF = tetra-n-butylammonium fluoride

i) TBAF, THF, rt.

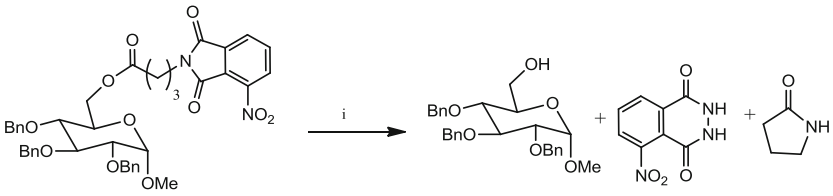


PG = Ac, Bz, Piv, Lev, Ms, Bn, All etc
R = OMe, OPh, OPMP, OPNP, STol, SPh, SEt etc

i) $TfOH\text{-}SiO_2$, MeCN



i) Zn, NH_4Cl, MeOH



i) H_2NNH_2-HOAc, DMF, 50°C.

Scheme 1.87 (continued)

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Chapter 2
O-glycoside Formation

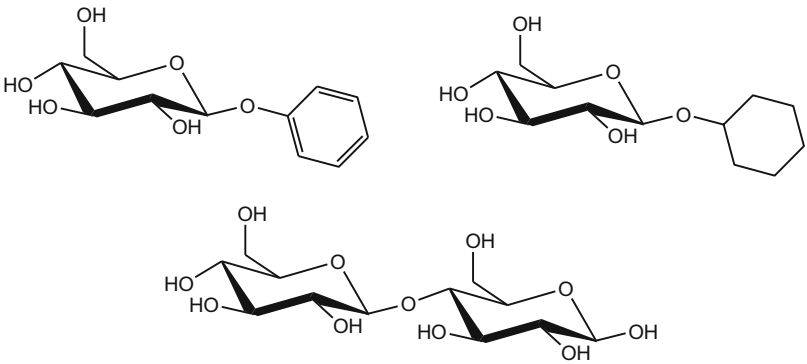
2.1 General Methods

When a monosaccharide (or a sugar fragment of any size) is condensed with either an aliphatic or aromatic alcohol, or another sugar moiety through oxygen, a glycoside bond is formed. General examples of *O*-glycosides are shown in Scheme 2.1.

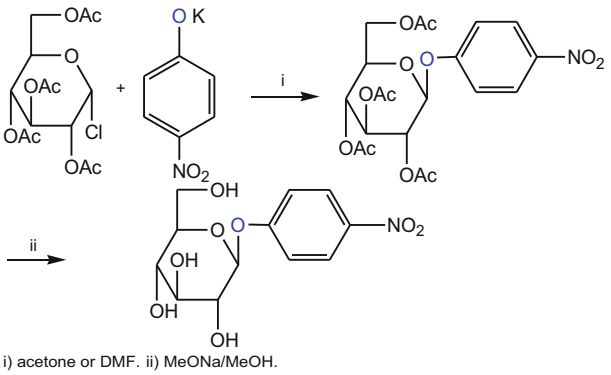
The most common coupling reaction methodologies used for preparing the vast majority of *O*-glycosides known thus far are as follows: [1]

Michael reaction
Fischer reaction
Koenigs–Knorr reaction
Helferich reaction
Fusion method
Imidate reaction
Glycal reaction
Sulfur reaction
Armed–disarmed approach
Unprotected anomeric carbon
Unprotected glycosylations
Miscellaneous leaving groups
Solid phase approach

M. Brito-Arias, *Synthesis and Characterization of Glycosides*,
DOI 10.1007/978-3-319-32310-7_2

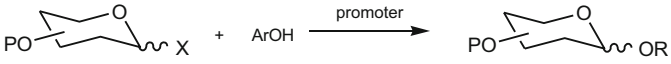


Scheme 2.1 Examples of *O*-glycosides



Scheme 2.2 Synthesis of paranitrophenyl-β-D-glucopyranosyl tetraacetate

2.1.1 Michael Reaction



Promoter	Conditions
NaH	THF
K_2CO_3, NaOH	Acetone

This pioneering methodology for *O*-glycosylation consists of the condensation reaction between 2,3,4,6-tetraacetyl-α-D-glucopyranosyl chloride and potassium phenoxide to generate the acetylated derivate that undergoes basic hydrolysis to give phenyl-β-D-glucopyranoside (Scheme 2.2). Since its original methodology, some modifications have been introduced especially for aromatic glycosides.

Some of the main features associated with this methodology are:

Preserves the pyranose or furanose ring
Drives the addition of the aromatic aglycon to the anomeric position
Uses protecting groups which are easily removed in basic medium
Produces exclusively the β-*O*-glycoside as a result of neighboring group participation

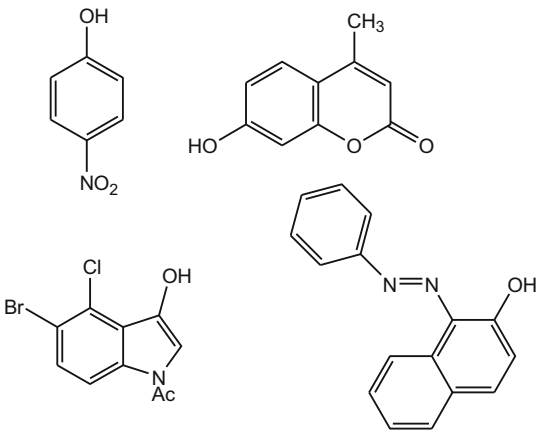
This reaction has been employed for the preparation of *O*-glycosides that are used as substrates for detection and measurement of enzymatic activity of most of the known glycosidases.

Using this methodology, several chromophores have been attached to most of the common monosaccharides. After *O*-glycoside cleavage by the enzyme, the release of the chromophore will indicate the sites and eventually will quantify the enzymatic activity. Some of the chromophores currently used for these purposes are represented in Scheme 2.3.

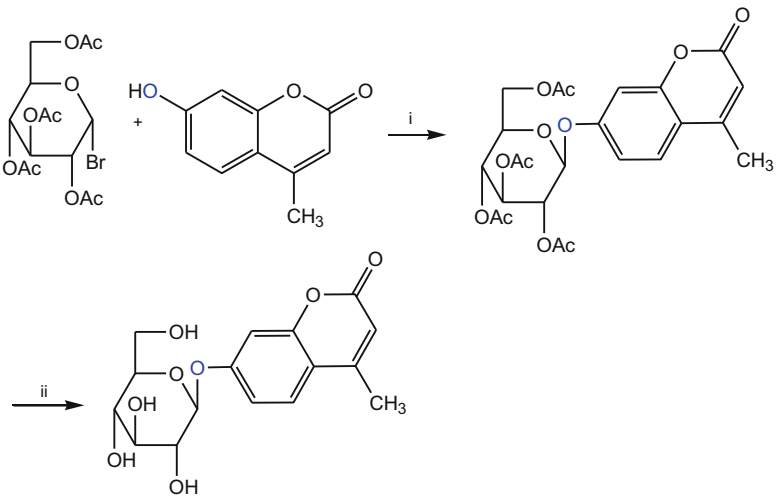
The highly fluorescent *O*-glycoside substrate 7-hydroxy-4-methylcoumarin-β-D-glucopyranose is prepared by condensation between acetobromoglucose and 4-methylumbelliferone in the presence of potassium carbonate in acetone. The intermediate is deacetylated under basic conditions to form umbelliferyl β-D-glucopyranoside (Scheme 2.4).

Anderson and Leaback [2] were able to prepare 5-bromo indoxyl-β-D-*N*-acetylglucopyranoside, a histochemical substrate for enzymatic detection of chitinase by condensing 3,4,6-triacetyl-β-D-*N*-acetylglucopyranoside chloride with 5-bromo-hydroxy-*N* acetyl indole at 0 °C under nitrogen atmosphere (Scheme 2.5).

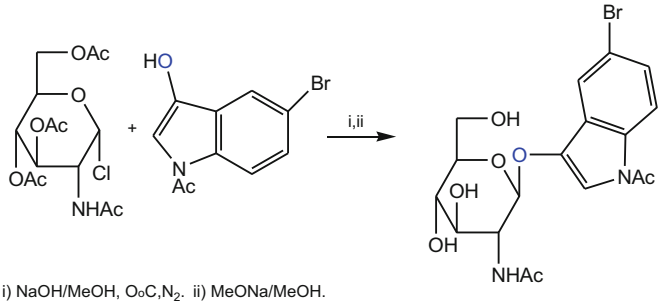
An alternative method for preparing the indoxyl glycosides was described more recently consisting in the coupling reaction between fucosyl bromide donor and indoxylic acid allyl ester under basic medium providing the *O*-glycosides in 84 % yield as β-anomer. This protocol was extended in the synthesis of sialic acid indoxyl glycosides (Scheme 2.6) [3].



Scheme 2.3 *O*-glycoside chromophores used for enzymatic detection

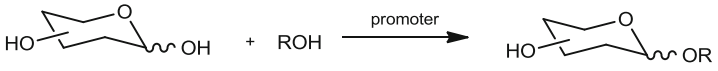


Scheme 2.4 Michael approach for preparation umbelliferyl-*O*-glycoside



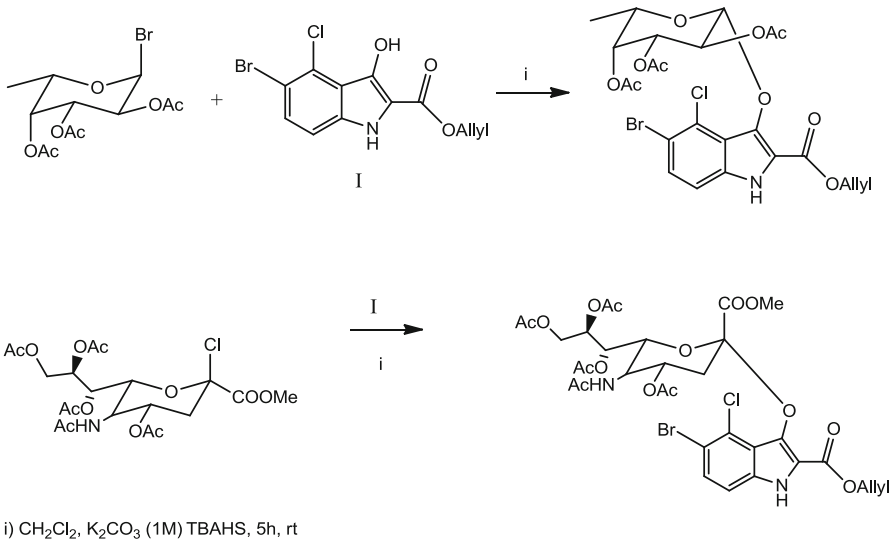
Scheme 2.5 Synthesis of indole *O*-glycoside derivative

2.1.2 Fischer Reaction



Promoter	Conditions
HCl gas	CH_2Cl_2, r.t.
pTsOH	CH_2Cl_2, r.t.

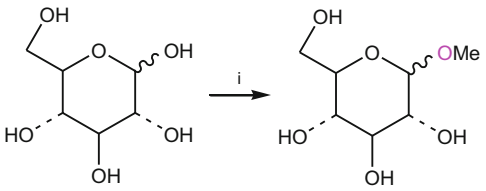
This straightforward strategy is used specially for the preparation of simple *O*-glycosides and the advantage of this methodology is that it does not require the use of protecting groups and simply by combining the free sugar with an alcohol



i) CH_2Cl_2, K_2CO_3 (1M) TBAHS, 5h, rt

Scheme 2.6 Alternative method for preparing the indoxyl glycosides

Scheme 2.7 Fischer *O*-glycoside reaction



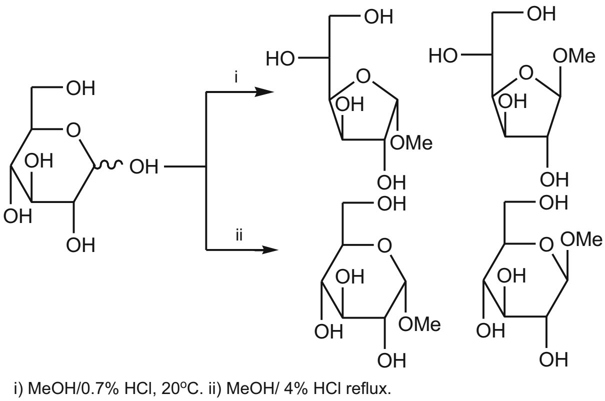
i) MeOH-HCl(g).

under acidic condition we furnish the corresponding *O*-glycoside. However, contrary to the previous method, this procedure is not stereo selective and therefore it provides a mixture of anomers. Also it has been found satisfactory only for small aliphatic alcohols (Scheme 2.7).

The addition of a controlled stream of dry HCl during a period of around 10 min at room temperature generally is the condition of choice. However, the use of Lewis acid, ion exchange resin and more recently triflic acid have been also reported providing good yields [4].

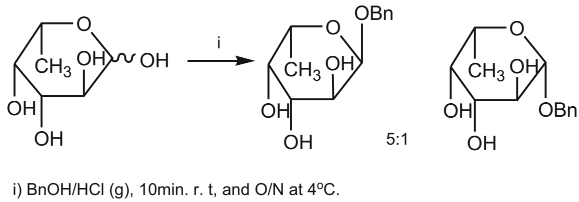
It is worth mentioning that besides the main product, a mixture of isomers has been detected, suggesting that a rather complex mechanism is involved. It is also seen that the amount of these isomers depends importantly on the condition reactions employed (Scheme 2.8).

The Fischer methodology has been applied successfully for the synthesis of benzyl *O*-glycosides. L-Fucose was converted into benzyl fucopyranoside [5] by treatment with benzyl alcohol under saturation with HCl at 0 °C, to furnish the α and β anomers (ratio 5:1) in 80 % yield (Scheme 2.9).

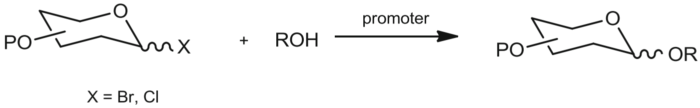


Scheme 2.8 Fischer *O*-glycoside isomers

Scheme 2.9 Fischer conditions for preparation of benzyl L-fucose

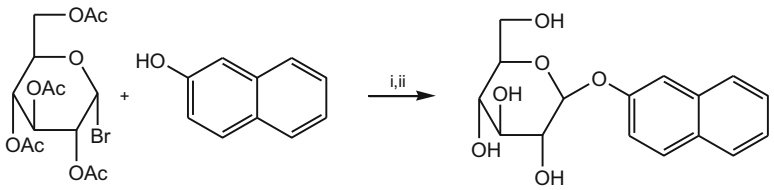


2.1.3 Koenigs–Knorr Reaction



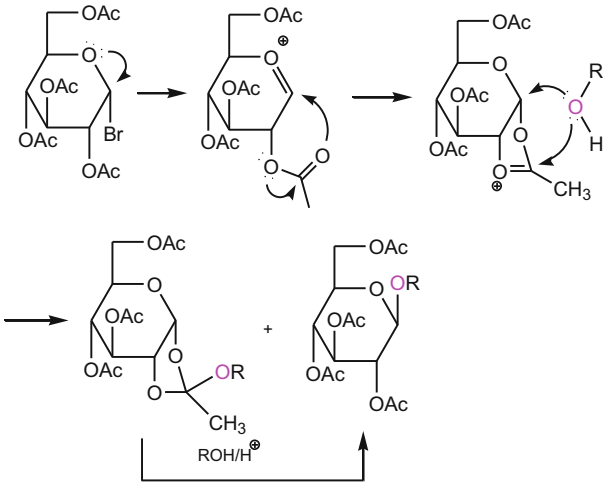
Promoter	Conditions
Ag_2CO_3	PhH, drierite (drying agent), I_2
Ag_2O	s-collidine (acid scavenger).
	CH_2Cl_2, 23 °C, borinic ester, ref. [6]
$AgNO_3$	HgO (acid scavenger)
$AgClO_4$	Ag_2ClO_3 (acid scavenger), THF or toluene, r.t.
AgOTf	CH_2Cl_2, r.t.
Silver silicate	CH_2Cl_2, 4 Å MS, –60 °C ref. [7]

This reaction reported in 1901 is still one of the most useful reactions for preparing a wide variety of *O*-glycosides [8]. It is useful for coupling reactions with either alkyl or aromatic alcohols as well as for coupling between sugars. The methodology requires silver salts as catalyst and among them the oxide, carbonate, nitrate, and



i) Ag_2O or Ag_2CO_3/PhH, drierite, I_2. ii) MeONa/MeOH.

Scheme 2.10 Koenigs–Knorr reaction

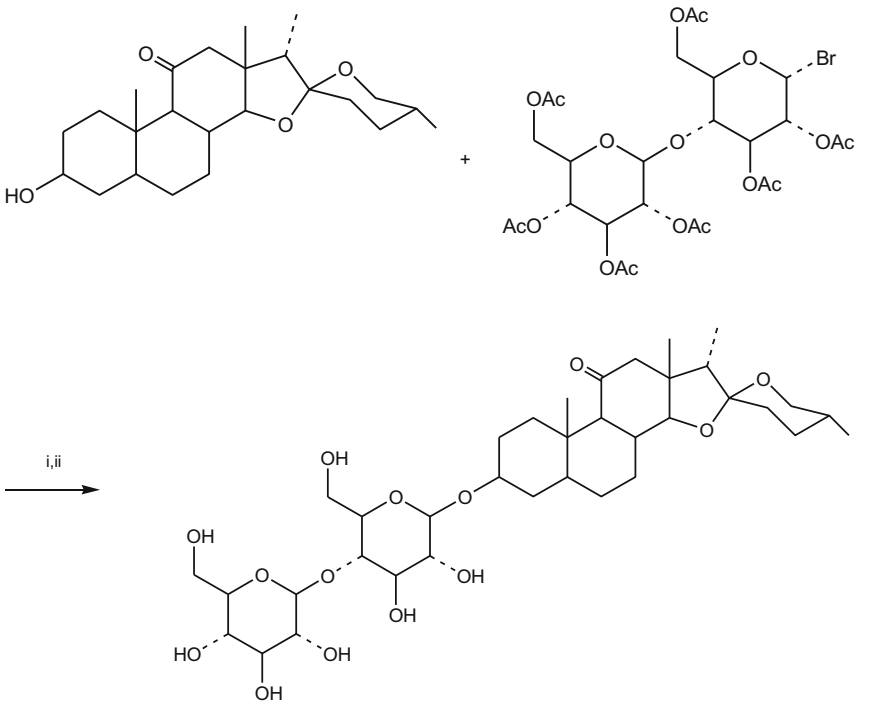


Scheme 2.11 Proposed mechanism for Koenigs–Knorr glycosidic reaction

triflate silver salts are the most commonly employed (Scheme 2.10). Also a drying agent such as calcium sulfate (drierite), calcium chloride, or molecular sieves is recommended. Improved yields are obtained with iodide, vigorous stirring, and protection against light during the course of the reaction.

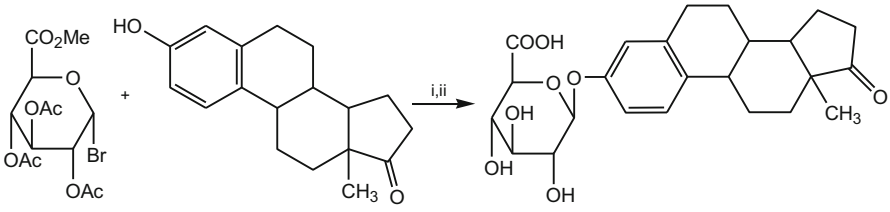
The stereochemistry observed is 1,2 trans type in most of the cases reported, as a consequence of neighboring group participation. When the protecting group is acetate at C (2), there is an intra molecular nucleophilic displacement of the leaving group, generating an orthoester [9]. This intermediate is responsible for the incorporation of the alcohol on the β-position (Scheme 2.11). Only until recently a method for preparing 1,2-cis glycosides has been developed involving the use of (1S)-phenyl-2-(phenylsulfanyl)ethyl moiety at C-2 of a glycosyl donor to give a quasi-stable anomeric sulfonium ion. The sulfonium ion is formed as a trans-decalin ring system. Displacement of the sulfonium ion by a hydroxyl leads to the stereoselective formation of α-glycosides [10].

This versatile methodology can be applied for preparation of alky, aryl, and oligosaccharide *O*-glycosides. A steroidal glycoside cholesterol absorption inhibitor



i) ZnF_2,CH_3CN. ii) NaOMe

Scheme 2.12 Synthesis of steroidal glycoside



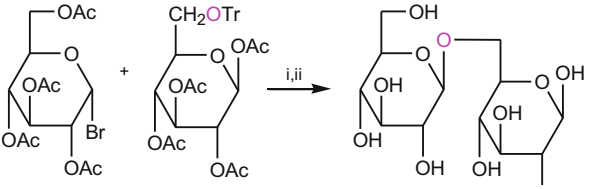
i) Cd_2CO_3. ii) MeONa/MeOH

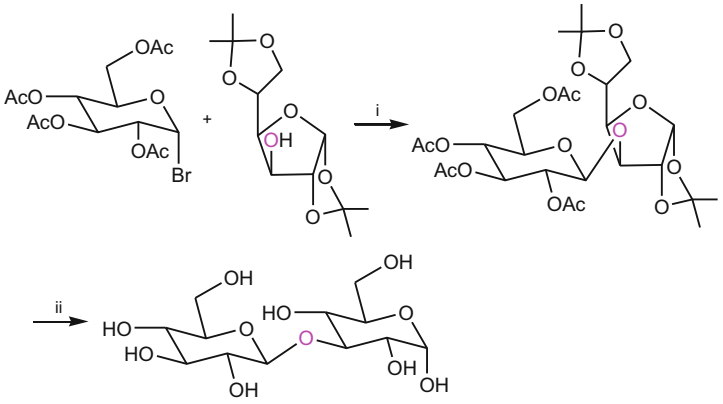
Scheme 2.13 Synthesis of a steroidal *O*-glycoside

was prepared by condensation between acetobromocellobiose and (3β,5α, 25R)-3-hydroxyspirostan-11-one with anhydrous ZnF_2 as catalyst in acetonitrile to provide the steroidal glycoside in 93 % yield (Scheme 2.12) [11].

The steroidal glycoside estrone-β-D-glucuronide was prepared by condensation between methyl tri-*O*-glucopyranosylbromide uronate and estrone, employing cadmium instead of silver carbonate (Scheme 2.13) [12]. For recent developments for the synthesis of *O*-glucuronides [13].

Scheme 2.14 Synthesis of gentobiose





Scheme 2.15 Synthesis of laminaribiose

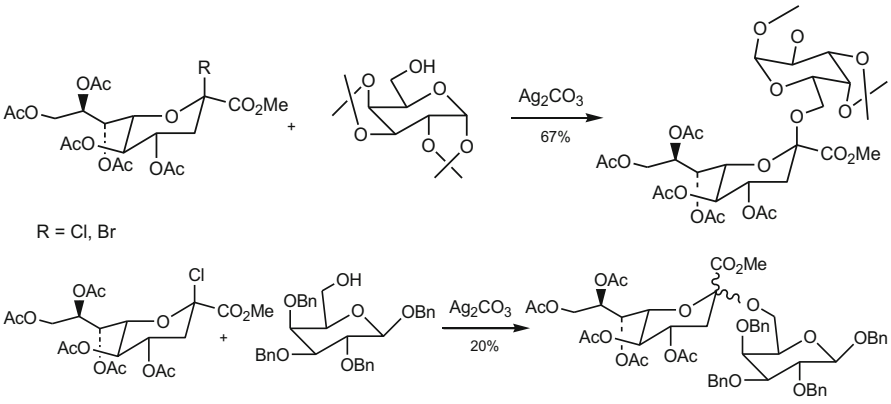
The syntheses of various disaccharides have been reported under Koenigs-Knorr conditions. Gentobiose octaacetate was prepared through condensation of acetobromoglucose with 1,2,3,4-tetra-*O*-acetyl-*O*-trityl-β-D-glucopyranose in nitromethane using silver perchlorate as catalyst (Scheme 2.14) [14].

Bächli and Percival [15] reported the synthesis of laminaribiose by reacting 1,2,5,6-diisopropylidenglucose with acetobromoglucose in the presence of silver carbonate, iodine, and drierite to produce an acetonide intermediate which upon treatment with oxalic acid and sodium methoxide furnished the 1,3-disaccharide (Scheme 2.15).

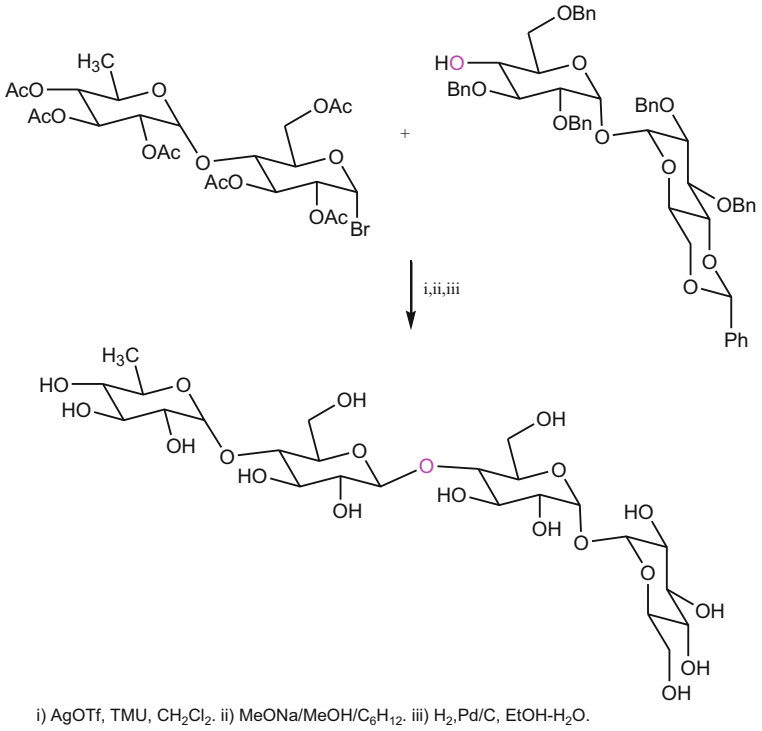
The synthesis of various disaccharides containing *N*-acetylneuraminic acid (Neu5Ac) was achieved by using acetochloro and acetobromo neuraminic acids as glycosyl donors with active glycosyl acceptors under Ag_2CO_3-promoted reactions conditions (Scheme 2.16) [16, 17].

These conditions are also suitable for preparing short oligosaccharides such as the one presented in Scheme 2.17. The donor sugar acetobromogentobiose is coupled to the acceptor intermediate using silver triflate as glycosidation catalyst [18].

Total synthesis of bleomycin group antibiotic has been achieved by Katano and Hecht [19]. Thus, glycoside coupling reaction of protected disaccharide glycosyl donor with histidine derivative using silver triflate as glycoside promoter provided bleomycin key intermediate in 21 % (Scheme 2.18).

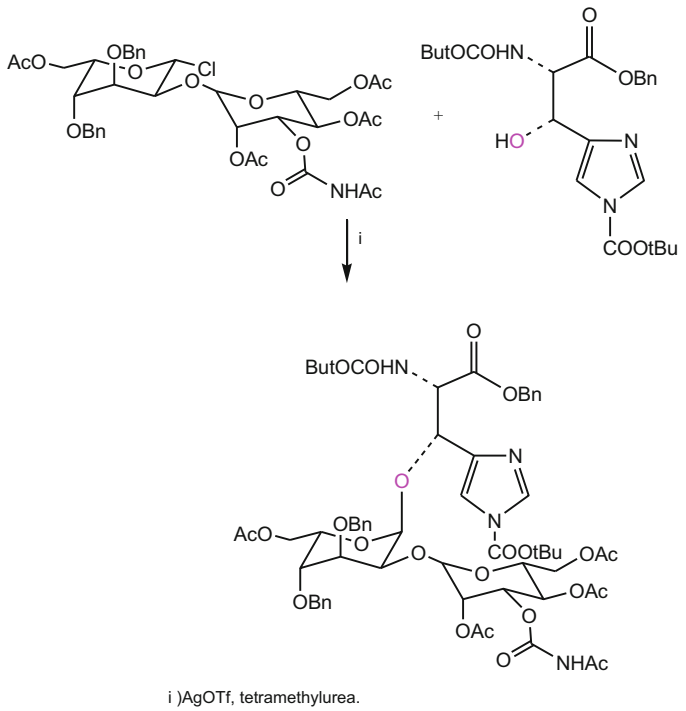


Scheme 2.16 Silver carbonate promoted synthesis of Neu5Ac(2→6) disaccharides

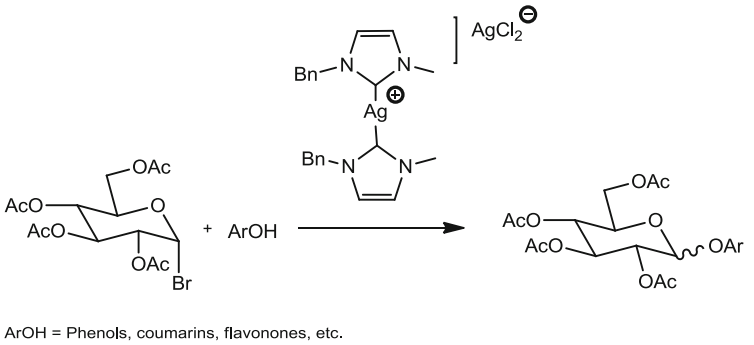


Scheme 2.17 Synthesis of tetrasaccharide

O-glycosidation reactions promoted via silver N-heterocyclic carbene complexes formed in situ in ionic liquids have been implemented. Good to excellent yields were obtained using Ag–NHC complexes derived from imidazolium halide salts to promote the glycosidation reaction (Scheme 2.19) [20].

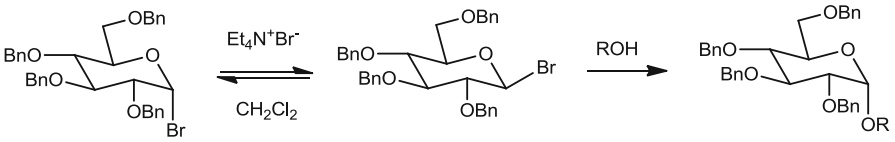


Scheme 2.18 Glycosylation reaction for preparation of bleomycin precursor



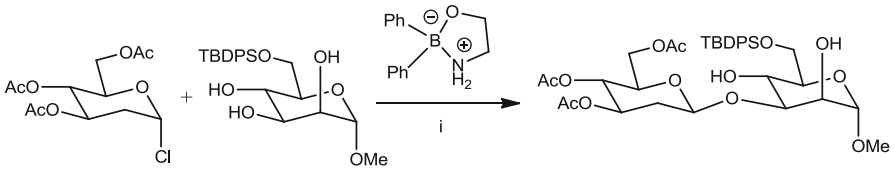
Scheme 2.19 O-glycosidation reactions promoted via silver N-heterocyclic carbene complexes

On the other hand it has been found that 1,2-cis glycosides can be synthesized from α-glycosyl bromide with aliphatic alcohols in the presence of tetraethylammonium bromide, under mild conditions reporting high yields. The α-stereoselectivity can be explained by an equilibrium between the glycosyl bromide promoted by the tetraethylammonium bromide and the nucleophilic attack on the oxonium ion generated during the interconversion (Scheme 2.20) [21].



R = Me, iPr, t-Bu-

Scheme 2.20 Preparation of α-glycosyl bromide with aliphatic alcohols in the presence of tetraethylammonium bromide

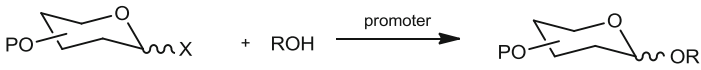


i) Ag_2O, CH_2Cl_2

Scheme 2.21 Glycosylation reaction in the presence of silver oxide and borinic acid derived catalyst

Deoxy aceto chloro glucose has been also used as glycosyl donors under silver oxide conditions providing disaccharides in high yields. Moreover, the use of borinic acid derived catalyst enhance the regioselective and β-selective reactions with acceptors having unprotected cis-1,2- and 1,3-diol groups (Scheme 2.21) [22].

2.1.4 Helferich Reaction

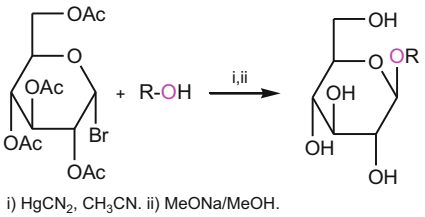


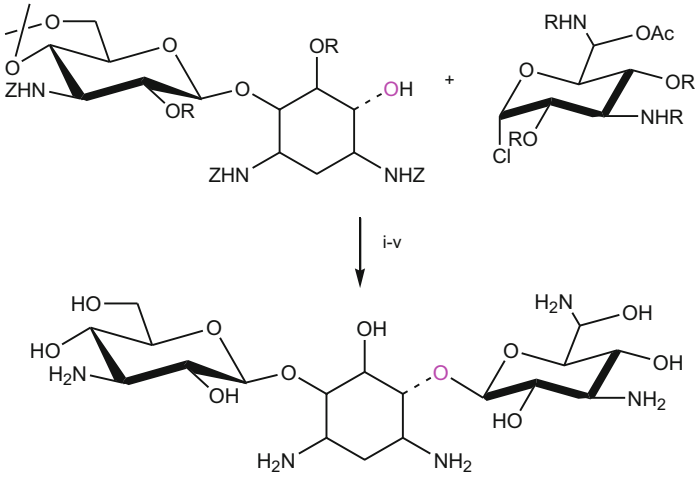
X = Br, Cl

Promoter	Conditions
$Hg(CN)_2$	CH_3CN
$HgBr_2$	CH_3CN
HgI_2	CH_3CN
ZnI_2	MS, CH_2Cl_2

This methodology is considered a modification of the previous one, and the main change being the use of mercury and zinc salts instead of silver. Also more polar solvents are used such as acetonitrile or nitromethane (Scheme 2.22). The yields reported for this reaction are up to 70 %, or higher; however, a mixture of anomers is often observed.

Scheme 2.22 Helferich general reaction



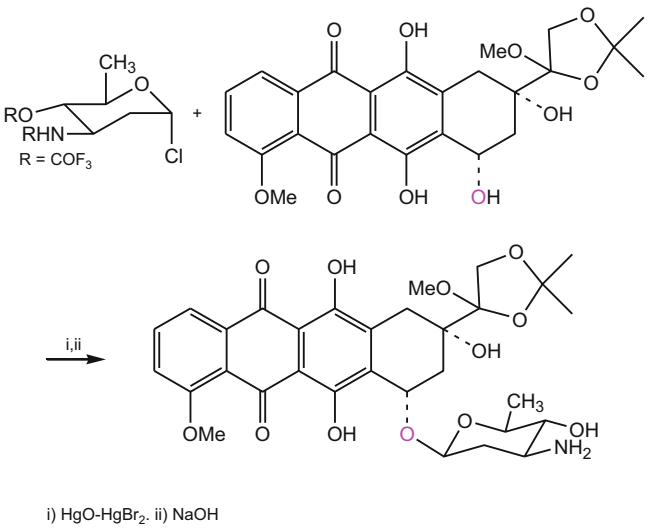


Scheme 2.23 Synthesis of a kanamycin A derivative

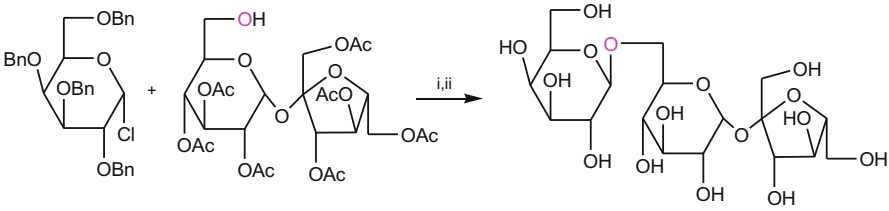
By following this strategy, Umezawa et al. [23, 159, 160] prepared kanamycin A by condensing 6-*O*-[2-*O*-benzyl-3-(benzyloxycarbonylamino)-3-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranosyl]-*N*,*N*′-di(benzoyloxycarbonyl)-2-deoxyestreptamine, as glycosyl acceptor with 2,3,4-tri-*O*-benzyl-6-(*N*-benzylacetamido)-6-deoxy-α-D-glycopyranosyl chloride, as glycosyl donor. The catalyst employed was mercury (II) cyanide (Scheme 2.23).

The antitumoral *O*-glycoside epirubicine was prepared under Helferich conditions [24] using the acetonide form of adriamycinone and 2,3,6-trideoxy-3-trifluoroacetamido-4-*O*-trifluoroacetyl-α-L-arabinohexopyranosyl chloride, and a mixture of mercury (II) oxide and bromide as shown in Scheme 2.24.

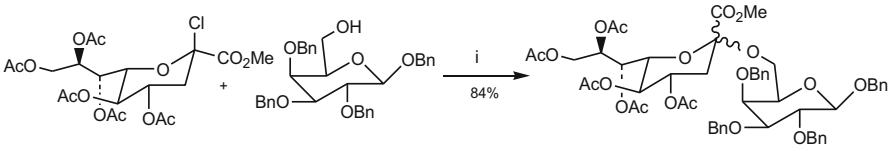
Other coupling reactions between sugars under Helferich conditions have been as well described [25]. For example the case of trisaccharide raffinose prepared by condensation between tetra-*O*-benzyl-α-D-galactopyranosyl chloride as donor and 2,3,4,1′,3′,4′,6′-hepta-*O*-acetyl sucrose as acceptor (Scheme 2.25).



Scheme 2.24 Synthesis of epirubicin



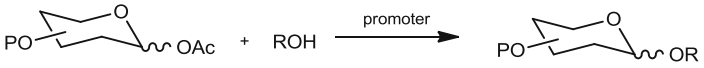
Scheme 2.25 Synthesis of raffinose derivative



Scheme 2.26 Helferich conditions for the preparation of sialic disaccharide

Helferich conditions have been used for preparing disaccharides containing Neu5Ac(2→6)Gal and Glc in good yields, although with low stereocontrol (α:β 3:4) (Scheme 2.26).

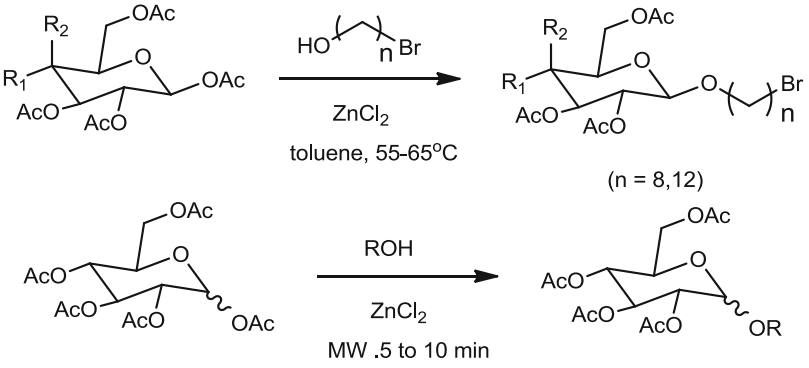
2.1.5 *Acetate Donors*

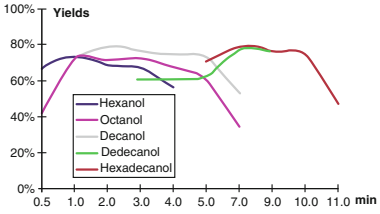


Promoter	Conditions
$ZnCl_2$	Heat, or MW
$ZnCl_2$	120 °C
$SnCl_4$	5–10 °C
TsOH	120 °C

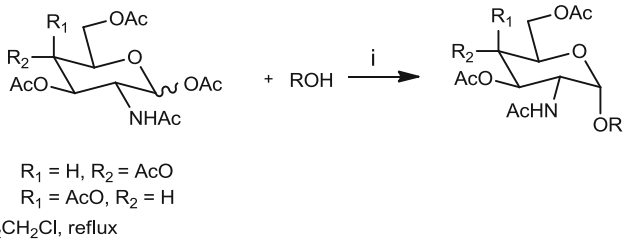
This method has been used for preparing long chain and aromatic glycosides under different acid promoters such as $ZnCl_2$, $SnCl_4$, $FeCl_3$, TsOH, or zeolite. Particularly the use of $ZnCl_2$ as promoter has been successfully utilized to attach long chain alcohol to peracetate saccharides with moderate heating or microwave conditions to produce amphipathic glycosides in moderate to good yields as mainly the 1,2-*trans*-glycosides or as a mixture of anomers (Scheme 2.27) [26, 27].

A one-step procedure for the preparation of α-*O*-glycosamine pentaacetylated glycosides with yields up to 70 % and high α-stereoselectivity was achieved by condensation between commercially available D-glycosamine pentaacetates and fluorogenic coumarins, substituted phenols, and protected serine acceptors under ferric chloride conditions (Scheme 2.28) [28].



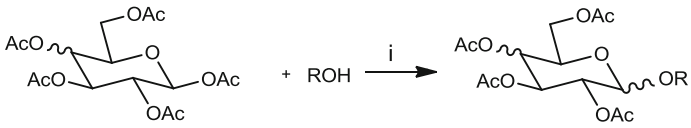


Scheme 2.27 Preparation of long chain and aromatic glycosides under different acid promoters



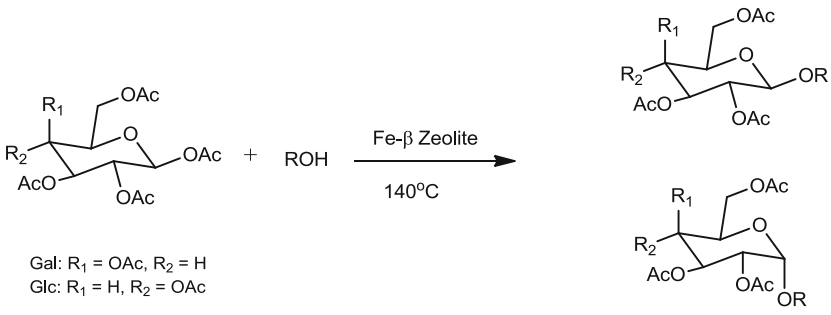
R_1 = H, R_2 = AcO
R_1 = AcO, R_2 = H
i) $FeCl_3$, $ClCH_2CH_2Cl$, reflux

Scheme 2.28 Preparation of α-*O*-glycosamine pentaacetylated glycosides



i) $SnCl_4$+CF_3CO_2Ag or $SnCl_4$

Scheme 2.29 O-glycosidation protocol under $SnCl_4$ or silver triflate an $SnCl_4$ conditions



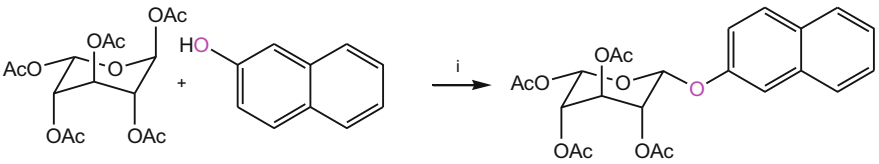
Gal: R_1 = OAc, R_2 = H
Glc: R_1 = H, R_2 = OAc
ROH = Cetyl alcohol, dodecyl alcohol, octanol, 2-ethylhexanol

Scheme 2.30 Heterogeneous catalysts for the preparations of alkyl glycosides

Another simple method for *O*-glycosidation under $SnCl_4$ or silver triflate an $SnCl_4$ is described reporting high yields as a mixture of anomers depending on the as bulkiness, presence of electron-withdrawing groups or polyethoxy motifs (Scheme 2.29) [29].

The application of zeolites as heterogeneous catalysts for the preparations of alkyl glycosides is an alternative method due to the acid strength and larger pore openings and channel intersections. Thus, the Fe-β zeolite gave the maximum yield of 63 % of cetyl galactopyranoside as a mixture of anomers (Scheme 2.30) [30].

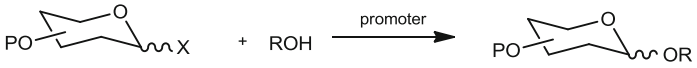
This methodology has been also useful to synthesize 1-naphthyl 2,3,4,6-tetra-*O*-acetyl-α,β-L-idopyranoside by mixing 1,2,3,4,6-penta-O-acetyl-α-L-idopyranose, 1-naphthol, zinc chloride and heating up to 120 °C during 1 h (Scheme 2.31) [31].



i) $ZnCl_2$,120ºC, 1h.

Scheme 2.31 Preparation of naphthyl *O*-glycosides with peracetylated sugars with naphthols under $ZnCl_2$ catalyst

2.1.6 *Imidate Reaction*



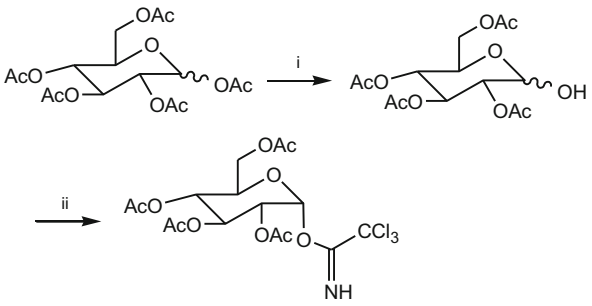
X = $OC(NH)CCl_3$

X = $OC(NPh)CF_3$

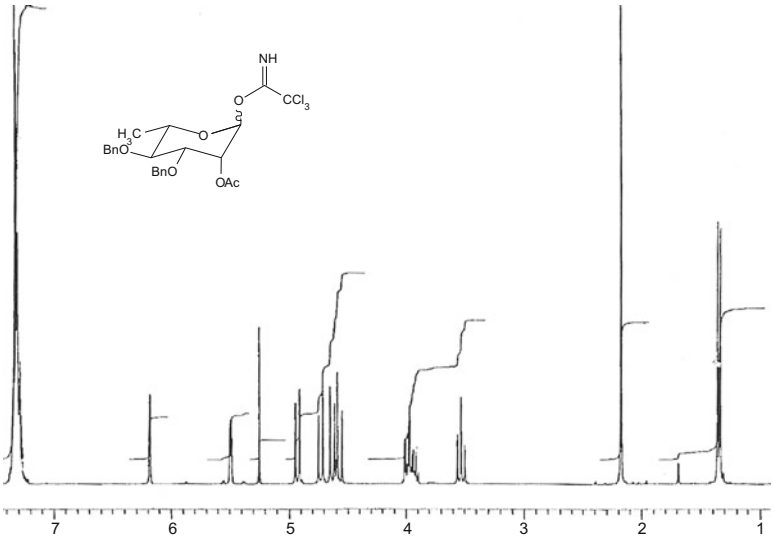
Imidate	Promoter	Conditions
$OC(NH)CCl_3$	AgOTf	CH_2Cl_2, 0 °C → r.t.
$OC(NH)CCl_3$	TMSOTf	CH_2Cl_2 or MeCN, 0 °C
$OC(NH)CCl_3$	BF_3-OEt_2	CH_2Cl_2 or MeCN, −20 °C
$OC(NH)CCl_3$	NaH	CH_2Cl_2
$OC(NH)CCl_3$	$PhBF_2$	CH_2Cl_2, −78 °C, ref. [32]
$OC(NH)CCl_3$	Chiral Brønsted acid catalyst	toluene ref. [33]
$OC(NH)CCl_3$	2 mol% $Pd(PhCN)_2Cl_2$, 4 mol% AgOTf	CH_2Cl_2, −78 °C, ref. [34]
$OC(NPh)CF_3$	TBSOTf	4 Å MS, toluene, −40 °C

This protocol is attributed to Schmidt and coworkers [35, 161] who introduced trichloroacetimidate as a good leaving group for preparation of *O*-glycosides. A significant number of simple and complex *O*-glycosides involving the imidate coupling reaction have been described. This strategy involves the use of trichloroacetonitrile that in the presence of a base is incorporated on the anomeric hydroxyl group to generate trichloroacetimidate (Scheme 2.32). It should be noted that the resulting imidate derivative is air sensitive and should be used in coupling reactions immediately following preparation. Imidate formation might be spectroscopically detected by ^{1}H NMR through a signal appearing down field at 6.2 ppm [36].

Once the imidate if formed, it can be subjected to nucleophilic attack to provide the corresponding *S*-, *N*-, *C*-, or *O*-glycoside, depending on the chosen nucleophile. The use of a catalyst such as $BF_3.OEt_2$, TMSOTf, or AgOTf is necessary to carry out the reaction to completion (Scheme 2.33). Although the unquestionable applicability of this approach, an undesirable side reaction has been encountered with glycosyl trichloroacetimidates in the presence of Lewis acid catalysis via the Chapman rearrangement [35, 161].



i) Bn-NH_2, HCl, THF. or NH_2NH_2 ii) Cl_3CN, $CsCO_3/CH_2Cl_2$, r.t.

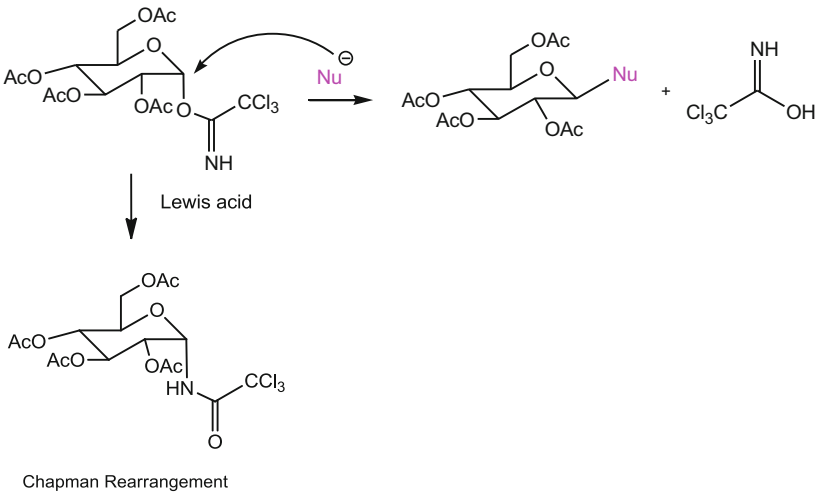


Scheme 2.32 Preparation of glycosyl imidate and ^{1}H NMR of imidate rhamnosyl derivative

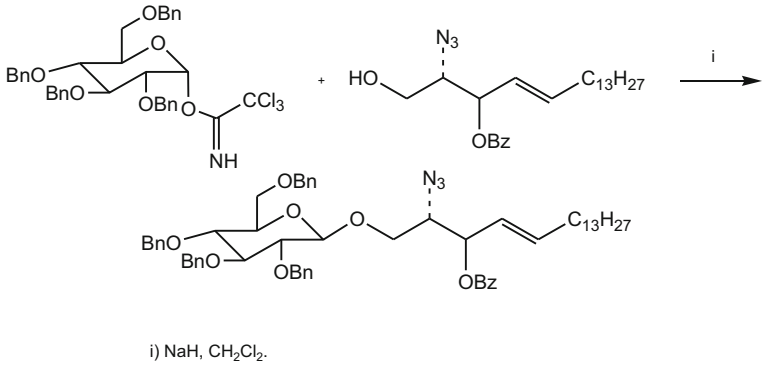
Hasegawa et al. [37] has prepared the ganglioside shown in Scheme 2.34 using 2,3,4,6-tetrabenzylglucopyranosyl-α-acetimidate with the lipophilic alcohol, to generate a ganglioside.

The total synthesis of calicheamicin α and dynemicin A has been described by Danishefsky's group [38], and involves glycosylation of calicheamicinone congener with the complex glycosyl imidate using $BF_3.OEt_2$ as Lewis acid catalyst (Scheme 2.35).

Naturally occurring herbicides known as tricolorin A, F and G were isolated from the plant *Ipomoea tricolor* and since then synthesized involving glycoside coupling reactions. The first total synthesis of tricolorin A was performed by Larson and Heathcock [39], involving three coupling reactions steps with imidate intermediates used as glycosyl donors (Scheme 2.36). The lactonization key step for the preparation of the synthesized tricolorins has been achieved either under macrolactonization



Scheme 2.33 Nucleophilic displacement of imidate leaving group

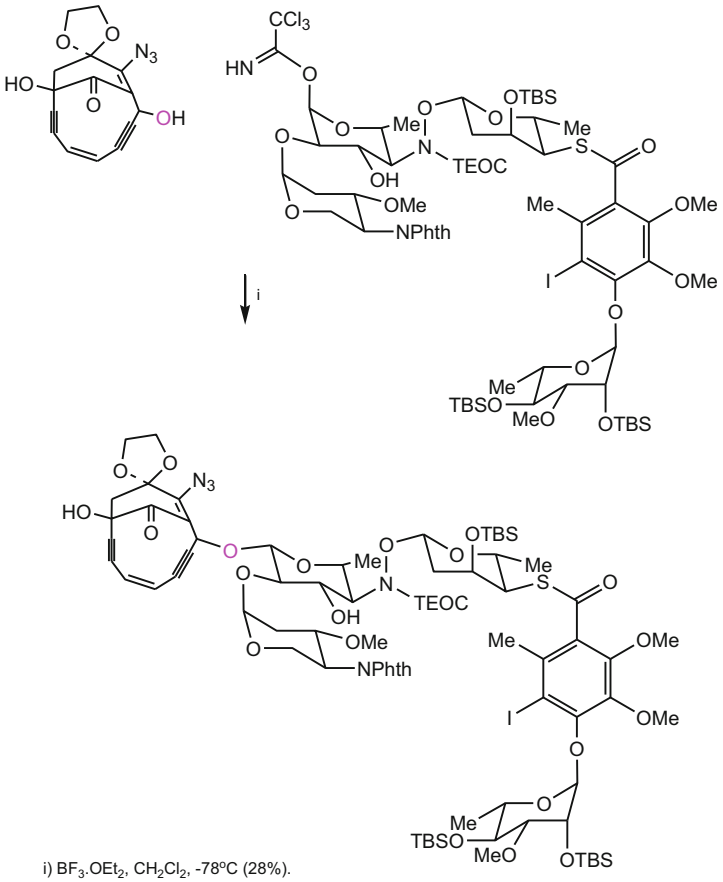


Scheme 2.34 Coupling reaction for the preparation of ganglioside

conditions reported by Yamaguchi [40, 41] and also under ring closure methathesis conditions [36].

Another hetero-trisaccharide resin glycoside of jalapinolic acid known as tricolorin F has been synthesized involving coupling reactions with imidates as glycosyl donors. In this way disaccharide and trisaccharide were prepared sequentially. The resulting tricoloric acid C derivative was deprotected and subjected to lactonization under Yamaguchi conditions to produce protected macrolactone. Final removal of acetonide and benzyl protecting groups provided Tricolorin F (Scheme 2.37) [41].

A convergent approach for obtaining a tumoral antigen fragment of Lewis^x has been developed by Boons et al. [42, 162] Condensation of the imidate glycosyl donor and the trisaccharide glycosyl acceptor provided the hexasaccharide, which

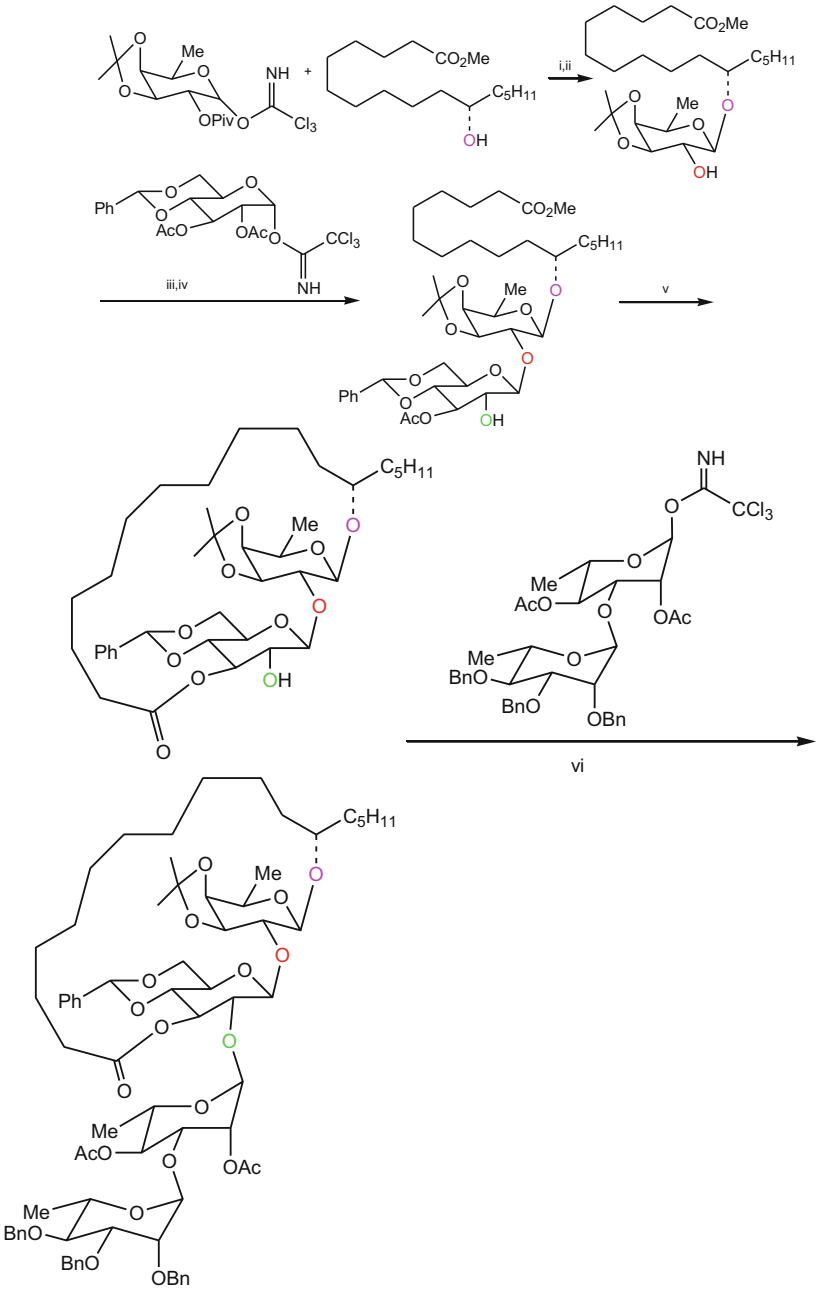


Scheme 2.35 Glycosylation of calicheamicinone congener

was further allowed to react with trichloroacetimidate to generate a hexasaccharide glycosyl donor. The final coupling reaction with the disaccharide using $BF_3.OEt_2$, furnished the tumoral fragment Lewis^X (Scheme 2.38).

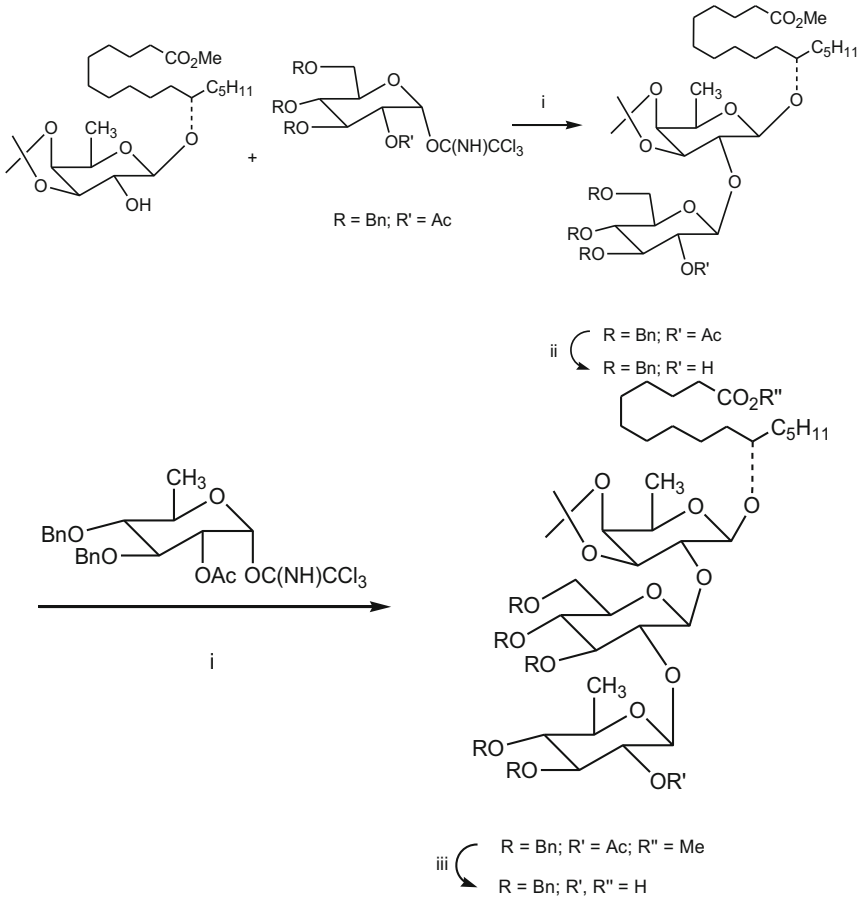
Selectins (E, P, and L) are mammalian C-type lectins involved in the recognition process between blood cells or cancer cells and vascular endothelium. L-selectins plays a key role in the initial cell-adhesive phenomena during the inflammatory process, whereas E-selectins binds strongly to sialyl Lewis^A and Lewis^X [43, 44, 163–165]. It has been found that the tetrasaccharide sialyl Lewis^X is the recognition molecule and the preparation of sialyl Lewis^X confirmed the hypothesis that sulfation increase the affinity for L-selectins [45]. The chemical synthesis of 3e- and 6e-monosulfated and 3e,6e-disulfated Lewis^X pentasaccharides has been prepared according to the Scheme 2.39.

Likewise, thioaryl donors can also be suitably converted to acetimidates for performing glycoside coupling reactions. This is the case of arabinosyl thio derivative



i) AgOTf, CH_2Cl_2. ii) MeONa/MeOH. iii) AgOTf, CH_2Cl_2. iv) a) MeONa/MeOH. b) 1eq. Ac_2O, DMAP, CH_2Cl_2, Et_3N. v) a) LiOH, THF, H_2O. b) 2,4,6-trichlorobenzoyl chloride, Et_3N, MAP, benzene. vi) AgOTf, CH_2Cl_2.

Scheme 2.36 Synthesis of tricolorin A precursor

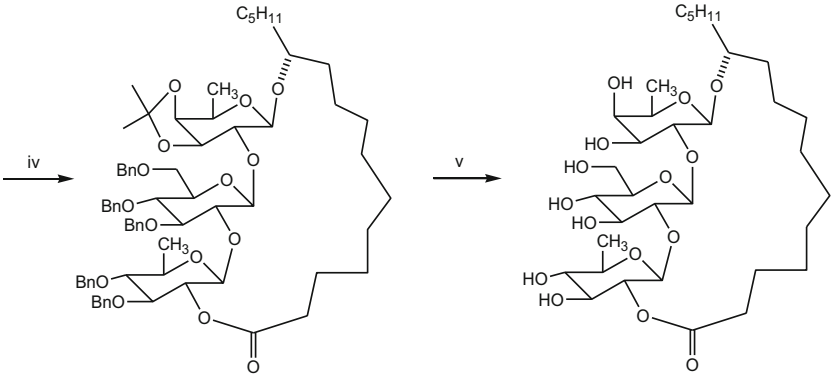


Scheme 2.37 Synthesis of tricolorin F

which is deprotected under NBS-pyridine conditions forming the lactol in 80 % yield as a mixture of anomers (2:1). Treatment with NaH, followed by addition of Cl_3CCN provided the desired trichloroacetimidate intermediate. This strategy has been successfully applied in the syntheses of cytotoxic marine natural products eleutherobin (Scheme 2.40) [46].

Fluorogenic aglycones such as 4-methylumbelliferyl have been attached to peracetylated imidates providing the alpha anomer only when TMSOTf was used as promoter at −20 °C (Scheme 2.41). The resulting glycoside was further used for preparing a 4-MU α-T-anitgen [47].

In order to understand the α-stereoselectivity the authors proposed that the imidates in the presence of TMSOTf generate an oxocarbenium triflate ion pair which in turn will accept the nucleophilic attack, favoring an alpha glycoside formation due to the extra stability arising from through-space electrostatic interaction between the axially disposed C-4 acetyl function and ring oxygen atom of the corresponding α-glycosyl oxonium ion (Scheme 2.42).



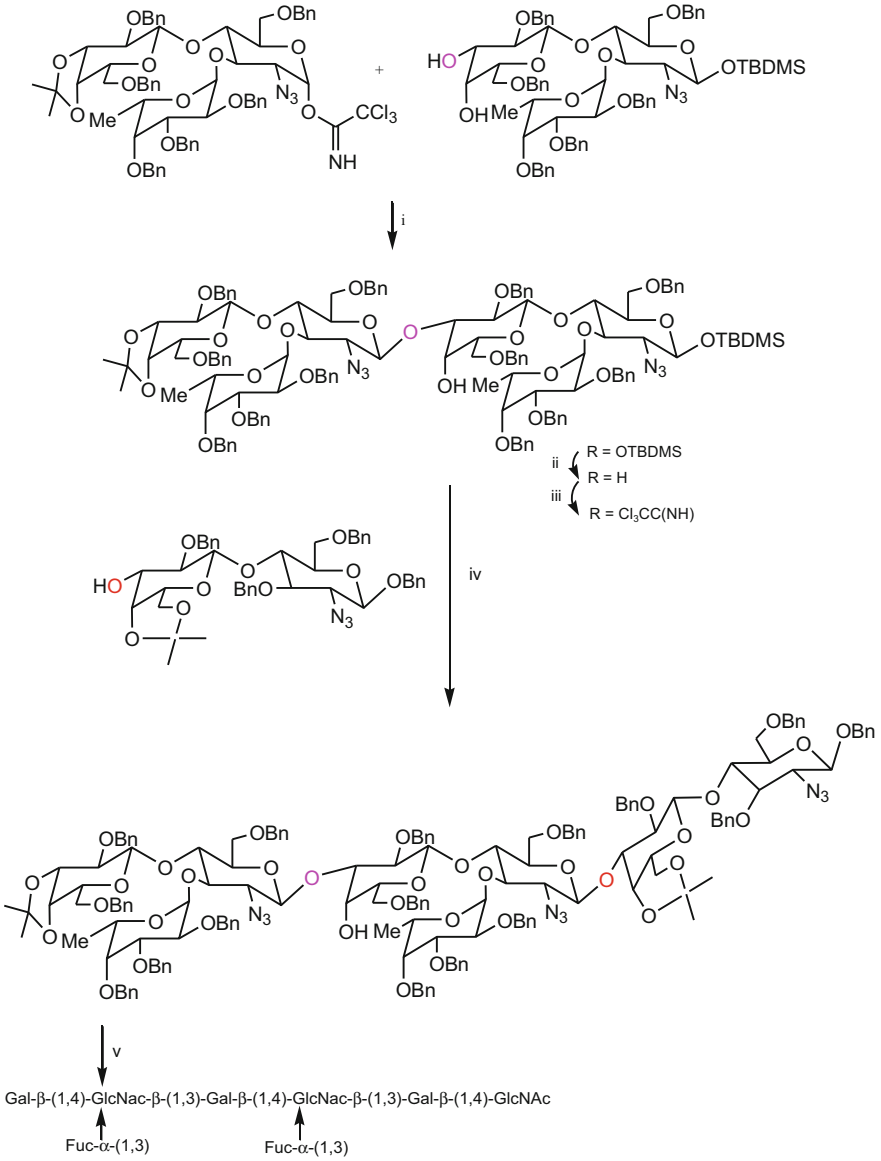
i) $BF_3.Et_2O$, CH_2Cl_2, -20°C, 1 h; (ii) NaOMe, MeOH, 6h, rt. iii) KOH, MeOH-H_2O, 4 h, reflux. iv) 2,4,6-trichlorobenzoyl chloride, Et_3N, DMAP, PhH. v) 10% HCl-MeOH, $Pd(OH)_2$-C 10%, MeOH.

Scheme 2.37 (continued)

Another approach leading to the preparation of amino acid glycosides with enhanced α-stereoselectivity was described involving trichloroacetimidate donors with non-participating protecting groups with protected amino acids using the heterogeneous catalyst, $HClO_4$–SiO_2, reporting high yields (Scheme 2.43) [48].

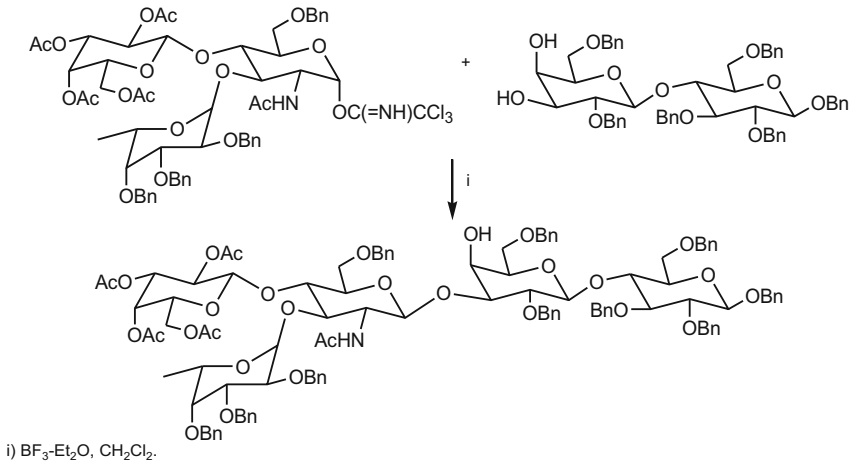
An additional utility of trichloroacetimidates as leaving group is its ability to be transformed to ureas with α-stereoselectivity via nickel-catalyzed [1,3]-rearrangement and subsequent treatment with secondary amines under the conditions described in Scheme 2.44 [49].

Another approach involving imidates was assayed with trifluoroacetimidate as leaving group and a disaccharide acceptor, using CH_2Cl_2 as solvent and TBSOTf as the promoter. Under these conditions different α:β ratios were observed, however by lowering the temperature from −20 °C to −40 °C and improved α:β ratio was obtained while keeping the good yields (Scheme 2.45) [50].



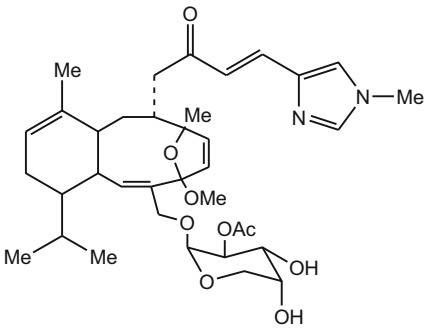
i) $BF_3.OEt_2$, CH_2Cl_2. ii) TBAF, AcOH. iii) Cl_3CCN, DBU. iv) $BF_3.OEt_2$, CH_2Cl_2.
v) a) AcOH. b) H_2, Pd-C.

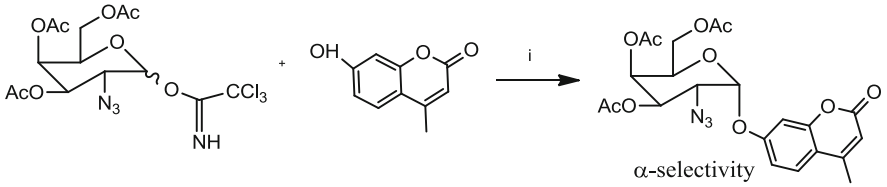
Scheme 2.38 Convergent synthesis of Lewis^X fragment



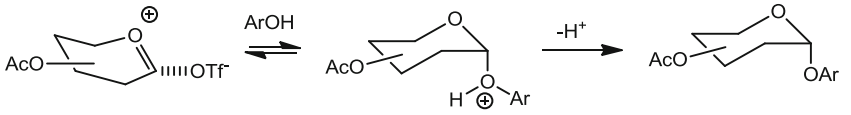
Scheme 2.39 Coupling reaction for the preparation of Lewis^X pentasaccharide intermediate

Scheme 2.40 Cytotoxic marine glycoside eleutherobin

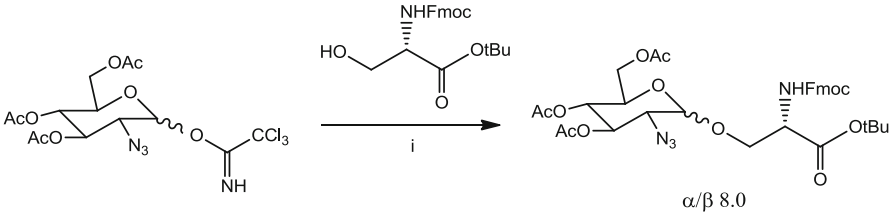




Scheme 2.41 Synthesis of α-4-methylumbelliferyl glycosides

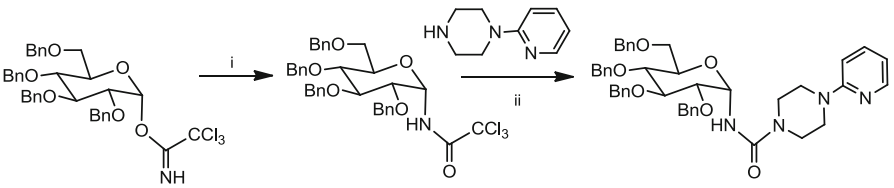


Scheme 2.42 Proposed oxocarbenium triflate ion intermediates leading to α-stereoselectivity



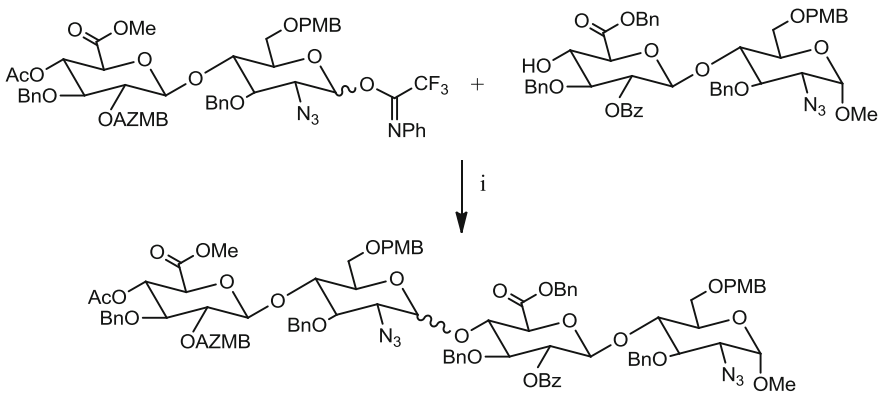
i) $HClO_4$-SiO_2, CH_2Cl_2-dioxane, 0°C

Scheme 2.43 Preparation of α-amino acid glycosides from imidates



i) $Ni(dppe)Cl_2$, AgOTf, CH_2Cl_2, 25°C. ii) Cs_2CO_3, DMF

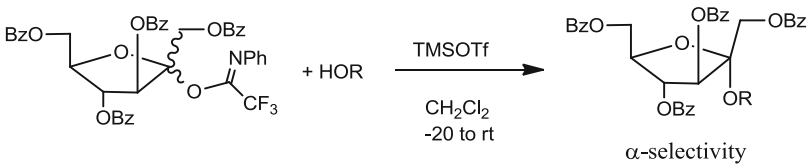
Scheme 2.44 Preparation of glycosyl ureas from imidates



i) TBSOTf,4 Å MS, toluene, -40 °C, 71%

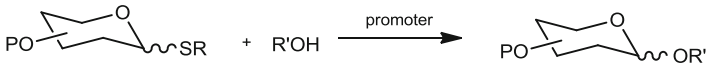
Scheme 2.45 Synthesis of tetrasaccharides from phenyl trifluoroacetimidate as glycosyl donor

Likewise, fructofuranosides having *N*-phenyl trifluoroacetimidate as leaving group formed α-*O*-glycosides for different aglycons such as admantanol, protected sugars, phenols, and flavonoids, when TMSOTf is used as promoter at low temperature (Scheme 2.46) [51].



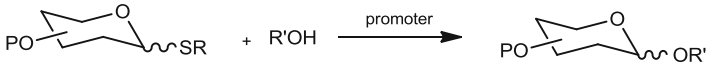
Scheme 2.46 Preparation of fructofuranosyl glycosides from *N*-phenyl trifluoroacetimidate as leaving group

2.1.7 Sulfur Reaction



R = Me, Et

Promoter	Conditions
NIS-TfOH	0 °C → r.t.
$HgCl_2$	CH_2Cl_2 or MeCN, 0 °C
$CuBr_2$-Bu_4NBr-AgOTf	CH_2Cl_2 or MeCN, −20 °C
MeOTf	Et_2O, r.t.
MeSOTf	Et_2O, r.t.
AgOTf-Br_2	CH_2Cl_2
DMTST	MeCN, −15 °C
NBS-TfOH	EtCN, −78 °C

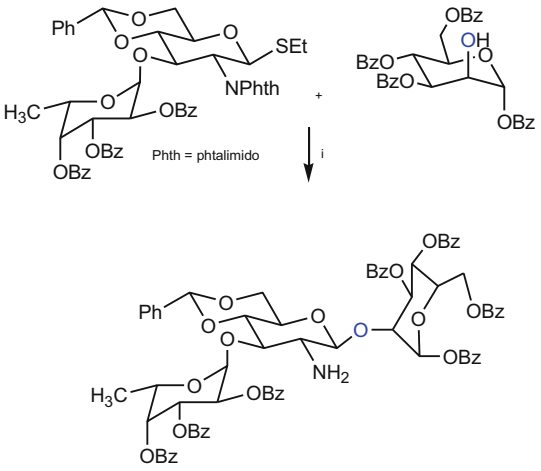


R = Ph, Tol

Promoter	Conditions
NIS/TfOH	MeCN
NBS	CH_2Cl_2, r.t.
BSP	CH_2Cl_2, MS, r.t
DMTST	CH_2Cl_2
MeOTf	CH_2Cl_2
MeSOTf	CH_2Cl_2
(a) Ph_2SO, Tf_2O (b) TBAI	CH_2Cl_2, MS, −78 °C, ref. [52]
NIS, AgOTf	CH_2Cl_2, MS, −45 °C ref. [53]

Thioglycosides are useful glycosyl donors widely used in the preparation of *O*-glycosides. An example of their applicability for the preparation of saccharide synthesis is represented in Scheme 2.47. Thus, the synthesis of trisaccharide intermediate was obtained by combining the thioglycoside donor with a monosaccharide

Scheme 2.47 Thioglycoside coupling reaction for preparation of a trisaccharide intermediate



i) $CF_3SO_3CH_3$, Et_2O, MS, rt. ii) a) NH_2-NH_2.H_2O, EtOH reflux. b) Ac_2O, Py

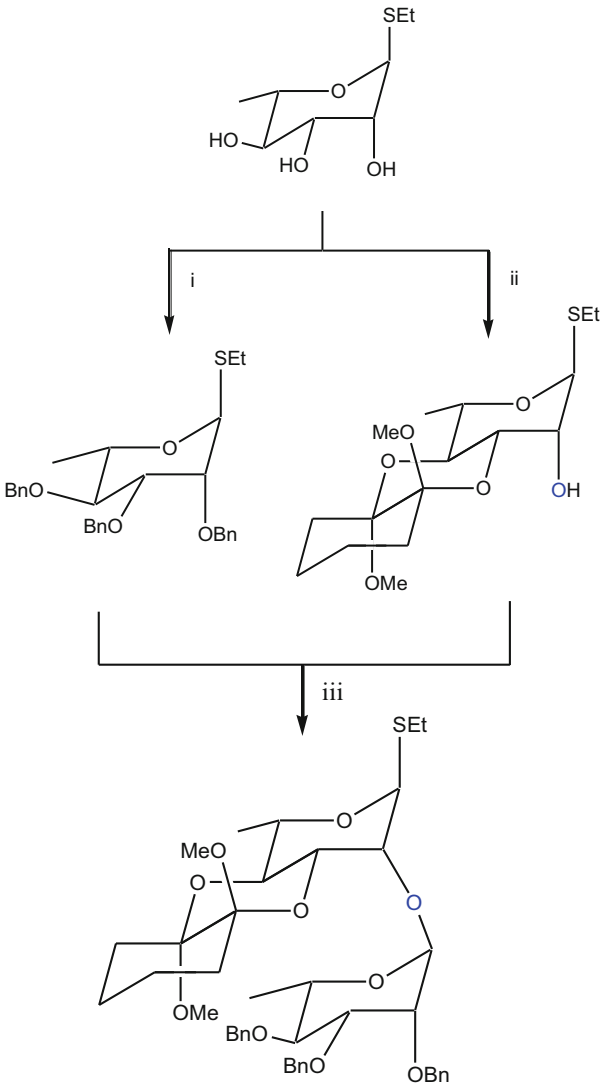
acceptor in the presence of methyltriflate, to provide the target trisaccharide in 72 % yield [54].

A convergent synthesis of the trisaccharide unit belonging to an antigen polysaccharide from streptococcus has been performed by Ley and Priepke [55]. In this approach rhamnosylalkylsulfur was used as the glycosyl donor, and cyclohexane-1,2-diacetal as the protecting group (Scheme 2.48).

Thioalkyl donors are also useful derivatives for the preparation of biologically important natural sugars known as sialic acids [23, 159, 160]. An efficient procedure for introducing thioalkyl groups as leaving groups involves the conversion of acetate into thiomethyl by treatment with methylthiotrimethylsilane in the presence of TMS-triflate. *O*-glycosylation reaction proceeds between the thioglycosylsialic donor and a glycosyl acceptor (bearing an -OH group available), using a catalyst such as *N*-iodosuccinimide-TfOH as promoter (Scheme 2.49) [56].

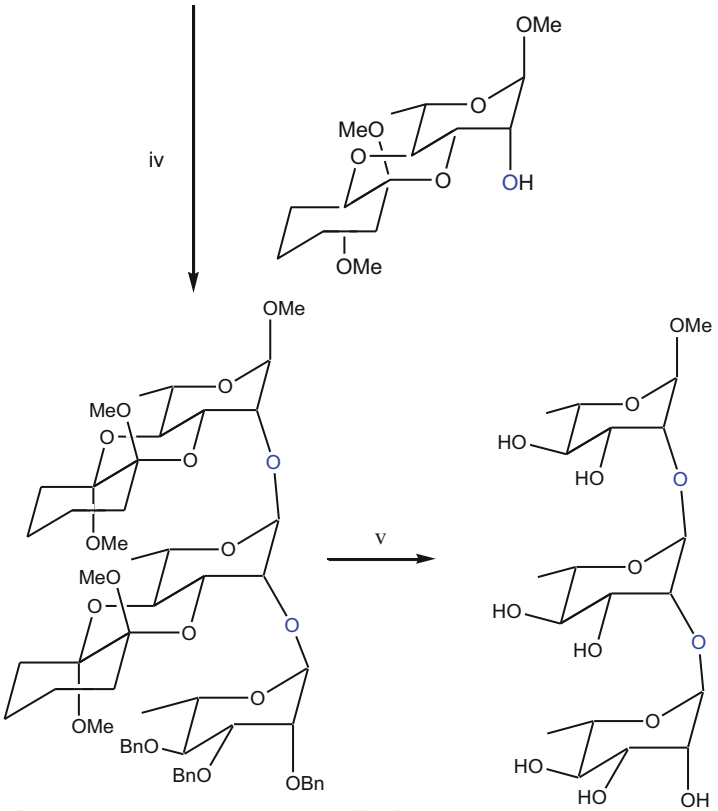
The synthesis of aryl 2-deoxy-D-glycopyranosides from 2-deoxy-1-thioglycosides and differently substituted phenols and naphthols under *N*-iodosuccinimide/triflic acid conditions is reported. The analysis of the reaction mixtures was followed by HPLC technique showing that the α-anomers are the major product (Scheme 2.50) [57].

2-thiophenyl glycosides were used as glycosyl donor for preparing complex oligosaccharides containing sialyl moieties. A remarkable convergent approach was described for preparing a sialyl octasaccharide consisting in the initial glycosidic reaction between 2-thiophenyl Neu5Ac donor and trisaccharide intermediate to produce the expected tetrasaccharide in 45 % having an α(2 → 6)-linkage. The resulting tetrasaccharide was coupled with dimeric sialyl donor to yield hexasaccharide in 42 %. Acetal hydrolysis was followed by coupling reaction with Neu5Acα(2 → 3) GalSMe donor to give the octasaccharide in 85 % yield (Scheme 2.51) [58].



Scheme 2.48 Synthesis of an antigen polysaccharide fragment

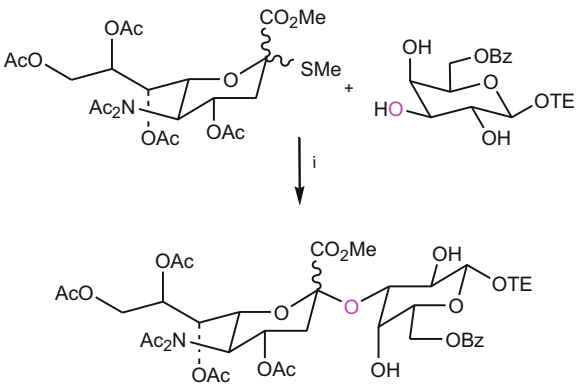
Crich and Li [59] introduced the use of 1-(Benezenesulfinyl)piperidine/triflic anhydride as promoter conditions for preparing *O*-glycosides from thioglycoside donors. These conditions were applied for preparing Salmonella type E1 core trisaccharide (Scheme 2.52). This method has been adopted as an alternative approach known as "iterative or preactivation" glycosylation which consist in treatment of the thioglycoside with 1-benzenesulfinyl piperidine (BSP) or morpholine analog (BSM) and triflic anhydride at low temperature, and the resulting "glycosyl triflate"



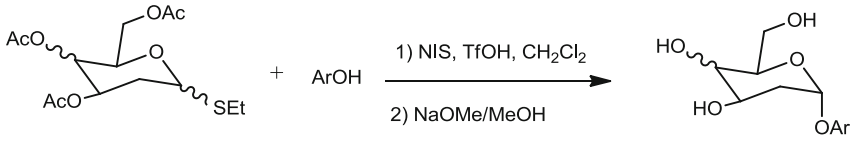
i) BnBr, NaH, DMF. ii) 1,1,2,2-tetramethoxycyclohexane. iii) IDCP, 4ÅMS. iv) NIS. v) AcOH-H_2O. vi) H_2,Pd/C, EtOH.

Scheme 2.48 (continued)

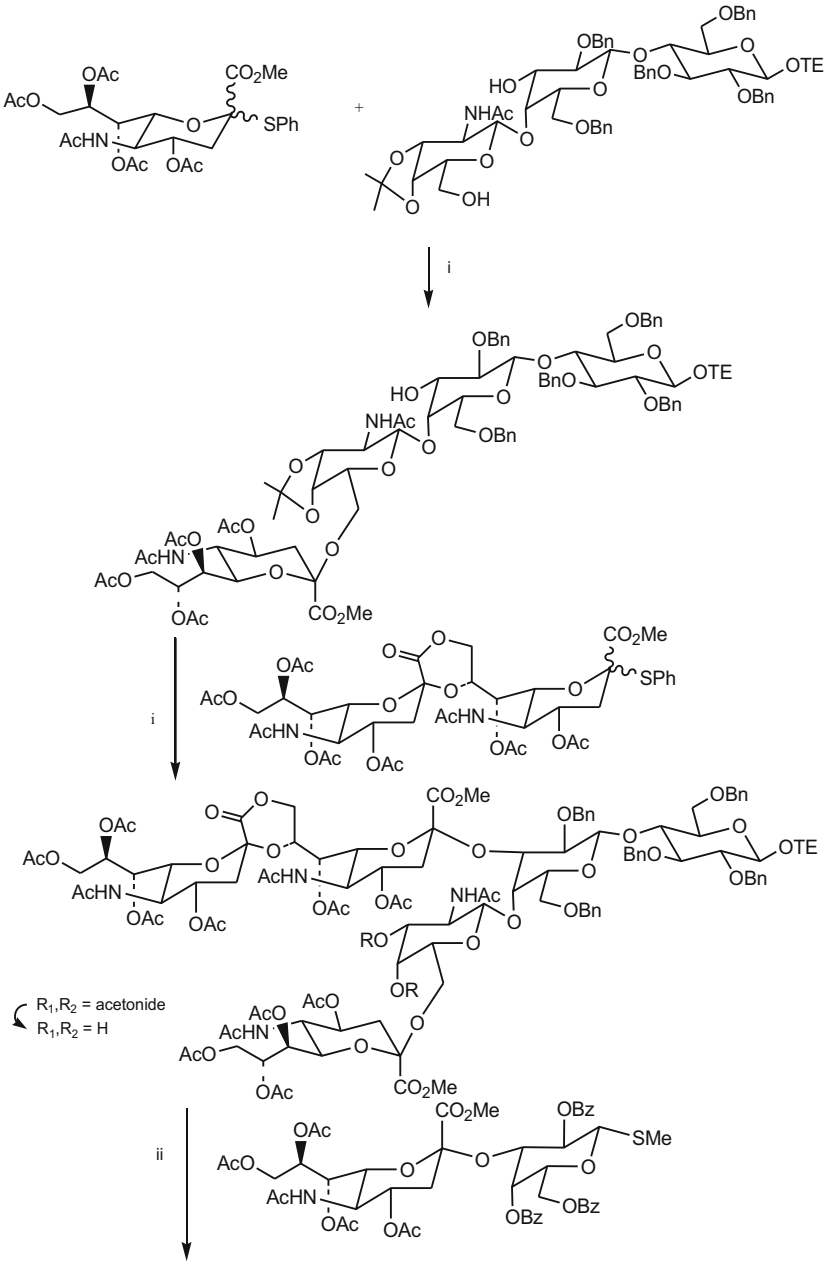
Scheme 2.49 Thioalkyl donor for the preparation of sialic acids



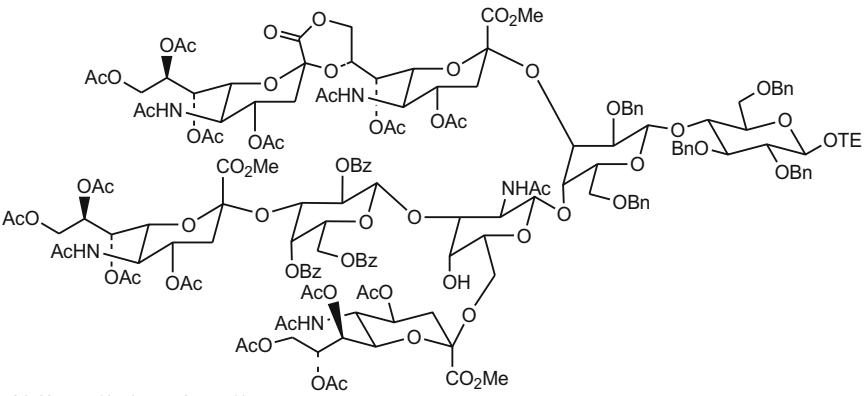
i) NIS/TfOH, MeCN, -40°C.



Scheme 2.50 Synthesis of aryl 2-deoxy-D-glycopyranosides from 2-deoxy-1-thioglycosides

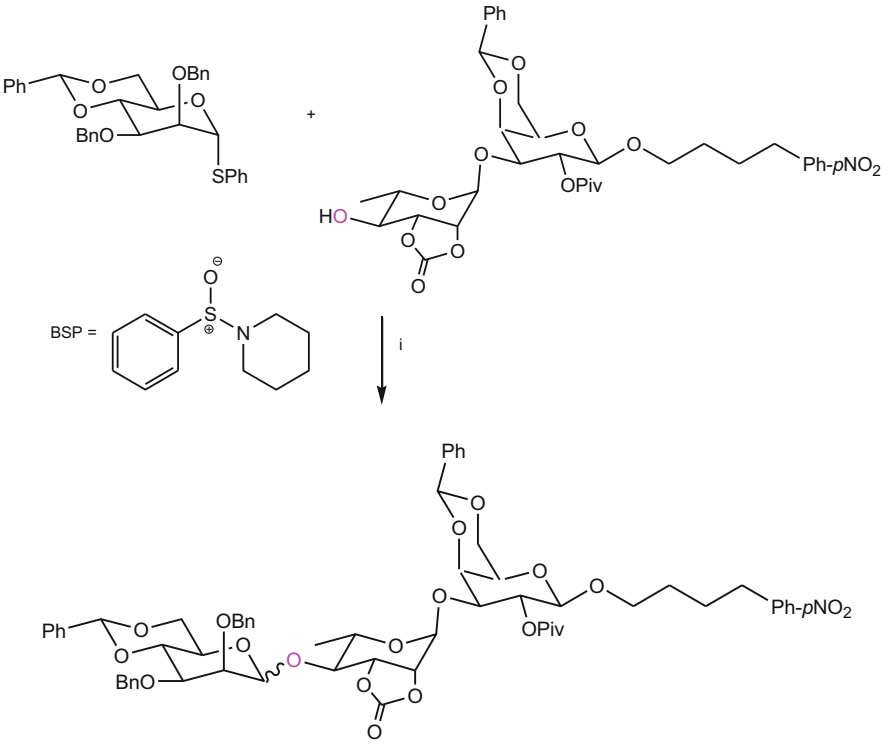


Scheme 2.51 Convergent synthesis of sialyl oligosaccharide



i) NIS/TfOH, 45%. ii) DMTST, 85%.

Scheme 2.51 (continued)



i) a) BSP, m.s., CH_2Cl_2, r.t. b) Tf_2O, -60°C to 0°C 1h.

Scheme 2.52 Preparation of Salmonella type E_1 core trisaccharide under BSP-Tf_2O conditions

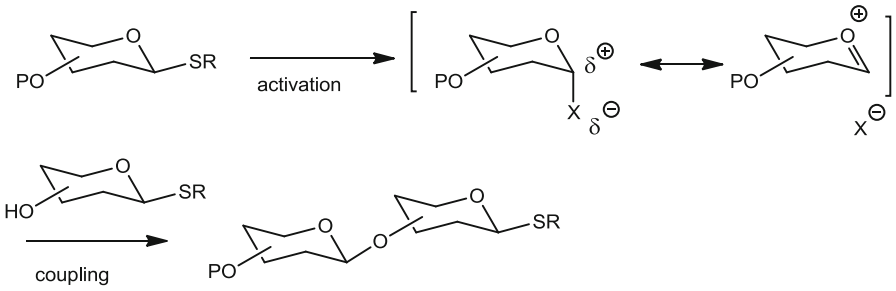
intermediate treated with a thioglycosides acceptor having a free alcohol suitable for attachment [60].

This method has been extended as an alternative approach known as "iterative or preactivation" glycosylation which consist in the treatment of the thioglycoside with 1-benzenesulfinyl piperidine (BSP) or morpholine analog (BSM) and triflic anhydride at low temperature, and the resulting "glycosyl triflate" intermediate treated with a thioglycosides acceptor having a free alcohol suitable for coupling reaction (Scheme 2.53) [60, 61].

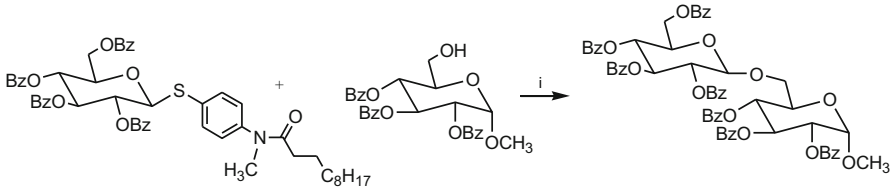
Highly fluorinated thiols have been developed and used as donors in the preparation of disaccharides. The reactivity of these novel fluorinated thiols were examined using different acceptors. Thus, disaccharide formation under glycosidic conditions provided the disaccharides in high yields (Scheme 2.54) [62].

Thioglycosides have been used as donor models for glycosylations with imidazolium-based ionic liquids promoters under *N*-iodosuccinimide conditions. Thus it was observed that tetra-*O*-benzyl-1-thio-β-D-glucopyranoside as donor and 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose as glycoside acceptor gave the disaccharide in almost 1:1 α/β ratio in 84 % yield. This methodology claims to have the ability of recycling the ionic liquid promoter which make it attractive as a cost effective protocol (Scheme 2.55) [63].

An study using protected thio gluco and galactoside bearing and acetate group at 6-position was conducted to determine the influence of solvent in the stereoselectivity of the glycosylation reaction with small and reactive acceptors has been carried

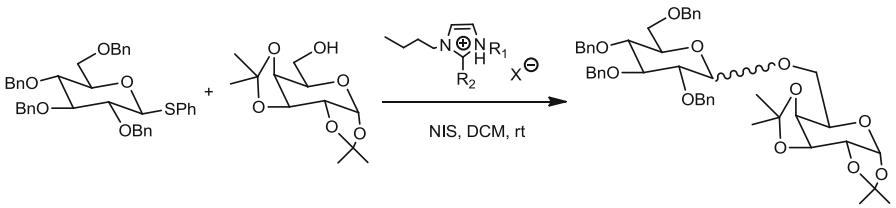


Scheme 2.53 Iterative or preactivation protocol

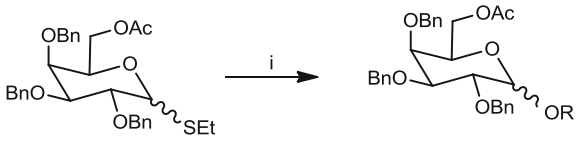


i) NIS (2eq.), AgOTf (0.2eq.), CH_2Cl_2.

Scheme 2.54 Highly fluorinated thiols glycosyl donor for glycosidation

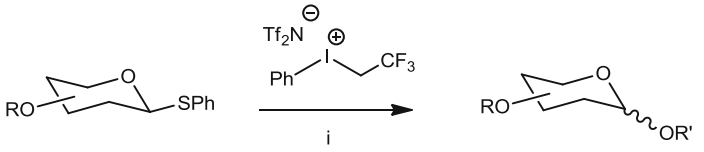


Scheme 2.55 glycosylations with imidazolium-based ionic liquids promoters under *N*-iodosuccinimide



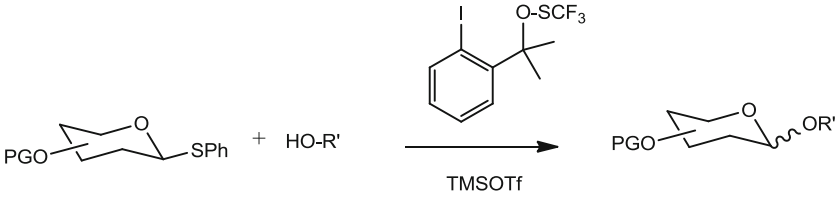
i) NIS, TfOH, MS, Et_2O, -60°C

Scheme 2.56 α-stereoselectivity under NIS/TfOH activation



i) R'OH, TTBP, CH_2Cl_2, rt

Scheme 2.57 *O*-glycoside formation with an air- and water-stable iodonium salt

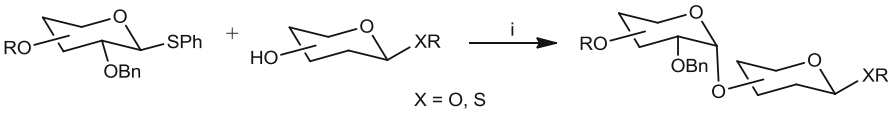


Scheme 2.58 O-glycosylation under thioperoxide-TMSOTf conditions

out, observing a high α-stereoselectivity when using NIS/TfOH as activator and ethyl ether as the solvent at −60 °C. Other solvents did not improve the *α*/*β* ratio, although yields were high (Scheme 2.56) [64].

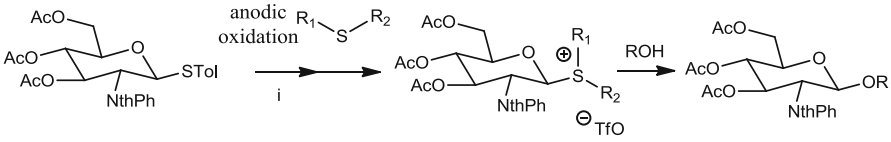
Fully substituted and deoxy thioglycoside donors were converted to cholesterol and disaccharide *O*-glycosides by reaction with an air- and water-stable iodonium salt phenyl(trifluoroethyl)-iodonium triflimide as an activator for glycosylation reporting 68–97 % yield as a mixture of isomers (Scheme 2.57) [65].

Thioperoxide in combination with trimethylsilyl trifluoromethanesulfonate (TMSOTf) was designed as thioglycosides activators as it can be seen in the *O*-glycoside synthesis of disaccharides reporting high yields and β stereoselectivity or as a mixture of anomers (Scheme 2.58) [66].



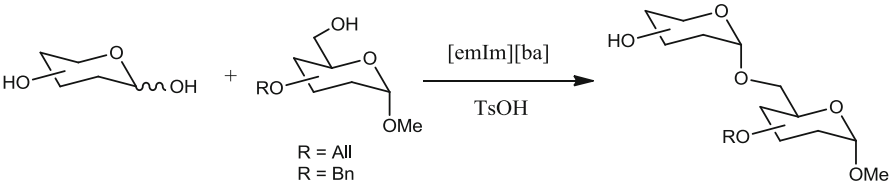
i) Ph_2SO, Tf_2O, N-methylmaleimide ii) BuN^+I^-

Scheme 2.59 1,2-Cis glycosylation under Ph_2SO/Tf_2O conditions



i) Bu_4NOTf, CH_2Cl_2, -78%°C

Scheme 2.60 O-glycosylation method via electrochemically generated glycosyl triflate



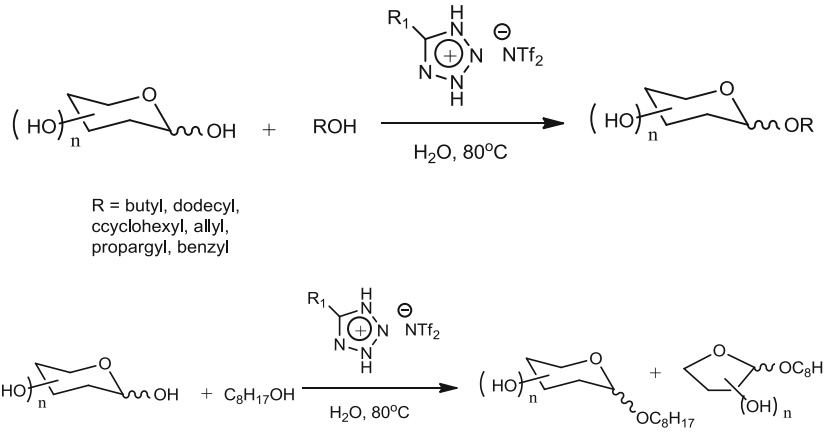
Scheme 2.61 Unprotected glycosylation in the presence of acidified liquid ion solvents

Another report for preparing 1,2-cis-R-glycosides from thioglycosyl donors without directing groups involved activating conditions of Ph_2SO/Tf_2O at low temperature. It was observed that the use of tetrabutylammonium iodide (TBAI) and *N*-methylmaleimide leds to a increase of yield accompanied by high 1,2-cis stereoselectivity (Scheme 2.59) [67].

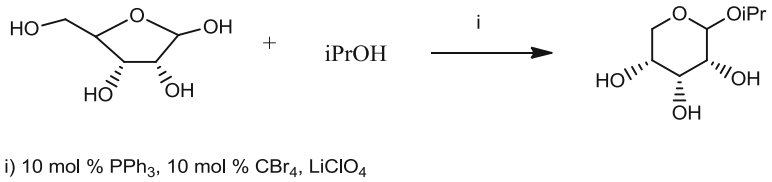
Toluylglycoside was chosen as a glycosyl donor for preparing glycosyl sulfonium ions, via electrochemically generated glycosyl triflate, which in turn served for preparing β-disaccharides from moderate to good yields depending on the temperature at which glycosylation was performed (Scheme 2.60) [68].

2.1.8 Unprotected Glycosylations

Attempts for preparing straight glycosylations using unprotected sugars with a variety of aglycons such as aliphatic, aromatic and other sugars have been implemented in the presence of different promoters. For instance simple benzyl glycosides and disaccharides of glucose, mannose and *N*-acetylgalactosamine were obtained in 1-ethyl-3-methylimidazolium benzoate with Amberlite IR-120 (H+) resin or *p*-toluenesulfonic acid as promoters in modest yields (Scheme 2.61) [69].



Scheme 2.62 Unprotected glycosylation in the presence of Brønsted acid ionic liquids (BAILs)

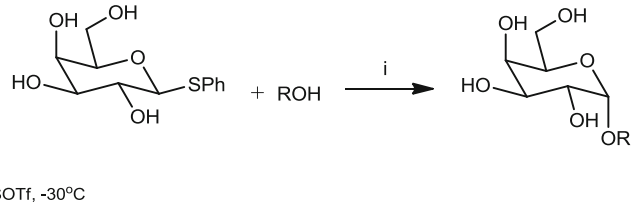


Scheme 2.63 Unprotected glycosylation via the Apple reaction

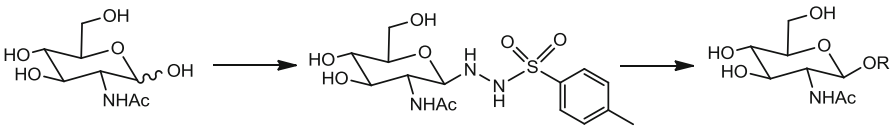
Brønsted acid ionic liquids (BAILs) have been designed as promoters for glycosylations of unprotected sugars due to their ability to adjust solubility properties by different cation–anion combinations. Under these conditions the yields reported range from 19 to 67 depending on the alcohol assayed, providing mainly the α-anomer. It has been observed that the reaction between different aldose monosaccharides and octanol produces a mixture of pyranosides and furanosides as a mixture of anomers (Scheme 2.62) [70].

Glycosylation of unprotected ribose with a variety of alcohols, have been carried out by following a variation of the Apple reaction which substitute a hydroxyl group by a bromine in situ, under triphenylphosphine and tetrabromomethane conditions. An improvement in the reaction was observed when lithium perchlorate was used in arabinose, xylose, and lyxose providing good yields although the glycosides were obtained in the pyranoid form with different α/β ratios (Scheme 2.63) [71].

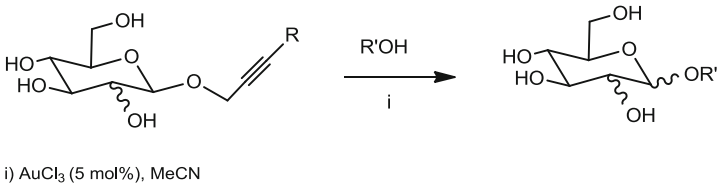
Previously this group was able to prepare isopropyl glycosides by direct glycosylation reaction of unprotected riboside with isopropanol in the presence of mandelic acid and titanium tert-butoxide [72]. On the other hand, Meng et al. [73] reported the 1,2-cis-alkyl glycosidation protocol with unprotected phenyl 1-thioglycosyl donors with a variety of alcohol acceptors under the activation of *N*-iodosuccinimide–trimethylsilyl triflate (although other Lewis acids such as TfOH or $BF_3.OEt_2$ provide good yields). The desired product was obtained in 75–76% yields and with high α stereoselectivity (Scheme 2.64).



Scheme 2.64 Unprotected glycosylation with unprotected phenyl 1-thioglycosyl donors



Scheme 2.65 Unprotected glycosylation by using *p*-toluenesulfonylhydrazide as donor



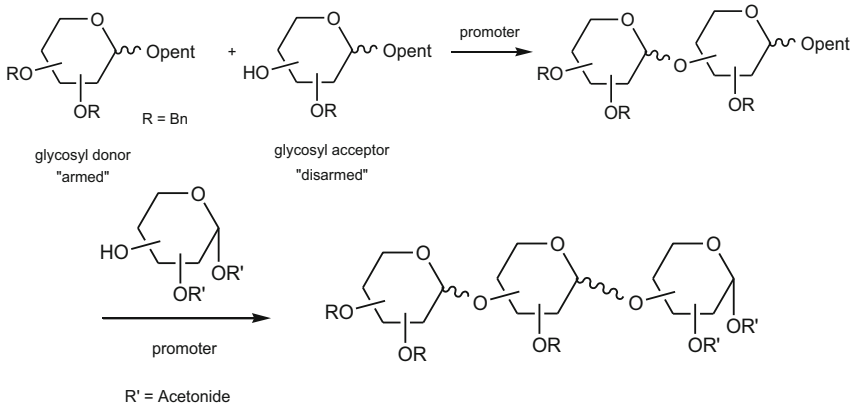
Scheme 2.66 Unprotected glycosylation from 2-butynyl glycosyl donors in the presence of gold (III) activation

Another protecting group free glycosidations was proposed by using *p*-toluenesulfonylhydrazide as leaving group followed by coupling reaction with alcohols in the presence of NBS in DMF at room temperature, providing the *O*-glycoside in good yields 70–87 % mainly as a β-isomer (Scheme 2.65) [74].

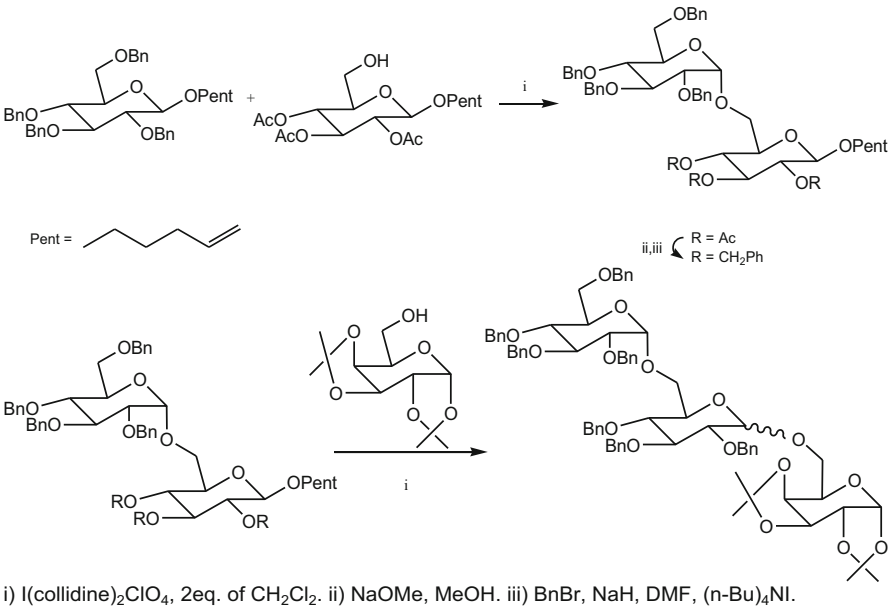
Gold (III) activation of unprotected glycosyl donors bearing 2-butynyl as leaving group has been used in combination with primary alcohols and protected saccharides as acceptors, providing the corresponding *O*-glycosides as a mixture of anomers in moderate yields (Scheme 2.66) [75].

2.1.9 Armed–Disarmed Method

This versatile approach has been attributed to Mootoo and Fraiser-Reid [76], and considers the use of a glycosyl donor in the classical sense coined with the term "armed saccharide" (because the reducing end is armed for further coupling reaction), and an acceptor in this case "disarmed saccharide" which contains both a free alcohol and a leaving group sufficiently resistant for the ongoing coupling reaction. The resulting disaccharide now becomes and armed disaccharide which in turn is



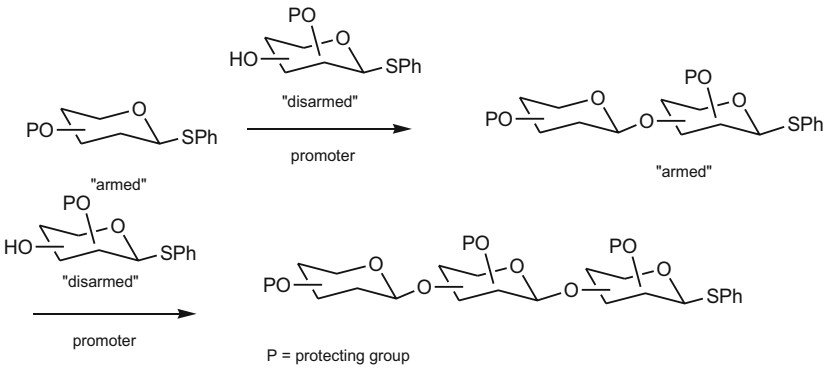
Scheme 2.67 General scheme for the armed–disarmed approach



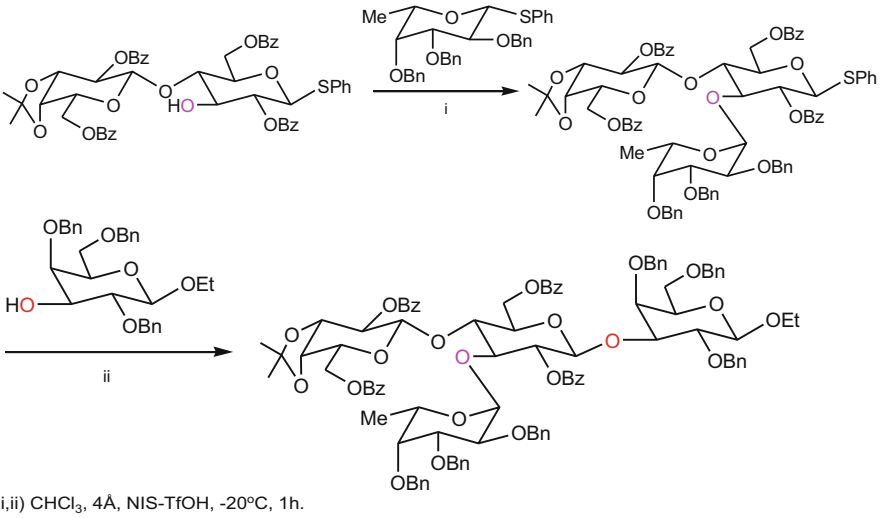
Scheme 2.68 Armed–disarmed approach

reacted with another glycosyl acceptor or disarmed sugar to produce the oligosaccharide chain elongation (Scheme 2.67).

This method was first implemented in the preparation of 1–6 linked trisaccharide shown in Scheme 2.68. As it can be observed the disarmed sugar intermediates function as glycosyl acceptor bearing the hydroxyl group at position 6 available for establishing a glycosidic linkage with the armed unit.



Scheme 2.69 General scheme of the armed–disarmed approach with thioglycosyl sugars

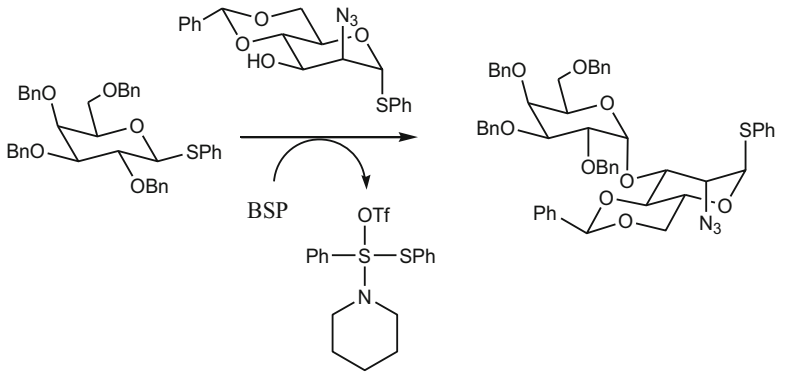


Scheme 2.70 Preparation of Lewis^x tetrasaccharide using armed–disarmed coupling method

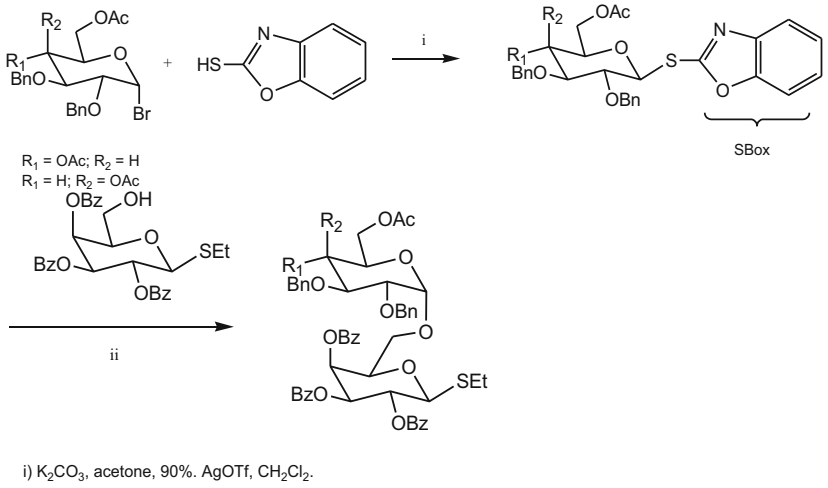
Despite the usefulness of pentenyl as protecting group, clear preference in the use of thioglycoside donors as armed and disarmed donors is often observed (Scheme 2.69) [77].

This concept was applied successfully in the stereocontrolled synthesis of Le^x oligosaccharide derivatives by using two glycosylation steps as described by Yoshida et al. [78]. The first coupling between "armed" thiophenyl fucopyranosyl derivative and "disarmed" thiophenyl lactose derivative under NIS-TfOH conditions provided trisacccharide which was subjected without purification to second condensation with different acceptors, one of which is indicated in Scheme 2.70.

The construction of α-linked mannoside disaccharide was achieved under the armed–disarmed approach by using armed thiogalactoside donor activated by BSP/Tf_2O and condensed with disarmed thiomannoazide intermediate bearing a



Scheme 2.71 Synthesis of α-linked mannosyl disaccharide following an armed–disarmed strategy

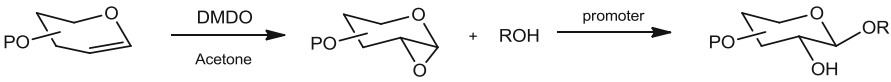


Scheme 2.72 Armed–disarmed synthesis using S-benzoxazol (SBox) as disarmed glycosyl donor

free hydroxyl group. Addition of triethyl phosphate prior to the aqueous work up led to the generation of the expected α-linked disaccharide in 74 % (Scheme 2.71) [77].

Recently S-benzoxazol thio glycoside (SBox) was synthesized and introduced as alternative glycosyl donor for preparing disaccharides under the armed–disarmed approach. Thus, the SBox glycosyl donor was used as armed donor and condensed with disarmed thioglycoside to provide the target disaccharide (Scheme 2.72) [79].

2.1.10 Glycal Reaction



Promoter	Conditions
$ZnCl_2$	THF

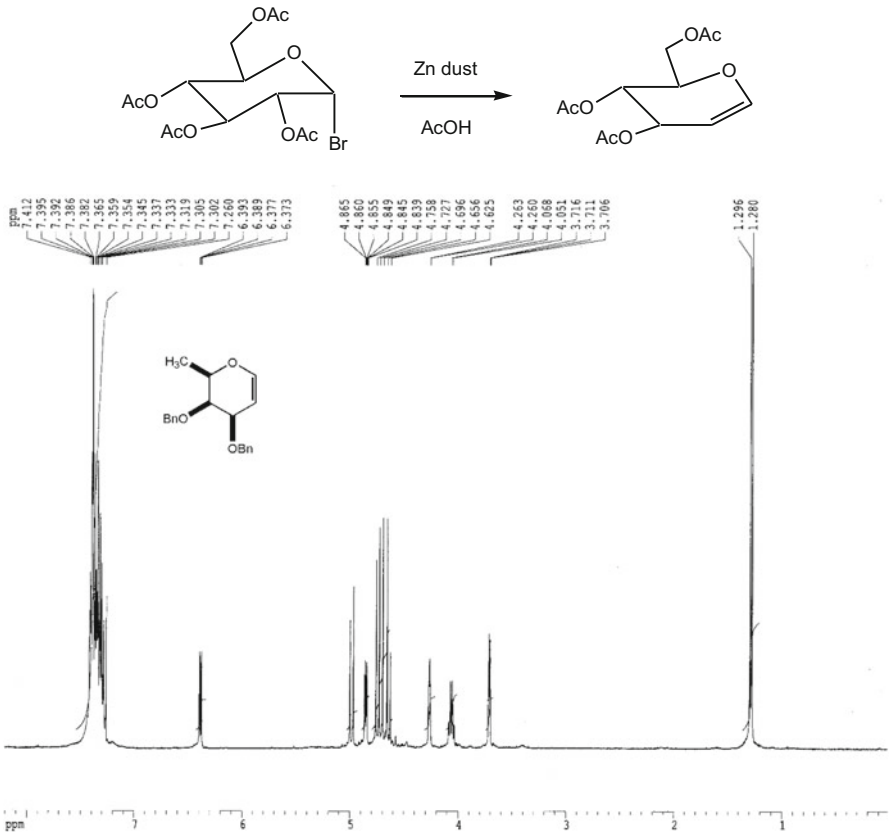
The glycals are unsaturated sugars with a double bond located between C1 and C2. These useful intermediates were discovered by Fischer and Zach in 1913 [80] and their utility in the preparation of building blocks for oligosaccharide synthesis is increasingly important. Different routes for the preparation of triacetyl glucals have been examined by Fraser-Reid et al. [81], involving the Ferrier rearrangement. Moreover, a suitable one-pot preparation of glucals has been more recently described, starting from reducing sugars by Shull et al. [82] The general procedure for preparing these valuable intermediates is based on the reductive removal of a halogen and neighboring acetate group through the use of zinc in acetic acid (Scheme 2.73). The completion of this reaction can be followed by ^{1}H NMR, where the presence of a signal around 6.3 ppm as double of double with $J_{1,2}=6.2$ Hz, $J_{1,3}=0.3$ Hz is expected for H-1, and a multiple shifted upfield for H-2.

More recently the use of alternative catalysts such as titanium complex, Li/NH_3, Sodium, Cr (II) and vitamin B-12 as catalysts has been described as improved method, for preparing especially acid sensitive glycals.

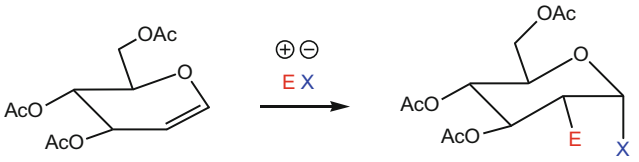
As for any double bond, these unsaturated sugars may undergo electrophilic addition, which takes place at the C2 position leaving a positive charge at C1, which instantly reacts with the conjugate base. This reaction is particularly useful for the preparation of 2-deoxypyranosides (Scheme 2.74).

A more extended application for glycoside bond formation has been developed recently. Such strategies consist of the conversion of glycals into Brigl's epoxide, and then further treatment with nucleophiles to effect ring opening. The oxidation of the double bond has been successfully achieved with dimethyl dioxirane (DMDO) in acetone (Scheme 2.75).

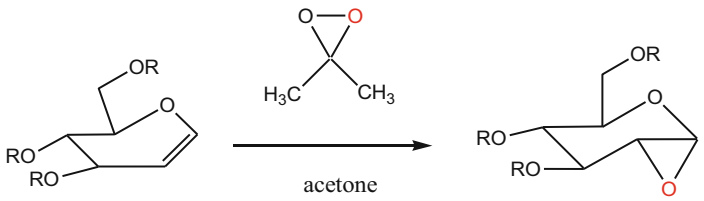
The standard procedure for generation of DMDO was developed by Murray and Jeyaraman [83], and optimized by Adam et al. [84]. Such procedure involves the use of potassium monoperoxysulfate as oxidizing agent, and the reaction conditions require temperatures below 15 °C and efficient stirring. The DMDO–acetone solution generated must be immediately distilled under moderate vacuum. The concentrations of DMDO are in the order of 0.09–0.11 M (5 %), and it is used as acetone solution. The transformation of the glycal to the epoxide can be verified by ^{1}H NMR, where it is observed the disappearance of the signal at 6.3 ppm for H-1 double bond, and it is expected the presence of a signal at 5.0, as double for H-1 and at 3.1 as double of double for H-2 (Scheme 2.76).



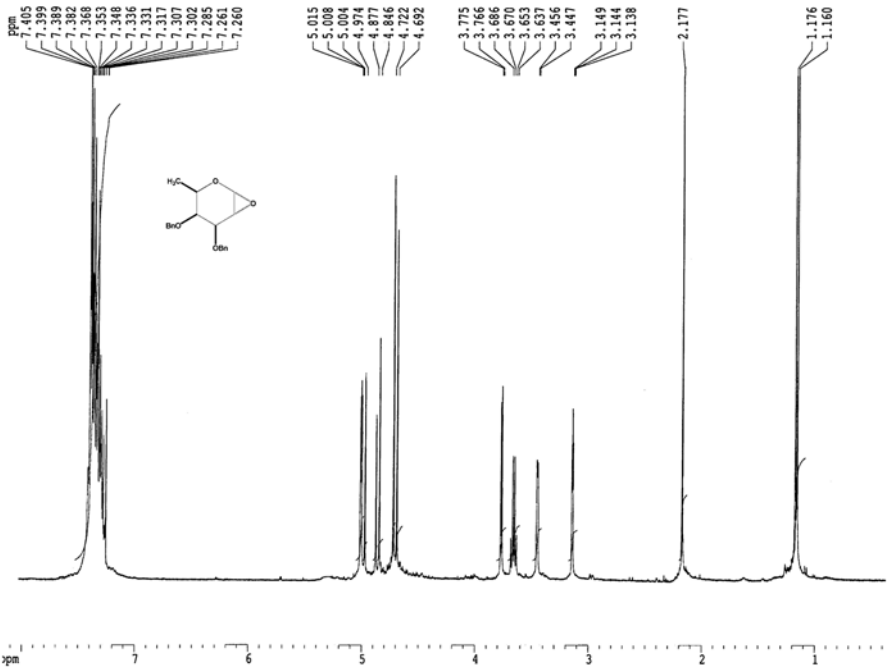
Scheme 2.73 Fischer–Sachs glucal and ^{1}H NMR of benzylfucopyranosyl glycal



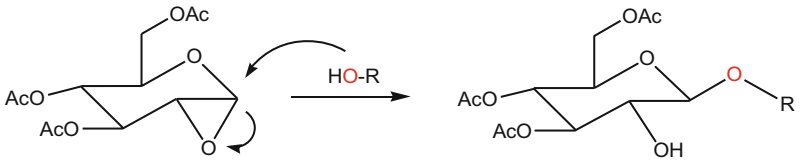
Scheme 2.74 Electrophilic addition



Scheme 2.75 Brigl epoxide formation



Scheme 2.76 ^{1}H NMR spectra of 1,2-anhydro-3,4-di-*O*-benzyl-α-D-fucopyranose (and traces of acetone)

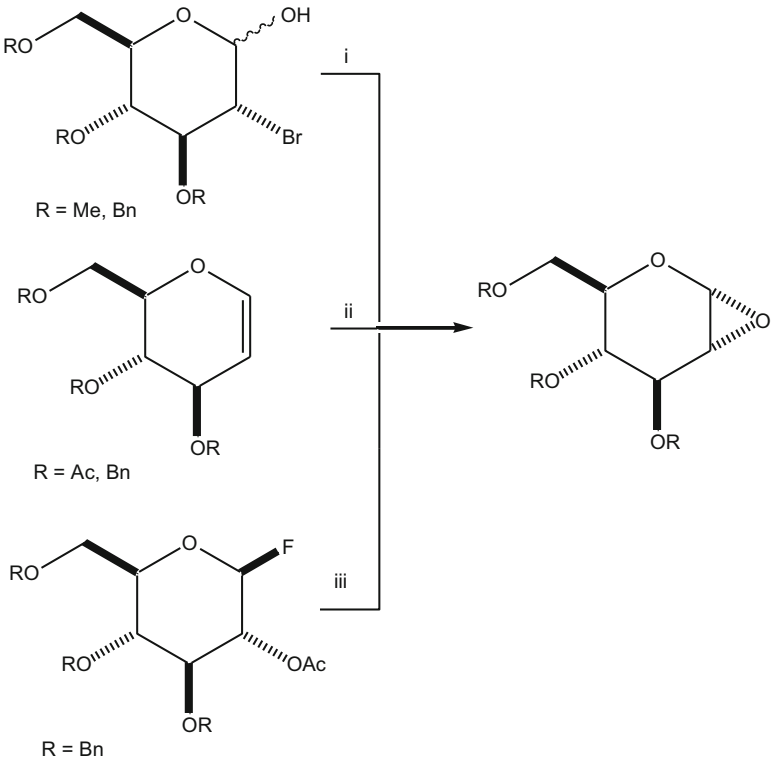


Scheme 2.77 Ring opening for β-glycoside formation

The stereo selectivity of epoxide formation is protecting group dependent, observing in the case of acetate protecting group a mixture of epoxide anomers, and preferentially the α-anomers if the protecting groups are benzyl, or methyl groups (α:β ratio 20:1). As expected, the epoxide ring opening by nucleophiles occurs with inversion of configuration, providing β-glycosides exclusively (Scheme 2.77).

Likewise, alternative epoxide conditions from glycals have been assayed besides DMDO treatment. Among them, cyclization of a bromohydrin [85], *m*-chloroperoxybenzoic acid-potassium fluoride complex oxidation of the glycal [86], and potassium tertbutoxide oxidation of fluoride glycosyl donor [87] has been described (Scheme 2.78).

The potential of 1,2-anhydro sugars as glycosyl donor for the preparation of β-linked saccharides was established by Halcomb and Danishefsky [88] and such

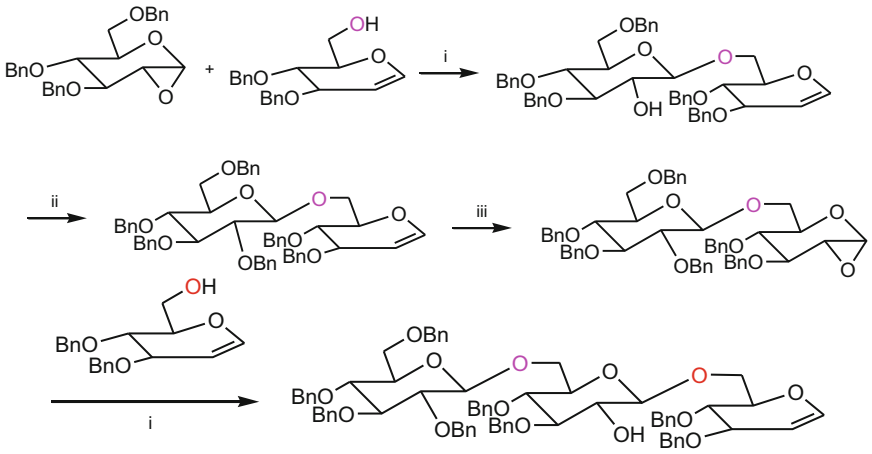


Scheme 2.78 Alternative glycal-epoxidations

strategy consist in the treatment of the glucal having available a hydroxyl group at position 6, with the sugar epoxide under Lewis acid conditions ($ZnCl_2$) at low temperature. The resulting glucal disaccharide generated as a single coupling product was further converted to the epoxide which eventually lead to the next coupling reaction with another glucal acceptor (Scheme 2.79).

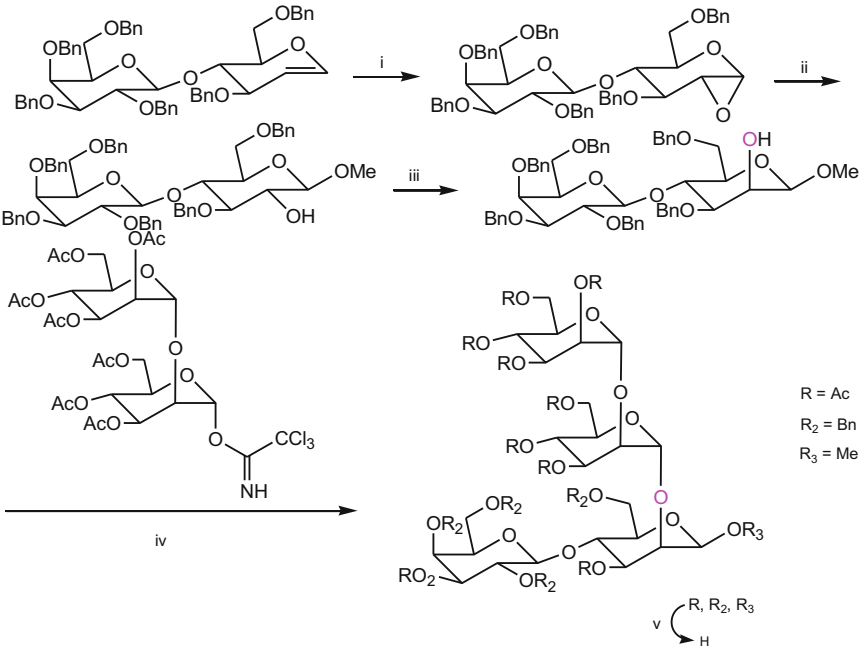
The tetrasaccharide Cap Domain of the antigenic lipophosphoglycan of *Leishmania donovani* has been prepared under the glycal approach by Upreti and Vishwakarma [89]. Thus, the preparation of the hexa-*O*-benzyl-lactal under standard procedures was followed by oxirane formation with dimethyl dioxirane to generate the corresponding oxirane. Methanolysis ring opening and gluco → manno conversion generated the disaccharide intermediate. This was coupled to the mannobiose donor to produce the tetrasaccharide, which after deprotection lead to the tetrasaccharide Cap domain (Scheme 2.80).

Brigl's epoxide has been exploited successfully for the preparation of glycosylated peptides such as collagen type II derived glycosides carrying β-Gal and αGlc-1,2-βGal side chains [90, 166]. Galactosyl glycal is reacted with DMDO–acetone



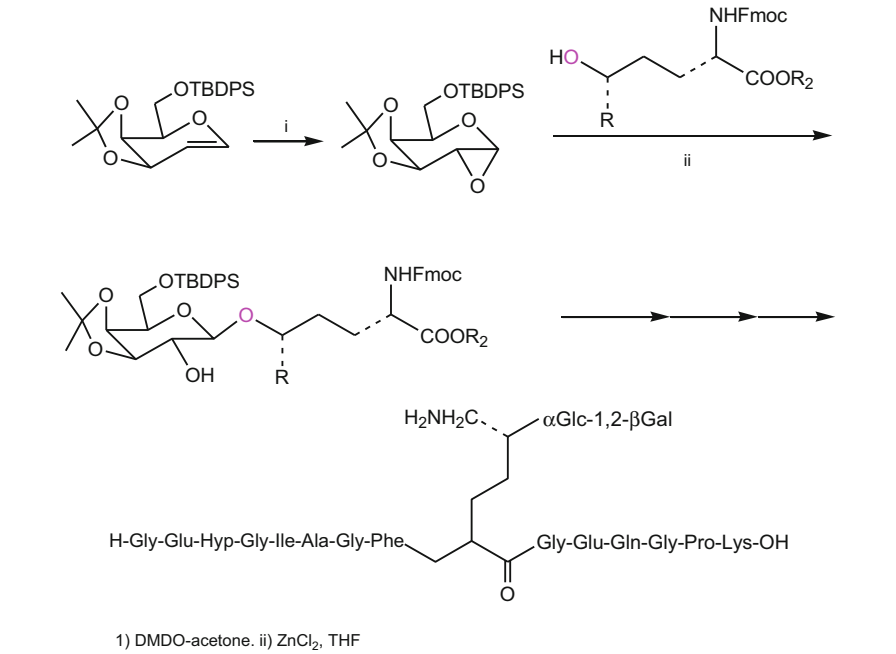
i) $ZnCl_2$/THF, -78ºC to r.t. ii) NaH, BnBr. iii) DMDO-acetone.

Scheme 2.79 Epoxide glycal as glycosyl donors

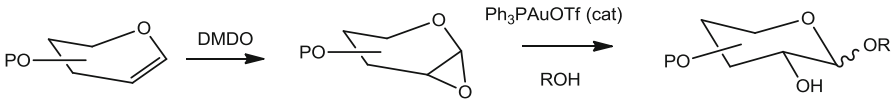


i) DMDO,CH_2Cl_2, 0ºC, 2h. ii) MeOH, rt, 4h. iii) a) $(COCl)_2$, DMSO, CH_2Cl_2, -78ºC. b) $NaBH_4$, rt, 4h.
iv) TMSOTf, CH_2Cl_2, -30ºC, 45min. v) a) $Pd(OH)_2$, H_2,4h. b) NaOMe, MeOH. c) Ac_2O/AcOH/H_2SO_4.

Scheme 2.80 Synthesis of a tetrasaccharide using an epoxide disaccharide as glycosyl donor



Scheme 2.81 Amino acids glycosidation



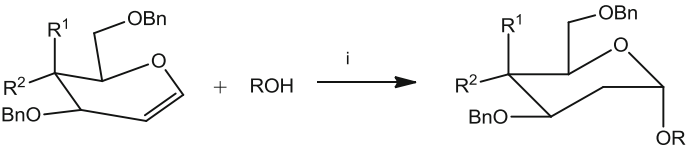
Scheme 2.82 O-glycosylation from anhydro glycals promoted by gold complex

solution and the resulting epoxide reacted with hydroxylysine and $ZnCl_2$ as promoter (Scheme 2.81). General procedures for preparation of glycosidic bond of glycopeptides can be reviewed in the comprehensive study reported by Kunz [91].

A Gold (I)-catalyzed glycosidation approach was developed by reaction of anhydro glycals with protected sugar acceptors or cholesterol, using as promoter $Ph_3PAuNTf_2$ producing the glycosylation product as a mixture of anomers in moderate to good yields (Scheme 2.82) [92].

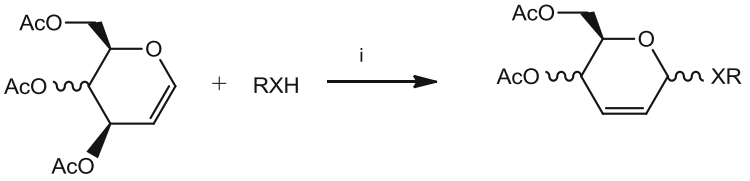
Glycals can lead to 2-deoxy-*O*-glycosides by treatment of protected D-glucal and D-galactal with the alcohol in the presence of trimethylsilyl iodide and triphenylphosphine to produce the *O*-glycoside favoring the α-selectivity (Scheme 2.83) [93].

Likewise the preparation of unsaturated *O*- and *S*-glycosides can be accomplished properly by glycosidic reaction of glycal triacetate with alcohol or thiol under erbium triflate-catalysis, observing that in dry CH_3NO_2 during 2 h the higher yields of the Ferrier product (90 %) mainly as the α-isomer (Scheme 2.84) [94].



R^1 = OBn, R^2 = H
R^1 = H, R^2 = OBn
i) TMSI-PPh_3, DCM

Scheme 2.83 Preparation of 2-deoxy-*O*-glycosides from glycals promoted by TMSI-PPh_3

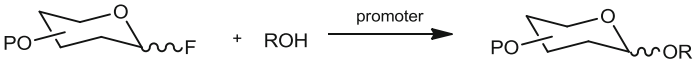


R = alkyl, aryl; X = O, S
i) $Er(OTf)_3$, dry CH_3NO_2, 2h

Scheme 2.84 Preparation of unsaturated *O*- and *S*-glycosides under erbium triflate-catalysis

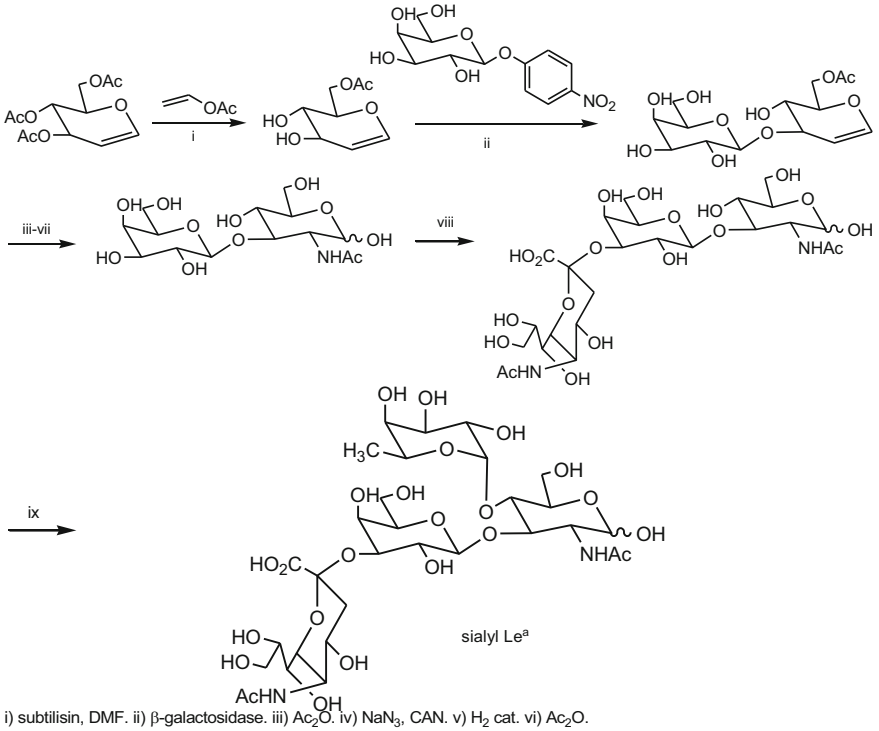
This methodology has been extended for the preparation of E-selectin ligand tetrasaccharide sialyl Lewis^X (SLe^x), which is located at the terminus of glycolipids present on the surface of neutrophils. The chemoenzymatic sequence consisted in the reaction of the 6-acetylated glucal with β-galactosidase transferase to produce disaccharide which was subjected to further transformations according to the pathway presented in Scheme 2.55 (Scheme 2.85) [95].

2.1.11 *Fluorine Reaction*



Promoter	Conditions
$SnCl_2$-$AgClO_4$	Et_2O, −15 → r.t.
Cp_2HfCl_2-AgOTf	CH_2Cl_2, −25 °C
$SnCl_2$-AgOTf	CH_2Cl_2, 0 °C

Fluorine is considered a poor leaving group, and its use for glycoside bond formation has been more restricted than chlorine and bromine, although display higher thermal and chemical stability. Nonetheless several *O*-glycoside synthesis involving



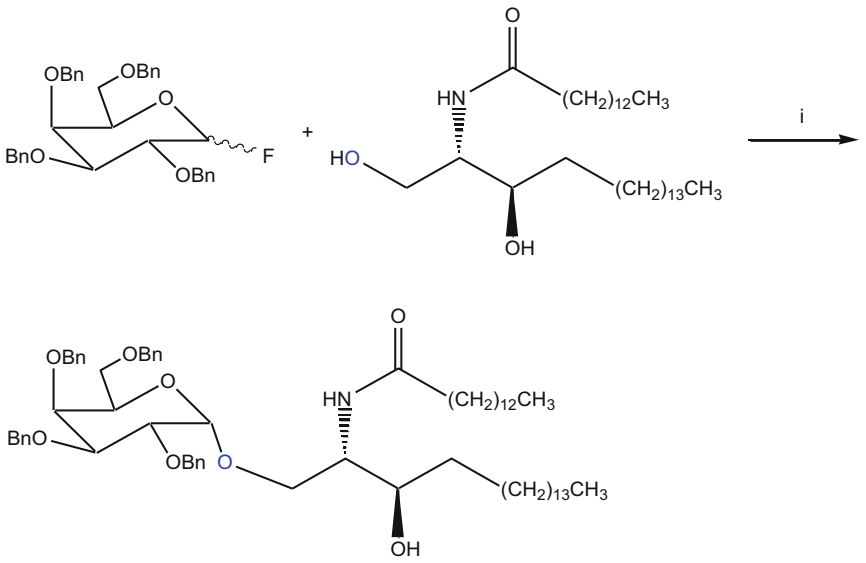
Scheme 2.85 Chemoenzymatic synthesis of tetrasaccharide sialyl Le^a

glycosyl donors with fluorine as leaving group has been described, specially for the preparation of α-*O*-glycosides with high stereoselectivity [96].

Based in the use of fluorine glycosyl donors, the synthesis of the marine algae α-agelaspines was carried out through the condensation of perbenzylated galactopyranosyl fluorine as anomeric mixture with the long chain alcohol in the presence of a mixture of $SnCl_2$ and $AgClO_4$ as catalyst (Scheme 2.86) [97].

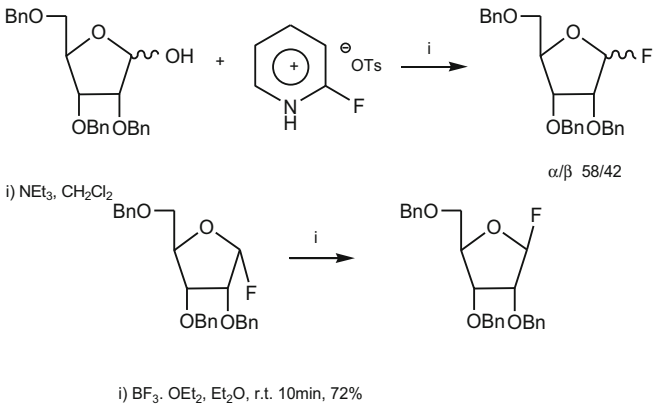
A general procedure for the preparation of ribofuranosyl fluorides and their use as glycosyl donors for *O*-glycosylation with α-stereocontrol was developed by Mukaiyama et al. [98], and consist in the conversion of 2,3,5-tri-*O*-benzyl-D--ribofuranoside that react under mild conditions with 2-fluoro-1-methylpyridinium tosylate at room temperature to give an anomeric mixture (α:β 58:42) in 84 % yield. These two fluorines could be either separate or interconverted by treating the α-anomer with boron trifluoride etherate in ether at room temperature (Scheme 2.87).

It has been observed that the glycosylation reaction between the glycosyl fluorine and different alcohols under Lewis acid conditions provides mainly α-riboglucosides in high yield as it is shown in Scheme 2.88



i) $SnCl_2$, $AgClO_4$/THF. ii) H_2, Pd-$BaSO_4$/THF.

Scheme 2.86 Fluorine monosaccharide as glycosyl donor

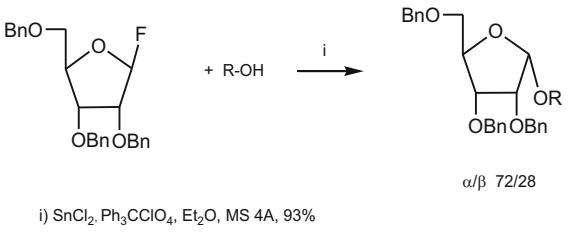


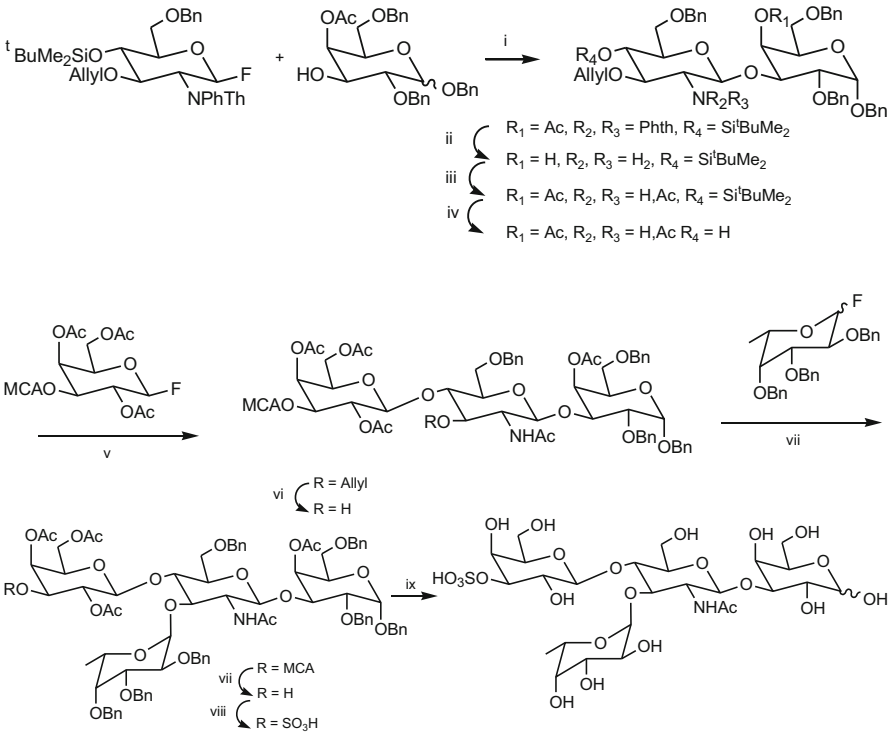
i) BF_3. OEt_2, Et_2O, r.t. 10min, 72%

Scheme 2.87 The Mukaiyama protocol for preparation of ribofuranosyl fluoride

Sulfated Le^x and Le^a-type oligosaccharide selectin ligands were synthetically prepared as described below. Thus, glycosyl donor and acceptor were condensed under Mukaiyama conditions ($AgClO_4$-$SnCl_2$) to form the β-glycoside in 90 % yield. The sulfated tetrasaccharide was formed by reaction of tetrasaccharide acceptor with SO_3.NM_3 complex in anhydrous pyridine (Scheme 2.89) [99].

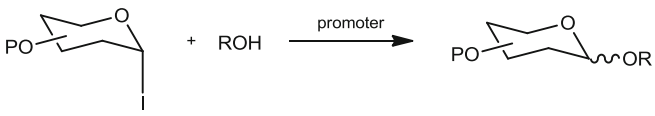
Scheme 2.88
N-glycosylation reaction using ribofuranosyl fluorine





Scheme 2.89 Total synthesis of sulfated Le^x

2.1.12 Iodine Reaction



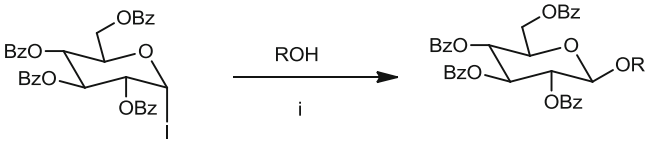
Promoter	Conditions
NBS (1.2), TMSOTf (0.4), TMU (0.2)	5 °C to rt, 4 h
$ZnCl_2$ (1.4)	rt, 12 h

Promoter	Conditions
NBS (1.2), Cu(OTf)2 (0.12)	5 °C to rt, 28 h
Bu_4NI	DIPEA, PhH, 4 Å MS
NIS, I_2, TMSOTf	3 Å MS, DCE

Glycosyl iodides have been increasingly adopted as glycosyl donors for the synthesis of *O*-, *S*, and *C* glycosides, on one side because of the introduction of suitable reagents for iodination such as iodotrimethylsilane (Me_3SiI), and hexamethyldisilane (HMDS) with molecular iodine, and on the other because of the feasibility for generating either α and β glycosides (Scheme 2.90) [100].

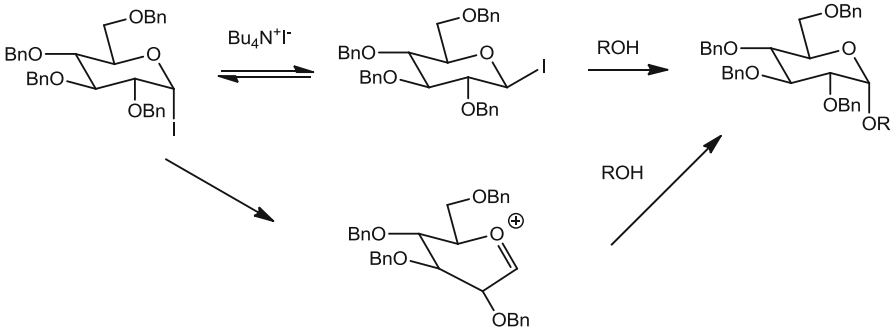
In general the stereocontrol on glycosylations depends on a combination of factors mainly the protecting group at C-2 position, the nature of the leaving group and the promoter conditions. It is well accepted that there are two possible mechanism S_N1-like and S_N2-like which define the final *α*/*β* ratio or the major anomer produced. Usually the intermediate oxacarbenium ion has poor stereochemical control, because it can be attacked from both the α- and β-side while in the S_N2-type the protected glycosyl donor is activated by an electrophile and the leaving group is displaced by the nucleophile being in this case the sugar acceptor or any other aglycone (Scheme 2.91) [101–103].

The nature of the aglycones linked to glycosyl iodide donors are diverse and among them morphine, uridine diphosphate, and steroidal alcohols have been glycosylated with promoters such as and Bu_4NF, NBS-I_2-TMSOTf (Scheme 2.92) [104–108].

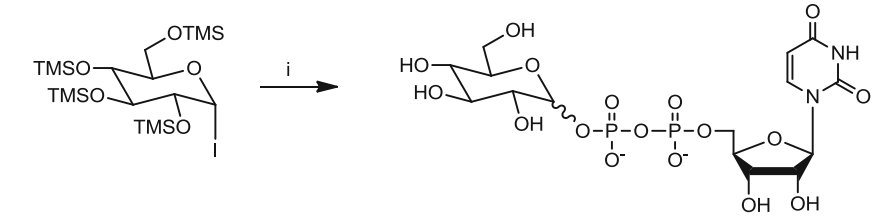


i) NBS with ZnI_2 (cat)

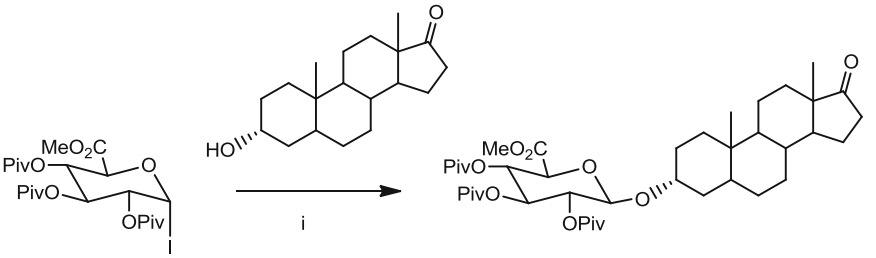
Scheme 2.90 O-glycosylation from protected glycosyl iodides under NBS-ZnI_2 conditions



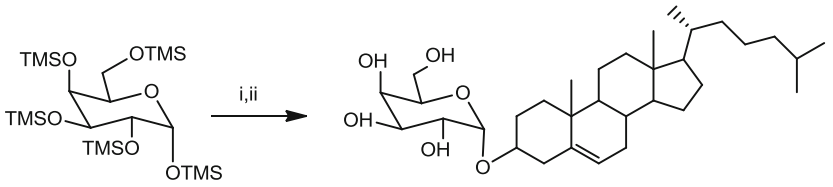
Scheme 2.91 Schematic representation of α-glycosylation stereocontrol involving glycosyl iodides



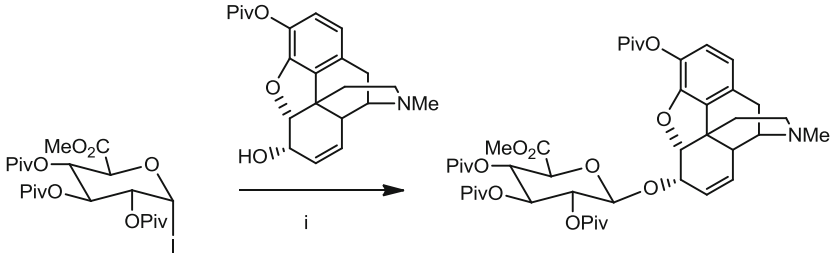
i) UDP(Bu_4N. ii) Bu_4NF, III) alkaline phosphatase



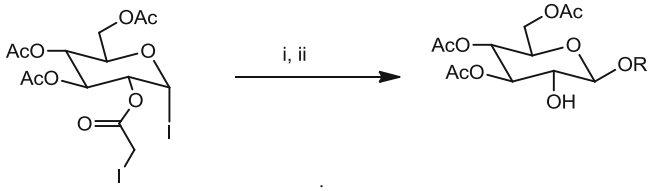
i) NBS, I_2, TMSOTf, 3 A MS, DCE



i) Cholesterol, TBAI (3 eq), DIPEA, 4 A MS, CH_2Cl_2, rt, 2d. ii) Dowex 50, MeOH



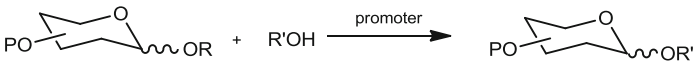
i) CH_2Cl_2, I_2, 4A MS



i) ROH, AgOTf. ii) thiourea

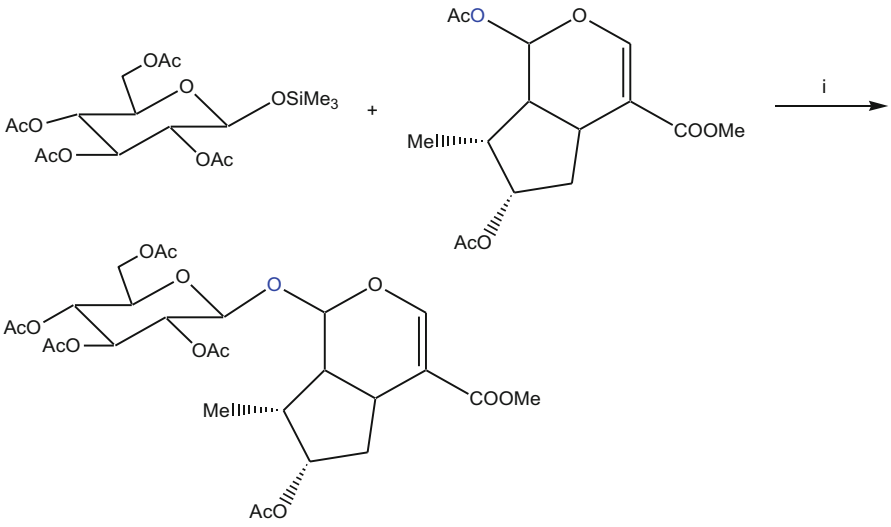
Scheme 2.92 Example of *O*-glycosylations from glycosyl iodides in the presence of different promoters

2.1.13 Silyl Reaction



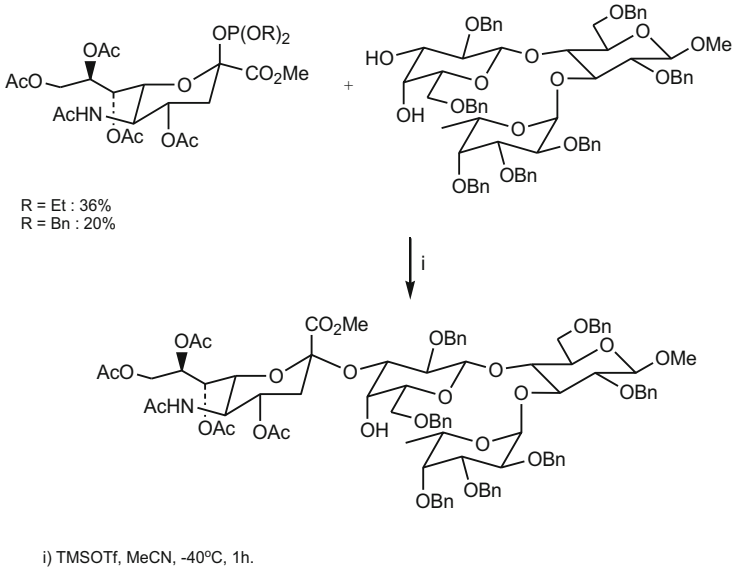
R	Promoter	Conditions
Me_3Si	TMSOTf or $BF_3.Et_2O$	CH_2Cl_2, −5 °C
$^tBuMe_2Si$	TMSOTf	CH_2Cl_2–acetone, −35 °C

Silyl groups are best known as versatile protecting groups, and their use as leaving groups for glycoside bond formation has been more limited. An example of glycoside formation involving a silyl group as leaving group is reported for the preparation of luganol *O*-glycoside [109]. In this work, the glycosyl donor is combined with luganine in the presence of trimethylsilyltriflate at low temperature (Scheme 2.93). It is worth mentioning that stereoselectivity is dependent on C-2 neighboring group participation. When acetate is the C-2 protecting group, the β-anomer is obtained, while if the protecting group is benzyl, the α-anomer is preferred.



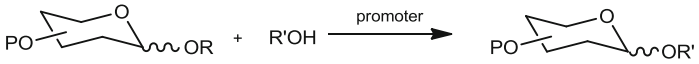
i) $TfOSiMe_3$, -40°C.

Scheme 2.93 Sialyl derivatives as glycosyl donors



Scheme 2.94 Phosphorous glycosyl donors for oligosaccharide synthesis

2.1.14 Phosphate Reaction

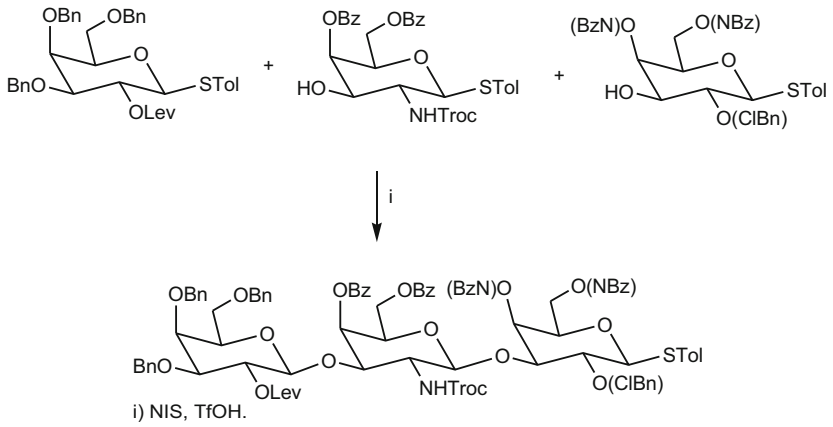


R	Promoter	Conditions
$P(=O)(OPh)_2$	TMSOTf	CH_2Cl_2, –5 °C
$P(=S)(Me)_2$	$TrClO_4$	
$P(=O)(NMe_2)_2$	TMSOTf	CH_3CN, –40 °C
$P(=NTs)(NMe_2)_2$	BF_3-Et_2O	CH_2Cl_2

Phosphorous glycosyl donors are another option for preparing oligosaccharides. These donors have been used for the preparation of sialyl oligosaccharides however the yield reported were moderate. This is the case of the preparation of sialyl tetrasaccharide derivative which was carried out by condensation between sialyl phosphate and trisaccharide acceptor under TMSOTf as catalyst (Scheme 2.94) [110, 111].

2.1.15 Pool Strategy

This term applies to define a one-step reaction used to build up two β-linkages simultaneously from three sugar intermediates [112]. This approach has been described for the preparation of the glycosyl ceramide Globo H hexasaccharide



Scheme 2.95 One-pot reaction for two β-linkages formation

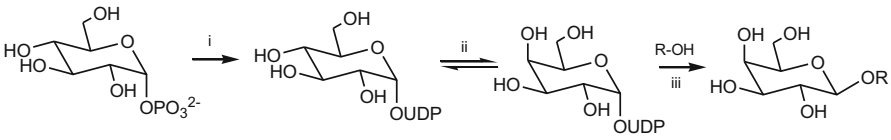
identified as an antigen on prostate and breast cancer cells. The synthesis consisted in the initial synthesis of the trisaccharide building block from the one-pot reaction of the three suitable sugar intermediates under *N*-iodosuccinimide and triflic acid conditions in 67 % yield (Scheme 2.95).

2.1.16 Enzymatic Approach

Enzymes in organic chemistry has become an essential tool for the synthesis of important target molecules and in many cases they are considered the first choice specially for those key steps involving stereospecifically controlled reaction conditions. In general enzymes are considered efficient catalysts which perform the desired transformation under mild conditions with high selectivity and specificity, usually avoiding epimerization, racemization and rearrangements processes. Besides there is a current need of developing economical and environment friendly processes for synthesis. However still some aspects needs close attention in order to fulfill thoroughly the requirements specially for high scale production. Thus, many enzymes are unstable, high cost, difficult to handle, and requires expensive cofactors.

Glycosyltransferases are important enzymes involved in essential processes related to oligosaccharide biosynthesis and they have found also very useful as biocatalyst for the chemoenzymatic synthesis of interesting oligosaccharides and nucleotides [113, 114]. They have been classified as Leloir if they are involved in the biosynthesis of most of N- and O-linked glycoproteins in mammalians, and require monophosphates and diphosphates as glycosyl donors, and non-Leloir enzymes which utilize sugar phosphates as substrates.

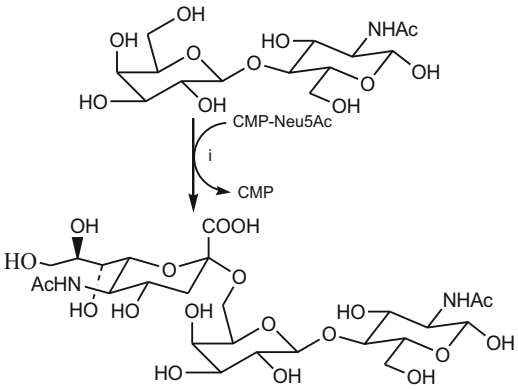
Glycosylations with galactosyltransferases can be performed through the use of glucose-1-phosphate as donor. A general sequence consists in the conversion by using UDP-Glc pyrophosphorylase to give UDP-glucose. Epimerization with UDP-glucose epimerase forms UDP-galactose which is used for glycosylation with galactosyltransferase (Scheme 2.96) [115].



i) UTP,UDP-Glcpyrophosphorylase. ii) UDP-Glc4-epimerase. iii) Gal transferase.

Scheme 2.96 Glycosylation with galactosyltransferases

Scheme 2.97 Synthesis of sialyl trisaccharide mediated by sialyl glycosyltransferase



i) α-(2-6)-sialyltransferase

The use of phosphorylase enzymes emerge as a potentially useful enzymatic tool for glycosylation, and an array of these enzymes such as glucan, sucrose, glucosyl glycerol, laminaribiose, nigerose, and maltose phosphorylases, have been isolated and identified from different microorganisms and considered for synthesis even at industrial scale synthesis [116].

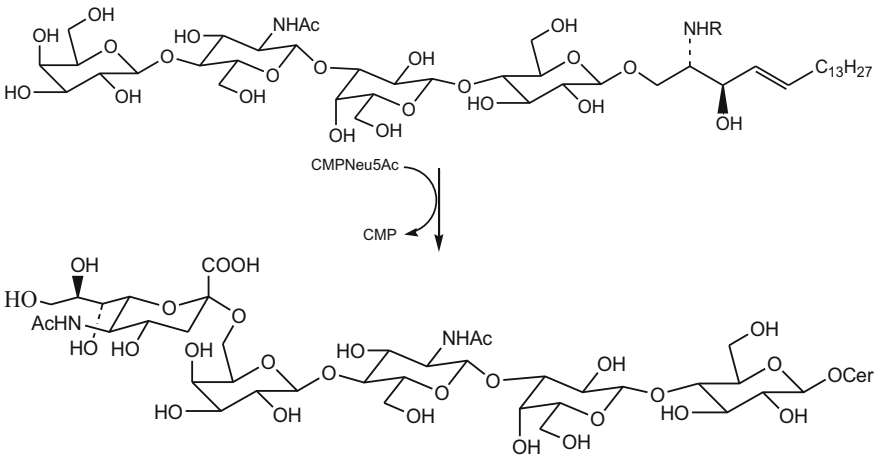
Several chemoenzymatic synthesis of α(2→6) and α(2→3)-oligosaccharides have been reported through the use of sialyltransferases for glycosidic coupling reactions. One described approach involves the in situ regeneration of CMP-Neu5Ac, requiring catalytic amount of CMP-Neu5Ac (Scheme 2.97) [117].

Sialyltransferases also proved to be efficient biocatalysts in the preparation of gangliosides, being involved in (2→6) linkage formation between the tetrasaccharide ceramide and CMP-Neu5Ac (Scheme 2.98) [118].

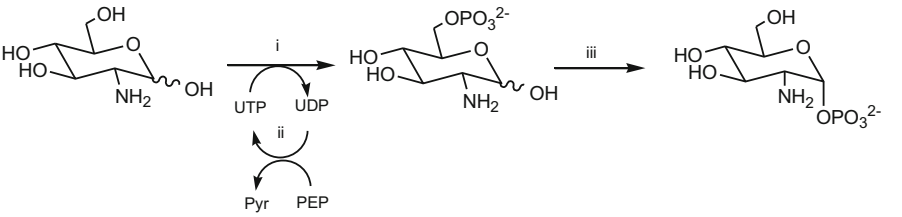
Glucosamine may be enzymatically transformed to glucosamine 6-phosphate by treatment with hexokinase from yeast, and ultimately to glucosamine 1-phosphate by the action of phosphoglucomutase (Scheme 2.99) [119].

UDP-glucuronic acid was prepared from UDP glucose by the action of UDP-Glc dehydrogenase along with NAD. This cofactor was regenerated with lactate dehydrogenase in the presence of pyruvate (Scheme 2.100) [120].

CMP-N-acetylneuraminic acid has been prepared form CTP and NeuAc under catalysis by CMP-NeuAc synthetase. In a cascade representation, it is observed that CTP is synthesized from CMP with adenylate kinase and pyruvate kinase (Scheme 2.101) [121].

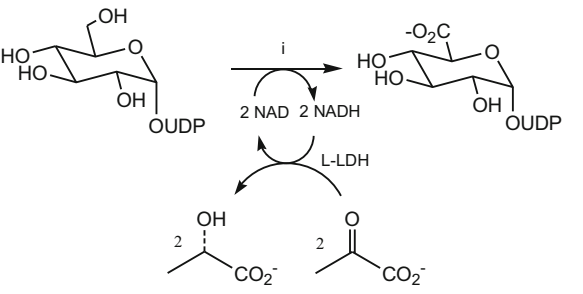


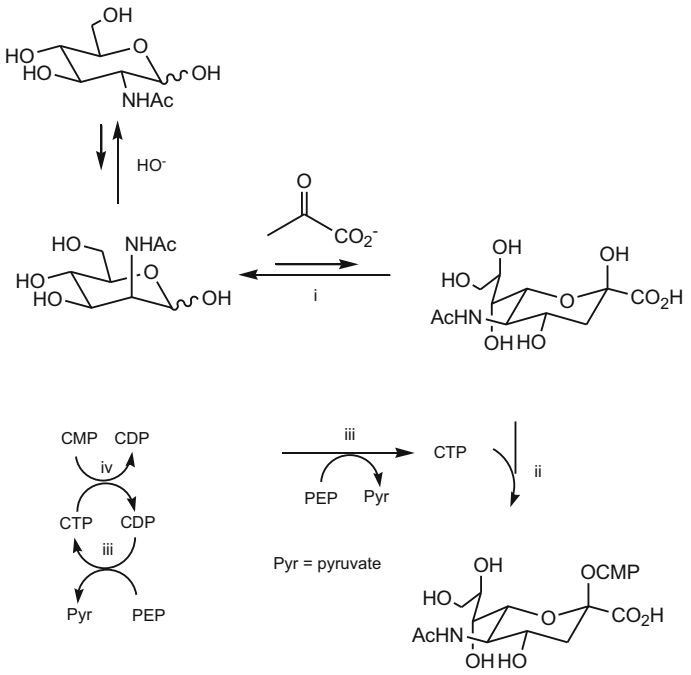
Scheme 2.98 Enzymatic synthesis of ganglioside



Scheme 2.99 Enzymatic preparation of glucosamine 6- and 1-phosphate

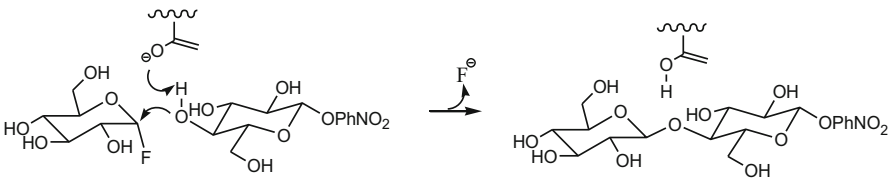
Scheme 2.100 Enzymatic preparation of UDP-glucuronide





i) UDP-NeuAc aldolase. ii) CMP-NeuAc synthetase. iii) pyruvate kinase. iv) adenylate kinase.

Scheme 2.101 Synthesis of CMP-N-acetylneuraminic acid

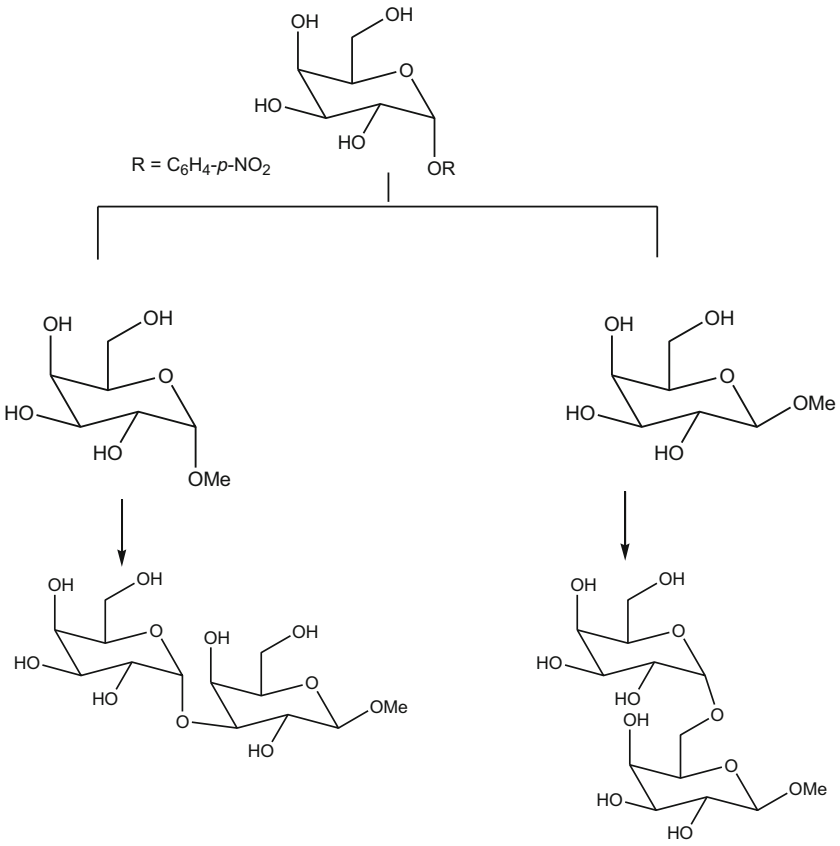


Scheme 2.102 Glycosynthase-catalyzed oligosaccharide synthesis

2.1.16.1 Enzymatic Synthesis of Oligosaccharides

Mutated glycosidase also known as glycosynthase AbgGlu358Ala in combination with activated glycosyl donors and suitable acceptors can generate synthetic oligosaccharides. Thus, for this transformation the conditions selected were α-glycosyl fluoride as glycosyl donor and *p*-nitrophenyl as glycosyl acceptor in the presence of ammonium bicarbonate buffer. The proposed mechanism of glycosynthase-catalyzed reaction is illustrated in Scheme 2.102 [122].

The Regioselective preparation of α-1,3 and α-1,6 disaccharides by using α-glycosidase as biocatalyst has been described. Thus, by combining

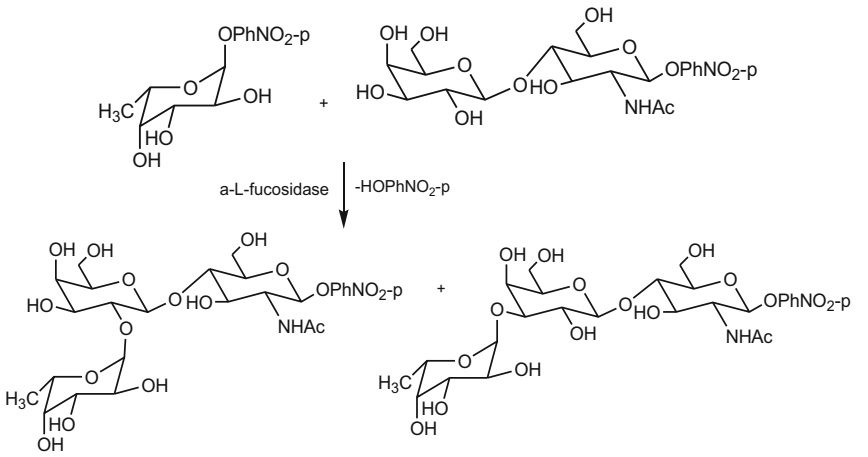


Scheme 2.103 Example of microbial catalyzed coupling reaction

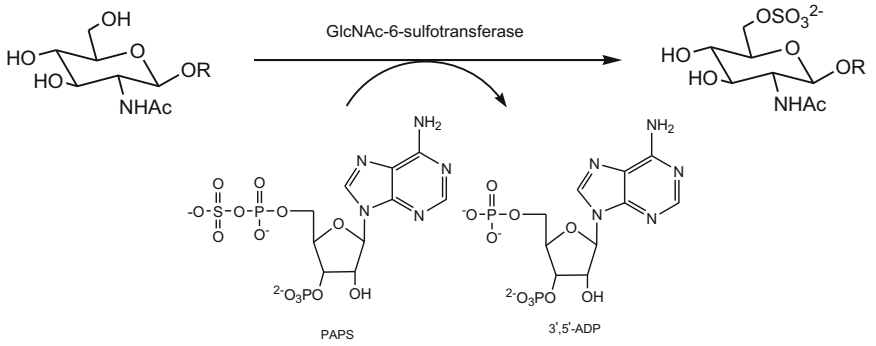
p-nitrophenyl-α-galactose functioning as glycosyl donor, with the glycosyl acceptor methoxygalactose, the expected 1,3- and 1,6-disaccharide were obtained in the form of α- and β-anomers (Scheme 2.103) [123].

A transglycosylation reaction mediated by α-L-fucosidase from *Alcaligenes* sp. was performed by combination of *p*-nitrophenylglycosides donors, with different acceptors such as *N*-acetylglucosamine, lactose, D-GlcNAc, and D-Glc, providing the corresponding *p*-nitrophenyl glycosides of disaccharides and trisaccharides containing a (1 → 2)-, (1 → 3)-, (1 → 4)-, or (1 → 6)-linked to the α-L-fucosyl group. In the general procedure illustrated in Scheme 2.76 the *p*-nitrophenyl fucoside donor was combined with *p*-nitrophenyl lactosamine acceptor, being incubated with α-L-fucosidase at 50 °C to produce the 2- and 3-linked trisaccharides (Scheme 2.104) [124].

Sulfotransferases provides a versatile method for the preparation of glycoside sulfates. A recent report describes the use of 3′-phosphoadenosine-5′-phosphosulfate (PAPS), and GlcNAc-6-sulfotransferase as catalyst (Scheme 2.105) [125].



Scheme 2.104 Transglycosylation reaction for the preparation of 2- and 3-linked trisaccharides

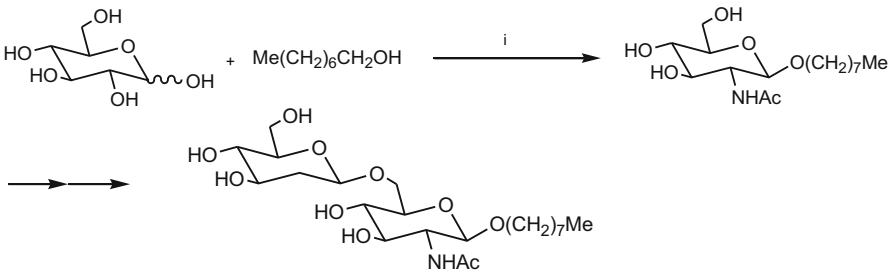


Scheme 2.105 Transfer of the sulfuryl group from PAPS to the glycoside

A chemoenzymatic synthesis of rhodiooctanoside isolated from Chinese medicines was described. The synthesis was carried out by direct β-glucosidation between 1,8-octanediol and D-glucose using immobilized β-glucosidase from almonds with the synthetic propolymer ENTP-4000 to generate the glycoside in 58 % yield (Scheme 2.106) [126].

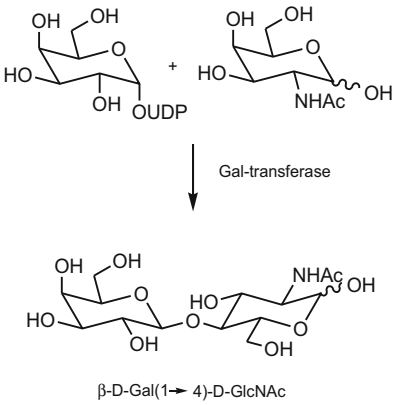
Lactosamine was prepared using and enzymatic approach consisting in the preparation of UDP glucose and condensation with *N*-acetyl glucosamine (GlcNAc) in the presence of galactosyl transferase (Scheme 2.107) [127].

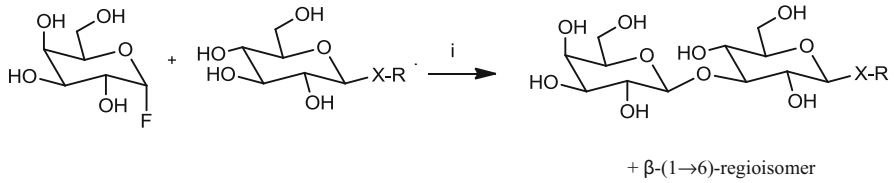
Unprotected glycosyl fluorides also have been used as donors for the enzymatic synthesis of disaccharides. For instance, glycosynthase and glycosidase mutants obtained from *Thermotoga maritima* and *Thermus thermophilus* have been used effectively for the regioselective synthesis of disaccharides (1 → 3) in higher of 80 % yield (Scheme 2.108) [128].



Scheme 2.106 Chemoenzymatic synthesis of rhodiooctanoside

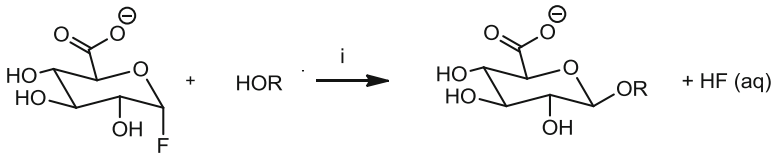
Scheme 2.107 Enzymatic synthesis of lactosamine





Scheme 2.108 Enzymatic glycosylation from unprotected glycosyl fluorides

Another example of enzymatic glycosylation using unprotected fluorides donors was achieved by using α-D-glucuronyl fluoride with engineered *Escherichia coli* glucuronylsynthase, providing β-glucuronides in moderated to good yield depending on the alcohol acceptor employed (Scheme 2.109) [129].



i) glucuronylsynthase phosphate buffer, pH 7.5

Scheme 2.109 Enzymatic glycosylation from unprotected glycosyl fluorides

2.1.17 Solid Phase Methodology

Perhaps what remains as the most challenging task for sugar chemistry is the synthesis of complex oligosaccharides such as that found in bacterial membranes or wall cells, and that are usually in the form of glycopeptides. Different types of monosaccharides can be present as constitutive parts such as glucose, galactose, mannose, *N*-acetylglucosamine, sialic acid and L-fucose. Also, the order of linkage and stereoselectivity between them is rarely conserved.

The different nature, stereoselectivity and linkage sequence have been a formidable obstacle for the development of general procedures of the type used for peptides and oligonucleotides which can be prepared on machine synthesizers with high efficiency.

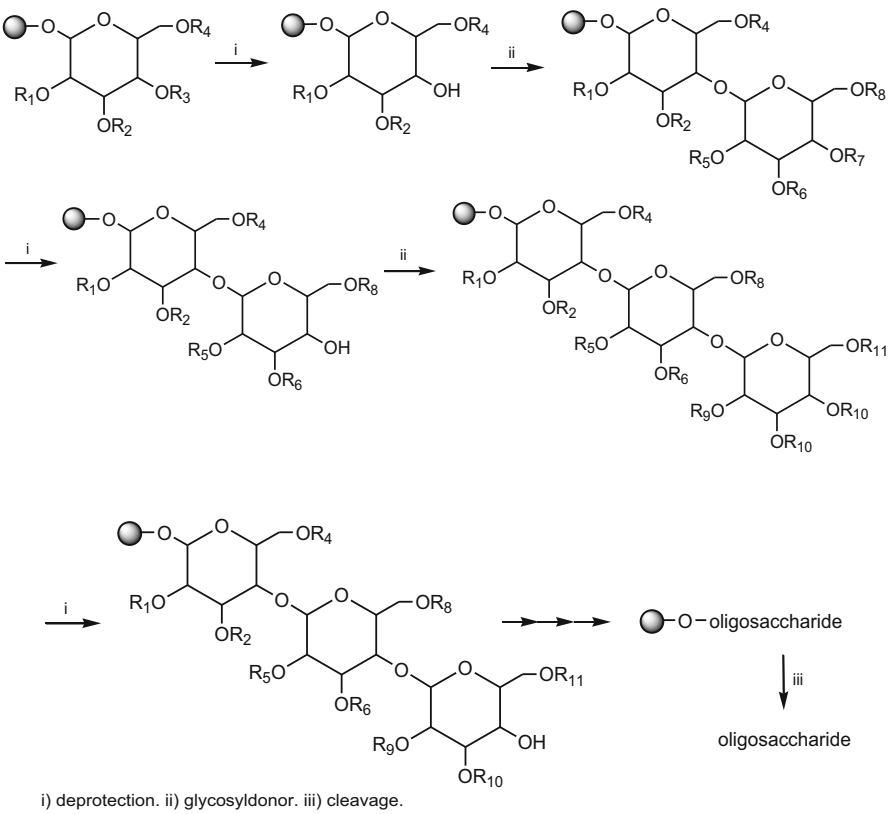
The main advantage of the solid phase methodology is the coupling of sugar units to the resin, which allows easy washing away of the non reacted reagents, avoiding tedious purifications steps.

Nonetheless despite the difficulties, interesting progress has been made for preparing oligosaccharides [130, 167, 168], and glycopeptides [131], suggesting that in the solid phase technology for complex sugars will be affordable.

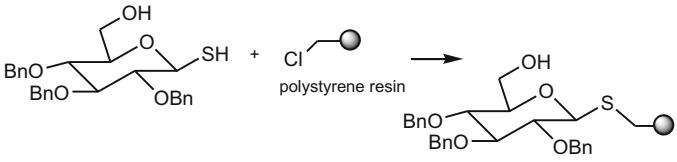
The solid phase approach involves three elements namely the glycosyl donor, glycosyl acceptor and the resin which is properly activated with a group susceptible for attachment either with the glycosyl donor or acceptor depending on the strategy of choice. Although it appears obvious, it is important to remain that the linkage between the resin and the sugar should be easily cleaved under compatible conditions for the glycoside bond.

According to a comprehensive review [132], the synthetic strategies are classified into: (a) donor-bound, (b) acceptor-bound, and (c) bidirectional Strategies.

One general approach involves the initial attachment of a glycosyl donor (halides, trichloroacetimidate, sulfoxides, phosphate (one is repeated), thio, allyl and glycals) to the resin (polystyrene-base). The attached sugar is selectively deprotected depending on the required position (1,2- 1,3- 1,4- 1,6-), transforming the resin–sugar complex in a sugar acceptor which will be coupled to the next glycosyl donor to produce a second linkage. By repeating this sequence an elongated chain is obtained. The final release and full deprotection will produce the free oligosaccharide (Scheme 2.110) [133].



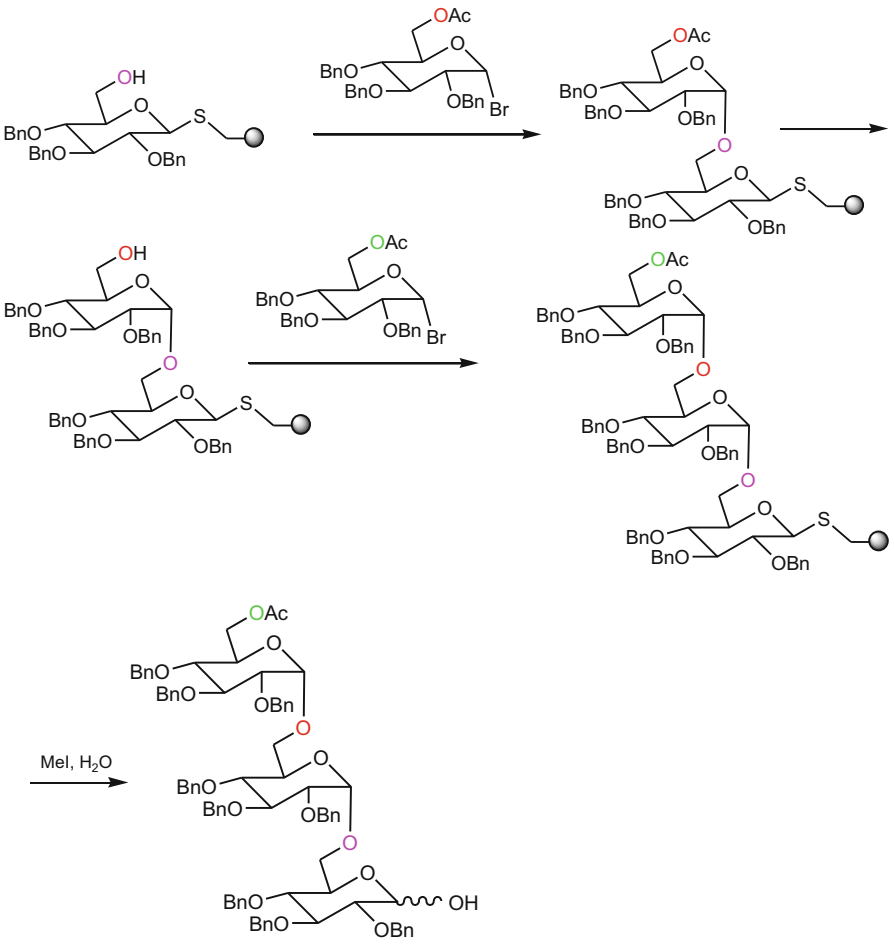
Scheme 2.110 General scheme for solid-phase oligosaccharide synthesis 1,4-linkage case



Scheme 2.111 Example of donor bound strategy for solid-phase glycosylation reactions

An example of the donor bound strategy is the bounding of sulfur glycoside to polystyrene resin to form a sulfur linkage between the donor and the resin (Scheme 2.111). Suitable hydroxyl group from the donor will serve as linkage site with de next sugar unit for chain elongation.

It should be noted that the glycosyl donor also contains a position available for the linkage with the next sugar. In other words the glycosyl donor once attached to the resin becomes a glycosyl acceptor, as can be seen for the next coupling sequence (Scheme 2.112) [132].

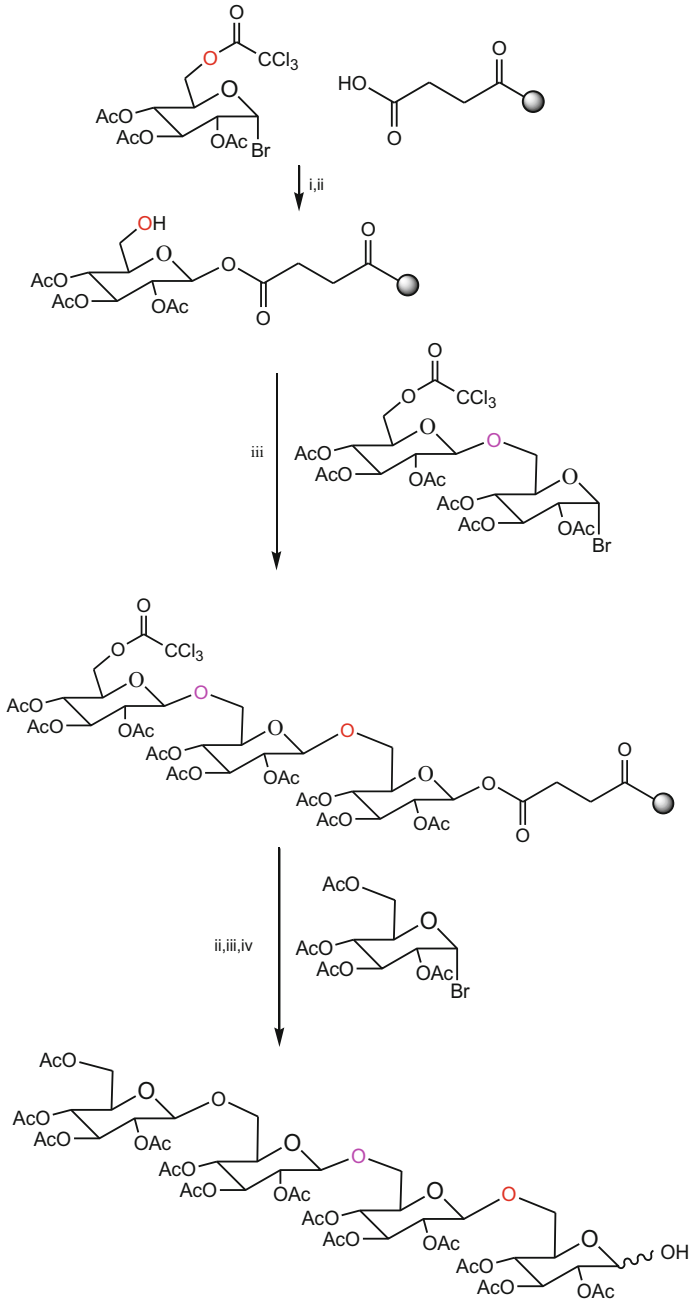


Scheme 2.112 Sulfur mediated solid-phase coupling reaction

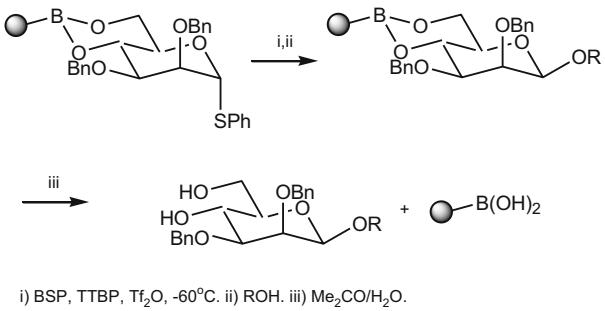
The synthesis of β-(1 → 6) gentotetraose was accomplished by using a benzoyl propionate as resin linker. The glycosyl donor chosen was acetobromoglucose functionalized with trichloroacetate group as a temporary protecting group at position 5. Glycosylation reactions were effected under Helferich conditions and cleavage from resin was performed with hydrazinium acetate (Scheme 2.113).

Polymer solid phase has been also exploited successfully by Crich et al. [134], for the synthesis of sensitive β-mannosides, using a variation of sulfhoxide method, consisting in the transformation of sulfoxide to triflic group as leaving group. The subsequent addition of alcohol acceptor to the donor attached to the Wang resin will result in the glycoside β-mannoside formation (Scheme 2.114).

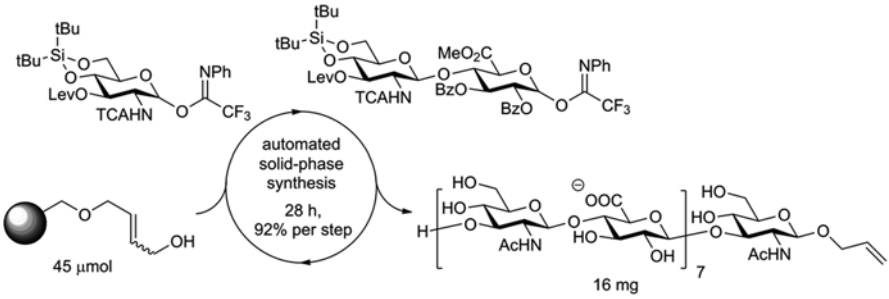
The *N*-phenyl trifluoroacetimidate donor was incorporated as a building block for solid-phase assembly as described in Scheme 2.115, starting from the coupling



Scheme 2.113 Solid-phase coupling promoted by Helferich conditions



Scheme 2.114 Solid-phase synthesis of β-mannoside glycoside

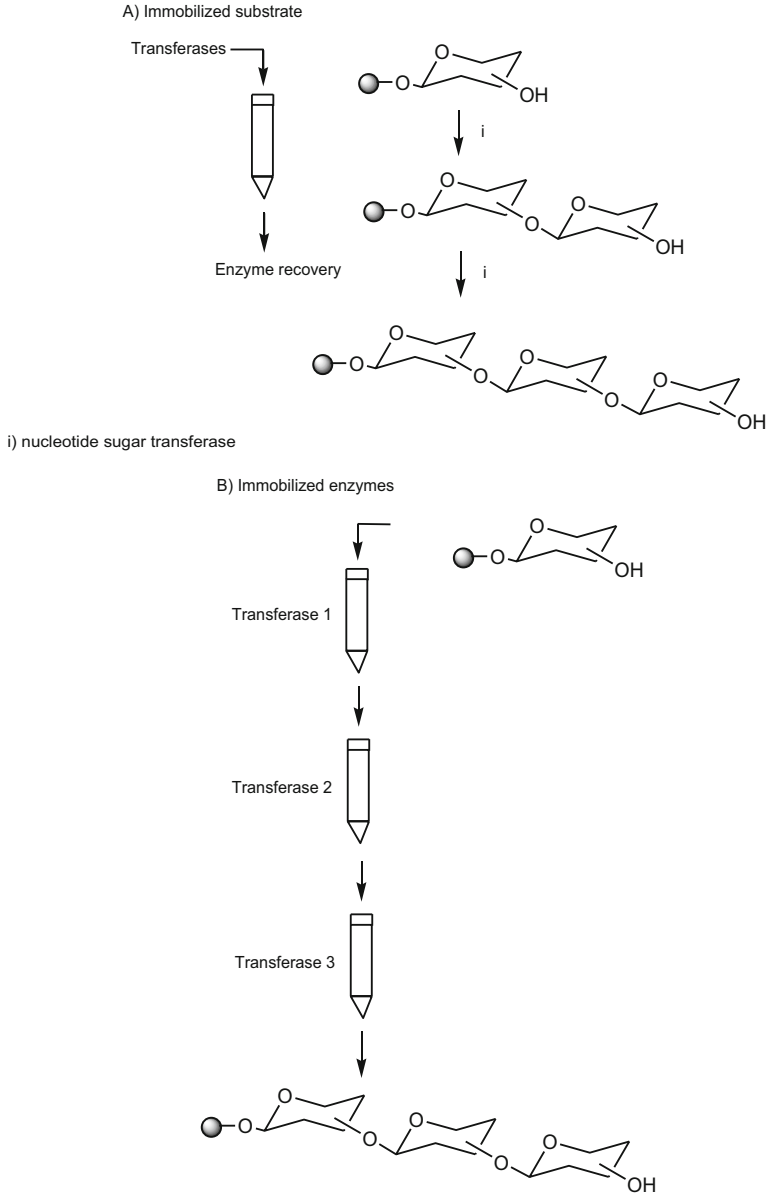


Scheme 2.115 Solid-phase assembly by using *N*-phenyl trifluoroacetimidate donors

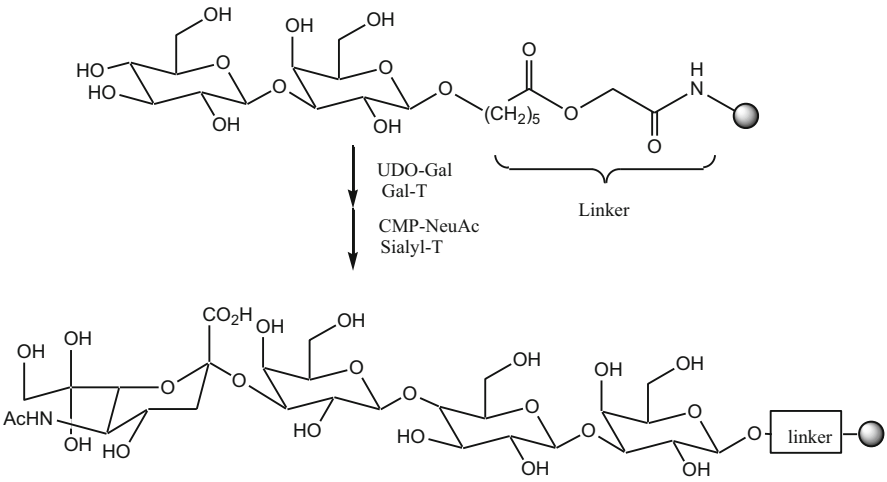
with a resin under TfOH conditions, and subsequent condensation with S-phenylglucuronic acid, to furnish dimer which was transformed into imidate donor until reaching a building block at multigram scale (Scheme 2.115) [135].

The enzymatic solid-phase oligosaccharide synthesis relies mainly by the use of glycosyltransferases, glycosidases, and glycosynthases. By taking advantage on their high stereoselectivity and regioselectivity, various oligosaccharides and glycopeptides have been prepared usually under mild conditions without the need of using protecting groups. Unfortunately the enzymatic approach is still in some cases unaffordable due to its high cost for large scale processes, lower yields provided and their limited capability for recognizing a broad range of sugars specially those not common. Two general approaches have been proposed for the preparation of oligosaccharides through the solid-phase approach (Scheme 2.116) [136].

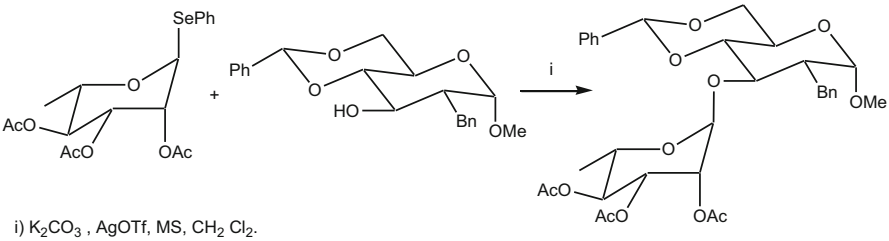
A solid-phase enzymatic approach for extending the oligosaccharide chain was described by Gijsen et al. [136] in which a disaccharide-linker fragment attached to a resin was coupled with the glycosyltransferases UDP-galactose and CMP-NeuAc in the presence of galactosyltransferases and sialyltransferase as enzymatic catalyst. Final treatment with hydrazine was used to release the tetrasaccharide from the solid support (Scheme 2.117).



Scheme 2.116 Two general approaches for immobilized solid-phase oligosaccharide synthesis



Scheme 2.117 Enzymatic-solid phase glycosylation reaction



Scheme 2.118 Phenylselenosugars as glycosyl donors

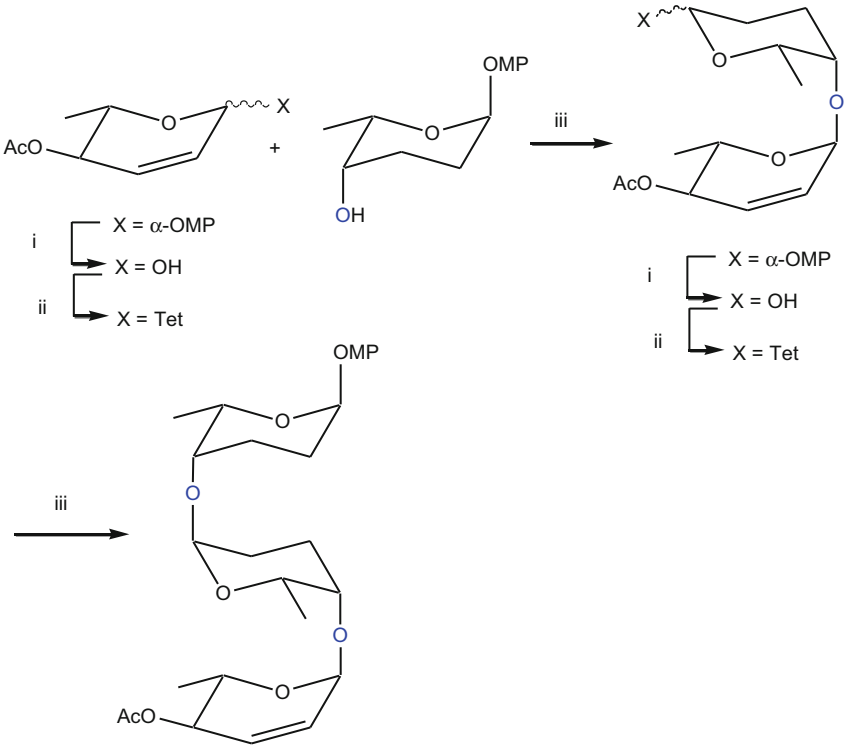
2.1.18 Miscellaneous Glycosylations

2.1.18.1 Selenosyl Donors

The use of selenoglycosides as glycosyl donors and acceptor in glycosylation reactions has also been described by Metha and Pinto [137]. A typical glycosidation procedure with phenylselenoglycoside donors involves the glycosyl acceptor, 4-Å molecular sieves, silver triflate, and potassium carbonate in dichloromethane (Scheme 2.118).

2.1.18.2 Tetrazol as Leaving Group

Tetrazol has also been tested as a leaving group for the preparation of an antibiotic fragment [138]. A coupling reaction with the methoxyphenyl glycosyl acceptor was catalyzed with $(Me_3)_3OBF_4$ as shown in Scheme 2.119.



i) CAN. ii) 1H-tetrazole. iii) $(CH_3)_3OBF_4$, MS.

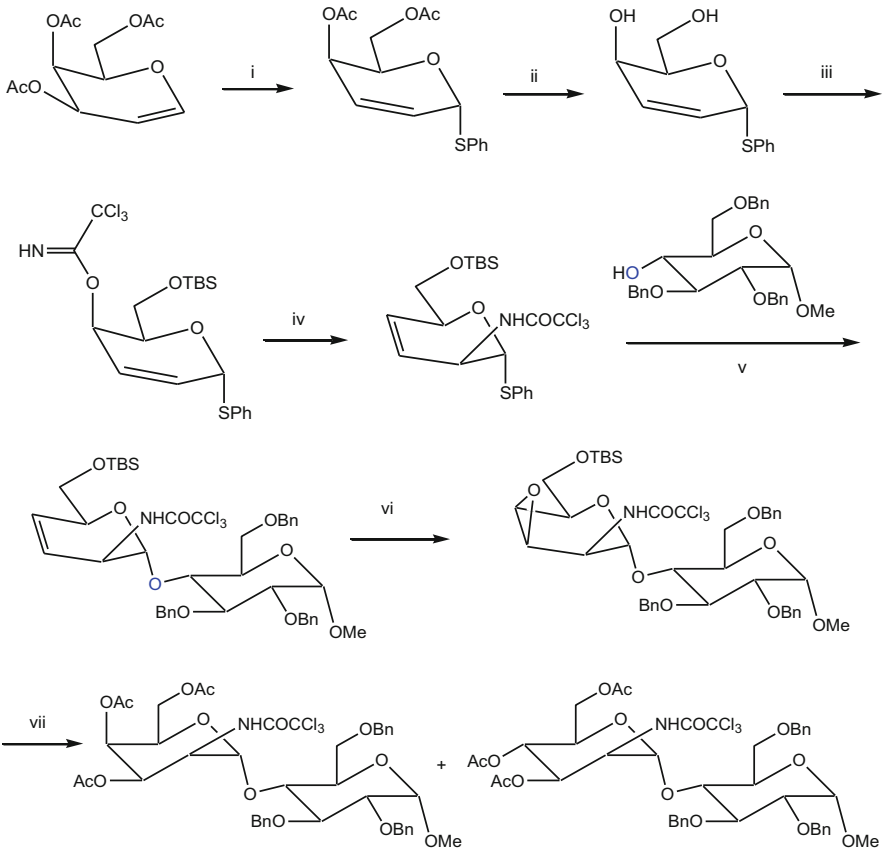
Scheme 2.119 The use of tetrazol as a leaving group

2.1.18.3 Sigmatropic Glyosylations

2-aminodisaccharides were obtained by an elegant [3,3] sigmatropic rearrangement, by Takeda et al. [139] The addition of thiophenol to an unsaturated C-1 in the presence of Lewis acid, was followed by a sigmatropic rearrangement with an imidate group which migrates from C-4 to C-2. Disaccharide formation was catalyzed with $Pd(CH_3CN)_2$-AgOTf complex in dichloromethane (Scheme 2.120).

2.1.18.4 Zinc Promoted Glycosylation

The total synthesis of the cyclic glycolipid arthrobacilin A, a cell growth inhibitor was achieved by Garcia and Nizhikawa [140], under zinc *p-tert*-butylbenzoate salt as glycoside catalyst, obtaining the β-galactoside glycoside in 73 % along with α-isomer in 27 % (Scheme 2.121).



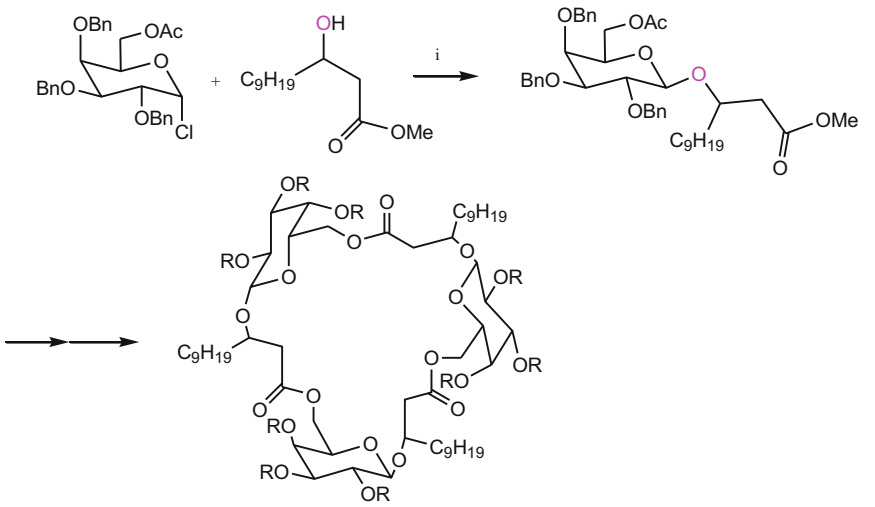
i) PhSH, $SnCl_4/CH_2Cl_2$ .ii) MeONa/MeOH. iii) a) TBSCl-imidazol/CH_2Cl_2. b) Cl_3CCN-NaH/CH_2Cl_2. iv) xylene, reflux. v) $Pd(CH_3CN)_2$-AgOTf,MS 4A/CH_2Cl_2 . vi) m-CPBA/CH_2Cl_2 . viii) Ac_2O-AcOH/ $BF_3.OEt_2$.

Scheme 2.120 Sigmatropic rearrangement

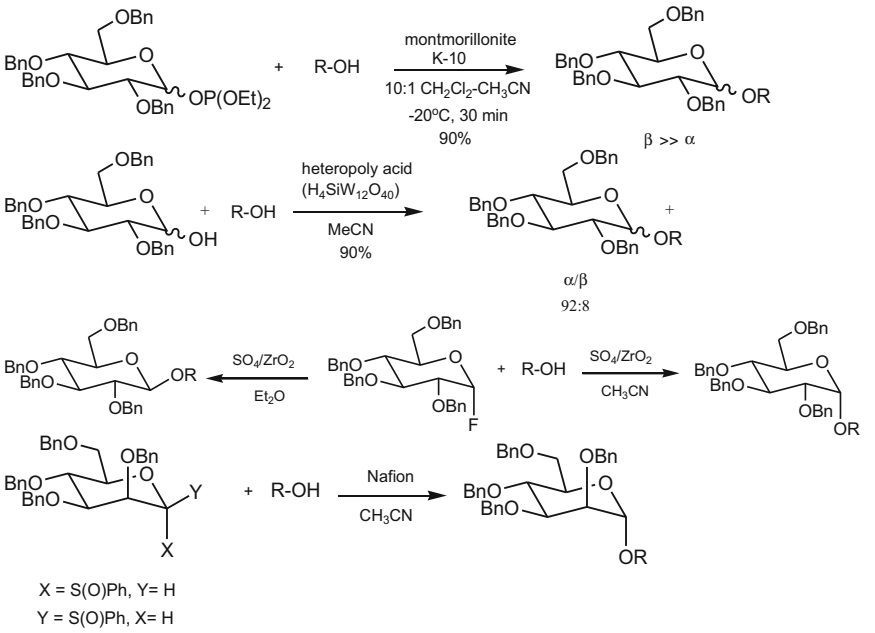
2.1.18.5 Heterogenous Catalysis

Stereocontrolled α- and β-glycosylations by using environmentally benign heterogenous catalyst has been developed as a novel approach for stereoselective formation of β-*O*-glycosidic linkages. Polymeric materials such as montmorillonite K-10 [141], heteropoly acid ($H_4SiW_{12}O_{40}$) [142], sulfated zirconia (SO_4/ZrO_2) [143], and perfluorinated solid-supported sulfonic acids (Nafion resins) [144] have been assayed successfully providing series of stereocontrolled *O*-glycosides in high yield (Scheme 2.122).

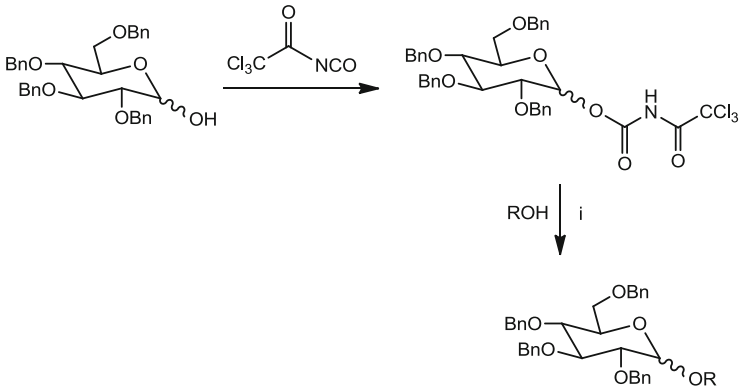
Glycosyl *N*-trichloroacetylcarbamate obtained from reaction of tetrabenzyl glucopyranoside hemiacetals with trichloroacetyl isocyanate was used as glycosyl



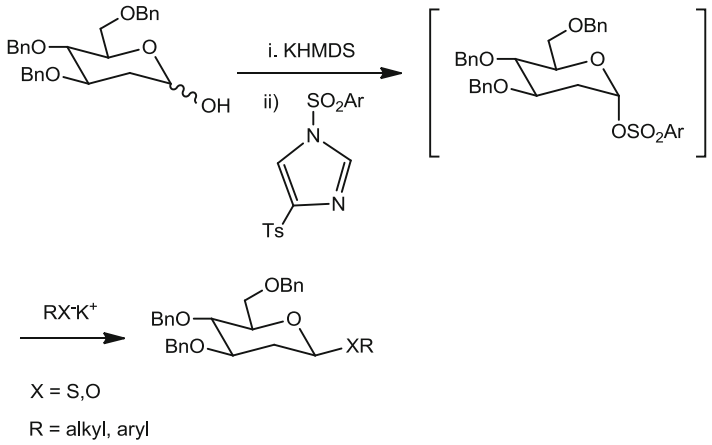
Scheme 2.121 Glycosylation reaction for preparation of arthrobacilin A



Scheme 2.122 Stereocontrolled O-glycosidations using heterogeneous polymeric materials



Scheme 2.123 O-glycosylation via *N*-trichloroacetylcarbamate

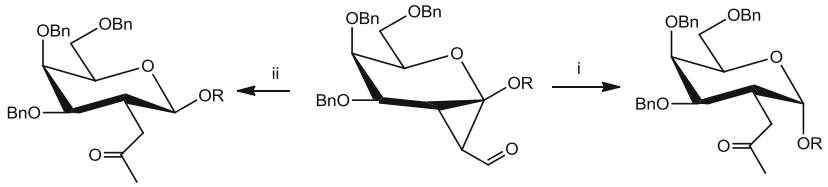


Scheme 2.124 Preparation of β-glycosides via glycosyl sulfonate formation

donors. Various Lewis acids were tested for α-selective glycosylation observing that the promoters TMSOTf and $TMSClO_4$ yield the best results (Scheme 2.123) [145].

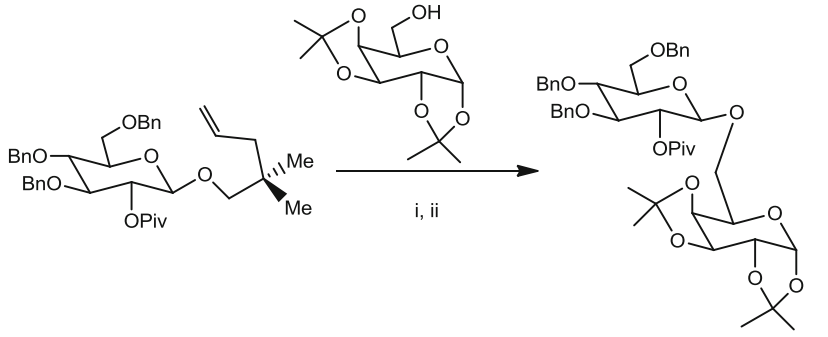
N-Sulfonyl imidazole has been used as activating agent for preparing 2-deoxy monosaccharides through deprotonation of the anomeric hydroxyl group with KHMDS at low temperature. Further reaction with *N*-sulfonyl imidazole resulted in the glycosyl sulfonates intermediate generated in situ which was finally reacted with the desired nucleophile to produce the β-glycoside in moderate to good yields (Scheme 2.124) [146, 147].

On the other hand 1,2-cyclopropaneacetylated sugar has been proposed as glycosyl donors for O-glycosylations, allowing stereoselective control depending on the catalyst employed. Thus, β-anomeric products were obtained with $BF_3.OEt_2$ as catalyst, whereas TMSOTf-catalyzed glycosylation prefers the α-anomeric products (Scheme 2.125).



i) TMSOTf, ROH, CH_2Cl_2, MS, 0°C to rt. ii) $BF_3.Et_2O$, ROH, CH_2Cl_2, MS, -20°C to rt

Scheme 2.125 Stereocontrolled glycosylations from 1,2-cyclopropaneacetylated sugar as glycosyl donors



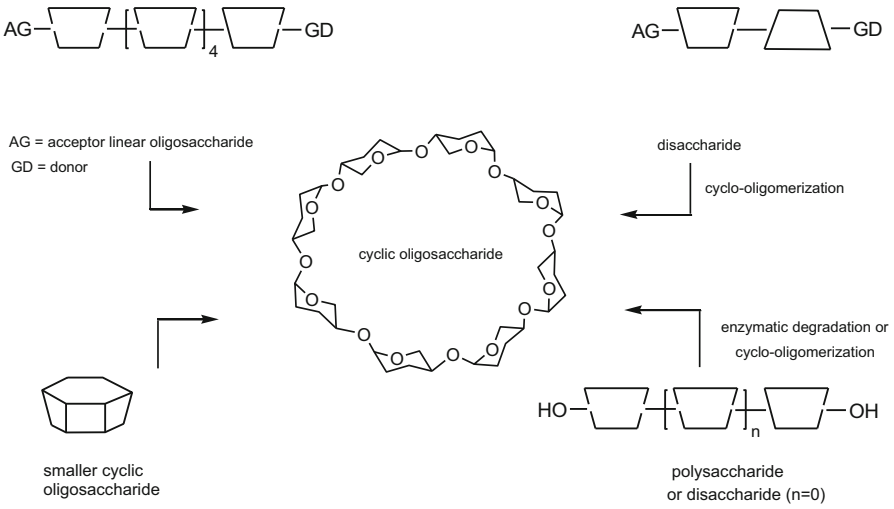
i) ROH, NBS, TESOTf (cat.), CH_2Cl_2, -78°C, 1h

Scheme 2.126 Preparation of protected β-1,6 disaccharide form Gem-dimethyl 4-*n*-pentenyl glycosides

Gem-dimethyl 4-*n*-pentenyl glycosides were proposed as glycosyl donors for glycosylation and hydrolysis of the anomeric carbon when using NBS as the sole stoichiometric activator with yield reported around 80 % mainly with β selectivity (Scheme 2.126) [148].

2.1.19 Cyclic Oligosaccharides

The synthesis of cyclic oligosaccharides involves the preparation of linear saccharides which ultimately are joined together to form a cyclic macromolecule. There are two main approaches proposed based on the cycloglycosylation step. The first involves the preparation of a long chain having and each end the donor and acceptor functionalities that will be interconnected through a glycosidic bond at a final step, and the second involving the polycondensation of smallest repeating unit called "saccharide monomers." It has been observed that the latter strategy is considered less laborious; however, it produces cyclic oligomers of different size since under these conditions the ring formation step is not controllable.



Scheme 2.127 The four suggested approaches to the synthesis of cyclic oligosaccharides

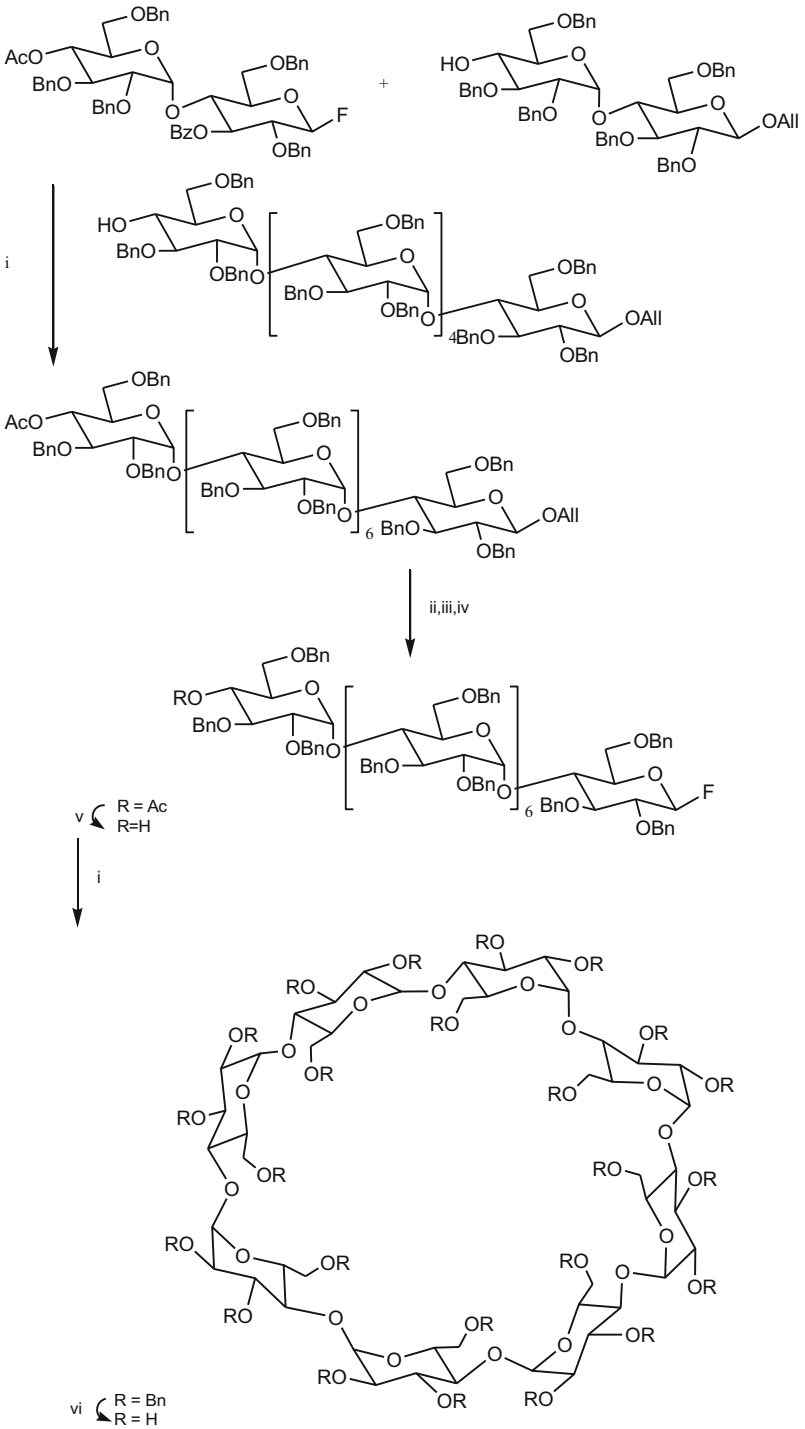
The chemical synthesis of cyclic oligosaccharides has been mainly driven to obtain cyclic (1 → 4)-linked oligopyranosides, however (1 → 3), and (1 → 6) linked cycloforms are also described. In the case of (1 → 2)-linked oligosaccharides, the ring closure require about 17 or more glucopyranoside residues because (1 → 2)-linkage composed of pyranoside connected by one equatorial and one axial bond assumes rigid conformations and cannot cyclize [149].

The pioneering total synthesis of cyclic oligosaccharide α-Cyclodextrin was carried out by Ogawa's group in 1985 [150] and since then alternative chemical or enzymatic methodologies appeared for preparing cyclic oligosaccharides. Nowadays the industrial production of cyclodextrins relies on the enzymatic conversion of prehydrolyzed starch into a mixture of cyclic and acyclic oligomers.

A full report about cyclic oligosaccharides [150] proposes four approaches to the synthesis of cyclic oligosaccharides developed during the last 10 years. (1) the stepwise preparation of a linear precursor that is subjected to cycloglycosylation; (2) the one-pot polycondensation/cycloglycosylation of a small "oligosaccharide monomer" typically, a disaccharide or trisaccharide that can yield a range of macrocycles of different sizes; (3) the enzyme-assisted synthesis of natural or unnatural cyclic oligosaccharides; (4) the ring opening of cyclodextrins followed by oligosaccharide chain elongation and cycloglycosylation (Scheme 2.127).

Despite the significant advances observed in cyclic oligosaccharide synthesis, their preparation is time consuming, producing the target compounds with low regioselective and stereoselective in low yields. The total synthesis of α-CD and γ-CD was described according to Scheme 2.128 [151, 152].

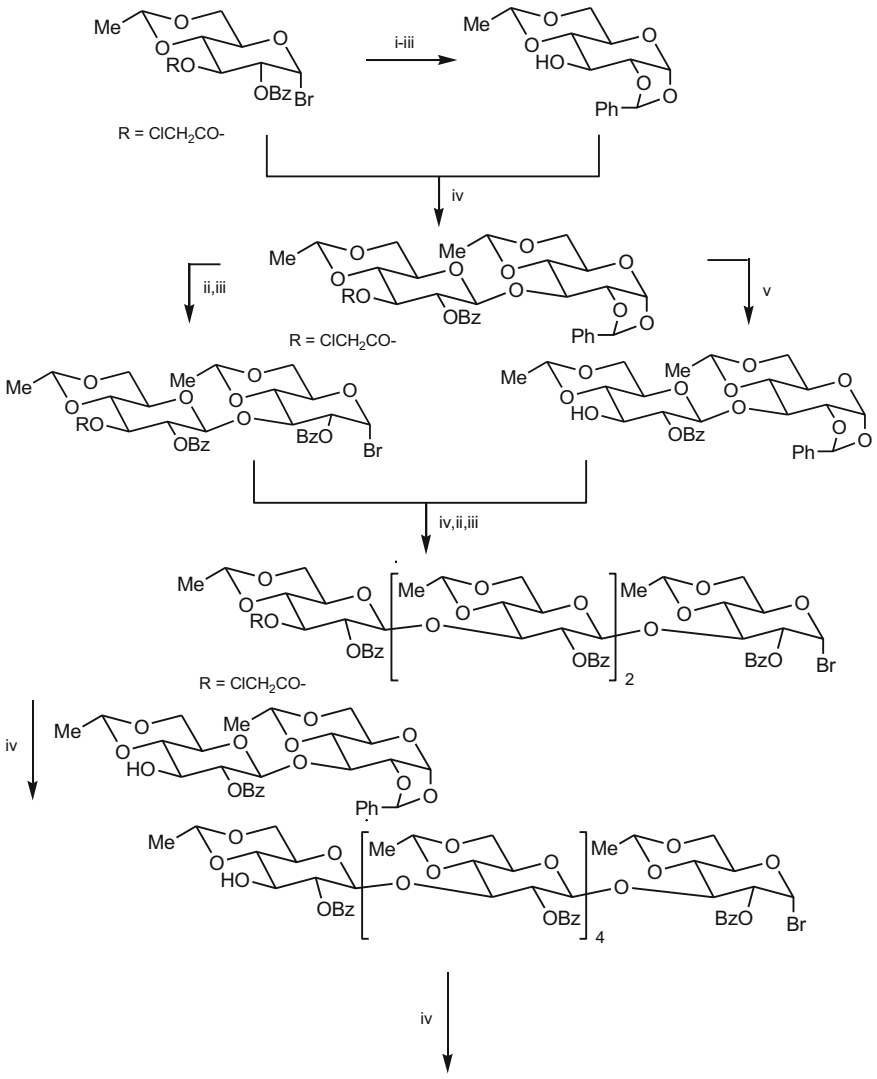
In 1990, the chemical synthesis of β-(1 → 3) linked hexasaccharide was reported. The chemical approach involved the glycosidic reaction between benzylidene acceptor and protected glycosyl bromide as glycosyl donor, under silver triflate-promoter conditions. As it can be seen in Scheme 2.89, the construction of



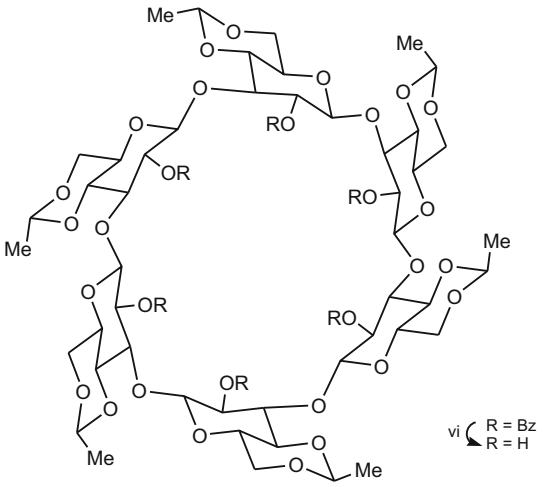
Scheme 2.128 Chemical synthesis of cyclic α(1 → 4)-oligosaccharide γ-CD

the linear oligosaccharide and its final cycloglycosylation was performed by using glycosyl bromides which were prepared by photolytic brominolysis of 1,2-*O*-benzylidene glucose with $BrCCl_3$ (Scheme 2.129) [153].

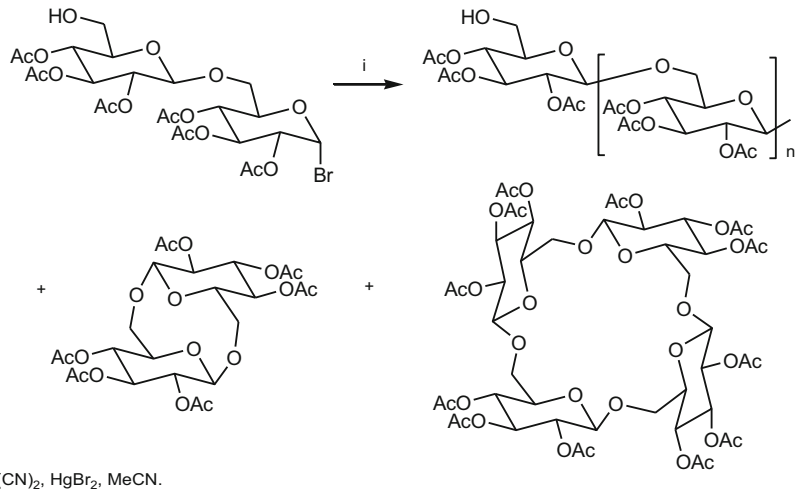
The formation of (1→6)-glycopyranosidic linkages might produce cyclic disaccharides, trisaccharides, and tetrasaccharides. An early synthesis of β-(1→6)-glucopyranan under Helferich conditions, generated along with the linear oligomer, a cyclic disaccharide and tetrasaccharide in 12% and 6% respectively (Scheme 2.130) [154].



Scheme 2.129 Synthesis of cyclic β-(1→3)-linked oligosaccharide

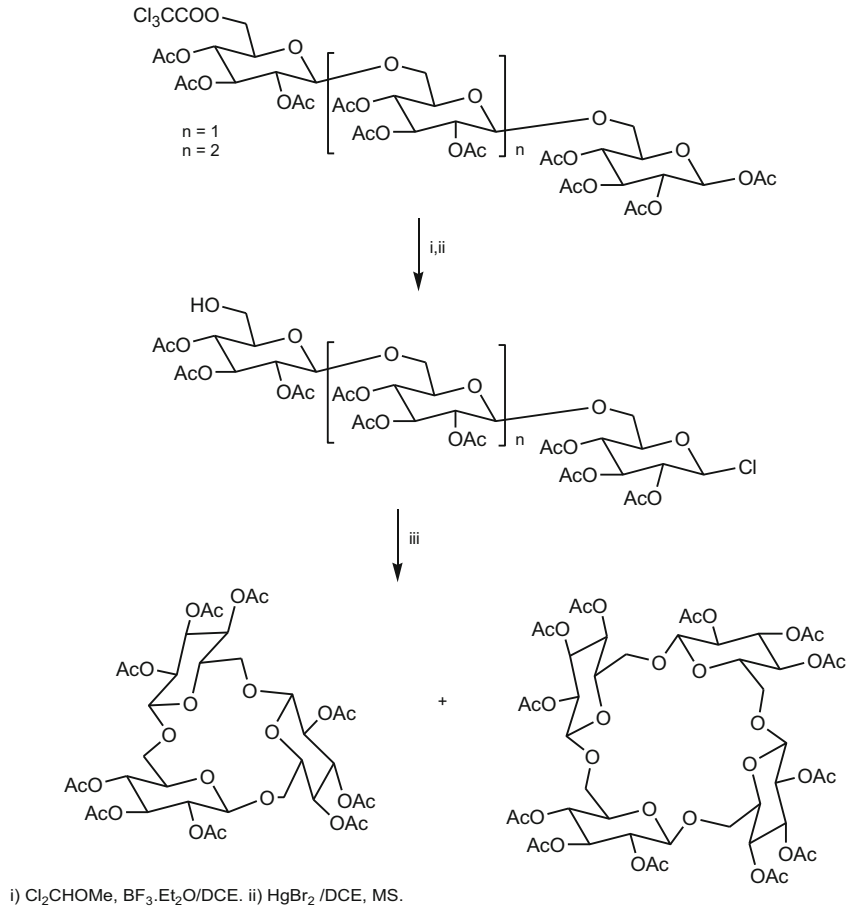


Scheme 2.129 (continued)



Scheme 2.130 Preparation of linear, and cyclic β(1 → 6) disaccharides and tetrasaccharides

An improved synthesis of cyclotetraoside was described by the same group 10 years later, consisting in the preparation from the peracetylated tetrasaccharide into the tetrasaccharide derivative having both the acceptor and the donor components. The final cyclization was performed under Helferich conditions providing a mixture of trisaccharide and tetrasaccharide in 22 % and 25 % yield respectively (Scheme 2.131) [118, 155].

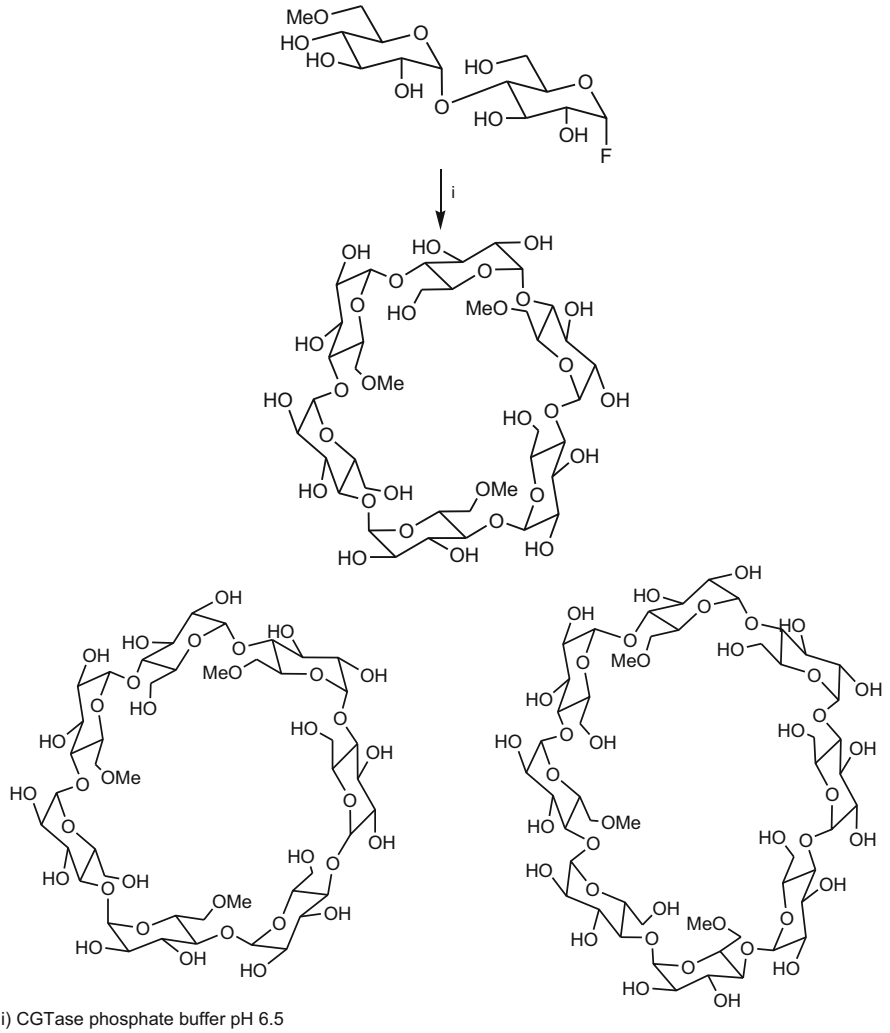


Scheme 2.131 Improved synthesis of cyclic β(1 → 6) trisaccharides and tetrasaccharides

2.1.19.1 Chemoenzymatic and Enzymatic Synthesis

The use of enzyme is as mentioned for many *O*- or *N*-glycosides the parallel possibility for preparing cyclic oligosaccharides. The limitation continue to be the availability and affordability; however, some enzymes such as glycosidases and cycloglycosyltransferases (CGTases) which are involved in the preparation of cyclodextrins from starch and other α-(1 → 4)-glucans are accessible and more versatile [155].

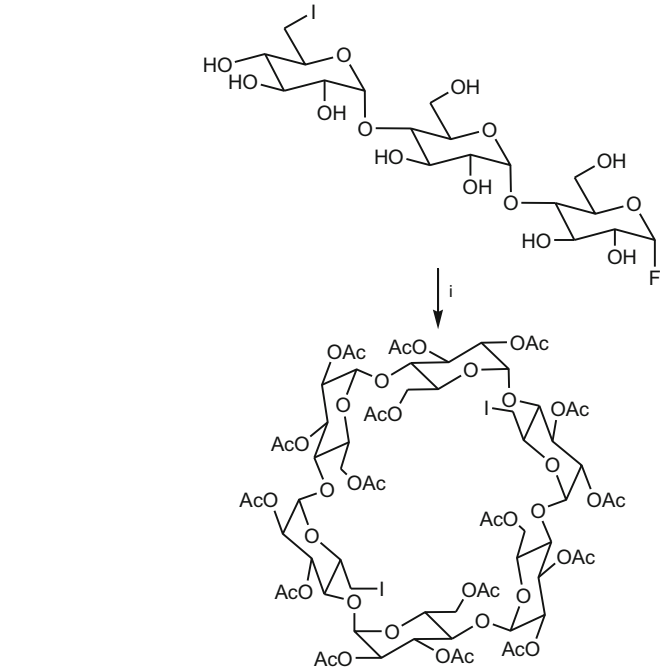
The feasibility of the chemoenzymatic approach was established in the preparation of cyclic β(1 → 4) hexasaccharides, heptasaccharides, and octasaccharides, from 6-*O*-methylmaltosyl fluoride when incubated with CGTase. Thus, a mixture of 6^{I}, 6^{III}, 6^{V}-tri-*O*-methyl-α-CD (42 %), 6^{I}, 6^{III}, 6^{V}-tetra-*O*-methyl-γ-CD (16 %) and in less proportion 6^{I}, 6^{III}, 6^{V}-tri-*O*-methyl-β-CD were obtained (Scheme 2.132) [136, 156].



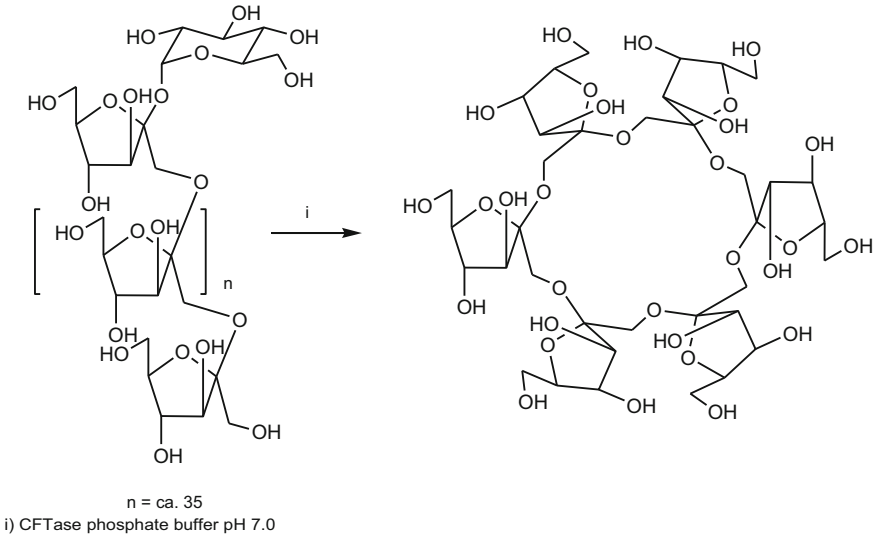
Scheme 2.132 Synthesis of 6^{I}, 6^{III}, 6^{V}-tri-*O*-methyl-α-CD, 6^{I}, 6^{III}, 6^{V}-tetra-*O*-methyl-γ-CD and 6^{I}, 6^{III}, 6^{V}-tri-*O*-methyl-β-CD

Furthermore, under the same conditions it was possible to prepare from the maltotriosyl fluoride the cyclic α(1 → 4) hexasaccharide (6^{I}, 6^{II}-dideoxy-6^{I},6^{II}-diiodo-α-CD) in 38 % (Scheme 2.133) [118, 157].

An alternative option for the enzymatic preparation of cyclic oligosaccharides besides CGTases is glycosidases which exerts its action on polysaccharides. This possibility is exploited in the preparation of cyclic fructins by conversion of β-(1 → 2)-fructofuranan by bacterial fructotransferases isolated from *Bacillus circulans* (Scheme 2.134) [158].

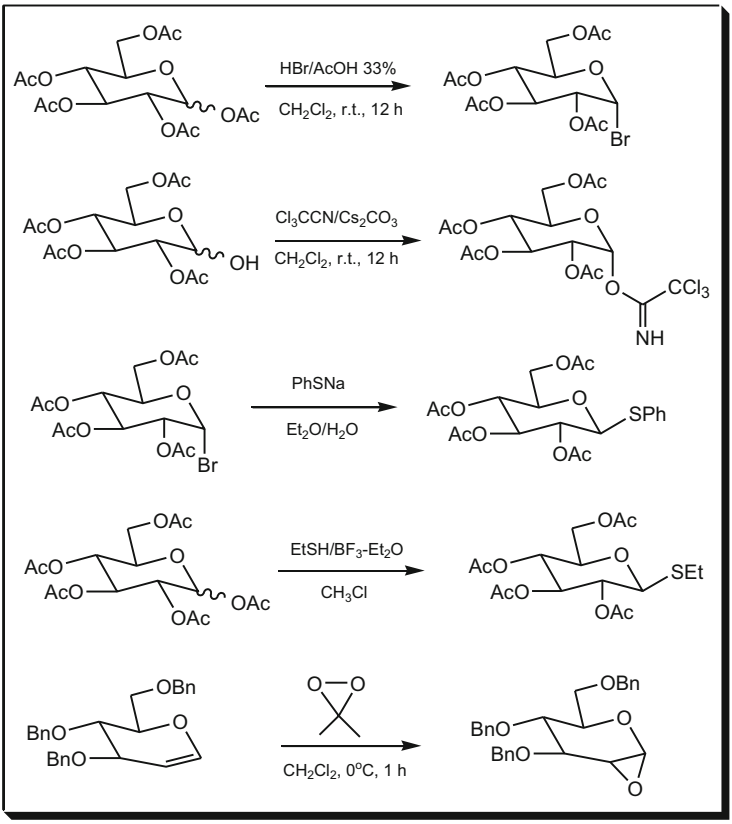


Scheme 2.133 Enzymatic synthesis of 6^{I}, 6^{II}-dideoxy-6^{I},6^{II}-diiodo-α-CD



Scheme 2.134 Enzymatic synthesis of cycloinulooligosaccharides

2.1.19.2 Summary for Preparing Conventional Glycosyl Donors



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Chapter 3
N-glycosides

N-glycosides are generated when a sugar component is attached to an aglycon, through a nitrogen atom, establishing as a result a C–N–C linkage. Nucleosides are among the most relevant *N*-glycosides since they are essential components of DNA, RNA, cofactors, and a variety of antiviral and antineoplastic drugs.

Usually for nucleosides, a pyrimidine or purine base is linked to the anomeric carbon of a furanoside ring. The nucleosides responsible for the formation of the genetic material DNA and RNA are: adenine, guanine, cytosine, and thymine, the latter replaced with uracil in the case of RNA (Scheme 3.1). Nucleosides can be classified into natural nucleosides such as those involved in the genetic storage of information, naturally modified nucleosides, and synthetic nucleosides.

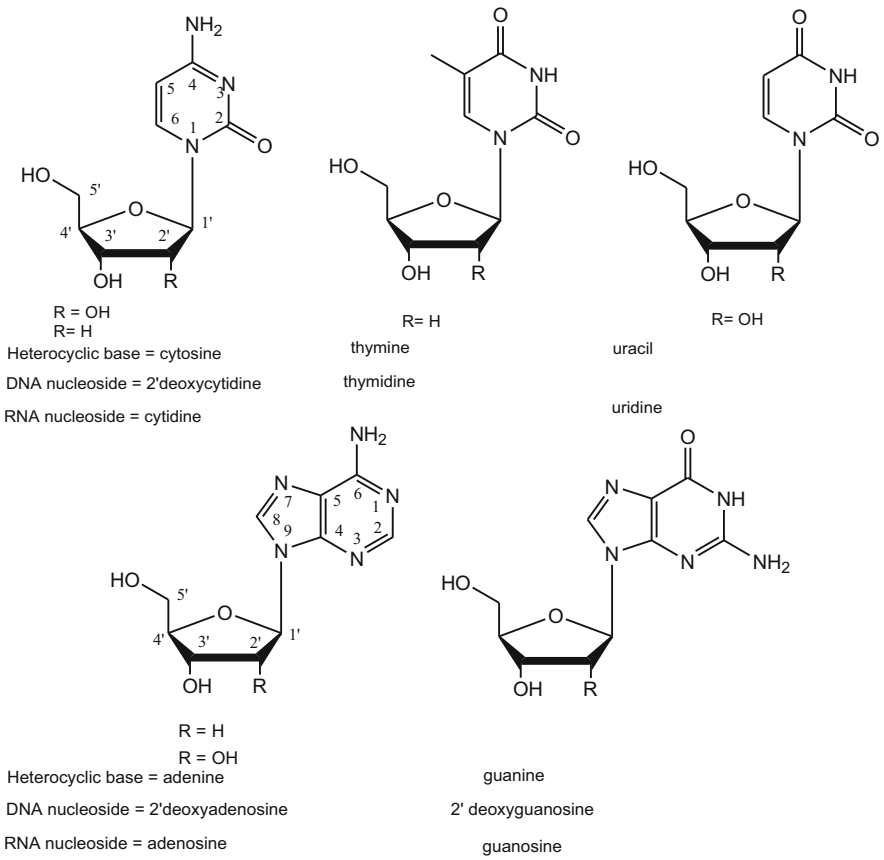
Naturally modified nucleosides include a significant and diverse number of compounds, some of them with slight changes mostly at the base, or major structural modifications done by enzymes. So far most of them have unknown biochemical function [1], nonetheless they have been strongly associated with antiviral, antitumoral, and growth regulation processes (Scheme 3.2).

Representative examples of natural modified nucleosides include queuosine (Q) and Wye base (W) which have been found in the tRNA of some plants and bacteria, and they play an important role in the inhibition of tumor processes. Derived from this relevant biological function the total synthesis of these unique nucleosides has been reported for Q [2–4] and W [5].

Moreover, the synthesis of complex nucleoside antibiotics has been reviewed [6, 7]. The analysis was focused on the challenging synthetic methods for carbohydrate and nucleoside chain elaboration, glycosidation, methods for controlling stereochemistry and for joining subunits. As a result, the total synthesis of polyoxin J [8], sinefungin [9], thuringiensin [10], tunicamycin V [11], nikkomycin B, [12] octosyl acid A [13], hikizimycin [14], and capuramycin [15] was completed (Scheme 3.3).

Important cofactors playing a key rule as biological catalysts required by the enzymes for the optimal performance of biochemical transformations are nucleotides.

M. Brito-Arias, *Synthesis and Characterization of Glycosides*,
DOI 10.1007/978-3-319-32310-7_3

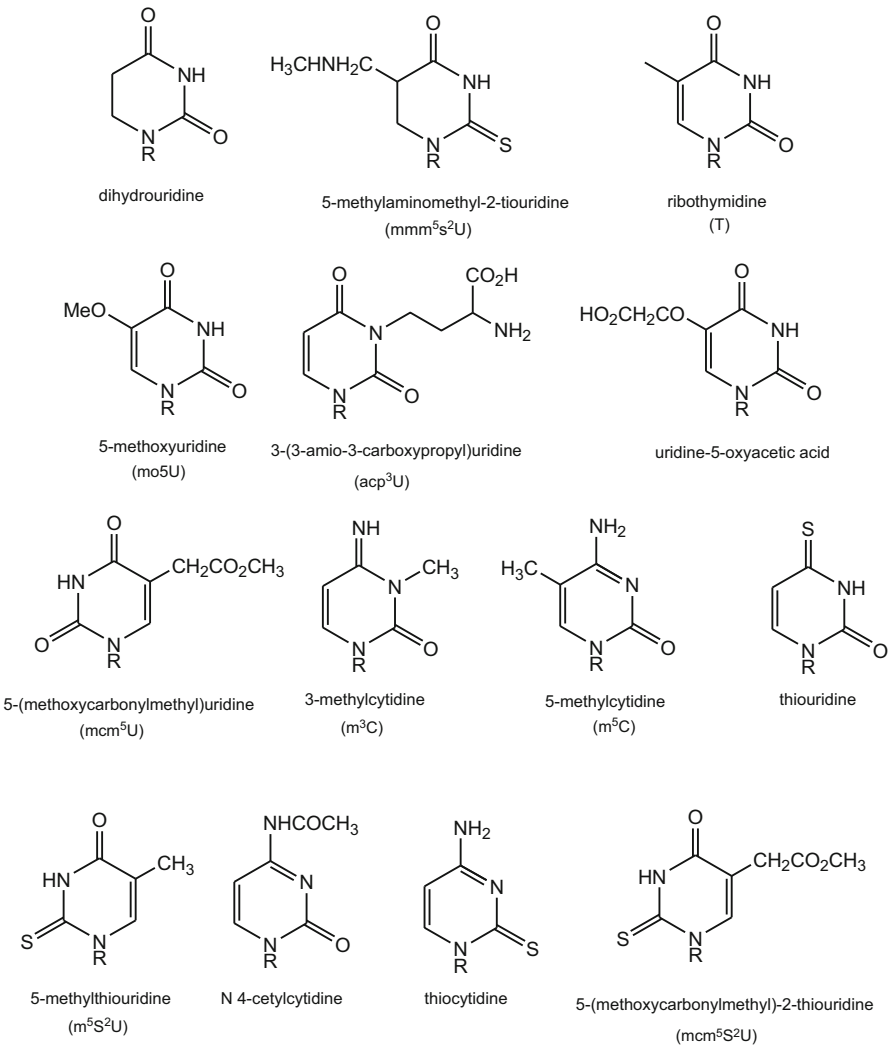


Scheme 3.1 DNA and RNA nucleosides

Such is the case of Adenosine triphosphate ATP and Nicotinic acid adenine dinucleotide NAD that are constituted by an adenosine nucleoside combined with phosphate for the former, and phosphate and nicotinamide for the latter (Scheme 3.4).

3.1 Nucleoside Formation

Considering a disconnection analysis there are two major general routes for nucleoside syntheses [16]. The first is based on the attachment between the aglycon base and the protected sugar activated with a good leaving group at the anomeric position. Under these conditions, the stereoselectivity is conditioned by the protecting group attached at position 2. The second general procedure considers the coupling reaction between a base precursor and the sugar derivative which contains the free amine linked to the anomeric carbon. The ring closure generally takes place after

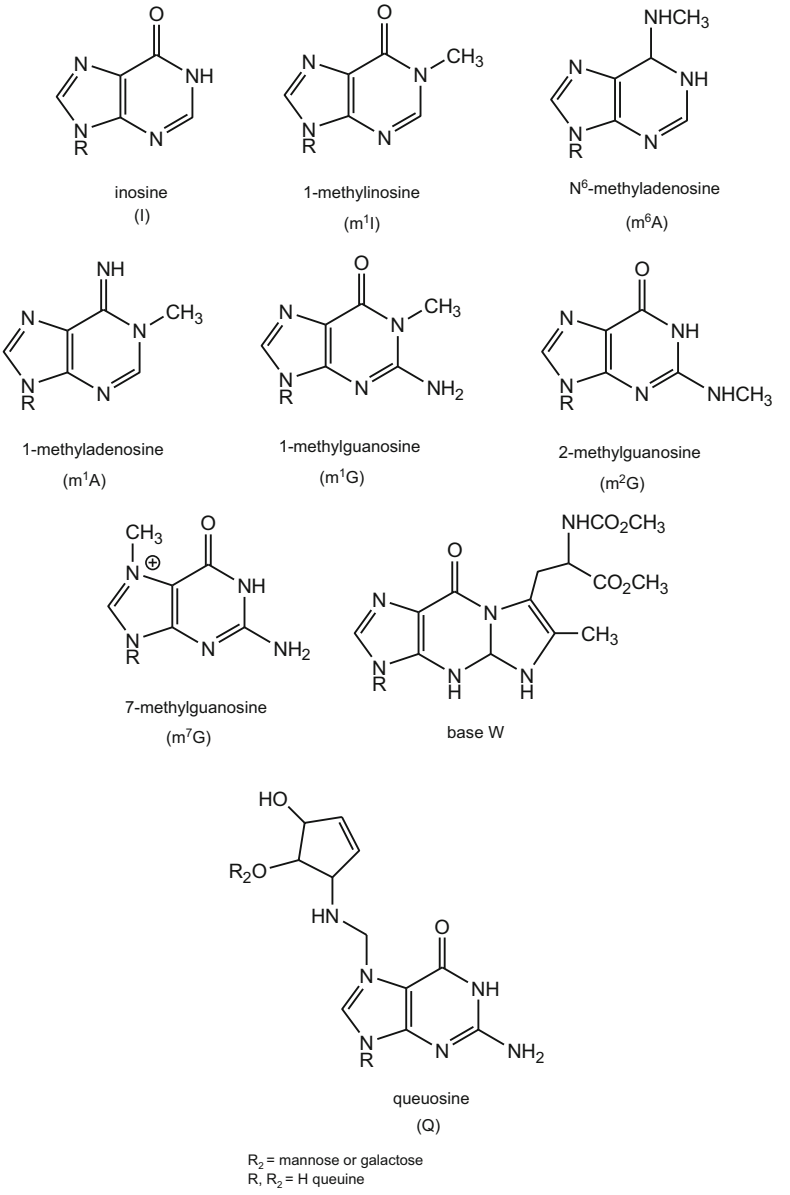


Scheme 3.2 Naturally modified nucleosides

the glycosidation reaction and the configuration is predetermined by the nitrogen attached to the anomeric carbon. The latter approach has been most efficiently used for preparing carbocyclic nucleosides (Scheme 3.5).

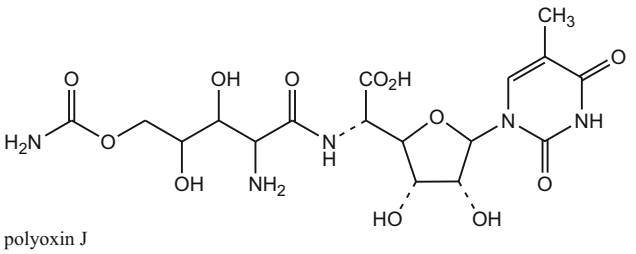
3.2 Protecting Groups

It has been mentioned in the previous chapter that protecting groups are important components for most of the general methodologies designed for establishing glycosidic bonds. Usually the methods for glycoside formation require prior protection of

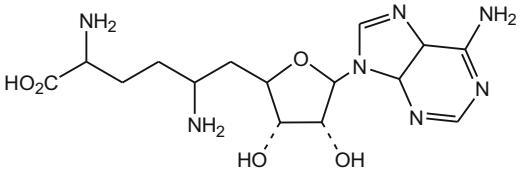


Scheme 3.2 (continued)

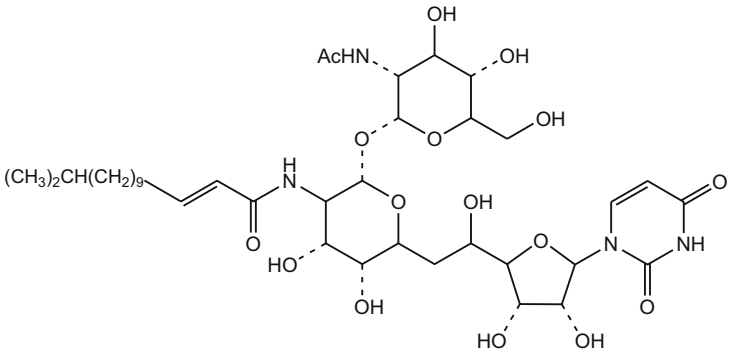
those elements (usually heteroatoms) within the molecule that are needed to remain unaltered. Also important is the fact that the cleavage of the protecting group should be carried out under preferentially mild conditions and in the case of complex nucleosides the installation and removal of the protecting groups for nitrogen, oxygen,



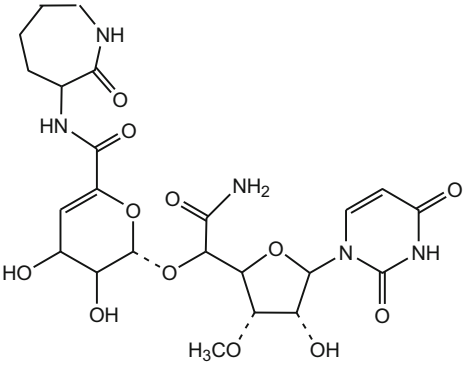
polyoxin J



sinefungin

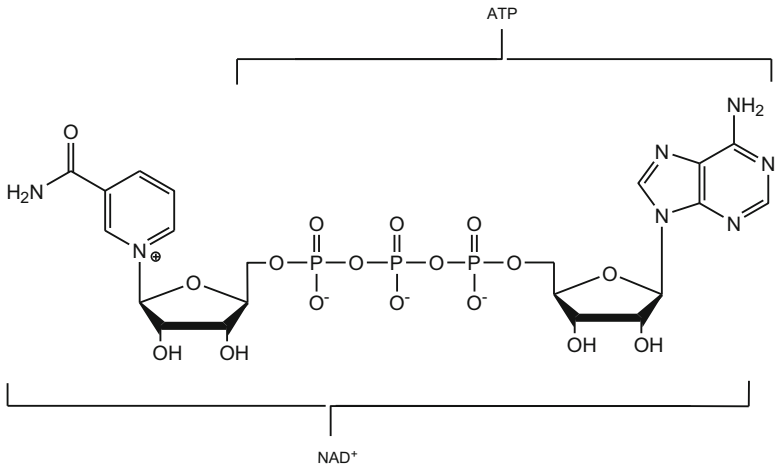


tunicamycin V

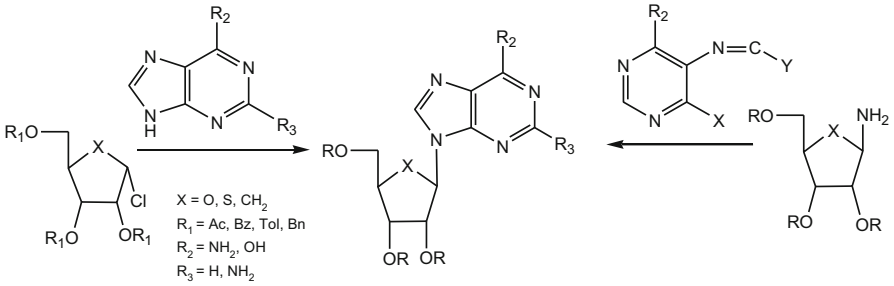


capuramycin

Scheme 3.3 Complex nucleoside antibiotics



Scheme 3.4 Structure of nucleoside cofactors ATP and NAD



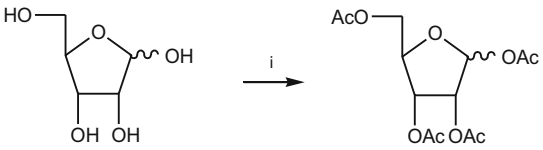
Scheme 3.5 General procedures for *N*-glycoside formation

and sulfur should be accomplished under compatible conditions. The protection and deprotection of nucleosides can be done by chemical or enzymatic means. Some of the most commonly used protecting groups used in the preparation of *O*-glycosides are also useful in the synthesis of nucleosides (Scheme 3.6).

3.2.1 Ribofuranoside Protecting Groups

Enzymes have been found to be interesting alternatives for installing protecting groups on nucleosides. Some of the enzymes used for this purpose are *subtilisin* mutant (8350) [18, 19] and lipases mainly from *Pseudomonas* and *Candida* strains [20, 21]. Representative protections of purine and pyrimidine nucleosides are indicated in Scheme 3.7.

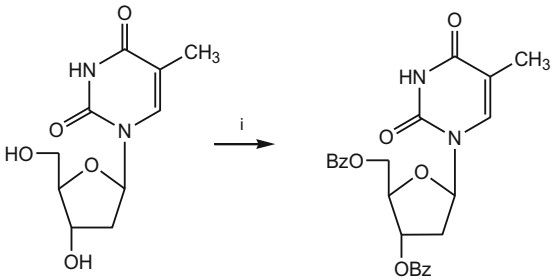
Acetate (CH_3CO-)



i) Ac_2O, CH_2Cl_2, DMAP, r.t.

cleavage: (1) NaOMe, MeOH.
(2) Aqueous NH_3, dioxane.

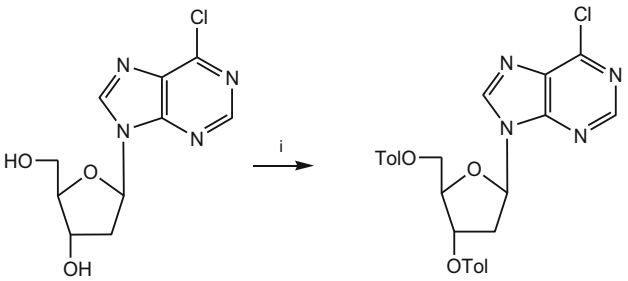
Benzoyl (PhCO-).



i) Bz-Cl, pyridine.

cleavage: (1) R-NH_2, EtOH, 100°C.
(2) EtOH, KOH, reflux, 3h.
(3) NH_3, MeOH

Toluyl (p-MePhCO-)

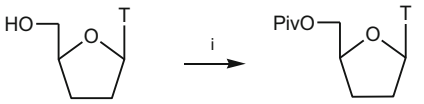


i) Tol-Cl, pyridine.

cleavage: NH_3, MeOH, 100°C, 78%.

Scheme 3.6 Common ribose protecting groups [17]

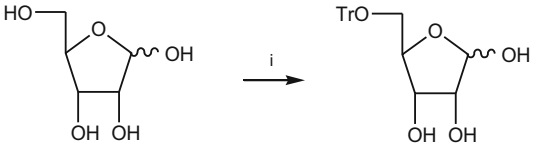
Pivaloyl (Me_3CCOCl)



i) Piv-Cl, pyridine.

cleavage: NaOMe, MeOH.

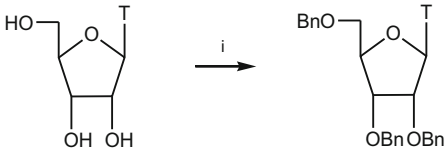
Trityl (Ph_3C-)



i) Tr-Cl, pyridine, r.t.

cleavage: (1) 80%, AcOH, 60ºC.
(2) HCO_2H, Et_2O.

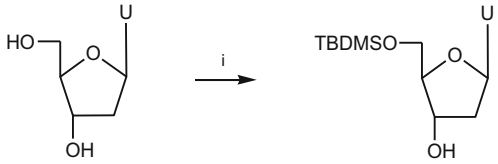
Benzyl ($PhCH_2$-)



i) BnBr, NaH, DMF.

cleavage: $H_2/Pd(OH)_2$, EtOH.

Tertbutyldimethylsilyl ($^tBuMe_2Si$-)

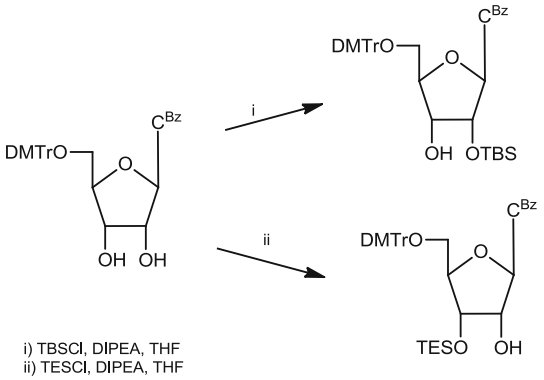


i) TBDMS-Cl, pyridine, r.t.

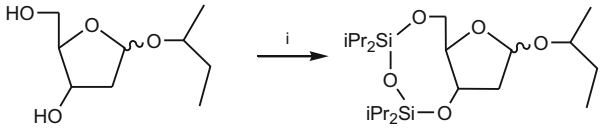
cleavage: (1) tetrabutylammonium fluoride (TBAF).
(2) pTsOH,MeOH,H_2O,7h.

Triethylsilane (TES-), *tert*-butyldimethylsilyl (TBS-)

Scheme 3.6 (continued)



Tetraisopropylsilyl ([(^{i}Pr)$_2$Si]$_2$O-)



i) $Pr_2iSi(Cl)OSi(Cl)Pr_2i$, imidazole, THF, rt, 90 min.

cleavage: Bu_4NF, THF.

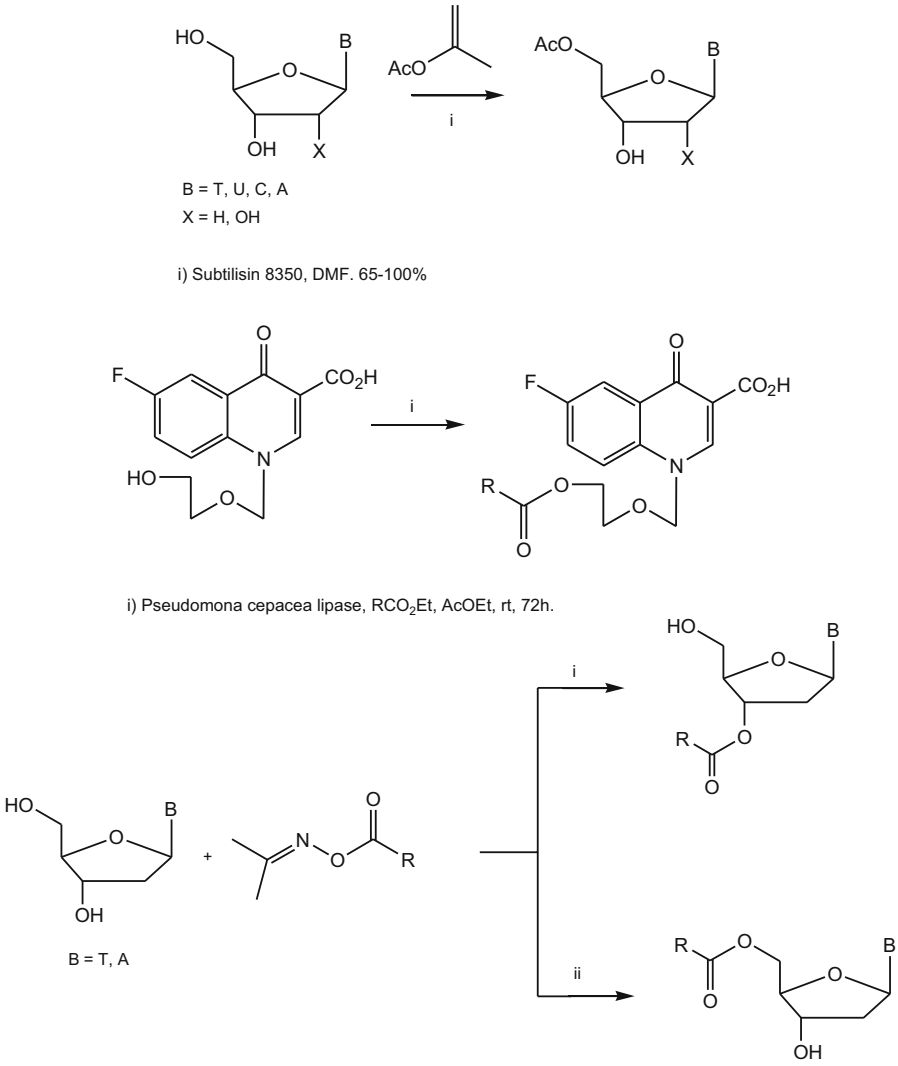
Scheme 3.6 (continued)

By using the appropriate lipase it is possible to achieve regioselective acyl protections on nucleosides. For instance, the enzymatic transesterification reaction of 5′-fluorouridine with n-octanoic anhydride catalyzed with *Candida Antarctica* (CAL), *Pseudomonas* sp. (PS), (KIWI-56), and *Mucor javanicus* (M) lipases was performed, producing 5′-, 3′-, and 2′-acylnucleosides, respectively (Scheme 3.8) [22].

Regioselective removal of certain protecting groups such as acetates attached to the ribosyl moiety of nucleosides might be carried out by enzymes. For instance *subtilisin* strain selectively hydrolyzes the 5′-position of purine and pyrimidine tri-*O*-acylated esters to produce 2′,3′-di-*O*-acylribonucleosides in 40–92 % (Scheme 3.9) [23].

On the other hand, diastereoselective deacetylation of peracetylated 2′-deoxyribofuranosyl thymine was carried out using wheat germ lipase (WGL) and porcine liver esterase (PLE), forming pure β-anomer thymidine in 29 % and 31 %, respectively (Scheme 3.10) [24].

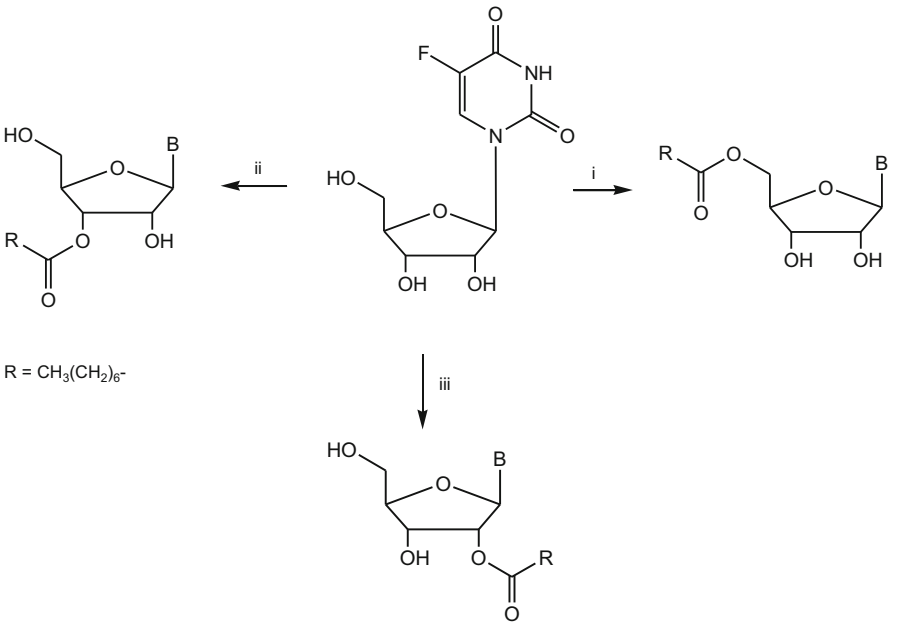
When porcine pancreas lipase (PPL) in phosphate buffer is used for deacetylation of 3′,5′-di-*O*-acetylthymine, the removal of the acetyl group at the 5′- position is achieved, leading to the 3′-*O*-acetylthymidine (Scheme 3.11) [25].



Scheme 3.7 Enzymatic regioselective acylation by oximeacetates and lipases

Other suitable selective protections and deprotections useful for chemical manipulations which might occur at the ribosyl moiety are illustrated in Scheme 3.12.

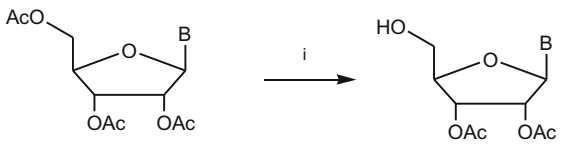
Regioselective protections and deprotections is often a critical step especially for the preparation of complex nucleosides. Some suitable deprotections of complex nucleosides which do not alter the original composition of the structure have been described (Scheme 3.13) [6, 7].



i) Candida antarctica lipase (CAL), 90%. ii) Pseudomona sp.lipase (PS), 92%.
iii) Mucor javanicus lipase (M), 42%.

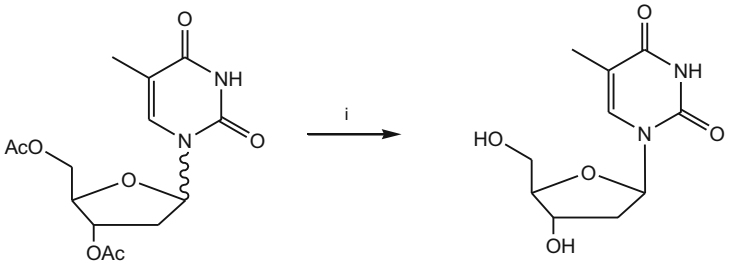
Scheme 3.8 Regioselective acyl protection by lipase

Scheme 3.9 Selective enzymatic 5′-acetyl deprotection



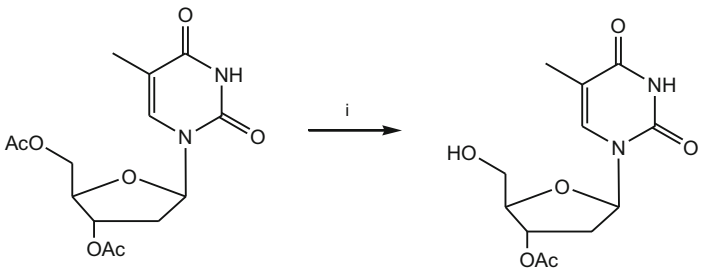
B = U, C, A, G, N-2AcG, H

i = Subtillisin or PPL, organic solvent, phosphate buffer, pH 7.



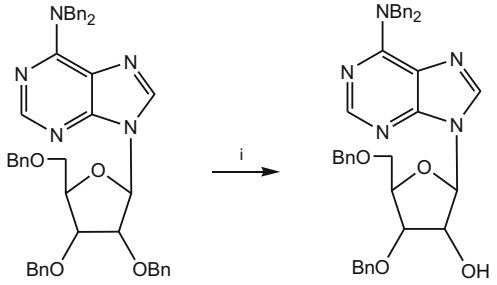
i) WGL, phosphate buffer, 29%. or PLE, phosphate buffer, 31%.

Scheme 3.10 Lipase-catalyzed deacetylation of anomeric nucleoside

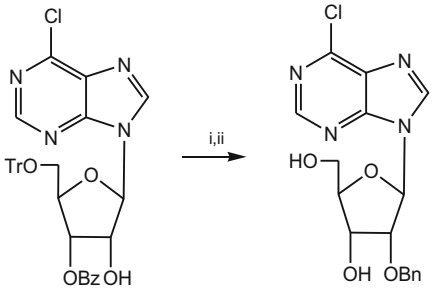


i) PPL, phosphate buffer, 98%.

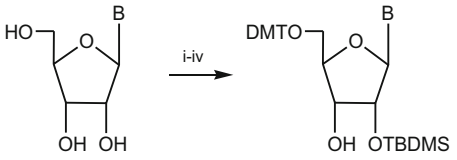
Scheme 3.11 Selective enzymatic 5′-deacetylation of 3′,5′-di-*O*-acetyl thymidine



i) t-BuMgBr, PhH, 80°C, 69%



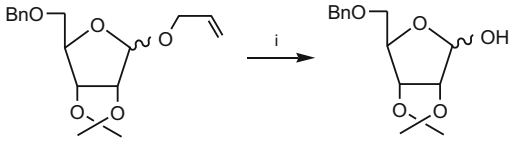
i) BnOH, $Me_2NCON = NCONMe_2$. ii) NH_3/MeOH



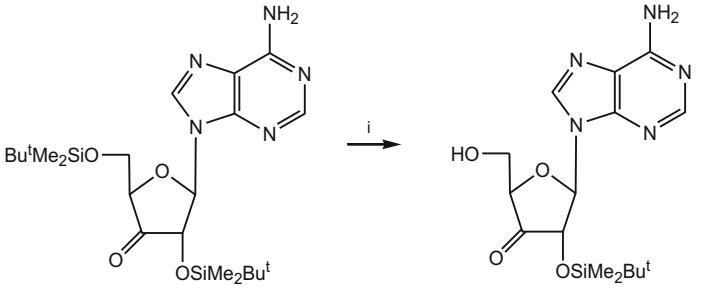
B = G, A, C, U

i) t-$Bu_2Si(OTf)_2$, Im, DMF, 0°C. ii) t-$BuMe_2SiCl$, Im, DMF,60°C, 80-87%. iii) HF-Py, CH_2Cl_2,0°C, 90%
iv) DMT-Cl, Py, 0°C, 90%.

Scheme 3.12 Miscellaneous chemical protection and deprotection [25–29]

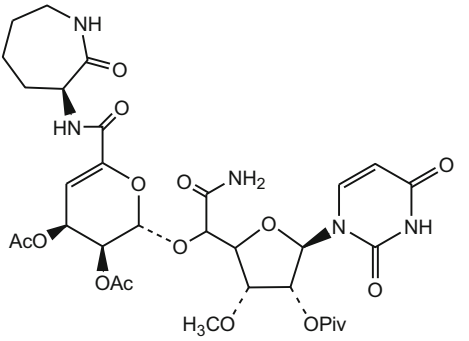


i) DIBAL, $NiCl_2$, Et_2O, 0°C, 55%

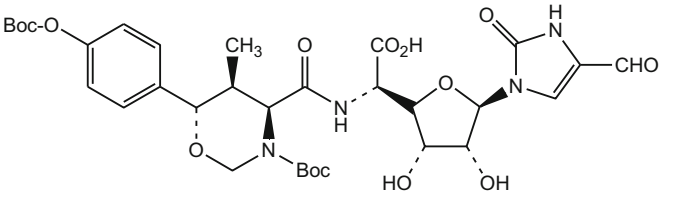


i) CF_3COOH-H_2O (9:1), 0°C, 95%.

Scheme 3.12 (continued)

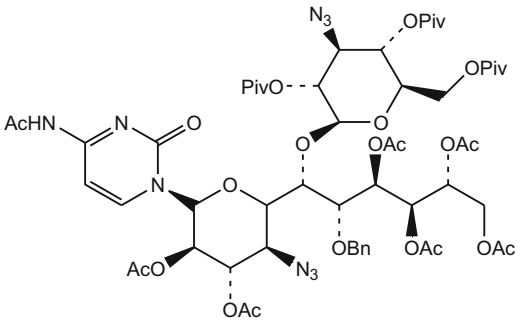


1. NaOH, aqMeOH, 2.5h (cleavesO-Ac and O-Piv)

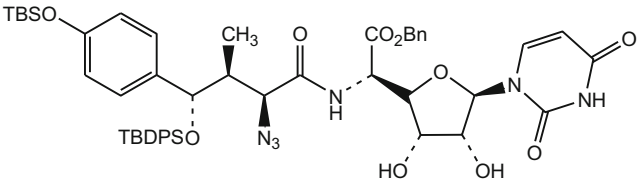


1. TFA, 0°C, 15min. (cleaves O- and N-BOC)
2. H_2O, thenlyophilize (cleaves acetal)

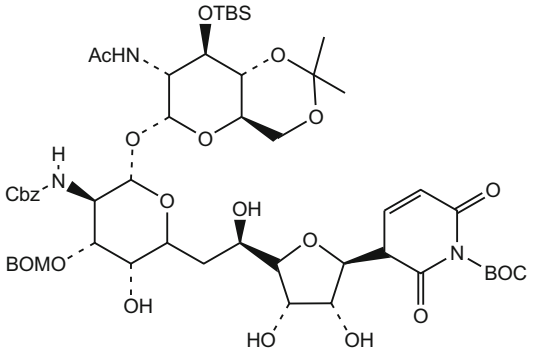
Scheme 3.13 Suitable deprotection of complex nucleosides



1. DDQ, CH_2Cl_2, 58°C, 43h (cleaves O-Bn)
2. n-Bu_4NOH, MeOH, reflux, 2h (cleaves acyls)
3. H_2, Lindlar, H_2O (reduces azide groups)



1. n-Bu_4NF, THF, 30min. (cleaves 2 O-SiR_3)
2. H_2, 10 % Pd-$BaSO_4$, aq. MeOH, 30min. (cleaves benzyl ester and reduces -N_3)



1. 10% HCO_2H, Pd, 1.5h (cleaves O-BOM, N-Cbz)
2. 13% HCO2H, MeOH, 40°C, 5h (cleaves N-BOC, acetonide)
3. HF, MeOH, CH_3CN (cleaves O-TBS)

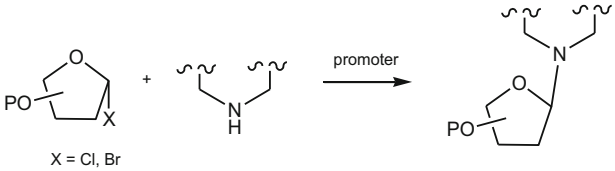
Scheme 3.13 (continued)

3.3 General Methods

- Michael reaction
- Fischer–Helferich reaction
- Davol–Lowy reaction
- Silyl mediated reaction
- Sulfur mediated reaction
- Imidate mediated reaction
- Mitsunobu reaction
- Palladium mediated reaction
- Microbial/enzymatic approach

3.3.1 Michael Reaction

3.3.1.1 General Scheme and Conditions

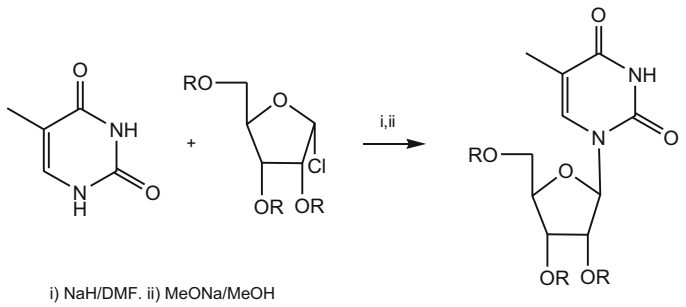


Promoter	Conditions
NaH	DMF
K_2CO_3	DMF
KOH-TBA	CH_2Cl_2

It is a classical procedure for preparing nucleosides, and it can be considered a modified *O*-glycoside approach. In this way, the sugar derivative is an R-*O*-furanosyl halide where R can be acyl, benzoyl, benzyl, tosyl, or silyl, and the halogen is commonly chlorine instead of bromine, since it has proved to be more stable for furanose derivatives than its counterpart. The nitrogen base (purine or pyrimidine) is reacted under basic conditions, usually NaH or K_2CO_3 in DMF (Scheme 3.14).

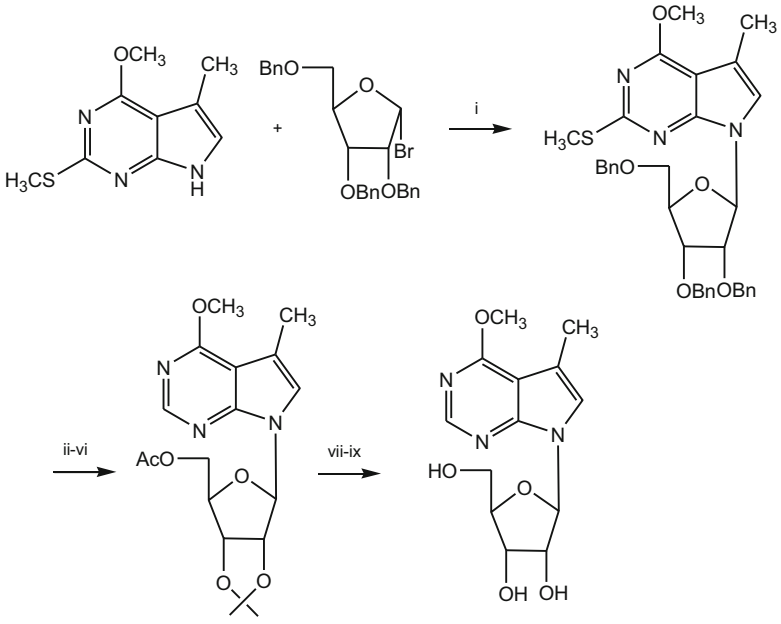
A variety of antibiotics have been prepared according to this method, as in the case of the nucleoside known as methyltubercidine. For achieving this goal, the 7-deazaguanine was used as nitrogen base which was condensed to 2,3,5-tri-*O*-benzylribofuranosyl bromide under NaH/DMF conditions to form a 1:1 anomeric mixture of *N*-glycosides (Scheme 3.15) [30].

More recently Battaharya [31] reported the synthesis of fluoroarabinotubercidine, toyocamicine, and sangivamicine, under the current *N*-glycoside formation procedure. Other deazapurines have been described by Seela et al. [32] involving the condensation between the purine base and protected ribosyl halides under basic conditions.



i) NaH/DMF. ii) MeONa/MeOH

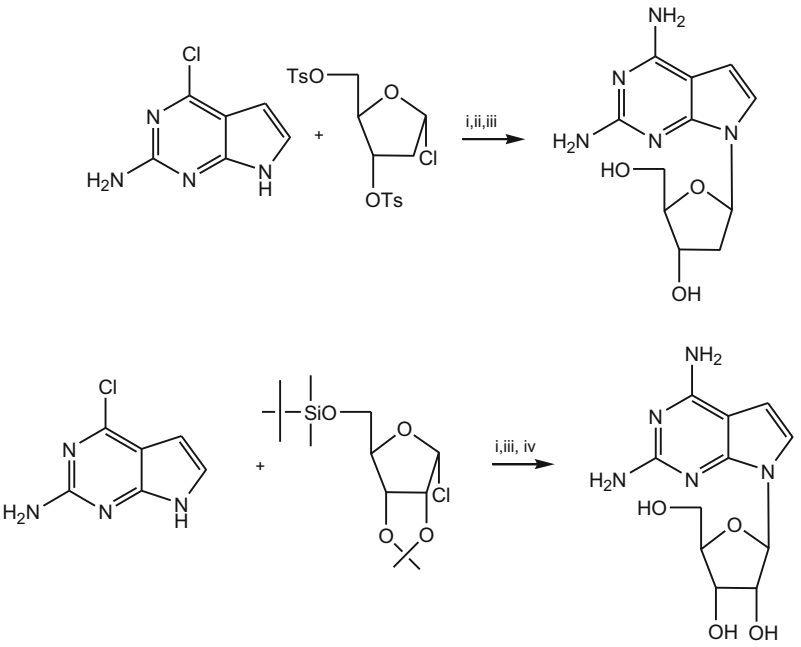
Scheme 3.14 Michael modification



i) NaH/DMF. ii) Ni/EtOH-PhH. iii) HCl/dioxane. iv) H_2,Pd-C. v) a) acetone/p-TsOH. b) Ac_2O/Py. vii) $POCl_3$. viii) NH_3/MeOH. ix) F_3CCOOH/H_2O.

Scheme 3.15 Synthesis of methyltubercidine

According to Seela [33] and Kazimierczuk [34] the stereoselective glycosylation of the sodium salts of halopurines, with 2-deoxy-3,5-di-*O*-*p*-tolouyl-α-D-*erytro*-pentofuranosyl chloride gave β-nucleosides via Walden inversion. This was demonstrated in the preparation of 2-amino 2′-desoxytubercidine and 2-aminotubercidine by condensation of 3,5-di-*O*-(*p*-tolyl)-α-D-pentafuranosylchloride and 5-*O*-[(1,1-dimethylethyl)dimethylsilyl]-2,3-*O*-(1-methylethyliden)-α-D-ribofuranosylchloride with the halopurine under Michael conditions. Final ammonia treatment provided the target deazanucleoside (Scheme 3.16).



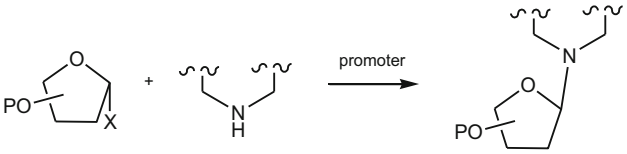
i) KOH, TBA/CH_2Cl_2. ii) MeONa/MeOH. iii) NH_3/MeOH. iv) CF_3COOH/H_2O.

Scheme 3.16 Synthesis of 2-aminotubercidine and 2-amino-2′-deoxytubercidine

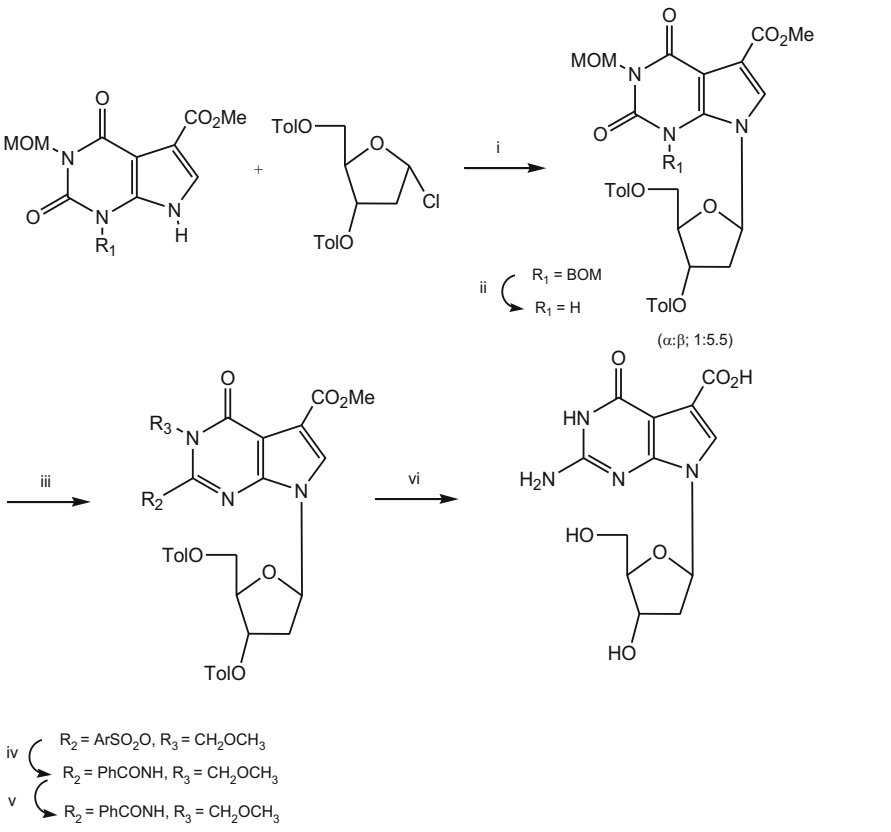
The 7-deazapurine nucleoside cadeguomycin isolated from strain of the actinomycete culture filtrate *Streptomyces hygroscopicus* was also synthesized under this approach. Thus, coupling reaction between protected 7-deazapurine derivative and 1-chloro-2-deoxy-3,5-ditoluyl-α-D-erythro-pentofuranose was effected with preference for the β-isomer. Subsequent transformations provided the target molecule 2′-deoxycadeguomycin (Scheme 3.17) [35].

3.3.2 Fischer–Helferich Reaction

3.3.2.1 General Scheme and Conditions



Promoter	Conditions
Silver salts	xylene



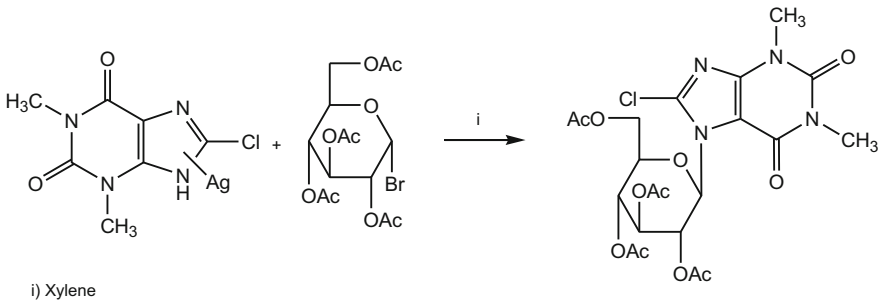
i) NaH,CH_3CN, 25°C.ii)H_2/Ph$(OH)_2$, MeOH,25°C. iii) NaH, THF, TIPBS-Cl. iv) $PhCONH_2$, NaH, THF. v) TFA, $CH_2C\ l_2$.

Scheme 3.17 Synthesis of 7-deazapurine nucleoside 2-deoxycadeguomycin

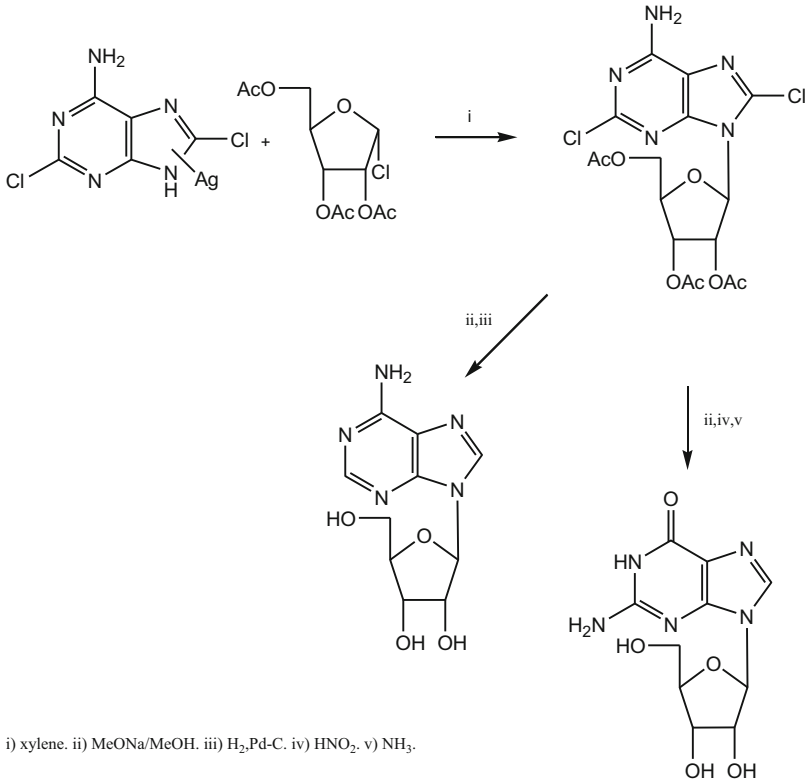
This general procedure consists in the use of an acylfuranoside or acylpyranoside, which is reacted with the silver or mercury salts of a nitrogen base. The original reaction involves the condensation between silver salt of theophylline and aceto-bromoglucose in hot xylene, giving preferentially the N-7 regioisomer (Scheme 3.18).

The feasibility of this method is observed in the synthesis of adenosine and guanosine by condensation of tri-*O*-acetyl-α-D-ribofuranosyl chloride with the silver salt of 2,8-dichloroadenine to generate an intermediate which under the conditions described below can generate either adenosine or guanosine (Scheme 3.19) [36].

The stereochemistry of this reaction can be predicted by applying the "trans rule" proposed by Tipson [37, 38] and extended by Baker. The rule establishes that the condensation between the purine or pyrimidine salt and the acyl-*O*-glycosyl halide will generate a nucleoside with C1-C2 trans configuration regardless of the initial configuration of C1-C2 of the sugar.

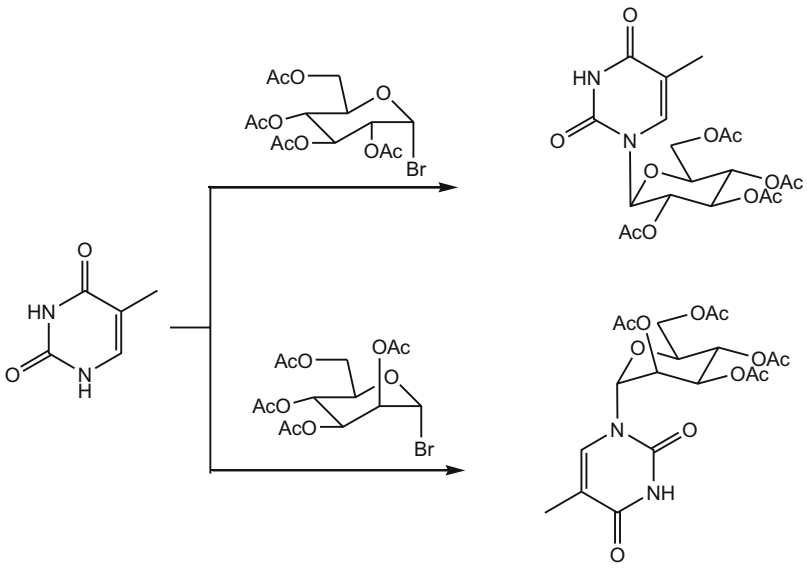


Scheme 3.18 Fischer–Helferich method



Scheme 3.19 Synthesis of adenosine and guanosine

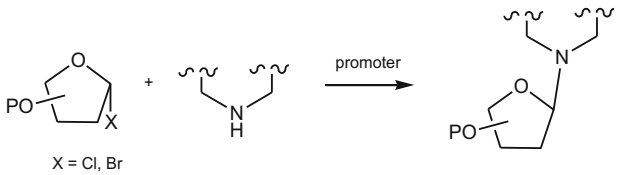
The trans rule is demonstrated in the preparation of thymidine acetoglucopyranose and mannopyranose, where -OH at position 2 for the former is equatorial, and for the latter axial. By following the rule, the coupling reaction generates β- and α-anomers, respectively, both of them having a trans disposition between substituents at positions 1 and 2 (Scheme 3.20).



Scheme 3.20 Tipson's trans rule

3.3.3 The Davol–Lowy Reaction

3.3.3.1 General Scheme and Conditions

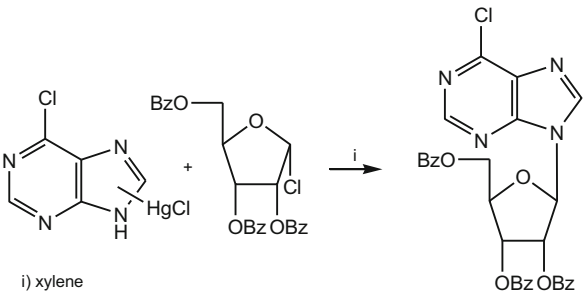


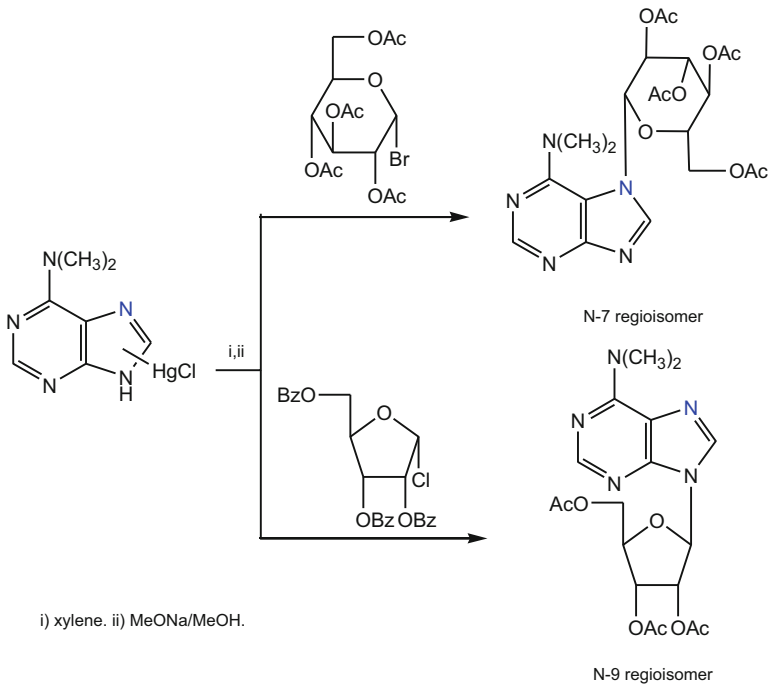
Promoter	Conditions
$Hg(CN)_2$	CH_3NO_2, reflux
$Hg(CN)_2$	Xylene

This method has been also considered a modified Fischer–Helferich procedure and involves the use of mercury chloride instead silver salts. Under these conditions the useful intermediate chloropurine nucleoside has been prepared under mild conditions (Scheme 3.21).

The nature of the glycosyl halide is important for determine the regioselectivity of the glycosidic linkage. If the condensation reaction occurs between purines and acetobromoglucose the N-7 regioisomer is obtained preferentially. On the other

Scheme 3.21
Davol–Lowy method

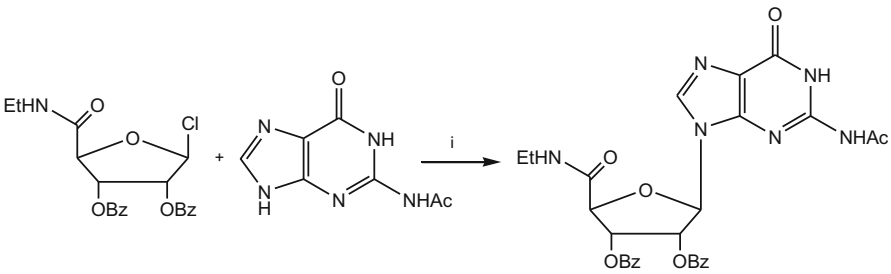




Scheme 3.22 Preparation of N-7 and N-9 regioisomers

hand, if acetoribosyl chloride is condensed with the same purine, the N-9 regioisomer is the major product observed (Scheme 3.22).

Another purine nucleoside prepared under these conditions is shown in Scheme 3.23, consisting in the coupling reaction between protected guanine and protected furanosyl chloride in nitromethane under refluxing conditions produced the corresponding *N*-glycoside in 50 % yield [39].

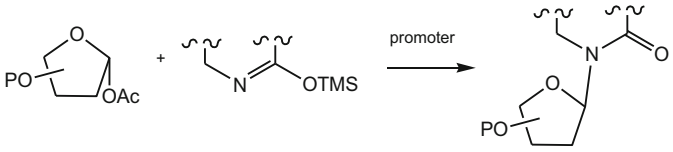


i) $Hg(CN)_2$, CH_3NO_2, reflux, 16 h.

Scheme 3.23 Glycosidation reaction for preparation of guanine derivative

3.3.4 Silyl Coupling Reaction

3.3.4.1 General Scheme and Conditions

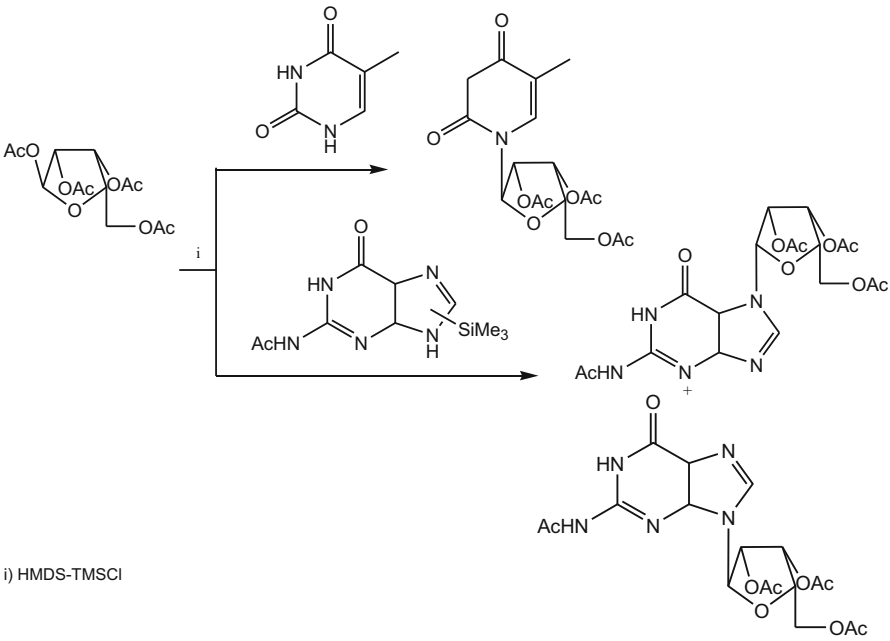


Promoter	Conditions
TMS-OTf	CH_3CN, 0 °C → r.t
TMS-OTf	$PhNO_2$, 127 °C
$SnCl_4$	CH_3CN
HMDS-TMDS	
$(MeSi)_2NAc$	
$CF_3(CF_2)_3SO_3K$/HMDS-TMSCl	
HMDS/$(NH_4)_2SO_4$	

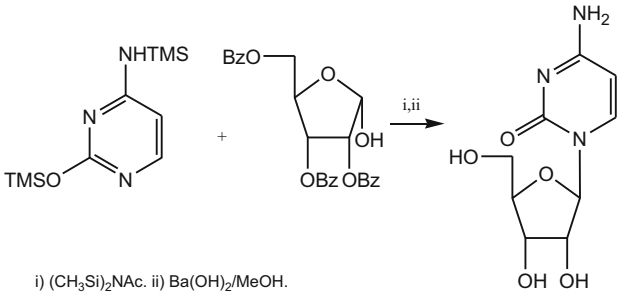
Various types of silyl agents have been tested as either protecting groups and or *N*-glycoside promoters. Among them trimethylsilyl chloride (TMS-Cl), bis(trimethylsilyl) acetamide, trimethylsilyltriflate, and hexamethyldisilazane are representative examples.

De Clercq et al. [40] prepared purine and pyrimidine α-D-lyxofuranosyl nucleosides employing HMDS, TMS, and TMSF as silyl coupling agents. Nucleoside α-D-lyxofuranosyl thymine was prepared by condensation between 1,2,3,5-tetra-*O*-acetyl-α-D-lyxose and thymine in the presence of HMDS-TMSCl mixture (Scheme 3.24).

Likewise cytidine has been synthesized in 95 % through condensation of silyl cytidine obtained from cytosine with bis [trimethylsilyl] acetamide, and sugar derivative 2,3,5-tri-*O*-benzoylribose, as represented in Scheme 3.25.



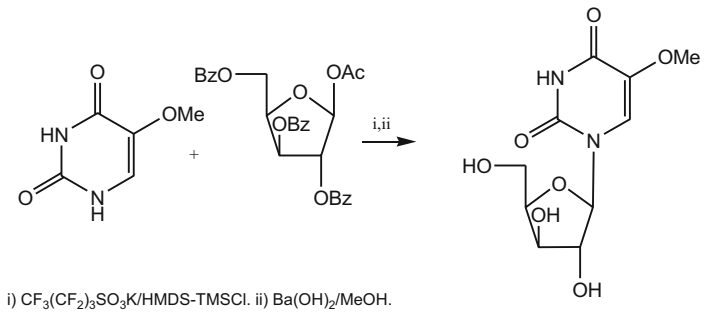
Scheme 3.24 Preparation of α-D-lyxofuranosyl thymine and guanine protected nucleosides



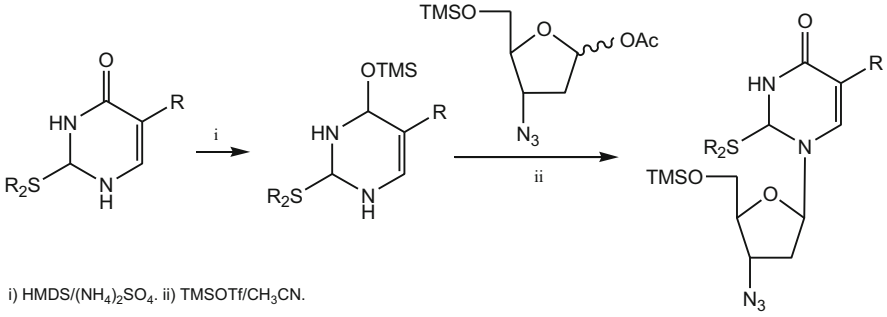
Scheme 3.25 Silyl mediated coupling reaction

Hilbert and Johnson [41] developed a procedure for preparing nucleosides employing a mixture of hexamethyldisilane (HMDS), trimethylsilane chloride and potassium nonaflate. According to this procedure 5-methoxyuridine was prepared by condensing 5-methoxyuracil, with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (Scheme 3.26).

A widespread silyl-based methodology was developed by Vorbrüggen [42, 43] which is based in the use of persilylated purines or pyrimidines, which are condensed with peracylated sugars in the presence of Lewis acid catalysis. Usually silylation of the base is achieved with hexamethyldisilazane (HMDS) or N,O-bis(trimethylsilyl)



Scheme 3.26 Hilbert and Johnson approach



Scheme 3.27 Vörbruggen's synthesis of AZT thioderivatives

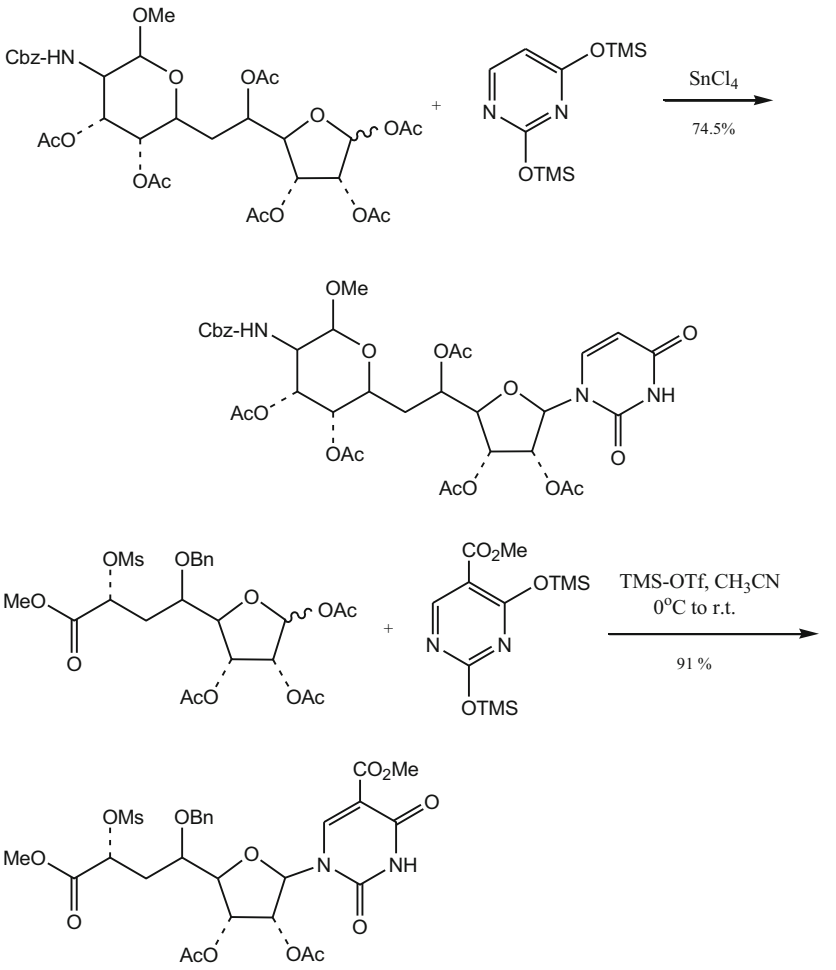
acetamide, the latter less difficult to remove during the workup process. Among the Lewis acids employed as catalysts, trimethylsilyl triflate (TMSOTf) has been the most suitable condensing agent for this reaction.

AZT alkylthioanalogs have been synthesized under the method reported by Vorbrüggen. This condition requires hexamethyldisilane for activation of the anomeric center, and trimethylsilyltriflate as condensing agent (Scheme 3.27).

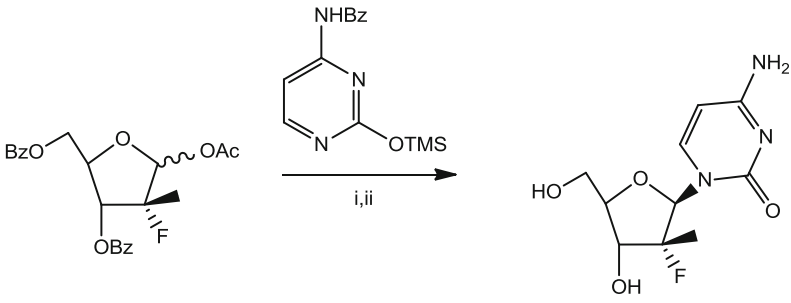
Vörbruggen-type coupling reaction has been method of choice in the *N*-glycoside bond formation of various complex nucleosides such as octosyl acid A, tunicaminyl-uracil, sinefungin, and hikizimycin. Some of the general conditions reported for the accomplishment of the mentioned synthesis are described in Scheme 3.28 [6, 7].

Likewise by following a variant of this protocol Wang et al. were able to prepare 2′-deoxy-2′-fluoro-2′-C-methylcytidine (PSI-6130), a potent and selective inhibitor of HCV NS5B polymerase. Thus, the N-glycosylation step was carried out by coupling reaction between 2′-deoxy-2′-fluoro-2′-methyl ribose acetate and silylated *N*-benzoylcytosine tin(IV) chloride as a catalyst (Scheme 3.29) [44].

The N-glycosylation of protected (triethylsilyl)ethynyl furanoside with 2-fluoroadenine to produce after deprotection and 2-deoxygenation the remarkably

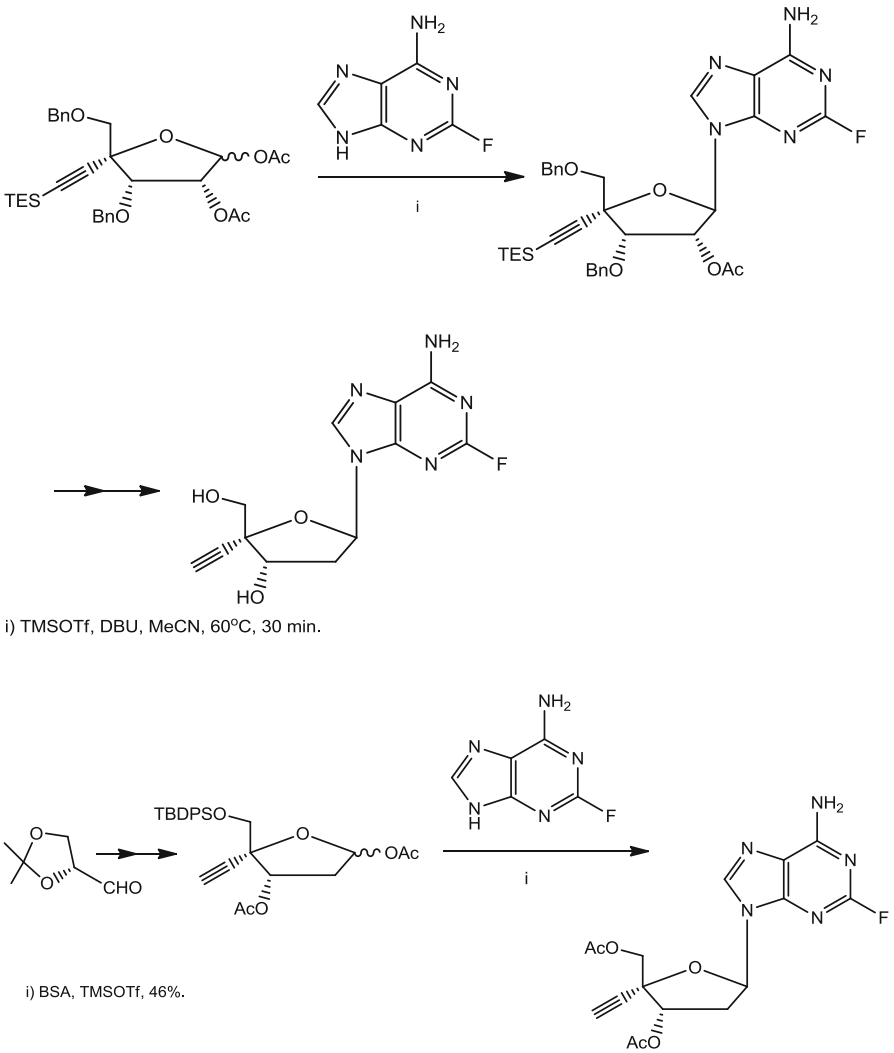


Scheme 3.28 Vörbruggen-type coupling reactions



i) $SnCl_4$, PhCl, 65°C. ii) NH_3, MeOH, rt

Scheme 3.29 Synthesis of antiviral 2′-deoxy-2′-fluoro-2′-C-methylcytidine (PSI-6130)

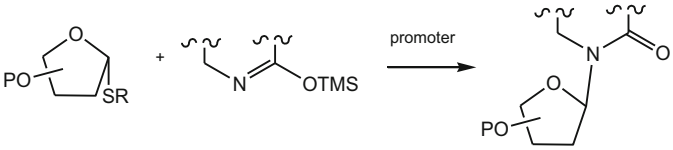


Scheme 3.30 Methods for preparing anti-HIV 4′-Ethynyl-2-fluoro-2′-deoxyadenosine (EfdA)

potent anti-HIV nucleoside 4′-ethynyl-2-fluoro-2′-deoxyadenosine (EfdA) was performed with TMSOTf and DBU in MeCN. Another approach for preparing this modified nucleoside was described by following a 12-step sequence starting from (R)-glyceraldehyde acetonide in 18 % overall yield (Scheme 3.30) [45, 46].

3.3.5 *Sulfur Mediated Reaction*

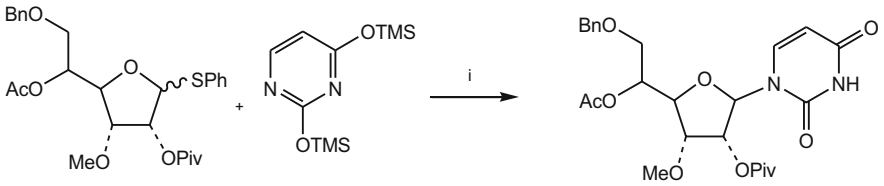
3.3.5.1 General Scheme and Conditions



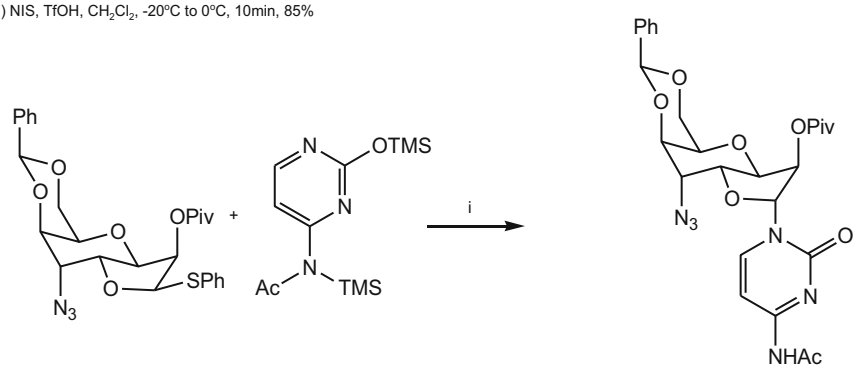
R = Ph, (=O)Ph

Promoter	Conditions
NIS-OTf	CH_2Cl_2
TMS-OTf	DCE r.t.
Br_2	DMF

Derived from their extensive use in the preparation of *O*-glycosides, the sulfur glycosyl donors have become another standard procedure for N-glycosylations. The conditions reported for the coupling reactions involves the sulfur glycosyl donor, the silyl protected heterocycle acceptor and usually *N*-iodosuccinimide, triflic acid as catalyst (Scheme 3.31) [47].



i) NIS, TfOH, CH_2Cl_2, -20°C to 0°C, 10min, 85%



i) NIS, TfOH, CH_2Cl_2, 1h, 95%

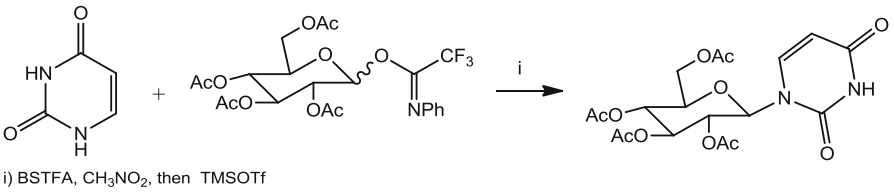
Scheme 3.31 *N*-glycoside formation via sulfur glycosyl donor

3.3.6 *Imidate Mediated Reaction*

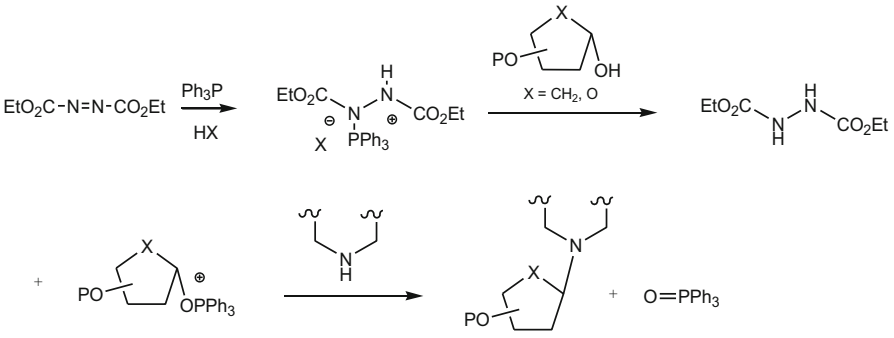
The imidate reaction is by far a method established for preparation of *O*-glycosides; however, some N-glycosylation has been achieved by following this protocol. An interesting novel step is the incorporation of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) as a silylating reagent when glycosyl trifluoroacetimidates were used as donors, providing the β-nucleoside in 80 % yield (Scheme 3.32) [48].

3.3.7 *Mitsunobu Reaction*

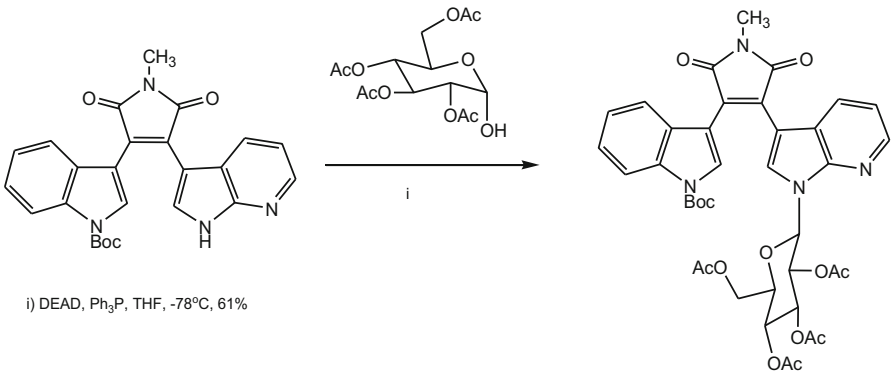
This reaction has been selected as another strategy for preparing N- and carbocyclic nucleosides. The mechanism involves a nucleophilic substitution displacement with inversion of the configuration between species bearing poor leaving groups with nucleophiles. The reaction mechanism involves the initial reaction of triphenylphosphine (Ph_3P) with diethylazodicarboxylate (DEAD) to produce a dipolar intermediate which will react with an alcohol to form an alkoxy phosphonium salt and diimide. Then the nucleophile will displace triphenylphospine oxide to give the substitution product (Scheme 3.33) [49].



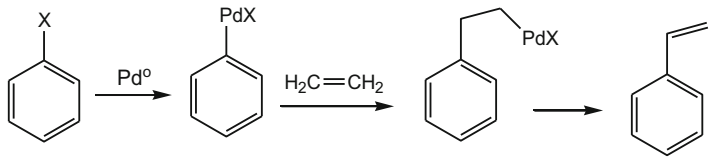
Scheme 3.32 Synthesis o glucopyranosyl pyrimidine from glycosyl trifluoroacetimidates



Scheme 3.33 Mitsunobu reaction for the construction of glycosidic bond



Scheme 3.34 Mitsunobu reaction for preparation of *N*-glycosides



Scheme 3.35 Heck reaction

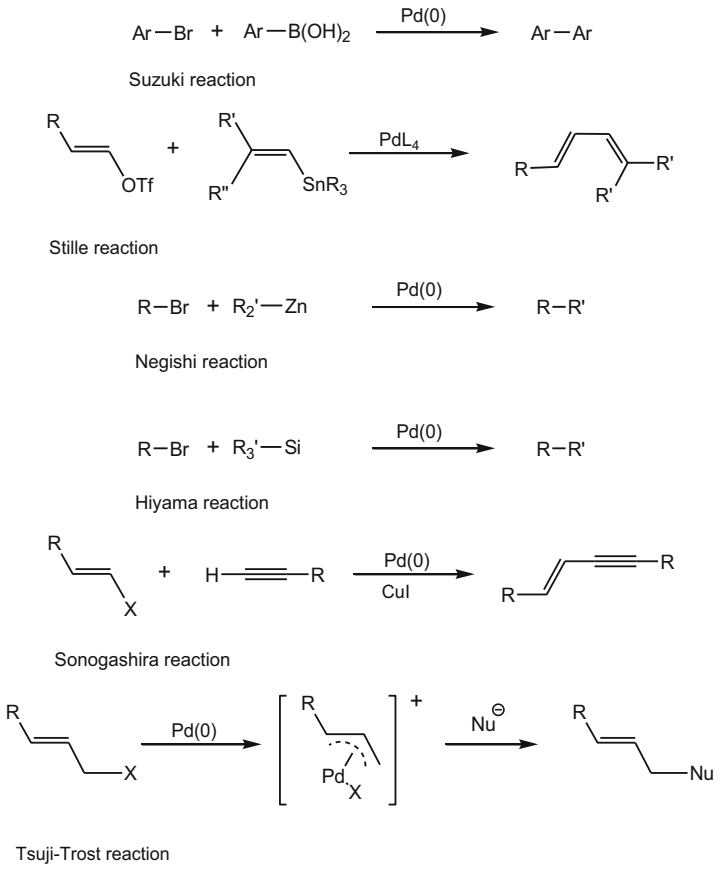
This procedure was used successfully for preparing the *N*-glycoside shown in Scheme 3.34 by reacting 2,3,4,6-tetraacetyl glucose with the heterocyclic base under the Mitsunobu conditions [50].

3.3.8 Palladium Mediated Reaction

Palladium catalysis is a well-established and versatile methodology for the preparation of nucleosides. Also known as the Heck reaction, it was developed initially for C–C bond formation and consists in the coupling of an aryl halide with activated olefin in the presence of palladium (0) as catalyst (Scheme 3.35) [51].

More recently other palladium mediated reaction have been developed with great potential for heterocycle coupling reaction with furanosides, to produce an interesting variety of nucleosides. The group of reactions includes the Suzuki (organoboranes) [52], Stille (organostannanes) [53], Negishi (zincated) [54], Sonogashira (alkyne-CuI) [55], Hiyama (organosilicon) [56], and Tsuji–Trost [57, 58] (Scheme 3.36).

Early reports in the use of Heck-type reactions for the preparation of nucleosides were described by Bergstrom [59–61]. More recently a comprehensive overview about palladium mediated reactions for *N*-glycoside bond formation or modifications at the base or the sugar moieties were described. A general scheme summarizing such possibilities is shown in Scheme 3.37 [62].

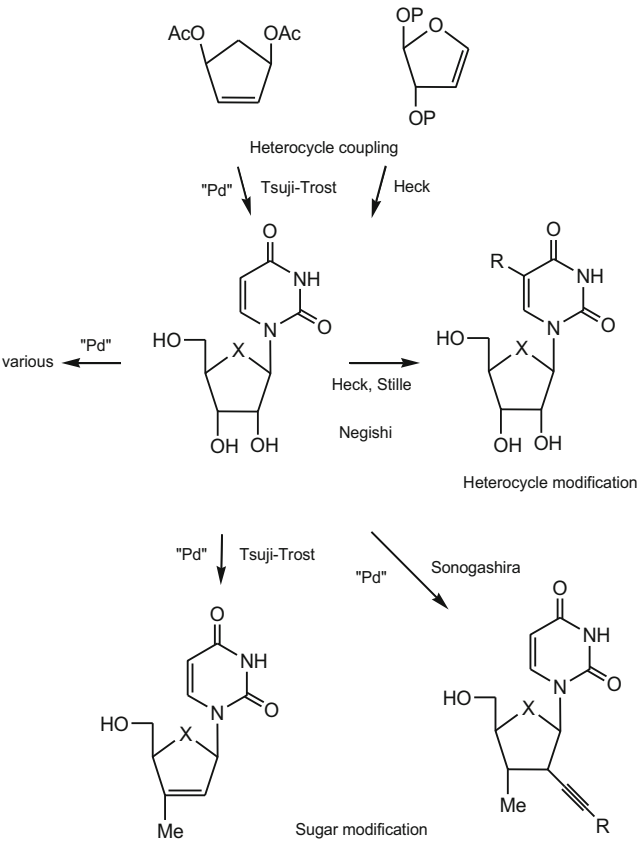


Scheme 3.36 Palladium mediated coupling reactions

Palladium-catalyzed reaction was applied for a N-heterocyclic glycosylation, by using glycal type donors with methyl isatin through a classic Ferrier rearrangement, in the presence of dppb ligand which improved the yield to 50 % (Scheme 3.38) [63].

3.3.9 Ortho-alkynylbenzoates Protocol

This method consist in the coupling reaction between ribofuranosyl ortho-alkynylbenzoate as donor and purines or pyrimidines in the presence of $Ph_3PAuNTf_2$ providing the *N*-glycosides in high β-selectivity. This method can be successfully applied in the preparation of complex nucleosides such as antibiotic A201A, and tunicamycin (Scheme 3.39) [64, 65].

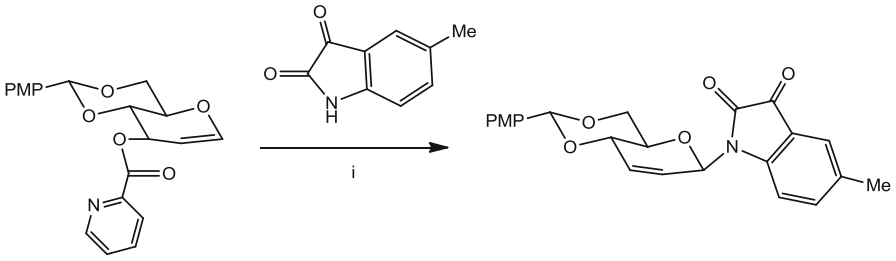


Scheme 3.37 Palladium-assisted modifications

3.3.10 Microbial/Enzymatic Approach

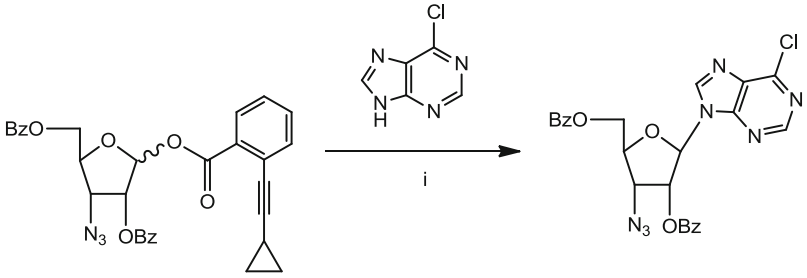
The synthesis of nucleosides by enzymatic methods is another extended possibility, and for this purpose the enzyme nucleoside phosphorylase has been selected as one of the most appropriate one. Usually the conversion proceeds by the reversible formation of a purine or pyrimidine nucleoside and inorganic phosphate from ribose-1-phosphate (R-1-P) and a purine or pyrimidine base. The general approach consists in the reaction of R-1-P as glycosyl donor which is condensed with purine or pyrimidine analogs. Following this method any heterocycle recognized by this enzyme can be glycosylated (Scheme 3.40).

The enzyme synthetase phosphoribosyl pyrophosphate PRPP was used for nucleotide synthesis of UMP. The sequence involves the conversion of ribose-6-phosphate with PRPP synthetase to produce phosphoribosyl pyrophosphate which

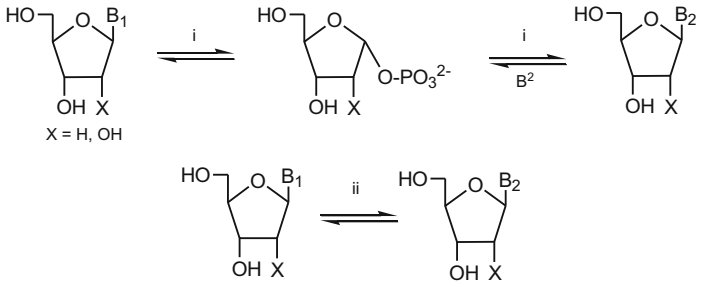


i) $Pd(PPh_3)_4$ DPPB, THF, 70°C

Scheme 3.38 Synthesis of glycosyl isatin through a classic Ferrier rearrangement



Scheme 3.39 Ortho-alkynylbenzoates method catalyzed by gold complex

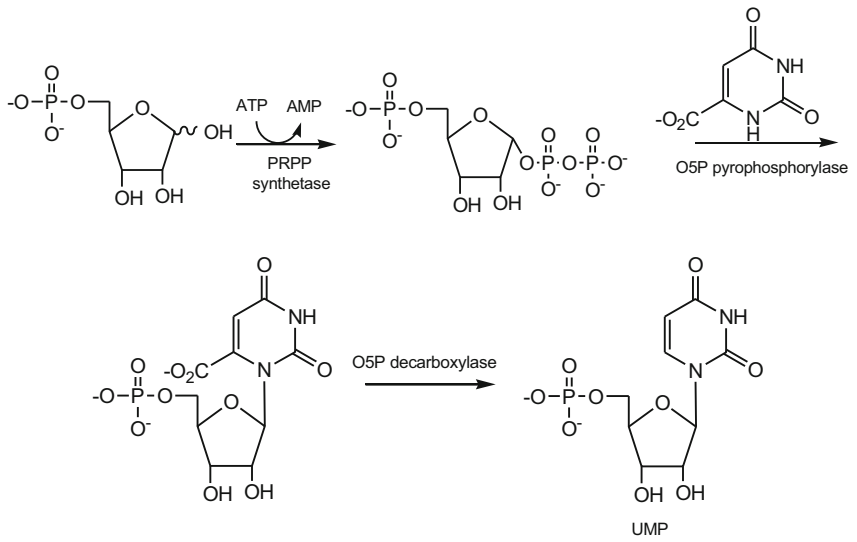


i) nucleoside phosphorilase. ii) trasribosylase.

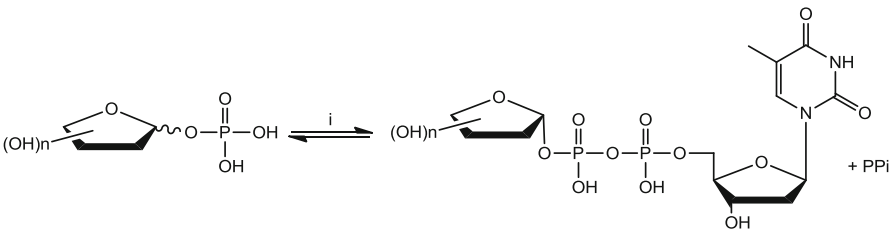
Scheme 3.40 General scheme for enzyme-mediated nucleoside synthesis

was condensed with orotate in the presence of O5P-Pyrophosphorylase to yield the nucleotide intermediate orotidine 5′-phosphate which after decarboxylation produced by the action of O 5P-decarboxylase the nucleotide Uridine monophosphate (Scheme 3.41) [66].

Bacterial α-D-glucopyranosyl-1-phosphate thymidylyltransferase was assayed as a catalyst for the synthesis of furanosyl nucleotides. Thus, five furanosyl-1-phosphates were evaluated as potential substrates for the bacterial thymidylyltransferase to produce only the β-anomer (1,2-*cis*-phosphate) of the sugar nucleotide as confirmed by proton NMR (Scheme 3.42) [67].



Scheme 3.41 Enzyme catalyzed synthesis of nucleotide



i) thymidylyltransferase CPs2L, deoxythymidine 5'-triphosphate, Mg^{2+}

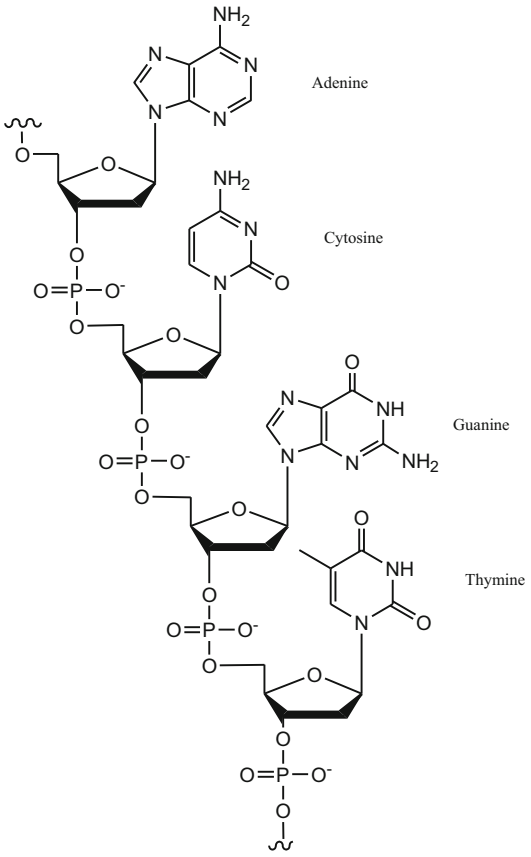
Scheme 3.42 Enzyme catalyzed synthesis of nucleotide by thymidylyltransferase

3.4 Oligonucleotide Synthesis

Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA) are very important natural polymers responsible for the processing of the genetic information of all organisms.

The basic repetitive unit known as nucleotide is composed of a nucleotide base, a sugar moiety, and a phosphate. The combinatorial pattern of the four different nucleosides constituted by the heterocyclic bases cytosine, thymine, guanine, and adenine is the base of DNA structure. In RNA strands uracil replace thymine and the furanoside is ribose instead of 2-deoxyribose. The phosphate group is attached at position 3′ of one sugar unit and the 5′ position of the next one forming a 3′–5′ elongation chain (Scheme 3.43).

Scheme 3.43 Fragment of single strand of DNA structure



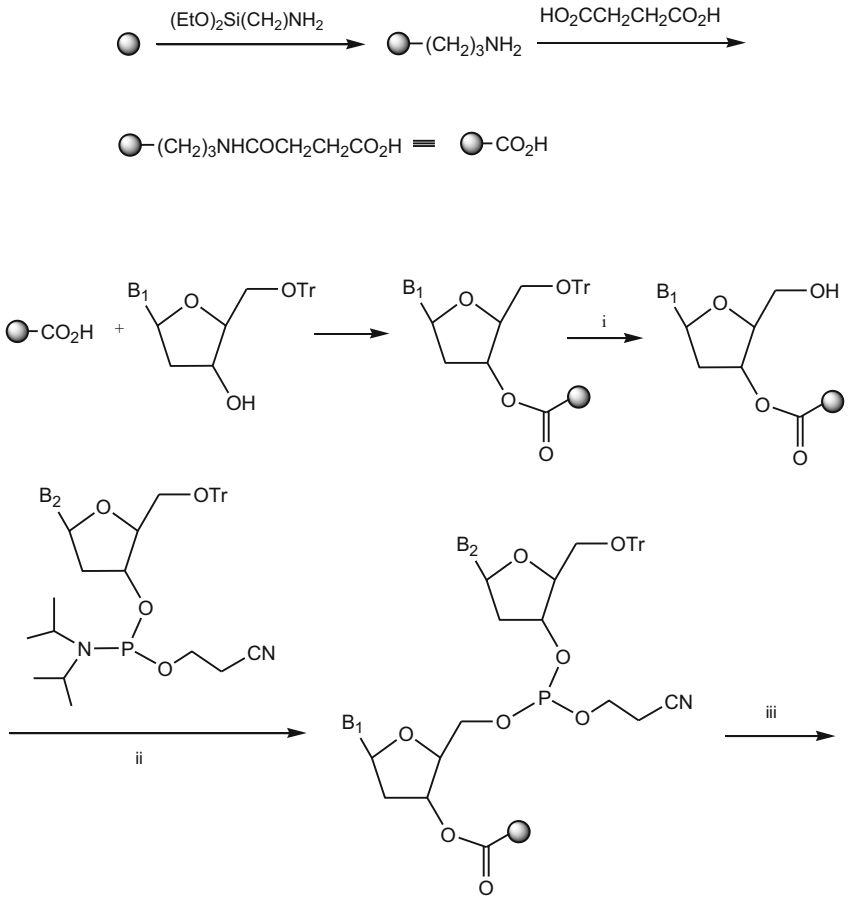
Oligonucleotide synthesis does not involve *N*-glycoside bond formation, but requires the design of nucleoside donors and nucleoside acceptors, following the same principle that applies for glycoside coupling reactions where suitable protecting groups, glycosyl donors and acceptors are required.

Solid phase procedures appear to be of great advantage for the coupling of nucleosides, and unlike for oligosaccharide solid phase chemistry, the attachment positions are always the same (3′ and 5′). The sequence of reactions that occurs in oligonucleotide synthesis starts on the attachment of 3′-OH position of 5′-protected nucleoside to a resin. Next, is deprotection of 5′-OH and subsequent attachment to a nucleoside donor which contains a phosphate precursor which in turn will be converted to phosphate group.

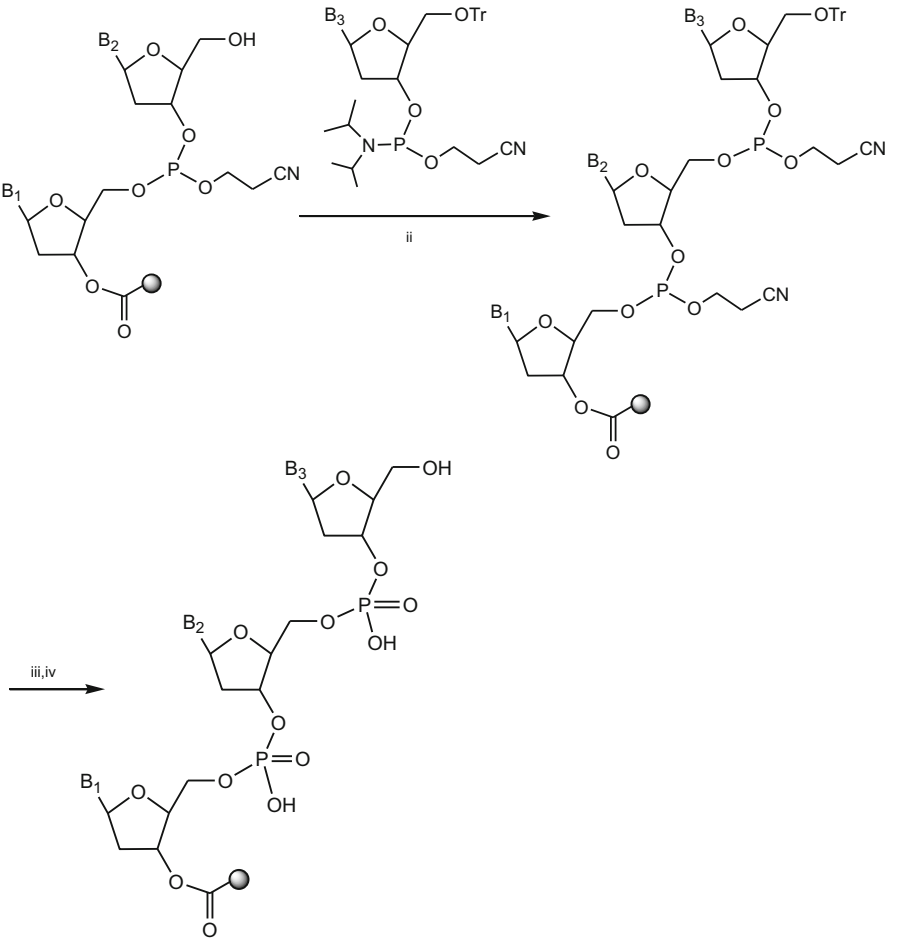
There are mainly two procedures for oligonucleotide synthesis: The phosphoramidite and the phosphonate method [16, 68].

3.4.1 Phosphoramidite Method

This methodology involves the use of the air-sensitive reagent 2-cyanoethyl tetraisopropylphosphorodiamidite $\{[(CH_3)_2CH]_2N\}POCH_2CH_2CN$ or 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite $(iPr)_2NP(Cl)OCH_2CH_2CN$ for activation of nucleoside donor [69]. This intermediate can be obtained by treatment of PCl_3 with 2 eq of diisopropylamine, and 1 eq of cyanoethylethanol. The general phosphoramidite approach, is outlined in Scheme 3.44, and begins with a nucleoside previously protected at the 5′-OH position with 4,4′-dimethoxytrityl group (Tr-), also attached to a silica support. The trityl group is then removed from the 5-OH position and allowed to react with a nucleoside donor protected at position 5-OH with trityl group and activated at position 3′ with 2-cyanoethyl diisopropylphophoroamidite. The coupling reaction being the critical step is catalyzed by tetrazol, and the process



Scheme 3.44 Phosphoramidite oligonucleotide strategy



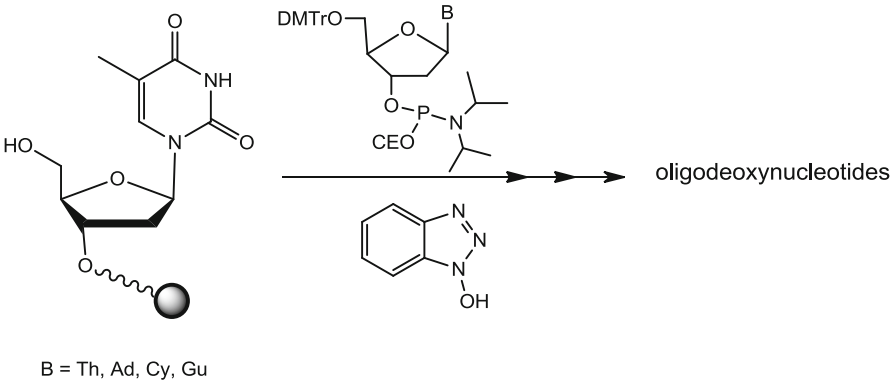
i) Cl_3CCOOH. ii) tetrazol. iii) Cl_3CCOOH. iv) a) I_2/H_2O. b) NH_4OH

Scheme 3.44 (continued)

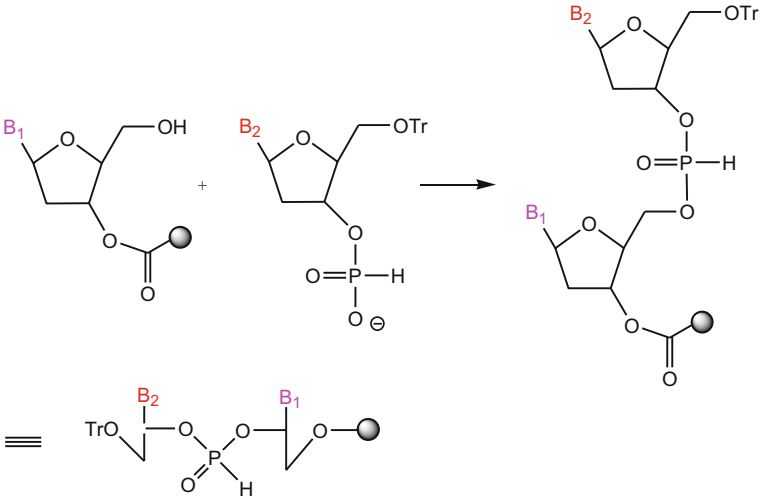
is repeated for the installation of subsequent nucleoside unit. Once the oligonucleotide chain is formed, the phosphoramidite group is transformed to phosphate with I_2-H_2O and released from resin with ammonia.

3.4.2 HOBt Solid Phase Synthesis

This protocol involves the initial attachment of a deoxy nucleoside with a highly cross-linked polystyrene resin and then reacted with a second phosphoramidite nucleoside in the presence of 1-hydroxybenzotriazole (HOBt) as the promoter to the solid-phase



Scheme 3.45 HOBt solid phase synthesis



Scheme 3.46 Phosphonate method

synthesis. Further deprotection with I_2-MeOH, tricholoracetic acid, and ammonia provides the desired oligonucleotides in good yields (Scheme 3.45) [70, 71].

3.4.3 Phosphonate Method

In this method the nucleoside donor is functionalized as a phosphotriester sugar derivative which reacts with nucleoside acceptor at 5-OH position which is available for linkage. An advantage of this method is the possibility of introducing substituents to the phosphate position giving place to the preparation of modified oligonucleotides Scheme 3.46.

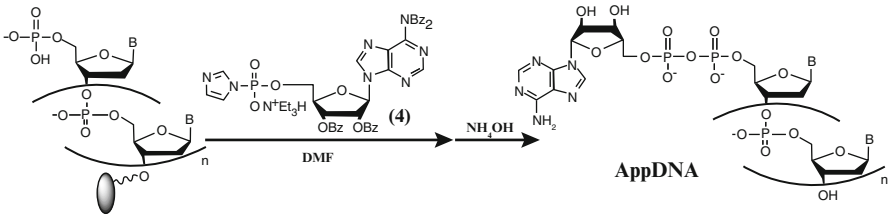
3.4.4 *Phosphorimidazolides Method*

This method propose a coupling reaction between a phosphate nucleoside attached to a resin and adenosine 5′-phosphorimidazolidate, to produce the corresponding protected AppDNA, which if finally debenzoylated with ammonia (Scheme 3.47) [72].

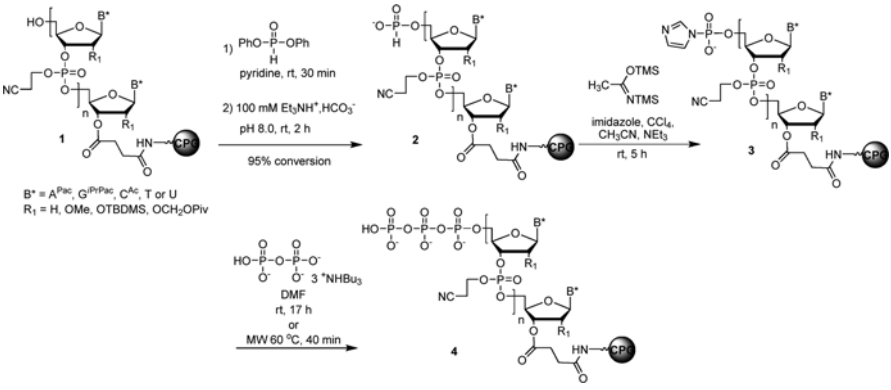
Another example on the applicability of this method is observed in the solid-phase preparation of the solid-phase dinucleotide triphosphate. This report consisted in the treatment of resin bounded phosphoramidite dinucleoside with a solution of diphenyl phosphite in pyridine, followed by hydrolysis, forming the solid-supported Hp-ON. Next the intermediate was oxidized to an activated 5′-phosphoroimidazolidate and subsequently treated with excess of (tri-*n*-butylammonium) pyrophosphate forming solid-phase nucleoside triphosphate (Scheme 3.48) [73].

3.4.5 *Modified Oligonucleotides*

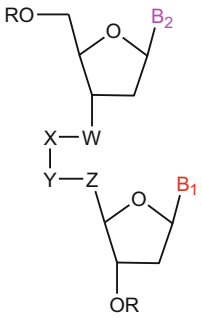
Modified oligonucleotides are another important application of solid phase oligonucleotide synthesis. It is known that natural oligonucleotides used as therapeutic strategy against viral infections as *antisense* for targeting RNA sequences may



Scheme 3.47 Phosphorimidazolides approach



Scheme 3.48 Another example of the phosphorimidazolides approach



W	X	Y	Z
O	-P(O)S-	O	CH_2
O	-P(O)CH_3-	O	CH_2
O	-P(O)NHR-	O	CH_2
O	-P(O)OR-	O	CH_2
NH	-P(O)O-	O	CH_2

Scheme 3.49 Modified oligonucleotides

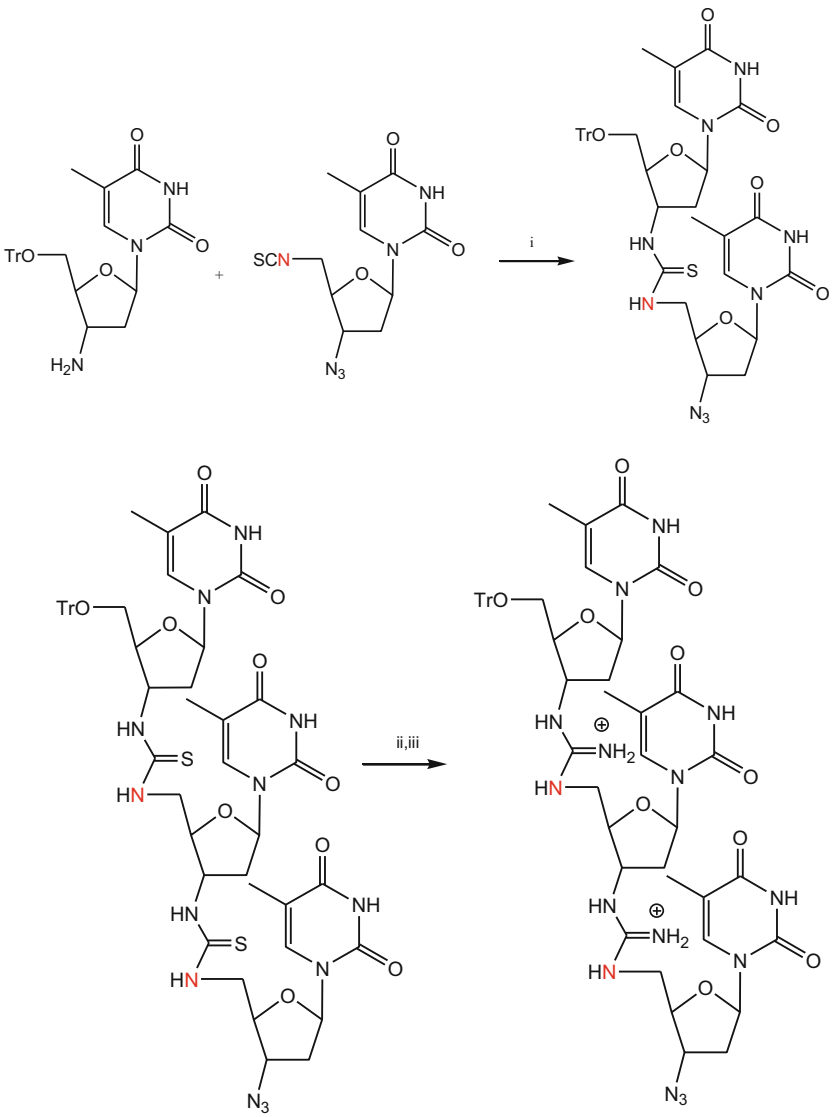
undergo enzymatic hydrolysis by endonucleases. Series of modified oligonucleotides carrying the modification either on the base, sugar or phosphate moiety provides ideally endonuclease resistance as well as high affinity for complementary RNA sequences.

Phosphodiester bond is the primary target for endonuclease breakage; therefore, the effort has been focused mainly on the modification of this segment of the chain. As a result of this, a first generation of modified phosphorous oligonucleotides such as phosphorothioates, methylphosphonates, phosphoramidates, phosphotriesters, and phosphodithioates were synthesized. Although these phosphorous derivatives showed increased resistance to endonuclease activity, the affinity for complementary sequences was decreased [74–76] For instance the synthesis of the antisense oligomer phosphorothioate analog of a 28-nucleotide homo-oligodeoxycytidine (S-dC_{28}) was achieved, and tested as a potent inhibitor of HIV in vitro, showing significant inhibition of reverse transcriptase activity and syncytium formation between HIV-1 producing cells and $CD4^+$ [77].

A second generation proposed the replacement of phosphodiester group by a bioisoster such as amides, urea, and carbamate (Scheme 3.49). In general the observations reveal better enzymatic hydrolysis resistances, but again poor affinity toward RNA complementary sequences.

Alternatively Dempcy et al., [78] reported the synthesis of modified guanidine–thymidine oligonucleotide following the procedure depicted in Scheme 3.50. The reactions involved are the condensation between 3′-amino-5′-*O*-trityl-3′-deoxythymidine and 3′-azido-5′-isothiocyano-3′,5′-deoxythymidine, to generate $5' \rightarrow 3'$ thiourea–nucleoside dimer. Reduction followed by coupling reaction of dimer with the latter nucleoside produced a chain elongation reaction. Guanidine conversion was done with aminoiminosulfonic acid and ammonium hydroxide, forming guanidinium thymidyl pentamer.

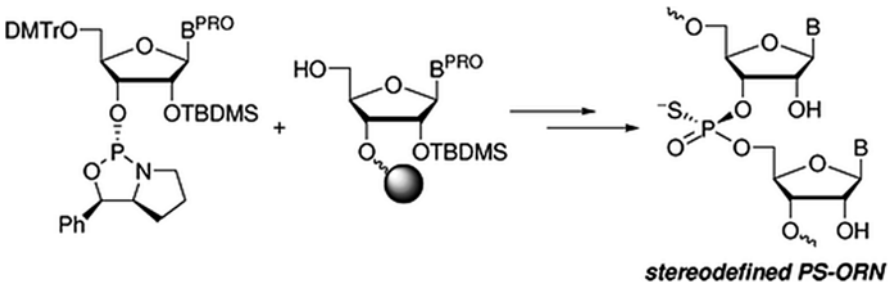
Another type of modified oligonucleosides more recently described correspond to the oligoribonucleoside phosphorothioates (PS-ORNs) which were prepared by using ribonucleoside 3′-*O*-oxazaphospholidine derivatives as monomer unit and submitted to react under activating conditions with protected 5′-OH nucleoside anchored to a highly cross-linked polystyrene (Scheme 3.51) [79].



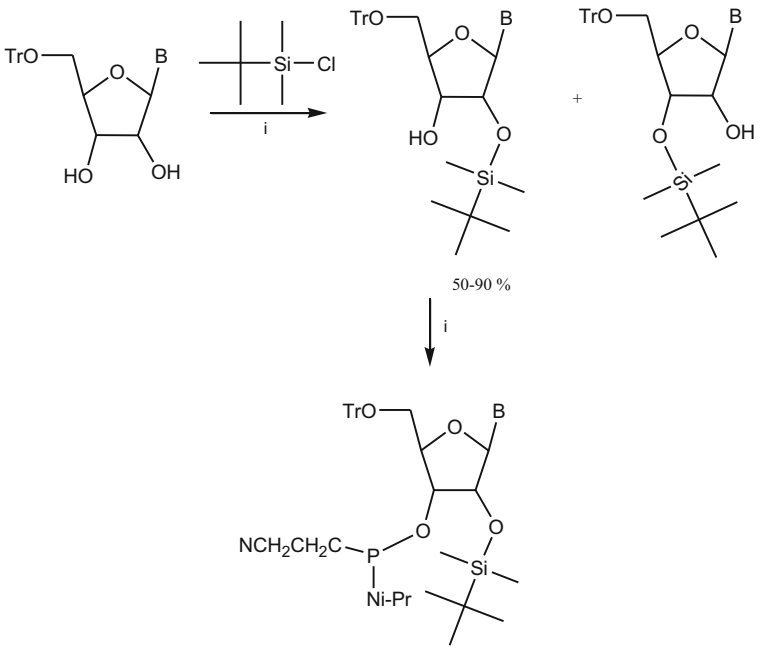
i) DMF. ii) H_2S. iii) 3´-azido-5´-isothiocyano-3´,5´-deoxythymidine. iv) a) TFA.
b) $H_2NC(=NH)SO_2H$. c) NH_4OH.

Scheme 3.50 Preparation of guanidinium oligonucleotides

The unit assemble for oligoribonucleotide synthesis is to some extend similar to deoxyribonucleotides synthesis; however, an additional consideration should be taken into account, which is the suitable protection of position 2-OH of ribose. The use of silyl protecting group, is one of the best choices so far reported, in particular



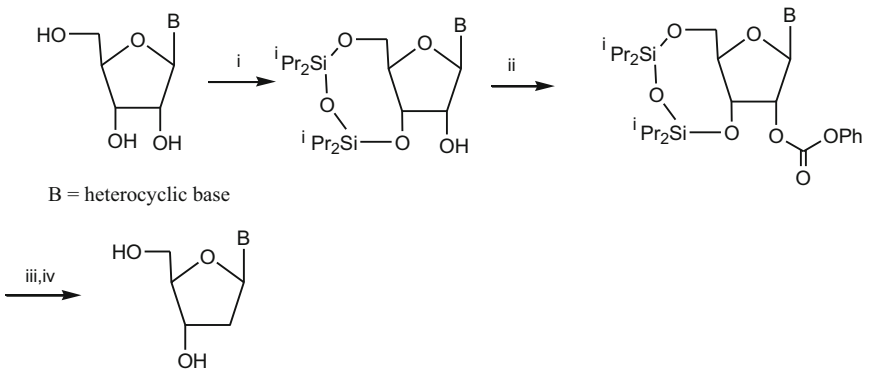
Scheme 3.51 Preparation of oligoribonucleoside phosphorothioates (PS-ORNs)



Scheme 3.52 Ribose protecting groups for oligoribonucleotide synthesis

the hindered *tert*-butyldimethyl silyl (TBDS) group. The protection of tritylribonucleoside produced a mixture of isomers, being the 2-OH silyl derivative generated in between 50 and 90 % yield. Final removal of this protecting group is usually achieved with 1 M tetrabutylammonium fluoride in THF (Scheme 3.52).

Some other choices for 2-OH protection are: tertahydropyran-1-il, 4-methoxytetrahydropyran-4-il and modified ketal of 1-(2-fluorophenyl)-4-methoxypiperidin-4- il (Fpmp); however, it has been found that acid conditions for removal of these protecting groups are not compatible with trityl protecting group.



i) $[(iPr)_2SiCl]_2O$, Py. ii) ClC(S)OPh, DMAP, MeCN. iii) Bu_3SnH, AIBN, $PhCH_3$.
iv) Bu_4NF, THF.

Scheme 3.53 Barton–McCombie procedure for the preparation of 2′ deoxynucleosides

Simultaneous protection of position 3′ and 5′ can be achieved by using the silyl protecting group tetraisopropyldisiloxychloride (TIPS-Cl) in pyridine. This type of protection has been useful in the conversion of adenosine to 2′-deoxyadenosine under the conditions reported by Barton and McCombie [80] (Scheme 3.53).

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Chapter 4
Nucleoside Mimetics

Modified nucleosides are useful therapeutic agents being currently used as antitumor, antiviral, and antibiotic agents. Despite the fact that a significant variety of modified nucleosides display potent and selective action against cancer, viral and microbial diseases, the challenge still attracts full attention since most of them do not discriminate between normal and tumor cell and in viral infections resistant strains usually appear during the course of the treatment.

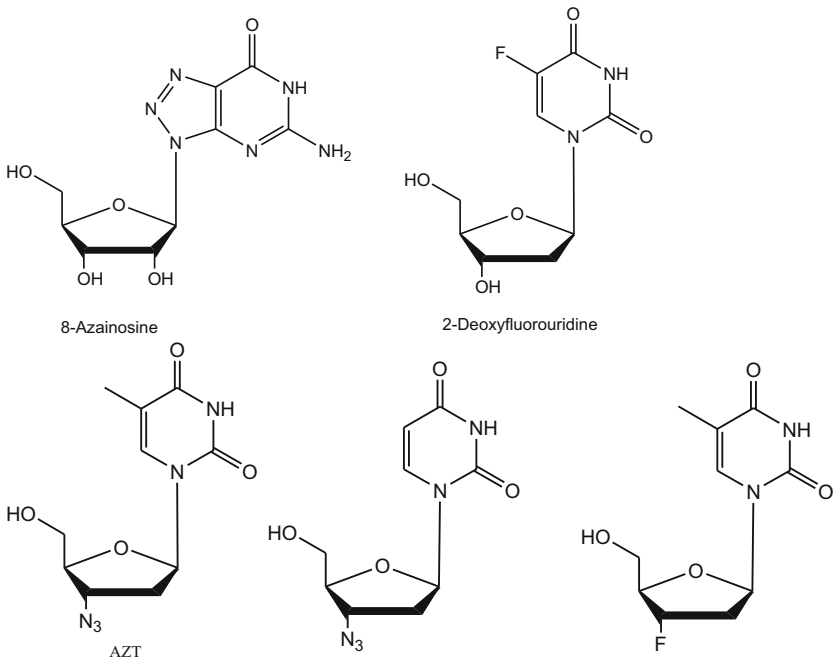
Synthetic acyclic and carbocyclic *C*-nucleosides and modified *N*-nucleosides have shown remarkable action against AIDS, Hepatitis, and herpes infections among others. Some of the nucleosides used as approved drugs are: acyclovir, carbovir being the treatment of choice against herpes, AZT, ddI, ddC, ddG, abacavir, which in combination with protease inhibitors are indicated in the treatment against HIV, and *C*-nucleoside ribavirin in the treatment against hepatitis [1, 2].

Representative examples of chemotherapeutic agents modified at the heterocyclic base, the sugar fragment, L and *C*-nucleosides, carbocyclic and acyclic nucleosides are depicted in Scheme 4.1.

A significant number of synthetically modified nucleosides have been designed as antiretroviral drugs in the therapy of human immunodeficiency virus (HIV) infection. During retroviral infection, the viral RNA is used as template for proviral DNA synthesis, a process mediated by viral DNA polymerase better known as reverse transcriptase. Thus, the process involves the initial formation of a RNA–DNA hybrid which is then degraded by an RNAse to release the DNA strand that will be the template for the synthesis of the double stranded viral DNA, a process also catalyzed by reverse transcriptase [3].

The proposed mechanism of action of modified agents such as AZT during viral infection involves the interruption of the viral replication process that occurs between the virus and host, particularly the replication inhibition inside T cells, monocytes, and macrophages.

M. Brito-Arias, *Synthesis and Characterization of Glycosides*,
DOI 10.1007/978-3-319-32310-7_4



Scheme 4.1 Representative synthetically modified nucleosides

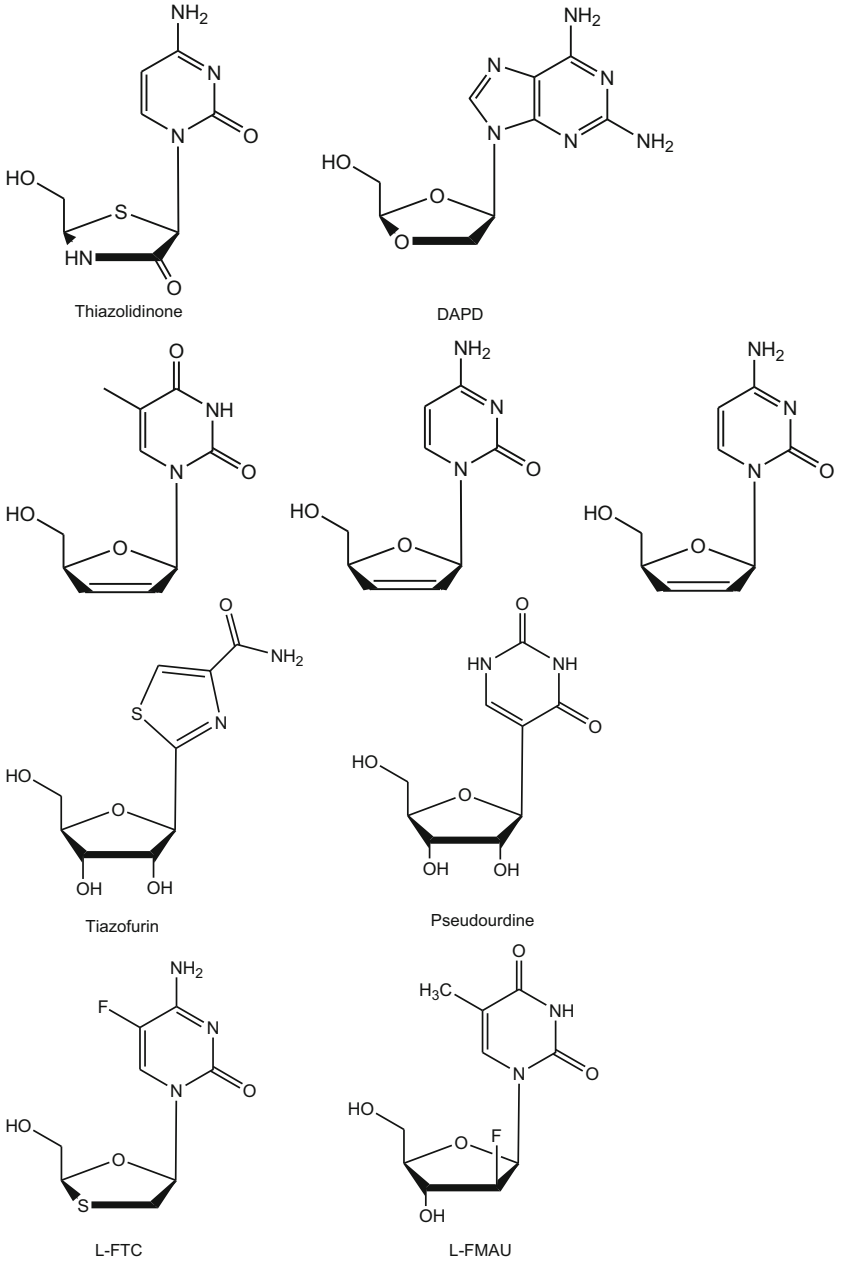
When the modified nucleoside is introduced into the cell, a sequential 5′-phosphorylation process mediated by kinases occurs on the furanoside ring which is subsequently incorporated into the DNA as triphosphate (Scheme 4.2).

An important collection of active nucleosides mimetics has been synthesized and classified for better understanding as follows: [4]

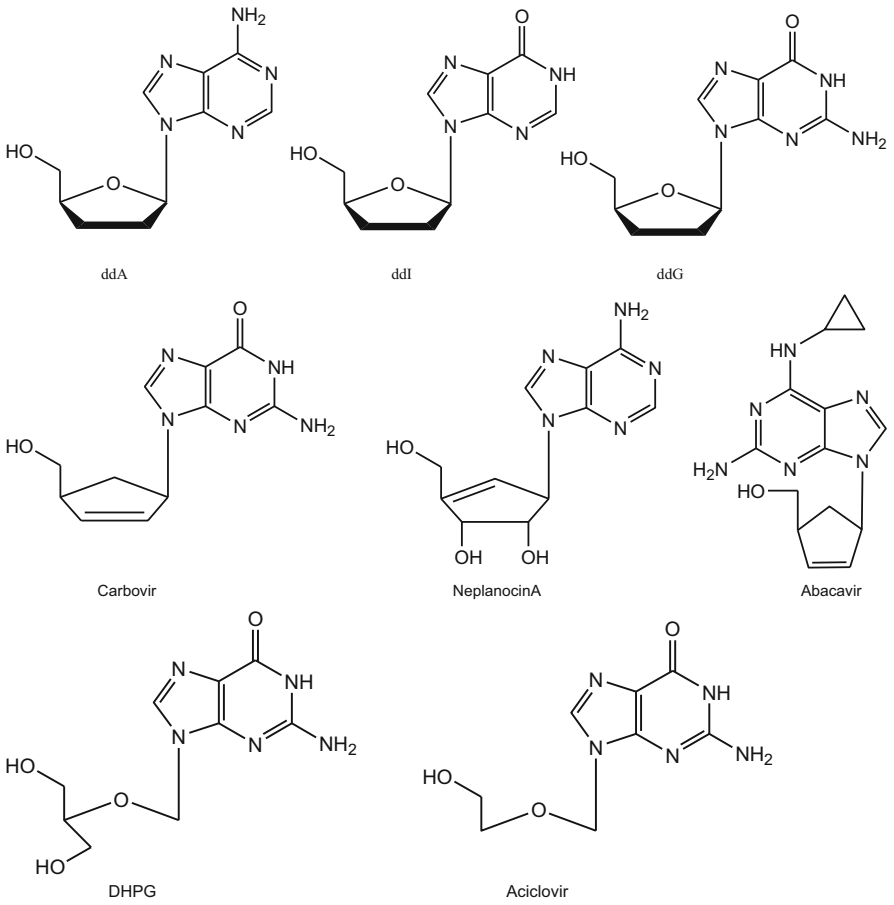
Modified *N*-nucleosides
L-nucleosides (D-isomers)
C-nucleosides
Carbocyclic nucleosides
Acyclic nucleosides
Thionucleosides

4.1 Modified *N*-nucleosides

A broad number of modified *N*-nucleosides have been developed and tested on clinical trials, some of them being highly promising. The chemical manipulations have been made at the heterocyclic base, the sugar of both. Some representative examples of chemical modifications leading to key intermediates or active nucleosides are:



Scheme 4.1 (continued)



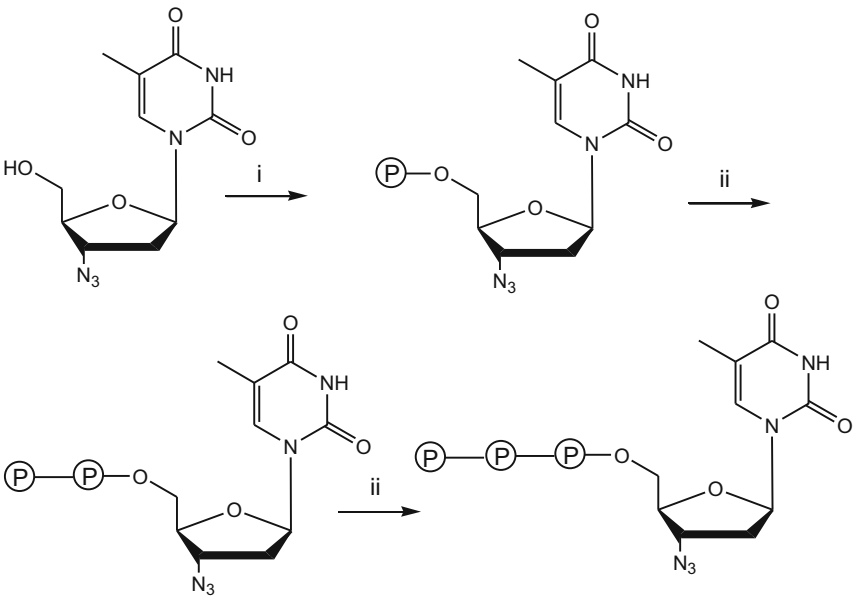
Scheme 4.1 (continued)

4.1.1 Heterocycle Modifications

4.1.1.1 C-5 Substituted Pyrimidines

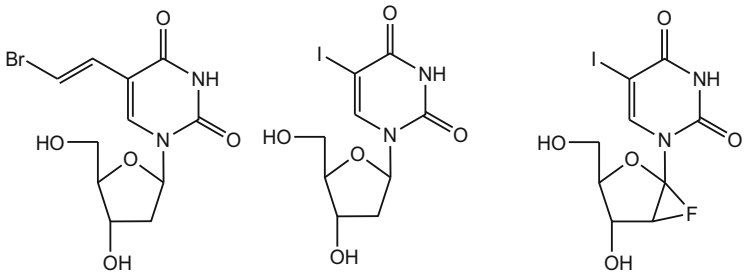
Several nucleoside analogs bearing modifications at the 5-position have been found to be active as antiviral and anticancer drugs. Examples of this are BVDU, IDU, and FIAU (Scheme 4.3) [5].

Palladium mediated transformations are a suitable strategy for introducing substituents at C-5. Some of the reactions implemented for this purpose are the Sonogashira [6, 7], Stille [8, 9], Heck [10, 136], and Hiyama [11] (Scheme 4.4).



i) Timidinkinase. ii) Timidilatokinase. iii) Nucleosidediphosphatekinase.

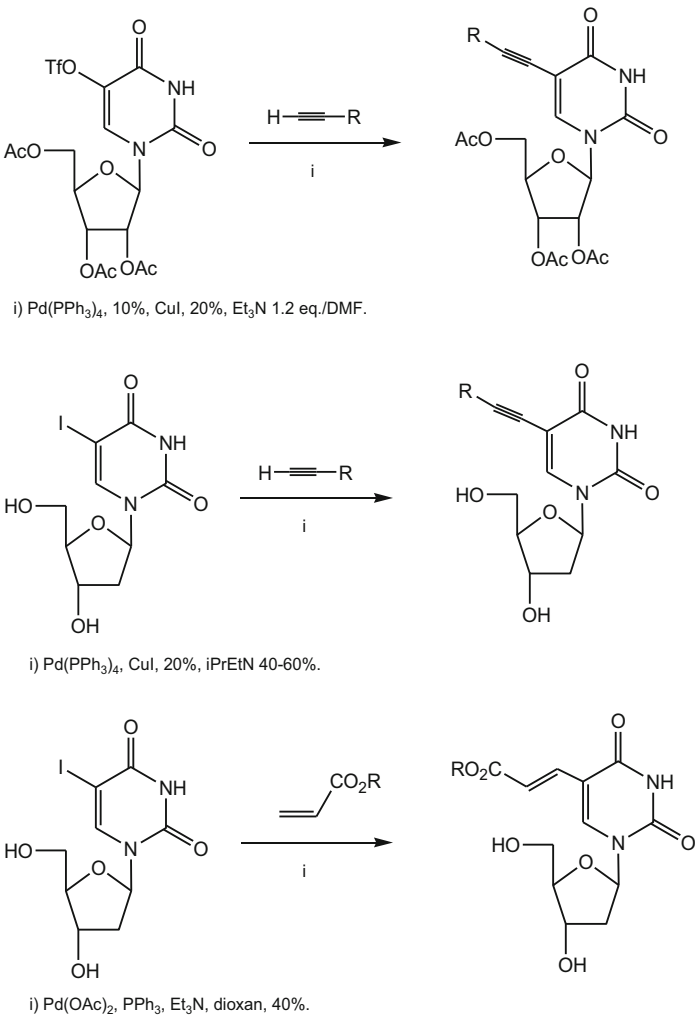
Scheme 4.2 Phosphorylation of AZT



Scheme 4.3 Active C-5 substituted pyrimidines

4.1.1.2 C-6 Substituted Pyrimidines

By following palladium-mediated substitutions, a more limited number of C-6 substituted pyrimidines have been described in comparison with C-5. For instance, by applying the Stille reaction it has been possible to prepare C-6 substituted aryl, vinyl, alkynyl derivatives (Scheme 4.5) [12].

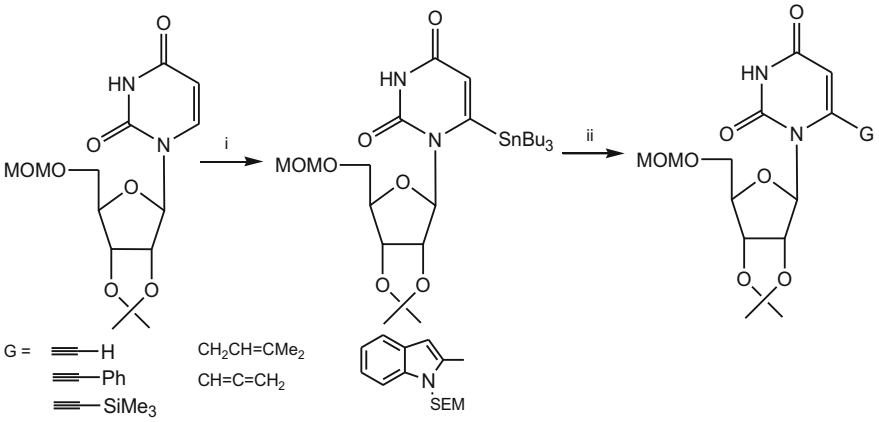


Scheme 4.4 Palladium mediated substitutions at C-5 pyrimidine position

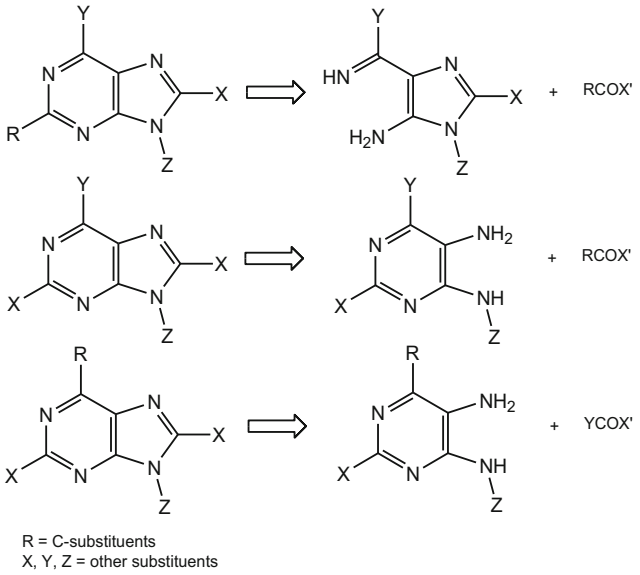
4.1.1.3 Purine Formation

The conventional methods of preparation of C-C purines are based on heterocyclization [13, 14]. The classical procedures involve:

(a) 2-C-C-purines cyclization of 4-aminoimidazole-5-carboxamides or nitriles with carboxylic acid equivalents.
(b) 8-C-C-purines from 5,6-diaminopyrimidines and carboxylic acid derivatives; and for 6-C-C-purines from 4-alkyl or 4-aryl-substituted 5,6-diaminopyrimidines (Scheme 4.6) [15].

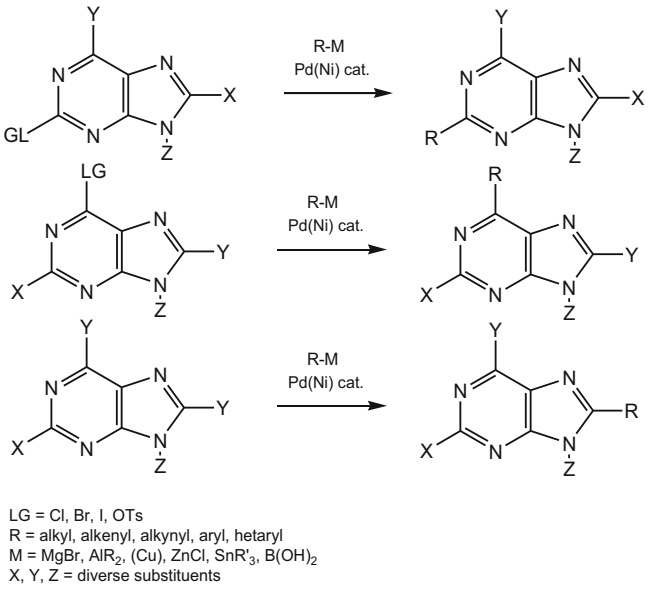


Scheme 4.5 Palladium-mediated substitution of 6-C substituted pyrimidines



Scheme 4.6 Conventional methods of preparation of C-C purines

Other explored methods involve radical [16, 17] or nucleophilic substitution [18], sulfur extrusion [19], and Wittig type reactions [20, 21]. Despite their usefulness, other methods based on the use of organometallic complex are getting particular significance especially in the synthesis of substituted purines (Scheme 4.7) [15].



Scheme 4.7 General scheme between purines and organometallic compounds

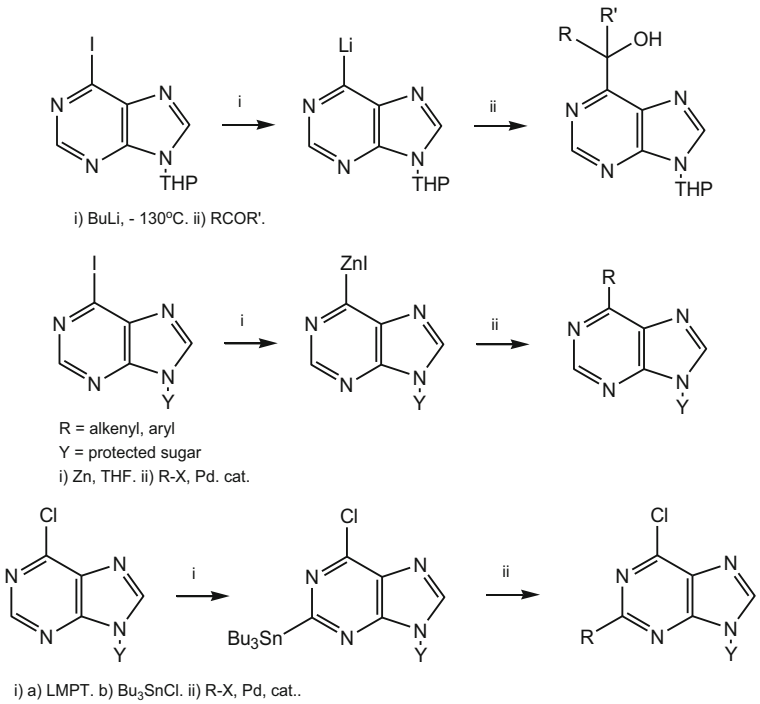
Usually the cross-coupling reactions involving organometallic compounds includes organolithium [22], magnesium [23], aluminum [24], cuprates [25], zinc [26], stannanes [27], and boron [28] reagents, in the presence of palladium catalyst and the purine base bearing a good leaving group usually halides or tosyl (Scheme 4.8).

Deazapurines are pyrrolo[2,3]pyrimidines of natural or synthetic source with significant antitumor, antiviral and antibacterial activities. Some compounds included in this class are tubercidin, toyocamycin, sangivamycin, and the hypermodified nucleoside queuosine. A flexible route for the preparation of pyrrolo[2,3] pyrimidines (7-deazapurines) has been developed, consisting in the condensation of protected uracil with ethyl *N*-(*p*-nitrophenethyl)glycinate and subsequent treatment with acetic anhydride and amine base with heating to provide 5-(acetyloxy) pyrrolo[2,3-d]-pyrimidine-2,4-dione in 74 % yield (Scheme 4.9) [29].

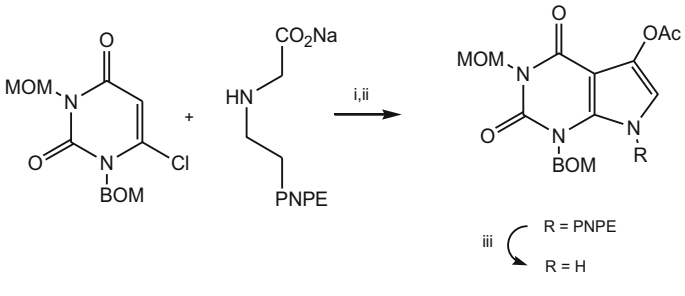
4.1.2 Sugar Modifications

4.1.2.1 2′-3′-dideoxysugars

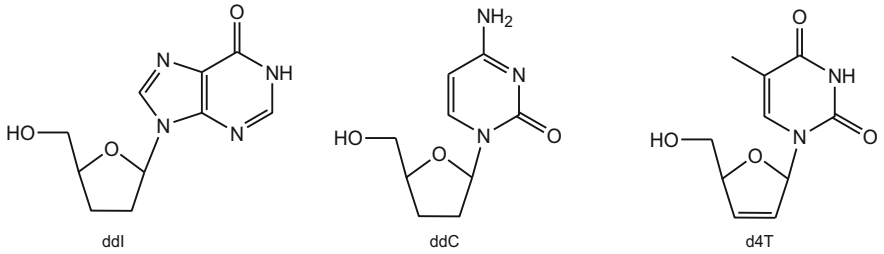
A significant number of saturated and unsaturated dideoxysugars have been synthesized and tested as antiviral or anticancer drugs. Remarkably, ddI and ddC are approved drugs for the treatment of AIDS [3], and others such as d4T being currently under clinical studies (Scheme 4.10) [30, 31].



Scheme 4.8 Cross-coupling reactions for purine modification



Scheme 4.9 Synthesis of 7-deazapurine analogs

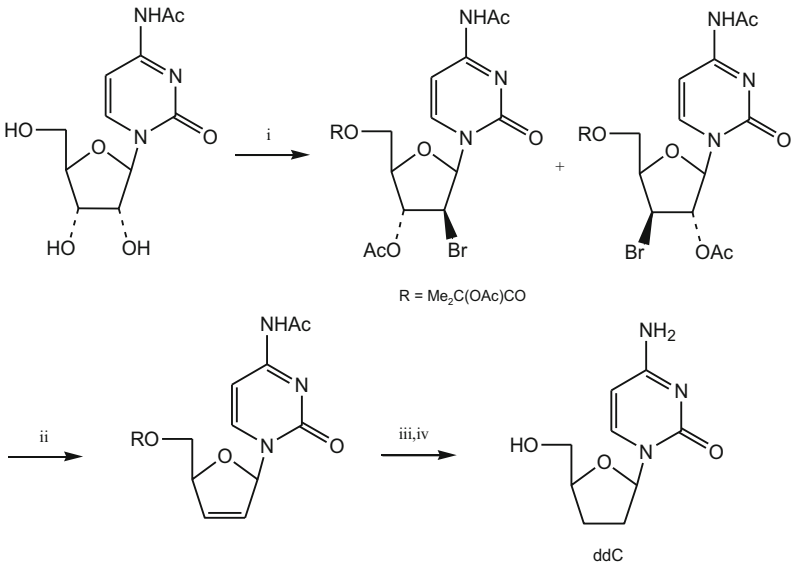


Scheme 4.10 Anti-AIDS 2′3′-dideoxy nucleosides

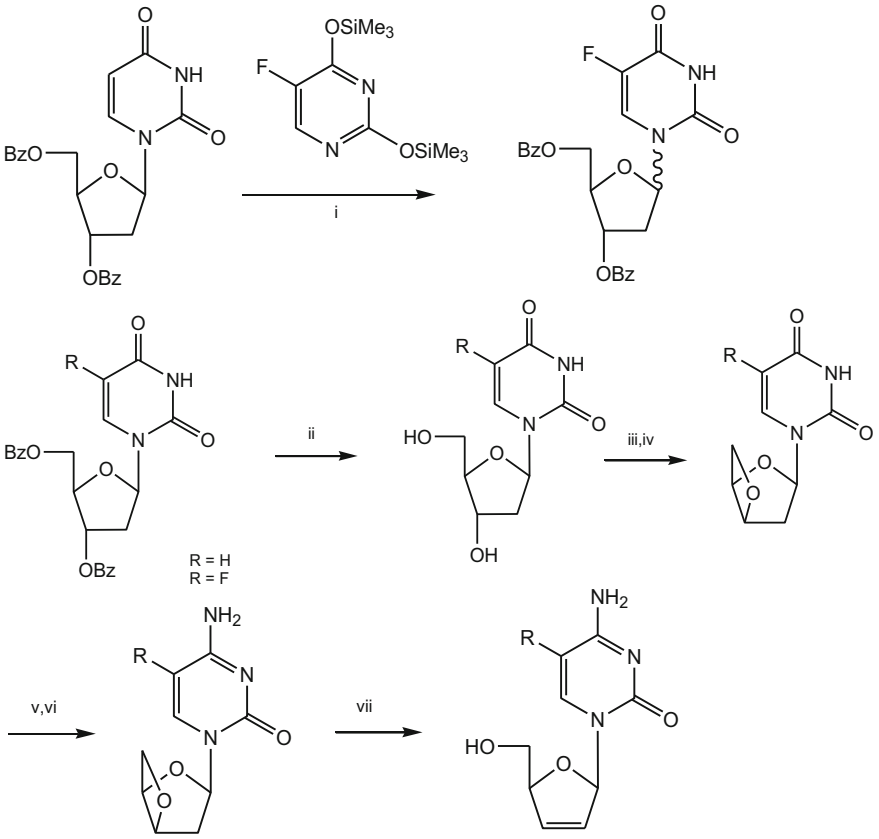
A method for preparing ddC was described involving bromoacetylation with HBr in acetic acid of N^4-acetylcytidine followed by reductive elimination with zinc–cooper couple in acetic acid to provide the corresponding 2′3′-unsaturated derivative. Final hydrogenation over 10 % palladium on charcoal gave ddC in 95 %. accompanied by some N-C cleavage in 5 % (Scheme 4.11) [32]. Similar reaction conditions were used for preparing 2′3′-dideoxyadenosine in 81 % yield from adenosine [33].

The design and synthesis of potent inhibitors for human hepatitis B Virus (HBV) 2′,3′-dideoxy-2′3′-didehydro-β-L-cytidine (β-L-d4C) and 2′,3′-dideoxy-2′3′-didehydro-β-L-5-fluorocytidine (β-L-Fd4C) nucleosides was carried out according to the pathway shown in Scheme 4.12 [34]. The key starting material 3′,5′-dibenzoyl-2′-deoxy-β-L-uridine was submitted to transglycosilation reaction with silylated 5-fluorouracil using TMSOTf as catalyst, providing an anomeric mixture separated by chromatography. After benzoyl deprotection, the anomeric nucleosides were treated with mesyl chloride followed by base to form cyclic ethers. Further transformation at the pyrimidine ring was followed by potassium *tert*-butoxide treatment to furnish β-L-d4C and β-L-Fd4C.

Other methods designed for the preparation of 2′3′-unsaturated and saturated deoxyfuranosides are based on: (a) Corey–Winter reaction involving cyclic thionocarbonate; [35–37], (b) Eastwood olefination process in which a five-membered cyclic orthoformate suffer a fragmentation to give in the presence of acetic anhydride the desired olefin (successfully applied in the preparation of ddU) [38, 39],



Scheme 4.11 Synthesis of anti-AIDS ddC



i) $CF_3SO_3SiMe_3$, CH_3CN. ii) NH_3/MeOH. iii) MsCl, Py. iv) 1N NaOH, EtOH/H_2O. v) 1,2,4-triazole, p-$ClC_6H_4OPOCl_2$, Py. vi) NH_4OH, dioxane. vii) *t*-BuOK, DMSO.

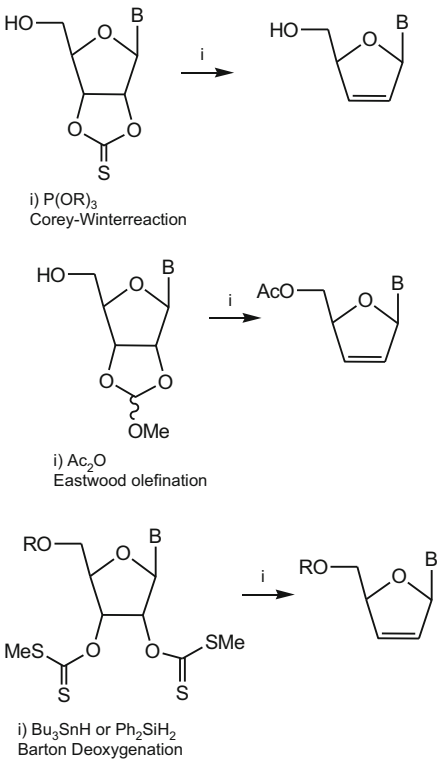
Scheme 4.12 Synthesis of anti-hepatitis B virus β-L-d4C and β-L-Fd4C

and (c) Barton deoxygenation involving the cyclic thionocarbonate or the bisxanthate, and then treated with tributyltin hydride [40, 41], or alternatively diphenylsilane [42] (Scheme 4.13).

The synthesis of modified nucleosides from natural nucleosides is another useful alternative for preparing pharmaceutically active dideoxy nucleosides. The potent antiviral inhibitors ddC, ddG, d4C, and d4G have been obtained from the corresponding protected natural nucleosides, as shown in Scheme 4.14 [43].

The chemoenzymatic approach has been also explored for the synthesis of 2′,3′ dideoxynucleosides. Such is the case of the antiviral 2′,3′-dideoxyguanosine which was synthesized from guanosine in 40 % overall yield using as a key step the commercially available mammalian adenosine deaminase (ADA) (Scheme 4.15) [44].

An strategy for preparing D- and L-2′-fluoro-2′3′-unsaturated nucleosides has been described and their anti-HIV activity evaluated. This approach requires 1-acet



Scheme 4.13 Alternative procedures for preparing 2′3′-unsaturated nucleosides

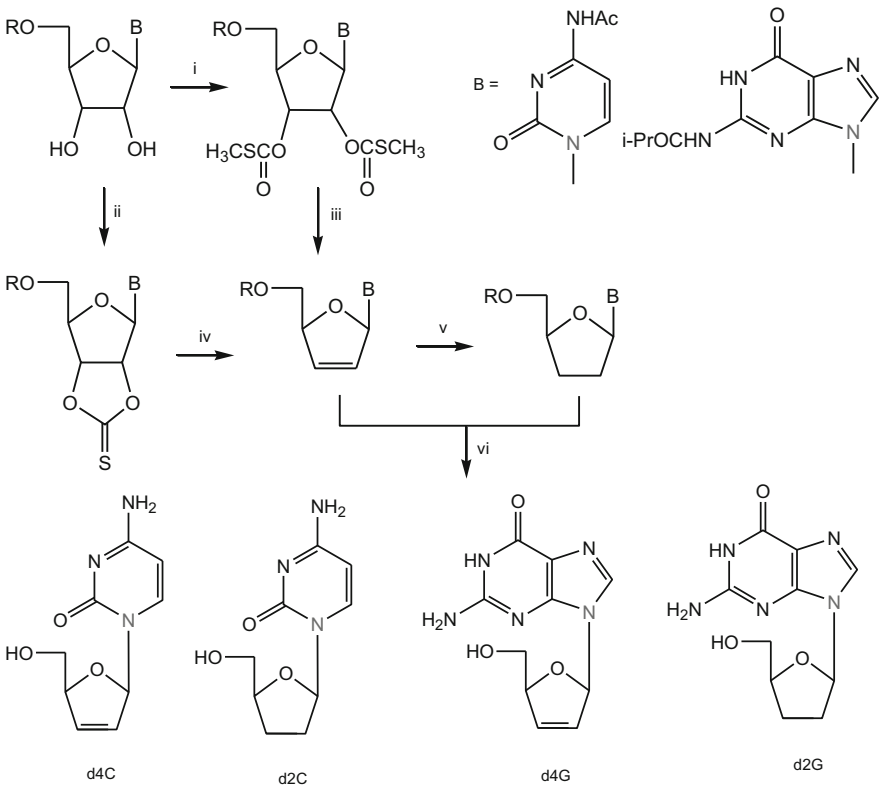
yl-5-*O*-benzoyl-2,3-dideoxy-3,3-difluoro-D-ribofuranose as key starting material which was condensed under Vörbruggen's conditions with purines and pyrimidines to provide the corresponding nucleosides. The resulting nucleosides were subjected to β-elimination to generate the fluoro unsaturated nucleosides (Scheme 4.16) [45].

4.1.2.2 2′-deoxynucleosides

The Barton deoxygenation provides another useful method for preparing 2′- and 3′-deoxynucleosides (obtained as a mixture), and involves as a key step the hydride reduction of the cyclic thionocarbonate with tributyltin hydride [42]. On the other hand, 2′-monotosylate nucleoside when treated with excess of lithium triethylborohydride produces the 2′-deoxy-3′β-hydroxy nucleoside in high yield (Scheme 4.17) [46].

2′-deoxynucleosides have been obtained from starting materials of different composition such as α,β-unsaturated aldehydes [47] chiral epoxy alcohols [48], butenolides [49, 50] and polyfunctionalized acetals among others [51].

The remarkable 2′-deoxynucleoside AZT widely prescribed as anti-AIDS drug was originally prepared from thymidine by Horwitz and coworkers [52], and since



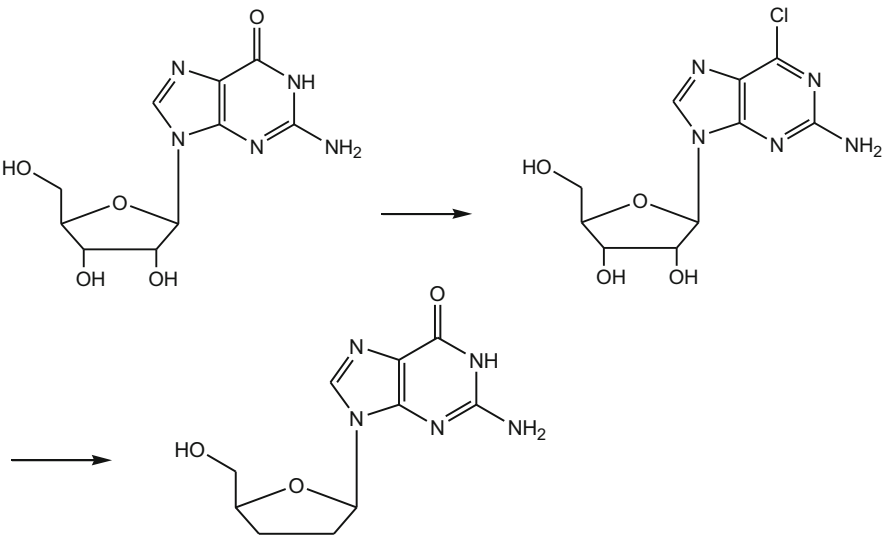
i) NaOH, CS_2/CH_3I. ii) $(Im)_2C{=}S$. iii) Bu_3SnH. iv) $(EtO)_3P$.v) H_2, Pd-C. vi) NH_3/MeOH.

Scheme 4.14 Antiviral modified nucleosides from natural sources

then, several other synthesis have been developed, some of them starting with either a nucleoside, or a sugar derivative [53–56], and others relaying on the use of non-carbohydrate starting materials [56, 57].

The procedure developed by Chu et al. [50] consisted in the use of mannitol as staring material which was subsequently transformed to provide the protected key intermediate 3′-azide-2′-deoxyribofuranose. The next step involved the coupling reaction with silylated thymine under Vörbruggen's conditions to produce an anomeric mixture of nucleosides in 66 %. Final desilylation and separation by chromatography column provided AZT in overall yield of 25 % from the furanoside intermediate (Scheme 4.18).

Another possibility was described by Hager and Liotta involving the coupling reaction between the azido diol intermediate and silylated thymine under Vörbruggen conditions to yield a diastereomeric mixture of azido diol nucleoside. Finally when exposed to concentrated acidic conditions the open form is converted into the β-anomer of AZT in 67 % yield (Scheme 4.19) [57].



i) adnosine deaminase, phosphate buffer, pH 6.5.

Scheme 4.15 Chemoenzymatic synthesis of 2′,3′-dideoxyguanosine

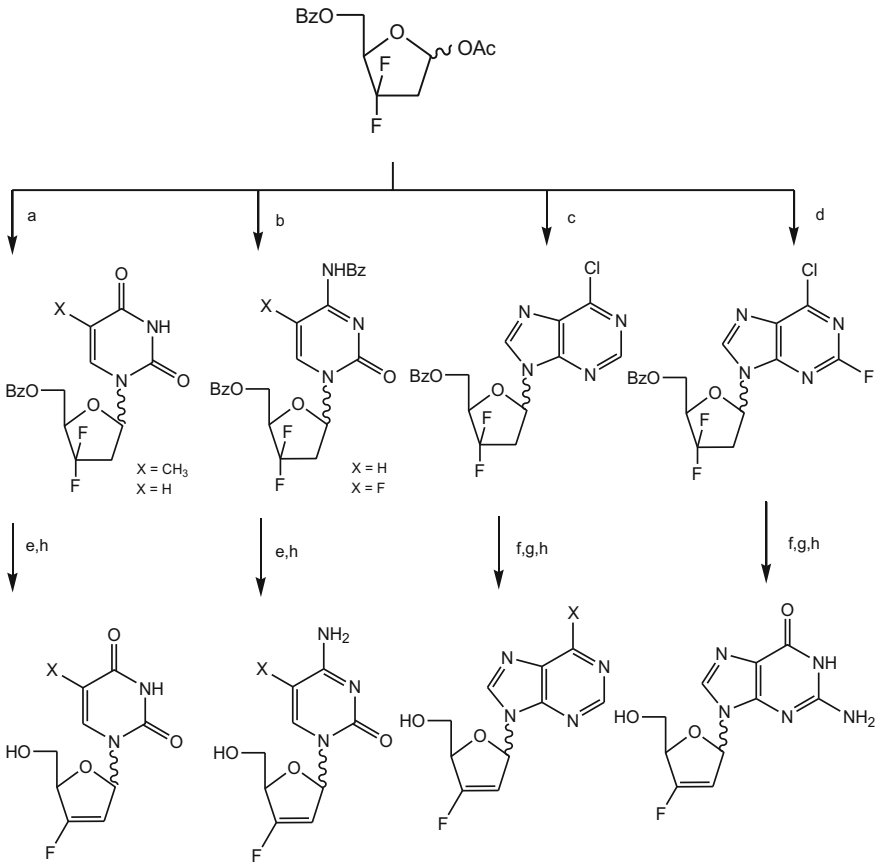
Transglycosidic reaction mediated by a deoxyribosyl transferase obtained from *E. coli* has been used in the synthesis of 3′-azido-2′,3′-dideoxyguanosine. The enzymatic reaction occurs between AZT which acts as glycosyl donor and substituted 2-amino-6-purines to generate the desired purine nucleoside and thymine as by-product (Scheme 4.20) [58].

4.1.2.3 3′-deoxynucleosides

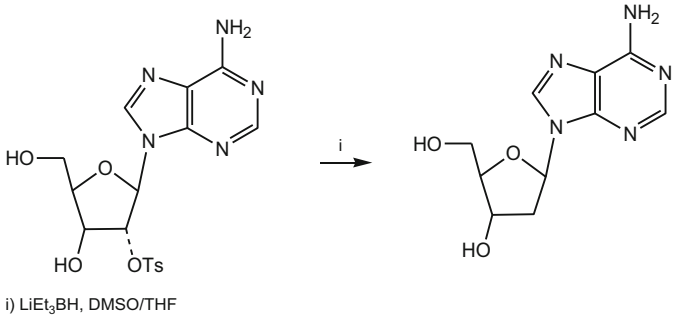
These deoxynucleosides may be readily prepared from 3′-*O*-tosylate via a [1,2]-hydride shift from C3′ to C2′ position with accompanying inversion of the C2′ center providing a 3′-ketone which was stereoselectively reduced by the hydride to produce 3′-deoxynucleoside (Scheme 4.21) [2, 46].

Also 3′-deoxyguanosine was synthesized by an enzymatic transglycosylation of 2,6-diaminopurine using 3′-deoxycytidine as a donor of the sugar moiety. The diaminopurine nucleoside was transformed to 3′-deoxyguanosine by the action of adenosine deaminase (Scheme 4.22) [59].

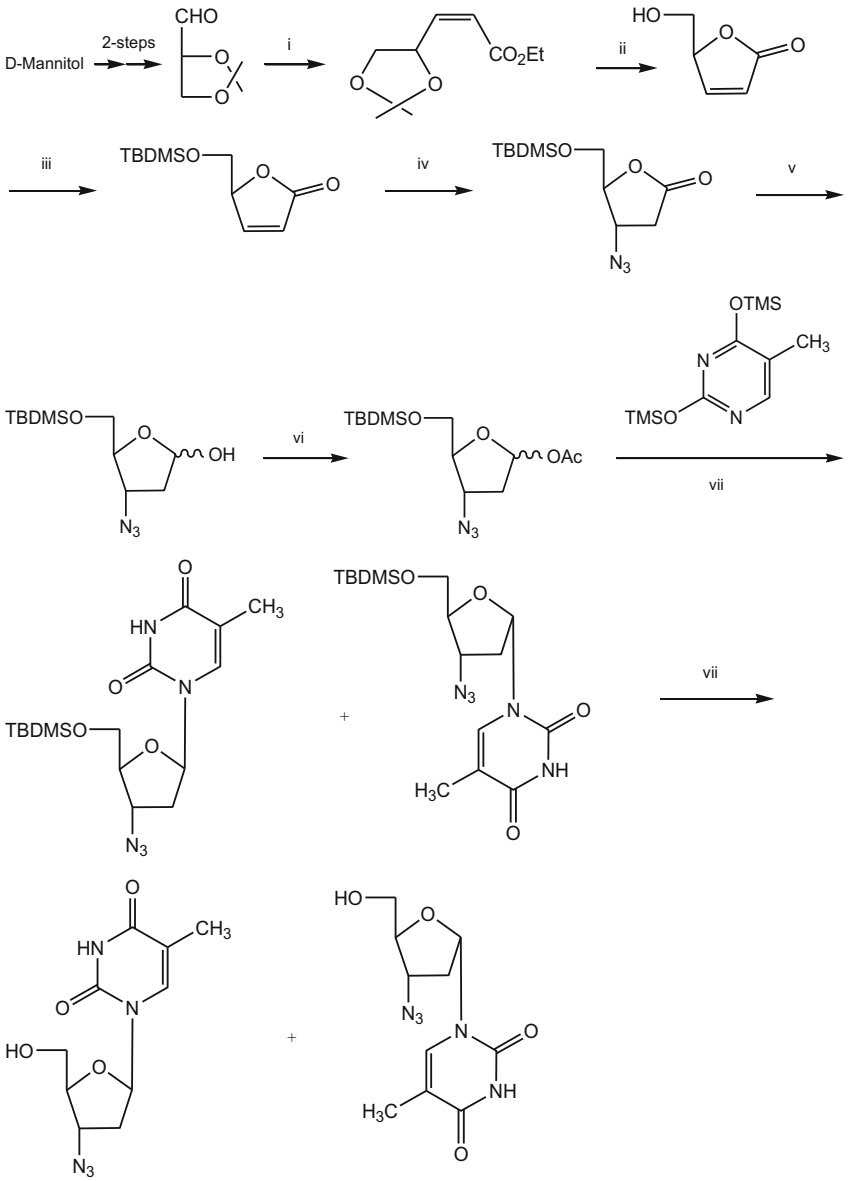
Lodenosine [9-(2,3-dideoxy-2-fluoro-β-D-threo-pentofuranosyl)] adenine (FddA) is a reverse transcriptase inhibitor with activity against HIV. This purine analog was evaluated as one of the most selective inhibitors in a series of 2′3′-dideoxyadenosines, although less active than ddA. An efficient method was developed starting from chloropurine riboside which was tritylated and selectively benzoylated at 3′-position. Before fluorination the 2′-hydroxyl group was converted to imidazolesulfonate or



Scheme 4.16 Preparation of D- and L-2′-fluoro-2′3′-unsaturated nucleosides

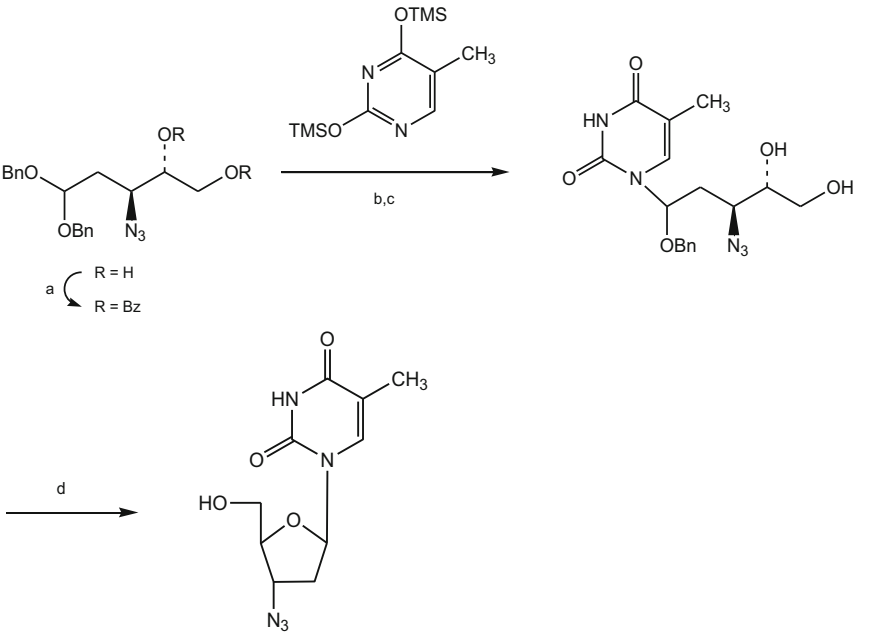


Scheme 4.17 The Barton deoxygenation for preparing 2′-deoxynucleosides



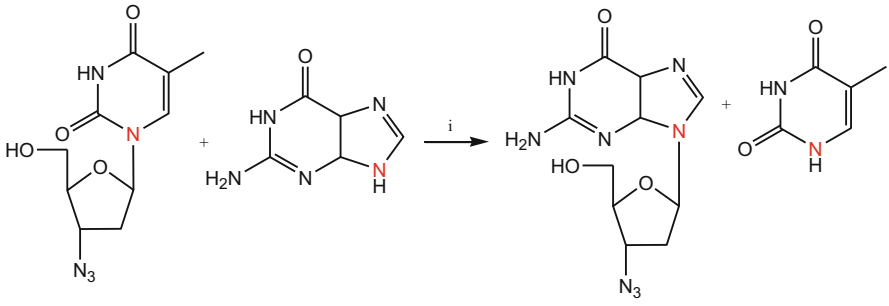
i) $Ph_3P{=}CHCO_2Et$, MeOH, 0°C. ii)HCldil. iii) t-Bu$(Me)_2$SiCl. imidazole, DMF. iv) LiN_3, THF, AcOH, H_2O. v) DIBAL, CH_2Cl_2, -78°C. vi)A c_2O,Py. vii) TMS-triflate, $ClCH_2CH_2Cl$. viii) n-Bu_4NF, THF.

Scheme 4.18 Synthesis of AZT from mannitol



i) PhCOCl (2.2equiv.), NEt_3, DMAP, CH_2Cl_2. b) $(CH_3)_3SiOTf$, $ClCH_2CH_2Cl$. c) NaOH (2equiv.), MeOH.
b) 4.7 NH_2SO_4 in MeOH.

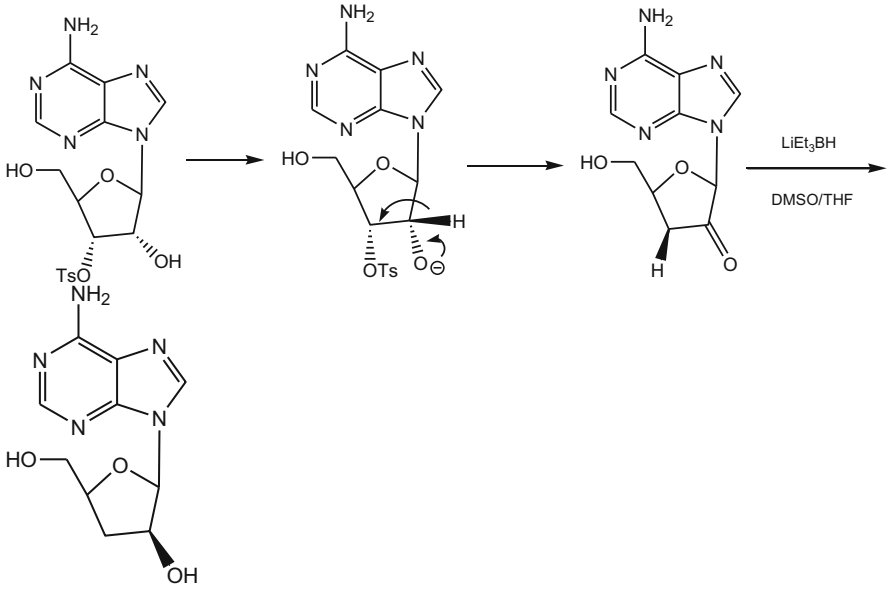
Scheme 4.19 Synthesis of AZT from azido diol intermediate



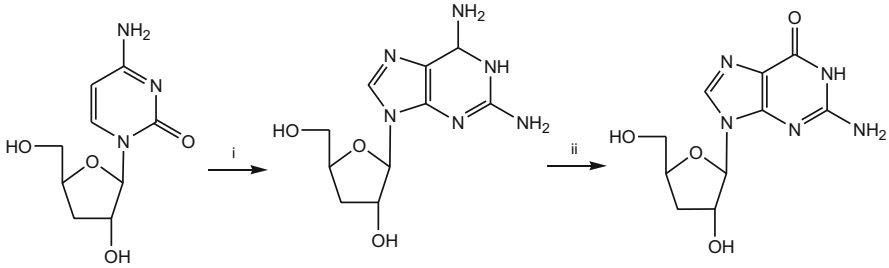
i) glycosyltransferases, pH6.0, 50°C.

Scheme 4.20 Enzymatic synthesis of 3′-azido-2′,3′-dideoxyguanosine

trifluoromethanesulfonate. Fluorination proceeds smoothly with 6 equiv. of $Et_3N.{}_3HF$ at reflux in 88% yield. Simultaneous 6-amination and 3′-debenzoylation was done with ammonia in high yield. Elimination of the 3′-hydroxy group was carried out under the Barton-McCombie procedure involving the formation of the 3′-O-thiocarbonyl followed by silane treatment. Final removal of trityl group provided FddA (Scheme 4.23) [60].



Scheme 4.21 Method for preparation of 3′-deoxynucleoside



i) 2,6-diaminopurine, *E. coli* BM-11 and BMT-4D/1A, K-phosphate buffer, 52°C, 26 h, 64%. ii) Adenosine deaminase (ADase), r.t., 16h, 68%.

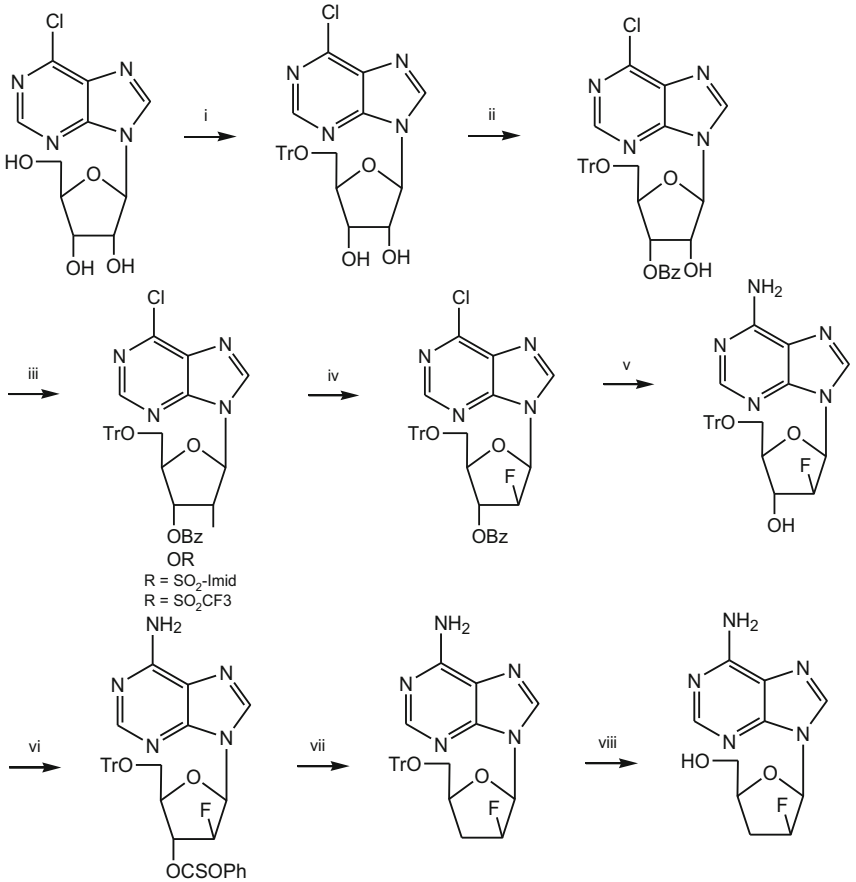
Scheme 4.22 Enzymatic synthesis of 3′-deoxyguanoside

4.1.2.4 4′-substituted Nucleosides

4′-substituted nucleosides have attracted much attention because of the discovery of potent anti-HIV agents 4′-azido- and 4′-cyano thymidine (Scheme 4.24).

One procedure involves the epoxidation of the exoglycal with dimethyldioxirane and ring opening of the resulting 4′,5′-epoxynucleosides to produce with high stereoselectivity the 4′-C-branched nucleosides (Scheme 4.25) [61].

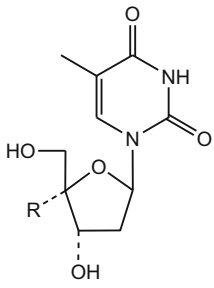
Likewise, others 4′-substituted nucleosides such as 4′-C-Ethynyl-β-D-arabino- and 4′-C-Ethynyl-2′-deoxy-β-D-ribopentofuranosyl pyrimidines have been reported by a different approach outlined in Scheme 4.26 [62].



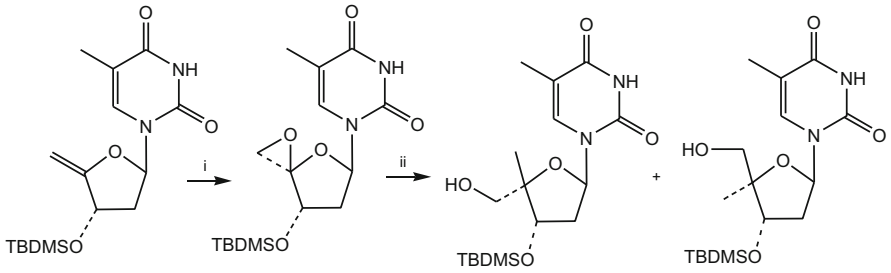
i) TrCl-iPrNH, DMF, 79%. ii) a) BzCl-Py, toluene. b) cat. Et_3N, toluene, 70%. iii) a) SO_2Cl_2-Py, CH_2Cl_2, b) imidazole. or CF_3SO_2Cl, DMAP, toluene. iv) Et_3.3HF, Et_3N, 70 and 78%. v) NH_3-MeOH, toluene 98%. vi) ClC(S)(OPh), DMAP, CH_3CN, 92%. vii) Ph_2SiH_2, AIBN, dioxane, 81%. viii) 80% AcOH, 100°C, 85%.

Scheme 4.23 Preparation of antiviral 2′3′-fluoro dideoxyadenosine FddA

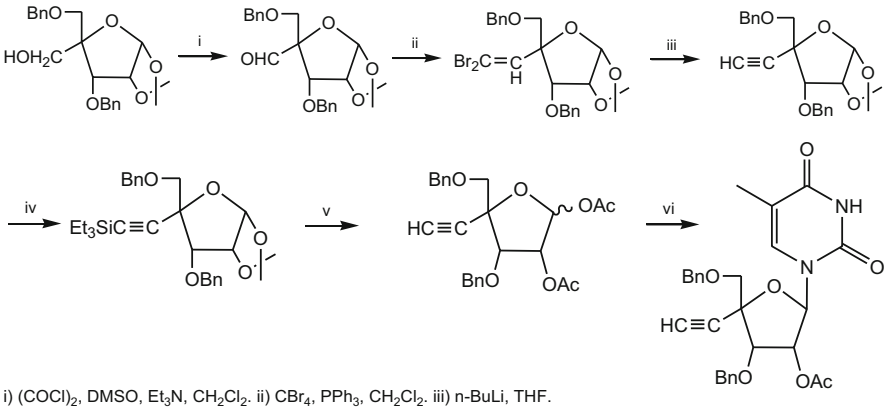
Scheme 4.24 Structure of potent anti-HIV 4′-substituted nucleosides



R = N_3, CN



Scheme 4.25 Ring opening of 4′,5′-epoxynucleosides

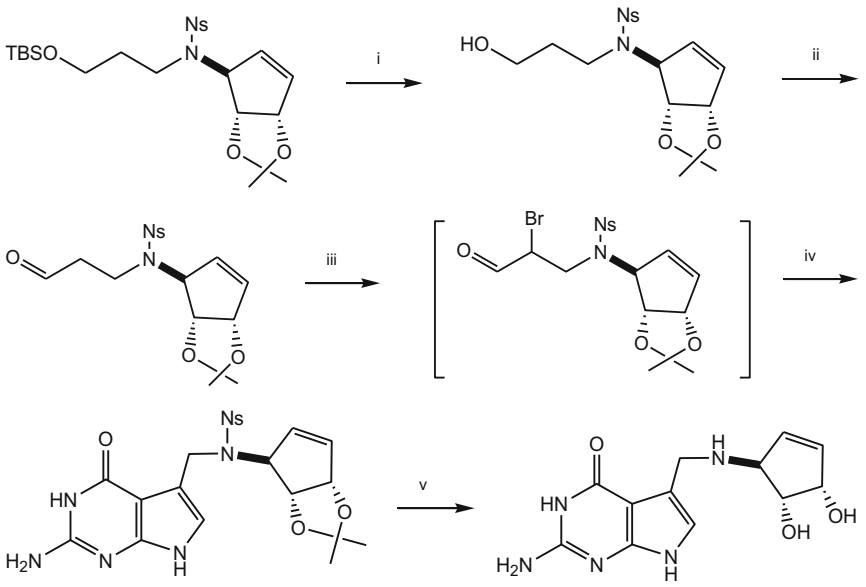


Scheme 4.26 Synthesis of 4′-C-Ethynyl-β-D-arabino- and 4′-C-Ethynyl-2′-deoxy-β-D-ribopentofuranosyl pyrimidines

4.1.3 Complex Nucleosides

The hypermodified Q base Queuine found in tRNA of plants and animals has been strongly associated with tumor growth inhibition. Three different approaches for preparing queuine have been described [63–65], the more recent in 11 steps from ribose. Completion of the synthesis involved the condensation of bromo aldehyde intermediate with 2,3-diamino-6-hydroxypyrimidine to give the desired heterocyclic product in 45 %. Final removal of protecting groups provided Q base (Scheme 4.27).

Capuramycin is a complex nucleoside antibiotic isolated from *Streptomyces griseus* 446-S3, which exhibit antibacterial activity against *Streptococcus pneumoniae* and



i) TBAF, THF, 87%. ii) TEMPO, NaOCl, KBr, CH_2Cl_2, 88%. iii) TMSBr, DMSO, MeCN. iv) NaOAc, H_2O/MeCN, 45%. v) a) $HSCH_2CH_2OH$, DBU, DMF, 46%. b) HCl, MeOH, 84%.

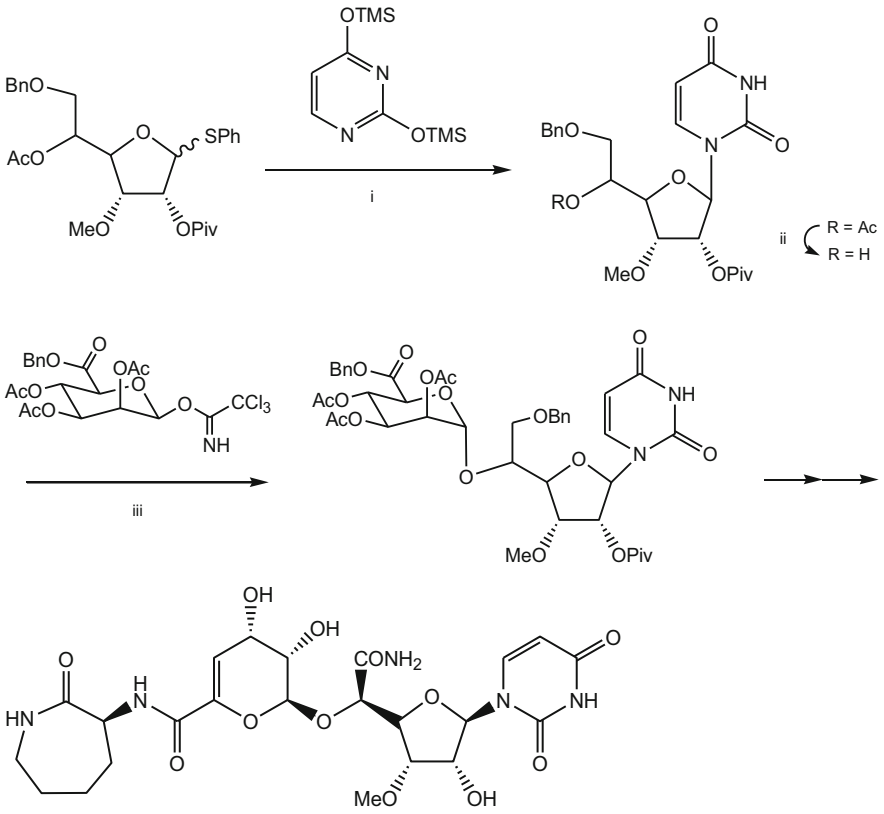
Scheme 4.27 Synthesis of hypermodified base Queuine

Mycobacterium smegmatis ATCC 607. The total synthesis was reported by Knapp and Nandan [66] consisting in the glycosylation reaction between the key thioglycoside donor and silylated pyrimidine to produce the corresponding L-*talo*-uridine. The next glycosidic coupling reaction was carried out with L-*talo*-uridine and imidate glycosyl donor under TMS-OTf conditions to provide the disaccharide nucleoside. Further transformations lead to the target molecule (Scheme 4.28).

Due its promising role as anti-tuberculosis drug, further efforts for preparing capuramycin and other analogs have been deployed as described in a more recent concise total synthesis [67].

Moreover, capuramycin has been also chemically transformed in an attempt to extend the antibacterial spectrum. Thus, radical oxygenation gave unexpected lactone in moderate yield via an intramolecular radical Ar-C glycosylation-lactonization reaction (Scheme 4.29) [68].

Synthetic studies of unique class tunicamycin antibiotics leading to the preparation of (+)-tunicaminyluracil, (+)-tunicamycin-V, and 5′-*epi*-tunicamacyn-V were described by Myers et al. [69] The key features are the development and application of a silicon-mediated reductive coupling of aldehydes, the allylic alcohols to construct the undecose core of the natural product, and the development of an efficient procedure for the synthesis of the trehalose glycosidic bond within the antibiotic (Scheme 4.30).



i) NIS, TfOH, CH_2Cl_2, -20°C. ii) NaOMe, MeOH, 77%. iii) TMS-OTf, CH_2Cl_2, -25°C, 16h, 85%.

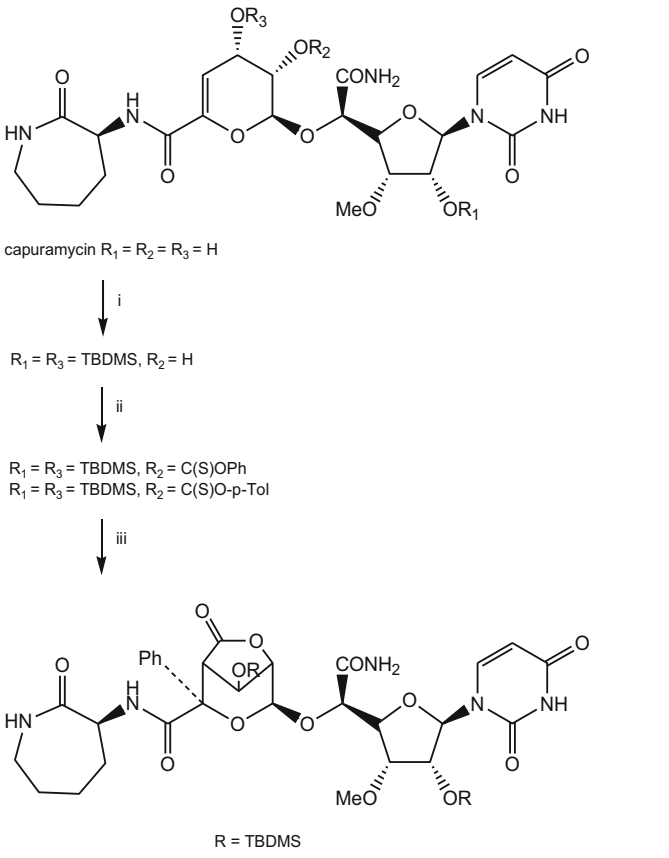
Scheme 4.28 Synthesis of Capuramycin

An alternative approach for the synthesis of tunicamycins is reported in a stereoselective approach, the key reactions being the Mukaiyama aldol reaction, intramolecular acetal formation, gold(I)-catalyzed O- and N-glycosylation, and final N-acylation (Scheme 4.31) [70].

4.1.3.1 Fused Heterocyclic Nucleosides

Selective and potent anti-Varicella Zoster Virus (VZV) bicyclic furanopyrimidine deoxynucleosides were synthesized. The bicyclic formation was performed by palladium-catalyzed coupling of aryl acetylenes with 5-iodo-2′-deoxyridine providing the desired fused furan nucleoside (Scheme 4.32) [71].

Triciribine is a tricyclic nucleoside with antineoplastic and antiviral properties, synthesized in an improved fashion from 6-bromo-5-cyanopyrrolo [2,3-d] pyrimidin-

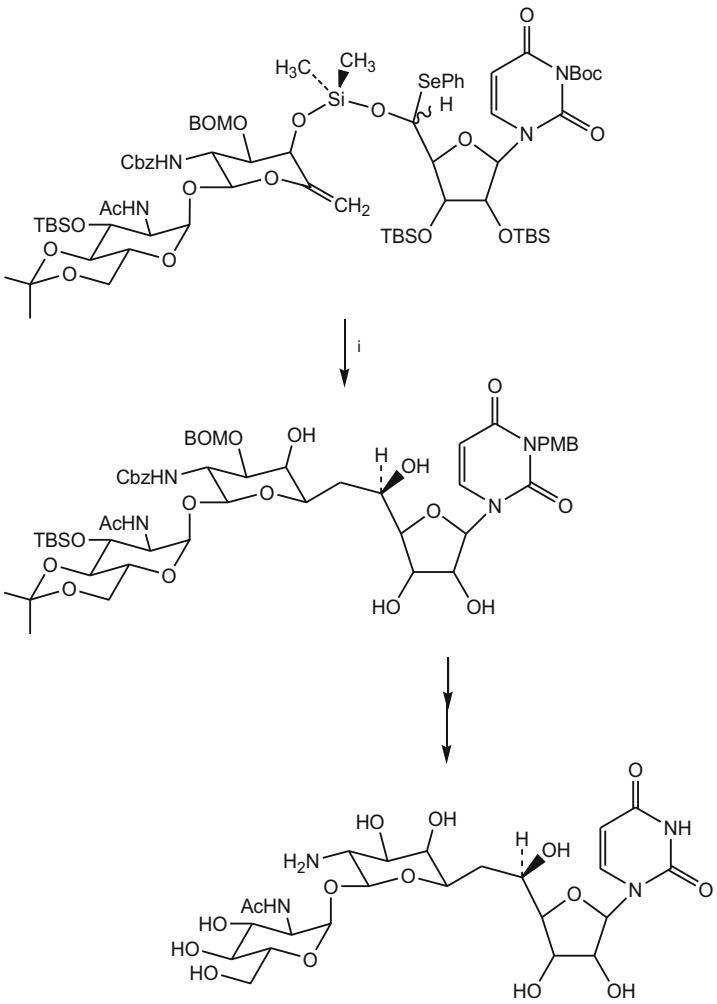


Scheme 4.29 Chemical transformations of capuramycin

4-one intermediate. A series of transformations including *N*-glycoside coupling reaction provided 4-amino-5-cyano-7-[2,3,5-tri-*O*-benzoyl-β-D-ribofuranosy] pyrrolo [2,3-d] pyrimidine that was then converted to the desired tricyclic nucleoside (Scheme 4.33) [72].

4.2 *C*-nucleosides

These modified nucleosides are structurally distinct to their counterparts *N*-nucleosides because of the presence of a C-C linkage instead of C-N between the furanoside and the heterocyclic aglycon. Their source could be either naturally occurring (pyrazomycin, showdomycin, formycin) or synthetic (thiazofurin),

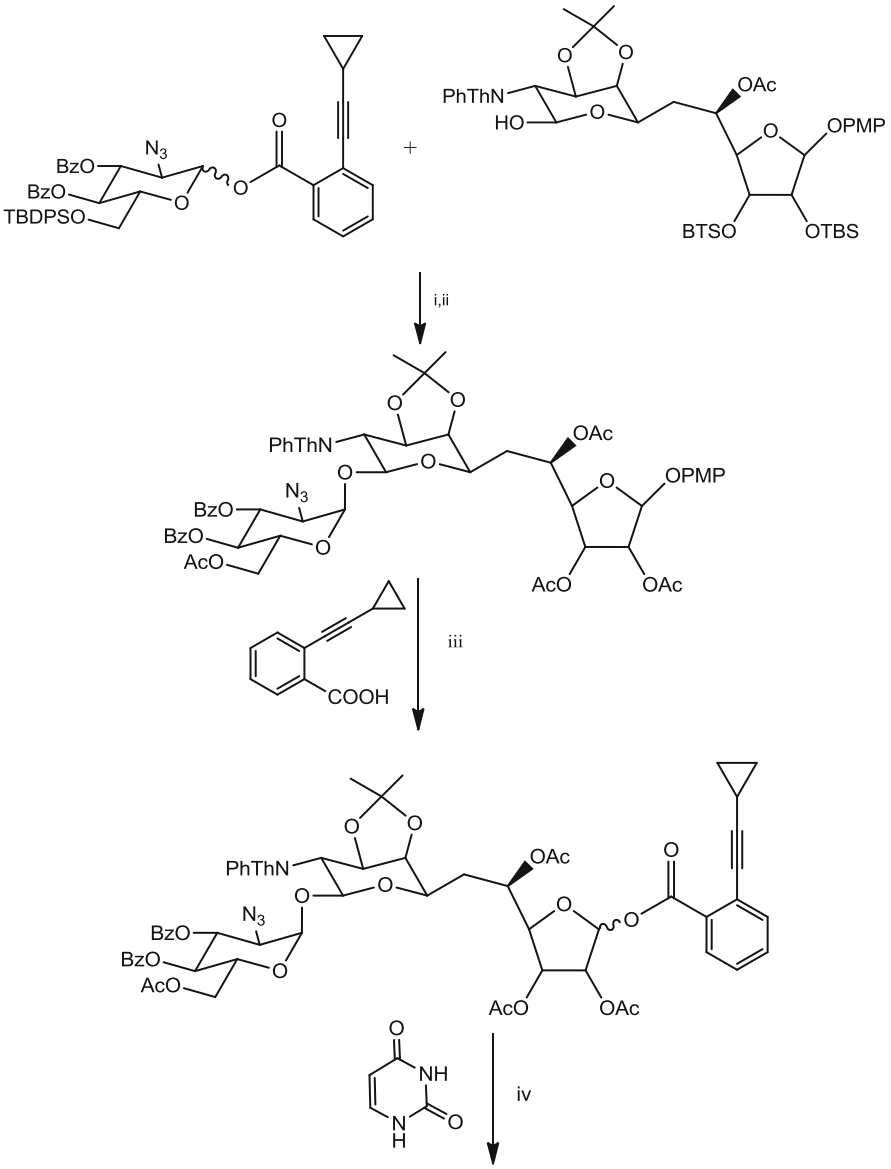


Scheme 4.30 Key step for the synthesis of Tunicamycin antibiotic

having in either case significant antitumor and antiviral activity. Also, some of them have been found in tRNA codons (pseudouridine) and others (tiazofurin and oxazofurin) designed as competitive inhibitor of cofactor nicotinamide adenine dinucleotide (Scheme 4.34).

An early approximation for the preparation of *C*-nucleosides proposed two basic possibilities depending on the nature of the atoms surrounding the C–C bond (Scheme 4.35) [73].

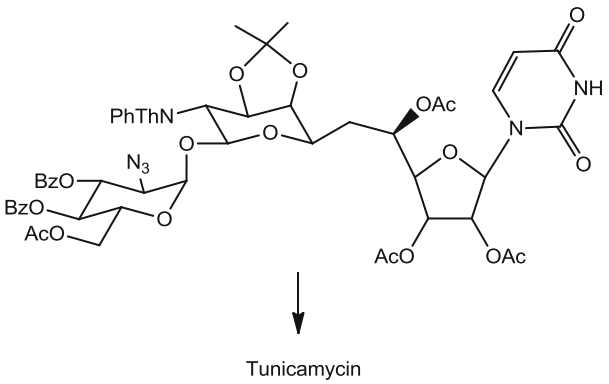
(a) If there is one heteroatom adjacent to the *C*-glycosidic bond, for example tiazofurin, formycin, Pyrazomycin.
(b) If there is no heteroatom adjacent to the *C*-glycosidic bond.



Scheme 4.31 Synthesis of tunicamycins mediated by gold complex catalysis

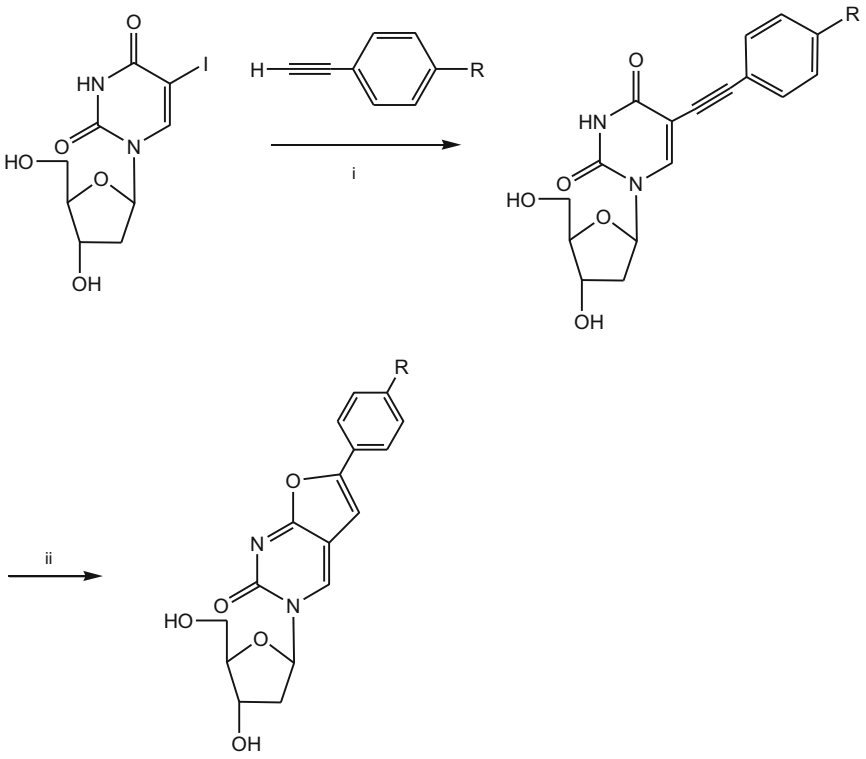
Alternatively other authors consider three general pathways for preparing *C*-nucleosides depending on the precursor employed as starting material [74].

An early synthesis of modified *C*-nucleoside from naturally occurring pseudouridine was carried out via ring opening with ozone to generate intermediate which was treated with thiosemicarbazone to provide 6-azathiopseudouridine. Treatment with iodomethane in acid medium produces the desired *C*-nucleoside as shown in Scheme 4.36 [75].



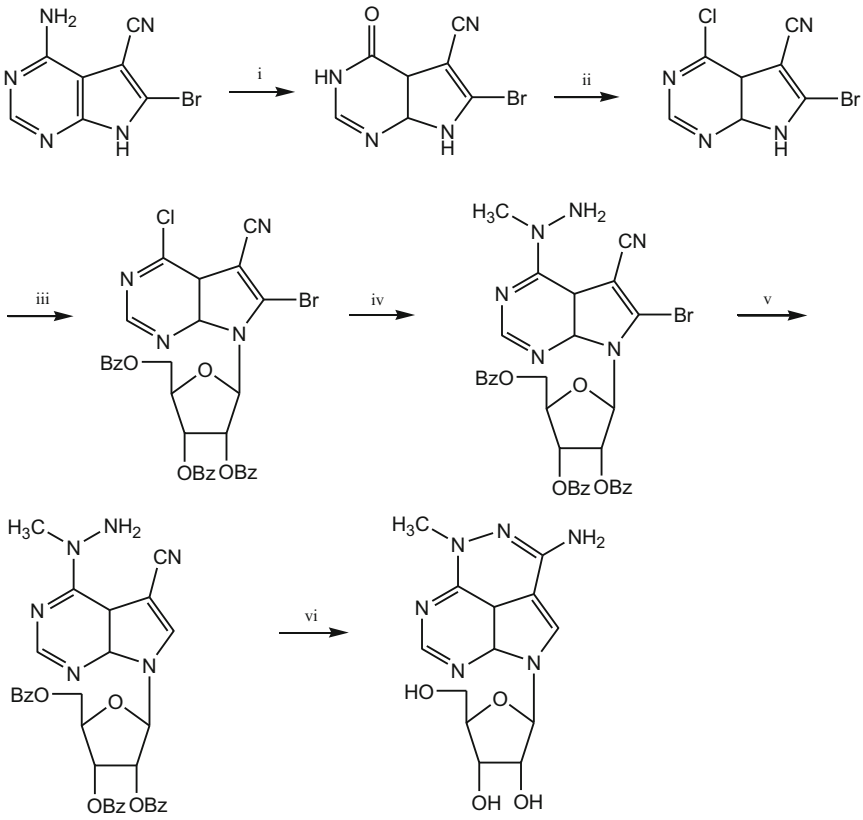
i)[$Ph_3PAuNTf_2$], toluene, AW MS, RT.i) a) HF.Pyr, THF, 60°C. b) Ac_2O, Pyr, DMAP, RT.iii) a) CAN, THF/H_2O, RT. b) EDCl, DMAP, DIPEA, DCM, RT. iv) a) BSTFA, CH_3CN, 50°C. b) [$Ph_3PAuNTf_2$], $ClCH_2CH_2Cl$, RT.

Scheme 4.31 (continued)



i) $Pd(PPh_3)_4$, iPr_2EtN, CuI, DMF, r.t, 19h. ii) Et_3N/MeOH. CuI, Δ, 4h.

Scheme 4.32 Synthesis of bicyclic furano pyrimidine



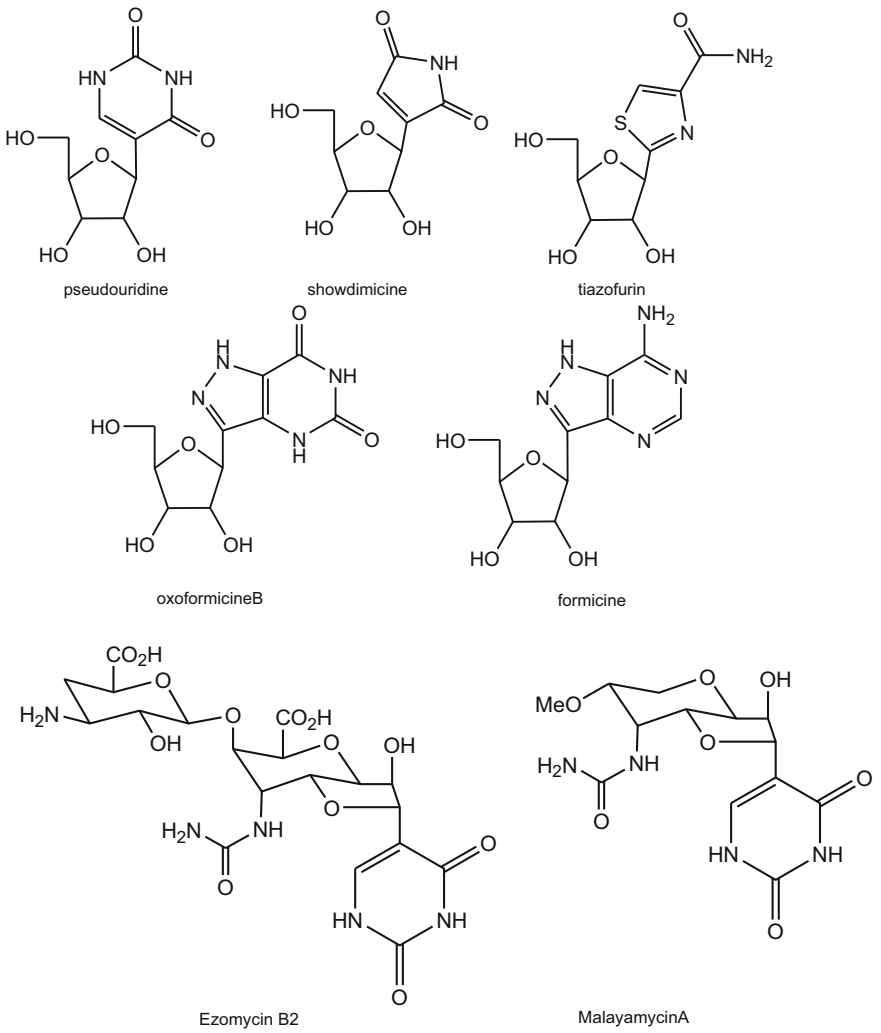
i) $NaNO_2$, AcOH, H_2O, ii) $POCl_3$. iii) BSA, CH_3CN then 1-O-acetyl-2,3,5-tri-O-benzoyl-b-D-ribofuranoside, TMSOTf. iv) NH_2NHCH_3, EtOH, $CHCl_3$. v) HCO_2NH_4, 10% Pd-C, EtOH, reflux. vi) NaOMe, MeOH, reflux.

Scheme 4.33 Synthesis of tricyclic nucleoside Triciribine

The synthesis of the *C*-nucleoside pseudouridine was reported by Asburn and Binkley [76], involving the condensation between 5-*O*-acetyl-2,3-*O*-isopropylidene-D-ribonolactone and 2,4-dibenzyloxypyrimidin-5-il lithium to provide the condensation product which was subjected to hydride reduction and hydrogenolysis to yield pseudouridine (Scheme 4.37).

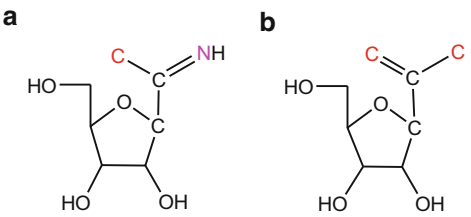
Antitumor *C*-nucleoside tiazofurin was synthesized by Robins et al. [77], from 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl cyanide which undergoes ring closure under conditions described in Scheme 4.38.

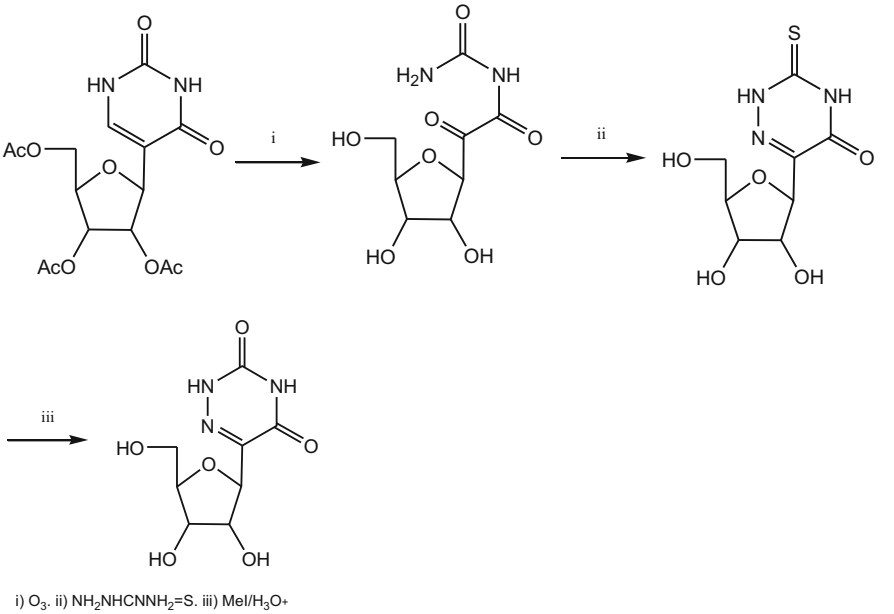
A new report for the synthesis of Tiazofurin is described, avoiding the use of H_2S gas which is unsafe on large-scale production. The synthesis initiate with the preparation of 1-cyano-2,3-*O*-isopropylidene-5-*O*-benzoyl-β-D-ribofuranose which was reacted with cysteine ethyl ester hydrochloride to give thiazoline derivative in 90 %. Further steps including oxidative aromatization under MnO_2 in



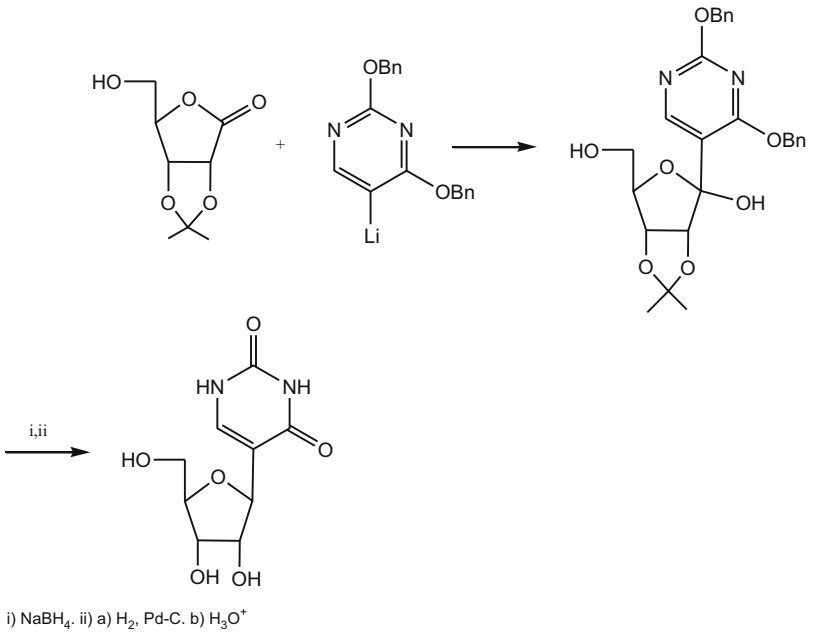
Scheme 4.34 Biologically active *C*-nucleosides

Scheme 4.35 *C*-nucleosides partial representations, with and without heteroatom attached to the *C*-glycosidic bond

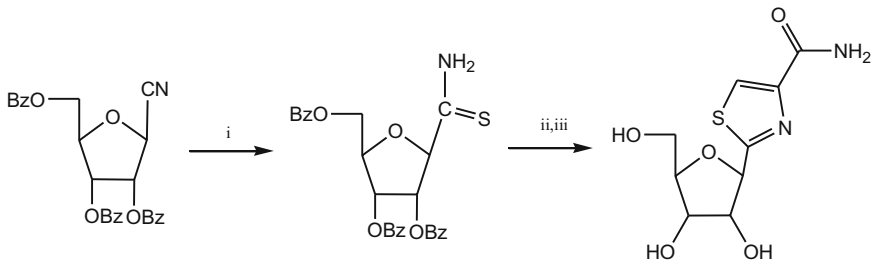




Scheme 4.36 Preparation of 6-azapseudouridine

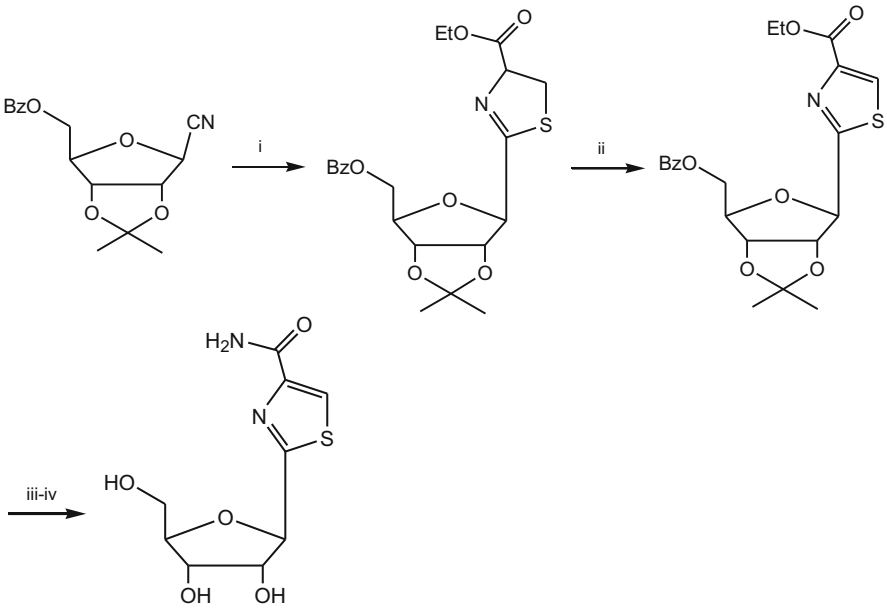


Scheme 4.37 Preparation of pseudouridine



i) H_2S, 4-DMAP. ii) ethylbromopyruvate. iii) NH_3/MeOH.

Scheme 4.38 Synthesis of tiazofurin



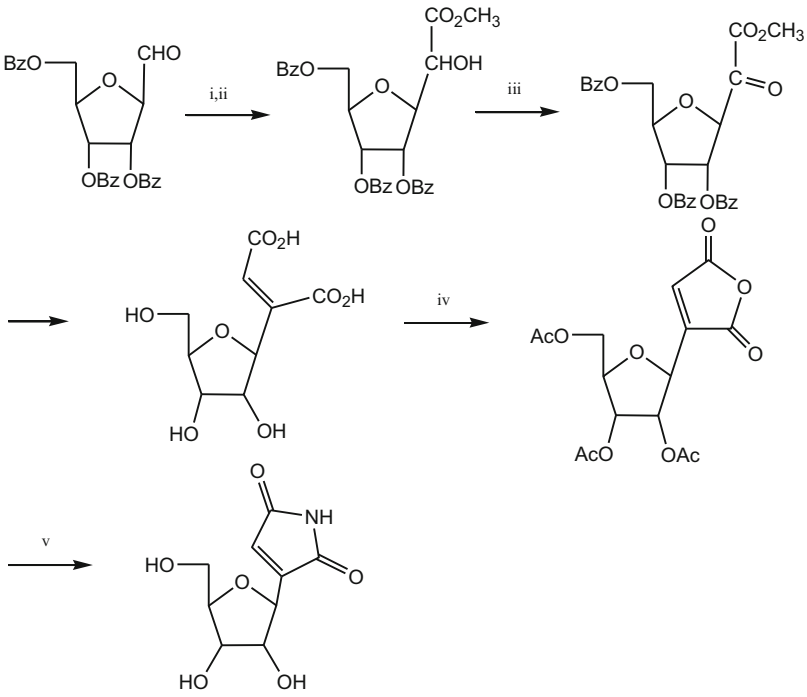
i) Cysteine ether ester hydrochloride/TEA. ii) MnO_2/Ph, reflux. iii) 90% TFA. iv) MeOH/NH_3

Scheme 4.39 A new synthetic methodology for tiazofurin

benzene and acetonide deprotection with iodide in methanol produced the desired *C*-nucleoside (Scheme 4.39) [78].

Another biologically important *C*-nucleoside known as showdomycin was prepared by Trumnlitz and Moffat [79]. The aldehyde used as starting material was converted first to an α-hydroxyacid and then to α-ketoacid. Wittig reaction on this intermediate and Lewis acid catalysis produced ring closure (Scheme 4.40).

Pyrazine riboside derivative was synthesized by treatment of glycine riboside with formaldehyde and cyanide (Strecker conditions) to generate cyanide intermediate as a mixture of isomers. Sulfenylation and sodium methoxide treatment produce the *C*-nucleoside (Scheme 4.41) [80].



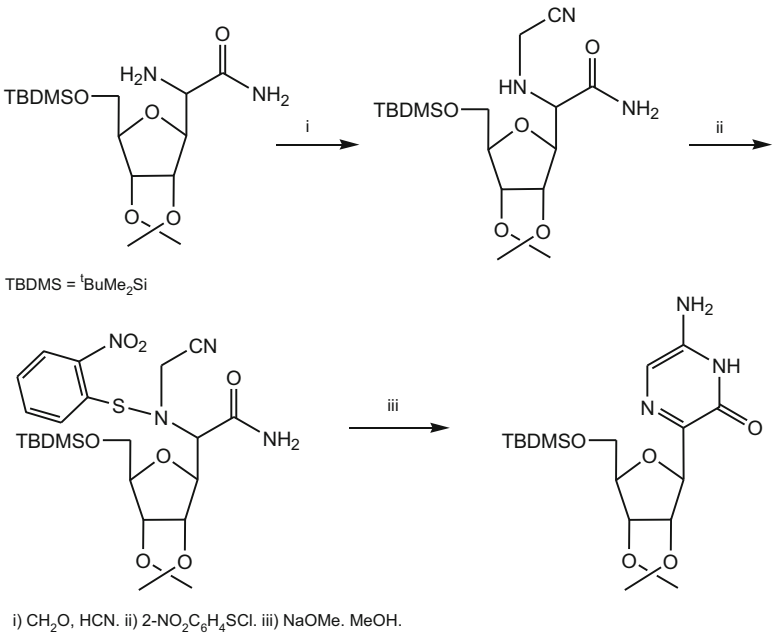
i) NaCN. ii) a) MeOH-H_3O^+. b) Me_2SO-DCC. iii) $Ph_3P{=}CHCONH_2$. iv) Ac_2O. v) a) NH_3. b) EPP. v) H^+.

Scheme 4.40 Preparation of showdomycin

Analogs of antiviral *C*-nucleoside Formycin have been synthesized by using the palladium-mediated glycosidic reaction between the furanoid glycal and the iodinated heterocycle. Similar conditions were used for preparing the pyrimidine analogs (Scheme 4.42) [81].

Radical cyclization of ribo-phenylselenoglycoside tethered with propargyl moieties on C-5 hydroxyl group provided cyclic intermediates potentially useful for the synthesis of *C*-nucleoside derivatives. Propargyl intermediate was prepared from ribo-phenylselenoglycoside via two-step sequence and then under radical reaction conditions (Bu_3SnH/AIBN) transformed to the cyclic intermediates in high yields. Further ring opening produce aldehyde intermediate which was subjected to coupling reaction with 1,2-phenylenediamine to produce the pyrazine *C*-glycoside (Scheme 4.43) [82].

Polyhalogenated quinoline *C*-nucleosides were synthesized as potential antiviral agents. The key step reaction for quinolin-2-one ring formation consisted in the condensation between 2-aminophenone allose derivative and keteneylidene(triphenyl)-phosphorane in benzene under reflux to provide the desired 6,7-dichloroquinolin-2-one nucleoside in 50 % yield (Scheme 4.44) [83].



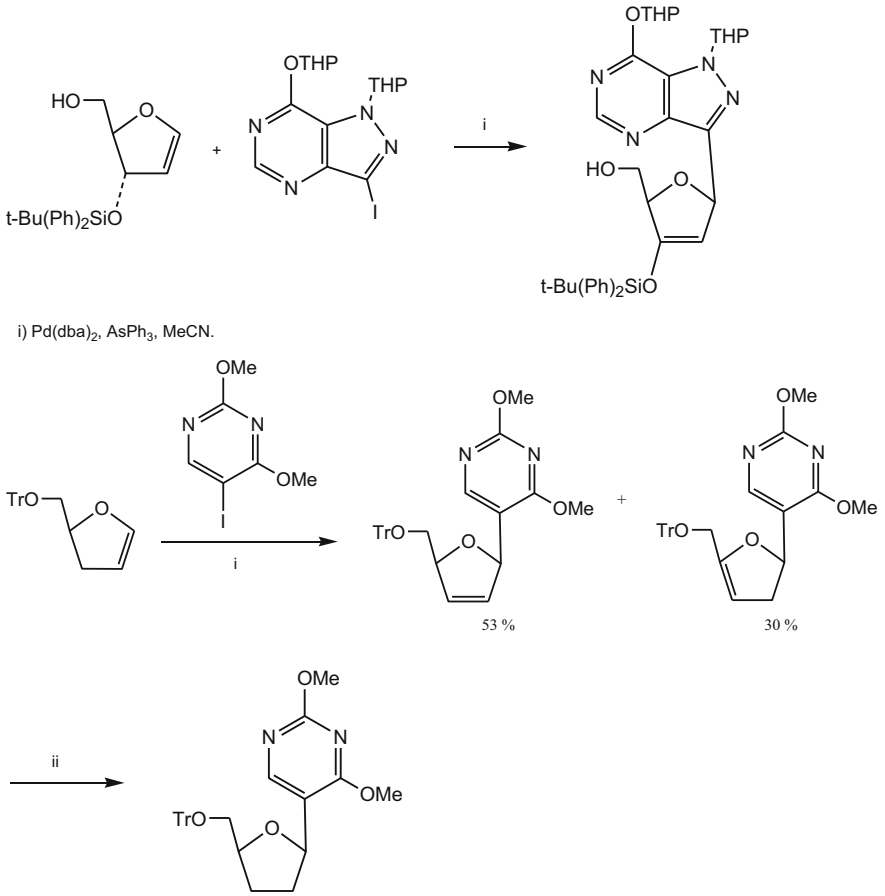
Scheme 4.41 Synthesis of *C*-nucleoside by pyrazine ring formation

The novel bicyclic *C*-nucleoside malayamycin A from *Streptomyces malaysiensis* was elegantly synthesized from D-ribonolactone which was transformed to the target molecule according to the pathway indicated in Scheme 4.45 [84].

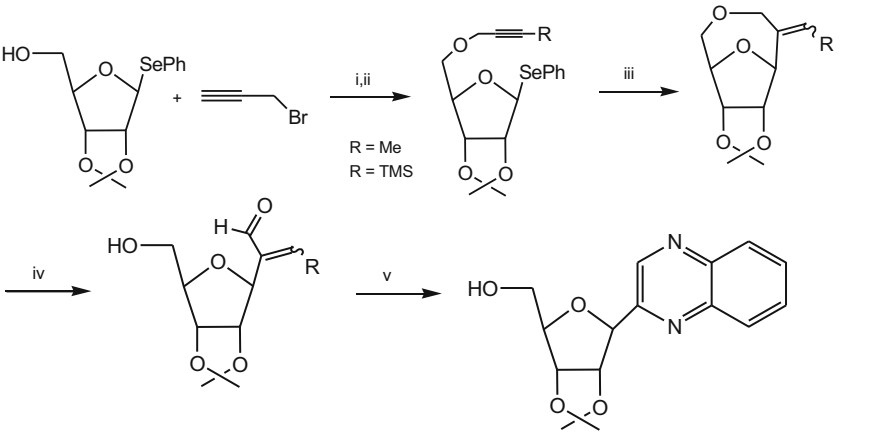
4.3 Carbocyclic Nucleosides

This class of modified nucleosides in which the furanose ring has been replaced by a cycloalkane ring (mainly cyclopentane) has been prepared by chemical or enzymatic methods. Besides their potent antitumor and antiviral activity for some of them, they have also shown high resistance to phosphorylases.

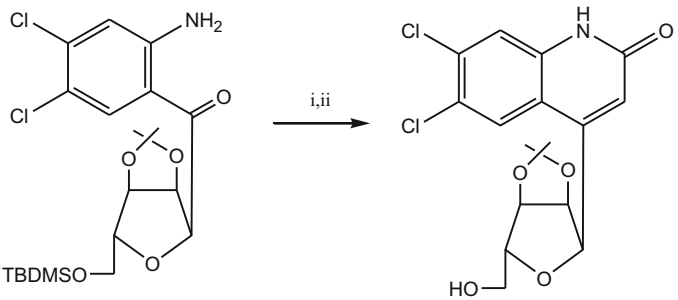
The use of enzymes particularly lipases for protections and deprotections is an important strategy for preparing carbocyclic nucleosides. This approach has been advantageous especially for the resolution of enantiomeric forms, leading to high enantiomeric purity. Constrained three [85] and four [86] member ring carbocyclic nucleosides have been obtained by applying chemoenzymatic methodologies involving lipase for enantiomeric resolution and stereoselective deprotections. In the case of more abundant five member rings the use of lipases for enzymatic resolution and regioselective deprotections have been under intense study. Special attention has been paid to cyclopentenyl diacetates which have been used as building blocks for the preparation of important carbocyclic nucleosides such as Neplanocin and Aristeromycin. To achieve this goal, the hydrolase enzyme acetyl-cholinesterase



Scheme 4.42 Palladium-mediated synthesis of *C*-nucleoside formycin analogs

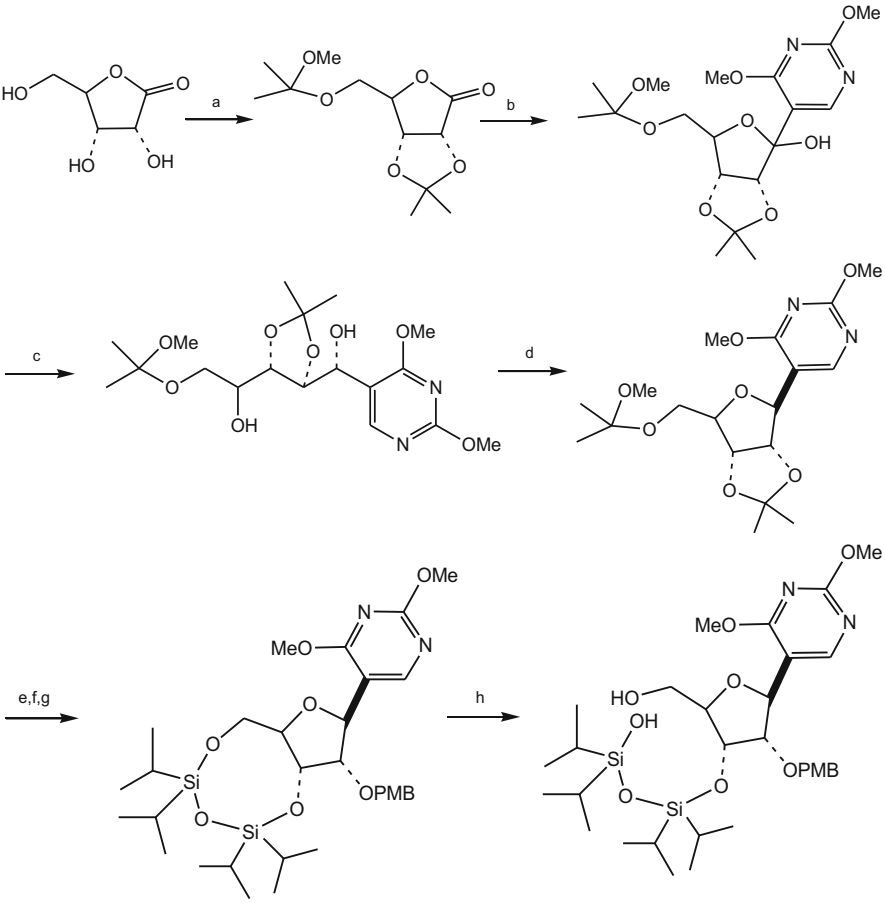


Scheme 4.43 *C*-nucleoside derivative formation via radical cyclization

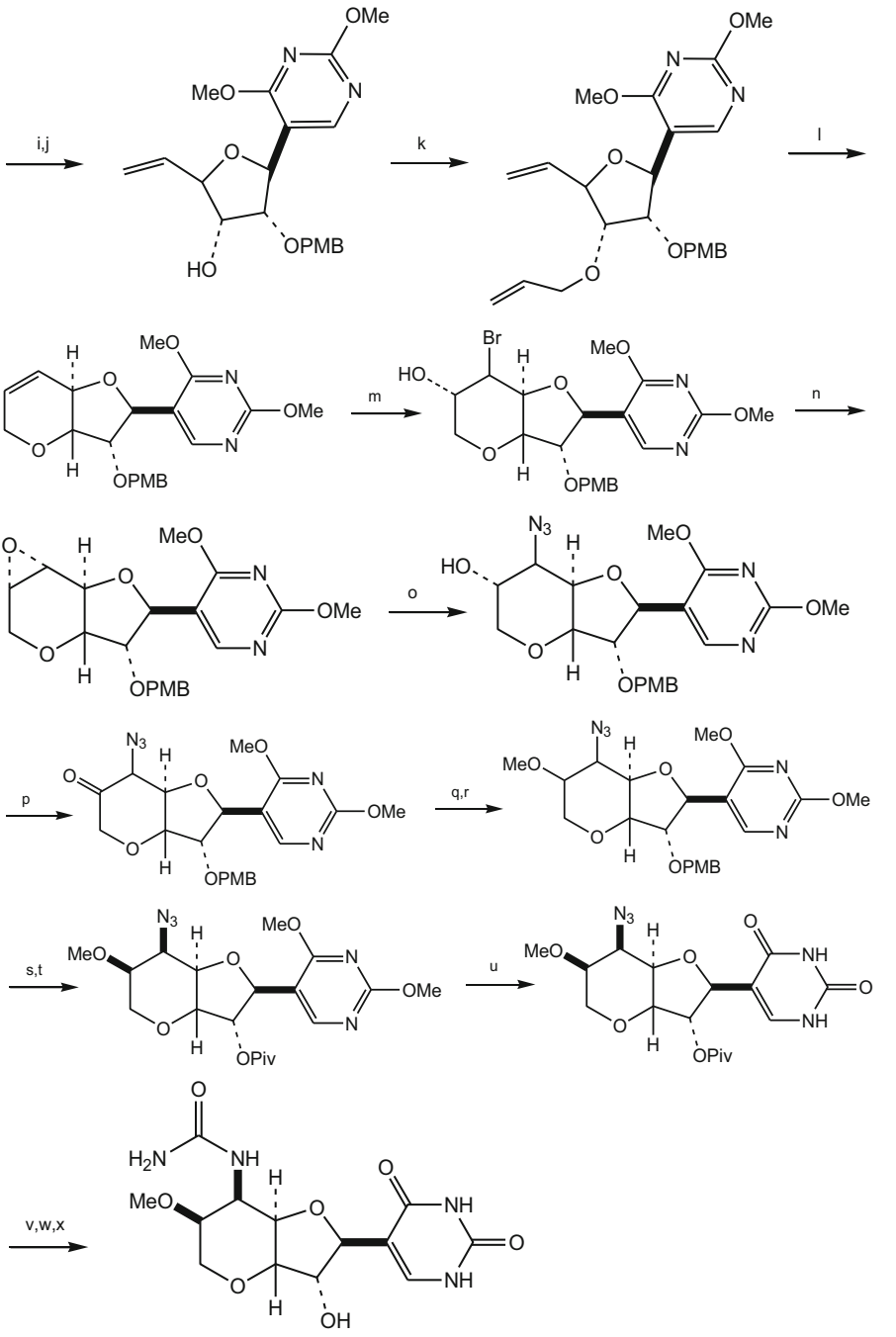


i) $Ph_3P{=}C{=}C{=}O$, PhH, reflux. ii) TBAF, THF, rt.

Scheme 4.44 Quinolin-2-one *C*-nucleoside formation via Wittig reaction



Scheme 4.45 Total synthesis of *C*-nucleoside Malayamycin A

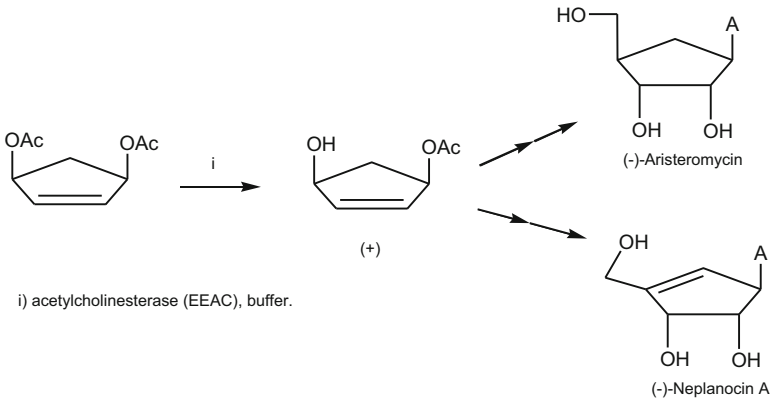


Scheme 4.45 (continued)

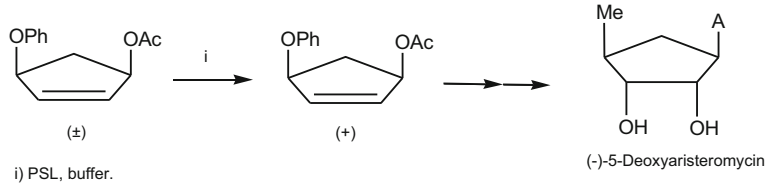
(EEAC) [86] showed high efficiency for obtaining the desired enantiomer (1R,4S)-4-hydroxy-2-cyclopentenyl derivative in enantiomeric excess (ee) up to 96% (Scheme 4.46) [87–89].

Racemic cyclopentenyl derivatives have been used as starting material in the preparation of the antiviral carbocyclic nucleoside (−)-5′-deoxyaristeromycin. The key step reaction was the enzymatic resolution with *Pseudomonas* sp. lipase (PSL) of the racemic mixture providing the (+)-enantiomer which was transformed chemically to the desired carbocyclic nucleoside (Scheme 4.47).

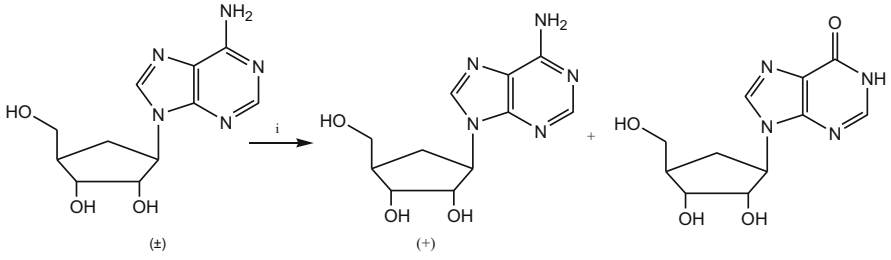
The separation of racemic carbocyclic nucleosides by enzymatic means has been reported as an alternative approach. Thus, racemic aristeromycin was treated with adenosine deaminase (ADA) to give (−)-carbocyclic inosine and pure dextrorotatory enantiomer (Scheme 4.48) [90].



Scheme 4.46 Enantiomeric resolution of prochiral cyclopentene diacetate



Scheme 4.47 Enzymatic resolution of racemic cyclopentene building blocks



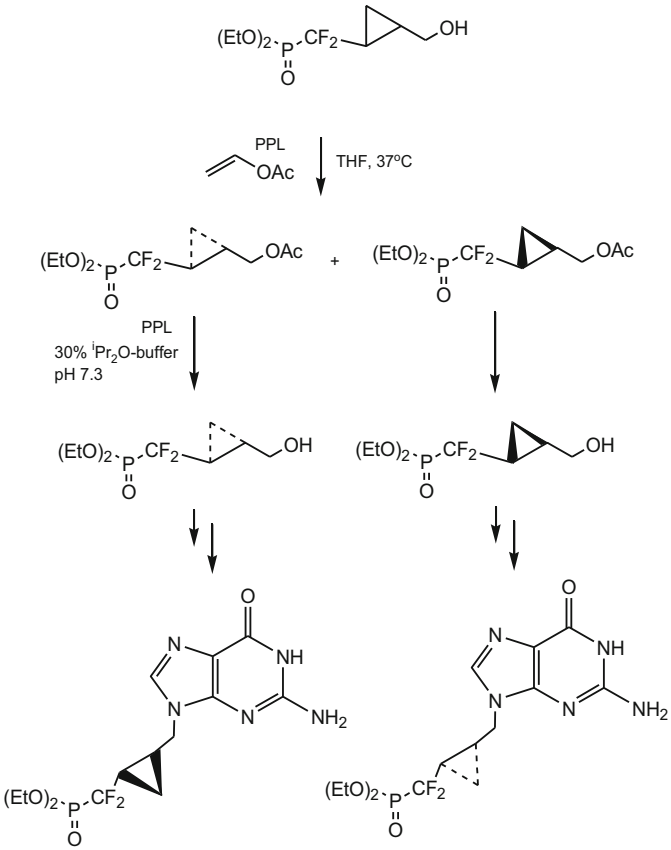
Scheme 4.48 Enzymatic resolution of carbocyclic nucleoside

4.3.1 Cyclopropane Carbocyclic Nucleosides

Conformationally constrained cyclopropane nucleosides have been prepared following a chemoenzymatic approach [85]. Thus, the racemic resolution of *trans*-1-(diethoxyphosphyl)difluoromethyl-2-hydroxymethylcyclopropane followed by acetate hydrolysis was carried out with porcine pancreas lipase (PPL) to yield (+)- and (−)-cyclopropanes in high enantiomeric excess. Further transformation lead to the preparation of the target cyclopropane nucleoside (Scheme 4.49).

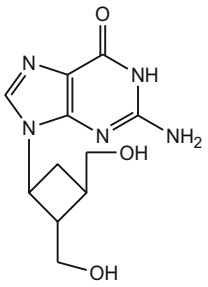
4.3.2 Cyclobutane Carbocyclic Nucleosides

Lubocavir is a synthetic potent inhibitor of DNA polymerase, active against cytomegalovirus [91] (Scheme 4.50).

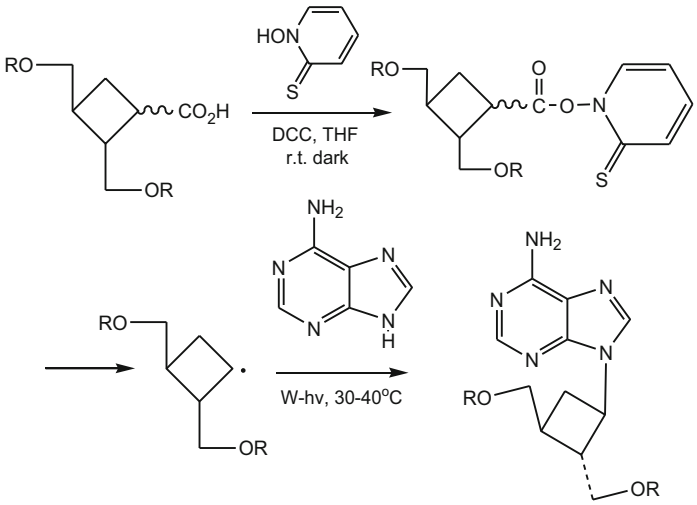


Scheme 4.49 Chemoenzymatic syntheses of cyclopropane nucleosides

Scheme 4.50 Structures of carboxetan carbocyclic nucleoside



Lobucavir



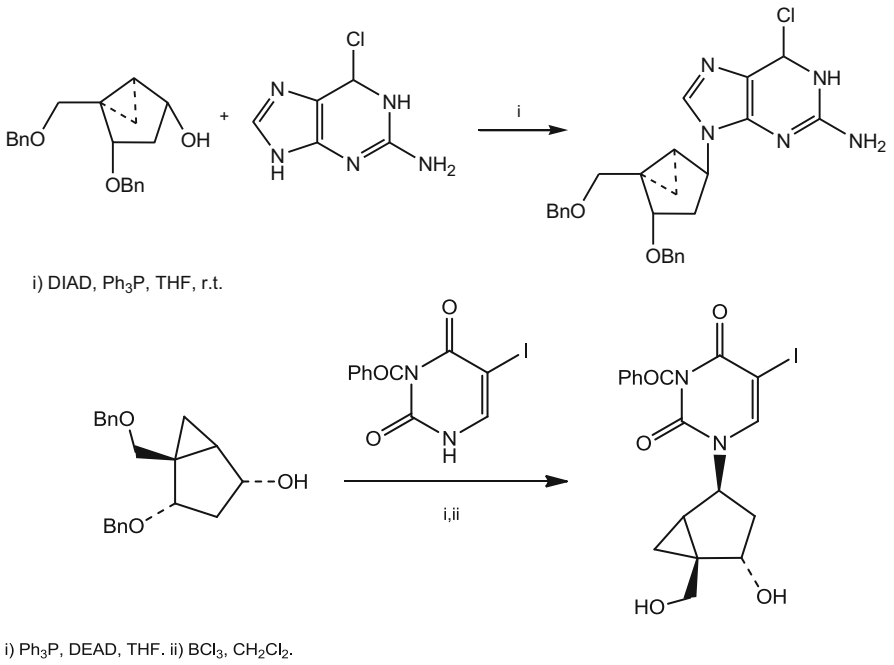
Scheme 4.51 The Barton decarboxylation method for the preparation of carbocyclic *C*-nucleosides

The carbocyclic four-membered *C*-nucleoside cyclobut-A was prepared following the Barton decarboxylation method. The method is based on the reaction between carboxylic acids and heteroaromatic compounds (Scheme 4.51) [92].

Other carbocyclic oxetanocin analogs have been prepared from oxetanocin A [93] 3,3-diethoxy-1,2-cyclobutanedicarboxylate [94], and enantiomeric cyclobutanone intermediates [95] as starting materials.

4.3.3 Cyclopentane Carbocyclic Nucleosides

The Mitsunobu reaction has become a valuable alternative approach for preparing cyclopentane carbocyclic nucleosides. This has been demonstrated in the preparation of conformationally locked carbocyclic AZT triphosphate analogs under these



Scheme 4.52 Synthesis of conformationally locked carbocyclic purine and pyrimidines under the Mitsunobu approach

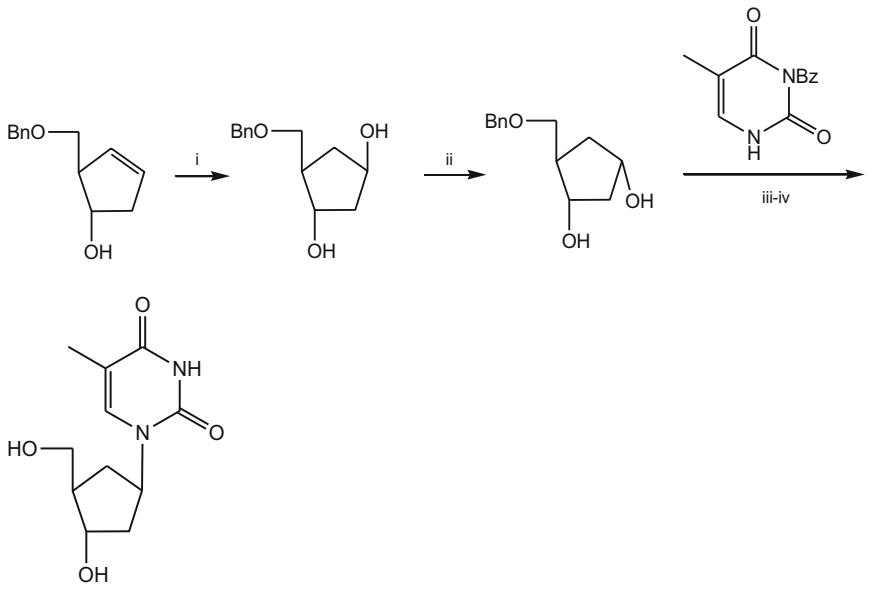
versatile conditions [96]. The standard procedure usually takes place with diethyl or diisipropylazocarboxylate (DEAD or DIAD) with triphenylphosphine $(Ph)_3P$ in THF to yield carbocyclic purines or pyrimidines nucleosides in high yield (Scheme 4.52) [97].

Another example on the applicability of this method was observed in the preparation of the carbocyclic thymidine nucleoside. It is worth mentioning that the desired stereochemistry of the hydroxyl group is obtained also through the Mitsunobu reaction (Scheme 4.53) [98].

4.3.4 Palladium Mediated

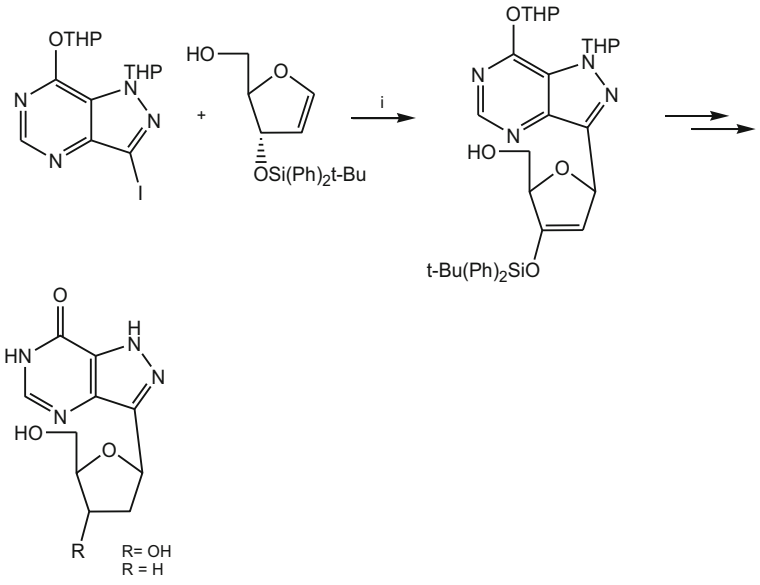
Based on the widespread palladium-coupling methodologies, several dideoxy, carbocyclic and *C*-nucleosides have been efficiently prepared. For instance the antiviral *C*-nucleosides 2′-deoxyformycin B was prepared by condensation reaction between the heterocycle iodide intermediate and the glycal, under $Pd(dba)_2$ as palladium catalyst in 62 % yield (Scheme 4.54) [99].

Solid phase synthesis of carbovir analogs under palladium catalysis was recently reported [100]. The carbocyclic derivative was linked to the Wang resin and then coupled with chloropurines under Pd(0) catalyst (Scheme 4.55).



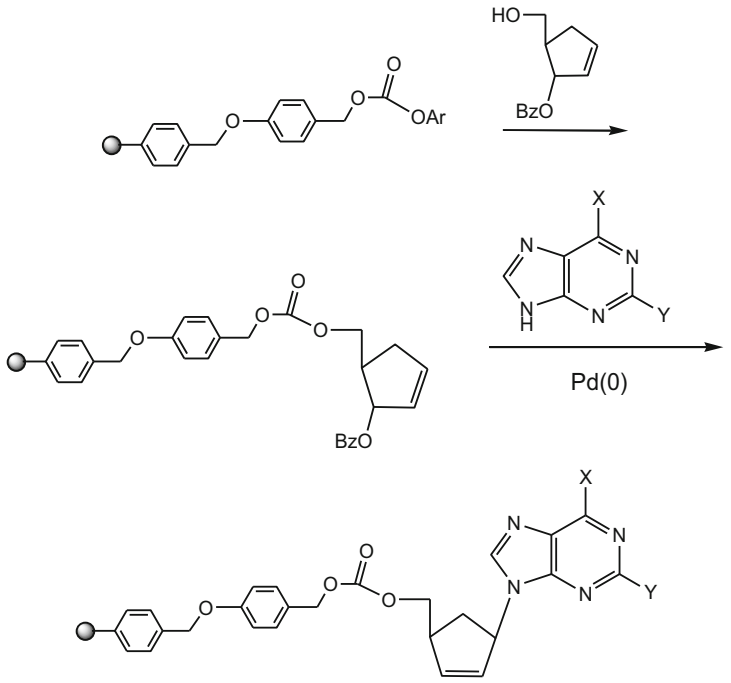
i) NaH, BnBr. ii) 9-BBN, H_2O_2/NaOH, 87%. iii) PPh_3, DIAD. iv) a) NaOH/MeOH. b) H_2, Pd/C, 90%

Scheme 4.53 The Mitsunobu reaction for preparation of the carbocyclic thymidine nucleoside

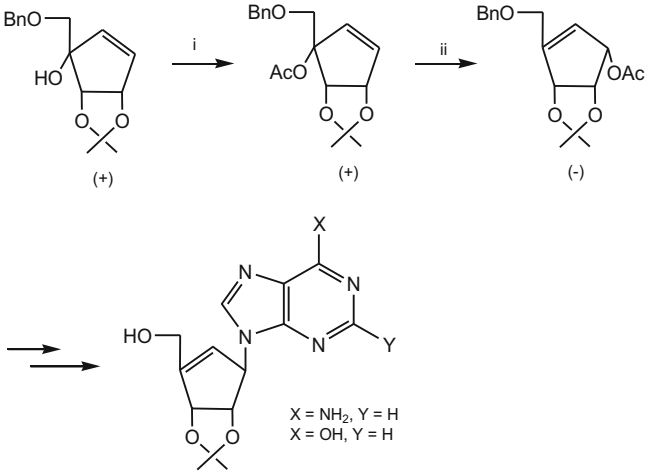


i) $Pd(dba)_2$, $AsPh_3$, CH_3CN.

Scheme 4.54 Palladium-mediated 2′-deoxyformycin B and 2′,3′-dideoxyformycin B

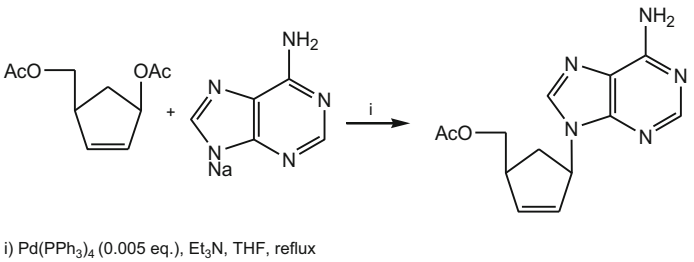


Scheme 4.55 Solid-phase synthesis of carbocyclic nucleosides under palladium catalysis

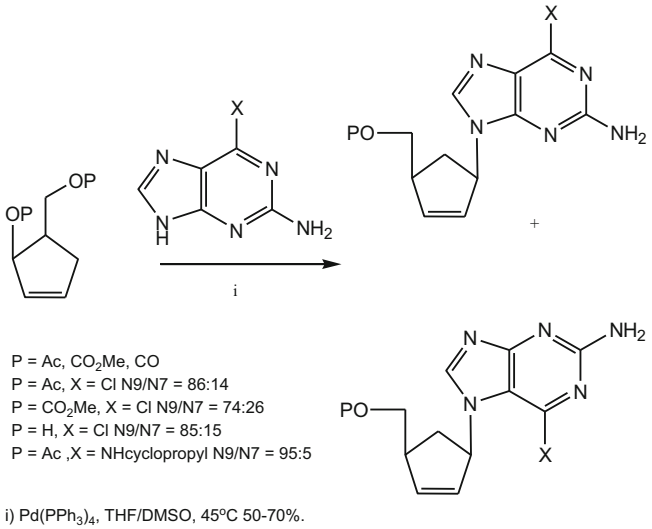


Scheme 4.56 Tsuji-Trost reaction for the preparation of neplanocin A

The Tsuji-Trost approach was used to prepare (−)-neplanocin A and its analog [101]. This synthesis proceeds via an allylic rearrangement of the hydroxyl group from the (+)-allylic alcohol to the (−)-allylic acetate (Scheme 4.56).



Scheme 4.57 Palladium catalyzed synthesis of aristeromycin

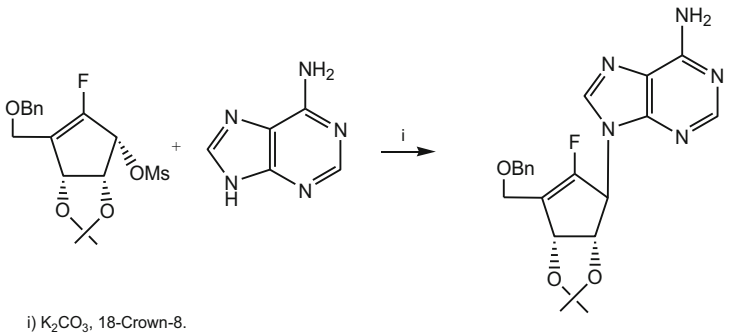


Scheme 4.58 Palladium-catalyzed coupling with purine base

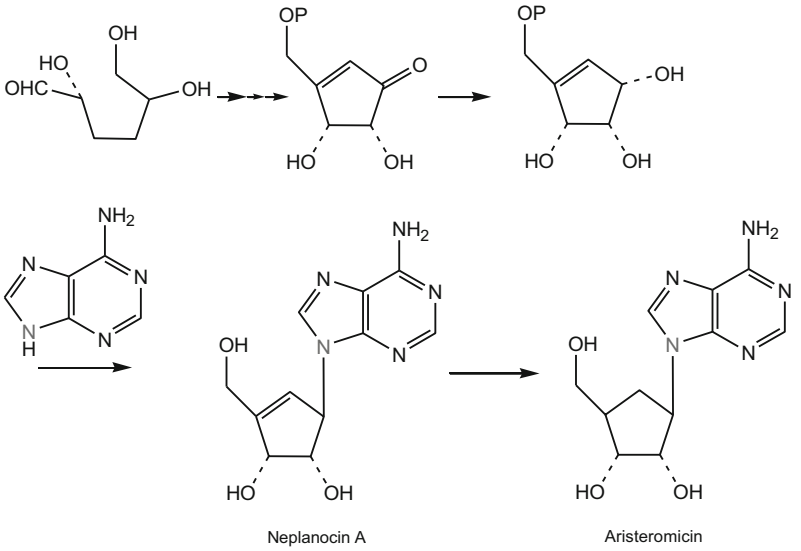
Carbocyclic nucleoside aristeromycin with antitumor and antiviral activity was prepared by condensation of the carbocyclic diacetate intermediate with the sodium salt of adenosine base under Pd(0) in 75 % yield (Scheme 4.57) [102].

Palladium mediated coupling of purine base with carbocyclic acetates, carbonates or benzoates lead to a mixture of N-7 and N-9 isomers. The regioselectivity of purine alkylations depends on the size and nature of the ligands, the most typical being Ph_3P, BINAP, $P(OMe)_3$, $P(OiPr)_3$, $P(OPh)_3$ (Scheme 4.58) [103].

Another straightforward methodology for preparing carbocyclic nucleosides involves the direct condensation of mesylated carbocyclic intermediate with the heterocyclic base in the presence of potassium carbonate and crown ethers as coupling conditions (Scheme 4.59) [104].



Scheme 4.59 Preparation of carbocyclic nucleosides with mesylated carbocyclic intermediates

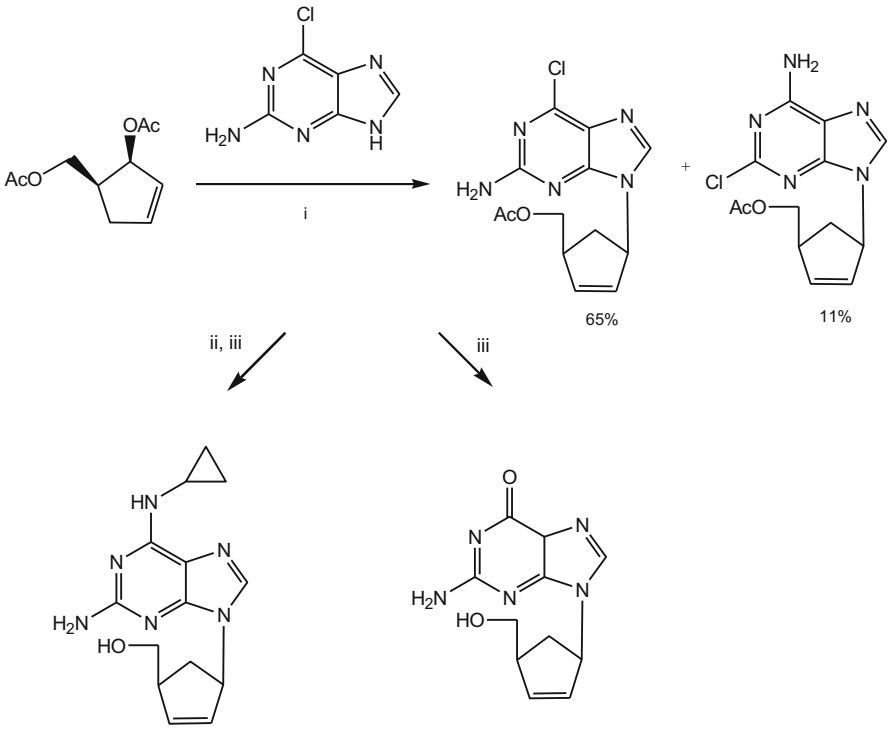


Scheme 4.60 Biosynthetic pathway of neplanocin A and aristeromycin

4.3.5 Enzymatic Synthesis

Likewise, carbocyclic nucleosides aristeromycin and neplanocin A can be biosynthetically prepared by using a mutant strain of *S. citricolor* as it is observed in Scheme 4.60.

The cyclopropylamino carbocyclic nucleosides (–)-abacavir is a potent anti-HIV with promising results on clinical trials [105]. An improved synthesis has been described by Crimmins et al. [106], involving the treatment of key carbocyclic 2-amino-6-chloropurine intermediate with cyclopropylamine producing Abacavir along its parent anti-HIV carbocyclic nucleoside (–)-Carbovir (Scheme 4.61).



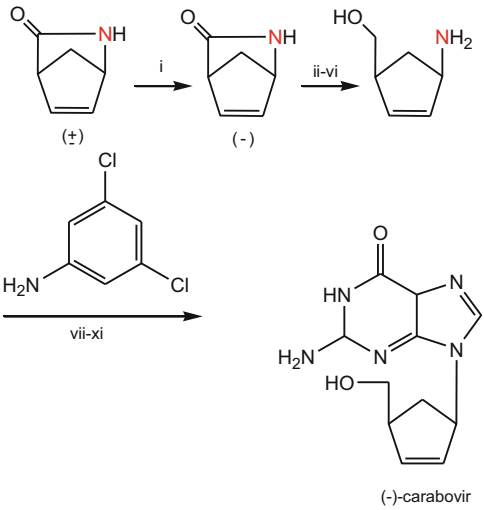
i) NaH, $Pd(PPh_3)_4$, 1:1 THF:DMSO. ii) cyclopropylamine, EtOH. iii) NaOH, H_2O.

Scheme 4.61 Synthesis of anti-HIV (−)-abacavir and (−)-carbovir

4.3.5.1 Base Ring Formation

Another useful strategy used for preparing carbocyclic nucleosides involves the use of intermediates in which the amino group is already attached to the sugar moiety and once the coupling reaction is achieved, a ring closure process takes place to generate the expected nucleoside. According to this procedure Roberts et al. [107] prepared the potent antiviral inhibitor (−)-carbovir which posses similar activity than AZT against HIV in MT-4 cells. Thus, the starting material (±)-2-azabiciclo [2.2.1] hept-5-en-3-one was submitted to microbial treatment with *Pseudomonas solanacearum* to provide enantiomerically pure (−) isomer. The enantiomerically pure carbocyclic amine was then conjugated to 2-amino-4,6-dichloropyrimidine to produce the carbocyclic precursor which was ultimately cyclized to provide the desired (−)-carbovir (Scheme 4.62).

Antileukemia carboxylic nucleoside Neplanocin A has been synthesized by Marquez et al., using the ring closure approach mentioned above. Thus, condensation of pyrimidine intermediate with isopropylideneaminocyclopentenediol furnished an



i) P.solanacearum NCIB 40249. ii HCl-H_2O. iii $(MeO)_2CMe_2$. iv) Ac_2O/Py. v) Ca $(BH_4)_2$/THF. vi) HCl-H_2O/EtOH. vii) PrNEt, nBuOH. viii) 4-Cl-$C_6H_4N_2$+Cl--AcOH, AcONa/H_2O. ix) Zn,AcOH/EtOH-H_2O. x) $(EtO)_3CH$/HCl. xi) NaOH/H_2O.

Scheme 4.62 Synthesis of (−)-carbovir

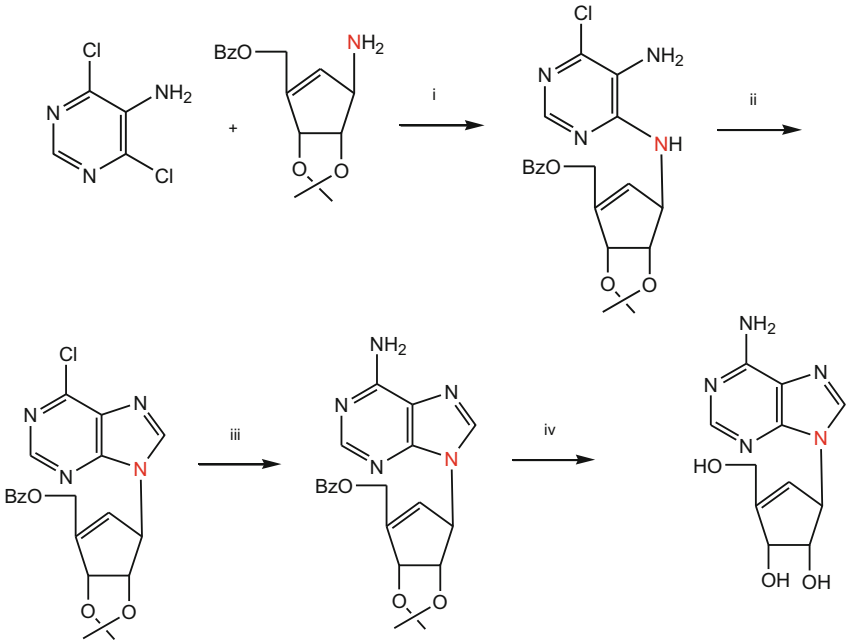
intermediate which was further cyclized to the purine base with triethylorthoformate. Final conversion to adenine ring with ammonia and protecting group removal gave rise to neplanocin A (Scheme 4.63) [108].

Likewise, this procedure was applied for the preparation of the close related pyrimidine analog by condensation of the previous carbocyclic amine with the unsaturated ether to produce the pyrimidine precursor who was transformed to thiopyrimidine and then to carbocyclic cytosine as it can be observed in Scheme 4.64. This compound has been found to be active against leukemia type L1210 in vivo [109].

An antiviral carbocyclic purine nucleoside was also reported [110] by following a ring closure step for purine formation. Condensation between pyrimidine intermediate and carbocyclic amine provided condensation product which is activated with diazonium salt for amino introduction. Ring closure was achieved with triethyl orthoformate in acid medium (Scheme 4.65).

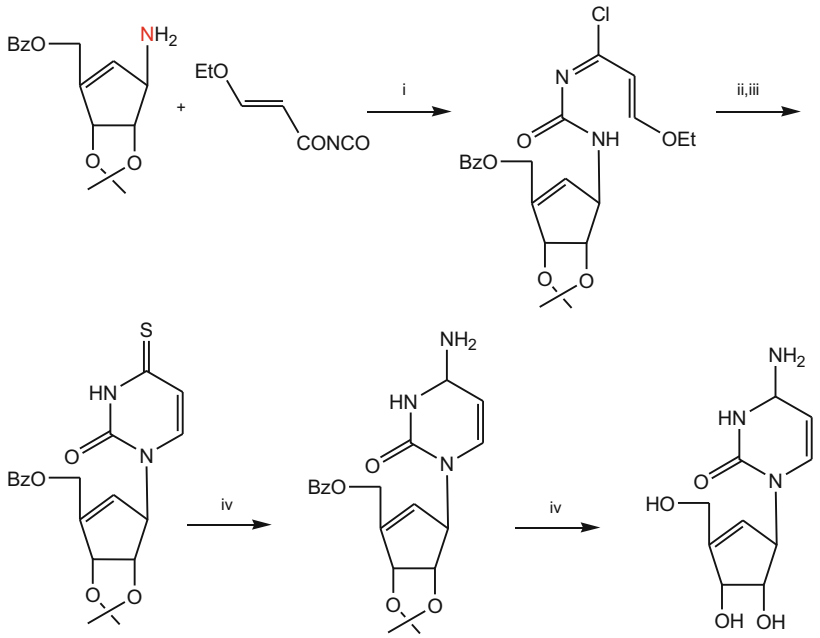
4.3.6 Carbocyclic C*-nucleosides*

This class of *C*-nucleosides in which a methylene group replaces the furan oxygen ring has not shown significant biological activity so far; however, there is an interest to synthesize *C*-nucleoside with natural heterocycle moieties in a stereocontrolled fashion. A recent stereocontrolled synthesis of carbocyclic *C*-nucleosides has been



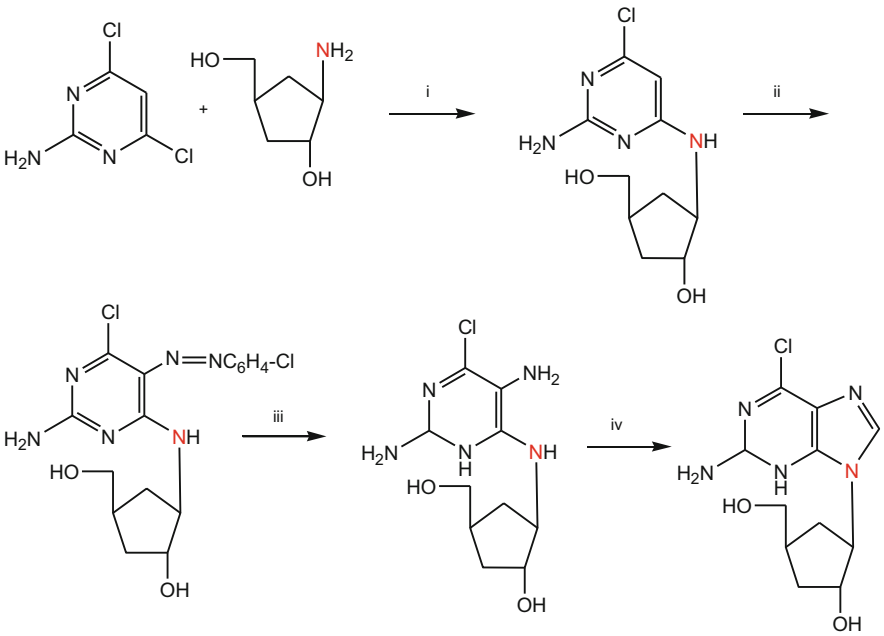
i) EtN_3/EtOH. ii) $HC(OEt)_3$, Ac_2O. ii) NH_3/MeOH. iii) BCl_3/CH_2Cl_2-MeOH

Scheme 4.63 Synthesis of neplanocin A



i) PhH. ii) DMF, NH_4OH. iii) Lawesson. iv) NH_3liq. v) a) BCl_3/CH_2Cl_2-MeOH. b) DowexH^+

Scheme 4.64 Synthesis of carbocyclic pyrimidine nucleoside



i) Et_3N/EtOH. ii) 4-Cl-$C_6H_4N_2^+Cl^-$, Na_2CO_3, AcOH/H_2O. iii) Zn/AcOH. iv) $CH(OEt)_3$-HCl/DMF.

Scheme 4.65 Ring closure approach for preparation of carbocyclic purine

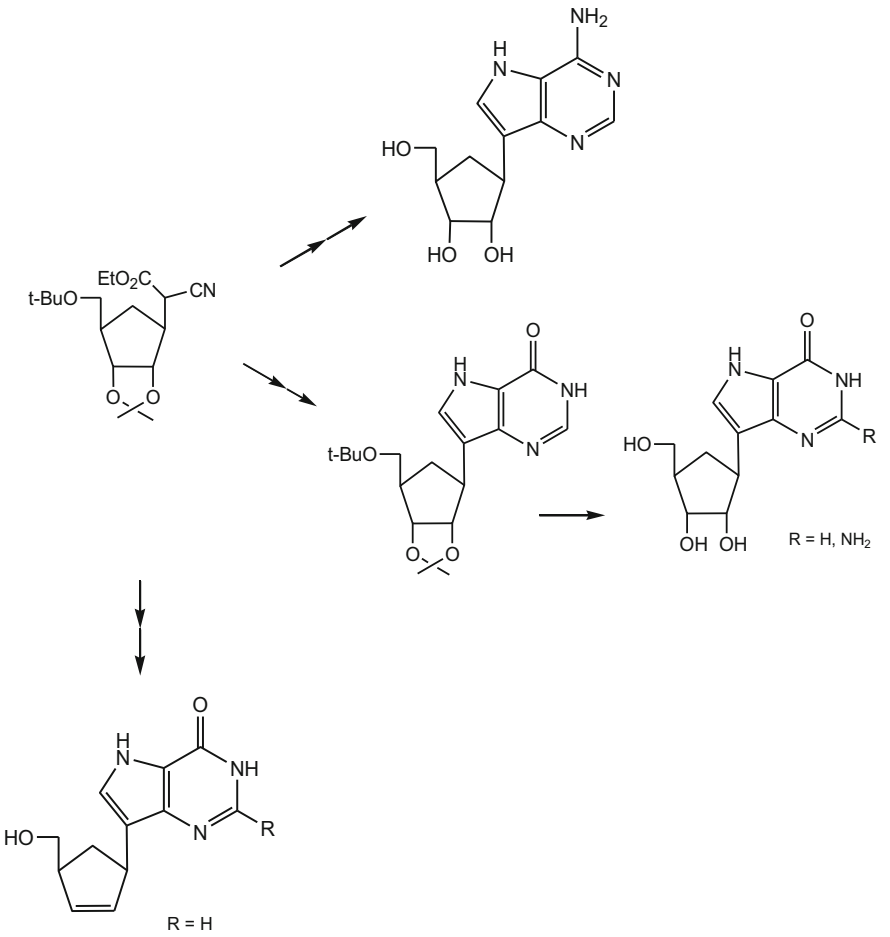
proposed involving as key starting material the cyano carbocyclic intermediate which was condensed to 9-deazapurine to produce saturated and unsaturated carbocyclic 9-deazapurine nucleosides (Scheme 4.66) [111].

4.4 Acyclic Nucleosides

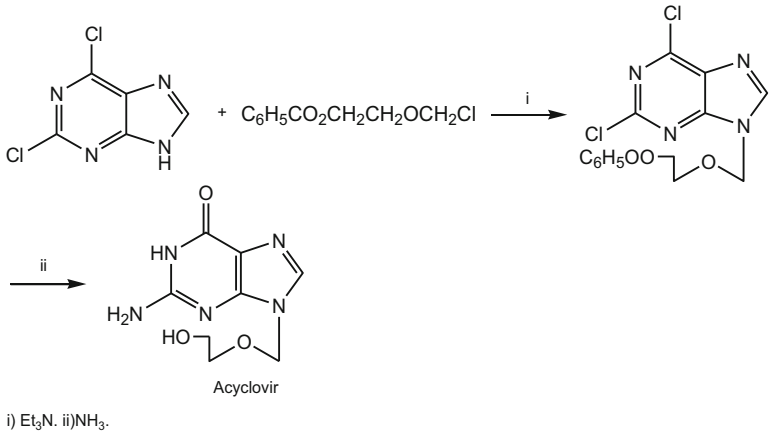
Since the discovery of acyclovir as an anti-herpes drug, important efforts have been made toward the synthesis of analogs of acyclovir and other acyclic nucleosides. A comprehensive review made by Chu and Cutler [112] summarizes the major achievements carried out for preparing acyclonucleosides defined as those heterocyclic compounds containing one or more hydroxyl groups on the alkyl side chain.

At least three representative synthesis of acyclovir have been made, the first by Schaeffer et al. [113] involving a condensation reaction of dichloropurine with ether chloride intermediate, and further purine transformation to generate 9-(2-hydroxyethoxymethyl)guanine (acyclovir) (Scheme 4.67).

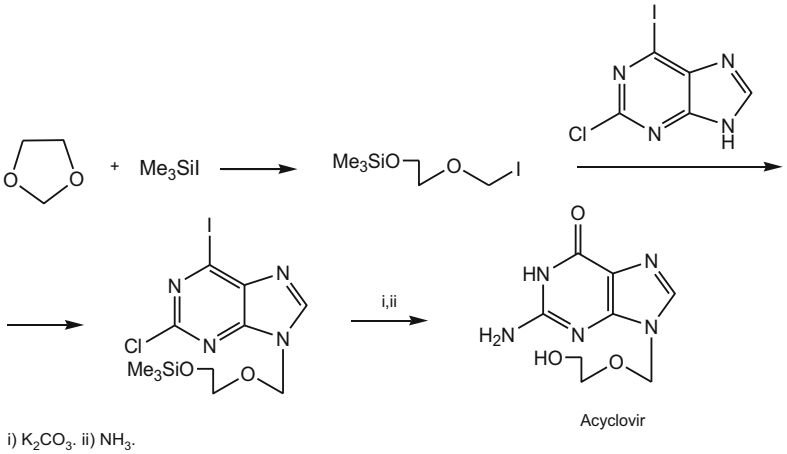
An improved version introduced by Barrio et al. [114, 115] consists in the initial reaction of 1,3-dioxolane with trimethylsilyl iodide to produce the side chain which was then condensed with the halogenated purine, to yield after hydrolysis and ammonolysis the target acyclovir (Scheme 4.68).



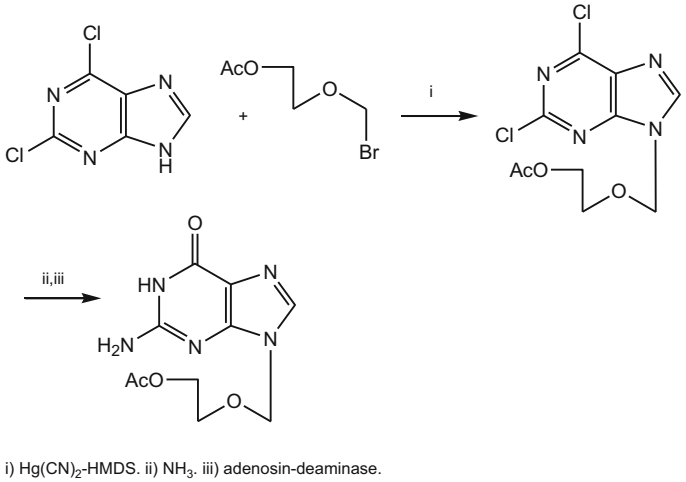
Scheme 4.66 Stereocontrolled syntheses of carbocyclic 9-deazapurine nucleosides



Scheme 4.67 First synthesis of acyclovir



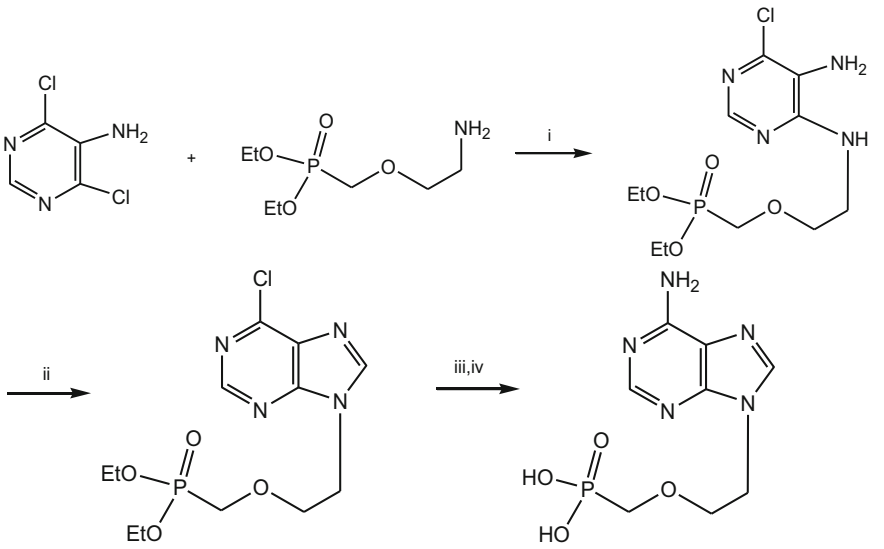
Scheme 4.68 Improved synthesis of acyclovir



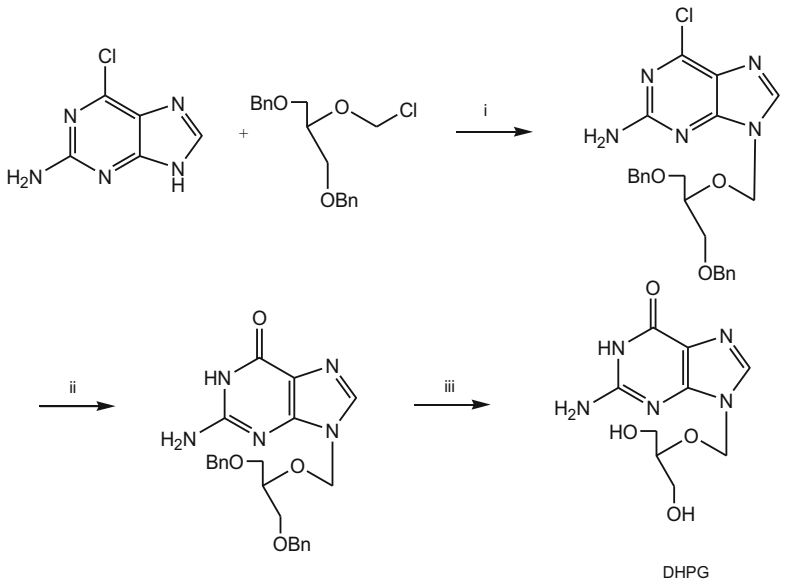
Scheme 4.69 Acyclovir synthesis

Robins and Hatfield [116] employed a chemoenzymatic approach for preparing acyclovir consisting initially in the use of mercury salts and hexamethyldisilane (HMDS) and in the final step an enzymatic conversion. Thus, the procedure involves the condensation between 2,6-dichloropurine and the bromoether, providing regioisomer N-7 shown in Scheme 4.69. Further amination and final transformation to guanine with the enzyme adenosin-deaminase produces the desired antiviral compound.

The phosphonate acyclic nucleoside 9-(2-phosphonomethoxyethyl)adenine (PMEA) was found to be a good antiviral analog with prolonged action [117]. A regio-defined synthesis base on the purine ring formation was described involving



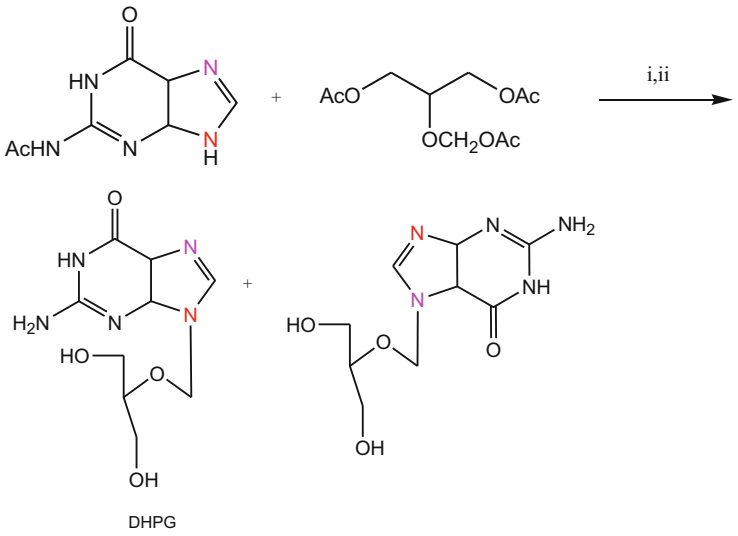
Scheme 4.70 Synthesis of phosphonate acyclic adenine PMEA



i) HMDS. ii) NaOMe, $HSCH_2CH_2OH$. iii) H_2, Pd-C

Scheme 4.71 Synthesis of antiviral acyclic nucleoside DHPG

the initial attachment of the phosphonate amine intermediate by nucleophilic substitution to the 5-amino-4,6-dichloropyrimidine base, and then ring formation followed by amination to produce the desired phosphonate acyclic adenine PMEA (Scheme 4.70) [118].



Scheme 4.72 Alternative synthesis of DHPG

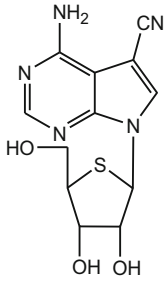
The effectiveness of acyclovir as antiviral drug encouraged different group to synthesize more potent acyclic analogs. As a result of this efforts, the acyclic nucleoside 9-[(1,3-dihydroxy-2-prpoxy)methyl]guanine (DHPG) [119] was prepared and tested as antiviral nucleoside, showing similar potency as acyclovir against simple herpes but stronger against encephalitis and vaginitis herpes.

Various report of DHPG were described, one of them involving the use of hexamethyldisilazane (HMDS) as condensing agent (Scheme 4.71) [112].

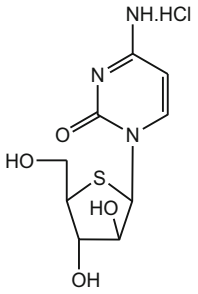
An alternative route for preparing DHPG involved the condensation reaction of acetylguanine base and triacetate derivative in the presence of ethanesulfonic acid, at temperatures ranging from 155 to 160 °C. As result two regioisomers were obtained from which one of those was converted to the desired antiviral compound Scheme 4.72 [112].

4.5 Thionucleosides

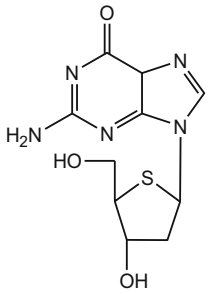
Nucleosides having the sugar ring oxygen replaced by sulfur are known as thionucleosides. The synthesis and therapeutic evaluation mainly as antiviral and anticancer drugs of these nucleoside mimics has been reviewed [120]. A comparative analysis of thionucleosides and nucleosides showed that sulfur replacement in some cases produced equivalent or higher potency [9, 121], and do not undergo enzymatic cleavage of the glycosidic bond, although it has been also observed increased toxicity as in the case of β-4′-thiothymidine [122] Some thionucleosides displaying antiviral and/or anticancer activity are shown in Scheme 4.73.



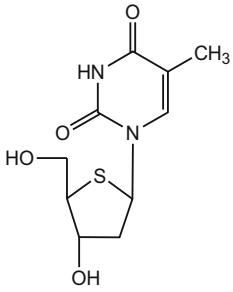
Thiotoyocamycin
Leukemia growth inhibitor



Thioarabinofuranosylcytosine
KB cell growth inhibitor

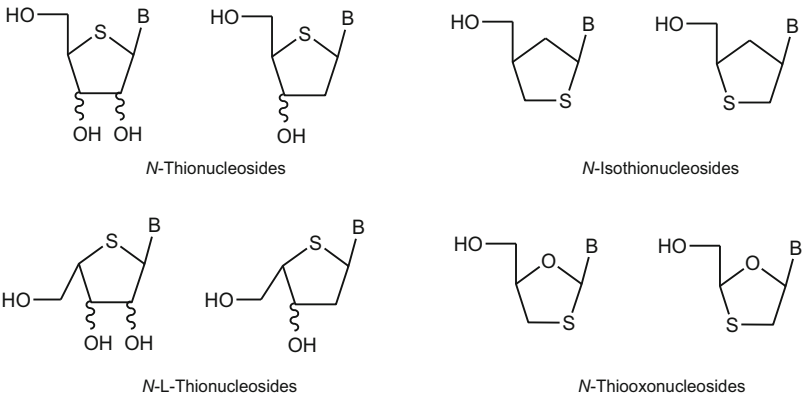


2'-Deoxy thioguanosine
Antiviral against HBV and HCMV



Thiothymidine
Carcinoma growth inhibitor

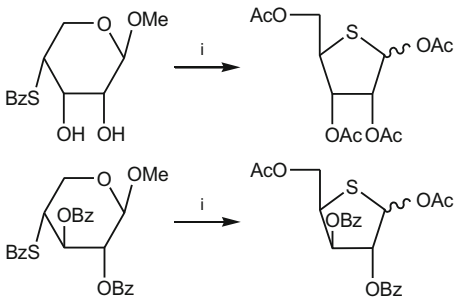
Scheme 4.73 Some active *N*-thionucleosides



Scheme 4.74 Classification of *N*-thionucleosides

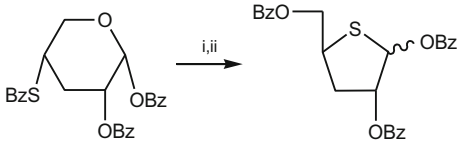
Based on their structural features *N*-thionucleosides defined also as thioribosyl sugars are classified into four groups (Scheme 4.74):

Scheme 4.75 Early synthesis of thioribofuranosyl derivatives



i) Ac_2O/AcOH, conc. H_2SO_4, 97%

Scheme 4.76 Preparation of benzoylated thioribofuranoside



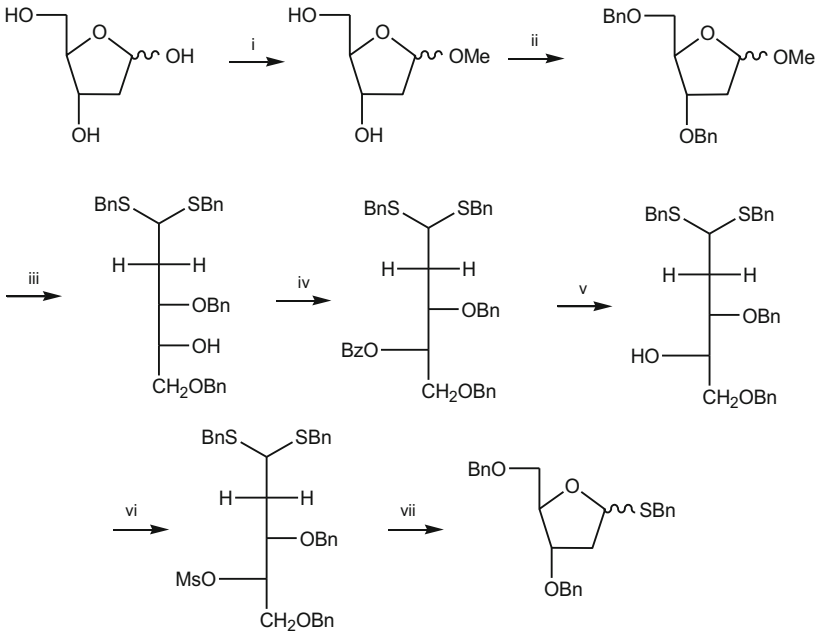
i) Na/aq. NH_3. ii) BzCl/Py

4.5.1 Preparation of Thioribofuranosyl Intermediates

A number of approaches oriented to replace or insert a sulfur atom instead or besides the cyclic oxygen into the ribose ring have been described. One of the earliest methods for preparing thioribosyl acetates was described by Reinst et al. [123, 124] involving as key steps the conversion of the 4-thiobenzoyl pyranoside into the thioribofuranosyl acetate (Scheme 4.75).

Short time later another report introduced the use of sodium in liquid ammonia followed by benzoylation to yield tribenzoylated thioribofuranoside as a mixture of anomers (α:β, 1:3) (Scheme 4.76) [125].

The thioribosyl derivative benzyl 3,5-di-*O*-benzyl-2-deoxy-1,4-dithio-D-*erythro*-pentofuranoside has been prepared and used as glycosyl donor in various thionucleoside synthesis [125–127]. The synthesis started from 2-deoxy ribose which was transformed to the methylbenzyl derivative by following a standard procedure and then treated with benzylmercaptan in acid to produce the dithiobenzylated derivative. Next, was to invert the hydroxyl group at 4-position by using the Mitsunobu protocol to generate the intermediate with the desired stereochemistry. Final tosyl protection and NaI-$BaCO_3$ treatment provided the desired thiosugar (Scheme 4.77) [126].



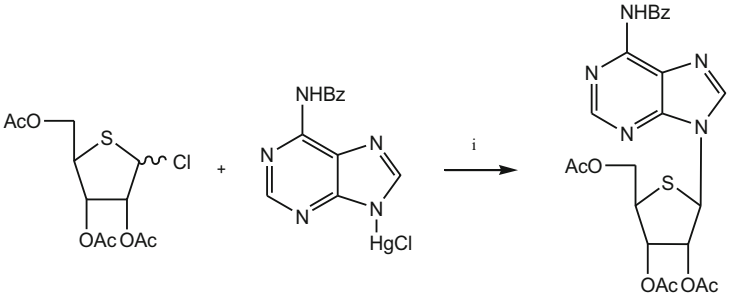
i) MeOH, HCl. ii) NaH, Bu_4NI, BnBr/THF. iii) BnSH,HCl. iv) PPh_3, $PhCO_2H$, DEAD/THF. v) NaOMe/MeOH. vi) MsCl/Py. vii) NaI, $BaCO_3$, acetone

Scheme 4.77 Synthesis of benzyl 3,5-di-*O*-benzyl-2-deoxy-1,4-dithio-D-*erythro*-pentofuranoside

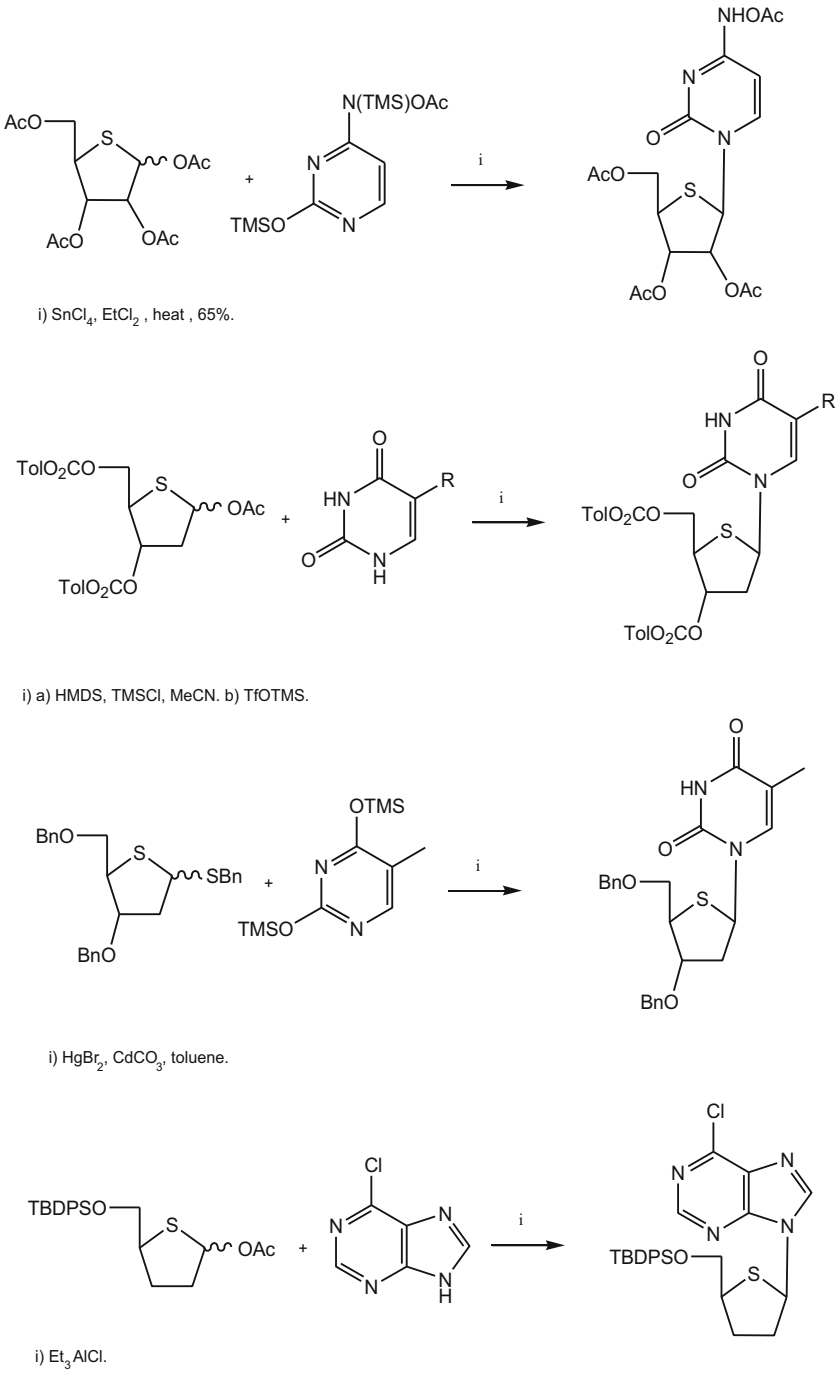
4.5.2 Glycosidic Bond Formation

The general methods for preparing *N*-thionucleosides are similar as for *N*-nucleosides; however, variations from slight to significant can be found specially in the preparation of four ring thietanocin or thiolane analogs [127, 128] Thus, according to a comprehensive review [120], the earliest reports for *N*-thionucleoside formation used chloromercury salt of purine and chlorine or benzoyl thioriboside as glycosyl donor, while more recently the silyl approach has been preferred (Scheme 4.78).

4.5.2.1 Chloromercuration Promoted Coupling Reactions

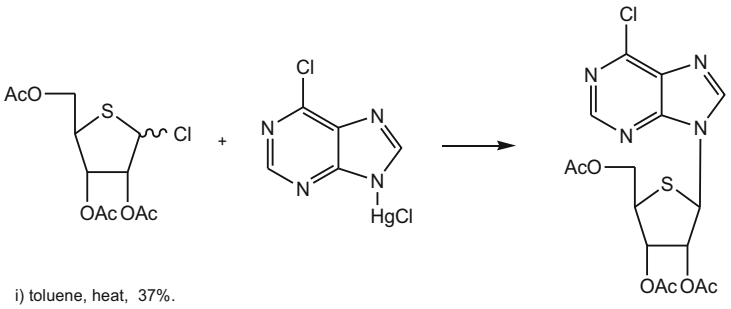


i) toluene



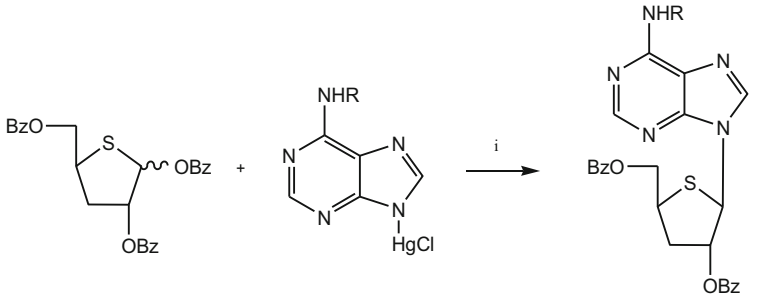
Scheme 4.78 Common glycosylation reactions for the preparation of thionucleosides [122, 129–132]

Ref. [123].



i) toluene, heat, 37%.

Ref. [129].

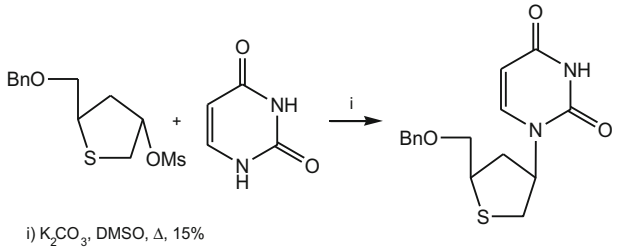


i)$TiCl_4$, $EtCl_2$, heat.

Ref. [130].

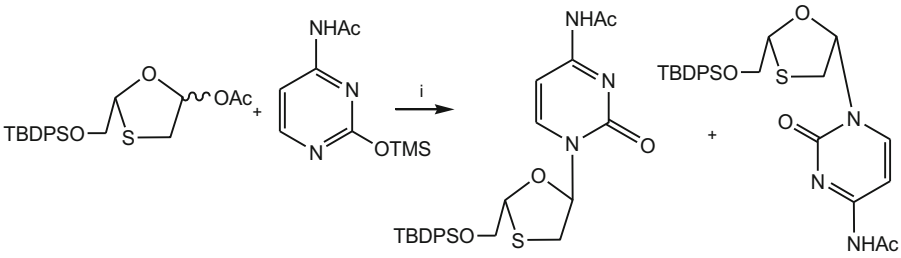
4.5.2.2 Silyl-Mediated Coupling Reactions

The preparation of potential anti-HIV *N*-isothionucleosides was described starting from glucose. The key coupling reaction proceeds in low yield between the pyrimidine base and the mesyl tetrahydrothiophene derivative under potassium conditions (Scheme 4.79) [133].



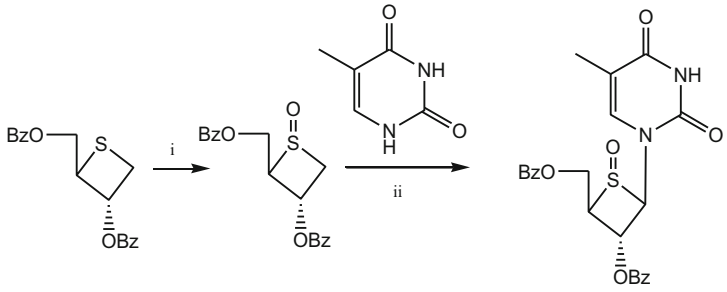
i) K_2CO_3, DMSO, Δ, 15%

Scheme 4.79 Preparation of *N*-isothionucleoside



i) TMSOTf, CH_2Cl_2, 64%

Scheme 4.80 Preparation of *N*-thioxonucleosides



i) TMSOTf, Et_3N, ZnI_2, toluene, 30 %.

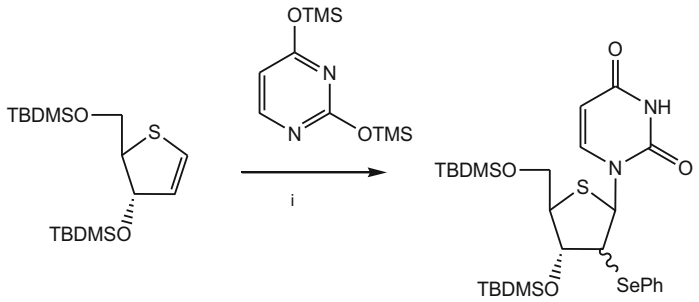
Scheme 4.81 Synthesis of thymidine thietane nucleoside

N-thioxonucleosides are another class of *N*-thionucleosides tested as anti-HIV agents. The conditions employed for performing the coupling reaction were TMSOTf as Lewis acid catalyst, providing a mixture of anomers (α:β, 1:2) in 64 % (Scheme 4.80) [134].

Thietane nucleoside was synthesized starting from the benzoyl thietane derivative which prior to the coupling reaction was treated with peroxide to produce the sulfoxide derivative. Then under Lewis acid conditions a Pummerer rearrangement process takes place to produce in the presence of thymine the expected thietane nucleoside (Scheme 4.81) [128].

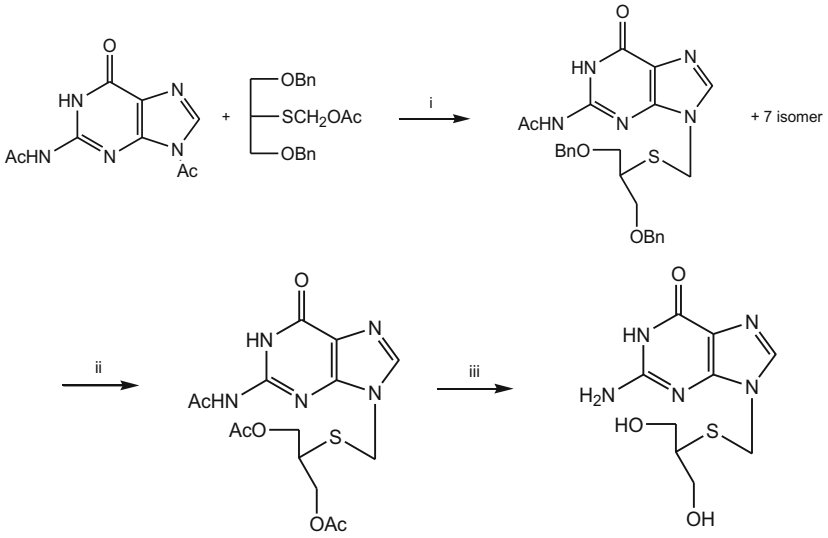
More recently the stereoselective synthesis of β-4′-thionucleosides based on electrophilic glycosilation of 4-thiofuranoid glycals has been described. Thus, the condensation of TBDMS-4-thioglycal with silylated uracil in the presence of PhSeCl as electrophile furnished thionucleosides in 88 % as a mixture of anomers (α:β; 1:4) (Scheme 4.82) [135].

The thio analog of antiviral DHPG with comparable activity to DHPG against HSV-1 and human cytomegalovirus was synthesized according to the scheme shown below (Scheme 4.83) [112].



i) PhSeCl 88 %.

Scheme 4.82 Synthesis β-4′-thionucleosides based on electrophilic glycosidation of 4-thiofuranoid glycols



i) $(p\text{-}NO_2C_6H_4)_2P(O)OH$. ii) $(C_2H_5)O\text{-}BF_3$,Ac_2O. iii) NH_3.

Scheme 4.83 Synthesis of thio analog of DHPG

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Chapter 5
C-glycosides

C-glycosides have attracted much attention, considering that many of them have demonstrated their effectiveness as therapeutic agents. The increasing significance of *C*-glycosides is that the conformational differences compared to *O*- or *N*-glycosides are minimal, and that they are resistant to enzymatic or acidic hydrolysis since the anomeric center has been transformed from acetal to ether [1]. A glycoside is defined as *C*-glycoside when what is supposed to be the anomeric carbon of a sugar is interconnected to the aglycon, generating a new C–C bond. According to Levy and Tang [2] the term *C*-glycoside describes those structures in which a common structural motifs the presence of carbon functionality at what would otherwise be the anomeric position of a sugar or derivative. Structurally *C*-glycosides can be constituted by aliphatic, or aromatic aglycon, and the sugar can be pyranose or furanose. A variety of natural product *C*-glycosides have been described. Examples of *C*-glycosides isolated from different plant genera or insects and characterized spectroscopically are: Carminic acid (cochineal), Aloin (Aloe vera), Scoparin (*Cytisus scoparius*), Saponarin (*Saponaria officinalis*), flavonoid phytoalexins such as Cucumerins (*Cucumis sativus*) and Naringenin (grapefruit), [3] *C*-glucosyl xanthones [4], and complex benzoquinone Altromycin B [5] (actinomycetes), among others (Scheme 5.1).

Moreover, much effort and creativity have been devoted to the preparation of complex *C*-glycosides with potent antibiotic activity. That is the case of Aurodox [6], Lasalocid A [7], Herbicidin [8], and the hyperfunctionalized molecules Spongistatin [9], and Palytoxin [10] (Scheme 5.2).

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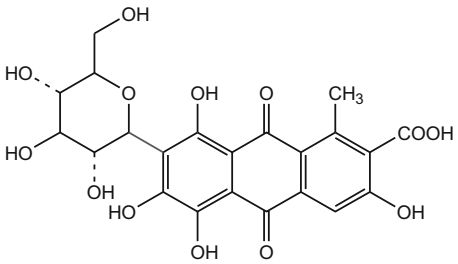
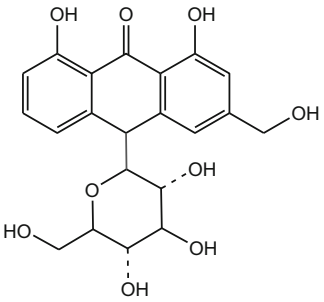


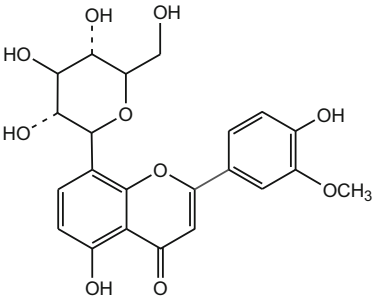
Carminic acid



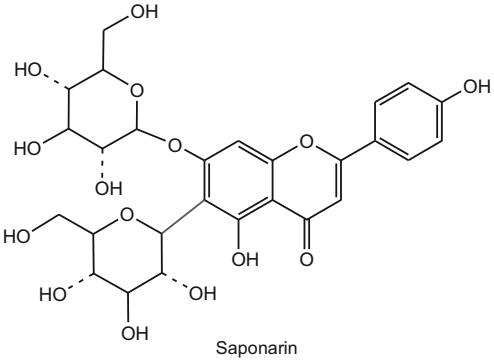
Aloin

cochineal

Aloe vera



Scoparin



Saponarin

Scheme 5.1 Some naturally occurring *C*-glycosides

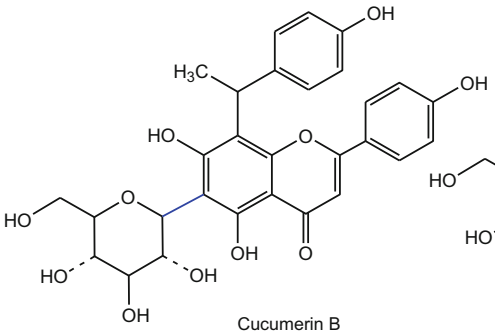
5.1 Synthetic Approaches for the Preparation of *C*-Glycosides

Based on comprehensive studies [2, 11–14], the general strategies for *C*-glycosides can be overviewed as follows:

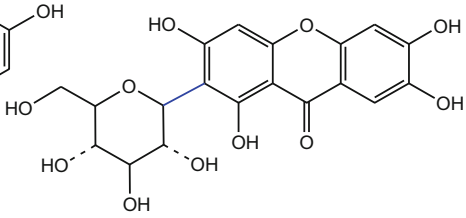
- Electrophilic glycosyl donors
- Concerted reactions

Cytisus scoparius

Saponaria officinalis

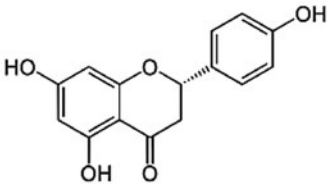


Cucumerin B



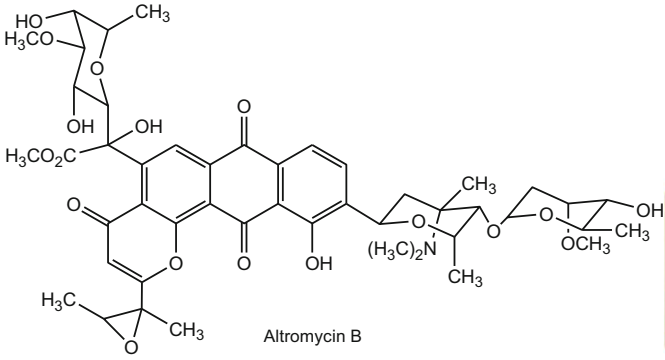
C-Glucosylxanthones

Cucumis sativus



Naringenin

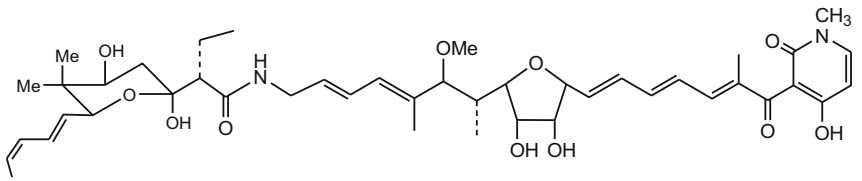
grapefruit



Altromycin B

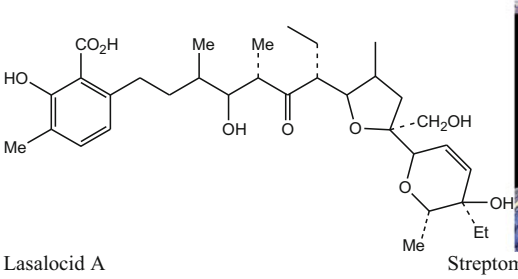
Actinomycetes

Scheme 5.1 (continued)

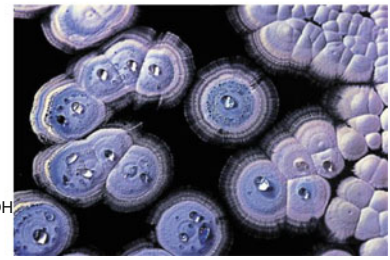


Aurodox

Streptomyces sp



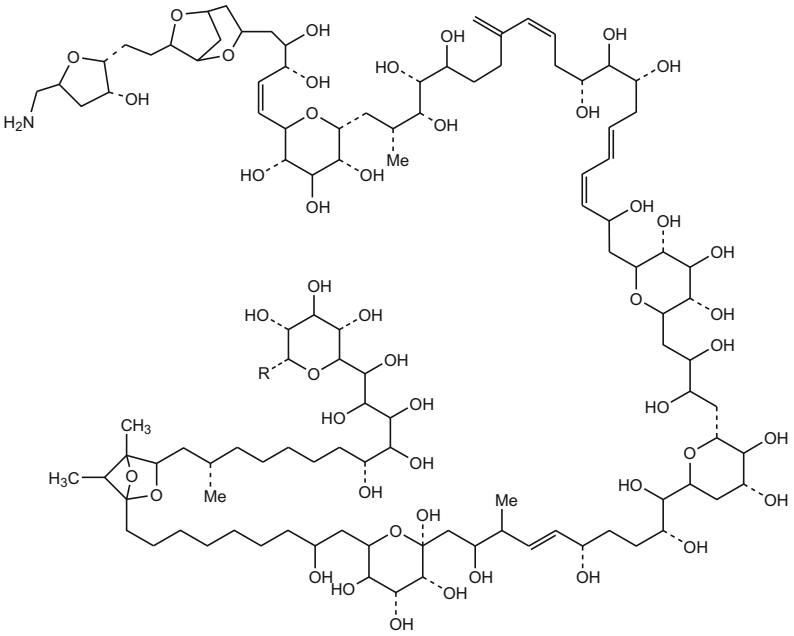
Lasalocid A

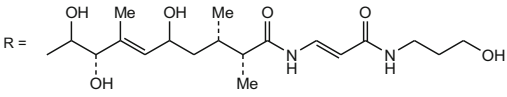


Streptomyces

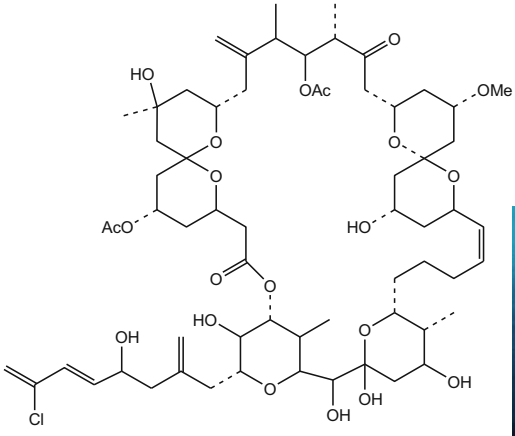
Scheme 5.2 Complex *C*-glycoside antibiotics

- Wittig approximation
- Palladium mediated reactions
- Mitsunobu reaction
- Nucleophilic sugars or anomeric anions intermediates.
- Cross-metathesis reaction
- Samarium promoted reaction
- Ramberg–Bäcklund reaction
- Free radical approaches
- Exoglycals
- The tether approach
  - With unprotected sugars

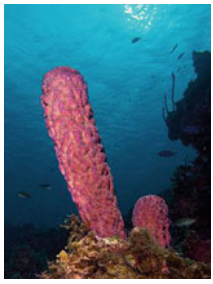




Palytoxin



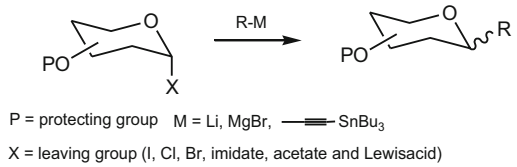
Spongistatin 1



Scheme 5.2 (continued)

5.1.1 Electrophilic Glycosyl Donors

5.1.1.1 Glycosyl Donors Bearing Good Leaving Groups



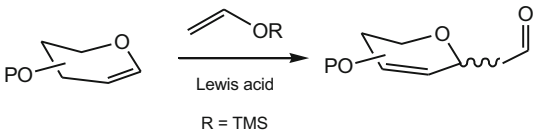
A general approach for the *C*-glycosidic bond formation is based on nucleophilic carbon addition on the electrophilic center of a glycosyl donor. The most extensively used glycosyl donors divided in four main groups (good leaving groups, sugar lactones, glycals, and 1,2 anhydrosugars) are used as electrophilic donors to generate *C*-glycosidic bonds when reacted with organocuprates, organotin, organozinc, cyanide, allylic Grignard, vinyl silyl reagents, and activated aromatic compounds among others [11].

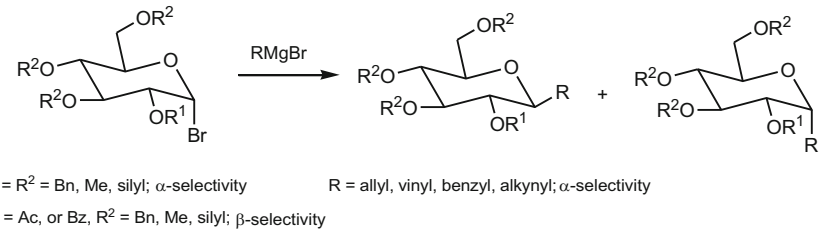
Some of the reactions described that have been used for preparing useful intermediates or *C*-glycosides are shown in Scheme 5.3

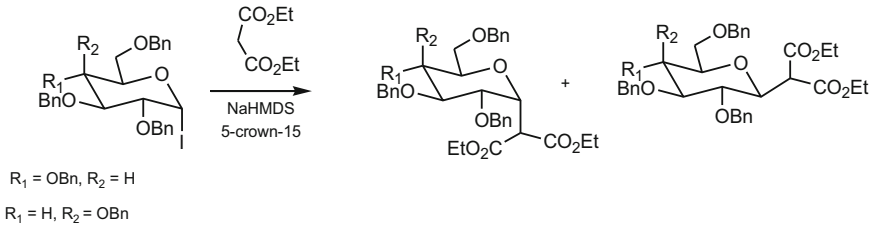
It is worth mentioning that for aryl C-glycosylations, there is a dependence on the electron density of the aromatic ring and the protecting groups at the glycosyl moiety [18]. Moreover, depending on the reaction conditions, there is a competing parallel process that ultimately will drive the reaction either to the *O*- or to the C-glycoside formation. This affirmation was demonstrated in the preparation of *C*- and *O*-flavonoid glycosides by Oyama et al., which treated glycosyl fluoride with flavan under Lewis acid conditions. It was observed that $BF_3.ET_2O$ and 2,6-di-tert-butyl-4-methyl pyridine (DTBMP) resulted predominantly in the formation of the 5-*O*-β-glycoside, while if the reaction is carried out only with BF_3-Et_2O, the *C*-glycoside is obtained (Scheme 5.4).

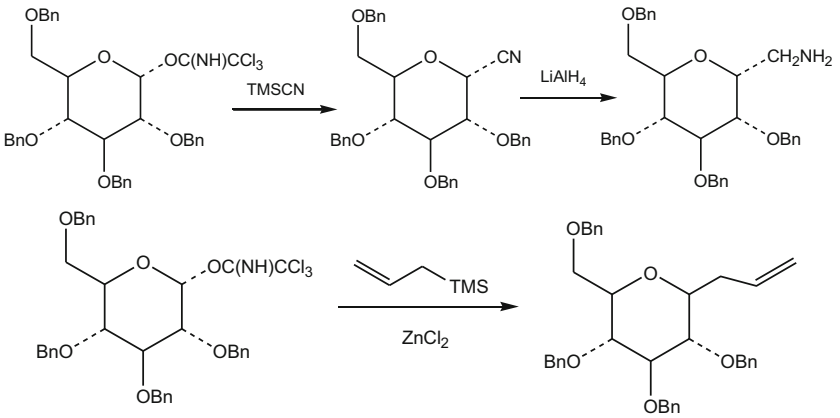
5.1.1.2 Other Electrophilic Glycosyl Donors

Additionally, the introduction of other electrophilic centers at the anomeric position has extended the possibilities for preparation of *C*-glycosides by using electrophilic sugars. Some of these electrophilic sugars are: lactols, anomeric esters, glycals, anhydrides, and lactones.

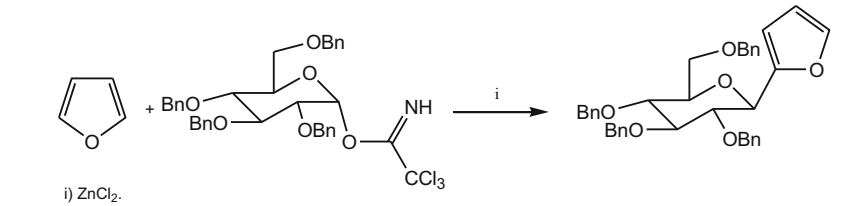


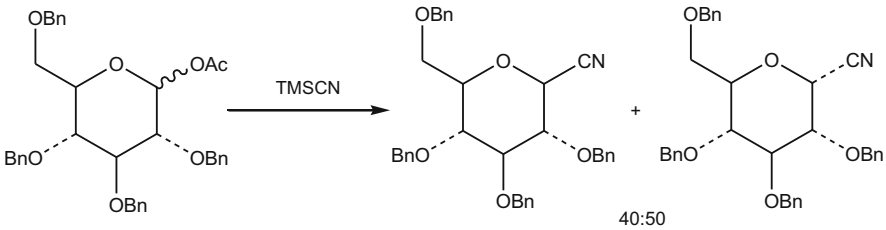


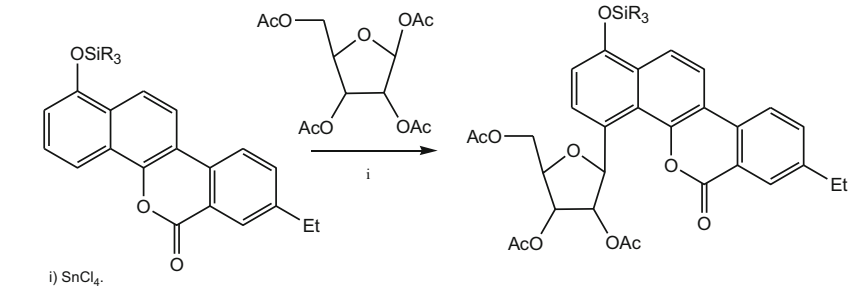


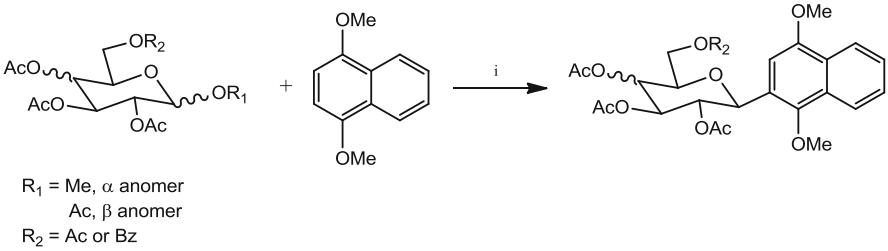


Scheme 5.3 Preparation of *C*-glycosides or intermediates from electrophilic glycosyl donor with good leaving groups [15–17]

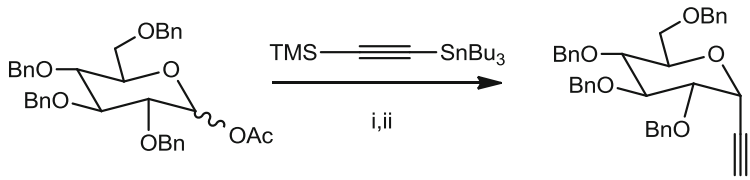








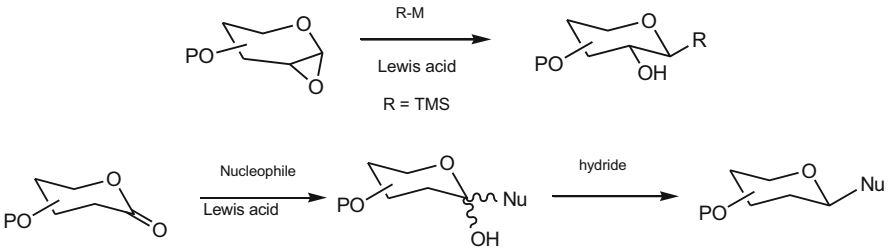
i) $AgOTf/SnCl_4$, CH_2Cl_2



i) TMSOTf, CH_2Cl_2. ii) K_2CO_3, MeOH

Scheme 5.3 (continued)

Scheme 5.4 C-glycosylations involving glycosyl donors with leaving group



Some of the reactions carried out for preparing *C*-glycoside intermediates involving these alternative glycosyl donors are shown in Scheme 5.5. In 1,2-anhydrosugars the stereoselectivity is 1,2-trans type and involves a typical S_N2 process. On the other hand glycals exhibit high stereoselectivity, and in glycosyl acetates the stereocontrol relies on the electronic and steric properties of the nucleophiles.

5.1.2 Concerted Reaction and Ring Formation

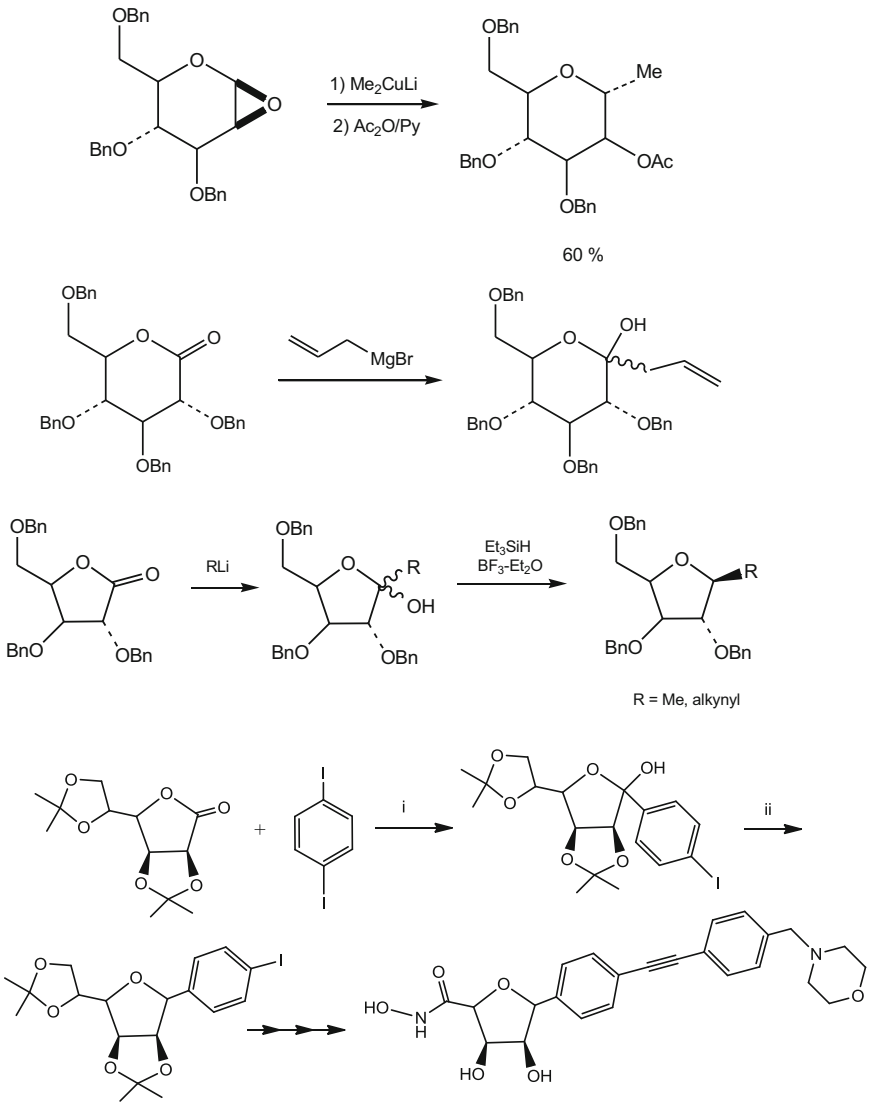
This type of reactions include sigmatropic rearrangements and cycloaddition transformations. As an example of the applicability of the sigmatropic rearrangement for preparation of *C*-glycosides, Ireland [7] reported the synthesis of Lasalocid A, consisting in the coupling of acid derivative with protected glycal as a result of enolate addition and Claisen rearrangement. A transformation series of this precursor will give rise to Lasalocid A (Scheme 5.6).

To exemplify the effectiveness of cycloadditions for preparation of *C*-glycosides, Schmidt et al. [23] prepared *p*-methoxyphenyl 2,3,4,6-tetraacetyl *C*-glucopyranose, by following a Diels–Alder approach. The reaction between heterodiene and dienophile produced cycloadduct that was successively transformed to give the desired product (Scheme 5.7).

Protected monosaccharide is reacted with Wittig ilide to produce a ring opening unsaturated intermediate, which was cyclized to produce a mixture of α,β *C*-glycosides. The α form could be converted to the β form under sodium methoxide conditions (Scheme 5.8) [24].

Cation-mediated cyclization reactions of silyl enols ether-containing thioglycosides give bicyclic ketotetrahydrofurans. Treatment with sodium amalgam in buffered methanol yields the expected dihydropyran which was transformed to the diol intermediate, and after separation converted to the bis-acetonides (Scheme 5.9) [25].

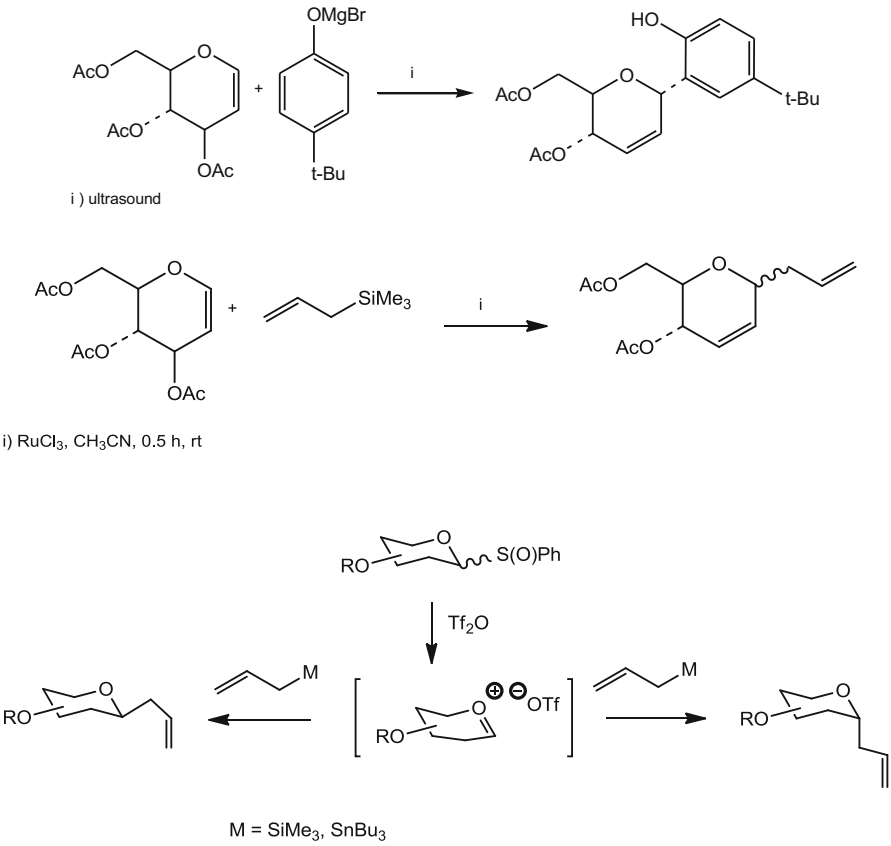
Ring closure of polyalcohol has been proposed as a suitable strategy for preparing *C*-glycosides [26]. Condensation between iodine pyranoside intermediate and an aldohexose will result in the condensation product which undergoes cyclization to give the mixture of *C*-disaccharides showed in Scheme 5.10.



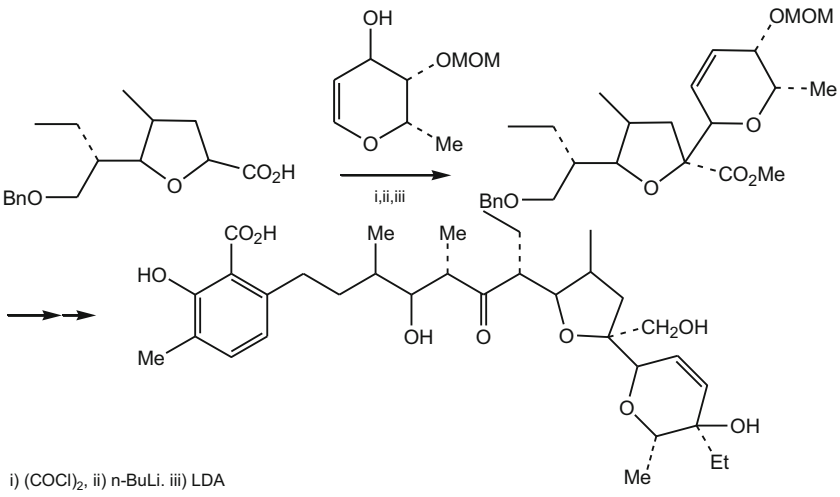
Scheme 5.5 *C*-glycoside formation with electrophilic sugars [19–22]

5.1.3 Palladium Mediated Reactions

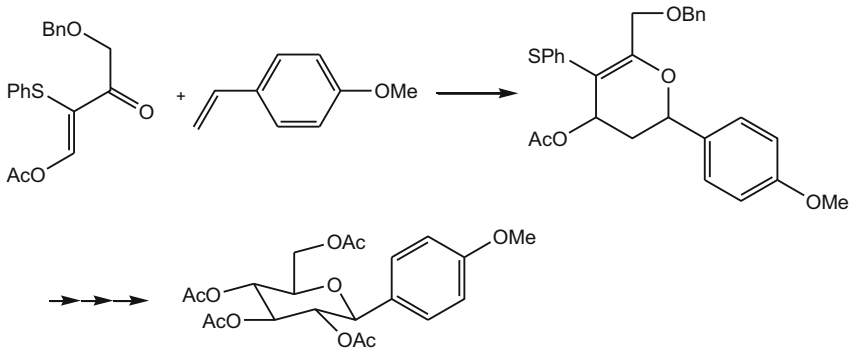
Heck type reactions have been successfully assayed for preparing interesting *C*-glycosides. Such is the case of vineomycinone B2 prepared by palladium catalyzed condensation between TBS protected glycal with anthracene derivative [27]. Further transformations will generate *C*-glycoside vineomycinone B2 (Scheme 5.11).



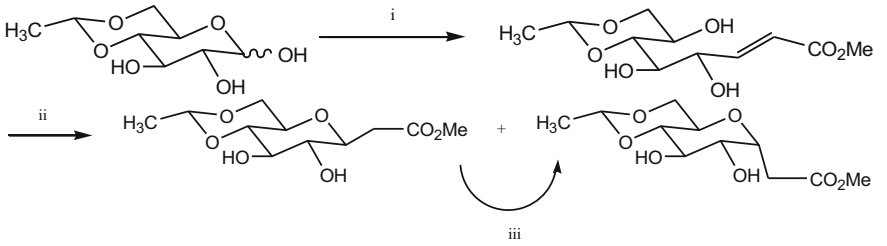
Scheme 5.5 (continued)



Scheme 5.6 Synthesis of Lasalocid A

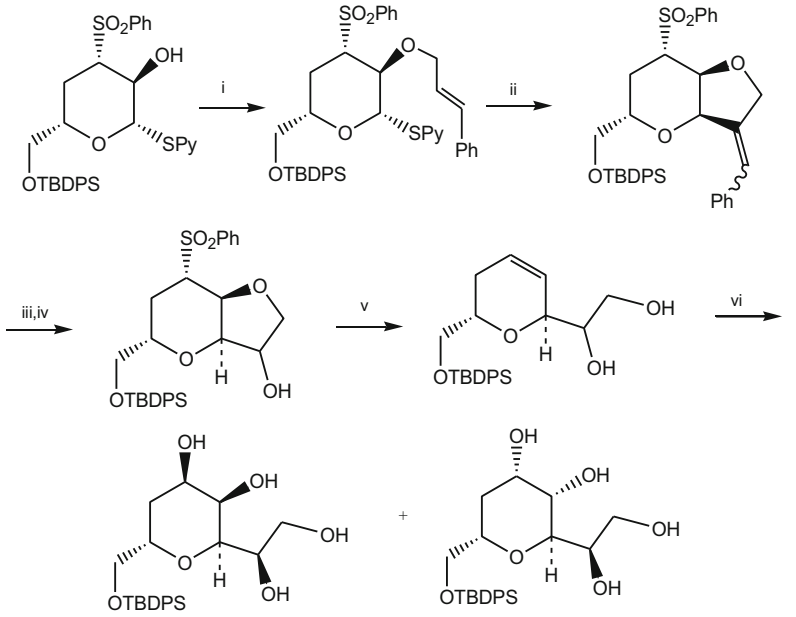


Scheme 5.7 The Diels-Alder reaction for *C*-glycoside formation



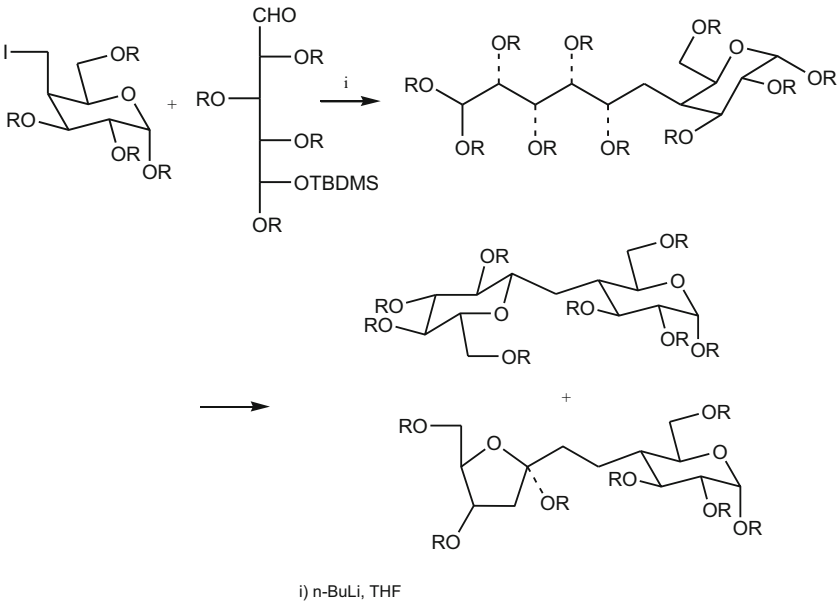
i) $Ph_2P{=}CHCO_2Me$. ii) KOH/MeOH. iii) MeONa/MeOH.

Scheme 5.8 Wittig reaction for *C*-glycoside formation

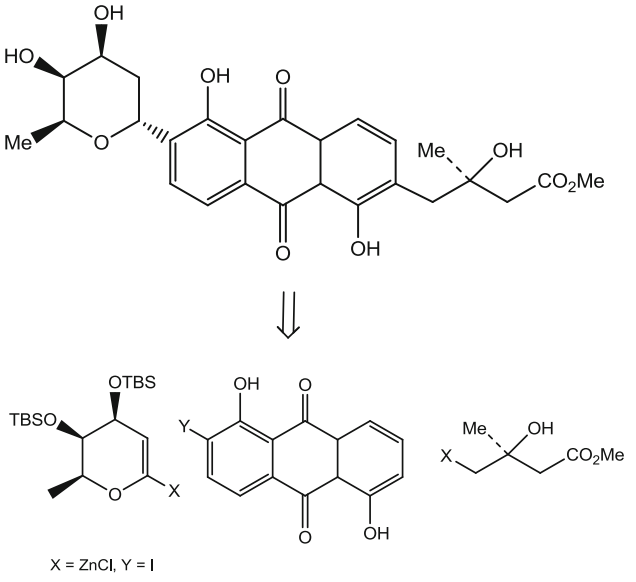


i) $PhCH{=}CHCH_2Br$, CH_2Cl_2, 50% aq. NaOH, r.t. ii) $AgOSO_2CF_3$, MS, CH_2Cl_2, r.t. then DBU. iii) O_3, CH_2Cl_2, -78°C, then PPh_3, -78°C to r.t. iv) $NaBH_4$, MeOH, 0°C. v) 6% Na(Hg), Na_2HPO_4, MeOH, 0°C. vi) OsO_4, NMO, 9:1 acetone-H_2O, r.t.

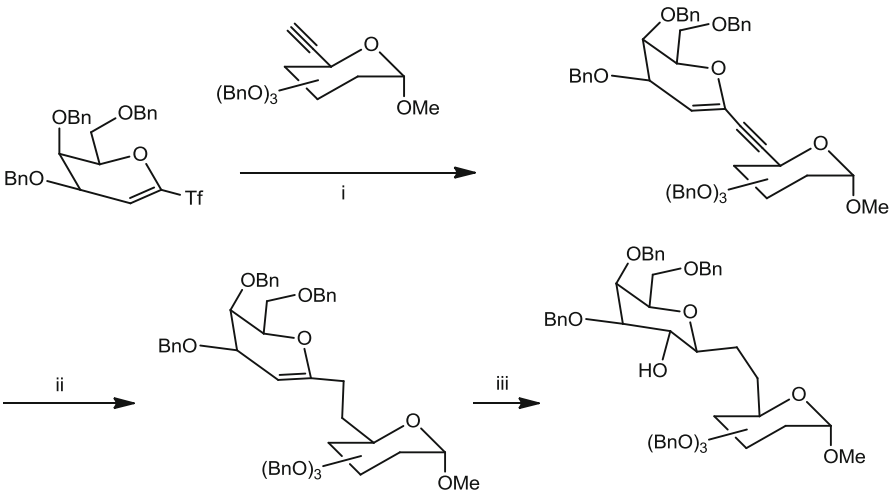
Scheme 5.9 Formation of tetrahydrofurans and application to the synthesis of 2-octulopyranosides



Scheme 5.10 *C*-disaccharide formation from aldohexoses



Scheme 5.11 Synthesis of vineomycinone B2 methyl ester



i) $Pd(PPh_3)_2Cl_2$ 5 mol%, CuI 10 mol%, NEt_3, rt. ii) Raney-nickel, H_2 (1bar), THF/MeOH, rt
iii) a) DMDO, CH_2Cl_2, -78°C to rt. b) DIBAL, CH_2Cl_2, -78°C to rt

Scheme 5.12 Synthesis of (1 → 6)-linked *C*-glycosidic disaccharides from glucal triflate

The synthesis of (1 → 6)-linked *C*-glycosidic disaccharides were suitably prepared starting from glucal triflate as glycosyl donor which was coupled with alkynyl glycosides under palladium mediated conditions, generating the pseudodisaccharides which was reduced with Raney-nickel under hydrogen atmosphere and the glycal epoxidated with dimethyldioxirane and finally transformed to the pyranoside ring by hydride reduction (Scheme 5.12) [28].

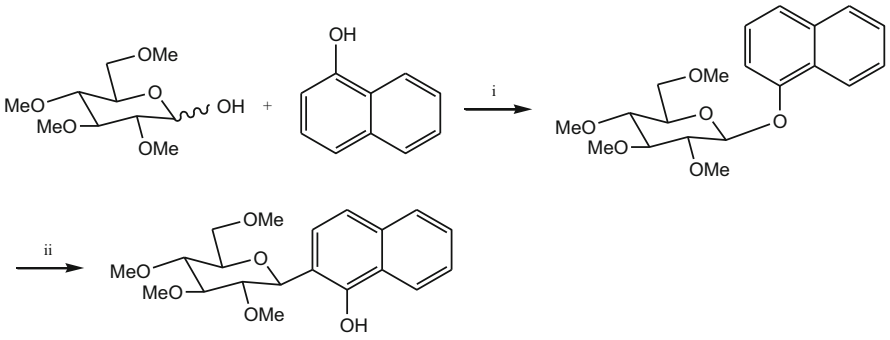
Other palladium-mediated coupling includes Stille (palladium-catalyzed vinyl substitution) [29], and Suzuki cross-coupling reactions [30].

5.1.4 Mitsunobu Reaction

Mitsunobu reaction is an additional useful reaction for preparing *C*-glycosides (Scheme 5.12). When tetra-*O*-methyl glucopyranose is reacted with 1-naphthol in the presence of Mitsunobu conditions (diethylazidodicarboxylate and triphenylphosphine), the resulting product is the *O*-glycoside which is rearranged with BF_3-Et_2O to the corresponding *C*-glycoside (Scheme 5.13) [31].

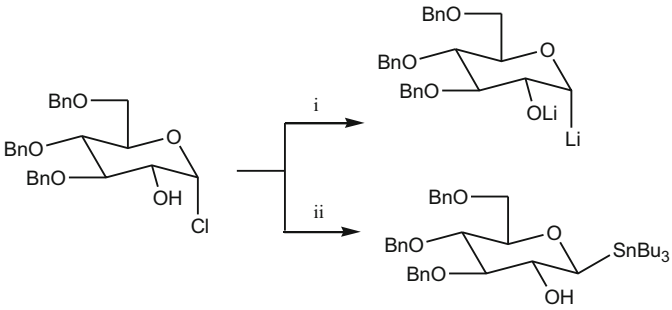
5.1.5 Nucleophilic Sugars

Anomeric carbons are considered electrophilic sites by nature, however it is possible to invert this reactivity by using metallic bases. The resulting carbanion character is known as *umpolung* reactivity and allows the species to behave as nucleophiles.



i) DEAD, Ph_3P. ii) $BF_3.Et_2O$.

Scheme 5.13 Mitsunobu reaction for aromatic *C*-glycoside formation



i) BuLi-LiN. ii) Bu_3SnLi.

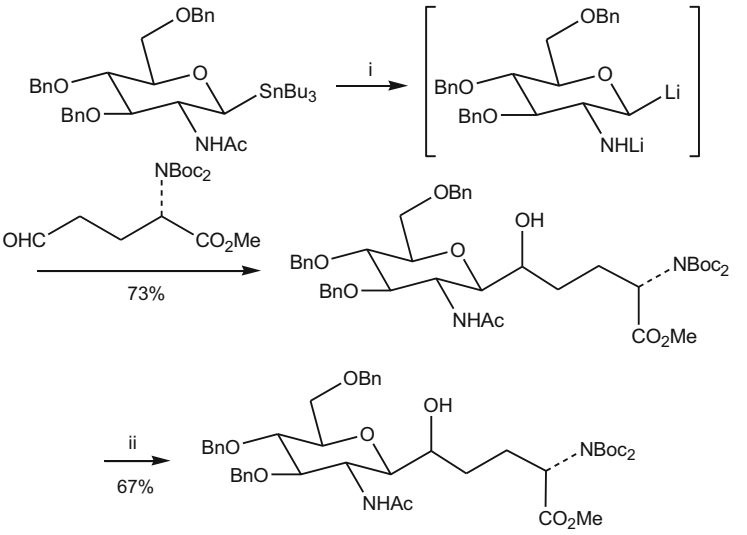
Scheme 5.14 Preparation of lithium and stannane glycosyl anions

A variety of glycosyl donors have been converted to lithium or stannane glycosyl anions (Scheme 5.14) [11].

By using this possibility, the synthesis of the *C*-glycosyl asparagine analog has been completed by Kessler and coworkers [32]. The transformation of the stannane to the lithium donor was followed by the coupling reaction with the aldehyde glutamic acid derivative to provide the β-D-linked *C*-glycoside. Removal of Boc protecting group and dehydroxylation reaction under Barton–McCombie condition provided the target molecule (Scheme 5.15).

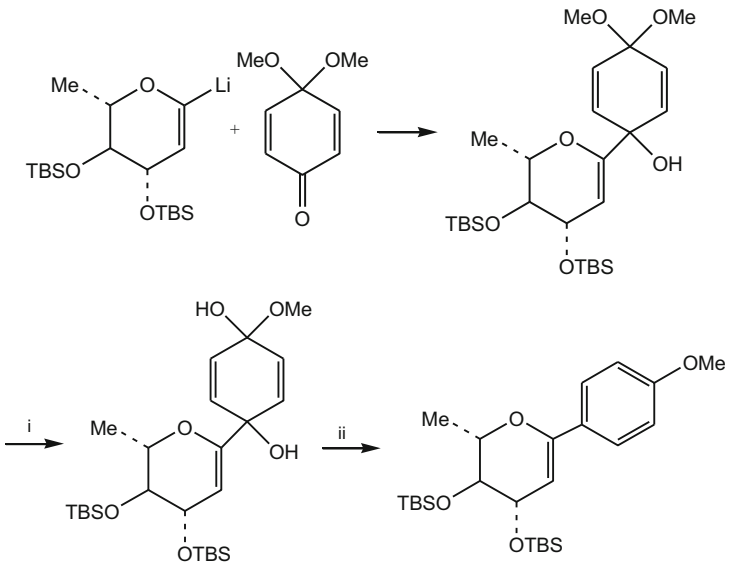
Another accomplishment following this umpolung strategy was the preparation of the aromatic *C*-glycoside shown in Scheme 5.16. Hence, lithium glycal (obtained from glycal treatment with lithium diisopropylamide) was reacted with quinolic ketal to yield addition product, which was transformed to the aromatic *C*-glycal [12, 14, 33].

Aldol condensations between glycosyl donors containing active methylene carbons and glycosyl acceptors has been also proposed as a suitable approach for preparing *C*-disaccharides. Martin et al. [34] described a procedure for preparing (1,6)- and (1,1)-linked *C*-disaccharides based on the nitroaldol condensation



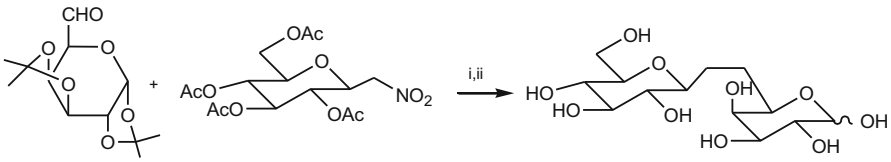
i) a)MeLi. b) BuLi. ii) a) $MgClO_4$. b) deoxygenation.

Scheme 5.15 Preparation of *C*-analogs of glycosyl asparagines from anionic glycosyl donors



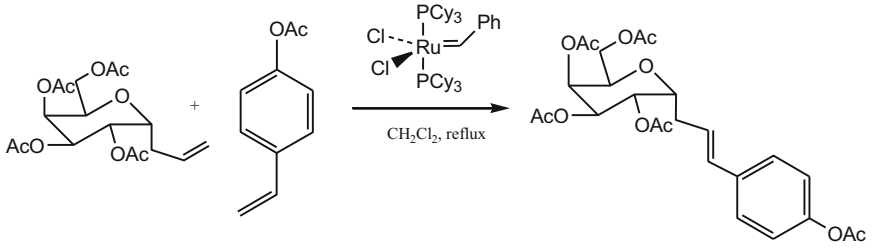
i) DIBAL/CH_2Cl_2. ii) $POCl_3$/Py.

Scheme 5.16 *C*-glycoside formation using lithium glycal nucleophilic donor



i) KF, MeCN, DCH-18-crown-6. ii) a) Ac_2O, Py, $CHCl_3$. b) $NaBH_4$, MeOH, CH_2Cl_2, 0°C. c) Bu_3SnH, AIBN, reflux. d) NaOMe, MeOH. e) H_3^+O.

Scheme 5.17 *C*-disaccharide formation with glycosyl donors containing active methylene carbons



Scheme 5.18 Cross-metathesis reaction for *C*-glycoside formation

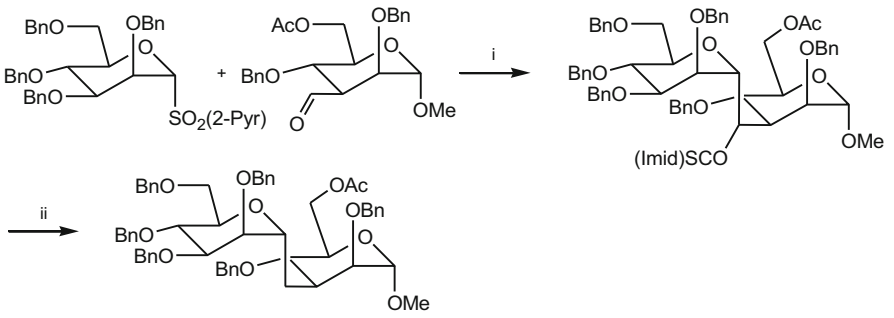
between the glycosylnitromethane peracetate and the galactose-derived aldehyde to provide after dehydration, reduction of the double bond and radical denitration the desired *C*-disaccharide (Scheme 5.17).

5.1.6 Cross-Metathesis Reaction

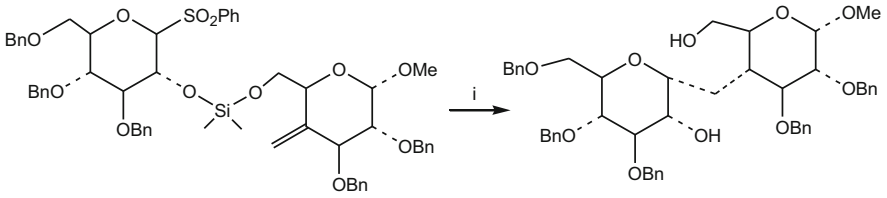
Cross-metathesis reaction is an emerging methodology for C–C bond formation. The air stable Grubbs ruthenium complex [35, 36] has become an attractive catalyst for the olefin cross-metathesis reactions and has been also applied successfully for the preparation of pseudosaccharides. The coupling reaction between C-allyl α-D-galactopyranoside and 4-acetoxystyrene led to the formation of the cross-metathesis product (Scheme 5.18) [37].

5.1.7 Samarium Promoted Reaction

The synthesis of a *C*-glycoside analog of α-1,3-mannobiose has been reported via SmI_2-promoted C-glycosilation. The general approach is based on the Barbier-type coupling [38] and involves the use of pyridyl sulfone glycosyl donor with a sugar aldehyde in the presence of SmI_2 as catalyst. This procedure has been exploited successfully for the preparation of disaccharides under the tether approach (Scheme 5.19) [39].

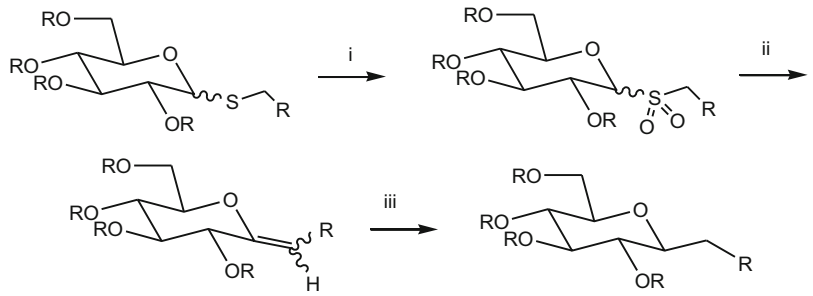


i) a) SmI_2 (2.8 eq), THF, 20°C. b) $(Imid)_2CS$ (15 eq), CH_3CN, reflux, 35% ii) F_5PhOH, Ph_3SnH, AIBN, toluene, reflux, 65%.



i) SmI_2, PhH,HMPA, 60°C. b) aq.HF.

Scheme 5.19 Samarium promoted C-glycosylation

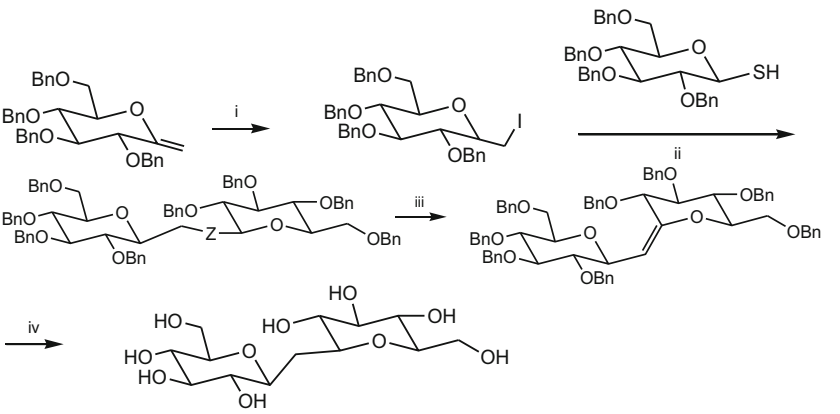


i) mCPBA, Na_2HPO_4/CH_2Cl_2, 77%. ii) KOH/Al_2O_3, $CBrF_2CBrF_2$/tBuOH, 50°C.iii) H_2, $Pd(OH)_2$/EtOAc.

Scheme 5.20 The Ramberg–Bäcklund approach for *C*-glycoside formation

5.1.8 The Ramberg–Bäcklund Reaction

This novel procedure introduced by Franck et al. is becoming a practical and versatile approach for the preparation of biologically active *C*-glycosides such as aromatic [5], amino acids [40, 41] or glycerolipids [42]. The reaction sequence for *C*-glycoside formation consist in the initial *S*-glycoside formation, transformation to the sulfone derivative, Ramberg–Bäcklund rearrangement involving sulfone extrusion, and hydrogenolysis (Scheme 5.20).



Scheme 5.21 Synthesis of *C*-isotrehalose

Another *C*-disaccharide was prepared by transformation of benzylated exo-glycal to the iodide derivative, which in turn was coupled with the sulfur glycosyl donor. Further transformation to the sulfone and Ramberg–Bäcklund rearrangement produced the unsaturated disaccharide which was finally reduced under Pearlman conditions to provide disaccharide in 70 % yield (Scheme 5.21) [43].

5.1.9 Free Radical Approach

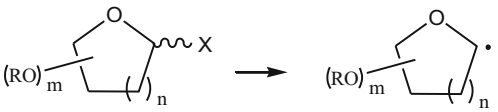
This approach is based on the generation of free radical at the anomeric carbon by using glycosyl donors which are subjected to stannous treatment of free radical conditions which in turn will react with mainly exoglycals to produce a *C*-glycosidic linkage. The general methods leading to anomeric radicals formation are summarized in Scheme 5.22 [44].

The coupling reaction between acetobromoglucose and the unsaturated lactone shown in Scheme 5.23 will result in the *C*-disaccharide formation, where a free radical mechanism promoted by a mixture of AIBN-Bu_3SnH is involved [45].

Anomer radicals may also generate rearranged products as a result of 1,2-migration particularly for the case of acetoxy and phosphate groups. This feature has been exploited successfully for preparing 2-deoxy sugars from commercially available sugars (Scheme 5.24) [46].

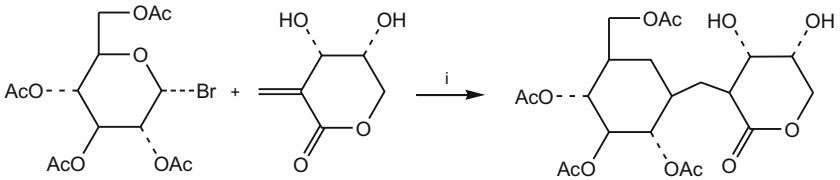
5.1.10 Exoglycals

Exo-glycals have been described as another possibility for preparing *C*-glycosyl derivatives. The term exo-glycal is given to those unsaturated sugars with exocyclic double bonds. The most representative of these compounds are 1,2- and 5-6-unsaturated sugars (Scheme 5.25) [47].



a) Bu_3SnH (Hg-hν or Δ)
X = NO_2, I, Br, Cl, SeAr, SAr
b) $Bu_3SnSnBu_3H$ (Hg-hν)
X = NO_2, I, Br, Cl, SeAr, SAr
c) $(C_2H_5)_3B$ (air) or N-acetoxy-2-thiopyridone (W-hν)
X = TeAr
d) Ester of N-Hydroxy-2-thiopyridone (W-hν)
X = COOH
e) (Diacyloxyiodo)arenes (Hg-hν orΔ)
X = COOH
f) Hg-hν
X = COBu-tetc
g) W-hν
X = $Co(dmgH)_2Py$

Scheme 5.22 General methods leading to anomeric radicals formation



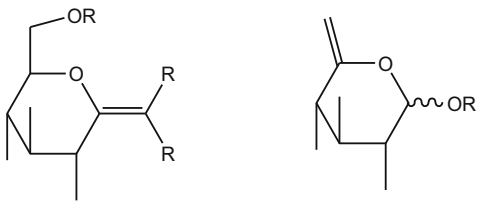
i) AIBN, Bu_3SnH.

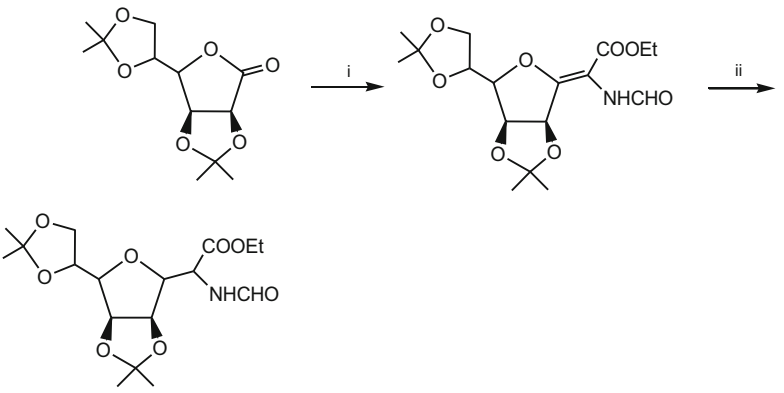
Scheme 5.23 Free radical coupling reaction

Scheme 5.24 Synthesis of 2-deoxy sugars by reductive 1,2-migration of glycosyl bromide

X = Br
R = OAc, OBz, $OPO_3(Ph)_2$

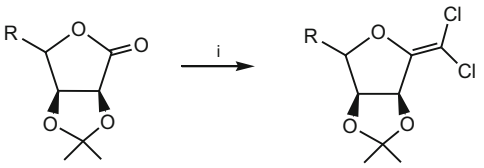
Scheme 5.25 1,2- and 5,6- unsaturated sugars



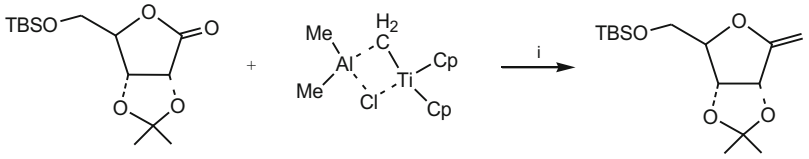


i) a) $EtOOCCH_2NC$, KH.b) AcOH. ii) a) H_2, Pd/Cb) H_2O.

Scheme 5.26 First synthesis of 1,2-unsaturated sugar



i) $P(NMe_2)_3$, CCl_4,- 30-0°C.



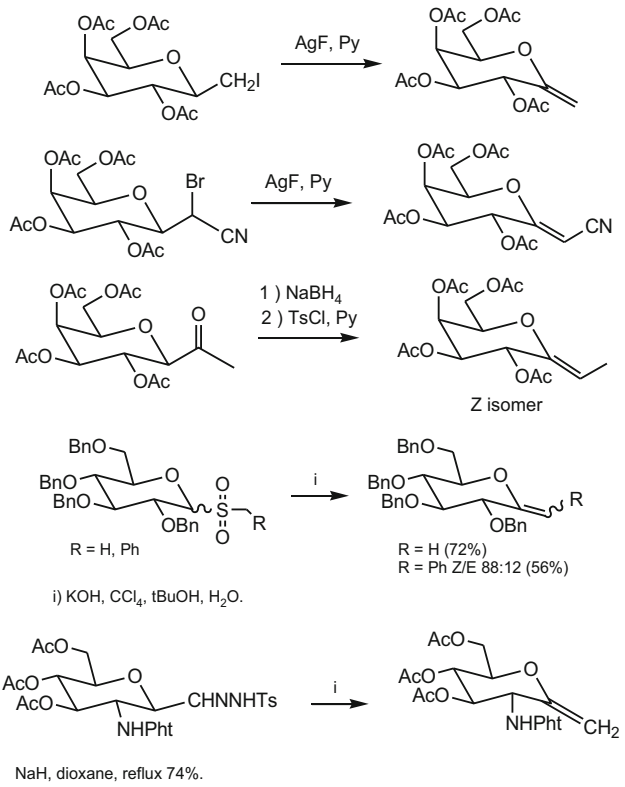
i) THF, Tol, -40°C.

Scheme 5.27 Early methods for preparation of exo-glycals

They were first prepared by reacting lactones with ethyl isocyanoacetate and subsequent hydrogenolysis [48, 49] (Scheme 5.26). This reaction has not been exploited extensively due to sugar oxazole formation.

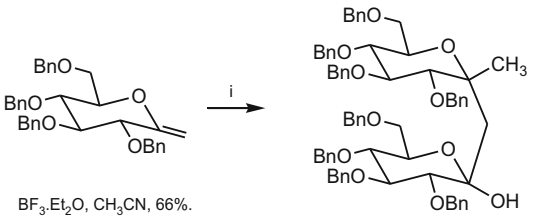
More recently two methods have been reported for direct olefination of lactones. One is based on phosphorous Wittig type reaction [50] and the other by direct methylenation using the Tebbe reagent [51] (Scheme 5.27).

Alternative methods for the preparation of exo-glycals include β-elimination of halides [52, 53], dehydration (Grignard nucleophilic addition, sulfone extrusion (Ramberg–Bäcklund olefination) [54], and tosyl hydrazones (Bamford–Stevens conditions) [55] among others (Scheme 5.28).



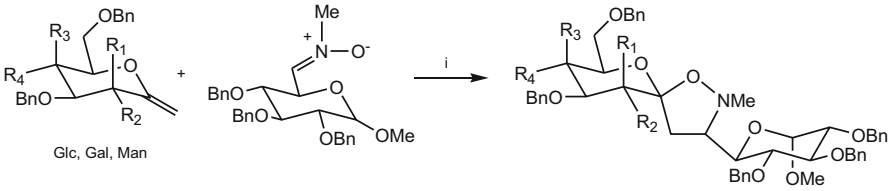
Scheme 5.28 Alternative methods for the preparation of exo-glycals

Scheme 5.29 Preparation of *C*-disaccharide from exo-glycals



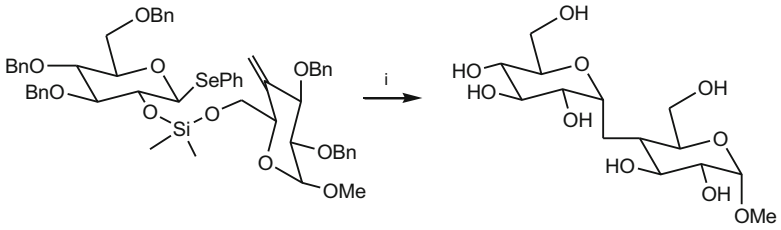
The synthesis of several *C*-disaccharides by using exo-glycals has been described. Such is the case of the preparation of *C*-disaccharide by reaction of two molecules of the *C*-methylene intermediate under Lewis acid conditions (Scheme 5.29). The reaction was proposed to proceed via oxonium cation [56].

A 1,3-dipolar cycloaddition of exo-methylene sugar with glycosyl nitrone has been proposed as an approach for the formation of amino-*C*-ketosyl disaccharides (Scheme 5.30) [57].



i) $PhCH_3$, reflux.

Scheme 5.30 Dipolar cycloaddition of exo-glycals



i) a) Bu_3SnH, AIBN, PhH, 60°C. b) HF, THF.c) H_2,Pd/C, MeOH, AcOEt.

Scheme 5.31 *C*-glycoside construction under the tether approach

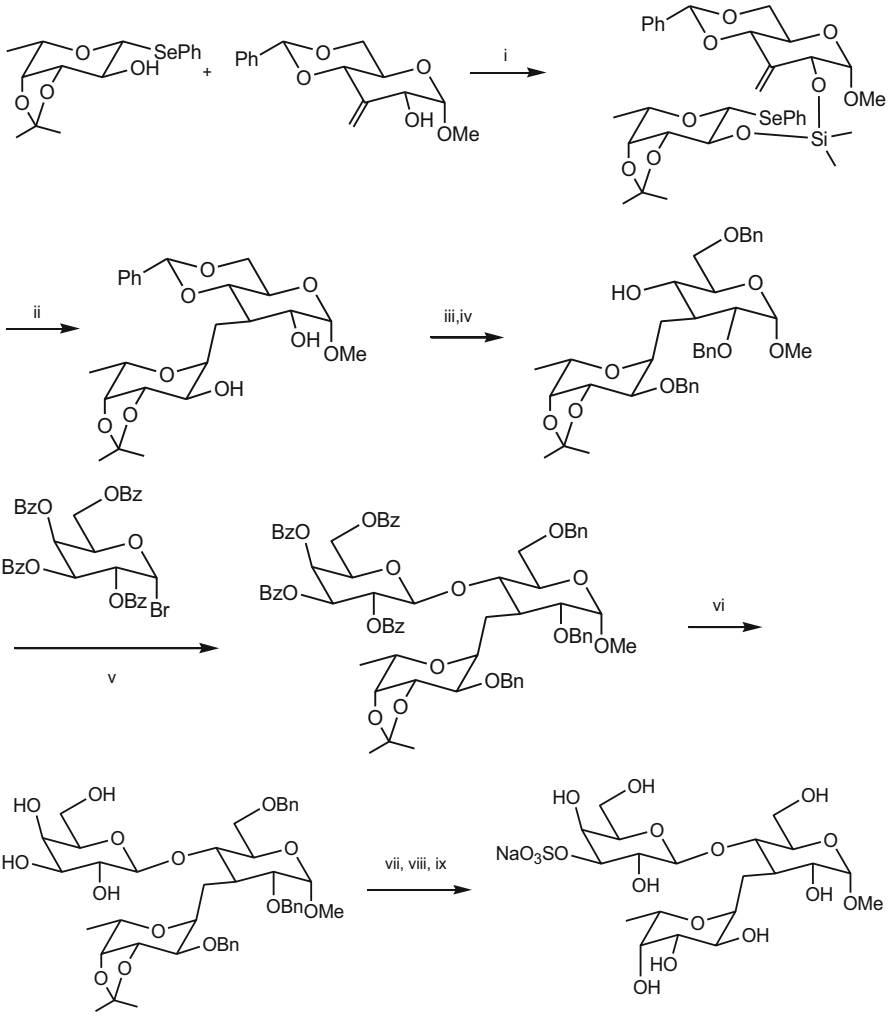
5.1.11 The Tether Approach

Various approaches for *C*-glycoside construction are comprehensively reviewed focusing mainly on the methylene formation [58]. The strategies presented are based on the concept that a nucleophilic anomeric donor is condensed with an exomethylene sugar to produce a *C*-disaccharide linkage [59]. According to this strategy methyl α-*C*-isomaltoside was prepared from the silaketal connected precursor as shown in Scheme 5.31.

The tether approach considers the preliminary formation of a temporary attachment usually involving a silyl protecting group, as tether which is cleaved after formation of he desired C–C bond. The general conditions involve the use of selenoglucopyranosides [60] or phenylsulfoxides [45, 61] as glycosyl donors. An important application of this methodology can be seen in the preparation of O-C mixed sulfated trisaccharide (Scheme 5.32) [13].

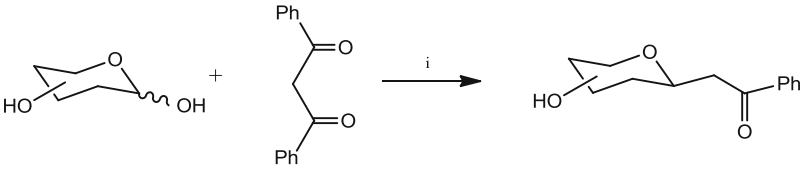
5.1.12 Unprotected Sugars

Direct coupling reaction between unprotected aldoses and aglycones such as dibenzoylmethane gave aryl ketone β-*C*-glycosides in good yields when treated with sodium bicarbonate base and a mixture of ethanol and water and subjected to microwave irradiation (Scheme 5.33) [62].



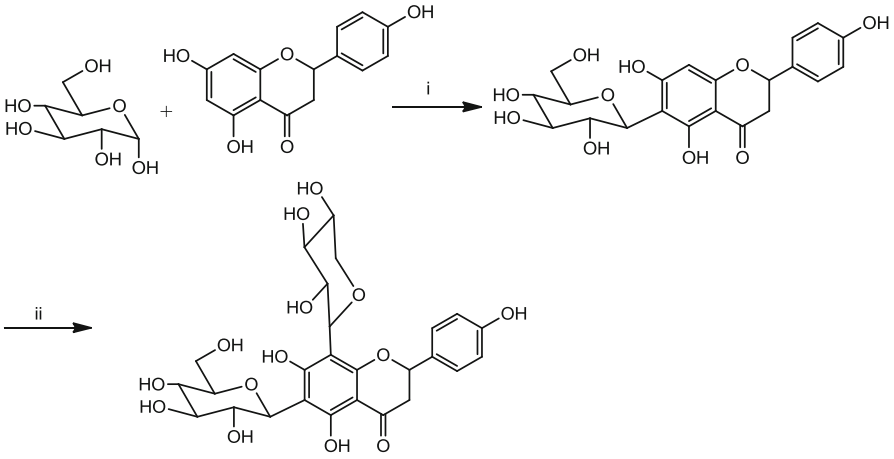
i) a) BuLi;Me_2SiCl_2 (4.4equiv.), THF, -78ºCto20ºC. b) imidazole,THF, r.t. ii) Bu_3SnH (2 equiv.), AIBN, PhMe, 110ºC, 17h. then Bu_4NF, THF, r.t., 60%. iii) BnBr, NaH, DMF, 100%. iv)$NaBH_3CN$, HCl, 70%. v) AgOTf, collidine, MS. 80%. vi) MeONa/MeOH, 97%. vii) Bu_2SnO, MeOH. viii)SO_3/Me_3N, 70%, 3steps. ix) H_2, Pd/C, 100%.

Scheme 5.32 Preparation of sulfated *C*-trisaccharide under the tether methodology



i) $NaHCO_3$, EtOH-H_2O (4:1), MW

Scheme 5.33 Synthesis of aryl ketone β-*C*-glycosides under microwave irradiation



i) $Sc(OTf)_3$, CH_3CN/H_2O (2:1), reflux, 12h 22% yield. ii) D-Xylose, $Sc(OTf)_3$, $EtOH/H_2O$ (2:1), 80°C

Scheme 5.34 Synthesis of *C*-glycoside flavonoid Vicenin-3 from unprotected monosaccharides

The *C*-glycoside flavonoid Vicenin-3 was prepared with high regioselectivity by condensation of naringenin with unprotected D-glucose and D-xylose in the presence of scandium trifluoromethanesulfonate although providing moderate yields (Scheme 5.34) [63].

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Chapter 6
Glycoconjugates

Carbohydrates covalently attached to proteins and lipids constitute three types of glycoconjugates: proteoglycans, glycoproteins, and glycolipids. Although in the first two cases the types of linkages are the same, chemically proteoglycans behave as polysaccharides and glycoproteins having much less carbohydrate content as proteins. The third important class of glycoconjugates, where carbohydrate residues are covalently attached to a lipidic component, has been classified into four types depending on the lipidic nature: glycoglycerol, glycosyl polyisoprenol pyrophosphates, fatty acid esters, and glycosphingolipids [1].

The most common monosaccharides residues found in glycoconjugates are D-galactose, D-mannose, *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine, L-fucose, D-xylose, and sialic acids (Scheme 6.1).

6.1 Biological Function and Structural Information

Glycoproteins and glycolipids are major components of the outer surface of mammalian cells. The former has been implicated in several essential events such as immune defense, viral replication, cell–cell adhesion, inflammation, and cell growth, while the latter has been implicated in cell–cell recognition, growth, differentiation, and interaction with proteins of viral and bacterial pathogens.

The first recognition of carbohydrates as biological signals is attributed to the discovery of hepatic Gal/GalNAc-binding receptor [2]. Subsequently Man-6-phosphate receptor for lysosomal enzymes and Man-receptor from alveolar macrophages were reported and investigated [3, 4].

In cellular immune system, some specific glycoproteins are implicated in the folding, quality control, and assembly of peptide-loaded major histocompatibility complex antigens and the T cells receptor complex. Furthermore, the oligosaccharides linked to glycoproteins provide protease protection, ER-associated retrograde transport of misfolded proteins, loading of antigenic peptides into MHC class I, and

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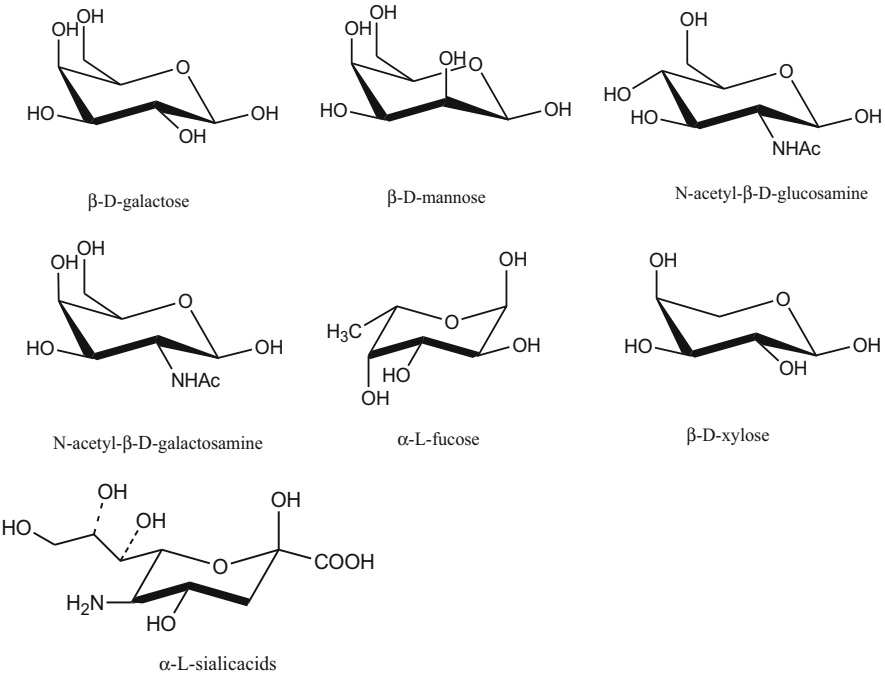


Scheme 6.1 Monosaccharides residues of glycoproteins

influence the range of antigenic peptides generated in the endosomal pathway for presentation by MHC class II [5].

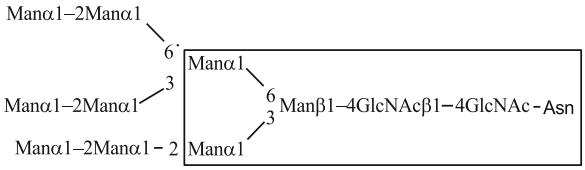
In addition, enveloped viruses such as human immunodeficiency virus (HIV) evade immune response by exploiting the host glycosylation machinery to protect potential antigenic epitopes [6]. They also use the host secretory pathway to fold and assemble their often heavily glycosylated coat proteins.

Another important fact to mention is that normal cells and tumor cells have evident differences in glycoprotein content of their cell membranes. Altered glycoproteins of the tumor membranes such as Thomsen–Friedenreich (T antigen) are tumor-associated antigens and belong to the class of *O*-glycoproteins [7–9].

6.1.1 Classification of Glycocoproteins

Based on the type of the glycosidic bond formed between the sugar and the protein residues, glycoproteins are divided into N- and O-glycans. The first type involves a glycosidic linkage between asparagine and *N*-acetylglucosamine and the second involves an *O*-glycosidic linkage between the sugar residue (fucose, galactose, *N*-acetylgalactosamine, and *N*-acetylglucosamine) and the oxygen in the side chain serine, threonine, or hydroxyl lysine.

First type



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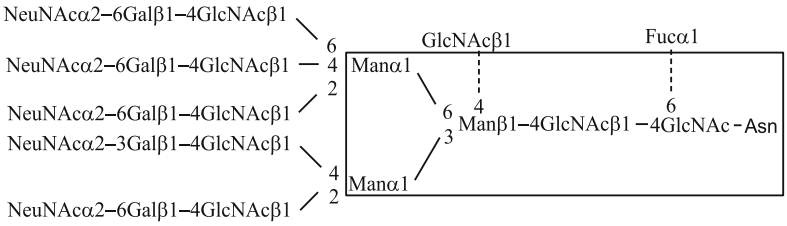

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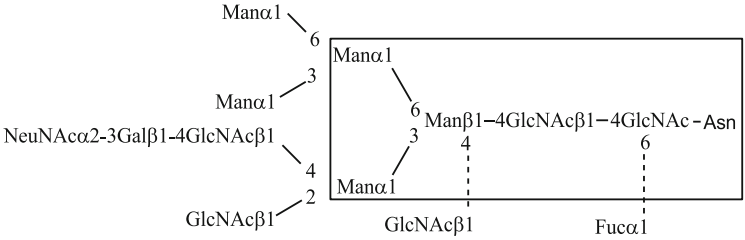
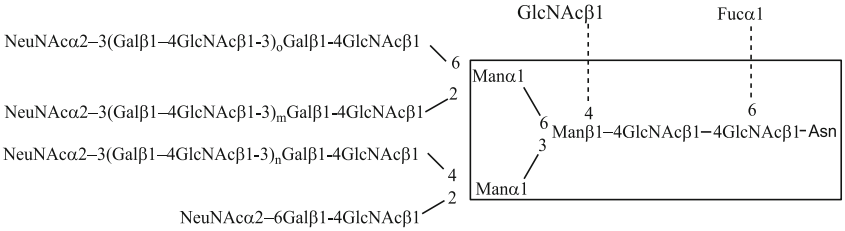


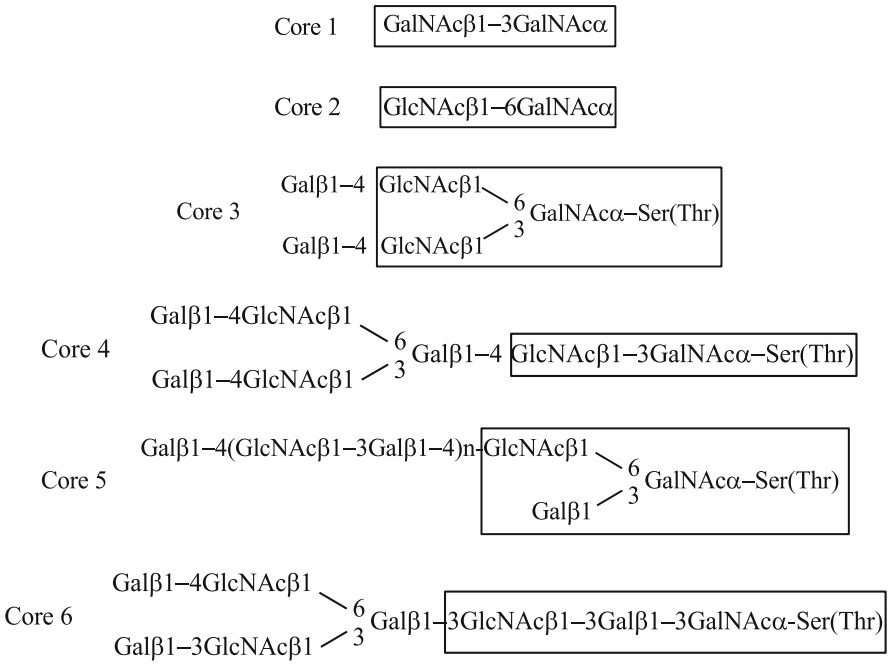
Fourth type



Scheme 6.2 Four groups of N-linked glycans

It is known that N-linked glycans contain the pentasaccharide Manα1-6(Manα1-3) Manβ1-4GlcNAcβ1-4GlcNAc as a common core, and they have been classified into four main groups on the basis of the structure and the location of glycan residues added to the trimannosyl core: oligomannose, complex, hybrid, poly-*N*-acetylglucosamine (Scheme 6.2) [10].

O-glycans do not present common core structures and until now they have been classified into at least six groups according to different core structures (Scheme 6.3).



Scheme 6.3 Core structures in O-linked glycans

6.1.2 Recognition Sites

There are two main classes of glycosidic linkage depending on the type of glycosidic bond formed between the sugar residue and the protein: the O-linked glycans involving the amino acids serine, threonine, and hydroxyl lysine, and N-linked glycans involving the amino acid asparagine in the form of tripeptide with sequence AsnXSer (where X is any amino acid except proline).

Thorough studies with sugar analogs indicate that presumably the most important of the substituents is the equatorial OH-group on carbon 3. Also important is the OH-group on carbon 4 which can be either axial or equatorial depending on the type of glycoprotein. Regarding C-2, there is certain tolerance; however, the size of the group should not be too large. Finally C-6 and the anomeric carbon apparently do not play a significant role in the binding (Table 6.1) [11].

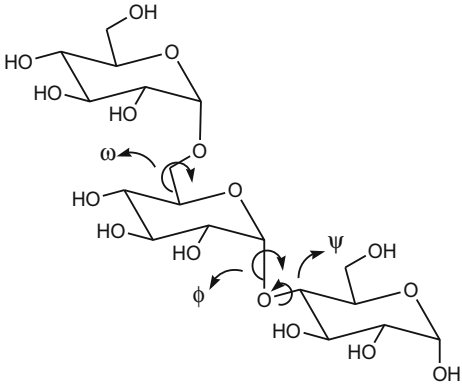
6.1.3 Structural Information of Glycoproteins

A better understanding about the conformation of glycoproteins has been reached by using NMR, molecular dynamics (MD), and in some cases X-ray diffraction techniques. The high motion of oligosaccharides mainly across the glycosidic

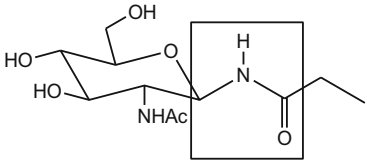
Table 6.1 Sugar requirements for three different glycoproteins

	Rat hepatic	Chicken hepatic	MBP-A
1		$\alpha \approx \beta$ large substituents tolerated, negative group	
	Detrimental	Enhancing	Tolerable
2	Eq. N-Ac enhance binding	Eq. N-Ac enhance binding	No effect by N-Ac
3		Eq. OH required	
4	Axial OH required	Eq. OH required	Eq. OH required
5		Large substituents accepted	

Scheme 6.4 Angles of rotation of carbohydrates



Scheme 6.5 Planarity of the Asn-GlcNAc glycosidic linkage



linkage (Scheme 6.4) has limited the unambiguous conformational determinations in glycoproteins; however, the conformations from the MD simulations are in good agreement with the values from NMR studies. It has been observed that ω-angle prefers *Gauche* conformation by solvation effects with ϕ-angle largely determined by the anomeric effect, and the ψ-angle highly influenced by non-bonded interactions [12].

The linkage between the sugar residue and the amino acid asparagine (N-linked glycans) is planar along the C1-NH-C=O glycosidic linkage and flexible along the CO-CH_2-CH- bonds (Scheme 6.5).

Based on the considerations that *N*-glycosidic linkage is rigid for the amide group and flexible for the side chain angles, the conformational motion of the glycoproteins depends on the flexibility of the asparagine side chain. This flexibility will have considerable effect on the volume occupied by the sugar and the shielding effects of the carbohydrate over the protein surface.

Hydrogen bond and van der Waals interactions showed for some cases stacked conformations, and distances across a carbohydrate residue (from O-1 to O-4) of 5.4 Å and for the first three residues of the core of an N-linked oligosaccharide extend to approximately 16 Å from head to tail [12].

6.2 Carbohydrate-Binding Proteins

Carbohydrate-binding proteins are defined as those proteins that interact through non-covalent bonds with carbohydrates. Of particular interest are lectins which bind reversibly to monosaccharides and oligosaccharides with high specificity, and are apparently devoid of catalytic activity [13].

Carbohydrate-binding proteins are widespread macromolecules found in viruses, bacteria, plants, and animals and act as recognition determinants including clearance of glycoproteins from the circulatory system, control of intracellular traffic of glycoproteins, recruitment of leukocytes to inflammatory sites, adhesion of infectious agents to host cells, and cell interactions in the immune system in malignancy and metastasis [13].

Depending on the affinity showed toward the type of monosaccharide they can be classified into mannose, galactose/*N*-acetylgalactosamine, *N*-acetylglucosamine, L-fucose, and *N*-acetylneuraminic acid. Due to their high specificity, lectins specific for galactose do not recognize glucose or mannose, nor *N*-acetylglucosamine with *N*-acetylgalactosamine, but mannose-specific animals lectins do recognize fucose.

Lectins also exhibit high specificity for disaccharides, trisaccharides, and tetrasaccharides and some interact only with oligosaccharides. Moreover different lectins specific for the same oligosaccharide may recognize different regions of its surface. Some of the lectins and their affinity ligands are shown in Table 6.2.

High resolution studies involving the protein sequence determination and three-dimensional analysis have given insight about the structure and molecular interaction between the sugar ligands and the proteins. As result of this structural analysis, it has been observed on the basis of common structural features that lectins fall into three main categories:

(a) Simple
(b) Mosaic or multidomain
(c) Macromolecular assemblies

Simple lectins are most of known plant lectins (legumes, cereals, Amaryllidaceae, Moraceae, Euphorbiaceae), animal lectins (galectins or formerly S-lectins), and Pentraxins, and contains a small number of nonidentical subunits of molecular weight below 40 kDa.

Mosaic or multidomain lectins include viral hemagglutinins and animal lectins C (endocytic lectins, collectins, selectins), P, and I types. Their molecular weight is variable and they are formed by different protein domains, only one of them with the carbohydrate binding site.

Table 6.2 Lectins and affinity ligands

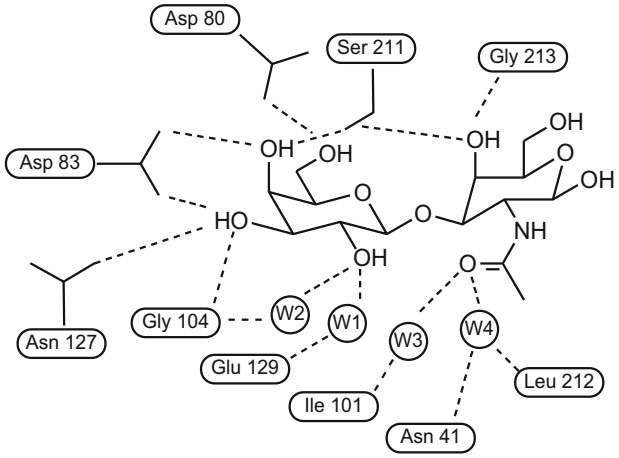
Family	Lectin	Abbreviation	Ligand
Legumes (plant lectins)	Concanavalin	ConA	MeαMan
			MeαGlc
			Manα3(Manα6)Man
	Erythrina corallodendron	EcorL	Galβ4Glc
	Fava bean	Favin	MeαMan
	Griffonia simplicifolia	GSIV	Fucα2Galβ3(Fucα4)GlcNAc
	Red kidney bean	PHA	Complex pentasaccharide
	Lathyrus ochrus	LOL I,II	Manα3Manβ4GlcNAc, complex octasaccharide
	Lentil	LCL	MeαMan, MeαGlc
	Pea	PSL	Manα3(Manα6)Man
	Peanut	PNA	Galβ4Glc
	Soybean	SBA	Biantennary pentasaccharide
Cereals	Wheat germ	WGA	NeuAc(α2-3)Galβ4Glc
			GlcNAcβ4GlcNAc
			sialoglycopeptide
Amaryllidaceae	Snow drop	GNA	MeαMann
			mannopentaose
Moraceae	Artocarpus integrifolia	Jacalin	MeαGal
Galectins (animal lectins)	Human hart	Galectin 1	Galβ4GlcNAc
			octasaccharide
	Rat liver	Galectin 2	Galβ4Glc

Macromolecular assemblies are common in bacteria and usually present in the form of fimbriae which are filamentous, heteropolymeric organelles present on the surface of bacteria [14].

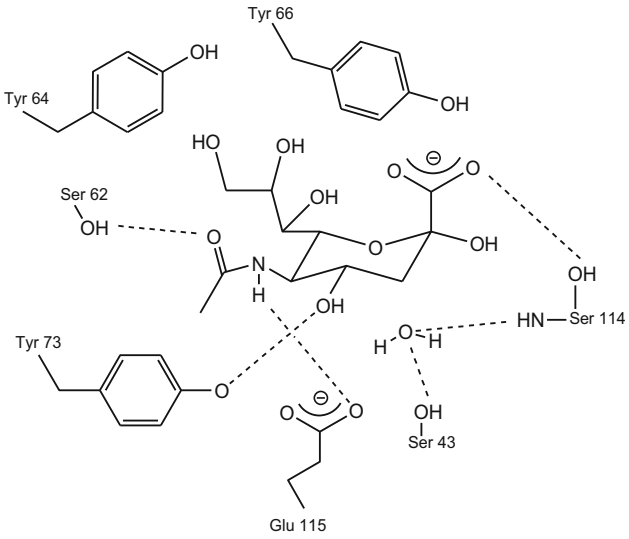
Most plants lectins recognize and interact with terminal nonreducing units of oligosaccharides and polysaccharides, glycoproteins, and glycolipids. Anomeric preference is an important finding observed for different carbohydrate-binding proteins; for instance all mannose/glucose binding lectins display great preference for the α-anomeric forms [15]; however, lectins from *Ricinus communis* bind preferentially to β-galactosidases, while other lectins show no difference in binding to anomers of GalNAc and GlcNAc. A considerable amount of structural information about carbohydrate-binding proteins such as the complete amino acid sequences for various lectins is available [13, 16].

6.2.1 Combining Sites

Lectins combine with carbohydrates mainly through weak forces such as hydrogen bonding, coordination with metal ions and hydrophobic interactions. The hydrogen bridge interaction is established between the carbohydrate hydroxyl groups and the



Scheme 6.6 Gal(β1 → 3)GalNAc in the combining site of peanut agglutinin

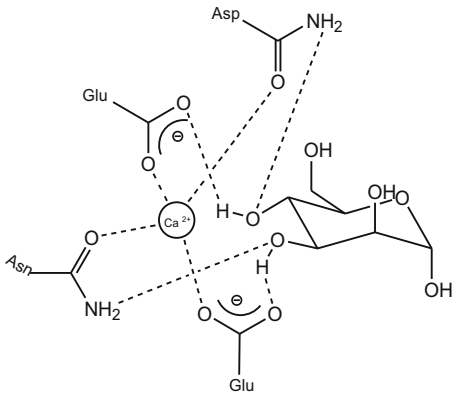


Scheme 6.7 Sialyllactose in the combining site of wheat germ agglutinin

amino groups. Additionally, contacts between the carbohydrate and the protein are mediated by water bridges (Scheme 6.6) [17].

Although carbohydrates are essentially polar molecules, there is a significant share of nonpolar or hydrophobic interactions which occur between the *N*-acetyl group of amino sugars and the glycerol moiety of neuraminic acid, and the aromatic amino acids phenylalanine, tyrosine, and tryptophan. In the combining site of wheat germ agglutinin with sialyllactose several van der Waals contacts stabilize the orientation of the sugar ring through nonpolar stacking interactions with the aromatic side chain of Tyr64, and Tyr66 that interacts through non-polar with the glycerol tail of the *N*-acetyl neuraminic acid (Scheme 6.7) [18].

Scheme 6.8 Mannose binding protein C with bound mannose



Several classes of lectins are ion dependent for their functional interaction with the ligands. Divalent ions such as calcium and manganese participate in the stabilization of the amino acid positions that interact with the sugars. The Ca^{2+} ion establishes a coordination bond with the carbonyl group of asparagine and with one carboxylate oxygen of an acidic amino acid. The Mn^{2+} does not coordinate any residues that interact directly with the protein, but is involved in fixing the Ca^{2+} position (Scheme 6.8) [16, 19].

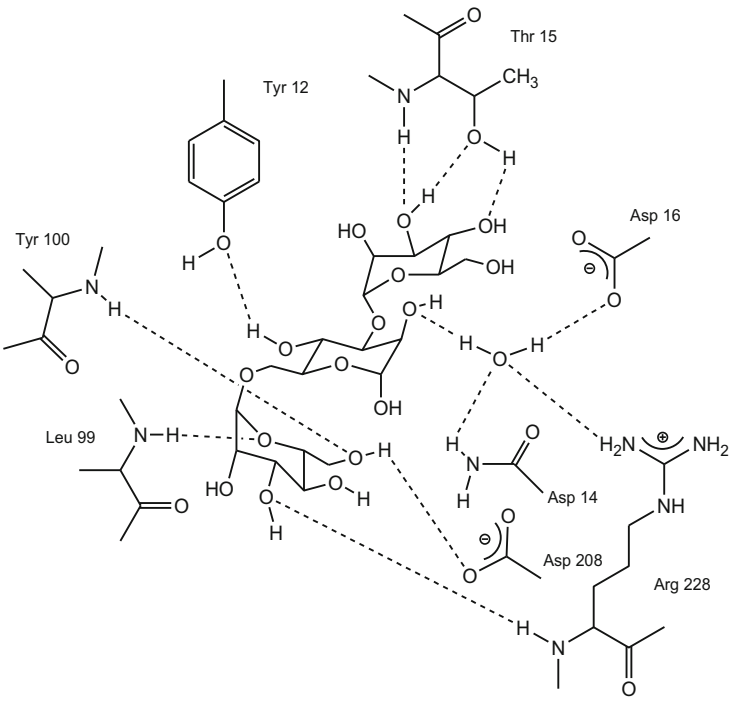
In the interaction of concanavalin A with the branched trisaccharide Man(α1-6)[Man(α1-3)]Man, several hydrogen bond contacts between the hydroxyl group of the sugar and the amino acid residues are observed. Some of these interactions are bifurcated or involve water and contribute importantly to the recognition process (Scheme 6.9) [13, 20].

Carbohydrate-binding proteins are classified into two types: calcium dependent (C-type glycoproteins), and thiol reagent dependent (S-type). The former are structurally more diverse (although the binding region known as carbohydrate recognition domain CRD is highly conserved) and more specific to organs and tissues, while the latter are structurally more conserved and are more widespread among the organs and tissue examined [21]. Other carbohydrate-binding proteins that do not fall into these two categories are fibronectin and laminin, serum immunoglobulins, mannose-phosphate receptor, viral hemagglutinins, and serum amyloid protein.

Another important class of carbohydrate binding proteins are known as **selectins** (classified as E-, P-, and L-selectins) and are defined as nonenzymatic and nonimmune proteins involved in the leukocyte recruitment to sites of inflammation [22, 23]. It has been found that the tetrasaccharide sialyl $Lewis^X$ is the recognition molecule and the use of synthetic sialyl $Lewis^X$ confirmed the hypothesis that sulfation increase the affinity for L-selectins [24].

6.3 Glycopeptide Synthesis

The design of glycopeptides requires a combination of sugar and peptide chemistry, a substantial part being the installation of the *O*- or *N*-glycosidic bond [25, 26] The synthetic approach is in principle designed on the basis of the glycosidic bond

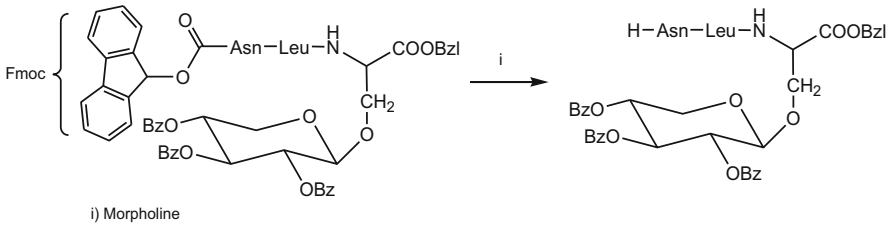


Scheme 6.9 Trimannoside binding site of Concanavalin A

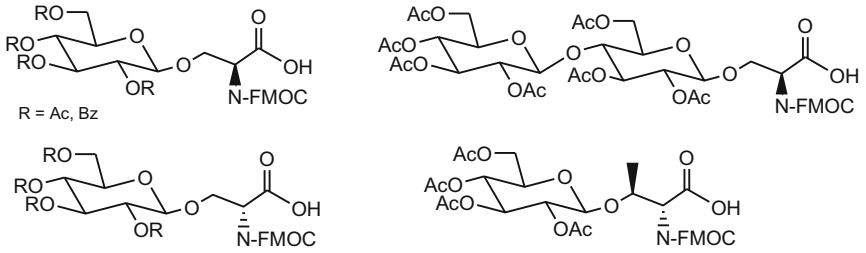
required. Thus, while in the case of *O*-glycopeptides, the synthetic methods relies on the common strategies for the preparation of *O*-glycosides, for the preparation of *N*-glycopeptides the strategy of choice involves the coupling between the amino glycosyl donor with aspartate in the presence of a condensing agent or by enzymatic catalysis.

Compatibility between the protecting groups and the glycosidic bond when they are subjected to different reaction conditions such as acid or base conditions is a sensitive issue. For instance it is known that the glycosidic bond in acetals is acid sensitive; however, in the case of *O*-glycosyl serine and threonine they conversely present base-sensitivity. The introduction of selective protecting groups for amino acid functionalities which can be cleaved under mild conditions without affecting the glycoside bond or protecting groups attached to the sugar moiety is a feasible approach. Widely employed protecting groups for this purpose are the Fmoc protecting group (9-fluorenyl)methoxycarbonyl), Pyroc (2-(pyridyl)ethoxycarbonyl), and Aloc (allyloxycarbonyl) for the peptide and MPM (4-methoxy-benzyl ether) for the sugar region. The conditions needed for the cleavage of the mentioned protecting group in the presence of other functionalities are indicated in Scheme 6.10 [27, 28].

The synthesis of N-α-FMOC amino acid glycosides was carried out with O'Donnell Schiff bases or with N-α-FMOC amino protected serine or threonine and



Scheme 6.10 Peptide protecting group Fmoc and removal conditions



Scheme 6.11 N-α-FMOC-amino acid glycosides

the appropriate glycosyl bromides under Koenigs–Knorr modified conditions [29]. The α-FMOC-protected glycosides were incorporated into 22 enkephalin glycopeptides analogs (Scheme 6.11).

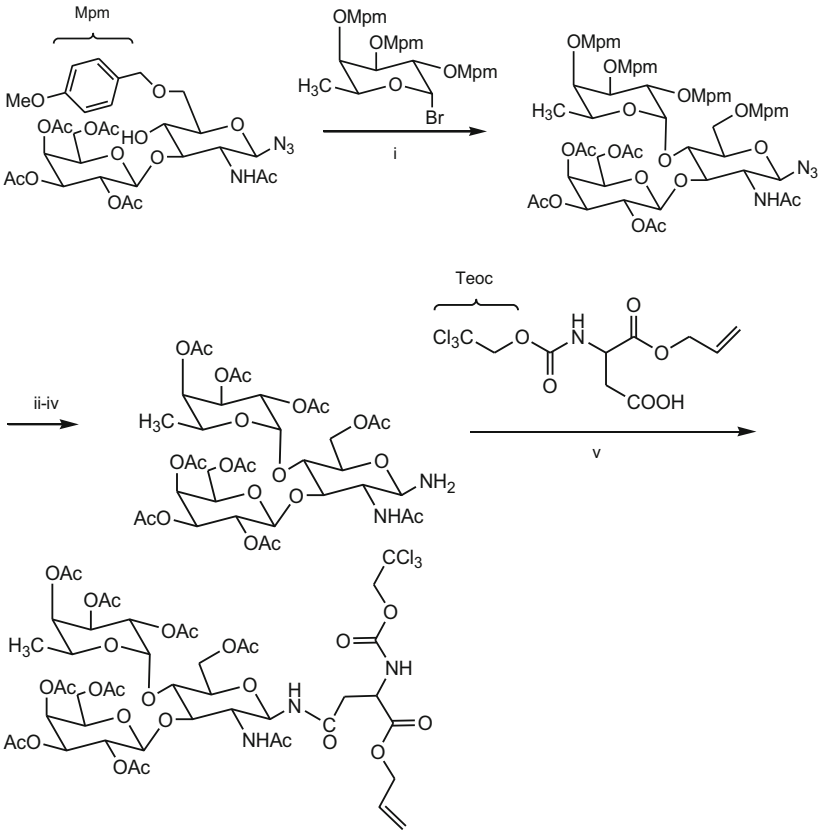
Pyroc is another protecting group useful in peptide chemistry. It is stable to acids, bases, and hydrogenolysis, but sensitive to morpholine. The allylic protecting group Aloc is also stable to acids, bases and can be removed under of Pd(0) catalysis or weak base as morpholine [28].

A tumor associated antigen Lewis[a] was synthesized by applying a combination of compatible sugar and peptide protecting groups. For this method the azide group was used as anomeric amine precursor (Scheme 6.12) [29].

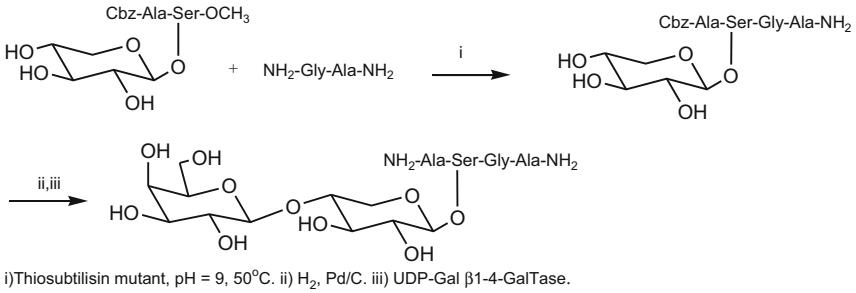
Enzymes have been useful for peptide elongation using an engineered *subtilisin* and disaccharide bond formation with glycosyltransferase as shown in Scheme 6.13 [30].

A novel chemoenzymatic synthesis of eel calcitonin glycopeptide analog having natural N-linked oligosaccharides such as disialo biantennary complex-type as model compound for glycoproteins has been described. Natural oligosaccharides are added by a transglycosylation reaction using endo-β-*N*-acetylglucosaminidase from *Mucor hiemalis* (Scheme 6.14) [31].

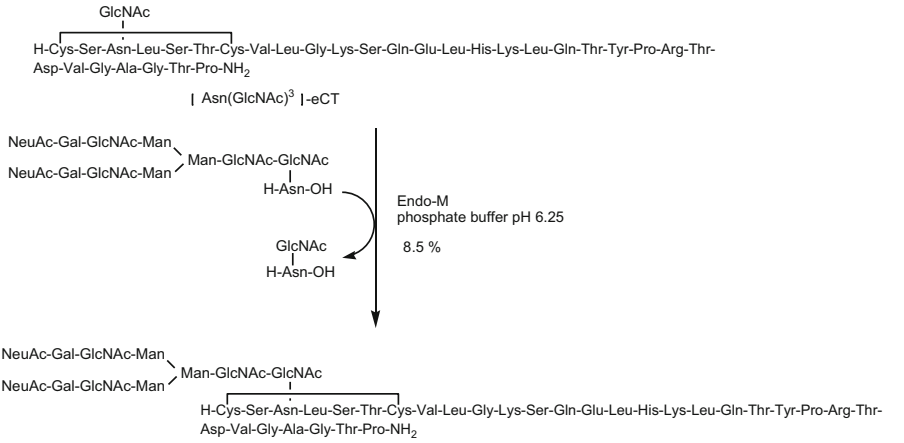
According to Sears and Wong [32] there are three basic approaches for preparing glycopeptides with complex glycans. (1) A converged method consisting in the independent preparation of the sugar and peptide components, and later assembled. (2) The preparation of the sugar attached to an amino acid using glycopeptide chemistry and simultaneously peptide linked to a glycal. (3) Solid-phase synthesis of the glycopeptide and chemoenzymatic elaboration of the glycal (Scheme 6.15).



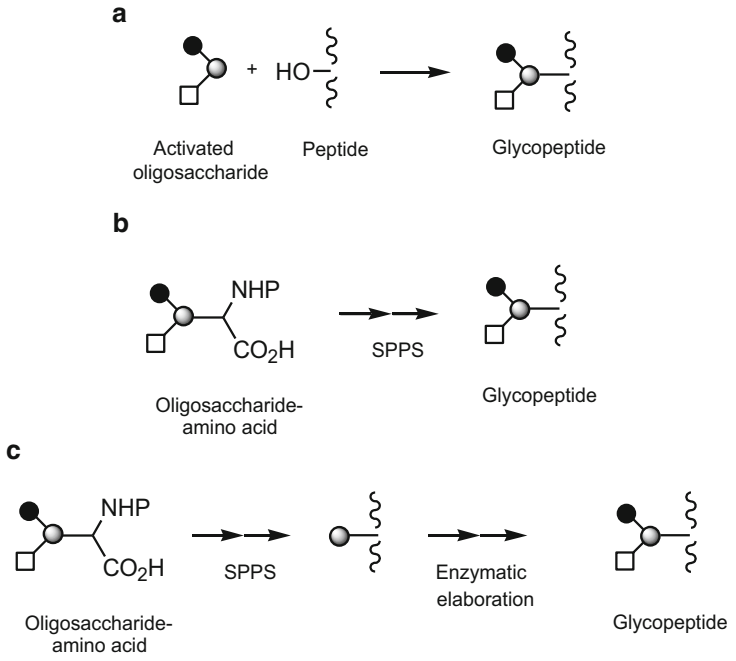
Scheme 6.12 Synthesis of glycopeptide Lewis[a]



Scheme 6.13 Chemoenzymatic synthesis of glycopeptide



Scheme 6.14 A transglycosylation reaction for preparation of glycopeptide



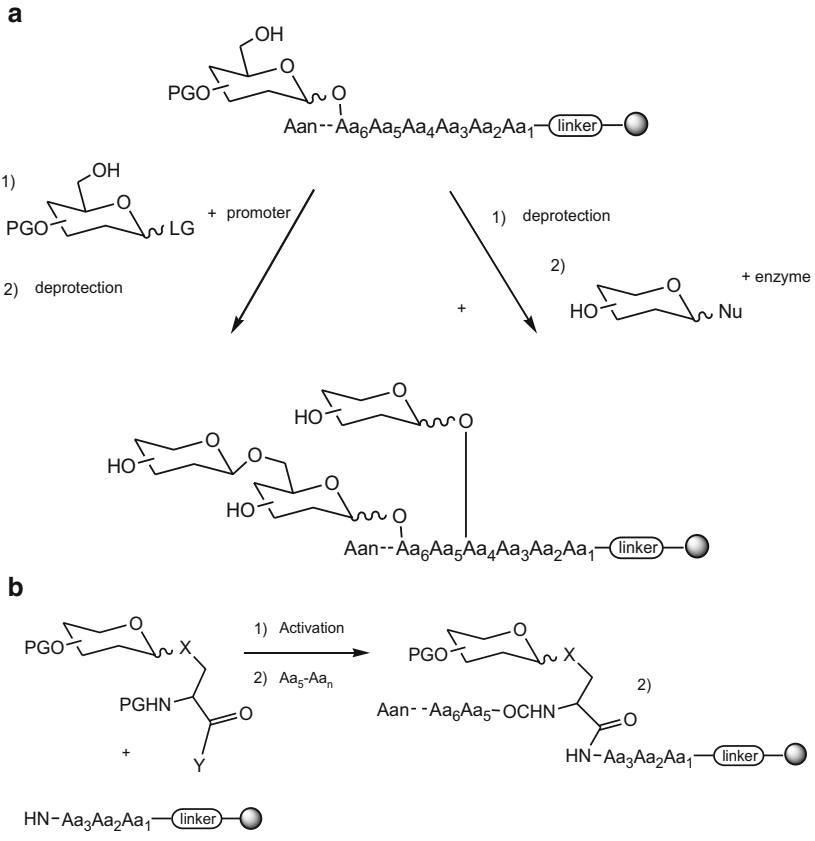
Scheme 6.15 Proposed general approaches for glycopeptide synthesis

6.4 Glycoprotein Synthesis

Glycoproteins are essential macromolecules involved in a wide range of functions related to cellular recognition processes. Natural glycoproteins usually exist as a mixture of glycoforms, and have been found difficult to isolate for their structural characterization and for understanding more about their function [33, 34].

As mentioned, glycoproteins can be obtained by fermentation process; however, this natural approximation produces a population of many different glycoforms as result of the participation of many glycosidases and transferases for a given protein, although the mixture can be useful for preparing a homogeneous core which in turn might be re-elaborated enzymatically [32]. The synthetic preparation of glycoproteins can be considered to some extent in glycopeptide chemistry, although the complexity is undoubtedly superior. The synthesis of glycoproteins has received a considerable attention, resulting in studies involving a combination of chemical and enzymatic methods [34–40].

A general strategy proposed by Duus et al. [41] considers the assembly of glycosylated amino acid building blocks in solid-phase peptide synthesis according to the general scheme shown in Scheme 6.16.



Scheme 6.16 Strategies for glycopeptide synthesis

According to a comprehensive review the strategies described so far for chemical glycoprotein synthesis are: (a) indiscriminate glycosylation, (b) chemoselective and site-specific glycosylation, and (c) site-selective glycosylation [42, 43].

6.4.1 Indiscriminate Glycosylation

This nonselective approach consists in the preparation of sugars bearing functionalities that under proper conditions may react with a protein. Some of the sugar derivatives used for this purpose are shown in Scheme 6.17.

6.4.2 Chemoselective and Site-Specific Glycosylation

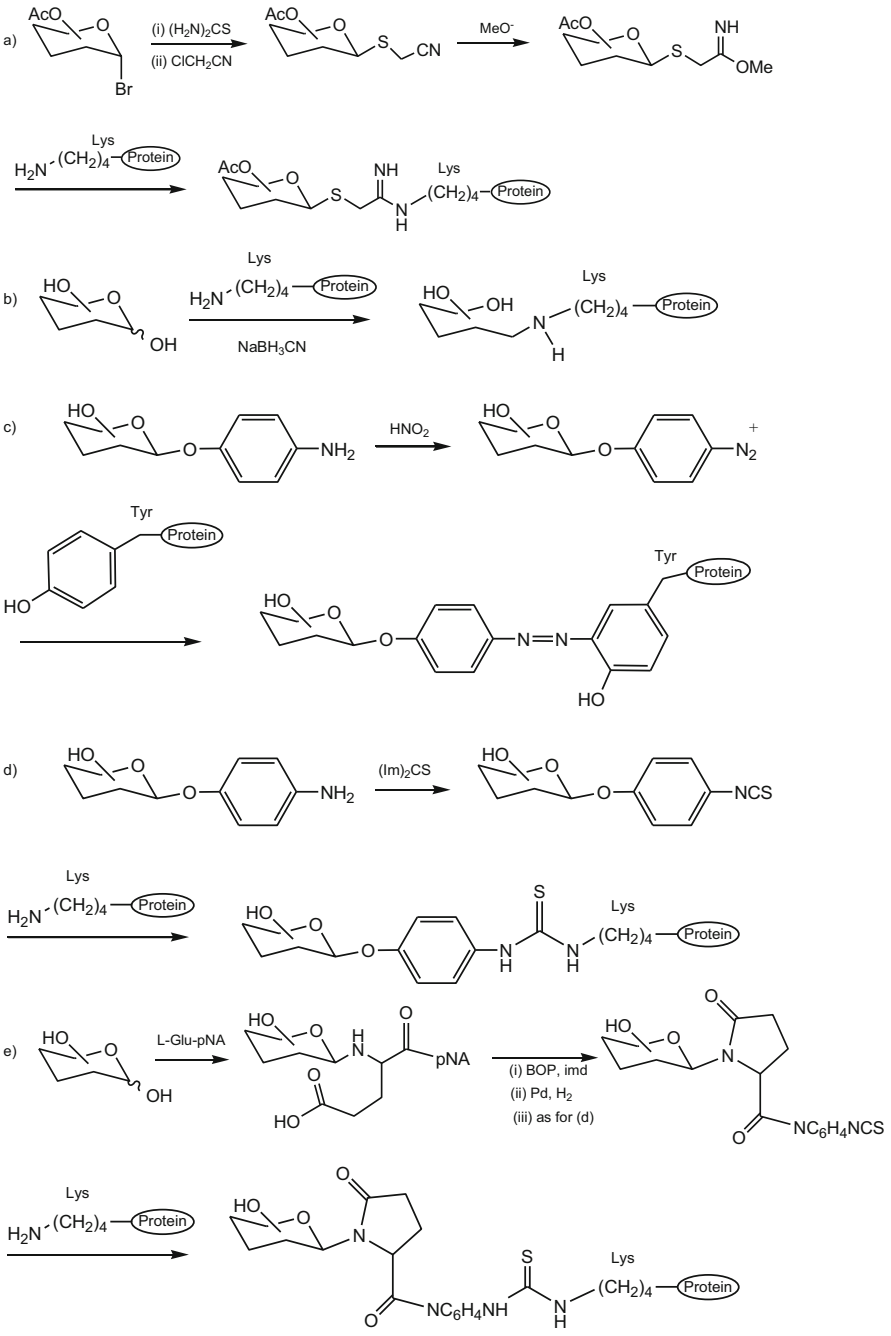
This approach intends to direct selectively the glycosidic linkage by using chemical and enzymatic tools. Such selectivity has been attempted under a strategy termed chemoselective ligation, and some enzymes involved in this strategy are galactose oxidase [53], horseradish peroxidase. Examples of these step reactions are indicated in Scheme 6.18.

6.4.3 Site-Selective Glycosylation

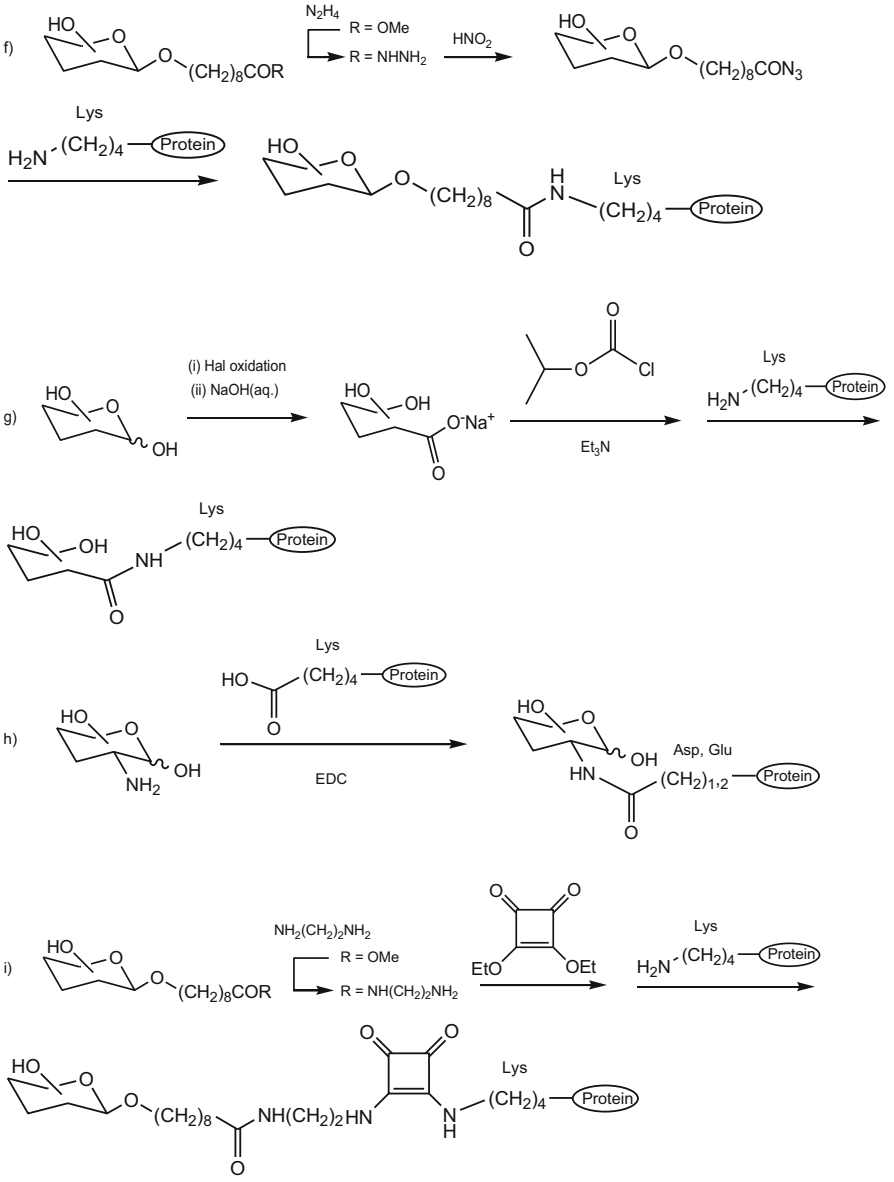
This possibility implies the choice of site selectivity on the glycan. In order to reach this goal a combined site-directed mutagenesis and chemical modification has been performed [62, 63]. This strategy involves the introduction of cysteine as chemoselective tag at preselected positions within a given protein and then reaction of its thiol group with glycomethanethiosulfonate (Scheme 6.19).

6.4.4 Lansbury Aspartylation

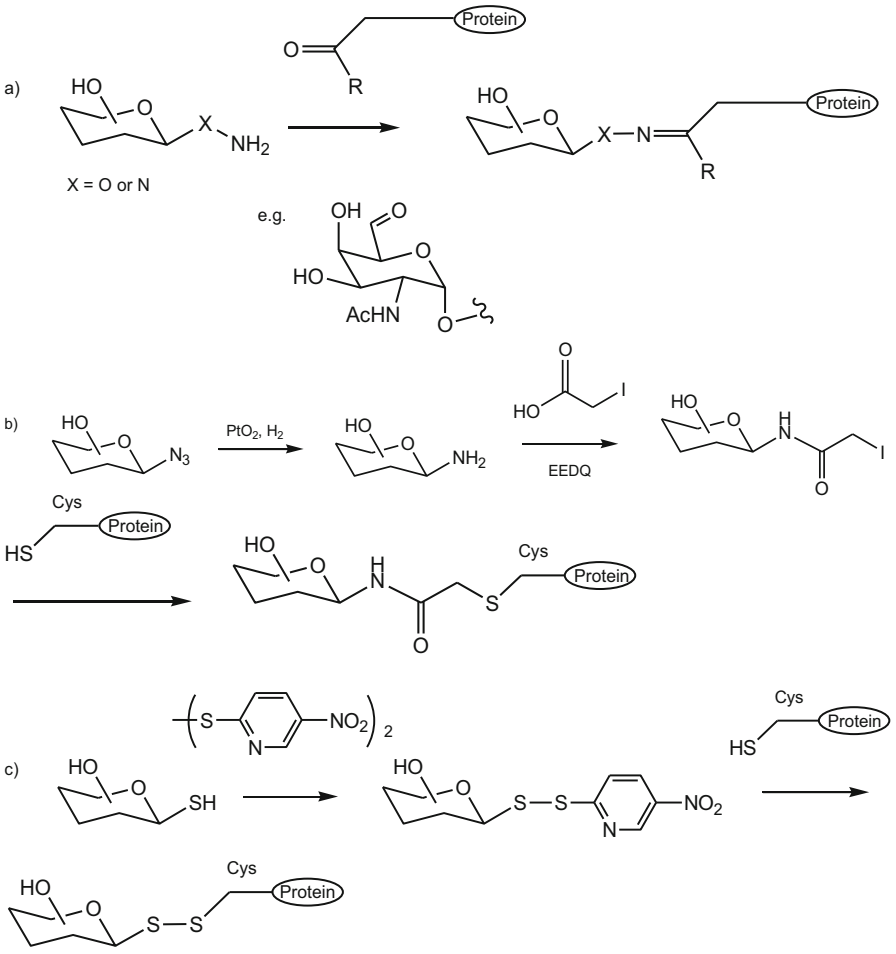
This reaction describes a nucleophilic attack of an amino saccharide with an unprotected amino saccharide. During the course of this reaction, cyclic aspartimides are also formed depending on the peptide sequence [64].



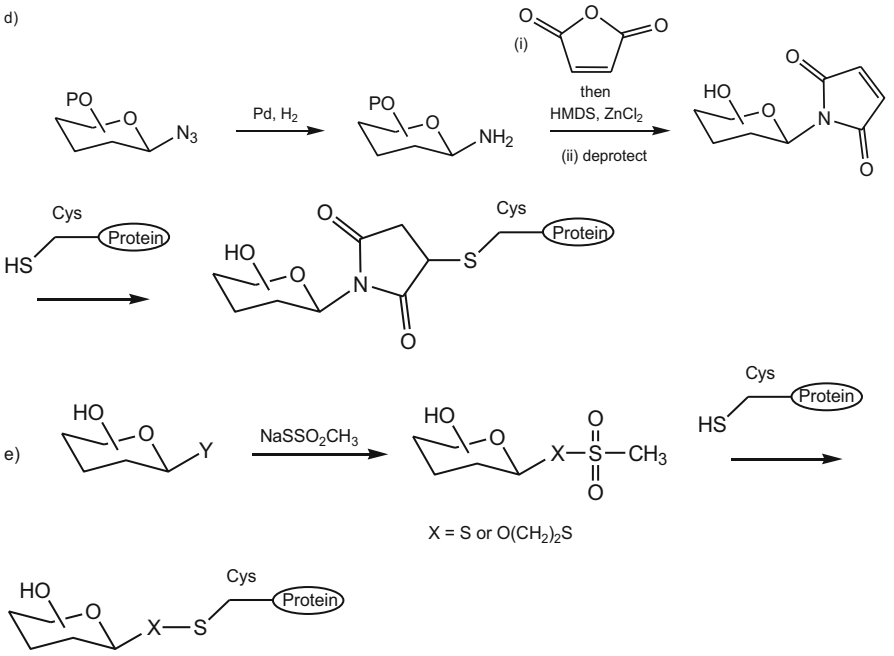
Scheme 6.17 Indiscriminate glycoprotein syntheses [44–52]



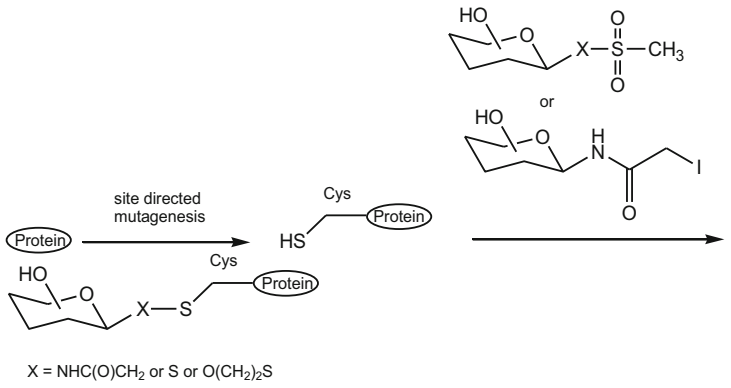
Scheme 6.17 (continued)



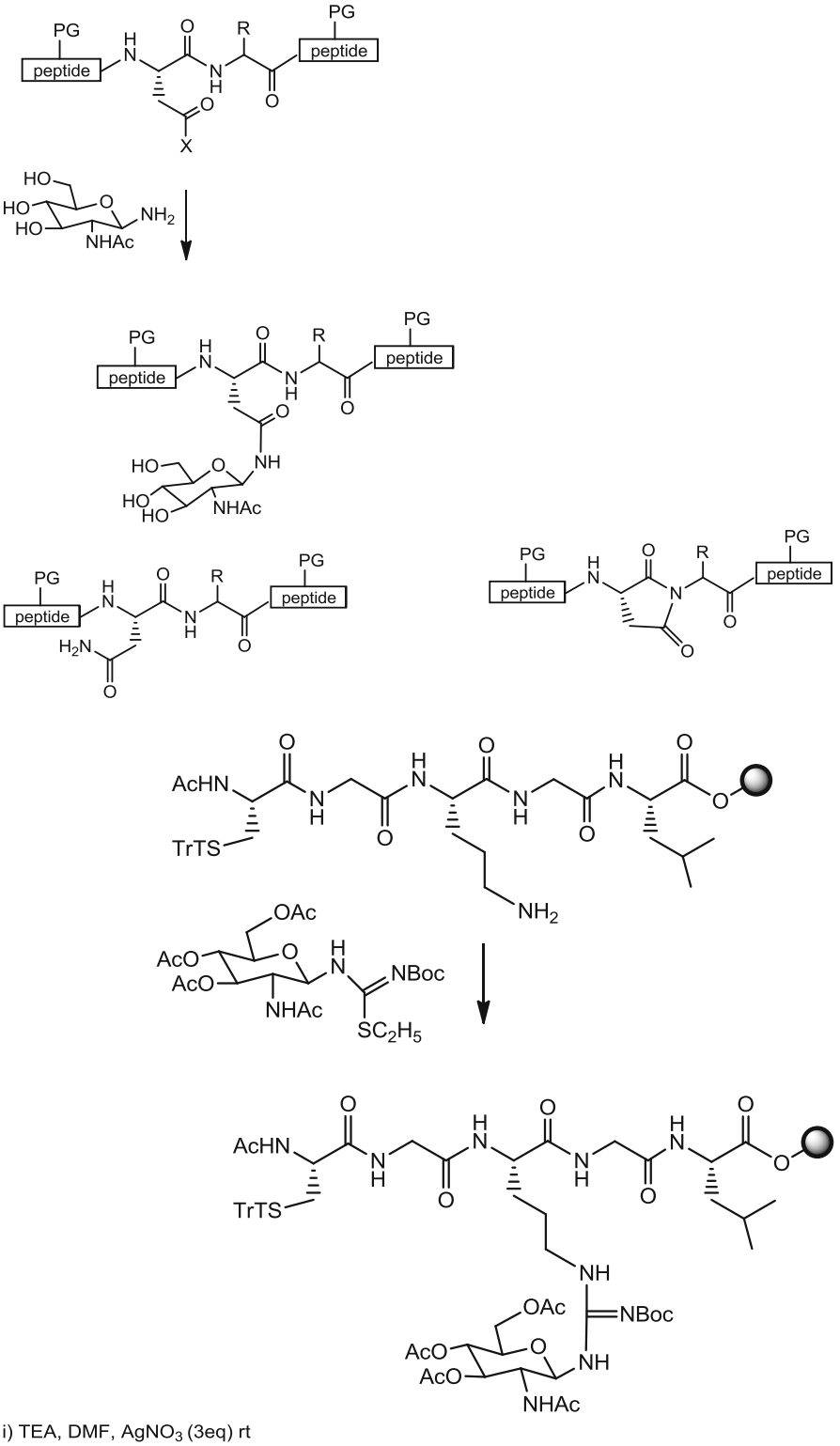
Scheme 6.18 Chemoselective and site specific glycoprotein syntheses [54–62]



Scheme 6.18 (continued)



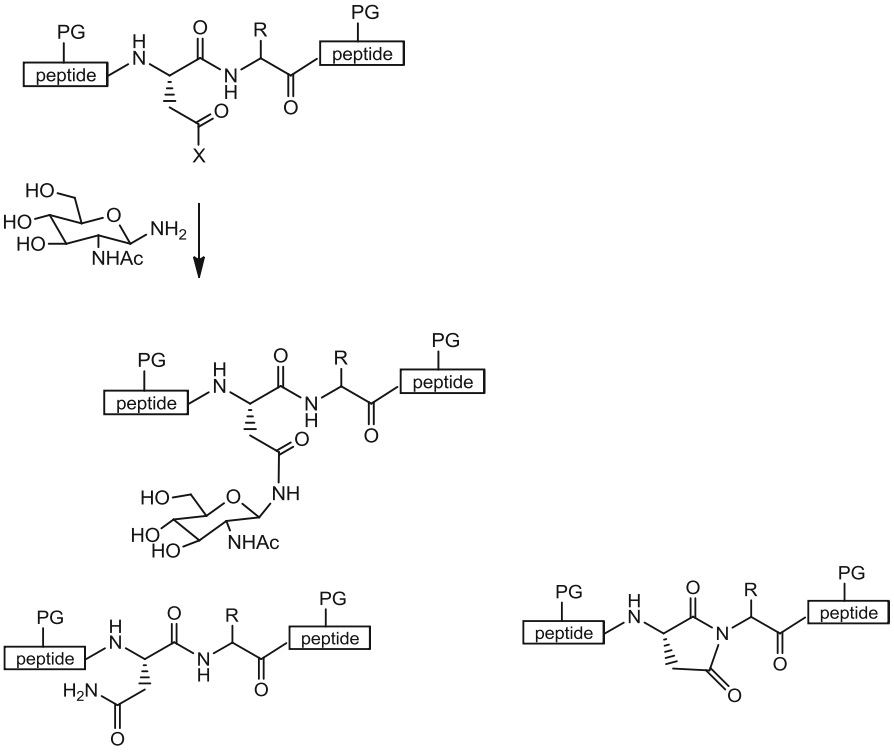
Scheme 6.19 Site-selective glycoprotein syntheses

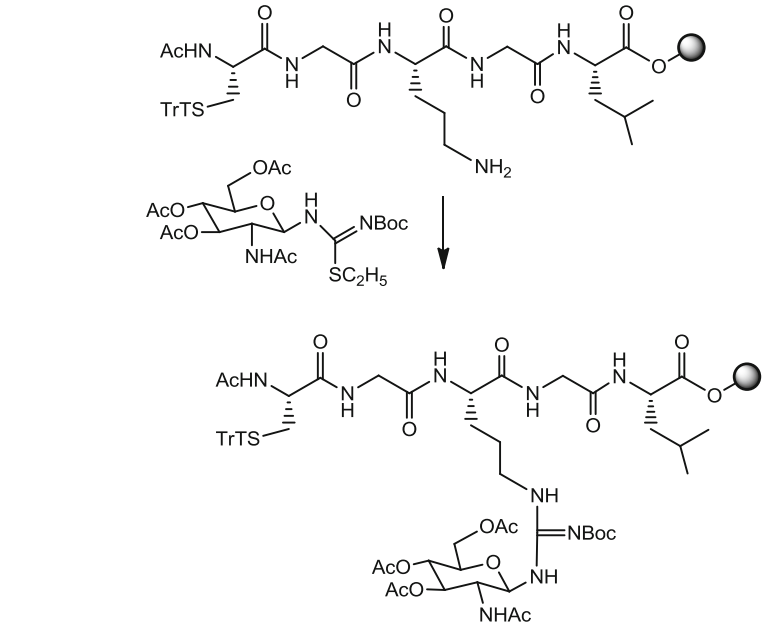


i) TEA, DMF, $AgNO_3$ (3eq) rt

6.4.5 *Guanylation Reaction*

This method considers the coupling of a glycosyl donor bearing an *S*-alkyl-isothiourea as a leaving group with a free amine attached at the peptide moiety under silver promoted condition, producing as a result a guanidine group between the sugar and the peptide [65].



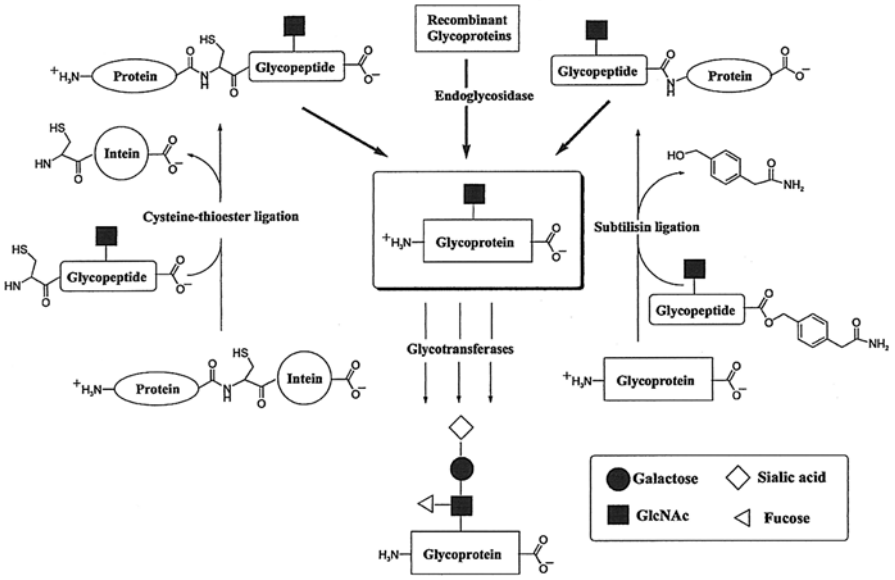


i) TEA, DMF, $AgNO_3$ (3eq) rt

6.4.6 *Enzymatic Synthesis*

Three basic strategies are considered for obtaining glycoproteins following an enzymatic approach: elaboration of glycans through the use of glycosyltransferases [66–69], trimming of glycans by purification of glycoform mixtures through selective enzymatic degradation [70], and alteration of glycans or glycoprotein remodeling, consisting in combined trimming of existing glycan structures followed by elaboration to alternative ones. Theses methods were used for preparing an unnatural glycoform of ribonuclease B by using endoH degradation and elaboration with galactosyltransferase, fucosyltransferase, and sialyltransferase system to construct an sLex glycoform [71]. Other approaches for the assembling of peptides are "native peptide ligation" [72] and endoglycosidase-catalyzed transglycosylation [31].

Recent advances on glycoprotein synthesis proposes an in vitro approach involving the following sequential steps, (a) remodeling of recombinant glycoproteins by using glycosidases and glycosyltransferases, (b) ligation of synthetic glycopeptides by enzymatic or chemical methods, (c) intein-mediated coupling of glycopeptides to larger proteins expressed as intein-fusion proteins, (d) ligation of glycopeptides to larger proteins containing N-terminal cysteine expressed as TEV protease cleavable fusion proteins, (e) in vitro translation, and (f) pathway reengineering in yeast system to produce human type N-linked glycoforms (Scheme 6.20) [73].



Scheme 6.20 Strategies for glycoprotein synthesis in vitro

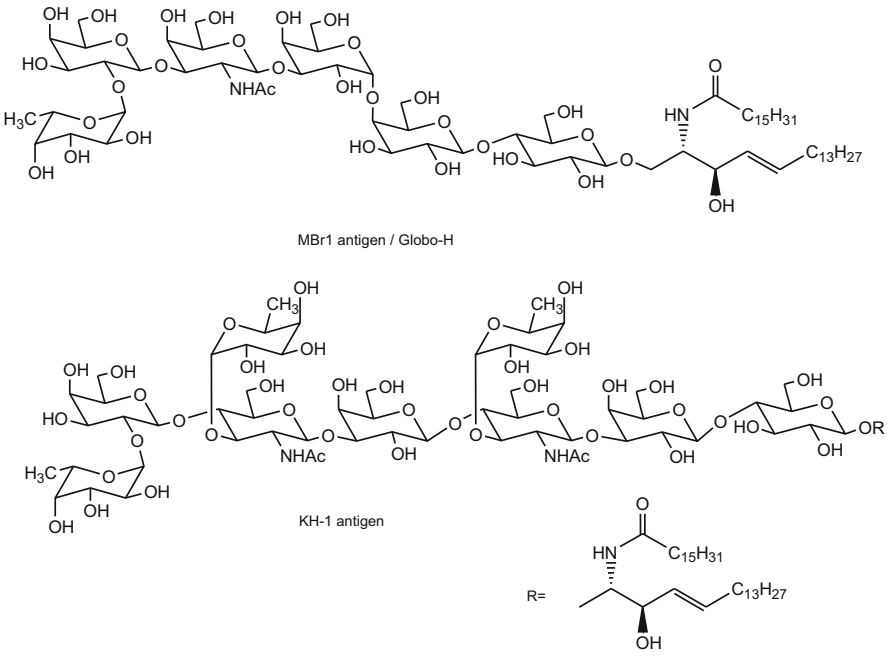
6.5 Synthesis of Antigenic Glycoconjugates

The preparation of complex glycoconjugates has been a current strategy for the design of synthetic vaccines, and usually involves the preparation of the oligosaccharide moiety which provides the immune specificity by chemical or enzymatic methods, and further attachment through a linker with an immunogenic protein. There has been a continuous effort for developing glycoconjugates containing antigens such as MBr1 antigen Globo-H, the blood group determinant and ovarian cancer antigen Lewis^y, N3 antigens associated with gastrointestinal cancer, the adenocarcinoma antigen KH-1, and the small cell lung carcinoma antigen fucosyl GM1 among others (Scheme 6.21) as a promising alternative to develop potentially useful carbohydrate-based anticancer vaccines accessible for clinical program. The synthetic approach becomes justified if we consider that cancer and normal cells growing in tissue culture generally show minimal level of expression of such antigens [33].

6.5.1 Glycosphingolipid and Gangliosides

6.5.1.1 Synthesis of Glycosphingolipid and Gangliosides

The chemical synthesis of most of these complex oligosaccharides represent a formidable challenge, and requires a convenient combination of strategies that allows suitable manipulations using appropriate protecting groups, glycosyl donors, acceptors, and coupling reactions conditions.



Scheme 6.21 Carbohydrate structures of tumor associated antigens

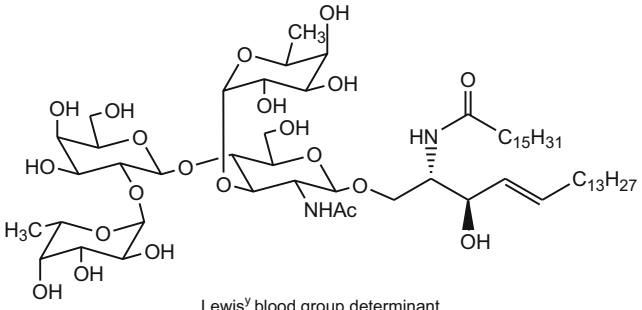
For instance the synthesis of glycolipid KH-1 was achieved by Desphande et al. [33] based on the glycal methodology (Scheme 6.22).

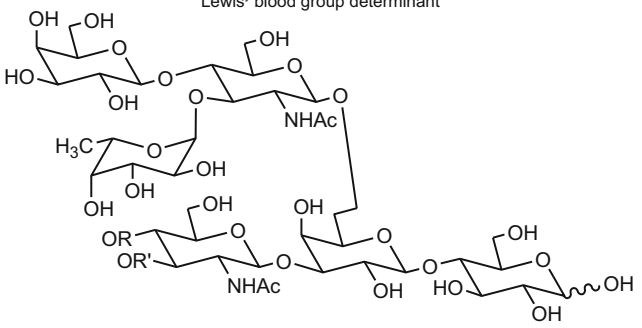
Likewise, the synthesis of the water-soluble galactosphingolipid analog that binds specifically to recombinant gp 120 was prepared by condensation of *C*-glucosyl aldehyde with Wittig reagent providing the oxazolidone which was transformed into the *C*-glycosylamino acid. By following a subsequent standard protocol represented in Scheme 6.23 the target glycolipid was constructed [34].

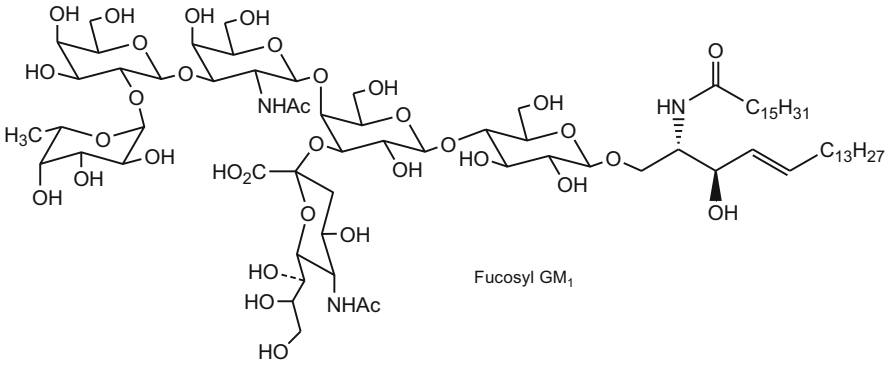
Glycoside ceramides are important molecules involved in apoptosis or active cell death. In leukemia cell lines C2 ceramide induces apoptosis via sphingomyelin pathway. It has been observed that α-galactosylceramides having more than ten carbons in fatty acid chain have immune stimulatory activities. Thus, the α-Gal-C2 was synthesized by direct glycosylation of C2-Cer with galactosyl fluoride donor in the presence of silver perchlorate as condensing agent (Scheme 6.24) [74].

The convergent synthesis is a procedure consisting in the parallel preparation of fragments or building block that will be connected through a coupling reaction, prior to deprotection. This procedure was applied successfully for preparation of glycosylphosphatidyl inositols (GPI) which are involved in the attachment of glycoproteins with eukaryotic cells (Scheme 6.25) [36].

The potential of carbohydrates as antibiotics, antiviral and anticancer substances has been established [37, 38]. Besides, their involvement in fertilization, embryogenesis, regulation of the immune system tissue repair, neuronal development,

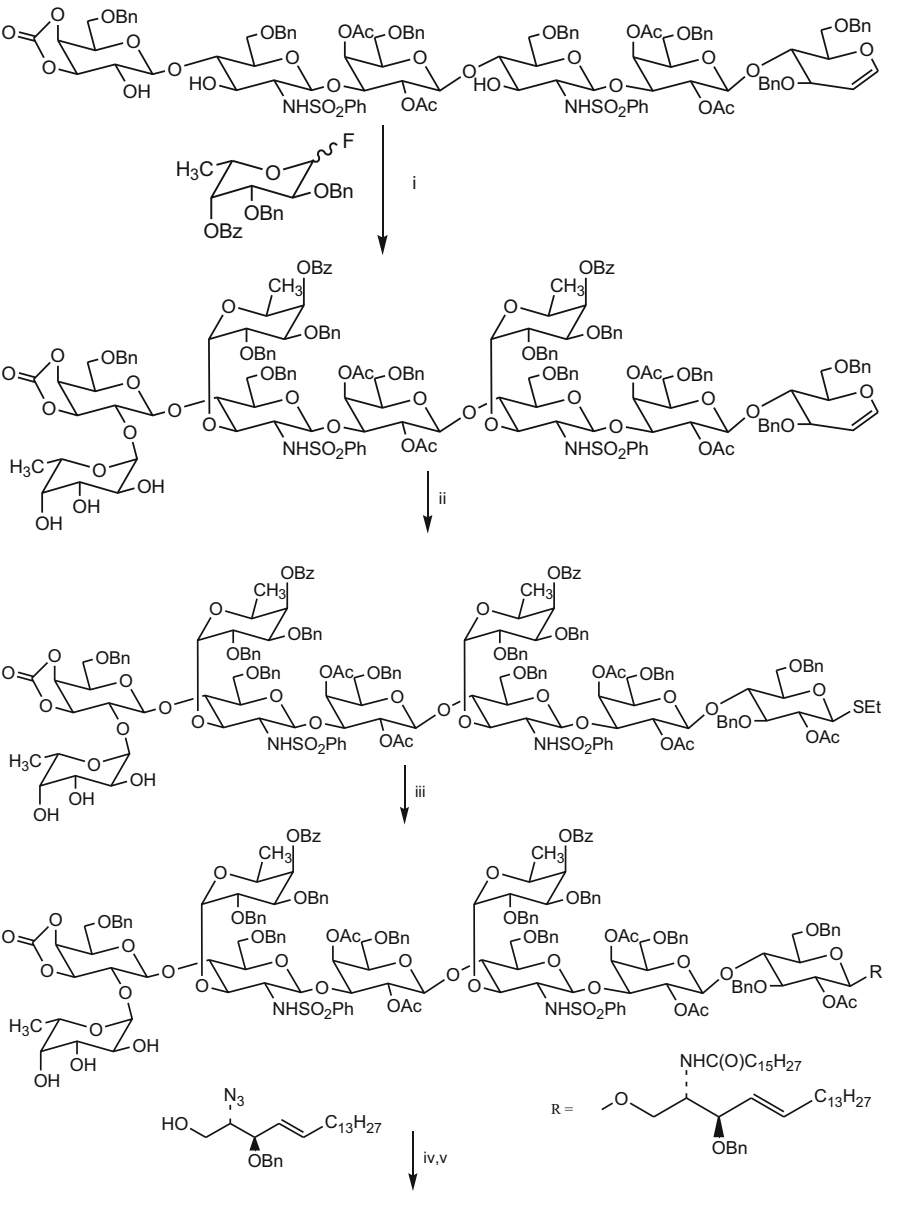






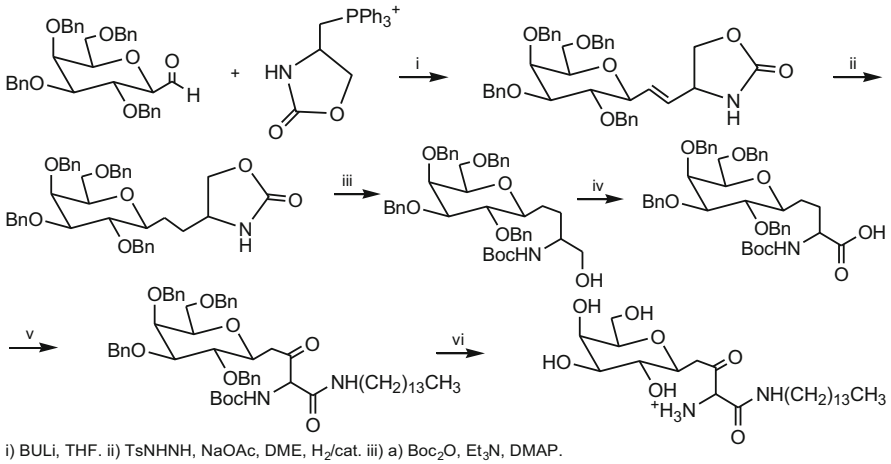
Scheme 6.21 (continued)

intracellular pathways, and cancer transformation among others has been demonstrated [12]. There is an increasing understanding of how carbohydrates behave biologically between normal and disease states and with this accurate information, novel carbohydrates and therapeutic approaches are developed [37]. For instance novel glycoside sulfates have been reported as novel potentially useful drugs (Scheme 6.26) [37, 38].

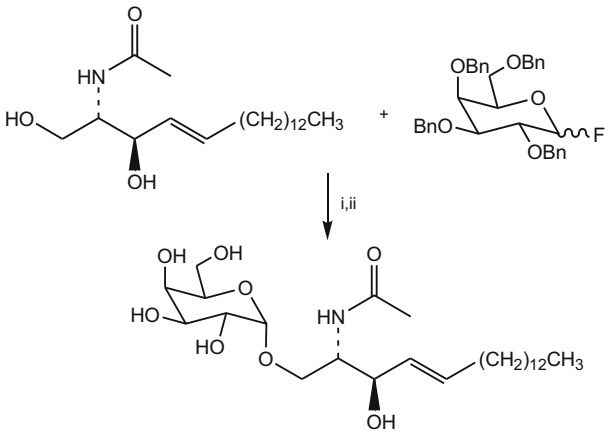


i) Sn(OTf), PhMe/THF (10:1), 4Å MS. ii) a) DMDO, CH_2Cl_2. b) EtSH, CH_2Cl_2, H^+ (cat.). c) Ac_2O, Py, CH_2Cl_2. iii) MeOTf, Et_2O/CH_2Cl_2 (2:1) 4 Å M.S. iv) a) H_2/Pd-$CaCO_3$, palmitic anh. EtOAc. v) a) Na/NH_3, THF, then MeOH. b) Ac_2O, Et_3N, DMAP, CH_2Cl_2. c) MeONa, MeOH.

Scheme 6.22 Synthesis of KH-1 antigen

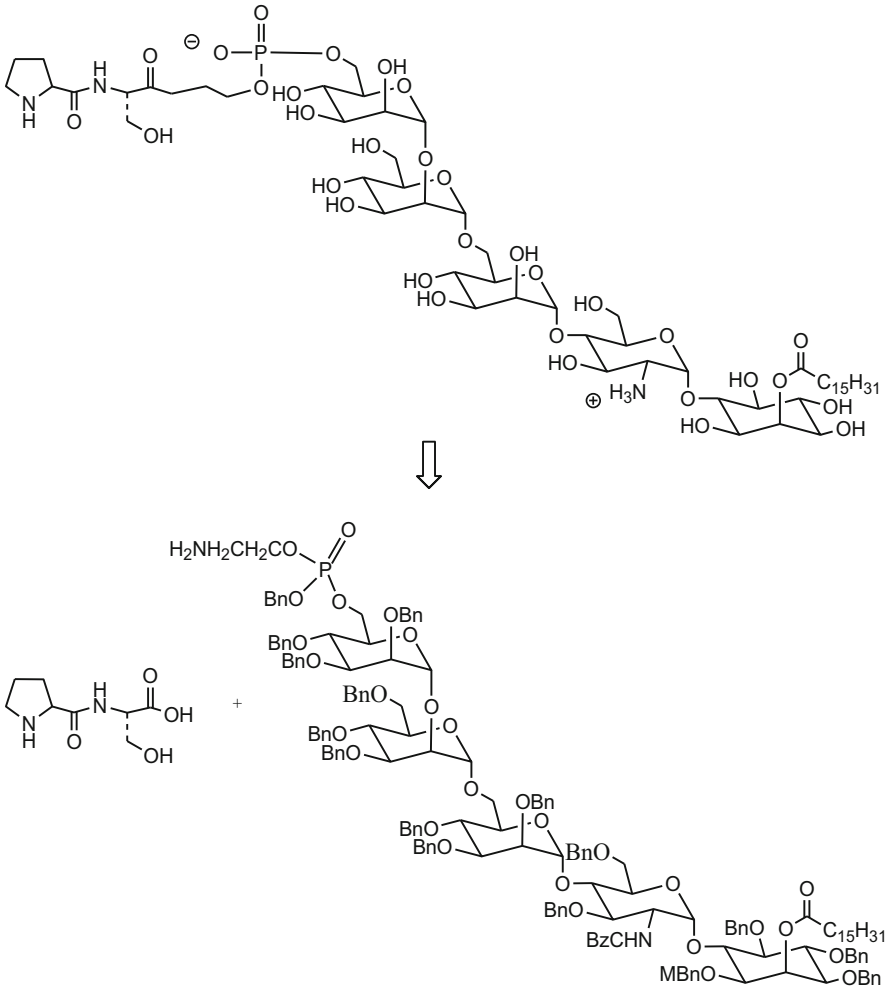


Scheme 6.23 Synthesis of galactosphingolipid analog

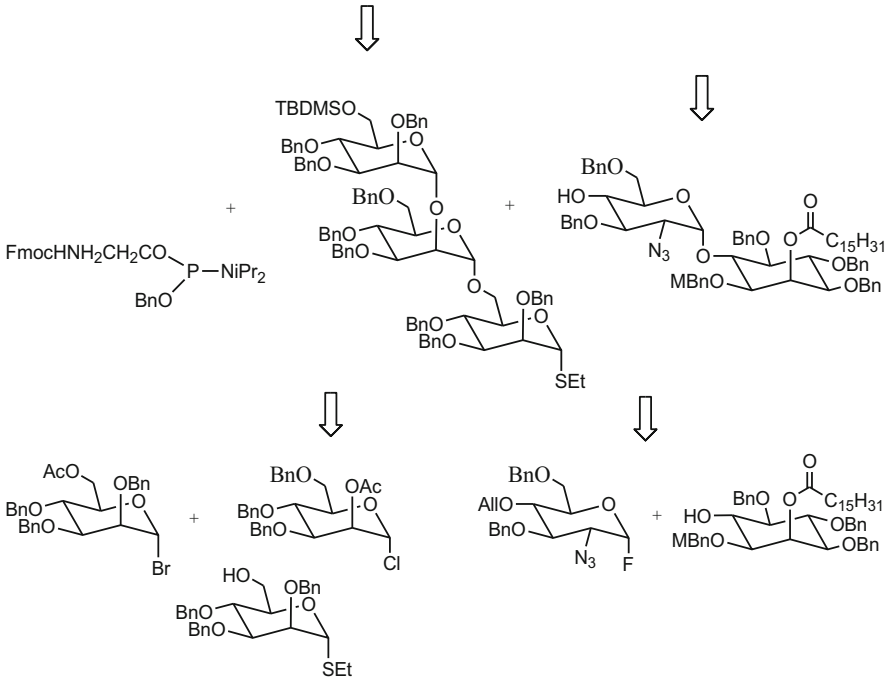


Scheme 6.24 Synthesis of glycosylceramide

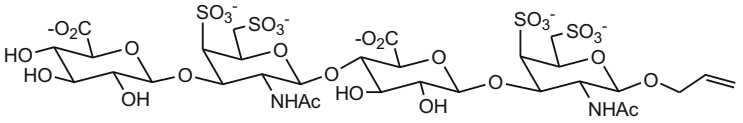
A variety of glycosphingolipid have been synthesized such as galacturonic sphingolipid from *Sphingomonas yanoikuyae* [75], immunostimulant C-glycosphingolipid [76], *Mycobacterium tuberculosis* sulfolipids SL-1, Ac2SGL analogs [77] and pentasaccharide moieties of ganglioside GAA-7 [78] and ganglioside GM3 [79].



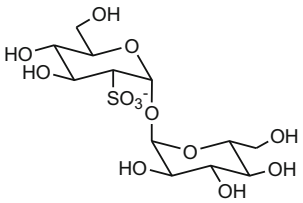
Scheme 6.25 Retrosynthesis for the preparation of GPI-anchored peptide using convergent synthesis



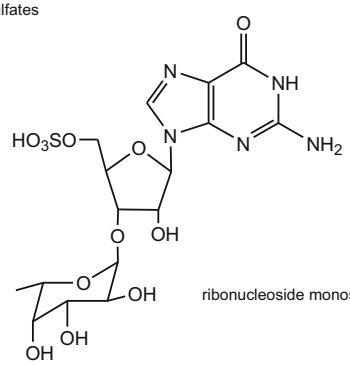
Scheme 6.25 (continued)



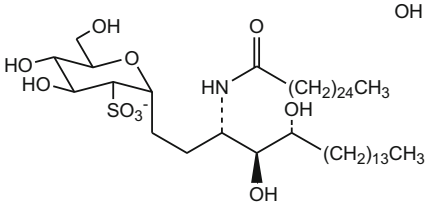
Chondroitin sulfates



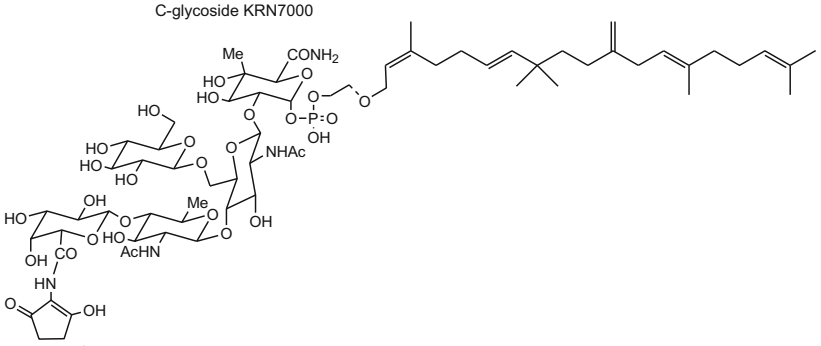
tetrahalose-2-sulfate



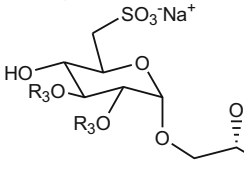
ribonucleoside monosulfate



C-glycoside KRN7000



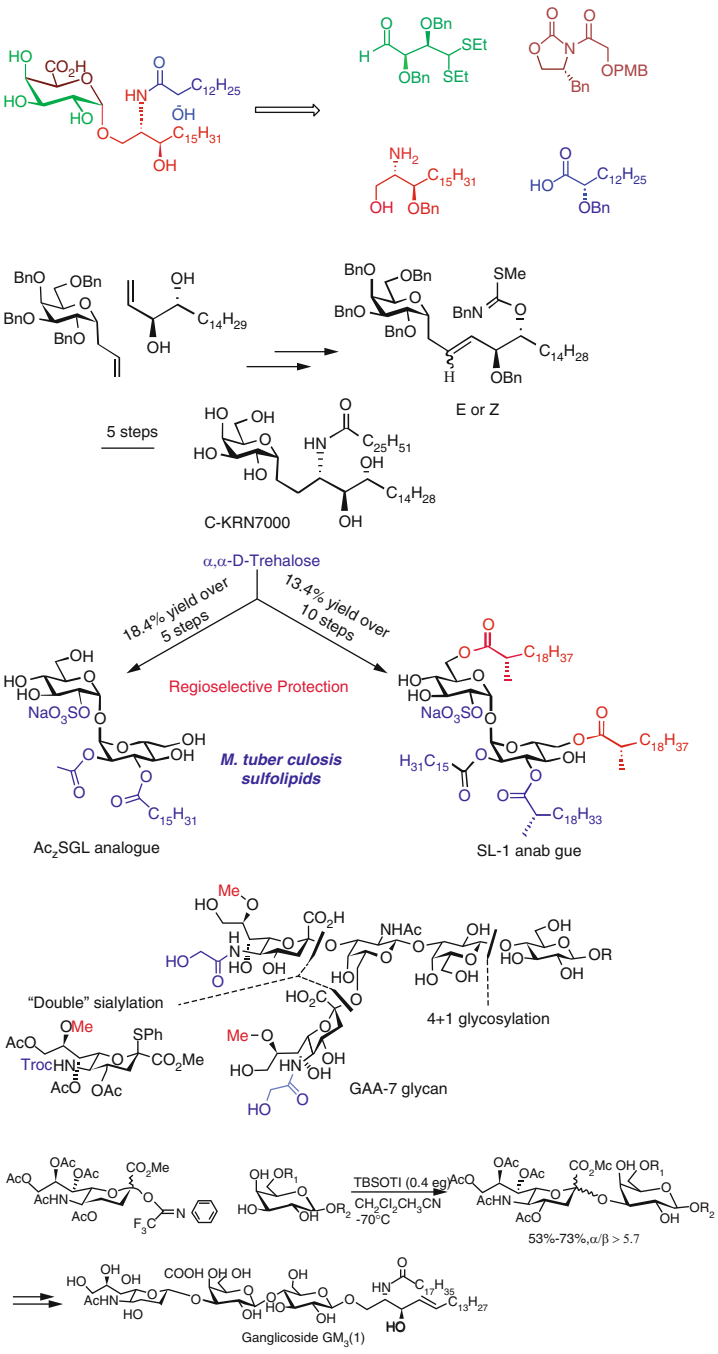
Menomycin A



R_1 = oleoyl R_2 = palmitoyl R_3 = H
R_1 = linoleoyl R_2 = palmitoyl R_3 = H
R_1 = palmitoyl R_2 = palmitoyl R_3 = H
R_1 = linoleoyl R_2 = palmitoyl R_3 = palmitoyl

Scheme 6.26 Novel glycoside sulfates and phosphates as potential drugs

Scheme: Synthesis of antigenic glycosphingolipids and ganglioside

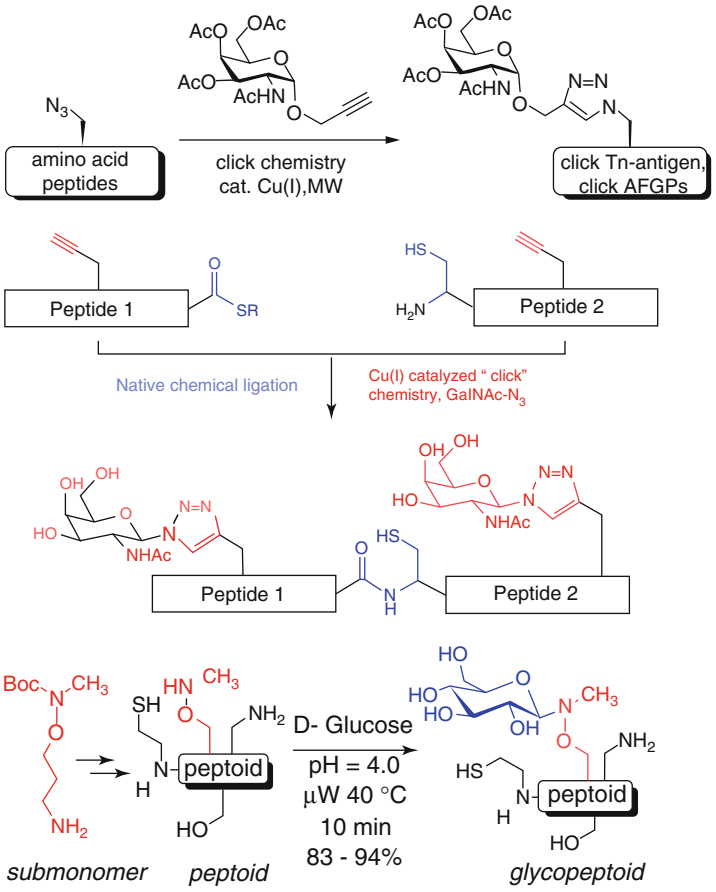


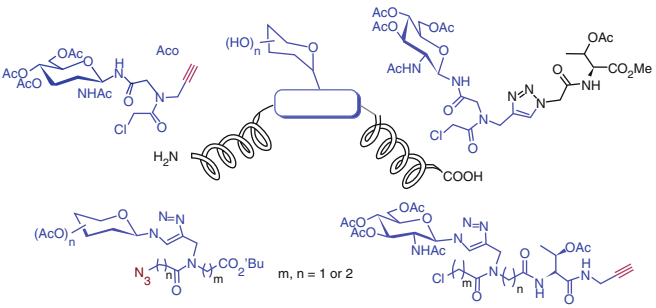
6.6 Glycopeptoids

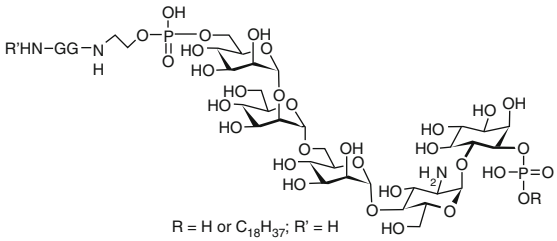
Glycopeptoids correspond to sugar moieties linked to short peptides which eventually can function as linkers for proteins. In cells the glycosylation of proteins is a posttranslational process with a number of important implications such as protein folding, stabilization, trafficking, recognition, immune defense, cell growth, inflammation, metastasis, bacterial and viral infections. It is known that aberrant glycosylation of cell surface glycoproteins is a common feature on numerous tumor cell types and they may undergo adaptive regulation of their cell surface through glycosylation in order to acquire a survival advantage.

A number a glycopeptoids have been prepared by using different approaches such as click chemistry [80, 81], chemoselective chemistry [82, 83], orthogonal native chemical ligation [84], metal-promoted glycosylative ligation [85], stereoselective synthesis [86], and cross-metathesis assisted solid-phase synthesis [87].

Scheme: Some approaches for the synthesis of glycopeptoids

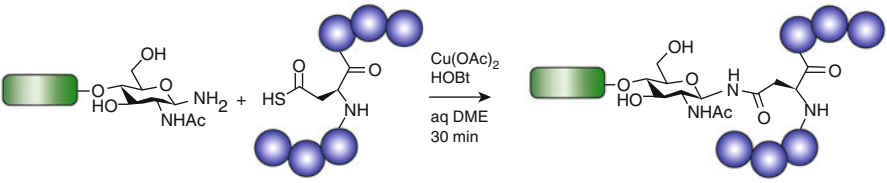


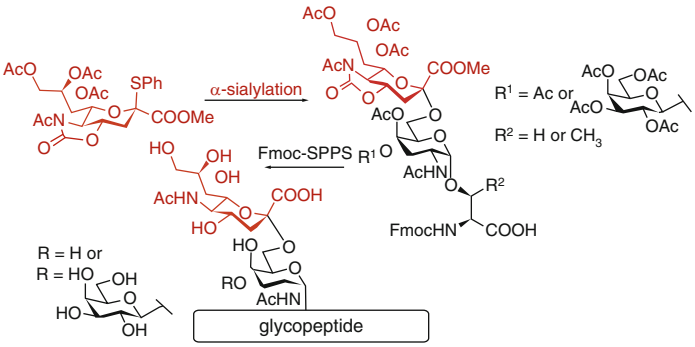


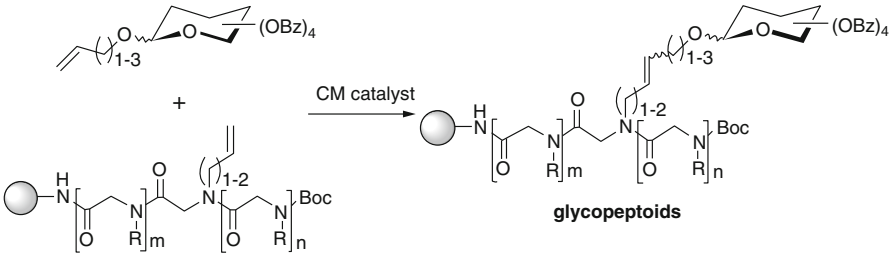


soratase A, CD52 polypeptide-LPKTGGS-OH or
Tris buffer CD24 polypeptide-LPKTGGS-OH

Analogs of CD52 antigen: R = H or $C_{18}H_{37}$R' = AcNH-GQNDTSQTSSPSLPKT-
Analogs of CD24 antigen:
R = H or $C_{18}H_{37}$; R' = NH_2-SETTTGTSSNSSQSTSNSGLAPNPTNATTKALPKT-







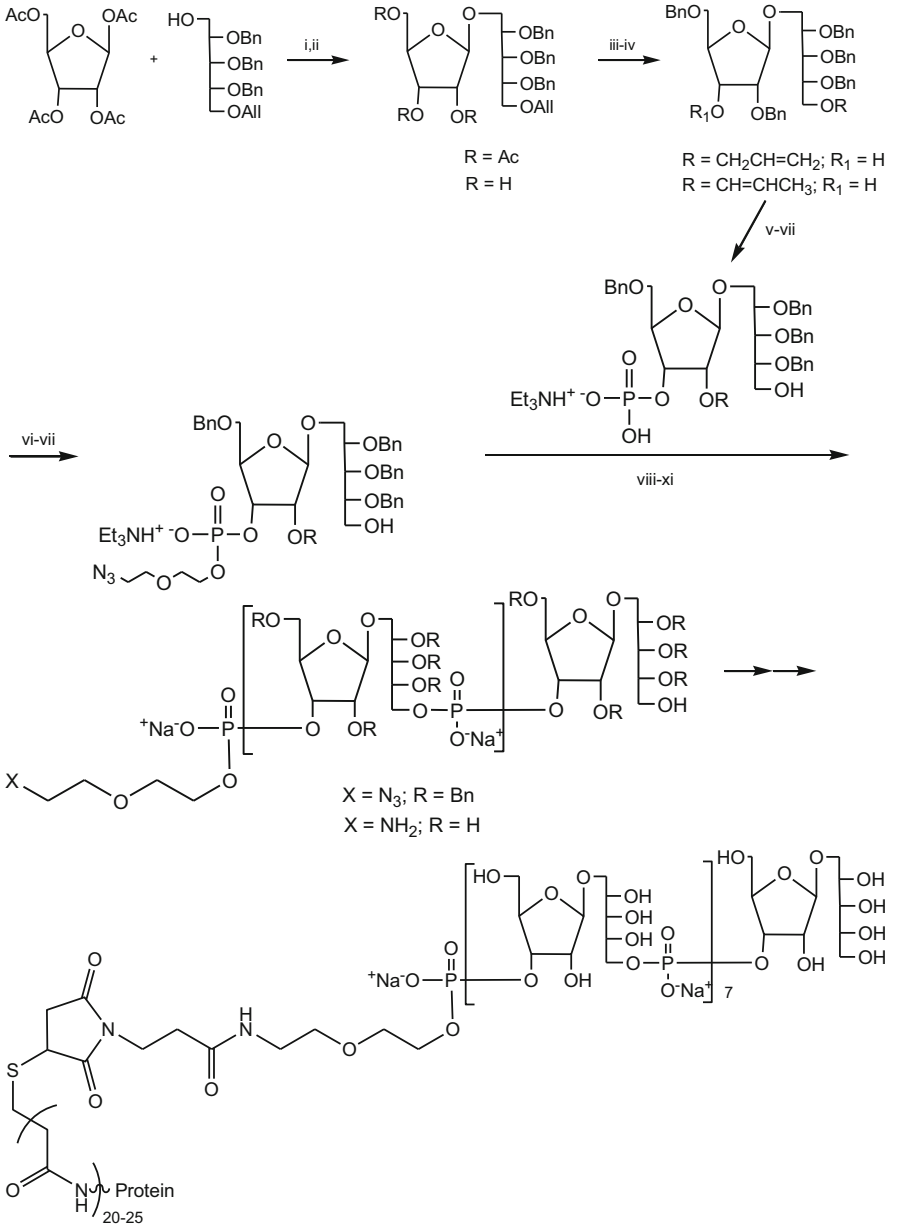
6.7 Synthetic Vaccines

Recent developments on carbohydrate chemistry made possible the design and escalation of new immunogenic carbohydrates. A newly developed synthetic carbohydrate attached to a protein carrier was reported by Verez-Bencomo and Fernández-Santana, and currently administered against *Haemophilus influenzae* type b disease. The chemical synthesis of oligomeric polyribosylribitol phosphate is described in Scheme 6.27 [88].

Another alternative therapeutic strategy for inducing immune response through the use of synthetic carbohydrate vaccines has been proposed by Danishefsky et al., involving the attachment of different tumor antigenic agents (Globo H, STn, Tn, Lewis^y,) coupled to a linker, and the linked antigens to a protein carrier (Scheme 6.28) [89].

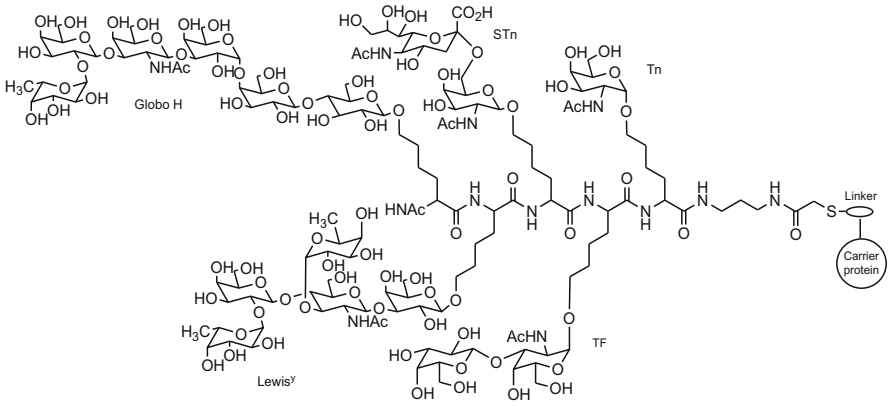
It has been mentioned that carbohydrate based agents such as glycoproteins and polysaccharides obtained from synthetic routes constitute an emerging and promising strategy for the preparation of vaccines [39, 40, 90, 91]. This possibility has become available due to the remarkable progress in the chemical and enzymatic preparation of oligosaccharides.

Other synthetic glycoproteins described are mucin MUC1 which strongly induces immune response against breast tumor tissues [92], glycosylated erythropoietin (EPO) [93, 94], enteropathogenic *Escherichia coli* (EPEC) type III [95, 96], and human interleukin-2 [97] among others. The methods employed for the attachment between the glycosyl and the peptide fragments were done by using solid-phase synthesis, native chemical ligation (NCL) [98], disulfide-linked precursors through a desulfurization process [99], Fmoc-modified amino acids, thiazolidine protection of the N-terminal, and reverse polarity protection strategy (Scheme 6.29) [64, 100–105].

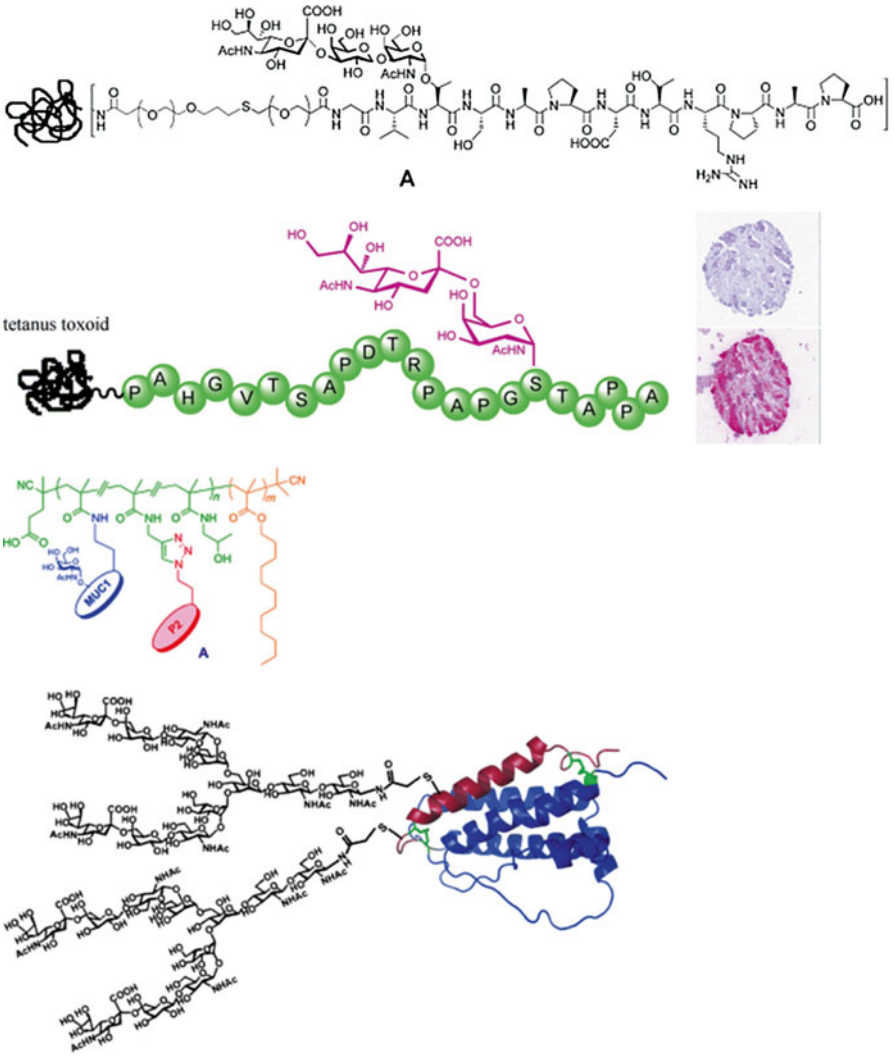


i) BF_3Et_2O, CH_2Cl_2. ii) CH_3ONa, CH_3OH. iii) BnCl, BuSnO, NaH, Bu_4NI. iv) tBuOK, DMSO, 100°C.
v) PCl_3, imidazole, CH_3CN. vi) $N_3(CH_2)_2O(CH_2)_2OH$, I_2. vii) AcOH-H_2O, 80°C. viii) PivCl, Py.
ix) Py-H_2O. x) H_2, Pd-C, EtOH-H_2O-EtOAc-AcOH, 1.5 atm. xi) cation exchange resin on Sephadex SP-C25.

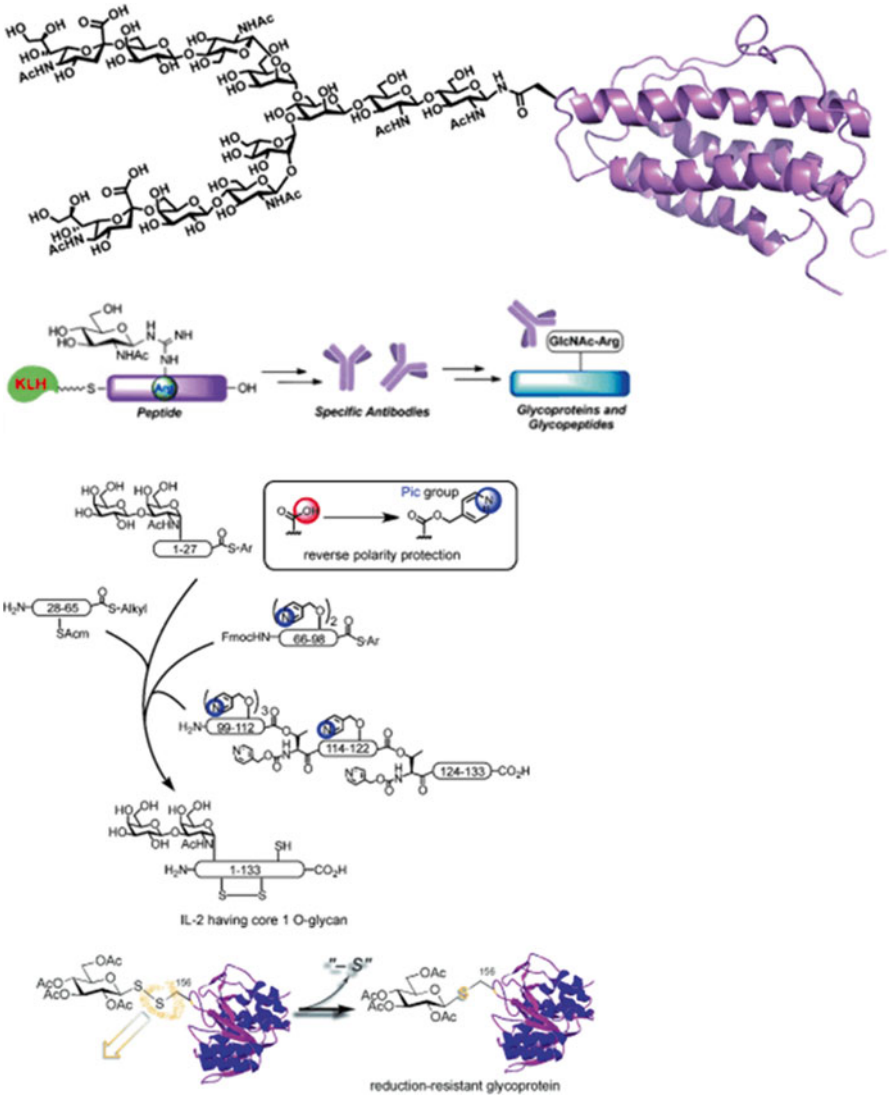
Scheme 6.27 Synthetic carbohydrate conjugate vaccine Quimi-Hib



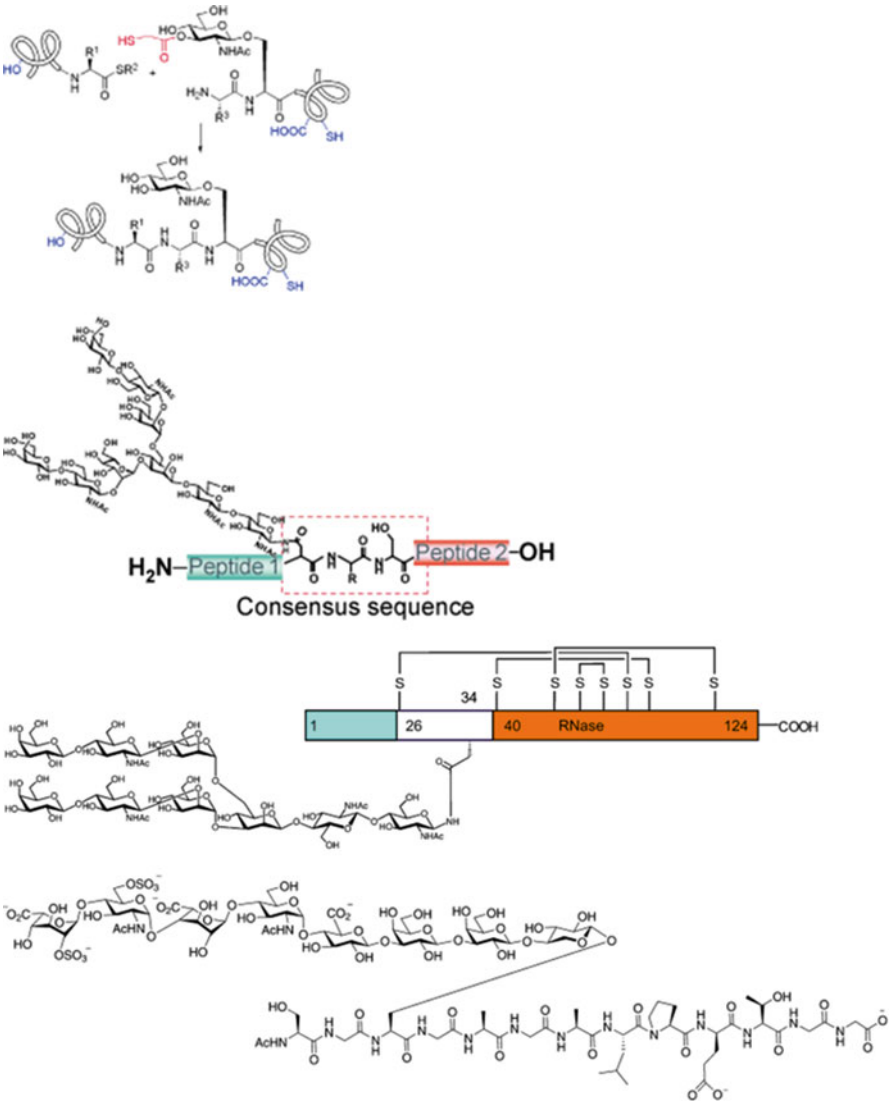
Scheme 6.28 Various tumor antigenic agents coupled to a linker developed as potential synthetic vaccines



Scheme 6.29 Examples of synthetic immunogenic glycoproteins [93–97]



Scheme 6.29 (continued)



Scheme 6.29 (continued)

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Chapter 7
Hydrolysis of Glycosides

The glycosidic bond might be degraded by chemical and/or enzymatic agents. Comparative studies revealed that chemical hydrolysis is nonspecific and on the other hand the enzymatic is regiospecific and stereospecific. The glycosides are chemically susceptible to acid conditions and only in some cases to basic conditions. In general the acid sensitivity is attributed to the sugar moiety and the basic non-stability to the aglycon nature.

7.1 Acidic Hydrolysis

When a glycoside is subjected to acid conditions, a process called acetolysis takes place. This phenomenon is more clearly seen on *O*-glycosides where even weak acid conditions can be sufficient for *O*-glycoside breakage. Some simple glycosides such as β methyl-2,3,4,6-tetra-*O*-methyl-D-glucopyranose are hydrolyzed under diluted HCl conditions to yield a hydroxy-2,3,4,6-tetra-*O*-methyl-D-glucopyranose. Likewise β ethyl-glucopyranose is hydrolyzed to a mixture of anomers (Scheme 7.1).

In general *S*-glycosides are more resistant than their counterparts *O*-glycosides to acidic medium; however, the former can be hydrolyzed under the conditions described in Scheme 7.2.

Disaccharides can be readily hydrolyzed under weak acidic conditions, producing their constitutive monomers in equivalent quantities (Table 7.1).

Depending on the strength of the hydrolytic conditions, polysaccharides undergo fragmentation, producing oligosaccharides, disaccharides, and monomers. The degradation degree relies on acid concentration, branching, and solubility. Thus, cellulose, being the most abundant natural polysaccharide in nature, requires high acid concentrations in order to be fully degraded to glucose. On the contrary some other polysaccharides at lower acid concentrations produce dimers and monomers (Table 7.2).

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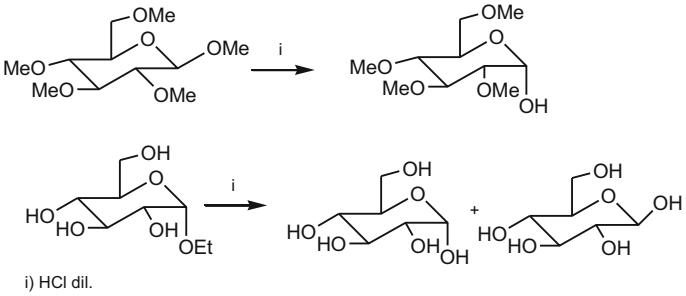


Scheme 7.1 Acid hydrolysis of simple *O*-glucosides

Scheme 7.2 Hydrolysis of thioglycosides

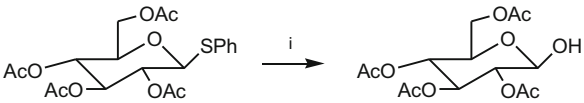


Table 7.1 Acid hydrolysis of disaccharides

Disaccharide	Hydrolysis product
(+)-Sucrose	D-(+)-glucose
	D-(−)-fructose
(+)-Lactose	D-(+)-glucose
	D-(+)-galactose
(+)-Cellobiose	D-(+)-glucose
	D-(+)-glucose

Table 7.2 Acid hydrolysis of polysaccharides

Polysaccharide	Partial hydrolysis	Total hydrolysis
Cellulose	1,4-cellobiose	D-glucose
Laminarin	1,3-laminaribiose	D-glucose
Curdlan	1,3-laminaribiose	D-glucose
Chitin	1,4-*N*-acetyl glucosamine	2-amino-2-deoxy-D-glucose
Mannan	1,4-mannobiose	D-glucose
Pullulan	1,4-maltotriose	D-glucose

Partial hydrolysis is important in certain cases in which disaccharides are not either affordable materials or easily obtained ones through synthetic means. Such is the case of 1,3-laminaribiose synthetically obtained in poor yields (9.5 %) [1], but readily available from polysaccharide curdlan [2].

Lewis acid hydrolysis of cellulose and methyl glycosides has been explored usually accompanied by heating. Thus, the conditions founded for achieving this goal were magnesium chloride in water with heating at 105 °C in either sealed or open vial [3].

7.2 Basic Hydrolysis

Some glycosides have been shown to be partially sensitive against basic conditions, besides their naturally high acid sensitivity. It is been experimentally founded that three classes of *O*-glycosides might be subject to basic hydrolysis [4].

(a) Phenolic glycosides
(b) Enolic glycosides
(c) β-substituted alcohol glycosides

7.2.1 Phenolic Glycosides

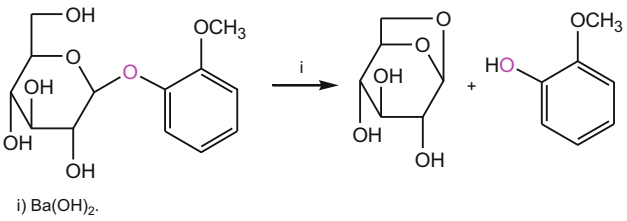
A typical example of phenolic glycoside decomposition under basic conditions is observed in the treatment of salicin with barium hydroxide giving as result a cyclic acetal and the release of the aglycon (Scheme 7.3).

7.2.2 Enolic Glycosides

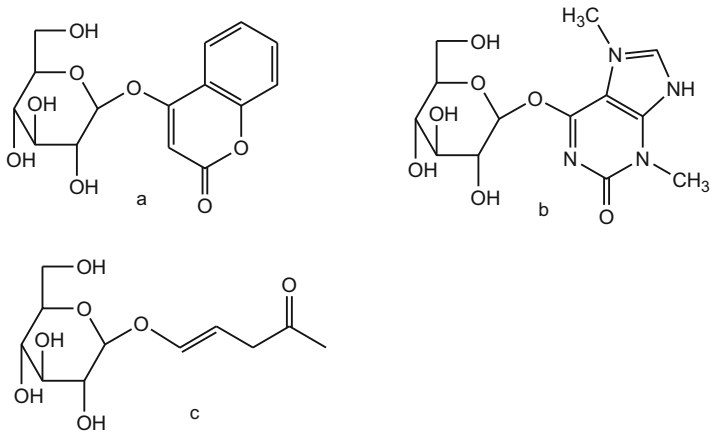
Within this type of glycosides, there are three varieties to be considered, which are: (a) 4-hydroxycoumarins, (b) purine and pyrimidine glycosides, and (c) simple enols (Scheme 7.4).

7.2.3 β-substituted Alcohol Glycosides

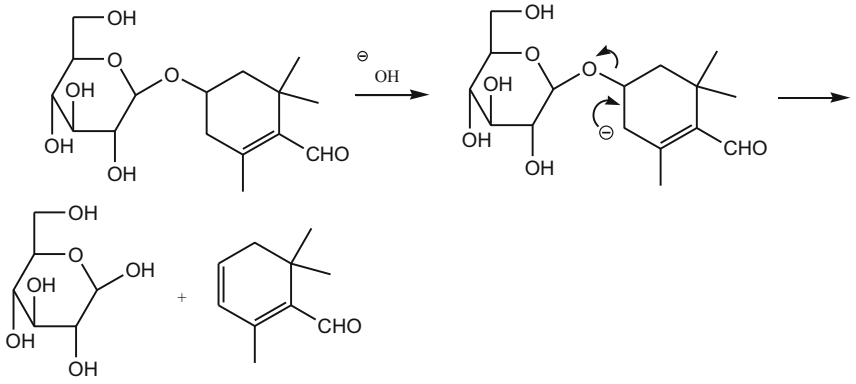
Glycoside picrocine is hydrolyzed in diluted potassium hydroxide solution, through a mechanism that involves a intermediate carbanion formation to give a conjugated unsaturated product and glucose as breakage product (Scheme 7.5).



Scheme 7.3 Basic hydrolysis of phenolic glycosides



Scheme 7.4 Basic hydrolysis of enolic glycosides



Scheme 7.5 Basic hydrolysis of β-substituted alcohol glycosides

Table 7.3 Degradation products of disaccharides under basic conditions

Disaccharide	Hydrolysis conditions (KOH) (N)	Temperature (°C)	Product
Cellobiose	1.5	50	Lactic acid
Gentobiose	2	50	Lactic acid
Lactose	0.2	100	D-galactose
Maltose	0.15	25	Phenylhydrazone of D-mannose

Contrary to acid hydrolysis of disaccharides where degradation products are their constitutive units, in most of the cases for basic conditions, non-sugar derivatives are produced (Table 7.3, Scheme 7.3).

7.3 Enzymatic Hydrolysis

β-glycosides are the natural substrates for hydrolytic enzymes known as β-glycosidases. So far, at biochemical level, the rule of most glycosidases is not totally well understood; however, some of them have been related to feeding, detoxification processes or even as a defense mechanism against herbivorous pathogens through release of thiocyanates, cyanides, and phytohormones. It has been established that there is a specific glycosidase for each aldopyranose, the sugar composition being responsible for the recognition pattern. Some of the best studied hydrolyses are the β-glycosidases and among them β-glucosidases, β-glucuronidases, β-glucanases, β-chitinases, all of them with important biological and economical implications [5].

7.3.1 β-glucosidases

There is strong evidence indicating that their action is mainly directed toward the defense mechanism and growth regulation. For instance cyanogenic glycosides are hydrolyzed, for the releasing of cyanide ions as a defense mechanism against animals. In humans the equivalent of β-glucosidase is called glucocerebrosidase (with low genomic homology to the plant counterpart) and catalyzes the degradation of glucosylceramide inside lysosome. The lack or deficiency of this enzyme produces the Gaucher disease characterized by accumulation of glucosylsphingosine and glucosylceramides.

7.3.2 β-glucanases, β-chitinases

The natural substrates for these oligosaccharide hydrolytic enzymes are laminarin and chitin, respectively, being present in fungi, yeast, and insects. Some of the processes related to the activity of these enzymes are: seed degradation, cellular elongation control, growth regulation, pollen growth regulation, digestion, and fertilization. Moreover, within the context of the defense mechanisms, these enzymes can be able to digest the fungi cellular wall, and also to release oligosaccharides that induce the production of antimycotic substances called phytoalexins.

7.3.3 β-cellulase

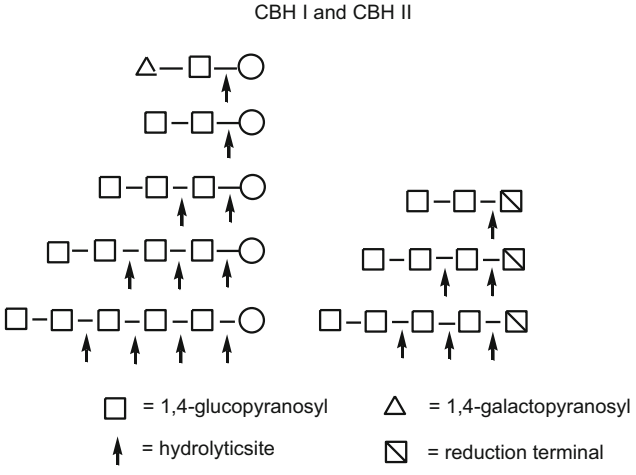
Cellulose is the most abundant natural polysaccharide on earth. Cellulytic enzymes particularly cellobiohydrolases CBHI, CBHII, EGI, and EGII found in fungi *Trichoderma reesei* have been thoroughly studied for determining the three-dimensional structure, the genomic sequence, receptors, and substrate specificity.

7.3.4 β-glucuronidase

In animals this enzyme is responsible for the detoxification processes, coupling mainly aromatic compounds and eliminating them as glucuronides. In plants there is not detectable β-glucuronidase activity; however, the development of the GUS gene fusion containing *E. coli* β-glucuronidase has been widely used as a gene marker [6]. Transgenic plants containing exogenic information fused to the β-glucuronidase gene marker can be conveniently monitored by using fluorogenic histochemical glucuronides.

7.3.5 Glycosidase Enzymatic Activity Detection

Detection can be achieved not only qualitatively, but also quantitatively, and for doing so high and low molecular weight substrates have been designed. Claeyssens [7] demonstrated hydrolytic specificity of cellulases CBH I and CBH II through the use of synthetic fluorogenic substrates containing the highly fluorescent coumarin umbelliferone or *p*-nitrophenol, in the form of *O*-glucosides. The cleavage of the glycoside releases the chromophore which can be easily measured in a fluorometer or spectrophotometer. The synthetic design of monosaccharides, disaccharides, trisaccharides, and tetrasaccharides attached to the mentioned chromophores has been of great advantage to determine the specificity during enzymatic cleavage (Scheme 7.6).



Scheme 7.6 Enzymatic specificity on low molecular weight substrates

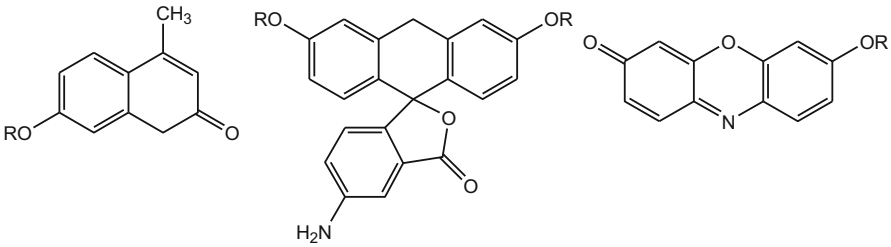
7.3.6 β-1,4-glucanases

The utilization of polysaccharides covalently attached to dyes has been reported. The complex Ostatin Brilliant Red-hydroxyethylcellulose (OBR-HEC)) is applied as a specific substrate for EG, Remazol Brilliant Blue-xylan (RBB-X)) the specific substrate for β-1,4-xylanases.

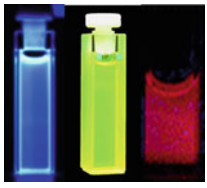
Likewise β-1,3-glucanases are detected by using an electrophoresis technique on polyacrylamide gels utilizing laminarin as substrate. The generated fragments are reacted further with azoic stain 2,3,5-triphenyltetrazolium to produce a color complex [8]. Despite their high sensitivity, this method cannot distinguish between endoglucanase and exoglucanase.

7.3.7 *Fluorescent* O-*Glycosides*

As mentioned before, fluorogenic aglycons are very useful molecules to monitor enzymatic activity. In principle, the fluorescent compound does not exhibit fluorescence in the glycoside form, and exerts its fluorescence when released as a result of the enzymatic activity (Scheme 7.7). Some of the fluorescent compounds widely used for enzymatic detections are: umbelliferone, fluorescein, and resorufin, having been coupled to most of the biologically important sugars as *O*-glycosides.

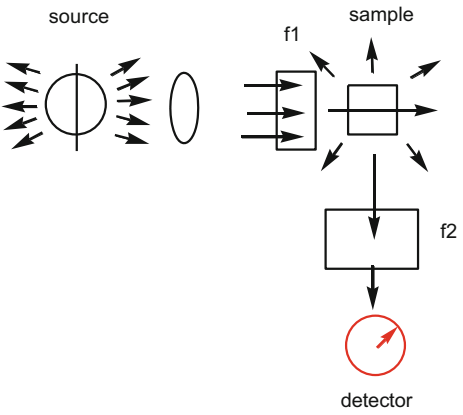


R = glucose, galactose, glucucronic acid, N-acetylglucosamine.

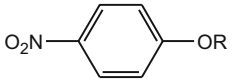


Scheme 7.7 Fluorescent *O*-glycosides and fluorescence emission after hydrolysis for (**a**) umbelliferone, (**b**) fluorescein, (**c**) resorufin

Scheme 7.8 Basic diagram of a fluorometer



Scheme 7.9 Absorption glycosides



The generated fluorescence is quantified in fluorometers constituted basically by a radiation source, and two monochromatic mirrors (f1 and f2). The first one selects the light for producing fluorescence activation, and the second transmits selectively fluorescence emission. A detector will measure the intensity of the fluorescence generated (Scheme 7.8).

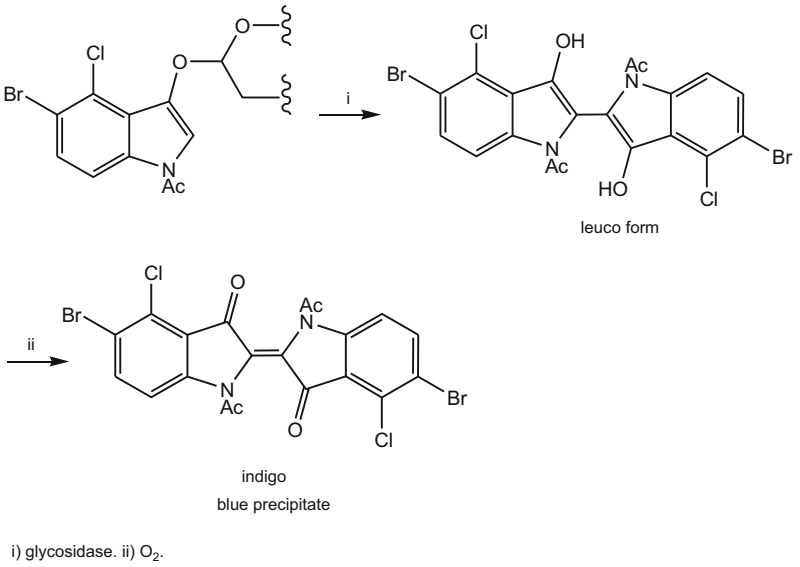
7.3.8 O-glycosides Measured by Absorption

Quantification of enzymatic activity following absorption detection is based in the use of synthetic *p*-nitrophenol in the form of *O*-glycosides as substrate (Scheme 7.9). The releasing of the aglycon from the sugar moiety produces slight yellow color measured as absorbance.

7.3.9 Histochemical O-Glycosides

Generally a histochemical substrate to be consider as a good candidate, should be such that in the form of *O*-glycosides it is water soluble and when the enzyme hydrolyzes the glycosidic bond releases the aglycon, which precipitates immediately. A compound that closely fulfills these requirements is 5-bromo-4-chloro-*N*-acetyl-3-indoxyl (X-gal, X-gluc, etc.) which has been attached to most of the biologically important monosaccharides, commonly identified as X-gal, X-gluc, etc. (Scheme 7.10).

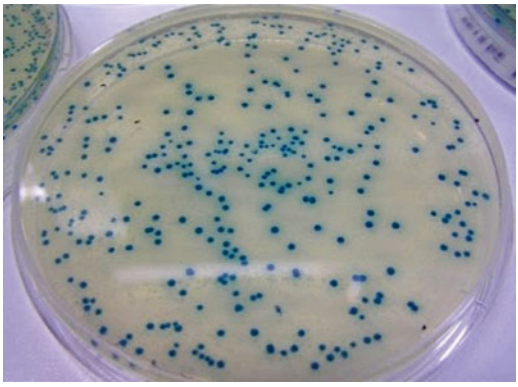
These chromophoric *O*-glycosides has been extensively used for detection of hydrolase activity and in molecular biology as screenable gene markers used to



i) glycosidase. ii) O_2.

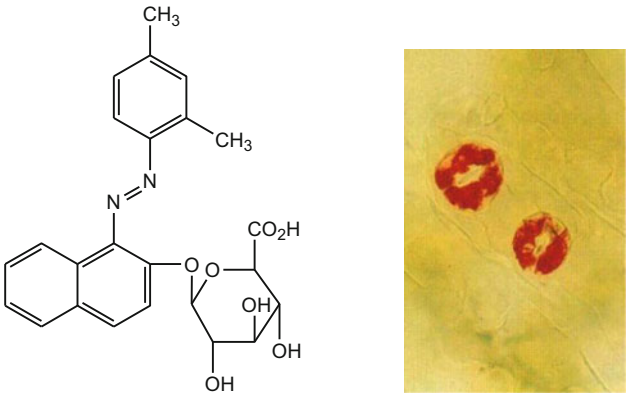
Scheme 7.10 5-bromo-4-chloro-indoxyl aldopyranose hydrolysis

Scheme 7.11 Genetically transformed bacteria cells containing lacZ gene expressing for β-galactosidase activity detected with X-Gal

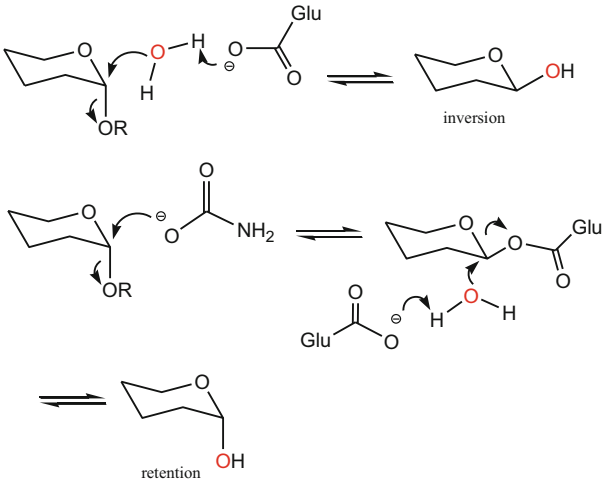


determine if a sequence has been successfully inserted in a cell known as the lacZ gene which encodes for β-galactosidase (Scheme 7.11). Although this is commercially available it is highly sensitive producing and easily detectable blue precipitate, it shows some diffusion before the monomers undergo dimerization in the presence of oxygen, to produce the blue indigo precipitate.

Alternatively, phenylazo naphthol *O*-glycosides (Scheme 7.12) known as Sudan glucuronides have been tested as a histochemical substrate for enzymatic detection of gene marker β-glucuronidase in transgenic plants [9, 10].



Scheme 7.12 Phenylazo naphthol glucuronides as histochemical substrates

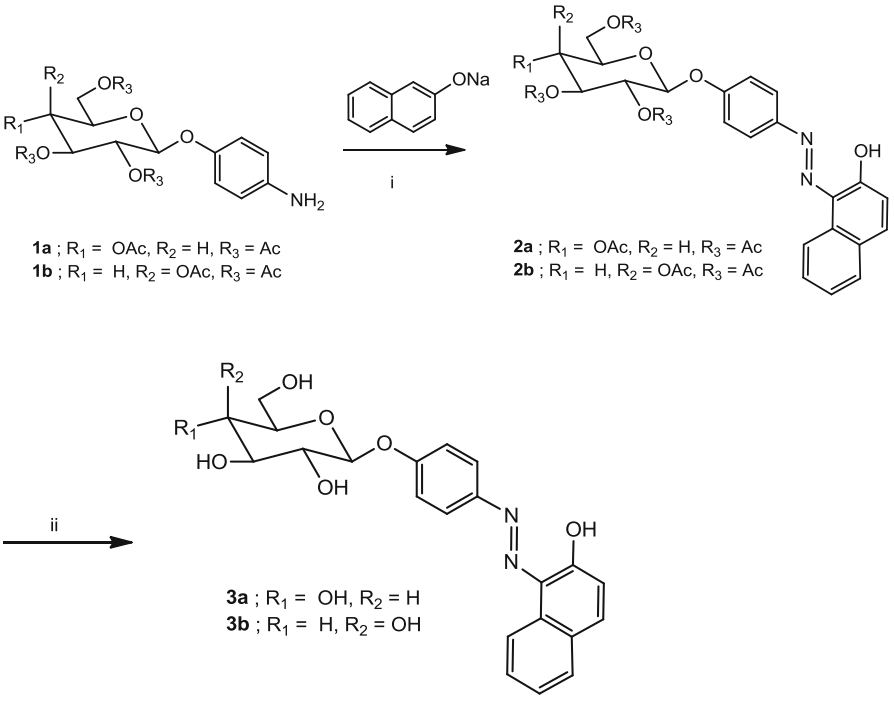


Scheme 7.13 Schematic representation of retention and inversion hydrolysis mechanism

The water-soluble Sudan glucuronide releases the phenylazo naphthol stain after enzymatic hydrolysis which can be seen in the sites of enzymatic activity as red crystals (Scheme 7.13). The mechanism and stereochemistry of enzymatic hydrolysis may occur with either inversion or retention of the configuration at the anomeric center. The first type of hydrolysis is carried out by the so-called inverting glycosidase, and the second by retaining glycosidase, with the vast majority of β-glucosidases being of the latter type. This has been proved through NMR studies, by measuring the chemical shift and magnitude of the coupling constant of the anomeric carbon. The most accepted mechanism involves protonation of substrate, participation of carboxylate attached to enzyme, glycoside–enzyme intermediate formation, and displacement as shown in Scheme 7.15 [12].

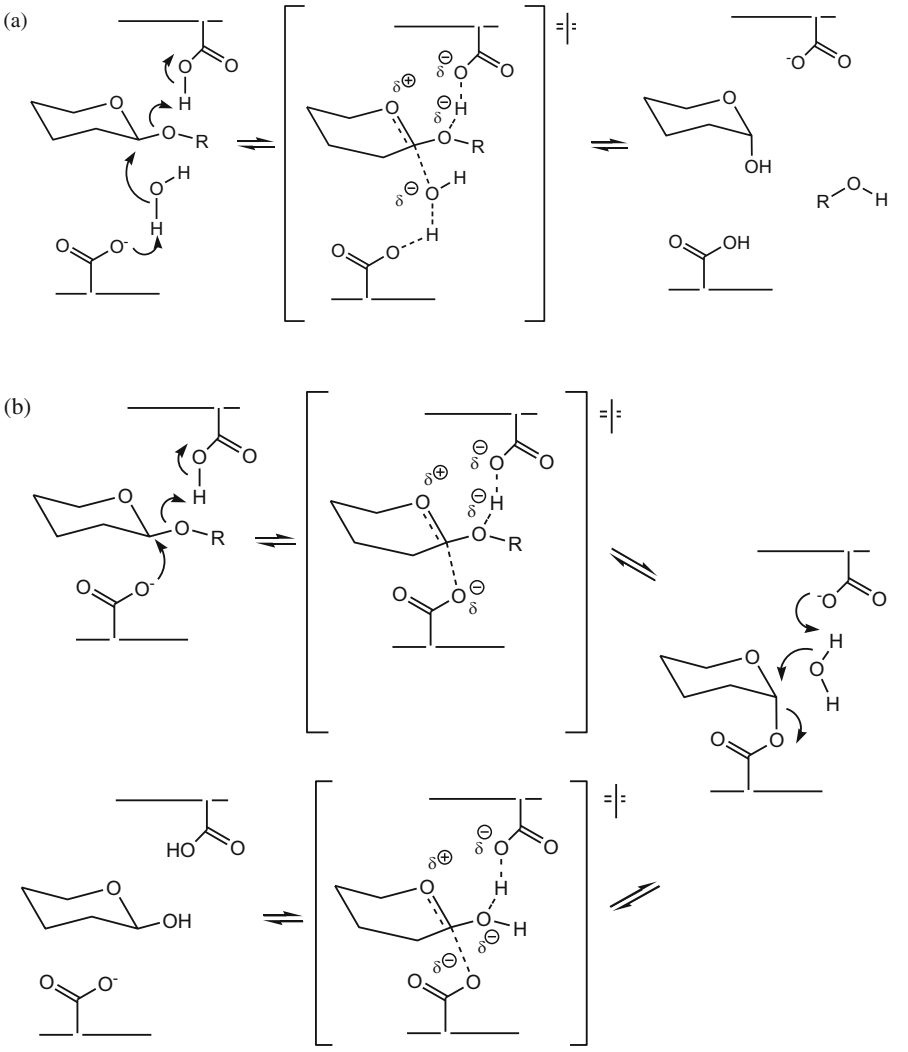
A suitable method for preparing azoic glycosides from aminophenyl glycoside precursor was performed under mild diazonium salt conditions, providing the corresponding protected azoic glycoside which after final deacetylation produce the azoic glycoside (Scheme 7.14) which was evaluated as substrates for detection of enzyme activity showing two maximum absorptions at 410 and 455 nm [11].

The partially water soluble Sudan glucuronide, releases the fenilazo naphthol stain after enzymatic hydrolysis which can be seen in the sites of enzymatic activity as red crystals. The mechanism and stereochemistry of enzymatic hydrolysis may occur with either inversion or retention of the configuration at the anomeric center. The first type of hydrolysis is carried out by the called inverting glycosidase, and the second by retaining glycosidase, being the vast majority of β-glucosidases of the later type. This has been proved through NMR studies, by measuring the chemical shift and magnitude of the coupling constant of the anomeric carbon. The most accepted mechanism involves, protonation of substrate, carboxylate participation attached to enzyme, intermediate formation glycoside-enzyme, and displacement as shown in Scheme 7.15 [12].



Conditions and reagents : (i) $NaNO_2$, AcOH, H_2O-THF, ^oC 30 min, then rt 1h. (iii) MeONa/MeOH, rt 30 min.

Scheme 7.14 General method for the preparation of azoic glycosides



Scheme 7.15 Schematic representation of inverting (**a**) and retaining (**b**) glycosidase mechanism

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Chapter 8
Nuclear Magnetic Resonance of Glycosides

8.1 NMR of Glycosides

Nuclear magnetic resonance (^{1}H, ^{13}C NMR), X-ray diffraction, and mass spectrometry are considered the most important analytical methods for structural elucidation. Characterization by means of ^{1}H, ^{13}C NMR, monodimensional and bidimensional spectroscopy is a powerful tool for structural assignment of simple and complex glycosides. Pioneering studies [1–4, 48–50] on simple monosaccharides were essential for understanding, through the chemical shifts and coupling constants, the conformational behavior of sugars.

Some basic considerations derived from the studies mentioned above that apply to simple saccharides are:

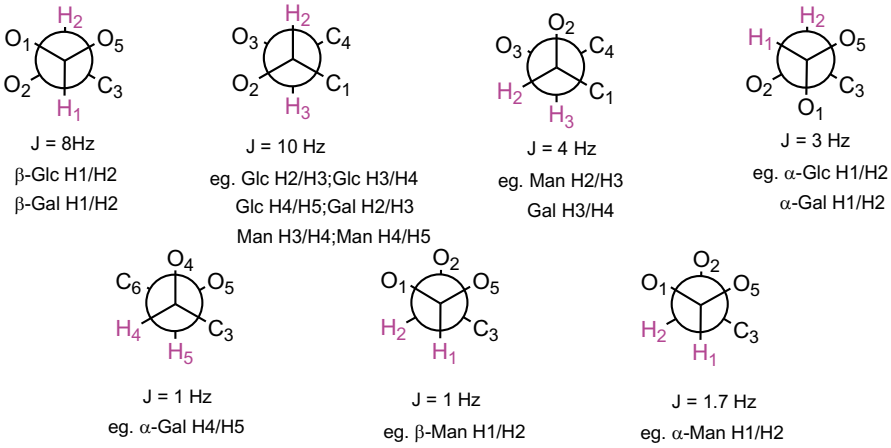
Pyranoside rings of the D-series generally prefer to assume conformation $^{4}C_{1}$ and those of the L-series the conformation $_{1}C^{4}$. The anomeric proton usually resonates at lower field than methine protons, whereas methylene protons resonate at somewhat higher fields.

In D-pyranoses with $^{4}C_{1}$ conformation, the α-anomer resonance is downfield compared to the β-anomer, and the value of the coupling constant between H-1 and H-2 at three bond distance $^{3}J_{1\text{-}2}$ determine if the anomeric proton is equatorial or axial, and therefore if the glycoside is α or β. Usually for axial–axial interactions the observed values are 8–10 Hz and for axial–equatorial or equatorial–equatorial 2–3 Hz. Thus for β-glucose, $^{3}J_{1,2} = 8$ Hz, $^{3}J_{2,3} = {}^{3}J_{3,4} = {}^{3}J_{4,5} = 10$ Hz, H-1 appears as doublet, and H-2, H-3, H-4 appear as 10 Hz triplets, and H-5 appears as double double doublet as it is coupled to the two H6s.

The α-galactose presents $^{3}J_{1,2} = 3$ Hz, $^{3}J_{2,3} = 10$ Hz, $^{3}J_{3,4} = 4$ Hz, $^{3}J_{4,5} < 1$ Hz. H-1 appears as 3 Hz doublet, H-2 and H-3 as double of doublets, H-4 as doublet, and H-5 as triplet for coupling with two H6s. The different possible arrangements are for better understanding represented in Newman projection (Scheme 8.1).

Equatorial protons are positioned at lower field than chemically equivalent axial protons except in those cases were there is a carbonyl group adjacent to H-equatorial,

M. Brito-Arias, *Synthesis and Characterization of Glycosides*,
DOI 10.1007/978-3-319-32310-7_8



Scheme 8.1 Newman projections showing the arrangements of hydrogens in $^{4}C_{1}$ and $_{4}C^{1}$ chair conformations and the expected coupling constants

or when there is a synaxial interaction with H-axial, in which a deshielding effect is observed [49].

The magnitude of coupling constant $^{3}J_{\text{H-H}}$ besides torsion angle dependence may be affected by other factors such as substituent electronegativity, bond length, and bond distance. Solvent effects on $^{3}J_{(\text{HH})}$ appear to be relatively minor, except in cases where solvent-induced conformational changes occur [5].

The ^{13}C chemical shift may also reveals along with de ^{1}H NMR the anomeric configuration, but the one bond ^{13}C-^{1}H coupling constants can be remarkably useful to determine the anomeric configuration in pyranoses. For instance, the $^{1}J_{\text{CH}}$ for the α-anomer is 170 Hz and for the β-anomer 160 Hz, and for the L-isomer the reverse [6].

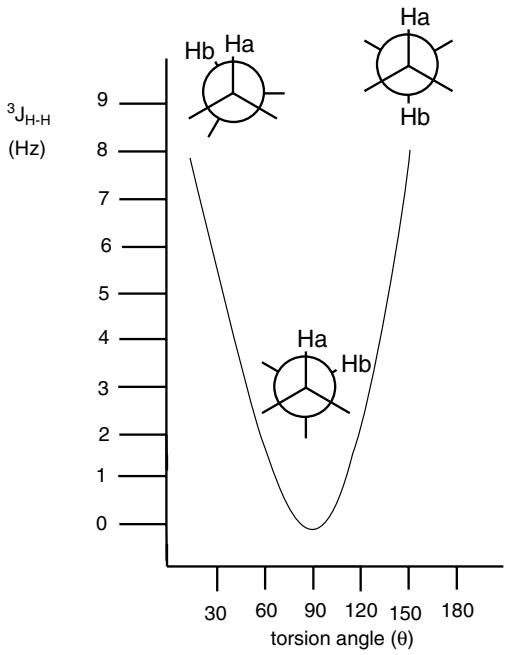
The chemical shift values of the ring protons are dependent of the groups attached to the hydroxyl groups. For instance a characteristic shift of ring-proton resonances to lower field occurs when the hydroxyl group is esterified with acetyl, sulfate, or phosphate where normally downfield shifts ~0.2–0.5 ppm are observed. If the protecting group is acetate, for non-aromatic solvents C-6 resonates at lower field, followed by C-2, C-4 and at highest field the 3-acetoxyl signal [4]. The proton magnetic resonance of 4,6-*O*-benzylidene pyranosides have been measured and the values of the coupling constants $J_{1,2}$, $J_{1,3}$, $J_{2,3}$, and $J_{3,4}$ support the assignment of the chair conformation to the pyranoid ring [2].

The coupling constants $^{3}J_{\text{H-H}}$ values on saturated systems can be predicted by applying the Karplus equation [7], which correlate the dihedral angle θ values with the magnitude of the coupling constant $^{3}J_{\text{H-H}}$.

$$^{3}J_{\text{H H}} = A + B\cos\theta + C\cos 2\theta$$

where θ is the dihedral angle between H1-C1-C2-H2, $A = 4.22$, $B = -0.5$, and $C = 4.5$ Hz for C–C bond distance 1.543 Å.

Scheme 8.2 Relationship between coupling constant and torsion angle



Coupling constants for vicinal protons at three bond distances are two or three times bigger when they are eclipsed or antieclipsed (0° or 180°) to each other than when they are synclinal or gauche (60°) (Scheme 8.2).

Karplus analysis is more accurate when comparative studies are performed between structurally similar compounds. For the study of conformational differences between structurally similar molecules the Karplus equation adopts the form of:

$$^3J_{\mathrm{H\,H}} = K\cos 2\theta$$

Where K is dependent on H1-C1-C2-H2 fragment, when θ is having values between 50 and 70°, or 110 and 130°, slight variations are observed, while for values close to 0, 90, and 180°, no observable changes are detected.

The effect of the relative orientation and electronegativity of substituents on the magnitude of ^3J(aa), ^3J(ae), and ^3J(ee) has been predicted by a simple set of additivity constants. The step followed in the derivation of the additivity constants considers that antiperiplanar substituents exert a negative and gauche substituents a positive effect on J. The resulting data were fitted equation $^3J = {}^3J^0 + \Sigma\Delta J(\mathrm{x})$ where $^3J^0$ represents the reference value. Some of the additivity constants ΔJ(x) for a given substituent are given in Table 8.1 [5].

A computer program known as ALTONA was developed for the calculation of dihedral angles from ^{1}H NMR. This program calculates plots of H-C-C-H dihedral angles from proton-proton NMR vicinal coupling constants using an empirically

Table 8.1 Additivity constants $\Delta J(x)$ for a substituent X

X	$\Sigma\ \Delta J$(ae)(x) or $\Sigma\Delta J$ (ee)(x)		$\Sigma\Delta J$(aa)(x)
	X anti	X gauche	X gauche
H,C	0.0	0.0	0.0
I,S	–0.3	+0.1	–0.3
Br	–0.9	+0.3	–0.7
N	–1.1	+0.3	–0.6
N_3	–1.4	+0.4	–1.1
Cl	–1.2	+0.4	–1.0
O	–1.8	+0.5	–1.4
F	–2.5	+0.7	–2.0

Table 8.2 ^{1}H chemical shifts and couplings ($^3J_{H\text{-}H}$) of peracetylated α- and β-D-glucopyranoses measured in $CDCl_3$ at 30 °C

Compound	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
α-glucopyranosyl pentaacetate	6.33	5.10	5.47	5.14	4.11	4.09	4.26
	3.71	10.29	9.49	10.35	2.32, 4.14		
β-glucopyranosyl pentaacetate	5.71	5.13	5.25	5.12	3.83	4.11	4.28
	8.33	9.58	9.39	10.11	2.25, 4.57		
	C-1	C-2	C-3	C-4	C-5	C-6	
α-glucopyranosyl pentaacetate	92.77	72.15	73.43	70.32	72.10	61.27	
β-glucopyranosyl pentaacetate	96.59	74.81	76.43	70.27	76.61	61.42	

generalized Karplus-type equation, which takes into account the electronegativity and the orientation of the substituents attached to the considered fragment [8].

The Complete assignment of the ^{1}H and ^{13}C NMR spectra of fully acetylated α and β glucopyranosides was determined and the ^{1}H chemical shifts and proton–proton coupling constants were refined by computational spectral analyses (Table 8.2) [9].

Also 1-thioaldopyranosides having the configurations β-D-xylo, α-L-arabino, β-D-ribo, β-D-gluco, and β-D-galacto were determined in different solvents, observing that the H-1 signal in these derivatives appears ~0.35 ppm to higher field than its position in the 1-oxygenated analogs [10].

Also detailed studies of ^{1}H NMR spectra of a series of hexopyranosyl halides have been accomplished. The first order assignments revealed several stereospecific dependencies, mainly upon the orientation of the halogen substituent with respect to the pyranose ring and the relative orientation of other substituents attached to the ring [11, 12, 50].

^{1}H and ^{13}C chemical shifts and *J*-coupling patterns for common D-aldohexoses, D-aldopentoses and some methyl monosaccharides are described in Tables 8.3, 8.4, and 8.5 [13, 14].

A wide number and variety of *O*-glycosides and to a less extent *C*-glycosides islolated from natural sources have been reported and their NMR analysis described.

Table 8.3 ^{1}H chemical shifts and couplings ($^{3}J_{H\text{-}H}$) of D-aldohexoses and aldopentoses measured at 400 MHz in D_2O

Compound	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
α-glucose, Ref. [8]	5.09	3.41	3.61	3.29	3.72	3.72	3.63
	3.6	9.5	9.5	9.5	3.81	2.8	5.7, 12.8
	5.21	3.51	3.69	3.93	2.3, 5.4	3.82	3.74
	3.8	9.8	9.1	9.9			
β-glucose, Ref. [8]	4.51	3.13	3.37	3.30	3.35	3.75	3.60
	7.8	9.5	9.5	9.5	3.44	2.8	5.7, 12.8
	4.62	3.22	3.46	3.38	2.2, 5.9	3.87	3.70
	7.9	9.4	9.1	9.9			
α-galactose	5.16	3.72	3.77	3.90	4.00	3.70	3.62
	3.8	10.0	3.8	1.0		6.4	6.4
β-galactose	4.48	3.41	3.56	3.84	3.61	3.70	3.62
	8.0	10.0	3.8	1.0		3.8	7.8
α-mannose	5.05	3.79	3.72	3.52	3.70	3.74	3.63
	1.8	3.8	10.0	9.8		2.8	6.8, 12.2
β-mannose	4.77	3.85	3.53	3.44	3.25	3.74	3.60
	1.5	3.8	10.0	9.8		2.8	6.8, 12.2

Table 8.4 ^{1}H chemical shifts and couplings ($^{3}J_{H\text{-}H}$) of D-aldopentoses measured at 400 MHz in D_2O

Compound	H-1	H-2	H-3	H-4	H-5a	H-5b
α-xylose	5.09	3.42	3.48	3.52	3.58	3.57
	3.6	9.0	9.0		7.5	7.5
β-xylose	4.47	3.14	3.33	3.51	3.82	3.22
	7.8	9.2	9.0		5.6	10.5, 11.4
α-arabinose	4.40	3.40	3.55	3.83	3.78	3.57
	7.8	9.8	3.6		1.8	1.3, 13.0
β-arabinose	5.12	3.70	3.77	3.89	3.54	3.91
	3.6	9.3	9.8		2.5	1.7, 13.5
α-ribose	4.75	3.71	3.83	3.77	3.82	3.50
	2.1	3.0	3.0		5.3	2.6, 12.4
β-ribose	4.81	3.41	3.98	3.77	3.72	3.57
	6.5	3.3	3.2		4.4	8.8, 11.4
α-lyxose	4.89	3.69	3.78	3.73	3.71	3.58
	4.9	3.6	7.8		3.8	7.2, 12.1
β-lyxose	4.74	3.81	3.53	3.73	3.84	3.15
	1.1	2.7	8.5		5.1	9.1, 11.7

The chemical shifts and coupling constants of some of them are described just as representative examples in Table 8.6.

Nuclear Overhauser effects (NOE) is a dipole-dipole relaxation experiment and has been one of the most useful experiments for the structural assignments of glyco-

Table 8.5 ^{13}C chemical shifts of some aldoses

Compound	C-1	C-2	C-3	C-4	C-5	C-6
α-glucose, Ref. [8]	92.9	72.5	73.8	70.6	72.3	61.6
	92.77	72.15	73.43	70.32	72.10	61.27
β-glucose, Ref. [8]	96.7	75.1	76.7	70.6	76.8	61.7
	96.59	74.81	76.43	70.27	76.61	61.42
α-galactose	93.2	69.4	70.2	70.3	71.4	62.2
β-galactose	97.3	72.9	73.8	69.7	76.0	62.0
α-mannose	95.0	71.7	71.3	68.0	73.4	62.1
β-mannose	94.6	72.3	74.1	67.8	77.2	62.1
α-arabinose	101.9	82.3	76.5	83.8	62.0	
β-arabinose	96.0	77.1	75.1	82.2	62.0	
α-ribose	97.1	71.7	70.8	83.8	62.1	
β-ribose	101.7	76.0	71.2	83.3	63.3	

sides on the basis of shielding and deshielding effects [20]. Glycosylation sites can be identified by comparison of ^{1}H NMR spectral data of the peracetylated and the nonprotected sugar, since free OH groups causes significant downfield shift (in the range of 1–0.5 ppm) The approach known as "structural-reporter-group" has been introduced to identify individual sugars or sequences of residues and can be used to identify structural motifs or specific sugars and linkage compositions found in relevant databases [14].

For complex molecules the interpretation is often problematic, especially due to the presence of internal motion. Some of the difficulties encountered for NMR structural assignment for oligosaccharides are: [21]

The limited number of C,H dipolar couplings measured across a single bond.
The distribution of C,H bond vectors is not isotropic due to the geometry of the pyranose ring.
Due the flexibility of the glycosidic bond that connects the different sugars moieties, different alignment tensors can be observed.

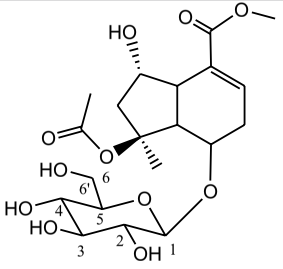
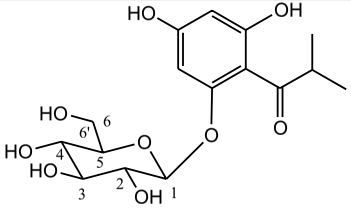
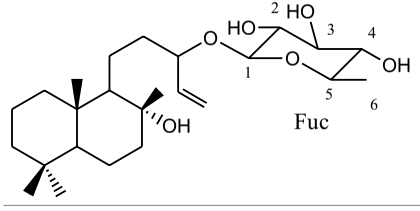
More recently the use of a novel procedure known as "residual dipolar coupling" has been introduced by Tian and Prestegard as an alternative approach for studying the conformational and the motional properties of oligosaccharides [22]. The approach is based on the solution for each ring of an order matrix that combines different types of couplings, $^{1}D_{CH}$, $^{2}D_{CH}$, and D_{HH}.

Dipolar coupling arise from through space spin–spin interactions and is dependent of both internuclear distance (r) and an angle between the magnetic field and the internuclear vector (θ) as described by the equation

$$D_{ij} = \xi_{ij}\left(\frac{3\cos^2\theta - 1}{2}\right)\left(1/r^3\right)$$

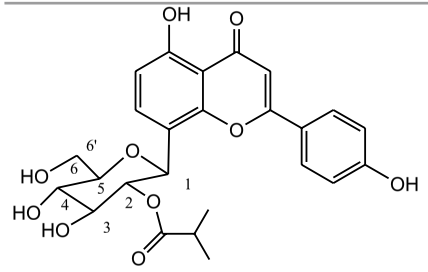
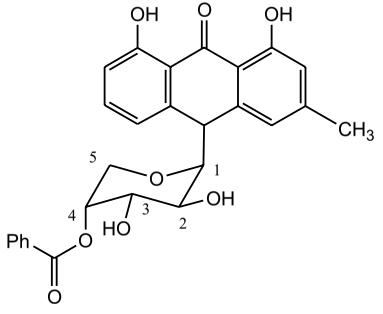
Where ξ_{ij} is a constant that depends on the properties of nuclei i and j.

Table 8.6 ^{1}H chemical shifts and couplings ($^{3}J_{H\text{-}H}$) of natural *O*- and *C*-glycosides

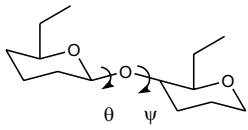
Natural glycoside	H-1	H-2	H-3	H-4	H-5	H-6, H-6′	Ref
 Glc	4.63 d (8.0)	3.17 dd (8.0, 9.0)	3.36 t (9.0)	3.26 t (9.0)	3.30 m	3.66 dd (6.0, 12.0); 3.89 dd (2.0, 12.0)	[15]
 Glc	5.04 d (7.5)	3.50 dd (9.0, 7.5)	3.44 m	3.38 t (9.0)	3.44 m	3.71 dd (5.5, 12.3), 3.91 dd (2.0, 12.3)	[16]
 Fuc	4.32 d (8.0)	3.59 dd (8.0, 10.0)	3.54 br dd (3.0, 10.0)	3.63 br d (3.0)	3.58 dq (1.0, 6.0)	1.21 d	[17]

(continued)

Table 8.6 (continued)

Natural glycoside	H-1	H-2	H-3	H-4	H-5	H-6, H-6′	Ref
 Glc	4.88 d (10.0)	5.35 t (10.0)	3.49 m	3.49 m	3.33 m	3.58 dd (5.5, 12.5), 3.78 m	[18]
	3.47 dd (1.9, 9.2)	3.66, t (9.2)	3.76 dd (2.8, 9.2)	5.21 br s	3.37 d (13.1), 3.88 dd (1.8, 13.1)		[19]

Scheme 8.3 Torsion angles around the glycosidic bond



Direct measurements of dipolar interactions can be achieved by dissolving molecules in oriented media such as crystals composed of bicelles or phage. Despite the fact that molecular tumbling remains fast in these media, the sampling of orientations is no longer isotropic, and consequently the dipolar coupling do not average to zero and splittings are observed between the dipolar coupled spin pairs.

The knowledge of the molecular geometry of a fragment and the measurement of five or more interdependent residual couplings from the fragment allows the determination of the Saupe order matrix elements (S_{ij}) from a set of linear equations relating dipolar couplings to the known geometry factors and the unknown order tensor elements.

$$D_{\text{resid}} \propto \sum_{ij} S_{ij} \cos\theta_i \cos\theta_j$$

Where θ_{ij} are the angles between the internuclear vectors.

Determination of the Saupe order matrices for individual rigid fragments of a molecule allow both structural characterization and assessments of internal motions between fragments [11].

NMR studies carried out by De Bruyn [23], using as models series of disaccharides provided valuable information about conformational behavior from the chemical shifts and the torsion angles present around the glycosidic bond (Scheme 8.3). Also it has been reported that the ^{13}C chemical shifts for the glycoside and the aglycone carbon can be directly correlated with one of the torsion angles psi (ψ) defined by the bonds C(1)-O(1)-C(4)-H(4) [20].

The sign of θ and ψ has been previously calculated through the method known as hard sphere exoanomeric effect [24] which predicted the relative stability of the different conformers around the torsion angles, considering the bond length, bond angle and atomic size. It has been observed that for a number of disaccharides there is a variation of the chemical shifts as a function of ψ, compared with the values of their corresponding monosaccharides (Table 8.7).

The development of Karplus relationship for three-bond C-O-C-C spin-coupling constants by Bose et al. [25], suggest that $^3J_{COCC}$ obeys a Karplus relationship similar to that observed for $^3J_{HH}$,$^3J_{HC}$, and other vicinal spin-coupling constants. However the precise form of this relationship that is the shape and amplitude of the Karplus curve is unknown. Also in this work, $^3J_{COCH}$ values have been measured to asses the phi (ϕ) and the psi (ψ) torsion angles (Scheme 8.4) and Karplus relationships have been reported for this vicinal coupling [26].

Another report describes the calculation of $^2J_{HH}$, $^3J_{HH}$, $^1J_{CH}$, $^2J_{CH}$, $^3J_{CH}$, $^1J_{CH}$, for the exocyclic CH_2OH group and the $^3J_{CXCH}$ for the X-glycosidic linkage, as a function

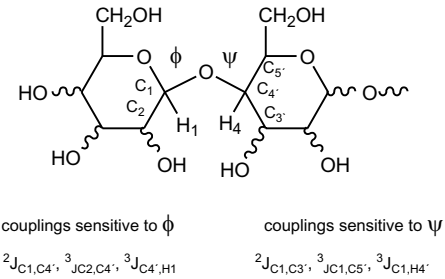
Table 8.7 Chemical shifts and $J_{H,H}$ coupling constants (Hz) of disaccharides and anomers of glucopyranose in D_2O at 30 °C

	H1′	H2′	H3′	H4′	H5′	H6a	H6b	H1	H2	H3	H4	H5	H6a	H6b
α-1, Ref. [8]	5.10	3.56	3.80	3.47	3.86	3.85	3.78	5.45	3.64	3.83	3.47	4.03	3.85	3.76
	5.08	3.54	3.77	3.44	3.93	3.82	3.75	5.42	3.62	3.80	3.44	3.84	3.83	3.78
	3.8	9.9	8.9	10.0	2.34, 5.3			3.5	9.7	9.0	10.1	2.35, 4.4		
β-1, Ref. [8]	5.41	3.55	3.80	3.47	3.86	3.78	3.78	4.81	3.39	3.59	-	-	3.90	3.56
	5.37	3.53	3.73	3.45	4.01	3.80	3.77	4.78	3.37	3.56	3.40	3.44	3.88	3.70
	3.8	9.9	9.1	10.1	2.54, 4.6			7.9	9.4	9.1	9.9	2.22, 5.9		
α-2, Ref. [8]	5.38	3.56	3.77	3.47	4.02	3.82	3.82	5.24	3.63	3.86	3.67	-	-	-
	5.35	3.55	3.74	3.43	3.99	3.82	3.76	5.21	3.61	3.83	3.63	3.84	3.81	3.70
	3.9	9.9	9.1	10.1	2.39, 4.78			3.8	9.7	8.9	10.3	2.33, 5.2		
β-2, Ref. [8]	5.36	3.57	3.76	3.45	4.02	3.82	3.82	4.67	3.36	3.64	3.64	3.48	-	-
	5.34	3.54	3.73	3.44	4.00	3.80	3.78	4.65	3.31	3.62	3.62	3.45	3.87	3.70
	3.9	9.9	9.1	10.1	2.31, 4.3			8.0	9.3	8.7	9.8	2.27, 5.8		
α-3 Ref. [9]	5.41	3.59	3.68	3.42	3.72	3.74	3.74	5.23	3.58	3.97	3.64	3.93	3.82	3.82
	5.39	3.56	3.68	3.40	3.71	3.84	3.75	5.21	3.55	3.95	3.63	3.92	3.83	3.79
	3.9	9.9	9.1	10.0	2.3, 5.2			3.8	9.9	8.9	10.0	2.2, 4.8		
β-3, Ref. [8]	5.41	3.58	3.69	3.42	3.74	3.77	3.77	4.66	3.28	3.77	3.62	3.60	3.92	3.77
	5.39	3.56	3.66	3.40	3.70	3.84	3.75	4.63	3.26	3.75	3.62	3.58	3.89	3.75
	3.9	9.9	9.2	10.0	2.2, 5.2									

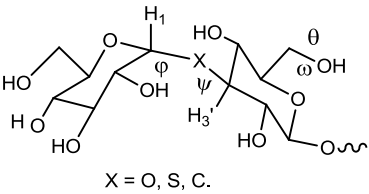
α-4, Ref. [8]	4.63	3.37	3.52	3.42	3.46	3.75	3.75	5.45	3.65	3.87	3.47	3.87	3.84	3.77
	4.61	3.35	3.49	3.40	3.43	3.88	3.72	5.42	3.62	3.84	3.44	3.82	3.82	3.75
	7.9	9.4	9.1	9.9	2.26, 5.6			3.6	9.7	9.2	10.0	2.3, 5.2		
β-4, Ref. [8]	4.77	3.32	3.50	3.37	3.44	3.91	3.70	4.70	3.50	3.68	3.42	3.45	3.87	3.71
	7.9	9.4	9.1	9.8	2.22, 6.3			7.9	9.3	9.3	9.9	2.1, 5.7		
α-5, Ref. [8]	4.73	3.37	3.54	3.42	3.49	3.73	3.73	5.23	3.73	3.92	3.52	3.88	3.85	3.79
	4.70	3.35	3.51	3.40	3.48	3.90	3.71	5.22	3.70	3.89	3.50	3.85	3.82	3.76
	7.9	9.5	9.1	9.9	2.26, 6.2			3.7	9.7	8.9	9.9	2.26, 4.92		
β-5, Ref. [8]	4.63	3.37	3.54	3.42	3.49	3.73	3.73	4.74	3.44	3.74	3.49	3.49	3.90	3.74
	4.72	3.34	3.50	3.39	3.47	3.90	3.70	4.66	3.42	3.72	3.50	3.47	3.88	3.72
	7.9	9.5	9.1	9.9	2.29, 6.22			8.0	9.3	8.9	10.0	2.07, 5.56		
α-6, Ref. [8]	4.50	3.31	3.49	3.41	3.47	3.90	3.72	5.21	3.56	3.81	3.62	3.93	3.87	3.84
	7.9	9.5	9.1	9.9	2.25, 5.8			3.7	9.8	9.1	9.6	2.24, 4.61		
β-6, Ref. [8]	4.51	3.32	3.52	3.42	3.50	3.75	3.75	4.67	3.29	3.60	3.65	3.58	3.97	3.82
	4.49	3.30	3.49	3.40	3.47	3.90	3.72	4.64	3.27	3.61	3.63	3.58	3.94	3.79
	7.9	9.4	9.1	9.9	2.27, 5.9			7.9	9.3	9.0	9.8	2.19, 5.14		
α-7, Ref. [8]	5.17	3.63	3.83	3.43	3.80	3.84	3.74							
	3.8	9.9	9.1	10.0	2.33, 5.43									
β-7, Ref. [8]	4.63	3.40	3.51	3.39	3.46	3.86	3.70							
	7.9	9.5	9.1	9.9	2.3, 5.7									

1 = kojibiose; 2 = nigerose; 3 = maltose; 4 = sophorose; 5 = laminaribiose; 6 = cellobiose; 7 = trehalose

Scheme 8.4 Couplings sensitive to ϕ and ψ torsion angles

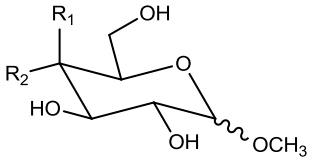


Scheme 8.5 The glycosidic torsion angles ϕ and ψ in disaccharides



of ω, θ, and glycosidic torsion angle ϕ (Scheme 8.5). The glycosidic torsion angles ϕ and ψ are usually determined from NOE measurements between protons near to the linkage (H1 and H3′).

The ^{1}H–^{13}C coupling constants of methyl α- and β-pyranosides of D-glucose and D-galactose were measured by one-dimensional and two-dimensional ^{1}H–^{13}C heteronuclear zero and double quantum, phase sensitive J-HMBC spectra, in order to assign the complete set of coupling constants ($^{1}J_{CH}$, $^{2}J_{CH}$, $^{3}J_{CH}$, $^{2}J_{HH}$, and $^{3}J_{HH}$) within the exocyclic hydroxymethyl group. As a result of this spin–spin couplings constants of α- and β-pyranosides of D-glucose and D-galactose were generated as shown in Table 8.8 [27].



D-glucopyranose R_1 = H, R_2 = OH
D-galactopyranose R_1 = OH, R_2 = H

Comparative conformational studies using a combination of NMR spectroscopy and molecular mechanics of lactose disaccharide (βGal[1–4]Glc) and its *C*-analog showed that for the former the population in solution is about 90 % *syn* and 10 % *anti*, while for the latter the conformation is more flexible in the forms 55 %, 40 %, and 5 % *syn*, *anti*, and *gauche-gauche*, respectively [28].

^{1}H NMR spectra of oligosaccharides follow in many cases complex patterns due to extensive overlap within the region δ 3.0–4.2; however, the use of pyridine-d_5 improves the signal dispersion, increasing the resolution especially in overcrowded

Table 8.8 Experimental ^{1}H–^{1}H and ^{13}C–^{1}H spin–spin couplings constants

Coupling	β-D-methyl glucose	α-D-methyl glucose	β-D- methyl galactose	α-D-methyl galactose
$^{3}J_{H1,H2}$	8.0	3.8	7.9	3.9
$^{3}J_{H2,H3}$	9.4	9.8	9.9	10.3
$^{3}J_{H3,H4}$	9.2	9.1	3.5	3.5
$^{3}J_{H4,H5}$	9.7	10.0	1.1	1.2
$^{3}J_{H5,H6R}$	6.0	5.4	7.9	8.2
$^{3}J_{H5,H6S}$	2.3	2.3	4.4	4.2
$^{3}J_{H6R,H6S}$	−12.3	−12.3	−11.8	−11.7
$^{1}J_{C4,H4}$	144.8	144.4	146.2	146.5
$^{1}J_{C5,H5}$	141.7	144.3	140.8	143.5
$^{1}J_{C6,H6R}$	143.2	143.2	145.5	145.1
$^{1}J_{C6,H6S}$	144.5	144.2	142.9	142.5
$^{2}J_{C4,H3}$	−4.7	−4.7	1.6	1.6
$^{2}J_{C4,H5}$	−2.8	−2.9	3.3	3.0
$^{2}J_{C5,H4}$	−4.0	−3.8	1.0	1.1
$^{2}J_{C5,H6R}$	−2.5	−1.9	−5.0	5.1
$^{2}J_{C5,H6S}$	−1.1	−1.4	0.4	1.0
$^{2}J_{C6,H5}$	−2.3	−1.4	−5.5	−5.2
$^{3}J_{C4,H2}$	1.1	1.0	0.7	0.9
$^{3}J_{C4,H6R}$	1.0	1.1	1.9	1.0
$^{3}J_{C4,H6S}$	2.4	2.9	4.0	3.7
$^{3}J_{C5,H3}$	1.1	1.0	–	0.5
$^{3}J_{C6,H4}$	3.6	3.6	1.0	1.0

regions. The localization of anomeric protons is a valuable tool for recognizing the number of monosaccharide residues.

A number of one- and two-dimensional methods provides thorough information to assert the complete assignment unambiguously. One-dimensional NMR analysis provides useful information about the chemical shifts and scalar couplings of well resolved signals such as anomeric protons (δ 4.4–5.6) and methyl groups for 6-deoxy monosaccharides (fucose, quinovose, rhamnose) at (δ 1.1–1.3). The effect on the proton chemical shift of glycosylation is a typical deshielding of the proton across the glycosidic bond and the two neighboring positions of the aglycone. This behavior is due to repulsion between hydrogens and due to the effect of the lone pair of the oxygen to the hydrogens [29].

Conformational analysis on more complex glycosides is based mainly on the inter-residue ^{1}H-^{1}H Nuclear Overhauser effects (NOE) [30]. and also ^{13}C-^{1}H long-range coupling constants across the glycosidic linkage for studying the preferred conformation of oligosaccharides in solution. Selective irradiation of the anomeric proton reveals inter-residual contacts with aglycone protons. In this way $1 \rightarrow 2$, $1 \rightarrow 3$, $1 \rightarrow 4$, and $1 \rightarrow 6$ combinations as well as α and β linkages may be determined [31, 51–53]. Long-range ^{1}H-^{1}H couplings involving four bonds between anomeric

and aglycone protons ($^4J_{HCOCH}$) are usually very small that could be detected but not measured [32, 54].

Two-dimensional NMR is a reliable method for determining inter-ring connectivity. Through space dipolar interactions between the anomeric and the trans glycosidic proton can be detected in the form NOE signals and represent the basis for linkage and sequence analysis [33], also the interglycosidic connectivities are established on the basis of long-range ($^3J_{CH}$) by HMBC studies [27]. The usefulness of this method has been later demonstrated in a number of structural elucidations [34, 55–58].

Bidimensional homonuclear techniques such as TOCSY experiment have been useful for the NMR characterization of the naturally occurring complex glycosides such as glycoresin tricolorin E [35, 59], allowing the total assignment of the sugar region, including the anomeric protons for each of the four monosaccharides established (Scheme 8.6).

Likewise, the complete ^{1}H and ^{13}C assignments of a synthetic octasaccharide fragment of the *O*-specific polysaccharide of *Shigella dysenteriae* type 1 by using 2D TOCSY at 600 MHz was described. In the contour plot it is possible to observe the connectivity between the sugar units and the detailed assignment of the protons [36]. Moreover, a 2D selective TOCSY-DQFCOSY experiment for identification of individual sugar components in oligosaccharides is described, assuming that unambiguous sequential assignment of the proton signals for individual components is reached [37].

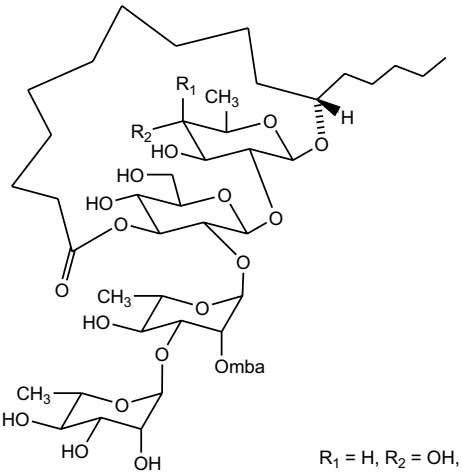
High resolution ^{1}H NMR spectroscopy has been applied in the structural analysis of glycoproteins. The initial efforts to assign all the anomeric and non anomeric protons were done by using spin decoupling and nuclear Overhauser spectroscopy [38].

Nuclear magnetic resonance of carbohydrate related to glycoconjugates have been analyzed. One of the first high resolution studies was reported back in 1973 on intact glycolipids in a 220-MHz magnet [39]. Subsequent studies on underivatized and permethylated glycosphingolipids in dimethylsulfoxide-d_6 and chloroform, respectively, allowed to assign all the anomeric protons and a number of nonanomeric proton resonances [40, 60].

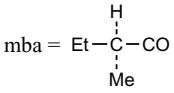
Early studies on high resolution NMR spectra of glycans chain in D_2O allowed to assign the anomeric and non anomeric protons as well as the coupling constants of sugar residues found in glycoproteins [38, 41]. More recently the complete resolution of acetyl protected sialic acid glycopeptides was achieved by using NOESY and DQF-COSY technique [6].

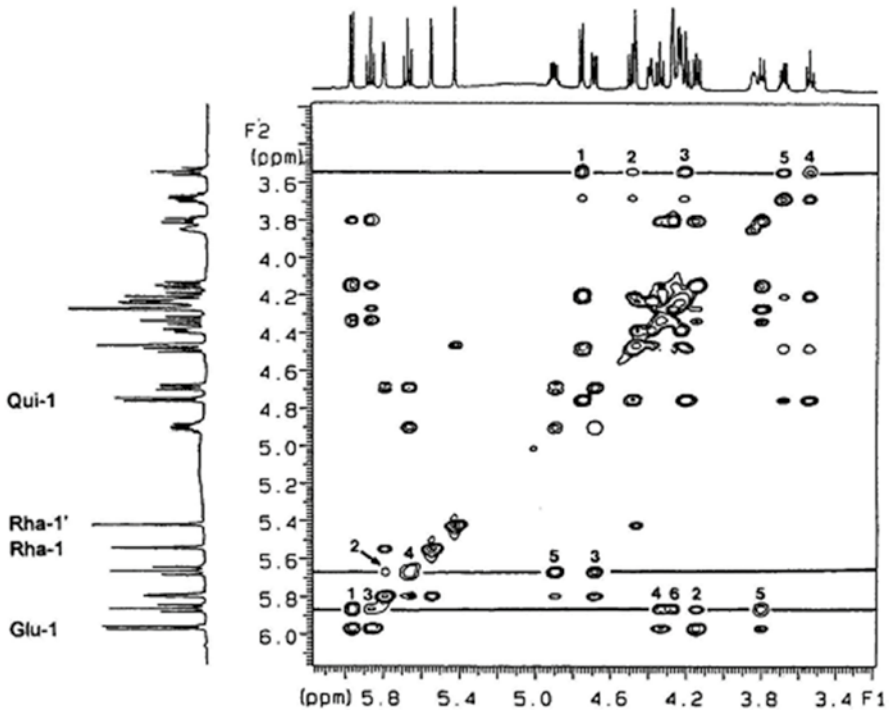
For the NMR-analysis of carbohydrate-protein complexes the transfer nuclear overhauser effect (trNOE) experiment seems to be a promising alternative [42, 61]. Recent advances on conformational analysis of oligosaccharides allows to determine the interresidue interactions based on the dihedral angles ϕ and ψ along the interglycosidic linkage [43, 62]. In this connection, recent conformational advances on E-selectin-sialyl Lewis^x complex has led to the determination of the bioactive conformation of the silayl Lewis^x tetrasaccharide [44].

NMR spectroscopy of glycoproteins has been achieved by using a combination of homo- and heteronuclear experiments at natural abundance [45, 63, 64]. Increased



R_1 = H, R_2 = OH, R_3 = mba





Scheme 8.6 Expanded region of TOCSY spectrum for characterization of tricolorin E

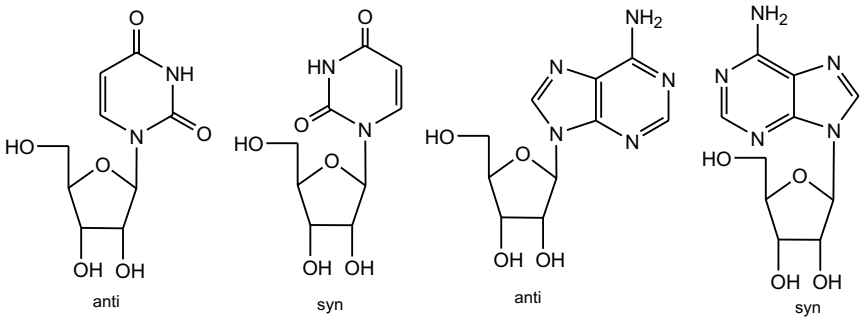
refinement is possible when a ^{15}N-labeled sample was used and the mobility of the glycan chain could be assesed by the measurements of ^{13}C line widths obtained fro the high resolution HSQC spectra [43, 62].

8.2 NMR of *N*-glycosides

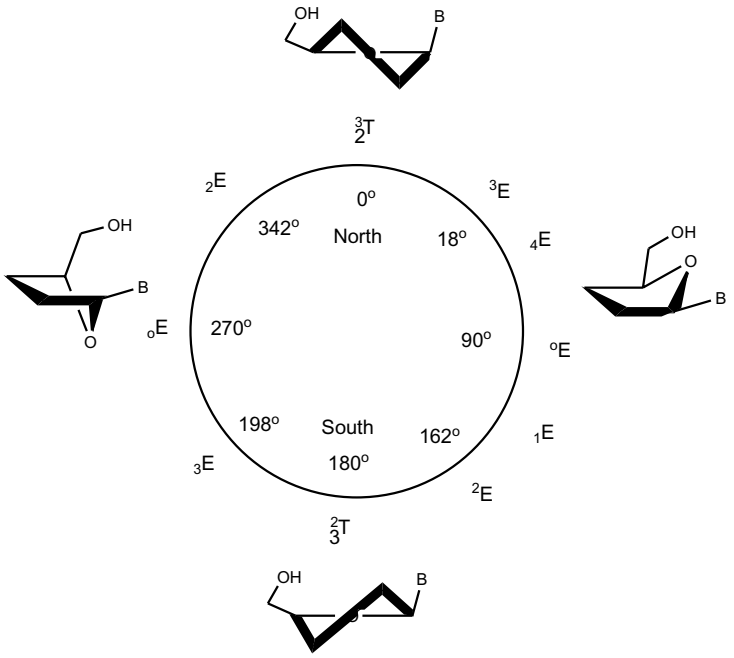
The conformational analysis of *N*-glycosides has been extensively studied on the basis of chemical shifts and coupling constant determinations mainly around the C-N linkage. Torsion angles symbolized as χ for furanosides rings, are also dependent on the Karplus equation, and similarly plays an important rule for the conformational analysis of five member rings [46]. For purines the angle χ is formed between O4′-C1-N9-C4 atoms, and O4′-C1-N1-C2 for pyrimidines. When torsion angles O4′-C1 N9-C4 for purines, and O4′-C1-N1-C2 for pyrimidines are eclipsed, then $\chi = 0°$. Positive angles of χ occur for rotation clockwise for N9-C4 for purines and N1-C2 for pyrimidines. The conformation *syn* in nucleosides, correspond to the angle $\chi = 0 \pm 90°$, and *anti* to $180 \pm 90°$ (Scheme 8.7).

Regarding furanoside rings, there are different non planar conformation possibly assumed in terms of five endocyclic torsion angles symbolized as ν_o, ν_1, ν_2, ν_3, ν_4, corresponding to the bonds O4′-C1′, C1′-C2′, C2′-C3′, C3′-C4′, and C4′-O4′. The two most common conformations founded are the envelope (E), referring to four atoms on the plane, and twist (T) for three atoms on the plane. The puckering of the furanoside rings of nucleosides is explained by Sorenssen et al. [47] Unmodified nucleosides are present as an equilibrium between the C-3′-endo conformation, located around $P = 18°$, and the C-2′-endo conformation centered around $P = 162°$ (Scheme 8.8).

Besides the torsion angle described for the *N*-glycosidic bond, there are for the case of oligosaccharides, additional torsion angles symbolized as ω,ω',ϕ,ϕ',ψ, and ψ' corresponding to the bonds P-O5, P-O3′, O5′-C5′, O3′-C3′, C5′-C4′, C4′-O3′ respectively (Scheme 8.9).

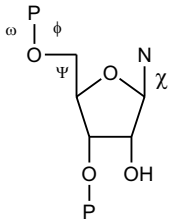


Scheme 8.7 *Syn-anti* conformations for purines and pyrirmidines



Scheme 8.8 Pseudorotacional cycle of the furanoside ring in nucleosides

Scheme 8.9 Torsion angles for oligosaccharides



The vicinal coupling constants at 3 bond distance are dependent of the dihedral angle θ, and the relationship determined by the Karplus equation.

$$^{3}J_{\mathrm{H\ H}} = A\cos 2\theta - B\cos\theta + C$$

Where A, B and C are constants and their values are given in Table 8.9.

The exchange of –OH for -OPO_3, does not affect sensibly the Karplus relationship, therefore the values are valid for both nucleosides or oligonucleosides, however, as mentioned, ^{3}J there is a dependence of other factors such as bond length, bond angle, electronegativity, and substituent orientation. Some of the values reported for ribose and deoxyribose are presented in Table 8.10.

The analysis of the *C*-nucleosides β-pseudouridine (β-ψ) and α-pseurouridine (α-ψ) in aqueous solution has been described and the observed coupling constants given in Table 8.11 [48].

Table 8.9 Karplus *A*, *B*, *C* constant values for nucleotide molecular fragments

	Torsion angle	*J* (Hz)	*A*	*B*	*C*
HO-CH-CH-OH	Sugar ring	J1′2′, J2′3′, J3′4′	10.2	0.8	0
H-C-C-H	ψ	J4′5′, J4′5″	9.7	1.8	0
H-C-O-H	ϕ'	J2′OH2′	10.4	1.5	0.2
	ϕ	J3′OH3′			
		J5′OH5′			
H-C-O-P	ϕ'	J3′P	18.1	4.8	0
	ϕ	J5′, J5″			

Table 8.10 $^3J_{2'3'}$ (Hz) values for furanoside ring in nucleosides

Nucleoside	$^3J_{1'2'} + {}^3J_{3'4'}$ (Hz)	$^3J_{2'3'}$ (Hz)
β-D-ribonucleosides		
pyrimidine (anti)	9.9 (±0.2)	5.3 (±0.2)
pyrimidine (syn)	10.3 (±0.2)	6.2 (±0.2)
purine (anti)	9.7 (±0.3)	5.2 (±0.1)
purine (syn)	9.6 (±0.3)	5.5 (±0.2)
β-D-deoxyribonucleosides		
pyrimidine (anti)	10.6 (±0.2)	6.7 (±0.2)
pyrimidine (syn)	11.0 (±0.1)	8.0 (±0.1)
purine (anti-syn)	6.3 (±0.1)	6.3 (±0.1)
C-nucleoside pyrimidic	10.6 (±0.3)	5.2 (±0.2)
C-nucleoside puric	10.1 (±0.3)	5.0 (±0.2)

Table 8.11 Coupling constant (Hz) for β- and α-pseudouridine at 30°

Coupling constant	β-ψ	α-ψ
$J_{61'}$	0.8	1.3
$J_{1'2'}$	5.0	3.3
$J_{2'3'}$	5.0	4.2
$J_{3'4'}$	5.2	7.9
$J_{4'5'B}$	3.2	2.4
$J_{4'5'C}$	4.6	5.7
$J_{5'B5'C}$	−12.7	−12.7

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Chapter 9
X-Ray Diffraction of Glycosides

X-ray crystallography is a powerful tool for obtaining molecular information regarding bond lengths, bond angles, hydrogen bond interactions, and torsion angles, which are necessary elements for understanding the conformation of glycosides. Improved diffractometers, faster computational processors, and mathematical programs have made possible the structural resolution of simple and complex substances of glycosidic nature particularly those with noncentrosymmetric space groups.

Early studies on simple glycosides allowed to confirm that the sugar residue is pyranoid (and not acyclic), assuming two possible chair conformations ($^4C_1$ and $_4C^1$), usually orienting the substituent to the equatorial position [1, 2].

In hydrogen bond interactions on pyranoid residues some of the invariable facts are: (a) the ring-oxygen atom is always a hydrogen bond acceptor; (b) each hydroxyl group is associated with two hydrogen bonds, one as the donor and one as the acceptor; (c) in disaccharides there might be intramolecular hydrogen bonding between two residues; (d) the hydrogen bond O-O distance has values around 2.68–3.04 Å.

Crystallographic observations on the anomeric effect demonstrated that the bond shortening and preferred *gauche* conformation of the glycosidic bonds in pyranoses are a consequence of an electronic distribution in the hemiacetal and acetal moiety of these molecules [3].

On the other hand, the primary alcohols can be present in three staggered orientations, defined as gg, gt, and tg referring to torsion angles O5-C5-C6-O6 and the second to C4-C5-C6-O6 [$g \gg \pm 60°$, $t \gg 180°$]. An alternative nomenclature refers to O5-C5-C6-O6 as $+g = gt$, $-g = gg$, $t = tg$.

The general standard molecular dimensions for pyranosides are described in Table 9.1, with the C-C bond length being in the range of 1.523–1.526, C-C-C angles 110.4–110.5°, and usually shorter glycosidic bond 1.398 for axial and 1.385 for equatorial disposition (Table 9.1) [4].

The distortion degree from the ideal chair conformation has been studied by Cremer and Pople [5], who by following a mathematical approximation were

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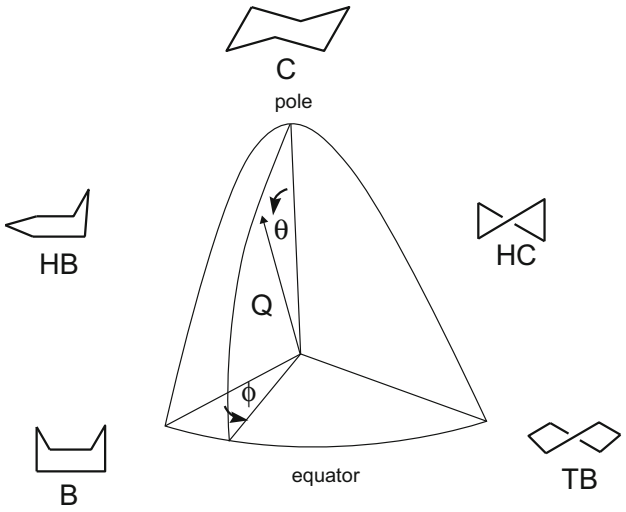
DOI 10.1007/978-3-319-32310-7_9

Table 9.1 Standard molecular dimensions for $^4C_1$ chair conformations in pyranosides

Bond type	Bond lengths (Å)	Bond lengths (Å)	Angle type	Bond angle (°)	Bond angle (°)	4-atom ring	Torsion angles (°)
C-C ring	1.526	1.523	C-C-C ring	110.4	110.5		
C-C exo	1.516	1.514	C-C-C exo	112.5	112.7		
C-O exo	1.420	1.426	C-C-O ring	110.0	110.0		
			C-C-O exo	109.7	109.6		
C5-O5 axial	1.434	1.436	C5-O5-C1	114.0	114.0	C-C-C-C	53
C1-O5 axial	1.419	1.419	O5-C1-O1	112.1	111.6	C-C-C-O	56
C1-O1 axial	1.398	1.415	C5-O5-C1	112.0	112.0	C-C-O-C	60
C5-O5 eq.	1.426	1.436	O5-C1-O1	108.0	107.3	C-C-C-C	53
C1-O5 eq.	1.428	1.429				C-C-C-O	57



Scheme 9.1 One octant of the sphere on which the conformations of six-membered rings can be mapped for a constant Q

able to propose three puckering parameters described as spherical polar set Q (total puckering amplitude), and the angles θ and ϕ, describing the distortion suffered by six-member rings from the ideal chair conformation. The chair corresponds to $\theta=0°$, $\phi=0°$; boat for $\theta=90°$, $\phi=0°$; and twist boat for $\theta=90°$, $\phi=90°$ (Scheme 9.1). The pyranoside ring varies slightly and in terms of Cremer and Pople puckering parameters, the range of values is $Q=0.55$–0.58 Å with θ within 5° of 0 or 180° [4].

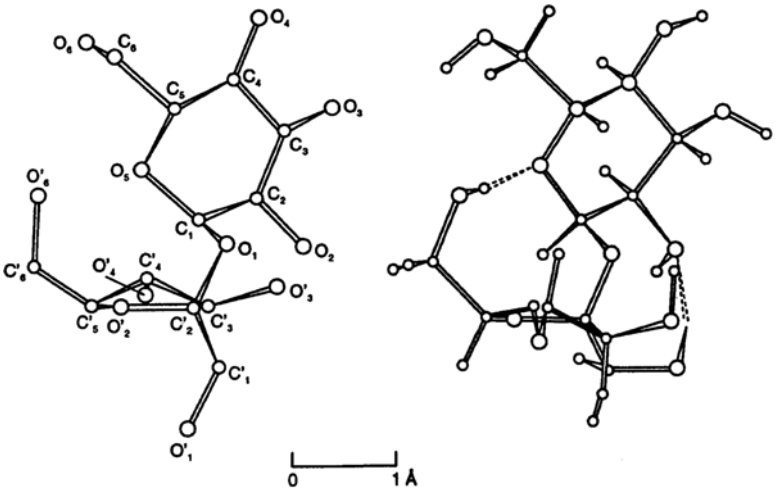
9.1 X-ray Diffraction of *O*-Glycosides

One of the pioneering studies about sugar X-ray analysis was presented by Levy and Brown [6] reporting the structure of sucrose, and sucrose NaBr.H_2O. Through these studies it was observed that although they were energetically equivalent, their chair conformations were different, due to slight hydrogen bridge interactions on the furanoside moiety (Scheme 9.2).

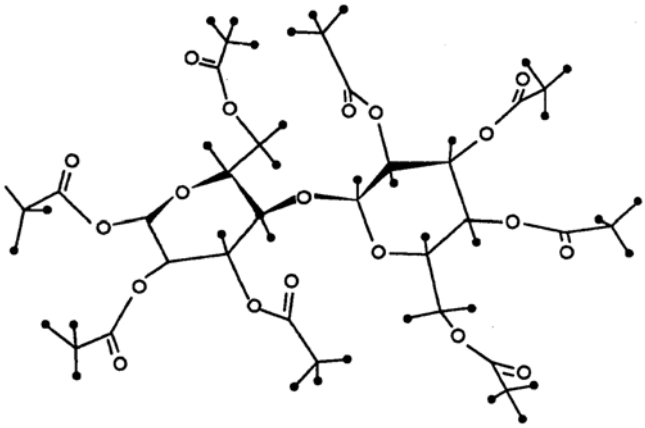
Another disaccharide characterized by X-ray crystallography was octa-*O*-acetyl-β-D-cellobiose which presents space group $P2_1,2_1,2_1$, with both pyranoside residues in $^4C_1$ chair conformation slightly more distorted in comparison with cellobiose. Moreover, the torsion angles determined were −77° for O5-C1-C4′, and 104° for C1-O1-C4-C5 (Scheme 9.3). The sign value indicates according with the Klyne and Prelog notation to the right if positive and to the left if negative [7].

The crystal structure of benzyl 2,3,4-tri-*O*-acetyl-β-D-fucopyranoside is described [8], presenting a monoclinic system, space group $P2_1$, with bond distances C-O 1.423 Å, C-C 1.513 Å, and shorter C-O 1.380 Å for equatorially anomeric bond. The angle disposition for the endocyclic bond C1-O5-C5 is of 112.4 (3)°, with this value being typical for chair conformation $^4C_1$ in pyranoside with substituents positioned at equatorial positions. The perspective view of the molecule shows equatorial disposition for all substituents except position 4 that remains axial (Scheme 9.4).

Disaccharide phenylmethyl-*O*-(2,3-di-*O*-acetyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1 → 2)-3,4-*O*-isopropylidene-β-D-fucopyranoside shows for



Scheme 9.2 X-ray diffraction of sucrose and sucrose NaBr.H_2O



Scheme 9.3 Chair conformation for octa-*O*-acetyl-β-D-cellobiose

fucopyranoside moiety a distorted chair due to the five-member ring acetonide at O3 and O4 positions, with Cremer and Pople puckering parameters of $Q = 0.556$ (3), $\theta = 159.9$ (3)°, and $\phi = 220.8$ (8)°. In contrast for the glucopyranosyl moiety with a six-member ring benzylidene ring attached at positions O4 and O6, the chair conformation is less distorted with Cremer and Pople puckering parameters of $Q = 0.597$ (3), $\theta = 170.5$ (3)°, and $\phi = 156.0$ (16)° (Scheme 9.5) [9].

The solid state crystal structure of glycoresin tricolorin A was solved by using an intense synchrotron radiation to collect data. The crystals belong as usual to the $P2_1$ having cell dimensions $a = 14.025(1)$, $b = 33.337(1)$, $c = 25.512(1)$ Å, $\beta = 91.07(1)°$. The energy maps were calculated as a function of two glycosidic linkage torsion angles defined as $\phi = \Theta$ (O5-C1-O1-Cx) and $\psi = \Theta$ (C1-O1-Cx-C(x + 1)), indicating a higher level of conformational freedom along ψ axis.

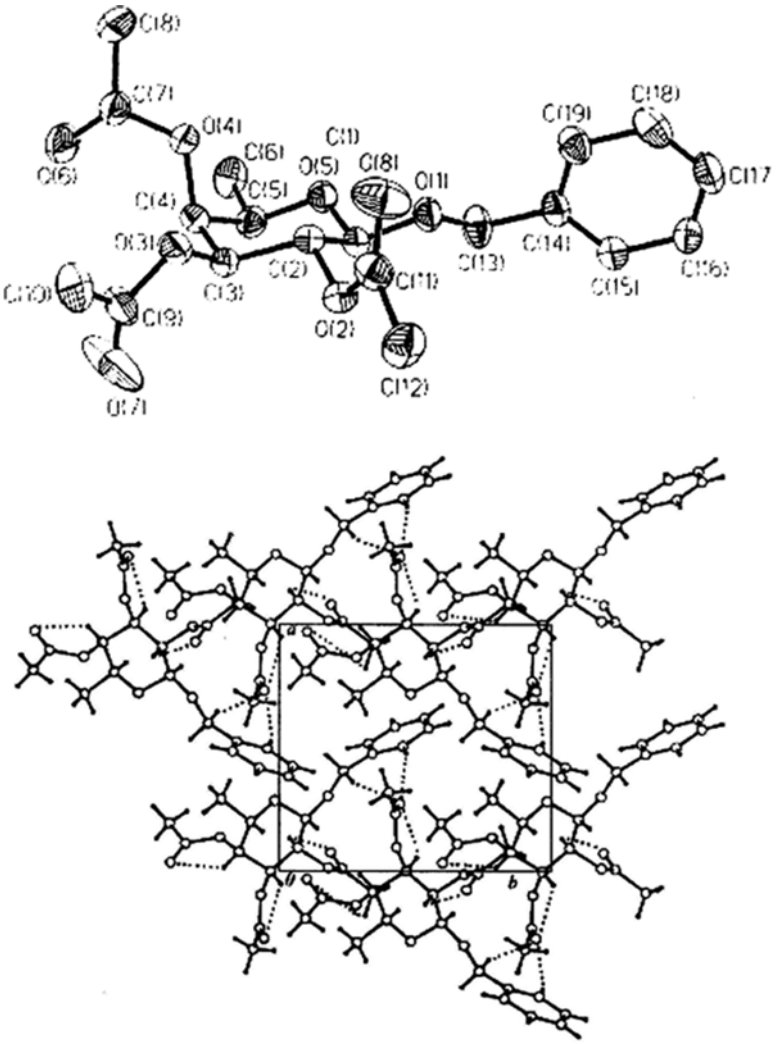
The size of the crystal unit cell demonstrates the presence of four independent tricolorin A molecules per asymmetric unit and the refined structure showed the presence of 18 water molecules forming a channel along the hydrophilic region (Scheme 9.6) [10].

Other selected pyranosides analyzed by X-ray diffraction and their parameters determined are shown in Table 9.2.

9.2 X-ray Diffraction of Nucleosides

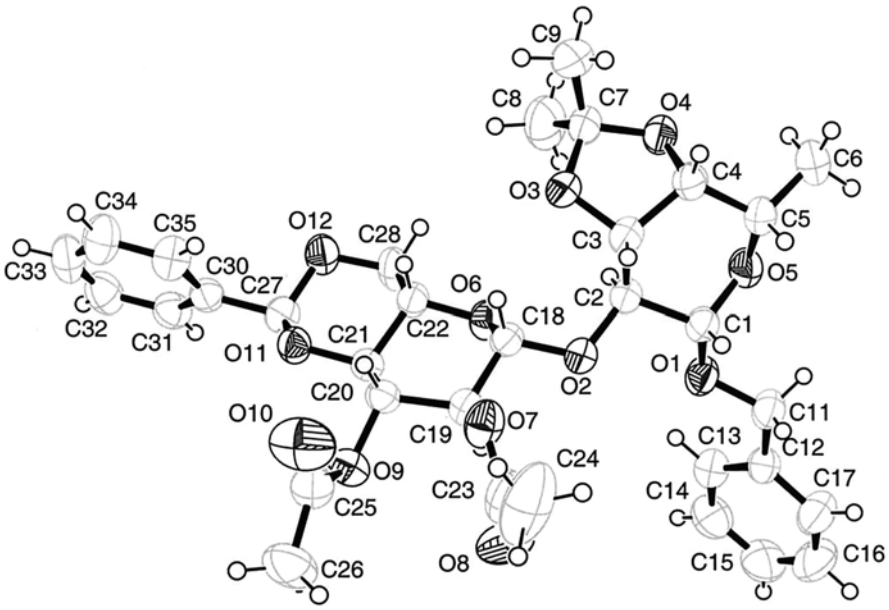
A number of *N*-glycosides and *C*-glycosides has been solved by X-ray analysis, presenting as common features space group $P2_12_12_1$ or $P2_1$, the furanoside ring in the twist conformation, and symmetric system monoclinic or orthorhombic.

For instance the hypermodified nucleoside queuosine presents a space group $P2_12_12_1$, cell dimensions $a = 26.895$, $b = 7.0707$, $c = 23.883$ Å, and symmetric system



Scheme 9.4 Thermal ellipsoid drawing and packing diagram showing the hydrogen bonding along [001] of phenyl methyl 2,3,4,-tri-*O*-acetyl-β-D-fucopyranoside

orthorhombic (Scheme 9.7). The three-dimensional structure determined by X-ray has been also helpful to understand the recognition process at the tRNA level. Thus, based on this information it is possible to determine that the bulky group cyclopentenediol due to the trans disposition assumed is not involved in any codon–anticodon interaction, therefore suggesting that another type of interaction has taken place [19].

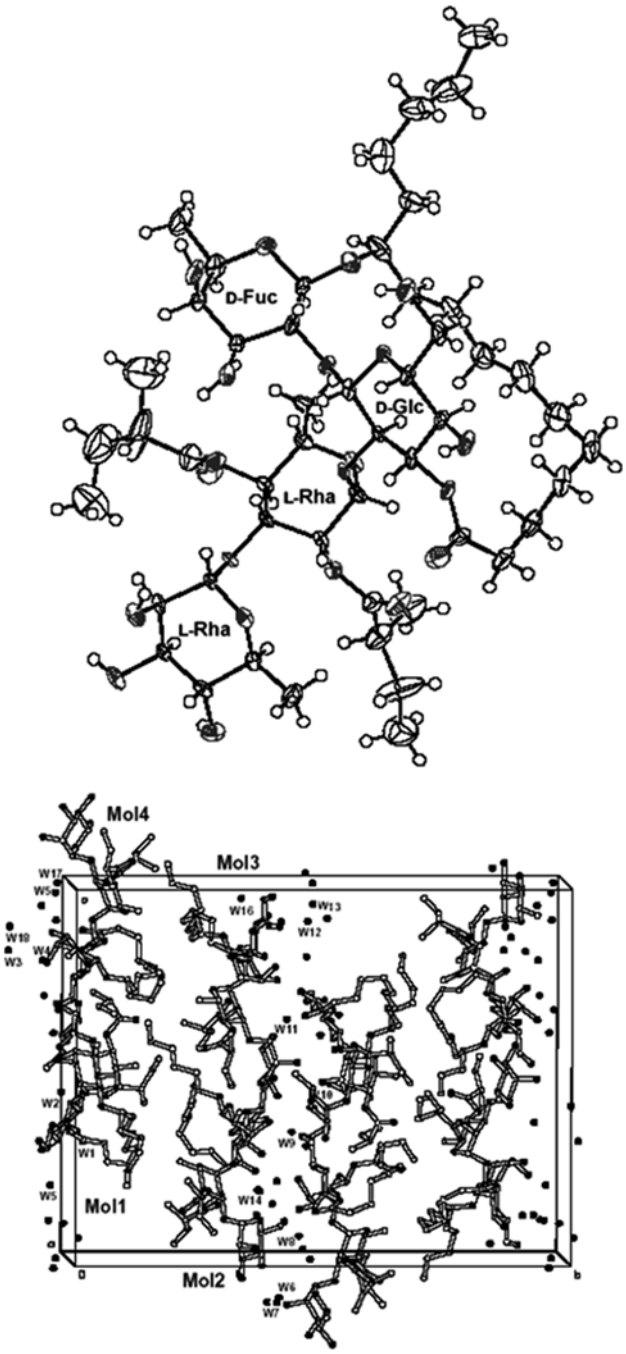


Scheme 9.5 Perspective Ortep view of phenylmethyl glucosyl fucopyranosyl derivative showing the distortion degree between 5- and 6-member fused rings on chair conformation

The unusual conformation of α-D anomer of 5-aza-7-deaza-2′-deoxyguanosine has been reported by Seela et al. [20] In this work it is described that the title compound adopts a high-anti conformation with the C1′-C2′ and N9-C8 bonds nearly eclipsed with torsion angle C1′-C2′-N9-C8 = 30.3 (4)°. It can be also observed that for 2′-deoxy-α-D-ribonucleosides the C2′ endo sugar puckering with either a half chair or envelope conformation is preferred (Scheme 9.8).

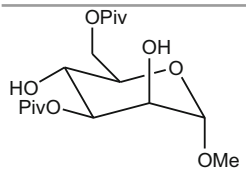
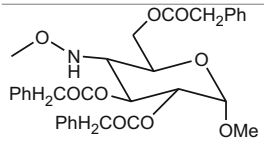
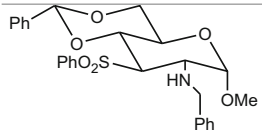
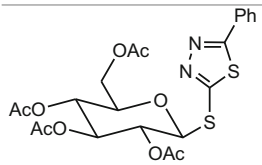
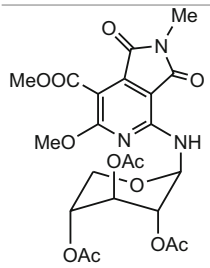
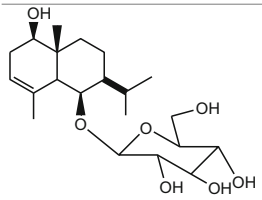
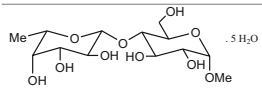
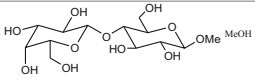
The solid-state conformation of constrained carbocyclic nucleosides (N)-methano-carba-AZT and N-(S)-methano-carba-AZT was determined by X-ray diffraction. As expected with the prediction, their thermal ellipsoid presented a rigid pseudoboat conformation for the bicycle [3.1.0] hexane system, which makes them assume nearly perfect $_2$E and $_3$E envelope conformations in the pseudorotational cycle (Scheme 9.9) [21].

Other selected *N*-nucleosides that have been analyzed by X-ray diffraction and their parameters determined are shown in Table 9.3.

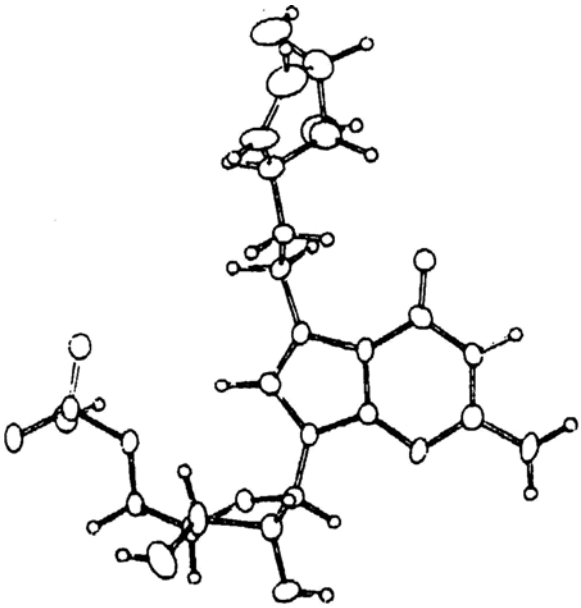


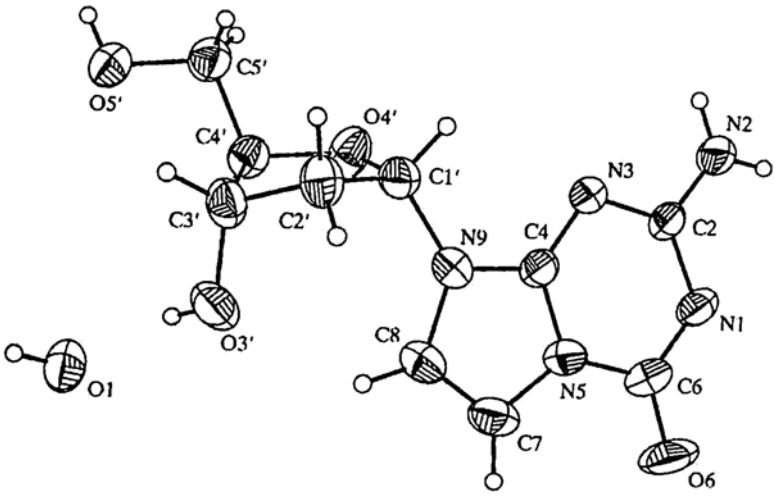
Scheme 9.6 ORTEP representation of tricolorin A and graphical representation of the unit cell

Table 9.2 X-ray diffraction parameters of some selected pyranosides

Structure	Symmetry cell	Symmetry space	Conformation	Ref
	Orthorhombic	P 2_1 2_1 2_1	$^4C_1$	[11]
	Monoclinic	P 2_1	$^4C_1$	[12]
	Monoclinic	P2_1	Chair for α-anomer and boat β-anomer	[13]
	Orthorhombic	P 2_1 2_1 2_1	$^4C_1$	[14]
	Monoclinic	C 2	$_4C^1$	[15]
	Orthorhombic	P 2_1 2_1 2_1	$^4C_1$	[16]
	Monoclinic	C 2	$_4C^1$ $^4C_1$	[17]
	Monoclinic	P2_1	$_4C^1$ $^4C_1$	[18]

Scheme 9.7 Perspective view of hypermodified nucleoside queuosine





Scheme 9.8 Perspective view of α-D anomer of 5-aza-7-deaza-2′-deoxyguanosine

Scheme 9.9 X-ray structure of (N)-*methano*-carba-AZT

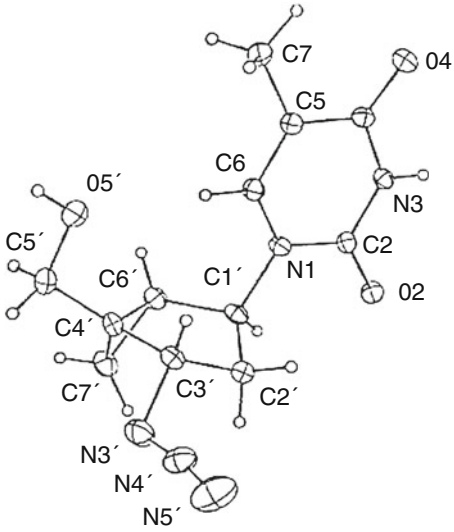
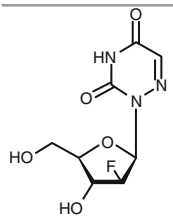
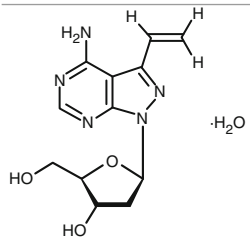
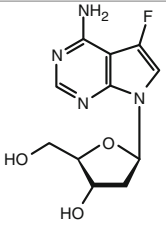
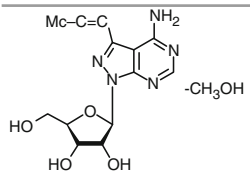
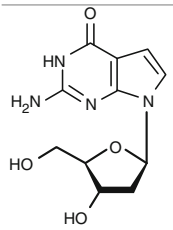
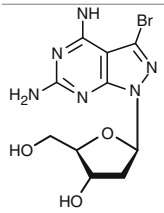
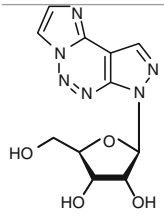
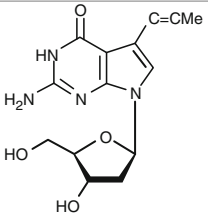
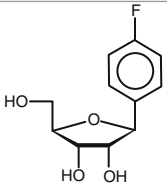
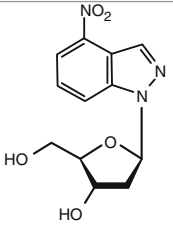


Table 9.3 *N*-glycosides and their X-ray diffraction parameters

Structure	Symmetry cell	Symmetry space	Sugar puckering	Conformation	Ref
	Monoclinic	P 2_1	$^3T_2$	*anti* [χ=−125.37 (13)°]	[22]
	Orthorhombic	P 2_1 2_1 2_1	Unsymmetrical twist	*anti*	[23]
	Orthorhombic	P2_1 2_1 2_1	$^2T_3$	*anti* and high *anti* [χ=−101.1 (3)°]	[24]

(continued)

Table 9.3 (continued)

Structure	Symmetry cell	Symmetry space	Sugar puckering	Conformation	Ref
	Orthorhombic	$P2_1\ 2_1\ 2_1$	$^3T_2$	*anti* and high-*anti* [$\chi = -101.8$ (5)°]	[25]
	Orthorhombic	$P2_1\ 2_1\ 2_1$	$^3T_4$	*anti* [$\chi = -106.5$ (3)°]	[26]
	Orthorhombic	$P2_1\ 2_1\ 2_1$	$^3T_2$	*anti*	[27]
	Orthorhombic	$P2_1\ 2_1\ 2_1$	$_3T^2$	*anti* and high *anti* [$\chi = -103.5$ (3)°].	[28]
	Monoclinic	$P\ 2_1$	$^2T_3$	*anti* [$\chi = -117.1$ (5)°]	[29]
	orthorhombic	$P\ 2_1\ 2_1\ 2_1$	C1′-*exo*, C2′-*endo* twist and C2′-*endo* envelope	*anti*	[30]
	Orthorhombic	$P2_1\ 2_1\ 2_1$	S-type	*anti* [torsion angle = −105.3 (2)°]	[31]

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Chapter 10
Mass Spectrometry of Glycosides

High-resolution mass spectrometry has become another valuable tool for characterization of simple and complex glycosides. The method is based on the collision of a high-energy electron against a sample under study producing as result a cation radical fragment known as the molecular ion, which should match with the molecular weight of the molecule. The mass spectrum also registers a number of fragments with the most intense base peak assigned a relative intensity of 100. Mass spectrometry can be applied as high and low ionization experiments, the former the most suitable for glycosides electron impact and the latter for fast atom bombardment (FAB) and electrospray ionization routine experiments for characterization of glycosides. In terms of sensitivity of the measurement this instrumental method requires a small amount of sample, even in the order of nanogram quantities.

The fragmentation patterns of acetyl protected pentoses and hexoses were studied and their main *m*/*z* fragment established. For instance for methyl-β-D-xylopyranoside triacetate the main fragment follows the two alternative routes shown in Scheme 10.1 [1].

High ionization experiments such as electron impact have been found to be a suitable approach for the determination of molecular weights through their corresponding molecular ions of protected glycosides such as peracetylated *O*-glycosides of low molecular weight. For instance, by using electron impact it was possible to determine the molecular weight, and the common fragmentation patterns of *m*/*e* 331 and 169 (100) of the phenylazo naphthol-β-D-glucopyranoside pentaacetate (Scheme 10.2) [2].

However, for most of non-protected glycosides high ionization does not provide reliable information and commonly decomposition is observed due to thermal unstability. The introduction of shift ionization techniques such as fast atom bombardment (FAB) and electrospray ionization has produced great progress for the structural characterization of simple and complex glycosides. This important analytical procedure is specially useful for determining the molecular weight through detection of the molecular ion, as well as sugar sequencing. The choice of the matrix and the solubility of the sample are essential aspects to consider for obtaining the

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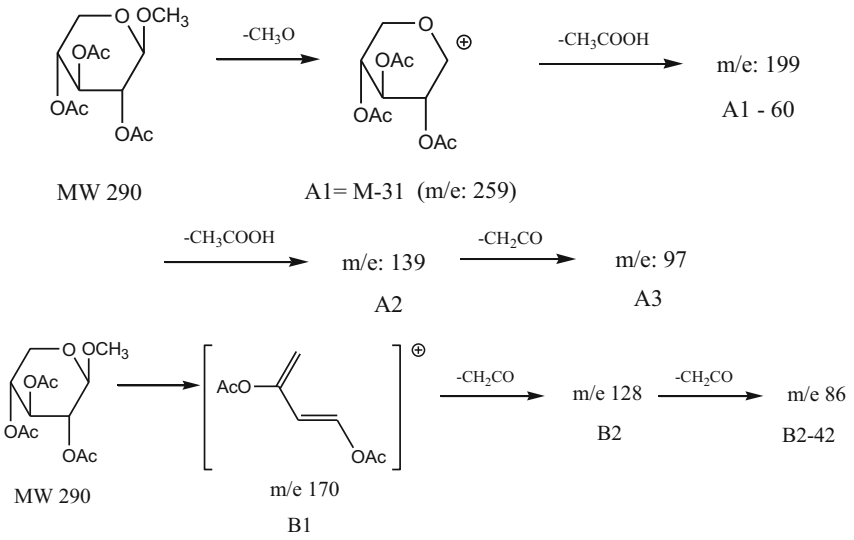
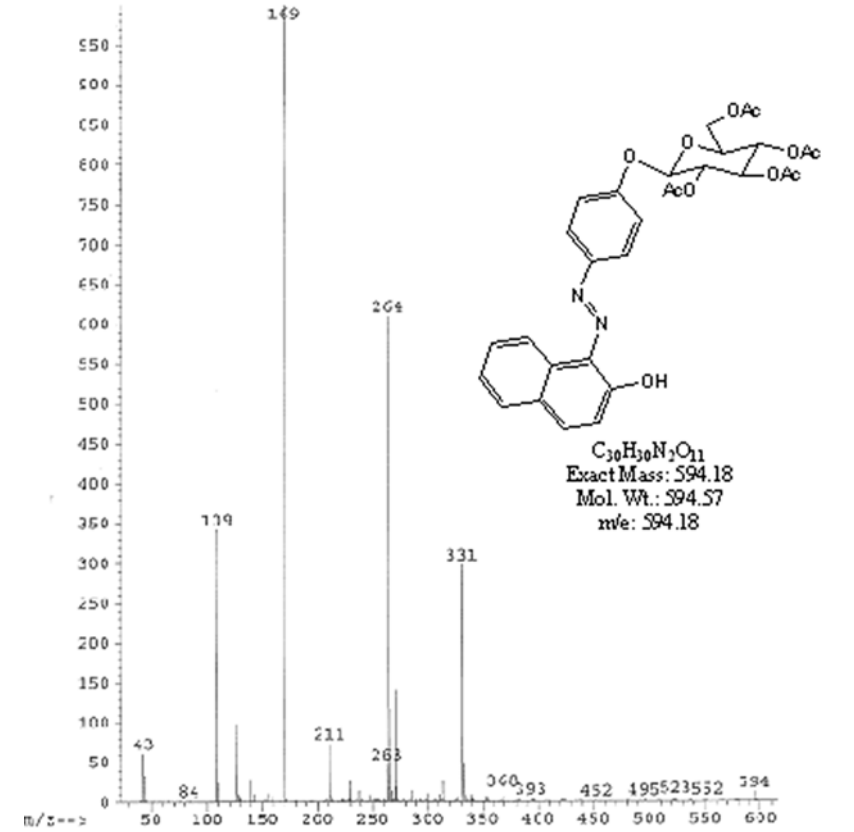


Scheme 10.1 Fragmentation pattern of methyl-β-D-xylopyranoside



Scheme 10.2 Mass spectrum of phenylazo naphthol glucoside

best resolution. Glycerol is the matrix most commonly used, and it is the best choice for underivatized carbohydrates and glycopeptides. Some other matrices used alternatively for hydrophobic samples are thioglycerol, tetraethyleneglycol, and triethanolamine [3].

The use of derivatives also plays an important rule and may improve the spectral interpretation and the sensitivity. The most commonly used derivatives are the per-*O*-acetyl and the per-*O*-methyl. Usually for the former the fragmentation pathways are less specific and furnish more information, although the spectrum is more difficult to interpret.

For the assignment of the molecular ion it is important to recognize the pseudomolecular ions produced during a FAB experiment, which can be positive-ion and negative-ion mode. In the positive-ion mode the usually present signals are $[M+H]^+$, $[M+NH_4]^+$, $[M+Na]^+$, and $[M+K]^+$, and for the negative $[M-H]^-$, and for those molecules that cannot lose a proton $[M+Cl]^-$, or $[M+SCN]^-$.

Some of the most common fragmentation pathways produced by polysaccharides and glycoconjugates are represented in Scheme 10.3 [4]:

10.1 FAB Fragmentation Patters

Likewise, application of different ionization techniques in the study of natural glycosides has been performed and consequently it has been possible to assign the main potential fragmentation sites in *O*- and *C*-glycosides (Scheme 10.4) [5].

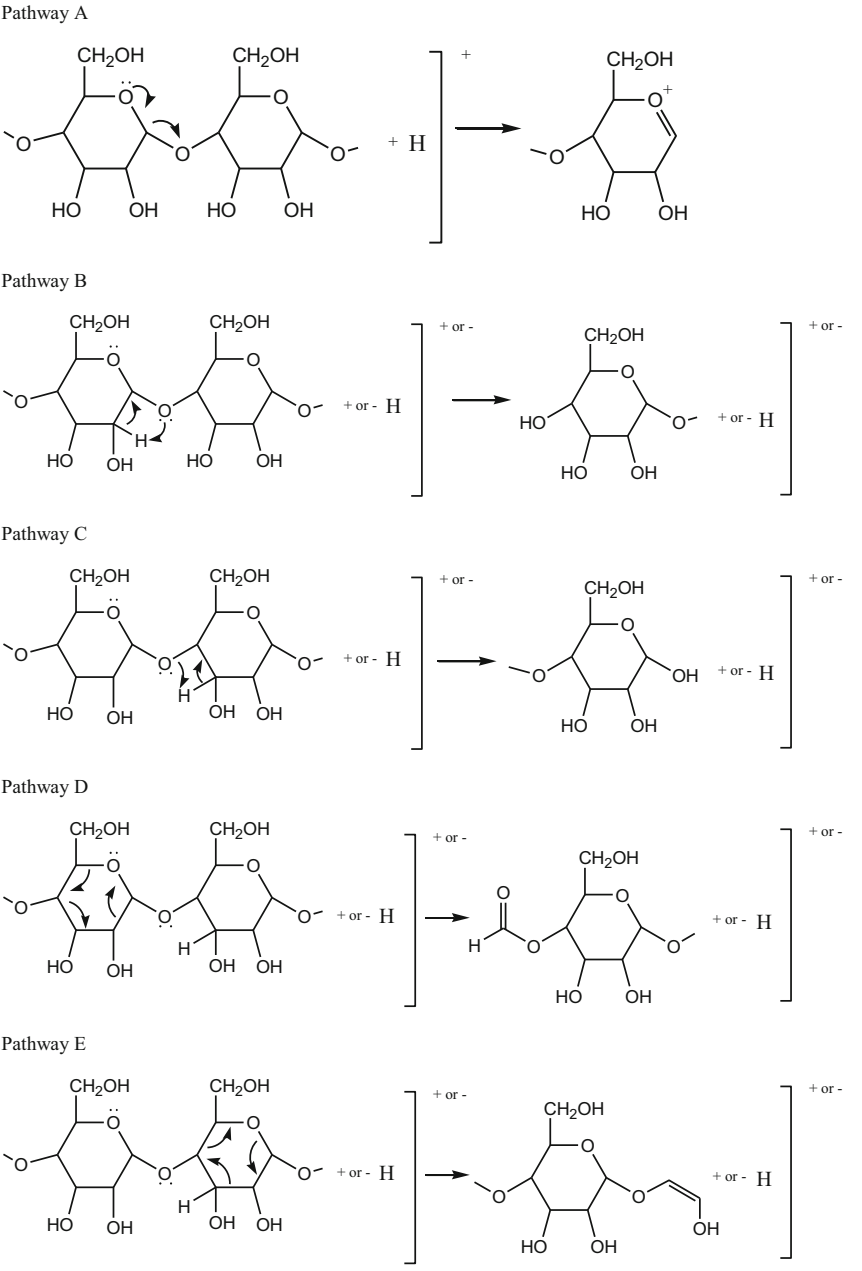
Negative ion FAB-MS in triethanolamine of synthetically prepared glycoresin composed by fucose, glucose, and quinovose attached to jalapinolic acid (Scheme 10.5), shows $[M-H]^-$ peak (*m*/*z* 1216) in agreement with the expected molecular weight [2].

Mass spectrometry has been also applied successfully for glycoprotein structural determination of primary structure. The first glycoprotein primary structure was determined through electron impact and chemical ionization [6]; however, soft ionization methods of fast atom bombardment (FAB), electrospray (ES), or matrix-assisted laser desorption ionization (MALDI) are used in most of the glycoprotein structural determinations.

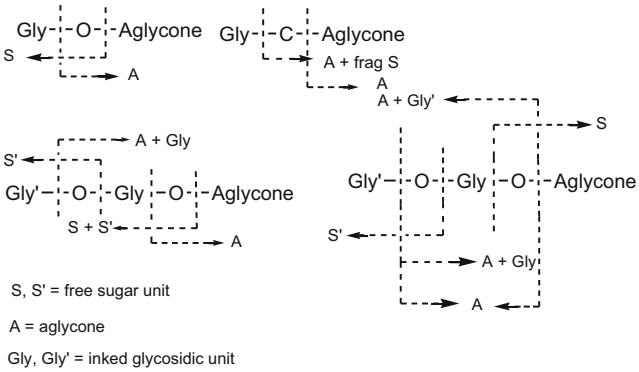
FAB is particularly useful for analyzing the permethyl derivatives of oligosaccharides released from glycoproteins by chemical or enzymatic methods. When the atom or ion beam collides with the matrix, a substantial number of sample molecules are ionized producing positively charged species called quasimolecular ions $[M+H]^+$ and $[M+Na]^+$ [7].

In order to optimize fragment ion information of glycoproteins, three approaches are currently being used: inducing fragmentation by collisional activation, monitoring natural ionization-induced fragmentation, and selecting derivatives that enhance and direct fragmentation.

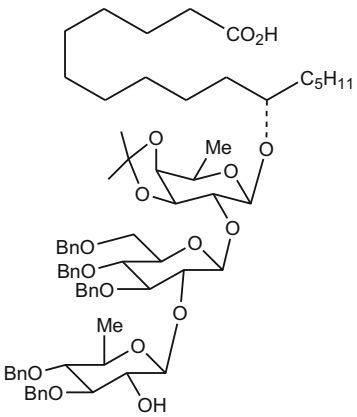
During collisional activation of collected fractions from an enzymatic digest, the first step is to identify in the MS mode the fractions containing sugar fragment-ions.

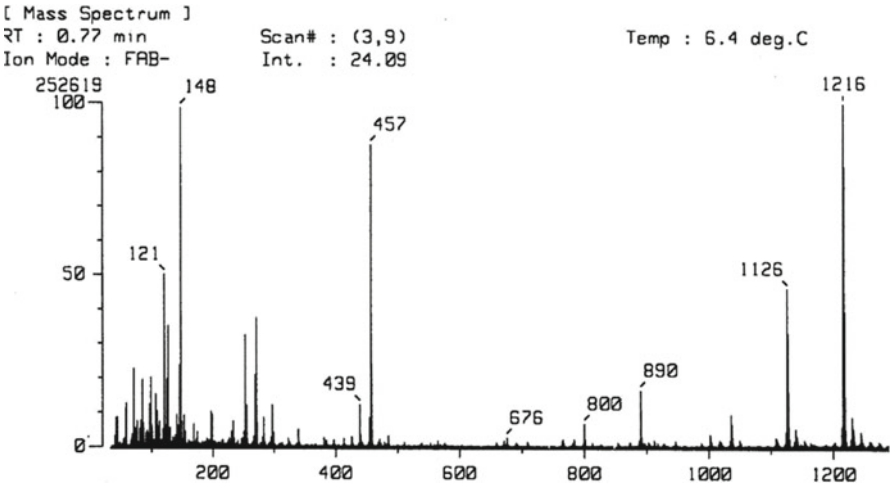


Scheme 10.3 The most common fragmentation pathways

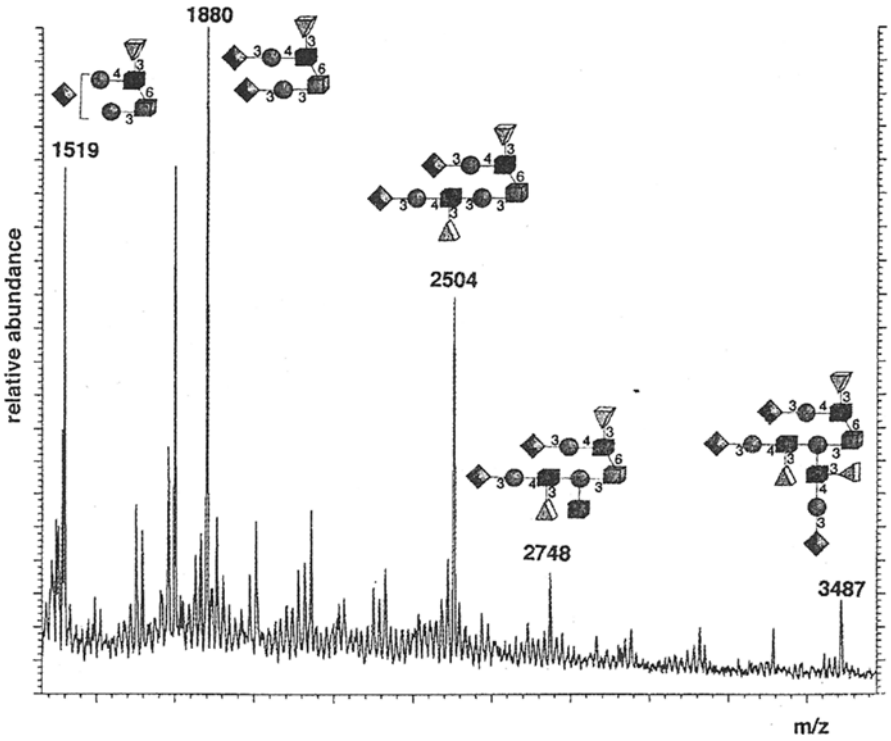


Scheme 10.4 Main potential fragmentation sites in *O*- and *C*-glycosides





Scheme 10.5 FAB-MS negative mode of synthetically prepared protected glycoresin



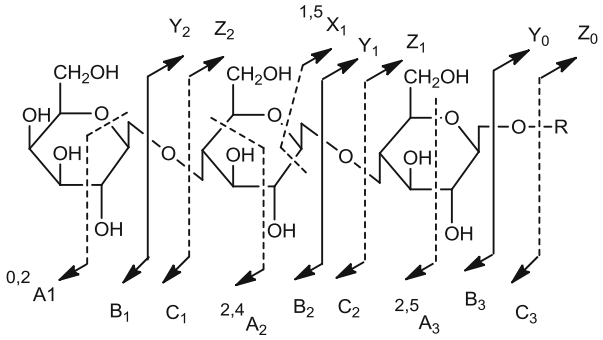
Scheme 10.6 Partial FAB mass spectrum of permethylated *O*-oligosaccharides from glycoprotein uromodulin

Then switching to the MS/MS mode of a doubly or triply charged ion a composite spectrum containing fragmentation of saccharide and peptide is obtained. Since glycosidic bonds are weaker than peptide bonds, the basic oligosaccharide sequence is determined [8].

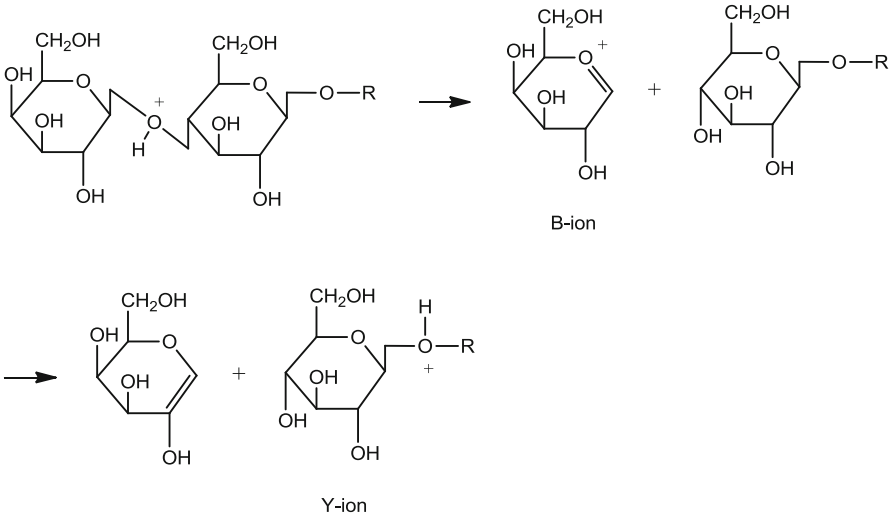
The natural fragmentation approach relies on the fragmentation created by internal energy transfer to the ion during the ionization process, and now it is becoming most limited in use that the previous one [4].

Derivatization methods are likewise divided into tagging of reducing ends and protection of most of all of the functional groups. The first type facilitates chromatographic purifications and enhances the formation of reducing end fragment ions. The second type involves primarily the permethylation, which form abundant fragment ions arising from cleavage on the reducing side of each HexNAc residue.

The permethylation of Tamm–Horsfall glycoprotein was effected and the FAB mass spectrum obtained, showing molecular ions for core 2-type structures carrying up to three sialyl Lex moieties (Scheme 10.6) [9].

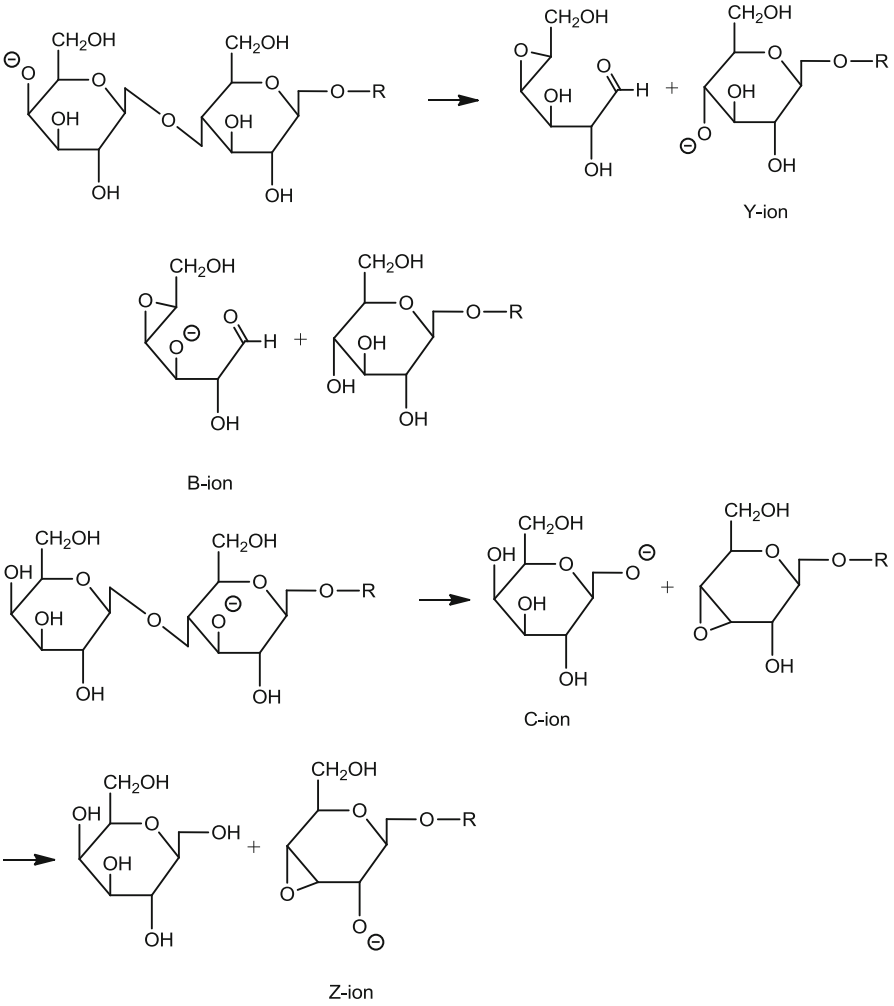


Scheme 10.7 Domon–Costello types of carbohydrate fragmentation.

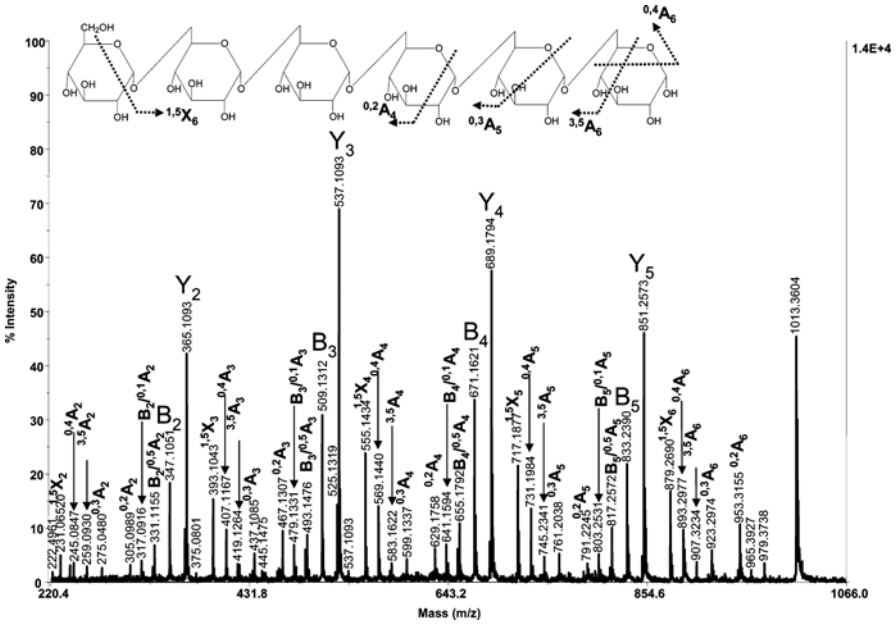


Scheme 10.8 Genesis of Bi and Yj ions in the positive ion mode.

A mass spectra pattern known as Domon–Costello (Scheme 10.7) was introduced to describe the ion fragments observed in glycoconjugates (glycosphingolipids, glycopeptides, glycosides, and carbohydrates). Thus, Ai, Bi, and Ci labels were used to designate fragments containing a terminal (nonreducing end) sugar unit (Scheme 10.8), whereas Xj, Yj, and Zj represent ions (Scheme 10.9) still containing the aglycone (or the reducing sugar unit). In addition, subscripts indicate the position relative to the termini analogous to the system used in peptides, and superscripts indicate cleavages within carbohydrate rings [10].



Scheme 10.9 Negative ion geneses of (a) Bi and Yj, and (b) Ci and Zj.



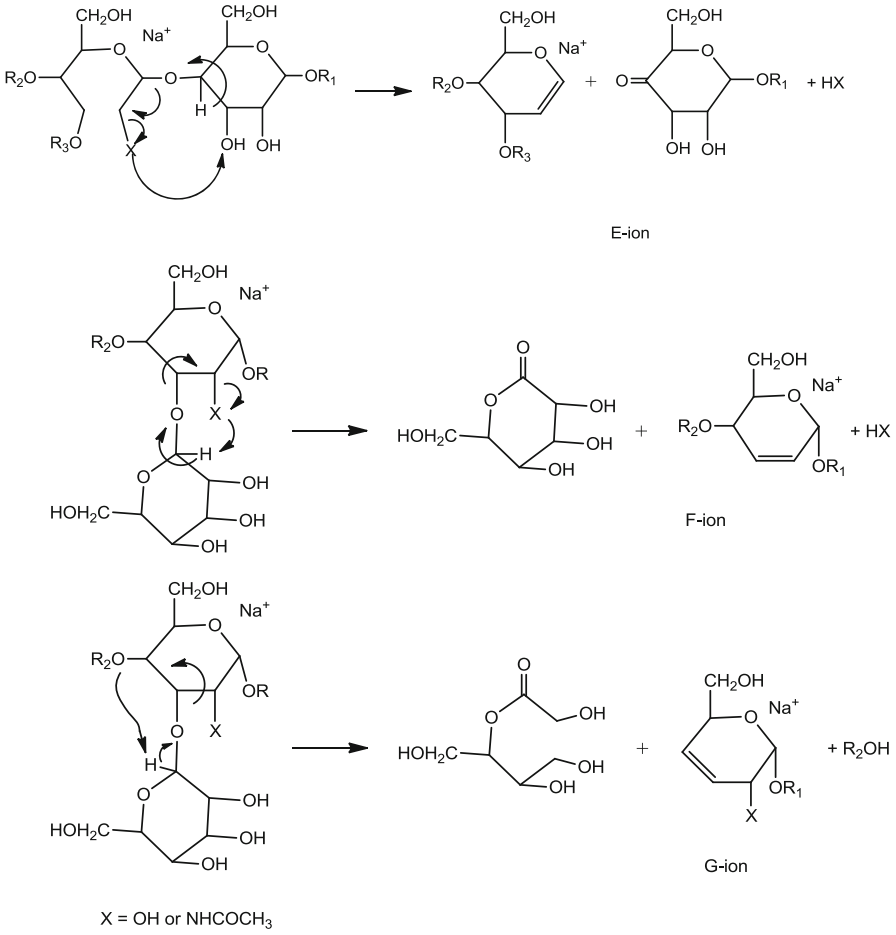
Scheme 10.10 The MALDI/TOF mass spectra of maltohexaose

10.2 The Domon–Costello Fragmentation

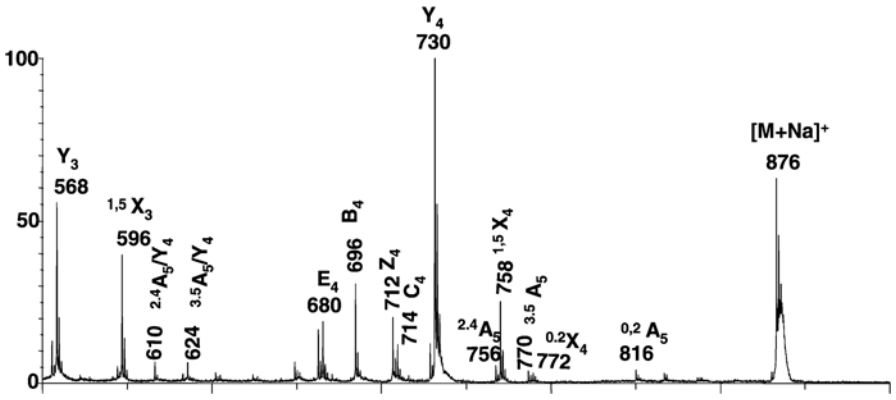
The Domon–Costello nomenclature was successfully applied to determine the ion fragments generated after a single collision of a dextran sample formed by six glucose molecules. The MALDI/TOF singly charged showed besides the $[M+Na]^+$ ion, the most intense Y-ions, less intense B-ions, and several other fragments (Scheme 10.10) [10].

Other ion fragments found in FAB, ESI, and in MALDI-TOF mass spectra of the $[M+Na]^+$ mode not described previously were described by Spina et al., derived from six-atom ring rearrangements named as E, F, and G ions (Scheme 10.11) [11].

The usefulness of this analytical tool has been established in the sequence determination of oligosaccharides present in human milk LNFP I–IV, having a sequence fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glc by using MALDI-TOF/TOF-MS/MS (Scheme 10.12) [12]. Also, a number oligosaccharides and glycoconjugates from different biological source have been analyzed by ESI and MALDI mass spectrometry as shown in Table 10.1.



Scheme 10.11 Six-atom ring rearrangement E, F, G ions



Scheme 10.12 MALDI-MS/MS spectrum of $[M+Na]^+$ ion of LNFP I

Table 10.1 Selected mass spectrometry analysis of oligosaccharides and glycoconjugates

Carbohydrate	MS analysis	Biological source	Ref
Sulfated polysaccharide	ESI	*Ascophyllum nodosum*	[13]
Oligosaccharides	Electrospray	Milk	[14]
Rhamnolipids	MALDI-TOF	Pseudomonas spp.	[15]
Acidic N-linked glycans	MALDI-TOF MS	Prepuberal pigs	[16]
Permethylated serum N-glycans	MALDI-LIT	Serum proteins	[17]
Arabino-oligosaccharides	ESI	Sugar beet arabinan	[18]
Neutral and sialylated glycans	MALDI-TOF	IgG and Fc-fusion protein	[19]
β-chain of human choriogonadotropin	ESI	Human serum IgG	[20]
N-glycans	MALDI-TOF	Bovine fetuin	[21]

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