RADIOIONIDATION REACTIONS FOR PHARMACEUTICALS

Compendium for Effective Synthesis Strategies

Heinz H. Coenen, John Mertens
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by

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PREFACE

Founded in 1971, COST is an intergovernmental framework for European Cooperation in the field of scientific and technical research, allowing the coordination of nationally funded research on a European level. COST actions cover basic and precompetitive research, as well as activities of public utility. The goal of COST is to ensure that Europe holds a strong position in the field of scientific and technical research for peaceful purposes, by increasing European cooperation and interaction.

COST consists in actions, the duration of which is generally 4 years. Two actions, COST B3 entitled "Development of New Radiotracers and Methods of Quality Assurance for Nuclear Medicine Applications" and COST B12 entitled "Radiotracers for In Vivo Assessment of Biological Functions", which have been devoted to radiopharmaceutical chemistry and radiopharmaceutical validation, were successfully completed.

Radiochemical methodology constitutes the most important base for successful in vivo functional imaging in nuclear medicine. For single-photon emission tomography (SPET) imaging, radioiodination methodology allows the development of potent radiotracers, and several of them are presently in clinical routine.

Many previous publications have tackled the specific problems of radioiodination, but the time for a state-of-the-art book seemed right now since this field has advanced over the last 30 years to reach a level where guidelines and expert systems can be suggested for the main methodological aspects.

This book has emerged from an original idea during the course of COST Action B3 and was developed during the course of COST Action B12 with the help of many recognised European experts in the field. Sincere thanks to all of them for their very precious support, advice and contributions.

The authors gratefully acknowledge the support of COST, which, all along the duration of the actions, has encouraged the preparation and the finalisation of a collective textbook.

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RADIOIODINATION COMPENDIUM

Summary

Several of the around 30 radioisotopes of iodine find wide application in life sciences research both in vitro and in vivo.

Most nuclear medicine departments possess one or more imaging devices for single-photon emission tomography (SPET). Molecules of biological interest, especially for functional imaging of metabolism and neurotransmission functions using SPET, are often labelled with ^{123}I. In this handbook, the major production routes of the six most relevant radioiodine isotopes are reviewed and various methods for labelling molecules of biological interest with radioiodine are described, including their basic reaction mechanisms. The influence of iodine introduction on physico-chemical, pharmaceutical and pharmacological properties of radiopharmaceuticals are discussed and corresponding examples of radioiodinated pharmaceutical preparations are given. Finally, an expert system providing guidelines for choosing the most appropriate method of radioiodination according to the chemical structure of the molecule of interest for tracer use is proposed.

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Chapter 1

INTRODUCTION

The use of "smart" radiopharmaceuticals for the imaging of the biochemical changes that come with any disease has allowed a worldwide recognition of the usefulness of in vivo imaging in medicine and biology. An effective labelling of compounds of biological interest is required for the preparation of these radiopharmaceuticals, and therefore the radiolabelling methodology is considered as one of the pillars on which nuclear medicine rests.

Isotopic labelling in which an element occurring naturally in an organic compound is replaced by a radioactive isotope of the same element has been in practice for a long time. Compounds of biological interest were first labelled using β^- emitters such as tritium, ^{14}C, ^{32}P or ^{35}S, and represent tools often referred to as "biological indicators". As the radiation emitted by these radionuclides does not allow external in vivo detection, nuclear medicine has to adapt itself to other radionuclides with more appropriate nuclear properties such as positron emitters or single-photon emitters.

Among the "organic" positron emitters ^{11}C, ^{13}N, ^{15}O, ^{18}F and ^{76}Br, in most of the cases ^{11}C is the only radionuclide allowing an isotopic radiolabelling. Concerning the single-photon emitters usable for in vivo functional imaging, their corresponding stable isotopes do not generally occur in the compound. Under these conditions, the only solution for the radiochemist is to switch over from isotopic to non-isotopic or analogous labelling. The most prominent example for the analogous approach is the labelling with ^{123}I. The reason to use ^{123}I as an "organic" labelling atom is because of: (i) its ideal nuclear properties; (ii) its covalent bond to carbon; (iii) the relatively modest structural alteration it induces and (iv) the wide variety of radiochemical methods available for its attachment to organic molecules.

The former class of radiolabelled molecules is mostly used in positron emission tomography (PET), whereas the latter class forms the basis of planar scintigraphy and single-photon emission tomography (SPET). However, some

β^+-emitting radioisotopes of iodine, namely ^{120}I, ^{122}I and ^{124}I, are also available for PET studies.

1.1 Authentic Labelling

The natural elements found in living organisms are essentially those of organic chemistry, carbon, hydrogen, oxygen and nitrogen, assembled into a variety of molecules. The manufacturing of radionuclides of these elements for nuclear medicine, all by means of an accelerator of charged particles (such as a cyclotron), and the subsequent preparation of radioactive organic molecules were accomplished in the 1960s and 1970s. This research work allows the preparation of radiopharmaceuticals that incorporate radionuclides with half-lives of the order of minutes and has led to important PET applications in biology and nuclear medicine.

However, PET has not yet been developed as a routine clinical technique applicable on a large scale. Furthermore, the most widely used radiopharmaceuticals in PET are not labelled by substitution of one of their atoms by a corresponding radioactive isotope (isotopic labelling), but by introducing a hetero-radionuclide (analogous labelling) in the molecule of interest. These radiopharmaceuticals have been, for example, successfully applied to the studies of metabolic pathways 2-[^{18}F]fluoro-deoxyglucose (^{18}F-FDG) and neurotransmission processes 6-[^{18}F]fluoro-3,4-dihydroxy-L-phenylalanine (^{18}F-FDOPA).

1.2 Labelling with Metallic Radioisotopes

The combination of the artificial radionuclide technetium-99m (^{99m}Tc) and the gamma camera has led to the rapid development of nuclear medicine. Apart from ^{99m}Tc, other radionuclides such as gallium-67, indium-111 and thallium-201 have been in use in nuclear medicine since the beginning of the 1970s. The half-lives of these radioisotopes are long enough and led to the development of commercially available radiopharmaceuticals, which are today the cornerstone of clinical diagnostic nuclear medicine.

1.3 Labelling Methods for Radioiodine

Few of these radiopharmaceuticals, however, participate in biochemical processes as PET radiopharmaceuticals do. To develop such investigations on a clinical basis using SPET, it is generally admitted that radiopharmaceuticals labelled with iodine radioisotopes, and more particularly with ^{123}I, have to be

used. Since iodinated compounds are generally not among the naturally occurring substrates, it is mandatory to have a valid concept based on well-known biochemical data, as for example in the case of the metabolism of fatty acids, for the development of such radiopharmaceuticals.

Presently, many successful examples can be found in the literature. For metabolic studies, for example, [^{123}I]iodofatty acids have been applied to study beta-oxidation of myocardial cells, α-methyl-[^{123}I]iodotyrosine for the transport of amino acids in tumours while [^{123}I]iodobenzamide, [^{123}I]β-CIT and [^{123}I]iodopargylline can be mentioned as examples for receptor, transporter or enzyme imaging, respectively.

The purpose of this handbook is to facilitate the radiolabelling of molecules of biological and medical interest with ^{123}I to be able to produce radiopharmaceuticals that could eventually play a major role in nuclear medicine. Nowadays, ^{123}I is generally produced commercially with automated devices and with high purity. Furthermore, advances in radiochemistry have reduced the production costs considerably. Finally, many iodinated compounds have already found practical use in nuclear medicine.

Radioiodination techniques can be divided according to the nature of the process into physico-chemical, chemical and enzymatic methods. Many methods have been described, but it appears that only a few chemical techniques allow radiopharmaceutical preparation with a good labelling yield and a high specific radioactivity. Based on the reaction types used, chemical radioiodination processes can principally be divided into nucleophilic and electrophilic substitution reactions.

Labelling reactions follow the physico-chemical laws but occur at sub-stoichiometric conditions. Generally, the concentration of the radioactive entity in the reaction medium is 10^6 to 10^9 times lower than that of the molecule to be labelled. This explains the high labelling yields obtained in reversible substitution reactions, as the backward reaction is also 10^6 to 10^9 times lower than the forward reaction. These sub-stoichiometric conditions also apply for side reactions with impurities, pseudo indifferent salts or the solvent, rendering a labelling reaction more critical than conventional chemical reactions with higher concentrations of reagents.

As a wide range of efficient electrophilic and nucleophilic techniques exist for the radioiodination of pharmaceuticals, the following aspects also have to be considered during selection:

- site of incorporation of a radioiodine, leading to a minimal change in the biological activity;
- knowledge of the pharmacological and toxicological properties of the iodinated compound;
- in vivo stability;

- achievement of the labelling within a few hours and preferentially in one step;
- high labelling yields (of multi-mega-Bequerel amounts) due to the high cost of ^{123}**I**;
- knowledge of the specific radioactivity for successful in vivo diagnosis in patients.

Chapter 2

IODINE RADIONUCLIDES

2.1 Physical Properties and Areas of Application

While about 30 artificial radioisotopes of iodine have been recognised, only one stable isotope, iodine-127, is found in nature. Three of the radioisotopes (^{123}I, ^{125}I and ^{131}I) have been used widely for labelling small and large molecules. The decay characteristics of several iodine radioisotopes (cf. Table 2.1) lead to their uses in biochemical and pharmaceutical research, radioimmunoassay and nuclear medicine.

In practice, iodine-125 ($t_{1/2} = 60$ d, γ emission 35 keV) is used for in vitro experiments (in particular for preliminary studies in the development of a potential radiopharmaceutical imaging agent) and potentially useful for therapy, iodine-123 ($t_{1/2} = 13.2$ h, γ emission 159 keV) is suited for nuclear medicine imaging agents used in diagnosis by scintigraphy and SPET and iodine-131 ($t_{1/2} = 8$ d, γ emission 364 keV (83%) and β^- emission 606 keV (90%)) is rather used for human therapy.

Three other positron-emitting isotopes (^{120}I, ^{122}I and ^{124}I) have recently been added to the list of useful radioiodines. ^{120}I ($t_{1/2} = 1.4$ h), a relatively short-lived isotope, appears to have a great potential for PET imaging purposes, while ^{124}I ($t_{1/2} = 4.18$ d) is suitable for quantitative dosimetric evaluation of therapeutic ^{131}I-labelled radiopharmaceuticals and to quantify ^{123}I-labelled diagnostic tracers with slow pharmacokinetics using PET. ^{122}I is very short-lived ($t_{1/2} = 3.6$ min) and has found only limited application.

2.2 Radiochemical and Radionuclidic Purity

The introduction of an iodine atom into a molecule is generally realised by nucleophilic or electrophilic substitution on a carbon atom. However, all

Table 2.1: Nuclear properties and application areas of some radioiodines [1,2]

Radionuclide	$t_{1/2}$	Mode of decay (%)	E_{β}(max) keV	Main γ-rays [keV] (%)	Application
^{120}I	1.4 h	β^+ (56) EC (44)	4000	601 (58.0) 1523 (11.2)	PET
^{122}I	3.6 min	β^+ (77) EC (23)	3120	564 (18.0)	PET
^{123}I	13.2 h	EC (100)		159 (83.0)	SPECT
^{124}I	4.18 d	β^+ (22) EC (78)	2140	603 (61.0) 723 (10.0) 1691 (10.4)	PET; Therapy control
^{125}I	59.4 d	EC (100)	Auger electrons	35.5 (6.7) 284 (6.1)	RIA; Auger electron therapy
^{131}I	8.02 d	β^- (100)	606	364 (81.2) 637 (7.3)	Therapy

production methods deliver the radioisotopes in the form of iodide. Commercially, $Na^{125}I$ solutions are available in 10^{-2} M NaOH or in phosphate buffer, while $Na^{131}I$ and $Na^{123}I$ are available in 10^{-2} M NaOH for labelling purposes.

Radiochemical purity is defined as the fraction of radioisotope that is present in the specified chemical form. Depending on the isolation process after nuclide production and on storage, radioiodide can be contaminated by different radioiodinated species. Mostly these are oxidised forms ($^*I_2$, $^*IO_2^-$, $^*IO_3^*$ and $^*IO_4^-$), which is due to the low oxidation potential of iodide and radiolytic processes [3,4]. Since the radiochemical purity of the radioiodide hampers the radiochemical yields of nucleophilic and electrophilic labelling methods, it has to be controlled by means of thin layer chromatography (TLC) or high-performance liquid chromatography (HPLC). Recently, a simple process was described to transform all the radioactivity into the form of radioiodide using hydrazine as a reducing agent [5].

Radionuclidic purity is defined as the fraction of total radioactivity that is present as the specified radionuclide. In general, high radionuclidic purity of an ^{123}I-labelled radiopharmaceutical is needed: (i) to avoid unnecessary radiation dose to the subject of the SPET investigation; (ii) to minimise any degradation of the quality of the image and (iii) to limit errors on measurements of the biological process. The radionuclidic purity of ^{123}I depends essentially on the nuclear reaction used and the time of use after the end of the radionuclide preparation.

Thus the choice of the production reaction is of crucial importance. For example, in some laboratories ^{123}I is produced by the $^{124}Te(p,2n)^{123}I$ nuclear reaction with 23 to 26 MeV protons. When using this pathway, the level of

^{124}I impurity is rather high (see below) due to the (p,n) side reaction. Therefore, this reaction route is not allowed in some countries for production of ^{123}I for human use. If applied, it is highly recommended to use ^{123}I within the first day of production, since the percentage of longer-lived radiocontaminant ^{124}I increases according to its longer half-life of 4.2 d.

The levels of radionuclidic impurities are generally determined by gamma-ray spectrometry with appropriate detectors (e.g. Ge(Li), HPGe or NaI). Such measurements are necessary to ensure that radionuclidic contaminants are within acceptable limits at the time of radiopharmaceutical administration. Typical purity levels are given later for the individual isotopes.

2.3 Specific Radioactivity

The specific radioactivity (A_s) must be assessed to control the mass of radiopharmaceutical that will actually be delivered or used. It is defined as the radioactivity per mass of a labelled compound. Conventionally, however, the molar activity, i.e. radioactivity per mol, is termed as specific radioactivity. The maximum specific activity (carrier-free state) for a radionuclide is attained when there is no dilution by other isotopes of the same element.

The theoretical maximum specific activity (Table 2.2) of a radionuclide is a function of the half-life of the nuclide and is calculated using the fundamental equation of the radioactive decay:

$$A = (\ln 2/t_{1/2}) \times N = (0.6931/t_{1/2}) \times N$$

where A is the radioactive decay rate (Bq), $t_{1/2}$ is the half-life (second) and N is the number of atoms of the radionuclide. N is converted to the equivalent number of moles by dividing by Avogadro's number ($N^\circ = 6.023 \times 10^{23}$ atoms/mol). So, the theoretical maximum specific activity related to one mol ($A_{s\,max}$) is given by:

$$A_{s\,max} = A/(N/N^\circ) = (0.6931 \times 6.02 \cdot 10^{23})/t_{1/2}\ [\text{Bq/mol}]$$

$$\text{or} \quad A_{s\,max} = 4.1725 \times 10^8/t_{1/2}\ [\text{GBq/}\mu\text{mol}]$$

$$\text{or} \quad A_{s\,max} = 1.1277 \times 10^7/t_{1/2}\ [\text{Ci/}\mu\text{mol}]$$

Table 2.2: Maximum specific activity of iodine radionuclides of biological interest

	^{120}I	^{122}I	^{123}I	^{124}I	^{125}I	^{131}I
Half-life	1.4 h	3.63 min	13.2 h	4.18 d	59.4 d	8.04 d
$A_{s\,max}$						
Bq/μmol	$8.25 \cdot 10^{16}$	$1.92 \cdot 10^{21}$	$8.77 \cdot 10^{18}$	$1.15 \cdot 10^{18}$	$8.14 \cdot 10^{16}$	$5.99 \cdot 10^{17}$
Ci/μmol	$2.23 \cdot 10^3$	$51.8 \cdot 10^3$	237.0	31.2	2.2	16.2

The relatively short half-lives of the iodine radionuclides result in low masses per mega-Bequerel in their pure Carrier-Free form. For example, 3.7 GBq of ^{123}I (i.e. 100 mCi) and 37 MBq of ^{125}I (i.e. 1 mCi) are equivalent to 0.42 and 0.46 nmol, respectively. In practice, although it is possible to approach the Carrier-Free state (c.f.: meaning that the radionuclide is not contaminated with any other radioactive or stable nuclide of the same element), it is difficult to exclude the stable form of the nuclide in most cases. Thus, the no-carrier-added (n.c.a.) terminology should be applied to a radionuclide or a radioactive compound to which no carrier of the same element or compound has been intentionally or otherwise added during its preparation, i.e. maximum achievable A_s under practical conditions.

The influence on the specific radioactivity of a given radionuclide of the other coproduced radioisotopes is presented below for one production process of ^{123}I. When ^{123}I is obtained from ^{123}Xe prepared by a (p,5n) reaction on natural iodine, ^{123}I is contaminated at the end of the preparation step by 0.1% and 0.15% of ^{121}I and ^{125}I, respectively. Thus, for example, 3.7 GBq of ^{123}I (i.e. 100 mCi), which corresponds to 0.42×10^{-9} atom-gram are contaminated by 3.7 MBq of ^{121}I (i.e. 0.1 mCi) corresponding to 6×10^{-14} atom-gram and 5.55 MBq of ^{125}I (i.e. 0.15 mCi) corresponding to 0.069×10^{-9} atom-gram. The dilution or contamination factor is then 1.16.

This dilution, though non-negligible, is, however, marginal in comparison to the contamination with the stable isotope ^{127}I under practical conditions. This indicates that the theoretical specific radioactivity cannot be reached in the case of side production of other isotopes. As the contaminating isotopes are stable or will disappear with their own half-lives, which are longer than that of ^{123}I for the major contaminating radioisotopes, the specific radioactivity of ^{123}I will decrease with time.

2.4 Production of Iodine Radionuclides

2.4.1 Production of ^{120}I

Iodine-120 is produced at a cyclotron and two nuclear routes have been utilised:

(a) ^{122}Te(p,3n)^{120}I

The optimum energy range for this reaction is $E_p = 37 \rightarrow 32$ MeV and the expected thick target yield of ^{120}I is 3.6 GBq/μA h [6]. The associated major impurities are ^{120m}I (25%, $t_{1/2} = 53$ min) and ^{121}I (22%, $t_{1/2} = 2.1$ h).

(b) ^{120}Te(p,n)^{120}I

The suitable energy range for this reaction is $E_p = 15 \rightarrow 9$ MeV and the expected thick target yield of ^{120}I is 2 GBq/μA h [2]. The only impurity observed is ^{120m}I at a level of ~5%. For production of ^{120}I, a thin $^{120}TeO_2$ target was irradiated and radioiodine separated via the dry distillation method described below for ^{124}I and ^{123}I. A batch yield of about 700 MBq has been reported.

Availability and Purity

Highly pure ^{120}I has been produced so far only in one laboratory [2]. The level of ^{120m}I impurity was <5%. The radionuclidic and radiochemical purity were also high. This radioisotope appears to have a great potential. Intensified efforts to upgrade its production therefore seem worthwhile.

2.4.2 Production of ^{122}I

Iodine-122 is obtained via the generator system

$$^{122}\text{Xe} \xrightarrow[20.1\,\text{h}]{\text{EC}} {}^{122}\text{I}.$$

The parent ^{122}Xe is generally produced via the ^{127}I(p,6n)^{122}Xe reaction at a proton energy of about 70 MeV [7]. An alternative route ^{124}Xe(p,p2n)^{122}Xe at $E_p = 43$ MeV has also been suggested [8]. The thick target yield of ^{122}Xe in both the processes amounts to about 500 MBq/μA h. Batch yields of about 10 GBq have been reported.

Availability and Purity

High purity (>99%) ^{122}I has been produced so far only via the above-mentioned generator system and only in one laboratory [7]. The radioisotope has found some application in developing remote radioiodinations. In general, however, it appears too short-lived for worthwhile applications.

2.4.3 Production of ^{123}I

Iodine-123 is the most suitable cyclotron-produced radioisotope for single-photon emission computed tomography (SPECT). It is commercially available and widely used. About 25 nuclear reactions have been suggested

and investigated for its production [9]. In practice, however, four routes are followed.

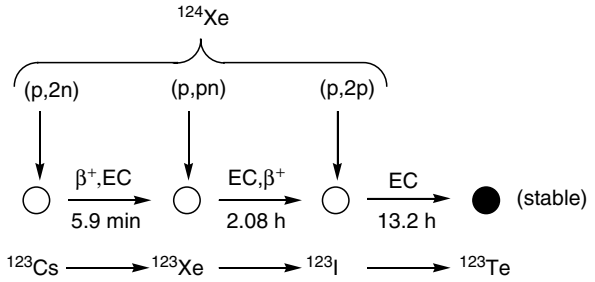
(a) $^{127}\mathbf{I(p,5n)^{123}Xe} \xrightarrow[\mathbf{20.1\,h}]{\mathbf{EC,\beta+}} {}^{123}\mathbf{I}$

This is a high-energy route and the suitable energy range is $E_p = 65 \rightarrow 45$ MeV [9]. The radioxenon produced is removed from the target with a He stream either on-line or in a batch process at the end of the irradiation. It is then frozen and allowed to decay for 2 to 8 h in a closed vessel. Thereafter radioxenon is regained cryogenically and the vessel is rinsed with water containing some reducing agent. The yield of ^{123}I is high ($\sim$400 MBq/μAh) and batches of up to 20 GBq have been reported. The major drawback of the process is the long-lived ^{125}I ($t_{1/2} = 59.4$ d) impurity at a level of about 0.25% at EOB.

(b) $^{124}\mathbf{Xe(p,x)^{123}I}$

This is a medium-energy route and demands the use of highly enriched ^{124}Xe as target material [10,11]. The production sequence is given below (from Ref. [11]).

The radionuclide ^{123}I can be formed directly via the (p,2p) reaction as well as indirectly via the decay of ^{123}Xe. The contribution of the (p,2p) reaction is small (<10%), the major route thus being the decay of ^{123}Xe. The precursor ^{123}Xe itself is formed directly via the (p,pn) reaction and via the decay of ^{123}Cs that is produced by the (p,2n) reaction. Cross section measurements showed that, of all the processes involved here, the (p,2n) reaction leading to the formation of ^{123}Cs is the strongest.



The production of ^{123}I via this route involves very sophisticated gas targetry since enriched ^{124}Xe is rather expensive. The optimum energy range for production is $E_p = 29 \rightarrow 23\,MeV$. Both low-pressure long targets and

medium-pressure shorter targets are utilised. Since no long-lived impurity is formed, irradiation times are not limited to a few hours (in fact, irradiations up to saturation of ^{123}I have been done). At the end of irradiation, ^{123}I is obtained through decay of cryogenically separated ^{123}Xe (together with the target gas ^{124}Xe) and rinsing the target with very dilute alkaline water. The collected radioiodine is concentrated via evaporation and purified via ion-exchange chromatography.

The combined yield of ^{123}I from the two sources is high ($\sim$300 MBq/μA h) and batches of about 100 GBq are routinely produced. To date, this is the best method of ^{123}I production since no detectable radionuclidic impurity is present. Therefore it is recommended or even demanded for ^{123}I production for clinical use.

(c) ^{124}Te(p,2n)^{123}I

This is also a medium-energy route and demands the use of highly enriched ^{124}Te as target material [9]. The most suitable energy range for production is $E_p = 26 \rightarrow 21$ MeV. It is a very high yield reaction ($\sim$800 MBq/μA h), but the level of the ^{124}I impurity is rather high (0.5% to 1.2%).

The production process utilising this route consists of irradiating $^{124}TeO_2$, highly enriched in ^{124}Te, with protons of suitable energy. Thereafter radioiodine is chemically separated via dry distillation (see below for ^{124}I). Batch yields of about 10 GBq have been reported.

(d) ^{123}Te(p,n)^{123}I

This is a low-energy route but demands the use of highly enriched $^{123}TeO_2$ as target material. The suitable energy range for production is $E_p = 14.5 \rightarrow 11.0$ MeV and the expected thick target yield is 137 MBq/μA h [12]. The level of impurities is low. The production process utilising this route is exactly the same as described earlier for the ^{124}Te(p,2n)^{123}I reaction. The only differences are the utilisation of a $^{123}TeO_2$ target (instead of $^{124}TeO_2$) and a lower proton energy range in the target. In recent years more effort has been devoted to this reaction, and batch yields of about 20 GBq have been reported. The level of the ^{124}I impurity is largely dependent on the enrichment of the ^{123}Te target used. In case of a highly enriched target, no significant impurity is observed.

Availability and Purity

Iodine-123 is commercially available in dilute NaOH solution as [^{123}I]iodide. The radioactive concentration lies at 11 to 20 GBq/ml. The chemical and radiochemical purity are high. The radionuclidic purity is extremely high if the

^{124}Xe(p,x)-process is used. In most countries, therefore, only radioiodine produced via this route is admissible for human use. However, in several parts of the world, rather remote from the main centres of production, ^{123}I produced via other routes is still used. The route ^{123}Te(p,n)^{123}I is very interesting for local application. The radionuclidic purity is high (depending on the enrichment of the ^{123}Te target used) and even a small-sized cyclotron is capable of producing this radionuclide.

2.4.4 Production of ^{124}I

Iodine-124 is both a diagnostic and a therapeutic radionuclide. It finds application as a useful tracer as well as for quantitation of ^{123}I-labelled SPET radiopharmaceuticals via PET. A few ^{124}I-labelled compounds have been applied in tumour therapy.

^{124}I is produced at a cyclotron, and two nuclear routes are often used:

(a) ^{124}Te(d,2n)^{124}I

The most suitable energy range for this reaction is $E_d = 16 \rightarrow 6$ MeV and the expected thick target yield of ^{124}I amounts to 22 MBq/μA h [13–15]. The level of ^{125}I impurity amounts to ~2%, and the levels of other radionuclidic impurities depend upon the enrichment of the ^{124}Te target used.

(b) ^{124}Te(p,n)^{124}I and ^{125}Te(p,2n)^{124}I

The most suitable energy range for the ^{124}Te(p,n)^{124}I reaction is $E_p = 14 \rightarrow 9$ MeV and the expected thick target yield of ^{124}I is 18 MBq/μAh [12,16–18]. The level of ^{125}I impurity here is <0.1% and if 99.9% enriched target material is used, no other radionuclidic impurity is observed [18].

In addition to the above-mentioned two reactions on ^{124}Te, cross section data for yet another reaction, namely ^{125}Te(p,2n)^{124}I, have also been reported [19]. The optimum energy range appears to be $E_p = 22 \rightarrow 15$ MeV. The yield of ^{124}I would be 90 MBq/μAh, which is about four times higher than via the presently used (d,2n)- and (p,n)-processes. The expected level of ^{125}I impurity is 0.9%. The practical applicability of this reaction, however, remains to be demonstrated.

The commonly used production process of ^{124}I consists of irradiating $^{124}TeO_2$, highly enriched in ^{124}Te, with deuterons or protons of suitable energy. Thereafter radioiodine is chemically separated via dry distillation at 750°C, i.e. just above the melting point of $^{124}TeO_2$. The activity is either

collected in a dilute solution of NaOH or at first in a capillary trap and then eluted with dilute NaOH. In the latter case the total volume is smaller.

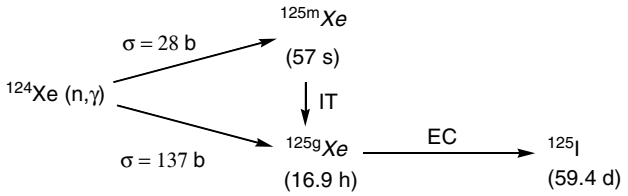
Availability and Purity

Iodine-124 has been produced to date in several laboratories in quantities of 0.2 to 1 GBq. These quantities are sufficient for research purposes but not for large-scale application. The demand for this radioisotope is increasing. So far no commercial supplier has undertaken the responsibility to produce ^{124}I in large quantities.

The activity available from research laboratories in about 0.5 ml of very dilute NaOH solution is in the form of [^{124}I]iodide. The chemical and radiochemical purity are high [17,18]. The radionuclidic purity depends upon the nuclear route adopted. The purest form is obtained in the case of the $^{124}Te(p,n)^{124}I$ reaction, with $<0.1\%$ ^{125}I as impurity [18].

2.4.5 Production of ^{125}I

Iodine-125, a reactor-produced radionuclide, is commercially available in large quantities [20,21]. Its production follows the (n,γ) reaction on ^{124}Xe in the following sequence:



The irradiation target is natural xenon gas containing 0.096% ^{124}Xe. It is filled in a zircaloy-2 capsule to a pressure of about 100 bars. On irradiation in a nuclear reactor, several radionuclides of xenon are produced. Fortunately, only the decay of ^{125}Xe leads to radioiodine ^{125}I. The other radioxenons decay either to stable xenon or to some caesium isotopes. It needs, however, to be pointed out that long irradiations are disadvantageous. Iodine-125 itself has a neutron capture cross section of 900 barns, and consequently during a long irradiation, part of the ^{125}I formed will be converted to ^{126}I. In practice, the irradiation time amounts to a few days. Thereafter the irradiated gas is allowed to decay for several days.

For isolating radioiodine, the irradiated capsule is cooled and Xe gas is allowed to escape. The inner walls of the capsule are then rinsed with dilute NaOH solution. In order to eliminate long-lived ^{135}Cs and ^{137}Cs, which may be present in small amounts, the solution is passed through a cation-exchange column. The radioiodide remains in solution.

Availability and Purity

Iodine-125 is commercially available in dilute NaOH solution as [^{125}I]iodide. The radioactive concentration lies at 4 to 11 GBq/ml and the specific radioactivity amounts to >75 GBq/μmol. The chemical and radiochemical purity are high. The radionuclidic purity is also high; only some ^{126}I ($t_{1/2}$ = 13.1 d) is unavoidable (see above). Its tolerable content lies at about 0.2%.

2.4.6 Production of ^{131}I

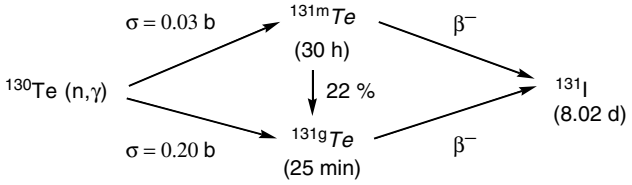
Iodine-131 is also a reactor-produced radionuclide and is commercially available in large quantities [20,21]. There are two routes for its production:

(a) Fission of ^{235}U

Since the chain yield of mass 131 is fairly high (2.885%) and the radioiodines with mass higher than 131 are short-lived, ^{131}I is easily obtained in a rather pure form. The irradiated $^{235}UAl_3$ is first stored for 24 h to allow decay of short-lived products. Thereafter it is treated with NaOH whereby radioactive inert gases (Kr and Xe) are emitted; on filtration, uranium and some fission products are removed. The filtrate is then acidified with HNO_3. On heating, radioiodine is distilled over and collected in a trap. The rest of the reaction mixture is treated further for separation of ^{99}Mo and other fission products.

(b) (n,γ) reaction

The (n,γ) reaction on ^{130}Te leads to the formation of ^{131m}Te and ^{131g}Te, both of which eventually decay to ^{131}I. The reaction sequence is thus:



The target material for irradiation is either Te-metal or TeO_2, depending on whether a wet chemical separation procedure is employed or a dry distillation method is used. In general, the latter method is preferred. The irradiated TeO_2 is allowed to decay for about 3 days so that a greater part of ^{131m}Te

is transformed into ^{131}I. The distillation of radioiodine is then done in a stream of air at 750°C, i.e. just above the melting point of TeO_2, and it is collected in a trap.

The cross sections for the formation of both ground and metastable states of ^{131}Te are rather low, so the expected overall yield of ^{131}I via the (n,γ) reaction is much lower than that via the fission process.

Availability and Purity

Iodine-131 is commercially available in dilute NaOH solution as [^{131}I]iodide. The radioactive concentration lies at >2 GBq/ml. Although production is done at a reactor, n.c.a. ^{131}I is formed via the indirect route and the specific radioactivity amounts to ~100 GBq/μmol. The chemical and radiochemical purity are high. The radionuclidic purity is also high for both processes. Some ^{129}I ($t_{1/2} = 1.57 \times 10^7$ a) may, however, be present at the ppb level. In the case of fission process, the chain yield of mass 131 is four times higher than that of 129, but in the (n,γ) reaction the cross sections of equally abundant ^{128}Te and ^{130}Te are almost equal. The level of ^{129}I in the case of (n,γ)-produced ^{131}I may thus be slightly higher.

$Na^{131}I$ of high specific activity in dilute NaOH solution may undergo self-radiolysis and oxidation to iodate and other chemical forms. Addition of a reducing agent (e.g. $Na_2S_2O_3$) prevents this and preserves the isotope in the form of iodide. The reducing agent may, however, interfere in the use of ^{131}I for labelling organic compounds, especially with in situ oxidation methods. An excess of reducing agent can be avoided by newer technologies using either hydrazine [5] or even more elegantly acid-treated platinum [22].

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Chapter 3

IODINATED RADIOPHARMACEUTICALS

3.1 Physico-chemical Properties

3.1.1 Carbon–iodine Bond

Bond Distances

The distances between atoms in a molecule are characteristic properties of the molecule and give us information on bond strength if we compare the same bond in different molecules. Bonds become weaker as we move down the periodic table since bond distances must increase because the number of inner electrons increases. Let us compare the four carbon–halogen bonds. On the same molecular site the stability of the bond decreases in the order: fluorine, chlorine, bromine, iodine. The bond distances are shortened by increasing *s* character of the carbon: sp^3 C−I = 2.13 Å, sp^2 C−I = 2.09 Å (ethylenic series), sp^2 C−I = 2.05 Å (aromatic series), *sp* C−I = 1.99 Å [1]. For vinyl, aryl and alkynyl structures compared with saturated structures, other explanations have also been offered for the shorter carbon–iodine bond distances. For instance, when a *p* orbital is on an atom adjacent to a double bond, there are three parallel *p* orbitals that overlap. A typical example is vinyl iodide. Although the *p* orbital of the iodine atom is filled, it still overlaps with the double bond. The double bond character of carbon–iodide bond is also confirmed by the canonical forms of resonance: $C{=}C{-}I \leftrightarrow C^{-}{-}C{=}I^{+}$. Analogous considerations can be made for the aryl–iodine bond and alkynyl–iodine bond. Double bonds are both shorter and stronger than the corresponding single bond. In general, shorter bonds are stronger bonds.

Energy, Stability

The energy necessary to cleave a bond to produce the constituent radicals is called the bond homolytic dissociation energy. For example, for the reaction $R-I \rightarrow R^{\bullet} + I^{\bullet}$ the energy is written $D(R^{\bullet}/I^{\bullet})$. When the cleavage of the bond yields the constituent ions the energy is called the bond heterolytic dissociation energy. For the reaction $R-I \rightarrow R^{+} + I^{-}$ the energy is written $D(R^{+}/I^{-})$. In Table 3.1 values of energy $D(R^{\bullet}/I^{\bullet})$ and $D(R^{+}/I^{-})$, respectively, are reported for different iodinated compounds. The table shows that the stronger carbon–iodine bonds, according to the higher homolytic dissociation energies, are observed for unsaturated structures C_6H_5-I and $CH_2=CH-I$ than for saturated compounds CH_3-I and C_2H_5-I. Considering heterolytic dissociation energies, similar comments can be made on the same compounds except for CH_3-I and $HC\equiv CCH_2-I$.

In conclusion, there is a correlation of bond strengths with bond distances that points out the choice for a vinyl–iodine bond, an aryl–iodine bond or even a methylene–iodine bond in saturated hydrocarbons in order to radioiodinate a compound with the maximum of stability. While for vinyl–iodine and aryl–iodine bonds the energies are sufficiently high to avoid hydrolysis, this is not generally true for saturated methylene–iodine bonds.

For example, it has been observed for cocaine derivatives used as dopamine transporter inhibitors that *N*-(3-iodopropyl)-2β-carbomethoxy-3β-(4′-iodophenyl)nortropane was subject to spontaneous partial hydrolysis in aqueous solution [3], whereas the *N*-(3-iodopropenyl)-derivative was found to be stable under similar conditions. The same was true for the chlorinated analogue (*E*)-*N*-(3-iodoprop-2-enyl)-2β-carbomethoxy-3β-(4′-chlorophenyl)-nortropane (*E*-IPT) [4].

Table 3.1: Bond homolytic dissociation energy $D(R^{\bullet}/I^{\bullet})$ and bond heterolytic dissociation energy $D(R^{+}/I^{-})$ calculated from Born–Haber thermodynamic cycles [2]. Energy is measured at 298 K under standard conditions. 1 kcal = 4.184 kJ

R–I	$D(R^{\bullet}/I^{\bullet})$kJ.mol^{-1}	$D(R^{+}/I^{-})$ kJ.mol^{-1}
CH_3-I	234.72	889.52
C_2H_5-I	222.59	722.58
$(CH_3)_2CH-I$	222.17	638.48
$(CH_3)_3C-I$	210.87	562.33
$CH_2=CHC\ H_2-I$	184.51	671.95
$HC\equiv CCH_2-I$	206.27	748.10
$C_6H_5CH_2-I$	167.36	566.93
$CH_2=CH-I$	263.59	826.34
C_6H_5-I	269.45	790.36

However, a relatively stable methylene–iodine bond was found in vivo with iodoethyl amides and ethers as in the case of *N*-(iodoethyl)spiperone [5] and *O*-(iodoethyl)-compounds [6]. Indeed, a recent systematic study on differently substituted *O*-(iodoethyl)-ethers confirmed their stability in contrast to other iodoalkylderivatives [7].

Steric Hindrance and Electronic Density

In first approximation, the changes in physico-chemical properties of an iodinated compound compared to the non-iodinated analogue could be attributed to the steric hindrance effect induced by the introduction of iodine. A model of comparison uses van der Waals radii of substituents, which permit to evaluate molecular steric interactions that could occur during ligand–target recognition. Indeed, the interactions between two non-bound atoms without electric charge can be attractive or repulsive. According to London strengths when the two atoms come close, the interaction which is weak at large distance becomes more and more attractive when the distance tends to the sum of their van der Waals radii, but the interaction changes to repulsion at smaller distance. This explanation is applicable to intermolecular interactions.

The van der Waals radii of various substituents are reported in Table 3.2. The radius increases with the atomic number in the halogen series. The radius is similar for the iodine atom (2.15 Å) and the methyl group (2.00 Å), but it is almost twice of the hydrogen radius (1.20 Å). Although that parameter has to be taken into account when replacing a hydrogen atom, a halogen atom or a methyl group by an iodine atom, the electronic density of the halogen atom bound to a carbon atom should probably be the more determining factor.

In order to illustrate the steric hindrance, the electrophilic substitution on R91150 (Figure 3.1), a $5HT_{2A}$ receptor antagonist, is given as an example. In the 4-amino-2-methoxybenzamide group, the 3-position is more activated than the 5-position for direct electrophilic substitution; thus a mixture of 3- and 5-radioiodinated compounds could be expected. Nevertheless, the electrophilic substitution occurs nearly entirely at the 5-position. This is due to steric hindrance by the freely rotating methoxy group on the 2-position. Less than 0.5% of the compound is labelled at the 3-position.

Table 3.2: van der Waals radius [8] and electronegativity [9] of some atoms and organic groups. Carbon electronegativity is 2.50 [10]

	H	F	Cl	Br	I	CH_3	CF_3
van der Waals radius (Å)	1.20	1.35	1.80	1.95	2.15	2.00	
Electronegativity	2.28	3.95	3.03	2.80	2.47	2.30	3.35

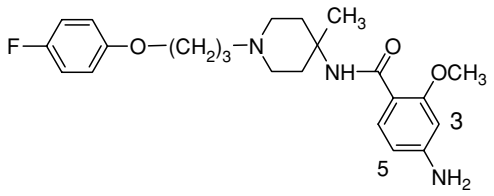

Figure 3.1: Structure of the $5HT_{2A}$ antagonist R91150

The electronic density of the halogen atom bound to a carbon atom results from the polarisation of the carbon–halogen bond. Indeed, the charge of the electron pair of a covalent bond is generally shared more by one atom than the other according to the probability of the presence of the electrons. This tendency of an atom to attract the electrons is called electronegativity. This concept was extended to characteristic groups. Electronegativity values of various substituents are reported in Table 3.2 in comparison to iodine. For a carbon–halogen bond, the electronic density decreases with the electronegativity in the halogen series from fluorine to iodine. The field created around the fluorine atom will be stronger than around the iodine atom. These field differences affect the intermolecular hydrogen–halogen interactions. In the case of fluorine this interaction is strong and this halogen constitutes a hydrophilic centre in vivo. For example, the first D-glucose analogue available for studying regional cerebral glucose metabolism was labelled with fluorine-18 [11] while radioiodinated analogues of D-glucose were described only some years later [12–18], none of the latter being substrates for hexokinase. On the other hand, iodine is not hydrated in aqueous media and constitutes a hydrophobic centre. Generally, iodine should be introduced on a hydrophobic part of the molecule to minimise the modifications of its biological behaviour.

In fact, for a better understanding of the structure–activity relationship towards developing specific radioiodinated ligands, it is necessary to take the whole physico-chemical parameters into account. As an example, mazindole analogues [19,20] were prepared for the SPECT investigation of the presynaptic dopamine transporter in the human brain. As expected, the lipophilicity of the congeners of mazindole increases with the atomic number of the halogen, but the biological efficacy, the inhibition of dopamine reuptake, decreases from chlorine to bromine, to iodine and to fluorine.

3.1.2 Lipophilicity

Incorporation of a radiohalogen, and especially of a voluminous iodine atom, can change considerably the pharmacology of the original molecule due

to: (i) a change of the torsion angle; (ii) a change of the electronic density and (iii) the increase of lipophilicity. It plays a fundamental role in the distribution volume (VD) of a radiotracer, on its binding to plasma proteins and on its tissular non-specific binding. Increase of lipophilicity then can impede accurate specific imaging.

Theoretical lipophilicity estimation is based on the calculation of the solvent accessible area (SAA) for a molecule, which is related to the Hammet [21] electronic and the Taft [22] steric substitution constant.

The determination of lipophilicity is based on the partition between a hydrophilic phase and a lipophilic phase. This parameter is fairly easily determined, either using calculated fragmental values or using simple partition methodology. Practical measurements generally based on octanol/water partition are expressed as the Hansch and Léo log *P* parameter [23] or as the k' value of reversed phase HPLC [24,25], which under well-defined conditions is directly related to lipophilicity. The octanol/water partitition, however, is limited with log *P* values above 3 due to many experimental problems.

As it can be seen in Figure 3.2 for the example of ketanserin analogues, SAA values and k' values are highly correlated. Moreover, the lower lipophilicity values of the 4-halogenated compounds compared to the 6 analogues can easily be explained using the clustered lipophilic compensation concept.

As shown in Figure 3.3, the site of substitution of the halogen will rule the increase of lipophilicity. Figure 3.3 shows that incorporation of the iodine atom in clustered lipophilic compensation (CLC) position, i.e. the halogen clustered within the electronic cloud of 4-*F*-phenylketone consisting of the aromatic resonance of the electronegative fluorine in the aromatic plane and

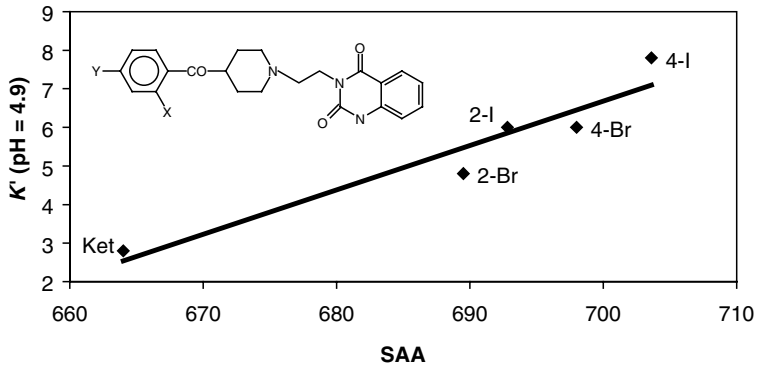

Figure 3.2: Theoretical and practical estimation of the lipophilicity of halogenated ketanserin analogues [26]

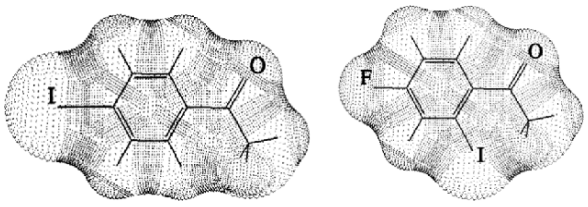

Figure 3.3: Spatial SAA representation of 4-F-2-I-alkylphenylketone in CLC position and 4-I-alkylphenylketone, respectively

the polar C=O group perpendicular to that plane, yields the lowest modification of the SAA and then the lowest increase of lipophilicity [27].

Theoretical estimation of the log P of a molecule is easily obtained by adding the substituent constants π [29] or the hydrophobic fragmental constants f [28] of its constituents and substituents.

For comparison of the effect of halogens on lipophilicity, the increments of differential change of lipophilicity upon their introduction into aliphatic and aromatic positions of given molecules are listed in Table 3.3.

Table 3.3: Hydrophobic fragmental constants [29] of radiopharmaceutically interesting halogens and hydrogen in aliphatic and aromatic bonds

	H	F	Br	I
Aliphatic	0.19	−0.51	0.24	0.59
Aromatic	0.19	0.43	1.17	1.46

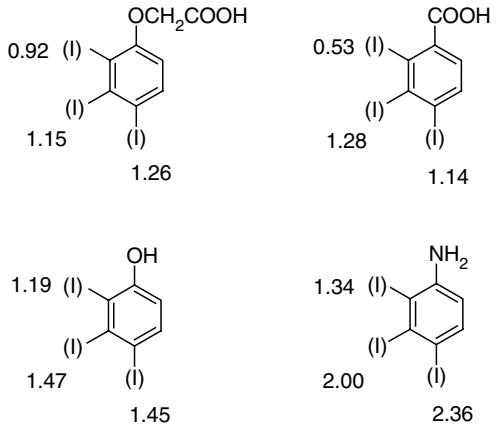

Figure 3.4: Different values of the substituent constant for iodine as a function of substituents on a benzene ring. The π value for iodine on unsubstituted benzene is 1.19 [31]

When comparing the averaged "differential lipophilicity increments" in Table 3.3, it was noted [30] that the introduction of all halogens in an aromatic rather than an aliphatic position leads almost to a tenfold increase in lipophilicity. The same is true when iodine replaces fluorine in both aliphatic and aromatic positions of a molecule ($\Delta \log P \approx 1$).

However, π values in different molecules are highly dependent on electronic interactions (inductive effect, steric effect, branching, conformational effect) as illustrated in Figure 3.4 for iodine bound in isomeric positions of various benzene derivatives.

3.2 Pharmaceutical Properties

As any other pharmaceutical, iodinated radiopharmaceuticals for human investigations call for safety evaluation, quality assurance and quality control procedures. Consequently, for each new radiopharmaceutical, the basic quality criteria must be met.

3.2.1 Chemical and Radiochemical Purity

Chemical purity is defined as the fraction of the compound in the formulated radiopharmaceutical that is in the desired molecular form.

Radiochemical purity may be defined as the "*fraction of a specific radionuclide that is present in the desired chemical form and in the specified molecular position*". The main reason for seeking radiochemical purity is to avoid unnecessary errors on measurements in vivo.

Radiochemical impurities may come from chemistry:

- incomplete reactions;
- side reactions;
- reactions with impurities or solvents;
- incomplete removal of protective groups;
- failed or incomplete preparative separation;
- chemical change during storage (e.g. by radiation damage).

Almost all labelling methods start from aqueous radioiodide solutions. If scavengers are added in these solutions to prevent oxidation of iodide, attention must be paid that they do not interfere with the labelling reaction. For nucleophilic exchange labelling reactions, traces of bromides or chlorides must be absent. In case of electrophilic substitution, traces of highly activated organic impurities can consume a large part of radioiodine and generate labelled side products.

3.2.2 Stability In Vitro and Shelf-life

During labelling and storage, the main factors responsible for the degradation of radioiodinated tracers are:

- radiation decomposition (especially at high specific activity and high concentration); structural alterations are caused by high levels for radiation during radioiodination and storage. The energy deposition is responsible for excitation and ionisation of the molecule. The ionised and excited molecules will dissociate and different chemical forms will result;
- loss of radioiodide due to oxygen, light, heat, solvent and pH;
- chemical damage due to radioiodide, reagents and impurities in radiopharmaceutical solutions.

Since it is difficult to predict product stability, the stability of the radiopharmaceutical must be experimentally determined in its final injectable formulation at typical activity levels over the time span of its anticipated storage prior to use. Another important consideration when defining the usable shelf-life of a radiopharmaceutical is the fact that the specific activity decreases exponentially with a half-life equal to the physical half-life of the nuclide. So, the administration of a given activity amount of ^{123}I-labelled pharmaceutical that has been stored for 24 h would result in a fourfold increase in the mass dose of the pharmaceutical.

An aspect that has found less attention in radiopharmaceutical chemistry is the fate of a radioiodinated molecule upon decay of its label. A brief estimation for the most often used radioiodine isotopes I-123, -125 and -131 shows that recoil energies (e.g. 0.2 and 0.3 keV for I-131) accompanying their corresponding decay processes will not lead to bond rupture. However, conversion of iodine-131 to xenon-131 will definitively lead to bond breakage. Much drastic is the effect upon EC decay of iodine-123 and -125 followed by an Auger effect. This leads to molecule fragmentation as, for example, shown for [^{125}I]iodouracil [32], indicating the therapeutic potential of this radionuclide. Thus, presence of minute amounts of possibly pharmacologically active des-iodocompounds should only be expected with iodine-131.

In practice, when expensive ^{125}I- or ^{131}I-labelled tracers are stored for a long time (often several months for I-125), a regular HPLC control and purification is required. Also when an n.c.a. ^{123}I-labelled tracer is used for in vivo diagnostics one half-life or more after the labelling procedure, radiolytic effects must be taken into account.

3.2.3 Pharmacological Characteristics

After the administration (injection) of a radiopharmaceutical in a living subject (human or animal), two different phases can be distinguished:

- a pharmacokinetic phase including the absorption, distribution, metabolism and elimination (clearance) phases;
- a pharmacodynamic phase describing the interaction of the radiopharmaceutical with its specific target (receptor, transporter, enzyme, etc.).

The quality of a tracer for functional imaging will be mostly related to the balance of performance of the tracer in these two phases. This makes what is called development of ''smart radiopharmaceuticals''.

The distribution of a radiopharmaceutical is usually expressed by its volume of distribution (VD) and indicates the virtual blood volume calculated from the (remaining) concentration in blood and from the total administered doses of activity. It is a theoretical number that assumes the radiopharmaceutical is at uniform concentration in the tissue and in the circulation (blood flow). Thus, the higher the VD is, the bigger is the tissular concentration.

The main factors that play a role in the distribution of radiopharmaceuticals, and then for their VD, are:

- the ability of crossing the cell membranes (lipophilicity, ionisation, etc.);
- the binding to plasma proteins (only the free or unbound fraction is available for crossing the membrane);
- the binding to blood cells (erythrocytes, lymphocytes, platelets);
- the pulmonary filter (the lung is the first organ crossed by the radiopharmaceutical after a peripheral intravenous injection and lung parenchyma cells are known to retain basic molecules, acting as a ''reservoir'');
- the stereospecific binding of the radiopharmaceutical to ''specific'' cell proteins such as receptors, transporters or enzymes and
- the non-specific binding to cell membrane components, such a ''binding'' being essentially dependent on the lipophilicity of the radiopharmaceutical.

The vast majority of radiopharmaceuticals cross the lipid membrane by passive diffusion along the electrochemical concentration gradient and then lipophilicity plays a fundamental role in their distribution characteristics (VD). The neutral form of the radiopharmaceutical is definitively much more liposoluble than its ionised form and the ionisation constant also plays an important role in its distribution characteristics.

3.2.4 Pharmaceutical Considerations

In the production of a radioiodinated pharmaceutical for SPET investigations, the criteria of sterility, apyrogenecity, isotonicity and of a neutral pH should be confirmed using the appropriate procedure, meeting the requirements of the national and/or European pharmacopoeia.

All radiopharmaceuticals for human use and long-term experiments in animals should be sterile and apyrogenic. Sterile labelled solutions can easily be prepared by filtration of the radiopharmaceutical through a membrane filter of low porosity (0.22 μm) and by following aseptic procedures in a laminar flow hood.

Pyrogens or endotoxins are soluble polysaccharides or proteins produced by microorganisms. They cannot be removed by filtration. After i.v. administration, pyrogens produce symptoms of fever, joint pain, sweating and headache. The only way to prevent pyrogenic contamination is to perform all chemical procedures meticulously, according to the pharmacopoeia specifications.

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Chapter 4

METHODS OF RADIOIODINATION

From the aspects discussed in Chapter 3, it is obvious that the substitution of an atom (hydrogen, halogen, etc.) or a polyatomic group (methyl, trifluoromethyl, etc.) by an iodine atom in a pharmacologically active compound could modify its biological behaviour. For that reason and because of the short lifetime of the radioisotopes of iodine, the development of new radioiodinated tracers creates challenges for design and synthesis as well.

In general, classical iodination methods used in organic synthetic chemistry can be used in radiochemistry. However, these methods have to be adapted taking into account several parameters such as the half-life of the radionuclide and the small-scale concentrations used. The methods of radioiodination were reviewed in literature with major emphasis on methods, mechanistic aspects or labelled compounds [1–6]. The main reactions of radiolabelling with iodine belong either to nucleophilic or electrophilic substitutions. This chapter is divided accordingly with a separate subchapter on labelling of macromolecules. In spite of unavoidable minor repetitions, this appeared necessary to account for the properties of those compounds and considerations of special methods. Direct radioiodination (replacement of hydrogen atom by a radioiodine atom) represents an exception almost for electrophilic substitution in arenes. The other reactions need the introduction of a "good" leaving group into the molecule that could be easily replaced by an iodine atom. The choice of the labelling site is determined by biological, chemical and structural considerations while a particular attention must be paid to the stability of the carbon–iodine bond. After making the choice, a regioselective labelling reaction should be used to introduce the iodine atom at the desired position. A mixture of iodinated isomers should be avoided for the preparation of an established radiopharmaceutical in order to prevent possibly tedious separation and purification procedures. Moreover, the high costs of radioiodine isotopes make site-specific labelling with high yields obligatory.

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4.1 Nucleophilic Substitution

4.1.1 General

In nucleophilic substitution reactions the attacking reagent (in this case the nucleophile is an iodide anion) brings an electron pair to the substrate (the electrophile) to form the new bond, and the leaving group (the nucleofuge) goes away with an electron pair. Several distinct mechanisms are possible depending on the substrate (aliphatic or aromatic), the leaving group and the reaction conditions (solvent, temperature, etc.). Radioiodination via nucleophilic substitution is most often performed with aliphatic and aromatic compounds according to the nature of the leaving group.

4.1.2 Halogen Exchange in Aliphatic Compounds

In aliphatic compounds, the reactions occur at a saturated carbon atom and correspond to the most common S_N1 and S_N2 mechanisms. The parameters affecting these reaction types such as properties of the compound, nucleophile, leaving group, solvent temperature, kinetics and stereochemistry are discussed in detail in basic teaching books. The two reaction mechanisms are exemplified by the following reactions, where $X(\neq H)$ is the nucleofugic group. The main difference is that the bond of the leaving group is broken before (S_N1) or after (S_N2) the bond formation of the nucleophile.

$$S_N1:\ RX + I^- \rightleftharpoons R^+ + X^- + I^- \rightleftharpoons RI + X^-$$

$$S_N2:\ RX + I^- \rightleftharpoons [X - R - I]^- \rightleftharpoons RI + X^-$$

Generally, sulphonates are better nucleofuges than halides. The most commonly used sulphonates for radioiodination are triflates (CF_3SO_3R), mesylates (CH_3SO_3R), tosylates (p-$CH_3C_6H_4SO_3R$), brosylates (p-$BrC_6H_4SO_3R$) and nosylates (p-$NO_2C_6H_4SO_3R$). The examples of application are too many to be listed here. Since for practical reasons a halogen is often chosen as leaving group (the order of halogen nucleofugality with radioiodide as nucleophile is I > Br > Cl), only halogen exchange is considered as a prototype reaction in this chapter.

For the S_N2 reaction ($RX + I^-$) the charge in the transition state ($X^{\delta-\cdots}R\cdots I^{\delta-}$) is dispersed within the starting material. The transition state is more solvated in polar aprotic solvents than in protic ones, while the original charged nucleophile is less solvated in aprotic solvents. So switching from a protic solvent such as methanol to a polar aprotic solvent such as dimethyl sulphoxide should induce a great increase in the substitution rate. The reaction is hindered by polar solvents. However, an increase in polarity of aprotic solvents also corresponds to a small decrease in the substitution rate. For example, the radioiodination of 17-iodoheptadecanoic acid from its brominated precursor is compared in the two different solvents, acetone (reflux) and dimethylformamide (80°C) [1].

Since for most of the reactions the S_N1 substitution rates go up while the S_N2 susbstitution rates go down by increasing the polarity of the solvent, it is quite possible for the same reaction to proceed by an S_N1 mechanism in one solvent and by an S_N2 mechanism in another one. For the S_N2 reaction, both the nucleophile I^- and the substrate RX are involved in the rate-determining step (the only step in this case). The reaction should be first order for each component, second order overall, and should satisfy the rate expression: rate $= k[RX][I]$.

Isotopic Exchange

The simplest way to introduce a radioiodine atom in a small organic molecule is to substitute a stable iodine atom already incorporated in the molecule by a radioactive iodine atom. Since most of the iodine atoms in the final product after labelling are iodine-127 atoms, compounds with very high specific radioactivity cannot be prepared by the iodine-exchange reaction. Nevertheless, the modest specific radioactivity achievable by this method allows to obtain radiopharmaceuticals, which can be used to satisfactorily perform quite a number of nuclear medicine investigations.

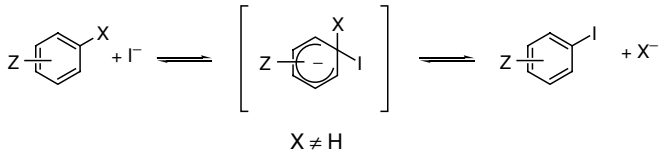
This method was used to prepare the radioiodinated steroid NM-145 by heating an acetone solution of its iodinated precursor with radioactive sodium iodide [2–5]. Other hormones such as diethylstilbestrol [6] and steroid NP-59 [7] were labelled according to this method. This radioiodination method was also applied to label 2-deoxy-2-iodo-D-glucose (IDG) [8], 4-iodoantipyrine (4-IAP) [9,10], fatty acids [7,11,12] and acyclonucleosides (IAzAcN) [13,14].

Radioiodine-for-Bromine Exchange

When bromine is used as a leaving group for radioiodination, the advantage of such an iodine-for-bromine exchange (non-isotopic exchange) reaction is that a very high specific activity can be obtained, provided the radioiodinated compound is efficiently separated from its brominated precursor. A great deal of work has been done in this area to develop the labelling procedures. For example, long-chain fatty acids radioiodinated on the terminal carbon were prepared and used for heart imaging purposes [1,7,9,15–19]. In some cases, the radioiodination has been performed on a secondary CH–I bond. For example, 16α-radioiodoestradiol (IE_2) was obtained from 16β-bromoestradiol in boiling acetonitrile [20,21]. Also sonication was used to improve the radioiodination rate during the fast low-temperature preparation of 17-[^{123}I]iodo-heptadecanoic acid from its brominated precursor [19]. Radioiodination of 4-IAP [10,22] and of the derivatives of [^{125}I]16α-iodoestradiol [23] was performed according to this method.

4.1.3 Halogen Exchange in Aromatic Compounds

Nucleophilic substitution proceeds slowly on an aromatic carbon. For successfully performing a nucleophilic substitution reaction on an aromatic substrate the reaction must be either activated by electron-withdrawing groups, preferably with −M and −I effect in ortho- or para-position to the leaving group, or catalysed by, for example, metal salts. These reactions can be performed either in a solvent, in a melt or under solid-state conditions. The general reaction mechanism of the S_NAr reaction is shown below, indicating that a tetrahedral carbon is formed in the S_N1 transition state in contrast to the pentavalent carbon in the S_N2 transition state.



In S_NAr reactions the substitution occurs at the same carbon, i.e. it is position selective. In contrast, the elimination–addition mechanism via aryne intermediates, starting from appropriate arylhalides and strong bases, leads to positional isomers and other side products. So far, this latter method is not used for radioiodination purposes. The other alternative of nucleophilic aromatic substitution, which proceeds via an S_N1 type reaction by decomposition of diazonium compounds, will be discussed below. The basic features of nucleophilic aromatic substitution are discussed elsewhere in more detail [24].

Isotopic Exchange

For many aromatic compounds, the exchange with sodium radioiodide in a solvent under reflux conditions gives only very poor radiolabelling yields besides generally low specific activity products. An alternative method is then to perform the isotopic exchange in a melt or under solid-state conditions.

A solid-state procedure was developed for the labelling of *meta*-iodobenzylguanidine (MIBG), a peripheral norepinephrine reuptake blocker. According to the Mangner method [25–27], the substrate should be heated with the sodium radioiodide in ammonium sulphate at 140°C to 150°C for 30 min.

Radioiodination by such isotopic exchange was used for a large variety of tracers: rose Bengal [28,29], iodohippuric acids [30,31], quinoline derivatives (4,3-DMQ) [32–34], hypaque [35], iodobenzyl quaternary ammonium compounds [36], *N*-isopropyl-*p*-iodoamphetamine (IMP) [37], iodobenzylpropanediamine derivatives (HIPDM) [38,39], 2-iodospiperone [40], iodo-phenylpropylpiperazine derivatives [41,42], vesamicol derivatives [43–45] and iodo-PK 11195 [46].

Radioiodine-for-Bromine or -Chlorine Exchange

The S_NAr reactions can be accelerated by using particular drastic conditions. For example, the radioiodination of a potential MAO-A ligand, Ro 11-9900, an iodinated analogue of moclobemide, was easily achieved under solid-state conditions in ammonium sulphate using the brominated precursor and [^{125}I]NaI at 210°C for 40 min [47].

The iodine-for-bromine exchange technique was successfully used for the radioiodination of benzodiazepines [48,49], L-6-iododopa (6-ID) [50] and benzamide [51].

Compared to bromine or iodine, chlorine is not a good leaving group. In arenes, a nucleophilic substitution of iodine-for-chlorine is generally very difficult to obtain. Nevertheless, such an iodine-for-chlorine exchange was successfully used for the preparation of radioiodinated PK 11195 and 5-iodo-2′-deoxyuridine [52] by heating the chlorinated precursor with sodium radioiodide according to the Van Dort method [53,54].

4.1.4 Copper-assisted Halogen Exchange

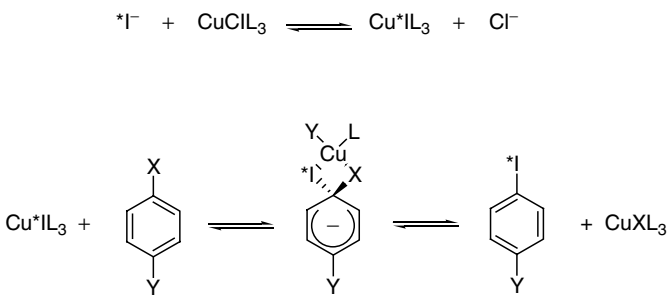
Cu(I)-assisted Radioiodination

Given the relatively low reactivity of nucleophilic aromatic halogen substitution, early attempts to use copper metal or copper salts as catalysts of nucleophilic iodination of arenes were quite successful [55]. Therefore, this method became important for aromatic radioiodination in recent years, making use of different methodologies of performance such as applying Cu^{2+}, Cu^{+} or Cu^{2+} together with various reducing agents like sodium disulphite [56] or Sn(II) salts [57,58].

Also various solvents are useful for the method, such as water [59,60], acetic acid, DMSO [58,61] or mixtures with ethanol [60,62]. When EtOH is used in mixed solvent reactions, addition of sodium sulphite avoids the formation of volatile Et*I, leading to a considerable loss of radioiodine.

An effective method allowing labelling of water-soluble molecules is the use of Cu^{2+} salts with an excess of reducing agent (e.g. Sn^{2+}) in aqueous solutions at pH 2.0 to 2.2 [60,63]. For more lipophilic compounds (e.g. phenyl fatty acids) an ethanol/water mixture of 7/3 (vv) proved useful [60,63]. For basic lipophilic compounds an aqueous solution of $<10\%$ acetic acid avoids side products. Here, especially hydrohalic acids should be avoided in order to prevent competing chlorination or bromination reactions [64,65]. Labelling of more than 20 aryl compounds was successfully performed in pure mineral acids or mixed solvents therewith [63,66].

According to the wide variety of reaction conditions employed for the Cu-assisted radioiodination, many different mechanisms were proposed in the literature. The first authors describing this reaction assumed radical reactions [67,68], which have to be ruled out for statistical reasons at least at n.c.a. conditions. A nucleophilic substitution via an ipso-complex of the dihaloarene intermediate in the form of tetracoordinated Cu(I) as suggested in 1978 appears more probable [62] and generally accepted by most authors [58,61,69–71] as outlined in the following scheme:



Based on mechanistic studies, Mertens *et al.* [72] propagate a primary complexation of Cu in a higher oxidation state with the carbon–halogen bond of the precursor with subsequent nucleophilic substitution by radioiodide. Some observations make even a pure nucleophilic mechanism appear doubtful. Moerlein [58] found a decrease of substitution yield with less electron-withdrawing (activating) substituents but an increase with electron-donating (deactivating) substituents and also lowest yields with fluorine as nucleofugic group, both effects contradicting nucleophilic substitution. This is also true for the "false" order of reactivity of ortho- > meta- > para-substituted benzamides [61,73].

On the other hand, several facts confirm the necessity of copper(I) to accelerate the aromatic exchange. Several methods are described where Cu(I) salts are directly used in either acetic acid [69–71,73] or dimethyl sulphoxide (DMSO) [74], or a reducing agent is needed in water to prevent disproportionation of Cu(I) to Cu(0) and Cu(II) [63,64]. Also a significant delay of increasing radiochemical yields (RCYs) with reaction time was firstly explained by the time needed for heating the reaction solution [75]. However, in comparative experiments with Cu(I) and Cu(II) salts under identical conditions, this could clearly be attributed to the time delay due to the necessary reduction in case of Cu(II) salts [70].

The use of Cu(I) as a catalyst in the presence of an excess of reducing agent can considerably improve the nucleophilic substitution yield. For example, radioiodinated MIBG can be prepared with a 99.7% RCY [66] at 100°C for 30 min in the presence of Cu(I), while the solid-state procedure in the presence of ammonium sulphate as a catalyst gives a considerably lower RCY at 140°C to 150°C [25,26].

This method was successfully transferred for the nucleophilic non-isotopic labelling of 2-iodopargyline, an MAO-B inhibitor, by using a brominated precursor heated with sodium radioiodide in the presence of Cu(I) as a catalyst (according to the Mertens method) [76,77]. Moreover, Cu(I)-assisted radioiodination under ultrasonication [78] was used for the radiolabelling of 2-iodoketanserin.

The literature mentions a vast number of tracers, which have been radioiodinated using the copper-assisted method: iodomazindol [79], hippuric and benzoic acid derivatives [67], MIBG [27], iodohippuric acid, IMP, HIPDM [66], iodotropapride [80], piperazine derivatives [81], benzamides [61,69], iodofluvoxamine [82], iodobenzylamine derivatives [83], deltorphin and dermorphin analogues [84], 2α-carbomethoxy-3β-(4′-iodo-phenyl)tropane (β-CIT) [85], vesamicol derivatives [86] and a spiperone analogue (IBSP) [87]. A selection from the wide variety of reaction conditions used for Cu(I)-assisted radioiodination is given in Table 4.1.

Table 4.1: Selection of reaction conditions for Cu(I)-assisted radioiodination

Precursor	c_{pre}(mM)	c_{Cu}(mM)	n_{pre}/n_{Cu}	Solvent	Reference
N-isopropyl-*p*-iodoamphetamine	19.6–21.0	0.65–0.81	22.6	110–150 µl H_2O/50 µl AcOH	[63]
15-(*p*-iodophenyl)-9-methylpenta-decanoic acid	2.18–4.35	0.5	4.35–8.7	400 µl EtOH/H_2O 7/3	[60]
m-iodobenzyl-guanidine	51.4–60.0	3.71–4.33	13.8	50 µl AcOH/10µl H_2O	[56]
N-(2-diethyl-aminoethyl)-*p*-iodo-benzamide	57.8	4.00	14.4	500 µl citrate-buffer pH 4	[88]
p-substituted bromo-arenes	65.0	6.50	10.0	155 µl DMSO/3% H_2O	[58]
N-aminoalkylated bromobenzamides	23.0	0.10	250	55 µl AcOH	[70]
m-iodobenzyl-guanidine	6.56–13.1	1.04	6.3–12.6	500 µl H_2O	[66]
iodoestradiols	0.12	20	0.0061	500 µl CH_3CN/EtOH 1/1	[62]

Cu(II)-assisted Radioiodination

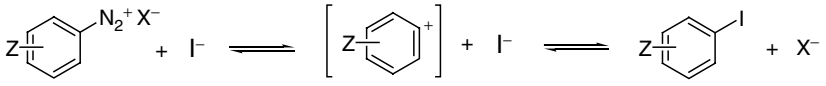
When labelling 2-iodohippuric acid, traces of 2-iodobenzoic acid, which are present in the commercial form of the iodohippuric acid, impair the RCY. The presence of copper(II) salts suppressed this problem. Here, the role of the copper salts has been explained by a coordination of the 2-iodobenzoic acid impurity, preventing its preferential radioiodolabelling. If a 2-iodohippuric acid with a purity higher than 99.9% is used, copper salts are not required anymore [89,90].

The use of Cu(II) has been later on generalised to the radioiodination of D-glucose derivatives [91,92], IMP [93], MIBG [27], benzamides [88,94–96], 2′-iododiazepam [97] and pyridinecarboxamide derivatives [98].

4.1.5 Radioiodo-dediazonisation

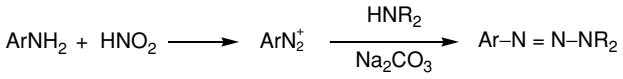
The treatment of aromatic diazonium salts with sodium radioiodide was one of the first methods to label the corresponding aromatic compound with radioiodine. The performance of such a Sandmeyer-type reaction implies that the appropriate aniline be available and that the other functional groups in the molecule do not react with (or be protected towards) the nitrous acid used to form the diazonium salt.

The iodo-dediazonisation proceeds according to an aromatic S_N1 mechanism, which first forms a reactive aromatic carbocation via release of nitrogen.



The Gatterman reaction is a variation of the iodo-dediazonisation procedure, which employs copper–bronze to catalyse the reaction. The problems with the dediazonisation method arise from the initial formation of an aryl cation or aryl radical, which will react with all free electron pairs in the vicinity (e.g. of solvent molecules), thus leading to many side products. High concentration of the diazonium salt is mandatory to limit the competition with the solvent. Nevertheless, the radioiodo-dediazonisation was earlier successfully applied for the preparation of a series of tracers even under n.c.a. conditions as summarised in earlier reviews together with a detailed discussion of the mechanistic features of the reaction [99,100]. Recently, the method was used to prepare a radioiodinated derivative of spiperone [40] and various benzamides [88,101] from their corresponding diazonium precursors.

For this kind of reaction the diazonium salt has to be prepared in situ during the radioiodination process, i.e. in the presence of an excess of nitrous acid. The Wallach reaction allows to avoid this problem. In this method the diazotised amine is trapped by the formation of a triazene with an appropriate secondary amine according to the following reaction. The triazene will be isolated and can be stored in dark.



The triazene is decomposed either by protic acids [29,102] or Lewis acids like trimethylsilyl halides [103]. This method was used to introduce an iodine radioisotope into the aromatic ring of phenyl-substituted fatty acids [104,105], phenoxyethanamine derivatives [88], benzamides [96,106], benzodiazepines [107] and phenyl-2-aminopropane derivatives [108].

4.1.6 References

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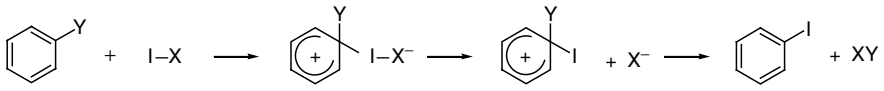
4.2 Electrophilic Substitution

4.2.1 General Considerations

Electrophilic radioiodination is a process in which formally a positively charged iodine (I^+) attacks a system with high electron density such as an aromatic ring or a double bond. As a result a covalent carbon–iodine bond is formed with loss of a positively charged leaving group. The leaving group (the electrofuge) must necessarily depart without its electron pair. The most important leaving groups are those that can best exist without the pair of electrons necessary to fill the outer shell, i.e. the weakest Lewis acids. The most common leaving group is the proton. In aliphatic systems, the proton can be a leaving group, but the reactivity depends on the acidity. Since metallic ions are easily able to bear positive charges, organometallic moieties are especially susceptible to electrophilic substitution.

For electrophilic substitution at least four possible major mechanisms are distinguished, which are called SE_1, SE_2, SE_i and the arenium ion mechanism, which is observed with arenes and abbreviated SE_{Ar} [1]. The first three concern substitution in aliphatic compounds and are discussed in detail. The vast majority of electrophilic radioiodination reactions, however, concerns aromatic substitution.

Due to the high energy of formation, iodine cannot exist in the oxidation state +1 in condensed phase. Rather, the attacking species is a dipole ($^{\delta-}X–I^{\delta+}$) in which iodine is positively polarised and where X is an electron-withdrawing group or a solvent molecule.

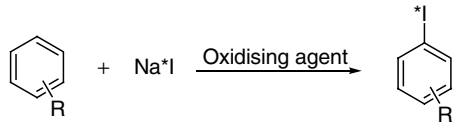


The mechanistic features of the electrophilic aromatic substitution have been discussed in detail [2,3].

For the development of radioiodinated tracers, it is important to take into account that aromatic and vinylic iodo compounds possess the highest chemical and often in vivo stability. In vinyl iodides the strength of the C−I bond is at least as high as that of the corresponding aryl iodides and identical labelling methods can be applied for the synthesis of both types of iodo compounds.

Aliphatic radioiodinated tracers, which are almost exclusively labelled via nucleophilic substitution, play only a minor role. Thus, nearly all the electrophilic labelling methods developed up to now have focussed on radioiodoarenes. Molecular radioiodine (radio-I_2) is not a suitable labelling reagent at the n.c.a. level, because it is highly volatile, rather unreactive and of course a

carrier-added reagent. However, the low oxidising potential of iodide ($I^- \leftrightarrow 1/2I_2 + e^-$ $\varepsilon_0 = 0.5355$) allows for the direct formation of an electrophilic species in which iodine is formally oxidised to "I^+". Mainly two methods, i.e. direct iodo-deprotonation and iodo-demetallation, both based on in situ oxidation of iodide, are suitable for radioiodination on the n.c.a. level and have gained importance.



4.2.2 Oxidising Reagents

Iodine Monohalide (ICl, IF)

For generation of these species a solution of sodium radioiodide is either treated with ICl, leading to a radioiodo–iodo exchange, or oxidised with the elemental halogen (F_2, Cl_2). The higher electron density of chlorine and fluorine results in a strong polarisation of the covalent chlorine– or fluorine–iodine bond in the interhalogen formed. Thus, the positive partial charge on the radioiodine atom renders it very electrophilic.

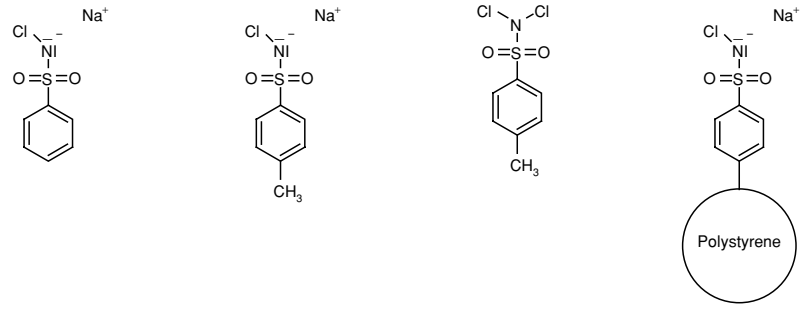
A disadvantage of ICl is its high oxidation potential and the necessity of carrier iodide addition. N.c.a. ^{123}I-IC1 can be obtained by the radioactive decay of ^{123}Xe-xenon to ^{123}I-iodine in an HCl–gas atmosphere. This procedure is very sophisticated and costly and thus never achieved practical importance [4–6].

A newer alternative reagent is radioiodomonofluoride [7–9], obtained by the reaction of fluorine gas with radioiodide in perfluorinated solvents such as perfluorohexane or trifluoroacetic acid (TFA). The reagent is directly used without the need for any purification or isolation procedures. Thus the aromatic compound to be labelled is added to the solution of *I–F. Using this reagent, n.c.a. radioiodination of weakly activated arenes, e.g. anisole, is possible with a RCY of about 65%.

N-*Chloroamides*

Another way to achieve electrophilic radioiodination is the application of sodium salts of *N*-chlorosulphonic acid amides such as the earliest used chloramine-T (CAT, *N*-chloro-*p*-toluenesulphonic acid) [10], chloramine-B (CAB, *N*-chloro-benzenesulphonic acid) or dichloramine-T (DCT, *N, N*-dichloro-*p*-toluenesulphonic acid).

In aqueous solution these compounds are often discussed to slowly release hypochlorous acid (HOCl), which oxidises iodide under formation of an iodonium ion (H_2OI^+). However, in strongly acidic aqueous medium, interhalogen species are probably formed from the sodium salts, whereas under neutral or slightly basic conditions, the corresponding radioiodoanalogues of the *N*-haloamides are postulated [11,12]. In organic solvents DCT is used for in situ oxidation.



Chloramine-B Chloramine-T Dichloramine-T Iodobeads

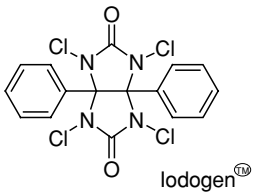
The relatively strong oxidising conditions and possible chlorination reactions often lead to by-product formation. It is recommended to work at low temperatures and to keep the reagent concentration as low as possible [4,13,14].

In order to limit these drawbacks, polymer-bound CAB, namely Iodobeads™, which is an immobilised CAT on polystyrene beads, may be used [15]. By adding the beads to the reaction mixture, the liberation of the oxidising species is retarded and its concentration is kept very low. Moreover, the contact time between the precursor and the oxidising agent is shorter. While the conditions for radioiodination with CAT and the Iodobeads are otherwise similar, a separation of the latter oxidant is possible by simple filtration, thus avoiding the use of a reducing agent. Since the monochlorosulphonamides as ionic compounds are insoluble in most organic solvents, their use is restricted to aqueous reaction media. The use of DCT makes it possible to work in organic solvents. The oxidation strength and labelling results of DCT differ only marginally from those of CAT [12,16].

Iodogen™

Iodogen™ is the trademark for 1,3,4,6-tetrachloro-3α,6α-diphenylglycouril, a compound containing four functional chlorine atoms [17]. Originally Iodogen™ was developed for the mild n.c.a. radioiodination of proteins, peptides and cell membranes. In these cases the obtained RCYs are

comparable with those using CAT. Since Iodogen™ is virtually insoluble in aqueous solutions, a two-phase technique has been developed that uses Iodogen™ as thin layer on the walls of the reaction vessel. For this purpose, a solution of Iodogen™ in a volatile organic solvent such as chloroform is transferred to the reaction flask and the solvent is evaporated under a flow of inert gas. An aqueous solution of the precursor for labelling and radioiodide are added and the labelling reaction is allowed to proceed for a few minutes. The reaction is stopped simply by removing the aqueous reaction mixture from the flask [4,17]. This procedure has found wide application in the n.c.a. radioiodination of compounds containing activated aromatic groups such as anilines and phenols. Because of its mildness it minimises oxidative side reactions with sensitive molecules and polyiodination.



Iodogen™

A variation of the two-phase Iodogen™ method consists of performing the radioiodination in TFA. Under these homogenous conditions, various differently substituted weakly activated anisole derivatives could be radioiodinated with RCYs up to 90% [18,19].

N-*Halosuccinimides*

Another group of *N*-halo compounds comprises the *N*-halosuccinimides: *N*-chloro-tetrafluorosuccinimide (NCTFS), *N*-chlorosuccinimide (NCS) and *N*-bromosuccinimide (NBS). NCTFS and NCS in combination with radioiodide give rise to RCYs and substitution patterns closely comparable to those obtained with CAT. For example, using TFA anhydride as a solvent, anisole and also toluene can be radioiodinated with RCYs of about 70% and 30%, respectively [20]. With NBS as oxidant, generally lower RCYs are obtained and simultaneously radical side reactions are observed [14,16,20].

A very recent approach uses the reagent combination NCS/Na*I in trifluoromethanesulphonic acid (triflic acid) as a very potent system for the direct electrophilic n.c.a. radioiodination of non-activated and strongly deactivated arenes. Under proper reaction conditions, even direct n.c.a. radioiodination of the strongly deactivated nitrobenzene is possible with an RCY of 70% [21]. For the first time, this approach allowed the direct electrophilic radioiodination of deactivated substrates at the n.c.a. level. The only prerequisite is the stability of the aromatic compound in triflic acid. The

feasibility could be demonstrated, for example, for complex precursors as substituted benzamides, phenylpentadecanoic acid and the D_4-ligand L-750,667 [21].

Peracids

The advantage of using peracids as oxidants is due to the fact that no chlorinated by-products can be formed and that the formation of by-products resulting from (over)oxidation is strongly reduced. Thus, peracids lend themselves especially to the radioiodination of sensitive substrates. It was suggested that the corresponding hypohalous acid (HOX) is generated through peracetic acid, which can be formed prior to addition to the substrate.

The peracid itself is generally formed in situ from hydrogen peroxide and an organic acid (e.g. formic or acetic acid); thus the concentration of the oxidant is kept very low. On the contrary, the direct use of preformed peracid often results in oxidative damage of the aromatic compound.

The RCYs obtainable with the peracid method are generally lower than those obtained using *N*-halooxidants [14,22]. For example, arenes like anisole can be radioiodinated with modest RCYs of about 25%.

Other Oxidants

Apart from the above described methods there exist some other less often used reagents for in situ oxidation with electrophilic labelling:

- iodate;
- metal ions;
- enzymes;
- electrochemical methods.

The iodate method uses potassium iodate or sodium iodate as an oxidant. Since iodate is a mild oxidant, this method can be used for the radioiodination of sensitive substrates such as proteins. As a matter of fact this method is not really carrier-free, but still a relatively high specific activity can be obtained with n.c.a. radioiodide [23–25], since iodate is not completely reduced by n.c.a. radioiodide.

Inorganic metal salts (Tl^{3+}, Ag^{+} and Ce^{4+}) have been used for the in situ formation of heteropolar, electrophilic iodine species. Cerium (IV) salts have the advantage of high chemoselectivity [26]. Thus, chloride ions, eventually present in the reaction mixtures, are not oxidised and the formation of chlorinated by-products is excluded [14]. Radioiodination using thallium tristrifluoroacetate as an oxidant in TFA has been examined in detail [27,28]. Strongly and weakly activated arenes like anisole, toluene and benzene could

be radioiodinated with RCYs of 82%, 66% and 35%, respectively. The strong oxidation potential of the Tl(III)/TFA system limits its application to substrates that are stable against strong oxidants. Furthermore, it is essential that the highly toxic metal salt be removed quantitatively from the labelled products to be used in vivo.

Very sensitive molecules, e.g. biomolecules such as proteins, can be labelled by the use of enzymes. The oxidation of radioiodide in this case is performed by peroxidases, e.g. lactoperoxidase [4,28,29].

The formation of electrophilic radioiodo species can also be performed using anodic electrochemical oxidation of radioiodide. The advantage of this method is its mildness without the formation of by-products. However, because of the high cost of the technical set-up, the rather complex performance and often low RCYs, the electrochemical method has not found wide application [4,30,31].

4.2.3 Direct Electrophilic Radioiodination (Radioiodo-deprotonation)

This method is generally limited to arenes, which are activated for electrophilic substitution. In given cases the strong oxidising conditions may cause problems. All the labelling reagents discussed above oxidise radioiodide in situ to a reactive, electrophilic iodine species suitable for substitution on electron-rich arenes. In general, direct electrophilic radioiodination gives rise to high RCYs and is simple to perform. One disadvantage of this labelling method is the formation of isomeric product mixtures, which may be difficult to separate. The orientation of substitution follows the known rules based on I- and M-effects, i.e. the most activating groups are ortho and para directing. Under certain circumstances a regioselective reaction may occur, depending on secondary substituents, and may lead to the desired radiotracer, e.g. in the case with the amino acid α-methyltyrosine [18,32–34] and iodobenzamides [35–37]. Hydrogen atoms in saturated alkanes are very unreactive towards iodonium species, but if the hydrogen is located in α-position to an electron-withdrawing group such as a carbonyl moiety, electrophilic substitution can often be carried out. An example is given by the direct radioiodination of testosterone, which yields the 2-iodotestosterone with a 10% RCY [38].

With respect to the question of the reacting iodination species, the often simply assumed iodonium cation must be ruled out as indicated above and by the fact that equal results could be obtained at least for positional orientation, whatever the oxidising agent used. Results of substitution yields and selectivity differ, however, with the type of oxidant and solvent used. Correspondingly, various species are under discussion to be the electrophile such as

Table 4.2: Comparison of radioiodination yields (%) of selected arenes using various oxidising agents and solvents [21]

	Trifluoroacetic acid anhydride			Trifluoroacetic acid	Trifluoromethane-sulphonic acid
Oxidising agent Reaction time	NCTFS 4 h	NCS 4 h	CAT 10 min	$T1(CF_3CO_2)_3^a$ 15 min	NCS 5–60 min
Anisole	69 ± 5	72 ± 4	75 ± 8	83 ± 5	39 ± 5
Toluene	47 ± 8	30 ± 6	49 ± 8	89 ± 7	84 ± 7
Benzene	~4	~1	~3	34 ± 2	81 ± 3
Chlorobenzene	—[b]	—[b]	—[b]	0	76 ± 5
Nitrobenzene	—[b]	—[b]	—[b]	0	38 ± 1

Reaction conditions: 0.5 mmol arene, 15μmol oxidant, 0.5 ml solvent, room temperature.
[a]7.5 μmol arene, 6.75 μmol metal salt, 0.3 ml solvent.
[b]Not determined.

interhalogens, (protonated) hypoiodites or *N*-iodoamides (-imides). A summary of the influence of solvents and oxidants on the reactivity of electrophilic iodo-deprotonation is given in Table 4.2 for the example of differently activated benzenes [21].

4.2.4 Demetallation Techniques (Radioiodo-demetallation)

In this case organometallic compounds are needed as precursors for electrophilic radioiodination, which are often difficult to synthesise. The most suitable organometallics are trialkylstannyl, trialkylsilyl or boronic acid derivatives. The advantage of this method lies in the possibility to regioselectively radioiodinate activated and deactivated arenes under very mild conditions. Most often, the radioiodo-demetallation can be conducted as a final step (one-step radiosynthesis).

The main drawback of the n.c.a. radioiodination via direct electrophilic substitution as described above is the general restriction to activated arenes and the formation of positional isomers, which are often difficult to separate and lower the yield of the desired regiospecific product. One possibility for the regioselective radioiodination of arenes at the n.c.a. level is the use of organometallic precursors. Performing labelling reactions on these substrates is technically very simple and gives rise to high RCYs without the simultaneous formation of regioisomers. According to the electropositive character of the metals used and the resulting polarisation of the carbon–metal bond, it has a lower binding energy than the carbon–hydrogen bond. Thus, the carbon–metal bond is much more activated for an electrophilic attack than the

carbon–hydrogen bond. Consequently, the use of organometallic precursors allows for short reaction times, mild reaction conditions and regioselectivity [14,22].

Table 4.3 shows that the electronegativity of the metals M is much lower than that of carbon (2.50). The polarity of the C–M covalent bond is weak, but much higher than that of the C–H bond. Moreover, cleavage of C–H bond requires more energy than that of the C–M bonds. These characteristics explain in part why electrophilic substitution occurs at the carbon of the C–M bond and not on the C–H bond of the aromatic ring. The differences in energy and bond length for a particular atom M indicated in the table cause a difference in reactivity according to the nature of the organometallic compound used as precursor. For example, the stretching of the C–M bond can further facilitate the attack of the electrophilic reagent for steric considerations. When the elements of the group IVA of the periodic table, silicon, germanium and tin are compared, the order of reactivity observed is Si < Ge < Sn, which in part is due to the variation of the bond length. In fact, the literature shows that there is no ideal organometallic precursor for iododemetallation when criteria such as ease of synthesis, stability, reactivity, toxicity and purification are considered together.

Radioiodo-deboronation

Regarding the commonly used metals that find application in radiohalogenations via electrophilic demetallation, boron has an exceptional position. It has been known for some time that organoboranes react with molecular iodine under basic conditions to produce alkyl or vinyl iodides with retention of configuration. Although the carbon–boron bond is hardly polar and has a high bonding energy, the empty 2*p*-orbital of the boron complex opens a kinetic pathway for an electrophilic attack. Furthermore, the small covalent radius of boron gives rise to a large steric influence of the attached ligands on the labelling reaction. One advantage of organoboranes is their good

Table 4.3: Physico-chemical characteristics of atoms M and bonds C–M [12]

Element M	Electronegativity χ	Covalent bond (Å)	Bond energy C–M ($kJ \cdot mol^{-1}$)	Ionic character C–M bond (%)
H	2.20	0.37	418.8	2
B	2.01	0.79	322.7	6
Si	1.74	1.18	301.5	16
Sn	1.72	1.40	226.1	16
Hg	2.00	1.50	113.1	6
Tl	2.04	1.70	150.8	5

handling; they are generally crystalline compounds, stable in air and moisture [14,39,40].

Organoboranes can be obtained through hydroboration of unsaturated bonds following Markovnikov's rule, since boron is more positive than hydrogen. The electrophilic iodo-species react with the organoborane in a regioselective and stereoselective S_E-reaction e.g. under elimination of the alkylborate moiety to form iodoarenes:

$$(R-CH_2-CH_2)_3B \xrightarrow{{}^*I^+X^-} R-CH_2-CH_2-{}^*I + (R-CH_2-CH_2)_2BOH$$

$$ArB(OR)_2 \xrightarrow{{}^*I^+X^-} Ar^*I + (RO)_2BOH$$

The speed of the iodination reaction is increased by an increasing number of alkyl substituents attached to the boron atom [14,41]. The highest RCYs in deboronation reactions, reported so far, were obtained using CAT as an oxidant [42].

This technique is not only useful for labelling of alkyl compounds, provided the corresponding alkene derivative is available, but labelled vinyl iodides can also be made from the appropriate alkynes via vinylboronic intermediates. The vinylborane obtained by this way is the *E* isomer. Radioiodination via organoborane chemistry has been used, for example, to synthesise a series of (*E*)-17α-iodovinylestradiols [39]. Arylboranes were also prepared for radiolabelling aromatic compounds such as iodobenzenes [43] and iodothiophene derivatives [44].

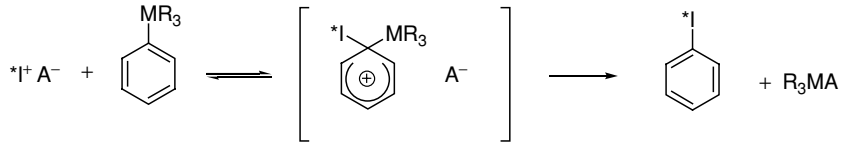
Radioiodo-demetallation with Group IV Metals

In addition to silicon and germanium, mainly tin is finding extensive application for the synthesis of organometallic precursors. The criteria of n.c.a. radioiodination via demetallation reactions have been examined in detail for all the three metals using different solvents. The highest RCYs of about 90% have been obtained with activated and deactivated arenes using trimethyltin precursors in acetic acid or methanol, while non-polar solvents such as tertachloromethane are less suitable for this purpose. That organosilicon and organogermanium compounds are less often used compared to organotin compounds can be attributed to the fact that, especially in the case of organosilicon compounds, the metal–carbon bond is stronger than the carbon–tin bond. This leads to more stable precursors but also to lower reactivity and hence RCYs. This limits the applicability of silicon and germanium organometallics [14,22,45–47].

Radioiodination of organometallic precursors proceeds under similar conditions as the above described direct electrophilic substitution. CAT, DCT or,

in order to circumvent the formation of chlorinated by-products, peracids are commonly used for the in situ oxidation of radioiodide [22,47].

Due to their positive inductive effect, alkyl groups attached to the metal increase the electron density at the aromatic or vinylic carbon bound to the metal, thus facilitating the electrophilic attack of "I^+". Moreover, the electron-withdrawing effect of the trialkylmetal moiety suppresses possible halodeprotonation reactions. The presence of a given anion (A^-) influences the stability of the leaving group and facilitates the formation of the radioiodoarene (Scheme 4.1).



Scheme 4.1: Schematic mechanism of the iodo-demetallation reaction (M = Si, Ge and Sn)

Although the synthesis of organometallic precursors sometimes is tedious, difficult and often comprises several synthetic reaction steps, labelling via demetallation is superior to all other radioiodination reactions and has found broad application for the regioselective n.c.a. radioiodination of complex pharmaceuticals [48–50]. The literature describes a plethora of radioiodinations using organotin precursors [51–57].

- *Radioiodo-desilylation*

Radioiodo-desilylation is preferentially carried out in protic solvents under acidic conditions (AcOH, TFA or acidic methanol) at moderate temperatures. However, for less activated aryl rings harsh conditions are required. Aryltrialkylsilane can be prepared by reacting appropriate trialkylchlorosilane with an aromatic Grignard reagent or an organolithium derivative. The chemical functions must be protected if they react with these organometallic reagents. Another method is based on a reaction of an arylhalide (ArX) with R_3SiLi or R_3SiNa without protection of the functions such as CN, NH_2, OH, CHO, COR′ and COOR′. A third most commonly used way directly leads to $ArSiR_3$ from ArX and hexaalkyldisilane in the presence of $Pd(PPh_3)_4$ as catalyst. This last method can be realised without protection of functions such as amine, alcohol, acid, ester, amide, ketone, etc. An application of this method is the radioiodine labelling of oligonucleotides for imaging of mRNAs. The procedure first implies the synthesis of a radioiodinated prosthetic group from the corresponding trimethylsilyl precursor [58].

- *Radioiodo-degermylation*

The halodemetallation reactions were successfully applied on organogermanium compounds [46], which are synthesised in a similar way as the tin analogues. Good yields were obtained with activated arenes as well as with non-activated arenes, using a wide variety of solvents and CAT as oxidising agent. Germanium precursors of weakly activated arenes can be radioiodinated with high RCYs whereas in the case of silicon the parent arene must be strongly activated. Thus, arylgermanium precursors have intermediate properties, with higher stability compared to organotin and higher reactivity compared to organosilyl compounds.

- *Radioiodo-destannylation*

The addition of tin hydrides to carbon–carbon triple bonds is an efficient way of obtaining functionally substituted vinyltins, especially when given substituents would preclude the use of conventional organometallics [59]. The addition often follows a homolytic chain reaction pathway promoted by initiators such as azobisisobutyronitrile (AIBN) or UV irradiation. However, if an electron-withdrawing substituent is present on the triple bond, in suitable solvents and under appropriate experimental conditions, the reaction can change to a nucleophilic hydride addition, which occurs with a reversed regioselectivity. Hydrostannation follows an *anti*-stereochemistry, but under suitable conditions (such as tin hydride in slight excess and free-radical initiation) the kinetic adduct tends to isomerise into the more stable *E* isomer. The preparation of aryltins can be performed according to similar methods described for arylsilanes.

The halodemetallation of vinyltins normally occurs with second-order kinetics and retention of the configuration with the vinyl precursor. It is a smooth and general route to vinyl bromides and iodides. The problem of stereoselective labelling concerns in particular the vinylic substitution. For example, *E* and *Z* *N*-(3-iodopropenyl)-2β-carbomethoxy-3β-(4′-chlorophenyl)nortropanes (IPT) were iodinated and radioiodinated from their corresponding tin precursors [60]. These two isomers display different biological properties according to the double-bond configuration bearing the iodine atom. The most dominant role of destannylation, however, is its use for postion-specific radioiodination of arenes, for which it has become the labelling reaction of choice (cf. Section 5.2.2).

Radioiodo-demetallation with Mercury and Thallium

Mercury, thallium and lead have also been used for the synthesis of organometallic precursors. Hg, Tl and Pb cations in their highest oxidation

state (Hg^{2+}, Tl^{3+} and Pb^{4+}) are isoelectronic and possess xenon configuration, a fact that gives rise to a comparable chemical behaviour. The electronegativity of these three metals is essentially identical and all are capable of forming polar carbon–metal bonds. The stability of the organometal compounds decreases in the order Hg(II) > Tl(III) > Pb(IV) and is inversely proportional to the redox potential of the metals [61]. Compared to thallium and mercury, organolead compounds are extremely unstable and their isolation is, apart from a few exceptions, impossible. Thus organolead compounds do not play any significant role as radioiodination precursors. Since all the metal compounds mentioned above are strongly toxic, care must be taken to remove even traces of the precursors and the metal salts formed during product isolation.

- *Radioiodo-demercuration*

In contrast it is possible to use aromatic mercury compounds as precursors for radioiodinations [40,62]. Generally, the easily accessible aromatic mercuric chloride compounds are used, which react with carrier-added iodide to form the labelled product under simultaneous displacement of mercury halide [14]. For example, using chloromercuribenzene as a precursor, radioiodobenzene has been obtained with an RCY of 80% to 90% [47].

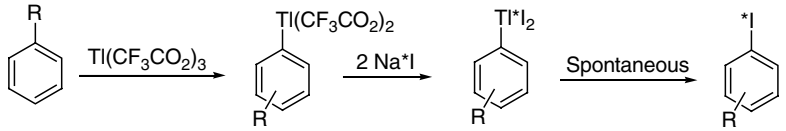
The organomercury compounds are often obtained by transmetallation, direct electrophilic substitution on aromatic rings or mercuration of alkenes [63]. Organoboranes are useful for preparing organomercuries by transmetallation, in particular the vinylmercuries. Both vinylmercuries and arylmercuries can be readily radioiodinated with high RCYs. The substitution iodine for mercury is regiospecific. As examples, a prosthetic group for protein iodination, the radioiodinated *N*-succinimidyl-3-iodobenzoate [64] and 6-iodocholesterol [65] were synthesised from the corresponding organomercuric precursor. The mercury compounds offer several advantages over other organometallics: stability, easy access and high reactivity.

- *Radioiodo-dethallation*

Radioiodo-dethallation is again increasingly being used. Thallation of numerous arenes using thallium(III)trifluoracetate (TTFA) in TFA gives rise to arylthallium(III) bistrifluoroacetates in good yields. Activated arenes react at room temperature in short reaction times, whereas higher reaction temperatures and longer reaction times are required for deactivated arenes [66–70]. If the aromatic system contains side chains bearing substituents with free electron pairs ($-CO_2H$, $-CO_2Me$, $-OH$, $-OR$, etc.), the formation of transition complexes with those substituents is most likely and the obtained products are exclusively ortho-thallated isomers [71–73]. Arenes having other

ring substituents do not exhibit such a high regioselectivity, however, due to steric reasons a preference for thallation in the para-position is generally observed. In the case of strong electron withdrawing substituents, the substitution group is predominantly meta. This method was also successful to label α-cellulose with radioiodine from a thallium derivative of benzhydryl cellulose [74].

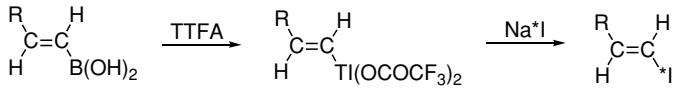
The arylthallium compounds are transformed into the corresponding radioiodoarenes via an intermediate thallium–(radio)iodo complex through reaction with carrier-added radioiodide (Scheme 4.2) [75–78].



Scheme 4.2: Schematic mechanism of radioiodination via arylthallium compounds

Although most of the described radioiodinations via arylthallium compounds have been performed with the addition of iodide carrier, this method has recently been examined in detail with regard to its application under n.c.a. conditions. For example, it has been demonstrated that anisole, toluene and benzene can be radiolabelled via organothallium precursors at the n.c.a level with RCYs ranging from 40% to 70% [27,79,80]. This method has been used for the carrier-added radioiodination of various pharmaceuticals [81–86].

The intermediate compounds of bis-(trifluoroacetate) thallates can also evolve from the in situ action of TTFA on borovinylic derivatives (Scheme 4.3). As a result, the radioiodination of vinyl compounds has an easy access and exhibits high yields. This technique is favourable in the case of molecules that are not stable in acid media and in the presence of oxidising agents.



Scheme 4.3: Vinyl iodides via vinyl thallates

4.2.5 References

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4.3 Macromolecule Labelling

4.3.1 Protein Radioiodination

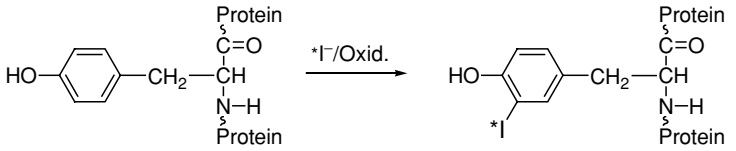
Methods for radioiodination of proteins, in general, should be mild and rapid, and should give high RCYs [1]. Furthermore, the radioiodinated protein obtained should generally have a high specific activity and should be inert to dehalogenation [2]. Labelling is often conducted as the reaction of an in situ-prepared electrophilic radioiodine species with the functional groups of the native protein. Such direct labelling is the most favoured iodination method if the protein is stable against the necessary in situ oxidation conditions [3]. Since this is often not the case, alternative methods have been proposed [2], namely:

- engineering an iodine-accepting group on the protein or
- conjugation of a small radioiodinated molecule with the protein.

Direct Radioiodination of Proteins

In proteins, functional groups such as the highly activated phenolic ring of tyrosine residues and to a lesser extent the benzene ring of phenylalanine residues, the imidazole ring of histidine residues, the indole ring of tryptophan residues and the sulfhydryl groups of cysteine residues [4] are reactive towards electrophilic radioiodine. For direct labelling, radioiodination is performed using the electrophilic methods with mild oxidative reagents and conditions as described in Section 4.2. Electrophilic reactions require the production of an electrophilic radioiodine species through the oxidation of iodide ions. The oxidant: (i) should be compatible with aqueous solutions; (ii) should not denature the protein and (iii) should permit an easy purification of the radiolabelled protein. The separation can be facilitated by assembling the peptide before iodination on a solid support using, for example, the fluorenylmethoxycarbonylamino-coupling protocol [5].

Under various reaction conditions (e.g. low pH) differing percentages of the tyrosyl, histidyl and cysteinyl moieties have been observed to react with radioiodine [6]. However, using commonly employed reaction conditions (i.e. pH 7.4, CAT as oxidant) a very large percentage of the radioiodine will be present on the phenolic tyrosine residues as shown in the following equation:



Some of the above mentioned oxidising reagents have been used for in situ generation of electrophilic radioiodine species in the presence of proteins:

- *Iodine monochloride (ICl)*

As a historically first agent for the labelling of proteins, radioiodine monochloride was prepared with a low specific activity by isotopic exchange with radioactive NaI. This labelling is quick and undesirable secondary reactions are limited. In addition, ICl is particularly stable in aqueous medium, allowing a tight control of the electrophilic substitution reaction. This method, originally proposed by McFarlane [7], has been subject to different modifications [8–11].

- *Chloramine-T*

Sodium *N*-chlorotoluene sulphonamide or CAT, a mild oxidising agent at room temperature under neutral or basic conditions, has been extensively used for high specific radioactivity labelling of peptides and proteins. However, the proteins to be labelled are in intense contact with CAT, which leads possibly to chlorination, polymerisation, formation of macro aggregates and oxidation of methionine. This drawback is minimised by using the lowest possible CAT to protein ratio [12,13]. In order to reduce the reaction with the oxidising reagent, *Iodobeads* were developed, where CAB is attached to a solid support made of derivatised non-porous polystyrene beads [14,15].

- *Iodogen*™

1,3,4,6-Tetrachloro-3α, 6α-diphenylglycouril, another mild oxidising chlorine compound, which does not need reducing agents, allows high labelling yield. Since iodogen is insoluble in aqueous media, its contact with the molecule to be labelled is limited and secondary reactions as well as protein degradation are minimised [16]. Moreover, as iodogen is readily separated from the radiolabelled protein, it has been proven to be very attractive for labelling macromolecules such as peptides and monoclonal antibodies [3]. Recently, a novel procedure for efficient coupling of high doses of ^{131}I to monoclonal antibodies, temporarily contacted with a minimum amount of iodogen, has been developed. Very high RCY and purity and full preservation of antibody integrity and immunoreactivity were achieved [17].

- *Oxidative enzymes*

As a very mild method, enzymatic radioiodination of proteins and peptides has been performed in situ with peroxidase and hydrogen peroxide. In the presence of very small amounts of hydrogen peroxide (added to the solution containing the peptide [18,19] or in situ generated [20]), the enzyme lactoperoxidase catalyses the oxidation of radioiodide to ''active iodine'', i.e. either HOI or I_2 [21]. The kinetics of radioiodination is pH dependent, and generally a pH of 5 to 6 is used. As the proteins are not exposed to strong oxidising media, immunological and biological properties of the molecules are maintained. During radioiodination, lactoperoxidase itself is iodinated, increasing the iodine loss and making the purification of the labelled protein from the labelled enzyme more tricky. This problem is overcome by using peroxydase (lactoperoxidase [22,23] or myeloperoxidase [24]) covalently bound to an insoluble matrix such as agarose that can be readily removed from the reaction mixture, which also prevents pyrogens.

Engineering an iodine-accepting group

The technique involves the grafting of an iodine-acceptor group (i.e. phenol or imidazole) at a specific site of the protein before performing the electrophilic radioiodination. This method is commonly used for the preparation of iodinated haptens. A review of the different substrates that can be iodinated and of the organic reactions that have been used can be found in the literature [24,25].

A conjugate with specific binding to the epidermal growth factor (EGF) receptor was, for example, prepared from EGF coupled to dextran by reductive amination in which the free amino group on the N-terminal of EGF was reacted with the aldehyde group on the reductive end of the dextran chain. Tyrosines were later on introduced to the activated dextran part of the conjugate. Finally, the EGF–dextran–tyrosine conjugate was, with high efficiency, iodinated with the CAT method [26].

Indirect Radioiodination via Conjugates

The direct incorporation of radioiodine for labelling of a macromolecule can be doomed to failure: (i) if the molecule does not possess functional groups that can be iodinated; or (ii) if those groups are present but are inaccessible to iodinating agents or (iii) if the molecule is sensitive to the oxidative reagents used. Moreover, radioiodination with direct labelling methods has a high risk of damaging the immunoreactivity of antibodies. For these situations various techniques have been developed to overcome the labelling problem. This alternative to direct labelling consists in the conjugation of the macromolecule with a prosthetic group that has been previously radioiodinated. In this approach, the protein to be labelled is treated with an iodinated molecule of high specific radioactivity, which has been activated for conjugation and will form a covalent bond with the protein of interest. A variation of this technique involves the preparation of a radioiodinated amino acid that will be used in the peptide synthesis [27]. The principal functional groups on proteins that can be used for conjugation reactions with prosthetic groups are amines, sulfhydryls and oxidised sugars. Many reviews describe the chemistry of protein modification through conjugation of small molecules [28].

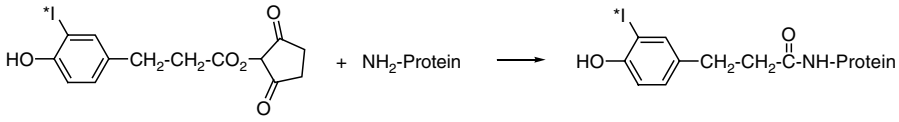
- *Amine-reactive conjugates*

The most common functional group used for conjugation of radioiodinated molecules to proteins is the amine group, generally the ε-amino group of lysine residues.

– Active esters

Coupling of protein amines with active esters provides small-molecule conjugations with stable amide linkages. For conjugates of radioiodinated compounds the first and still most commonly used active ester is the Bolton–Hunter reagent [29]. This is radioiodo-labelled *N*-succinimidyl-3-(4-hydroxyphenyl) propionate [30].

The principle of the labelling as originally reported [15,31] is simple and involves two steps: (i) preparation of the Bolton–Hunter reagent with radioactive iodine and CAT as oxidant and (ii) subsequent coupling with a free amine group of the protein (a lysine for example):



The Bolton–Hunter reagent, however, falls short with respect to in vivo enzymatic deiodination by deiodinases. In spite of this and the higher radiotoxicity of iodine-124, an improved Bolton–Hunter method was recently used for the preparation of an ^{124}I-labelled VEGF antibody [32].

In order to achieve a stable label, 3- and 4-radioiodinated benzoic acid and phenylalkyl carboxylic acid esters were developed for conjugation [33–36]. Among similar radioiodinated prosthetic groups a pyridine derivative, *N*-succinimidyl-5-[^{131}I]iodo-3-pyridine carboxylate [37], which also provides conjugates, is found to be very stable towards in vivo deiodination, and also *N*-succinimidyl-3- (tri-*n*-butylstannyl radioiodo)benzoate [36] must be mentioned.

– Imidate esters

Imidate esters react with protein amines to form amidine bonds. An imidate ester of phenol, known as the Wood reagent, has been used as a protein conjugate for radioiodination [38]. This reagent, the mono- or diradioiodo derivative of methyl-4-hydroxybenzimidate, is supposed to have the same advantages of labelling conditions as the Bolton–Hunter reagent with the additional advantage of retention of the positive charge on the amidine functionality [39].

– Aldehydes

Protein amines react with aldehydes to form imines (Schiff bases), which can subsequently be reduced to form secondary amines. Conjugates containing phenolic rings for radioiodination and aldehyde functionalities for conjugation

have been developed. The use of radioiodinated (4-hydroxyphenyl)acetaldehyde analogues for protein labelling and efficient incorporation of radioiodine in the protein even at low concentration of proteins has been reported [40].

Another example is the use of labelled aldehyde derivatives of carbohydrates (compared to the dextran–tyramine conjugate described above). An important example is the iodo–tyramine–cellobiose conjugate method [41,42]. This is of course applicable only to larger proteins, but results in a much higher in vivo stability of the iodine label compared with direct radioiodination, as demonstrated for various monoclonal antibodies [43–45].

– Isothiocyanates

Protein amines react with isothiocyanates to form thiourea bonds. 3-Iodophenyl isothiocyanate has been used for the radioiodinaton of monoclonal antibodies [46,47].

– Activated halides

Protein amines react with α-carbonyl halides to form alkylated amines. The conjugation of (radioiodophenyl)-α-bromoacetamide to an antibody via amino groups has been reported, for example [48].

• *Sulfhydryl-reactive conjugates*

The second most used functional group on a protein for formation of conjugates is the thiol group of cysteine residues. Proteins that do not contain native thiols can be reacted with compounds to generate them. Thiols are nucleophiles, like amines, and will react with most of the functional groups used for conjugation of amines.

– Activated halides

The reaction of activated halides is not specific, as thiol groups can also readily displace activated halides by nucleophilic substitution forming thioethers. A high activity radioiodination of antibodies using the α-iodoacetyl derivatised tyramine has been reported [49].

– Maleimides

Maleimides derivatives react selectively with thiol groups to form thioethers. *N*-(m-[^{125}I]iodophenyl) maleimide has, for example, been used to label rabbit immunoglobin G (IgG) and bovine serum albumin [50].

- *Carbohydrate-reactive conjugates*

Some glycoproteins, such as monoclonal antibodies, have their carbohydrate groups attached away from the biologically active binding site, making these groups attractive for site conjugation of radiochemical species. So, an oxidation of the oligosaccharide moieties using sodium periodate can be used to form aldehyde functionalities, which will react with radioiodinated amines, hydrazines or hydroxylamines [2].

- *Non-specific conjugates*

When the labelling on a particular type of residue is not needed, non-specific conjugation reagents such as diazonium salts [51], arylazides [52] or diazirines [53], which react non-selectively with aryl-, sulphhydryl-, amine- or histidyl-groups, respectively, have been proposed.

4.3.2 Oligonucleotide Radioiodination

The use of antisense oligonucleotides as in vivo diagnostic agents would bring molecular imaging at the level of gene expression [54]. However, oligonucleotides are non-canonical radiopharmaceuticals and much progress is needed to use them for in vivo imaging. Most of the labelling methods published so far used the tailing technique, which involves the addition of a radiolabelled prosthetic group to the 3′- or 5′-end of the oligonucleotide.

In analogy to the conjugate method with proteins, radiolabelling is performed in two steps:

1. radiosynthesis of a prosthetic group designed for high reactivity and stable incorporation of radioiodine (i.e. *N*-(radioiodobenzyl)-2-bromoacetamide [55], tributyl-stannyl-radioiodobenzamide [56] or 5-radioiodo-2′-deoxyuridine analogue [57]) and
2. regioselective conjugation of the synthon to the oligonucleotide. Several methods for capping oligonucleotides with ^{125}I- or ^{123}I- labelled synthons [55,57] have been proposed.

A low-yield incorporation of an ^{125}I-labelled nucleotide by the enzymatic synthesis of an oligonucleotide has also been reported [58].

4.3.3 References

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Chapter 5

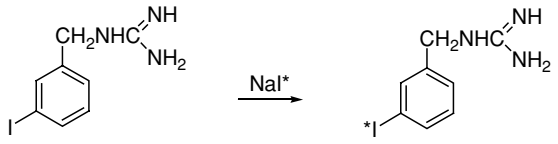
IODINATED RADIOPHARMACEUTICALS

5.1 Examples of Nucleophilic Labelling

5.1.1 Interhalogen Exchange

Meta-iodobenzylguanidine

m-Iodobenzylguanidine (MIBG) is used in diagnosis (labelled with ^{123}I) and therapy (labelled with ^{131}I) of neuroblastoma and pheochromocytoma. Also its application in myocardial imaging of noradrenergic innervation has been reported (see also Section 5.2.2).



Procedure

To a dry mixture of 7 mg MIBG sulphate, 4 mg ammonium sulphate, 3 mg ascorbic acid, 0.05 ml of a 1% copper sulphate solution and 11 to 22 GBq of [^{131}I]NaI in 0.1 M NaOH were added (only 2 mg precursor was used if labelling was done with up to 5 GBq [^{123}I]NaI).

The container was tightly closed and heated to 160°C and left at this temperature for 25 min. The solution was cooled and passed over an anion-exchange resin (Dowex 1×8, chloride form) into a penicillin vial, which contained about 1 g of silver wool. This allowed to keep the solution for a few days and dispensing it according to needs. Physiological phosphate buffer was used for dilution.

The anion-exchange resin was rinsed with 70% ethanol and physiological saline immediately before use.

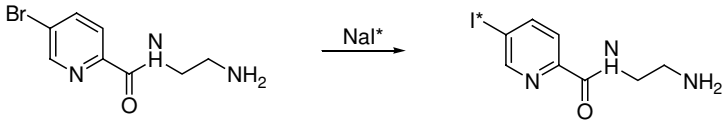
An overview with 18 references on labelling procedures and 30 references on methods of quality control was published by Wafelman *et al.* [1].

N.c.a. procedure

In order to achieve MIBG labelled with high specific activity, another procedure starts with the bromo-precursor *meta*-bromobenzylguanidine (MBBG) making direct use of Cu(I) for non-isotopic exchange [2]. Radioiodide and 25 μg $Na_2S_2O_5$ were evaporated to dryness in a 5-ml vial. About 250 μg MBBG in 50 μl and 0.5 μg CuCl in 5 μl acetic acid were added and heated for 10 min at 180°C. Separation of the halogen derivatives was done on a reverse-phase column (Merck Purospher RP18 – 5 μ) with 0.01 M NaH_2PO_4/CH_3CN (95/5) as eluent, capacity factors: k'(MBBG) = 5.5; k'(MIBG) = 9.5.

RO 43-0463

The compound is a reversible inhibitor of monoamine oxidase B and shows higher accumulation in the thalamus and cerebellum than in the frontal cortex and white matter. In temporal lobe epilepsy a high accumulation in the ipsilateral putamen was observed [3].



Procedure

Two mg of the bromo-precursor were mixed with about 1 GBq [^{123}I]NaI in 1 ml of 0.1 M NaOH, 0.5 mg copper sulphate, 9.8 mg phosphoric acid, 5 mg ascorbic acid and heated to 165°C for 1 h.

The product was purified over HPLC using the following conditions:

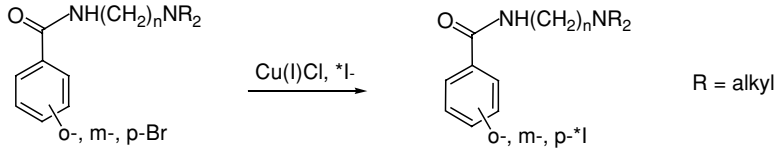
Column:	Lichrosorb RP18
Solvent:	0.36 M H_3PO_4, 0.01 M$(NH_4)_2HPO_4$ (in water) and 15% ethanol

The collected product fraction was neutralised with an equal volume of 0.45 M NaOH.

Retention k' (capacity factors): iodide = 0.4; bromo-precursor = 1.6; RO 43-0463 = 5.6.

Benzamides

The iodinated benzamides (alkyl- or piperidinyl-substituted) have a potential to visualise melanomas [4,5] (see also iodobenzamide (IBZM) and epidepride, which are differently substituted benzamides for brain receptor imaging; Sections 5.2.1 and 5.2.2) (Scheme 5.1).



Scheme 5.1: Non-isotopic exchange on ring-substituted bromo-benzamides is performed by Cu(I) assistance [6]

Procedure

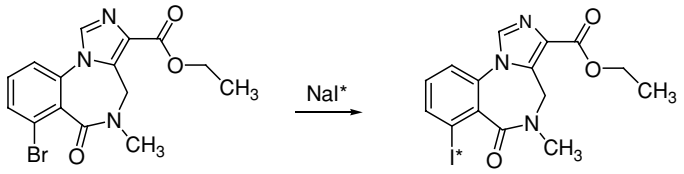
Radioiodide and 20 μg $Na_2S_2O_5$ were evaporated to dryness in a 5 ml conical vial. Bromo-precursor (250 μl) and 0.5 μg CuCl in 50 and 5 μl acetic acid, respectively, were added and heated for 20 min at 180°C. HPLC separation was achieved on a Kromasil RP18 (5 μ) column with acetonitrile/H_2O/Et_2N (30/70/0.2) as eluent.

For example, $n = 2$ and R = C_2H_5: k' (bromo-precursor) = 30; k' (product) = 41.

Iomazenil

Iomazenil is used to image the benzodiazepine-binding site of the gamma-aminobutryic acid (GABA) A receptors. The compound has been applied in many situations, e.g. imaging of viable tissue in the areas of reduced perfusion or neuronal density in Alzheimer's disease, panic disorder, schizophrenia, etc.

The method was used to prepare iomazenil labelled with ^{125}I (for autoradiography) and ^{123}I (for in vivo application) [7]. High yields were obtained only if fresh [^{123}I]NaI solutions were used. Hydrolysis of the ester gives the labelled free acid as a by-product.



Stability tests of the finished product gave better results with physiological glucose solution compared to physiological saline.

Procedure

Up to 11 GBq [^{123}I]NaI (40 MBq in the case of ^{125}I) in 0.1 N NaOH were evaporated to dryness. In parallel 1.5 mg of the bromo-precursor were dissolved in 0.1 ml glacial acetic acid, added to the [^{123}I]NaI and heated for 1 h at 155°C. Then the mixture was cooled, dissolved in 5 ml water and purified by HPLC. The collected product fraction was evaporated to dryness, dissolved in 5% glucose solution (isotonic) and passed over a silver wool column to eliminate the free iodide formed during evaporation.

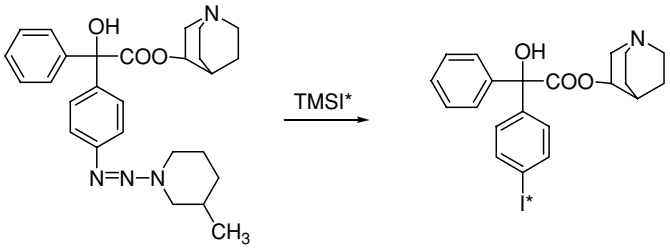
HPLC conditions

Column:	RP18 (8 × 250) Knauer Lichrosorb (10 μm)
Solvent:	methanol/water 45/55
Capacity factors (k'):	0.0 (iodide), 1.63 (free acid), 2.75 (bromo-precursor), 4.00 (iomazenil)

5.1.2 Other Methods

IQNB

The compound 3-iodoquinuclidinyl benzilate (IQNB) was first presented as a heart agent and later for brain imaging; it is a potent ligand to visualise the density of muscarinic cholinergic receptors.



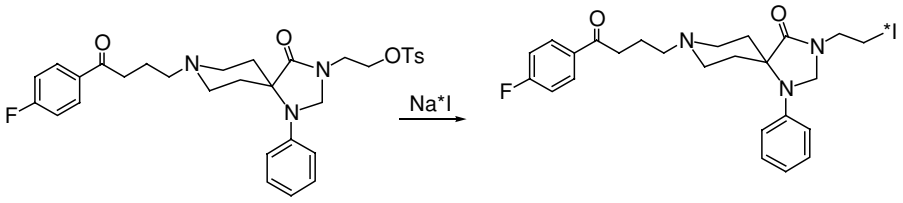
Procedure

This is to our knowledge the only example of application of an iododediazotation method (Wallach-type reaction) for routine production of a radiopharmaceutical [8,9]. Many types of iodide and solvents were used and the best RCY (20%) was reported with trimethylsilyl iodide (TMSI) in acetonitrile: 10 mM triazene precursor, 40 mM TMSI in 5 μl NaOH and 10 mM methanesulphonic acid were mixed to a final pH 6.0 to 6.5. The reaction was run for 1 h at 75°C.

Iodoethylspiperone

This compound has been designed to visualise the dopamine D_2 receptor [10].

Due to the low stability of the iodine–aliphatic carbon bond compared to an iodine–aromatic carbon bond, however, such compounds are not used in nuclear medicine.



Procedure

In the first step, the tosylethyl precursor was prepared in a one-pot synthesis from ethylene glycol di-*p*-toluene sulfonate and the potassium salt of spiperone.

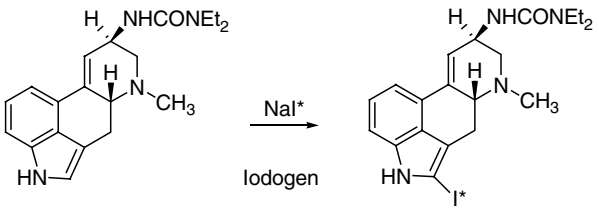
In the second step, radiolabelling was performed via substitution of the tosyl group by a radioactive iodine by refluxing the precursor in acetone for 2 h in the presence of sodium iodide. The labelled iodoethylspiperone was then extracted with ethyl acetate. RCY was 80% [10].

5.2 Examples of Electrophilic Labelling

5.2.1 Direct Iodination

Iodo-lisuride

Iodo-lisuride is used to image dopamine D_2 receptors. Main applications are Parkinson's disease and psychiatric disorders.

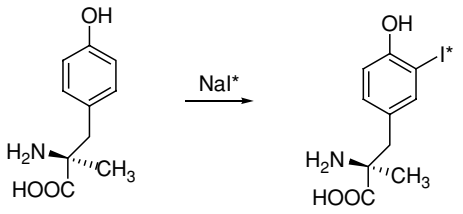


Procedure

Iodogen (1 μmol) was deposited on the walls of a conical vial by evaporating a dichloromethane solution under a stream of nitrogen. Four μmol lisuride in 200 μl of acetic acid (1 M) and [^{123}I]NaI were added. After 1 h at room temperature the labelled iodo-lisuride was purified. The solution was poured on a C18 cartridge and the polar products were washed off with 5 ml water. The lipophilic products were eluted with 5 ml chloroform and absorbed on a silica cartridge. The labelled iodo-lisuride was then eluted selectively with 3 ml chloroform–methanol (95/5) while the cold precursor remained on the column [11].

3-Iodo-α-methyl-L-tyrosine

3-Iodo-α-methyl-L-tyrosine (IMT) is used for tumour diagnosis, specifically glioma or more generally brain tumour differentiation. A review on its radiosynthesis, properties and clinical application was recently given [12].



Procedure using $NaIO_3$ as oxidation agent (carrier-added product) [13,14]

L-α-Methyltyrosine in HCl was mixed with cold NaI and [^{123}I]NaI. After addition of $NaIO_3$ the reaction mixture was heated to 50°C and the brown colour disappeared. The colourless solution was checked with HPLC (Nucleosil C18, $H_2O/EtOH/CH_3COOH = 90/9/1$). If the purity is less than 97%, the reaction was allowed to proceed for another 30 min, or $Na_2S_2O_5$ was added and the reaction mixture buffered with 0.6 M phosphate buffer. RCY was >95%.

N.c.a. procedure with Iodogen [15]

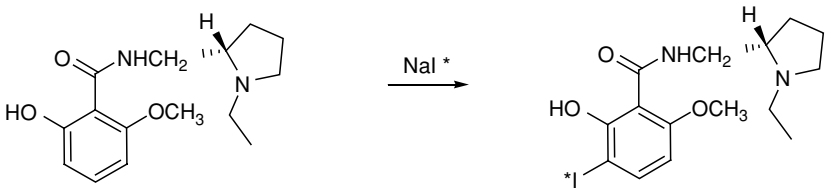
To 0.1 mg dried Iodogen 300 μl of a 0.0034 molar solution of L-α-methyltyrosine in borate buffer solution (pH 8) were added and the reaction started by addition of the radioiodide solution (typically 1.48 MBq ^{123}I). After 10 min at room temperature with occasional shaking, the reaction was stopped by removing the solution from the vessel with a syringe. The specific activity was 167 TBq/mmol and the RCY 80%.

Purification

HPLC analysis was done on LiChrosorb RP18 (250×4 mm) and H_2O/EtOH/AcOH 87.5/10/2.5 containing 2.5 g NH_4OAc/1000 ml eluent. Capacity factor (k') for IMT = 2.2.

IBZM

IBZM binds to the dopamine D_1/D_2 receptors and is used to investigate patients with Parkinson's disease or psychiatric disorders.



BZM IBZM

Procedure [16]

Peracetic acid (4.20 μmol) in a volume of 100 μl was added to a mixture of BZM (0.18 μmol in 50 μl EtOH), [^{125}I]- or [^{123}I]NaI (10 μl, 1 to 2 MBq), and buffer (ammonium acetate, pH 4) solution in a sealed vial (total volume was 0.45 ml). The reaction was allowed to proceed at room temperature for 2 min. The oxidation reaction was terminated by addition of an excess amount of the reducing agent sodium bisulphite (0.1 ml, 200 mg/ml) and neutralised with saturated sodium bicarbonate (0.5 to 1.0 ml). Solvent extraction was done using ethyl acetate (3×1 ml). The combined organic layers were dried by passing through an anhydrous sodium sulphate column.

N.c.a. synthesis gave 90% to 95% labelling yield and 93% to 95% radiochemical purity of IBZM. Specific activities were 75 and >185 TBq/mmol for [^{125}I]IBZM and [^{123}I]IBZM, respectively.

Purification [17]

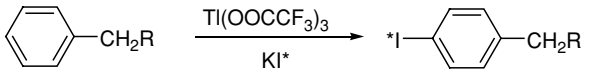
HPLC analysis and purification were performed on C18 column with a mixture of 90% MeOH, 10% 0.1 M NH_4OH as the eluent. Capacity factors: BZM ($k' = 2.7$) and IBZM ($k' = 4.4$).

5.2.2 Radioiodo-demetallation

Fatty Acid

Radiolabelled analogues are used for heart imaging as fatty acids are an important energy source for the heart. The iodinated fatty acid is taken up and trapped in the myocardium; depending on the substituent it may be metabolised.

After the first publications on fatty acids in the 1970s, the labelling of many different fatty acid analogues, such as the given example [18], was carried out.



R = $(CH_2)_{12}$-C$(CH_3)_2$-COOH

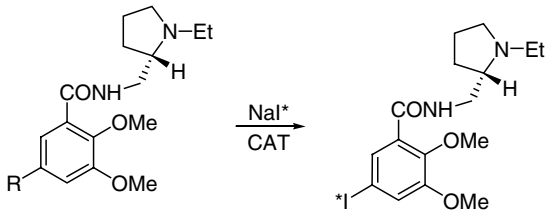
Procedure

The precursor (3.5 mg) was dissolved in 2 ml TFA and 10.8 mg thallium trifluoroacetate was added. The mixture was stirred at room temperature under red light. Then ^{123}I, ^{125}I or ^{131}I was mixed with 1 ml of a 0.01 M KI solution, added to the substrate solution and refluxed for 15 min.

The product was purified over an SiO_2 column using as eluents 5 ml petrol ether first, then 5 ml benzene and finally 10 ml chloroform. The product appeared in the chloroform phase.

Epidepride

Epidepride binds with a nanomolar affinity to the dopamine D_2 receptor and is used in patients with Parkinson's disease or psychiatric disorders. It may be potentially interesting for the diagnosis of brain tumours (macroprolactinoma).



R = Bu_3Sn or Me_3Sn

The reaction with Bu_3Sn leads to an RCY of only about 30% and a high amount of by-products. The Me_3Sn-precursor gives an RCY >95% and a purity >98% [19].

Procedure [20]

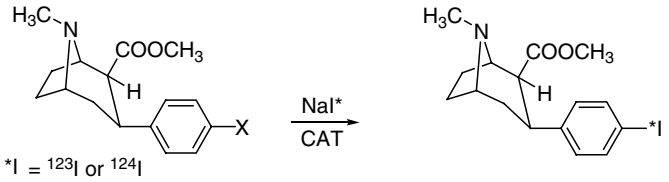
To a solution of about 1 GBq [^{123}I]NaI in 0.76 ml, 0.1 N NaOH was added 0.2 mM NaI (0.025 ml, 5.0 nmol) followed by 1.7 mM tributyltin-precursor (0.025 ml, 0.43 nmol) made by dissolving 50 mg of the precursor in 50 ml ethanol. Concentrated HCl (0.025 ml, 0.30 mmol) was added at 23°C. An aqueous 2 mM solution of CAT (0.025 ml, 50 nmol), freshly prepared by dissolving 13 mg in 25 ml sterile water, was added. After 2 min 0.1 M sodium metabisulphite (0.025 ml, 2500 nmol, 96 mg $Na_2S_2O_5$ in 5 ml water) was added. The reaction mixture was neutralised with 14 N ammonia and the product was extracted with ether.

Purification

Reversed-phase HPLC (Waters 8NVCN4HP, 0.8 × 10 cm column) with a 1:4 mixture of 0.10 M sodium phosphate (pH 6.3) and ethanol (96%) as mobile phase is used for purification. The flow rate was 2 ml/min and the retention time of the epidepride was between 17 and 20 min.

Beta-CIT

β-CIT binds to the dopamine transporter [21] and is mainly used for the diagnosis of Parkinson's disease [22–24].



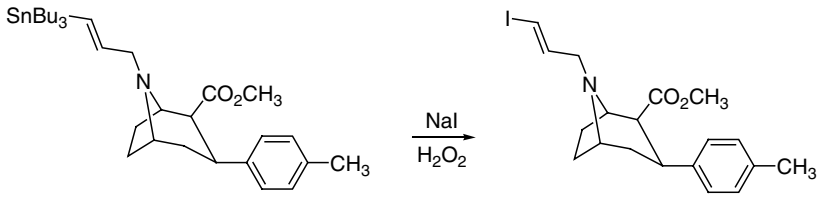
Different labelling procedures have been tested such as a copper-assisted halogen exchange with X = Br or an iodo-destannylation technique; the best yield was obtained with X = $Sn(CH_3)_3$.

Procedure (de-stannylation) [25]

Tin precursor (0.02 mg) was dissolved in 0.025 ml ethanol, then HCl, Na*I in 0.01 N NaOH and 0.015 mg CAT were added and stirred for 3 min at room temperature. The purification was performed using HPLC with a Kromasil 5-C18 (4.6 × 250 mm) column and an acetonitrile/water/diethylamine 60/40/0.2 mixture as eluent.

PE2I

(*E*)-*N*-(3-iodoprop-2-enyl)-2β-carbomethoxy-3β-(4′-methylphenyl)nortropane (PE2I) binds to the dopamine transporter [26] and is mainly used for the diagnosis of Parkinson's disease.



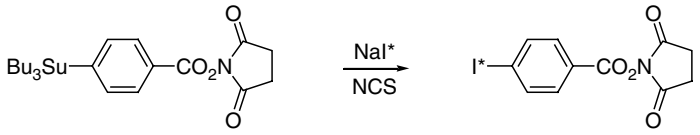
Procedure

Both [^{125}I]- and [^{123}I]PE2I were prepared by iodo-destannylation of the tributyltin-precursor. To a vial containing 50 μg of the stannyl-precursor, 50 μl EtOH, 50 μl HCl (0.1 N), 37 MBq [^{125}I]NaI (in 10 μl NaOH 0.1 N, specific activity 75 TBq/mmol) or 74 MBq [^{123}I]NaI (in 100 μl NaOH 0.1 N, specific activity >185 TBq/mmol) and 50 μl of 3% w/v hydrogen peroxide were added. The reaction was allowed to stand at room temperature for 15 to 30 min, quenched with 100 μl $Na_2S_2O_5$ (300 mg/ml), basified with saturated $NaHCO_3$ and extracted with ethyl acetate (3 × 1 ml). The combined ethyl acetate extracts were evaporated under a nitrogen stream, and the residue was dissolved in 200 μl of the HPLC mobile phase.

The radioiodinated compound was purified by HPLC using a reverse-phase column C18 and a mixture of $MeOH/H_2O/Et_3N$ 75/25/0.2 as a mobile phase (flow rate = 1 ml/min). The fraction eluted at the retention time of PE2I was collected and passed through a SEP-PAC C18 column. The radioiodinated product was then eluted with 2 × 1 ml EtOH and evaporated under a stream of nitrogen. The stannyl-precursor of PE2I provided [^{125}I]PE2I or [^{123}I]PE2I with a labelling yield greater than 50%. After purification by HPLC, radioiodinated compounds were obtained with high radiochemical purity of >95%. These n.c.a. compounds displayed high specific activities of 75 and >185 TBq/mmol for [^{125}I]PE2I and [^{123}I]PE2I, respectively.

Succinimide-activated Para-iodobenzoic Acid

This product is not used as a radiopharmceutical, but as a labelling agent. It forms amide bonds with free amino groups, like the terminal amine or lysine in peptides and proteins [27].

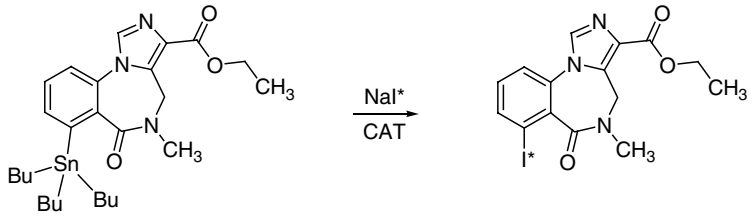


Procedure

Into a reaction vial fitted with a septum, 50 μl of a 0.25 mg/ml solution of the precursor in 1% HOAc/MeOH, 10 μl of a 1.0 mg/ml solution of NCS in MeOH and 10 μl of phosphate buffered saline were placed. [^{125}I]- or [^{131}I]NaI (up to 200 MBq, volume not to exceed 10 μl) was added. After 5 min at room temperature the reaction was quenched by addition of 10 μl of a 0.72 mg/ml aqueous solution of $NaHSO_3$. The methanol was evaporated by passing a gentle stream of nitrogen through the reaction vial.

Iomazenil

For imaging application cf. Section 5.1.1 (iomazenil).



Compared to other compounds the destannylation method to label iomazenil needs harsher conditions. This method gives 50% to 70% RCY and usually small amounts of impurities if Iodogen or CAT are used as oxidising agents. H_2O_2 and peracetic acid proved less useful. In addition to inactive benzodiazepine volatile iodobutane is formed as a by-product. At elevated temperature the yield seems to increase; however, the data are not unequivocal [28].

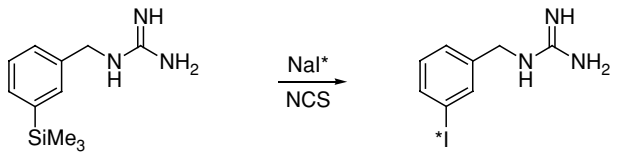
Procedure [29]

Tin-precursor (1.8 mg) and 1.4 mg Iodogen™ were mixed with 2 ml ethanol, placed for 3 min in a sonicator and filtered through a 0.22 μm polytetrafluoroethylene (PTFE) filter to remove undissolved solids. A 2 ml solution of 1:1:1 EtOH/2 M NH_4Cl/0.1 M H_3PO_4 was added to the reaction solution (pH 3) containing a positive starch KI test paper. Sodium iodide (in the case of ^{125}I: 0.02 ml, 50 MBq in 0.01 M NaOH, or in the case of ^{123}I: 4 ml, 6 GBq adjusted to pH 3 with H_3PO_4) was added and the solution allowed to

stand at ambient temperature for 2 h. Then 1 ml 0.5 M $NaHSO_3$ was added, followed by 10 ml water. The obtained solution was filtered through a SEP-PAC C18 cartridge (which was pretreated with sequential wash of 10 ml portions of water, ethanol, tetrahydrofurane, ethanol, water), which was then washed with 10 ml water followed by 1:1:1 EtOH/2 M NH_4Cl/0.1 M H_3PO_4.

MIBG

For imaging application see Section 5.1.1.



Procedure

[^{123}I]MIBG was pepared by electrophilic substitution under oxidative conditions from 3-trimethylsilylbenzylguanidine, a precursor found to be very stable [30]. N.c.a. radioiododesilylation was performed at room temperature using NCS as oxidant inTFA. RCYs of 90% were routinely obtained within 5 min after RP-HPLC purification [31].

5.3 References

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Chapter 6

EXPERT SYSTEM

6.1 General Considerations

The aim of the expert system is to propose a method that allows to choose objectively the most appropriate radioiodination method for the actual molecules of interest and for the new molecules in order to save time and money.

Depending on the molecule various conditions must be considered.

- The site of introduction of the radioiodine atom is already known, as well as the physico-chemical properties of the compound involved.
- The site of introduction of the radioiodine atom must still be evaluated taking the following points into account:

(a) the chemical accessibility of different sites (situated on an aryl or a heterocyclic group with aromatic properties),
(b) the increase of lipophilicity, possibly depending on the site of substitution,
(c) the decrease of affinity for the biological specific binding site due to the presence of a big iodine atom in the vicinity of a group important for the biochemical interaction (the pharmacophore),
(d) in cases where deactivation does not allow direct electrophilic substitution or in case of instability under the reaction conditions necessary for nucleophilic exchange, radioiodo-demetallation becomes the method of choice,
(e) if Cu(I)-assisted nucleophilic exchange can be used, it is a roundabout route to the demetallation method, as most often a brominated precursor is already available,
(f) the radioiodo-destannylation is the most widely applicable method.

A guideline is presented in this chapter describing an analytical approach to obtain the most suitable method for radioiodination. As labelling in benzene moieties is most attractive for stability reasons, the first point to be considered is the possibility of electrophilic or nucleophilic substitution. Therefore some theoretical aspects of aromatic substitution are described below.

6.2 Activating Effects of Aromatic Substituents

The aim of this chapter is to briefly summarise the basic theory and rules for nucleophilic and electrophilic substitutions in order to facilitate the guideline evaluation of the compound of interest.

6.2.1 Aromatic Nucleophilic Substitution (SN_{Ar})

Effect of Substituent

Aromatic nucleophilic substitutions are accelerated by electron-withdrawing groups, especially in the ortho and para positions to the leaving group, and are hindered by electron-donating groups. Table 6.1 contains a list of groups arranged approximately in the order of activating or deactivating ability. Benzene rings that lack activating substituents are generally not useful substrates for the SN_{Ar} mechanism. In this case one of the methods making use of a catalyst, described earlier, can possibly be applied. Activating groups, by withdrawing electron density, are able to stabilise the intermediates. The favoured position for nucleophilic attack is the one that leads to the more stable carbanion intermediate.

Effect of Leaving Group

An approximate order of leaving group ability is $F > NO_2 > Cl, Br, I$. However, it depends strongly on the structure of the nucleophile. The halogens, apart from F, have reactivities fairly close together. The order is usually $Cl > Br > I$, but not always. The leaving group order can be quite different. The first step of the SN_{Ar} mechanism, i.e. attack of nucleophile, is usually rate determining. In this case F and NO_2 are good leaving groups. But F is the poorest leaving group of the halogens when the second step of the SN_{Ar} mechanism is rate determining.

Table 6.1: Groups listed in approximate descending order of activating ability in SN_{Ar}

Activates halide exchange at room temperature	N_2^+	Activates reaction with strong nucleophiles at 40–60°C	COOR
			COOH
			SO_3^-
Activates reaction with strong nucleophiles at room temperature	$R_1R_2N^+=R_3$ (heterocyclic)		Br
			Cl
Activates reaction with strong nucleophiles at 80–100°C	NO		I
	NO_2		COO^-
	$R_1R_2N=R_3$ (heterocyclic)		H
			F
Activates reaction with strong nucleophiles at room temperature	SO_2Me		CMe_3
	NMe_3^+		Me
	CF_3		OMe
	CN		NMe_2
	CHO		OH
			NH_2

Effect of Attacking Nucleophile

It is not possible to construct an invariant nucleophilicity order, because different substrates and different conditions lead to different orders of nucleophilicity. Important parameters can be hydration, dissociation and basicity. An overall approximate order is $OH^- > NH_3 > I^- > Br^- > Cl^- > H_2O > ROH$.

6.2.2 Aromatic Electrophilic Substitution (SE_{Ar})

An important effect of substituent groups on aromatic substitution is their inductive property. An electron-attracting group will exert an electrostatic force, such as to destabilise a positively charged intermediate, while an electron-donating group will have the opposite effect.

Selectivity

The selectivity relationship is based on the principle that reactivity of a species varies inversely with selectivity. Table 6.2 shows how electrophiles can be arranged in the order of selectivity as measured by two indices: (i) their selectivity in attacking toluene rather than benzene and (ii) their selectivity between the meta and para position in toluene. An electrophile more selective in one respect is also more selective in the other.

Table 6.2: Relative rates and product distributions of some electrophilic substitution reactions on toluene and benzene

	Relative rate	Product distribution (%)	
Reaction	$k_{toluene}/k_{benzene}$	Meta	Para
Bromination	605.0	0.3	66.8
Chlorination	350.0	0.5	39.7
Benzoylation	110.0	1.5	89.3
Nitration	23.0	2.8	33.9
Mercuration	7.9	9.5	69.5
Isopropylation	1.8	25.9	46.2

Pattern of Orientation in SE_{Ar}

The orientation data in Table 6.3 are expressed as the percentage of ortho, meta and para isomers formed, and the rate data are the overall rates relative to benzene.

With respect to the activation and orientation in an electrophilic reaction, substituents already present on the aromatic ring fall into one of the following three categories:

1. substituents which activate all the ring positions relative to benzene, but are more activating for ortho and para positions (ortho and para orientation with activation), e.g. $-OH$, $-OCH_3$, $-NR_2$, $-NHCOCH_3$, ...
2. substituents which deactivate all the ring positions, but deactivate the ortho and para positions less (ortho and para orientation with deactivation), e.g. Cl, Br, CH_2Cl, ...

Table 6.3: Orientation and relative reactivity in the nitration of some mono-substituted benzene derivatives

Substituent	Orientation (%)			
R	Ortho	Meta	Para	Relative reactivity
$-CH_3$	56.5	3.5	40.0	24.0
$-C(CH_3)_3$	12.0	8.5	79.5	15.7
$-CH_2Cl$	32.0	15.5	52.5	0.302
$-Cl$	29.6	0.9	68.9	0.033
$-Br$	36.5	1.2	62.4	0.030
$-NO_2$	6.4	93.2	0.3	$\sim 10^{-7}$
$-CO_2C_2H_5$	28.3	68.4	3.3	0.0003
$-CF_3$		100.0		Low
$-N^+(CH_3)_3$		89.0	11.0	Low

Table 6.4: Orientation and reactivity effects of ring substituents in SE_{Ar}

Ortho and para orientation with activation	Ortho and para orientation with deactivation	Meta orientation with deactivation
$-O^-$	$-CH_2Cl$	$-NO_2$
–OH	$-F$[a]	$-N^+H_3$
–OR	–Cl	$-N^+R_3$
$-OC_6H_5$	–Br	$-P^+R_3$
$-NH_2$	–I	$-S^+R_3$
$-NR_2$	$-CH=CHNO_2$	$-I^+C_6H_5$
$-NHCOCH_3$		$-CF_3$
–alkyl (e.g. CH_3)		$-CCl_3$
–aryl (e.g. C_6H_5)		$-SO_3H$
		$-SO_2H$
		$-CO_2H$
		$-CO_2R$
		$-CONH_2$
		–CHO
		–COR
		$-C\equiv N$

[a]Slight activation in para position.

3. substituents which deactivate all the positions, but deactivate the ortho and para positions more than the meta position (meta orientation with deactivation), e.g. $-NO_2$, $-COOC_2H_5$, $-N^+(CH_3)_3$, $-CF_3$, ...

Table 6.4 shows a comprehensive list of substituents, which fall into one of the three categories.

Chapter 7

GUIDELINES FOR RADIOIODINATION

7.1 Introduction

This chapter is intended to help the radiochemist who is confronted with the radioiodination of a new molecule. In this situation two main questions arise:

- Where to introduce the radioiodine?
  - on an aromatic ring
  - on an aliphatic chain
- How to perform the radiolabelling?

The following examples, based on some well-known radiopharmaceuticals, illustrate how to proceed to get answers to these questions and to choose an efficient labelling method for the given molecule.

7.1.1 Reactivity of the Aromatic Ring

Each substituted group R must be checked for possible activation or deactivation towards nucleophilic or electrophilic substitution, respectively, using the theory and tables described in Chapter 6. Groups causing steric hindrance for the approach of the active iodine entity must be taken into consideration. Lipophilicity (solubility) can be a limiting factor for the amount of substrate molecule needed to obtain the maximum labelling yield. Both nucleophilic and electrophilic substitution can require rather strong acidic or basic conditions. Nucleophilic exchange requires higher temperatures most of the time. A check of the stability of the substrate molecule under the conditions supposed to be applied must be carried out.

When using a catalyst, e.g. Cu(I) or Cu(II), complexation or precipitation can occur. The oxidising agent needed for electrophilic substitution can be soft (Iodogen) or strong (DCT).

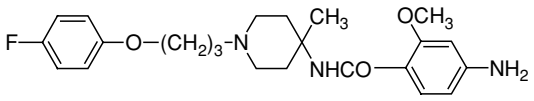
The aim of the guideline is to check those parameters to facilitate a decision about the method of choice.

The way to use the guideline checklist is illustrated by two examples of radiopharmaceuticals from daily practice.

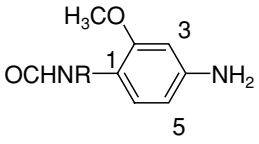
7.2 Examples

7.2.1 Compound: R91150

This compound is a $5HT_{2A}$ receptor ligand.
Molecular Structure:



Define Aromatic Ring of Choice:



Preference: 3-I or 5-I
Minimum increase of lipophilicity

1. Direct electrophilic substitution (radioiodo-deprotonation)

Effect of Substituents

	Define groups (care of protonated forms)	Activation		Deactivation	
		Strong	Moderate	Strong	Moderate
1. R_1 =	R–NHCO				6
2. R_2 =	OCH_3	3 & 5			
3. R_3 =	H				
4. R_4 =	NH_2	3 & 5			
5. R_5 =	H				
6. R_6 =	H				
Resulting activated site:		3 & 5			

2. Nucleophilic non-isotopic exchange

Effect of Substituents

	Define groups (care of protonated forms)	Activation		Deactivation	
		Strong	Moderate	Strong	Moderate
1. R_1 =	R–NHCO		6		
2. R_2 =	OCH_3				3 & 5
3. R_3 =	H				
4. R_4 =	NH_2			3 & 5	
5. R_5 =	H				
6. R_6 =	H				

Resulting activated site: none

3. Consideration for both electrophilic or nucleophilic substitution

Steric Hindrance

	Group	Hindrance of position		Group	Hindrance of position
R_1 =	R–NHCO	6	R_4 =	NH_2	3 & 5
R_2 =	OCH_3	3	R_5 =	H	
R_3 =	H		R_6 =	H	

Resulting site for substitution: 5 (considering activation and steric hindrance)
Substitution on other sites such as double bonds or arylic analogues: none

4. Physico-chemical properties

Character of the Compound

Is the compound:	Yes/no		Yes/no		Yes/no
an acid	—	a base	Yes	neutral	—

Solubility of the Compound in (qualify with good/poor/no if possible)

H_2O (neutral)	Poor	Acetic acid	Good
H_2O (acidic)	Good	Tetrahydrofurane	?
H_2O (basic)	No	Acetonitrile	
Methanol	Good	[a]	
Ethanol	Good	[a]	

[a]Other solvents, specify.

Stability of the Compound in Solution at Room Temperature

		Yes/no			Yes/no
Acid	Strong	Yes	Base	Strong	Yes
	Medium	Yes		Medium	Yes
Oxidising conditions		Yes	Reducing conditions		Yes

Stability of the Compound in Solution at High Temperature (. . . °C)

		Yes/no			Yes/no
Acid	Strong	Yes	Base	Strong	Yes
	Medium	Yes		Medium	Yes
Oxidising conditions		Yes	Reducing conditions		Yes

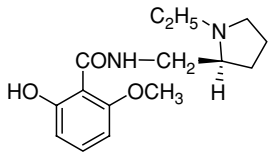
Stability while heated without solvent (melt): up to . . . °C

5. Conclusion

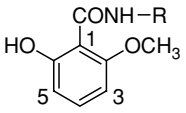
Electrophilic substitution possible: . . . . . Yes . . . . . (Yes/no)
Nucleophilic substitution possible: . . . . . No . . . . . (Yes/no)
Choice of oxidising agent: . . . . . H_2O_2/CH_3COOH . . . . .
Choice of reducing agent: . . . . . — . . . . .
Choice of solvent (or melt): . . . . . CH_3COOH . . . . .
Choice of catalyst: . . . . . — . . . . .

7.2.2 Compound: IBZM

This compound is a dopamine D_2 receptor ligand.
Molecular Structure:



Define Aromatic Ring of Choice:



1. Direct electrophilic substitution (radioiodo-deprotonation)

Effect of Substituents

	Define groups (care of protonated forms)	Activation		Deactivation	
		Strong	Moderate	Strong	Moderate
1. R_1 =	R–NHCO				4
2. R_2 =	OCH_3		3 & 5		
3. R_3 =	H				
4. R_4 =	H				
5. R_5 =	H				
6. R_6 =	OH	5 & 3			
Resulting activated site:		3 & 5			

2. Nucleophilic non-isotopic exchange

Effect of Substituents

	Define groups (care of protonated forms)	Activation		Deactivation	
		Strong	Moderate	Strong	Moderate
1. R_1 =	R–NHCO		4		
2. R_2 =	OCH_3				5 & 3
3. R_3 =	H				
4. R_4 =	H				
5. R_5 =	H				
6. R_6 =	OH			5 & 3	
Resulting activated site:		4			

3. Consideration for both electrophilic or nucleophilic substitution

Steric Hindrance

	Group	Hindrance of position		Group	Hindrance of position
R_1 =	R–NHCO		R_4 =	H	
R_2 =	OCH_3	3	R_5 =	H	
R_3 =	H		R_6 =	OH	

Resulting site for substitution: 5 (considering activation and steric hindrance)
Substitution on other sites such as double bonds or arylic analogues: none

4. Physico-chemical properties

Character of the Compound

Is the compound:	Yes/no		Yes/no		Yes/no
an acid	No	a base	Yes	neutral	—

Solubility of the Compound in (yes or no; qualify with good/poor if possible)

H_2O (neutral)	No	Acetic acid	Yes
H_2O (acidic)	Yes	Tetrahydrofurane	Yes
H_2O (basic)	No	Acetonitrile	Yes
Methanol	Yes	[a]	
Ethanol	Yes	[a]	

[a]Other solvents, specify.

Stability of the Compound in Solution at Room Temperature

		Yes/no			Yes/no/?
Acid	Strong	No	Base	Strong	No
	Medium	Yes		Medium	Yes
Oxidising conditions		Yes	Reducing conditions		?

Stability of the Compound in Solution at High Temperature: 100°C

		Yes/no			Yes/no/?
Acid	Strong	No	Base	Strong	No
	Medium	No		Medium	Yes (?)
Oxidising conditions			Reducing conditions		?

Stability while heated without solvent (melt): up to . . . °C

5. Conclusion

Electrophilic substitution possible: . . . . . Yes . . . . . (yes/no)
Nucleophilic substitution possible: . . . . . No . . . . . (yes/no)
Choice of oxidising agent: . . . . . Chloramine-T (CAT), peracetic acid
Choice of reducing agent: . . . . . .
Choice of solvent (or melt): . . . . . Ethanol/water (buffer) . . . . . .
Choice of catalyst: . . . . . .

7.3 Blank Checklist

Chemical Family
Molecular Structure
Define Group of Choice

1. Direct electrophilic substitution (radioiodo-deprotonation)

Effect of Substituents

	Define groups (care of protonated forms)	Activation		Deactivation	
		Strong	Moderate	Strong	Moderate
1. R_1 =					
2. R_2 =					
3. R_3 =					
4. R_4 =					
5. R_5 =					
6. R_6 =					

Resulting activated site:

2. Radioiodo-demetallation

Effect of Substituents

	Leaving group (care of protonated forms)	Other groups	Group to protect
1. R_1 =			
2. R_2 =			
3. R_3 =			
4. R_4 =			
5. R_5 =			
6. R_6 =			

Resulting labelling site:

3. Direct nucleophilic non-isotopic exchange

Effect of Substituents

	Define groups (care of protonated forms)	Activation		Deactivation	
		Strong	Moderate	Strong	Moderate
1. R_1 =					
2. R_2 =					
3. R_3 =					
4. R_4 =					
5. R_5 =					
6. R_6 =					

Resulting activated site:

4. Consideration for both electrophilic or nucleophilic substitution

Steric Hindrance

Group	Hindrance of position	Group	Hindrance of position
R_1 =		R_4 =	
R_2 =		R_5 =	
R_3 =		R_6 =	

Resulting site for substitution: (considering activation and steric hindrance)
Substitution on other sites such as double bonds or arylic analogues:

5. Physico-chemical properties

Character of the compound

Is the compound:	Yes/no	Yes/no	Yes/no
an acid		a base	neutral

Solubility of the Compound in (qualify with good/poor/no if possible)

H_2O (neutral)	Acetic acid
H_2O (acidic)	Tetrahydrofurane
H_2O (basic)	Acetonitrile
Methanol	[a]
Ethanol	[a]

[a]Other solvents, specify.

Stability of the Compound in Solution at Room Temperature

	Yes/no		Yes/no
Acid	Strong Medium	Base	Strong Medium
Oxidising conditions		Reducing conditions	

Stability of the Compound in Solution at High Temperature: . . . °C

	Yes/no		Yes/no
Acid	Strong Medium	Base	Strong Medium
Oxidising conditions		Reducing conditions	

Stability while heated without solvent (melt): up to . . . °C

6. Conclusion

Direct electrophilic substitution possible: . . . . . (yes/no)
Demetallation possible . . . . . (yes/no)
Nucleophilic substitution possible: . . . . . (yes/no)
Choice of oxidising agent: . . . . .
Choice of reducing agent: . . . . .
Choice of solvent (or melt): . . . . .
Choice of catalyst: . . . . .

7. Reference . . . . . .