Universitat Ramon Llull

DOCTORAL THESIS

Title: **Development of New Strategies for the Synthesis of Radiotracers Labeled with Short-Lived Isotopes: Application to ^{11}C and ^{13}N.**

Presented by: **Vanessa Gómez-Vallejo**

Centre: **Escola Tècnica Superior IQS**

Department: **Organic Chemistry**

Directed by: **Dr. Jordi Llop Roig – Dr. José Ignacio Borrell Bilbao**

C. Claravall, 1-3
08022 Barcelona
Tel. 936 022 200
Fax 936 022 249
E-mail: urlsc@sec.url.es
www.url.es

1. INTRODUCTION

1.1. PET: POSITRON EMISSION TOMOGRAPHY

1.1.1. Overview

Positron emission tomography (PET) is one of the most sensitive and specific technique for imaging molecular pathways *in vivo* and in a non-invasive way. It is based on the administration of compounds labeled with short-lived positron-emitting radionuclides to obtain three-dimensional images of functional processes in animals and/or humans. Typical isotopes used as radionuclides in PET are fluorine-18, carbon-11, nitrogen-13 and oxygen-15 (see Table 1.1 for physical properties). These radionuclides are incorporated into biologically active compounds that play a specific role in living organisms.[1]

Radio-nuclide	Half-life (min)	Decay mode / decay product	Max. Energy (MeV)	Most Probable Energy (MeV)	Max. Range (mm)
Fluorine-18	109.8	97% β^+ 3% EC* Oxygen-18	0.69	0.202	2.4 mm
Carbon-11	20.4	100 % β^+ Boron-11	0.96	0.326	4.1 mm
Nitrogen-13	9.98	100 % β^+ Carbon-13	1.19	0.432	5.4 mm
Oxygen-15	2.05	100 % β^+ Nitrogen-15	1.70	0.650	8.0 mm

Table 1.1: Physical characteristics of fluorine-18, carbon-11, nitrogen-13 and oxygen-15. *EC: Electronic capture. Obtained from: Welch, M.J; Redvanly, C.S; Handbook of radiopharmaceuticals: Radiochemistry and applications; *Wiley*, **2005**.

The spontaneous decay of a positron emitter produces a positron, which travels a certain distance (depending on its energy) to finally react with one electron of a surrounding atom. This process is called annihilation; as a result, two gamma photons are emitted (511 keV each, emitted at 180º to each other, Figure 1.1). The generation of these gamma rays is the basis of positron emission tomography. When a tracer containing a positron emitter is administered to an organism, the high-energy gamma rays produced, which have a high penetration power, escape from the body and are detected by an

external ring of detectors as a coincident event (Figure 1.2). The detection of hundreds of thousands of such coincident events permits the reconstruction of a 3D image that contains information about the distribution of the radiolabeled tracer within the organism.

The most commonly used PET detection system consists of an array of scintillation crystals. These crystals are usually made of high density materials to enhance interaction with gamma rays and are optically coupled to several photomultipliers. The intrinsic resolution is determined by the number of detectors on the array and the size of the individual detectors. Hence, resolution can be enhanced by reducing the dimension of the detector crystals.

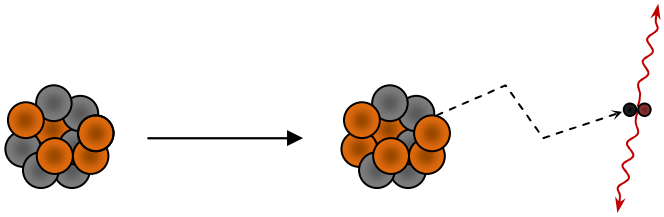

Figure 1.1: Positron annihilation process: spontaneous radioactive decay of positron emitters (carbon-11 in the example) produces the emission of a positron, whose energy is lost until reaction with one electron of a surrounding atom leads to the annihilation process, with consequent formation of two 511 keV gamma rays, 180º to each other. In the example, carbon-11 is converted into boron-11.

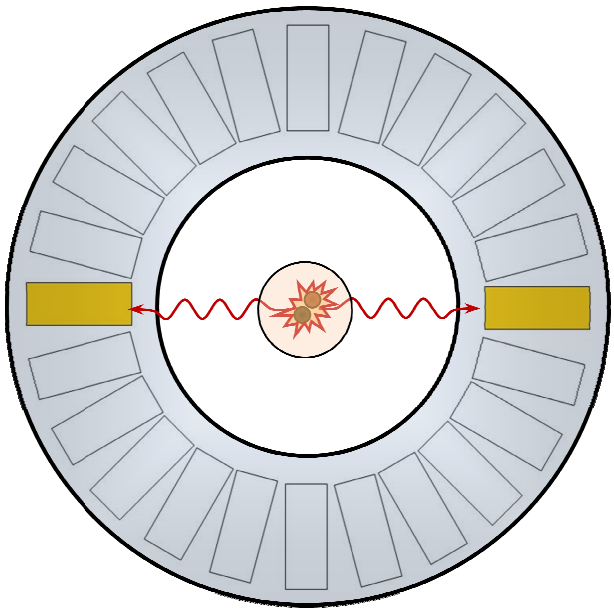

Figure 1.2: Positron annihilation produces the formation of two 511 keV gamma rays, 180º to each other. These two gamma rays escape from the body due to their high penetration power and are detected by an external ring of detectors (sited into the PET camera) as coincident events.

Several physical restrictions (as positron range, Compton scattering, angulation, accidental coincidences and detector dead-time)[2] impose a spatial resolution of 2-3 mm in clinical (human dedicated) scanners and 0.5-1 mm in preclinical (small animal dedicated) scanners.[3] Spatial resolution of PET scanners is lower than that obtained with anatomical imaging techniques like magnetic resonance imaging (MRI) or computed tomography (CT), but information about biological and/or physiological processes can be obtained at a molecular level (see Figure 1.3).[4]

Data from PET cameras are obtained as sinograms that are reconstructed into tomographic images after correction for attenuation and detector efficiency by using different methods. From the reconstructed images, time-activity curves can be derived. In many PET studies, the objective is not just to visualize a specific activity but to obtain quantitative or semi-quantitative data. Two values are typically calculated: (i) %ID/g, percent injected dose per gram of tissue and (ii) SUV, standardized uptake value (Equation 1).

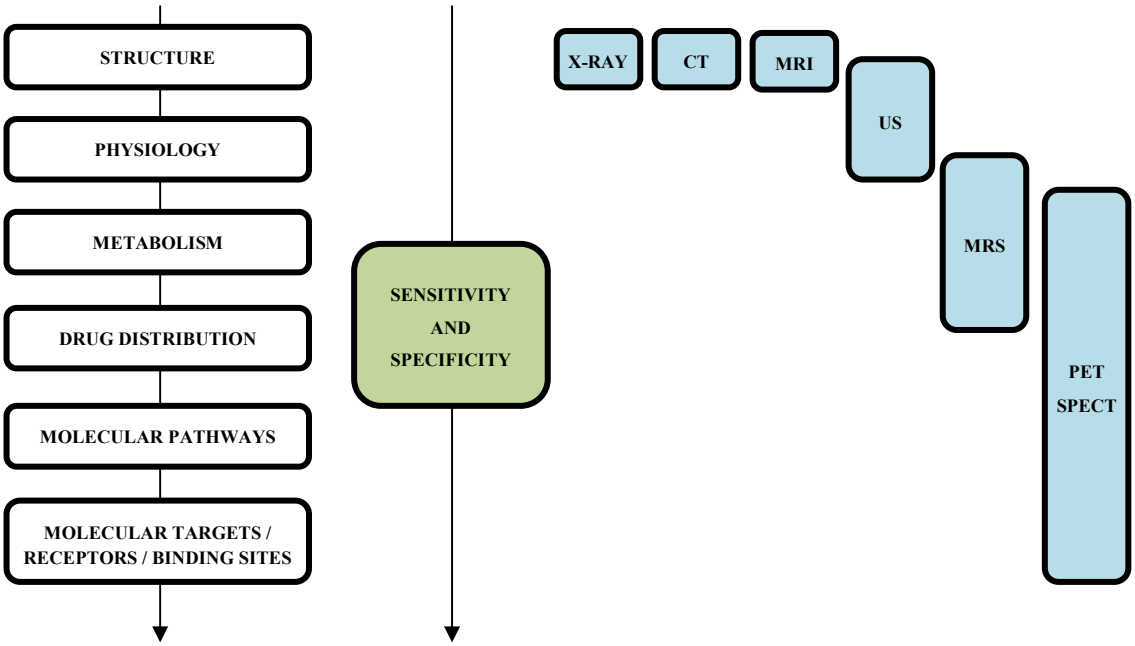

Figure 1.3: Spectrum of medical imaging. The sensitivity range covers from millimolar (top) to picomolar (bottom). X-ray, CT and MRI give accurate structural information with high resolution. Nuclear imaging techniques (PET and SPECT) have lower resolution but offer pharmacokinetic and pharmacodynamic information. CT, computed tomography; MRI, magnetic resonance imaging; US, ultrasound imaging; MRS, magnetic resonance spectroscopy; PET, positron emission tomography; SPECT, single photon emission computerized tomography. Adapted from: Price, P; PET as a potential tool for imaging molecular mechanisms of oncology in man; *Trends Mol. Med*; **2001**, 7(10): 442-446.

$$SUV = \frac{\text{Activity } (^{\mu Ci}/_{g})}{\text{injected dose } (^{\mu Ci}/_{g}) / _{\text{weight (Kg)}}}$$

Equation 1: Standardized uptake value (SUV).

PET was first used for oncologic studies in the 1970s, and nowadays it is a clinically established technology and suited for translational applications. The high sensitivity of the method allows the use of trace concentrations that are below the pharmacological effective dose (typical synthesis scales are in the order of 0.2 μmol). This fact has an impact on the regulatory approval process of PET radiotracers (extended to the safety and toxicology), and facilitates the availability of such radiotracers for clinical studies.[5]

Although PET technology can be applied to approach a large variety of physiological, biological and/or medical questions, both in the preclinical and in the clinical areas, the development of (new) labeled species is required. This development requires a multidisciplinary approach regarding: (i) target selection, (ii) organic synthesis, (iii) radiolabeling, (iv) *in vitro* and *in vivo* evaluation and (v) kinetic modeling of the data.

The first step in the development of new PET radiotracers is the identification of the target which generally consists of an enzyme, a transporter, a receptor or any other biomolecule that allows high affinity binding of the compound. Taking into account the limited resolution of PET (specially compared to other imaging techniques), the volume of the affected tissue should be compatible with the resolution of PET cameras. In some cases the high affinity of the radiotracer is a limiting factor and some modifications are needed to reduce this affinity; such is the case of displacement studies.

Once the target has been identified, rational design of the adequate radiotracer based on structural biology and high throughput screening of libraries of compounds is used to select the best ligand/s.

Moreover, and before performing studies with the labeled specie, there are some *in vitro* experiments that can be done using the non radioactive compound, such as metabolism, binding and affinity. Compounds with the best results during *in vitro* assays (concerning pharmacokinetics and target affinity) are selected for *in vivo* studies.

The first key factor to be considered when planning *in vivo* studies is the choice of the radionuclide, whose physical characteristics will define the applicability of the labeled specie. Thus, the energy of the positron has an effect on image resolution, while half-life of the radionuclide should be long enough to perform the radiolabeling, purification, formulation and quality control of the final product. Moreover, half-life of the radioisotope should match the biological half-life of the radiotracer, in order to allow a complete pharmacokinetic study while dose exposure is minimized.

Once the radiotracer is ready for use, some *in vitro* assays (e.g., determination of binding and stability in plasma) may be done before performing *in vivo* studies. Typically, first *in vivo* studies for a newly developed radiotracer consist of a biodistribution assay for the determination of its pharmacokinetic properties. If the resolution of the technique is not enough, *ex vivo* autoradiography studies can be performed to visualize the distribution of the radiotracer, although in this case longitudinal studies within the same subject (animal) are not possible.

1.1.2. PET isotopes: general aspects

Among all radionuclides, only a few of them have the adequate physical properties to become suitable candidates for the preparation of radiotracers. In the particular case of positron emitters, there are four radioisotopes which have been historically used: carbon-11, nitrogen-13, fluorine-18 and oxygen-15. The wide historical use of these isotopes is due to (i) they can be produced in relatively high yields in commercially available cyclotrons, (ii) they can be easily introduced in a biomolecule, (iii) their decay mode is close to 100% positron emission (Table 1.1) and (iv) their stable isotopes are present in all organic molecules; this last statement is not true in the case of fluorine, but in many cases the substitution of an hydroxyl group (or hydrogen atom) by a fluorine atom does not dramatically alter the biological behavior of the molecule; thus fluorine can be used to prepare analogs of molecules with specific biological roles.

Many positron-emitters can be generated in a cyclotron, where a specific target is irradiated with high energy protons (8-19 MeV) to produce a nuclear reaction with subsequent formation of the positron emitter. The number of reactions that take place in the target depend on many factors. Thus, the energy of the proton beam and the chemical composition of the target (including impurities) define the radioisotopes produced during beam. On the other hand, the chemical form of the final product can be

modified by changing either the chemical composition or the physical state of the target during irradiation. Other factors like the presence of radical scavengers and/or moderators in the target can modulate chemical reactions taking place within the target.

Among all PET isotopes, fluorine-18 is probably the most widely used, especially for clinical applications. Fluorine-18 forms strong covalent bonds with carbon atoms and can be incorporated into a large variety of organic molecules; moreover, substitution of a hydrogen atom by a fluorine atom causes little steric alterations in the molecule; in some particular cases, the biological properties of the radiotracer are even improved with respect to the original molecule (such is the case of [^{18}F]FDG, a radiotracer used in the clinical environment for diagnostic of some types of cancer). Fluorine-18 has a small positron range and its half-life is relatively long (109.8 min) allowing the preparation of complex molecules with acceptable radiochemical yields. The relatively long half-life permits the commercialization of radiotracers as diagnostic tools in the clinical environment.

Out of the above mentioned clinical environment, other positron emitters like carbon-11 and nitrogen-13 have a huge potential for the synthesis of radiotracers. The manufacturing process of radiotracers with these two positron emitters presents some difficulties because of their short half-life (20.4 and 9.98 min, respectively). Carbon-11 can be obtained in cyclotrons in different chemical forms, depending on the irradiated material and the environment during irradiation; however, two chemical forms are, by far, the most commonly used radioactive precursors for labeling molecules: [^{11}C]CO_2 (obtained by irradiating N_2/O_2 mixtures) and [^{11}C]CH_4 (obtained by irradiation of N_2/H_2 mixtures). Nitrogen-13 is obtained by irradiation of water solutions and is usually produced as [^{13}N]NH_4^+ after irradiation of diluted ethanol aqueous solutions; this radiochemical specie has direct application in the clinical environment as perfusion marker.

Oxygen-15 is the shortest-lived positron emitting isotope of oxygen. Its half-life is 2.05 minutes, and it is historically one of the first artificial radioisotopes produced with low energy deuterons on a cyclotron. It does not have many applications due to its short half-life and it is mainly used directly as produced (inhaled) or to synthesize [^{15}O]H_2O.

1.1.3. PET isotopes: production

Principles of cyclotron

A cyclotron (See Figure 1.4 for general schematic) is a particle accelerator in which an electric field is used to accelerate ions, such as H^- or D^-, and a magnetic field is applied to "guide" them. The electric field is generated by the application of an electric potential difference to two electrodes (called dees and counter-dees), which are connected to the alternating current source (radiofrequency generator). Negative ions are generated in the ion source (placed in the center of the cyclotron) by applying a high voltage to hydrogen (or deuterium) gas. Negative ions are extracted from the center of the cyclotron by applying an electrical field. When the dee is positively charged, the counter dee is negatively charged. Thus, the ion is accelerated towards the dee by the electric field. Once the ion enters the hole of the dee it experiences only the magnetic field. When the ion leaves the dee, the polarity on the dees is reversed, so that the ion is accelerated again to the counter dee. The same process is repeated in the other dee, but the orbit radius is higher as the speed of the ion is higher. This process continues and the ion is spiraling outward towards the border of the magnetic field. When the radius reaches a certain value, the negative ions hit a stripping foil (usually graphite) which removes the electrons. The charged ion, now positive, experiences the same force in the same direction but with opposite sense (Figure 1.5), leaves the magnetic field and continues to an external target position, where collision with the target material takes place and radioactive atoms are generated (see Figure 1.6 for physical target structure).

Before the collision with the target, the ion gets an energy which is equal to the sum of all individual accelerations in the gaps between the dees and the counter dees. The final kinetic energy is determined by the nature of the ion and the size of the magnet.

Negative particle cyclotrons have some advantages with respect to positive particle ones: (i) the ability to easily have a variable energy cyclotron, by moving the extraction foil to different radii, (ii) almost 100% extraction, due to the extraction through the stripping foil, and (iii) being able to extract multiple beams simultaneously, by inserting the extraction foils in a way that only intercept part of the beam, allowing the remainder to continue its acceleration to the next foil.[6]

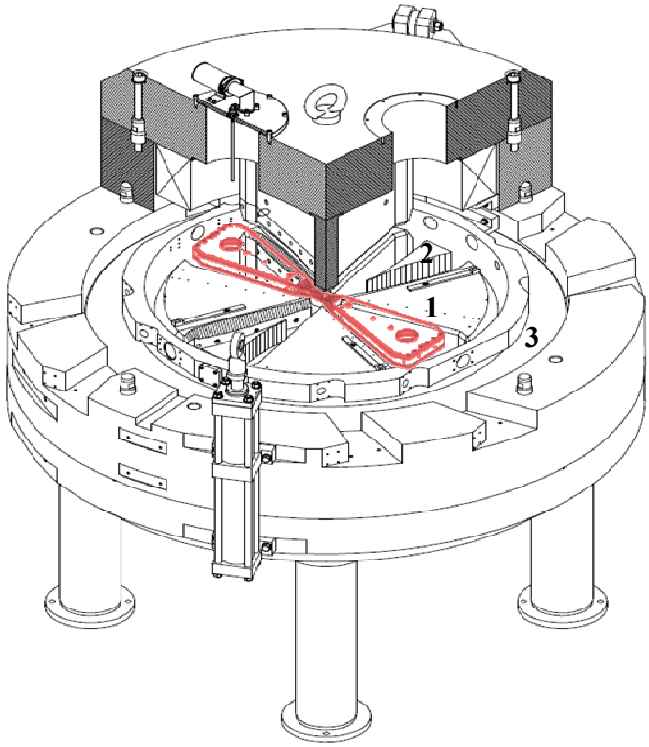

Figure 1.4: General view of the main body of an IBA Cyclone 18/9 cyclotron. Some parts (ion sources, strippers and targets) have been removed from the drawing. The electrical current passing through the coils (3) that surround the four wedge-shaped steel sectors (1) creates the magnetic field. Dees (highlighted in red) are placed between the steel sectors and counter dees (not shown in the drawing) are placed on the edges of the four steel sectors close to the dees. For dual particle cyclotrons (acceleration of H^- and D^-), small steel sectors called flaps (2) can be introduced or removed to modulate the magnetic field when particle is changed from H^- to D^-. Adapted from: IBA users manual.

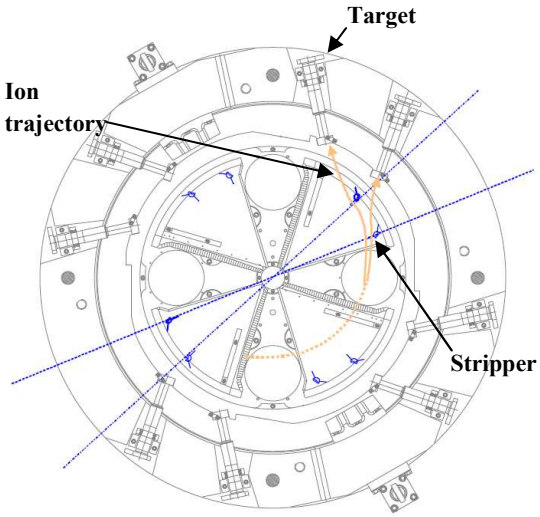

Figure 1.5: Negative ions are accelerated until they hit a stripping foil, which removes the electrons. The ion charge, now positive, experiences the same force in the same direction but with opposite sense, leaving the cyclotron and hitting the selected target. Adapted from: IBA users manual.

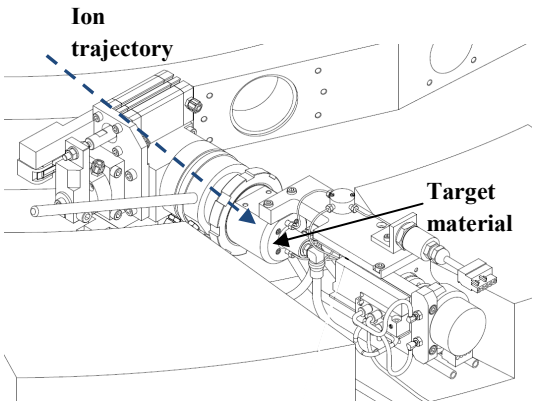

Figure 1.6: Liquid target for the production of [^{18}F]F^- in an IBA Cyclone 18/9 cyclotron. Positive ions deflected by the stripper collision with the target material to produce the radioisotope. Adapted from: IBA users manual.

Most important characteristics for a cyclotron dedicated to clinical and preclinical research in PET are:

- Two particles can be accelerated (proton-deuteron). Protons are needed for the production of carbon-11, nitrogen-13 and fluorine-18 and deuterons are required for the production of oxygen-15. Currently, methods for the production of oxygen-15 using protons instead of deuterons are being developed with promising results.[7]
- Fixed energy. The same energy can be used for the production of carbon-11, nitrogen-13 and fluorine-18. A lower deuteron energy for the production of oxygen-15 is enough. The energy of the beam can be modulated by the insertion of energy degraders (eg., aluminum foils) into the trajectory of the accelerated ions. Many applications are found in the irradiation of solid targets for the production of non-conventional isotopes, like Ti-45, Zr-89, etc.
- Simple design and operation.

Targets

The target is a container where the target material to be irradiated (usually gas or liquid, but solid materials are also used) is introduced. There are some key parameters which have to be considered for target body design:

- The threshold energy for the reaction, to say, the minimal energy needed to generate the radioactive atom.
- The energy where the maximum cross-section (probability for the nuclear reaction to occur) is obtained.
- The physical form of the target material: gas, liquid or solid. Heat transfer properties and potential effects due to heating while irradiation takes place should be carefully considered for each particular case.
- The chemical form of the target material.
- The physical form of the product.
- The chemical form of the product.
- The ease of separation of the product from the target.

Targets specially designed for the production of the most common positron emitters (fluorine-18, carbon-11, nitrogen-13 and oxygen-15) are implemented in commercially

available cyclotrons and its design to improve its performance has been optimized for many years.

Production of fluorine-18

There are different nuclear reactions to produce fluorine-18 (see Table 1.2). However, the most widely used reactions are $^{20}Ne(d,\alpha)^{18}F$ and $^{18}O(p,n)^{18}F$, because both reactions require moderate particle energy and low current beam to give useful yields.

Despite only the above mentioned nuclear reactions are routinely used, the variety in the design of fluorine-18 targets is very large. The first nuclear reaction ($^{20}Ne(d,\alpha)^{18}F$) is used to prepare carrier-added (CA) [^{18}F]fluorine (as $^{18}F_2$) and the second one is used to produce no-carrier-added (NCA) [^{18}F]fluorine as $^{18}F^-$ (no-carrier-added is the preparation of a radioactive isotope which is essentially free from stable isotopes of the element. It is also called carrier free). In the last years, the nuclear reaction $^{18}O(p,n)^{18}F$ has been also used for the production of carrier added [^{18}F]fluorine as $^{18}F_2$.[8]

Nuclear reaction	Useful Energy Range (MeV)	Natural Abundance (%)
$^{18}O(p,n)^{18}F$	4 - 14	0.2
$^{16}O(^{3}He,p)^{18}F$	1 - 15	99.7
$^{16}O(^{3}He,n)^{18}Ne{:}^{18}F$	15 - 40	99.7
$^{16}O(^{4}He,np)^{18}F$	20 - 40	99.7
$^{16}O(^{4}He,2n)^{18}Ne{:}^{18}F$	10-52	99.7
$^{20}Ne(d, \alpha)^{18}F$	0-15	90.5
$^{20}Ne(p,2pn)^{18}F$	30-40	90.5
$^{20}Ne(^{3}He, \alpha\ p)^{18}F$	10-40	90.5

Table 1.2: Nuclear reactions used for obtaining fluorine-18. Natural abundance refers to the irradiated stable isotope for the production of ^{18}F. Obtained from: Welch, M.J; Redvanly, C.S; Handbook of radiopharmaceuticals: Radiochemistry and applications; *Wiley*, **2005**.

Although nowadays the NCA procedure is the routine process to obtain fluorine-18, which is mostly used to produce 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG), a well-established radiotracer used in clinical applications, mainly, for oncologic diagnostic, for some applications carried added [^{18}F]fluorine can be an advantage to label certain molecules by electrophilic substitution to do *in vivo* PET studies, [9] and thus the reaction $^{20}Ne(d,\alpha)^{18}F$ is used. Moreover, in some cases, the synthetic route to obtain the radiotracer is easier if the chemical specie [^{18}F]F_2 is used, and thus [^{18}F]F_2 has also some important applications.

Regarding the production of $^{18}F^-$, in the last decades several targets made from silver or titanium have been used, either applying high or low pressure during irradiation. Silver targets have a high thermal conductivity, but they have the disadvantage that colloids can be formed during irradiation, which can lead to increase the target pressure, decreasing thus the reaction yield. On the other hand, titanium is inert, but effective cooling is a disadvantage. In addition, the radioisotope vanadium-48 is produced as a byproduct, which contaminates the enriched water and causes high radiation levels in the cyclotron bunker by accumulation in the target foils. In the recent years, niobium, an inert metal with higher thermal conductivity than titanium, is the most widely used material for targets designed for the production of $^{18}F^-$.

When the reaction $^{20}Ne(d,\alpha)^{18}F$ is used, it is necessary to add non radioactive F_2 to the target because F_2 is very reactive and can be absorbed into the target walls and transfer lines (from the target to the synthesis modules in the radiochemistry laboratory). This essential step is called passivation and it is critical to obtain good yields.

Production of carbon-11

Carbon-11, due to its short half-life, is not easy to transport from manufacturing centers to surroundings hospitals. Thus, carbon-11 began to receive increasing attention as a useful nuclide in medical application in the sixties due to the widespread installation of cyclotrons in hospitals.

The nuclear reactions able to produce carbon-11 are shown in Table 1.3. By far, the most common reaction is $^{14}N(p,\alpha)^{11}C$ using nitrogen gas as irradiated material. This reaction produces carbon-11 with high yields and, with the addition of oxygen, all carbon-11 is obtained in the chemical form of [^{11}C]CO_2. The presence of oxygen in the

target mixture generates unwanted [^{13}N]-labeled nitrogen oxides which are not useful because they are converted into [^{13}N]N_2 in the target.[10] With the addition of hydrogen instead of oxygen, [^{11}C]CH_4 can be produced.

Nuclear reaction	Useful Energy Range (MeV)	% Natural Abundance
$^{11}B(p,n)^{11}C$	5-20	80.1
$^{10}B(d,n)^{11}C$	3-12	19.9
$^{12}C(p,pn)^{11}C$	20-50	98.9
$^{14}N(p,\alpha)^{11}C$	7-15	99.6
$^{14}N(d,n\ \alpha)^{11}C$	10-15	99.6
$^{12}C(^{3}He,\ \alpha)^{11}C$	7-15	98.9

Table 1.3: Nuclear reactions used for obtaining carbon-11. Natural abundance refers to the irradiated stable isotope for the production of ^{11}C. Obtained from: Welch, M.J; Redvanly, C.S; Handbook of radiopharmaceuticals: Radiochemistry and applications; *Wiley*, **2005**.

Production of nitrogen-13

There are several reactions leading to the production of nitrogen-13 (see Table 1.4). The generation of nitrogen-13 in cyclotrons has been focused in the production of the chemical specie [^{13}N]NH_4^+. This is a blood flow/perfusion marker and is used in cardiac studies to determine areas of ischemic or infarcted tissues.[11] In this case, nitrogen-13 can be generated by bombarding mixtures of water/ethanol with high energy protons ($^{16}O(p,\ \alpha)^{13}N$ nuclear reaction).[12] The function of ethanol as a scavenger and reducing agent increases the amount of radioactivity generated as well as the relative amount of [^{13}N]NH_4^+ with respect to [^{13}N]NO_2^- and [^{13}N]NO_3^-. In the absence of this scavenger, the relative amount of [^{13}N]NH_4^+ decreases drastically to <20%.[13] This is sometimes interesting in order to obtain [^{13}N]NO_2^- and [^{13}N]NO_3^- to be used as radioactive precursors.

Nuclear reaction	Useful Energy Range (MeV)	% Natural Abundance
$^{13}C(p,n)^{13}N$	4-9	1.1
$^{12}C(d,n)^{13}N$	1-6	98.9
$^{16}O(p, \alpha)^{13}N$	8-15	99.8
$^{10}B(\alpha,n)^{13}N$	4-6	19.9
$^{11}B(\alpha,2n)^{13}N$	6-10	80.1
$^{14}N(p,pn)^{13}N$	14-30	99.6

Table 1.4: Nuclear reactions used for obtaining nitrogen-13. Natural abundance refers to the irradiated stable isotope for the production of ^{13}N. Obtained from: Welch, M.J; Redvanly, C.S; Handbook of radiopharmaceuticals: Radiochemistry and applications; *Wiley*, **2005**.

Production of oxygen-15

There are several nuclear reactions for the production of oxygen-15 (see Table 1.5).

Nuclear reaction	Useful Energy Range (MeV)	% Natural Abundance
$^{16}O(p,pn)^{15}O$	20-26.5	99.8
$^{15}N(p,n)^{15}O$	4-10	0.4
$^{14}N(d, n)^{15}O$	2-10	99.6
$^{12}C(^{4}He,n)^{15}O$	12-18	98.9

Table 1.5: Nuclear reactions used for obtaining oxygen-15. Natural abundance refers to the irradiated stable isotope for the production of ^{15}O. Obtained from: Welch, M.J; Redvanly, C.S; Handbook of radiopharmaceuticals: Radiochemistry and applications; *Wiley*, **2005**.

The most commonly used reaction is $^{14}N(d, n)^{15}O$; in this case, N_2/O_2 mixtures are irradiated with high energy protons. When these mixtures of nitrogen and oxygen are bombarded, nitrogen oxides can be produced directly, but $[^{15}O]O_2$ is the most abundant specie. Further reaction with hydrogen under catalytic conditions (e.g., palladium) yields $[^{15}O]H_2O$, a blood flow marker.[14]

Production of non conventional isotopes

In the recent years, especial targets have been developed to be coupled to commercially available cyclotrons for the production of non conventional isotopes. Thus, solid materials (generally metals or metal oxides) can be irradiated with protons whose energy can be modulated by introducing energy degraders. Some examples of the production of non conventional positron emitters are shown in Table 1.6.

Isotope	Nuclear reaction	Max. energy (MeV)
^{124}I	$^{124}Te(p, n)^{124}I$	2.15
^{86}Y	$^{86}Sr(p, n)^{86}Y$	3.15
^{64}Cu	$^{64}Ni(p, n)^{64}Cu$	0.66
^{45}Ti	$^{45}Sc(p, n)^{45}Ti$	1.04
^{89}Zr	$^{89}Y(p, n)^{89}Zr$	0.90

Table 1.6: Nuclear reactions used for obtaining non conventional positron emitters. Obtained from: Welch, M.J; Redvanly, C.S; Handbook of radiopharmaceuticals: Radiochemistry and applications; *Wiley*, **2005**.

As can be seen, there are many alternatives for the selection of the radioisotopes, the way to generate them and the chemical form in which they can be obtained. However, efforts are focused nowadays towards the optimization of the existing methods for the generation of the most widely used radioactive precursors.

1.1.4. The concept of radiotracer

A radiotracer can be defined as a substance containing a radioactive atom to allow easier detection and measurement. The radioactive atom defines the physical properties of the radiotracer, while the chemical structure of the molecule attached to the radioisotope defines the biological properties of this radiotracer. Radiotracers have many applications, but their applicability is especially relevant in drug development and evaluation of response to specific therapies.[15]

Many drugs fail in development stages due to unfavorable pharmacokinetic properties. They do not reach their target organ because of poor absorption and membrane

permeability, or because they are rapidly excreted so that the total exposure time is too short to achieve reasonable plasma level, or they are rapidly metabolized to a pharmacologically inactive metabolite. Because of these, it is obvious that detailed knowledge on the drug biodistribution is of key importance in the development of novel therapeutic and diagnostic tools.

In experimental animals, such as mice and rats, studies on drug distributions are commonly carried out using whole-body autoradiography. The desired labeled compound is administered to animals which are sacrificed at different time points following compound administration. Autoradiography is quantitative and provides high spatial resolution and high sensitivity, but it has two principal drawbacks: i) drug distribution can only be assessed postmortem and ii) the method does not provide any information on drug metabolism because it is not possible to know if the radioactive group is bound to the parent compound or not. While this *ex vivo* technique provides valuable information about the pharmacokinetic properties of the drug, *in vivo* techniques allow obtaining information about absorption, distribution, metabolism and elimination (ADME). Consequently, there is an increasing realization that radiotracer drug imaging, mainly with PET, has a major role in drug development both in clinical and preclinical scenarios, which may reduce the substantial costs currently incurred.

The use of the appropriate ligands for molecular imaging with PET will depend on the development of adequate tracers and efficient procedures for their radiolabeling. In the ideal situation, radiotracers interact only with the target molecule of interest and no non-specific accumulation is observed. However, the ideal radiopharmaceutical does not exist. Some common criteria, not dependent on the PET application of the radiotracer, are:

- High affinity for its target (low K_d). This characteristic allows obtaining high-contrast PET images. The main mechanism of the radiotracer should be the accumulation in the target tissue. The required affinity depends on the concentration of the target.
- High specificity. It is necessary because interaction of the radiotracer with other types of molecules will interfere with the desired radioactive signal detected by the PET camera.

- "Easy" radiolabeling. The radiolabeling procedure needs to generate a radiotracer with a good radiochemical yield. There are some parameters that should be considered, including position to be labeled, reaction time, choice of radioisotope and specific radioactivity.
- Rapid metabolism of the radiotracer is not desirable. Labeled metabolites can bind to other molecules or play a role in unknown biochemical processes and the final result is a non-specific accumulation of the radioactivity.
- Lipophilicity of the radiotracer. This parameter has an important relevance because it determines the ability of the molecule to cross cell membranes and barriers like blood-brain-barrier (BBB). The lipophilicity is usually expressed as the partition coefficient between *n*-octanol and water (log P). Log P > 1.5-2 is needed to cross lipid bilayers. Higher values (> 3) cause a high non-specific binding and hydrophobic interactions with lipids and proteins are provoked. Sometimes, the presence of carrier systems to help in crossing the cell membrane or other barriers is beneficial to obtain increased levels of the tracer in the cell, which means a higher contrast in images.
- Clearance of non-specifically bound radioactivity in the time scale of measurement for PET is necessary to distinguish between specific and non-specific uptake.
- Low toxicity. Although small amounts of mass are injected (usually in the microgram scale), in most countries the authorities require toxicity tests in rodents and Ames test[16] for mutagenicity using doses 1000-10000 times higher than PET doses.[17,18]

1.1.4.1. Important considerations related to radiotracers

Half-life of the radionuclide

An important consideration in the production of PET radiotracers is the half-life of the radionuclide. Ideally, a radiotracer should be produced within 2-3 half-lives of the radionuclide to maintain high radiochemical yield and specific radioactivity. In addition, due to the low mass of radioactive agent, the stoichiometrical ratio between the non-radioactive starting material (precursor for labeling) and the radioactive precursor is usually chosen in the range between 10000 and 10. As a consequence, the starting

radiolabeled reagent is consumed fast because of pseudo-first order reaction kinetics, independently of its concentration.

Specific radioactivity

The specific radioactivity can be defined as the ratio between the labeled and the non labeled compound. Although theoretical specific radioactivity values for PET isotopes are very high (see Table 1.7) these values are usually very far (10-10000 times higher) from specific radioactivity values obtained once the radiotracer has been synthesized. This decrease in specific radioactivity is due to a dilution process with the non radioactive isotope, usually occurring during radionuclide production and/or manipulation and preparation of the radiotracer. When this dilution process is significant and specific radioactivity is far from the theoretical value, the physical decay of the radionuclide does not only reduce the activity of the radiolabeled compound but also reduces the specific radioactivity by a factor 2 for every half-life. Thus, specific radioactivity of a radiotracer decreases with time.

Nuclide	**Theoretical specific radioactivity (TBq/µmol)**
C-11	341.1
N-13	699.3
O-15	3394.0
F-18	63.3

Table 1.7: Theoretical specific radioactivities for some radioisotopes. Obtained from: Welch, M.J; Redvanly, C.S; Handbook of radiopharmaceuticals: Radiochemistry and applications; *Wiley*, **2005**.

Recent advances in PET applications are associated to an increasing demand for novel and specific radiotracers labeled, mainly, with carbon-11 and fluorine-18, and in some applications, such as the study of the behavior of bioactive or toxic molecules, dose exposure studies and the visualization of low density receptors in the brain, the concept of specific radioactivity becomes especially relevant because the total quantity of tracer

administered (labeled + unlabeled) is related to the specific radioactivity and the amount of radioactivity injected.

The competition between the labeled and the unlabeled compounds may have a negative effect on the concentration of the radioactivity in the target tissue, and it can definitely produce undesired pharmacodynamic and/or toxic effects, as well as receptors saturation. Hence, a high specific activity should provide a sufficient contrast in images between the target tissue and its surrounding. In addition, in the case of dosimetry studies, a selective use of different specific radioactivity values can help in finding an optimal value.[19,20]

Importantly, specific radioactivity can be always reduced by diluting the radiotracer with non radioactive compound, while it cannot be increased. Thus, synthesis procedures yielding high specific radioactivity values permit the preparation of the radiotracer with a pre-determined specific radioactivity by simple addition of the right amount of non labeled specie.

Specific radioactivity values of cyclotron produced radioisotopes are usually dependent on several factors: the material they are in contact with during irradiation and transfer to the synthesis module, the quality of the irradiated material and the amount of radioactivity produced during irradiation. Stable isotopes leaking from the surface of target holder and/or tubings, or present in the irradiated material, may thus contaminate the radionuclides, thereby reducing the specific activity.[21]

The synthetic process followed for the preparation of the radiotracers has also an important impact in the specific radioactivity. In the particular case of carbon-11, competing side reactions with environmental carbon-12 sources (especially when $[^{11}C]CO_2$ is used as radioactive precursor) and its short half-life make high specific activity radiotracers labeled with carbon-11 some of the most synthetically challenging to prepare.

Microdose concept

Drug development is a long (10-15 years), complex and expensive process (the average costs is around 1 billion $). The US Food and Drug Administration (FDA) estimates that a drug entering in a clinical trial today has only 8% possibilities of reaching the market.

The sensitivity of PET makes the detection of radiotracers at concentrations in the low picomolar range possible (which means that the total amount of radiotracer administered is typically less than 1 μg). The potential toxicological risk to humans at this mass scale is very limited. These PET studies in which tiny amounts of radiotracer are used are known as PET microdosing studies, Phase 0 clinical trials or First-time-in-humans. Accelerator mass spectrometry (AMS) is another technique based on the administration of radiotracers that is suitable for microdosing studies. Microdosing studies have no therapeutic or diagnostic purpose but they establish whether a novel compound has appropriate pharmacokinetic and pharmacodynamic profiles in humans, which means that they evaluate the administration, distribution, metabolism and elimination (ADME) and/or distribution properties or receptor selectivity profile (pharmacodynamic endpoint) of new drugs earlier in the development process of a novel drug and at the same time of the preclinical stage, which could be a major advantage in the design and decision concerning clinical development of an agent.[22]

The Committee for Human Medicinal Products (CHMP) of the European Agency for the Evaluation of Medicinal Products (EMEA) defined microdose as the dose of one-hundredth ($1/100^{th}$) of the pharmacologically active dose (up to a maximum of 100 μg, or 30 nmol for protein products). Preclinical toxicology studies should demonstrate that a dose 100 times the proposed human dose does not induce adverse effects. These toxicological studies required in animals in order to do a microdose study are extended single-dose studies in only one appropriate mammalian specie with a control group, with comparative *in vitro* data. [23]

Microdosing appears to be adequate for compounds (small organic molecules, peptide and protein therapeutics) with linear pharmacokinetics, small molecules with short half-lives or compounds that are metabolized and have a rapid dissociation of binding to

their target. In contrast, microdosing is not appropriate for molecules with nonlinear pharmacokinetics and/or high-affinity binding to their targets.

One of the ideal isotopes to perform microdosing studies is carbon-11, because the radiotracer can be prepared with the same chemical structure as the compound under evaluation. Moreover, its short half-life enables to repeat studies on the same subject within the same day. It is therefore possible to measure the baseline and effect of drug treatment on receptor occupancy in the same subject on the same day, thereby eliminating inter-subject variability and facilitating conclusive studies in a limited number of volunteers.[24,25]

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1.2. LABELING STRATEGIES FOR SHORT LIVED ISOTOPES

1.2.1. General Aspects

Radiolabeling is the chemical reaction in which the radionuclide is incorporated in the desired molecule to yield the radiotracer (Figure 1.7). Many synthetic routes to prepare PET radiotracers have been developed during the last decades. Mainly, developed radiotracers incorporate carbon-11 and fluorine-18; the radioactive atoms are incorporated in the target molecules by organic chemical reactions, usually in organic solvents. The half-lives of nitrogen-13 and oxygen-15 are too short to perform radiochemical syntheses of more than one reaction step, and thus the number of radiotracers synthesized with these positron emitters is considerably lower.

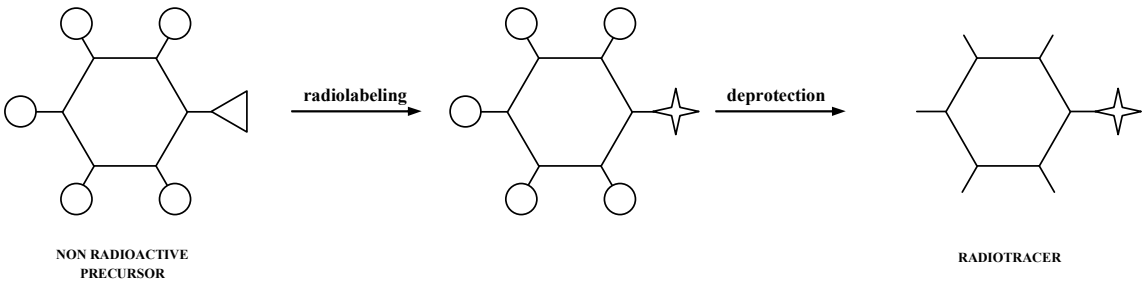


Figure 1.7: General process for the production of a radiotracer. The non radioactive precursor usually has an active center (with a good leaving group, triangle in the figure) while all other reactive groups are protected (circles in the figure). The radioisotope (star in the figure) is introduced in a first step. After reaction, the protective groups are removed to yield the desired radiotracer.

In some cases, before the radiolabeling step, it is necessary to protect reactive groups of the target molecule to avoid the introduction of the radioisotope at unwanted positions or to avoid negative effects of some functional groups on the labeling efficiency. In this case, the process requires a deprotection step after the labeling for removal of the protecting groups. Due to the short half-life of PET radionuclides, methods to increase the radiochemical yields and/or allowing shorter reaction times have been developed such as microwave assisted reactions and microfluidics technology.

In a basic research radiochemistry laboratory, where the main focus is the development of new labeled species, versatility of the equipment and the capacity to implement new synthesis strategies is very important. In contrast, the production of large scale commercial radiopharmaceuticals such as [^{18}F]FDG, requires the implementation of

robust processes in mono-dedicated synthesis modules (to avoid cross contamination), linked to high capacity cyclotrons and targets. To avoid cross contamination and reduce preparation time, routine production of PET radiopharmaceuticals for clinical use is increasingly performed using single use kits which contain all the necessary reagents and solvents.

1.2.2. Radiochemistry with fluorine-18

The main advantage of fluorine-18 is its half-life (109.8 min), which allows longer synthesis routes than other radioisotopes. Moreover, distribution of the radiotracers from high-scale production centers to surrounding hospitals is feasible.

Most used reactions with $[^{18}F]F^-$ (no-carrier-added) involve S_N2 reactions in aliphatic compounds and S_NAr reactions in homo-aromatic and hetero-aromatic compounds with a suitable leaving group. Electrophilic substitution reactions using $[^{18}F]F_2$ can also be applied but, in this case, final radiotracers have low specific radioactivity. Electrophilic substitution reactions include additions across double bonds, reactions with carbanions and fluorodehydrogenation and fluorodemetallation.

1.2.2.1. Nucleophilic fluorination

Nucleophilic fluorination allows obtaining a large variety of radiotracers with high radiochemical yields and high specific radioactivities. There are two main options to perform nucleophilic fluorinations:

- Direct substitution of an appropriate leaving group by $[^{18}F]F^-$ in the desired precursor, which can be followed by hydrolysis of protective groups.
- Preparation of an intermediate fluorinating agent by nucleophilic substitution, followed by a second reaction to finally perform a deprotection step.

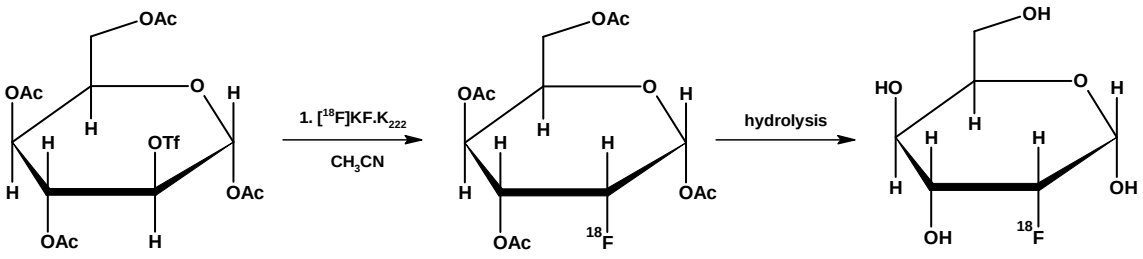
After the production of $[^{18}F]F^-$ in the cyclotron, the $[^{18}F]F^-$ anion is in aqueous solution; under these conditions, $[^{18}F]F^-$ is a poor nucleophile but a strong base. In order to isolate $[^{18}F]F^-$, it is trapped in an anion exchange resin contained in a cartridge, previously conditioned with K_2CO_3 or other potassium salts, like $KHCO_3$ (when the decrease of the basicity for the reaction medium is required) to improve trapping yields (>95%). In this step, $K[^{18}F]$ is formed, which is not soluble in organic polar non protic solvents (suitable for S_N2 reactions). Because of the lack of solubility, a phase-transfer catalyst is

needed, such as tetraalkylammonium salts or aminopolyethers. In addition, a few (2-3) azeotropic evaporations with dry acetonitrile are performed to remove any trace of water, which could decrease the nucleophilicity of the anion. [1,2]

Once [^{18}F]F$^-$ has been isolated, a solution of the precursor in a polar, non protic solvent is added and the reaction mixture is usually heated (10-30 min, 80-160ºC). Typical solvents are acetonitrile, dimethyl sulfoxide (DMSO) and dimethyl formamide (DMF), although tertiary alcohols or amyl alcohols have been recently used with promising results.[3] The advantage of using acetonitrile as the solvent is that it is easily removed by evaporation. On the other side, DMSO and DMF allow the reaction to take place at high temperature in closed reactors at low pressure. The use of microwave ovens is gaining relevance in fluorine radiochemistry, because shorter reactions times lead to higher yields with limited formation of undesired byproducts.

Regarding the precursor, in aliphatic substitutions sulfonic esters such as triflates, tosylates, mesylates and nosylates are suitable leaving groups. Sometimes bromide or iodide are used, but lower radiochemical yields are obtained. Aromatic substitutions need to be carried out with electron-withdrawing groups (nitro- or trimethylammonium) in the *para* or *ortho* position of the substituted moiety.[4]

The most widely used nucleophilic reaction for the incorporation of fluorine-18 into a precursor to yield a radiotracer is applied to the production of [^{18}F]FDG. The reaction pathway is shown in Scheme 1.1.



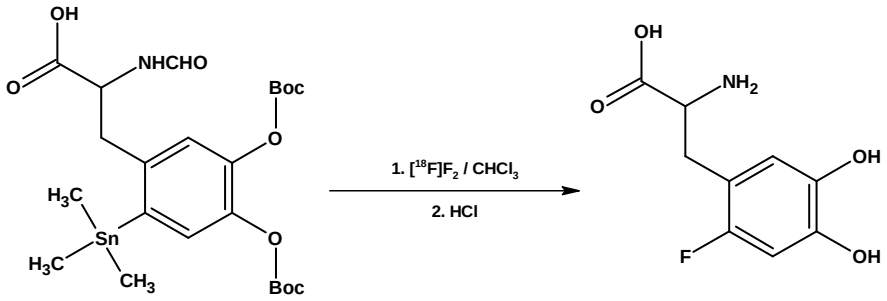
Scheme 1.1: Synthesis of [^{18}F]FDG. Mannose triflate reacts with [^{18}F]F$^-$ in acetonitrile. After fluorination is completed, the protective groups (acetyl) are eliminated by hydrolysis under acidic or basic conditions. Adapted from: Dollé, F; Roeda, D; Kuhnast, B; Lasne, M.C; Fluorine and Health, *Elsevier*, **2008**.

1.2.2.2. Electrophilic fluorination

In electrophilic fluorinations, fluorine is reacted with an electron-donating reactant such as an alkene, aromatic ring or carbanion to form a carbon-fluorine covalent bond.

Possible electrophilic fluorinations include additions to double bonds or electrophilic aromatic substitutions of a trialkyl tin or mercury group. The synthetic procedure is simple and involves bubbling of the gas into the substrate solution. Taking into account the extremely high reactivity of F_2 and, consequently, the difficulties associated to $[^{18}F]F_2$ production, electrophilic fluorinations are not used very often. The oxidizing strength of fluorine often leads to exothermic radical chain reactions with the formation of side products. It was found that its reactivity could be moderated by dilution with an inert gas and by performing the reaction in a strong acidic medium.[5,6]

One of the most important electrophilic fluorinations in radiochemistry is the preparation of $[^{18}F]$FDOPA,[7] radiotracer used for studying the presynaptic dopamine metabolism, for example in the diagnostic of Parkinson disease. The synthesis pathway is following a direct electrophilic substitution of the stannyl precursor, followed by acid hydrolysis of the protection groups and purification by HPLC. The synthesis pathway is shown in Scheme 1.2.



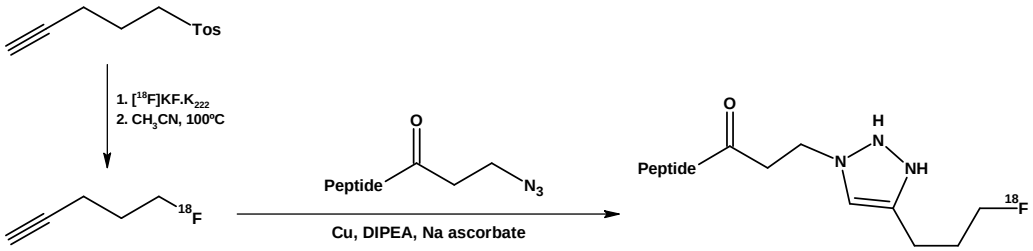
Scheme 1.2: Synthesis of $[^{18}F]$FDOPA. The stannyl precursor is reacted with $[^{18}F]F_2$ in $CHCl_3$ and then the protective groups are eliminated by hydrolysis under acid conditions. The reaction crude is purified by HPLC.

1.2.2.3. Other fluorination strategies

Indirect $[^{18}F]$fluorination with suitable prosthetic groups is an interesting alternative to nucleophilic and electrophilic substitutions, because reactions can be usually performed under mild conditions limiting thus, the formation of byproducts.

One recently developed strategy is the so called click chemistry, or formation of triazole rings by the cycloaddition of alkynes to azides (Scheme 1.3). This reaction has been exploited in radiochemistry due to the speed, selectivity and simplicity under mild reaction conditions. A possible limitation of this method is that copper catalyst can

decompose under acidic conditions. Furthermore, the biological effect of including triazole rings into biomolecules should be investigated.

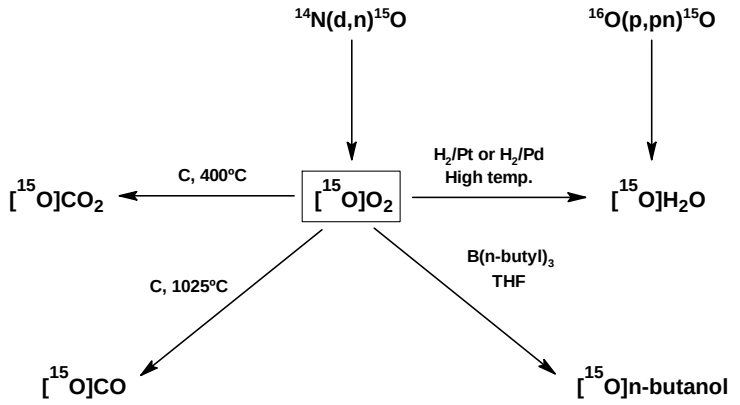


Scheme 1.3: Example of a click reaction in radiochemistry for the introduction of a ^{18}F atom into a peptide. Obtained from: Miller, P.W; Long, N.J; Vilar, R; Gee, A.D; Synthesis of ^{11}C, ^{18}F, ^{15}O and ^{13}N Radiolabels for positron emission tomography, *Angew. Chem.*; **2008**, 47: 8998-9033.

Another strategy consists of using enzymes for the introduction of the radioactive label. This is also an attractive strategy because enzymatic reactions are specific. However, this approach is more used in carbon-11 chemistry.[8]

1.2.3. Radiochemistry with oxygen-15

Due to its short half-life, oxygen-15 is not commonly used in PET applications.Its short half-life is an important drawback to perform radiochemical syntheses including more than one reaction step. Simple chemical products such as $[^{15}O]CO_2$ and $[^{15}O]O_2$ can be obtained directly from the cyclotron and used without further reaction or purification steps (see Scheme 1.4).

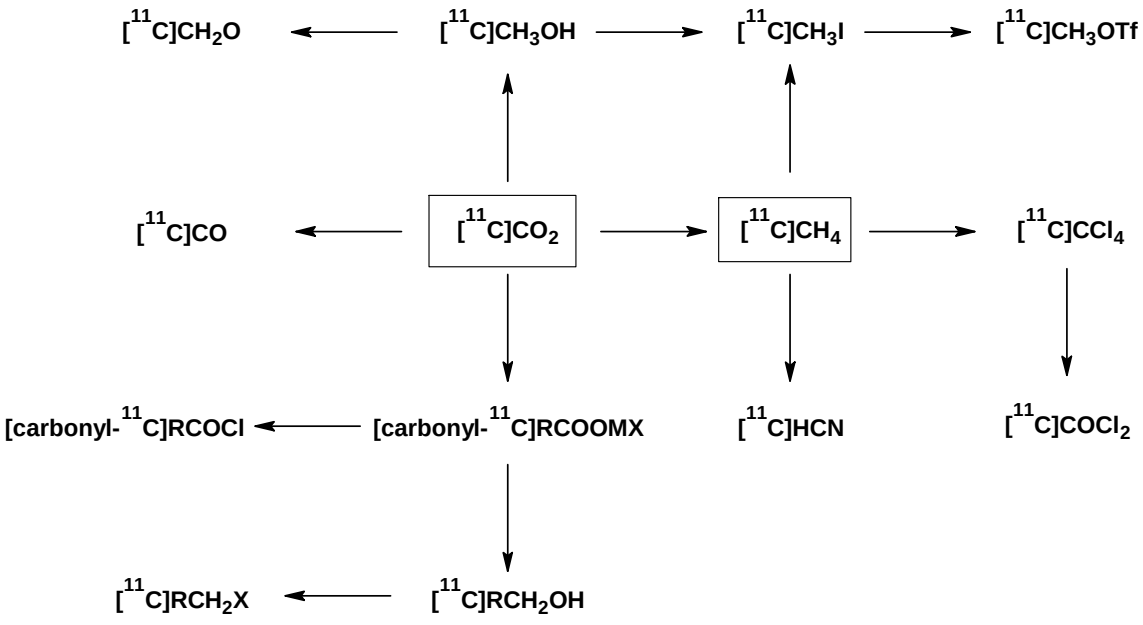


Scheme 1.4: Synthesis of common oxygen-15 compounds for PET applications. Obtained from: Miller, P.W; Long, N.J; Vilar, R; Gee, A.D; Synthesis of ^{11}C, ^{18}F, ^{15}O and ^{13}N Radiolabels for positron emission tomography, *Angew. Chem.*; **2008**, 47: 8998-9033.

[^{15}O]H_2O can be also synthesized in an on-line process with high efficiency. Some alcohols (see Scheme 1.4) have been also prepared by direct reaction of [^{15}O]O_2 with trialkyl boranes.[9]

1.2.4. Radiochemistry with carbon-11

Carbon-11 is the radioisotope with a wider range of reported labeling strategies. It can be obtained in commercially available cyclotrons as [^{11}C]CO_2 and [^{11}C]CH_4, and one of the simplest labeling strategies consists of performing Grignard-type reactions by using [^{11}C]CO_2. However, very often, the radioactive specie generated in the cyclotron (either [^{11}C]CO_2 or [^{11}C]CH_4) is converted into a more suitable specie for further reaction with the adequate precursor. The variety of reactions that can be made from these precursors is very large and is shown in Scheme 1.5.



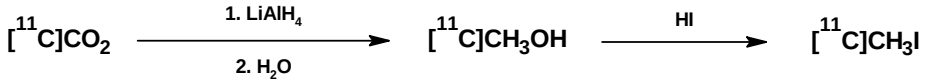
Scheme 1.5: Most important ^{11}C precursors used in the synthesis of ^{11}C-labeled compounds produced from either [^{11}C]CO_2 or [^{11}C]CH_4. Obtained from: Miller, P.W; Long, N.J; Vilar, R; Gee, A.D; Synthesis of ^{11}C, ^{18}F, ^{15}O and ^{13}N Radiolabels for positron emission tomography, *Angew. Chem.*; **2008**, 47: 8998-9033.

1.2.4.1. Methyl Iodide as methylating agent

Production of [^{11}C]CH_3I and direct methylations

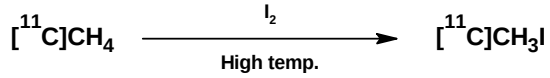
The most commonly used radioactive precursor for introducing carbon-11 into organic molecules is, by far, [^{11}C]CH_3I; with this radioactive precursor, a methylation reaction is performed in order to incorporate [^{11}C]CH_3- into the target molecule. This

[^{11}C]methylating agent can be prepared by the "wet" method[10] or by the gas-phase reaction.[11] The "wet" method starts with the transformation of [^{11}C]CO_2 into [^{11}C]CH_3OH via reduction with $LiAlH_4$ and a final iodination with hydriodic acid (Scheme 1.6). These steps can be easily carried out, but some precautionary measures should be taken. After the trapping of [^{11}C]CO_2 in $LiAlH_4$, the solvent (tetrahydrofuran, THF) should be removed using vacuum, inert gas flow and heat. Later, the aluminium salt must be cooled again in order to add concentrated hydriodic acid without producing an exotermic reaction. Once HI has been added, the mixture is again heated to distill [^{11}C]CH_3I using an inert gas stream. The main difficulty is to maintain the specific activity of the radioactive precursor as high as possible. This is especially critical in the manipulation of $LiAlH_4$. Attention should be paid also in maintaining inert atmosphere during the whole procedure.



Scheme 1.6: Schematic pathway for the production of [^{11}C]CH_3I from [^{11}C]CO_2.

An alternative strategy for obtaining high specific activity values is the use of [^{11}C]CH_4 instead of [^{11}C]CO_2 in the synthesis of the [^{11}C]methylating agent [^{11}C]CH_3I. The amount of CH_4 in the atmosphere is considerably lower than the amount of CO_2 and thus the contamination is strongly restricted. When [^{11}C]CH_4 is obtained from the cyclotron, a gas-solid iodination reaction of [^{11}C]CH_4 at high temperature provides [^{11}C]CH_3I as [^{11}C]methylating agent. The iodination step is carried out in a quartz tube which contains I_2 vapor at 720 ºC. The high temperature allows the dissociation of iodine molecule and the reaction with the [^{11}C]CH_4 (see Scheme 1.7).[12] The disadvantage of this methodology is the low efficiency in the radical reaction, so the gas must be recirculated several times to obtain acceptable yields.



Scheme 1.7: Schematic pathway for the production of [^{11}C]CH_3I from [^{11}C]CH_4.

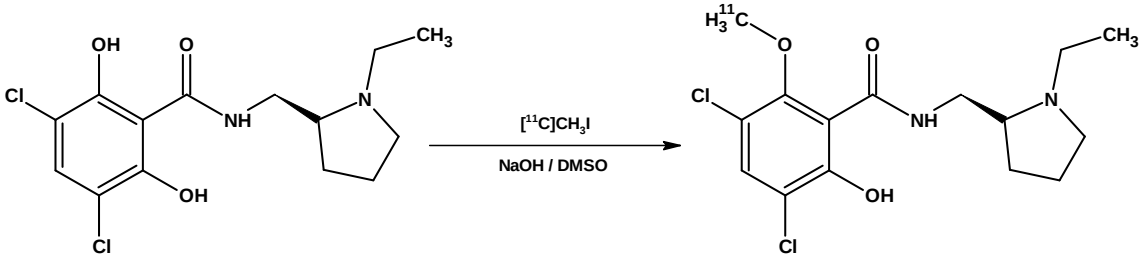
When methylation using [^{11}C]CH_3I is slow, [^{11}C]methyl triflate can be used to obtain better radiochemical yields in milder conditions, due to its higher reactivity. [^{11}C]methyl triflate is prepared by passing gaseous [^{11}C]methyl iodide (either prepared by the "wet" or the "gas-phase" method) through a silver triflate column at high temperature.[13] Other alkylating agents have been developed, such as [^{11}C]ethyl iodide, [^{11}C]propyl iodide, [^{11}C]butyl iodide and [^{11}C]benzyl iodide,[14] although reactions are usually slower due to steric hindrance.

Methylation reactions including [^{11}C]CH_3I or [^{11}C]CH_3OTf are nucleophilic substitutions of methyl iodide with a precursor, usually an amine, alcohol or thiol group. The simplicity and the short reaction times that this step requires have converted this strategy into one of the most commonly used for the production of ^{11}C-labeled compounds.

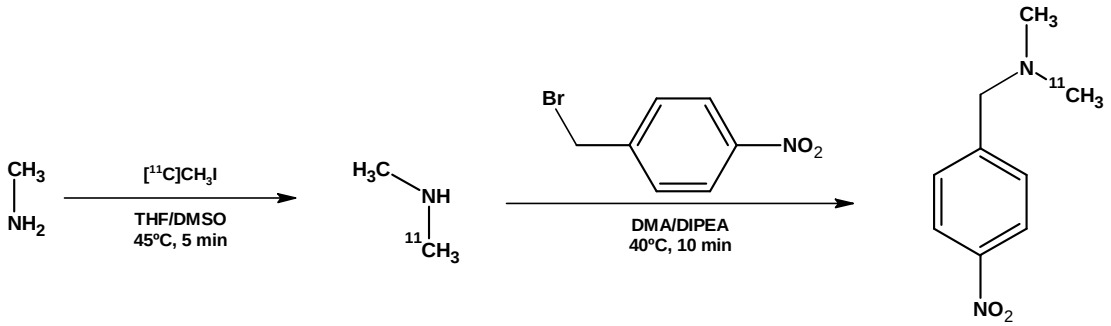
The development of *captive solvent methods*, where the radioactive synthon is trapped in a solution of the non radioactive precursor, is a clear example. Automated continuous flow reaction and purification systems using narrow-bore stainless-steel or plastic/polymer loops as reactors have found an increased use in simple [^{11}C]methylation reactions because their ease use and versatility. These methods are called "in loop" methods, and they involve coating the inside surface of the loop with micromolar amounts of non radioactive precursor in a suitable solvent and then passing a gaseous stream of [^{11}C]CH_3I or [^{11}C]CH_3OTf through the loop.

These methods have been used to label a large variety of biological compounds due to their good radiochemical yields, purities and their short reaction times.[15] The efficiency of this methodology is excellent, taking into account that surface areas of the reaction loops are relatively small and the fact that they can be carried out without cooling or heating the system. Another advantage is that the loop minimizes the use of solvents and precursor material which may contribute to the increased efficiency of the reactions. In addition, minimal losses of labeled product occur as a result of transfers because the crude reaction mixture can be introduced directly into an integrated HPLC system for purification or, even better, in a cartridge to perform purification through a solid-phase support.[16] A typical methylation reaction for the production of [^{11}C]raclopride, which can be carried out by using the *captive solvent method*, is shown in Scheme 1.8.

Indirect [^{11}C]methylation strategies using [^{11}C]CH_3I and [^{11}C]CH_3Otf have been also developed. One example is the preparation of [^{11}C]dimethylamine as intermediate, which can be used for the preparation of [^{11}C]methylated compounds with dimethylamine functional groups. Although direct labeling using [^{11}C]CH_3I can be achieved, an indirect method using [^{11}C]dimethylamine and bromide precursors shows some advantages (Scheme 1.9).[17]



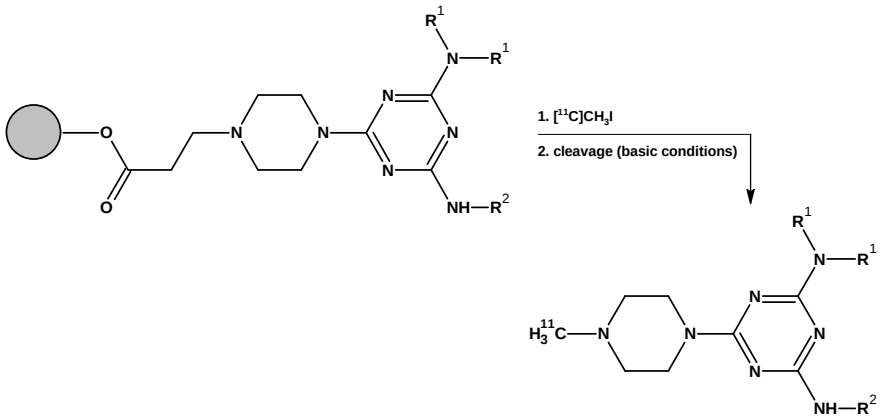
Scheme 1.8: Synthesis of [^{11}C]raclopride. The non radioactive precursor is reacted with [^{11}C]CH_3I in DMSO under basic conditions. The reaction crude is purified by HPLC.



Scheme 1.9: Radiosynthesis of [^{11}C]dimethylamine and reaction with bromomethyl-4-nitrobenzene to yield the ^{11}C-labeled radiotracer. Obtained from: Miller, P.W; Long, N.J; Vilar, R; Gee, A.D; Synthesis of ^{11}C, ^{18}F, ^{15}O and ^{13}N Radiolabels for positron emission tomography, *Angew. Chem.*; **2008**, 47: 8998-9033.

Another strategy to achieve the [^{11}C]methylation is the so-called "safety-catch" linker method (Scheme 1.10). This method is based in using a solid-support, like polymers, to carry out reactions. The precursor is attached to a solid support and then treated with the radiolabeling agent. The unlabeled compound remains with the polymer and thus simplifies purification. Most commonly used resins include REM and Kenner.[18]

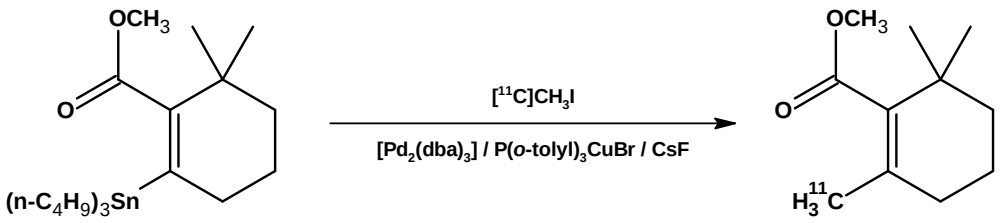
The "safety-catch" process provides high purity ^{11}C-labeled compounds avoiding HPLC purification. Moreover, the method is based on standard combinatorial synthesis and could potentially be used for rapid preparation of ^{11}C-labeled drug candidates for screening.



Scheme 1.10: ^{11}C-methylation of the resin-attached precursor molecule followed by release of the labeled molecule under basic conditions. Obtained from: Miller, P.W; Long, N.J; Vilar, R; Gee, A.D; Synthesis of ^{11}C, ^{18}F, ^{15}O and ^{13}N Radiolabels for positron emission tomography, *Angew. Chem.*; **2008**, 47: 8998-9033.

Palladium-Mediated [^{11}C]Methylation

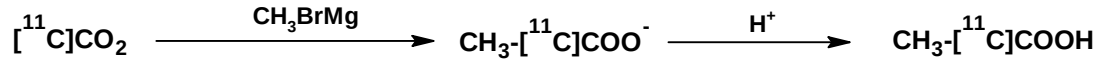
In the last decades, many groups have developed a large number of reaction strategies for the formation of C-C, C-O and C-N bonds via palladium-catalyzed reactions. These strategies have been applied to the preparation of ^{11}C-labeled compounds, although, in the radiochemistry scientific community, the commonly accepted term for this kind of reactions is "palladium-mediated reaction" instead of "palladium-catalyzed reaction" due to the excess of palladium complex compared to the amount of radioactive precursor. Stille-type reaction has been the most widely used (Scheme 1.11).[19] The use of stannanes is compatible with many functional groups while the polarity of the molecule is not very high, helping in the purification step via chromatographic separation. One important drawback of using stannanes is the toxicity, which may cause problems for *in vivo* studies. Another well studied palladium-mediated reaction in radiochemistry is the Suzuki method, which has higher radiochemical yields than the Stille reaction, especially when microwave is used.[20]



Scheme 1.11: Stille cross-coupling reaction for the formation of ^{11}C-labeled alkenes using [^{11}C]CH_3I. Obtained from: Miller, P.W; Long, N.J; Vilar, R; Gee, A.D; Synthesis of ^{11}C, ^{18}F, ^{15}O and ^{13}N Radiolabels for positron emission tomography, *Angew. Chem.*; **2008**, 47: 8998-9033.

1.2.4.2. Grignard reactions

As stated before, [^{11}C]CO_2 can be used directly after obtention from the cyclotron by reacting with organometallic Grignard reagents to form [^{11}C]carboxymagnesium halides and then transformed into the [^{11}C]carboxylic acids. These can be converted into more reactive species (e.g., acid chloride) and react with amines to form [carbonyl-^{11}C]amides and [^{11}C]ureas.[21] An important example of the applicability of this synthethic strategy is found in the preparation of [^{11}C]acetate, a radiotracer used in the evaluation of myocardial oxygen metabolism and diagnosis of prostate cancer (Scheme 1.12).[22]



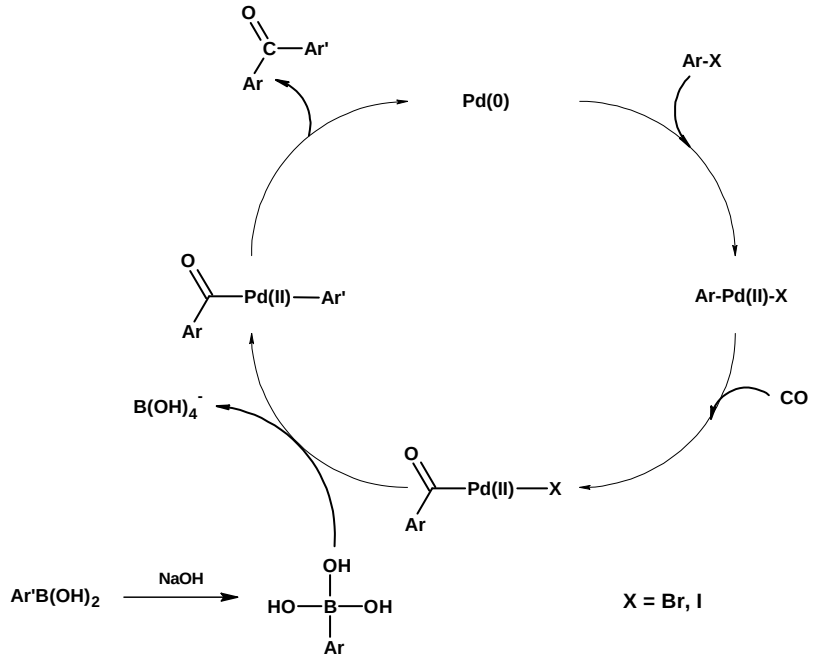
Scheme 1.12: Schematic pathway of [^{11}C]acetate synthesis.

1.2.4.3. [^{11}C]carbonylation reactions

The interest in labeling molecules with [^{11}C]CO is due to two main reasons: (i) the wide variety of carbonyl-containing biologically interesting molecules which can be synthesized through carbonylation reactions and (ii) the possibility to obtain [^{11}C]CO through the reduction of [^{11}C]CO_2 over zinc or molybdenum in an on-line process. The most widely used method is the palladium-mediated [^{11}C]carbonylation process.[23] Carboxylic acids,[24] esters,[25] amides[26] and imides[27] are some of the products which can be synthesized via palladium-mediated carbonylation of olefins, alkynes and organic halides using carbon monoxide at atmospheric pressure.

The catalytic cycle consists of three steps: (i) oxidative addition of the organohalide to Pd^0, (ii) insertion of [^{11}C]CO into the carbon-palladium bond and (iii) nucleophilic attack on the carbonyl carbon and reductive elimination (Scheme 1.13).

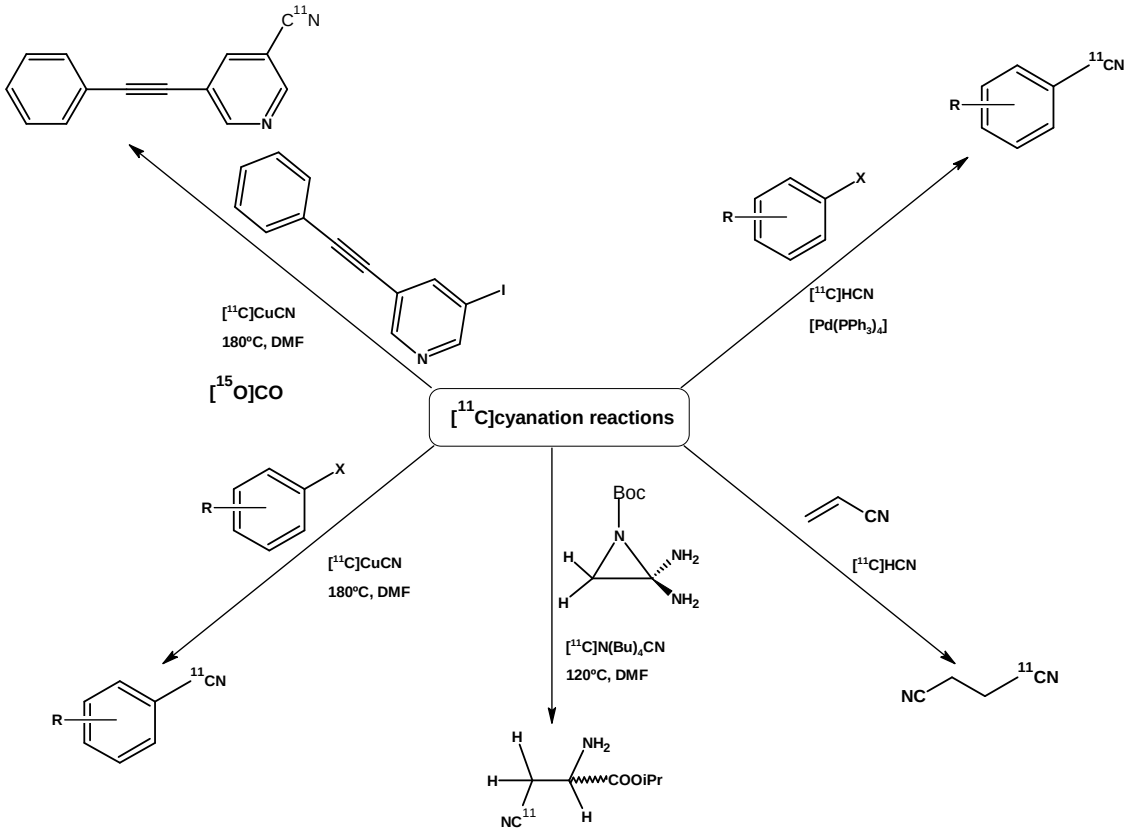
The limited use of [^{11}C]CO as radioactive precursor in the insertion of carbon-11 is due to the poor reactivity of carbon monoxide as a result of its low solubility in organic solvents at low CO pressures. Recently, different strategies to improve [^{11}C]CO trapping have been developed with promising results.[28]



Scheme 1.13: Mechanism of palladium-catalyzed carbonylation of organohalides.

1.2.4.4. Other reactions for the incorporation of carbon-11

Although [^{11}C]alkylation by using [^{11}C]alkyl iodide or triflate and palladium-mediated reactions (either for the introduction of [^{11}C]CO or [^{11}C]CH_3-) and Grignard reactions using [^{11}C]CO_2 are by far the most commonly used strategies for the preparation of ^{11}C-labeled radiotracers, other synthetic routes have been also used with interesting potential applications. [^{11}C]phosgene has been developed as a synthon for ^{11}C-labeling;[29] although it is a very reactive specie which could be applied for fast and efficient incorporation of carbon-11 into molecules, there are still technical difficulties regarding its production in a reliable and reproducible way. Another radioactive precursor is [^{11}C]HCN, which is the starting material for [^{11}C]cyanation reactions and it is usually prepared by the reduction of [^{11}C]CO_2 to [^{11}C]CH_4 using H_2 over nickel, and further conversion into [^{11}C]HCN by reaction with NH_3 over platinum.[30] [^{11}C]HCN can be used directly or may be converted into [^{11}C]CuCN to react with aryl halides (Scheme 1.14) for the preparation of nitrile compounds.[31]



Scheme 1.14: Summary of [^{11}C]cyanation reactions. Obtained from: Miller, P.W; Long, N.J; Vilar, R; Gee, A.D; Synthesis of ^{11}C, ^{18}F, ^{15}O and ^{13}N Radiolabels for positron emission tomography, *Angew. Chem.*; **2008**, 47: 8998-9033.

1.2.5. Radiochemistry with nitrogen-13

As in the case of oxygen-15, nitrogen-13 is an attractive radionuclide for labeling biomolecules because their stable isotopes (oxygen-16 and nitrogen-14, respectively) are in large variety of bioactive molecules. However, the extremely short half-life of nitrogen-13 (9.98 min) has imposed limitations in the radiosynthesis of labeled species and putative clinical application; on the other hand, the development of ^{13}N-labeled radiotracers would allow performing repeated PET procedures on the same subject within one day due to the fast decay.

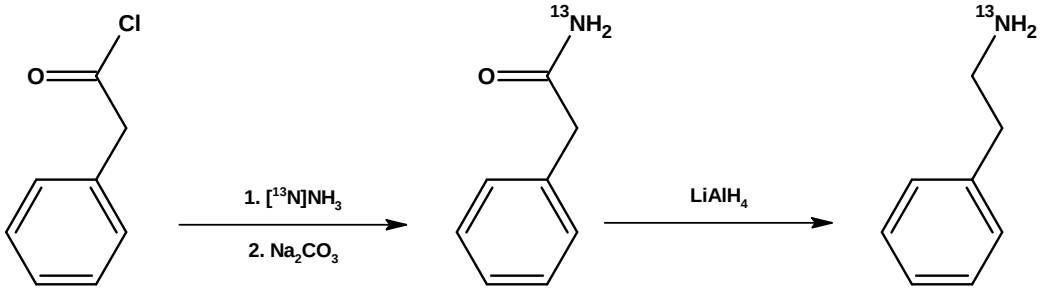
Nowadays, main applications of nitrogen-13 include: (i) direct use as [^{13}N]NH_4^+ to measure myocardial blood flow[32] and (ii) production of ^{13}N-labeled amino acids. Actually, an extensive range of ^{13}N-labeled aminoacids have been prepared (Table 1.8) and reported in the literature.

^{13}N-labeled amino acid	Precursor	^{13}N reagent	Enzyme
L-[^{13}N]alanine	Pyruvate	[^{13}N]NH_3	GAD[33]
L-[^{13}N]leucine	α-ketoisocaprate	[^{13}N]NH_3	GAD[34]
L-[^{13}N]methionine	α-keto-γ-methiol-butyrate	[^{13}N]NH_3	GAD[34]
L-[^{13}N]Phenylalanine	Phenylpiruvate	[^{13}N]NH_3	PAD[35]
L-[^{13}N]Tyrosine	*p*-hydroxy-phenylpiruvate	[^{13}N]NH_3	PAD[35]

Table 1.8: Examples of ^{13}N-labeled amino acids reported in the literature.

These compounds are of particular interest for the determination of protein synthesis rates, particularly in tumors, and are usually produced through biosynthetic methods using enzymes, which enable rapid region and stereospecific syntheses to be performed under mild aqueous reaction conditions.

Apart from the production of amino acids, nitrogen-13 has been also used for the production of other ^{13}N-labeled species, such as amines. Several strategies have been used, like reaction via [^{13}N]imines using [^{13}N]ammonia as radioactive precursor,[36] or the reaction of acid chlorides with [^{13}N]ammonia to yield the corresponding amides, which are later reduced to the amines using $LiAlH_4$ (Scheme 1.15).[37] Other routes for the radiosynthesis of ^{13}N-labeled amines follow amination of organoboranes.[38]



Scheme 1.15: Synthesis of [^{13}N]-β-phenethylamine through the corresponding amide and reduction with $LiAlH_4$. Adapted from: Tominaga, T.; Inoue, O.; Suzuki, K.; Yamasaki, T.; Hirobe, M.; Synthesis of ^{13}N-labeled amines by reduction of ^{13}N-labeled amides; *Int. J. Appl. Radiat. Isot.;* **1986**, 37: 1209-1212.

Nitrogen-13 has been also used for the preparation of other ^{13}N-labeled species, although literature reporting these works is not very extensive. *N*-nitrosoureas have been labeled with nitrogen-13 with moderate radiochemical yields starting from the radioactive precursor $[^{13}N]NO_2^-$, obtained from the reduction of $[^{13}N]NO_3^-$.[39] This method was optimized later by using more concentrated carrier solutions to prepare antibiotics in good yields.[40] More recently, a resin supported methodology for the preparation of ^{13}N-labeled nitrosamines and ^{13}N-labeled nitrosothiols was reported[41] although many details about the procedure were omitted and reaction conversions and radiochemical yields were not specified.

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2. JUSTIFICATION AND OBJECTIVES

2.1. JUSTIFICATION OF THE WORK

The scientific work carried out during this PhD thesis has been performed in two different centers. The first part was carried out at Institut d'Alta Tecnologia PRBB-Fundación Privada (IAT-PRBB), while the second part was developed at CIC biomaGUNE.

IAT-PRBB is a full profit company offering molecular imaging services based on Positron Emission Tomography (PET) and Magnetic Resonance Imaging (MRI). The main goals of the company are: (i) to collaborate with industry and/or research groups offering knowledge and state-of-the-art equipment in the field of molecular imaging, to solve (using a multimodal approach) medical or biological questions using imaging technologies, and (ii) to produce [^{18}F]FDG in a high scale fashion for self use in clinical applications and to be distributed to nearby hospitals. In this scenario, the radiochemistry department was involved in clinical (human) and pre-clinical (experimental animals, mainly rodents) trials, usually sponsored by pharmaceutical companies or, occasionally, by research groups. In these projects, efforts were not focused in the development of new labeled chemical entities, but in the development (or refinement) of protocols for the manufacture of known tracers using fast, efficient and reproducible processes in a GMP environment.

In one of these above mentioned clinical trials, a large number of patients had to be scanned with L-[methyl-^{11}C]methionine ([^{11}C]methionine), a radiotracer used for the assessment of brain tumors when [^{18}F]FDG is not appropriate (due to high uptake by normal brain tissue). Due to several reasons (specified in section 3.1), a method based on the *captive solvent method* had to be developed to produce [^{11}C]methionine efficiently. The method should permit the production under GMP environment and the final radiotracer should accomplish specifications according to the Spanish Pharmacopoeia. From this work, the first paper entitled "*New method for routine production of L-[methyl-^{11}C]methionine: in loop synthesis"* was published in *Journal of Labelled Compounds and Radiopharmaceuticals*.

Also during this period, several clinical trials with new labeled chemical entities were started. Due to the relatively high toxicity of some of the radiotracers (which presented minor adverse effects even when administered in the microgram scale) specific radioactivity for radiotracers synthesized through the "wet" method became an issue.

With the aim of improving specific radioactivity and to find out which were the major sources of non radioactive carbon, a deep analysis of the different elements involved in the whole synthetic process was carried out. The second paper entitled "*Specific activity of [^{11}C]CH_3I synthesized by the ''wet'' method: Main sources of non-radioactive carbon*" was published in *Applied Radiation and Isotopes* as a result of this work. Using the results of this work, two more publications were prepared in which the process to obtain optimal specific radioactivity values was applied to the preparation of two tracers. These works have not been included in the compendium of the present PhD thesis but are attached as annexes (Annex I and II).

CIC biomaGUNE, the center where the second part of this PhD thesis was developed, is a non-profit research organization created to promote basic scientific research and technological innovation. Thus, the main goals of the radiochemistry laboratory at CIC biomaGUNE are (i) the development of new imaging agents with potential applications in PET and SPECT and (ii) the development of new radioactive precursors with application in the manufacture of complex labeled structures.

Taking advantage of prospective studies carried out by some members of our research group related to the preparation of ^{13}N-labeled compounds, this work was continued and research was focused in the development of new labeling strategies for the production on ^{13}N-labeled species. As it has been stated in the introduction, not much work has been published up to date related to the use of nitrogen-13 as labeling agent, although there is a great variety of biologically active compounds containing nitrogen in their structure. The possibility to label such compounds with a positron emitter would mean an excellent tool for giving further understanding of their specific biological and/or physiological role *in vivo*.

Most of the work historically carried out with nitrogen-13 has been focused in the production and further use of [^{13}N]NH_4^+ as labeling agent. In the current PhD thesis, the work has been focused in increasing the variety of radioactive precursors containing nitrogen-13 and its automated and efficient production. Specifically, efforts have been oriented to improve the production of [^{13}N]NO_3^- and [^{13}N]NO_2^- and further application to the radiosynthesis of ^{13}N-labeled nitrosothiols and nitrosamines. From this work, two papers concerning the radiosynthesis of ^{13}N-labeled nitrosothiols (entitled, respectively, *"Synthesis of S-[^{13}N]nitrosoglutathione (^{13}N-GSNO) as a new potential PET imaging*

agent" and "Fully automated synthesis of ^{13}N-labeled nitrosothiols", published in *Applied Radiation and Isotopes* and *Tetrahedron Letters*, respectively) and one paper regarding the synthesis of ^{13}N-labeled nitrosamines (entitled "*Efficient system for the preparation of [^{13}N]labeled nitrosamines"* published in *Bioorganic and Medicinal Chemistry Letters*) have been published. Very recently, a new strategy for the preparation of ^{13}N-labeled azo compounds (with potential application as β-amiloyd imaging agents) has been developed. From this work, one paper (entittled "*A convenient synthesis for the preparation of ^{13}N-labeled azo compounds: A new route for the preparation of PET probes for in vivo amyloid imaging*") has been written and is ready for submission to *European Journal of Medicinal Chemistry*.

2.2. OBJECTIVES

The objectives of this PhD thesis are:

1. To develop a synthetic strategy for the automated, robust and efficient production of L-[methyl-^{11}C]methionine.
2. To study the main sources of non-radioactive carbon in the production of [^{11}C]CH_3I through the "wet" method.
3. To establish general guidelines for the optimization of specific radioactivity values in the production of ^{11}C-labeled structures through methylation via the "wet" method.
4. To develop a fast, efficient and reproducible method for the production of [^{13}N]NO_2^- from cyclotron generated [^{13}N]NO_3^-.
5. To develop synthetic strategies for the synthesis of ^{13}N-labeled nitrosothiols.
6. To develop synthetic strategies for the synthesis of ^{13}N-labeled nitrosamines.
7. To develop synthetic strategies for the synthesis of ^{13}N-labeled azo compounds.
8. To design and implement an automatic remote controlled synthesis module for the production of ^{13}N-labeled structures using [^{13}N]NO_2^- as radioactive precursor.

3. RESULTS AND DISCUSSION

3.1. PAPER 1: IN LOOP SYNTHESIS OF L-[METHYL-^{11}C]METHIONINE

3.1.1. Introduction

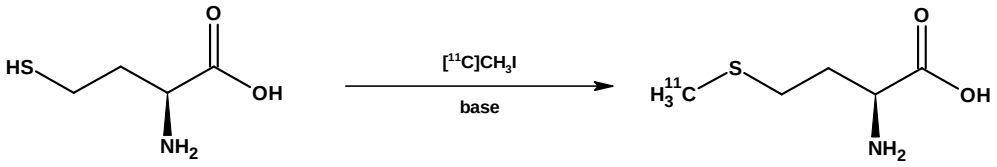
In many radiosynthesis with carbon-11, the radioactive precursor (as [^{11}C]CH_3I or [^{11}C]CH_3OTf) is introduced (via gas-phase) into the precursor solution. Many attempts to streamline the process have revolved around the idea of eliminating the traditional "*solution in reaction vial*", where the [^{11}C]precursor is distilled into a vessel containing solvent, precursor and base (or catalyst, when is needed). In this methodology, cooling of the reactor is usually required for trapping [^{11}C]precursor. After that, the reactor has to be heated in order to perform the synthesis and finally the reaction is quenched. When HPLC is needed for purification, the vessel content has to be transferred to the HPLC system.[1]

An innovative technique was described to avoid manual steps and changes of temperature in the reactor, as well as to avoid the need of transfering the reaction mixture from the vial to the purification system.[2] This method is called *captive solvent chemistry*. The main objective was to develop a solid support to trap the [^{11}C]precursor and the reagents together, eliminating the need of a reaction vessel with a septa and needles. In the *captive solvent chemistry*, reactions could be conveniently carried out in a thin tubing loop. Commonly, this tubing is directly attached to the HPLC injector for easy purification after reaction.

In recent years, this technique has been implemented in the synthesis of a variety of PET radiotracers.[3] This method has two important advantages: (i) good efficiency of [^{11}C]CH_3I trapping into the loop without cooling and (ii) rapid methylation reactions without heating.[4] Until 2004, only DMF and DMSO had been reported as solvents for *in loop* methylation reactions. This fact is mainly due to: (i) [^{11}C]methylation reaction is a nucleophilic substitution and polar aprotic solvents are suitable to carry out these reactions, and (ii) [^{11}C]CH_3I is distilled under an inert gas stream towards the reaction loop (pre-charged with a solution of the precursor) and the loop is open to the atmosphere during reaction. Thus, high boiling point solvents are anticipated to be ideal to prevent evaporation during [^{11}C]CH_3I trapping and reaction with the precursor. However, sometimes these solvents are not suitable for the radiosynthesis of some specific compounds, as reported by Klein and Holschbach 2001,[5] since the sulfur atom

in DMSO is a nucleophilic center and the solvent itself may compete with the non-radioactive precursor.

In the particular case of the here reported work, the *captive solvent method* needed to be applied to the preparation of L-[methyl-^{11}C]methionine (Scheme 3.1). L-[methyl-^{11}C]methionine is one of the most widely used amino acids to image brain tumors. In brain tumors, the BBB can be functionally altered resulting in an increase of active amino acid transport. With L-[methyl-^{11}C]methionine, quantitative measurements of the specific biochemical properties of tumor cells (at cellular level) or alteration of the BBB may be obtained.[6,7] Because of the important role of L-[methyl-^{11}C]methionine in PET, different routine methods have been developed in order to achieve high reproducibility and to make easy its production and purification. First improvements were made by Pascali *et al.*,[8] who published the possibility to synthesize this radiotracer in a C-18 solid-phase extraction cartridge. More recently, Mitterhauser and co-workers[9] reported an automated system avoiding HPLC purification, which considerably increased the final yields.



Scheme 3.1: Schematic process for the synthesis of L-[methyl-^{11}C]methionine.

Three main reasons forced the application of the *captive solvent method* to the preparation of L-[methyl-^{11}C]methionine: (i) a clinical trial consisting of obtaining brain scans in patients using this radiotracer, had to be carried out. In some occasions, several patients had to be scanned within one day; (ii) the only automated technology available in the IAT-PRBB radiochemistry laboratory was designed to run reactions in an HPLC loop, and (iii) the process used until that moment in the laboratory (semi-manual) followed the guidelines described in the Spanish Pharmacopoeia;[10] thus the non radioactive precursor was dissolved in ethanol and the reaction was carried out in a reactor in the presence of a strong base. In this scenario, changing the reaction solvent could involve time consuming and a long and costly validation process, especially concerning the quality control assays for the final radiotracer.

With the aim of overcoming this full validation process, a new process for the production of L-[methyl-^{11}C]methionine following the *in loop* synthesis and using ethanol as solvent was developed, although ethanol has a low boiling point and risk of evaporation during [^{11}C]CH_3I distillation was foreseen.

3.1.2. Results and discussion

In spite of the problems associated to the use of a solvent with low boiling point, a method in which [^{11}C]CH_3I was trapped successfully in a 2 mL stainless-steel loop filled with water/ethanol 1:1 could be developed. In a typical production, [^{11}C]CH_3I was produced in a Bioscan Module starting from [^{11}C]CO_2 generated in the cyclotron IBA 18/9 via de nuclear reaction $^{14}N(p,\alpha)^{11}C$. [^{11}C]CO_2 was almost quantitatively trapped in a molecular sieve column and then released by heating into a lithium aluminium hydride solution (0.1 M in dry THF) to form the alumminum salt. After evaporation of the solvent, hydriodic acid (57% aqueous solution) was added. [^{11}C]CH_3OH was formed in this step, undergoing under heating [^{11}C]CH_3I which was distilled into the loop, previously charged with the precursor solution (1 mg L-homocysteine solved in 80 µL of 0.5 M sodium hydroxide solution in water/ethanol 1:1). After complete trapping of [^{11}C]CH_3I, the reaction was carried out in 1 min at room temperature. The purification step consisted of pushing the reconstituted final solution with 7.5 mL of physiologic saline solution through a C18 cartridge (where unreacted [^{11}C]CH_3I was trapped). Finally, sodium phosphate was added to neutralize sodium hydroxide. To perform the last step (purification and formulation) an automated module was designed and implemented (see Figure 2, Paper 1).

The *in loop* method allowed the preparation of L-[methyl-^{11}C]methionine with good radiochemical yield (38.4 ± 4.1 % EOS). Specific radioactivity values were 28.9 ± 12 GBq/µmol EOS, and the fact that the reaction was carried out in low boiling point solvents did not decrease neither the yield nor the reproducibility of the reaction. Quality control confirmed the chemical and radiochemical purity of the radiotracer, and all analytical parameters were within the specifications of Spanish Pharmacopoeia. Surprisingly, ethanol concentration in the final radiotracer was lower than expected (1360 ± 270 mg/L), due to partial evaporation while [^{11}C]CH_3I was distilled. Enantiomeric purity was determined by chiral HPLC and was > 99% in all cases (Figure 1, Paper 1).

3.1.3. Conclusions

The reaction of [^{11}C]CH_3I (produced via the "wet" method) with a solution of the non radioactive precursor (L-homocysteine) in water/ethanol under basic conditions permits the synthesis of L-[methyl-^{11}C]methionine in good radiochemical yields (38.4 ± 4.1%, EOS) in a fully automated process by using the *captive solvent method*. The simplicity of the process and the implementation of an adequate cleaning sequence between consecutive runs permits multiple productions within one day with minimal radiological exposure.

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PAPER 1: New method for routine production of L-[methyl-^{11}C]methionine: *in loop* synthesis

Gómez, V; Gispert, J.D; Amador, V; Llop, J; New method for routine production of L-[methyl-^{11}C]methionine: in loop synthesis; *J. Label. Compd. Radiopharm.*; **2008**; 51 (1), 83-86. DOI: 10.1002/jlcr.1483.

3.2. PAPER 2: IMPROVING SPECIFIC RADIOACTIVITY

3.2.1. Introduction

As it was reported in the paper entitled "*New method for routine production of L-[methyl-^{11}C]methionine: in loop synthesis*", the *captive solvent method* is suitable for the production of L-[methyl-^{11}C]methionine with good radiochemical yields. This methodology was extended to the preparation of other ^{11}C-labeled radiotracers which required purification by HPLC.

However, and as can be seen in the results obtained in the previous work (Paper 1) relatively low values of specific radioactivity values (average of 28.9 ± 12 GBq/µmol, EOS) were obtained in the production of L-[methyl-^{11}C]methionine, with total production time of 12 minutes. These values led to the conclusion that specific radioactivity (corrected to the end of bombardment) was 43.5 ± 18 GBq/µmol. Specific radioactivity of a radiotracer decreases as radioactivity does; thus, if production time is increased (e.g., due to the need of purification via HPLC, or longer reaction times due to less reactivity of the non radioactive precursor) specific radioactivity of the final radiotracer will decrease. With specific radioactivities around 40 GBq/µmol (EOB), the specific radioactivity of the radiotracer would be around 20, 10 and 5 GBq/µmol for production times of 20, 40 and 60 minutes, respectively.

Specific radioactivity is not an issue in the particular case of L-[methyl-^{11}C]methionine because it is a protein incorporation marker. However, this parameter becomes essential when receptor occupancy studies are carried out and/or when high toxicity molecules are used. The radiochemistry laboratory of IAT-PRBB was involved in two clinical studies in which specific radioactivity had special relevance:

- The first clinical trial was related to striatal D_2 receptor occupancy. In this study, the synthesis of [^{11}C]raclopride (a radiotracer useful to determine the density of striatal dopamine D_2 receptors) with reproducible and high specific radioactivity values was required.
- The second clinical trial was a *First Time in Humans* assay. This study consisted of the implementation of a manufacturing procedure for the preparation of the recently developed GlyT-1 PET radioligand to be applied in: (i) quantification of

GlyT-1 density, (ii) determination of drug-induced receptor occupancy and (iii) elucidation of the role played by GlyT-1 in schizophrenia. Due to the already known toxicity of the radiotracer (minor adverse effects were observed at doses higher than 3 μg in humans), the specific activity became, again, an important parameter.

As stated in the introduction (section 1.2.4.1) there are some alternatives to the "wet" method to produce high specific radioactivity ^{11}C-labeled radiotracers. A well known procedure involves using [^{11}C]CH_4 as starting material instead of [^{11}C]CO_2 to produce [^{11}C]CH_3I through the gas-phase method.[1] Nevertheless, the technology to run the reaction by using this methodology was not available in the IAT-PRBB laboratory, while a very well implemented system to produce [^{11}C]CH_3I by using the "wet" method had been implemented; thus, there was a need to search for the main sources of non radioactive CO_2 in the production of [^{11}C]CH_3I via the "wet" method in our configuration with the aim of minimizing them and obtaining radiotracers with the best possible specific radioactivity.

Although the "wet" method is, in general terms, the preferred method to perform [^{11}C]methylation reactions (it is a well established reaction and offers good radiochemical yields) and the fact that increasing specific radioactivity is always highly desirable, from the early 1980s only a few works have evaluated the factors that potentially affect the presence of the non-radioactive specie, and there are still some controversial about the sources of non-radioactive carbon. Iwata *et al*.[2] concluded that carbon-12 comes from THF traces after the evaporation step. However, Zhang and Suzuki[3] pointed towards the $LiAlH_4$ solution as a main source of carbon-12, while Matarrase *et al.*[4] asserted that the major contribution comes from the [^{11}C]CO_2 production in the cyclotron. These differences suggest that the sources of contamination are extremely dependent on each particular configuration (cyclotron and targets, transfer lines, synthesis module, synthesis protocol, etc.) which differs from site to site.

3.2.2. Results and discussion

With the aim of minimizing the sources of non-radioactive carbon in the particular configuration of IAT-PRBB, we planned different experiments were planned in order to assess the contribution of each individual element to the final amount of unlabeled specie. Thus, partial runs to investigate the contribution to the decrease in specific

activity of (i) the synthesis module (including reagents and molecular sieve), (ii) target gas and (iii) irradiation process were performed.

When the contamination due to the synthesis module and reagents was investigated, only 15.5 ± 2.7 nmol of non-radioactive CH_3I were obtained (Figure 2a, Paper 2). This value is lower than the one reported by Iwata *et al*. (0.2 μmol), which suggested that in our configuration the quality of the reagents was appropriate and the evaporation step was correctly optimized. In a second step, the contribution of absorbed CO_2 in the molecular sieve was evaluated. In these experiments, 45.8 ± 11.7 nmol of CH_3I were obtained (Figure 2a, Paper 2). These experiments were performed after preconditioning the molecular sieve column at 250 ºC for one hour to release any absorbed CO_2 before starting the syntheses. Without preconditioning, values above 300 nmol were found. In a third step, the contribution from the nitrogen/oxygen gas mixture, the target chamber and the transfer line were investigated. The individual contribution of these factors was 32.7 ± 24 nmol (Figure 2b, Paper 2). In the last step, the contribution of the irradiation process was assessed. In this case, 459.4 ± 209.8, 294.9 ± 253.8, 74.6 ± 84.5 and 159.8 ± 87.5 nmol of CO_2 (Figure 2b, Paper 2) were generated during first, second, third and fourth-tenth μAh of bombardment, respectively (target current: 24 μA).

In view of these results and taking into account that non radioactive CO_2 was coming, mainly, from the target and from the molecular sieve column (when it was not pre-conditioned) general procedures to be performed routinely before starting a production were implemented, including:

- Loading/unloading the target in order to purge all the CO_2 present in the target chamber and/or in the transfer line.
- Loading/bombarding/unloading the target to eliminate, as a result of the combustion, all the carrier carbon compounds present in the target chamber.
- Keeping the target pressurized between productions to prevent the external contamination.
- Preconditioning the molecular sieve for one hour with the aim of desorbing the CO_2.
- Cleaning and drying carefully the synthesis module to avoid atmospheric contamination.

Performing these simple steps before any single production of ^{11}C-labeled compounds via the "wet" method allowed us to produce [^{11}C]CH_3I with high specific activity (above 200 GBq/µmol EOS).

This substantial improvement in the specific radioactivity was essential to run two different projects, one in the clinical and one in the preclinical environment.

In the first study, [^{11}C]raclopride scans were performed to evaluate antipsychotic-induced D_2 receptor occupancy values in twenty schizophrenic patients and ten healthy volunteers. Subjects were also scanned with [^{123}I]IBZM in random order on different days within a week to compare the results obtained by PET and by SPECT, respectively. To minimize differences among different scans, specific radioactivity of both radiotracers had to be maintained constant. [^{123}I]IBZM was purchased from Amersham Health, but [^{11}C]raclopride was prepared by [^{11}C]methylation of the precursor desmethyl-raclopride with [^{11}C]CH_3I, which was synthesized through the "wet" method. By applying the general procedures stated above, not only high but also reproducible values of specific radioactivity (23.2 ± 3.2 GBq/µmol, administration time >50 min after EOB) were obtained. The results from this study were published in a paper entitled "*Within-subject comparison of striatal D_2 receptor occupancy measurements using [^{123}I]IBZM SPECT and [^{11}C]Raclopride PET*" (Annex I).

In the second study, inflammatory response after transient focal cerebral ischemia was determined in rats with PET using the radiotracer [^{11}C]PK11195 (a peripheral benzodiazepine receptor ligand), which was also prepared by methylation of the non radioactive precursor with [^{11}C]CH_3I, synthesized through the "wet" method. Due to the (expected) low PBRs density in the rat brain, specific radioactivity had to be maintained as high and constant as possible. Again, specific radioactivity values of 25.0 ± 2.8 GBq/µmol (administration time >50 min after EOB) were obtained. The results from this study were published in a paper entitled "*Imaging brain inflammation with [^{11}C]PK11195 by PET and induction of the peripheral-type benzodiazepine receptor after transient focal ischemia in rats*" (Annex II).

3.2.3. Conclusions

The main contribution to non-radioactive CO_2 contamination in the final product is attributable to the production of this specie during irradiation, probably due to

combustion of carbon carriers due to high temperature and pressure. The application of some routine protocols before, during and after synthesis processes allows the preparation of ^{11}C-labeled radiotracers with high specific activity (~200 GBq/μmol, EOB).

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PAPER 2: Specific activity of [^{11}C]CH_3I synthesized by the ‘‘wet’’ method: Main sources of non-radioactive carbon

Gómez-Vallejo, V.; Llop, J.; Specific activity of [^{11}C]CH_3I synthesized by the “wet” method: main sources of non-radioactive carbon; *Appl. Radiat. Isot.*; **2009**; 67, 111-114. DOI: 10.1016/j.apradiso.2008.09.012.

3.3. PAPERS 3-4: SYNTHESIS OF ^{13}N-LABELED NO DONORS

3.3.1. Introduction

Nitric oxide ($NO^{\bullet}$) is a free radical which easily diffuses across cell membranes. The presence of the unpaired electron gives paramagnetic properties to the molecule, prevents its dimerization and increases its reactivity with a variety of atoms and free radicals.

$NO^{\bullet}$ is produced *in vivo* by a group of enzymes called nitric oxide synthases (NOS). There are three main isoforms of the NOS enzyme: endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS). Generation of $NO^{\bullet}$ by eNOS and nNOS occurs in short times (seconds) but only relatively small quantities (picomolar range) of $NO^{\bullet}$ are produced. In contrast, the activity of iNOS is transcriptionally regulated by cytokines and other pro-inflammatory stimuli; maximum induction of iNOS takes several hours and much higher (nanomolar) levels of $NO^{\bullet}$ are generated.[1,2] The fact that $NO^{\bullet}$ can be produced by two kind of enzymes (constitutive and inducible) causes that $NO^{\bullet}$ plays different roles (like neurotransmission, immune regulation, vascular smooth muscle relaxation and inhibition of platelet aggregation) depending on the origin of its formation.

The importance of $NO^{\bullet}$ has promoted a lot of efforts in the development of new drugs able to generate $NO^{\bullet}$ directly or indirectly for therapeutic purposes. These drugs are called $NO^{\bullet}$ donors and they may play a role in several physiological and pathophysiological processes.

There is evidence of therapeutic benefit with $NO^{\bullet}$ donor drugs. Thus, organic nitrates, which breakdown to form $NO^{\bullet}$ within the coronary and systemic blood vessels, have been used as vasodilators for a long time. However, they create tolerance, which limits their use. *S*-nitrosothiols are a promising class of $NO^{\bullet}$ donor drugs which break down to form $NO^{\bullet}$ and the corresponding disulphide. The therapeutic benefit of *S*-nitrosothiols has been reported in ischemic heart disease patients, and they are known to act as anti-platelet agents. Moreover, they do not generate tolerance.

In spite of the potential applications of *S*-nitrosothiols as $NO^{\bullet}$ donor drugs, the exact mechanisms underlying the role developed by this molecule have not been fully

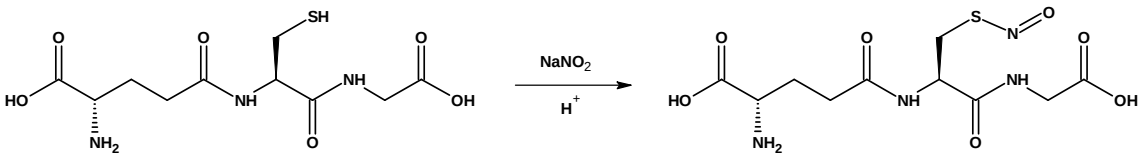
elucidated. Consequently, it would be desirable to have a technique able to detect and quantify the presence of *S*-nitrosothiols and their metabolites *in vivo* and at trace levels.

Because of this, and taking into account that GSNO is one of the most stable *S*-nitrosothiols and that it can be formed *in vivo*, a synthesis process for the preparation of [^{13}N]GSNO, which might allow an *in vivo* characterization of the mechanism of action of exogenously added *S*-nitrosothiols was developed.

With that purpose, the generation of ^{13}N-labeled species in the cyclotron depending on the chemical composition of irradiated species was first studied. Once an adequate precursor for the synthesis of ^{13}N-labeled nitrosothiols ([^{13}N]NO_3^-) was obtained with reasonable yields, its reduction (to [^{13}N]NO_2^-) and further reaction with the adequate non radioactive precursor (Glutathione, GSH) to yield [^{13}N]GSNO were optimized. These results were published in the paper entitled "*Synthesis of S-[^{13}N]nitrosoglutathione (^{13}N-GSNO) as a new potential PET imaging agent"*. The synthetic route was later applied to the production of other *S*-[^{13}N]nitrosothiols and an automatic remote controlled synthesis box was designed and implemented. The results were published in the paper entitled "*Fully automated synthesis of ^{13}N-labeled nitrosothiols*".

3.3.2. Results and discussion

GSNO was first synthesized by the reaction of Glutathione (GSH) with sodium nitrite ($NaNO_2$) in acidic media (Scheme 3.2). To reproduce this synthesis in radioactive conditions, the production of [^{13}N]GSNO needs to be performed by following four steps: (i) generation of nitrogen-13 in the cyclotron as an adequate precursor ([^{13}N]NO_3^- + [^{13}N]NO_2^-) (ii) chemical reduction of [^{13}N]NO_3^- to [^{13}N]NO_2^-; (iii) reaction of [^{13}N]NO_2^- with GSH and (iv) purification of the radiotracer.



Scheme 3.2: Schematic process for the synthesis of GSNO by nitrosation of the thiol under acidic conditions.

The generation of nitrogen-13 in cyclotrons has been traditionally focused on the production of $[^{13}N]NH_4^+$, a radiotracer for myocardial perfusion, through the nuclear reaction $^{16}O(p,\alpha)^{13}N$ by bombarding water with high energy protons. In this case, ethanol is used as scavenger to increase the relative amount of $[^{13}N]NH_4^+$ with respect to $[^{13}N]NO_3^-$ and $[^{13}N]NO_2^-$ and the amount of total radioactivity obtained. In the current work, efforts were focused towards decreasing the relative amount of $[^{13}N]NH_4^+$, and thus some experiments using different quantities of scavenger (0.0, 0.5, 1.0 and 2.0 mM aqueous ethanol solutions) were performed. In all the cases, the relative amount of $[^{13}N]NO_2^-$ was less than 5%, but 86.1 ± 1.1% of radioactivity was obtained as $[^{13}N]NO_3^-$ when purified water was used as irradiated material (see Figure 1A, Paper 3).

In view of these experimental data, purified water was considered to be optimal for the production of $[^{13}N]NO_3^-$ + $[^{13}N]NO_2^-$. However, a reduction step was mandatory to reduce $[^{13}N]NO_3^-$ to $[^{13}N]NO_2^-$. This reduction step was carried out by using a glass column filled with cadmium powder. The composition and length of the column were also optimized to produce a virtually $[^{13}N]NO_3^-$ free solution in the shortest time (see Table 2, Paper 3).

Reaction between GSH and $[^{13}N]NO_2^-$ in acidic media to yield [^{13}N]GSNO was also optimized in terms of acid concentration and reaction time. Generally, higher hydrochloric acid concentrations gave faster reaction rates. Acid concentration of 125 mM (aqueous solution) gave good radiochemical conversion in 1 minute (88.8 ± 1.1%, see Figure 4, Paper 3).

Due to the presence of radioactive impurities (mainly $[^{13}N]NH_4^+$, $[^{13}N]NO_2^-$ and $[^{13}N]NO_3^-$) a purification step was introduced by using solid phase extraction (SPE) cartridges. Good radiochemical yields (24.2 ± 2.0%, end of synthesis) and radiochemical purities (96.4 ± 0.6%, Table 3, Paper 3) were obtained. No consideration was necessary for the non-radioactive precursor in the purification step, because reported clinical trials state that 600-1200 mg of GSH injected intravenously in healthy volunteers and/or patients produced no toxicological effects.[3]

Despite the good radiochemical yields obtained for the preparation of [^{13}N]GSNO in this first work, the routine preparation of ^{13}N-labeled nitrosothiols required: (i) the development of an easy-to-automate synthesis process, (ii) the design and

implementation of an automatic remote controlled synthesis module and (iii) the incorporation of a purification process suitable for the elimination of undesired byproducts or unreacted precursor. With this aim, a solid-phase-supported synthesis method was developed. The main difference with respect to the previous work consisted of trapping the $[^{13}N]NO_2^-$ solution (after reduction step) in an anion exchange cartridge to perform there the nitrosation reaction in a second step. By using this approach, the reaction mixture could be easily pushed into an HPLC system for purification. An automatic remote controlled box was designed and implemented (Figure 3.1). In Table 3.1, the sequence followed for the preparation of the radiotracers is reported.

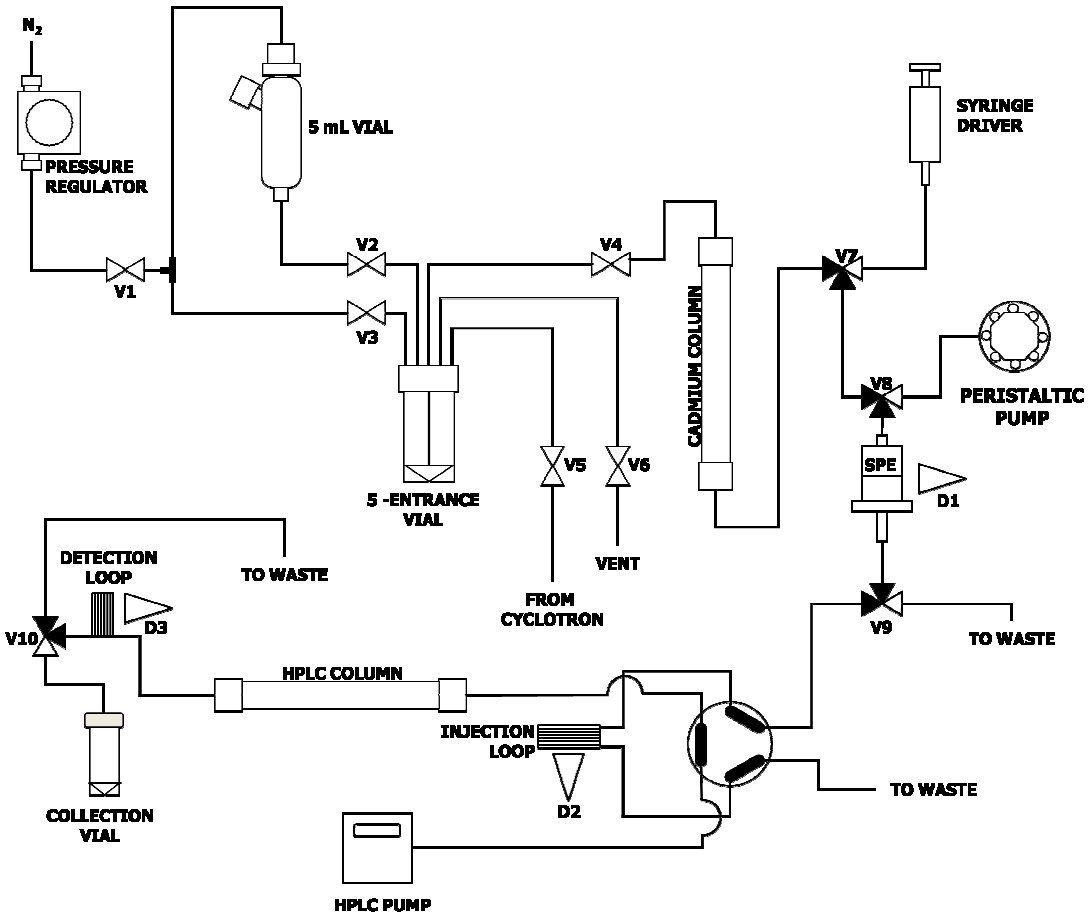


Figure 3.1. Schematic drawing of the automatic remote controlled synthesis module implemented for the synthesis of *S*-[^{13}N]nitrosothiols. V1-V10 are 2 and 3-way valves. All 2-way valves are normally closed. Normally Open paths in 3-way valves are shown in black. Radiation detectors were positioned in the SPE cartridge (D1), injection loop (D2) and detection loop (D3).

The synthesis box showed in Figure 3.1 permitted the manufacture of five *S*-[^{13}N]nitrosothiols (*S*-[^{13}N]nitroso-1-Adamantanethiol, *S*-[^{13}N]nitroso-cyclohexanethiol, *S*-[^{13}N]nitroso-1-Propanethiol, *S*-[^{13}N]GSNO and *S*-[^{13}N]nitroso-2-(N-Acetyl-D-penicillamido)-2-deoxy-1,3,4,6-tetra-O-acetyl-β-D-glucopyranose or [^{13}N]-RIG-200) with high radiochemical yields (from 33.8% to 60.6%, decay corrected, Table 2, Paper 4) in short times (< 13 min, including purification). The purification step (HPLC) ensured chemical and radiochemical purity of the final tracers (Table 2, Paper 4). Stability of the radiotracer was checked by HPLC. In all cases, radiochemical purity was above 95% at 48 min after EOS (Table 3, Paper 3).

Table 3.1. Valve opening sequence and explanation of the synthesis process step by step. Coding for 2-way valves: O, open; C, closed; coding for 3-way valves: L, left; R, right; U, up; D, down.

STEP[a]	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	ACTION
1	**C**	**C**	**C**	**C**	**O**	**O**	**L**	**L**	**L**	**U**	Transfer of ^{13}N solution from cyclotron
2	**O**	**C**	**O**	**O**	**C**	**C**	**R**	**L**	**L**	**U**	Transfer of ^{13}N solution to cadmium column
3	**O**	**C**	**O**	**O**	**C**	**C**	**L**	**L**	**R**	**U**	Elution of ^{13}N solution through SPE cartridge
4	**O**	**O**	**C**	**O**	**C**	**C**	**R**	**L**	**R**	**U**	Fill cadmium column with water
5	**O**	**O**	**C**	**O**	**C**	**C**	**L**	**L**	**R**	**U**	Elution of rinsing water through SPE cartridge
6	**O**	**C**	**O**	**O**	**C**	**C**	**L**	**L**	**R**	**U**	Dry SPE with inert gas
7	**C**	**C**	**C**	**C**	**C**	**C**	**R**	**L**	**R**	**U**	Load precursor in the SPE cartridge[b]
8	**C**	**C**	**C**	**C**	**C**	**C**	**R**	**L**	**R**	**U**	Reaction occurs[c]
9	**C**	**C**	**C**	**C**	**C**	**C**	**L**	**R**	**L**	**U**	Reaction mixture pushed to loop[d]
10	**C**	**C**	**C**	**C**	**C**	**C**	**L**	**R**	**L**	**U**	Start HPLC[e]
11	**C**	**C**	**C**	**C**	**C**	**C**	**L**	**R**	**L**	**D**	Collection of desired fraction[f]

[a] The 5 mL collection vial must be loaded with purified water (2 mL) and the syringe driver must be loaded with the precursor solution before starting the process.[b] Syringe driver is started.[c] Syringe driver is stopped.[d] Peristaltic pump is started.[e] Peristaltic pump is stopped, Rheodyne valve is switched to inject position, HPLC pump is started.[f] When signal is detected in radioactive detector.

Isolated purified fractions were analyzed by HPLC. Co-elution with reference standard compounds confirmed the presence of the desired radiotracers. Although UPLC-MS (ESI-TOF) is not appropriate for the identification of nitrosocompounds (due to its instability) the presence of some labeled species could be confirmed by this technique. GSNO was detected as protonated molecule (m/z = 377.08) and RIG-200 was detected as sodium adduct (m/z = 572.15).

3.3.3. Conclusions

The *in vial reaction* of the radioactive precursor [^{13}N]NO_2^- with GSH in aqueous acidic media leads to the formation of [^{13}N]GSNO with good radiochemical conversion. The introduction of a purification step by solid phase extraction cartridges allows obtaining radiochemically pure radiotracers (RCP > 95%, EOS).

The application of the resin supported method permits the synthesis of ^{13}N-labeled nitrosothiols with high radiochemical conversion values (from 60.3 to 74.5%) and high radiochemical yields (from 33.8% to 60.6%). Chemically and radiochemically pure radiotracers can be obtained by incorporating a purification step by means of semipreparative HPLC.

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PAPER 3: Synthesis of *S*-[^{13}N]nitrosoglutathione (^{13}N-GSNO) as a new potential PET imaging agent

Llop, J.; **Gómez-Vallejo, V.**; Bosque, M.; Quincoces, G.; Peñuelas, I; Synthesis of *S*-[^{13}N]nitrosoglutathione (^{13}N-GSNO) as a new potential PET imaging agent; *Appl. Radiat. Isot*.; **2009**; 69, 95-99. DOI: 10.1016/j.apradiso.2008.09.014.

PAPER 4: Fully automated synthesis of ^{13}N-labeled nitrosothiols

Gómez-Vallejo, V.; Kato, K.; Oliden, I.; Calvo, J.; Baz, Z.; Borrell, J.I.; Llop, J.; Fully automated synthesis of ^{13}N-labeled nitrosothiols; *Tetrahedron Lett.*; **2010**; 51, 2990-2993. DOI: 10.1016/j.tetlet.2010.03.122.

3.4. PAPER 5: SYNTHESIS OF ^{13}N-LABELED NITROSAMINES

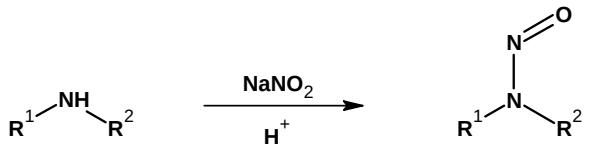
3.4.1. Introduction

In continuation of the work carried out for the synthesis of ^{13}N-labeled nitrosothiols, the application of the radioactive precursor [^{13}N]NO_2^- to the preparation of other ^{13}N-labeled compounds was approached. Despite (in theoretical terms) any organic molecule can be labeled with carbon-11, and although carbon-11 has some advantages with respect to nitrogen-13 as a positron emitter (longer half-life, better known chemistry), developing new strategies for ^{13}N-labeling could be very useful especially concerning the preparation of radiotracers labeled in different positions, which could allow obtaining additional information of specific biological and/or physiological processes.

One clear example of this concept is found in nitrosamines. Nitrosamines are present in the human environment, like in cosmetics, tobacco and drugs, and have also been detected in food items, such as cured meat, bacon, fish and beer. The carcinogenic potential of nitrosamines was first reported by Magee and Barnes[1] in 1956. After that, many other nitrosamines have been tested in preclinical studies and about 90% of them showed potent carcinogenic effects. However, and in spite of the vast literature, the exact mechanisms underlying these above mentioned carcinogenic effects are not fully elucidated. Although *in vitro* and *ex vivo* experiments have been performed to understand the carcinogenic mechanism of tobacco-specific nitrosamines, no *in vivo* studies could be done due to the lack of a technique able to detect, *in vivo* and on real time, the biodistribution of nitrosamines and their metabolites.

Thus, the radioactive precursor [^{13}N]NO_2^- was applied to the preparation of ^{13}N-labeled nitrosamines via *N*-[^{13}N]nitrosation of secondary amines. *N*-nitrosation of amines is a well explored reaction in organic synthesis, and different nitrosating agents including nitrous acid (HNO_2), nitrosyl chloride (NOCl), dinitrogen trioxide (N_2O_3), dinitrogen tetroxide (N_2O_4), nitrosonium tetrafluoroborate and alkyl nitrites have been applied widely for the synthesis of nitrosamines. Another common strategy is the *in situ* generation of the nitrosating agent by treating the amine with nitrite solution in mineral acid (Scheme 3.3). However, there are several drawbacks in this synthetic strategy under radioactive conditions. Mainly: (i) the low nucleophilicity of secondary amines which make the reaction slow in the time scale of nitrogen-13 half-life and (ii) the low

amount (sub-micro molar scale) in which the radioactive precursor ($[^{13}N]NO_2^-$) can be produced in the cyclotron. Thus, new approaches needed to be developed to successfully label nitrosamines.



Scheme 3.3: Synthesis of nitrosamines by treatment of secondary amines with nitrite in acid media.

3.4.2. Results and discussion

First attempts to synthesize ^{13}N-labeled nitrosamines were performed following direct nitrosation of amines by reaction of $[^{13}N]NO_2^-$ with the amine in acidic conditions (like shown in Scheme 3.3). Under non-radioactive conditions, and for a wide range of temperatures (0 ºC ≤ T ≤ 100 ºC) and acid concentrations (1 ≤ pH ≤ 4.5) very low chemical conversions (under 5% in all the cases) were obtained after 10 min of reaction.

Because of these poor results, the resin-supported methodology (in which trapped $[^{13}N]NO_2^-$ was reacted with secondary amines dissolved in aqueous acidic media) was assayed, with similar results. There was thus the need to develop an alternative strategy for the fast, efficient and reproducible preparation of *N*-[^{13}N]nitrosamines.

The method implemented was a combined approach: the first step consisted of trapping $[^{13}N]NO_2^-$ quantitatively in an anion exchange solid phase extraction cartridge. The cartridge was then dried, washed with tetrahydrofuran (THF) and dried again. In a second step, a freshly prepared solution containing Ph_3P/Br_2/secondary amine (1.2: 1.2: 1 ratio) in dry dichloromethane was passed through the cartridge.

This method allowed the efficient preparation of *N*-[^{13}N]nitrosopiperidine, *N*-[^{13}N]nitrosomorpholine, *N*-[^{13}N]nitrosodiisopropylamine and *N*-[^{13}N]-nitrosodiethylamine, with radiochemical conversions above 45% in all cases (see Table 1, Paper 5).

The purification was carried out by elution of the reaction mixture through a C-18 SPE cartridge to selectively retain the desired radiotracer. After rinsing with purified water, *N*-[^{13}N]nitrosamines were eluted with ethanol/water solutions. Unreacted $[^{13}N]NO_2^-$

(major radiochemical impurity) and bromotriphenylphosphonium bromide (major chemical impurity) were removed. Radiochemical yields were between 34.0 and 40.7% (decay corrected), in a synthesis time < 10 min (Table 1, Paper 5). Radiochemical purity was above 99% in all cases.

3.4.3. Conclusions

The reaction of resin trapped $[^{13}N]NO_2^-$ with secondary amines in the presence of Ph_3P and Br_2 allows the synthesis of the corresponding *N*-$[^{13}N]$nitrosamines with excellent radiochemical conversion and good radiochemical yields.

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PAPER 5: Efficient system for the preparation of [^{13}N]labeled nitrosamines

Gómez-Vallejo, V.; Kato, K.; Hanyu, M.; Minegishi, K.; Borrell, J.I.; Llop, J.; Efficient system for the preparation of [^{13}N]labeled nitrosamines; *Bioorg. Med. Chem. Lett.*; **2009**; 19, 1913-1915. DOI: 10.1016/j.bmcl.2009.02.066.

3.5. PAPER 6: SYNTHESIS OF ^{13}N-LABELED AZO COMPOUNDS

3.5.1. Introduction

Some neurodegenerative disorders, like Alzheimer's disease (AD), are associated to the depositions of senile plaques (SPs) and neurofibrillary tangles (NFTs), which contain β-amyloid (Aβ) peptides and highly phosphorylated tau proteins in specific regions of the brain.[1] Especially in AD, the Aβ far overweighs the other amyloid related protein components in a total mass basis in most brain regions,[2] and its neurotoxicity is dependent on aggregation of Aβ into β-sheet fibrils.[3]

The above mentioned β-sheet structure of Aβ deposits has historically facilitated the detection of such deposits. Thus, Congo Red (Figure 3.2) has been used for decades to identify amyloid deposits in post-mortem tissue, due to its intense color and selective affinity for β-sheet structures.[4] However, the detection of β-amyloid plaques in the brain by using Congo Red can be only performed in postmortem studies.

Currently, there are a lot of efforts dedicated to the development of PET and SPECT imaging probes able to cross the Blood Brain Barrier (BBB) and bind to β-Amyloid deposits *in vivo*. The possibility to perform *in vivo* studies to visualize on real time the deposition of β-Amyloid plaques would give further understanding about the evolution of the pathology. Moreover, these tracers could be used as probes for the early diagnose of the pathology and would accelerate the discovery of potential effective therapeutic agents. Thus, in the recent years, a number of groups have developed promising PET probes (Figure 3.3) including [^{11}C]-2-4'(methylaminophenyl)-6-hydroxybenzothiazole ([^{11}C]PIB),[5] [^{11}C]-4-*N*-methylamino-4'hydroxystilbene ([^{11}C]SB-13),[6] [^{18}F]-4-(*N*-methylamino)-4′-(2-(2-(2-fluoroethoxy)ethoxy)ethoxy)-stilbene ([^{18}F]BAY94-9172),[7] [^{11}C]-2-(2-(2-dimethylaminothiazol-5-yl)ethenyl)-6-(2-(fluoro)ethoxy)benzoxazole ([^{11}C]BF-227),[8] and [^{18}F]-2-(1-(2-(*N*-(2-fluoroethyl)-*N*-methyl-amino)naphthalene-6-yl)ethylidene)malo-nonitrile ([^{18}F]-FDDNP).[9]

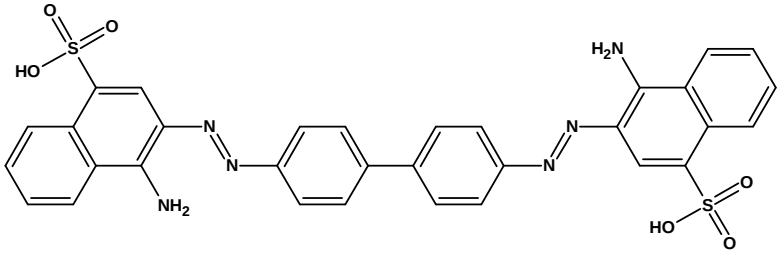

Figure 3.2. Chemical structure of Congo Red, used for the identification of amyloid deposits in post-mortem tissue.

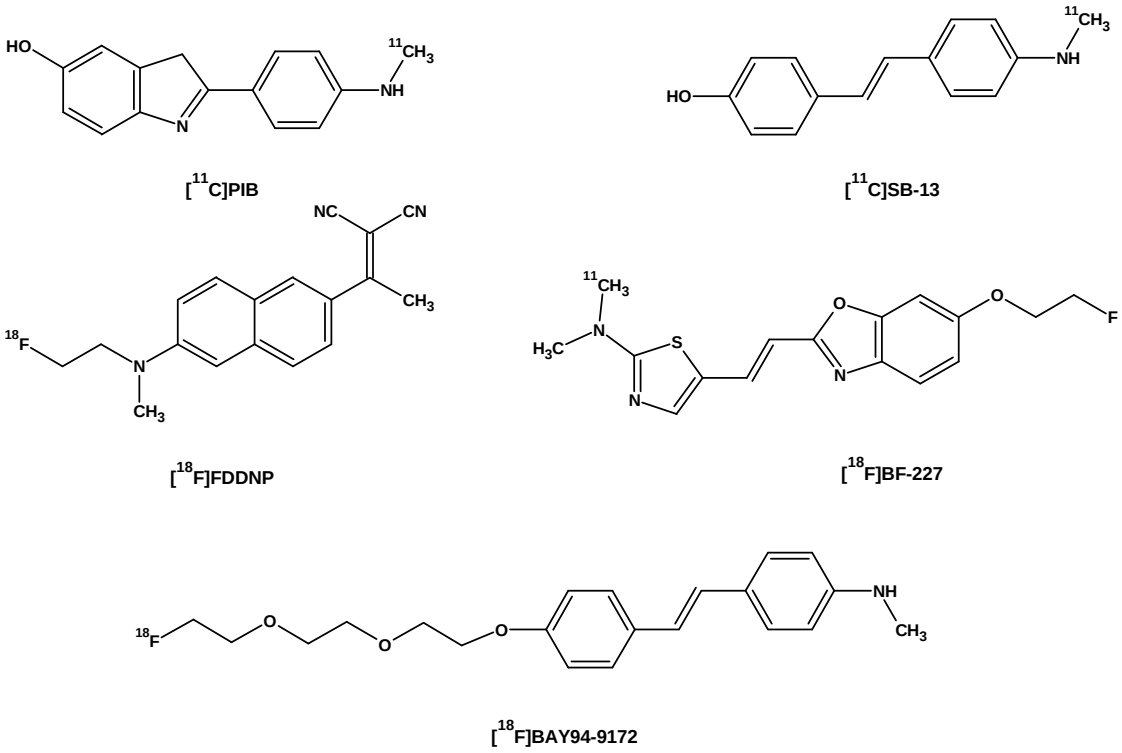

Figure 3.3. Chemical structure of some PET tracers developed for the *in vivo* detection of Aβ plaques: [^{11}C]PIB, [^{11}C]SB-13, [^{18}F]BAY94-9172, [^{11}C]BF-227, and [^{18}F]-FDDNP.

Following the previous work concerning the potential applications of the radioactive precursor [^{13}N]NO_2^- for the preparation of ^{13}N-labeled compounds, and taking advantage of the well-known chemical reaction for the synthesis of azo compounds via diazonium salt formation (Figure 3.4), the synthesis of ^{13}N-labeled azo compounds (which could be potentially used for the *in vivo* detection of Aβ plaques in neurodegenerative disorders) was approached. In non radioactive conditions, the synthetic procedure includes two steps. In a first step, the diazonium salt is formed by the reaction of an aromatic primary amine with NO_2^- in acidic conditions. The second step involves the reaction of the diazonium salt with the adequate aromatic moiety to yield the final compound, which is usually isolated by precipitation.

When applying the synthesis route in radioactive conditions (this is, using [^{13}N]NO_2^- as radioactive precursor) modifications have to be introduced because precipitation is not possible. Thus, a labeling strategy was developed and applied to the preparation of five

different azo compounds. Some modifications to the synthesis box presented in section 3.3.2 were implemented to automatically perform the above mentioned synthesis procedure (see Figure 3 and Table 3, Paper 6).

3.5.2. Results and discussion

The synthesis of ^{13}N-labeled azo compounds was approached by a three steps methodology (see Figure 3.5). In the first step, [^{13}N]NO_3^- was reduced to [^{13}N]NO_2^-, which was trapped in an anion exchange resin cartridge (SPE). In a second step, [^{13}N]NO_2^- was reacted *in situ* (into the SPE) with the aromatic primary amine (dissolved in acidic media) to yield the corresponding ^{13}N-labeled diazonium salt. The labeled compound was then pushed into a vial containing the aromatic non radioactive precursor B. The reaction between the labeled and the unlabeled specie (see reaction times and temperatures, Table 1, Paper 6) led to the formation of the desired azo compound, which was purified by semipreparative HPLC.

Isolated purified fractions were analyzed by HPLC. Co-elution with reference standard compounds confirmed the presence of the desired radiotracers. The presence of labeled species could be confirmed by UPLC-MS (ESI-TOF).

This method allowed the efficient preparation of 4-phenyl-[^{13}N]-azophenol, p-phenyl-[^{13}N]-azoaniline, 4-(Dimethylamino)-[^{13}N]-azobenzene, 4-[4-(Dimethylamino)phenyl-[^{13}N]-azo]benzenesulfonic acid and 4-(4-hydroxy-phenyl-[^{13}N]-azo)-benzenesulfonic acid with radiochemical conversions above 40% in all cases.

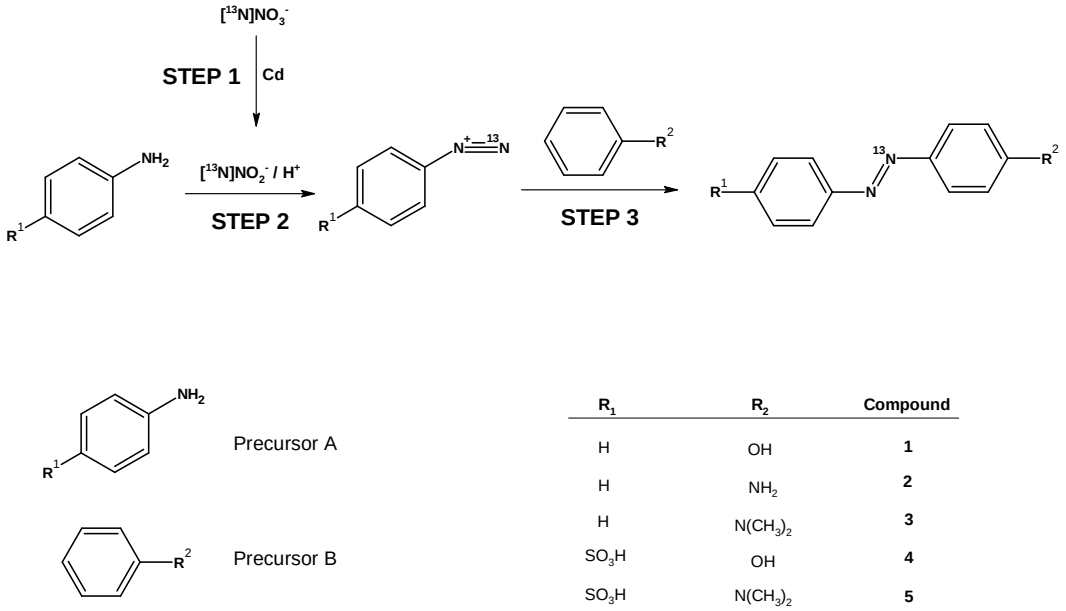

Figure 3.5. Synthesis of ^{13}N-labeled azo compounds through a three steps procedure.

Radiochemical yields were between 20.44 and 47.21% (decay corrected, Table 2, Paper 6). The stability of the resulting radiotracers was checked at 25ºC. Radiochemical purity values >99.9% were obtained 60 minutes after the end of the synthesis in all cases. Specific radioactivity values were checked by HPLC. Values between 4.6 and 8.8 (corrected to the end of the synthesis, Table 2, Paper 6) were obtained.

3.5.3. Conclusions

The reaction of resin trapped $[^{13}N]NO_2^-$ with primary aromatic amines allows the synthesis of the corresponding ^{13}N-labeled diazonium salts. Further reaction with aromatic non radioactive precursors (amines or alcohols) leads to the formation of azo compounds with good radiochemical conversions, acceptable radiochemical yields (20.4 – 47.2%, decay corrected) and excellent radiochemical purity values (> 99.9%).

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PAPER 6: Convenient synthesis of ^{13}N-labeled azo compounds: a new route for the preparation of amyloid imaging PET probes

Gómez-Vallejo, V.; Borrell, J.I.; Llop, J.; A convenient synthesis of ^{13}N-labeled azo compounds: a new route for the preparation of amyloid imaging PET probes; *Eur. J. Med. Chem.*; **submitted**.

4. CONCLUSIONS

4.1. CONCLUSIONS

1- The reaction of [^{11}C]CH_3I (produced via the "wet" method) with a solution of the non radioactive precursor (L-homocysteine) in water/ethanol under basic conditions permits the synthesis of L-[methyl-^{11}C]methionine in good radiochemical yields (38.4 ± 4.1%, EOS) in a fully automated process by using the *captive solvent method*. The simplicity of the process and the implementation of an adequate cleaning sequence between consecutive runs permits multiple productions within one day with minimal radiological exposure.

2- The main contribution to non-radioactive CO_2 contamination in the final product is attributable to the production of this specie during irradiation, probably due to combustion of carbon carriers due to high temperature and pressure. The application of some routine protocols before, during and after synthesis processes allows the preparation of ^{11}C-labeled radiotracers with high specific activity (~200 GBq/μmol, EOB).

3- The *in vial reaction* of the radioactive precursor [^{13}N]NO_2^- with GSH in aqueous acidic media leads to the formation of [^{13}N]GSNO with good radiochemical conversion. The introduction of a purification step by solid phase extraction cartridges allows obtaining radiochemically pure radiotracers (RCP > 95%, EOS).

4- The application of the resin supported method permits the synthesis of ^{13}N-labeled nitrosothiols with high radiochemical conversion values (from 60.3 to 74.5%) and high radiochemical yields (from 33.8% to 60.6%). Chemically and radiochemically pure radiotracers can be obtained by incorporating a purification step by means of semipreparative HPLC.

5- The reaction of resin trapped [^{13}N]NO_2^- with secondary amines in the presence of Ph_3P and Br_2 allows the synthesis of the corresponding *N*-[^{13}N]nitrosamines with excellent radiochemical conversion and good radiochemical yields.

6- The reaction of resin trapped [^{13}N]NO_2^- with primary aromatic amines allowed the synthesis of the corresponding ^{13}N-labeled diazonium salts. Further reaction with aromatic non radioactive precursors (amines or alcohols) lead to the formation of azo compounds with good radiochemical conversions, acceptable radiochemical yields (20.4 – 47.2%, decay corrected) and excellent radiochemical purity values (> 99.9%).

ANNEX I:

Within-subject comparison of striatal D_2 receptor occupancy measurements using [^{123}I]IBZM SPECT and [^{11}C]Raclopride PET

Catafau, A.M.; Suarez, M.; Bullich, S.; Llop, J.; Nucci, G.; Gunn, R.G.; Brittain, C.; Laruelle, M.; On behalf of the Barcelona Clinical Imaging in Psychiatry Group (BCIPG); Within-subject comparison of striatal D_2 receptor occupancy measurements using [^{123}I]IBZM SPECT and [^{11}C]Raclopride PET; *NeuroImage*; **2009**; 46, 447-458. doi:10.1016/j.neuroimage.2009.02.005.

ANNEX II:

Imaging brain inflammation with [^{11}C]PK11195 by PET and induction of the peripheral-type benzodiazepine receptor after focal ischemia in rats

Rojas, S.; Martín, A.; Arranz, M.J.; Pareto, D.; Purroy, J.; Verdaguer, E.;Llop, J.; **Gómez, V.**;Gispert, J.D.; Millán, O.; Chamorro, A.; Planas, A.M.; Imaging brain inflammation with [^{11}C]PK11195 by PET and induction of the peripheral-type benzodiazepine receptor after focal ischemia in rats; *Journal of Cerebral Blood Flow & Metabolism*; **2007**, 27(12), 1975-1986. DOI: 10.1038/sj.jcbfm.9600500.