Chemistry of PET Radiopharmaceuticals: Labelling Strategies

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Abstract

Positron emission tomography (PET) is an imaging technology developed to use compounds labelled with positron-emitting radioisotopes as molecular probes to image and measure biochemical processes of mammalian biology in vivo. Since this area is rapidly developing, the demand for rapid synthetic methods for radiolabelling the molecule of interest is one of the main challenges for the radiochemists. This chapter will provide information about the most common radiolabelling strategies as well as the more recent developments in the synthesis of PET radiopharmaceuticals labelled with fluorine-18, carbon-11, nitrogen-13 and oxygen-15. Since gallium-68 has gained enormous importance in radiopharmacy in the last 10 years, a chapter will highlight the important role of radiolabelling with gallium-68 in clinical radiopharmacy.

4.1 Introduction

Positron emission tomography (PET) is an imaging technology developed to use compounds labelled with positron-emitting radioisotopes as molecular probes to image and measure biochemical processes of mammalian biology in vivo. While imaging tests like X-rays can show

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what the structures inside your body look like, a PET scan produces images that show how your organs work. For instance, a PET scan can show how blood flows to your heart, what areas of your brain are more active or less active and what lesion are metabolically altered or express a certain type of receptors.

These imaging techniques rely on the use of exogenous radioactive probes able to provide a detectable signal. These probes can be designed to be tissue- or receptor-specific and provide a detailed picture of the targeted structure or biological processes under study. Despite the great wealth of information that such probes can provide, the development of the exogenous probes represents an important challenge for organic chemists and radiochemists.

The aim of this chapter is to provide an overview of the most common chemical approaches for the synthesis of PET radiopharmaceuticals in clinical radiopharmacy, starting from the "classic" approach regarding ^{18}F and ^{11}C radiopharmaceuticals to the more recent development of ^{68}Ga and "metal-based" radiopharmaceuticals.

4.2 Positron Emission Tomography: Radionuclides for Labelling PET Radiopharmaceuticals

PET is a non-invasive molecular imaging technique that is used to study and visualise human physiology by the detection of probes labelled by positron-emitting radionuclides. Since some of the positron-emitting radionuclides are low atomic mass elements (e.g. C, N and O) found in biomolecules, it is possible to directly label molecules of interest without interfering with their biological activity. This ability differentiates PET from other techniques, which make use of relatively large molecule which, when attached to the targeting species, can modify its bioactivity.

The development of 2-deoxy-2-^{18}F-fluoro-D-glucose (FDG) for studying energy metabolism [1] together with the establishment of a reliable synthesis [2] and the subsequent demonstration of the high usefulness of FDG tracer to identify metastatic sites in cancer patients were major breakthroughs leading to the development of PET as an indispensable tool in clinical nuclear medicine. Since then, fluorine-18 ($t½ = 110$ min) is the most widely used radionuclide in PET, and it is often referred to as the "radionuclide of choice" because of its favourable physical and nuclear characteristics.

^{18}F can be produced by medical cyclotron in two different molecular forms: the elemental form, $^{18}F_2$, and the ionic form, ^{18}F-fluoride or $^{18}F^-$.

$^{18}F_2$ is obtained from the nuclear reactions of $^{20}Ne(d,\alpha)^{18}F$ or $^{18}O(p,n)^{18}F$ and represents the most common reagent for electrophilic fluorination. Nucleophilic $^{18}F^-$ is commonly produced by the nuclear reaction $^{18}O(p,n)^{18}F$ from enriched $H_2{}^{18}O$. Although nucleophilic fluorination is currently the most common synthetic approach for ^{18}F-radiolabelling, electrophilic fluorination has played an important and historic role in the development of ^{18}F-labelled molecules for PET imaging.

Carbon-11 is an attractive and important positron-emitting radionuclide for labelling molecules of biological interest because of the ubiquitous presence of carbon in natural products and drug compounds. ^{11}C is currently produced either by 11 or 18 MeV medical cyclotron. Production occurs by proton irradiation of pure ^{14}N, which emits an α particle to give ^{11}C, according to the nuclear reaction $^{14}N(p,\alpha)^{11}C$.

The two major ^{11}C precursors used in synthesis are $^{11}CO_2$ and $^{11}CH_4$, which are formed when either small amounts of oxygen or hydrogen, respectively, are present in the target.

Besides ^{11}C and ^{18}F, the radionuclides ^{15}O and ^{13}N are attractive choices for labelling since their stable isotopes are ubiquitous in biologically active organic molecules. The extremely short half-lives of ^{13}N ($t½ = 10$ min) and particularly ^{15}O ($t½ = 2$ min) have imposed limitations with regard to radiosynthetic methods for these two isotopes. ^{15}O is commonly produced in a cyclotron by the reaction $^{14}N(d,n)\ ^{15}O$, whereby irradiation of N_2 with an O_2 content of less than 5 % gives the common precursor $^{15}O_2$.

^{13}N is produced in the cyclotron by the nuclear reaction $^{16}O(p,\alpha)^{13}N$. Since ^{13}N is available as nitrate or nitrite in water (^{13}NOx), subsequent reduction with Devarda's alloy yields the most commonly used ^{13}N source, $^{13}NH_3$ [3–5]. Direct in-target production of $^{13}NH_3$ is usually carried out by addition of ethanol as a scavenger to the target water [6] or the use of methane gas [7]. Using traditional PET isotopes, due to their often short half-lives and rapid clearance, only early time points are available for imaging, leaving the investigation of biological processes, which occur over the duration of hours or days, difficult to explore.

With the continuing development of biological targeting agents, such as proteins, peptides, antibodies and nanoparticles, which demonstrate a range of biological half-lives, the need to produce new radionuclides with half-lives complementary to their biological properties has increased. As a result, the production and radiochemistry of radiometals such as Zr, Y and Cu have been investigated as radionuclide labels for biomolecules since they have the potential to combine their favourable decay characteristics with the biological characteristics of the targeting molecule to become a useful radiopharmaceutical.

Different Cu radionuclides (^{60}Cu half-life, 0.4 h; ^{61}Cu half-life, 3.3 h; ^{64}Cu half-life, 12.7 h) can be cyclotron-produced by "p,n" nuclear reactions using the corresponding enriched Ni isotope(s) as target material: $^{60}Ni(p,n)\ ^{60}Cu$, $^{61}Ni(p,n)\ ^{61}Cu$ and $^{60}Ni\ (p,n)\ ^{64}Cu$. ^{86}Y (half-life 14,7 h) and ^{89}Zr can be produced by a cyclotron via "p,n" nuclear reactions as follows: $^{86}Sr(p,n)^{86}Y$ and $^{89}Y(p,n)^{89}Zr$. Besides radiometals, the radiohalogen ^{124}I can also be used in a variety of PET research applications, such as protein and antibody iodinations, as well as in the design and synthesis of new PET tracers because of its conveniently long half-life ($t½ = 4.2$ days), and well-established labelling chemistry is [8, 9].

An alternative production of positron-emitting radionuclides is via a generator. This is the case for radioisotopes such as ^{68}Ga, ^{82}Rb and ^{62}Cu, produced by $^{68}Ge/^{68}Ga$, $^{82}Sr/^{82}Rb$ and $^{62}Zn/^{62}Cu$ generators, respectively. Generators have the advantage of allowing clinical studies without an on-site cyclotron, or if cyclotron beam time is not available, and they may provide radionuclides and radioactive probes at any time on demand.

^{68}Ga is of great interest as a positron emitter because of some important advantages. It has a physical half-life of 67.71 min, which is compatible with the pharmacokinetics of most radiopharmaceuticals of low molecular weight such as peptides, antibody fragments, aptamers and oligonucleotides.

The impressive success of utilising ^{68}Ga-DOTA-octreotides and PET/CT [10, 11] for staging neuroendocrine tumours (NET) paved the way not only to the clinical acceptance of this particular tracer for imaging NET but also to the realisation of the great potential of the $^{68}Ge/^{68}Ga$ generator for modern nuclear medicine in general. The most important PET radionuclides produced both by cyclotron and generator are summarised in Table 4.1.

4.3 Radiolabelling with Fluorine-18

^{18}F is the most often used radionuclide for diagnostic PET imaging since the decay properties of ^{18}F provide significant advantages. Among the routinely produced positron emitters, the relatively longer half-life of ^{18}F ($T½ = 109.8$ min) poses less constraints on synthesis time and permits longer imaging protocols to investigate processes of slower tracer kinetic up to about 6 h.

Moreover, the relatively longer half-life of ^{18}F also permits the distribution of ^{18}F radiopharmaceuticals to clinical centres that can be reached within a few hours of transport. In recent years, however, there has been a huge increase in the number of biologically active fluoro-organic drugs; the reason for this is directly due to the beneficial effects of simple substitution of an H atom by an F atom on the physical and/or biological properties of the molecule.

Tagging a molecule with ^{18}F in place of a hydrogen atom often does not change its size or shape, and generally metabolically stable compounds are obtained. The unknown effects of introducing an "unnatural" fluorine atom, however,

Table 4.1 Positron-emitting radionuclides

Nuclide	Half-life	Nuclear reaction	Decay mode	$\beta^{\pm}_{mean}$ (KeV)	Target material	Product
Cyclotron-produced PET radionuclides						
^{18}F	109.8 m	$^{18}O(p,n)^{18}F$	β^+ (96.7 %) EC(3.3 %)	249.8	$H_2{}^{18}O$	$^{18}F^-$
		$^{20}Ne(d,\alpha)^{18}F$ $^{18}O(p,n)^{18}F$			Ne/F_2 $^{18}O_2$	$^{18}F_2$
^{11}C	20.33 m	$^{14}N(p,\alpha)^{11}C$	β^+ (99.77 %) EC(0,23 %)	386	N_2+O_2	$^{11}CO_2$
^{13}N	9.96 m	$^{16}O(p,\alpha)^{13}N$	β^+ (99.8 %) EC(0.2 %)	492	H_2O	$^{13}NH_3$
^{15}O	122.24 s	$^{14}N(d,n)^{11}C$	β^+ (99.9 %) EC(0.1 %)	735	N_2+O_2	$^{15}O_2$
^{60}Cu	23.7 m	$^{60}Ni(p,n)^{60}Cu$	β^+ (93 %) EC(7 %)	970	^{60}Ni	^{60}Cu
^{61}Cu	3.33 h	$^{61}Ni(p,n)^{61}Cu$	β^+ (61 %) EC(39 %)	500	^{61}Ni	^{61}Cu
^{64}Cu	12.7 h	$^{64}Ni(p,n)^{64}Cu$	β^+ (17.6 %) EC(43.9 %) β^-(38.5 %)	278 190	^{64}Ni	^{64}Cu
^{86}Y	14.74 h	$^{86}Sr(p,n)^{86}Y$	β^+ (31.9 %) EC(68.1 %)	660	$SrCO_3$	^{86}Y
^{89}Zr	78.41 h	$^{89}Y(p,n)^{89}Zr$	β^+ (22.74 %) EC(77.26 %)	396	Natural ^{89}Y	^{89}Zr
^{124}I	4.18 days	$^{124}Te(p,n)^{124}I$	β^+(22.7 %) EC(77.3 %)	820	^{124}TeO	$^{124}I_2$
Generator-produced PET radionuclides						
^{62}Cu	9.67 m	$^{62}Zn/^{62}Cu$ generator	β^+ (97.83 %) EC (2.17 %)	1319		
^{82}Rb	1.27 m	$^{82}Sr/^{82}Rb$ generator	β^+ (95.43 %)	1472		
^{68}Ga	67.71 m	$^{68}Ge/^{68}Ga$ generator	β^+ (88.91 %) EC(11.09 %)	829.5		

Data source: National Nuclear Data Centre, Brookhaven National Laboratory, based on ENSDF and the Nuclear Wallet Cards

render an analogous compound with potentially changed physicochemical properties and with possibly altered biochemical, pharmacological and toxicological features. This necessitates a careful evaluation of new ^{18}F-labelled compounds with respect to their anticipated use if they are not identical with drugs of known pharmacology [12].

With a few exceptions, radiofluorinations can be classified as either electrophilic or nucleophilic. The electrophilic reactions mainly use molecular fluorine ($^{18}F_2$) of moderately low specific radioactivity, or reagents prepared from it, and include additions to alkenes, reactions with carbanions and especially fluorodehydrogenation and fluorodemetallation. The nucleophilic reactions usually involve no-carrier-added (high specific radioactivity) fluoride ($^{18}F^-$) as its $K^{18}F$-K222 complex and include S_N2-type substitutions in the aliphatic series and S_NAr-type substitutions in the aromatic and heteroaromatic series.

4.3.1 Electrophilic Fluorination

There are *two* major processes for ^{18}F-electrophile production:

1. Historical method consists in using the $^{20}Ne(d,\alpha)^{18}F$ nuclear reaction [13, 14], where the target gas consists of natural abundance

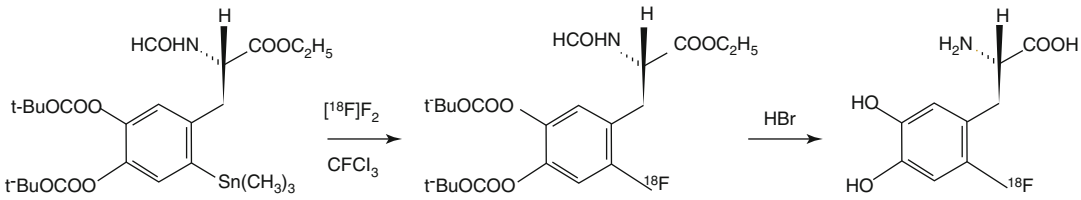

Fig. 4.1 Synthesis of 6-^{18}F-fluoro-L-DOPA

Ne containing 0.1–2 % F_2 as carrier. In this system, the carrier fluorine exchanges with ^{18}F produced by the nuclear reaction to yield ^{18}F-^{19}F molecules. Because of the large excess of $^{19}F_2$ molecules present, the resulting specific activity is very low.

Recovery from this target system is rather slow, ranging from about 50–70 % depending upon conditions such as beam current, length of irradiation and correlates with the carrier concentration. $^{18}F_2$ gas produced in the cyclotron can be directly used for electrophilic fluorination or alternatively be used as a progenitor of other fluorination reagents.

2. The two bombardment method, using $^{18}O(p,n)^{18}F$ nuclear reaction and O_2 gas for production of elemental fluorine $^{18}F_2$ [15], offers the opportunity to produce larger quantities, however, at the expense of being more complicated.

In this approach, the first bombardment is done on passivated nickel target charged with >95 % enriched O_2 and irradiated with 10 MeV protons to give ^{18}F. ^{18}F sticks to the target walls, while $^{18}O_2$ is recovered. Refilling the target with a noble gas (Ne or Kr)/$^{19}F_2$ mixture and a second irradiation allows radiolitically induced isotopic exchange reactions between the adsorbed ^{18}F and the molecular $^{19}F_2$ to generate the $^{18}F_2$. Specific activity is low and can be modulated by decreasing the $^{19}F_2$ concentration in the mixture which unfortunately leads to a decrease of $^{18}F_2$ yield.

Fluoride is a violently reactive gas that erratically reacts with organic molecules to give poor regioselectivity and mixtures of products that result from the addition across the double bond [16]. Considerable steps usually need to be taken to control the very reactive $^{18}F_2$ species. The use of fluorine diluted with an inert gas gives a more controllable reagent that can react selectively with organic compounds.

Another alternative for the use of the reactive $^{18}F_2$ electrophile is to convert it to the less reactive electrophilic moiety, acetyl hypofluorite (AcOF) [16, 17]. This method can be applicative for a direct labelling of small molecules [18] or peptides [17].

Other derivatives that have been used as electrophilic fluorinating reagents are ^{18}F Fluoropyridones [19, 20] and ^{18}F-fluoro-N sulfonamides [21]. These reagents can be used to fluorinate electron-rich substrates (such as alkenes and aryl compounds) by either direct electrophilic substitution or by demetallation reactions using organometallic reagents such as organomercury and organotin reagents. The widest use of ^{18}F-electrophile in clinical radiopharmacy is represented by the synthesis of ^{18}F-DOPA by regioselective, electrophilic fluorodestannylation reaction [22, 23] (Fig. 4.1).

$^{18}F_2$ gas with much higher specific activity can be produced with a "post-target" method developed by Bergman and Solin [24]. This could lead to three orders of magnitude higher improved specific activity of the tracer, but it is extremely difficult to implement in clinical radiopharmacy.

In conclusion, because of the relatively low specific activity caused by the carrier-added method of $^{18}F_2$ production and the poor specificity of labelling with electrophilic reagents, electrophilic ^{18}F-fluorinations are less favoured nowadays, and the general trend is to move to nucleophilic substitution reactions.

4.3.2 Nucleophilic Fluorination

Nucleophilic ^{18}F-fluorination reactions are routinely used to efficiently produce some of the most important PET radiotracers: virtually all

^{18}F-labelled radiopharmaceuticals used in clinical practice are obtained by this synthetic approach. Nucleophilic $^{18}F^-$ is commonly produced by the nuclear reaction $^{18}O(p,n)\ ^{18}F$ from enriched $H_2{}^{18}O$. The present technology for the production of $^{18}F^-$ consists of irradiating a small volume of enriched ^{18}O-H_2O in a metal target with protons of energies from near threshold (approximately 3 MeV) up to energy of the cyclotron, although energies above 13 MeV add little to yield while increasing the heat load on the target. Typical beam currents for research are on the order of 20–40 μA while beam currents for commercial production facilities are in the 60–100 μA range.

The new niobium target has proven to be a low maintenance target with reliable production and a good quality of $^{18}F^-$. Water targets in general can produce higher specific activity $^{18}F^-$ which is mandatory to achieve high specific activity tracers. Specific activities of 185 GBq/μmole or more (theoretical 63 TBq/μmol) have been achieved in routine production [25]. The choice of materials and careful handling are necessary to maintain high specific activity in the final product since stable fluorine can be found in many substances [26].

$^{18}F^-$ from the target is then trapped on an ion-exchange column which allows the recovery of $H_2{}^{18}O$. The trapped $^{18}F^-$ is then eluted from the ion-exchange resin using potassium carbonate in a water/acetonitrile solution. The aqueous $^{18}F^-$ obtained is, however, a poor nucleophile because of its high degree of solvation. The addition of the phase-transfer reagent kryptofix-222 (K222), followed by the removal of water has proven to be crucial in improving the reactivity of the ^{18}F fluoride ion for nucleophilic substitution reactions. The cryptand K222 forms a strong complex with the potassium cation (Fig. 4.2) and leaves the $^{18}F^-$ fluoride ion exposed ("naked") and highly nucleophilic when dissolved in a polar non-protic solvent such as DMF, DMSO, or acetonitrile.

Tetrabutylammonium (TBA) is a phase transfer catalyst alternative to K222. Comparisons between the reactivity of the two catalysts seem to support the hypothesis that TBA fluoride gives

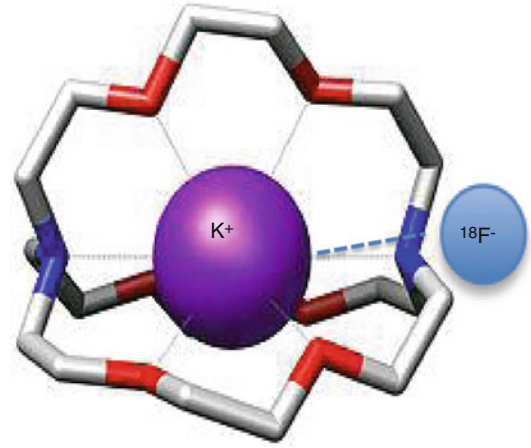

Fig. 4.2 Complexation of a potassium ion (*purple*) by the cryptand kryptofix-222 (K222); *light blue*: fluoride ion

greater yields of fluorinated products in short reaction times (<10 min) [27]. Conversely K222 could cope to metallic impurities from target better than tetraalkylammonium complexes [28].

In addition to ^{18}F-fluoride activation, the reacting precursor molecule is required to have a suitable leaving group and, in the case of aromatic rings, be suitably activated. In contrast to the wide variety of electrophilic reagents that have been developed and used with varying success, there is only one nucleophilic fluorinating reagent: fluoride ion. Nucleophilic fluorination can be performed both on aliphatic (S_N2) and aromatic compounds (S_NAr).

4.3.2.1 Aliphatic Nucleophilic Fluorination

Nucleophilic displacement that is usually used for aliphatic fluorination reactions involves the S_N2 substitution of ^{18}F ion and alkyl substrate containing good leaving groups such as halogens or sulfonic ester. Unlike aromatic substitution reactions, activating groups are not required.

Sulfonates are more reactive than halogens as a leaving group. There is a variety of sulfonate leaving groups available for aliphatic nucleophilic fluorination, such as p-toluenesulfonate (tosylate), methanesulfonate (mesylate), trifluoromethanesulfonate (triflate) and p-nitrosulfonate (nosylate). Among the sulfonates, reactivity increases from tosylate to mesylate and nosylate

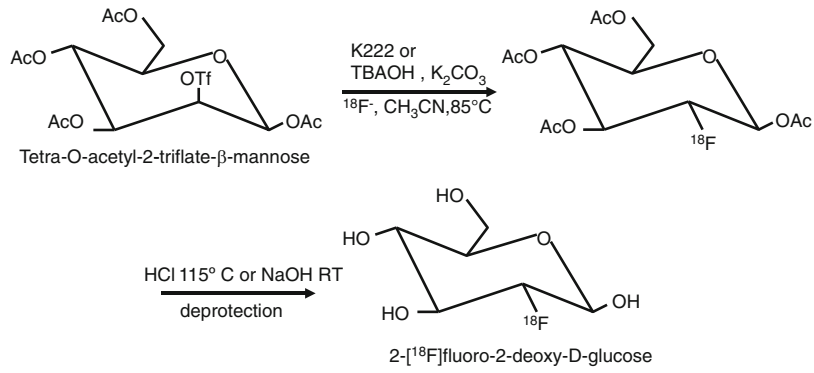

Fig. 4.3 Synthesis of ^{18}F-fluoro-2-deoxy-D--glucose (^{18}F-FDG)

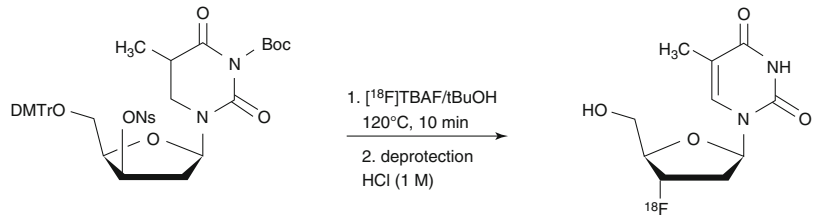

Fig. 4.4 Synthesis of ^{18}F-FLT by reaction in tert-butyl alcohol of the protected using [18F] TBAF as phase transfer catalyst

and to triflate that is the most reactive group for ^{18}F-labelling. Nevertheless, due to its high reactivity, triflate compounds may be unstable during the fluorination reaction temperatures; they cannot tolerate any water and are subject to side reaction such as elimination.

Depending on the stability of the precursor for labelling, the reactivity and simplicity of the incorporation, aliphatic fluorination can be formed by either direct labelling [2, 29] or formation of ^{18}F-fluoroalkyl agents [30, 31]. The main drawback of direct labelling method is the need to protect any potentially competing sites of nucleophilic attack in the molecule (principally acid, alcohol or amine groups), thus resulting in additional synthesis and purification steps.

A good example of aliphatic nucleophilic ^{18}F substitution is the synthesis of ^{18}F-FDG [2] in which the acetyl-protected sugar tetra-O-acetyl-2-triflate-β-mannose is used during the direct ^{18}F-fluorination step. A deprotection of the ester groups completes the synthesis of ^{18}F-FDG. The synthesis of ^{18}F-FDG is now fully automated and can be achieved in approximately 30 min with radiochemical yields greater than 70 % (Fig. 4.3). A wide offer of synthesis modules, also equipped with disposable cassette that includes all the reagents, are available on the market.

Besides ^{18}F-FDG, many radiopharmaceuticals have been synthesised by S_N2 fluorination reactions involving aliphatic substitution such as ^{18}F-fluoro-3-deoxy-L-thymidine (^{18}F-FLT) [32, 33], ^{18}F-fluoroestradiol (^{18}F-FES) [34, 35], ^{18}F-fluoromisonidazole (^{18}F-FMISO) [36] and anti-1-amino-3-^{18}F-fluorocyclobutane-1-carboxylic acid (^{18}F-FACBC) [37], a promising tracer for staging prostate carcinoma.

Even if protic solvents, such as alcohols, are generally not used for nucleophilic substitution reactions because of their ability to solvate the nucleophile and retard its reactivity, the use of ionic solvents, such as tertiary alcohols, as a reaction media for the nucleophilic fluorination with alkali metal fluoride has been described [38, 39]. The protic medium is reported to suppress the formation of by-products and increase the rate of nucleophilic fluorination. An example is the significant improvement in the synthesis yield of ^{18}F-FLT [40] compared to previously described methods using ^{18}F-KF/K222 labelling procedure in aprotic solvents (Fig. 4.4).

^{18}F-fluoroalkyl agents are synthesised by S_N2 reaction of ^{18}F-fluoride with dihalo or disulfonate

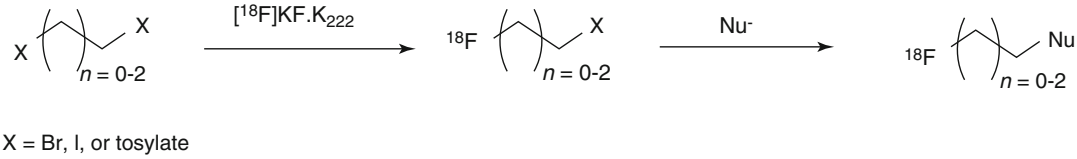

Fig. 4.5 Synthesis of a simple ^{18}F-fluoroaliphatic agents

alkyl starting materials with no-carrier-added fluoride ion in the presence of K_2CO_3/K222 complex or tetrabutylammonium hydroxide in organic solvents such as acetonitrile, o-dichlorobenzene or tetrahydrofuran [30, 31]. ^{18}F-fluoroalkylating agents with methyl, ethyl and propyl carbon backbones together with suitable leaving groups for reaction with nucleophilic species have been prepared (Fig. 4.5).

The large excess of the alkyl starting material compared to the $^{18}F^-$ allows exclusive formation of the mono ^{18}F-fluoroalkyl halide or sulfonate. After the formation of ^{18}F-fluoroalkyl agent, it can be used for the second alkylation reaction. ^{18}F-fluoroalkyl agents can be purified using gas chromatography separation or distillation from the reaction mixture into disposable C_{18} cartridges [31]. Purification of ^{18}F-fluoroalkyl agent before the next alkylation reaction provides more chemically and radiochemically pure product and eliminates non-volatile impurities, therefore increasing the radiochemical yield of the second alkylation reaction.

The most known aliphatic substitution reaction using ^{18}F-fluoroalkyl agents is the radiosynthesis of ^{18}F-fluorocholine, in which dibromomethane is fluorinated to generate ^{18}F-fluorobromomethane, which reacts with dimethylethanolamine to produce ^{18}F-fluorocholine [41]. In some cases, ^{18}F-fluorobromomethane can be converted to the more reactive synthon ^{18}F-fluoromethyltriflate, which would react more efficiently with 2-dimethylethanolamine to give ^{18}F-fluorocholine [31].

4.3.2.2 Aromatic Nucleophilic Fluorination

Introduction of no-carrier-added ^{18}F into aromatic ring is mostly limited to substitution on activated arenes. The presence of "activating" electron-withdrawing groups, such as cyano, trifluoromethyl, aldehydes, ketones and nitro, in the ortho and para positions on the aromatic ring decreases the electron density allowing for a sufficient activation for the nucleophilic substitution [16]. The leaving group most widely used in aromatic nucleophilic fluorination are nitro, quaternary trimethylammonium, alogens and sulphonates such as tosylate, mesylate and triflate. Due to low specific activity of the final product, isotopic exchange, ^{19}F-fluoride to ^{18}F-fluoride, is not used.

Direct aromatic nucleophilic fluorination has been used for obtaining high radiochemical yields and specific activities of ^{18}F-labelled compounds by a simple one-pot method [42]; however, not all the precursors for the labelling can handle high temperature and basic conditions of the fluorination; they may decompose during radiosynthesis. More often, the labelled compounds can be obtained by milder indirect ^{18}F-labelling methods. An example is the use of small ^{18}F-labelled reactive precursors bearing a reactive functional group that can form part of the intrinsic structure of the molecule or act as a "prosthetic label" to the molecule of interest such as proteins or other biomolecules. These ^{18}F-fluoroaromatic groups can be used as ^{18}F-precursor molecules by reacting rapidly and under mild conditions after the initial direct ^{18}F-fluorination step. A range of ^{18}F-fluoroaromatic precursors is shown in Fig. 4.6.

Nitrobenzene derivatives and ^{18}F-fluorobenzaldehydes are the most widely used precursors in the preparation of simple ^{18}F-fluoroaromatic compounds. The nitro group is both an activating group (in the ortho or para positions) and a leaving group under the right conditions. The strongly electron withdrawing nature of the nitrile group provide a high radiochemical yield of ^{18}F-fluorobenzonitrile precursors; moreover, the nitrile group can further be transformed

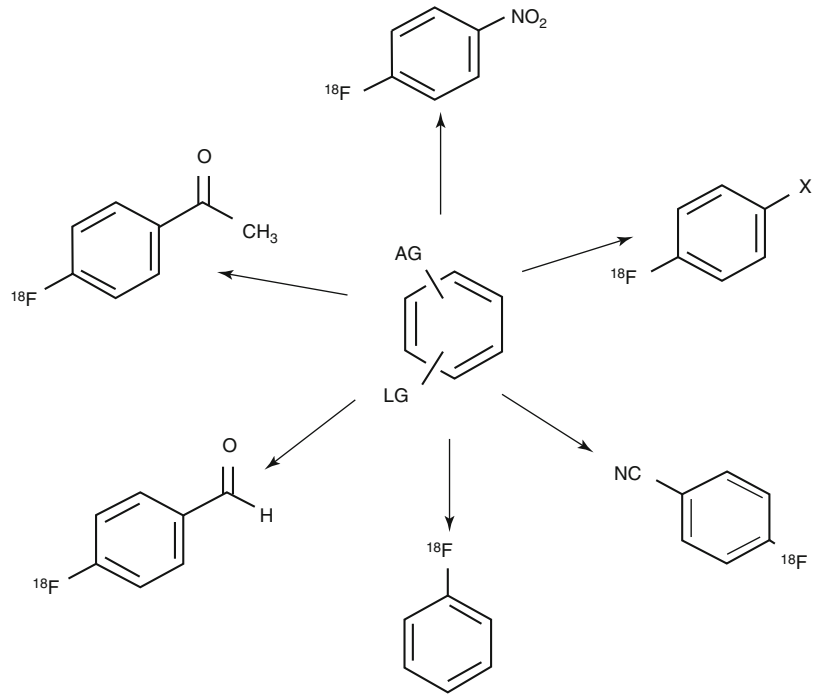

Fig. 4.6 ^{18}F-fluoroaromatic precursors synthesised by direct nucleophilic ^{18}F-substitution (*AG* activating group, *LG* leaving group, *X* I or Br)

into reactive groups such as N-(4-^{18}F-fluorobenzyl)-2-bromoacetamide for the ^{18}F-labelling of peptides and oligonucleotides (see Sect. 4.3.3) [43].

The increased use and versatility of palladium-catalysed cross-coupling reactions in organic chemistry have had an important effect in the field of radiochemistry. The new chemical methods hold the unrealised potential of changing radiotracer design and development since the synthesis of relatively simple ^{18}F-fluoroaryl precursors has, until recently, been surprisingly quite problematic. Recently it has been described the process of the successful translation of a modern Pd-mediated fluorination reaction and the application to PET imaging. Transformation of ^{18}F-fluoride into an electrophilic fluorination reagent provides access to ^{18}F-aryl bonds that would be challenging to synthesise via conventional radiochemistry methods [44].

Aromatic nucleophilic substitution can also be done using diaryliodonium salts and, without the need of electron withdrawing groups, fluoride can be introduced into the arene. The introduction of ^{18}F-fluoride into dihomoaryliodonium salts precursor gives ^{18}F-fluoroarenes and the corresponding iodoarenes [45] and represents an extremely useful alternative for the synthesis of a range of simple ^{18}F-fluoroaromatic compounds in good radiochemical yields and in short reaction times that would be otherwise unobtainable by traditional methods. Very recently, it has been demonstrated that a novel synthetic approach to synthesise ^{18}F-DOPA via nucleophilic substitution of a diaryliodonium salt precursor with ^{18}F-fluoride [46] yielded a product with SA of three orders of magnitude higher than the product obtained by the traditional electrophilic destannylation with $^{18}F_2$ with comparable biological behaviour and imaging properties in neuroendocrine tumour model [47]. The simplicity of the synthesis method, compared with the conventional electrophilic approach along with the possibility of injecting a dose three orders of magnitude lower in comparison with the conventional product, thus dramatically reducing the risk of pharmacologic effects due to the co-administration of ^{19}F-DOPA, appears very promising.

4.3.3 ^{18}F-Labelling of Biomolecules

Biomolecules such as peptides, proteins, affibodies, antibodies and oligonucleotides can be

labelled with fluoride-18 and evaluated for their potential as diagnostic imaging agents. Considering the relatively short ^{18}F half-life, labelling biomolecules with ^{18}F needs a careful consideration of the tracer kinetics since a fast clearance from the blood and high accumulation in target tissue should be required. Out of the biomolecules mentioned above, peptides fit these demands, with rapid clearance from the blood and high concentrations in target tissue. Moreover, the small size of peptides usually makes them relatively easy to synthesise with chemical modification, if needed, and they can often tolerate harsh chemical conditions for radiolabelling.

Larger biomolecules, such as antibodies, have slow pharmacokinetics (slow clearance from the blood) and high nonspecific binding, and they usually have lower uptake in target tissue and sometimes dependent on protein concentrations in the short time frame of the imaging.

Direct nucleophilic fluorination with ^{18}F-fluoride is not generally appropriate with larger peptides and proteins because of the high temperatures, organic solvents and basic conditions needed to obtain a good radiochemical yield. The strategy for labelling peptides and proteins for PET studies is based on the introduction of ^{18}F radionuclide by reaction with suitable prosthetic group under mild reaction condition. There is no general protocol for the synthesis of labelled peptides for PET, and often several labelling procedures need to be explored and optimised to find the best method for a particular peptide.

Many of these prosthetic ^{18}F-groups have been synthesised for targeting amino, carboxylic acid, or sulfhydryl functional groups within the peptide. N-terminal primary amino groups and lysine residues in proteins or peptides have received the greatest attention.

To date, the most common ^{18}F-prosthetic group for labelling biomolecules through the reactive amine group of lysine is the N-succinimidyl-4-^{18}F-fluorobenzoate (^{18}F-SFB) [48]. ^{18}F-SFB can be synthesised in different routes, starting from different precursors, but it requires a time-consuming three-step synthesis. Recently, however, significant advances have been taken to automate its synthesis [49]. Coupling of ^{18}F-SFB with peptides or proteins can be performed under mild pH and temperature conditions in aqueous media (pH 8–9). Acylation with ^{18}F-SFB was shown to be a convenient labelling method in terms of in vivo stability and radiochemical yield.

4-^{18}F-fluorobenzaldehyde (^{18}F-FBA) has also proven to be a versatile labelling reagent that is significantly easier to prepare than ^{18}F-SFB. Chemoselective ^{18}F-labelling, with high radiochemical yields and under mild reaction conditions, of peptides having an amino-oxyl functional group (via the formation of an oxime group) can be achieved using ^{18}F-FBA [50].

Another possible method involves labelling a thiol group (e.g. in cysteine) using ^{18}F-prosthetic group maleimide (^{18}F-maleimide) and its derivatives (^{18}F-FBABM) [51]. The radiosynthesis of ^{18}F-maleimide and its derivatives can also be done through the formation of other ^{18}F-prosthetic groups such as 4-^{18}F-fluorobenzaldehyde or ^{18}F-FBA that could be further reacted with different maleimide precursors to give various derivatives of ^{18}F-maleimide prosthetic groups [51]. 4-^{18}F-fluorobenzaldehyde is capable of forming bond with hydrazino group in the biomolecule, to form hydrazone [52].

^{18}F-glycosylation reactions of amino acids and peptides using chemoselective ^{18}F-fluoro-glycosylated derivatives of ^{18}F-FDG have been reported to be an effective way of introducing an ^{18}F-label [53]. The glycosylation of biomolecules, such as peptides or proteins, has been frequently shown to improve the in vivo kinetics and stability in blood, to enhance bioavailability and BBB permeability and to accelerate the clearance of such glycoconjugates in vivo. Moreover, it has been shown by that glycosylation of peptides with subsequent radiolabelling opens the way to radiotracers with improved in vivo properties. The area of ^{18}F-glycosylation reactions has recently and comprehensively been reviewed [54].

The reaction of 1,3-dipolar cycloaddition (Huisgen reaction), flexible ^{18}F-labelling chemistry known as “click chemistry” and its use in radiochemistry were reported in 2006 [55] for the preparation of ^{18}F-labelled peptide fragments. Especially the Cu(I)-catalysed variant of the Huisgen 1,3-dipolar cycloaddition of terminal

alkynes and azides (Cu-catalysed azide-alkyne cycloaddition, CuAAC) offers a very powerful reaction with high specificity and excellent yields under mild conditions [56].

^{18}F-labelled alkynes were prepared by the ^{18}F-nucleophilic substitution reaction of an alkyne tosylate. The large stoichiometric excess of the CuI catalyst and azide compared to the ^{18}F-alkyne results in good to excellent radiochemical yield for the conjugation step within 10 min at room temperature under basic conditions. The labelled compounds were obtained in high purity by using a simple purification method based on a C_{18} cartridge followed by evaporation of the eluent solvent and excess ^{18}F-fluoroalkyne. As a result, numerous PET tracers have been synthesised using CuAAC in a widespread spectrum of structural varieties of the prosthetic group within the last decade.

In 2007, it has been reported for the first time [57] ^{18}F-PEG derivatives as new ^{18}F-labelled prosthetic click groups. These compounds showed a reduced volatility and increased polarity compared with other ^{18}F-labelled prosthetic groups like ^{18}F-FEA or ^{18}F-fluoroalkynes. ^{18}F-labelled PEGylated prosthetic groups have been widely employed by for labelling peptides and nanoparticles [58–60]. ^{18}F-gluco derivatives for CuAAC-radiolabelling have been developed in order to improve the in vivo behaviour of peptides with respect to blood clearance and stability [54, 61].

However, the need of cytotoxic copper during CuAAC has led to the necessity of alternative fast and copper-free click reaction strategies for radiofluorination and additionally enabling pretargeting approaches in living systems. This has led to the development of copper-free click-labelling reactions which have been focused on derivatives of cyclooctynes and dibenzocyclooctynes [62] or on the possibility to perform Cu-free click reactions given by the inverse electron demand of the Diels-Alder cycloaddition between a cyclooctene and a tetrazine [63]. A detailed review on the development of click chemistry for ^{18}F-labelling has been recently published [64].

The field of click cycloadditions has a major impact in ^{18}F-labelling chemistry. Very mild reaction conditions, excellent efficiency and protection group chemistry not needed are particularly suitable for ^{18}F-labelling especially for complex and sensitive biomolecules such as peptide, proteins and oligonucleotides.

Silicon has a high affinity for F, allowing facile introduction of ^{18}F under mild conditions facilitating direct ^{18}F-labelling to Si-conjugated biomolecules. In silicon-based ^{18}F-fluoride acceptor (Si-FA) moieties, the Si atom is associated with an aromatic group, and ^{18}F-labelling of the Si is achieved by isotopic exchange or substitution of an OH group. The ^{18}F-Si-FA is then conjugated to the biomolecule. Since the side groups attached to the Si atom affect the stability of the ^{18}F-Si bond in the Si-FA moiety to hydrolysis, it has been demonstrated that tert-butyl groups dramatically improved the stability of the ^{18}F-Si complex in the labelling of peptide moieties [65, 66].

Efficient protein labelling by conjugation with ^{18}F-Si-FA has also been demonstrated [67, 68]. The authors [68] suggested that the ^{18}F-labelling of serum albumin for blood pool imaging procedure could be adapted to a simple kit method, avoiding time-consuming purification or toxic catalysts. The use of a boronic ester as a captor of aqueous ^{18}F-fluoride has been suggested as a means of labelling biomolecules in one step for PET imaging. Boroaryl compounds can form stable boron trifluorides, facilitating ^{18}F-fluorination of boronic acids or esters in the presence of ^{18}F-fluoride/KHF2 mixtures. However, optimisation of radiolabelling conditions, as well as determination of the best electron-withdrawing substituents on the aromatic ring to achieve a practically applicable ^{18}F-labelling rate and tracers with ^{18}F-B bonds stable to hydrolysis, is strongly required. Further in vivo studies are needed to fully determine the potential of boron-based fluoride acceptor molecules for ^{18}F-labelling of macromolecules.

Radiolabelling of peptides and macromolecules with metal nuclides, for example, ^{68}Ga, ^{99m}Tc, ^{111}In, is carried out by a simple chelation step. These labelling procedures are easier than the ^{18}F and ^{11}C nucleophilic substitution reactions, do not require the use of the complex instrumentation and could be translated to a kit formulation.

A new method for ^{18}F-labelling was published by McBride et al. [69], which reported on the

direct labelling of chelate-attached peptide with aluminium fluoride ($Al^{18}F$). Initially, ^{18}F-fluoride is attached to aluminium to form $Al^{18}F$, which is further reacted with peptides that contain macrocyclic chelator group such as NOTA (1,4,7-triaza cyclononane-1,4,7-triacetic acid), to form a stable complex of $Al^{18}F$-NOTA-peptide. This method is characterised by short synthesis time, aqueous environment, absence of toxic phase transfer catalysts and lower peptide concentration required for efficient labelling in comparison with ^{18}F-N-succinimidyl 4-(fluoromethyl) benzoate succinyl method [70].

^{18}F-fluoride should be purified with a QMA and the optimum pH adjusted with acetic acid to pH 4.0 and incubated with conjugated peptide at 100°. It has been also demonstrated [71] that ^{18}F-fluoride used directly without QMA purification produced similar labelling yields as QMA-purified ^{18}F-fluoride.

Optimisation of the factors that influence labelling yield including the Al^{3+}/peptide ratio, the presence of hydrophilic organic solvents and antioxidants allowed the translation of the labelling procedure to a kit form [72].

The aluminium fluoride approach has been applied to the labelling of several peptides such as RGD peptides for imaging of integrin αvβ3 [73], bombesin derivatives [74], prostate-specific membrane antigen (PSMA) ligands [75], anti-CEA antibodies [76] and in vivo labelling of serum albumin for PET [77].

The chelation of $Al^{18}F$ with NOTA, NODA or other macrocyclic chelator-conjugated peptides represents the most promising novel approach for convenient ^{18}F-labelling of peptides and biomolecules since it is rather simple and can be developed in kit form, opening up the possibility of carrying out ^{18}F-labelling without the need for expensive PET chemistry facilities.

4.4 Radiolabelling with Carbon-11

Carbon-11 is an attractive PET radionuclide because is an ubiquitous element in biomolecules; thus, ^{11}C-labelled molecules will behave the same, chemically and biologically, as their unlabelled equivalent, preventing any doubts about the effect of introducing an "artificial" PET radionuclide (such as introducing an ^{18}F atom) may have on the biological properties of the compound of interest. Moreover, the possibility to choose from different labelling positions in the same molecule provides the possibility to refine the radiopharmaceutical in terms of metabolic stability and nonspecific background ratio [78]. The short life of ^{11}C also enables comparative PET studies with the same ^{11}C-tracer or with ^{11}C-tracer and ^{18}F-tracer (multitracer studies) in a short time frame with more favourable patient dosimetry [79].

On the other hand, the production of these radiopharmaceuticals must be performed in PET facilities with on-site cyclotrons and should be as fast as possible to reduce the loss of activity due to decay. Although the half-life of ^{11}C is rather short (20.4 min) and limits multistep synthesis, a diverse array of reactions has been applied and developed for the introduction of ^{11}C into target molecules.

One limitation is the small number of ^{11}C-precursors available that can be used directly in synthesis or converted into more reactive secondary precursors prior to the final radiolabelling step. $^{11}CO_2$ and $^{11}CH_4$, which are formed by $^{14}N(p,\alpha)^{11}C$ reaction (see Sect. 4.2) when either small amounts of oxygen or hydrogen, respectively, are present in the target, are the main ^{11}C-precursors used for the synthesis. Almost all ^{11}C-labelled compounds for PET are made from these two major synthons (Fig. 4.7).

$^{11}CO_2$ is produced using a mixture of nitrogen with trace amount to 2 % of oxygen, while $^{11}CH_4$ is produced using a mixture of nitrogen with 5–10 % of hydrogen as gas target. Another way to produce $^{11}CH_4$ is the reduction of $^{11}CO_2$ with hydrogen on a nickel catalyst at high temperature [80]. $^{11}CO_2$ can be recovered from cyclotron and purified by means of cryogenic trapping with liquid nitrogen or by trapping on molecular sieves [81]. $^{11}CH_4$ can be recovered and purified with a Porapak N trap [80]. The use of in-target-produced $^{11}CH_4$ improves the specific activity (SA) [82, 83] but requires a long time to reach

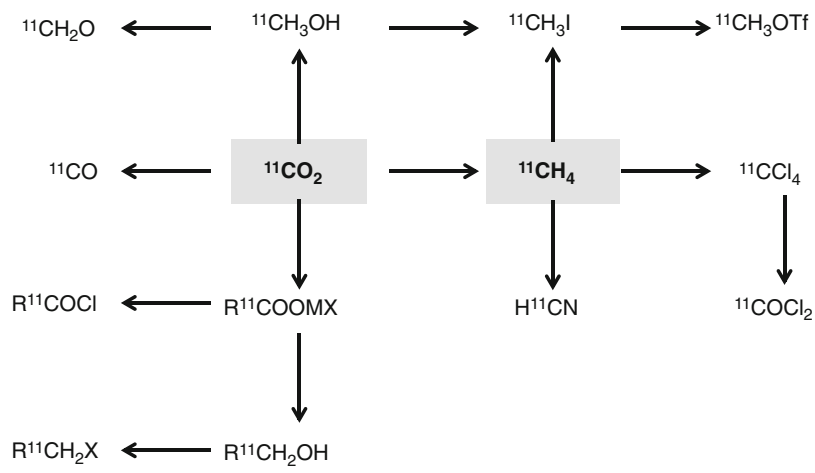

Fig. 4.7 Precursors used in the synthesis of ^{11}C-labelled compounds produced from the two major synthons $^{11}CO_2$ or $^{11}CH_4$

maximum yield, and, in general, total obtained activity is lower compared to $^{11}CO_2$ target [83].

The development of technology has had a pivotal role in the diffusion of ^{11}C tracers with relevant applications mainly in clinical oncology [84] and neurology. The use of fully automated synthesis modules [85–87] also combined with automated HPLC purification [88], microfluidic reactors [89, 90], "on-column" synthesis [91] and automated "loop" synthesis [92, 93] and other technological approaches, have enhanced the speed, efficiency, reliability and safety of radiosynthesis, leading to a final product characterised by pharmaceutical quality.

4.4.1 ^{11}C-Methylation Reactions

^{11}C-methylation leads to the incorporation of $^{11}CH_3$ methyl group into a target compound; it represents the most frequently used method for the introduction of ^{11}C into organic molecules.

^{11}C-methyl iodide ($^{11}CH_3I$) is the most commonly used methylating agent and can be prepared by using two different methodologies: the "wet chemistry", which is based on $^{11}CO_2$ reduction by $LiAlH_4$ and followed by iodination with hydroiodic acid [81, 94, 95], and the "gas-phase chemistry", which synthesises $^{11}CH_3I$ from radical iodination of $^{11}CH_4$ by molecular iodine [80, 96] (Fig. 4.8).

Compared to "gas phase chemistry", "wet chemistry" method generally provides $^{11}CH_3I$ in

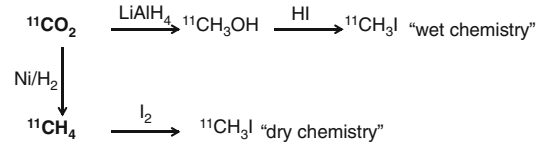

Fig. 4.8 Production of $^{11}CH_3I$ via $LiAlH_4$/HI method (*wet chemistry*) or via iodination of $^{11}CH_4$ (*dry chemistry*)

higher yields (almost twofold higher) and in shorter synthesis time. However, the use of reagents like HI and $LiAlH_4$ makes more difficult the management of the synthesis and cleaning procedures. Moreover, lower $^{11}CH_3I$ SA values are in general obtained since $LiAlH_4$ is a carrier of cold CO_2. Average SA values reached with this method are within 1–5 Ci/μmol decay corrected (DC) at the end of synthesis (EOS). Lowering $LiAlH_4$ amount, using freshly distilled solvent, low target volume and high purity gas are strongly recommended to increase $^{11}CH_3I$ SA [81].

On the contrary, an advantage of the "gas phase chemistry" is the elimination of $LiAlH_4$, which contributes to the higher SA of $^{11}CH_3I$ (even more than 15 Ci/μmol DC at EOS [80]), a clear advantage of this method when higher SA radiopharmaceuticals are needed. Furthermore, elimination of $LiAlH_4$ and HI facilitates cleaning procedures and allows back-to-back syntheses of $^{11}CH_3I$ without adding or changing reagents.

The alternative methylating agent $^{11}CH_3$-methyl triflate ($^{11}CH_3OTf$) has become more

important and more widely used in recent years because of its greater reactivity and volatility [97]; these properties make it ideally suited to rapid methylation reactions [98, 99].

^{11}C-methyl triflate is prepared by passing gaseous $^{11}CH_3$ through a column of silver triflate at 200 °C [97]. The introduction of the $^{11}CH_3$ group into a target molecule is generally carried out by nucleophilic substitution reactions of methyl iodide with a precursor amine, alcohol or thiol group to form the labelled primary or secondary amine, ether or thioether (N-, O- and S-methylation reactions). The synthetic methods used to carry out methylation reactions are relatively straightforward and usually involve simply trapping $^{11}CH_3I$ in a solution of the target precursor and heating for a short time. For some oncological tracers like ^{11}C-choline [91] or ^{11}C-methionine [100] that have an huge impact in clinical PET, methylation reactions are carried out also at room temperature using "on-column" approach.

The "loop" methods involve coating the inside surface of the loop with micromolar amounts of reagent precursor in a suitable solvent and then passing a gaseous stream of $^{11}CH_3I$ or $^{11}CH_3OTf$ through the stainless-steel or plastic/polymer loops as reaction chambers. This is an example of "captive method" where, as previously described in the "on-column" method, the solution of the target precursor is coated on a solid device and the $^{11}CH_3OTf$ is trapped. These methods have found increased use in simple ^{11}C-methylation reactions because of their ease of use, reproducibility and versatility.

The simplicity and speed of the methylation reaction has made it the primary method for the production of ^{11}C-labelled compounds. Many ^{11}C-methylation procedures have been reported not only for oncological tracers like ^{11}C-choline and ^{11}C-methionine but also for the production of ^{11}C tracers for imaging amyloid plagues (^{11}C-PIB [101]), dopamine receptors (^{11}C-raclopride [102], ^{11}C-*N*-methylspiperone [103]), opiate receptors (^{11}C-carfentanil [104]), benzodiazepine receptors (^{11}C-flumazenil [105]) and many others.

The dimethylamine functional group is a common component of the chemical structure of numerous drugs, thus representing an attractive moiety for ^{11}C-labelling. ^{11}C dimethylamine provides an attractive alternative method for the preparation of ^{11}C-methyl compounds with dimethylamine functional groups that avoid the direct use of ^{11}C-methyl iodide [106]. Many other reactions are used for ^{11}C-labelling particularly in research PET radiochemistry. Although the application of these labelling strategies in clinical radiopharmacy is still to be fully established, they should be briefly mentioned.

The vast developments in traditional synthetic chemistry where palladium catalysts are used for the formation of C-C, C-O and C-N bonds have led to a wider application of the palladium-catalysed reactions for C-C bond formation in the synthesis of ^{11}C compounds for PET. Palladium(0)-mediated Stille-type coupling reactions have been the most widely studied of the palladium coupling reactions for the introduction of ^{11}C methyl groups into organic molecules [107]. A review on the application of cross coupling reaction for the preparation of PET radiotracers has been published [108].

4.4.2 ^{11}C-Carbonylation Reactions

Labelling target molecules with ^{11}CO is an attractive strategy which came about for at least three reasons: first, the huge number of carbonyl-containing biologically interesting molecules that have the potential to be synthesised through carbonylation reactions; second, the use of ^{11}CO might be favourable because lower atmospheric concentration of stable carbon monoxide compared with carbon dioxide may result in higher SA of the tracer; third, the ready availability of ^{11}CO through the reduction of $^{11}CO_2$ over zinc or molybdenum.

The most widely applied ^{11}C-carbonylation method is the palladium-mediated carbonylation reaction [109]. Rhodium-mediated carbonylation reactions provide an alternative route for the introduction of ^{11}CO into organic molecules [110]. An exhaustive review of the ^{11}CO chemistry for the labelling of PET tracer covering all the aspects of transition-metal-catalysed carbonylation with ^{11}C has been published [111].

4.4.3 Reactions with Organometallic Grignard Reagents

$^{11}CO_2$ can be treated with organometallic Grignard reagents to form ^{11}C-carboxymagnesium halides and then transformed into ^{11}C-carboxylic acids. Acetate is an important metabolite in the synthesis of cholesterol and lipids. ^{11}C-acetate was initially employed for the study of myocardial metabolism [112, 113] and more recently in oncology for the imaging of prostate cancer [114, 115]. ^{11}C-acetate has been also employed in the study of hepatocarcinoma (HCC) [116, 117], lung cancer [118] and brain tumours [119].

^{11}C-acetate is synthesised by means of ^{11}C-carboxylation reaction of Grignard reagent methylmagnesium chloride or bromide (CH_3MgCl, CH_3MgBr) by cyclotron produced $^{11}CO_2$. Unlike ^{11}C-methylation reactions, the target product $^{11}CO_2$ is directly employed in the labelling step without any further chemical conversion. ^{11}C-carboxylation is then followed by hydrolysis and purification of the product. As regards the synthesis method, $^{11}CO_2$ can be bubbled directly in the Grignard reagent or can be flushed and reacted into a loop of different tubing materials containing methyl magnesium bromide coated onto the internal surface of the loop [93].

^{11}C-palmitate was identified as a valuable radiopharmaceutical for the assessment of myocardial metabolism and function [120]. A method for automated preparation on a commercial synthesis module of ^{11}C-palmitate and ^{11}C-acetate based on Grignard reaction has been described [121].

^{11}C-carboxylic acids obtained by reaction of $^{11}CO_2$ with Grignard reagents can also be converted into the more reactive acid chloride species and treated with amines to form [carbonyl-^{11}C] amides. This method has been used for ^{11}C-labelling at the carbonyl position of the $5HT_{1A}$ receptor ligand WAY100635 [122, 123].

4.5 Radiolabelling with Oxygen-15 and Nitrogen-13

Oxygen-15 and nitrogen-13 represent attractive choice for labelling since their stable isotopes are ubiquitous in biologically active organic molecules. Due to the extremely short half-lives, radiochemical syntheses of more than one reaction step are rarely performed. Simple chemical products such as $C^{15}O_2$, $H_2{}^{15}O$ and $^{13}NH_3$ can be obtained directly from the cyclotron target and used as such or rapidly converted into other simple products (e.g. $C^{15}O_2$ and $C^{15}O$).

$^{15}O_2$ is commonly produced in a cyclotron by the reaction $^{14}N(d,n)^{15}O$ by irradiation of N_2/O_2 mixture ($O_2 < 5\,\%$). $^{15}O_2$ could also be produced by the reaction $^{15}N(p,n)^{15}O$. This reaction could be used in any cyclotron, since it does not need deuterons option, but, on the other side, a ^{15}N recycling system to overcome the high cost of the enriched gas should be implemented. A common application of oxygen-15 is the study of regional cerebral blood flow by using ^{15}O-labelled water [124, 125]. ^{15}O-labelled water is obtained by conversion of $^{15}O_2$ into $H_2{}^{15}O$ by reduction over a platinum or palladium [126, 127] catalyst at high temperature. Other two ways to form ^{15}O-labelled water are available: (1) by the conversion of $^{15}O_2$ into $C^{15}O_2$, which is instantaneously converted after inhalation into $H_2{}^{15}O$ in the lungs by the carbonic anhydrase enzyme, and (2) by bombardment of $H_2{}^{16}O$ with protons according to the $^{16}O(p,pn)^{15}O$ nuclear reaction [128]. This yields $H_2{}^{15}O$ that can be administered intravenously.

The most commonly used ^{13}N source, $^{13}NH_3$, can be obtained by post target reduction of $^{13}NO_x$ (see Sect. 4.2) or by direct in-target production in presence of a scavenger such as ethanol (6) or methane (7). The 10-min half-life of ^{13}N precludes extensive synthetic reactions. In addition, the high positron range (maximum energy of 1.19 MeV, maximum range in water of 5.4 mm) usually leads to low resolution images, especially when compared with those obtained with ^{18}F-labelled radiotracers. Therefore, ^{13}N routine application in PET is limited to simple procedures, such as using ^{13}N-ammonia to measure myocardial blood flow [7, 129]. The developments in ^{13}N chemistry, including different production routes of primary precursors and their applications to the preparation of more complex ^{13}N-labelled molecules as well as current situation and future perspectives, have recently been reviewed [130].

4.6 Radiolabelling with Gallium-68

Fluorine-18, carbon-11, oxygen-15 and nitrogen-15 are radionuclides produced with a cyclotron and their use demands an on-site cyclotron. The half-life of the ^{18}F isotope is long enough to allow transportation of doses to sites several hours away.

An alternative production of positron-emitting radionuclides is via a generator. This is the case for radioisotopes such as ^{68}Ga, ^{82}Rb, ^{62}Cu (Tab.1.2); among them, ^{68}Ga has gained enormous importance in radiopharmacy in the last 10 years. The explosive growth of publications reflecting the success of ^{68}Ga applications is remarkable; rough estimation demonstrates that the number of ^{68}Ga-related scientific articles published during 2011–2012 stands for over 45 % of all publications since 1956 [131].

Gallium-68 is of great interest as a positron emitter because of some important advantages. It has a physical half-life of 67.71 min, which is compatible with the pharmacokinetics of most radiopharmaceuticals of low molecular weight such as antibody fragments, peptides, aptamers and oligonucleotides. ^{68}Ga decays to 88.91 % by positron emission and to 11.09 % via electron capture into stable ^{68}Zn. The average positron energy per disintegration is 829.5 keV (Tab.1.2) which is higher, for example, than that of ^{18}F and potentially leads to a somewhat lower resolution. Moreover, there is a well-established coordination chemistry of Ga^{3+} that allows the development of agents resistant to in vivo transchelation of Ga^{3+}.

The long half-life ($T½$.270.95 days) of the parent ^{68}Ge combined with the half-life of ^{68}Ga ($T½$.67.71 min) makes this pair almost ideal for a generator strategy. The development of the $^{68}Ge/^{68}Ga$ generator has been reviewed in several articles [132–135].

However, there are still some drawbacks for the direct use of the ^{68}Ga eluate in the preparation of radiopharmaceuticals. Among them are measurable activities of the long-lived ^{68}Ge (breakthrough), the high eluate volume and high HCl concentration. In addition, metallic impurities such as Zn^{2+}, generated from the decay of ^{68}Ga, Ti^{4+} or other residuals from the column material, as well as Fe^{3+}, could be present in the eluate. Thus, dedicated procedures to process the eluate from the radionuclide generator to remove the ^{68}Ge breakthrough, to purify from the metal impurities and to minimise the labelling volume of ^{68}Ga radiopharmaceuticals have been described. An anion exchange chromatography-based post-processing has been developed [136]. This strategy separates ^{68}Ge but does not allow for a direct loading of $^{68}Ga^{3+}$ on the anion exchange resin from 0.1 N HCl since it introduces an additional dilution step in 5.5 M HCl, and it does not provide purification of $^{68}Ga^{3+}$ from e.g. Zn^{3+} and Fe^{3+}. Another approach to overcome problems like eluate volume, acidic pH and content of ^{68}Ge and chemical impurities is to fractionate the initial generator eluate [137]. Contents of ^{68}Ge and metallic impurities are minimised because of the lower eluate volume used but in principle not chemically removed prior to the ^{68}Ga-labelling steps.

Cation exchange chromatography-based post-processing procedure consists in the direct transfer of the initial 0.1 N HCl ^{68}Ga eluate to a cation exchanger [138] and a selective elution with acetone/HCl mixtures. This procedure leads to almost complete removal of metallic impurities including ^{68}Ge breakthrough. More details on the purification of the $^{68}Ge/^{68}Ga$ generator eluate are extensively described in a recent review [134].

In aqueous solution, the only stable oxidation state of gallium is +3, where the free hydrated Ga^{3+} ion is stable only under acidic conditions. In the pH range of 3–7, it can hydrolyse to insoluble $Ga(OH)_3$, while at physiological pH, its solubility is high due to the almost exclusive formation of $[Ga(OH)_4]^-$ ions. Ga^{3+} is quite similar to the high spin Fe^{3+} ion with respect to its coordination chemistry; both are 3+ charged with similar ionic radii and the same major coordination number of six.

The Ga^{3+} ion is classified as a hard Lewis acid, forming thermodynamically stable complexes with ligands that are hard Lewis bases, containing oxygen, nitrogen and sulphur donor atoms, such as carboxylate, phosphonate, hydroxamate and amine but also softer functional groups, such

as phenolate and thiol groups, were found to be appropriate. The main requirements for a Ga^{3+} chelate in order to be suitable as a radiopharmaceutical are the thermodynamic stability towards hydrolysis and the kinetic inertness during the period of clinical use in order to avoid ligand exchange with the blood serum protein transferrin. Human transferrin also has a high binding affinity for Ga^{3+} given by log $K_{ST} = 20.3$ [139]. Thus, the complexes should be more stable than the Ga^{3+}-transferrin complex or kinetically inert in order not to exchange with this protein. On the other hand, hydrolysis and formation of the $Ga(OH)_3$ can be avoided in the presence of stabilising weak ligands such as acetate, citrate or HEPES, in the preparation of the complexes.

Several bifunctional chelators that present a functionality that allows covalent coupling to a targeting vector besides binding the metal cation have been proposed and coupled to biomolecules for gallium labelling. They should meet the following criteria:

1. They should chelate the radiometal rapidly and sufficiently when linked to a macromolecule.
2. The chelate should be kinetically stable to demetallation over a pH range of 4–8 and stable in the presence of other serum cations (Ca^{2+}, Zn^{2+}, Mg^{2+}).

One of the most known chelators used for radiometals in +3 oxidation state is the macrocyclic chelator 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA). DOTA and its derivatives are readily obtained from straightforward and convenient synthetic routes and available from commercial suppliers. Complexes obtained from DOTA, DO3A and its derivatives and DO2A and its derivatives have been shown to be sufficiently stable to avoid the loss of the Ga^{3+} core under physiological conditions.

Multiple applications of DOTA and its congeners have been reported in literature; a renaissance of ^{68}Ga radiopharmacy has come with the development of small tumour-affine peptides, most notably those targeting somatostatin receptors for the imaging of neuroendocrine tumours (NET) [136, 140].

The more promising compound was [^{68}Ga-DOTA,Tyr3]-octreotide (^{68}Ga-DOTA-TOC). It showed higher affinity for somatostatin receptor subtype 2 than [^{111}In/^{90}Y]-DOTATOC and also a 2.5-fold higher tumour uptake in a mouse model bearing the sst2-positive AR4-2 J tumour [141]. Other small molecules were labelled with ^{68}Ga, e.g. different somatostatin-based peptides, like DOTA-lanreotide [142], [^{68}Ga-DOTA,1-Nal3]octreotide (^{68}Ga-DOTA-NOC) [143, 144] and [^{68}Ga-DOTA, Tyr3, Thr8] octreotide (^{68}Ga-DOTATATE) [145]. Structural formulae of DOTA-TOC, DOTA-NOC and DOTA-TATE are showed in Fig. 4.9.

These compounds now represent the gold standard in the imaging of NET. It has been demonstrated that ^{68}Ga-DOTANOC PET/CT either affected stage or caused a therapy modification in more than half the patients, thus confirming the clinical role of PET in the management of NET [10]. Because of huge diffusion of these ^{68}Ga-labelled somatostatin analogues in clinical practice, specific guidelines to assist nuclear medicine physicians in recommending, performing, reporting and interpreting the results of somatostatin (SST) receptor PET/CT imaging using ^{68}Ga-DOTA-conjugated peptides have been published [11].

Though readily available and so widespread, DOTA is not intrinsically the most appropriate chelator for to Ga^{3+}, as its K_{ML} and pM values suggest. The thermodynamic stability constant of the Ga^{3+} complex of the tetraaza tetraacetic acid chelator DOTA is much lower (logK = 21.33) [146] than that of the triaza triacetic acid chelator NOTA (logK = 30.98) [147] due to the larger dimensions of its cavity. In contrast to DOTA, the smaller congener NOTA is much more suited for Ga, as its smaller 1,4,7triazacyclononane (TACN) ring apparently allows the formation of multiple five-membered chelate rings with one central Ga^{3+} core, without the intramolecular strain accompanied by the Ga-DOTA complex. NOTA forms slightly distorted octahedral complexes with Ga^{3+}; due to the facial arrangement of donors, the energetic barrier for complexation is significantly lower than with DOTA. Therefore, NOTA readily forms stable complexes with Ga^{3+} already at

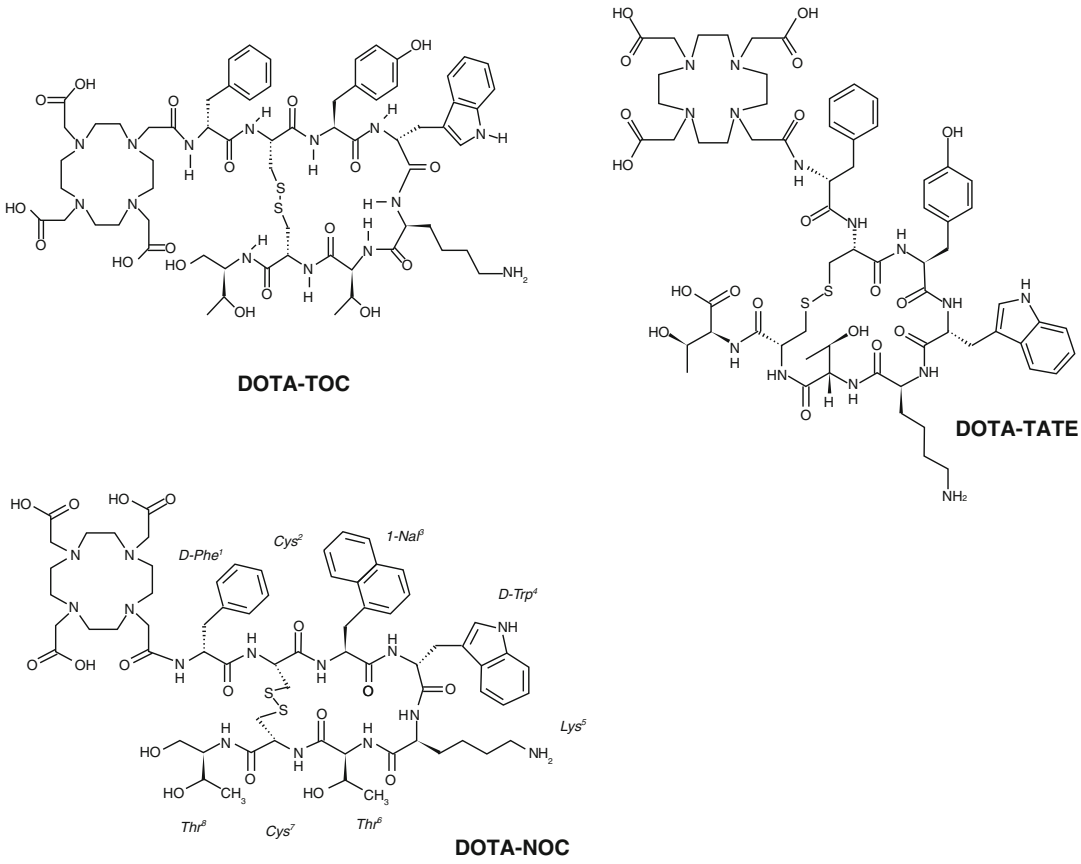

Fig. 4.9 Structural formulae of *DOTA-TOC*, *DOTA-NOC* and *DOTA-TATE*

moderate temperature while the formation of complex Ga-DOTA requires high temperature (80–100 °C) which can be dangerous for compounds like proteins and large molecular weight peptides.

NOTA does not offer the same opportunity of using a spare carboxylate or amine function for conjugation, thus needing chemical modifications to obtain bifunctional derivatives. Because of the huge potential of ^{68}Ga for medical application, several bifunctional derivatives of NOTA have been reported during the last decades [148–151] as well as the effect of different chelators in regard to pharmacokinetics, tumour uptake and retention [152].

Recently, it has been demonstrated that the chelators 1,4,7-triazacyclononane-1,4,7-tris [methyl(2-carboxyethyl)phosphinic acid] (TRAP-Pr) [153] and 1,4,7-triazacyclononane-1-[methyl(2-carboxyethyl) phosphinic acid]-4,7-bis[methyl(2-hydroxymethyl) phosphinic acid] (NOPO) [154] possess markedly improved affinity to Ga^{3+} and higher ^{68}Ga-labelling efficiency. Compared to DOTA and NOTA, quantitative incorporation of $^{68}Ga^{3+}$ into chelates requires smaller concentration of these chelators, thus obtaining TRAP and NOPO-based radiopharmaceuticals with extremely high specific activities [154, 155]. Furthermore, ^{68}Ga-labelling of triazacyclononanetriphosphinates can be performed at lower temperatures and over a broad pH range (0.5–5). Other factors influencing the performance of ^{68}Ga-labelling reactions should be taken into account. One of them is the presence of other metal ions in the ^{68}Ge/^{68}Ga generator eluate. These can compete with $^{68}Ga^{3+}$ for the chelator, thus diminishing the labelling yield, which is particularly problematic in view of the low concentration of the carrier-free $^{68}Ga^{3+}$ in the eluate. Structural

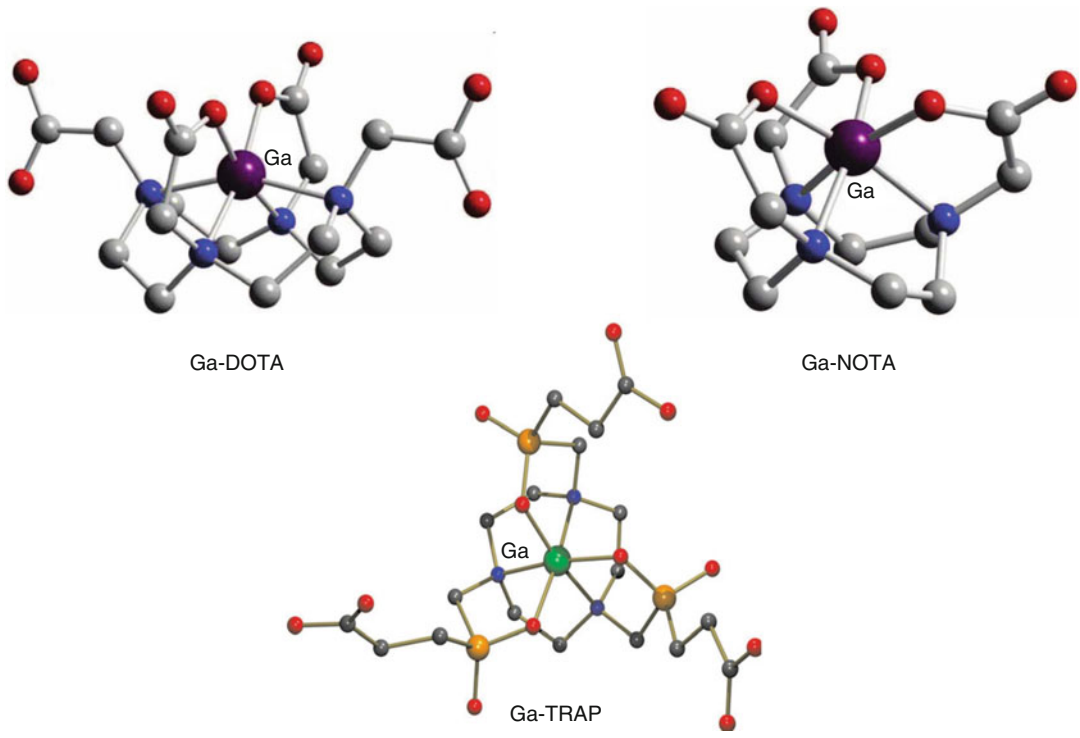

Fig. 4.10 Structural formulae of *Ga-DOTA*, *Ga-NOTA* and *Ga-TRAP* chelates

formulae of Ga-DOTA, Ga-NOTA and Ga-TRAP chelates are shown in Fig. 4.10

For example, the total amount of metal contaminants (Ga, Ge, Zn, Ti, Sn, Fe, Al and Cu) in the eluate of a SnO_2-based generator was reported to be <10 ppm (<3 ppm Zn^{2+}; <1 ppm for each of the other ions) [156]. The most remarkable feature of TRAP, triazacyclononane-phosphinate ligands, seems to rely in their selectivity for Ga^{3+}, rapid Ga^{3+} complexation kinetics with extraordinarily high thermodynamic stability and kinetic inertness of the respective ^{68}Ga chelates in comparison with other class of chelates. These compounds allow also preparation of ditopic Ga^{3+}/Gd^{3+} complex for application as bimodal imaging agent for PET/MRI [157]. Bifunctional derivatives of NOTA, TRAP and NOPO provide high potential for the development of ^{99m}Tc-kit-like formulations.

N,N′-Bis[2-hydroxy-5-(carboxyethyl)-benzyl]ethylenediamine- N,N′- diacetic acid (HBED-CC) was recently proposed as an efficient acyclic 68Ga chelator with fast complexing kinetics and a high in vitro as well as in vivo complex stability [158, 159].

Besides the efficient Ga^{3+} complexing characteristics, HBED-CC was selected because of its lipophilic nature. It was found that the "active binding site" of prostate specific membrane antigen (PSMA) is composed of two structural motifs, one representing a lipophilic pocket and the other interacting with urea-based inhibitors [160].

The simple replacement of HBED-CC by the prominent radiometal chelator DOTA was shown to dramatically reduce the in vivo imaging quality of the respective ^{68}Ga-labelled PSMA-targeted tracer proving that HBED-CC contributes intrinsically to the PSMA binding of the Glu-urea-Lys(Ahx) pharmacophore [161]. ^{68}Ga-labelled Glu-urea-Lys(Ahx)-HBED-CC ([68Ga]Ga-PSMA-HBED-CC) represents a successful novel PSMA inhibitor radiotracer which has recently demonstrated its relevance in the detection of prostate cancer [162, 163].

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