Chapter 2

State of the art of radiochemistry for positron emitting radioisotopes

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ABSTRACT

Carbon-11, Fluorine-18, Zirconium-89 and Gallium-68 labeled radiopharmaceuticals are compounds used in positron emission tomography (PET). The first three are cyclotron-generated and Gallium-68 is mainly a generator-sourced isotope. Their radioisotopic half-life time matches biodistribution of most commonly used molecular probes to which they are covalently bound or chelated. The growing interest in the fluorine-18 radionuclide for PET undoubtedly results from its convenient half-life \(T_{1/2} = 109.8 \text{ min}\) and clean decay profile. The special interest of carbon-11 \(T_{1/2} = 20.38 \text{ min}\) is due to its prevalence in almost all (drug) molecules, allowing isotopologue labeling of the bioactive molecule as well as the possibility to radiolabel at different positions. Radiochemistry includes the production of these short-lived radionuclides in a cyclotron and the development of rapid synthetic methods for the introduction of these nuclides into a desired molecule, as well as the development of fast purification, analysis and formulation techniques. Radiochemistry is the driving force in molecular PET imaging and the field is still changing significantly. Radiochemistry of carbon-11 and fluorine-18 is dominated by nucleophilic substitution of a heteroatom with a methylating agent \((^{11}\text{CH}_3\text{I}, ^{11}\text{CH}_3\text{OTf})\) or a good leaving group with a fluoride source (i.e. tosylate, triflate, halogen with \([^\text{^{18}}\text{F}]\text{KF/K222} \text{ or }[^\text{18}\text{F}]\text{TBAF}\)). In recent years however, radiochemistry has developed immensely with novel reactions and approaches. From cross-coupling reactions, \([^{11}\text{C}]\text{CO}_2\) fixation reactions, organolithium reagents, chemical and electrochemical \([^{11}\text{C}]\text{CO}_2\) decomposition, \([^{11}\text{C}]\)-carbonylation reactions, the usage of boronic acid ester and iodonium precursors, \[^{18}\text{F}]-\text{deoxyfluorination of phenols via Ru }\pi\text{-complexes, fluorodestannylation and preparation of }^{18}\text{F}-\text{building blocks.}\n
On the other hand, gallium-68 and zirconium-89 radiochemistry heavily relies on chelation with suitable compounds. Most of the chelators are built with carboxylic and amine groups that can form coordination bonds with radiometal. Their structures (called chelators) are constantly being improved to achieve the optimal chelation properties. In the past decade zirconium-89 peaked scientist interest, due to growing utility of monoclonal antibodies as PET tracers. \(^{89}\text{Zr} \text{ half-life time } \(T_{1/2} = 78.41 \text{ h}\) matches the biological half-life time of antibodies, whereas \(^{68}\text{Ga} \text{ (}T_{1/2} = 67.71 \text{ min}\) matches with antibody fragments, proteins, peptides and small molecular weight compounds. This chapter presents a summary of most common properties and reactions used with those four radioisotopes, as well as new developments in the field of radiochemistry for \(^{11}\text{C}\) and \(^{18}\text{F}\) and new chelator synthesis for \(^{68}\text{Ga}\) and \(^{89}\text{Zr}\).

KEYWORDS

PET, radiopharmaceuticals, radiochemical synthesis, fluorine-18, carbon-11, gallium-68, zirconium-89
**INTRODUCTION**

Positron-emission tomography (PET) is a non-invasive nuclear imaging technique that provides functional information on biochemical and physiological processes at the molecular level. It offers high sensitivity, fast imaging time and requires small amounts of used molecular probe in comparison to other imaging techniques. Unlike anatomical imaging modalities such as magnetic resonance imaging (MRI) and computed tomography (CT), PET can monitor changes in biological processes before clinical symptoms of a disease are observed. PET is therefore capable of early detection and diagnosis of diseases as well as monitoring treatment efficiency. The majority of clinically used PET tracers are small molecules radiolabeled with the short-lived radionuclides such as $^{18}$F and $^{11}$C. A short half-life allows for repeated imaging at short intervals, with an acceptable radiation dose for the patient. Due to their fast decay, $^{13}$N (10 min.) and $^{18}$O (2 min.) are barely used as radioisotopes. To date, $^{11}$C and $^{18}$F are the most commonly used radionuclides with their half-lives allowing for multistep syntheses, however often the isotope is introduced in the final step. $^{11}$C-Radiochemistry commonly uses $^{11}$C-methylation reactions using $[^{11}\text{C}]$methyl iodide ($[^{11}\text{C}]\text{CH}_3\text{I}$) or $[^{11}\text{C}]$methyl triflate ($[^{11}\text{C}]\text{CH}_3\text{OTf}$). Fluorine-18 has been effectively used for the labeling of numerous bioactive molecules via nucleophilic substitution reactions between $[^{18}\text{F}]$fluoride and aliphatic and aromatic substrates. In the past decades, the radiochemistry field has grown immensely resulting in the application of organic chemistry reactions in the radiochemistry field. For example, cross-coupling reactions, $[^{11}\text{C}]\text{CO}_2$ fixating agents and secondary reagents e.g. $[^{11}\text{C}]$phosgene and carbon monoxide ($^{11}$CO) in $^{11}$C-radiochemistry and iodonium precursors for the radiolabeling of electron-rich arenes with $[^{18}\text{F}]$fluoride have been developed. It is important to acknowledge that the handling of both radionuclides brings practical challenges. For example, protection from radiation, production of minute amounts of the nuclide, development of rapid synthesis methods and the use of fast purification, analysis and formulation techniques. Modern PET facilities are equipped with “hot cells” (basically lead shielded fume hoods) to carry out radiolabeling procedures via automated systems. The efficient production restricts exposure of the chemist to radiation as much as possible. In contrast to $^{11}$C- and $^{18}$F-radiochemistry, $^{68}$Ga- and $^{89}$Zr-PET tracer production is based on complexing of the chosen radiometal into the chelator connected by covalent bond with bioactive pharmacophore. Therefore, the manufacturing process is limited to ligand-chelate complex synthesis and labeling with radiometal. Commonly those procedures are relatively simple and can be translated into kit-like automated systems. Currently, advancements in $^{68}$Ga- and $^{89}$Zr radiochemistry focuses on synthesis of new and improved chelators, allowing better chelation strength and easier radiolabeling. In this chapter the focus will be on the most common radiochemical reactions used to synthesize carbon-11 and fluorine-18, as well as gallium-68 and zirconium-89 labeled radiotracers, highlighting recent developments and the problems and challenges currently faced.
CARBON-11

Carbon-11 is an almost pure positron emitter (98.1% $\beta^+$, 0.19% EC) that decays to the stable boron-11 isotope with a half-life of 20.4 minutes. The natural abundance of carbon in organic substrates provides many options for PET tracer design, if a radiolabeling method is available. The short half-life provides both advantages and disadvantages. Synthetic procedures need to be fast with typical reaction times of minutes, fast HPLC purification and introduction of carbon-11 as the last step of the synthesis route. As main advantages a PET scan with a $^{11}$C-labeled compound results in a relatively low radiation dose for the patient and it is possible to do two PET scans per day in the same subject. That provides an optimal situation for a test-retest scenario or comparison of baseline PET data with PET data after a pharmacological intervention.

Synthesis of $^{11}$C- building blocks

$^{11}$C is produced in a cyclotron with a nuclear reaction between a nitrogen gas target and high energy protons. In the presence of oxygen, $[^{11}\text{C} ]$carbon dioxide ($^{11}\text{CO}_2$) is obtained; in the presence of hydrogen - $[^{11}\text{C} ]$methane ($^{11}\text{CH}_4$). $[^{11}\text{C} ]$CH$_4$ can also be obtained via the H$_2$/Ni reduction of $^{11}\text{CO}_2$ at 350 °C. From these primary precursors it is relatively easy to synthesize the secondary precursors which are used in the $^{11}$C-labeling (Scheme 1).

![Scheme 1. $^{11}$C-production and conversion to primary and secondary $^{11}$C-precurors.](image-url)

[^11]C-carbon monoxide ([^11]CO) is also produced via the “gas phase” or “wet method”. The “gas phase” method is based on the reduction of ^11CO₂. This was first achieved with activated charcoal at 800 °C and heated zinc columns at 400 °C. Using activated charcoal resulted in low molar activity and traditional zinc column were prone to accidental overheating due to working close to the melting point of zinc (420 °C). Therefore researchers looked for better way to synthesize ^11CO. Zeisler et al showed that [^11]CO₂ can be reduced with molybdenum at 850 °C to [^11]CO in around 70 % yield. Further improvements were made by using a fused silica supported zinc column at 485 °C, which yields [^11]CO in about 90% yield. “Wet” methods are based on chemical decomposition of [^11]C-formyl chloride, or [^11]C-silacarboxylic acids, treatment of [^11]C-carbon dioxide with fluoride-activated disilanes, and electrochemical reduction of ^11CO₂. The “wet” methods to produce [^11]CO have not been used often until now.


[^11]C-methylation is the most commonly used ^11C-radiolabeling method. Chemical groups that can be methylated are amines, amides, phenols, thiols and, less frequently, aliphatic alcohols and carboxylic acids. The reaction is based on deprotonation of the heteroatom using a base such as potassium carbonate or sodium hydroxide, depending on the proton’s acidity, followed by a fast nucleophilic attack on ^11CH₃I or ^11CH₃OTf. Because the triflate moiety is a better leaving group than iodide, ^11CH₃OTf is generally more reactive resulting in shorter reaction times and lower temperature. Currently about 90% of the ^11C-labeled tracers are produced via ^11C-methylation reaction, some examples are shown in Scheme 2.
Scheme 2. $^{11}$C-radiopharmaceuticals produced through $^{11}$C-methylation.

Although $^{11}$C-methylation of heteroatoms provides the easiest method of incorporating $^{11}$C into biologically active compounds, it is also a metabolically labile position in vivo. Since metabolism might lead to undesirable radiolabeled metabolites, $^{11}$C-methylation of a carbon atom rather than a heteroatom via transition-metal mediated cross-coupling reactions provides an interesting alternative. For example, Rejc et al. reported the radiolabeling of $[^{11}\text{C}]$thymidine using a Negishi coupling reaction with $^{11}$CH$_3$I in the presence of zinc and a palladium catalyst.\textsuperscript{19} Moreover, for some tracer a methylation reaction on an heteroatom is not possible and a methylation on a carbon atom is required, e.g. $[^{11}\text{C}]$UCB-J, a SV2A targeting tracer which is used to measure synaptic density with PET.\textsuperscript{20} Since the applied tin, palladium and other metal catalyst for cross-coupling reactions are toxic, a careful purification and quality control is required. Consequently, the application of these methods into translational research is still not fully explored and incorporation of $^{11}$C in different position has attracted the attention of the scientific community.

$^{11}$CO$_2$-fixation

Grignard and organolithium reagents

The incorporation of $^{11}$CO$_2$ into organic molecules can occur via organometallic reagents, which are well-known in organic chemistry. They contain at least one bond between a metal and the $^{11}$C-carbon atom. This bond changes
the polarization towards the carbon, making it nucleophilic as opposed to the methylation reagents. Organometallic reagents are highly reactive and able to easily form new carbon-carbon bond. In $^{11}$C-radiolabeling methods Grignard reagents and organolithiums are often used (Scheme 3).\textsuperscript{21} $^{11}$CO$_2$ reaction with Grignard reagents results in the $^{11}$C-radiolabeling of carboxylic acids, amides, amines and acyl halides. This method allowed the synthesis of $[^{11}\text{C}]$acetic acid for use in cardiology diagnostics as well as the production of $[^{11}\text{C}]$WAY100635, an important serotonin receptor ligand (Scheme 3A).\textsuperscript{22} The importance of $[^{11}\text{C}]$methyllithium ($^{11}$CH$_3$Li) can be illustrated by the synthesis of $[^{11}\text{C}]$acetone, an useful intermediate for further radiolabeling.\textsuperscript{21} Firstly $^{11}$CO$_2$ is transformed into the lithium salt of acetal, which is then hydrolyzed to a carbon-11 radiolabeled ketone (Scheme 3B). Although $^{11}$CH$_3$Li offers access to several $^{11}$C-labeled compounds, its formation and handling remains challenging. In addition, the high reactivity due to bond polarization diminish selectivity and group tolerance.

**Scheme 3. Synthesis via $^{11}$CCO$_2$ fixation reaction using organometallic reagents.** A) Synthesis of $[^{11}\text{C}]$WAY100635 via $[^{11}\text{C}]$CO$_2$ fixation with Grignard reagents. B) Synthesis of synthetic intermediate $[^{11}\text{C}]$acetone with methyllithium.
New developments in $^{11}$CO$_2$ fixation

In recent years, $^{11}$CO$_2$ fixation reactions have made use of fixing bases such as triethylamine (TEA), 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) or 2-tert-Butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (BEMP) instead of organometallic reagents. By substituting the $^{11}$CO$_2$-base complex with an amine of choice in a transamination step, $^{11}$C-labeled urea, carbamate and oxazolidinones moieties can be obtained (Scheme 4).\textsuperscript{21,23-25} The resulting groups are not electrophilic enough to react further, but can be activated by phosgene, phosphoryl chloride or thionyl chloride. In the synthesis of $^{11}$C-urea, the fixing base is already the amine of choice - lithium bis(trimethylsilyl)amide (LHMDS). and further activation is not needed. Reaction is finished by hydrolysis with aqueous ammonium chloride.\textsuperscript{21} With this method the selective GSK-3\beta inhibitor $[^{11}$C-carbonyl]AR-A014418\textsuperscript{26}, the FAAH inhibitor $[^{11}$C]URB694\textsuperscript{27} and the MAO-B inhibitor $[^{11}$C]SL25.1188\textsuperscript{28} were prepared (Scheme 4A-C).
Scheme 4. The synthesis of $^{11}$C-labeled motives using a fixation base and an activating agent such as phosphoryl chloride.

Clinically relevant $[^{11}\text{C} ]$ureas (A), $[^{11}\text{C} ]$carbamates (B) and $[^{11}\text{C} ]$oxazolidinones (C) synthesized via $^{11}$C-fixation.

In addition, a copper-catalyzed method for the $^{11}$C-C bond formation has been reported using boronic acid esters in order to overcome the limitations of organometallic reagents. Their stability to air and moisture as well as their high functional group tolerance makes them easier to manipulate (Scheme 5A).²⁹

$^{11}$C-carbonylation reactions
$^{11}$CO is an appealing synthon for introducing carbon-11 at a carbonyl position (C=O). Similar to $^{11}$CO$_2$ fixation, $^{11}$CO can be used to synthesize $^{11}$C-labeled ureas, carboxylic acids, but also amides, aldehydes, ketones and esters. The incorporation of $^{11}$CO is often palladium mediated, but other transition metals such as rhodium and nickel have also been used. The mechanism requires the presence of a nucleophile, a good leaving group like halogen, tosylate, boronic acid esters and a catalyst that can assist the $^{11}$C-carbonylation reaction. The synthesis of $[^{11}$C-carbonyl]raclopride shows the added value of $^{11}$CO labeling compared to the traditionally produced $[^{11}$C]raclopride via $O$-methylation (Scheme 5B).

**Scheme 5. New developments in the use of $^{11}$CO$_2$ fixation, $^{11}$CO and secondary $^{11}$C-synthons.** A) Synthesis of a $^{11}$C-labeled oxytocin receptor ligand via copper-mediated $^{11}$CO$_2$ fixation. B) Synthesis of $[^{11}$C-carbonyl]raclopride via a palladium-mediated cross-coupling reaction with $[^{11}$C]CO.

$^{11}$C-cyanation

Apart from discussed above primary and secondary labeling precursors, another important and worth mentioning is $[^{11}$C]HCN, since nitriles are moieties present in biologically active substances and they are suitable group for further conversion into amides, amines and carboxylic acids. Production of $[^{11}$C]cyanides starts with $[^{11}$C]CH$_4$ that reacts with ammonia over platinum column at high temperature (950 °C). If preferred, $[^{11}$C]hydrogen cyanide can also be converted into its salt form. A very interesting, reaction to introduce $^{11}$C into molecule is through Strecker reaction. Basis for that reaction comes with cyanide substitution of good leaving group like halogen, sulfonate salt etc. Later hydrolysis results in conversion into carboxylic moiety. One variation of clinical usage of this reaction is tracer for prostate cancer $[^{11}$C-carbonyl]sarcosine (Scheme 6).

$[^{11}C$-carbonyl]sarcosine
RCY: 3%
$A_m \geq 55.5$ GBq/μmol
**FLUORINE-18**

For several decades, fluorine-containing pharmaceuticals have been attracting attention due to their enhanced pharmacokinetic properties, such as membrane permeability, *in vivo* metabolic stability and potency.\(^{33}\) Currently, about 20% of the marketed drugs contain at least one fluorine atom or a fluorinated functional group (e.g. trifluoromethyl, CF\(_3\)).\(^{34}\) These so-called fluoro-pharmaceuticals are of great interest for positron emission tomography (PET) imaging.\(^{35}\) Fluoro-pharmaceuticals offer the possibility of labeling many biologically active compounds with fluorine-18 without alteration of the original structure, thereby giving access to a wide range of potential new PET tracers.

Fluorine-18 is one of the most commonly used positron-emitting radionuclide due to its favorable chemical and nuclear properties. A convenient half-life of 109.7 minutes (allowing multistep synthesis, extended imaging protocols and transport to off-site PET facilities), a clean decay profile (97% positron emission) and a low positron energy (maximum 0.635 MeV) result in high-resolution PET images.\(^{4,36}\)

### Synthesis of \(^{18}\)F-sources

As starting material for all \(^{18}\)F-radiolabeling methodologies either electron-rich (nucleophilic) or positively charged (electrophilic) fluorine-18 is used. For the productions of nucleophilic \(^{18}\)F-fluoride, oxygen-18 enriched water (\([^{18}\text{O}]\text{H}_2\text{O}\)) is used as the target (Scheme 7A) and is bombarded with 11-18 MeV protons for the \(^{18}\text{O}(p,n)^{18}\text{F}\) nuclear reaction, resulting in \([^{18}\text{F}]\text{fluoride}\) solution in water. The strongly hydrated \([^{18}\text{F}]\text{fluoride}\) ions are trapped on an anion-exchange resin and eluted with an aqueous acetonitrile (ACN) solution containing an inorganic base and phase transfer catalyst (PTC). Typically, a combination of K\(_2\)CO\(_3\) and Kryptofix (K\(_{2.2.2}\)) is used to complex with the \([^{18}\text{F}]\text{fluoride}\) ions and enhance its solubility and reactivity in anhydrous solvents. After repeated azeotropic drying with ACN, the residual anhydrous \([^{18}\text{F}]\text{KF/K}_{2.2.2}\) complex is taken up in a polar aprotic solvent suitable for the subsequent labeling reaction.\(^{37}\) Although suitable for big scale productions (> 370 GBq/batch) with high molar activity (> 100 GBq/μmol), the overall procedure can be time-consuming in the context of \(^{18}\)F-radiochemistry (around 10–15 min) and can lead to significant losses of radioactivity (up to 30%) due to natural decay and unspecific absorption on the reactor surface during azeotropic drying. Most importantly, substantial amounts of base are needed to isolate \([^{18}\text{F}]\text{fluoride}\) from the cartridge because of the difficult exchange between the trapped \([^{18}\text{F}]\text{fluoride}\) anions and the anions in the eluent. The high amounts of base potentially limits the synthetic utility of \([^{18}\text{F}]\text{fluoride}\).\(^{37,38}\)

Electrophilic \([^{18}\text{F}]\text{fluorine}\) is typically produced by the same nuclear reaction as for nucleophilic \([^{18}\text{F}]\text{fluoride}\), but using gaseous \([^{18}\text{O}]\text{O}_2\) as the target instead and \([^{18}\text{F}]\text{F}_2\) as a carrier gas (Scheme 7B).\(^{36,39}\) Produced \([^{18}\text{F}]\text{F}_2\) can be used as is or alternatively is transformed into other electrophilic fluorination reagents, including \([^{18}\text{F}]\text{XeF}_2\), \(O-^{18}\text{F}\) fluorinated reagents (\([^{18}\text{F}]\text{CH}_3\text{COOF},\ [^{18}\text{F}]\text{FClO}_3\)) and more recently \(N-^{18}\text{F}\) fluorinated reagents (\([^{18}\text{F}]\text{NFSi},\ [^{18}\text{F}]\text{Selectfluor}\)).\(^{36,37,40}\) All of the electrophilic \(^{18}\text{F}\)-type reagents have a low molar activity with a theoretical maximum radiochemical yield (RCY) of 50%, because every F\(_2\) consists of a \(^{18}\text{F}\) atom and a \(^{19}\text{F}\) atom.
The above listed $^{18}$F-labeled reagents are used to introduce $^{18}$F into biologically active compounds via two reaction pathways: nucleophilic and electrophilic substitution reactions.

**A Nucleophilic $^{18}$F nuclide**

$$H_2^{18}O + p \rightarrow ^{18}F^-$$

Scheme 7. Nuclear reactions to produce [$^{18}$F]fluoride, [$^{18}$F]fluorine gas and the electrophilic $^{18}$F-fluorinating reagents derived from [$^{18}$F]F$_2$.

**B Electrophilic $^{18}$F nuclide**

$$^{18}O_2 + p \rightarrow ^{18}F^-$$

Nucleophilic $^{18}$F-fluorination reactions

The nucleophilic substitution reaction involves the addition of a highly negatively charged [$^{18}$F]fluoride ion, the nucleophile, to an electron deficient carbon atom in an organic compound by displacing a leaving group, either on an aromatic (unsaturated) carbon or an aliphatic (saturated) carbon. Those are so-called aromatic or aliphatic nucleophilic $^{18}$F-fluorinations, respectively.

**Nucleophilic aliphatic substitution**

Following the S$_{N}$2 mechanism the aliphatic substitution of halogens or sulfonate esters with [$^{18}$F]fluoride is the classical method for introducing $^{18}$F in high radiochemical yield (RCY) and with high molar activity ($A_m$) (Scheme 8). Choosing an adequate leaving group is a critical step in the radiosynthetic design. In general, sulfonate esters are more reactive than halogens as a leaving group with the triflate group being the most reactive in $^{18}$F-fluorination reactions. A variety of molecules have been radiolabeled with $^{18}$F in a typically two-step process, where the $^{18}$F-substitution is followed by removal of protecting groups as illustrated by the state-of-the-art radiosynthesis of 2-[$^{18}$F]fluoro-2-deoxyglucose ([$^{18}$F]FDG). In the presence of K$_{222}$ as phase transfer catalyst and ACN as solvent, [$^{18}$F]fluoride replaces the triflate group at the C2-position of the protected mannose precursor (Scheme 8A). After the nucleophilic replacement and hydrolysis of the acetyl groups, [$^{18}$F]FDG is obtained in high radiochemical yield (RCY) and typical molar activities of 37-74 GBq/µmol. One challenge in the aliphatic $^{18}$F-fluorination reaction is control of the product stereochemistry. To this end, regio- and stereospecific
ring-opening reactions of epoxides and aziridines by \(^{18}\text{F}\)fluoride have been developed for the radiosynthesis of PET tracers, including clinically used fluoroestradiol (\(^{18}\text{F}\)FES, Scheme 8B)\(^{39,43}\).

### Nucleophilic aliphatic substitution

\[
\begin{align*}
\text{Nucleophilic aliphatic substitution} \\
\text{LG} - \begin{array}{c} \text{R}_1 \end{array} \begin{array}{c} \text{R}_2 \end{array} \begin{array}{c} \text{R}_3 \end{array} ^{18}\text{F}^- \rightarrow & \left[ \begin{array}{c} \text{LG} \end{array} - \begin{array}{c} \text{R}_1 \end{array} \begin{array}{c} \text{R}_2 \end{array} \begin{array}{c} \text{R}_3 \end{array} \right] ^{18}\text{F}^- \\
& \rightarrow \begin{array}{c} \text{R}_1 \end{array} \begin{array}{c} \text{R}_2 \end{array} \begin{array}{c} \text{R}_3 \end{array} \text{LG} \end{align*}
\]

\[
\text{LG} = \text{OTf} \rightarrow \text{OTs} = \text{OMs} > \text{I} > \text{Br} > \text{Cl} \\
\text{\(18\text{F}^-\)} = \text{\([^{18}\text{F}]\text{KF/K}_{222}\)}, \text{\([^{18}\text{F}]\text{TBAF}, \text{\([^{18}\text{F}]\text{TEAF}, \text{etc.}\) etc.}
\]

A

\[
\begin{align*}
\text{A} & \quad \text{AcO} \quad \text{OTf} \\
\text{AcO} & \quad \text{AcO} \\
\text{OAc} & \quad \text{OAc}
\end{align*}
\]

1) \([^{18}\text{F}]\text{KF/K}_{222}, \text{MeCN, 80 }^\circ\text{C, 5 min}
\]

2) \(1\text{M HCl, 130 }^\circ\text{C, 15 min}

\[\text{\([^{18}\text{F}]\text{FDG}\)}

\[
\text{RCY: } 44 \pm 4\% \text{ n.d. } (n=7) \\
\text{Am: } 37-74 \text{ GBq}/\text{μmol}
\]

B

\[
\begin{align*}
\text{B} & \quad \text{OH} \\
\text{OH} & \quad \text{OH} \\
\text{HO} & \quad \text{HO}
\end{align*}
\]

1) \([^{18}\text{F}]\text{TBAF, MeCN, 110 }^\circ\text{C, 15 min}
\]

2) \(1\text{M H}_{2}\text{SO}_{4}, \text{MeCN, 100 }^\circ\text{C, 15 min}

\[\text{\([^{18}\text{F}]\text{FES}\)}

\[
\text{RCY: } 30-35\% \text{ d.c. } (n=3) \\
\text{Am: } 182-232 \text{ GBq}/\text{μmol}
\]

Scheme 8. Common strategies for the nucleophilic aliphatic \(^{18}\text{F}\)-fluorination. A) Synthesis of \([^{18}\text{F}]\text{FDG via }^{18}\text{F}-\text{aliphatic substitution of 1,3,4,6-tetra-O-acetyl-2-O-triflate-β-D-mannose; basic hydrolysis also possible. B) Two-step-one-pot radiosynthesis of }^{18}\text{F}\text{FES by regioselective substitution of the cyclic sulfate precursor.}

### Conventional nucleophilic aromatic substitution

Historically, nucleophilic aromatic substitution (S\(\text{N}\)Ar) reactions involved the thermal decomposition of aryl diazonium salts, the so-called Balz-Schiemann and Wallach methodologies.\(^{37}\) However, low RCYs and molar activities and extensive by-product formation led to the development of the conventional S\(\text{N}\)Ar reaction, which involves displacement of a suitable leaving group from an electron deficient benzene ring (Scheme 9). The relatively electron deficient center in the precursor is created by a strong inductive or resonance electron withdrawing group in the ortho- or para-position to the leaving group. Sufficient activation of the aryl ring i.e. stabilization of the intermediate Meisenheimer complex is commonly achieved by NO\(_2\), CF\(_3\), CN or carboxyl functional groups.\(^{37,39,40}\) In the early days halides were used as leaving groups for the introduction of \([^{18}\text{F}]\)fluoride via halogen or isotope exchange reactions. Although, in general, from the halides fluoride shows the best reactivity, aromatic exchange of \(^{19}\text{F}\) by \(^{18}\text{F}\) will result in low molar activity PET tracers, which may preclude its imaging application. Therefore, alternative leaving groups are used where the nitro and quaternary

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trimethylammonium are current state of the art.\textsuperscript{40} For example, important clinical tracers such as [\textsuperscript{18}F]flumazenil, [\textsuperscript{18}F]FPEB and 6-\textsuperscript{[18}F]fluoro-L-DOPA have successfully been labeled with fluorine-18 via S\textsubscript{N}Ar displacement of a nitro or trimethylammonium precursor, even with meta-positioned activating groups (Scheme 9A).\textsuperscript{44-46} In addition, nitrogenous heteroarenes such as pyridines are more electron deficient than their corresponding homoarenes and therefore amenable to substitution with fluorine-18 in the absence of an activating group.\textsuperscript{37,39} For example, the selective NACHRs tracer [\textsuperscript{18}F]flubatine uses the non-activated, Boc-protected trimethylammonium precursor (Scheme 9A). The nucleophilic substitution, product purification and formulation provided [\textsuperscript{18}F]flubatine in good RCYs (60±5\%) and with high molar activity (approximately 350 GBq/μmol) and has been validated for clinical application.\textsuperscript{47,48}

\textbf{Traditional nucleophilic aromatic substitution}

\begin{center}
\begin{tikzpicture}
\node at (0,0) {$\text{EWG}$} edge[bend left] node [midway, above] {$\text{18}^F^-$} node [midway, below] {$\text{LG}$} {$\text{EWG}$ \hspace{1cm} \text{X}} edge[bend right] node [midway, above] {$\text{X}$} node [midway, below] {$\text{18}^F^-$} edge[bend right] node [midway, above] {$\text{LG}$} {$\text{EWG}$ \hspace{1cm} \text{X}};
\end{tikzpicture}
\end{center}

\begin{itemize}
\item EWG = NO\textsubscript{2}, CN, CF\textsubscript{3}, carbonyl groups, etc.
\item X = C or N
\item LG = \textsuperscript{1}NMe\textsubscript{3}, NO\textsubscript{2}, halogen, etc.
\end{itemize}

\begin{center}
\textbf{Scheme 9. Conventional aromatic nucleophilic substitution (S\textsubscript{N}Ar) with [\textsuperscript{18}F]fluoride. A) Clinically relevant fluoro(hetero)arenes synthesized via \textsuperscript{18}F-substitution on NO\textsubscript{2} and \textsuperscript{1}NMe\textsubscript{3} leaving groups.}
\end{center}

\begin{itemize}
\item [\textsuperscript{18}F]flumazenil
  \begin{itemize}
  \item LG = NO\textsubscript{2}, X = C
  \item RCY: 6.4 ± 0.7\% n.d.c. (n=8)
  \item $A_m$: >185 GBq/μmol
  \end{itemize}
\item [\textsuperscript{18}F]FPEB
  \begin{itemize}
  \item LG = NO\textsubscript{2}, X = C
  \item RCY: 2.6 ± 1\% (n=114)
  \item $A_m$: 5.3 ± 2.5 GBq/μmol
  \end{itemize}
\item [\textsuperscript{18}F]flubatine
  \begin{itemize}
  \item LG = \textsuperscript{1}NMe\textsubscript{3}, X = N
  \item RCY: 60 ± 5\% (n=25)
  \item $A_m$: >350 GBq/μmol
  \end{itemize}
\end{itemize}

\textit{\textbf{18}\textsuperscript{F}-fluorination of onium salts}

Although clinically relevant PET tracers can be successfully labeled via the conventional nucleophilic aromatic substitution, radiolabeling of compounds with non-activating aromatic rings is less efficient and only low isolated radiochemical yields can be obtained. Consequently, new precursors carrying different functional groups have been developed, with the discovery of onium salts as crucial step. Generally, onium salt precursors are electrophilic at the heteroatom causing the [\textsuperscript{18}F]fluoride anion to regio-selectively attack the benzene ring with the remaining onium group serving as the leaving group.\textsuperscript{49} A variety of onium salt precursors have been reported
including compounds with an electrophilic sulfur (sulfonium), oxygen (uronium) and iodine (iodonium) functionality (Scheme 10). For example, sulfonium salt precursors such as substituted dibenzothiophene sulfonium groups have been used for the efficient $^{18}$F-radiolabeling of electron-neutral and -rich arenes under mild reaction conditions as illustrated by $[^{18}\text{F}]$FPEB (Scheme 10A). Alternatively, the $^{18}$F-fluorination of phenols proceeds via an uronium salt, in which the oxygen has been transformed into an electron-withdrawing functionality (Scheme 10A). By using a ruthenium catalyst to form an electron-deficient $\eta^6$-$\pi$-complex, the $^{18}$F-radiolabeling of electron-rich phenols can be obtained as shown by $[^{18}\text{F}]$bavarostat.

The most extensively studied onium salt precursors are the iodonium salts. Diaryliodonium salts bearing an electron-rich aromatic substituent e.g., para-methoxyphenyl, allow for the selective and highly efficient $^{18}$F-fluorination of a non-activated aryl ring. Additional regioselectivity in this substitution is caused by the preferential substitution of $[^{18}\text{F}]$fluoride into the aromatic ring with a substituent ortho to the iodonium moiety, the so-called “ortho-effect” (Scheme 10). Diaryliodonium salt precursors have been widely applied in the synthesis of several important PET tracers. For example, $[^{18}\text{F}]$Flumazenil was synthesized from its iodonium precursor in 67 ± 4% RCY, >99% radiochemical purity and with a molar activity of 370–450 GBq/μmol compared to ~6% RCY obtained using the conventional SnAr reaction. Similarly, 6-$[^{18}\text{F}]$fluorodopamine has been labeled from the corresponding diaryliodonium salt precursors in up to 35% RCY with high molar activities (>74 GBq/μmol) under optimized reaction conditions (Scheme 10A), while under conventional SnAr a RCY of only 9% could be obtained.

More recently, the use of iodonium salt precursors has been extended by the development of electron-rich arylidonium ylides. The use of spirocyclic iodonium ylides allows for the efficient and regioselective synthesis of electron-rich arenes, which has been a limitation of the diaryliodonium salts. For example, complex radiopharmaceuticals such as $[^{18}\text{F}]$mFBG and $[^{18}\text{F}]$FDPA have been efficiently $^{18}$F-fluorinated using this method (Scheme 10B). It is noteworthy that $[^{18}\text{F}]$FPEB synthesized from its spirocyclic iodonium ylide precursor was fully automated, resulting in a 20% RCY and a molar activity of 666 GBq/μmol. The final product demonstrated a substantial improvement in the RCY and molar activity compared to the traditional SnAr method (RCY <5%) and has been validated for human use.
Scheme 10. Aromatic nucleophilic $^{18}$F-fluorination on sulfonium, diaryliodonium and uronium salt precursors.

**A)** Recent applications of the $^{18}$F-fluorination of onium salts. **B)** One-step radiosynthesis of $[^{18}\text{F}]$FDPA from the adamantyl auxiliary-based spirocyclic iodonium ylide precursor.
Electrophilic $^{18}$F-fluorination reactions

Electrophilic fluorination is a chemical reaction in which an electron rich organic compound attacks the electron-poor $[^{18}\text{F}]$fluorine atom - the electrophile (Scheme 11). There are two types of electrophilic substitution reactions: aromatic and aliphatic $^{18}$F-fluorinations. The most studied electrophilic aliphatic radiofluorination is undoubtedly the addition of $[^{18}\text{F}]\text{F}_2$ to an alkene. The first synthesis of $[^{18}\text{F}]\text{FDG}$ was carried out by electrophilic fluorination of 3,4,6-tri-$O$-acetyl-$D$-glucal, albeit in low RCY (8%) and accompanied by undesired regioisomers (Scheme 11A)\textsuperscript{62}. More recently, the enantioselective $^{18}$F-fluorination of aldehydes has been reported employing $[^{18}\text{F}]\text{NFSI}$ as the electrophilic $^{18}$F-source\textsuperscript{63}. In the presence of a chiral imidazolidinone catalyst, good radiochemical conversions to (2S,4S)-4-$[^{18}\text{F}]$fluoroglutamic acid of 65±3% were obtained with high enantioselectivity (>99% ee) (Scheme 11B).

\begin{center}
\textbf{Scheme 11.} Electrophilic aliphatic substitution. A) Synthesis of $[^{18}\text{F}]\text{FDG}$ via electrophilic fluorination of 3,4,6-tri-$O$-acetyl-$D$-glucal by $[^{18}\text{F}]\text{F}_2$. B) Enantioselective radiosynthesis of 4-(2S,4S)-$[^{18}\text{F}]$fluoroglutamic acid via the organomediated $\alpha$-$[^{18}\text{F}]$-fluorination of aldehydes.
\end{center}

The most representative electrophilic aromatic $^{18}$F-fluorination is the synthesis of 6-$[^{18}\text{F}]$fluoro-$L$-DOPA. The direct electrophilic aromatic substitution of 3,4-dihydroxy-phenyl-$L$-alanine with $[^{18}\text{F}]\text{F}_2$, and later milder electrophilic fluorinating reagents such as $[^{18}\text{F}]\text{XeF}_2$ and $[^{18}\text{F}]\text{AcOF}$, resulted in low RCY and poor regioselectivity with the 2- and 5-regioisomers produced in addition to the desired 6-isomer (Scheme 12A)\textsuperscript{64}. Since then, the regioselective synthesis of 6-$[^{18}\text{F}]$fluoro-$L$-DOPA has been performed from prefunctionalized organometallic
Precursors, such as organomercuric, -stannane and -silver precursors. For example, electrophilic $^{18}$F-fluorination of $N$-(trifluoroacetyl)-3,4-dimethoxy-6-trifluoroacetoxy-mercurio-L-phenylalanine ethyl ester with $[^{18}F]$AcOF obtained 6-$[^{18}F]$fluoro-L-DOPA in 12±1% d.c. RCY (Scheme 12B). Currently, the electrophilic radiofluorination of 6-$[^{18}F]$fluoro-L-DOPA relies on the demetallation of a stannane precursor with $[^{18}F]$F$_2$ followed by a single deprotection step (Scheme 12C). More recently, 6-$[^{18}F]$fluoro-L-DOPA has been synthesized from a neopentyl glycol boronate ester precursor with $[^{18}F]$Selectfluor in the presence of a silver catalyst in 19±12% RCY with a molar activity of 2.6 GBq/μmol (Scheme 12D). Although these demetallation reactions generally provide enantiomerically pure products, they suffer from low molar activity and stringent purification to eliminate any metals from the final PET tracer and therefore have not been widely applied in clinical productions.

Scheme 12. 6-$[^{18}F]$fluoro-L-DOPA synthesis. A) unselective direct fluorination, B) selective fluorodemercuration, C) fluorodestannylation and D) silver-mediated $^{18}$F-fluorination on arylboron precursors.

New developments in $^{18}$F-fluorination reactions

Lately, a number of reports describing transition-metal-mediated $^{18}$F-fluorination reactions have appeared in the literature that allow the generation of highly functionalized PET tracers under mild conditions. Significant advances in the field of aromatic radiofluorination have been achieved by using copper catalysts. Substrates like mesityl(aryl)iodonium salts, aryl boronates and arylstannanes form high-valent aryl–Cu$^{II}$-$^{18}$F species that can undergo rapid reductive elimination to yield electron-rich, -neutral, and -deficient $^{18}$F-arenes with a large variety of functional groups (Scheme 13). The Cu-mediated $^{18}$F-fluorination has been applied in the synthesis of several PET tracers including the automated production of enantiomerically pure 6-$[^{18}F]$fluoro-L-DOPA from the boronic ester precursor in good RCYs and with high molar activities (Scheme 13A).
Scheme 13. Cu-mediated aromatic radiofluorination of aryl boronates, arylstannanes and aryl(mesityl)iodonium salts with $^{18}$F-fluoride. A) One-pot radiosynthesis of enantiomerically pure 6-$^{18}$F-L-DOPA by Cu-mediated $^{18}$F-fluorination of the aryl boronic pinacol ester precursor with high molar activity.

In addition, the integration of modern synthetic organic chemistry and radiochemistry is demonstrated by the enantioselective $^{18}$F-fluorination of $[^{18}F]$THK-5105 (85% ee) and $[^{18}F]$FMISO (90% ee) with chiral $[^{18}F]$(salen)CoF (Scheme 14A).59 Finally, new concepts to synthesize $^{18}$F-labeled arenes make use of electrophilic $^{18}$F-reagents with high molar activity via the umpolung of $^{[18}F]$fluoride. For example, highly fluorophilic metal complexes (Pd$^{IV}$ or Ni$^{II}$) have been shown to capture $[^{18}F]$fluoride and subsequently transfer the $[^{18}F]$fluoride via electrophilic fluorination to electron-rich aromatic compounds.70,71,72 This concept has been successfully applied in the synthesis of $[^{18}F]$paroxetine, an antidepressant selective serotonin reuptake inhibitor, on a 37 GBq scale (>1% RCY) with a molar activity of up to 481 GBq/μmol (Scheme 14B).73 However, the application of these electrophilic fluorinating reagents has been limited due to the complex precursor synthesis and demanding reaction conditions.
Scheme 14. \(^{18}\text{F}\)-metal complexes for late stage fluorination. A) Co-mediated enantioselective \(^{18}\text{F}\)-fluorination of \(^{18}\text{F}\)FMISO. B) Radiosynthesis of \(^{18}\text{F}\)paroxetine via palladium-mediated, electrophilic late-stage fluorination.

Last, but not least it is worth to mention different types of \(^{18}\text{F}\)-fluorination utilized for labelling of biomolecules. In case of biomolecules such as peptides or proteins, harsh conditions used in direct labeling are unsuitable. Therefore, high temperatures and use of organic solvents are replaced with mild aqueous conditions of indirect labeling with fluorine-18 containing building blocks, e.g. N-succinimidyl 4-[\(^{18}\text{F}\)]fluorobenzoate ([\(^{18}\text{F}\)]SFB).\(^{74}\) Mild conditions of \(^{18}\text{F}\)-labeling can also be achieved by incorporating “fluorophilic” elements (silica, boron or aluminum) into biomolecules and consequent formation of metal-fluoride bond (Scheme 15). This allows to perform labeling reactions in aqueous solvents like buffers and temperatures below 100 °C, as shown in an example of \(^{18}\text{F}\)AlF-NOTA-octreotide (Scheme 15A) and \(^{18}\text{F}\)SIALTATE (Scheme 15B).\(^{75-78}\)
Scheme 15. Mild $^{18}$F-labeling of biomolecules through incorporation of "fluorophilic" elements. Exemplary radiosynthesis of clinically relevant $^{18}$F-tracers: $[^{18}F]$AlF-NODA-octreotide (A) and $[^{18}F]$SIFAlin-TATE (B); Aux = PEG, Asp, or carbohydrates.
GALLIUM-68

Gallium-68 has a half-life of 67.7 minutes and is a pure positron emitter with high positron energy (maximum 1.899 MeV).\textsuperscript{117} This radioisotope can be produced either via the cyclotron route using the nuclear reaction \textsuperscript{68}Zn(\(\rho\),n)\textsuperscript{69}Ga or \textsuperscript{66}Zn(\(\alpha\),n)\textsuperscript{68}Ga and through the generator route utilizing \textsuperscript{68}Ge/\textsuperscript{68}Ga decay. Usage of generator is currently preferred due to absence of zinc in starting material, which can compete with gallium in chelation to the molecule. Additional advantages are the small size of generator, user-friendly operating program for elution and the fact that the generator can be present in off-site PET facility. Germanium-68 decays by electron capture to gallium-68 with a half-life of 270.95 days, which means that the generator can be used up to 9 months to yield sufficient \textsuperscript{68}Ga for clinical applications.\textsuperscript{81,117,80} At this moment, the generator derived way of \textsuperscript{68}Ga production is definitely the most favored way, nevertheless, it is limited to the low activity levels (≤ 3.7 GBq) and the long latency between elutions (~4 hours).\textsuperscript{81} Introduction of radiometal production using liquid targets has opened an attractive alternative for \textsuperscript{68}Ga production. In this approach, \textsuperscript{68}Zn as a target material is dissolved in a strong acid, diluted and loaded into the liquid target. Typically target material consists of \textsuperscript{68}Zn(NO\textsubscript{3})\textsubscript{2} or \textsuperscript{68}ZnCl\textsubscript{2} in a concentration range 1.0-1.7 M. In contrast with standard solid targets, liquid targets offer faster target material preparation and post-irradiation procedures. It is also possible to use the same target for several irradiations. Additionally, target loading and downloading is considerably simpler. Although these techniques have its disadvantages, e.g. lower target material density and therefore lower production yields, recent publications demonstrate that obtained activities are sufficient for clinical use (up to 5-6 GBq of \textsuperscript{68}Ga per batch).\textsuperscript{82-84}

Chemistry behind incorporating gallium-68 into radiopharmaceuticals is different than those discussed with \textsuperscript{11}C and \textsuperscript{15}F. As a radiometal, \textsuperscript{68}Ga requires a chelator – a compound with an ability to form multiple covalent and coordinating bonds. Chelating agents are commonly organic compounds, which include cyclic or linear moiety with a high affinity towards metal. It involves formation of two or more coordination bonds between the ligand (chelate) and single central atom (metal ion).\textsuperscript{85} Second key component of chelator is a group that allows covalent binding to a chosen pharmacophore. Commonly utilized chemistry for this covalent binding is an amide bond formation. The classical method for amide synthesis is ester aminolysis through an active ester moiety (e.g. succinimide) that is highly susceptible towards a nucleophile attack like amine group of lysine amino acid residue.\textsuperscript{86} Amide bond formation can also occur by employing chemistry of nucleophilic attack on isothiocyanates, which leads towards thioureas and thiocarbamates formation (Scheme 16). Among many available moieties, \textit{N}-hydroxysuccinimidy (NHS), isothiocyanate (NCS), hydroxybenzotriazole (HOBt) and tetrafluorophenyl (TFP) are the most commonly used. There are multiple chelators that are frequently used in labeling with gallium-68, the most common ones are depicted in Scheme 16A.\textsuperscript{87-89} Typically, development of those chelators have source in known iron-chelating compounds that are used in nature by microbes and have medical application in iron-related diseases. A class of nature-derived chelators are siderophores, with desferrioxamine being a prime example.
Scheme 16. Reactions used for covalent binding of gallium-68 chelators to chosen pharmacophore. A) Most commonly used chelators for $^{68}$Ga; the majority of them depicted with modification (isothiocyanate, tetrafluorophenol ester etc.) allowing for further conjugation to peptides and proteins.
The most known radiopharmaceutical radiolabeled with gallium-68 is a tracer for neuroendocrine tumors as shown below (DOTA-TATE and DOTA-TOC, two variants of a molecule binding to the somatostatin receptor – with the highest activity to SSR2) (Scheme 17).90-93 Another example is gallium-68 labeled molecule targeting prostate-specific membrane antigen (PSMA) which is applied to image prostate cancer (Scheme 18A).94,95 Due to high energy of the emitted positrons, resulting in low resolution PET images, current direction in nuclear medicine is to translate ^68Ga-tracers into ^18F-tracers (^68Ga- and ^18F-PSMA, Scheme 18).96

Scheme 17. Structure and general method for DOTA chelator ^68Ga labeling and two derivatives of tracer for neuroendocrine tumor. (A) DOTA-TATE and (B)DOTA-TOC.
Scheme 18. Labeling strategies for PSMA molecule. (A) $[^{68}\text{Ga}]$PSMA-11 and (B) $[^{18}\text{F}]$PSMA-1007.

Current developments in field of gallium-68 radiochemistry involve new generation chelators that allows milder condition and shorter times of radiolabeling and superior chelation strength resulting in high kinetic stability. Commonly used 1, 4, 7, 10-tetraazacyclododecane-1, 4, 7, 10-tetraacetic acid (DOTA) chelator requires harsh conditions of pH 4 and heating over 80 °C to achieve desired yields. Another golden standard - 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) can be utilized for radiolabeling in room temperature. However, acidic conditions are still needed. Those aggressive labeling conditions can be problematic for heat and pH sensitive biomolecules, where protein folding is crucial for its binding. Amino-acid based chelators such as N,N-bis[(6-carboxypyridin-2-yl)methyl]glycine (H3Dpaa) could be a solution, since recent publications show promising results of efficient gallium-68 chelation under physiological conditions. Deferiprone based derivatives also proved to have attractive properties, as representative chelator - CP2567 that has reported radiolabeling times of less than 5 minutes at pH ~ 6.5.

Aside from chelation of $^{68}$Ga to bispecific chelators that can be used directly as a PET agent or conjugated to biomolecule, coordination directly to biomolecules has also been explored. This chelator-free approach relies on chelating abilities of biomolecule, such as transferrin (Scheme 19A), adsorption properties of nanoparticles (Scheme 19B) or trapping of gallium-68 inside a nanoparticle core (core doping, Scheme 19C). First approach is rather straightforward and has been already explored in 1980. It is based on intravenous injection of $^{68}$Ga in a simple form such as $^{68}$Ga-citrate. Subsequently $^{68}$Ga is transchelated to transferrin present in the bloodstream, which is possible due to strong affinity of gallium to transferrin. This tracer has application in imaging pulmonary vascular permeability, tumor and inflammatory abscesses and bacterial infection such as $S.\text{aureus}$.
Two other routes for chelator-free $^{68}$Ga labeling are based on direct incorporation into the surface or within the core of nanoparticle (NP). Usage of nanoparticle such as superparamagnetic iron oxide nanoparticles (SPIONs) results in formation of hybrid probe for PET/MRI. Incorporation directly into the surface of NP is effortless and can be accomplished in a single step. However, radioisotope desorption from NP surface needs to be addressed carefully. A solution for this drawback can be an incorporation of radiometal within the core of nanoparticle. This process, called core-doping happens simultaneously with the nanoparticle formation and can be accelerated up by using microwave-assisted protocols. Core-doping enhance stability of the tracer, as it prevents in vivo transmetallation. Nanoparticle based $^{68}$Ga tracers are used as a multimodal contrast agent.$^{103,104}$ An interesting version of gallium encapsulation is Galligas, graphite based encapsulation that allows aerosol delivery of $^{68}$Ga for PET imaging of lung function.$^{105}$

**Scheme 19. Chelator-free approaches for $^{68}$Ga labeling.** A) Coordination of metal by biomolecule illustrated by crystal structure adapted in UCFS Chimera from PDB entries of differic bound human serum transferrin (3QYT); magenta shows position of complexed metal atom. B) Adsorption of radiometal on the surface of the nanoparticle demonstrated by polyethylene glycol coated SPIONs; yellow shows position of $^{68}$Ga atom. C) Core-Doping of radiometal by nanoparticle exemplified by $^{68}$Ga-C-IOPN-RGD; yellow shows position of $^{68}$Ga atom.
It is worth noting that a good chelator should have specific affinity towards one specific metal to avoid chelation of contaminants, e.g., previously mentioned zinc (as a starting material of cyclotron target or as a daughter nuclide of gallium-68 decay). Other metal contaminations, e.g., iron present in the environment iron, should be avoided and can be easily prevented by usage of metal-free materials. Nonetheless, flexibility in chelating different metals can be beneficial. Those are chelators, to which not only gallium can be chelated, but other important radiometals, i.e., copper-64, lutetium-177 or MRI contrast agents metals like gadolinium. Such feature allows generation of universal product – a pharmacophore that can be used in different diagnostic and therapy methods. Among this group of compounds are phosphonic acid arms based ligands (p-SCN-PhPr, $^{68}$Ga and $^{64}$Cu$^{106}$ or desferal based compounds (DFO, $^{68}$Ga and $^{89}$Zr$^{106}$-109).
ZIRCONIUM-89

Zirconium, with half life time of 78.41 h, is a long lived PET radioisotope that is used increasingly in nuclear medicine. Zirconium-89 is obtained from a cyclotron via the nuclear reaction $^{88}$Y($\alpha$,n)$^{89}$Zr or $^{99}$Y(d.,2n)$^{99}$Zr. Due to its long half-life time it can be used to radiolabel molecules with comparable biological half-life time, such as monoclonal antibodies or fragments of antibodies (e.g. Fab fragments). It offers higher resolution, sensitivity and more accurate image quantification than SPECT radioisotopes used in antibody labeling (e.g., $^{111}$In). Zirconium-89 labeled tracers are being extensively used in clinical immunoPET imaging.

Similarly to gallium-68, zirconium chemistry relies on chelators. Standardly used one is deferoxamine (DFO) that was first used clinically as a chelating agent in patients with iron overdose, hemochromatosis or other iron related conditions. To date it is the only clinically used chelator for $^{89}$Zr based PET tracers. The production process of chemistry and radiolabeling is depicted in Scheme 20. Addition of iron to DFO chelator is helpful in evaluation of successful chelator-antibody amide bond formation, by iron UV detection at 430 nm.

Scheme 20. Synthesis scheme of DFO-TPF chelator conjugation to antibody and zirconium-89 labeling. Additional coordination bonds created by water molecules shown in grey.

New developments in $^{89}$Zr radiochemistry are associated with a generation of new chelators that can improve zirconium coordination. Chelation strength can be correlated to tracer stability and it is particularly important for clinical applications. Insufficient stability of zirconium-based tracer in vivo can result in unnecessary higher radioactive burden and increased background signal due to accumulation of free $^{89}$Zr in bones. Enhanced binding can be achieved by creating more coordination bonds, which is shown by example of hydroxamate ligands: hexadentate (DFO) vs octadentate (DFO*, DFO2) (Scheme 21).
Although, the majority of zirconium-89 chelators are hydroxamate-based compounds, a series of other chelators have had good success as well. An alternative to $^{89}$Zr-DFO tracers - polyazamacrocycle ligands (Scheme 21) has been reported to allow radiolabeling in ambient conditions and have remarkable stability in transchelation, competition and in vitro test challenges.\textsuperscript{119}

Albeit long half-life time of $^{89}$Zr allows flexible timing of radiosynthesis and radiotracer handling, advances in shortening synthesis time are vital as well. Short synthesis times grants shorter working time of a radiochemist and quicker delivery of a tracer to the patient. This can be achieved by using one pot, light assisted radiosynthesis with azide modified chelator (Scheme 22). Basis for following synthesis can be found in a scope of click chemistry reactions that are modular, stereospecific, wide in scope, give very high yields and generate only inoffensive by-products removable by nonchromatographic methods. Additionally, click reactions include
simple reaction conditions (ideally oxygen and water insensitive) and easily available reagents.\textsuperscript{120} Those characteristics are fitting for zirconium radiolabeling of antibodies and the method described by Klingler et al. gives several advantages. The reaction is rapid and the time in which the chelator is conjugated to the protein and labeled with zirconium-89 can be less than 15 minutes. Secondly, photosynthesis is chemoselective towards lysine side-chain, due to formed ketenimine intermediate. Photoactivation occurs at wavelength 365 nm that is not absorbed by antibodies; thus it is not damaging to protein structure and function. Additionally, this approach allows full automation of radiosynthesis in contrast with conventional methods. However, potential conjugation of chelator to lysine residue within the binding site and large batch of used protein need to be consider as probable disadvantages of this method.\textsuperscript{121}

\begin{center}
\begin{tikzpicture}
\node (a) at (0,0) {\textbf{Scheme 22. Multicomponent photoradiosynthesis reaction mechanism. A) Radiosynthesis of }\textsuperscript{89}Zr-DFO-azepin-onartuzumab.};
\end{tikzpicture}
\end{center}
CONCLUSIONS AND PERSPECTIVES

Lastly, but not least it is important to acknowledge the fact that each of presented radioisotope has its advantages and disadvantages. Carbon-11 chemistry brings many practical challenges. Automatization of $^{11}$C-tracers production is needed due to high activity that radiochemists need to work with, due to low (in comparison with $^{18}$F) activity yields. Also, as a result of typical molar activities being usually on a high level (magnitude of 40–750 GBq/μmol) and administration of a PET-tracer to patient is less than 1 GBq, the injected amount of carbon-11 tracer is in nano- or even picomolar scale. This means that synthesis needs to be performed in an environment protected against possible contaminations with stable isotopes of carbon and purification done in a very attentive manner (methane and carbon dioxide from the atmosphere). $^{18}$F gives a little bit more flexibility due to its longer half-life, however, like $^{11}$C small scale of produced tracer needs careful quality control. In comparison with carbon and fluorine, gallium and zirconium radiochemistry is rather simple and straightforward. Additionally, $^{68}$Ga labeling can be easily performed with kits, in an automated way. However, energy of the positrons for $^{68}$Ga is relatively high and obtained resolution from PET scans is worse than those from $^{11}$C and $^{18}$F scans. Therefore, there is a visible trend in translation of $^{68}$Ga-tracers into their corresponding $^{18}$F molecules (e.g. $^{68}$Ga-PSMA and $^{18}$F–PSMA). $^{89}$Zr is one of few PET isotopes with half life time long enough for radiolabeling bigger molecules like antibodies. Long half-life time allows for flexible radiolabeling procedures, tracer handling and waiting time between injection and PET scan. Waiting time is needed for proper biodistribution of the tracer and accordingly contributes to achieving high signal to noise ratio PET images. Together with the rise of antibody therapy, immunoPET and zirconium-89 radiolabeling field is expanding as well. Although automation of $^{89}$Zr labeling is not a current practice, as it is in case of $^{68}$Ga, it is definitely in a perspective of a near future. Nonetheless, $^{89}$Zr emits also high energy gamma photons, that are not part of PET image, but contribute to radioactivity exposure. Therefore, a greater radioactivity burden must be recognized, while administering a tracer with this radioisotope to the patients.

Radiochemistry is undoubtedly one of the most rapidly developing research fields and there is still a lot of possible reactions to be translated from conventional organic and inorganic chemistry. This chapter has demonstrated that not only simple reactions can be successfully utilized in tracer development, but also complex organic chemistry reactions. In addition to research exploration, complex reactions found their way into GMP production (e.g. Cu-mediated fluorination of BPin precursor). Similarly to the organic chemistry, the inorganic chemistry has also had a great influence on radiochemistry development. This field of science not only helped to understand bond creation and quantification of optimal number of coordinating bonds between radiometals and chelators, but also unlock novel chelator design and synthesis. For every new tracer stability examination is crucial, due to possibility of degradation, dissociation and in vivo metabolism. Suboptimal tracer stability can be improved by radiolabeling in different positions of tracer molecule or usage of other chelators. Those synthetic pathways are available due to advancement made in radiochemistry. Without no reasonable doubt a combination of incorporation of reactions well known outside of radiochemistry field, automatization and module development will contribute to exponential growth of radiochemistry in the near future.
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