

Challenges for Developing PET Tracers: Isotopes, Chemistry, and Regulatory Aspects

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KEYWORDS

• Carbon 11 • Fluorine 18 • PET regulatory • Chemistry

PET continues to be the primary functional imaging technique for conducting translational research studies aimed at identifying the molecular basis of human disease. Although PET imaging studies of the central nervous system (CNS) initially focused on global or regional changes in brain function such as glucose utilization, cerebral blood flow, and oxygen metabolism, a major effort in PET research in the past 20 years has focused on the development of specific probes that are capable of studying the change in neurotransmitter receptors, second messenger systems, and neuronal networks in the brain. As our knowledge of the diversity of neurotransmitter systems continues to grow, so will the need to develop molecular imaging probes with an even higher target specificity than first-generation PET radiotracers. For example, before the cloning of dopamine receptors, when PET imaging studies of the dopamine D₂ receptor began in the mid-1980s, it was believed that there were only 2 types of dopamine receptors: D₁ and D₂. It is now known that there are 2 different families of dopamine receptors: D₁-like (consisting of the D₁ and D₅ receptors) and D₂-like (consisting of the D₂, D₃, and D₄ receptors).¹ There is a growing body of evidence that within the D₂-like family of receptors, D₂ and D₃ receptors are regulated in an opposing manner under conditions of increased or decreased dopaminergic tone. For example, autoradiography studies have shown that there is

a 15% increase in D₂ receptors in the caudate and putamen and a 45% decrease in D₃ receptors in the ventral striatum of postmortem brain samples of Parkinson disease.² Similar results have been reported in the study of chronic exposure to cocaine on D₂ and D₃ receptor function. That is, D₂ receptors are reduced in autoradiography³ and PET imaging studies of rhesus monkeys that have self-administered cocaine,⁴ and in chronic cocaine abusers.^{5,6} However, the autoradiography studies conducted by Staley and Mash⁷ reported an upregulation of D₃ receptors in human cocaine overdose victims compared with age-matched controls. Because PET radiotracers such as [¹¹C]raclopride, [¹⁸F]fallypride, and [¹¹C]PHNO have a high affinity for D₂ and D₃ receptors, newer radiotracers having a higher affinity and selectivity for D₃ versus D₂ receptors are needed to study the role of the D₃ receptor in CNS disorders. This same argument can be applied to many other CNS receptor systems, including the serotonergic system (7 different families for receptors and at least 13 different receptor subtypes),⁸ glutamate receptors (2 different classes, ionic and metabotropic, with at least 11 different subtypes),⁹ and the adrenergic receptors (6 different subtypes of α adrenergic receptors and 3 different subtypes of β receptors).¹⁰ As the need for more subtype-selective radiotracers grows, the success criteria for these newer radiotracers are expected to be higher than PET chemists have

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striven to achieve in the past. For example, as a PET radiotracer becomes more subtype selective, the target density to be imaged by the tracer within the CNS (ie, receptor density or B_{max}) invariably decreases. The development of high-affinity, high-selectivity, and low-nonspecific-binding radiotracers will likely be required to provide a suitable signal-to-noise ratio in PET imaging studies. Factors such as improved specific activity also play a key role in the ability to image receptors, and other protein targets that have a low expression in the CNS.

This article does not review the advances in radiotracer design with respect to subtype-selective PET imaging probes; that topic is covered in other articles in this and other issues of *PET Clinics*. This article provides an overview of the basic principles of PET radiochemistry and describes recent advances in PET radiosynthesis to assist the development of subtype-selective PET radiotracers needed to address the next level of scientific inquiry within clinical neuroscience research. The general principles of radiotracer design used by many chemists in the development of a PET radiotracer are also described, especially when this involves the incorporation of fluorine 18 (¹⁸F) into a molecule that does not possess a fluorine atom. A description of the current regulatory guidelines relevant to the production of PET radiotracers for human use studies is also provided.

RADIONUCLIDES USED IN PET

The 4 positron-emitting radionuclides most frequently used in PET are oxygen 15 (¹⁵O), nitrogen 13 (¹³N), carbon 11 (¹¹C), and ¹⁸F. There are several reasons why these radionuclides are routinely used in PET imaging applications. The first is that most of these radionuclides can be substituted into biologically active molecules with a hot-for-cold substitution. That is, ¹¹C can be substituted for nonradioactive ¹²C in a biologically active molecule without altering the biologic

properties of the molecule. In this example, ¹¹C is a true radiotracer because the structure of the biologically active molecule has not been altered to introduce the PET radiolabel. The same concept applies to ¹⁵O and ¹³N; however, the short half-lives of ¹⁵O and ¹³N have limited their use in PET radiotracer development. Consequently, these radionuclides are largely used as perfusion tracers: [¹⁵O]water for brain perfusion studies and [¹³N]ammonia for heart perfusion studies.

An exception to the principle of hot-for-cold substitution is ¹⁸F; although fluorine is the most abundant halogen in soil samples, the abundance of naturally occurring organofluorine compounds is low and limited to fluorinated carboxylic acids such as fluoroacetate, fluorocitrate, and fluorinated fatty acids produced in numerous plant species indigenous to Africa, Australia, and South America.¹¹ There are no naturally occurring organofluorine compounds in animals. However, fluorine is a frequently used substituent in drug development, and there are several fluorine-containing drugs targeting neurotransmitter receptors in the CNS.¹² These include the antidepressants fluoxetine and paroxetine, the tricyclic antipsychotic fluphenazine, the butyrophenones haloperidol and spiperone, and the atypical antipsychotics risperidone and setoperone. These fluorine-containing drugs have served as a direct source of leads for PET radiotracers involving the principle of an ¹⁸F for ¹⁹F substitution.^{13–19} In addition to these examples, it is often possible to incorporate fluorine into a nonfluorine-containing lead compound without losing activity at the target protein; 1 example is the dopamine D₂ imaging agent fluorocleobopride, an analogue of the dopamine antagonist cleopride.²⁰

A second reason for the prominent use of these radionuclides in PET imaging studies is that each can be produced in high yield and high specific activity with a low energy (ie, 11–17 MeV), medical cyclotron using either the p,n (¹⁵O, ¹³N, ¹⁸F) or p,α (¹¹C) nuclear reaction (Table 1). Because the

Table 1 Radionuclides commonly used in PET					
Radionuclide	Half-life (min)	Nuclear Reaction	Average β + Energy (keV)	β + Range mm (H ₂ O)	Maximum Specific Activity (Ci/μmol)
Oxygen 15	2	¹⁵ N(p,n) ¹⁵ O	735	8.2	91730
Nitrogen 13	10	¹³ C(p,n) ¹³ N	491	5.39	18900
Carbon 11	20	¹⁴ N(p,α) ¹¹ C	385	4.1	9220
Fluorine 18	110	¹⁸ O(p,n) ¹⁸ F	242	2.39	1710

Routine specific activity: ¹¹C, ~10 Ci/μmol; [¹⁸F]fluoride, 2–10 Ci/μmol.

target material is either gas (^{15}O , ^{11}C) or liquid (^{13}N , ^{18}F), the radionuclides are readily transferred from the cyclotron target to a hot cell in the radiochemistry laboratory, where the radionuclide can be incorporated into a biologically active molecule. There are also several commercially available automated chemistry systems that are capable of converting these PET radionuclides, obtained as low molecular weight species from the cyclotron target, into either a chemically reactive species or a radiolabeled prosthetic group. Automated chemistry systems are typically designed to conduct 2 to 3 organic reactions in series, followed by either a resin-based or high-performance liquid chromatography (HPLC) purification step, leading to the synthesis of a PET radiotracer that is suitable for imaging studies in preclinical imaging studies in animal models or in clinical research studies. Although simplistic in theory, the adaptation of a commercially available automated PET chemistry system to enable the synthesis of structurally diverse PET radiotracers, and the routine review and optimization of the synthesis to ensure reproducible radiochemical yields and high specific activity, requires considerable expertise in organic chemistry and chemical engineering. Quality assurance testing to confirm that the radiotracer meets criteria for radiochemical and chemical purity, specific activity, sterility, pyrogen levels, and presence of residual solvents for use in humans also requires expertise in analytical chemistry and radiopharmacy. Therefore, most PET centers actively engaged in clinical research consist of a collaboration between organic/medicinal chemists, chemical engineers, analytical chemists, and radiopharmacists working in concert to meet the significant time constraints imposed by the short-lived radioisotopes routinely used in PET.

Because ^{11}C and ^{18}F are the 2 main radionuclides used in the development of PET radiotracers for imaging brain function, this article focuses on a review of the basic principles and recent developments in the radiosynthesis of ^{11}C - and ^{18}F -labeled compounds.

^{11}C Radiochemistry

^{11}C is produced in the cyclotron target using the $^{14}\text{N}(\text{p},\alpha)^{11}\text{C}$ nuclear reaction. The target material is nitrogen (N_2) containing trace quantities of oxygen (0.5% O_2) to convert the ^{11}C into $^{11}\text{C}\text{CO}_2$.^{21,22} The simplest reaction using ^{11}C involves the transfer of $^{11}\text{C}\text{CO}_2$ from the target to a chemistry module or a reaction vessel containing a Grignard reagent that reacts with $^{11}\text{C}\text{CO}_2$ to give the corresponding ^{11}C -labeled

carboxylic acid. The most prominent examples of this process are the metabolic tracers ^{11}C acetate and ^{11}C palmitate (**Fig. 1**), which are prepared by the direct venting of $^{11}\text{C}\text{CO}_2$ into a solution of either methylmagnesium bromide or 1-pentadecylmagnesium bromide in an anhydrous solvent such as tetrahydrofuran or ether.²³ There are 2 examples in which $^{11}\text{C}\text{CO}_2$ has been used in the synthesis of a PET radiotracer for imaging CNS receptors: (1) the serotonin 5-hydroxytryptamine_{1A} (5-HT_{1A}) antagonist ^{11}C Way-10065^{24,25}; and (2) the radiolabeled dopamine D₂/D₃ agonist, ^{11}C (+)-PHNO, which is primarily used to image the high affinity state of the dopamine D₂ and D₃ receptors.^{26–28} In each case, $^{11}\text{C}\text{CO}_2$ is vented through a solution of the Grignard reagent (cyclohexyl magnesium chloride for Way-10065 and ethylmagnesium bromide for (+)-PHNO) to give ^{11}C -labeled carboxylic acid; conversion to the corresponding acid chloride results in the formation of ^{11}C -labeled acid chloride, which is reacted with the secondary amine (see **Fig. 1**) to give the corresponding amide. Synthesis of ^{11}C (+)-PHNO requires the additional reduction of the amide with lithium aluminum hydride resulting in the formation of ^{11}C (+)-PHNO.²⁶

The most common chemical transformation of $^{11}\text{C}\text{CO}_2$ is into ^{11}C methyl iodide ($^{11}\text{C}\text{CH}_3\text{I}$) (**Fig. 2**), a highly reactive species that can be used to introduce ^{11}C into biologically active molecules via alkylation of N-, O-, or S-nucleophiles (**Fig. 3**). The initial method for preparing $^{11}\text{C}\text{CH}_3\text{I}$ involved passing $^{11}\text{C}\text{CO}_2$ through a solution of lithium aluminum hydride (LiAlH_4) and tetrahydrofuran (see **Fig. 3**), followed by quenching with hydriodic acid. This method has been largely replaced with the gas phase method of synthesizing ^{11}C methyl iodide, which involves the catalytic reduction of $^{11}\text{C}\text{CO}_2$ to ^{11}C methane ($^{11}\text{C}\text{CH}_4$), followed by iodination with molecular iodine (I_2) to produce $^{11}\text{C}\text{CH}_3\text{I}$. An alternative method for preparing $^{11}\text{C}\text{CH}_3\text{I}$ involves the direct production of $^{11}\text{C}\text{CH}_4$ by using a target gas composition of 10% H_2/N_2 in the target, followed by iodination with molecular I_2 (see **Fig. 2**). The rationale for using the in-target production of $^{11}\text{C}\text{CH}_4$ is the lower amount of methane in the atmosphere (~1.6 ppm) relative to CO_2 (~300 ppm); in theory, this should result in improved specific activity of $^{11}\text{C}\text{CH}_3\text{I}$. However, the inexplicable low recovery for the in-target production of $^{11}\text{C}\text{CH}_4$ has prevented the widespread use of this method for the synthesis of $^{11}\text{C}\text{CH}_3\text{I}$. Average specific activities of ^{11}C -labeled radiotracers using $^{11}\text{C}\text{CH}_3\text{I}$ have been reported in the range of 2 to 10 Ci/ μmol (74–370 GBq/ μmol). This specific activity is lower than the

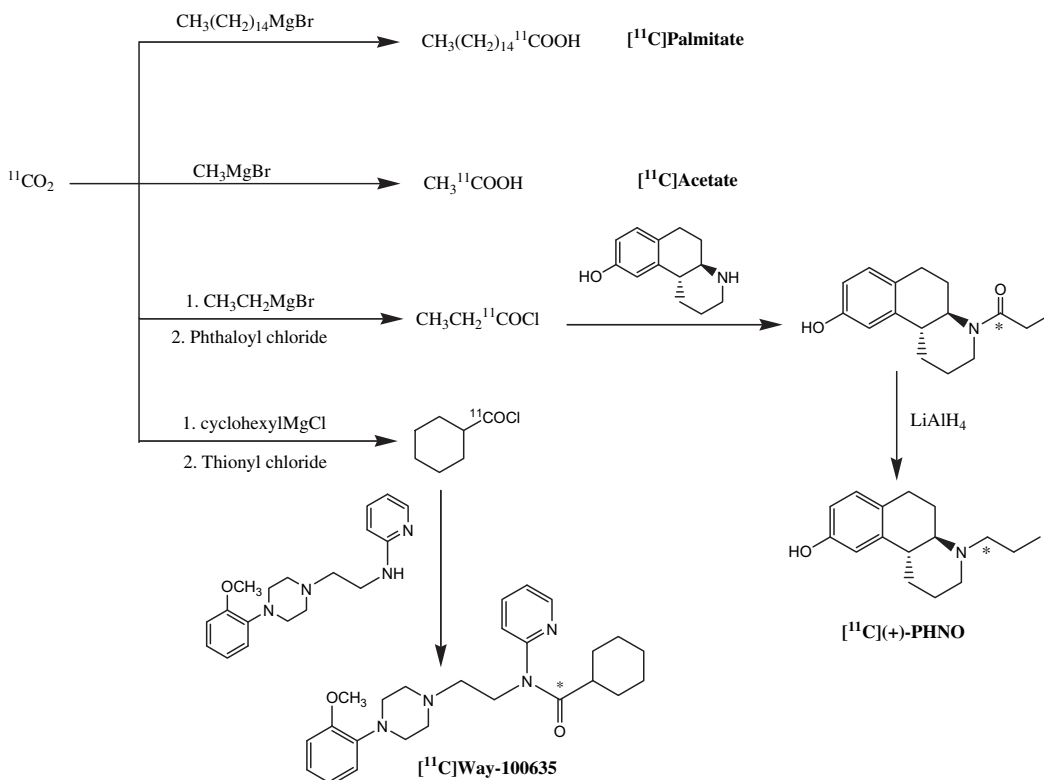


Fig. 1. Synthesis of [^{11}C]palmitate, [^{11}C]acetate, [^{11}C](+)-PHNO and [^{11}C]Way-100635 using [^{11}C]CO₂.

maximum theoretic specific activity of ^{11}C (see **Table 1**). Therefore, for an ^{11}C -labeled radiotracer having a specific activity of 10 Ci/mmol, only 1 in 1000 tracer molecules contain ^{11}C , with the remaining containing ^{12}C .

Another highly reactive form of ^{11}C is [^{11}C]methyltriflate ([^{11}C]CH₃OTf). [^{11}C]CH₃OTf is formed by passing gaseous [^{11}C]CH₃I over a column containing silver triflate (AgOTf) at 200°C (see **Fig. 2**).²¹ The advantage of [^{11}C]CH₃OTf is that this method generally requires lower reaction temperatures, shorter reaction times, and lower amounts of the *des*-methyl precursor for labeling than is usually required to label a compound using [^{11}C]CH₃I. Because HPLC retention times of the *des*-methyl precursor are similar to the ^{11}C -labeled *N*-methyl

product, reducing the amount of precursor for the labeling reaction can simplify the HPLC purification of the radiotracer as a result of a reduced tendency of peak broadening by the *des*-methyl precursor. Although this time saving may seem trivial, reducing the synthesis time from 30 minutes to 25 minutes leads to an approximately 20% increase in specific activity of a ^{11}C -labeled radiotracer simply by reducing the loss of activity by radioactive decay. Examples comparing radiolabeling with [^{11}C]CH₃I and [^{11}C]CH₃OTf are shown in **Fig. 4**.^{22,29–31} Perhaps the best example of the advantage of [^{11}C]CH₃OTf over [^{11}C]CH₃I is the synthesis of the β amyloid imaging agent [^{11}C]PiB. The synthesis of [^{11}C]PiB from [^{11}C]CH₃I requires the addition of a base such as

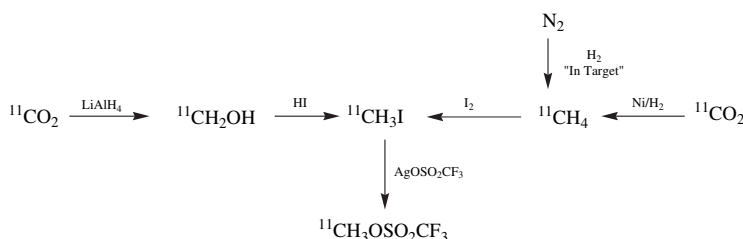


Fig. 2. Synthesis of [^{11}C]CH₃I and [^{11}C]CH₃OTf.

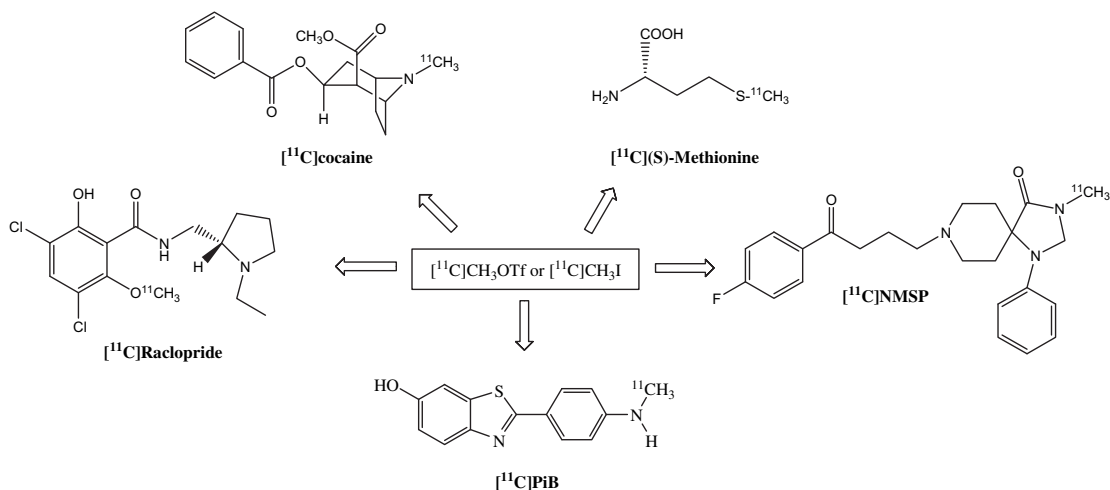


Fig. 3. Examples of ^{11}C -labeled radiotracers synthesized using $[^{11}\text{C}]\text{CH}_3\text{I}$ or $[^{11}\text{C}]\text{CH}_3\text{OTf}$.

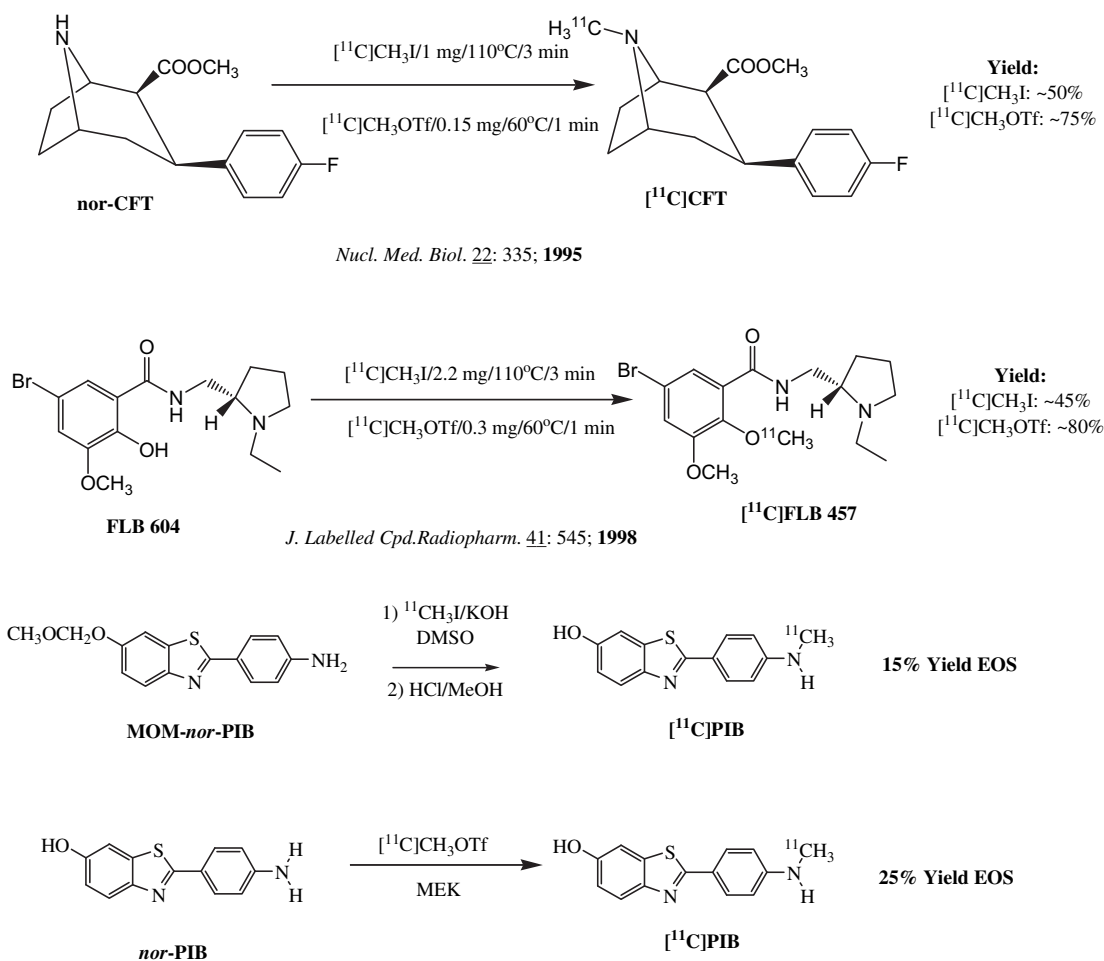


Fig. 4. Comparison of ^{11}C -radiolabeling using $[^{11}\text{C}]\text{CH}_3\text{I}$ and $[^{11}\text{C}]\text{CH}_3\text{OTf}$.

sodium hydroxide because of the low reactivity of the amine nitrogen (see **Fig. 4**). This process requires the protection of the hydroxyl group of the precursor as the corresponding methoxymethyl (MOM) ether to prevent the unwanted reaction of $[^{11}\text{C}]\text{CH}_3\text{I}$ at the oxygen atom. Consequently, a separate acid-catalyzed deprotection step is required to prepare $[^{11}\text{C}]\text{PiB}$ following N-alkylation of the amine group with $[^{11}\text{C}]\text{CH}_3\text{I}$.^{32,33} In comparison, because $[^{11}\text{C}]\text{CH}_3\text{OTf}$ has a higher chemical reactivity than $[^{11}\text{C}]\text{CH}_3\text{I}$, the reaction at the aniline nitrogen occurs without the addition of a base catalyst, thereby eliminating the need to protect the phenol group as the corresponding MOM ether. This 1-step synthesis using $[^{11}\text{C}]\text{CH}_3\text{OTf}$ is the preferred method for making $[^{11}\text{C}]\text{PiB}$ because of its shorter synthesis time and higher radiochemical yield.^{34–36}

¹⁸F Radiochemistry

Two forms of ^{18}F are used in the synthesis of PET radiotracers: nucleophilic and electrophilic. Nucleophilic fluoride is produced via the $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$ nuclear reaction using $[^{18}\text{O}]\text{water}$ as the target material. This process results in the formation of $[^{18}\text{F}]\text{HF}$, which is transferred from the target to either a reaction vessel containing a base such as potassium carbonate (to make $[^{18}\text{F}]\text{KF}$) or tetrabutylammonium hydroxide (to give $[^{18}\text{F}]\text{TBAF}$). Alternatively, the radioactivity can be passed through an anion exchange resin, which traps the activity as $[^{18}\text{F}]\text{fluoride}$. The activity is then removed from the anion exchange resin by elution

with an aqueous solution of base (usually potassium carbonate or tetrabutylammonium hydroxide) to give $[^{18}\text{F}]\text{fluoride}$ as a salt (ie, $[^{18}\text{F}]\text{KF}$ or $[^{18}\text{F}]\text{TBAF}$). An advantage of $[^{18}\text{F}]\text{TBAF}$ is that it can be directly solubilized into organic solvents such as acetonitrile and dimethylsulfoxide, whereas $[^{18}\text{F}]\text{KF}$ requires the addition of the crown ether [2.2.2]kryptofix to solubilize the reactivity.

$[^{18}\text{F}]\text{Fluoride}$ is incorporated into biologically active molecules via 2 types of reaction mechanisms: $\text{S}_{\text{N}}2$ and $\text{S}_{\text{N}}\text{Ar}2$. The $\text{S}_{\text{N}}2$ mechanism occurs when an appropriate leaving group is displaced from an aliphatic (ie, sp^3) carbon atom (**Fig. 5**). The $\text{S}_{\text{N}}\text{Ar}2$ mechanism occurs when the leaving group is displaced from an aromatic (ie, sp^2) carbon atom such as a benzene ring. Although fluoride is a relatively unreactive nucleophile, high yields of an ^{18}F -labeled radiotracer can be obtained with the $\text{S}_{\text{N}}2$ mechanism if the precursor contains a good leaving group such as a triflate ($\text{R} = \text{CF}_3$) or mesylate ($\text{R} = \text{CH}_3$) group. In the $\text{S}_{\text{N}}\text{Ar}2$ mechanism, the para position must be activated by an electron-withdrawing group to increase the rate of reaction so that an acceptable yield of the labeled compound can be obtained within the time constraints imposed by ^{18}F . Examples of activating groups are the cyano (CN), nitro (NO_2), keto ($\text{RC} = \text{O}$) and aldehyde ($\text{HC} = \text{O}$) groups (see **Fig. 5**). Although the nitro group was historically the first leaving group used in radiofluorinations using the $\text{S}_{\text{N}}\text{Ar}2$ mechanism, the trimethylammonium group is more commonly used today because of its rapid rate of reaction and

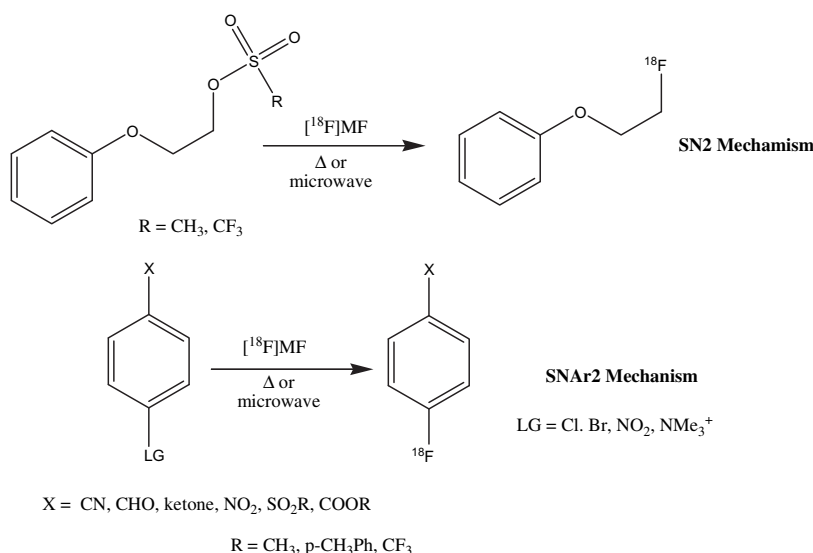


Fig. 5. Reaction mechanism for introducing ^{18}F into organic molecules. M refers to a positive cation such as K^+ , Cs^+ , or $(\text{Bu})_4\text{N}^+$.

the ease of eliminating unreacted precursor resulting from the high polarity of the charged trimethylammonium group.³⁷ There are 2 methods used for increasing the rate of reactivity of [¹⁸F]fluoride: heating the reaction mixture (from 90 to 160 °C) and microwave irradiation. Microwave irradiation is rapidly becoming the preferred method of nucleophilic [¹⁸F]fluoride incorporation because the reaction time needed to achieve a high radiochemical yield of the product is typically faster than the simple thermal method of incorporation.

Although activation of the paraposition within 1 of these groups facilitates the incorporation of [¹⁸F]fluoride, it does not guarantee a high radiochemical yield. An example of this is shown in **Fig. 6**. Whereas [¹⁸F]fluoride is readily incorporated into the nitroprecursor, resulting in a high radiochemical yield of [¹⁸F]setoperone, similar reaction conditions result in only a low yield of [¹⁸F]*N*-methylspiperone even although both substrates have a ketone as the activating group in the paraposition.^{19,38} The low yield of [¹⁸F]*N*-methylspiperone is likely caused by keto-enol tautomerization, which leads to the formation of the nonreactive enol (see **Fig. 6**). Because of the long half-life of ¹⁸F (110 minutes), a multistep synthesis of [¹⁸F]NMSP was developed³⁹ (**Fig. 7**), which afforded yields suitable for human imaging studies.⁴⁰

A second example of the S_NAr2 reaction mechanism for incorporating ¹⁸F into biologically active molecules involves the displacement of a leaving group in either the 2 or 6 position of a pyridine ring. Because pyridine is a π -deficient heteroaromatic ring system, it is prone to nucleophilic attack at the 2, 4, and 6 positions. Therefore, a pyridine ring does not require the presence of an electron-withdrawing group to activate the ring system to nucleophilic attack by [¹⁸F]fluoride. This strategy has been used extensively in the synthesis of ligands for imaging nicotinic $\alpha_4\beta_2$ receptors (**Fig. 8**).^{41–44}

An alternative strategy for introducing high specific activity, nucleophilic [¹⁸F]fluoride into ligands is to use an ¹⁸F-labeled prosthetic group. This method uses the same basic principle as labeling a molecule with [¹¹C]CH₃I, which is essentially an ¹¹C-labeled prosthetic group. In this approach, ¹⁸F is incorporated into an organic molecule, which is then used to alkylate a precursor leading to an ¹⁸F-labeled radio-tracer.¹⁷ As with [¹¹C]CH₃I, the most common reaction is the alkylation of a nitrogen atom. The following different ¹⁸F-labeled prosthetic groups have been used to date: [¹⁸F]2-fluoroethoxytriflate or [¹⁸F]2-fluoroethyltosylate^{45,46}; and, [¹⁸F]4-fluorobenzyl iodide (or bromide).^{47–49} Examples

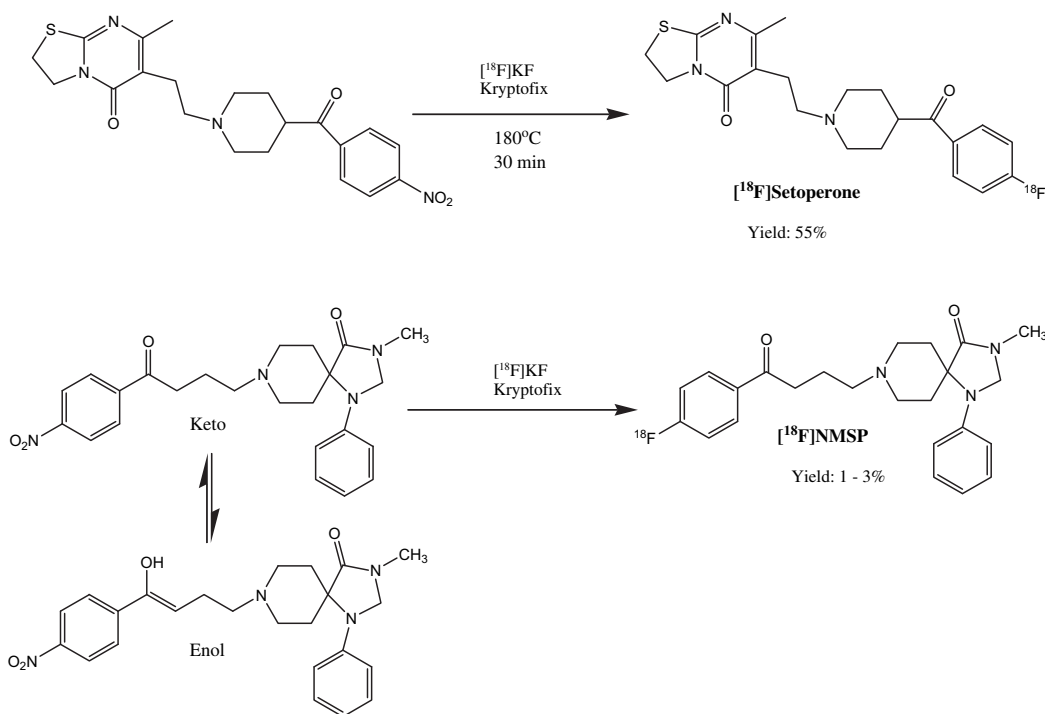


Fig. 6. Examples of nucleophilic incorporation of [¹⁸F]fluoride. Note the difference in radiochemical yield even although both substrates are activated by a ketone group in the para position.

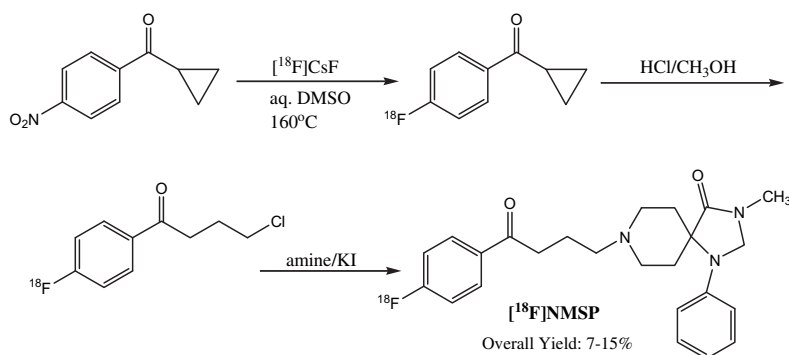


Fig. 7. Synthesis of [^{18}F]NMSP by the PET group at Brookhaven National Laboratory.

of the use of these prosthetic groups in radiotracer synthesis are shown in **Figs. 9** and **10**.

A recent development in PET radiochemistry is the use of copper-assisted 1,3-dipolar cycloaddition reactions to prepare ^{18}F -labeled compounds. The method, often referred to as click chemistry, uses either an ^{18}F -labeled fluorinated acetylene or an ^{18}F -labeled organic azide as the prosthetic group (**Fig. 11**). The first click radiolabeling reactions used simple [^{18}F]fluoroalkynes⁵⁰ and [^{18}F]2-fluoroethylazide.⁵¹ Reported radiochemical yield using this strategy has been high, often in excess of 80%.^{50,51} This labeling strategy has been used

most often in the radiolabeling of peptides because it avoids the need to protect the multiple functional groups in a polypeptide that could react with the prosthetic groups currently used in producing ^{18}F -labeled peptides (**Fig. 12**). Several second-generation ^{18}F -labeled click synthons have been introduced recently⁵²⁻⁵⁴ and are shown in **Fig. 11**. A review of the use of click chemistry in PET radiotracer design was recently published by Glaser and Robbins.⁵⁵

A second method for introducing ^{18}F into biologically active molecules is through the use of an electrophilic fluorination reaction with [^{18}F]F₂.

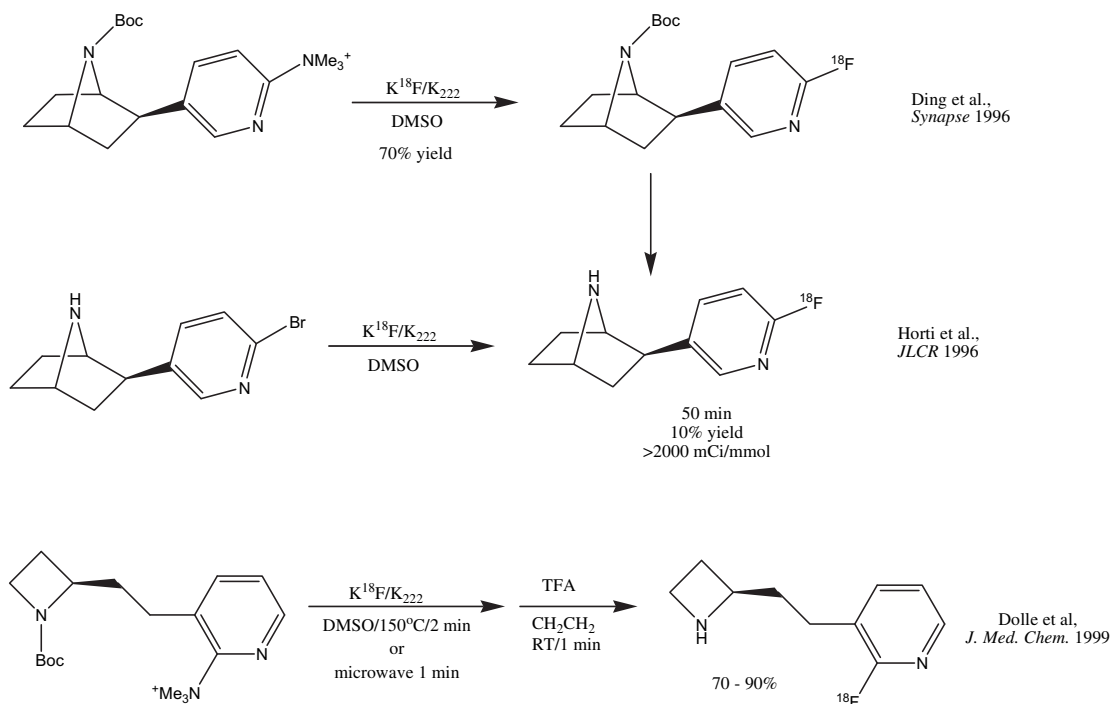


Fig. 8. Synthesis of ^{18}F -labeled radiotracers involving the direct introduction of ^{18}F into the 2-position of a pyridine ring.

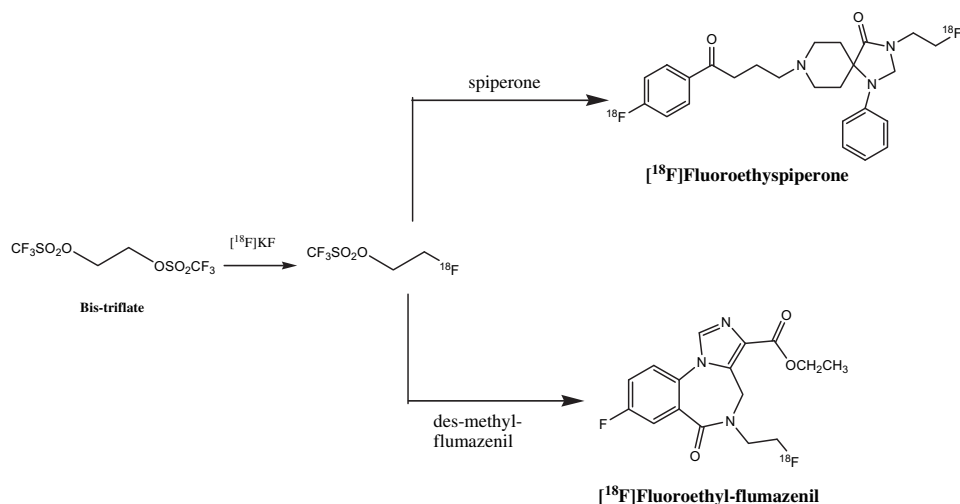


Fig. 9. Radiosynthesis using the [F^{18}]2-fluoroethoxytriflate as a prosthetic group.

There are 2 different ways to produce electrophilic [F^{18}] F_2 . The first method, which requires a cyclotron designed to accelerate deuterons, is the $^{20}\text{Ne}(\text{d},\alpha)^{18}\text{F}$ nuclear reaction (**Fig. 13**). The neon target gas contains ~2% F_2 to complete the

in-target production of [F^{18}] F_2 . The second method for producing [F^{18}] F_2 involves the $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$ nuclear reaction. This method, which is generally used on proton-only cyclotrons, requires a multistep process to produce

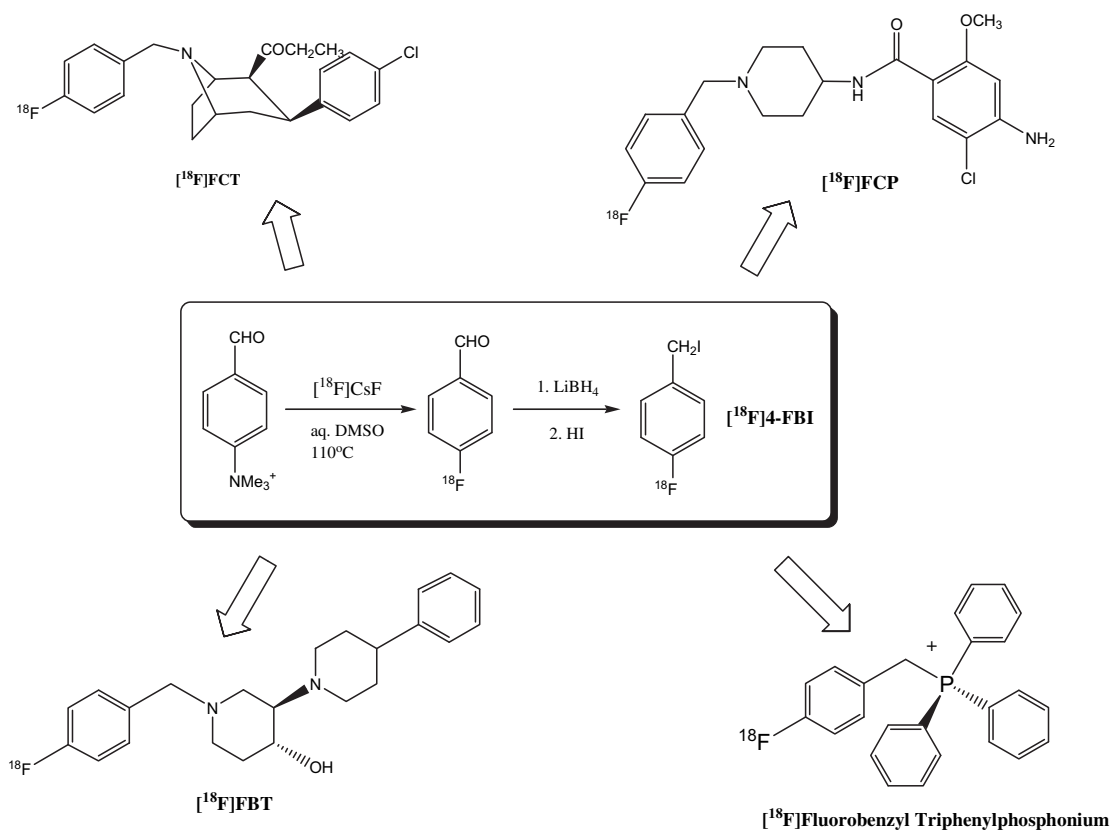


Fig. 10. Examples of ^{18}F radiolabeling using the prosthetic group, [F^{18}]4-fluorobenzyl iodide. (Data from Refs. 47–49)

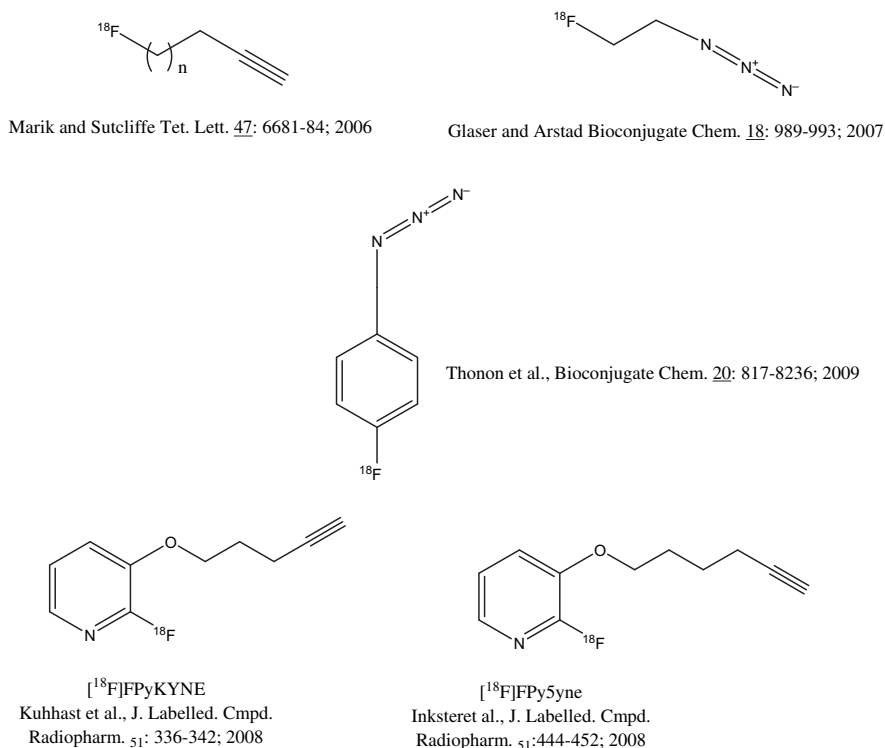


Fig. 11. Prosthetic groups used in click labeling with ^{18}F .

$[^{18}\text{F}]\text{F}_2$.⁵⁶ The first step involves irradiation of 2% F_2/Ar mixture to passivate the target. The target is then filled with $[^{18}\text{O}]\text{O}_2$, and a second irradiation is conducted to produce ^{18}F -labeled species that are deposited on to the interior surface of the target. This deposition of the ^{18}F on to the target surface enables the recovery of the $[^{18}\text{F}]\text{O}_2$ target gas. Once the $[^{18}\text{O}]\text{O}_2$ has been recovered, the target is filled with 2% F_2/Ar and a third irradiation is conducted to recover the $[^{18}\text{F}]\text{F}_2$. Electrophilic fluorination with $[^{18}\text{F}]\text{F}_2$ is the preferred method for making $[^{18}\text{F}]\text{fluoro-L-dihydroxyphenylalanine}$ ($[^{18}\text{F}]\text{FDOPA}$)⁵⁷ and $[^{18}\text{F}]\text{fluoro-meta-tyrosine}$,⁵⁸ radiotracers that measure presynaptic dopaminergic terminal density (see **Fig. 13**). Other PET radiotracer synthesized using $[^{18}\text{F}]\text{F}_2$ include 2 β -carbomethoxy-3 β -(4- $[^{18}\text{F}$]-fluorophenyl)tropane, $[^{18}\text{F}]\text{CFT}$,⁵⁹ which labels the dopamine transporter (another marker of dopamine terminal density) and 2-(2-nitro-1H-imidazol-1-yl)-N-(2,2,3,3,3-pentafluoropropyl)-acetamide (EF-5) labeled with ^{18}F -fluorine, $[^{18}\text{F}]\text{EF-5}$, a radiotracer for imaging hypoxia.⁶⁰ The main drawback to using electrophilic fluorination for introducing ^{18}F is low specific activity that is achieved using $[^{18}\text{F}]\text{F}_2$, because of the requirement of adding unlabeled F_2 to recover the radioactivity from the target. However, a method for producing electrophilic $[^{18}\text{F}]\text{F}_2$ from nucleophilic

$[^{18}\text{F}]\text{fluoride}$ was reported by Bergman and Solin in 1997.⁶¹ Although the addition of unlabeled F_2 is still required to form $[^{18}\text{F}]\text{F}_2$, a significant increase in specific activity of $[^{18}\text{F}]\text{FDOPA}$ and $[^{18}\text{F}]\text{CFT}$ have been reported using this method of electrophilic radiofluorination.⁶¹

FACTORS DETERMINING THE ABILITY OF PET RADIOTRACERS TO CROSS THE BLOOD-BRAIN BARRIER

There are several factors to contend with in the design of a radiotracer for imaging targets within the CNS. A description of these factors of prerequisites for CNS PET radiotracers can be found in the review by Ametamey and colleagues.⁶² A brief summary is provided in the next section.

Affinity and Selectivity for the Target Receptor or Enzyme

Ideally, a PET probe should possess a high (ie, nanomolar) affinity and high selectivity (>100-fold) for the target protein versus other proteins in the CNS. However, the exact affinity of a suitable PET ligand for its target protein depends on a variety of factors, including the density of the receptor or protein target, the presence of endogenous ligands that could compete with the

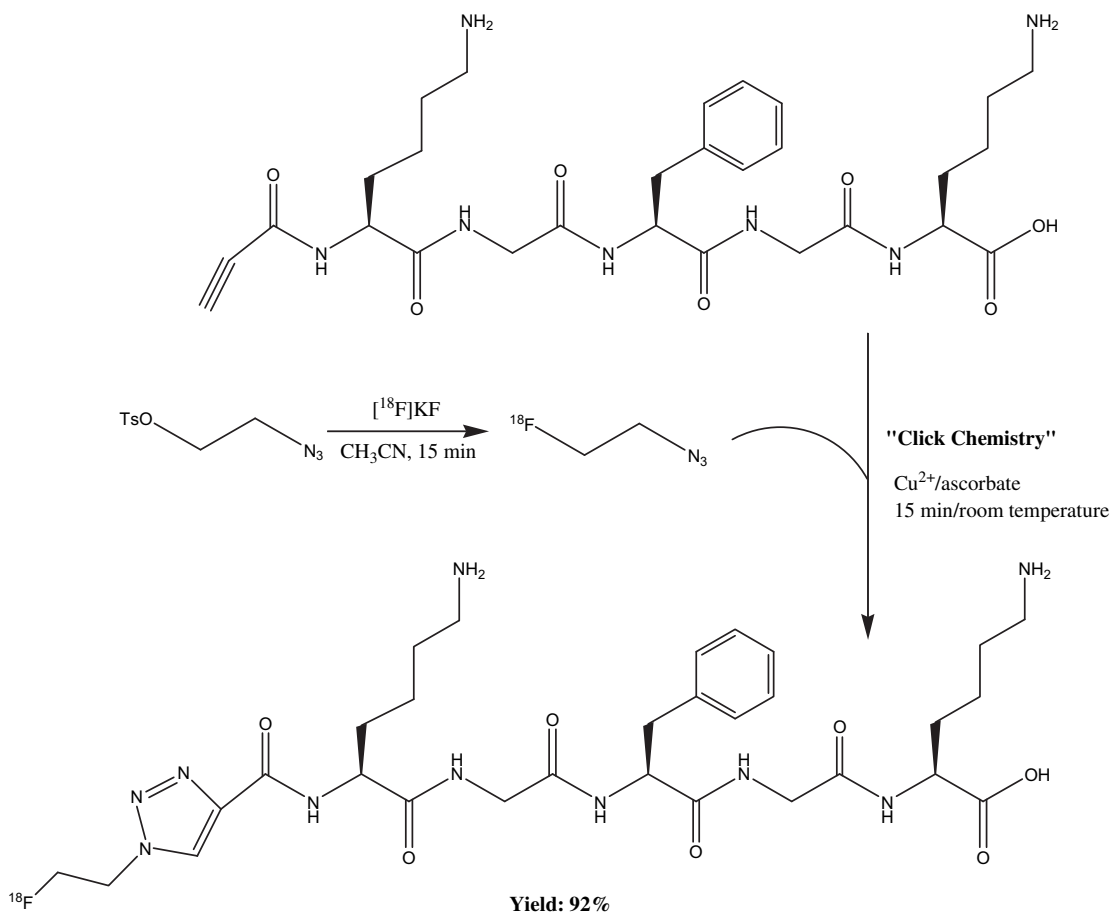


Fig. 12. Click chemistry and an example of its use in ^{18}F -labeled peptide chemistry. (Data from Glaser M and E Arstad. "Click labeling" with 2- ^{18}F fluoroethylazide for positron emission tomography. *Bioconjug Chem* 2007;18(3):989–93.)

radiotracer for ligand binding site on the protein, and the level of nonspecific binding of the tracer. Tracers having a high affinity (ie picomolar) for the target receptor often bind irreversibly to the receptor. That is, there is no washout of radiotracer from regions of the brain expressing the receptor during the data acquisition period of PET. The uptake of tracers displaying irreversible kinetics in vivo can be dependent on and influenced by differences in cerebral blood flow. Therefore, kinetically irreversible tracers often require correction for differences in cerebral blood flow (ie, ^{15}O water study or K_1 estimates by analyzing the early part of the tissue-time activity curves) to obtain accurate measures of the density or binding potential of the receptor or protein under investigation. A prime example of this concept is the development of PET radiotracers for imaging striatal versus extrastriatal D_2/D_3 receptors. ^{11}C Raclopride, which has ~ 5 nM affinity for D_2 and D_3 receptors, is a suitable

radiotracer for imaging striatal D_2/D_3 receptors. There is a high density of D_2/D_3 receptors in the caudate and putamen and the tracer binds reversibly to D_2/D_3 receptors. However, it is not generally useful for imaging extrastriatal D_2/D_3 receptors, which are expressed in lower density in dopamine receptor-enriched extrastriatal regions versus the caudate and putamen. The short half-life of ^{11}C and rapid dissociation of ^{11}C raclopride from dopamine receptors results in a low signal-to-noise ratio in regions with a low density of D_2/D_3 receptors. The best radiotracers for imaging extrastriatal D_2/D_3 receptors are ^{18}F fallypride and ^{18}F FLB-457, which have a picomolar affinity for D_2 and D_3 receptors, and a low level of nonspecific binding. These properties (picomolar affinity and low nonspecific binding) make it possible to obtain stable measures of extrastriatal receptors expressing in brain regions containing a low density of D_2 and D_3 receptors. The irreversible binding of ^{18}F fallypride and ^{18}F FLB-457 to D_2/D_3

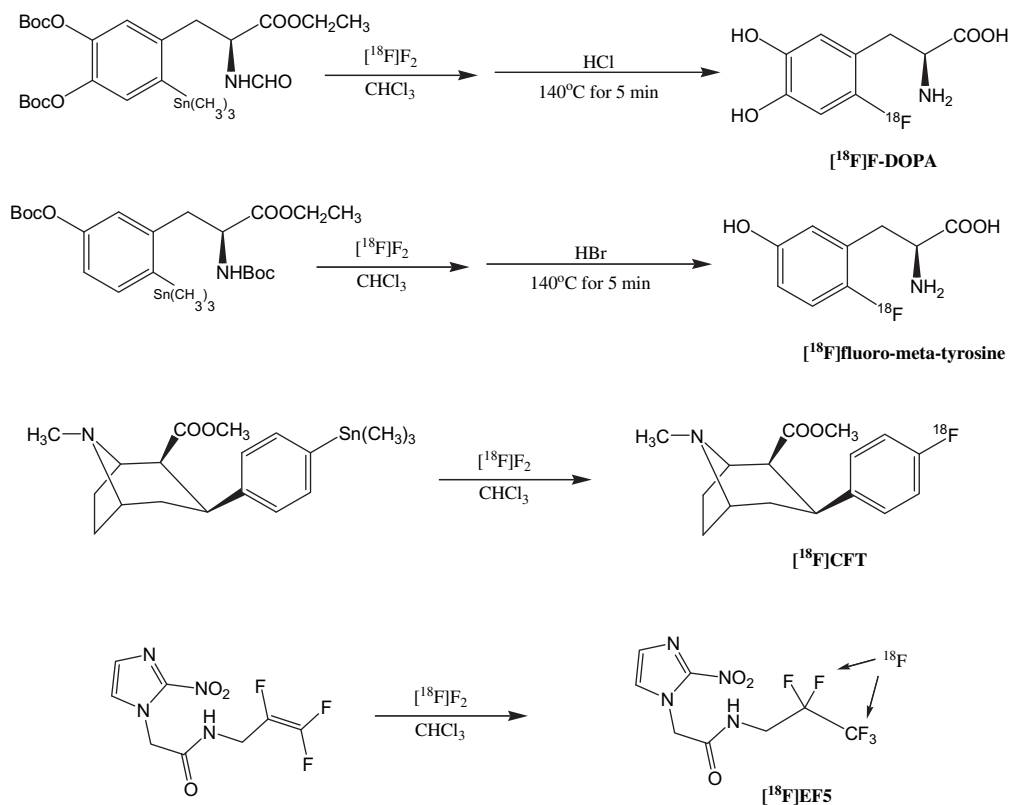


Fig. 13. The synthesis of PET radiotracers using electrophilic fluorination with $[^{18}\text{F}]\text{F}_2$.

receptors in the striatum, which has a high density of D_2 and D_3 receptors, requires a correction for differences in cerebral blood flow to obtain an accurate measure of the D_2/D_3 receptor binding potential.

Specific Activity

Specific activity is also a factor that must be considered for PET imaging studies in which there is a saturable level of receptor or target protein. The lower the target density (eg, B_{max}), the higher the specific activity needed to ensure that the principles of radiotracer binding are maintained (ie, only a small percentage of sites are occupied by the radioligands). Because specific activity changes with time, the more useful measurement in PET radiotracer formulations is the amount of mass contained in the dose. For ^{11}C - and ^{18}F -labeled radiotracers, 1 to 10 μg of cold compound are usually synthesized because of the presence of carrier in the target materials and reagents. The amount of mass that can be injected in the patient depends on the density of the target being imaged, as well as the pharmacology or toxicology of the compound. Typically 0.5 to 5 μg of carrier

can be administered to human patients without any adverse side-effects.

Lipophilicity and Other Physicochemical Properties

Because most CNS-based radiotracers cross the blood-brain barrier by a passive diffusion mechanism, the main physicochemical property of a PET radiotracer that determines its ability to enter the brain and label the target receptor or enzyme is the lipophilicity of the compound. Lipophilicity is operationally defined as the logarithm of the octanol-water partition coefficient of the compound (ie, the $\log P$ value). There is a parabolic relationship between the brain uptake and the $\log P$ of a PET radiotracer. The optimal range of $\log P$ values for a CNS radiotracer is 2.0 to 3.5, but radiotracers with $\log P$ values higher than 4.0 are also capable of crossing the blood-brain barrier, albeit at a lower value. Increasing the $\log P$ value also results in a higher amount of nonspecific binding of the radiotracer, which can be problematic for imaging targets with a low expression in the brain. Other factors that can influence the ability of a compound to cross the blood-brain barrier by a passive diffusion mechanism are the

molecular weight and number of hydrogen donors or acceptors in the molecule. Compounds with a molecular weight greater than 600 g/mol or less than 6 H bonds have a lower tendency to cross the blood-brain barrier. In addition, radiotracers displaying a high level of protein binding have a low free fraction and typically do not show high uptake in brain.

Metabolism

Because PET measures only the radioactive decay of a positron emitter, one must confirm that a PET radiotracer for imaging targets within the CNS does not undergo peripheral metabolism in a manner that results in the formation of radiolabeled metabolites capable of crossing the blood-brain barrier. The formation of lipophilic radiolabeled metabolites complicates the use of graphical or compartmental methods of radiotracer modeling because one must know the level of signal in the brain caused by the metabolite in addition to the parent compound, which is technically challenging. Consequently, most radiotracers used in quantitative imaging studies of CNS targets are metabolized in a manner that does not lead to the formation of lipophilic radiolabeled metabolites capable of crossing the blood-brain barrier. The metabolism rate of the radiolabeled compound can also affect the brain uptake of a radiotracer. Radiotracers that are rapidly metabolized (completely between 5 and 10 minutes after injection) typically have a low brain uptake because PET radiotracers usually reach peak uptake in brain between 10 and 30 minutes after injection.

FACTORS USED IN THE DESIGN OF PET RADIOTRACERS

One of the advantages of $[^{11}\text{C}]\text{CH}_3\text{I}$ and $[^{11}\text{C}]\text{CH}_3\text{OTf}$ in the development of PET radiotracers is that there are many lead compounds in the literature that have an *N*-methyl group in the parent structure that simplifies the development of an ^{11}C -labeled radiotracer. In this case, an ^{11}C -labeled radiotracer can be prepared by reacting the corresponding *des*-methyl precursor with $[^{11}\text{C}]\text{CH}_3\text{I}/[^{11}\text{C}]\text{CH}_3\text{OTf}$. Consequently, the ^{11}C -labeled analogue is a true radiotracer because the ^{11}C is incorporated in the parent compound. This situation is occasionally true with ^{18}F -labeled radiotracers, and $[^{18}\text{F}]\text{setoperone}$ and $[^{18}\text{F}]\text{NMSP}$ are examples where an ^{18}F for ^{19}F substitution leads to a straightforward preparation of an ^{18}F -labeled radiotracer. However, in many cases this is not an option, and an ^{11}C - or ^{18}F -labeled radiotracer must be designed using a lead compound

whose structure must be altered in a way to incorporate the ^{11}C or ^{18}F radiolabel. In this case, the radiolabel must be introduced into the lead compound in a manner that does not reduce the affinity of the ligand for the target macromolecule. Some of the logic used by radiochemists in the design of PET radiotracers using a lead compound in which there is no simple method (ie, hot-for-cold atom substitution) for accomplishing this goal is discussed later.

Table 2 provides several substituents used to design PET radiotracers in which the structure of the lead compound must be altered to incorporate a positron-emitting radionuclide. The left-hand column of **Table 2** shows the primary radionuclides and common labeling strategies for incorporating ^{11}C or ^{18}F into a lead compound. The second column shows the Hansch lipophilicity constant (π) value for each substituent; the π value is a measure of the relative lipophilicity of the substituent and determines the effect of adding this substituent on the overall logP of the molecule.⁶³ For example, replacing an H atom of a lead compound with a methyl group will increase the logP value by 0.56 units. The third column lists the Hammett substituent constant (σ_p), which is a measure of the electron-withdrawing or electron-donating properties of the substituent. A negative σ_p value indicates the substituent donates electrons to an aromatic ring system, whereas a positive σ_p constant indicates that this is an electron-withdrawing substituent. The molar refractivity (MR) of the substituent is a measure of the steric bulk of each functional group.

Substitution of an H atom of a primary or secondary amine with an $[^{11}\text{C}]\text{methyl}$ group is

Table 2
Substituent constants for radionuclides and labeling strategies used in PET radiotracer development

Substituent	π	σ_p	MR
H	0.00	0.00	0.1
OH	-0.67	-0.37	2.85
CH ₃	0.56	-0.17	0.57
F	0.14	0.06	0.1
I	1.12	0.18	1.39
Br	0.86	0.23	0.89
CH ₂ CH ₂ F	0.85	-0.15	0.93
CH ₂ CH ₂ CH ₂ F	1.59	-	1.48

Data from Hansch C, Leo A, Hoekman D. Exploring QSAR: hydrophobic, electronic, and steric constants. In: Heller SR, editor. Computer applications in chemistry, vol. 2. Washington, DC: American Chemical Society; 1995. p. 348.

a commonly used strategy provided that there is enough steric tolerance in the region of the nitrogen atom (MR for a CH₃ group = 0.57 vs 0.1 for an H atom). Substitution of a CH₃ for an H atom also increases the lipophilicity of the PET radiotracer by 0.56 π units, which results in a higher degree of nonspecific binding relative to the parent compound.

The synthesis of ¹⁸F-labeled compounds often involves the substitution of an F for H, which rarely alters the affinity and physicochemical properties of the parent compound because the substituent constants of an F atom are similar to those of an H atom. However, F is also capable of serving as a hydrogen bond acceptor; therefore, a second case that has been used in the design of ¹⁸F-labeled radiotracers is a substitution of an F atom for an OH group.

The use of a 2-fluoroethyl or 3-fluoropropyl group for introducing ¹⁸F into a lead compound is increasing in popularity because the radiochemical yields of an SN2 reaction are generally high. However, the substituent constants in terms of lipophilicity (π) and steric bulk (MR) limits the options in which these groups can be substituted into a lead compound. For example, the substitution of a 2-fluoroethyl or 3-fluoropropyl group for an H atom is not optimal because it would result in a large increase in the lipophilicity (ie, logP) of the compound, and also introduces a quantity of steric bulk into the region of the molecule in which the substitution occurs. A 2-fluoroethyl or 3-fluoropropyl for methyl substitution can work provided that (1) the increase in lipophilicity does not increase the nonspecific binding to the point that the tracer does not have a suitable signal-to-noise ratio for imaging purposes, and (2) the increased steric bulk does not reduce the affinity of the ligand for the target protein. A more appropriate substitution is a 2-fluoroethyl group for a Br atom, and a 3-fluoropropyl group for an I atom. Note the similarity in the substituent constants for the 2-fluoroethyl group and a Br atom, and the 3-fluoropropyl group for an I atom. In addition, because many CNS-active compounds contain either a Br or I atom in the parent structure, this strategy often leads to the development of a successful PET radiotracer without the need to conduct a rigorous structure-activity relationship study. Examples of the different strategies for PET radiotracer design are shown in **Fig. 14**.

PRECLINICAL EVALUATION OF NEW RADIOTRACERS

The initial evaluation of a PET radiotracer for imaging receptors or proteins within the CNS

typically involves studies in rodents (usually rats) aimed at determining whether the radiotracer crosses the blood-brain barrier and labels the target protein in vivo. For receptor-based radiotracers, blocking studies are usually performed with a gold standard blocking agent to determine the specificity of the radiotracer for the target receptor. Because the metabolism of radiotracers in rodents is different from that of humans, ex vivo metabolism studies in rodents are typically not performed because the data may not be predictive of what occurs in primates. The poor performance of a PET radiotracer in rodents does not guarantee a similar poor performance in higher species such as nonhuman primates, largely because of species differences in metabolism; it is the authors' standard practice to conduct at least 1 imaging study in nonhuman primates before eliminating a radiotracer from further investigation.

The best animal model for evaluating new PET radiotracers for imaging CNS function is macaque monkeys (ie, rhesus or cynomolgus monkeys). Because macaque monkeys are phylogenetically closer to humans than rodents, radiotracers that produce promising PET imaging results in monkeys typically succeed in clinical research studies in humans. These preclinical studies include a battery of tests such as evaluating the between-subject and test/retest variability of the radiotracer, blood metabolism, and in vivo blocking or displacement studies to confirm specificity of the radiotracer for its molecular target.

A unique feature of conducting PET imaging studies in macaque monkeys is that there are several nonhuman primate models of behavior and disease that are predictive of, and often parallel, different neurologic and behavioral conditions in humans. Examples of these models include the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of Parkinson disease,^{64,65} cocaine self-administration,^{3,4} nonhuman primate models of socially derived stress,^{66,67} and age-related cognitive impairment.⁶⁸ These animal models not only serve as a screening tool for evaluating new radiotracers for subsequent clinical research studies but also provide a unique resource for designing PET imaging studies capable of addressing specific neurobiological questions that cannot be conducted in human imaging studies. An example of this was the study by Nader and colleagues,⁶⁷ in which a rhesus monkey model of cocaine self-administration was used to assess the effect of acute and chronic exposure to cocaine on dopamine receptor function in cocaine-naïve animals.

Once a PET radiotracer has shown promising results in preclinical PET imaging studies

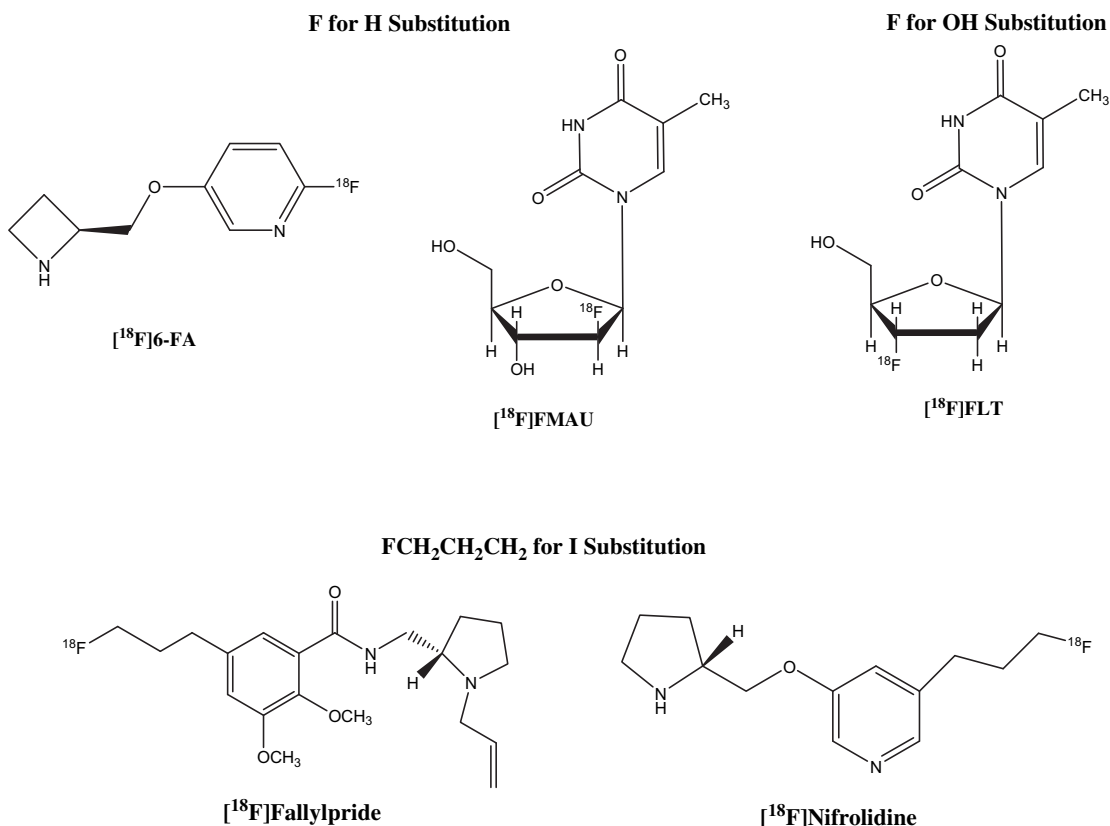


Fig. 14. Examples of ¹⁸F radiotracer development.

conducted in nonhuman primates, then a series of steps must be taken before its use in human subjects. Unlike animal research, which is governed by the Department of Agriculture and the American Association for Laboratory Animal Care, PET imaging studies in humans are under the regulatory control of the US Food and Drug Administration (FDA). A brief description of the regulatory criteria for producing PET radiotracers for clinical imaging studies is presented in the next section.

REGULATION OF PET RADIOTRACER PRODUCTION FOR USE IN HUMANS: HISTORICAL PERSPECTIVE AND CURRENT STATUS

From 1963 to 1975 the FDA exempted all radioactive drugs from compliance with new drug requirements, as long as they complied with the Atomic Energy Commission regulations. In 1975 the FDA lifted this exemption, and together with the newly formed Nuclear Regulatory Commission, required that all radioactive drug manufacturers be subject to the requirements of the Food Drug and

Cosmetic Act (FD&C Act). The FDA also required that all human research studies involving radiopharmaceuticals (RaPh) be carried out under an investigational new drug (IND) application. In addition, in 1975, the Radioactive Drug Research Committee (RDRC) regulations were established in Title 21 Code of Federal Regulations part 361.1; these regulations clarified under what situations certain radioactive drugs would be eligible for use in basic research studies involving human subjects, without requiring an IND. To establish an RDRC at an Academic Institution, members are appointed by the Institution. The committee is composed of physicians and scientists who are required to cover specific specialty areas. Each RDRC is approved by the FDA and must report at least annually regarding its activities.

The RDRC permits basic research in humans, but does not allow first-in-human studies. In addition, no clinical decisions are allowed under RDRC regulated research. The pharmacology of the drug must be known in humans and generally recognized as safe and effective. No observed pharmacologic effects can be noted from the mass dose administered. This is referred to as the no observed

effect level (NOEL). Specific radiation dose limits must be adhered to for adults, and children can receive only 10% of the allowable adult limits. These regulations have not been updated since 1975, and there are problems meeting the radiation dose limits for pediatric studies. The established pediatric limits often make it impossible to study children under RDRC regulated studies. To acquire acceptable image statistics, pediatric PET imaging studies can require administration of higher dosages than would be allowed under RDRC dose limits for children. This situation can be problematic for grant funding that requires inclusion of pediatric subjects in the research. In such instances, an IND must then be filed with the FDA to proceed with pediatric studies. **Fig. 15** shows the regulatory process, modified from VanBrocklin.⁶⁹

In 1976, Wolf and colleagues at Brookhaven National Laboratories synthesized [¹⁸F]fluorodeoxyglucose (FDG), a radiotracer that was used in PET imaging for human research studies.⁷⁰ FDG is widely used in hospitals and research centers throughout the world to diagnose cancer and evaluate neurologic and cardiac diseases. PET drugs are unique RaPh because of the short half-life of most PET radionuclides. Cyclotrons used to produce the PET radionuclides were originally established for research purposes in university settings. Each batch of a PET drug was prepared and administered for research purposes only on the day of preparation for a single patient injection. Regulation of these research studies usually proceeded under the RDRC or through IND submission at respective universities. During the 1980s and 1990s, interest in FDG for possible clinical use increased. During this time the FDA did

not regulate PET drugs. This situation was similar to the traditional nuclear medicine RaPh before 1975, which were originally exempted from FDA oversight, and regulated only by the Atomic Energy Commission. In 1995 the FDA issued Draft Guidelines on the Manufacture of PET Drug Products, and issued the first Proposed Rule—Current Good Manufacturing Practice (cGMP) for Finished PET Radiopharmaceuticals. This proposed rule met significant concern from the newly regulated PET community, and congress was mobilized by its constituency. By 1997 the FDA Modernization Act (FDAMA) was finalized, which required the FDA to develop appropriate current good manufacturing practice standards for PET drugs, which would be separate from cGMP for traditional drugs. On September 20, 2005 the FDA issued Proposed Rule (21 CFR Part §212): *Current Good Manufacturing Practice for PET Drugs* (70 F.R 55,038),⁷¹ and revised Draft Guidance: *PET Drug Products: Current Good Manufacturing Practice* (70 F.R. 55145).⁷² Five years later, on December 10, 2009, FDA published the Final Rule (74 F.R. 65409) and Guidance, “PET Drugs—Current Good Manufacturing Practice (CGMP)”.⁷³ The Final Rule will become effective on December 12, 2011. This will require that either a New Drug Application (NDA) or an Abbreviated New Drug Application (ANDA) must be filed by each manufacturer, before the effective date, for the existing FDA approved PET drugs, F-18 fluorodeoxyglucose, F-18 fluoride, and N-13 ammonia.

Until the effective date, PET drug compounding (manufacturing) remains subject to the provisions of Section 121 of FDAMA, requiring that the compounding (manufacturing) of PET drugs must conform to the requirements of the United States Pharmacopeia (USP) Chapter 823 *Radiopharmaceuticals for Positron Emission Tomography—Compounding*,⁷⁴ and if available, USP monographs. The Final Rule, will allow a PET drug producer to follow 21 CFR Part 212 or continue to use USP chapter 823 for the production of investigational PET drugs produced under an IND (phase 1 and 2) and research drugs produced under the authority of the RDRC. If a PET drug producer intends to seek marketing approval for a PET drug, phase 3 studies of the drug should be in accordance with the PET CGMP requirements in 21 CFR Part 212.

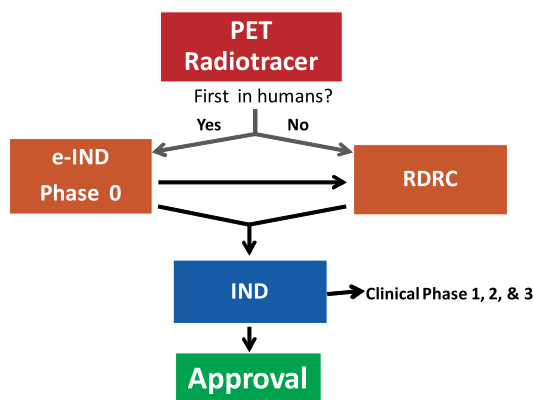


Fig. 15. FDA regulatory process for new PET radiotracers. (Data from VanBrocklin HF. Radiopharmaceuticals for drug development: United States regulatory perspective. Current radiopharmaceuticals 2008;1(1):2–6.)

THE EXPLORATORY IND MECHANISM AND ITS EFFECT ON FIRST-IN-HUMAN STUDIES

Because PET RPh are true tracers, they contain a minimal mass quantity and have no expected pharmacologic or toxicologic effects. Yet in the

past they were required to provide the same safety evaluation as traditional drugs. This process required significant monetary resources; the cost of the pharmacologic and toxicology studies alone for a traditional IND ranged between US\$400,000 and US\$500,000.⁶⁹

Because of the significant decline during the past 15 years in numbers of submissions to the FDA for new molecular entities and biologics, the FDA developed a plan called the Critical Path Initiative, which served as a framework for facilitating drug discovery and development.⁷⁵ One of the initiatives the FDA implemented in 2006 was to develop guidance describing the flexibility that existed in the IND program to allow testing of multiple candidate drugs in the early clinical phases.⁷⁶ For PET drug development, this has significantly affected imaging probe development. The Exploratory Investigational New Drug (eIND) embraces the concept of microdosing, which was initially introduced in the European Union in 2004 by a position paper.⁷⁷ The microdose mass is one-hundredth of the dose of a test substance calculated to yield a pharmacologic effect. The maximum allowable mass dose is 100 µg or less, or for protein products 30 nmoles or less. If the mass requirement is met, there are reduced pharmacology and toxicology requirements. This system also significantly reduces the cost to bring a new drug to market. For the eIND toxicology study only 1 mammalian species is required, and both sexes must be studied. The administered mass dose must be at least 100 times the anticipated mass dose to be administered in the human. The length of the toxicology study must be 14 days, with interim necropsy on day 2 and on day 14. A limited subject enrollment of 5 to 30 subjects is allowed. Under the eIND only the phase 0 studies are performed, and if the candidate drug is successful, and further study is indicated, a transition to the traditional IND phase 1 must be completed. This limited IND facilitates first-in-human imaging studies for drugs and biologics, and bridges the gap between the preclinical and clinical trials. It is ideal for proof-of-concept studies, and testing of multiple candidate drugs to identify a lead candidate to move forward through the IND process. As molecular imaging advances, these changes in the regulatory environment should stimulate the development of RaPh in a more cost-effective manner.

USP CHAPTER 823 QUALITY CONTROL REQUIREMENTS FOR PET RAPH

PET drug production involves use of short-lived cyclotron-produced radionuclides, which are

then incorporated into the final drug product. These radiolabeled drugs require significant considerations because of the half-lives (which vary from minutes to hours) and decay emissions (positrons and 511 keV photons) requiring significant radioprotection. In addition the short half-lives necessitate rapid quality control methods, to assure the batch is acceptable for intravenous injection in humans. Because sterility testing requires 14 days to complete, the product must be released before the completion of sterility testing. This system requires additional aseptic process controls are in place to ensure the quality of the final product.

As mentioned earlier, PET drugs must be compounded (manufactured) according to USP Chapter 823. This regulation requires that written specifications must be in place for the identity, purity, and quality of all components used for any PET drug compounding procedure, which includes definition of the appropriate storage. All shipments of components must follow a log-in process and expiration dates must be recorded. If there is no expiration date, one must be assigned. For acceptance of a lot of sterile filters, a sample filter must be tested for integrity. Manufacturers' certificates of analysis can be used to demonstrate compliance with the established written specifications.

Chapter 823 also requires written acceptance criteria be established for each PET drug defining the identity, purity, and quality of the finished PET drug. If a USP monograph exists, these standards are the minimum acceptance criteria. Written and verified procedures must be established for each PET drug that incorporates 0.22 µm sterile membrane filtration for parenteral administration or 0.45 µm particulate filtration for PET drugs intended for inhalation.

Controls must be maintained of computers and related automated equipment to ensure that changes in compounding software are instituted by authorized personnel. It is also required to document and verify changes and assure that only the current version of the software is being used in PET compounding. Copies of outdated software must be kept to provide a complete history from the time of initiation of a synthesis.

Verification studies must be performed on a minimum of 3 consecutive batches of a new PET drug to show that the product meets established acceptance criteria. In addition, whenever there is a change in the compounding procedure, computer software program, or component specifications that have the potential to alter the identity, quality, or purity of the drug product, 3 consecutive verification studies must again be performed.

Expiration dating and storage conditions for the PET drug must be established on the results of stability testing. The test samples must be withdrawn from the product stored in the container used for the PET drug. The PET drug must meet all acceptance criteria at expiration.

Before initiating the PET synthesis, the sterile filter and vent filters must be inserted through the final PET product vial septum in a class 100 environment (laminar flow hood or isolator). The internal surfaces of the class 100 hood must be disinfected with a sterile disinfectant such as 70% isopropyl alcohol before use. Microbiological testing should be performed periodically using microbiological contact plates for the surfaces and settle plates or a dynamic air sampler.

The PET drug must be compounded according to a written verified standard operating procedure, and a batch record must be maintained for each batch of drug produced. Lot numbers of the PET drug must initial all the critical steps, indicating that they were performed. Any unplanned deviation or unexpected results must be documented and investigated. This documentation should be in writing. All analytical data must be included with each batch record.

For PET drugs with a half-life of 20 minutes or greater the following quality control testing requirements must be completed on the batch, or for PET drugs with a half-life of 20 minutes or less the quality control should be performed on the initial subbatch, before release, according to established written procedures. The quality control subbatch is the first batch of a PET drug made from a single lot of components, for example [¹⁵O]water or [¹³N]ammonia. The following are the test procedures required.

1. For all PET drugs intended for parenteral administration a membrane filter integrity test must be completed after final filtration. The bubble point test is commonly performed, which requires use of a pressure gauge and a source of air pressure connected to the transfer set attached to the filter used for the synthesis. The pressure is increased until the validated bubble point is reached. This test must be completed before release for human use, except in the case of [¹⁵O]water, in which the integrity test may be completed after injection.
2. The pH must be measured, usually using pH paper.
3. The PET drug must be visually inspected using adequate shielding for an as-low-as-reasonably-achievable technique, to ensure the solution that is clear and free of particulate matter.
4. Radiochemical purity and identity are analyzed using thin-layer or high-pressure liquid radiochromatography. A nonradioactive standard must be chromatographed with the PET drug to allow identification of the compound, using an authentic standard for comparison with the radiolabeled drug. If a USP standard is available, it must be used. The FDA, cGMP for PET (21 CFR Part §212.70) Final Rule states that conditional final release cannot be granted if tests for radiochemical purity and identity are not performed. Radiochemical purity analysis may require duplication of quality control equipment.
5. Radionuclidic identity: identity testing involves decay analysis in a dose calibrator for a defined period. The half-life is determined mathematically using linear regression.
6. Specific activity analysis is required for PET drugs with mass-dependent localization or toxicity concerns. An example of a PET drug requiring specific activity analysis is a radio-tracer for imaging receptor function such as [¹¹C]raclopride.
7. Residual solvent analysis and other toxic chemicals (such as Kryptofix-222) must be analyzed to determine compliance with established acceptance criteria. Gas chromatographic methods can be developed for the measurement of residual solvents that allow precision and linearity across the range of concentration levels required by the USP Chapter 467.⁷⁸ There is a USP-approved method for Kryptofix analysis in the FDG monograph. In addition there are other methods, such as the Mock method,⁷⁹ using iodoplatinate thin-layer chromatography strips to perform a color end-point test, which can be validated for Kryptofix analysis.⁷⁸
8. Bacterial endotoxin testing (BET) must be performed. A 60-minute bacterial endotoxin test (gel-clot) or other recognized test such as the photometric test must be performed on each batch. The acceptable endotoxin limit is 175 endotoxin units/V. The V is usually defined as the batch volume of the PET drug.

Postrelease sterility testing must be performed for each batch of PET drug. The USP 823 currently requires that the product must be inoculated in fluid thioglycolate and soybean-casein digest media within 24 hours after the end of synthesis. The PET cGMP Final Rule has increased this time to 30 hours. If the microbiological test fails, an investigation needs to be performed to determine the cause, and the corrective actions that will be taken. It is good to maintain all these records in writing to

assure an adequate record. After a record of successful sterility tests is established for a particular PET drug, only the first lot prepared each day needs to be subjected to a sterility test, unless the second batch of that PET drug uses any components that differ from the first batch.

The USP monographs require that radionuclidic purity should be determined, but do not specify the time interval. γ -Ray spectroscopy using a suitable γ counting device such as a multichannel analyzer can be used to determine the presence of any γ photon energy other than that characteristic of positron-emitting radionuclides, which would include 511 keV, 1.02 MeV, or Compton scatter.

All aseptic operations used in producing PET drugs, including assembly of sterile components, compounding, filtration, and manipulation of sterile solutions, must be performed by operators, qualified to work with aseptic techniques. Operators must be qualified by passing three growth promotion tests, which require process simulations using microbiological growth medium. After simulation, the media are incubated at the appropriate temperature for 14 days with periodic examination for the evidence of bacterial growth. The absence of growth is necessary for an acceptable test result. Simulations are performed in triplicate to qualify a new operator. After initial qualification, 1 simulation must be performed each year, or each time procedures are changed. All aseptic operations are performed by operators wearing appropriate laboratory clothing.

USP CHAPTER 797 OVERVIEW

In the case of production of RaPh for PET, USP Chapter 823 supersedes Chapter 797 *Pharmaceutical Compounding—Sterile Preparations*.⁸⁰ On release of a PET drug as a finished drug product from a production facility, the further handling, manipulation, or use of the product is considered compounding, and the contents of Chapter 797 are applicable. Chapter 797 sets out practice standards to help ensure that compounded sterile preparations (CSPs) are of high quality. These standards help to ensure that these compounded products do not inflict harm on patients as a result of contaminated preparations, and assigns risk levels according to potential for microbial contamination during compounding. Although PET production is not required to follow Chapter 797, it outlines practices required for PET drug dispensing, and a general overview of the chapter is discussed in the following section.

One of the sections of Chapter 797 is Radio-pharmaceuticals As CSPs. This section describes

RaPh, compounded from sterile components in closed sterile containers, with a volume of 100 mL or less, and a single-dose injection of not more than 30 mL, as low-risk level CSPs. Low-risk CSPs must have a 12-hour or less expiration time or beyond-use time.

Low-risk RaPh are compounded from sterile components in an International Organization for Standardization (ISO) class 5 (enclosures allow 100 particles of 0.5 μ m and larger per cubic foot of air) primary engineering control (PEC) with not more than 2 entries into the original sterile vial. The ISO class 5 PEC must be located in an ISO class 8 (enclosures allow 100,000 particles of 0.5 μ m and larger per cubic foot of air) area or a segregated compounding area. The area may be demarcated by a line on the floor, or be a separate room. The room must have no unsealed windows, and no connection to outside, or areas of high traffic flow. There must be no sink next to the ISO class 5 PEC. Personnel must be appropriately gowned and gloved in the work area.

Immediate-use provision is intended only for those situations when there is a need for emergency or immediate patient administration of a CSP. Immediate-use CSPs are exempt from low-risk RaPh requirements if the RaPh can be prepared, dispensed, and administered within 1 hour of preparation. An example of an immediate-use practice in PET would be the dispensing of an ^{11}C -labeled PET RaPh, prepared for a single patient administration.

Chapter 797 outlines aseptic training requirements to compound low-risk RaPh, and they can be used as guidance for setting up training for preparation of PET drugs. Training can be achieved using audiovisual instruction sources, produced by expert personnel, as well as written publications. Personnel are required to pass a written examination after training, and initially complete media-fill testing, using aseptic manipulations, to demonstrate compounding skills. This testing must be repeated at least annually. This training differs from the Chapter 823 requirements for completion of 3 media-fill tests before being initially authorized to compound PET drugs.

Written training procedures are required for appropriate gowning and gloving, including procedures for hand washing. Gowning requirements include dedicated shoes or shoe covers, head cover, masks and eye protection, gowns with fitted sleeves, and sterile gloves disinfected with sterile 70% isopropanol. These training procedures must be documented through observational audits and gloved fingertip sampling using microbiological contact plates. Garb exposed in patient care areas may not reenter the compounding area.

USP Chapter 797 requires the ISO class 5 PEC must be cleaned using appropriate disinfectants (such as 70% sterile isopropyl alcohol alternated on a defined schedule with a disinfectant effective against spore-forming bacteria) at the beginning of each shift, and checked regularly using contact and air-settling plates. The value of viable microbial monitoring of gloved fingertips and surfaces of components and the compounding environment is to identify and correct unacceptable work practice. Chapter 797 recommended action levels for microbial contamination are in an ISO class 5 environment, fingertip samples should be greater than 3 colony-forming units (cfu) per plate and surface sample testing (contact plate) should also be greater than 3 cfu per plate. For ISO class 8 surface sampling action levels should be greater than 100 cfu per plate. If these levels are exceeded work practices and cleaning procedures should be reevaluated. An investigation should be initiated until the source of the problem is eliminated, the area is cleaned (or task), and resampling is successfully performed. This investigation may require review of hand hygiene, garbing, and gloving.

Because most PET drugs are administered intravenously, it is important to emphasize the aseptic process without placing undue burden on that process with additional regulatory requirements. All PET drug batches are released after most quality control requirements are completed. Although sterility testing is not complete the final filter is tested for integrity and the BET test is completed, giving an indication of the sterility of the final PET drug. The mass of drug contained in a PET drug is usually in the μg range, compared with therapeutic drugs, which allows an additional safety factor for PET drugs.

SUMMARY

The development of ^{11}C - and ^{18}F -labeled probes for use with PET continues to be an active area of research. ^{11}C will continue to be a useful radio-tracer in clinical research studies because the short half-life of this radionuclide (20.4 minutes) permits imaging sessions in which multiple tracers can be administered (following a short delay to allow for radioactive decay and biologic clearance). Therefore, 2 or more targets comprising a biochemical pathway or neurotransmitter system can be studied in the same imaging session when using ^{11}C -labeled radiotracers. ^{18}F will continue to be the radionuclide of choice for imaging studies using a single radiotracer. Advances in ^{18}F -labeling strategies such as the development of click chemistry will likely have an effect on the future development of ^{18}F -labeled radiotracers.

Recent changes in the regulatory requirements such as the creation of the eIND and phase 0 studies in humans are expected to lead to a dramatic increase in the number of PET radio-tracers making the translational jump from preclinical research studies in animal models of disease to clinical research studies in patients. However, the continued success in translational PET research studies using new PET radiotracers depends on the strict adherence of PET radio-chemistry programs to the requirements stated in the USP Chapter 823 *Radiopharmaceuticals for Positron Emission Tomography—Compounding*, or the new 35 CFR Part 212.

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