Bioconjugate Chemistry

Review

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Fluorine-18 Radiochemistry, Labeling Strategies and Synthetic Routes

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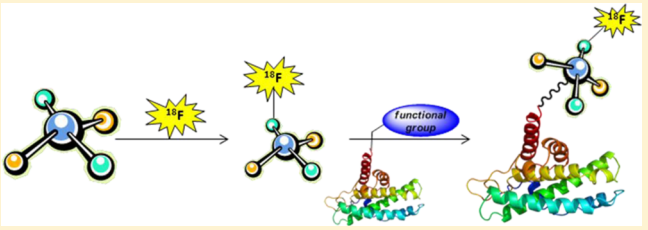
ABSTRACT: Fluorine-18 is the most frequently used radioisotope in positron emission tomography (PET) radiopharmaceuticals in both clinical and preclinical research. Its physical and nuclear characteristics (97% β^+ decay, 109.7 min half-life, 635 keV positron energy), along with high specific activity and ease of large scale production, make it an attractive nuclide for radiochemical labeling and molecular imaging. Versatile chemistry including nucleophilic and electrophilic substitutions allows direct or indirect introduction of ^{18}F into molecules of interest. The significant increase in ^{18}F radiotracers for PET imaging accentuates the need for simple and efficient ^{18}F-labeling procedures. In this review, we will describe the current radiosynthesis routes and strategies for ^{18}F labeling of small molecules and biomolecules.



1. INTRODUCTION

Positron emission tomography (PET) is a nuclear medicine imaging technology that provides moderate-resolution, sensitive images of the biodistribution of a radiotracer in vivo.[1] The technique has sufficient speed of acquisition to allow determination of pharmacokinetics of radiotracer uptake and distribution. The combination of PET imaging with a validated radiopharmaceutical can allow the images to provide interpretation of a biological function. In addition to applications for diagnosis of diseases,[1] PET imaging can provide important insights for both drug discovery and development and for potentially limiting side effects due to off-target binding.[2] The most important component to the future utility of PET technology is the development of novel, specific, validated radiotracers for clinically relevant targets and methods for their efficient preparation.

PET is based on the administration of radiolabeled molecules with positron emitting nuclides such a ^{15}O, ^{13}N, ^{11}C, and ^{18}F, with relatively short half-lives of 2.037, 9.965, 20.39, and 109.8 min, respectively.[2] Of the nuclides mentioned above, ^{18}F has the most ideal half-life for labeling of radiopharmaceuticals (small organic molecules, peptides, aptamers, and proteins) and has a unique and diverse chemistry for introduction into various molecules. In the discipline of medicinal chemistry, fluorine is a favorable atom in drug development due to its physical properties including small van der Waals radius (1.47 Å), high electronegativity, and ability to form a strong bond with carbon (C−F energy bond of 112 kcal/mol), which in comparison to a carbon−hydrogen bond (C−H = 98 kcal/mol) is more thermally stable and oxidation resistant.[3,4] Fluorine can act as a bioisostere with hydrogen (size and valence electrons) and oxygen (size and electronegativity).[4] As a result of its significance in the pharmaceutical field, several selective fluorination reagents for nucleophilic (F^-) and electrophilic (F^+) incorporation have been developed and have become commercially available (Figure 1).[5,6]

In the field of radiochemistry, fluorine-18 also gained high interest due to its favorable nuclear and physical characteristics, including high positron decay ratio (97%), relatively short half-life (109.7 min), and low positron energy (maximum 0.635 MeV). The positron energy results in a short diffusion range (<2.4 mm) that favorably increases the resolution limits of the PET images.[7] Because of these nuclear properties and the ability to synthesize fluorine-18 in large quantities, there have been many radiosynthetic methods developed for incorporation of this radionuclide into biologically important and interesting molecules ranging from drug-like molecules to antibodies and oligonucleotides. Because of the short half-life, emphasis has been placed on developing radiosynthetic schemes that introduce the radionuclide in a late stage of the synthetic pathway. Some of its distinctive radiochemistry will be further discussed in this review.

1.1. Fluorine-18 Production. Fluorine-18 is produced with a cyclotron primarily by proton (^{1}H) irradiation of ^{18}O, a stable naturally occurring isotope of oxygen. When the target is liquid $H_2{}^{18}O$, an aqueous solution of ^{18}F-fluoride ion is obtained; when the target is $^{18}O_2$ gas, $^{18}F-F_2$ gas is obtained. $^{18}F-F_2$ is also prepared from deuteron-irradiation of Ne. The production method used is dependent on the desired subsequent chemical reactions; ^{18}F-fluoride is produced for use as a nucleophile, while ^{18}F-fluorine is produced for use in electrophilic methods.[8−11] The key differences between these two chemical forms are the specific activity (SA = radioactivity/

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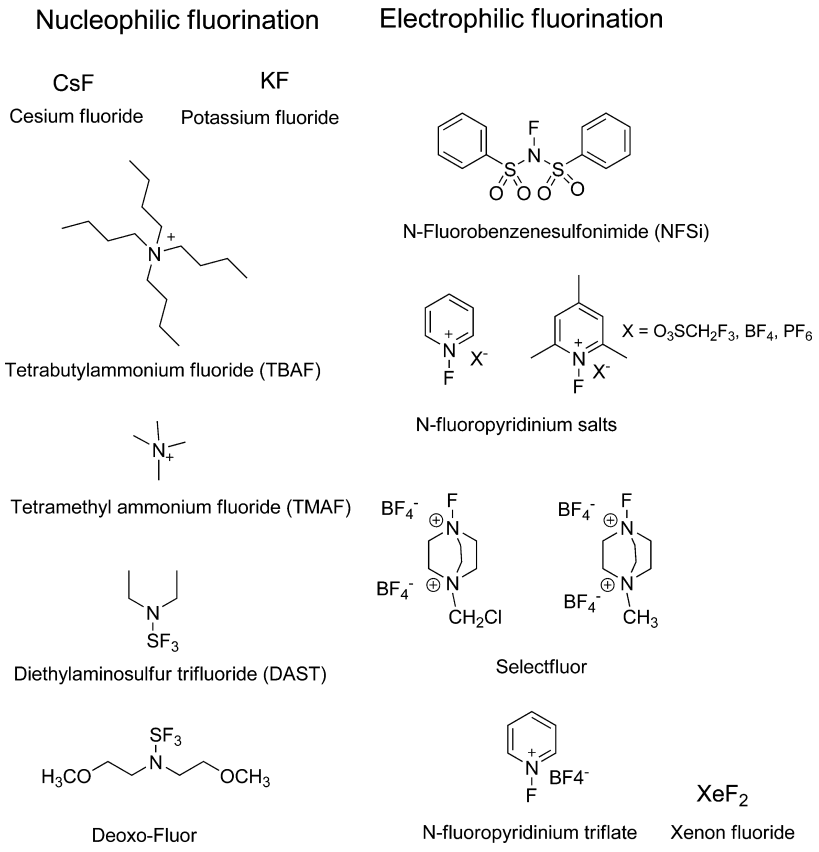

Figure 1. Commercial fluorinating reagents for nucleophilic and electrophilic substitutions.

mol) of the produced ^{18}F isotope. Nucleophilic ^{18}F-fluoride is produced by the efficient nuclear reaction $^{18}O(p,n)^{18}F$ to give a high amount of radioactivity (>370 GBq/batch). Nucleophilic ^{18}F-fluoride is produced with specific activity in the range of 10^2 GBq/μmol.[9] Electrophilic $^{18}F-F_2$ has much lower specific activity (100−600 MBq/μmol) because fluorine-19 gas must be added as a carrier to extract the $^{18}F-F_2$ from the target.[9] The SA becomes crucial when working on low capacity systems (i.e., ligand−receptor binding). The addition of a carrier ($^{19}F-F_2$) leads to increased mass of the final radiotracer, which may result in receptor saturation and reduction of PET signal from specific binding.[12] High mass may also cause pharmacological effects.[9] The high yield from cyclotron production along with higher SA that is crucial for PET imaging of receptor−ligand interaction dictates that most of the fluorine-18 reactions in nuclear medicine use nucleophilic ^{18}F-fluoride.

2. NUCLEOPHILIC FLUORINATION

Although fluoride ion is a strong nucleophile, in aqueous solution it forms hydrogen bonds with the surrounding water molecules and becomes unreactive for nucleophilic substitution.[13] To achieve nucleophilic fluorination, the ^{18}F-fluoride must be substantially dehydrated by evaporation of the water and subsequent displacement reactions conducted in polar aprotic organic solvents. The solubility and nucleophilicity of fluoride ion in organic solvents is enhanced by the addition of a phase transfer catalyst (PTC) (such that the cryptand Kryptofix$_{222}$ complexes potassium) or by the addition of bulky tetrabutylammonium cation. Radiofluorinations are typically conducted in the presence of poorly nucleophilic bases (typically carbonate or bicarbonate ions). Thus, the aqueous solution of ^{18}F-fluoride obtained from the cyclotron target is treated with the desired salt (cation and, if necessary, a complexing agent, and desired anion) prior to evaporation of the water;[6,13] water removal is assisted by azeotropic distillation of water using CH_3CN.

Once the process of drying is complete, fluoride can be introduced by S_N2 mechanism into aliphatic positions or via nucleophilic aromatic substitution (S_NAr) into aromatic molecules. A wide range of precursors, leaving groups, and reaction conditions can be utilized for ^{18}F-fluoride nucleophilic substitution (Figure 2). Dipolar aprotic solvents such as dimethyl sulfoxide (DMSO), *N*,*N*-dimethylformamide (DMF), dimethylacetamide and CH_3CN are preferred solvents.[6] On the other hand, there are several publications that describe nucleophilic substitution using the polar protic solvents *t*-butanol and *t*-amylalcohol[13−16] and some in which the addition of a low percentage of water enhances the yields.[17] Optimization of conditions for a particular radiochemical synthesis will require exploration of solvent, temperature, counterion, and concentration.

2.1. Aliphatic Nucleophilic Fluorination. Aliphatic nucleophilic fluorination involves the S_N2 substitution of ^{18}F-fluoride into precursors that contain a leaving group. Choosing the best leaving group is a critical step of the radiosynthetic design and should take into consideration the reactivity of the leaving group and the stability of the precursor to basic conditions of the fluorination reaction.[18] The reactivity of leaving groups has been studied in great detail under various conditions. The order of leaving ability is Cl < Br < I < 4-methylbenzenesulfonate (tosylate) ∼ methanesulfonate (mesylate) < 4-nitrobenzenesulfonate (nosylate) < trifluoromethanesulfonate (triflate).[2] The better the leaving group, the more

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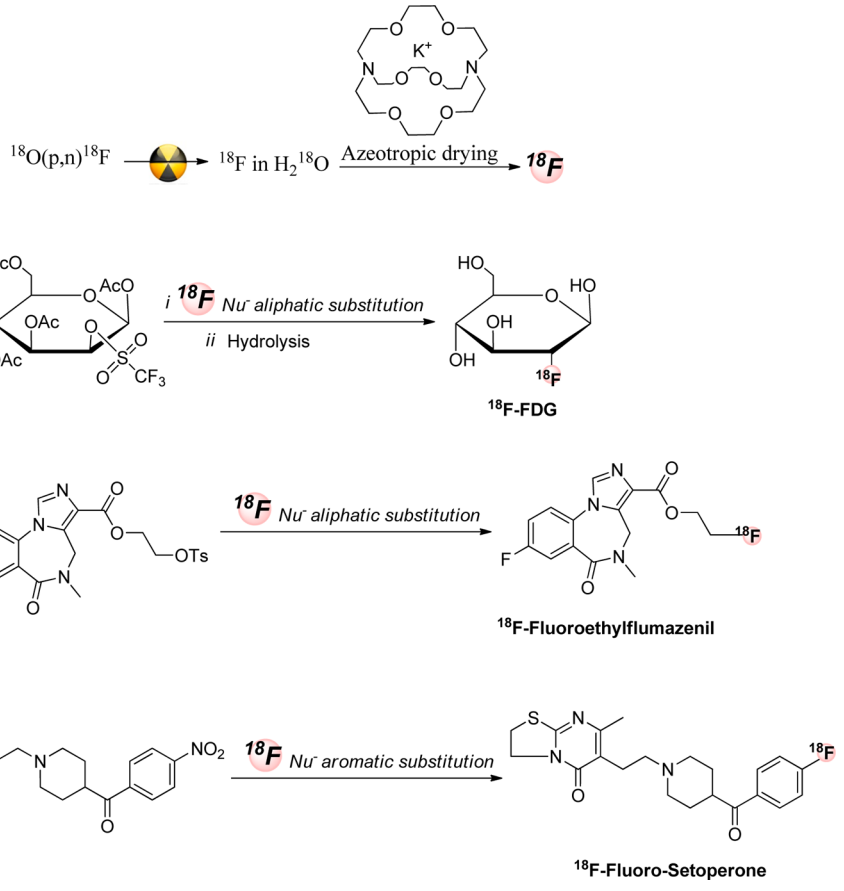

Figure 2. Examples of ^{18}F-aliphatic and aromatic nucleophilic substitution.

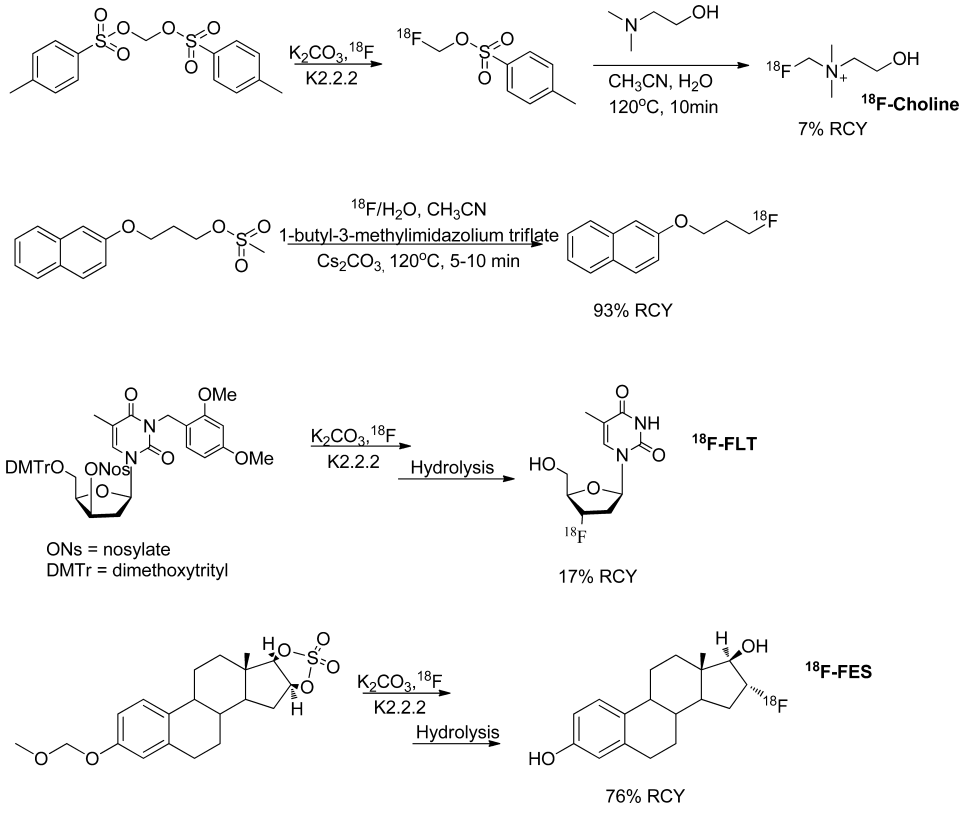

Figure 3. Synthetic routes of known radiotracers by ^{18}F-aliphatic nucleophilic mechanism.[15,16,31,140]

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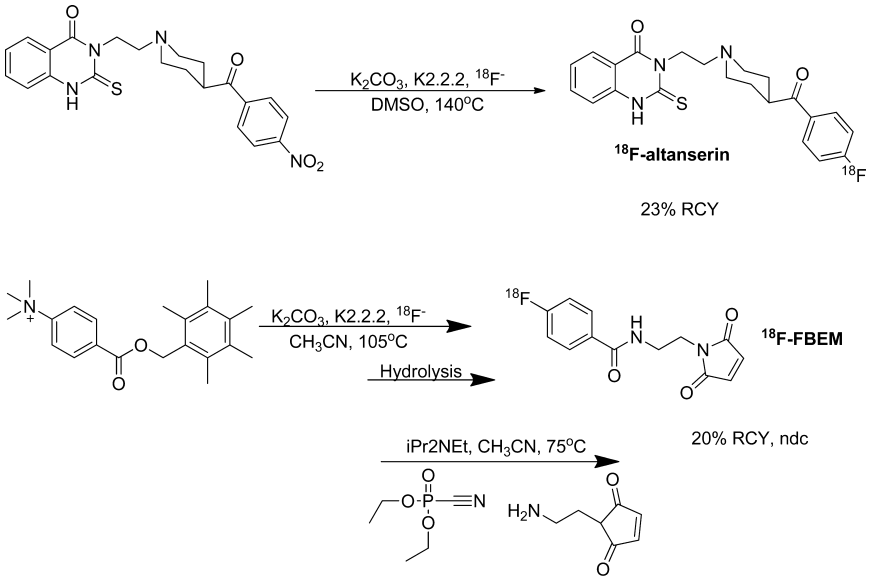

Figure 4. ^{18}F-Aromatic nucleophilic substitution on NO_2 and $^{+}NMe_3$ leaving groups.[40,89]

likely a competing elimination reaction will occur under basic conditions.[9] The position of the leaving group also has an effect on the efficiency of the substitution (primary benzylic > primary aliphatic ≫ secondary aliphatic).[9]

The typical anions added to the radiofluorination reaction result in basic conditions and often lead to undesired formation of byproducts resulting from decomposition of the base-sensitive precursors. To avoid the aforementioned side reactions, several parameters need to be optimized: (1) ratio of PTC-to-base-to-precursor, (2) selection of a less basic counteranion such as oxalate, bicarbonate, etc., (3) reaction temperature, and (4) the choice of leaving group. More reactive leaving groups are more sensitive to elimination side reaction, especially with increasing temperatures.[9] With careful evaluation of these parameters, nucleophilic aliphatic radiofluorinations are usually efficient in terms of radiochemical yield (RCY), require moderate temperature (room temperature to 100 °C), and need relatively short reaction time (usually up to 15 min).[19]

Depending on the stability of the precursor, the presence of the various functional groups and the reactivity of the leaving group, desired products of aliphatic fluorinations can be obtained in one or two steps. Typically, the first step is the substitution of the leaving group by ^{18}F and then, if necessary, removal of protecting groups or conversion of the labeled intermediate into the desired product (Figure 3).[18,20] Many of the radiopharmaceuticals used for human subject PET imaging, for example, ^{18}F-FDG, ^{18}F-FLT, ^{18}F-FMISO, ^{18}F-choline, ^{18}F-FES, ^{18}F-fluoroacetate, ^{18}F-fluoro-α-methyltyrosine, *O*-(2-^{18}F-fluoroethyl)-L-tyrosine (^{18}F-FET), and ^{18}F-fluoro furanyl norprogesterone (^{18}F-FFNP), are synthesized via S_N2 displacement of an aliphatic leaving group by ^{18}F-fluoride.[16,21−32]

2.2. Nucleophilic Aromatic Fluorination. ^{18}F nucleophilic aromatic substitution requires sufficient activation of the phenyl ring, which can be achieved by electron withdrawing group(s) (such as $-NO_2$, $-CN$, $-CF_3$, or carbonyl groups) in the *ortho* or *para* position to the leaving group. Aromatic nucleophilic substitution is conducted in a polar aprotic solvent and requires higher temperatures than aliphatic substitution (typically above 100 °C) (Figure 4). Aromatic exchange of ^{19}F by ^{18}F is feasible in the presence of carbonate and Kyrptofix$_{222}$ or anhydrous tetrabutylammonium fluoride (TBAF) in DMSO.[33,34] However, it will result in a lower specific activity radiotracer, which may preclude imaging application in low binding capacity systems.

The most common and efficient leaving groups for no-carrier-added nucleophilic aromatic substitutions are trimethylammonium salt and nitro group.[35] Lower temperatures (100−110 °C) are normally used for an aromatic fluorination on trimethylammonium group compared with a nitro group (120−180 °C). Therefore, acetonitrile is often used as solvent in a closed reactor system for trimethylammonium displacement. Because of the higher temperatures required for substitution of nitro group, DMF or DMSO are used as solvent. The choice of activating group, leaving group, and protecting group(s) must be considered during synthetic design in order to facilitate conversion of the initial radiofluorinated intermediate into the final product.

A number of ^{18}F-radiopharmaceuticals have been synthesized by aromatic nucleophilic substitution and used in clinical trials, including 6-^{18}F-fluorodopamine, (−)-6-^{18}F-fluoronorepinephrine, and 6-^{18}F-altanserin.[36−40] However, the requirement that the aromatic ring must be electron deficient in order to achieve labeling severely limits the scope of aromatic nucleophilic substitution. Because of this, a great deal of effort has been expended to find easier, more general, and more efficient methods to achieve aromatic substitution.

2.2.1. Fluorination of Heteroarenes. Heteroarenes containing a nitrogen are more electron deficient than the corresponding aromatic hydrocarbon and, thus, are amenable to direct substitution for ^{18}F-fluoride without an additional activating group.[41] In comparison to a monosubstituted benzene, the substituted pyridine has lower LUMO energy at *ortho* and *para* position than benzene, which allows direct ^{18}F substitution with high radiochemical yields, using NO_2, $N^{+}Me_3$, Br, I, or Cl as a leaving group at 2- and 4-positions (Figure 5). ^{18}F-labeling on heteroarenes is conducted in the presence of potassium carbonate and Kyrptofix$_{222}$ with DMSO or DMF as solvent at high temperature (120−150 °C).[41] Labeling of heteroarene moieties has been applied for small organic

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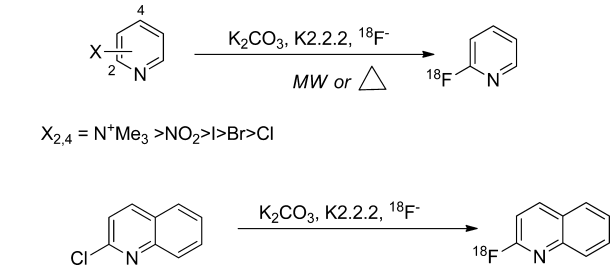

Figure 5. ^{18}F-Labeling on pyridine and quinoline derivatives.

molecules as well as prosthetic groups for labeling biomolecules.[42−46]

2.2.2. Balz−Schiemann Reaction. When radiochemists began to explore radiofluorination of aromatic rings, they adapted literature procedures that had been used successfully for nonlabeled fluorine incorporation. One attempt to label aromatic rings with $^{18}F^-$ in the absence of S_NAr activating groups and in a regiospecific manner was done using the Balz−Schiemann reaction, which involves the thermal decomposition/pyrolysis of aryl diazonium tetrafluoroborate salt in the presence of ^{18}F-fluoride to give the corresponding ^{18}F-fluoroarene (Figure 6).[12,47] The Balz−Schiemann reaction

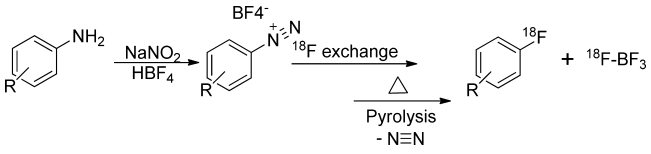

Figure 6. Balz−Schiemann reaction mechanism.

proceeds through the formation of a carbocation intermediate, which may be trapped by any nucleophilic species and results in the formation of numerous products.[9] The use of tetrafluoroborate as a counteranion theoretically limits the RCY to a maximum of 25% and decreases significantly the SA because of the exchange between ^{18}F-fluoride and the four fluorine atoms of BF_4^-. Because of these limitations, the reaction has seen limited application in this field and, accordingly, was used sparsely in published research. Weinreich and co-workers described the labeling of 5-^{18}F-fluoro-D/L-DOPA (an electron-rich arene) using the Balz−Schieman reaction of the corresponding diazonium tetrafluoroborate salt. This reaction involves six steps for the preparation of the precursor and two steps for the ^{18}F-labeling; pyrolysis of the diazonium tetrafluoroborate at 120 °C in xylene, followed by hydrolysis and final purification on a chiral column to give the desired 5-^{18}F-fluoro-L-DOPA. The labeling resulted in much lower RCY than the theoretical one (10%).[48]

2.2.3. Wallach Reaction. Another approach rarely used for regiospecific labeling of electron-rich arene employs the Wallach reaction that involves acid catalyzed, thermal decomposition of aryl-triazenes in the presence of ^{18}F-fluoride (Figure 7), leading to the corresponding fluoroarenes.[12,49,50] A diazonium salt is believed to be an intermediate in the reaction mechanism. The reaction mechanism is also S_N1 type, and the

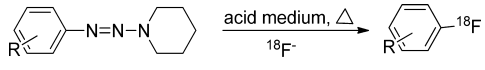

Figure 7. Wallach labeling reaction.

initially formed aryl cation can react with any available nucleophilic species, potentially yielding high amounts of side products and providing lower RCY in comparison to other labeling routes.[9] Tewson et al. demonstrated the feasibility of the decomposition of aryl-piperidyl triazines in the presence of ^{18}F-CsF to provide high specific activity aryl fluorides with RCY greater than 50%. The method was used for the no-carrier-added radiosynthesis of the neuroreceptor ligand, ^{18}F-haloperidol.[50]

2.2.4. Diaryliodonium Salts. Because of the limitations of the Balz−Schiemann and Wallach reactions, new approaches have been sought. A much more efficient method to introduce an ^{18}F into arenes has been accomplished using nucleophilic substitution of diaryliodonium salts.[51−53] The regioselectivity of this fluorination reaction is guided by the electronic and steric features of the two aryl rings (Figures 8A,B).[12,35,54] In the case of asymmetrical diaryliodonium salts, the nucleophilic substitution occurs on the more electron deficient aromatic component. In addition, the regioselectivity of the substitution is subject to an observed "*ortho*" effect. The *ortho* effect preferentially directs the ^{18}F-fluoride substitution toward the aromatic ring which has substituent *ortho* to the iodonium moiety. Moreover, the RCYs increase when an additional *ortho*-substituent is introduced into the ring.[9] The *ortho* effect is believed to be encouraged because, during the nucleophilic attack, an iodine-centered trigonal bipyramidal intermediate is formed and the sterically limiting *ortho*-substituted ring in the equatorial position favors the introduction of ^{18}F into this moiety.[54,55]

The degree of reactivity and the selectivity of radiofluorination of asymmetrical diaryliodonium salts was reported by Chun et al., which had shown that the selectivity for an *ortho* substituted product depends on the *ortho* substituents in the following order: 2,6-di-Me > 2,4,6-tri-Me > Br > Me > Et ∼ iPr ≫ H > OMe. The *ortho* effect is not purely dependent on the substituent bulk/steric influence but can be enhanced by the presence of one or more *ortho* hydrophobic groups (e.g., *alkyl*). The microenvironment created by these substituents is sufficiently lipophilic to support loose binding of ^{18}F to the hypervalent iodine atom and then nucleophilic attack onto the adjacent lipophilic *ortho*-substituted ring. The electronic nature of the *ortho* substituents is also a factor in the product selectivity. The highly electron donating substituent *ortho*-OMe (an opposing substitutent) directs fluorination away from its ring, while the *ortho*-Br (a reinforcing substituent) enhances selection of its ring compared with *ortho*-Me.[56]

These reactions can be conducted in a microfluidic reactor in one single step.[17] They typically required high temperature (140−200 °C) and the presence of base and Kryptofix$_{222}$. In most cases, the ^{18}F substitution is very efficient and results in high radiochemical yield (as determined by HPLC; isolated yields were not reported). The addition of a high concentration of a radical scavenger such as 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO) significantly increased the labeling yield, perhaps by preventing the decomposition of the iodonium salt precursors before the fluorination reaction is complete (Figure 8C).[57]

The challenge of applying this methodology in radiochemistry is the design, synthesis, and purification of the required precursor. The entire molecule must be constructed with iodine in the ultimate location of the radioactive fluorine, and the molecule must either be substituted *ortho* to the desired site of fluorination or electron poor in relation to a thiophene

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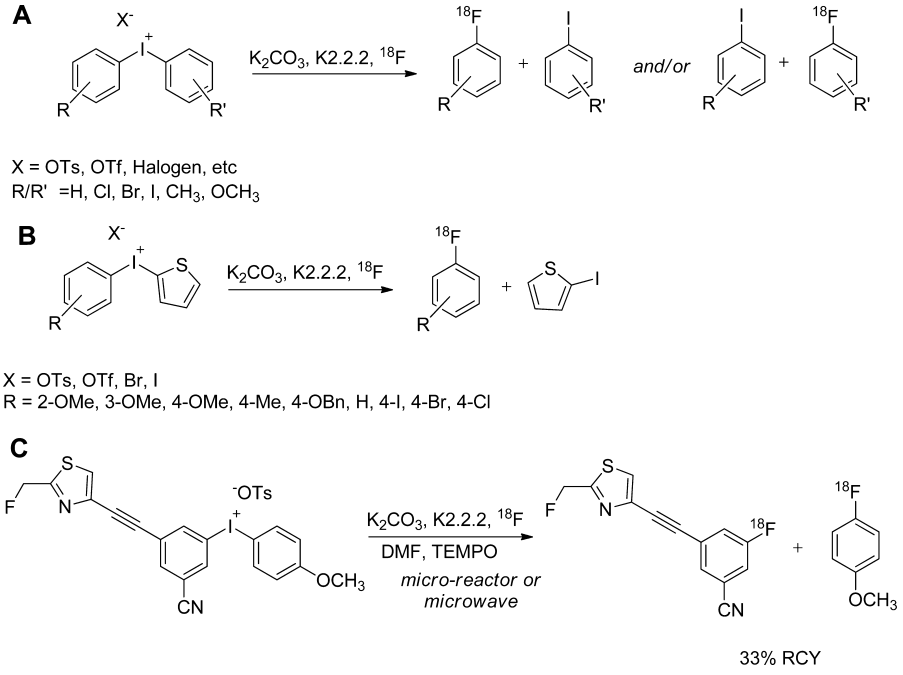

Figure 8. ^{18}F-Aromatic nucleophilic substitution on diaryliodonium salts. (A) ^{18}F-Labeling on dihomoaryliodonium.[54] (B) ^{18}F-Labeling of aryl(2-thienyl)iodonium salts. (C) ^{18}F-Labeling on 3-cyano-5-((2-(fluoromethyl)thiazol-4 yl)ethynyl)phenyl)(4-methoxyphenyl) iodonium salt.[57]

or 4-methoxybenzene. This precursor structure must be compatible with the oxidative procedures for preparing the iodonium salt. Attempts to label more complicated small organic molecules with this method have resulted in much lower isolated RCY.[57] Nevertheless, the method has promise for aromatic radiofluorination. Despite the increased scope provided to nucleophilic aromatic radiofluorinations by this method, it has, up to this point, seen limited applications to preparation of novel ^{18}F-fluoro aromatic tracers for biological imaging.

3. ELECTROPHILIC FLUORINATION

As discussed under the heading "fluorine-18 production", the production methods for electrophilic ^{18}F−F_2 require the addition of carrier ^{19}F−F_2 in order to extract the radioactivity from the cyclotron target. The maximum theoretical RCY of ^{18}F−F_2 is limited to 50%, because on every ^{18}F atom there is a ^{19}F atom as well. The produced ^{18}F−F_2 can be used as is or converted to less reactive and more selective fluorination agents such as acetylhypofluorite (^{18}F-CH_3COOF) for labeling.[58,59] The more selective agents can be produced in the gas phase and carefully bubbled through the appropriate procurer solution.

One effort to improve the SA of ^{18}F−F_2 utilized a unique method for the conversion of ^{18}F-fluoride into ^{18}F-fluorine gas in an electrical discharge chamber.[58,59] The ^{18}F-fluoride was first converted to ^{18}F-fluoromethane by nucleophilic substitution on methyl iodide.[58,59] The conversion to ^{18}F-fluoromethane was very facile (less than 6 min) and with high RCY (75%).[58,59] The authors reported a SA of 5.5 TBq/μmol (148 Ci/μmol) for ^{18}F-fluoride which decreased to 2.5 TBq/μmol (67.5 Ci/μmol) for ^{18}F-fluoromethane. Then the $CH_3{}^{18}F$ was cooled and either purified by gas chromatography or used as produced and transferred to an electric discharge chamber containing the desired amount of carrier ^{19}F-fluorine.[58,59] The SA and yield of ^{18}F−F_2 were dependent on the amount of ^{18}F-fluoromethane and carrier ^{19}F−F_2, along with the efficiency of the exchange reaction between the two.[58,59] The authors reported a maximum exchange of 60%, as the amount of carrier ^{19}F−F_2 increased.[58,59] The calculated SA of the final ^{18}F-labeled radiopharmaceuticals, which was reported by the authors, was 15 GBq/μmol (0.4 Ci/μmol) at the end of synthesis.

Electrophilic ^{18}F−F_2 and its derivatives allow labeling of electron rich aromatic rings and alkenes, but because the regioselectivity is low, a mixture of fluoro isomers is obtained that presents challenging purification needs (Figure 9A).[9,60,61] The regioselectivity of the ^{18}F-fluorine can be increased by using organometallic precursors (Figure 9B); aryltrimethyltin is superior to arylmercury, aryltrimethylsilane, and aryltrimethyl-

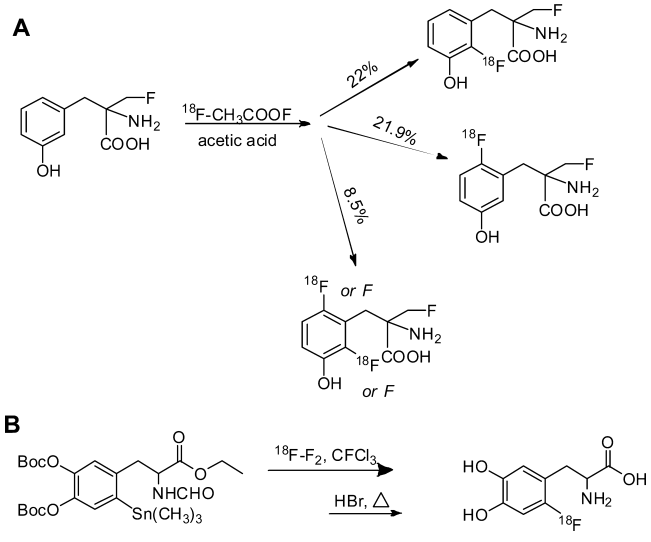

Figure 9. (A) $^{18}F_2$-Electrophilic substitution on 6-^{18}F-fluoro-*m*-tyrosine.[60] (B) Synthesis of ^{18}F-FDOPA via $^{18}F_2$-electrophilic substitution on aryltrimethyltin precursor.

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germanium.[9,62] As a direct outcome of improving the selectivity of ^{18}F-fluorine, fewer byproducts form and higher RCY of the desired ^{18}F product is realized.[9,62]

One interesting class of mild electrophilic fluorination reagents are the N-fluoro compounds. Gouverneur's group described the preparation and use of two such reagents: (1) ^{18}F-*N*-fluorobenzenesulfonimide (^{18}F-NFSi), a mild fluorination reagent used for the labeling of ethers, allylsilanes, and silyl enols,[63] and (2) Selectfluor, prepared with high SA ^{18}F−F_2 via electrical discharge chamber as described above, for labeling a variety of small molecules (Figure 10).[59,64] The availability of high specific activity ^{18}F-Selectfluor as ^{18}F-fluorination reagent may significantly advance electrophilic ^{18}F-radiochemistry of electron rich arenes.[59] Although utility of ^{18}F-Selectfluor is very promising for those applications that can tolerate addition of some carrier ^{19}F, the preparation of the electrical discharge chamber is not trivial. Consequently, the method has not been widely adopted in the field. Therefore, most of the clinically used radiopharmaceuticals, such as ^{18}F-2-fluoro-L-tyrosine, ^{18}F-6-fluoro-3,4-dihydroxy-L-phenylalanine (^{18}F−F-DOPA), are prepared by electrophilic reaction using low SA ^{18}F−F_2.

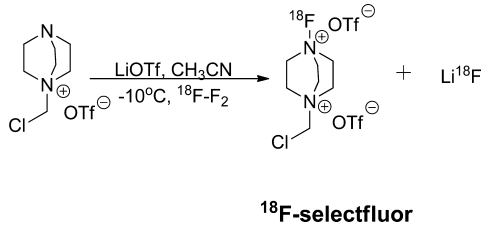

Figure 10. Labeling of fluorination reagent ^{18}F-selectfluor using high specific activity ^{18}F−F_2.[59]

4. TRANSITION METAL MEDIATED RADIOFLUORINATION

Transition metal mediated cross-coupling reactions that result in coupling of aromatic rings or the addition of nucleophiles to aromatic rings has been a significant area of research in organic chemistry. Applications of this methodology to fluorination have been reported recently.[65,66] These reactions have a wide range of tolerable functional groups and, therefore, may increase the diversity of substrates that can be labeled with ^{18}F-fluoride.[5]

Ritter and co-workers reported on the development and synthesis of an organopalladium-based fluorination reagent derived from fluoride and its application in labeling small aromatic molecules via late-stage fluorination (Figure 11A).[65] Because of its high oxidative state (IV) in this type of complex, palladium functions as an oxidant and transfers the substrate to nucleophilic fluoride while being reduced to a lower oxidation state.[65] The authors chose octahedral palladium(IV) in order to avoid undesired nucleophilic attack at the transition metal.[65] Within the formed palladium−fluorine complex, the fluorine is partially negatively charged and the palladium is positively charged, forming a polar complex.[65] Overall, this late-stage fluorination reaction requires two steps: capture of fluoride by the palladium complex and then its transfer via electrophilic fluorination to the appropriate aryl molecule.[65] As in most fluorination reactions, nearly anhydrous conditions are required.

Recently, the same group reported the syntheses of aryl and alkenyl fluorides from organometallic nickel complexes[66] in a late-stage fluorination. This fluorination reaction could be conducted in a single synthetic step using aqueous fluoride, without the need of azeotropic dehydration of ^{18}F-fluoride (Figure 11B). This late-stage fluorination method was applied to label small molecules, but its simplicity and the ability to use aqueous fluoride-18 (1% of the reaction volume is water) may portend future application for labeling more complex bioactive molecules for medical applications.

5. RADIOLABELING OF BIOMOLECULES

Bioactive molecules, such as peptides, proteins, and oligonucleotides, are often used as molecular imaging agents because of their target specificity. Typically, these molecules do not have

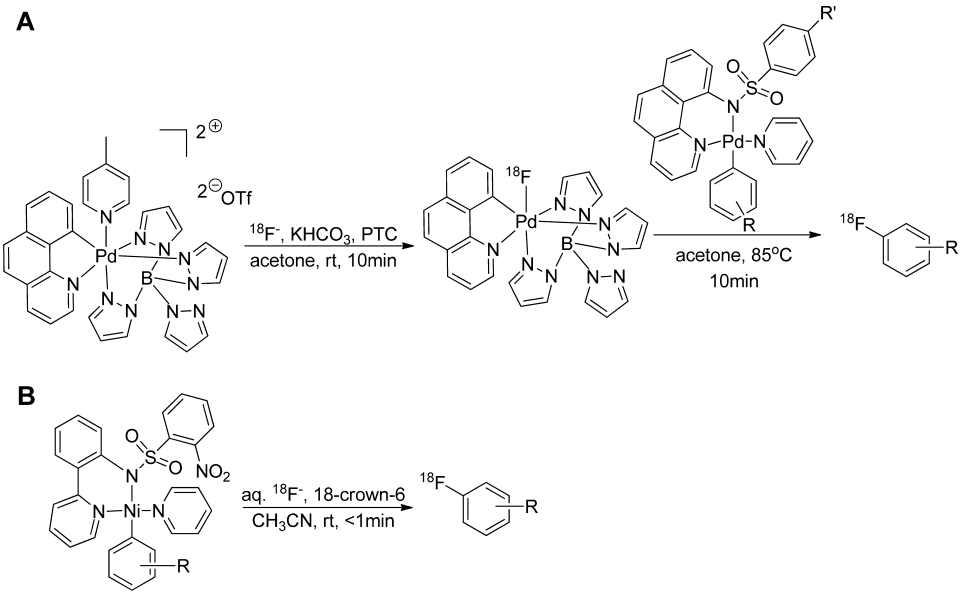

Figure 11. ^{18}F-Labeling by late-stage fluorination reaction. (A) ^{18}F_2-Electrophilic fluorination via organometallic palladium complex.[65] (B) ^{18}F-Fluoride reaction with nickel−aryl complex.[66]

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good stability to the common reaction conditions used in radiofluorination reactions, although there are exceptions. The radiolabeling of these molecules may be conducted by direct methods or indirect methods. Direct methods are those in which the fluoride is reacted directly with the molecule/biomolecule, which may have been previously modified to facilitate radiolabeling, and only subsequent purification is required to obtain the final product. Indirect methods require the prior radiosynthesis of a prosthetic group and subsequent bioconjugation to a molecular entity that has been modified for site specific reaction. In the sections that follow, we will discuss some direct and indirect methods that have been applied to radiolabeling biomolecules. This discussion will include preparation of unique prosthetic groups and methods to conjugate these prosthetic groups to the biomolecules.

5.1. Direct Labeling Methods for Biomolecules. A few successful attempts to introduce ^{18}F directly into small peptides have been reported in the literature.[67−72] We have reported on a one-step labeling strategy of peptide with ^{18}F-fluoride by displacing an aromatic nitro group in an arene which is activated toward nucleophilic substitution by an *ortho* trifluoromethyl group. We applied this labeling method to cyclic RGD monomer and dimeric peptides. One downside of this labeling is the difficulty of separating the nitro-containing peptide precursors from the desired ^{18}F-labeled products. Thus, the specific activity of the labeled peptide is related to the amount of precursor and radioactivity which are used.[71] This method would not be expected to be generally applicable as the reaction conditions utilized high temperatures and basic conditions that may not be tolerated by most biomolecules.

Another example of such labeling, described by Hazari et al., was the synthesis of Si^{18}F−dipropargyl glycerol scaffold, based on silicon-fluoride acceptor (Figure 12).[73] The precursor for

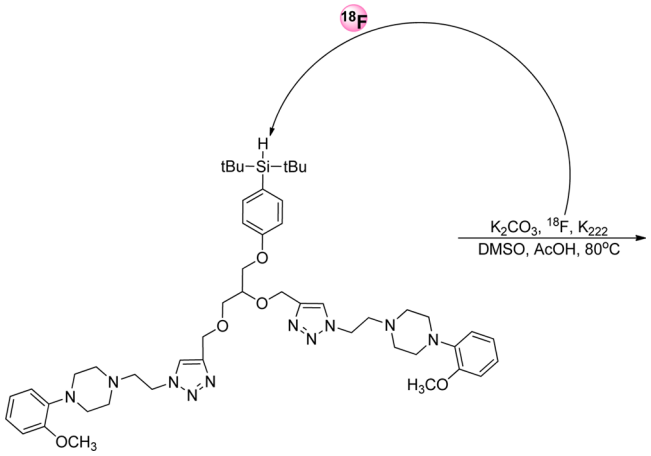

Figure 12. Direct ^{18}F-labeling on the conjugated Si−dipropargyl glycerol prosthetic group.[73]

the labeling was prepared by conjugation of 4-(2,3-bis(prop-2-ynyloxy)propoxy)phenyl)-di-*tert*-butylsilane (SiH−dipropargyl glycerol) to 1-(2-azidoethyl)-4-(2-methoxyphenyl)piperazine through alkyne−azide Huisgen cycloaddition. ^{18}F-labeling was conducted in the presence of K_2CO_3 and Kryptofix$_{222}$ following azeotropic drying. The fluorination reaction was done in DMSO with 1% glacial acetic acid at 80 °C for 15 min.[73] The desired labeled product was obtained in RCY of 50−60% following a 1 h synthesis time and applied for serotonin receptor PET imaging studies.

Perrin's group has described the labeling of RGD peptides using ^{18}F-aryltrifluoroborate.[67−70] The labeling was done in the presence of potassium (mainly in its carbonate form) without the addition of PTC. The K^{18}F solution was concentrated to near dryness. Thereafter, carrier ^{19}F-fluoride was added to the mixture, followed by the peptide, which contains a boronate ester/borimidine and HCl acidic buffer at pH 2 (Figure 13).[68−70] The acidification was required for the formation of

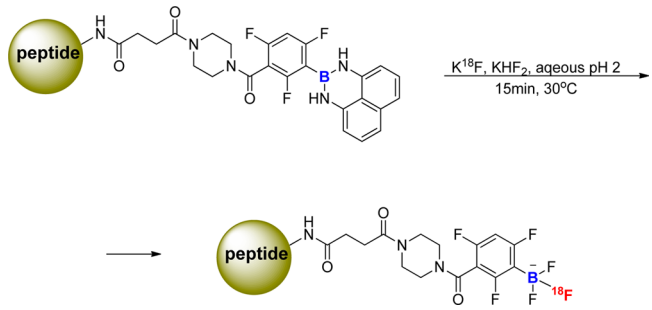

Figure 13. Labeling peptides using ^{18}F-aryltrifluoroborate.[68]

three B−F bonds of an ^{18}F-labeled aryltrifluoroborate anion.[67−70] The efficiency of this methodology was confirmed by large-scale production of a ^{18}F-aryltrifluoroborate-RGD peptide through direct ^{19}F−^{18}F exchange.[70] However, the use of low pH may not be amenable for majority of biomolecules and may disrupt their biological activity.

In 2009, McBride et al. hypothesized that ^{18}F, which was known to bind and form stable complexes with many metals, would form a stable NOTA Al−^{18}F complex.[74] The ensuing experiments resulted in successful chelation of Al^{18}F into peptide conjugated with *p*-SCN-Bn-1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) (Figure 14).[74] The labeling was performed at pH 4 and required neither adding PTC nor azeotropic drying of water. The labeling is typically done at 100 °C for 15 min with a small amount of conjugated peptide and resulted in relatively high RCY.[75] Although this is a promising methodology for relatively quick labeling of chelator-conjugated molecules, the conditions, such as excessive heating and low pH, may limit the scope of biomolecules that can be labeled by this approach. Since the initial report and subsequent work by the originators, this labeling methodology has been used by several groups for the labeling of small molecules, peptides, and proteins.[76−86] Wan et al. reported recently on the first human study using a new lyophilized kit for the labeling of Al^{18}F RGD dimer peptide.[83]

5.2. Indirect Labeling Methods for Biomolecules. As discussed above, direct substitution methods usually require some nonphysiological conditions of pH or temperature. Most peptides, proteins, oligonucleotides, etc., do not tolerate such conditions and may undergo hydrolysis. Hence, labeling of biomolecules is often accomplished using a prosthetic group.[2,87] The biomolecules are attached to the prosthetic groups mostly through amine- or thiol-reactive groups via acylation, alkylation, amidation, imidation, oxime, hydrazone formation,[2] or using click chemistry (described below). The choice of prosthetic group is critical for radiotracer development, as they may adversely alter the physical and physiological characteristics of the labeled molecule.[88] Methods for the site specific introduction of reactive thiols and other unique reactive functional groups must be utilized to develop novel, active, biological radiotracers.

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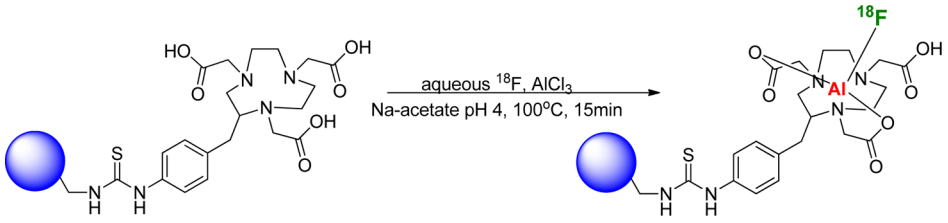

Figure 14. Labeling through $Al^{18}F$ methodology.[74]

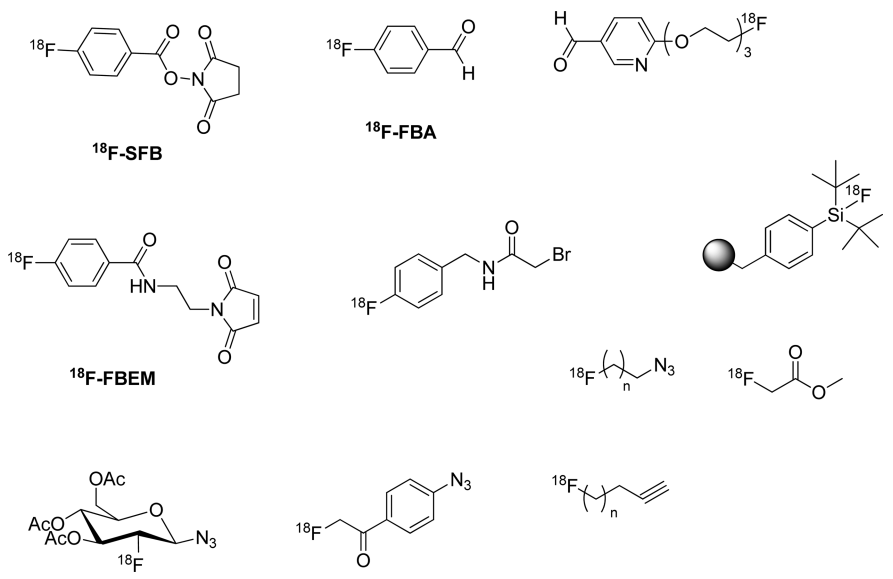

Figure 15. Overview of common ^{18}F-labeled prosthetic groups.

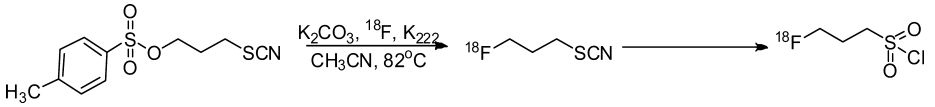

Figure 16. Radiosynthesis of 3-^{18}F-fluoropropanesulfonyl chloride prosthetic group.[88]

^{18}F-labeled prosthetic groups are prepared in a reaction employing from one to three synthetic steps and require subsequent purification processes to remove fluorination reagents (base and PTC), unreacted precursor, and other byproducts that can affect the conjugation with the biomolecule.[89] Typically, the labeled biomolecule undergoes an additional purification process to obtain the desired product with high SA.[87] The relatively short half-life of ^{18}F is a challenge when designing a radiosynthesis that includes several synthetic and purification steps.

Despite the half-life limitations, numerous prosthetic groups have been developed for conjugation with biomolecules or small molecules using nucleophilic procedures described previously in this review (Figure 15).[9,90−95] A great deal of work has been published using *N*-(hydroxysuccimidyl)-^{18}F-fluorobenzoate (SFB) for coupling to amine functionalities on peptides and proteins and various ^{18}F-fluorinated maleimide analogues for reaction with free thiols.[89,96] Fluorinated *N*-hydroxysuccinimidyl esters and maleimides are typically synthesized in two or three labeling steps. Simplifications of their syntheses using one-pot reaction have been reported and appear to be routine in many laboratories.[89,96−98] These prosthetic groups are of low volatility such that they can be concentrated without significant loss of radioactivity, thus, allowing scale up to clinical doses.

Another prosthetic group with specificity for amino functionalities, 3-^{18}F-fluoropropanesulfonyl chloride, was first reported by Kiesewetter and co-workers for labeling target molecules by forming sulfonamide derivatives.[99] Loser et al. recently described the preparation of this same molecule using a two-step ^{18}F-labeling procedure via the intermediate 3-^{18}F-fluoropropyl thiocyanate (Figure 16).[88] The resulting 3-^{18}F-fluoropropanesulfonyl chloride was reacted with different primary and secondary aliphatic and aromatic amines at room temperature with or without various bases.[88] RCY (calculated from radio-thin layer chromatography) were high (77−89%) for most of the reactions without the addition of base.[88]

Another interesting paper, recently published by Gao et al., described the fluorination of proteins and peptides via 4-fluorophenylboronic acid prosthetic group using an aqueous Suzuki−Miyaura coupling reaction with an optimized palladium ligand.[100] This reaction requires three labeling steps, ^{18}F nucleophilic fluorination on a diaryliodonium precursor, conversion to the 4-fluorophenylboronic acid prosthetic group, and palladium-catalyzed cross-coupling reaction with an appropriate small molecule, peptide, or larger biomolecule (Figure 17).[100] The site specificity of the radiolabeling is achieved only by site specific introduction of an aryl iodide into the biomolecule by either chemical methods or genetic modification. Thus, the site of labeling can be selected so

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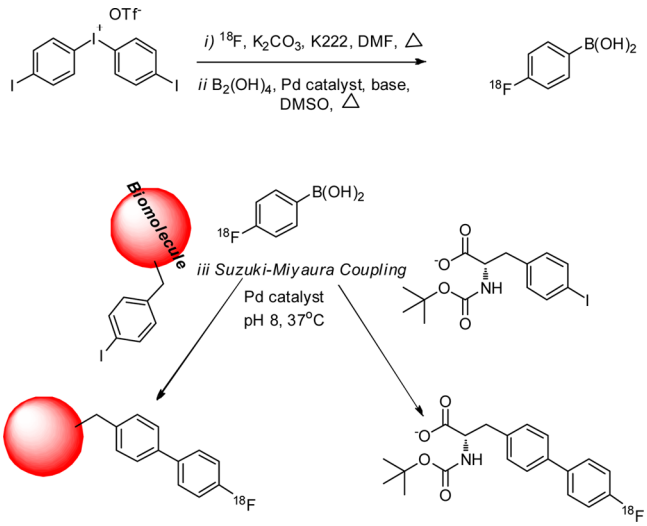

Figure 17. Suzuki–Miyaura coupling via 4-^{18}F-fluorophenylboronic acid prosthetic group.[100]

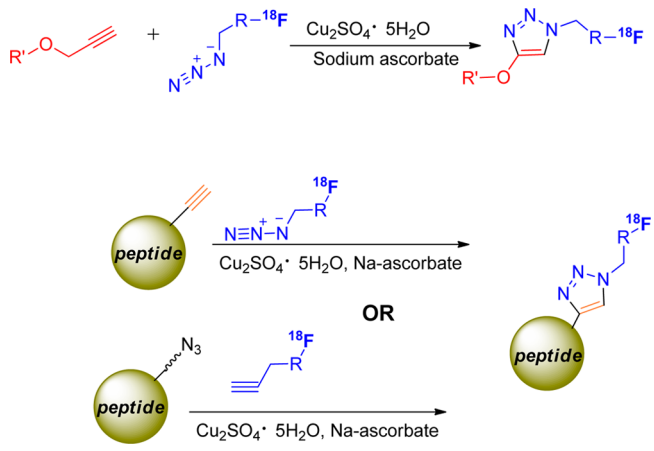

Figure 18. Examples of copper(I)-catalyzed azide–alkyne cycloaddition.

that the functional binding site of the protein is not impaired. The reported procedure produced the 4-^{18}F-fluorophenylboronic acid in relatively low yield (5–10% corrected). The subsequent coupling yields with a functionalized peptide were on the order of 30%, but, unfortunately, coupling yields with larger proteins were quite low. Significant optimization will be required if this method is to become useful for labeling biomolecules.

5.3. Click Chemistry/Bioorthogonal Reactions. Bioorthogonal reactions are defined as chemical reactions that take place in living tissue but do not interact with the biological system and because of that, allow investigation of the various biological processes. In addition to being highly selective, the bioorthogonal reactions are typically rapid and can be conducted in biological media. Consequently, a number of bioorthogonal reaction strategies have been developed and used for incorporation of ^{18}F-labeled prosthetic groups into drug-like and biomolecules (indirect labeling). To select a click chemistry approach, consideration must be given to the reaction rate, stoichiometry, concentration, and mass of biomolecule required for achieving good radiochemical yields and minimizing purification procedures. In this section, we will discuss the reactions that have been applied in ^{18}F chemistry.

5.3.1. Copper(I)-Catalyzed Azide–Alkyne Cycloaddition. The Huisgen cycloaddition is a 1,3-dipolar cycloaddition of an azide and an acyclic alkyne to yield a 1,2,3-triazole. As originally described, excessive heat and prolonged reaction time are required to overcome the activation barrier of triazole formation from alkyne bond.[101] Research led by K. Barry Sharpless first described the catalysis of the Huisgen cycloaddition by Cu(I) and created the name "click chemistry" to describe this type of reaction.[102] Meldal[103] and Sharpless[102] groups exploited this reaction, described as copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC) for the formation of various five-membered heterocycles (Figure 18). The scope of the reaction is quite large and, because alkynes and azides are typically inert in biological systems,[104] qualifies as a bioorthogonal reaction.[105] The orthogonal nature of cycloaddition reactions does not require protection of other functional groups and can be done in the presence of water and oxygen.[106] Primary, secondary, and tertiary alkyl azides, aryl azides, and an azido sugar were reacted in the copper(I)-catalyzed cycloaddition with alkynes to give a variety of 1,4-substituted [1,2,3]-triazoles in peptide backbones or side chains as a mixture of two possible regioisomers.[103,104,106−108] It is widely accepted that Cu(I) coordinates first to the terminal alkyne to form a copper(I) acetylide.[102,103,109] Recently, Worrell et al. suggested a mechanism which involves two copper atoms within the cycloaddition step.[105]

The high efficiency and selectivity of this reaction has been exploited for ^{18}F labeling.[104,109−111] A variety of ^{18}F-labeled small molecules containing azides or alkynes have been prepared, and peptides and other biomolecules have been decorated with the corresponding reactive moiety. The cycloaddition reaction can be catalyzed with Cu(I) sources such as CuBr and CuI but is typically done with $CuSO_4$, which becomes Cu(I) in situ in the presence of a reducing agent such as sodium ascorbate.

Ramenda et al. described the radiosynthesis of *N*-propargyl-*N*-methyl^{18}F-fluorobenzenesulfonamide as a prosthetic group for Cu(I)-mediated [3 + 2] cycloaddition reactions. Its applicability was evaluated by conjugation to a peptide, human serum albumin (HSA) protein, and a RNA oligonucleotide.[110,112] ^{18}F labeling of peptide was evaluated using different amounts of peptide (0.1–0.4 mg) and in the presence of $CuSO_4$ and sodium ascorbate in borate buffer. As more peptide was used, higher RCY was achieved.[112] For HSA labeling, the use of $CuSO_4$ and sodium ascorbate did not result in the desired labeled protein. The authors speculated that the sodium ascorbate induced partial or complete reduction of the disulfide bonds in the protein.[112]

The successful labeling of both HSA and RNA oligonucleotide was done using CuBr and oligotriazole tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)-methyl]-amine (TBTA), which is used to prevent the reoxidation of Cu(I) to Cu(II) (Figure 19).[112] The stable Cu(I)–TBTA complex allows the cycloaddition reaction between azide-modified biomolecule and the ^{18}F-arylalkynyl sulfonamide while avoiding generation of hydroxyl radicals and subsequent oxidative decomposition.[112]

5.3.2. Strain-Promoted Alkyne–Azide Cycloaddition. Strain-promoted alkyne–azide cycloaddition involves the reaction of azide with the bent triple bond of the cyclooctyne to give a triazole without the need for Cu(I) catalyst (Figure 20).[101,113] In this reaction, the activation barrier is significantly reduced by the bent geometry of the triple bond of the eight-membered ring.[101] Increased reactivity toward terminal azides

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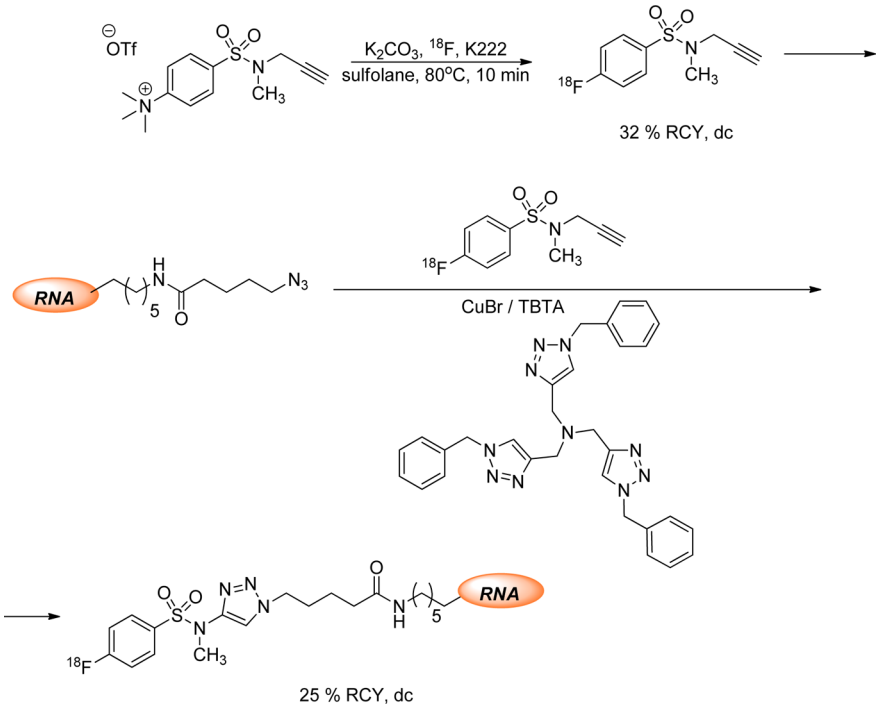

Figure 19. Synthesis of ^{18}F-aryl sulfonamide as a building block for Cu(I)-mediated [3 + 2] cycloaddition reaction with RNA.[112]

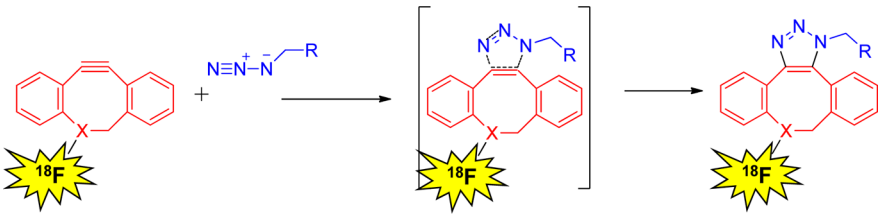

Figure 20. Strain-promoted alkyne−azide cycloaddition mechanism.

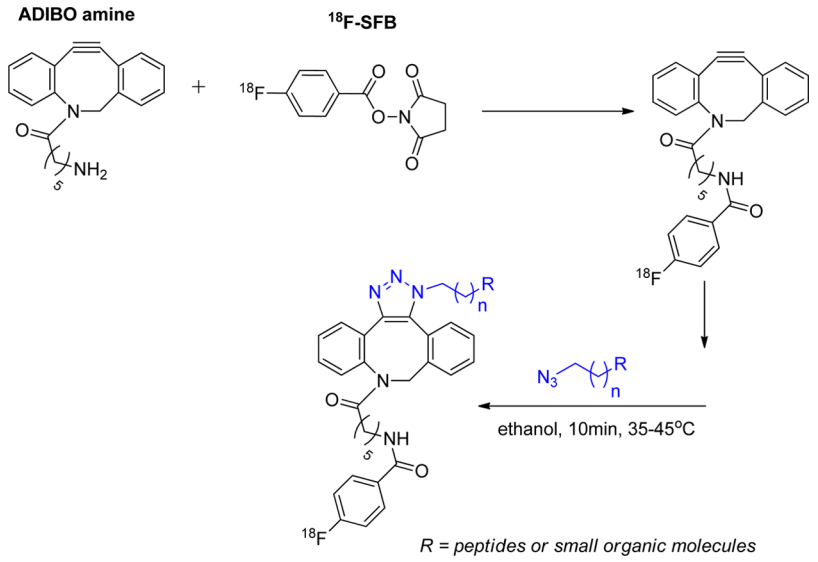

Figure 21. 1,3-Dipolar cycloaddition of ^{18}FB-azadibenzocyclooctyne (ADIBO) with alkyl azide without Cu(I) catalyst.[113,122]

can be achieved by modifying the cyclooctyne with electron withdrawing groups or conformationally restricted aromatic rings adjacent to the triple bond. More importantly, the elimination of toxic copper as a catalyst allows use of this alkyl−azide cycloaddition in living systems.[109,113−116]

In recent years, several cyclooctynes and dibenzocyclooctynes have been used in strain-promoted reactions for ^{18}F-labeling.[113,117−121] As in the case of CuAAC reaction, two radioactive triazole regioisomers are formed and are detected by HPLC.[113,122] These regioisomers might have different bio-

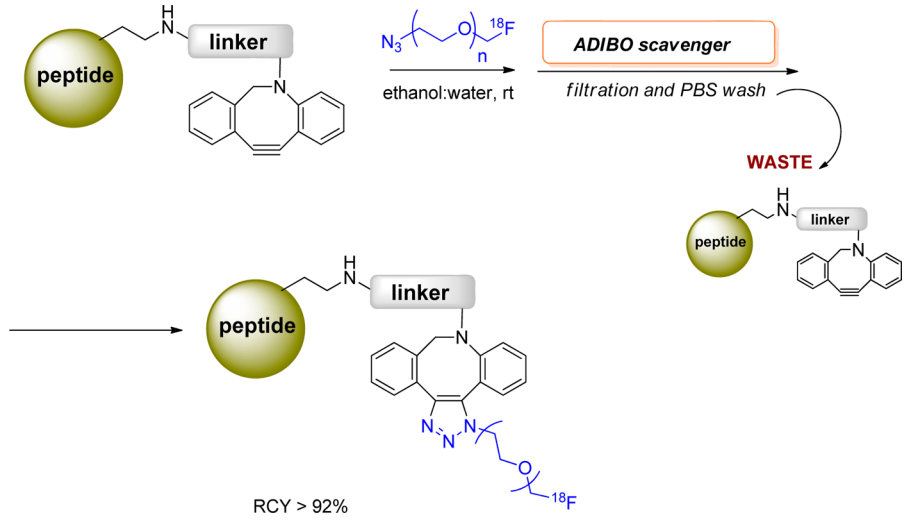

Figure 22. Strain-promoted alkyne−azide cycloaddition under nontoxic physiological conditions.[121]

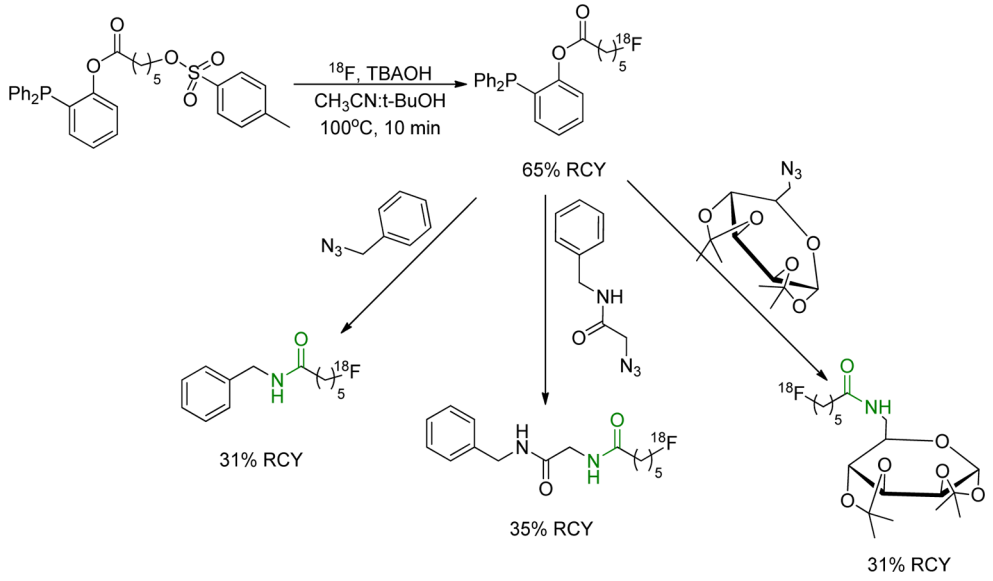

Figure 23. Staudinger ligation between ^{18}F-6-fluorohexanoyl-phosphane and benzyl azide, azidoacetic acid, and a 6-azido-galactose.[19]

logical properties in vivo such as target binding affinity and pharmacokinetics. One example for such cycloaddition, reported by Carpenter et al., described the 1,3-dipolar cyloaddition of ^{18}F-FB-azadibenzocyclooctyne (ADIBO) with alkyl azides (Figure 21). ^{18}F-Fluoride was initially introduced into the cyclooctyne prosthetic group. Subsequent strain-promoted cyclization with small molecules was conducted. This reaction provided the desired product in high RCY (>70% yield of the cycloaddition reaction) in less than 1 h reaction time. In addition, the triazole product showed high stability in rat serum.[122] The same group also applied this methodology for labeling peptides that had been site selectively functionalized with an azide. This cycloaddition reaction was conducted for 10 min at 35−45 °C, with a peptide substrate concentration of 1 mg (0.37 μmol) in 150 μL of ethanol and resulted in an isolated RCY of 12% based on the quantity of [^{18}F]ADIBO (Figure 21).[113] Sachin et al. described ^{18}F labeling of ADIBO peptide precursors with an ^{18}F-azide synthon under physiological conditions with quantitative RCY. One of the advances described in this work was the replacement of HPLC purification with a unique azide-containing scavenger resin that effectively removed unreacted ADIBO peptide precursor from the desired radiolabeled triazole product (Figure 22).[121] This intriguing methodology can be easily transferred into a commercial kit, making it readily available for labeling biomolecules for clinical applications.

5.3.3. Staudinger Ligation. Staudinger reaction occurs between azide and phosphine via an iminophosphorane, which is stable in organic solvents but is rapidly hydrolyzed in aqueous solution, to give the primary amine and phosphine oxide.[123−125] Mechanistic studies revealed the potential to perform ligation reactions. An intramolecular electrophilic trap, such as an ester moiety, could capture the nucleophilic aza-ylide intermediate with the formation of a stable covalent adduct that upon rearrangement forms a stable amide bond; no hydrolysis of the aza-ylide is required.[126] This method was first demonstrated in 2000 by Saxon and Bertozzi, in which coupling of azide and modified triarylphosphine yielded an amide bond.[126]

Although this method has proven to be very useful, the resulting amides contain a very lipophilic triphenylphosphonium oxide moiety as part of the product. As a result, the

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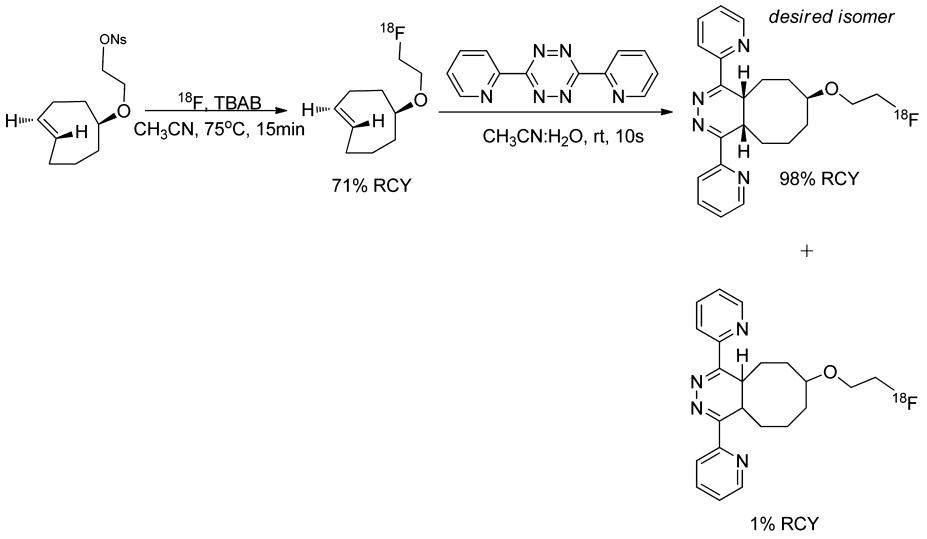

Figure 24. Tetrazine–*trans*-cyclooctene ligation between 3,6-di(2-pyridyl)-*s*-tetrazine and ^{18}F-*trans*-cyclooctene.[139]

Bertozzi group and the Raines group independently developed the "traceless" Staudinger ligation for chemoselective synthesis in which the triphenylphosphonium oxide moiety is lost during the ligation reaction.[127,128] The phosphine, stabilized by two phenyl groups, is linked to an acyl group via an ester or thioester. The intermediate aza-ylide attacks the carbonyl group, cleaving the ester or thioester bond. Subsequent hydrolysis gives an amide bond and releases the phosphine oxide moiety.[127]

For ^{18}F-labeling of biomolecules, traceless Staudinger ligation does not require metal catalyst and allows a simple separation of the ^{18}F-labeled amide from the phosphine oxide by-product.[129,130] It has been applied for indirect ^{18}F-labeling via the synthesis of a prosthetic group followed by conjugation with phosphine derivatives and formation of amide bond.[19,130−133] One example, published by Pretze et al., described the ligation between ^{18}F-6-fluorohexanoyl-phosphane moiety and several azides such as benzyl azide, azidoacetic acid, and a 6-azido-galactose derivative (Figure 23).[19] The Staudinger ligation has two major limitations. The first is the relatively slow kinetics that requires high concentration of the phosphine to obtain usable reaction rates for radiochemical synthesis. The second is the fact that the phosphine precursor is subject to oxidation that will demolish its reactivity.

5.3.4. Tetrazine Ligation. Tetrazine ligation is a bioorthogonal cycloaddition based on inverse electron demand Diels–Alder reaction of *s*-tetrazine and *trans*-cyclooctene moieties.[134] The inverse demand Diels–Alder reaction followed by a retro-Diels–Alder reaction with release of nitrogen yields either dihydropyradazines or pyradazines depending on the dienophile reactant (alkynes or alkenes).[134,135] This reaction is characterized by fast reaction rate and, therefore, no need for excess amount of reactants or catalyst, which makes for a fast and efficient bioconjugation at low concentrations.[134,135] It also tolerates a broad range of functionality and is not dependent on the solvent, i.e., can be performed in organic solvents, water, cell media, or cell lysate without reducing the high yield.[134]

Several publications described the ^{18}F incorporation via tetrazine–*trans*-cyclooctene ligation (TTCO-ligation) by indirect (the use of prosthetic groups) or direct routes.[135−139] Li et al. described the TTCO-ligation between 3,6-di(2-pyridyl)-*s*-tetrazine and ^{18}F-*trans*-cyclooctene (^{18}F-TCO) (Figure 24). The TTCO ligand was completely consumed within 10 s and resulted in high RCY (98%). The concentration of tetrazine was 21 μM and the 18F-TCO ranged from 0.2−2 μM. There was also a very small amount of side product (Figure 24), which is due to the slow rearrangement of the product with formation of regio- and stereoisomers.[139] The feasibility of TTCO was further evaluated by conjugation of ^{18}F-TCO with tetrazinyl-maleimide-conjugated to c(RGDyC) peptide and vascular endothelial growth factor (VEGF) protein, containing free cysteines (Figure 25). The click chemistry was done in

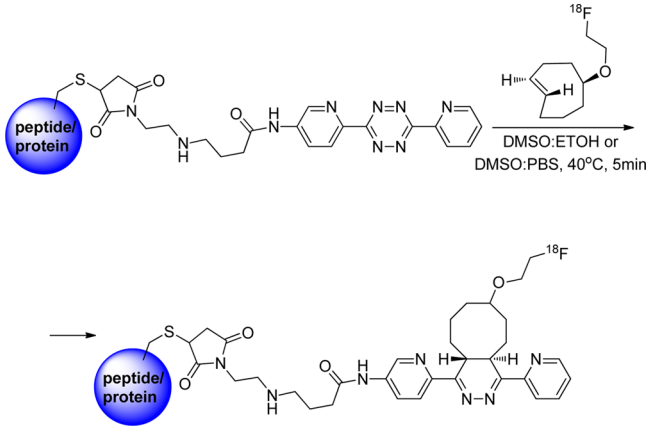

Figure 25. Tetrazine–*trans*-cyclooctene ligation of ^{18}F-*trans*-cyclooctenes with biomolecules.[138]

DMSO and ethanol or DMSO and phosphate buffer to give the desired product with high RCY (quantitative yield for the RGD and 75% for VEGF, using 80−100 μM and 6 μM substrate concentrations, respectively).[138] Unfortunately, the complementary reaction using an ^{18}F-tetrazine was unsuccessful. Attempts to incorporate [^{18}F]fluoride via aromatic and aliphatic nucleophilic substitutions into tetrazine precursors resulted in very low yields (<1%) because the tetrazine precursors proved unstable under basic fluorination conditions.[139]

This method seems to be the most promising of the click methods in terms of high yield and very fast reaction rates. A number of the chemical entities for the click chemistry pairs have been made commercially available. However, the

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precursor for ^{18}F-TCO is not yet commercially available. The TCO precursor is prepared by a photochemical isomerization with kinetic trapping;[134] both procedures are rarely practiced in radiochemistry laboratories. This precursor will need to be a commercial product in order for the method to be thoroughly studied. Questions remain as to the volatility of ^{18}F-TCO, which will determine the ability to concentrate the radioactive component for synthesis of sufficient material for clinical use.

6. CONCLUSIONS

Fluorine-18 labeling chemistry has developed tremendously over the last decades. Because of higher specific activity, most syntheses use no-carrier-added nucleophilic fluoride-18 rather than electrophilic fluorine. Some of the ^{18}F-labeling strategies require time-consuming and challenging radiosynthesis, and, thus, are not clinically viable.

Numerous labeling methods to incorporate ^{18}F-fluoride into aliphatic or aromatic substrates have been developed, attempting to achieve easier and more time-efficient radiosyntheses, with high specific activity and high yield of desired products. Traditional nucleophilic aromatic radiofluorinations require electronically deficient rings; however, newer methods using aryl iodonium salts or palladium and nickel complexes have provided efficient radiofluorination of electron rich arenes. These approaches expand the scope of radiofluorination substrates and place the challenge on the ability to synthesize the required precursor molecules.

The need for easy and rapid labeling techniques of biomolecules, using biocompatible conditions, is still not fully answered. Typically, biomolecules are functionalized with a reactive functional group, (i.e., amine, azide, alkyne, thiol, metal chelator) and radiolabeled through reaction with the appropriate prosthetic group or radiometal. The use of click chemical reactions, including strain-promoted alkyne−azide cycloaddition and tetrazine ligation, for rapid labeling of biomolecules has been heavily exploited. The development of the method for conjugation of ^{18}F-AlF with NOTA has allowed ^{18}F-fluoride to be utilized in chelation labeling for small and large biomolecules. In addition, the development of purification resins (such as the azide resin) for separation of the undesired precursors may further promote the use of click chemistry in clinical translation of novel ^{18}F-labeled radiotracers.

This review highlights the great arsenal of useful radiofluorination strategies available to prepare the next generation of ^{18}F radiotracers for PET that will provide patients with more sensitive diagnosis, faster and more accurate evaluation of therapeutics, and ultimately improved outcomes.

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Notes

The authors declare no competing financial interest.

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