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Review

Oxime formation for fluorine-18 labeling of peptides and proteins for positron emission tomography (PET) imaging: A review

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ABSTRACT

Positron emission tomography (PET) is a powerful technology for medical and biological imaging and the scope of PET applications is expanding rapidly. The development of suitable PET tracers is at a central position in PET technology. An increasing number of peptides and proteins have been developed for the clinic due to their special properties which small molecule drugs do not have. These advances have provoked interest in the research community to develop radiolabeled peptides and proteins for diagnosis and therapy. Fluorine-18 is a short-lived isotope of fluorine with superior properties for PET-imaging. A majority of presently used radiopharmaceuticals in PET are labeled with fluorine-18. This review focuses on a promising strategy, oxime formation of aminooxy-functionalized peptides with ^{18}F-containing aldehydes, for fluorine-18 labeling of peptides/proteins. At present only a few ^{18}F-containing prosthetic groups are available for oxime formation. The radiosynthesis of the ^{18}F-containing aldehydes and key factors influencing conjugation efficiency of ^{18}F-containing aldehydes with peptides are addressed.

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1. Introduction

The element fluorine has had a profound impact on the pharmaceutical industry, with a significant proportion of the drugs on the market and in research pipelines containing fluorine. Ten of the top 30 best selling medicines in the US in 2008 contained at least one fluorine atom [1]. Fluorine-18 (^{18}F) is a radioactive isotope of fluorine and of major importance in nuclear pharmacy [2,3]. The half-life of ^{18}F is 109.7 min and ^{18}F has a very clean positron decay process (97% β^+ emission) [4]. ^{18}F is the most often used radionuclide for clinical positron emission tomography (PET) imaging and 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG) is the most used PET tracer. PET is a sensitive, noninvasive, and quantitative technology for medical and biological imaging. The prerequisite for carrying out PET imaging is to inject a PET tracer to the patient before scanning [5]. Therefore the development of suitable PET tracers has a central position in medical PET applications.

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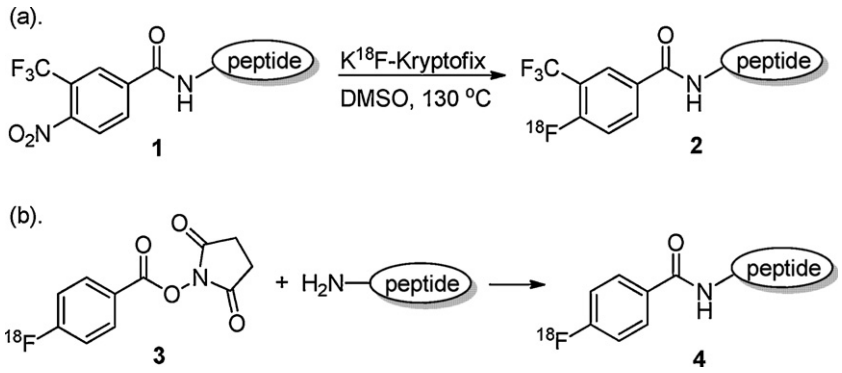


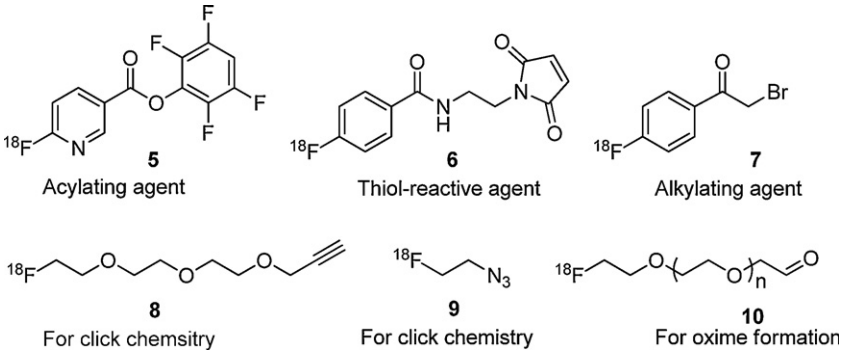
Scheme 1. (a) Direct approach for ^{18}F-fluorination of peptides [13] and (b) labeling of peptides with prosthetic groups (indirect approach for ^{18}F-fluorination of peptides).

Since each organ expresses its unique peptide receptors both in health and in disease, the corresponding peptide ligand (sometimes called the homing peptide) may act as a probe for specific targeting [6]. Numerous peptide drugs are in widespread clinical use, e.g., Exendin and Octreotide analogues. Radiolabeled natural peptides are considered to be promising tracers for diagnosis of major diseases and for monitoring physiological changes [7,8]. There are both direct and indirect approaches for fluorine-18 labeling of peptides. In a direct approach, a covalent C–F bond is formed between the peptide and ^{18}F-fluoride. In an indirect approach, a prosthetic group is produced by incorporation of ^{18}F-fluoride into a bifunctional agent and is subsequently conjugated to a peptide. Albeit it is a less commonly used method than indirect labeling, direct labeling methods have been continuously developed and some exciting results have appeared (Scheme 1a) [9–13]. For example, nucleophilic substitution of a nitro group with K^{18}F-Kryptofix complex was successfully used for the labeling of peptide **1** [13]. The nitro group is a well known leaving group used for aromatic nucleophilic substitution and the *ortho*-trifluoromethyl group facilitates the substitution by decreasing electron density in the aromatic ring [14]. The synthesis was achieved in 40 min and the specific activity of labeled peptide **2** was 79 ± 13 GBq/μmol.

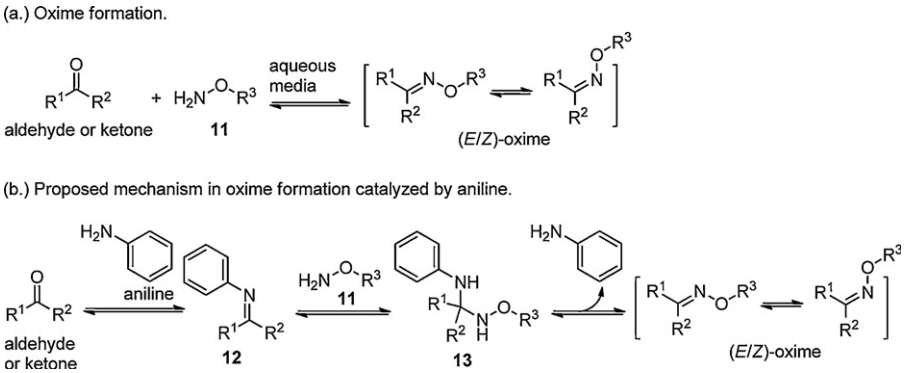
So far, in a majority of cases peptide labeling is achieved by using ^{18}F-containing prosthetic groups (indirect labeling approach, Scheme 1b). Several types of prosthetic groups based on different chemical reactions are available (Scheme 2) [8]. Among acylation agents, *N*-succinimidyl 4-[^{18}F]fluorobenzoate (SFB, **3**) is frequently used to acylate free amino groups in peptides [15–18]. Recently Olberg et al. developed the acylating agent, nicotinic acid tetrafluorophenyl ester **5** (Scheme 2), and the radiosynthesis of prosthetic group **5** is straightforward [19]. When an acylating agent will react with any/all free amino groups available, the peptide precursor should bear only one amino group in order to label the peptide in a specific position. The prosthetic group bearing a maleimide (e.g., compound **6**) is highly thiol-reactive and thus is a commonly used reagent for site-specific labeling of cysteine-containing peptides [20]. 4-[^{18}F]Fluorophenacyl bromide (compound **7**) and 2-bromoketone represent an alternative methodology for peptide labeling based on thiol chemistry, albeit to a lesser extent compared to maleimide-thiol chemistry [21]. Click chemistry (1,3-dipolar cycloaddition) is a very attractive method for bioconjugations, as it is highly compatible with biomolecules and the reactions can be carried out under physiological conditions with high efficiency [22]. In PET applications, it is possible to employ either an alkyne (e.g., compound **8**) or an azide (e.g., compound **9**) as the ^{18}F-prosthetic group for peptide labeling [23,24].

Oxime formation between an aldehyde or a ketone and an aminooxy-bearing compound **11** is widely used for conjugation of biomolecules, as it is a highly chemoselective reaction which can be carried out in aqueous media (Scheme 3a) [25]. In oxime bond formation reactions, other functional groups in biomolecules are tolerated without a need for protection/deprotection processes. Similar to other types of imine formation, oxime formation is a reversible reaction [26,27]. However, due to α-effect nitrogen in the aminoxy group [28], reaction equilibrium favors oxime bond formation and in many cases the reactions can reach completion. The reaction efficiency of oxime bond formation can be dramatically enhanced by a number of catalysts (e.g. aniline, Scheme 3b) [25,26,29]. Aniline forms a Schiff base **12** with an aldehyde or ketone, and **12** undergoes rapid transimination to oxime via the formation of intermediate **13**. The formed oxime exists in *E*- and *Z*-forms in solution and is stable under physiological conditions. The ratio of *E*- to *Z*-form of an oxime partially depends on the size of substitutes at the C=N double bond. Because the two isomers equilibrate quickly in solution, it is not practically useful to isolate *E*- and *Z*-forms from each other [30].

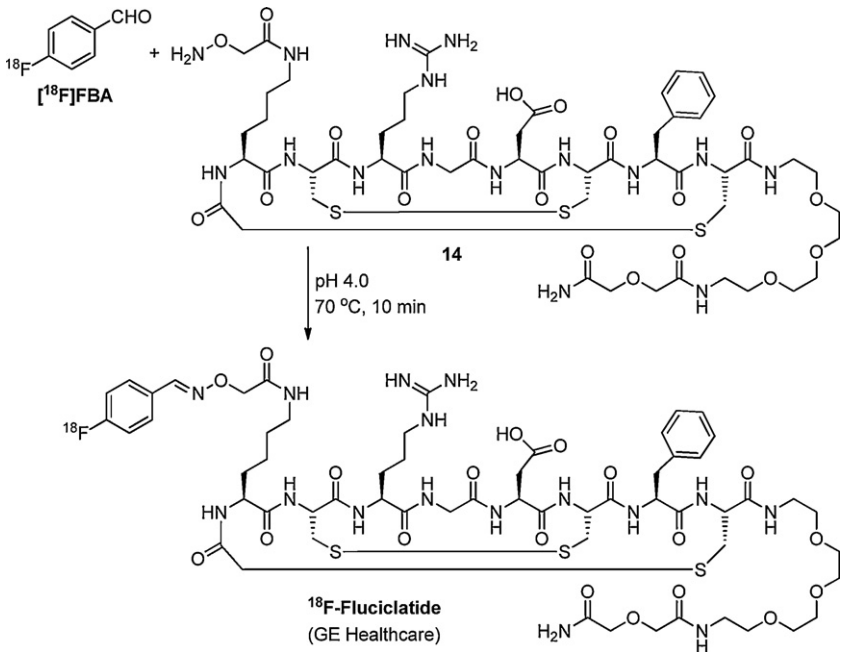
Oxime formation is useful in PET tracers synthesis, because it has high chemoselectivity, straightforward reaction protocol and it



Scheme 2. Examples of prosthetic groups for peptide labeling with fluorine-18.



Scheme 3. Formation of oxime bond without (a) and with (b) aniline as a catalyst [26].



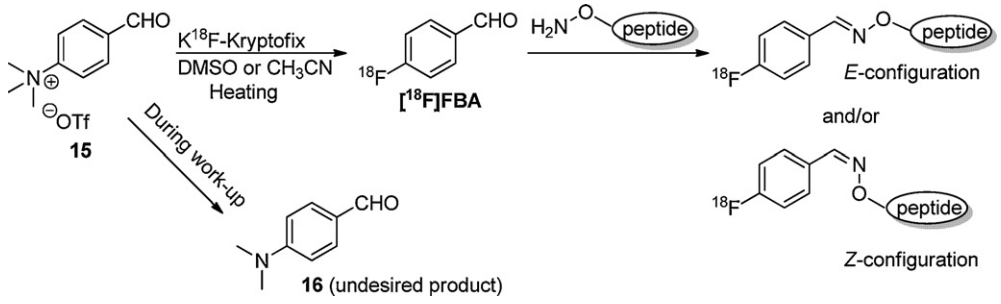
Scheme 4. Synthesis of [^{18}F]fluciclatide by oxime formation [33].

is amendable for automation. Accordingly, some prosthetic groups (e.g. compound **10** in Scheme 2) have been developed for peptide labeling by oxime bond formation [8]. At present, GE Healthcare has the ^{18}F-fluorinated peptide tracer, [^{18}F]fluciclatide, in clinical trials [31,32]. [^{18}F]Fluciclatide is a conjugate between 4-[^{18}F]fluorobenzaldehyde ([^{18}F]FBA) and peptide **14** bearing an aminooxy functionality at the N-terminus (Scheme 4) [33]. This review focuses on the application and practical issues in using ^{18}F-fluorinated aldehydes (e.g., compound **10** and [^{18}F]FBA) as prosthetic groups for peptide labeling.

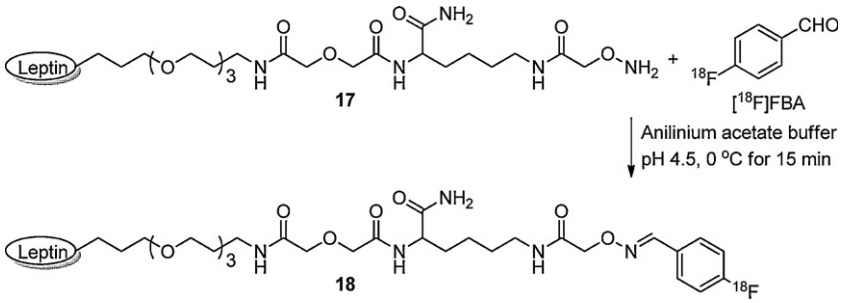
2. Prosthetic groups for oxime formation

2.1. 4-[^{18}F]Fluorobenzaldehyde ([^{18}F]FBA)

[^{18}F]FBA is usually synthesized from the precursor 4-formyl-*N*,*N*,*N*-trimethylanilinium triflate **15** in the presence of K^{18}F-Kryptofix (Scheme 5) [33]. In aqueous solution, fluoride is a weak nucleophile because fluoride is highly hydrated. Thus, Kyptofix is added to form a complex with K^{18}F to increase the nucleophilicity of [^{18}F]fluoride. Before the fluorination reaction, K^{18}F-Kryptofix is



Scheme 5. Radiosynthesis of [^{18}F]FBA.



Scheme 6. ^{18}F-Labeling of leptin with [^{18}F]FBA in the presence of aniline as a catalyst [41].

well dried by azeotropic distillation with acetonitrile. The fluorination of **15** with K^{18}F-Kryptofix can be carried out in anhydrous dimethyl sulfoxide (DMSO) [34], dimethylformamide (DMF) [35], and acetonitrile under heating (e.g., at 100 °C) [36]. For subsequent oxime formation reaction, it is pivotal that [^{18}F]FBA does not contain any impurity that acts as a competing aldehyde source (e.g., compound **16** in Scheme 5). In a recent publication, compound **16** was identified as a competing aldehyde, causing the failure of the conjugation of the protein annexin V-128 to [^{18}F]FBA via a maleimide linker [36]. [^{18}F]FBA was separated from **16** by using a Phenomenex Prodigy ODS HPLC column by isocratic elution (45% ethanol in water at 50 °C) [36]. [^{18}F]FBA has also been separated from precursor **15** and possibly **16** by using a combination of a LiChrolut SCX-cartridge (Waters) and a Sep-Pak C_{18} cartridge or by HPLC purification [37]. Because [^{18}F]FBA is a volatile compound, special radiosafety precautions should be used when handling [^{18}F]FBA, especially at elevated temperature [36].

In the radiosynthesis of [^{18}F]fluciclatide, [^{18}F]FBA is conjugated to peptide **14** in ammonium acetate buffer at pH 4 and 70 °C. The reaction was very fast and the conversion based on consumption of [^{18}F]FBA was 95% in a 10 min reaction time. [^{18}F]Fluciclatide was obtained with a radiochemical yield of 23 ± 5% (n = 3, decay corrected) and the radiochemical purity after a preparative HPLC purification was 96%. The specific activity of [^{18}F]fluciclatide ranged between 76 and 170 GBq/μmol at the end of synthesis [32,33]. In a recent development, a solid phase extraction (SPE) strategy was adopted for purification of [^{18}F]fluciclatide by using low cost and single use cartridges, which can make [^{18}F]fluciclatide even more attractive for routine production [38].

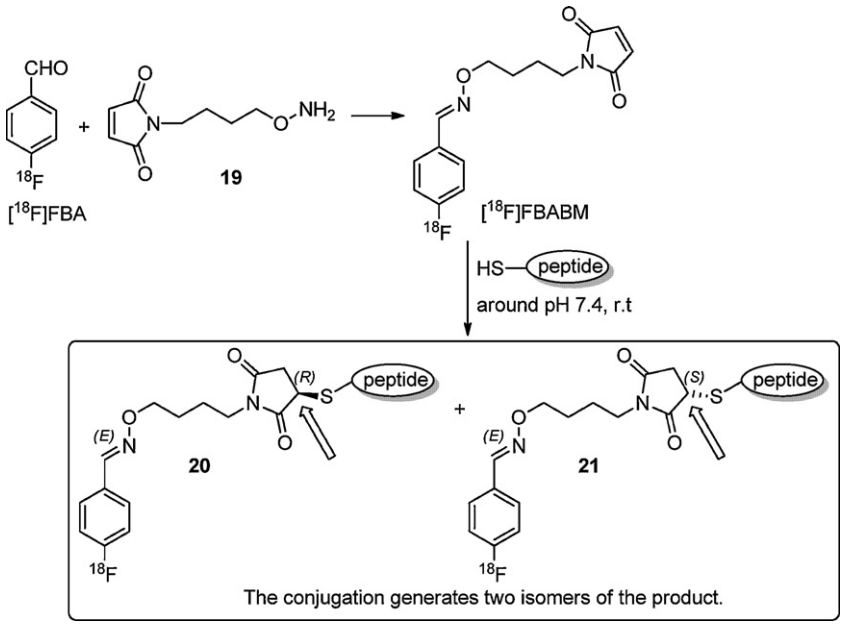
[^{18}F]Fluciclatide has been used for preclinical studies in a Lewis lung tumor mouse model [33]. It seems that the use of [^{18}F]FBA as a prosthetic group increases the lipophilicity of the peptide, higher liver uptake being observed in comparison with other tracers studied. Very recently, [^{18}F]fluciclatide has been successfully used in a treatment-response study in a U87-MG xenograft tumor mouse model, where reduced uptake of [^{18}F]fluciclatide was observed after two days of therapy with Sunitinib [39]. Sunitinib is an antiangiogenic drug, and the results show that the decreased tumor uptake of [^{18}F]fluciclatide is specific. So far, clinical phase I and phase II trials have been carried out on cancer patients, although little data has been published yet. [^{18}F]Fluciclatide is suggested to be a safe PET tracer for clinical use and to be useful for detection of both primary and metastatic breast cancer lesions. The PET imaging study in a limited number of human subjects shows that [^{18}F]fluciclatide is quite stable in vivo and the blood clearance is rapid [32,39,40].

In addition to [^{18}F]fluciclatide, a number of other peptides have been conjugated with [^{18}F]FBA [34,37,41–43]. In general, the conjugations require low pH (e.g., pH 2.5 or below) and heating (60–70 °C) to improve reaction efficiency. Short peptides can withstand relatively harsh labeling conditions because peptides, in general, lack tertiary structure. However, in labeling of long peptides (proteins) the combination of low pH and elevated temperature becomes an issue. As an example, the initial attempts failed to conjugate [^{18}F]FBA with leptin due to precipitation of the aminooxy-functionalized leptin **17** at elevated temperature [41]. As mentioned above, aniline is a well-known catalyst for oxime formation [25,26,29]. Accordingly, the conjugation of functionalized leptin **17** with [^{18}F]FBA has been achieved in anilinium acetate buffer (pH 4.5, 100 mM) at 0 °C, the conversion being typically over 50% in 15 min of reaction (Scheme 6). The total synthesis time was about 120 min starting from the production of ^{18}F-fluoride. The specific activity of product **18** was 93–167 GBq/μmol and the radiochemical purity was over 95%. Notably, it was possible to use a small amount of protein **17** (30 nmol, <0.24 mM) in a typical labeling experiment. The labeled protein **18** had appropriate stability both in vitro and in vivo. High contrast in PET imaging with **18** was obtained in preclinical studies in *ob*/*ob* mice. Protein **18** bound specifically in the cortex of the kidney, which indicated that the linker and the ^{18}F-label did not markedly alter leptin's biological function [41].

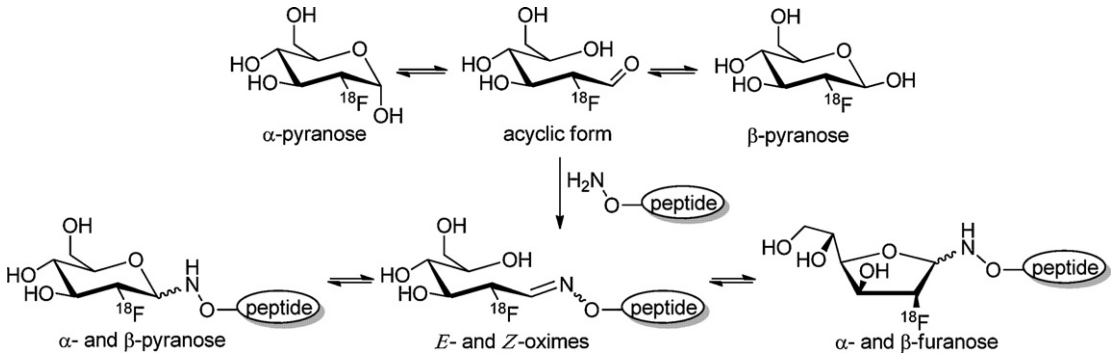
Use of a bifunctional linker, as exemplified by compound **19** (Scheme 7), provides an alternative method for labeling peptides and proteins at low concentration and under mild reaction conditions [^{18}F]FBA reacts with linker **19**, affording the formation of [^{18}F]FBABM exclusively in the *E*-configuration at the oxime bond. Subsequently [^{18}F]FBABM is conjugated to cysteine-containing peptides/proteins at pH 7.4 and room temperature [35,36,44]. To achieve site-specific labeling, only one chemically reactive thiol group should be present in the peptide/protein. Maleimide-thiol chemistry is a widely used method in conjugation chemistry [25]. In theory, when the thiol group attacks the C=C double bond from both sides in the maleimide, it results in two diastereomers **20** and **21**. However, no reports in the literature have described diastereomers that have been separately formulated for PET studies. The existence of stereoisomers may complicate the interpretation of imaging results; different isomers can possibly have different biodistribution patterns in vivo. In addition, the presence of different isomers becomes a potential issue when a PET tracer is developed for clinical use, as approved drugs are predominantly in single enantiomer/isomer form. In 1992–1993, the US Food and Drug Administration (FDA) and the European Union published guidelines for the approval of new stereoisomeric drugs [45,46]. The approval of drugs in a mixture of stereoisomeric forms is possible only if pharmacokinetics and pharmacodynamics of each stereoisomer have been fully assessed [47].

2.2. [^{18}F]FDG as aldehyde source

2-[^{18}F]Fluoro-2-deoxyglucose ([^{18}F]FDG) is practically available at every PET center worldwide which has provoked much interest in the use of [^{18}F]FDG as a prosthetic group for bioconjugations. [^{18}F]FDG is presumed to exist in both the cyclic



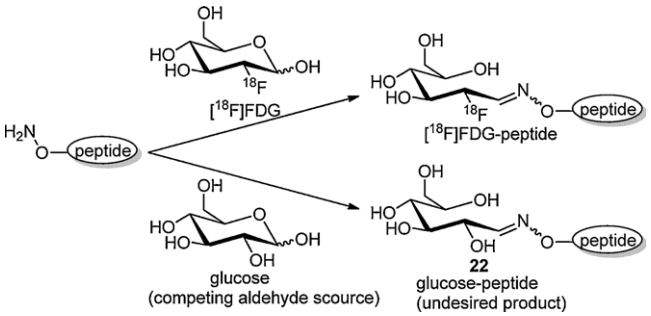
Scheme 7. Peptide labeling with [^{18}F]FBABM.



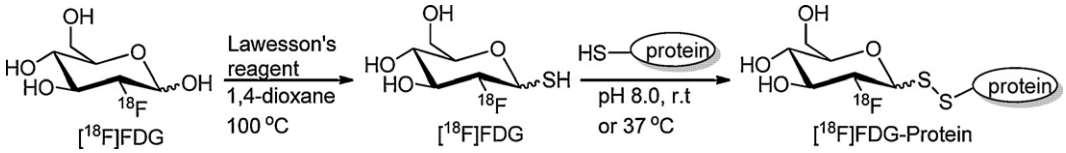
Scheme 8. Proposed product profile in [^{18}F]FDG conjugations with peptide [30,51].

and acyclic forms in solution, based on the knowledge of mutarotation of sugars [30]. The acyclic form of [^{18}F]FDG bears an aldehyde group available for oxime formation in the presence of an oxyamine-containing peptide (Scheme 8). Based on limited data from the literature, low pH and relatively high temperature are required in oxime formation with [^{18}F]FDG. Namavari et al. have conjugated a linear RGD peptide with [^{18}F]FDG in the presence of trifluoroacetic acid (0.4%) and ethanol (16%) in saline at 100 °C; the decay-corrected radiochemical yield was 27.5% [48]. According to a similar protocol, a cyclic RGD peptide and [^{18}F]FDG conjugate was obtained in 41% decay-corrected radiochemical yield [48]. In another case, the conjugation of a cyclo(RGDfK[(Boc)Aoa]) peptide with [^{18}F]FDG was performed in a mixture of water and DMSO at pH 2.5 and 130 °C. The duration of the conjugation reaction was typically 20 min and the decay-corrected radiochemical yield was between 56% and 93% [49]. Peptide conjugation with [^{18}F]FDG in a mixture of methanol and water was also successfully achieved using a reaction temperature of 80 °C. In a 30 min reaction, the radiochemical yield was 63% for the aminooxy-functionalized hexapeptide when the peptide concentration in the reaction mixture was 2.5 mg/mL (~2.9 mM) [30], a large molar excess with respect to [^{18}F]FDG. In all cases mentioned above, an organic solvent (ethanol, DMSO or methanol) was added to the conjugation reaction mixture. However, it is not clear whether the added organic solvent facilitated the conjugation or if it was used only to enhance the solubility.

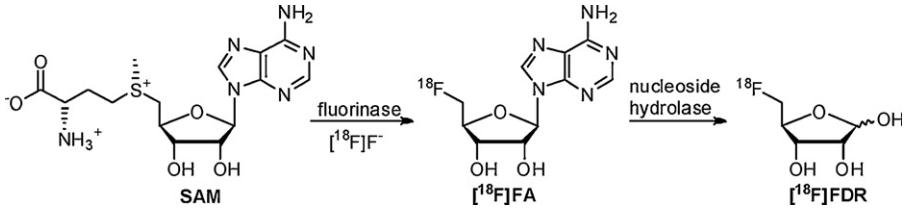
[^{18}F]FDG, routinely produced for clinical use, contains a significant amount of glucose [49]. Because glucose can also form an oxime with aminooxy-functionalized peptides, the conjugation reaction results in the formation of the undesired peptide conjugate **22** (Scheme 9). The conjugation of peptide with [^{18}F]FDG may even fail if too much glucose is present as a competing aldehyde source. Thus, [^{18}F]FDG must be separated



Scheme 9. Glucose is a competing aldehyde source in peptide conjugation with [^{18}F]FDG [49].



Scheme 10. Protein labeling with thionated [^{18}F]FDG [52].



Scheme 11. Enzymatic radiosynthesis of [^{18}F]FDR [55].

from glucose, particularly in scale-up experiments. Hultsch et al. have successfully used a YMC-Pack Polyamine II HPLC column to isolate [^{18}F]FDG for subsequent peptide labeling [49]. The conjugation of glucose-free [^{18}F]FDG with peptide was carried out in a solution of DMSO (10%) in water at 130 °C and pH 2.5.

Besides the pH value, reaction temperature, and the presence of competing glucose, the concentration of the peptide precursor can also influence the conjugation efficiency with [^{18}F]FDG. Concentrated peptide solutions result in more efficient conjugation [30], a trend which is sustained in the use of other types of ^{18}F-containing prosthetic groups [50]. In a comparison study, when the concentration of a peptide precursor was increased from 2.5 mg/mL to 7.5 mg/mL, the conversion was increased from 15% to 88% after a 30 min reaction at 80 °C [30]. HPLC or cartridge purification is usually required to purify ^{18}F-labeled peptides in order to achieve acceptable chemical purity. The product profile in [^{18}F]FDG conjugation is relatively complex, due to the presence of various forms of the sugar unit. According to the few published HPLC traces, at least three radioactive products can be detected with identical mass, according to mass spectroscopy analyses [30].

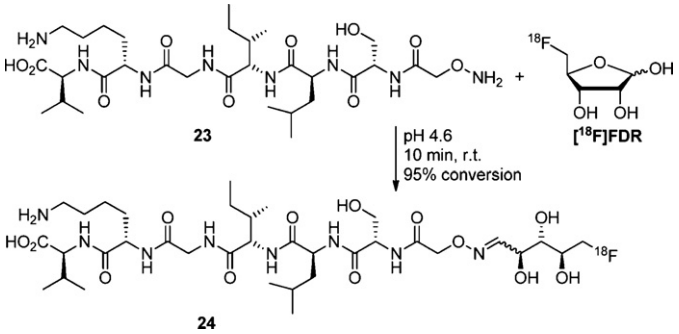
In addition to the direct use of [^{18}F]FDG for oxime formation, a number of methods have been developed to modify [^{18}F]FDG itself to some extent for subsequent labeling [51,52]. One interesting approach is to effect thionation of [^{18}F]FDG with Lawesson's reagent, and the thionated [^{18}F]FDG is subsequently conjugated site-specifically to cysteine-containing proteins by disulfide- and thioether bond formation (Scheme 10). This reaction sequence represents a novel and practical strategy for cysteine-containing protein ^{18}F-labeling and well-established protocols exist for generating single reactive cysteine residues in proteins [52].

2.3. 5-[^{18}F]Fluoro-5-deoxyribose ([^{18}F]FDR) as an aldehyde source

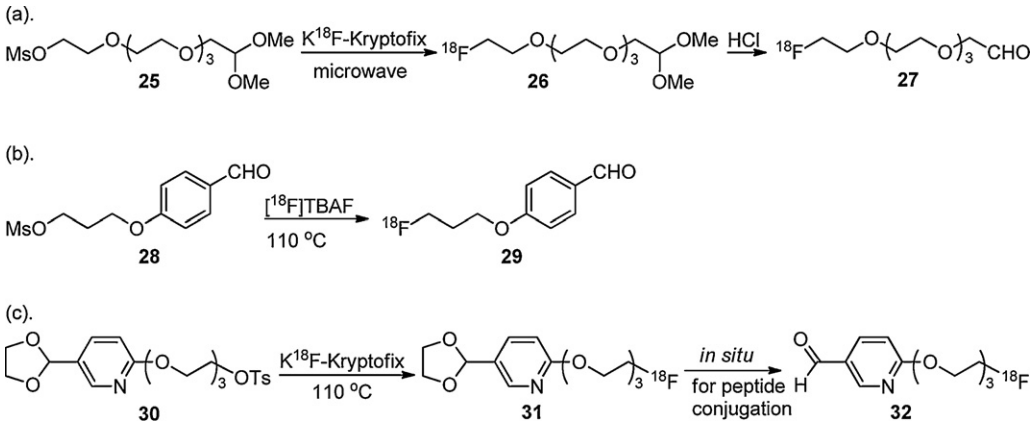
The incorporation of a carbohydrate unit into a peptide may improve in vivo pharmacokinetics for the peptide. A good example is the [^{18}F]galacto-RGD peptide which has been successfully used in clinical studies [53,54]. At present, however, broad clinical use of [^{18}F]galacto-RGD is hindered because of its complex labeling procedure [40]. Thus, it is important to search for alternative ^{18}F-labeled carbohydrates which are readily accessible and can be conjugated to peptides with ease.

A unique enzymatic protocol has been developed to produce 5-[^{18}F]fluoro-5-deoxyribose ([^{18}F]FDR) [55]. The fluorinase enzyme catalyzes direct nucleophilic fluorination, and *S*-adenosyl-L-methionine (SAM) is transformed into 5-[^{18}F]fluoro-5-deoxyadenosine ([^{18}F]FA) in the presence of no-carrier-added aqueous [^{18}F]fluoride (Scheme 11). Subsequently, the nucleoside hydrolase enzyme transforms [^{18}F]FA into [^{18}F]FDR. These two steps can be carried out in one pot and the reactions proceed at room temperature and near neutral pH. The use of no-carrier-added aqueous [^{18}F]fluoride for fluorination is important because the radiosynthesis can then be easily automated. Fluorinase-catalyzed ^{18}F-fluorination has great potential in the field of PET tracer development. A number of organic compounds have been labeled with fluorine-18 using this strategy [56–58].

As an extension of the previous work, 5-[^{18}F]fluoro-5-deoxyribose ([^{18}F]FDR) has been used as an efficient prosthetic group for peptide conjugation [59]. As an example, [^{18}F]FDR was conjugated with the aminooxy-functionalized hexapeptide agonist (compound **23**) of the human protease activated receptor 2 (PAR-2) in sodium acetate buffer (pH 4.6), with 95% conversion at room temperature in 10 min (Scheme 12). This reaction could also be conducted at pH 6.0. The investigators proposed that the location of the fluorine at C-5 of the 5-membered ring might facilitate the formation of the acyclic form of [^{18}F]FDR. To study the product profile, aminooxy-functionalized glutathione was used as a model peptide. NMR analyses indicated that the [^{18}F]fluororibose unit in the conjugate was exclusively in the ring-opened form, and the ratio of *E*- to *Z*-isomer was 4 to 1 in D_2O at room temperature. Accordingly, an automated radiosynthesis of [^{18}F]FDR was developed by using GE TracerLab module. ^{18}F-Fluorination of the tosylated precursor with K^{18}F-Kryptofix was followed by HCl-catalyzed hydrolysis. After a Chromabond cartridge purification, the radiochemical purity of [^{18}F]FDR was greater than 98%. The total synthesis time was 50 min and decay-corrected radiochemical yield was 35.0 ± 5% (n = 6).



Scheme 12. Peptide conjugation with [^{18}F]FDR as a prosthetic group [59].



Scheme 13. Other ^{18}F-containing aldehydes as prosthetic groups [33,61].

2.4. Other ^{18}F-aldehydes

Different prosthetic groups will induce different pharmacokinetics of the tracers in vivo [60]. For this reason, new prosthetic groups (e.g., ^{18}F-aldehydes) are continuously being developed. A mesylated compound **25** was used for nucleophilic fluorination in the presence of K^{18}F-Kryptofix with the aid of microwave heating, affording the formation of intermediate **26**. The subsequent hydrolysis of **26** gave aliphatic ^{18}F-aldehyde **27** (Scheme 13a). Compound **27** was conjugated to peptide **14** and the conjugate was used for tumor imaging. The compound **27** conjugated peptide demonstrated good tumor uptake and a good target to background ratio. A high proportion of renal excretion was also observed, at least partially due to the hydrophilic nature of prosthetic group **27** [33].

For nucleophilic fluorination reactions, K^{18}F-Kryptofix is a commonly used reagent, as this preparation protocol is well established at all PET centers. However, in some cases K^{18}F-Kryptofix does not work well. For example, attempts failed to transform compound **28** into aldehyde **29** in the presence of K^{18}F-Kryptofix in acetonitrile at 80 °C. Alternatively, *tert*-butyl ammonium [^{18}F]fluoride ([^{18}F]TBAF) was used for labeling. In the presence of [^{18}F]TBAF in DMSO using microwave heating at 110 °C for 5 min, compound **29** was obtained in 57% yield (Scheme 13b). Evaluation of the compound **29** conjugated peptide in vivo led to the proposal that compound **29** putatively increased hydrophobicity of the peptide, and thus influenced the overall excretion pattern of the PET tracer [33].

Compound **31** was prepared from precursor **30** by using a standard K^{18}F-Kryptofix protocol. The aldehyde group in **30** was protected to avoid side reaction during the ^{18}F-fluorination step [61]. The aldehyde protecting group was removed in situ, generating aldehyde **32** under the acidic reaction conditions for peptide conjugation, e.g., in TFA/ethanol/H_2O at 70 °C. The radiochemical yields varied from 21% to 35%, and synthesis time was from 71 to 137 min depending on if either solid phase extraction or an HPLC method was used for the purification. In some cases it was possible to reduce the amount of peptide precursors (<0.1 mg) in the conjugation reactions. As the radiosynthesis of **32** was straightforward and compound **32** might be less hydrophobic than [^{18}F]FBA, this protocol might be the method of choice in bioconjugations with prosthetic **32**.

3. Conclusions

In contrast to conventional organic synthesis, radiosynthesis of PET tracers has special challenges regarding radiosafety, radioprotection, and a limited availability of synthons. This review has focused on the currently available ^{18}F-containing aldehydes for peptide and protein labeling by oxime formation, giving a general account of the major strategies available for ^{18}F-labeling of peptides and proteins. So far, [^{18}F]FBA is probably the most frequently used aromatic ^{18}F-aldehyde for peptide conjugation, and the production of [^{18}F]FBA is feasible in most radiochemistry laboratories. Although minor concerns have appeared about the hydrophobic and volatile nature of [^{18}F]FBA, the [^{18}F]FBA conjugated peptide, [^{18}F]fluciclatide, has already found its way to clinical trials. Due to the ready availability of [^{18}F]FDG in every PET center, the labeling of peptides using [^{18}F]FDG as an aldehyde source is an attractive methodology that does not require derivatization. However, low pH and relatively high temperature are usually required to improve conjugation efficiency with [^{18}F]FDG. These conditions may limit the application of [^{18}F]FDG in labeling large proteins and antibodies with defined tertiary structures. Additionally, the relatively complex product profile in [^{18}F]FDG conjugations [51], is an issue to be addressed, particularly when maleimide-thiol chemistry (generating both *R*- and *S*-absolute configurations upon double bond addition) is used in combination with oxime formation (generating of *E*- and *Z*-configurations at the oxime bond). The newly identified prosthetic group [^{18}F]FDR can be produced in a facile manner. Based on the initial studies, [^{18}F]FDR has shown its potential for efficient conjugation with aminooxy-functionalized peptides under mild conditions, the product profile being simple and clear. In addition, few other ^{18}F-aldehydes have appeared for peptide labeling (Section 2.4). From a PET imaging point of view, it has not been possible to conclude which one is the superior prosthetic group for peptide labeling in general. Each prosthetic group may find its utility in a specific application for PET imaging, as evaluated by the individual preclinical and possible clinical imaging outcomes.

Oxime formation is one of the simplest chemical reactions that are amenable for automation. Automated production is mandatory in radiosynthesis due to radiosafety issues. Importantly, automated production ensures the quality of radiopharmaceuticals for clinical use according to Good Manufacturing Practice (GMP). PET imaging characteristics of a peptide tracer are determined not only by the peptide itself but also by the prosthetic groups used for labeling. Therefore, there is a need for novel ^{18}F-aldehydes that can be explored and evaluated in vivo, and contributions from mainstream fluorine chemists are obviously needed in this endeavor.

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