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Peptides Radiofluorination: Main Methods and Highlights

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Abstract

Peptides have an important role in organism and its high quantity present in tumors leading to development of radiolabeled peptides for tumor-specific imaging. Once the traditional methodologies used for radiofluorination do not work with peptides, due to their harsh conditions, other radiolabeling strategies had to be developed to supply the need. Direct radiofluorination is either an inefficient method, and the use of bidirectional groups, or prosthetic groups, is needed to enable the binding between the radionuclide fluorine-18 and a peptide functionalized. New peptides radiolabeling strategies have been developed sourcing increase the synthesis yield, its chemoselectivity, and the binding stability, and reduce the total process time and the number of steps required. The progress of radiofluorination methodologies led to development of the amidation, acylation, imidation, and alkylation techniques, the use of thiol groups, photochemical conjugation, chemoselective reactions, and "click chemistry", in addition to use of FDG molecule and heteroatoms as linkers. This paper presents the main strategies used for peptides radiofluorination, presenting their positive and negative points, and the prosthetic groups most used in each method.

Keywords

Radiofluorination, Radiolabeling, Peptides, Prosthetic Groups, Fluorine-18

1. Introduction

Peptides have an important role in organism, regulating growth, cellular function, and intercellular communication in normal and tumoral tissues, through receptors [1] [2] [3] [4]. In general, tumor cells present a higher quantity of receptors for peptides than normal cells, enabling the use of these peptides for visualization of tumors [2] [3] [5] [6] [7]. Because of that, the research of radiolabeled

peptides for tumor-specific imaging has increased in the last decades [5] [6] [7].

The use of radiolabeled peptides has advantages over other ligands like small molecules, proteins, and antibodies due to small size, has a favorable pharmacokinetics, with rapid clearance from blood and non-target tissues; they have high receptor binding affinity and high tumor penetration, with low toxicity and without immunogenic effects; they can have your structure easily modified to increase their affinity, modify their biodistribution profile or change the route of excretion; they can be easily synthetized with low costs and GMP grade, and easily radiolabeled enabling the formulation of synthesis kits [1]-[7]. Peptides have either a low biologic half-life by reason of their low stability to enzymatic degradation, that can be improved by molecular modification with introduction of unnatural aminoacids, amidation, acetylation, glycosylation and PEGylation, or by modification in the radiolabeling strategy with introduction of chelating agents [1] [2] [3] [5] [7] [8].

Radiolabeling of peptides is mainly represented by binding with radiometals, as the ease of the technique. Although there are several radiometals available, none of them has the ideal characteristics for the PET routine. The radionuclide that comes closest to the ideal is the ^{18}F, since its low positron energy (0.635 MeV), its half live of 109.8 minutes, and its clean decay profile (97%), leading to a high-resolution PET imaging [9] [10] [11] [12].

Direct labeling of peptides with ^{18}F is not possible. The traditional labeling method, nucleophilic substitution, demands extreme conditions, such as strong bases and high temperatures, that lead to denaturing of the peptide [2] [7] [11] [12]. The electrophilic substitution results in products with low specific activity, mainly due to the low regioselectivity of the ^{18}F-labeling position, leading to multiple isomers [11]. Because of that, the peptides labeling is only possible through bifunctional groups, also referred to as prosthetic groups [2] [7] [11] [12].

Prosthetic groups are bifunctional agents, with two different sites; one site allows labeling of radionuclide (^{18}F) while the other has functional groups that covalently bond with the peptide. These groups are developed to tolerate harsh conditions, such as high temperatures, being thermodynamically and kinetically stable and preventing the degradation of the radiolabeled product [2].

The use of a prosthetic group requires its previous synthesis, which adds multiple steps until the end of the radiolabeling process, increasing the total time required for the synthesis of the radiopharmaceutical and reduces its radiochemical yield [12]. Therefore, the optimal synthesis methodology must consist of two main steps: the first is a high yield labeling of a stable prosthetic group with ^{18}F; the second is a chemoselective coupling of the radiolabeled prosthetic group with a functionalized unprotected peptide [11].

Peptide functionalization is a strategy to increase the chemoselectivity and the stability of the prosthetic group-peptide linkage, directing where the covalent bond should be formed. Peptides can be functionalized with groups such as amine, aminooxy, hydrazine, alkyne, azide and others (**Figure 1**), depending on the synthesis strategy chosen [2].

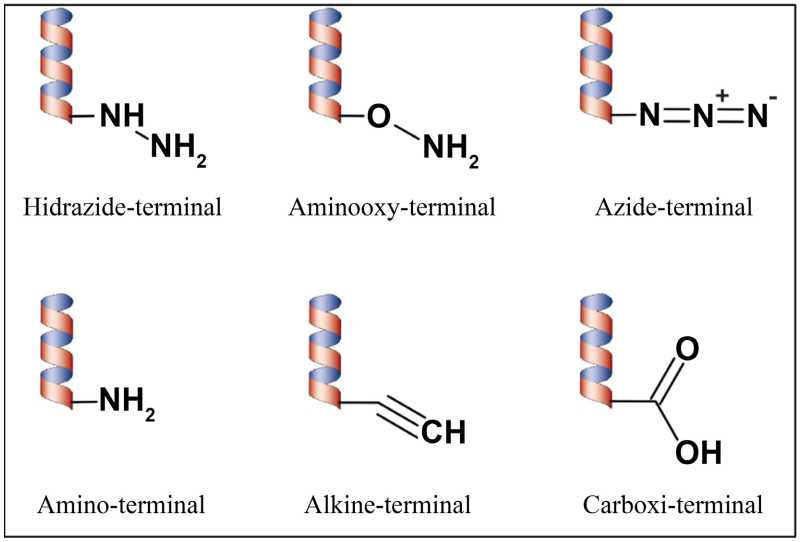


Figure 1. Groups used to peptide functionalization.

In the last decades, new peptides radiolabeling strategies have been developed, sourcing increase the synthesis yield, its chemoselectivity, and the binding stability, in addition to reducing the total process time and the number of steps required, always in search of the ideal methodology. This sourcing led to development of the amidation, acylation, imidation, and alkylation techniques, the use of thiol groups, photochemical conjugation, chemoselective reactions, and 'click chemistry', in addition to use of FDG molecule and heteroatoms as linkers.

2. Methods of Peptide Radiofluorination

The most traditional method for peptides radiofluorination is based on the nucleophilic groups present in peptides, as amidation, acylation and imidation [7] [12] [13]. The acylation method uses a radiolabeled prosthetic group with an activated ester and a primary amine on peptide [7] [11] [12]. Using an opposite approach, the amidation methodology uses a radiolabeled amine for coupling with an activated carboxylic group on the peptide, forming an amide group [7] [11]. Once nucleophilic groups such as amino, carboxyl, and hydroxyl, can be present in peptides, this method requires the protection of the amine groups on side chains [7] [13] [14]. In this regard, the addition of the radiolabeled and activated prosthetic group can be done during peptide synthesis, while the side chain groups are still protected, using the "solid phase radiolabeling approach" [11] [14]. The mainly eletrophic prosthetic groups that can be used in these methodologies are [^{18}F]fluorobenzoate ([^{18}F]SFB), 4-nitrophenyl 2-[^{18}F]fluoropropionate ([^{18}F]NPFP), N-succinimidyl 8-[4'-([^{18}F]fluorobenzyl)amino]suberate ([^{18}F]SFBS), 3-[^{18}F]fluoro-5-nitrobenzimidate ([^{18}F]FNB), and others [11] [15] [16]. Despite these methodologies present good conjugation yields and high in vivo stability, use of [^{18}F]fluoromethylbenzoates and other similar substituted aromatic compounds can result in decomposition, because of the linkage low stability, and use of deprotected groups on side chains can lead to undesired side reac-

tions [11] [17].

The alkylation is a similar method, also based on nucleophilic groups in peptides, that uses free sulfhydryl groups present in peptides, mainly in cysteine residues, with thiol-reactive prosthetic groups [2] [7] [11] [12] [15]. Once the natural sulfhydryl groups are not abundant in peptides, the use of thiol-reactive prosthetic groups enable the modification in specific sites of the peptides, to increase the chemoselectivity [11] [14]. In alkylation, the conjugation prosthetic group-peptide can be made using a labeled prosthetic group with a thiol-functionalized biomolecule or using labeled fluorothiol group with a haloacetylated peptide precursor, in a reverse strategy [11]. A prosthetic group generally used in alkylation methodology is 4-[^{18}F]fluorophenacyl bromide ([^{18}F]FPB) [16].

Thiol groups can be used in other fluorolabeling strategy based on thiol-maleimide coupling chemistry [2] [11] [16]. This approach uses the Michael addition chemistry to couple a maleimide reagent group with peptide containing a thiol and an amine group, resulting in highly effective products [11]. The prosthetic groups 1-[3-(2-[^{18}F]fluoropyridin-3-yloxy)propyl]pyrrole-2,5-dione ([^{18}F]FPyMe), and N-2-(4-[^{18}F]fluorobenzamido)ethymaleimide ([^{18}F]FBEM) are used in this methodology [2].

The labeling methodology of photochemical conjugation was based on well-established techniques used in biochemistry, such as photoaffinity labeling. This methodology uses compounds which generate reactives species after a photolysis reaction. In general procedures, phenylnitrenes can form substituted azepines by nucleophilic addition. To use this method for radiofluorination, the prosthetic group 4-azidophenacyl-[^{18}F]fluoride ([^{18}F]APF) is used. [^{18}F]APF irradiation with UV light of 365 nm, in the presence of peptides or proteins, results in a radiochemical conjugation product with high yields. However, once the prosthetic group APF has a lipophilic profile, the protein/peptide binding is affected, becoming highly unspecific. In addition, to achieve a satisfactory yield, high concentrations of the protein/peptide are required, improving the cost of the synthesis [11].

As the synthesis of the prosthetic groups used in the methodologies previously mentioned are multistep procedures, new methodologies sourcing simplifies the process and decrease the number of steps needed were developed [2] [11] [16].

The first developed strategy was chemoselective reactions. This strategy uses aldehydes, alkynes, or azides derivatives, labeled with ^{18}F, to bind to a hydroxyl amino (aminooxy) group present in a peptide, forming an oxime bond [2] [11] [18]. This methodology demonstrated to be a highly efficient and selective reaction, used for proteins, peptides, carbohydrates, and oligonucleotides fluorination [18]. In a similar strategy, the prosthetic group can be used to bind with a hydrazino-group (hydrazinonicotinic acid-NYNIC) present in the peptide, forming a hydrazone bond [11] [12] [19]. Some prosthetic groups used are 4-[^{18}F] fluorobenzaldehyde ([^{18}F]FBA) and (p-(di-t-butyl[^{18}F]fluorosilyl)benzaldehyde) ([^{18}F]SiFA-A) [2] [18]. All these groups are produced in one step synthesis, form an effective bond with excellent pharmacokinetic properties and allow the use of

unprotected peptides, just with an aminooxy functionalization [2] [11] [19]. Despite the high efficiency of this strategy, it is highly dependent on pH, peptide concentration, reaction time and temperature [11].

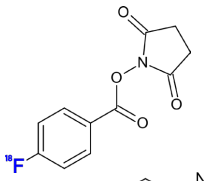
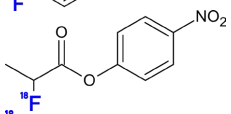
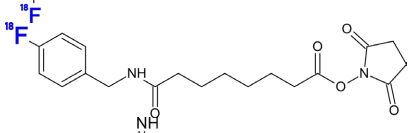
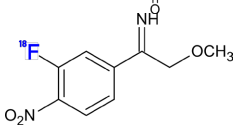
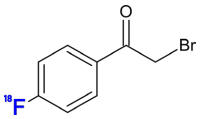
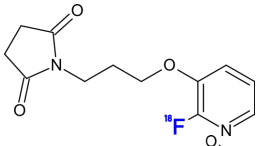
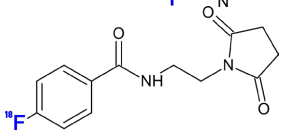
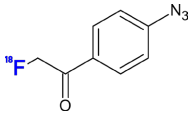
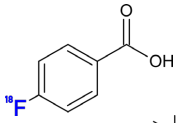
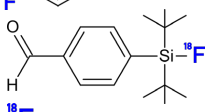
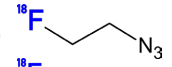
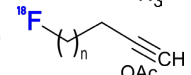
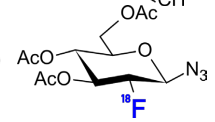
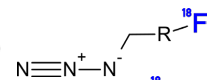
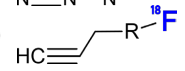
All methodologies and strategies discussed above have one problem in common: products with high lipophilicity. This characteristic leads to a high non-specific uptake in the liver, to a low tumor uptake and to a hepatobiliary excretion, that limit the use of the labeled product [2] [11] [20].

Another developed strategy to simplify the process and decrease the product lipophilicity was the Huisgen cycloaddition or "click chemistry". This reaction is a copper(I)-catalyzed Huisgen 1,3-dipolar cycloaddition (CuAAC) between azides and alkynes, a highly regioselective reaction resulting in 1,4-disubstituted 1,2,3-triazoles [7] [14] [16]. This methodology is a two-step approach that can be done through reaction of [^{18}F]fluoroalkynes with azido-functionalized peptides or of PEGylated [^{18}F]fluoroazides with alkynyl-functionalized peptides [14] [21]. The obtained products are relatively stable, with a large dipole moment, and with an increased polarity due the nitrogen atoms that can form hydrogen bonds [14]. Although the "click chemistry" was an efficient, stereospecific, and high-yielding technique, the copper cytotoxicity was a complicated obstacle [16] [22] [23]. The duration of the reaction transformation is high and may take hours to complete; to avoid this problem, the use of a microwave can be done [14].

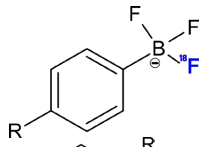
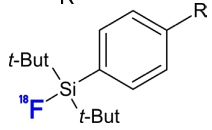
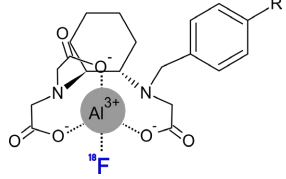
To reduce the lipophilicity of radiolabeling peptides, modification into peptide backbones can be done by introduction of sugar moieties, improving the pharmacokinetic profile of the radiopharmaceutical, with lipophilicity decreasing and leading to renal excretion, and without affecting the receptor affinity [2] [4]. For this strategy, glycosylation e polyethylene glycol (PEG) conjugations can be used [2]. In glycosylation, the radiopharmaceutical [^{18}F]fludeoxyglucose ([^{18}F]FDG) is used as a prosthetic group, in a highly selective reaction [24]. Beyond the advantages presented, the use of [^{18}F]FDG as a prosthetic group increases the peptide absorption, the quality of PET images, in view of the high tumor-tissue rate, and the metabolic stability [12] [25] [26] [27] [28]. The labeling occurs between an aldehyde function and an aminooxy group present in an unprotected peptide, forming an oxime bond [12] [24]. The reaction done in aqueous solution leads to an equilibrium between two mutarrotational forms of [^{18}F]FDG: α-ciclic and β-pyranose; an acyclic intermediary, with an aldehyde group is selectively used to the peptide labeling [12] [24] [29] [30]. Once a high quantity of peptide is required to achieve a satisfactory yield in this methodology, a modification in the [^{18}F]FDG structure can be done to increase the labeling yield with a lower peptide quantity: substitution of carbon-1 by a carbonyl group, remaining the closed structure of the [^{18}F]FDG backbone and avoiding the formation of isomeric structures [31] [32]. Although all the advantages presented, the principal negative point of this method is the difficulties presented to separate the glucose-peptide conjugate [12].

The newest methodology of radiofluorination used is a one-step labeling procedure using heteroatoms, such as B, Al and Si, and macrocyclic ligands [13]

Table 1. Strategies for peptides radiofluorination.

Methodologies	Ligants compounds	Structures
Acylation, Amidation, Imidation, (Prosthetic group with an activated ester and a primary amine)	(1) [^{18}F]fluorobenzoate ([^{18}F]SFB), (2) 4-nitrophenyl 2-[18F]fluoropropionate ([^{18}F]NPFP), (3) N-succinimidyl 8-[4'-([^{18}F]fluorobenzyl)amino]suberate ([^{18}F]SFBS), (4) 3-[^{18}F]fluoro-5-nitrobenzimidate ([^{18}F]FNB	(1)  (2)  (3)  (4) 
Alkylation (Uses free sulfhydryl groups)	(5) 4-[^{18}F]fluorophenacyl bromide ([^{18}F]FPB)	(5) 
Thiol groups (Based on thiol-maleimide coupling chemistry)	(6) 1-[3-(2-[^{18}F]fluoropyridin-3-yloxy) propyl]pyrrole-2,5-dione ([^{18}F]FPyMe), (7) N-2-(4-[^{18}F]fluorobenzamide)ethylmaleimide ([18F]FBEM	(6)  (7) 
Photochemical conjugation	(8) 4-azidophenacyl-[^{18}F]fluoride ([^{18}F]APF)	(8) 
Chemoselective reactions	(9) 4-[^{18}F]fluorobenzaldehyde ([^{18}F]FBA), (10) (p-(di-t-butyl[^{18}F]fluorosilyl)benzaldehyde) ([^{18}F]SiFA-A), (11) [^{18}F]fluoroethylazide, (12) [^{18}F]fluoroalkynes (13) [^{18}F]glycosyl azide	(9)  (10)  (11)  (12)  (13) 
Click chemistry	(14) [^{18}F]fluoroalkynes (15) [^{18}F]fluoroazides	(14)  (15) 

Continued

Heteroatoms (B, Al and Si, and macrocyclic ligands)	(16) cyclo-RGD-boronic ester (17) cyclo-RGD- BF_4^- (18) silicon-based fluoride acceptor (SiFA) (19) Aluminium-[^{18}F]fluoride ([^{18}F]AlF)	(16), (17)  (18)  (19) 

[33]. Initially, the radiolabeling with alkyltrifluoroborates and ^{18}F was developed, using aqueous solution and at room temperature [34]. The compound produced demonstrated to have hydrolytic stability and a rapid clearance [35] [36] [37]. However, the labeling step required a carrier fluoride, leading to low specific activities [38]. Therefore, other precursors were used, as cyclo-RGD-boronic ester and cyclo-RGD-BF4-, achieving high specific activities [39] [40].

Using Si, [^{18}F]fluorosilanes can be produced from sterically-hindered silanols and silyl hydrates, to be used in a one-step labeling procedure [41]. Peptides labeled with this methodology have an excellent hydrolytic stability, but with a lipophilic profile and a hepatobiliary excretion [42] [43] [44]. The silicon-based fluoride acceptor (SiFA) allows "one-step" and "two-steps" radiolabeling procedures, with a simple SPE purification, high specific activities, and radiochemical yields, but resulting in the same lipophilic profile [45] [46] [47]. The lipophilicity can be decreased by modification in the fluoride acceptor structure with addition of polyethylene glycol (PEG) spaces, carbohydrates, or quaternary ammonium function [48] [49].

The aluminum-[^{18}F]fluoride ([^{18}F]AlF) complex can be used for peptide radiolabeling. This methodology requires the use of macrocyclic ligands, such 2,2',2"-(1,4,7-triazacyclononane-1,4,7-triyl)triacetic acid (NOTA) and 1,4,7-triazacyclononane-1,4-diacetate (NODA), achieving more stable [^{18}F]AlF complexes and allowing to use elevate temperatures in the syntheses [33] [50]. The [^{18}F]AlF complex formation is highly pH dependent, with an optimal range between 4 and 5; acid and basic conditions leading to other compounds formation, to [^{18}F]HF and insoluble species, respectively. Besides that, metallic impurities can interfere in the yield reaction, requiring high purity reagents, and the use of co-solvents is obligatory, increasing the radiochemical yield and the solubility of [^{18}F]AlF complex [50]. This methodology forms thermodynamically stable and kinetically inert chelates, leading to very stable products, inclusive in physiological conditions, with radiochemical yields [44] [50].

Table 1 shows the main strategies for radiolabeling peptides with ^{18}F.

3. Conclusion

Several methodologies were developed looking for an ideal protocol for peptides radiofluorination, with short steps and favorable for the development research or pre-clinical and clinicals application procedures. Although there are many interesting strategies, efforts still have been made to achieve this objective, since the ideal method remains in the peptide profile and the nature of the aminoacids residues present in its structure. Modifications in the peptide structure can be necessary to have satisfactory radiolabeling and now the use of prosthetic groups has been the more attractive strategy to achieve increased radiochemical yields and specific activities. Besides, the nature of prosthetic groups can result in more stable bonds, increasing the physiological stability of the final compound and allowing the use of high temperatures in the steps synthesis on the conjugation peptide-prosthetic groups.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Ambrosini, V., Fani, M., Fanti, S., Forrer, F. and Maecke, H.R. (2011) Radiopeptide Imaging and Therapy in Europe. *Journal of Nuclear Medicine*, **52**, 42S-55S. https://doi.org/10.2967/jnumed.110.085753

[2] Jamous, M., Haberkorn, U. and Mier, W. (2013) Synthesis of Peptides Radiopharmaceuticals for the Therapy and Diagnosis of Tumor Diseases. *Molecules*, **18**, 3379-3409. https://doi.org/10.3390/molecules18033379

[3] Opalinska, M., Hubalewska-Dydejczyk, A. and Sowa-Staszczak, A. (2017) Radiolabeled Peptides: Current and New Perspectives. *The Quarterly Journal of Nuclear Medicine and Molecular Imaging*, **61**, 153-167. https://doi.org/10.23736/S1824-4785.17.02971-5

[4] Richter, S., Wuest, M., Bergman, C.N., Way, J.D., Krieger, S., Rogers, B.E. and Wuest, F. (2015) Rerouting the Metabolic Pathway of ^{18}F-Labeled Peptides: The Influence of Prosthetic Groups. *Bioconjugate Chemistry*, **26**, 201-212. https://doi.org/10.1021/bc500599m

[5] Fani, M., Maecke, H.R. and Okarvi, S.M. (2012) Radiolabeled Peptides: Valuable Tools for the Detection and Treatment of Cancer. *Theranostics*, **2**, 481-501. https://doi.org/10.7150/thno.4024

[6] Knight, L.C. (2003) Chapter 23: Radiolabeled Peptides for Tumor Imaging. In: Welch, M.J. and Redvanly, C.S., Eds., *Handbook of Radiopharmaceuticals: Radiochemistry and Applications*, Wiley, Chichester, 643-684. https://doi.org/10.1002/0470846380.ch23

[7] Wynendaele, E., Bracke, N., Stalmans, S. and Spiegeleer, B.D. (2014) Development

of Peptide and Protein Based Radiopharmaceuticals. *Current Pharmaceutical Design*, **20**, 2250-2267. https://doi.org/10.2174/1381612813196660663

[8] Li, X., Cai, H., Wu, X., Li, L., Wu, H. and Tian, R. (2020) New Frontiers in Molecular Imaging Using Peptide-Based Radiopharmaceuticals for Prostate Cancer. *Frontiers in Chemistry*, **8**, Article 583309. https://doi.org/10.3389/fchem.2020.583309

[9] McBride, W.J., Sharkey, R.M., Karacay, H., D'Souza, C.A., Rossi, E.A., Laverman, P., Chang, C.-H., Boergman, O.C. and Goldenberg, D.M. (2009) A Novel Method of ^{18}F Radiolabeling for PET. *Journal of Nuclear Medicine*, **50**, 991-998. https://doi.org/10.2967/jnumed.108.060418

[10] Van der Born, D., Pees, A., Poot, A.J., Orru, R.V.A., Windhorst, A.D. and Vugts, D.J. (2017) Fluorine-18 Labelled Building Blocks for PET Tracer Synthesis. *Chemical Society Reviews*, **46**, 4709-4773. https://doi.org/10.1039/C6CS00492J

[11] Wester, H.J. and Schottelius, M. (2007) Chapter 4: Fluorine-18 Labeling of Peptides and Proteins. In: Schubiger, P.A., Lehmann, L. and Friebe, M., Eds., *PET Chemistry: The Driving Force in Molecular Imaging. Ernst Schering Research Foundation Workshop*, Springer, Berlin, 79-111. https://doi.org/10.1007/978-3-540-49527-7_4

[12] Wuest, F., Hultsch, C., Berndt, M. and Bergmann, R. (2009) Direct Labelling of Peptides with 2-[^{18}F]Fluoro-2-Deoxy-D-Glucose ([^{18}F]FDG). *Bioorganic and Medicinal Chemistry Letters*, **19**, 5426-5428. https://doi.org/10.1016/j.bmcl.2009.07.108

[13] Chin, J. (2013) Methods for Carbon-11 and Fluorine-18 Labeling of Peptides as PET Radiopharmaceuticals: Direct Labeling with [^{11}C]Methyl Triflate on Cysteine Residues and [^{18}F]Fluoride on the Cationic Silicon-Based Fluoride Acceptor (SIFA) Moiety. Department of Chemistry, McGill University, Montreal.

[14] Marik, J. and Sutcliffe, J.L. (2006) Click for PET: Rapid Preparation of [^{18}F]Fluoropeptides Using CuI Catalyzed 1,3-Dipolar Cycloaddiation. *Tetrahedron Letters*, **47**, 6681-6684. https://doi.org/10.1016/j.tetlet.2006.06.176

[15] Guhlke, S., Coenen, H.H. and Stöcklin, G. (1994) Fluoroacylation Agents Based on Small N.C.A. [^{18}F]Fluorocarboxylic Acids. *Applied Radiation Isotopes*, **45**, 715-727. https://doi.org/10.1016/0969-8043(94)90252-6

[16] Li, Z.-B., Wu, Z., Chen, K., Chin, F.T. and Chen, X. (2007) Click Chemistry for ^{18}F-Labeling of RGD Peptides and Micropet Imaging of Tumor Integrin $Av\beta3$ Expression. *Bioconjugate Chemistry*, **18**, 1987-1994.https://doi.org/10.1021/bc700226v

[17] Wester, H.-J., Hamacher, K. and Stöcklin, G. (1996) A Comparative Study of N.C.A. Fluorine-18 Labeling of Proteins Via Acylation and Photochemical Conjugation. *Nuclear Medicine and Biology*, **23**, 365-372. https://doi.org/10.1016/0969-8051(96)00017-0

[18] Poethko, T., Schottelius, M., Thumshirn, G., Hersel, U., Herz, M., Henriksen, G., Kessler, H., Schwaiger, M. and Wester, H.-J. (2004) Two-Step Methodology for High-Yield Routine Radiohalohenation of Peptides: 18F-Labeled RGD and Octreotide Analogs. *Journal of Nuclear Medicine*, **45**, 892-902.

[19] Bruus-Jensen, K., Poethko, T., Schottelius, M., Hauser, A., Schwaiger, M. and Wester, H.-J. (2006) Chemoselective Hydrazone Formation between HYNIC-Functionalized Peptides and 18F-Fluorinated Aldehydes. *Nuclear Medicine and Biology*, **33**, 173-183.https://doi.org/10.1016/j.nucmedbio.2005.10.010

[20] Glaser, M. and Arstad, E. (2007) "Click Labeling" with 2-[^{18}F]Fluoroethylazide for Positron Emission Tomography. *Bioconjugate Chemistry*, **18**, 989-993. https://doi.org/10.1021/bc060301j

[21] Gill, H.S. and Marik, J. (2011) Preparation of ^{18}F-Labeled Peptides Using the Copper(I)-Catalyzed Azide-Alkyne 1,3-Dipolar Cycloaddition. *Nature Protocols*, **6**,

1718-1725. https://doi.org/10.1038/nprot.2011.390

[22] Ala, A., Walker, A.P., Ashkan, K., Dooley, J.S. and Schilsky, M.L. (2007) Wilson's Disease. *The Lancet*, **369**, 397-408. https://doi.org/10.1016/S0140-6736(07)60196-2

[23] Brewer, G.J. (2010) Copper Toxicity in the General Population. *Clinical Neurophysiology*, **121**, 459-460. https://doi.org/10.1016/j.clinph.2009.12.015

[24] Maschauer, S. and Prante, O. (2014) Sweetening Pharmaceutical Radiochemistry by ^{18}F-Fluoroglycosylation: A Short Review. *Biomed Research International*, **2014**, Article ID: 214748. https://doi.org/10.1155/2014/214748

[25] Albert, R., Marbach, P., Bauer, W., Briner, U., Fricker, G., Bruns, C. and Pless, J. (1993) SDZ CO 611: A Highly Potent Glycated Analog of Somatostatin with Improved Oral Activity. *Life Science*, **53**, 517-525. https://doi.org/10.1016/0024-3205(93)90703-6

[26] Haubner, R., Kuhnast, B., Mang, C., Weber, W.A., Kessler, H., Wester, H.-J. and Schwaiger, M. (2004) [^{18}F]Galacto-RGD: Synthesis, Radiolabeling, Metabolic Stability, and Radiation Dose Estimates. *Bioconjugate Chemistry*, **15**, 61-69. https://doi.org/10.1021/bc034170n

[27] Kihlberg, J. and Ahman, J. (1995) Glycosylated Peptide Hormones: Pharmacological Properties and Conformational Studies of Analogues of [1-Desamino 8-D-Arginine] Vasopressin. *Journal of Medical Chemistry*, **38**, 161-169. https://doi.org/10.1021/jm00001a021

[28] Schottelius, M., Wester, H.-J., Reubi, J.C., Senekowitsch-Schmidtke, R. and Schwaigner, M. (2002) Improvement of Pharmacokinetics of Radioiodinated Tyr^3-Octreotide by Conjugation with Carbohydrates. *Bioconjugate Chemistry*, **13**, 1021-1030. https://doi.org/10.1021/bc0200069

[29] Hultsch, C., Schottelius, M., Auernheimer, J., Alke, A. and Wester, H.-J. (2009) ^{18}F-Fluoroglucosylation of Peptides, Exemplified on *cyclo*(RGDFK). *European Journal of Nuclear Medicine and Molecular Imaging*, **36**, 1469-1474. https://doi.org/10.1007/s00259-009-1122-0

[30] Namavari, M., Cheng, Z., Zhang, R., De, A., Levi, J., Hoerner, J.K., Yaghoubi, S.S., Syud, F.A. and Gambhir, S.S. (2009) A Novel Method for Direct Site-Specific Radiolabeling of Peptides Using [^{18}F]FDG. *Bioconjugate Chemistry*, **20**, 432-436. https://doi.org/10.1021/bc800422b

[31] Price, N.P.J., Bowman, M.J., Gall, S.L., Berhow, M.A., Kendra, D.F. and Lerouge, P. (2010) Functionalized C-Glycoside Ketohydrazones: Carbohydrate Derivatives that Retain the Ring Integrity of the Terminal Reducing Sugar. *Analytical Chemistry*, **82**, 2893-2899. https://doi.org/10.1021/ac902894u

[32] Thapa, U. (2014) Fluoride-18 Labeling and Simultaneous Glycosylation of the Model Peptide Demobesin 1 by the Novel Prosthetic Group, Keto-[^{18}F]FDG. Ph.M. Dissertation, Rheinischen Friedrich-Wilhelms-Universität Bonn, Bonn.

[33] Allott, L., Pieve, C.D., Turton, D.R. and Smith, G. (2017) A General [^{18}F]ALF Radiochemistry Procedure on Two Automated Synthesis Platforms. *Reaction Chemistry and Engineering*, **2**, 68-74. https://doi.org/10.1039/C6RE00204H

[34] Ting, R., Adam, M.J., Ruth, T.J. and Perrin, D.M. (2005) Arylfluoroborates and Alkylfluorosilicates as Potential PET Imaging Agents: High-Yielding Aqueous Biomolecular 18F-Labeling. *Journal of the American Chemical Society*, **127**, 13094-13095. https://doi.org/10.1021/ja053293a

[35] Harwig, C.W., Ting, R., Adam, M.J., Ruth, T.J. and Perrin, D.M. (2008) Synthesis and Characterization of 2,6-Difluoro-4-Carboxyphenylboronic Acid and a Biotin

Derivative Thereof as Captors of Anionic Aqueous [^{18}F]-Fluoride for the Preparation of [^{18}F/^{19}F]-Labeled Aryltrifluoroborates with High Kinetic Stability. *Tetrahedron Letters*, **49**, 3152-3156. https://doi.org/10.1016/j.tetlet.2008.03.021

[36] Ting, R., Lo, J., Adam, M.J., Ruth, T.J. and Perrin, D.M. (2008) Capturing Aqueous [^{18}F]-Fluoride with an Arylboronic Ester for PET: Synthesis and Aqueous Stability of a Fluorescent [^{18}F]-Labeled Aryltrifluoroborate. *Journal of Fluorine Chemistry*, **129**, 349-358. https://doi.org/10.1016/j.jfluchem.2008.01.011

[37] Ting, R., Harwig, C.W., Lo, J., Li, Y., Adam, M.J., Ruth, T.J. and Perrin, D.M. (2008) Substituent Effects on Aryltrifluoroborate Solvolysis in Water: Implications for Suzuki–Miyaura Coupling and the Design of Stable ^{18}F-Labeled Aryltrifluoroborates for Use in PET Imaging. *Journal of Organic Chemistry*, **73**, 4662-4670. https://doi.org/10.1021/jo800681d

[38] Elizarov, A.M., Van Dam, R.M., Shin, Y.S., Kolb, H.C., Padgett, H.C., Stout, D., Shu, J., Huang, J., Daridon, A. and Heath, J.R. (2010) Design and Optimization of Coin-Shaped Microreactor Chips for PET Radiopharmaceutical Synthesis. *Journal of Nuclear Medicine*, **51**, 282-287. https://doi.org/10.2967/jnumed.109.065946

[39] Liu, Z., Li, Y., Lozada, J., Pan, J., Lin, K.-S., Schaffer, P. and Perrin, D.M. (2012) Rapid, One-Step, High Yielding ^{18}F-Labeling of an Aryltrifluoroborate Bioconjugate by Isotope Exchange at Very High Specific Activity. *Labelled Compounds and Radiopharmaceuticals*, **55**, 491-496. https://doi.org/10.1002/jlcr.2990

[40] Liu, Z., Li, Y., Lozada, J., Schaffer, P., Adam, M.J., Ruth, T.J. and Perrin, D.M. (2013) Stoichiometric Leverage: Rapid ^{18}F-Aryltrifluoroborate Radiosynthesis at High Specific Activity for Click Conjugation. *Angewandte Chemie International Edition*, **52**, 2303-2307. https://doi.org/10.1002/anie.201208551

[41] Mu, L., Höhne, A., Schubiger, P.A., Ametamey, S.M., Graham, K., Cyr, J.E., Dinkelborg, L., Stellfeld, T., Srinivasan, A., Voigtmann, U. and Klar, U. (2008) Silicon-Based Building Blocks for One-Step ^{18}F-Radiolabeling of Peptides for PET Imaging. *Angewandte Chemie International Edition*, **47**, 4922-4925. https://doi.org/10.1002/anie.200705854

[42] Höhne, A., Mu, L., Honer, M., Schubiger, P.A., Ametamey, S.M., Graham, K., Stellfeld, T., Borkowski, S., Berndorff, D., Klar, U., Voigtmann, U., Cyr, J.E., Friebe, M., Dinkelborg, L. and Srinivasan, A. (2008) Synthesis, ^{18}F-Labeling, and *in Vitro* and *in Vivo* Studies of Bombesin Peptides Modified with Silicon-Based Building Blocks. *Bioconjugate Chemistry*, **19**, 1871-1879. https://doi.org/10.1021/bc800157h

[43] Höhne, A., Yu, L., Mu, L., Reiher, M., Voigtmann, U., Klar, U., Graham, K., Schubiger, P.A. and Ametamey, S.M. (2009) Organofluorosilanes as Model Compounds for ^{18}F-Labeled Silicon-Based PET Tracers and Their Hydrolytic Stability: Experimental Data and Theoretical Calculations. *Chemistry—A European Journal*, **15**, 3736-3743. https://doi.org/10.1002/chem.200802437

[44] Laverman, P., McBride, W.J., Sharkey, R.M., Eek, A., Joosten, L., Oyen, W.J.G., Goldenberg, D.M. and Boerman, O.C. (2010) A Novel Facile Method of Labeling Octreotide with ^{18}F-Fluorine. *Journal of Nuclear Medicine*, **51**, 454-461. https://doi.org/10.2967/jnumed.109.066902

[45] Iovkova, L., Wängler, B., Schirrmacher, E., Schirrmacher, R., Quandt, G., Boening, G., Schürmann, M. and Jurkschat, K. (2009) Para-Functionalized Aryl-di-tert-butylfluorosilanes as Potential Labeling Synthons for ^{18}F Radiopharmaceuticals. *Chemistry—A European Journal*, **15**, 2140-2147. https://doi.org/10.1002/chem.200802266

[46] Schirrmacher, R., Bradtmöller, G., Schirrmacher, E., Thewsm, O., Tillmanns, J., Siessmeier, T., Buchholz, H.G., Bartenstein, P., Wängler, B., Neimeyer, C.M. and

Jurkschat, K. (2006) ^{18}F-Labeling of Peptides by Means of an Organosilicon-Based Fluoride Acceptor. *Angewandte Chemie International Edition*, **45**, 6047-6050. https://doi.org/10.1002/anie.200600795

[47] Schirrmacher, E., Wängler, B., Cypryk, M., Bradtmöller, G., Schäfer, M., Eisenhut, K.J. and Schirrmacher, R. (2007) Synthesis of P-(Di-Tert-Butyl[^{18}F]Fluorosilyl) Benzaldehyde ([^{18}F]SIFA-A) with High Specific Activity by Isotopic Exchange: A Convenient Labeling Synthon for the ^{18}F-Labeling of N-Amino-Oxy Derivatized Peptides. *Bioconjugate Chemistry*, **18**, 2085-2089. https://doi.org/10.1021/bc700195y

[48] Kostikov, A.P., Chin, J., Orchowski, K., Niedermoses, S., Kovacevic, M.M., Aliaga, A., Jurkschat, K., Wängler, B., Wängler, C., Wester, H.-J. and Schirrmacher, R. (2012) Oxalic Acid Supported Si-18F-Radiofluorination: One-Step Radiosynthesis of N-Succinimidyl 3-(Di-tert-butyl[^{18}F]fluorosilyl)benzoate ([^{18}F]SIFB) for Protein Labeling. *Bioconjugate Chemistry*, **23**, 106-114. https://doi.org/10.1021/bc200525x

[49] Wängler, B., Quandt, G., Iovkova, L., Schirrmacher, E., Wängler, C., Boening, G., Hacker, M., Schmoeckel, M., Jurkschat, K., Bartenstein, P. and Schirrmacher, R. (2009) Kit-Like ^{18}F-Labeling of Proteins: Synthesis of 4-(Di-tert-butyl[^{18}F]fluorosilyl)benzenethiol (Si[^{18}F]FA-SH) Labeled Rat Serum Albumin for Blood Pool Imaging with PET. *Bioconjugate Chemistry*, **20**, 317-321. https://doi.org/10.1021/bc800413g

[50] Archibald, S.J. and Allott, L. (2021) The Aluminium-[^{18}F]Fluoride Revolution: Simple Radiochemistry with a Big Impact for Radiolabelled Biomolecules. *EJNMMI Radiopharmacy and Chemistry*, **6**, Article No. 30. https://doi.org/10.1186/s41181-021-00141-0