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Fluoride-18 Radiolabeling of Peptides Bearing an Aminooxy Functional Group to a Prosthetic Ligand via an Oxime Bond

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Abstract

We have developed a novel F-18 prosthetic ligand named fluoro-PEG-benzaldehyde (FPBA) **1**. [¹⁸F]-FPBA **1** is formed in situ from its radiolabeled precursor [¹⁸F]**6**. Compound **6** is efficiently synthesized in four steps starting from commercially available 6-bromo-3-pyridine carbaldehyde **2**. [¹⁸F]-FPBA was evaluated as a prosthetic ligand to radiolabel three cyclic peptides bearing an aminooxy functional group at the *N*-terminus position. Acetal [¹⁸F]**6** is purified by either solid-phase extraction (SPE) or reverse-phase HPLC with the overall radiochemical yields (RCY) and radiochemical purity (RCP) in very close agreement. The SPE purification process has the advantage of shorter reaction times (71–87 min for entire reaction sequence), while the use of the reverse-phase HPLC purification process allows the use of up to fifty times less of the expensive synthetic peptides (~ 50 nmol) in the oxime coupling reaction. We have demonstrated an efficient methodology in the production of [¹⁸F]-FPBA **1** and demonstrated its use as a prosthetic ligand for the labeling of peptides possessing an aminooxy functional group.

Keywords

Fluorine-18; Prosthetic ligand; Aminooxy; Oxime; Peptide; PET

Positron emission tomography (PET) increasingly plays a major role in clinical diagnosis and basic research.¹ PET images can quantitatively detect changes in cellular functions and physiological pathways. Various PET ligands labeled with F-18 have been used to study specific receptors and transporter sites. Once bound to the target sites, the decay of positrons produces two high-energy photons (511 keV, 180° apart), which are detected by coincidence

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Supporting Information

Supporting information for the synthesis, HPLC, and MALDI of oximes **7-ox**, **8-ox**, and **9-ox** along with the radiosynthesis of prosthetic ligand [¹⁸F]**6**, oximes [¹⁸F]**7-ox**, [¹⁸F]**8-ox**, and [¹⁸F]**9-ox**, and the corresponding HPLCs are available free of charge via the internet.

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circuitry of a PET imaging scanner. PET imaging agents can be used on the picomolar scale while still maintaining high-resolution images.

Fluorine-18 is one isotope that is widely used for PET imaging due to its physical and nuclear characteristics.² There are two labeling methods used to substitute the fluorine-18 atom into a molecule. This process is traditionally accomplished by either electrophilic substitution or nucleophilic displacement.^{1–5} The use of the fluoride anion [¹⁸F]F[–], i.e. nucleophilic displacement, has the advantages of high specific activity^{3, 4} and more control over the site at which the fluoride anion will react. The conditions used for the S_N2 type reaction of [¹⁸F]F[–] are strongly basic, and are therefore not suitable for compounds containing acidic protons, such as those functional groups found in proteins and peptides. Prosthetic ligands, compounds containing a pre-existing fluorine-18 labeled moiety, are typically used to indirectly attach the fluorine-18 radionuclide onto a desired protein or peptide.^{1, 5}

There have been recent advances in the direct F-18 labeling of peptides^{6–10} that do not include the use of a prosthetic ligand. In one such case, Chen and coworkers were able to directly label a modified peptide in one step.¹⁰ The precursor contains a 4-NO₂-3-CF₃ arene moiety, with the nitro group acting as a leaving group and the 18F-fluoride displacing it through nucleophilic aromatic substitution. Although the reaction takes place in a timely manner (40 min), there are some drawbacks to this methodology, which include harsh reaction conditions that can denature a protein or peptide, incompatibility with certain functional groups (particularly alcohols), and the use of a relatively large amount (10 × molar excess) of starting material compared to our current method. Recently, a novel in situ labeling of peptides, based on the formation of Al-F bond via 1,4,7-triazacyclononane-Al peptide complexes, have been reported.¹¹ The Al-F bond is readily formed under a mild condition. Especially useful and unique, is the fact that the peptide-Al-¹⁸F complexes can be prepared without drying, i.e. directly in an aqueous solution.

The approach of anchoring a prosthetic ligand by way of an oxime bond has been previously reported in the literature with the use of aldehydes.^{12–14} This methodology takes advantage of the highly chemoselective nature of the oxime bond formation between aminoxy groups and aldehydes or ketones under an aqueous media. Several groups have quite efficiently used [¹⁸F]FB-CHO as their prosthetic ligand to be anchored on to a target molecule by way of an oxime bond.^{15–17} Although it is only one-step to produce [¹⁸F]FB-CHO in high RCY, the compound is volatile and can increase lipophilicity in the target ligand, altering the ligand's pharmacokinetics.¹⁸ Recently [¹⁸F]FDG has been reported to chemoselectively form an oxime bond with aminoxy groups,^{19–21} taking advantage of the fact that, at elevated temperature, FDG exists in equilibrium between its cyclic and linear forms. The ¹⁸F-FDG conjugated peptides have been successfully reported.

Previously, we described the synthesis and optimization of a novel F-18 prosthetic ligand **1** (Figure 1).²² Compound **1** was used to radiolabel two model aminoxy complexes, by way of oxime coupling, to provide the desired fluorine-18 radiotracers. The biodistribution in male ICR mice for both oxime labeled complexes were compared to that of the known β-amyloid plaque indicator, [¹⁸F]-AV-45, florbetapir.^{23, 24} These oximes showed promising results, but even with their larger size, they still retained some capability to cross the blood-brain barrier (BBB). We have demonstrated a general protocol for the fluorine-18 labeling with a new prosthetic ligand that is tolerant towards several functional groups and is formed via a chemoselective oxime coupling.^{25, 26} We herein report our findings on the use of our novel fluorine-18 prosthetic ligand, named [¹⁸F]-fluoro-PEG-benzaldehyde ([¹⁸F]-FPBA) **1**, towards labeling peptides bearing an aminoxy functional group. The peptides chosen for

this study are designed based on the amino acid sequence found in a known fibrin-binding MRI contrast-imaging agent, EP-2104R.²⁷

The synthesis of prosthetic ligand [¹⁸F]-FPBA **1** has been previously reported by our group.²² The synthesis begins with the protection of the aldehyde functionality of 6-bromo-3-pyridine carbaldehyde to afford acetal **2**²⁸ (Scheme 1). A pegylated chain is then added onto the molecule and the alcohol function group is transformed into tosylate **5**. The 'standard' fluorine-19 prosthetic precursor **6** is then prepared with the use of tetra-*n*-butylammonium fluoride hydrate in refluxing tetrahydrofuran. Fluorine **6** was used to determine the formation of the radiolabeled derivative by HPLC analysis.

Three cyclic peptides were purchased from Bachem (Figure 2). Each peptide has similarities to the reported fibrin-binding MRI contrast agent, EP-2104R.²⁷ All three of these synthetic peptides possess the aminooxy functionality at the *N*-terminus of the peptide sequence.

Each peptide was subjected to an acidic media (TFA) in the presence of ligand **6** (Scheme 2). The acidic media served to facilitate the de-blocking of the acetal functional group as well as promoting the formation of the oxime bond between the aldehyde and aminooxy groups. The reactions were monitored and purified by HPLC. The purified oxime was characterized by MALDI mass spectrometry (see Supplementary Data). It is important to note that the reactions conditions were tolerant of the disulfide bridge found in each cyclic peptide as determined from mass acquired from the MALDI mass spectrometry and the same reaction conditions were used to form the radiolabeled peptides.

With the formation of the desired oximes in hand, we next turned our attention to the labeling of these synthetic peptides with the use of our prosthetic ligand [¹⁸F]-FPBA **1**. The prosthetic precursor [¹⁸F]**6** was formed in one step from tosylate **5** (Scheme 3). It is extremely crucial in the radiolabeling step to have the aldehyde protected as the acetal to avoid side products.²² The aldehyde functionality is then de-blocked in situ during the oxime coupling reaction rendering [¹⁸F]-FPBA **1**.

The prosthetic ligand, compound **1**, was successfully coupled to each peptide (Scheme 4). The reaction conditions appear compatible with the rest of the functional groups located on the peptide, including the disulfide bridge. The reaction occurs in an ethanol/aqueous media facilitate with the use of trifluoroacetic acid (TFA). The acidic media de-blocks the acetal group and enables the oxime coupling reaction to occur. The final product is purified using an activated OASIS HLB 3cc cartridge (SPE). The crude sample is loaded onto the cartridge via an aqueous media. The cartridge is then flushed with ethyl acetate. This washing removes any of the un-reacted [¹⁸F]-FPBA **1**. The OASIS HLB 3cc cartridge is finally eluted with 1 % TFA/ethanol solution to provide the desired radiolabeled oxime peptide.

The prosthetic precursor [¹⁸F]**6** was tested in the coupling reaction with each peptide using two different purification methods. In the first purification process, the crude reaction mixture was loaded onto an OASIS HLB 3cc cartridge via water. This was then washed with water (3×), leaving the organics loaded onto the OASIS cartridge. The cartridge was then eluted with ethanol (0.7 mL) to provide [¹⁸F]**6**. This solution was then subjected to an aqueous acidic media (TFA/H₂O) at elevated temperatures (70 °C) using varying amounts of peptides, ranging from 2.5 – 5.0 mg, to form the desired radiolabeled oxime peptides (Table 1, entries 1 – 6). The overall reaction is very efficient and as depicted in Table 1, increasing the amount of peptide increases the overall RCY.

Due to the large amount of peptide needed with SPE purification for the oxime coupling, it was suggested to purify the prosthetic precursor through a reverse-phase HPLC. This method should remove other organic materials which have similar functional groups found

in [^{18}F]-FPBA **1** that would compete in the oxime coupling reaction. After the reaction was complete and cooled to room temperature, it was diluted in 2.5 mL of acetonitrile and injected onto a semi-prep HPLC (for conditions see Supplementary Data). The desired samples were collected and concentrated to a smaller volume (with the use of an OASIS HLB 3cc cartridge) in 1% TFA/ethanol solution. As little as 0.1 mg of peptide ($50 \times$ less) could then be used in the coupling reaction of prosthetic ligand [^{18}F]-FPBA **1**. From the data collected, similar RCY were achieved using the HPLC purification process as compared to the SPE purification (see Table 1). Excellent RCP were achieved with the use of a semi-prep HPLC; however the overall reaction time sequence had doubled, due to the use of an HPLC.

Similar to EP-2104R, the peptides selected for this conjugation studies have demonstrated high binding affinity to the fibrin clots. We expect that the ^{18}F -labeled peptides will be useful for PET imaging of specific binding of atherosclerosis in the myocardial blood vessels. Biological evaluations of these ^{18}F -peptides reported in this paper are currently ongoing.

In conclusion, we have demonstrated an effective method of radiolabeling aminooxy-functionalized peptides with a newly developed prosthetic ligand, [^{18}F]-fluoro-PEG-benzaldehyde ([^{18}F]-FPBA) **1**. The radiolabeled ligand, [^{18}F]**6**, was successfully synthesized in a total of four steps, starting with readily available 6-bromo-3-pyridine carbaldehyde **2**. The radiolabeling of tosylate **5** was achieved in high radiochemical yields ($71 \pm 2\%$, $n = 3$) with excellent radiochemical purity ($> 99\%$, based on HPLC analyses) in short reaction time (10 min). With the use of semi-preparative HPLC, small amounts (as little as 0.1 mg) of the peptide are needed to form the desired radiolabeled oxime in RCY similar to those obtained with the use of SPE purification on the prosthetic precursor, [^{18}F]**6**. The final radiolabeled peptide is purified by SPE, making this methodology very efficient and practical. The overall reaction sequence ranged from 71 – 137 min over two steps, depending on the purification method used for the ligand [^{18}F]**6**.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Tolmachev V, Stone-Elander S. *Biochem Biophys Acta*, Gen Subjects. 2010; 1800:487.
2. Cherry SR. *J Clin Pharmacol*. 2001; 41:482. [PubMed: 11361044]
3. Ametamey SM, Honer M, Schubiger PA. *Chem Rev*. 2008; 108:1501. [PubMed: 18426240]
4. Mach RH, Schwarz SW. *PET Clinics*. 2010; 5:131.
5. Kuhnast B, Dolle F. *Curr Radiopharm*. 2010; 3:174.
6. Hohne A, Mu L, Honer M, Schubiger PA, Ametamey SM, Graham K, Stellfeld T, Borkowski S, Berndorff D, Klar U, Voigtman U, Cyr JE, Friebe M, Dinkelborg L, Srinivasan A. *Bioconjugate Chem*. 2008; 19:1871.
7. Becaude J, Mu L, Karamkam M, Schubiger PA, Ametamey SM, Graham K, Stellfeld T, Lehmann L, Borkowski S, Berndorff D, Dinkelborg L, Srinivasan A, Smits R, Koksche B. *Bioconjugate Chem*. 2009; 20:2254.

8. Mu L, Hohne A, Schubiger PA, Ametamey SM, Graham K, Cyr JE, Dinkelborg L, Stellfeld T, Srinivasan A, Voigtmann U, Klar U. *Angew Chem, Int Ed.* 2008; 47:4922.
9. Roehn U, Becaud J, Mu L, Srinivasan A, Stellfeld T, Fitzner A, Graham K, Dinkelborg L, Schubiger AP, Ametamey SM. *J Fluorine Chem.* 2009; 130:902.
10. Jacobson O, Zhu L, Ma Y, Weiss ID, Sun X, Niu G, Kiesewetter DO, Chen X. *Bioconjugate Chem.* 2011; 22:422.
11. McBride WJ, Sharkey RM, Karacay H, D'Souza CA, Rossi EA, Laverman P, Chang CH, Boerman OC, Goldenberg DM. *J Nucl Med.* 2009; 50:991. [PubMed: 19443594]
12. Wescott, CR.; Nair, SA.; Kolodziej, A.; Beltzer, JP. Application: WO, WO Patent 2000-US20612, 2001009188. 2001. p. 114
13. Glaser M, Morrison M, Solbakken M, Arukwe J, Karlsen H, Wiggen U, Champion S, Kindberg GM, Cuthbertson A. *Bioconjugate Chem.* 2008; 19:951.
14. Carrasco MR, Alvarado CI, Dashner ST, Wong AJ, Wong MA. *J Org Chem.* 2010; 75:5757. [PubMed: 20704452]
15. Poethko T, Schottelius M, Thumshirn G, Herz M, Haubner R, Henriksen G, Kessler H, Schwaiger M, Wester HJ. *Radiochim Acta.* 2004; 92:317.
16. Poethko T, Schottelius M, Thumshirn G, Hersel U, Herz M, Henriksen G, Kessler H, Schwaiger M, Wester HJ. *J Nucl Med.* 2004; 45:892. [PubMed: 15136641]
17. Harki DA, Satyamurthy N, Stout DB, Phelps ME, Dervan PB. *Proc Natl Acad Sci U S A.* 2008; 105:13039. [PubMed: 18753620]
18. Colombo M, Bianchi A. *Molecules.* 2010; 15:178. [PubMed: 20110882]
19. Namavari M, Cheng Z, Zhang R, De A, Levi J, Hoerner JK, Yaghoubi SS, Syud FA, Gambhir SS. *Bioconjugate Chem.* 2009; 20:432.
20. Wuest F, Hultsch C, Berndt M, Bergmann R. *Bioorg Med Chem Lett.* 2009; 19:5426. [PubMed: 19665892]
21. Hultsch C, Schottelius M, Auernheimer J, Alke A, Wester HJ. *Eur J Nucl Med Mol I.* 2009; 36:1469.
22. Carberry P, Lieberman BP, Ploessl K, Choi SR, Haase DN, Kung HF. *Bioconjugate Chem.* 2011; 22:642.
23. Choi SR, Golding G, Zhuang Z, Zhang W, Lim N, Hefti F, Benedum TE, Kilbourn MR, Skovronsky D, Kung HF. *J Nucl Med.* 2009; 50:1887. [PubMed: 19837759]
24. Lin KJ, Hsu WC, Hsiao IT, Wey SP, Jin LW, Skovronsky D, Wai YY, Chang HP, Lo CW, Yao CH, Yen TC, Kung MP. *Nucl Med Biol.* 2010; 37:497. [PubMed: 20447562]
25. Adams JP. *J Chem Soc, Perkin Trans.* 2000:125.
26. Singh Y, Edupuganti OP, Villien M, Defrancq E, Dumy P. *C R Chim.* 2005; 8:789.
27. Overoye-Chan K, Koerner S, Looby RJ, Kolodziej AF, Zech SG, Deng Q, Chasse JM, McMurphy TJ, Caravan P. *J Am Chem Soc.* 2008; 130:6025. [PubMed: 18393503]
28. Zoppellaro G, Ivanova A, Enkelmann V, Geies A, Baumgarten M. *Polyhedron.* 2003; 22:2099.

Figure 1.

Novel prosthetic ligand [¹⁸F]-fluoro-PEG-benzaldehyde ([¹⁸F]-FPBA) **1**.

Scheme 1.

Synthetic pathway for the preparation of ligand **6**. Reagents and conditions: (i) ethylene glycol, *p*-TsOH, benzene, reflux (98 %); (ii) triethylene glycol, Cs_2CO_3 , DMF, microwave, 150 °C, 50 min (61 %); (iii) *p*-TsCl, Et_3N , DMAP, CH_2Cl_2 (82 %); (iv) TBAF. H_2O , THF, reflux (57 %).

Scheme 2.

General coupling between ligand **6** and peptides **7**, **8**, and **9**. Reagents and conditions: (i) peptide **7**, **8**, or **9**, TFA, EtOH/H₂O, 70 °C, 30 min.

Scheme 3.

Radiolabeling of ligand **[¹⁸F]6**. Reagents and conditions: (i) K[²²²]¹⁸F, K₂CO₃, ACN, 110 °C, 10 min (RCY = 71 ± 2 %; RCP = 99 ± 1 %; n = 3, for SPE purification).

Scheme 4.

General coupling between ligand [¹⁸F]**6** and peptides [¹⁸F]**7-ox**, [¹⁸F]**8-ox**, and [¹⁸F]**9-ox**.

Reagents and conditions: (i) TFA, EtOH/H₂O, 70 °C, 2 min, then peptide **7**, **8**, or **9**, EtOH/H₂O, 70 °C, 30 min.

Table 1

Comparison of the formation of oximes using different purification methods

entry	peptide	quantity (mg)	purification	% RCY [*]	% RCP [*]	time elapsed (min) [*]
1	8	2.5	SPE	21	97	79
2	8	3.0	SPE	28	96	74
3	8	5.0	SPE	32	98	80
4	7	3.0	SPE	25	97	71
5	7	5.0	SPE	35	93	78
6	9	3.0	SPE	26	90	87
7	8	<0.1	HPLC	24	> 99	137
8	7	<0.1	HPLC	25	> 99	127
9	9	<0.1	HPLC	21	> 99	135

0.8 mg of tosylate **5** used.^{*} RCY, RCP and time for entire radiolabeling sequence.