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Fluorine-18 Radiochemistry: A Novel Thiol-Reactive Prosthetic Group, [^{18}F]FBAMPy

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Abstract

A novel thiol-reactive bifunctional agent, an analogue of fluorobenzaldehyde-*O*-[6-(2,5-dioxo-2,5-dihydro-pyrrol-1-yl)-hexyl]oxime, (FBAM) has been synthesized. The new prosthetic group, [^{18}F]-FBAMPy, replaces the 4-fluorophenyl moiety with a 2-fluoropyridinyl moiety leading to increased polarity (FBAM analytical HPLC R_f = 6.4 min; FBAMPy R_f = 4.8 min) while retaining the sulfur-reactive pendant. By altering the polarity of the molecule, this new prosthetic group should have significant impact in coupling it with small peptides and other biomolecules.

Keywords

Radiochemistry, Prosthetic Group, PET, Fluorine-18

1. Introduction

Biomolecules are increasingly useful in the diagnosis of disease due to their known interactions *in vivo*. Diseases can be diagnosed or located by employing antibodies or antibody fragments that have been radiolabeled in combination with positron emission tomography (PET) [1]. However, labeling biomolecules such as peptides presents difficulties due to the number and diversity of the functional groups present. Direct incorporation of the short lived isotope fluorine-18 into biomolecules requires harsh reaction conditions which may destabilize sensitive biomolecules such as proteins, peptides, nucleotides, etc. [2]. In order to circumvent this difficulty, techniques for peptide labeling using prosthetic groups have been developed.

Prosthetic groups, also called bifunctional labeling agents, are small organic molecules that can be easily radiolabeled and then conjugated to sensitive biomolecules under very mild conditions. Generally, prosthetic groups target amines or thiols and in a very few cases carboxylic acids present in the biomolecule (**Figure 1**). A

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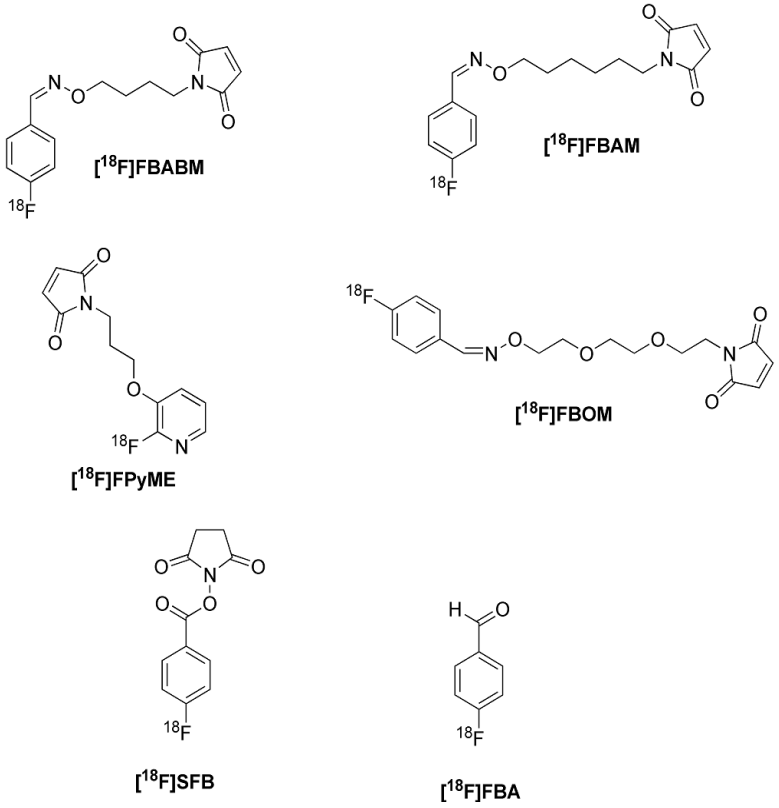
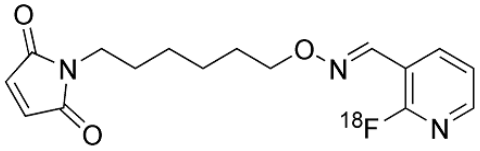


Figure 1. Various thiol- and amine-reactive bifunctional agents.

wide choice of prosthetic groups is necessary to accomplish radiolabeling quickly and successfully as there is a range of biomolecules known in the literature.

Amines are the most abundantly available functional groups in peptides or proteins and so they are commonly used to conjugate small ^{18}F-labeled bifunctional agents. *N*-Succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB) is widely used for conjugation with primary amines at physiological conditions [3]-[6]. However, the synthesis of [^{18}F]SFB is cumbersome, involving a laborious three-step process. Additionally, in some cases, the steric hindrance of *N*-terminal region of peptides has been known to cause threefold to fourfold lower radiochemical yields [7]-[9]. The unsuitability of amine reactive prosthetic groups for certain peptides has generated interest in developing thiol reactive prosthetic groups that promise to be more selective. Furthermore, thiol-reactive prosthetic groups react rapidly and require fewer equivalents of protein compared to amine reactive prosthetic groups [1] [10]. In addition to naturally occurring proteins containing cysteine residues, mutant proteins incorporating a single cysteine have been developed for labeling [11]. Numerous sulfur reactive prosthetic groups have been developed, that contain a maleimide unit which reacts with sulfur very rapidly and a pendant holding the radioactive isotope. [^{18}F]Fluorobenzaldehyde-*O*-(2-{2-[2-(pyrrol-2,5-dione-1-yl)ethoxy]-ethoxy}-ethyl)oxime ([^{18}F]FBOM) was found to be suited for labeling hydrophilic peptides under aqueous conditions [2]. [^{18}F]Fluorobenzaldehyde-*O*-[6-(2,5-dioxo-2,5-dihydro-pyrrol-1-yl)-hexyl]oxime ([^{18}F]FBAM) is well suited for high molecular weight molecules, however its lipophilicity causes it to be absorbed at undesired locations. [10] Both [^{18}F]FBOM and [^{18}F]FBAM have been used to label glutathione [2]. A four carbon variant of [^{18}F]FBAM, *N*-[4-[(4-[^{18}F]fluorobenzylidine)aminooxy]butyl]maleimide ([^{18}F]FBABM), is used for labeling Annexin V, which is used to detect apoptosis [12]. A maleimide ether with a pyridine unit, 1-[3-(2-[^{18}F]fluoropyridin-3-yloxy)propyl]pyrrole-2,5-dione ([^{18}F]FPyME), has been developed and tested on several peptides [13]. This work combines the six carbon chain heterobifunctional linker used in the synthesis of [^{18}F]FBAM with a pyridine moiety to create a new molecule, [^{18}F]FBAMPy (**1**), for the radiochemist's repertoire of prosthetic groups which may provide an alternative to [^{18}F]FBAM in cases where the lipophilicity of [^{18}F]FBAM leads to poor results.



[^{18}F]FBAMPy (1)

2. Results and Discussion

The preparations of the requisite maleimide precursor and the cold standard **7** of the title compound is described in the **Figure 2**. *tert*-Butyl *N*-[6-hydroxyloxyl)oxy]carbamate, **2**, was prepared according to the literature procedure [14]. The carbamate **2** was converted to the requisite bifunctional precursor **4** in two steps. The introduction of the maleimide group was accomplished *via* Mitsunobu reaction to obtain the maleimide derivative **3** in a 72% yield. Deprotection of the Boc group to obtain the precursor **4**, in quantitative yield, was carried out using HCl/EtOAc. 2-Fluoronicotinaldehyde, **6**, was prepared by the lithiation of 2-fluoropyridine, **5**, using (trimethylsilylmethyl)lithium, followed by formylation with *N*-formylpiperidine. Condensation of 2-fluoronicotinaldehyde, **6**, with maleimide precursor **4** in DMF provided the standard **7**.

Radiosynthesis to obtain the title compound, **1**, was performed in two steps both in a microreactor and in a vial starting from 2-bromonicotinaldehyde, **8** [**Figure 3**]. Thus aldehyde **8** was allowed to react with azeotropically dried [^{18}F]fluoride to generate 2-[^{18}F]fluoropyridine-3-carboxaldehyde, **9**, which was converted to [^{18}F]FBAMPy, **1**, by condensing with maleimide precursor **4** in the presence of methanolic-HCl. Precursor and isotope solutions are dispensed from storage loops on the NanoTek system by syringe pumps, pump 1 (P1) being the tracer precursor and pump 3 (P3) being the isotope solution. The operations of the NanoTek module have been further described in the literature [15]. Fluoride drying was performed in the concentrator module of the commercially available NanoTek system using drying macros. Azeotropically dried fluoride was dissolved in DMF. For both microfluidic and vial synthesis, the concentration of the precursor **8** is 4 mg in 1 mL of DMF. For microfluidic synthesis the optimum reaction parameters, utilizing the Discovery mode, were found to be T = 220˚C, combined flow rate = 90 µL/min, and P3:P1 ratio = 1:2. The incorporation yields at different flow rates are presented in **Table 1**. The yields are in the range of 24% - 53%. The microfluidic synthesis was completed by bubbling the solution from the reactor directly into a solution of 10 mg of the maleimide precursor **4** in methanol (1 mL) and 2*N* HCl (1 mL) in concentrator module 2, followed by heating the vial at 75˚C for 20 minutes.

Vial synthesis was performed by adding a solution of 2-bromonicotinaldehyde (4 mg in 1 mL DMF) to anhydrous fluoride in a vial and heating at 160˚C for 20 minutes. A 40% of incorporation of the isotope was observed. The condensation of labeled aldehyde **9** with the precursor **4** was accomplished under the same conditions as described previously. The final product was purified using semi-preparative HPLC followed by Sep-Pak separation to obtain the product in low boiling solvent for use in peptide labeling experiments. The identity of the product was confirmed by analytical HPLC and by co-elution with the standard (**Figure 4**; R_f = 4.8 min). The retention time for [^{18}F]FBAM, under identical HPLC conditions, is 6.4 minutes.

3. Materials and Methods

All chemicals and solvents were purchased from Aldrich and Acros and used as received. 2-Fluoronicotinaldehyde was prepared according to a literature procedure [16]. No-carrier-added [^{18}F]F^-, (740 - 1110 MBq, specific activity >74 GBq/µmole) produced from recycled [^{18}O]water, was obtained from PETNet (Knoxville, TN). Radio thin-layer chromatography was performed with radiation detection using a BioScan AR-2500 radio-TLC reader and WinScan 1.3 software. SilicaTLC plates to test completeness of fluorination of 2-bromonicotinaldehyde were developed in ethyl acetate. Analytical radio-HPLC analyses were performed on an Agilent 1200 series instrument employing a 254 nm UV detector and a Phenomenex C-18 column, 5 µm, 4.6 × 250 mm, using a 80:20 mixture of acetonitrile: 0.1*M* ammonium formate. Microfluidic fluorine-18 labeling was performed in 100 µm × 2 m reactors using the Discovery mode of the Advion Nano Tek Microfluidic Synthesis System controlled by Nano Tek LF 1.4 Software. Samples were collected in plastic, capped sample vials upon exit from the reactor for analysis. Vial labeling was performed in a 5 mL tapered bottom vial containing 4 mg/mL precursor in 1 mL DMF following the addition of the entire loop of dried fluoride in DMF.

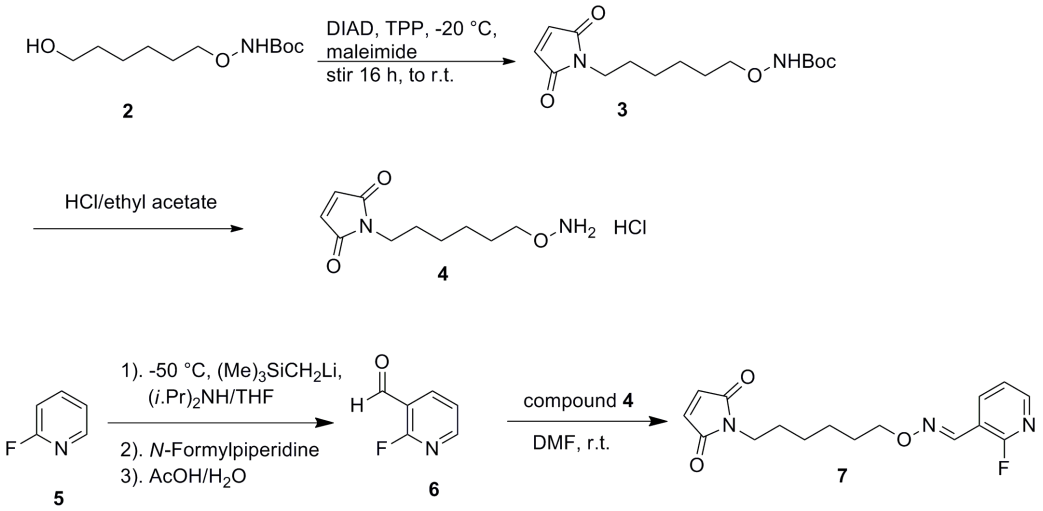


Figure 2. Synthesis of precursor, **4**, and nonradioactive FBAMPy, **7**.

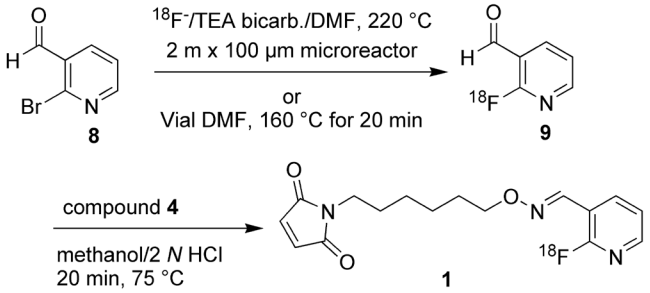


Figure 3. Radiosynthesis of [^{18}F]FBAMPy, **1**.

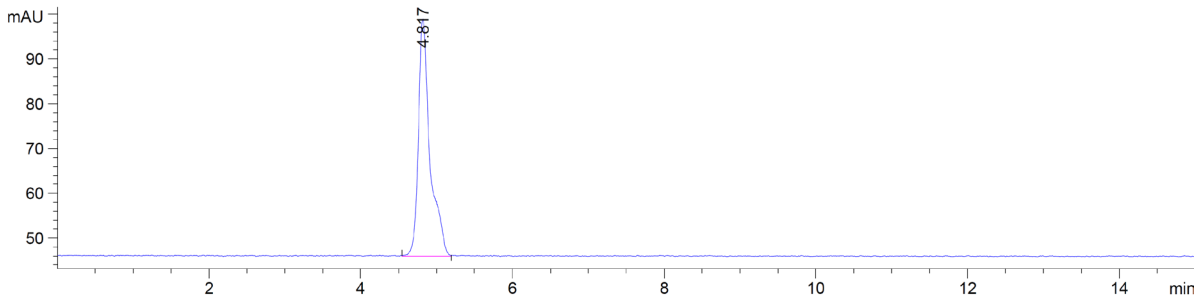


Figure 4. Radio-HPLC of **1**, after semi-preparative HPLC purification.

Table 1. Conditions for microfluidic labeling of 2-bromonicotinaldehyde, **8**, and yields of **9**.

Precursor Concentration (mg/mL)	Temperature (˚C)	P3 Flow Rate (µL/min)	P1 Flow Rate (µL/min)	Incorporation Yield (%)
4	220	10	10	32
4	220	30	60	53
4	220	40	40	48
4	220	50	50	48
4	220	50	150	46
4	220	30	90	47
8	220	60	30	24

3.1. 2-Fluoronicotinaldehyde

(Trimethylsilylmethyl)lithium (34.6 mL 0.7*M* solution in hexanes; 24.2 mmol) was added to a three necked 100 mL round bottomed flask along with a magnetic stir bar, under argon purge. After cooling to −50˚C, a solution of 2-fluoropyridine/THF/diisopropylamine (1.5 mL; 20.6 mmol/25 mL/0.13 mL; 0.93 mmol) was added over ~1 min. After stirring for 3 h, *N*-formylpiperidine (2.35 mL; 21.2 mmol) was added. The solution was stirred overnight, while allowing solution to warm to room temperature, and then quenched with acetic acid/water (3 mL/7 mL). The aqueous layer was separated, washed with ether and the combined organic layers dried over anhydrous $MgSO_4$ and the solvent removed to give brown oil. The product was purified using flash chromatography (15% ethyl acetate/hexane) to give 0.833 g 2-fluoronicotinaldehyde (32.4%). ^{1}H NMR (250 MHz, Chloroform-*d*) δ 10.33 (s, 1H), 8.49 (m, 1H), 8.33 (m, 1H), 7.42 (m, 1H).

3.2. 1-(6-(Aminooxy)Hexyl)-1*H*-Pyrrole-2,5-Dione Hydrochloride

Triphenylphosphine (4.5 g; 17.2 mmol) was dissolved in THF (20 mL) prior to addition of diisopropyl diazene-1,2-dicarboxylate (4.16 g; 20.6 mmol) at 0˚C. Additional THF (15 mL) was added if a solid formed. This solution was stirred for 30 minutes followed by addition of *tert*-butyl (6-hydroxyhexyl)oxycarbamate (4.00 g; 17.2 mmol) in THF (15 mL) at −20˚C. Subsequently, maleimide (1.66 g; 17.2 mmol) was added under an argon purge and the reaction was stirred for 16 h while warming to room temperature. Purification was accomplished by flash chromatography, first employing a 20% ethyl acetate/hexane eluent followed by a second flash chromatography column with 1% methanol/methylene chloride eluent. Deprotection using HCl in ethyl acetate gave the desired product. ^{1}H NMR (300 MHz, Chloroform-*d*) δ 6.96 (s, 2H), 3.96 (t, 2H), 3.50 (br), 3.34 (t, 2H), 1.47 (m, 4H), 1.22 (m, 4H).

3.3. 2-Fluoronicotinaldehyde *O*-(6-(2,5-Dihydro-1*H*-Pyrrol-1-yl)Hexyl) Oxime (FBAMPy)

2-Fluoronicotinaldehyde (31 mg; 0.25 mmol) and 1-(6-(aminooxy)hexyl)-1*H*-pyrrole-2,5-dione hydrochloride (42 mg; 0.17 mmol) were separately dissolved in 1 mL DMF, then mixed, and stirred for 30 minutes. This solution was then diluted with water to form a white, cloudy solution which was extracted with diethyl ether. The solution was washed with water and then concentrated to yield the product as a colorless oil. This sample was used for spectroscopy and elemental analysis as well as an authentic sample for coinjection verification. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.22 - 8.10 (m, 2H), 7.14 (dddd, J = 7.4, 5.0, 1.7, 0.7 Hz, 1H), 6.61 (s, 1H), 4.11 (t, J = 6.6 Hz, 2H), 3.45 (t, J = 7.3 Hz, 2H), 1.71 - 1.14 (m, 8H). Elemental analysis: Theory: C 60.18%; H 5.68%; N 13.16% Found: C 60.47%; H 5.64%; N 13.10%

3.4. Microfluidic Radiosynthesis of [^{18}F]FBAMPy

Cyclotron produced fluoride (~50 mCi) was trapped on a QMA cartridge and eluted with a tetraethylammonium bicarbonate solution and dried azetropically using the drying macro of the Advion Nano Tek System. Anhydrous fluoride was dissolved in dry DMF (0.5 mL) and loaded into a loop on pump 3. A solution of 2-bromonicotinaldehyde (2 mg in 0.5 mL of DMF) was loaded into a loop on pump 1. Using the batch mode, the reagents were allowed to react in a micro-reactor (2 m × 100 μ) at 220˚C with a combined flow rate of 90 μL/min. The labeled product, 2-[^{18}F]fluoronicotinaldehyde, from the reactor was delivered to a vial, in the second concentrator module, containing the precursor **4** (10 mg in 0.5 mL of methanol and 0.5 mL of 2*N* HCl). The resulting mixture was stirred at 75˚C for 20 min. The reaction mixture was diluted with water (10 mL) and passed through C_{18} Sep-Pak cartridge and the crude product was eluted with acetonitrile (2 mL). Semipreparative HPLC purification was performed using a Luna C_{18} column, 5 μ, 10 × 250 mm; 4 mL/min. (A: CH_3CN, B: 0.1 M ammonium formate; 0 - 5 min 40% A and 60% B; 5 - 15 min 30% A - 70% B; 15 - 30 min 70% A - 30% B). The fraction between 25 - 26 min was collected and diluted with water (10 mL) and passed through C_{18} Sep-Pak cartridge. The product was eluted from the cartridge using ethyl ether (2 mL) to obtain pure [^{18}F]FBAMPy (13 -17 mCi; 30% ± 4% decay corrected; n = 3). The radiochemical purity of the product was determined to be ≥95%. The total time to complete the synthesis was 80 min.

3.5. Vial Synthesis of [^{18}F]FBAMPy

To anhydrous fluoride obtained as described in the previous section (~50 mCi) was added a solution of 2-bro-

monicotinaldehyde (4 mg in 1 mL dry DMF) and heated in a sealed vial for 20 min at 160˚C. To the cooled reaction mixture was added a solution of **4** (10 mg in 0.5 mL of methanol and 0.5 mL of 2*N* HCl) and stirred at 75˚C for 20 min. The pure product was isolated and characterized as described above to obtain in a 20% decay corrected yield.

4. Conclusion

A novel, thiol-reactive prosthetic group [^{18}F]FBAMPy, has been designed by combining attributes of the known prosthetic groups [^{18}F]FBAM and [^{18}F]FPyME. The synthesis was carried out in a micro-reactor as well as in a vial. The observed decay corrected yields were 30% ± 4% and 20% respectively with a radiochemical purity of ≥95%.

Acknowledgements

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References

[1] Berndt, M., Pietzsch, J. and Wuest, F. (2007) Labeling of Low-Density Lipoproteins Using the F-18-Labeled Thiol-Reactive N-[6-(4-[F-18]fluorobenzylidene)aminooxyhexyl]maleimide. *Nuclear Medicine and Biology*, **34**, 5-15. http://dx.doi.org/10.1016/j.nucmedbio.2006.09.009

[2] Wuest, F., Kohler, L., Berndt, M. and Pietzsch, J. (2009) Systematic Comparison of Two Novel, Thiol-Reactive Prosthetic Groups for F-18 Labeling of Peptides and Proteins with the Acylation Agent Succinimidyl-4-[F-18]fluorobenzoate ([F-18]SFB). *Amino Acids*, **36**, 283-295. http://dx.doi.org/10.1007/s00726-008-0065-2

[3] Vaidyanathan, J., Vaidyanathan, T.K., Wang, Y. and Viswanadhan, T. (1992) Thermoanalytical Characterization of Visible-Light Cure Dental Composites. *Journal of Oral Rehabilitation*, **19**, 49-64. http://dx.doi.org/10.1111/j.1365-2842.1992.tb01590.x

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

[5] Zijlstra, S., Gunawan, J. and Burchert, W. (2003) Synthesis and Evaluation of a F-18-Labelled Recombinant Annexin-V Derivative, for Identification and Quantification of Apoptotic Cells with PET. *Applied Radiation and Isotopes*, **58**, 201-207. http://dx.doi.org/10.1016/S0969-8043(02)00302-0

[6] Wust, F., Hultsch, C., Bergmann, R., Johannsen, B. and Henle, T. (2003) Radiolabelling of Isopeptide N-epsilon-(gamma-glutamyl)-L-lysine by Conjugation with N-succinimidyl-4-[F-18]fluorobenzoate. *Applied Radiation and Isotopes*, **59**, 43-48. http://dx.doi.org/10.1016/S0969-8043(03)00161-1

[7] Yang, C.Y., Gu, Z.W., Yang, M.L., Lin, S.N., Garcia-Prats, A.J., Rogers, L.K., *et al*. (1999) Selective Modification of ApoB-100 in the Oxidation of Low Density Lipoproteins by Myeloperoxidase *in Vitro*. *Journal of Lipid Research*, **40**, 686-698.

[8] Fu, X.Y., Mueller, D.M. and Heinecke, J.W. (2002) Generation of Intramolecular and Intermolecular Sulfenamides, Sulfinamides, and Sulfonamides by Hypochlorous Acid: A Potential Pathway for Oxidative Cross-linking of Low-Density Lipoprotein by Myeloperoxidase. *Biochemistry-US*, **41**, 1293-1301. http://dx.doi.org/10.1021/bi015777z

[9] Hoppmann, S., Steiniger, B., Strobel, K., Haase, C. and Pietzsch, J. (2006) *In Vivo* Catabolism of Hypochlorite-Modified Low Density Lipoproteins (LDL): Insights from Small Animal Positron Emission Tomography Studies. In: Pietzch, J., Ed., *Research Developments in Pathological Biochemistry. Protein Oxidation and Disease*, Research Signpost, Trivandrum, 197-219.

[10] Kniess, T., Kuchar, M. and Pietzsch, J. (2011) Automated Radiosynthesis of the Thiol-Reactive Labeling Agent N-[6-(4-[F-18]fluorobenzylidene)aminooxyhexyl]maleimide ([18(F)]FBAM). *Applied Radiation and Isotopes*, **69**, 1226-1230. http://dx.doi.org/10.1016/j.apradiso.2011.03.043

[11] Kapty, J., Kniess, T., Wuest, F. and Mercer, J.R. (2011) Radiolabeling of Phosphatidylserine-Binding Peptides with Prosthetic Groups N-[6-(4-[F-18]fluorobenzylidene)aminooxyhexyl]maleimide ([F-18]FBAM) and N-succinimidyl-4-[F-18]fluorobenzoate ([F-18]SFB). *Applied Radiation and Isotopes*, **69**, 1218-1225. http://dx.doi.org/10.1016/j.apradiso.2011.05.012

[12] Li, X.H., Link, J.M., Stekhova, S., Yagle, K.J., Smito, C., Krohn, K.A., *et al*. (2008) Site-Specific Labeling of Annexin V with F-18 for Apoptosis Imaging. *Bioconjugate Chemistry*, **19**, 1684-1688. http://dx.doi.org/10.1021/bc800164d

[13] de Bruin, B., Kuhnast, B., Hinnen, F., Yaouancq, L., Amessou, M., Johannes, L., *et al*. (2005) 1-[3-(2-[F-18]fluoropyridin-3-yloxy)propyl]pyrrole-2,5-dione: Design, Synthesis, and Radiosynthesis of a New [F-18]fluoropyridine-based Maleimide Reagent for the Labeling of Peptides and Proteins. *Bioconjugate Chemistry*, **16**, 406-420. http://dx.doi.org/10.1021/bc0497463

[14] Jones, D.S., Hammaker, J.R. and Tedder, M.E. (2000) A Convenient Synthesis of N-(tert-butyloxycarbonyl)aminooxy Ethers. *Tetrahedron Letters*, **41**, 1531-1533. http://dx.doi.org/10.1016/S0040-4039(99)02331-X

[15] Lu, S., Giamis, A.M. and Pike, V.W. (2009) Synthesis of [18F]Fallypride in a Micro-Reactor: Rapid Optimization and Multiple-Production in Small Doses for Micro-PET Studies. *Current Radiopharmaceuticals*, **2**, 49-55. http://dx.doi.org/10.2174/1874471010902010049

[16] Woltermann, C.J. and Sutton, D.E. (2006) Using Alkylmetal Reagents for Directed Metalation of Azaaromatics. US Patent No. 7638635 B2.