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ORIGINAL ARTICLE

Systematic comparison of two novel, thiol-reactive prosthetic groups for ^{18}F labeling of peptides and proteins with the acylation agent succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB)

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Abstract A systematic comparison of 4-[^{18}F]fluorobenzaldehyde-*O*-(2-{2-[2-(pyrrol-2,5-dione-1-yl)ethoxy]-ethoxy}-ethyl)oxime ([^{18}F]FBOM) and 4-[^{18}F]fluorobenzaldehyde-*O*-[6-(2,5-dioxo-2,5-dihydro-pyrrol-1-yl)-hexyl]oxime ([^{18}F]FBAM) as prosthetic groups for the mild and efficient ^{18}F labeling of cysteine-containing peptides and proteins with the amine-group reactive acylation agent, succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB), is described. All three prosthetic groups were prepared in a remotely controlled synthesis module. Synthesis of [^{18}F]FBOM and [^{18}F]FBAM was accomplished via oxime formation through reaction of appropriate aminooxy-functionalized labeling precursors with 4-[^{18}F]fluorobenzaldehyde. The obtained radiochemical yields were 19% ([^{18}F]FBOM) and 29% ([^{18}F]FBAM), respectively. Radiolabeling involving [^{18}F]FBAM and [^{18}F]FBOM was exemplified by the reaction with cysteine-containing tripeptide glutathione (GSH), a cysteine-containing dimeric neurotensin derivative, and human native low-density lipoprotein (nLDL) as model compounds. Radiolabeling with the acylation agent [^{18}F]SFB was carried out using a dimeric neurotensin derivative and nLDL. Both thiol-group reactive prosthetic groups show significantly better labeling efficiencies for the peptides in comparison with the acylation agent [^{18}F]SFB. The obtained results demonstrate that [^{18}F]FBOM is especially suited for the labeling of hydrophilic cysteine-containing peptides, whereas [^{18}F]FBAM shows superior labeling performance for higher molecular weight compounds as exemplified for nLDL apolipoprotein constituents. However, the acylation agent [^{18}F]SFB is the preferred prosthetic group for labeling nLDL under physiological conditions.

Keywords ^{18}F-labeled prosthetic groups · Peptides · LDL · Apolipoproteins · Positron emission tomography (PET)

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Introduction

Radiolabeled peptides and proteins, including antibodies and antibody fragments, have been the subject of intense research efforts for targeted diagnostic imaging and radiotherapy in nuclear medicine over the last 30 years. Peptides and proteins play an important role as key regulators of cell growth and cellular function in living organisms. The ongoing interest in radiolabeled peptides and proteins as specific molecular probes mainly stems from the elevated numbers of high affinity receptors for peptides and proteins as found in many neoplastic and inflammatory tissues.

The use of radiolabeled peptides and proteins for receptor imaging and tumor targeting in vivo has become an established method in diagnostic nuclear medicine. Tumor visualization through specific peptide receptor targeting started about 15 years ago when radiolabeled somatostatin analogs were introduced into nuclear medicine for in vivo imaging of human tumors (Krenning et al. 1993). To date, hundreds of radiolabeled peptides have been synthesized and tested. Many radiolabeled peptides have been introduced into clinics, making the radiolabeled peptide approach as one of the most promising fields in diagnostic nuclear oncology. Moreover, various peptide- and protein-based radiotherapeutics, such as ^{90}Y-rituximab (Zevalin®), ^{131}I-tositumomab (Bexxar®) and the somatostatin receptor

binding ^{90}Y-DOTATOC, have been successfully introduced into oncological endoradionuclide therapy (Bodei et al. 2004; Chinn et al. 1999; de Jong et al. 1997; Goldenberg 2001; Jacene et al. 2007; Wiseman et al. 1999). Other applications of radiolabeled peptides and proteins in nuclear medicine and related fields aim at the visualization of inflammatory processes; for example, atherosclerotic plaque formation or progression of acute synovitis in rheumatoid arthritis (Carrió et al. 1998; Jamar et al. 1997).

The majority of peptides and proteins for molecular imaging purposes using single photon computed tomography (SPECT) or positron emission tomography (PET) have been labeled with γ- or β^+-emitting radiometals (e.g., ^{99m}Tc, ^{111}In, ^{68}Ga, ^{64}Cu) or radioiodine isotopes (e.g., ^{123}I, ^{124}I). Recently, a paper by Cai et al. and a subsequent perspective referring to the latter report, have emphasized the importance of ^{18}F as an ideal radionuclide for immuno-PET with antibody fragments, especially for routine clinical PET imaging (Cai et al. 2007; Shively 2007).

Despite the obvious advantages of ^{18}F like its ease of production at high specific activity, low β^+ energy (0.64 MeV) and favorable half-life (109.8 min), direct no-carrier-added (n.c.a.) ^{18}F-labeling of peptides and proteins with (^{18}F]fluoride is usually not possible, albeit a recent report claims that direct labeling of peptides with [^{18}F]fluoride seems to be possible (Becaud et al. 2007). Radiolabeling reactions with n.c.a. [^{18}F]fluoride require quite harsh reaction conditions which will cause denaturation of sensitive compounds like peptides and proteins.

For these reasons, peptide and protein labeling with ^{18}F is accomplished by means of prosthetic groups, also referred to as bifunctional labeling agents. The used prosthetic groups differ in the complexity of their radiosynthesis, labeling yield, and efficiency and chemoselectivity of conjugation to biomacromolecules. The conjugation of the prosthetic group can either be accomplished via acylation, amidation and imidation of amine groups, alkylation of thiol groups, photochemical conjugation and chemoselective reactions like formation of oximes and hydrazones (Wester and Schottelius 2007 and references therein), or more recently, click chemistry (Glaser et al. 2007; Li et al. 2007; Marik and Sutcliffe 2006; Ramenda et al. 2007).

Among the arsenal of available prosthetic groups for ^{18}F-labeling of peptides and proteins, the acylation agent, succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB), is the most widely and most frequently used prosthetic group (Vaidyanathan and Zalutsky 2006; Wester et al. 1996; Wuest et al. 2003). The laborious and time-consuming radiosynthesis of [^{18}F]SFB has been improved over the years, and several remotely controlled synthesis protocols have been reported, making [^{18}F]SFB more readily available today. However, despite the enormous improvements in terms of synthesis and availability, in some instances the use of the amine-reactive agent [^{18}F]SFB for peptide or protein labeling may not be optimal, or can even fail. Labeling with [^{18}F]SFB often requires fairly large amounts of peptide or protein to achieve sufficient radiochemical yields, which is not always applicable. Moreover, steric hindrance of amine groups may also limit the scope of [^{18}F]SFB.

With respect to a regio- and chemoselective radiolabeling of peptides and proteins, fine-tuning of reaction conditions and/or the application of novel, site-specific labeling agents are required. In this line, thiol-reactive prosthetic groups are particularly attractive labeling agents (Berndt et al. 2007; de Bruin et al. 2005; Dolle et al. 2003; Shiue et al. 1989; Toyokuni et al. 2003). Free thiol groups, which are not typically found in most peptides and proteins, enable site-specific modifications by means of thiol-reactive prosthetic groups.

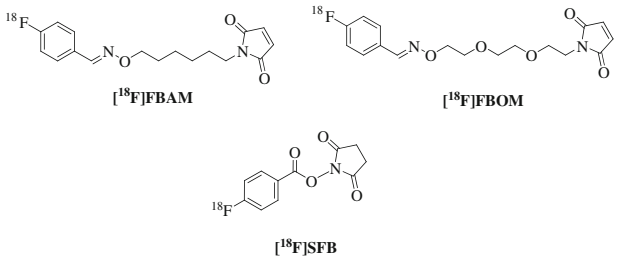
In this report, we present scopes and limitations of two thiol-reactive ^{18}F-labeled prosthetic groups ([^{18}F]FBAM and [^{18}F]FBOM) developed in our laboratory for peptide and protein conjugation in comparison with the most prominent prosthetic group, the amine-reactive acylation agent, [^{18}F]SFB (Scheme 1).

Radiolabeling with [^{18}F]FBAM and [^{18}F]FBOM was exemplified by the reaction with cysteine-containing tripeptide glutathione (GSH), a cysteine-containing dimeric neurotensin derivative, and human native LDL (nLDL). Radiolabeling with [^{18}F]SFB was performed with a dimeric neurotensin derivative and nLDL.

Materials and methods

General

All reagents and solvents were purchased from commercial suppliers and used without further purification unless otherwise specified. Nuclear magnetic resonance spectra were recorded on a Varian Unity 400 MHz spectrometer. ^{1}H and ^{13}C chemical shifts are given in ppm and were referenced with the residual solvent resonances relative to



Scheme 1 Prosthetic groups: [^{18}F]FBAM, [^{18}F]FBOM, and [^{18}F]SFB

tetramethylsilane (TMS). Mass spectra were obtained on a Quattro/LC mass spectrometer (MICROMASS) by electrospray ionization. MALDI-TOF mass spectra were recorded on a Bruker autoflex II TOF/TOF mass spectrometer. Column chromatography was performed on MERCK silica gel (mesh size 230–400 ASTM). Reactions were monitored by thin-layer chromatography (TLC) on Merck silica gel F-254 aluminum plates, with visualization under UV (254 nm). Melting points were determined with a Galen IIITM melting point apparatus from Cambridge Instruments. Elemental analyses were conducted using a LECO CHNS-932 apparatus. Cysteine-containing neurotensin dimer **7**, neurotensin dimer **8**, FBAM-7, and 4-*N*,*N*,*N*-trimethylammonium benzaldehyde triflate were prepared according to literature procedures (Berndt et al. 2007; Hultsch et al. 2007). Radiosyntheses of [^{18}F]FBAM and [^{18}F]SFB were accomplished in a remotely controlled synthesis module as previously described (Berndt et al. 2007; Mäding et al. 2005).

Chemical syntheses

*2-[2-[Iodoethoxy)ethoxy]ethanol (**2**)*

A solution of 2-[2-(chloroethoxy)ethoxy]-ethanol **1** (10.6 g, 63.0 mmol) and NaI (19.0 g, 126 mmol) was refluxed in acetone (60 ml) for 19 h. The solvent was evaporated under reduced pressure. The residue was re-dissolved in a small amount of water. After extraction with ethyl ether, the organic layer was washed with $NaHSO_3$ and dried over Na_2SO_4. The solvent was removed under reduced pressure to yield 13.9 g (85%) of the desired compound **2** as a pale yellow oil. ^{1}H-NMR ($CDCl_3$, 400 MHz): $\delta = 3.27$ (t, J = 6.8 Hz, 2 H, CH_2), 3.61–3.64 (m, 2 H, CH_2), 3.65–3.70 (m, 4 H, CH_2), 3.73–3.78 (m, 4 H, CH_2).

*Tert.-Butyl-N-[(6-hydroxyhexyl)oxy]carbamate (**3**)*

1,8-Diazabicyclo[5.4.0]-undec-7-ene (DBU) (6.85 g, 45.0 mmol) was added in a dropwise manner to a mixture of 2-[2-[iodoethoxy)ethoxy]ethanol **2** (11.7 g, 45.0 mmol) and *tert*-butyl hydroxycarbamate (4.93 g, 37.0 mmol). Methylene chloride (20 ml) was added, and the mixture was stirred at 70°C for 24 h. The mixture was washed with 1 N HCl and brine. The organic layer was dried over Na_2SO_4, and the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel, 2% MeOH/CH_2Cl_2) to give compound **3** as a pale yellow oil. Yield: 5.75 g (62%). ^{1}H-NMR ($CDCl_3$, 400 MHz): $\delta = 1.48$ (s, 9 H, $C(CH_3)_3$), 3.61–3.64 (m, 2 H, CH_2), 3.67–3.69 (m, 4 H, CH_2), 3.72–3.77 (m, 4 H, CH_2), 4.01–4.04 (m, 2 H, NH–O–CH_2).

*Tert.-Butyl-N-({[6-(1-maleimidyl)ethoxy]ethoxy}ethoxy) carbamate (**4**)*

PPh_3 (1.00 g, 3.80 mmol), maleimide (0.37 g, 3.80 mmol) and *tert*-butyl *N*-[(6-hydroxyhexyl)oxy]carbamate **3** (1.00 g, 3.80 mmol) were dissolved in dry THF (20 ml). Diisopropyl azodicarboxylate (DIAD) (1.09 ml, 5.40 mmol) was added slowly at room temperature. The reaction mixture was stirred at ambient temperature for 24 h. The resulting clear solution was concentrated under reduced pressure, and the crude product was purified by column chromatography (silica gel, 50% EtOAc/petroleum ether) to afford 0.54 g (42%) of compound **4** as a pale yellow oil. ^{1}H-NMR ($CDCl_3$, 400 MHz): $\delta = 1.44$ (s, 9 H, $C(CH_3)_3$), 3.56–3.64 (m, 2 H, CH_2), 3.94–3.96 (m, 2 H, CH_2), 6.67 (s, 2 H, =CH). ^{13}C-NMR ($CDCl_3$, 100 MHz): $\delta = 28.2$, 37.0, 67.8, 69.2, 69.8, 70.5, 75.2, 81.5, 134.1, 156.7, 170.7. LR–MS (ESI positive): m/z = 367.2 ($[M + Na]^+$). Anal. calculated for $C_{15}H_{24}N_2O_7$: C 52.32, H 7.02, N 8.13; found: C 50.63, H 7.02, N 7.70.

*1-({[(Aminooxy)ethoxy]ethoxy}ethyl)1H-pyrrol-2,5-dione hydrochloride (**5**)*

A solution of carbamate **4** (0.20 g, 0.60 mmol) in 3 N HCl/EtOAc (5 ml, 50:50) was stirred at room temperature for 30 min. The solvent was removed under reduced pressure, and the residue was re-dissolved in 5 ml of methanol. Diethyl ether (100 ml) was added and the resulting white precipitate was collected by filtration. The precipitate was re-dissolved in 3 ml of THF for purification. Diethyl ether (100 ml) was added and the resulting white precipitate was collected by filtration to afford 0.11 g (74%) of compound **5** as the HCl-salt. mp 94.2–96.3°C ^{1}H-NMR (CD_3OD, 400 MHz): $\delta = 3.63$–3.65 (m, 6 H, CH_2), 3.69–3.72 (m, 2 H, CH_2), 3.79–3.81 (m, 2 H, CH_2), 4.18–4.20 (m, 2 H, CH_2), 6.84 (s, 2 H, = C–H). ^{13}C-NMR (CD_3OD, 100 MHz): $\delta = 37.1$, 67.8, 69.6, 70.0, 70.6, 73.5, 134.1, 170.6. LR–MS (ESI positive): m/z = 245.2 ($[M + H]^+$) Anal. calculated for $C_{10}H_{17}ClN_2O_5$: C 42.79, H 6.10, N 9.98; found: C 42.23, H 6.13, N 9.90.

*4-Fluorobenzaldehyde-O-(2-{2-[2-(pyrrol-2,5-dione-1-yl)-ethoxy]ethoxy}-ethyl)-oxime (FBOM) (**6**)*

4-Fluorobenzaldehyde (0.05 g, 0.40 mmol) was added to a solution of aminooxy compound **5** (0.06 g, 0.20 mmol) in DMF (10 ml). The reaction mixture was stirred at room temperature for 30 min. The solution was diluted with water and extracted with diethyl ether. The organic layer was washed with brine and dried (Na_2SO_4). The solvent was removed under reduced pressure, and the residue was

purified by column chromatography (silica gel, 50% EtOAc/petroleum ether) to afford 0.06 g (90%) of the desired compound **6** as a colorless oil. ^{1}H-NMR ($CDCl_3$, 400 MHz): $\delta = 3.62$–3.65 (m, 6 H, CH_2), 3.71–3.76 (m, 4 H, CH_2), 4.28–4.30, (m, 2 H, CH_2), 6.68 (s, 2 H, = C–H), 7.05 (m, 2 H, 2-H), 7.54–7.58 (m, 3-H), 8.09 (s, N = CH). ^{13}C-NMR ($CDCl_3$, 100 MHz): $\delta = 37.1$, 67.8, 69.6, 70.0, 70.6, 73.5, 115.8 (d, J = 22.0 Hz), 128.4 (d, J = 3.4 Hz), 128.8 (d, J = 8.3 Hz), 134.1, 147.8, 163.6 (d, J = 250.0 Hz), 170.6. Anal. calculated for $C_{17}H_{19}FN_2O_5$: C 58.28, H 5.47, N 8.00, found: C 58.61, H 5.80, N 7.61.

Dimeric neurotensin derivative (FBOM-7)

Cystein-containing neurotensin dimer **7** (Hultsch et al. 2007) (2 mg, 1.1 μmol) was dissolved in phosphate buffer (30 μL, pH 7.4). Maleimide **6** (0.5 mg, 1.4 μmol) in 30 μL of acetonitrile was added, and the mixture was stirred at 60°C for 2 h. Purification of the reaction mixture was accomplished by semi-preparative HPLC onto a Zorbax 300 SB-C18 column (9.4 × 250 mm, 5 μm) at a flow rate of 2 ml/min. Solvent A: CH_3CN; solvent B: 0.1% TFA. The following gradient was used: 0 min 20% A, 35 min 80% A. The peak eluting between 19–20 min was collected. Lyophilization gave the desired product FBOM-7 as a white powder. Yield: 1.3 mg, 54%. M_W $C_{101}H_{157}FN_{28}O_{24}S$ calculated 2,198.61, found MALDI-TOF 2,199.90 $[M + H]^+$.

Radiochemical syntheses

No-carrier added aqueous [^{18}F]fluoride ion was produced in a IBA CYCLONE 18/9 cyclotron by irradiation of [^{18}O]H_2O via the $^{18}O(p,n)^{18}F$ nuclear reaction. Syntheses of 4-[^{18}F]fluorobenzaldehyde and compound [^{18}F]FBOM were carried out in an automated nucleophilic fluorination module (Nuclear Interface, Münster). HPLC analyses were carried out with a Phenomenex RP-18 column (LUNA C18(2) 4.6 × 250 mm, 5 μm) using an indicated isocratic eluent from a gradient pump (Jasco PU-980 Intelligent HPLC Pump) with a flow rate of 1 ml/min. The products were monitored by a UV detector (Jasco UV-1575 intelligent UV/VIS detector) at 254 nm and by γ-detection with a scintillation detector GABI (X-RAYTEST). Semi-preparative HPLC was performed with a Phenomenex RP-18 column (LUNA C18(2) 10 × 250 mm, 10 μm) column using isocratic elution with CH_3CN/0.1 M ammonium formate (50/50) at a flow rate of 3.0 ml/min. Radio-TLC analyses of glutathione coupling reaction with [^{18}F]FBOM and [^{18}F]FBAM was performed on Merck RP18 F-254 aluminum plates. [^{18}F]FBAM and [^{18}F]SFB were prepared according to literature procedures (Berndt et al. 2007; Mäding et al. 2005).

4-[^{18}F]fluorobenzaldehyde-O-(2-{2-[2-(pyrrol-2,5-dione-1-yl)ethoxy]-ethoxy}-ethyl)oxime ([^{18}F]FBOM)

The radiosynthesis started with the preparation of 4-[^{18}F]fluorobenzaldehyde ([^{18}F]FBA). Cyclotron-produced [^{18}F]HF (6.54 GBq) was dried in a remotely controlled synthesis apparatus for nucleophilic radiofluorinations. Then, 4-*N*,*N*,*N*-trimethylaminobenzaldehyde triflate (10 mg, 36.5 μmol) dissolved in DMF (1.0 ml) was added, and the reaction mixture was heated at 80°C for 15 min. After cooling the reaction vessel to 40°C, 15 mg (50 μmol) of aminooxy compound **5** in 1 N HCl/MeOH (1 ml, 50:50) was added to the reaction mixture containing [^{18}F]FBA. After stirring for 15 min at 50°C, the crude product was subjected onto a semi-preparative HPLC column. The product fraction (21–23 min) was collected, diluted with water (20 ml), and passed through a Waters Sep-Pak-tC-18 cartridge. The cartridge was washed with water (10 ml) and [^{18}F]FBOM was eluted with diethyl ether (1 ml). The solvent was evaporated in a gentle stream of nitrogen to afford 730 MBq (19%, decay-corrected) of [^{18}F]FBOM within 80 min, including HPLC purification. The specific activity was determined to be 60 GBq/μmol. Radio-HPLC-analysis: CH_3CN/0.1 M ammonium formate solvent (50/50), $t_R = 10.8$ min. Radio-TLC: $R_f = 0.1$, EtOAc/hexane (50/50).

Determination of lipophilicity (LogP)

Lipophilicity (logP) of [^{18}F]FBOM, [^{18}F]FBAM, and [^{18}F]SFB was determined at pH 7.4 according to the method reported by Wilson et al. (2001).

Radiolabeling of glutathione (GSH) with [^{18}F]FBOM and [^{18}F]FBAM

[^{18}F]FBOM or [^{18}F]FBAM in water (25 μl, 3–5 MBq) was added to 1 ml of GSH solutions (1.0 mg/ml–10 ng/ml) in phosphate buffer (pH 7.2). The mixture was incubated at 20°C. Conversion of the prosthetic groups was monitored by radio-TLC after 5, 30, and 60 min. Radio-TLC: $R_f = 0.0$, 50% EtOAc/petroleum ether.

Radiolabeling of the cysteine-containing neurotensin dimer **7** with [^{18}F]FBOM and [^{18}F]FBAM

Peptide **7** was dissolved in phosphate buffer (30 μl, pH 7.4) and 2–4 MBq of [^{18}F]FBOM or [^{18}F]FBAM in 10 μl of acetonitrile was added. The final peptide concentrations were 2.5 mg/ml, 0.5 mg/ml, 0.25 mg/ml, and 50 μg/ml, respectively. The reaction mixtures were incubated at room temperature. After 5, 20, and 30 min, aliquots were taken

and analyzed via radio-TLC. Radio-TLC: $R_f = 0.0$, 50% EtOAc/petroleum ether.

Radiolabeling of the dimeric neurotensin **8** with [^{18}F]SFB

Neurotensin dimer **8** was dissolved in borate buffer (75 µl, pH 8.4) and [^{18}F]SFB in acetonitrile (25 µl, 2–5 MBq) was added. The peptide concentration was 10 and 0.5 mg/ml, respectively. The reaction mixture was kept at 40°C for 30 min. The radiochemical yield was determined by radio-HPLC. The gradient was 0 min 30% CH_3CN, 10 min 30% CH_3CN, 30 min 40% CH_3CN. $t_R = 20.0$ min.

Radiolabeling of human native LDL with [^{18}F]FBOM, [^{18}F]FBAM, and [^{18}F]SFB

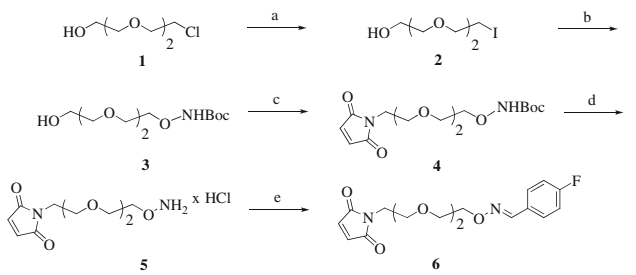
Human native total LDL (density 1.006–1.063 g/ml) was isolated as previously described (Pietzsch et al. 2004). Apolipoprotein and lipid constituents of nLDL were assayed as published elsewhere (Berndt et al. 2007). The LDL particles were free of albumin and other contaminating plasma proteins. All LDL particles have been isolated from donors who were homozygous for apoE isoform (genotype) 3. Prior to the radiolabeling of pooled nLDL, [^{18}F]FBOM, [^{18}F]FBAM or [^{18}F]SFB were dried in a gentle stream of nitrogen to remove the solvents (Et_2O or CH_3CN). To dried [^{18}F]FBOM, [^{18}F]FBAM or [^{18}F]SFB, 1 ml of nLDL (0.12 mg apoB-100/ml) in PBS (10 mM sodium phosphate, 150 mM sodium chloride, pH 7.2, containing 0.1% propylene glycol vol/vol) was added. The reaction mixture was incubated at room temperature for 20 min. The reaction mixture (1 ml) was eluted over a size-exclusion column (Econo-Pac 10DG containing Bio-Gel P6, 10 ml bed volume, Bio-Rad) with a flow rate of 2.5 ml/min using PBS as the eluent to separate the radiolabeled nLDL fractions from unreacted [^{18}F]FBOM, [^{18}F]FBAM or [^{18}F]SFB, and other radioactive by-products. Radiochemical purity was assessed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), as reported elsewhere (Berndt et al. 2007).

Results

Chemistry and radiochemistry

Aminooxy compound **5** as bifunctional labeling precursor for the synthesis of [^{18}F]FBOM was prepared in a four-step synthesis sequence. Synthesis of labeling precursor **5** and reference substance **6** is depicted in Scheme 2.

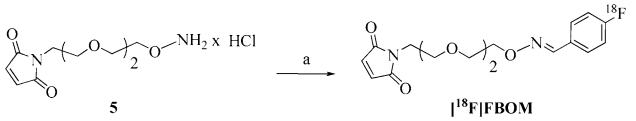
The synthesis sequence started with the reaction of commercially available 2-[2-(chloroethoxy)ethoxy]ethanol



Scheme 2 Synthesis of labeling precursor **5** and reference compound **6**: *a* NaI, acetone, 19 h, reflux, 85%; *b* BocNHOH, DBU, 24 h, 70°C, 62%; *c* PPh_3, DIAD, maleimide, THF, r.t., 16 h, 42%; *d* 3 M HCl, ethyl acetate, r.t., 30 min, 74%; *e* 4-fluorobenzaldehyde, DMF, r.t., 30 min, 90%

1 with NaI according to a Finkelstein reaction. A reaction time of 19 h resulted in nearly complete conversion of starting material **1** to afford the desired product **2** in very good 85% isolated yield. Treatment of iodide **2** with *tert*-butyl hydroxycarbamate in the presence of DBU as a base gave the *N*-Boc-protected aminooxy compound **3** at a yield of 62% after purification by means of flash chromatography. The maleimide-group was introduced via Mitsunobu reaction. It is known that the amount of starting material and the addition order of the other reagents are crucial for the reaction (Walker 1994, 1995). Hence, a 1.4-fold excess of diisopropyl azodicarboxylate (DIAD) was slowly added to a solution containing equimolar amounts (1.0 equiv.) of triphenylphosphine, maleimide and alcohol **3**. Following this procedure according to a Mitsunobu-esterification, the desired compound **4** was obtained in 42% isolated yield. Removal of the Boc-protecting group was achieved by treatment of compound **4** with HCl. The labeling precursor **5** was obtained at 74% yield as HCl salt. Reference compound **6** was prepared by the condensation of aminooxy compound **5** with 4-fluorobenzaldehyde. The reaction proceeded at room temperature in DMF to afford the corresponding oxime **6** in very good 90% yield. NMR analysis revealed exclusive formation of the single isomer. The observed chemical shift of the oxime proton at 8.09 ppm is indicative of formation of the *E*-isomer (Berndt et al. 2007; Karabatsos et al. 1967; Toyokuni et al. 2003).

The radiosynthesis of [^{18}F]FBOM was carried out in a nucleophilic radiofluorination module (Nuclear Interface, Münster) and involved a two-step/one-pot reaction sequence (Scheme 3).



Scheme 3 Radiosynthesis of [^{18}F]FBOM: *a* 4-[^{18}F]fluorobenzaldehyde, 1 N HCl/MeOH (1:1), 50°C, 15 min

The synthesis commenced with the preparation of 4-[^{18}F]fluorobenzaldehyde. The reaction was carried out in DMF at 80°C. These reaction conditions enabled preparation of 4-[^{18}F]fluorobenzaldehyde in sufficient radiochemical yields while avoiding formation of by-products. Performance of the reaction at higher temperatures (120°C) led to the formation of larger amounts of not further specified by-products. 4-[^{18}F]Fluorobenzaldehyde was directly used in the oxime formation step without further purification. Coupling of 4-[^{18}F]fluorobenzaldehyde with aminooxy compound **5** was carried out in a 1:1 mixture of 1 N HCl/MeOH (1 ml) at 50°C for 15 min to give the desired prosthetic group [^{18}F]FBOM. The reaction mixture was purified by means of semi-preparative HPLC. The total synthesis time of [^{18}F]FBOM was 80 min. The radiochemical yield (decay-corrected) was 19% yield, and the specific activity was determined to be 60 GBq/μmol at the end of synthesis. The radiochemical purity of the final product was greater than 98% as verified by radio-HPLC and radio-TLC analysis.

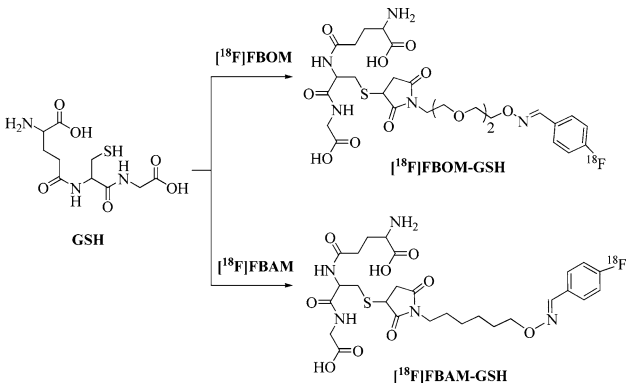
Lipophilicity (logP) of [^{18}F]FBOM, [^{18}F]FBAM, and [^{18}F]SFB

Peptide, and in particular, protein labeling should preferentially be performed under physiological condition to avoid decomposition and alteration or even loss of biological activity. This especially implies the performance in aqueous media at physiological pH at ambient temperature. Reactions in aqueous media require good solubility of all reaction partners in water. Beside many other factors, a molecule's lipophilicity (logP) is an important parameter that governs its solubility in aqueous media and, therefore, its reactivity. This is of special importance for radiolabeled compounds at high specific activity as expected for [^{18}F]FBAM, [^{18}F]FBOM, and [^{18}F]SFB, since undesired adsorption effects onto reaction vials and traces of impurities may drastically reduce reactivity of the radiotracer. The lipophilicity of compounds [^{18}F]FBAM, [^{18}F]FBOM, and [^{18}F]SFB was determined at pH 7.4 using the shake-flask method (Wilson et al. 2001), being 2.71 ± 0.21 for [^{18}F]FBAM, 0.84 ± 0.04 for [^{18}F]FBOM, and 1.75 ± 0.17 for [^{18}F]SFB, respectively.

Radiolabeling of GSH and cysteine-containing neurotensin dimer **7** with [^{18}F]FBOM and [^{18}F]FBAM

The labeling properties of [^{18}F]FBOM and [^{18}F]FBAM as thiol group-reactive prosthetic groups were first assessed by the reaction with cysteine-containing tripeptide GSH (Scheme 4).

The labeling of GSH at various concentrations (1 mg/ml to 10 ng/ml) was performed in PBS (pH 7.2) at room temperature for 5, 30, and 60 min. The radiochemical yield was determined by radio-TLC and refers to the percentage of radioactivity area of the desired product ([^{18}F]FBOM-GSH and [^{18}F]FBAM-GSH) relative to the total radioactivity area (Table 1).



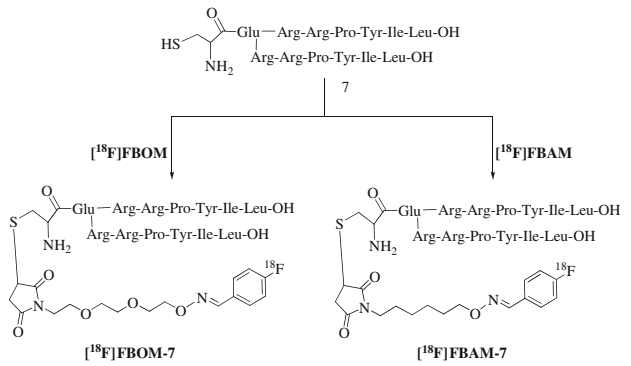
Scheme 4 Radiolabeling of GSH with [^{18}F]FBOM and [^{18}F]FBAM

For GSH concentrations ≥10 μg/ml, almost complete conversion (>95%) of [^{18}F]FBOM and [^{18}F]FBAM into the corresponding GSH derivatives ([^{18}F]FBOM–GSH and [^{18}F]FBAM–GSH) was accomplished within 5 min. At a GSH concentration of 1 μg/ml, conversion was completed after 30 min. When GSH concentrations ≥1 μg/ml are used, both prosthetic groups show comparable labeling efficiency. However, the use of lower GSH concentrations (0.1 μg/ml) reveals differences. While the radiochemical yield increased over time in the case of [^{18}F]FBOM, reaching 70% after 60 min, the radiochemical yield remains nearly constant in the case of [^{18}F]FBAM, reaching 13, 20, and 20% after 5, 30, and 60 min, respectively. At very low GSH concentrations (0.01 μg/ml), both prosthetic groups show less than 5% labeling yield at all time points studied.

To further explore the labeling properties of [^{18}F]FBOM and [^{18}F]FBAM with thiol groups, both prosthetic groups were conjugated with cysteine-containing neurotensin dimer **7** (Scheme 5), which was synthesized in our group (Hultsch et al. 2007).

Table 1 Dependency of the radiochemical yield (%) on GSH concentration during conjugation with [^{18}F]FBOM (A) and [^{18}F]FBAM (B)

GSH concentration	5 min		30 min		60 min	
	A	B	A	B	A	B
1 mg/ml	>95	>95	>95	>95	>95	>95
0.1 mg/ml	>95	>95	>95	>95	>95	>95
10 μg/ml	>95	>95	>95	>95	>95	>95
1 μg/ml	75	66	>95	>95	>95	>95
0.1 μg/ml	16	13	40	20	70	20
0.01 μg/ml	≤5	≤5	≤5	≤5	≤5	≤5



Scheme 5 Radiolabeling of cysteine-containing neurotensin derivative **7** with [^{18}F]FBOM and [^{18}F]FBAM

The labeling of neurotensin dimer **7** was performed in a 1:1 mixture of phosphate buffer (pH 7.2) and MeOH at 60°C for 5, 15, and 30 min. The labeling yields were monitored by radio-TLC analyses, and the determined radiochemical yield refers to the percentage of radioactivity area of the desired products ([^{18}F]FBOM-7 and [^{18}F]FBAM-7) relative to the total radioactivity area. The results are summarized in Table 2.

Both prosthetic groups showed nearly complete conversion into the desired conjugates, [^{18}F]FBOM-7 and [^{18}F]FBAM-7, within 5 min at a peptide concentration of 2.5 mg/ml, which equal 1.35 µmol/ml. At lower peptide concentrations (0.5 mg/ml and 0.25 mg/mg), [^{18}F]FBOM showed almost complete conversion after 5 min, whereas [^{18}F]FBAM gave lower radiochemical yield of about 80%, which remained constant over time (5, 20, and 30 min). At very low peptide concentration of 50 µg/ml, the use of [^{18}F]FBOM provided increasing radiochemical yields over time, reaching 75% after 30 min. On the other hand, [^{18}F]FBAM gave significantly lower radiochemical yield of 22–30% at 5, 20, and 30 min. This result is consistent with the results obtained for the radiolabeling of [^{18}F]FBOM and [^{18}F]FBAM with GSH. Both experiments confirm the well-known dependency of the radiochemical yield on the peptide concentration. The obtained results reveal that [^{18}F]FBOM as thiol-reactive prosthetic group shows superior labeling performance towards cysteine-containing peptides in comparison with [^{18}F]FBAM.

Table 2 Dependency of the radiochemical yield on the peptide concentration for radiolabeling of neurotensin dimer **7** with [^{18}F]FBOM (A) and [^{18}F]FBAM (B)

Concentration of neurotensin dimer **7**	5 min		20 min		30 min	
	A	B	A	B	A	B
2.5 mg/ml	>95	>95	>95	>95	>95	>95
0.5 mg/ml	>95	81	>95	82	>95	82
0.25 mg/ml	>95	81	>95	80	>95	80
50 µg/ml	30	22	48	33	75	30

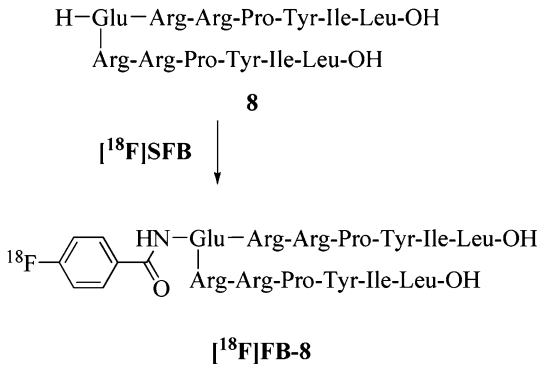
Radiolabeling of neurotensin dimer **8** with [^{18}F]SFB

As already shown in previous reports, the radiochemical yield for acylation reactions with the popular prosthetic group, [^{18}F]SFB, also depends strongly on the used peptide concentration. To further evaluate the labeling properties of [^{18}F]FBOM and [^{18}F]FBAM as highly reactive and efficient prosthetic groups, we set out radiolabeling experiments of a dimeric neurotensin derivative with the most frequently employed prosthetic group, [^{18}F]SFB. The radiolabeling of neurotensin dimer **8** using [^{18}F]SFB is given in Scheme 6.

The use of 0.5 mg/ml of peptide **8** resulted in a radiochemical yield of only 5% after incubation at pH 8.4 for 30 min at 40°C. This is significantly lower compared to the results observed for the radiolabeling of cysteine-containing neurotensin dimer **8** with [^{18}F]FBOM and [^{18}F]FBAM. Although a 20-fold increase in peptide concentration (10 mg/ml) gave better radiochemical yields of 33%, this finding clearly demonstrates an important drawback of [^{18}F]SFB as prosthetic group for peptide labeling. Reasonable radiochemical yields are only achievable when fairly large amounts of peptide are used. This might be, in some circumstances, a crucial factor for the application of [^{18}F]SFB, especially when peptides with limited availability and/or solubility are used.

Radiolabeling of human native LDL with [^{18}F]FBOM, [^{18}F]FBAM, and [^{18}F]SFB

The radiolabeling of human nLDL particles with the prosthetic groups [^{18}F]FBOM, [^{18}F]FBAM, and [^{18}F]SFB was performed in PBS buffer (pH 7.2) at ambient temperature for 20 min. The obtained results (radiochemical yield, synthesis time, and specific activity) are summarized in Table 3.



Scheme 6 Radiolabeling of neurotensin dimer **8** with [^{18}F]SFB

Table 3 Results of radiolabeling of nLDL particles with [^{18}F]FBOM, [^{18}F]FBAM, and [^{18}F]SFB

Prosthetic group	Radiochemical yield (%)	Synthesis time (min)	Specific activity (GBq/μmol)
[^{18}F]FBOM	5	45	30–50
[^{18}F]FBAM	20	45	50–300
[^{18}F]SFB	32	45	200–500

After incubation of nLDL with the prosthetic groups [^{18}F]FBOM, [^{18}F]FBAM or [^{18}F]SFB, the corresponding radiolabeled lipoprotein particles ([^{18}F]FBOM–LDL, [^{18}F]FBAM–LDL, and [^{18}F]FB–LDL) were separated from unreacted prosthetic groups and other non-specified radioactive by-products via size-exclusion chromatography. The fractions containing radiolabeled nLDL were collected and analyzed with sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The overall radiochemical purity of separated radiolabeled LDL particles ([^{18}F]FBOM–LDL, [^{18}F]FBAM–LDL, and [^{18}F]FB–LDL) was ≥96% as confirmed by SDS-PAGE analysis. The ^{18}F radioactivity distribution in LDL apolipoprotein constituents and in the lipid layer is given in Table 4.

Discussion

Due to the increasing relevance of peptides and proteins for molecular imaging purposes over the last decades, numerous reports have been published on the labeling of peptides and proteins with the short-lived positron emitter ^{18}F. The preference of ^{18}F-labeled radiotracers is mainly based on the ideal nuclear characteristics of ^{18}F such as low positron energy (0.64 MeV) and its convenient 109.8 min half-life. Moreover, the choice of ^{18}F as an ideal PET radionuclide for peptide and protein labeling would also meet the needs and experiences of PET clinicians, who are familiar with the equipment and the interpretation of PET scans with conventional PET radiotracers like [^{18}F]FDG, [^{18}F]FLT or [^{18}F]F-DOPA. However, reaction conditions required for the incorporation of [^{18}F]fluoride into molecules are quite harsh (e.g., strong basic conditions, elevated temperatures). With special respect to reaction conditions compatible with structural and functional integrity of peptides and proteins, most methods described in the literature rely on the use of prosthetic groups for introducing ^{18}F. Various prosthetic groups have been developed and used for peptide and protein labeling. Most prosthetic groups take advantage of the presence of accessible thiol and/or amine groups present in peptides and proteins, capable of being linked with corresponding thiol- or amine-reactive prosthetic groups.

The present work summarizes our experiences in peptide (GSH and neurotensin derivatives) and protein (human nLDL) labeling with the novel thiol-reactive prosthetic groups, [^{18}F]FBOM and [^{18}F]FBAM, in comparison with the popular and versatile amine-reactive acylation agent, [^{18}F]SFB. Moreover, labeling with [^{18}F]SFB is considered to be a validated method for the incorporation of the positron emitter ^{18}F into peptides and proteins as published by our and other groups.

The frequent use of prosthetic groups for peptide and protein labeling requires their reliable and reproducible synthesis in reasonable radiochemical yields, preferentially by means of a remotely controlled synthesis module. All

Table 4 ^{18}F radioactivity distribution in LDL apolipoproteins and in the lipid layer

LDL constituent	^{18}F radioactivity (%)			1 versus 3	2 versus 3	1 versus 2
	[^{18}F]FBOM-LDL^{1}	[^{18}F]FBAM-LDL^{2}	[^{18}F]FB-LDL^{3}			
ApoB-100[a]	86 ± 4.2	83 ± 4.6	97 ± 2.1	$P < 0.05$	$P < 0.01$	ns
ApoE[a]	11.2 ± 3.6	8.4 ± 3.2	0.4 ± 0.2	$P < 0.01$	$P < 0.01$	ns
ApoA-I[a]	n.d.	n.d.	n.d.	–	–	–
Lipid layer[b]	1.1 ± 0.6	9.8 ± 4.2	1.7 ± 0.3	ns	$P < 0.01$	$P < 0.1$

Human total LDL (density 1.006–1.063 g/ml) used in this study showed the following composition: total cholesterol 2.75 ± 0.48 mmol/l; triglycerides 0.26 ± 0.08 mmol/l; phospholipids 0.54 ± 0.21 mmol/l; apolipoprotein (apo) B-100 0.95 ± 0.21 g/l; apoE 0.08 ± 0.03 g/l; apoA-I 0.02 ± 0.01 g/l. The LDL particles were free of albumin and other contaminating plasma proteins. All LDL particles have been isolated from donors who were homozygous for apoE isoform (genotype) 3. All data are means ± SD ($n = 6$); statistical analyses (Mann–Whitney U tests, P) were performed using the SPSS 12.1 software package

n.d. Not detectable, *ns* not significant

[a] 18F radioactivity distribution among LDL apolipoproteins (apo) was determined by radioluminographic evaluation of the SDS-PAGE pattern of each individual radiolabeled LDL using a BAS 5000 scanner (FUJIX, Tokyo, Japan). Electrophoretic band intensities were quantified using AIDA (advanced image data analyzer) software version 4.14 (Raytest, Berlin, Germany)

[b] Noncovalently associated ^{18}F radioactivity in the LDL lipid layer was determined after extraction with an ice-cold chloroform/methanol mixture (70/30)

three prosthetic groups described in this work can conveniently be prepared in a synthesis module. The synthesis of acylation agent [^{18}F]SFB is accomplished via a three-step/two-pot synthesis sequence involving SPE purification of the final product. Thiol-reactive prosthetic groups, [^{18}F]FBOM and [^{18}F]FBAM, are prepared in a two-step/one-pot reaction including HPLC purification of the final product. Syntheses of the labeling precursors (*tert*.-butyl 4-*N,N,N*-trimethylammoniumbenzoate triflate, 4-*N,N,N*-trimethylammoniumbenzaldehyde triflate, aminooxy-compound **5** and *N*-(6-aminoxyhexyl)-maleimide) needed for the radiosynthesis of [^{18}F]SFB, [^{18}F]FBOM, and [^{18}F]FBAM are straightforward, and provide the desired compounds in sufficient quantities suitable for routine synthesis of the prosthetic groups on a daily basis.

The results of the remotely controlled radiosyntheses of [^{18}F]SFB, [^{18}F]FBOM, and [^{18}F]FBAM are given in Table 5.

The similiar two-step/one-pot reaction sequence for the preparation of [^{18}F]FBOM and [^{18}F]FBAM [(1) synthesis of 4-[^{18}F]fluorobenzaldehyde, (2) oxime formation with the corresponding aminooxy compound] allows the use of the same synthesis module (including synthesis program) for the radiosynthesis of both prosthetic groups. Hence, time-consuming alterations in the module set-up and synthesis program can be avoided when either [^{18}F]FBOM or [^{18}F]FBAM should be prepared. However, the synthesis of [^{18}F]SFB requires a dedicated synthesis module. Once the module has been set-up and programmed, it is solely suited for the preparation of [^{18}F]SFB.

The radiosynthesis of [^{18}F]SFB, [^{18}F]FBOM, and [^{18}F]FBAM was accomplished within a total synthesis time of 70–80 min, compatible with the 109.8 min half-life of ^{18}F. The determined specific activity is higher for [^{18}F]FBOM and [^{18}F]FBAM (60 and 76 GBq/μmol, respectively) as for [^{18}F]SFB (35 GBq/μmol), presumably due to subsequent HPLC purification of the thiol-reactive prosthetic groups. However, the determined specific radioactivity of all prosthetic groups should be sufficiently high for subsequent labeling reactions via alkylation of thiol groups ([^{18}F]FBOM and [^{18}F]FBAM), and acylation of amine groups ([^{18}F]SFB). The radiochemical yield of 29% for [^{18}F]FBAM is comparable to that of a related ^{18}F-labeled oxime (35%) reported by Toyokuni et al. (2003). Less lipophilic [^{18}F]FBOM, however, gave lower radiochemical yields of 19%. The lower radiochemical yield of [^{18}F]FBOM can be explained with the more hydrophilic character of the compound. The hydrophilic character of [^{18}F]FBOM (logP = 0.84) resulted in a lower retention of the compound on the SPE cartridge after HPLC purification, which led to a loss of product during the final SPE purification step. On the other hand, more lipophilic [^{18}F]FBAM (logP = 2.71) was retarded more efficiently on the SPE cartridge, and more product could be eluted from the cartridge after HPLC purification. The obtained radiochemical yield of [^{18}F]SFB is in the range reported in the literature (Mäding et al. 2005; Pietzsch et al. 2004), whereas the determined specific activity (35 GBq/μmol) is higher compared to data from the literature (11 GBq/μmol) (Vaidyanathan and Zalutsky 2006).

The possibility to perform the syntheses in remotely controlled synthesis modules allows the safe handling of large amounts of radioactivity, resulting in the convenient preparation of several hundreds MBq to a couple of GBq amounts of the desired prosthetic groups [^{18}F]SFB, [^{18}F]FBOM, and [^{18}F]FBAM.

The thiol-reactive prosthetic groups, [^{18}F]FBOM and [^{18}F]FBAM, and the acylation agent, [^{18}F]SFB, were used in various labeling experiments to evaluate their labeling efficiency towards peptides and proteins. Beside the reaction of [^{18}F]FBOM and [^{18}F]FBAM with cysteine-containing tripeptide GSH as a model, all three prosthetic groups were used for the radiolabeling of appropriately functionalized neurotensin derivatives and human nLDL particles. LDL particles are considered as a well-characterized model system for the radiolabeling of proteins with the short-lived positron emitter ^{18}F (Berndt et al. 2007; Pietzsch et al. 2004). The interest in using neurotensin derivatives stems from their potential for targeting neurotensin receptor expressing tumors such as ductal pancreatic carcinomas (Bergmann et al. 2002).

Results on the labeling efficiency of [^{18}F]FBOM, [^{18}F]FBAM, and [^{18}F]SFB with peptides (GSH, neurotensin dimers **7** and **8**) clearly showed the advantage of thiol-reactive prosthetic groups, [^{18}F]FBOM and [^{18}F]FBAM, compared to [^{18}F]SFB. Both thiol-reactive prosthetic groups required substantially less amounts of peptide compared to [^{18}F]SFB. A concentration of 0.25 mg/ml (0.13 μmol/ml) of cysteine-containing neurotensin dimer **7** gave excellent 95% of product [^{18}F]FBOM-7 and 81% of

Table 5 Summary of remotely controlled radiosyntheses of [^{18}F]FBOM, [^{18}F]FBAM, and [^{18}F]SFB

Prosthetic group	Radiochemical yield (%)	Synthesis steps	Synthesis time (min)	Specific activity (GBq/μmol)
[^{18}F]FBOM	19	2	80	60
[^{18}F]FBAM	29	2	70	76
[^{18}F]SFB	25–38	3	70	35

product [^{18}F]FBAM-7 after 5 min. In contrast, reaction of [^{18}F]SFB with a 40× higher amount of neurotensin dimer **8** (10 mg/ml, 5.5 μmol/ml) afforded only a moderate radiochemical yield of 33% for [^{18}F]SFB-8 after 30 min. The need of using fairly large amounts of peptide **7** to achieve acceptable radiochemical yields of radiolabeled peptide [^{18}F]SFB-8 agrees with experiences reported in the literature when other peptides have been labeled with the acylation agent [^{18}F]SFB (Bergmann et al. 2002; Hultsch et al. 2007; Wuest et al. 2003). Better results in terms of minimizing the amount of peptide while reaching reasonable radiochemical yields in peptide labelings with ^{18}F are achieved with chemoselective oxime or hydrazone formation through conjugation of corresponding aminooxy compounds or hydrazines as labeling precursors with 4-[^{18}F]fluorobenzaldehyde (Wester and Schottelius 2007). More recent reports upon the application of Click-chemistry for peptide labeling, however, also showed that rather large peptide amounts are required to achieve reasonable radiochemical yields (Glaser et al. 2007; Marik and Sutcliffe 2006; Li et al. 2007; Ramenda et al. 2007). On the other hand, thiol-group reactive prosthetic groups like [^{18}F]FBOM and [^{18}F]FBAM, as shown in this report, require significantly lower amounts of cysteine-containing peptides to achieve high radiochemical yields in comparison with other methods for peptide labeling, such as acylation with [^{18}F]SFB, Click-chemistry, and chemoselective oxime and hydrazone formation. Moreover, the straightforward synthesis of aminooxy-functionalized maleimide-containing labeling precursors (compound **5** and *N*-(6-aminooxy-hexyl)-maleimide) and the facile and reliable, remotely controlled radiosynthesis of [^{18}F]FBOM and [^{18}F]FBAM are additional important aspects which make [^{18}F]FBOM and [^{18}F]FBAM excellent prosthetic groups for highly efficient radiolabelings of cysteine-containing peptides with the positron emitter ^{18}F. Both prosthetic groups show the same labeling efficiency at peptide concentrations of 10 μg/ml (GSH) and 2.5 mg/ml (neurotensin dimer **7**). However, at very low peptide concentrations, differences in the labeling efficiency became visible for [^{18}F]FBOM and [^{18}F]FBAM. [^{18}F]FBOM provided good radiochemical yields of 70% after 60 min using a GSH concentration of as little as 0.1 μg/ml, whereas [^{18}F]FBAM gave a radiochemical yield of only 20% under the same reaction conditions (Table 1). This trend could also be observed in the radiolabeling of cysteine-containing neurotensin dimer **7** with [^{18}F]FBOM and [^{18}F]FBAM. A peptide concentration of 50 μg/ml afforded the desired [^{18}F]FBOM-based coupling product [^{18}F]FBOM-7 in 75% radiochemical yield after 30 min. Conversely, the radiochemical yield of the corresponding [^{18}F]FBAM-based coupling product, [^{18}F]FBAM-7, was only 30% using the same reaction conditions (Table 2). Since the radiochemical purity and the specific activity are in the same range for both prosthetic groups (Table 4), this finding may be explained by the lipophilicity differences of [^{18}F]FBOM (logP = 0.84) and [^{18}F]FBAM (logP = 2.71). The hydrophilic character of [^{18}F]FBOM supports radiolabeling reactions in aqueous media, and potential adsorption effects on reaction vial walls and impurities seem to have no detrimental influence on the labeling efficiency. On the other hand, more lipophilic [^{18}F]FBAM seems to be more susceptible to undesired adsorption effects, especially at low concentrations of the potential reaction partner (GSH or neurotensin dimer **7**), which presumably led to lower radiochemical yields. Hence, our results suggest that less lipophilic [^{18}F]FBOM is an excellent prosthetic group for ^{18}F labeling of thiol group-containing peptides, especially at very low peptide concentrations as exemplified in this work with GSH and cysteine-containing neurotensin dimer **7**.

The labeling properties of [^{18}F]FBOM and [^{18}F]FBAM in comparison with [^{18}F]SFB were further studied by the radiolabeling of various apolipoproteins of native human low density lipoprotein (nLDL) particles as a model system. The general structure of human nLDL comprises a lipid core consisting of cholesteryl esters and triglycerides, a surface lipid layer of phospholipids and unesterified cholesterol, and various amphiphilic apolipoproteins embedded or attached in the overall lipophilic microenvironment of the lipoprotein particle (Scott 1989; Segrest et al. 2001). The major protein of human LDL is apolipoprotein (apo) B-100; however, LDL particles can also contain traces of other apolipoproteins, particularly, the exchangeable apolipoproteins apoE and apoA-I.

ApoB-100 represents the structural protein of LDL. There is only one copy of the protein on each LDL particle. The metabolic fate of each LDL particle is paired for life with that of its apoB-100 molecule; therefore, efficient radiolabeling of apoB-100 is a prerequisite for studies aiming at, for example, the investigation of LDL kinetics in animal models of disease using PET (Berndt et al. 2007; Pietzsch et al. 2004). Mature human apoB-100 (M_r = 516.000, without carbohydrate content) consists of a single polypeptide chain of 4,536 amino acids. In principle, ^{18}F-radiolabeling of apoB-100 can be performed through acylation of both its chemically accessible *N*-terminal glutamate residue and lysine side chain residues with [^{18}F]SFB, respectively, or through alkylation of free cysteine residues with maleimide-containing prosthetic groups such as [^{18}F]FBOM and [^{18}F]FBAM. However, chemical accessibility of the 357 lysine residues can be partially constricted by steric hindrance. Lund-Katz et al. (1988) found about 225 lysines exposed on the surface of LDL, with the remaining 132 lysines buried and unavailable for

methylation reactions. For the 25 cysteine residues, it has been proposed that at least 16 of them are involved in intramolecular disulfide bridge formation (Yang et al. 1994). As a consequence, the acylation agent [^{18}F]SFB has approximately 25× more potential sites for conjugation, compared to the thiol-reactive prosthetic groups, [^{18}F]FBOM and [^{18}F]FBAM. However, all labeling reactions were carried out at pH 7.2. Below a pH of 7.2, nearly all lysine side chain residues are supposed to be protonated, hence not being available for [^{18}F]fluorobenzoylation with [^{18}F]SFB. As a consequence, the chemically accessible *N*-terminal glutamate residue of apoB-100 is the most privileged site for conjugation. Lysine side chain residues become the preferred site of conjugation with [^{18}F]SFB only under basic conditions (e.g., pH 8.4). For peptide labeling with [^{18}F]SFB, the pH is usually adjusted to 8.2–8.4. In many cases, the more complex structure of proteins, as also found in human nLDL particles, requires performance of radiolabeling reaction under physiological conditions, being 37°C, aqueous media, and physiological pH.

The obtained good radiochemical yield of 32% for [^{18}F]SFB labeling of nLDL at pH 7.2 suggests that the *N*-terminus in apoB-100 is readily accessible for [^{18}F]fluorobenzoylation with [^{18}F]SFB under physiological reaction conditions. This is in good agreement with the literature and our own observations (Pietzsch et al. 2004; Segrest et al. 1994). However, the open accessibility of the *N*-terminus in apoB-100 for acylation reaction with [^{18}F]SFB is undoubtedly crucial, but other aspects such as lipophilicity of the prosthetic group itself seem to have an even more important influence on the labeling yield. In this line, the determined lipophilicity (logP 1.75) of [^{18}F]SFB seems to be especially well-suited and beneficial for the radiolabeling of LDL. The lipophilicity of [^{18}F]SFB allows conjugation reactions in aqueous media while showing no significant incorporation into the lipid layer of LDL particles. The incorporation of more lipophilic prosthetic groups into the lipid part of LDL (>30%) was shown to be a limiting factor for the use of lipophilic iodinated Bolton–Hunter-type reagents (Shepherd et al. 1976).

The importance of lipophilicity of the prosthetic group on LDL labeling is further supported by our results obtained with thiol-reactive prosthetic groups [^{18}F]FBOM and [^{18}F]FBAM. Although both prosthetic groups showed excellent labeling properties for cysteine-containing peptides even at very low peptide concentrations, the obtained radiochemical yield for LDL labeling are lower as observed for [^{18}F]SFB, being 20% for [^{18}F]FBAM and only 5% for [^{18}F]FBOM. This finding is somewhat surprising taking into account the number of still nine free cysteine residues in LDL apoB-100 as potential accessible conjugation sites at pH 7.2.

However, lipophilicity of [^{18}F]FBAM causes substantial incorporation of the prosthetic group into the lipid layer of the LDL particle, thus leading to lower radiochemical yields. The importance of lipophilicity is even more pronounced in the case of [^{18}F]FBOM (logP 0.84). The hydrophilic character of the prosthetic group certainly supports reaction in aqueous media; however, the very low radiochemical yield of 5% indicates that [^{18}F]FBOM seems to have only limited access to the free cysteine residues. All 14 cysteine residues within the chemically accessible *N*-terminal region of apoB-100 are linked in disulfide bridges (Yang et al. 1994). In this line, it can be speculated that the free cysteine residues are arranged in domains embedded in a more lipophilic microenvironment, thus being shielded to the hydrophilic compound [^{18}F]FBOM.

Although the concentration of apoE and apoA-I is very low in LDL particles, potential radiolabeling of these protein constituents also has to be considered (Berndt et al. 2007). However, under the conditions employed, the amount of radioactivity assigned to radiolabeled apoE was about 10× lower than that assigned to radiolabeled apoB-100. Radiolabeling of apoA-I was virtually negligible. Both [^{18}F]FBAM and [^{18}F]FBOM showed a better efficacy for radiolabeling of LDL apoE than [^{18}F]SFB. As an explanation, the *N*-terminal domain of apoE, which is the target for [^{18}F]fluorobenzoylation at pH 7.2, is postulated to become buried in the interior of the lipoprotein particle and, thus, is chemically not accessible (Fisher et al. 2000). On the other hand, the single free cysteine residue in apoE isoform 3 proved to be a good target for conjugation with both [^{18}F]FBAM and [^{18}F]FBOM. Human apoA-I showed no radiolabeling at pH 7.2, neither by [^{18}F]SFB nor by both [^{18}F]FBAM and [^{18}F]FBOM. For radiolabeling with [^{18}F]FBAM and [^{18}F]FBOM, respectively, wild-type apoA-I can be used as a negative control, because it does not contain any cysteine residues. For labeling with [^{18}F]SFB, it can be speculated that the *N*-terminal residue is, comparably to apoE, chemically not accessible.

Summary and conclusions

The labeling of peptides and proteins with the short-lived positron emitter ^{18}F remains a special challenge for radiopharmaceutical chemists. Within the arsenal of available prosthetic groups for peptide and protein labeling, various thiol-reactive agents have been shown to be especially useful. In this report, we have summarized our experiences in the labeling of GSH and cysteine-containing neurotensin derivatives as examples for peptides, and human nLDLs as examples for proteins with the two novel thiol-reactive prosthetic groups, [^{18}F]FBOM and [^{18}F]FBAM. The results on the radiosynthesis and labeling

efficiency were compared to experiments performed with the most prominent and most frequently used prosthetic group for peptide and protein labeling, the acylation agent [^{18}F]SFB.

[^{18}F]FBOM and [^{18}F]FBAM can conveniently be prepared in a remotely controlled synthesis module, enabling their reproducible preparation even in high radioactivity amounts on a routine base. The preparation of the required labeling precursors is straightforward and can be accomplished within a few synthesis steps involving standard organic chemistry.

Thiol-reactive prosthetic groups, [^{18}F]FBOM and [^{18}F]FBAM, showed excellent labeling properties for cysteine-containing peptides in comparison with [^{18}F]SFB. At very low peptide concentrations, [^{18}F]FBOM is superior to [^{18}F]FBAM. This finding makes [^{18}F]FBOM the preferred prosthetic group for labeling cysteine-containing peptides when very low peptide concentrations are used.

Our results on the radiolabeling of human LDL, however, indicate that [^{18}F]FBAM, and especially [^{18}F]FBOM, are not better suitable for labeling of LDL particles in comparison to [^{18}F]SFB. However, in some instances, the use of the amine-reactive agent [^{18}F]SFB can fail. As an example, steric hindrance of the *N*-terminal region of apoB-100 occurs due to oxidative modification of apoB-100 by myeloperoxidase or hypochlorous acid (Yang et al. 1999). This type of modification caused 3- to 4-fold lower radiochemical yields of [^{18}F]fluorobenzoylated hypochlorite-modified LDL (<10%; corrected for decay, related to [^{18}F]SFB) when compared with [^{18}F]FB–nLDL and [^{18}F]FB–oxLDL, respectively (Hoppmann et al. 2006). Beside oxidation, other types of modification such as enzymatic and nonenzymatic glycosylation, homocysteinylation as well as conformational properties should be considered as a cause for diminished or failed conjugation of [^{18}F]SFB with the *N*-terminus of the protein of interest. Correspondingly, this also has to be considered for lysine side chain residues that are very susceptible for structural changes due to oxidative or glycoxidative attack (Pietzsch and Bergmann 2004).

In this line, a direct comparison of [^{18}F]FBOM and [^{18}F]FBAM favors [^{18}F]FBAM for the radiolabeling of LDL. The obtained results emphasize the importance of lipophilicity of the prosthetic group for labeling LDL. A lipophilic prosthetic group like [^{18}F]FBAM causes undesired incorporation into the lipid part of the LDL, whereas hydrophilic prosthetic group [^{18}F]FBOM (logP 0.84) seems to have only limited access to the free cysteine residues due to the lipophilic environment of the LDL particle's proximity.

However, both prosthetic groups should also be tested for the radiolabeling of other proteins, including antibodies and antibody fragments, to further evaluate their scope and limitations. Based on the results presented in this study, it can be concluded that [^{18}F]FBOM and [^{18}F]FBAM should play an important role for the labeling of cysteine-containing peptides.

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References

Becaud J, Karramkam M, Mu L, Schubiger PA, Ametamey S, Smits R, Koksch B, Graham K, Cyr JE, Dinkelborg L, Suelzle D, Stellfeld T, Brumby T, Lehmann L, Srinivasan A (2007) Development of a new direct method for 18F-labeling of peptides. J Label Compd Radiopharm 50:S215

Bergmann R, Scheunemann M, Heichert C, Mäding P, Wittrisch H, Kretzschmar M, Rodig H, Tourwé D, Iterbeke K, Chavatte K, Zips D, Reubi JC, Johannsen B (2002) Biodistribution and catabolism of (18)F-labeled neurotensin(8–13) analogs. Nucl Med Biol 29:61–72

Berndt M, Pietzsch J, Wuest F (2007) Labeling of low-density lipoproteins using the 18F-labeled thiol-reactive reagent N-[6-(4-[18F]fluorobenzylidene)aminooxyhexyl]maleimide. Nucl Med Biol 34:5–15

Bodei L, Cremonesi M, Grana C, Rocca P, Bartolomei M, Chinol M, Paganelli G (2004) Receptor radionuclide therapy with 90Y-[DOTA]0-Tyr3-octreotide (90Y-DOTATOC) in neuroendocrine tumours. Eur J Nucl Med Mol Imaging 31:1038–1046

Cai W, Olafsen T, Zhang X, Cao Q, Gambhir SS, Williams LE, Wu AM, Chen X (2007) PET imaging of colorectal cancer in xenograft-bearing mice by use of an 18F-labeled T84.66 anti-carcinoembryonic antigen diabody. J Nucl Med 48:304–310

Carrió I, Pieri PL, Narula J, Prat L, Riva P, Pedrini L, Pretolani E, Caruso G, Sarti G, Estorch M, Berná L, Riambau V, Matías-Guiu X, Pak C, Ditlow C, Chen F, Khaw BA (1998) Noninvasive localization of human atherosclerotic lesions with indium 111-labeled monoclonal Z2D3 antibody specific for proliferating smooth muscle cells. J Nucl Cardiol 5:551–557

Chinn PC, Leonard JE, Rosenberg J, Hanna N, Anderson DR (1999) Preclinical evaluation of 90Y-labeled anti-CD20 monoclonal antibody for treatment of non-Hodgkin's lymphoma. Int J Oncol 15:1017–1025

de Bruin B, Kuhnast B, Hinnen F, Yaouancq L, Amessou M, Johannes L, Samson A, Boisgard R, Tavitian B, Dollé F (2005) 1-[3-(2-[18F]fluoropyridin-3-yloxy)propyl]pyrrole-2,5-dione: design, synthesis, and radiosynthesis of a new [18F]fluoropyridine-based maleimide reagent for the labeling of peptides and proteins. Bioconjug Chem 16:406–420

de Jong M, Bakker WH, Krenning EP, Breeman WA, van der Pluijm ME, Bernard BF, Visser TJ, Jermann E, Béhé M, Powell P, Mäcke HR (1997) Yttrium-90 and indium-111 labelling, receptor binding and biodistribution of [DOTA0,d-Phe1,Tyr3] octreotide, a promising somatostatin analogue for radionuclide therapy. Eur J Nucl Med 24:368–371

Dolle F, Hinnen F, Lagnel B, Boisgard R, Sanson A, Russo-Marie F et al (2003) Radiosynthesis of a [^{18}F]fluoropyridine-based maleimide reagent for protein labelling. J Label Compd Radiopharm 46:S15

Fisher CA, Narayanaswami V, Ryan RO (2000) The lipid-associated conformation of the low density lipoprotein receptor binding domain of human apolipoprotein E. J Biol Chem 275:33601–33606

Glaser M, Arstad E (2007) "Click labeling" with 2-[18f]fluoroethylazide for positron emission tomography. Bioconjug Chem 18:989–993

Goldenberg DM (2001) The role of radiolabeled antibodies in the treatment of non-Hodgkin's lymphoma: the coming of age of radioimmunotherapy. Crit Rev Oncol Hematol 39:195–201

Hoppmann S, Steiniger B, Strobel K, Haase C, Pietzsch J (2006) In vivo catabolism of hypochlorite-modified low density lipoproteins (LDL): insights from small animal positron emission tomography studies. In: Pietzsch J (ed) Research developments in pathological biochemistry. Protein oxidation and disease. Research Signpost, Kerala, pp 197–219

Hultsch C, Berndt M, Bergmann R, Wuest F (2007) Radiolabeling of multimeric neurotensin(8–13) analogs with the short-lived positron emitter fluorine-18. Appl Radiat Isot 65:818–826

Jacene HA, Filice R, Kasecamp W, Wahl RL (2007) Comparison of 90Y-ibritumomab tiuxetan and 131I-tositumomab in clinical practice. J Nucl Med 48:1767–1776

Jamar F, Chapman PT, Manicourt DH, Glass DM, Haskard DO, Peters AM (1997) A comparison between 111In-anti-E-selectin mAb and 99Tcm-labelled human non-specific immunoglobulin in radionuclide imaging of rheumatoid arthritis. Br J Radiol 70:473–481

Karabatsos G, His N (1967) Structural studies by nuclear magnetic resonance-XI: conformations and configurations of oxime *O*-methyl ethers. Tetrahedron Lett 23:1079–1095

Krenning EP, Kwekkeboom DJ, Bakker WH, Breeman WA, Kooij PP, Oei HY, van Hagen M, Postema PT, de Jong M, Reubi JC et al (1993) Somatostatin receptor scintigraphy with [111In-DTPA-D-Phe1]- and [123I-Tyr3]-octreotide: the Rotterdam experience with more than 1,000 patients. Eur J Nucl Med 20:716–731

Li ZB, Wu Z, Chen K, Chin FT, Chen X (2007) Click chemistry for (18)F-labeling of RGD peptides and microPET imaging of tumor integrin alphavbeta3 expression. Bioconjug Chem 18:1987–1994

Lund-Katz S, Ibdah JA, Letizia JY, Thomas MT, Phillips MC (1988) A ^{13}C NMR characterization of lysine residues in apolipoprotein B and their role in binding to the low density lipoprotein receptor. J Biol Chem 263:13831–13838

Mäding P, Füchtner F, Wuest F (2005) Module-assisted synthesis of the bifunctional labelling agent *N*-succinimidyl 4-[(18)F]fluorobenzoate ([(18)F]SFB). Appl Radiat Isot 63:329–332

Marik J, Sutcliffe JL (2006) Click for PET: rapid preparation of [^{18}F]fluoropeptides using Cu(I) catalyzed 1,3-dipolar cycloaddition. Tetrahedron Lett 47:6881–6884

Pietzsch J, Bergmann R (2004) Analysis of 6-hydroxy-2-aminocaproic acid (HACA) as a specific marker of protein oxidation: the use of *N*(*O*,*S*)-ethoxycarbonyl trifluoroethyl ester derivatives and gas chromatography/mass spectrometry. Amino Acids 26:45–51

Pietzsch J, Bergmann R, Rode K, Hultsch C, Pawelke B, Wuest F, van den Hoff J (2004) Fluorine-18 radiolabeling of low-density lipoproteins: a potential approach for characterization and differentiation of metabolism of native and oxidized low-density lipoproteins in vivo. Nucl Med Biol 31:1043–1050

Ramenda T, Bergmann R, Wuest F (2007) Synthesis of 18F-labeled neurotensin(8–13) via copper-mediated 1,3-dipolar [3 + 2] cycloaddition reaction. Lett Drug Des Discov 4:279–285

Scott J (1989) The molecular and cell biology of apolipoprotein-B. Mol Biol Med 6:65–80

Segrest JP, Jones MK, Mishra VK, Anantharamaiah GM, Garber DW (1994) apoB-100 has a pentapartite structure composed of three amphipathic alpha-helical domains alternating with two amphipathic beta-strand domains. Detection by the computer program LOCATE. Arterioscler Thromb 14:1674–1685

Segrest JP, Jones MK, De Loof H, Dashti N (2001) Structure of apolipoprotein B-100 in low density lipoproteins. J Lipid Res 42:1346–1367

Shepherd J, Bedfordm DK, Morgan HG (1976) Radioiodination of human low density lipoprotein: a comparison of four methods. Clin Chim Acta 66:97–109

Shiue CY, Wolf AP, Heinfeld JF (1989) Synthesis of ^{18}F-labelled *N*-(p [18F]fluorophenyl)maleimide and its derivatives for labelling monoclonal antibody with ^{18}F. J Label Compd Radiopharm 26:287–289

Shively JE (2007) F-18 labeling for immuno-PET: where speed and contrast meet. J Nucl Med 48:170–172

Toyokuni T, Walsh JC, Dominguez A, Phelps ME, Barrio JR, Gambhir SS et al (2003) Synthesis of a new heterobifunctional linker, *N*-[4-(aminooxy)butyl]maleimide, for facile access to a thiol-reactive F-18-labeling agent. Bioconjug Chem 14:1253–1259

Vaidyanathan G, Zalutsky MR (2006) Synthesis of *N*-succinimidyl 4-[^{18}F]fluorobenzoate, an agent for labeling proteins and peptides with ^{18}F. Nat Protoc 1:1655–1661

Walker MA (1994) The Mitsunobu reaction—a novel method for the synthesis of bifunctional maleimid linkers. Tetrahedron Lett 35:665–668

Walker MA (1995) A high yielding synthesis of *N*-alkyl maleinides using a novel modification of the Mitsunobu reaction. J Org Chem 60:5352–5355

Wester HJ, Schottelius M (2007) Fluorine-18 labeling of peptides and proteins. Ernst Schering Res Found Workshop 62:79–111

Wester HJ, Hamacher K, Stöcklin G (1996) A comparative study of N.C.A. fluorine-18 labeling of proteins via acylation and photochemical conjugation. Nucl Med Biol 23:365–372

Wilson AA, Jin L, Garcia A, DaSilva JN, Houle S (2001) An admonition when measuring the lipophilicity of radiotracers using counting techniques. Appl Radiat Isot 54:203–208

Wiseman GA, White CA, Witzig TE, Gordon LI, Emmanouilides C, Raubitschek A, Janakiraman N, Gutheil J, Schilder RJ, Spies S, Silverman DH, Grillo-López AJ (1999) Radioimmunotherapy of relapsed non-Hodgkin's lymphoma with zevalin, a ^{90}Y-labeled anti-CD20 monoclonal antibody. Clin Cancer Res 53:3281s–3286s

Wuest F, Hultsch C, Bergmann R, Johannsen B, Henle T (2003) Radiolabelling of isopeptide *N* epsilon-(gamma-glutamyl)-L-lysine by conjugation with *N*-succinimidyl-4-[^{18}F]fluorobenzoate. Appl Radiat Isot 59:43–48

Yang C, Gu ZW, Yang M, Gotto AM Jr (1994) Primary structure of apoB-100. Chem Phys Lipids 67–68:99–104

Yang CY, Gu ZW, Yang M, Lin SN, Garcia-Prats AJ, Rogers LK, Welty SE, Smith CV (1999) Selective modification of apoB-100 in the oxidation of low density lipoproteins by myeloperoxidase in vitro. J Lipid Res 40:686–698