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Research Article

^{18}F-FESB: synthesis and automated radiofluorination of a novel ^{18}F-labeled pet tracer for β-amyloid plaques

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Summary

1-(2′-[^{18}F]-fluoroethoxy)-2,5-*bis*(4′-methoxystyryl)benzene (^{18}F-FESB) was synthesized in ~76% radiochemical yield (specific activity > 58.6 GBq or 1.58 Ci/μmol) in an Advanced Cyclotron Systems' automated synthesis unit by nucleophilic substitution of 1-(2′-toluenesulfonylethoxy)-2,5-*bis*(4′-methoxystyryl)benzene and purified using reversed phase column chromatography. When performed in the presence of ionic fluid, either 1-butyl-3-methylimidazolium tetrafluoroborate (Bmimtetrafluoroborate; BmimBF_4) or 1-ethyl-3-methylimidazolium trifluoromethanesulfonate (Emimtriflate; EmimTFMS), radiochemical yields of ^{18}F-FESB ranged from 17 to 76%. The radiochemical yields were consistently lower (~3–7%) in the absence of these ionic fluids. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: ^{18}F-labeled styryl benzene derivatives; automated synthesis; HPLC purification; PET marker of β-amyloid plaques; ionic fluids

Introduction

In spite of recent advances in the development of new radiopharmaceuticals for the clinical evaluation of Alzheimer's disease (AD), positron emission tomography (PET) and single photon emission computed tomography (SPECT) are not currently in routine use as diagnostic modalities.[1] Structural

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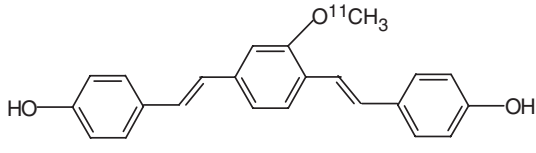
magnetic resonance imaging (MRI) and blood flow studies depend on gross changes in brain structure and function, and are 'secondary markers' since they diagnose functional alterations that have already occurred in the brain and may therefore not be ideal for following patients over time.[2,3] [^{18}F]fluorodeoxyglucose, a glucose analog, is a PET tracer that is used to evaluate metabolic disorders and, therefore, is an indirect tool to detect the presence and progression of AD.[4] However, the important roles of amyloid cascades and neurofibrillary tangles (NFTs) in the pathogenesis of AD necessitate the development of a biomarker that facilitates early diagnosis of the disease, allows clinico-pathological correlations of amyloid deposition at an early stage and is receptor specific so that it serves as a true diagnostic tool for anti-amyloid therapies.[5–7]

A recent study suggests that anti-amyloid therapies, when co-investigated in combination with positron PET or SPECT amyloid-imaging tracers, could facilitate *in vivo* evaluation of the efficacy of therapy in the aging human brain.[8] The efficacies of [^{11}C]-labeled Congo Red and [^{99m}Tc]-Chrysamine-G analogs[9] as markers of β-amyloid plaques suffer from marginal brain entry[10–13] which consequently makes the detection of the disease difficult. Recently, a study with ^{11}C-labeled [1,4-*bis*(4′-hydroxystyryl)-2-[^{11}C]methoxybenzene ([^{11}C]-methoxy-XO4), synthesized in ~6.7% radiochemical yield,



^{11}C-Methoxy XO4

Tg 2576APPsw and PS1(M146L) transgenic mice showed that it binds specifically to β-amyloid plaques.[14] Another ^{11}C-labeled analog of (*E,E*)-1-(3′,4′-dihydroxystyryl)-4-(3′-methoxy-4′-hydroxystyryl)benzene, synthesized in ~2.3% radiochemical yield, has been evaluated for its specific binding to amyloid cells and is found to exhibit high brain uptake and selective binding affinity to Aβ(1-40) fibrils *in vitro*.[15] Recently, a fluorine containing ^{13}C-labeled styrylbenzene derivative has been developed as a contrast agent for MRI to locate AD pathologies *in vivo*.[16]

The first human study of a novel amyloid-imaging PET tracer, named Pittsburgh Compound-B (PIB), in a limited number of patients diagnosed with mild AD, further supports the role of a PET AD diagnostic radiotracer in AD therapy management. PIB typically showed marked retention in the areas known to contain large amounts of amyloid deposits in AD, most prominently in frontal cortex (1.94-fold, $p = 0.0001$), parietal (1.71-fold, $p = 0.0002$),

temporal (1.52-fold, $p = 0.002$), and occipital (1.54-fold, $p = 0.002$) cortex and the striatum (1.76-fold, $p = 0.0001$) in comparison to healthy patients.[17]

Low radiochemical yields but promising efficacy and pharmacokinetic properties of ^{11}C-XO4 and other PET diagnostic agents in providing quantitative information on amyloid deposits in living subjects support the development of an ^{18}F-labeled XO4 PET. Such a tracer would be expected to possess similar binding to β-amyloid proteins and would have a longer observation window due to the longer decay time. The present work describes a structural modification of XO4 where radioactive fluorine is incorporated nucleophilically to produce a high specific activity radiopharmaceutical without a major alteration in the basic structure of XO4. We now report the synthesis of 1-(2′-fluoroethoxy)-2,5-*bis*(4′-methoxystyryl)benzene, **7**, and its ^{18}F-labeled analog **8**.

Experimental

Materials and methods

2,5-Dimethylphenol was purchased from Sigma-Aldrich Chemical Company Inc., USA. All chemicals were reagent grade and were used without further purification unless otherwise noted. Emimtriflate (EmimTFMS) and Bmimtetrafluoroborate ($BmimBF_4$) were purchased from Fluka, Switzerland. Thin-layer chromatography (TLC) was carried out on Kieselgel 60 F254 (Merck) silica gel coated micro TLC plates (2.5×7.5 cm). Column chromatography was performed using silica gel 60 (230–400) Mesh ASTM, 0.040–0.063 mm supplied by Rose Scientific Ltd. (Edmonton, Canada). IR spectra were recorded on a Perkin-Elmer 700 spectrometer. ^{1}H, ^{13}C and ^{19}F NMR spectra were obtained using a Bruker AM-300 spectrometer. The chemical shifts are quoted in δ ppm with respect to tetramethylsilane (^{1}H and ^{13}C) and hexafluorobenzene (C_6F_6, as an external standard for ^{19}F) and the coupling constants (J) are reported in Hertz. ^{13}C-NMR spectral assignments were aided by the J-MOD technique. All NMR spectra were acquired by preparing the samples in $CDCl_3$ solution. When necessary, high-resolution mass spectra (HRMS) were obtained *in lieu* of elemental analysis and were acquired on a Kratos MS50 MASPEC system (Manchester, UK).

The automated synthesis unit (ASU) employed for the manufacture of ^{18}F-FESB was purchased from Advanced Cyclotron Systems (Richmond, Canada). It performs unit operations including the movement of liquids and gases (vacuum, pressure), valve actuation and heating systems on a time-dependent basis through a well-defined flow path, all under computer (CPU) control. Feedback control, monitoring and diagnostic functions are built into the operation using various sensor-based systems. The purification of the synthesized radiopharmaceutical involved the use of a Beckman HPLC system

Table 1. Radiofluorination reaction parameters for ^{18}F-FESB

Reaction #	Ionic fluid[a]	Amount (μl)	Reaction time (min)	Purified RCY (%)	Specific activity (Ci/μmol)
1	$BmimBF_4$	20	12	16.9	> 0.45
2	$BmimBF_4$	20	8	43.2	> 2.19
3	$BmimBF_4$	50	9	76.9	> 1.58
4	EmimTFMS	50	8	39.6	> 1.57
5	EmimTFMS	50	8	25.5	> 0.70
6	EmimTFMS	50	8	42.5	> 0.49
7	None	n/a	4	3.0	NC
8	None	n/a	8	7.0	NC

[a] Acetonitrile (2.0 ml) and anhydrous DMF (50 μl) were used as solvent in reactions 1–8. n/a = not applicable; NC = not calculated.

equipped with guard column, reversed phase C-18 (10ODS3; 25 × 0.9 cm) Whatman column, UV detector (wavelength 370 and 254 ηm) and a radiodetector. Impure radiolabeled mixture was purified using ethanol–water (75:25, v/v) as an eluent at a flow rate of 1.5 ml/min. The purified ^{18}F-FESB was collected using a sterile manifold that was attached to the terminal HPLC outlet and equipped with a sterile neutral alumina cartridge (to remove any traces of unreacted fluoride) and a Millipore LG (0.45 μm pore size filter). The specific activity was interpolated from a calibration curve plotting peak area as a function of cold FESB (1 μg–10 ηg). The minimum detectable amount for FESB peak under present chromatographic conditions was 10 ηg. The radiochemical yield of ^{18}F-FESB reached to ~77% (specific activity > 58.6 GBq or 1.58 Ci/μmol) when Bmimtetrafluoroborate (50 μl) was used in the radiosynthesis (Table 1). However, the radiochemical yield of ^{18}F-FESB varied between 16.9 and 76.9% depending on the nature and amount of the ionic fluid used and the labeling time.

Chemistry

2-[(Tert-butyldiphenylsilyl)oxy]-1,4-dimethylphenol **1**

2,5-Dimethylphenol (3.73 g, 30.5 mmol) and imidazole (2.1 g, 30.9 mmol) were added to anhydrous dimethylformamide (DMF) (18 ml) under stirring at 22°C. After 10 min, *tert*-butyldiphenylsilyl chloride (TBDPS chloride, 9.3 ml, 36.3 mmol) was added to this solution and stirring was continued for an additional 16 h at this temperature. The TLC at this time showed complete disappearance of the starting phenol and a new product appeared at a higher R_f value. The reaction mixture was poured into water (200 ml) and the water layer was extracted with methylene chloride (150 ml × 3). The combined organic phase was washed with water (50 ml), dried over anhydrous sodium sulfate (Na_2SO_4), filtered and evaporated. The impure residue was purified by

silica gel column chromatography using hexanes–ethyl acetate (20:1, v/v) as eluent to afford colorless crystals (9.67 g, 87.9%) of **1**; mp 47–48°C; ^{1}H-NMR ($CDCl_3$) δ 7.79–7.76 (m, 4H, Ar*H* of TBDPS), 7.50–7.41 (m, 6H, Ar*H* of TBDPS), 7.07 (d, $J = 7.6$ Hz, 1H, *H-5″* of benzene), 6.64 (d, $J = 7.6$ Hz, 1H, *H-6″* of benzene), 6.28 (s, 1H, *H-3″* of benzene), 2.40 and 1.99 (two s, 6H, each for 3H of two CH_3), 1.16 (s, 9H, C(CH_3)$_3$); ^{13}C-NMR ($CDCl_3$) δ 153.5, 136.0, 135.4, 133.2, 130.4, 129.7, 127.7, 125.1, 121.4, 119.3, 26.7 (s, C(*C*H$_3$)$_3$), 20.9 (s, *C*H$_3$Ph), 19.7(s, *C*(CH$_3$)$_3$), 16.8 (s, *C*H$_3$Ph); analytically calculated for $C_{24}H_{28}OSi$: C, 79.95; H, 7.83. Found: C, 79.36; H, 8.02.

1-[(Tert-butyldiphenylsilyl)oxy]-2,5-bis(bromomethyl)benzene **2**

Benzoyl peroxide (23.4 mg) was added to a solution of **1** (4.37 g, 11.7 mmol) and *N*-bromosuccinimide (4.6 g, 25.8 mmol) in carbon tetrachloride (CCl_4) (26 ml) and the mixture was heated under reflux for 110 min. Afterwards, the reaction mixture was cooled, filtered and the solvent was evaporated on a rotary evaporator. The impure viscous residue was purified on a silica gel column using hexanes–methylene chloride (100:3; v/v) as eluent to give a white solid (3.0 g, 48.2%); mp 91–92°C; ^{1}H-NMR ($CDCl_3$) δ 7.77–7.72 (m, 4H, Ar*H* of TBDPS), 7.53–7.27 (m, 6H, Ar*H* of TBDPS), 7.30 (d, $J =$ 7.6 Hz, 1H, *H-5″* of benzene), 6.86 (d, $J = 7.6$ Hz, 1H, *H-6″* of benzene), 6.40 (s, 1H, *H-3″* of benzene), 4.70 (s, 2H, BrCH_2), 4.05 (s, 2H, BrCH_2), 1.17 (s, 9H, C(CH_3)$_3$); ^{13}C-NMR ($CDCl_3$) δ 153.7, 139.2, 135.4, 135.3, 131.8, 131.6, 127.9, 121.6, 119.8, 118.6, 118.0, 32.6, 28.9, 26.5, 19.6; analytically calculated for $C_{24}H_{26}Br_2OSi$: C, 55.61; H, 5.06. Found: C, 53.95; H, 4.70.

1-[(Tert-butyldiphenylsilyl)oxy]-2,5-bis(diethylphosphonatomethyl)benzene **3**

A mixture of **2** (1.0 g, 1.89 mmol) and triethylphosphite (0.63 g, 3.8 mmol) was stirred and heated at 160°C for 4 h. The reaction mixture was cooled to 22°C and excess triethylphosphite was evaporated under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane–methanol (100:2; v/v) as eluent that afforded **3** as a yellow oil (0.74 g, 61.0%); ^{1}H-NMR ($CDCl_3$δ)- 7.72–7.68 (m, 4H, Ar*H* of TBDPS), 7.44–7.31 (m, 6H of Ar*H* of TBDPS), 7.29 (d, $J = 7.8$ Hz, 1H, *H-5″* of benzene), 6.79 (d, $J = 8.0$ Hz, 1H, *H-6″* of benzene), 6.40 (s, 1H, *H-3″* of benzene), 4.12–3.94 (m, 4H, 2 × OCH_2), 3.79–3.58 (m, 4H, 2 × OCH_2), 3.39 (d, $J_{gem} = 22$ Hz, 2H, PCH_2), 2.67 (d, $J_{gem} = 22$ Hz, 2H, PCH_2), 1.24 (t, $J = 6.9$ Hz, 6H, 2 × CH$_2$CH_3),1.08 (s, 9H, C(CH_3)$_3$), 1.02 (t, $J = 6.9$ Hz, 6H, 2 × CH$_2$CH_3); ^{13}C-NMR ($CDCl_3$δ)- 153.0–153.2 (m), 135.4, 132.3, 131.2–131.1 (m), 129.9, 127.7, 122.4, 120.4, 120.2, 120.1, 61.8, −61.7, 33.27 (d, $J = 137.33$ Hz, *C*H$_2$P), 26.84 (d, $J = 140.62$Hz, *C*H$_2$P), 26.57 (CH$_3$ of t-butyl)19.5, 16.49–16.26 (m); analytically calculated for $C_{32}H_{49}O_7P_2Si$: C, 60.74; H, 7.33. Found: C, 59.56; H, 7.29.

2,5-Bis(4′-methoxystyryl)phenol **4**

Sodium hydride (0.92 g, 23 mmol, 60% in mineral oil) was added to a solution of **3** (3.0 g, 4.65 mmol) in anhydrous diomethoxyethyl ether (10 ml) at 0°C. After stirring the mixture at 22°C for 30 min, *p*-anisaldehyde (1.26 g, 9.26 mmol) was added to it and the stirring was continued for an additional 16 h at 85°C. The reaction mixture was cooled and then poured into 50 ml of water. The product was extracted in dichloromethane (150 ml × 3) and the combined organic phase was washed with water (50 ml), dried over anhydrous Na_2SO_4, filtered and evaporated. The impure product, so obtained, was purified on a silica gel column using hexanes–ethyl acetate(8:1) as eluent that gave **4** as a yellow solid (1.21 g, 73.8%); mp 238–240°C; ^{1}H-NMR ($CDCl_3\delta$)- 9.76 (br, 1H, O*H*), 7.81 (d, $J = 8.3$ Hz, 2H, aromatics), 7.54 (d, $J = 8.2$ Hz, 1H, *H-5″* of benzene), 7.49–7.45 (two d, $J = 8.4$Hz, 4H, Ar*H*), 7.35 (s, 1H, *H-3″* of benzene), 7.24 (d, $J = 8.0$ Hz, 1H, *H-6″* of benzene), 7.21 (d, $J = 16.3$, 2H, C*H* = C*H*), 7.11–6.92 (m, 6H, 4H, aromatics and 2H of C*H* = C*H*), 3.77 (s, 3H, OCH_3) and 3.40 (s, 3H, OCH_3); ^{13}C-NMR ($CDCl_3\delta$)- 158.9, 154.7, 137.2, 130.4, 129.6, 127.1–127.8 (m), 126.3, 126.0, 123.4, 121.1, 117.6, 114.4, 113.2, 55.2; HR-MS (M^+) m/z calculated for $C_{24}H_{22}O_3$ 358.15689, measured 358.15755.

1-(2′-Bromoethoxy)-2,5-bis(4′-methoxystyryl)benzene **5**

Sodium hydride (22 mg, 0.55 mmol, 60% in mineral oil) was added to a solution of **4** (100 mg, 0.28 mmol) in anhydrous DMF (1 ml) at 0°C. The temperature of the reaction mixture was slowly brought up to 22°C and stirring was continued for an additional 10 min. 1-Bromoethyltoluenesulfonate[18] (114 mg, 0.36 mmol) was added to this reaction mixture at this time and the mixture was allowed to stir at this temperature for 48 h. Afterwards, the reaction mixture was poured into cold water (15 ml) and the solution was extracted with dichloromethane (20 ml × 3). The combined organic phase was washed with water (10 ml), dried over anhydrous Na_2SO_4 and filtered. The solvent was removed in vacuo and the residue was purified by silica gel column chromatography using hexanes–ethyl acetate (9:1) as eluent to afford **5** as a yellow solid (55 mg, 42.2%); mp 137–140°C; ^{1}H-NMR ($CDCl_3$)- 7.61 (d, $J = 7.9$ Hz, 1H, *H-5″* of benzene), 7.51 (two d, $J = 8.5$ and 8.8 Hz, 4H, *H-3′* and *H-5′* of two anisyl moieties), 7.35 (d, $J = 16.8$ Hz, 1H, *vinyl H*), 7.30 (s, 1H, *H-3″* of benzene), 7.26 (d, $J = 17.5$ Hz, 1H, *vinyl H*), 7.22 (d, $J = 8.0$ Hz, 1H, *H-6″* of benzene), 7.14 (d, $J = 15.9$ Hz, 1H, *vinyl H*), 7.05 (d, $J = 16.5$ Hz, 1H, *vinyl H*), 6.95 (d, $J = 8.5$ Hz, 4H, *phenyl*), 4.46 (t, $J = 5.1$ Hz, 2H, CH_2), 3.94 (t, $J = 5.2$ Hz, 2H, CH_2), 3.77 (s, 6H, 2 × OCH_3); HS-MS (M^+) m/z calculated for $C_{26}H_{25}BrO_3$ 464.098706, measured 464.09644.

1-(2′-Toluenesulfonylethoxy)-2,5-bis(4′-methoxystyryl)benzene **6**

Sodium hydride (0.22 g, 5.5 mmol, 60% in mineral oil) was added to a solution of **4** (0.95 g, 2.7 mmol) in anhydrous DMF (8 ml) at 0°C. The temperature of the reaction was slowly raised to 22°C over a duration of 30 min and, then, 1,2-ditoluenesulfonylethane[19] (2.42 g, 6.5 mmol) was added to this reaction mixture. The stirring was continued at this temperature for 4 days, and then the reaction mixture was poured into cold water (100 ml). The water layer was extracted with dichloromethane (150 ml × 3), the combined organic phase was washed with water (100 ml) and dried over anhydrous Na_2SO_4. The solution was filtered and the solvent was removed on a rotary evaporator. The impure product was purified by silica gel column chromatography using hexanes–ethyl acetate (9:1) as eluent that afforded a yellow solid. Recrystallization from dichloromethane–hexanes gave 0.73 g (48.2%) of pure **6**; mp 156–159°C; ^{1}H-NMR ($CDCl_3\delta$)- 7.82 (d, $J = 8.2$ Hz, 2H, *H-2* and *H-6* of toluene), 7.55 (d, $J = 8.2$ Hz, 1H, *H-5″* of benzene), 7.47–7.49 (m, 4H, aromatic), 7.26 (s, 1H, *H-3″* of benzene), 7.24 (d, $J = 15.9$ Hz, 1H, *vinyl H*), 7.11 (d, $J = 9.4$ Hz, 1H, *H-6″* of benzene), 7.08 (d, $J = 16.8$ Hz, 1H, *vinyl H*), 7.04 (d, $J = 16.8$ Hz, 1H, *vinyl H*), 6.92 (d, $J = 16.2$ Hz, 1H, *vinyl H*), 6.94–6.89 (m, 6H, *4H′*of anisyl and *H-3* and *H-5* of toluene) 4.48 (t, $J = 4.3$Hz, 2H, CH_2), 4.30 (t, $J = 4.3$ Hz, 2H, CH_2), 3.85, 3.83 (two s, 6H, 2 × OCH_3), 2.37 (s, 3H, $ArCH_3$), 2.37 (s, 3H, tolyl CH_3); ^{13}C-NMR ($CDCl_3\delta$)- 159.3, 159.1, 155.3, 145.2, 137.7, 132.5, 132.3, 130.7, 129.9, 127.9, 126.4, 124.9, 121.0, 120.0, 118.9, 113.9, 110.0, 102.7, 66.7, 55.3, 21.7; LR-MS (MNa^+) *m/z* calculated for $C_{33}H_{32}O_6S$ 579.18, measured 579.20.

1-(2′-Fluoroethoxy)-2 5-bis(4′-methoxystyryl)benzene **7**

A solution of **6** (70 mg; 0.14 mmol) in anhydrous DMF (700 µl), methylene chloride (700 µl) and acetonitrile (2 ml) was heated to 35°C gently under stirring and an inert atmosphere, and at this point, 1-ethyl-3-methylimidazolium trifluoromethanesulfonate (700 µl; 3.7 mmol) was added to the reaction mixture. The contents were stirred and transferred to the reaction vial containing vacuum dried tetra-butyl ammonium fluoride (175 mg; 0.7 mmol). The reaction flask was flushed with argon and the temperature of the reaction vessel was raised to 90°C. The heating was continued for 60 min and, then, the solvent was removed by rotary evaporation. The impure product was subjected to flash column chromatography using gradients of hexanes: ethyl acetate, (starting with 3.5:1 and ending with 2:1 ratios) as eluent to obtain 4.6 mg (9%) of pure **7** as a semi solid along with unreacted precursor **6** (35 mg); ^{1}H-NMR ($CDCl_3\delta$)- 7.58 (d, $J = 7.9$ Hz, 1H, Ar*H, H-5″*of benzene), 7.45 (two d, $J = 7.9$ Hz, 4H, Ar*H′* of anisyl moiety), 7.40 (d, $J = 16.5$ Hz, 1H, *vinyl H*), 7.38 (s, 1H, *H-3″* of benzene), 7.14 (d, $J = 16.8$ Hz, 1H, *vinyl H*), 7.07 (d, $J = 16.5$ Hz, 1H, *vinyl H*), 7.04 (d, $J = 9.2$ Hz, 1H, *H-6″* of benzene), 7.02

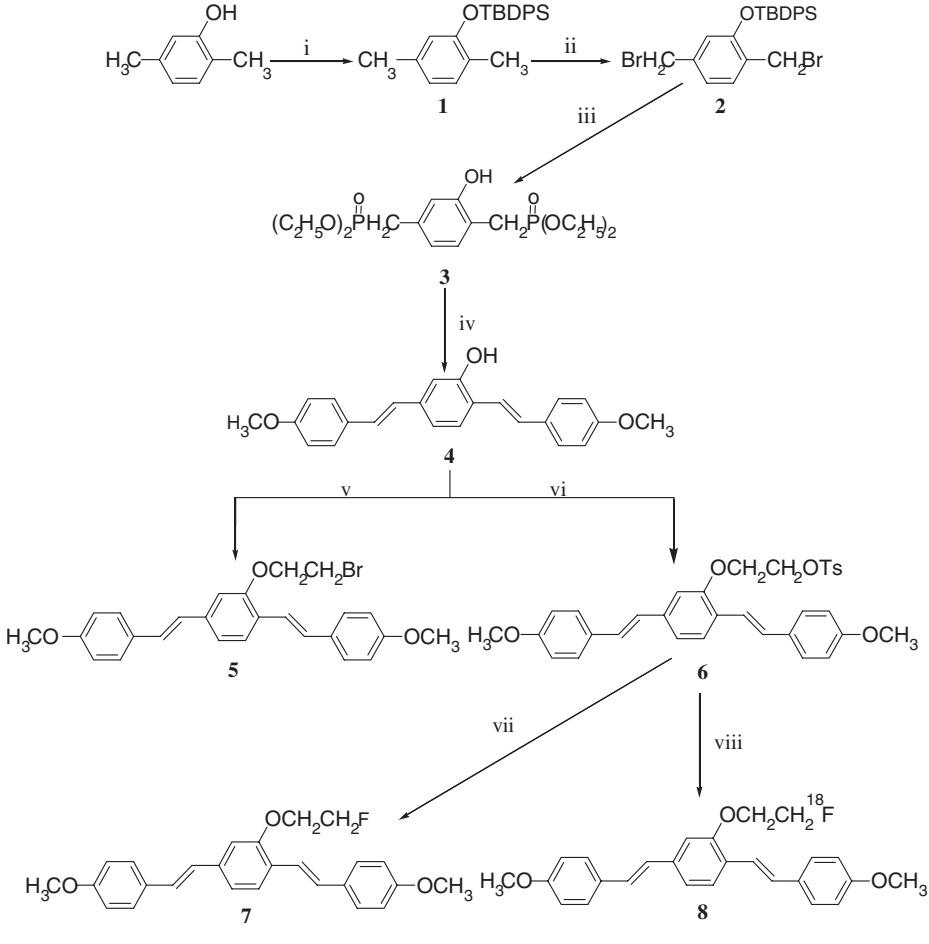
(d, $J = 16.8$ Hz, 1H, *vinyl H*), 6.91 (d, 4H, aromatic), 4.83 (d, $J_{F-CH_2} = 47.3$ Hz, of d, $J_{gem} = 10.0$ Hz, of t, $J_{CH_2-CH_2} = 4.3$ Hz, 1H, CH_2F), 4.58 (d, $J_{F-CH_2} = 47.3$ Hz, of d, $J_{gem} = 10.0$ Hz, of t, $J_{CH_2-CH_2} = 3.7$ Hz, 1H, CH_2F), 4.36 (d, $J_{F-OCH_2} = 29.3$ Hz, of d, $J_{gem} = 10.0$ Hz, of t, $J_{CH_2-CH_2} = 3.7$ Hz, 1H, OCH_2), 4.28 (d, $J_{F-OCH_2} = 23.9$ Hz, of d, $J_{gem} = 10.0$ Hz, of t, $J_{CH_2-CH_2} = 4.0$ Hz, 2H, OCH_2), 3.85 (s, 6H, $2 \times$ OCH_3); ^{19}F NMR ($CDCl_3\delta + C_6F_6$)- 25.58 (dddd, $J_{CH_2-F} = 47.3$ Hz, $J_{OCH_2-F} = 24.0$ and 29.3 Hz); ^{13}C-NMR ($CDCl_3\delta$)- 159.3, 159.1, 155.3, 145.2, 137.7, 132.5, 132.3, 130.7, 129.9, 127.9, 126.4, 124.9, 121.0, 120.0, 118.9, 113.9, 110.0, 102.7, 80.5 (d, $J_{F-CH_2} = 173.6$ Hz, *C*H$_2$F), 68.36 (d, $J_{F-OCH_2} = 20.9$ Hz, O*C*H$_2$), 66.7, 55.3, 21.7; EI-MS (M^+) m/z calculated for $C_{26}H_{25}FO_3$ 404.1787, measured 404.1776 (100%).

Radiochemistry: automated radiosynthesis of 1-(2′-[^{18}F]-fluoroethoxy)-2,5-bis(4′-methoxystyryl) benzene [^{18}F]-FESB

The synthesis of ^{18}F-FESB was carried out in the ASU housed in a commercially available (Comecer, Castelbolognese, Italy) radioisotope isolator unit designed to confer a Class 100 operating environment during ASU operation. The inlet and exhaust lines of the ASU are attached to nitrogen. The inlet pressure of nitrogen is regulated at 5–8 psi during the synthesis that in conjunction with the evacuation system controls the movement of reagents and maintains inert atmosphere during the synthesis. Time-controlled selective valves are operated by the Lookout for Win32 Version 4.5.1 software that is provided by National Instruments, USA. All required reagents were installed onto the ASU prior to the start of the synthesis. Acetonitrile, eluent (K-2.2.2/K_2CO_3, 22 mg/7 mg dissolved in 0.8 ml, 56:44, v/v, acetonitrile/water solution), tosylate precursor (3.8–5.0 mg, 6.8–9 μmol) pre-dissolved in 2 ml acetonitrile and a solution of ethanol/water (50:50, 1.5 ml) were prepared in color coded crimped vials that were then placed at their respective points of attachment in the ASU. The reactor vial was placed on the heating block and the vacuum pump and the trap were attached to the ASU. The synthesis was initiated after confirming all necessary prerequisites for the radiosynthesis.

The currently employed radiochemical method for the synthesis of ^{18}F-FESB was optimized and adapted for automated radiopharmaceutical processing. No-carrier-added (NCA) radiofluoride was first adsorbed from its $H_2^{18}O$ target solution onto the (K_2CO_3 conditioned) anion exchange resin (QMA) and $H_2^{18}O$ ($\sim$98%) was recovered for potential future reprocessing and re-use. This was followed by adding eluent (K-2.2.2, 22 mg and potassium carbonate, 7 mg in 0.45 ml acetonitrile and 0.35 ml water) onto the QMA cartridge to elute K^{18}F from the resin rapidly, at room temperature, into the reaction vial. Using preset heat (95°C), vacuum (0.8 bar) conditions and nitrogen carrier gas flow (5–8 psi, 20–30 ml/min), the solvent was removed.

Two azeotropic distillations using repeated additions of dry acetonitrile (1 ml each) were used to remove the residual traces of water. At the end of this process, tosylate precursor, pre-dissolved in anhydrous acetonitrile (2 ml) and $BmimBF_4$ (20–50 µl) or EmimTFMS (50 µl) was added to the reaction vial. Nucleophilic substitution of the tosylate by radiofluoride (potassium–Kryptofix–radiofluoride complex) in anhydrous acetonitrile at 85°C, depending on the nature and amount of the ionic fluid and the reaction time, afforded radiofluorinated FESB (^{18}F-FESB) in 17–76% radiochemical yield (Scheme 1). The impure ^{18}F-FESB reaction mixture was dissolved in 1.0 ml



i=TBDPSCl, Imidazole; ii=NBS/CCl_4; iii=$P(OC_2H_5)_3$; iv=NaH/p-anisaldehyde, v=NaH/p-$O_2NC_6H_4O_2SOCH_2CH_2Br$; vi=$(CH_2OSO_2C_6H_4CH_3)_2$; vii=Emimtriflate/$Bu_4NF$/90°C/60 min and viii = ionic fluid/$K^{18}F$/K-2.2.2./85°C/8–12 min

Scheme 1. Synthesis route to 1-(2′-fluoroethoxy)-2,5-*bis*(4′-methoxystyryl)benzene (FESB), 7, and ^{18}F-FESB, 8

of 50% aqueous ethanol and then dispensed from the ASU into a shielded product vial.

This radiofluorinated reaction mixture solution, dispensed from the ASU, was injected onto HPLC and purified using ethanol–water (75:25, v/v) as an eluent at a flow rate of 1.5 ml/min. Pure ^{18}F-FESB, eluted from the HPLC column at a retention time of 19.5 min (>90% purity; specific activity 16.5–81.2 GBq or 0.45–2.19 Ci/μmol) and was collected in a sterile multi-dose vial (20 ml) containing sterile saline (10 ml) after passing through a sterile neutral alumina cartridge. Depending on the nature of ionic fluid and the reaction time used in the fluorination ^{18}F-FESB was isolated with specific activity ranging between 16.5 and 81.2 GBq/μmol. The results are presented in Table 1.

Results and discussion

The synthesis of 1-(2′-fluoroethoxy)-2,5-*bis*(4′-methoxystyryl)benzene **7** and its tosylate precursor started with 1-*O*-*tert*-butyldiphenylsilyl-2,5-dimethylphenol **1** (Scheme 1). 2,5-Dimethylphenol was silylated with *tert*-butyldiphenylsilane in anhydrous pyridine since it withstands the hard acidic conditions that exist during the bromination step. Bromination of the methyl groups at C-2 and C-5, using *N*-bromosuccinimide, proceeded through free a radical mechanism that was assisted by benzoyl peroxide, to afford 1-[(*tert*-butyldiphenylsilyl)oxy]-2,5-*bis*(bromomethyl)benzene **2** in 48% yield. Reaction of the bromomethyl product **2** at 85°C, with triethylphosphite gave 1-[(*tert*-butyldiphenylsilyl)oxy]-2,5-*bis*(diethylphosphonatomethyl)benzene **3** in satisfactory yield (61%). The formation of **3** was supported by observing a strong phosphorous coupling ($J_{P-CH_2} = 137.3-140.6$ Hz) with methylenic carbons in the ^{13}C NMR spectrum. The conversion of this diethylphosphonate derivative **3** to the corresponding 2,5-*bis*(4-methoxystyryl)phenol, **4**, progressed smoothly (74% yield). This reaction required strongly basic conditions and, therefore, led to desilylation of the molecule, rendering the hydroxyl group at C-1 aromatic position of the benzene unprotected and free for substitution with an alkyl chain. The formation of the 2,5-*bis* styryl linkage was confirmed by the resonances from the newly formed vinyl protons. The introduction of the toluene sulfonyloxyethyl chain at the 1′-OH group was initially attempted by condensing **4** with 1-bromoethyltoluenesulfonate in the presence of sodium hydride, but this led to condensation between toluenesulfonyl and the OH moiety, resulting in the formation of 1-(2′-bromoethoxy)-2,5-*bis*(4′-methoxystyryl)benzene **5** (42%). To overcome the formation of this unexpected product, 1,2-*bis*-toluenesulfonyloxyethane was used as tosylating reagent. Tosylation of the free hydroxyl group using 1,2-*bis*-toluenesulfonyloxyethane in anhydrous DMF afforded 1-(2′-toluenesulfonylethoxy)-2,5-*bis*(4′-methoxystyryl)benzene **6** as a yellow solid in 48% yield.

The synthesis of 1-(2′-fluoroethoxy)-2, 5-*bis*(4′-methoxystyryl)benzene **7** and its radiofluorinated ^{18}F-analog **8** started from **6** and involved nucleophilic substitution of the toluenesulfonyl group in **6** with fluoride. The tosylate **6**, on reaction with tetrabutyl ammonium fluoride at 100°C, gave 1-(2′-fluoroethoxy)-2,5-*bis*(4′-methoxystyryl)benzene **7** (9%). It was observed that more than half of the tosylated precursor did not undergo fluorination and was recovered during the chromatographic purification of **7**. The relatively low chemical yield for **7** may also partially be due to the formation of 1-fluoroethyltoluenesulfonate that was present as a secondary fluorinated product. The formation of this side product could be due to competitive fluorination at α-ethyl carbon of the ethylsulfonate chain. Continuous electronic delocalization resonances associated with 3′-*O* would exert a strong *–I* effect and might be a potential factor for this secondary fluorination. Substitution of fluorine in **7** is further evident by *F–H* (47.3 Hz) and *F–C* (173.6 Hz) coupling constants in its ^{1}H and ^{13}C NMR spectra. ^{19}F NMR spectra also displayed a dddd at a chemical shift of δ 25.58 with desired *F–H* coupling constants that collapsed upon proton decoupling. Fluorine coupling with 3-ethoxy protons and carbon was also observed in related spectra.

The synthesis of ^{18}F-labeled **8** was initially attempted using the conventional Kryptofix 2.2.2/K^{18}F complex at elevated temperatures, but this provided only partial success. Recent reports on ionic fluid-assisted radiofluorination[20,21] indicate that a significant increase in the reactivity of fluoride ion could be expected due to unmasking of the fluoride ion that is most likely a factor for poor labeling efficiency in radiofluorination reactions. Incorporation of an appropriate ionic fluid in our radiofluorination reactions supported this observation and a significant improvement in the labeling efficiency of ^{18}F-FESB was achieved (Table 1). The radiochromatogram of radiofluorinated mixture (Figure 1) featured a number of radioactive peaks that eluted between 7 and 10 min, in addition to ^{18}F-FESB at 19.5 min. The retention times for Emimtriflate and Bmimtetrafluoroborate are 7.3 and 6.9 min, respectively, therefore, the radioactive peaks appearing between 7 and 10 min in the radiochromatogram of the labeled ^{18}F-FESB mixture correspond, most likely, to unreacted fluoride, and radiofluorinated $BmimBF_4$ or EmimTFMS salts formed due to the exchange of radioactive fluorine with ionic fluid. An uncharacterized radioactive component appearing between 11 and 18 min is visible as a hump in the chromatogram (Figure 1) but the purity of ^{18}F-FESB is not compromised during HPLC purification of the reaction mixture by collecting ^{18}F-FESB after 30 s of the peak start. Normally, fluoride ion acts as a strong base that initiates elimination of the leaving (tosylate) group, and is 'masked', and therefore is a weak nucleophile.[22] The competition of these competing reactions results in relatively low radiochemical yield of the desired product. We observed that the radiofluorination of **6** to form **8** proceeded in

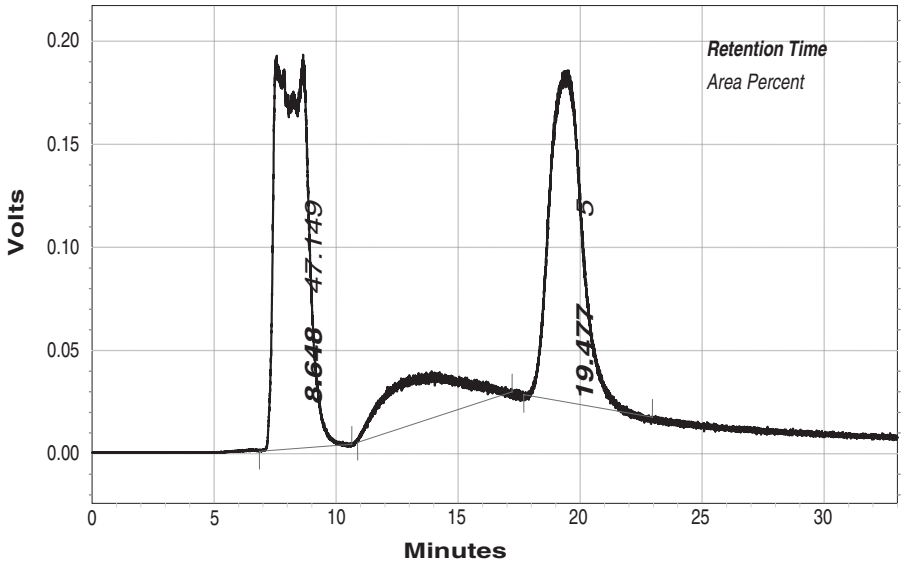


Figure 1. HPLC elution profiles of ^{18}F-FESB on a Partisil 10 ODS-3 reversed phase column using ethanol:water (75:25; 1.5 ml/min) as eluent. ^{18}F-FESB appears at a retention time of 19.5 min

low radiochemical yield (3–7%), but the use of $BmimBF_4$ or EmimTFMS as ionic solvent during radiofluorination increased the radiochemical yield to 76%. It was also observed that ^{18}F-FESB was obtained in better radiochemical yields with higher specific activity when the radiofluorination was assisted by $BmimBF_4$ (reactions 2 and 3 compared with reactions 4–6, Table 1) indicating that during the radiofluorination $BmimBF_4$ presumably undergoes less radiolysis in comparison to EmimTFMS. This radiolysis is further supported by the fact that use of a larger amount of ionic fluid led to ^{18}F-FESB with lower specific activities (compare reaction 2 with 3–6). Also, an optimum labeling time was found to be 8 min for this product since a longer reaction time led to possible decomposition resulting in to lower radiochemical yield (reaction 1, Table 1).

Conclusion

The synthesis of ^{18}F-FESB, a fluorinated Congo Red derivative, has been developed using an ASU. It was observed that use of 'ionic fluids' in the radiochemical synthesis significantly enhanced the labeling efficiency of the product. An attempt to correlate radiochemical yield of **8** to varying amounts of the ionic fluids $BmimBF_4$ or EmimTFMS were inconclusive. The biological properties of ^{18}F-FESB are currently under investigation.

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