Steroids 76 (2011) 1331–1340

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Synthesis of [^{11}C]PBR06 and [^{18}F]PBR06 as agents for positron emission tomographic (PET) imaging of the translocator protein (TSPO)

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ARTICLE INFO

Article history:
Received 27 April 2011
Received in revised form 27 June 2011
Accepted 29 June 2011
Available online 3 July 2011

Keywords:
[^{11}C]PBR06
[^{18}F]PBR06
The translocator protein 18 kDa (TSPO)
Positron emission tomography (PET)
Neuroinflammation imaging
Tumor imaging

ABSTRACT

The translocator protein 18 kDa (TSPO) is an attractive target for molecular imaging of neuroinflammation and tumor progression. [^{18}F]PBR06, a fluorine-18 labeled form of PBR06, is a promising PET TSPO radioligand originally developed at NIMH. [^{11}C]PBR06, a carbon-11 labeled form of PBR06, was designed and synthesized for the first time. The standard PBR06 was synthesized from 2,5-dimethoxybenzaldehyde in three steps with 71% overall chemical yield. The radiolabeling precursor desmethyl-PBR06 was synthesized from 2-hydroxy-5-methoxybenzaldehyde in five steps with 12% overall chemical yield. The target tracer [^{11}C]PBR06 was prepared by *O*-[^{11}C]methylation of desmethyl-PBR06 with [^{11}C]CH_3OTf in CH_3CN at 80 °C under basic condition and isolated by HPLC combined with SPE purification with 40–60% decay corrected radiochemical yield and 222–740 GBq/μmol specific activity at EOB. On the similar grounds, [^{18}F]PBR06 was also designed and synthesized. The previously described Br-PBR06 precursor was synthesized from 2,5-dimethoxybenzaldehyde in two steps with 78% overall chemical yield. A new radiolabeling precursor tosyloxy-PBR06, previously undescribed tosylate congener of PBR06, was designed and synthesized from ethyl 2-hydroxyacetate, 4-methylbenzene-1-sulfonyl chloride, and *N*-(2,5-dimethoxybenzyl)-2-phenoxyaniline in four steps with 50% overall chemical yield. [^{18}F]PBR06 was prepared by the nucleophilic substitution of either new tosyloxy-PBR06 precursor or known Br-PBR06 precursor in DMSO at 140 °C with K[^{18}F]F/Kryptofix 2.2.2 for 15 min and HPLC combined with SPE purification in 20–60% decay corrected radiochemical yield, >99% radiochemical purity, 87–95% chemical purity, and 37–222 GBq/μmol specific activity at EOB. Radiosynthesis of [^{18}F]PBR06 using new tosylated precursor gave similar radiochemical purity, and higher specific activity, radiochemical yield and chemical purity in comparison with radiosynthesis using bromine precursor.

1. Introduction

The translocator protein 18 kDa (TSPO), also known as peripheral-type benzodiazepine receptor (PBR), is a protein which has high affinity cholesterol- and drug-binding protein in the outer mitochondrial membrane [1]. TSPO is also found in many tissues such as lung, liver, heart, spleen, kidney, adrenals, brain, glial cells, masts cell and macrophages, however, TSPO is expressed at the highest levels under normal conditions only in those steroid synthesized tissues [2]. TSPO is involved in a variety of cellular functions, including cholesterol import into mitochondria (a key involvement in steroidogenesis), apoptosis, cell proliferation, differentiation, anion transport, porphyrin transport, heme synthesis, and regulation of mitochondrial function [3]. It has been extensively reported that the most characterized functional role of the TSPO is its ability to regulate the rate-limiting translocation of cholesterol from the outer to the inner mitochondrial membrane before its transformation by cytochrome P-450 into pregnenolone, which leads to the production of various steroids [4–6]. The overexpression of TSPO has been linked in multiple disorders, including cancer, brain injury, neurodegeneration, and ischemia–reperfusion injury [7]. High-affinity TSPO ligands can stimulate steroid production and become promising therapeutic tools [8]. Recent reports have indicated that TSPO ligand PK11195 can be used as a therapeutic agent for neurological and psychiatric disorders, and cancers [3,9,10]. TSPO is an attractive target for molecular imaging of neuroinflammation like in Alzheimer's disease and in tumor progression using the biomedical imaging technique positron emission tomography (PET) [11]. The clinically useful prototypical TSPO-selective PET radioligand is [^{11}C]PK11195; however, it is reported to have many limitations such as low uptake and sensitivity [12]. These limitations have motivated investigators to search for new TSPO PET radioligands. Recently numerous new radioligands have been developed, and promising candidates progressing to human PET studies include [^{11}C]DAA1106, [^{18}F]FEDAA1106, [^{11}C]PBR28,

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0039-128X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.steroids.2011.06.012

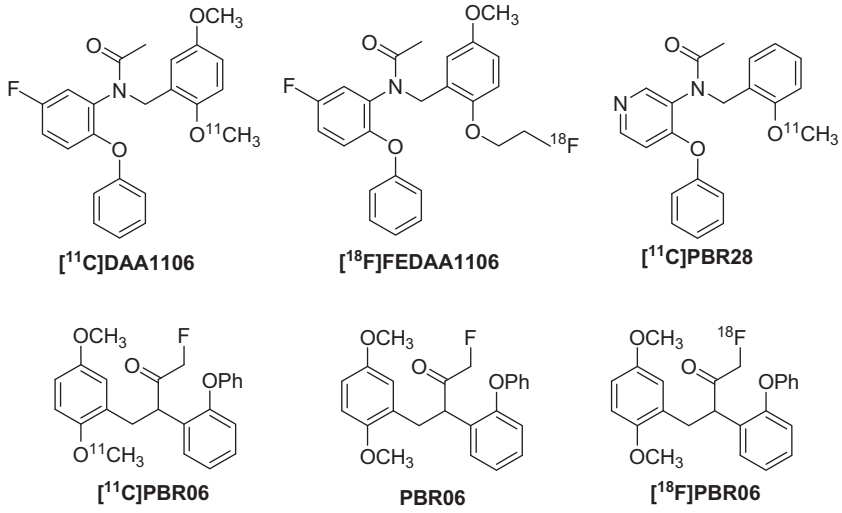


Fig. 1. Chemical structures of TSPO radioligands.

and [^{18}F]PBR06 (*N*-(2,5-dimethoxybenzyl)-2-[^{18}F]fluoro-*N*-(2-phenoxyphenyl)acetamide) [13–16], as indicated in Fig. 1. The link between PET imaging of TSPO and the importance of this target for steroids has been strengthened, and a high-affinity TSPO-specific PET radioligand may provide significant information for brain neurosteroidogenesis, detecting progression of disease and efficacy of TSPO ligand therapy [17,18].

[^{18}F]PBR06, a fluorine-18 labeled form of PBR06, is a promising PET brain TSPO radioligand originally developed and characterized by Briard et al. at National Institute of Mental Health (NIMH) [19], which displayed high PBR (TSPO) binding affinities (K_i = 0.180, 0.318 and 0.997 nM in brain homogenates from rat, monkey and human, respectively) [19,20]. Wishing to study this compound in our laboratory, we synthesized the radiolabeling precursors and standards following the literature methods. However, the published production of [^{18}F]PBR06 using the reaction of [^{18}F]fluoride ion with the corresponding bromide precursor resulted in low specific activity, radiochemical yield and chemical purity of [^{18}F]PBR06 in our hands, due to difficult separation of the fluorine-18 labeled product from the bromine precursor, and poor leaving group of the bromide precursor. While studying the reported methods for [^{18}F]PBR06 production, we designed the fully automated synthesis of [^{11}C]PBR06 (Fig. 1), a carbon-11 labeled form of PBR06, for the first time. Compared to fluorine-18 tracers (half-life 110 min), carbon-11 tracers (half-life 20 min) have some advantages in back-to-back same-day PET studies such as allowing a patient to receive multiple C-11 injections to shorten the diagnostic process when multiple tracer studies are required, and in reducing the radiation exposure for both radiopharmaceutical production staff and environment [21,22]. We also discovered a new labeling precursor, the previously undescribed tosylate congener of PBR06 with better tosyloxy leaving group, and investigated a fully automated synthesis of [^{18}F]PBR06.

2. Materials and methods

2.1. General

All commercial reagents and solvents were purchased from Sigma–Aldrich and Fisher Scientific and used without further purification. [^{11}C]methyl triflate ([^{11}C]CH_3OTf) was prepared according to a literature procedure [23]. Melting points were determined on a MEL-TEMP II capillary tube apparatus and were uncorrected. ^{1}H NMR spectra were recorded on Bruker Avance II 500 MHz NMR spectrometers using tetramethylsilane (TMS) as an internal standard. Chemical shift data for the proton resonances were reported in parts per million (ppm, δ scale) relative to internal standard TMS (δ 0.0), and coupling constants (J) were reported in hertz (Hz). The high resolution mass spectra (HRMS) were obtained using a Thermo Electron Corporation MAT 95XP-Trap spectrometer. Chromatographic solvent proportions are indicated as volume:volume ratio. Thin-layer chromatography (TLC) was run using Analtech silica gel GF uniplates (5 × 10 cm^2). Plates were visualized under UV light. Preparative TLC was run using Analtech silica gel UV 254 plates (20 × 20 cm^2). Normal phase flash column chromatography was carried out on EM Science silica gel 60 (230–400 mesh) with a forced flow of the indicated solvent system in the proportions described below. All moisture- and/or air-sensitive reactions were performed under a positive pressure of nitrogen maintained by a direct line from a nitrogen source. Analytical high performance liquid chromatography (HPLC) was performed using a Prodigy (Phenomenex) 5 μm C-18 column, 4.6 × 250 mm; 3:1:1 CH_3CN/MeOH/20 mM, pH 6.7 phosphate (buffer solution) mobile phase; flow rate 1.5 mL/min; and UV (254 nm) and γ-ray (PIN diode) flow detectors. Semi-preparative HPLC was performed using a YMC-Pack ODS-A, S-5 μm, 12 nm, 10 × 250 mm C-18 column; 3:1:1 CH_3CN/MeOH/20 mM, pH 6.7 phosphate (buffer solution) mobile phase; 4.0 mL/min flow rate; UV (254 nm) and γ-ray (PIN diode) flow detectors. C18 Plus Sep-Pak cartridges were obtained from Waters Corporation (Milford, MA). Sterile Millex-FG 0.22 μm filter units were obtained from Millipore Corporation (Bedford, MA).

2.2. 2-(Benzyloxy)-5-methoxybenzaldehyde (**1**)

To a stirred solution of 2-hydroxy-5-methoxybenzaldehyde (10.0 g, 65.7 mmol) in DMF (100 mL) was added K_2CO_3 (10.9 g, 78.9 mmol), followed by benzyl bromide (8.2 mL, 69.0 mmol). The reaction mixture was stirred at 45 °C for 5 h. After cooling to room temperature (RT), water was added. The resulting mixture was acidified to pH 7.0 with ice-cold 3% aqueous HCl and extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (10:1 hexanes/EtOAc) to afford **1** (14.33 g, 90%) as a white solid, mp 46–47 °C (lit [24], 48–49 °C). ^{1}H NMR ($CDCl_3$): δ 10.51 (s, 1H, CHO), 7.43–7.38 (m, 4H, ArH), 7.34–7.33 (m, 2H, ArH),

7.11 (dd, J = 9.0, 3.5 Hz, 1H, ArH), 7.00 (d, J = 9.0 Hz, 1H, ArH), 5.15 (s, 2H, $ArCH_2O$), 3.80 (s, 3H, OCH_3).

2.3. N-(2-(Benzyloxy)-5-methoxybenzyl)-2-phenoxyaniline (**2**)

A suspension of compound **1** (3.0 g, 12.4 mmol) and 2-phenoxyaniline (2.3 g, 12.4 mmol) in MeOH (20 mL) was stirred at RT for 2 h, and then MeOH was removed *in vacuo*. After the residue was heated at 95 °C for 2 h under nitrogen atmosphere, the mixture was cooled to RT and diluted with MeOH (20 mL). $NaBH_4$ (1.87 g, 49.6 mmol) was added portionwise at 0 °C. The reaction mixture was allowed to warm to RT and stirred for 1 h. The mixture was cooled to 0 °C, 5% aqueous acetic acid (100 mL) was added dropwise. After stirring at RT for 30 min, the resulting mixture was extracted with EtOAc. The combined organic layers were washed with saturated aqueous $NaHCO_3$, brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (12:1 hexanes/EtOAc) to afford **2** (2.25 g, 49%) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 7.35–7.21 (m, 7H, ArH), 7.00–6.94 (m, 2H, ArH), 6.91 (d, J = 8.5 Hz, 2H, ArH), 6.85 (d, J = 2.5 Hz, 1H, ArH), 6.81 (dd, J = 8.0, 1.0 Hz, 1H, ArH), 6.77 (d, J = 8.5 Hz, 1H, ArH), 6.71 (d, J = 8.0 Hz, 1H, ArH), 6.66 (dd, J = 7.5, 3.0 Hz, 1H, ArH), 6.59 (dt, J = 8.0, 1.0 Hz, 1H, ArH), 4.93 (s, 2H, $ArCH_2O$), 4.37 (s, 2H, NCH_2), 3.62 (s, 3H, OCH_3). HRMS (EI, *m/z*): calcd for $C_{27}H_{25}NO_3$ (M^+) 411.1829; found 411.1821.

2.4. N-(2-(Benzyloxy)-5-methoxybenzyl)-2-bromo-N-(2-phenoxyphenyl)acetamide (**3**)

To a stirred solution of compound **2** (2.1 g, 5.1 mmol) and NEt_3 (0.86 mL, 6.12 mmol) in CH_2Cl_2 (10 mL) was added bromoacetyl bromide (0.49 mL, 5.62 mmol) dropwise at 0 °C. The reaction mixture was allowed to warm to RT and stirred for 3 h. The resulting mixture was poured into water and extracted with CH_2Cl_2. The combined organic layers were washed with 0.5 N aqueous HCl, saturated aqueous $NaHCO_3$, brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (5:1 hexanes/EtOAc) to afford **3** (1.42 g, 86%) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 7.32–7.16 (m, 8H, ArH), 7.12–7.08 (m, 2H, ArH), 7.05 (d, J = 2.5 Hz, 1H, ArH), 6.96 (dt, J = 7.5, 1.0 Hz, 1H, ArH), 6.81 (dd, J = 8.5, 1.0 Hz, 1H, ArH), 6.78 (d, J = 7.5 Hz, 2H, ArH), 6.74–6.69 (m, 2H, ArH), 5.23 (d, J = 14.5 Hz, 1H, NC*H*H), 4.81–4.72 (m, 3H, NCH*H* + $ArCH_2OAr$), 3.78 (dd, J = 24.0, 11.0 Hz, 2H, CH_2Br), 3.67 (s, 3H, OCH_3). HRMS (EI, *m/z*): calcd for $C_{29}H_{26}BrNO_4$ (M^+) 531.1040; found 531.1024.

2.5. N-(2-(Benzyloxy)-5-methoxybenzyl)-2-fluoro-N-(2-phenoxyphenyl)acetamide (**4**)

Method A: A mixture of compound **3** (770 mg, 1.45 mmol) and dry KF (253 mg, 4.35 mmol) in diethylene glycol (8 mL) was heated rapidly to 150 °C in a preheated oil-bath. After stirring for 5 h at 150 °C, the reaction mixture was cooled to RT and quenched with water. The resulting mixture was extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (5:1 hexanes/EtOAc) to afford **4** (384 mg, 56%) as a pale yellow oil.

Method B: To a stirred solution of 2-fluoro-*N*-(2-phenoxyphenyl)acetamide (**9**) (200 mg, 0.82 mmol) in DMF (3 mL) was added NaH (60% dispersion in mineral oil, 50 mg, 1.25 mmol) at 0 °C. After the mixture was allowed to warm to RT and stirred for 30 min, a solution of 1-(benzyloxy)-2-(bromomethyl)-4-methoxybenzene (**7**) (280 mg, 91.4 mmol) in DMF (1 mL) was added dropwise. The reaction mixture was stirred at RT for 4 h, and then poured into ice-water. The resulting mixture was extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (from 5:1 to 3:2 hexanes/EtOAc) to afford **4** (360 mg, 48%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$): δ 7.31–7.28 (m, 3H, ArH), 7.26–7.18 (m, 3H, ArH), 7.15–7.09 (m, 2H, ArH), 7.10 (t, J = 7.5 Hz, 1H, ArH), 7.04 (d, J = 2.5 Hz, 1H, ArH), 6.97–6.91 (m, 2H, ArH), 6.79 (d, J = 8.0 Hz, 1H, ArH), 6.75–6.69 (m, 4H, ArH), 5.22 (d, J = 14.5 Hz, 1H, NC*H*H), 4.82–4.64 (m, 5H, NCH*H* + $ArCH_2OAr$ + CH_2F), 3.66 (s, 3H, OCH_3). HRMS (EI, *m/z*): calcd for $C_{29}H_{26}FNO_4$ (M^+) 471.1840; found 471.1834.

2.6. 2-Fluoro-N-(2-hydroxy-5-methoxybenzyl)-N-(2-phenoxyphenyl)-acetamide (desmethyl-PBR06, **5**)

A solution of compound **4** (300 mg, 0.64 mmol) in MeOH (10 mL) was hydrogenated over 10% Pd–C (50 mg) at 60 psi for 6 h. The catalyst was filtered through a layer of Celite, and then the solvent was removed *in vacuo*. The crude product was purified by silica gel column chromatography (2:1 hexanes/CH_3COCH_3) to afford **5** (128 mg, 53%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 8.56 (br s, 1H, OH), 7.36–7.32 (m, 3H, ArH), 7.18–7.15 (m, 1H, ArH), 7.13–7.08 (m, 2H, ArH), 6.91 (dd, J = 7.5, 1.0 Hz, 1H, ArH), 6.86–6.84 (m, 3H, ArH), 6.76 (dd, J = 9.0, 3.0 Hz, 1H, ArH), 6.24 (d, J = 3.0 Hz, 1H, ArH), 4.93 (d, J = 14.5 Hz, 1H, NC*H*H), 4.77 (d, J = 47.0, 5.5 Hz, 1H, CH_2F), 4.53 (d, J = 14.5 Hz, 1H, NCH*H*), 3.62 (s, 3H, OCH_3). HRMS (EI, *m/z*): calcd for $C_{22}H_{20}FNO_4$ (M^+) 381.1371; found 381.1387.

2.7. (2-(Benzyloxy)-5-methoxyphenyl)methanol (**6**)

To a solution of compound **1** (3.0 g, 12.4 mmol) in CH_2Cl_2 (8 mL) and MeOH (40 mL) was added $NaBH_4$ (650 mg, 17.2 mmol) portionwise at 0 °C. The reaction mixture was allowed to warm to RT and stirred for 5 h, and then the solvents were removed *in vacuo*. Water was added and the mixture was extracted with EtOAc. The combined organic layers were washed with saturated aqueous NH_4Cl, brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (2:1 hexanes/EtOAc) to afford **6** (2.90 g, 96%) as a white solid, mp 48–49 °C. ^{1}H NMR ($CDCl_3$): δ 7.41–7.37 (m, 4H, ArH), 7.35–7.31 (m, 1H, ArH), 6.90 (d, J = 3.0 Hz, 1H, ArH), 6.87 (d, J = 9.0 Hz, 1H, ArH), 6.77 (dd, J = 9.0, 3.0 Hz, 1H, ArH), 5.06 (s, 2H, $ArCH_2OAr$), 4.69 (s, 2H, $ArCH_2OH$), 3.77 (s, 3H, OCH_3), 2.17 (s, 1H, CH_2OH).

2.8. 1-(Benzyloxy)-2-(bromomethyl)-4-methoxybenzene (**7**)

To a stirred solution of compound **6** (2.2 g, 8.19 mmol) in CH_2Cl_2 (20 mL) was added a solution of PBr_3 (0.77 mL, 8.19 mmol) in CH_2Cl_2 (5 mL) dropwise at 0 °C. The reaction mixture was allowed to warm to RT and stirred for 5 h. The resulting mixture was poured into ice-water and extracted with CH_2Cl_2. The combined organic layers were washed with cold saturated aqueous $NaHCO_3$, brine, dried over anhydrous Na_2SO_4, and filtered. The solvent was removed *in vacuo* to afford **7** (2.67 g, 97%) as a brown solid, which was used without further purification. ^{1}H NMR ($CDCl_3$): δ 7.47 (d, J = 7.5 Hz, 2H, ArH), 7.39 (t, J = 7.5 Hz, 2H, ArH), 7.33–7.30 (m, 1H, ArH), 6.92 (d, J = 3.0 Hz, 1H, ArH), 6.85 (d, J = 9.0 Hz, 1H, ArH), 6.79 (dd, J = 9.0, 3.0 Hz, 1H, ArH), 5.10 (s, 2H, $ArCH_2OAr$), 4.57 (s, 2H, $ArCH_2Br$), 3.76 (s, 3H, OCH_3). HRMS (EI, *m/z*): calcd for $C_{15}H_{15}BrO_2$ (M^+) 306.0250; found 306.0235.

2.9. 2-Bromo-N-(2-phenoxyphenyl)acetamide (**8**)

To a stirred solution of 2-phenoxyaniline (1.0 g, 5.4 mmol) and NEt_3 (0.83 mL, 5.94 mmol) in CH_2Cl_2 (5 mL) was added bromoacetyl bromide (0.52 mL, 5.94 mmol) dropwise at 0 °C. The reaction mixture was allowed to warm to RT and stirred for 2 h. The resulting mixture was poured into water and extracted with CH_2Cl_2. The combined organic layers were washed with 0.5 N aqueous HCl, saturated aqueous $NaHCO_3$, brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (1:3 hexanes/CH_2Cl_2) to afford **8** (1.42 g, 86%) as pale yellow solid, mp 49–51 °C. ^{1}H NMR ($CDCl_3$): δ 8.78 (br s, 1H, NH), 8.39 (dd, J = 8.5, 1.5 Hz, 1H, ArH), 7.38–7.34 (m, 2H, ArH), 7.17–7.13 (m, 2H, ArH), 7.08–7.03 (m, 3H, ArH), 6.90 (dd, J = 8.0, 1.0 Hz, 1H, ArH), 3.99 (s, 2H, CH_2Br).

2.10. 2-Fluoro-N-(2-phenoxyphenyl)acetamide (**9**)

A mixture of compound **8** (1.0 g, 3.28 mmol) and dry KF (480 mg, 8.26 mmol) in diethylene glycol (10 mL) was heated rapidly to 150 °C in a preheated oil-bath. After the reaction mixture was stirred at 150 °C for 2 h, it was cooled and diluted with water. The resulting mixture was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (1:3 hexanes/CH_2Cl_2) to afford **9** (384 mg, 48%) as a brown solid, mp 74–76 °C (lit [11] 75–77 °C). ^{1}H NMR ($CDCl_3$): δ 8.59 (br s, 1H, NH), 8.45 (dd, J = 8.5, 1.5 Hz, 1H, ArH), 7.38–7.30 (m, 2H, ArH), 7.17–7.12 (m, 2H, ArH), 7.08–7.03 (m, 3H, ArH), 6.88 (dd, J = 8.5, 1.5 Hz, 1H, ArH), 4.90 (d, J = 47.0 Hz, 2H, CH_2F).

2.11. N-(2,5-Dimethoxybenzyl)-2-phenoxyaniline (**10**)

To a stirred solution of 2-phenoxyaniline (5.0 g, 27.0 mmol) in MeOH (30 mL) was added a solution of 2,5-dimethoxy-benzaldehyde (4.93 g, 29.7 mmol) in MeOH (25 mL) at RT. The reaction mixture was heated at reflux for 2 h, and then MeOH was removed *in vacuo*. The residue was heated at 120 °C for 1 h under nitrogen atmosphere, and then it was cooled to RT and diluted with MeOH (40 mL). $NaBH_4$ (4.5 g, 119.0 mmol) was added portionwise at 0 °C. The reaction mixture was allowed to warm to RT and stirred for 1 h. The mixture was cooled to 0 °C, 5% aqueous acetic acid (100 mL) was added dropwise. After stirring at RT for 30 min, the resulting mixture was extracted with EtOAc. The combined organic layers were washed with saturated aqueous $NaHCO_3$, brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (12:1 hexanes/EtOAc) to afford **10** (8.87 g, 98%) as a pale yellow solid, mp 48–49 °C. ^{1}H NMR ($CDCl_3$): δ 7.30–7.25 (m, 2H, ArH), 7.05–6.98 (m, 2H, ArH), 6.94–6.92 (m, 2H, ArH), 6.85–6.83 (m, 2H, ArH), 6.78–6.70 (m, 3H, ArH), 6.64 (dt, J = 7.5, 1.0 Hz, 1H, ArH), 4.34 (s, 2H, NCH_2), 3.70 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3).

2.12. 2-Bromo-N-(2,5-dimethoxybenzyl)-N-(2-phenoxyphenyl)acetamide (Br-PBR06, **11**)

To a stirred solution of compound **10** (1.2 g, 3.58 mmol) and NEt_3 (0.55 mL, 3.94 mmol) in CH_2Cl_2 (5 mL) was added bromoacetyl bromide (0.34 mL, 3.94 mmol) dropwise at 0 °C. The reaction mixture was allowed to warm to RT and stirred for 2 h. The resulting mixture was poured into water and extracted with CH_2Cl_2. The combined organic layers were washed with 0.5 N aqueous HCl, saturated aqueous $NaHCO_3$, brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (5:1 hexanes/EtOAc) to afford **11** (1.30 g, 80%) as a clear oil. ^{1}H NMR ($CDCl_3$): δ 7.35–7.32 (m, 2H, ArH), 7.23–7.20 (m, 1H, ArH), 7.16–7.11 (m, 2H, ArH), 6.98 (dt, J = 7.5, 1.5 Hz, 1H, ArH), 6.95 (d, J = 8.0 Hz, 1H, ArH), 6.91–6.89 (m, 2H, ArH), 6.85 (dd, J = 8.0, 1.0 Hz, 1H, ArH), 6.72 (dd, J = 8.5, 3.0 Hz, 1H, ArH), 6.67 (d, J = 9.0 Hz, 1H, ArH), 5.19 (d, J = 14.5 Hz, 1H, NC*H*H), 4.69 (d, J = 14.0 Hz, 1H, NCH*H*), 3.79 (d, J = 7.0 Hz, 2H,CH_2Br), 3.67 (s, 3H, OCH_3), 3.53 (s, 3H, OCH_3).

2.13. N-(2,5-Dimethoxybenzyl)-2-fluoro-N-(2-phenoxyphenyl)acetamide (PBR06, **12**)

A mixture of compound **11** (400 mg, 0.88 mmol) and dry KF (192 mg, 3.30 mmol) in diethylene glycol (5 mL) was heated rapidly to 150 °C in a preheated oil-bath. After the reaction mixture was stirred at 150 °C for 6 h, it was cooled and diluted with water. The resulting mixture was extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (5:1 hexanes/EtOAc) to afford **12** (315 mg, 91%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$): δ 7.34 (t, J = 8.0 Hz, 2H, ArH), 7.22–7.19 (m, 1H, ArH), 7.15 (t, J = 7.5 Hz, 1H, ArH), 7.03–6.95 (m, 2H, ArH), 6.93 (d, J = 3.0 Hz, 1H, ArH), 6.87 (d, J = 7.5 Hz, 2H, ArH), 6.83 (dd, J = 8.5, 1.0 Hz, 1H, ArH), 6.72 (dd, J = 9.0, 3.0 Hz, 1H, ArH), 6.66 (d, J = 8.5 Hz, 1H, ArH), 5.16 (d, J = 14.0 Hz, 1H, NC*H*H), 4.76 (d, J = 14.0 Hz, 1H, NCH*H*), 4.75 (dd, J = 47.0, 3.5 Hz, 2H, CH_2F), 3.66 (s, 3H, OCH_3), 3.50 (s, 3H, OCH_3).

2.14. Ethyl 2-(tosyloxy)acetate (**13**)

To a stirred solution of ethyl glycolate (5.0 g, 48.0 mmol) and tosyl chloride (9.15 g, 48.0 mmol) in anhydrous Et_2O (40 mL) was added NEt_3 (96.0 mmol, 13.4 mL) dropwise at 0 °C. After the reaction mixture was stirred at 0 °C for 2 h, water was added and the phase separated. The aqueous phase was extracted with Et_2O. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (5:1 hexanes/EtOAc) to afford **13** (9.33 g, 75%) as a white solid, mp 42–43 °C. ^{1}H NMR ($CDCl_3$): δ 7.84 (d, J = 8.0 Hz, 2H, ArH), 7.36 (d, J = 8.0 Hz, 2H, ArH), 4.58 (s, 2H, OCH_2CO), 4.19 (q, J = 7.0 Hz, 2H, OCH_2CH_3), 2.46 (s, 3H, $ArCH_3$), 1.24 (t, J = 7.0 Hz, 3H, OCH_2CH_3).

2.15. 2-(Tosyloxy)acetic acid (**14**)

To a stirred suspension of compound **13** (5.0 g, 19.4 mmol) in EtOH (20 mL) was added 5% aqueous NaOH (16 mL). After the reaction mixture was stirred at RT for 5 h, EtOH was removed *in vacuo*, and the residue was cooled with ice-water and acidified with 5% aqueous HCl (25 mL). The solid was collected by filtration, rinsed with water. The crude product was recrystallized from EtOAc/hexanes to afford **14** (3.49 g, 78%) as a white solid, mp 136–138 °C. ^{1}H NMR (CD_3OD): δ 7.82 (d, J = 8.0 Hz, 2H, ArH), 7.44 (d, J = 8.0 Hz, 2H, ArH), 4.59 (s, 2H, OCH_2CO), 2.45 (s, 3H, $ArCH_3$).

2.16. 2-Chloro-2-oxoethyl 4-methylbenzenesulfonate (**15**)

A mixture of compound **14** (3.0 g, 13.0 mmol) and thionyl chloride (10 mL) was heated at reflux for 2 h. The excess thionyl chloride was removed *in vacuo*, and the residue was dried under high vacuum to afford **15** (2.97 g, 92%) as pale yellow oil, which was used without further purification. ^{1}H NMR (CD_3Cl): δ 7.82 (d, J = 8.5 Hz, 2H, ArH), 7.38 (d, J = 8.5 Hz, 2H, ArH), 4.90 (s, 2H, OCH_2CO), 2.47 (s, 3H, CH_3).

*2.17. 2-((2,5-Dimethoxybenzyl)(2-phenoxyphenyl)amino)-2-oxoethyl 4-methylbenzenesulfonate (tosyloxy-PBR06, **16**)*

To a stirred solution of compound **10** (500 mg, 1.49 mmol) and NEt_3 (0.24 mL, 1.72 mmol) in CH_2Cl_2 (2 mL) was added a solution of compound **15** (415 mg, 1.67 mmol) in CH_2Cl_2 (2 mL) dropwise at 0 °C. After the reaction mixture was allowed to warm to RT and stirred for 3 h, water was added and the phases were separated. The aqueous phase was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (1.5:1 hexanes/EtOAc) to afford **16** (747 mg, 92%) as a pale yellow solid, mp 109–110 °C. ^{1}H NMR (CD_3Cl): δ 7.80 (d, J = 8.0 Hz, 2H, ArH), 7.34–7.29 (m, 4H, ArH), 7.22–7.18 (m, 1H, ArH), 7.15 (t, J = 7.5 Hz, 1H, ArH), 7.01–6.95 (m, 2H, ArH), 6.87 (d, J = 3.0 Hz, 1H, ArH), 6.83 (d, J = 7.5 Hz, 2H, ArH), 6.79 (dd, J = 8.5, 1.0 Hz, 1H, ArH), 6.71 (dd, J = 9.0, 3.0 Hz, 1H, ArH), 6.64 (d, J = 9.0 Hz, 1H, ArH), 5.05 (d, J = 14.5 Hz, 1H, CON*H*H), 4.71 (d, J = 14.5 Hz, 1H, CONH*H*), 4.57 (s, 2H, OCH_2CO), 3.64 (s, 3H, OCH_3), 3.48 (s, 3H, OCH_3), 2.42 (s, 3H, CH_3). HRMS (EI, *m/z*): calcd for $C_{30}H_{29}NO_7S$ (M^+) 547.1659; found 547.1654.

*2.18. 2-Fluoro-N-(2-[^{11}C]methoxy-5-methoxybenzyl)-N-(2-phenoxyphenyl)acetamide ([^{11}C]PBR06, [^{11}C]**12**)*

[^{11}C]CO_2 was produced by the ^{14}N(p,α) ^{11}C nuclear reaction in small volume (9.5 cm^3) aluminum gas target (CTI) from 11 MeV proton cyclotron on research purity nitrogen (+1% O_2) in a Siemens radionuclide delivery system (Eclipse RDS-111). In a small reaction vial (5 mL), the desmethyl-PBR06 precursor **5** (0.5–1.0 mg) was dissolved in CH_3CN (400 μL). To this solution was added NaH (~1 mg) with a microspatula. No carrier-added (high specific activity) [^{11}C]CH_3OTf that was produced by the gas-phase production method [23] from [^{11}C]CO_2 through [^{11}C]CH_4 and [^{11}C]CH_3Br with silver triflate (AgOTf) column was passed into the reaction vial at RT, until radioactivity reached a maximum (~2 min), and then the reaction vial was isolated and heated at 80 °C for 3 min. The contents of the reaction vial were diluted with $NaHCO_3$ (1 mL, 0.1 M), and injected onto the semi-preparative HPLC column with 3 mL injection loop for purification. The product fraction was collected in a recovery vial containing 30 mL water. The diluted tracer solution was then passed through a C-18 Sep-Pak Plus cartridge, and washed with water (5 mL × 5). The cartridge was eluted with EtOH (1 mL × 2) to release [^{11}C]PBR06 ([^{11}C]**12**). The eluted product was then sterile-filtered through a Millex-FG 0.22 μm membrane into a sterile vial and formulated with 10 mL saline. Total radioactivity was assayed and total volume was noted for tracer dose dispensing. Retention times in the semi-preparative HPLC system were: t_R **5** = 5.35 min, t_R **12** = 7.12 min, t_R [^{11}C]**12** = 7.12 min. Retention times in the analytical HPLC system were: t_R **5** = 3.28 min, t_R **12** = 4.49 min, t_R [^{11}C]**12** = 4.49 min.

*2.19. N-(2,5-Dimethoxybenzyl)-2-[^{18}F]fluoro-N-(2-phenoxyphenyl)-acetamide ([^{18}F]PBR06, [^{18}F]**12**)*

No-carrier-added (NCA) aqueous H[^{18}F]F was produced by ^{18}O(p,n) ^{18}F nuclear reaction using a Siemens Eclipse RDS-111 cyclotron by irradiation of $H_2{}^{18}O$ (2.5 mL). H[^{18}F]F (7.4–18.5 GBq) in [^{18}O]water plus 0.1 mL K_2CO_3 solution (1.7 mg) and Kryptofix 2.2.2 (10 mg) in 1.0 mL CH_3CN with additional 1 mL CH_3CN were placed in the fluorination reaction vial (10-mL V-vial) and repeated azeotropic distillation (17 min) was performed at 110 °C to remove water and to form the anhydrous K[^{18}F]F-Kryptofix 2.2.2 complex. The precursor Br-PBR06 **11** or tosyl-PBR06 **16** (1 mg) dissolved in DMSO (1.0 mL) was introduced to the reaction vessel and heated at 140 °C for 15 min to affect radiofluorination. After cooling to ~90 °C, the contents of the reaction vial were diluted with $NaHCO_3$ (1 mL, 0.1 M), and injected onto the semi-preparative HPLC column with 3 mL injection loop for purification. The product fraction was collected in a recovery vial containing 30 mL water. The diluted tracer solution was then passed through a C-18 Sep-Pak Plus cartridge, and washed with water (5 mL × 5). The cartridge was eluted with EtOH (1 mL × 2) to release [^{18}F]PBR06 ([^{18}F]**12**). The eluted product was then sterile-filtered through a Millex-FG 0.22 μm membrane into a sterile vial and formulated with 10 mL saline. Total radioactivity was assayed and total volume was noted for tracer dose dispensing. Retention times in the semi-preparative HPLC system were: t_R **11** = 8.18 min, t_R **16** = 9.43 min, t_R **12** = 7.12 min, t_R [^{11}C]**12** = 7.12 min. Retention times in the analytical HPLC system were: t_R **11** = 5.70 min, t_R **16** = 7.31 min, t_R **12** = 4.48 min, t_R [^{11}C]**12** = 4.48 min.

3. Results and discussion

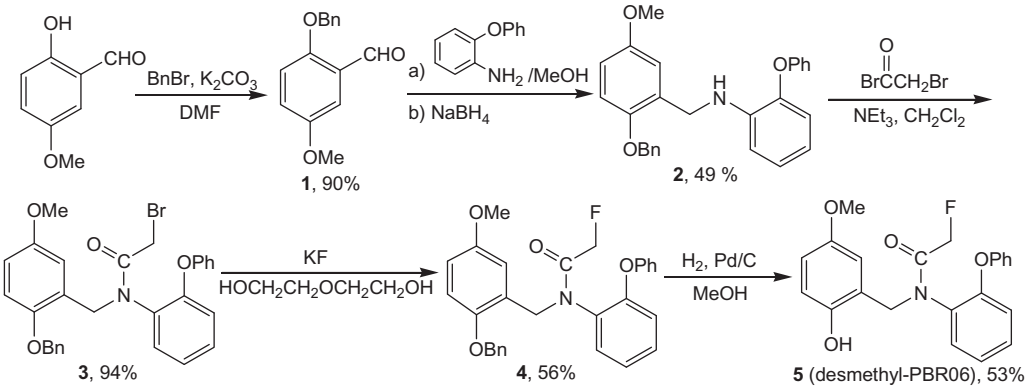
3.1. Chemistry

The synthetic approach designed for the preparation of the desmethyl-PBR06 precursor **5** for carbon-11 labeling is outlined in Scheme 1. The phenolic hydroxyl group of 2-hydroxy-5-methoxybenzaldehyde was protected as benzyl ether by reacting with benzyl bromide in DMF in the presence of K_2CO_3 to afford benzaldehyde **1** in 90% yield [24]. Condensation of 2-phenoxyaniline with **1** followed by reduction with $NaBH_4$ in MeOH achieved **2** in 49% yield, which was bromoacetylated with bromoacetyl bromide in CH_2Cl_2 in the presence of NEt_3 to give acetamide **3** in 94% yield. Acetamide **4** was obtained by nucleophilic substitution of **3** with dry KF in diethylene glycol in 56% yield. The benzyl group of **4** was removed by catalytic hydrogenation with 10% Pd/C in MeOH to provide the precursor **5** in 53% yield.

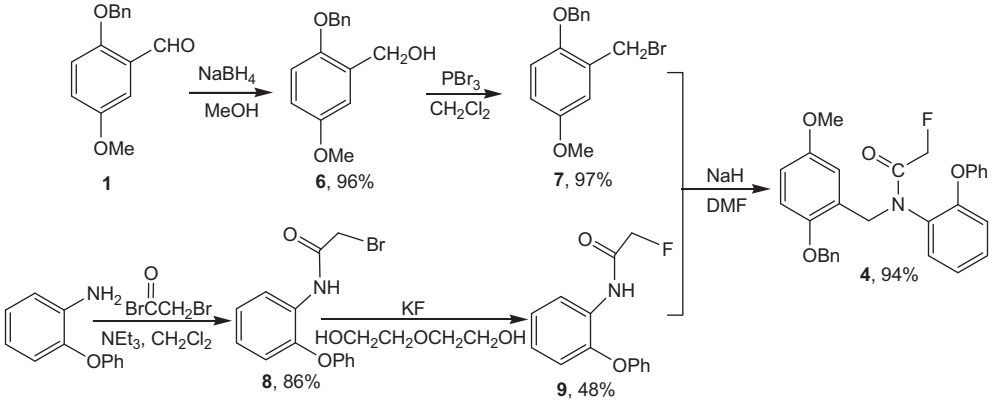
Compound **4** could also be achieved by another approach as shown in Scheme 2. Reduction of **1** to alcohol **6** was performed with $NaBH_4$ in a mixture of CH_2Cl_2 and MeOH in 96% yield. Benzyl alcohol **6** was converted to benzyl bromide **7** with PBr_3 in CH_2Cl_2 in 97% yield. Bromoacetylation of 2-phenoxyaniline with bromoacetyl bromide in CH_2Cl_2 in the presence of NEt_3 generated *N*-bromoacetyl aniline **8** in 86% yield, which was then treated with dry KF in diethylene glycol to afford *N*-fluoroacetyl aniline **9** in 48% yield [19]. Coupling reaction of **7** with **9** in DMF in the presence of NaH gave **4** in 94% yield.

The PBR06 standard **12** was synthesized according to the literature method with modification [19]. As indicated in Scheme 3, 2-phenoxyaniline was condensed with 2,5-dimethoxybenzaldehyde followed by reduction with $NaBH_4$ in MeOH to give phenoxyaniline **10** in 98% yield. Higher yield at shorter reaction time was achieved for the condensation performed in MeOH at reflux initially for 2 h, followed by solvent removal and then further heated to 120 °C for 1 h. Bromoacetylation of **10** yielded the Br-PBR06 precursor **11** for fluorine-18 labeling in 80% yield. Fluorination of **11** using dry KF in diethylene glycol provided the reference standard **12** in 91% yield.

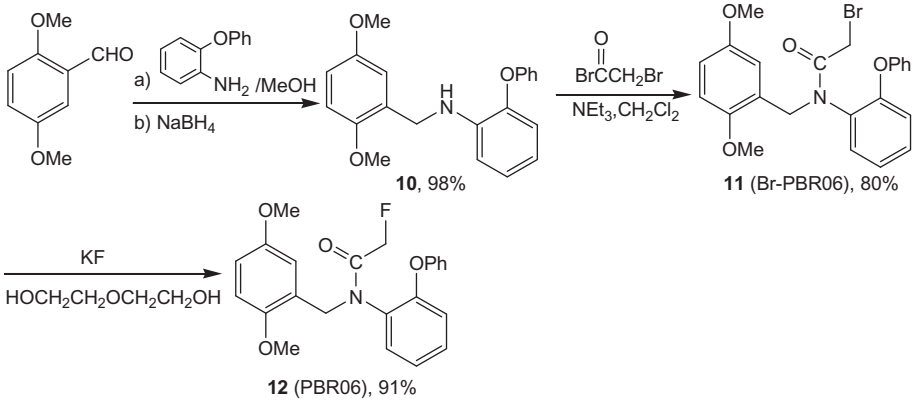
To improve the radiochemical yield of ^{18}F-labeling reaction and chemical purity of [^{18}F]fluorinated product, it became necessary to design and synthesize the appropriate radiolabeling precursor bearing suitable leaving group for nucleophilic substitution of [^{18}F]F^-, and possessing suitable polarity difference to labeled product for separation and purification process. Therefore, we turned our attention to developing a new tosylate precursor for [^{18}F]-labeled PBR06. The tosyloxy-PBR06 precursor **16** was prepared by a four-step sequence of reactions delineated in Scheme 4. Coupling reaction of ethyl glycolate with tosyl chloride



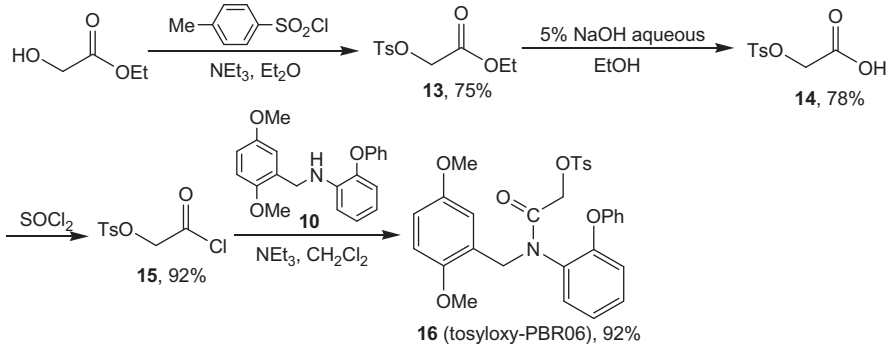
Scheme 1. Synthesis of desmethyl-PBR06 (**5**).



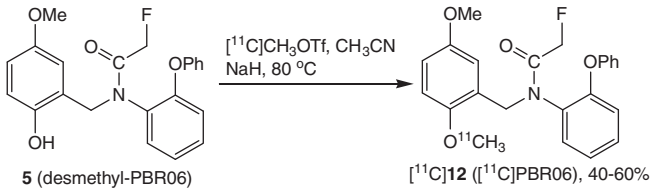
Scheme 2. Synthesis of an intermediate **4**.



Scheme 3. Synthesis of Br-PBR06 (**11**) and PBR06 (**12**).



Scheme 4. Synthesis of tosyloxy-PBR06 (**16**).



Scheme 5. Synthesis of [^{11}C]PBR06 ([^{11}C]**12**).

in Et_2O in the presence of NEt_3 afforded compound **13** in 75% yield. Ethyl ester **13** was hydrolyzed with 5% aqueous NaOH in EtOH to give compound **14** in 78% yield, which was converted to acyl halide **15** with thionyl chloride [25] in 92% yield. The desired tosylate precursor **16** was achieved by coupling reaction of **10** with **15** in CH_2Cl_2 in the presence of NEt_3 in 92% yield.

3.2. Radiochemistry

Synthesis of the target tracer [^{11}C]PBR06 ([^{11}C]**12**) is indicated in Scheme 5. The desmethyl-PBR06 precursor **5** was labeled by [^{11}C]CH_3OTf [23,26] through *O*-[^{11}C]methylation [27,28] at 80 °C under basic condition (NaH) and isolated by a semi-preparative HPLC method (C-18 column) and a solid-phase extraction (SPE) method (C-18 Plus Sep-Pak cartridge) (a second purification or isolation process) [29] to produce the corresponding pure radiolabeled compound [^{11}C]**12** in 40–60% radiochemical yield, decay corrected to end of bombardment (EOB), based on [^{11}C]CO_2. [^{11}C]CH_3OTf is a proven methylation reagent with greater reactivity than commonly used [^{11}C]methyl iodide ([^{11}C]CH_3I) [27,30], and thus, the radiochemical yields for [^{11}C]PBR06 is relatively high. Addition of $NaHCO_3$ to quench the radiolabeling reaction and to dilute the radiolabeling mixture prior to the injection onto the semi-preparative HPLC column for purification gave better separation of [^{11}C]PBR06 from its phenolic precursor [29,31]. The radiosynthesis was performed in a home-built automated multi-purpose [^{11}C]-radiosynthesis module, allowing measurement of specific radioactivity during synthesis [32,33]. The overall synthesis, purification and formulation time was 30–40 min from EOB. The specific radioactivity was in a range of 222–740 GBq/μmol at EOB. Chemical purity and radiochemical purity were determined by analytical

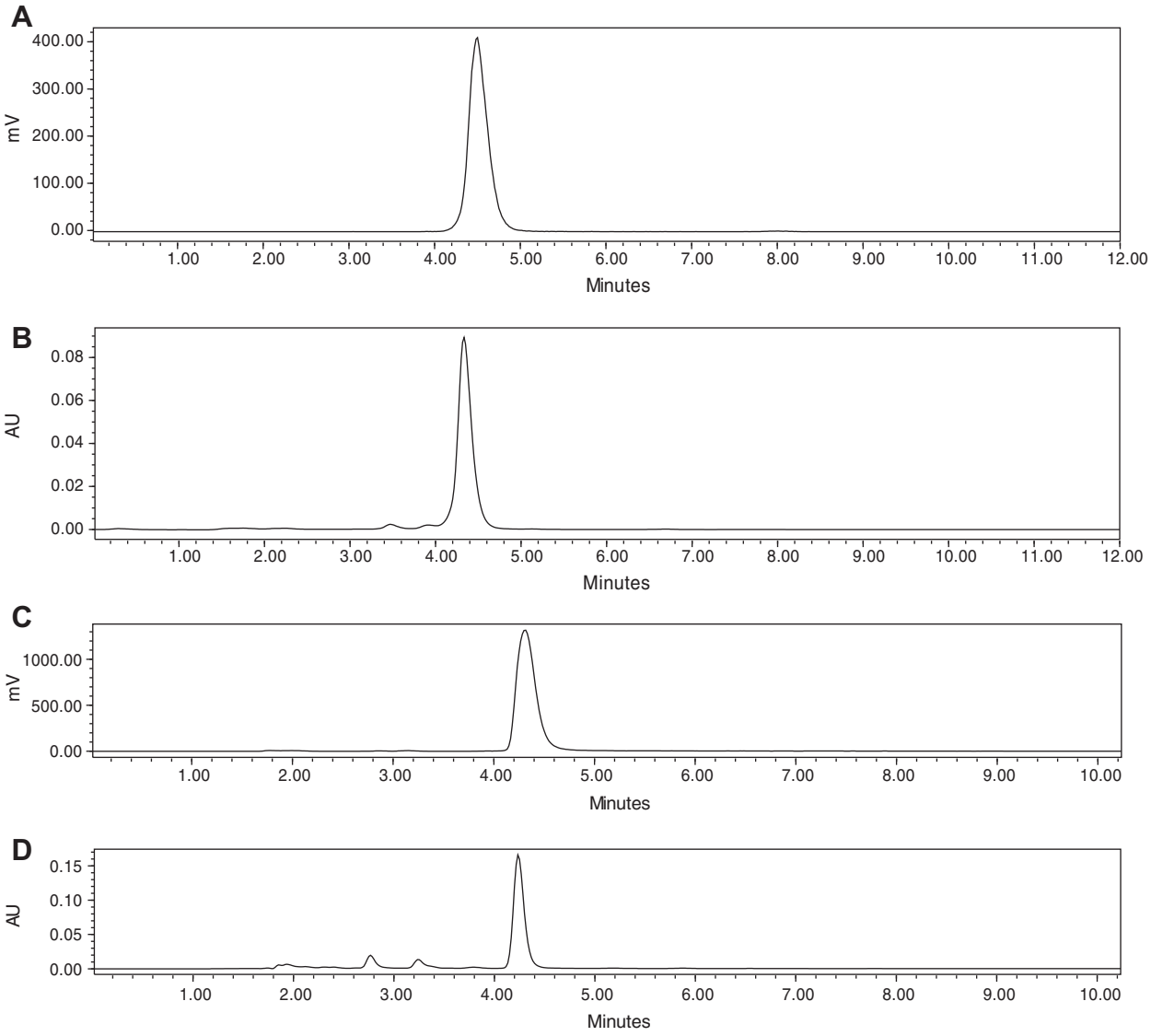
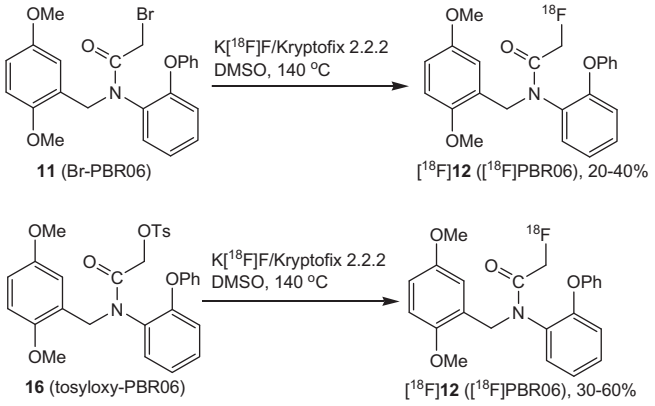


Fig. 2. A representative analytical HPLC chromatographic profile for the tracer [^{11}C]PBR06 produced with either Sep-Pak purification or solvent evaporation. Analytical radioactive (A) and UV (B) HPLC traces for [^{11}C]PBR06 produced with Sep-Pak purification, t_R [^{11}C]PBR06 = 4.49 and 4.33 min, respectively. Analytical radioactive (C) and UV (D) HPLC traces for [^{11}C]PBR06 produced with solvent evaporation, t_R [^{11}C]PBR06 = 4.31 and 4.24 min, respectively.



Scheme 6. Synthesis of [^{18}F]PBR06 ([^{18}F]**12**).

HPLC [34]. The chemical purity of the precursor desmethyl-PBR06 and reference standard PBR06 was >96%. The radiochemical purity of the target tracer [^{11}C]PBR06 was >99% determined by radio-HPLC through γ-ray (PIN diode) flow detector, and the chemical purity of [^{11}C]PBR06 was >95% determined by reverse-phase HPLC through UV flow detector. A C-18 Plus Sep-Pak cartridge was used to significantly improve the chemical purity of the tracer solution. Initial HPLC purification was employed to separate the labeled product from its un-reacted excess precursor and other labeled by-products. The second SPE purification with Sep-Pak [29,34] was employed, instead of rotary evaporator, to remove potential impurities from the HPLC co-elution with the precursor and from the residual solvents including HPLC mobile phase solvents and module set-up cleaning solvents. Rotary evaporation was unable to perform in this regard. Moreover, it could result in the decomposition of the labeled product such as desmethylation during the heating. The chemical purity of the [^{11}C]PBR06 tracer solution with Sep-Pak purification was increased higher 10–20% than that without Sep-Pak purification. Representative analytical HPLC chromatograms for [^{11}C]PBR06 produced with either Sep-Pak purification or solvent evaporation are shown in Fig. 2.

Synthesis of the target tracer [^{18}F]PBR06 ([^{18}F]**12**) is outlined in Scheme 6. The Br-PBR06 **11** or tosyloxy-PBR06 **16** precursor was labeled with K[^{18}F]F/Kryptofix 2.2.2 through nucleophilic

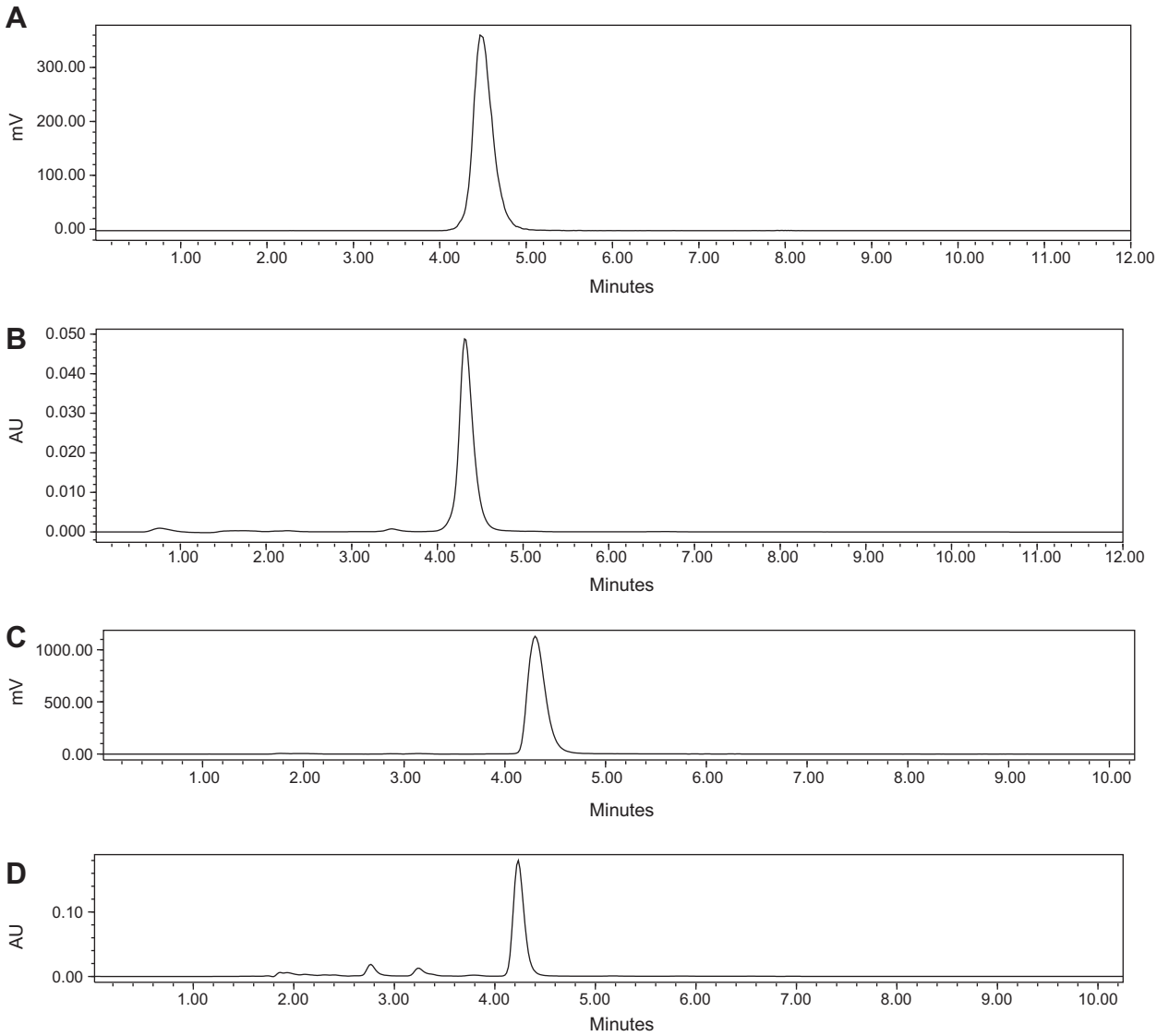


Fig. 3. A representative analytical HPLC chromatographic profile for the tracer [^{18}F]PBR06 produced by both tosyloxy-PBR06 and Br-PBR06. Analytical radioactive (A) and UV (B) HPLC traces for [^{18}F]PBR06 produced using tosyloxy-PBR06, t_R [^{18}F]PBR06 = 4.48 and 4.32 min, respectively. Analytical radioactive (C) and UV (D) HPLC traces for [^{18}F]PBR06 produced using Br-PBR06, t_R [^{18}F]PBR06 = 4.30 and 4.23 min, respectively.

substitution and isolated by a semi-preparative HPLC method with C-18 column and a SPE method with a C-18 Plus Sep-Pak cartridge (a second purification or isolation process) [29] to produce the corresponding pure radiolabeled compound [^{18}F]**12** in 20–40% and 30–60% decay-corrected radiochemical yield from K[^{18}F]F, respectively. Likewise, addition of $NaHCO_3$ to quench the radiolabeling reaction and to dilute the radiolabeling mixture prior to the injection onto the semi-preparative HPLC column for purification gave better separation of [^{18}F]PBR06 from its Br-PBR06 or tosyloxy-PBR06 precursor [29,31]. The radiosynthesis was performed using a self-designed automated multi-purpose [^{18}F]-radiosynthesis module. The overall synthesis, purification and formulation time was 50–60 min from EOB. The specific radioactivity was 37–222 GBq/μmol at EOB. No-carrier-added [^{18}F]fluoride ion in [^{18}O]water was trapped with or without a QMA cartridge. If the cyclotron-produced [^{18}F]fluoride ion was trapped without the use of a cartridge [29,35], it would significantly increase the specific activity of the prepared [^{18}F]PBR06. The reason was that there was a low-level contamination of QMA anionic resins with fluoride ion [35]. The amounts of bromide or tosylated precursor used were ~1 mg. A large amount of precursor would increase the radiochemical yield of [^{18}F]PBR06, but decrease the chemical purity of the [^{18}F]PBR06 tracer solution due to precursor contamination, especially for Br-PBR06 precursor. The reaction solvent and temperature were either CH_3CN/120 °C or DMSO/140 °C. Radiolabeling procedure with DMSO at 140 °C resulted in higher radiochemical yield [36]. Chemical purity and radiochemical purity were determined by analytical HPLC [34]. The chemical purity of the Br-PBR06 and tosyloxy-PBR06 precursors and reference standard PBR06 was >96%. The radiochemical purity of the target tracer [^{18}F]PBR06 was >99%, and the chemical purity of [^{18}F]PBR06 was 87–95% determined by HPLC methods. Likewise, a C-18 Plus Sep-Pak cartridge was used to significantly improve the chemical purity of the tracer solution as aforementioned. The chemical purity of the [^{18}F]PBR06 tracer solution with Sep-Pak purification was increased higher 10–20% than that without Sep-Pak purification. We noticed Briard et al. [19] have reported an experimental radiosynthesis of [^{18}F]PBR06 using 18-crown-6 with $KHCO_3$ in 97% radiochemical yield, and they have previously shown for a model compound that 18-crown-6 is preferred to replace Kryptofix 2.2.2 in ^{18}F-labeling reaction because the precursor alkylates Kryptofix 2.2.2 [37]. However, this method is not practical and useful for the tracer routine production, since the starting [^{18}F]fluoride ion was <74 mBq (2 mCi), and it is difficult to scale up to and to repeat in automated production of ^{18}F-tracers. An automated production of [^{18}F]PBR06 using Kryptofix 2.2.2 with K_2CO_3 and a commercial module (TRACERlab EX_{F-N}) was also reported by Briard et al. in the same paper, but it only gave 12% isolated radiochemical yield [19]. Furthermore, their Investigational New Drug (IND) Application 23195 for [^{18}F]PBR06 at NIMH submitted to the US Food and Drug Administration (FDA) (a copy is available at: http://pdsp.med.unc.edu/snidd/) shows they actually adopt automated production of [^{18}F]PBR06 (12% using Kryptofix 2.2.2) in their CMC ([^{18}F]PBR for injection: Chemistry, Manufacturing and Controls). This method is comparable to our radiosynthesis performed in a self-designed automated [^{18}F]-radiosynthesis module. Obviously, the radiochemical yield of [^{18}F]PBR06 was significantly increased in our method, especially for new tosylated precursor (30–60% isolated radiochemical yield) we developed. Specific activity of [^{18}F]PBR06 produced by tosylated precursor was higher than that by the bromide precursor, because there might be a pseudo carrier in the bromide precursor. In summary, the radiosynthesis of [^{18}F]PBR06 using new tosylated precursor gave similar radiochemical purity, and higher specific activity, radiochemical yield and chemical purity in comparison with the bromide precursor. Representative analytical HPLC chromatograms for [^{18}F]PBR06 produced by both tosyloxy-PBR06 and Br-PBR06 are shown in Fig. 3.

4. Conclusions

Improved and efficient syntheses of PBR06 and its Br-PBR06 precursor (for F-18 labeling) have been developed. New desmethyl-PBR06 precursor (for C-11 labeling) and tosyloxy-PBR06 precursor (for F-18 labeling) have been designed and synthesized for the first time. Desmethyl-PBR06 was labeled with [^{11}C]CH_3OTf, and isolated by semi-preparative HPLC combined with SPE purification to provide [^{11}C]PBR06, a carbon-11 labeled form of PBR06, for the first time, in high radiochemical yield with excellent specific activity and shorter reaction times. Previously undescribed tosyloxy-PBR06 precursor was labeled with K[^{18}F]F/Kryptofix 2.2.2 through nucleophilic substitution, and isolated by semi-preparative HPLC combined with SPE purification to produce [^{18}F]PBR06, a fluorine-18 labeled form of PBR06, in higher radiochemical yield, chemical purity and specific activity, compared to previously described Br-PBR06 precursor. An automated self-designed multipurpose [^{11}C]- and [^{18}F]-radiosynthesis module for the synthesis of [^{11}C]PBR06 and [^{18}F]PBR06 has been built, featuring the measurement of specific activity by the on-the-fly technique. New and improved results in the synthetic methodology, radiolabeling, preparative separation and analytical details for PBR06, desmethyl-PBR06, Br-PBR06 and tosyloxy-PBR06 precursors, [^{11}C]PBR06 and [^{18}F]PBR06 have been addressed. These methods are efficient and convenient. It is anticipated that the approaches for the design, synthesis and automation of new tracer and radiolabeling precursors, and improvements to increase radiochemical yield, chemical purity and specific activity of the tracers described here can be applied with advantage to the synthesis of other ^{11}C- and ^{18}F-radioligands for PET imaging. These chemistry results warrant future preclinical and clinical PET studies of [^{11}C]PBR06 and [^{18}F]PBR06 in animals and humans to image cancer and neuroinflammation. This work will provide useful information for other investigators who will perform *in vitro* and *in vivo* biological evaluations of these [^{11}C] and [^{18}F] tracers and develop new PET tracers for imaging the TSPO *in vivo*.

Acknowledgments

This work was partially supported by the Breast Cancer Research Foundation. ^{1}H NMR spectra were recorded on a Bruker Avance II 500 MHz NMR spectrometer in the Department of Chemistry and Chemical Biology at Indiana University Purdue University Indianapolis (IUPUI), which is supported by a NSF-MRI Grant CHE-0619254. The referees' criticisms and editors' comments for the revision of the manuscript are greatly appreciated.

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