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Research Article

Radiosynthesis of 3-(3-[^{18}F]fluoropropoxy)-4-(benzyloxy)-*N*-[(1-dimethylaminocyclopentyl)methyl]-5-methoxybenzamide, a potential PET radiotracer for the glycine transporter GlyT-2

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Summary

The recently described selective and potent GlyT2 antagonist, 4-benzyloxy-3, 5-dimethoxy-N-[(1-dimethylaminocyclopentyl) methyl]benzamide ($IC_{50} = 16$ nM) provided an important additional tool to further characterize GlyT2 pharmacology. In order to identify an effective PET radioligand for *in vivo* assessment of the GlyT-2 transporter, 3-(3-[^{18}F]fluoropropoxy)-4-(benzyloxy)-N-((1-dimethylaminocyclopentyl) methyl)-5-methoxybenzamide ([^{18}F]**3**), a novel analog of 4-benzyloxy-3,5-dimethoxy-N-[(1-dimethylaminocyclopentyl) methyl]benzamide was synthesized using a one-pot, two-step method. The NCA radiofluorination of 1,3-propanediol di-*p*-tosylate in the presence of K_2CO_3 and Kryptofix-222 in acetonitrile gave 81% 3-[^{18}F]fluoropropyl tosylate, which was subsequently coupled with 4-benzyloxy-3-hydroxy-5-methoxy-N-[(1-dimethylaminocyclopentyl) methyl]-benzamide in the same reaction vessel. Solvent extraction and HPLC (Eclipse XDB-C8 column, 80/20/0.1 MeOH/H_2O/Et_3N, 3.0 ml/min) gave [^{18}F]**3** in 98.5% radiochemical purity. The radiochemical yield was determined to be 14.0–16.2% at EOS, and the specific activity was 1462 ± 342 GBq/μmol. The

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time of synthesis and purification was 128 min. The final product was prepared as a sterile saline solution suitable for *in vivo* use.

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Key Words: glycine transporter 2(GlyT2); PET; fluorine-18; radiotracer transporter 2(GlyT2)

Introduction

Glycine is an obligatory co-agonist at N-methyl-D-aspartate receptor (NMDA-R), and recent clinical studies have demonstrated efficacy of exogenous glycine in treating negative symptoms of psychosis. As extracellular glycine is regulated by high-affinity glycine transporters, pharmacological inhibition of such transporters represents a novel mechanism for increasing glycine within the brain.[1] Clinical studies with high dose glycine therapy, which improves outcome of schizophrenic patients with negative symptoms, supports the idea that augmenting NMDA transmission by increasing glycine levels can benefit psychotic patients.[2] Glycine transporters have been cloned and are at present classified into two distinct gene families, glycine transporter 1 (GlyT1) and glycine transporter 2 (GlyT2). These have now been further divided into three subtypes of GlyT-1 (a, b, and c) and two splice variant versions of GlyT2 (a and b).[3–5] Immunocytochemical studies showed that the GlyT1 transporter has a wide distribution throughout the central nervous system (CNS) whereas the GlyT2 transporter has a similar distribution to strychnine-sensitive glycine receptor (ssGlyR), being confined to the spinal cord and brain stem.[6,7] Alberati D *et al.*[8] reported a novel class of GlyT1 inhibitors. But, the molecules are devoid of activity at the GlyT2 isoform. Recently, the first potent and selective GlyT2 inhibitor was reported, 4-benzyloxy-3,5-dimethoxy-N-[(1-dimethylaminocyclopentyl) methyl]benzamide **1** (Figure 1), which provides an important tool to further characterize GlyT2 pharmacology.[9,10] This compound had a high affinity for GlyT2 ($IC_{50} = 16$ nM) and negligible affinity ($IC_{50} > 100\ \mu$M) for GlyT1. Haradahira1 *et al.*,[11] reported a synthesis of C-11 labeled **1** as a potential PET radioligand, however, *in vivo* studies have not been published with this compound. Therefore, we synthesized a benzamide analog substituted with a fluoropropyl group as potential PET GlyT2 radiotracer. Our approach to a radiolabeled analog was to replace one of the methoxy groups with a fluoroalkyl ether. 3-(3-[^{18}F]Fluoropropoxy)-4-(benzyloxy)-N-((1-dimethylaminocyclopentyl) methyl)-5-methoxybenzamide ([^{18}F]**3**) was synthesized in a one-pot two-step process by reacting the mono-demethylated phenolic precursor **2** (Figure 1) with 3-[^{18}F]fluoropropyl tosylate.

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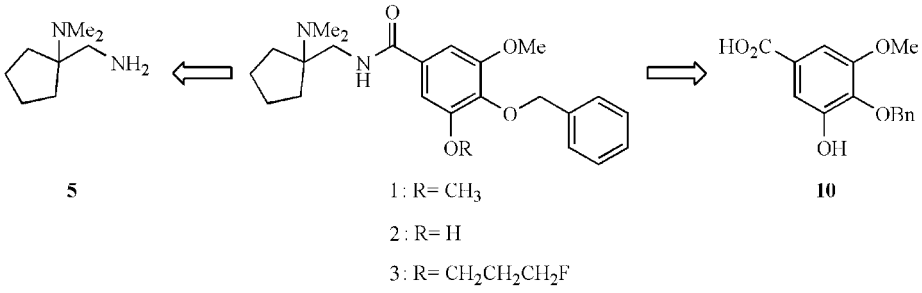

Figure 1. Known GlyT2 ligand 4-benzyloxy-3,5-dimethoxy-N-[(1-dimenthylaminocyclopentyl) methyl]benzamide 1 and novel analogues 2, 3

Results and discussion

Synthetic chemistry

The fluoropropyl analog **3** was prepared in a convergent synthesis from the retrosynthetic components **5** and **10** (Figure 1). The key challenge was generating the unsymmetrically substituted phenolic synthon **10**. Initial attempts to selectively remove one methoxy group were unsuccessful, as the benzyl ether was more labile to cleavage than the methyl ethers under every condition tried. We therefore built the molecule starting from methyl gallate (Scheme 1). Selective 4-*O*-benzylation of methyl gallate has been reported;[12] however, in our hands, only low yields of the desired isomer were obtained. The successful route converted methyl gallate to the triacetoxy ester **6** with acetic anhydride and pyridine. Treatment of the ester with potassium carbonate in the presence of potassium iodide and benzyl chloride in refluxing acetone resulted in selective hydrolysis and concomitant Williamson alkylation to give the 4-*O*-benzyl ester **7** in 72 % yield. Acid hydrolysis at 0°C resulted in preferential cleavage of the aryl acetate esters groups to give methyl 4-*O*-benzylgallate **8** in 90% yield. Careful reaction with one molar equivalent of methyl iodide with K_2CO_3 in DMF at 0°C resulted in the desired unsymmetrical monomethyl ether **9**, separated from the dimethyl byproduct by flash chromatography. Saponification of the methyl ester **9** gave the unsymmetrically substituted acid **10**.

The second half of the convergent synthesis proceeded in a straightforward sequence from cyclopentanone. Condensation with potassium cyanide and dimethylamine gave the amino nitrile **4**, which was then reduced with *in situ* generated aluminum hydride to give the diamine **5**. Final assembly of the ligand was accomplished by Williamson reaction between phenol **9** and 3-fluoropropyl tosylate (synthesized in two steps from 3-chloro-1-propanol by reaction with potassium fluoride in ethylene glycol followed by esterification with *p*-toluenesulfonyl chloride in pyridine) in DMF using NaH as base to give

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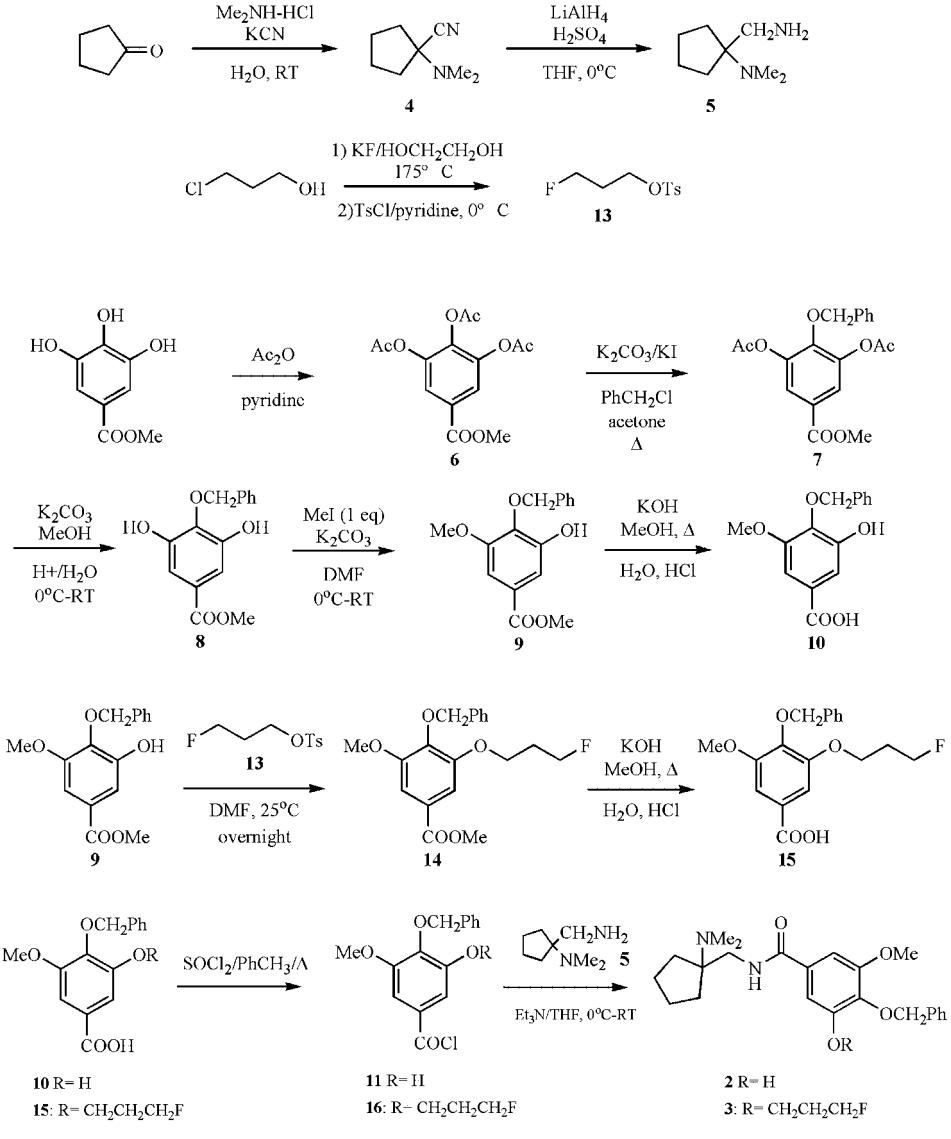

Scheme 1. Chemical Synthesis of fluoro analog 3 and phenolic precursor 2

the ester **14**. The methyl ester was hydrolyzed (acid **15**), then converted to the acid chloride **16** and reacted with diamine **5** to give the target ligand **3**. For synthesis of the phenolic precursor **2**, acid **10** was converted to the acid chloride with thionyl chloride **11** and reacted with diamine **5** in THF overnight at room temperature.

Radiochemistry

[^{18}F]**3** was synthesized in a two-step one-pot process as shown in Scheme 2. The labeling intermediate 3-[^{18}F]fluoropropyl tosylate was prepared by the

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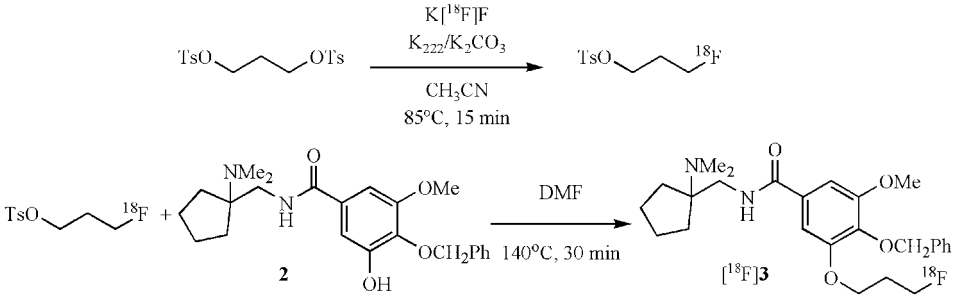

Scheme 2. Radiosynthesis of [^{18}F]3

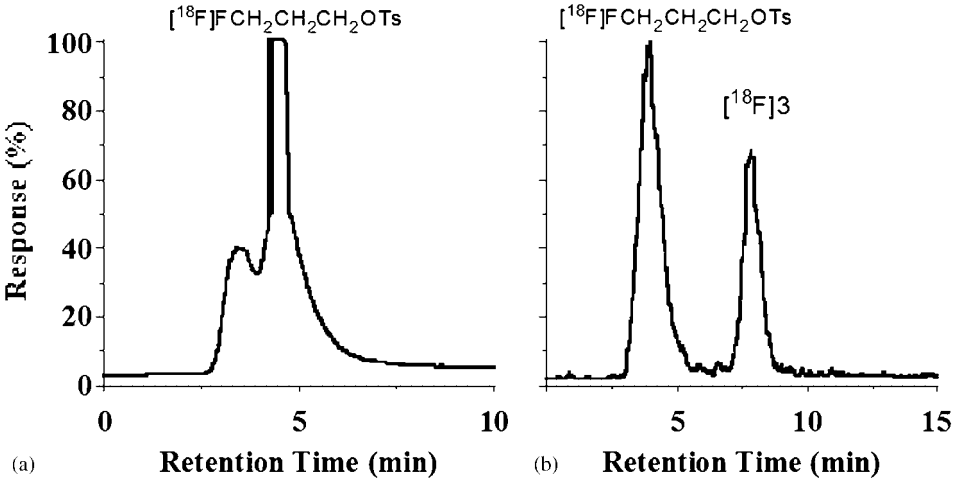

Figure 2. Radio-HPCL of reaction mixtures of [^{18}F]fluoropropyl tosylate (a) and [^{18}F]3 (b)

reaction of [^{18}F]F^- with 1,3-propanediol di-*p*-tosylate in the presence of K_2CO_3 and Kryptofix-222, and provided 3-[^{18}F]fluoropropyl tosylate in 81% labeling yield. HPLC analysis (Figure 2(a)) indicated that 3-[^{18}F]fluoropropyl tosylate was stable in acetonitrile for up to 60 min. For preparation of [^{18}F]**3**, the labeled radioactive intermediate was not isolated but was subjected directly to the alkylation reaction in the same pot after evaporation of CH_3CN.

Alkylation was carried out by heating the 3-[^{18}F]fluoropropyl tosylate with **2** in DMF in the presence of NaH at 140°C for 30 min. HPLC analysis of the reaction mixture showed two radioactive peaks (t_R 4.6 and 7.8 min), corresponding to the retention times of 3-[^{18}F]fluoropropyl tosylate and [^{18}F]**3**, respectively (Figure 2(b)). Sampling at intermediate time points indicated initial rapid rate of alkylation followed by slow increase up to 30 min (Figure 3).

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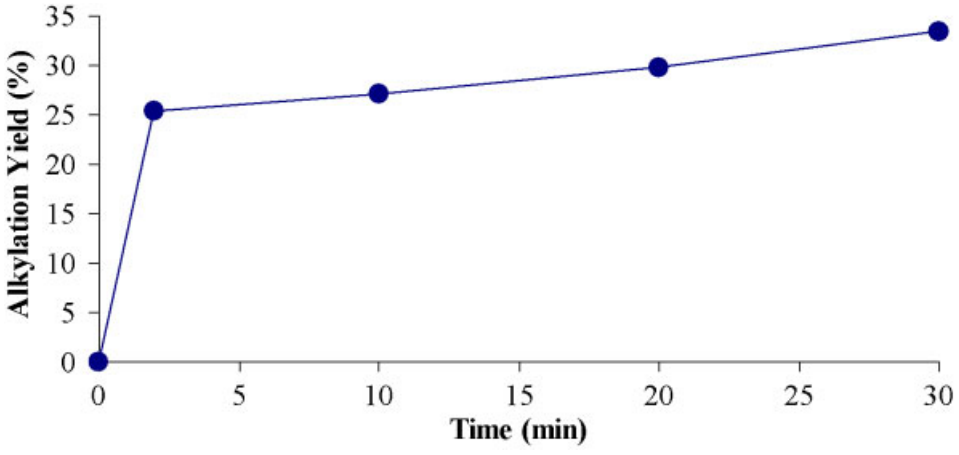

Figure 3. Rate of alkylation of precursor 2 with 3-[^{18}F]fluoropropyl tosylate (decay-corrected)

Two pre-HPLC purification methods were examined: solid phase extraction (SPE) and liquid–liquid extraction (LLE). For SPE, the reaction mixture was added to water and the aqueous mixture was passed through a C-18 Sep-Pak cartridge. The Sep-Pak was washed with water and the radioactive products were eluted with methanol. The results showed poor recovery, with the radioactivity in the methanol extract less than that in the water. On the other hand, ethyl acetate extraction of [^{18}F]**3** from the aqueous reaction mixture provided 40% recovery. After evaporating ethyl acetate, the residue was dissolved in methanol and the crude product was purified by semi-preparative HPLC. The overall radiochemical yield of [^{18}F]**3** was 14–16% with a total synthesis time of about 128 min. The radiochemical purity of [^{18}F]**3** was $>98\%$ and the specific activity was 1462 ± 342 GBq/μmol as determined by HPLC UV analysis; the stability test showed it was stable during 60 min.

3-[^{18}F]fluoropropyl tosylate was selected to react with compound **2**, the reason for this selection was that 3-[^{18}F]fluoropropyl tosylate has been developed as a useful alkylating reagent for introducing F-18 into substrates containing amine, phenol and amide functional groups. In previous work,[13] in one-pot method an alkylating agent, [^{18}F]fluoropropyl tosylate, was used without intermediate purification. We chose 12 mg 1,3-propanediol di-*p*-tosylate as F-18 labeling precursor based on Teija Koivula *et al.*'s report,[14] the best radiochemical yield in the preparation of [^{18}F]fluoropropyl tosylate can be achieved using 10–15 mg of 1,3-propanediol-di-*p*-tosylate precursor. Other reason we select excess 1,3-propanediol di-*p*-tosylate was that the success of the 'one-pot' synthesis of [^{18}F]FEM-IMPY and [^{18}F]FPM-IMPY,[15] where excess 1,3-propanediol bistosylate does not interfere with the radiosynthesis. We also hope that an excess of the fluoropropyl tosylate was

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used for the alkylation to avoid the presence of any unreacted compound **2** in the crude product.

In this work we did not optimize mole ratio of ditosylate and compound **2**, a lower relative yield of [^{18}F]**3** from 3-[^{18}F]fluoropropyl tosylate was achieved (Figure 3), HPLC analysis elutes the product accompanied with impure byproduct, these results imply that excess ditosylate may compete with the 3-[^{18}F]fluoropropyl tosylate for the precursor and which may have caused a lower radiochemical yield of [^{18}F]**3**.

Experimental

General

Melting points (m.p.) were uncorrected; ^{1}H-NMR spectra were recorded on a Bruker 400 or 500 MHz spectrometer with tetramethylsilane as an internal standard. ^{13}C NMR spectra were taken on a Bruker 400 or 500 MHz spectrometer. Elemental analysis was performed at Atlantic Microlab, Inc. (Norcross, GA). Flash chromatography was done on Merck Kieselgel gel 60 F254. Reagents were purchased from Sigma-Aldrich, St. Louis, MO.

Analytical HPLC was performed on Waters Nova-Pak®C18 4 μm, 4.6 × 250 mm. (Waters Inc., USA); mobile phase, MeOH/H_2O/Et_3N (80/20/0.1, v/v/v), flow rate, 1.0 ml/min. The labeled product was separated by semi-preparative HPLC using Eclipse XDB-C8 9.4 × 250 mm. column (Agilent Technologies Inc. USA), with the same mobile phase at a flow rate of 3.0 ml/min. HPLC analysis and purification were performed with a HPLC pump (Varian Prostar 210 solvent delivery module, Varian Associates. Inc., USA), an in-line Varian Prostar 320 UV-detector (254 nm), and a G-M radioactivity detector (Ludlum Model 2600 Spectrometer, Ludlum Measurements. Inc., USA). HPLC data were recorded by a ProStar/Dynamax System dual channel control/interface module (Varian. Inc., USA) connected to a microcomputer with Star Chromatography Workstation Version 5 software.

Synthetic chemistry

*1-(Dimethylamino)cyclopentanecarbonitrile (**4**).* A solution of KCN (6.5 g, 0.1 mol) in 50 ml H_2O was added over 10 min to a stirred, cooled suspension of $HNMe_2 \cdot HCl$ (8.15 g, 0.1 mol) and cyclopentanone (8.4 g, 0.1 mol). The mixture was stirred overnight at room temperature and extracted with Et_2O. The organic layer was washed with H_2O, dried over $MgSO_4$ and then evaporated under reduced pressure to give a colorless oil **4** (7.15 g, 52%, lit. 87%). [16] ^{1}H NMR($CDCl_3$) δ: 2.27 (s, 6H, $CH_3 \times 2$), 2.1 (m, 2H), 1.8–1.6 (m, 6H); ^{13}C NMR ($CDCl_3$) δ: 119.6 (CN), 69.4 (C_1), 41.9($CH_3 \times 2$), 38.4 (C_2 and C_5), 23.2(C_3 and C_4).

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*1-Aminomethyl-1-dimethylamino-cyclopentane (**5**).* To a stirred suspension of $LiAlH_4$ (12.4 g, 0.32 mol) in 200 ml dry THF, cooled to 0°C under N_2, was added dropwise a solution of H_2SO_4 (8.2 ml, 0.16 mol) in dry THF(30 ml). The mixture was stirred for 2 h and then allowed to rest overnight at room temperature. To this suspension, a solution of **4** (13.8 g, 0.10 mol) in 80 ml THF was added dropwise at 0°C. The mixture was warmed at 40–50°C for 3 h, then cooled and quenched with H_2O. The mixture was filtered and the filtrate was concentrated under reduced pressure. The oily residue was purified by bulb-to-bulb distillation to give **5** (6.31 g, 45%, lit. 56%);[16] ^{1}H NMR ($CDCl_3$) δ: 2.6 (s, 2H, NCH_2), 2.2 (s, 6H, CH_3 x 2), 1.8–1.3 (m, 8H, cyclopentyl); ^{13}C NMR ($CDCl_3$) δ: 70.1 (C_1), 48.6 (CNH_2), 39.6 ($CH_3 \times 2$), 30.1 (C_2), 30.9 (C_5), 25.9 (C_3 and C_4).

*Methyl 3,4,5-triacetoxybenzoate (**6**).* Methyl 3,4,5-triacetoxybenzoate was prepared as previously described[12] with minor modifications. A mixture of methyl gallate (5.00 g, 27.15 mmol) and acetic anhydride (9.98 g, 97.7 mmol) in pyridine (25 ml) was stirred 2 h at 0°C to room temperature. The reaction mixture was then poured into 1.2 M HCl and extracted with EtOAc (3×75 ml). The combined EtOAc extracts were washed with aqueous $NaHCO_3$ until the wash solution was basic, washed with brine (50 ml), dried ($MgSO_4$,), and evaporated in vacuo. Recrystallization (EtOH) gave 7.90 g (93%) of **6** as a white solid, m.p. 119–121°C (lit 126.5–128°C).[17]

*Methyl 3,5-diacetoxy-4-benzyloxybenzoate (**7**)*

A mixture of methyl 3,4,5-triacetoxybenzoate **6** (4.00 g, 12.90 mmol), K_2CO_3 (5.40 g, 38.70 mol), KI (0.330 g, 2.0 mmol), and benzyl chloride (3.26 g, 25.80 mmol) was heated in acetone (200 ml) under reflux for 18 h. The reaction mixture was then cooled, poured into water (300 ml), and extracted with Et_2O (3×100 ml). The combined Et_2O extracts were washed with brine (3×100 ml), dried ($MgSO_4$), and evaporated in vacuo. Recrystallization (EtOH) gave 3.98 g (72%) of **7**, m.p. 104–106°C (lit 94–96°C).[17] ^{1}H NMR ($CDCl_3$) δ: 7.7 (s, 2H), 7.4 (m, 5H), 5.1 (s, 2H), 3.8 (s, 3H), 2.1 (6H); ^{1}H NMR ($CDCl_3$) δ: 7.69 (2H, s, H_2, H_6), 7.36 (5H, s, CH_2Ph, ArH); 5.05 (2H, s, CH_2); 3.89 (3H, s, CO_2Me), 2.20(6H, s, 3-OAc, 5OAc).

*Methyl 4-benzyloxy-3,5-dihydroxybenzoate (**8**).* To a solution of **7** (1.60 g, 4.46 mmol) in MeOH (80 ml) was added a solution of K_2CO_3 (2.80 g) in water (40 ml) at 0°C in 10 min. After being stirred at room temperature for 20 min, the solvent was evaporated and the residue was acidified (pH = 2) with 1 M HC1, then extracted with EtOAc (3×100 ml). The combined EtOAc extracts were washed with brine (2×100 ml) and H_2O (1×100 ml), dried ($MgSO_4$), and evaporated *in vacuo*. Recrystallization ($CHC1_3$/hexane) gave 1.06 g (90%)

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of **8**, m.p. 127–129°C (lit. m.p.:133–134°C).[17] ^{1}H NMR ($CDCl_3$) δ: 7.3 (m, 5H), 7.2 (s, 2H), 5.6 (s, 1H), 5.1 (s, 2H), 3.8 (s, 3H).

*Methyl 4-benzyloxy-3-hydroxy-5-methoxybenzoate (***9***)*. Methyl 4-benzyloxy-3,5-dihydroxybenzoate (**8**) (2.02 g; 7.4 mmol) and 1.96 g K_2CO_3 were suspended in 10 ml DMF. Then, 0.994 g (7.00 mmol) CH_3I was added at 0°C and the resulting mixture was stirred at RT for 5 h. After removing the solvent, the residue was treated with H_2O and extracted with Et_2O. The Et_2O extract was dried ($MgSO_4$) and the solvent removed on the rotary evaporator. The residue was purified by chromatography (ether/hexane 1/1) to afford 1.43 g (yield 67.5%) of white solid, m.p. 124–126 °C. ^{1}H NMR ($CDCl_3$) δ: 7.3 (m, 5H), 7.2 (m, 2H), 5.8 (s, 1H), 5.1 (s, 2H), 3.8 (s, 3H), 3.7(s, 3H).

*4-Benzyloxy-3-hydroxy-5-methoxybenzoic acid (***10***)*. Methyl 4-benzyloxy-3-hydroxy-5-methoxybenzoate **9** (405 mg, 1.4 mmol) was heated at reflux in 10 ml of 30% aqueous KOH and methanol for 1 h. Excess solvent was removed on the rotary evaporator and 20 ml H_2O was added. 12 M HCl was added dropwise to the mixture to pH 1. The white precipitate was washed with H_2O and dried to give 317 mg (82.4%) **10** as a white solid, m.p. 133–135°C. ^{1}H NMR ($CDCl_3$) δ 7.2–7.3 (m, 7H), 5.2 (s, 1H), 5.1 (s, 2H), 3.8 (s, 3H).

*4-Benzyloxy-3-hydroxy-5-methoxybenzoyl chloride (***11***)*. A mixture of acid **10** (317 mg, 1.15 mmol) and thionyl chloride (1.19 g, 10 mmol) in toluene (5 ml) was heated at reflux until no more gas was given off (2 h), then the reaction mixture was concentrated under reduced pressure. To remove excess of thionyl chloride, the residue was repeatedly dissolved in toluene (2×10 ml) and evaporated to dryness to give acid chloride **11** as an oil, which was used directly in subsequent reactions.

*3-Fluoro-1-propanol (***12***)*. Compound **12** was prepared from 3-chloro-1-propanol as described.[18] A mixture of 18.9 g. (0.2 mol) of 3-chloro-1-propanol, 23.3 g. (0.4 mol) of KF, and 30 g of ethylene glycol was heated at 175–180°C with vigorous stirring. During the reaction the product was continuously distilled off between 130 and 160°C. After 3 h, 8.17 g of a colorless liquid was collected. The crude 3-fluoro-1-propanol was distilled twice. Yield 8.17 g (54%). ^{1}H NMR($CDCl_3$) δ 1.8 (m, 2H), 3.3 (m, 1H), 3.7 (m, 2H), 4.4–4.5 (m, 2H).

*3-Fluoropropyl p-toluenesulfonate (***13***)*. A mixture of *p*-toluenesulfonyl chloride (9.8 g, 50.2 mmol) and pyridine (12 g) was stirred at 0°C while 3-fluoro-1-propanol (3.92 g, 50 mmol) was added slowly. Stirring was continued for 1 h more, resulting in a white precipitate. The mixture was diluted with H_2O, 12 M HCl was added until no pyridine odor was perceptible,

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and the resultant mixture was extracted several times with Et_2O. The combined extracts were washed successively with H_2O, aqueous $NaHCO_3$ and finally with H_2O again. After drying ($MgSO_4$) and removal of Et_2O, distillation of the residue yielded 3-fluoropropyl *p*-to1uenesulfonate (7.63 g, 65.9%,), as a colorless liquid. ^{1}H NMR($CDCl_3$) δ 1.8 (m, 2H), 2.3 (s, 3H), 4.0 (m, 2H), 4.2 (m, 2H), 7.2(m, 2H), 7.6(m, 2H).

*Methyl 3-(3-fluoropropoxy)-4-(benzyloxy)-5-methoxybenzoate (***14***).* A mixture of compound **9** (560 mg, 1.9 mmol), 928 mg (4 mmol) 3-fluoropropyl *p*-toluenesulfonate and NaH (50 mg, 4 mmol) in 10 ml DMF was stirred at room temperature overnight. The solvent was removed under vacuum, then the residue was dissolved in DMF and purified by chromatography (ether/hexane, 1/1), to give 425 mg (50%) oil. ^{1}H NMR ($CDCl_3$) δ 2.0 (m, 2H), 3.8 (s, 6H), 4.0 (m, 2H), 4.3 (m, 2H), 5.0 (s, 2H), 7.3 (m, 5H), 7.4(m, 2H).

*3-(3-Fluoropropoxy)-4-(benzyloxy)-5-methoxybenzoic acid (***15***).* This compound was synthesized from 570 mg (1.64 mmol) compound **14** according to the method described for **10** to give 370 mg (68%) oil. ^{1}H NMR($CDCl_3$) δ 2.0 (m, 2H), 3.7 (s, 3H), 3.9 (m, 2H), 4.4–4.5 (m, 2H), 5.0 (s, 2H), 7.3 (m, 5H), 7.4(m, 2H).

*3-(3-Fluoropropoxy)-4-(benzyloxy)-5-methoxybenzoyl chloride (***16***).* This compound was synthesized from compound **15** according to the method described for **11** as oil, used directly in the next reaction.

*3-(3-Fluoropropoxy)-4-(benzyloxy)-N-((1-(dimethylamino) cyclopentyl) methyl)-5-methoxybenzamide (***3***).* A solution of 3-(3-fluoropropoxy)-4-(benzyloxy)-5-methoxybenzoyl chloride **16** (409 mg, 1.16 mmol) in anhydrous THF(10 ml) was added dropwise over 15 min to a solution of 1-aminomethyl-1-dimethylamino-cyclopentane **5** (210 mg, 1.5 mmol) and Et_3N (151 mg, 1.5 mmol) in THF (5 ml) at 0°C. The reaction was allowed to warm to room temperature and stirred overnight. The precipitate was removed and the residue was purified by chromatography (EtOAc/MeOH, 1/9), to give 174 mg (34.3%) oil. It was dissolved in MeOH, then excess HCl in Et_2O was added. Evaporation to dryness yielded the hydrochloride **3** as a gum. ^{1}H NMR ($CDCl_3$) δ 1.4–1.6 (m, 8H), 1.9(m, 2H), 2.1 (s, 6H), 3.4(s, 2H), 3.7 (s, 3H), 4.0(m, 2H), 4.4–4.5(m, 2H), 5.0 (s, 2H), 7.1–7.3 (m, 7H).

*4-Benzyloxy-3-hydroxy-5-methoxy-N-[(1-dimethylaminocyclopentyl)methyl]-benzamide (***2***)*

This compound was synthesized from **5** (168 mg, 1.2 mmol) and **11** (2.46 g, 8.5 mmol) according to the method described for **3**. Yield 188 mg (41.0%) as oil; HCl salt: gum. ^{1}H NMR($CDCl_3$) δ 1.3 (m, 2H), 1.5 (m, 4H), 1.7 (m, 2H),

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2.2 (s, 6H), 3.2(s, 2H), 3.8 (s, 3H), 5.2 (s, 2H), 5.7 (s, 1H), 6.9 (m, 2H), 7.3–7.4 (m, 5H). Analytically Found C, 65.90; H, 7.39; N, 6.22. Calculated for $C_{23}H_{31}ClN_2O_4$: C, 63.51; H, 7.18; N, 6.44.

Radiochemistry

Two-step/one-pot radiofluorination and alkylation. [^{18}F]Fluoride was produced with an RDS-112 11 MeV negative ion cyclotron by the reaction ^{18}O(p,n)^{18}F using 150 µl [^{18}O]H_2O (97.2 at% ^{18}O) in a silver target. The aqueous solution of [^{18}F]fluoride was transferred to a 12-ml test tube (red-top Vacutainer®, BD) containing a mixture of K_2CO_3 (4 mg) and kryptofix-222 (20 mg) in CH_3CN/H_2O, then dried by azeotropic distillation with acetonitrile (4 × 0.5 ml) at 120°C (oil bath) under a stream of Ar(g). After cooling to room temperature, the residue was dissolved in 0.5 ml CH_3CN, 12 mg 1,3-propanediol di-*p*-tosylate was added, the tube was sealed, and heated at 85°C for 15 min. Aliquots (10 µl) were analyzed by analytical HPLC. The radiofluorination labeling yield was calculated from the area% of the radioactive peak at t_R 4.6 min, the proof of identity of the products in the HPLC is by comparison with reference compounds. Solvent was evaporated under a stream of Ar(g). This residue was used directly for the alkylation.

A solution of 4-benzyloxy-3-hydroxy-5-methoxy-N-[(1-dimethylamino-cyclopentyl) methyl]benzamide HCl salt (**2**) (5 mg) in *N,N*-dimethylformamide (DMF, 0.5 ml) and NaH (1.3 mg) was added, the tube was capped, then heated to 140°C. Aliquots (10 µl) were analyzed by analytical HPLC. The alkylation yield was calculated from the area% of the radioactive peak at t_R 7.8 min. Upon completion of alkylation (after 30 min of reaction), DMF was evaporated in vacuo at 80°C. The residue was dissolved in ethyl acetate (2 ml) and extracted with H_2O (3 ml), and then the organic layer was passed through a plug filled with Na_2SO_4. The solvents were removed and the residue was re-dissolved in 300 µl of MeOH and purified by semi-preparative HPLC, collecting the fraction corresponding to [^{18}F]**3**. After removing solvent on a rotary evaporator, the residue was dissolved in EtOH and diluted with normal saline to give a final solution of 10% ethanol-saline.

Conclusion

To develop a PET radiotracer for imaging glycine transporter 2(GlyT2), a novel benzamide analog substituted with a fluoropropyl group for GlyT2 was synthesized. The PET radiotracer [^{18}F]**3** was synthesized by the alkylation of the precursor **2** with 3-[^{18}F]fluoropropyl tosylate, the radioligand was obtained with high specific activity and radiochemical purity and in sufficient radiochemical yield for PET studies with animals.

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