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Research Article

Efficient synthesis of 6-chloro-3-((2-(*S*)-azetidinyl)methoxy)-5-((*E*)-2-(2-[^{18}F]fluoropyridin-4-yl)vinyl)pyridine ([^{18}F]NIDA 52289), a very high affinity radioligand for nicotinic acetylcholine receptors

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Summary

6-Chloro-3-((2-(*S*)-azetidinyl)methoxy)-5-((*E*)-2-(2-[^{18}F]fluoropyridin-4-yl)vinyl)pyridine ([^{18}F]NIDA 52289), a very high affinity radioligand for studying nicotinic acetylcholine receptors (nAChRs) by positron-emission tomography, was synthesized through Kryptofix 222 assisted no-carrier-added nucleophilic [^{18}F]fluorination of 6-chloro-3-((1-(*tert*-butoxycarbonyl)-2-(*S*)-azetidinyl)methoxy)-5-((*E*)-2-(2-bromopyridin-4-yl)vinyl)pyridine, followed by acidic deprotection. The overall radiochemical yield of the radiosynthesis was 10% (non-decay-corrected), the specific radioactivity was in the range of 93–326 GBq/μmol (2.5–8.8 mCi/μmol) and the radiochemical purity was greater than 99%. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: nicotinic acetylcholine receptors; A-85380; positron-emission tomography; ^{18}F

Introduction

In the past few years, synthesis of epibatidine[1] and A-85380[2] (Figure 1)-based radioligands for the high specific uptake imaging of nAChRs in animals by positron-emission tomography (PET)[3–6] has been reported. In 2002, the first high specific uptake PET imaging of nAChR in human subjects was performed by using the newly developed radiotracer 2-[^{18}F]fluoro-A-85380 (Figure 1).[7,8] Owing to the moderate affinity of 2-[^{18}F]fluoro-A-85380 (K_D 46 pM[9]), it is only suitable for quantification of brain regions with the highest density of nAChRs, i.e., thalamus.[10] In addition, 2-[^{18}F]fluoro-A-85380 displays very

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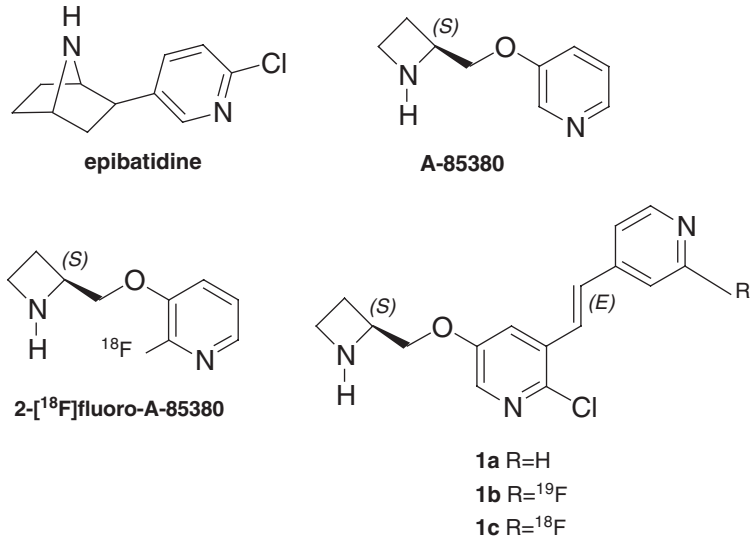


Figure 1. High-affinity nAChR ligands

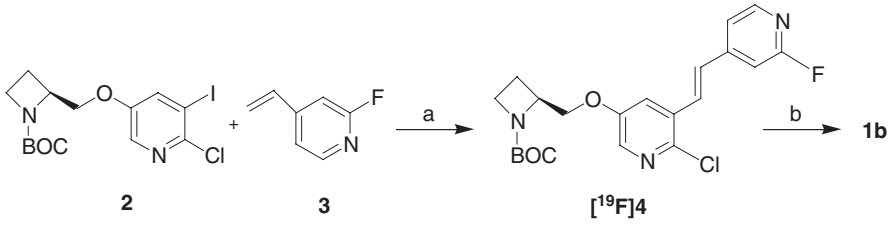
slow brain kinetics which makes this ligand less than ideal for receptor occupancy studies in monkeys and humans. There could be several reasons for the slow kinetics including high hydrophilicity of 2-[^{18}F]fluoro-A-85380 ($c\log D_{7.4} = -1.99$).[11] It is well known that the rate of passive transport of small molecules across blood–brain-barrier (BBB) exhibits a bell-shaped correlation versus apparent lipophilicity ($\log D_{7.4}$) of the molecule with the highest rate of permeability in the range of $\log D_{7.4} = 0$–3.[12,13] Therefore, under the conditions of passive transport, the hydrophilic molecules tend to display a slower rate of permeability. Based on the above-mentioned data we assumed that a more lipophilic radiotracer than 2-[^{18}F]fluoro-A-85380 might display improved brain kinetics in PET studies. However, for a lipophilic compound, we should expect a higher non-specific binding and lower binding potential than those of hydrophilic 2-[^{18}F]fluoro-A-85380. For that reason, a higher binding affinity is necessary for the lipophilic compound in order to improve the binding potential.[14]

In the further development of high-affinity ligands for nAChRs, a series of 2-chloro-5-vinylpyridinyl-A-85380 derivatives was reported by our lab.[11] One member of that series, 6-chloro-3-((2-(*S*)-azetidinyl)methoxy)-5-((*E*)-2-(4-pyridinyl)vinyl)pyridine (**1a**) (Figure 1), was of particular interest because of its very high binding affinity (K_i 9 pM) and a higher lipophilicity than that of 2-F-A-85380. However, this ligand is not suitable for further PET application due to the lack of a site for radiolabeling. Here we describe the F-18 radiolabeling of a fluorinated derivative of **1a**, 6-chloro-3-((2-(*S*)-azetidinyl)methoxy)-5-((*E*)-2-(2-fluoropyridin-4-yl)vinyl)pyridine (NIDA 52289, **1b**,

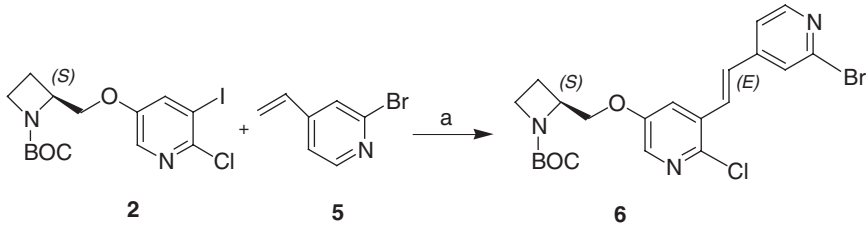
Figure 1), a more lipophilic ligand ($c\log D_{7.4} = 0.24$) that displays a substantially higher affinity at $\alpha4\beta2$-nAChRs (K_i 3.1 pM)[15] than those of 2-[^{18}F]fluoro-A-85380 or epibatidine ($K_D = 15$ pM).[16]

Results and discussion

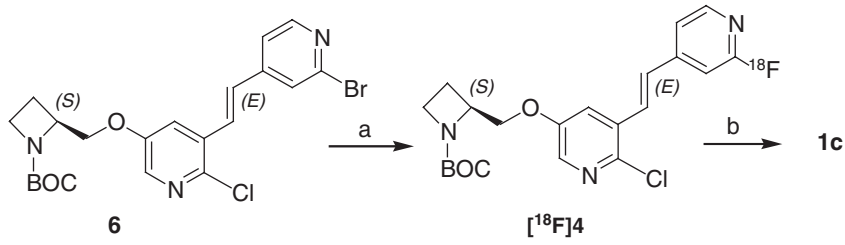
The standard compound **1b** was prepared by Heck coupling reaction of the iodopyridyl ether **2**[11] and vinyl-heteroarene derivative **3**[15] followed by removal of t-BOC protective group (Scheme 1). The precursor for the radiosynthesis, 6-chloro-3-((1-(*tert*-butoxycarbonyl)-2-(*S*)-azetidinyl)methoxy)-5-((*E*)-2-(2-bromopyridin-4-yl)vinyl)pyridine (**6**), was also prepared by Heck coupling reaction of **2** and **5**[15] (Scheme 2). NMR spectra of compounds **1b**, **4** and **6** show proton spin-coupling constants (J_{ab}) value of 16 Hz for the olefinic vicinal hydrogens. Such a value of J_{ab} is characteristic for *E*-protons (12–18 Hz) whereas *Z*-protons exhibit the J_{ab} value in the range of 6–12 Hz.[17] Formation of *E*-alkenes is typical for Heck-type coupling involving vinylarenes.[18,19] The preparation of [^{18}F]-labeled **1c** (Figure 1) was accomplished by the no-carrier-added nucleophilic halogen-exchange radiofluorination of compound **6** using a general procedure described previously,[4] followed by removal of *tert*-BOC-protective group (Scheme 3). Kryptofix 222-assisted radiofluorination of **6** in DMSO at 180–190°C yielded the corresponding 6-chloro-3-((1-(*tert*-butoxycarbonyl)-2-(*S*)-azetidinyl)methoxy)-5-((*E*)-2-(2-[^{18}F]



Scheme 1. Reagents: (a) 1,2,2,6,6-pentamethylpiperidine, Pd $(OAc)_2$, $(p\text{-to-lyl})_3$P, acetonitrile. (b) TFA



Scheme 2. Reagents: (a) 1,2,2,6,6-pentamethylpiperidine, Pd $(OAc)_2$, $(p\text{-to-lyl})_3$P, acetonitrile



Scheme 3. Reagents: (a) [^{18}F]fluoride/Kryptofix 222, DMSO. (b) TFA/CH_2Cl_2

fluoropyridin-4-yl)vinyl)pyridine [^{18}F]**4** in 15–18% radiochemical yield after semi-preparative high performance liquid chromatography (HPLC) purification. In additional experiments, [^{18}F]**4** co-eluted with [^{19}F]**4**.

In the course of reverse phase HPLC purification of [^{18}F]**4**, an unidentified radioactive by-product with greater retention time (18–21 min) than that of [^{18}F]**4** (14–16 min) has been observed. The peak ratio [^{18}F]**4**/by-product was in the range of 7–10. Since chlorine atom connected with the internal pyridine ring can potentially undergo nucleophilic radiofluorination, we conclude that the by-product might be, perhaps, 2-[^{18}F]fluoro-5-((1-(*tert*-butoxycarbonyl)-2-(*S*)-azetidinyl)methoxy)-3-((*E*)-2-(2-bromopyridin-4-yl)vinyl)pyridine. The calculated lipophilicity of the by-product ($\log P = 4.65$) is greater than that of [^{18}F]**4** ($\log P = 4.37$), which is in agreement[20] with retention times of both radiolabeled compounds in reverse phase (RP) HPLC. Removal of *tert*-BOC-protective group worked too slowly when TFA was added directly to the solution of [^{18}F]**4** in the HPLC mobile phase. Therefore, the mobile phase was rotary-evaporated and the protective group was removed by refluxing a solution of [^{18}F]**4** in a methylene chloride/trifluoroacetic acid (TFA) mixture for 15 min followed by evaporation of the solvent. After semi-preparative HPLC purification of the deprotected residue, the expected radiotracer **1c** was prepared with a non-decay-corrected (n.d.c.) overall radiochemical yield of 10%. The average time of the synthesis was 120 min. The specific radioactivity of the final product prepared in six runs from 7 to 19 GBq (200–500 mCi) of starting [^{18}F]fluoride was in the range of 93–326 GBq/μmol (2.5–8.8 mCi/μmol) (n.d.c) and the radiochemical purity was greater than 99%.

Experimental

Materials and methods

All reagents and solvents used were ACS or HPLC grade and were purchased from Aldrich Chemical Co. (Milwaukee, WI). HPLC analysis and purification were performed with two HPLC pumps (model 600/610, Waters, Minneapolis, MN), an in-line Waters UV-detector (254 nm), and a single 2-in NaI crystal flow-count radioactivity detector (Bioscan 3200, Washington, DC). HPLC

chromatograms were recorded by a Dynamax dual channel control/interface module (Rainin/Varian, Palo Alto, CA) connected to a Macintosh computer with Dynamax v.1.4.2 software. A dose calibrator (model CRC-35R, Capintec, Ramsey, NJ) was used for all radioactivity measurements. [^{18}F]Fluoride was prepared using an RDS111 cyclotron (CTI, Knoxville, TN). The radiofluorination was performed using an automated radiochemistry module CPCU (CTI, Knoxville, TN). The bromo-precursor **6**, the standard [^{19}F]**4** and **1b** were prepared in our lab.[15] Elemental analysis was performed at Quantitative Technologies, Inc (QTI). The newly synthesized final compound gave a satisfactory elemental analysis result (C, H, N, $\pm$ 0.4%). Dissociative partition coefficient value ($c \log D$) of compound **1b** was calculated for octanol-water system at pH 7.4 using ACD/log D Suite software (Toronto, Canada).

Chemistry

2-*Chloro*-5-((1-(*tert-butoxycarbonyl*)-2-(*S*)-*azetidinyl*)*methoxy*)-3-((*E*)-2-(2-*fluoropyridin*-4-*yl*)*vinyl*)*pyridine*([^{19}F]**4**). 2-Chloro-3-iodo-5-((1-(*tert*-butoxycarbonyl)-2-(*S*)-azetidinyl)methoxy)pyridine (**2**)[11] (467 mg, 1.10 mmol) and 2-fluoro-4-vinylpyridine (**3**)[15] (135 mg, 1.10 mmol) were dissolved in anhydrous acetonitrile (5 ml). 1,2,2,6,6-pentamethylpiperidine (343 mg, 2.21 mmol), palladium(II) acetate (50 mg, 0.22 mmol) and tri-*o*-tolylphosphine (15 mg, 0.22 mmol) were then added to the solution. The mixture was stirred at 100°C for 12 h while the reaction was monitored by HPLC using SymmetryShield RP18 column, 3.5 µm, 4.6 × 150 mm (Waters, Minneapolis, MN) (50:50 acetonitrile:water, 2 ml/min). The reaction mixture was filtered and concentrated under the vacuum. Gradient flash LC purification (90:10 to 50:50 hexane:ethyl acetate) produced the compound [^{19}F]**4** (252 mg, 0.66 mmol) in 60% yield. ^{1}H NMR ($CDCl_3$/TMS) δ: 8.24 (d, J = 5.2 Hz, 1 H), 8.10 (d, J = 2.9 Hz, 1 H), 7.62 (m, 1 H), 7.57 (d, J = 16.3 Hz, 1 H), 7.33 (dt, J = 1.5, 3.7 Hz, 1 H), 7.07 (d, J = 16.3 Hz, 1 H), 7.03 (s, 1 H), 4.54 (m, 1 H), 4.43 (m, 1 H), 4.21 (dd, J = 2.6, 10.2 Hz, 1 H), 3.90 (m, 2 H), 2.35 (m, 2 H), 1.42 (s, 9 H).

2-*Chloro*-5-((2-(*S*)-*azetidinyl*)*methoxy*)-3-((*E*)-2-(2-*fluoropyridin*-4-*yl*)*vinyl*)-*pyridine* (**1b**), *salt with TFA*. TFA (0.5 ml, 6.5 mmol) was added to a solution of [^{19}F]**4** (61 mg, 0.17 mmol) in dichloromethane (2 ml). The mixture was stirred overnight at room temperature and the solvent was removed by rotary-evaporation at 50–60°C to reveal **1b** · TFA (120 mg, 0.17 mmol) as viscous yellow oil with quantitative yield. ^{1}H NMR ($CDCl_3$/TMS) δ: 8.28 (d, J = 5.4 Hz, 1 H), 8.14 (d, J = 2.9 Hz, 1 H), 7.69 (d, J = 2.9 Hz, 1 H), 7.55 (d, J = 16.4 Hz, 1 H), 7.36 (d, J = 5.3 Hz, 1 H), 7.07 (d, J = 16.4 Hz, 1 H), 7.06 (s, 1 H), 4.98 (m, 1 H), 4.42 (m, 2 H), 4.20 (m, 2 H), 2.80 (m, 2 H). Elemental analysis: Calcd for $C_{16}H_{15}N_3ClFO \cdot 3.15\ TFA \cdot 0.9\ H_2O$: C, 38.52; H, 2.87; N, 6.04. Found: C, 38.81; H, 2.68; N, 5.79.

2-*Chloro*-5-((1-(*tert-butoxycarbonyl*)-2-(*S*)-*azetidinyl*)*methoxy*)-3-((*E*)-2-(2-*bromopyridin*-4-*yl*)*vinyl*)*pyridine* (**6**). Compound **2**[11] (467 mg, 1.10 mmol) and 2-bromo-4-vinylpyridine, **5**[15] (202 mg, 1.10 mmol) were dissolved in anhydrous acetonitrile (5 ml). 1,2,2,6,6-pentamethylpiperidine (343 mg, 2.21 mmol), palladium(II) acetate (50 mg, 0.22 mmol) and tri-*o*-tolylphosphine (15 mg, 0.22 mmol) were then added to the solution. The mixture was stirred at 100°C for 48 h while the reaction was monitored by HPLC using SymmetryShield RP18 column, 3.5 μm, 4.6 × 150 mm (Waters, Minneapolis, MN) (50:50 acetonitrile:water, 2 ml/min). The reaction mixture was filtered and concentrated under the vacuum. Gradient flash LC purification (90:10 to 50:50 hexane:ethyl acetate) produced the compound **6** in 72% yield. MS, m/z, $(M+H)^+$ 480.3622, calcd. for $C_{21}H_{24}BrClN_3O_3$: $(M+H)^+$ 480.0690; ^{1}H NMR ($CDCl_3$/TMS) δ: 8.38 (d, J = 5.2 Hz, 1 H), 8.10 (d, J = 2.9 Hz, 1 H), 7.61 (m, 2 H), 7.55 (d, J = 16.4 Hz, 1 H), 7.38 (dd, J = 1.4, 5.2 Hz, 1 H), 7.01 (d, J = 16.6 Hz), 4.54 (m, 1 H), 4.43 (m, 1 H), 4.21 (dd, J = 2.4, 10.6 Hz, 1 H), 3.90 (m, 2 H), 2.36 (m, 2 H), 1.42 (s, 9 H).

Radiochemistry

6-*Chloro*-3-((2-(*S*)-*azetidinyl*)*methoxy*)-5-((*E*)-2-(2-[^{18}F]*fluoropyridin*-4-*yl*)*vinyl*)-*pyridine* (**1c**). An aqueous solution of the [^{18}F]fluoride (prepared by 11 MeV proton irradiation of 98% enriched $H_2^{18}O$), 25 mg of Kryptofix 222, and 4.5 mg potassium carbonate was added to a 10 ml reaction vessel. The mixture was heated in an oil bath at 120–130°C under a stream of argon while water was evaporated azeotropically using addition of acetonitrile. A solution of compound **6** (3 mg) in anhydrous dimethylsulfoxide (0.9 ml) was added into the reaction vessel and heated at 175°C for 15 min. The reaction mixture was cooled, diluted with 1 ml water, injected onto the semi-preparative Hamilton PRP-1 HPLC column, 10 μm, 7 × 305 mm (Reno, NV) and eluted with a mixture of CH_3CN:H_2O 61:39 at a flow rate of 6 ml/min. The radioactive peak with a retention time of 14–16 min corresponding to intermediate [^{18}F]**4** was collected into a flask with 1 ml TFA (to avoid distillation of [^{18}F]**4**) and the solvent was removed on a rotary evaporator (60–80°C). The residue was dissolved in a mixture of 2 ml TFA and 8 ml CH_2Cl_2 and heated at 60–80°C for 15 min. The solvent was evaporated again on a rotary evaporator, the residue was re-dissolved in 2 ml of the mobile phase (CH_3CN:CH_3OH:CF_3COOH 23:77:0.2), injected onto the second semi-preparative HPLC column (Hamilton PRP-1, 10 μm, 7 × 305 mm) and eluted at a flow rate of 6 ml/min. The radioactive peak with a retention time of 11.5–12.5 min corresponding to **1c** was collected, and the solvent was removed on a rotary evaporator. The product was dissolved in saline (5 ml). The radiochemical yield was in the range of 9–12%.

An aliquot of the final solution of known volume and radioactivity was applied to an analytical Hamilton PRP-1 HPLC column, 7 μm, 4.1 × 250 mm. A mobile phase (CH_3CN:CH_3OH:CF_3COOH 23:77:0.2) at a flow rate of 2 ml/min was used to elute the radioligand, which had a retention time of 9.5 min. The radiochemical purity was greater than 99%. The area of the UV absorbance peak at 254 nm corresponding to carrier product was measured and compared to a standard compound (**1b**) curve relating mass to UV absorbance. The radiochemical product also co-eluted with a sample of non-radiolabeled NIDA 52289, **1b**.

Conclusion

In summary, an efficient radiosynthesis of a very high-affinity-radiofluorinated ligand for nAChRs has been developed. The radioligand was obtained with high specific activity and radiochemical purity and in sufficient radiochemical yield for PET studies with animals. [^{18}F]NIDA 52289 could be of interest for studying nAChRs with PET.

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