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Research Article

Synthesis of 6-chloro-3-((2-(*S*)-azetidinyl)methoxy)-5-(2-[^{18}F]fluoropyridin-4-yl)pyridine ([^{18}F]NIDA 522131), a novel potential radioligand for studying extrathalamic nicotinic acetylcholine receptors by PET

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Summary

6-Chloro-3-((2-(*S*)-azetidinyl)methoxy)-5-(2-[^{18}F]fluoropyridin-4-yl)pyridine ([^{18}F]NIDA 522131), a potential radioligand for studying extrathalamic nicotinic acetylcholine receptors by positron-emission tomography, was synthesized via no-carrier-added nucleophilic [^{18}F]fluorination of 6-chloro-3-((1-(*tert*-butoxycarbonyl)-2-(*S*)-azetidinyl) methoxy)-5-(2-iodopyridin-4-yl)vinyl)pyridine, followed by acidic deprotection. The overall radiochemical yield of the radiosynthesis was 4–8% (non-decay-corrected), the specific radioactivity was in the range of 167–335 GBq/μmol (4500–9000 mCi/μmol) and the radiochemical purity was greater than 99%. Preparation of [^{18}F]NIDA522131 via corresponding bromo-derivative **2** is also described. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: nicotinic acetylcholine receptors; nucleophilic halogen-exchange; positron emission tomography; ^{18}F

Introduction

Non-invasive PET imaging of cerebral $\alpha4\beta2$ nicotinic acetylcholine receptors (nAChRs) is of substantial interest for elucidating the role of nAChRs in the normal and altered states.[1] In 2002, the first high specific uptake PET imaging of nAChR in human subjects was performed[2,3] by using the newly developed radiotracer 2-[^{18}F]fluoro-A-85380[4,5] (K_i 61 pM,[6] Figure 1). Because of the moderate binding potentials (BP) of 2-[^{18}F]fluoro-A-85380, this ligand allows

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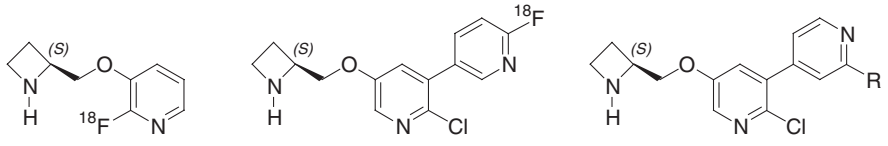


Figure 1. High affinity nAChR ligands

only quantitative imaging of nAChRs in thalamus (BP *ca.* 2), a brain region with the highest density of the receptor.[7–9] In order to develop radioligands suitable for quantification of PET data in the regions with lower density of nAChRs such as cortex and striatum, compounds with much higher binding affinity are required.[10]

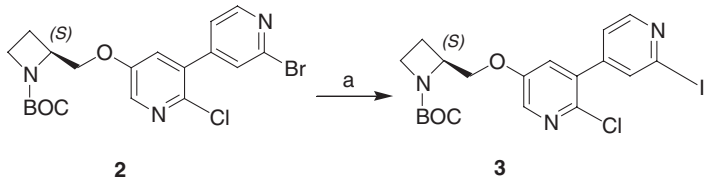
Recently, 5-substituted derivatives of 6-halogeno-3-((2-(*S*)-azetidinyl)-methoxy)pyridine, a series of high affinity ligands for nAChRs, was reported by our lab.[10] One ligand of that series, 6-chloro-3-((2-(*S*)-azetidinyl)methoxy)-5-(2-[^{18}F]fluoropyridin-5-yl)pyridine ([^{18}F]NIDA52189) was of particular interest as a radiotracer for imaging $\alpha4\beta2$-nAChR because its binding potential in monkey brain was substantially greater than that of 2-[^{18}F]fluoro-A-85380.[10] Currently, [^{18}F]NIDA52189 is being studied as a radiotracer for quantitative imaging of extrathalamic $\alpha4\beta2$-nAChR.

An isomer of NIDA52189, 6-chloro-3-((2-(*S*)-azetidinyl)methoxy)-5-(2-fluoropyridin-4-yl)pyridine (**1a**, NIDA 522131) (Figure 1) displays extremely high binding affinity (K_i 4.8 pM) and relatively low lipophilicity ($\log D = -0.92$). Our preliminary *in vivo* study showed that [^{18}F]NIDA 522131 (**1b**) in monkey brain specifically labels $\alpha4\beta2$-nAChR with binding potential value at least as high as that of [^{18}F]NIDA52189.[11]

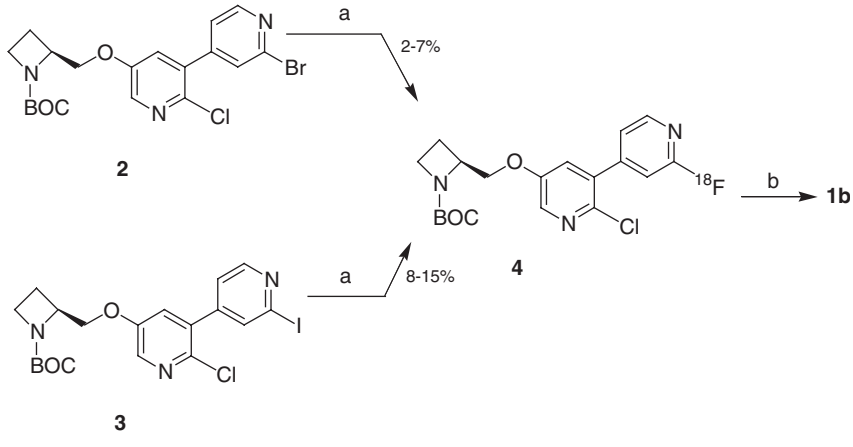
In this paper, we describe a radiosynthesis of **1b** through no-carrier-added nucleophilic [^{18}F]fluorination of two precursors: 2-chloro-5-((1-(*tert*-butoxycarbonyl)-2-(*S*)-azetidinyl)methoxy)-3-(2-bromopyridin-4-yl)pyridine (**2**)[10] and 2-chloro-5-((1-(*tert*-butoxycarbonyl)-2-(*S*)-azetidinyl)methoxy)-3-(2-iodopyridin-4-yl)pyridine (**3**).

Results and discussion

The syntheses of the standard compound **1a** (Figure 1) and the bromo-precursor **2** (Scheme 1) have been described previously by our group.[10] The preparation of [^{18}F]-labelled **1b** (Figure 1) was performed initially by the no-carrier-added nucleophilic Kryptofix 222 – assisted halogen-exchange radiofluorination of compound **2** followed by removal of *tert*-BOC-protective group using a general procedure described previously[12] (Scheme 2). Incorporation of radiofluoride via precursor **2** was optimized with respect of reaction temperature (optimal temperature 185°C, temperature range



Scheme 1. Reagents: (a) KI, CuI, DMSO



Scheme 2. Reagents: (a) [^{18}F]fluoride/Kryptofix 222/K_2CO_3, DMSO. (b) TFA/CH_2Cl_2

170–195°C) yielding the intermediate **4**, 2-chloro-5-((1-(*tert*-butoxycarbonyl)-2-(*S*)-azetidinyl)methoxy)-3-(2-[^{18}F]fluoropyridin-4-yl)pyridine, in 2–7% radiochemical yield. The intermediate **4** and the final [^{18}F]NIDA522131 were both purified by semi-preparative high-performance liquid chromatography (HPLC). However, even after such purification, both the intermediate **4** and the final **1b** were contaminated with a substantial amount of non-radiolabelled unidentified by-products. The overall radiochemical yield of contaminated **1b** obtained via the bromo-precursor **2** was in the range of 0.5–4%. The amounts of Kryptofix 222 and potassium carbonate have not been optimized in this study.

Since the bromo-precursor did not give satisfactory yield and purity of **1b**, we attempted to use the iodo-precursor **3**. Previously, we found that 2-iodopyridine derivatives can be radiofluorinated successfully with high radiochemical yield.[12] Compound **3** was synthesized through copper-assisted iodo–bromo-exchange reaction (Scheme 1). We did not observe any potential reaction of the iodo–chloro-exchange as was expected from previous studies.[13] Radiofluorination of compound **3** at 170°C under the same reaction conditions as for bromo-precursor **2** gave a 8–15% radiochemical yield of intermediate **4** that was free of non-labelled contaminants after a preparative

HPLC purification. Deprotection of **4** followed by second HPLC separation yielded radioligand **1b** with overall non-decay-corrected (n.d.c.) radiochemical yield of 4–8% and free of non-radiolabelled contaminants. The specific radioactivity of the final product was in the range of 167–335 GBq/μmol (4500–9000 mCi/μmol) (n.d.c) from 13–17 GBq (350–450 mCi) of starting [^{18}F]fluoride and the radiochemical purity was greater than 99%. The average time of the synthesis was 140 min.

It is noteworthy to mention that radiofluorination of both precursors **2** and **3** gave radiolabelled unidentified by-products with retention time on reverse-phase HPLC greater than that of compound **4**. We assume that both by-products were the result of [^{18}F]fluorine–chlorine-exchange reaction. As determined by HPLC, the radiochemical yields of the by-products were in the range of 0.3–1 and 1–2%, respectively. In both cases, the radiolabelled by-products and intermediate **4** were readily separated by semi-prep HPLC.

Experimental

Materials and methods

All reagents and solvents used were purchased from Aldrich Chemical Co. (Milwaukee, WI). HPLC analysis and purification were performed with two HPLC pumps (model 600/610, Waters, Milford, MA), 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). High-resolution mass-spectrometry analysis was performed at Emory University Mass Spectrometry Center. ^{1}H NMR spectra were recorded on a Bruker AM 300 (300 MHz) instrument; chemical shifts (δ) were recorded in parts per million (ppm) downfield from TMS. Flash chromatography was conducted using silica gel (230–400 mesh, Merck).

Chemistry

2-Chloro-5-((1-(tert-butoxycarbonyl)-2-(S)-azetidinyl)methoxy)-3-(2-iodopyridin-4-yl)pyridine (**3**). Compound **2**[10] (45.0 mg, 0.10 mmol), copper (I) iodide (1.0 g, 5.2 mmol) and potassium iodide (0.9 g, 5.4 mmol) were dissolved in anhydrous DMSO (1.7 ml). The mixture was stirred at 135°C while the reaction completion was monitored by HPLC (60:40 acetonitrile: 1.0 N aqueous ammonium formate, Symmetry C-18 analytical column (4.6 × 150 mm),

2 ml/min). The retention time for the reactant and the product were 3.9 and 4.3 min, respectively. The reaction was completed after 2.5 h and the mixture was poured into water (20 ml), filtered through celite and extracted with ethyl acetate (3×20 ml). Concentration under the vacuum produced 31 mg crude **3** as a yellow oil. The crude product was purified further via gradient flash column chromatography (9:1 hexane:ethyl acetate, 7:3 hexane:ethyl acetate). The collected product fraction was concentrated by rotary-evaporation. Product **3** was obtained as a pale yellow oil (30 mg, 60%). MS, m/z, M^+ 501.0305, calcd. for $C_{19}H_{21}O_3N_3I^{35}Cl$: M^+ 501.0316; ^1H NMR ($CDCl_3$/TMS) δ: 8.46 (d, $J = 4.6$ Hz, 1 H), 8.20 (d, $J = 3.0$ Hz, 1 H), 7.82 (s, 1 H), 7.40 (dd, $J = 1.6$, 5.1 Hz, 1 H), 7.25 (d, $J = 4.6$ Hz, 1 H), 4.53 (m, 1 H), 4.38 (m, 1 H), 4.16 (dd, $J = 2.8$, 10.0 Hz), 3.88 (m, 2 H), 2.34 (m, 2 H), 1.40 (s, 9 H).

Radiochemistry

6-Chloro-3-((2-(S)-azetidinyl)methoxy)-5-(2-[^{18}F]fluoropyridin-4-yl)pyridine (**1b**). 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 **3** (3 mg) in anhydrous dimethylsulfoxide (0.9 ml) was added into the reaction vessel and heated at 170°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 53:47 at a flow rate of 6 ml/min. The radioactive peak with a retention time of 17–21 min corresponding to intermediate **4** was collected into a flask with 1 ml TFA (to avoid distillation of **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 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 165:835:2), 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 16–18 min corresponding to **1b** was collected, and the solvent was removed on a rotary evaporator. The product was dissolved in saline (5 ml). 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_3-COOH 170:830:2) 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 with a standard compound (**1a**) curve relating mass to UV absorbance.

Conclusion

In summary, a successful radiosynthesis of a potential radioligand for studying extrathalamic nAChRs, [^{18}F]NIDA 522131, has been developed via a corresponding iodo-precursor and is superior to that via bromo-precursor. The radioligand was obtained with high specific activity and radiochemical and chemical purity.

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References

1. Villemagne VL, Musachio JL, Scheffel U. *Neuronal Nicotinic Receptors* 1999; 235–250.
2. Kimes AS, Horti AG, London ED, Chefer SI, Contoreggi C, Ernst M, Friello P, Koren AO, Kurian V, Matochik JA, Pavlova O, Vaupel DB, Mukhin AG. *FASEB J* 2003; **17**: 1331–1333.
3. Bottlaender M, Valette H, Roumenov D, Dolle F, Coulon C, Ottaviani M, Hinnen F, Ricard M. *J Nucl Med* 2003; **44**: 596–601.
4. Horti AG, Koren AO, Ravert HT, Musachio JL, Mathews WB, London ED, Dannals RF. *J Label Compd Radiopharm* 1998; **41**: 309–318.
5. Dolle F, Valette H, Bottlaender M, Hinnen F, Vaufrey F, Guenther I, Crouzel C. *J Label Compd Radiopharm* 1998; **41**: 451–463.
6. Brown LL, Kulkarni S, Pavlova OA, Koren AO, Mukhin AG, Newman AH, Horti AG. *J Med Chem* 2002; **45**: 2841–2849.
7. Hall M, Zerbe L, Leonard S, Freedman R. *Brain Res* 1993; **600**: 127–133.
8. Marutle A, Warpman U, Bogdanovic N, Nordberg A. *Brain Res* 1998; **801**: 143–149.
9. Chefer SI, London ED, Koren AO, Pavlova OA, Kurian V, Kimes AS, Horti AG, Mukhin AG. *Synapse* 2003; **48**: 25–34.
10. Zhang Y, Pavlova OA, Chefer SI, Hall AW, Kurian V, Brown LL, Kimes AS, Mukhin AG, Horti AG. *J Med Chem* 2004; **47**: 2453–2465.
11. Zhang Y, Mukhin AG, Pavlova OA, Chefer SI, Vaupel DB, Brown LL, Hall AW, Kurian V, Horti AG. Abstracts of Papers, *225th ACS National Meeting*, New Orleans, LA, USA, March 23–27, 2003.
12. Horti AG, Scheffel U, Koren AO, Ravert HT, Mathews WB, Musachio JL, Finley PA, London ED, Dannals RF. *Nucl Med Biol* 1998; **25**: 599–603.
13. Hama Y, Nobuhara Y, Aso Y, Otsubo T, Ogura F. *Bull Chem Soc Jpn* 1988; **61**: 1683–1686.

