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Research Article

N1'-(*p*-[^{18}F]Fluorobenzyl)naltrindole (*p*-[^{18}F]BNTI) as a potential PET imaging agent for DOP receptors

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Summary

The *N1*'-(*p*-fluorobenzyl)naltrindole **5** has been synthesized by reaction of 3-*O*-benzyl NTI **3** with *p*-fluorobenzylbromide under phase transfer catalysis. The subsequent 3-*O*-benzyldeprotection of **4** in HBr/CH_3COOH gave the target compound **5** in three steps from naltrindole **2**. *p*-FBNTI **5** is a novel delta opioid receptor antagonist ($K_i = 0.00312$ nM) and antagonizes the delta opioid (DOP) agonist, DPDPE, with a $K_e = 1.55$ nM in the mouse vas deferens preparation. Using the same synthetic strategy the synthesis of *p*-[^{18}F]BNTI **10** was undertaken. The final yield was 4% and the specific activity varied in a range of 250–400 mCi/μmol. Copyright © 2006 John Wiley & Sons, Ltd.

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Key Words: FBNTI; delta opioid receptors; fluorine-18; positron emission tomography

Introduction

Opioid receptors (ORs) belong to the superfamily of G protein-coupled receptors (GPCRs). The existence of at least four types of opioid receptors (MOP, KOP, DOP and NOP) is well established.[1,2]

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Pharmacological and radioligand binding studies have led to the proposal for opioid receptors subtypes. However, the cloning of ORs has confirmed only four main types that are coupled to inhibitory G proteins.[3] The DOP receptors are one of the four major opioid receptors that are present in the central nervous system and in the periphery. In the central nervous system DOP receptors are found in the neocortex, striatum, olfactory areas, substantai nigra, amygdala and the nucleus accumbens.[4] Activation of these receptors results in many physiological and behavioral effects ranging, from modulation of antinociception, mood, sensory system, motor integration and cognative functions. In addition, delta opioid receptors are also present in several immunocompetent cells, which suggest they may play an important role in regulating the immune system.[5]

Pharmacological studies of DOP receptors have suggested the existence of at least two subtypes (delta1 and delta2)[6,7] which has not been substantiated by cloning.[8,9] These pharmacological results can be explained on the basis of two possibilities. The first is that DOP receptors form heterodimers[10–13] with other receptors and these heterodimers have different phenotypical behaviors.[14] The second possibility is that the putative subtypes of delta opioid receptors represent different affinity states of the same receptors due to dimerization and or higher-order oligomerization.[15]

To address these issues, a full characterization of delta opioid receptors is necessary. The *in vivo* distribution profiles can provide information about the DOP receptors in general and their changes in pathological conditions in particular. One useful tool for this is positron emission tomography (PET) which is a non-invasive technique.[16] PET utilizes specifically designed radiotracers with affinity and selectivity for target receptors. Ligand development is the crucial part of PET studies. Therefore, we were interested in preparation a selective DOP receptor ligand to aid the mapping delta opioid receptors *in vivo*. As *N1*'-benzylnaltrindole (BNTI) **1** is known to be a delta2 selective DOP receptors antagonist,[17] we chose to synthesize *p*-fluorobenzylnaltrindole (*p*-FBNTI) **5** based on its close structural similarity to BNTI **1**, because *p*-FBNTI **5** was expected to have similar binding profile for DOP receptors.

We have prepared *p*-FBNTI[18,19] **5** and determined that it bound specifically to delta opioid receptors. *p*-FBNTI **5** antagonized DPDPE with a $K_e = 1.55$ nM in the mouse vas deferens prep. The synthesis of corresponding radiolabeled *p*-[^{18}F]BNTI **10** was undertaken because the ^{18}F ($t_{1/2} = 110$ min) positron emitter should be good candidate for PET study of DOP receptors in the central nervous system. The following report summarizes our results.

Results and discussion

The synthesis of *p*-fluorobenzylnaltrindole **5** started with protection of naltrindole[20] **2** as 3-*O*-benzylnaltrindole **3** prior to fluorobenzylation. The

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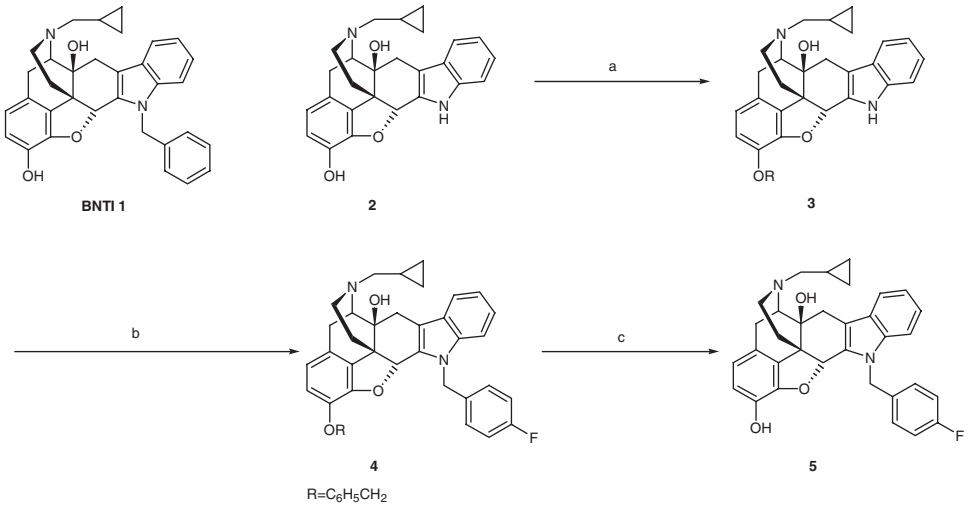

Scheme 1. Synthesis of *N1*'-(*p*-fluorobenzyl)naltrindole *p*-FBNTI 5

synthetic route involved the transfer of *p*-fluorobenzyl bromide under phase transfer catalysis to the indole nitrogen of NTI **2**. The reaction was conducted in the presence of a catalytic amount of tetrabutylammonium hydrogen sulfate (TBAHS) and NaOH (50%) in methylene chloride,[21] affording [3-*O*-benzyl, *N1*'-(fluorobenzyl)]NTI **4** in a yield of 95% which was characterized clearly by ^{1}H and ^{13}C-NMR analyzes. Elemental analysis of compound and mass spectra confirmed unequivocally its constitution. Using HBr in acetic acid the 3-*O*-benzyl protective group was removed selectively. This deprotection method was very effective and the reaction was complete in 10 min, affording *p*-fluorobenzyl NTI **5** in over 90% yield. The structure of the product was assigned with the aid of NMR spectroscopic data. High-resolution mass and elemental analysis also confirmed clearly the identity of the compound (Scheme 1).

In its biological evaluation, the *in vitro* characteristics of *p*-FBNTI **5** showed high affinity for delta opioid receptors ($K_i = 0.00312$ nM). Weak binding was observed for the μ and κ compounds [^{3}H]DAMGO ($K_i = 87.4 \pm 33.8$ nM) and [^{3}H]U69593 ($K_i > 1000$ nM*l*).[18,19] The binding assay (Inhibition of ^{3}H-Diprenorphine in HEK 293 cells) showed the following selectivity ratios of $\mu/\delta = 13814$ and $\kappa/\delta = 41987$ (see Figure 1).

The key compound *p*-[^{18}F]fluorobenzaldehyde **7** was prepared in a yield of $58.8 \pm 11.6\%$ by reaction of $^{18}F^{-}$ with *p*-trifluoromethylammonium-benzaldehyde triflate **6**.[22–25] This starting material was prepared according to a known procedure.[22] Reduction of **7** with $LiBH_4$ and subsequent treatment

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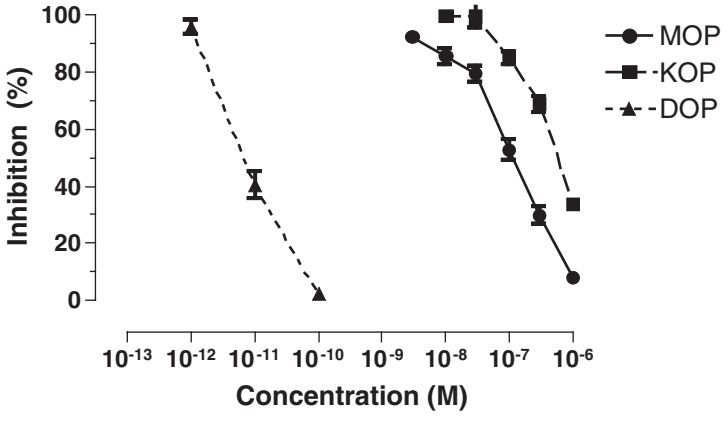

Figure 1. Binding of 5 to opioid receptor-transfected HEK 293 cells

with HBr (48%) gave *p*-[^{18}F]fluoro benzyl bromide **8** in a yield of 68.5%. Compound **8** was transferred via N_2-flow in pentane into a flask containing phase transfer catalyst tetrabutylammonium hydrogen sulfate (TBAHS), KOH and (*3-O*-benzyl NTI) **3** in methylene chloride/toluene (1:1). This reaction gave the intermediate **9**, which on treatment with HBr/CH_3COOH provided the final product **10**. The yield was 4%, and the specific activity varied between 250 and 400 mCi/μmol decay corrected to EOB. The average time for the radiochemical synthesis was 3.5 h from the end of bombardment. Because of its very high DOP selectivity, *p*-[^{18}F]BNTI **10** complements existing PET imaging agents such as *N1*'-([^{11}C]methyl)naltrindole[20] and *N1*'-([^{18}F]fluoroethyl)naltrindole.[26] Application of *p*-[^{18}F]BNTI **10** *in vivo* studies will help to image DOP receptors in human brain and their changes as a consequence of diseases. Imaging DOP receptors and their distribution *in vivo* using positron emission tomography will be very helpful to understand interaction of DOP receptors with other GPCRs in general, and with MOP and KOP receptors in particular.[27] In this regard, a bivalent opioid ligand labeled with positron emitter should visualize the opioid heterodimers and their changes *in vivo* with neuronal cross-reactivity.

Experimental

Solvents and chemicals were purified prior to use. Melting points were determined with Mel-Temp Laboratory Devices and uncorrected. IR spectra were recorded with a Perkin-Elmer 1600 FTIR. ^{1}H NMR (300.007 MHz) and ^{13}C NMR (75.462 MHz) were obtained with a Varian Gemini-300 instrument. Chemical shifts are reported in ppm (δ) relative to internal Me_4Si in $CDCl_3$. High-resolution fast bombardment mass spectroscopy (HRFABMS) was done at the University of Minnesota Mass Spectroscopy Facility. Elemental analysis

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was determined by M-H-W Laboratory in Phoenix, Arizona. Analytical TLC was done on Baker-flex, silica gel IB2-F plates. The analytical HPLC equipment consisted of Waters 600E pumps, Waters 490E UV absorbance detector (254 nm), a NaI (TI) crystal (2'') Beckman 170 Radioisotope detector and Hewlett–Packard 3396A integrators. Preparative HPLC consisted of spectral series UV 100 in series (254 nm) with NaI detector for monitoring radioactivity. Analytical (Waters Bondapak C-18, 3.9×300 mm) and semi preparative (Alltech, Adsorbospere C-18, HS 7U, 10×300 mm) reverse-phase HPLC columns were used. A Hewlett–Packard 3396A integrator recorded the HPLC chromatograms. Radioactivity was measured with a Capintec radioisotope dose calibrator. NTI **1** was prepared from naltrexone as described in the literature.[28,29]

(Cyclopropylmethyl)-6,7-dehydro-4,5-epoxy-3-benzyloxy-14-hydroxy-6,7-2',3'-indolomorphinan (3-O-benzyl NTI) **3**

A solution of NTI **2** (1.1 g, 2.45 mmol) in dry acetone (25 ml) containing K_2CO_3 (10 equivalent) and benzyl bromide (461 mg, 2.7 mmol, 1.1 eq.) was refluxed for 5 h, and then the mixture was cooled to room temperature and filtered. The mother liquor was portioned between water and methylene chloride where the compound was extracted with methylene chloride (3×50 ml) and dried over anhydrous Na_2SO_4. The solvent was evaporated and the compound was purified by gravity column chromatography [SiO_2, CH_2Cl_2/MeOH (20:1)] to give the final product as a white solid.

Yield: 1.27 g (85%); mp > 115°C decomposed.; IR (Film, $CHCl_3$) υ 3500–3100 (w), 3050 (w), 2950 (s), 2850 (m), 1505 (s), 1438 (m) cm^{-1}; ^{1}H-NMR($CDCl_3$) δ 8.1 (s, 1 H, NH), 7.42 (d, $J = 7.74$ Hz, 1 H, Ar), 7.7–7.4 (m, 6 H, Ar), 7.3 (t, $J = 7.0$ Hz, 1 H, Ar), 7.05 (t, $J = 7.86$ Hz, 1 H, Ar), 6.6 (AB, $J_{AB} = 8.25$ Hz, 2 H, Ar), 5.68 (s, 1 H, H_5), 5.05 (AB, $J_{AB} = 11.9$ Hz, 2 H, benzylic protons), 3.49–2.2 (m, 9 H, aliphatic protons), 1.77 (dd, $J = 3.99$ Hz, $J = 2.0$ Hz, 2 H, aliphatic protons), 1.3 (s, 1 H, OH), 0.9 (m, 1 H), 0.6 (d, 8.19 Hz, cyclopropyl protons), 0.2 (d, $J = 4.68$ Hz, 2 H, cyclopropyl protons).; ^{13}C-NMR ($CDCl_3$) δ 144.1, 141.2, 136.9, 136.7, 131.2, 129.3, 128.1, 127.9, 127.7, 126.6, 126.1, 121.8, 118.8, 118.3, 115.3, 111.3, 110, 84.5, 72.1, 70.2, 61.6, 52.6, 47.2, 43.4, 31.1, 31, 22.8, 22.1, 9.3, 3.9, 3.6; (FABHRMS): 505.2491, $C_{33}H_{33}N_2O_3$ requires $(M+H)^+ = 505.2491$. Analytically calculated for $C_{33}H_{33}N_2O_3$ C, 78.54: H, 6.39: N, 5.55. Found C, 78.65: H, 6.53: N, 5.29.

(Cyclopropylmethyl)-6,7-dehydro-4,5-epoxy-3-benzyloxy-14-hydroxy-6,7,2',3'-[1'-(p-fluorobenzyl)]-indolomorphinan [3-O-benzyl, N1'-(p-fluorobenzyl)]NTI **4**

A solution of 3-*O*-benzyl NTI **3** (148 mg, 0.29 mmol) in methylene chloride (15 ml) containing tetrabutylammonium hydrogen sulfate (20 mg) was mixed

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with NaOH (50%, 15 ml). To this reaction mixture was added *p*-fluorobenzyl bromide (60.8 mg, 1.1 eq.) dissolved in methylene chloride (5 ml) and this mixture was stirred for 2 h at room temperature. The reaction mixture was diluted with ice-water and the organic layer was removed. The aqueous layer was extracted with methylene chloride (2×25 ml) and the combined extracts were dried over anhydrous sodium sulfate. After filtration and evaporation of the solvent, the residual compound was purified by flash chromatography [SiO_2, CH_2Cl_2/MeOH (20:1)]. A white powder was obtained which was crystallized from CH_2Cl_2/pentane.

Yield: 132 mg (74.5%); mp > 94°C decomposed.; IR (Film, $CHCl_3$) υ 3450–3400(br, OH), 3060 (w), 2900 (s), 2850 (m), 1625 (m), 1587 (m), 1500 (s) cm^{-1}; ^{1}H-NMR($CDCl_3$) δ 7.48 (d, $J = 7.41$ Hz, 1 H, Ar), 7.4–7.0 (m, 11 H, Ar), 6.48 (t, $J = 8.5$ Hz, 1 H, Ar), 6.5 (AB, $J_{AB} = 8.25$ Hz, 2 H, Ar), 5.72 (s, 1 H, H_5), 5.6 (AB, $J_{AB} = 16.5$ Hz, 2 H, benzylic protons), 4.98 (AB, $J_{AB} = 4.95$ Hz, 2 H, benzylic protons), 3.49–2.2 (m, 9 H, aliphatic protons), 1.85 (dd, $J = 3.99$ Hz, $J = 2.0$ Hz, 2 H, aliphatic protons), 1.3 (s, 1 H, OH), 0.95 (m, 1 H), 0.6 (d, $J = 7.6$ Hz, cyclopropyl protons), 0.2 (d, $J = 4.17$ Hz, 2 H, cyclopropyl protons); ^{13}C-NMR ($CDCl_3$) δ 142, 138, 137.9, 132($J_{C-1,F} = 236$ Hz), 129.3, 127.3 ($^3J_{C-3,F} = 9$ Hz), 126.8, 126.5, 125.7, 125.4, 121.6, 118.2, 118.1, 117.7, 115.5, 114.5, 114.5(d, $^2J_{C-2,F} = 22.5$ Hz), 110.1, 109, 83.4, 71.6, 70.6, 61.3, 58.6, 47.2, 45.9, 42.7, 30.8, 28.2, 22.3, 8.6, 3.2, 3.0; (FABHRMS): 613.2880, $C_{40}H_{37}N_2O_3F$ requires $(M+H)^+ = 613.2854$. Analytically calculated for $C_{40}H_{37}N_2O_3F$ C, 78.40: H, 6.08: N, 4.57. Found C, 78.32: H, 6.19: N, 4.56.

N1'-(p-fluoromethyl)-17-(cyclopropylmethyl)-6,7-didehydro-4,5-epoxy-3,14-dihydroxyindolo[2',3',6,7]morphinan (FBNTI).HBr.1/2H_2O **5**

A solution of **4** (735 mg, 1.2 mmol) was dissolved in acetic acid/ HBr (5 ml, 1:1). The entire mixture was heated to 90°C within 2 h. Subsequently, this mixture was poured into saturated $NaHCO_3$ solution. The resulting gum-like material was washed with water until the filtrate was neutral. The solid material then was dissolved in ethyl acetate (5 ml) and allowed to precipitate as a white powder. The powder was washed with cold ethyl acetate and ether. The HPLC analysis {MeOH/$(NH_4)_2PO_4$ [85:15], flow rate 1 ml/min, $t_R = 9.1$ min} showed the product analytically pure.

Yield: 535 mg (85.3%); mp > 180°C decomposed.; IR (Film, $CHCl_3$) υ 3500–3050(br, OH), 2950 (m), 2900 (m), 2850 (m), 1600 (m), 1550 (m), 1500 (s), 1495 (s) cm^{-1}; ^{1}H-NMR($CDCl_3$) δ 9.3 (s(br), 1H, OH), 7.49 (d, $J = 7.62$ Hz, 1H, Ar), 7.15–6.9 (m, 7H, Ar), 6.6 (AB, $J_{AB} = 8.25$ Hz, 2H, Ar), 5.70 (s, 1H, H_5), 5.4 (AB, $J_{AB} = 16.7$ Hz, 2H, benzylic protons), 4.98, 3.8–2.2 (m, 9H, aliphatic protons), 1.77 (dd, $J = 3.99$ Hz, $J = 2.0$ Hz, 2H, aliphatic protons), 1.3 (s, 1H, OH), 0.9 (m, 1H), 0.58 (d, $J = 7.6$ Hz, cyclopropyl protons), 0.2 (d, $J = 5.1$ Hz, 2H, cyclopropyl protons); ^{13}C-NMR ($CDCl_3$) δ 142.5, 138.7, 137,

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132($J_{C-1,F}$ = 236 Hz), 129.9, 127.6 ($^3J_{C-3,F}$ = 8.2 Hz), 126.6, 125.4, 122.8, 119.4, 119.2, 119.1, 116.7, 115.4(d, $^2J_{C-2,F}$ = 22.5 Hz), 111.4, 109.7, 85, 72.6, 62.3, 59.5, 46.5, 43.8, 45.9, 31.5, 29.1, 23.2, 9.5, 4.2, 3.9, 1.82; (FABHRMS): 523.2384, $C_{33}H_{31}N_2O_3F$ requires $(M+H)^+ = 523.2389$. Analytically calculated for $C_{33}H_{32}N_2O_3BrF.1/2H_2O$ C, 64.71: H, 5.43: N, 4.61. Found C, 64.71: H, 5.52: N, 4.35.

Receptor binding assays

The modified receptor binding assays[30] were applied as follows: Competition binding experiments were performed using HEK 293 cells genetically modified to produce wild-type MOP, KOP, or DOP receptors. Ten concentrations of the tested compounds (50 μL) were added to test tubes containing 0.1 nM ^{3}H-diprenorphine ($\approx 0.5–1.0 \times K_D$) (50 μL) and whole cells (75 mm^2 plate, 80–90% confluent) suspended in 12.5 ml HEPES buffer (25 mM, pH = 7.4) (400 μL). Final volume was 500 μL. Non-specific binding was measured using 10 μM naloxone. Assays were incubated at room temperature for 90 min and then filtered using a Brandel M-48 tissue harvester through Whatman GF/C filter paper that was pre-soaked in 0.25% poly(ethyleniminie). Filters were washed three times with ice-cold HEPES buffer (see above), and the radioactivity counted using a Beckmann LS 6500 liquid scintillation counter. All measurements were performed in triplicate. IC_{50} values were calculated using PRISM software utilizing non-linear regression of the data normalized to fit a sigmoidal dose–response curve with a variable slope (100% defined at concentration = 0 (total binding) and 0% defined at the value of non-specific binding). K_i values were determined from the Cheng-Prusoff[31] equation assuming a single-site-binding model. Values reported are mean IC_{50} and $K_i \pm$ standard error of the mean (SEM) of three or more independent experiments.

Radiolabeling procedure

In preparation for the synthesis of *N1'-p*-([^{18}F]fluorobenzyl naltrindole **10**, the synthesis of *p*-[^{18}F]fluorobenzyl bromide **8** was undertaken as depicted in Scheme 2. Potassium [^{18}F]fluoride was obtained by bombarding [^{18}O]H_2O (Isotec, 1.2 mL, 98% isotopic abundance) with 15 MeV protons and transferred under helium pressure to hot cell through a polyethylene tubing (1/16'' i.d.) in a Reacti-vial containing ~5 mg potassium carbonate (99.995% pure). This solution was concentrated to 0.1 ml. Krytofix^R [2.2.2] (~5–7 mg) in acetonitrile (1 ml) was added and the mixture transferred under helium pressure to a vessel purged with argon gas. The potassium [^{18}F]fluoride was dried via azeotropic removal of water with acetonitrile (3 × 0.5 ml) at 120°C. The precursor, *p*-trifluoromethylammonium-benzaldehyde triflate **6**, dissolved in 0.3 ml DMSO was added to the reaction vessel containing the [^{18}F]fluoride,

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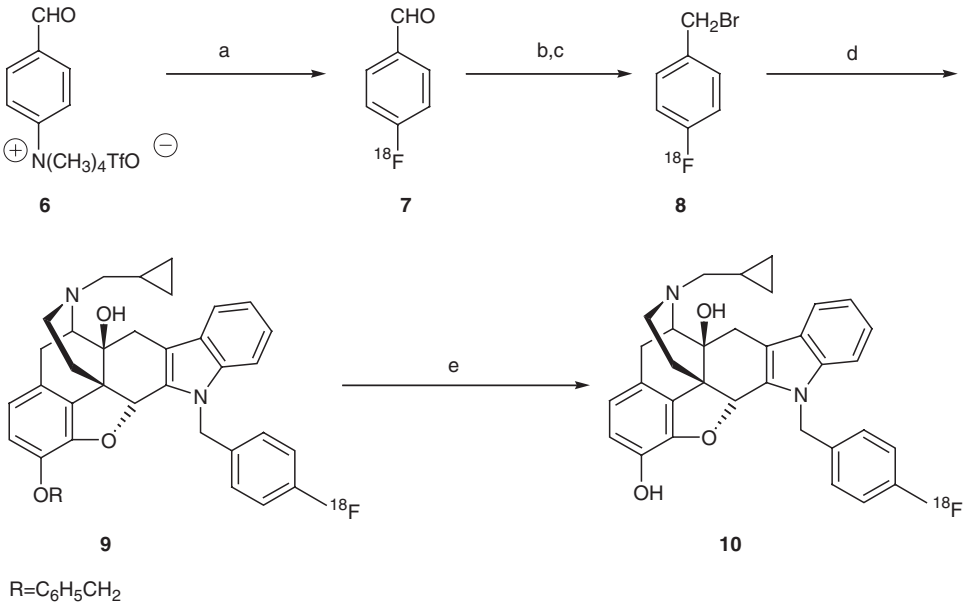

a) ^{18}F-/K222/K_2CO_3; b) THF, $LiBH_4$; c)HBr(48%); d) CH_2Cl_2, tetrabutylammonium hydrogen sulfate, NaOH (50%), *3-O*-benzylnaltrindole **3** ; e) i.CH_3COOH/HBr(1:1), 90 °C, ii. $NaHCO_3$

Scheme 2. Synthesis of *N1*'-(*p*-[^{18}F]fluorobenzyl)naltrindole *p*-[^{18}F]BNTI 10

and the mixture was heated at 85°C for 5 min. After cooling for 2 min at room temperature, the mixture was quenched with 5 ml water and passed through a C-18 Sep-Pak (Waters, Milford, MA) which was sequentially washed with 5 ml 0.01 M HCl and water. The radioactive *p*-[^{18}F]fluorobenzaldehyde **7** was eluted with pentane and passed through an anhydrous Na_2SO_4 column into a flask containing 0.3 ml $LiBH_4$ (2 M in THF). This mixture was stirred for 3 min at room temperature. Finally, the solvent was evaporated at 90°C. The residual material was cooled with methanol/ice and treated with HBr(48%, 2 ml) and then heated at 90°C for 10 min. The resulting mixture was cooled with ice-water for 3 min, diluted with water, and then passed through a C-18 Sep-Pak (Waters, Milford, MA) which was rinsed with a solution of $NaHCO_3$ (3 ml, 1 M), and the radioactive *p*-[^{18}F] fluorobenzyl bromide **8** then was eluted with pentane (3 ml), and the eluent passed through a anhydrous Na_2SO_4/K_2CO_3 column into a flask containing TBAHS (5 mg), KOH (100 µl, 30 M) in methylene chloride/toluene (1:1, 4 ml). To this mixture (3-*O*-benzyl NTI) **3** was added and the entire mixture was heated at 40°C until the pentane was evaporated. Then this mixture was heated at 90°C for 15 min. On cooling, water was added to the reaction mixture, and the product was extracted with methylene chloride and transferred to a round bottom flask. Most of

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methylene chloride was evaporated, and then a mixture of acetic acid and HBr (48%) [1:1, 2 ml] was added to the flask and heated at 90°C for 15 min. This mixture then was quenched with saturated sodium bicarbonate solution and the product was extracted with ether.

The final purification of *p*-[^{18}F]BNTI **10** was achieved via a semi preparative reverse-phase HPLC column using acetonitrile/$(NH_4)_2HPO_4$ (4 mM) in a mixture of 40:60 (flow rate 5 ml/min) for 15 min then the solvent mixture changed to 60:40. The fraction containing *p*-[^{18}F]BNTI 10 ($t_R = 32$ min) was collected and evaporated to dryness. The residual was dissolved in saline containing 10% ethanol and transferred to a sterile, pyrogen-free bottle. The retention time of the *p*-[^{18}F]BNTI **10** was 9.2 min using methanol/dibasic ammonium phosphate [85:15] with a flow rate 1 ml/min. Radiochemical purity and specific activity were determined by analytical HPLC. Specific activity was calculated by relating the area of the UV absorbance peak of carrier *p*-FBNTI **5** in an aliquot of *p*-[^{18}F]BNTI **10** of known radioactivity to the peak area of a standard sample of *p*-FBNTI. *p*-[^{18}F]BNTI **10** of $>99\%$ radiochemical purity was obtained at the end of synthesis with the specific activity varied in a range of 250–400 mCi/μmol, and radiochemical yield of 4% based upon initial [^{18}F]-fluoride activity.

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