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SYNTHESIS OF (*E*)-N-(3-BROMOPROP-2-ENYL)-2β-CARBOMETHOXY-3β-(4'-TOLYL) NORTROPANE (PE2Br) AND RADIOLABELLING of [^{76}Br]PE2Br : A POTENTIAL LIGAND FOR EXPLORATION OF THE DOPAMINE TRANSPORTER BY PET.

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SUMMARY

In order to study the dopamine transporter by PET, we prepared (*E*)-N-(3-bromoprop-2-enyl)-2β-carbomethoxy-3β-(4'-tolyl) nortropane (PE2Br) and its radiobrominated analogue. PE2Br and [^{76}Br]PE2Br were synthesized by bromodestannylation of the tributylstannyl derivative using either N-bromosuccinimide in THF or [^{76}Br]NH_4Br with peracetic acid as oxidant respectively. After purification by HPLC, [^{76}Br]PE2Br was obtained with a radiochemical yield of 80%, a radiochemical purity higher than 98% and a specific radioactivity of 20 MBq/nmol. *Ex vivo* autoradiographic studies in rats showed that 1 hour after i.v. injection of [^{76}Br]PE2Br the highest accumulation in the brain was observed in the striata whereas no

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accumulation was observed in any other brain region. These results can be interpreted in terms of specific binding of [^{76}Br]PE2Br to the dopamine transporter.

INTRODUCTION

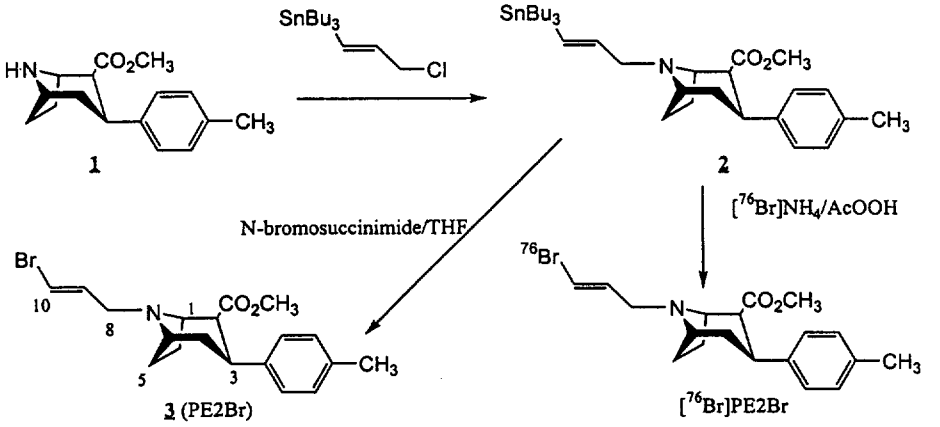
Disturbances of the central dopaminergic system are involved in numerous pathological conditions such as Parkinson's disease and Alzheimer's disease. Exploration of the dopamine transporter, localized on dopaminergic nerve endings, by functional brain imaging using Single Photon Emission Tomography (SPET) or Positron Emission Tomography (PET) is of great value for the diagnosis, and the follow up of therapy effects. In the human brain, cocaine is known to bind to the dopamine (DAT), serotonin (5HTT) and norepinephrine (NET) transporters (1). Cocaine and several cocaine derivatives have been labelled with ^{11}C in order to image the DAT *in vivo*. Among them 2β-carbomethoxy-3β-(4'-iodophenyl) tropane (β-CIT) has been proposed and used in clinical studies (2, 3). However this ligand also has high affinity for the 5HTT (5) and, moreover, maximal specific binding to the DAT in the brain is obtained only 20 hours post-injection (4). Other cocaine derivatives have therefore been developed. [^{11}C]N-(3-fluoropropyl)-2β-carbomethoxy-3β-(4'-iodophenyl) tropane ([^{11}C]CIT-FP) and [^{11}C] N-(2-fluoroethyl)-2β-carbomethoxy-3β-(4'-iodophenyl) tropane ([^{11}C]CIT-FE) present improved specific binding to the DAT in the striatum of the monkey brain compared to β-CIT. However their kinetic properties are not appropriate to the short half-life of ^{11}C ($T_{½}$ = 20 min) (6, 7). We have recently developed a SPET radioligand, (*E*)-N-(3-iodoprop-2-enyl)-2β-carbomethoxy-3β-(4'-tolyl) nortropane (PE2I), possessing fast kinetics, allowing maximal specific binding 1 hour post-injection, and high specificity for the DAT (8). We therefore synthesized the bromo analogue of PE2I, (*E*)-N-(3-bromoprop-2-enyl)-2β-carbomethoxy-3β-(4'-tolyl) nortropane (PE2Br), in order to develop a PET radiotracer with high specificity and early in vivo specific binding to the DAT. We report here the synthesis, the radiosynthesis and preliminary biological evaluation of PE2Br labelled with ^{76}Br, a positron emitter with a long half-life ($T_{½}$ = 16hours), so that radiopharmaceutical characterization and preclinical evaluation is easily accomplished.

RESULTS AND DISCUSSION

Chemistry

(*E*)-N-(3-bromoprop-2-enyl)-2β-carbomethoxy-3β-(4'-tolyl) nortropane **3** (PE2Br) and its radiobrominated analogue were synthesized as described in scheme 1. The 2β-carbomethoxy-3β-(4'-tolyl) tropane was prepared by 1-4 Michael addition, at -40°C, of the tolylmagnesium bromide to ecgonidine methyl ester according to previously reported methods (9, 10). N-demethylation of the tropane derivative was accomplished by conversion to its carbamate using 2,2,2-trichloroethylchloroformate followed by zinc-acetic acid reduction to supply 2β-carbomethoxy-3β-(4'-tolyl) nortropane **1** according to the procedure previously described by Clark et al (11). (*E*)-3-*n*-tributylstannylprop-2-enyl chloride, prepared in two steps from propargyl alcohol, then reacted with the nortropane derivative **1** to yield the tributylstannyl precursor **2** according to a previously described procedure (12). The bromodestannylation reaction was performed in THF using N-bromosuccinimide yielding 60% PE2Br (13). This reaction achieved at 0°C using one equivalent of N-bromosuccinimide afford only to bromodestannylation versus electrophilic aromatic substitution since no aromatic brominated compound could be detected by NMR and HPLC. Moreover this stereoselective reaction

Scheme 1: Synthesis of (*E*)-N-(3-bromoprop-2-enyl)-2β-carbomethoxy-3β-(4'-tolyl) nortropane PE2Br and [^{76}Br]PE2Br.



afford to the same (*E*)-configuration as the stannyl precursor **2**. Indeed, the double bond proton coupling constant was in accordance with the (*E*) rather than the (*Z*)-configuration ($^3J_{AB}$ = 13.6 Hz).

Radiochemistry

[^{76}Br](*E*)-N-(3-bromoprop-2-enyl)-2β-carbomethoxy-3β-(4'-tolyl) nortropane was prepared at room temperature by bromodestannylation of the tributylstannyl derivative **2** in ethanol using peracetic acid as an oxidant. The lipophilic character of the stannyl precursor allowed easy purification of [^{76}Br]PE2Br on HPLC (retention time 15 min). [^{76}Br]PE2Br was obtained with a radiochemical yield of 80%. The radiochemical purity was greater than 98% and the specific activity was 20 MBq/nmol.

Ex vivo autoradiographic studies

Ex vivo autoradiographic images of the radioactivity distribution in horizontal slices of a rat brain passing through the frontal cortex, the striatum, the thalamus, the hippocampus and the cerebellum demonstrated that a preferential localization of radioactivity can be observed in the striatum one hour after injection of [^{76}Br]PE2Br. No significant uptake of [^{76}Br]PE2Br was observed in the other brain regions. The ratios of concentrations of radioactivity in brain structures to the cerebellum were 3.9, 1.2, 1.2 and 1.0 for the striatum, frontal cortex, thalamus and hippocampus, respectively. These ratios are in accordance with specific in vivo binding of [^{76}Br]PE2Br to the DAT over both 5HTT and NET.

EXPERIMENTAL

NMR spectra were recorded on a Brüker DPX Avance 200 spectrometer (200 MHz for ^{1}H, 50.3 MHz for ^{13}C) using $CDCl_3$ as solvent. Chemical shifts were expressed in ppm in relation to TMS as an internal standard. Mass spectra were obtained on a GC-MS Hewlett Packard 5989A spectrometer (electronic impact at 70eV). The thin layer chromatographic (TLC) analyses were performed using 60F-254 silica gel plates (Merck) and visualization was

performed under UV or in an iodine chamber. Flash chromatography was used for routine purification of reaction products using silica gel 230-400 mesh (Merck). All chemicals and solvents were of commercial quality and were purified following standard procedure.

(E)-N-(n-tributylstannylprop-2-enyl)-2β-carbomethoxy-3β-(4'-tolyl) nortropane 2:

(*E*)-3-*n*-tributylstannylprop-2-enyl chloride (260 mg, 0.71 mmol) was added to a solution of 2β-carbomethoxy-3β-(4'-tolyl) nortropane **1** (184 mg, 0.64 mmol) in absolute EtOH (2.5 mL) containing Et_3N (0.1 mL) and a catalytic amount of KI. The mixture was refluxed for 16 h. The reaction mixture was then concentrated under reduced pressure, and the residue was purified by flash chromatography (petroleum ether 40-65°C / AcOEt / Et_3N, 85 / 15 / 1) to yield pure (*E*)-N-(*n*-tributylstannylprop-2-enyl -2β-carbomethoxy-3β-(4'-tolyl) nortropane **2** as a colourless oil (418 mg, 81%).

^{1}H NMR ($CDCl_3$): δ = 0.82 [t, 9H, ^3J = 7.0 Hz, ($C\underline{H}_3CH_2CH_2CH_2)_3Sn$]; 1.18-1.59 [m, 21H, ($CH_3C\underline{H}_2C\underline{H}_2C\underline{H}_2)_3Sn$, H-4α, H-6α, H-7α]; 1.96 (m, 2H, H-6β, H-7β); 2.21 (s, 3H, Ph-CH_3); 2.56 (td, 1H, $^3J_{3\text{-}4\beta} = {}^2J_{4\alpha\text{-}4\beta}$ = 12.5 Hz, $^3J_{4\beta\text{-}5}$ = 3.0 Hz, H-4β); 2.75-2.98 (m, 3H, H-2, H-3, H-8); 3.11 (dd, 1H, ^2J = 13.6 Hz, $^3J_{8'\text{-}9}$ = 3.8 Hz, H-8') ; 3.37 (m, 1H, H-5); 3.42 (s, 3H, O-CH_3); 3.62 (m, 1H, H-1); 5.75-6.01 (ABXX', 2H, $^3J_{9\text{-}10}$ = 19.2 Hz, $^3J_{8\text{-}9}$ = 6.1 Hz, $^3J_{8\text{-}9}$ = 3.8 Hz, H-9, H-10); 6.97 (d, 2Har, ^3J = 8.0 Hz); 7.10 (d, 2Har, ^3J= 8.0 Hz).

^{13}C NMR ($CDCl_3$): δ = 9.3 [($CH_3CH_2CH_2\underline{C}H_2)_3Sn$, $^1J_{Sn\text{-}C}$ = 327, 342 Hz]; 13.6 [($\underline{C}H_3CH_2CH_2CH_2)_3Sn$]; 20.9 (Ph-$CH_3$); 25.7, 26.0 (C-6, C-7); 27.2 [($CH_3\underline{C}H_2CH_2CH_2)_3Sn$, $^3J_{Sn\text{-}C}$= 54 Hz]; 29.0 [($CH_3CH_2\underline{C}H_2CH_2)_3Sn$, $^2J_{Sn\text{-}C}$= 22 Hz]; 33.9 (C-3); 34.0 (C-4); 50.9 (C-2); 52.6 (O-CH_3); 60.3 (N-CH_2); 61.2 (C-5); 61.5 (C-1); 126.5 (2 CHar); 128.5 (2 CHar); 130.1 ($Bu_3Sn\underline{C}$, $^1J_{Sn\text{-}C}$ = 366, 384 Hz); 135.0 (Car); 139.9 (Car); 146.8 (C-8); 171.9 (CO_2).

MS (EI): m/z = 532 (17); 298 (100); 266 (65); 177 (29); 122 (49); 41 (96).

(E)-N-(3-bromoprop-2-enyl)-2β-carbomethoxy-3β-(4'-tolyl) nortropane 3:

To a solution of (*E*)-N-(*n*-tributylstannylprop-2-enyl)-2β-carbomethoxy-3β-(4'-tolyl) nortropane **2** (150 mg, 0.26 mmol) in 5 mL of THF was added N-bromosuccinimide (42 mg,

0.23 mmol). The reaction mixture was stirred at room temperature for 60 min. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (Et_2O/Et_3N, 95/5) to afford the (*E*)-N-(3-bromoprop-2-enyl)-2β-carbomethoxy-3β-(4'tolyl) nortropane **3** (PE2Br) as a waxy substance (60 mg, 62%).

^1H NMR ($CDCl_3$) : δ = 1.57-1.68 (m, 3H, H-4α, H-6α, H-7α); 1.89-2.01 (m, 2H, H-6β, H-7β); 2.22 (s, 3H, Ph-CH_3); 2.52 (td, 1H, $^3J_{3\text{-}4\beta} = {}^2J_{4\alpha\text{-}4\beta} = 12.6$ Hz, $^3J_{4\beta\text{-}5} = 2.8$ Hz, H-4β); 2.75-2.96 (m, 4H, H-2, H-3, N-CH_2); 3.33 (m, 1H, H-5); 3.44 (s, 3H, O-CH_3); 3.58 (m, 1H, H-1); 6.01-6.18 (ABXX', 2H, $^3J_{trans}$ = 13.6 Hz, H-9, H-10); 7.00 (d, 2Har, ^3J = 8.3 Hz); 7.08 (d, 2Har, ^3J= 8.3 Hz).

^{13}C NMR ($CDCl_3$) : δ = 20.9 (Ph-CH_3); 25.7, 25.9 (C-6, C-7); 33.7 (C-3); 33.9 (C-4); 51.0 (O-CH_3); 52.6(C-2); 55.3 (C-8); 61.3 (C-5); 62.2 (C-1); 106.8 (C-10); 127.1 (2CHar); 128.6 (2CHar); 135.2, 135.9 (2Car); 139.7 (C-9); 171.4 (CO_2).

MS (EI): m/z = 379 (15); 377 (15); 298 (65); 189 (35); 188 (21); 187 (37); 186 (19).

Radiochemistry

[^{76}Br] was produced by irradiation of natural arsenic (1.7 g) with a beam of 30 MeV [^{3}He] ions. After decay of ^{75}Br ($T_{½}$ = 1.6 hours), the target was dissolved in 40 mL concentrated sulfuric acid at 180°C. After cooling to 50°C, 4 g of chromic acid in 12 mL water were added. The radioactive bromine was removed under a nitrogen stream and trapped as bromide in 2 mL 1M ammonia that was later taken to dryness.

100 µg of (*E*)-N-(*n*-tributylstannylprop-2-enyl)-2β-carbomethoxy-3β-(4'-tolyl) nortropane **2** in 100 µL of ethanol and 100 µL of 1.6% peracetic acid in concentrated acetic acid were added to a vial containing no-carrier-added [^{76}Br]NH_4. After 30 min at room temperature the labelling process was stopped, on evaporation under reduced pressure, the radiolabelled compound was isolated by HPLC on a RP18 column (µ-Bondapak-C18, 7.8 x 300 mm, Waters) using H_3PO_4 0.01 M/acetonitrile (60/40) as mobile phase (flow rate of 3 mL/min). Detection was monitored with an UV detector (Waters) at 254 nm and a Geiger-Müller

radioactivity detector. The solvent was evaporated under reduced pressure at room temperature and the residue was dissolved in a mixture of saline/ethanol (95/5) and filtered through a 0.22 μm sterile membrane (Millipore FG).

Ex vivo autoradiographic studies

The *in vivo* specific localization of [^{76}Br]PE2Br was studied by autoradiography after i.v. injection of 3.5 MBq of the radiotracer in a rat. The rat was sacrificed 1 h after injection. The brain was removed, frozen (-70°C), cut in 20 μm thickness horizontal section with a cryomicrotome (Leitz 1720) and transferred to cooled glass plates. The slices were placed on phosphor imager plates for 16 h exposure. The images of radioactivity distribution were analyzed using a computerized densitometric system and image analysis software (Molecular Dynamics).

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