Synthesis of a Novel ^{123}I-Labelled Derivative of GBR12783, a Potential Agent for SPECT Imaging of Dopamine-Reuptake Sites.

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SUMMARY

[^{123}I]1-[2-(Diphenylmethoxy)ethyl]-4-[3-(*m*-iodophenyl)-2-propenyl]piperazine ([^{123}I]**2**), a potential SPECT imaging agent for dopamine reuptake sites was efficiently synthesized in 4 steps from 1-[2-(diphenylmethoxy)ethyl]piperazine (**3**). A key step in the synthesis was the selective AlH_3-mediated reduction of 1-[2-(diphenylmethoxy)ethyl]-4-[*m*-bromocinnamoyl] piperazine (**4**) to 1-[2-(diphenylmethoxy)ethyl]-4-[3-(*m*-bromophenyl)-2-propenyl]piperazine (**5**). The ^{123}I label of [^{123}I]**2** was introduced in the final step in up to 90% radiochemical yield by treatment of the organotin precursor 1-[2-(diphenylmethoxy)ethyl]-4-[3-[(*m*-(tributyl stannyl)phenyl]-2-propenyl]piperazine (**6**) with Na^{123}I in aqueous CH_3CO_3H.

Key Words: [^{123}I]1-[2-(Diphenylmethoxy)ethyl]-4-[3-(*m*-iodophenyl)-2-propenyl] piperazine ([^{123}I]**2**), SPECT, dopamine reuptake, organotin, 1-[2-(diphenylmethoxy) ethyl]-4-[3-[(*m*-(tributyl stannyl)phenyl]-2-propenyl]piperazine (**6**).

INTRODUCTION

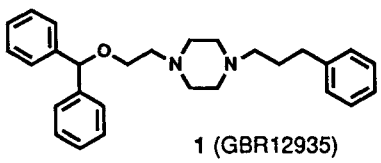
The dopamine (DA) reuptake complex is the major site of action of the drug of abuse, cocaine [1]. This site represents a potential target for the treatment of cocaine abuse as well as diseases such as Parkinson's and schizophrenia [2]. In addition to cocaine, several other classes of compounds also mediate their pharmacological effects at this site. These include arylcyclohexylamines such as PCP [3] and BTCP [4], piperazines such as GBR12935 (**1**) [5], and nomifensine [6].

Cocaine produces its behavioural effects through a robust inhibition of DA-reuptake at presynaptic nerve terminals in the striatum and other brain areas containing dopaminergic neurons.

Positron emission tomography (PET) and single photon emission computed tomography (SPECT) are related non-invasive techniques that have provided a

0362–4803/93/060493–08$09.00

Received 30 November, 1992
Revised 25 January, 1993



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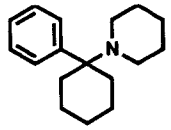
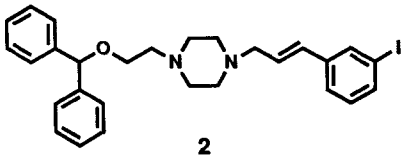


PCP



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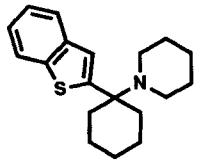


BTCP

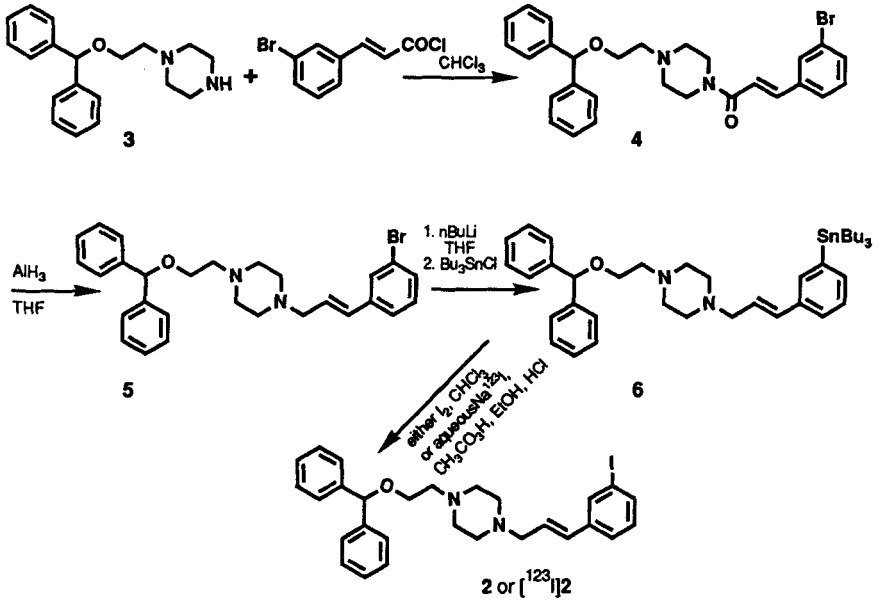
breakthrough in the visualization and quantitation of receptor binding in conscious subjects [7]. Application of these techniques to the study of the DA-reuptake system in normal versus abnormal subjects has the potential to provide clinical correlates which will ultimately allow diagnosis and treatment of certain illnesses involving the DA-system. As part of our program to gain further insight into the involvement of DA-reuptake sites in cocaine addiction, we report here the synthesis and radiolabelling to high specific activity of the GBR12935 based SPECT ligand **2**. The key radiolabelling step was very efficiently accomplished using iodine/tin exchange methodology [8]. Our choice for a SPECT as opposed to PET ligand was based upon the greater technical simplicity, lower cost, and greater adaptability of SPECT to the small hospital setting.

[^{123}I]3-Iodo-6-methoxybenzamide has been previously reported as a SPECT agent for dopamine receptors [9]. [^{11}C]Nomifensine [10] and [^{18}F]GBR13119 [11] have been reported as PET ligands for DA-reuptake sites. Very recently, a derivative of GBR12935 labelled on the diphenylmethoxy moiety was reported as a potential SPECT imaging agent for DA-reuptake sites [12]. Van Dort et al [13] reported the radiosynthesis of ^{18}F and ^{125}I labelled GBR12935 which are saturated derivatives of **2** . We decided upon incorporation of the double bond as in **2** since this has been found to confer higher binding affinity to DA reuptake sites [5]. More recently, the ^{123}I-labelled cocaine derivative [^{123}I]-2β-carbomethoxy-3β-(4-iodophenyl)tropane was successfully employed to image these sites in primates [14].

SYNTHESIS

1-[2-(Diphenylmethoxy)ethyl]piperazine (**3**), synthesized as described previously [5], was coupled with *m*-bromocinnamoyl chloride to give cinnamide **4** in quantitative yield. Treatment of **4** with AlH_3 in THF [15] at room temperature afforded **5** in 86% recrystallized yield with no side-products corresponding to 1,4-addition of hydride. Use of $LiAlH_4$ in this reduction was unsuccessful since it resulted in halogenolysis of the bromine atom. Enamine **5** was stannylated by treatment with n-butyllithium in THF at -78 °C followed by reaction with tributyltin chloride. Treatment of **6** with excess iodine in chloroform at room temperature afforded the target compound **2** in 73% yield. Similarly, treatment of **6** with carrier-free Na^{123}I [16] in the presence of CH_3CO_3H afforded [^{123}I]2

Scheme 1: Synthesis of [^{123}I]1-[2-(Diphenylmethoxy)ethyl]-4-[3-(*m*-iodophenyl)-2-propenyl]piperazine ([^{123}I]2)



in up to 90% radiochemical yield and >99% radiochemical purity(HPLC). The product was purified by reverse phase HPLC and stored at -80 °C in either EtOH or acetonitrile prior to use.

DISCUSSION

The synthetic route employed herein allowed an efficient (4-step) synthesis of [^{123}I]**2** starting from piperazine derivative **2**. AlH_3 reduction of cinnamide **4** proved to be a key step in this synthesis since it allowed selective 1,2-reduction of the cinnamide and at the same time preserved the aromatic bromine atom. Intermediate **4** was very amenable to bromine/lithium exchange in spite of the presence of competing benzylic and allylic protons in the molecule.

Organotin **6** proved to be an effective precursor to both unlabelled and ^{123}I-labelled **2**. This precursor provided another important advantage in that it was readily separated from the product both by TLC and reverse phase HPLC. [^{123}I]**2** is presently being evaluated for its capacity to label DA-reuptake sites in primates [17]. The results of these studies will be reported elsewhere.

EXPERIMENTAL

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Elemental analyses were performed at Atlantic Microlabs, Atlanta, GA. Chemical-ionization mass spectra (CIMS) were obtained using a Finnigan 1015 mass spectrometer. Electron-ionization mass spectra (EIMS) and high resolution mass

measurements (HRMS) were obtained using a VG-Micro Mass 707F mass spectrometer. ^{1}H-NMR spectra were recorded from $CDCl_3$ solutions using a Varian XL-300 spectrometer. Thin layer chromatography of unlabelled compounds was performed on 250 μm Analtech (Newark, DE, USA) GHLF silica gel plates. Solvent system A refers to chloroform-methanol-concentrated aqueous ammonia (90:9:1). Solvent system B refers to chloroform-methanol-concentrated aqueous ammonia (150:5:0.5). All steps involving the use of ^{123}I were initially performed with unalabelled materials and the products were confirmed spectroscopically. Chromatographic separations of radioisotopes were accomplished on 250 μm analytical plates (GTLC, Macherey-Nagel, Germany). Radioactivity was detected on TLC plates using a Bioscan system 200 imaging scanner. Radioactivity determinations were made using a Radcal model 4050 Radionuclide calibrator (Radcal corporation). Na^{123}I was purchased from Nordion International Inc., Vancouver, Canada and used immediately upon receipt.

1-[2-(Diphenylmethoxy)ethyl]-4-[*m*-bromocinnamoyl] piperazine (4).

A solution of *m*-bromocinnamic acid (11.35 g, 50 mmol) in thionyl chloride (100 mL) was boiled under reflux overnight. The solvent was evaporated *in vacuo* and the residue was freed of traces of thionyl chloride by addition and evaporation of toluene (3 x 50 mL). A solution of this acid chloride in chloroform (150 mL) was added dropwise at room temperature to a stirred solution of 1-[2-(diphenylmethoxy)ethyl] piperazine base (**3**) (obtained from 25 g, 47 mmol of **3**•bismaleate [5] by partitioning the salt between 10% aqueous NaOH/chloroform) in chloroform (200 mL). TLC (solvent system A) indicated the reaction to be complete after 1 h. The solvent was evaporated *in vacuo* and the residue was partitioned between excess dilute aqueous ammonia solution (200 mL) and chloroform (200 mL). The organic layer was dried (Na_2SO_4) and the solvent was evaporated *in vacuo* to give the product 24 g (quantitative) as a brown oil. **4**•maleate crystallized from isopropanol as off-white crystals (25.6 g, 88%): mp 159-160 °C; ^{1}H-NMR ($CDCl_3$) δ 7.67 (m, 1H, ArH), 7.58, 6.86 (ABq, J=15.4 Hz, 2H, COCH=CH), 7.47 (dm, J=7.9 Hz, 1H, ArH), 7.41 (dm, J=7.9 Hz, 1H, ArH), 7.21-7.37 (complex m, 11H, ArH), 5.37 (s, 1H, OCHPh$_2$), 3.73 (m, 2H, piperazineCH_2), 3.64 (m, 2H, piperazineCH_2), 3.62 (t, J=5.7 Hz, 2H, OCH_2CH_2N), 2.71 (t, J=5.7 Hz, 2H, OCH$_2CH_2$N), 2.56 (m, 4H, piperazineCH_2); CIMS (MH$^+$ calcd for $C_{28}H_{29}BrN_2O_2$): 507. Found: 507; Anal (calcd for $C_{32}H_{33}BrN_2O_6$): C 61.84, H 5.35, N 4.51%. Found: C 61.58, H 5.41, N 4.33%.

1-[2-(Diphenylmethoxy)ethyl]-4-[3-(*m*-bromophenyl)-2-propenyl]piperazine (5).

To a stirred solution of AlH_3 in THF [15] (100 mL of a 1.0 M solution, 100 mmol) at room temperature was added, dropwise, a solution of **4** (base obtained from 12.42 g, 20 mmol of **4**•maleate) in THF (30 mL). TLC (solvent system A) indicated the reaction to be complete after 30 min at room temperature. The reaction was quenched by carefully pouring it into 15% aqueous NaOH (300 mL). The aqueous mixture was extracted with chloroform (300 mL) and the organic layer was dried (Na_2SO_4) and evaporated *in vacuo*

to give the crude **5** as an oil (9.8 g, quantitative). **5**•bismaleate (12.5 g, 86%) crystallized from 2-propanol: mp 186-187 °C; ^{1}H-NMR ($CDCl_3$) δ 7.51 (m, 1H, ArH), 7.22-7.37 (complex m, 12H, ArH), 7.17 (t, J=7.8 Hz, 1H, ArH), 6.46 (distorted d, J=16 Hz, 1H, CH=CH-Ar), 6.27 (dt, J=16, 6.4 Hz, 1H, CH=CH-Ar), 5.37 (s, 1H, $OCHPh_2$), 3.61 (t, J=5.9 Hz, 2H, OCH_2CH_2N), 3.16 (d, J=6.4 Hz, 2H, NCH_2CH=CH), 2.71 (t, J=5.9 Hz, 2H, OCH_2CH_2N), 2.56 (m, 8H, piperazineCH_2); EIMS (M^+ calcd for $C_{28}H_{31}BrN_2O$): 490, 492. Found: 490, 492; HRMS (M^+ calcd for $C_{28}H_{31}BrN_2O$): 490.1620. Found: 490.1592 (M^+); Anal (calcd for $C_{36}H_{39}BrN_2O_9$): C 59.75, H 5.43, N 3.87%. Found: C 60.60, H 5.50, N 3.94%.

1-[2-(Diphenylmethoxy)ethyl]-4-[3-[(*m*-(tributylstannyl)phenyl]-2-propenyl] piperazine (6).

To a stirred solution of **5** (base obtained from 1.45 g, 2 mmol of **5**•bismaleate by partitioning with 15% aqueous NaOH/chloroform) in dry THF (20 mL) at -78 °C was added dropwise, via syringe, a solution of n-butyllithium (1.6 mL of a 1.6M solution, 2.6 mmol) in hexane. The reaction mixture was stirred at this temperature for 25 min and then tri-(n-butyl)tin chloride (0.6 mL, 2.2 mmol) was added dropwise. The reaction mixture was stirred for a further 30 min at -78 °C and then warmed to room temperature and quenched with saturated ammonium chloride (1.2 mL). Water (20 mL) was added and the mixture was extracted with CH_2Cl_2 (50 mL). The organic extract was dried (Na_2SO_4) and the solvent was evaporated *in vacuo* to give the crude product (1.4 g, quantitative) as a pale yellow oil. Further purification was achieved by flash column chromatography on silica gel eluting with solvent system B to give **6** (1.19 g, 85%) as a colorless oil: ^{1}H-NMR ($CDCl_3$) δ 7.45 (m, 1H, ArH), 7.21-7.38 (complex m, 13H, ArH), 6.51 (distorted d, J=16 Hz, 1H, CH=CH-Ar), 6.26 (dt, J=16, 6.6 Hz, 1H, CH=CH-Ar), 5.37 (s, 1H, $OCHPh_2$), 3.60 (t, J=6.1 Hz, 2H, OCH_2CH_2N), 3.15 (d, J=6.6 Hz, 2H, NCH_2CH=CH), 2.70 (t, J=6.1 Hz, 2H, OCH_2CH_2N), 2.57 (m, 8H, piperazineCH_2), 1.54 (quintet, J_{app}=7.3 Hz, 6H, $SnCH_2CH_2CH_2CH_3$), 1.33 (sextet, J_{app}=7.6 Hz, 6H, $SnCH_2CH_2CH_2CH_3$), 1.05 (t, J_{app}=8.1 Hz, 6H, $SnCH_2CH_2CH_2CH_3$), 0.88 (t, J_{app}=7.2 Hz, 9H, $SnCH_2CH_2CH_2CH_3$); Anal (calcd for $C_{40}H_{58}N_2OSn$): C 68.47, H 8.33, N 3.99%. Found: C 68.57, H 8.35, N 3.97%.

1-[2-(Diphenylmethoxy)ethyl]-4-[3-(*m*-Iodophenyl)-2-propenyl]piperazine (2).

To a stirred solution of **6** (280 mg, 0.14 mmol) in hydrocarbon stabilized chloroform (5 mL) was added a 0.1M solution of iodine in hydrocarbon stabilized chloroform (10 mL). The reaction was allowed to proceed overnight at room temperature, and then treated with a 1M solution of KF in MeOH (1 mL, 1 mmol), 5% aqueous $Na_2S_2O_5$ (2 mL), and finally water (2 mL). The chloroform layer was separated, dried (Na_2SO_4), and the solvent was evaporated *in vacuo* to give the crude product. Purification by flash column chromatography eluting with solvent system B afforded pure **2** (158 mg, 73%) as a colorless oil. Treatment of **2** with maleic acid in 2-propanol resulted in a crystalline salt: mp 190-191 °C; ^{1}H-NMR ($CDCl_3$) δ 7.72 (m, 1H, ArH), 7.55

(dm, J=7.8 Hz, 1H, ArH), 7.19-7.37 (complex m, 11H, ArH), 7.03 (t, J=7.8 Hz, 1H, ArH), 6.42 (distorted d, J=16 Hz, 1H, CH=CH-Ar), 6.26 (dt, J=16, 6.5 Hz, 1H, CH=CH-Ar), 5.37 (s, 1H, OCHPh$_2$), 3.60 (t, J=6.0 Hz, 2H, OCH$_2$CH$_2$N), 3.14 (d, J=6.5 Hz, 2H, NCH$_2$CH=CH), 2.70 (t, J=6.0 Hz, 2H, OCH$_2$CH$_2$N), 2.40-2.68 (m, 8H, piperazineCH$_2$); CIMS (MH$^+$ calcd for $C_{28}H_{31}IN_2O$): 539. Found: 539; Anal (calcd for $C_{36}H_{39}IN_2O_9$): C 56.11, H 5.10, N 3.64%. Found: C 56.18, H 5.14, N 3.61%.

[^{123}I]1-[2-(Diphenylmethoxy)ethyl]-4-[3-(*m*-iodophenyl)-2-propenyl] piperazine ([^{123}I]2).

Aqueous 0.32% peracetic acid (100 μL) (diluted from 32%w/v commercially available reagent from Aldrich Chemical Co., Milwaukee, WI) was added in one portion to a mixture of **6** (100 μL of a solution containing 1 mg of **6** in ethanol (1 mL), 0.142 micromoles), ethanol (200 mL), 1M HCl (40 μL) and no carrier added Na^{123}I (5 mCi)[16] in a sealed vial. The reaction was allowed to stand at room temperature for 5 min and then quenched by the addition of excess $NaHSO_3$ (20 mg). A solution of $NaHCO_3$ (25 mg) in water 1 mL was added in order to render the reaction mixture basic. The mixture was extracted with ethylacetate (3 x 1 mL) and the combined organic extract was washed with water (1 mL) and the organic layer was evaporated to dryness under a stream of nitrogen gas. The residue was dissolved in ethanol (100 μL) and the product was purified by reverse phase HPLC (Hamilton Company C-18 column, 5 μm particle size, PRP-1, 250 mm x 4.1 mm) eluting with acetonitrile-water-trifluoroacetic acid (40:60:0.1). Elution was isocratic at a flow rate of 1 mL/min. [^{123}I]**2** exhibited a retention time of 6 min while the precursor **6** displayed a retention time of >15 min. Fractions containing the product were collected and extracted with ethylacetate (3 x 1 mL) to yield [^{123}I]**2** (3.5-4 mCi, 70-80%, >99% radiochemically purity). The solvent was evaporated under a stream nitrogen and the product was redissolved ethanol (100 μL) and diluted withi 0.9% saline (1-2 mL). This solution was filtered through a sterile 0.22 μm Millipore filter prior to use in autoradiographic and imaging studies.

ACKNOWLEDGEMENT

XSH Acknowledges financial help from the Fogarty Foundation (NIH). This work was supported in part by the National Alliance for Research on Schizophrenia through the Laureate Psychiatric Clinic and Hospital Investigator Award to D. R. Weinberger (CDB, NIMH). The authors offer their sincere appreciation to Noel Whittaker and Wesley White of the Laboratory of Analytical Chemistry, NIDDK, NIH for obtaining mass spectra of compounds reported in this paper.

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16. The supplier of $Na^{123}I$ (Nordion International, Vancouver, Canada) manufactures no carrier added material which at the instant of synthesis has a specific activity of 240000 Ci/mmol. In the synthesis of **2**, the radiolabelling was accomplished 48h after production of the isotope. This sets the maximum feasible specific activity at 19000 Ci/mmol for the product.

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