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Synthesis of a Selective Sigma Receptor Radioligand for SPECT: [^{123}I]-1-(2-Hydroxyethyl)-4-(4-iodophenoxymethyl)piperidine

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Summary

[^{123}I]-1-(2-Hydroxyethyl)-4-(4-iodophenoxymethyl)piperidine has been prepared as a novel sigma receptor radioligand for tumour imaging by SPECT. This ligand was found to be selective *in vitro* for the sigma-1 receptor (Ki = 2.3 nM) when tested in a variety of neuroreceptor binding assays. The lipophilicity of this ligand is appropriate for crossing the blood/brain barrier (log $P_{7.5}$ = 4.0). Radioiodination was accomplished by no-carrier-added iododestannylation using chloramine-T in acetic acid. The specific activity of the radiotracer >10,000 mCi/μmole. The radiochemical yield was 63-77% EOS and the radiochemical purity was >99%.

Keywords: sigma receptors, radioligands, SPECT, tumour imaging, I-123

Introduction

In recent years there has been an effort to develop radioligands for *in vivo* evaluation of sigma (σ) receptor densities due to various proposed applications in nuclear medicine. While the specific uses of sigma receptor radioligands in neurology are yet to be identified, such agents might be useful in oncology for the *in vivo* tomographic detection of malignant melanoma and other types of tumours. *In vitro* binding assays using selected sigma receptor radioligands have revealed that several rodent and human tumour cells, including mouse melanoma, human malignant melanoma and tumours of the breast, kidney, colon, lung, and brain, exhibit sigma-1 and sigma-2 receptors (1-5).

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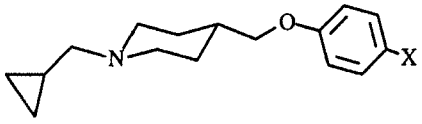
A few radiolabeled compounds with affinity for sigma receptors have been reported to localize in melanoma in mouse models (5, 6), and one sigma ligand has been used to image malignant melanoma in humans (7).

Several compounds have been reported as possible sigma receptor radioligands for single photon emission computed tomography (SPECT). These include [^{123}I]-iodobenzamides (8), [^{123}I]-iodobenzovesamicol (9), and [^{125}I]-iodophenyl-3-(adamantyl)guanidine (10). Described herein is the synthesis 1-(2-hydroxyethyl)-4-(4-iodophenoxymethyl)piperidine, **1**, and its ^{123}I-labeled analog, measurement of its lipophilicity using high performance liquid chromatography and characterisation of the ligand by *in vitro* receptor binding assays.

Compound **1** was designed based in part on the pharmacology of the high affinity sigma receptor ligand 1-(cyclopropylmethyl)-4-(4-fluoro-2'-oxoethyl)piperidine (DuP 734) and related analogs. It was reported that substution of 4-(phenoxymethyl)piperidine derivatives of Dup 734 at the *para* position is possible while maintaining the affinity of these ligands for sigma receptors (Table I) (11). We therefore synthesised a *para*-substituted iodoaryl derivative with the aim of developing a sigma receptor ligand which could be radioiodinated with ^{125}I for autoradiographic studies or with ^{123}I for tomographic imaging. The cyclopropylmethyl group of DuP 734 was replaced with a hydroxyethyl group to reduce the lipophilicity of the iodinated ligand.



		Ki (nM)		
Receptor	X =	CN	NO_2	F
Sigma		10	13	2
Serotonin 5HT2		2818	215	9
Dopamine D2		>10,000	>10,000	381

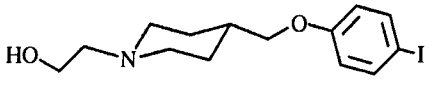


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Table I. *In Vitro* Characteristics of some *para*-substituted (phenoxymethyl)piperidines. Data taken from reference (11).

Materials and Methods

Proton NMR spectra were recorded on a JEOL 400 MHz FT-NMR spectrometer. Chemical shifts were recorded in ppm (δ) from an internal tetramethylsilane standard in deuterochloroform and coupling constants (J) are reported in hertz (Hz). High resolution fast atom bombardment mass spectroscopy (HRMS) was performed using a ZAB-EQ mass spectrometer at the Department of Chemistry, The University of Tennessee (Knoxville, TN). Melting points were recorded using a Gallenkamp melting point apparatus and are uncorrected. Elemental analysis was performed by Atlantic Microlabs Inc. (Norcross Georgia). Gravity chromatography was performed using silica gel (Fluka, 70-230 mesh, ASTM) using the solvent systems as indicated in the text. For mixed solvent systems, the ratios are given with respect to volumes.

All reagents were purchased from commercial sources and were used without further purification. Sodium ^{123}I-iodide was obtained from the National Medical Cyclotron (Sydney, Australia) as a solution in 0.1 N sodium hydroxide. Radioactivity was measured using a Capintec CRC-15R dose calibrator. HPLC purification of the radioligand was performed using a Waters 510 HPLC pump, a Waters 440 UV detector, and a Berthold LB506 radiation detector. The column used for radioligand purification was a reverse-phase base-deactivated column (Activon, Goldpak Exsil, ODS B, 1 x 25 cm) and the mobile phases are indicated in the text below.

(*tert*-Butoxycarbonyl)-4-hydroxymethylpiperidine **2**: 4-Hydroxymethylpiperidine (6.50 g, 56.4 mmol) was dissolved in ethanol-free dichloromethane (80 ml) and to this was added in several portions di-*tert*-butyl dicarbonate (13.97 g, 62.1 mmol). The resulting cloudy, colorless mixture was stirred at room temperature for 30 minutes and then the solvent was removed *in vacuo* to provide a clear, light yellow oil. The product was purified by column chromatography (ethyl actetate/ethanol, 17:3) to provide 1-(*tert*-butoxycarbonyl)-4-hydroxymethylpiperidine (11.8 grams, 54.4 mmol, 96%) as a white, crystalline solid, mp 73°C; ^{1}H NMR: δ 1.03-1.17 (m, 2H), 1.42 (s, 9H), 1.55-1.73 (m, 3H), 2.56-2.73 (m, 2H), 3.34 (t, 1H, J = 5.40), 3.41 (t, 2H, J = 5.40), 4.00-4.14 (m, 2H); MS m/z 216; anal. calcd for $C_{11}H_{21}NO_3$: C, 61.37; H, 9.83; N, 6.51. Found: C, 61.46; H, 9.82; N, 6.56.

(*tert*-Butoxycarbonyl)-4-(methansulfonyloxymethyl)piperidine **3**: 1-(*tert*-Butoxycarbonyl)-4-hydroxymethylpiperidine (3.60 g, 16.6 mmol) was dissolved in ethanol-free dichloromethane (50 ml) and to this was added anhydrous triethylamine (8.10 ml, 58.0 mmol) followed by methanesulfonyl chloride (1.54 ml, 19.6 mmol). This mixture was stirred at 0°C for 60 minutes, turning dark brown in color and containing a yellow precipitate that formed as the reaction progressed. The reaction mixture was made basic with aqueous potassium carbonate (0.1M, 100 ml) and the product extracted into dichloromethane (2 x 100 ml). The combined organic extracts were dried over anhydrous magnesium sulfate and the solvent removed *in vacuo* to give a pale yellow oil. The product was purified by column chromatography (hexanes/ethyl acetate 2.5:1.0) to provide a clear, colorless oil (4.51g, 15.3 mmol, 92.9%) which solidified upon standing to a white, crystalline solid, mp 76-77°C; ^{1}H-NMR: δ 1.15-1.28 (m, 2H), 1.46 (s, 9H), 1.82 (d, 2H, J = 11.40), 1.90-1.97 (m, 1H), 2.67-2.77 (m, 2H), 3.02 (s, 3H), 4.07 (d, 2H, J = 6.55), 4.12-4.25 (m, 2H); MS m/z 294; Anal. calcd for $C_{12}H_{23}NO_5S$: C, 49.12; H, 7.90; N, 4.77; S, 10.93. Found: C, 49.19; H, 7.93; N, 4.83; S, 10.84.

(tert-Butoxycarbonyl)-4-(4-iodophenoxymethyl)piperidine **4**: 4-Iodophenol (3.0 g, 13.5 mmol) was dissolved in anhydrous N,N-dimethyl formamide (30 ml) and to this was added sodium hydride (542 mg, 13.5 mmol) in several portions over a 10 minute period. The resulting light-brown mixture was stirred at room temperature for an additional 10 minutes and then 1-(*tert*-butoxycarbonyl)-4-(methanesulfonyloxymethyl)piperidine (1.0 g, 3.4 mmol) was added in one portion. This solution was heated at 60°C for 24 hours and then cooled to room temperature. Next the reaction mixture was diluted with aqueous sodium hydroxide (1.0 M, 200 ml) and the product extracted into dichloromethane (3 x 100 ml). The organic layers were combined, dried over magnesium sulfate, and the solvent removed *in vacuo* to provide a dark-brown oil. The product was purified by column chromatography (hexanes/ethyl acetate 2.5:1.0) to provide a white solid (1.2 g, 85%), mp = 80-81 °C; ^{1}H-NMR: δ 1.20-1.33 (m, 2H), 1.46 (s, 9H), 1.80-1.90 (m, 2H), 1.98-2.10 (m, 1H), 2.68-2.80 (m, 2H), 3.75 (d, 2H, J = 6.40), 6.65 (d, 2H, J = 9.00), 7.52 (d, 2H, J = 9.00); anal. calcd for $C_{17}H_{24}NO_3I$: C, 48.91; H, 5.80; N, 3.36. Found: C, 48.99; H, 5.82; N, 3.29.

4-(4-Iodophenoxymethyl)piperidine **5**: 1-(*tert*-Butoxycarbonyl)-4-(4-iodophenoxymethyl)-piperidine (200 mg, 0.5 mmol) was stirred with 3 ml of a mixture of dichloromethane and trifluoroacetic acid

(2:1) for 30 minutes at room temperature. Next, the solution was made basic by the addition of aqueous potassium carbonate (1M, 200 ml) followed by aqueous potassium hydroxide (0.1M, 50 ml). The product was extracted into dichloromethane (3 x 100 ml) and the organic extracts were combined, dried over magnesium sulfate and the solvent removed *in vacuo* to provide the product (140 mg, 92%) as an off-white solid, mp = 111-114 °C; ^{1}H-NMR: δ 1.20-1.36 (m, 2H), 1.72-1.98 (m, 4H), 2.59-2.75 (m, 2H), 3.12 (d, 2H, J = 9.21), 3.74 (d, 2H, J = 6.41) 6.66 (d, 2H, J = 9.00), 7.53 (d, 2H, J = 9.00); MS m/z: 318; anal. calcd for $C_{12}H_{16}NOI$: C, 45.42; H, 5.09; N, 4.42. Found: C, 45.52; H, 5.12; N, 4.36.

1-Hydroxyethyl-4-(4-iodophenoxymethyl)piperidine **1**: 4-(4-Iodophenoxymethyl)piperidine (430 mg, 1.4 mmol) was dissolved in ethanol-free dichloromethane (10 ml) and to this was added potassium carbonate (755 mg, 5.7 mmol) and 2-bromoethanol (407 µl, 1.5 mmol). The resulting mixture was stirred at room temperature for 26 hours and then diluted with aqueous sodium hydroxide solution (40 ml). The product was extracted into dichloromethane (3 x 25 ml) and the organic layers were combined, dried over anhydrous magnesium sulfate and the solvent evaporated to provide a crude yellow solid. The product was purified by column chromatography (silica; absolute ethanol) to provide the product as a white solid (328 mg, 67%), mp = 139-141 °C; ^{1}H-NMR: δ 1.40-1.55 (m, 2H), 1.84-1.90 (m, 3H), 1.95-2.10 (m, 2H), 2.73 (t, 2H, J = 4.10), 3.20 (d, 2H, J = 11.60), 3.73 (t, 2H, J = 4.10), 3.83 (d, 2H, J = 5.80), 6.66 (d, 2H, J = 9.00), 7.54 (d, 2H, J = 9.00); MS m/z: 362; anal. calcd for $C_{14}H_{20}NO_2I \cdot H_2O$: C, 44.34; H, 5.84; N, 3.68, I, 33.46. Found: C, 44.31; H, 5.73; N; 3.68, I, 33.55.

1-Hydroxyethyl-4-[4-(*tri-n*-butyltin)phenoxymethyl]piperidine **6**: 1-Hyroxyethyl-4-(4-iodophenoxy-methyl)piperidine (160 mg, 0.44 mmol) was dissolved in toluene (7 ml) and to this was added bis-(*tri*-n-butyltin) (550 µl, 1.33 mmol) and tetrakis(triphenylphosphine)palladium (0) (20 mg, 0.18 mmol). The resulting yellow mixture was stirred at reflux under an argon atmosphere for 48 hours and then cooled to room temperature. The black precipitate that formed as the reaction progressed was removed by passing the reaction mixture through filter aid (1 gram). The eluant was loaded directly onto a silica gel column and the product (rf = 0.15) was eluted with ethyl acetate/ethanol (1:1) to provide a clear colorless oil (123 mg, 53%), ^{1}H-NMR: δ 0.83-1.12 (m, 15H), 1.25-1.40 (m, 7H), 1.45-1.65 (m, 8H), 1.85-2.10 (m, 4H), 2.25-2.35 (m, 2H), 2.72 (t, 2H,

J = 4.20), 3.20 (d, 2H, J = 6.0), 3.70-3.86 (m, 4H), 6.88 (d, 2H, J = 8.55), 7.36 (d, 2H, H = 8.55); FAB HRMS 522.2695 $(M+H)^+$; calcd for $C_{26}H_{47}N_2OSn$: 522.2703.

Analysis of **6** by HPLC using a reverse-phase base deactivated column (Activon, Goldpak Exsil, ODS B, 1 x 25 cm; flow rate = 2.0 ml/min; ret. time = 12.1 min) and a mobile phase of methanol revealed that the stannane was 96% pure. This HPLC method was used as a final purification to obtain milligram quantities of **6** in >99% purity for use in subsequent radioiodination reactions.

Synthesis and Purification of [^{123}I]-1-(2-hydroxyethyl)-4-(4-iodophenoxymethoxy)piperidine ^{123}I-**1**: To a solution of sodium [^{123}I]iodide (13.10 mCi) in aqueous sodium hydroxide solution (0.1 N, 50 µl) in a 3.0 ml Wheaton vial was added acetic acid (20 µl), chloramine-T dihydrate (0.5 mg) dissolved in a solution of methanol and water (80:20, 50 µl), followed immediately by a solution of 1-[2-hydroxyethyl]-4-[4-(*tri*-n-butyltin)phenoxymethyl]piperidine, **6**, in ethanol (1.2 mg, 200 µl). After standing 1 minute, the reaction mixture was quenched with aqueous sodium metabisulfite (1.0 M, 20 µl) and then made basic by the addition of potassium carbonate (75 mg). The mixture was decanted from the solid salts and the product purified by HPLC (mobile phase: methanol/water 80:20) to provide 9.3 mCi (70% EOS) of the desired radiotracer. The radiochemical purity of the product was >99% as determined by HPLC analysis. The purified radioligand co-eluted with the standard (ret. time = 13.8 min) when a spiked aliquot of the purified product was analyzed using identical HPLC conditions.

Specific Activity Determination: The specific activity of the product was determined by HPLC analysis using a base-deactivated reverse-phase analytical column (Goldpak ODS-B, 4.6 x 250 mm, 10 µm) and a mobile phase consisting of methanol and 0.1 M ammonium acetate buffer (55:45, pH = 6.7), with a flow rate of 1.0 ml/min. The limit of detection of **1** was determined by plotting the mass of **1** injected versus UV detector response at 232 nm. The detection limit was determined to be the response of the detector providing a peak height 2.5 times the noise level. From extrapolation, this response corresponded to 7.0 X 10^{-12} mol of **1** injected. Upon analysis of 210 µCi of ^{123}I-**1** (in methanol/water 80:20) using the HPLC method described above, the specific activity of **1** was determined to be >10,000 mCi/µmol.

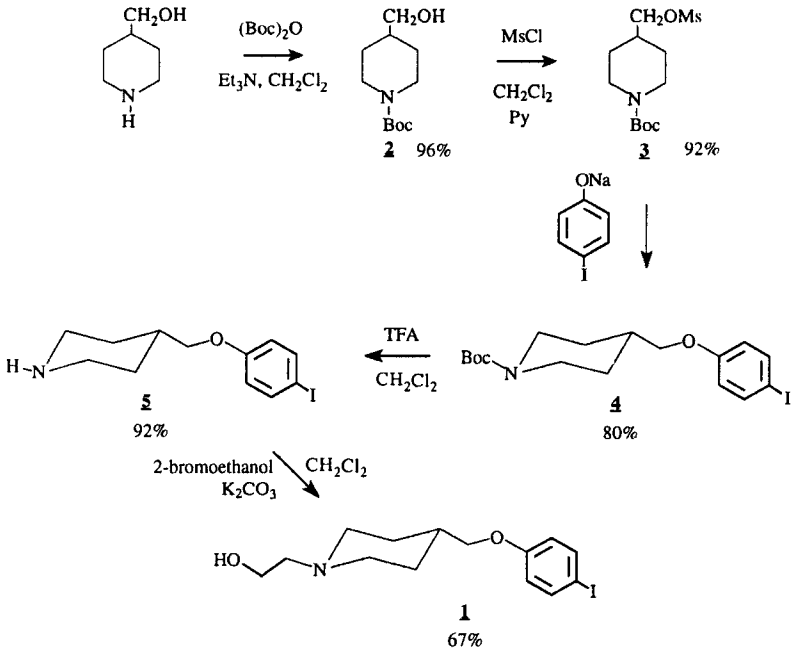
Lipophilicity estimations: The lipophilicity of **1** was examined by determination of the log $P_{7.5}$ value using a HPLC method previously described (12). Briefly, samples were analysed using a C18

column (Goldpak Exsil 10μm, 4.6 x 250mm) and a mobile phase of MeOH and phosphate buffer (85:15 v/v, pH = 7.5) at 1.0 ml/min. The log P value of **1** was determined by comparison of the retention time of the compound to that of standards having known log P values. The standards used in our study were catechol, aniline, benzene, bromobenzene, ethyl benzene, trimethylbenzene and hexachlorobenzene dissolved in an appropriate solvent. Relative retention times, RRT (to catechol), were calculated, and a calibration curve of log P vs. log RRT was generated. The calibration equations were polynomial with r^2 of 0.994 or greater. All sample injections were done in triplicate and the results averaged to provide the final values. Using this method, the log $P_{7.5}$ value for **1** was 4.0, indicating that this ligand should readily cross the blood brain barrier (13, 14).

Ligand Binding Assays: Compound **1** was tested through the NIMH/NovaScreen Drug Discovery & Development Program (Contract No. NIMH-2003). Briefly, competitive binding assays were performed in either 250 or 500 μl volumes containing, by volume, 80% receptor preparations, 10% radioligand and 10% of **1** (non-specific binding determinant/4% DMSO (total binding determinant)). All compounds were solubilised in neat DMSO and diluted with water to a final concentration of 0.4% DMSO for use in the assay. Assays were terminated by rapid vacuum filtration over glass fiber filters (Whatman) followed by rapid washing with cold buffer. Radioactivity was determined by either liquid scintillation counting or gamma spectrometry. Data was reduced by a software program proprietary to NOVASCREEN. References for specific receptor binding assays are indicated in Table II.

Results and Discussion:

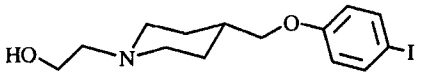
The target compound, 1-hydroxyethyl-4-(4-iodophenoxymethyl)piperidine, **1**, was synthesized as is indicated in Scheme I following published procedures with slight modification (11). 4-Hydroxymethylpiperidine stirred with di-*tert*-butyl dicarbonate in dichloromethane to provide **2** in 96% yield. Compound **2** was dissolved in ethanol-free dichloromethane and reacted with methanesulfonyl chloride and anhydrous triethylamine to provide **3** in 92% yield. The sodium salt of 4-iodophenol condensed with **3** to provide 1-(*tert*-butoxycarbonyl)-4-[(4-iodophenoxy)methyl]piperidine, **4**, in 80% yield. After deprotection of **4** with trifluoroacetic acid, the resulting amine **5** was alkylated with 2-bromoethanol to provide compound **1** in 67% yield.



Scheme I. Synthesis of 1.

The aryl stannane precursor 6 was synthesised in 53% yield by reacting 1 with bis-(*tri*-n-butyltin) for 24 hours in toluene at reflux in the presence of a palladium catalyst (Scheme II) (15). TLC analysis of the reaction mixture indicated that 1 had been depleted after 48 hours reaction time. Compound 6 was purified by column chromatography [silica; ethyl acetate/ethanol (1:1); rf = 0.15] to provide a clear, colorless oil. The stannane was further purified by HPLC to ensure that all of the starting material 1 was removed. The final purity of 6 was >99% as determined by HPLC and the compound was stable for at least 6 months when stored at -4 °C.

Compound 1 was screen *in vitro* in receptor binding assays and was dermined to have good affinity *in vitro* for the sigma-1 receptor, moderate affinity for the sigma-2 receptor, serotonin $5HT_2$ and $5HT_3$ receptors and negligable affinity for other neuroreceptors examined (Table II). The lipophilicity of 1 was estimated by determining the log $P_{7.5}$ value using an HPLC method as previously described. The log P7.5 value of 1 was found to be 4.0 and, therefore, the ligand should readily cross the blood brain barrier (11,12). In light of these *in vitro* results, ^{123}I-1 was prepared for further *in vitro* and *in vivo* evaluation.



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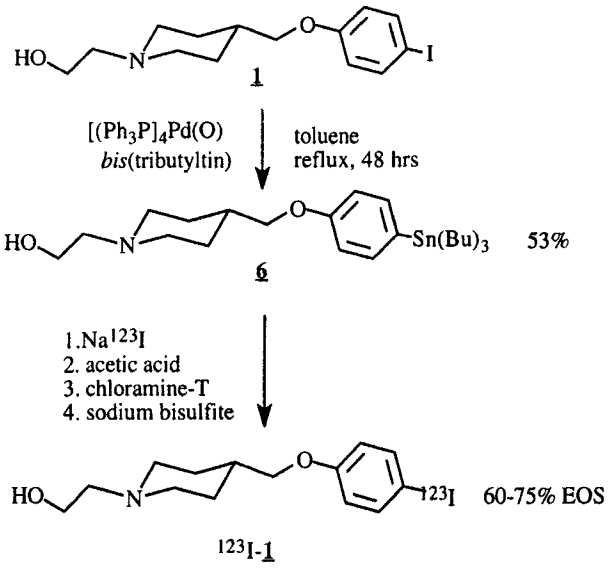
Receptor	Ki (nM)	Reference
Sigma-1	2.3	17
Sigma-2	139	17
Serotonin 5HT2	335	18
Serotonin 5HT3	818	19
Dopamine D1	>10,000	20
Dopamine D2	>10,000	21
NMDA	>10,000	22
PCP	>10,000	23
Muscarinic M1, M2, M3	>10,000	24, 25
Alpha-1	>10,000	26
Alpha-2	>10,000	26
Beta	>10,000	27

Table II. *In Vitro* Characterisation of 1

Radioiodination of 1-(2-hydroxyethyl)-4-[4-(*tri*-n-butyltin)phenoxymethyl]piperidine, 6, was accomplished by electrophilic iododestannylation at acidic pH using chloramine-T dihydrate as the oxidant (16). Upon termination of the reaction by the addition of sodium metabisulfite, the reaction mixture was made basic decanted from the solid salts. The product purified by HPLC to provide ^{123}I-1 in yield of 63-77% EOS (Figure I). A total of four radiolabeling experiments were performed. The starting activity varied between 4.0-15.2 mCi and the averge yield was 69.5 +\- 6.3%. The radiochemical purity of the product was >99% as determined by HPLC analysis and the radioligand co-eluted with the standard when a spiked aliquot of the purified product was analyzed using identical chromatographic conditions.

The specific activity of the product was determined by HPLC analysis. The limit of detection of 1 was determined by plotting the mass of 1 injected versus UV detector response at 232 nm. Upon analysis of 210 μCi of ^{123}I-1, no response corresponding to 1 was noted in the UV trace and the specific activity of 1 was determined to be >10,000 mCi/μmol.

To obtain suitable preparations of ^{123}I-1 for use *in vivo*, the eluted radioactive peak corresponding to ^{123}I-1 was collected, the mobile phase removed *in vacuo* and the product redissolved in saline. The resulting solution was passed through a sterile filter into an evacuated sterile vial and diluted with sterile saline to provide a solution that contained approximately 10 μCi of ^{123}I-1 per 100 μl solution. Preparations of this type are suitable for evaluation in rodents and up to 10.0 mCi of the radiotracer was prepared in this manner.



Scheme II. Preparation of ^{123}I-1 from 1.

Conclusions

(2-Hydroxyethyl)-4-(4-iodophenoxymethyl)piperidine is a novel sigma-1 receptor ligand having appropriate lipophilicity for crossing the blood/brain barrier. The corresponding SPECT radiotracer, [^{123}I]-1-(2-hydroxyethyl)-4-(4-iodophenoxymethyl)piperidine, has been prepared in high specific activity and good radiochemical purity in sufficient quantities to permit *in vitro* and pre-clinical *in vivo* evaluations of the ligand.

Acknowledgments

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