PREPARATION OF [^{123}I]- AND [^{125}I]EPIDEPRIDE: A DOPAMINE D-2 RECEPTOR ANTAGONIST RADIOLIGAND

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SUMMARY

(*S*)-(-)-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-5-[^{123}I]iodo-2,3-dimethoxybenzamide (TDP 517) (proposed generic name, [^{123}I]epidepride) is the iodine-123 substituted analogue of isoremoxipride (FLB 457), both of which are very potent dopamine D-2 antagonists (epidepride K_D 0.024 nM). [^{123}I]Epidepride was radioiodinated in 60-70% radiochemical yields in 35 min from the corresponding 5-(tributyltin) derivative using Na^{123}I with a specific radioactivity of 3000 Ci/mmol, and oxidized *in situ* with chloramine-T. The aryltin precursor was prepared from non-labelled epidepride by palladium-catalyzed stannylation using bis(tri-*n*-butyltin) in triethylamine. Alternatively, using no carrier-added Na^{125}I as the radioisotope, [^{125}I]epidepride at 2000 Ci/mmol specific radioactivity was prepared in 86% radiochemical yield and 99% radiochemical purity after purification by reverse phase HPLC in ethanolic phosphate buffer.

Keywords: Iodine-123, iodine-125, radioiodination, substituted benzamide, dopamine D-2 receptor antagonist, iododestannylation

INTRODUCTION

Neuroleptic agents have affinity for dopamine receptors and are therefore potential candidates for radioligands and brain imaging agents. With the discovery of potent substituted benzamides, such as eticlopride [1] and emonapride (YM-09150-2) [2], radioligands with drug-receptor dissociation constants (K_D) of <0.1 nM became available. [^{3}H]Emonapride (K_D 20-60 pM) labels dopamine D-2 receptors in the rat frontal cortex [3], an area of the brain that has become the focus of interest because of its implication in the action of neuroleptic agents [4]. Thus, the possibility of detecting clinically relevant blockade of dopamine receptors in extrastriatal areas of the brain, where the dopamine D-2 receptor density is one to two orders of magnitude less than that in the striatum [3], has precipitated a need for potent, receptor-selective radioligands with the inherent quality of being radiolabeled with a high specific radioactivity [4]. Ligands containing iodine-125, having 27.4 keV gamma photons with maximum specific activity of 2,170 Ci/mmol,

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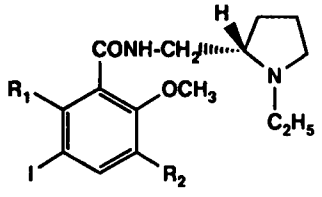
are suitable for receptor binding studies. Ligands containing iodine-123, having 159 keV gamma photons and maximum specific activity of 237,400 Ci/mmol, are suitable for non-invasive visualizion of neurotransmitter receptors in the human brain by single photon tomography (SPECT) [5]. Initial studies in man with the iodine-123 substituted analogue of raclopride, [^{123}I]IBZM, have demonstated selective uptake in basal ganglia compared to cerebellum of 1.5 to one at 1 h post injection [6]. An iodinated derivative of emonapride, [^{125}I]IBZ (spectramide) has shown striatum-to-cerebellum uptake ratio of 4 to one in the mouse brain [7]. These moderate uptake ratios would probably limit the use of [^{123}I]IBZM or [^{123}I]IBZ in quantifying extrastiatal dopamine receptors in man by SPECT.



Iodobenzamide (IBZM, FLA 961); R_1 = OH, R_2 = H
Epidepride (NCQ 219, TDP 517); R_1 = H, R_2 = OCH_3

The discovery of a series of substituted benzamides, structurally related to remoxipride, that are very potent antagonists of apomorphine-induced behavior in the rat [8], has presented the potential for developing new and more potent radioligands. One of these compounds, the iodine substituted analogue of isoremoxipride, (*S*)-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-5-iodo-2,3-dimethoxybenzamide (NCQ 219, TDP 517, **1**) (proposed generic name: epidepride), has the ability to displace [^{3}H]spiperone in rat striatal homogenate with an IC_{50} value of 0.7 nM [9]. [^{125}I]Epidepride binds specifically and selectively to dopamine D-2 receptors in the rat striatum with a dissociation constant (K_D) of 0.024 nM [10, 11]. Uptake into the rat brain in vivo demonstrated a peak striatum-to-cerebellum regional selectivity of 234 to one [11]. The remarkably high potency of epidepride can be explained by the combination of optimal aromatic and aliphatic substituents; the eutomeric (*S*)-(1-ethyl-2-pyrrolidinyl) side chain, a lipophilic iodine atom in the aromatic 5-position, and an electron-donating and sterically small methoxy group in the 3-position [12]. This results in high amine basicity (pKa 9.0) and a moderate apparent lipophilicity (log k_w 2.0) at pH 7.5, which mimics the in vivo condition at which the molecules exist mostly in their protonated form [13].

We now report the synthesis of the corresponding tri-*n*-butyltin derivative (TDP 526) in a high yield from epidepride (**1**) [11, 12] and its utilization in rapid and facile preparations of [^{125}I]epidepride and [^{123}I]epidepride for receptor binding studies and SPECT imaging, respectively, of dopamine D-2 receptors in the basal ganglia and in extrastriatal brain areas.

MATERIALS AND METHODS

HPLC: the semi-preparative system consisted of a Rheodyne 4125 injector (0.5 mL loop), a Kontron 420 pump and Kontron 432 scanning UV detector (wavelength 235 nm) in series with a NaI(Tl) gamma scintillation detector (Ortec 276) and ten 2 cm loops of 1/16th inch AWG-19 teflon tubing. The column for product purifications was Waters Radial-Pak 0.8 x 10 cm cyano reversed

phase column (8NVCN4HP) with 0.10 M sodium phosphate (pH 6.3) - 96% ethanol (1:4) mobile phase, protected by a Waters Resolve C18 Guard Pak. Flow rate was 2.0 mL/min.

TLC: the analytical system consisted of Whatman K6F silica gel 60 A plates (250 um, 5 x 10 cm), and eluted with EtOAc - EtOH - 14 N NH_4OH (200:20:1). Spots were detected by their quenching of the 254 nm fluorescence under illumination by a UVP Mineralight model UVGL-25, and by reacting with iodine vapors at 23 °C. The radioactive spots are detected and recorded by a Bioscan 200 imaging scanner equipped with automatic plate reader.

^1H NMR: Fourier-transform spectra were recorded in [^2H]$CHCl_3$ on a Bruker 300 MHz instrument with 1% tetramethylsilane as the internal chemical shift reference.

Unlabelled epidepride (1): (*S*)-(-)-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-5-iodo-2,3-dimethoxybenzamide (**1**) [9, 12], was prepared in 28% overall yield from *o*-vanillin by iodination of its mercury salt according to the method of Profft and Pannach [14], subsequent *O*-alkylation with dimethyl sulfate, followed by oxidation of the produced iodobenzaldehyde with $AgNO_3$ according the conditions used by Pettit and Piatak for the corresponding bromo analogue [15]. The desired compound **1** was then obtained in 82% yield by reacting the corresponding 5-iodo-2,3-dimethoxybenzoyl chloride with (*S*)-2-(aminomethyl)-1-ethylpyrrolidine, prepared from racemic diamine (Aldrich) by resolving its di-D-(-)tartrate in 50% aqueous ethanol [16]. The optical purity of the diamine, as the di-D-tartrate ($[\alpha]_D^{23}$ -38°), was 97%. ^1H-NMR: δ 8.34 (b, 1, NH), 8.02 (d, 1, *J* = 2.6 Hz), 7.28 (d, 1, *J* = 2.6 Hz), 3.93 (s, 3, OMe), 3.89 (s, 3, OMe), 3.79 - 1.65 (m, 11, pyrrolidine-H), 2.88 and 2.24 (dt, 2, N-Et), 1.12 (t, 3, N-Et) ppm. TLC: R_f 0.21.

Precursor (*S*)-5-(tri-*n*-butyltin)-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-2,3-dimethoxybenzamide (2): To a solution of (*S*)-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-5-iodo-2,3-dimethoxybenzamide (**1**) [9, 12] (0.42 g, 1.0 mmol) in dry Et_3N (20 mL) was added solid $(Ph_3P)_4Pd$ (Aldrich) (0.06 g, 0.05 mmol) followed by Bu_6Sn_2 (Aldrich) (0.58 g, 1.0 mmol). The mixture was heated to reflux temperature (bath 87 °C) for 3.5 h. After cooling, the solvent was removed by evaporation and the residual oil (0.84 g)was subjected to chromatographic separation on silica gel (28 g, 0.063-0.20 mm) in hexane-EtOAc (1:1) to give 0.46 g (79%) of compound **2**. ^1H-NMR: δ 8.62 (b, NH), 7.74 (d, 1, *J* = 0.9 Hz, H-6), 7.12 (d, 1, *J* = 0.9 Hz, H-4), 3.98 (s, 3, OMe), 3.91 (s, 3, OMe), 3.8-1.6 (m, 11, pyrrolidinyl), 1.54 (t, 6, 1-Bu), 1.29 (dt, 12, 2+3-Bu), 1.08 (t, 3, N-Et), 0.89 (t, 9, 4-Bu) ppm. TLC: R_f 0.29.

Preparation of (*S*)-5-Chloro-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-2,3-dimethoxybenzamide (3): Treatment of 2,3-dimethoxybenzoic acid (1.8 g, 0.01 mol) with SO_2Cl_2 (1.0 mL, 0.012 mol) in $CHCl_3$ (50 mL) gave the 5-chlorobenzoic acid derivative (2.2 g) [9], which was converted to 1.9 g (62%) the benzamide **3** [8] as an oil via its acid chloride as described for compound **1**. ^1H-NMR: δ 8.34 (b, 1, NH), 7.69 (d, 1, *J* = 2.6 Hz), 6.99 (d, 1, *J* = 2.6 Hz), 3.90 (s, 3, OMe), 3.88 (s, 3, OMe), 3.8-1.6 (m, 11, pyrrolidine-H), 1.12 (t, 3, N-Et) ppm. TLC: R_f 0.20. HPLC: Retention time 14.4 min at 2.0 mL/min.

Radiolabeling with I-123: To a solution of [^{123}I]NaI (34.8 mCi, Medi-Physics) in 0.76 ml 0.1 N NaOH was added 0.2 mM NaI (0.025 mL, 5.0 nmol) followed by 1.7 mM (*S*)-5-

(tri-*n*-butyltin)-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-2,3-dimethoxybenzamide (**2**) (0.025 mL, 43 nmol), made by dissolving 50 mg of **2** in 50 mL EtOH. Concentrated HCl (0.025 mL, 0.30 mmol) was added at 23 °C. An aqueous 2 mM solution of *N*-chloro-4-toluenesulfonamide sodium monohydrate, Chloramine-T (0.025 mL, 50 nmol) , freshly prepared by dissolving 13 mg in 25 mL sterile water, was added. After 2 min, 0.1 M sodium metabisufite (0.025 mL, 2500 nmol), prepared by dissolving 96 mg $Na_2S_2O_5$ in 5 mL sterile water, was added. The reaction mixture was neutralized by addition of 14 N NH_4OH (0.050 mL, 0.7 mmol) and the product was extracted with ether (2 x 0.3 mL), the organic layer being removed with a Pasteur pipet. The combined extracts contained 32.5 mCi (86%, decay corrected). The solvent was removed with a gentle stream of nitrogen and replaced with 0.05 mL of ethanol. Purification on reverse phase HPLC gave 23 mCi (66%) of pure [^{123}I]-**1** in 5.5 mL buffer at 17-20 min retention time at a flow rate of 2.0 mL/min. Comparison of the UV peak at 235 nm of 4.2 ug (10 nmol) of unlabelled **1** showed a peak area corresponding to 7.1 nmol of product giving a specific radioactivity of 2670 Ci/mmol at 3.5 h after synthesis. Radiochemical purity >98%.

Radiolabeling with I-125: To a solution of [^{125}I]NaI (9.4 mCi, Cintichem) in 28 uL of 0.03 mM NaOH (pH 9.5) was added 1.7 mM ethanolic (*S*)-5-(tri-n-butyltin)-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-2,3-dimethoxybenzamide (**2**) (10 uL, 17 nmol), previously prepared by dissolving 50 mg of **2** in 50 mL EtOH, followed by 0.2 N HCl (10 uL, 2000 nmol) at 23 °C. An aqueous 5 mM solution of N-chloro-4-toluenesulfonamide sodium monohydrate, Chloramine-T (10 uL, 50 nmol), freshly prepared by dissolving 25 mg in 25 mL sterile water, was added. After 2 min, a solution of 0.1 M sodium metabisufite (10 uL, 1000 nmol), prepared by dissolving 96 mg $Na_2S_2O_5$ in 5 mL sterile water, was added. The reaction mixture was neutralized by addition of 1.0 N NH_4OH (5 uL, 5000 nmol) and the crude raction product was injected into a reverse phase HPLC column (Waters Novapak). At 14.7 min, a peak corresponding to approximately 6% of the

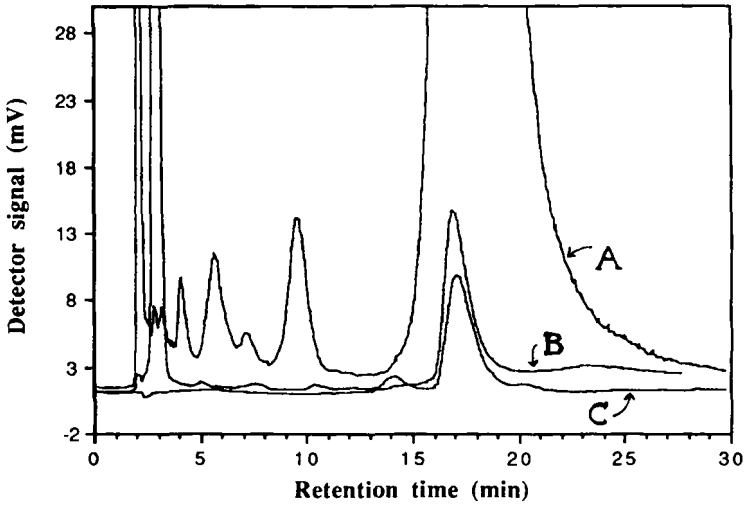


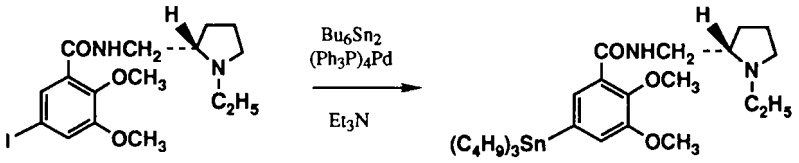
Figure 1. Overlay chromatograms of A. Radioactivity of 6.24 mCi crude ether-extracted [^{125}I]epidepride. B. 20 uL of 0.2 mM (4.0 nmol) epidepride. C. UV absorption at 235 nm of no carrier-added crude ether-extracted [^{125}I]epidepride.

chloro derivative **3**, was seen (Figure 1).. Collection of the radioactive peak at 16.2-19.5 min gave 6.24 mCi (66%) of pure [^{125}I]-**1** in 6.3 mL buffer. Comparison of the UV peak at 235 nm of 40 uL of 0.1 mM (1.68 ug, 4.0 nmol) of unlabelled **1** showed a peak at 17.5 min with a peak area corresponding to 3.1 nmol of product (Figure 1) giving a specific radioactivity of >2000 Ci/mmol. Reinjection of a 50 uL sample of the collected fraction showed a radiochemical purity >99.5%.

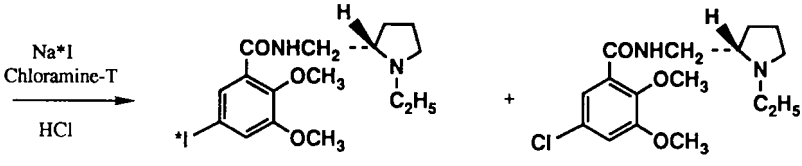
RESULTS AND DISCUSSION

The reaction of [^{123}I]NaI with the tributyltin derivative was made more reliable by the addition of traces (nmol) of iodine carrier. In preliminary experiments with no-carrier-added [^{123}I]NaI, radiochemical yields of only 40% were obtained. For brain imaging studies, specific activities ranging from 2,000 to 8,000 Ci/mmol were considered to be of sufficiently high specific activity to being able to accurately quantify the receptor binding without saturation.

The reported method for synthesis of a suitable precursor for the radioiodination of epidepride from isoremoxipride is inefficient [9] and, in our hands, failed to give acceptable yields. Direct iodination of the corresponding desiodo derivative, as was successfully applied in the radiolabelling of IBZM [6, 17], gave only the 6-iodobenzamide as detected by NMR and HPLC. We therefore used the corresponding tri-*n*-butyltin derivative in an iododestannylation reaction [18]. The iododestannylation reaction conditions were originally developed by Seitz the for production of [^{125}I]iodotamoxifen [19] and have been successfully applied in the preparation of a number of iodine substituted radioligands [20]. We adopted a slightly modified method according to the preparation of [^{125}I]iodopride [16], starting with the unlabelled iodo compound in a stannylation reaction according to the method of McBride et al. [18, 21]. The stannylation and iododestannylation reactions with epidepride were consistently analoguous to those experienced with iodopride [16]. Iodopride is a close structural analogue of epidepride, its structure differing only by lacking the 3-methoxy substituent [6, 11, 16].



epidepride , **1** **2**



[^{125,123}I]-**1**, [^{125,123}I]epidepride **3**

Treatment of (*S*)-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-5-iodo-2,3-dimethoxybenzamide (epidepride, **1**) with an equal amount of bis(tributyltin) in refluxing triethylamine and in the presence of catalytic amounts of tetrakis(triphenylphosphine)palladium(0) gave an almost quantitative yield of (*S*)-5-(tri-*n*-butyltin)-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-2,3-dimethoxybenzamide (**2**) together with tri-*n*-butyltin iodide [18, 21]. The aryltin derivative **2** is stable under basic conditions, having a carbon-tin bond energy of 50 kcal/mol [18], and can be extracted with ether. It was found advantageous, however, to apply the crude reaction mixture, or in the case of multigram batches, the residue after rotevaporation of the solvent, on a short silica column in hexane to remove the very lipophilic tri-*n*-butyltin iodide. Subsequent elution with ethyl acetate, optionally with increasing amounts of ammonialkalic ethanol, gave compound **2** as a viscous oil.

The complete conversion of the iodine substituent could be monitored by NMR. The aromatic part of the ^{1}H NMR spectrum of **2** shows a narrow meta-doublet centered at δ 7.85 ppm, with characteristic side bands from coupling with the 16% natural abundance of tin isotopes with odd atomic mass numbers. This signal was assigned to the proton in the 6-position. The corresponding signal in the spectrum of **1** is centered at 8.04 ppm [9]. Attempted preparation of **2** from the bromo analogue of **1**, isoremoxipride (FLB 457) [8], by the same reaction conditions, required long reaction time and was still incomplete after 24 h (data not shown). Formation of the unwanted chlorine substituted analogue **3** in the radiolabelling reaction could be supressed, either by shortening the reaction time from addition of chloramine-T to quenching with metabisufite to no more than a few minutes, or by keeping the excess of precursor **2** as low as possible. Attempted short-time oxidation of the iodide with hydrogen peroxide or peracetic acid as described for the preparation of [^{123}I]IBZM [17, 22] gave poor yields. We preferred the chloramine-T method since separation and removal of compound **3** in amounts ranging from 5% to 50% by HPLC proved expedient. It should be noted, that the reported [^{3}H]spiperone binding activity of compound **3**, IC_{50} 0.4 nM [9], is probably in error by one order of magnitude (unpublished observation).

Dysfunction of extrastriatal dopamine receptors has been linked with both the therapeutic response of neuroleptic therapy and to the etiology of schizophrenia [23]. Less potent ligands such as [^{3}H]raclopride are able to visualize dopamine D-2 receptors in extrastriatal areas of the rat and monkey brain by autoradiography [4], but not those of the living human brain using [^{11}C]raclopride and PET [24]. The potential SPECT ligands, [^{123}I]IBZM and [^{123}I]spectramide, seem to be lacking sufficient regional uptake specificity [6, 7]. With the availibility of ultra-potent radioligands such as [^{125}I]epidepride and its corresponding 6-hydroxy derivative, [^{125}I]-NCQ 298 ([^{125}I]ioxipride) [11, 25], extrastriatal dopamine D-2 receptors can be quantified in vitro [10] and possibly in vivo. Preliminary SPECT studies of the human brain with [^{123}I]epidepride have recently demonstrated exellent imaging potential of this substituted benzamide [26]. Because epidepride is less lipophilic than ioxipride, log k_w 2.03 vs. log k_w 2.48, [^{123}I]epidepride displays a higher regional specificity than [^{123}I]ioxipride [25, 26]. The easy preparation [12, 14] of [^{125}I]epidepride and [^{123}I]epidepride would make it the radioligand of choice for studying the dopamine D-2 receptor in vitro as well as in the basal ganglia and in extrastriatal regions in vivo.

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