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IODINE-123 LABELED DERIVATIVES OF METHYLPHENIDATE: POTENTIAL SPECT RADIOPHARMACEUTICALS FOR BRAIN DOPAMINE TRANSPORTERS

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SUMMARY

Since *dl-threo*-[^{11}C]methylphenidate (Ritalin) and especially the more active enantiomer, *d-threo*-[^{11}C]methylphenidate, have favorable properties for PET studies,we prepared two radioiodinated analogs of methylphenidate, p-[^{123}I]iodomethylphenidate and m-[^{123}I]iodo-p-hydroxymethylphenidate with a view to evaluating them as potential SPECT tracers. To prepare p-[^{123}I]iodomethylphenidate, the p-tributyltin derivative was prepared from the previously reported p-bromomethylphenidate and reacted under acidic conditions with I-123 iodide plus chloramine-T at room temperature for 90 seconds. The predominant radioactive product was obtained in 85% radiochemical yield and >10 Ci/μmol specific radioactivity after HPLC purification. It had the same HPLC retention time as a spectroscopically characterized non-radioactive p-iodomethylphenidate standard prepared *via* nitration of methylphenidate and diazotization, after protection of the secondary amino group by benzoylation. A second radioiodinated methylphenidate derivative, m-[^{123}I]iodo-p-hydroxymethylphenidate was prepared in 80% radiochemical yield by direct iodination of the known p-hydroxymethylphenidate. In this case the non-radioactive standard was prepared by iodination of p-hydroxyritalinic acid using I_2 and iodic acid, followed by esterification.

Key words: dopamine transporter, iodine-123, SPECT, cocaine, Ritalin.

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INTRODUCTION

Methylphenidate (Ritalin) is a psychomotor stimulant drug which is widely used in the treatment of attention-deficit hyperactivity disorder [1]. The therapeutic properties of methylphenidate are thought to be mediated by its binding to a site on the DA transporter, resulting in inhibition of DA reuptake and enhanced levels of synaptic dopamine [2]. Methylphenidate also inhibits reuptake of norepinephrine [3]. Methylphenidate has two chiral centers, but its pharmacological activity is believed to be due largely to the *d-threo* isomer [3]. Carbon-11 labeled *d-threo*methylphenidate has recently been synthesized and demonstrated to be an effective radiotracer for striatal dopamine transporters, using positron emission tomography (PET) in the baboon and human brain [4, 5, 6, 7, 8]. A number of other PET radioligands for the dopamine transporter have been evaluated, including: cocaine [9], the cocaine analog WIN 35,428 [10], nomifensine [11], and GBR 13119 [12].

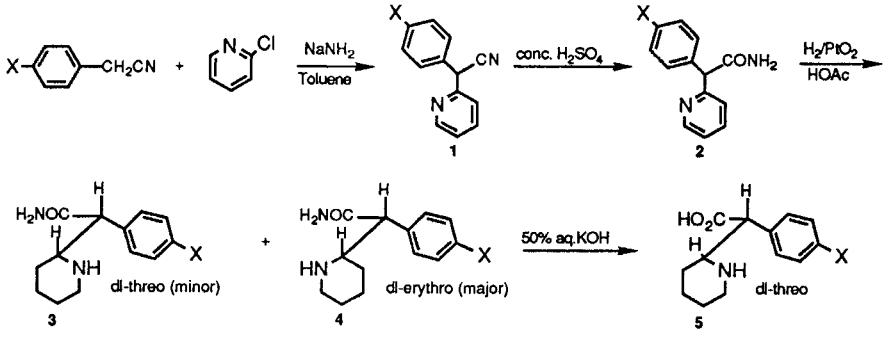
Work on SPECT radioligands has been more limited, in terms of structural diversity of target molecules, most of which have been based on the cocaine and related phenyltropane ("WIN") skeletons [see, e.g. 13, 14]. Although we determined previously that p-iodococaine was unlikely to have utility as a radiopharmaceutical [15], the related compound RTI-55, where the benzoyl ester is replaced by a phenyl group, and which has a much higher affinity for the dopamine transporter [16], has been used extensively in animal and human SPECT imaging experiments [17, 18]. Several candidate radiopharmaceuticals structurally related to RTI-55 have also recently been prepared and evaluated [13, 14]. Our objective was to prepare radioiodine substituted derivatives of methylphenidate so that these would be available in radioiodinated form for evaluation as SPECT radiopharmaceuticals.

RESULTS AND DISCUSSION

Preparation of Starting Materials and Chromatographic Standards

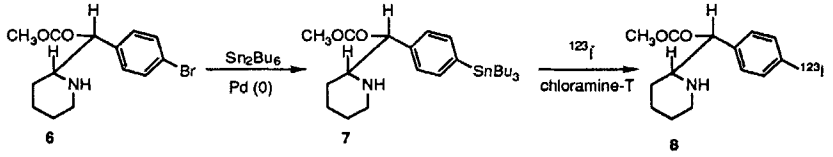
The general approach to synthesis of non-radioactive methylphenidate derivatives needed as starting materials and chromatographic standards followed Panizzon's [19] original methods with minor modifications (Scheme I).

Scheme I: Panizzon's Synthesis of Methylphenidate and Derivatives.



Scheme I was used more recently by Patrick et al. to prepare the methylphenidate metabolite p-hydroxymethylphenidate [20]. Our yields of methylphenidate and its derivatives by this route were not excellent, but we did not optimize reactions, or at this time attempt to develop novel synthetic routes, since our aim was to determine whether the methylphenidate structure possessed potential for development of SPECT radioligands. We had previously reported the synthesis of p-bromomethylphenidate **6** [21], and we therefore used this compound to prepare p-tributylstannylmethylphenidate **7**, via a palladium catalyzed coupling reaction [22]. The organotin compound was then used as the starting material for p-[^{123}I]iodomethylphenidate **8** (Scheme II) in an oxidative radiohalodestannylation reaction [23].

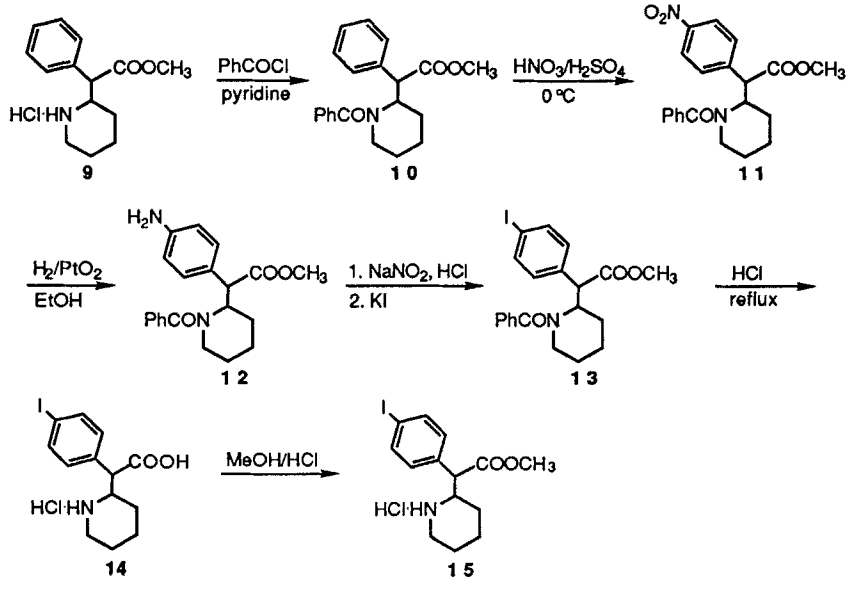
Scheme II: p-Tributylstannylmethylphenidate and I-123 p-Iodomethylphenidate.



6 **7** **8**

Unfortunately, several attempts to use this route on a millimolar scale to prepare non-radioactive p-iodomethylphenidate failed. The p-p' coupled dimer was the only compound isolated from reaction mixtures. The reason for this failure is unclear. Attempts to prepare p-iodomethylphenidate from 4-iodophenylacetonitrile (Scheme I) also failed, because deiodination occurred during the hydrogenation procedure. We therefore adopted an alternative synthesis based on nitration and subsequent diazotization of methylphenidate (Scheme III). Protection of the secondary amino group by benzoylation was necessary, to avoid oxidation reactions during treatment with nitrous acid.

Scheme III: Synthesis of p-Iodomethylphenidate.



9 **10** **11** **12** **13** **14** **15**

A second iodinated methylphenidate derivative, m-iodo-p-hydroxymethylphenidate **18**, was prepared by iodination of the previously reported p-hydroxyritalinic acid **16**, an intermediate in Patrick's [20] route to p-hydroxymethylphenidate (Scheme IV), and subsequent esterification.

Preparation of Iodine-123 Labeled Compounds

Electrophilic radioiodinations were conducted using chloramine-T as oxidizing agent. Net yields of labeled methylphenidate derivatives **8** and **20** after HPLC purification were 80-90% (Table 1). Compound **8** was prepared directly from p-hydroxymethylphenidate (**19**). It was found that identical reaction conditions, with only minor changes to the HPLC solvent mixture, could be used to prepare the radioiodinated cocaine derivatives p-[^{123}I]iodococaine [15] and [^{123}I]RTI-55. Each of the radioactive compounds shown in

Scheme IV: Synthesis of m-Iodo-p-Hydroxymethylphenidate.

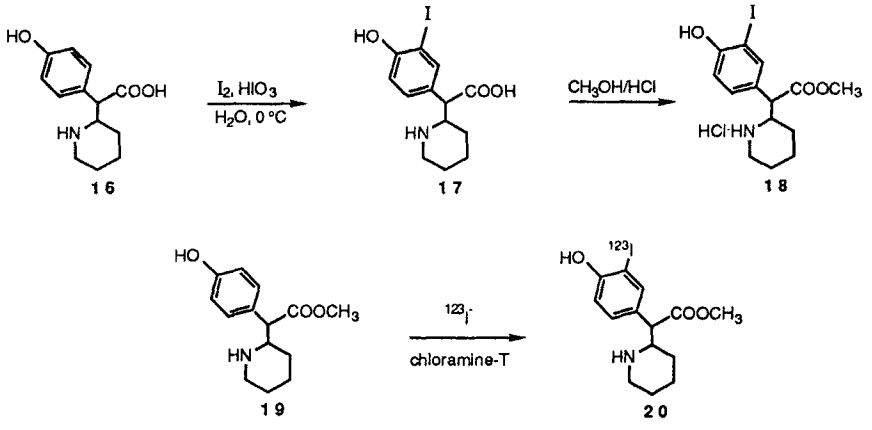


Table 1 had the same retention time as commercial or spectroscopically characterized non-radioactive material. The radioactive peaks were well separated from starting materials and other contaminants. The latter included peaks which eluted 2-5 minutes before product peaks which we tentatively assigned as the related chlorinated compounds. This procedure gives a higher radiochemical yield, in a shorter time, than our originally reported route to [^{123}I]iodococaine [15]. Radiochemical purities of both labeled methylphenidate derivatives were >95% after storage at 4 °C overnight in physiological saline solution.

Since the specific radioactivity of [^{123}I]iodide purchased from Nordion International is >50,000 Ci/mmol, the specific radioactivity of the products is determined by adventitious introduction of non-radioactive iodide. In some but not all experiments, UV absorbing peaks with the same retention time as labeled product were detectable on HPLC's. The areas of these peaks were compared with standards and found to correspond to <100 pmol of "carrier" iodinated compound. Thus, when labeling reactions were conducted with 1 mCi of [^{123}I]iodide, specific activities at the end of synthesis were > 10,000 Ci/mmol.

Table 1. Labeling and Purification Data for I-123 Labeled Compounds.

Compound	Precursor	Yield (%)	CH_3CN (%)	t_R (minutes) at 1 mL/min		
				Product	Starting material	Chloro-cpd.*
p-Iodomethylphenidate	Bu_3Sn cpd	85	40	25	>50	21
m-Iodo-p-hydroxy-methylphenidate	p-hydroxy-MP	80	40	16	12	?
RTI-55 (β-CIT)	Me_3Sn cpd	90	60	21	25	16
p-Iodococaine	Bu_3Sn cpd	90	50	19	50	17

key: t_R = retention time. Values in the CH_3CN (%) column are the percentage of CH_3CN in the mobile phase, the remainder being 40 mM pot. phosphate, pH 7.4. The stationary phase was an Alltech Econosil cyanopropyl column (10 micron; 250 x 4.6 mm).
* tentative assignment.

Preliminary Biological Evaluation

Both the iodine-substituted methylphenidate derivatives reported here inhibited binding of the dopamine transporter radioligand [^{3}H]WIN 35,428 to membranes prepared from rat striatum (SJ Gatley, R Chen, D Pan, Y-S Ding; Life Sci, in press). IC_{50} values were about 20 nM and 40 nM for p-iodomethylphenidate and m-iodo-p-hydroxymethylphenidate, respectively. Thus both compounds are more potent than methylphenidate itself (75 nM), but considerably less potent than RTI-55 (1.5 nM). Our work therefore tends to confirm the idea that radioiodinated compounds related structurally to methylphenidate could be developed as SPECT radiopharmaceuticals. However, based on experience with other receptor and transporter binding radiopharmaceuticals, a compound with a higher affinity would probably be desirable. Thus although we reported in a recent abstract [24] that uptake of p-[^{123}I]iodomethylphenidate in mouse brain was regionally specific, stereo-selective and could be inhibited by methylphenidate, the radioactivity washed out of the brain within one hour. SPECT experiments would require more more persistent uptake.

EXPERIMENTAL

General. Synthetic reagents were purchased from the Aldrich Chemical Co. (Milwaukee). Methylphenidate hydrochloride (**9**), p-hydroxyritalinic acid (**16**) and p-hydroxymethylphenidate (**19**) were prepared by the methods of Panizzon [19] and Patrick [20]. Iodine-123 was obtained from Nordion International as 1-10 μL of a solution in 0.01N NaOH. Starting material for [^{123}I]RTI-55 as well as non-radioactive RTI-55 (listed in the catalog as trimethylstannyl-β-CIT and β-CIT) were purchased from Research Biochemicals Inc. (Natick, MA). Starting material for [^{123}I]p-iodococaine was prepared as previously described [15] and purified by HPLC. A Brucker 300 MHz instrument was used for ^{1}H NMR spectra and a Finnegan-Mat 5100 for GC-MS. Analytical and preparative HPLC were performed using 4.6 x 250 mm and 10 x 250 mm Alltech columns containing Econosil cyanopropyl or octadecylsilane stationary phases. Knauer HPLC pumps and UV

detectors were employed. Radioactive peaks were detected by leading the column output through a loop of tubing held near a lead-shielded Geiger or scintillation probe connected to suitable counting electronics. Detector outputs were recorded and displayed using a Vision-4 system (Autochron Inc, College Station, PA). Flash column chromatography was performed with Merck silica gel 60 (200-400 mesh).

p-Tributylstannylmethylphenidate (7)

To a solution of *dl-threo*-p-bromomethylphenidate **6** (310 mg, 1.0 mmol) and hexabutylditin (1.73 g, 3 mmol) in toluene (40 mL) was added tetrakis(triphenylphosphine) palladium (58 mg, 0.05 mmol) under a nitrogen atmosphere. The reaction mixture was stirred at 60 °C for 5 hr. in a dark environment. Silica gel TLC (elution with ethyl acetate) indicated that about 90% of the starting material had been converted to *p*-tributylstannylmethylphenidate (Rf 0.65), with two minor byproducts. This mixture was used in labeling experiments with I-123. Several attempts to isolate *p*-tributylstannylmethylphenidate yielded the p-p' coupled dimer (verified by NMR).

dl-threo-N-Benzoylmethylphenidate (10)

To a solution of *dl-threo*-methylphenidate **9** (2.3 g, 10.4 mmol) and pyridine (0.83 g, 10.4 mmol) in an icebath was added 1.2 g (11 mmol) of benzoyl chloride. The newly formed brown solid was consecutively rinsed with water (20 mL), 5% hydrochloric acid (20 mL), water (20 mL), 5% aqueous sodium hydroxide (20 mL), and water (20 mL). The residue was then recrystallized from ethanol-water solution. The *dl-threo-N*-benzoylmethylphenidate **10** (2.7 g, 82%) was collected as a brown solid.
^{1}H NMR ($CDCL_3$) δ 1.21-1.80 (m, 6H), 3.07 (m, 1H), 3.40 (t, 1H), 3.59-3.73 (m, 1H), 3.66 (s, 3H), 4.28 (d, 1H), 7.14-7.56 (m, 10H).

dl-threo-N-Benzoyl-p-nitromethylphenidate (11)

To 230 mg (0.51 mmol) of *dl-threo-N*-benzoylmethylphenidate **10** was added 1 mL of an ice cold mixture of HNO_3 and H_2SO_4 (1:1, v/v). The solution was stirred at room temperature for 5 hr. and then 2 g of ice was added. A white solid was formed. The supernatant was decanted and the residue was rinsed with cold water twice. The solid was recrystallized from ethanol-water solution. The *dl-threo-N*-benzoyl-p-nitromethylphenidate **11** (190 mg, 72%) was collected as a white solid.
^{1}H NMR ($CDCL_3$) δ 1.33-1.70 (m, 6H), 3.42 (t, 1H), 3.62-3.76 (m, 1H), 3.69(s, 3H), 4.38 (d, 1H), 5.61 (d, 1H), 7.30-7.45 (m, 5H), 7.72, 8.26 (dd, 4H).

dl-threo-N-Benzoyl-p-aminomethylphenidate (12)

To *dl-threo-N*-benzoyl-p-nitromethylphenidate **11** (1.5 g, 3.0 mmol) in 20 mL of ethanol was added 10 mg of PtO_2. The solution was hydrogenated at a pressure of 5 psi for 20 minutes. The solution was filtered and the solvent was removed using a rotary evaporator. The residue was recrystallized from ethanol-water solution. The *dl-threo-N*-benzoyl-p-aminomethylphenidate **12** (830 mg, 60%) was collected as white solid.

***dl-threo-N*-Benzoyl-p-iodomethylphenidate (13)**

To a mixture of *dl-threo-N*-benzoyl-p-aminomethylphenidate **12** (730 mg, 2.1 mmol), concentrated HCl (1 mL), water (1 mL) and ice (1 g) in ice bath was added a solution of 160 mg (2.3 mmol) of sodium nitrite in 1 mL of water. A starch-iodide test paper showed a positive blue color. A solution of 350 mg (2.1 mmol) of potassium iodide in 1 mL of water was added and the mixture was stirred overnight at room temperature. The solution was adjusted to pH 9 with 1N NaOH and extracted with ethyl acetate (50 mL x 4). The combined extracts were dried over anh. $MgSO_4$ and concentrated by rotary evaporation. The residue was purified by flash chromatography (EtOAc/Hex, 1:3). The *dl-threo-N*-benzoyl-p-iodomethylphenidate **13** (480mg, 50%) was collected as a white solid.
^1H NMR ($CDCl_3$) δ 0.84-1.40 (m, 6H), 3.50 (t, 1H), 3.62-3.68 (m, 1H), 3.66(s, 3H), 4.22 (d, 1H), 5.52 (d, 1H), 7.38-7.46 (m, 5H), 7.28, 7.71 (dd, 4H).

***dl-threo*-p-Iodoritalinic acid (14)**

A solution of *dl-threo-N*-benzoyl-4-iodomethylphenidate **13** (300 mg) in 10 mL of 6N HCl was refluxed for 24 hr. Benzoic acid was removed by extraction with ether (15 mL x 2). The aqueous solution was concentrated to 4 mL. White crystals of *dl-threo*-4-iodoritalinic acid **14** were obtained by recrystallization from ethanol/diethylether (185 mg, 75%).
^1H NMR (CD_3OD) δ 1.33-1.89 (m, 6H), 3.07 (t, 1H), 3.41 (m, 1H), 3.75(m, 2H), 7.11, 7.76 (dd, 4H).

***dl-threo*-p-Iodomethylphenidate (15)**

dl-threo-p-Iodoritalinic acid **14** (155 mg, 0.41 mmol) was dissolved in 20 mL of methanol. Hydrogen chloride gas was passed through the solution which was then refluxed for 5 hr. The solution was concentrated to 5 mL. Diethyl ether was added dropwise until the product crystallized. The *dl-threo-p*-iodomethylphenidate **15** (105 mg, 66%, m.pt. 208°) was collected as white needle-shape crystals of the hydrochloride. HPLC gave a single UV peak with t_R = 25 min (conditions given in Table 1).
^1H NMR ($CDCl_3$) δ 1.34-2.09 (m, 6H), 2.90 (m, 1H), 3.60-3.72 (m, 2H), 3.80(s, 3H), 4.34 (d, 1H), 7.04, 7.69 (dd, 4H).

***dl-threo*-2-(p-Hydroxy-m-iodophenyl)-2-(2'-piperidyl) acetic acid (17)**

A solution of *dl-threo*-2-(p-Hydroxylphenyl)-2-(2'-piperidyl) acetic acid **16** (58 mg, 0.18 mmol) and iodine (19 mg, 0.073 mmol) in 5 mL of water was mixed with iodic acid (13 mg, 0.072 mmol) and stirred at 0°C for 48 hr. The reaction mixture was applied to a semi-preparative HPLC column (Alltech Associates Econosil cyanopropyl column, 250 x 7.8 mm, eluted with a solution of 10% CH_3CN-90% aqueous 40 mM KH_2PO_4). The product fractions were combined and dried by rotary evaporation.

***dl-threo*-m-iodo-p-hydroxymethylphenidate (18)**

The above crude product **17** was dissolved in 30 mL of methanol and HCl gas bubbled through the solution. The solution was refluxed for 4 hr, and methanol was removed using

a rotary evaporator. The residue was dissolved in 30 mL of 5% aqueous sodium bicarbonate solution and extracted with diethyl ether (30 mL x 4). The ethereal solutions were combined and dried over anhydrous magnesium sulfate. The ether was removed and the white solid was recrystallized in methanol-ether solution. Yield 70 mg (60%) m.pt. 231°C. HPLC gave a single UV peak with t_R = 16 min (conditions given in Table 1). ^1H NMR (CD_3COCD_3) δ 1.24-1.75 (m, 6H), 2.61 (dt, 1H), 3.03 (M, 2H), 3.38 (m, 1H), 3.60 (S, 3H), 6.91 (d, 1H), 7.19 (d, 1H), 7.70 (s, 1H).

Preparation and analysis of p-[^{123}I]iodomethylphenidate (8) and m-[^{123}]iodo-p-hydroxymethylphenidate (20)

An aqueous solution (1mg/mL) of chloramine-T was prepared immediately before the labeling reaction. To 1-20 μL of Na^{123}I in dilute NaOH was added 20 μg of the indicated starting material (either p-tributylstannylmethylphenidate **7** or p-hydroxymethylphenidate **16**) dissolved in 20 μL acetonitrile. The mixture was shaken, and 15 μL of 0.5M H_3PO_4 and 15 μL chloramine -T solution were added in turn. The reaction vial was capped and shaken, and after 90 seconds 10 μL of 5% aq. $NaSO_3$ was added. The reaction mixture was then applied directly to an HPLC column (10 micron 250 x 4.6 mm cyanopropyl) eluted with mixtures of acetonitrile and 40 mM potassium phosphate buffer, pH 7.4. The major radioactive peaks were collected. Mobile phase compositions and radiotracer retention times are given in Table 1. When radioactive peaks were reanalyzed by HPLC, recoveries were determined by comparison of collected radioactivity with that in a duplicate aliquot of the sample.

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