SYNTHESIS OF THE RACEMATE AND INDIVIDUAL ENANTIOMERS OF [^{11}C]METHYLPHENIDATE FOR STUDYING PRESYNAPTIC DOPAMINERGIC NEURON WITH POSITRON EMISSION TOMOGRAPHY

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SUMMARY

Carbon-11 labeled *dl-threo*-methylphenidate (methyl-2-phenyl-2-(2-piperidyl)acetate, Ritalin), a psychostimulant drug widely used to treat attention deficit hyperactivity disorder, was prepared in two steps: O-methylation of the N-protected *dl-threo*-ritalinic acid derivative with [^{11}C]methyl iodide followed by deprotection. The same strategy was applied for the preparation of C-11 labeled individual enantiomers of *threo*-methylphenidate from N-protected *d-threo-* or *l-threo*-ritalinic acid. The subsequent C18 sep-pak and reverse-phase HPLC purification resulted in ca. 40% radiochemical yield with a total synthesis time of 40 minutes and an average specific activity of 1.5 Ci/μmole (at EOB).

Key words: methylphenidate, Ritalin, carbon-11, positron emission tomography (PET), dopamine uptake site.

INTRODUCTION

An important link has been established between the brain dopamine system and motor function, mood and reinforcement [1, 2, 3, 4]. For example, the dopaminergic neurons display a special vulnerability [5]. There is a progressive decrease in dopaminergic neurons at a rate of 0.5 % / year in normal aging and at an accelerated rate (to about 1% / year) in Parkinson's disease [6]. It has also been established that drugs abused by humans preferentially increase synaptic dopamine in the mesolimbic system [7] and that cocaine binding sites on dopamine transporters on the presynaptic dopaminergic neuron are related to self administration of cocaine [8]. The need to better understand the association between changes in the brain dopamine system and its relationship to neurological and psychiatric disorders and to

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track changes which occur in normal aging, in neurodegenerative disease and drug abuse and drug therapy has stimulated the development of a number of different radiotracer methodologies. For example, 6-[^{18}F]fluoroDOPA [9] and [^{11}C]L-DOPA [10] has been used to probe dopamine metabolism and has been used widely in PET studies in Parkinson's disease [11, 12]. Other molecular targets include the vesicular transporter [13] and the neuronal dopamine transporter [14]. Many PET and SPECT radiotracers for the neuronal dopamine transporter have been described including [^{11}C]nomifensine [15, 16, 17], [^{11}C]cocaine [18, 19], [^{18}F]GBR 13119 [20], [^{11}C]WIN 35428 [21, 22, 23, 24] and [^{123}I] β-CIT [25].

Methylphenidate (methyl-2-phenyl-2-(2-piperidyl)acetate, Ritalin) is a mild central nervous system stimulant used primarily in the treatment of attention-deficit disorder (ADD) [26]. The psychostimulant and therapeutic properties of methylphenidate are thought to be mediated by its binding to a site on the dopamine transporter, resulting in inhibition of dopamine reuptake and enhanced levels of synaptic dopamine.

Methylphenidate (MP) has two chiral centers (four stereoisomers), and is marketed as the *dl-threo* form (Fig. 1). However, it is known that the pharmacological specificity for the dopamine transporter and its pharmacological activity resides almost entirely in the *d-threo* -enantiomer [27]. The potency (IC_{50}) of *dl-threo*-MP in displacing [^{3}H]*dl-threo*-MP from striatal synaptosomal membrane binding sites is 0.21 μM as compared to 0.088 μM for the *d-threo*-enantiomer and 1.2 μM for the *l-threo*-enantiomer [28]. In order to examine *in vivo* pharmacokinetics and pharmacological profiles of the racemate and individual enantiomers of methylphenidate and to

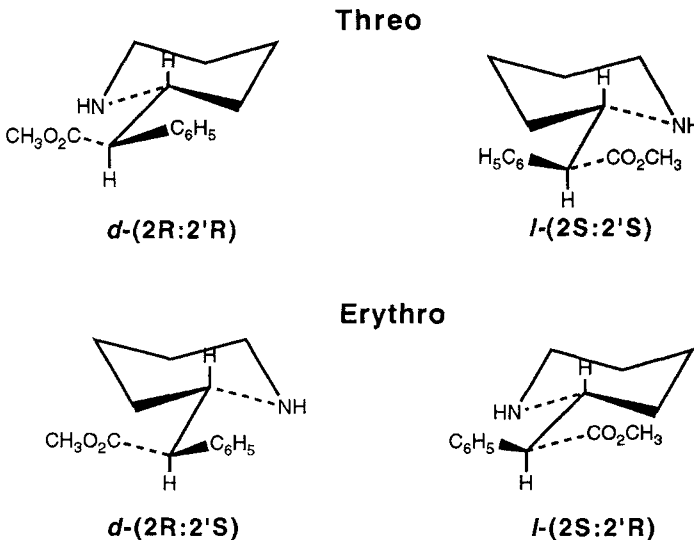


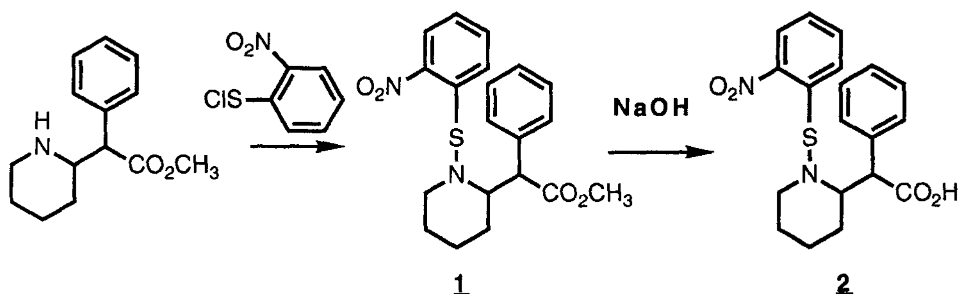
Figure 1: Stereochemistry of methylphenidate isomers

evaluate their suitability as radiotracers for the presynaptic dopaminergic neuron, we have developed the synthesis of carbon-11 labeled *dl-threo* , *d-threo,* and *l-threo* methylphenidate. We report here the synthesis, purification, and quality control of [^{11}C]*dl-threo, d-threo* and *l-threo* methylphenidate suitable for human study with PET. An abstract of the preliminary results has appeared previously [29].

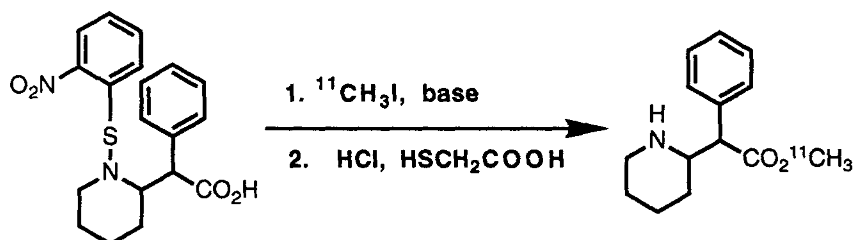
RESULTS AND DISCUSSION

Our first approach to [^{11}C]*dl-threo*-MP involved direct alkylation of ritalinic acid (obtained from Ciba Geigy Corp.) with [^{11}C]H_3I. However, the potential for both N and O alkylation of the ritalinic acid precursor required that a suitable N-protected derivative be developed. The o-nitrophenylsulfenyl group (NPS) was chosen as the protective group due to its facile preparation from either free amino acids or alkyl esters of amino acids and its rapid removal under very mild conditions without affecting the ester functional group [30]. Thus, [^{11}C]MP was prepared via a two-step synthesis: O-methylation of the N-protected *dl-threo*-ritalinic acid derivative with [^{11}C]methyl iodide followed by acid hydrolysis of the amine protective group (Scheme 2). The same strategy was applied to prepare C-11 labeled individual enantiomers using N-protected *d-threo* or *l-threo* ritalinic acid derivatives as the precursors. The N-protected ritalinic acid derivatives (the precursors for C-11 labeling) were prepared by treatment of the corresponding unlabeled *dl-threo, d-threo,* or *l-threo* methylphenidate with o-nitrophenylsulfenyl chloride followed by base hydrolysis of the methyl ester group (Scheme 1).

Scheme 1: Synthesis of precursor to [^{11}C]methylphenidate



Scheme 2: Synthesis of [^{11}C]methylphenidate



In the process of developing a rapid, stereoselective synthetic method suitable for preparation of C-11 (a positron emitter with a half-life of 20 .4 minutes) labeled methylphenidate, we encountered several challenges: (1) finding suitable conditions for acid hydrolysis of the amine protective group; (2) purification of the crude product; and (3) finding a reliable chiral HPLC system to verify the stereochemical purity of the starting materials and the final products. For the acid hydrolysis step, the reaction was not complete within 5 min at room temperature when 0.1 mL of 1 M HCl in anhydrous ether was used alone. However, an increased amount of HCl (0.15-2.0 mL) or higher temperature (60° C) resulted in the formation of side products. Attempts to cleave the protective group by nucleophiles alone, such as 2-mercaptopyridine [31] or mercaptoacetic acid [32] were not successful. However, we found that the combination of HCl (0.1 mL) and mercaptoacetic acid (2.5 µL) [33] accomplished the removal of the NPS group (3 min at room temperature) with no detection on HPLC of C-11 labeled N-NPS methylphenidate in the reaction mixture.

In our hands, the base hydrolysis step (the reaction conditions have not been optimized) to prepare the precursor for radiolabeling resulted in isomerization with a range of 5-15% of conversion from *threo* to *erythro*. For example, N-protected *d-threo*-ritalinic acid (2) would isomerize to *l-erythro*-2. The procedure for C-11 labeling, on the other hand, maintained the stereochemical integrity of the molecule. A chromatographic separation of *erythro* and *threo* methylphenidate in the final reaction mixture was therefore required. A variety of HPLC solvent systems with either silica columns or C-18 columns were tested. In all cases, there was no separation when the reaction mixture was injected directly onto HPLC column without any work-up. However, after treatment with a mixture of sodium borate, H_2O and NaOH [34] and a C-18 sep-pak work-up, *erythro* and *threo* methylphenidate were well separated on a ODS2 column with a solvent system CH_3CN : 0.17 M ammonium formate = 70 : 30. The retention times for *erythro* and *threo* methylphenidate were 12 and 15 min, respectively at a flow of 3.0 mL/min. The total synthesis time was 40 minutes with ca. 40% radiochemical yield and a specific activity of 1.0-2.6 Ci/µmole (at EOB).

The individual enantiomers *d-threo,* or *l-threo* methylphenidate used as the starting material for the preparation of the precursor to $[^{11}C]$*d-threo,* or *l-threo* methylphenidate were prepared using a literature method [27] for the preparative resolution of *dl-threo*-methylphenidate (obtained from Ciba-Geigy). In order to ensure the enantiomeric purity of the starting material after the resolution and the stereochemical purity of the final product $[^{11}C]$*d-threo,* or *l-threo* methylphenidate, an HPLC system able to separate all four isomers was required. The first approach using α_1-acid glycoprotein-bonded stationary phase (Anspec Co., Chiral Amino Acid column, 4.6 x 250 mm, 5 µm) with a solvent system 1 mM $Bu_4N^+Br^-$ in 0.02 M phosphate buffer (pH 7.0) [35] afforded a 1.8 min baseline separation of *d-threo* and *l-threo* methylphenidate. However, the system lost separation capability after several injections. A reliable, efficient chiral separation of all isomers was achieved on a Chiralpak AD column (DAICEL, 10 x 250 mm) using hexane: isopropyl alcohol:

diethylamine = 98: 2: 0.1 as the solvent. The retention times for *l-erythro, d-erythro*, *l-threo,* and *d-threo* methylphenidate were 7.56, 7.76, 11.04, and 15.12 min, respectively at a flow of 4.0 mL/min. The radiochemical purity as well as stereochemical purity were determined after each radiosynthesis (see Experimental), both being over 98% pure in all cases. Moreover, the extent of isomerization which occurred during the base hydrolysis step was determined by the same chiral HPLC column. Prior to the HPLC analysis, the N-protected ritalinic acid derivatives (2) were converted back to the corresponding methyl esters by treatment with diazomethane [36]. The retention times for N-NPS protected *d-erythro, l-erythro, d-threo,* and *l-threo* methylphenidate under the HPLC conditions described above were 12.7, 14.5, 17.6, and 28.4 minutes, respectively.

The fact that methylphenidate is an approved drug facilitates approval for human studies and is also important since the unlabeled drug can be administered to humans, permitting the assessment of non-specific binding. It is also noteworthy that the primary metabolite of methylphenidate is ritalinic acid [37] which would not be labeled and would not contribute to the PET image.

In summary, we have carried out the first C-11 labeled synthesis of methylphenidate, a drug which is widely used in the treatment of hyperactive children. The availability of these labeled drugs makes it possible to examine drug pharmacokinetics in the human brain and to optimize drug dosing and timing in the therapeutic application of methylphenidate and in research protocols involving methylphenidate challenge. Studies are underway to evaluate the labeled *dl, d* and *l* enantiomers of methylphenidate, to determine the selectivity of [^{11}C]methylphenidate binding to the dopamine transporter and its sensitivity to changes in synaptic dopamine and neuronal loss [38].

EXPERIMENTAL

o-Nitrophenylsulphenyl chloride, (R)-(-) and (S)-(+) 1,1'-binaphthyl-2,2'-diyl hydrogen phosphate were purchased from Aldrich Chemical Co. ^{1}H NMR spectra were obtained in $CDCl_3$ on a Bruker 300 MHz NMR spectrometer and reported in ppm downfield from tetramethylsilane. Mass spectra (MS) were recorded with a Finnegan-Mat GC-MS 5100 mass spectrometer using electron impact ionization at 70 eV. Purification and analyses of radioactive mixtures were performed with a Knauer HPLC pump, in-line UV detector (254 nm), and a NaI crystal radioactive detector. Peak areas were measured using two Hewlett-Packard 3390A recording integrators. Details of semipreparative, analytical and chiral HPLC conditions are described in the following experimentals.

Preparation of Precursors to [^{11}C]Methylphenidate:

For the preparation of the precursor to [^{11}C]*dl-threo, d-threo,* or *l-threo* methylphenidate, the corresponding unlabeled *dl-threo*, *d-threo,* or *l-threo*

methylphenidate was used as the starting material and the same synthetic procedure (Scheme 1) was followed as described below. *dl-threo*-Methylphenidate was obtained from Ciba-Geigy Corp. The individual enantiomers were prepared according to a literature method for the preparative separation of *dl-threo*-methylphenidate using (R)-(-) and (S)-(+) 1,1'-binaphthyl-2,2'-diyl hydrogen phosphate as the resolving reagents [27].

To a solution of methylphenidate hydrochloride (134.9 mg, 0.5 mmol) in 20 mL of chloroform, 0.14 mL of triethylamine and o-nitrophenylsulphenyl chloride (95 mg, 0.5 mmol) were added. The solution was allowed to stand at room temperature for 6 hours and then successively washed with water, dilute acetic acid (5%), aqueous potassium hydrogen carbonate solution, and again with water, followed by drying with $MgSO_4$, and evaporation to dryness. The crude product (N-NPS-methylphenidate **1**) was purified by column chromatography with silica gel. Yield: 189 mg, 98%. ^{1}H NMR ($CDCl_3$): 7.2-8.3 (aromatic H's, 9H), 4.07 (d, J = 11.2 Hz, 1H), 3.2-3.8 (multiplets, 3H), 3.11 (s, 3H), 1.1-1.9 (multiplets, 6H). MS, m/e (rel. intensity): 386 (M^+, 2), 291 (2), 237 (100), 154 (53), 138 (12), 121 (12), 106 (16), 98 (30), 96 (12), 91 (12), 85 (16), 84 (17), 83 (22), 55 (10).

To N-NPS methylphenidate **1** (140 mg, 0.36 mmol) in 15 mL of 60% $EtOH/H_2O$ at 60° C, was added 5% NaOH (5 mL). The mixture was stirred at 60° C for 0.5 hour and the EtOH was removed under vacuum. The solution was then diluted with water, washed with ether and acidified with 3 N HCl. The acidified solution was extracted several times with ether. The ethereal extracts was repeatedly washed with water, dried and evaporated. The crude product (N-protected ritalinic acid **2**) was purified by column chromatography with silica gel. Yield: 100 mg, 75%. ^{1}H NMR ($CDCl_3$): 6.6-8.1 (aromatic H's, 9H), 4.04 (d, J = 10.8 Hz, 1H), 3.1-3.8 (multiplets, 3H), 1.2-1.9 (multiplets, 6H). MS, m/e (rel. intensity): 372 (M^+, 0.4), 308 (1.1), 237 (32), 154 (30), 136 (35), 106 (13), 98 (23), 91 (100), 85 (16), 84 (31), 83 (21), 65 (18), 55 (15).

Preparation of [^{11}C]Methylphenidate

For the preparation of [^{11}C]*dl-threo, d-threo,* or *l-threo* methylphenidate, the corresponding N-protected *dl-threo , d-threo,* or *l-threo* ritalinic acid **2** was used as the starting material and the same synthetic procedure (Scheme 2) was followed as described below.

Carbon-11 labeled methyl iodide prepared as described previously [39] was purged into a solution of N-protected ritalinic acid **2** (2 mg) dissolved in a mixture of 0.4 mL of DMF and 2.7 µL of 2 N NaOH in a reaction vessel cooled by a dry ice-acetonitrile bath. When trapping of the labeled methyl iodide was complete, the reaction mixture was heated to 80° C for three minutes. The mixture was then cooled in an ice bath, followed by sequential addition of 0.1 mL of 1 M HCl in anhydrous ether and 2.5 µL of mercaptoacetic acid in 0.1 mL of DMF. The resulting mixture was stirred at room temperature for 3 minutes and then diluted with a mixture of saturated sodium borate (1 mL), sodium hydroxide (2 N, 20 µL) and H_2O (0.5 mL). After passing the

mixture through a C18 sep-pak, the C18 sep-pak was rinsed with CH_3CN (1.4 mL). The CH_3CN rinse was injected onto a semipreparative HPLC column (Phenomenex ODS2, 10 x 250 mm). Using a solvent mixture consisting of CH_3CN:0.17 mM ammonium formate (70:30) and a flow rate of 3.0 mL/min. The desired product (retention time = 14 min) was collected and evaporated to dryness under reduced pressure. The residue was taken up in 3 mL of sterile water and the resulting solution was passed through a 0.22 μm Millipore filter into a sterile, pyrogen free vial.

Determination of Radiochemical Purity, Specific Activity, and Stereochemical Purity:

Radiochemical Purity: The radiochemical purity of the product was determined by TLC and by analytical HPLC (see conditions below for assaying Specific Activity). An authentic sample of *dl-threo*-methylphenidate obtained from Ciba Geigy Corp. was used as a chromatographic standard and either co-spotted or co-injected along with a small amount of the radioactive product and the congruence of mass and radioactivity was ascertained. For the TLC analyses, Macherey-Nagel polygram sil G/UV_{254} plastic-back TLC plates were used. A short wavelength ultraviolet lamp and NaI well counter or automatic TLC scanner (Berthold Automatic TLC Linear Analyzer) were used as UV and radioactivity detectors. The R_f value for methylphenidate was 0.58 in a solvent mixture of cyclohexane: toluene: diethylamine (75:15:10).

Specific Activity: An aliquot of the final product was assayed for specific activity by analytical HPLC (Phenomenex ODS2, 25 x 0.46 cm, CH_3CN:0.17 M ammonium formate (70:30)). A Knauer UV detector (254 nm) and Atomic sodium iodide scintillation probe fitted with survey meter model 3 (Ludlum Measurements, Sweet water,Texas) were used to detect mass and radioactivity. [^{11}C]Methylphenidate eluted at 6.7 min at the flow of 1.0 mL/min.

Stereochemical Purity: An aliquot of the final product was assayed for stereochemical purity by chiral HPLC (Daicel Chiralpak AD, 25 x 1.0 cm, hexane: isopropyl alcohol: diethylamine = 98: 2: 0.1). The retention times for *l-erythro, d-erythro, l-threo, d-threo* methylphenidate were 7.56, 7.76, 11.04, 15.12 min, respectively.

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