SYNTHESIS OF SUICIDE INHIBITORS OF MONOAMINE OXIDASE: CARBON-11 LABELED CLORGYLINE, L-DEPRENYL AND D-DEPRENYL

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SUMMARY

The suicide inhibitors of monoamine oxidase type A and B, clorgyline and L-deprenyl have been labeled with carbon-11 by [^{11}C]methylation of the norbases with [^{11}C]H_3I. The less active enantiomer of deprenyl (D-deprenyl) was also labeled using this procedure. The synthesis time was 35 minutes, the radiochemical yield was 25-40% and the specific activity was 0.8-2.0 Ci/μmol (calculated to EOB). Procedures for synthesis of the precursor norbases as well as the synthesis of unlabeled clorgyline, L-deprenyl and D-deprenyl are given.

Key Words: [^{11}C]Clorgyline, [^{11}C]-L-Deprenyl, [^{11}C]-D-Deprenyl, Monoamine Oxidase A and B, Suicide Inhibitors

INTRODUCTION

The enzyme monoamine oxidase (MAO), which catalyzes the oxidative deamination of endogenous neurotransmitter amines as well as a variety of exogenous amines, has been subdivided, on the basis of substrate and inhibitor selectivity, into two types: MAO-A and MAO-B (1,2). The substituted N-methyl propargylamines clorgyline (N-[3-(2,4-dichlorophenoxy)propyl]-N-methyl-2-propynylamine, 3) and L-deprenyl(L-N,α-dimethyl-N-2-propynyl phenethylamine, 5) have been shown to be selective irreversible inhibitors of MAO-A and MAO-B respectively (3,4). Because of the specificity of their inhibition, these inhibitors have been widely used in studies of the two forms of the enzyme and have provided the basis for the development of an extensive data base on MAO A and B. These two forms of MAO have been the subject of a large number of studies directed toward

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determining whether either might be of significance to the etiology, diagnosis or treatment of medical and psychiatric disorders (5,6).

Since the MAO inhibitors (MAOIs) clorgyline and L-deprenyl act as suicide inhibitors deactivating the enzyme by the formation of a covalent bond to its active site, it might be possible to label selectively each form of MAO *in vivo* and to determine quantitatively the patterns of distribution of MAO using PET if the MAOIs were labeled with a positron emitting nuclide. Furthermore, since L-deprenyl is a much more active inhibitor than its enantiomer, D-deprenyl (7), the use of each ^{11}C-labeled enantiomer in serial PET studies would provide a powerful mechanistic probe.

As a continuation of a program to investigate the synthesis and use of positron emitter labeled radiotracers to probe functional MAO activity *in vivo* (8-10), we report here the synthesis of N-[^{11}C]-methyl labelled clorgyline, L-deprenyl and D-deprenyl as well as the precursors required in their synthesis. Preliminary reports of the biological behavior of these compounds have been presented (11,12).

RESULTS AND DISCUSSION

Clorgyline and deprenyl have been labeled with C-14 in the methylene position of the propargyl group by Fowler (10) and in the N-methyl position of clorgyline by Williams (13). During the course of this work a similar approach to the one taken here was reported by Ishiwata *et al.* (14) who described the synthesis and biological behavior of N-[^{11}C]methyl-labelled pargyline, another irreversible inhibitor of MAO. A different method to use N-[^{11}C]-methyl amines to study MAO has been reported by Inoue and coworkers (15) who use their tracers as substrates for MAO, the products of which are metabolically trapped within the brain; a method which might be complementary to the direct labelling of the enzyme.

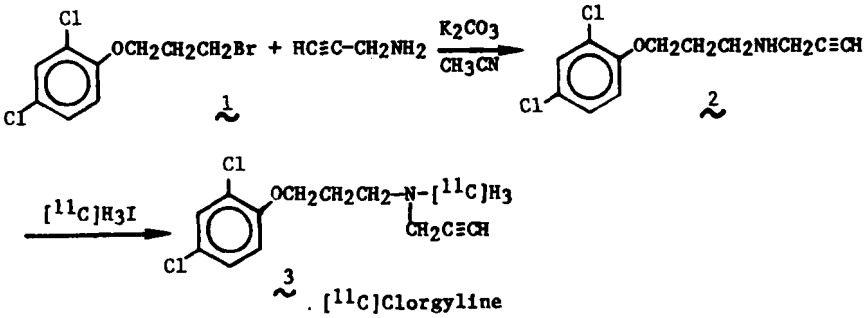
In this work clorgyline and L-deprenyl have been synthesized from the corresponding desmethyl compounds by alkylation with [^{11}C]methyl iodide, the production of which has been described elsewhere (16,17,18), using techniques that are similar to many other [^{11}C] methylations. The [^{11}C] MAOIs were obtained in good yield (25-40% EOB) in a reasonable synthesis

time (35 min) and in a state of high chemical and radiochemical purity through the use of preparative high performance liquid chromatography (HPLC). It was found that yields were improved significantly by the use of an excess of the free base of the amine, rather than the hydrochloride salt and an organic base, such as 2,2,6,6-tetramethyl piperidine, which inevitably competitively consumed much of the $[^{11}C]H_3I$. In addition the use of a solvent mixture of DMF:DMSO (4:1) in the methylation step increased the radiochemical yield.

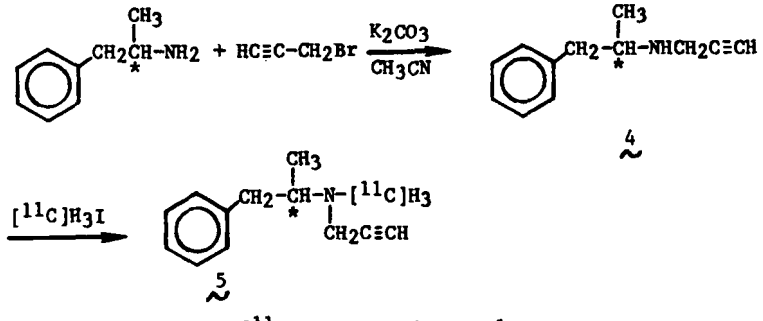
In the preparative reactions a two fold excess of amine over alkyl bromide was employed in an attempt to minimize dialkylation of the amine. In addition to the methods described below, clorgyline and deprenyl were synthesized on a preparative scale by the reactions of the nor compounds with methyl iodide.

Scheme 1. Synthesis of ^{11}C-Labeled MAOI's and Their Precursors

$[^{11}C]$Clorgyline



$[^{11}C]$L- or D-Deprenyl



MATERIALS AND METHODS

Propargyl amine, N-methyl propargyl amine, propargyl bromide (80% in toluene) and 2,4-dichlorophenol were purchased from Aldrich. L-Amphetamine was purchased from Sigma, D-amphetamine was purchased from K and K Laboratories and 1,3-dibromopropane from Eastman.

Amines were converted to their hydrochloride salts by the gradual addition of a solution of dry HCl in ether to an ethereal solution of the amine, followed by centrifugation. NMR spectra were run on a Bruker 300 MHz instrument using $CDCl_3$ as a solvent and TMS as an internal standard. Optical rotations were run on a Rudolf polarimeter.

Chromatography: Progress of the reactions was followed by high performance liquid chromatography using a C-18 reversed phase column (4.6 x 250 mm) and UV detection at 254 nm. The solvent system for the deprenyl series was (70:30) methanol: 0.05 N ammonium formate; for the clorgyline series the ratio was 80:20. Flow in each case was 1 ml/min. Preparative scale purification was achieved by conventional liquid column chromatography (12 cm x 5 cm) using silica gel 60 (230-400 mesh) (Merck). The content of the column eluent fractions was monitored by thin layer chromatography using plastic plates precoated with silica gel 60F (Merck), the plates being developed with the same solvent as had been used to eluate the column.

3-(2,4-Dichlorophenoxy)propylbromide (1,19). 2,4-Dichlorophenol (20.5 g, 0.126 mol), 1,3-dibromopropane (49.1 g, 0.243 mol) and a solution of 5 g NaOH in 20 ml H_2O were refluxed for 75 min (oil bath 135°). A solution of 4.5 g of NaOH in 32 ml H_2O was added and the mixture was heated on a steam bath for 1 1/2 hr. The layers were separated, the organic layer was extracted with water and then subjected to vacuum distillation (3-4 mm). A 19.6 g forecut was collected from room temperature to 50°. The product (20.5 g, 57%) distilled from 134-137° (lit. 140°/3 mm) (19). HPLC analysis (MeOH: .05 N NH_4HCO_2 80:20) showed the material to be 87% pure. Retention times of the impurities were 4.8 and 8.8 min while the product was 11.6 min. Extraction with water had no effect on the

composition. As the impurities were found to be inert during the subsequent reactions, the material was used without further purification.

General Procedure for the Reaction of Amines with Alkyl Bromides.

To a solution containing 11 mmole of alkyl bromide and 22 mmole of amine in 30 ml of acetonitrile was added a solution of 12 mmole K_2CO_3 in 3 ml of H_2O. The resulting clear two phase mixture was stirred at room temperature until HPLC analysis demonstrated that all of the alkyl bromide had been consumed. During this time precipitated KBr had caused the aqueous layer to thicken to a gummy solid. The acetonitrile was decanted, treated with fresh K_2CO_3, filtered and evaporated on a rotary evaporator. Absolute ethanol was added to the residue and evaporated. The residue, which consisted of a yellow to brown free flowing liquid and a fine white solid, was treated with a minimum volume of ethyl ether sufficient to dissolve the liquid and this solution was applied to the silica gel column. After a forecut was collected, 10 ml fractions of a column eluate were taken. The fractions containing the desired product, as determined by tlc, were combined and evaporated to give the alkylated amine as the free base.

N-[3-(2,4-Dichlorophenoxy)propyl]-2-propynyl amine (norclorgyline 2).

2.3 ml of 87% pure 3-(2,4-dichlorophenoxy) propylbromide (2.99 g; 0.011 mole) and 1.6 ml propargylamine (1.38 g; 0.025 mole) in 30 ml CH_3CN and 1.65 g k_2CO_3 in 3 ml H_2O were stirred at room temperature for 5 days.

HPLC retention time (min) norclorgyline 5.8.

Norclorgyline eluted from the silica column in 200-320 ml of ether.

Yield 1.5 g (30%). mp hydrochloride salt 145-147° .

NMR ($CDCl_3$) δ 6.83 - 7.36 (m, 3H, aromatic H's), 4.10 (t, 2H, J = 6, $-CH_2O-$), 3.45 (d, 2H, J = 2, $>NCH_2C{\equiv}CH$), 2.93 (t, 2H, J = 6.6, $>NCH_2CH_2-$); 2.22 (t, 1H, J = 2, $>NCH_2C{\equiv}CH$), 2.02 (p, 2H, J = 6.3, $>NCH_2CH_2CH_2-$), 1.56 (s, 1H, $>NH$). Anal. $C_{12}H_{13}Cl_2NO{\cdot}HCl$

Calculated: C 48.92, H 4.79, N 4.75

Found: C 48.98, H 4.74, N 4.62

N-[3-(2,4-Dichlorophenoxy)propyl]-N-methyl-2-propynylamine. (Clorgyline 3).

2.9 g of 87% 3-(2,4-dichlorophenoxy) propyl bromide and 2.0 ml

N-methylpropargylamine (1.64 g; 0.024 mole) were treated as above. The reaction mixture was stirred for 2 days.

Clorgyline eluted in 110-180 ml.

HPLC retention time (min): clorgyline 8.4.

Yield 1.8 g (76%). mp hydrochloride salt 97-99° (lit (21) 98.5-100°).

l-α-Methyl-N-2-propynyl phenethylamine. (Nordeprenyl 4). A mixture of l-amphetamine (2.9 g, 0.022 mol) and propargyl bromide (80% in toluene; 1.3 g, 0.011 mol) in 30 ml CH_3CN and 1.65 g K_2CO_3 in 3 ml H_2O was stirred overnight.

HPLC retention times (min): propargyl bromide 3.8, toluene 8.7, amphetamine 4.4, nordeprenyl 5.6, and a second product presumed to be the dipropargylated amphetamine 9.3.

Care was taken when evaporating solutions of nordeprenyl in light of the reported volatility of deprenyl (20).

The eluting solvent for the silica column was ether: hexane 1:1. Nordeprenyl eluted in fractions 180-290 ml. Yield 1.37 g (72%) Mp (hydrochloride salt 158-160°).

NMR ($CDCl_3$) δ 7.19 - 7.34 (m, 5H, aromatic H's), 3.37 - 3.51 (qd, 2H, $>NC\underline{H}_2C{\equiv}CH$), 3.12 - 3.20 (m, 1H, $ArCH_2C\underline{H}$), 2.61 - 2.75 (m, 2H, $ArC\underline{H}_2^-$), 2.18 (t, 1H, J = 2.4, $-C{\equiv}C\underline{H}$) 1.52 (s, 1H, >NH), 1.06 (d, 3H, J = 6Hz, $C\underline{H}_3CH<$). Anal. $C_{12}H_{15}N{\cdot}HCl$

Calculated: C 68.72, H 7.69 N 6.68

Found: C 68.57, H 7.61, N 6.70

D-Amphetamine (α_D^{25} = + 22.8; lit (7) + 25.5) and L-amphetamine (α_D^{25} = -23.6; lit (7) - 26.5) were converted to the N-formyl-1-phenyl-2-amino-propanes according to the procedure of Cavallits and Grey (22) and reduced to the corresponding N-α-dimethylphenethylamines with $LiAlH_4$ (23).

L-N,α-Dimethylphenethylamine mp 168-171° (lit (7) 171.5 - 172.5°) α_D^{25} -14.40° lit (7) - 16.33°.

D-N,α-Dimethylphenethyl amine mp 167-69° (lit (7) 171.5 - 172.5°) α_D^{25} + 17.6° (lit (7) + 15.95°.

D- and L-Deprenyl

To a solution of N,α-dimethylphenethylamine (1.9 g; 12.8 mmole) and propargyl bromide (1.52 g; 12.8 mmole) in 30 ml of acetonitrile was added a solution of 12 mmole of K_2CO_3 in 3 ml of H_2O and the mixture was stirred at room temperature for 1-1/2 hours. The organic layer was decanted and then acidified with concentrated HCl. The solvent was evaporated and the residue taken up in 1 N HCl. This solution was extracted with ether, which was discarded, then made basic with 10 N NaOH and extracted again with ether. This ether layer was extracted with 1 N HCl. The HCl was evaporated. Absolute ethanol was added to the residue and evaporated. The residue was dissolved in a minimum volume of absolute ethanol. Ethyl ether was added to this solution until crystalization occurred. Yield 1.8 g (63%).

L-Deprenyl mp 141 - 142.5° (lit (7) 140.5 - 142) α_D^{25} - 10.67 (lit (7) - 11.56).

D-Deprenyl mp 137.5 - 139 (lit (7) 140 - 141°) α_D^{25} + 11.05 (lit (7) + 9.53).

[^{11}C]-Clorgyline (3), [^{11}C]-L-Deprenyl, and [^{11}C]-D-Deprenyl (5).

[^{11}C]O_2 was obtained as previously described (26). It was converted to [^{11}C]methanol by purging through 0.2 ml of 1 M $LiAlH_4$ in tetrahydrofuran (THF). When the trapping was complete the mixture was heated and the THF was evaporated with a stream of N_2. To the residue was added 0.5 ml 58% HI. The vessel was closed and heated to 160° under reflux. After a vigorous reflux had been established the vessel was opened to a stream of N_2 which carried the [^{11}C]methyl iodide into a cooled solution of 0.3 ml of CH_3CN and 0.2 ml of a mixture of DMF:DMSO (4:1) containing the appropriate desmethyl precursor (free base) at -40°. For clorgyline 2 µl of the free base was used and for L-deprenyl and D-deprenyl 10 µl of the free base was used. The solution was heated in the closed vessel to 125° for 5 minutes. Then 0.5 ml of water was added and the solution injected onto a C-18 preparative HPLC column (Spherisorb ODS 2, 10 x 250 nm). For [^{11}C]clorgyline the solvent was MeOH: 0.05 N NH_4HCO_2, 80:20; flow 5 ml/min,

retention time 9 min. For [^{11}C]-L- and D-deprenyl the solvent was MeOH: 0.05 N NH_4HCO_2, 70:30; flow 5 ml/min retention time 11 min. The fraction containing the product was evaporated in the presence of 2 ml of 2% HCl (conc) in ethanol. To the residue was added 2 ml EtOH and this was evaporated. The residue was dissolved in 3 ml saline:H_2O (3:1) and 0.3 ml was retained for HPLC analysis and determination of specific activity. The remainder of the solution was passed through a 22 μm millipore filter into a vial containing 0.1 ml of 1 M $NaHCO_3$.

The radiochemical yields were 25-40% with a synthesis time of 35 minutes. The specific activities were about 0.8 - 2.0 Ci μmol^{-1} at EOB.

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