Synthesis and Selective Monoamine Oxidase B-Inhibiting Properties of 1-Methyl-1,2,3,6-tetrahydropyrid-4-yl Carbamate Derivatives: Potential Prodrugs of (*R*)- and (*S*)-Nordeprenyl

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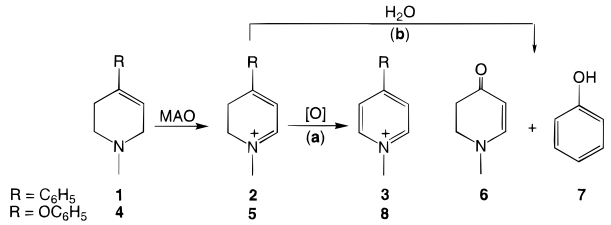
The results of previous studies have established that the monoamine oxidase-catalyzed oxidation of 1-methyl-1,2,3,6-tetrahydropyridyl derivatives bearing heteroatom substituents at C-4 generates 2,3-dihydropyridinium intermediates that undergo spontaneous hydrolysis to release the C-4 substituent and form the amino enone 1-methyl-2,3-dihydro-4-pyridone. We have attempted to adapt this metabolic pathway to the preparation of amine-containing prodrugs that may target the central nervous system which is rich in monoamine oxidase A and B. In this paper we report the synthesis and the *in vitro* and *in vivo* metabolic fate of the tetrahydropyridyl carbamate derivatives which are designed to release (*S*)- and (*R*)-nordeprenyl. These carbamates are selective monoamine oxidase A substrates. An *ex vivo* assay has shown that the *R*-enantiomer is an effective and selective inhibitor of brain mitochondrial monoamine oxidase B.

Introduction

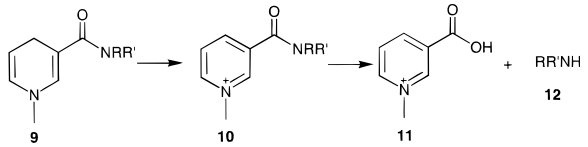
The excellent monoamine oxidase B (MAO-B) substrate properties of the parkinsonian-inducing cyclic tertiary allylamine 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine [MPTP (**1**)] have led to extensive structure−enzyme substrate activity studies on a variety of related tetrahydropyridine derivatives. The reaction pathway involves initial α-carbon oxidation to yield the dihydropyridinium species $MPDP^+$ (**2**) which, following subsequent oxidation, is converted to the neurotoxic 1-methyl-4-phenylpyridinium species MPP^+ (**3**) (Scheme 1, path a).[1,2] The 4-phenoxy analog **4** proved to be a better MAO-B substrate than MPTP but was not toxic *in vivo*, presumably because the intermediary dihydropyridinium metabolite **5** undergoes rapid hydrolysis (Scheme 1, path b) to yield the amino enone **6** and phenol (**7**) rather than oxidation to form the putative neurotoxic pyridinium species **8**.[3,4]

This behavior suggested the possibility of using the tetrahydropyridyl moiety as a "carrier" for prodrugs.[5] Since MAO-A and MAO-B, the two well-documented forms of this flavoenzyme system, are localized in specific cell types in the central nervous system,[6] this approach offered the possibility of selectivity targeting these cells with tetrahydropyridyl derivatives which would be converted, in an MAO-catalyzed reaction, to the corresponding dihydropyridinium species that would release the active drug. A similar concept has been explored extensively by Bodor and his colleagues with prodrugs constructed by attaching an amine-containing drug moiety to a 1-methyl-1,4-dihydropyridine carrier via a carbonyl group at C-3.[7] Oxidation of the dihydropyridine **9** leads to the corresponding pyridinium metabolite **10** which, because of the electropositive character at the amide carbonyl functionality, undergoes rapid hydrolysis to give the *N*-methylpyridinium analog **11** of nicotinic acid and the drug **12** (Scheme 2).

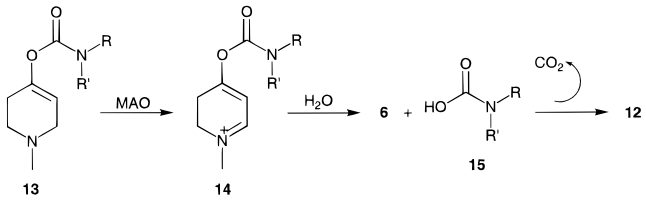
Scheme 1. MAO-B-Catalyzed Oxidation of MPTP (**1**) and 4-Phenoxy-1-methyl-1,2,3,6-tetrahydropyridine (**4**)



Scheme 2. Bioactivation of a 1,4-Dihydropyridine Prodrug



Scheme 3. Bioactivation Pathway for Tetrahydropyridyl Carbamates



In order to adapt the tetrahydropyridine carrier to construct potential amine-containing prodrugs, a carbamate linkage (**13**) was employed to circumvent the hydrolytic instability of the enamine functionality that results from direct attachment of the amino group and the tetrahydropyridyl carrier. The MAO-catalyzed oxidation of **13** would generate the dihydropyridinium intermediate **14** which, following 1,4-hydrolytic cleavage and decarboxylation of the resulting carbamic acid **15**, would release the amine drug **12** (Scheme 3). Preliminary studies established that model carbamates (**13**: R, R′ = alkyl or aryl groups) were moderate MAO-B substrates but only with small groups attached to the carbamoyl nitrogen atom.[3] Subsequent studies, how-

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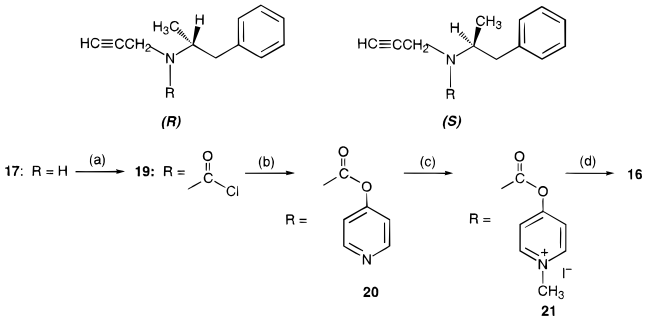
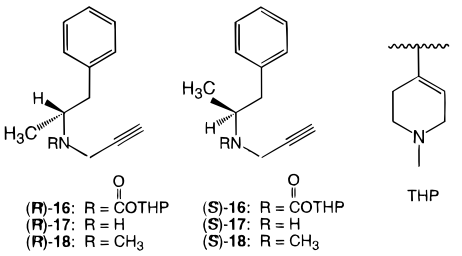
Scheme 4. Synthetic Route to the Tetrahydropyridyl Carbamates (*R*)- and (*S*)-**16**[a]



[a] (a) $(Cl_3CO)_2$ in CH_2Cl_2; (b) 4-hydroxypyridine in CH_3CN containing Reillex 402; (c) CH_3I in Et_2O; (d) $NaBH_4$ in CH_3OH.

ever, showed that larger groups could be accommodated by the A form of the enzyme.[8]

These results led to an examination of the tetrahydropyridyl carbamate derivative (*R*)-**16** of (*R*)-nordeprenyl [(*R*)-**17**]. This choice was based on the known potent and selective MAO-B inactivator properties of (*R*)-deprenyl [(*R*)-**18**], a putative neuroprotectant used in



the treatment of early stage Parkinson's disease (PD).[9] The target amine, (*R*)-nordeprenyl, also is an effective and selective inactivator of MAO-B which, in the rodent, appears to be about as potent as (*R*)-deprenyl *in vivo*.[10] Concern has been raised recently about the therapeutic advantages of (*R*)-deprenyl since its use appears to lead to increased mortality in treated PD patients.[11] The proposed prodrug approach involving the selective release of the active agent in the central nervous system might help to circumvent this outcome. In this study we also have examined the (*S*)-tetrahydropyridyl carbamate derivative (*S*)-**16** of (*S*)-nordeprenyl [(*S*)-**17**] in order to provide some insight into the stereochemical features of the interactions of these types of compounds with MAO-A and MAO-B.

Results and Discussion

Chemistry. The syntheses of (*R*)-nordeprenyl [(*R*)-**17**] and (*S*)-nordeprenyl [(*S*)-**17**] were readily accomplished using established procedures by *N*-propargylation of (*R*)- and (*S*)-amphetamine.[12] Treatment of these secondary amines with triphosgene gave the corresponding chloroformates (*R*)- and (*S*)-**19** which were converted to the pyridyl carbamates (*R*)- and (*S*)-**20** (Scheme 4) by reaction with 4-hydroxypyridine. The HCl liberated in these reactions was neutralized effectively by the polyvinylpyridine acid sponge Reillex 402. Subsequent treatment of (*R*)- and (*S*)-**20** with iodomethane gave the pyridinium methiodides (*R*)- and (*S*)-**21** which were converted to the desired tetrahydropyridyl carbamates (*R*)- and (*S*)-**16** with $NaBH_4$ reduction.

Enzymology. Initial studies focused on characterizing the *in vitro* MAO-A- and MAO-B-inactivating properties of the (*R*)- and (*S*)-propargylamines (*R*)- and (*S*)-**17**. The time and inhibitor concentration dependent loss of MAO-A activity was examined with 1-methyl-4-phenoxy-1,2,3,6-tetrahydropyridine (**4**) as substrate. MPTP (**1**) served as substrate for the MAO-B inactivation studies. (*R*)-Nordeprenyl displayed excellent MAO-B inactivation properties, but estimates of k_{inact} and K_I could not be obtained since the rates of inactivation even at low inhibitor concentrations (10 μM) were too fast to measure. These results are in agreement with other studies which have shown that (*R*)-nordeprenyl is a potent MAO-B inhibitor.[10] (*S*)-Nordeprenyl [(*S*)-**17**] also inactivated MAO-B in a time dependent process but was considerably less potent than the *R*-enantiomer. Although linear plots of the natural log of the percent remaining enzyme activity vs time were obtained at inhibitor concentrations of 10−50 μM, the plot of $1/k_{obs}$ vs 1/[inhibitor] did not yield Michaelis−Menton values, possibly because the rates that could be estimated were below K_I. In contrast to the MAO-B-inhibiting properties of these nordeprenyl isomers, no evidence of MAO-A inhibition was observed with either enantiomer even at high (1 mM) concentrations of (*S*)- and (*R*)-**17**.

The MAO-A and MAO-B substrate properties of the (*R*)- and (*S*)-carbamates at 1 mM were examined spectrophotometrically by recording repeated scans (500−250 nm) over a 120 min incubation period. No new chromophore was detected during the incubations with MAO-B, behavior which is consistent with previous results showing the lack of MAO-B substrate properties of related tetrahydropyridyl carbamates.[3] On the other hand, the spectral analysis of the MAO-A incubation mixtures documented the time dependent appearance of a chromophore corresponding to the amino enone **6** indicating that both (*R*)- and (*S*)-**16** are substrates for this form of the enzyme. Double-reciprocal plots of 1/initial rates of oxidation vs 1/[substrate] provided estimates of k_{cat} (88 and 36 min^{-1}) and K_M (0.3 mM in both cases) for the *R*- and *S*-enantiomers, respectively. The corresponding k_{cat}/K_M ratios [295 min^{-1} mM^{-1} for (*R*)-**16** and 120 min^{-1} mM^{-1} for (*S*)-**16**] established that these compounds are moderate MAO-A substrates.

***In Vivo* Studies.** Since (*R*)-nordeprenyl displayed potent MAO-B inhibition properties, we speculated that the MAO-A-catalyzed bioactivation of (*R*)-**16** would lead to the inhibition of MAO-B *in vivo*. Furthermore, since MAO-A is not inactivated by (*R*)-nordeprenyl, the inhibition should be selective for the B form of the enzyme. The poorer MAO-B inhibitor properties of (*S*)-nordeprenyl and the poorer substrate properties of (*S*)-**16** suggested that the (*S*)-carbamate would be a less effective inhibitor of MAO-B than the (*R*)-carbamate.

Characterization of the *in vivo* enzyme inhibitor properties of (*R*)- and (*S*)-**16** required an assay that would provide estimates of changes in brain MAO-A and MAO-B activities in drug-treated animals relative to control animals. Several *ex vivo* assays for brain MAO activity have been reported in the literature,[13−15] and the subject has been reviewed.[16] We elected to develop a spectrophotometric assay that exploited the excellent substrate properties (k_{cat}/K_M = 1625 min^{-1} mM^{-1} for MAO-A and k_{cat}/K_M = 1800 min^{-1} mM^{-1} for MAO-B) of 1-methyl-4-(1-methyl-2-pyrryl)-1,2,3,6-tetrahydropyri-

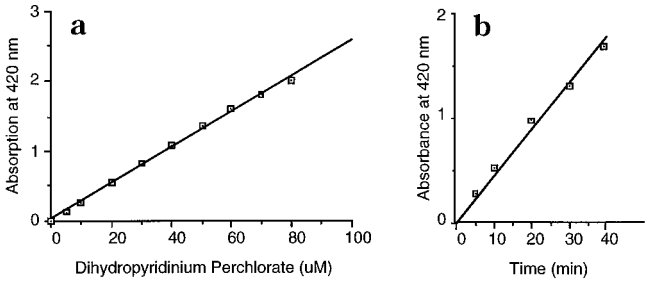
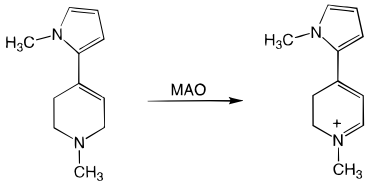


Figure 1. (a) Linear relationship between the absorbance at 420 nm and the concentration of synthetic 1-methyl-4-(1-methyl-2-pyrryl)-2,3-dihydropyridinium perchlorate (**23**·HC-lO$_4$). (b) Linear production of 1-methyl-4-(1-methyl-2-pyrryl)-2,3-dihydropyridinium metabolite (**23**) following a 30 min preincubation at 37 °C in the presence of mouse brain mitochondrial membranes.

dine (**22**). The dihydropyridinium metabolite **23** formed in this reaction is stable, has a high ϵ value (25 000 M^{-1}),[17] and absorbs maximally at 420 nm, a wavelength far removed from biological background absorbances.



22 **23**

Mitochondria were isolated by differential centrifugation from brain homogenates and lysed in chilled water.[18] The lysed mitochondrial membrane fragments were suspended in 10 mM phosphate buffer, pH 7.4, and aliquots of this suspension were used to estimate MAO activities. Studies also were carried out in which mitochondrial membranes were subjected to a sucrose gradient purification step. Since this step did not improve the precision of the assay, it was not included.

Preincubation of the homogenate at 37 °C for 30 min with either 3×10^{-7} M (*R*)-deprenyl or 3×10^{-8} M clorgyline followed by incubation for 30 min with **22** provided estimates of the contribution to substrate oxidation from both MAO-A and MAO-B, respectively. Total MAO activity was estimated by preincubating the membranes in phosphate buffer only. Remaining 'residual enzyme activity', which proved to be from 6% to 13% of the total activity as observed by others,[19] was measured following preincubation with both 3×10^{-7} M (*R*)-deprenyl and 3×10^{-8} M clorgyline. Since these membrane suspensions were turbid, it was necessary to treat the postincubation mixtures with 5% acetic acid in acetonitrile and to read the optical density following centrifugation. The linearity between the concentration of **23** and the optical density at 420 nm was verified by constructing a standard curve with synthetic **23** (Figure 1a). The linearity of the rate of metabolite formation during the 30 min incubation period also was verified by assaying the concentration of **23** vs time over a 40 min incubation period (Figure 1b). This behavior of these membrane preparations gave us confidence that we could estimate rates of enzyme catalysis by measuring the concentration of the dihydropyridinium metabolite **23** at 30 min.

Preliminary toxicity studies suggested that an ip dose of 25 μmol/kg/day $\times$ 3 days of (*S*)-**16** would be well tolerated. A single 11 μmol/kg ip dose of (*R*)-deprenyl, which has been shown to cause 90% depletion of brain MAO-B activity in the rat,[19] was used as a positive control; untreated animals provided base-line MAO-A and MAO-B activities. The animals were sacrificed on day 4 of the experiment, that is, 24 h after receiving the third and final dose of the (*S*)-carbamate and the single dose of (*R*)-deprenyl. The results are summarized in Table 1. The values for the control mice show that the majority of brain MAO activity is due to MAO-B and only about 15% to MAO-A. These values compare favorably with those reported in the literature.[19] The corresponding measurements with the (*R*)-deprenyl-treated positive control animals show a reduction of total MAO activity to almost 20% of the control level. Furthermore, essentially all of the lost activity is due to inhibition of MAO-B. The enzyme activities in the (*S*)-carbamate-treated animals, however, are essentially identical with those observed in the untreated control animals. Consequently, at the dose employed in this experiment, the (*S*)-carbamate does not inhibit brain MAO.

The results of the *in vivo* experiments with the (*R*)-carbamate (*R*)-**16** are summarized in Table 2. Preliminary toxicity studies showed that a dose of 124 μmol/kg/day $\times$ 3 days was well tolerated. In this series of experiments a second positive control [120 μmol of (*R*)-nordeprenyl/kg/day $\times$ 3 days] was included to provide a direct comparison with the active drug that should be released following bioactivation of the (*R*)-carbamate. The control mice received sterile saline only. The (*R*)-deprenyl-treated mice were administered only sterile saline on days 1 and 2 followed by 11 μmol of drug/kg on day 3. All mice were sacrificed on day 4, the brains were excised, and the mitochondrial membranes were prepared and assayed for MAO activity.

The values for the total MAO activity as well as for the MAO-A, MAO-B, and residual activity for the control animals were essentially the same as the corresponding values for the control animals observed in the first experiment (Table 1) as were the values for the (*R*)-deprenyl-treated animals. Under these dosing conditions, (*R*)-nordeprenyl was as effective as (*R*)-deprenyl in inhibiting brain MAO-B activity as measured by the reduction of the ratio of MAO-B/MAO-A from 3.06 to 0.66 [(*R*)-deprenyl treatment] and 0.71 [(*R*)-nordeprenyl treatment]. Again, no effect was observed on MAO-A activity. Treatment with (*R*)-**16** also led to the selective loss of brain MAO-B activity (reduction to 23% of control level) with complete retention of brain MAO-A activity (1.10 vs 1.13 nmol of product/min/mg of protein). Thus, with these doses, the (*R*)-carbamate is as active and selective an inhibitor of brain MAO-B as is (*R*)-deprenyl. More detailed studies will be required to determine the relative activities of these compounds since it appears that the extent of inhibition achieved with all three compounds in these studies may be near maximal. Furthermore, the apparent lack of inhibitory activity of the (*S*)-carbamate may be attributed to the lower dose used with this enantiomer.

The possibility that the prodrug properties observed with the (*R*)-carbamate may lead to the selective inhibition of brain MAO-B activity with a sparing of peripheral MAO-B activity is currently under investigation.

Table 1. MAO Activity[a] of Brain Mitochondrial Membranes Obtained from Mice following the Indicated Treatments

treatment ($n = 3$)	total MAO activity	MAO-A activity	MAO-B activity	residual activity	MAO-B/A activity
control[b]	3.76 ± 1.19	0.58 ± 0.55	3.45 ± 0.89	0.21 ± 0.08	5.95
(*R*)-deprenyl[c]	0.84 ± 0.61	0.64 ± 0.57	0.46 ± 0.06	0.24 ± 0.06	0.72
carbamate (*S*)-**16**[d]	3.75 ± 0.25	0.76 ± 0.39	3.35 ± 0.16	0.20 ± 0.10	4.40

[a] Units: nmol of 1-methyl-4-(1-methylpyrrol-2-yl)-2,3-dihydropyridinium metabolite (**23**) formed/min/mg of protein. [b] Untreated. [c] 11 μmol/kg on day 3 only. [d] 25 μmol/kg/day × 3 days.

Table 2. MAO Activity[a] of Brain Mitochondrial Membranes Obtained from Mice following the Indicated Treatments

treatment ($n = 6$)	total MAO activity	MAO-A activity	MAO-B activity	residual activity	MAO-B/A activity
saline[b]	3.55 ± 0.54	1.13 ± 0.14	3.46 ± 0.87	0.46 ± 0.09	3.06
(*R*)-deprenyl[c]	1.36 ± 0.11	1.06 ± 0.11	0.70 ± 0.23	0.44 ± 0.11	0.66
(*R*)-nordeprenyl[d]	1.31 ± 0.15	1.07 ± 0.09	0.68 ± 0.24	0.46 ± 0.10	0.71
carbamate (*R*)-**16**[e]	1.36 ± 0.08	1.10 ± 0.11	0.78 ± 0.15	0.45 ± 0.11	0.63

[a] Units: nmol of 1-methyl-4-(1-methylpyrrol-2-yl)-2,3-dihydropyridinium metabolite (**23**) formed/min/mg of protein. [b] 0.1 mL of saline once daily × 3 days. [c] 0.1 mL of saline on days 1 and 2 followed by 11 μmol of drug/kg on day 3. [d] 120 μmol/kg/day × 3 days. [e] 124 μmol/kg/day × 3 days.

Experimental Section

Chemistry. (*R*)-Deprenyl and clorgyline were obtained from Research Biochemicals Inc., Natic, MA. All other chemicals were reagent or HPLC grade. Unless otherwise noted, reactions were run under N_2. Proton NMR spectra were recorded on a Bruker WP200 or 270 spectrometer. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS), and spin multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). Gas chromatography−electron ionization mass spectrometry (GC−EIMS) was performed on a Hewlett-Packard (HP) model 5890 gas chromatograph equipped with an HP-1 fused silica gel capillary column (12 m × 0.2 mm, 0.33 μm film thickness) connected to an HP 5870 mass selective detector. Data were acquired on an HP 5970 ChemStation. Helium was employed as the carrier gas (40 mL/min), and oven parameters were 100 °C for 1 min followed by 25 °C/min to 275 °C. Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses, performed by Atlantic Microlabs of Norcross, GA, were within 0.4% of the theoretical values calculated for C, H, and N.

(*R*)-*N*-(1-Methyl-2-phenylethyl)-*N*-propargylcarbamoyl Chloride [(*R*)-19]. To a solution of (*R*)-nordeprenyl [(*R*)-**17**; 1.90 g, 11.0 mmol][12] and triethylamine (1.67 g, 16.5 mmol) in 40 mL of CH_2Cl_2 was added dropwise triphosgene (1.41 g, 4.75 mmol) in 20 mL of CH_2Cl_2 at 0 °C with stirring. After stirring at 0 °C for 2.5 h, the reaction mixture was washed sequentially with cold aqueous 10% HCl, cold aqueous $NaHCO_3$, and cold saturated salt solution. The crude product obtained after drying ($MgSO_4$) and removing solvent was filtered through a column filled with 30 g of Flurosil with 250 mL of CH_2Cl_2 to give 2.45 g (95%) of the carbamoyl chloride (*R*)-**19** as an oil: ^{1}H NMR ($CDCl_3$) δ 7.26 (m, 5H, ArH), 4.50 (2q, 1H, N-C*H*), 4.04 (m, 2H, CH_2 of benzyl), 3.95 (2m, 2H, NH-CH_2), 2.30 (t, 1H, alkyne), 1.56 (bs, 1H, NH_2), 1.35 (d, 3H, CH_3). The (*S*)-enantiomer [(*S*)-**19**] was prepared in the same way.

4-Pyridyl (*R*)-*N*-(1-Methyl-2-phenylethyl)-*N*-propargylcarbamate [(*R*)-20]. The above crude carbamoyl chloride (2.42 g, 10.27 mmol) in 25 mL of acetonitrile was added dropwise to a mixture of 4-hydroxypyridine (0.92 g, 9.76 mmol) and Reillex 402 (9.2 g, 8.1 mequiv) in 50 mL of refluxing acetonitrile with vigorous stirring. After an additional 6.5 h, the mixture was filtered, the solvent was removed under reduced pressure, and the resulting oil was chromatographed (silica gel, 40 g, eluent: ethyl acetate) to yield (*R*)-**20** (2.01 g, 70.0%): GC−EIMS *m*/*z* 294 ($M^{\bullet+}$, 1), 203 (100), 91 (65), 78 (26), t_R = 7.52 min; ^{1}H NMR ($CDCl_3$) δ 8.55 (dd, 2H, C2, C6), 7.21−7.29 (m, 5H, Ph-H), 6.90 and 7.08 (dd, 2H, C3, C5), 4.43 (m, 1H, N-C*H*), 4.00 (m, 2H, Ph-CH_2), 2.93 (m, 2H, N-CH_2), 2.04 (s, 1H, propargyl-H), 1.35 and 1.44 (dd, 3H, CH_3); HRCIMS calcd for $(C_{18}H_{19}N_2O_2)H^+$ 295.144 653, found 295.144 684. The (*S*)-enantiomer [(*S*)-**20**] was prepared in the same way.

(*R*)-1-Methyl-4-[[*N*-(1-methyl-2-phenylethyl)-*N*-propargylcarbamoyl]oxy]pyridinium Iodide [(*R*)-21]. Iodomethane (2.70 g, 1.90 mmol) was added dropwise to a solution of (*R*)-**20** (1.40 g, 4.75 mmol) in 50 mL of dry Et_2O. The reaction mixture was heated under reflux for 3 days, and then the supernatant was decanted off to yield the pyridinium methiodide (*R*)-**21** (1.59 g, 76.8%) as a light yellow solid which was too hygroscopic to obtain a melting point: UV (100 mM phosphate buffer, pH 7.4) λ_{max} 227 nm; ^{1}H NMR (DMSO-d_6) δ 8.89 and 8.94 (dd, 2H, C2, C6), 7.58 and 7.79 (dd, 2H, C3, C5), 7.23−7.29 (m, 5H, Ph-H), 4.25 (d, 3H, N^+-CH_3), 3.28−3.37 (m, 3H, Ph-CH_2, N-CH), 2.91 (m, 2H, N-CH_2), 2.49, (s, 1H, propargyl-H), 1.28 and 1.36 (dd, 3H, CH_3); HRCIMS calcd for $(C_{19}H_{23}N_2O_2I - CH_3I)H^+$ 295.144 653, found 295.145 599. The (*S*)-enantiomer [(*S*)-**21**] was prepared in the same way.

Oxalate Salt of 4-(1,2,3,6-Tetrahydropyridyl) (*R*)-*N*-(1-Methyl-2-phenylethyl)-*N*-propargylcarbamate [(*R*)-16]. The above pyridinium methiodide (1.59 g, 3.65 mmol) in 45 mL of dry MeOH was treated portionwise with stirring at 0 °C with $NaBH_4$ (0.21 g, 5.47 mmol). The reaction mixture was stirred for an additional 25 min at 0 °C, the solvent was removed, and the residue was treated with 15 mL of cold H_2O and extracted twice with ethyl acetate to obtain 1.4 g of crude (*R*)-**16**: GC−EIMS *m*/*z* 312 ($M^{\bullet+}$, 2), 119 (46), 112 (27), 91 (100), t_R = 7.65 min. The corresponding oxalate salt, prepared in Et_2O, was recrystallized from acetonitrile/ether to yield 1.14 g (77.6%) of a hygroscopic solid: mp 114−115 °C; ^{1}H NMR (DMSO-d_6) δ 7.18−7.30 (m, 5H, Ph-H), 5.16 and 5.33 (unresolved, 1H, C5), 4.24 (m, 1H, N-CH), 4.00 and 4.07 (d, 2H, Ph-CH_2), 3.61 (unresolved, 2H, C6), 3.18 (unresolved, 2H, C2), 2.81 (m, 2H, N-CH_2), 2.70 (s, 3H, N^+-CH_3), 2.33 (unresolved, 2H, C3), 2.15 (d, 1H, propargyl-H), 1.19 and 1.25 (dd, 3H, CH_3). Anal. ($C_{21}H_{26}N_2O_6$) C, H, N. The (*S*)-enantiomer [(*S*)-**16**] was prepared in the same way: mp 112−113 °C. Anal. ($C_{21}H_{26}N_2O_6$) C, H, N.

Enzymology. MAO-A and MAO-B were prepared from human placenta and bovine liver, respectively, according to the method of Salach[20] with the following variations. The phospholipase A was obtained commercially (Sigma, St. Louis, MO) rather than from the crude venom. The MAO-A preparation was not subjected to the Sephadex purification step and the MAO-B preparation to the gradient purification step. The activity of the MAO-B isozyme was determined by measuring the initial rate of formation of $MPDP^+$ (**2**; λ_{max} = 343 nm, ε = 16 000 M^{-1}) from 5 mM MPTP (**1**) at 30 °C and was based on the reported k_{cat} (204 min^{-1}) for this reaction.[8] The final enzyme concentration was 9 nmol/mL, and the preparation was stable when stored at −15 °C over the period of this study. The specific MAO-A activity was estimated at 37 °C by measuring the initial rate of oxidation of 1 mM 1-methyl-4-phenoxy-1,2,3,6-tetrahydropyridine (**4**) to its dihydropyridinium metabolite **5** (k_{cat} = 130 min^{-1})[4] by monitoring the amino enone hydrolysis product **6** (324 nm, ε = 15 300 M^{-1}).[21] This MAO-A enzyme preparation was not stable; therefore the

activity was monitored on a bimonthly basis, and the appropriate estimated activities (17−8 nmol/mL) were used in the calculations. Due to the viscosity of the MAO A preparation, it was diluted with 3 vol of phosphate buffer just prior to analysis.

Enzyme studies on the tetrahydropyridyl carbamates (*R*)- and (*S*)-**16** and (*R*)- and (*S*)-nordeprenyl were carried out at 37 °C. The MAO-B inactivation properties of the nordeprenyl enantiomers were examined as follows: Aliquots (50 μL) of stock solutions in 100 mM phosphate buffer, pH 7.4, were mixed with 50 μL of the 9.0 μM MAO-B preparation to yield inhibitor concentrations ranging from 10 to 100 μM. The resulting mixtures were incubated with gentle agitation in a water bath. At specific time points 10 μL aliquots of each incubation mixture were added to a 1 mL quartz cuvette containing 490 μL of 5 mM MPTP (pre-equilibrated to 37 °C in 100 mM sodium phosphate buffer, pH 7.4). The initial rates of MPTP oxidation were determined by monitoring the absorbance of $MPDP^+$ for 120 s.[21] The MAO-A inactivation properties of the nordeprenyl enantiomers were examined in a similar manner except that the rates of oxidation of the 1-methyl-4-phenoxy-1,2,3,6-tetrahydropyridine (**4**) were used to monitor remaining enzyme activity.

To evaluate the MAO-A and MAO-B substrate properties of the (*S*)- and (*R*)-tetrahydropyridyl carbamates, incubations were carried out in 500 μL of MAO-A (0.17−0.08 μM) or MAO-B (0.09 μM) and a range of substrate concentrations (0.25−2.0 mM in 100 mM sodium phosphate buffer, pH 7.4) in a 1 mL quartz cuvette. The initial rates of oxidation of these compounds were estimated by monitoring the absorbance of the amino enone **6** at 324 nm because the intermediate carbamoyldihydropyridinium species were too unstable to be detected. No evidence of pyridinium formation ($\lambda_{max} = 227$ nm) was observed. The k_{cat} and K_M values were calculated from plots of 1/initial velocity vs 1/[substrate].

Preparation of Mouse Brain Mitochondrial Membranes. ICR white mice (control animals or animals treated as described below) were sacrificed by cervical dislocation and decapitated, and the whole brains were removed. Each brain was homogenized in 10 vol of a solution 250 mM in sucrose and 10 mM in phosphate buffer, pH 7.4. Cellular debris was pelleted by centrifugation for 10 min at 600*g*. The mitochondria were pelleted from the decanted supernatant at 6500*g* for 20 min. This pellet was resuspended in 5 mL of sucrose buffer and again centrifuged at 6500*g* for 20 min. After removal of the supernatant fraction, the mitochondria were lysed in 5 mL of ice-cold water using a glass and Teflon homogenizer. This homogenate was centrifuged at 105000*g* for 30 min, and the supernatant was removed. The resulting pellet was resuspended in 5 mL of ice-cold water, homogenized, and centrifuged at 105000*g* for 30 min. The supernatant was again removed, and the remaining pellet was homogenized in 1 mL of 10 mM phosphate buffer, pH 7.4, to yield a preparation with a protein concentration of approximately 2 mg/mL. Protein concentrations were determined on the lysed mitochondrial homogenate by the Coomassie brilliant blue dye binding method of Bradford.[22] All points in the standard curve were determined in triplicate, and sample assays were performed in duplicate.

Determination of Mitochondrial MAO Activity. Aliquots of the above preparation (150 μL) containing either no inhibitor (control), 3×10^{-8} M clorgyline, 3×10^{-7} M deprenyl, or clorgyline plus deprenyl were preincubated for 30 min following which 100 μL of a 5 mM solution of 4-(1-methyl-2-pyrryl)-1-methyl-1,2,3,6-tetrahydropyridinium hydrochloride (**22**)[17] in phosphate buffer was added. The incubation was continued for an additional 30 min, and then the reaction was quenched by the addition of 250 μL of 5% acetic acid in acetonitrile. The denatured protein was removed by centrifugation at 16000*g* for 10 min. The absorption of the supernatant fraction containing the dihydropyridinium metabolite **23**[17] was read at 420 nm with a Beckman DU-50 spectrophotometer against a blank consisting of homogenate in phosphate buffer denatured with 5% acetic acid in acetonitrile (1/1, v/v). Assays were performed in duplicate.

Animal Studies. ICR male mice (25−35 g; Harlan, Dublin, VA) were housed 1−6/cage in the Laboratory Animal Resource Facility at 21−23 °C with free access to standard laboratory chow and tap water on a 12 h day/night cycle. All compounds were dissolved in sterile saline, and injections were administered intraperitoneally in a volume of 100 μL.

(*S*)-Tetrahydropyridyl Carbamate Studies. Three protocols (each with $n = 3$) were employed as follows: (1) untreated negative control, (2) (*R*)-deprenyl-treated positive control [saline on days 1 and 2 and 11 μmol of (*R*)-**18**/kg on day 3], and (3) drug-treated [25 μmol of (*S*)-**16**/kg/day for 3 days]. All mice were sacrificed on day 4, 24 h after the last injection.

(*R*)-Tetrahydropyridyl Carbamate Studies. Four protocols (each with $n = 6$) were employed as follows: (1) untreated negative control, (2) (*R*)-deprenyl-treated positive control [saline on days 1 and 2 and 11 μmol of (*R*)-**18**/kg on day 3], (3) (*R*)-nordeprenyl-treated [120 μmol of (*R*)-**17**/kg/day for 3 days], and (4) drug-treated [124 μmol of (*R*)-**16**/kg/day for 3 days]. All mice were sacrificed on day 4, 24 h after the last injection.

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