Synthesis of Novel MPTP Analogs as Potential Monoamine Oxidase B (MAO-B) Inhibitors

Amit S. Kalgutkar and Neal Castagnoli, Jr.*

Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

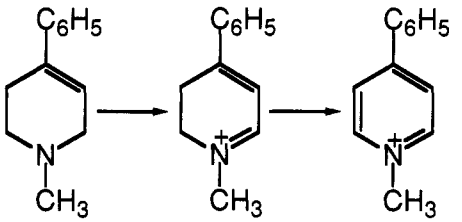
Received May 29, 1992

The nigrostriatal toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is an excellent substrate and a weak inactivator of the flavoenzyme monoamine oxidase B (MAO-B). In an attempt to develop novel mechanism-based inactivators of MAO-B, we have synthesized analogs of MPTP bearing a variety of functional groups at either the N or the C(4) position and have examined their interactions with a purified MAO-B preparation isolated from beef liver. The substituents selected include allyl, propargyl, ethenyl, ethynyl, and cyclobutyl, that is, functionalities which were considered potential sources of enzyme generated electrophilic or radical intermediates that might alkylate and inactivate the enzyme. None of the C(4)-substituted compounds displayed significant enzyme inhibitor properties although some proved to be good substrates. In the N-substituted MPTP series only the 4-phenyl-1-propargyl analog was a good inhibitor. The time- and concentration-dependent inhibition of MAO-B displayed by this compound is consistent with a mechanism-based inactivation pathway and the catalytic mechanism currently held for monoamine oxidases. The results of these studies provide additional insights into the steric features of the active site of MAO-B and predict that the area in which the C(4) substituent of the tetrahydropyridine ring resides lacks a reactive nucleophilic group.

Introduction

The 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine derivative MPTP (1, Scheme I) is a potent and selective neurotoxic agent which destroys the dopaminergic neurons of the substantia nigra in primates and produces a syndrome similar to that of idiopathic Parkinson's disease.[1-3] A variety of studies has established that the monoamine oxidase B (MAO-B) catalyzed oxidation of MPTP leading to the dihydropyridinium intermediate $MPDP^+$ (2) and subsequently to the pyridinium metabolite MPP^+ (3) is an obligatory process linked to the degeneration of the nigrostriatal neurons.[4-7] The excellent substrate properties of MPTP for MAO-B and, to a lesser extent, MAO-A[8] were unexpected since all previously reported substrates for these flavoenzymes were open-chain aliphatic amines. The observation that MAO-B inhibitors protect experimental animals against the neurodegenerative effects of MPTP by inhibiting its oxidation to MPP^+ [9] has led to clinical trials designed to evaluate their neuroprotective potential. Preliminary results of these studies have been encouraging.[10] Results from animal experiments also indicate that inhibition of MAO-B may lead to other beneficial effects such as improved cognition.[11] In view of the therapeutic potential of MAO-B inhibitors, we have initiated experiments designed to exploit the unique interactions of tetrahydropyridines with MAO through the development of nontoxic derivatives that will protect against the toxicity of MPTP and, perhaps, related endogenous[12] or exogenous[13] agents which may contribute to the aging and early demise of dopaminergic neurons.

Scheme I. MAO-B Catalyzed Oxidation of MPTP (1)



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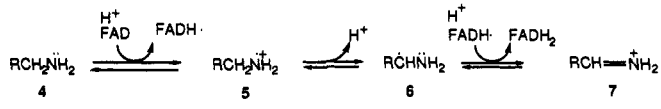
Scheme II. Postulated Pathway for MAO-B Catalysis



Numerous amines have been reported to function as mechanism-based inactivators of MAO-B.[14-18] The proposed inactivation pathways are based on the currently accepted catalytic mechanism for this enzyme (Scheme II), namely, one-electron transfer from the nitrogen lone pair electrons of the substrate **4** followed by proton loss from the α-carbon atom of the resulting aminium radical **5** to form a carbon-centered radical **6** and a second one-electron transfer to yield the iminium species **7**. The inactivation of MAO-B by open-chain aliphatic amines is thought to be mediated by enzyme-generated electrophilic iminium intermediates related to **7** which form a covalent linkage with the flavin cofactor.[19,20] *N*-Cyclopropylamine and *N*-cyclobutylamine derivatives appear to inactivate this enzyme via ring-opened carbon-centered radicals, derived from aminium radical species related to **5**, which alkylate an active-site sulfhydryl group.[21-23] MPTP also is a time- and concentration-dependent inhibitor of MAO-B (k_{inact} = 0.034 min^{-1} at 5 mM).[24] The inactivation pathway, however, is not well understood. Deuterium isotope effect measurements argue for the involvement of species derived from MPDP$^+$ [25] while results obtained with model dihydroyridinium compounds argue for the direct involvement of MPDP$^+$ itself.[26]

Our initial studies on the design of tetrahydropyridine derivatives with potential MAO inactivating properties focused on the *N*- and *C*-4-cyclopropyl analogs **8** and **9**, respectively.[27] Although compound **9** proved to be an excellent substrate for MAO-B, it displayed very weak inactivating properties, possibly because of the unexpected stability of the dihydropyridium intermediate **10**, which may preclude formation of a reactive radical intermediate. In contrast, the *N*-cyclopropyl derivative was a very poor substrate but a good concentration- and time-dependent inhibitor of MAO-B (k_{inact} = 0.7 min^{-1}; K_I = 180 μM). These results are consistent with the enzyme-mediated formation of the ring-opened carbon-centered radical **11**, which could react covalently with an active-site functionality essential for the catalytic process as suggested for other cyclopropylamine inactivators.[21,22]

In the present study we have examined the MAO-B substrate and inhibitor properties of a variety of MPTP analogs, the majority of which bear a functional group with the potential to react with a nucleophilic center at the active site following initial processing by the enzyme. Results of published structure–activity studies[8,27-33] have led us to limit the scope of this investigation to 1-substituted-4-phenyl-1,2,3,6-tetrahydropyridine and 1-methyl-4-substituted-1,2,3,6-tetrahydropyridine derivatives. The 1-substituted-4-phenyl series includes the *N*-allyl (**12**) and *N*-propargyl (**13**) analogs (see Scheme IV), which were considered potential sources for the electrophilic eniminium and yniminium intermediates, **14** and **15**, respectively (see structures **11**, **14**, **15**, and **18**). The *N*-propyl derivative **16** was prepared in an attempt to estimate the rate of the MAO-B catalyzed oxidation of a tetrahydropyridine derivative with a three-carbon chain attached to the nitrogen atom that would not lead to an analogous electrophilic intermediate. The *N*-cyclobutyl analog **17** (see Scheme IV) was prepared as an extention of our previous studies with the expectation that its MAO-B catalyzed bioactivation would lead to the ring-opened carbon-centered radical **18**.[23] The *N*-cyclopentyl derivative **19** was prepared as a strain-free cycloalkylamine analog which should not form the corresponding carbon-centered radical. The 4-ethenyl- and 4-ethynyl-1-methyl-1,2,3,6-tetrahydropyridine derivatives **20–23** were selected as potential substrates that would generate electrophilic metabolic intermediates with bioalkylating potential positioned at C(4) of the tetrahydropyridine ring. The inactivation pathway was envisioned to proceed via the corresponding dihydropyridinium intermediates. The ethynyl analog **21** was considered particularly attractive since the corresponding dihydropyridinium intermediate

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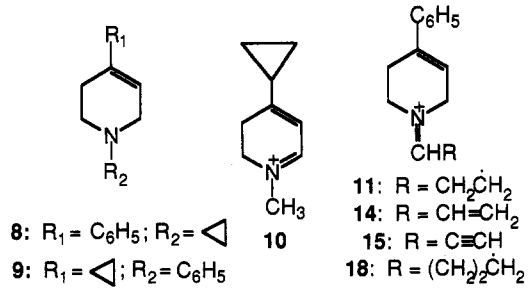


53 could lead to resonance stabilized protein adducts such as 26 via rearrangement of the aminoallene species 25 (Scheme III).

Results and Discussion

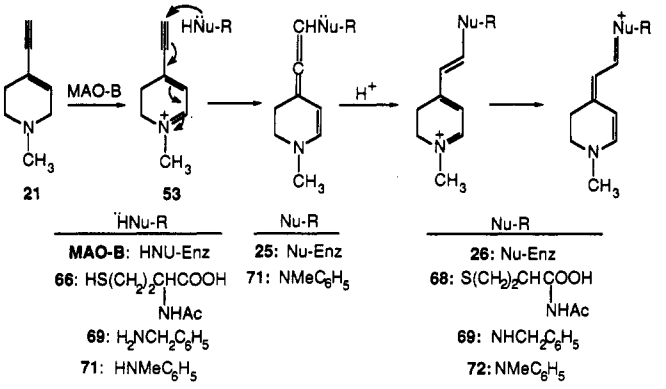
Chemistry. With the exception of the *N*-cyclobutyl analog 17, all of the 1-substituted-4-phenyltetrahydropyridine derivatives were synthesized from 4-phenylpyridine (27). N-Alkylation of 27 with allyl iodide, propargyl bromide, propyl iodide, and cyclopentyl bromide yielded the pyridinium intermediates 28, 29, 30, and 31 as the corresponding halide salts (Scheme IV). Compound 31 was not obtained in pure form since it formed a stable ternary complex consisting of 2 mol of 31 and 1 mol of 27. Treatment of this complex and as well as the other pyridinium intermediates 28–30 with sodium borohydride yielded the desired tetrahydropyridines 19, 12, 13, and 16 respectively. As part of our enzyme kinetic studies, we also required the 4-phenyl-1-propyl-2,3-dihydropyridinium species 33, which was prepared from the tetrahydropyridine derivative 16 by treatment of the corresponding *N*-oxide 32 with trifluoroacetic acid anhydride (see Scheme V).[34]

Attempted alkylation of 27 with cyclobutyl bromide failed to yield the corresponding *N*-cyclobutylpyridinium species 34, and therefore the synthesis of the *N*-cyclobutyltetrahydropyridine analog 17 was approached via a condensation reaction involving cyclobutylamine, 2-phenylpropene (35), and formaldehyde (Scheme VI).[35] The crude reaction mixture was shown by GCMS and ^{1}H NMR analysis to consist of a mixture of the expected 1,3-oxazine 36, the isomeric alcohol 37, and the desired tetrahydropyridine 17. Acid treatment of this mixture gave a good yield of 17.

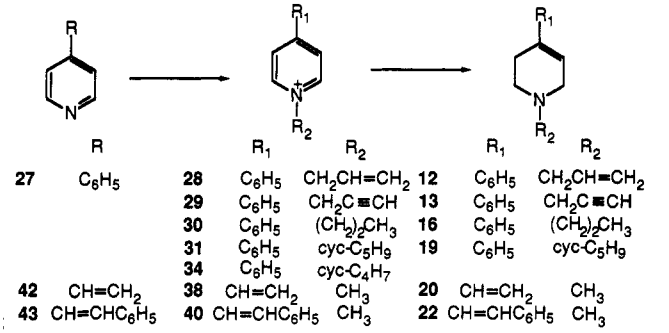
The MPTP analogs in the C(4) series, compounds 20–23, were obtained from the 1-methylpyridinium derivatives 38–41, respectively (Schemes IV, VII, and VIII). Methylation of commercially available 4-ethenylpyridine (42) gave the corresponding 1-methylpyridinium intermediate 38, which yielded 20 when treated with 1 mol of sodium borohydride (excess hydride reagent resulted in further reduction of the exocyclic double bond). The analogous reaction sequence with the previously reported (*E*)-4-(2-phenylethenyl)pyridine (43)[36] gave the known tetrahydropyridine 22.

The synthesis of the 4-ethynyltetrahydropyridine analog 21 was approached via a palladium-catalyzed cross coupling reaction.[37] Coupling of ethyne itself to heteroaromatics has not been reported, but monosubstituted ethynes are known to undergo such reactions with a variety of sp^2 centers using Pd^0 or Pd^{II} catalysts.[38–42] We were able to obtain the 1-methyl-4-[(trimethylsilyl)ethynyl]pyridinium species 45 by coupling 4-bromopridine (44) with trimethylsilylethyne in the presence of an in situ generated palladium catalyst and CuI to generate 4-[(trimethylsilyl)-ethynyl]pyridine, which was treated with iodomethane to afford 45 (Scheme VII).[43] Hydrolysis of 4-[(trimethylsilyl)-ethynyl]pyridine with aqueous K_2CO_3 in MeOH gave the

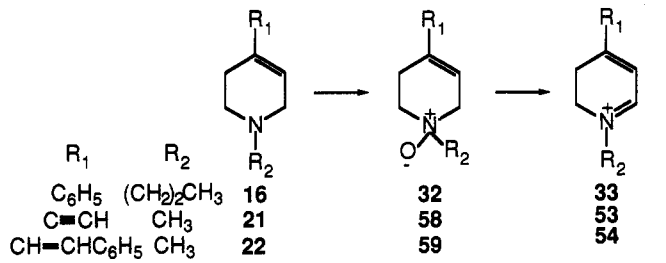
Scheme III. Proposed MAO-B Inactivation Pathway for 4-Ethynyl-Substituted Tetrahydropyridines



Scheme IV. Synthesis of Tetrahydropyridine Derivatives



Scheme V. Synthesis of Dihydropyridinium Derivatives



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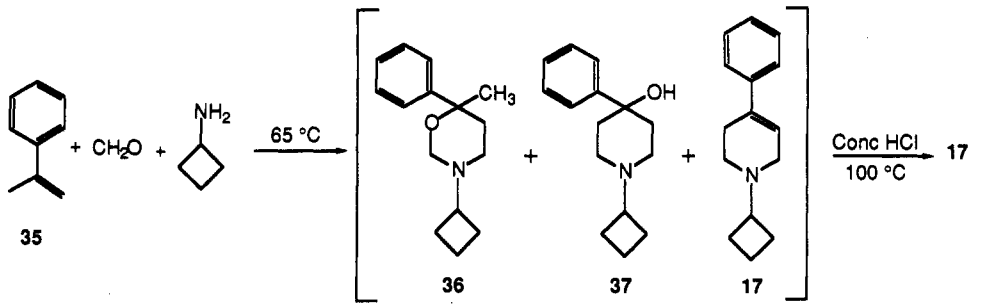
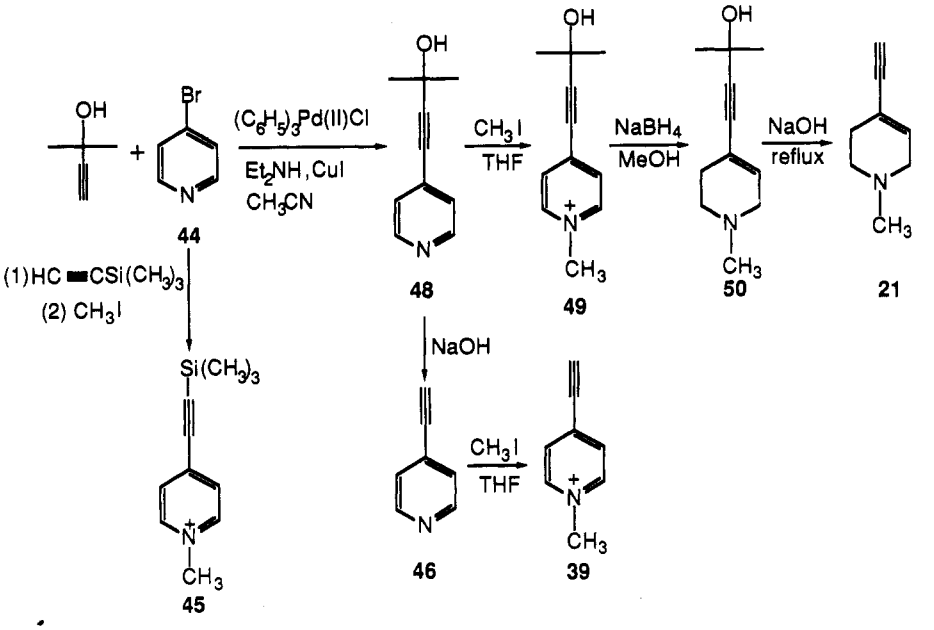
Scheme VI. Synthetic Route to 1-Cyclobutyl-4-phenyl-1,2,3,6-tetrahydropyridine



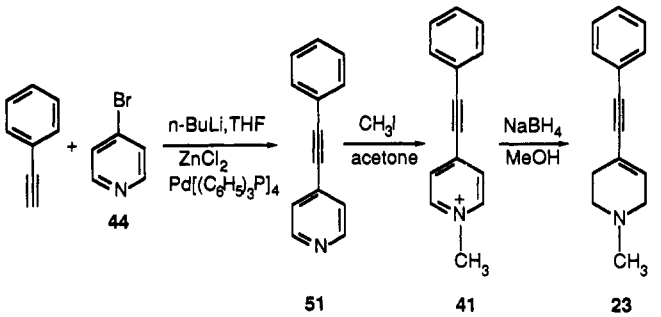
Scheme VII. Synthetic Pathway to 4-Ethynyl-1-methyl-1,2,3,6-tetrahydropyridine



desired 4-ethynylpyridine (**46**). Since methylation of **46** under standard conditions (iodomethane in acetone) led to extensive decomposition, an alternative pathway to compound **21** involving the palladium-catalyzed coupling of commercially available 2,2-dimethyl-3-butyn-2-ol (**47**) with 4-bromopyridine (**44**) to furnish 2-methyl-4-(4-pyridyl)-3-butyn-2-ol (**48**) was pursued.[37] Methylation of **48** yielded the protected pyridinium product **49**, which was reduced in high yield to the tetrahydropyridine derivative **50**. Hydrolysis of **50** yielded the desired 4-ethynyl-1-methyltetrahydropyridine (**21**). Hydrolysis of **48** also provided an alternative route to 4-ethynylpyridine (**46**) in excellent yield. Subsequent reaction conditions using THF led to the successful synthesis of the pyridinium product **39**, which precipitated immediately upon formation from the reaction mixture.

The phenylethynyl derivative **23** was obtained using a modified Pd-catalyzed cross coupling reaction previously applied to heteroaromatics such as halofurans.[44] The Pd-catalyzed coupling between 4-bromopyridine (**44**) and phenylethyne in the presence of *n*-butyllithium, zinc chloride, and $Pd[(C_6H_5)_3P]_4$ afforded 4-(1-phenylethynyl)-pyridine (**51**), which subsequently was methylated to yield the corresponding pyridinium species **41**. Reduction of

Scheme VIII. Synthetic Pathway to 1-Methyl-4-(phenylethynyl)-1,2,3,6-tetrahydropyridine



41 with $NaBH_4$ gave the desired tetrahydropyridine **23** (Scheme VIII).

Finally, the MAO-B substrate properties of the tetrahydropyridine derivatives **20–22** required the synthesis of the corresponding dihydropyridinium species, **52–54**, for metabolite structure confirmation. Treatment of the *N*-oxides **58** and **59** obtained by *m*-CPBA oxidation of the corresponding tetrahydropyridines **21** and **22** with trifluoroacetic anhydride yielded the desired dihydropyridinium products **53** and **54**, respectively (Scheme V). The route to the 4-ethenyldihydropyridinium system **52** proceeded by *m*-CPBA N-oxidation of the readily available piperidinol derivative **55**, obtained from a Grignard reaction between 1-methyl-4-piperidone (**56**) and vinylmagnesium bromide, followed by the treatment of the resulting *N*-oxide **57** with trifluoroacetic anhydride (Scheme IX).

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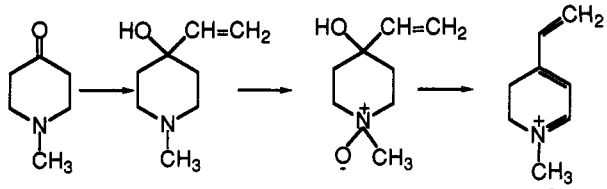
Scheme IX. Synthesis of 4-Ethenyl-1-methyl-2,3-dihydropyridinium Perchlorate (52)



56 55 57 52

Table I. Kinetic Parameters for the MAO-B Catalyzed Oxidation of MPTP and Analogs

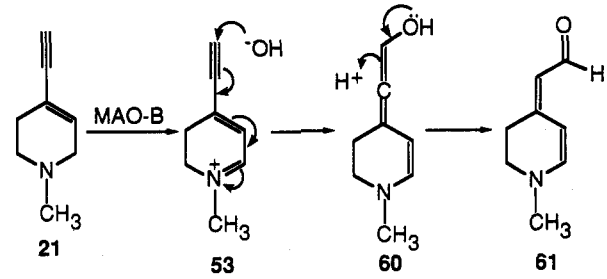
compd	V_{max}[a]	K_M[b]	V_{max}/K[c]
MPTP (1)	204	390×10^3	523×10^6
16	7	2000×10^3	3.5×10^6
20	190	80×10^3	2370×10^6
21	684	861×10^3	790×10^6
22	10	954×10^3	10×10^6

[a] Nanomoles/minute per unit of MAO-B. [b] nM. [c] Liters/minute per unit of MAO-B.

Enzymology. The substrate and inhibitor properties of the candidate compounds were examined with purified beef liver MAO-B. We first screened these molecules for their substrate properties by performing repeated 400 to 220 nm scans of 2 mM solutions of each compound in the presence of 0.02 unit of MAO-B over a period of 1 h. MPTP was included as a reference against which to compare the properties of the test compounds. The results of these studies are summarized in Table I. Under these conditions MPTP undergoes rapid oxidation to the corresponding dihydropyridinium species $MPDP^+$ (λ_{max} 343 nm), which is converted slowly to the pyridinium metabolite MPP^+ (λ_{max} 290 nm).[4] The 4-ethenyl (**20**) and 4-ethynyl (**21**) analogs also underwent rapid oxidation to generate the corresponding dihydropyridinium species **52** (λ_{max} 309) and **53** (λ_{max} 308), which were identified by comparison of their spectral properties with those of the synthetic standards. For reasons which remain unclear, the 4-ethenyl-1-methyl-2,3-dihydropyridinium metabolite **52** was stable under the incubation conditions while the ethynyl derivative **53** behaved like $MPDP^+$ and underwent slow oxidation to the pyridinium product **39** (λ_{max} 264).[45] The synthetic ethynyldihydropyridinium species **53** behaved in an identical fashion. Furthermore, contrary to reports on $MPDP^+$,[25] the rate of this oxidation was not affected by MAO-B, suggesting that the conversion of **53** to **39** is an autoxidative process.

In addition to the ethynylpyridinium species, a second product with λ_{max} 375 nm was observed in the UV scans of the MAO-B incubation mixture with compound **21**. When incubated under these conditions, the synthetic dihydropyridinium compound also displayed the same chromophore, which we tentatively assign to the aldehyde derivative **61**. The proposed pathway leading to the formation of the **61** may involve nucleophilic attack by water on the reactive ethynyldihydropyridinium system **53** (Scheme X). This is analogous to the Schuster–Meyer rearrangement[46] which involves hydration of a propargylic cation intermediate followed by rearrangement of the

Scheme X. Proposed Conversion of the Ethynyldihydropyridinium Metabolite **53** to Aldehyde **61**



21 53 60 61

resulting allenol to yield an acrolein derivative. In the present case, hydrolysis of **53** generates an aminoallene (**60**) which would rearrange to afford the more stable aldehyde **61**.

Of the remaining compounds, only the phenylethenyl derivative **22** and the *N*-propyl derivative **16** showed any significant turnover under these incubation conditions. Scans of **22** clearly showed the formation of a metabolite with λ_{max} 375 nm corresponding to the synthetic dihydropyridinium derivative **54**. Upon standing, the absorption maximum shifted to 350 nm, which corresponds to the spectrum of the synthetic pyridinium species **40**. An earlier report[36] that the phenylethenyl compound **22** is not a substrate for MAO-B may be due to the use of a mitochondrial enzyme preparation instead of the purified enzyme studied here. Similar results were obtained with the *N*-propyl derivative **16**, which underwent slow oxidation to the dihydropyridinium intermediate **33** (λ_{max} 342 nm). This dihydropyridinium intermediate also underwent further oxidation to the pyridinium species (λ_{max} 294). On the basis of these results, previous reports suggesting the lack of substrate properties of MPTP analogs bearing N-substituents larger than an ethyl group must be viewed with caution.[33] On the other hand, we could find no evidence of turnover of the remaining tetrahydropyridines. It is possible, therefore, that electronic factors may contribute to the nature of the interactions between tetrahydropyridine derivatives and MAO-B since the *N*-allyl and *N*-propargyl derivatives appeared to be completely stable under the incubation conditions that led to a measurable rate of oxidation of the *N*-propyl derivative.

More quantitative data on the characteristics of the four tetrahydropyridine MAO-B substrates were obtained by examining the dependence of oxidation rates on substrate concentration. Linear semilog plots of initial rates vs concentration provided data which were analyzed by double-reciprocal plots. Typical of these plots is that for 4-ethenyl-1-methyl-1,2,3,6-tetrahydropyridine (**20**) shown in Figure 1. The V_{max}, K_M, and V_{max}/K_M values calculated from the Lineweaver–Burk plots are summarized in Table I together with the corresponding values for MPTP.

The 4-ethenyl (**20**) and 4-ethynyl (**21**) compounds displayed substrate properties comparable to those of MPTP while the 2-phenylethenyl analog **22** proved to be a weak substrate and the corresponding phenylethynyl analog **23** a very poor substrate with undetermined kinetic parameters. The good substrate properties of the ethenyl and ethynyl analogs relative to the corresponding phenylethenyl and phenylethynyl analogs are likely to reflect steric interactions in the active site. The excellent substrate properties of the 4-benzyl and 4-(2-phenylethyl) analogs[36] emphasize the importance of the geometry in

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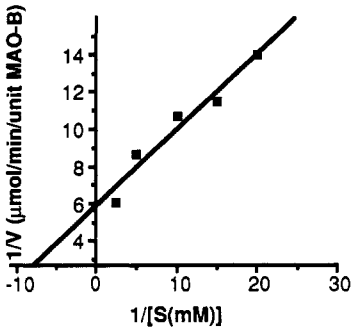

Figure 1. Plots of $1/V$ versus $1/[S]$ for the MAO-B catalyzed oxidation of 4-ethenyl-1-methyl-1,2,3,6-tetrahydropyridine.

this region of the substrate molecules. The observation that significant bulk at the meta but not the para position of the phenyl group of MPTP[33] is tolerated is suggestive of a hydrophobic region which may accommodate the 4-benzyl and the 4-(2-phenylethyl) groups but not the more rigid phenyl-substituted ethenyl and ethynyl substituents.

The MAO-B inhibiting properties of these compounds were determined by assessing the rate of loss of enzyme activity (as measured by the initial rate of oxidation of 5 mM MPTP) vs time at several concentrations of the potential inhibitor.[27] Linear semilog plots were obtained for those compounds displaying significant inhibitor properties. Figure 2a presents data for 4-phenyl-1-propargyl-1,2,3,6-tetrahydropyridine (**13**) which are typical of these plots and from which the observed rate of loss of enzyme activity (k_{obs}) could be calculated for each concentration of inhibitor. These characteristics of time- and concentration-dependent inhibition of enzyme activity are typical of those associated with mechanism-based inactivators that irreversibly inhibit enzyme activity by alkylating an active-site functionality essential for substrate turnover.[47] The double-reciprocal plot of $1/k_{obs}$ vs 1/inhibitor concentration (Figure 2b) provided estimates of k_{inact} (maximum rate of inactivation) and K_I (the concentration of inhibitor at which the rate of oxidation of MPTP was 50% of its maximal rate).

The most efficient inhibitor of MAO-B in this series is the propargyl derivative **13**. On the basis of published evidence for the inactivation pathway associated with other propargylamines,[20] compound **13** presumably inactivates MAO-B via the pathway shown in Scheme XI. Initial one-electron transfer generates the aminium radical species **62**, which then looses a proton from the methylene group of the propargyl moiety to form the carbon centered radical **63**. A second one-electron transfer leads to the highly electrophilic ethynyliminium species **15**, which presumably inactivates the enzyme by reacting with an active-site nucleophilic group. This pathway is analogous to the previously proposed mechanism by which other tertiary propargylic amines, via covalent binding of the ethynyliminium intermediate with the flavin prosthetic group to form a flavocyanine, are thought to inactivate MAO.[19,48-50] In the present case, one might expect that the aminium radical **62** also would yield the ring allylic carbon-centered radical **64** and hence the dihydropyridinium intermediate **65**. However, no evidence for the formation of **65** under standard conditions of incubation as well as in the presence of a 40-fold increase in the enzyme concentration could be obtained. The fact that the *N*-propyl analog **16** undergoes ring α-carbon oxidation to yield the expected dihydropyridinium metabolite **33** (Table I) at a measurable rate demonstrates the feasibility of such a pathway with a three-carbon group attached to nitrogen.

Although many of the other candidate compounds in these two series were found to be time- and concentration-dependent inhibitors of MAO-B (Table II), none of them proved to be very efficient. In view of the moderately effective inactivating properties of the *N*-propargyl derivative, the weak inactivating properties of the *N*-allyl analog **12** was somewhat surprising. In contrast with the corresponding *N*-cyclopropyl derivative **8**, the *N*-cyclobutyl analog **17** showed no significant activity at 200 μM. The limited solubility of **17** precluded a more definitive characterization of its interactions with MAO-B. Furthermore, despite their good substrate properties and the potential electrophilic reactivity of the corresponding dihydropyridinium metabolic intermediates, neither the ethenyl (**20**) nor the ethynyl (**21**) analog displayed significant inhibitor properties. The lack of inhibitor properties was not due to poor reactivity of the 4-ethynyldihydropyridinium species **53**. We examined the susceptibility of **53** to attack with several model nucleophiles including *N*-acetylcysteine (**66**) and benzylamine (**67**). When treated with **66** or **67** at room temperature, the UV chromophore of **53** (λ_{max} 308 nm) was replaced by chromophores with λ_{max} 414 nm for **66** and λ_{max} 451 nm for **67**. These spectral characteristics are consistent with the adducts **68** and **69** (see Scheme III) that would result from a pathway similar to that shown in Scheme VI for the hydrolysis of **53**. This reaction pathway was studied in a greater detail using *N*-methylaniline (**70**) as the nucleophile. The reaction was monitored by ^{1}H NMR using 0.5 M **53** and **70** in CD_3CN. Evidence of reaction (loss of the characteristic downfield signals of **53**) was immediate. Upon workup a fluffy orange solid was isolated which was characterized by ^{1}H NMR, fast atom bombardment mass spectrometry (M^+ 227), and elemental analysis as 4-[2-(*N*-methylanilino)ethenyl]-1-methyl-2,3-dihydropyridinium perchlorate (**72**). The UV spectral characteristics of **72** (λ_{max} 464 nm, ϵ 51 000) are comparable to those of structurally related cyanines.[51-54] The pathway to **72** may involve initial formation of the highly reactive aminoallene **71**, which could rearrange to the resonance-stabilized product **72**.

Overall these results lead us to conclude that there is little opportunity to design effective tetrahydropyridine-

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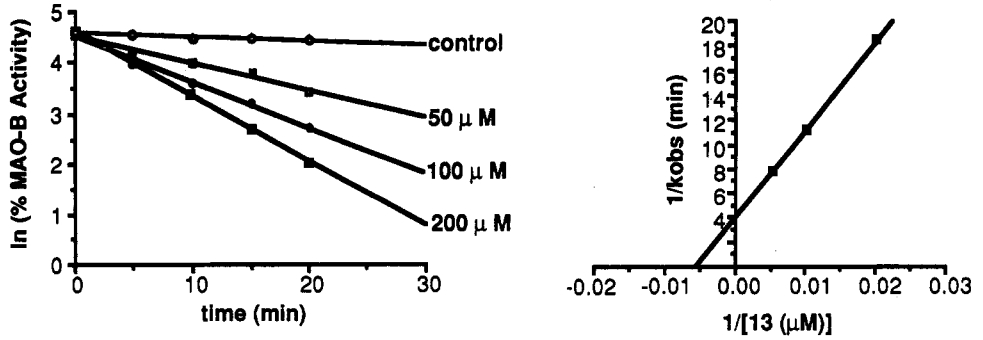

Figure 2. Kinetic studies on the inactivation of MAO-B by 4-phenyl-1-propargyl-1,2,3,6-tetrahydropyridine. Left panel (a) shows the time- and concentration-dependent inactivation curves. Right panel (b) is a double-reciprocal plot of constructed from the linear semilog plots shown in (a).

Scheme XI. Proposed Inactivation Pathway of MAO-B by 13

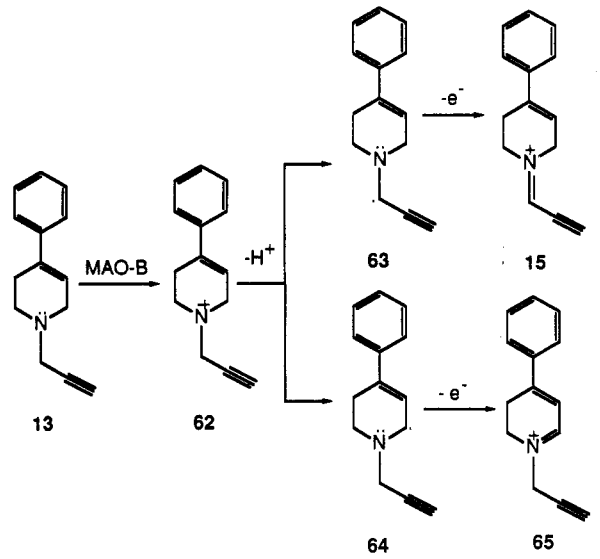

Table II. MAO-B Inactivation Properties of N- and C(4)-Substituted MPTP Analogues[a]

compd	k_{inact} (min^{-1})	K_I (μM)	compd	k_{inact} (min^{-1})	K_I (μM)
MPTP (1)	0.034	5000	**19**	0.045	173
12	0.029	268	**20**	0.08	330
13	0.24	172	**21**	0.05	154
16	0.06	5000	**22**	0.05	312
17	not active[b]		**23**	0.32	820

[a] The kinetics of inactivation were determined as described in the Experimental Section. The inhibitor concentrations used in these experiments depended on the activity of the individual compound and ranged from 50–100 μM for 13 to 300–1500 μM for 16. [b] The limited solubility of 17 precluded a thorough study of its inhibitor properties.

based MAO-B inactivators that rely on enzyme-generated electrophilic moieties located in the C(4) position of the tetrahydropyridine ring. Furthermore, the poor fit of tetrahydropyridine derivatives bearing a large N-substituent, as illustrated by the high K_M value for the propyl derivative **16** and the lack of substrate or inhibitor properties of the *N*-cyclobutyl derivative **17**, limits the design opportunities at this region of the molecule. Finally, literature reports documenting the lack of substrate properties for tetrahydropyridine derivatives bearing substituents other than at the N and C(4) positions seem to rule out other variations on this theme.[33] We conclude, therefore, that the 1,2,3,6-tetrahydropyridine system is unlikely to yield pharmacologically useful inactivators of MAO-B.

Experimental Section

Chemistry. Melting points were determined using a Thomas-Hoover melting point apparatus and are uncorrected. Unless stated otherwise, reagents were obtained from commercial sources and were used directly. Reactions were conducted under a dry nitrogen atmosphere. THF and diethyl ether were distilled from $LiAlH_4$. UV–vis spectra were recorded on a Beckman DU Series 50 spectrophotometer. ^1H NMR spectra were recorded on either a Bruker 270 MHz or a 200 MHz spectrometer; chemical shifts are reported in parts per million (ppm) relative to internal TMS except where noted. The following abbreviations are used to describe peak patterns when appropriate: b = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, db = doublet of doublets. Gas chromatography/mass spectrometry (GCMS) was performed using an Hewlett Packard (HP) 5890 capillary GC coupled to an HP 59970 MS chem station. The capillary column used in all the cases was an HP-1 (12.5 m × 200 mm × 0.33 μm film thickness). Elemental analyses, performed by Atlantic Microlab, Inc., Norcross, GA, were within 0.4% of the theoretical values calculated for C, H, and N. The *N*-alkyl-4-phenylpyridinium halides **28·I**, **29·Br**, **30·I** were prepared as described previously.[55]

General Procedure for the Synthesis of the Oxalate Salts of N-Substituted-4-phenyl-1,2,3,6-tetrahydropyridine Derivatives 12, 13, and 16. Sodium borohydride (5.52 mmol) was added in portions to a stirred solution of the appropriate *N*-alkyl-4-phenylpyridinium halide (2.76 mmol) in 30 mL of dry methanol at 0 °C. The mixture was stirred for an additional 60 min, and the solvent was subsequently removed under reduced pressure. The residue was taken up in 15 mL of H_2O and the solution extracted with diethyl ether (3 × 50 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated to 50% of the original volume. Treatment with oxalic acid (2.76 mmol) in 10 mL of diethyl ether precipitated the crude oxalate salt which was recrystallized from the appropriate solvent to afford a white crystalline solid.

Oxalate salt of 1-allyl-4-phenyl-1,2,3,6-tetrahydropyridine (12): 80% from ethanol; mp 174–6 °C; ^1H NMR (DMSO-d_6) δ 2.8 (m, 2 H, C-3 CH_2), 3.45 (m, 2 H, C-2 CH_2), 3.7 (m, 4 H, CH_2), 5.5 (m, 2 H, olefinic CH_2), 5.95 (m, 1 H, olefinic CH), 6.35 (bs, 1 H, C-5 olefinic H), 7.45 (m, 5 H, ArH); GCMS (temperature program, 100 °C for 1 min followed by a ramp of 20 °C/min for 8 min) showed a single peak (t_R = 5.35 min) M^+ 199. Anal. ($C_{16}H_{19}NO_4 \cdot 0.25H_2O$) C, H, N.

Oxalate salt of 4-phenyl-1-propargyl-1,2,3,6-tetrahydropyridine (13): 85% from ethanol; mp 143–5 °C; ^1H NMR (DMSO-d_6) δ 2.56 (m, 2 H, CH_2), 3.04 (m, 2 H, C-3 H CH_2), 3.54 (s, 1 H, acetylenic H), 3.6 (m, 2 H, C-2 CH_2), 3.8 (m, 2 H, C-6 CH_2), 6.23 (bs, 1 H, olefinic H), 7.36 (m, 5 H, ArH); GCMS (temperature program, 100 °C for 1 min followed by a ramp of 25 °C/min) showed a single peak (t_R = 5.5 min) M^+ 197. Anal. ($C_{16}H_{17}NO_4 \cdot 0.25H_2O$) C, H, N.

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Oxalate salt of 4-phenyl-1-propyl-1,2,3,6-tetrahydropyridine (16): 86% from 2-propanol; mp 174–5 °C; ^{1}H NMR (DMSO-d_6) δ 1.0 (t, 3 H, CH_3), 1.8 (m, 2 H, CH_2), 2.8 (bd, 2 H, C-3 CH_2), 3.0 (q, 2 H, CH_2), 3.4 (m, 2 H, C-2 CH_2), 3.8 (m, 2 H, C-6 CH_2), 6.2 (bs, 1 H, C-5 olefinic H), 7-3-7.5 (m, 5 H, ArH); GCMS (temperature program, 100 °C for 1 min followed by a ramp of 20 °C/min) showed a single peak (t_R = 4.93 min) M^+ 201. Anal. ($C_{16}H_{21}NO_4$) C, H, N.

1-Cyclobutyl-4-phenyl-1,2,3,6-tetrahydropyridine Hydrochloride (17·HCl). A solution containing cyclobutylamine (1.0 g, 14.06 mmol), acidified with 50% HCl to pH 6, and 37% aqueous formaldehyde (2.32 g, 77.6 mmol) was heated to 65 °C for 10 min at which time 2-phenylpropene (**35**, 0.832 g, 7.0 mmol) was added. After heating at 65 °C for an additional 6 h, methanol (50 mL) was added and the reaction mixture was allowed to stir at room temperature overnight. The methanol was removed under reduced pressure to afford a yellow oil which according to GCMS analysis was composed of a mixture of the 1,3-oxazine **36** ($M^{\cdot+}$ 234), the isomeric alcohol **37** ($M^{\cdot+}$ 234), and the desired tetrahydropyridine **17** (M^+ 217). This oil in concentrated HCl (15 mL) was heated at 100 °C for 1 h. The excess acid was removed under reduced pressure and the resulting yellow oil in 0.1 N HCl was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic extracts were dried ($MgSO_4$) and filtered, and the solvent was removed under reduced pressure to yield 0.81 g of a yellow solid which recrystallized from acetonitrile/diethyl ether to afford 0.7 g (42%) of **17**·HCl as an off-white crystalline solid: mp 214–7 °C dec; ^{1}H NMR ($CDCl_3$) δ 2.0–2.3 (m, 6 H, CH_2), 2.85 (m, 2 H, C-3 CH_2), 3.5 (m, 4 H, C-2 and C-6 CH_2), 4.1 (m, 1 H, cyclobutylmethine), 6.0 (bs, 1 H, C-5 olefinic H), 7.5 (m, 5 H, ArH), 8.4 (bs, 1 H, NH); GCMS (temperature program, 100 °C for 1 min followed by a ramp of 25 °C/min) showed a single peak (t_R = 6.3) M^+ 213. Anal. ($C_{15}H_{20}NCl·0.75H_2O$) C, H, N.

1-Cyclopentyl-4-phenyl-1,2,3,6-tetrahydropyridine Hydrochloride (19·HCl). A solution of 4-phenylpyridine (**27**, 1.0 g, 6.4 mmol) and bromocyclopentane (3.8 g, 26 mmol) in acetone (50 mL) was heated under reflux for 72 h, and the resulting precipitated white solid (1.75 g) was filtered and washed with acetone and was shown by ^{1}H NMR to be a 2:1 mixture of the pyridinium species **31** and **27**, respectively: mp 122–4 °C; ^{1}H NMR ($CDCl_3$) δ 1.8–2.6 (m, 8 H, cyclopentyl H), 5.5 (m, 1 H, cyclopentylmethine), 7.6 (m, 5 H, ArH), 8.22 (d, 2 H, C-2 and C-6 H), 9.55 (d, 2 H, C-3 and C-5 H), 7.7 (m, ArH related to **27**), 7.8 (d, C-2 and C-6 related to **27**), 8.9 (d, C-3 and C-5 H related to **27**). To this solid (0.75 g, 2.4 mmol) in methanol (25 mL) was added portionwise sodium borohydride (0.18 g, 4.8 mmol) with stirring at 0 °C. The reaction mixture was stirred at room temperature for 1 h following which the methanol was removed under reduced pressure. An aqueous solution of the residue was extracted with diethyl ether (3 × 20 mL), and the combined organic layers were extracted with 1 N HCl (2 × 10 mL). This aqueous solution was extracted with CH_2Cl_2 (2 × 15 mL), and the organic extract was dried ($MgSO_4$), filtered, and evaporated to yield a white solid which was recrystallized from ethanol to yield 0.21 g (38%) of **19**·HCl as a white crystalline solid: mp 255–6 °C; ^{1}H NMR ($CDCl_3$) δ 1.6–2.2 (m, 8 H, cyclopentylmethylenes), 2.6 (m, 2 H, C-3 CH_2), 3.1–3.6 (m, 4 H, C-2 and C-6 CH_2), 4.2 (m, 1 H, cyclopentylmethine), 5.99 (d, 1 H, C-5 olefinic H), 7.28–7.3 (m, 5 H, ArH); GCMS (temperature program, 50 °C for 1 min followed by a ramp of 25 °C/min for 10 min) showed a single peak (t_R = 6.92 min) M^+ 227. Anal. ($C_{16}H_{22}ClN$) C, H, N.

***m*-CBA Salt of 4-Phenyl-1-propyl-1,2,3,6-tetrahydropyridine *N*-Oxide (32).** To a solution of 4-phenyl-1-propyl-1,2,3,6-tetrahydropyridine free base (**16**) [obtained from the corresponding oxalate salt (0.4 g, 1.37 mmol)] in CH_2Cl_2 (20 mL) was added 60% *m*-CPBA (0.26 g, 1.5 mmol) at 0 °C. After stirring for 2 h at 0 °C, the solvent was removed under reduced pressure to afford 0.29 g (64%) of the white crystalline *N*-oxide as its *m*-CBA salt. The analytical sample was obtained by recrystallization from acetone/diethyl ether: mp 92–93 °C; ^{1}H NMR ($CDCl_3$) δ 2.1 (m, 2 H, CH_2), 2.6–3.2 (m, 2 H, C-3 CH_2), 3.7–4.6 (m, 5 H, C-2, C-6 and N-propyl CH_2), 6.0 (d, 1 H, C-5 olefinic H), 7.3–8.0 (complex m, 8 H, ArH). Anal. ($C_{21}H_{24}ClNO_3$) C, H, N.

4-Phenyl-1-propyl-2,3-dihydropyridinium Perchlorate (33·ClO$_4$). The above oxide (0.27 g, 0.72 mmol) in CH_2Cl_2 was applied to a basic alumina column which was eluted with CH_2Cl_2. The solvent was removed under reduced pressure and the resulting *N*-oxide free base in CH_2Cl_2 (15 mL) was treated at 0 °C with trifluoroacetic anhydride (0.89 g, 3.9 mmol) in 5 mL of CH_2Cl_2. After stirring at 0 °C for 1 h, the solvent was removed and the resultant yellow oil (0.43 g) was crystallized from methanol (15 mL) containing 0.11 mL of 70% perchloric acid to yield 100 mg (45%) of shiny yellow crystals: mp 96–8 °C; UV (MeOH) λ_{max} 343 nm (ε 16 750); ^{1}H NMR (DMSO-d_6) δ 1.1 (t, 3 H, CH_2), 1.8 (m, 2 H, CH_2), 3.2 (t, 2 H, C-3 CH_2), 3.8–4.0 (m, 4 H, C-2 and *N*-propyl CH_2), 7.1 (d, 1 H, C-5 H), 7.4–7.6 (m, 5 H, ArH), 8.7 (d, 1 H, C-6 H). Anal. ($C_{14}H_{18}ClNO_4$) C, H, N.

4-Ethenyl-1-methylpyridinium Iodide (38). A mixture of iodomethane (5.06 g, 35.6 mmol) and 4-ethenylpyridine (**42**, 2.5 g, 23.7 mmol) in acetone (30 mL) was stirred at room temperature for 30 min. The precipitated bright yellow solid was recrystallized from hot acetone to yield 5.28 g (90%) of shiny yellow crystals: mp 148–149 °C; UV (MeOH) λ_{max} 265 nm (ε 15 250); ^{1}H NMR ($CDCl_3$) δ 4.6 (s, 3 H, CH_3), 6.0 (db, 1 H, J = 17 Hz), 6.4 (db, 1 H, J = 11 Hz), 6.9 (m, 1 H, vinylic H), 8.15 (d, 2 H, C-3, C-5 H), 9.35 (d, 2 H, C-2, C-6 H). Anal. ($C_{18}H_{10}NI·0.5H_2O$) C, H, N.

Oxalate Salt of 4-Ethenyl-1-methyl-1,2,3,6-tetrahydropyridine (20). To a solution of 4-ethenyl-1-methylpyridinium iodide (**38**, 0.7 g, 2.8 mmol) in 30 mL of methanol was added sodium borohydride (0.105 g, 2.8 mmol) portionwise at 0 °C with stirring. The reaction mixture was stirred at 0 °C for 30 min following which the methanol was removed under reduced pressure. The residue was treated with water (10 mL) and extracted with diethyl ether (2 × 30 mL). The combined organic extracts were dried ($MgSO_4$) and filtered, and oxalic acid (0.2 g, 2.2 mmol) in diethyl ether (10 mL) was added to precipitate the oxalate salt of **20** as a white solid which was recrystallized from 2-propanol to yield 0.28 g (47%) of white fluffy crystals: mp 147–149 °C; ^{1}H NMR (DMSO-d_6) δ 2.4 (bd, 2 H, C-3 CH_2), 3.0 (s, 3 H, CH_3), 3.5 (m, 2 H, C-2 CH_2), 3.7 (m, 2 H, C-6 CH_2), 5.4 (d, 1 H, J = 17 Hz), 5.5 (d, 1 H, J = 11 Hz), 6.0 (bs, 1 H, C-5 olefinic H), 6.6 (dd, 1 H, vinylic H); GCMS (temperature program, 50 °C for 1 min followed by a ramp of 25 °C/min) for 10 min showed a single peak (t_R = 2.06) M^+ 123. Anal. ($C_{10}H_{15}NO_4·0.5H_2O$) C, H, N.

Oxalate Salt of (*E*)-1-Methyl-4-(2-phenylethenyl)-1,2,3,6-tetrahydropyridine (22). Sodium borohydride (0.095 g, 2.52 mmol) was added portionwise to a solution of (*E*)-1-methyl-4-(2-phenylethenyl)pyridinium iodide[36] (**40·I**, 0.41 g, 1.26 mmol) in methanol (25 mL) at 0 °C with stirring. The reaction mixture was allowed to stir at room temperature for 30 min following which the methanol was removed under reduced pressure. The residue was treated with water (10 mL) and extracted with diethyl ether (3 × 15 mL). The combined organic extracts were dried ($MgSO_4$) and filtered, and oxalic acid (0.11 g, 1.22 mmol) in diethyl ether (10 mL) was added to precipitate the oxalate salt of **22**, which was recrystallized from methanol to afford 0.26 g (72%) of a white crystalline solid: mp 222–4 °C; GCMS (temperature program, 50 °C for 1 min followed by a temperature gradient of 25 °C/min for 10 min) showed a single peak (t_R = 6.254) M^+ 199; ^{1}H NMR (DMSO-d_6) δ 2.6 (bd, 2 H, C-3 CH_2), 2.8 (s, 3 H, CH_3), 3.3 (m, 2 H, C-2 CH_2), 3.75 (bs, 2 H, C-6 CH_2), 5.9 (bs, 1 H, C-5 olefinic H), 6.6 (d, 1 H, J = 16 Hz), 7.0 (d, 1 H, J = 16 Hz), 7.3–7.5 (m, 5 H, ArH). Anal. ($C_{16}H_{19}NO_4$) C, H, N.

1-Methyl-4-[(trimethylsilyl)ethynyl]pyridinium Iodide (45·MeI). A mixture of **44** (2.5 g, 13.3 mmol), triphenylphosphine (0.524 g, 2 mmol), cuprous iodide (0.126 g, 0.66 mmol), triethylamine (7 mL), and acetonitrile (70 mL) was degassed with argon for 15 min. (Trimethylsilyl)ethyne (2.61 g, 26.6 mmol) was added followed by (0.149 g, 0.66 mmol) of palladium acetate. The mixture was stirred under argon for 6 h at room temperature, and the residue obtained after evaporating the solvent was partitioned between water and CH_2Cl_2. The organic layer was dried (Na_2SO_4) and evaporated, and the residue was chromatographed on basic alumina using CH_2Cl_2 to afford 1.1 g (47%) of **45** as a yellow oil. The product was treated with iodomethane (2.2 g, 16 mmol) to yield the corresponding pyridinium salt (**45**) as fine yellow crystals (1.8 g, 45%): mp 180–182 °C; ^{1}H NMR (DMSO-d_6) δ 0.38 (s, 9 H, TMS-H), 4.29 (s, 3 H, CH_3), 8.15 (d, 2 H, C-2 and C-6 H), 8.9 (d, 2 H, C-3 and C-5 H). Anal. ($C_{11}H_{16}INSi$) C, H, N.

4-(3-Hydroxy-3-methylbut-1-ynyl)-1-methylpyridinium Iodide (49·I). A mixture of iodomethane (5.27 g, 37.2 mmol) and 2-methyl-4-(4-pyridyl)-3-butyn-2-ol (**48**, 2 g, 12.4 mmol)[37] in dry THF (20 mL) was stirred overnight under nitrogen. Recrystallization of the resulting precipitate from methanol/diethyl ether afforded 3.3 g (89%) of product: mp 114–118 °C; ^{1}H NMR (DMSO-d_6) δ 1.51 (s, 6 H, $(CH_3)_2$), 4.32 (s, 3 H, CH_3), 5.8 (s, 1 H, acetylenic H), 8.0–8.1 (d, 2 H, J = 17 Hz), 8.95–8.97 (d, 2 H, J = 17 Hz); UV (MeOH) λ_{max} 264 (ϵ 12 200). Anal. ($C_{11}H_{14}NOI$) C, H, N.

Oxalate Salt of 4-(3-Hydroxy-3-methylbut-1-ynyl)-1-methyl-1,2,3,6-tetrahydropyridine (50). To a solution of **49** (0.2 g, 0.66 mmol) in MeOH (20 mL) was added sodium borohydride (49 mg, 1.32 mmol) at 0 °C. After stirring at 0 °C for 40 min, the solvent was removed under reduced pressure, the residue was dissolved in water (10 mL), and an aqueous solution of the residue was extracted with diethyl ether (3 × 15 mL). The combined organic layers were dried (Na_2SO_4) and filtered, and oxalic acid (65 mg, 0.67 mmol) in diethyl ether (5 mL) was added to precipitate the oxalate salt which was recrystallized from methanol/diethyl ether to afford 90 mg (75%) of the desired product: mp 158–9 °C; GCMS (temperature program, 50 °C for 1 min followed by a temperature ramp of 25 °C/min) showed a single peak at (t_R = 4.549 min) M^+ 179; ^{1}H NMR (DMSO-d_6) δ 1.36 (s, 6 H, $(CH_3)_2$), 2.33 (bs, 2 H, C-3 CH_2), 2.8 (s, 3 H, CH_3), 3.0–3.1 (t, 2 H, C-2 CH_2), 3.55 (bs, 2 H, C-6 CH_2), 5.9 (bs, 1 H, C-5 olefinic H). Anal. ($C_{13}H_{19}NO_5$) C, H, N.

Oxalate Salt of 4-Ethynyl-1-methyl-1,2,3,6-tetrahydropyridine (21). A mixture of NaOH (0.3 g, 7.6 mmol) and **50** (1.37 g, 7.6 mmol) in dry toluene (40 mL) was heated under reflux for 2.5 h. After cooling, water (10 mL) was added and the resulting mixture was acidified with 0.1 N HCl. The toluene layer was discarded, and the aqueous solution was neutralized with aqueous K_2CO_3 and then extracted with diethyl ether (2 × 25 mL). The combined organic extracts were dried (Na_2SO_4) and filtered, and oxalic acid (0.68 g, 7.6 mmol) was added to the solution to precipitate the oxalate salt which was recrystallized from methanol/diethyl ether to afford 0.92 g (59%) of the desired product: mp 159–161 °C; GCMS (temperature program, 50 °C for 1 min followed by a temperature ramp of 25 °C/min for 10 min) showed a single peak (t_R = 3.071 min) M^+ 121; ^{1}H NMR (DMSO-d_6) δ 2.39 (bs, 2 H, C-3 CH_2), 2.71 (s, 3 H, CH_3), 3.1 (t, 2 H, C-2 CH_2), 3.63 (bs, 2 H, C-6 CH_2), 4.0 (s, 1 H, acetylenic H), 6.08 (bs, 1 H, C-5 olefinic H), 9.0 (bs, 2 H, oxalate H). Anal. ($C_{10}H_{13}NO_4$) C, H, N.

4-Ethynylpyridine (46). A mixture of NaOH (0.476 g, 11.9 mmol) and **48** (1.9 g, 11.9 mmol) in dry toluene (50 mL) was heated under reflux for 2 h. The reaction mixture was cooled and acidified with 0.1 N HCl. The aqueous solution was separated and neutralized with aqueous K_2CO_3 and extracted with diethyl ether (3 × 15 mL). The combined organic extracts were dried (Na_2SO_4) and filtered, and the solvent was removed under reduced pressure to afford a light brown solid which was recrystallized from ethyl acetate/diethyl ether to yield 0.8 g (65%) of **46**: mp 94–6 °C (lit.[37] mp 95–6 °C).

4-Ethynyl-1-methylpyridinium Perchlorate (39·ClO_4). A solution of **46** (0.4 g, 3.8 mmol) and iodomethane (1.6 g, 11.4 mmol) in dry THF (15 mL) was stirred at room temperature for 6 h during which time the methiodide of **39** precipitated as a dark brown solid. This material 0.43 g (50%) was treated with 0.7 mL of 70% ethanolic perchloric acid to afford **39** as the perchlorate salt in an overall yield of 40%: mp 161–3 °C; ^{1}H NMR (DMSO-d_6) δ 4.5 (s, 3 H, CH_3), 5.38 (s, 1 H, acetylenic H), 8.18–8.21 (d, 2 H, C-3 and C-5 H), 8.98–9.01 (d, 2 H, C-2 and C-6 H); UV (MeOH) λ_{max} 264 (ϵ 12 220). Anal. ($C_8H_8NClO_4$) C, H, N.

1-Methyl-4-(1-phenylethynyl)pyridinium Iodide (41·I). *n*-Butyllithium (15.63 mL of a 1.6 M solution in hexane, 25 mmol) was added to a solution of phenylethyne (2.4 g, 23.6 mmol) in 25 mL of freshly distilled, dry THF at 0 °C. After the reaction mixture was stirred for 2 h, the contents of the flask were added to a solution of anhydrous zinc chloride (3.4 g, 25 mmol) in 25 mL of dry THF. After stirring for 1 h, the phenylacetylide–zinc chloride complex was added dropwise to a solution of 4-bromopyridine free base [obtained from 4-bromopyridine hydrochloride (41·HCl, 1.166 g, 5.9 mmol)] in 40 mL of dry THF containing palladium tetrakistriphenylphosphine (50 mg, 0.05 mole). The resulting reaction mixture was heated under reflux overnight, and after cooling, the reaction was quenched by the addition of 200 mL of saturated aqueous ammonium chloride. The organic layer was separated, washed with water (3 × 100 mL), and extracted with 1.0 M HCl (3 × 100 mL). After washing with $CHCl_3$ (100 mL), the pH of the aqueous extract was adjusted to 11 with 1.0 M NaOH and the resulting solution was extracted with diethyl ether (2 × 100 mL). The organic extract was dried ($MgSO_4$) and filtered, and the solvent was removed under reduced pressure to afford a yellow oil 0.89 g (84%). Treatment of this oil in 20 mL of acetone with iodomethane (8.41 g, 59.2 mmol) gave a yellow solid precipitate which was recrystallized from 2-propanol to yield 1.35 g (71%) of 41·I as yellow crystals: mp 151–3 °C; ^{1}H NMR (DMSO-d_6) δ 4.3 (s, 3 H, CH_3), 7.5–7.7 (m, 5 H, ArH), 8.3 (d, 2 H, J = 6.64 Hz), 9.01 (d, 2 H, J = 6.71 Hz); UV (H_2O) λ_{max} 328 (ϵ 17 000). Anal. ($C_{14}H_{12}NI·0.89H_2O$) C, H, N.

Oxalate Salt of 1-Methyl-4-(1-phenylethynyl)-1,2,3,6-tetrahydropyridine (23). To a solution of 1-methyl-4-(1-phenylethynyl)pyridinium iodide (41·I, 0.7 g, 2.1 mmol) in methanol (20 mL) was added sodium borohydride (0.158 g, 4.2 mmol) portionwise at 0 °C with stirring. The reaction mixture was stirred at room temperature for 1 h, and then the solvent was removed under reduced pressure. The residue was taken up in water (15 mL) and extracted with diethyl ether (3 × 20 mL). The organic extract was dried ($MgSO_4$), and filtered, and oxalic acid (0.175 g, 1.9 mmol) in diethyl ether (5 mL) was added to precipitate the oxalate salt of **23**, which was recrystallized from hot 2-propanol to afford 0.46 g (76%) of fine white crystals: mp 202–4 °C; ^{1}H NMR (DMSO-d_6) δ 2.5 (bd, 2 H, C-3 CH_2), 2.7 (s, 3 H, CH_3), 3.2 (m, 2 H, C-2 CH_2), 3.7 (m, 2 H, C-6 CH_2), 6.2 (bs, 1 H, C-5 olefinic H), 7.5 (m, 5 H, ArH); GCMS (temperature program, 50 °C for 1 min followed by a ramp of 25 °C/min for 10 min) showed a single peak at (t_R = 6.3) M^+ 197. Anal. ($C_{16}H_{17}NO_4·0.75H_2O$) C, H, N.

***m*-CBA Salt of 4-Ethynyl-1-methyl-1,2,3,6-tetrahydropyridine *N*-Oxide (58).** A solution of the tetrahydropyridine free base **21** obtained from the corresponding oxalate salt (0.41 g, 1.94 mmol) and 60% *m*-CPBA (0.365 g, 2.1 mmol) was stirred at 0 °C for 1 h. Removal of the solvent under reduced pressure gave a yellow oil which was stirred in diethyl ether to yield the desired product as a crystalline *m*-CBA salt in 61% yield: mp 77–8 °C; ^{1}H NMR ($CDCl_3$) δ 7.2–8.0 (m, 4 H, ArH), 6.0 (s, 1 H, C-5 H), 3.9–4.5 (dd, 2 H, C-6 H), 3.6–3.9 (m, 2 H, C-2 H), 3.5 (s, 3 H, NCH_3), 3.1 (s, 1 H, acetylenic H), 2.5–3.0 (m, 2 H, C-3 H). Anal. ($C_{15}H_{16}ClNO_3$) C, H, N.

***m*-CBA Salt of (*E*)-1-Methyl-4-(2-phenylethenyl)-1,2,3,6-tetrahydropyridine *N*-Oxide (59).** This compound was obtained in a identical fashion in 74% yield: mp 114–116 °C; ^{1}H NMR ($CDCl_3$) δ 7.26–8.0 (m, 9 H, ArH), 6.7–6.8 (d, 1 H, olefinic H), 6.5–6.6 (d, 1 H, olefinic H), 5.74 (s, 1 H, C-5 olefinic H), 4.1–4.58 (m, 2 H, C-6 CH_2), 3.7–3.9 (m, 2 H, C-2 CH_2), 3.5 (s, 3 H, CH_3), 2.7–2.95 (m, 2 H, C-3 CH_2). Anal. ($C_{21}H_{22}ClNO_3$) C, H, N.

4-Ethynyl-1-methyl-2,3-dihydropyridinium Perchlorate (53·ClO_4). This compound was obtained in 61% yield following the procedure for the preparation of **52**·ClO_4: mp 112 °C dec; UV (CH_3CN) λ_{max} 308 (ϵ 6000); ^{1}H NMR (DMSO-d_6) δ 8.57 (bs, 1 H, C-6 H), 6.77 (bs, 1 H, C-5 H), 5.34 (s, 1 H, acetylenic H), 3.8–3.9 (t, 2 H, C-2 CH_2), 3.62 (s, 3 H, CH_3), 2.77–2.84 (t, 2 H, C-3 CH_2). Anal. ($C_8H_{10}ClNO_4·0.5H_2O$) C, H, N.

(*E*)-1-Methyl-4-(2-phenylethenyl)-2,3-dihydropyridinium Perchlorate (54·ClO_4). This compound was obtained in 82% yield following the procedure for the preparation of **52**·ClO_4: mp 172–4 °C; UV (MeOH) λ_{max} 364 (ϵ 24 000); ^{1}H NMR (DMSO-d_6) δ 8.5 (d, 1 H, C-6 H), 7.2–7.7 (m, 7 H, ArH and olefinic H), 6.6 (bs, 1 H, C-5 H), 4.0 (t, 2 H, C-2 CH_2), 3.6 (s, 3 H, CH_3), 3.0 (t, 2 H, C-3 CH_2). Anal. ($C_{14}H_{16}ClNO_4$) C, H, N.

4-Ethenyl-1-methylpiperidin-4-ol Oxalate (55). A solution of 1-methyl-4-piperidone (**56**, 2.26 g, 20 mmol) in diethyl ether (50 mL) treated with vinylmagnesium bromide (30 mL, 1.0 M solution in THF, 30 mmol) was heated under reflux for 1.5 h. The reaction was quenched with 1.0 M NaOH, and the resulting mixture was extracted with diethyl ether (2 × 75 mL). The combined organic extracts were dried ($MgSO_4$) and filtered, and

oxalic acid (1.7 g, 18.8 mmol) in diethyl ether (15 mL) was added to precipitate a colorless oil which solidified upon standing to afford 2.4 g (52%) of the desired product. An analytical sample was prepared by recrystallization from acetone/diethyl ether: mp 125–7 °C; ^{1}H NMR (CD_3OD) δ 1.6–1.9 (bd, 4 H, C-3 and C-5 CH_2), 2.8 (s, 3 H, CH_3), 3.2 (m, 4 H, C-2 and C-6 CH_2), 5.1 (d, 1 H, J = 11 Hz), 5.2 (d, 1 H, J = 18 Hz), 5.9 (m, 1 H, olefinic H); GCMS (temperature program, 50 °C for 1 min followed by a temperature ramp of 25 °C/min for 10 min) showed a single peak (t_R = 2.57 min) M^+ 141. Anal. ($C_{10}H_{17}NO_5$) C, H, N.

4-Ethenyl-1-methyl-2,3-dihydropyridinium Perchlorate (52·ClO$_4$). A solution of 60% *m*-CPBA (0.4 g, 2.33 mmol) and the free base of **55** (0.3 g, 2.2 mmol) was stirred at 0 °C for 1 h. The solvent was removed under reduced pressure and the oily residue was chromatographed on basic alumina with CH_2Cl_2/MeOH (90:10) to yield the *N*-oxide **57** as a white solid (0.22 g, 68%): mp 172–4 °C; ^{1}H NMR ($CDCl_3$) δ 5.9–6.0 (dd, 1 H, C-5 H), 5.3 (dd, 1 H, J = 17 Hz), 5.1 (dd, 1 H, J = 10 Hz), 4.6 (bs, 1 H, OH), 3.6 (m, 2 H, C-6 CH_2), 3.3 (s, 3 H, CH_3), 3.1 (m, 2 H, C-2 CH_2), 1.5–2.5 (m, 4 H, C-3 and C-5 CH_2). TFAA (1.33 g, 6.35 mmol) was added dropwise to a CH_2Cl_2 solution of **57** (0.2 g, 1.27 mmol) under a nitrogen atmosphere at 0 °C. After the reaction mixture was stirred at 0 °C for 1 h, the solvent was removed under reduced pressure and the residue was treated with 0.2 mL of 70% methanolic perchloric acid solution to afford **52** as the perchlorate salt in 67% yield: mp 95 °C; ^{1}H NMR (DMSO-d_6) δ 2.89 (t, 2 H, C-3 CH_2), 3.61 (s, 3 H, CH_3), 3.9 (t, 2 H, CH_2), 5.8 (d, 1 H, J = 10 Hz), 6.09 (d, 1 H, J = 17 Hz), 6.5 (d, 1 H, C-5 H), 6.7–6.8 (dd, 1 H, olefinic H), 8.6 (d, 1 H, C-6 H); UV (H_2O) λ_{max} 309 (ε 6000). Anal. ($C_8H_{12}ClNO_4 \cdot 0.25H_2O$) C, H, N.

4-[2-(*N*-Methylanilino)ethenyl]-1-methyl-2,3-dihydropyridinium Perchlorate (72·ClO$_4$). A reaction mixture containing **53**·ClO_4 (0.1 g, 0.45 mmol) and *N*-methylaniline (**70**, 48 μL, 0.45 mmol) in CD_3CN (1 mL) was allowed to stand for 6 h at room temperature following which the solvent was removed under reduced pressure and the residue was crystallized from CH_2Cl_2/diethyl ether to afford 60 mg (41%) of a fluffy red solid: mp 147–8 °C; UV (CH_3CN) λ_{max} 464 nm (ε 51 000); ^{1}H NMR (DMSO-d_6) δ 2.9 (t, 2 H, C-3 H), 3.3 (s, 3 H, CH_3), 3.5 (s, 3 H, CH_3), 3.6 (t, 2 H, C-2 H), 5.9 (bs, 2 H, olefinic H), 7.5 (m, 5 H, ArH), 8.0 (dd, 2 H, olefinic H); FABMS (M^+ 227). Anal. ($C_{15}H_{19}ClN_2O_4$) C, H, N.

Enzymology Studies. The isolation and purification of MAO-B from beef liver was carried out using the methodology reported earlier by Salach.[56] Protein content was determined using the Sigma diagnostic Lowry protein assay kit. The activity of the enzyme was determined spectrophotometrically at 250 nm (benzaldehyde) using benzylamine (2 mM) as substrate on a Beckman DU 50 spectrophotometer using initial rate measurements (30–120 s). A unit of activity (equivalent to 3.7 nmol of protein) is defined as the amount of enzyme required to convert 1 μmol of benzylamine to benzaldehyde in 1 min.

Substrate and Inactivation Studies with MAO-B. General Methods. All UV spectral data were obtained on a Beckman DU-50 spectrophotometer. Stock solutions of the test compounds were prepared in phosphate buffer (100 mM, pH 7.4) and diluted with the same buffer to give concentrations of 0.05, 0.067, 0.10, 0.20, 1.0, and 2.5 mM. A 480-μL aliquot of each solution was added to a sample cuvette which then was placed in a spectrophotometer that was maintained at 37 °C. After a 2-min equilibration period, 0.05 unit of MAO-B in 20 μL of buffer was added, and the rates of oxidation were determined by monitoring the increment in absorbance of the corresponding dihydropyridinium product formed during the enzyme catalyzed reaction. MAO-B inhibition studies were conducted as follows: A 250 mM stock solution of the test compound in 100 mM sodium phosphate buffer (pH = 7.4) was diluted with buffer to obtain solutions of 0.1–2 mM. MAO-B (0.5 unit) was added, and incubations were carried out with gentle agitation in a water bath incubator at 37 °C. A 50-μL aliquot was removed at 0, 5, 10, 15, and 20 min and was added to a sample cuvette containing 5 mM MPTP in phosphate buffer (450 μL, pH = 7.4). The rate of $MPDP^+$ formation was determined by monitoring the absorbance at 343 nm every 3 s for 2 min.

Acknowledgment. This study was supported by the National Institute of Neurological and Communicative Disorders and Stroke (NS 28792) and the Harvey W. Peters Center for the Study of Parkinson's Disease.

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