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Design, Synthesis, and Biological Evaluation of Novel 4-Substituted 1-Methyl-1,2,3,6-tetrahydropyridine Analogs of MPTP

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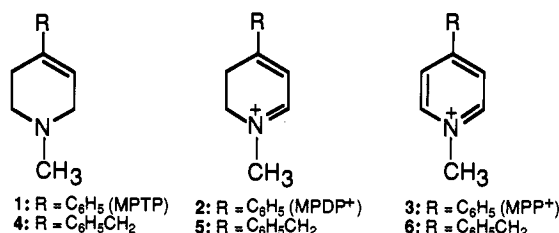
The exceptionally good MAO-B substrate properties of several 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) derivatives have prompted studies to evaluate the corresponding properties of tetrahydropyridines bearing heteroatom-linked groups at C-4. The 1-methyl-4-phenoxy-1,2,3,6-tetrahydropyridine analog proved to be an excellent MAO-B substrate. Unlike analogs bearing hydrocarbon substituents at C-4, the resulting dihydropyridinium metabolite did not undergo further oxidation to the pyridinium compound but rather underwent hydrolytic cleavage. This observation has led to studies designed to explore the possibility of developing novel, nontoxic derivatives of MPTP bearing potential pharmacologically active leaving groups at C-4. In this paper we report the results of synthetic and metabolic studies on a series of tetrahydropyridine analogs of MPTP with oxygen, sulfur, and carbamoyloxy derivatives on C-4 which serve as model compounds to evaluate the scope of this prodrug concept.

Introduction

The discovery that the cyclic tertiary allylamine 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, 1, Chart I) causes the selective destruction of nigrostriatal neurons and a parkinsonian syndrome in man and subhuman primates has resulted in extensive studies on its mechanism of action.[1] It is now generally accepted that the neurotoxic effects of 1 are dependent on its extraneuronal MAO-B-catalyzed oxidation to give the dihydropyridinium intermediate 2 which then spontaneously oxidizes to the pyridinium species MPP^+ (3).[2,3] Additional requirements for toxicity include the active transport of MPP^+ first into the striatal nerve terminals via the dopamine (DA) uptake system[4] and finally into the inner mitochondrial membrane[5] where it inhibits mitochondrial respiration leading to ATP depletion[6] and cell death.

Numerous studies have been pursued to delineate the structural features present in 1 that are associated with its unusual MAO-B substrate properties.[7] Among the several analogs that have been made, the 4-benzyl derivative 4 was found to be an even better MAO-B substrate than MPTP.[8,9] The observation that 4 was not neurotoxic in an MPTP responsive mouse model could be related to the inefficient conversion of the dihydropyridinium metabolite 5 to the neurotoxic pyridinium species 6. Since the ease with which 5 undergoes deprotonation at the benzylic position could account for the poor autoxidation of 5 to 6,[10] we decided to examine the interactions of the corresponding 4-phenoxytetrahydropyridine derivative 7 with MAO-B. Compound 7 (Scheme I) was expected to display good MAO-B substrate properties and to undergo further conversion to the suspected neurotoxic pyridinium metabolite 9.

Chart I



Results and Discussion

The synthesis of 7 was readily accomplished by reduction of the corresponding phenoxypyridinium species 9 which in turn was obtained by treatment of 4-chloro-1-methylpyridinium iodide (10) with phenol in the presence of potassium carbonate (Scheme I). Incubation of 7 with purified MAO-B isolated from beef liver led to the rapid formation of a compound with λ_{max} of 324 nm which we tentatively assigned to the expected dihydropyridinium metabolite 8. The formation of this product was com-

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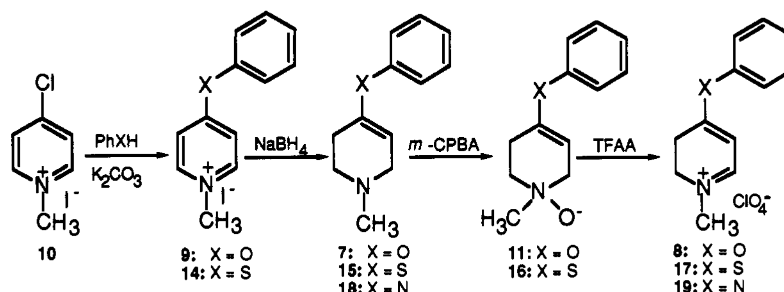
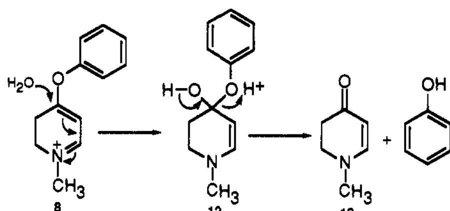
Scheme I



Scheme II



pletely inhibited by pretreatment of the enzyme preparation with 10^{-6} M deprenyl, a potent inactivator of MAO-B.[11] The absence of a species absorbing maximally at 225 nm, the λ_{max} for the synthetic pyridinium species 9, suggested that the 324-nm absorbing product was stable to oxidation.

In order to confirm the structure of the metabolite, an authentic sample of dihydropyridinium species 8 was prepared by *m*-chloroperoxybenzoic acid (*m*-CPBA) oxidation of 7 to yield the corresponding *N*-oxide 11 followed by reaction of 11 with trifluoroacetic anhydride as depicted in Scheme I.[12] The structure of 8 as its perchlorate salt was confirmed by analysis of its ^{1}H NMR spectrum which displayed the expected signals including the diagnostic resonances at 8.4 and 5.4 ppm for the C-6 azomethine proton and the C-5 olefinic proton, respectively. The λ_{max} for synthetic 8 was found to be 316 nm. Upon standing, however, this value shifted to 324 nm, the same absorbance observed in the MAO-B incubation mixture of the tetrahydropyridine 7. Examination of a fresh incubation mixture of 7 a few minutes after the addition of enzyme revealed formation of the dihydropyridinium metabolite (λ_{max} 316 nm) and its rapid conversion to the 324-nm absorbing species. A consideration of its potential reactivity suggested the possibility that 8 was undergoing hydrolytic cleavage via the hemiketal 12 to yield phenol and the amino enone 13 (Scheme II). This proposal was documented fully by GC-EI mass spectral analysis of an extract of the incubation mixture which displayed two major components, one of which (R_t = 1.38 min) was identified as phenol by comparison with an authentic sample. The second component (R_t = 2.99 min) had a molecular ion (the base peak) at m/z 111 which corresponds to the molecular weight of the amino enone 13. Comparison of the GC-EI mass spectrum of the synthetic amino enone, obtained by oxidation[13] of 1-methyl-4-piperidone with mercuric acetate in acetic acid,[14] with that of the metabolite unambiguously established its identity.

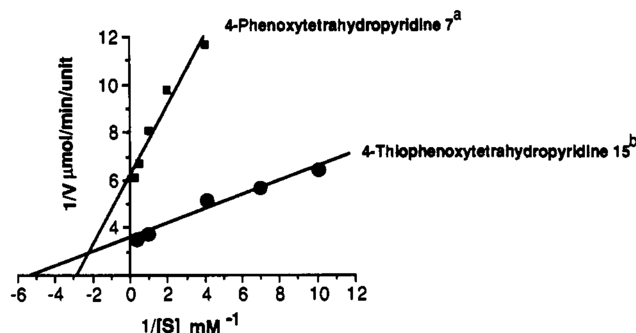


Figure 1. Lineweaver–Burk plot ($1/V$ vs $1/[S]$) corresponding to the MAO-B-catalyzed oxidation of tetrahydropyridine analogs: (a) monitored at 324 nm (λ_{max} of amino enone 13); (b) monitored at 352 nm (λ_{max} of dihydropyridinium species 17).

Table I. Kinetic Parameters Associated with the MAO-B-Catalyzed Oxidations of Various 4-Substituted 1-Methyl-1,2,3,6-tetrahydropyridine Derivatives

compd	C(4) substituent on tetrahydropyridine	V_{max} (nmol/min per unit of MAO-B)[a]	K_M (μM)	V_{max}/K_m (μL/min per unit of MAO-B)
1	C_6H_5	204	390	523
4	$C_6H_5CH_2$	222	83	2674
7	OC_6H_5	161	234	688
15	SC_6H_5	274	80	3425
35	$OCON(CH_3)_2$	401	7100	57
36	$OCONCH_3C_6H_5$	255	3850	67

[a] One unit is defined as the amount of enzyme required to convert one micromole of benzylamine to benzaldehyde in 1 min.

The rapid hydrolysis of the initially formed phenoxydihydropyridinium metabolite complicated our attempts to assess the kinetic parameters associated with the MAO-B-catalyzed oxidation of the phenoxytetrahydropyridine substrate. Apparent V_{max} and K_M values were obtained from a double reciprocal plot (Figure 1) of the initial rate data (30–180 s after addition of enzyme) corresponding to amino enone formation (monitored spectrophotometrically at 324 nm) at various substrate concentrations. The results (Table I) show that the binding of the phenoxy compound to MAO-B (K_M = 234 μM) is comparable to that of MPTP (K_M = 390 μM),[9] consistent with results obtained with other flexible tetrahydropyridine analogs of MPTP.[15]

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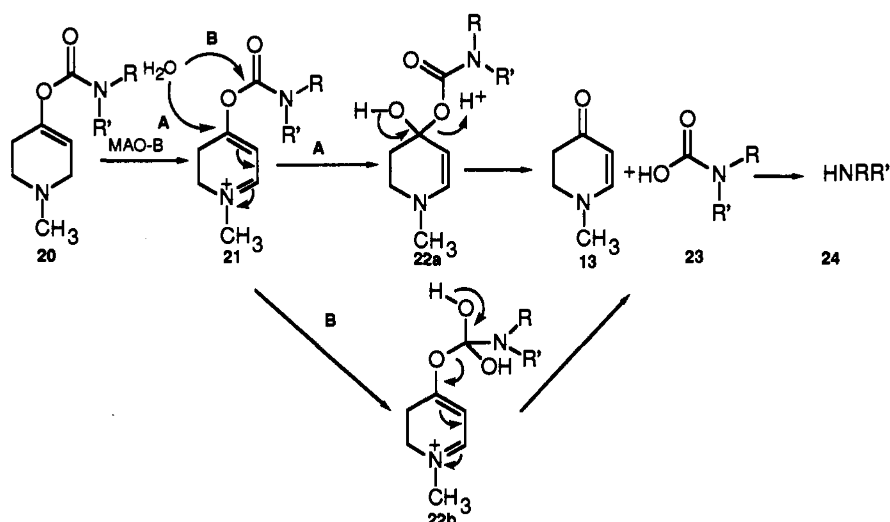
Scheme III

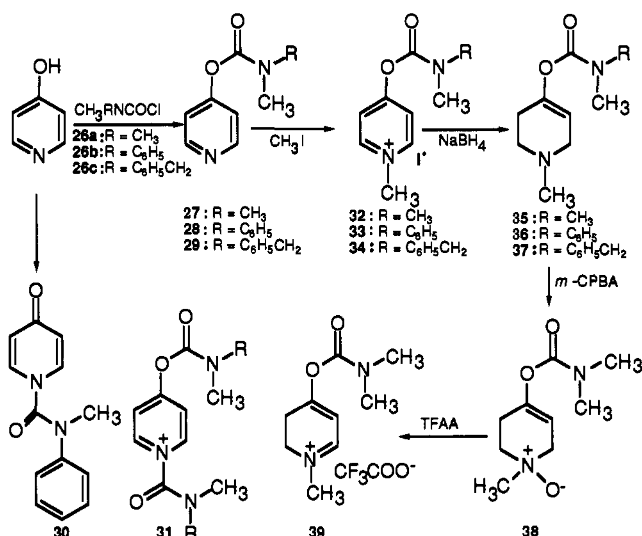


The excellent MAO-B substrate properties of **7** and the susceptibility of the 1,2-dihydropyridinium metabolite **8** to hydrolytic cleavage suggested the possibility of designing prodrugs in which a pharmacologically active moiety is attached to C-4 of the 1,2,3,6-tetrahydropyridine system via a heteroatom. Subsequent MAO-B-catalyzed oxidation to the dihydropyridinium metabolite could result in liberation of this pharmacophore via hydrolytic cleavage as was observed with the phenoxy compound. In order to assess the scope of this prodrug concept, we have extended our synthetic targets to tetrahydropyridine derivatives in which a sulfur- or nitrogen-containing moiety is incorporated into the group attached to the C-4 position.

The thiophenoxy derivative **15** was synthesized via sodium borohydride reduction of the corresponding pyridinium intermediate **14** which in turn was prepared by condensation of thiophenol with **10** (Scheme I). The V_{max} (274 nmol/min per unit) and K_M (80 μM) values for this MPTP analog were similar to those of the 1-methyl-4-benzyl analog **4** (Table I). The dihydropyridinium metabolite **17**, however, was stable under the incubation conditions to hydrolysis and also did not undergo oxidation to the pyridinium species **14** as is the case with $MPDP^+$.[16] Synthetic **17**, prepared by treatment of the corresponding *N*-oxide **16** with trifluoroacetic anhydride, displayed identical physicochemical properties to those of the metabolite. The stability of **14** to hydrolysis may be rationalized on the basis of the decreased electronegativity of the sulfur atom relative to the oxygen atom of the phenoxy compound. On the other hand, no obvious explanation for the oxidative stability of this compound relative to $MPDP^+$ is apparent.

The possibility of examining the substrate properties of C-4 amino substituted analogs (**18**) of MPTP was attractive since the expected hydrolytic instability of the corresponding dihydropyridinium metabolites (**19**) offered the possibility of designing prodrugs of pharmacologically active amines. A direct approach to evaluate this possibility was considered unfeasible since the enamine functionality present in **18** would be expected to hydrolyze rapidly under physiological conditions. Consequently we elected to prepare the more stable carbamoyloxy system **20** with the anticipation that the corresponding MAO-B-generated dihydropyridinium metabolite **21** would hydrolyze to give the desired amine **24** via the carbamic acid intermediate **23** as shown in Scheme III.

Scheme IV



Our approach to the model carbamates **35–37** (Scheme IV) required preparation of the corresponding (carbamoyloxy)pyridine intermediates **27–29** via reaction of 4-hydroxypyridine (**25**) with the appropriate carbamoyl chloride. Treatment of **25** with 1 mol equiv of the *N*-phenyl-*N*-methylcarbamoyl chloride (**26b**) gave as the principal product a compound isomeric with the desired carbamate **33** which was characterized as the urea derivative **30**. The ^{1}H NMR spectrum of this neutral material showed coupled doublets at δ 7.5 and δ 6.0 which were assigned to the olefinic protons at $C_{2,6}$ and $C_{3,5}$, respectively. This problem could be overcome by using a 2 to 1 molar ratio of the carbamoyl chloride **26** to 4-hydroxypyridine to insure a quantitative conversion to the desired carbamate even though some of the product might also be in the form of the bis adduct **31**. Upon workup the *N*-carbamoyl group presumably undergoes hydrolysis to yield the desired product. In this way yields in excess of 50% of the intermediate *N,N*-dimethyl- and *N*-methyl-*N*-phenylcarbamates (**27** and **28**, respectively), were obtained.

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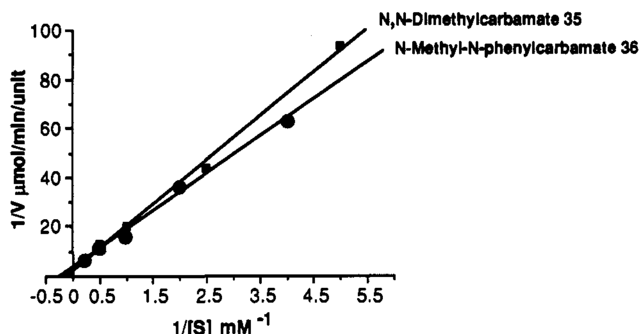

Figure 2. Lineweaver–Burk plot (1/*V* vs 1/[S]) corresponding to the MAO-B-catalyzed oxidation of the carbamates analogs as monitored by amino enone formation.

The *N*-benzyl-*N*-methylcarbamate **29** was prepared by the same route except that the corresponding carbamoyl chloride **26c** was not commercially available and had to be synthesized by treating *N*-methyl-*N*-benzylamine with triphosgene in the presence of triethylamine.[17]

The above pyridyl carbamates were treated with iodomethane to give the corresponding pyridinium iodides (**32–34**) which were converted to the final tetrahydropyridine products (**35–37**) with sodium borohydride.[18] All of the tetrahydropyridine products exhibited weak molecular ions when analyzed by GC-EIMS. The *N,N*-dimethyl- and the *N*-phenyl-*N*-methyl carbamates (**35** and **36**, respectively) showed the respective carbamoyloxy ions as their base peaks (*m/z* 72 and 134, respectively). On the other hand the *N*-benzyl derivative **37** gave the characteristic tropylium ion (*m/z* 91) as the most intense fragment ion. The ^{1}H NMR spectra all displayed the characteristic olefinic signal near δ 5.5 ppm.

Incubations of the tetrahydropyridyl carbamate analogs **35–37** with MAO-B resulted in the rapid formation of a chromophore with λ_{max} 324 consistent with the enzyme-catalyzed formation and subsequent spontaneous hydrolysis of the corresponding [(*N,N*-dialkylcarbamoyl)oxy]-dihydropyridinium metabolites. As with the phenoxy analog, 10^{-6} M deprenyl[11] completely inhibited the oxidation of the carbamates. Estimates of V_{max} and K_M values were obtained by constructing double reciprocal plots of the initial rates of amino enone formation vs time at a series of substrate concentrations as in the case of the phenoxy analog (Figure 2). The *N,N*-dimethyl analog **35** and the *N*-phenyl-*N*-methyl analog **36** proved to be excellent substrates (V_{max} 401 nmol/min per unit of MAO-B and 255 nmol/min per unit of MAO-B, respectively). The K_M values (7100 μM for **35** and 3850 μM for **36**) show that these compounds have a lower affinity for the enzyme than the phenoxy and the thiophenoxy analogs. It is conceivable that unfavorable interactions between the polar carbamoyl group and the enzyme may interfere with substrate binding. In contrast to the good substrate properties of compounds **35** and **36**, the *N*-benzyl analog **37** was stable in the presence of MAO-B. Consequently, steric constraints appear to limit the size of the substituent at C-4 that is tolerated in the active site. Similar conclusions have been reached by Efange in his studies on a series of MPTP substrate analogs.[15]

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The pathway for the hydrolysis of the 4-(carbamoyloxy)-tetrahydropyridines to the amino enone **13** was investigated with the aid of $H_2{}^{18}O$. We reasoned that attack of water at the electropositive C-4 carbon atom of the dihydropyridinium ring would lead to the tetrahedral hemiketal intermediate **22a** (Scheme III) with incorporation of ^{18}O into the amino enone (pathway A) whereas a pathway proceeding via intermediate **22b** would yield amino enone containing no ^{18}O (pathway B). In order to characterize this hydrolytic pathway we undertook the synthesis of the dihydropyridinium intermediate **39**. The synthetic standard was prepared as described earlier via oxidation of the tetrahydropyridine **35** (with *m*-CPBA) followed by treatment of the resulting *N*-oxide **38** with trifluoroacetic anhydride in dichloromethane. Instability of the dihydropyridinium product precluded its isolation in pure form and therefore the crude product was treated with water enriched with 20.5% ^{18}O. Analysis of the resulting mixture by GC-EIMS revealed the parent ion for the amino enone **13** at *m/z* 111 (^{16}O) and a second ion at *m/z* 113 (^{18}O) the intensity of which was 20% that of the *m/z* 111 ion. In a parallel experiment we showed that the oxygen atom of the amino enone does not undergo spontaneous exchange with the 20% ^{18}O-enriched water. These results are fully consistent with a hydrolytic pathway involving initial 1,4-addition of water to the dihydropyridinium species and subsequent cleavage of the resulting hemiketal to yield the amino enone and, following decarboxylation of the carbamic acid **23**, the secondary amine group as shown in pathway A in Scheme III.

In summary, the above results suggest that tetrahydropyridine analogs of MPTP bearing C-4 heteroatom-linked substituents with limited molecular volumes exhibit good substrate properties for MAO-B. However unlike MPTP, the corresponding MAO-B-catalyzed dihydropyridinium species do not convert to the pyridinium product but instead may undergo hydrolytic cleavage to form the corresponding amino enone and release the heteroatom-linked moiety. The scope of this pathway suggests that good substrate properties are retained with oxygen-, sulfur-, and nitrogen-containing groups in the tetrahydropyridine system linked either directly or, as illustrated with the nitrogen-containing model compounds, via a carbonyloxy group. This system may be exploited to develop prodrugs which may target the MAO-B-rich cells in the central nervous system. The preliminary results reported here, however, suggest that the spatial features of the active site of MAO-B will limit the size of the tetrahydropyridyl C-4 substituent and therefore the opportunities of drug development.

Experimental Section

All chemicals were reagent or HPLC grade. Proton NMR spectra were recorded on a Bruker WP 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 chromatography connected to an HP 5970 EI mass spectrometer with a HP series computer and an HP methylsilicon capillary column (12 m × 0.2 mm) employing helium as the carrier gas (40 mL/min). Enzyme reaction rates were monitored on a Beckman Model DU-50 spectrophotometer. Melting points were obtained on a Thomas-Hoover melting point apparatus and are uncorrected. Microanalyses were performed by the Atlantic Microlab, Inc., Norcross, GA.

1-Methyl-4-phenoxypyridinium Iodide (9). Potassium carbonate (3.7 g, 26.8 mmol) was added to a mixture of 4-chloropyridine hydrochloride (1.0 g, 6.7 mmol) phenol (10 mL, 11.4 mmol) and iodomethane (2.08 mL, 33.5 mmol), and the resulting solution was stirred at room temperature for 24 h. The reaction mixture was concentrated in vacuo, and the residue obtained was recrystallized from absolute ethanol to give 1.0 g (48%) of the desired product **9**: mp 221–224 °C; UV (water) λ_{max} 225 (ϵ = 19 600 M^{-1}); ^1H NMR (DMSO-d_6) δ 4.25 (s, 3 H, N CH_3), 7.3–7.7 (m, 7 H, ArH, C 3 and C 5), 8.85 (d, 2 H, C 2 and C 6). Anal. ($C_{12}H_{12}NOI$) C, H, N.

The Oxalate Salt of 1-Methyl-4-phenoxy-1,2,3,6-tetrahydropyridine (7). Sodium borohydride (0.18 g, 4.8 mmol) was added in small portions to a solution of 1-methyl-4-phenoxypyridinium iodide (**9**, 0.5 g, 1.6 mmol) in dry methanol (50 mL) at 0 °C for 15 min. The solvent then was removed under reduced pressure, and the residue was triturated with anhydrous ether (20 mL). The solution was filtered through a plug of florosil, and the filtrate was added to a solution of oxalic acid (0.145 g, 1.6 mmol) in anhydrous ether (20 mL) at 0 °C. The solid obtained was filtered and recrystallized from absolute ethanol/ether to afford 0.3 g (67%) of **7**: mp 171–173 °C, UV (water) λ_{max} 215 nm (ϵ = 15 100 M^{-1}); GC-EIMS (free base, m/z, %) [GC temperature program; 80 °C for 1 min, then 20 °C/min up to 260 °C (t_R 5.92 min)] 189 ($M^{\cdot+}$); ^1H NMR (DMSO-d_6) δ 2.4 (t, 2 H, C 3), 2.8 (s, 3 H, N CH_3), 3.3 (t, 2 H, C 2), 3.65 (d, 2 H, C 6), 4.85 (d, 1 H, C 5), 7.5–7.0 (m, 5 H, ArH). Anal. ($C_{14}H_{17}NO_5$) C, H, N.

***m*-Chlorobenzoate Salt of 1-Methyl-4-phenoxy-1,2,3,6-tetrahydropyridine *N*-Oxide (11).** *m*-CBPA (380 mg, 2.2 mmol) was added to a solution of 1-methyl-4-phenoxy-1,2,3,6-tetrahydropyridine (**7**, 330 mg, 1.7 mmol) in dry dichloromethane (25 mL) at 0 °C under a nitrogen atmosphere. The solution was stirred at 0 °C for 1 h, and the solvent was removed in vacuo. The residue obtained was triturated with dry ether at 0 °C to afford 0.540 g (88%) of white crystals of the **11** as the *m*-chlorobenzoic acid salt: mp 107–109 °C; ^1H NMR ($CDCl_3$) δ 2.6 (m, 1 H, C 3), 3.05 (m, 1 H, C 3), 3.62 (s, 3 H, N CH_3), 3.8 (q, 1 H, C 2), 4.2–4.0 (m, 2 H, C 2 and C 6), 4.6 (t, 1 H, C 5), 7.0–8.1 (m, 9 H, ArH). This salt was converted to the desired dihydropyridinium product without further characterization.

1-Methyl-4-phenoxy-2,3-dihydropyridinium Perchlorate (8). Trifluoroacetic anhydride (0.43 mL, 3.0 mmol) was added to a solution of 1-methyl-4-phenoxy-1,2,3,6-tetrahydropyridine *N*-oxide (**11**, 125 mg, 0.61 mmol) in dry dichloromethane (60 mL) at 0 °C under a nitrogen atmosphere. The solution was stirred for 1 h, and 70% perchloric acid (90 mg, 0.61 mmol) was added and the stirring was continued for 1 h. The solvent was removed in vacuo, and the resulting oil was triturated with dry ether. The solid thus obtained was recrystallized from anhydrous acetonitrile/ether to yield 0.120 g (68%) of **8**: mp 134–135 °C; UV (phosphate buffer, pH 7.4) λ_{max} 316 nm (ϵ = 12 260 M^{-1}); ^1H NMR (DMSO-d_6) δ 2.6 (t, 2 H, C 3), 3.25 (s, 3 H, N CH_3), 3.65 (t, 2 H, C 2), 5.4 (d, 1 H, C 5), 6.7–7.3 (m, 5 H, ArH), 8.4 (d, 1 H, C 6). Anal. ($C_{12}H_{14}ClNO_5$) C, H, N.

1-Methyl-2,3-dihydro-4-pyridone (13).[13] To a solution of 1-methyl-4-piperidone (2 mL, 16.3 mmol) in 5% acetic acid (150 mL) was added mercuric acetate (15.6 g, 48.8 mmol), and the reaction mixture was heated to about 90 °C for 18 h with stirring. The reaction mixture was cooled in an ice bath and was saturated with hydrogen sulfide. The resulting mercuric sulfide was filtered and washed with 5% acetic acid. The combined filtrates were stirred vigorously with dichloromethane (200 mL) while an excess of 25% sodium hydroxide solution was added carefully in the cold. The dichloromethane was promptly separated, and the aqueous layer was extracted with dichloromethane (3 × 200 mL). The combined extracts were dried (magnesium sulfate) and filtered. The solvent was removed in vacuo, and the crude product was purified by column chromatography (alumina, ethyl acetate) to give 1.6 g (60%) of crude product. This product was purified further by silica gel preparative thin-layer chromatography (PTLC) with ethyl acetate/methanol (4:1) to give **13** as a yellow oil: GC-EIMS (m/z, %) [GC temperature program; 50 °C for 1 min, then 25 °C/min up to 275 °C (t_R 3.16 min)] 111 ($M^{\cdot+}$, 100), 82 (70), 55 (80); ^1H NMR ($CDCl_3$) δ 2.25 (t, 2 H, C 5), 3.04 (s, 3 H, N CH_3), 3.44 (t, 2 H, C 2), 4.98 (d, 1 H, C 5), 6.98 (d, 1 H, C 6).[13]

1-Methyl-4-thiophenoxypyridinium Iodide (14). A solution of 4-chloro-1-methylpyridinium iodide[19] (**10**, 1.0 g, 3.9 mmol), thiophenol (0.4 mL, 3.9 mmol), and triethylamine (0.82 mL, 5.85 mmol) in dry acetone (50 mL) was heated under reflux for 7 h. The solvent was evaporated in vacuo, and the resulting residue, dissolved in saturated sodium bicarbonate (20 mL), was extracted with chloroform (3 × 20 mL). The combined organic fractions were dried (magnesium sulfate) and the solvent removed under reduced pressure. Recrystallization of the resulting solid from acetonitrile afforded 0.71 g (55%) of **14**: mp 171–173 °C; GC-EIMS (free base, m/z, %) [GC temperature program; 40 °C for 1 min, then 20 °C/min up to 260 °C (t_R 7.586)] 187 ($M^{\cdot+}$, 100); ^1H NMR ($CDCl_3$) δ 4.52 (s, 3 H, N CH_3), 7.40 (d, 2 H, C 3, and C 5), 7.54–7.70 (m, 5 H, ArH), 9.0 (d, 2 H, C 2 and C 6). Anal. ($C_{12}H_{12}INS$) C, H, N.

The Oxalate Salt of 1-Methyl-4-thiophenoxy-1,2,3,6-tetrahydropyridine (15). This compound was prepared in 75% yield by following the procedure described for **7**: mp 159–161 °C; GC-EIMS (free base, m/z, %) [GC temperature program; 80 °C for 1 min, then 20 °C/min up to 260 °C (t_R 5.807 min)] 205 ($M^{\cdot+}$), 96 (100); ^1H NMR (DMSO-d_6) δ 2.4 (m, 2 H, C 3), 2.8 (s, 3 H, N CH_3), 3.3 (t, 2 H, C 2), 3.75 (s, 2 H, C 6), 5.85 (s, 1 H, C 5), 7.4 (m, 5 H, ArH). Anal. ($C_{16}H_{17}NO_4S$) C, H, N.

1-Methyl-4-thiophenoxy-2,3-dihydrohydropyridinium Perchlorate (17). This compound was prepared by following the procedure described for **8**. The *m*-chlorobenzoate salt **16** was prepared in 49% yield and was converted without purification to the desired dihydropyridinium **17**: mp 98–100 °C; UV (phosphate buffer, pH = 7.4) λ_{max} 352 nm (ϵ = 15 200 M^{-1}); ^1H NMR (DMSO-d_6) δ 3.0 (t, 2 H, C 5), 3.5 (s, 3 H, N CH_3), 3.8 (t, 2 H, C 6), 5.7 (d, 1 H, C 3), 7.3–7.7 (m, 5 H, ArH), 8.4 (d, 1 H, C 2). Anal. ($C_{12}H_{14}ClNO_4S$) C, H, N.

1-Methyl-4-[[(*N*,*N*-dimethylamino)carbonyl]oxy]pyridinium Iodide (32). A solution of 4-hydroxypyridine **25** (3.8 g, 40 mmol) and *N*,*N*-dimethylcarbamoyl chloride (**26a**) (8.56 g, 80 mmol) in acetonitrile (80 mL) was heated under reflux and nitrogen for 12 h. The reaction mixture then was concentrated in vacuo to give an oily residue which was treated with saturated sodium bicarbonate solution (30 mL). The resulting solution was extracted with dichloromethane (30 mL), and the combined organic layers were washed with water (2 × 30 mL), dried (sodium sulfate), and evaporated in vacuo to give an oil. Flash chromatography of the residue over silica gel and elution with ethyl acetate/methanol (9:1) gave the *N*,*N*-dimethylcarbamate **27** (2.19 g, 33%) as an oil. The carbamate thus obtained was dissolved in anhydrous tetrahydrofuran (30 mL), and iodomethane (10.9 g, 4.81 mL, 76 mmol) was added. The mixture was stirred for 36 h at room temperature, and the precipitate obtained was filtered and recrystallized with acetonitrile/ether to give **32** (2.9 g, 73%): mp 131–132 °C; UV (water) λ_{max} 230 nm (ϵ = 18 300 M^{-1}); GC-EIMS (free base, m/z, %) [GC temperature program; 50 °C for 1 min, then 25 °C/min up to 275 °C (t_R 5.297 min)] 166 ($M^{\cdot+}$, 10), 72 (100); ^1H NMR ($CDCl_3$) δ 3.1 (s, 3 H, $CONCH_3$), 3.2 (s, 3 H, $CONCH_3$), 4.6 (s, 3 H, NCH_3), 7.9 (d, 2 H, C 3 and C 5); 9.3 (d, 2 H, C 2 and C 6). Anal. ($C_9H_{13}N_2O_2I$) C, H, N, I.

1-[[(*N*-Methyl-*N*-phenylamino)carbonyl]oxy]-4-pyridinone (30). The compound was prepared by the procedure described for **27** (refer to procedure for **32**) except that 1 equiv of *N*-methyl-*N*-phenylcarbamoyl chloride (**26b**) was used. The product obtained was purified by column chromatography, followed by recrystallization with ethyl acetate/hexane to afford 51% of **30**: mp 132–133 °C; UV (methanol) λ_{max} 272 nm (ϵ = 20 517 M^{-1}); GC-EIMS (m/z, %) [GC temperature program; 100 °C for 1 min, then 25 °C/min up to 275 °C (t_R 6.757 min)] 228 ($M^{\cdot+}$, 30), 134 (100), 106 (56), 77 (40); ^1H NMR ($CDCl_3$) δ 3.5 (s, 3 H, $CONCH_3$), 6.0 (d, 2 H, C 3 and C 5), 7.0–7.3 (m, 5 H, ArH), 7.5 (d, 2 H, C 2 and C 6). Anal. ($C_{13}H_{12}N_2O_2$) C, H, N.

1-Methyl-4-[[(*N*-methyl-*N*-phenylamino)carbonyl]oxy]pyridinium Iodide (33). This compound was prepared by the procedure described for **32** except that the intermediate **28** was column purified using ethyl acetate as a solvent. The solid (**33**) was obtained in 67% yield after recrystallization from methanol/

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ethyl acetate: mp 111–112 °C; UV (water) λ_{max} 226 nm (ϵ = 24 800 M^{-1}); GC-EIMS (free base, m/z, %) [GC temperature program; 100 °C for 1 min, then 25 °C/min up to 275 °C (t_R 5.948 min)] 228 ($M^{•+}$, 22), 134 (100), 106 (57), 77 (38); ^{1}H NMR ($CDCl_3$) δ 3.4 (s, 3 H, $CONCH_3$), 4.6 (s, 3 H, NCH_3), 7.3–7.5 (m, 5 H, ArH), 7.75 (d, 2 H, C 3 and C 5), 9.2 (d, 2 H, C 2 and C 6). Anal. ($C_{14}H_{15}N_2O_2I$) C, H, N, I.

(*N*-Methyl-*N*-benzylamino)carbonyl Chloride (26c). To an ice-cold solution of *N*-methyl-*N*-benzylamine (6.05 g, 49.9 mmol) and triethylamine (5.8 g, 57.3 mmol) in dichloromethane (75 mL) was added dropwise a solution of triphosgene (5.0 g, 16.8 mmol) in dichloromethane (25 mL). The reaction mixture was allowed to stir at room temperature for 2 h and then was washed with water (50 mL), 5% hydrochloric acid (50 mL), saturated sodium bicarbonate solution (50 mL), and finally brine (50 mL). The organic layer was dried (sodium sulfate) and then evaporated in vacuo to give the desired carbamoyl chloride **26c** (6.22 g, 68%) as an oil: GC-EIMS (m/z, %) [GC temperature program; 100 °C for 1 min, then 25 °C/min up to 275 °C (t_R 6.250 min)] 183 ($M^{•+}$, 8), 147 (26), 118 (10), 91 (100), 65 (14). The compound was used for the next step without further characterization.

1-Methyl-4-[[(*N*-methyl-*N*-benzylamino)carbonyl]oxy]-pyridinium Iodide (34). This compound was prepared by the procedure described for **32** except that intermediate **29** was column purified by using ethyl acetate as a solvent. Pure **34** was obtained in 80% yield after recrystallization from acetonitrile and ether: mp 121 °C; UV (water) λ_{max} 230 nm (ϵ = 22 050 M^{-1}); GC-EIMS (free base, m/z, %) [GC temperature program; 100 °C for 1 min, then 25 °C/min up to 275 °C (t_R 6.975 min)] 242 ($M^{•+}$, 19), 148 (12), 91 (100), 65 (10); ^{1}H NMR ($CDCl_3$) δ 3.0 (d, 3 H, $CONCH_3$), 4.65 (m, 5 H, NCH_3, $ArCH_2$), 7.2–7.5 (m, 5 H, ArH), 8.0 (m, 2 H, C 3 and C 5), 9.3 (d, 2 H, C 2 and C 6). Anal. ($C_{15}H_{17}N_2O_2I$) C, H, N, I.

The Oxalate Salt of 1-Methyl-4-[[(*N,N*-dimethylamino)-carbonyl]oxy]-1,2,3,6-tetrahydropyridine (35). This compound was prepared in 95% yield by following the procedure described for **7** except that the compound was recrystallized with methanol/ether: mp 167 °C; GC-EIMS (free base, m/z, %) [GC temperature program; 50 °C for 1 min, then 25 °C/min up to 275 °C (t_R 4.036 min)] 184 ($M^{•+}$, 1), 112 (10), 96 (20), 72 (100); ^{1}H NMR (DMSO-d_6) δ 2.4 (m, 2 H, C 3), 2.7 (s, 3 H, NCH_3), 2.8 (s, 3 H, $CONCH_3$), 2.9 (s, 3 H, $CONCH_3$), 3.2 (t, 2 H, C 2), 3.7 (m, 2 H, C 6), 5.5 (t, 1 H, C 5), 8.2 (br s, 2 H, oxalate). Anal. ($C_{11}H_{18}N_2O_6$) C, H, N.

The Oxalate Salt of 1-Methyl-4-[[(*N*-methyl-*N*-phenylamino)carbonyl]oxy]-1,2,3,6-tetrahydropyridine (36). This compound was prepared in 64% yield by following the procedure described for **7** except that the compound was recrystallized from methanol/ether: mp 133–134 °C; UV (water) λ_{max} 224 nm (ϵ = 6100 M^{-1}); GC-EIMS (free base, m/z, %) [(GC temperature program; 100 °C for 1 min, then 25 °C/min up to 275 °C (t_R 6.543 min)] 246 ($M^{•+}$, 1), 134 (80), 106 (57), 96 (100); ^{1}H NMR (DMSO-d_6) δ 2.4 (m, 2 H, C 3), 2.7 (s, 3 H, N CH_3), 3.2 (m, 5 H, C 2 and NCH_3), 3.7 (m, 2 H, C 6), 5.5 (t, 1 H, C 5), 7.3–7.5 (m, 5 H, ArH). Anal. ($C_{16}H_{20}N_2O_6$) C, H, N.

The Oxalate Salt of 1-Methyl-4-[[(*N*-methyl-*N*-benzylamino)carbonyl]oxy]-1,2,3,6-tetrahydropyridine (37). This compound was prepared in 79% yield by following the method described for **7** except that the compound was recrystallized from acetonitrile/ether: mp 73 °C; UV (water) λ_{max} 215 nm (ϵ = 21 400 M^{-1}); GC-EIMS (free base, m/z, %) [GC temperature; 100 °C for 1 min, then 25 °C/min up to 275 °C (t_R 8.642 min)] 260 ($M^{•+}$, 2), 202 (4), 112 (22), 91 (100), 70 (28); ^{1}H NMR (MeOH-d_4) δ 2.4 (m, 2 H, C 3), 2.7 (d, 6 H, $CONCH_3$ and NCH_3), 3.3–3.4 (m, 2 H, C 2), 3.7 (m, 2 H, C 6), 4.3 (d, 2 H, $ArCH_2$), 5.5 (t, 1 H, C 5), 7.1–7.3 (m, 5 H, ArH). Anal. ($C_{17}H_{22}N_2O_6$) C, H, N.

***m*-Chlorobenzoate Salt of 1-Methyl-4-[[(*N,N*-dimethylamino)carbonyl]oxy]-1,2,3,6-tetrahydropyridine *N*-Oxide (38).** This compound was prepared in 42% yield by following the procedure described for **11**: mp 90–91 °C; UV (water) λ_{max} 205 nm (ϵ = 21 600 M^{-1}); ^{1}H NMR (DMSO-d_6) δ 2.4 (m, 1 H, C 3), 2.7 (m, 1 H, C 3), 2.8 (s, 3 H, $CONCH_3$), 2.9 (s, 3 H, $CONCH_3$), 3.5 (s, 3 H, NCH_3), 3.8 (m, 2 H, C 2), 4.3 (m, 2 H, C 6), 5.5 (t, 1 H, C 5), 7.4–7.5 (m, 2 H, ArH), 8.0 (m, 2 H, ArH). Anal. ($C_{16}H_{21}N_2O_5Cl{\cdot}0.5H_2O$) C, H, N.

Preparation and Hydrolysis of 39 with $H_2{}^{18}O$. This compound was prepared by following the procedure described for **8** except that the solution was evaporated in vacuo and the residue was stirred for 10 min with water enriched with 20.5% ^{18}O (1 mL). The solution was then neutralized and extracted with chloroform (1 mL). The organic layer was dried (sodium sulfate) and analyzed by GC-EIMS. The mass spectra of the corresponding amino enone was identical to that of **13** except for the parent ion at m/z 113 corresponding to $M^{•+}$ ^{18}O-enriched product.

Enzyme Preparation and Assay. MAO-B was isolated from bovine liver mitochondria by the method of Salach.[20] Protein content was determined using a Sigma diagnostic Lowry protein assay kit (16.9 mg/mL). The activity of the enzyme was determined spectrophotometrically at 250 nm (benzaldehyde) using benzylamine (2 mM) as a substrate (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 one micromole of benzylamine to benzaldehyde in 1 min. Enzyme incubations were performed at 37 °C in 0.1 M sodium phosphate buffer (pH 7.4) in a final volume of 510 μL containing 0.05 unit of MAO-B/mL. Solutions of the substrates were prepared in the same buffer, and the reactions were initiated by the addition of substrate to give final substrate concentrations of 0.1–3.0 mM. The formation of amino enone **13** (for **7**, **35**, and **36**) and the formation of the dihydropyridinium **17** were monitored spectrophotometrically at 324 nm (λ_{max} of **13**) and 352 (λ_{max} of **17**), respectively. Initial rates were calculated from product yield over the 30–180-s time period. The V_{max} and the K_M values were then calculated by the Lineweaver–Burk plot.[21]

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