# Studies on the Monoamine Oxidase-B-Catalyzed Biotransformation of 4-Azaaryl-1-methyl-1,2,3,6-tetrahydropyridine Derivatives

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The substrate properties of a series of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridinyl (MPTP) analogues in which the C-4 phenyl group has been replaced with various 4-azaaryl moieties have been examined in an effort to evaluate the contribution of electronic, polar, and steric parameters to the MAO-B-catalyzed oxidation of this type of cyclic tertiary allylamine to the corresponding dihydropyridinium metabolite. No significant correlation could be found with the calculated energy of the C−H bond undergoing cleavage. A general trend, however, was observed between the magnitude of the log *P* value with the magnitude of $k_{cat}/K_m$. The results indicate that the placement of a polar nitrogen atom in the space occupied by the phenyl group of MPTP leads to a dramatic decrease in substrate properties. Enhanced substrate properties, however, were observed when benzoazaarenes replaced the corresponding five-membered azaarenes. These results are consistent with our previously published molecular model of the active site of MAO-B.

## Introduction

The flavoenzymes monoamine oxidase-A and -B (MAO-A and -B) catalyze the oxidative deamination of endogenous neurotransmitters such as dopamine and serotonin[1−3] and a variety of xenobiotics. A novel structural type displaying MAO substrate properties is the 1-methyl-4-substituted-1,2,3,6-tetrahydropyridinyl system **A** shown in Scheme 1. (The only classes of cyclic tertiary amines reported to be substrates for MAO are the six (1,2,3,6-tetrahydropyridinyl) and five (pyrrolinyl) systems; both α-carbon oxidations occur at allylic centers.) These compounds undergo allylic, α-carbon oxidation to form, via the C-6 radicals **B**, the generally unstable dihydropyridinium intermediates **C** that are oxidized further to the pyridinium metabolites **D**. An important member of this series of compounds is the parkinsonian inducing agent 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, **1**) which is biotransformed to the dihydropyridinium species $MPDP^+$ (**2**) and subsequently to the neurotoxic pyridinium metabolite $MPP^+$ (**3**) (Scheme 1).

The MAO catalytic pathway has been a topic of debate. Arguments in support of a hydrogen atom-transfer (HAT) pathway (**4** → **6** → **7**, Scheme 2)[5−7] and a single-electron-transfer (SET) pathway (**4** → **5** → **6** → **7**)[8−11] have been advanced. Particularly persuasive evidence supporting the SET pathway is the mechanism-based inactivation properties of cyclopropylaminyl derivatives such as *N*-benzylcyclopropylamine (**8**). MAO-B-catalyzed bioactivation of **8** proceeds through the cyclopropylaminyl radical cation **9** and the ring-opened primary carbon radical **10** that bioalkylates an active site functionality of MAO-B.[10]

Studies with a variety of MPTP analogues have provided evidence both supporting and challenging the SET pathway.[5,12−15] Consistent with the SET pathway, the 4-phenyl-1-cyclopropyl analogue **11** (Chart 1) is an efficient inactivator of MAO-B.[12] The 4-benzyl analogue **12**, however, proved to be both a good substrate and an inactivator of this enzyme.[5] Furthermore, the 4-thiophenoxy (**13**), 4-phenoxy (**14**), and 4-(2-methylphenyl) (**15**) analogues[14] are devoid of inactivator properties but are good to excellent MAO-B substrates with $k_{cat}/K_m$ values for conversion to the dihydropyridinium metabolites ranging from 635 (**15**) to 1980 (**13**) $min^{-1}$ $mM^{-1}$. When subjected to model SET reaction conditions, all of the cyclopropyltetrahydropyridinyl derivatives underwent ring-opening reactions.[16] No evidence of α-carbon oxidation was observed. These results plus the report that cyclopropylaminyl radical cations undergo ring opening at rates too fast to measure[17] suggested that the MAO-B-mediated α-carbon oxidation of these compounds did not proceed via cyclopropylaminyl radical cations. On the other hand, the alignment of the half-filled p-orbitals of the aminyl radical cations with the p-type orbitals of the cyclopropyl carbon−carbon bonds required for efficient ring opening of these systems could be prevented by steric constraints imposed by the active site.

The structural features of the active sites of MAO-A and -B are not well-defined. In the absence of X-ray crystallographic data,[18−20] SAR studies have been pursued to gain an appreciation of how steric, electronic, and polar properties influence the interactions of substrates and inhibitors with these enzymes.[13,15,21−27] We have focused our SAR work on analogues of MPTP (Chart 1). The poor MAO-B substrate properties of the 4-pyridinyl analogues **16**−**18** ($k_{cat}/K_m$ = 6−60 $min^{-1}$ $mM^{-1}$) compared to MPTP ($k_{cat}/K_m$ = 1471 $min^{-1}$ $mM^{-1}$) point to the possible importance of electronic and/or polar factors.[15] The poor substrate properties of the 4-(pyrrol-2-yl) analogue **19** ($k_{cat}/K_m$ = 155 $min^{-1}$ $mM^{-1}$) versus the 4-(1-methylpyrrol-2-yl) analogue **20** ($k_{cat}/K_m$ = 1800 $min^{-1}$ $mM^{-1}$) may reflect an influence of steric

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**Scheme 1.** MAO-B-Catalyzed Oxidation of MPTP

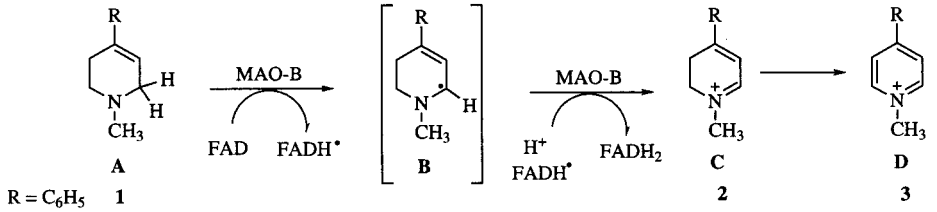
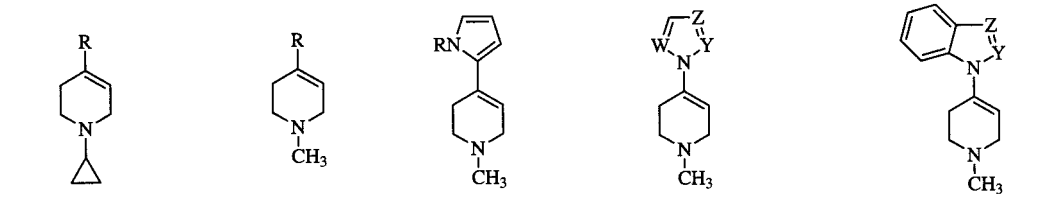



**Chart 1.** Structures of 1,2,3,6-Tetrahydropyridinyl Derivatives Discussed in the Text




**11**: R = $C_6H_5$
**12**: R = $C_6H_5CH_2$
**13**: R = $C_6H_5S$
**14**: R = $C_6H_5O$
**15**: R = 2-$CH_3C_6H_4$

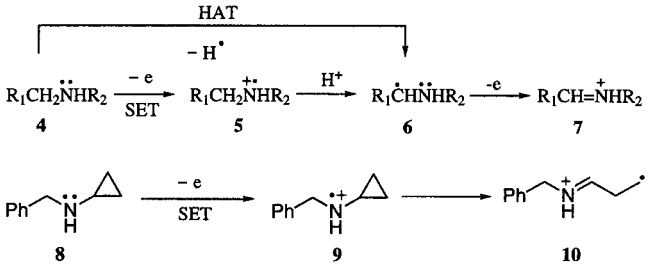
**16**: R = 2-$C_5H_4$
**17**: R = 3-$C_5H_4$
**18**: R = 4-$C_5H_4$

**19**: R = H
**20**: R = $CH_3$

**21**: W = Y = Z = CH
**22**: W = Y = CH; Z = N
**23**: W = CH; Y = Z = N
**24**: W = Y = N; Z = CH

**25**: Y = Z = CH
**26**: Z = N; Y = CH
**27**: Y = Z = N
**28**: Y = N; Z = CH

**Scheme 2.** Proposed HAT and SET Pathways for the MAO-Catalyzed Oxidation of Amines




factors.[15] In the present paper, results from studies with two series (**21**−**24** and **25**−**28**) of 4-azaaryl MPTP analogues are summarized. The geometry within each series is constant, while the polar and electronic features of members of each series differ. We have determined the MAO-B substrate properties of these compounds using MAO-B purified from beef liver and have examined the relationships between these substrate properties and the corresponding log *P* values. Additionally we have calculated the heats of formation of the putative C-6 radical intermediates **B** in an attempt to identify possible electronic contributions of the azaaryl groups on the catalytic pathway.

## Results and Discussion

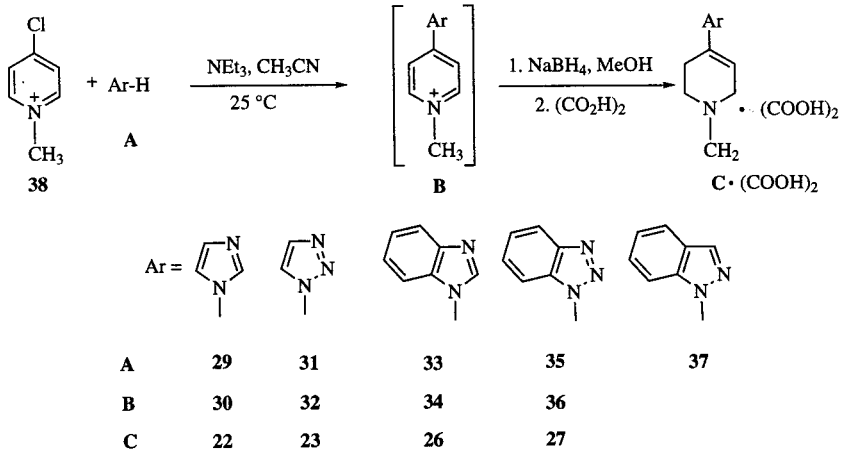
**Chemistry.** All of the 1-methyl-4-azaaryl-1,2,3,6-tetrahydropyridinyl derivatives **C** were prepared by $NaBH_4$ reduction of the corresponding 1-methylpyridinium intermediates **B** (Scheme 3).[28] Various approaches, principally involving nucleophilic aromatic substitution chemistry, were employed to obtain the required methylpyridinium intermediates.

The basic properties of imidazole **29**, 1*H*-[1,2,3]triazole **31**, benzimidazole **33**, benzotriazole **35**, and indazole **37** suggested the possibility that these azaarenes might be adequately nucleophilic to participate in nucleophilic aromatic substitution chemistry with the 4-chloro-1-methylpyridinium species **38** serving as the electrophilic substrate. The reaction with indazole **37** failed. The remaining basic azaarenes, however, were sufficiently nucleophilic to undergo reaction with **38** at room temperature to generate the pyridinium intermediates **30**, **32**, **34**, and **36** in good yield. These compounds proved to be hygroscopic and were reduced without purification to the desired tetrahydropyridinyl target compounds **22**, **23**, **26**, and **27**. The final products were converted to their stable oxalate salts.

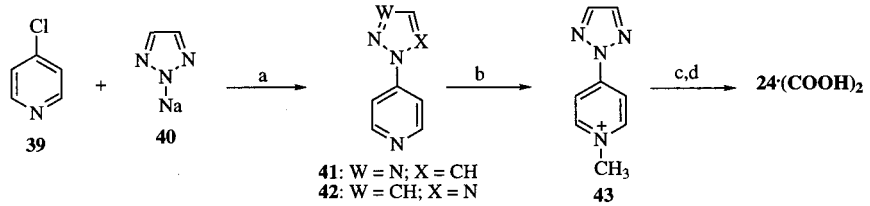
The success achieved with the chloromethylpyridinium substrate led us to examine 4-chloropyridine **39** as a possible electrophile for these condensation reactions. As expected, **39** did not react with 1*H*-[1,2,3]-triazole **31**, even at elevated temperatures. The corresponding triazolylsodium salt **40**, however, did undergo reaction at 100 °C. GC−EIMS analysis of the reaction mixture indicated the presence in low yield of two isomeric triazolylpyridinyl products ($M^{\bullet+}$ 146 Da) in a ratio of 2/1 (Scheme 4). The major isomer gave a fragment ion at $m/z = 118$, corresponding to a loss of $N_2$ (28 amu), which is characteristic of 1*H*-triazolyl derivatives.[29] The second isomer gave a fragment ion at $m/z = 119$, corresponding to a loss of CHN (27 amu), as expected for a 2*H*-triazolyl isomer. These compounds were separated by $SiO_2$ column chromatography, and the structures were established by $^1H$ NMR spectroscopy. The major isomer, 4-1*H*-[1,2,3-triazolyl]pyridine **41**, was not processed further since the required tetrahydropyridinyl product **23** had already been prepared. The minor isomer proved to be the symmetrical 2*H*-[1,2,3]triazolyl product **42** which displayed the expected three sets of signals for the aromatic protons. Methylation of **42** afforded the pyridinium intermediate **43** which, upon reduction with $NaBH_4$, gave the tetrahydropyridinyl product **24**.

The marginal success obtained in this reaction indicates that 4-chloropyridine is not adequately reactive to be of general utility in these types of substitution reactions. A literature report on the coupling reaction between 4-fluoropyridine **44** and indole **45**[30] prompted us to examine this method to prepare the indolylpyridinyl (**46**) and 4-(1-indazolyl)pyridinyl (**47**) derivatives (Scheme 5). The reactions were carried out with the sodium salt of the azaarenes **45** and **48** in DMF at high

**Scheme 3.** Synthesis of 4-Azaaryl-1-methyl-1,2,3,6-tetrahydropyridinyl Derivatives **22**, **23**, **26**, and **27**

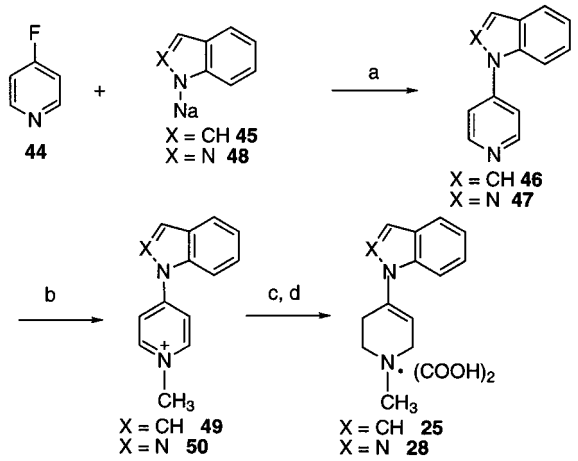



**Scheme 4.** Synthesis of 4-2*H*-[1,2,3]Triazolyl Derivative **24**[a]




[a] Key: (a) DMF, 100 °C, 31% yield of **41** + **42**; (b) $CH_3I$, acetone, 25 °C, 90% yield; (c) $NaBH_4$, $CH_3OH$; (d) oxalic acid, $CH_3OH$, $Et_2O$, 70% yield.

**Scheme 5.** Synthetic Routes to the Indazolyl (**28**) and Indolyl (**25**) Tetrahydropyridinyl Derivatives[a]




[a] Key: (a) DMF, 100 °C, 90% yield for **46**, 60% yield for **47**; (b) $CH_3I$, acetone, 25 °C, 90% yield for **49**, 91% yield for **50**; (c) $NaBH_4$, $CH_3OH$; (d) oxalic acid, $CH_3OH$, $Et_2O$, 70% yield for **25**, 64% yield for **28**.

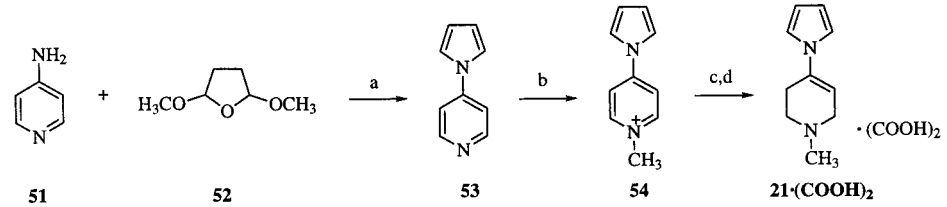
temperature. Subsequent reaction with iodomethane followed by $NaBH_4$ reduction of the resulting pyridinium species **49** and **50** gave the desired tetrahydropyridinyl products **25** and **28**.

The remaining target substrate was 4-(1-pyrrolyl)-1,2,3,6-tetrahydropyridine (**21**). The synthesis of **21** was achieved by the condensation of 4-aminopyridine (**51**) with 2,5-dimethoxytetrahydrofuran (**52**) under acidic conditions (Scheme 6).[31] The resulting pyrrolylpyridinyl product **53** was treated with $CH_3I$ to give the pyridinium intermediate **54**. Compound **54** was reduced with $NaBH_4$ to yield the tetrahydropyridinyl product **21** that was purified as its oxalate salt (Scheme 6).

Quantitative estimations of the rates of substrate turnover (**A** → **C**, Scheme 1) required molar extinction coefficients for the corresponding 1,2-dihydropyridinium metabolites **C**. For the five-membered heterocyclic system we used the $\epsilon$ value (16 000 $M^{-1}$)[15] of the dihydropyridinium metabolite (**C**: R = 1-methylpyrrol-2-yl) derived from 1-methyl-4-(1-methylpyrroyl-2-yl)-1,2,3,6-tetrahydropyridine (**20**, see Chart 1). The dihydropyridinium metabolite **55** of the indazolyl analogue **27** was prepared, and its $\epsilon$ value was used for all of the benzo-fused ring structures. The synthetic approach was based on an established route to this system[32] which proceeds via the corresponding *N*-oxide **56** (Scheme 7). Treatment of **56** with trifluoroacetic anhydride gave the dihydropyridinium product **55**, presumably via the corresponding trifluoroacetyloxy intermediate **57**. Dihydropyridinium derivatives such as **55** are unstable unless fully protonated, and therefore the final product was purified as its perchlorate salt. The $\epsilon$ value for **55** was estimated to be 21 000 $M^{-1}$.

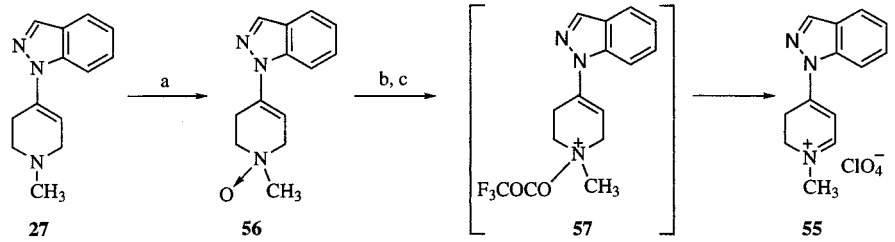
**Enzymology.** UV scans of 10 mM solutions of the 1-methyl-4-imidazolyl analogue **20** and 4-triazolyl analogues **23** and **24** in the presence of 0.16 $\mu$M MAO-B showed that the substrate properties of these compounds were too poor to generate useful kinetic data. The 1-methyl-4-pyrrol-1-yl (**21**), 4-benzimidazolyl (**26**), 4-benzotriazolyl (**27**), and 4-indazolyl (**28**) analogues

**Scheme 6.** Synthesis of 4-(Pyrrol-1-yl) Derivative **21**[a]



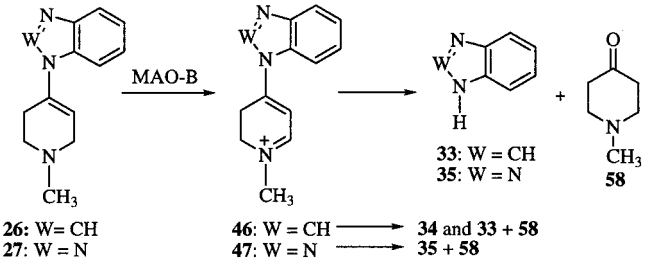

[a] Key: (a) AcOH, reflux, 90% yield; (b) $CH_3I$, acetone, 25 °C, 90% yield; (c) $NaBH_4$, $CH_3OH$, 99% yield; (d) oxalic acid, $CH_3OH$, $Et_2O$, 70% yield.

**Scheme 7.** Synthesis of the Indazolyldihydropyridinium Species **55**[a]




[a] Key: (a) *m*-CPBA, $CHCl_3$, 0 °C, 96% yield; (b) $(CF_3CO)_2O$, $CH_2Cl_2$; (c) $HClO_4$, 73% yield.

**Scheme 8.** Fate of **26** and **27** upon Incubation with MAO-B




proved to be better substrates. UV scans of 0.5 mM solutions of these tetrahydropyridines in the presence of 0.08 $\mu$M MAO-B showed the time-dependent formation of chromophores near 360 nm as expected for the corresponding dihydropyridinium metabolites. Linear initial velocities versus substrate concentration plots and linear double-reciprocal plots were obtained for all four compounds. Closer examination of the UV scans of incubation mixtures containing **26** or **27** and MAO-B revealed that the spectra corresponding to the dihydropyridinium metabolites **46** and **47** (Scheme 8) shifted with time from a $\lambda_{max}$ of 360 nm toward a $\lambda_{max}$ of 325 nm, suggesting their further oxidation to the pyridinium species **34** and **36**, respectively. In the absence of pure synthetic standards, the possible formation of pyridinium products in these reactions was examined by GC−EIMS following treatment of the incubation mixtures with $NaBD_4$.[33] The detection of **26-*2,6-d*$_2$** confirmed the suspected formation of the benzimidazolylpyridinium species **34**. The absence of **27-*2,6-d*$_2$** in the corresponding experiment with the triazolyl substrate indicated that the dihydropyridinium metabolite **47** derived from **27** had experienced an alternative fate. The possibility that **47** and (possibly) **46** had undergone hydrolytic cleavage to yield benzotriazole **35** and benzimidazole **33**, respectively, and the aminoenone **58** ($\lambda_{max}$ = 324 nm), analogous to 4-aryloxydihydropyridinium metabolites,[34] was examined with the aid of an HPLC-diode array assay. The detection of **58** in these assays established that both dihydropyridinium metabolites **46** and **47** spontaneously hydrolyze to **58** and the corresponding azaarenes. The rates of hydrolysis, however, were slow, and therefore estimations of the initial rates of conversion of the tetrahydropyridinyl substrates to their dihydropyridinium metabolites could be obtained during the first 120 s of the incubation.

**Table 1.** MAO-B Kinetic Data and Calculated (ACD Log*P*) log *P* Values for Various 1-Methyl-4-azaaryl-1,2,3,6-tetrahydropyridinyl Analogues

| compd | C-4 substituent | $k_{cat}$ ($min^{-1}$) | $K_m$ (mM) | $k_{cat}/K_m$ ($min \cdot mM)^{-1}$ | log *P* |
|---|---|---|---|---|---|
| **21** | 1-pyrrolyl | 268 | 0.65 | 410 | 2.17 |
| **22** | 1-imidazolyl | | | | 1.31 |
| **23** | 1*H*-triazolyl | | | | 1.13 |
| **24** | 2*H*-triazolyl | | | | 1.13 |
| **25** | 1-indolyl | 414 | 0.11 | 3350 | 3.61 |
| **26** | 1-benzimidazolyl | 200 | 0.90 | 220 | 2.54 |
| **27** | 1-benzotriazolyl | 54 | 0.08 | 675 | 2.36 |
| **28** | 1-indazolyl | 120 | 0.1 | 850 | 2.85 |
| **1** | phenyl | 271 | 0.2 | 1470 | 2.74 |
| **16** | 2-pyridinyl | 56 | 0.9 | 60 | 1.24 |
| **17** | 3-pyridinyl | 67 | 1.9 | 35 | 1.44 |
| **18** | 4-pyridinyl | 14 | 2.4 | 6 | 1.24 |
| **19** | 2-pyrrolyl | 85 | 1.80 | 46 | 1.27 |
| **20** | $NCH_3$-2-pyrrolyl | 360 | 0.2 | 1800 | 1.73 |

Table 1 presents the MAO-B substrate properties and log *P* values of MPTP and the 4-azaaryl analogues that have been studied to date. In the discussion that follows, we have taken $k_{cat}/K_m$ as the measure of the overall efficiency of enzyme catalysis. The electron-rich pyrrolyl analogue **19** is a good substrate, while the activities of the more electron-deficient imidazolyl (**20**) and triazolyl (**21** and **22**) analogues are too slow to measure. This is somewhat reminiscent of the poor substrate properties of the pyridinyl analogues **16**−**18** compared to those of MPTP,[15] suggestive of electronic and/or polar effects. Since the cleavage of the C6−H bond in this class of biotransformation (**A** → **B**, Scheme 1) is accompanied by a primary isotope effect,[6] we speculated that stabilization of the C-6 radical **B**, generated by either C−H bond homolysis (HAT pathway) or deprotonation of the aminyl radical cation (SET pathway), by electron-donating substituents at C-6 could lead to a decrease in $\Delta G^{\ddagger}$ and an increase in $k_{cat}$. The calculated C6−H bond energies [$\Delta\Delta H_f$(C6H−C6 radical)] for all the

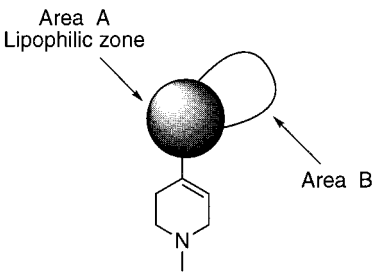



**Figure 1.** Areas of substrate interactions defined by azaaryl analogues of MPTP.

N-linked analogues, including the benzoazaarenes, however, show no clear relationship to substrate turnover and vary by less than 0.75 kcal/mol. (Calculations are not reported for the pyridinyl series **16**−**18** nor for the pyrrolyl derivatives **19** and **20**, because they are not N-linked. In addition, the six-membered ring in the pyridinyl series induces a new steric parameter which may influence the stabilization of the C-6 radical intermediate by the C-4 substituent. There is very little steric interaction between five-membered rings and the hydrogen atoms at positions C-3 and C-5 of the tetrahydropyridinyl ring. The six-membered rings, on the other hand, do interact with these C-3 and C-5 hydrogen atoms. Comparison of stabilization energy then would become hazardous, as different factors operate for the various series of compounds.) Consequently, it appears unlikely that electronic factors contribute significantly to the substrate properties of these systems. The fact that only cyclic tertiary *allyl*amines are substrates for MAO suggests that electronic factors still may be important in determining the substrate behavior of these systems.

In contrast to the five-membered azaarenes, all of the benzoazaarenes are MAO-B substrates. As shown in Table 1, all of these fused ring systems also are more lipophilic than the corresponding monocyclic analogues. In fact, a good trend exists between the log *P* and $k_{cat}/K_m$ values with the most lipophilic compound, the 1-indolyl analogue **23**, being the most lipophilic and the best substrate. Compounds with $k_{cat}/K_m$ values of less than 60 $(min \cdot mM)^{-1}$ have log *P* values below 1.5. On the basis of this analysis, lipophilicity is judged to be an important factor contributing to the efficiency of substrate turnover in this series of compounds.

The direct correlation between the MAO-B substrate properties of azaaryltetrahydropyridinyl derivatives and their lipophilic character parallels those effects observed with phenyl-substituted MPTP derivatives.[25] On the other hand, some polar compounds, such as the dopamine (log *P* 0.12), are excellent MAO-B substrates. Therefore the poor substrate properties observed with some of these azaaryl derivatives may describe regions of unfavored polar interactions. Results with the five-membered azaaryl and pyridinyl analogues suggest that nitrogen atoms with localized nonbonding lone pairs of electrons located in area A of the model shown in Figure 1 may form hydrogen bonds with active site proton donors that disfavor effective binding of the substrate. The improved substrate properties of the benzoazaaryl analogues suggest a region of favorable interactions indicated by area B in the model. This depiction is consistent with the improved substrate properties of MPTP analogues bearing a variety of polar and nonpolar substituents at C-2 and C-3 on the phenyl ring[24] and by the excellent substrate properties of 4-aryloxy[34] and 4-benzyl[35] analogues of MPTP. Substituents in this area might enhance the interactions with the active site and "lock" the nitrogen of the tetrahydropyridine in a more suitable position for catalysis. (The structure shown in Figure 1 places the off-axis groupings on the same side as the tetrahydropyridinyl double bond. This representation is arbitrary since these results provide no information on the preferred orientation of the molecule in the active site. Calculations show that the syn structure (off-axis substituent on the side of the double bond as in Figure 1) and the anti structure differ in energy by 0.4 kcal/mol or less.)

Studies currently in progress focus on the region identified as area B. We are particularly interested in studying MPTP analogues bearing polar functionalities that may occupy the same regions of the active site that accommodate the phenolic OH groups of the catecholamines and serotonin.

## Experimental Section

**Caution!** MPTP is a known nigrostriatal neurotoxin, and therefore compounds of this class should be handled using disposable gloves in a properly ventilated hood. Detailed procedures for the safe handling of MPTP have been reported.[27]

**General Methods.** All nonaqueous reactions were carried out using glassware that had been flame-dried under an inert atmosphere of dry nitrogen. All common reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI) or Lancaster Synthesis Inc. (Windham, NH) and were used without further purification. The following compounds, 4-fluoropyridine (**32**),[36,37] 4-(1-indolyl)pyridine (**33**),[30] and 4-chloro-1-methylpyridinium iodide (**37**),[38] were synthesized as described previously. The synthesis of compound **41** will be described separately.[39] THF was distilled from sodium/benzophenone ketyl and acetone from $K_2CO_3$. UV absorption spectra were recorded on a Beckman DU-7400 spectrophotometer. Proton NMR spectra were recorded on a Brukers WP 270 or AM-360 or a Varian 400-MHz spectrometer. Chemical shifts ($\delta$) are reported relative to tetramethylsilane as an internal standard. Spin multiplicities are given as s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). Coupling values *(J)* are given in hertz (Hz). Gas chromatography−electron ionization mass spectrometry (GC−EIMS) was performed on a Hewlett-Packard 5890 GC fitted with an HP-1 capillary column which was coupled to a Hewlett-Packard 5870 mass selective detector. Data were acquired using an HP 5970 ChemStation. Unless otherwise stated, the temperature program employed was as follows: 125 °C for 1 min, then 25 °C/min to 275 °C. Normalized peak heights are reported as a percentage of the base peak. Melting points were performed on a Thomas-Hoover melting point apparatus and are uncorrected. Microanalyses were performed by Atlantic Microlab, Inc., Norcross, GA. The lipophilicity parameter log *P* was calculated using the ACD/Log*P* software (ACD/Labs). Experimetal and calculated log *P* values were essentially identical for **1** (2.71 vs 2.74) and **18** (1.25 vs 1.24).[25] Calculations were performed on a Macintosh G3, using the semiempirical method AM1 embedded in MacSpartan Plus software. Unrestricted Hartree−Fock method was used for calculations on radical species.

**4-(Pyrrol-1-yl)pyridine (53).** Under anhydrous conditions, a mixture of 4-aminopyridine (**51**; 9.4 g, 100 mmol) and 2,5-dimethoxytetrahydrofuran (**52**; 16.5 g, 125 mmol) in 100 mL of glacial AcOH (100 mL) was heated to reflux for 4.5 h. An EtOAc (199 mL) solution of the residue obtained after removing the AcOH was washed extensively with aqueous 10% NaOH (8 × 50 mL) followed by $H_2O$ and brine. The organic phase was dried ($MgSO_4$), the solvent was removed, and the

residue was filtered through a column of $Al_2O_3$ using $CH_2Cl_2$ as an eluent to give 13.7 g (90.4 mmol, 90% yield) of **53** as a white solid: mp 78 °C. Anal. ($C_9H_8N_2$) C, H, N.

**1-Methyl-4-(pyrrol-1-yl)pyridinium Iodide (54).** A mixture of **53** (1.73 g, 12 mmol) and $CH_3I$ (5.07 g, 36 mmol) in 120 mL of anhydrous acetone was stirred in the dark for 24 h. The solid iodide salt was collected and washed with $Et_2O$ to give pure **54** (3.1 g, 10.8 mmol, 90% yield) as a white solid: mp 197−198 °C. Anal. ($C_{10}H_{11}IN_2$) C, H, N.

**Oxalate Salt of 1-Methyl-4-(pyrrol-1-yl)-1,2,3,6-tetrahydropyridine (21).** Reduction of **54** with an excess of $NaBH_4$ in MeOH for 1 h followed by removal of the solvent and extraction with $CH_2Cl_2/H_2O$ gave a crude residue of **21** free base, which under treatment in $Et_2O$ with an excess of oxalic acid gave the corresponding oxalate that was recrystallized from MeOH to yield **21** (600 mg, 2.7 mmol, 70% yield) as a white crystalline solid: mp 185−186 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 7.13 (dd, $J$ = 2.4 and 2.0 Hz, 2H), 6.16 (dd, $J$ = 2.4 and 2.0 Hz, 2H), 5.82 (m, 1H), 3.72 (dd, $J$ = 2.4 and 5.6 Hz, 2H), 3.46 (t, $J$ = 6.4 Hz, 2H), 2.85 (m, 2H), 2.78 (s, 3H); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 165.1, 133.6, 118.3, 110.2, 50.5, 49.6, 42.2, 24.0; MS (*m*/*z*, rel int) 162 (27), 161 (19), 118 (47), 96 (100), 94 (20), 42 (47); UV (nm, MeOH) 207, 246. Anal. ($C_{12}H_{16}N_2O_4$) C, H, N.

**1-Methyl-4-(indol-1-yl)pyridinium Iodide (49).** A mixture of **46** (1.94 g, 10 mmol) and $CH_3I$ (4.2 g, 30 mmol) in 100 mL of anhydrous acetone was stirred in the dark for 28 h. The solid iodide salt was collected and washed with $Et_2O$ to give pure **49** (3.05 g, 9.1 mmol, 91% yield) as a white solid: mp 210−212 °C. Anal. ($C_{12}H_{13}IN_2$) C, H, N.

**Oxalate Salt of 1-Methyl-4-(indol-1-yl)-1,2,3,6-tetrahydropyridine (25).** Same procedure as described for the synthesis of **21** was followed which yielded a white solid from MeOH/$Et_2O$ in 91% yield: mp 209−210 °C; $^1$H NMR ($CD_3OD$) $\delta$ 7.59 (2H, t, $J$ = 8.0 Hz), 7.35 (1H, d, $J$ = 3.3 Hz), 7.20 (1H, t, $J$ = 8.0 Hz), 7.09 (1H, t, $J$ = 8.0 Hz), 6.59 (1H, dd, $J$ = 3.35 Hz, $J$ = 0.8 Hz), 5.97 (1H, bs), 4.03 (2H, bs), 3.64 (2H, t, $J$ = 4.0 Hz), 3.05 (3H, s), 2.99 (2H, bs); $^{13}$C NMR ($CD_3OD$) $\delta$ 160.5, 135.6, 131.8, 128.9, 128.6, 127.2, 123.7, 123.6, 121.8, 121.6, 115.8, 52.4, 50.1, 41.5, 24.4; GC ($t_R$ 5.57 min)−EIMS *m*/*z* (rel int) 212 (22%), 180 (2), 168 (25), 143 (2), 130 (7), 96 (100), 89 (9), 53 (9); UV (nm, MeOH) 337. Anal. ($C_{16}H_{18}N_2O_4$) C, H, N.

**1-Methyl-4-(indazol-1-yl)pyridinium Iodide (50).** The procedure described for **54** yielded a yellow solid in 90% yield: mp 261−263 °C. Anal. ($C_{13}H_{12}IN_3$) C, H, N.

**Oxalate Salt of 1-Methyl-4-(indazol-1-yl)-1,2,3,6-tetrahydropyridine (28).** Reduction of **50** as described for **21** yielded a white crystalline solid in 68% yield after recrystallization from MeOH: mp 174−175 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 8.28 (1H, d, $J$ = 0.9 Hz), 7.85 (m, 2H), 7.49 (1H, ddd, $J$ = 1.2 Hz, 6.9 Hz, 8.0 Hz), 7.25 (1H, ddd $J$ = 0.6 Hz, 6.9 Hz, 7.9 Hz), 6.12 (1H, m), 3.87 (2H, m), 3.42 (2H, d, $J$ = 6.1 Hz), 3.03 (2H, m), 2.84 (3H, s); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 164.1, 137.8, 134.9, 134.0, 127.3, 124.8, 121.7, 121.4, 111.3, 108.8, 50.3, 49.5, 41.9; MS (*m*/*z*, rel int) 213 (40), 185 (24), 157 (36), 144 (18), 130 (9), 96 (27), 70 (100); UV (nm, MeOH) 208, 244, 301. Anal. ($C_{15}H_{17}N_3O_4$) C, H, N.

**General Procedure for the Synthesis of the 4-Azaaryl-1,2,3,6-tetrahydropyridinyl Derivatives 22, 23, 26, and 27.** A mixture of 4-chloro-1-methylpyridinium iodide (**38**) (2 equiv), the appropriate azaarene (2 equiv), and freshly distilled triethylamine (3 equiv) was stirred in $CH_3CN$ at room temperature under nitrogen for 14 h. The solvent was evaporated, and the residue was treated with a saturated solution of $K_2CO_3$. This mixture was extracted with $CH_2Cl_2$ (3 × 60 mL), and the combined organic extracts were washed with brine and dried over $MgSO_4$. Evaporation of the solvent gave the crude, hygroscopic pyridinium product which was treated directly with $NaBH_4$ (2.5 equiv) in 25 mL of MeOH at 0 °C. After stirring for 15 min, the solvent was removed under reduced pressure and the residue in 20 mL of $H_2O$ was extracted with $CH_2Cl_2$ (2 × 25 mL). The combined organic layer was dried over $MgSO_4$, filtered, and evaporated, and the residue in 25 mL of dry $Et_2O$ was treated with oxalic acid (3 equiv). The precipitated oxalate salt was recrystallized from $CH_3CN$/MeOH.

**Oxalate Salt of 4-(1-Imidazolyl)-1-methyl-1,2,3,6-tetrahydropyridine (22).** This compound was obtained in 30% yield: mp 153−154 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 9.88 (bs, 2H), 8.14 (bs, 1H), 7.62 (bs, 1H), 7.09 (bs, 1H), 6.07 (bs, 1H), 3.82 (bs, 2H), 3.43 (bs, 2H), 2.90 (bs, 2H), 2.84 (s, 3H); GC ($t_R$ = 4.26 min)−EIMS *m*/*z* (%) 163 ($M^{\cdot+}$, 27), 119 (26), 96 (100), 94 (28); UV (0.1 M sodium phosphate buffer, pH 7.4) $\lambda_{max}$ = 257 nm ($\epsilon$ = 490 $M^{-1}$). Anal. ($C_9H_{13}N_3 \cdot 1.1C_2H_2O_4$) C, H, N.

**Bisoxalate Salt of 4-(1-Benzimidazolyl)-1-methyl-1,2,3,6-tetrahydropyridine (26).** This compound was obtained in 22% yield: mp 146 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 8.43 (bs, 1H), 7.69−7.72 (m, 2H), 7.25−7.33 (m, 2H), 6.13 (bs, 1H), 3.94 (bs, 2H), 3.49−3.52 (m, 2H), 2.98 (bs, 2H), 2.90 (s, 3H); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 163.5, 143.9, 142.5, 132.9, 131.3, 123.8, 123.0, 120.3, 113.5, 112.2, 50.6, 49.6, 41.9, 25.2; GC ($t_R$ = 7.06 min)−EIMS *m*/*z* (%) 213 ($M^{\cdot+}$, 22), 169 (26), 96 (100). Anal. ($C_{17}H_{19}N_3O_8$) C, H, N.

**Oxalate Salt of 4-(1-Benzotriazolyl)-1-methyl-1,2,3,6-tetrahydropyridine (27).** This compound was obtained in 45% yield: mp 190−191.5 °C dec; $^1$H NMR of free base ($CDCl_3$) $\delta$ 8.07−8.10 (d, $J$ = 6.5 Hz, 1H), 7.69−7.71 (d, $J$ = 6.5 Hz, 1H), 7.48−7.53 (m, 1H), 7.37−7.41 (m, 1H), 6.17−6.18 (m, 1H), 3.28−3.30 (m, 2H), 3.02−3.06 (m, 2H), 2.84 (m, 2H), 2.50 (s, 3H); $^{13}$C NMR of free base ($CDCl_3$) $\delta$ 146.0, 134.0, 132.0, 127.8, 124.2, 120.1, 117.1, 111.0, 53.3, 51.7, 45.4, 28.4; GC ($t_R$ = 6.72 min)−EIMS *m*/*z* (%) 214 ($M^{\cdot+}$, 2), 186 (20), 157 (20), 143 (70), 130 (75), 94 (25), 77 (100), 53 (95); UV (0.1 M sodium phosphate buffer, pH 7.4) $\lambda_{max}$ = 266 nm ($\epsilon$ = 7000 $M^{-1}$) and 293 nm ($\epsilon$ = 6200 $M^{-1}$). Anal. ($C_{14}H_{16}N_4O_4$) C, H, N.

**Oxalate Salt of 1-Methyl-4-(1*H*-[1,2,3]triazolyl)-1,2,3,6-tetrahydropyridine (23).** This compound was obtained in 27% yield: mp 238−239 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 8.0 (s, 2H), 6.43−6.45 (m, 1H) 3.72 (bs, 2H), 3.29 (m, 2H), 2.98 (bs, 2H), 2.74 (s, 3H); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 164.3, 135.9, 133.8, 108.3, 50.1, 49.2, 41.9, 22.8; GC ($t_R$ = 2.38 min)−EIMS *m*/*z* (%) 164 ($M^{\cdot+}$, 67), 163 (35), 110 (52), 96 (52), 94 (100), 70 (55); UV (0.1 M sodium phosphate buffer, pH 7.4) $\lambda_{max}$ = 254 nm ($\epsilon$ = 12 000 $M^{-1}$). Anal. ($C_{10}H_{14}N_4O_4$) C, H, N.

**4-(1*H*-[1,2,3]Triazolyl)pyridine (41) and 4-(2*H*-[1,2,3]-Triazolyl)pyridine (42).** Under anhydrous conditions, 1,2,3-triazole (7.7 mmol, 0.53 g) in DMF (35 mL) was treated portionwise with NaH (8.05 mmol, 0.19 g). The freshly prepared free base of 4-chloropyridine (**39**; 8 mmol, 1.0 g) in solution in DMF (15 mL) was then added, and the reaction mixture was heated under reflux for 14 h. After cooling to 25 °C, a saturated aqueous solution of $NH_4Cl$ (10 mL) was added. The mixture was washed with hexane (2 × 40 mL), neutralized with a saturated aqueous solution of $K_2CO_3$ (15 mL), and extracted with $Et_2O$ (4 × 50 mL). The combined organic phase was dried over $MgSO_4$ and evaporated under reduced pressure. Column chromatography ($SiO_2$, EtOAc) of the resulting mixture gave **41** as a pale-yellow solid in 9% yield: mp 125.5−127 °C. Anal. ($C_7H_6N_4$) C, H, N. A second fraction from the column yielded **42** as a pale-yellow solid in 22% yield: mp: 128−130 °C. Anal. ($C_7H_6N_4$) C, H, N.

**1-Methyl-4-(2*H*-[1,2,3]triazolyl)pyridinium Iodide (43).** Methylation of **42** as described for **53** gave **43** as a yellow solid in 82% yield: mp 255−256 °C dec. Anal. ($C_8H_9IN_4$) C, H, N.

**Oxalate Salt of 1-Methyl-4-(2*H*-[1,2,3]triazolyl)-1,2,3,6-tetrahydropyridine (24).** Treatment of **43** as described for the synthesis of **21** gave **24** as a white solid in 25% yield: mp 189−190 °C; $^1$H NMR ($CD_3OD$, 360 MHz) $\delta$ 7.82 (2H, s), 6.02 (1H, bs), 3.97 (2H, bs), 3.63 (2H, t, $J$ = 4.0 Hz), 3.05 (3H, s), 2.98 (2H, bs); $^{13}$C NMR ($CD_3OD$, 90 MHz) $\delta$ 121.3, 114.2, 52.4, 50.1, 41.5, 24.4; GC−EIMS *m*/*z* (rel int) 164 ($MH^{\cdot+}$, 22%), 136 (100), 115 (12), 96 (42); UV (MeOH) $\lambda_{max}$ 290, 265 nm. Anal. ($C_{10}H_{14}N_4O_4$) C, H, N.

**1-Methyl-4-(1*H*-indazolyl)-2,3-dihydropyridinium Perchlorate (55).** To a solution of 1-methyl-4-(1*H*-indazolyl)-1,2,3,6-tetrahydropyridine (**28**; 250 mg, 1.2 mmol) in 15 mL of anhydrous $CHCl_3$ at 0 °C was added *m*-chloroperoxybenzoic acid (*m*-CPBA; 50%, 384 mg, 1.1 mmol) in one portion. After

stirring for 15 min, the reaction mixture was concentrated in vacuo. The residue was purified by column chromatography on basic alumina, eluting with $CH_3Cl$/MeOH (9.5:0.5) to give 246 mg (1.1 mmol, 96%) of the *N*-oxide **56** as a yellow oil: $^1$H NMR (DMSO-$d_6$, 360 MHz) $\delta$ 8.27 (d, $J$ = 0.8 Hz, 1H), 7.84 (m, 2H), 7.47 (ddd, $J$ = 1.2 Hz, $J$ = 6.9 Hz, $J$ = 8.2 Hz, 1H), 7.23 (ddd, $J$ = 1.0 Hz, $J$ = 6.9 Hz, $J$ = 8.0 Hz, 1H), 5.97 (d, $J$ = 8.0 Hz, 1H), 4.26 (m, 1H), 3.81 (m, 1H), 3.58 (m, 1H), 3.44 (m, 1H), 3.34 (m, 1H), 3.21 (s, 3H), 2.88 (m, 1H); $^{13}$C NMR (DMSO-$d_6$, 90 MHz) $\delta$ 137.8, 134.6, 133.7, 127.1, 124.7, 121.5, 121.3, 111.3, 108.7, 79.2, 65.6, 62.2, 59.0; UV (MeOH) $\lambda_{max}$ 208, 244, 299 nm. HR-CIMS Calcd for $C_{15}H_{15}N_3O$: 229.1215123. Found: 229.12247.

A mixture of **56** (105 mg, 0.46 mmol) and trifluoroacetic anhydride (115 mg, 0.55 mmol) in 2 mL of $CH_2Cl_2$ was stirred for 15 min at 0 °C. Addition of a solution of perchloric acid (134 mg, 0.92 mmol) in 4 mL of MeOH followed by stirring for 1 h gave 105 mg of a yellow crystalline product (0.34 mmol, 73%): mp 174−175 °C; $^1$H NMR (DMSO-$d_6$, 360 MHz) $\delta$ 8.74 (d, $J$ = 0.8 Hz, 1H), 8.55 (dddd, $J$ = 1.0 Hz, $J$ = 1.0 Hz, $J$ = 1.0, $J$ = 5.6 Hz, 1H), 8.18 (dddd, $J$ = 0.8 Hz, $J$ = 0.8 Hz, $J$ = 0.8 Hz, $J$ = 8.6 Hz, 1H), 8.01 (ddd, $J$ = 1.0 Hz, $J$ = 1.0 Hz, $J$ = 8.0, 1H), 7.72 (ddd, $J$ = 1.2 Hz, $J$ = 7.1 Hz, $J$ = 8.4 Hz, 1H), 7.51 (ddd, $J$ = 0.6 Hz, $J$ = 7.2 Hz, $J$ = 7.9, 1H), 6.89 (d, $J$ = 5.5 Hz, 1H), 4.05 (m, 2H), 3.83 (m, 2H), 3.63 (s, 3H); $^{13}$C NMR (DMSO-$d_6$, 90 MHz) $\delta$ 163.4, 157.9, 154.5, 142.2, 138.4, 129.8, 127.4, 125.0, 122.9, 114.2, 98.5, 47.3, 45.4; UV (MeOH) $\lambda_{max}$ 382 nm ($\epsilon$ = 21 000 $M^{-1}$). Anal. ($C_{13}H_{14}N_3O_4Cl \cdot 0.7H_2O$) C, H, N. HR-CIMS Calcd for $C_{13}H_{14}N_3$: 212.1187726. Found: 212.118454.

**Enzyme Studies.** MAO-B was isolated from bovine liver mitochondria according to the method of Salach and Weyler[40] with minor modifications as described previously.[41] The purified enzyme concentration was calculated to be 8 nmol/mL. All enzyme assays were performed at 37 °C on a Beckman DU-7400 spectrophotometer. Stock solutions (2−10 mM) of the tetrahydropyridinyl derivatives were prepared in 100 mM sodium phosphate buffer, pH 7.4. In preliminary experiments, the potential MAO-B substrate properties of each test compound (0.25−10 mM) were examined by recording repeated scans (500−250 nm) in the presence of 0.08−0.16 $\mu$M MAO-B. For kinetic analyses, initial rates of oxidation of the tetrahydropyridinyl derivatives were determined at four substrate concentrations, which bracketed the $K_m$ value. A mixture of each test compound (50−500 $\mu$M) and MAO-B (0.08−0.16 $\mu$M) was added to a sample cuvette, and the initial rates of oxidation were estimated by monitoring the increase in absorbance of the corresponding dihydropyridinium metabolite every 5 s for 2 min. The $K_m$ and $k_{cat}$ values were calculated from Lineweaver−Burke plots. Duplicate analyses gave $k_{cat}/K_m$ values that differed by 10% or less.

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**Supporting Information Available:** Spectroscopic data on all synthetic intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

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