Chapter 4. 1,4-Disubstituted Tetrahydropyridines

The unexpected substrate properties of the MPTP analog 4-benzyl-1-cyclopropyl-1,2,3,6-tetrahydropyridine (**54**) have prompted us to consider catalytic pathways other than the SET pathway for the MAO-B catalyzed oxidation of cyclic tertiary allylamines. In particular, we have raised the question of whether or not the putative aminyl radical cation proposed by the SET pathway is an obligatory intermediate. If this is the case, then the general expectation that the relative rates of cyclopropylaminyl radical cation ring opening will be considerably faster than α-proton loss may be in question. A direct loss of $H^{\cdot}$ from the substrate (see Scheme 18) that would bypass the radical cation intermediate, however, would be consistent with the substrate behavior of **54**.

In an effort to assess possible steric and electronic factors which might contribute to the unexpected MAO-B substrate properties of 4-benzyl-1-cyclopropyl-1,2,3,6-tetrahydropyridine (**54)**, we synthesized the structurally related 1-cyclopropyl-4-phenoxy (**69**) and 4-thiophenoxy (**70**) analogs and examined their interactions with MAO-B. Previous studies had established that the 4-phenoxy- (**71**)[98] and 4-thiophenoxy-1-methyl-1,2,3,6-tetrahydropyridine (**72**)[114] analogs are excellent MAO-B substrates.

4.1. 4-Phenoxy- and 4-Thiophenoxy-1-cyclopropyl-1,2,3,6-tetrahydropyridine

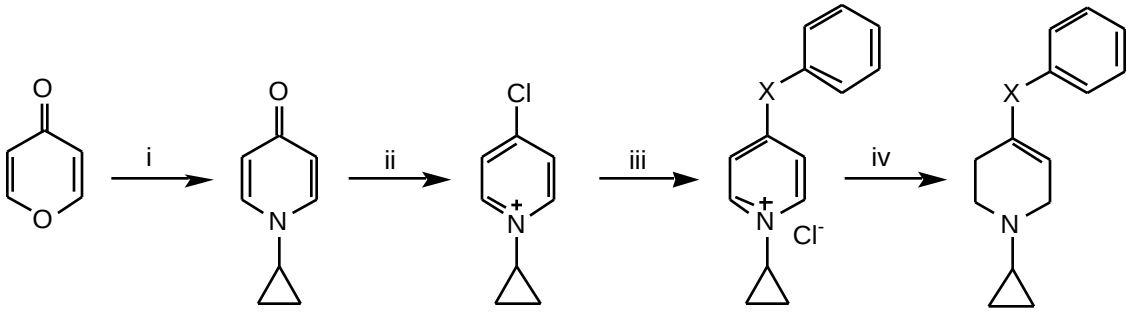
4.1.1. Chemistry

*Syntheses of the 4-phenoxy- and 4-thiophenoxy-1-cyclopropyl derivatives **69** and **70** started with the preparation of γ-pyrone (**73**)[115] which

* The syntheses of the 4-phenoxy- and 4-thiophenoxy-1-cyclopropyl analogs were carried out in collaboration with Dr. John Rimoldi.

was obtained from the thermal decarboxylation of chelidonic acid.[116] Condensation of **73** with cyclopropylamine gave the pyridone **74** which, upon reaction with thionyl chloride, generated 4-chloro-1-cyclopropylpyridinium chloride (**75**). This subsequent nucleophilic substitution reaction of **75** with the appropriate arenol in the presence of triethylamine generated the corresponding 4-aryloxy-1-cyclopropylpyridinium compounds **76** and **77**. Due to their hygroscopicity and susceptibility to hydrolysis, these pyridinium intermediates were not isolated but rather were reduced directly with sodium borohydride in methanol to yield the requisite tetrahydropyridine derivatives **69** and **70** (Scheme 19).

Scheme 19. Synthesis of 4-Phenoxy- and 4-Thiophenoxy-1-cyclopropyl-1,2,3,6-tetrahydropyridines

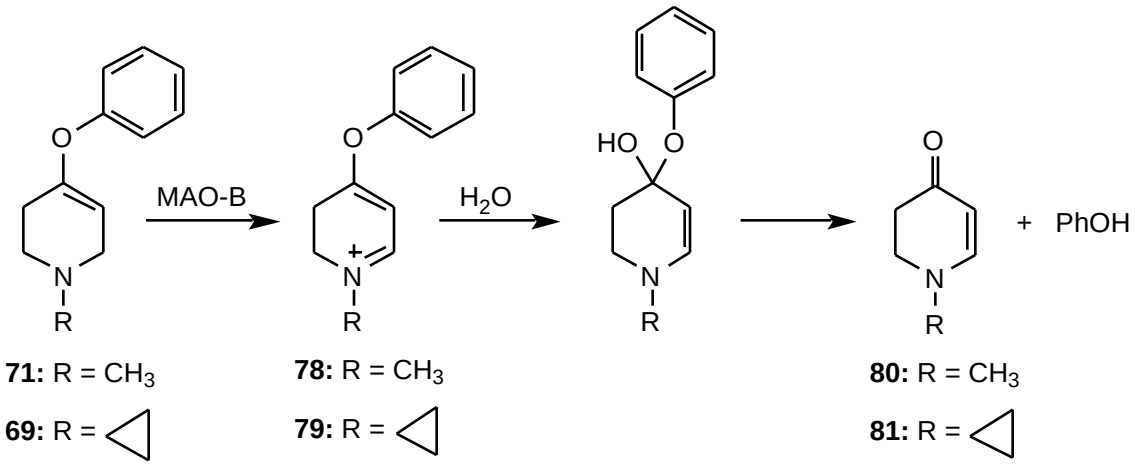


Reagents: (i) cyclopropylamine, H_2O; (ii) $SOCl_2$; (iii) HXAr, NEt_3, CH_3CN; (iv) $NaBH_4$, CH_3OH

4.1.2. Enzymology

Metabolic screening of the phenoxy analog **69** clearly documented its substrate properties. The repeated UV scans showed the formation of a chromophore with λ_{max} 332 nm. The species responsible for this chromophore was tentatively identified by UV analysis as 1-cyclopropyl-2,3-dihydro-4-pyridone (**81**) by comparison with the synthetic sample (λ_{max} 328 nm). GC-EIMS analysis of an ethyl acetate extract of a mixture composed of 50 µL of the MAO-B preparation and 100 µL of 1 mM **69** that had been incubated for 10 minutes at 37 ˚C displayed a peak with retention time 5.31 min and the following mass spectral characteristics: *m/z* 137 (M^+), 109 (M^+ - CO), 108 (M^+ - CHO), 94, 81, 80 and 54. Identical GC-EIMS behavior observed with the synthetic sample confirmed the structure of the MAO-B generated product as **81**. Based on these data, the metabolic fate of **69** is analogous to that observed with the 1-methyl-4-phenoxy[98] analog **71** (Scheme 20).

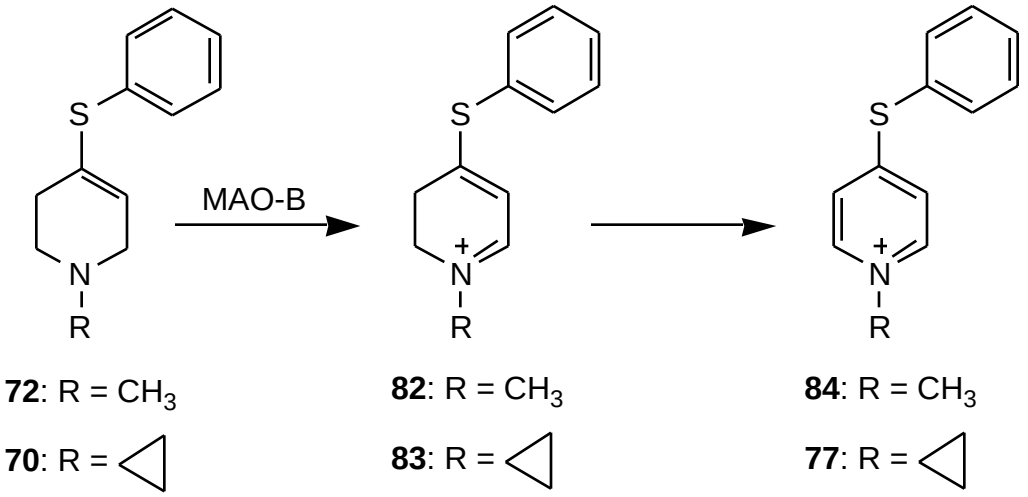
Scheme 20. Metabolic Fate of the Tetrahydropyridines **69** and **71**



Enzyme kinetic data of substrate turnover performed by monitoring the 332 nm chromophore established that this analog is an excellent MAO-B substrate. Taking V_{max}/K_m as an overall estimate of the efficiency of catalysis, the substrate properties of **69** (1650 $min^{-1}mM^{-1}$) were found to be comparable to the 4-benzyl derivative **54** (2025 $min^{-1}mM^{-1}$). Unlike the benzyl compound, however, this phenoxy analog showed no enzyme inactivation properties even at high (1 mM) concentrations.

Incubation of the 4-thiophenoxy analog **70** with MAO-B led to the formation of a chromophore with λ_{max} 365 nm which, unlike the phenoxydihydropyridinium species **79**, did not shift to 332 nm, the chromophore for aminoenone **81**, but instead slowly shifted to λ_{max} 305 nm. We suspected from the known behavior of the corresponding N-methylthiophenoxydihydropyridinium derivative **82**[114] that, instead of undergoing hydrolysis, the relatively stable 1-cyclopropyl-4-thiophenoxydihydropyridinium metabolite **83** had undergone slow oxidation to yield the corresponding pyridinium species **77** (Scheme 21). The UV spectrum of synthetic **77**[118] and the final spectrum of the incubation mixture (λ_{max} 305 nm) confirmed the identity of this product as **77** which presumably was formed by autoxidation and/or disproportionation[117] of the dihydropyridinium intermediate **83**. Apparently, the weaker electronegativity of the sulfur vs the oxygen atom is responsible for the hydrolytic stability of this system. Like the 4-phenoxy derivative **69**, the thiophenoxy analog **70** is a good MAO-B substrate (1900 $min^{-1}mM^{-1}$) and displays no time or concentration dependent MAO-B inactivation properties.

Scheme 21. Metabolic Fate of Tetrahydropyridines **70** and **72**

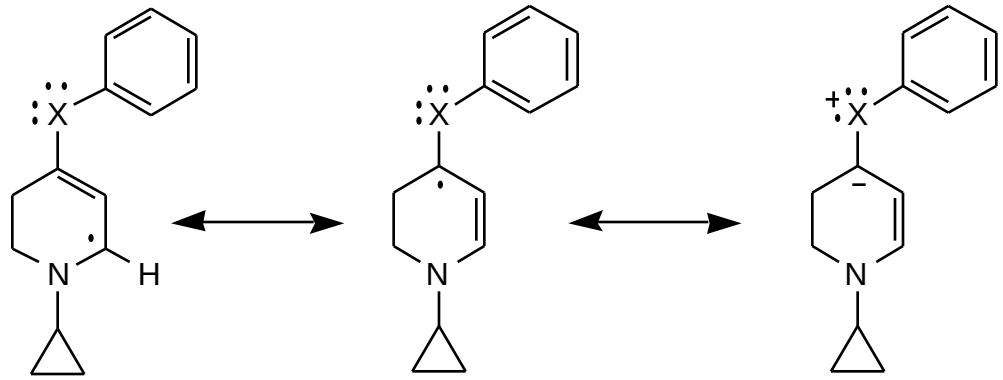


72: R = CH_3 **82**: R = CH_3 **84**: R = CH_3

70: R = **83**: R = **77**: R =

If we assume that the three cyclopropyl analogs, 4-benzyl (**54**), 4-phenoxy (**69**) and 4-thiophenoxy (**70**), all have similar geometries, the differences in their enzymatic properties must be explained by a factor other than sterics. The 4-benzyl analog displayed good MAO-B substrate properties but was an efficient MAO-B inactivator. Although the 4-phenoxy and 4-thiophenoxy showed good substrate properties, there was no evidence of enzyme inactivation. One explanation may be due to the differences in electronics of these three substituents. The phenoxy and thiophenoxy groups should better stabilize the carbon centered radical intermediates **69a** and **70a** than the benzyl group through resonance contribution from the heteroatom (Scheme 21a). We investigated further the influence of electronic effects on substrate/inactivator properties.

Scheme 21a. Resonance Stabilization of Radicals **69a** and **70a**



69a: X = O
70a: X = S

4.2. C-4 Heteroaromatic MPTP Analogs

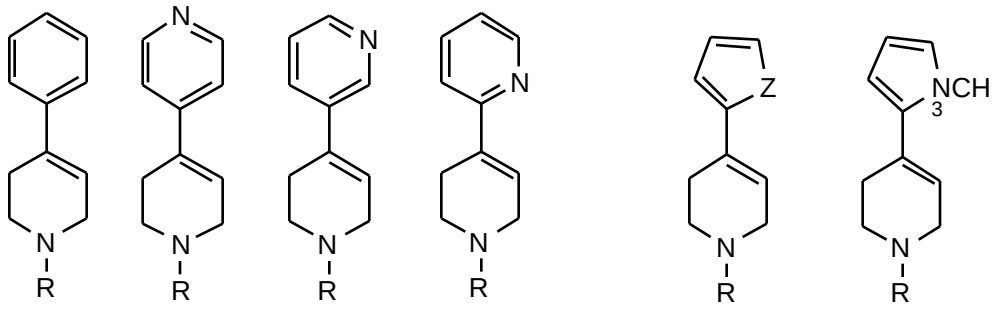
We examined the MAO-B substrate and inactivator properties of a series of 1-methyl- and 1-cyclopropyltetrahydropyridine derivatives bearing C-4 heteroaromatic substituents. Since all of these compounds are 4-aryltetrahydropyridine derivatives with the potential to assume similar conformations with the active site of the enzyme, we speculated that the inactivation properties of the N-cyclopropyl derivatives would not be so influenced by differences in the extent to which the orbitals may align for ring opening as might be encountered with the benzyl and aryloxy analogs. On the other hand, the putative allylic radical intermediates should be stabilized by electron rich heterocyclic aromatic groups at C-4, in which case the N-methyl analogs might be expected to display a relatively wide range of substrate properties. This expectation is based on the assumption that the catalytic step leading to the putative allylic radical intermediate is rate determining as indicated by the primary isotope effect observed in the MAO-B catalyzed

oxidation of MPTP[81] and the 4-benzyl-1-cyclopropyl analog **54**. We anticipated that the results from these types of comparative studies would help to assess the intermediacy of allylic radicals in these α-carbon oxidation reactions.

4.2.1. Chemistry

The principal focus of these studies was to examine the stereoelectronic effects of C-4 substituents on the MAO-B catalyzed oxidations of 1,4-disubstituted tetrahydropyridine derivatives. Our approach included a comparison of the substrate properties of the N-1 methyl analogs **44** and **85**-**91** with the inactivator properties of the N-1 cyclopropyl analogs **49** and **92**-**98** (see Chart 1). *The preparation of these compounds required a variety of synthetic approaches which are discussed below.

Chart 1



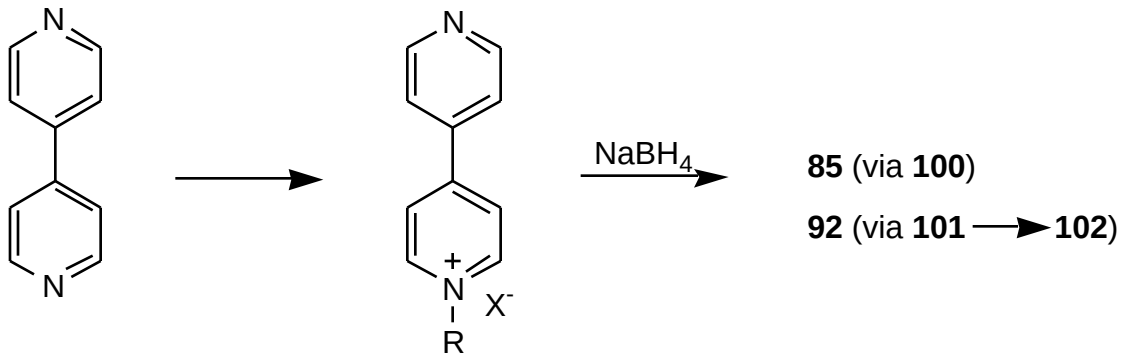
					Z =	S	O	NH	
R = CH_3	**44**	**85**	**86**	**87**		**88**	**89**	**90**	**91**
R = 	**49**	**92**	**93**	**94**		**95**	**96**	**97**	**98**

The synthesis of the 1-methyl-4-(4-pyridyl) analog **85** (Scheme 22) was

* The syntheses of the 4-heteroaromatic analogs were carried out in collaboration with Dr. Sandeep Nimkar.

achieved by monomethylation of the commercially available 4,4'-bipyridine (**99**) followed by $NaBH_4$ reduction of the resulting known methiodide **100**.[117] Preparation of the corresponding N-cyclopropyl analog **92** required an alternative route due to the poor reactivity of halocyclopropanes. The synthetic sequence, adapted from our earlier work[118] and based on the Zincke reaction,[119] also started from 4,4'-bipyridine which was converted to the N-(2,4-dinitrophenyl)pyridinium intermediate **101**. Treatment of **101** with cyclopropylamine gave the N-cyclopropylpyridinium species **102** which was converted to the desired tetrahydropyridine **92** with $NaBH_4$.

Scheme 22. Preparation of the 4-(4-Pyridyl)tetrahydropyridine Species **85** and **92**



99

100: R = CH_3; X^- = I^-

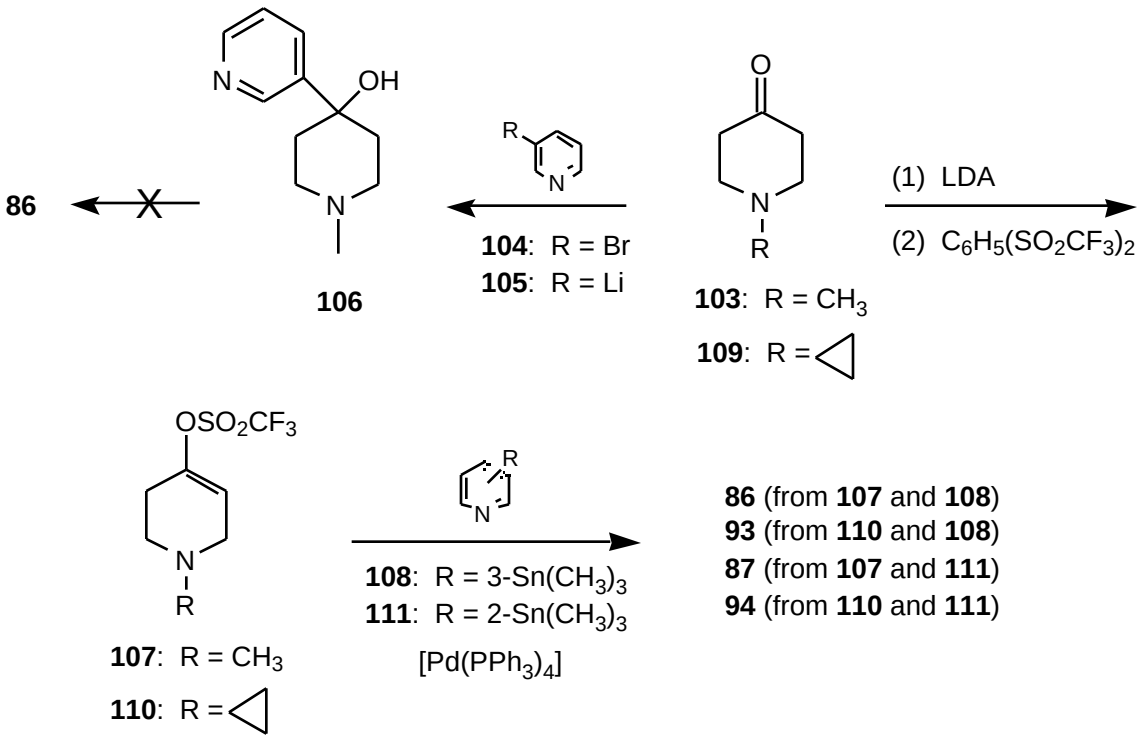
101: R = 2,4-$(NO_2)_2C_6H_3$; X^- = Cl^-

102: R =  ; X^- = Cl^-

Our initial approach to the synthesis of the 1-methyl-4-(3-pyridyl)tetrahydropyridine analog **86** (Scheme 23) proceeded via 4-(3-pyridyl)-

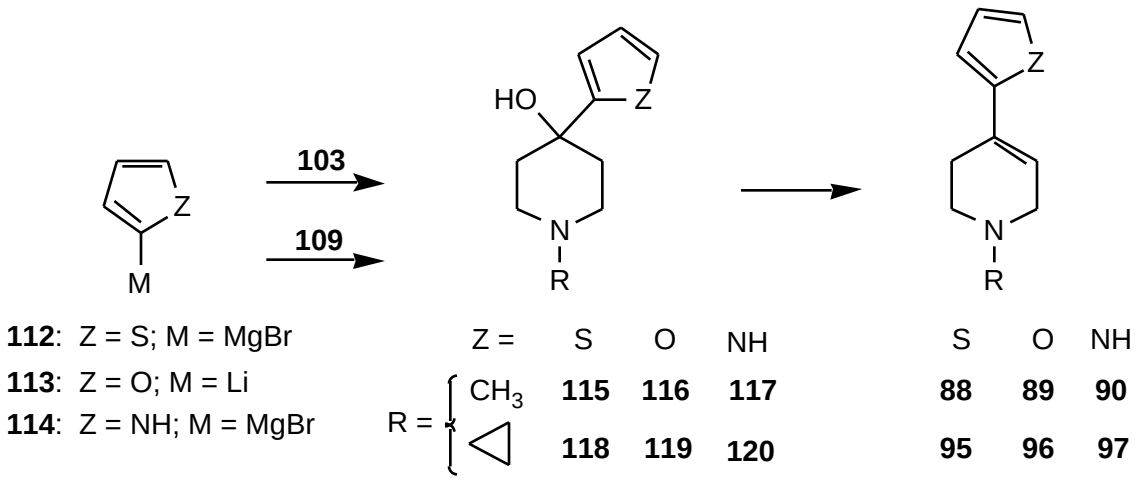
4-piperidinol (**106**) that was obtained via condensation of 1-methyl-4-piperidone (**103**) with the lithium reagent **105** prepared from 3-bromopyridine (**104**). Attempts to bring about the acid catalyzed dehydration of **106**, however, failed under a variety of conditions presumably because protonation of the two basic nitrogen atoms prevents protonation of the hydroxy group that is required for the dehydration step. Attempts to functionalize the piperidinol OH group as a mesylate or tosylate also failed. An alternative approach to **86** proceeded by treatment of **103** with lithium diisopropylamide (LDA) followed by reaction of the lithium enolate with phenyltrifluoromethanesulfonimide [$C_6H_5N(SO_2CF_3)_2$] to generate the tetrahydropyridyl triflate **107**.[120] A Stille-type cross coupling reaction[121] between **107** and the known 3-trimethylstannylpyridine (**108**)[122] in the presence of $Pd(PPh_3)_4$ gave the desired tetrahydropyridine **86** which was isolated as its stable oxalate salt in 46% overall yield. The analogous reaction sequence starting from 1-cyclopropyl-4-piperidone (**109**)[118] and proceeding via the corresponding tetrahydropyridyl triflate **110** provided the N-cyclopropyl analog **93**. Similarly, the 1-methyl- and 1-cyclopropyl-4-(2-pyridyl)-1,2,3,6-tetrahydropyridines **87** and **94**, respectively, were prepared from the corresponding cross coupling reactions between the tetrahydropyridyl triflates **107** and **110** and 2-trimethylstannylpyridine (**111**).[122] It is worth noting that this synthetic approach provides a novel pathway for the preparation of a variety of 4-aryl susbstituted tetrahydropyridines that might not otherwise by available via classical organometallic chemistry.

Scheme 23. Synthetic Pathway to the 4-(3-Pyridyl)tetrahydropyridine Analogs **86** and **93** and 4-(2-Pyridyl)tetrahydropyridine Analogs **87** and **94**



The thienyl analogs **88** and **95**, the furanyl analogs **89** and **96** and the pyrrolyl analogs **90** and **97** were obtained by acid catalyzed dehydration of the corresponding 4-thienyl-4-piperidinols **115** and **118**, the 4-furanyl-4-piperidinols **116** and **119** and the 4-pyrrolyl-4-piperidinols **117** and **120**, respectively (Scheme 24). The intermediate carbinolamines were prepared by reactions between 1-methyl- or 1-cyclopropyl-4-piperidone and the Grignard (**112** or **114**) or lithium (**113**) reagents.

Scheme 24. Preparation of the Thienyl (**88** and **95**), Furanyl (**89** and **96**) and Pyrrolyl (**90** and **97**) Analogs

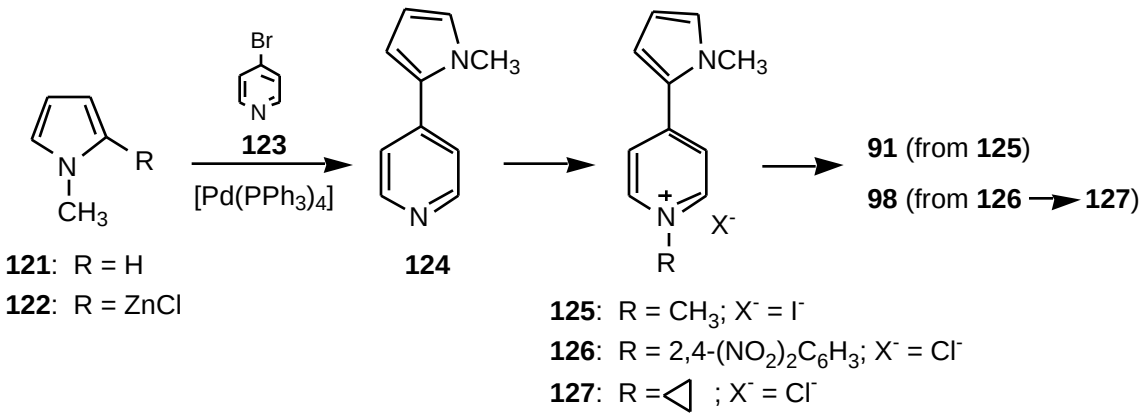


112: Z = S; M = MgBr
113: Z = O; M = Li
114: Z = NH; M = MgBr

R =	Z =	S	O	NH	S	O	NH
	CH_3	**115**	**116**	**117**	**88**	**89**	**90**
		118	**119**	**120**	**95**	**96**	**97**

The synthesis of 1-methyl-4-(1-methyl-2-pyrrolyl)-1,2,3,6-tetrahydropyridine (**91**) had been achieved previously[124] by a sequence starting with the $Pd(PPh_3)_4$ catalyzed cross coupling reaction between the pyrrolylzinc reagent **122** [prepared from 1-methylpyrrole (**121**)] and 4-bromopyridine (**123**) to give the pyrrylpyridine intermediate **124** (Scheme 25). Subsequent methylation of **124** and $NaBH_4$ reduction of the resulting methylpyridinium product **125** gave **91**. The corresponding N-cyclopropyl derivative **98** was prepared as part of the present effort by converting **124** to the N-(2,3-dinitrophenyl)pyridinium species **126** which, upon heating with cyclopropylamine, gave the N-cyclopropylpyridinium intermediate **127**. Subsequent reduction of **127** with $NaBH_4$ gave **98**. Key intermediates and final products, mostly as their stable but hygroscopic oxalate salts, were fully characterized (see Experimental Section).

Scheme 25. Synthetic Pathway to the 4-(1-Methyl-2-pyrrolyl)tetrahydropyridine Analogs **91** and **98**



121: R = H
122: R = ZnCl

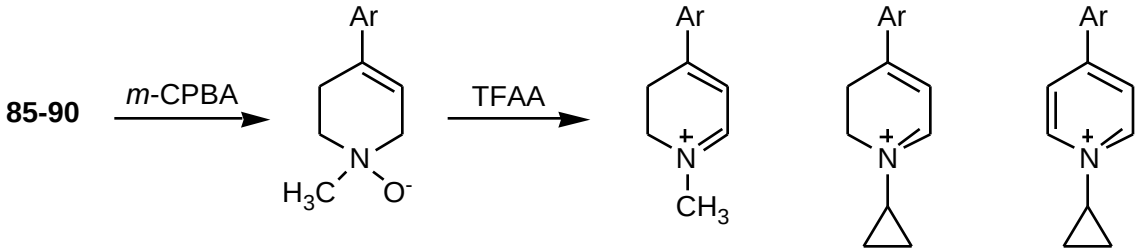
124

125: R = CH_3; X^- = I^-
126: R = 2,4-$(NO_2)_2C_6H_3$; X^- = Cl^-
127: R = ◁ ; X^- = Cl^-

The spectrophotometric assay used to measure the rates of substrate turnover required estimations of the molar extinction coefficients (ε-values) of the dihydropyridinium metabolites **45** and **60-65**. The ε-values for the dihydropyridinium species **45**, from MPTP[69,123] and **140**, from the N-methylpyrrolyl analog **91**[124] are known. Attempts to prepare the remaining dihydropyridinium derivatives via treatment of the corresponding N-oxides (**128-133**) with trifluoroacetic anhydride (Scheme 26) were only partially successful. The thienyl analog **137** could be obtained in pure form. In the case of the furanyl analog **138**, both the precursor N-oxide **132** and the dihydropyridinium species resisted crystallization. The ^1H and ^{13}C NMR spectra of the N-oxide suggested a pure product and consequently we estimated the ε-value for this compound by assuming that **132** underwent quantitative conversion to the dihydropyridinium product. The dihydropyridinium metabolites **134**, **135** and **136** derived from the isomeric 4-pyridyltetrahydropyridines **85**, **86** and **87** readily autoxidized[125] and pure

synthetic samples could not be obtained. Consequently, the known ε-values for the corresponding phenyldihydropyridinium species **45** was used in these calculations.

Scheme 26. Synthesis and Structures of the Dihydropyridinium and Pyridinium Derivatives Discussed in the Text



Ar =	4-Py;	3-Py;	2-Py;	2-Th;	2-Fu;	2-Pr;	1-Me-2-Pr;	Ph
A:	**128**	**129**	**130**	**131**	**132**	**133**		
B:	**134**	**135**	**136**	**137**	**138**	**139**	**140**	
C:	**141**	**142**	**143**	**144**	**145**	**146**	**147**	**53**
D:	**148**	**149**	**150**	**151**	**152**	**153**	**154**	**155**

4.2.2. Enzymology

UV scans of 3 mM solutions of the 1-methyl-4-pyridyl analogs **85**, **86** and **87** in the presence of 0.16 μM MAO-B revealed the time dependent formation of broad bands with maximal absorptions centering near 325 nm as expected for the dihydropyridinium metabolites **134**, **135** and **136**. Linear initial velocities vs substrate concentration plots and linear double reciprocal plots were obtained for all three compounds. The V_{max}/K_m values (Table 5) ranged from 6

to about 60 $min^{-1}mM^{-1}$, all less than 5% of the corresponding value for MPTP. Due to the rough estimate used for ε-values (estimated 10,000 M^{-1}) for the dihydropyridinium metabolites, the V_{max} values could be off by as much as 50%. Nevertheless, these pyridyl analogs are marginal substrates at best.

The 1-methyltetrahydropyridine analogs bearing a 5-membered heterocyclic group (**88-91**) proved to be better MAO-B substrates than the corresponding pyridyl derivatives. As with the pyridyl analogs, UV scans showed the time-dependent formation of chromophores expected for the corresponding dihydropyridinium metabolites (**137** from the thienyl analog **88**, **138** from the furanyl analog **89**, **139** from the pyrrolyl analog **90** and **140** from the N-methylpyrrolyl analog **91**). Compounds **88**, **89** and **91** all displayed K_m values (Table 5) of 0.2 mM, the same value obtained for MPTP in these studies, suggesting similar interactions of these analogs within the enzyme active site. The pyrrolyl analog **90**, however, showed a significantly higher K_m value. This increase in the binding constant K_m may be due to unfavorable polar interactions in the active site. SAR studies have demonstrated that the active site of MAO-B exhibits better catalytic properties for more lipophilic C-4 substituents.[99] Although the thienyl (**88**), the furanyl (**89**) and the pyrrolyl (**90**) analogs are better substrates than the pyridyl derivatives, they are nevertheless much weaker substrates than MPTP. On the other hand, the N-methylpyrrolyl analog **91** showed substrate properties (V_{max}/K_m = 1800 $min^{-1}mM^{-1}$) better than MPTP (V_{max}/K_m = 1350 $min^{-1}mM^{-1}$). A more notable difference is the dramatic increase in substrate properties of the N-methylpyrrolyl analog **91** relative to the pyrrolyl analog **90**. This may be explained by their differences in geometry, a topic that will be addressed in Chapter 5.

Table 5. Parameters Related to the MAO-B Catalyzed Oxidation of Various 1-Methyl-4-heteroaryl-1,2,3,6-tetrahydropyridine Derivatives and MPTP

Compound	**85**	**86**	**87**	**90**	**89**	**88**	**44**	**91**
V_{max} (min^{-1})	14	67	56	74	31	60	270	360
K_m (mM)	2.4	1.9	0.9	1.6	0.2	0.2	0.2	0.2
V_{max}/K_m ($min^{-1}mM^{-1}$)	6	35	60	46	155	300	1350	1800

Solutions (3 mM) of the cyclopropyl analogs **92**-**97** were incubated in the presence of 0.16 μM MAO-B and scanned spectrophotometrically. None of the analogs showed evidence of dihydropyridinium or pyridinium formation under these conditions. UV scans of the N-methylpyrrolyl analog **98** in the presence of MAO-B, however, revealed the time dependent formation of a chromophore at 420 nm corresponding to the dihydropyridinium metabolite **140**. Kinetic analysis of substrate turnover performed by monitoring the 420 nm chromophore established the V_{max}/K_m value of 290 $min^{-1}mM^{-1}$. The ε value (24,000 M^{-1}) was estimated using the value for the perchlorate salt of the 1-methyl-4-(1-methyl-2-pyrrolyl)-2,3-dihydropyridinium species **140**.[124] Closer examination by GC-EIMS analysis, however, revealed that an incubation mixture containing the 4-phenyl-1-cyclopropyl analog **49** and MAO-B that had been treated with $NaBD_4$ gave a peak with m/z corresponding to **49-d$_2$**. These data provided evidence for pyridinium formation. The 1-methyl-4-phenyl-2,3-dihydropyridinium intermediate **45** is unstable and undergoes oxidation to the pyridinium species **46**.[71] Thus, it is reasonable to assume that the dihydropyridinium intermediate generated from the MAO-B catalyzed oxidation

of **49** undergoes further oxidation to the pyridinium product (**155**). $NaBD_4$ reduction of the pyridinium generates **49-d$_2$**. Additionally, incubation of the heteroaromatic-1-cyclopropyl analogs **92**-**97** in the presence of MAO-B all showed evidence of pyridinium formation. The substrate properties of **49** and **92**-**97** were too poor to obtain the kinetic parameters V_{max} and K_m.

Studies on the inactivation properties showed that all of the 1-cyclopropyl analogs were time and concentration dependent inhibitors of MAO-B. Attempts to estimate the k_{inact}/K_I values for the thienyl and furanyl analogs **95** and **96**, respectively, failed because the rates of inactivation at concentrations still below K_I were too fast to obtain accurate values for the rates of loss of enzyme activity. At 100 μM inhibitor, enzyme activity was nearly depleted within 5 minutes. Additionally, the good substrate properties of the N-methylpyrrolyl analog **98** prevented accurate measurements of rates of inactivation vs inhibitor concentration. The k_{inact}/K_I values for the remaining 1-cyclopropyl analogs are reported in Table 6.

Table 6. Inactivation Kinetics and Partition Ratios for Various 4-Heteroaryl Substituted 1-Cyclopropyl-1,2,3,6-tetrahydropyridine Derivatives

Compound	**49**	**92**	**93**	**94**	**95**	**96**	**97**	**98**
k_{inact}/K_I ($min^{-1}mM^{-1}$)	1.0	0.1	0.3	0.3	---	---	0.5	---
Partition Ratio	17	4	9	8	10	8	20	400

The limited success with the inactivation studies led us to examine the

partition ratios (moles of product formed per unit time/moles of enzyme inactivated per unit time) of these compounds. With the exception of the pyrrolyl analog **98**, the spectrophotometric assay employed with the 1-methyl analogs could not be used to study the 1-cyclopropyl analogs because metabolite formation was too slow at the low enzyme concentrations used. At higher enzyme concentrations, the solutions were too turbid for spectrophotometric analysis. Consequently, we elected to examine the partition ratios with the aid of a GC-EIMS assay that provided an estimate of the total number of moles of product formed during the same period that the enzyme (4 μM) underwent complete inactivation. Although enzyme inactivation was complete after about 30 minutes, incubations were maintained for 3 hours to insure that all of the intermediate dihydropyridinium metabolites (**141**-**146** and **53**) had undergone quantitative oxidation to the corresponding pyridinium products (**148**-**153** and **155**). Confirmation of the complete oxidation of the intermediate dihydropyridinium metabolite **53** to the pyridinium product **155** was obtained with the aid of an HPLC-diode array assay. The partition ratio for analogs **49** and **92**-**97** ranged from 4-20 (Table 6). Clearly the inactivation pathway is the preferred pathway over product formation compared to the 4-benzyl-1-cyclopropyl analog **54** (partition ratio 1250).

Special problems were encountered in the case of the N-methylpyrrolyl analog **98**. The GC-EIMS experiment indicated that the principal metabolite present after the 3 hour incubation period was the intermediate dihydropyridinium species **147** rather than the pyridinium product **154**. HPLC-diode array analysis confirmed that only a small amount of **154** was present. Instead, the principal metabolic product was the dihydropyridinium intermediate

147 Consequently, the partition ratio for this compound was estimated by measuring the amount of substrate consumed during the inactivation time course with the aid of an HPLC-diode array assay. Consistent with the observed increase in substrate properties, it was found to be 400, approximately 20 times greater than the other heteroaromatic analogs **92**-**97**.

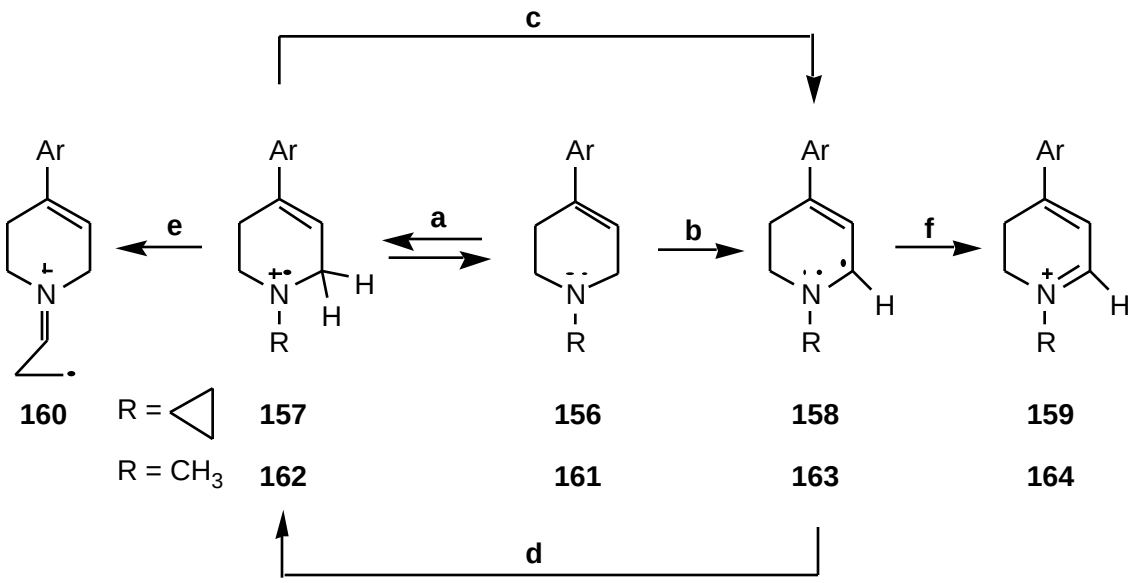
4.3. Discussion

Only a moderate substituent effect is observed on the inactivation characteristics of these 1-cyclopropyltetrahydropyridine derivatives and, with the exception of the pyrrolyl analog **98**, an even more moderate effect is observed on the corresponding partition ratios, behavior that in general, would be expected for the SET pathway (**156 -> 157 -> 160**, Scheme 27). In contrast to these results, the V_{max}/K_m values for the 1-methyl analogs vary over a range of 300 fold (6 to 1800 $min^{-1}mM^{-1}$) with the electron rich pyrrolyl analog **91** being the best substrate and the electron poor pyridyl analogs being the poorest substrates. The tendency for N-methyl analogs (**161**, Scheme 27) bearing electron releasing groups to undergo more efficient oxidation to the dihydropyridinium metabolites **164** may be rationalized according to the SET pathway in which the deprotonation step (step c) of the aminyl radical cation **162** to yield the allylic radical **163** is rate determining. The HAT pathway also predicts the observed electronic effects for the 1-methyl analogs since the energy barrier associated with a rate determining loss of a hydrogen atom from the substrate (step b) should decrease with increasing stabilization of the resulting allylic radical (**163**). This suggestion of an electronic effect on catalysis is quite tentative in part because of the limited number of compounds examined in this study and also because of the large differences observed in

the V_{max}/K_m values of the pyrrolyl vs the thienyl and furanyl analogs.

The inactivating properties of the 1-cyclopropyltetrahydropyridine derivatives (**156**, Scheme 27) can be readily explained by the SET pathway since the cyclopropylaminyl radical cation (**157**) would be expected to ring open rapidly to yield the bioalkylating species **160**. In order to account for the observed formation of 1-cyclopropyldihydropyridinium metabolites (**159**), the deprotonation step leading to the allylic radical **158** (step c) would have to compete kinetically with the ring opening step (step e). Conformational arguments (lack of orbital alignment) could be invoked to explain why some 1-cyclopropyltetrahydropyridine derivatives are such good substrates. If the inactivating properties of the 1-cyclopropyltetrahydropyridine derivatives mediated by the ring opened species **160**, then the pathway proceeding by HAT requires that the allylic radical (**158**) undergo rapid protonation (step d) to form the aminyl radical cation (**157**). A third possible interpretation of these data is based on the proposal involving partitioning of the tetrahydropyridines (**156** or **161**) between the SET and the HAT pathways.

Scheme 27. Proposed Pathways for the MAO-B Catalyzed Oxidation of 1,4-Disubstituted-1,2,3,6-tetrahydropyridine Derivatives



Chapter 5. Additional Studies on the MAO-B Catalyzed Oxidation of 1,4-Disubstituted Tetrahydropyridine Derivatives

5.1. Deuterium Isotope Effects

The deuterium isotope effect studies on the MAO-B catalyzed oxidation of 4-benzyl-1-cyclopropyl-1,2,3,6-tetrahydropyridine (**54**) revealed that the C-H bond cleavage step leading to dihydropyridinium formation was at least partially rate determining. The presence of deuterium in the substrate slowed the rate of product formation while it increased the rate of enzyme inactivation. Previous studies[81] had shown that the C-H bond cleavage step in the MAO-B catalyzed oxidation of MPTP is also rate determining. The observed isotope effect of MPTP-2,2,6,6-d_4 on V_{max}/K_m was nearly 8. These results prompted us to examine the deuterium isotope effects on the oxidation of additional 1,4-disubstituted tetrahydropyridine analogs. We wanted to establish if the rate determining C-H bond cleavage step is dependent on the structure of the substrate.

We elected to investigate the deuterium isotope effects of the 4-phenyl-1-cyclopropyl analog **49**. This compound was shown to have a very low partition ratio (17, see Chapter 4), demonstrating that enzyme inactivation is the preferred pathway compared to the 4-benzyl-1-cyclopropyl analog (partition ratio 1250). We were unable to measure the kinetic parameters V_{max} and K_m for the substrate turnover of **49**, however the k_{inact}/K_I value was found to be 1.0 $min^{-1}mM^{-1}$. We wanted to examine the influence of deuterium substitution on the substrate/inactivation pathways.

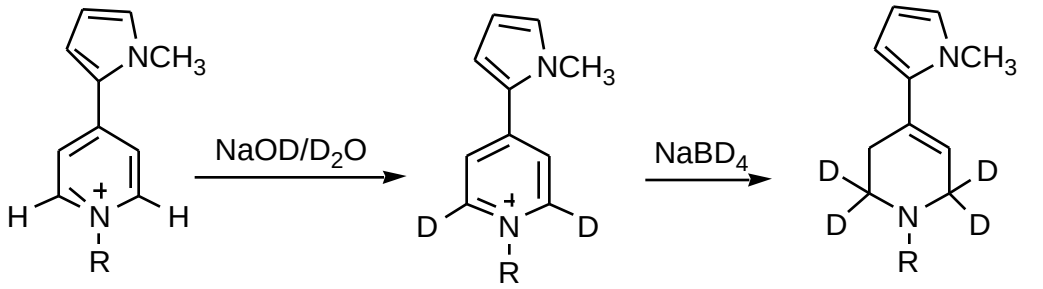
The 1-methyl- and 1-cyclopropyl-4-(2-methylpyrrolyl) analogs **91** and **98** displayed substate/inactivator characteristics dramatically different from the

other C-4 heteroaromatic analogs examined (see Chapter 4). Both the 1-methyl analog **91** and the 1-cyclopropyl analog **98** showed enhanced substrate properties. Compound **98** had a partition ratio of 400, while all of the other 4-heteroaromatic substituted 1-cyclopropyl analogs displayed partition ratios less than 20. In an attempt to gain additional information about the N-methylpyrrolyl analogs **91** and **98**, we examined the deuterium isotope effects on the enzymatic pathways.

5.1.1. Synthesis of the d_4 Analogs of 49, 91 and 98

Syntheses of the 1-methyl- and 1-cyclopropyl-4-(1-methyl-2-pyrrolyl)-1,2,3,6-tetrahydropyridine-2,2,6,6-d_4 analogs (**91-d_4** and **98-d_4**) were achieved by treatment of the pyridinium intermediates **125** and **127** with sodium deuteroxide in D_2O to form the intermediates **125-d_2** and **127-d_2** (Scheme 28). Reduction of these pyridinium intermediates with $NaBD_4$ gave the desired 2,2,6,6-d_4 analogs (**91-d_4** and **98-d_4**). Deuterium incorporation estimated by ^1H NMR and GC-EIMS was greater than 99%. The syntheses of the pyridinium intermediates are described in Chapter 4.

Scheme 28. Synthetic Pathway to the 4-(1-Methyl-2-pyrrolyl)tetrahydropyridine Analogs **91-d$_4$** and **98-d$_4$**



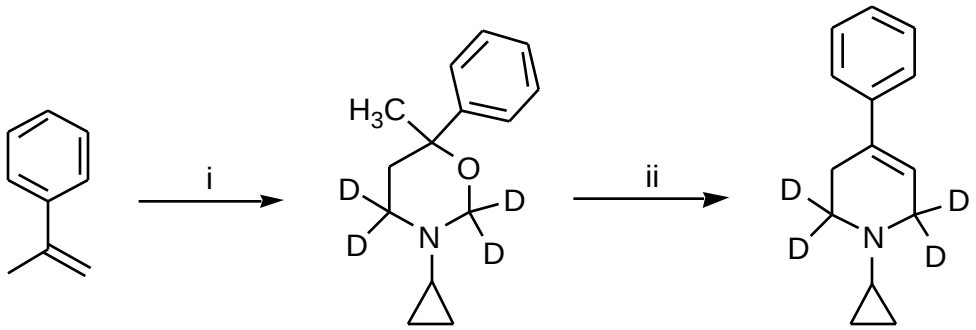
125: R = CH_3; X^- = I^-
127: R = ◁ ; X^- = Cl^-

125-d$_2$: R = CH_3; X^- = I^-
127-d$_2$: R = ◁ ; X^- = Cl^-

91-d$_4$: R = CH_3
98-d$_4$: R = ◁

The synthesis of 1-cyclopropyl-4-phenyl-1,2,3,6-tetrahydropyridine-2,2,6,6-d$_4$ (**49-d$_4$**) was carried out following the methodology reported earlier for the d$_0$ analog.[101] Reaction of α-methylstyrene (**165**) with paraformaldehyde-d$_2$ and cyclopropylamine gave the 1,3-oxazine-d$_4$ (**166-d$_4$**) (Scheme 29). Acid treatment of **166-d$_4$** gave the desired product **49-d$_4$** which showed virtually 100% deuterium incorporation as estimated by ^{1}H NMR and GC-EIMS.

Scheme 29. Synthetic Pathway to the 4-Phenyl-1-cyclopropyl Analog **49-d$_4$**



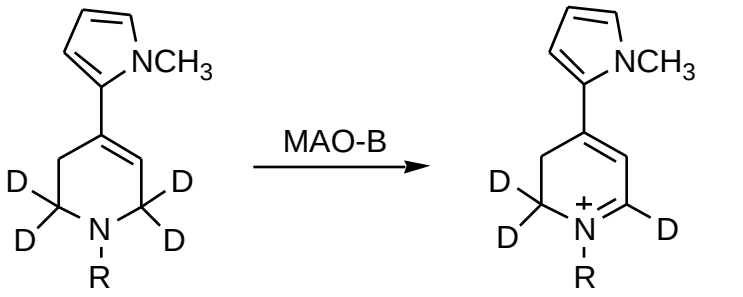
165 **166-d$_4$** **49-d$_4$**

Reagents: (i) 2 D_2CO, cyclopropylamine; (ii) conc. HCl

5.1.2. Enzymology

The 1-methyl-4-(2-methylpyrrolyl) analog **91** is an excellent MAO-B substrate (V_{max}/K_m = 1800 $min^{-1}mM^{-1}$).[124] The deuterated analog **91-d$_4$** also showed good substrate properties, however, the presence of deuterium slowed the rate of oxidation of the tetrahydropyridine substrate to the dihydropyridinium intermediate **140-d$_3$** (V_{max}/K_m = 1200 $min^{-1}mM^{-1}$, Scheme 29a). The K_m value for both the **91-d$_0$** and **91-d$_4$** analogs is 0.2 mM, demonstrating that deuterium substitution does not influence the binding of the substrate. The observed kinetic isotope effect on V_{max}/K_m is 1.5 indicating that C-H bond cleavage is at least partially rate determining.

Scheme 29a. Metabolic Fate of the 4-(2-methylpyrrolyl) Analogs **91-d$_4$** and **98-d$_4$**



91-d$_4$: R = CH_3

98-d$_4$: R = 

140-d$_3$: R = CH_3

147-d$_3$: R = 

Deuterium substitution of the 1-cyclopropyl-4-(2-methylpyrrolyl) analog **98** had a more pronounced effect on both the pathway leading to product formation and the inactivation pathway. The deuterated analog **98-d$_4$** showed poorer substrate properties (V_{max}/K_m = 70 $min^{-1}mM^{-1}$) than the corresponding proteo compound (V_{max}/K_m = 290 $min^{-1}mM^{-1}$). This decrease in substrate properties resulted from a slower rate of catalysis since the K_m value was the same for **98** and **98-d$_4$**. The observed isotope effect on V_{max}/K_m is 4.1. Attempts to measure the rates of inactivation of the proteo compound were unsuccessful due to its good substrate properties. Rates of MAO-B inactivation by the deuterated analog **98-d$_4$**, on the other hand, could be measured due to its decreased substrate properties. The k_{inact}/K_I was found to be 0.2 $min^{-1}mM^{-1}$. The partition ratio for **98-d$_4$** is 160 and the observed isotope effect is 2.5. The kinetic parameters for the MAO-B catalyzed oxidation and enzyme inactivation of these 1,4-disubstituted tetrahydropyridine analogs are summarized in Table

7.

Table 7. Kinetic Parameters for the MAO-B Catalyzed Oxidation/Inactivation of 1,4-Disubstituted-1,2,3,6-tetrahydropyridines

	Compound					
	49-d_0	**49-d_4**	**91-d_0**	**91-d_4**	**98-d_0**	**98-d_4**
k_{cat} (min^{-1})			360	240	58	14
K_m (mM)			0.2	0.2	0.2	0.2
k_{cat}/K_m ($min^{-1}mM^{-1}$)			1800	1200	290	70
$^D(k_{cat}/K_m)$			1.5		4.1	
k_{inact}/K_I ($min^{-1}mM^{-1}$)	1.0	1.2				0.2
$^D(k_{inact}/K_I)$	0.8					
Partition ratio (r)	22	3			400	160
$^D(r)$	7				2.5	

We also measured the deuterium isotope effects on enzyme inactivation and on the partition ratio of the 4-phenyl-1-cyclopropyl analog **49**. The deuterated analog **49-d_4** showed slightly better inactivation properties (k_{inact}/K_I = 1.2 $min^{-1}mM^{-1}$) than the corresponding proteo compound, resulting from "metabolic switching". The observed isotope effect on k_{inact}/K_I results in an inverse isotope effect of 0.8. The partition ratio, determined by GC-EIMS analysis, was found to be 3 which translates into a normal isotope effect of 7. The deuterated analog also showed very poor substrate properties and, like the proteo analog, enzyme inactivation is the favored pathway.

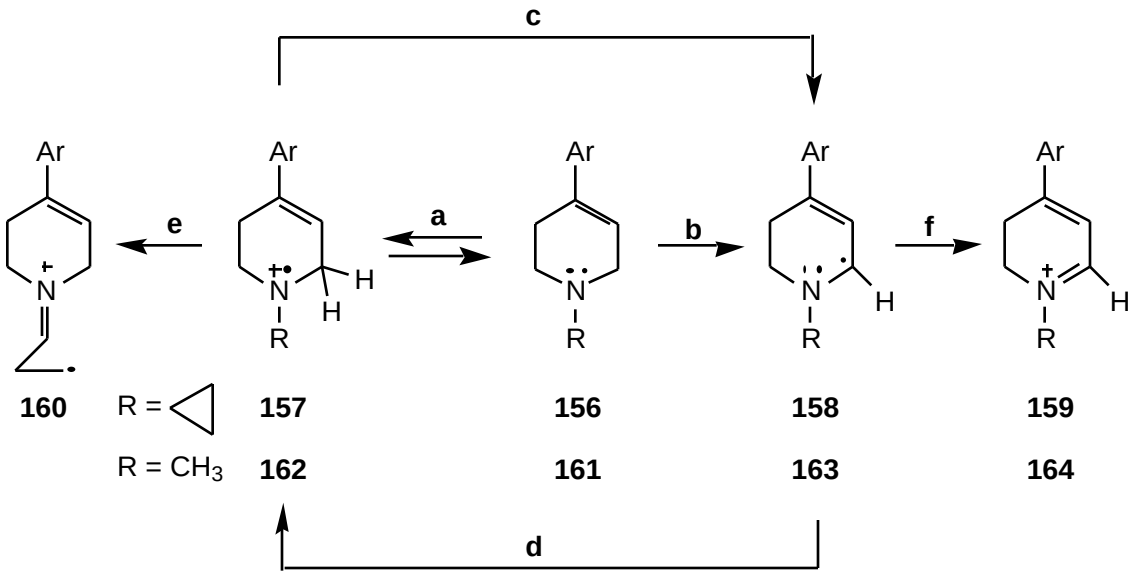
5.1.3. Discussion

We observed a normal isotope effect on dihydropyridinium formation for the 1-cyclopropyl-4-(2-methylpyrrolyl) analog **98**, an inverse isotope effect on enzyme inactivation, and a normal isotope effect on the partition ratio. Although we could not measure the Michaelis Menton parameters for the substrate properties of the 1-cyclopropyl-4-phenyl derivative **49**, we did observe a normal isotope effect on the partition ratio and an inverse effect on enzyme inactivation.

We interpret these results in terms of the pathways shown in Scheme 27. If the SET pathway (path a) was the sole pathway operating, one would expect to see an isotope effect on product formation, due to the C-H bond cleavage step (step c), no isotope effect or an inverse effect on enzyme inactivation, due to metabolic switching, and a normal isotope effect on the partition ratio. Similarly, in the case of HAT (path b), one would also expect to observe an isotope effect on product formation and no isotope effect or an inverse effect on enzyme inactivation (assuming the protonation step is slow). However, the partition ratio for the HAT pathway should be devoid of an isotope effect because the partitioning occurs after the initial hydrogen atom transfer and the following steps do not involve C-H bond cleavage. If partitioning occurs between electron transfer and HAT, one could not distinguish it from the SET pathway based on kinetic isotope effects. Based on these assumptions, the kinetic isotope effects we observed on the MAO-B catalyzed oxidation of various 1,4-disubstituted tetrahydropyridine derivatives cannot distinguish whether partitioning occurs at the tetrahydropyridine or the radical cation intermediate. Our results are consistent with a SET pathway proceeding through a radical cation intermediate or a partitioning pathway of the tetrahydropyridine between

electron transfer leading to enzyme inactivation and hydrogen atom transfer leading to product formation.

Scheme 27. Proposed Pathways for the MAO-B Catalyzed Oxidation of 1,4-Disubstituted-1,2,3,6-tetrahydropyridine Derivatives



5.2. Investigations of Steric Interactions

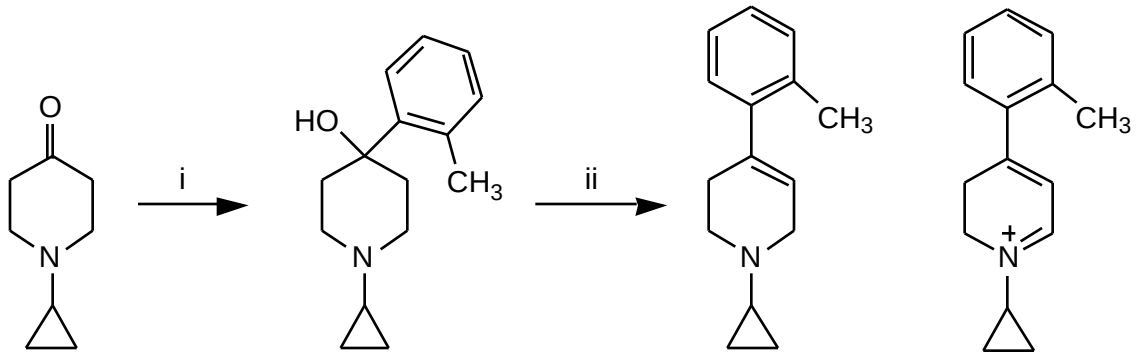
In an effort to gain further information on the structural requirement for substrates of 1-cyclopropyltetrahydropyridines, we focused our attention on steric factors. We observed a dramatic increase in the substrate properties of the 1-methyl-4-(2-methylpyrrolyl) analog **91** compared to the 1-methyl-4-pyrrolyl analog **90** (V_{max}/K_m = 1800 $min^{-1}mM^{-1}$ and 46 $min^{-1}mM^{-1}$, respectively). Although an increase in the K_m value of **91** contributes to its poorer substrate properties, there is nearly a 5 fold difference in the rates of catalysis between these two analogs. In addition, the 1-cyclopropyl-4-(2-methylpyrrolyl) analog **98** is a much better substrate than the 1-cyclopropyl-4-

pyrrolyl derivative **97.** The partition ratio showed a 20 fold increase in the rates of dihydropyridinium formation. We expect the electron donating properties of the 2-methylpyrrolyl and pyrrolyl substituents are similar. However, the conformation of these two analogs may be different due to an increase in the torsion angle in **91** produced by the addition of a methyl group. These findings opened the opportunity to explore the influence of steric interactions on MAO-B catalysis. We synthesized the 1-cyclopropyl-4-(2-methylphenyl) analog **167** and the 1-methyl- and 1-cyclopropyl-4-(2-methylfuranyl) analogs (**168** and **169**) to examine the influence of sterics on substrate/inactivation properties.

5.2.1. Chemistry

1-Cyclopropyl-4-(2-methylphenyl)-1,2,3,6-tetrahydropyridine (**167**) was synthesized by the reaction of 1-cyclopropyl-4-piperidone (**109**) with the aryl Grignard reagent to give the corresponding piperidinol **170**. The crude tertiary alcohol was subjected to acid catalyzed dehydration with HCl/HOAc to generate the desired tetrahydropyridine **167** (Scheme 30).

Scheme 30. Synthesis of 1-Cyclopropyl-4-(2-methylphenyl)-1,2,3,6-tetrahydropyridine



109 **170** **167** **171**

Reagents: (i) 2-CH_3-C_6H_4Br, Mg, Et_2O; (ii) HCl/HOAc

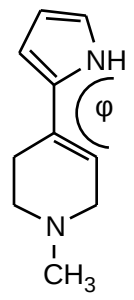
5.2.2. Enzymology

The MAO-B inactivation properties of the 4-phenyl-1-cyclopropyl derivative (**49**) have been previously investigated. This compound was shown to be a good mechanism based inactivator (k_{inact}/K_I = 1.0 $min^{-1}mM^{-1}$)[120] while the N-methyl analog (MPTP) is a good substrate of MAO-B. The 1-methyl-4-(2-methylphenyl) analog (**172**, V_{max}/K_m = 1275 $min^{-1}mM^{-1}$) displayed substrate properties even better than MPTP (V_{max}/K_m = 523 $min^{-1}mM^{-1}$).[126] The least expected result surfaced when 1-cyclopropyl-4-(2-methylphenyl)-1,2,3,6-tetrahydropyridine (**167**) was incubated with MAO-B. This analog gave rise to a chromophore (λ_{max} 338 nm) that was assigned to the dihydropyridinium species **171** by comparison with the UV spectral properties of crude synthetic **171**. Quantitative kinetic analysis established the V_{max} (176 min^{-1}) and K_m (0.28 mM) values for this oxidation. This compound, therefore, is a reasonably good MAO-B substrate (V_{max}/K_m = 635 $min^{-1}mM^{-1}$). Equally surprising was the

observation that **167** did not inhibit MAO-B. These results are in dramatic contrast to the corresponding characteristics observed with the 4-phenyl analog **49**, which showed very poor substrate properties but was a very good time and concentration dependent inactivator of MAO-B.

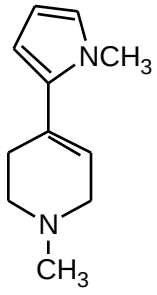
We observed a trend in both the MAO-B substrate and inactivation properties of these 1,4-disubstituted tetrahydropyridine derivatives. The 1-methyl analogs **90** and **44** showed an increase in the rates of dihydropyridinium formation when a methyl group was added to the C-4 substituent (**91** and **172**). The addition of this methyl group increases the torsion angle φ (see Chart 2). Perhaps a change in the configuration of these analogs allows better interaction with the enzyme active site which would explain the observed increase in substrate properties. The enzymatic properties of the 1-cycloproypyl analogs are influenced as well by the addition of a methyl group. The 4-pyrrolyl analog **97** displayed very poor substrate properties but showed excellent MAO-B inactivation properties. The 4-(2-methylpyrrolyl) analog **98**, however, showed a dramatic decrease in the rate of inactivation as well as an increase in the rate of product formation. The same trend was observed for the 4-phenyl and 4-(2-methylphenyl) analogs (**49** and **167**) but was even more pronounced. The MAO-B inactivation properties of **49** were lost by addition of a methyl group. Furthermore, the 4-(2-methylphenyl) analog **167** showed good substrate properties.

Chart 2



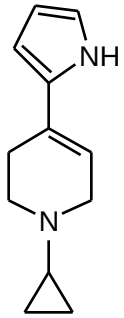
90

poor substrate



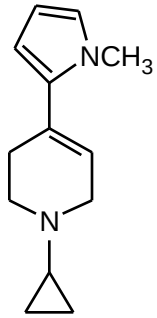
91

very good substrate



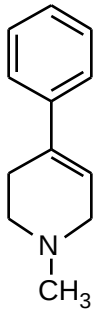
97

very poor substrate
very good inactivator



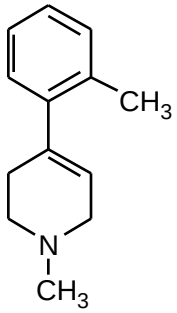
98

good substrate
good inactivator



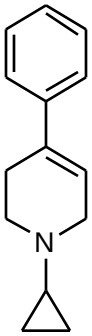
44

good substrate



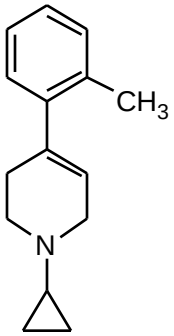
172

very good substrate



49

very poor substrate
very good inactivator

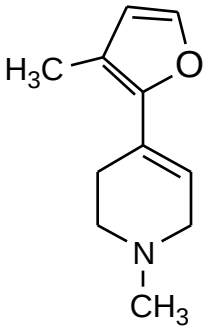


167

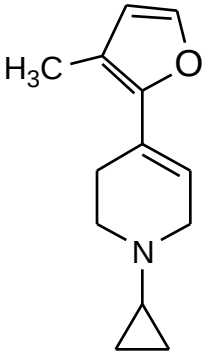
good substrate
no inactivation

These results led us to explore the possibility of converting good mechanism based inactivators into good substrates by adding a "twist" to the molecule. First we examined the interactions of 1-methyl-4-(2-methylfuranyl)-1,2,3,6-tetrahydropyridine[127] (**168**) with MAO-B. The substrate properties of **168** were dramatically increased compared to the 4-furanyl analog **89**. The V_{max} value for **89** is 31 min^{-1} while compound **168** showed a 10 fold increase (300 min^{-1}). In addition, the K_m value for **168** was substantially lower (0.03 mM) than **89** (0.2 mM). The ratio V_{max}/K_m indicated an increase in substrate

properties by 60 fold by addition of the methyl group, 155 for **89** and 10,000 for **168**.



168



169

Examination of the inactivation properties of the 4-furanyl analog **89** revealed that it is an extremely potent MAO-B mechanism based inactivator (see Chapter 4). Although the kinetic parameter k_{inact}/K_I could not be measured due to its rapid rates of inactivation, it clearly is the best cyclopropyl containing tetrahydropyridine analog that we have studied. Based on the above results, we predicted that the 1-cyclopropyl-4-(2-methylfuranyl)[127] analog **169** should be a good to excellent MAO-B substrate. UV scans of an incubation mixture containing **169** and MAO-B, however, showed no evidence of dihydropyridinium formation. In fact, the 4-(2-methylfuranyl) analog **169** displayed nearly identical behavior as the 4-furanyl analog **89**. It, too, revealed extremely potent time and concentration dependent inactivation of MAO-B. The kinetic parameter k_{inact}/K_I was estimated to be 4 $min^{-1}mM^{-1}$. The partition ratio, determined by GC-EIMS analysis, was found to be 25. The corresponding ratio for the 4-furanyl analog **89** is 8. These results were very surprising and clearly indicate that the system we are trying to understand is complex. It is difficult to imagine that any one factor such as electronics or sterics govern the enzymatic properties. Rather, it is more conceivable that a combination of many

parameters dictate the substrate and inactivation characteristics of these 1,4-disubstituted tetrahydropyridines.

5.3. Chemical Models

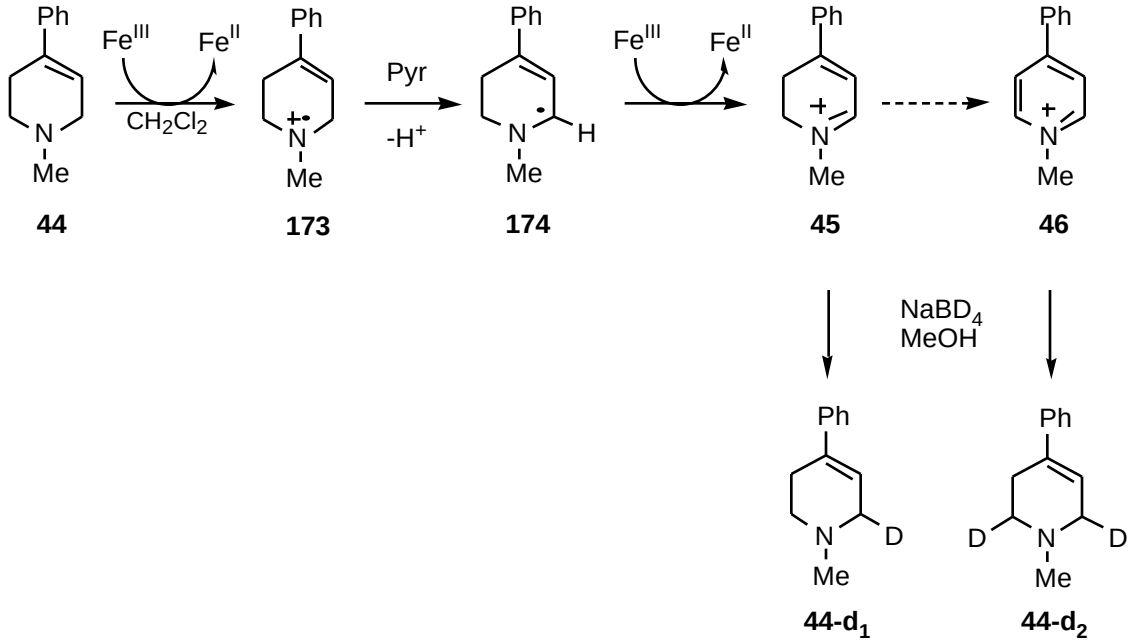
*In addition to examining the MAO-B substrate and inactivation properties of various 1,4-disubstituted tetrahydropyridine derivatives, we developed two chemical models based on reactions reported by Dinnocenzo.[110] The two enzymatic pathways of interest, single electron transfer (SET) and hydrogen atom transfer (HAT), were chemically modeled with reagents known to abstract selectively an electron or a hydrogen atom. We studied the interactions of various 1-methyl and 1-cyclopropyl analogs under SET and HAT conditions.

5.3.1. Single Electron Transfer

Electron abstraction from the nitrogen lone pair of MPTP (**44**) was induced by the phenanthroline ferric complex Fe^{3+}(1,10-phenanthroline)$_3$(PF_6^-)$_3$, a known outer-sphere one-electron oxidant,[128-130] to generate the radical cation intermediate **173**. Deprotonation at the C-6 position of the tetrahydropyridine ring was achieved with pyridine. Pyridine was chosen as a base because of its moderate basicity and resistance to oxidation. The strongly reducing α-amino radical **174** was oxidized to the iminium ion by a second equivalent of Fe^{III} complex to give $MPDP^+$(**45**) (Scheme 31).

* The chemical model studies were conducted in collaboration with Dr. Christelle Franot.

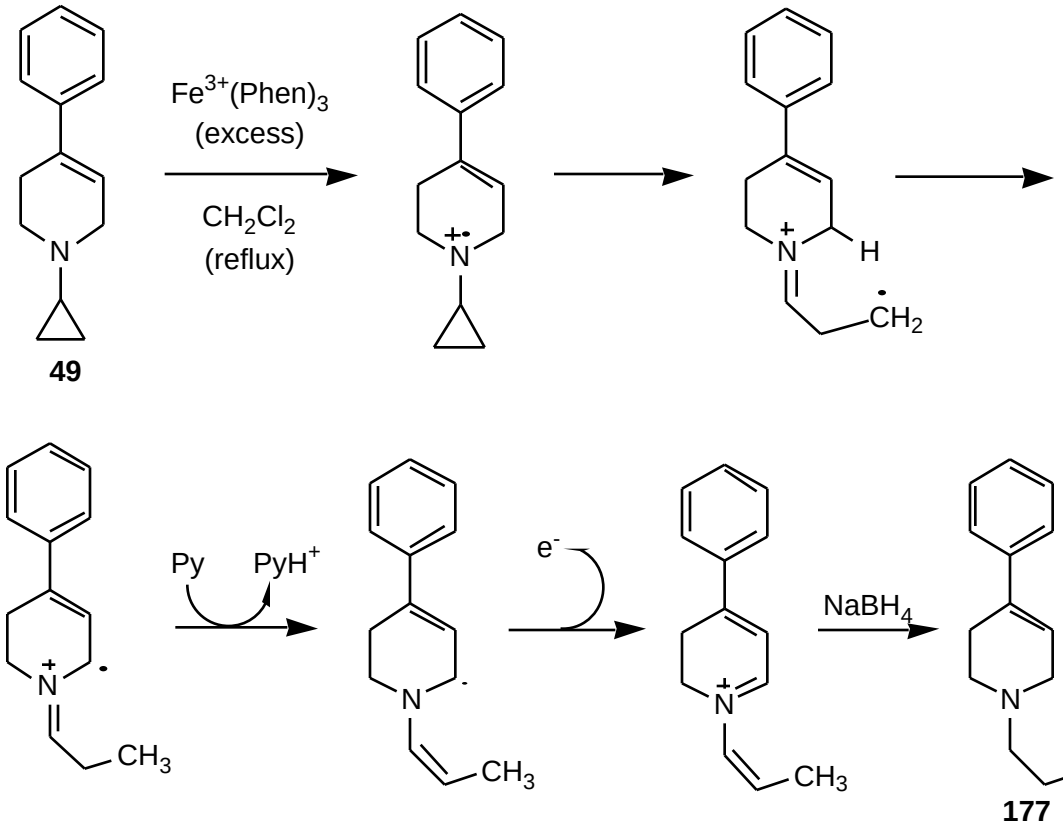
Scheme 31. Mechanism for the Reaction of MPTP (**44**) with Fe^{3+}(1,10-phenanthroline)$_3$ and Pyridine



Following reduction of **45** with sodium borodeuteride, GC-EIMS analysis showed a mixture of monodeuterated MPTP (**44-d$_1$**), the expected product, and dideuterated MPTP (**44-d$_2$**). It is well known that $MPDP^+$ is unstable and undergoes spontaneous conversion to MPP^+ and MPTP through disproportionation.[71] In order to avoid disproportionation, the concentration of $MPDP^+$ should remain below 1 μM, but this was not experimentally feasible. In addition to MPTP, 4-(2-methylpyrrolyl)- (**91**), 4-phenoxy- (**71**) and 4-(4-pyridyl)-1-methyl-1,2,3,6-tetrahydropyridine (**85**) were also examined under SET conditions. All of these analogs showed mixtures of the dihydropyridinium and pyridinium products as expected. The corresponding 1-cyclopropyl analogs **49** (4-phenyl), **98** [4-(2-methylpyrrolyl)], **69** (4-phenoxy) and **92** [4-(4-pyridyl)] were studied under the same conditions (Scheme 32). GC-EIMS analysis, however,

showed no evidence of dihydropyridinium or pyridinium formation although disappearance of the starting material was observed. Rather, a peak corresponding to the ring opened product (**177**) was detected. These results establish that cyclopropyl ring opening is faster than deprotonation of the radical cation.

Scheme 32. Reaction of the 1-Cyclopropyl-4-phenyl Analog (**49**) Under SET Conditions

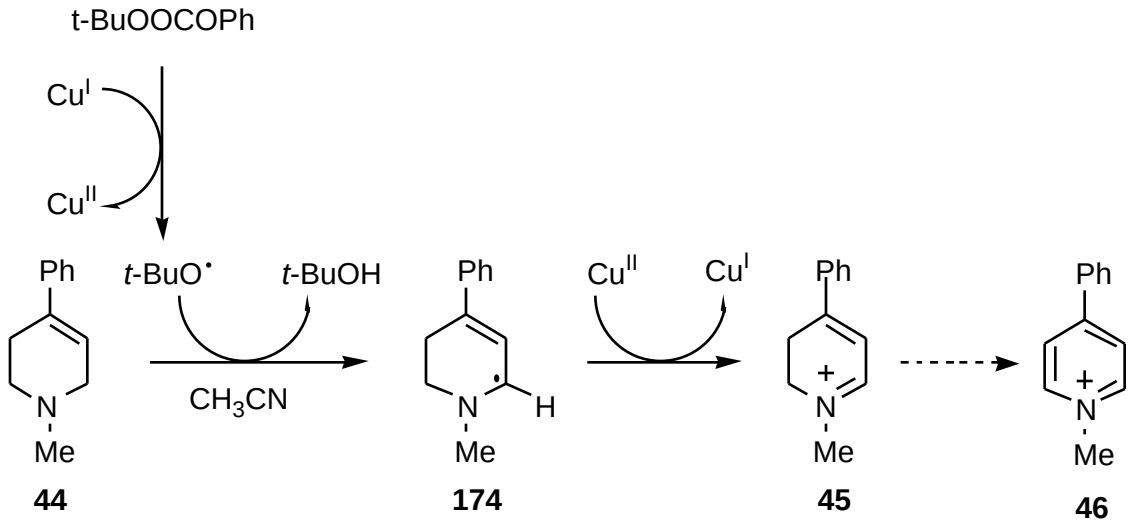


5.3.2. Hydrogen Atom Transfer

The HAT reaction was chemically modeled using the *tert*-butoxyl radical as the hydrogen atom abstracting agent. This radical was chosen because it

unambiguously abstracts hydrogen atoms from the α-carbon atoms of amines.[131] The *tert*-butoxyl radical was generated from *tert*-butyl peroxybenzoate in the presence of a catalytic amount of copper chloride at room temperature.[132-134] During the formation of the *tert*-butoxyl radical, Cu^{I} was oxidized to Cu^{II}. The *tert*-butoxyl radical reacted with MPTP (**44**) by hydrogen atom abstraction from the C-6 position of the tetrahydropyridine ring. The resulting α-amino radical **174** was then oxidized by Cu^{II} to generate $MPDP^{+}$ (**45**) (Scheme 33).

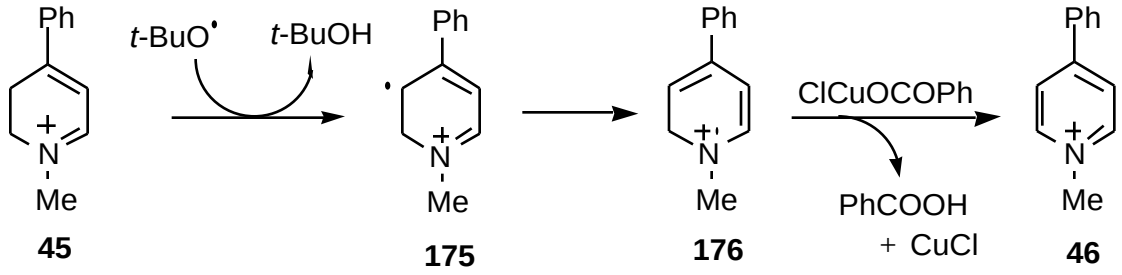
Scheme 33. Mechanism for the Reaction of MPTP (**44**) with *tert*-Butyl Peroxybenzoate and CuCl



Reduction of the reaction mixture with sodium borodeuteride followed by GC-EIMS analysis showed only formation of dideuterated MPTP (**44-d$_2$**), suggesting that conversion of $MPDP^{+}$ to MPP^{+} is faster under HAT conditions than SET conditions. One possible explanation is that disproportionation occurs more readily due to a high concentration (20 mM) of the reaction mixture.

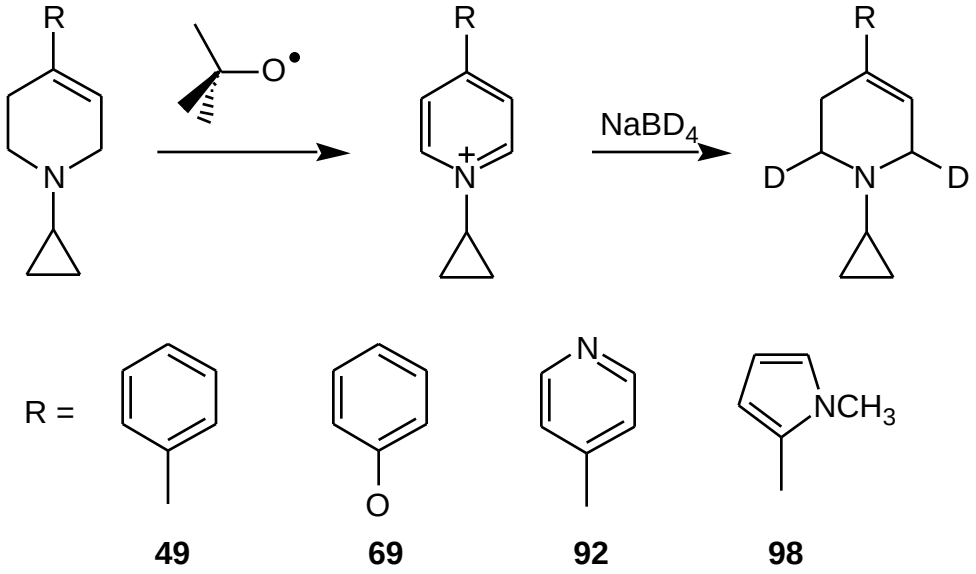
Another explanation is that a second hydrogen atom abstraction may occur from the C-3 position of $MPDP^+$ (**45**), followed by a rearrangement to give the radical cation **176** then oxidation by Cu^{II} to generate the pyridinium species **46** (Scheme 34).

Scheme 34. Possible Mechanism for the Conversion of $MPDP^+$ to MPP^+



Reaction of the 1-methyl analogs **71**, **85** and **91** under HAT conditions showed formation of the pyridinium species. However, unlike the SET reaction, the corresponding 1-cyclopropyl analogs **49**, **69**, **92** and **98** also showed pyridinium formation under HAT conditions (Scheme 35). There was no evidence for cyclopropyl ring opening.

Scheme 35. Reaction of Various 1-Cyclopropyl Analogs Under HAT Conditions



5.3.3. Discussion

Scheme 36 summarizes the results obtained from the chemical model studies. As shown in (a), reaction of the tetrahydropyridine substrate under HAT conditions showed dihydropyridinium and pyridinium formation irrespective of the N-substituent. All of the N-methyl and N-cyclopropyl analogs examined gave rise to the corresponding α-carbon oxidation products. The 1-cyclopropyl analogs revealed no evidence of the ring opened product.

Likewise, reaction of the N-methyl analogs under SET conditions also resulted in the oxidation products (36 b). These results provide evidence that upon generation of the radical cation intermediate, α-carbon deprotonation does occur.

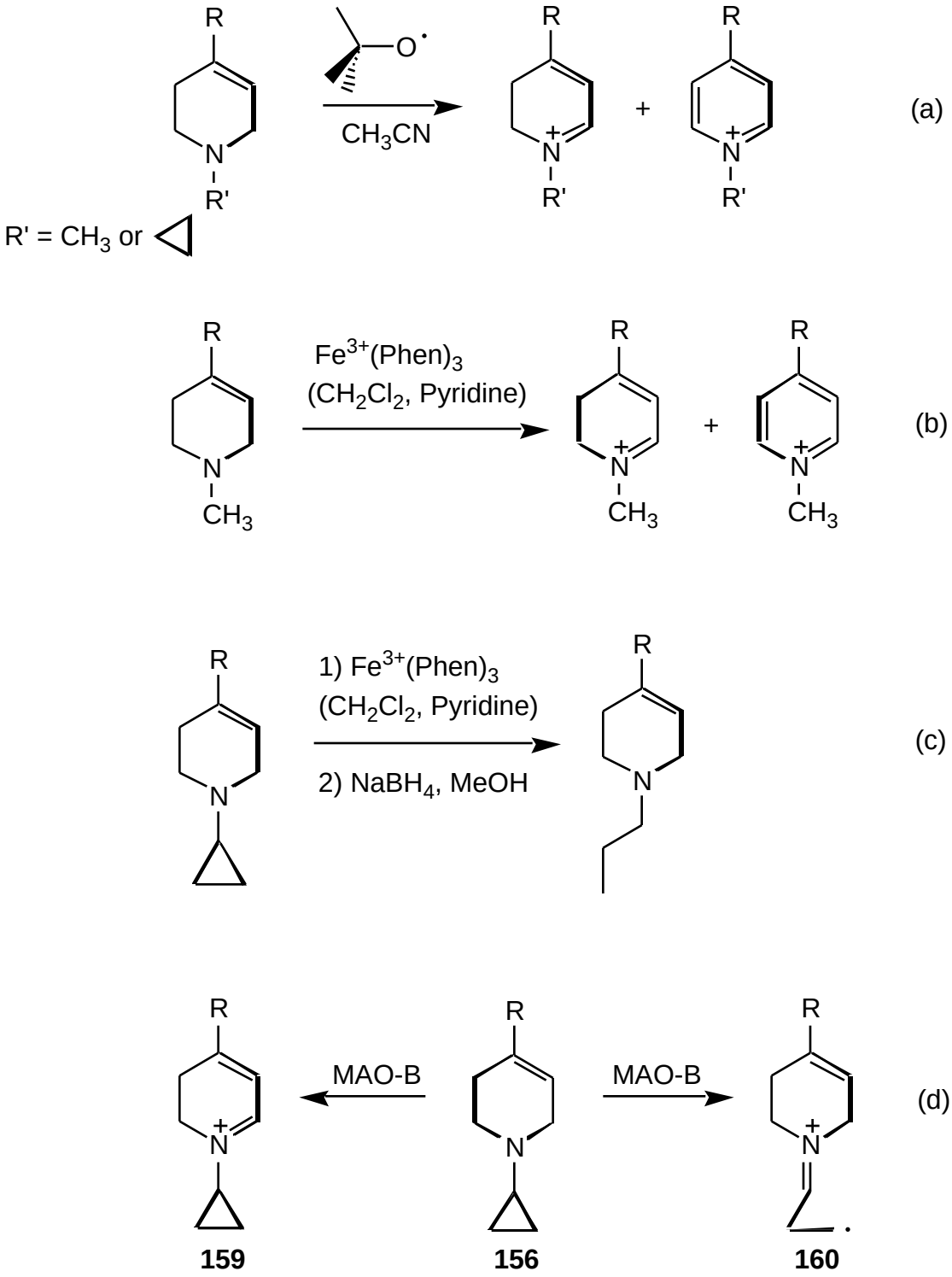
The N-cyclopropyl analogs, however, showed no evidence of α-carbon oxidation under SET conditions (36 c). Only the ring opened product was

observed. These results establish that, in solution, cyclopropyl ring opening is faster than deprotonation of the radical cation.

Incubation of the 1-methyl anlogs in the presence of MAO-B revealed formation of dihydropyridinium and pyridinium products. The behavior of the 1-cyclopropyl analogs, however, was dependent on the C-4 substituent (36 d). In some cases, only enzyme inactivation was observed resulting from the ring opened product, while in other cases, there was no evidence of enzyme inactivation but good substrate turnover. Some of the 1-cyclopropyl analogs showed mixed substrate/inactivator properties.

If a SET pathway is invoked solely, one must assume that the enzyme restricts some substrates such that cyclopropyl ring opening cannot occur in order to account for 1-cyclopropyldihydropyridinium formation. On the other hand, a pathway that does not proceed through aminyl radical cation formation, possibly a HAT pathway, would also explain the observed results. Results from the chemical model studies argue in favor of a mixed pathway with partitioning occuring at the tetrahydropyridine substrate between a SET pathway leading to enzyme inactivation and a HAT pathway leading to product formation.

Scheme 36. Summary of Enzymatic and Chemical Model Pathways



Chapter 6. Final Conclusions

One of the objectives of this project was to better understand the catalytic pathway of MAO-B. It was shown in our laboratory that the 1-cyclopropyl analog of the MAO-B substrate MPTP is an effecient time and concentration dependent inhibitor of MAO-B.[101] These results were consistent with the single electron transfer pathway proposed by Silverman which proceeds through an aminyl radical cation intermediate.

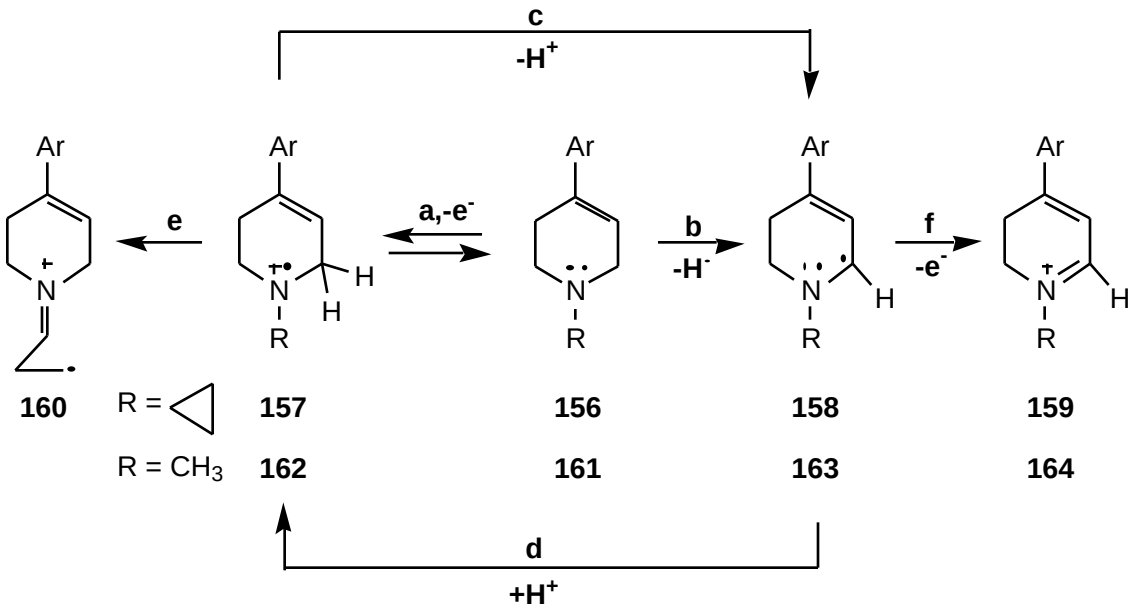
The unexpected substrate properties of various 1-cyclopropyltetrahydropyridine derivatives (**156**), however, have prompted us to consider catalytic pathways other than SET for the MAO-B catalyzed oxidation of cyclic tertiary allylamines. In particular, we have raised the question of whether or not the putative aminyl radical cation (**157**) generated by the SET step is an obligatory intermediate. In order to account for the observed formation of 1-cyclopropyldihydropyridinium metabolites (**159**), the deprotonation step leading to the allylic radical **158** (step c, Scheme 27) would have to compete kinetically with the ring opening step (step e).

An alternative pathway follows the suggestion of Edmondson in which the first step involves hydrogen atom abstraction (path b) to generate the stabilized allylic radical (**158**) directly. If the inactivating properties of the 1-cyclopropyltetrahydropyridine derivatives are mediated through the ring opened species **160**, then the allylic radical (**158**) must undergo rapid protonation (step d) to form the aminyl radical cation (**157**).

However, one could also account for the observed substrate and inactivation properties of **156** if partitioning were to occur between an electron transfer pathway leading to enzyme inactivation (path a) and a pathway

proceeding via direct carbon-hydrogen bond cleavage leading to product formation (path b).

Scheme 27. Proposed Pathways for the MAO-B Catalyzed Oxidation of 1,4-Disubstituted-1,2,3,6-tetrahydropyridine Derivatives



Results from the deuterium isotope effect studies are consistent with a partitioning that occurs from the tetrahydropyridine substrate between a SET pathway (path a) leading to enzyme inactivation and a HAT pathway (path b) leading to product formation. We observed a normal isotope effect on product formation, an inverse isotope effect on enzyme inactivation and a normal isotope effect on the partition ratio. These results indicate that partitioning occurs at the point of C-H bond cleavage. This bond breakage is involved only in product formation, not in inactivation.

It could be argued that the observed isotope effects are also consistent

with a partitioning that occurs after the initial single electron transfer step, i.e., partitioning from the aminyl radical cation. One would still predict a normal isotope effect on product formation, an inverse isotope effect on enzyme inactivation and a normal isotope effect on the partition ratio. If a SET pathway were invoked solely, the rates of α-carbon deprotonation must compete kinetically with the rates of cyclopropyl ring opening in order to account for cyclopropyldihydropyridinium formation. Ring opening of cyclopropylaminyl radical cations is reported to be very rapid,[103] thus, the deprotonation step must also be very fast. However, we observe a normal deuterium isotope effect on dihydropyridinium formation, indicating that the C-H bond cleavage step is at least partially rate determining.

Results from the chemical model studies indicate that if the aminyl radical cation is generated in solution, cyclopropyl ring opening always occurs. There is no evidence of α-carbon oxidation products when the cyclopropylaminyl radical cation is generated. However, many of the 1-cyclopropyl tetrahydropyridines that were examined enzymatically showed evidence of dihydropyridinium formation. If the MAO-B catalytic pathway proceeds through an aminyl radical cation intermediate, the only way to rationalize cyclopropyldihydropyridinium formation is to assume that the enzyme locks the substrate into a conformation such that cyclopropyl ring opening cannot occur.

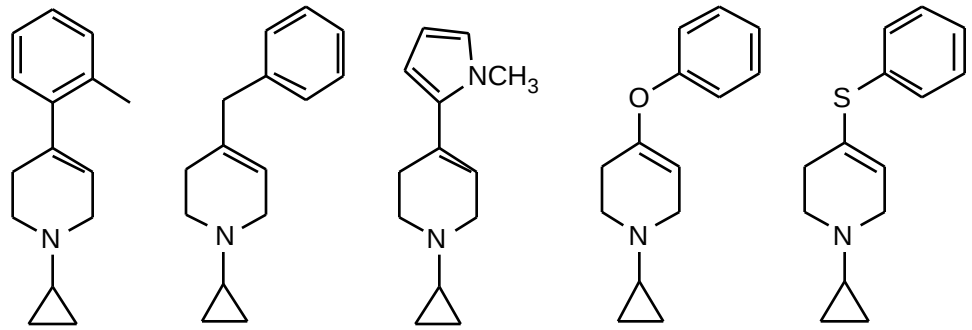
Opening of the cyclopropyl ring via a conformation which allows overlap of the half filled p-orbital of the radical cation with the p-like orbitals of the cyclopropyl carbon-carbon bonds is considered to be an energetically favored process because of the release of ring strain. Nevertheless, constraints imposed by the active site on the conformation of the tetrahydropyridine

substrate could restrict orbital alignments such that cyclopropyl ring opening is slowed due to poor orbital overlap. Consequently, a possible explanation for the good substrate and poor inactivator properties of these 1-cyclopropyltetrahydropyridine derivatives could be steric constraints imposed by the active site that prevent proper orbital alignment required for ring opening.

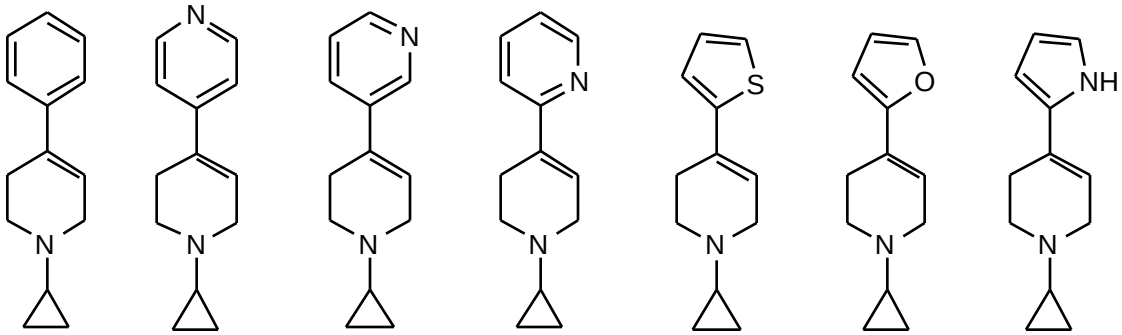
We have examined the substate/inactivator characteristics of various 1,4-disubstituted tetrahydropyridine derivatives and have found a wide range of enzymatic properties. As shown below in Chart 3, it is difficult, if not impossible, to predict the behavior of the 1-cyclopropyl analogs. In some cases we observe good substrate and poor inactivation properties while in other cases we observe good inactivation and poor substrate properties. It is difficult to imagine that the enzyme could be so selective in effectively constraining only certain substrates such that cyclopropyl ring cannot occur while other substrates with similar geometries may undergo rapid ring opening.

Chart 3

Good Substrates; Poor Inactivators



Good Inactivators; Poor Substrates



These results have led us to conclude that the cyclopropylaminyl radical cation may not be an obligatory intermediate in the MAO-B catalytic pathway. More consistent with the results is a partitioning of the 1-methyl and 1-cyclopropyltetrahydropyridine substrates between a HAT pathway (Scheme 27, path b) leading to product formation and a SET pathway (path a) leading to enzyme inactivation through the ring opened product.

Chapter 7. Experimental

7.1. Chemistry

Synthetic reactions were carried out under a nitrogen atmosphere. R (-)-deprenyl was obtained from Research Biochemicals Inc., Natic, MA. All other chemicals (Aldrich, Milwaukee, WI) were reagent or HPLC grade. Diethyl ether (Et_2O) and THF were distilled from sodium benzophenone ketyl. Dichloromethane (CH_2Cl_2) and acetonitrile (CH_3CN) were distilled from CaH_2. UV-Vis absorption spectra were recorded on a Beckman DU series 50 or 7400 spectometer and proton NMR spectra were recorded on a Bruker WP 270-MHz or Varian 400-MHz spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane as internal standard. Spin multiplicities are given as s (singlet), brs (broad singlet), d (doublet), t (triplet), or m (multiplet). Coupling values (J) are given in hertz. Column chromatography was performed using 230-425 mesh silica gel or 150 mesh basic alumina. Gas chromatography-electron ionization mass spectrometry (GC-EIMS) was performed on a Hewlett Packard (HP) 5890 GC fitted with an HP-1 capillary column (15 m x 0.2 mm i.d., 0.33 μm film thickness) which was coupled to an HP 5870 mass-selective detector. Data were acquired using an HP 5970 MS 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 percentage of the base peak. High-resolution electron ionization mass spectrometry (HR-EIMS) and high-resolution chemical ionization mass spectrometry (HR-CIMS) were performed on a VG 7070 HF instrument. Melting points were obtained on a Thomas-Hoover melting point apparatus and are uncorrected. Microanalyses were performed by Atlantic

Microlab, Inc., Norcross, GA.

Methoxycarbonylmethyltriphenylphosphonium bromide (**60**). To a stirred solution of triphenylphosphine (9 g, 34.4 mmol) in benzene was added methyl bromoacetate (5 g, 32.7 mmol) over a period of about 5 minutes. The temperature was maintained at 40-45 °C during this period and the phosphonium bromide began to crystallize out. The mixture was stirred overnight then filtered and the product washed first with benzene then with hexanes to yield 11 g of product as white crystals; 91%; mp 167-168 °C [lit.[111] mp 162-163 ˚C]; ^{1}H NMR (DMSO-d_6) δ 7.85 (m, C_6H_5, 15 H), 5.40 (d, CH_2, 2H), 3.60 (s, OCH_3, 3H).

Methyl acrylate-2,2-d_2 (**62**). A solution of anhydrous DMSO (60 mL) and NaH (2.32 g of 60% oil dispersion, 58 mmol) was stirred under N_2 at 65 °C until a clear solution resulted. To this solution **60** (24 g, 58 mmol) was added in portions with stirring over a period of 1 hour at room temperature. Paraformaldehyde-d_2 (2 g, 63 mmol) was cracked in a separate flask by heating the white powder to 200 °C and the resulting vapors were condensed into the reaction flask that was heated to 90 ˚C. The reaction mixture was stirred and maintained at 90 °C for 2 hours and then stirred overnight at room temperature. The product (2.66 g, 53%) was obtained by distillation under vacuum (50 torr) with the receiver cooled to -78 °C: ^{1}H NMR ($CDCl_3$) δ 6.12 (s, CH, 1H), 3.76 (s, OCH_3, 3H).

N,N-*Bis*methoxycarbonylmethylcyclopropylamine-d_4 (**63-d_4**). A mixture of **62-d_2** (5.6 g, 63.6 mmol) and cyclopropylamine (1.8 g, 32 mmol) in methanol (5 mL) was stirred at room temperature for 5 days. The reaction was monitored by GC-EIMS for the disapperance of the mono-substituted

product. The solvent was removed in vacuo and the resulting oil was distilled under vacuum to yield 6.6 g (89%) of product: bp 114-115 ˚C/50 torr; ^{1}H NMR ($CDCl_3$) δ 3.66 (s, OCH_3, 6H), 2.50 (s, NCD_2CH_2, 4H), 2.15 (m, $NCHCH_2$, 1H), 0.40 (m, $NCHCH_2$, 4H); GC (t_R = 3.17 min)-EIMS m/z (%), 233 ($M^{\circ+}$, 12), 174 (30), 160 (100), 89 (34), 59 (48).

1-Cyclopropyl-4-piperidone-2,2,6,6-d$_4$ (65-d$_4$). A solution of **63-d$_4$** (6.0 g, 26 mmol) in anhydrous THF (60 mL) was added dropwise to a suspension of NaH (1.56 g of 60% oil dispersion, 39 mmol) in THF. Methanol (1.5 mL) was added and the resulting mixture was heated under reflux for 36 hours. The solution obtained after adding 50% aqueous acetic acid to adjust the pH to 7 was extracted with ethyl acetate (4 x 30 mL). The combined extracts were dried over $MgSO_4$ and the solvent was removed in vacuo to yield crude **64-d$_4$** as a yellow oil. The crude product was then heated under reflux in 70 mL of 18% aqueous HCl for 5 hrs. After basification with 1 N NaOH, the product was extracted into ethyl acetate (4 x 30 mL). The combined extracts were dried over $MgSO_4$ and the solvent removed in vacuo to yield a yellow oil (1.9 g, 51%): ^{1}H NMR ($CDCl_3$) δ 2.41 (s, NCD_2CH_2, 4H), 1.78 (m, $NCHCH_2$, 1H), 0.53 (m, $NCHCH_2$, 4H); GC (temperature program: 80 °C for 2 min, then 10 °C/min to 275 °C; t_R = 4.44 min)-EIMS m/z (%), 143 ($M^{\circ+}$, 20), 100 (40), 99 (80), 85 (62), 70 (100), 55 (78).

4-Benzyl-1-cyclopropyl-4-piperidinol-2,2,6,6-d$_4$ (66-d$_4$). A solution of **65-d$_4$** (1.9, 13.3 mmol) in anhydrous THF was added dropwise to a solution of benzylmagnesium chloride (9.97 mL of 2.0 M solution in THF, 19.95 mmol) in THF (5 mL). The mixture was heated under reflux for 4 hours then at room temperature overnight. The mixture was made basic with 1 N NaOH and

extracted into dichloromethane (4 x 30 mL). The extracts were dried over $MgSO_4$ and the solvent evaporated to yield a yellow oil (2.4 g, 77%): GC (temperature program: 125 °C for 1 min, then 10 °C/min to 250 °C; t_R = 4.774 min)-EIMS m/z (%), 235 ($M^{\bullet+}$, 38), 205 (42), 144 (40), 101 (65), 85 (100), 72 (78).

1-Cyclopropyl-4-benzyl-1,2,3,6-tetrahydropyridinium-2,2,6,6-d_4 Oxalate (**54-d_4**). A solution of **66-d_4** (2.4 g, 10.2 mmol) and *p*-toluenesulfonic acid (2.3 g, 12.2 mmol) in benzene (175 mL) was heated under reflux overnight. Another 1.2 eq of acid (2.3 g, 12.2 mmol) was added and the heating was continued for 4 hours. The reaction mixture was concentrated to 80 mL and then was washed with $NaHCO_3$ (3 x 30 mL). The aqueous layer was extracted with ethyl acetate (3 x 30 mL) and the combined organic layers were dried over $MgSO_4$, filtered and the solvent removed in vacuo to yield a brownish oil (2.0 g, 91%). Treatment of the oil (1.7 g, 7.8 mmol) with oxalic acid (0.86 g, 8 mmol) in 20 mL of diethyl ether yielded a "gummy" precipitate that was recrystallized from acetone to afford a mixture of **54-d_4** and the isomeric 1-cyclopropyl-4-(phenylmethylene)piperidine-d_4 (**67-d_4**) as white crystals. GC-EIMS analyses indicated a product ratio of 4:1 (**54-d_4:67-d_4**). Several recrystallizations from acetone gave essentially pure **54-d_4**: mp 144-145 °C [**54** lit.[104] mp 148-149 °C]; ^{1}H NMR (DMSO-d_6) δ 7.1-7.4 (m, ArH, 5H), 5.45 (bs, NCD_2CH, 1H), 3.31 (s, $ArCH_2$, 2H), 2.49 (m, $NCHCH_2$, 1H), 2.10 (s, NCD_2CH_2, 2H), 0.6-0.8 (m, $NCHCH_2$, 4H); GC (temperature program: 125 °C for 1 min, then 10 °C/min to 250 °C; t_R = 4.14 min)-EIMS m/z (%), 217 ($M^{\bullet+}$, 65), 201 (100), 91 (55), 70 (40).

1-Cyclopropyl-4-pyridone (73). To γ-pyrone (6.1 g, 63.5 mmol) in

30 mL of water was added cyclopropylamine (4.35 g, 76.2 mmol). The solution was heated under reflux for 3 h and cooled to room temperature. After saturating the aqueous layer with K_2CO_3, the product was extracted with $CHCl_3$ (6 x 30 mL). The combined extracts were dried (Na_2SO_4) and concentrated in vacuo to yield the crude product as a yellow oil which was flash-vacuum filtered over basic alumina with 10% 2-propanol/90% CH_2Cl_2. Recrystallization from cold ethyl acetate and filtration under a N_2 atmosphere yielded 4.2 g (60%) of **73** as pale yellow hydroscopic needles: mp 62-64 °C; GC (t_R = 7.55 min)-EIMS m/z (%) 135 ($M^{\bullet+}$, 65), 107 (25), 106 (100), 80 (28), 67 (15), 54 (40); ^{1}H NMR ($CDCl_3$) δ 7.45 (dd, J = 7.7, 1.8, 2H, NC<u>H</u>=CH), 6.32 (dd, J = 7.7, 1.8, 2H, NCH=C<u>H</u>), 3.41 (m, 1H, NCH), 0.98-1.12 (m, 4H, NCHC<u>H</u>$_2$). Due to the hydroscopic nature of the pyridone, elemental analysis was performed on the corresponding hydrochloride salt (mp 184-186 °C): Anal. Calcd. for $C_8H_9NO{\cdot}HCl$: C, 55.99; H, 5.87; N, 8.16. Found: C, 56.09; H, 5.92; N, 8.13.

4-Chloro-1-cyclopropylpyridinium Chloride (75). A mixture of thionyl chloride (12 mL, 150 mmol) and pyridone **73** (2.2 g, 16.3 mmol) was heated under reflux for 4 h. The light brown residue obtained following removal of the excess thionyl chloride by rotary evaporation was dissolved in CH_2Cl_2 and the crude product was precipitated out with the addition of diethyl ether. Recrystallization from anhydrous CH_3CN yielded 2.3 g (75%) of **75** as hygroscopic, pale yellow needles: mp 218-220 °C dec; ^{1}H NMR (DMSO-d_6) δ 9.16 (d, J = 7.0, 2H, NC<u>H</u>=CH), 8.31 (d, J = 7.0, 2H, NCH=C<u>H</u>), 4.38 (m, 1H, NCH), 1.41 (m, 2H, NCHC<u>H</u>$_2$), 1.25 (m, 2H, NCHC<u>H</u>$_2$). Anal. Calcd. for $C_8H_9Cl_2N{\cdot}(1/6)H_2O$: C, 49.77; H, 4.87; N, 7.25. Found: C, 49.80; H, 5.04; N, 7.18.

1-Cyclopropyl-4-phenoxy-1,2,3,6-tetrahydropyridinium

Oxalate (69). A solution of 4-chloro-1-cyclopropylpyridinium chloride (**75**, 3.13 mmol), phenol (3.44 mmol) and triethylamine (4.70 mmol) in 30 mL of anhydrous CH_3CN was stirred at room temperature for 24 h. The reaction mixture then was evaporated to dryness and the residue in a stirred solution of CH_3OH (20 mL) was treated at 0 °C portionwise with $NaBH_4$ (1.5 g, 4.1 mmol). After stirring an additional 30 min at room temperature, the solvent was removed under vacuum and the residue in CH_2Cl_2 was washed with dilute aqueous $NaHCO_3$. The organic layer was washed successively with water and brine, dried over Na_2SO_4 and evaporated to yield the crude product. The oxalate salt was prepared by adding an ethereal solution of oxalic acid (1.5 equiv) to the tetrahydropyridine in ether. Recrystallization from CH_3CN yielded 0.52 g (55%) of **69**: mp 148-150 °C; GC (t_R = 7.35 min)-EIMS m/z (%) 215 ($M^{\bullet+}$, 30), 200 (100), 138 (10), 122 (25), 94 (47), 77 (37), 68 (52); ^{1}H NMR (DMSO-d_6) δ 7.36 (t, J = 7.7, 2H, PhH), 7.11 (t, J = 7.3, 1H, PhH), 7.00 (d, J = 7.7, 2H, PhH), 4.80 (bs, 1H, $NCH_2C\underline{H}$), 3.38 (bs, 2H, $NC\underline{H}_2CH$), 3.10 (t, J = 5.9, 2H, $NC\underline{H}_2CH_2$), 2.36 (bs, 2H, $NCH_2C\underline{H}_2$), 2.24 (m, 1H, NCH), 0.61 (bs, 4H, $NCHC\underline{H}_2$). Anal. Calcd. for $C_{16}H_{19}NO_5$: C, 62.94; H, 6.27; N, 4.59. Found: C, 62.75; H, 6.33; N, 4.51.

1-Cyclopropyl-4-thiophenoxy-1,2,3,6-tetrahydropyridinium

Oxalate (70). A solution of 4-chloro-1-cyclopropylpyridinium chloride (**75**, 3.13 mmol), thiophenol (3.44 mmol) and triethylamine (4.70 mmol) in 30 mL of anhydrous CH_3CN was stirred at room temperature for 24 h. The reaction mixture then was evaporated to dryness and the residue in a stirred solution of CH_3OH (20 mL) was treated at 0 °C portionwise with $NaBH_4$ (1.5 g, 4.1 mmol).

After stirring an additional 30 min at room temperature, the solvent was removed under vacuum and the residue in CH_2Cl_2 was washed with dilute aqueous $NaHCO_3$. The organic layer was washed successively with water and brine, dried over Na_2SO_4 and evaporated to yield the crude product. The oxalate salt was prepared by adding an ethereal solution of oxalic acid (1.5 equiv) to the tetrahydropyridine in ether. Recrystallization from CH_3CN yielded 0.52 g (52%) of **70**: mp 148-149 °C; GC (t_R = 8.46 min)-EIMS m/z (%) 231 ($M^{\circ+}$, 50), 216 (100), 198 (8), 147 (9), 122 (22), 106 (19), 80 (17), 53 (30); ^1H NMR (DMSO-d_6) δ 7.33 (m, 5H, PhH), 5.88 (bs, 1H, $NCH_2C\underline{H}$), 3.52 (bd, J = 2.8, 2H, $NC\underline{H_2}CH$), 3.08 (t, J = 5.8, 2H, $NC\underline{H_2}CH_2$), 2.28 (m, 3H, $NCH_2C\underline{H_2}$ and NCH), 0.61 (m, 4H, $NCHC\underline{H_2}$). Anal. Calcd. for $C_{16}H_{19}NO_4S$: C, 59.80; H, 5.96; N, 4.36. Found: C, 59.60; H, 5.92; N, 4.47.

1-Cyclopropyl-2,3-dihydro-4-pyridone (81). To a slurry of $LiAlH_4$ (0.155 g, 4.07 mmol) in 20 mL of anydrous THF was added pyridone **73** (1.1 g, 8.15 mmol) over 5 min at 0 °C. After stirring at this temperature for 1 h, the reaction was stopped by the careful addition of 10 mL of 15% NaOH and 5 mL of H_2O. The product was extracted into CH_2Cl_2, and the extract was washed several times with water, dried over Na_2SO_4, and concentrated in vacuo to yield a yellow oil. Column chromatography (neutral alumina-1% CH_3OH in $CHCl_3$) gave pure **81** (0.4 g, 36%) as a yellow oil: UV (0.1 M Na_3PO_4) λ_{max} = 328 nm (ε 10,000 M^{-1}); GC (t_R = 5.3 min)-EIMS m/z (%) 137 ($M^{\circ+}$, 15), 109 (22), 108 (26), 94 (25), 82 (25), 81 (50), 80 (45), 68 (33), 54 (100); ^1H NMR ($CDCl_3$) δ 7.14 (d, J = 7.6, 1H, $NC\underline{H}$=CH), 4.97 (d, J = 7.6, 1H, NCH=C$\underline{H}$), 3.50 (t, J = 7.6, 2H, $NC\underline{H_2}CH_2$), 2.65 (m, 1H, NCH), 2.44 (t, J = 7.6, 2H, $NCH_2C\underline{H_2}$), 0.68-0.80 (m, 4H, $NCHC\underline{H_2}$); EI-HRMS: Calcd for $C_8H_{11}NO$: 137.0841 (M^+). Found:

137.0845.

1-Methyl-4-(4-pyridyl)-1,2,3,6-tetrahydropyridinium Dihydrochloride (85). To a solution of 1-methyl-4-(4-pyridyl)pyridinium iodide[117] (**100**, 320 mg, 1.07 mmol) in CH_3OH at 0 °C was added excess $NaBH_4$ in several portions. The solution was stirred for 1 hour at ambient temperature, the solvent removed in vacuo and the residue purified by column chromatography (silica gel, EtOAc) to afford a residue that was purified by recrystallization of its dihydrochloride salt from CH_3OH/CH_3CN (149 mg, 0.61 mmol, 57%): mp 187-188 °C; ^1H NMR (DMSO-d_6) δ 11.65 (s, 2H, HCl), 8.86 (d, J = 6.50, 2H, ArH), 8.06 (d, J = 5.5, 2H, ArH), 6.93 (s, 1H, $NCH_2C\underline{H}$), 4.02 (dm, J = 3.3, 2H, $NC\underline{H}_2CH$), 3.60 (bs, 1H, $NC\underline{H}_{eq}HCH_2$), 3.25 (bs, 1H, $NCH\underline{H}_{ax}CH_2$), 2.90 (bs, 2H, $NCH_2C\underline{H}_2$), 2.82 (s, 3H, NCH_3); Anal. Calcd for $C_{11}H_{16}N_2Cl_2$: C, 53,45; H, 6.52; N, 11.33. Found: C, 53.41; H, 6.58; N, 11.38.

4-Hydroxy-1-methyl-4-(3-pyridyl)piperidine (106). To a solution of 3-bromopyridine (2 g, 12.66 mmol) in Et_2O (50 mL) at -78 °C was added dropwise n-BuLi (2.5 mM in hexanes, 6 mL, 15.2 mmol). The resulting yellow reaction mixture was stirred for 1 hour and then 1-methyl-4-piperidone (Aldrich) (**103**, 1.76 g, 15.5 mmol) in Et_2O (25 mL) was added dropwise. The reaction mixture was stirred for 1 hour at -78 °C and, following warming to room temperature, was treated with water (10 mL) and extracted with Et_2O (3 X 50 mL). The residue obtained after drying ($MgSO_4$) and removing the solvent was purified by column chromatography (silica gel, EtOAc) to give **106** (1.23 g, 6.41 mmol, 50%) as a yellow oil: ^1H NMR ($CDCl_3$) δ 8.48 (d, J = 2.26, 1H, ArH), 8.11 (dd, J = 4.80 and 1.40, 1H, ArH), 7.62 (dm, J = 6.10, 1H, ArH), 7.05 (dd, J = 8.0 and 4.8, 1H, ArH), 5.31 (bs, 1H, OH), 2.48 (dm, J = 10.87, 2H, $NC\underline{H}_{eq}H$), 2.29-

2.37 (m, 2H, NCH$\underline{H}_{ax}$), 1.93 (s, 3H, NCH_3), 1.88 (td, J = 13.26, 4.42, 2H, $NCH_2C\underline{H}_{eq}H$), 1.54 (dm, J = 12.74, 2H, $NCH_2CH\underline{H}_{ax}$); GC ($t_R$ = 6.84 min)-EIMS m/z (%), 192 ($M^{\bullet+}$, 35), 174 (17), 96 (22), 78 (37), 70 (100), 57 (72); HR-EIMS Calcd. for $C_{11}H_{16}N_2O$ ($M^{\bullet+}$): 192.126263. Found: 192.126572.

1-Methyl-4-trifluoromethanesulfonyloxy-1,2,3,6-tetrahydropyridine (107). A solution of 1-methyl-4-piperidone (**103**, 1 g, 8.84 mmol) in THF (16 mL) was added to lithium diisopropylamide (LDA) (9.72 mmol, prepared freshly from n-BuLi/diisopropylamine) in THF (20 mL) at -78 °C. After stirring for 2 hours, a solution of 1,1,1-trifluoro-N-phenyl-N-[(trifluoromethyl)sulfonyl]methanesulfonimide [N-phenyltrifluoromethane-sulfonimide (9.72 mmol)] in THF (10 mL) was added and the reaction mixture was stirred at 0 °C for 10 hours. After solvent removal, the residual yellow oil was purified by column chromatography (silica gel, 70% EtOAc/30% hexanes) to give **107** (1.56 g, 6.36 mmol, 72%) as a crude yellow oil which was not further purified: ^{1}H NMR ($CDCl_3$) δ 5.72-5.75 (bs, 1H, $NCH_2C\underline{H}$), 3.1 (dd, J = 6.2 and 3.0, 2H, $NC\underline{H}_2CH$), 2.70 (t, J = 5.8, 2H, $NC\underline{H}_2CH_2$), 2.49 (m, 2H, $NCH_2C\underline{H}_2$), 2.40 (s, 3H, NCH_3); GC (t_R = 4.0 min)-EIMS m/z (%), 245 ($M^{\bullet+}$, 21), 112 (49), 70 (100); HR-CIMS Calcd. for $C_7H_{10}F_3NO_3S$ (MH^+): 245.0335. Found: 245.0336.

1-Methyl-4-(3-pyridyl)-1,2,3,6-tetrahydropyridinium *Bis*oxalate (86). A mixture of the above tetrahydropyridyl triflate (**107**, 280 mg, 1.14 mmol), 3-trimethylstannylpyridine[122] (276 mg, 1.14 mmol), LiCl (350 mg, 8.26 mmol) and $Pd(PPh_3)_4$ (20 mg) in THF (15 mL) was heated under reflux for 12 hours. The solvent was removed in vacuo and the residue was purified by column chromatography (basic alumina, EtOAc). The *bis*oxalate

was recrystallized from CH_3OH/CH_3CN (186 mg, 0.53 mmol, 46%): mp 162 °C dec; ^1H NMR (DMSO-d_6) δ 8.73 (s, 1H, ArH), 8.52 (d, J = 4.69, 1H, ArH), 7.90 (d, J = 8.0, 1H, ArH), 7.42 (dd, J = 8.0 and 4.8, 1H, ArH), 6.32 (s, 1H, $NCH_2C\underline{H}$), 6.20 (s, 4H, COOH), 3.86 (bs, 2H, $NC\underline{H}_2CH$), 3.42 (bs, 2H, $NC\underline{H}_2CH_2$), 2.86 (s, 3H, NCH_3), 2.80 (bs, 2H, $NCH_2C\underline{H}_2$); ^{13}C NMR (DMSO-d_6) δ 163.39 (C), 149.34 (CH), 146.61 (CH), 134.18 (C), 132.76 (CH), 131.93 (C), 123.98 (CH), 118.71 (CH), 51.65 (CH_2), 49.84 (CH_2), 42.07 (CH_3), 23.87 (CH_2); GC (t_R = 5.08 min)-EIMS m/z (%), 174 ($M^{\bullet+}$, 46), 173 (41), 132 (22), 131 (37), 130 (100), 96 (48); UV (0.1 M Na_3PO_4) λ_{max} = 239 nm (ε 9,100 M^{-1}); Anal. Calcd. for $C_{15}H_{18}N_2O_8$: C, 50.85; H, 5.12; N, 7.91. Found: C, 50.88; H, 5.08; N, 7.93.

1-Methyl-4-(2-pyridyl)-1,2,3,6-tetrahydropyridinium *Bis*oxalate (87). A mixture of the above tetrahydropyridyl triflate (**86**, 190 mg, 0.78 mmol), 2-trimethylstannylpyridine[122] (187 mg, 0.78 mmol), LiCl (237 mg, 5.60 mmol) and $Pd(PPh_3)_4$ (20 mg) in THF (10 mL) was heated under reflux for 12 hours. The solvent was removed in vacuo and the residue was first filtered through celite with EtOAc and then subjected to column chromatography (basic alumina, EtOAc). The *bis*oxalate was recrystallized from CH_3OH/CH_3CN to yield a white solid (115 mg, 0.33 mmol, 42%): mp 182 °C dec; ^1H NMR (DMSO-d_6) δ 8.54-8.56 (m, 1H, ArH), 7.80 (dt, J = 7.63, 1.68, 1H, ArH), 7.60-7.62 (m, 1H, ArH), 7.30 (dd, J = 7.5 and 4.9, 1H, ArH), 6.69 (bs, 1H, $NCH_2C\underline{H}$), 3.84 (bs, 2H, $NC\underline{H}_2CH$), 3.35 (bs, 2H, $NC\underline{H}_2CH_2$), 2.82 (bs, 5H, NCH_3 and $NCH_2C\underline{H}_2$); ^{13}C NMR (DMSO-d_6) δ 148.92 (CH), 136.89 (CH), 133.99 (C), 122.84 (CH), 120.05 (CH), 119.13 (CH), 51.46 (CH_2), 49.73 (CH_2), 41.83 (CH_3), 22.80 (CH_2); GC (t_R = 7.24 min)-EIMS m/z (%), 174 ($M^{\bullet+}$, 74), 173 (22), 131 (22), 130 (100), 117 (87), 96 (19), 78 (17); UV (0.1 M Na_3PO_4) λ_{max} =

237 nm (ε 12,700 M^{-1}); Anal. Calcd. for $C_{15}H_{18}N_2O_8$: C, 50.85; H, 5.12; N, 7.91. Found: C, 50.88; H, 5.08; N, 7.93.

1-Methyl-4-(2-thienyl)-1,2,3,6-tetrahydropyridinium Oxalate (88). Condensation of the 2-thienyl Grignard reagent **112** [prepared from 2-bromothiophene, (500 mg, 3.06 mmol)] with 1-methyl-4-piperidone (**103**, 345 mg, 3.06 mmol) gave, after chromatography (silica gel, EtOAc) the piperidinol **115** (335 mg, 1.8 mmol) as an oil in 60% yield: ^{1}H NMR ($CDCl_3$) δ 7.18 (dd, J = 5.0 and 2.0, 1H, ArH), 6.94-6.96 (m, 2H, ArH), 2.85 (bs, 1H, OH), 2.62 (dm, J = 11.29, 2H, NC$\underline{H}_{eq}$H), 2.44 (dt, J = 11.75, 2.44, 2H, NCH$\underline{H}_{ax}$), 2.27 (s, 3H, NCH_3), 2.15 (td, J = 13.43, 4.42, 2H, $NCH_2C\underline{H}_{eq}H$), 1.91 (dm, J = 11.75, 2H, $NCH_2CH\underline{H}_{ax}$); ^{13}C NMR ($CDCl_3$) δ 126.71 (CH), 123.85 (CH), 121.88 (CH), 51.50 (CH_2), 46.06 (CH_3), 39.45 (CH_2); GC (t_R = 4.35 min)-EIMS m/z (%), 197 ($M^{\bullet+}$, 22), 178 (17), 113 (52), 96 (54), 70 (100), 57 (91), 78 (17).

The dehydration of **115** (150 mg, 0.76 mmol) in HCl/HOAc gave following chromatography (silica gel, 30% EtOAc/hexanes), **88** (109 mg, 0.61 mmol, 80%) as a yellow oil. The oxalate salt was recrystallized from CH_3CN to yield 160 mg (0.59 mmol, 78%) of a light green solid: mp 193-194 °C; ^{1}H NMR (DMSO-d_6) δ 10.5 (bs, 2H, COOH), 7.47 (d, J = 5.19, 1H, ArH), 7.16 (d, J = 3.66, 1H, ArH), 7.05 (ddd, J = 4.3, 3.7 and 0.8, 1H, ArH), 6.07 (bs, 1H, $NCH_2C\underline{H}$), 3.74 (bs, 2H, $NC\underline{H}_2CH$), 3.31 (t, J = 5.96, 2H, $NC\underline{H}_2CH_2$), 2.79 (s, 3H, NCH_3), 2.74 (bs, 2H, $NCH_2C\underline{H}_2$); ^{13}C NMR (DMSO-d_6) δ 164.48 (C), 142.67 (C), 128.78 (C), 127.79 (CH), 125.20 (CH), 123.69 (CH), 115.66 (CH), 51.05 (CH_2), 49.33 (CH_2), 41.90 (CH_3), 24.40 (CH_2); GC (t_R = 3.70 min)-EIMS m/z (%), 179 ($M^{\bullet+}$, 100), 178 (81), 150 (25), 135 (50), 97 (40); UV (0.1 M Na_3PO_4) λ_{max} = 275 nm (ε 9,500 M^{-1}); Anal. Calcd. for $C_{12}H_{15}NO_4S$: C, 53.52; H, 5.61; N, 5.20. Found:

C, 53.43; H, 5.60; N, 5.29.

4-(2-Furanyl)-4-hydroxy-1-methylpiperidine (116). A solution of furan (2 g, 29.38 mmol) in Et_2O (15 mL) was added dropwise at 0 °C to a solution of nBuLi (2 M in hexanes, 11.66 mL, 23.3 mmol) in dry Et_2O (10 mL). The reaction mixture was stirred for 20 minutes at 10-15 °C and the stirring was continued at ambient temperature for an additional 1 hour and then was cooled to -78 °C. A solution of 1-methyl-4-piperidone (**103**, 1.05 g, 9.29 mmol) in Et_2O (10 mL) was added to the furanlylithium reagent (**113**) over a period of 15 minutes. The reaction mixture was stirred for 2 hours and then saturated $NaHCO_3$ (20 mL) was carefully added. The aqueous layer was extracted with EtOAc (3 x 25 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent removed in vacuo to afford a solid which was recrystallized from EtOAc to afford **116** (1.30 g, 7.18 mmol, 77%) as white crystals: mp 136-137 °C; ^{1}H NMR ($CDCl_3$) δ 7.35 (d, J = 1.72, 1H, ArH), 6.31 (dd, J = 3.2 and 1.9, 1H, ArH), 6.19 (d, J = 2.75, 1H, ArH), 2.49 (m, 4H, NCH2), 2.26 (s, 3H, NCH3), 2.11 (td, J = 13.8, 7.11, 2H, $NCH_2C\underline{H}_{eq}H$), 1.93 (dm, J = 12.9, 2H, $NCH_2CH\underline{H}_{ax}$); ^{13}C NMR ($CDCl_3$) δ 141.51 (CH), 110.03 (CH), 104.28 (CH), 51.29 (CH_2), 46.08 (CH_3), 36.04 (CH_2); GC (t_R = 2.82 min)-EIMS m/z (%), 181 ($M^{\bullet+}$, 18), 162 (11), 113 (27), 96 (31), 70 (100), 57 (56); Anal. Calcd. for $C_{10}H_{15}NO_2$: C, 66.27; H, 8.34; N, 7.73. Found: C, 66.30; H, 8.39; N, 7.66.

1-Methyl-4-(2-furanyl)-1,2,3,6-tetrahydropyridinium Oxalate (89). A mixture of the piperidinol **116** (500 mg, 2.76 mmol), benzene (10 mL) and a catalytic amount of *p*-toluenesulfonic acid was heated under reflux for 3 hours and the water of the reaction was collected in a Dean-Stark trap. The residue obtained after removing the solvent was purified by column

chromatography (silica gel, 95% EtOAc/5% CH_3OH) to afford an oily product that was purified by recrystallization of its oxalate salt (279 mg, 1.10 mmol, 40%) from CH_3CN: mp 191-193 °C; ^1H NMR (DMSO-d_6) δ 7.67 (s, 1H, ArH), 6.52 (bs, 2H, ArH), 6.10 (bs, 1H, $NCH_2C\underline{H}$), 3.76 (bs, 2H, $NC\underline{H_2}CH$), 3.31 (t, J = 6.0, 2H, $NC\underline{H_2}CH_2$), 2.79 (s, 3H, NCH_3), 2.61-2.63 (m, 2H, $NCH_2C\underline{H_2}$); ^{13}C NMR (DMSO-d_6) δ 164.66 (C), 152.07 (C), 142.96 (CH), 124.82 (C), 114.03 (CH), 111.56 (CH), 106.64 (CH), 50.81 (CH_2), 49.05 (CH_2), 41.89 (CH_3), 22.09 (CH_2); GC (t_R = 2.52 min)-EIMS m/z (%), 163 ($M^{\bullet+}$, 100), 162 (76), 147 (6), 134 (39), 120 (28), 81 (61); UV (0.1 M Na_3PO_4) λ_{max} = 264 nm (ε 12,800 M^{-1}); Anal. Calcd. for $C_{10}H_{15}N \cdot 0.178\ H_2O$: C, 56.20; H, 6.03; N, 5.49. Found: C, 56.20; H, 5.97; N, 5.62.

4-(4-Pyridyl)-1-(2,4-dinitrophenyl)pyridinium Chloride (101). A mixture of 4,4'-bipyridine (**99**, 1.51 g, 9.66 mmol) and 1-chloro-2,4-dinitrobenzene (1.32 g, 6.52 mmol) in acetone (10 mL) was stirred at ambient temperature for 15 minutes and then under reflux for 15 hours. The solid which precipitated was collected, washed with ice cold acetone (100 mL), dried in vacuo and recrystallized from CH_3CN to afford 2.01 g (5.6 mmol, 86%) of **101** as a white solid: mp 149 °C; ^1H NMR (DMSO-d_6) δ 8.77 (d, J = 6.8, 2H, ArH), 8.08-8.30 (m, 6H, ArH), 7.64 (d, J = 8.7, 1H, ArH), 7.35 (dm, J = 4.53, 2H, ArH); UV (0.1 M Na_3PO_4) λ_{max} = 273 nm (ε 25,600 M^{-1}); Anal. Calcd. for $C_{16}H_{11}N_4O_4Cl \cdot 0.71\ H_2O$: C, 51.72; H, 3.37; N, 15.14. Found: C, 51.72; H, 3.41; N, 15.01.

1-Cyclopropyl-4-(4-pyridyl)pyridinium Chloride (102). To a solution of **101** (670 mg, 1.87 mmol) in 1-butanol (20 mL) was added cyclopropylamine at ambient temperature. A brown solid which precipitated

dissolved on heating. After heating under reflux for 4 hours the reaction mixture was allowed to cool to ambient temperature and the solvent removed in vacuo. An aqueous solution (25 mL) containing the residue was washed with CH_2Cl_2 and the water was removed in vacuo. Remaining traces of water were removed by co-evaporation with benzene (50 mL). The resulting yellow precipitate was recrystallized from 80% MeOH/20% H_2O to give 370 mg (1.59 mmol, 85%) of product as yellow crystals: mp 126 °C; ^{1}H NMR (DMSO-d_6) δ 8.43 (d, J = 5.8, 2H, ArH), 8.01 (dm, J = 4.5, 2H, ArH), 7.73 (d, J = 5.5, 2H, ArH), 7.21 (d, J = 4.5, 2H, ArH), 3.62 (m, 1H, NCH), 0.44-0.47 (m, 4H, NCHC$\underline{H}_2$); UV (0.1 M Na_3PO_4) λ_{max} = 264 nm (ε 17,300 M^{-1}); Anal. Calcd. for $C_{13}H_{13}N_2Cl \cdot 0.55\ H_2O$: C, 64.37; H, 5.86; N, 11.60. Found: C, 64.37; H, 6.05; N, 11.36.

1-Cyclopropyl-4-(4-pyridyl)-1,2,3,6-tetrahydropyridinium Dihydrochloride (92). To a solution of **102** (370 mg, 1.59 mmol) in CH_3OH (20 mL) at 0 °C was added an excess of $NaBH_4$. The reaction mixture was stirred for 1 hour at ambient temperature and the solvent was removed in vacuo. The residue in CH_2Cl_2 (30 mL) was washed with water (2 x 20 mL), the organic extract was dried ($MgSO_4$) and the solvent was removed in vacuo to give **92** which was characterized as its white *bis*HCl salt (329 mg, 1.21 mmol, 76%): mp 162 °C dec; ^{1}H NMR (DMSO-d_6) δ 9.5 (bs, 2H, HCl), 8.89 (d, J = 5.8, 2H, ArH), 8.07 (d, J = 6.0, 2H, ArH), 6.94 (bs, 1H, $NCH_2C\underline{H}$), 3.94-4.15 (m, 2H, $NC\underline{H}_2CH$), 3.33-3.66 (m, 2H, $NC\underline{H}_2CH_2$), 2.42-2.46 (m, 2H, $NCH_2C\underline{H}_2$), 1.53-1.66 (m, 1H, NCH), 0.49-0.56 (m, 4H, NCHC$\underline{H}_2$); UV (0.1 M Na_3PO_4) λ_{max} = 254 nm (ε 7,500 M^{-1}); Anal. Calcd. for $C_{17}H_{20}N_2O_8Cl_2 \cdot 0.30\ H_2O$: C, 52.94; H, 5.38; N, 7.29. Found: C, 52.94; H, 5.42; N, 7.27.

1-Cyclopropyl-4-trifluoromethanesulfonyl-1,2,3,6-

tetrahydropyridine (110). Compound **110** as a yellow oil (0.99 g, 3.6 mmol, GC yield 65%) was prepared from 1-cyclopropyl-4-piperidone (**109**, 800 mg, 5.76 mmol) and LDA (6 mmol, prepared freshly from nBuLi/diisopropylamine) and N-phenyltrifluoromethanesulfonimide (5.76 mmol) in the same manner as described for the 1-methyl analog **107**: ^{1}H NMR ($CDCl_3$) δ 6.1 (s, 1H, $NCH_2C\underline{H}$), 3.74 (bs, 2H, $NC\underline{H}_2CH$), 3.45 (bs, 2H, $NC\underline{H}_2CH_2$), 2.66 (bs, 2H, $NCH_2C\underline{H}_2$), 2.45 (m, 1H, $NC\underline{H}CH_2$), 0.77 (m, 4H, $NCHC\underline{H}_2$); GC (t_R = 4.21 min)-EIMS m/z (%), 271 ($M^{\bullet+}$, 3), 256 (6), 138 (100), 110 (48); HR-CIMS Calcd. for $C_9H_{12}F_3NO_3S$ (MH^+): 272.0568. Found: 272.0576.

1-Cyclopropyl-4-(3-pyridyl)-1,2,3,6-tetrahydropyridinium *Bis*oxalate (93). A mixture of the above tetrahydropyridyl triflate (**110**, 220 mg, 0.81 mmol), 3-trimethylstannylpyridine (**108**, 276 mg, 1.14 mmol), LiCl (350 mg, 8.26 mmol) and $Pd(PPh_3)_4$ (70 mg) in THF (15 mL) was heated under reflux for 12 hours. The solvent was removed in vacuo and the residue was purified by column chromatography (basic alumina, EtOAc). The product was further purified using basic alumina preparative TLC with EtOAc. The *bis*oxalate salt was recrystallized from a mixture of CH_3OH, CH_3CN and Et_2O to give a white solid (120 mg, 0.32 mmol, 39%): mp 171-172 °C dec; ^{1}H NMR (DMSO-d_6) δ 11.50 (bs, 4H, COOH), 8.66 (d, J = 1.7, 1H, ArH), 8.46 (dd, J = 4.7 and 1.5, 1H, ArH), 7.83 (dm, J = 8.0, 1H, ArH), 7.36 (ddd, J = 7.0, 4.7 and 0.8, 1H, ArH), 6.26 (bs, 1H, $NCH_2C\underline{H}$), 3.63 (bs, 2H, $NC\underline{H}_2CH$), 3.18 (t, J = 6.0, 2H, $NC\underline{H}_2CH_2$), 2.64 (bs, 2H, $NCH_2C\underline{H}_2$), 2.33-2.38 (m, 1H, NCH), 0.63-0.71 (m, 4H, $NCHC\underline{H}_2$); ^{13}C NMR (DMSO-d_6) δ 163.02 (C), 148.39 (CH), 146.04 (CH), 134.68 (C), 132.15 (CH), 131.67 (C), 131.15 (CH), 120.93 (CH), 51.46 (CH_2),

49.26 (CH_2), 37.65 (CH), 25.09 (CH_2), 4.53 (CH_2); GC (temperature program: 90 °C for 1 min, then 25 °C/min to 275 °C; t_R = 7.5 min)-EIMS m/z (%), 200 ($M^{\bullet+}$, 38), 185 (100), 144 (24), 131 (21), 117 (15); UV (0.1 M Na_3PO_4) λ_{max} = 243 nm (ε 10,200 M^{-1}); Anal. Calcd. for $C_{17}H_{20}N_2O_8$: C, 53.68; H, 5.30; N, 7.37. Found: C, 53.72; H, 5.37; N, 7.39.

1-Cyclopropyl-4-(2-pyridyl)-1,2,3,6-tetrahydropyridinium *Bis*oxalate (94). In a manner similar to that described for the 3-pyridyl analog **93**, the 1-cyclopropyl derivative **94** was prepared in 33% yield from the tetrahydropyridyl triflate **110** (150 mg, 0.55 mmol), 2-trimethylstannylpyridine (**111** 133 mg, 0.55 mmol), LiCl (175 mg, 4.14 mmol) and $Pd(PPh_3)_4$ (30 mg) in THF 10 mL). The product was characterized as its *bis*oxalate salt: mp 181-182 °C dec; ^{1}H NMR (DMSO-d_6) δ 8.53-8.55 (m, 1H, ArH), 7.78 (dt, J = 7.5 and 1.83, 1H, ArH), 7.56-7.58 (m, 1H, ArH), 7.27 (ddd, J = 7.5, 4.7 and 0.9, 1H, ArH), 6.68-6.70 (m, 1H, $NCH_2C\underline{H}$), 3.65 (bs, 2H, $NC\underline{H}_2CH$), 3.14-3.18 (m, 2H, $NC\underline{H}_2CH_2$), 2.69 (bs, 2H, $NCH_2C\underline{H}_2$), 2.33 (m, 1H, NCH), 0.63-0.67 (m, 4H, $NCHC\underline{H}_2$); ^{13}C NMR (DMSO-d_6) δ 163.20 (C), 155.45 (C), 148.87 (CH), 136.83 (CH), 134.29 (C), 122.55 (CH), 121.97 (CH), 119.01 (CH), 51.43 (CH_2), 49.47 (CH_2), 37.78 (CH), 23.96 (CH_2), 4.36 (CH_2); GC (temperature program: 60 °C for 1 min, then 25 °C/min to 275 °C; t_R = 5.36 min)-EIMS m/z (%), 200 ($M^{\bullet+}$, 4), 185 (7), 144 (30), 143 (100), 130 (14), 117 (25); UV (0.1 M Na_3PO_4) λ_{max} = 239 nm (ε 11,800 M^{-1}); Anal. Calcd. for $C_{17}H_{20}N_2O_8$: C, 53.68; H, 5.30; N, 7.37. Found: C, 53.78; H, 5.35; N, 7.39.

1-Cyclopropyl-4-hydroxy-4-(2-thienyl)piperidine (118). A 5 mL aliquot of a solution of 2-bromothiophene (631 mg, 3.87 mmol) in dry Et_2O (15 mL) was transferred to a 50 mL three neck flask and magnesium turnings (97

mg, 4 mmol) were added. The reaction was initiated by adding a crystal of iodine which was followed by the dropwise addition of the remaining 2-bromothiophene solution. The reaction mixture was stirred for 1 hour at ambient temperature and 1-cyclopropyl-4-piperidone (**109**, 592 mg, 4.26 mmol) was added dropwise in Et_2O (10 mL). After stirring for 15 hours, saturated NH_4Cl (10 mL) was added carefully followed by 10% HCl (5 mL). The aqueous layer was washed with Et_2O (2 x 25 mL) and then was made basic with 40% NaOH and extracted with EtOAc (3 x 25 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent was removed in vacuo. The residue was purified by column chromatography (silica gel, EtOAc) and the product was further purified by recrystallization from hexanes affording 681 mg (3.05 mmol, 79%) of **118** as a light yellow solid: mp 113-114 °C dec; ^{1}H NMR ($CDCl_3$) δ 7.18 (d, J = 4.5 and 1.3, 1H, ArH), 6.91-6.94 (m, 2H, ArH), 2.85 (dm, J = 11.6, 2H, NC$\underline{H}_{eq}$H), 2.65 (td, J = 11.6 and 2.6, 2H, NCH$\underline{H}_{ax}$), 2.27 (bs, 1H, OH), 2.07 (td, J = 13.7 and 4.47, 2H, $NCH_2C\underline{H}_{eq}H$), 1.89 (dm, J = 12.3, 2H, $NCH_2CH\underline{H}_{ax}$), 1.61-1.68 (m, 1H, NCH), 0.43 (m, 4H, NCHC$\underline{H}_2$); ^{13}C NMR ($CDCl_3$) δ 153.35 (C), 126.67 (CH), 123.79 (CH), 121.87 (CH), 70.25 (C), 49.54 (CH_2), 39.92 (CH_2), 38.39 (CH), 5.84 (CH_2); GC (t_R = 7.44 min)-EIMS m/z (%), 223 ($M^{\bullet+}$, 12), 194 (14), 111 (50), 97 (54), 82 (100), 68 (64); Anal. Calcd. for $C_{12}H_{17}NOS$: C, 64.54; H, 7.67; N, 6.27. Found: C, 64.54; H, 7.74; N, 6.32.

1-Cyclopropyl-4-(2-thienyl)-1,2,3,6-tetrahydropyridinium Oxalate (95). A solution of **118** (203 mg, 0.91 mmol) in HCl:HOAc (1:3 v/v, 15 mL) was heated under reflux for 15 hours. The reaction mixture at ambient temperature was basified with 30% NaOH and extracted with CH_2Cl_2 (4 x 25 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent was

removed in vacuo. The residue was purified by column chromatography (silica gel, 30% EtOAc/hexanes) to give the free base **95** (131 mg, 0.64 mmol, 70%) as a yellow oil. The oxalate salt was recrystallized from CH_3CN to afford 125 mg (0.42 mmol, 46%) of product as a light green solid: mp 181 °C; ^{1}H NMR (DMSO-d_6) δ 10.5 (bs, 2H, COOH), 7.40 (dd, J = 5.1 and 1.1, 1H, ArH), 7.10 (d, J = 3.4, 1H, ArH), 7.01 (dd, J = 5.1 and 2.6, 1H, ArH), 6.05-6.07 (m, 1H, $NCH_2C\underline{H}$), 3.60 (d, J = 2.6, 2H, $NC\underline{H_2}CH$), 3.18 (t, J = 6.1, 2H, $NC\underline{H_2}CH_2$), 2.60-2.63 (m, 1H, $NCH_2C\underline{H_2}$), 2.37-2.40 (m, 1H, NCH), 0.61-0.74 (m, 4H, $NCHC\underline{H_2}$); ^{13}C NMR (DMSO-d_6) δ 163.14 (C), 145.43 (C), 129.00 (C), 127.70 (CH), 124.67 (CH), 123.08 (CH), 117.73 (CH), 51.08 (CH_2), 49.10 (CH_2), 37.69 (CH), 25.70 (CH_2), 4.44 (CH_2); GC (t_R = 7.08 min)-EIMS m/z 205 (%), ($M^{\bullet+}$, 40), 190 (100), 135 (33), 97 (30); UV (0.1 M Na_3PO_4) λ_{max} = 277 nm (ε 9,900 M^{-1}); Anal. Calcd. for $C_{14}H_{17}NO_4S$: C, 56.93; H, 5.80; N, 4.74. Found: C, 57.04; H, 5.78; N, 4.69.

1-Cyclopropyl-4-(2-furanyl)-1,2,3,6-tetrahydropyridinium Oxalate (96). Condensation of the furanyllithium reagent **113**, prepared by treatment of furan (2.0 g, 29.4 mmol) with nBuLi (2 M in hexanes, 11.7 mL, 23.3 mmol) in dry Et_2O (15 mL), with 1-cyclopropyl-4-piperidone (**109**, 200 mg, 1.44 mmol) as described above for the synthesis of **118** gave, following recrystallization from EtOAc 185 mg (0.89 mmol, 62%) of the piperidinol **119**: mp 176 °C; ^{1}H NMR ($CDCl_3$) δ 7.35 (dd, J = 1.8 and 0.9, 1H, ArH), 6.32 (dd, J = 3.2 and 1.8, 1H, ArH), 6.19 (dm, J = 3.3, 1H, ArH), 2.72-2.74 (m, 4H, NCH_2), 2.03-2.13 (m, 2H, $NCH_2C\underline{H_{eq}}H$), 1.94 (dm, J = 11.9, 2H, $NCH_2CH\underline{H_{ax}}$), 1.65 (m, 1H, NCH), 0.42-0.47 (m, 4H, $NCHC\underline{H_2}$); GC (temperature program: 60 °C for 2 min, then 15 °C/min to 250 °C; t_R = 7.7 min)-EIMS m/z (%), 207 ($M^{\bullet+}$, 18), 192

(8), 178 (19), 97 (28), 96 (42), 95 (40), 82 (100), 68 (75), 54 (33). Acid catalyzed dehydration of this crude **119** (100 mg, 0.48 mmol) gave the free base **96** as an oil that was purified by recrystallization of its oxalate salt from CH_3CN to yield a white solid (97 mg, 0.35 mmol, 72%): mp 163 °C; ^{1}H NMR (DMSO-d_6) δ 7.62 (bs, 1H, ArH), 6.47-6.48 (m, 1H, ArH), 6.42 (d, J = 3.2, 1H, ArH), 6.10 (bs, 1H, $NCH_2C\underline{H}$), 4.50 (bs, 2H, COOH), 3.59 (bs, 2H, $NC\underline{H}_2CH$), 3.13 (t, J = 5.8, 2H, $NC\underline{H}_2CH_2$), 2.48 (bs, 2H, $NCH_2C\underline{H}_2$), 2.33 (bs, 1H, NCH), 0.62-0.67 (m, 4H, $NCHC\underline{H}_2$); ^{13}C NMR (DMSO-d_6) δ 162.76 (C), 152.16 (C), 142.93 (CH), 125.07 (C), 114.34 (CH), 111.56 (CH), 106.64 (CH), 50.44 (CH_2), 48.72 (CH_2), 37.92 (CH), 22.30 (CH_2), 3.76 (CH_2); GC (t_R = 7.0 min)-EIMS m/z (%), 189 ($M^{\bullet+}$, 50), 174 (100), 105 (15), 91 (26), 77 (23), 54 (26); UV (0.1 M Na_3PO_4) λ_{max} = 264 nm (ε 13,600 M^{-1}); Anal. Calcd. for $C_{14}H_{17}NO_5 \cdot 0.18\ H_2O$: C, 59.36; H, 6.17; N, 4.97. Found: C, 59.36; H, 6.16; N, 5.26.

4-(1-Methyl-2-pyrryl)-1-(2,4-dinitrophenyl)pyridinium Chloride (126). A solution of 4-(1-methyl-2-pyrryl)pyridine[124] (**124**, 640 mg, 4.10 mmol) and 2,4-dinitrochlorobenzene (820 mg, 4,10 mmol) in anhydrous acetone (10 mL) was heated under reflux for 24 hours. The reaction mixture was concentrated and the precipitated solid was collected, washed with ice cold acetone and recrystallized from CH_3OH/Et_2O to yield 610 mg (1.19 mmol, 41%) of product as dark yellow needles: mp 241-243 °C; ^{1}H NMR (CD_3OD) δ 9.22 (d, J = 2.5, 1H, ArH), 8.86 (dd, J = 8.8 and 2.5, 1H, ArH), 8.80 (d, J = 7.4, 2H, ArH), 8.27 (d, J = 3.9, 1H, ArH), 8.25 (dd, J = 7.4 and 2.4, 2H, ArH), 7.41 (m, 2H, ArH), 6.44 (dd, J = 6.8 and 2.5, 1H, ArH), 4.10 (s, 3H, NCH_3); Anal. Calcd. for $C_{16}H_{13}ClN_4O_4$: C, 53.27; H, 3.63; N, 15.53. Found: C, 53.24; H, 3.60; N, 15.46.

1-Cyclopropyl-4-(1-methyl-2-pyrryl)pyridinium Chloride (127). A solution of cyclopropylamine (133 mg, 2.33 mmol) and **126** (700 mg, 1.94 mmol) in 1-butanol (40 mL) was heated under reflux for 24 hours and then was evaporated to dryness in vacuo. The residue was recrystallized from CH_3CN/Et_2O to yield 208 mg (0.89 mmol, 38%) of product as pale yellow hygroscopic needles: mp 131-132 °C; ^1H NMR (CD_3OD) δ 8.69 (d, J = 6.8, 2H, ArH), 8.01 (d, J = 7.3, 2H, ArH), 7.20 (d, J = 2.1, 1H, ArH), 7.12 (dd, J = 5.7 and 1.7, 1H, ArH), 6.33 (dd, J = 6.8 and 2.6, 1H, ArH), 4.16 (m, 1H, $NC\underline{H}CH_2$), 3.98 (s, 3H, NCH_3), 1.33 (m, 4H, $NCHC\underline{H}_2$); UV (0.1 M Na_3PO_4) λ_{max} = 373 nm (ε 18,800 M^{-1}); Anal. Calcd. for $C_{13}H_{15}ClN_2 \cdot 0.78\ H_2O$: C, 62.90; H, 6.68; N, 11.29. Found: C, 62.89; H, 6.63; N, 11.26.

1-Cyclopropyl-4-(1-methyl-2-pyrryl)-1,2,3,6-tetrahydropyridinium Oxalate (98). To a solution of the above cyclopropylpyridinium chloride (**127**, 150 mg, 0.64 mmol) in CH_3OH (15 mL) was added $NaBH_4$ (61 mg, 1.60 mmol) at 0 °C. This mixture was allowed to warm to room temperature and was stirred for an additional 30 minutes. After removing the solvent, the residue was extracted with CH_2Cl_2 and the extract was washed with water several times. The product obtained following removal of the CH_2Cl_2 in vacuo was converted to its oxalate salt which was recrystallized from 2-propanol/Et_2O to yield 114 mg (0.39 mmol, 55%) of product as pale yellow needles: mp 179-180 °C; ^1H NMR (DMSO-d_6) δ 6.71 (s, 1H, ArH), 6.03 (s, 1H, ArH), 5.94 (t, J = 3.3, 1H, ArH), 5.72 (s, 1H, $NCH_2C\underline{H}$), 3.59 (s, 5H, CH_3, $NC\underline{H}_2CH$), 3.14 (t, J = 5.9, 2H, $NC\underline{H}_2CH_2$), 2.48 (s, 1H, $NC\underline{H}CH_2$), 2.35 (s, 2H, $NCH_2C\underline{H}_2$), 0.69 (m, 4H, $NCHC\underline{H}_2$); GC (temperature program: 60 °C for 1 min, then 25 °C/min to 275 °C; t_R = 9.1 min)-EIMS m/z

(%), 202 ($M^{\bullet+}$, 55), 187 (100), 159 (10), 132 (40), 118 (60), 94 (55), 77 (20), 68 (25); Anal. Calcd. for $C_{15}H_{20}N_2O_4$: C, 61.63; H, 6.90; N, 9.58. Found: C, 61.43; H, 6.98; N, 9.35.

1-Methyl-4-(2-thienyl)-1,2,3,6-tetrahydropyridine Oxide (131). To a solution of 1-methyl-4-(2-thienyl)-1,2,3,6-tetrahydropyridine (**88**, 200 mg, 1.12 mmol) in dry $CHCl_3$ (15 mL) at 0 °C was added *m*-chloroperoxybenzoic acid (*m*-CPBA, 50%, 390 mg, 2.23 mmol) in one portion. After stirring for 15 minutes the reaction mixture was concentrated in vacuo and the residue was purified by column chromatography (basic alumina, 95% $CHCl_3$/5% MeOH) to give **131** (149 mg, 0.76 mmol, 70%) as an oil: ^{1}H NMR ($CDCl_3$) δ 7.05 (d, J = 5, 1H, ArH), 6.82-6.88 (m, 2H, ArH), 5.80 (brs, 1H, $NCH_2C\underline{H}$), 3.88 (brs, 2H, $NC\underline{H}_2CH$), 3.36 (t, J = 6, 2H, $NC\underline{H}_2CH_2$), 3.09 (s, 3H, NCH_3), 2.91-2.96 (m, 1H, $NCH_2C\underline{H}_{eq}H$), 2.51-2.56 (m, 1H, $NCH_2CH\underline{H}_{ax}$); HR-CIMS Calcd. for $C_{10}H_{13}NSO$ (MH^+): 196.0796. Found: 196.0806.

1-Methyl-4-(2-thienyl)-2,3-dihydropyridinium Perchlorate (137). A mixture of the above N-oxide (149 mg, 0.76 mmol) and trifluoroacetic anhydride (177 mg) was stirred for 15 minutes at 0 °C in CH_2Cl_2 (3 mL) and then was added to perchloric acid (2.10 mmol) in methanol (10 mL). The resulting solid was recrystallized from methanol to give **137** (95 mg, 0.34 mmol, 45%) as yellow needles: mp 140-141 °C; ^{1}H NMR (CD_3OD) δ 8.35-8.38 (m, 1H, ArH), 7.92 (dd, J = 5.0 and 1.0, 1H, ArH), 7.85 (dd, J = 4.0 and 1.0, 1H, ArH), 7.35 (dd, J = 5.0 and 4.0, 1H, $NC\underline{H}CH$), 6.78 (d, J = 5.2, 1H, $NCHC\underline{H}$), 4.00 (t, J = 9.2, 2H, $NC\underline{H}_2CH_2$), 3.87 (s, 3H, NCH_3), 3.30-3.34 (m, 2H, $NCH_2C\underline{H}_2$); ^{13}C NMR (CD_3OD) δ 135.57 (CH), 133.96 (CH), 130.75 (CH), 111.53 (CH), 49.08 (CH_2), 46.98 (CH_3), 26.58 (CH_2); Anal. Calcd. for $C_{10}H_{13}NSO_4Cl$: C, 43.16; H,

4.71; N, 5.04. Found: C, 43.42; H, 4.41; N, 5.06.

1-Methyl-4-(2-furanyl)-2,3-dihydropyridinium Perchlorate (138). A solution of 1-methyl-4-(2-furanyl)-1,2,3,6-tetrahydropyridine (100 mg, 0.61 mmol) in dry CH_2Cl_2 (10 mL) was cooled to 0 °C in an ice bath, and *m*-CPBA (50%, 212 mg, 1.2 mmol) was added in one portion. After stirring for 15 min, the reaction mixture was concentrated in vacuo and the residue was purified by column chromatography (basic alumina, 95% $CHCl_3$/5% MeOH) to give **132** (82 mg, 0.46 mmol, 75%) as an oil which was pure according to TLC and NMR analysis: ^{1}H NMR ($CDCl_3$) δ 7.39 (brs, 1H, ArH), 6.40 (dd, J = 2.0 and 2.0, 1H, ArH), 6.32 (d, J = 4.5, 1H, ArH), 6.12 (m, 1H, $NCH_2C\underline{H}$), 4.08 (brs, 2H, $NC\underline{H}_2CH$), 3.53 (t, J = 6.3, 2H, $NC\underline{H}_2CH_2$), 3.26 (s, 3H, NCH_3), 2.99-3.05 (m, 1H, $C\underline{H}_{eq}H$), 2.60-2.66 (m, 1H, $CH\underline{H}_{ax}$); ^{13}C NMR ($CDCl_3$) δ 152.11 (C), 142.35 (CH), 124.67 (C), 113.95 (CH), 111.19 (CH), 106.29 (CH), 67.80 (CH_2), 64.00 (CH_2), 57.24 (CH_3), 23.72 (CH_2); HR-CIMS Calcd. for $C_{10}H_{13}NO$ (M-16$^+$): 163.0997. Found: 163.0993. A mixture of the N-oxide (11 mg) and trifluoroacetic anhydride (13 mg) was stirred for 15 min at 0 °C in 3 mL of CH_2Cl_2. This reaction mixture was added to 50 mL of methanolic perchloric acid, and 0.7 mL of the resulting solution was diluted to 10 mL with methanol to give a final concentration of the dihydropyridinium species **138** of 88 μM: UV (MeOH) λ_{max} = 382 nm (ε 23,000 M^{-1}).

1-Cyclopropyl-4-phenyl-1,2,3,6-tetrahydropyridinium-2,2,6,6-d_4 Oxalate (49-d_4). Paraformaldehyde-d_2 (1.28 g, 40 mmol) was added to a solution of cyclopropylamine (1.14 g, 20 mmol) in 40 mL of water (pH 6.0, adjusted with concentrated HCl). The reaction mixture was heated to 100 °C for 24 h, and after cooling to room temperature, α-methylstyrene (**165**, 1.18 g, 10

mmol) was added and heated to 65 °C for 24 h. The solvent was removed and 40 mL of concentrated HCl was added and the solution was heated to 100 °C for 2 h. The reaction mixture was made basic with KOH and the aqueous layer extracted with ethyl acetate (3 x 25 mL). The residue obtained after drying ($MgSO_4$) and removing the solvent was purified by flash column chromatography (silica gel, methylene chloride/ethyl acetate 9:1). The oxalate salt was prepared by dropwise addition of a saturated solution of oxalic acid in ether to a solution of the tetrahydropyridine in ether to afford **49-d$_4$** as fine white needles (2.0 g, 69%): mp 204-205 °C; ^{1}H NMR (DMSO-d_6) δ 7.36 (m, ArH, 5H), 6.14 (s, $NCD_2C\underline{H}$, 1H), 6.00 (bs, NH, 1H), 2.64 (s, NCD_2CH_2, 2H), 2.49 (m, $NC\underline{H}CH_2$, 1H), 0.7-0.8 (m, $NCHC\underline{H}_2$, 4H); GC (t_R = 6.1 min)-EIMS m/z (%) 203 ($M^{\cdot+}$, 50), 187 (100), 172 (7), 159 (9), 146 (18), 131 (38), 117 (27), 102 (7), 92 (15), 77 (15), 70 (52), 55 (33). Anal. Calcd for $C_{16}H_{15}D_4NO_4$: C, 65.51; H +D as H, 6.53; N, 4.77. Found: C, 65.10; H + D as H, 6.63; N, 4.66.

1-Cyclopropyl-4-(1-methyl-2-pyrryl)-1,2,3,6-tetrahydropyridinium-2,2,6,6-d$_4$ Oxalate (98-d$_4$). 1-Cyclopropyl-4-(1-methyl-2-pyrryl)pyridinium chloride (**127**, 0.5 g, 2.13 mmol) was added to 25 mL of a 0.1 M solution of sodium deuteroxide in D_2O. The resulting solution was stirred at room temperature for 48 h. This solution was then titrated with 20% DCl in D_2O to a pH of 6.5 and evaporated to dryness under reduced pressure. The residue was stirred in 50 mL of methanol and treated at 0 °C portionwise with $NaBD_4$ (0.5 g, 11.94 mmol). The reaction mixture was stirred for 1 hour at ambient temperature and the solvent was removed in vacuo. The residue in CH_2Cl_2 (30 mL) was washed with water (2 x 20 mL). The residue obtained after drying ($MgSO_4$) and removing the solvent was purified by flash

column chromatography (silica gel, ethyl acetate). The oxalate salt was recrystallized from acetonitrile to afford **98-d_4** as light yellow needles (0.1 g, 16%): mp 175-176 °C; ^{1}H NMR (DMSO-d_6) δ 6.72 (t, ArH, 1H), 6.09 (t, ArH, 1H), 5.95 (t, ArH, 1H), 5.71 (s, $NCD_2C\underline{H}$, 1H), 3.59 (S, $NC\underline{H}_3$, 3H), 2.48 (m, $NC\underline{H}CH_2$, 1H), 2.47 (s, $NCD_2C\underline{H}_2$, 2H), 0.78 (m, $NCHC\underline{H}_2$, 4H); GC (t_R = 4.83 min)-EIMS m/z (%) 206 ($M^{\circ+}$, 50), 190 (100), 175 (10), 150 (10), 134 (40), 120 (45), 96 (40), 70 (25), 55 (25). Anal. Calcd for $C_{14}H_{16}D_4N_2O_4$: C, 60.79; H + D as H, 6.80; N, 9.45. Found: C, 60.66; H + D as H, 6.80; N, 9.50.

1-Methyl-4-(1-methyl-2-pyrryl)-1,2,3,6-tetrahydropyridinium-2,2,6,6-d_4 Oxalate (91-d_4). 1-Methyl-4-(1-methyl-2-pyrryl)pyridinium iodide (**125**, 0.5 g, 1.67 mmol) was added to 50 mL of a 0.1 M solution of sodium deuteroxide in D_2O and stirred for 48 hours at room temperature. This solution was then titrated with 20% DCl in D_2O to a pH of 6.5 and evaporated to dryness under reduced pressure. The residue was stirred in 100 mL of methanol and treated at 0 °C portionwise with $NaBD_4$ (0.5 g, 11.94 mmol). The reaction mixture was stirred for 1 hour at ambient temperature and the solvent was removed in vacuo. The residue in CH_2Cl_2 (30 mL) was washed with water (2 x 20 mL), the organic extract dried ($MgSO_4$) and the solvent removed in vacuo. The oxalate salt was recrystallized from acetonitrile/ether to yield **91-d_4** as fine white needles (0.26 g, 58%): mp 120-121 °C; ^{1}H NMR (CD_3OD) δ 6.52 (t, ArH, 1H), 5.98 (m, ArH, 1H), 5.87 (m, ArH, 1H), 5.56 (s, $CD_2C\underline{H}$, 1H), 3.52 (s, methylpyrrole protons, 3H), 2.81 (s, methylpyridine protons, 3H), 2.60 (bs, NCD_2CH_2, 2H); GC (t_R = 6.04 min)-EIMS m/z (%) 202 ($M^{\circ+}$, 5), 188 (10), 158 (7), 145 (5), 131 (7), 117 (20), 97 (10), 77 (25), 70 (100), 56 (15).

1-Cyclopropyl-4-(2-methylphenyl)-1,2,3,6-tetrahydro-

pyridinium Perchlorate (167). A stirred solution of 1-bromo-2-methylbenzene (10 mmol) and Mg turnings (11 mmol) in anhydrous diethyl ether (20 mL) was treated with a crystal of I_2 to initiate the reaction. After stirring at room temperature for 1 h, the Grignard reagent was added dropwise to a solution of 1-cyclopropyl-4-piperidone (**109**, 10.5 mmol) in anhydrous diethyl ether (20 mL) at room temperature. The reaction mixture was stirred for an additional 1 h at room temperature and then treated with saturated aqueous NH_4Cl followed by 10% HCl (to pH 1) and the resulting solution was washed with diethyl ether. The aqueous phase was basified with 40% NaOH and extracted with CH_2Cl_2 to afford the crude piperidinol which, without purification, was heated under reflux for 20 h in HCl/HOAc (1:3 v/v, 30 mL). The reaction mixture was cooled to room temperature, basified with 40% NaOH and extracted with CH_2Cl_2 to afford the crude tetrahydropyridine product. The perchlorate salt was prepared by the addition of 10% methanolic $HClO_4$ to the free base followed by recrystallization from CH_3OH to yield 0.35 g (15%) of **167**: mp 214-215 °C; GC (t_R = 6.88 min)-EIMS m/z (%) 213 ($M^{\circ+}$, 48), 198 (100), 142 (21), 129 (52), 91 (17), 54 (37); ^1H NMR (DMSO-d_6) δ 7.05-7.15 (m, 4H, PhH), 5.49 (bs, 1H, $NCH_2C\underline{H}$), 3.75 (bs, 2H, $NC\underline{H_2}CH$), 3.34 (bt, 2H, $NC\underline{H_2}CH_2$), 2.68 (m, 1H, $NC\underline{H}CH_2$), 2.50 (m, 2H, $NCH_2C\underline{H_2}$), 2.19 (s, 3H, CH_3), 0.71-0.95 (m, 4H, $NCHC\underline{H_2}$); UV (0.1 M Na_3PO_4) λ_{max} = 208 nm (ε 8,100 M^{-1}); Anal. Calcd. for $C_{15}H_{20}ClNO_4$: C, 57.42; H, 6.42; N, 4.46. Found: C, 57.15; H, 6.41; N, 4.48.

7.2. Chemical Models.

SET reaction. To a solution of $Fe^{3+}(Phen)_3(PF_6^-)_3$ (103.1 mg, 0.1 mmoles) in 10 mL of methylene chloride was added at room temperature a

solution of the tetrahydropyridine (0.025 mmole) and pyridine (10 μL, 0.125 mmoles) in 5 mL of methylene chloride. The reaction mixture was stirred for 1h, then cooled to 0 °C and a solution of sodium borodeuteride in methanol was added. The reaction mixture was stirred for 30 min at ambient temperature, and the solvent was removed in vacuo. An aqueous solution containing the residue (10 mL) was washed with Et_2O, the organic extract was dried ($MgSO_4$), filtered and analyzed by GC-EIMS.

Hydrogen atom abstraction reaction. To a solution of the tetrahydropyridine (0.114 mmoles) and CuCl (0.3 mg, 1%) in acetonitrile (5 mL) was added *tert*-butylperbenzoate (0.1 mL, 0.57 mmoles) at room temperature. After 1h, the reaction mixture was cooled to 0 °C and a solution of sodium borodeuteride in methanol was added. The reaction mixture was strirred for 30 min at ambient temperature, and the solvent was removed in vacuo. An aqueous solution containing the residue (10 mL) was washed with ethyl acetate, the organic extract was dried ($MgSO_4$), filtered and analyzed by GC-EIMS. In order to follow the kinetics of the reaction, 50 μL of the reaction mixture was taken every 30 seconds and added to a solution of sodium borodeuteride in methanol and treated as above.

7.3. Enzymology

MAO-B Preparation and Determination of Activity. The isolation and purification of MAO-B from beef liver was carried out as reported earlier[136] with the following modifications. The phospholipase A used in the preparation of MAO-B was obtained commercially (Sigma, St. Louis, MO) rather than from the crude venom and the preparation was not subjected to the sucrose gradient purification step. A highly active and stable preparation was obtained and was

stored at -15 °C in 200-400 µL aliquots. Solutions of the enzyme in 50 mM sodium phosphate buffer, pH 7.4, containing 50% (w/v) glycerol, were transparent in the UV range of interest at concentrations of 0.08-0.16 µM.

The activity of MAO-B was determined spectrophotometrically at 30 °C on a Beckman DU 7400 spectrophotometer. The initial rates (120 s) of oxidation of 5 mM MPTP (**44**) to the dihydropyridinium species **45** (343 nm, ε 16,000 M^{-1})[137] were estimated. The turnover number for MPTP (204 min^{-1}) was taken from the literature. The final enzyme concentration was calculated to be 8 nmol/mL.

MAO-B Substrate Properties. All enzyme assays were performed at 37 °C on a Beckman DU-50 or 7400 series spectrophotometer. In preliminary experiments, the potential MAO-B substrate properties of each test compound (0.5-3 mM) were examined by recording repeated scans (500 to 250 nm) in the presence of 0.08-0.16 µM MAO-B. For kinetic analyses, initial rates of oxidation of the tetrahydropyridine derivatives were determined at four concentrations. Solutions (ranging from 8000 to 37.5 µM) of the substrates were prepared in 100 mM sodium phosphate buffer (pH 7.4). A 490-495 µL aliquot of each solution (pre-equilibrated to 37 °C) was placed in a sample cuvette. An aliquot of the MAO-B preparation (final concentration 0.08-0.16 µM) was added to the substrate solution. The initial rates of oxidation of each substrate were estimated by monitoring the absorbance of the corresponding dihydropyridinium metabolite every 5 s for 2-5 min, depending on the efficiency of the conversion. The K_m and V_{max} values were calculated from Lineweaver-Burke double reciprocal plots. Duplicate analysis gave V_{max}/K_m values that differed by 10% or less.

MAO-B Inactivation Studies. The inactivation studies were performed as follows: Standard solutions of the 1-cyclopropyl analogs (ranging from 1000 μM to 100 μM) in sodium phosphate buffer (100 mM, pH 7.4) were prepared. Each solution (50 μL) was treated with 50 μL of the stock MAO-B preparation (final enzyme concentration 4 μM) and the resulting mixtures were incubated with gentle agitation in a water bath at 37 °C. A 10 μL aliquot of each incubation mixture taken at timed intervals was added to a sample cuvette containing 490 μL of a 5 mM solution of MPTP (pre-equilibrated to 37 °C) in sodium phosphate buffer (100 mM, pH 7.4). The rate of MPTP oxidation was determined at 37 °C by monitoring the rate of formation of the dihydropyridinium metabolite **45** at 343 nm every 5 seconds for 2 minutes.

Determination of the Partition Ratio for 54-d_0 and 54-d_4. A 0.5 mL mixture of MAO-B (0.09 μM) and **54-d_0** or **54-d_4** (0.5 mM) was allowed to incubate at 37 °C for 1 minute during which time dihydropyridinium product **59-d_0** or **59-d_3** formation was recorded spectrophotometrically at 296 nm. Identical 1 minute incubation mixtures were assayed for remaining enzyme activity by adding an equal volume of 10 mM MPTP and determining the rate of $MPDP^+$ formation as usually performed in our inactivation studies. The ratio of nmol of product formed per nmol of enzyme inactivated was calculated from these measurements and the corresponding deuterium isotope effect on the partition ratio was obtained from the partition ratios for **54-d_0** and **54-d_4**.

GC-EIMS Assay for 54-d_0. A 500 μL mixture containing 0.5 mM **54-d_0** and 4.5 μM MAO-B was incubated at 37 ˚C. Aliquots (20 μL) removed at 0, 2, 4, 6, and 8 minutes were added to 480 μL of 10% aqueous K_2CO_3 maintained at 0 ˚C. The resulting basic solution was extracted with ethyl

acetate (1 mL) and the organic layer was dried over $MgSO_4$. A 1 µL sample was analyzed by GC-EIMS selected ion monitoring at m/z 213 (temperature program: 125 ˚C for 1 minute followed by a ramp of 25 ˚C/min for 5 minutes then a ramp of 50 ˚C/min to 300 ˚C). In a control experiment, MAO-B was preincubated with 10 µM deprenyl for 10 minutes at 37 ˚C and then treated as above.

Determination of the Irreversibility of the Inactivation of MAO-B by 54-d_0. A 200 µL mixture containing 2.3 µM MAO-B and 0.5 mM **54-d_0** in 100 mM sodium phosphate buffer pH 7.4 was incubated at 37 ˚C for 3 hours. After adding 100 µL of 0.3 % Blue Dextran solution in the same buffer the solution was applied to Sephadex G-25 (1.5 x 6 cm) previously equilibrated with 100 mM sodium phosphate buffer, pH 7.4, and eluted with the same buffer at a flow rate of 1.0 mL/min. The fraction containing the majority of the Blue Dextran was collected and assayed for enzyme activity using 5 mM MPTP. In a control experiment, 2.3 µM MAO-B was incubated with buffer for 3 hours at 37 ˚C and the procedure was repeated as above.

Determination of the Partition Ratios for 92-97. Partition ratios (moles of tetrahydropyridine consumed per unit time/moles of enzyme inactivated per unit time) for the 1-cyclopropyltetrahydropyridines **92-97** were determined as follows: a mixture (200 µL final volume) of each 1-cyclopropyltetrahydropyridine analog (1.5 mM for **92** and 500 µM for **93-97**) and MAO-B (4 µM) was incubated at 37 ˚C with gentle agitation for 3 hours by which time the enzyme was completely inactivated and the intermediate dihydropyridinium metabolites had oxidized to the corresponding pyridinium products. Each incubation mixture was treated with an excess of $NaBD_4$. The

resulting solutions were vortexed for a few minutes and extracted with EtOAc (2 x 500 µL), and 2 µL aliquots of the combined extracts were analyzed by GC-EIMS selected ion monitoring by integrating the ion intensities of the m/z values for the parent ions corresponding to the d_0, d_1, and d_2 species. Control values were obtained for the pure d_0 substrates in each case. The ion currents corresponding to the possible d_1 reduction products that would have resulted if any of the dihydropyridinium intermediates still remained in the incubation mixture were less than 5% of the d_2 species and were disregarded. The ratios of ion intensities of the tetrahydropyridine-2,6-d_2 reduction products (**49**-d_2 and **92**-d_2-**97**-d_2) to the sum of the ion currents for the d_0 and d_2 species were used to estimate the nanomoles of tetrahydropyridine substrate consumed in each incubation. These values, when divided by the nanomoles of enzyme inactivated (0.8 nmol/200 µL), gave the partition ratios. An independent estimate of the amount of 1-cyclopropyl-4-phenyl-1,2,3,6-tetrahydropyridine (**49**) consumed under these conditions by an HPLC-diode array assay (see below) gave comparable values for the partition ratio (19 by the HPLC method and 17 by the GC-EIMS method).

An HPLC-diode array assay was developed to estimate the partition ratio for the 1-cyclopropylpyrryl analog **98**. A Beckman 421A controller and 114M pump, Hewlett Packard 1040 UV-diode array detector controlled by a Hewlett Packard 85B computer, a Zorbax SB C8, 4.6 mm x 25 cm, 5 µm particle size column, and a mobile phase [40:60 CH_3CN/aqueous phase (0.6% glacial acetic acid, 1% triethylamine, pH 5)] at a flow rate of 1 mL/min were employed. Four wavelengths were used to monitor the compounds of interest: 244 nm for the starting tetrahydropyridine **98** (t_R = 5.7 min), 296 nm for the pyridinium

metabolite **154** (t_R = 4.3 min), 430 nm for the dihydropyridinium metabolite **147** (t_R = 4.7 min) and 418 nm for an unknown metabolite (t_R = 3.6 min). A standard curve prepared for the tetrahydropyridine in the mobile phase showed good linearity over a concentration range of 2-20 μM.

Timed (1, 11, 31, and 181 min) samples were prepared by diluting a 10 μL aliquot from the enzyme incubation mixture (480 μM **98** and 0.4 μM MAO-B, 37 ˚C) to 250 μL with mobile phase. The resulting solution was vortexed, centrifuged at 16000*g* for 1 min, and injected onto the HPLC column (total time elapsed was 2 min). Quantitative estimations of the tetrahydropyridine remaining in the incubation mixtures vs time were obtained by peak height measurements (244 nm) with the aid of the standard curve. Comparable results were obtained from three experiments.

By an analogous assay with the appropriate standard curves, we examined the loss of the 1-cyclopropyl-4-phenyl-1,2,3,6-tetrahydropyridine (**49**, 480 μM, λ_{max} = 244 nm, t_R = 6.7 min) and formation of the pyridinium metabolite (**155**, λ_{max} = 296 nm, t_R = 3.9 min) in an incubation mixture containing 4.0 μM MAO-B. The experiment was run twice with comparable results.

Chapter 8. References

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