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Stereochemical studies on the novel monoamine oxidase B substrates (1*R*,6*S*)- and (1*S*,6*R*)-3-methyl-6-phenyl-3-aza-bicyclo[4.1.0]heptane

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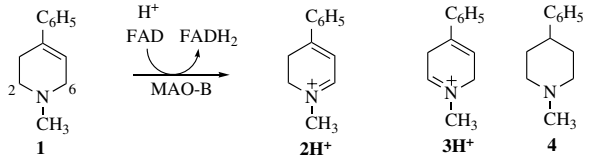
Abstract—Previous studies have established the unexpected monoamine oxidase-B (MAO-B) substrate properties of racemic 3-methyl-6-phenyl-3-aza-bicyclo[4.1.0]heptane, the 3,4-cyclopropyl analog of the achiral proneurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). The two stereocenters present in this compound provide an opportunity to examine the enantioselectivity and diastereoselectivity of the MAO-B-catalyzed ring α-carbon oxidation of cyclic tertiary amines to give the corresponding conjugated iminiumyl metabolites. This paper reports the results of such stereochemical studies using expressed human MAO-B as the catalyst.

1. Introduction

The mitochondrial flavoenzyme monoamine oxidase A and B (MAO-A and MAO-B) catalyze the two-electron α-carbon oxidation of a variety of primary and secondary amines including the aminyl neurotransmitters dopamine, serotonin and norepinephrine.[1,2] Reports of tertiary aminyl substrates are few[3,4] and, with the exception of the class of compounds under consideration in this study, no cyclic tertiary amine has been described as an MAO substrate. This exceptional class of compounds includes 5-membered[5,6] and 6-membered[7] cyclic tertiary allylamines that undergo ring α-carbon MAO-catalyzed oxidation to give cyclic iminiumyl metabolites.[8–13] An important example of this pathway is the MAO-B-catalyzed bioactivation of the parkinsonian inducing proneurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine [MPTP (**1**)] to give the conjugated dihydropyridiniumyl metabolite $\mathbf{2H^+}$.[14–17] This biotransformation is regiospecific in that oxidation at the homoallylic, unconjugated position to give the iminiumyl isomer $\mathbf{3H^+}$ is not observed.[18] The MAO-B substrate properties of MPTP are dependent on the presence of the 4,5-π bond of the heterocyclic moiety since the corresponding piperidinyl analog **4** is not an MAO substrate [19,20] (Scheme 1).

In view of the π-bond characteristics of the cyclopropyl group,[21–25] studies were undertaken recently to examine the human placental mitochondrial MAO-A and baboon liver mitochondrial MAO-B substrate properties of racemic 3-methyl-6-phenyl-3-aza-bicyclo[4.1.0]heptane



Scheme 1.

Keywords: Monoamine oxidase B; Cyclopropyl analog of MPTP; Enantioselectivity; Diastereoselectivity.
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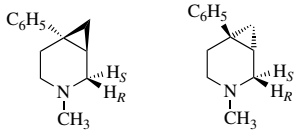
(**5ab**),[26] a fused cyclopropyl analog of MPTP. These studies established that **5ab** is biotransformed in the presence of MAO-B to the 3-methyl-6-phenyl-3-azabicyclo[4.1.0]hept-2-enyliminiumyl species **6ab**$^+$ (Scheme 2).[27]

Compound **5** bears two stereocenters at C(1) and C(6). Furthermore, C(2), the carbon atom reported to undergo MAO-B-catalyzed oxidation, is a pro-stereocenter. The present studies report the results of efforts to characterize the enantioselectivity (**5a** vs **5b**) and diastereoselectivity ($2H_R$ vs $2H_S$) of the expressed human MAO-B-catalyzed oxidation of **5**. Of particular interest is the opportunity to examine these stereochemical parameters with a substrate having fixed geometries at the positions β and γ to the nitrogen atom.



5a (1*R*,6*S*) **5b** (1*S*,6*R*)

2. Results and discussion

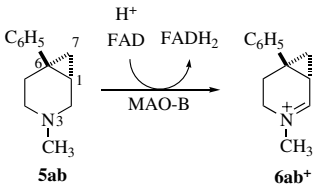
2.1. Synthesis

The resolution of **5ab** was accomplished by recrystallization of the diastereomeric salts obtained with (*R*)- and (*S*)-camphorsulfonic acid. The diastereomeric compositions of the intermediate stages of the resolution were monitored by ^{13}C NMR. The absolute configurations of the **5a** and **5b** were established by X-ray crystallography of the perchlorate salt of **5b.**

An examination of the potential diastereoselective loss of $2H_R$ versus $2H_S$ of **5a** and **5b** required the syntheses of the isotopoepimers **5a**-(**2*R***)-***d*$_1$ or **5a**-(**2*S***)-***d*$_1$ and **5b**-(**2*R***)-***d*$_1$ or **5b**-(**2*S***)-***d*$_1$. Characterization of the products obtained from the synthetic efforts (see below) was made possible by NMR studies that led to the identification of the $2H_R$ and $2H_S$ signals of **5**.

COSY, NOESY, HSQC and HMBC spectral studies led to assignments of the carbon and proton signals of **5** as summarized in Figure 1 and Table 1 with the (1*S*,6*R*)-enantiomer **5b**. A molecular model of **5b** is included for clarity.

Analysis of the spectral data that led to the critical assignments for the signals of $2H_R$ and $2H_S$ is as follows:



5ab **6ab**$^+$

Scheme 2.

the three carbon signals at δ 55.1, 45.1 and 51.9 ppm can be assigned with confidence to the three carbon atoms attached to nitrogen. Furthermore, the most deshielded of these three signals (δ 55.1 ppm) must be due to C(2) since it is disubstituted and since the cyclopropyl group is deshielding.[29] The three highest field proton signals in the spectrum (δ 1.42, 0.88 and 1.04 ppm) can be assigned to the three protons attached to the cyclopropyl group, C(1)H, C(7)H_a and C7H_b. The most deshielded of these three signals (δ 1.42 ppm) must be due to the methine proton, that is, C(1)H. The proton signals at δ 2.89 (dd, $J = 11.8_{gem}$ and 6.3_{vic} Hz) and 2.72 (dd, $J = 11.5_{gem}$ and 1.7_{vic} Hz) correlate with C(2) and therefore these signals must be C(2)H_R and C(2)H_S. The cis and trans relationships between C(1)H and these two signals should provide an opportunity to assign the pro-*R* and pro-*S* protons. The calculated (Chem 3D) dihedral angle for H(1)–C(1)–C(2)–H_R(2) is ~38°. The corresponding dihedral angle for H(1)–C(1)–C(2)–H_S(2) is ~74°. The Karplus relationship predicts a cis coupling with $J_{38°}$ of ~6 Hz and a trans coupling with $J_{74°}$ of ~2 Hz. The experimentally determined vicinal cis and trans coupling constants are 6.3 and 1.7 Hz. Therefore, the signal at δ 2.72 is assigned to C(2)H_R, the proton cis to C(1)H, and the signal at δ 2.89 to C(2)H_S, the proton trans to C(1)H. Several 2D correlations support these assignments (see Fig. 1). Particularly convincing is the NOESY observed between C(1)H and C(2)H_R but not C(2)H_S.

The preparation of the required monodeuterated substrates was approached by reduction of the iminiumyl double bond of **6**$^+$. Compounds **6a**$^+$ and **6b**$^+$ were obtained in pure form from **7a/8a** and **7b/8b**, respectively, by the reaction pathway reported previously for the synthesis of **6ab**$^+$ (Scheme 3, illustrated with **7a/8a**).[27] The deuteration was attempted with a variety of deuterium enriched reagents [$NaCND_3$, Li-9-BBN-D, LiAl(^{t}O-Bu)$_3$D] under a variety of conditions (H_2O, MeOH, THF).[30] The best diastereoselectivity was obtained with $NaBD_4$ in methanol at 0 °C (Scheme 3, the reaction is illustrated with intermediate **6a**$^+$). The product obtained from **6a**$^+$ displayed an NMR spectrum with the signal for $2H_R$ (δ 2.72 ppm) integrating for 0.75 protons and the signal for $2H_S$ (δ 2.89 ppm) integrating for 0.25 protons. Consequently deuteride attack occurred preferentially from the side opposite the cyclopropyl group to give 3 parts **5a**-(**2*S***)-***d*$_1$ and 1 part **5a**-(**2*R***)-***d*$_1$ (the '**5a-2-*d*$_1$** 75:25 isotopoepimeric mixture'). The corresponding '**5b-2-*d*$_1$** 75:25 isotopoepimeric mixture' was obtained starting with *N*-oxides **7b/8b**.

The inverse isotopoepimeric mixture, the '**5a-2-*d*$_1$** 25:75 isotopoepimeric mixture' was obtained by $NaBH_4$ reduction of **6a**$^+$**-2-*d*$_1$**. Intermediate **6a**$^+$**-2-*d*$_1$** was prepared by the reaction sequence leading to **6a**$^+$**-*d*$_0$** only starting with **5a-2,2-*d*$_2$** (Scheme 4). The synthesis of **5a-2,2-*d*$_2$** was achieved by cyclopropylation of the known MPTP analog[31] **1-6,6-*d*$_2$** followed by resolution of the resulting **5ab-2,2-*d*$_2$**. The conversion of **5a-2,2-*d*$_2$** to **6a**$^+$**-2-*d*$_1$** proceeded by treatment of the *N*-oxides **7a/8a-2,2-*d*$_2$** as described for **7a/8a-*d*$_0$**. $NaBH_4$ converted **6a**$^+$**-2-*d*$_1$** to a mixture in which **5a**-(**2*S***)-***d*$_1$** was the minor

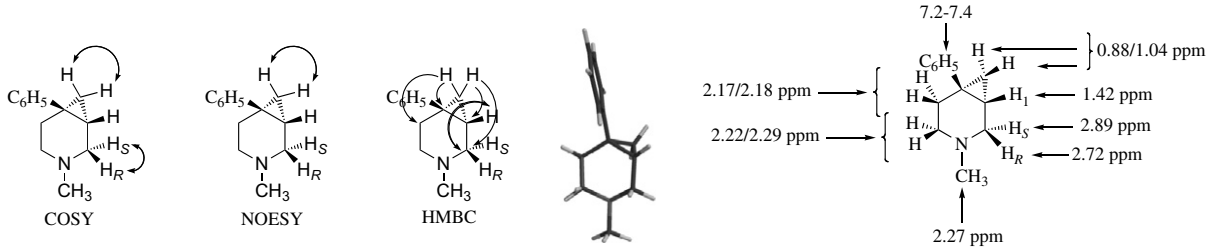
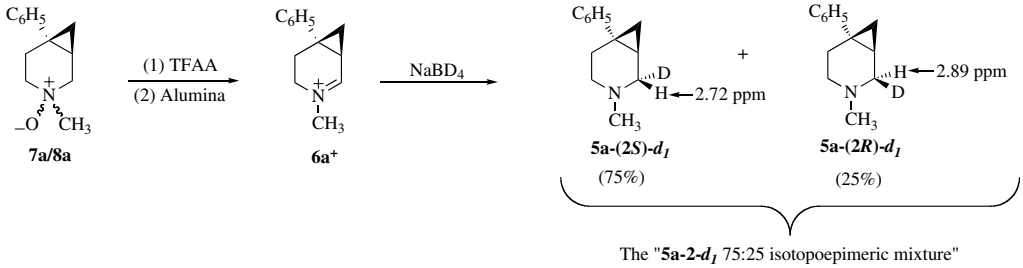


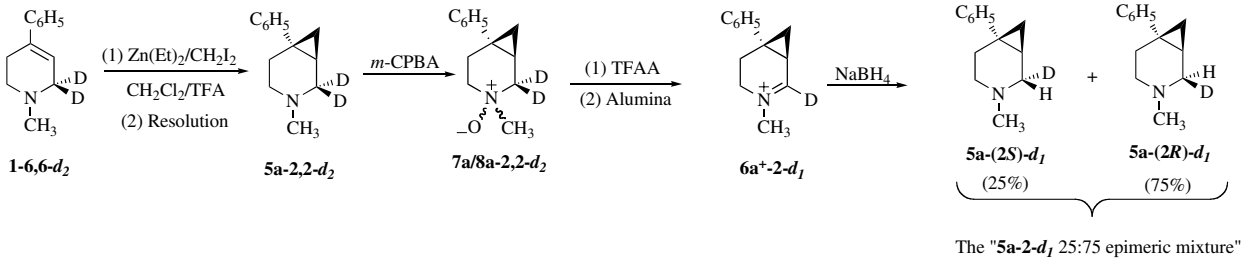
Figure 1. Summary of 2D correlations and chemical shift assignments for the proton signals of **5b**.

Table 1. Chemical shifts, proton–proton couplings and coupling constants for **5b**

Position	δ_C (ppm)	H	δ_H (ppm) (multiplicity)	J (Hz)
1	19.5 (CH)	C(1)H	1.42 (m)	
2	55.1 (CH_2)	$C(2)H_R$	2.72 (dd)	11.8_{gem}, 6.3_{vic}
		$C(2)H_S$	2.89 (dd)	11.5_{gem}, 1.7_{vic}
3	45.1	$N(3)CH_3$	2.27 (s)	
4	51.9 (CH_2)	$C(4)H_2$	2.22 (ddd)	
			2.29 (ddd)	
5	31.8 (CH_2)	$C(5)H_2$	2.17 (ddd)	
			2.18 (ddd)	
6	22.4 (q-C)			
7	18.1 (CH_2)	$(7)H_aH_b$	0.88 (dd)	4.3_{gem},[28] 5.3_{vic}
			1.04 (dd)	4.3_{gem}, 9.1_{vic}
1′	148 (q-C)			
2′,6′	128.8 (CH)	$C(2',3',4',5',6')H_5$	7.2–7.4 (m)	
3′5′	127.5 (CH)			
4′	125.7 (CH)			



Scheme 3.



Scheme 4.

isotopoepimer and **5a-(2*R*)-d_1** the major isotopoepimer (Scheme 4). NMR analysis confirmed the expected composition of 25% **5a-(2*S*)-d_1** and 75% **5a-(2*R*)-d_1**. The corresponding reaction sequence with **5b-2,2-d_2** was not pursued.

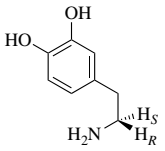
2.2. Enzymology

A few examples of enantioselective MAO-catalyzed conversions of chiral substrates[32,3,33] and mechanism-based inactivators[34–37] have been reported. The present paper

describes the results of studies on the enantioselectivity of the ring α-carbon oxidations of **5a** versus **5b**.

The substrate properties of the (1*R*,6*S*)-enantiomer **5a** and the (1*S*,6*R*)-enantiomer **5b** were examined spectrophotometrically (λ_{max} 258 nm) with the aid of a microtiter plate reader using expressed human MAO-B (50 nM). All plots of metabolite concentration versus time were linear for 30 min. Double reciprocal plots of the rates of metabolite formation versus the concentration of **5a** (100–1000 μM) gave a K_m value of 221 ± 27 μM and a V_{max} value of 135 ± 5 nmol **6a**$^+$ formed/min-nmol MAO-B (or min^{-1}). The corresponding values for **5b** (250–3000 μM) were 753 ± 22 μM and 127 ± 5 min^{-1}. The ratio of the V_{max}/K_m values for **5a**/**5b** is 617/127 = 4.9 (the average of 4 determinations for each enantiomer). When repeated with 250 nM MAO-B and a data collection period of 5 min, the average **5a**/**5b** ratio of V_{max}/K_m was 4.8. This reaction, therefore, is enantioselective with **5a** being the preferred substrate.

A high degree of α-carbon enantioselectivity has also been reported for prochiral MAO substrates. Examples include the mitochondrial MAO substrates dopamine (**9**),[38,39] benzylamine (**10**),[40] and 1-aminoheptane (**11**).[41] All conversions are reported to proceed with selective loss of the pro-*R* proton.



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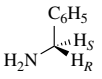

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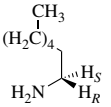

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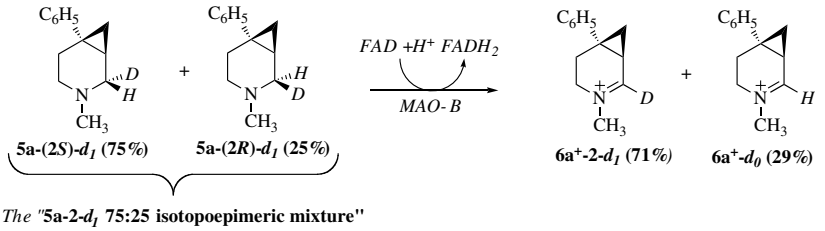
In the case of **5**, the C(2) protons are diastereotopic. The metabolic fate of the '**5a-2-*d*$_1$** 75:25 isotopoepimeric mixture' (1 mM) was examined in the presence of MAO-B (1 μM). LC-ESI/MS extracted ion chromatographic analyses following work-up of the 2 h incubation mixtures documented that less than 5% of the starting substrate remained. Consequently, even though studies on related tetrahydropyridinyl substrates have shown significant primary kinetic deuterium isotope effects,[42,43] under the conditions used in the present study, any such isotope effect should not have a significant influence on the distribution of deuterium in the products of this reaction. The deuterium content of **6a**$^+$ is summarized in Scheme 5. The percentages (average of 3 determinations) of the total integrated ion currents (*m*/*z* 186 + *m*/*z* 187) were 29% for **6a**$^+$-***d*$_0$** (*m*/*z* 186) and, when corrected for the ^{13}C-satellite peak of *m*/*z* 186, 71% for **6a**$^+$**-2-*d*$_1$** (*m*/*z* 187). Although the use of the mixture of monodeuterated isotopoepimers in this study may limit the accuracy of this analysis, these values clearly are consistent with a reaction that proceeds with the selective loss of the proton and deuteron cis to the cyclopropyl group in this '**5a-2-*d*$_1$** 75:25 isotopoepimeric mixture'.

Support for the proposed diastereoselectivity of this reaction was sought by examining the metabolic fate of the '**5a-2-*d*$_1$** 25:75 isotopoepimeric mixture'. The incubations were conducted with 2.4 μM MAO-B and 500 μM substrate. The increased ratio in the concentration of enzyme to substrate was prompted by a possible deuterium isotope effect referred to above since the major component of this mixture, **5a**-(**2*R***)-***d*$_1$**, bears the deuterium cis to the cyclopropyl group. LC-ESI/MS extracted ion chromatographic analyses again indicated that less than 5% of the starting substrate remained following the 2-h incubation period. The average values of 3 such incubations gave a ratio for **6a**$^+$-***d*$_0$**:**6a**$^+$**-2-*d*$_1$** of 68:32 (Scheme 6). This value, which is close to the inverse of the 29:71 ratio obtained with the '**5a-2-*d*$_1$** 75:25 isotopoepimeric mixture', is consistent with the conclusion that the MAO-B-catalyzed oxidation of **5a** is a highly diastereoselective process with 2H$_R$ being preferentially lost.

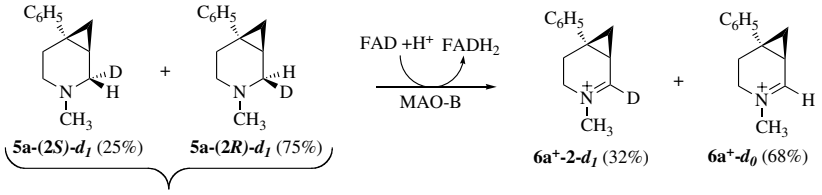
A final experiment examined the fate of the '**5b-2-*d*$_1$** 75:25 isotopoepimeric mixture'. LC-ESI/MS extracted ion chromatographic analyses of the 2-h incubation mixtures indicated only 88% consumption of substrate even though the enzyme:substrate ratio was 2.4:500. The average percentage (3 determinations) of the integrated ion current at *m*/*z* 186 attributable to **6b**$^+$-***d*$_0$** was 68%; the corresponding value for **6b-2-*d*$_1$** at *m*/*z* 187, when corrected for the ^{13}C-satellite contribution from *m*/*z* 186, was 32% (Scheme 7). This result documents that, as observed with the (1*R*,6*S*)-enantiomer **5a**, the 2H$_R$ proton is selectively lost in the MAO-B-catalyzed oxidation of the (1*S*,6*R*)-enantiomer **5b**. In the case of **5a**, however, the 2H$_R$ proton is cis to the cyclopropyl group while in **5b** the 2H$_R$ proton is trans to the cyclopropyl group. Consequently, although the configurations at C(1) and C(6) have an impact on the V_{max}/K_m values of **5a** versus **5b**, the configurations of these stereocenters do not alter the stereochemical course of the reaction since 2H$_R$ is lost selectively with both **5a** and **5b**. This behavior may prove useful when attempting to model the complex between MAO-B and cyclic tertiary aminyl substrates.

3. Summary and conclusions

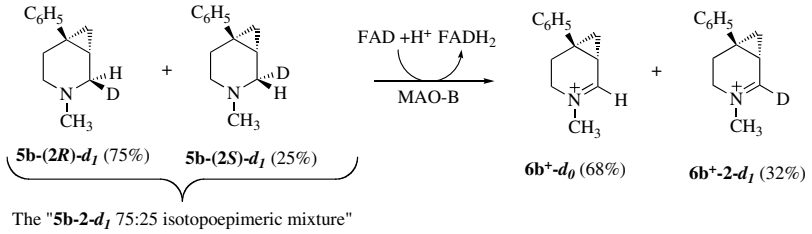
The results of these studies document that the MAO-B-catalyzed ring α-carbon oxidation of **5** is enantioselective with an almost 5-fold preference for the (1*R*,6*S*) enantiomer **5a** based on V_{max}/K_m values. A summary of the results of the diastereoselectivity of this reaction (2H$_R$ vs 2H$_S$) is presented in Scheme 8. In all cases the predominant pathway in the oxidation of **5** to **6**$^+$ involves the loss of 2H$_R$ even though 2H$_R$ is cis to the cyclopropyl group in **5a** and trans to the cyclopropyl group in **5b**. This outcome is consistent with the behavior of prochiral substrates in that substituents β to the nitrogen atom do not alter the stereochemical course of the reactions with selective loss of the α-pro-*R* proton observed with all substrates reported. The present results provide additional insight into the enzyme-mediated stereochemical control of this type of oxidation in



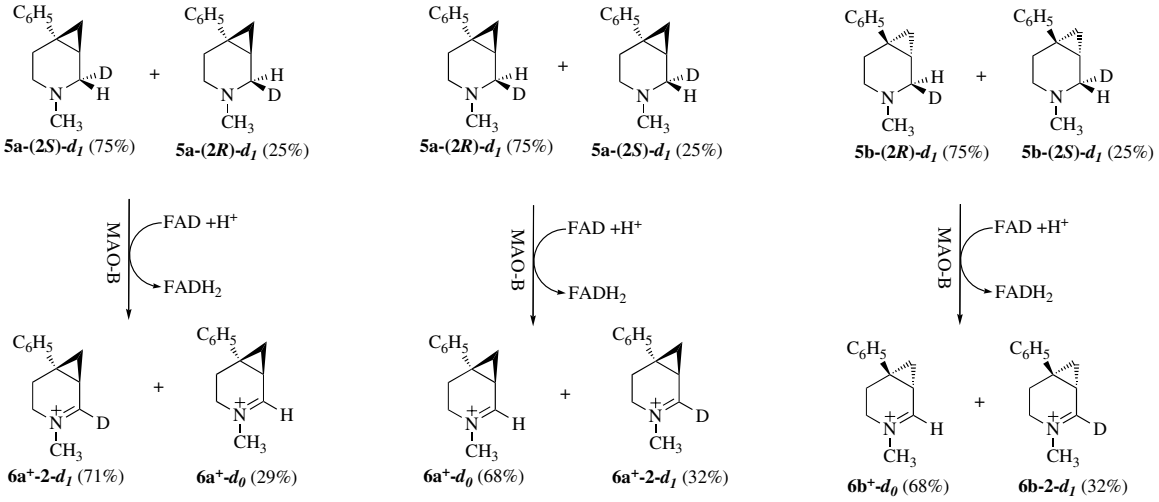
Scheme 5.



Scheme 6.



Scheme 7.



Scheme 8.

that, unlike previous examples, the geometries of the atoms β and γ to the nitrogen atom are fixed. It may be reasonable to speculate that the stereochemical course of the MAO-B-catalyzed oxidations of amines in general is dictated by the geometries of the nitrogen and α-carbon atoms and a structurally rigid enzyme–substrate complex. Molecular modeling studies that will take advantage of these results and the reported X-ray structure of the expressed human MAO-B used in this work[44–46] are being pursued.

4. Experimental

Important notice: some 1,4-disubstituted-1,2,3,6-tetrahydropyridines are known nigrostriatal neurotoxins

and should be handled using disposable gloves in a properly ventilated hood following good laboratory practices. Detailed procedures for the safe handling of MPTP have been reported.[47]

4.1. General methods

Proton and ^{13}C NMR spectra were recorded on a JEOL 500-MHz spectrometer. X-ray analysis of **5b·HClO$_4$** was performed on an Oxford Diffraction Xcalibur Gemini diffractometer equipped with a Sapphire 3 CCD detector.[48] Enzyme kinetic studies were performed on the SpectraMax 384 microplate reader and the data were evaluated with SoftMax PRO v. 4.7.1 software equipped with a pre-programmed Michaelis–Menten protocol. All assays were carried out at 37 °C in 50 mM potassium phosphate buffer (pH 7.0). Recombinant human MAO-B (50 nM or 250 nM) was used in the experiments to estimate the values of V_{max} and K_m for **5a** and **5b**. Plots of OD (420 nm) versus time were linear for the first 30 min at 50 nM MAO-B and for 5 min at 250 nM MAO-B. The diastereoselectivity studies on the monodeutero isotopoepimers were performed on 1 mM substrate and 1–2.4 μM MAO-B. Incubations (0.5 mL final volume) were conducted in 50 mM potassium phosphate buffer (pH 7.0) at 37 °C for 2 h at which time acetonitrile (0.5 mL) was added. After vortexing and centrifugation, the supernatant fraction was evaporated under vacuum and the residue in 0.5 mL methanol was filtered through a 1 μm syringe filter and 25 μL of the filtrate in 25 mL methanol was analyzed by LC-ESI/MS in the positive ion mode on an API Sciex 365 triple quadrupole tandem mass spectrometry (Applied Biosystems, Foster City, CA) under the following conditions: The auxiliary gas (N_2) flow-rate was 2 L/min, the curtain gas flow-rate was 1.3 L/min at 60 p.s.i. and the turbo ion spray temperature was 400 °C; the HPLC system consisted of an Agilent 1100 series HPLC pump, diode-array detector and a Zorbax SB-C18 column (2.1 × 150 mm, 5 μm). The mobile phase—methanol:10 mM ammonium formate (80:20, v/v), was delivered in an isocratic mode at a flow-rate of 0.2 mL/min. The deuterium contents of the iminiumyl metabolites were estimated by selected ion monitoring of the appropriate ions taking into account the ^{13}C contribution of the d_0 species to the d_1 species. The results recorded in Section 2 are the average of three such determinations for each of the three substrates analyzed.

4.1.1. (1*S*,6*R*)-3-Methyl-6-phenyl-3-azabicyclo[4.1.0]heptane HCl (5b·HCl). A solution of (1*R*)-(−)-10-camphorsulfonic acid (8.82 g, 38 mmol) in MeOH (20 mL) was added to a solution of the free base **5ab**[27] (14.23 g, 76 mmol) in ether (80 mL) at room temperature. The crystals (13.7 g, 86%) were collected and recrystallized five times from ether/MeOH to give (1*S*,6*R*)-(−)-3-methyl-6-phenyl-3-azabicyclo[4.1.0]heptane (1*R*)-10-camphorsulfonate [**5b·(*R*)-C$_{10}$H$_{16}$O$_4$S**] as white crystals (4.46 g, 28%): mp 177–178 °C; α_D = +13.8 (*c* 1.05 g/100 mL in MeOH); ^1H NMR (500 MHz, CD_3OD) δ 0.84 (s, 3H), 1.11 (s, 3H), 1.24 (m, 1H), 1.39 (m, 1H), 1.62 (m, 2H), 1.87 (d, *J* = 18 Hz, 1H), 2.02 (m, 2H), 2.33 (m, 1H), 2.42 (m, 2H), 2.64 (m, 1H), 2.77 (d, *J* = 15 Hz, 1H), 2.86 (s, 3H), 2.97 (bs, 1H), 3.28 (s, 1H), 3.96 (bs, 1H), 7.21 (m, 1H), 7.31 (m, 2H), 7.38 (m, 2H); ^{13}C NMR (125.8 MHz, CD_3OD) δ 15.1, 17.6, 18.8, 19.1, 22.5, 24.4, 26.5, 28.3, 42.0, 42.3, 42.7, 46.9, 49.3, 53.6, 58.3, 126.6, 127.4, 128.4, 145.0, 217.1. Anal. Calcd for $C_{23}H_{33}NO_4S$ (419.59): C, 65.84; H, 7.93; N, 3.34. Found: C, 65.78; H, 7.90; N, 3.34. An ethereal solution of the corresponding free base **5b** (1.9 g, 10 mmol) was treated with methanolic HCl (1.2 equiv) and the resulting solid was recrystallized from MeOH/ether to give 1.8 g (82%) of **5b·HCl**: mp 212–213 °C; α_D = +66.9 (*c* 0.69 g/100 mL in MeOH). Anal. Calcd for $C_{13}H_{18}ClN$ (223.74): C, 69.79; H, 8.11; N, 6.26. Found: C, 69.63; H, 8.04; N, 6.13.

4.1.2. (1*R*,6*S*)-3-Methyl-6-phenyl-3-aza-bicyclo[4.1.0]heptane HCl (5a·HCl). The solvent from the filtrate obtained after the first crystallization with the (1*R*)-(−)-10-camphorsulfonic acid was removed under reduced pressure and a saturated aqueous solution of K_2CO_3 (100 mL) was added. The aqueous phase was extracted with ether (3× 30 mL) and the combined organic layers were washed with water (3× 30 mL), brine (2× 30 mL), dried over Na_2SO_4 and filtrated. After removal of the solvent under reduced pressure the residue (7.7 g, 41 mmol) was dissolved in ether (80 mL) and a solution of (1*S*)-(+)-10-camphorsulfonic acid (9.5 g, 41 mmol) in MeOH (20 mL) was added at room temperature. The collected crystals were recrystallized five times from ether/MeOH to give **(1*R*,6*S*)-3-methyl-6-phenyl-3-azabicyclo[4.1.0]heptane (1*S*)-(+)-10-camphorsulfonate [5a·(*S*)-C$_{10}$H$_{16}$O$_4$S]** as white crystals (4.76 g, 28%): mp 177–178 °C; α_D = −11.5 (*c* 0.84 g/100 mL in MeOH). Anal. Calcd for $C_{23}H_{33}NO_4S$ (419.59): C, 65.84; H, 7.93; N, 3.34. Found: C, 65.83; H, 7.93; N, 3.35. An ethereal solution of the corresponding free base **5a** (1.8 g, 10 mmol) was treated with methanolic HCl (1.2 equiv) and the resulting precipitate was recrystallized from MeOH/ether to give **5a·HCl** (1.3 g, 78%) as white crystals: mp 212–213 °C; α_D = −70.8 (*c* 0.62 g/100 mL in MeOH). Anal. Calcd for $C_{13}H_{18}ClN$ (223.74): C, 69.79; H, 8.11; N, 6.26. Found: C, 69.41; H, 7.94; N, 6.04.

4.1.3. (1*S*,6*R*)-3-Methyl-6-phenyl-3-azabicyclo[4.1.0]heptane HClO$_4$ (5b·HClO$_4$). A methanolic solution of HClO4 (1 mL, 0.64 mmol) was added to an ethereal solution (2 mL) of the free base **5b** (100 mg, 0.53 mmol). The precipitate was filtrated and recrystallized from MeOH/ether to give **5b·HClO$_4$** (137 mg, 90%) as white crystals: mp 164–165 °C; ^1H NMR (500 MHz, CD_3OD) δ 1.12 (m, 1H), 1.22 (m, 1H), 1.60 (m, 1H), 2.40 (m, 2H), 2.86 (s, 3H), 2.95 (m, 1H), 3.14 (m, 1H), 3.30 (m, 1H), 3.98 (m, 1H), 7.20 (t, *J* = 7.5 Hz, 1H), 7.30 (t, *J* = 7.5 Hz, 2H), 7.36 (d, *J* = 7.5 Hz, 2H); ^{13}C NMR (125.8 MHz, CD_3OD) δ 15.0, 17.7, 22.5, 28.3, 41.9, 49.3, 53.9, 126.6, 127.4, 128.4, 145.0. Anal. Calcd for $C_{13}H_{18}ClNO_4$ (287.74): C, 54.26; H, 6.31; N, 4.87. Found: C, 54.42; H, 6.23; N, 4.90.

4.1.4. (1*R*,6*S*)-3-Methyl-6-phenyl-3-azabicyclo[4.1.0]hept-2-enyl perchlorate (6a$^+$-*d*$_0$·ClO$_4^-$). The procedure as described previously[27] for the synthesis of racemate **6ab$^+$**

was followed. Recrystallization of the crude product from MeOH/ether gave **6a**$^+$-d_0·ClO_4^- (386 mg, 30%) as white crystals: mp 126–127 °C; $\alpha_D = -255$ (*c* 1.41 g/100 mL in MeOH). Anal. Calcd for $C_{13}H_{16}ClNO_4$ (285.72): C, 54.65; H, 5.64; N, 4.90. Found: C, 54.48; H, 5.63; N, 4.91.

4.1.5. (1*S*,6*R*)-3-Methyl-6-phenyl-3-azabicyclo[4.1.0]hept-2-enyl perchlorate (6b$^+$-d_0·ClO_4^-). The same procedure gave **6b**$^+$-d_0·ClO_4^- (161 mg, 37%) as white crystals: mp 127–128 °C; $\alpha_D = +234$ (*c* 0.58 g/100 mL in MeOH). Anal. Calcd for $C_{13}H_{16}ClNO_4$ (285.72): C, 54.65; H, 5.64; N, 4.90. Found: C, 54.30; H, 5.54; N, 4.84.

4.1.6. (1*R*,6*S*)-3-Methyl-6-phenyl-3-azabicyclo[4.1.0]heptane HCl [5a-(2*S*)-d_1·HCl] from 6a$^+$-$d_0$$ClO_4^-$. $NaBD_4$ (15 mg) was added at 0 °C to a solution of **6a**$^+$-d_0 (50 mg, 0.17 mmol) in CD_3OD (1 mL). After 30 min water was added and the mixture was extracted with ether; ^{1}H NMR (500 MHz, CD_3OD) δ 0.82 (m, 1 H), 0.99 (m, 1H), 1.39 (m, 1H), 2.21 (m, 4H), 2.24 (m, 3H), 2.62 (m, 0.75), 2.80 (m, 0.25H), 7.15 (m, 1H), 7.28 (m, 4H). The free base was treated with methanolic HCl to give **5a-(2*S*)-d_1·HCl** (21 mg, 52%) as a white powder: mp 215–216 °C.

4.1.7. (1*S*,6*R*)-3-Methyl-6-phenyl-3-azabicyclo[4.1.0]heptane HCl [5b-(2*S*)-d_1·HCl] from 6b$^+$-d_0 · ClO_4^-. $NaBD_4$ (15 mg) was added at 0 °C to a solution of **6b**$^+$-d_0 (50 mg, 0.17 mmol) in CD_3OD (1 mL). After 30 min water was added and the mixture was extracted with ether; ^{1}H NMR (500 MHz, CD_3OD) δ 0.81 (m, 1H), 0.99 (m, 1H), 1.38 (m, 1H), 2.21 (m, 4H), 2.24 (m, 3H), 2.62 (m, 0.75), 2.79 (m, 0.25H), 7.15 (m, 1H), 7.28 (m, 4H). The free base was treated with HCl methanolic to give **5b-(2*S*)-d_1·HCl** (13 mg, 53%) as a white powder: mp 215–216 °C.

4.1.8. (1*R*,6*S*)-2,2-d_2-3-Methyl-6-phenyl-3-azabicyclo[4.1.0]-heptane HCl (5a-2,2-d_2·HCl). To a solution of racemic **5ab-2,2-d_2**[31] (7.11 g, 41 mmol) in ether (45 mL) was added a solution a (1*S*)-(+)-10-camphorsulfonate (4.72 g, 20 mmol) in MeOH (10 mL). The salt was recrystallized three times from MeOH/ether to give the (*S*)-(+)-10-camphorsulfonic acid salt as white crystals (1.53 g, 18%): mp 176–177 °C. This salt was treated with an aqueous solution of K_2CO_3 and the free base was extracted with ether. The free base (40 mg, 0.21 mmol) was treated with a methanolic solution of HCl to give **5a-2,2-d_2·HCl** (45 mg, 95%) as a white solid: mp 216–217 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 1.14 (m, 1H), 1.25 (m, 1H), 1.60 (m, 1H), 2.43 (m, 2H), 2.83 (s, 3H), 2.91 (m, 1H), 3.35 (m, 1H), 7.22 (m, 1H), 7.31 (m, 2H), 7.38 (m, 2H).

4.1.9. (1*R*,6*S*)-3-Methyl-6-phenyl-3-azabicyclo[4.1.0]hept-2-enyl perchlorate (6a$^+$-2-d_1·ClO_4^-). The same procedure as described previously[27] for the synthesis of racemate **6ab**$^+$ was followed. The solid was recrystallized from MeOH/ether to give **6a**$^+$-d_1·ClO_4^- (166 mg, 22%) as white crystals: mp 127–128 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 2.02 (m, 1H), 2.30 (m, 1H), 2.47 (m, 3H), 3.64 (s, 3H), 3.78 (m, 2H), 7.27 (m, 1H), 7.34 (m, 2H), 7.44 (m, 2H).

4.1.10. (1*R*,6*S*)-3-Methyl-6-phenyl-3-aza-bicyclo[4.1.0]heptane 5a-(2*S*)-d_1 from 6a$^+$-2-$d_1$$ClO_4^-$. $NaBH_4$ (12 mg) was added at 0 °C to a solution of **6a**$^+$-d_1 (46 mg, 0.16 mmol) in MeOH (1 mL). After 30 min water was added and the mixture was extracted with ether; ^{1}H NMR (500 MHz, CD_3OD) δ 0.81 (m, 1H), 0.97 (m, 1H), 1.37 (m, 1H) 2.22, (m, 4H), 2.24 (s, 3H), 2.62 (m, 0.25H), 2.77 (m, 0.75H), 7.17 (m, 1H), 7.28 (m, 4H). The free base was treated with methanolic HCl to give **5a-(2*S*)-d_1HCl** (22 mg, 60%) as a white powder: mp 214–215 °C.

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