Transformation of Monoamine Oxidase-B Primary Amine Substrates into Time-Dependent Inhibitors. Tertiary Amine Homologues of Primary Amine Substrates

Charles Z. Ding, Xingliang Lu, Kuniko Nishimura,‡ and Richard B. Silverman*

Department of Chemistry and Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, Illinois 60208-3113

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A family of *N*-methylated and *N,N*-dimethylated alkyl and arylalkylamines was prepared and more than half of the analogues were shown to be time-dependent pseudo-first-order inhibitors of monoamine oxidase-B. Some of the time-dependent inactivators were reversible and others were irreversible with respect to prolonged dialysis following inactivation. Partition ratios ranged from zero to 11 000. These results are rationalized in terms of a combination of an inductive effect and a stereoelectronic effect as a result of hindered rotation of an active site covalent adduct. A molecular mechanics calculation indicates that there is at least 10 kcal/mol of torsional energy to be overcome in order for the enzyme adduct to be released. These findings show that tertiary amine homologues of primary amine substrates of monoamine oxidase are time-dependent inhibitors, and this should be useful in the design of new inactivators of this enzyme.

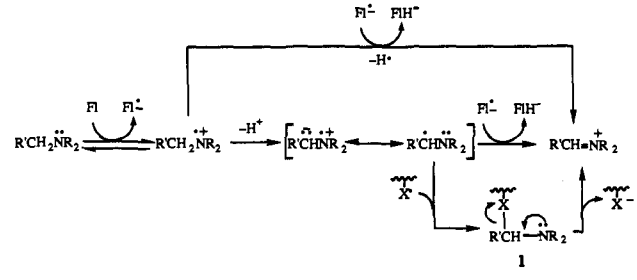
Inactivators of monoamine oxidase-B (EC 1.4.3.4; MAO-B) have been shown to be useful adjuncts in the treatment of Parkinson's disease.[1] A variety of structures have been utilized in the search for selective and potent inactivators of this isozyme.[2] Several years ago we observed that whereas cinnamylamine is an excellent substrate for MAO-B, the corresponding *N,N*-dimethyl analogue was an irreversible inactivator of the enzyme.[3] Recently, Williams et al.[4] reported that *N*-methylcinnamylamine also was a time-dependent inhibitor of MAO-B; dialysis did not cause reactivation of the inactivated enzyme. No explanations were offered by either of these groups as to the possible reasons for this observed inhibitory effect of *N*-methylcinnamylamine.

We thought that it would be informative and potentially useful in the design of new inactivators of MAO-B to determine the generality of the previously reported inhibitory effect of tertiary amines. Consequently, we have synthesized a variety of *N*-alkyl and *N,N*-dimethyl substrate analogues in order to determine if they have the same effect on the activity of the parent amine as did the secondary and tertiary cinnamylamine analogues. It appears that this *is* a general phenomenon and rationalizations for this consequence are discussed in this paper.

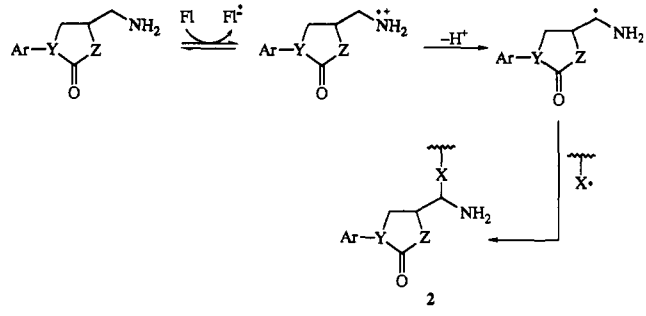
Results and Discussion

Alkyl and arylalkyl primary amines are excellent substrates for MAO.[5] Over the last dozen years or so we have provided evidence[6] for a mechanism of MAO that we[7] and the Krantz[8] group proposed (Scheme I shows our current mechanistic hypothesis). We have investigated a variety of new classes of inactivators of MAO,[9] such as the oxazolidinones,[10] dihydrofuran-2(3*H*)-ones,[11] and pyrrolidinones,[12] and conclude that when there is an electron-withdrawing group near the aminomethyl methylene group these compounds inactivate the enzyme (Scheme II). It is thought that the cause for inactivation is the stabilization

Scheme I



Scheme II



of the covalent intermediate (**2**, Scheme II) in the reaction as a result of the inductive effect of the electron-withdrawing atoms in the heterocyclic ring. Earlier evidence[6] indicates that the X group on the enzyme is a cysteine residue; a cysteine radical could be generated by hydrogen atom transfer from the cysteine thiol to the flavin semiquinone radical anion.

We now have found that in addition to the incorporation of electron-withdrawing heterocycles in substrates,[10-12] N-methylation and N,N-dimethylation of primary amine substrates (Figure 1) convert many of these substrates into pseudo-first-order, time-dependent inhibitors of MAO (Table I) with the exceptions of *N,N*-dimethylbenzylamine (**3**), *N*-methyl-2-phenylethylamine (**8**), *N*-methyl-3-phenylpropylamine (**10**), *N*-methyl-4-phenylbutylamine (**11**), and *N*-methylpentylamine (**12**). Full enzyme activity returns upon prolonged dialysis except in the cases of *N,N*-dimethyl-2-phenylethylamine (**4**) and *N,N*-dimethyl-3-

* Address correspondence to this author at the Department of Chemistry.
‡ Current address: Department of Life Chemistry, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, Yokohama, 227, Japan.

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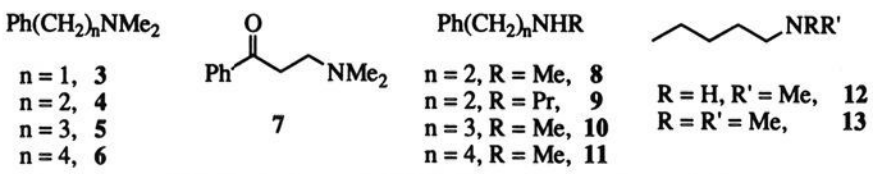


Figure 1. *N*-Methyl- and *N,N*-dimethylamine inhibitors.

Table I. Inhibition of Monoamine Oxidase-B by Secondary and Tertiary Amines

compd	inactivator?	K_I (mM)	k_{inact} (min^{-1})	reversible[a] or irreversible	K_m (mM)	k_{cat} (min^{-1})
3	no				*b*	*b*
4	yes	7.1	7.7×10^{-3}	irrev	1.49	84.2
5	yes	0.38	0.43	rev	0.18	12.6
6	yes	10.0	2.9×10^{-3}	rev	2.11	8.33
7	yes	0.35	1.73	irrev	*b*	*b*
8	no				*c*	*c*
9	yes	4.0	4.6×10^{-3}	rev	*c*	*c*
10	no				*c*	*c*
11	no				*c*	*c*
12	no				*c*	*c*
13	yes	2.1	1.6×10^{-2}	rev	*c*	*c*

[a] Reversible to dialysis. [b] Not a substrate. [c] Not determined.

oxo-3-phenylpropylamine (**7**). The presence of 2 mM β-mercaptoethanol in the incubation solution had no effect on the rate of inactivation by **4**, indicating that an electrophilic species released from the enzyme is not responsible for inactivation. On the basis of the mechanism of MAO (Scheme I) and the mechanism of inactivation of MAO by the heterocyclic analogues (Scheme II), it is hypothesized that inactivation by the secondary and tertiary amine analogues is the result of the formation of a stable covalent adduct similar in structure to the proposed covalent adducts during substrate turnover (**1**, R = Me; Scheme I) and in heterocyclic analogue inactivation (**2**, Scheme II). In the case of primary amine substrates, the covalent adducts (**1**, R = H; Scheme I) are unstable and readily decompose to the corresponding iminium ions (the product of MAO-catalyzed oxidation of amine substrates), whereas the N,N-dimethylated analogues, except for **3**, presumably do not. In order to show that these compounds are capable of inactivating MAO by the oxidation mechanism depicted by Scheme II, the oxidation properties of several of the compounds were determined. Compounds **4–6** were substrates for MAO, but **3** and **7** were not. There is no correlation between substrate and inactivation properties; compounds **4–7** are inactivators, and **3** is not. On the basis of the partition ratio for inactivation (k_{cat}/k_{inact}), compound **7** is the most efficient inactivator of the ones tested (partition ratio of 0), compound **5** is the next most efficient (partition ratio of 29), and **6** (partition ratio 2900) and **4** (partition ratio 11 000) are the least efficient inactivators.

The flavin spectrum was monitored during inactivation of MAO by compound **4**. After inactivation and dialysis the flavin was shown to be in the reduced form by UV vis spectroscopy; denaturation resulted in reoxidation of the flavin (data not shown). However, since the inactivator has not yet been synthesized with a radioactive label, it is not known if the inactivator remains bound to the enzyme under these denaturation conditions. Therefore, it cannot yet be concluded that attachment occurs at a site other than on the flavin.

The difference in stabilities of the adducts with primary and tertiary amines can be rationalized by consideration of their Newman projections (Scheme III). Adducts derived from primary amines would adopt conformation

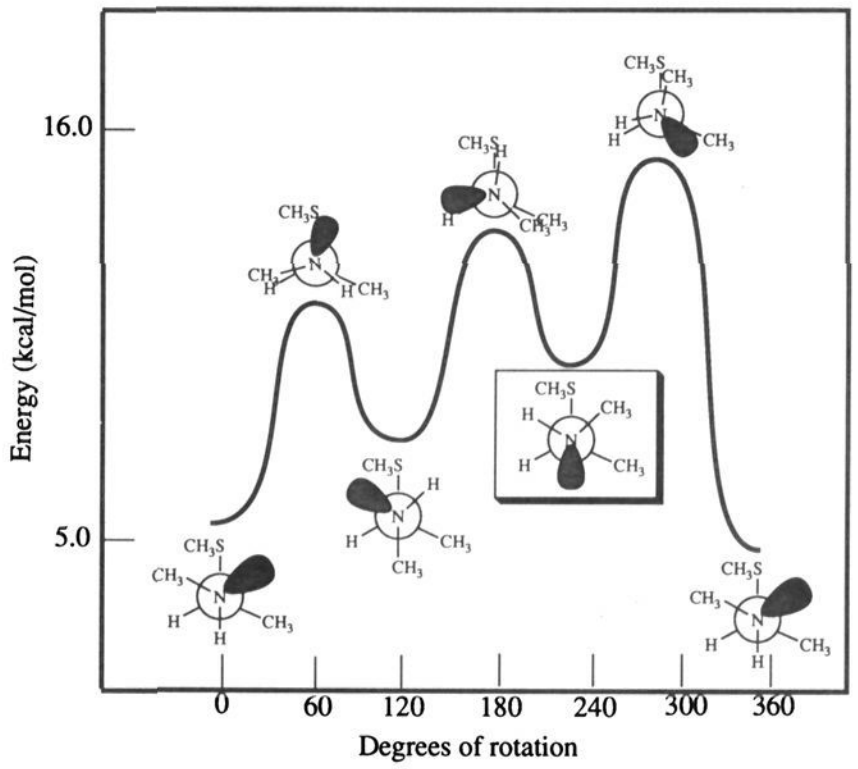
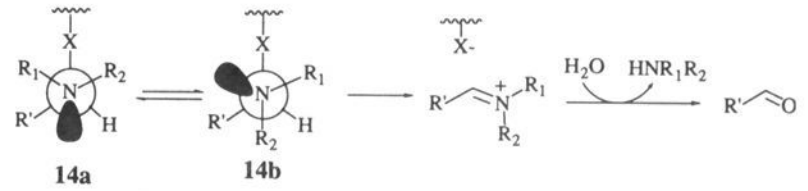


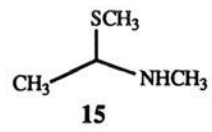
Figure 2. Energy diagram for the rotation of the C–N bond in **15**.[15]

Scheme III



14a ($R_1 = R_2 = H$) to take advantage of the anomeric stabilizing effect[13] with the nonbonded electrons of the nitrogen atom antiperiplanar to the C–X bond. This conformation readily expels the leaving group (the active site amino acid residue) to give the iminium ion and reduced enzyme, which is rapidly oxidized to active enzyme. In the case of secondary and tertiary amines the nonbonded electrons of the nitrogen would prefer conformation **14b** (either $R_1 = H$, $R_2 = Me$ or $R_1 = R_2 = Me$, depending upon whether it is secondary or tertiary) in order to minimize the gauche interactions.[14] This conformation orients the orbital containing the nonbonded electrons orthogonal to the C–X bond, and therefore, expulsion of the leaving group (the active site residue) becomes very difficult.

In order to estimate the energy barrier for rotation about the C–N bond of the proposed enzyme adduct, a molecular mechanics calculation[15] was performed on *N*-methyl-1-(methylthio)aminoethane (**15**), a model for an adduct



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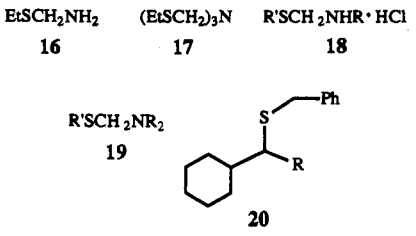
produced by attachment of a secondary amine substrate to a cysteine residue in the active site. The results of that calculation are depicted in Figure 2. The torsional energy barrier around the C–N bond to generate the appropriate conformer is 10–11 kcal/mol. This is the energy that must be overcome in order to have proper orbital alignment for expulsion of the leaving group (the cysteine residue). The *N,N*-dimethyl analogues should possess an even greater rotational energy barrier around the C–N bond than the *N*-methyl analogues and would decompose even slower.

This crude molecular mechanics calculation predicts that aminothiol acetals of primary amines should be unstable, those of secondary amines should be relatively

stable, and those of tertiary amines should be even more stable. This, in fact, is the case. A primary aminomethyl sulfide, such as aminomethyl ethyl sulfide (16) exists only



below −100 °C;[16] upon warming it rearranges to the more stable product 17, a tertiary aminomethyl sulfide, with elimination of ammonia. Reynolds and Cossar[17] reported the preparation of secondary aminomethyl sulfide hydrochlorides 18; however, the formation of hydrochloride salts ties up the nitrogen nonbonded electrons and blocks elimination. Whereas these hydrochloride salts were stable, the unprotonated species were not.[18] It has long been known that (*N*,*N*-dialkylamino)methyl sulfides (e.g., 19) could be prepared easily.[19] These tertiary aminomethyl sulfides are stable at room temperature. *N*,*N*-Diethylaminomethyl isopropyl sulfide (19, R′ = isopropyl; R = Et) is stable to hydrolysis with cold water and only slowly decomposes in hot water.[20] We have found that the tertiary aminomethyl sulfide 20 (R = piperidino) can be synthesized easily by the reaction of cyclohexanecarboxaldehyde with piperidine and benzyl mercaptan. However, under the identical reaction conditions the secondary aminomethyl sulfide 20 (R = cyclohexylamino) could not be synthesized.

The results summarized in Table I are *not* consistent with a simple stereoelectronic effect against bond rotation. However, if a combination of inductive effects and stereoelectronic effects are considered, then the results are more understandable. Consider the general adduct structure 1 (Scheme I). *N*,*N*-Dimethylbenzylamine (3) is not a time-dependent inhibitor. Although there is a torsional energy barrier to overcome, elimination of the active site residue is an exceedingly favorable process because of the carbenium ion stabilization by the phenyl ring which *accelerates* the rate of cleavage of the carbon–cysteine bond. The phenyl group in *N*,*N*-dimethyl-2-phenylethylamine (4), however, acts as an electron-withdrawing group, thereby destabilizing the formation of iminium product and stabilizing the adduct. The stereoelectronic effect also must be very important because 2-phenylethylamine and *N*-methyl-2-phenylethylamine (8) are excellent substrates, not inactivators. However, *N*-propyl-2-phenylethylamine (9) *is* a time-dependent inactivator, presumably because of the greater steric effect to rotation of the *n*-propyl group relative to that for the methyl group of 8. When the appropriate combination of inductive and stereoelectronic effects is present (such as in the case of 4), the adduct that forms is stable even to dialysis. Likewise, *N*,*N*-dimethyl-3-oxo-3-phenylpropylamine (7) has two strong electron-withdrawing groups (a carbonyl and a phenyl group) which provide the necessary inductive stabilization of the adduct in combination with the stereoelectronic effect of the dimethylamino group. This compound also forms an adduct that is stable to dialysis. When the phenyl group is more removed from the site of adduct formation, the inductive effect is diminished and the stability of the adduct also diminishes. This is consistent with the observation that *N*,*N*-dimethyl-3-phenylpropylamine (5), *N*,*N*-dimethyl-4-phenylbutylamine (6), and *N*,*N*-dimethylpentylamine (13) are time-dependent inhibitors, but the adducts formed are not stable to dialysis. Once the stereoelectronic effect is diminished by N-monoalkylation instead of N,N-dialkylation, the stability of the adduct formed also diminishes. *N*-Methyl-2-phenylethylamine (8), *N*-methyl-3-phenylpropylamine (10), *N*-methyl-4-phenylbutylamine (11), and *N*-methylpentylamine (12) do not inactivate MAO. These latter three compounds have both diminished stereoelectronic effects and diminished (or nonexistent) inductive effects of the phenyl group. *N*,*N*-Dimethylpentylamine (13) has no phenyl group to supply an inductive effect, but the stereoelectronic effect of the dimethylamino group is, apparently, sufficient to produce time-dependent inhibition; the adduct, however, is not stable to dialysis.

Conclusion

These studies suggest that tertiary amine homologues of primary amine substrates for MAO are, in general, time-dependent inhibitors of this enzyme and that this approach of modifying primary amine substrates should be useful in rational drug-design efforts. Structure–activity data suggest that in order for the N-monoalkylation to be effective it is necessary for an electron-withdrawing group to be in the β-position. The results have been rationalized in terms of a combination of an inductive effect and a stereoelectronic effect arising from hindered rotation about the C–N bond of the proposed enzyme adduct. If these results are as general as they appear to be, then this suggests that isozyme selective substrates for MAO-A and MAO-B may be candidates for conversion into isozyme-selective inactivators by N,N-dimethylation. This has yet to be determined.

Experimental Section

General. NMR spectra were recorded either on a Varian Gemini 300-MHz or on a Varian XL-400 400-MHz spectrometer. Chemical shifts are reported as δ values in parts per million downfield from Me_4Si as the internal standard in $CDCl_3$. Thin-layer chromatography was performed on EM/UV silica gel plates with a UV indicator. Melting points were obtained with a Fisher-Johns melting point apparatus and are uncorrected. All chemicals were purchased from Aldrich Chemical Co. and were used without further purification unless indicated otherwise. *N*,*N*-Dimethyl-3-oxo-3-phenylpropylamine hydrochloride (7) was purchased from Aldrich Chemical Co. and recrystallized from ethanol–ethyl acetate prior to use. Glassware was dried in an oven overnight when dry conditions were required. All air- and moisture-sensitive reactions were carried out in an atmosphere of inert gas (nitrogen or argon).

General Procedure for the Syntheses of N,N-Dimethylated Amines.[21] The amine (50 mmol) was added dropwise via a syringe to cooled formic acid (88%, 11.0 mL, 250 mmol), and to the resulting mixture was added formaldehyde (37%, 9.5 mL, 125 mmol). The resulting mixture was stirred at 0 °C for 1 h and then heated at 90 °C for 4 h. The mixture was then cooled down to 0 °C, 10 mL of deionized water was added followed by 8 mL of concentrated HCl. The volatile solvents were removed in vacuo, and the residue was crystallized two times from a mixture of ethanol, ethyl acetate, and hexane.

The following compounds were prepared by this method.

***N*,*N*-Dimethylbenzylamine hydrochloride (3):** 62%, white flakes; mp 174–175 °C (lit.[22] mp 175 °C); ^1H NMR (D_2O) δ 7.50 (br s, 5 H), 4.30 (s, 2 H), 2.80 (s, 6 H).

***N*,*N*-Dimethyl-2-phenylethylamine hydrochloride (4):** 38%; white flakes; mp 164–165 °C (lit.[23] mp 165 °C); ^1H NMR (D_2O) δ 7.30–7.50 (m, 5 H), 3.40 (t, 2 H), 3.05 (t, 2 H), 2.90 (s, 6 H); ^{13}C NMR δ 136.05, 129.23, 128.93, 127.54, 58.56, 42.87, 30.28.

N,N-Dimethyl-3-phenylpropylamine hydrochloride (5): 60%; white plates; mp 145–147 °C (lit.[24] mp 146–147 °C); ^{1}H NMR δ 7.30–7.45 (m, 5 H), 3.10 (t, 2 H), 2.85 (s, 6 H), 2.70 (t, 2 H), 2.00 (m, 2 H); ^{13}C NMR δ 141.0, 129.0, 128.5, 127.0, 57.5, 32.0, 42.5, 25.5.

N,N-Dimethyl-4-phenylbutylamine hydrochloride (6): 71%; a snowy white powder; mp 131–132 °C (lit.[25] mp 130–131 °C); ^{1}H NMR δ 7.25–7.45 (m, 5 H), 3.10 (t, 2 H), 2.85 (s, 6 H), 2.70 (t, 2 H), 1.70 (m, 4 H); ^{13}C NMR δ 142.0, 129.0, 128.5, 126.0, 58.0, 32.5, 34.0, 27.5.

N-Methyl-2-phenylethylamine Hydrochloride (8). The free amine (Aldrich) was converted into its hydrochloride salt and recrystallized from a two-solvent system where ethanol and ethyl acetate (1:5) was the first solvent and cyclohexane was the second to give the product as white plates; mp 162–164 °C (lit.[26] mp 156–158 °C).

N-Propyl-2-phenylethylamine Hydrochloride (9). To a solution of phenylacetaldehyde (2.4 g, 20 mmol) in 50 mL of methanol was added propylamine (2.0 mL, 24 mmol) at room temperature under an argon atmosphere. Then molecular sieves (4 Å, 5 g) were added, and stirring was maintained for 2 h. Sodium borohydride (0.7 g, 18 mmol) was added to the resulting mixture portionwise over a 1-h period, and the resultant mixture was stirred overnight. Excess sodium borohydride was destroyed by addition of a 10% aqueous HCl solution at 0 °C, and the aqueous solution was filtered. The filtrate was concentrated in vacuo, and aqueous solution was basified with 20% KOH. The basified solution was extracted with ether (2 × 30 mL), and the combined ether extracts were dried over magnesium sulfate. After filtration of the drying agent and removal of solvent the resultant liquid was dissolved in methanol and gaseous HCl was introduced. The methanol was removed and the residue was crystallized from ethyl acetate–cyclohexane to give white crystals (0.95 g, 24%): mp 204–206 °C; ^{1}H NMR δ 7.40 (m, 5 H), 3.22 (t, 2 H), 3.00 (m, 4 H), 1.60 (m, 2 H), 0.90 (t, 3 H). Anal. ($C_{11}H_{18}ClN$) C, H, N.

N-Methyl-3-phenylpropylamine Hydrochloride (10). A solution of *N*-methylcinnamylamine hydrochloride in ethanol was hydrogenated under 1 atm of hydrogen at room temperature in the presence of Pd/C for 18 h. The solid was removed by filtration, and solvent was removed in vacuo. The residue was recrystallized from a two-solvent system, in which the first solvent is a 1:5 mixture of ethanol and ethyl acetate and the second solvent is cyclohexane, to give white plates in a quantitative yield: mp 144–146 °C (lit.[27] mp 145.6–146.1 °C); ^{1}H NMR δ 7.25–7.40 (m, 5 H), 3.00 (t, J = 7.5 Hz, 2 H), 2.72 (t, J = 7.5 Hz, 2 H), 2.67 (s, 3 H), 2.02 (m, 2 H).

N-Methyl-4-phenylbutylamine Hydrochloride (11). 4-Phenylbutylamine (1.58 mL, 10 mmol) was dissolved in 50 mL of THF followed by the addition of potassium carbonate (2.7 g, 20 mmol) to the solution, then the mixture was cooled down to 0 °C under an argon atmosphere. To the resulting mixture was added methyl chloroformate (0.77 mL, 10 mmol) via syringe, and stirring was maintained for 2 h. The resulting mixture was diluted with benzene (50 mL) and filtered through a bed of Celite. The filtrate was concentrated in vacuo and the residue was dissolved in anhydrous THF. The THF solution was treated with $LiAlH_4$ (1.0 g, 25 mmol) portionwise at room temperature under argon. The reaction was allowed to proceed overnight, then the excess $LiAlH_4$ was destroyed by addition of water, and the solid was filtered through a bed of Celite which was washed with ethyl acetate. The filtrate was concentrated in vacuo, and the residue was dissolved in ethyl acetate (50 mL). The ethyl acetate solution was washed with 10% aqueous HCl solution (2 × 10 mL), and the aqueous solution was basified with 20% KOH and then extracted with ethyl acetate (2 × 25 mL). The organic layer was dried over $MgSO_4$, the solvent was removed, and the residue was dissolved in methanol. Gaseous HCl was bubbled through the solution to saturation. Removal of methanol, and recrystallization from a two-solvent system, in which the first solvent is a mixture of methanol and ethyl acetate (1:5) and the second solvent is cyclohexane, gave a crystalline solid (1.2 g, 60%), mp 125–126 °C (lit.[28] mp 125.5–127 °C).

N-Methylpentylamine Hydrochloride (12). Methylamine hydrochloride (17 g, 250 mmol) and NaOH (9.6 g, 240 mmol) were stirred in 100 mL of water cooled in an external ice bath. Valeryl chloride (6 mL, 50 mmol) was added dropwise, then the reaction mixture was stirred for 2 h and extracted with ether, and the organic layer was dried over $MgSO_4$. After the solvent was removed, dry THF was added to the residue, then $LiAlH_4$ was added in small portions until no more hydrogen was liberated. The mixture was heated to reflux for 24 h, then the reaction was quenched with saturated Na_2SO_4, and the solution was filtered through a pad of Celite. Hydrogen chloride was bubbled through the solution and the white precipitate that formed was recrystallized from ethanol–ether to give 4 g (47%) as white plates: mp 183–185 °C (lit.[29] mp 181–182 °C); NMR (DMSO) δ 0.88 (t, 3 H, 6 Hz), 1.1–1.4 (m, 4 H), 1.4–1.8 (m, 2 H), 2.45 (s, 3 H), 2.8 (t, 2 H, 7.5 Hz), 8.9–9.3 (br, 1 H). Anal. ($C_6H_{16}ClN$) C, H, N.

Benzyl Cyclohexylpiperidinomethyl Sulfide (20, R = piperidine). Cyclohexanecarboxaldehyde (2.3 g, 20 mmol) was cooled to 0 °C under an argon atmosphere. Piperidine (2.0 mL, 20 mmol) was added to the aldehyde via syringe followed by benzyl mercaptan (2.34 mL, 20 mmol). A solid was formed as soon as the three components were mixed. The solid was recrystallized from ethanol to give white crystals (5.5 g, 85%): mp 71–73 °C; ^{1}H NMR ($CDCl_3$) δ 7.30 (m, 5 H), 3.80 (s, 2 H), 3.43 (d, J = 10 Hz, 1 H), 2.60 (m, 2 H), 2.27 (m, 3 H), 1.90 (d, J = 12 Hz, 1 H), 1.40–1.80 (m, 10 H), 1.20 (m, 3 H), 0.85 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 139.69, 129.07, 128.94, 128.75, 83.03, 49.87, 41.93, 38.28, 31.73, 31.41, 26.87, 26.77, 26.36, 26.29, 26.00, 24.65. Anal. ($C_{16}H_{29}NS$) C, H, N.

Attempted Preparation of Benzyl Cyclohexyl(cyclohexylamino)methyl Sulfide (20, R = cyclohexylamine). The same procedure for the preparation of benzyl cyclohexylpiperidinomethyl sulfide was employed, except cyclohexylamine was substituted for piperidine. TLC and NMR analysis of the crude reaction mixture indicated a mixture of the three unreacted starting materials.

Enzyme and Assays. Bovine liver MAO-B was isolated according to the method of Salach.[30] MAO activity was assayed by a modified published procedure[31] in Tris buffer (100 mM, pH 9.0) at 25 °C with cinnamylamine (0.5 mM) as substrate. For the determination of the substrate kinetic constants (K_m and k_{cat}) the rate of formation of hydrogen peroxide was monitored as previously described.[9d] The change in the flavin spectrum after inactivation also was determined as previously reported.[9d]

General Procedure for Time-Dependent Inactivation Experiments. Solutions (180 μL each) of an inactivator hydrochloride at various concentrations and a control containing no inactivator in potassium phosphate buffer (100 mM, pH 7.40) in the presence of 2.0 mM β-mercaptoethanol (or glutathione) were preincubated at 25 °C. To these solutions was added MAO-B (20 μL of a 6 mg/mL solution). After being mixed, the samples were incubated at 25 °C, periodically agitated, and assayed for MAO activity by removal of 10 μL of the mixture and adding it to 490 μL of a 0.5 mM cinnamylamine solution in Tris buffer (100 mM, pH 9.0). The enzyme activity thus determined was corrected against a control containing no inactivator. Kinetic constants (K_I and k_{inact}) were determined as described by Kitz and Wilson.[32]

General Procedure for Reactivation Studies. MAO-B (50 μg) was incubated with an inactivator at an appropriate concentration in potassium phosphate buffer (100 mM, pH 7.4) in the presence of 2.0 mM glutathione at 25 °C for 18 h to give greater than 90% enzyme inactivation. The incubation mixture and a control (1000 μL each) were transferred to dialysis bags (Spectra/POR, 12 000-14 000, MW cutoff). These were dialyzed against 4 L of potassium phosphate buffer (100 mM, pH 7.40) in the presence of 0.1% Triton X-100 at room temperature. The MAO-B activity was assayed by periodical removal of 20 μL of the mixture and adding it to 480 μL of cinnamylamine solution (0.5 mM) in Tris buffer (100 mM, pH 9.0). The enzyme activity thus determined was corrected with a control that was not inactivated.

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References

(1) Tetrud, J. W.; Langston, J. W. The effect of deprenyl (selegiline) on the natural history of Parkinson's disease. *Science (Washington, D.C.)* **1989**, *245*, 519–522.

(2) Cesura, A. M.; Pletscher, A. The new generation of monoamine oxidase inhibitors. *Prog. Drug Res.* **1992**, *38*, 171–297.
(3) Hiebert, C. K.; Silverman, R. B. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) analogues. Inactivation of monoamine oxidase by conformationally-rigid analogues of N,N-dimethylcinnamylamine. *J. Med. Chem.* **1988**, *31*, 1566–1570.
(4) Williams, C. H.; Lawson, J.; Colette Backwell, F. R. Inhibition and inactivation of monoamine oxidase by 3-amino-1-phenyl-prop-1-enes. *Biochim. Biophys. Acta* **1992**, *1119*, 111–117.
(5) Dostert, P. L.; Strolin Benedetti, Tipton, K. F. Interactions of monoamine oxidase with substrates and inhibitors. *Med. Res. Rev.* **1989**, *9*, 45–89.
(6) Silverman, R. B. Electron transfer chemistry of monoamine oxidase. In *Advances in Electron Transfer Chemistry*, Vol. 2; Mariano, P. S., Ed.; JAI Press: Greenwich, CT, 1992; pp 177–213.
(7) Silverman, R. B.; Hoffman, S. J.; Catus, W. B., III A mechanism for mitochondrial monoamine oxidase-catalyzed amine oxidation. *J. Am. Chem. Soc.*, **1980**, *102*, 7126–7128.
(8) (a) Simpson, J. T.; Krantz, A.; Lewis, F. D.; Kokel, B. Photochemical and photophysical studies of amines with excited flavins. Relevance to the mechanism of action of the flavin-dependent monoamine oxidase. *J. Am. Chem. Soc.* **1982**, *104*, 7155–7161. (b) Krantz, A.; Kokel, B.; Sachdeva, Y. P.; Salach, J.; Detmer, K.; Claesson, A.; Sahlberg, C. Inactivation of mitochondrial monoamine oxidase by β,γ,β-allenic amines. In *Monoamine Oxidase: Structure, Function, and Altered Functions*; Singer, T. P.; Von Korff, R. W.; Murphy, D. L., Eds.; Academic Press: New York, 1979, p 51.
(9) (a) Silverman, R. B.; Hoffman, S. J. N-(1-Methyl)cyclopropylbenzylamine: A novel inactivator of mitochondrial monoamine oxidase. *Biochem. Biophys. Res. Commun.* **1981**, *101*, 1396–1401. (b) Silverman, R. B.; Yamasaki, R. B. Mechanism-based inactivation of mitochondrial monoamine oxidase by N-(1-methylcyclopropyl)-benzylamine. *Biochemistry* **1984**, *23*, 1322–1332. (c) Silverman, R. B.; Zieske, P. A. Mechanism of inactivation of monoamine oxidase by 1-phenylcyclopropylamine. *Biochemistry* **1985**, *24*, 2128–2138. (d) Silverman, R. B.; Zieske, P. A. 1-Benzylcyclopropylamine and (1-phenylcyclopropyl)methylamine, an inactivator and a substrate of monoamine oxidase. *J. Med. Chem.* **1985**, *28*, 1953–1957. (e) Silverman, R. B.; Zieske, P. A. 1-Phenylcyclobutylamine, the first in a new class of monoamine oxidase inactivators. Further evidence for a radical intermediate. *Biochemistry* **1986**, *25*, 341–346. (f) Silverman, R. B.; Banik, G. M. (Aminoalkyl)trimethylsilanes. A new class of monoamine oxidase inactivators. *J. Am. Chem. Soc.* **1987**, *109*, 2219–2220. (g) Silverman, R. B.; Vadnere, M. K. Aminoalkyltrimethylgermanes: the first organogermanium mechanism-based enzyme inactivators. A new class of monoamine oxidase inactivators. *Bioorg. Chem.* **1987**, *15*, 328–332. (h) Hiebert, C. K.; Silverman, R. B. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) analogues. Inactivation of monoamine oxidase by conformationally-rigid analogues of N,N-dimethylcinnamylamine. *J. Med. Chem.* **1988**, *31*, 1566–1570. (i) Banik, G. M.; Silverman, R. B. Mechanism of inactivation of monoamine oxidase B by (aminomethyl)trimethylsilane. *J. Am. Chem. Soc.* **1990**, *112*, 4499–4507.
(10) Gates, K. S.; Silverman, R. B. 5-(Aminomethyl)-3-aryl-2-oxazolidinones. A novel class of mechanism-based inactivators of monoamine oxidase B. *J. Am. Chem. Soc.* **1990**, *112*, 9364–9372.
(11) Ding, Z.; Silverman, R. B. 5-(Aminomethyl)-3-aryldihydrofuran-2(3*H*)-ones, a new class of monoamine oxidase-B inactivators. *J. Med. Chem.* **1992**, *35*, 885–889.
(12) Ding, C. Z.; Silverman, R. B. 4-(Aminomethyl)-1-aryl-2-pyrrolidinones, a new class of monoamine oxidase B inactivators. *J. Enz. Inhib.* **1992**, *6*, 223–231.
(13) Deslongchamps, P. *Stereoelectronic Effects in Organic Chemistry*, Pergamon Press: Oxford, England, 1983.
(14) Anderson, J. E.; Watson, D. G. Eclipsed conformations of 1,1,2-trisubstituted single bonds with space-demanding substituents. Examples from X-ray diffraction structure determinations *J. Am. Chem. Soc.* **1992**, *114*, 1517–1518.
(15) Chem 3D™ version 3.0 (Cambridge Scientific Computing Inc.) was used to calculate the rotational energy barrier.
(16) Braillon, B.; Lasne, M. C.; Ripoll, J. L.; Denis, J. M. Methanimine. *Nouv. J. Chim.* **1982**, *6*, 121–122.
(17) Reynolds, D. D.; Cossar, B. C. 1,3,5-Trisubstituted hexahydrotriazines as Mannich reagents. I. Preparation of secondary aminomethyl sulfide hydrochlorides. *J. Heterocycl. Chem.* **1971**, *8*, 597–604.
(18) Erlenmeyer, H.; Oberlin, 4-Thiazolidone. *Helv. Chim. Acta* **1947**, *30*, 1329–1355.
(19) (a) Renshaw, R. R.; Searle, D. E. Onium compounds. XVII. Thioesters of formocholine and their sulfones. *J. Am. Chem. Soc.* **1937**, *59*, 2056–2058; (b) Sakai, H.; Ito, K.; Sekiya, M. Reaction of N-(dialkylaminomethyl)amides and N-(α-dialkylaminobenzyl)-amides with sulfides and cyanide. *Chem. Pharm. Bull.* **1973**, *21*, 2257–2264; (c) Katritzky, A. R.; Szajda, M.; Bayyuk, S. The preparation of some N- and S-acetals of benzaldehyde and terephthalaldehyde. *Synthesis* **1986**, 804–807.
(20) McLeod, C. M.; Robinson, G. M. Pseudo-bases. III. Dialkylaminomethyl alkyl ethers and sulfides. *J. Chem. Soc.* **1921**, *119*, 1470–1476.
(21) Clarke, H. T.; Gillespie, H. B.; Weisshaus, S. Z. Action of formaldehyde on amines and amino acids. *J. Am. Chem. Soc.* **1933**, *55*, 4571–4587.
(22) Heilbron, I. *Dictionary of Organic Compounds*; Oxford University Press: Oxford, 1965; Vol. 2.
(23) Buck, J. S.; Baltzly, R.; Ide, W. S. β-Phenylethylamine derivatives. Tertiary and quaternary salts. *J. Am. Chem. Soc.* **1938**, *60*, 1789–1792.
(24) Brine, G. A.; Boldt, K. G.; Huang, P. T.; Sawyer, D. K.; Carroll, F. I. Carbon-13 nuclear magnetic resonance spectra of fentanyl analogs. *J. Heterocycl. Chem.* **1989**, *26*, 677–686.
(25) Pierson, M. E.; Lyon, R. A.; Titeler, M.; Kowalski, P.; Glennon, R. A. Design and synthesis of propranolol analogs as serotonergic agents. *J. Med. Chem.* **1989**, *32*, 859–863.
(26) Meyers, A. I.; Jagdmann, G. E., Jr. Enamidines. Versatile vehicles for homologation of carbonyl compounds. *J. Am. Chem. Soc.* **1982**, *104*, 877–879.
(27) Cope, A. C.; McElvain, S. M. *N*-Methyl-*N*-phenylalkylaminoalkyl benzoates and *p*-aminobenzoates. *J. Am. Chem. Soc.* **1931**, *53*, 1587–1594.
(28) Neale, R. S.; Walsh, M. R.; Marcus, N. L. The influence of solvent and chloramine structure on the free-radical rearrangement products of *N*-chlorodialkylamines. *J. Org. Chem.* **1965**, *30*, 3683–3688.
(29) Heilbron, I. *Dictionary of Organic Compounds*; Oxford University Press: Oxford, 1965; Vol. 4.
(30) Salach, J. I. Monoamine oxidase from beef liver mitochondria: simplified isolation procedure, properties, and determination of its cysteinyl flavin content. *Arch. Biochem. Biophys.* **1979**, *192*, 128–137.
(31) Tabor, C. W.; Tabor, H.; Rosenthal, S. M. Purification of amine oxidase from beef plasma. *J. Biol. Chem.* **1952**, *208*, 645–661.
(32) Kitz, R.; Wilson, I. B. Esters of methanesulfonic acid as irreversible inhibitors of acetylcholinesterase. *J. Biol. Chem.* **1962**, *237*, 3245–3249.