Mechanism of Inactivation of Monoamine Oxidase B by (Aminomethyl)trimethylsilane

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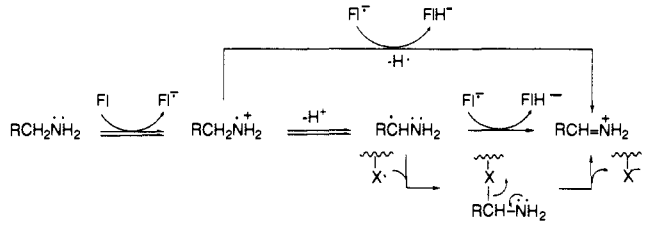
Abstract: The mechanism of inactivation of mitochondrial monoamine oxidase (MAO) by (aminomethyl)trimethylsilane (**1**) has been investigated. The carbon analogue of **1**, namely, neopentylamine, is a substrate for MAO, suggesting that the silicon atom is responsible for the inactivation properties of **1**, which exhibits all of the characteristics of a mechanism-based inactivator. The inactivated enzyme slowly releases the adduct and becomes reactivated in a time- and pH-dependent reaction. Inactivation by [1-^{3}H]-**1** results in the incorporation of 1.24 equiv of ^{3}H into the enzyme and the release of [^{3}H]formaldehyde. Inactivation of MAO by [1-$^2H_2$]-**1** exhibits a deuterium isotope effect of 2.3 on inactivation and releases both mono- and dideuterated formaldehyde. Inactivation by **1** in ^2H_2O gives mono- and undeuterated formaldehyde. [^{14}C-*methyl*]-**1** inactivates the enzyme with the incorporation of 3.29 equiv of radioactivity. The rate of release of ^{14}C from [^{14}C-*methyl*]-**1**-inactivated enzyme is approximately the same as the rate of release of ^{3}H from [1-^{3}H]-**1**-inactivated enzyme and the rate of return of enzyme activity. The flavin becomes reduced during inactivation but is reoxidized upon denaturation. After 22 h of dialysis 0.44 equiv of tritium remains bound to the oxidized enzyme. These results indicate that **1** undergoes oxidation by two different pathways, but only one leads to inactivation, namely, oxidation to the corresponding trimethylsilyl iminium ion (presumably by a one-electron mechanism) followed by attack of an active-site amino acid nucleophile at the imine carbon. A one-electron oxidation mechanism followed by desilylation is supported as the alternate pathway that does not lead to inactivation. The conclusions regarding the reaction of **1** with MAO are discussed in terms of the normal catalytic mechanism of the enzyme.

Monoamine oxidase (MAO; EC 1.4.3.4) is an important flavoenzyme in the catabolism of various biogenic and xenobiotic amines.[1] In 1968 it was shown that MAO exists in two isoenzymic forms, MAO A and MAO B;[2] these forms have been shown to be two distinct enzymes on the basis of the sequences of the cDNA clones from human[3,4] and rat[5] liver that encode the two enzymes. Apparently, the functional difference in these isoenzymes is the efficiency by which they oxidize the various amine neurotransmitters.

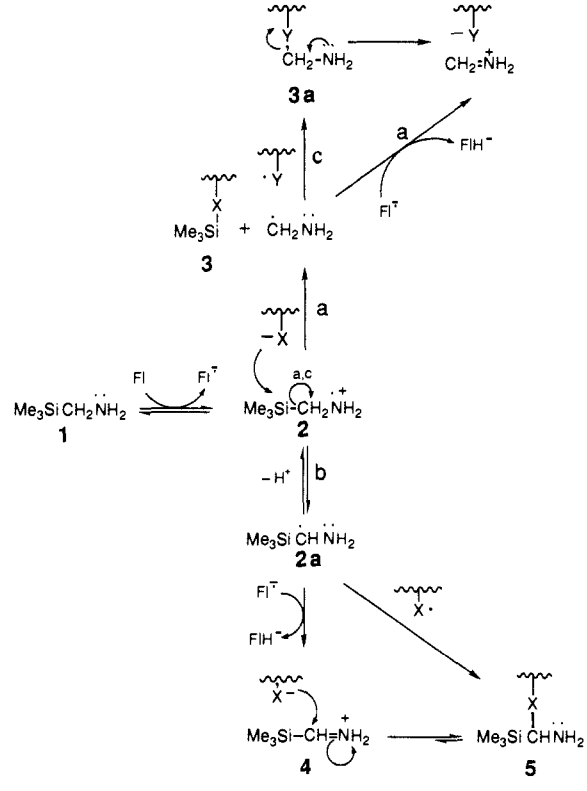
Compounds that inactivate MAO A exhibit an antidepressant effect, and those that inactivate MAO B are used as adjuncts to the treatment of Parkinson's disease.[6] We have been interested for several years in the elucidation of the mechanism of inactivation of MAO by various inactivators and in the design of new classes of MAO inactivators. In particular, the use of mechanism-based enzyme inactivators[7] has been most appealing because of the mechanistic information about the target enzyme that it affords. Our studies with cyclopropylamines[8-18] and cyclobutylamines[19,20] have supported inactivation mechanisms that involve radical intermediates. 1-Phenyl- and 1-benzoylcyclobutylamine were shown by ESR spectroscopy to produce radicals during their MAO-catalyzed oxidation.[20] On the basis of these studies, the mechanism for MAO-catalyzed amine oxidation in general is concluded to be that shown in Scheme I. One-electron transfer from the amine to the flavin gives the amine radical cation, which can become further oxidized by proton transfer followed either by a second electron transfer or by radical combination with an active site radical and subsequent β-elimination. A radical mechanism also was invoked to explain the inactivation of MAO by 5-(aminomethyl)-3-aryl-2-oxazolidinones.[21]

Several years ago Mariano and co-workers[22-25] showed that the electrophilicity of a silicon atom could be greatly increased when a strongly electron-deficient atom, particularly a radical cation, was generated β to the silicon. Since our previous work indicated that the first step in MAO-catalyzed amine oxidation was amine radical cation formation, this suggested that silicon-containing amine analogues may represent a new class of potential MAO inactivators. Consequently, a series of silicon-containing amines, the (aminoalkyl)trimethylsilanes, were synthesized and shown to be time-dependent inactivators of MAO.[26] Two general mechanisms were proposed for the inactivation of MAO by (aminomethyl)trimethylsilane (**1**; Scheme II, pathways a and b). Both

Scheme I. Proposed Mechanism for MAO-Catalyzed Amine Oxidation



Scheme II. Potential Pathways for Inactivation of MAO by **1**



mechanisms proceed in the first step by one-electron transfer from the amino group of **1** to the flavin coenzyme to give the amine

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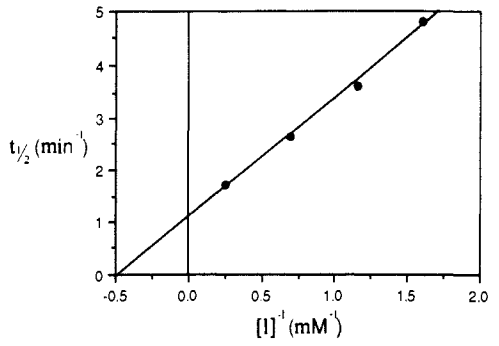

Figure 1. Kitz and Wilson[28] plot for inactivation of MAO by **1**. See Experimental Section for procedure.

radical cation **2**. The mechanism that is modeled after the work of Mariano and co-workers[22-25] (pathway a) involves active-site nucleophilic attack on the electron-deficient silicon leading to trimethylsilylation of the enzyme (**3**). The resulting aminomethyl radical would be further oxidized to the immonium ion of formaldehyde. In the second mechanism, intermediate **2** may act as a normal substrate for the enzyme and undergo proton removal (**2a**) and a second electron transfer (pathway b) to give intermediate **4**, which, as an analogue of an acylsilane, would be highly reactive[27] and could be attacked by an active-site nucleophile, leading to alkylation of the enzyme (**5**). By analogy to the mechanism in Scheme I, **2a** also could undergo combination with an active-site radical to give **5** directly. A third mechanism that could be considered (pathway c) involves combination of the aminomethyl radical formed in pathway a with some active-site radical, resulting in an aminomethylated enzyme (**3a**).

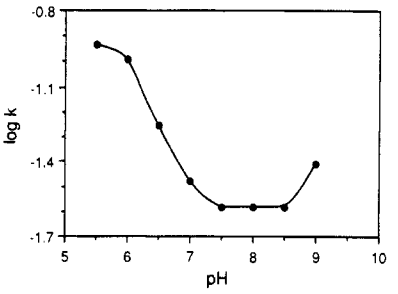

Figure 2. Plot of log k for reactivation of **1**-inactivated MAO at various pH values. See Experimental Section for procedure.

In this paper we report a detailed study of the mechanism of inactivation of MAO by (aminomethyl)trimethylsilane and conclude that although the mechanisms in pathways a and b of Scheme II appear to be relevant to the oxidation of **1**, pathway b is responsible for the inactivation of the enzyme.

Results

Substrate Activity of Neopentylamine. Neopentylamine was found to be a substrate, albeit a poor one, for MAO with a K_m of 15 mM and a k_{cat} of 1.9 min^{-1}. It also was a competitive inhibitor of the oxidation of benzylamine (K_i = 46 mM), but no inactivation of MAO was observed, even after incubation with 80 mM neopentylamine for 2 h.

Inactivation of MAO by (Aminomethyl)trimethylsilane (1). (Aminomethyl)trimethylsilane was a time-dependent inactivator of MAO with a K_I of 2.1 mM and a k_{inact} of 0.64 min^{-1}, as obtained by a Kitz and Wilson[28] plot (Figure 1). Inactivation of MAO by **1** was protected by a 10-fold excess of benzylamine, indicating that inactivation is an active-site-directed process. The addition of the electrophile trapping agent β-mercaptoethanol did not alter the rate of inactivation of MAO by **1**, suggesting that inactivation is not the result of the attachment of an electrophile that had escaped the active site prior to its interaction with it.

Time- and pH-Dependent Return of Enzyme Activity Following MAO Inactivation by 1. Subsequent to inactivation of MAO by **1**, it was found that enzyme activity slowly returned in a pseudo-first-order time- and pH-dependent process. The half-life for reactivation of MAO at pH 7.0 and 25 °C was 21 h. A plot of the log k of reactivation at various pH values (Figure 2) indicated that the rate of reactivation was fastest at low pH, leveled between pH 7.5 and 8.5, and then began to rise again at pH 9 (the highest pH measured).

Formation of [^{3}H]Formaldehyde during Inactivation of MAO by [1-^{3}H](Aminomethyl)trimethylsilane. The microdialysate of MAO that had been inactivated by [1-^{3}H](aminomethyl)trimethylsilane was treated with 2,4-dinitrophenylhydrazine, and then the organic products were analyzed by both normal- and reversed-phase HPLC. Only one radioactive product, which corresponded to the 2,4-dinitrophenylhydrazone of formaldehyde (T_R = 4.8 min, Figure 3A), was detected. The peak at 6.9 min is 2,4-dinitrophenylhydrazine. A nonenzymatic control was devoid of radioactive products (Figure 3B).

Partition Ratio for Inactivation of MAO by 1. A plot of the percent of MAO activity remaining versus the ratio of [**1**]/[MAO] used to inactivate the enzyme (Figure 4) showed that the partition ratios at pH 7.0 and 9.0 were 87 and 18, respectively.

Comparison of Rates of Inactivation of MAO by 1 and by [1-^{2}H$_2$]-1. Inactivation of MAO by **1** and [1-^{2}H$_2$]-**1** resulted in pseudo-first-order loss of enzyme activity. The k_{inact} values, obtained from Kitz and Wilson[28] replots of the kinetic data (Figure 5), were determined to be 1.10 min^{-1} and 0.48 min^{-1} for the nondeuterated and dideuterated compounds, respectively, with no effect on the K_I value (4.7 mM). These data, which were obtained in simultaneous experiments, indicate that there is a

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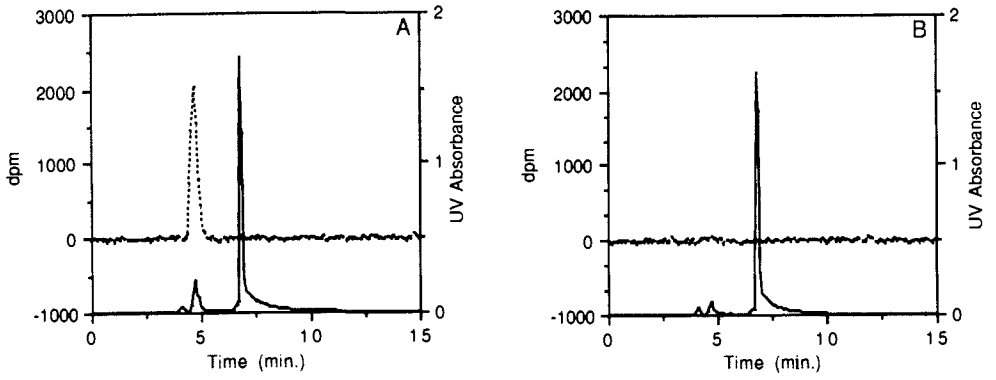

Figure 3. Normal-phase HPLC chromatogram of the 2,4-dinitrophenylhydrazone of [^{3}H]formaldehyde formed during inactivation of MAO by [1-^{3}H]-**1**. (A) In the presence of MAO. The dotted line is the radioactivity and the solid line is the UV absorbance. The peak at 4.8 min is the 2,4-dinitrophenylhydrazone of formaldehyde and the peak at 6.9 min is 2,4-dinitrophenylhydrazine. (B) In the absence of MAO. See Experimental Section for procedure.

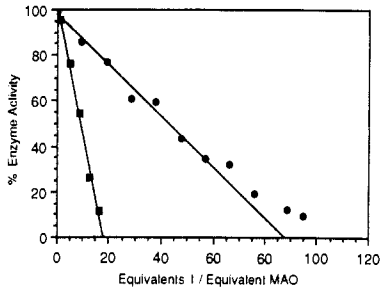

Figure 4. Partition ratio for the inactivation of MAO by **1** at pH 7.0 (●) and pH 9.0 (■). See Experimental Section for procedure.

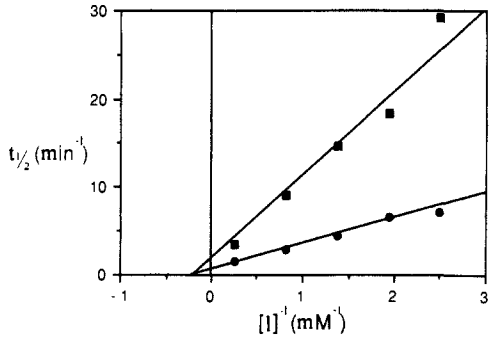

Figure 5. Kitz and Wilson[28] plots for the inactivation of MAO by **1** (●) and by [1-$^2H_2$]-**1** (■). See Experimental Section for procedure.

deuterium isotope effect on inactivation of 2.3. The K_I and k_{inact} values described in this experiment are different from those given above because the experiments were done 3 years apart with different batches of enzyme.

Formation of Deuterated Formaldehydes during Inactivation of MAO by [1-$^2H_2$]-1. The 2,4-dinitrophenylhydrazone of formaldehyde was isolated from the treatment of MAO inactivated by [1-$^2H_2$]-**1** with 2,4-dinitrophenylhydrazine. Mass spectral analysis of this product (Figure 6) indicated that it was a mixture of dideuterated (MW 212), monodeuterated (MW 211), and undeuterated (MW 210) hydrazones formed in the ratio of 16.8:58.7:24.5. The nonenzymatic control produced the nondeuterated (but not mono- or dideuterated) 2,4-dinitrophenylhydrazone of formaldehyde; formaldehyde was a contaminant, presumably from one of the reagents or solvents used in the experiment. This control and the finding that no tritiated formaldehyde was produced in the nonenzymatic control with [1-^{3}H]-**1** (vide supra) are evidence that the mono- and dideuterated products are enzyme-derived.

Formation of Deuterated Formaldehyde during Inactivation of MAO by 1 in ^2H_2O. The experiment described above was repeated with undeuterated **1**, but in deuterated water. Mass spectral analysis (Figure 7) of the 2,4-dinitrophenylhydrazone of formaldehyde isolated showed that monodeuterated and undeuterated hydrazones were formed. Because of the contamination of formaldehyde in the solvents, an exact ratio of monodeuterated to undeuterated hydrazones was not obtained.

Time-Dependent Release of Radioactivity from [1-^{3}H]- and [^{14}C-*methyl*]-1-Inactivated MAO. Inactivation of MAO by [1-^{3}H]- and [^{14}C-*methyl*]-**1** led to the attachment of 1.24 and 3.29 equiv of radioactivity, respectively, per molecule of MAO (on the basis of a molecular weight of 117 600[3]). Incubation of labeled MAO in pH 7.0 buffer at 25 °C resulted in a pseudo-first-order time-dependent release of radioactivity from the enzyme with half-lives

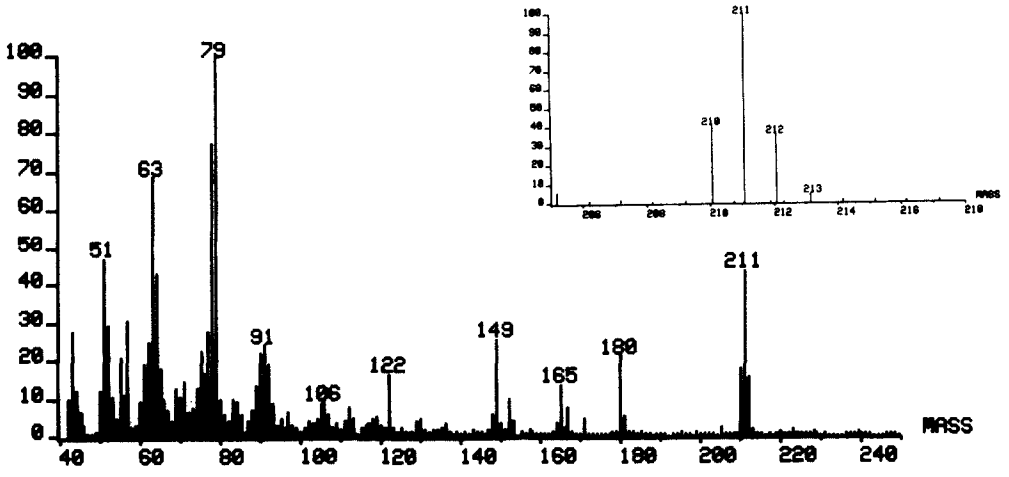

Figure 6. Mass spectrum of the mixture of formaldehyde 2,4-dinitrophenylhydrazones isolated from the workup after inactivation of MAO by [1-$^2H_2$]-**1** in H_2O. The insert is a blowup of the 205–218 mass region. See Experimental Section for procedure.

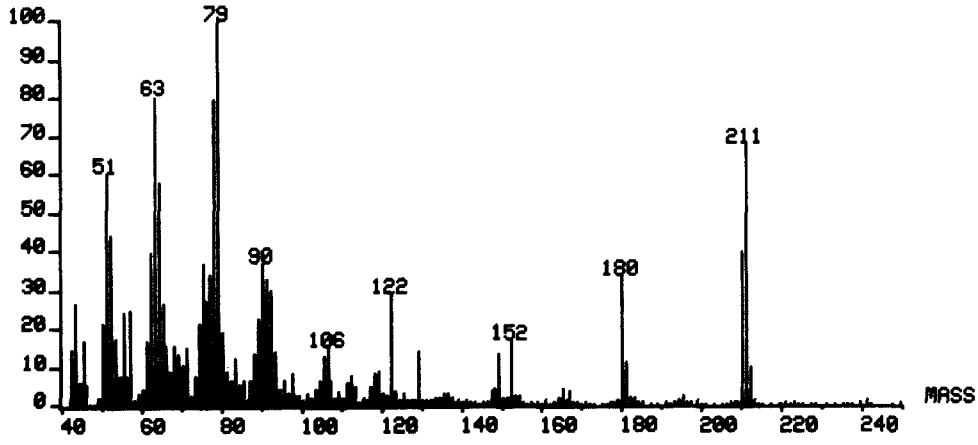

Figure 7. Mass spectrum of the mixture of formaldehyde 2,4-dinitrophenylhydrazones isolated from the workup after inactivation of MAO by **1** in ^2H_2O. See Experimental Section for procedure.

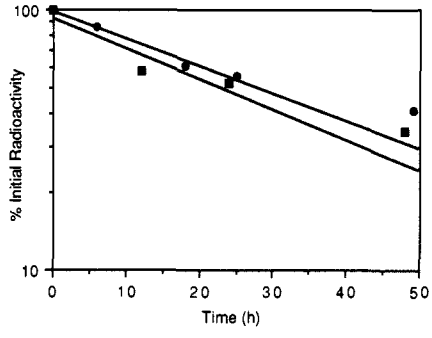

Figure 8. Time-dependent release of radioactivity from MAO inactivated by [1-^{3}H]-**1** (●) and [^{14}C-*methyl*]-**1** (■). The computer-fitted lines ignore the last time points in each case. See Experimental Section for procedure.

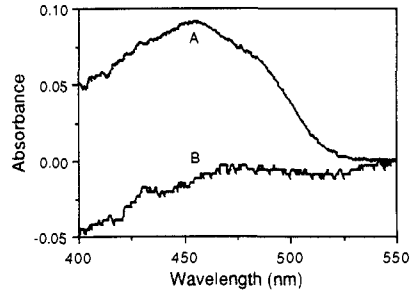

Figure 9. Flavin difference spectra (A) after inactivation of MAO by [1-^{3}H]-**1** and (B) upon denaturation of [1-^{3}H]-**1**-inactivated MAO. See Experimental Section for procedure. The spectra were corrected for dilution.

of 29 and 26 h for the ^{3}H- and ^{14}C-labeled enzymes, respectively (Figure 8). Upon urea denaturation and exhaustive dialysis of the labeled enzyme over 36 h at 4 °C, 0.38 equiv of ^{3}H and 1.52 equiv of ^{14}C remained attached to the protein.

Change in the Flavin Spectrum during Inactivation of MAO by [1-^{3}H]-1. Inactivation of MAO with [1-^{3}H]-**1** resulted in complete reduction of the flavin; upon denaturation of the inactive enzyme, the flavin rapidly became reoxidized (Figure 9). After dialysis in urea for 22 h at 4 °C, 0.44 equiv of tritium was still attached to the protein (in a second experiment, under slightly different conditions, 0.57 equiv of tritium remained bound). This suggests that an active-site amino acid, not the flavin, is labeled during inactivation.

Discussion

Our original design of (aminoalkyl)trimethylsilanes as a potential new class of MAO inactivators[26] was based on the photochemical studies of silanes by Mariano and co-workers.[22-25] In their studies it was shown that the silicon atom becomes highly electrophilic upon generation of a radical cation β to it. Since our work on the mechanism of MAO[8-20] indicates that it catalyzes the oxidation of amines by initial amine radical cation formation, it was thought that this first enzymatic step might activate silanes for attachment to MAO (Scheme II, pathway a). Although the hydrolytic stability of a trimethylsilylated heteroatom is poor, it was thought that inside the active site of an enzyme it may be protected against hydrolysis. The stability of the product formed from pathway b (**5**) was unknown; no example of this class of structures could be found in the literature. The product of pathway c (**3a**) should be quite unstable; its structure would be similar to one of the possible normal intermediates in MAO-catalyzed amine oxidation (see Scheme I) and, therefore, should break down rapidly to the iminium ion of formaldehyde as shown.

The other motive for designing **1** was that all of our previous work on mechanism-based inactivators as probes for the mechanism of MAO[8-20] involved the use of strained ring analogues (cyclopropylamines and cyclobutylamines). It could be argued that these compounds are not structurally similar enough to those of the normal substrates to give reactions that proceed by the same mechanism. Compound **1** is an acyclic compound that has the same general structure as neopentylamine (except for one atom), which, as shown here, is a substrate for MAO. If **1** undergoes desilylation, it would be strong support for a radical mechanism, since it would be difficult to rationalize this result by a two-electron mechanism.

Compound **1** exhibited all of the expected[7] criteria for a mechanism-based inactivator of MAO: time-dependent inactivation, saturation kinetics, protection by substrate, no return of enzyme activity upon dialysis at 4 °C, a 1:1 stoichiometry of labeling with [1-^{3}H]-**1**, conversion of the flavin to its reduced form upon inactivation (indicating that two electrons were transferred during inactivation), and no effect of an exogenous nucleophile (β-mercaptoethanol) on the rate of enzyme inactivation (suggesting that a reactive electrophile released from the enzyme is not responsible for the inactivation). Since **1** is an inactivator of MAO but neopentylamine is a substrate, the silicon atom in **1** must be responsible for its inactivation properties.

Three general pathways for inactivation of MAO by **1**, desilylation (pathways a and c) and deprotonation (pathway b), are shown in Scheme II. The adducts formed by any of these mechanisms should be relatively unstable, and indeed, after inactivation of MAO by **1**, a slow, time-dependent return of enzyme activity was observed, which also was pH dependent (Figure 2). Shirai et al.[29] studied the effect of pH on the hydrolysis of trimethylsilyl ethers and found a pH–rate profile similar to that of the pH–reactivation rate profile shown in Figure 2; i.e., the rate was fastest at low and high pH and slowest between about pH 7 and 9. The major difference in the nonenzymatic and enzymatic rate profiles, however, was the magnitude of the rate difference. In the range pH 5.5–9 (the range of our enzyme work), there was

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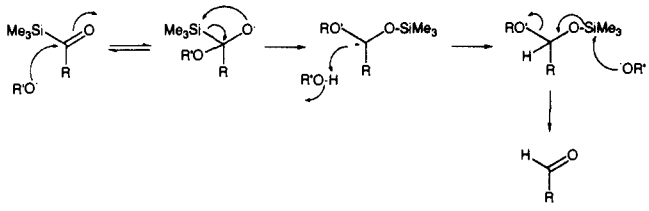
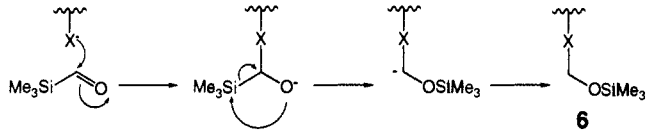
Scheme III. Brook Rearrangement[30]



Scheme IV. Potential Brook Rearrangement at the Active Site of MAO Leading to Inactivation by **1**



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a factor of 2–3 orders of magnitude between the fastest and slowest nonenzymatic hydrolysis rates, but only a factor of about 5 for the enzyme reactivation rate. Although there is a similarity to the shape of the enzymatic (Figure 2) and nonenzymatic[29] rate plots, a similar reactivation rate profile also would be predicted for the unknown compounds having the general structure depicted by **5** (Scheme II). Consequently, several experiments were carried out to differentiate these possibilities.

Because of the ability of fluoride ion to react with trimethylsilyl heteroatom compounds, adduct **3**, but not **5**, should be desilylated rapidly by fluoride ion. Incubation of MAO inactivated by **1** with fluoride ion had no effect on the rate of reactivation, suggesting either that the adduct is not a trimethylsilylated enzyme or that the fluoride ion was hydrated or otherwise unable to react with the adduct.

According to Scheme II, pathway a (or c), treatment of MAO with [1-^{3}H]-**1** should lead to inactivation with no incorporation of tritium into the enzyme and concomitant release of tritium into solution as [^{3}H]formaldehyde. Pathway b should give MAO containing tritium and no tritiated formaldehyde. What was observed was *both* predictions, namely, 1.24 equiv of tritium bound to the inactivated and dialyzed enzyme and the formation of [^{3}H]formaldehyde, detected as its 2,4-dinitrophenylhydrazone (Figure 3). This could be rationalized in two ways: either all pathways are operative or only pathway b is important, but formaldehyde is somehow formed by that pathway. Both rationalizations are reasonable because acylsilanes (the product of hydrolysis of **4**) are known to undergo hydrolysis to the aldehyde via the Brook rearrangement[30] (Scheme III; in this case, R = R′ = H). If **4** is released into solution, it can undergo this rearrangement. Alternatively, the Brook rearrangement could be initiated by active-site nucleophilic attack on the acylsilane, in which case the adduct that would be responsible for inactivation is **6** (Scheme IV). Upon hydrolysis, **6** would decompose to formaldehyde. In order for this rearrangement to occur at the active site, **4** must be hydrolyzed to the corresponding acylsilane prior to active-site nucleophilic addition; it is not clear that this is a favorable process inside the active site. Also, since **6** is a trimethylsilyl ether, fluoride ion should have reactivated the inactive enzyme, but it did not (vide supra); however, as indicated earlier, fluoride ion may not have gained access to this adduct. The partition ratio (the number of turnovers to product per inactivation event) for **1** was found to be 87 at pH 7.0 and 18 at pH 9.0 (Figure 4); therefore, multiple turnovers occur, and if **4** is formed, it is more likely to be released into solution than to be attacked by an active-site nucleophile. Pathway c would generate formaldehyde by β-elimination of **3a** as shown in Scheme II. Note that the formation of formaldehyde via pathways a and c is analogous to the formation of product from substrate radical via the two mechanisms: electron transfer or radical combination followed by β-elimination (see Scheme I).

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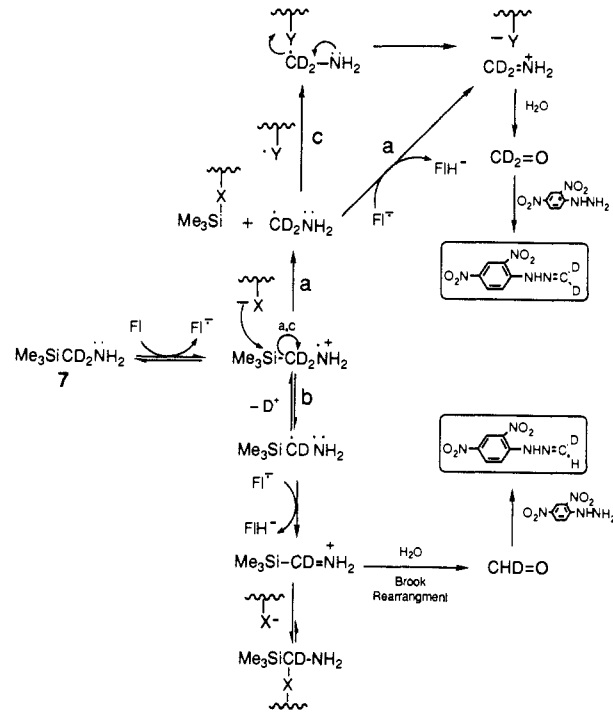
Scheme V. Pathways for Formation of Mono- and Dideuterated Formaldehydes during the Inactivation of MAO by **1**



Since an acylsilane is a likely product of the reaction of **1** with MAO and acylsilanes are known to undergo photochemically induced conversions to carbenes,[31] it was necessary to show that the inactivation of MAO by **1** was not the result of a secondary photochemical reaction. Complete inactivation of MAO by **1** was found to occur in the dark as well as in the light, indicating that inactivation is not a photochemical process.

In order to differentiate pathways a–c (Scheme II), [1-$^2H_2$]-**1** (**7**) was synthesized and used to inactivate MAO; Scheme V shows the potential outcomes of inactivation of MAO by **7**. Inactivation by pathway a or c would produce dideuterated formaldehyde (trapped as the 2,4-dinitrophenylhydrazone), whereas pathway b, following hydrolysis and Brook rearrangement, would give monodeuterated formaldehyde. Furthermore, since other substrates for MAO B such as tyramine and kynuramine,[32] benzylamine,[33] and dopamine[34] exhibit kinetic isotope effects on α-C–H bond cleavage, pathway b, but not pathway a or c, may exhibit a deuterium isotope effect on the rate of inactivation (a C–D bond is broken during inactivation by pathway b, but not by pathway a or c). Inactivation of MAO by **1** and [1-$^2H_2$]-**1** exhibited a deuterium isotope effect (k^H_{inact}/k^D_{inact}) of 2.3 with no effect on the K_I (Figure 5). This isotope effect is almost identical with the deuterium isotope effects reported for [1-$^2H_2$]tyramine[32] and for [1-$^2H_2$]dopamine[34] and indicates that pathway b is responsible for inactivation of MAO by **1**. Mass spectral analysis of the formaldehyde produced during the inactivation (isolated as the 2,4-dinitrophenylhydrazone) revealed that both monodeuterated (pathway b) formaldehyde and dideuterated (pathway a or c) formaldehyde were formed in the ratio of 3.5 to 1 (Figure 6). Therefore, both pathway a (or c) and pathway b are operative, but pathway b is responsible for inactivation and pathway a (or c) results in turnover to product without inactivation. The converse experiment, namely, the inactivation of MAO by **1** in ^2H_2O, produced a mixture of monodeuterated (pathway b; the Brook rearrangement leads to incorporation of a deuteron from the ^2H_2O)

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and undeuterated (pathway a or c) formaldehyde (Figure 7), confirming that the formaldehyde comes from pathway a (or c) and pathway b. This also indicates that the [^{3}H]formaldehyde observed during inactivation of MAO by [1-^{3}H]-**1** (vide supra) also came from pathway a (or c) and pathway b.

The observation of the product from pathway a (or c) is strong support for a radical mechanism. Recently, Mariano and co-workers[35] carried out a photochemical study with [(diethylamino)methyl]trimethylsilane that was analogous to the enzymatic one described here with (aminomethyl)trimethylsilane (**1**). The results of their study indicate that, upon generation of the amine radical cation, two processes occur, desilylation (equivalent to our pathway a or c) and deprotonation (equivalent to our pathway b). The ratio of these two pathways is solvent dependent (low-polarity nonhydroxylic solvents favor deprotonation and polar hydroxylic solvents favor desilylation) and base dependent (increased base strength favors deprotonation). Although the products of pathway b could be rationalized by any mechanism that generates intermediate **4** (Scheme II), the products of pathway a (or c) will not form without the generation of a highly electron-deficient amino group. Our earlier precedents for the generation of amine radical cations with cyclopropylamines[8-18] and cyclobutylamines[19,20] in conjunction with the model studies of Mariano and co-workers[22-25] strongly suggest that an amine radical cation intermediate is involved in the case of **1**. Since **1** is an acyclic molecule that is structurally related to a substrate (neopentylamine) for MAO, our results with **1** seem to validate the radical mechanism for MAO-catalyzed amine oxidation as the rule, not the exception. Also, since pathway b is favored over pathway a (or c), the Mariano model study[35] would suggest that the active site of MAO is more hydrophobic than it is hydrophilic. This is not surprising considering the nature of the substrates for MAO.

The chemistry of trimethylsilyl groups is, in many ways, that of a "super proton".[36] Therefore, removal of the trimethylsilyl group (pathway a, Scheme II) and removal of a proton (pathway b) from intermediate **2** are closely related processes and are not really divergent pathways. Alternatives to pathways a and b would be removal of the trimethylsilyl group or the hydrogen, respectively, as radicals to give directly the immonium ion of formaldehyde or **4**, respectively. However, on the basis of the model study work and discussions of Mariano and co-workers,[35] removal of the trimethylsilyl group or a hydrogen as a radical is not relevant to the oxidation of **1** (or to amine oxidation in general).

Inactivation of MAO by pathway b (Scheme II) should result in the incorporation of 1 equiv of tritium from [1-^{3}H]-**1** and 1 equiv of ^{14}C from [^{14}C-*methyl*]-**1** (label in the trimethylsilyl group); reactivation of inactivated labeled MAO should release both labels at the same rate with a concomitant return of enzyme activity. Inactivation of MAO with these inactivators led to the incorporation of 1.24 and 3.29 equiv of radioactivity, respectively. It is not known if the additional 2 equiv of ^{14}C radioactivity (presumably, the result of trimethylsilylated nucleophiles) is at the active site. Incubation of the labeled enzymes at pH 7.0 resulted in a pseudo-first-order release of both ^{3}H and ^{14}C with half-lives of 29 and 26 h, respectively (Figure 8), and return of enzyme activity with a half-life of 21 h (three separate experiments). The values for the rates of ^{3}H and ^{14}C release and for enzyme reactivation are similar enough to suggest that all three of these processes occur simultaneously, which, again, is consistent with the formation of adduct **5** (Scheme II), the product formed by pathway b.

Inactivation of MAO by [1-^{3}H]-**1** results in the reduction of the flavin. This is a common observation for inactivators of MAO, whether or not they become attached to the flavin cofactor. Upon denaturation of the inactivated enzyme, however, the flavin spectrum reverts to that of the oxidized form (Figure 9). This suggests that the attachment is to an amino acid residue, not to the flavin, that is, unless the adduct has hydrolyzed off the enzyme during denaturation. After denaturation and exhaustive dialysis of the inactivated enzyme, 0.44 (and 0.57, in a second experiment) equiv of tritium still was attached to the denatured protein. Although some hydrolysis of the adduct had occurred during dialysis for removal of the excess inactivator, it is apparent from Figure 9 that none of the flavin is in the reduced form. Therefore, attachment of the inactivator to an amino acid residue is a reasonable conclusion.

The fact that **1** acts as a substrate for MAO but leads to a covalent adduct supports a mechanism for MAO-catalyzed amine oxidation in general (see Scheme I) that involves one-electron transfer to the amine radical cation followed by proton abstraction and radical combination to give a covalent adduct. When R = trimethylsilyl, the adduct formed may be stabilized and β-elimination to the iminium ion product may be slowed down. It is possible that hydrogen atom transfer from the active-site cysteine residue to the flavin radical anion,[14] giving a cysteinyl radical, is involved in the normal catalytic process. This would result in the formation of a more labile adduct than if the flavin radical underwent radical combination with the substrate radical (Scheme I, X = S versus X = Fl), thereby catalyzing the substrate reaction further.

Recently, it has been reported by Danzin et al.[37] that a series of (aminoethyl)benzyldimethylsilanes are potent time-dependent inactivators of rat brain MAO; one of the compounds in the series showed a greater than 10^4 selectivity for MAO B relative to MAO A. This class of inactivators represents a potent new class of potential anti-Parkinsonian agents whose mechanism of MAO inactivation should be the same as that described here for **1**. Also, the mechanism of inactivation of MAO by (aminomethyl)trimethylgermane, another compound that we reported,[38] should be the same as that for **1**, but there may be a difference in the proportion of each of the pathways.

Experimental Section

Analytical Methods. Optical spectra and MAO assays were recorded on either a Perkin-Elmer Lambda 1 or Beckman DU-40 UV/vis spectrophotometer. NMR spectra were recorded on a Varian EM-390 90-MHz spectrometer. Chemical shifts are reported as δ values in parts per million downfield from Me_4Si as the internal standard in $CDCl_3$. Chloroform or dichloromethane was used as the internal standard for trimethylsilyl-containing $CDCl_3$-soluble compounds. An Orion Research Model 601 pH meter with a general combination electrode was used for pH measurements. Mass spectra were obtained on a VG Instruments VG70-250SE high-resolution spectrometer. HPLC was performed by using Beckman 110B pumps in series with a Beckman 163 variable wavelength detector and a Radiomatic Instruments Flo-One/Beta Model CR radioactivity detector utilizing Packard Radiomatic FLO-SCINT II or RPI 3a7OB scintillation cocktails for reversed- (C_{18} silica gel) and normal-phase (silica gel) HPLC, respectively. An Alltech Econosil C_{18} 10-μm 10 × 250 mm column was used for semipreparative HPLC, and either an Econosil C_{18} 10-μm 4.6 × 250 mm or an Econosil silica 10-μm 10 × 250 mm column was used for analytical HPLC. Liquid scintillation counting was done in a Beckman LS-3133T scintillation counter with 10 mL of RPI 3a7OB scintillation cocktail. [^{14}C]Toluene (4×10^5 dpm/mL) and [^{3}H]toluene (2.22×10^6 dpm/mL, corrected for first-order decay) from New England Nuclear were used as internal standards. All dialyses of radioactive compounds were carried out until radioactivity in the dialysate was the same as background. Amine hydrochlorides were visualized on TLC plates by spraying with a solution of ninhydrin (300 mg) with pyridine (2 mL) in acetone (100 mL) and then heating. Radiopurity of radioactive compounds was assessed by cutting TLC plates (Merck Kieselgel 60 plates without fluorescent indicator) into strips and counting each strip with scintillation cocktail in a scintillation counter. Aldehydes were converted to the corresponding 2,4-dinitrophenylhydrazones by using the standard reagent[39] and were recrystallized from absolute ethanol.

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Reagents. The syntheses of (aminomethyl)trimethylsilane hydrochloride,[40] benzophenone *N*-[(trimethylsilyl)methyl]imine,[41] and triphenyl(methoxymethyl)phosphonium chloride[42] were reported. Benzophenone (99+%), *n*-butyllithium (2.0 M in pentane), chloromethyl methyl ether, chloroform-*d*, citric acid, 18-crown-6, deuterium chloride (37 wt %, 99 atom % D), deuterium oxide (>99.96 atom % D and 99.8 atom % D), anhydrous diethyl ether, hexamethyldisilane, hydrazine monohydrate, leuco crystal violet, lithium bis(trimethylsilyl)amide (1.0 M in tetrahydrofuran), magnesium bromide etherate, ninhydrin, phthalimide, potassium fluoride, sodium deuterioxide (40 wt %), anhydrous tetrahydrofuran, tetramethylsilane, triethylaluminum (1.0 M in hexanes), and triphenylphosphine were purchased from Aldrich. Neopentylamine was purchased from Aldrich and converted to the corresponding hydrochloride salt by acidification with aqueous HCl, rotary evaporation to dryness, and recrystallization from absolute ethanol/anhydrous diethyl ether. Benzene, chloroform, dichloromethane, *N*,*N*-dimethylformamide, 2-propanol, anhydrous magnesium sulfate, magnesium turnings, methanol, potassium, potassium carbonate, potassium chloride, potassium hydroxide, potassium phosphate, anhydrous sodium carbonate, sodium hydroxide, and sodium phosphate were obtained from Mallinckrodt. (Chloromethyl)dimethylchlorosilane and (chloromethyl)trimethylsilane were purchased from Aldrich and Petrarch Systems. Absolute ethanol was purchased from Midwest Grain Co. of Illinois. Ultrapure urea was purchased from Schwarz/Mann Biotech. Catalase, 2,4-dinitrophenylhydrazine, horseradish peroxidase (Type II), sodium hydrosulfite, and tris(hydroxymethyl)aminomethane (Tris) were purchased from Sigma. [^{3}H]Water (5.0 Ci/mL) was obtained from Amersham. [^{14}C]Methyl iodide (1–15 mCi/mmol) was acquired from Pathfinder Laboratories. Diethyl ether and tetrahydrofuran for reactions were distilled from sodium with benzophenone ketyl indicator under nitrogen immediately prior to use. HPLC-grade acetonitrile, ethyl acetate, hexane, and water were obtained from Mallinckrodt and were filtered prior to use. Other organic solvents were dried over 3- or 4-Å molecular sieves. Hexamethyldisilane was distilled immediately prior to use. 18-Crown-6 was dried at 100 °C in vacuo overnight prior to use. All other chemicals were used without further purification. Distilled water was deionized or deionized and redistilled.

[1-^{3}H]Benzophenone *N*-[(Trimethylsilyl)methyl]imine. To anhydrous tetrahydrofuran (40 mL) under argon at −78 °C was added 2.0 M *n*-BuLi in pentane (5.5 mL, 11.0 mmol). Benzophenone *N*-[(trimethylsilyl)methyl]imine (2.67 g, 10.0 mmol) in tetrahydrofuran (5 mL) was added dropwise while being stirred with a magnetic stirrer. The intense blackish red anion was allowed to stir for 1 h at −78 °C; then [^{3}H]water (180 μL, 10 mmol) in tetrahydrofuran (2 mL) was added quickly with stirring. The solution was allowed to stir at room temperature for ca. 15 min and then was quenched with cold water (50 mL). The reaction mixture was extracted with diethyl ether (3 × 100 mL). The combined organic layers were washed with water (2 × 50 mL) and dried over anhydrous magnesium sulfate. Organic solvents were removed by rotary evaporation in vacuo to yield a clear, colorless oil.

[1-^{3}H](Aminomethyl)trimethylsilane Hydrochloride. The oily residue of [1-^{3}H]benzophenone *N*-[(trimethylsilyl)methyl]imine was dissolved in absolute ethanol (50 mL), hydrazine monohydrate (485 μL, 10.0 mmol) was added, and the mixture was allowed to stir at room temperature overnight. Water (50 mL) was added to the reaction mixture, which was extracted with diethyl ether (2 × 100 mL). The combined organic layers were washed with water (2 × 50 mL) and then extracted with 0.1 N HCl (2 × 100 mL). The combined acidic layers were washed with diethyl ether (2 × 50 mL). Water and HCl were removed by rotary evaporation in vacuo, followed by azeotropic removal of water with absolute ethanol. The resulting white solid was dissolved in water (20 mL) and diethyl ether (20 mL), basified with aqueous sodium hydroxide, and separated. The aqueous layer was extracted with diethyl ether (2 × 10 mL). Ether and methylamine side products were distilled from the combined organic layers at 50 °C. When distillation was complete, absolute ethanol (20 mL) was added, followed by concentrated HCl (1 mL). The solvent was removed to yield a white solid that was recrystallized from 2-propanol/anhydrous diethyl ether to yield shiny white plates. Chemical purity and radiopurity were checked by TLC in 12:5:3 1-butanol/water/acetic acid, which yielded one spot when visualized [R_f = 0.47; (aminomethyl)trimethylsilane hydrochloride standard, R_f = 0.47]. Radiopurity was determined to be 98% by TLC. The specific activity was determined to be 3.25 × 10^7 dpm/μmol.

[^{14}C-*methyl*](Aminomethyl)trimethylsilane Hydrochloride. Magnesium turnings (78 mg, 3.3 mmol) in anhydrous diethyl ether (2 mL) under argon were treated with methyl iodide (50 μL, 0.80 mmol). To this was added [^{14}C]methyl iodide (0.25 mCi) in anhydrous diethyl ether (2 mL), followed by an additional aliquot of methyl iodide (35 μL, 0.56 mmol). Finally, an additional aliquot of methyl iodide (9 μL, 0.14 mmol) was added. The mixture was heated for 2 min and then stirred for an additional 1 h. The solution was filtered through glass wool in a glass syringe via cannula; then (chloromethyl)dimethylchlorosilane (198 μL, 1.5 mmol) was added with stirring. After 30 min the reaction mixture turned cloudy and two layers appeared. The reaction was stirred for an additional 58 h. The reaction mixture was separated into halves. Half was transferred via cannula into a flask containing phthalimide (0.110 g, 0.75 mmol), anhydrous potassium carbonate (52 mg, 3.76 mmol), and *N*,*N*-dimethylformamide (2 mL). The solution was allowed to reflux for 24 h, at which point the reflux condenser was replaced with a distillation apparatus to remove the *N*,*N*-dimethylformamide. The residue was dissolved in water and extracted with diethyl ether (2 × 15 mL). The combined organic layers were washed sequentially with 0.2 M sodium hydroxide (5 mL) and brine (7 mL). The solution was then dried over anhydrous magnesium sulfate overnight. The solution was filtered and the solvent was removed to yield a white solid. Phthalimide contaminant was removed by column chromatography on silica gel (28 g) eluting with 1:3 ethyl acetate/hexane. After solvent removal, the white solid (0.0755 g, 0.324 mmol) was deprotected by stirring with hydrazine monohydrate (18 μL, 0.51 mmol) in absolute ethanol (1.5 mL) at reflux for 3 h. Concentrated hydrochloric acid (60 μL) was added, and the reaction was allowed to stir at reflux overnight. Hydrochloric acid (10%, 4 mL) was added, and the mixture was transferred to a centrifuge tube and centrifuged. The supernatant was filtered through glass wool, the precipitate was boiled with 6 N HCl (5 mL), cooled, and centrifuged, and the supernatant was filtered through glass wool. The precipitate was treated with an additional aliquot of 6 N HCl, and the combined solutions were evaporated to dryness. The white solid was redissolved in water and loaded onto an ion-exchange column (Dowex 50 H$^+$ form, 0.75 mL). The column was washed with ethanol (20 mL) and water (20 mL). The amine was eluted from the column with 3 N hydrochloric acid (62.5 mL). Solvent was removed to yield a white solid that was recrystallized from absolute ethanol/anhydrous diethyl ether to yield shiny white plates (40 mg, 88% yield). Chemical purity and radiopurity were checked by TLC in 12:5:3 1-butanol/water/acetic acid, which yielded one spot when visualized with ninhydrin. Radiopurity was determined to be 99.4% by TLC. Specific activity was determined to be 2.2 × 10^5 dpm/μmol.

[$^2H_2$]Triphenyl(methoxymethyl)phosphonium Chloride. A previously reported method[43] was modified to increase isotopic purity. Thus triphenyl(methoxymethyl)phosphonium chloride (17.12 g, 50.0 mmol) was dissolved in D_2O (25.00 g, >99.96 atom % D), and the solution was stirred under argon for 30 min and then filtered through a sintered glass funnel. Anhydrous sodium carbonate (2.65 g, 25.0 mmol) was added, and the solution was stirred under argon for 2 days. Following filtration and acidification with 37 wt % deuterium chloride, the solution was rotary-evaporated in vacuo to dryness. The residue was triturated with dichloromethane and recrystallized with hot ethyl acetate to give white crystals (9.19 g, 53% yield). The process was repeated with [$^2H_2$]triphenyl(methoxymethyl)phosphonium chloride (9.19 g, 26.7 mmol), D_2O (13.34 g, >99.96 atom % D), and anhydrous sodium carbonate (1.41 g, 13.3 mmol) to yield white crystals (7.65 g, 83%) after recrystallization: NMR ($CDCl_3$) δ 3.75 (s, 3 H), 7.80 (m, 15 H) [lit.[44] NMR (undeuterated) ($CDCl_3$) δ 3.67 (s, 3 H), 5.83 (d, 2 H), 7.80 (m, 15 H)]. Mass spectral analysis showed a peak at *m*/*z* 309 (M − Cl).

[$^2H_2$]Chloromethyl Methyl Ether. [$^2H_2$]Triphenyl(methoxymethyl)-phosphonium chloride (3.67 g, 10.7 mmol) was heated under argon in a microdistillation apparatus in a 220–230 °C oil bath. A clear, colorless liquid was distilled and collected (0.41 g, 47% yield): NMR ($CDCl_3$) δ 3.50 (s) [lit.[45] NMR (undeuterated) δ 3.53 (s), 5.47 (s)].

[$^2H_2$]Formaldehyde 2,4-Dinitrophenylhydrazone. To [$^2H_2$]chloromethyl methyl ether (1.45 mmol) was added water (1 mL) followed by 2,4-dinitrophenylhydrazine reagent (10 mL). The precipitate was filtered and recrystallized from hot absolute ethanol to yield fine yellow needles, mp 167.5–168 °C [lit.[46] mp (undeuterated) 167 °C].

[$^2H_2$]-*N*,*N*-Bis(trimethylsilyl)methoxymethylamine. The procedure for the preparation of *N*,*N*-bis(trimethylsilyl)methoxymethylamine was

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followed, substituting [$^2H_2$]chloromethyl methyl ether for chloromethyl methyl ether.[47] Thus [$^2H_2$]chloromethyl methyl ether (0.41 g, 5.0 mmol) was added to tetrahydrofuran (20 mL) at 0 °C. Lithium bis(trimethylsilyl)amide (1.0 M, 5 mL, 5.0 mmol) was added, and the mixture was stirred at 0 °C for 1 h and then at room temperature overnight. The mixture was quenched with water and extracted with diethyl ether, and the combined organic extracts were dried over anhydrous $MgSO_4$. The solution was filtered, and the solvent was removed by rotary evaporation in vacuo to yield a clear, colorless oil (0.55 g, 56% yield): NMR ($CDCl_3$) δ 0.12 (s, 18 H), 3.18 (s, 3 H) [lit.[47] NMR (undeuterated) ($CDCl_3$) δ 0.14 (s, 18 H), 3.17 (s, 3 H), 4.28 (s, 2 H)].

[1-$^2H_2$](Aminomethyl)trimethylsilane Hydrochloride. Potassium metal (0.163 g, 4.16 mmol) was treated with absolute methanol (3 mL) under argon. After reaction was complete, the solvent was removed under vacuum, and the potassium methoxide residue was dried in vacuo with a heat gun for 3 h. Tetrahydrofuran (5.5 mL) and 18-crown-6 (1.100 g, 4.16 mmol) were added. The addition of hexamethyldisilane (0.609 g, 4.16 mmol) in tetrahydrofuran (2 mL) to the potassium methoxide solution resulted in a lemon yellow solution indicative of the formation of (trimethylsilyl)potassium. To this stirred solution was added [$^2H_2$]-*N*,*N*-bis(trimethylsilyl)methoxymethylamine (0.55 g, 2.78 mmol) in tetrahydrofuran (4 mL) followed by magnesium bromide etherate (1.074 g, 4.16 mmol). After being stirred for 1 h, 30% NaOH was added and the mixture was extracted with diethyl ether. The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, and then filtered. Solvent was removed by rotary evaporation in vacuo to yield a clear, colorless oil. The residue was deprotected by refluxing overnight in methanol (30 mL) with a pinch of silica gel as catalyst. Concentrated HCl (2 mL) was added, and all solvents were removed by rotary evaporation in vacuo. The solid residue was basified with aqueous potassium hydroxide and steam-distilled into 6 M HCl. The resulting distillate was rotary-evaporated to dryness. Methylamine hydrochloride contaminant was removed by basifying the hydrochloride salt, extracting with diethyl ether, and removing the methylamine and diethyl ether by distillation through a 3-in. column of glass helices (oil bath temperature 70–80 °C). The residue was acidified, rotary-evaporated to dryness, and recrystallized from 2-propanol/anhydrous diethyl ether to give shiny white plates (0.085 g, 22% yield): NMR (D_2O) δ 0.05 (s). TLC yielded one spot after visualization [12:5:3 1-butanol/water/acetic acid, R_f = 0.59; (aminomethyl)trimethylsilane hydrochloride standard, R_f = 0.59].

Enzymes and Assays. Bovine liver MAO B was isolated according to the published method.[48] MAO activity was assayed by the method of Tabor et al.[49] Protein assays were done with either Pierce BCA protein assay reagent or Pierce Coomassie protein assay reagent, using bovine serum albumin for standard curves. All buffers and enzyme solutions were prepared with doubly distilled deionized water.

Effect of Neopentylamine on MAO Activity. An aliquot of neopentylamine solution (80 mM, 95 μL) in sodium phosphate buffer (100 mM, pH 7.0) was preincubated at 25 °C. Sodium phosphate buffer (100 mM, pH 7.0, 95 μL) was preincubated also for a control. MAO (219 μM, 5 μL) was added to both aliquots, and MAO activity was assayed in duplicate initially, after 1 h, and after 2 h.

Substrate Activity of Neopentylamine. All glassware was thoroughly washed with 50% nitric acid and then thoroughly rinsed with doubly distilled and deionized water. The general method of Mottola et al.[50] for the assay of H_2O_2 was used. Solutions of neopentylamine hydrochloride were prepared (101.01, 31.08, 18.36, 13.03, and 10.10 mM in 0.2 M sodium phosphate buffer, pH 7.00). Solutions of leuco crystal violet (0.050 g diluted to 100 mL with 1:195 concentrated hydrochloric acid/water), horseradish peroxidase (1.0 mg/mL in water), and sodium acetate buffer (2.0 M, pH 4.5) also were prepared. Aliquots of the neopentylamine solutions and aliquots of buffer as a control (2 × 495 μL) were preincubated at 25 °C. A stock solution of MAO was prepared by diluting 275 μM MAO (20 μL) with 0.2 M sodium phosphate buffer, pH 7.00 (80 μL). Aliquots (5 μL) of the MAO stock solution were added to each of the above neopentylamine and control solutions; then the solutions were allowed to incubate for 2 min before addition of 110 μL of leuco crystal violet solution, 56 μL of horseradish peroxidase solution, and 445 μL of sodium acetate buffer, with brief vortexing after each addition. Absorbance at 596 nm was read on an instrument zeroed on a mixture of the control MAO solution with 110 μL of leuco crystal violet solution, 56 μL of horseradish peroxidase solution, and 445 μL of sodium acetate buffer. Absorbance values were converted to molarity by using a reported extinction coefficient[50] corrected for pH difference.

Inhibition of MAO Oxidation of Benzylamine by Neopentylamine. Solutions of neopentylamine hydrochloride (161.6 mM) in potassium phosphate buffer (100 mM), potassium chloride (161 mM) in potassium phosphate buffer (100 mM), benzylamine (2.02 mM) in potassium phosphate buffer (100 mM), and potassium phosphate buffer (100 mM) were prepared; the pH was adjusted in each solution to 7.00 with potassium hydroxide. Solutions of neopentylamine (161.6, 121.2, 80.8, 40.4, and 0.0 mM final concentration) and benzylamine (2.02, 1.01, 0.667, 0.505, 0.404, and 0.0 mM final concentration) were prepared by dilution with potassium chloride/potassium phosphate and potassium phosphate buffers, respectively. Mixtures (1:1) of each of the neopentylamine solutions were prepared with each of the benzylamine solutions. Stock solutions of MAO (170 μM, 25 μL) in potassium phosphate buffer (100 mM, pH 7.00, 238 μL) and potassium chloride (161 mM) in potassium phosphate buffer (100 mM, pH 7.00, 238 μL) were prepared. The rate of change in the optical density at 250 nm for the above 1:1 neopentylamine/benzylamine mixtures (495 μL) plus stock MAO solution (5 μL) was monitored. Assays were performed in triplicate.

Time-Dependent Inactivation of MAO by (Aminomethyl)trimethylsilane. Solutions of (aminomethyl)trimethylsilane hydrochloride (4.21, 1.51, 0.92, and 0.66 mM) in potassium phosphate buffer (20 mM, pH 7.00) were preincubated at 25 °C. Aliquots of the solutions of (aminomethyl)trimethylsilane (95 μL) were incubated with MAO (125 μM, 5 μL) and aliquots were assayed periodically. A control with no inactivator was prepared also.

pH-Dependent Reactivation of (Aminomethyl)trimethylsilane-Inactivated MAO. MAO (170 μM, 80 μL) was incubated with (aminomethyl)trimethylsilane hydrochloride (50 mM) in 10 mM Tris buffer, pH 7.00 (120 μL), at 25 °C, and enzyme activity was checked periodically. A control of 10 mM Tris buffer, pH 7.00 (120 μL), and 170 μM MAO (80 μL) was prepared also. The MAO was completely inactivated relative to the control when checked after 4.75 h. The inactivated and control enzyme solutions were separately dialyzed against 10 mM Tris buffer, pH 7.00, at 4 °C for 1 h. The solutions were then removed from the dialysis tubing, and aliquots of each (10 μL) were incubated with aliquots (90 μL) of the following buffers preincubated at 25 °C: 100 mM citric acid at pH 5.5, 6.0, 6.5, and 7.0 and 100 mM Tris at pH 7.0, 7.5, 8.0, 8.5, and 9.0. Enzyme activity was assayed at 4-h intervals over a 24-h period. All calculations were normalized for changes in protein concentration after dialysis.

Effect of β-Mercaptoethanol on Rate of Inactivation of MAO by 1. Solutions of (aminomethyl)trimethylsilane (2.64 mM), β-mercaptoethanol (0.525 mM), and (aminomethyl)trimethylsilane (2.64 mM) with β-mercaptoethanol (0.525 mM) were prepared in potassium phosphate buffer (20 mM, pH 7.0). Aliquots (95 μL) of these solutions were preincubated at 25 °C. An aliquot of potassium phosphate buffer (20 mM, pH 7.0, 95 μL) was preincubated for a control. MAO (207 μM, 5 μL) was added to each, and the solutions were assayed periodically.

Effect of Benzylamine on Rate of Inactivation of MAO by 1. Solutions of (aminomethyl)trimethylsilane (5.27 mM), benzylamine (52.7 mM), and (aminomethyl)trimethylsilane (5.27 mM) with benzylamine (52.7 mM) were prepared in potassium phosphate buffer (20 mM, pH 7.0). Aliquots (95 μL) of these solutions were preincubated at 25 °C. An aliquot of potassium phosphate buffer (20 mM, pH 7.0, 95 μL) was preincubated for a control. MAO (207 μM, 5 μL) was added to each, and the solutions were assayed periodically.

Effect of Fluoride Ion on (Aminomethyl)trimethylsilane-Inactivated MAO. Two aliquots of (aminomethyl)trimethylsilane (211 μM, 95 μL) in Tris buffer (40 mM, pH 9.0) were preincubated at 25 °C. An aliquot of Tris buffer (40 mM, pH 9.0, 95 μL) was preincubated also as a control. MAO (156 μM, 5 μL) was added to each, and the inactivator solutions were found to be completely inactive relative to the control when assayed after 10 min. An aliquot of potassium fluoride (4.0 M, 5 μL) in Tris buffer (40 mM, pH 9.0) was added to the inactivated enzyme solution. Tris buffer (40 mM, pH 9.0, 5 μL) was added to the second inactivated enzyme solution and to the control. Enzyme activity was assayed periodically over several hours.

Formation of Tritiated Formaldehyde during Inactivation of MAO by [1-^{3}H](Aminomethyl)trimethylsilane. MAO (292 μM, 50 μL) was incubated with 25 mM [1-^{3}H](aminomethyl)trimethylsilane hydrochloride in 150 mM Tris buffer, pH 9.00 (500 μL), and with 35 mM [1-^{3}H]-(aminomethyl)trimethylsilane hydrochloride in 150 mM sodium phosphate buffer, pH 7.00 (500 μL), at 25 °C. Controls without inactivator were run simultaneously at one-tenth the scale. Enzyme at pH 7.00 and 9.00 was assayed after 30 and 5 min, respectively, and was found to be completely inactive. Aliquots of enzyme inactivated at pH 7.00 and 9.00 (275 μL each) were separately microdialyzed against cold phosphate or Tris buffer (3 mL) in an ice bath for 1 h. Nonenzymatic controls were

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similarly microdialyzed. The inactivated enzymes then were dialyzed exhaustively (6 × 300 mL changed hourly) against the appropriate buffer at 4 °C to determine the number of equivalents of radioactivity bound to MAO (an aliquot was assayed for protein concentration and another aliquot was counted for radioactivity). 2,4-Dinitrophenylhydrazine reagent (200 μL) was added to each microdialysate, and the solutions were allowed to react overnight at room temperature. Microdialysates were extracted with chloroform (2 × 10 mL); then the combined organic extracts were washed with water (2 × 5 mL) and evaporated. Samples were redissolved in the appropriate solvent and analyzed by using analytical normal- (1:1 ethyl acetate/*n*-hexane, 1.0 mL/min; RPI 3a7OB scintillation fluid, 1.0 mL/min) and reversed-phase (60:40 acetonitrile-/water, 0.25 mL/min; FLO-SCINT II scintillation fluid, 0.75 mL/min) HPLC.

Partition Ratio for Inactivation of MAO by (Aminomethyl)trimethylsilane. MAO (170.8 μM, 2.5 μL) was incubated with 47.5 μL of various concentrations of (aminomethyl)trimethylsilane hydrochloride (475.0, 427.5, 380.0, 332.5, 285.0, 237.5, 190.0, 142.5, 95.0, 47.5, and 0.0 μM) in 20 mM potassium phosphate buffer, pH 7.00, at 25 °C, and the enzyme activity was assayed after 1 h. A similar experiment was carried out at pH 9.00, in which MAO (156 μM, 10 μL) was incubated with 190 μL of various concentrations of (aminomethyl)trimethylsilane hydrochloride (315.8, 263.2, 210.5, 157.9, 105.3, 52.6, 10.5, and 0.0 μM) in 40.0 mM Tris buffer, pH 9.00, at 25 °C; the enzyme activity was assayed after 0.5 h.

Inactivation of MAO by 1 in the Dark. MAO (11 μM) in 100 mM sodium phosphate buffer, pH 7.00 (40 μL), at 25 °C was incubated with **1** (1.0 mM) in two separate tubes, one in ambient light and one in the dark. After 1 h, the solution in the light was assayed for enzyme activity in the light and the solution in the dark was assayed in the dark. Both were compared with the enzyme activity of a control incubated in the light without inactivator.

Inactivation of MAO by (Aminomethyl)trimethylsilane and [1-$^2H_2$]-(Aminomethyl)trimethylsilane. Aliquots (195 μL) of 4.10, 1.26, 0.746, 0.529, 0.410, and 0.0 mM (aminomethyl)trimethylsilane hydrochloride and [1-$^2H_2$](aminomethyl)trimethylsilane hydrochloride solutions in 50 mM sodium phosphate buffer, pH 7.00, were preincubated at 25 °C. MAO (292 μM, 5 μL) was added to each solution, and the enzyme activity was assayed periodically.

Formation of Deuterated Formaldehyde during Inactivation of MAO by [1-$^2H_2$](Aminomethyl)trimethylsilane. [1-$^2H_2$](Aminomethyl)trimethylsilane (10.53 mM) in 100 mM sodium phosphate buffer, pH 7.00, containing 200 μg/mL catalase (2850 μL) was incubated at room temperature with 292 μM MAO (150 μL). A control without inactivator [100 mM sodium phosphate buffer, pH 7.00, containing 200 μg/mL catalase (475 μL) plus 292 μM MAO (25 μL)] and a control without MAO [10.53 mM [1-$^2H_2$](aminomethyl)trimethylsilane in 50 mM sodium phosphate buffer, pH 7.00, containing 200 μg/mL catalase (4300 μL) with 50 mM sodium phosphate buffer, pH 7.00, (226 μL)] also were prepared. After 0.5 h, the enzyme was completely inactivated. 2,4-Dinitrophenylhydrazine reagent was added to the solutions of inactivated enzyme and to the control without MAO (2.0 and 1.5 mL to the solutions with and without MAO, respectively), and each was allowed to react for 15 min. Each solution was extracted with ethyl acetate (2 × 10 mL for the inactivated enzyme and 2 × 2 mL for the control), and the extracts were washed separately with water and brine (3 mL each for the inactivated enzyme and 1 mL each for the control). Extracts were concentrated and applied to preparative silica TLC plates and eluted with chloroform (100%). The silica gel containing the band corresponding to formaldehyde 2,4-dinitrophenylhydrazone was scraped from the plate, and the silica was triturated with ethyl acetate, filtered, and then washed with additional ethyl acetate. The solvent was removed by rotary evaporation in vacuo, and then samples were dissolved in acetonitrile and filtered. Samples were purified further by semipreparative reversed-phase HPLC (60:40 acetonitrile/water for 10 min, then 100% acetonitrile with a linear gradient for 2 min at 4 mL/min). The solvent was removed from the collected fractions, and then the residues were redissolved in acetonitrile and submitted for mass spectral analysis.

Formation of Deuterated Formaldehyde during Inactivation of MAO by 1 in ^2H_2O. Protons were exchanged with deuterons in (aminomethyl)trimethylsilane hydrochloride and monobasic sodium phosphate monohydrate by dissolving undeuterated samples in D_2O and rotary-evaporating in vacuo to dryness five times. Protons were exchanged with deuterons in catalase by dissolving catalase in D_2O, lyophilizing, and redissolving in D_2O. Sodium phosphate buffer (100 mM) was prepared by adjusting a solution of deuterated sodium phosphate in D_2O to pD 7.0 (pH meter reading = 6.6[51]) with sodium deuterioxide. A solution of catalase (200 μg/mL) in deuterated phosphate buffer was prepared by diluting an aliquot of the deuterium-exchanged catalase (10000 μg/mL, 100 μL) with phosphate buffer (100 mM, pD 7.0, 4900 μL). A solution of deuterium-exchanged (aminomethyl)trimethylsilane (10.53 mM) was prepared by diluting deuterium-exchanged (aminomethyl)trimethylsilane (4.51 mg) to 3.0 mL with catalase (200 μg/mL) in phosphate buffer (100 mM, pD 7.0). Protons were exchanged with deuterons in MAO by dialyzing MAO (171 μM, 160 μL) against deuterated phosphate buffer (100 mM, pD 7.0, 50 mL) for 0.5 h at room temperature. The deuterated inactivator solution (10.53 mM, 3000 μL) was incubated with deuterium-exchanged MAO (171 μM, 150 μL) for 45 min. Enzyme was completely inactive relative to a control of catalase (200 μg/mL) in sodium phosphate buffer (100 mM, pD 7.0) with deuterium-exchanged MAO (171 μM, 5 μL). 2,4-DNP reagent (1 mL) was added to the enzyme/inactivator solution, and the solution was allowed to stand overnight. The sample was extracted and purified as with the dideuterated inactivator (vide supra).

Time-Dependent Release of Radioactivity from [1-^{3}H](Aminomethyl)-trimethylsilane-Inactivated MAO. [1-^{3}H](Aminomethyl)trimethylsilane (10.53 mM) in 0.2 M sodium phosphate buffer, pH 7.00 (1900 μL), was incubated with 273 μM MAO (100 μL) at room temperature. After 1.5 h, the inactive enzyme was dialyzed exhaustively (10 changes of 300 mL of 0.2 M sodium phosphate buffer, pH 7.00, over 19 h at 4 °C). An aliquot (350 μL) was removed for a protein assay and scintillation counting. A second aliquot (150 μL) was diluted with an equivalent amount of 8 M urea in 0.2 M sodium phosphate buffer, pH 7.00, and dialyzed (4 changes of 300 mL of 8 M urea in 0.2 M sodium phosphate buffer, pH 7.00, over 36 h at 4 °C). The remainder was dialyzed at room temperature over a 2-day period, during which time aliquots were removed periodically for protein assays and scintillation counting.

Time-Dependent Release of Radioactivity from [^{14}C-*methyl*](Aminomethyl)trimethylsilane-Inactivated MAO. [^{14}C-*methyl*](Aminomethyl)-trimethylsilane hydrochloride (10.54 mM) in 0.2 M sodium phosphate buffer, pH 7.00 (5258 μL), was incubated with 273 μM MAO (277 μL) at room temperature. After 1.5 h, the inactive enzyme was dialyzed exhaustively (10 changes of 300 mL of 0.2 M sodium phosphate buffer, pH 7.00, over 28.5 h at 4 °C). An aliquot (1.2 mL) was removed for a protein assay and scintillation counting. A second aliquot (500 μL) was diluted with an equivalent amount of 8 M urea in 0.2 M sodium phosphate buffer, pH 7.00, and dialyzed (4 changes of 300 mL of 8 M urea in 0.2 M sodium phosphate buffer, pH 7.00, over 36 h at 4 °C). The remainder was dialyzed at room temperature over a 2-day period, during which time aliquots were removed periodically for protein assays and scintillation counting.

Flavin Difference Spectra of [1-^{3}H]-1-Inactivated MAO. An aliquot of [1-^{3}H](aminomethyl)trimethylsilane (10.53 mM) in sodium phosphate buffer (0.1 M, pH 7.00, 475 μL) was preincubated at 25 °C. An identical reaction without inactivator also was run as a control. MAO (219 μM, 25 μL) was added to each, and the solutions were incubated at 25 °C for 1 h, at which point enzyme activity was assayed. The enzyme with inactivator was found to be devoid of activity. A flavin difference spectrum was recorded, and then the enzymes were denatured by the addition of urea (9.0 M) in sodium phosphate buffer (0.1 M, pH 7.00, 1000 μL). Another flavin difference spectrum was recorded, and the enzyme solutions were dialyzed exhaustively (4 changes of 300 mL of 6 M urea in 0.1 M sodium phosphate buffer, pH 7.00, over 22 h at 4 °C). The enzyme solutions were removed from the dialysis tubing, and a third flavin difference spectrum was recorded. Aliquots of the protein were counted in a scintillation counter and assayed for protein concentration.

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Registry No. 1, 18166-02-4; [1-^{3}H]-**1**·HCl, 126543-81-5; [^{14}C-*methyl*]-**1j**·HCl, 126543-82-6; [1-$^2H_2$]-**1**·HCl, 126543-84-8; **7**, 126543-83-7; MAO, 9001-66-5; $^2H_2$, 7782-39-0; neopentylamine, 5813-64-9; [1-^{3}H]benzophenone *N*-[(trimethylsilyl)methyl]imine, 126543-80-4; [$^2H_2$]triphenyl(methoxymethyl)phosphonium chloride, 1779-52-8; [$^2H_2$]chloromethyl methyl ether, 4008-90-6; [$^2H_2$]formaldehyde 2,4-dinitrophenylhydrazone, 74421-01-5.

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