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Inactivation of Monoamine Oxidase B by Benzyl 1-(Aminomethyl)cyclopropane-1-carboxylate

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Abstract—Monoamine oxidase (MAO) is a flavoenzyme that catalyzes the oxidation of various biogenic and xenobiotic amines. Benzyl 1-(aminomethyl)cyclopropane-1-carboxylate (**1**) was designed as a diactivated cyclopropane mechanism-based inactivator of MAO (Silverman, R.B.; Ding, C.Z.; Borrillo, J.L.; Chang, J.T. *J. Am. Chem. Soc.* **1993**, *115*, 2982). [1,1-$^2H_2$]-**1** exhibits a deuterium isotope effect of 4.5 on inactivation, but in D_2O the isotope effect is only 2.3. [1-^{3}H]-**1** and [1-^{14}C]-**1** were synthesized; upon inactivation of MAO, 1.1 and 2.0 equiv of radioactivity, respectively, are incorporated into the enzyme. Tritium, as ^3H_2O, is released during inactivation with [1-^{3}H]-**1**. The flavin absorption spectrum changes from that of oxidized to that of reduced flavin after inactivation; denaturation of the inactivated enzyme shows a reduced flavin spectrum, suggesting the formation of a modified flavin. Tryptic digestion of the enzyme labeled with [1-^{3}H]-**1** or [1-^{14}C]-**1**, followed by HPLC analysis, monitoring at 450 nm (flavin), shows that the radioactivity comigrates with the 450 nm absorptions. The metabolites that are generated during inactivation are benzyl 1-formylcyclopropane-1-carboxylate, benzyl alcohol, and 1-formylcyclopropane-1-carboxylic acid; no ring-cleaved products were detected. The partition ratio, as determined from the ratio of nonamines to enzyme, is 110. These results are rationalized in terms of a single-electron transfer mechanism leading to the imine of benzyl 1-formylcyclopropane-1-carboxylate, which alkylates the flavin coenzyme. © 1997, Elsevier Science Ltd. All rights reserved.

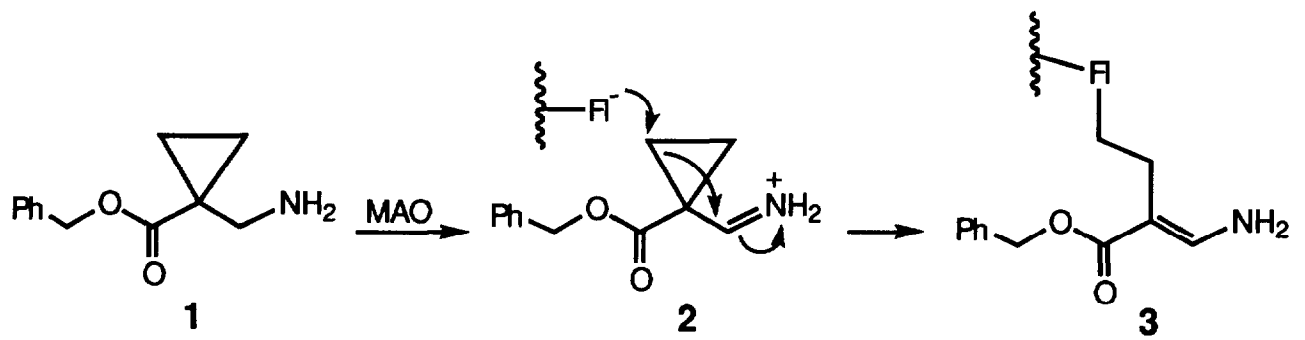
Introduction

Monoamine oxidase (EC 1.4.3.4; MAO) is a flavoenzyme that is important in the degradation of a variety of biogenic amines. Compounds that inhibit MAO exhibit either antidepressant activity, if they inhibit the A isozyme,[1] or antiparkinsonian activity, if they inhibit the B isozyme.[2] A few years ago we reported an approach to the inactivation of enzymes in which the inactivator was activated by enzyme-catalyzed conversion to a diactivated cyclopropane.[3] As an example of that approach benzyl 1-(aminomethyl)cyclopropane-1-carboxylate (**1**, Scheme 1) was shown to undergo MAO B-catalyzed oxidation to a product (**2**) that inactivated the enzyme by apparent active-site flavin modification (**3**).[3] The evidence for flavin modification was a change in the absorption spectrum during inactivation to that of reduced flavin, which remained reduced after denaturation. If the flavin were reduced, but not modified, then denaturation should have resulted in the reoxidation of the flavin. In this paper we provide further support for the proposed inactivation reaction and for attachment of **1** to the flavin.

Results

Benzyl 1-(aminomethyl)cyclopropane-1-carboxylate (1)

The synthesis of **1** was carried out by the route shown in Scheme 2. Ethyl cyanoacetate was converted to 1-cyanocyclopropanecarboxylic acid (**4**) under phase transfer conditions. The carboxylic acid was esterified



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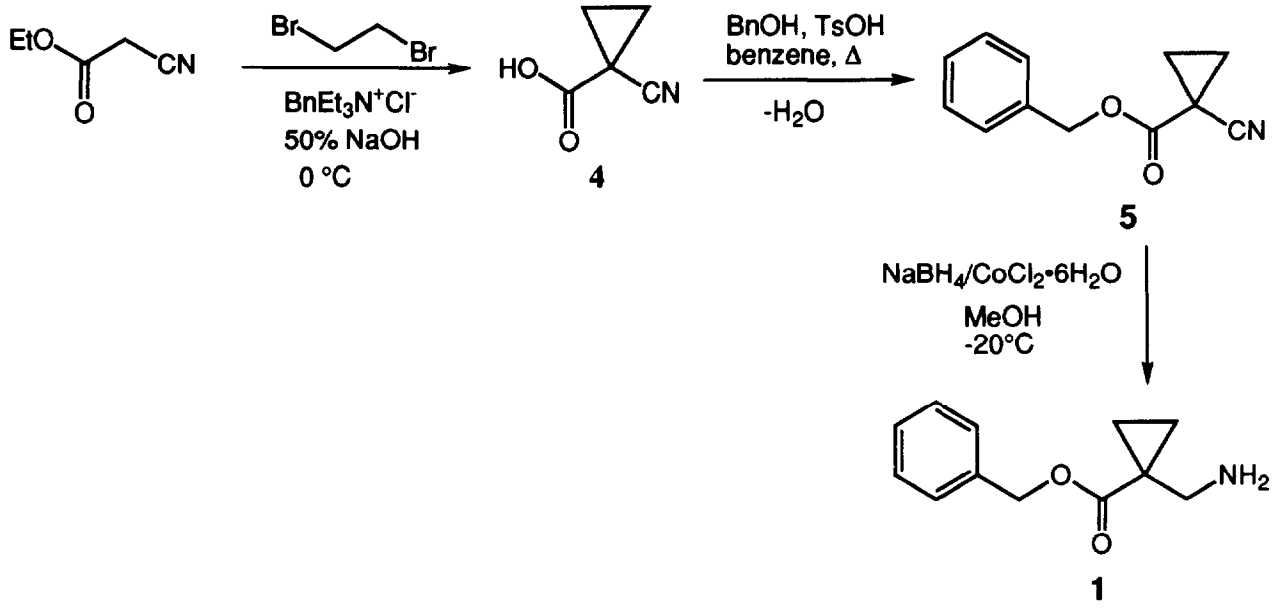
Scheme 1.



Scheme 2.

with benzyl alcohol (**5**), and the nitrile was reduced with sodium borohydride with a Co^{2+} catalyst.[4]

Syntheses of [1,1-$^2H_2$]-1 (6), [1-^{3}H]-1 (7), and [1-^{14}C]-1 (8)

Incorporation of deuterium into the aminomethyl group of **1** was carried out by reduction of **5** with $NaBD_4/CoCl_2 \cdot 6H_2O$ (**6**). When this reaction was carried out in methanol or THF/H_2O, the product had only about 70% incorporation of deuterium. When this reaction was performed in THF/D_2O, essentially quantitative incorporation of deuterium was observed. This suggests that the reaction is not a simple hydride reduction mechanism, and indicates that electron transfer may be important. The corresponding tritiated analogue (**7**) was synthesized by the same reaction, substituting NaB^3H_4 (in H_2O). The ^{14}C analogue (**8**) was prepared by the same route as the unlabeled analogue, except that ethyl [^{14}C]cyanoacetate was first synthesized from ethyl chloroacetate and K^{14}CN.

Deuterium isotope effect on inactivation of MAO by 6

The kinetic constants for inactivation of MAO B by **1** and **6** in H_2O and in D_2O are shown in Table 1. Deuteration has an effect on both K_I and k_{inact}. The k^H_{inact}/k^D_{inact} in H_2O is 4.5, indicating a strong deuterium isotope effect on the inactivation rate constant and suggesting that C-H bond cleavage occurs in a rate-determining step. There also is an effect on the K_I values; the K^H_I/K^D_I in H_2O is 2.8. Therefore, the $(k_{inact}/K_I)^H/(k_{inact}/K_I)^D$ (H_2O) = 1.6. When the inactivation was carried out in D_2O, the isotope effect was not as pronounced: k^H_{inact}/k^D_{inact} in D_2O = 2.3. The K^H_I/K^D_I in D_2O is similar to that in H_2O, namely, 2.2, and the $(k_{inact}/K_I)^H/(k_{inact}/K_I)^D$ (D_2O) = 1.1, which also is similar to that in H_2O.

Attachment of 7 and 8 to MAO during inactivation

Inactivation of MAO B with **7** followed by denaturation resulted in the incorporation of 1.1 equiv of tritium into the enzyme. The same reaction with **8**, however, led to the incorporation of 2.0 equiv of ^{14}C.

Release of tritium as ^3H_2O during inactivation of MAO by 7

From the total radioactivity of the volatiles obtained from bulb-to-bulb distillation divided by the radioactivity attached to MAO B it was calculated that 5 equiv of ^3H_2O were generated during inactivation.

Change in the flavin absorption spectrum during inactivation of MAO

Figure 1 shows the absorption spectrum of MAO B prior to inactivation, after inactivation, and after inactivation and denaturation. The oxidized flavin absorption between 350 and 500 nm diminishes to that of reduced flavin after inactivation. Upon denaturation there is essentially no change from the spectrum after inactivation.

Proteolytic digestion of MAO inactivated by 7 and 8, and HPLC separation of the labeled peptides

MAO inactivated with **8** was tryptic digested and the peptides were separated by reversed phase HPLC. As shown in Figure 2, the radioactively-labeled peptides comigrated with the 450 nm absorptions, indicating that the inactivator is associated with the flavin fragments. The same result was observed when **7** was used for inactivation (data not shown).

Metabolites generated during inactivation of MAO by 1

Three products were observed by TLC, but only two were in sufficient quantity for ^{1}H and ^{13}C NMR analysis. One product was identified as benzyl 1-(formyl)cyclopropane-1-carboxylate (**9**), the expected MAO-catalyzed oxidation product, and the other was benzyl alcohol. Isolation of benzyl alcohol indicates hydrolysis occurred, but it is not known if this occurred during isolation. Presumably, since benzyl alcohol was formed, 1-(formyl)cyclopropanecarboxylic acid (**10**), the hydrolysis product of **9**, was the third unidentified metabolite.

Metabolites generated during inactivation of MAO by 8

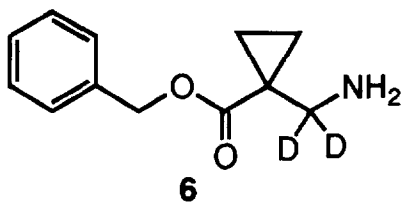
When MAO was inactivated with the ^{14}C-labeled analogue **8**, two radiolabeled metabolites were produced, **9** and **10**. No other metabolites were detected; 1,1-cyclopropanedicarboxylic acid (**11**) and formylcyclopropane (**12**) were synthesized as standards, and neither was detected.

Partition ratio determined from nonamine generation

Following inactivation of MAO by **8**, the radioactive nonamines generated were isolated and quantitated. Relative to the amount of enzyme used, there were 110 times as many nonamines; therefore, the partition ratio is 110.

Discussion

The results described here support an inactivation pathway that proceeds via a doubly-activated cyclopropane which becomes attached to the flavin coenzyme (Scheme 1). The [1,1-$^{2}H_2$] analogue (**6**) shows a deuterium isotope effect on inactivation ($k^{H}_{inact}/k^{D}_{inact}$) of 4.5, indicating that α-C-H bond cleavage is involved in a rate-determining step during inactivation. The observation that the $k^{H}_{inact}/k^{D}_{inact}$ in D_2O, however, is 2.3 suggests that there is another step that is important to the rate. One rationalization for the diminished isotope effect is shown in Scheme 3. Much evidence has been reported to support a single-electron transfer mechanism for MAO,[5] such as that shown in Scheme 3. This mechanism suggests that in D_2O there may be an



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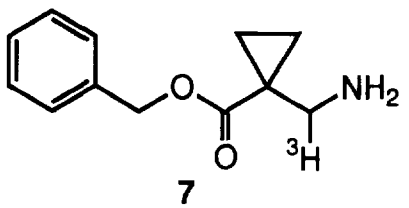

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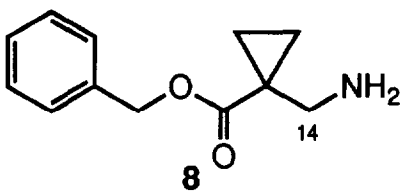

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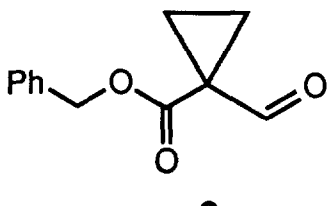

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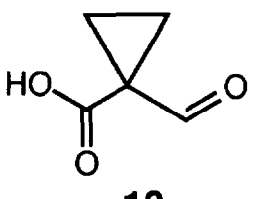

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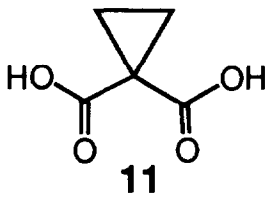

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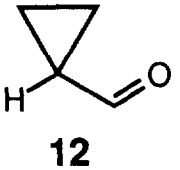

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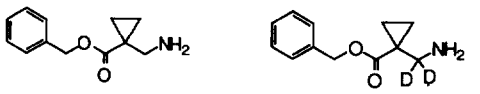
isotope effect on removal of the proton of the active site base which is responsible for removal of the proton from the inactivator. This isotope effect would attenuate the isotope effect of C-H bond cleavage of the inactivator and lead to a diminished isotope effect on inactivation. The difference in the $k^{H}_{inact}/k^{D}_{inact}$ in H_2O vs D_2O derives from the lower k_{inact} of **1** in D_2O. Further support for the formation of a diactivated cyclopropane is the isolation of benzyl 1-(formyl)cyclopropane-1-carboxylate (**9**), the hydrolysis product of the corresponding iminium ion intermediate (**2**) that is proposed to be responsible for inactivation. It was previously reported[3] that the presence of a good nucleophile (glutathione) in the inactivation buffer had no effect on the rate of inactivation, suggesting that inactivation occurs prior to release of **2** into solution. However, inactivation of MAO with the ^{14}C analogue **8** leads to the incorporation of 2 equiv of radioactivity into the enzyme. Therefore, in addition to active-site labeling there also must be peripheral labeling of the protein after release of **2** from the active site. The fact that the tritium-labeled analogue (**7**) is only incorporated to the extent of 1.1 equiv may be the result of release of tritium from the inactivator molecule, which, therefore, would lead to inactivation without radiolabeling. The isolation of ^3H_2O from the inactivation mixture supports the release of tritium from the inactivator.

Table 1. Deuterium isotope effect on inactivation[a]



K_I (H_2O)	0.20 mM	0.071 mM
k_{inact} (H_2O)	0.10 min^{-1}	0.022 min^{-1}
K_I (D_2O)	0.061 mM	0.028 mM
k_{inact} (D_2O)	0.055 min^{-1}	0.023 min^{-1}
	$k^{H}_{inact}/k^{D}_{inact}$ (H_2O) = 4.5	
	$k^{H}_{inact}/k^{D}_{inact}$ (D_2O) = 2.3	

[a]50 mM Tris, pH 9.0 or pD 8.6, 25 °C.

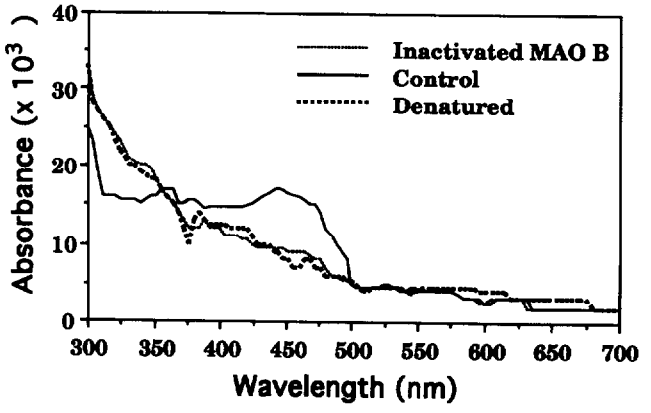


Figure 1. UV-vis spectra before (———) and after (.....) inactivation of MAO by **1** and after denaturation of the inactivated enzyme (----).

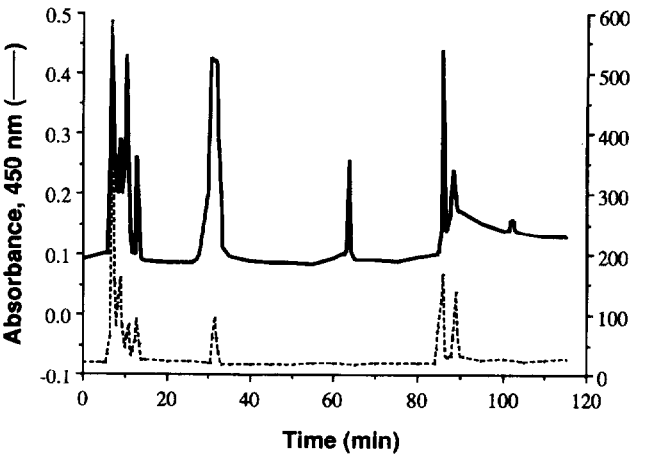
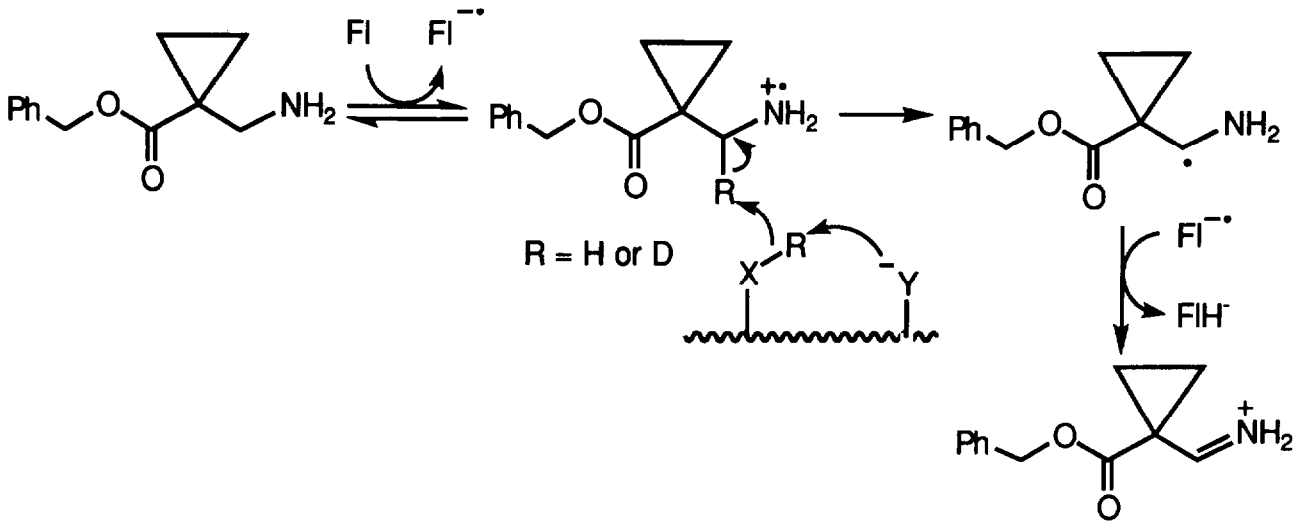


Figure 2. HPLC of the tryptic digest after inactivation of MAO by **8**.



Scheme 3.

Preliminary evidence in support of attachment of the inactivator to the flavin was reported previously;[3] the flavin absorption spectrum indicates a reduction of the flavin during inactivation which remains reduced even after denaturation. This is depicted in Figure 1. The fact that the flavin remains reduced after denaturation is strong evidence for a modification of the flavin. To test this further, MAO was inactivated with **7** or **8** and, following tryptic digestion of the radiolabeled enzyme, the resulting peptides were separated by HPLC with monitoring at 450 nm, a wavelength that would detect reduced flavin (Fig. 2). The fact that the radioactivity comigrated with seven out of eight absorption peaks, supports the proposed labeling of the flavin. The peak that is not radiolabeled may represent a small amount of enzyme that was inactivated by attachment of the inactivator to a site other than at the flavin or enzyme that was not inactivated.

The only metabolites detected after inactivation of MAO by **8** were **9** and **10**. It does not appear that cyclopropyl-cleaved metabolites were generated. The fact that **9** was stable to the inactivation conditions indicates that, although this is a relatively reactive compound,[6] only the enzyme nucleophiles appear to be sufficiently reactive to attack it. This is a useful property of an enzyme inactivator, whether an affinity labeling compound or a mechanism-based inactivator,[7] such as **1**, because it minimizes side reactions after activation.

By quantification of the nonamine metabolites that are generated during inactivation with a radiolabeled inactivator, it is possible to determine the partition ratio, the number of turnovers to product per inactivation event. With **8** it was found that 110 nonamine molecules were generated per enzyme molecule inactivated, i.e. the partition ratio is 110. It was previously reported,[3] by measuring the k_{cat} and k_{inact} values that the partition ratio (k_{cat}/k_{inact}) was 126. Given the completely different approaches to the measurement of the partition ratio and the time difference from when the experiments were carried out, these numbers are in agreement; the radiolabeled method reported here is more accurate.

The results described here support the formation of a modified flavin during inactivation of MAO by **1**. One possible mechanism of inactivation and structure of a modified flavin (**11**) that could be formed is shown in Scheme 4. Studies are currently underway to determine the actual structure of the modified flavin.

Experimental

Chemicals and analytical methods

NMR spectra were recorded either on a Varian 300-MHz or a Varian Unity Plus 400-MHz spectrometer. Chemical shifts are reported as δ values in parts per million down field 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. Mass spectra were obtained on a VG Instruments VG70–250SE high-resolution spectrometer. Column chromatography was performed with Merck silica gel (230–400 mesh). All chemicals were purchased from Aldrich Chemical Co. Biochemicals and enzymes were purchased from Sigma Chemical Co. and used without further purification. Potassium ^{14}C-cyanide (500 μCi, 45.6 mCi/mmol) was obtained from Sigma Chemical Co., and sodium [^{3}H]borohydride was purchased from American Radiolabeled Chemicals. Ether and THF were freshly distilled from sodium. Methylene chloride was distilled from CaH_2 before use. Glassware was dried in an oven overnight when dry conditions were required. All chemical reactions were carried out in an atmosphere of inert gas (nitrogen or argon).

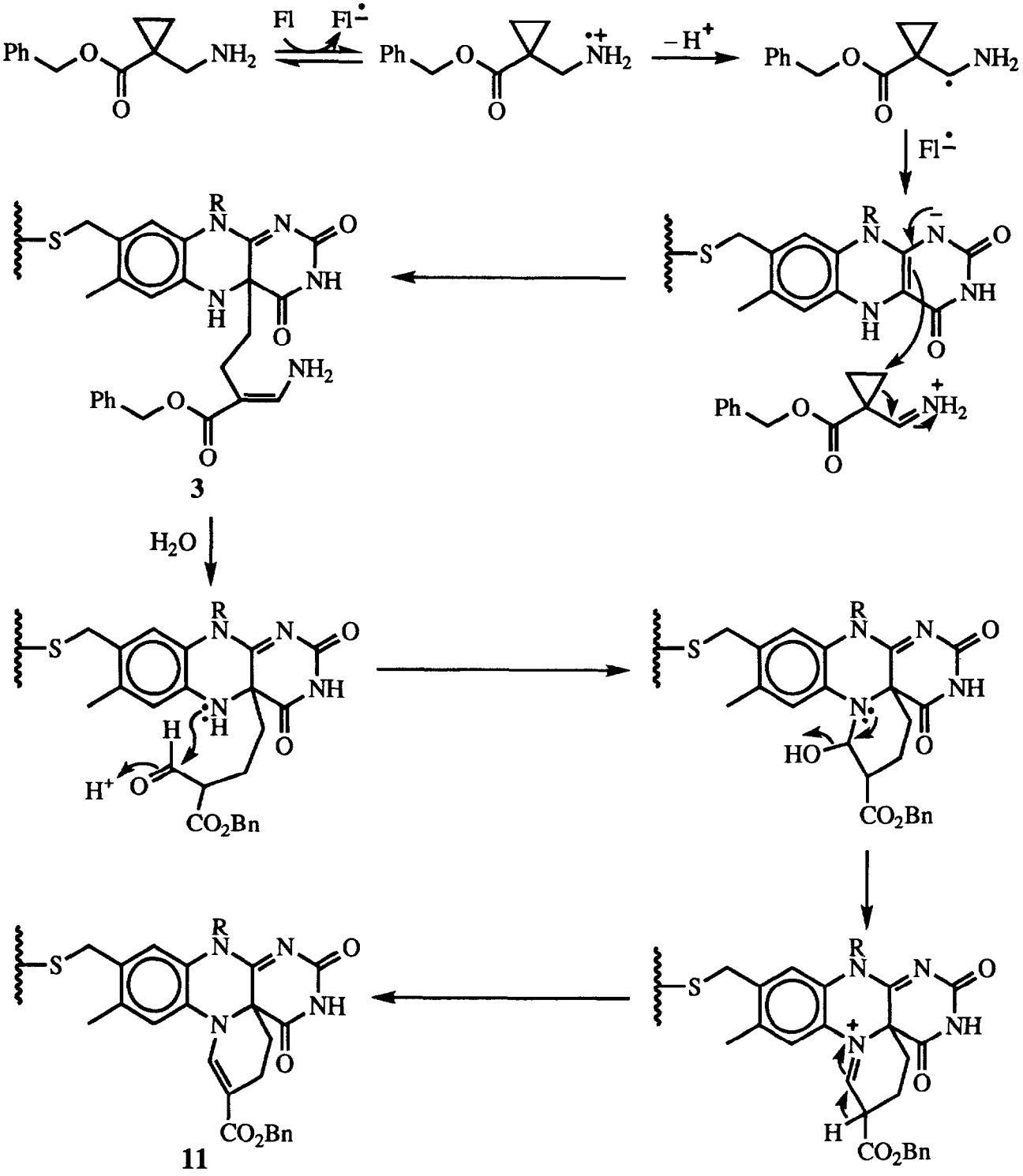
Ethyl [^{14}C]cyanoacetate. To the vial containing K^{14}CN (500 μCi, 45.6 mCi/mmol) was added KCN (0.0966 g, 1.48 mmol). This was dissolved in H_2O, transferred to the reaction vessel, and lyophilized overnight. A soln of ethyl chloroacetate (1.00 mmol) and 18-crown-6 (0.082 mmol) in CH_3CN (548 μL) was added, and the reaction was stirred at room temperature under N_2. After 48 h additional KCN (0.035 g, 0.53 mmol) was added to the orange mixture, which was allowed to stir 24 h more. The CH_3CN was removed under vacuum, and CH_2Cl_2 (8 mL) and water (2 mL) were added. The organic layer was washed with water (3 × 1 mL) and the combined aqueous washes were back extracted with CH_2Cl_2 (2 × 1 mL). The organic layer was dried (Na_2SO_4), filtered, and the solvent removed. The deep orange oil was purified on a silica gel column using 2:1 hexane:EtOAc as the solvent, giving ethyl [^{14}C]cyanoacetate as a light yellow oil (0.0898 g, 79%).

1-[^{14}C]Cyanocyclopropanecarboxylic acid ([1-^{14}C]-4). Following a modification of a literature procedure,[8] benzyltriethylammonium chloride (0.181 g, 0.796 mmol) was added to a stirred soln of 1.5 mL of 50% aq NaOH at 0 °C. A mixture of ethyl [^{14}C]cyanoacetate (0.0898 g, 0.794 mmol) and 1,2-dibromoethane (0.224 g, 1.19 mmol) was added dropwise via syringe at 0 °C, followed by addition of 1 mL of CH_2Cl_2. The reaction was stirred on ice under N_2 for 4.5 h. The CH_2Cl_2 was removed under vacuum, the basic aqueous layer was diluted with water (2 mL), and washed with ether (3 × 2 mL). The aq layer was acidified to pH 2 on ice using conc HCl and was extracted with ether (3 × 2 mL). The aq layer was then satd with NaCl and 3 more ether extractions were carried out. The combined ether extracts were dried over $MgSO_4$, filtered, and upon removal of the solvent under vacuum, a cream-colored solid remained (0.0501 g, 57%), which co-migrated on TLC with cyanocyclopropanecarboxylic acid.

Benzyl [1-^{14}C]cyanocyclopropanecarboxylate ([1-^{14}C]-5). To 1-[^{14}C]cyanocyclopropanecarboxylic acid (0.0354 g, 0.318 mmol) was added *p*-toluenesulfonic acid (10 mg) and 2 mL of benzene. Benzyl alcohol (0.0408 g, 0.376

mmol) was added, and the reaction was refluxed under N_2 using Dean–Stark conditions with $CaCl_2$ in the Dean–Stark trap. After 36 h of reflux, the benzene soln was extracted three times with 2 mL each of satd $NaHCO_3$, and then was dried over $MgSO_4$ and filtered. The yellowish oil remaining after removal of the solvent was a mixture of [1-^{14}C]-**5** and benzyl alcohol. The benzyl alcohol was removed azeotropically with H_2O under red pres, which left a light yellow oil ([1-^{14}C]-**5**) (0.0426 g, 67%).

Benzyl 1-(amino[^{14}C]methyl)cyclopropane-1-carboxylate (8). Following a procedure by Osby *et al.*,[9] catalytic $CoCl_2 \cdot 6H_2O$ (0.0088 g, 0.037 mmol) was added to [1-^{14}C]-**5** (0.0426 g, 0.211 mmol) in 1 mL of 2:1 THF:H_2O. The mixture was cooled on ice, and



Scheme 4.

$NaBH_4$ (0.0311 g, 0.821 mmol), which had been dissolved in a minimum amount of 2:1 THF:H_2O, was slowly added. Formation of a black precipitate along with evolution of H_2 gas was observed. The reaction was stirred at room temperature under N_2 for 20 h, using TLC to monitor the progress of the reaction. Additional $NaBH_4$ (1 equiv) was added. After 5 h a few drops of conc NH_4OH were added to bring the pH to 9–10. The precipitate was removed by centrifugation, and the supernatant retained. The precipitate was washed once with solvent, the supernatants combined, and the THF removed in vacuo. The aqueous layer was extracted with ether (3 × 2 mL), and the ether layer was extracted with 10% HCl (3 × 2 mL), again using TLC to detect amine in the ether layer. The water was removed by lyophilization, leaving a white solid which was recrystallized from EtOH:ether to give [1-^{14}C]-**1** (35 mg; >95% radiopure) with a specific activity of 1.36×10^6 dpm/μmol.

Benzyl 1-(amino[$^2H_2$]methyl)cyclopropane-1-carboxylate (6). This compound was synthesized on a 1 mmol scale as described above from benzyl 1-cyanocyclopropane-1-carboxylate with $NaBD_4$; ^{1}H NMR (D_2O): δ 7.39 (m, 5 H), 5.15 (s, 2 H), 1.41 (m, 2 H), 1.07 (m, 2 H).

Benzyl 1-(amino[^{3}H]methyl)cyclopropane-1-carboxylate (7). The same procedure as that described above for **8** was used, starting with unlabeled **5** (0.088 g, 0.44 mmol) and $CoCl_2 \cdot 6H_2O$ (0.014 g, 0.059 mmol) in 1 mL of 2:1 THF:H_2O. The mixture was cooled on ice, then NaB^3H_4 (25 mCi, 200 mCi/mmol, 0.125 mmol), to which had been added unlabeled $NaBH_4$ (0.00929 g, 0.246 mmol, 0.25 equiv), which was dissolved in a minimum amount of 2:1 THF:H_2O, was slowly added to the flask. Formation of a black precipitate along with evolution of H_2 gas was observed. The reaction was stirred at room temperature under N_2 for 7 h. Additional $NaBH_4$ (0.00792 g, 0.209 mmol, ~0.25 equiv) was added, and the reaction was stirred for 16 h, after which time the remaining $NaBH_4$ was added (0.0141 g, 0.372 mmol) and the reaction was monitored by TLC in 3:1:1 BuOH:H_2O:HOAc. After an additional 24 h of stirring, a few drops of conc NH_4OH were added to bring the pH to 9 or 10. Work up was as described for **8**. The product was obtained as a light green solid (0.0415 g, 39% yield), which was recrystallized from ethanol:ether; radiopurity 98%; specific activity 1.27×10^6 dpm/μmol.

1-Formylcyclopropanecarboxylic acid. This was synthesized by the procedure of Nicolaou;[10] ^{1}H NMR ($CDCl_3$): δ 10.3 (bd, 1 H), 10.2 (s, 1 H), 1.78 (m, 4 H). HRMS Calc. for $C_5H_6O_3$ 114.0316, Found 114.0302.

Enzyme and assays

Bovine liver MAO B was isolated and assayed as previously reported.[11]

Time-dependent inactivation experiments

To solns (480 μL, 0, 0.01, 0.03, 0.05, 0.07, 0.1, 0.2, 0.6, 1.0 mM) of benzyl 1-(amino-methyl)cyclopropane-1-carboxylate hydrochloride or analogues in 100 mM sodium phosphate buffer, pH 7.4 was added MAO B (20 μL, 2 mg/mL) at 25 °C. After being mixed, the samples were incubated and periodically assayed for MAO activity by removal of 20 μL of the solution and addition to 480 μL of a 1.04 mM benzylamine solution in 100 mM Tris buffer, 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.[12]

Release of tritium as ^3H_2O during inactivation of MAO B with 7

Compound **7** (600 μL of a 16 mM soln) in 50 mM sodium phosphate buffer, pH 7.4 was incubated with 100 μL of MAO B (178 μM) for 24 h at room temperature; the enzyme assay showed that MAO B was inactivated. The inactivation mixture was bulb-to-bulb distilled at dry ice temperature, and the volatiles (580 μL) were counted in a scintillation counter.

Incorporation of radioactivity into MAO B by 7 and 8

MAO B (178 μM, 100 μL) was incubated with 12.5 mM **7** or **8** in 100 mM sodium phosphate buffer, pH 7.4 (660 μL) at 25 °C. A control without inactivator was run simultaneously at one-third the scale. MAO B treated with inactivator was devoid of activity when checked after 3 h. Inactivated enzyme was dialyzed against 100 mM sodium phosphate buffer, pH 7.4 (500 mL) at 4 °C, and the dialysis buffer was changed 4 times over 36 h. The enzyme was removed from the dialysis tubing and was assayed for radioactivity and protein concentration, from which the number of equivalents of radioactivity attached per MAO B molecule was calculated. To the inactivated enzyme solution was added 336 mg of urea and 55.3 mg of ammonium bicarbonate at room temperature, and the solution was allowed to stand overnight to denature the enzyme. Dithiothreitol (45 mM, 75 μL) was added at 50 °C for 30 min, and the denatured enzyme solution was dialyzed at 4 °C vs 8 M urea in 400 mM ammonium bicarbonate (500 mL). The dialysis buffer was changed 3 times over 20 h. The denatured enzyme solution was assayed for radioactivity and protein concentration, from which the number of equivalents of radioactivity attached per MAO B molecule was calculated.

Attachment of 7 or 8 to the flavin coenzyme

The denatured enzyme described above was digested with pronase E (3.3 mg) or trypsin (0.2 mg) at 37 °C for 40 h. The peak fractions were isolated by reversed phase HPLC using a Vydac column eluting with a gradient over 1 h from 0.06% of TFA in water (solvent

A) to 0.052% of TFA in 80% of acetonitrile (solvent B) with a flow rate of 0.5 mL/min and detection at 450 nm. Each fraction was scintillation counted. Similar results were obtained with **7** and **8**.

Identification of the metabolites formed during inactivation of MAO B by 1

MAO B (200 μM, 100μL) was incubated with 80 mM benzyl 1-(aminomethyl)cyclo-propane-1-carboxylate in 100 mM sodium phosphate buffer, pH 7.4 (1000 μL) at 25 °C. A control without inactivator was run simultaneously at one-fifth the scale. After 3 h **1** had inactivated MAO B completely. The incubation solution was extracted with methylene chloride (4 × 5 mL). The components in the combined extracts were separated by preparative TLC, eluting with CH_2Cl_2. Three bands (R_f = 0.70, 0.52, 0.30) on the silica gel were scraped off and extracted with methylene chloride. Each extract was analysed by ^1H and ^{13}C NMR spectroscopy.

The band with R_f = 0.70 is benzyl 1-(formyl)cyclopropane-1-carboxylate: ^1H NMR($CDCl_3$): δ 1.65 (m, 2H), 1.71 (m, 2H), 5.25 (s, 2H), 7.38 (m, 5H), 10.42 (s, 1H); ^{13}C NMR($CDCl_3$): δ 22.9, 25.0, 34.0, 67.3, 127.9, 128.3, 128.6, 128.8, 171.0, 198.7 The band with R_f = 0.52 is benzyl alcohol: ^1H NMR($CDCl_3$): δ 1.22 (s, 1H), 4.70 (d, 2H), 7.30–7.38 (m, 5H); ^{13}C NMR ($CDCl_3$): δ 53.5, 127.1, 127.3, 128.6.

Identification of the metabolites formed during inactivation of MAO B by 8

MAO B (50 μL; 155 μM) was incubated with **8** (1.3 mg) in 100 mM sodium phosphate buffer, pH 7.4 (400 μL) until the remaining activity of MAO B was less than 10% compared to the control without inactivator. The inactivated enzyme solution was acidified, then extracted with CH_2Cl_2 (4 × 200 μL). The aqueous layer contained the inactivator; the organic layer was analyzed by TLC, and two radioactive spots were detected corresponding to **9** and **10**. The combined organic extracts were evaporated, and the residue was taken up in acetonitrile:water (6:4). This was analysed by reversed phase HPLC using a C-18 column, eluting with 40% water in acetonitrile, monitoring at 214 nm. The major radioactive metabolite co-migrates with 1-formylcyclopropanecarboxylic acid, as a result of hydrolysis during work up. No 1,1-cyclopropanedicarboxylic acid or cyclo-propanecarboxaldehyde was detected. The organic extract (200 μL) and remaining aqueous layer (200 μL) were each passed through a Dowex 50 × 12 column (8 × 75 mm) eluting first with water and then with 0.25 M ammonium hydroxide. The water eluent containing the nonamines was collected and was scintillation counted.

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