2-(Iodoethenyl)benzylamines as Potential Probes for Radical Intermediates Formed during Monoamine Oxidase Catalyzed Oxidations

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An attempt to trap radical intermediates during the monoamine oxidase (MAO) catalyzed oxidation of amines by intramolecular cyclization with an activated alkene that is built into the substrate was unsuccessful. (*E*)-2-(Iodoethenyl)benzylamine (**3a**) was shown to be a reversible inhibitor of MAO B, but the corresponding *Z* isomer (**3b**) was a good substrate. By GC-MS analysis, the expected radical trapping product, isoquinoline (**5**), was observed as one of two products in a 1:1 ratio. However, NMR analysis prior to GC-MS analysis showed no evidence of isoquinoline, suggesting that the isoquinoline was generated during gas chromatography. As a model for this GC-dependent reaction, the corresponding aldehyde, (*Z*)-2-(iodoethenyl)benzaldehyde (**14**), was treated with ammonia, and the product was analyzed by GC-MS; isoquinoline was detected. Likewise, the reaction of **14** with methylamine also produced isoquinoline by GC-MS analysis (but not by NMR analysis). These results are explained by electrocyclization of the corresponding imines at the elevated temperatures in the GC (Schemes 7 and 8). Substrate **3b** and aldehyde **14** were recovered when the enzyme reaction products were chromatographed, although they were not detected by GC-MS. These products could arise via hydrolytic decomposition of the corresponding imine (**17**; Scheme 9). GC-MS analysis of **17** produced isoquinoline and (*Z*)-2-(iodoethenyl)benzyl iodide (**18**) in a 1:1 ratio; these are the two products observed in a 1:1 ratio after incubation of MAO with **3b** and apparently arise from thermally induced electrocyclization and iodide ion cleavage (Scheme 9).

Introduction

Monoamine oxidase (MAO, EC 1.4.3.4), an integral membrane protein, is one of the enzymes responsible for the degradation of various biogenic and xenobiotic amines.[1] It catalyzes the oxygen-dependent conversion of amine substrates to the corresponding imines, which are nonenzymatically hydrolyzed to aldehydes.[2] MAO exists in two principal isozymic forms known as MAO A and MAO B.[3] Compounds that inhibit MAO A exhibit antidepressant effects,[4] and those that inhibit MAO B are useful adjuncts to L-dopa treatment for Parkinson's disease.[5] The catalytic mechanism for MAO has long been proposed to involve radical intermediates **1** and/or **2** (Scheme 1); the electron-transfer mechanism shown in Scheme 1[6] and a hydrogen atom abstraction mechanism to go directly to **2**[7] are favored pathways. Previous studies using cyclopropylamines, cyclobutylamines, and cubylcarbinylamines have provided evidence for an electron-transfer mechanism by observing ring-cleavage reactions that would be expected to accompany cycloalkylcarbinyl or -aminyl intermediates.

Related nonenzymatic amine oxidations via amine radical cations have been demonstrated under electrochemical,[8] photochemical,[9] and chemical oxidizing[10] conditions. It has been shown that amine radical cations[9c] can undergo ring cyclization to alkenes with rate constants on the order of 10^8 s^{-1}, and carbon radicals undergo cyclization to an alkene with rate constants on the order of 10^5-10^7 s^{-1}.[11]

We envisioned incorporating a rotation-constraining factor into an analogue of a substrate for MAO and placing a double bond nearby to trap either the potential amine radical cation or carbon radical intermediate; formation of this intermediate would be detected by

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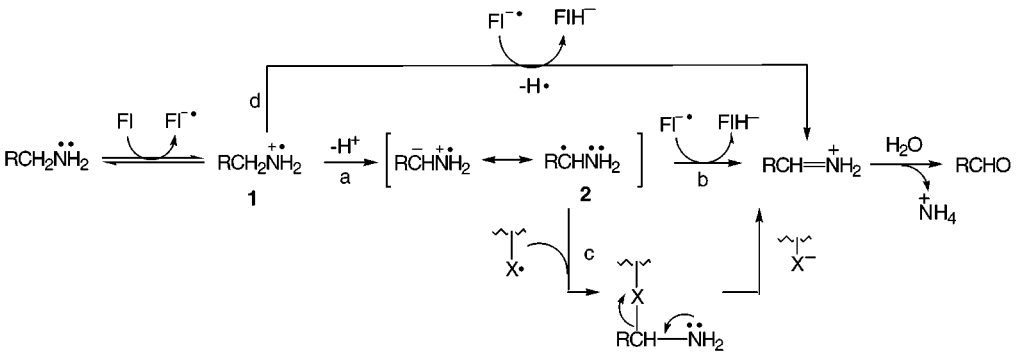
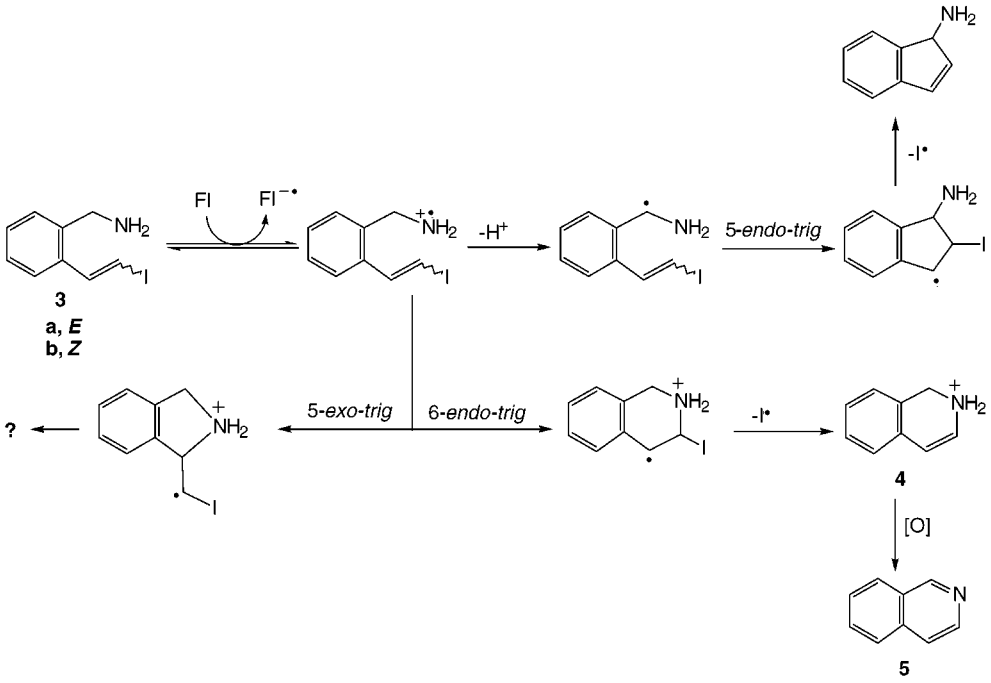
Scheme 1



Scheme 2



isolation of the cyclized product. Compound **3** was designed to lead to one of three possible cyclized products, depending upon whether the amine radical cation or the α-radical were trapped by either a 5-*exo-trig* or 6-*endo-trig* cyclization process; the 5-*endo-trig* cyclization is disfavored, according to the Baldwin rules[12] (Scheme 2). The 6-*endo-trig* cyclization would lead to a dihydroisoquinoline (**4**), which, upon further oxidation would give isoquinoline (**5**). Isoquinoline was, in fact, detected by monitoring the reaction by GC. However, it was subsequently determined that the isoquinoline was generated only by the cis isomer of **3** and only during the gas chromatography monitoring, not by the enzyme-catalyzed reaction. Although this approach was not able to detect the proposed enzyme-catalyzed intermediate, unexpected oxidized products were identified.

Results and Discussion

Synthesis of *o*-[(*E*)-Iodoethenyl]benzylamine *p*-Toluenesulfonate (3a). Amine **3a** was synthesized as shown in Scheme 3. Aldehyde **7** was synthesized in high yield from *o*-bromobenzaldehyde by palladium coupling.[13] Iodination of alkyne **9** was readily realized by radical addition of tributyltin hydride followed by iodine addition.[14] The alcohol was converted to **3a** by standard functional group transformations.

Synthesis of *o*-[(*Z*)-Iodoethenyl]benzylamine Hydrochloride (3b). The key intermediate **14** was prepared by a novel Wittig reaction (Scheme 4).[15] To avoid reaction with both aldehyde functionalities of *o*-phthalaldehyde, the ylide was generated and was added slowly to an excess amount of *o*-phthalaldehyde. Conversion of **15** to **3b** followed the same route that was used to prepare **3a**.

Synthesis of Condensation Product 17 and Metabolite 18. The condensation product **17** was prepared according to a procedure by Enholm et al.[16] (Scheme 5); compound **18** was prepared from the corresponding alcohol (Scheme 6).[17]

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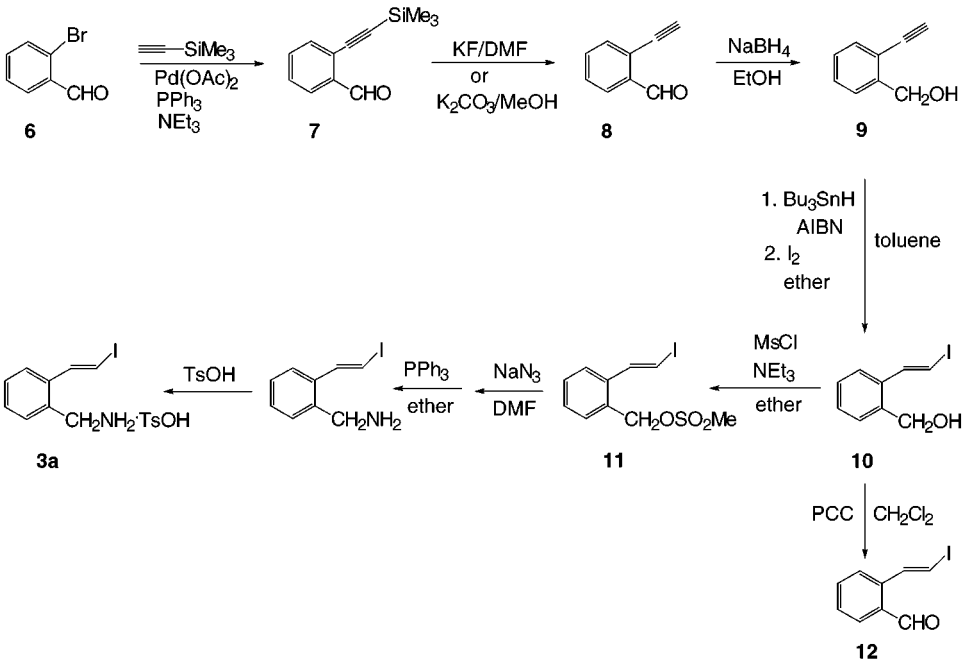
Scheme 3



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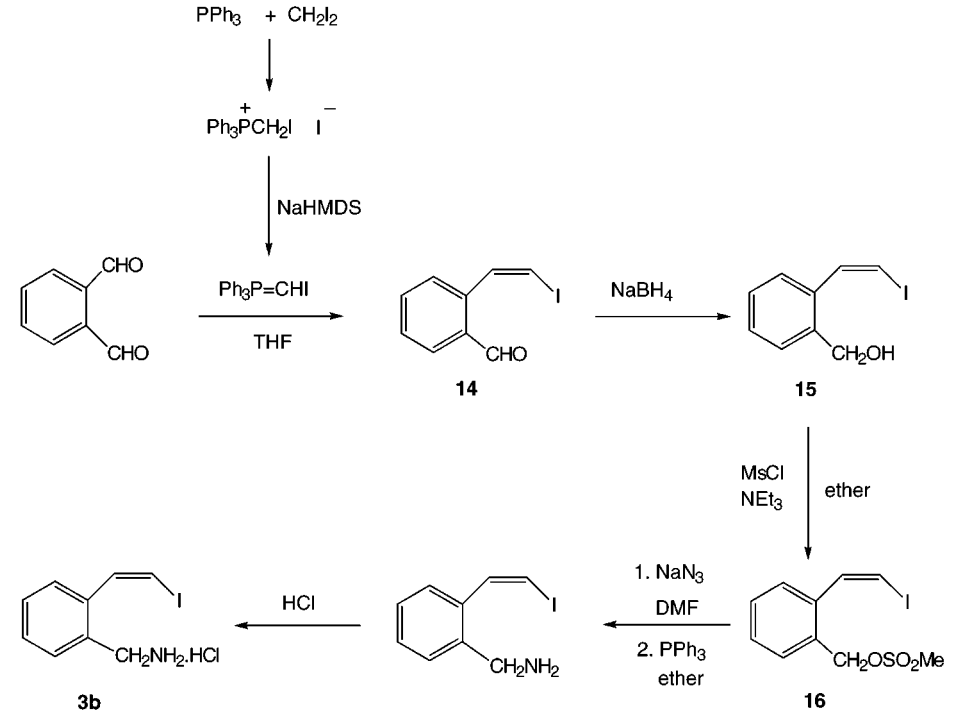
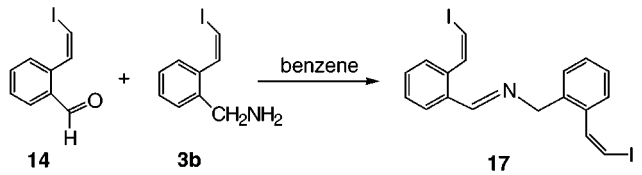


Scheme 5



Enzymatic Studies. Compound **3a** was synthesized and found to be a competitive inhibitor of MAO B with $K_i = 0.18$ mM (Figure 1). Trace amounts of isoquinoline (**5**) were detected by GC-MS when **3a** was incubated with MAO B. The 6-*endo-trig* mechanism in Scheme 2 could explain the generation of isoquinoline. However, formation of isoquinoline was not found to be strictly time-dependent, and after short time periods, the formation of isoquinoline ceased. A small contaminant appeared to be consumed during enzyme-catalyzed oxidation of **3a**. Mass spectrometric analysis revealed that the contaminant was an isomer of **3a**, suggesting the *Z* isomer **3b**. After **3b** was synthesized, it was subjected to MAO B oxidation and was found to be a substrate ($K_m = 0.24$ mM, $k_{cat}/K_m = 1490$ mM^{-1} min^{-1}). Two products were observed, one of them being isoquinoline (**5**), as identified by GC-MS analysis, and the other one was an unknown compound.

However, when the experiment was carried out on a large enzyme scale (36 μmol), an NMR spectrum of the

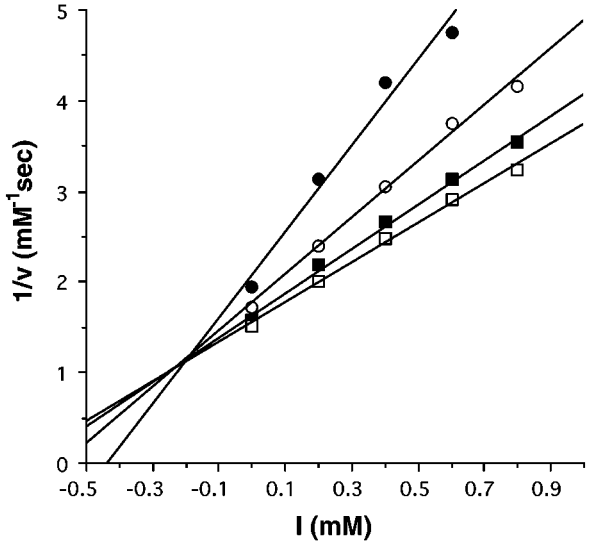
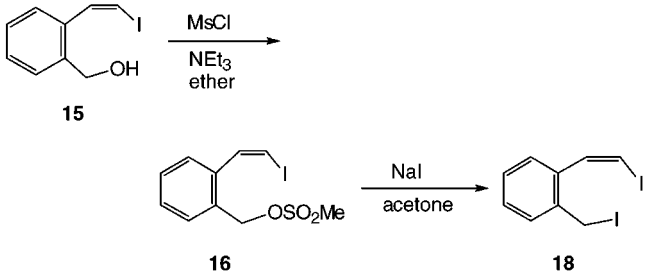
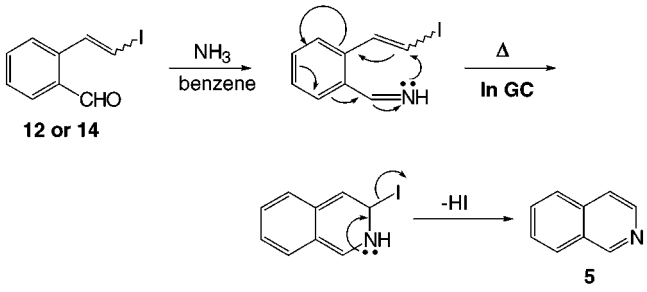


Figure 1. Dixon plot of the inhibition of MAO B by **3a** in 100 mM Tris buffer, pH 9.0 at 25 °C; (●) 0.2 mM; (○) 0.35 mM; (■) 0.5 mM; (□) 0.67 mM.

Scheme 6



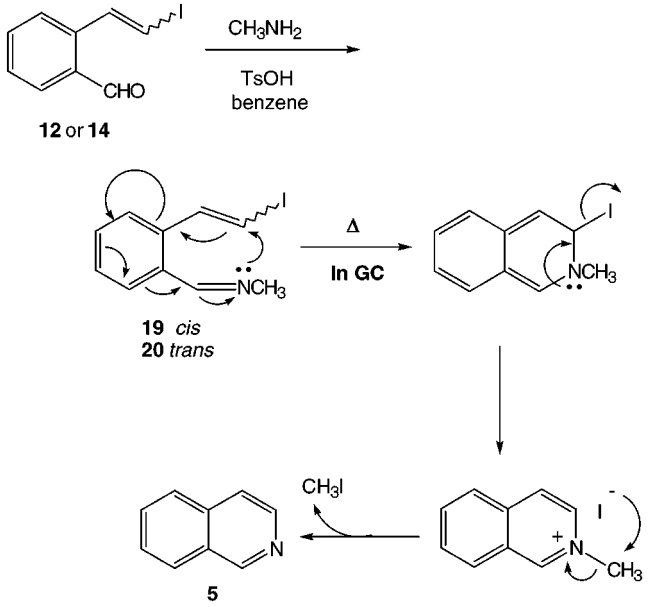
Scheme 7



total products indicated that no isoquinoline was present. Instead, it was shown that the products had the same NMR vinyl proton chemical shifts and coupling constants as those of the vinyl protons of the starting material. This suggests that the formation of isoquinoline occurred during the gas chromatography. As a test of this hypothesis, aldehydes **12** and **14** were treated with ammonia, and the incubation mixture was injected into the GC; isoquinoline was detected. Electrocyclization in the GC (Scheme 7) would rationalize that observation. Isoquinoline also was observed by GC-MS when both **12** and **14** were treated with methylamine; the isolated products (prior to GC analysis) were determined by NMR and MS to be the corresponding imines **19** and **20** (Scheme 8). This result also can be explained by a GC-induced electrocyclization, followed by demethylation.

The second product that was detected by GC was always found to be in the ratio of approximately 1:1 with **5** by GC-MS analysis throughout the reaction. This

Scheme 8



indicates that these two products might have come from the same precursor by decomposition. The substrate **3b** and the aldehyde **14** were recovered when the enzyme reaction products were chromatographed, although they were not detected by GC. These products (**3b** and **14**) could arise via hydrolytic decomposition of the corresponding imine (**17**; Scheme 9). The unknown second GC product, then, is proposed to be **18**. The structure of **17** was verified by GC, GC-MS, and NMR comparison with the independently synthesized compound, prepared as shown in Scheme 5. The structure of **18** was confirmed by GC and GC-MS using synthetic **18** (Scheme 6).

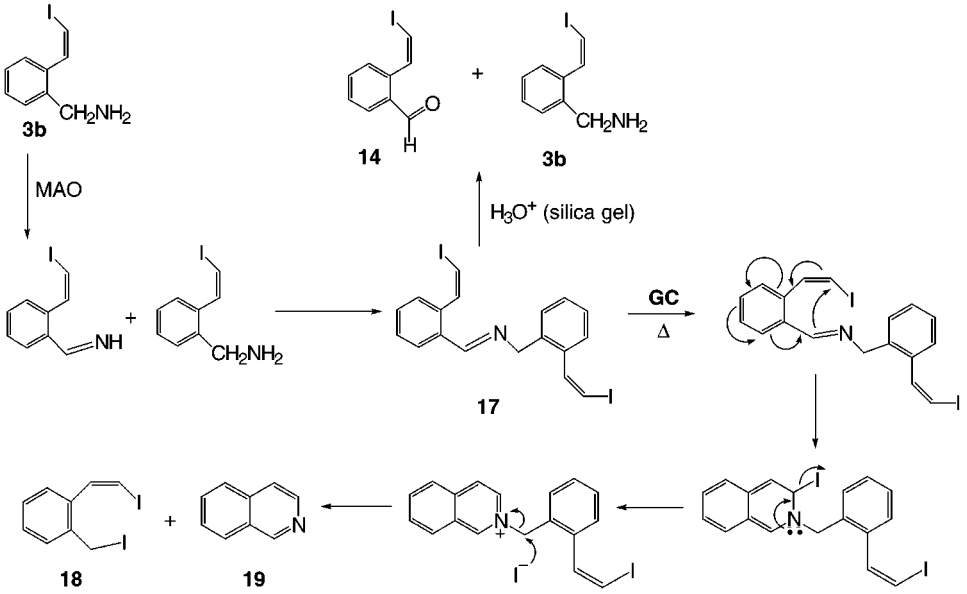
Conclusions

Compounds **3a** and **3b** were designed to trap potential radical intermediates of the MAO-catalyzed reaction intramolecularly. However, the rate of cyclization must be too slow relative to the rate of electron transfer, possibly because of constraints within the active site, and only "normal" oxidation to the corresponding imine results. An important finding from this study, however, is the artifactual formation of isoquinoline (**5**) during product analysis by gas chromatography. GC is an easy and fast technique for product study; however, it can produce secondary products unrelated to the system that it is being used to analyze, and therefore this may be a drawback for monitoring sensitive enzyme-catalyzed reactions. Although this general approach was not successful in the case of monoamine oxidase, it has the potential to be applied to the trapping of radical intermediates in other enzyme-catalyzed reactions.

Experimental Section

General Procedures. All reagents and solvents were purchased from Aldrich or Fisher and were used without further purification unless otherwise stated. Dichloromethane, triethylamine, and toluene were freshly distilled from CaH_2. THF and ether were freshly distilled from sodium metal. Glassware was oven-dried. Acetone was dried over 3 Å molecular sieves. All of the reactions were carried out under an atmosphere of inert gas.

Scheme 9



2-[(Trimethylsilyl)ethynyl]benzaldehyde (7) and 2-Ethynylbenzaldehyde (8). The procedure of Austin et al.[13] was utilized. A deaerated solution of 15.66 g (84.6 mmol) of 2-bromobenzaldehyde, 338 mg (1.29 mmol) of triphenylphosphine, and 167 mg (0.74 mmol) of palladium (II) acetate in 200 mL of anhydrous triethylamine was treated with 12.47 g (12.70 mmol) of ethynyltrimethylsilane under argon. The reaction mixture was rapidly heated to 80 °C for 6 h. After it was cooled, the mixture was filtered, and the filtrate was concentrated, mixed with water (100 mL), and extracted with dichloromethane (3 × 50 mL). The combined organic phases were dried over Na_2SO_4, concentrated, and distilled at 80−84 °C (0.1 Torr) to give 2-[(trimethylsilyl)ethynyl]benzaldehyde (**7**) as a yellow-brown solid (15.30 g, 90%): mp 47−48 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.57 (s, 1H), 7.91−7.933 (d, 1H), 7.43−7.58 (m, 3H), 0.30 (s, 9H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 193.00, 137.24, 134.80, 134.60, 129.93, 127.96, 127.90, 103.53, 101.14, 0.88; MS (EI) *m*/*z* 201 (M − H$^+$), 187, 161, 128; HRMS (EI) *m*/*z* calcd for $C_{12}H_{13}OSi$ (M − H$^+$) 201.0736, found 201.0733.

The aldehyde **7** (8.0 g, 39.6 mmol) was treated with potassium fluoride dihydrate (8.3 g, 88.2 mmol) in DMF (30 mL) under nitrogen at room temperature for 3 h. It was then poured into water (60 mL) and extracted with dichloromethane (3 × 30 mL), dried over Na_2SO_4, and concentrated to yield **8** (5.0 g, 97%) as a yellow solid. An analytical sample was prepared by sublimation at 15 Torr: mp 61−62 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.53 (s, 1H), 7.92−7.94 (d, 1H), 7.48−7.60 (m, 3H), 3.46 (s, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 192.57, 137.60, 134.99, 134.82, 130.30, 128.33, 126.57, 102.43, 85.36; MS (EI) *m*/*z* 130 (M$^+$), 102, 76, 51; HRMS (EI) *m*/*z* calcd for C_9H_6O 130.0419 (M$^+$), found 130.0415.

2-Ethynylbenzyl Alcohol (9). To a solution of **8** (2.56 g, 19.7 mmol) in anhydrous ethanol (30 mL) was added portionwise sodium borohydride (370 mg, 10 mmol) at 0 °C under nitrogen. The reaction mixture was stirred at 0 °C for 1 h before it was warmed to room temperature. The mixture was concentrated, diluted with 20 mL of water, and extracted with ether (3 × 15 mL). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, concentrated, and purified by column chromatography (15% EtOAc/85% hexanes) to give **9** (2.55 g, 97%) as a white solid: mp 61−62 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.46−7.55 (m, 2H), 7.30−7.38 (m, 2H), 4.79 (s, 2H), 3.38 (s, 1H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 143.22, 132.92, 129.30, 127.53, 127.40, 120.26, 82.03, 81.30, 63.90; MS (EI) *m*/*z* 132 (M$^+$), 103, 77, 51; HRMS (EI) *m*/*z* calcd for C_9H_8O 132.0575 (M$^+$), found 132.0570.

2-[(*E*)-2-Iodoethenyl]benzyl Alcohol (10). To a solution of hydroxy alkyne **9** (1.32 g, 10 mmol) and AIBN (87.2 mg, 0.53 mmol) in anhydrous toluene (30 mL) was added Bu_3SnH (2.91 g, 10 mmol) at room temperature under argon. The reaction mixture was heated at 100−105 °C for 3 h and stirred at room temperature overnight. The reaction mixture was concentrated to afford a light yellowish liquid.

To the crude vinyl stannane (4.8 g) in ether (20 mL) was added iodine (2.54 g, 10 mmol) dissolved in ether (10 mL). After 30 min, the reaction mixture was washed with 10% $Na_2S_2O_3$ (15 mL). The aqueous layer was washed with ether (15 mL). Organic layers were combined, washed with brine, dried over Na_2SO_4, concentrated, and chromatographed (17% EtOAc/83% hexane) to give **10** (1.04 g, 40%) as a white solid: mp 129−131 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.75−7.80 (d, 1H), 7.31−7.35 (m, 4H), 6.80−6.85 (d, 1H), 4.73 (s, 2H); ^{13}C NMR ($CDCl_3$, 300 MHz) 142.30, 136.89, 136.85, 128.65, 128.58, 128.44, 126.40, 118.94, 79.25, 63.40; MS (EI) *m*/*z* 260 (M$^+$), 133, 115, 105, 77, 51; HRMS (EI) calcd for C_9H_9IO (M$^+$) 259.9700, found 259.9699. Anal. Calcd for C_9H_9OI: C, 41.56; H; 3.49. Found: C, 41.68: H, 3.79.

2-[(*E*)-2-Iodoethenyl]benzylamine (3a). To a stirred solution of **10** (743 mg, 2.86 mmol) and triethylamine (438 μL, 3.14 mmol) in anhydrous ether (30 mL) at 0 °C under nitrogen was slowly added methanesulfonyl chloride (342 mg, 3 mmol). After 2 h, water (10 mL) was added to the reaction mixture. The organic layer was washed with brine, dried with Na_2SO_4, and concentrated to give **11** as a yellow syrup (940 mg, 97%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.70−7.75 (d, 1H), 7.37−7.44 (m, 4H), 6.86−6.91 (d, 1H), 5.30 (s, 1H), 2.93 (s, 1H).

A stirred suspension of the sulfonate ester **11** (300 mg, 0.9 mmol) in anhydrous DMF (15 mL) containing sodium azide (455 mg, 7 mmol) under nitrogen was heated to no greater than 50 °C for 3 h. The reaction mixture was cooled to room temperature, and water (30 mL) was added to bring excess sodium azide into solution. The mixture was extracted with ether (2 × 25 mL). The organic extracts were combined, washed with water (20 mL) and brine (20 mL), and dried over Na_2SO_4. Part of the solvent was removed, and to this ether solution at 0 °C was added triphenylphosphine (264 mg, 1 mmol). After 2 h, 100 μL of water was added to the reaction mixture. After 12 h, this ether solution was extracted with 1 N HCl (2 × 20 mL). The water layer was washed with ether (3 × 10 mL) followed by addition of potassium hydroxide pellets until strongly basic. The solution was then extracted with ether (3 × 20 mL). The organic phases were combined, washed with brine, dried over Na_2SO_4, and acidified with *p*-toluenesulfonic acid and then recrystallized from ether−ethanol to give **3a** as white crystals (200 mg, 51%): mp 185−190 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) 7.73−7.78 (d, 1H), 7.29−7.37 (m, 4H), 6.75−6.80 (d, 1H), 3.90 (s, 1H); ^{13}C NMR ($CDCl_3$,

300 MHz) 142.56, 139.49, 136.50, 128.72, 128.26, 127.49, 126.39; MS (EI) *m/z* 258 ($M - H^+$), 132, 115, 77; HRMS (EI) calcd for $C_9H_{10}NI$ ($M - H^+$) 259.9781, found 257.9785. Anal. Calcd for $C_{16}H_{18}NO_3SI$ (tosylate salt): C, 44.56; H, 3.91; N, 3.25. Found: C, 44.34; H, 3.93; N, 3.17.

2-[(*E*)-2-Iodoethenyl]benzaldehyde (12). To a vigorously stirred suspension of PCC (1.20 g, 5.57 mmol) in anhydrous dichloromethane (20 mL) was added vinyl iodide **10** (1.00 g, 3.9 mmol). The reaction mixture was stirred for 2 h before 20 mL of ether was added. The solution was filtered through a pad of silica gel and concentrated to give **12** as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.21 (s, 1H), 8.28−8.33 (d, 1H), 7.48−7.80 (m, 4H), 6.87−6.92 (d, 1H); ^{13}C NMR ($CDCl_3$, 300 MHz) 192.20, 141.85, 139.59, 134.00, 132.43, 132.05, 128.59, 127.52, 81.83; MS (EI) *m./z* 131, 103, 77, 51; HRMS (EI) calcd for C_9H_7OI (M^+) 257.9543, found 257.9538. The compound slowly decomposed, prohibiting elemental analysis.

2-[(*Z*)-2-Iodoethenyl]benzaldehyde (14). To a stirred suspension of (iodomethyl)triphenylphosphonium iodide[18] (1.80 g, 3.2 mmol) in anhydrous THF (15 mL) was added sodium hexamethyldisilazine (1 M, 3.2 mL) dropwise. After it was stirred for 1 min, the solution was cooled in a dry ice−acetone bath and HMPA (1 mL) was added. The above orange solution was transferred via cannula slowly to a solution of phthalic dicarboxaldehyde (1.72 g, 12.8 mmol) in THF (20 mL) at −78 °C. The cold bath was then removed, and stirring was continued for 30 min. Hexane (40 mL) was added to the reaction mixture. After filtration, the filtrate was washed with water (3 × 15 mL) and brine (20 mL), dried over Na_2SO_4, concentrated and chromatographed (11% EtOAc/89% hexane) to give **14** (350 mg, 42%), which was inseparable from its trans isomer. An analytical sample was obtained by column chromatographic separation of the corresponding alcohol **15** (see below) followed by PCC oxidation: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.20 (s, 1H), 7.94−7.96 (d, 1H), 7.78−7.80 (d, 1H), 7.53−7.68 (m, 3H), 6.89−6.92 (d, 1H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 192.33, 141.02, 138.24, 134.32, 133.71, 131.21, 130.34, 129.19, 86.80; HRMS (EI) calcd for C_9H_7OI (M^+) 257.9543, found 257.9534. The compound slowly decomposed, prohibiting elemental analysis.

2-[(*Z*)-2-Iodoethenyl]benzyl Alcohol (15). Compound **15** was prepared by the same method as **10** by $NaBH_4$ reduction of **14**: mp 54−55 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.27−7.49 (m, 5H), 6.72−6.75 (d, 1H), 4.64 (s, 2H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 138.25, 138.12, 136.78, 128.70, 128.54, 128.02, 127.71, 84.70, 63.44; MS (EI) *m/z* 260 (M^+), 133, 105, 77, 51; HRMS (EI) calcd for C_9H_9IO (M^+) 258.9699, found 259.9703. Anal. Calcd for C_9H_9OI: C, 41.56; H, 3.49; Found: C, 41.60; H, 3.48.

2-[(*Z*)-2-Iodoethenyl]benzylamine (3b). Compound **3b** was prepared from **15** by the same method used to make **3a** from **10**. It was analyzed as the HCl salt. The sulfonate ester **16** was first obtained: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.43−7.50 (m, 5H), 6.82−6.84 (d, 1H), 5.22 (s, 2H), 2.90 (s, 3H).

Compound **3b** (HCl salt): mp 202−204 °C dec; ^{1}H NMR (D_2O, 300 MHz) δ 7.48−7.54 (m, 5H), 6.96−6.99 (d, 1H), 4.16 (s, 2H); ^{13}C NMR (D_2O, 300 MHz) δ 138.64, 137.76, 129.82, 129.50, 129.32, 129.21, 129.00, 88.10, 40.59; MS (EI) *m/z* 258 (M^+), 132, 115, 77, 51; HRMS (EI) calcd for $C_9H_{10}IN$ (M^+) 257.9781, found 257.9788. Anal. Calcd for $C_9H_{11}NClI$ (HCl salt): C, 36.58; H, 3.75; N, 4.74. Found: C, 36.28; H, 3.76; N, 4.86.

Condensed Imine of 3b and 14 (17). To a solution of the free amine form of **3b** (16.62 mg, 0.064 mmol) in benzene (5 mL) was added aldehyde **14** (16.5 mg, 0.064 mmol) in 5 mL of benzene. The solvent was removed in vacuo, and benzene was added and evaporated a couple of times to get rid of the water. Compound **17** was obtained as a yellowish oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.48 (s, 1H), 8.02 (d, 1H), 7.60−7.62 (d, 1H), 7.52−7.55 (d, 1H), 7.32−7.49 (m, 7H), 6.78−6.81 (d, 1H), 6.71−6.74 (d, 1H), 4.67 (s, 2H); ^{13}C NMR ($CDCl_3$, 300 MHz) 160.36, 138.86, 138.65, 138.48, 136.91, 133.17, 130.33, 128.80, 128.64, 128.58, 128.49, 128.40, 127.67, 126.97; MS (EI) *m/z* 497 ($M - H^+$), 371, 245, 130, 116; HRMS (EI) calcd for $C_{18}H_{15}NI_2$ ($M - H^+$) 497.9216, found 497.9218.

2-[(*Z*)-2-Iodoethenyl]benzyl Iodide (18). To a solution of the sulfonate ester **16** (62 mg, 0.19 mmol; prepared by the same procedure as **11**) in anhydrous acetone (5 mL) was added a solution of sodium iodide (67.5 mg, 0.45 mmol) in acetone (10 mL). The solution was allowed to reflux overnight. The solvent was removed, water (10 mL) was added, and the solution was extracted with ether (3 × 10 mL). The organic layers were combined, dried, concentrated, and chromatographed to afford **18** as a white solid: mp 40−42 °C; the color changes rapidly upon exposure to light; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.36−7.42 (m, 3H), 7.28−7.30 (m, 2H), 6.83−6.86 (d, 1H), 4.38 (s, 2H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 138.72, 138.29, 137.55, 130.59, 130.43, 129.87, 129.17, 86.25, 5.33; MS (EI) *m/z* 370 (M^+), 254, 243, 116, 63; HRMS (EI) calcd for $C_9H_8I_2$ (M^+) 369.8716, found 369.8713.

***N*-{2-[(*Z*)-2-Iodoethenyl]benzylidene}methylamine (19).** A solution of **14** (10 mg, 0.04 mmol) and *p*-toluenesulfonic acid hydrate (1.5 mg, 0.008 mmol) in benzene (5 mL) was bubbled with methylamine for 3 min. The reaction mixture was stirred for 1 h, washed with saturated Na_2CO_3 (3 mL), dried over Na_2SO_4, and concentrated to afford **19** as a yellow oil (10 mg, 90%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.42 (d, 1H), 7.94−7.96 (m, 1H), 7.59−7.62 (d, 1H), 7.38−7.45 (m, 3H), 6.79−6.82 (d, 1H), 3.53 (d, 3H); MS (EI) *m/z* 272 (MH^+), 144, 103, 55; HRMS (EI) calcd for $C_{10}H_{11}IN$ (MH^+) 271.9936, found 271.9949.

N-{2-[(*E*)-2-Iodoethenyl]benzylidene}methylamine (20). Compound **20** was prepared in a manner identical with that for **19**, starting from **12**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.56 (d, 1H), 7.97−8.02 (d, 1H), 7.79−7.80 (d, 1H), 7.35−7.38 (m, 3H), 6.72−6.77 (d, 1H), 3.56 (d, 3H); MS (EI) *m/z* 270 ($M - H^+$), 229, 144, 131, 115, 103, 77; HRMS (EI) calcd for $C_{10}H_9NI$ ($M - H^+$) 269.9780, found 269.9766.

Enzyme and Assay. Beef liver MAO B was isolated as described previously[19] and stored as a concentrated solution (15−25 mg/mL) in sodium phosphate buffer (50 mM, pH 7.2) at 4 °C. The specific activity varied among preparations, ranging from 3.5 to 7 units/mg, where a unit of activity is the conversion of 1 μmol of benzylamine to benzaldehyde per minute at pH 9.0 and 30 °C.

General Procedure in Organic Solvent. Stock MAO (40 μL) was pipetted into a vial, frozen in a −78 °C dry ice−acetone bath, and lyophilized. The substrate solution (6 mM, 1000 μL) was syringed into the vial containing the dried enzyme; water was pipetted into the mixture to give a final water concentration of 0.5% (v/v). The mixture was sonicated for 10 s in an ultrasonic cleaning bath. For analysis, 1 μL aliquots were periodically removed and analyzed by capillary gas chromatography.

General Procedure for Inhibition of the MAO-Catalyzed Oxidation of Cinnamylamine. An MAO solution was prepared by diluting 15 μL of the stock MAO solution with 285 μL of Tris-HCl buffer (100 mM, pH 9.0). The amount of inhibition of the oxidation of various concentrations of cinnamylamine (0.2, 0.35, 0.50, and 0.67 mM in Tris-HCl buffer, 100 mM, pH 9.0) by various concentrations of inhibitor **3a** (0, 0.2, 0.4, 0.6, 0.8 mM in Tris-HCl buffer, 100 mM, pH 9.0) was determined by adding 10 μL of the above MAO solution to 490 μL of an inhibitor/substrate solution at 25 °C, followed by monitoring the increase in absorbance at 290 nm. Up to 30% anhydrous DMSO was used as the cosolvent to dissolve **3a**. An enzyme control was incubated in DMSO-containing buffer to factor out any effects of the cosolvent on the enzyme activity. The K_i value was determined by a Dixon plot.[20]

Metabolite Studies. To a solution of **3b** (12.4 mM) in Tris-HCl buffer (3.0 mL, 100 mM, pH 9.0) was added MAO B (220 μL, 17.75 mg/mL). Aliquots of 20 μL were periodically withdrawn, extracted with 40 μL of methylene chloride by

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spinning, concentrated by gently blowing nitrogen over the surface of the liquid, and analyzed by gas chromatography. When the starting material was consumed, the reaction mixture was extracted with dichloromethane (2 × 5 mL). The organic layers were combined, washed with brine, dried over Na_2SO_4, and concentrated for NMR analysis: ^{1}H NMR ($CDCl_3$, 300 MHz) 8.47 (s, 1H), 8.03−8.05 (d, 1H), 7.59−7.62 (d, 1H), 7.51−7.54 (d, 1H), 7.32−7.48 (m, 7H), 6.78−6.81 (d, 1H), 6.70−6.73 (d, 1H), 4.76 (s, 1H).

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