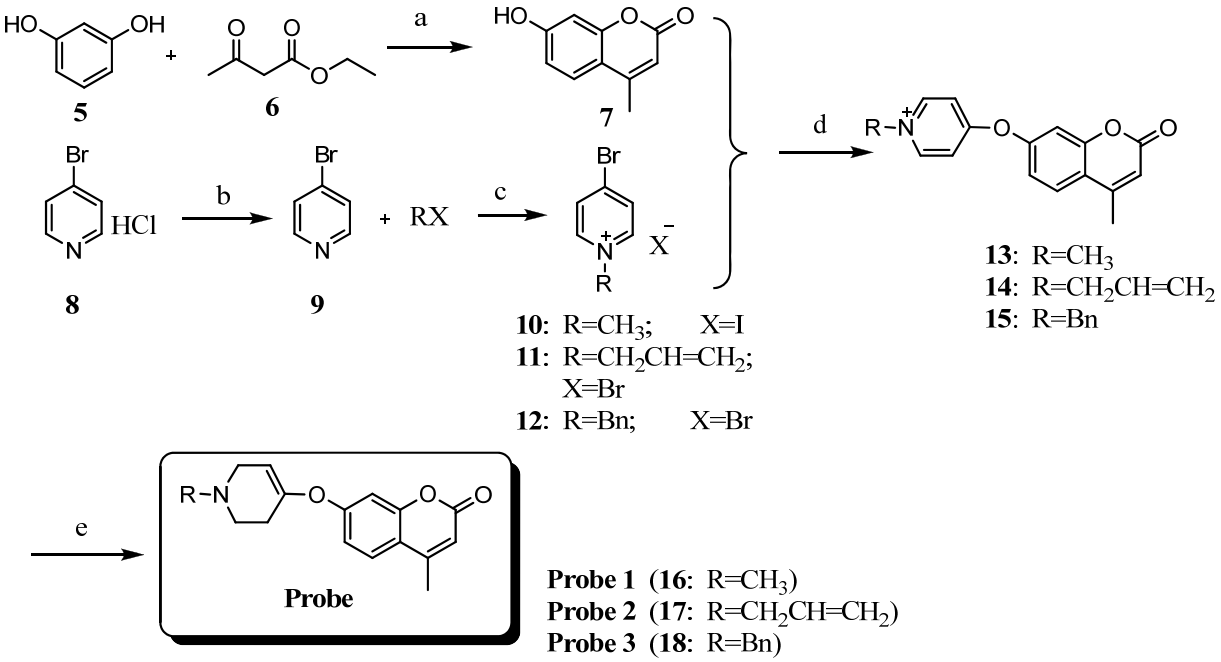
Electronic Supplementary Material (ESI) for Chemical Communications

An Activity-based Fluorogenic Probes for Sensitive and Selective Monoamine Oxidases-B detection

Experimental Section

Part I: Chemistry

Scheme S1: Syntheses of Probe 1, Probe 2 and Probe 3.



Scheme S1. (a) Concentrated sulfuric acid, CH_3CH_2OH, rt, 90.1 %. (b) $NaOH/H_2O$,0 ^oC, 90 %. (c) for 10: Iodomethane, CH_2Cl_2, 4 ^oC, 88 %; for 11: Allyl bromide, CH_2Cl_2, -20 ^oC, 82 %; for 12: Bian bromine, CH_2Cl_2, rt, 91 %. (d) for 13: Compounds 10, DMF/CH_3ONa, 75 %; for 14: Compounds 11, CH_3COCH_3/Et_3N, 61 %; for 15: Compounds 12, CH_3COCH_3 /Et_3N, 61 %. (e) $NaBH_4$/MeOH, 0 ^oC, 95 %.

4-Methyl-7-hydroxy-coumarin (7). Resorcinol **5** (11.0 g, 100 mmol) and sulfuric acid (10 mL) was dissolved under cooling in anhydrous EtOH (20 mL). Ethyl acetoacetate **6** (13 g, 100 mmol) was added dropwise over a period of 30 min. The mixture was allowed to warm up to room temperature and stirred further for 2 hours. The resulting solution was poured onto crushed ice, and the formed white precipitate was collected by filtration, washed with water, dichloromethane and dried under reduced pressure. Crude product was purified by crystallization from ethanol to afford a white needle-like solid product **7** (15.86 g, 90.1%). ^{1}H NMR (500 MHz, d^6-DMSO): δ 10.51 (s, 1H), 7.59 (d, *J* = 8.8 Hz, 1H), 6.80 (d, *J* = 2.4 Hz, 1H), 6.70 (s, 1H), 6.13 (s, 1H), 2.50 (s, 3H); ESI-MS *m/z* 177.2 $(M+1)^+$.

1-Methyl-4-bromo-pyridyl iodized salt (10). 4-Bromo-pyridine hydrochloride (**8**, 1.93 g, 10 mmol) was dissolved in 50 mL aqueous sodium hydroxide solution (0.008 g/ml), and the resulting solution was stirred for 10 min. The reaction mixture was extracted with CH_2Cl_2 (100 mL × 3), and the organic layer was washed with water (100 mL), dried over Na_2SO_4 and concentrated in vacuo to afford a pale yellow oil **9 (**1.40 g, 91 %). Next, compound **9 (**1.256 g, 8 mmol) was dissolved in 20 mL CH_2Cl_2, followed by the addition of iodomethane (16 mmol). The resulting mixture was stirred overnight at 4 ^{o}C. The formed yellow precipitate was collected by filtration, washed with n-hexane affording yellow product **10** (2.03 g, 88%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.95 (d, *J* = 7.0 Hz, 2H), 8.51 (d, *J* = 7.0 Hz, 2H), 1.59 (s, 3H); ESI-MS m/z 173.3 $(M+1)^+$.

1-Allyl-4-bromide-pyridine bromide salt (11). Compound **9 (**1.256 g, 8 mmol) was dissolved in 20 mL CH_2Cl_2, then allyl bromide (16 mmol) was added. The resulting mixture was stirred overnight at -20 ^{o}C. The formed reddish-brown precipitate was collected by filtration, washed with n-hexane affording reddish-brown product **11** (1.85 g, 82%). ^{1}H NMR (500 MHz，$CDCl_3$): δ 8.85 (d, *J* = 7.0 Hz, 2H), 8.42 (d, *J* = 7.0 Hz, 2H), 6.15-6.23 (m, 1H), 5.57-5.60 (m, 2H), 5.24 (d, *J* = 6.0 Hz, 2H); ESI-MS *m/z* 173.6 $(M+1)^+$.

1-Benzyl-4-bromo-pyridinium bromide salt (12). Compound **9 (**1.256 g, 8 mmol) was dissolved in 20 mL CH_2Cl_2, then benzyl bromide (16 mmol) was added. The resulting solution

was stirred for 8 h at room temperature, and the resultant golden yellow precipitate was collected by filtration, washed with n-hexane affording golden yellow product **12 (**2.33 g, 91 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.89 (d, J = 7.0 Hz, 2H), 8.46 (d, J = 7.0 Hz, 2H), 7.47 (d, J = 3.75 Hz, 2H), 7.41 (m, 3H), 3.18 (s, 2H); ESI-MS *m/z* 249.6 $(M+1)^+$.

4-Methyl-7-(1-methyl-1,2,3,6-tetrahydropyridin-4-yloxy)-2H-chromen-2-one(16, Probe 1). 4-Methyl-7-hydroxy-coumarin (0.088 g, 0.5 mmol) and sodium methoxide (0.0324 g, 0.6 mmol) were dissolved in 2 mL DMF. After the mixture was stirred for 10 min, the compound **10** was added and the resulting mixture was stirred overnight at room temperature. The resultant precipitate was collected by filtration, washed with ether to afford a yellow product **13 (**0.087 g, 0.22 mmol). To compound **13** (0.079 g, 0.2 mmol) in MeOH (10 mL) was added $NaBH_4$ (0.0432 g, 0.08 mmol) at 0 °C, and the reaction mixture was stirred for 10 min. After the organic solvent was removed under reduced pressure, water (30 mL) was added to the aqueous residue, extracted with AcOEt (30 mL×3), and dried over anhydrous $MgSO_4$. After evaporation of the solvent, the residue was purified by SiO_2 chromatography (CH_2Cl_2:MeOH = 20:1) to give compound **16** (0.015 g, 75 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.44 (d, *J* = 5.5 Hz, 2H), 6.89-6.92 (m, 3H), 6.01 (s, 1H), 5.09-5.11 (m, 1H), 2.97-2.99 (m, 2H), 2.62-2.64 (m, 2H), 2.35 (d, *J* = 1.1 Hz, 3H), 2.33 (d, *J* = 1.2 Hz, 3H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 18.7, 27.2, 45.3, 52.0, 52.0, 105.9, 107.5, 112.8, 115.0, 115.2, 125.7, 150.4, 152.3, 154.9, 159.3, 160.9; ESI-MS *m/z* 272.8 $(M+1)^+$. HRMS (EI) calcd for C16H18NO3: 272.1287; found: 272.1383. Anal. calcd for C16H17NO3 C, 70.57; H, 6.66; N, 5.14; found C, 70.35; H, 6.53; N, 5.26.

7-(1-Allyl-1,2,3,6-tetrahydropyridin-4-yloxy)-4-methyl-2H-chromen-2-one (17, Probe 2). To a mixture of 4-methyl-7-hydroxy-coumarin **7 (**0.022 g, 0.125 mmol) and compound **11** (0.0345 g, 0.125 mmol) in 3 mL of acetone was added Et_3N (80μl) at room temperature. After stirring for 2 hours, solid precipitate was collected by filtration to afford black product **14** (0.0362 g, 0.086 mmol). To compound **14** (0.0362 g, 0.086 mmol) in MeOH (10 mL) was added $NaBH_4$ (0.027 g, 0.50 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 10 min. After the organic solvent was removed under reduced pressure, water (30 mL) was added to the aqueous residue, extracted with AcOEt (30 mL × 3), and the combined organic layer was dried over $MgSO_4$. The crude residue was purified by SiO_2 chromatography (CH_2Cl_2:MeOH=20:1) to give white product **17** (0.022 g, 61 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.54 (d, *J* = 7.0 Hz, 1H), 7.01-7.03 (m, 1H), 6.98

(d, *J* = 7.5 Hz, 1H), 6.20 (s, 1H), 5.939-6.00 (m, 1H), 5.24-5.27 (m 2H), 5.16-5.17 (d, *J* = 3.0 Hz, 1H), 3.22-3.23 (m, 2H), 3.17 (s, 2H), 2.82-2.84 (m, *J* = 11.0 Hz, 2H), 2.42-2.44 (m, *J* = 7.0 Hz, 2H), 2.11 (s, 3H); ^{13}C NMR (500 MHz, $CDCl_3$): 18.8, 27.2, 49.5, 51.0, 62.0, 105.8, 107.7, 112.8, 115.1, 125.7, 127.3, 129.1, 138.0, 150.5, 152.4, 154.9, 159.4, 161.0; ESI-MS *m/z* 298.5 $(M+1)^+$. HRMS (EI) calcd for C18H21NO3: 298.1443; found: 298.1457. Anal. calcd for C18H20NO3 C, 72.46; H, 6.76; N, 4.69; found C, 72.57; H, 6.63; N, 4.76.

7-(1-Benzyl-1,2,3,6-tetrahydropyridin-4-yloxy)-4-methyl-2H-chromen-2-one (18，Probe 3). This compound was prepared according to the same procedure for the synthesis of 17 by using 1-Benzyl-4-bromo-pyridinium bromide salt **(12)** in place of 1-allyl-4-bromide-pyridine bromide salt **(11)**. Yield: 61 %. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.54 (d, *J* = 8.5 Hz, 1H), 7.42 (d, *J* = 3.75 Hz, 2H), 7.36 (d, *J* = 7.5 Hz, 2H), 7.02 (d, *J* = 3.66 Hz, 2H), 6.98 (d, *J* = 2.5 Hz, 1H), 6.20 (d, *J* = 1.0 Hz, 1H), 5.15 (d, *J* = 3.5 Hz, 1H), 3.18 (s, 2H), 2.83 (s, 1H), 2.41-2.43 (m, 2H), 2.11 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 161.0, 159.4, 154.9, 152.4, 150.5, 157.9, 129.1, 128.4, 127.3, 125.8, 115.1, 112.8, 107.7, 105.8, 77.4, 77.2, 76.9, 62.0, 50.9, 49.5, 27.2, 18.8; ESI-MS *m/z* 348.6 $(M+1)^+$. HRMS (EI) calcd for C22H23NO3: 348.1600; found: 348.1657. Anal. calcd for C22H22NO3 C, 75.84; H, 6.36; N, 4.02; found C, 75.67; H, 6.23; N, 4.26.

Part II: Enzymatic Assays

Enzymatic activity Assays. The detection of activity of enzymes with Probe were performed in 96-well fluorescence assay plates. The stock solutions of Probe 1 were prepared in DMSO (10mM) and diluted in enzyme assay buffer (100 mM Borate buffer, pH=8.4) to a final concentration 200 μM. MAO A, MAO B, diamine oxidase and heat-inactivated enzyme were added to a final protein concentration 40 μg/mL and incubation for 2 h at 37 ºC, respectively. Fluorescence emission spectra of probe 1-3 were recorded by using Molecular Devices Spectramax M2 Microplate Spectrofluorometer after 2 h. The excitation wavelength was 360 nm. Data represent the means of three separate experiments and error bars represents standard deviation.

Table S1 MAOs selectivity of **Probe 1-3**

	Probe 1	Probe 2	Probe 3
MAO A	1523±64	1213±51	1142±53
MAO B	33113±196	3884±114	3688±138
Diamine oxidase	1026±49	815±31	712±32
Inactivating enzyme	182±8	186±8	213±9
Control	156±6	154±7	160±7

Enzymatic Kinetics Assays. Enzyme kinetics experiments were performed in 96-well fluorescence assay plates. A series concentrations of Probe 1 was diluted in enzyme assay buffer (100 mM Borate buffer, pH = 8.4) to a final concentration containing (0-200 μM). The fluorescence intensity was collected at 460 nm (λ_{ex} = 360 nm) by using Molecular Devices Spectramax M2 Microplate Spectrofluorometer at 5 min intervals from 0 to 1 h at 37 ºC. Enzyme kinetics experiments with Probe 1 and MAO B were performed on three independent experiments.

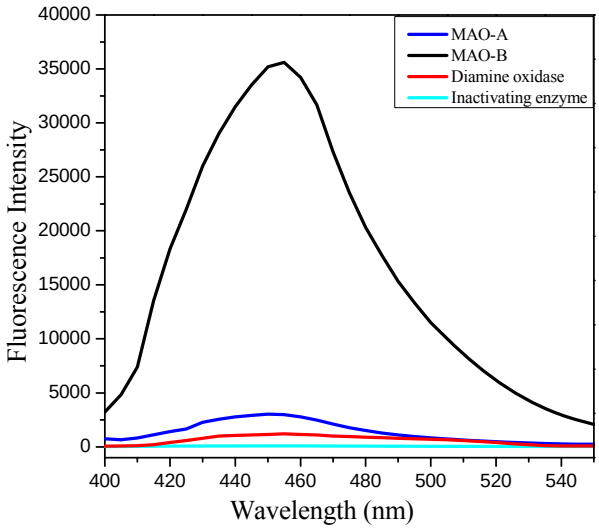


Figure S1. Emission spectra of probe 1. These spectra were recorded after 2 h reaction with each type of MAO A and MAO B /Diamine oxidase/Inactivating enzyme at 37 ^{o}C in enzyme assay buffer (100 mM borate buffer, pH=8.4). The excitation wavelength was 360 nm and enzyme concentration was 40 μg mL^{-1}.

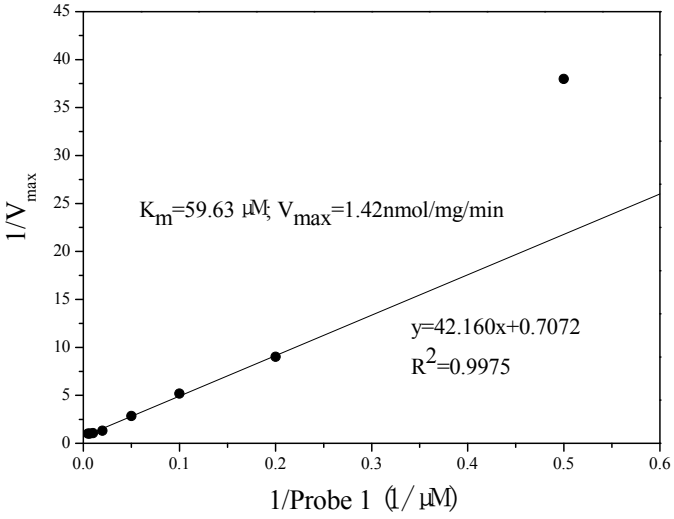


Figure S2. K_m values of Probe 1 with MAO. The K_m and V_{max} value MAO-B was assessed by a series concentrations of Probe 1 (0-200 μM) reaction with MAO-B (40 μg mL^{-1}) at 37 ^oC in enzyme assay buffer (100 mM borate buffer, pH=8.4). The fluorescence intensity was collected at 460 nm (λ_{ex} =360 nm).