Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information for

PET-depedent Fluorescence Sensing of Enzyme Reactions Using a Large and Tunable pK_a Shift of Aliphatic Amines

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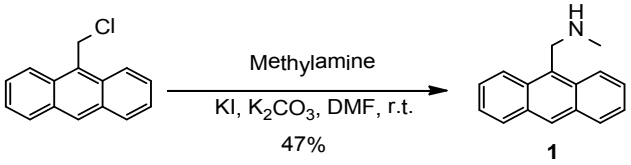
General Materials and Methods for Organic Synthesis.

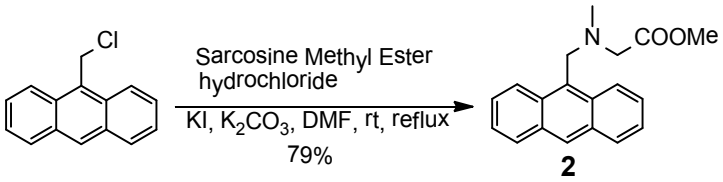
Unless otherwise noted, chemical reagents were purchased from commercial suppliers (Aldrich, Tokyo Chemical Industry (TCI), Wako Pure Chemical Industries and used without further purification. ^{1}H NMR spectra were recorded using a Varian UNITY-400 (400 MHz) spectrometer (Varian, Palo Alto, CA), and chemical shifts (δ, ppm) were referenced to residual solvent peak. ESI mass spectrometry was recorded using a Bruker micrOTOF II (Bruker Daltonics, Billerica, MA) spectrometer. HPLC purification was conducted with a HITACHI L-7100 (Hitachi, Japan).



Synthesis of 1

A mixture of 9-chloromethylanthracence (113 mg, 0.50 mmol), KI (42 mg, 0.25 mmol), K_2CO_3 (138 mg, 1.0 mmol), 40% methylamine methanol solution (0.10 mL, 0.98 mmol) in dry DMF (5 mL) was stirred for 3 h at rt. The mixture was acidified with HCl aq. (pH 4) and washed with AcOEt (20 mL×4). The aqueous layer was alkalized with NaOH aq. (pH 12) and extract with AcOEt (20 mL×3). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo to give **1** as a pale yellow solid (52 mg, 47%).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.65 (3H, s), 4.69 (2H, s), 7.43-7.54 (4H, m), 7.98–8.01 (2H, d, J = 8.8 Hz), 8.32-8.34 (2H, d, J = 8.8 Hz), 8.39 (1H, s). HRMS (ESI) calcd for $C_{16}H_{16}N$ $[M+H]^+$ 222.1283, found 222.1279.

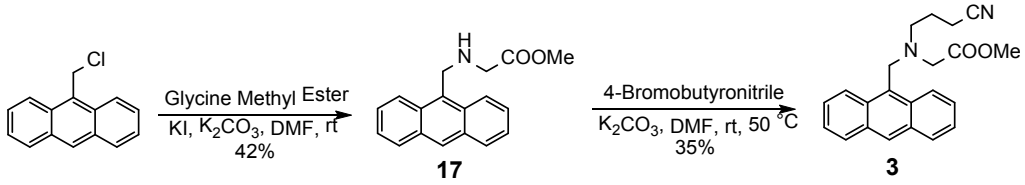


Synthesis of 2

A mixture of 9-chloromethylanthracence (227 mg, 1.0 mmol), KI (83 mg, 0.50 mmol), K_2CO_3 (276 mg, 2.0 mmol), sarcosine methyl ester hydrochloride (168 mg, 1.2 mmol) in dry DMF (5 mL) was stirred at rt for 2 h and further stirred with heating for 2 h. The mixture was cool to rt, diluted with saturated $NaHCO_3$ aq. (50 mL) and then

extracted with AcOEt (30 mL×3). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (SiO_2, hexane / AcOEt = 3 : 1) to give **2** as a yellow oil (232 mg, 79%).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.48 (3H, s), 3.39 (2H, s), 3.69 (3H, s), 4.68 (2H, s), 7.42-7.51 (4H, m), 7.97-7.99 (2H, d, *J* = 8.4), 8.41 (1H, s), 8.49-8.52 (2H, d, *J* = 9.2). HRMS (ESI) calcd for $C_{19}H_{19}NNaO_2$ $[M+Na]^+$ 316.1313, found 316.1312.



Synthesis of 17

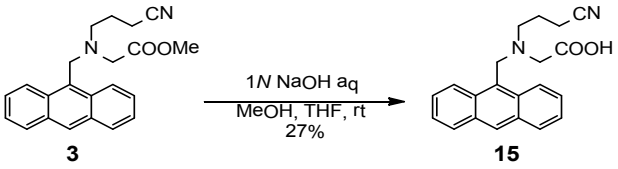
A mixture of 9-chloromethylanthracence (227 mg, 1.0 mmol), KI (83 mg, 0.50 mmol), K_2CO_3 (276 mg, 2.0 mmol), glycine methyl ester hydrochloride (151 mg, 1.2 mmol) in dry DMF (5 mL) was stirred for overnight at rt. The mixture was diluted with saturated $NaHCO_3$ aq. (50 mL) and extracted with AcOEt (30 mL×3). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was dissolved in AcOEt (100 mL) and extracted with 0.5*N* HCl aq. (50 mL×3). The combined aqueous layers were neutralized with $NaHCO_3$ aq. and extracted with AcOEt (50 mL×3). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (SiO_2, hexane / AcOEt = 1 : 1) to give **17** as a white solid (118 mg, 42%).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.62 (2H, s), 3.79 (3H, s), 4.75 (2H, s), 7.43-7.47 (2H, t, *J* = 7.2 Hz), 7.52-7.56 (2H, t, *J* = 7.4 Hz), 7.98-8.00 (2H, d, *J* = 8.0 Hz), 8.41-8.43 (3H, m). ESI-MS *m/z* 302.1 $[M+Na]^+$.

Synthesis of 3

A mixture of **17** (70 mg, 0.25 mmol), K_2CO_3 (69 mg, 0.50 mmol), 4-bromobutyronitrile (0.25 mL, 2.5 mmol) in dry DMF (2 mL) was stirred for overnight at rt. After addition of 4-bromobutyronitrile (0.25 mL, 2.5 mmol), the reaction mixture was further stirred for 10 h at 50 °C. The resulting mixture was diluted with saturated $NaHCO_3$ aq. (50 mL) and extracted with AcOEt (20 mL×3). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (SiO_2, hexane / AcOEt = 2 : 1) to give **3** as a white solid (30 mg, 35%).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.61-1.67 (2H, m), 2.04-2.08 (2H, t, *J* = 7.2 Hz), 2.87-2.90 (2H, t, *J* = 6.4 Hz), 3.38 (2H, s), 3.69(3H, s), 4.82 (2H, s), 7.43-7.47 (2H, t, *J* = 8.4 Hz), 7.50-7.54 (2H, t, *J* = 6.4 Hz), 7.98-8.00 (2H, d, *J* = 8.0 Hz), 8.43-8.45 (2H, m). HRMS (ESI) calcd for $C_{22}H_{22}N_2NaO_2$ $[M+Na]^+$ 369.1579, found 369.1578.

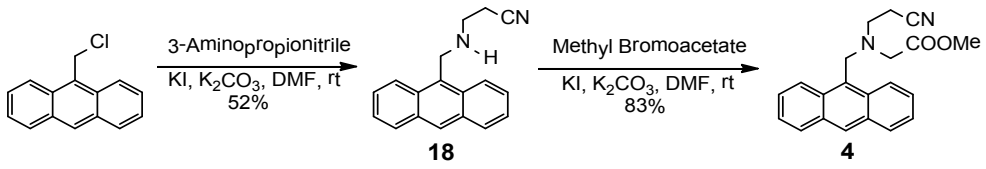


3 **15**

Synthesis of 15

A solution of **3** (19 mg, 0.055 mmol) in MeOH (1 mL)-THF (0.5 mL)-1*N* NaOH aq. (0.5 mL) was stirred for 45 min at rt. After dilution with water (100 mL), the mixture was acidified with 1*N* HCl aq. (~pH 5) and extracted with AcOEt (30 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was dissolved in acetone and then precipitated by addition of hexane. The precipitate was collected by filtration to give **15** as a pale yellow solid (5 mg, 27%)

^{1}H-NMR(400 MHz, $CDCl_3$): δ 1.82-1.89 (2H, m), 2.23-2.27 (2H, t, *J* = 7.2 Hz), 2.96-2.99 (2H, t, *J* = 7.2 Hz), 3.33 (2H, s), 4.82 (2H, s), 7.46-7.50 (2H, t, *J* = 7.2 Hz), 7.55-7.59 (2H, t, *J* = 8.4 Hz), 8.02-8.03 (2H, d, *J* = 7.6 Hz), 8.32-8.34 (2H, d, *J* = 8.8 Hz), 8.47 (1H, s) HRMS (ESI) calcd for $C_{21}H_{20}N_2NaO_2$ $[M+Na]^+$ 355.1412, found 355.1417.



18 **4**

Synthesis of 18

A mixture of 9-chloromethylanthracence (340 mg, 1.5 mmol), KI (125 mg, 0.75 mmol), K_2CO_3 (415 mg, 3.0 mmol), 3-aminopropionitrile (0.133 mL, 1.8 mmol) in dry DMF (7.5 mL) was stirred for overnight at rt. The mixture was diluted with saturated $NaHCO_3$ aq. (100 mL) and extracted with AcOEt (30 mL×3). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (SiO_2, hexane / AcOEt = 1 : 1) to give **18** as a pale a yellow oil (259 mg, 52%).

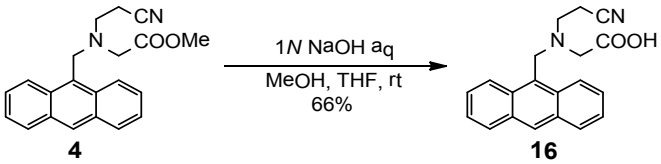
^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.55-2.59 (3H, t, *J* = 6.4 Hz), 3.12-3.15 (2H, t, *J* = 6.4 Hz), 4.79 (2H, s), 7.44-7.48 (2H, t, *J* = 7.6 Hz), 7.52-7.56 (2H, t, *J* = 7.6 Hz), 8.00-8.02

(2H, d, J = 8.4 Hz), 8.30-8.32 (2H, d, J = 8.4 Hz), 8.42 (1H, s). ESI-MS *m/z* 283.1 $[M+Na]^+$.

Synthesis of 4

A mixture of **18** (182 mg, 0.70 mmol), KI (66 mg, 0.40 mmol), K_2CO_3 (207 mg, 1.5 mmol), metyl bromoacetate (0.138 mL, 1.5 mmol) in dry DMF (5 mL) was stirred for overnight at rt. The mixture was diluted with saturated $NaHCO_3$ aq. (100 mL) and extracted with AcOEt (40 mL×3). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (SiO_2, hexane / AcOEt = 3 : 2) to give **4** as a pale yellow solid (192 mg, 83%).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.26-2.30 (2H, t, J = 7.2 Hz), 3.19-3.22 (2H, t, J = 7.2 Hz), 3.46 (2H, s), 3.68 (3H, s), 4.89 (2H, s), 7.44-7.47 (2H, t, J = 7.6 Hz), 7.51-7.54 (2H, t, J = 7.6 Hz), 7.98-8.00 (2H, d, J = 8 Hz), 8.42-8.46 (3H, m). HRMS (ESI) calcd for $C_{21}H_{20}N_2NaO_2$ $[M+Na]^+$ 355.1422, found 355.1422.

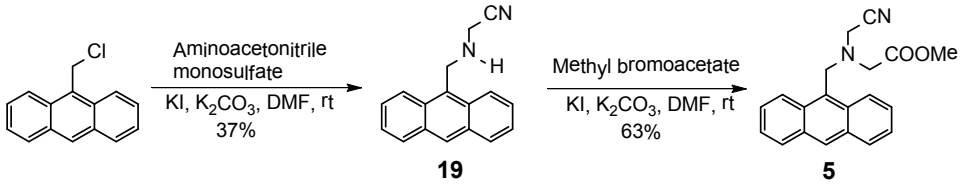


4 **16**

Synthesis of 16

A solution of **4** (33 mg, 0.10 mmol) in MeOH (3 mL)-THF (1 mL)-1*N* NaOH aq. (0.5 mL) was stirred for 20 min at 0 °C and then stirred for 40 min at rt. After addition of 1*N* NaOH aq. (0.5 mL), the reaction mixture was further stirred for 1 h at rt. After dilution with water (30 mL), the aqueous layer was washed with AcOEt-Hexane (2:1, 15 mL) and then acidified with 1*N* HCl aq. (~pH 5). Precipitate was collected by filtration to give 1**6** as a pale yellow solid (21 mg, 66%).

^{1}H-NMR(400 MHz, $CDCl_3$) : δ 2.46-2.49 (2H, t, J = 6.8 Hz), 3.20-3.23 (2H, t, J = 6.8 Hz), 3.41 (2H, s), 4.88 (2H, s), 7.46-7.50 (2H, t, J = 7.2 Hz), 7.56-7.60 (2H, t, J = 8 Hz), 8.02-8.03 (2H, d, J = 7.6 Hz), 8.36-8.38(2H, d, J = 8.8 Hz), 8.48 (1H, s). HRMS (ESI) calcd for $C_{20}H_{18}N_2NaO_2$ $[M+Na]^+$ 341.1266, found 341.1266.



19 **5**

Synthesis of 19

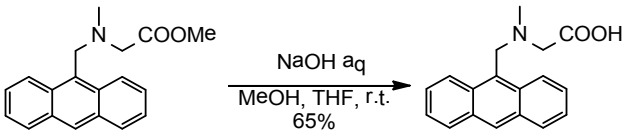
A mixture of 9-chloromethylanthracence (227 mg, 1.0 mmol), KI (83 mg, 0.50 mmol), K_2CO_3 (568 mg, 4.1 mmol), aminoacetnitrile monosulfate (231 mg,1.5 mmol) in DMF (2 mL) was stirred for overnight at rt. The mixture was diluted with saturated $NaHCO_3$ aq. (50 mL) and extracted with AcOEt (30 mL×3). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (SiO_2, hexane / AcOEt = 3 : 1) to give **19** as yellow solid (92 mg, 37%).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.73 (2H, s), 4.88 (2H, s), 7.45-7.49 (2H, t, *J* = 8.0 Hz), 7.54-7.58 (2H, t, *J* = 8.0 Hz), 8.00-8.02 (2H, d, *J* = 8.0 Hz), 8.35-8.37(2H, d, *J* = 8.8 Hz), 8.44 (1H, s). ESI-MS *m/z* 269.1 $[M+Na]^+$.

Synthesis of 5

A mixture of **19** (86 mg, 0.35 mmol), KI (33 mg, 0.20 mmol), K_2CO_3 (97 mg, 0.70 mmol), methyl bromoacetate (0.065 mL, 0.70 mmol) in dry DMF (3 mL) was stirred for overnight at rt. The mixture was diluted with saturated $NaHCO_3$ aq. (50 mL) and extracted with AcOEt (30 mL×3). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (SiO_2, hexane / AcOEt = 2 : 1) to give **5** as a pale yellow solid (70 mg, 63%).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.55 (2H, s), 3.69 (2H, s), 3.70 (3H, s), 4.80 (2H, s), 7.45-7.49 (2H, t, *J* = 7.6 Hz), 7.54-7.57 (2H, d, *J* = 8.0 Hz), 7.99-8.02 (2H, d, *J* = 8.4 Hz), 8.46-8.48 (2H, d, *J* = 7.6 Hz), 8.51 (1H, s). HRMS (ESI) calcd for $C_{20}H_{18}N_2NaO_2$ $[M+Na]^+$ 341.1266, found 341.1268.

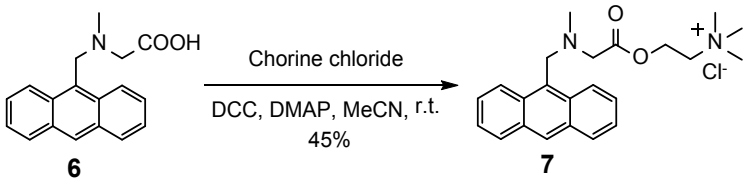


2 **6**

Synthesis of 6

A solution of **2** (189 mg, 0.64 mmol) in MeOH (7 mL)-THF (3 mL)-1*N* NaOH aq. (3.6 mL) was stirred for overnight at rt. After dilution with water (50 mL), the resultant mixture was evaporated to remove the organic solvent. The aqueous layer was washed with AcOEt and then acidified with 1*N* HCl aq. (~pH 5). Precipitate was collected by filtration to give **6** as a pale yellow solid (118 mg, 65%).

^{1}H-NMR (400 MHz, DMSO-d_6): δ 2.29 (3H, s), 3.33 (2H, s), 4.66 (2H, s), 7.49-7.56 (4H, m), 8.07-8.09 (2H, d, J = 8.8), 8.58-8.59 (3H, s+d). HRMS (ESI) calcd for $C_{18}H_{17}NNaO_2$ $[M+Na]^+$ 302.1157, found 302.1153.



6 **7**

Synthesis of 7

A mixture of **6** (70 mg, 0.25 mmol), 4-dimethylaminopyridine (6 mg, 0.05 mmol), DCC (62 mg, 0.3 mmol), Chorine chloride (35 mg, 0.25 mmol) in dry CH_3CN (3 mL) was stirred for 40 h at rt. After concentration in vacuo, the residue was diluted with water (40 mL). The aqueous layer was washed with $CHCl_3$ (20 mL×5) and then concentrated in vacuo. The residue was purified by reverse-phase column chromatography (H_2O / MeOH = 1 : 10) to give a yellow oil. The oil was dissolved in acetone and then precipitated by addition of hexane. The precipitate was collected by filtration to give **7** as a yellow solid (45 mg, 45% calcd as chloride salt).

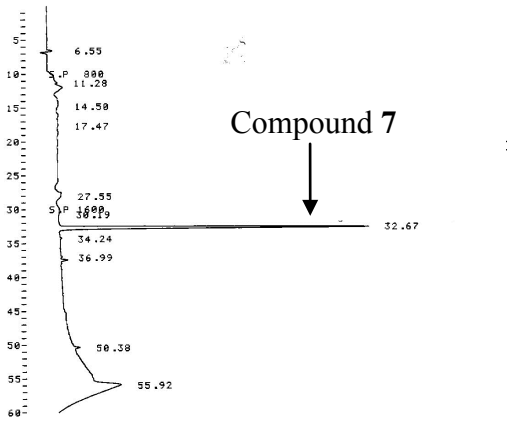
^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.31 (3H, s), 3.06 (9H, s), 3.63 (2H, br), 4.48 (2H, br), 4.62 (2H, s), 7.49-7.58 (4H, m), 8.08-8.10 (2H, d, J = 8.4 Hz), 8.54-8.56 (2H, d, J = 8.8 Hz), 8.58 (1H, s). HRMS (ESI) calcd for $C_{23}H_{29}N_2NaO_2$ $[M-Cl]^+$ 365.2224, found 365.2226.

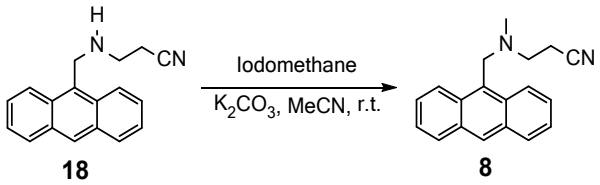
For the enzyme reaction, **7** was further purified by HPLC using the following conditions:

Column : YMC-Triart C18 250 mm×10 mm 1025001201

Gradient : CH_3CN(0.1%TFA) / H_2O(0.1%TFA) = 0 / 100 → 50 / 50(40 min) → 95 / 5(50 min)

Flow rate：3.0 mL/min　Detection：UV(220 nm)



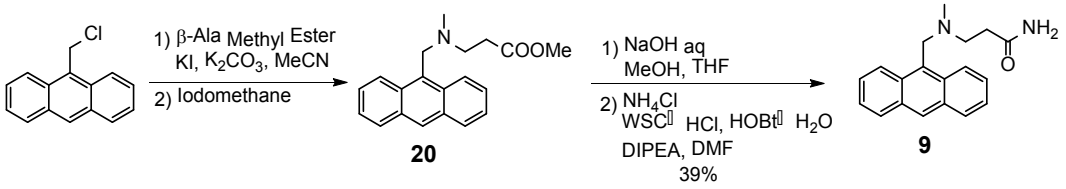


18 **8**

Synthesis of 8

A mixture of **18** (172 mg, 0.66 mmol), K_2CO_3 (182 mg, 1.32 mmol), MeI (50 μL, 0.80 mmol) in dry CH_3CN (3.0 mL) was stirred for overnight at rt. The mixture was diluted with saturated $NaHCO_3$ aq. (100 mL) and extracted with AcOEt (30 mL×3). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, hexane / AcOEt = 2 : 1) to give **8** as a yellow solid (135 mg, 74%).

^{1}H-NMR(400 MHz, $CDCl_3$): δ 2.35 (3H, s), 2.41-2.45 (2H, t, J = 6.8 Hz), 2.85-2.89 (2H, t, J = 6.8 Hz), 4.51 (2H, s), 7.43-7.47 (2H, m), 7.50-7.54 (2H, m), 8.00-8.01 (2H, d, J = 7.6 Hz), 8.41-8.44 (2H, d, J = 8.8 Hz), 8.43 (1H,s). HRMS (ESI) calcd for $C_{19}H_{18}N_2Na$ $[M+Na]^+$ 297.1368, found 297.1362.



20 **9**

Synthesis of 20

A mixture of 9-chloromethylanthracene (181 mg, 0.80 mmol), β-alanine methyl ester hydrochloride (140 mg, 1.0 mmol), KI (83 mg, 0.5 mmol), K_2CO_3 (415 mg, 3 mmol), in dry CH_3CN (3.0 mL) was stirred at rt. After stirring for 8 h, MeI (125 μL, 2.0 mmol) was added and the mixture and further stirred for 1.5 h at rt. After dilution with saturated $NaHCO_3$ aq. (100 mL), the resultant mixture was extracted with AcOEt (30

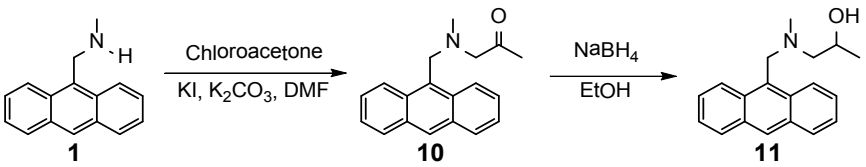
mL×3). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, hexane / AcOEt = 4 : 1) to give crude **20** as a yellow solid (62 mg).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.24 (3H, s), 2.52-2.55 (2H, t, *J* = 6.8 Hz), 2.90-2.94 (2H, t, *J* = 6.8 Hz), 3.48 (3H, s), 4.44 (2H, s), 7.41-7.50 (4H, m), 7.97-7.99 (2H, d, *J* = 8.4 Hz), 8.40-8.45 (3H, s+d). ESI-MS *m/z* 308.2 $[M+H]^+$.

Synthesis of 9

The crude of **20** (59 mg) in MeOH (4 mL)-THF (1.5 mL)-1*N* NaOH aq. (2 mL) was stirred for 1 h at rt. After neutralization with HCl aq., the mixture was concentration in vacuo. The residue was dissolved in EtOH and the insoluble salt was removed by filtration. The filtrate was concentrated in vacuo to give the corresponding carboxylate of **20**. A solution of the carboxylate, WSC・HCl (58 mg, 0.3 mmol), HOBt・H_2O (46 mg, 0.3 mmol), NH_4Cl (27 mg, 0.5 mmol), DIPEA (0.21 mL, 1.2 mmol) in dry DMF (2.5 mL) was stirred for overnight at rt. The mixture was diluted with saturated $NaHCO_3$ aq. (100 mL) and extracted with AcOEt (30 mL×3). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, AcOEt / MeOH = 20 : 1 → 10 : 1) to give **9** as a yellow solid (22 mg, 39%).

^{1}H-NMR-(400 MHz, $CDCl_3$): δ 2.33-2.36 (2H, t, *J* = 6.0 Hz), 2.40 (3H, s), 2.73-2.76 (2H, t, *J* = 6.8 Hz), 4.50 (2H, s), 4.73 (1H, br), 7.44-7.53 (4H, m), 7.62 (1H, br), 8.00-8.02 (2H, d, *J* = 8.0 Hz), 8.32-8.34 (2H, d, *J* = 9.6 Hz), 8.44 (1H, s). HRMS (ESI) calcd for $C_{19}H_{20}N_2NaO$ $[M+Na]^+$ 315.1473, found 315.1468.



1 **10** **11**

Synthesis of 10

A mixture of **1** (155 mg, 0.7 mmol), chloroacetone (0.1 mL, 1.2 mmol), KI (58 mg, 0.35 mmol), K_2CO_3 (197 mg, 1.4 mmol) in dry DMF (5 mL) was stirred for 1 h at rt. The mixture was diluted with saturated $NaHCO_3$ aq. (100 mL) and extracted with AcOEt (30 mL×3). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, hexane / AcOEt = 2 : 1) to give **10** as a pale yellow oil (179 mg, 92%).

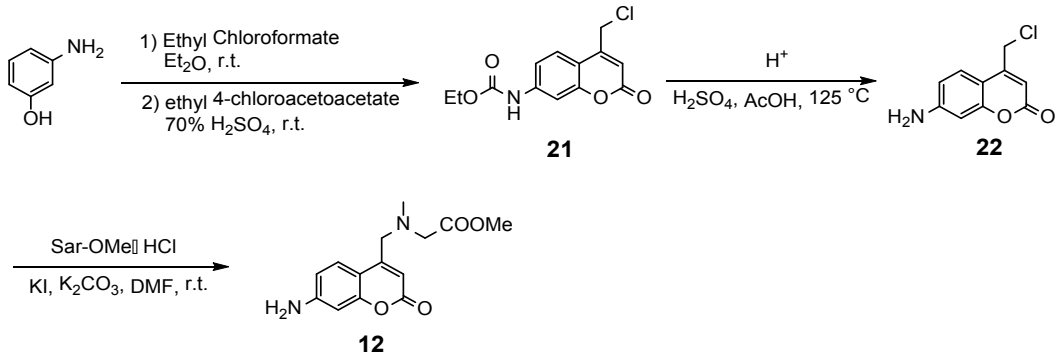
^{1}H-NMR(400 MHz, $CDCl_3$): δ 1.92 (3H, s), 2.44 (3H, s), 3.20 (2H, s), 4.58 (2H, s),

7.43-7.47 (2H, t, J = 7.4 Hz), 7.50-7.54 (2H, d, J = 7.6 Hz), 7.98-8.00 (2H, d, J = 8.8 Hz), 8.42 (1H, s), 8.48-8.50 (2H, d, J = 9.2 Hz). HRMS (ESI) calcd for $C_{19}H_{19}NNaO$ $[M+Na]^+$ 300.1364, found 300.1351.

Synthesis of 11

To a solution of **10** in dry EtOH (5 mL) was added portionwise $NaBH_4$ (378 mg, 10 mmol), and the mixture was stirred for 2 h at rt. After dilution with saturated $NaHCO_3$ aq. (100 mL), the resultant mixture was extracted with AcOEt (30 mL×3). The combined organic layers were washed with saturated $NaHCO_3$ aq. (100 mL) and dried over Na_2SO_4. The organic layer was concentrated in vacuo to give **11** as a pale yellow oil (118 mg, 90%).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.02-1.03 (3H, d, J = 6.0 Hz), 2.30 (3H, s), 2.47-2.48 (2H, d, J = 5.6 Hz), 3.83 (1H, br), 4.45-4.49 (1H, d, J = 12.8 Hz), 4.57-4.60 (1H, d, J = 12.4 Hz), 7.43-7.46 (2H, t, J = 7.0 Hz), 7.50-7.53 (2H, d, J = 7.6 Hz), 7.99-8.01 (2H, d, J = 7.6 Hz), 8.39-8.41 (2H, d, J = 8.4 Hz), 8.42 (1H, s). HRMS (ESI) calcd for $C_{19}H_{22}NO$ $[M+H]^+$ 280.1701, found 280.1692.



Synthesis of 21

To a solution of *m*-aminophenol (1.64 g, 15 mmol) in dry Et_2O (60 mL) was added dropwise ethyl chloroformate (1.43 mL, 15 mmol), and the mixture was stirred for 2 h at rt. After concentration in vacuo, the residue was dissolved in acetone and precipitated by addition of hexane. The precipitate was collected by filtration to give a white solid. This solid was dissolved in 70% H_2SO_4 and mixed with ethyl 4-chloroacetoacetate. After stirring for 3 h at rt, the mixture was poured into ice-water. The precipitate was collected by filtration and washed with EtOH to give **21** as a white solid (1.47 g, 35%).

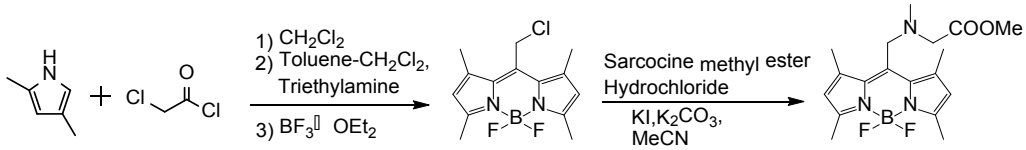
^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.24-1.28 (3H, t, J = 7.2 Hz), 4.14-4.19 (2H, m), 4.96 (2H,s), 6.50 (1H,s), 7.21-7.43 (1H, m), 7.58-7.59 (1H, d, J = 2.0 Hz), 7.74-7.76 (1H, d, J = 8.8 Hz), 10.16 (1H,s, br). ESI-MS *m/z* 282.1 $[M+H]^+$.

Synthesis of 22

A mixture of **21** (563 mg, 2.0 mmol) in AcOH (1.7 mL)-H_2SO_4 (1.7 mL) was stirred at 125 °C for 2 h. After cooling to rt., the mixture was poured into ice-cold water (50 mL). The resultant mixture was alkalized with NaOH aq. (pH 9) to form the precipitate. The precipitate was washed with cold water to give **22** as a yellow solid (365 mg, 87%).
^{1}H-NMR (400 MHz, DMSO-d_6): δ 4.86 (2H, s), 6.17 (1H, s), 6.20 (2H, s, br), 6.427-6.433 (1H, d, J = 2.4 Hz), 6.56-6.59 (1H, m), 7.46-7.48 (1H, d, J = 8.8 Hz). ESI-MS *m/z* 232.0 $[M+Na]^+$.

Synthesis of 12

A mixture of **22** (52 mg, 0.25 mmol), KI (25 mg, 0.15 mmol), K_2CO_3 (138 mg, 1.0 mmol), sarcosine methyl ester hydrochloride (70 mg, 0.50 mmol) in dry DMF (3.0 mL) was stirred for 2 h at rt. The mixture was diluted with saturated $NaHCO_3$ aq. (80 mL) and extracted with AcOEt (30 mL×3). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, hexane / AcOEt = 1 : 1→2 : 3) to give **12** as a yellow solid (30 mg, 44%).
^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.43 (3H, s), 3.37 (2H,s), 3.71 (3H, s), 3.78 (2H, s), 4.12 (2H,s ,br), 6.22 (1H, s), 6.53 (1H, s), 6.55-6.56 (1H, d, J = 2.4 Hz), 7.70-7.68 (2H, d, J = 8.4 Hz). HRMS (ESI) calcd for $C_{14}H_{16}N_2NaO_4$ $[M+Na]^+$ 299.1008, found 299.1002.



23 **13**

Synthesis of 23

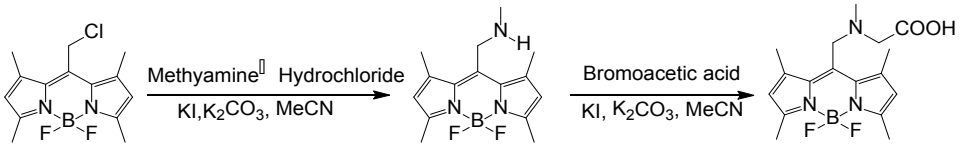
Compound **23** was prepared according to the synthetic method reported by Lippard et al.[S1]

Synthesis of 13

A mixture of **23** (30 mg, 0.10 mmol), KI (32 mg, 0.22 mmol), K_2CO_3 (30 mg, 0.22 mmol), sarcosine methyl ester hydrochloride (28 mg, 0.20 mmol) in dry CH_3CN (2.0 mL) was stirred for overnight at rt. After dilution with saturated $NaHCO_3$ aq. (50 mL), the mixture was extracted with AcOEt (30 mL×3). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash

column chromatography (SiO_2, hexane / AcOEt = 10 : 1) to give **13** as a red solid (18 mg, 50%).

^{1}H-NMR(400 MHz, $CDCl_3$): δ 2.44 (3H, s), 2.48 (6H, s), 2.50 (6H, s), 3.41 (2H, s), 3.66 (3H, s), 4.02 (2H, s), 6.04 (2H, s). HRMS (ESI) calcd for $C_{18}H_{24}BF_2N_2NaO_2$ [M +Na]$^+$ 386.1827, found 386.1826.



23 **24** **14**

Synthesis of 24

A mixture of **23** (152 mg, 0.51 mmol), KI (100 mg, 0.60 mmol), K_2CO_3 (276 mg, 2.0 mmol), methylamine hydrochloride (68 mg, 1.0 mmol) in dry CH_3CN (6.0 mL) was stirred for overnight at rt. After removal of the solvent in vacuo, the residue was diluted with saturated $NaHCO_3$ aq. (100 mL) and extracted with AcOEt (50 mL×3). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, hexane / AcOEt = 1 : 2) to give **24** as a red solid (24 mg, 16%).

^{1}H-NMR(400 MHz, $CDCl_3$): δ 2.45 (6H, s), 2.49 (6H, s), 2.53 (3H, s), 3.87 (2H, s), 6.04 (2H, s). ESI-MS *m/z* 292.2 [M+H]$^+$.

Synthesis of 14

A mixture of **24** (22 mg, 0.076 mmol), K_2CO_3 (21 mg, 0.15 mmol), bromoacetic acid (14 mg, 0.10 mmol) in dry CH_3CN (2.0 mL) was stirred for 6 h at rt. After removal of the solvent in vacuo, residue was diluted with saturated $NaHCO_3$ aq. (40 mL) and extracted with AcOEt (50 mL×3). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, AcOEt→ AcOEt / MeOH = 200 : 1 → 100 : 1) to give **14** as a red solid (9 mg, 34%).

^{1}H-NMR(400 MHz, $CDCl_3$): δ 2.45-2.52 (15H, m), 3.36 (2H, s), 4.03 (2H, s), 6.09 (2H, s). HRMS (ESI) calcd for $C_{17}H_{22}BF_2N_3NaO_2$ [M+Na]$^+$ 372.1671, found 372.1670.

Fluorescence pH titration

Fluorescent spectra were recorded on a Perkin-Elmer LS55 spectrometer. Fluorescence pH titration was conducted with a solution of the probe (1 □M) in 5 mM MES - 5 mM HEPES - 5 mM CHES buffer (25 mL) at 25 °C. The probe solution was first acidified with concentrated HCl solution, and then pH was adjusted by addition of a small amount of concentrated NaOH solution (ca. 27 M). The total volume of the NaOH solution added was less than 100 μL at the end of the titration. The fluorescence emission intensity change was measured at each pH conditions. The fluorescence titration curve was analyzed with a nonlinear least-square curve-fitting method fitted to Henderson-Hasselbalch equation to evaluate pK_a value.

Fluorescence Monitoring of the Enzyme reactions

Porcine Liver Esterase (Pig, EC 3.1.1.1) and AChE (Type VI-S, *Electrophorus electricus*, EC 3.1.1.7) were purchased from Sigma (St. Louis, MO, USA). Nitrile hydratase (EC 4.2.1.84) was kindly provided from Dia-Nitrix Co., Ltd. (Japan). Carbonyl redutase (Chiralscreen OH E078) was purchased from Daicel Corporation (Japan).

Generally, an assay solution containing the probe in 3 mL of assay buffer (50 mM HEPES, pH 7.4 or 20 mM phosphate, pH 7.0) was pre-incubated (25 °C or 37 °C) in a quartz cell. An appropriate amount of the enzyme was added to an assay solution, and the quartz cell was immediately set in the fluorescence spectrometer (Perkin-Elmer LS55). Fluorescence Intensity was measured automatically at each reaction time points.

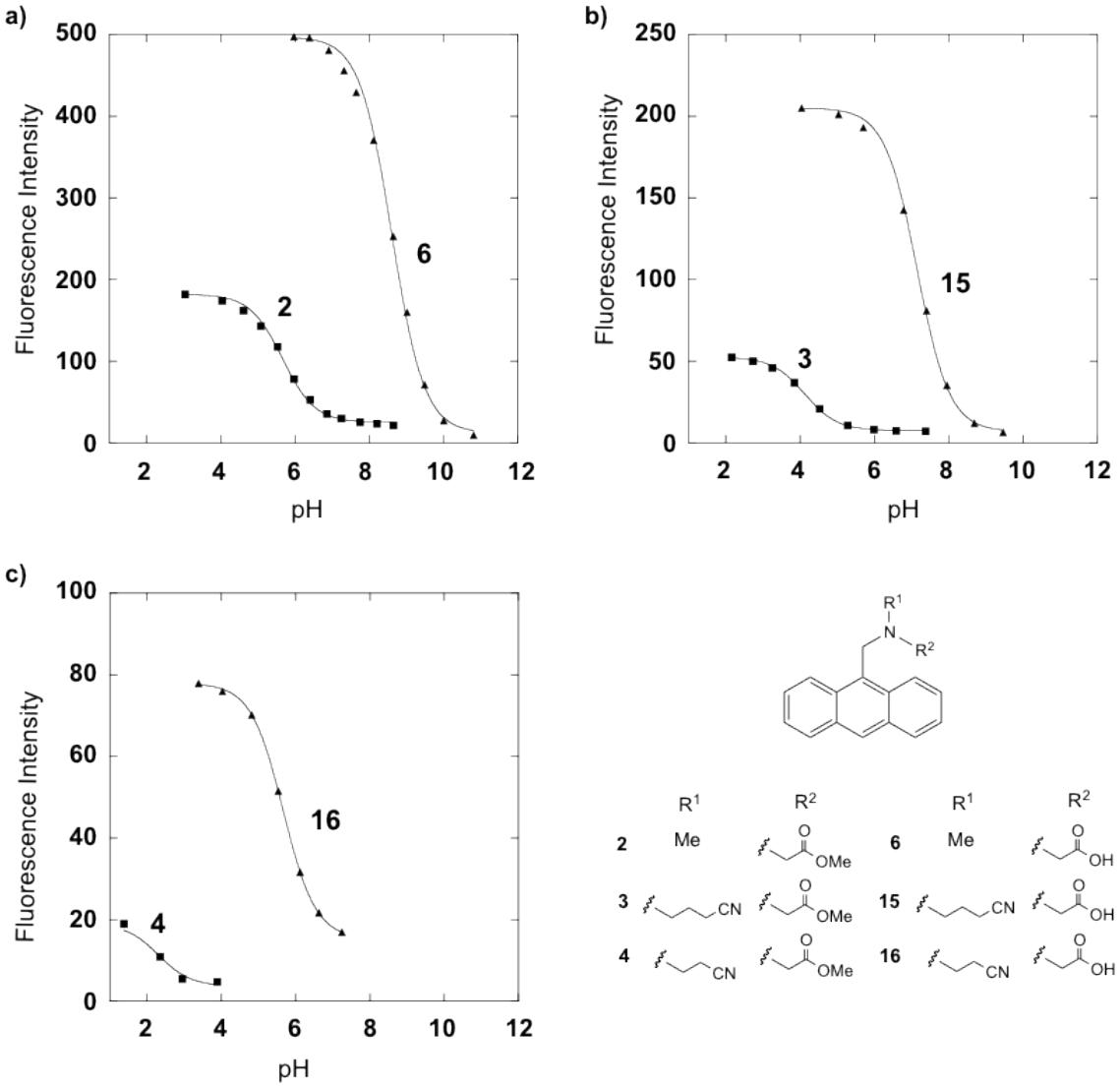


Fig. S1 pH-dependent fluorescent intensity changes of probe **2**, **3**, **4** and their corresponding carboxylates (**6**, **15**, **16**). Conditions: [probe] = 1 μM, 5 mM MES - 5 mM HEPES - 5 mM CHES buffer, λ_{ex} = 367 nm, 25 °C.

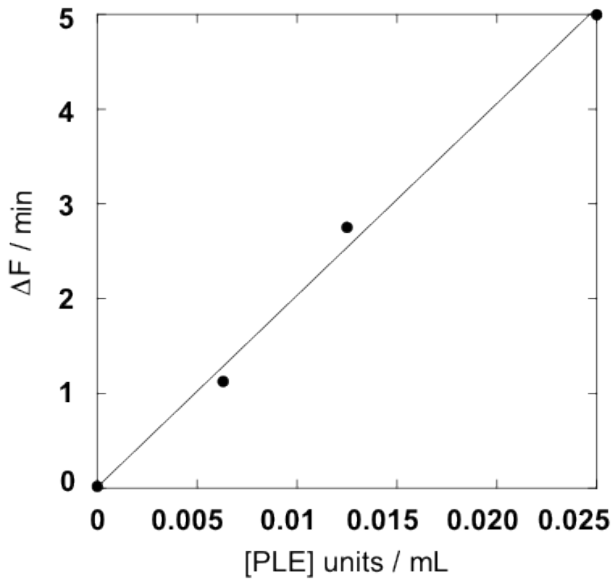


Fig. S2 Plot of the initial rate of the fluorescence increase (ΔF, min^{-1}) as a function of the amount of PLE. [PLE] = 0, 0.0063, 0.0125, 0.025 units/mL.

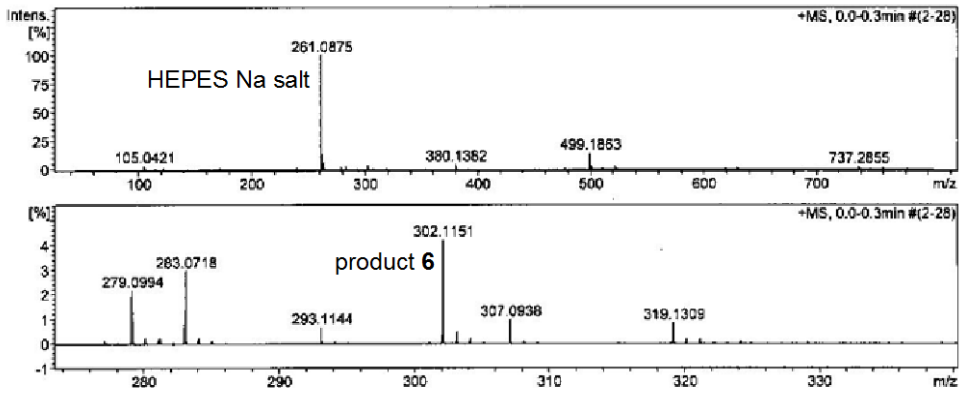


Fig. S3 ESI-MS detection of the PLE catalyzed hydrolysis of **2** to afford the product **6** (calcd. for $C_{18}H_{17}NNaO_2$ $[M+Na]^+$ 302.1157, found 302.1151).

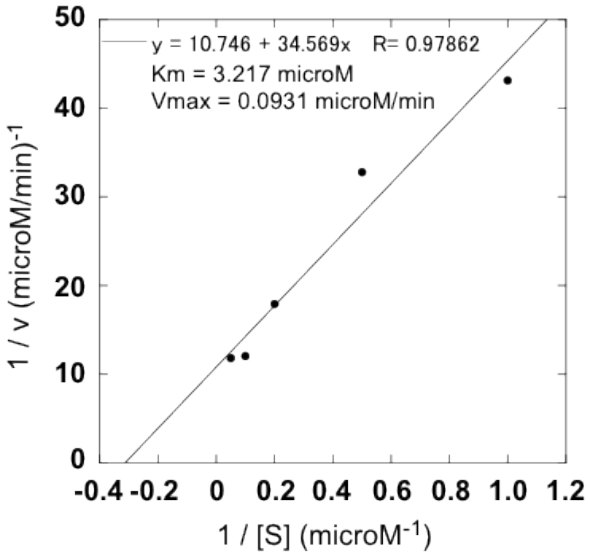


Fig. S4 Lineweaver-Burk plot of the PLE-catalyzed hydrolysis of **2**. [PLE] = 0.0125 units/mL. [**2**] = 1.0, 2.0, 5.0, 10, 20 microM. 50 mM HEPES buffer (pH 7.4), 37 °C.

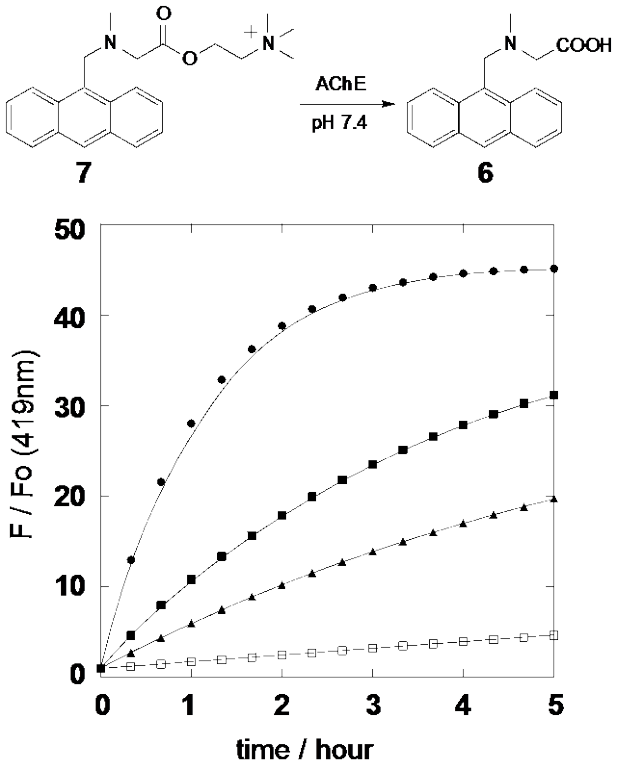


Fig. S5 Real-time fluorescent monitoring of the AChE catalyzed hydrolysis of **7**. Conditions: [**7**] = 1 μM, [AChE] = 19.92(●), 5.08(■), 2.54(▲), and 0 (□) units/mL, 50 mM HEPES buffer, pH 7.4, λ_{ex} = 367 nm, 37 °C.

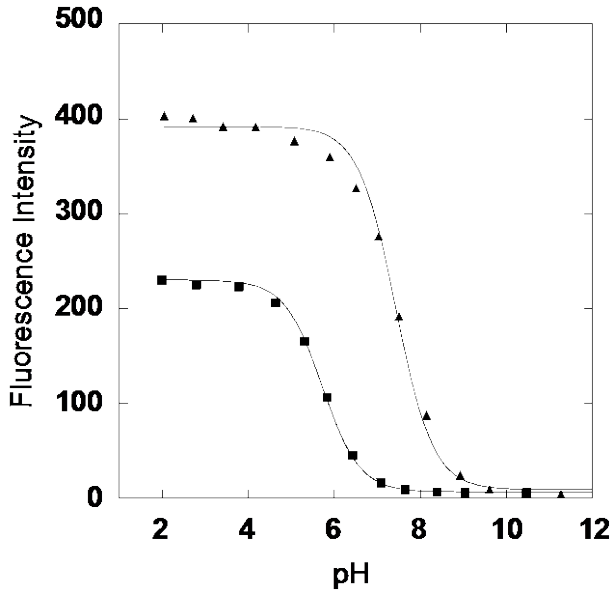


Fig. S6 pH-dependent fluorescent intensity changes of compound **8** (■) and **9** (▲). Conditions: [probe] = 1 μM, 5 mM MES - 5 mM HEPES - 5 mM CHES buffer, λ_{ex} = 367 nm, 25 °C.

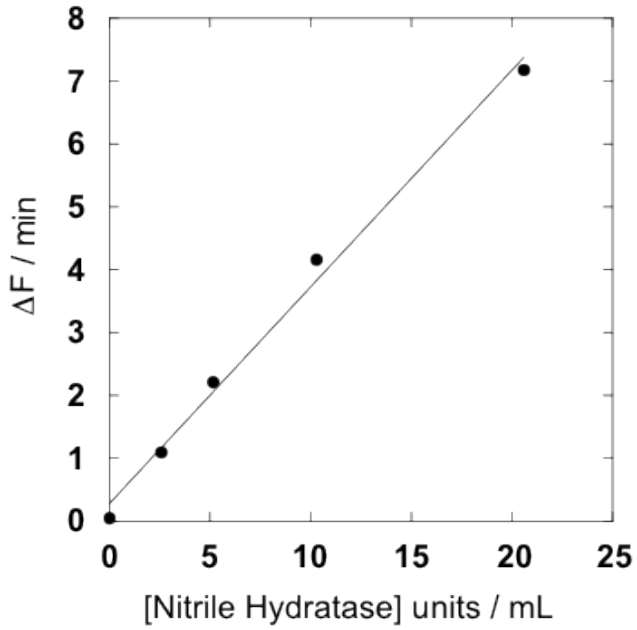


Fig. S7 Plot of the initial rate of the fluorescence increase (ΔF, min^{-1}) as a function of the amount of nitrile hydratase. [Nitrile Hydratase] = 0, 2.58, 5.16, 10.3, 20.6 units/mL.

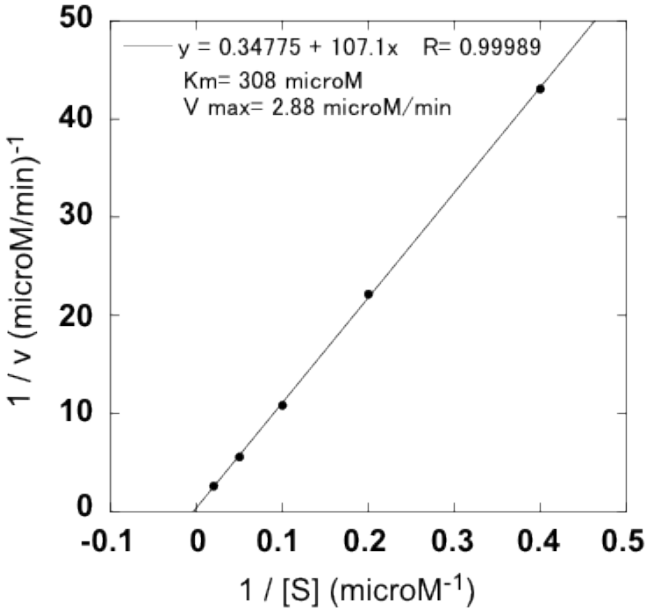


Fig. S8 Lineweaver-Burk plot of the Nitrile Hydratase-catalyzed reaction of **8**. [Nitirle Hydratase] = 10.6 units/mL. [**8**] = 2.5, 5.0, 10, 20, 50 microM. 20 mM Phosphate buffer (pH 7.0), 40 °C.

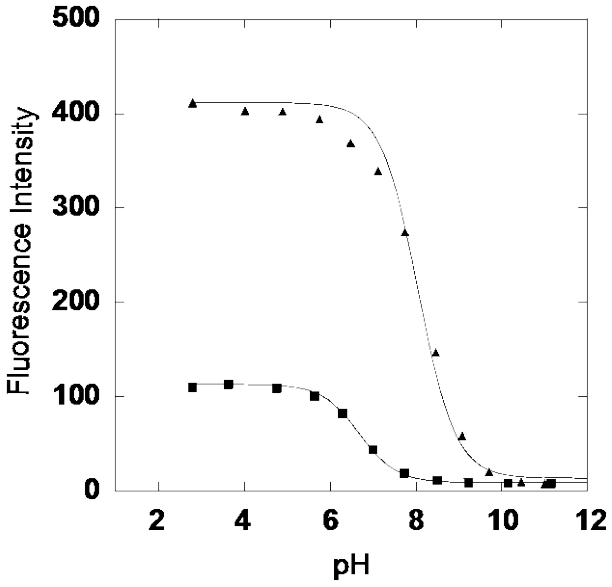


Fig. S9 pH-dependent fluorescent intensity changes of compound **10** (■) and **11** (▲). Conditions: [probe] = 1 μM, 5 mM MES - 5 mM HEPES - 5 mM CHES buffer, λ_{ex} = 367 nm, 25 °C.

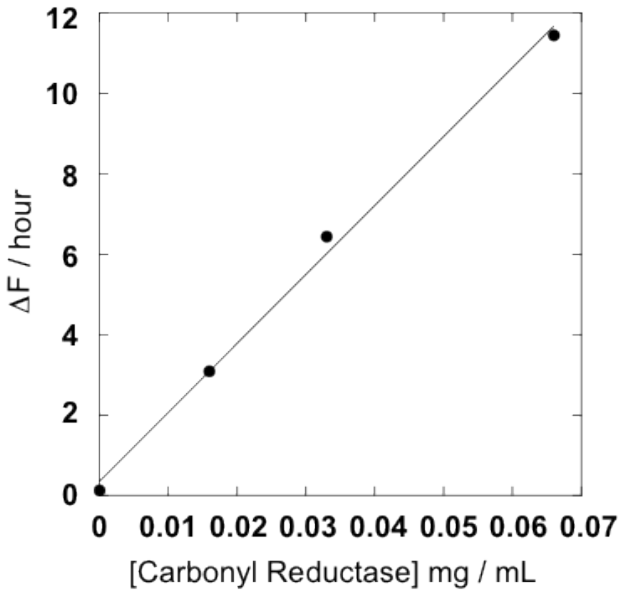


Fig. S10 Plot of the initial rate of the fluorescence increase (ΔF, hr^{-1}) as a function of the amount of carbonyl reductase. [Carbonyl Reductase] = 0, 0.016, 0.033, 0.066 units/mL.

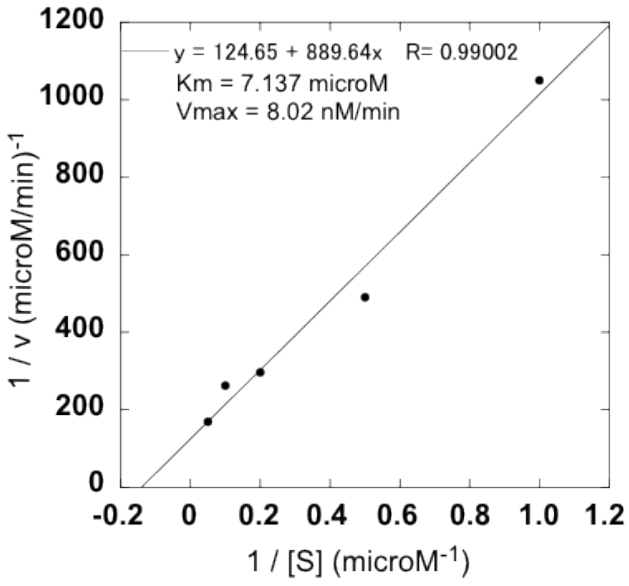


Fig. S11 Lineweaver-Burk plot of the Carbonyl Reductase-catalyzed reaction of **10**. [Carbonyl Reductase] = 0.033 mg/mL. [**10**] = 1.0, 2.0, 5.0, 10, 20 microM. 50 mM HEPES buffer (pH 7.4), 25 °C.

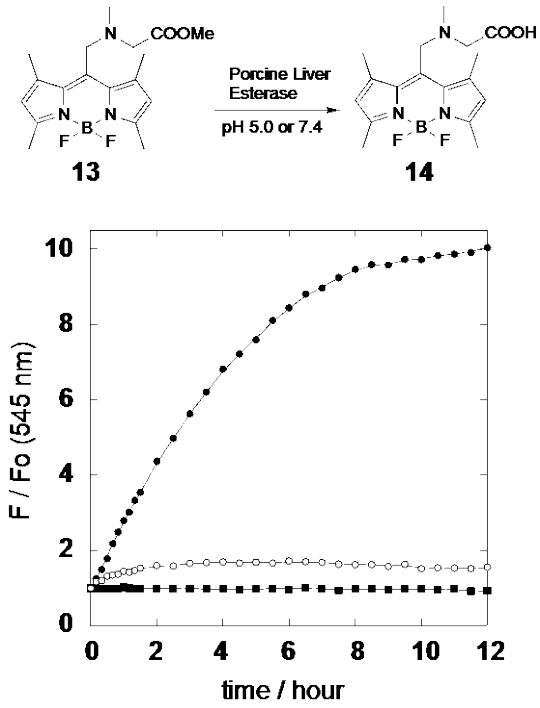


Fig. S12 Real-time fluorescent monitoring of the PLE catalyzed hydrolysis of **13**. Conditions: [**13**] = 1 μM, [PLE] = 0.166 (●), and 0 (■) units/mL, 50 mM KCl - 50 mM AcONa – HCl buffer, pH 5.0, [PLE] = 0.166(○) units/mL, 50 mM HEPES buffer, pH 7.4, λ_{ex} = 519 nm, 37 °C.

Cell Culture.

HeLa cells were cultured in high-glucose Dulbecco's Modified Eagle Medium (DMEM, 4.5 g of glucose/L) supplemented with 10% fetal bovine serum (FBS), penicillin (100 units/mL) and streptomycin (100 μg/mL) under a humidified atmosphere of 5% CO_2 in air. For all experiments, cells were harvested from subcon fluent (<80%) cultures using a trypsin-EDTA solution and then resuspended in fresh medium. A subculture was performed every 2−3 days.

Fluorescence Imaging in HeLa Cells.

HeLa cells cultured in DMEM were treated with 3 μM (final concentration) of **13** in Phenol Red free – DMEM (Gibco, 21063-029) for several hours at 37 °C. The cells were washed with HBS containing 0.5% DMSO (x1) followed by HBS (x2), and subjected to imaging analysis at the appropriate time (0, 4, 13 hr). For inhibition of esterase activity, the cells were treated with 0.5 mM (final conc.) of PMSF at every 2 hours before the imaging analysis. The fluorescence imaging was conducted with a fluorescence microscope (IX-71, Olympus) with a 60x oil-immersion objective lens. BODIPY (excitation: 531/40 nm, dichroic mirror: 570 nm, emission: >575 nm) and Lysotracker Red (excitation: 530-550 nm, dichroic mirror: 660 nm, emission: 692/40 nm) and differential interference contrast (DIC) images were collected and analyzed using Aqua Cosmos software (Hamamatsu Photonics).

pH-dependent Fluorescence Intensity Change in HeLa Cells.

HeLa cells cultured in DMEM were washed with HBS buffer, and treated with 3 μM (final concentration) of **13** in DMEM (phenol red (–)) for 13 hr at 37 °C. After washing with HBS containing 0.5% DMSO (x1) followed by HBS (x2), the cells were treated with chloroquine (200 μM final conc.) and were subjected to imaging analysis.

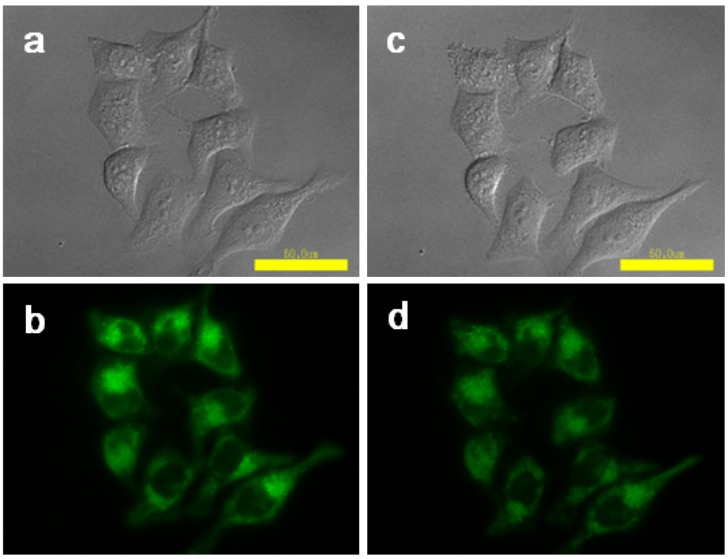


Fig. S13 Effect of chloroquine on fluorescence of **14** in HeLa cells. The cells pre-incubated with **13** for 13 h at 37 °C (a, b) were treated with chloroquine (200 μM) for 1 h at 25 °C, and then subjected to imaging analysis (c, d). Scale bars: 50 μm.

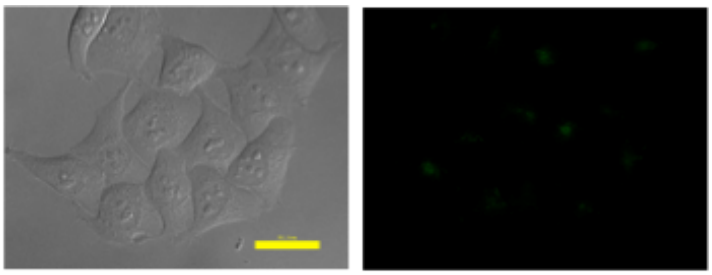


Fig. S14 Fluorescence imaging of HeLa cells upon treatment with **14.** The cells were incubated with **14** (3 μM) for 30 min at 37 °C, and then subjected to imaging analysis. The fluorescence due to BODIPY was scarcely observed inside cells, indicative of membrane impermeability of **14**. Scale bars: 50 μm.

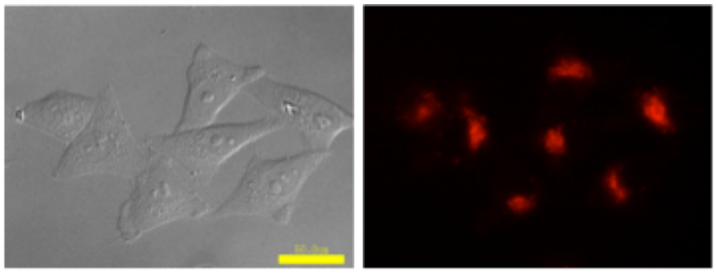
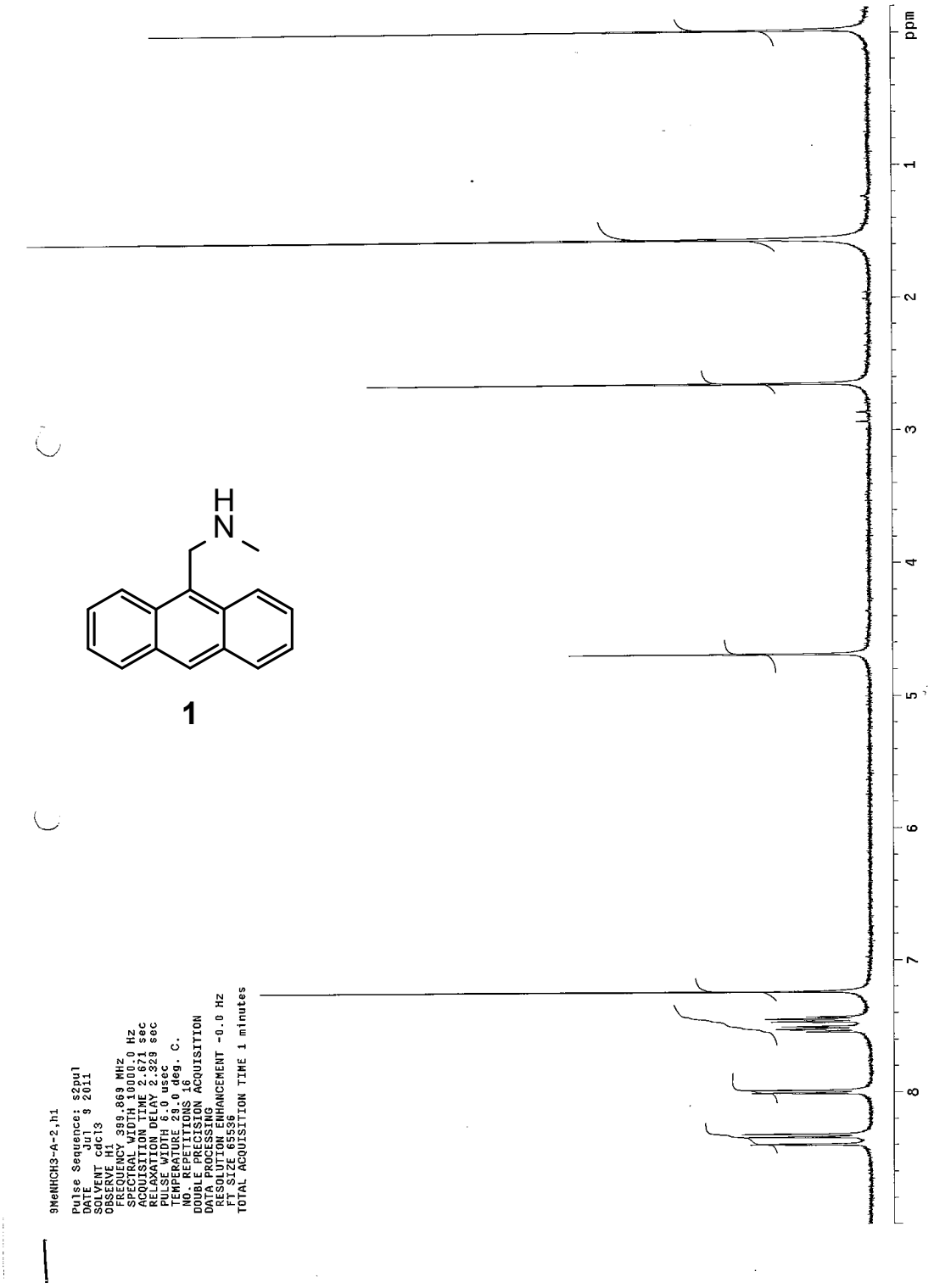


Fig. S15 Fluorescence imaging of HeLa cells by Lysotracker Red after the treatment with PMSF (13 hr). Scale bars: 50 μm.

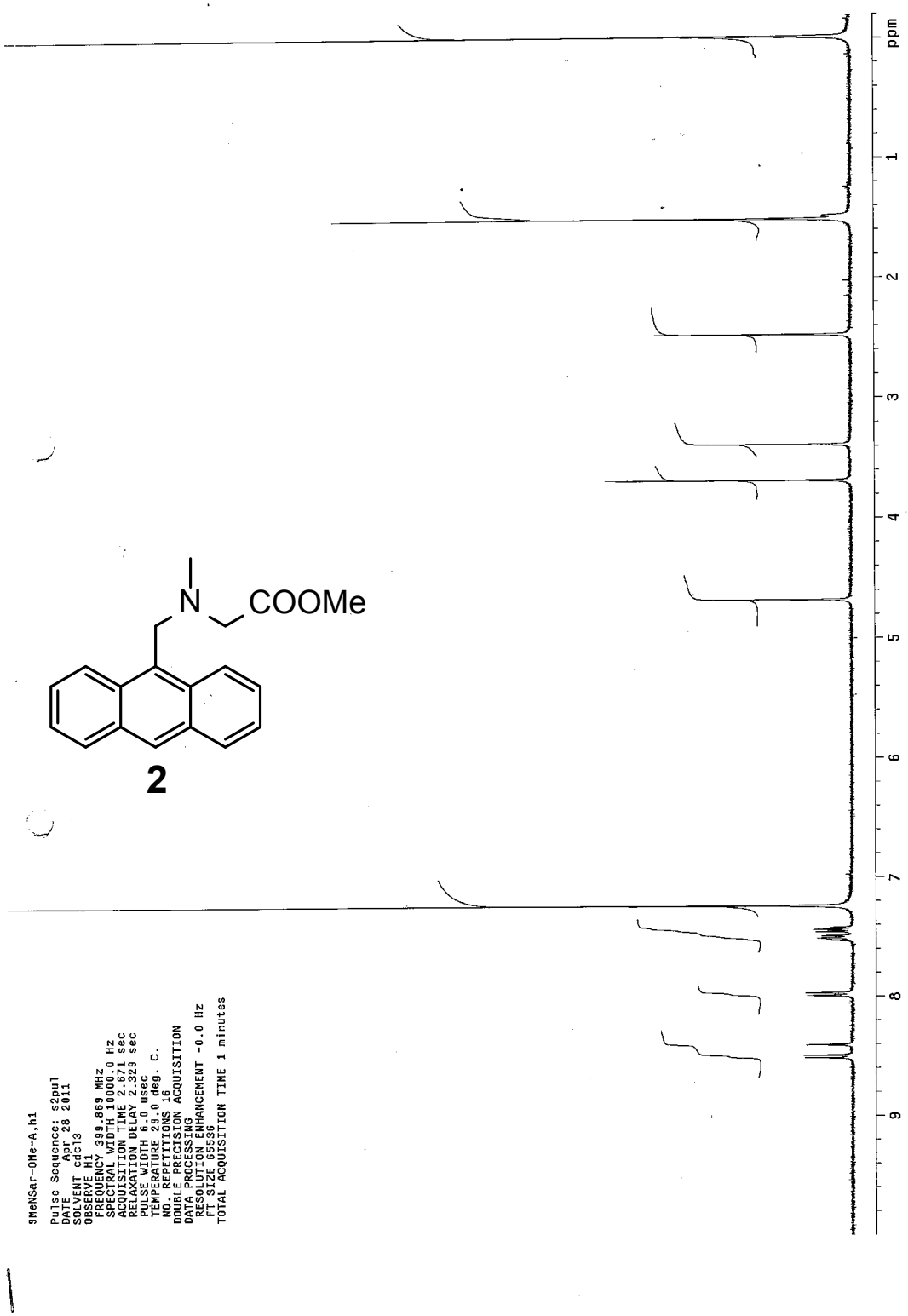
Reference

S1. J. Rosenthal, S. J. Lippard, *J. Am. Chem. Soc.,* 2010, **132**, 5536

^{1}H-NMR data of compound **1** ~**16**



1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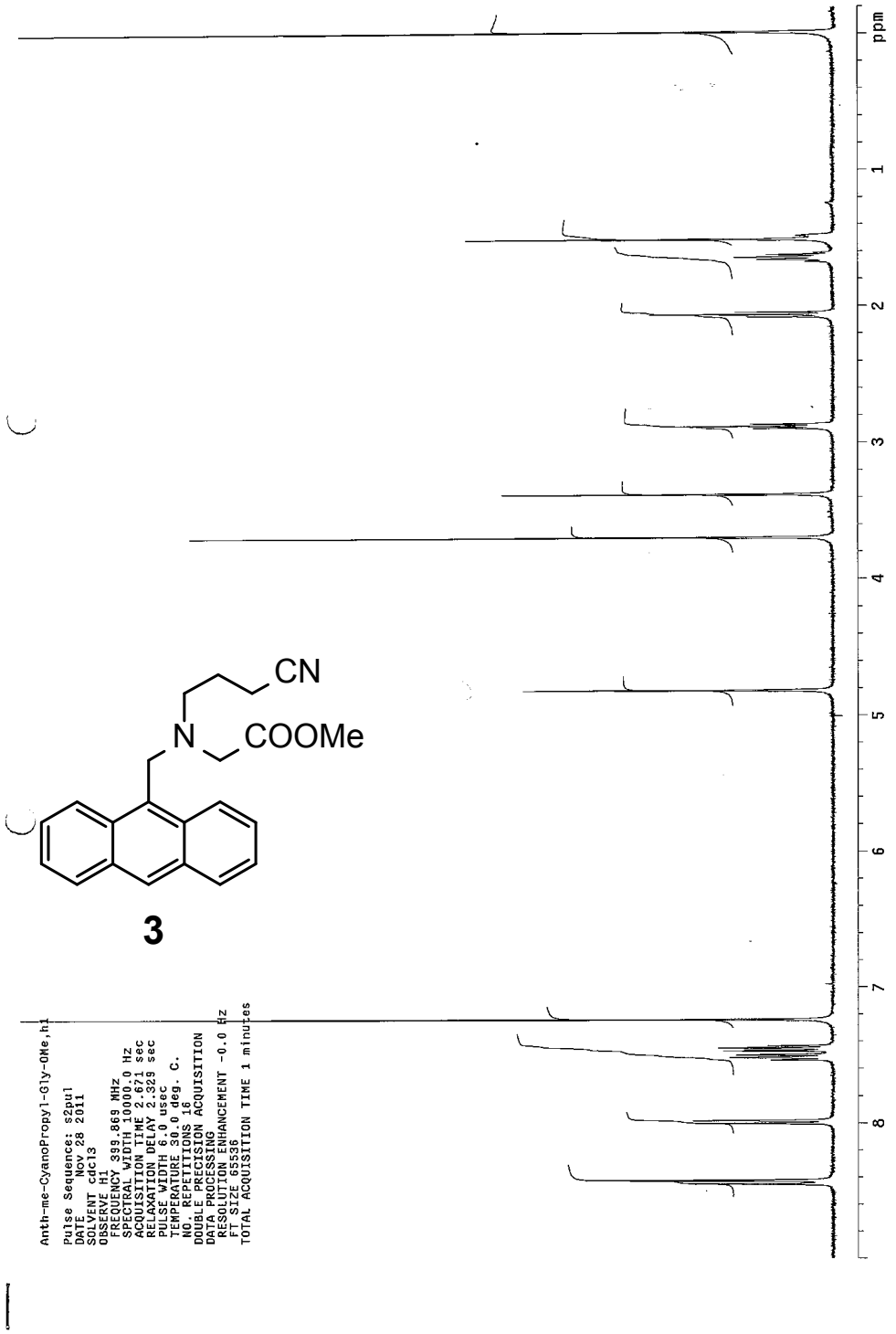
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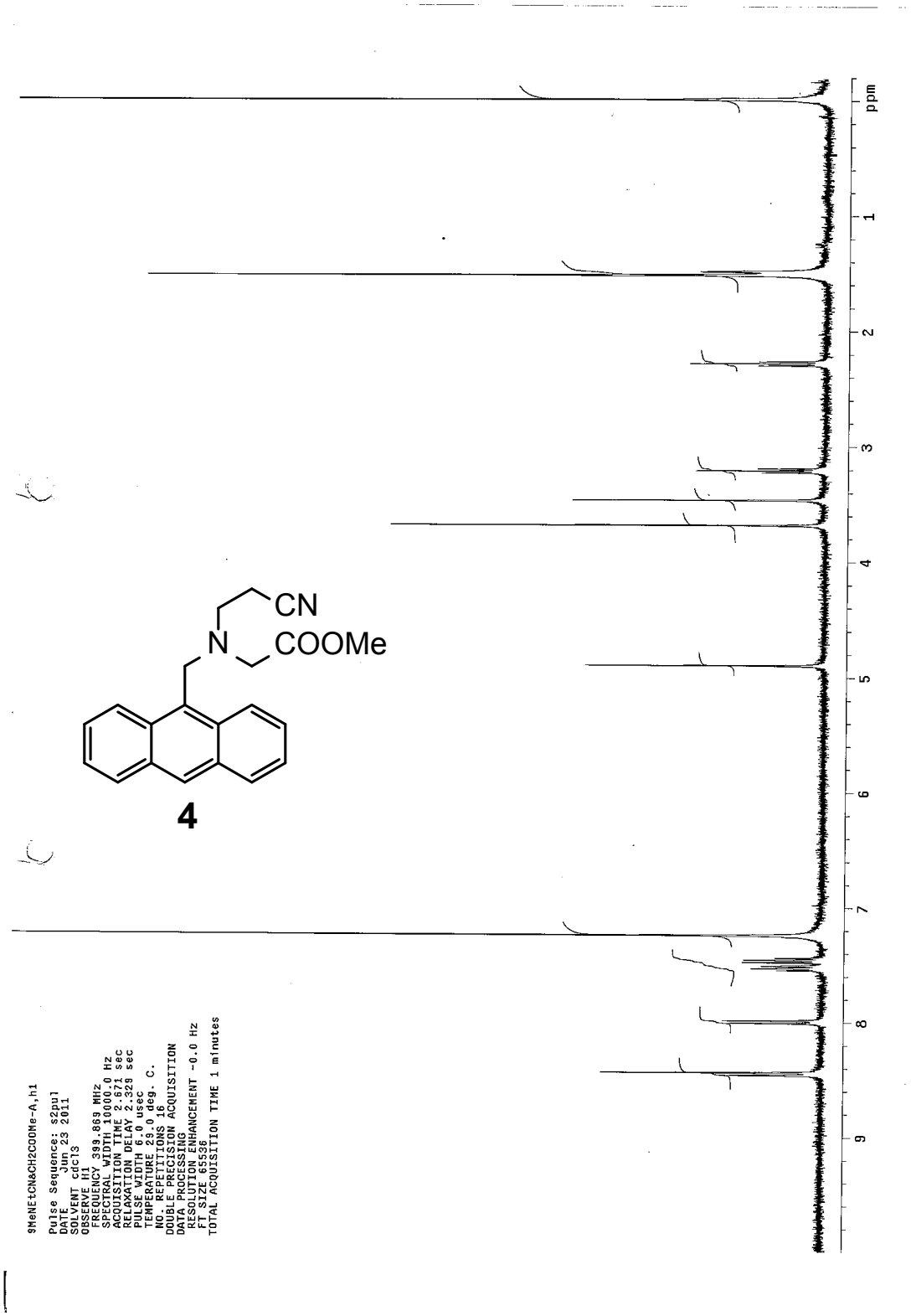
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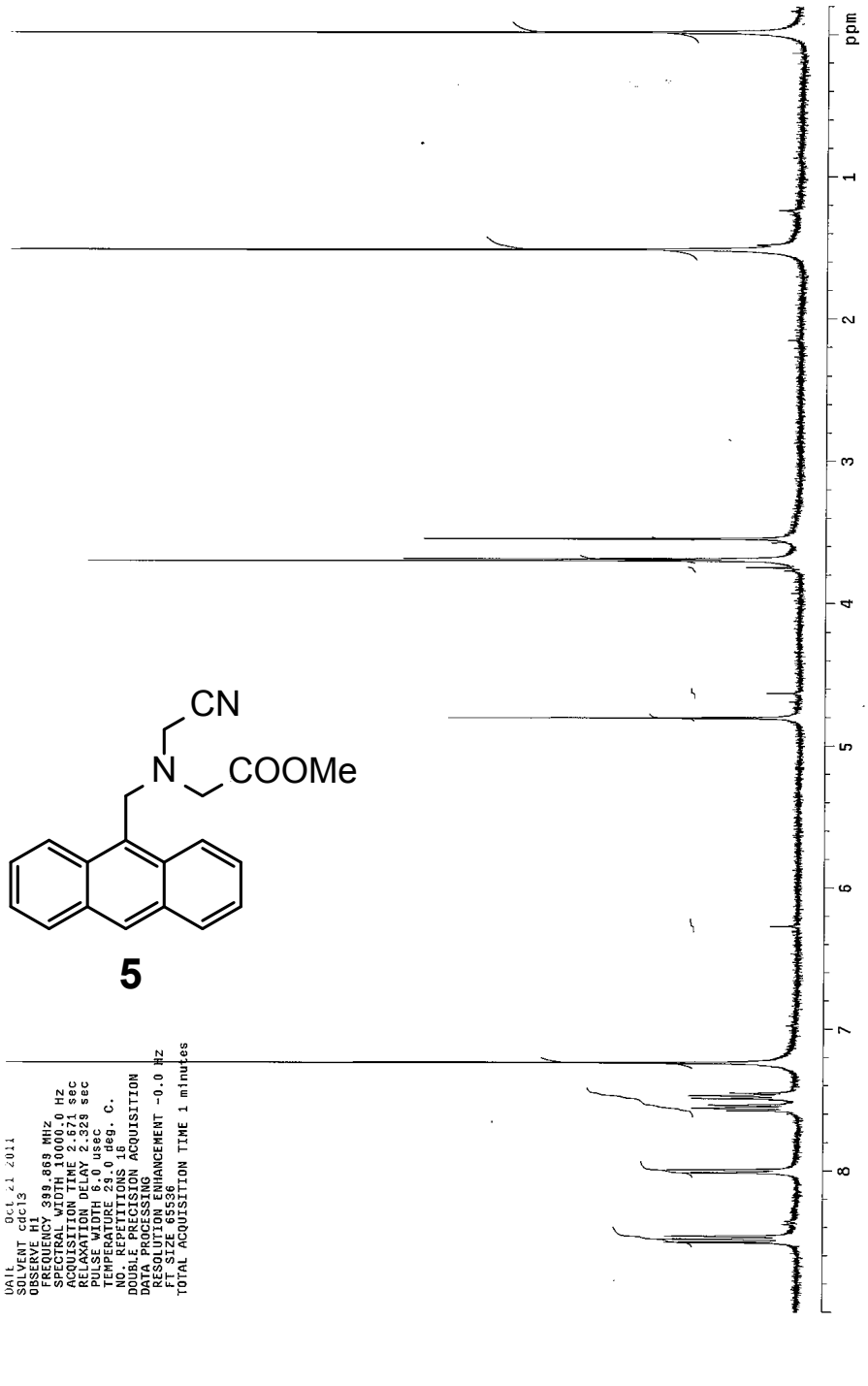
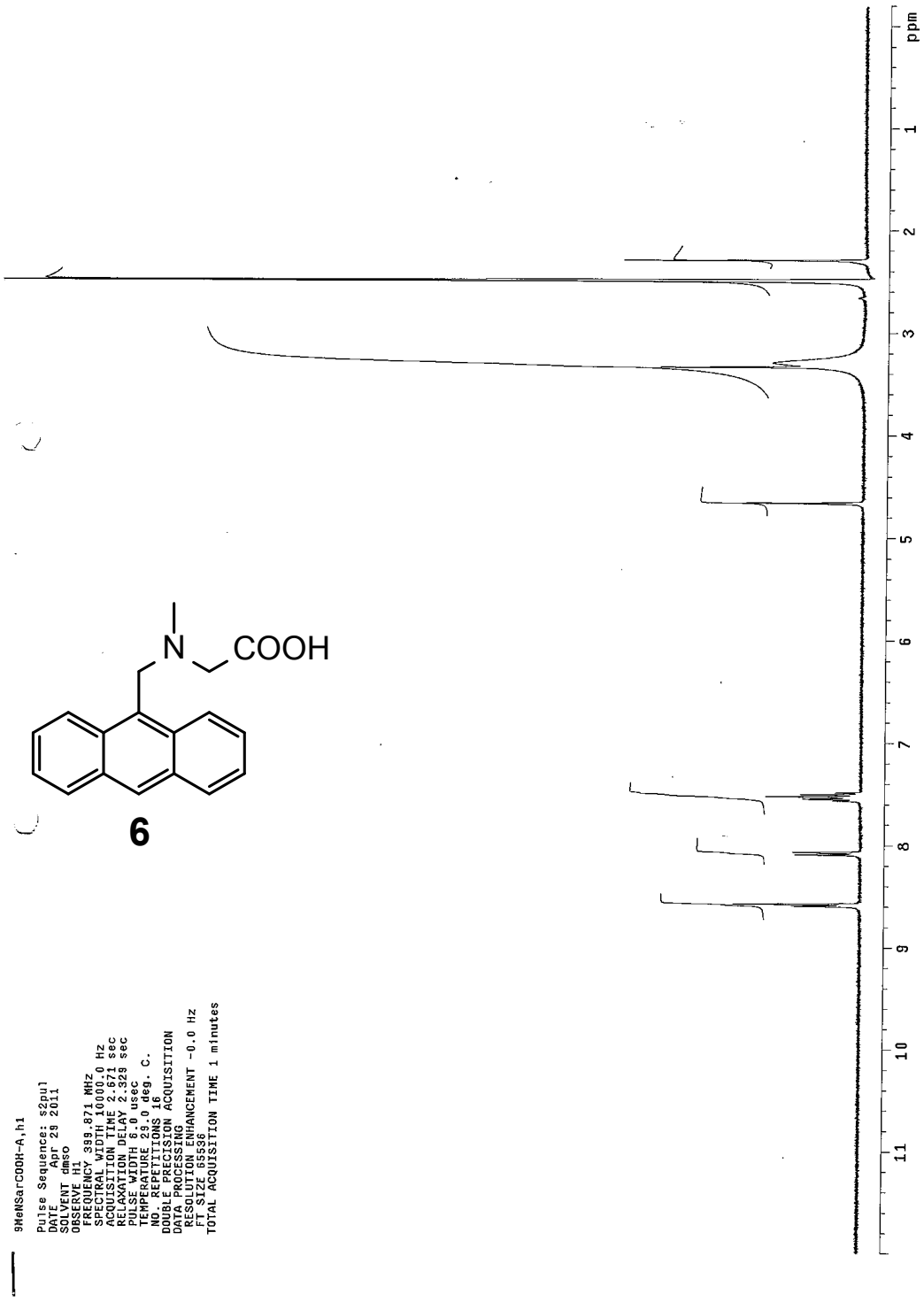
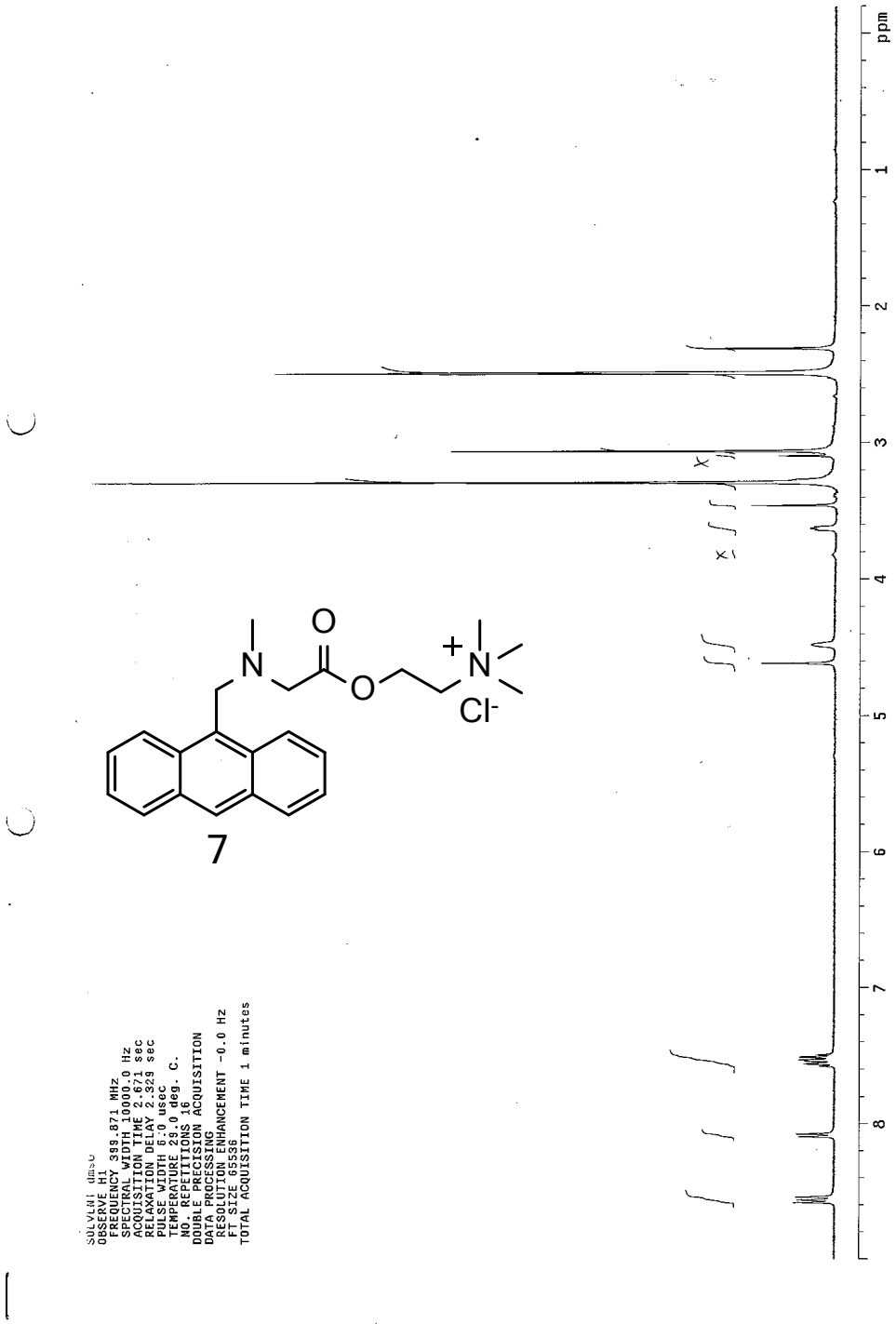
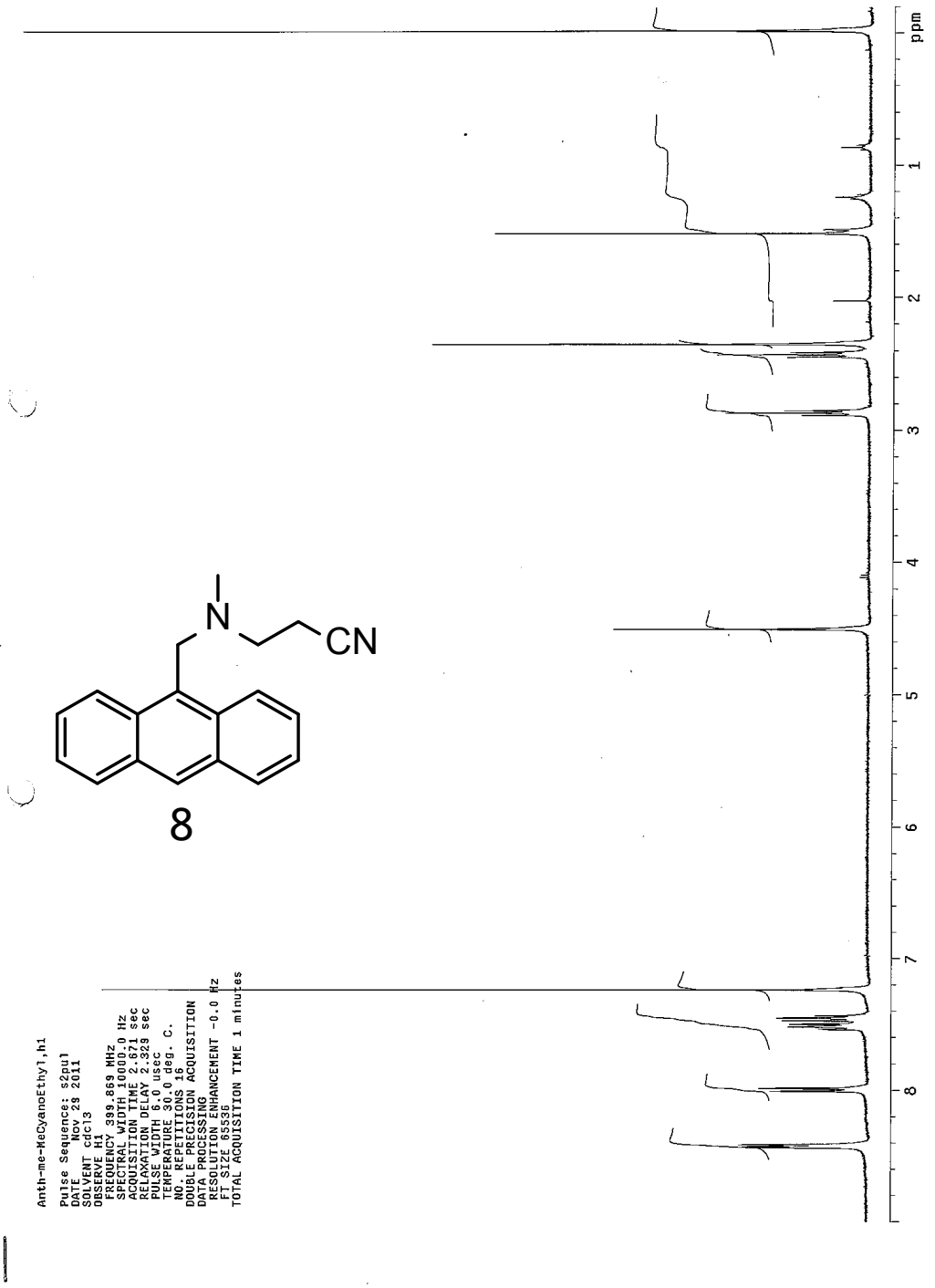
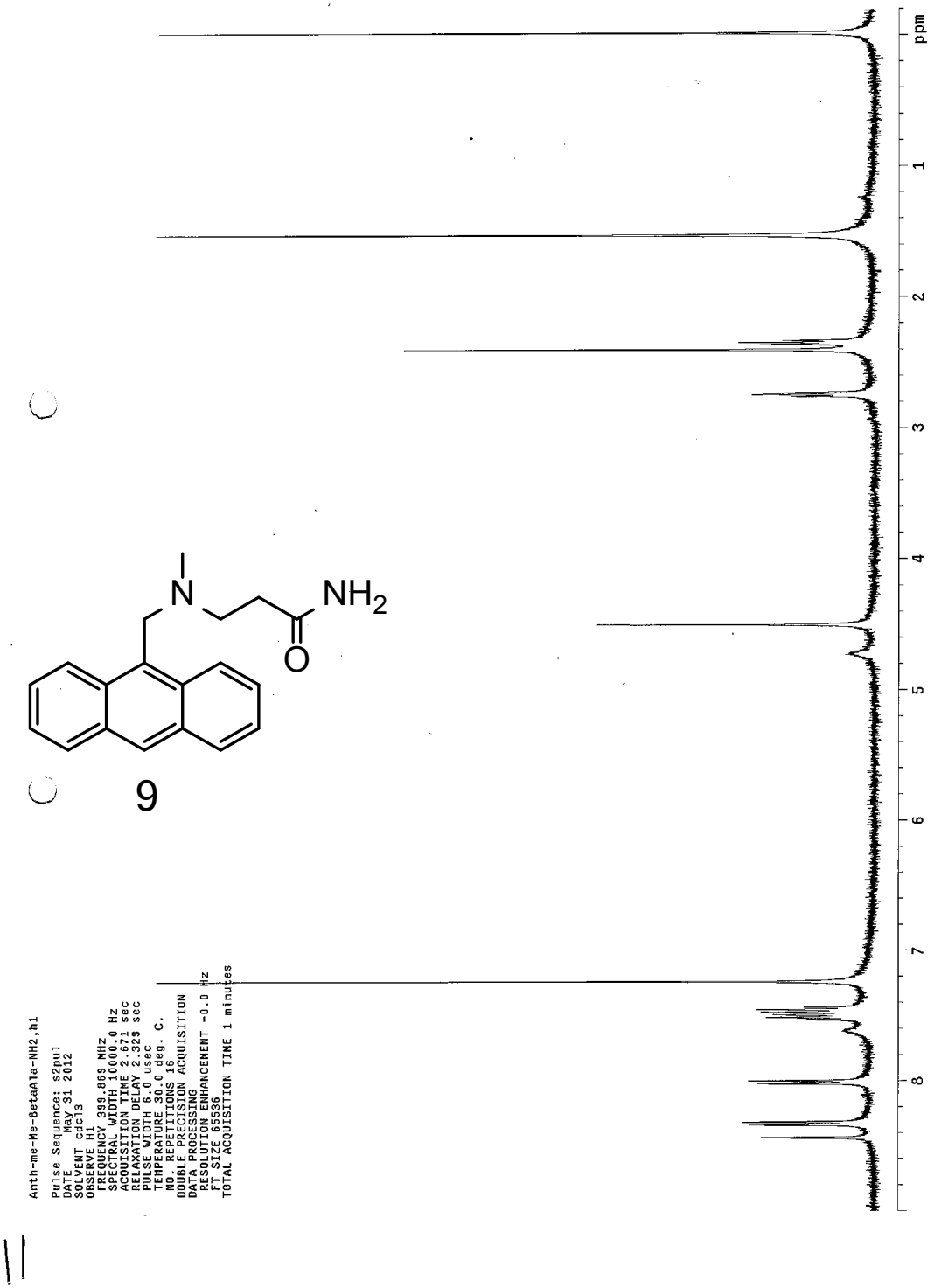
FT SIZE 65536
TOTAL ACQUISITION TIME 1 minutes

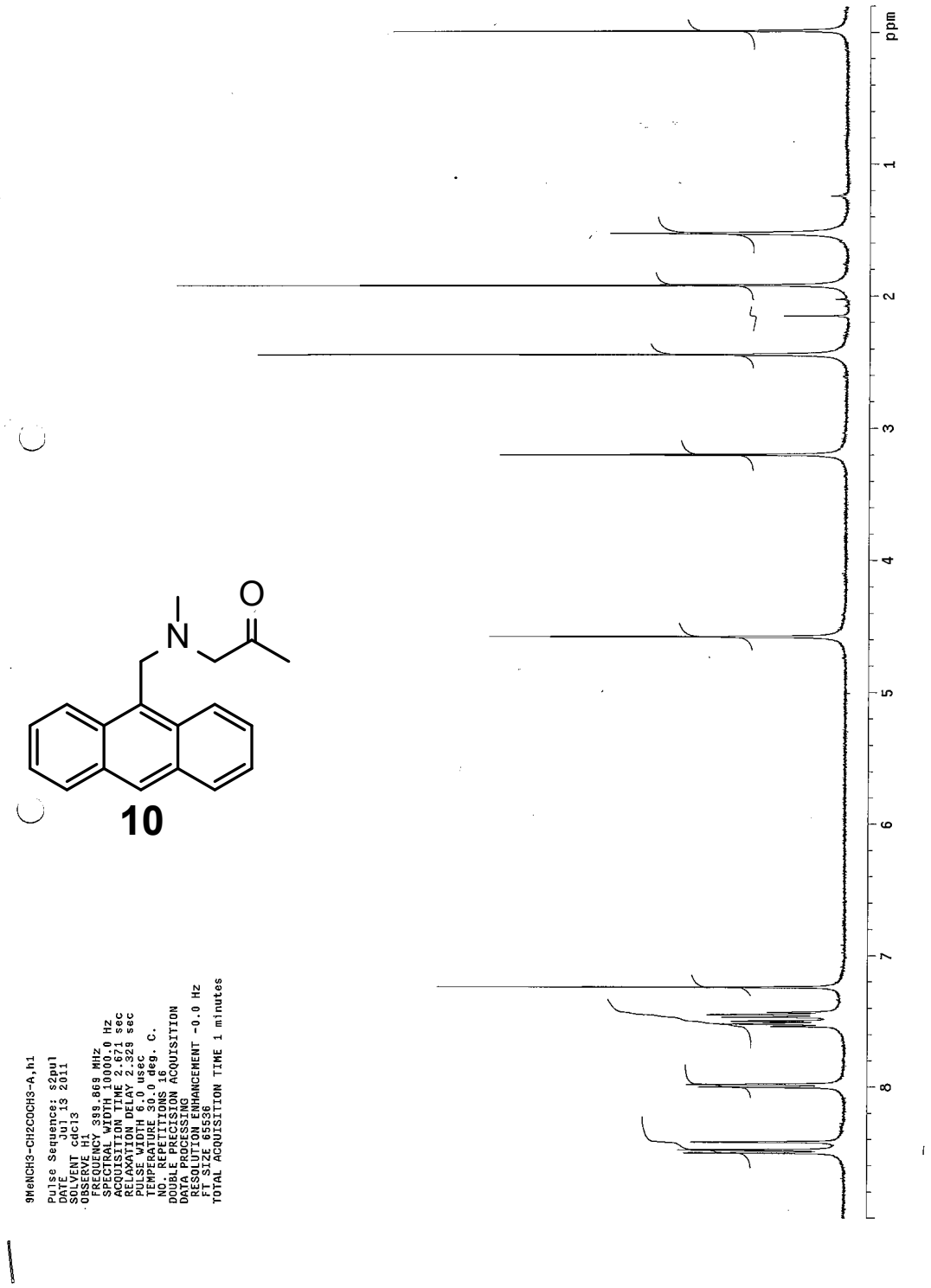

CN
N
COOMe
5
ppm
1
2
3
4
5
6
7
8
DATE Oct 21 2011
SOLVENT cdcl3
OBSERVE H1
FREQUENCY 399.869 MHz
SPECTRAL WIDTH 10000.0 Hz
ACQUISITION TIME 2.621 sec
RELAXATION DELAY 2.328 sec
PULSE WIDTH 6.0 usec
TEMPERATURE 29.0 deg. C.
NO. REPETITIONS 16
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
RESOLUTION ENHANCEMENT -0.0 Hz
FT SIZE 65536
TOTAL ACQUISITION TIME 1 minutes

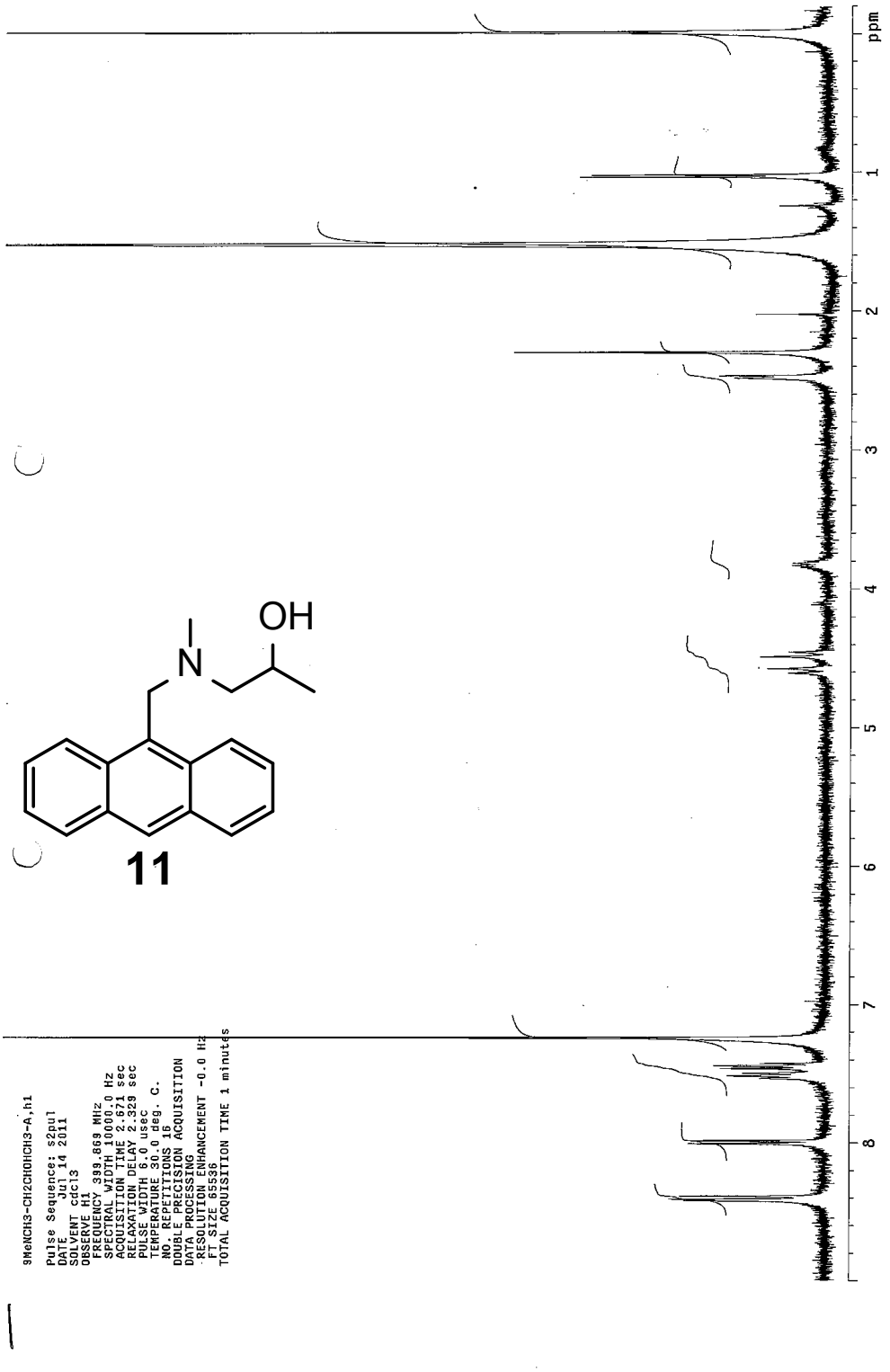

9MeNSarCOOH-A,h1
Pulse Sequence: s2pul
DATE Apr 28 2011
SOLVENT dmso
OBSERVE H1
FREQUENCY 399.871 MHz
SPECTRAL WIDTH 10000.0 Hz
ACQUISITION TIME 2.871 sec
RELAXATION DELAY 2.328 sec
PULSE WIDTH 6.0 usec
TEMPERATURE 23.0 deg. C.
NO. REPETITIONS 16
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
RESOLUTION ENHANCEMENT -0.0 Hz
FT SIZE 65536
TOTAL ACQUISITION TIME 1 minutes
N
COOH
6
ppm
1
2
3
4
5
6
7
8
9
10
11

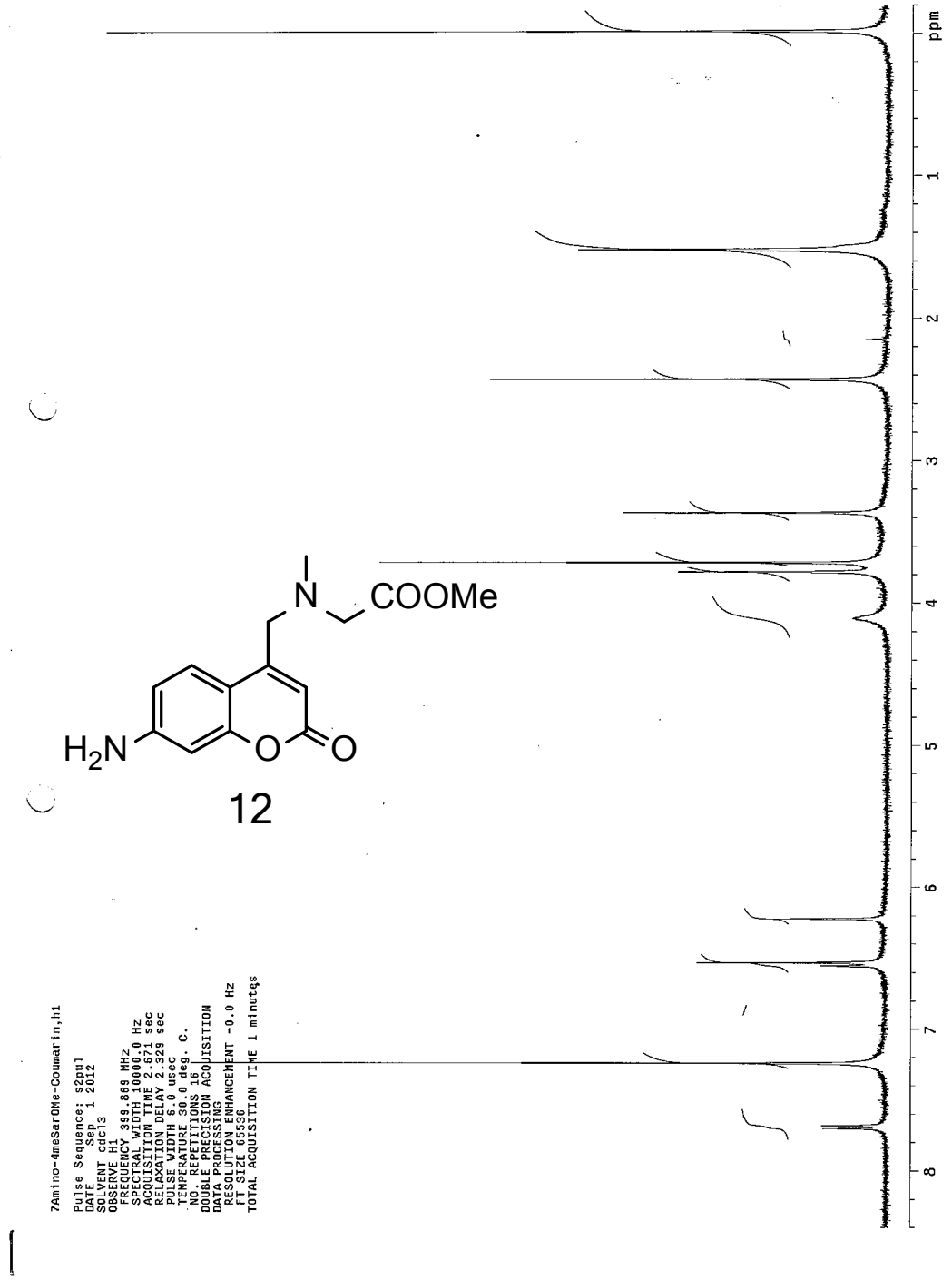

SOLVENT DMSO
OBSERVE H1
FREQUENCY 399.871 MHz
SPECTRAL WIDTH 10000.0 Hz
ACQUISITION TIME 2.671 sec
RELAXATION DELAY 2.329 sec
PULSE WIDTH 6.0 usec
TEMPERATURE 29.0 deg. C.
NO. REPETITIONS 16
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
RESOLUTION ENHANCEMENT -0.0 Hz
FT SIZE 65536
TOTAL ACQUISITION TIME 1 minutes
O
N
O
+N
Cl-
7
ppm
1
2
3
4
5
6
7
8

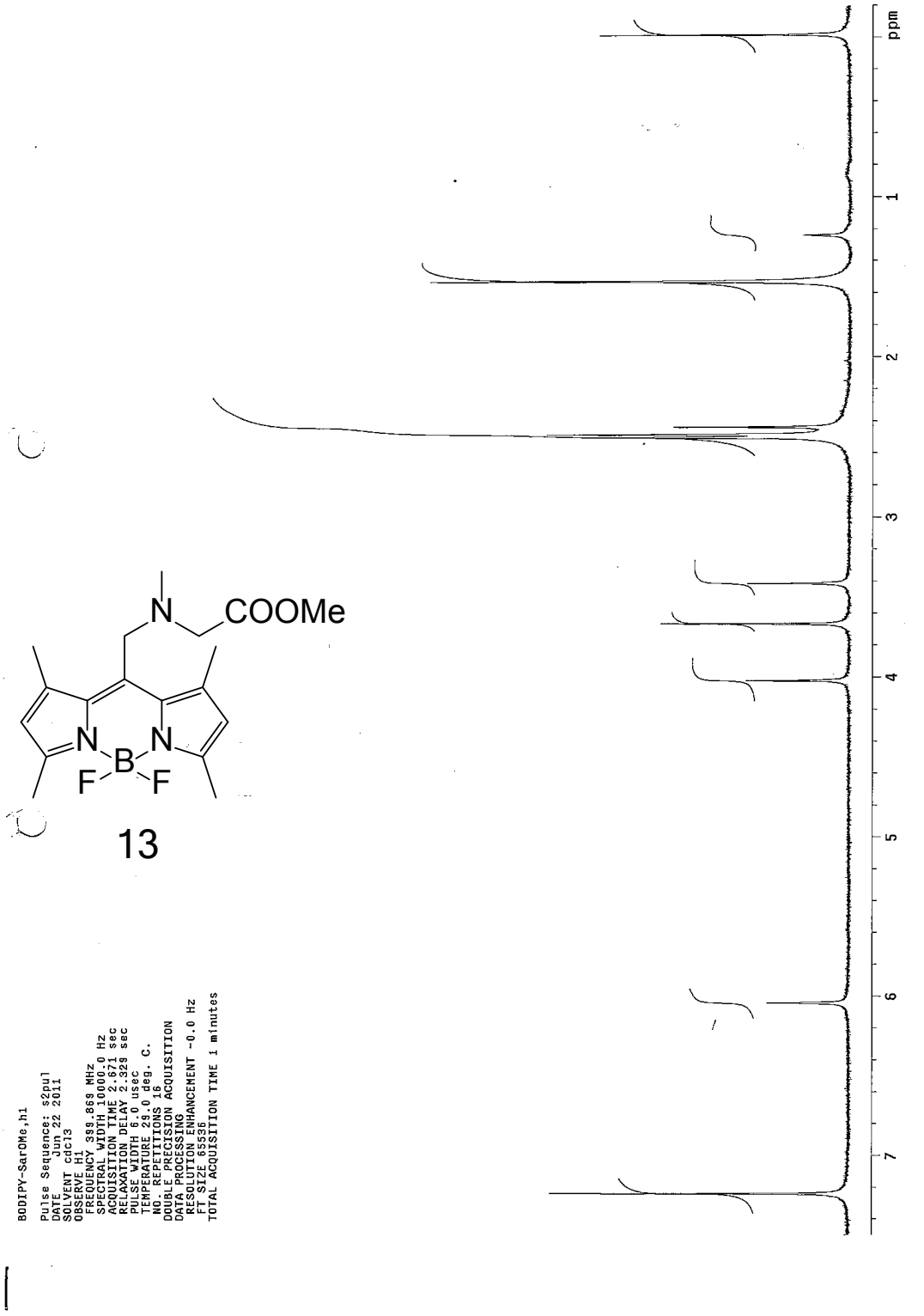

Anth-me-MeCyanoEthyl,h1
Pulse Sequence: s2pul
DATE Nov 29 2011
SOLVENT cdcl3
OBSERVE H1
FREQUENCY 399.869 MHz
SPECTRAL WIDTH 10000.0 Hz
ACQUISITION TIME 2.671 sec
RELAXATION DELAY 2.329 sec
PULSE WIDTH 6.0 usec
TEMPERATURE 30.0 deg. C.
NO. OF REPETITIONS 16
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
RESOLUTION ENHANCEMENT -0.0 Hz
FT SIZE 65536
TOTAL ACQUISITION TIME 1 minutes
N
CN
8
ppm
1
2
3
4
5
6
7
8

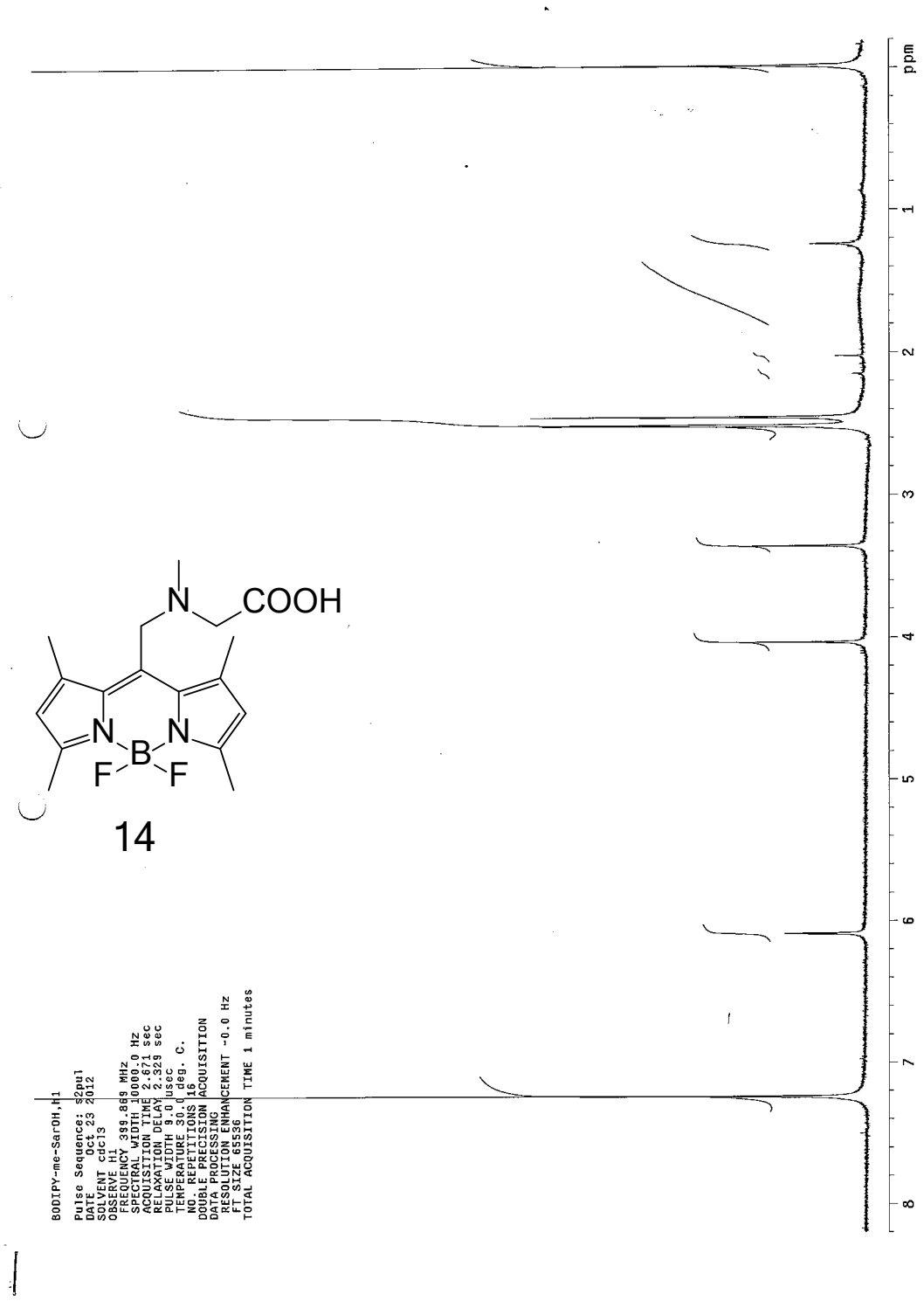

N
NH2
O
9
ppm
1
2
3
4
5
6
7
8
Anth-me-Me-BetaAla-NH2,h1
Pulse Sequence: s2pul
DATE May 31 2012
SOLVENT cdcl3
OBSERVE H1
FREQUENCY 399.869 MHz
SPECTRAL WIDTH 10000.0 Hz
ACQUISITION TIME 2.671 sec
RELAXATION DELAY 2.329 sec
PULSE WIDTH 6.0 usec
TEMPERATURE 30.0 deg. C.
NO. REPETITIONS 16
DUAL ACQUISITION ACQUISITION
DATA PROCESSING
RESOLUTION ENHANCEMENT -0.0 Hz
FT SIZE 65536
TOTAL ACQUISITION TIME 1 minutes

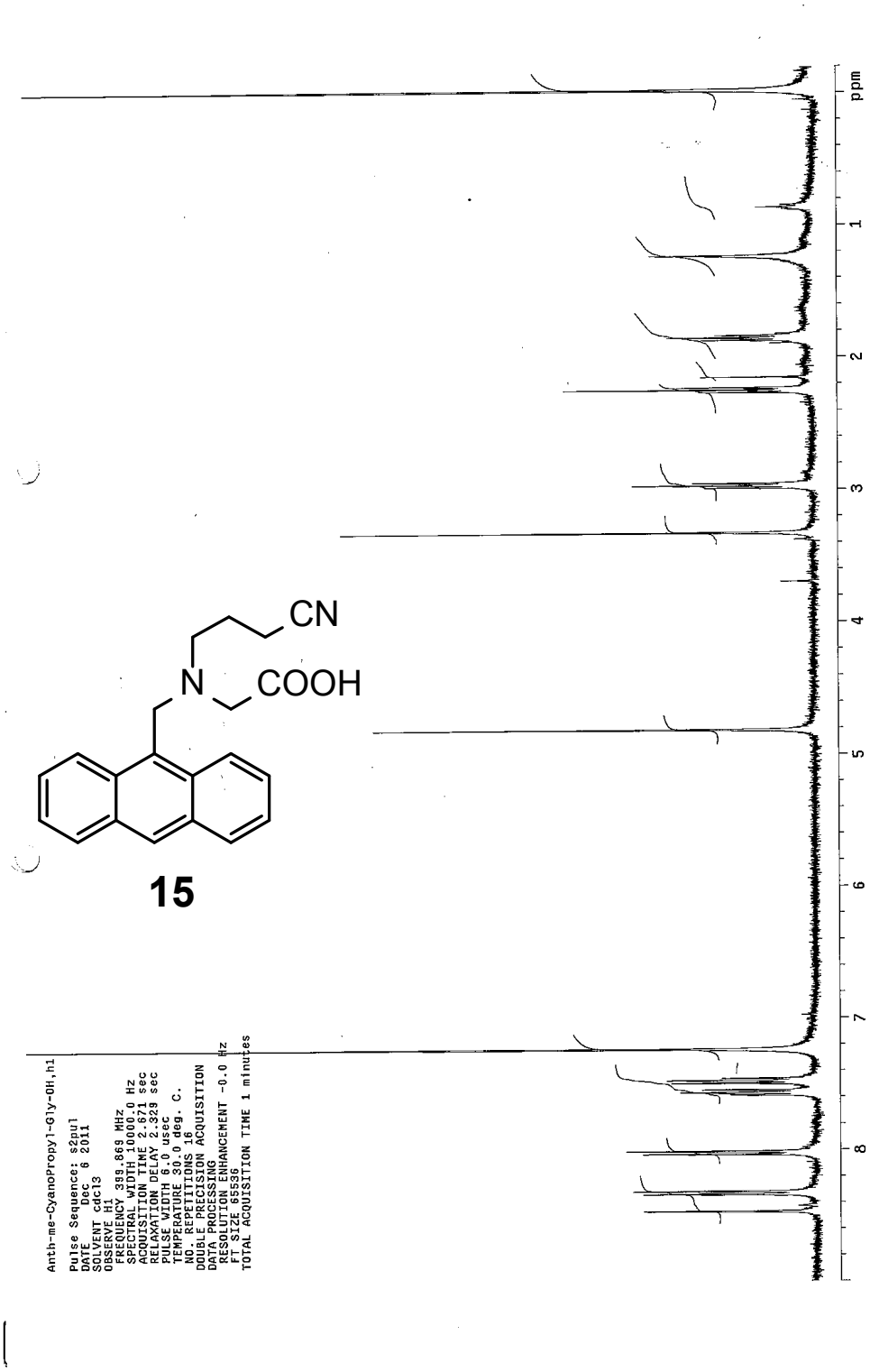

9MeNCH3-CH2COCH3-A,h1
Pulse Sequence: s2pul
DATE Jul 13 2011
SOLVENT cdcl3
OBSERVE H1
FREQUENCY 399.869 MHz
SPECTRAL WIDTH 10000.0 Hz
ACQUISITION TIME 2.671 sec
RELAXATION DELAY 2.328 sec
PULSE WIDTH 6.0 usec
TEMPERATURE 30.0 deg. C.
NO. REPETITIONS 16
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
RESOLUTION ENHANCEMENT -0.0 Hz
FT SIZE 65536
TOTAL ACQUISITION TIME 1 minutes
O
N
10
ppm
1
2
3
4
5
6
7
8

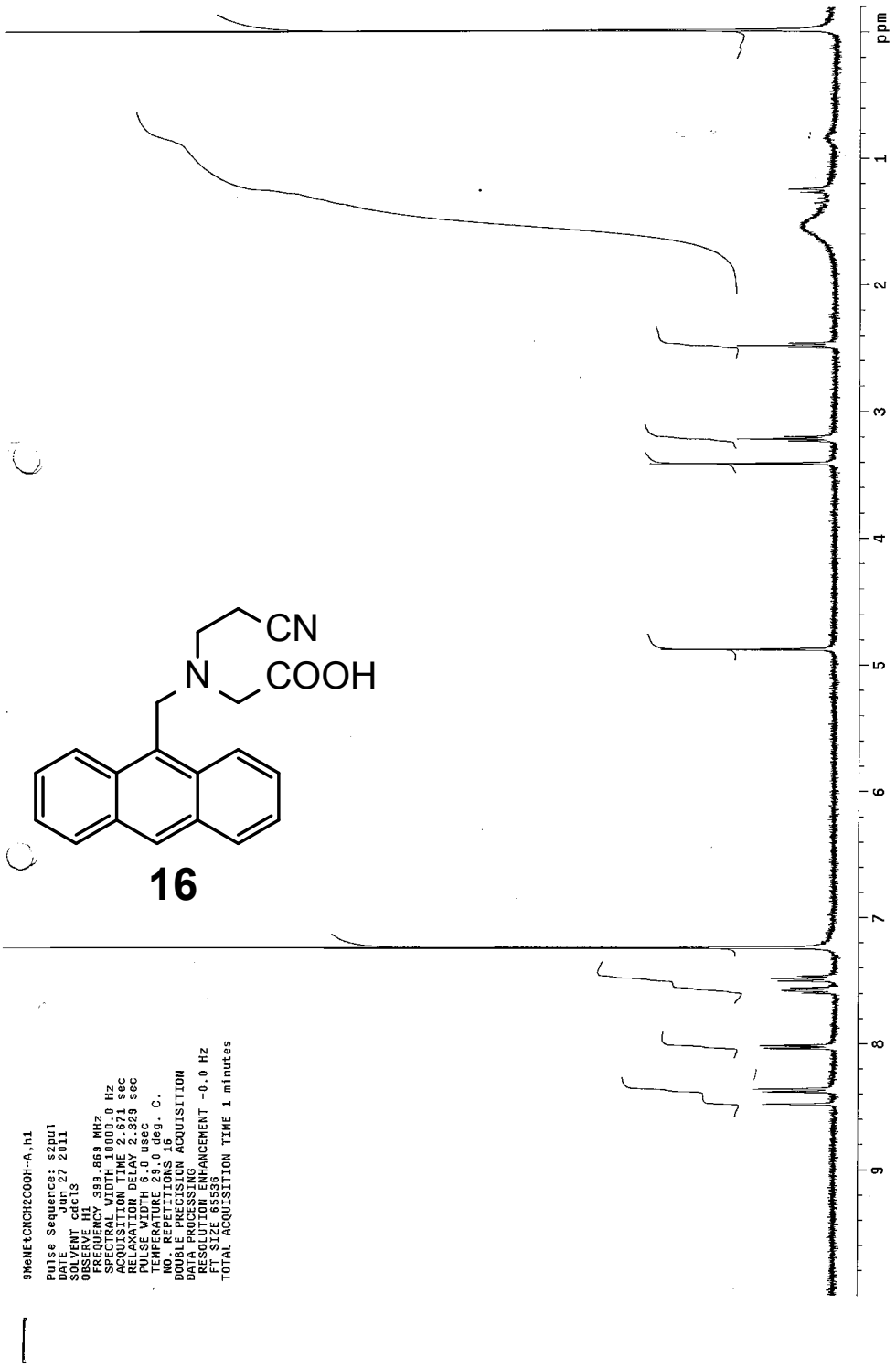

OH
N
11
9MeNCH3-CH2CHOHCH3-A.h1
Pulse Sequence: s2pul
DATE Jul 14 2011
SOLVENT cdcl3
OBSERVE H1
FREQUENCY 399.669 MHz
SPECTRAL WIDTH 10000.0 Hz
ACQUISITION TIME 2.671 sec
RELAXATION DELAY 2.329 sec
PULSE WIDTH 6.0 usec
TEMPERATURE 30.0 deg. C.
NO. REPETITIONS 16
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
RESOLUTION ENHANCEMENT -0.0 Hz
FT SIZE 65536
TOTAL ACQUISITION TIME 1 minutes
ppm
1
2
3
4
5
6
7
8


7Amino-4meSarOMe-Coumarin,h1
Pulse Sequence: s2pul
DATE Sep 1 2012
SOLVENT cdcl3
OBSERVE H1
FREQUENCY 399.869 MHz
SPECTRAL WIDTH 6000.0 Hz
ACQUISITION TIME 2.671 sec
RELAXATION DELAY 2.329 sec
PULSE WIDTH 6.0 usec
TEMPERATURE 30.0 deg. C.
NO. REPETITIONS 16
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
RESOLUTION ENHANCEMENT -0.0 Hz
FT SIZE 65536
TOTAL ACQUISITION TIME 1 minutes
N
COOMe
H2N
O
O
12
ppm
1
2
3
4
5
6
7
8

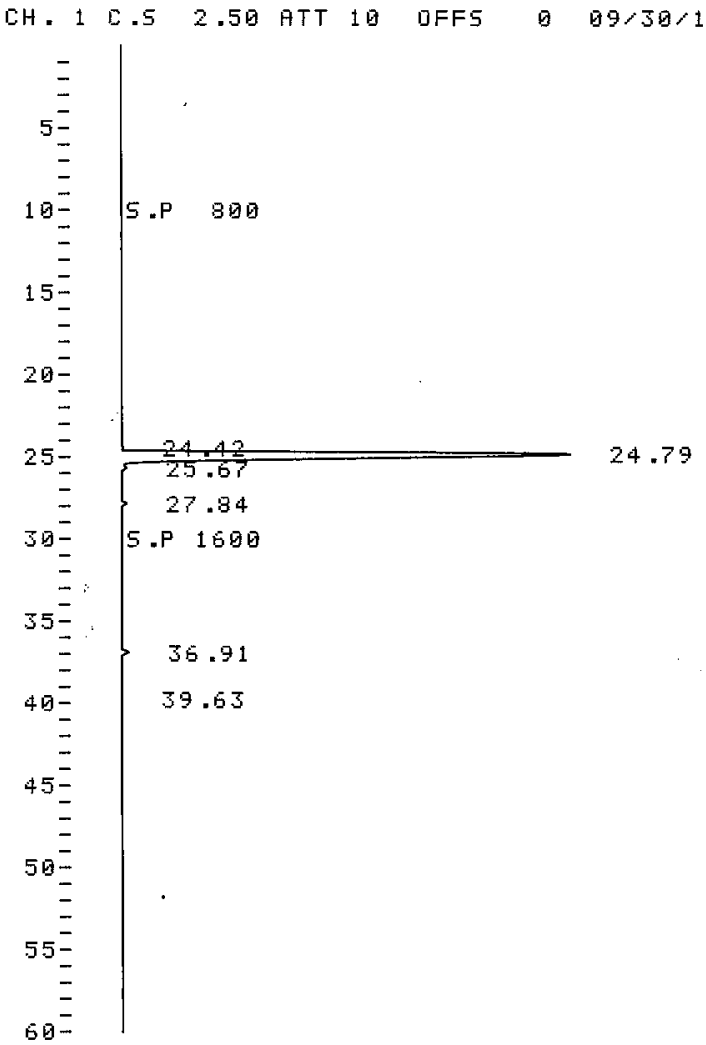

N
COOMe
N
B
N
F
F
13
BODIPY-SarOMe,h1
Pulse Sequence: s2pul
DATE Jun 22 2011
SOLVENT cdcl3
OBSERVE H1
FREQUENCY 399.868 MHz
SPECTRAL WIDTH 10000.0 Hz
ACQUISITION TIME 2.671 sec
RELAXATION DELAY 2.329 sec
PULSE WIDTH 6.0 usec
TEMPERATURE 20.0 deg. C.
NO. REPETITIONS 16
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
RESOLUTION ENHANCEMENT -0.0 Hz
FT SIZE 65536
TOTAL ACQUISITION TIME 1 minutes
ppm
1
2
3
4
5
6
7

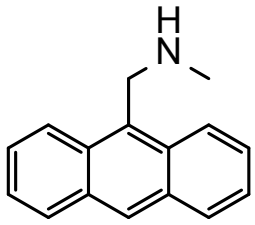

N
COOH
N
B
N
F
F
14
BODIPY-me-SarOH,H1
Pulse Sequence: s2pul
DATE Oct 23 2012
SOLVENT cdcl3
OBSERVE H1
FREQUENCY 399.889 MHz
SPECTRAL WIDTH 10000.0 Hz
ACQUISITION TIME 2.671 sec
RELAXATION DELAY 2.329 sec
PULSE WIDTH 8.0 usec
TEMPERATURE 30.0 deg. C.
NO. REPETITIONS 16
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
RESOLUTION ENHANCEMENT -0.0 Hz
FT SIZE 65536
TOTAL ACQUISITION TIME 1 minutes
ppm
1
2
3
4
5
6
7
8


CN
N
COOH
15
ppm
1
2
3
4
5
6
7
8
Anth-me-CyanoPropyl-Gly-OH,h1
Pulse Sequence: s2pul
DATE Dec 6 2011
SOLVENT cdcl3
OBSERVE H1 399.869 MHz
FREQUENCY 399.869 MHz
SPECTRAL WIDTH 1000.0 Hz
ACQUISITION TIME 2.671 sec
RELAXATION DELAY 2.329 sec
PULSE WIDTH 6.0 usec
TEMPERATURE 30.0 deg. C.
NO. REPETITIONS 16
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
RESOLUTION ENHANCEMENT -0.0 Hz
FT SIZE 65536
TOTAL ACQUISITION TIME 1 minutes


CN
N
COOH
16
ppm
1
2
3
4
5
6
7
8
9
9MeNEtCNCH2COOH-A,h1
Pulse Sequence: s2pul
DATE Jun 27 2011
SOLVENT cdcl3
OBSERVE H1
FREQUENCY 399.869 MHz
SPECTRAL WIDTH 10000.0 Hz
ACQUISITION TIME 2.371 sec
RELAXATION DELAY 2.329 sec
PULSE WIDTH 6.0 usec
TEMPERATURE 29.0 deg. C.
NO. REPETITIONS 16
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
RESOLUTION ENHANCEMENT -0.0 Hz
FT SIZE 65536
TOTAL ACQUISITION TIME 1 minutes

HPLC data of compound **1** ~**16**





1

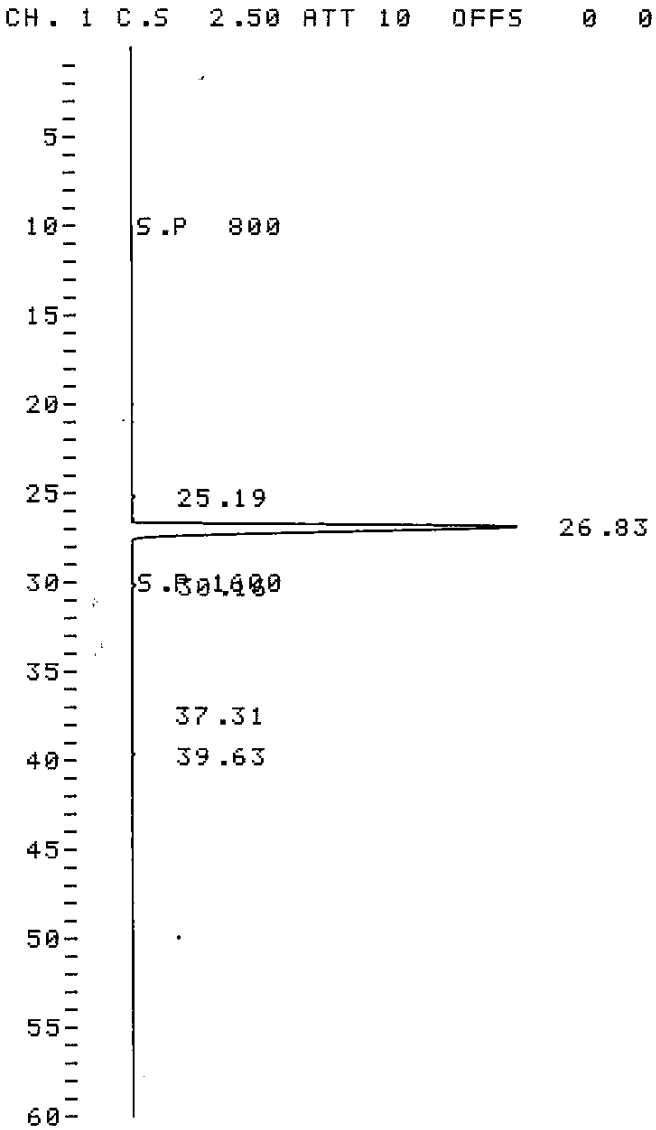
D-2500 09/30/13

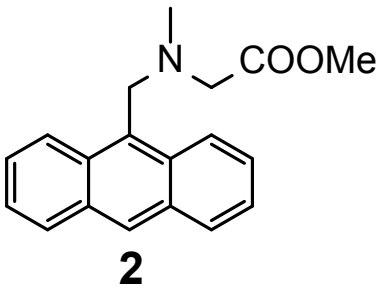
METHOD: TAG: 8 CH: 1

FILE: 1 CALC-METHOD: AREA% TABLE: 0 CONC: AREA

NO.	RT	AREA	CONC	BC
2	24.79	10583976	98.676	VV
4	27.84	51626	0.481	BB
5	36.91	90340	0.842	BB
TOTAL				
		10725942	100.000	
PEAK REJ :		50000		

Detection wavelength: 360 nm





2

D-2500 09/30/13

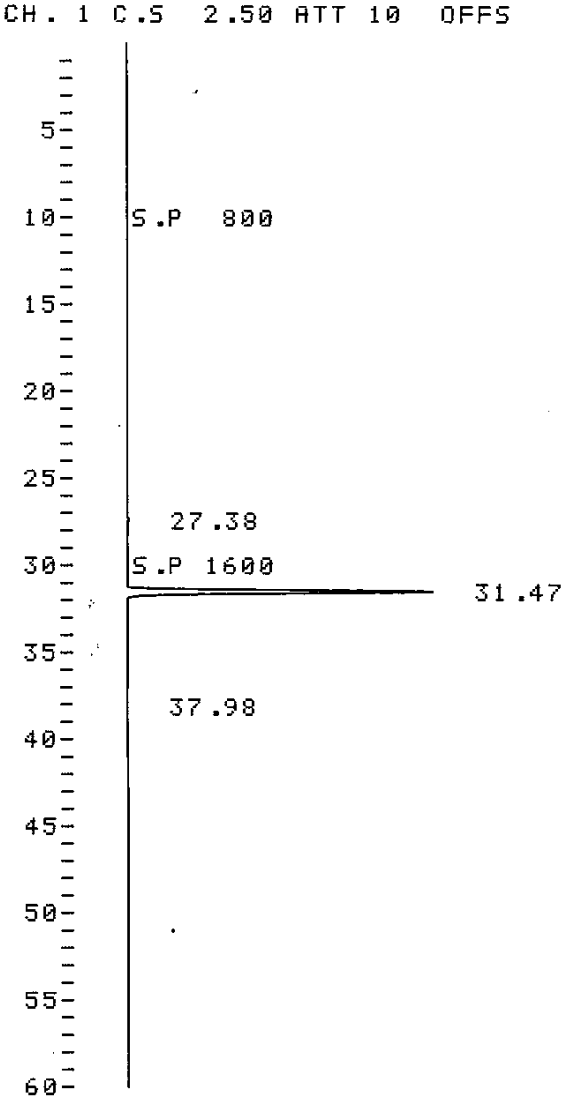
METHOD: TAG: 9 CH: 1

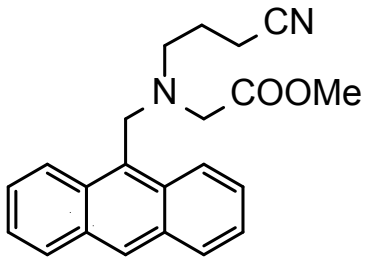
FILE: 1 CALC-METHOD: AREA% TABLE: 0 CONC: AREA

NO.	RT	AREA	CONC	BC
3	26.83	9166279	100.000	VB
TOTAL		9166279	100.000	

PEAK REJ : 50000

Detection wavelength: 360 nm





3

D-2500 09/30/13

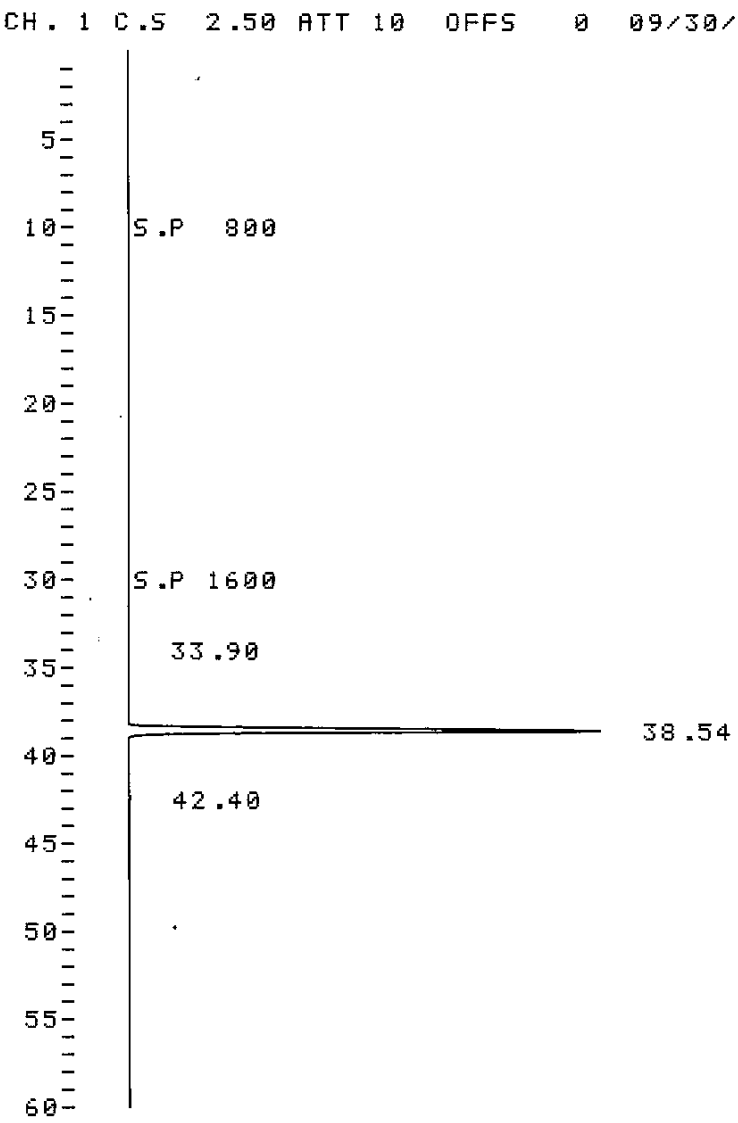
METHOD: TAG: 10 CH: 1

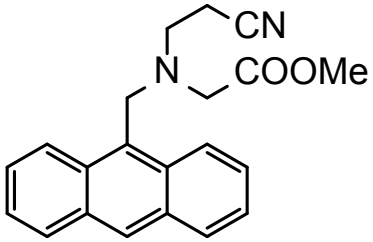
FILE: 1 CALC-METHOD: AREA% TABLE: 0 CONC: AREA

NO.	RT	AREA	CONC	BC
2	31.47	3715081	100.000	BB
TOTAL		3715081	100.000	

PEAK REJ : 50000

Detection wavelength: 360 nm





4

D-2500 09/30/13

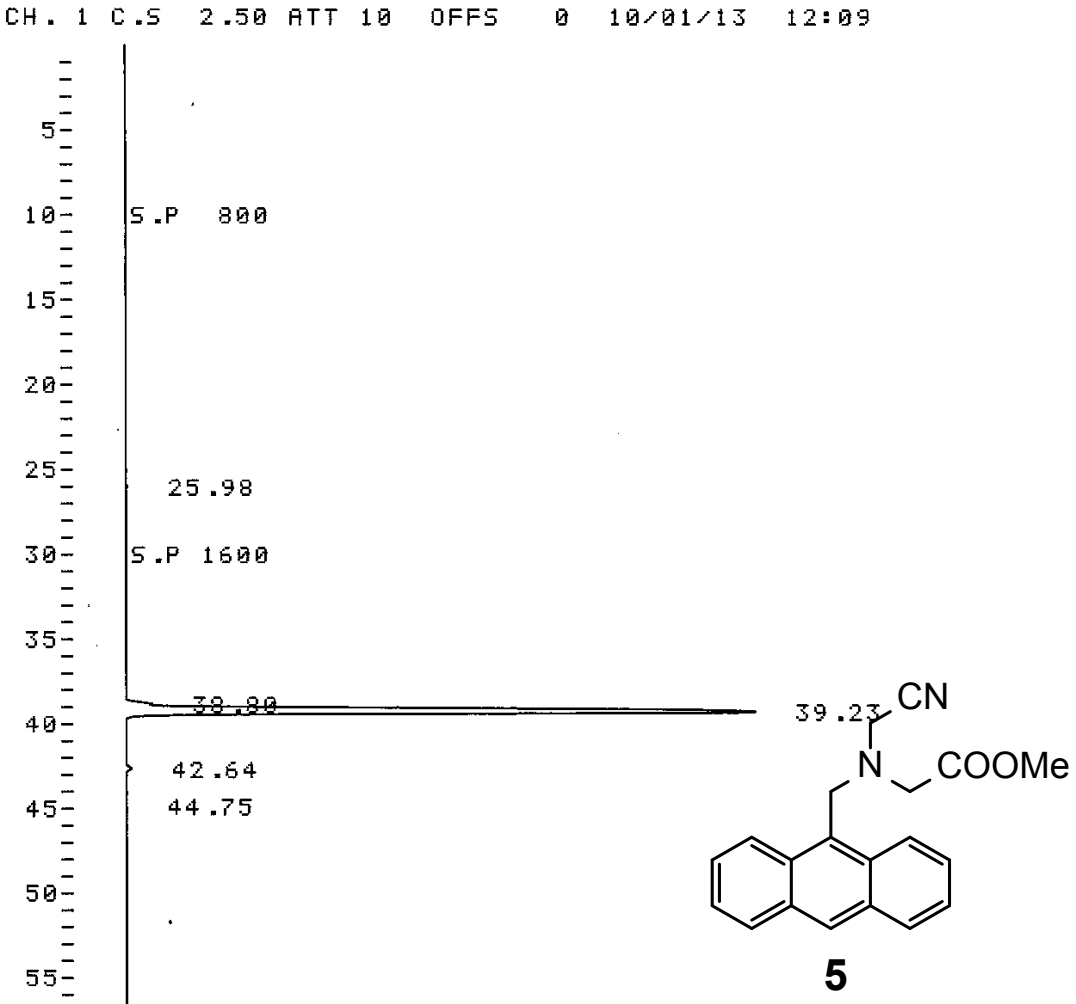
METHOD: TAG: 11 CH: 1

FILE: 1 CALC-METHOD: AREA% TABLE: 0 CONC: AREA

NO.	RT	AREA	CONC	BC
2	38.54	6464362	100.000	BB
TOTAL		6464362	100.000	

PEAK REJ : 50000

Detection wavelength: 360 nm



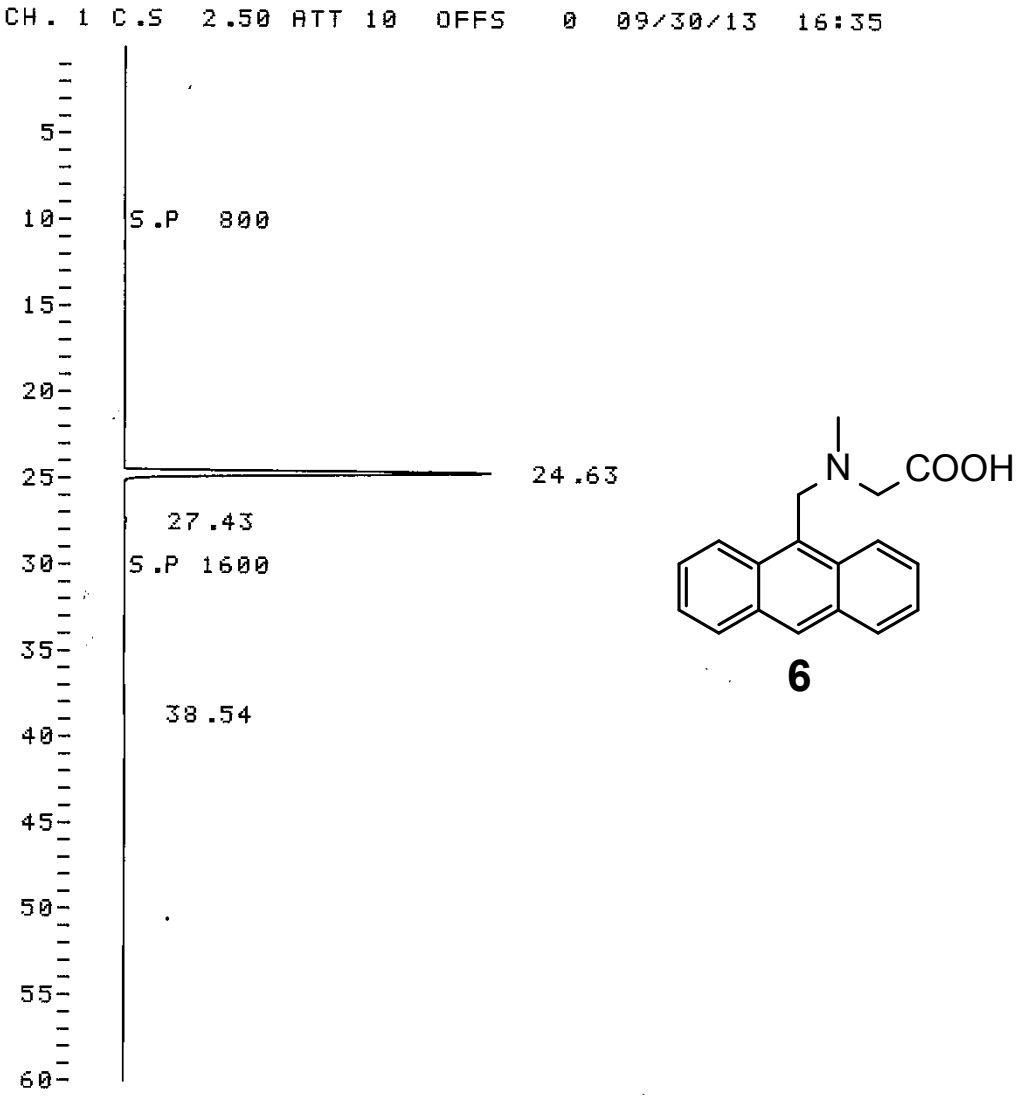
D-2500 10/01/13

METHOD: TAG: 9 CH: 1

FILE: 1 CALC-METHOD: AREA% TABLE: 0 CONC: AREA

NO.	RT	AREA	CONC	BC
2	38.80	274977	2.180	BV
3	39.23	12268590	97.264	VB
4	42.64	70084	0.556	BB
TOTAL		12613651	100.000	
PEAK REJ :		50000		

Detection wavelength: 360 nm



D-2500 09/30/13

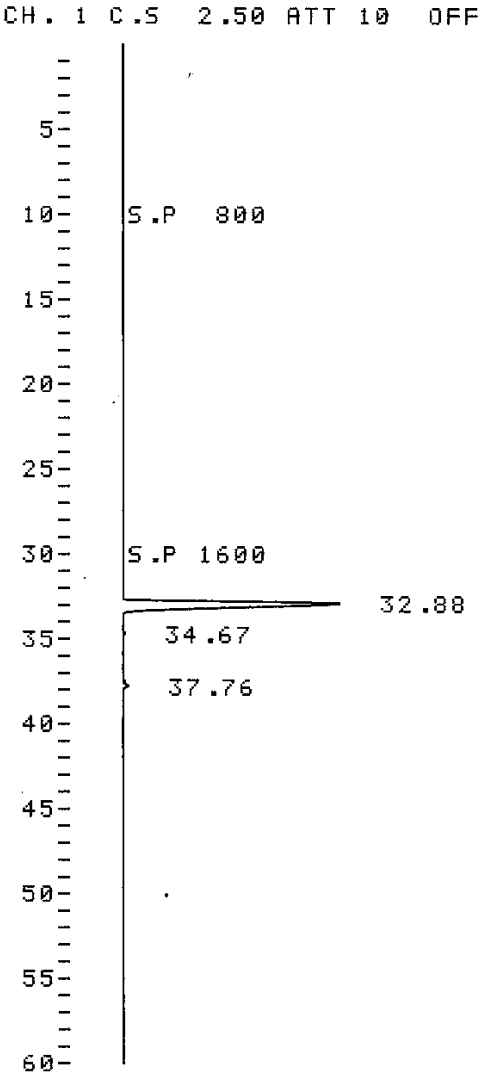
METHOD: TAG: 12 CH: 1

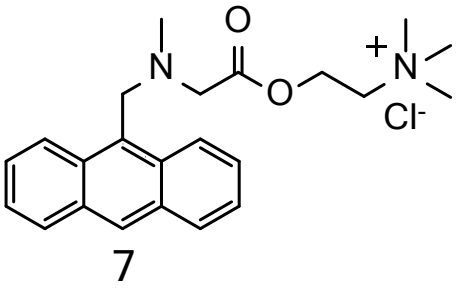
FILE: 1 CALC-METHOD: AREA% TABLE: 0 CONC: AREA

NO.	RT	AREA	CONC	BC
1	24.63	5369029	100.000	BB
TOTAL		5369029	100.000	

PEAK REJ : 50000

Detection wavelength: 360 nm





7

D-2500 10/11/13

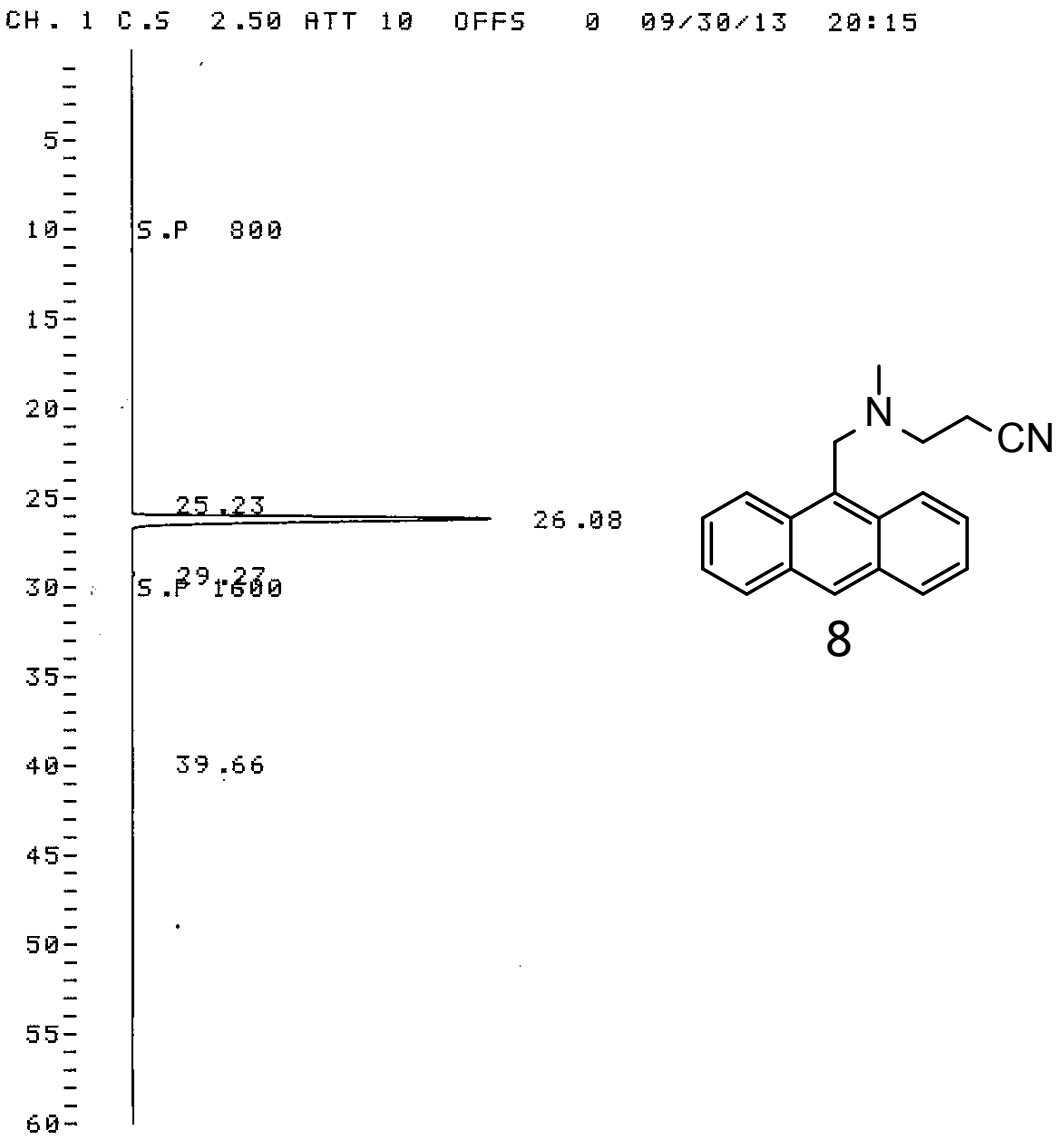
METHOD: TAG: 7 CH: 1

FILE: 1 CALC-METHOD: AREA% TABLE: 0 CONC: AREA

NO.	RT	AREA	CONC	BC
1	32.88	4580226	98.343	BV
3	37.76	77188	1.657	BB
TOTAL		4657414	100.000	

PEAK REJ : 50000

Detection wavelength: 360 nm



D-2500 09/30/13

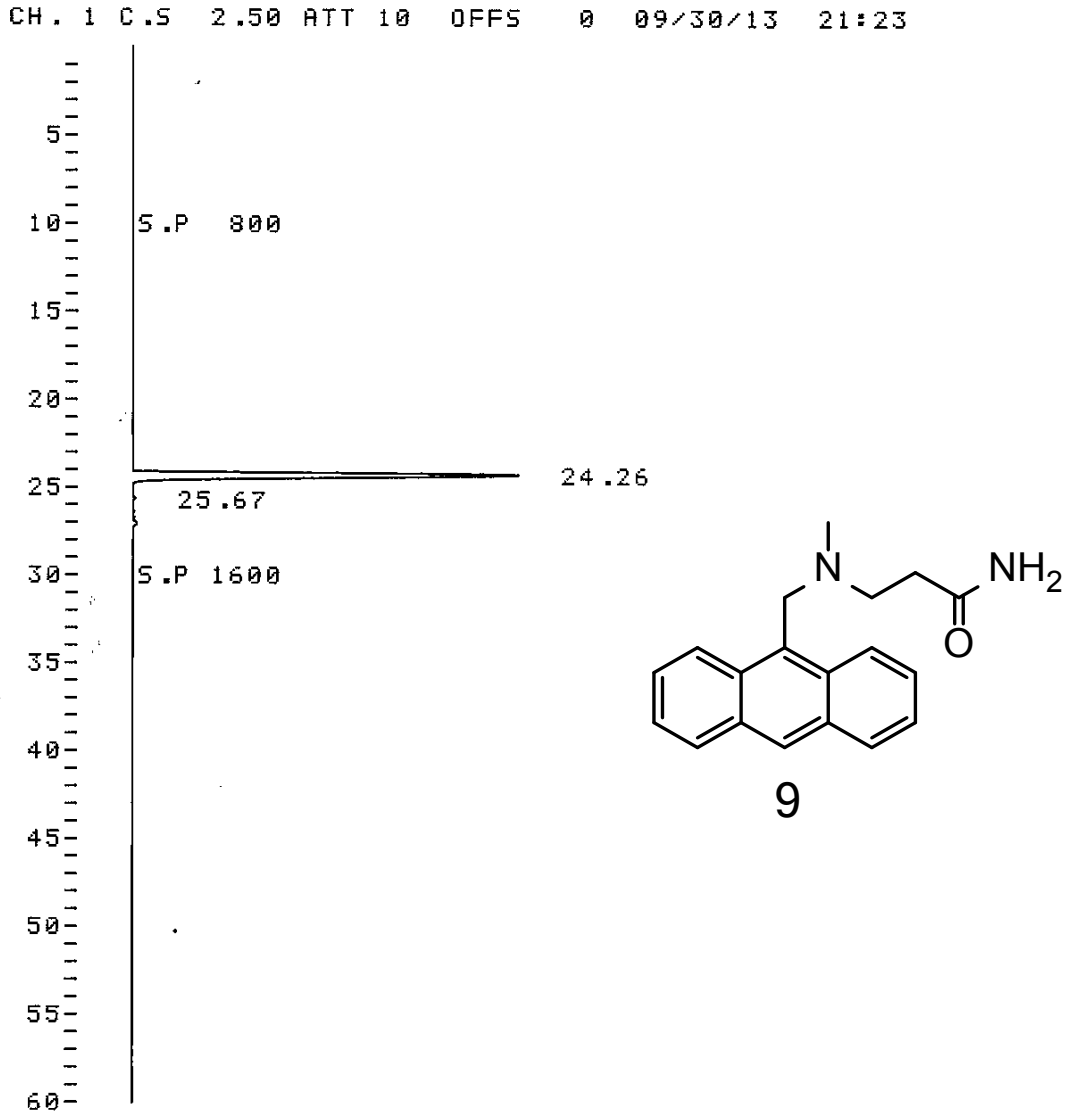
METHOD: TAG: 15 CH: 1

FILE: 1 CALC-METHOD: AREA% TABLE: 0 CONC: AREA

NO.	RT	AREA	CONC	BC
2	26.08	6743185	100.000	BB
TOTAL		6743185	100.000	

PEAK REJ : 50000

Detection wavelength: 360 nm



D-2500 09/30/13

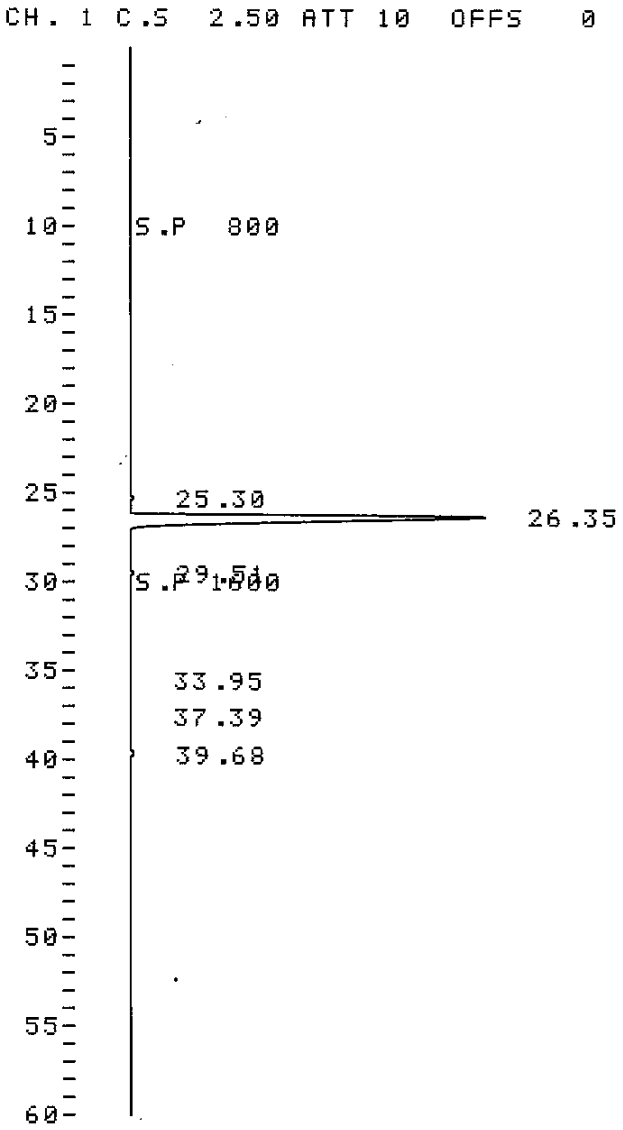
METHOD: TAG: 16 CH: 1

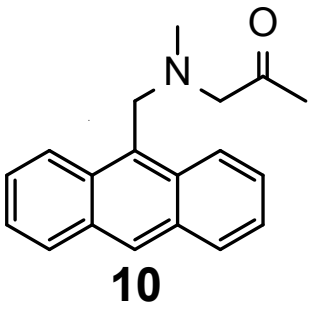
FILE: 1 CALC-METHOD: AREA% TABLE: 0 CONC: AREA

NO.	RT	AREA	CONC	BC
1	24.26	6166862	98.954	BB
5	27.07	65160	1.046	VB
TOTAL		6232022	100.000	

PEAK REJ : 50000

Detection wavelength: 360 nm





10

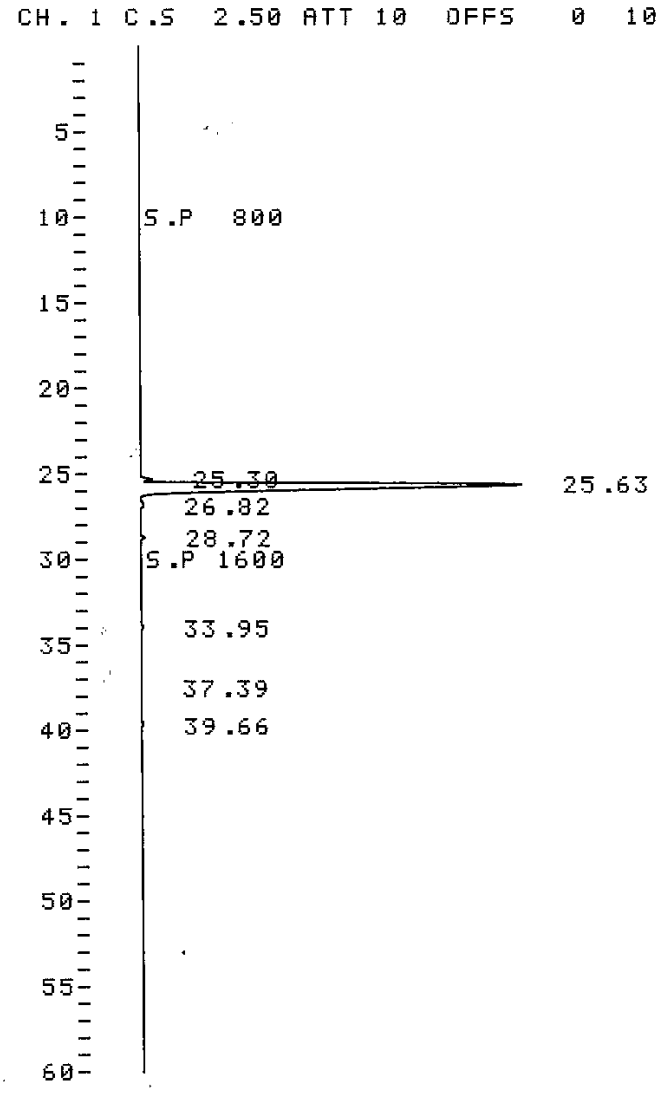
D-2500 10/01/13

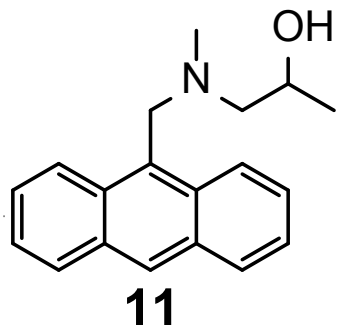
METHOD: TAG: 7 CH: 1

FILE: 1 CALC-METHOD: AREA% TABLE: 0 CONC: AREA

NO.	RT	AREA	CONC	BC
2	26.35	7485857	99.334	VB
6	39.68	50167	0.666	BB
TOTAL		7536024	100.000	
PEAK REJ :		50000		

Detection wavelength: 360 nm





11

D-2500 10/01/1

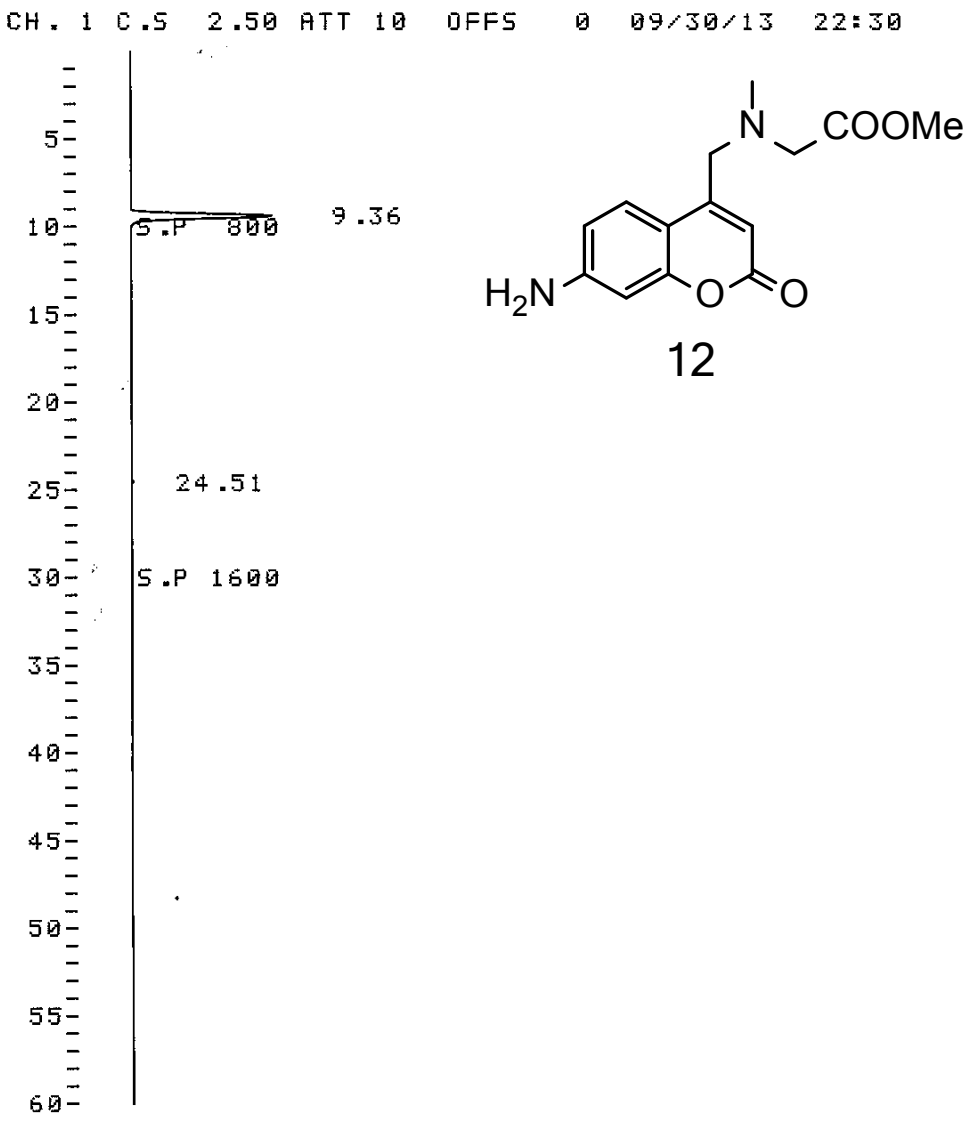
METHOD: TAG: 8 CH: 1

FILE: 1 CALC-METHOD: AREA% TABLE: 0 CONC: AREA

NO.	RT	AREA	CONC	BC
1	25.30	103541	1.236	BV
2	25.63	8274267	98.764	VV
TOTAL		8377808	100.000	

PEAK REJ : 50000

Detection wavelength: 360 nm



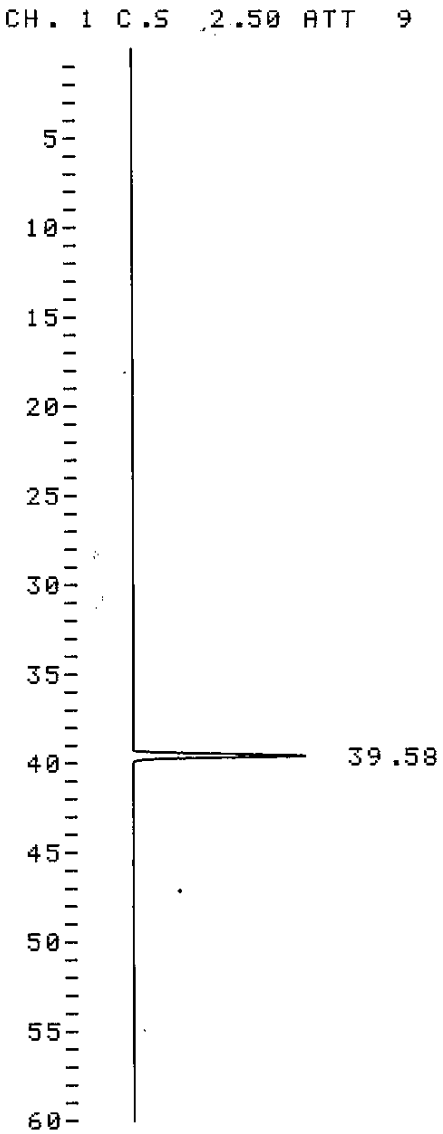
D-2500 09/30/13

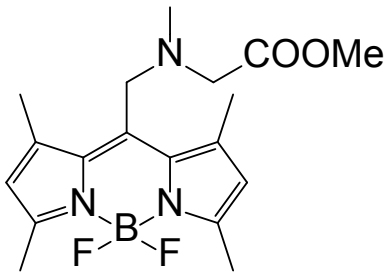
METHOD: TAG: 17 CH: 1

FILE: 1 CALC-METHOD: AREA% TABLE: 0 CONC: AREA

NO.	RT	AREA	CONC	BC
1	9.36	2436604	100.000	BB
TOTAL		2436604	100.000	
PEAK REJ :		50000		

Detection wavelength: 360 nm





13

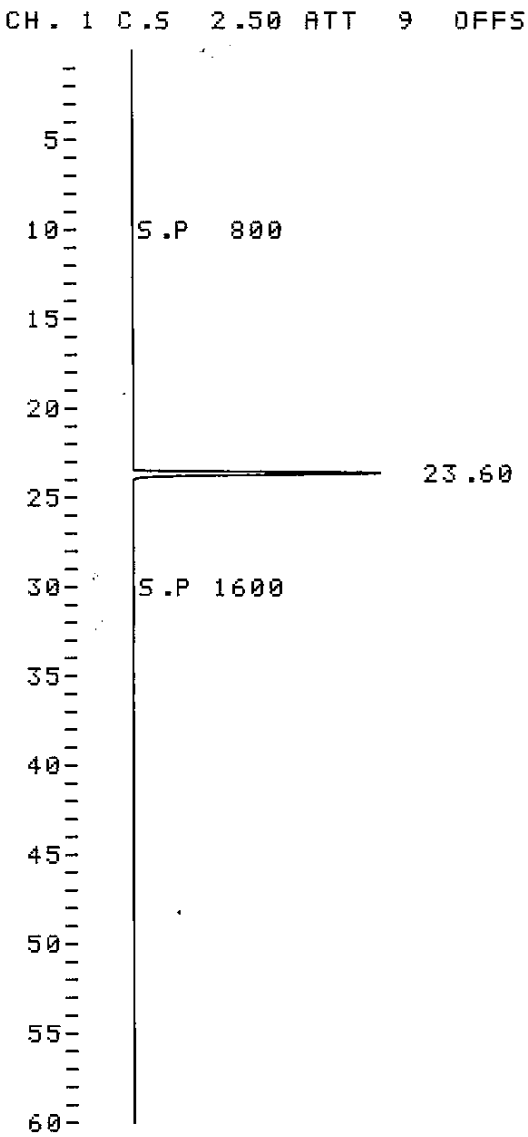
D-2500 10/01/13

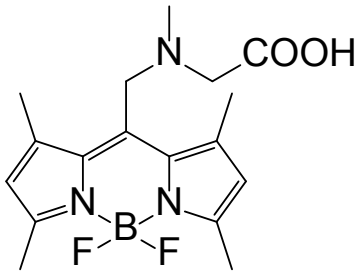
METHOD: TAG: 10 CH: 1

FILE: 1 CALC-METHOD: AREA% TABLE: 0 CONC: AREA

NO.	RT	AREA	CONC	BC
1	39.58	1080188	100.000	BB
TOTAL				
		1080188	100.000	
PEAK REJ :		50000		

Detection wavelength: 450 nm





14

D-2500 10/01/13

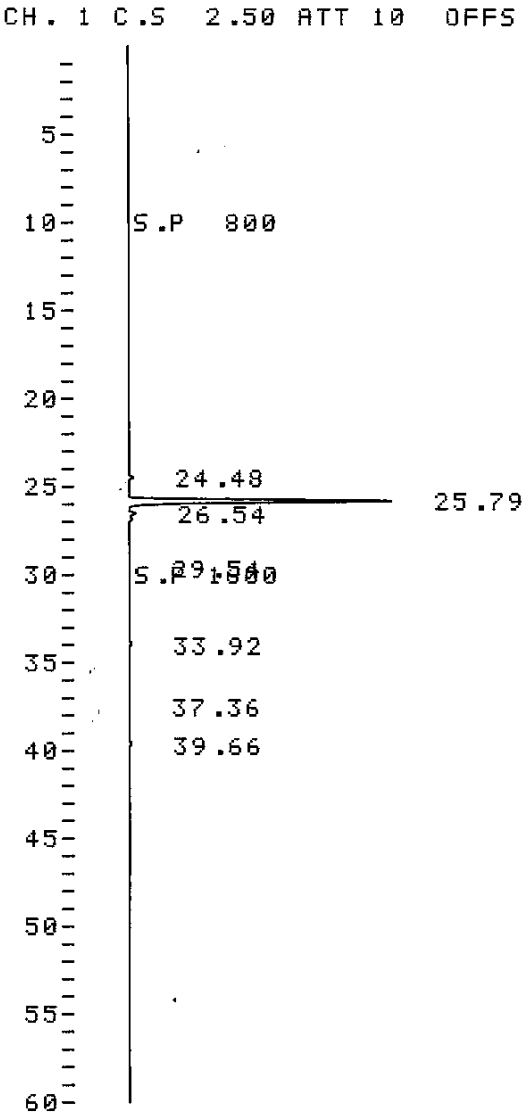
METHOD: TAG: 11 CH: 1

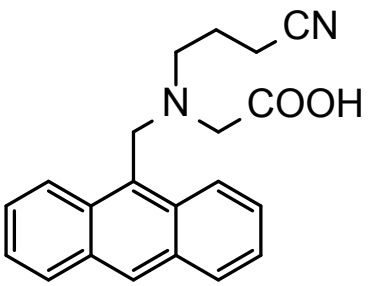
FILE: 1 CALC-METHOD: AREA% TABLE: 0 CONC: AREA

NO.	RT	AREA	CONC	BC
1	23.60	1258935	100.000	BB
TOTAL		1258935	100.000	

PEAK REJ : 50000

Detection wavelength: 450 nm





15

D-2500 09/30/13

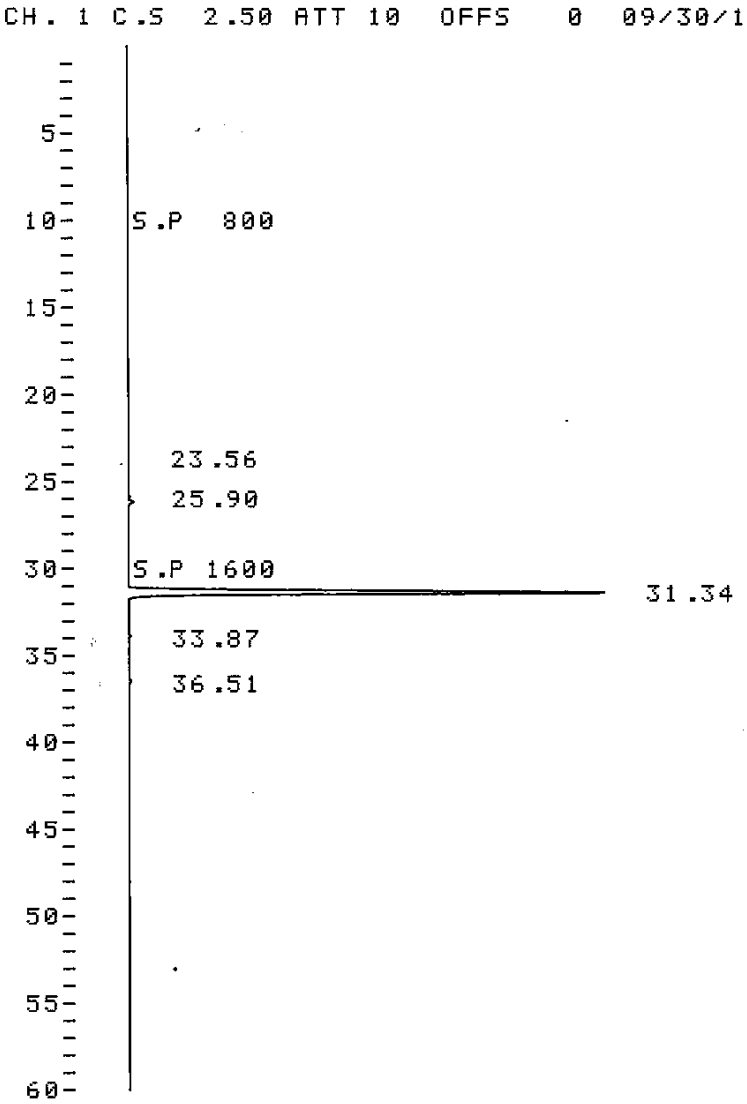
METHOD: TAG: 13 CH: 1

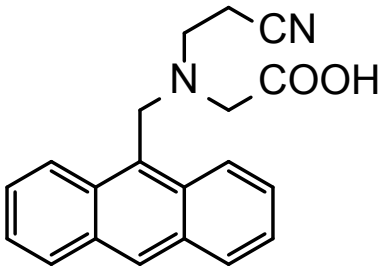
FILE: 1 CALC-METHOD: AREA% TABLE: 0 CONC: AREA

NO.	RT	AREA	CONC	BC
2	25.79	2988715	98.057	VV
3	26.54	59212	1.943	TBV
TOTAL		3047927	100.000	

PEAK REJ : 50000

Detection wavelength: 360 nm





16

D-2500 09/30/13

METHOD: TAG: 14 CH: 1

FILE: 1 CALC-METHOD: AREA% TABLE: 0 CONC: AREA

NO.	RT	AREA	CONC	BC
4	26.19	56946	0.949	VB
5	31.34	5945184	99.051	BB
TOTAL		6002130	100.000	

PEAK REJ : 50000

Detection wavelength: 360 nm