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Supporting Information

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Fluoromorphic Substrates for Fatty Acid Metabolism: Highly Sensitive Probes for Mammalian Medium-Chain Acyl-CoA Dehydrogenase

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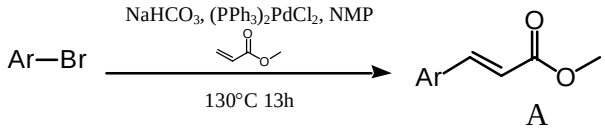
Materials and General methods

^{1}H and ^{13}C NMR spectra were recorded on Bruker 300 Fourier transform NMR spectrometers. Spectra were recorded in $CDCl_3$ solutions referenced to solvent residual peak, unless otherwise indicated. Low Resolution Mass Spectra were obtained on a JOEL JMS-HX110 HF mass spectrometer. Flash chromatography was performed on SILICYCLE silica gel (230-400 mesh). All chemicals were purchased from Aldrich or Sigma and used as received. All reactions were monitored by Thin Layer Chromatography.

Ultraviolet spectra were measured on a Molecular Devices SpectraMax Plus 384 UV/VIS spectrophotometer. Fluorescence measurements were taken on a Jobin Yvon Fluorolog fluorescence spectrofluorometer. Fluorescent measurements with 96-well plates were performed by the MicroMax 384 connected to a Jobin Yvon Fluorolog through F-3000 fiber optic cables.

Synthesis of Probes



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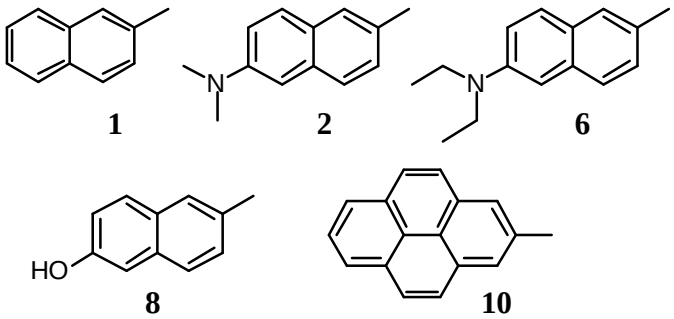

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Method A: General Procedure for the Synthesis of Methyl 3-aryl-acrylates from Aryl-bromides, Exemplified for Methyl 3-(6-dimethylamino-2-naphthyl)-acrylate (2A). 2-Bromo-6-dimethylamino naphthalene (1.0 g, 4.0 mmol), prepared according to literature,[1] $NaHCO_3$ (0.40 g, 4.8 mmol), $(PPh_3)_2PdCl_2$ (0.050 g, 0.072 mmol), methylacrylate (0.43 mL, 4.8 mmol), and 1-methyl-2-pyrrolidone (10 mL) were added to a sealed tube, placed under an Argon atmosphere, and heated at 130°C for 15h. The tube was cooled and the contents were poured into water (20 mL). The solids were collected by filtration and dissolved in CH_2Cl_2, washed with brine, dried ($MgSO_4$), and filtered through a celite pad. Recrystallization from hexanes resulted in 0.67 g of methyl 3-(6-dimethylamino-2-naphthyl)-acrylate (2A) (66% yield).

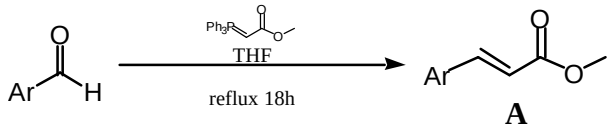
NMR ^{1}H (300 MHz, $CDCl_3$) δ ppm:

7.83 (d, J=15.9 Hz, 1H), 7.78 (s, 1H), 7.71 (d, J=9 Hz, 1H), 7.60 (q, J=8.7 Hz, 2H), 7.15 (dd, J_1=9 Hz, J_2=2.2 Hz, 1H), 6.88 (d, J=2.2 Hz, 1H), 6.41 (d, J=15.9 Hz, 1H), 3.85 (s, 3H), 3.10 (s, 6H).

NMR ^{13}C ((300 MHz, $CDCl_3$) δ ppm:

168.36, 149.84, 145.98, 136.55, 130.49, 130.06, 128.42, 127.23, 124.41, 116.79, 115.69, 106.43, 51.94, 41.00.

LRMS (FAB): 256 ($C_{16}H_{17}NO_2$, M+H).



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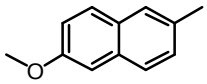

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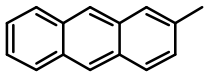

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Method B: General Procedure for the Synthesis of Methyl 3-aryl-acrylates from Aryl-aldehydes, Exemplified by Methyl 3-(6-methoxy-2-naphthyl)-acrylate (7A). A solution of 6-methoxy naphthaldehyde (0.5 g, 2.6 mmol) and methyl (triphenylphosphoranylidene)acetate (0.97 g, 2.9 mmol) in THF (10mL) was refluxed overnight under an argon atmosphere. Once the solution cooled, the solvent was evaporated and the remaining solids were treated with 20 mL diethyl ether. After stirring for 2 hours, the undissolved solids were removed. The filtrate was concentrated and purified by column chromatography using silica gel and hexanes-EtOAc 9:1 to obtain 0.58 g (92%) of methyl 3-(6-methoxy-2-naphthyl)-acrylate (7A).

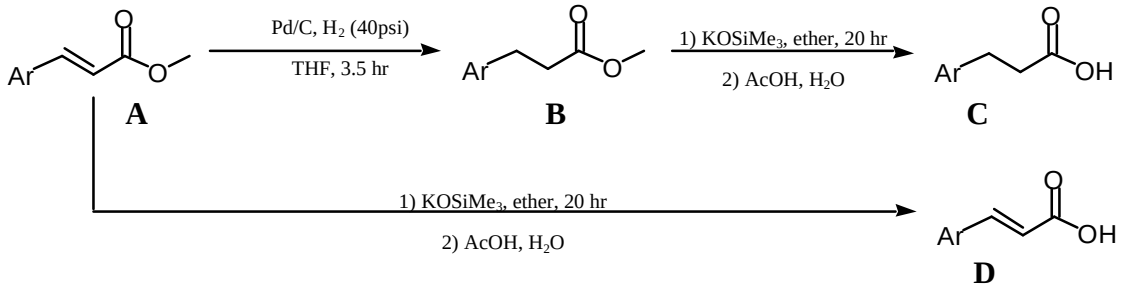
NMR ^{1}H (300 MHz, $CDCl_3$) δ ppm:

7.94-7.80 (m, 4H) 7.65 (dd, J_1=8.7 Hz, J_2=1.5 Hz, 1H), 7.2-7.14 (m, 2H), 6.52 (d, J=15.9 Hz, 1H), 3.96 (s, 3H), 3.85 (s, 3H)

NMR ^{13}C ((300 MHz, $CDCl_3$) δ ppm:

168.12, 159.22, 145.54, 136.10, 130.54, 130.22, 130.15, 129.05, 127.90, 124.56, 119.90, 117.13, 106.34, 55.79, 52.10

LRMS (FAB): 243 ($C_{15}H_{14}O_3$, M+H).



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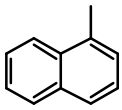

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General Procedure for the Synthesis of 3-Aryl-propionic Acids from Methyl 3-aryl-Acrylates. Exemplified for 3-(6-Dimethylamino-2-naphthyl)-propionic Acid (2C).

Methyl 3-(6-dimethylamino-2-naphthyl)-propionate (2B) Methyl 3-(6-dimethylamino-2-naphthyl)-acrylate (2A) (0.67 g, 2.63 mmol) and a catalytic amount of 10% Pd/C (0.0060 g) were combined and hydrogenated at 40 psi for 3.5 h. The reaction mixture was filtered through celite, and concentrated to yield 0.65 g pure methyl 3-(6-dimethylamino-2-naphthyl)-propionate (2B) (97% yield).

NMR ^{1}H (300 MHz, $CDCl_3$) δ ppm:
7.67 (d, J=9 Hz, 1H), 7.62 (d, J=8.4 Hz, 1H), 7.51 (s, 1H), 7.25 (dd, J_1=8.4 Hz, J_2=1.5 Hz, 1H), 7.18 (dd, J_1=9 Hz, J_2=2.7 Hz, 1H), 6.92 (d, J=2.1 Hz, 1H), 3.71 (s, 3H), 3.12 (t, J=6.9 Hz, 2H), 3.07 (s, 6H), 2.73 (t, J=6.9, 2H)

NMR ^{13}C ((300 MHz, $CDCl_3$) δ ppm:
174.0, 148.85, 134.48, 133.99, 128.69, 127.69, 127.36, 126.89, 126.46, 117.16, 106.97, 52.03, 41.43, 36.32, 31.37

LRMS (FAB): 258 ($C_{16}H_{19}NO_2$, M+H).

3-(6-Dimethylamino-2-naphthyl)-propionic acid (2C) Methyl 3-(6-dimethylamino-2-naphthyl)-propionate (2B) (0.65 g, 2.5 mmol) was added to a slurry of potassium trimethylsilanoate (0.33 g, 2.5 mmol) in 10 mL dry diethyl ether. The reaction mixture stirred under argon atmosphere for 20 h at RT. The resulting solid was filtered, washed with ether and dried. The solid was then dissolved in a small amount (~1 mL) of acetic acid and diluted with 70 mL water. After stirring for 1 h, the precipitate was collected to yield 0.34 g of 3-(6-dimethylamino-2-naphthyl)-propionic acid (2C) (63% yield).

NMR ^{1}H (300 MHz, D_2O) δ ppm:
7.72-7.64 (m, 2H), 7.56 (s, 1H), 7.33-7.24 (m, 2H), 7.18 (s, 1H), 2.94 (t, J=7.8 Hz, 2H), 2.85 (s, 6H), 2.49 (t, J=7.8 Hz, 2H)

NMR ^{13}C ((300 MHz, D_2O) δ ppm:
179.26, 148.30, 134.78, 133.85, 128.89, 127.94, 127.79, 127.15, 126.51, 117.42, 108.23, 41.90, 36.15, 31.05

LRMS (FAB): 244 ($C_{15}H_{17}NO_2$, M+H).

3-(2-Naphthyl)-propionoic acid (1C) was synthesized from 3-(2-naphthyl)-acrylic acid by hydrogenation as above for 2B.

NMR ^{1}H (300 MHz, DMSO-*d_6*) δ ppm:
δ 12.20 (s, 1H), 7.94-7.89 (m, 3H), 7.79 (s, 1H), 7.58-7.69 (m, 3H), 3.10 (t, J=7.7 Hz, 2H), 2.7 (t, J=7.7 Hz, 2H)

NMR ^{13}C (300 MHz, DMSO-*d_6*) δ ppm:
179.40, 144.15, 138.72, 137.26, 133.35, 133.07, 132.93, 132.85, 131.69, 131.92, 130.92, 40.71, 36.11

LRMS (FAB): 201 ($C_{13}H_{12}O_2$, M+H).

Methyl 3-(6-methoxy-2-naphthyl)-propionate (7B).

NMR ^{1}H (300 MHz, $CDCl_3$) δ ppm:
7.94-7.79 (m, 4H), 7.71 (dd, J_1=8.7 Hz, J_2=1.8 Hz, 1H), 7.27-7.21 (m, 2H), 6.59 (d, J=15.9 Hz, 1H), 4.03 (s, 3H), 3.92 (s, 3H).

NMR ^{13}C ((300 MHz, $CDCl_3$) δ ppm:
173.85, 157.74, 136.07, 133.59, 129.41, 127.86, 127.40, 126.73, 119.25, 106.01, 55.70, 52.07, 36.18, 31.33.

LRMS (FAB): 245 ($C_{15}H_{14}O_3$, M+H).

3-(6-Methoxy-2-naphthyl)-propionic acid (7C)

NMR ^{1}H (300 MHz, DMSO-*d_6*) δ ppm:
12.18 (s, 1H), 7.81-7.70 (m, 3H), 7.44-7.18 (m, 3H), 3.93 (s, 3H), 3.04 (t, J=6.9 Hz, 2H), 2.70 (t, J=6.9 Hz, 2H)

NMR ^{13}C ((300 MHz, DMSO-*d_6*) δ ppm: 174.67, 157.70, 136.85, 133.70, 129.68, 129.41, 128.47, 127.56, 126.82, 119.36, 106.62, 55.98, 36.11, 31.21

LRMS (FAB): 231 ($C_{14}H_{14}O_3$, M+H).

3-(6-Methoxy-2-naphthyl)-acrylic acid (7D)

NMR ^{1}H (300 MHz, DMSO-*d_6*) δ ppm:
12.38 (sb, 1H), 7.90-7.85 (m, 3H), 7.77 (d, J=16.2 Hz, 1H), 7.43 (d, J=2.4 Hz, 1H), 7.27 (dd, J_1=8.9 Hz, J_2=2.1 Hz, 1H), 6.66 (d, J=15.9 Hz, 1H), 3.97 (s, 3H)

NMR ^{13}C ((300 MHz, DMSO-*d_6*) δ ppm:
168.60, 159.23, 145.01, 136.17, 130.93, 130.45, 130.36, 129.10, 128.26, 125.41, 120.06, 119.15, 107.10, 56.18

LRMS (FAB): 289 ($C_{14}H_{12}O_3$, M+H).

Methyl 3-(2-anthracene)-acrylate (9A) was prepared from 2-anthracene carboxylic acid[2] using Method B.

NMR ^{1}H (300 MHz, $CDCl_3$) δ ppm:
8.43 (d, J=15 Hz, 2H), 8.09-7.99 (m, 4H), 7.91 (d, J-15.9 Hz, 1H), 7.66 (d, J=15, 1H), 7.53-7.50 (m, 2H), 6.59 (d, J=15.9 Hz, 1H), 3.87 (s, 3H)

NMR ^{13}C (300 MHz, $CDCl_3$) δ ppm:
179.26, 156.61, 144.25, 143.87, 143.50, 143.19, 142.99, 140.81, 139.99, 139.92, 139.24, 138.01, 137.88, 137.60, 134.06, 129.55, 63.44

LRMS (FAB): 263 ($C_{18}H_{13}O_2$, M+H).

2-Anthracene acrylic acid (9D)

NMR ^{1}H (300 MHz, DMSO-d_6) δ ppm:

12.44 (s, 1H), 8.76 (d, J=8.4 Hz, 2H), 8.31 (s, 1H), 8.10-8.07 (m, 2H), 7.88-7.78 (m, 2H), 7.56-7.53 (m, 2H), 6.71 (d, J=15.6, 1H)

NMR ^{13}C (300 MHz, DMSO-d_6) δ ppm:

168.53, 144.78, 132.85, 132.51, 132.44, 132.17, 131.81, 129.78, 129.07, 128.98, 128.18, 127.15, 127.00, 126.88, 123.80, 120.45

LRMS (FAB): 249 ($C_{17}H_{12}O_2$, M+H).

Methyl 3-(2-anthracene)-propionate (9B) was prepared from methyl 3-(2-anthracene)-acrylate (9A) according to literature procedure.[3] Methyl 3-(2-anthracence)-acrylate (50 mg, 0.17 mmol) was hydrogenated at 10 psi over 10% Pd/C (4 mg) in 20 mL anhydrous ethanol for 4.5 h. This was filtered through celite and concentrated. The crude product was dissolved in benzene (5 mL) and o-chloranil (42 mg, 0.17 mmol) was added. This mixture was refluxed, under argon for 3 h. After the reaction cooled it was washed with water, concentrated, and purified by column chromatography on silica gel with Hexanes/EtOAc 95:5 to yield 43 mg (87%).

NMR ^{1}H (300 MHz, $CDCl_3$) δ ppm:

8.39 (d, J=11.1 Hz, 2H), 8.01-7.94 (m, 3H), 7.79 (s, 1H), 7.49-7.43 (m, 2H), 7.33 (d, J=8.7 Hz, 1H), 3.72 (s, 3H), 3.18 (t, J=7.7 Hz, 2H), 2.80 (t, J=7.7 Hz), 2H

NMR ^{13}C ((300 MHz, $CDCl_3$) δ ppm:

173.75, 137.79, 132.31, 132.22, 131.89, 131.03, 128.87, 128.58, 128.50, 127.33, 126.46, 126.41, 126.06, 125.78, 125.55, 52.08, 35.70, 31.68

LRMS (FAB): 265 ($C_{18}H_{16}O_2$, M+H).

3-(2-anthracene)-propionic acid (9C)

NMR ^{1}H (300 MHz, DMSO-d_6) δ ppm:

12.18 (s, 1H), 8.49 (d, J=15.3 Hz, 2H), 8.07-7.99 (m, 3H), 7.82 (s, 1H), 7.50-7.41 (m, 3H), 3.03 (t, J=7.5 Hz, 2H)), 2.69 (t, J=7.5Hz, 2H)

NMR ^{13}C ((300 MHz, DMSO-d_6) δ ppm:

174.64, 138.94, 132.23, 131.76, 131.02, 128.95, 128.90, 128.80, 128.23, 126.61, 126.47, 126.35, 126.15, 126.13, 35.62, 31.55

LRMS (FAB): 251 ($C_{17}H_{14}O_2$, M+H).

2-Bromo-6-diethylamino naphthalene A mixture of 6-bromo-2-naphthol (10.0 g, 44.8 mmol), $Na_2S_2O_5$ (16.1 g, 85.1 mmol), NaOH (6.9 g, 224 mmol), and 40 mL water were combined in a sealed tube. Ethylamine hydrochloride (16.2 g, 224 mmol) was quickly added, the tube was sealed, and heated at 140°C for 5 days. After cooling, the mixture was poured into 80 mL NaOH (2M). The precipitate was collected and used without purification in the next step. 2-Bromo-6-ethylamino naphthalene (4.31 g, 17.2 mmol), and sodiumtriacetoxyborohydride (3.65 g, 17.2 mmol) were added to a flask and flushed with argon. CH_2Cl_2 (100 mL), acetaldehyde (1.93 mL, 17.2 mmol), and acetic acid (0.99 mL, 17.2 mmol) were added to the flask. The reaction stirred for 6 h, after which a second equivalent of sodiumtriacetoxyborohydride (3.65 g, 17.2 mmol) and acetaldehyde (1.93 mL, 17.2 mmol) were added, this stirred for 12 h. The reaction was diluted with CH_2Cl_2 (100 mL), washed with a saturated solution of $NaHCO_3$, water, and dried ($MgSO_4$). Purification by column chromatography on silica gel with hexanes-EtOAc 95:5 gave 2-bromo-6-diethylamino naphthalene in 66% yield.

NMR ^{1}H (300 MHz, $CDCl_3$) δ ppm:
7.88 (s, 1H), 7.67 (d, J=9.0 Hz, 1H), 7.56 (d, J=9.0 Hz, 1H), 7.48 (d, J=9.0 Hz, 1H), 7.18 (d, J=9 Hz, 1H), 6.91 (d, J=1.5 Hz, 1H), 3.56 (q, J=6.9 Hz, 4H), 1.33 (t, J=6.9 Hz, 6Hz)
NMR ^{13}C (300 MHz, $CDCl_3$) δ ppm:
146.48, 134.23, 129.70, 129.65, 128.40, 127.96, 127.62, 117.04, 114.79, 105.47, 44.91, 13.06
LRMS (FAB): 278 ($C_{14}H_{16}BrN$, M+H).

Methyl 3-(6-diethylamino-2-naphthyl)-acrylate (6A) was synthesized using Method A from 2-bromo-6-diethylamino naphthalene.
NMR ^{1}H (300 MHz, $CDCl_3$) δ ppm:
7.83-7.67 (m, 3H), 7.56-7.52 (m, 2H), 7.10-7.04 (m, 1H), 6.83 (s, 1H), 6.47 (d, J=15.8 Hz, 1H), 3.83 (s, 3H), 3.50 (q, J=6.9 Hz, 4H), 1.26 (t, J=6.9 Hz, 6H)
NMR ^{13}C (300 MHz, $CDCl_3$) δ ppm:
168.44, 147.35, 146.12, 136.95, 130.55, 130.28, 127.83, 126.92, 125.93, 124.32, 116.35, 115.23, 105.33, 51.91, 44.94, 13.10
LRMS (FAB): 284 ($C_{18}H_{21}NO_2$, M+H).

Methyl 3-(6-diethylamino-2-naphthyl)-propionate (6B)
NMR ^{1}H (300 MHz, $CDCl_3$) δ ppm:
7.62 (d, J=9.0 Hz, 1H), 7.56 (d, J=8.4 Hz, 1H), 7.47 (s, 1H), 7.44 (dd, J_1=8.4 Hz, J_2=1.8 Hz, 1H), 7.10 (dd, J_1=9.0 Hz, J_2=2.4 Hz, 1H), 6.87 (d, J=2.4 Hz, 1H), 3.69 (s, 3H), 3.47 (q, J=7.0 Hz, 4H), 3.07 (t, J=8.1 Hz, 2H), 2.71 (t, J=8.1 Hz, 2H), 1.24 (t, J=7.0 Hz, 6H)
NMR ^{13}C (300 MHz, $CDCl_3$) δ ppm:
174.02, 145.99, 132.34, 133.86, 128.87, 127.61, 126.75, 126.61, 126.45, 116.71, 105.99, 52.07, 44.99, 36.38, 31.38, 13.06
LRMS (FAB): 286 ($C_{18}H_{23}NO_2$, M+H).

3-(6-Diethylamino-2-naphthyl)-propionic Acid (6C)
NMR ^{1}H (300 MHz, MeOD-d_4) δ ppm:
7.61 (d, J=9.1 Hz, 1H), 7.54 (d, J=8.4 Hz, 1H), 7.47 (s, 1H), 7.21 (d, J=8.4 Hz, 1H), 7.12 (dd, J_1=9.1 Hz, J_2=2.2 Hz, 1H), 6.02 (s, 1H), 3.45 (q, J=7.0 Hz, 4H), 3.01 (t, J=7.8 Hz, 2H), 2.66 (t, J=7.8 Hz, 2H), 1.19 (t, J=7.0 Hz, 6H)
NMR ^{13}C (300 MHz, MeOD-d_4) δ ppm:
175.92, 134.41, 134.37, 145.56, 128.50, 127.42, 127.16, 126.36, 125.93, 116.72, 106.85, 44.89, 36.04, 31.08, 11.89
LRMS (FAB): 272 ($C_{17}H_{21}NO_2$, M+H).

3-(1-Naphthyl)-propionic acid (8C) was synthesized by hydrogenation of 3-(1-naphthyl)-acrylic acid.
NMR ^{1}H (300 MHz, DMSO-d_6) δ ppm:
8.13 (d, J=8.1 Hz, 1H), 7.99 (d, J=7.8 Hz, 1H), 7.85 (d, J=7.8 Hz, 1H), 7.66-7.56 (m, 2H), 7.52-7.44 (m, 2H), 3.39 (t, J=7.5, 2H), 2.76 (t, J=7.5, 2H)
NMR ^{13}C (300 MHz, DMSO-d_6) δ ppm:
174.69, 137.63, 134.26, 132.07, 129.51, 127.57, 127.01, 126.60, 126.50, 124.30, 35.47, 28.27

LRMS (FAB): 201 ($C_{13}H_{12}O_2$, M+H).

Benzyl 3-(6-hydroxy-2-naphthyl)-acylate was synthesized starting from 6-bromo-2-naphthol, substituting benzyl acrylate for methyl acrylate, using Method A .

NMR ^{1}H (300 MHz, $CDCl_3$) δ ppm:
10.03 (s, 1H), 8.07 (s, 1H), 7.81-7.76 (m, 3H), 7.69 (d, J=8.7 Hz, 1H), 7.45-7.31 (m, 5H), 7.13-7.09 (m, 2H), 6.71 (d, J=15.9 Hz, 1H), 5.23 (s, 2H)

NMR ^{13}C (300 MHz, $CDCl_3$) δ ppm:
171.92, 162.49, 150.95, 141.97, 141.39, 135.96, 134.10, 133.69, 132.97, 132.41, 129.79, 124.91, 121.87, 114.64, 71.12

LRMS (FAB): 229 ($C_{14}H_{12}O_3$, M+H).

3-(6-hydroxy-2-naphthyl)-propionic acid (5C) was synthesized from catalytic hydrogenation of benzyl 3-(6-hydroxy-2-naphthyl)-acrylate.

NMR ^{1}H (300 MHz, MeOD-d_4) δ ppm:
7.64 (d, J=8.7 Hz, 1H), 7.58-7.56 (m, 2H), 7.27 (dd, J_1=8.4 Hz, J_2=1.8 Hz, 1H), 7.07-7.02 (m, 2H), 3.37-3.32 (m, 1H), 3.03 (t, J=8.4 Hz, 2H), 2.68 (t, J=8.4 Hz, 2H)

NMR ^{13}C (300 MHz, MeOD-d_4) δ ppm:
175.86, 155.02, 135.48, 134.05, 129.02, 127.31, 126.43, 126.26, 118.27, 108.73, 35.77, 30.98

LRMS (FAB): 217 ($C_{13}H_{12}O_3$, M+H).

Methyl 3-(2-pyrene)-acrylate (10A) was synthesized starting from 2-bromopyrene using Method A

NMR ^{1}H (300 MHz, $CDCl_3$) δ ppm:
8.82 (d, J=15.7 Hz, 1H), 8.45 (d, J=9.3 Hz, 1H), 8.25-8.16 (m, 4H), 8.13-8.09 (m, 2H), 8.05-8.0 (m, 2H), 6.70 (d, J=15.7 Hz, 1H), 3.93 (s, 3H)

NMR ^{13}C (300 MHz, $CDCl_3$) δ ppm:
167.99, 141.96, 133.08, 131.67, 131.05, 130.10, 128.96, 128.49, 127.69, 126.66, 126.39, 126.19, 125.42, 125.36 125.24, 124.50, 122.81, 120.13, 52.27, 30.14

LRMS (FAB): 287 ($C_{20}H_{14}O_2$, M+H).

Methyl (2-pyrene)-propionate (10B)

NMR ^{1}H (300 MHz, $CDCl_3$) δ ppm:
8.27 (d, J=9.3 Hz, 1H), 8.19-8.10 (m, 4H), 8.03-7.98 (m, 3H), 7.90 (d, J=7.8 Hz, 1H), 3.74 (s, 3H), 3.72 (t, J=8.1 Hz, 2H), 2.90 (t, J=8.1 Hz, 2H)

NMR ^{13}C (300 MHz, $CDCl_3$) δ ppm:
185.07, 146.19, 143.10, 142.55, 141.90, 140.29, 139.33, 139.16, 138.70, 138.58, 137.60, 136.76, 136.61, 134.58, 63.42, 47.61, 40.37

LRMS (FAB): 289 ($C_{20}H_{16}O_2$, M+H).

3-(2-Pyrene)-propionic acid (10C)

NMR ^{1}H (300 MHz, $CDCl_3$) δ ppm:
8.16 (d, J=7.2 Hz, 2H), 8.10-7.94 (m, 7), 3.70 (t, J=8.2 Hz, 2H), 2.89 (t, J=8.2 Hz, 2H)

NMR ^{13}C (300 MHz, $CDCl_3$) δ ppm:

175.07, 147.29, 145.62, 142.73, 140.84, 140.36, 139.37, 139.05, 138.65, 137.49, 136.21, 42.91, 39.54

LRMS (FAB): 275 ($C_{19}H_{14}O_2$, M+H).

Preparation of Coenzyme A Derivatives:

The coenzyme A derivatives were prepared using the mixed anhydride method as reported by Fong and Schulz.[4] Briefly, a solution of coenzyme A (10 mg, 0.013 mmol), and $NaHCO_3$ in degassed water (3 mL) was prepared and dry THF (2 mL) was added. The carboxylic acid (0.067 mmol) was added to a second flask and flushed with argon. It was then dissolved in dry THF (2mL) and freshly distilled triethylamine (0.067 mmol) and ethylchloroformate (0.067 mmol) were added. After ten minutes the reaction was quickly filtered through a pipet with a glass wool plug. This was added to the coenzyme A solution over 10 minutes, adding extra water to prevent phase separation. After 1 hour the solution was acidified to pH 3 and the THF was removed under reduced pressure.

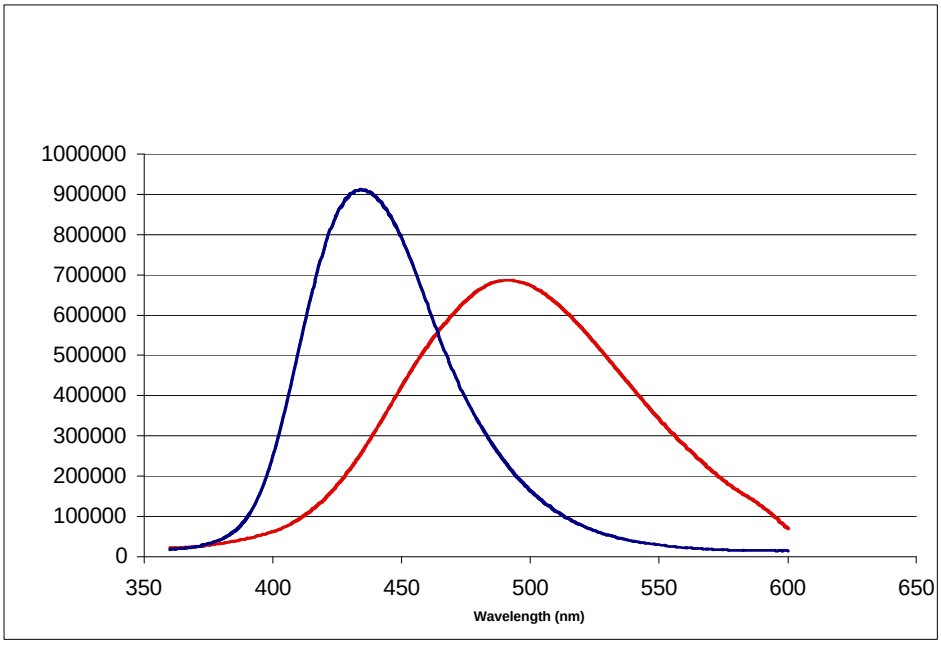
The solutions were purified by HPLC using water (with 0.1% TFA) and acetonitrile, while monitoring at 254 nm. Fractions containing product were determined by MS or fluorescence and the acetonitrile was removed under reduced pressure. Compounds were found to be greater than 95% pure by HPLC. Concentration of coenzyme A derivatives was determined as previously reported.[5] The thioester bond was quantitatively cleaved with 1 M hydroxylamine (pH 7) and the free thiols were reacted with 5,5'-dithiobis(2-nitrobenzoic acid). The absorbance in 100 mM potassium phosphate buffer (pH 8) was recorded at 412 nm (ε=13,700 $M^{-1}cm^{-1}$). CoA-derivatives were stored in water at –20°C and the concentration was checked periodically for degradation. The derivatives are stable for over a month at -20°C, even with repeated thawing.

Photochemical Characterization:

Fluorescence Spectra of Selected Probes 1, 7, and 9:

All compounds were excited at their excitation maxima. Compounds **1** and **3** were excited at 340 nm, compounds **7** and **11** were excited at 350 nm, and compounds **9** and **12** were excited at 356nm. Fluorescence emission spectra were recorded with 50 μM solutions in 100 mM potassium phosphate buffer (pH 8.0).

A/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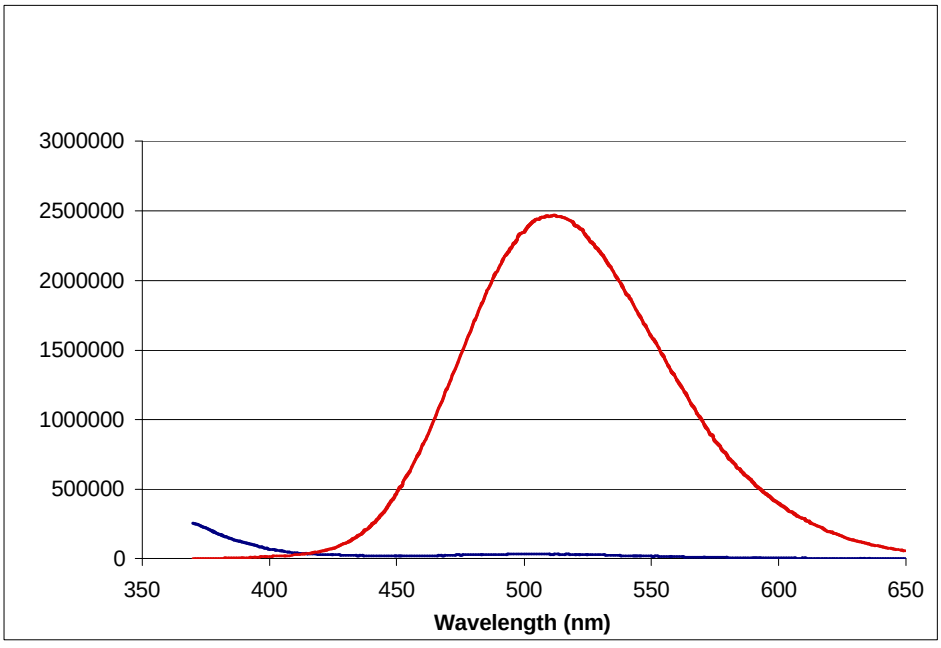
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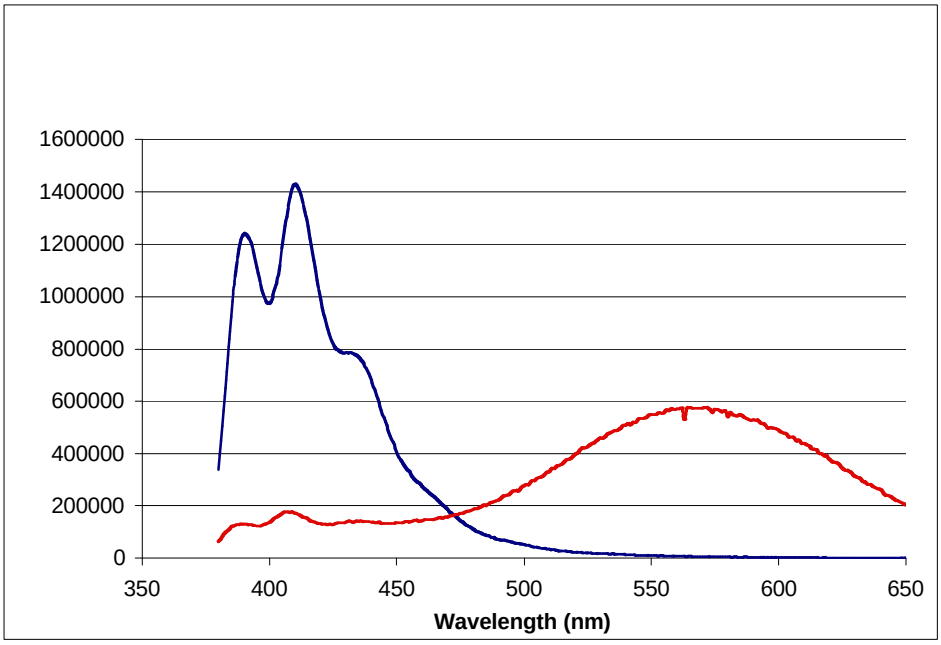

Figure SS1: Fluorescent Spectra of Active Probes. All spectra are 50 μM probe in 100 mM potassium phosphate buffer (pH 8). A/ Fluorescent spectra of **1** and **3** (λ_{ex}=340 nm). B/ Fluorescent spectra of **7** and **11** (λ_{ex}=350 nm). C/ Fluorescent spectra of **9** and **12** (λ_{ex}=356 nm).

Photophysical Characterization

Extinction coefficients reported are the average of triplicate measurements of the lowest energy wavelength transition at three different concentrations. Fluorescence quantum yields are the average of three independent quantum yield determinations and are determined by excitation at 260 or 350 nm using 9, 10-diphenylanthracene in cyclohexane as the standard.[6]

Table SS1: Photophysical Properties of Probes.

	Saturated					Unsaturated			
	λ_{max} (nm)	ε ($M^{-1}cm^{-1}$)	λ_{em} (nm)	ϕ		λ_{max} (nm)	ε ($M^{-1}cm^{-1}$)	λ_{em} (nm)	ϕ
1	260	28000±6000	436	0.026[b]	**3**	325	69000±8000	492	0.021[b]
7	232	17000±4000	-	-	**11**	345	26000±400	510	0.043[a]
9	256	9300±200	410	0.134[a]	**12**	350	18000±6000	567	0.014[a]

‡ In 100 mM potassium phosphate buffer (pH 7.2). [a] relative to 9, 10-diphenyl anthracene as a standard (excited at 350 nm); [b] relative to 9, 10-diphenyl anthracene as a standard (excited at 260 nm).

Protocols for Enzymatic Assays

Procedure for Enzymatic Screening of Probes 1, 2, 5-10:

Rat medium-chain acyl-CoA dehydrogenase (rMCAD) was provided by Professor Horst Schulz (City University of New York); pig MCAD (pMCAD), pig short-chain acyl-CoA dehydrogenase (SCAD), and human long-chain acyl-CoA dehydrogenase (LCAD) were

provided by Professor Jung-Ja Kim (Medical College of Wisconsin). Activity was checked by DCPIP/PMS assay[7] using butanoyl-CoA, octanoyl-CoA, or palmitoyl-CoA as the substrate for SCAD, MCAD, and LCAD, respectively. Enzymatic assays were performed in duplicate on probes using the DCPIP/PMS assay or ferricenium hexafluorophosphate ($FerPF_6$) assay[8] according to the following protocol.

For the DCPIP/PMS assay the following solution was prepared: 2,6-dichloroindolephenol (28 μM), N-ethyl maleimide (0.2 mM), and 100 mM potassium phosphate buffer pH 7.6 (Buffer A). This was stored in an amber bottle, under argon. To initiate the assay, substrate (50 μL) was added to a quartz cuvette which contained Buffer A, KCN (8.2 μL, 2.93 mg/mL), 0.5 – 6 μL of enzyme (concentrations of 2 – 7 mg/mL), with a total volume of 730 μL. The absorbance decrease at 600 nm was recorded for 3 minutes (Rate A). Then, 35 μL of 20 mg/mL phenazine methosulfate was added and the absorbance decrease at 600 nm was recorded (Rate B). A control experiment at each concentration of enzyme was run as above, omitting substrate. The rate was determined as (ε^{600} 21,300 $M^{-1}cm^{-1}$):

Rate = Rate B – Rate A – control

For $FerPF_6$ assay a solution of $FerPF_6$ in 10 mM HCl was prepared daily and the concentration was determined by measuring the absorbance at 617 nm (ε^{617} 410 $M^{-1}cm^{-1}$). The assay was started by the addition of 0.5 – 6 μL enzyme (concentrations 2 – 7 mg/mL) to a quartz cuvette containing Buffer B (700 μL; potassium phosphate buffer (100mM, pH 7.2) with 0.1mM EDTA), $FerPF_6$ (200 μM), and substrate (50 μM). The absorbance decrease was monitored at 300 nm (ε^{300} 4,300 $M^{-1}cm^{-1}$) for three minutes. The rate was taken as this rate minus a control when substrate was omitted. In some instances an increase in absorbance was observed, due to the product absorbing at 300 nm. Unactive probes were considered those that did not give a significant rate by either of the above assays. The formation of product was corroborated by HPLC analysis.

Determination of Steady State Kinetic Parameters for MCADs

Michaelis Menten constant (K_m) and catalytic rate (k_{cat}) of the fluorogenic substrates were determined as follows: to a STARNA semi-micro fluorometer cell (equipped with a stir bar and with 4 polished walls) was added (1) Buffer B (final volume 700 μL), (2) $FerPF_6$ (200 μM), (3) substrate (to achieve assay concentrations of $5K_M$ to $K_M/5$) and (4) 2 μL of diluted MCAD or homogenate (1:2 to 1:20, depending on the kinetics of a particular isozyme's reduction of a substrate). Fluorescence arising from the formation of product was monitored over the course of 3 minutes (excitation and emission band pass slits both at 3 nm, lamp 750 V, λ_{ex} 350 nm for **1** and **7**, λ_{ex} 356 nm for **9**, λ_{em} 492, 510, or 567 for **1**, **7**, and **9**, respectively). The rate of product formation, expressed in units of nanomoles per minute, were calculated according to previously published procedures[9]:

$$\text{initial rate} = \frac{\left[n_{st} \times \left(\frac{F_t - F_0}{F_{st}} \right) \right]}{t} \qquad (1)$$

where F_t and F_0 represent the fluorescence at times t and 0 minutes, n_{st} is the nanomoles of product in a known concentration of product, and F_{st} is the fluorescence resulting from n_{st} of

product. Kinetic parameters were approximated by GraFit (Erithacus Software, Surrey, UK) nonlinear regression analysis program to fit the untransformed data to a hyperbolic function as originally described. Reported enzymatic kinetic parameters are the average of three independent determinations from three different preparations of enzyme. Validity of fluorescent assay was confirmed by a chromogenic assay where the formation of product was monitored spectrophotometrically (according to Table 1). Kinetic parameters for chromogenic and fluorescent assays were similar. For example, for probe **1** with rMCAD k_{cat} was found to be 311 ± 60 min^{-1}, by the UV assay, while the fluorescent assay yielded a k_{cat} of 370 ± 10 min^{-1}.

Preparation of Tissue Homogenate

Tissue homogenate was prepared as already reported.[7] Briefly, rat liver (gift of Horst Schulz, City University of New York), was minced and then homogenized at 0°C with 5 vol of isolation buffer (mannitol (210 μM), sucrose (70 μM), Tris (10 μM) and EDTA (0.1 mM), adjusted to pH 7.4 with HCl) for 2 minutes. This was treated with Triton X-100 (0.2% v/v) and stirred at 0°C for 15 minutes. After, being clarified by centrifugation (12,000 xg for 10 min at 4°C), the resultant clear homogenate was assayed for MCAD as above.

Competitive Substrate Assays

Competitive substrate assays were carried out as described above for $FerPF_6$, except in a 96-well plate with a total volume of 200 μL. To a black 96-well plate were added: (1) Buffer B (total volume 200 μL), (2) $FerPF_6$ (200 μM), (3) butanoyl-CoA, octanoyl-CoA, or palmitoyl-CoA (50 μM), and (4) homogenate (87 μg protein). After 30 seconds **1**, **7**, or **9** (10 μM) was added and the fluorescence was monitored for 3 minutes.

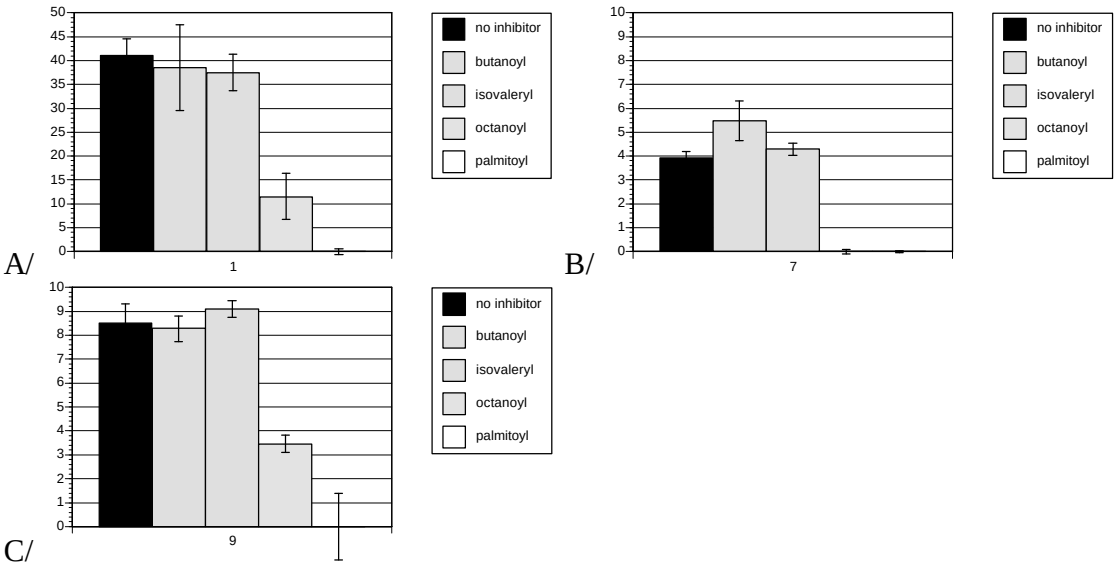

Figure SS2: Competitive substrate assays for fluorescent probes **1**, **7,** and **9**. Competitive substrate (50 μM butanoyl-CoA, isovaleryl-CoA, octanoyl-CoA, or palmitoyl-CoA) was preincubated with rat liver homogenate, 10 μM fluorescent probe (**1**, **7,** or **9)** was added and the fluorescence was monitored (490, 510, or 567 nm, respectively). Zero activity is defined as a decrease in fluorescence, which was similar to the decrease in fluorescence when fluorescent substrate was omitted. A/ Probe **1,** B/ Probe **7**, C/ Probe **9**. Mean (n = 3) ± S.D.

Activity vs. Protein Concentration

Assays with varying amounts of protein were carried out as described above for $FerPF_6$, except in a 96-well plate with a total volume of 200 µL. To a black 96-well plate were added: (1) Buffer B (total volume 200 µL), (2) $FerPF_6$ (200 µM), (3) **1**, **7**, or **9** (10 µM), and (4) homogenate (0.4-50 µg protein). Fluorescence was collected every 30 seconds for the first three minutes and every 10 minutes after that for 60 minutes.

The time scale of the reaction must be changed depending on the amount of protein present in order to only monitor the initial rate of the reaction, under steady-state kinetic assumptions, thus activity verses protein concentration plots are only linear within narrow regions. For example probe **1** activity is linear with 4-16 µg of protein on the three minute time scale (Figure SS3A) and linear with 0.4-4 µg protein on the sixty minute time scale (Figure SS3B). Probes **7** and **9** show linearity over larger regions of protein concentration due to their lower turnover rate.

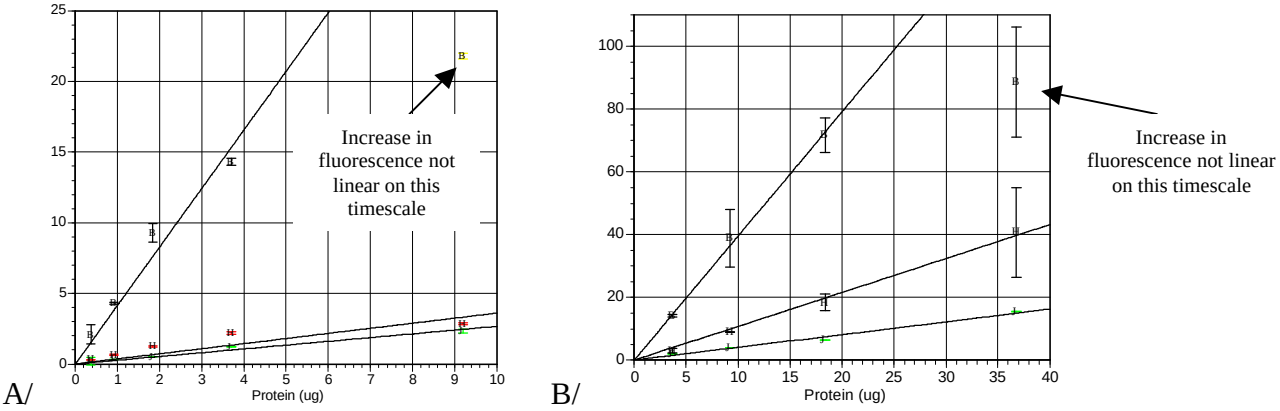

Figure SS3: Activity versus Protein Concentration. Fluorescent substrate (**1** (■), **7** (●), **9** (▲), 10 µM) was added to buffer containing $FerPF_6$ (200µM) and rat liver homogenate; the fluorescence was monitored (490, 510, or 567 nm, respectively) every 30 seconds for the first 3 minutes and every 10 minutes after for 60 minutes. A/ Fluorescence was followed for 60 minutes. B/ Fluorescence was followed for 3 minutes. Mean (n=3) ± S.D.

[1] C. Balo, F. Fernandez, X. Garcia-Mera, C. Lopez, *Org. Preps. and Proc. Int.* **2000**, *32*, 367.
[2] H. C. Brown, C. G. Rao, S. U. Kulkarni, *Syn.-Stut.* **1979**, 704.
[3] P. Arjunan, N. Shymasundar, K. D. Berlin, D. Najjar, M. G. Rockley, *J. Org. Chem.* **1981**, *46*, 626.
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[5] G. L. Ellman, *Arch. Biochem. Biophys.* **1959**, *82*, 70.
[6] D. F. Eaton, *J. Photochem. Photobiol. B* **1988**, *2*, 523.
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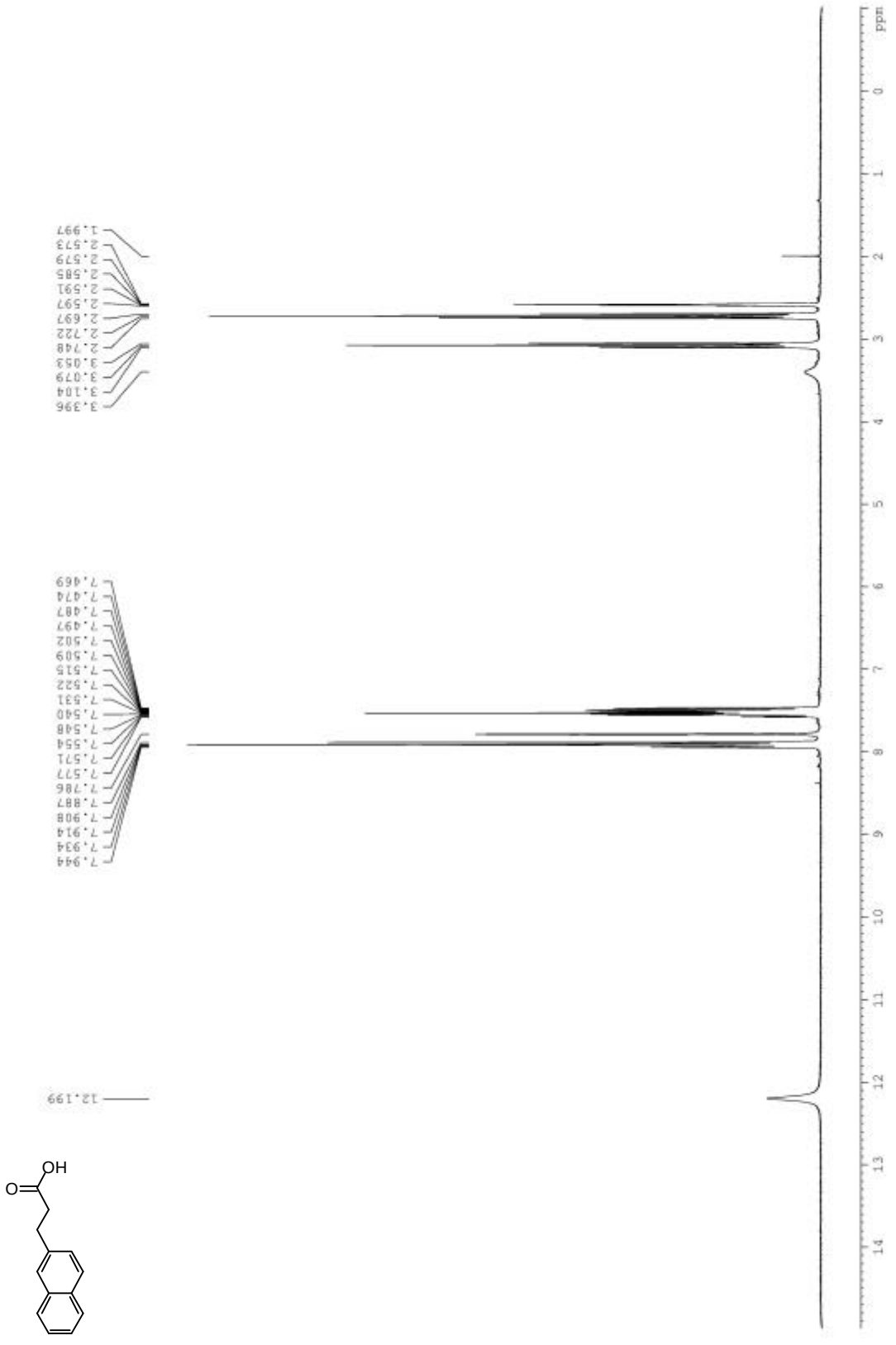
[8] T. C. Lehman, D. E. Hale, A. Bhala, C. Thorpe, *Anal. Biochem.* **1990**, *186*, 280.
[9] J. Wierzchowski, W. P. Dafeldecker, B. Holmquist, B. L. Vallee, *Anal. Biochem.* **1989**, *178*, 57.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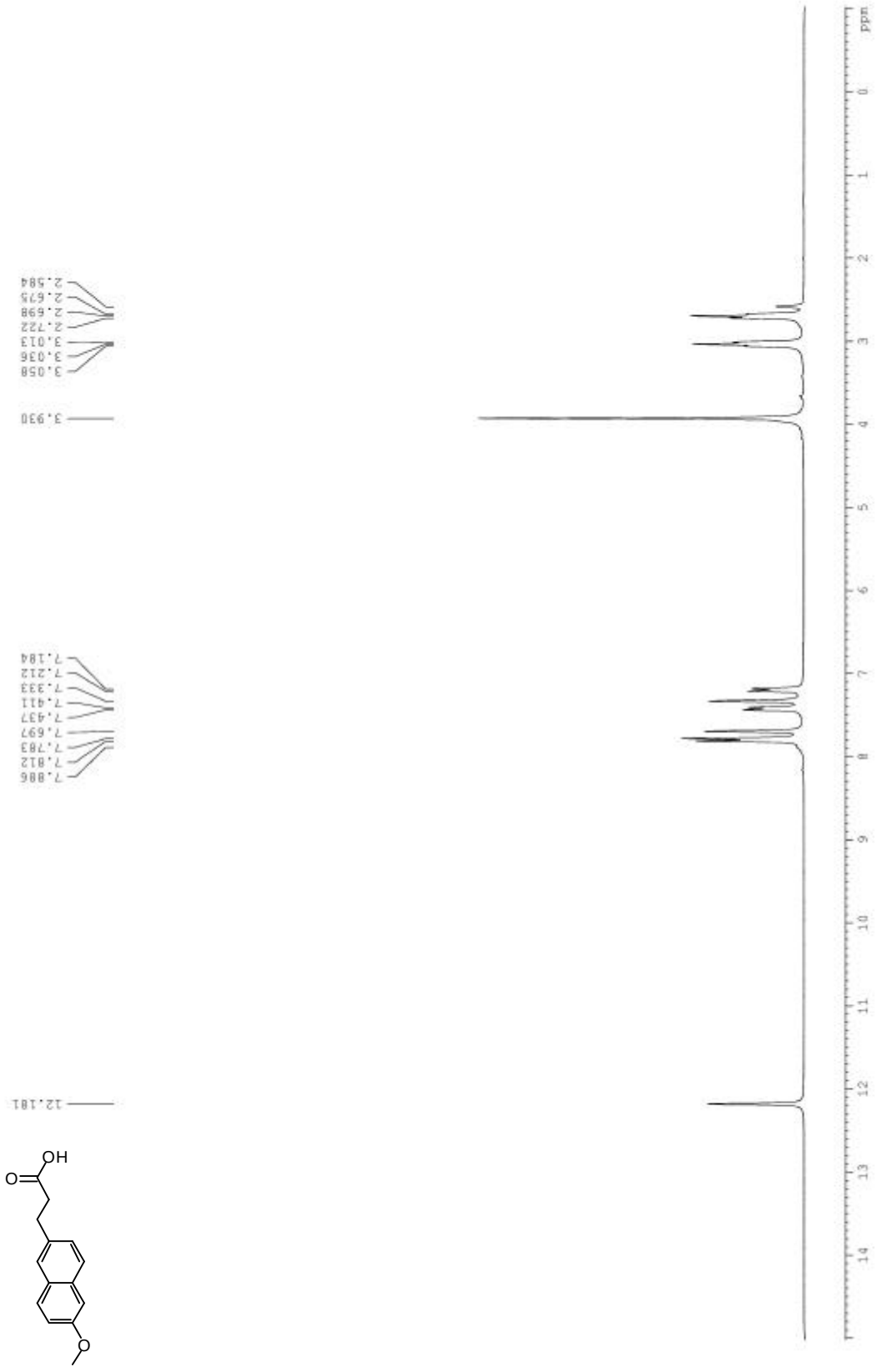
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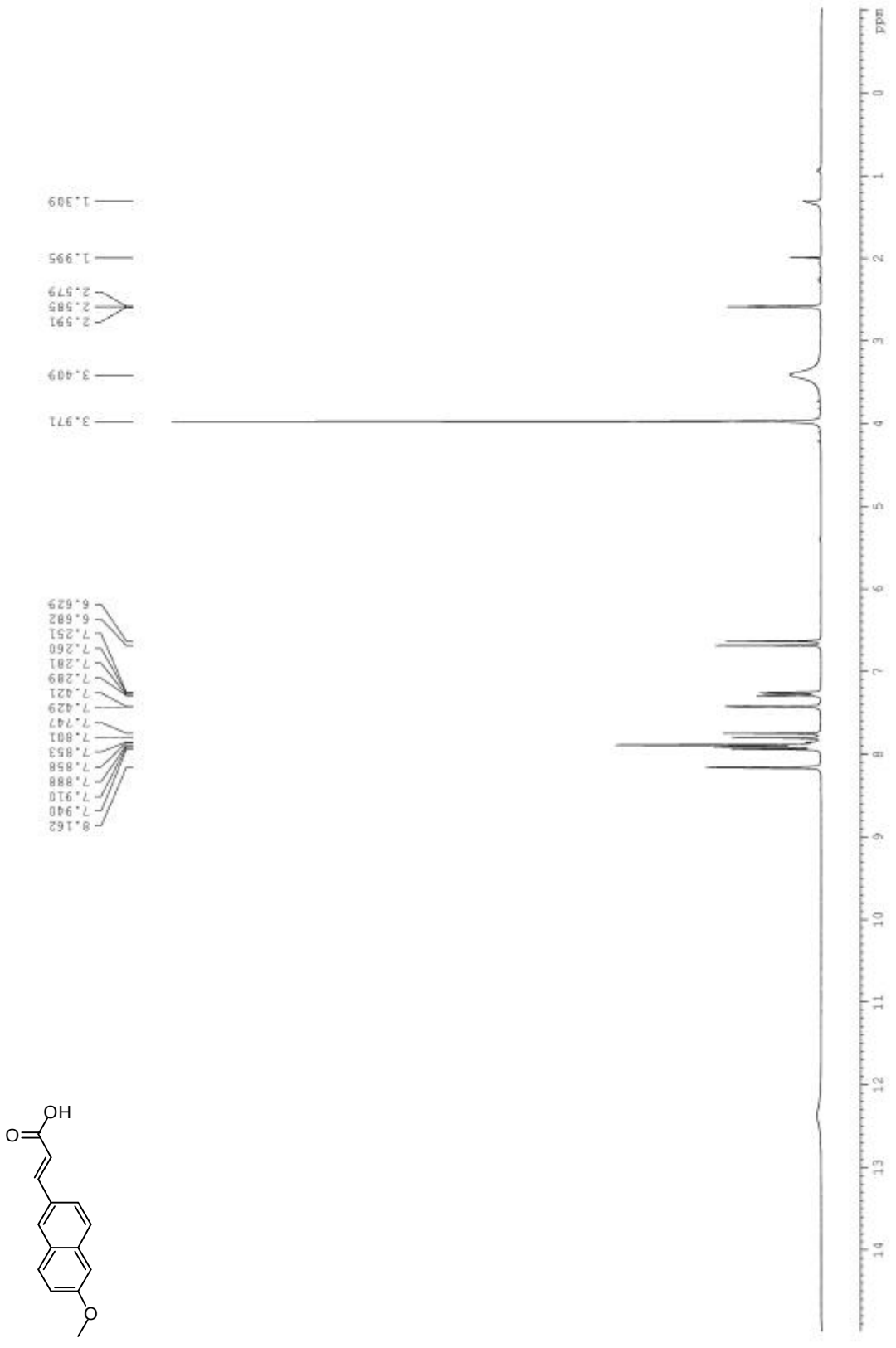
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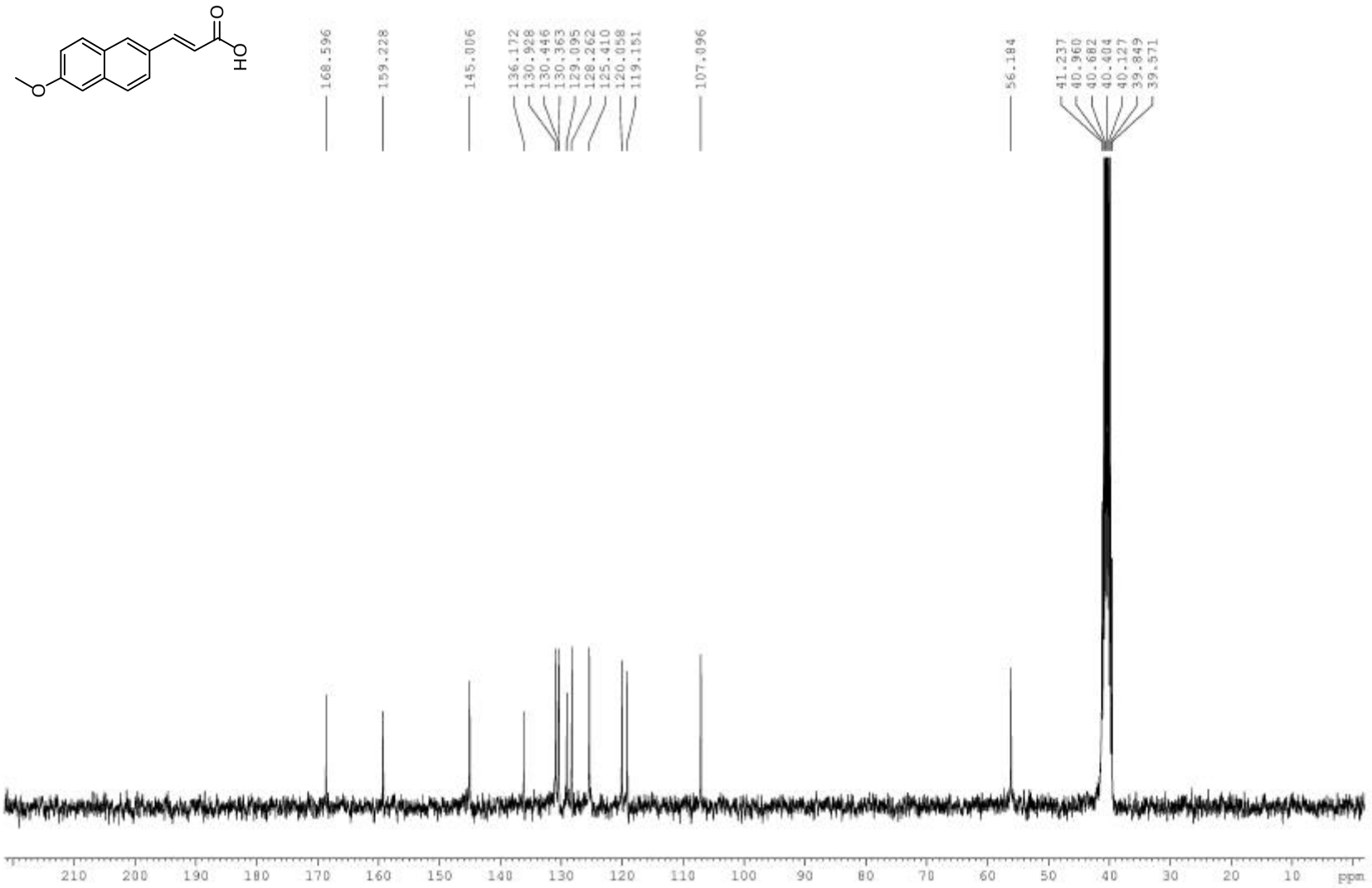
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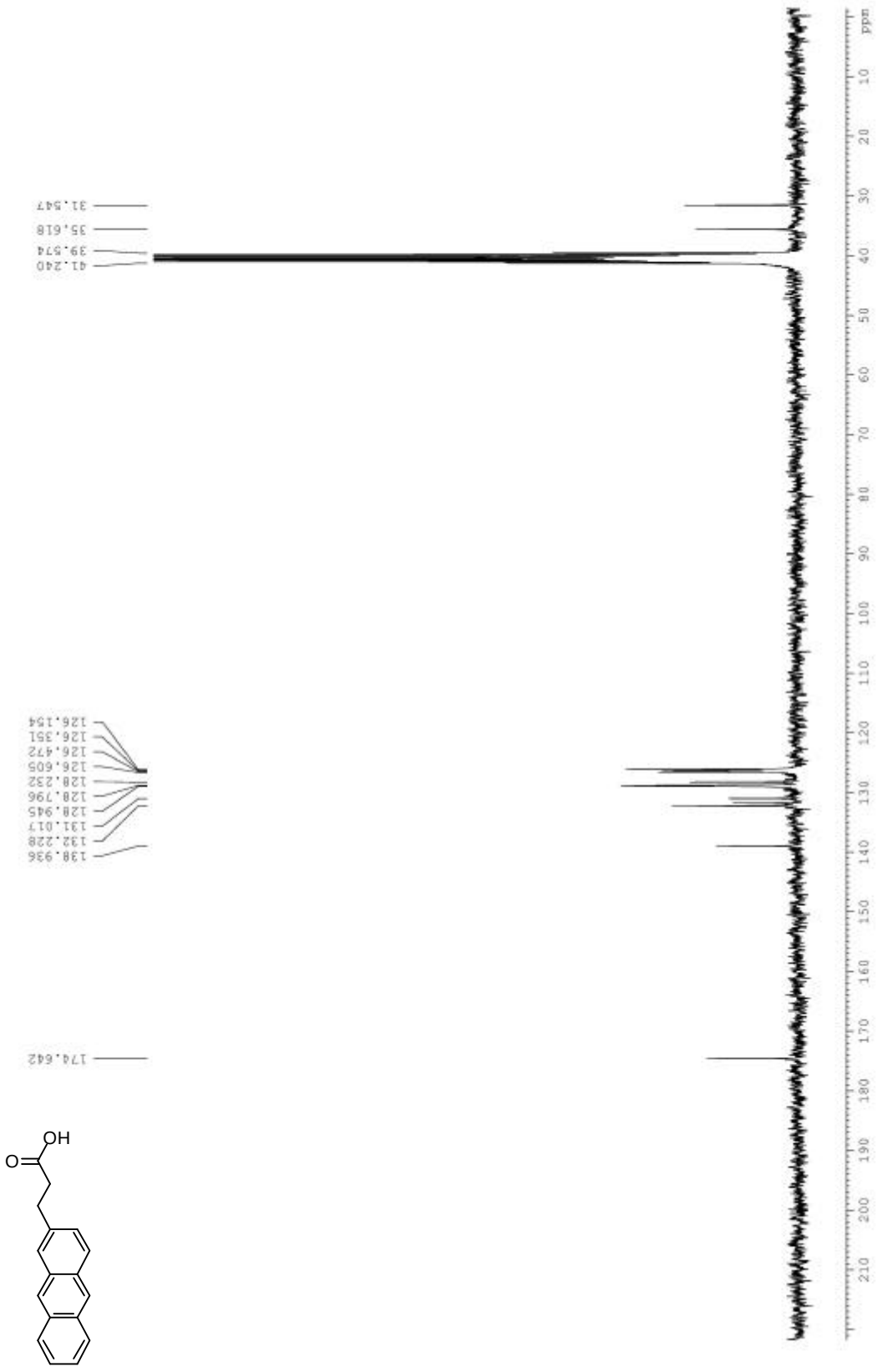
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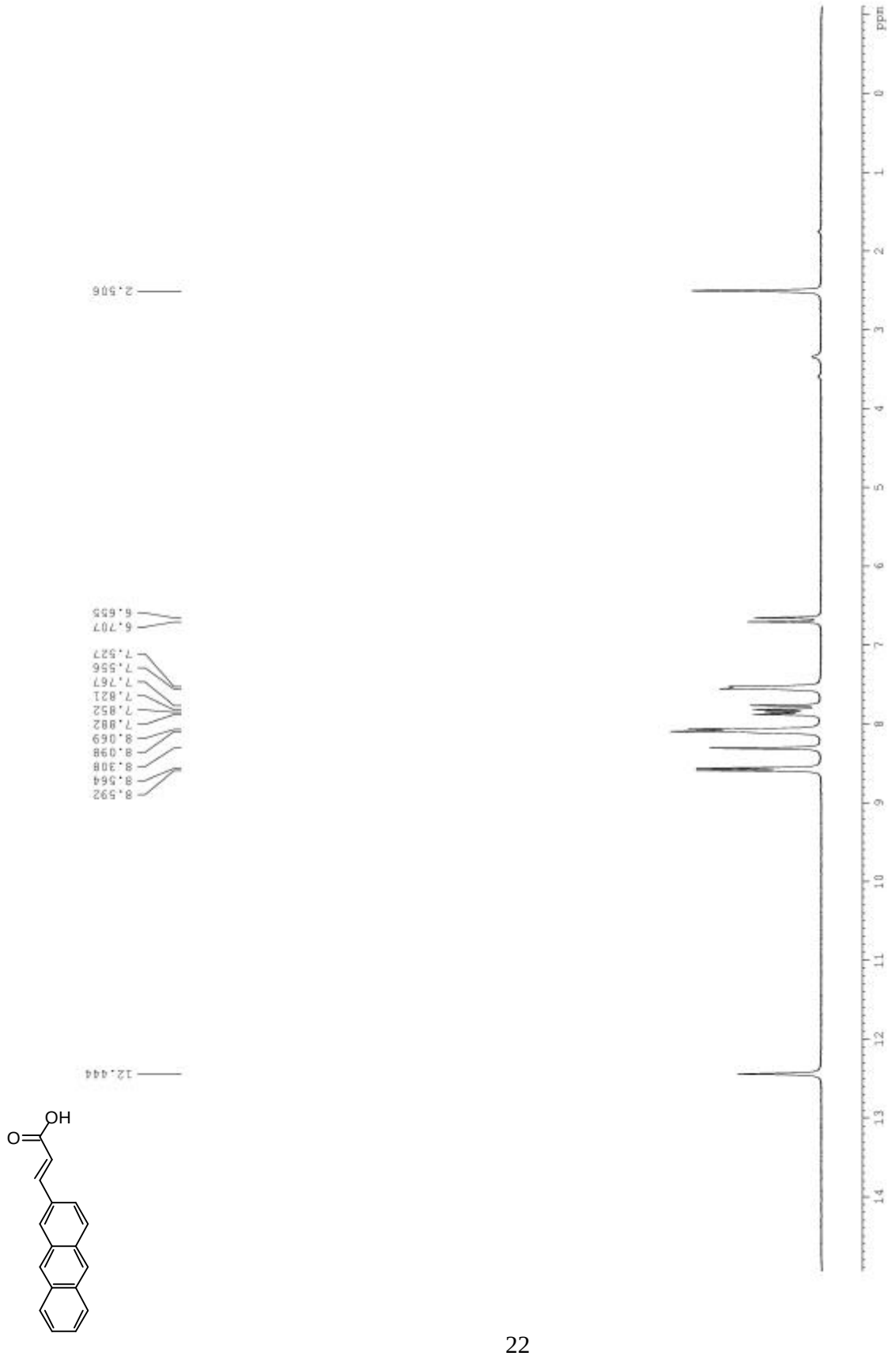
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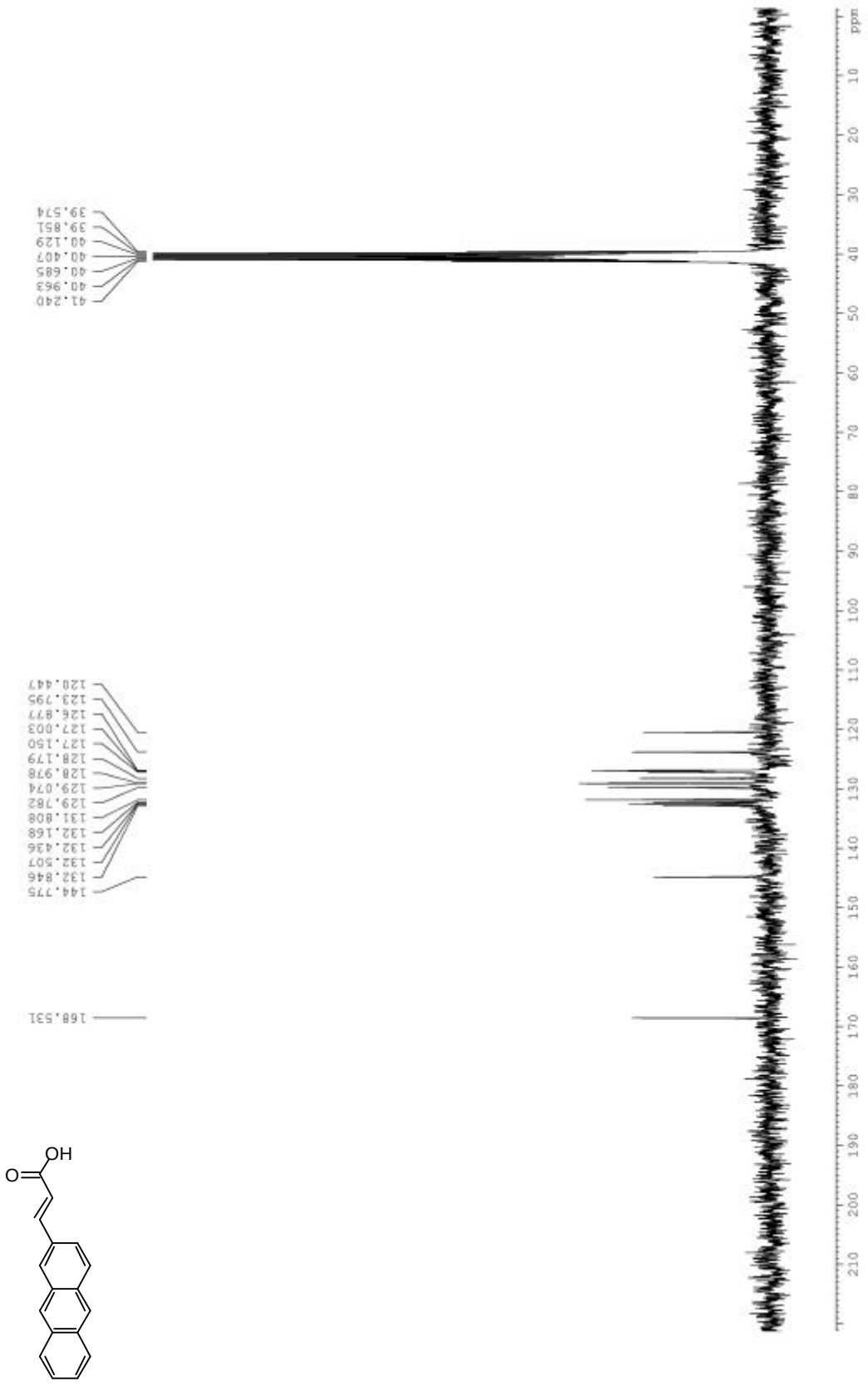
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