New Tools for Molecular Imaging of Redox Metabolism: Development of a Fluorogenic probe for 3β-Hydroxysteroid Dehydrogenases

Dominic J. Yee, Vojtech Balsanek, Dalibor Sames*

Department of Chemistry, Columbia University, New York NY 10027
sames@chem.columbia.edu

Supporting Information

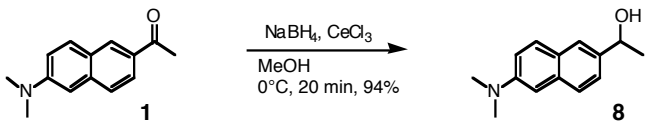
Materials and General methods

^{1}H and ^{13}C NMR spectra were recorded on Bruker 300 or 400 Fourier transform NMR spectrometers. Spectra were recorded in $CDCl_3$ solutions referenced to TMS or the solvent residual peak unless otherwise indicated. IR spectra were taken as neat for liquids on NaCl plates or as KBr pellets for solids using a Perkin-Elmer 1600 FTIR spectrometer. High 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 and used as received. All reactions were monitored by Thin Layer Chromatography.

Ultraviolet spectra were measured on a Cary 100 UV-Visible spectrophotometer and recorded in EtOH solutions. Recorded λ_{max} is that of the longest wavelength transition. Fluorescence measurements were taken on a Jobin Yvon Fluorolog fluorescence spectrofluorometer in potassium phosphate pH 7.0 buffer unless otherwise indicated. Quantum yields were measured relative to 9, 10 diphenylanthracene in EtOH[1] for probes **1-4** and alcohols **8, 11, 13,** and **15**, or Coumarin 6 in EtOH[2] for probes **5-7** and alcohols **19, 21,** and **22**. Reported quantum efficiencies are the average of at least three independent preparations of the probes and their cognate alcohols.

Synthesis of Probes 1-7 and the Corresponding Alcohols

Synthesis of probe 1



1-(6-Dimethylamino-naphthalen-2-yl)-ethanone (1).

This compound was prepared by a literature procedure and spectral data are consistent with those previously published[3].

1-(6-Dimethylamino-naphthalen-2-yl)-ethanol (8).

$CeCl_3.7H_2O$ (116 mg, 0.31 mmol) was added to a solution of **1** (50 mg, 0.23 mmol) in MeOH (10 ml) at 0°C, followed by addition of $NaBH_4$ (46 mg, 1.22 mmol). After 20 minutes, the reaction was quenched with a saturated aqueous solution of NH_4Cl and extracted with $CHCl_3$. Organic layer was dried over $MgSO_4$, evaporated and the crude product was purified by column chromatography on silica gel (CH_2Cl_2-EtOAc 98:2) to provide pure alcohol (47 mg, 94%).

NMR ^{1}H (300 MHz, $CDCl_3$) □ ppm:

7.67 (d, 1H, J1=9.0 Hz); 7.63 (bs, 1H); 7.63 (d, 1H, J1=8.5 Hz); 7.37 (dd, 1H, J1=8.5 Hz, J2=1.7 Hz); 7.15 (dd, 1H, J1=9.0 Hz, J2=2.5 Hz); 6.90 (d, 1H, J1=2.5 Hz); 4.99 (m, 1H); 3.03 (s, 6H); 1.79 (d, 1H, J1=3.5 Hz), 1.59 (d, 3H, J1=6.4 Hz).

NMR ^{13}C (300 MHz, $CDCl_3$) □ ppm:

148.7; 139.3; 134.5; 128.7; 126.6; 126.5; 124.2; 123.6; 116.7; 106.5; 70.6; 40.9; 24.9.

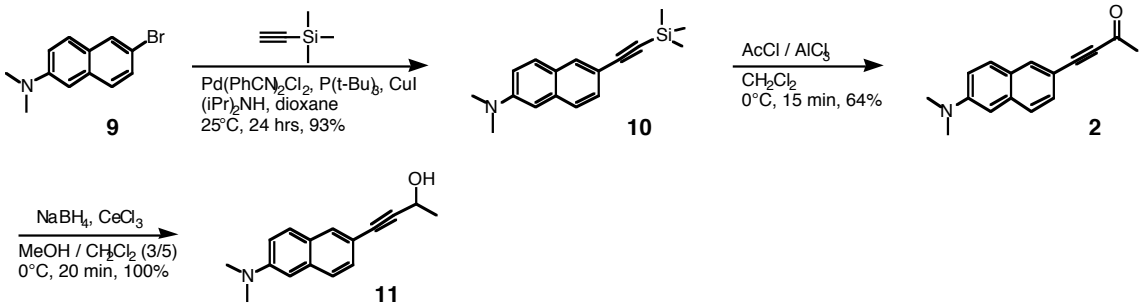
IR (NaCl, cm^{-1}): 3358, 2969, 2875, 1632, 1606, 1507, 1444, 1382, 1334, 1171, 1069, 968, 845, 804, 676.

HRMS (FAB): 215.1308 ($C_{14}H_{17}ON$, M; calc 215.1310).

UV (EtOH): λ_{max} = 348 nm.

Fluorescence (potassium phosphate pH 7.0): λ_{em} = 429 nm, Φ_f = 0.07.

Synthesis of probe 2



Dimethyl-(6-trimethylsilanylethynyl-naphthalen-2-yl)-amine (10).

This compound was prepared by the procedure of Buchwald and Fu[4] from bromide **9**, which was obtained from 2-bromo-6-naphthol according to literature[5]. $Pd(PhCN)_2Cl_2$ (4.6 mg, 0.012 mmol), CuI (1.5 mg, 0.008 mmol), **9** (100 mg, 0.400 mmol), dioxane (1 ml), diisopropylamine (68 □l, 0.024 mmol) and (trimethylsilyl)acetylene (110 □l, 0.800 mmol) were mixed in a vial under argon and allow to stir 24 hrs at room temperature. The resultant mixture was diluted with EtOAc, washed with brine and dried over $MgSO_4$. Following solvent evaporation and product purification by column chromatography using silica gel and hexanes-EtOAc 98:2, **10** was yielded (99 mg, 93%).

NMR ^{1}H (300 MHz, $CDCl_3$) □ ppm:

7.81 (bs, 1H); 7.61 (d, 1H. J1=9.1 Hz); 7.52 (d, 1H, J1=8.5 Hz); 7.36 (dd, 1H, J1=8.5 Hz, J2=1.6 Hz); 7.11 (dd, 1H, J1=9.1 Hz, J2=2.5 Hz); 6.83 (d, 1H, J1=2.5 Hz); 3.05 (s, 6H); 0.27 (s, 9H).

NMR ^{13}C (300 MHz, acetone-d) δ ppm:

150.4; 135.8; 132.3; 129.5; 129.3; 127.0; 126.8; 117.6; 116.5; 107.4; 106.5; 92.9; 40.6; 0.1.

IR (NaCl, cm^{-1}): 2960, 2901, 2812, 2147, 1629, 1598, 1247, 894, 850, 838, 809.

HRMS (FAB): 267.1442 ($C_{17}H_{21}NSi$, M; calc 267.1443).

4-(6-Dimethylamino-naphthalen-2-yl)-but-3-yn-2-one (2).

AcCl (13 μl, 0.18 mmol) was added to a solution of **10** (43 mg, 0.16 mmol) in CH_2Cl_2 (2 ml) at 0°C, followed by addition of $AlCl_3$ (107 mg, 0.80 mmol). After 15 minutes, the reaction was quenched with H_2O and extracted with EtOAc. After the organic layer was dried over $MgSO_4$, the solvent was removed, and the residue was purified by column chromatography on silica gel (hexanes-EtOAc 98:2) to yield ketone **2** (55 mg, 64%).

NMR ^{1}H (300 MHz, $CDCl_3$) δ ppm:

7.96 (bs, 1H); 7.66 (d, 1H, J1=9.1 Hz); 7.56 (d, 1H, J=8.5 Hz); 7.41 (dd, 1H, J1=8.5 Hz, J2=1.6 Hz); 7.14 (dd, 1H, J1=9.1 Hz, J2=2.5 Hz); 6.82 (d, 1H, J1=2.5 Hz); 3.09 (s, 6H); 2.46 (s, 3H).

NMR ^{13}C (300 MHz, $CDCl_3$) δ ppm:

184.6; 149.8; 135.9; 134.6; 129.3; 129.1; 126.3; 125.5; 116.5; 111.9; 105.4; 93.1; 88.5; 40.4; 32.7.

IR (NaCl, cm^{-1}): 2892, 2817, 2180, 1667, 1625, 1507, 1354, 1280, 1190, 1168, 896, 851, 810.

HRMS (FAB): 237.1138 ($C_{16}H_{15}ON$, M; calc 237.1154).

UV (EtOH): λ_{max} = 389 nm.

Fluorescence (potassium phosphate pH 7.0): 448 nm, Φ_f = 0.00.

4-(6-Dimethylamino-naphthalen-2-yl)-but-3-yn-2-ol (11).

Reduction of **2** (20 mg, 0.084 mmol) in MeOH-CH_2Cl_2 3:5 (5ml) followed a procedure analogous to that used for the preparation of **8.** Column chromatography on silica gel (CH_2Cl_2) afforded alcohol **11** (20 mg, 100%).

NMR ^{1}H (300 MHz, $CDCl_3$) δ ppm:

7.77 (bs, 1H); 7.61 (d, 1H, J1=9.1 Hz); 7.54 (d, 1H, J1=8.5 Hz); 7.33 (dd, 1H, J1=8.5 Hz, J2=1.5 Hz); 7.12 (dd, 1H; J1=9.1 Hz, J2=2.4 Hz); 6.83 (d, 1H, J1=2.4 Hz); 4.78 (m, 1H); 3.05 (s, 6H); 1.88 (d, 1H, J1=4.8 Hz); 1.57 (d, 3H, J1=6.5 Hz).

NMR ^{13}C (300 MHz, $CDCl_3$) δ ppm:

149.1; 134.5; 131.4; 128.8; 128.7; 126.1; 126.0; 116.6; 115.3; 105.9; 89.9; 85.0; 59.0; 40.6; 24.5.

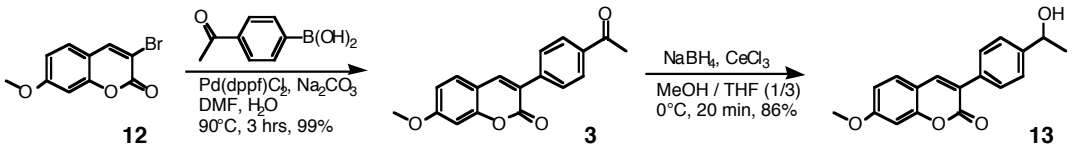
IR (NaCl, cm^{-1}): 3346, 2982, 2930, 2882, 1628, 1598, 1505, 1389, 1101, 1072, 1035, 893, 848, 809.

HRMS (FAB): 239.1305 ($C_{16}H_{17}ON$, M; calc 239.1310).

UV (EtOH): λ_{max} = 361 nm.

Fluorescence (potassium phosphate pH 7.0): 440 nm, Φ_f = 0.08.

Synthesis of probe 3



3-(4-Acetyl-phenyl)-7-methoxy-coumarin (3).

Bromide **12** (400 mg, 1.57 mmol), obtained by bromination of 7-methoxycoumarin, was mixed with 4-acetylphenylboronic acid (283 mg, 1.72 mmol), $PdCl_2$dppf (40 mg, 0.047 mmol), Na_2CO_3 (831 mg, 7.84 mmol), H_2O (3.92 ml) and DMF (16 ml) under argon. The resulting mixture was heated to 90°C and stirred until completion (3 hrs). The cooled mixture was then diluted with water and extracted with CH_2Cl_2. Combined organic fractions were dried over $MgSO_4$. Following the evaporation of solvent, the residue was purified by column chromatography on silica gel (CH_2Cl_2) to afford desired product **3** (456 mg, 99%).

NMR ^{1}H (300 MHz, $CDCl_3$) δ ppm:
8.00 (m, 2H); 7.83 (m, 2H); 7.77 (bs, 1H); 7.46 (d, 1H, J1=8.4 Hz); 6.88 (m, 2H); 3.90 (s, 3H); 2.64 (s, 3H).

NMR ^{13}C (300 MHz, $CDCl_3$) δ ppm:
197.6; 163.1; 160.5; 155.6; 141.0; 139.6; 136.6; 129.2; 128.5; 128.4; 123.5; 113.1; 113.1; 100.4; 55.9; 26.7.

IR (NaCl, cm^{-1}): 3070, 2962, 1710, 1670, 1613, 1505, 1442, 1360, 1275, 1198, 1122, 1022, 929, 859, 829, 776.

HRMS (FAB): 295.0967 ($C_{18}H_{15}O_4$, M+1; calc 295.0970).

UV (EtOH): λ_{max} = 348 nm.

Fluorescence (potassium phosphate pH 7.0): 462 nm, Φ_f = 0.00.

3-[4-(1-Hydroxy-ethyl)-phenyl]-7-methoxy-coumarin (13).

Reduction of **3** (42 mg, 0.14 mmol) in MeOH-THF 1:3 (15ml) proceeded as described for the preparation of **8.** Column chromatography on silica gel (eluent gradient: CH_2Cl_2 to CH_2Cl_2-EtOAc 8:2) afforded alcohol **13** (36 mg, 86%).

NMR ^{1}H (300 MHz, $CDCl_3$) δ ppm:
7.75 (s, 1H); 7.67 (m, 2H); 7.44 (m, 3H); 4.95 (m, 1H); 3.89 (s, 3H); 1.85 (d, 1H, J1=3.4 Hz); 1.52 (d, 3H, J1=6.4 Hz).

NMR ^{13}C (300 MHz, $CDCl_3$) δ ppm:
162.5; 160.9; 155.2; 146.1; 139.8; 134.0; 128.8; 128.4; 125.4; 124.4; 113.3; 112.7; 100.3; 70.0; 55.7; 25.1.

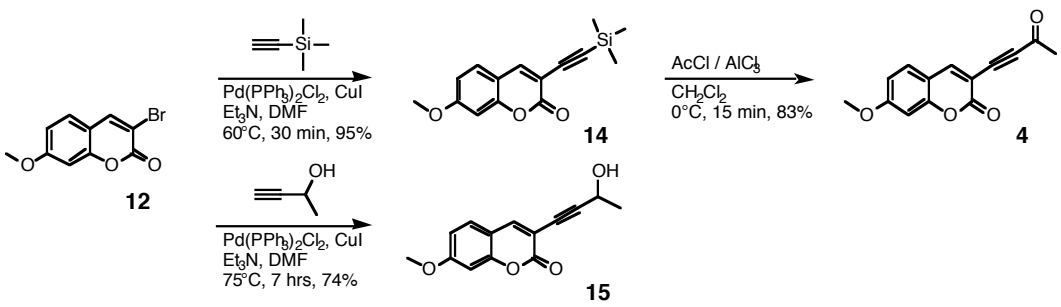
IR (NaCl, cm^{-1}): 3415, 2971, 1719, 1611, 1057, 1443, 1364, 1271, 1202, 1163, 1120, 1089, 1026, 832.

HRMS (FAB): 297.1112 ($C_{18}H_{17}O_4$, M+1; calc 297.1127).

UV (EtOH): λ_{max} = 342 nm.

Fluorescence (potassium phosphate pH 7.0): 429 nm, Φ_f = 0.12.

Synthesis of probe 4



7-Methoxy-3-trimethylsilanylethynyl-coumarin (14).

$PdCl_2(PPh_3)_2$ (28 mg, 0.04 mmol), CuI (8 mg, 0.04 mmol), Et_3N (278 µl, 2.00 mmol) and (trimethylsilyl)acetylene (138 µl, 1.50 mmol) were added to a solution of bromide **12** (255 mg, 1.00 mmol) in dry DMF (10 ml) under argon. The resulting solution was heated to 60°C and allowed to react 30 minutes. The mixture was then cooled, diluted with water, and extracted with CH_2Cl_2. The organic fractions were then combined and dried over $MgSO_4$. Removal of solvent *in vacuo* and purification of the residue by column chromatography on silica gel (CH_2Cl_2) afforded product **14** (259 mg, 95%).

NMR ^{1}H (300 MHz, $CDCl_3$) δ ppm:
7.82 (s, 1H); 7.32 (d, 1H, J1=8.6 Hz); 6.83 (dd, 1H, J1=8.6 Hz, J2=2.4 Hz); 6.78 (d, 1H, J1=2.4 Hz); 3.86 (s, 3H); 0.26 (s, 9H).

NMR ^{13}C (300 MHz, acetone-d) δ ppm:
164.6; 159.5; 156.5; 147.5; 130.4; 113.8; 113.2; 109.3; 101.3; 100.2; 99.8; 56.5; -0.2.

IR (NaCl, cm^{-1}): 3040, 2961, 2840, 1721, 1600, 1441, 1368, 1272, 1247, 1034, 973, 831, 807, 765.

HRMS (FAB): 272.0869 ($C_{15}H_{16}O_3Si$, M; calc 272.0869).

7-Methoxy-3-(3-oxo-but-1-ynyl)-coumarin (4).

Compound **14** (103 mg, 0.38 mmol) was converted into ketone **4** by the procedure used for the preparation of **2**. Column chromatography of the crude product on silica gel (CH_2Cl_2) provided **4** (76 mg, 83%).

NMR ^{1}H (300 MHz, $CDCl_3$) δ ppm:
8.00 (bs, 1H); 7.40 (d, 1H, J1=8.7 Hz); 6.88 (dd, 1H, J1=8.7 Hz, J2=2.3 Hz); 6.81 (d, 1H, J1=2.3 Hz); 3.90 (s, 3H); 2.47 (s, 3H).

NMR ^{13}C (300 MHz, $CDCl_3$) δ ppm:
184.1, 164.7; 158.9; 156.3; 149.8; 129.7; 113.8; 112.1; 106.0; 100.9; 92.2; 84.1; 56.0; 32.6.

IR (NaCl, cm^{-1}): 3046, 2197, 1725, 1664, 1617, 1596, 1557, 1504, 1368, 1273, 1250, 1152, 1116, 1019, 836.

HRMS (FAB): 242.0572 ($C_{14}H_{10}O_4$, M+1; calc. 242.0579) .

UV (EtOH): λ_{max} = 368 nm.

Fluorescence (potassium phosphate pH 7.0): 416 nm, Φ_f = 0.00.

3-(3-Hydroxy-but-1-ynyl)-7-methoxy-coumarin (15).

Alcohol **15** was prepared by Sonogashira coupling of bromide **12** (100 mg, 0.39 mmol) and but-3-yn-2-ol (32 µl, 0.43 mmol) under conditions similar to that used for the

preparation of **14**. After 7 hours at 75°C, the reaction was complete. The crude alcohol was purified by column chromatography on silica gel (CH_2Cl_2-EtOAc 95:5) to afford product **15** (96 mg, 74%).

NMR ^{1}H (300 MHz, $CDCl_3$) δ ppm:

7.81 (bs, 1H); 7.35 (d, 1H, J1=8.6 Hz); 6.86 (dd, 1H, J1=8.6 Hz, J2=2.4 Hz); 6.81 (d, 1H, J1=2.4 Hz); 4.79 (m, 1H); 3.88 (s, 3H); 2.26 (d, 1H, J1=5.2 Hz); 1.56 (d, 3H, J1=6.6 Hz).

NMR ^{13}C (300 MHz, $CDCl_3$) δ ppm:

163.3; 160.1; 155.2; 145.5; 128.8; 113.2; 112.4; 108.6; 100.7; 96.7; 77.9; 58.7; 55.8; 24.0.

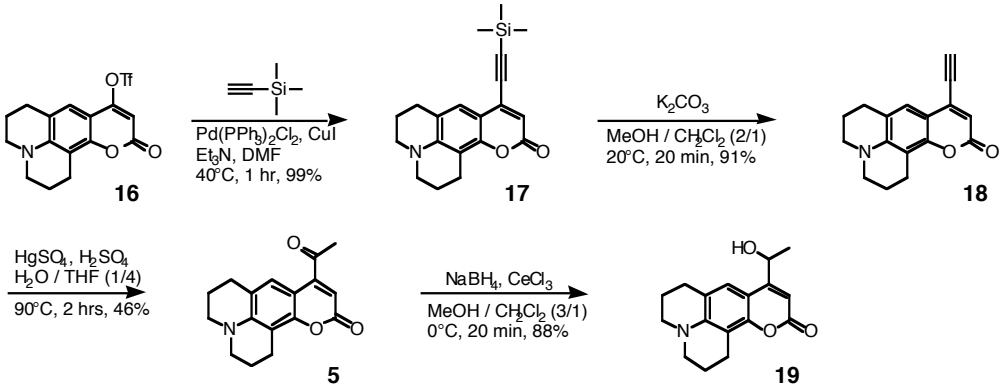
IR (NaCl, cm^{-1}): 3414, 2983, 2939, 2843, 1733, 1618, 1506, 1365, 1269, 1121, 1024, 768.

HRMS (FAB): 244.0744 ($C_{14}H_{12}O_4$, M; calc 244.0736).

UV (EtOH): λ_{max} = 346 nm.

Fluorescence (potassium phosphate pH 7.0): 420 nm, Φ_f = 0.18.

Synthesis of probe 5



8-Trimethylsilanylethynyl-2,3,5,6-tetrahydro-1*H*, 4*H*-11-oxa-3a-aza-benzo[*de*]anthracen-10-one (17).

Triflate **16** (707mg, 1.82 mmol), obtained from 8-hydroxyjulolidine according to the literature[6], was coupled with (trimethylsilyl)acetylene (377 µl, 2.72 mmol) under conditions described for the preparation of **14**. The reaction was complete after 1 hr at 40°C. Column chromatography on silica gel (CH_2Cl_2) provided desired product **17** (607 mg, 99%).

NMR ^{1}H (300 MHz, $CDCl_3$) δ ppm:

7.16 (s, 1H); 6.11 (s, 1H); 3.26 (m, 4H); 2.83 (m, 4H); 1.97 (m, 4H); 0.31 (s, 9H).

NMR ^{13}C (300 MHz, $CDCl_3$) δ ppm:

161.8; 151.1; 146.1; 137.0; 123.5; 118.3; 110.8; 107.6; 106.6; 106.3; 98.8; 49.9; 49.4; 27.6; 21.4; 20.4; 20.2; -0.4.

IR (NaCl, cm^{-1}): 2946, 2848, 1701, 1612, 1546, 1511, 1421, 1367, 1310, 1245, 1184, 843.

HRMS (FAB): 338.1574 ($C_{20}H_{24}O_2NSi$, M+1; calc 338.1576).

8-Ethynyl-2,3,5,6-tetrahydro-1*H*, 4*H*-11-oxa-3a-aza-benzo[*de*]anthracen-10-one (18).

Powdered K_2CO_3 (600 mg) was added to a solution of **17** (580 mg, 1.72 mmol) in MeOH-CH_2Cl_2 2:1 (30 ml). The mixture was stirred at room temperature until the reaction was complete (20 min). Reaction mixture was diluted with $CHCl_3$, filtered, and washed with brine. The resultant organic layers were combined and dried over $MgSO_4$, after which the solvent was removed *in vacuo*. Purification by column chromatography on silica gel (eluent gradient: CH_2Cl_2 to CH_2Cl_2-EtOAc 95:5) afforded terminal alkyne **18** (416 mg, 91%).

NMR ^{1}H (300 MHz, $CDCl_3$) δ ppm:
7.19 (s, 1H); 6.16 (s, 1H); 3.58 (s, 1H); 3.27 (m, 4H); 2.87 (m, 2H); 2.78 (m, 2H); 1.97 (m, 4H).

NMR ^{13}C (300 MHz, $CDCl_3$) δ ppm:
161.6; 151.1; 146.3; 136.3; 123.4; 118.5; 111.7; 107.6; 106.4; 87.5; 78.0; 49.9; 49.5; 27.5; 21.3; 20.4; 20.2.

IR (NaCl, cm^{-1}): 3221, 2931, 2838, 2103, 1699, 1616, 1519, 1428, 1371, 1311, 1176, 826.

HRMS (FAB): 266.1193 ($C_{17}H_{16}O_2N$, M+1; calc 266.1181).

8-Acetyl-2,3,5,6-tetrahydro-1*H*, 4*H*-11-oxa-3a-aza-benzo[*de*]anthracen-10-one (5).

$HgSO_4$ (112 mg, 0.38 mmol) was added to a solution of **18** (100 mg, 0.38 mmol) in THF (8 ml), followed by addition of conc. H_2SO_4 (105 μl, 1.88 mmol) in H_2O (2ml). The reaction mixture was heated in a sealed tube at 90°C for 2 hrs. After cooling to room temperature, a spatula tip of $NaHCO_3$ was added and the mixture was evaporated to dryness. $MgSO_4$ was added and the residual solids were washed thoroughly with $CHCl_3$. The solvent was the evaporated and the residue purified by column chromatography on silica gel (CH_2Cl_2-Et_2O 95:5) yielding ketone **5** (49 mg, 46%).

NMR ^{1}H (300 MHz, $CDCl_3$) δ ppm:
7.18 (s, 1H); 6.13 (s, 1H); 3.27 (m, 4H); 2.88 (m, 2H); 2.74 (m, 2H); 2.55 (s, 3H); 1.96 (m, 4H).

NMR ^{13}C (300 MHz, $CDCl_3$) δ ppm:
200.4; 162.1; 152.1; 150.8; 146.2; 123.2; 118.7; 106.8; 106.8; 103.7; 49.9; 49.4; 29.7; 27.6; 21.3; 20.4; 20.3.

IR (NaCl, cm^{-1}): 2933, 2844, 1694, 1611, 1544, 1525, 1434, 1373, 1352, 1311, 1232, 1170, 1148.

HRMS (FAB): 283.1195 ($C_{17}H_{17}O_3N$, M; calc 283.1208).

UV (EtOH): λ_{max} = 418 nm.

Fluorescence (potassium phosphate pH 7.0): 520 nm, Φ_f = 0.00.

8-(1-Hydroxy-ethyl)-2,3,5,6-tetrahydro-1*H*, 4*H*-11-oxa-3a-aza-benzo[*de*]anthracen-10-one (19).

Reduction of **5** (16 mg, 0.056 mmol) in MeOH-CH_2Cl_2 3:1 (5ml) proceeded by previously described procedures (used for preparation of **8)**. Column chromatography on silica gel (eluent gradient: CH_2Cl_2 to CH_2Cl_2-EtOAc 9:1) afforded alcohol **19** (14 mg, 88%).

NMR ^{1}H (300 MHz, $CDCl_3$) δ ppm:

7.01 (s, 1H); 6.24 (s, 1H); 5.14 (m, 1H); 3.26 (m, 4H); 2.87 (m, 2H); 2.77 (m, 2H); 2.07 (d, 1H, J1=3.8 Hz); 2.10 (m, 4H); 1.55 (d, 3H, J1=6.5 Hz).

NMR ^{13}C (300 MHz, $CDCl_3$) δ ppm:

163.0; 159.4; 151.4; 145.6; 121.0; 118.0; 107.1; 105.9; 103.8; 65.9; 49.9; 49.5; 27.8; 23.6; 21.5; 20.6; 20.5.

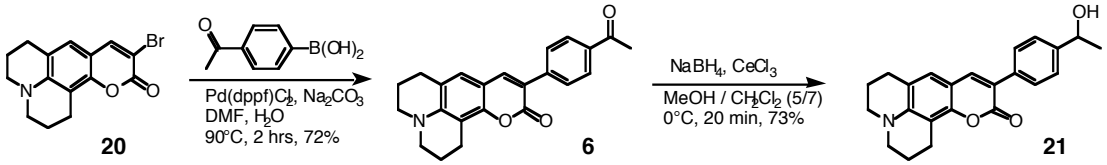
IR (NaCl, cm^{-1}): 3396, 2936, 2843, 1688, 1611, 1554, 1520, 1433, 1372, 1311, 1183, 1133.

HRMS (FAB): 286.1437 ($C_{17}H_{20}O_3N$, M+1; calc 286.1443).

UV (EtOH): λ_{max} = 398 nm.

Fluorescence (potassium phosphate pH 7.0): 509 nm, Φ_f = 0.21.

Synthesis of probe 6



9-(4-Acetyl-phenyl)-2,3,5,6-tetrahydro-1*H*, 4*H*-11-oxa-3a-aza-benzo[*de*]anthracen-10-one (6).

Bromide **20** (100 mg, 0.31 mmol), obtained by bromination of coumarin 6H, was coupled with 4-acetylphenylboronic acid (77 mg, 0.46 mmol), under similar conditions as those used for preparation of **3**. Reaction was complete after 2 hrs at 90°C. Column chromatography on silica gel (eluent gradient: CH_2Cl_2 to CH_2Cl_2-EtOAc 95:5) provided desired ketone **6** (81 mg, 72%).

NMR ^{1}H (300 MHz, $CDCl_3$) δ ppm:

7.96 (m, 2H); 7.81 (m, 2H); 7.68 (s, 1H); 6.91 (s, 1H); 3.29 (m, 4H); 2.93 (m, 2H); 2.77 (m, 2H); 2.61 (s, 3H); 1.98 (m, 4H).

NMR ^{13}C (300 MHz, $CDCl_3$) δ ppm:

197.7; 161.4; 151.5; 146.3; 141.7; 141.0; 135.6; 128.3; 128.0; 125.4; 118.7; 118.0; 108.7; 106.1; 50.0; 49.6; 27.4; 26.6; 21.4; 20.4; 20.2.

IR (NaCl, cm^{-1}): 2941, 2845, 1699, 1677, 1616, 1594, 1563, 1518, 1360, 1306, 1269, 1213, 1171.

HRMS (FAB): 359.1527 ($C_{23}H_{21}O_3N$, M; calc 359.1521).

UV (EtOH): λ_{max} = 435 nm.

Fluorescence (potassium phosphate pH 7.0): 511 nm, Φ_f = 0.01.

9-[4-(1-Hydroxy-ethyl)-phenyl]-2,3,5,6-tetrahydro-1*H*, 4*H*-11-oxa-3a-aza-benzo[*de*]anthracen-10-one (21).

Reduction of **6** (15 mg, 0.041 mmol) in MeOH-CH_2Cl_2 5:7 (6ml) by the procedure used for preparation of **8** and recrystallization from $CHCl_3$-hexanes afforded alcohol **21** (11 mg, 73%).

NMR ^{1}H (300 MHz, $CDCl_3$) δ ppm:

7.66 (m, 2H); 7.58 (s, 1H); 7.40 (m, 2H); 6.88 (s, 1H); 4.92 (q, 1H, J1=6.4 Hz); 3.28 (m, 4H); 2.92 (m, 2H); 2.76 (m, 2H); 1.98 (m, 4H); 1.81 (bs, 1H); 1.51 (d, 3H, J1=6.4 Hz).

NMR ^{13}C (300 MHz, $CDCl_3$) δ ppm:

161.9; 151.2; 145.8; 145.1; 140.8; 135.3; 128.3; 125.3; 125.1; 119.6; 118.5; 109.0; 106.3; 70.2; 50.0; 49.6; 27.5; 25.1; 21.5; 20.6; 20.3.

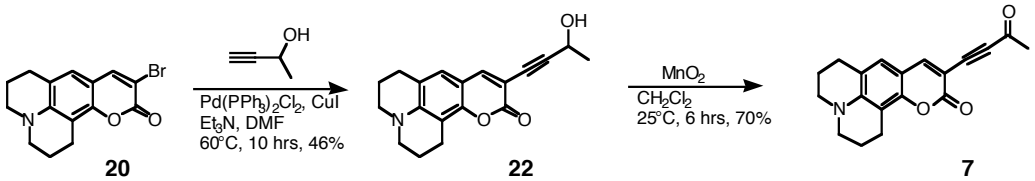
IR (NaCl, cm^{-1}): 3408, 2930, 2844, 1694, 1615, 1599, 1564, 1519, 1309, 1209, 1170, 839, 748.

HRMS (FAB): 361.1673 ($C_{23}H_{23}O_3N$, M; calc 361.1678).

UV (EtOH): λ_{max} = 422 nm.

Fluorescence (potassium phosphate pH 7.0): 509 nm, Φ_f = 0.14.

Synthesis of probe 7



20 **22** **7**

9-(3-Hydroxy-but-1-ynyl)-2,3,5,6-tetrahydro-1*H*, 4*H*-11-oxa-3a-aza-benzo[*de*]anthracen-10-one (22).

Alcohol **22** was prepared by Sonogashira coupling of bromide **20** (100 mg, 0.31 mmol) and but-3-yn-2-ol (26 µl, 0.34 mmol) as described for the preparation of **14**. The reaction was stopped after 10 hrs at 60°C. Column chromatography on silica gel (eluent gradient: CH_2Cl_2 to CH_2Cl_2-EtOAc 9:1) provided **22** (45 mg, 46%).

NMR ^{1}H (300 MHz, $CDCl_3$) δ ppm:

7.60 (s, 1H); 6.78 (s, 1H); 4.77 (m, 1H); 3.28 (m, 4H); 2.87 (m, 2H); 2.75 (m, 2H); 2.14 (d, 1H, J1=4.9 Hz); 1.97 (m, 4H); 1.54 (d, 3H, J1=6.6 Hz).

NMR ^{13}C (300 MHz, $CDCl_3$) δ ppm:

161.6; 151.3; 146.6; 146.4; 125.0; 118.9; 108.1; 106.4; 102.8; 94.7; 79.3; 58.9; 50.1; 49.7; 27.4; 24.1; 21.3; 20.4; 20.2.

IR (NaCl, cm^{-1}): 3397, 2934, 2849, 1709, 1692, 1616, 1594, 1518, 1360, 1309, 1290, 1169, 765.

HRMS (FAB): 309.1365 ($C_{19}H_{19}O_3N$, M; calc 309.1365).

UV (EtOH): λ_{max} = 429 nm.

Fluorescence (potassium phosphate pH 7.0): 508 nm, Φ_f = 0.35.

9-(3-Oxo-but-1-ynyl)-2,3,5,6-tetrahydro-1*H*, 4*H*-11-oxa-3a-aza-benzo[*de*]anthracen-10-one (7).

To alcohol **22** (30 mg, 0.097 mmol) dissolved in dry CH_2Cl_2 (3 ml) was added powdered MnO_2 (150 mg) at room temperature. The resulting suspension was stirred until the reaction was complete (6 hrs). The subsequent mixture was filtered through Celite, dried *in vacuo*, and purified by column chromatography on silica gel (eluent gradient: CH_2Cl_2 to CH_2Cl_2-EtOAc 98:2) to afford **7** (21 mg, 70%).

NMR ^{1}H (300 MHz, $CDCl_3$) δ ppm:

7.77 (s, 1H); 6.82 (s, 1H); 3.33 (m, 4H); 2.86 (m, 2H); 2.75 (m, 2H); 2.44 (s, 3H); 1.97 (m, 4H).

NMR ^{13}**C** (300 MHz, $CDCl_3$) □ ppm:
184.3; 160.6; 152.3; 150.2; 148.2; 125.9; 119.4; 108.1; 106.2; 98.8; 92.4; 87.9; 50.2; 49.8; 32.5; 27.3; 21.0; 20.1; 20.0.

IR (NaCl, cm^{-1}): 2937, 2844, 2170, 1714, 1657, 1620, 1586, 1520, 1358, 1295, 1154, 760.

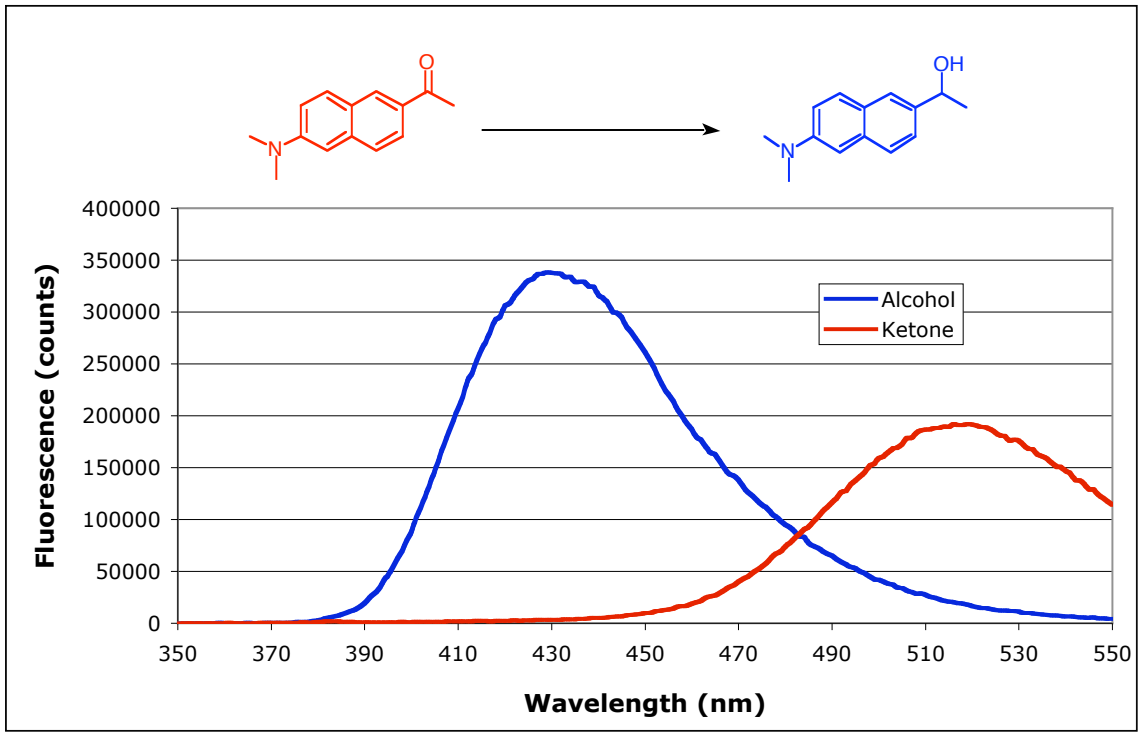
HRMS (FAB): 308.1295 ($C_{19}H_{18}O_3N$, M+1; calc 308.1287).

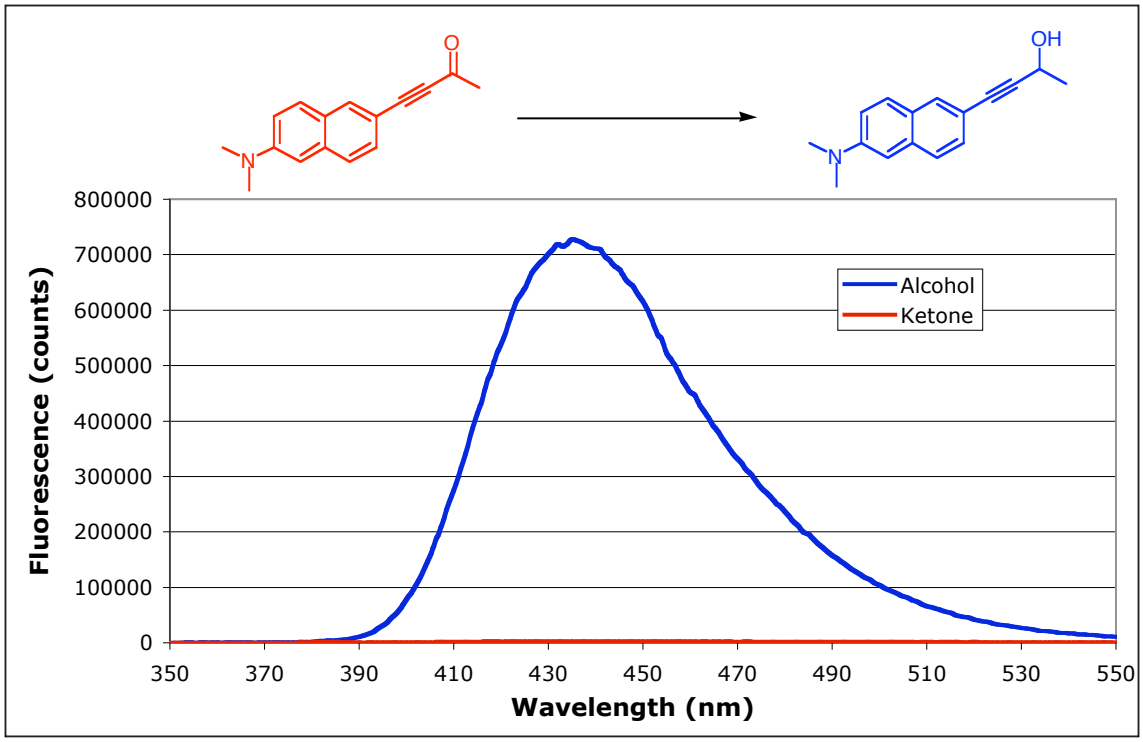
UV (EtOH): λ_{max} = 464 nm.

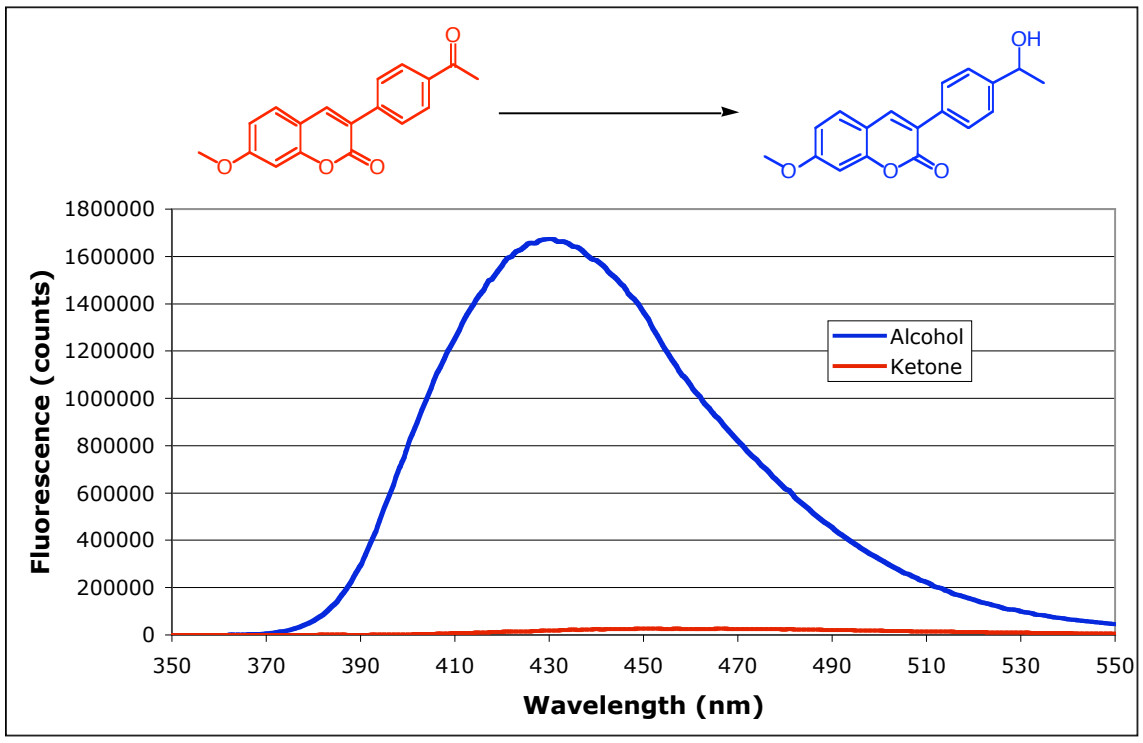
Fluorescence (potassium phosphate pH 7.0): 512 nm, Φ_f = 0.01.

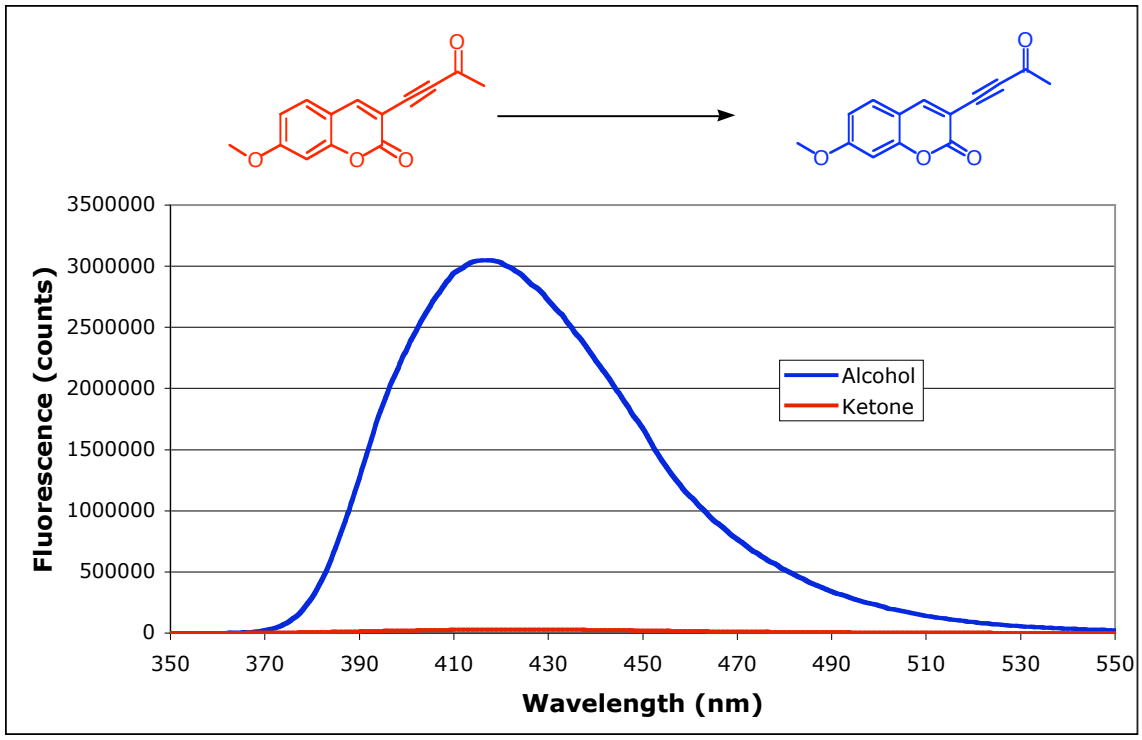
Fluorescence Spectra of Selected Probes 1-7:

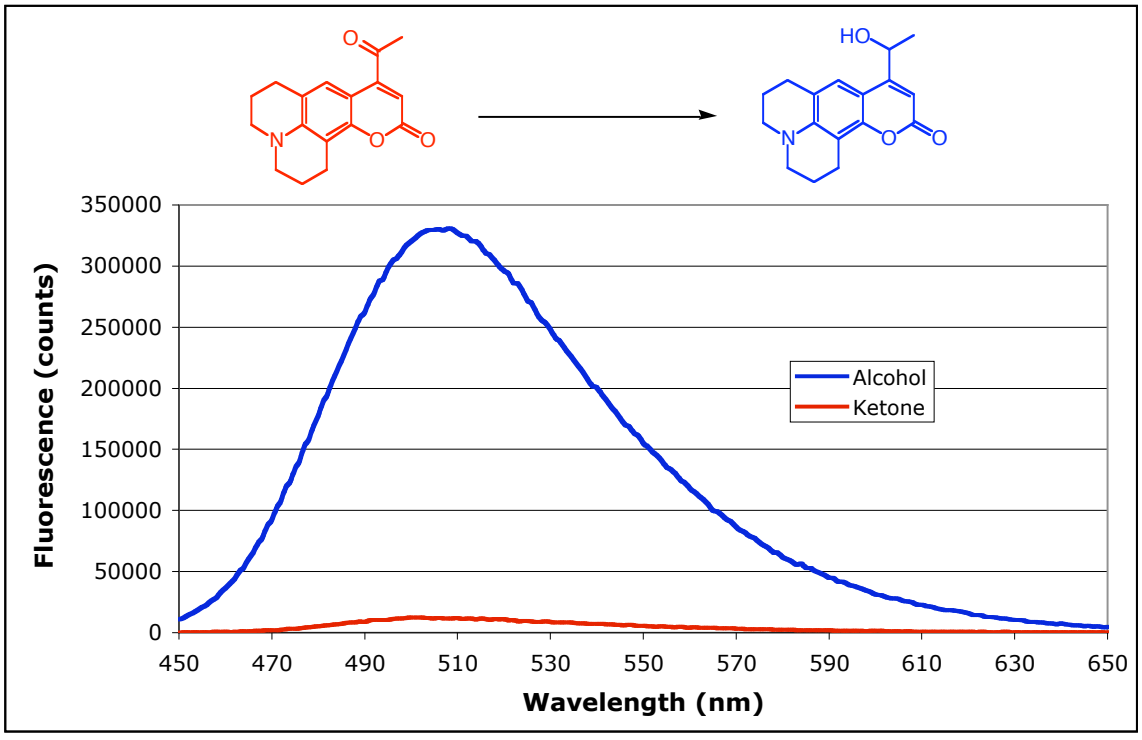
Compounds 1-4 were excited at 340 nm, while compounds 5-7 were excited at 440 nm. Fluorescence emission spectra were recorded with 10 □M solutions (<1% DMSO v/v) in 100 mM potassium phosphate buffer (pH 7.0).

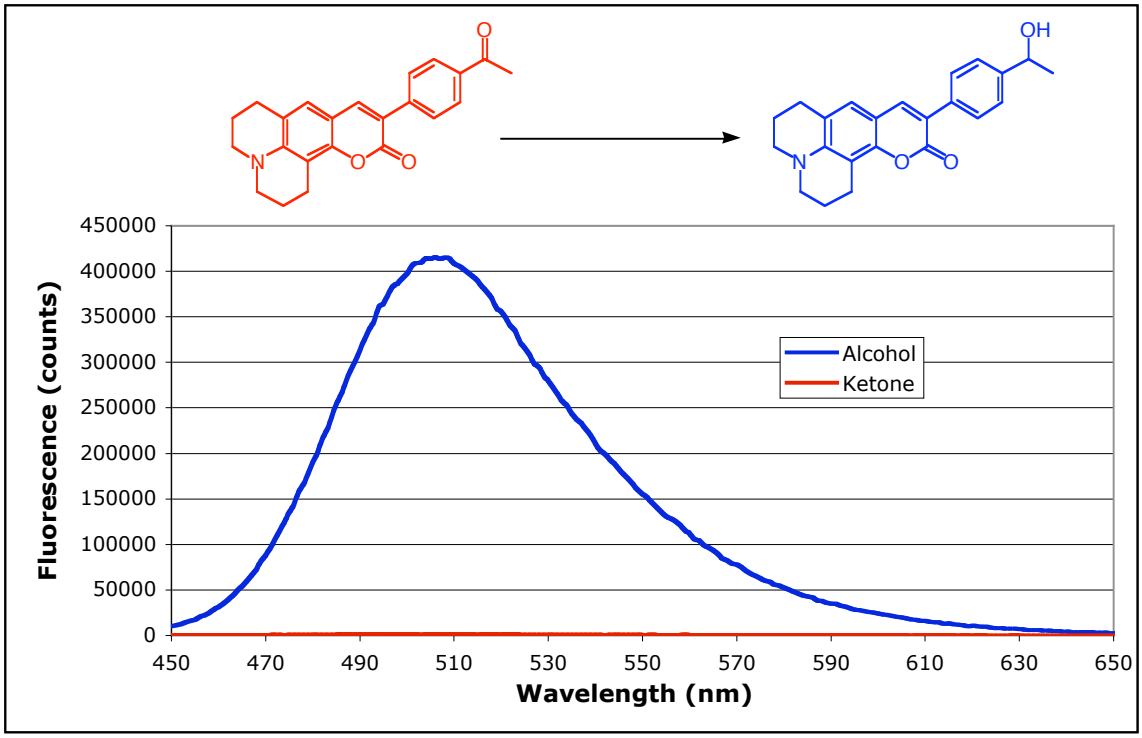


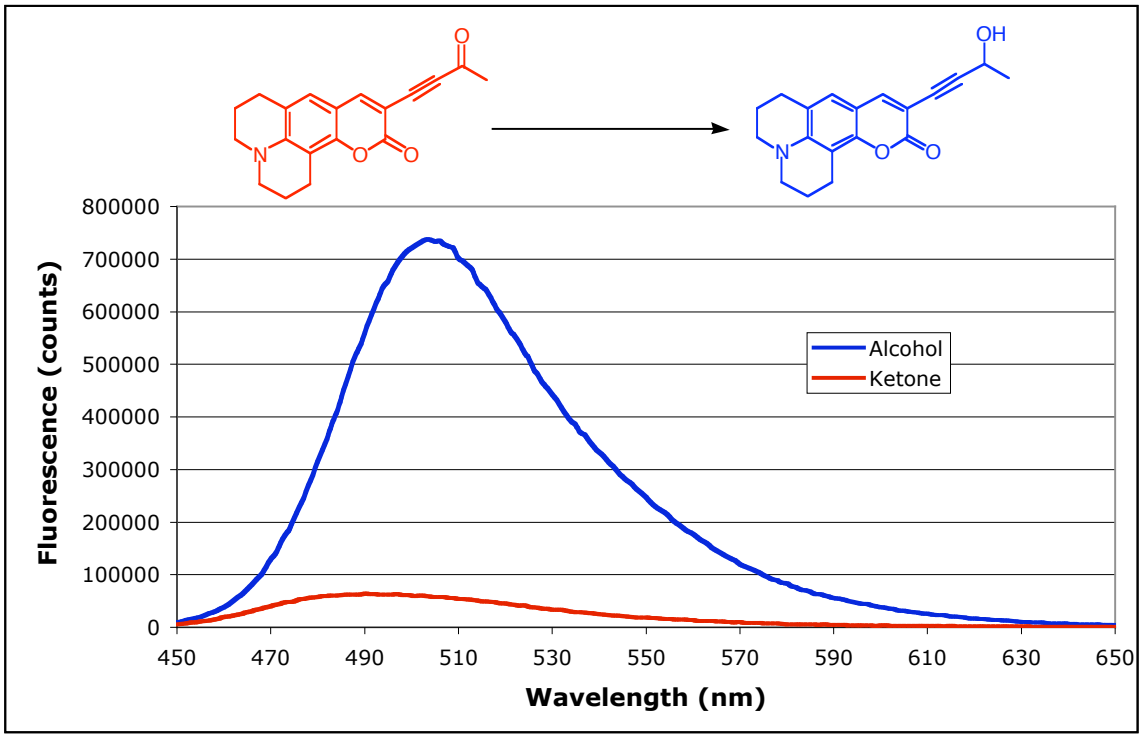

O
N
OH
N
800000
700000
600000
500000
400000
300000
200000
100000
0
Fluorescence (counts)
Alcohol
Ketone
350
370
390
410
430
450
470
490
510
530
550
Wavelength (nm)


O
O
O
O
OH
O
O
O
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
Fluorescence (counts)
Alcohol
Ketone
350
370
390
410
430
450
470
490
510
530
550
Wavelength (nm)

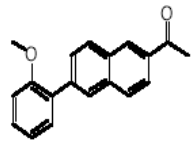
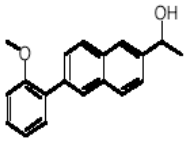
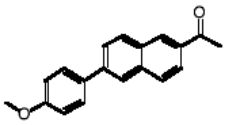
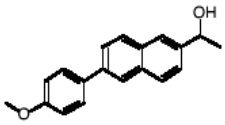
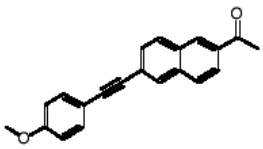
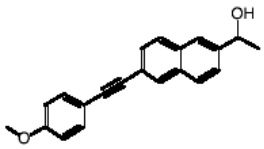
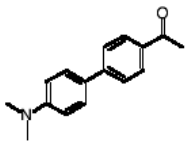
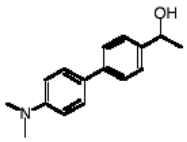
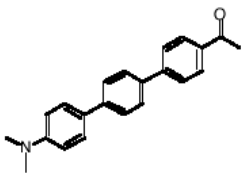
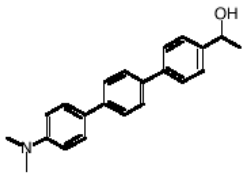
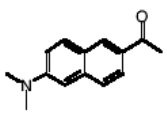
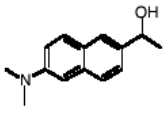

O
O
O
O
O
O
O
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
Fluorescence (counts)
Alcohol
Ketone
350
370
390
410
430
450
470
490
510
530
550
Wavelength (nm)

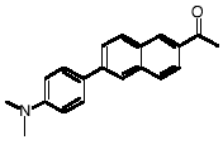
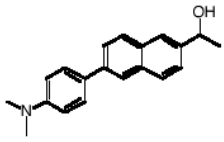
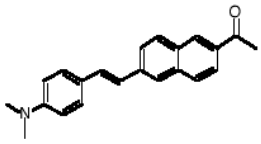
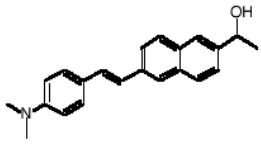
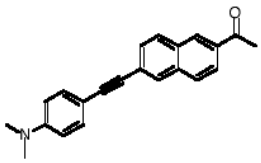
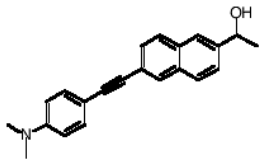
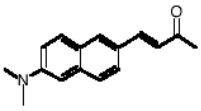
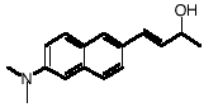
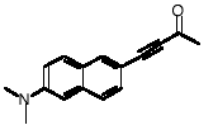
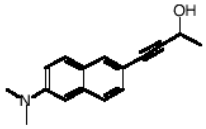
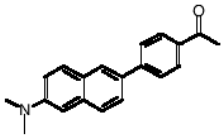
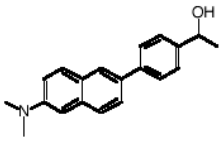

O
N
O
O
HO
N
O
O
350000
300000
250000
200000
150000
100000
50000
0
Fluorescence (counts)
Alcohol
Ketone
450
470
490
510
530
550
570
590
610
630
650
Wavelength (nm)

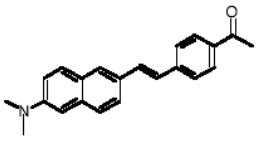
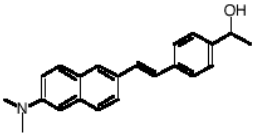
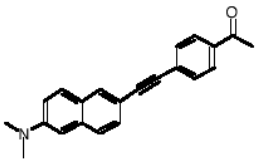
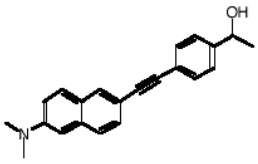
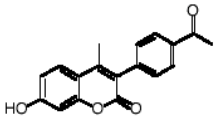
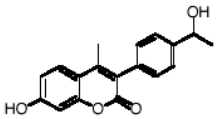
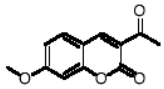
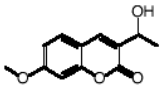
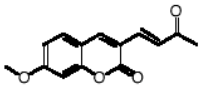
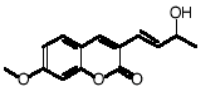
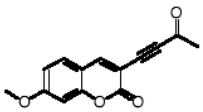
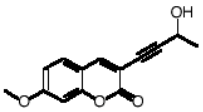

O
OH
N
O
O
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
Fluorescence (counts)
Alcohol
Ketone
450
470
490
510
530
550
570
590
610
630
650
Wavelength (nm)

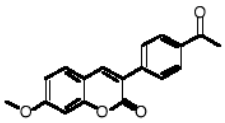
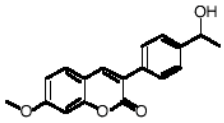
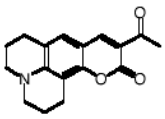
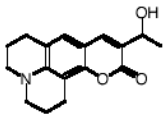
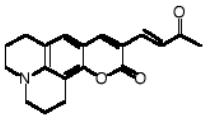
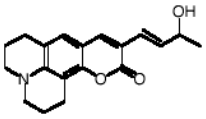
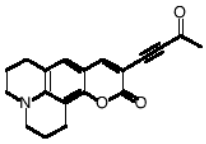
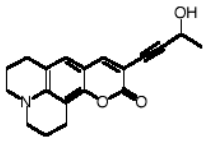
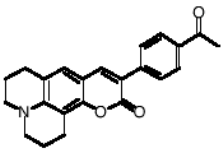
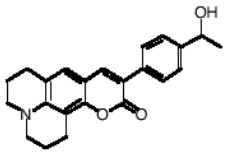
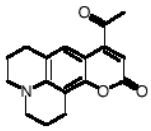
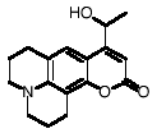

O
OH
N
O
O
800000
700000
600000
500000
400000
300000
200000
100000
0
Fluorescence (counts)
Alcohol
Ketone
450
470
490
510
530
550
570
590
610
630
650
Wavelength (nm)

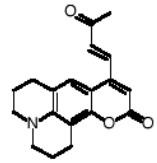
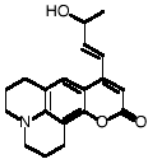
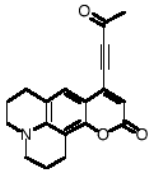
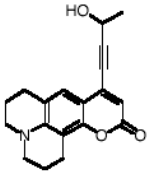
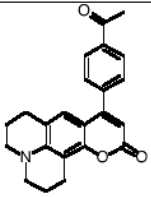
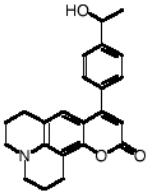
Photochemical Characterization of All Synthesized Compounds:

Ketone	Abs. Max (nm)	Fluor. Max (nm)	Alcohol	Abs. Max (nm)	Fluor. Max (nm)
	312	349		274	366
	316	354		279	380
	334	454		346	411#
	360	410		295	404
	346	392		318	474
	**368**	**521**		**348**	**429**

	361	-			316	498
	396	416			347	512#
	377	516			335	461
	395	-			318	453
	**389**	**448**			**361**	**440**
	364	-			315	447

	**392**	**452**			**348**	**510#**
	378	-			331	-
	333	668			329	461
	359	429			320	400
	322	443			278	455
	**368**	**416**			**346**	**420**

	**348**	**462**			**342**	**429**
	449	504			378	501*
	465	587			416	519^
	**464**	**512**			**429**	**508**
	**435**	**511**			**422**	**509**
	**418**	**520**			**398**	**509**

	-	-			402	502#
	458	539			433	450^
	410	474			405	550#

low quantum yield, ^ reactivity with cellular reductants, * no change in wavelength of emission

Protocols for Enzymatic Assays

Procedure for Enzymatic Screening of Selected Probes 1-7:

Horse Liver alcohol dehydrogenase (Lot Number 51K7520), *Thermoanaerobium brockii* $NADP^+$ dependent alcohol dehydrogenase (Lot Number 033K4093), *Pseudomonas testosteroni* 3-hydroxysteroid dehydrogenase (Lot Number 053K8624), and *Bacillus sphaericus* 12-hydroxysteroid dehydrogenase (Lot Number 70K16621) were purchased from Sigma. Yeast alcohol dehydrogenase (Lot Number 93122920), glycerol dehydrogenase (Lot Number 92110122), (D)-lactate dehydrogenase (Lot Number 92419236), (L)-lactate dehydrogenase (Lot Number 92801821), NAD^+, $NADP^+$, NADH, and NADPH were purchased from Roche. Enzyme activity was confirmed by compliance to supplier's quality control assays prior to usage. Rat and human 3-hydroxysteroid dehydrogenases were provided by Professor Trevor Penning (University of Pennsylvania School of Medicine) and human amyloid- peptide binding alcohol dehydrogenase was supplied by Professor Shi Du Yan (Columbia University School for Physicians and Surgeons).

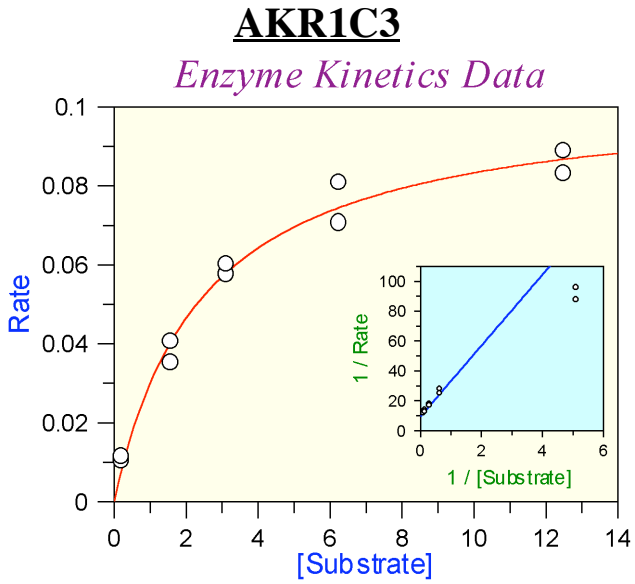
Enzymatic assays were performed in triplicate on selected fluorogenic substrates according to the following protocol. To each well of a FALCON 96-well black flat bottom plate was added (1) 40 L of 500 mM potassium phosphate buffer pH 7.0, (2) 113 L of double deionized water, (3) 25 L of 2 mM NADH (except for *Pseudomonas testosteroni* 3-hydroxysteroid dehydrogenase, rat 3-hydroxysteroid dehydrogenase, and *Thermoanaerobium brockii* $NADP^+$ dependent alcohol dehydrogenase, in which cases 2 mM of NADPH was used), (4) 2 L of a 3-5 mM solution of substrate in DMSO, and (5) 20 L of a 40-50 g/mL solution of enzyme. Reaction volumes were mixed thoroughly after addition of cofactor, substrate, and enzyme and allowed to react 12 hours at 25°C. Scanning of the 96-well plate was performed by the MicroMax 384 connected to a Jobin Yvon Fluorolog through F-3000 fiber optic cables.

Determination of Kinetic Parameters for AKR1C3

Fluorogenic substrate **5** reduction was monitored on a Hitachi F-4500 fluorimeter in Starna quartz cuvettes fluorometrically in 1 mL systems containing 100 mM potassium phosphate pH 6.0 containing excess of NADPH cofactor (250 M) and various amounts of the substrate (0.1953-50 M) dissolved in 4% acetonitrile. Aqueous assay components were added first, followed by addition of 20 L of acetonitrile as a cosolvent, and then addition of 20 L of the substrate in acetonitrile (total acetonitrile in the assay did not exceed 4%). Cuvettes were mixed thoroughly after addition of cofactor, cosolvent, and substrate. Reactions were initiated by the addition of 4 L of dilute AKR1C3 (115 g/mL) and were corrected for nonenzymatic rates. All reactions were followed by monitoring the increase in fluorescence of the product alcohol for 5 minutes at λ_{em} 510 nm with λ_{ex} 440 nm (Excitation and emission band pass slits both at 2.5 nm, lamp 900 V) at 37°C. The initial velocities, expressed in units of nanomoles per minute, were calculated according to previously published procedures[7]:

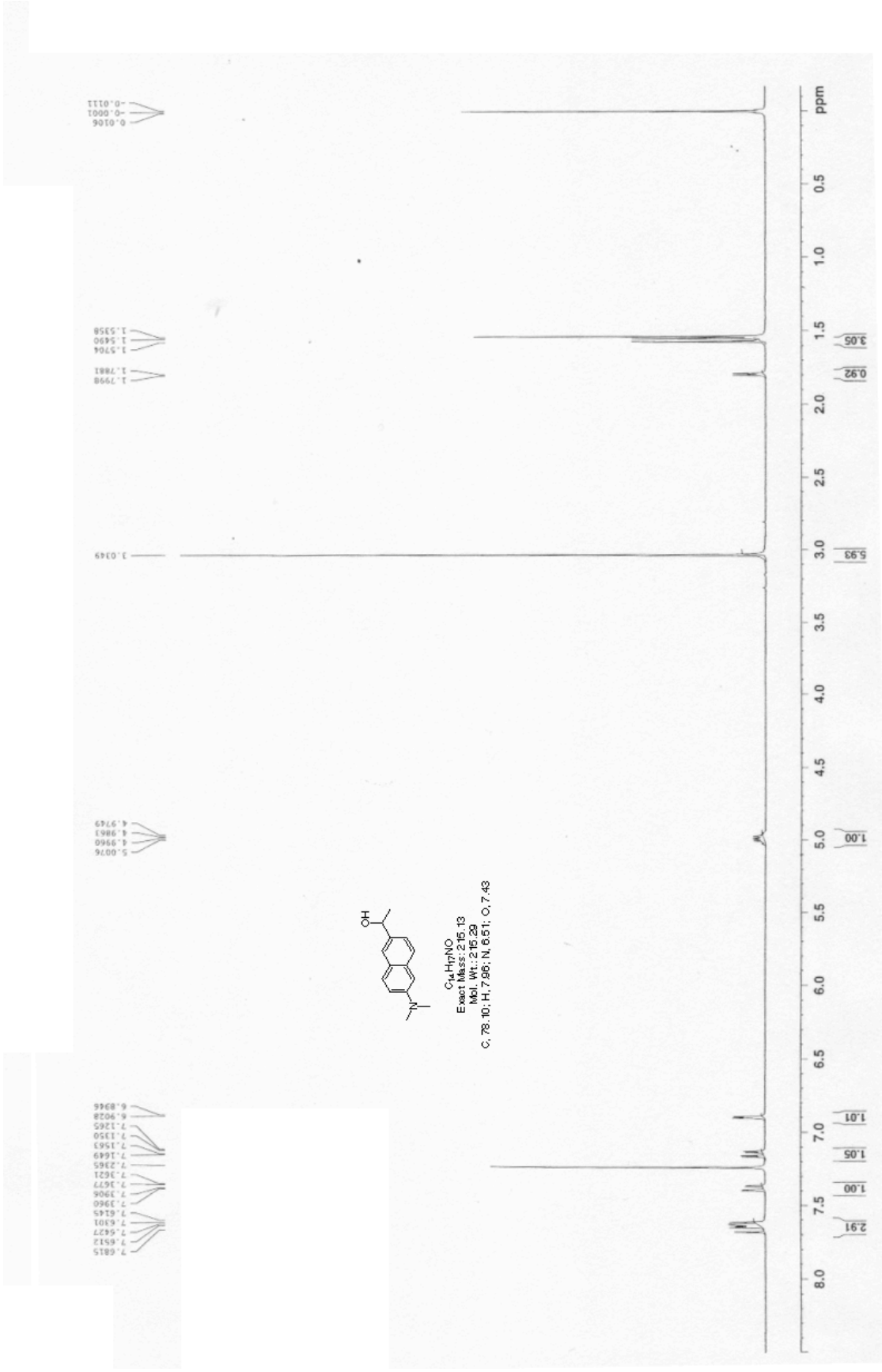
$$\text{initial rate} = [n_{st} \times (F_t - F_0)/(F_{st})] / t \qquad (1)$$

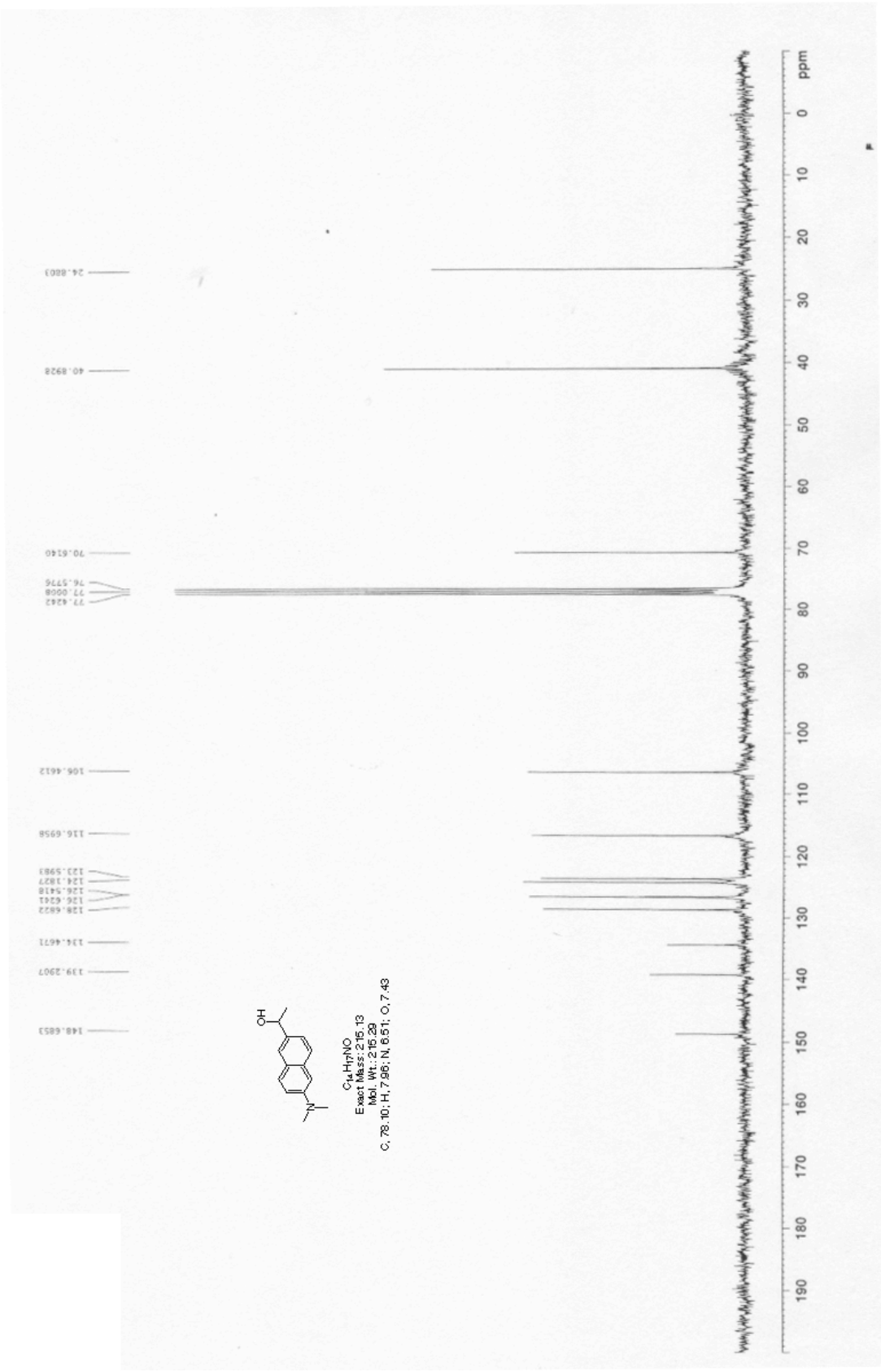
where F_t and F_0 represent the fluorescence at time t and 0, n_{st} is the nanomoles of the product standard, and F_{st} is the fluorescence resulting from n_{st} of product. Kinetic constants were approximated using the GraFit (Erithacus Software, Surrey, UK) non-linear regression analysis program to fit the untransformed data to a hyperbolic function as originally described[9], yielding estimated values of k_{cat}, K_m, and their associated standard errors.

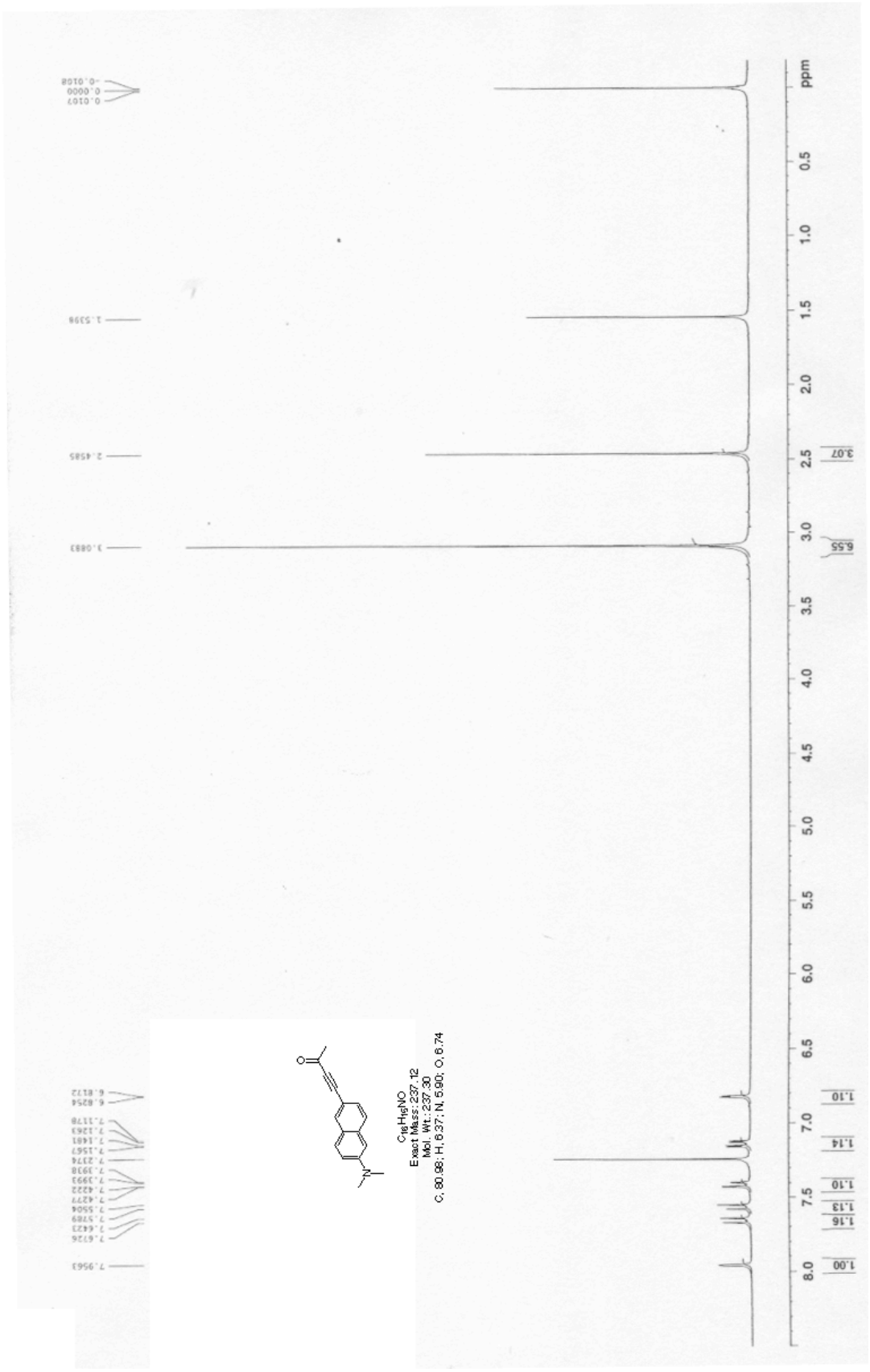


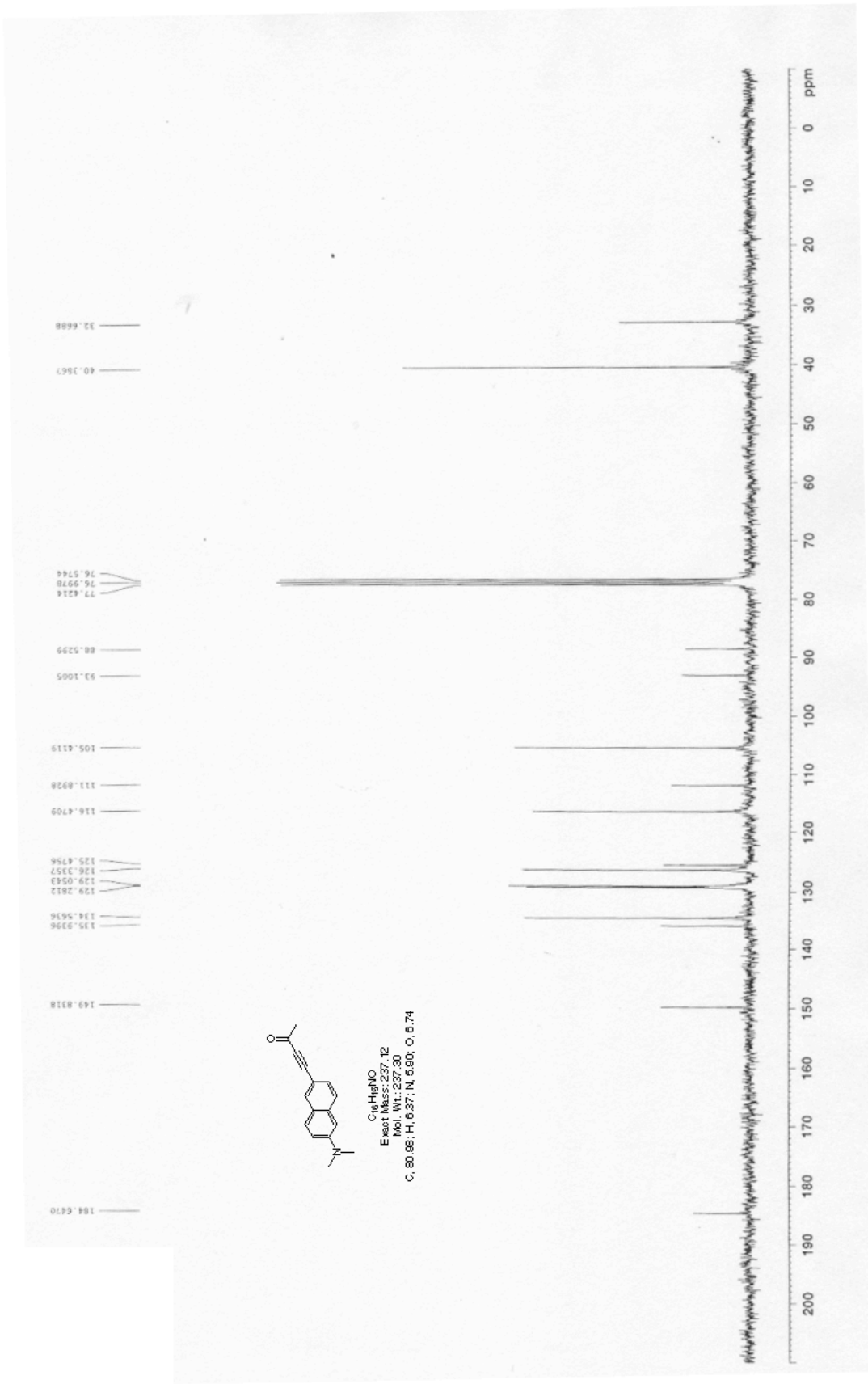
Parameter	Value	□	Std. Error
Vmax	0.1039		0.0049 nmol/min
Km	2.4637	□	0.3511 uM
kcat	8.244		0.389 min^{-1}
kcat/Km	335		min^{-1}/ mM^{-1}
Spec. activity	0.226		0.011 umol/min/mg

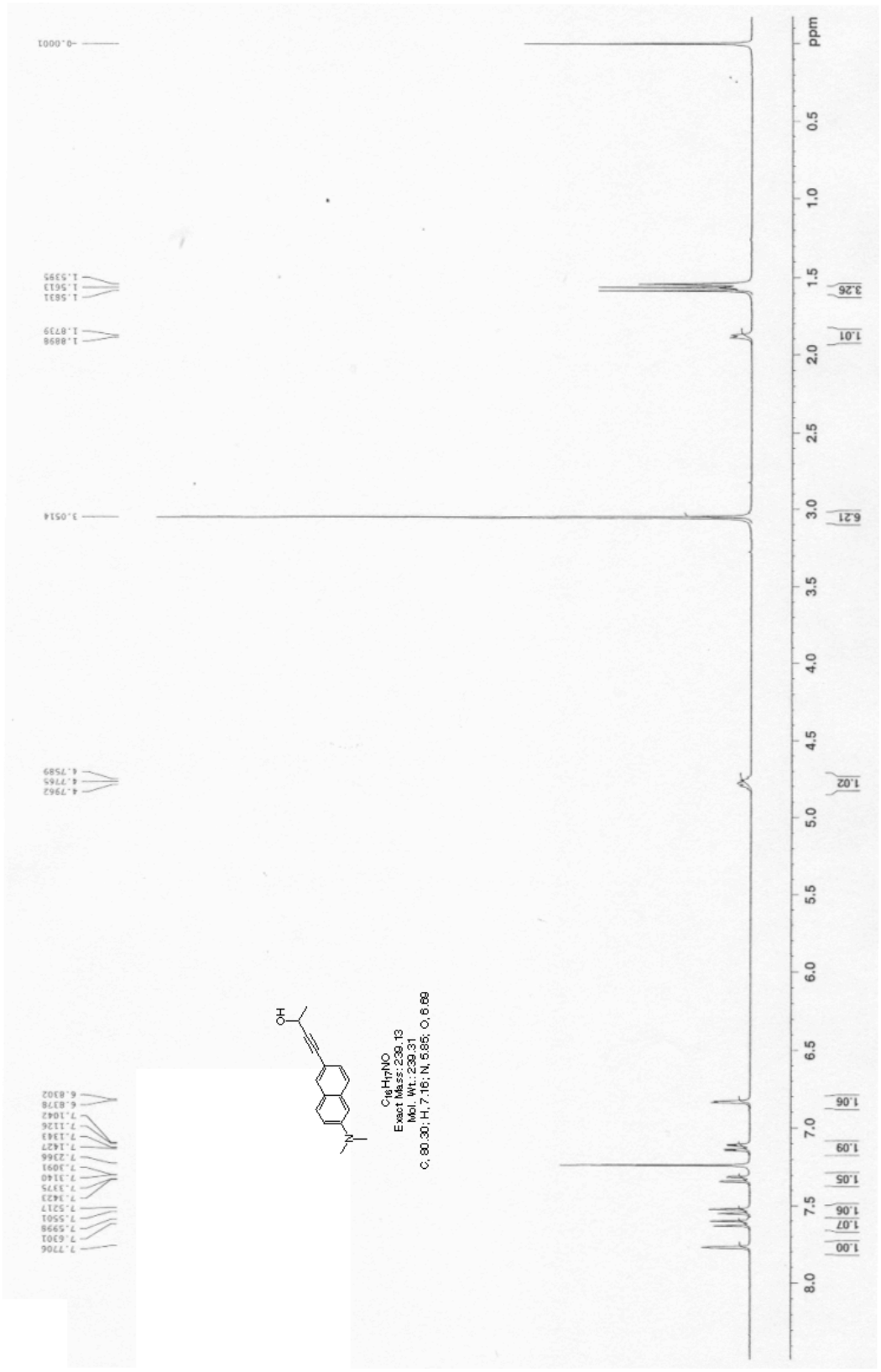
AKR1C3 kinetic data was also performed by HPLC separation of the fluorogenic substrate and its product alcohol and measurement of ketone to alcohol ratios. This data was found to correlate well with kinetic parameters determined fluorometrically[10].

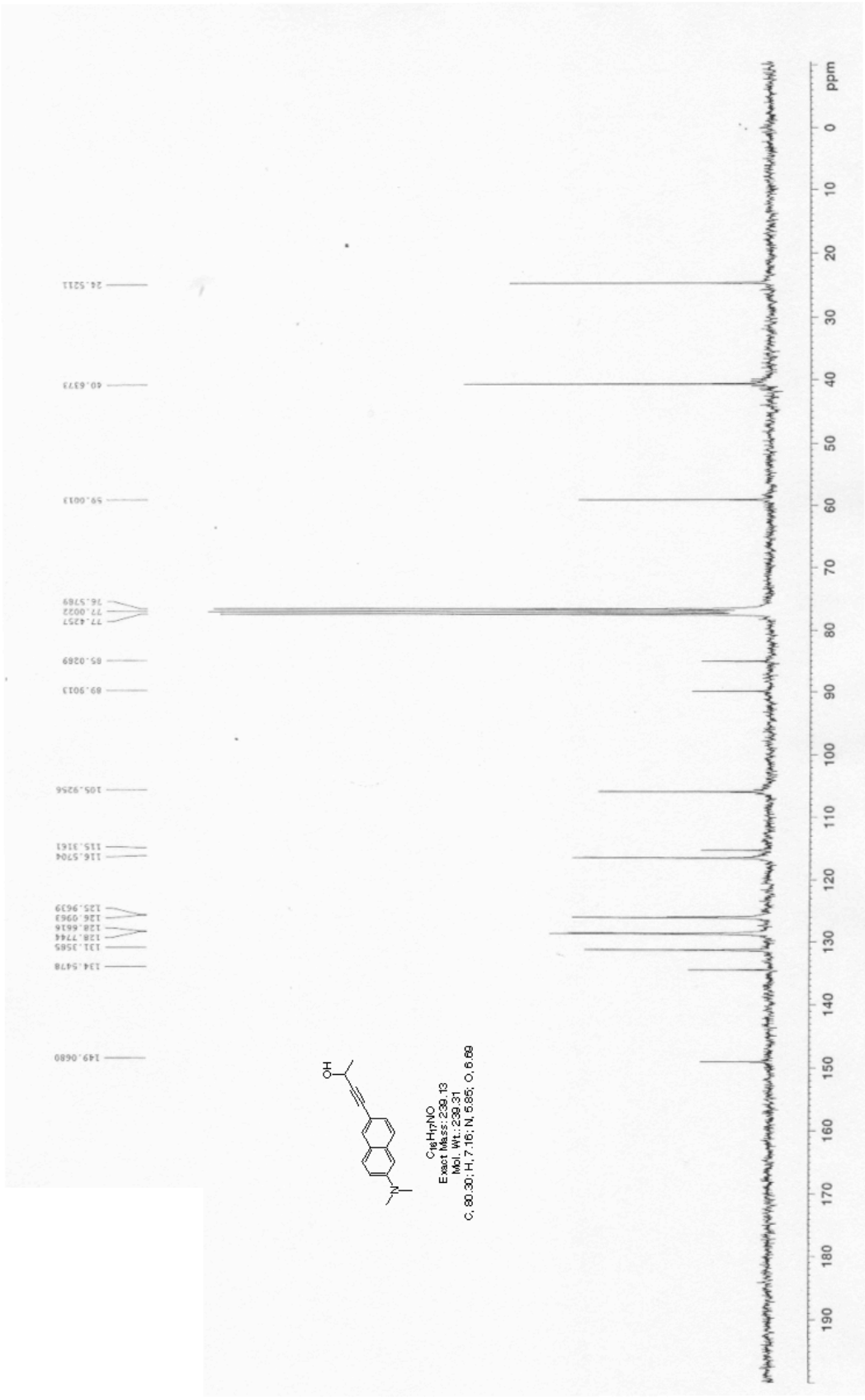

C14H17NO
Exact Mass: 215.13
Mol. Wt.: 215.29
C, 78.10; H, 7.96; N, 6.51; O, 7.43
ppm

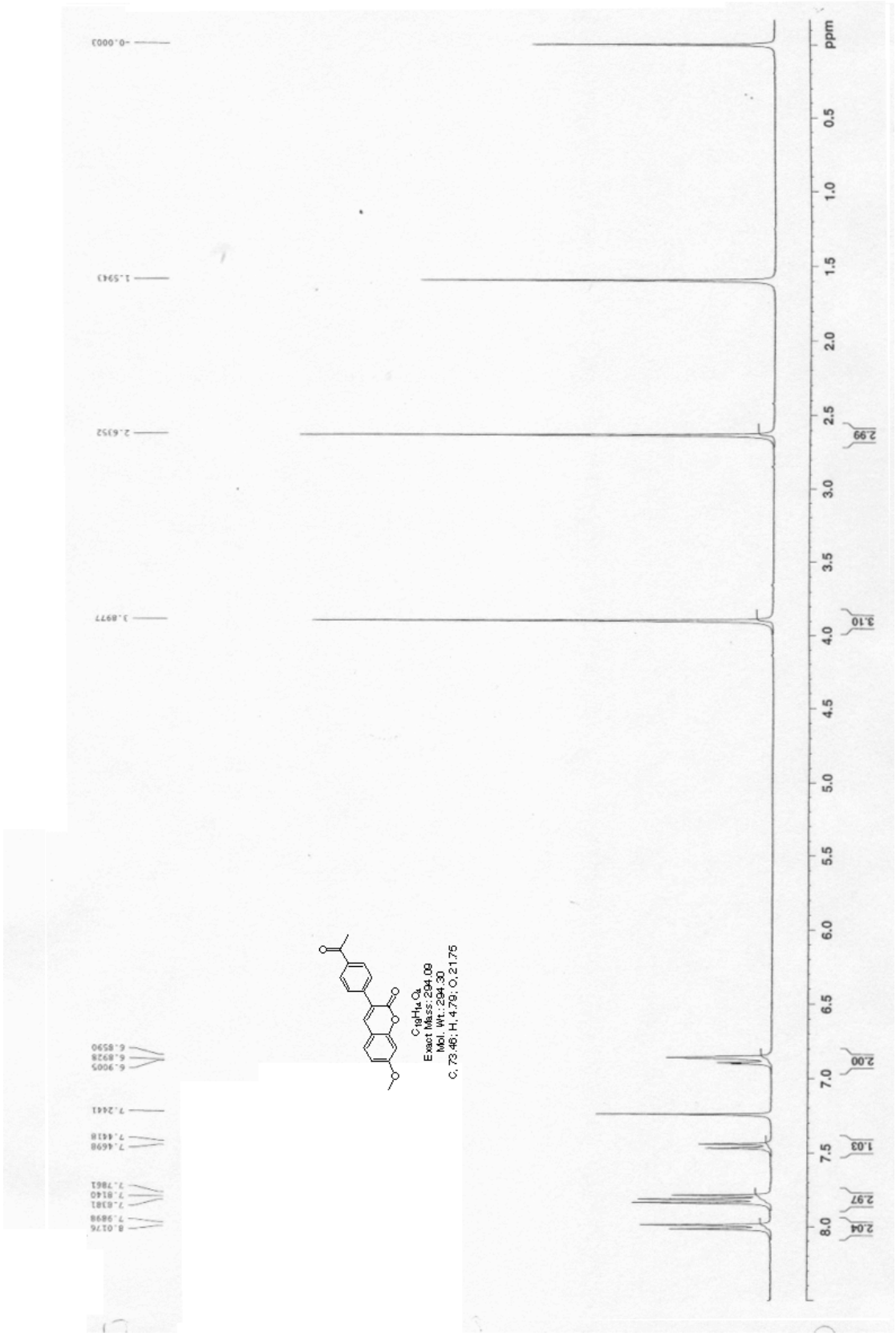

24.8803
40.8928
70.8140
76.5776
77.0008
77.4242
106.4612
116.6958
123.5983
124.1827
126.5418
126.6241
128.6852
134.4671
139.2907
148.6853
OH
N
C14H17NO
Exact Mass: 215.13
Mol. Wt.: 215.29
C, 78.10; H, 7.96; N, 6.51; O, 7.43
ppm
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190

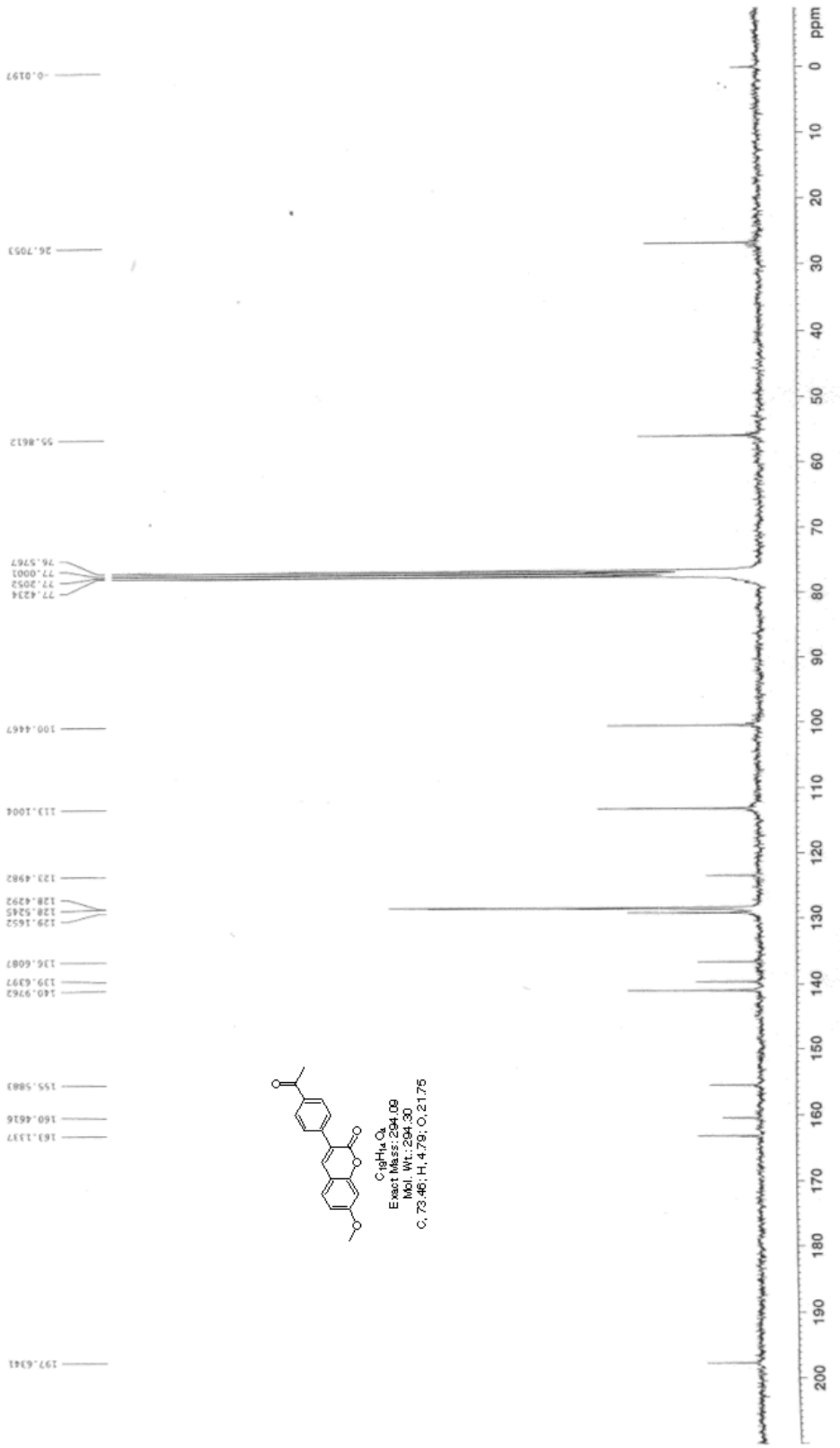

C16H15NO
Exact Mass: 237.12
Mol. Wt.: 237.30
C, 80.98; H, 6.37; N, 5.90; O, 6.74
ppm

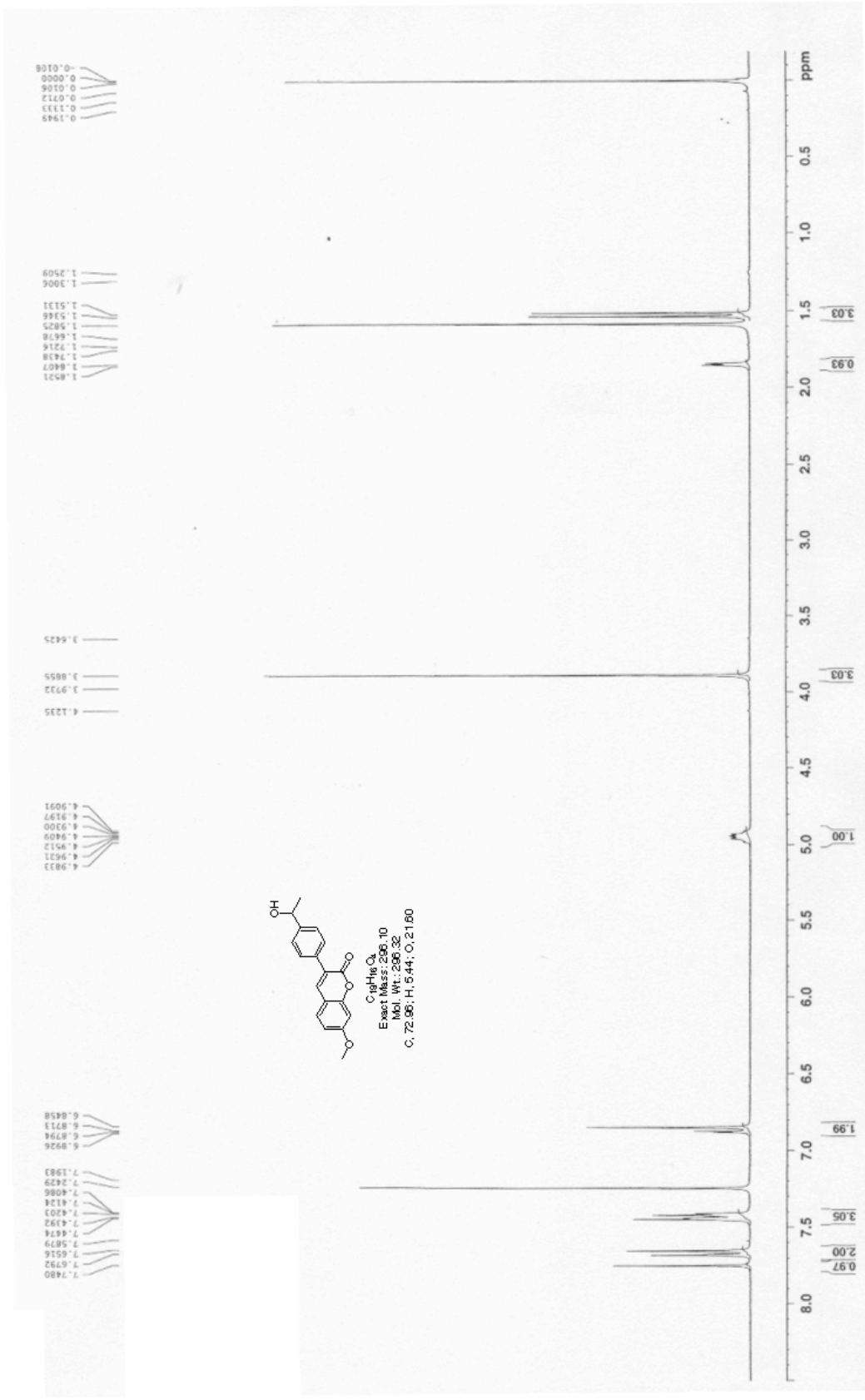

C16H15NO
Exact Mass: 237.12
Mol. Wt.: 237.30
C, 80.98; H, 6.37; N, 5.90; O, 6.74
ppm

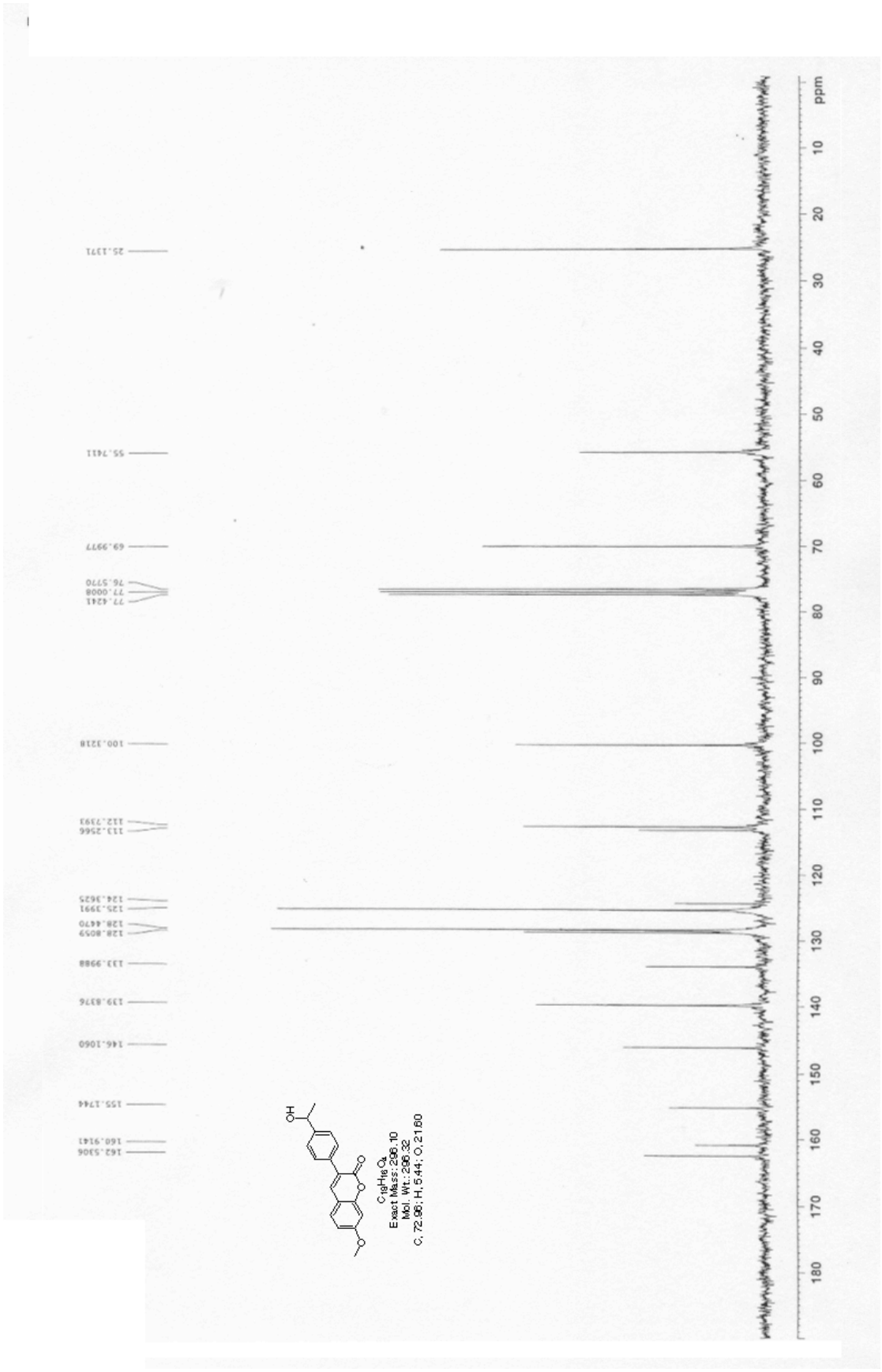

C16H17NO
Exact Mass: 239.13
Mol. Wt.: 239.31
C, 80.30; H, 7.16; N, 5.85; O, 6.69
ppm

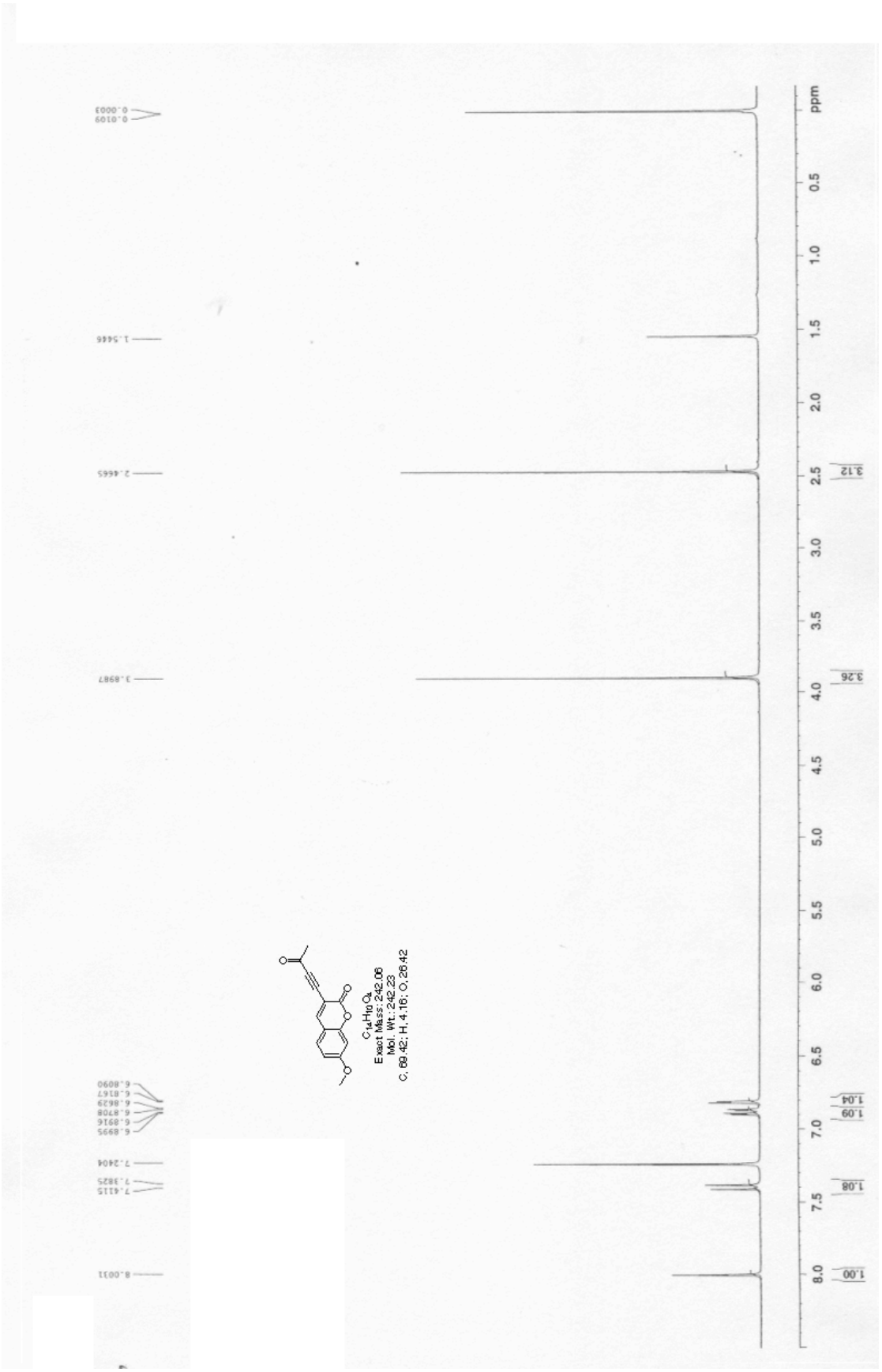

24.5211
60.6373
59.0013
77.4257
77.0022
76.5789
85.0269
89.9013
105.9256
115.3161
116.5704
125.9639
126.0963
128.6616
128.7744
131.3585
134.5478
149.0680
OH
N
C18H17NO
Exact Mass: 239.13
Mol. Wt.: 239.31
C, 80.30; H, 7.16; N, 5.85; O, 6.69
ppm
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190

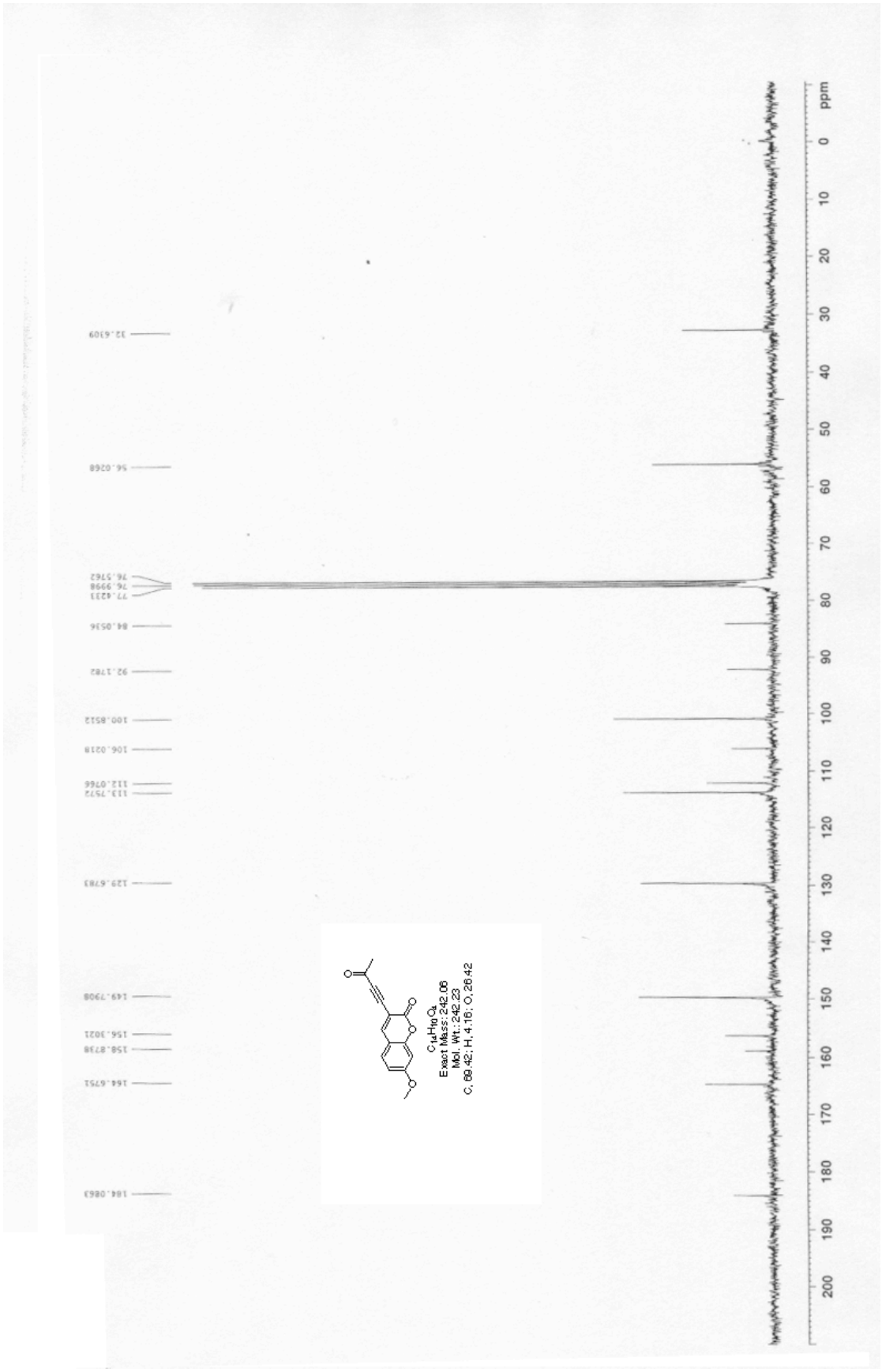

C19H14O4
Exact Mass: 294.09
Mol. Wt.: 294.30
C, 73.46; H, 4.79; O, 21.75
ppm

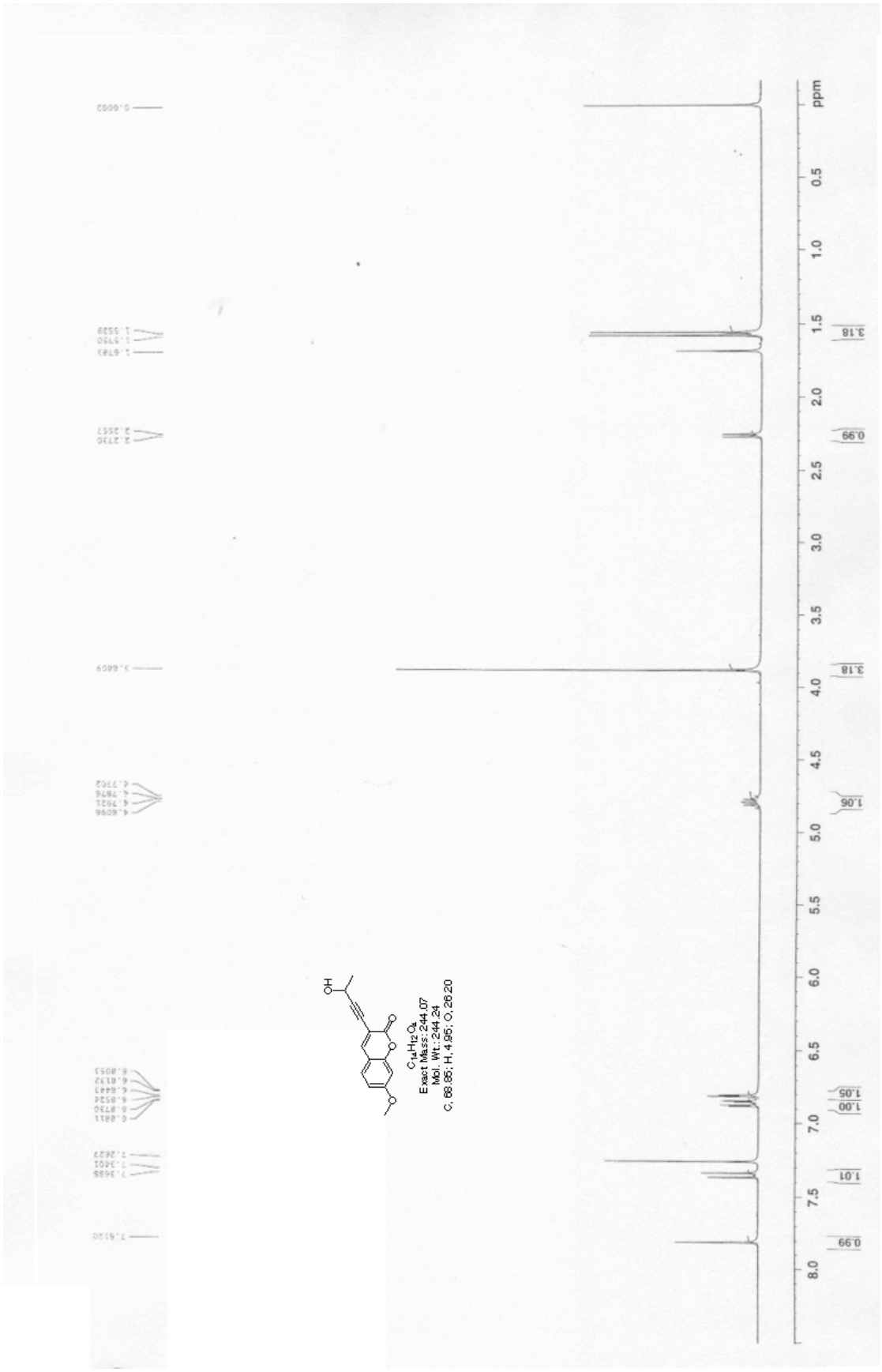

C19H14O4
Exact Mass: 294.09
Mol. Wt.: 294.30
C, 73.46; H, 4.79; O, 21.75
ppm

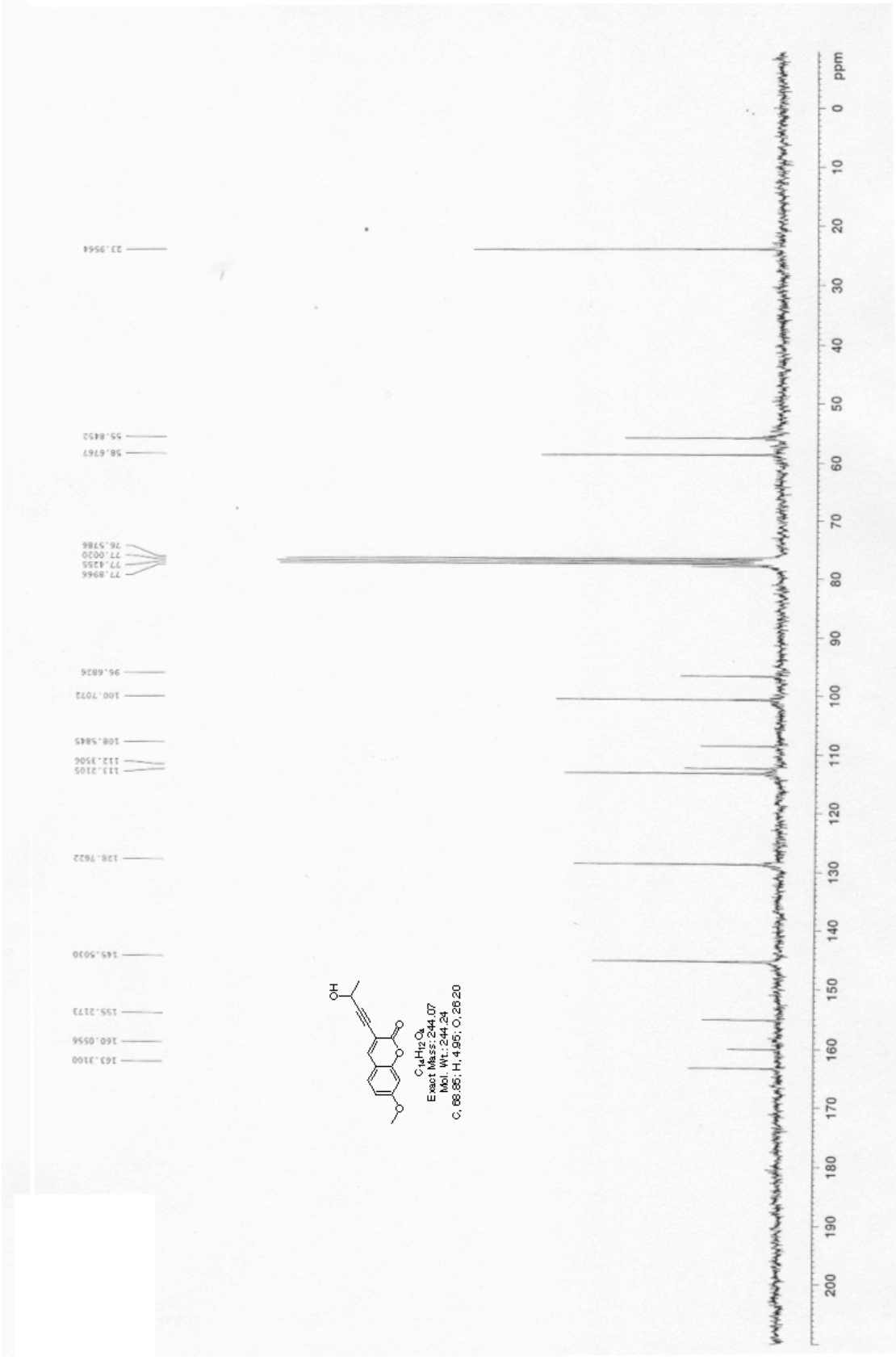

C19H18O4
Exact Mass: 298.10
Mol. Wt.: 298.32
C, 72.96; H, 5.44; O, 21.60
ppm

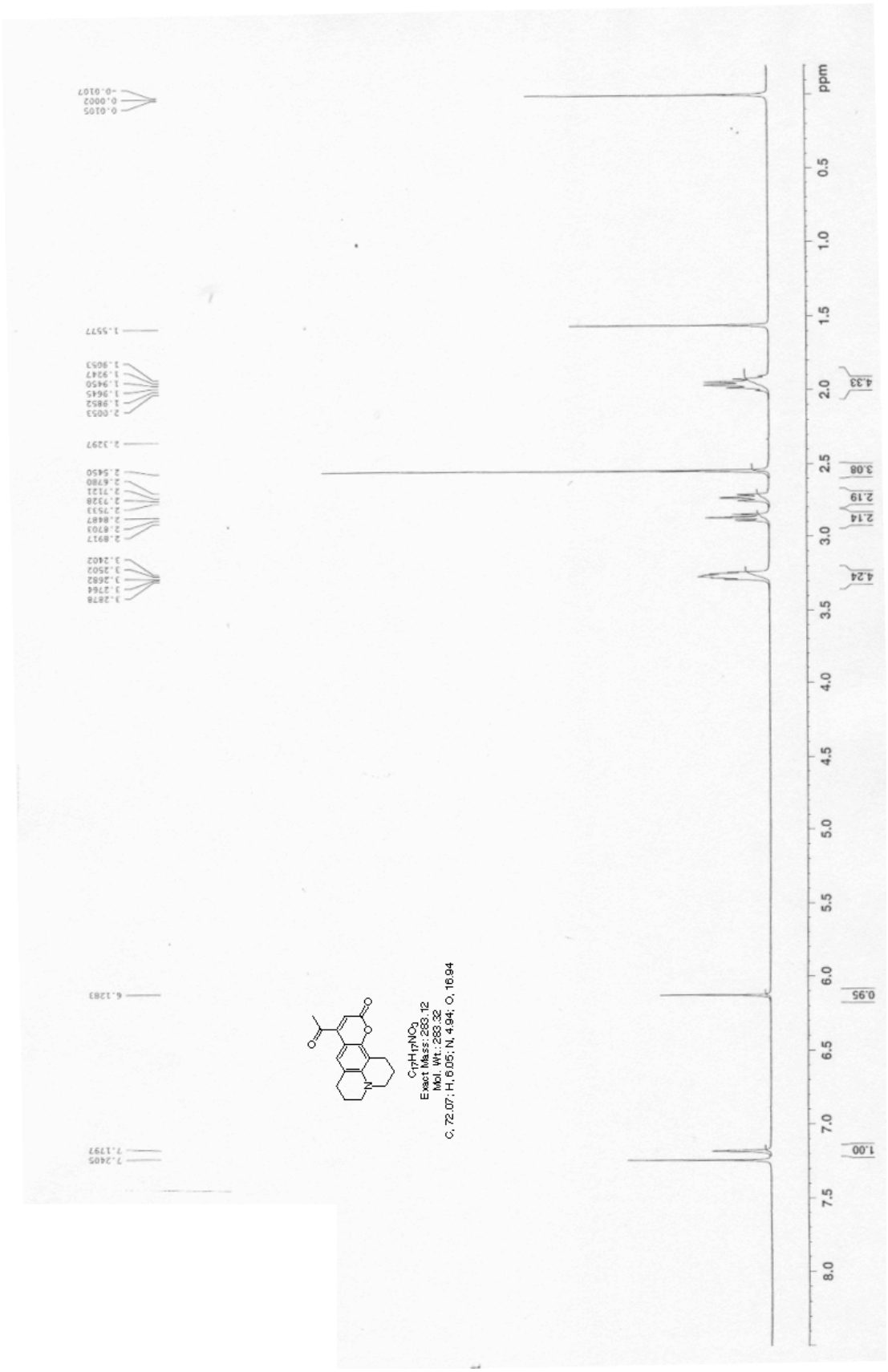

25.1371
55.7411
69.9977
76.5770
77.0008
77.4241
100.3218
112.7393
113.2566
124.3625
125.3991
128.4670
128.8059
133.9988
139.8376
146.1060
155.1744
160.9141
162.5306
OH
C19H16O4
Exact Mass: 296.10
Mol. Wt.: 296.32
C, 72.96; H, 5.44; O, 21.60
ppm

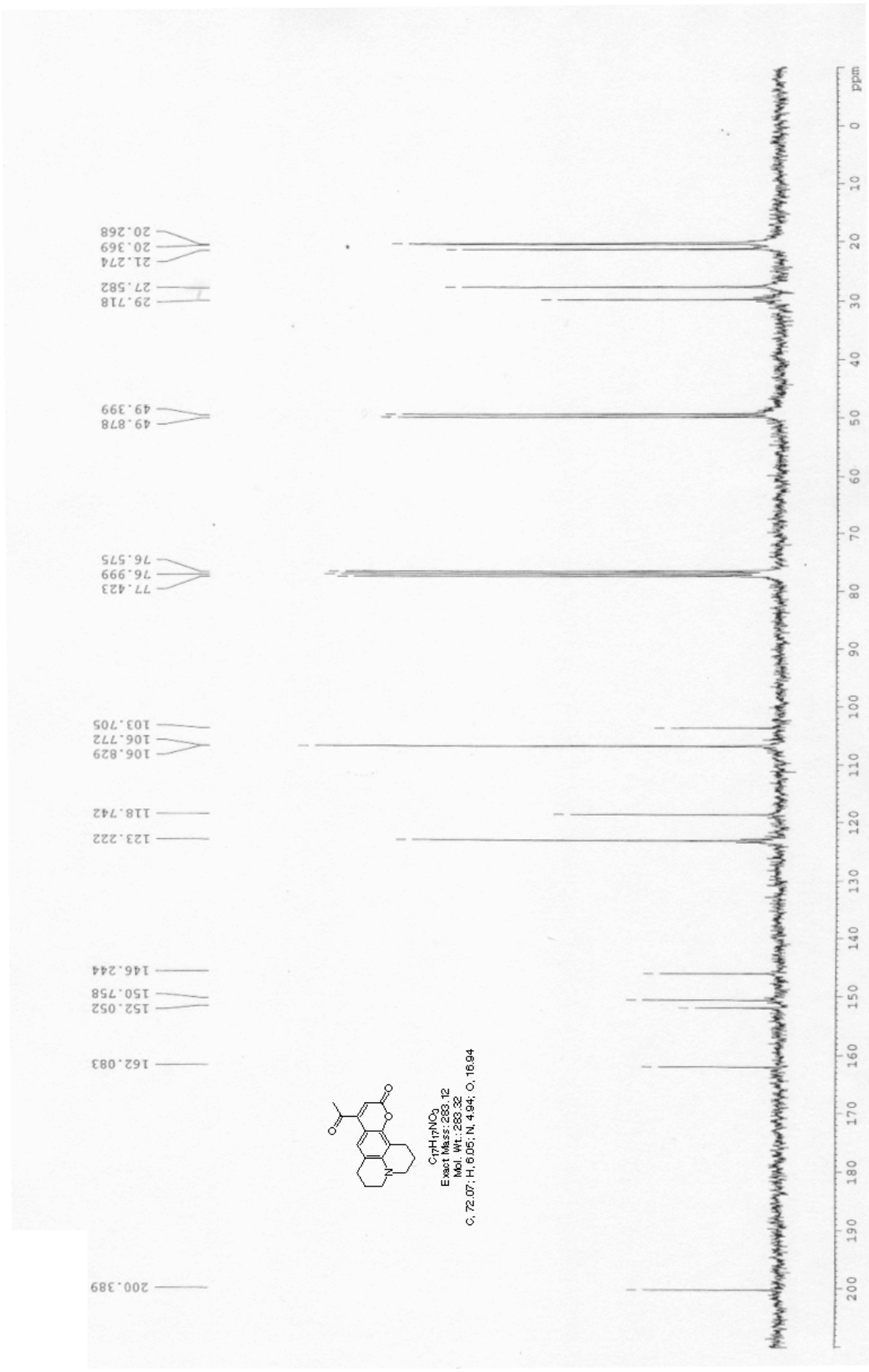

C14H10O4
Exact Mass: 242.06
Mol. Wt.: 242.23
C, 69.42; H, 4.16; O, 26.42
ppm

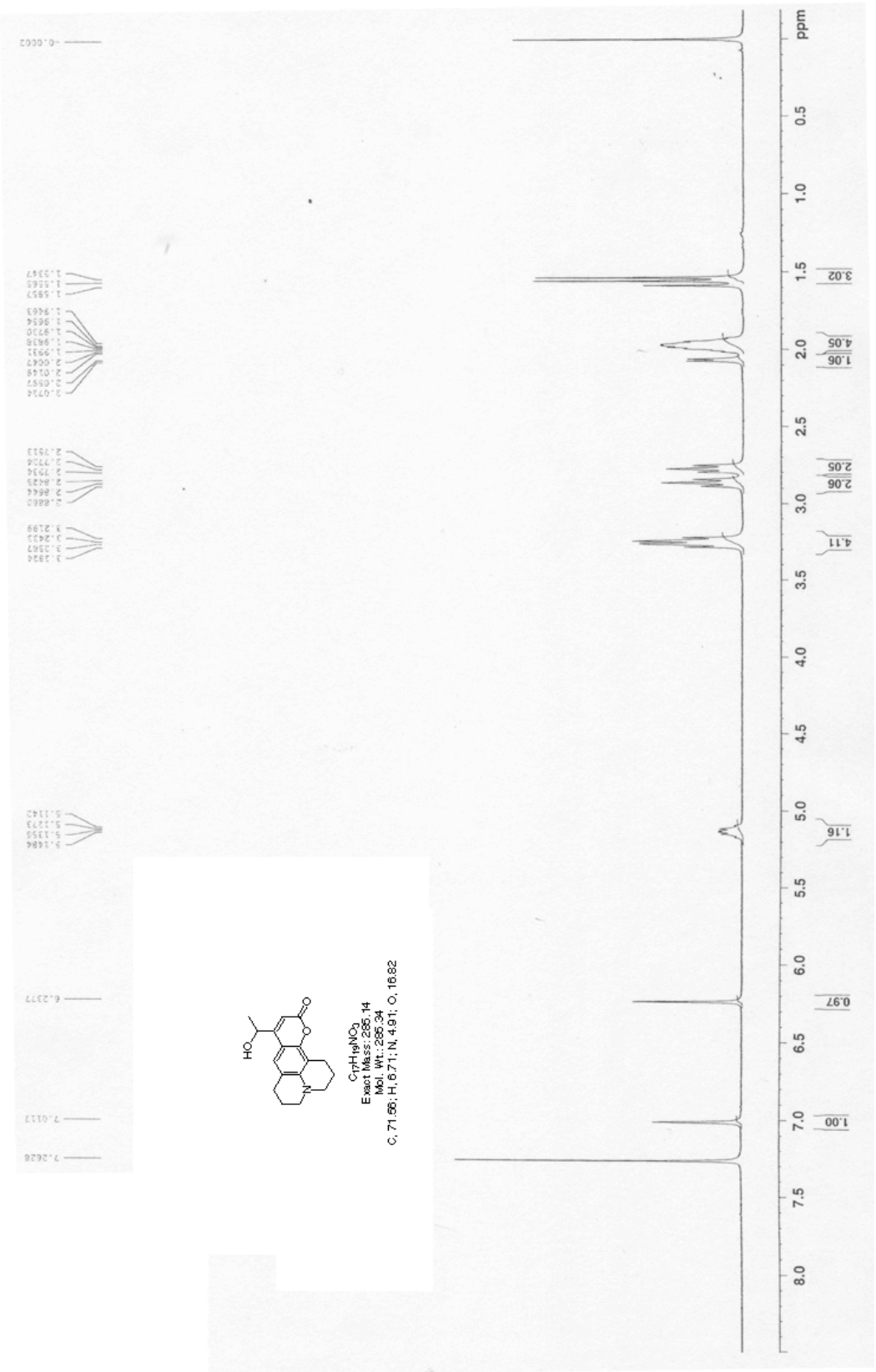

C14H10O3
Exact Mass: 242.06
Mol. Wt.: 242.23
C, 69.42; H, 4.16; O, 26.42
32.6309
56.0268
76.5762
76.9998
77.4233
84.0536
92.1782
100.8512
106.0218
112.0766
113.7572
129.6783
149.7908
156.3021
158.8738
164.6751
184.0863
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

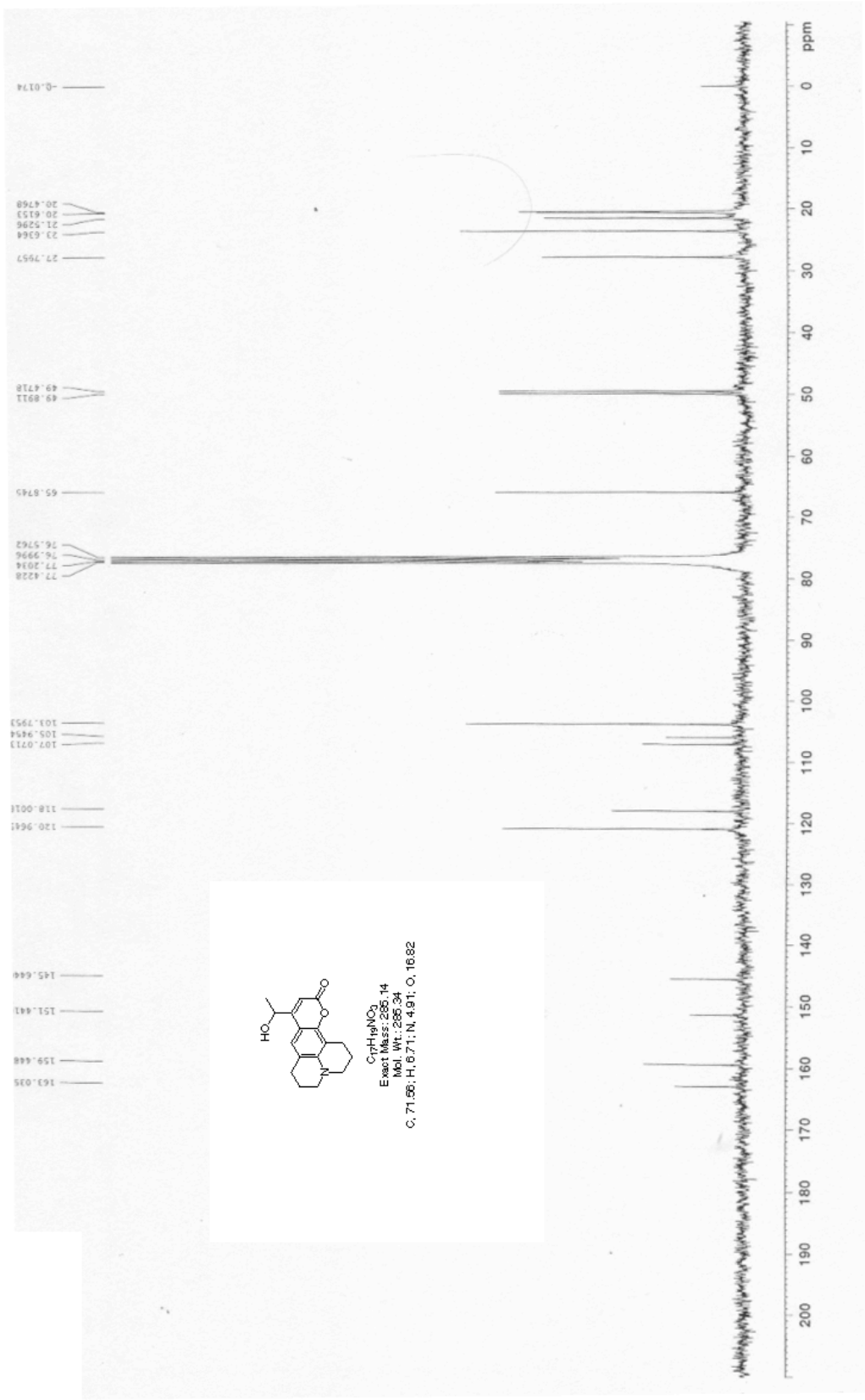

OH
O
O
O
C14H12O4
Exact Mass: 244.07
Mol. Wt.: 244.24
C, 68.85; H, 4.95; O, 26.20
ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
3.18
0.99
3.18
1.06
1.05
1.00
1.01
0.99

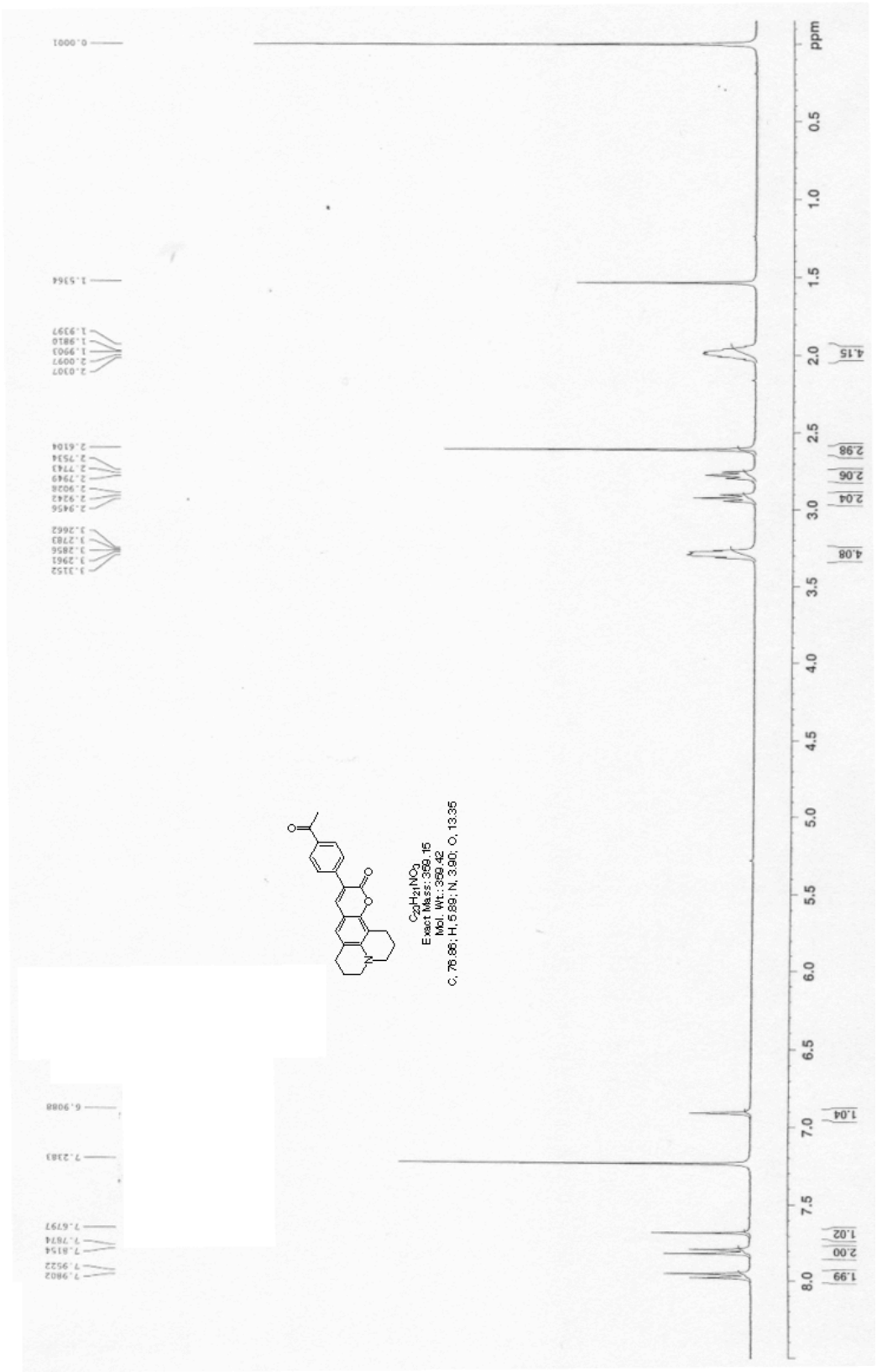

23.9564
55.8452
58.6767
76.5786
77.0020
77.4255
77.8965
96.6826
100.7072
108.5865
112.3506
113.2105
128.7622
145.5030
155.2173
160.0556
163.3100
C14H12O4
Exact Mass: 244.07
Mol. Wt.: 244.24
C, 68.85; H, 4.95; O, 26.20
ppm

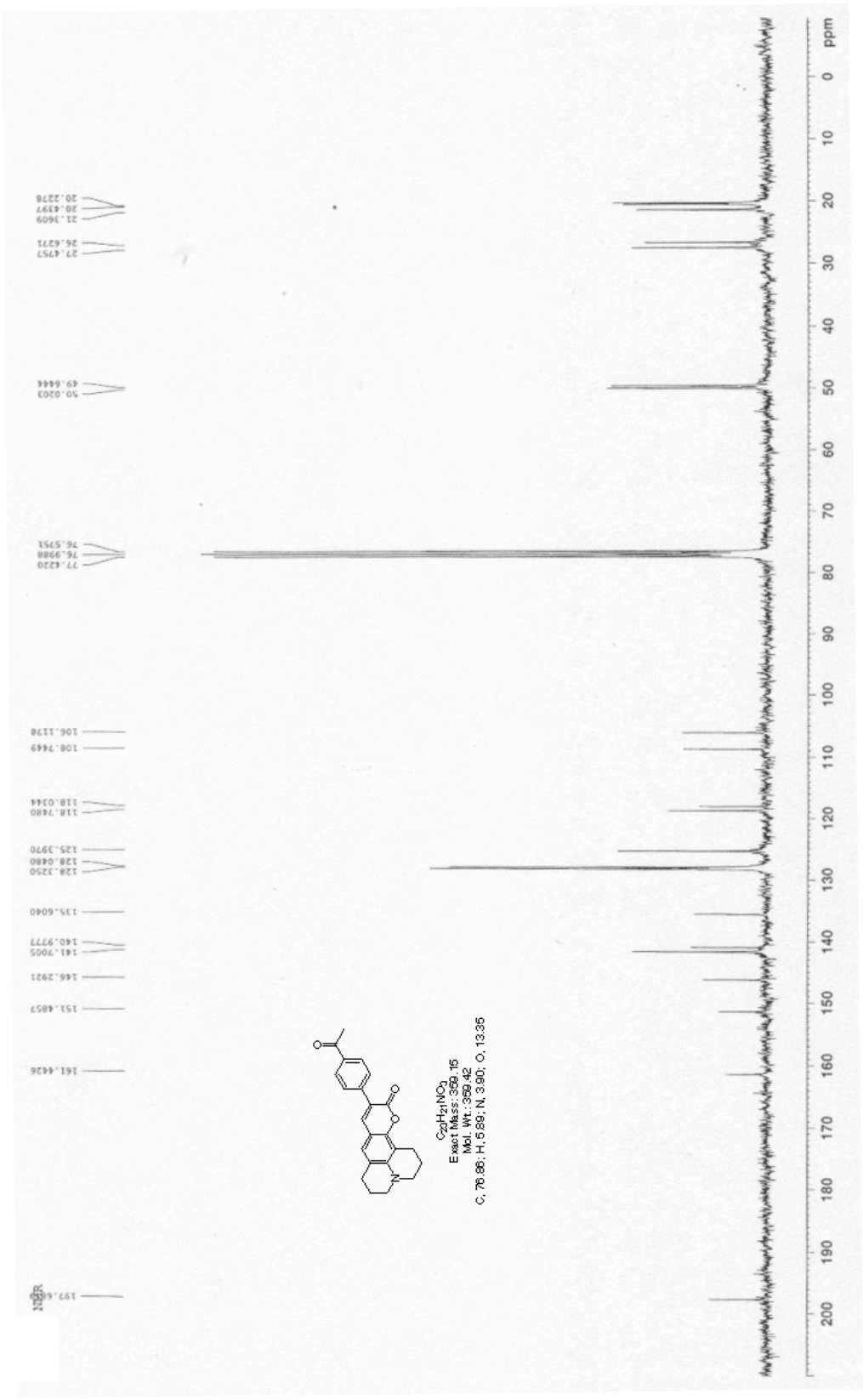

C17H17NO3
Exact Mass: 283.12
Mol. Wt.: 283.32
C, 72.07; H, 6.05; N, 4.94; O, 16.94
ppm


20.268
20.369
21.274
27.582
29.718
49.878
49.399
76.575
76.999
77.423
103.705
106.772
106.829
118.742
123.222
146.244
150.758
152.052
162.083
200.389
C17H17NO3
Exact Mass: 283.12
Mol. Wt.: 283.32
C, 72.07; H, 6.05; N, 4.94; O, 16.94
ppm

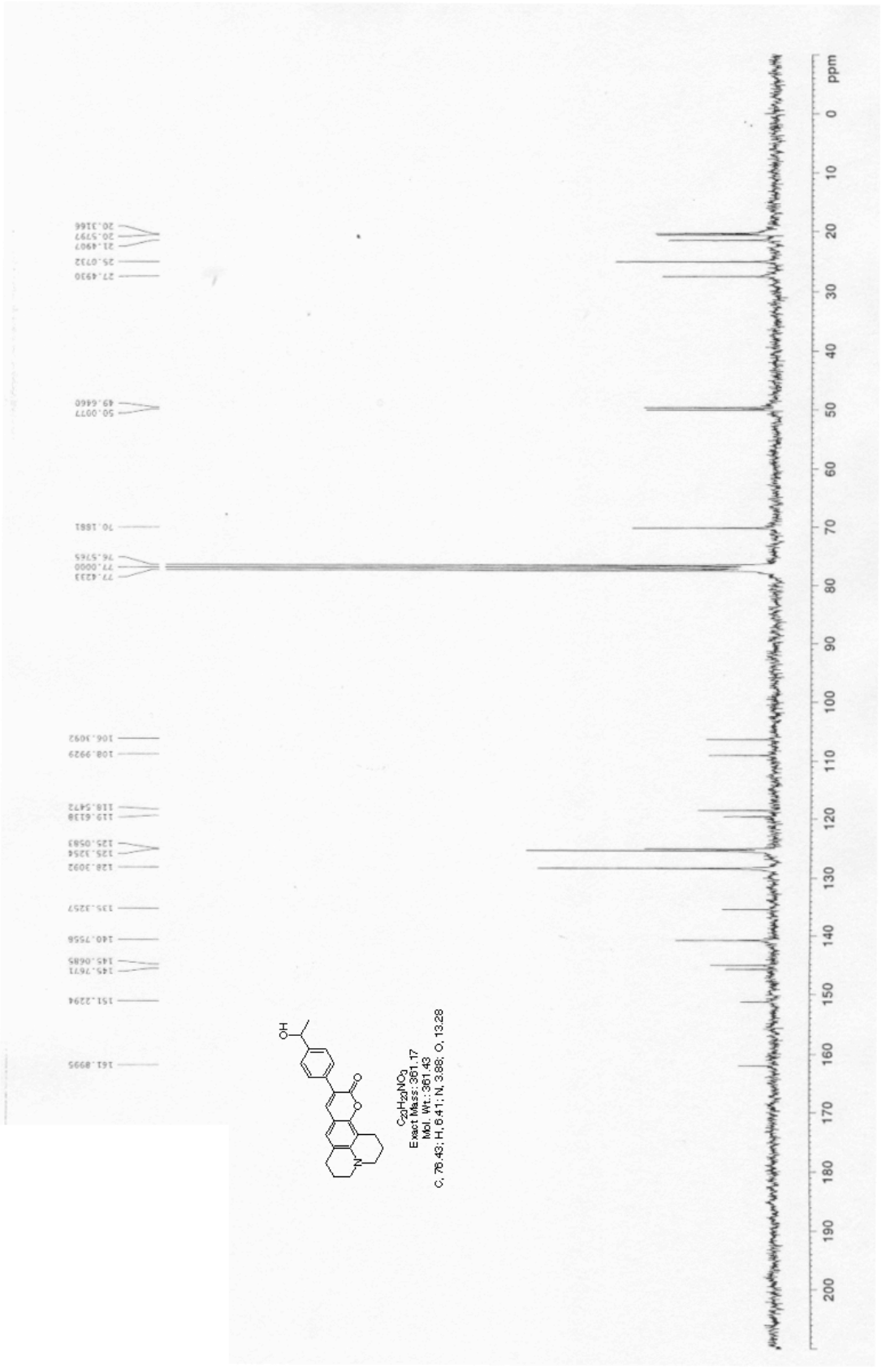

C17H19NO3
Exact Mass: 285.14
Mol. Wt.: 285.34
C, 71.56; H, 6.71; N, 4.91; O, 16.82
ppm

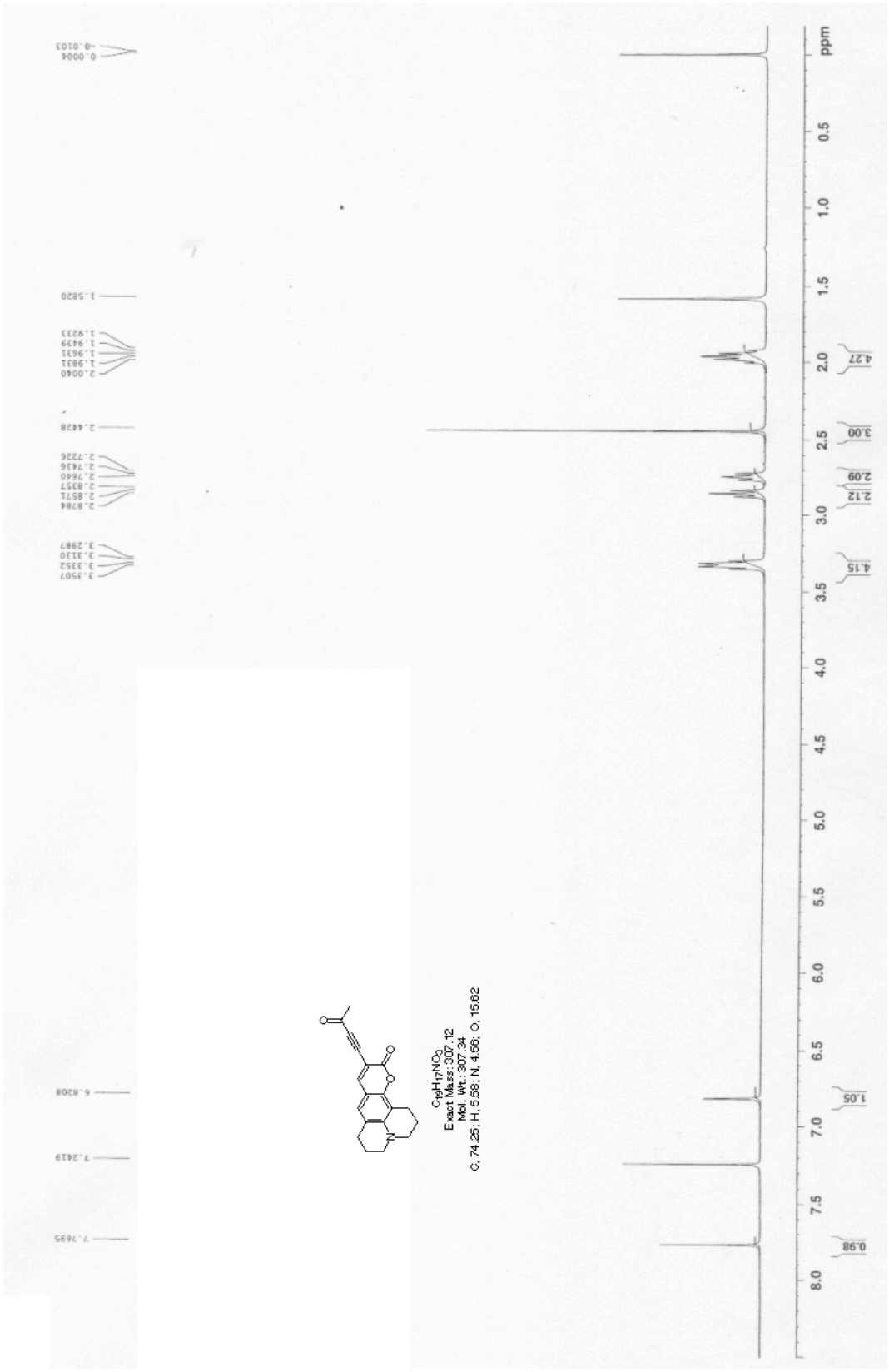

C17H19NO3
Exact Mass: 285.14
Mol. Wt.: 285.34
C, 71.56; H, 6.71; N, 4.91; O, 16.82
HO
O
O
N
ppm
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200

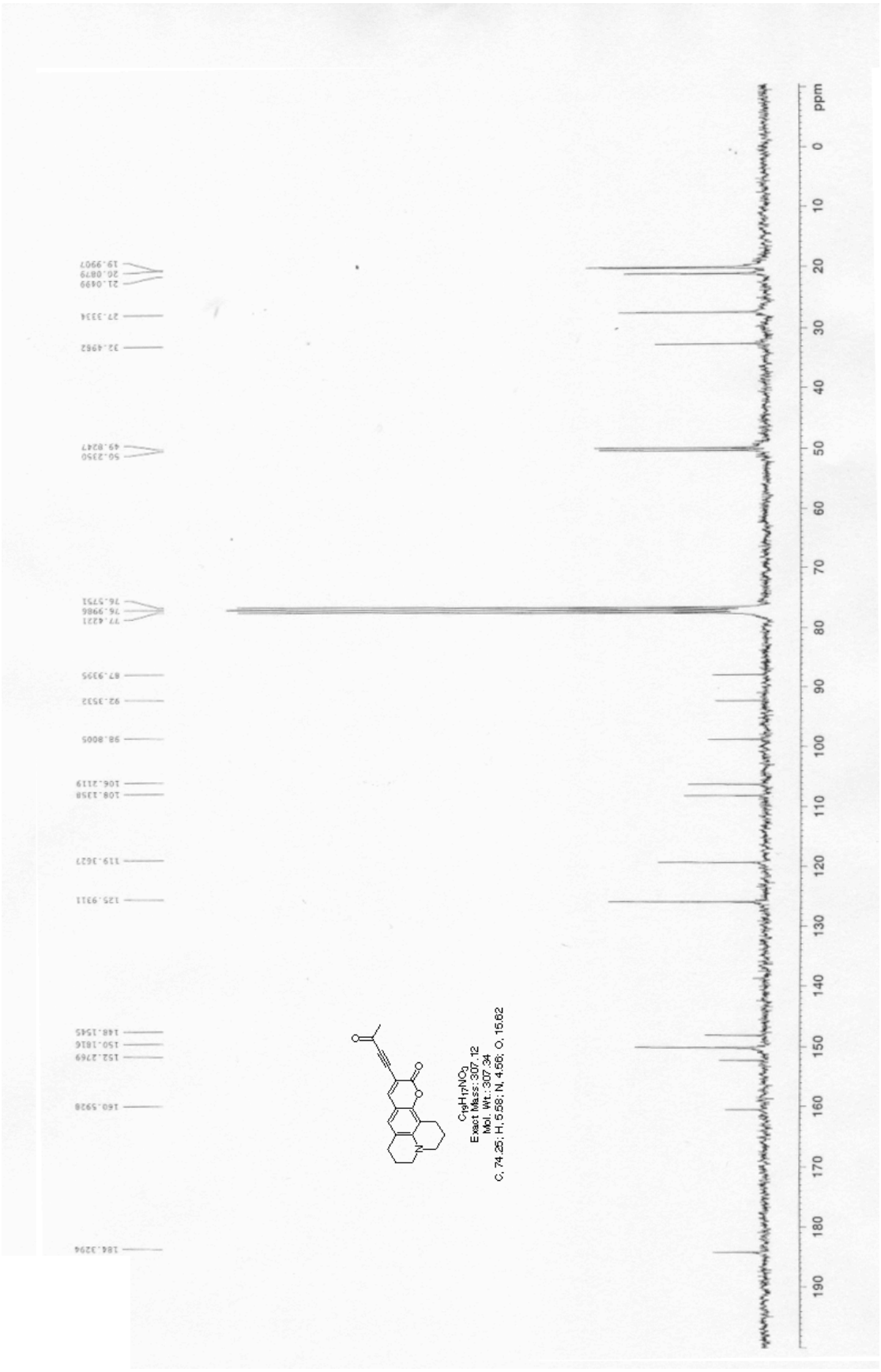

C22H21NO3
Exact Mass: 359.15
Mol. Wt.: 359.42
C, 76.86; H, 5.89; N, 3.90; O, 13.35
ppm

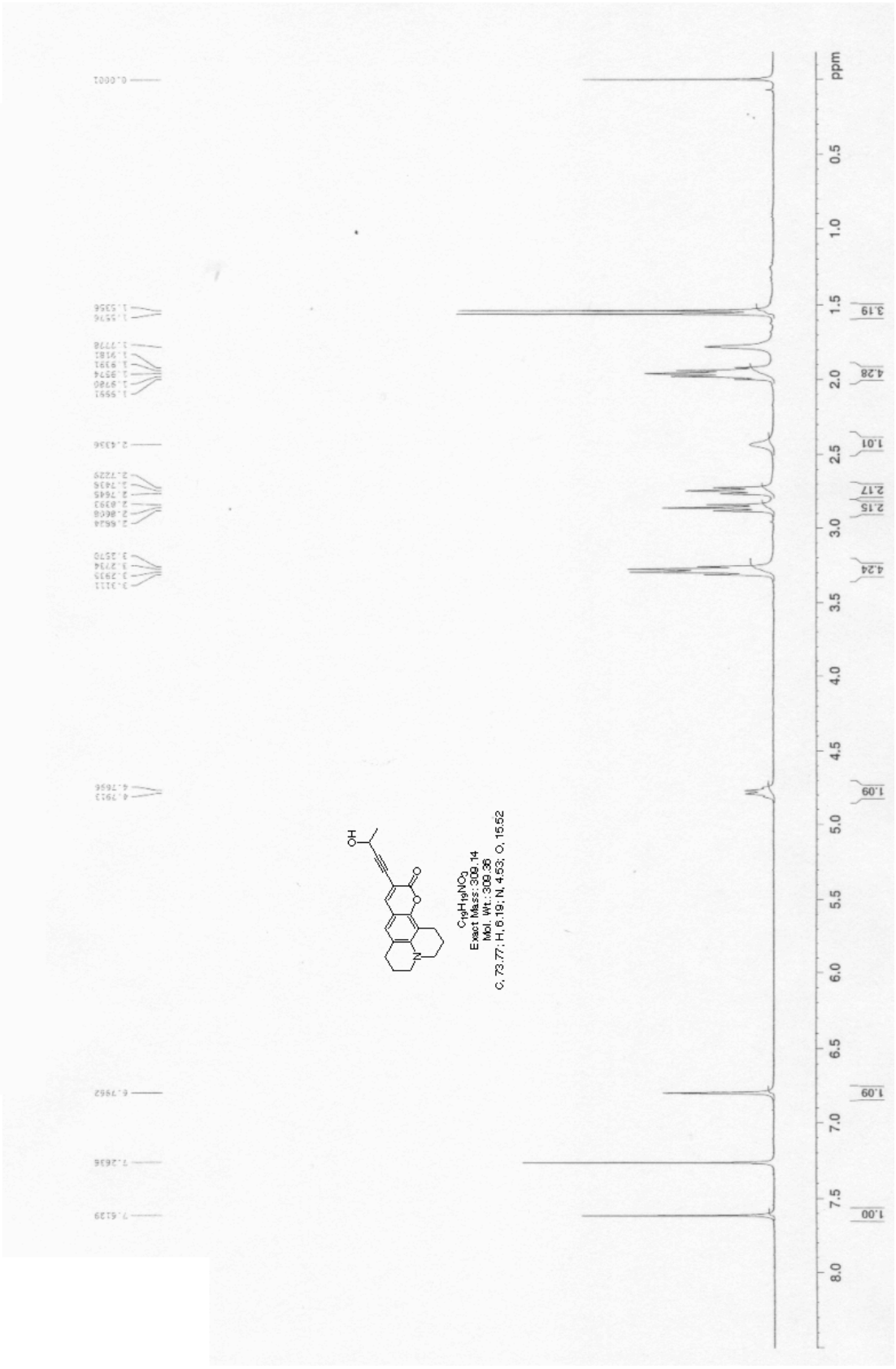

C22H21NO3
Exact Mass: 359.15
Mol. Wt.: 359.42
C, 76.86; H, 5.89; N, 3.90; O, 13.35
ppm

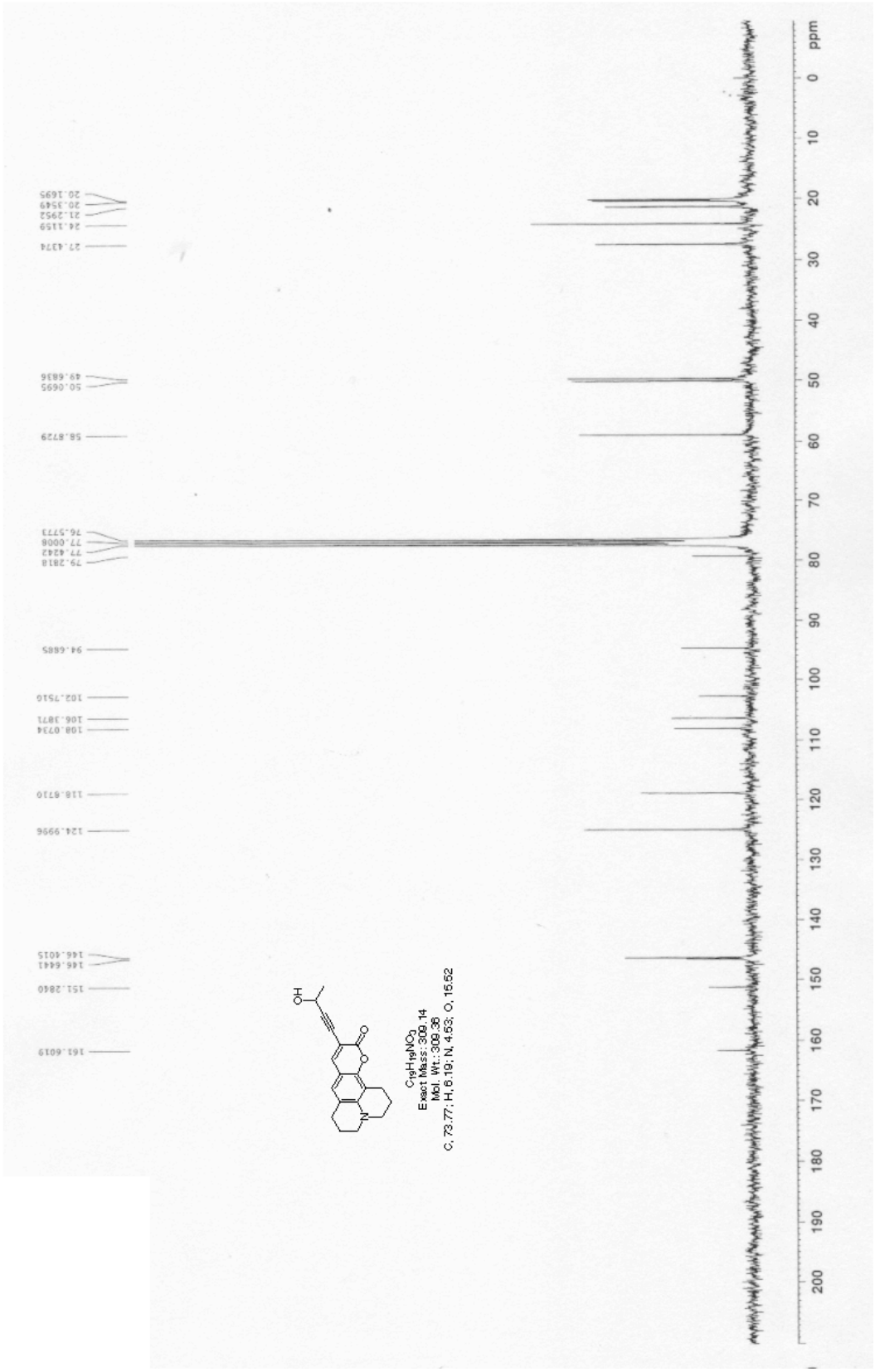

C23H23NO3
Exact Mass: 361.17
Mol. Wt.: 361.43
C, 76.43; H, 6.41; N, 3.88; O, 13.28
ppm


C22H23NO3
Exact Mass: 361.17
Mol. Wt.: 361.43
C, 76.43; H, 6.41; N, 3.88; O, 13.28
OH
O
O
N
ppm
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
20.3166
20.5797
21.4907
25.0732
27.4930
49.6460
50.0077
70.1881
76.5765
77.0000
77.4233
106.3092
108.9929
118.5472
119.6138
125.0583
125.3254
128.3092
135.3257
140.7558
145.0685
145.7671
151.2294
161.8995


C19H17NO3
Exact Mass: 307.12
Mol. Wt.: 307.34
C, 74.25; H, 5.58; N, 4.56; O, 15.62
ppm


C19H17NO3
Exact Mass: 307.12
Mol. Wt.: 307.34
C, 74.25; H, 5.58; N, 4.56; O, 15.62
ppm


OH
O
O
N
C19H19NO3
Exact Mass: 309.14
Mol. Wt.: 309.36
C, 73.77; H, 6.19; N, 4.53; O, 15.52
ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
3.19
4.28
1.01
2.17
2.15
4.24
1.09
1.09
1.00


20.1695
20.3549
21.2952
24.1159
27.4374
49.6836
50.0695
58.8729
76.5773
77.0008
77.4242
79.2818
94.6885
102.7516
106.3871
108.0734
118.8710
124.9956
146.4641
146.6015
151.2860
161.6019
OH
O
O
N
C19H19NO3
Exact Mass: 309.14
Mol. Wt.: 309.36
C, 73.77; H, 6.19; N, 4.53; O, 15.52
ppm
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200

References

1. Heinrich, G.; Schoof, S.; Gusten, H. *J. Photochem.* **1974/75**, *3*, 312-320.

2. Reynolds, G. A.; Drexhage, K. H. *Opt. Commun.* **1975**, *13*, 222.

3. Jacobson, A.; Petric, A.; Hogenkamp, D.; Sinur, A.; Barrio, J. R. *J. Am. Chem. Soc.* **1996**, *118*, 5572-5579.

4. Hundertmark, T.; Littke, A. F.; Buchwald, S. L.; Fu, G. C. *Org. Lett.* **2000**, *2*, 1729-1731.

5. Balo, C.; Fernandez, F.; Garcia-Mera, X.; Lopez, C. *Org. Prep. Proced. Int.* **2000**, *32*, 367-372.

6. Coleman, R. S.; Madaras, M. L. *J. Org. Chem.* **1998**, *63*, 5700-5703.

7. Wierzchowski, J.; Dafeldecker, W. P.; Holmquist, B.; Vallee, B. L. *Anal. Biochem.* **1989**, *178*, 57-62

8. http://www.erithacus.com/grafit

9. Wilkinson, G. N. *Biochem. J.* **1961**, *80*, 324-332

10. Yee, D. J.; Balsanek, V.; Sames, D. *unpublished results*.