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Propargylamine-Isothiocyanate Reaction: Efficient Conjugation Chemistry in Aqueous Media

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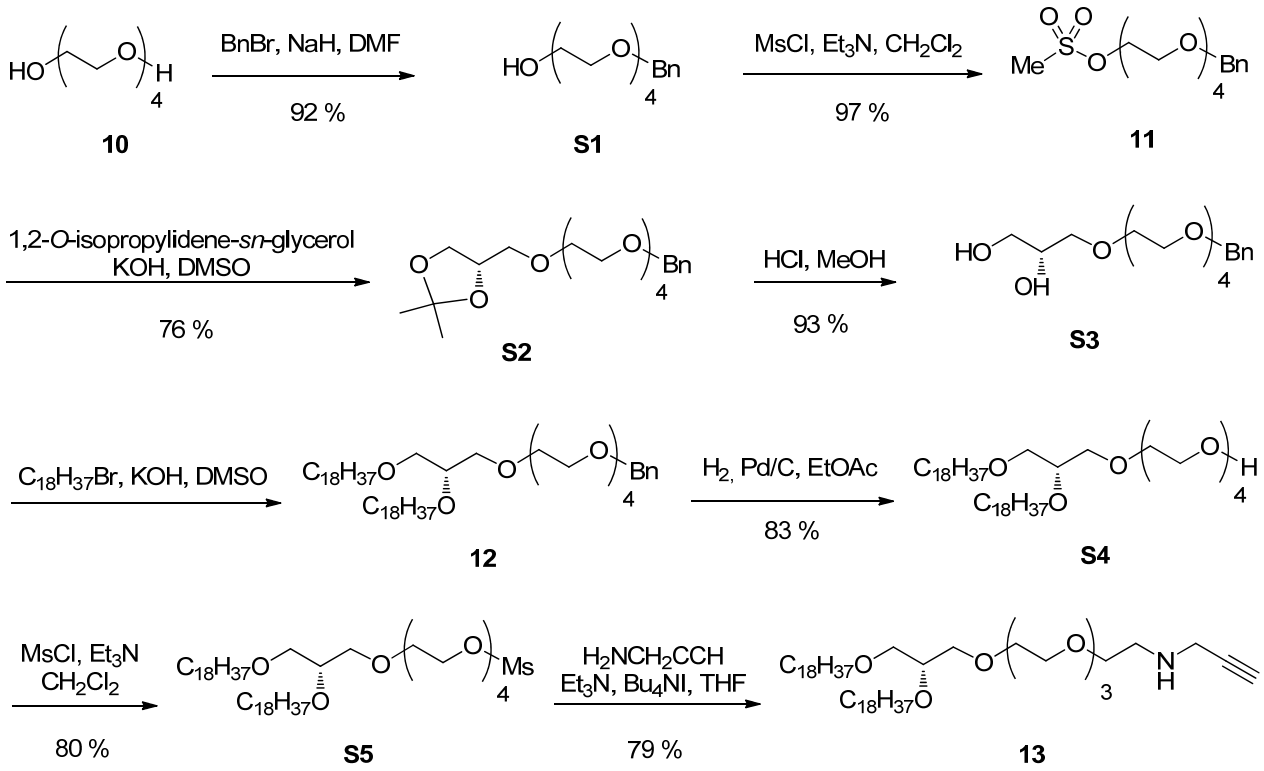
SUPPORTING INFORMATION



Scheme S1. Detailed reaction sequence **10**→**13**.

EXPERIMENTAL PROCEDURES

Chemical Synthesis. *General.* Starting materials, reagents, and solvents were purchased from Sigma-Aldrich Chemical Co. and used without further purification. CH_2Cl_2 was dried over 4 Å molecular sieves and THF was dried over sodium/benzophenone and distilled before use. Evaporation of solvents was done under reduced pressure (*in vacuo*). Thin layer chromatography (TLC) was performed on Merck aluminum sheets precoated with silica gel 60 F_{254}. Compounds were visualized by UV irradiation at 254 nm and/or by charring after dipping in a solution of 6.25 g of $(NH_4)_6Mo_7O_{24}$ and 1.5 g of $Ce(SO4)_2$ in 250 mL of 10% aqueous H_2SO_4, in an ethanolic solution of phosphomolybdenic acid (48 g/L) or in a solution of 10 mL of p-anisaldehyde, 10 mL of a concentrated aqueous solution of H_2SO_4 and 250 mL EtOH. Column chromatography was performed using Matrex 60 Å silica gel. The purity of the tested compounds was found to be >95% by HPLC. Normal-phase HPLC was performed on a Waters Alliance HPLC equipped with a diode array detector, using a LiChrospher Si 60 column and eluting with water/isopropanol/hexane mixtures or for chiral analysis a Chiralcel AS-H or OD-H column and eluting with hexane/2-propanol mixtures. RP-HPLC was obtained using a Shimadzu LC-2010C analytical HPLC by employing a XTerra RP8 5µm (4.6*150mm) column and eluting with water/acetonitrile mixtures containing 0.1% TFA. Preparative HPLC purification was performed on a C18 Phenomenex Luna column (5 µm, 100 Å, 250 mm × 20 mm) using an Agilent 1260 LC system equipped with a diode array UV detector and an evaporative light scattering detector (ELSD). A gradient with eluent III (water−MeCN−TFA, 95:5:0.1) and eluent IV (0.1% TFA in acetonitrile) rising linearly from 0% to 95% of IV during t = 5−45 min was applied at a flow rate of 20 mL/min (gradient C).
Analytical UPLC/MS (ESI) analysis was performed on a Waters AQUITY RP-UPLC system equipped with a diode array detector using an AQUITY UPLC BEH C-18 column (d 1.7 µm, 2.1 x 50 mm; column temp: 65 °C; flow: 0.6 mL/min). Eluents A (0.1% HCO_2H in water) and B (0.1% HCO_2H in acetonitrile) were used in a linear gradient (5% B to 100% B) in a total run time of 5.2 min. The LC system was coupled to a SQD mass spectrometer. The LC system was coupled to a Micromass LCT orthogonal time-of-flight mass spectrometer equipped with a Lock Mass probe operating in positive electrospray mode. NMR spectra were recorded using a Bruker AC 200 MHz spectrometer, a Varian Mercury 300 MHz spectrometer, a Bruker Ascend 400 MHz spectrometer, a Varian Unity Inova 500 MHz spectrometer or a Bruker Ascend 500 MHz spectrometer. Chemical shifts were measured in ppm and coupling constants in Hz, and the field is indicated in each case. IR analysis was carried out on a Bruker Alpha FT-IR spectrometer and optical rotations were measured with a Perkin-Elmer 341 polarimeter, units for $[\propto]^{20}_{D(589)}$ are 10^{-1} deg cm^{-2} g^{-1} HRMS was recorded on an Ionspec Ultima Fourier transform mass spectrometer. Melting points were measured by a Buch & Holm melting point apparatus and given in degrees Celsius (°C) uncorrected.

2-(Fluorescein-5-yl)imino-5-methylene-thiazolidine 4

FITC (0.200 g, 0.514 mmol, 1 equiv.) and propargyl amine (0.056 g, 1.03 mmol, 2 equiv.) were dissolved in 5 mL of *t*-BuOH:H_2O (2:3) and the reaction mixture was stirred for 6 hours at 22 °C. The reaction mixture was diluted with acetic acid/acetate buffer (15 mL, pH = 4.7) and extracted with EtOAc (6×15 mL). The combined organic phases were dried over anhydrous Na_2SO_4, concentrated *in vacuo* and purified by column chromatography (heptane:EtOAc 2:3 + 1% AcOH)

resulting in 0.18 g (81%) of **4** as a yellow solid. R_f = 0.20 (heptane:EtOAc 2:3 + 1% AcOH). Mp.: 160–165 °C (decomp.). IR (KBr): ν 3066, 2924, 1738, 1597, 1502, 1462, 1386, 1314, 1260, 1207, 1178, 1111, 914, 850 cm^{-1}. ^1H NMR (300 MHz, CD_3OD): δ 8.19 (1H, bs), 7.66 (1H, dd, *J* = 1.8 Hz, 8.1 Hz), 7.07 (1H, dd, *J* = 0.5 Hz, 8.1 Hz), 6.66 (2H, d, *J* = 2.4 Hz), 6.64 (2H, d, *J* = 8.7), 6.54 (2H, dd, *J* = 2.4 Hz, 8.7 Hz) 5.27–5.32 (1H, m), 5.18–5.23 (1H, m), 4.79 (2H, bs). ^{13}C NMR (75 MHz, DMSO-*d6*): δ 168.8, 159.4 (2C), 152.9, 151.9 (2C), 147.0, 145.3, 142.9, 129.1 (2C), 127.1, 125.3, 124.3, 112.5 (2C), 112.1, 109.8 (2C), 103.1, 102.2 (2C), 83.3, 48.6. HRMS (ESI^+) $C_{24}H_{17}N_2O_5S$ [M+H] calcd. m/z 445.0858, found m/z 445.0867.

2-(Fluorescein-5-yl)imino-3-(2-hydroxyethyl)-5-methylene-thiazolidine 5

FITC (0.200 g, 0.514 mmol, 1 equiv.) and 2-prop-2-ynylamino-ethanol (0.102 g, 1.03 mmol, 2 equiv.) were dissolved in 8 mL of *t*-BuOH:H_2O (1:1), Et_3N was added (0.04 mL) and the reaction mixture was stirred for 2 hours at 23 °C. The reaction mixture was diluted with acetic acid/acetate buffer (20 mL, pH = 4.7) and extracted with EtOAc (4×40 mL). The combined organic phases were dried over anhydrous Na_2SO_4, concentrated *in vacuo* and purified by column chromatography (heptane:EtOAc 1:3 + 1% AcOH) resulting in 0.20 g (78%) of **5** as a yellow solid. R_f = 0.256 (heptane/EtOAc 1:3 + 1% AcOH). Mp.: 165–170 ◦C (decomp.). IR (KBr): ν 3153, 3067, 2962, 2925, 2874, 1738, 1588, 1460, 1385, 1309, 1248, 1207, 1177, 1110, 1071, 849 cm^{-1}. ^1H NMR (300 MHz, CD_3OD): δ 7.44 (1H, dd, *J* = 0.5 Hz, 1.9 Hz), 7.28 (1H, dd, *J* = 1.9 Hz, 8.2 Hz), 7.07 (2H, dd, *J* = 0.5 Hz, 8.2 Hz), 6.67 (2H, d, *J* = 2.3 Hz), 6.62 (2H, d, *J* = 8.7), 6.54 (2H, dd, *J* = 2.3 Hz, 8.7 Hz) 5.32 (1H, dd, *J* = 2.2 Hz, 3.9 Hz), 5.17 (1H, dd, *J* = 2.2 Hz, 3.9 Hz), 4.57 (2H, t, *J* = 2.2 Hz), 3.86 (2H, t, *J* = 5.3 Hz), 3.70 (2H, t, *J* = 5.4 Hz). ^1H NMR (300 MHz, DMSO-*d6*): δ 10.15 (2H, br. s), 7.29 (1H, d, *J* = 2.0 Hz), 7.22 (1H, dd, *J* = 2.0 Hz, 8.1 Hz), 7.12 (1H, dd, *J* = 8.1 Hz), 6.66 (2H, d, *J* = 1.5 Hz), 6.61–6.52 (4H, m), 5.36 (1H, d, *J* = 1.3 Hz), 5.25 (1H, d, *J* = 1.5 Hz), 4.91 (1H, br. s), 4.55 (2H, t, *J* = 1.5 Hz), 3.67 (2H, br. s), 3.59 (2H, t, *J* = 5.6 Hz). ^{13}C NMR (75 MHz, DMSO-*d6*): δ 168.6, 159.5 (2C), 156.4, 153.0, 151.9 (2C), 146.3, 136.6, 130.0, 129.0 (2C), 128.9, 127.3, 124.6, 115.9 (2C), 112.6 (2C), 109.9, 106.4 (2C), 102.2, 83.5, 58.3, 56.7, 48.3. HRMS (ESI^+) $C_{26}H_{21}N_2O_6S$ [M+H] calcd. m/z 489.1120, found m/z 489.1118.

***N*-(Fluorescein-5-yl)-*N*'-(2-hydroxyethyl)thiourea 6**

FITC (0.100 g, 0.257 mmol, 1 equiv.) and ethanolamine (0.0314 g, 0.514 mmol, 2 equiv) were dissolved in 5 mL H_2O. The reaction mixture was stirred for 2 hours at 23 °C. The reaction mixture was diluted with acetic acid/acetate buffer (20 mL, pH = 4.7) and extracted with EtOAc (4×40 mL). The combined organic phases were dried over anhydrous Na_2SO_4, concentrated *in vacuo* resulting in 0.108 g (93%) of **6** as a yellow solid. Mp.: 170–175 °C (decomp.). IR (KBr): ν (cm −1) 3331, 3155, 3064, 2996, 2950, 2853, 1735, 1597, 1539, 1490, 1459, 1394, 1370, 1308, 1271, 1236, 1212, 1171, 1117, 1075, 874, 852, 761, 680. ^1H NMR (300 MHz, CD_3OD): δ 8.18 (1H, d, *J* = 1.9 Hz),

7.77 (1H, dd, *J* = 1.9 Hz, 8.2 Hz), 7.15 (1H, d, *J* = 8.2 Hz), 6.67 (2H, d, *J* = 8.7 Hz), 6.67 (2H, d, *J* = 2.4), 6.54 (2H, dd, *J* = 2.4 Hz, 8.7 Hz) 3.76 (4H, br. s). ^{1}H NMR (300 MHz, DMSO-*d6*): δ 10.08 (3H, br.s), 8.31 (1H, br. s), 7.77 (1H, br. s), 7.74 (1H, d, *J* = 8.3 Hz), 7.17 (1H, d, *J* = 8.2 Hz), 6.66 (2H, d, *J* = 2.2), 6.61 (2H, d, *J* = 8.6 Hz), 6.65 (2H, dd, *J* = 2.2 Hz, 8.6 Hz), 4.89 (1H, br. s), 3.58 (4H, br. s). ^{13}C NMR (50 MHz, DMSO-*d6*): δ 180.5, 168.5, 159.6 (2C), 151.9 (2C), 146.8, 141.4, 129.1, 129.0 (2C), 126.6, 124.0, 116.4, 112.7 (2C), 109.8 (2C), 103.0 (2C), 102.2, 83.7, 59.1, 46.4. HRMS (ESI^{+}) $C_{23}H_{19}N_2O_6S$ [M+H] calcd. m/z 451.0964, found m/z 451.1001.

Competition experiment with propargyl amine 2 and ethanolamine in organic/aqueous solvent mixtures

2-Prop-2-ynylamino-ethanol (**2**) (19.1 mg, 0.193 mmol, 1.2 equiv.) and ethanolamine (11.8 mg, 0.193 mmol, 1.2 equiv.) were dissolved in 2 mL of *t*-BuOH:H_2O (1:1) and added to FITC (50 mg, 0.128 mmol, 1 equiv.). The reaction mixture was stirred for 2 hours at 23 °C. The reaction mixture was diluted with acetic acid/acetate buffer (5 mL, pH = 4.7) and extracted with EtOAc (4×10 mL). The combined organic phases were dried over anhydrous Na_2SO_4, concentrated *in vacuo* resulting in 48.9 mg (78%) of **5** and 4.63 mg (8%) of the thiourea **6** as determined by NMR analysis.

2-Prop-2-ynylamino-ethanol 7

Ethanolamine (1.148 g, 18.8 mmol, 5 equiv.) was dissolved in EtOH (8 mL) at 0 °C and 1-bromobut-2-yne (0.5 g, 3.76 mmol, 1 equiv.) was added slowly under stirring and the reaction mixture was left for 16 hours at 22 °C. The reaction mixture was concentrated *in vacuo* and purified by column chromatography (pentane:CH_2Cl_2:MeOH 4:1:1) resulting in 0.2984 g (71%) of **7** as a yellow oil. R_f = 0.40 (CH_2Cl_2:MeOH 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.72 (2H, t, *J* = 5.6 Hz), 3.42 (2H, quartet, *J* = 2.2 Hz), 2.85 (2H, t, *J* = 5.6 Hz), 1.83 (3H, t, *J* = 2.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 80.1, 75.8, 60.3, 49.8, 37.9, 3.4.

***N*-(Fluorescein-5-yl)-*N'*-(2-hydroxyethyl)-*N'*-but-2-yn-1-ylthiourea 8 and 5-ethylene-2-(fluorescein-5-yl)imino-3-(2-hydroxyethyl)-thiazolidine 9**

FITC (0.200 g, 0.514 mmol, 1 equiv.) and 2-but-2-ynylamino-ethanol (0.1142 g, 1.027 mmol, 2 equiv.) were dissolved in 8 mL of H_2O:*t*-BuOH (5:3). The reaction mixture was stirred for 3 hours at 22 °C. The reaction mixture was diluted with acetic acid/acetate buffer (20 mL, pH = 4.7) and extracted with EtOAc (4×40 mL). The combined organic phases were dried over anhydrous Na_2SO_4, concentrated *in vacuo* purified by column chromatography (heptane:EtOAc 1:3 + 1% AcOH) resulting in 0.134 g (52%) of **8** and 0.088 g (34%) of the thiourea **9**.

The experiment was repeated with a reaction time of 24 hours, which resulted in the isolation of **9** as the sole product in 82% yield.

8: Mp.: 160–165 ◦C (decomp.). IR (KBr): ν (cm^{-1}) 3118, 2931, 2816, 2182, 1750, 1576, 1505, 1455, 1334, 1252, 1210, 1181, 1111, 1024, 994, 861. ^{1}H NMR (300 MHz, CD_3OD): δ 7.62 (1H, d, *J* = 1.5 Hz), 7.41 (1H, dd, *J* = 2.0 Hz, 8.2 Hz), 7.18 (1H, dd, *J* = 0.5 Hz, 8.2 Hz), 6.71 (2H, d, *J* = 8.8 Hz), 6.69–6.66 (2H, m), 6.59–6.54 (2H, m), 4.16 (2H, t, *J* = 5.0 Hz), 4.01 (2H, t, *J* = 5.0 Hz), 3.98–3.84 (2H, m), 1.42 (3H, dd, *J* = 6.4 Hz, 10.6 Hz). ^{13}C NMR (75 MHz, DMSO-*d6*): δ 187.9, 168.6, 157.9 (2C), 152.1 (2C), 151.5, 140.5, 129.6, 129.1 (2C), 125.6, 119.9, 115.3 (2C), 112.9, 109.9 (2C), 102.2 (2C), 72.2, 62.6, 58.3, 57.7, 49.6. MALDI-TOF MS $C_{27}H_{23}N_2O_6S$ [M+H] calcd. m/z 503.13, found m/z 503.15.

9: Mp.: 165–170 ◦C (decomp.). IR (KBr): ν (cm^{-1}) 3117, 2952, 2816, 1753, 1606, 1505, 1454, 1249, 1182, 1112, 994, 852 - cf. appendix N. ^{1}H NMR (300 MHz, CD_3OD): δ 7.46 (1H, dd, *J* = 0.5 Hz, 2.0 Hz), 7.31 (1H, dd, *J* = 2.0 Hz, 8.2 Hz), 7.10 (1H, dd, *J* = 0.5 Hz, 8.2 Hz), 6.67 (2H, d, *J* = 2.3 Hz), 6.65 (2H, d, *J* = 8.7 Hz), 6.56 (2H, dd, *J* = 2.3 Hz, 8.7), 5.68 (1H, tq, *J* = 2.1 Hz, 6.8 Hz), 4.51 (2H, t, *J* = 2.1 Hz), 3.95 (2H, t, *J* = 5.4 Hz), 3.69 (2H, t, *J* = 5.4 Hz), 1.67 (3H, td, *J* = 2.1 Hz, 6.8 Hz). ^{13}C NMR (75 MHz, CD_3OD): δ 171.5, 161.4 (2C), 159.7, 154.8, 154.2 (2C), 148.3, 131.7, 130.3 (2C), 129.7, 129.3, 125.9, 118.3, 116.9, 113.7 (2C), 111.8 (2C), 103.6 (2C), 88.0, 60.6, 58.1, 49.7, 16.3. MALDI-TOF MS $C_{27}H_{23}N_2O_6S$ [M+H] calcd. m/z 503.13, found m/z 503.16.

13-Phenyl-3,6,9,12-tetraoxatridecan-1-ol S1

To a solution of tetraethyleneglycol (10.30 mmol, 2 g) in anhydrous DMF (10 mL) cooled to 0 °C under Ar, was added NaH (60% in mineral oil 10.29 mmol, 412 mg) in four portions over 10 min and the resulting suspension was stirred at 0 °C for 10 min, then at 20 °C for additional 10 min. BnBr (2.06 mmol, 245 µL) was then added dropwise and the reaction mixture was stirred at 20 °C for 20 h. The reaction mixture was poured into ice water (50 mL) and extracted with Et_2O (3×50 mL). The combined organic phases were dried ($MgSO_4$), concentrated *in vacuo* and purified by flash chromatography (heptane /EtOAc 1:1 to 0:1) to afford **S1** as a colorless oil in 92% yield (540 mg), and unreacted tetraethyleneglycol was recovered (7.10 mmol, 1.380 g). R_f = 0.2 (EtOAc/heptane 2:1). ^{1}H NMR (300 MHz; $CDCl_3$): δ 7.35-7.27 (m, 5H, $C_6\underline{H_5}$), 4.57 (s, 2H, $C\underline{H_2}$-Ph), 3.70-3.58 (m, 16H, O-$C\underline{H_2}$-$C\underline{H_2}$-O), 2.54 (t, *J* = 5.5 Hz, 1H, O$\underline{H}$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.2 ($\underline{C_6}H_5$), 128.3 (2C, $\underline{C_6}H_5$), 127.7 (2C, $\underline{C_6}H_5$), 127.5 ($\underline{C_6}H_5$), 73.2 ($\underline{C}H_2$-Ph), 72.5 (O-$\underline{C}H_2$-$\underline{C}H_2$-O-), 70.6 (4C, O-$\underline{C}H_2$-$\underline{C}H_2$-O-), 70.3 (O-$\underline{C}H_2$-$\underline{C}H_2$-O-), 69.4 (O-$\underline{C}H_2$-$\underline{C}H_2$-O-), 61.7 ($\underline{C}H_2$-OH).

13-Phenyl-3,6,9,12-tetraoxatridecanyl methanesulfonate 11

To a solution of **S1** (7.52 mmol, 2.14 g) in CH_2Cl_2 (15 mL) was added Et_3N (13.53 mmol, 1.9 mL). The reaction mixture was cooled to -30 °C and MsCl (12.03 mmol, 930 µL) was added dropwise.

The mixture was stirred for 10 min at that temperature then allowed to reach 20 °C slowly over 1 h and stirred for an additional 14 h. Sat. aq. NH_4Cl (20 mL) was added, the organic phase was separated, and the aqueous phase was washed with CH_2Cl_2 (3×20 mL). The combined organic phases were washed with H_2O (20 mL), sat. aq. $NaHCO_3$ (20 mL), and brine (20 mL) and dried over $MgSO_4$ to afford 2.64 g of **11**, which was used without further purification. R_f = 0.42 (EtOAc/heptane 9:1). ^{1}H NMR (300 MHZ; $CDCl_3$): δ 7.34-7.26 (m, 5H, $C_6\underline{H_5}$), 4.56 (s, 2H, $C\underline{H_2}$-C_6H_5), 4.37-4.34 (m, 2H, $C\underline{H_2}$-OMs), 3.76-3.73 (m, 2H, $C\underline{H_2}$-CH_2-OMs), 3.65-3.64 (m, 12H, (O-$C\underline{H_2}$-$C\underline{H_2}$)$_3$-OBn), 3.05 (s, 3H, SO_2-$C\underline{H_3}$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.2, 128.3 (2C), 127.7 (2C), 127.5, 73.2, 70.6 (3C), 70.5 (2C), 69.4, 69.3, 69.0, 37.7; HRMS (ESI^+) $C_{16}H_{26}NaO_7S$, [M+Na^+] calcd. m/z 385.1297, found m/z 385.1294.

1,2-*O*-Isopropylidene-3-*O*-(13-phenyl-3,6,9,12-tetraoxatridecyl)-*sn*-glycerol S2

To a flame dried round bottomed flask was added powdered KOH (17.95 mmol, 1 g) and anhydrous DMSO (20 mL) under Ar, and the resulting suspension was stirred at 20 °C for 1 h. A solution of (*S*)-(+)-1,2-isopropylideneglycerol (10.77 mmol, 1.42 g), **11** (7.18 mmol, 2.6 g) in anhydrous DMSO (20 mL) was added, and the reaction mixture was stirred at 40 °C for 20 h. Aq. NH_4Cl (50% w/w, 40 mL) was added, the aqueous phase was extracted with EtOAc (3×50 mL), the combined organic phases were washed with H_2O (100 mL), dried over $MgSO_4$, concentrated *in vacuo* and purified by flash chromatography (heptane/EtOAc 1:1) to afford 2.1 g of **S2** as a colorless oil (76%). R_f = 0.18 (heptane/EtOAc 1:1). $[\propto]_D^{20}$= +3.51 ° (c = 1.51, $CHCl_3$). ^{1}H NMR (300 MHz; $CDCl_3$): δ 7.34-7.24 (m, 5H, C_6H_5), 4.56 (s, 2H, $C\underline{H_2}$-C_6H_5), 4.32-4.22 (m, 1H, CH_2-$C\underline{H}$-CH_2), 4.04 (dd, *J* = 8.3, 6.4 Hz, 1H, $C\underline{H_a}H_b$-CH-CH_2-OTEG), 3.72 (dd, *J* = 8.3, 6.4 Hz, 1H, $CH_a\underline{H_b}$-CH-CH_2-OTEG), 3.69-3.60 (m, 16H, (O-$\underline{CH_2}$-$\underline{CH_2}$)$_4$), 3.57 (dd, *J* = 10.0, 5.7 Hz, 1H, CH_2-CH-$C\underline{H_a}H_b$-OTEG), 3.48 (dd, *J* = 10.0, 5.5 Hz, 1H, CH_2-CH-$CH_a\underline{H_b}$-OTEG), 1.41 (s, 3H, $C\underline{H_3}$), 1.35 (s, 3H, $C\underline{H_3}$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.5 (aromatic), 128.6 (2C, aromatic), 128.0 (2C, aromatic), 127.8 (aromatic), 109.6 (CH_3-$\underline{C}$-CH_3), 74.9 (CH_2-$\underline{C}$H-CH_2), 73.5 ($\underline{C}H_2$-C_6H_6), 72.6 ((O-CH_2-CH_2)$_4$), 71.2 ((O-CH_2-CH_2)$_4$), 70.9 (2C, (O-CH_2-CH_2)$_4$), 70.8 (3C, (O-CH_2-CH_2)$_4$), 70.7 ((O-CH_2-CH_2)4), 69.6 ($\underline{C}H_2$-CH-CH_2-OTEG), 67.0 (CH_2-CH-$\underline{C}H_2$-OTEG), 27.0 (CH_3), 25.6 (CH_3); IR (neat): 2985, 2866, 1454, 1370, 1252, 1212, 1095, 1049, 843, 738, 698 cm^{-1}. HRMS (ESI^+) $C_{21}H_{34}NaO_7$ [M+Na^+] calcd. m/z 421.2202, found m/z 421.2193.

3-*O*-(13-Phenyl-3,6,9,12-tetraoxatridecyl)-*sn*-glycerol S3

To a solution of **S2** (11.4 mmol, 4.5 g) in MeOH (100 mL), was added HCl (1M in MeOH, 100 mL), and the reaction mixture was stirred at 20 °C for 18 h, after what the solvent was removed *in vacuo*, and **S3** was isolated by flash chromatography (EtOAc/MeOH 90:10 to 75:25) as a clear oil in 93% yield (3.8 g). R_f = 0.2 (EtOAc). $[\propto]_D^{20}$= -1.27 ° (c =2.68, $CHCl_3$). ^{1}H NMR (300 MHz; CD_3OD): δ 7.33-7.21 (m, 5H, $C_6\underline{H_5}$), 4.52 (s, 2H, $C\underline{H_2}$-C_6H_5), 3.75-3.40 (m, 21H, $C\underline{H_2}$-$C\underline{H}$-$C\underline{H_2}$- and O-$C\underline{H_2}$-$C\underline{H_2}$-O), 3.28 (s (br), 2H, OH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.2 ($\underline{C_6}H_5$), 128.3

($\underline{C}_6H_5$, 2C), 127.7 ($\underline{C}_6H_5$, 2C), 127.6 ($\underline{C}_6H_5$), 73.2 ($\underline{C}H_2$-Ph), 72.9 (TEG + glycerol), 70.7 (TEG + glycerol), 70.6 (TEG + glycerol, 2C), 70.5 (TEG + glycerol, 4C), 70.4 (TEG + glycerol), 69.3 (TEG + glycerol), 63.9 ($\underline{C}H_2$-OH). IR (neat): 3419 (br.), 2867, 1453, 1350, 1296, 1249, 1090, 1040, 924, 753 cm^{-1}. HRMS (ESI^+) $C_{18}H_{31}O_7$ [$M+H^+$] calcd. m/z 359.2070, found m/z 359.2076.

1,2-Di-*O*-octadecyl-3-*O*-(13-phenyl -3,6,9,12-tetraoxatridecyl)-*sn*-glycerol 12

In a flame-dried 25 mL round bottom flask, were added **S3** (0.84 mmol, 300 mg) and DMSO (5 mL), followed by KOH (4.19 mmol, 235 mg), and the resulting mixture was stirred at 20 °C for 3 h in order to solubilise KOH fully. When all a homogeneous solution was obtained, 1-bromooctadecane was added slowly and the reaction mixture was stirred for 22 h. NH_4Cl (10 mL) was then added and the aqueous phase was washed with EtOAc (3×20 mL), the combined organic phases were dried ($MgSO_4$), concentrated *in vacuo*, and **12** was obtained after purification by flash chromatography (heptane/EtOAc 1:0 to 1:3) in 68% yield (490 mg) as a white solid. Mp.: 36.0-36.5 °C. R_f = 0.69 (EtOAc). $[\propto]_D^{20}$= +0.16 ° (c = 1.26, $CHCl_3$). ^{1}H NMR (300 MHz; $CDCl_3$): δ 7.34-7.23 (m, 5H, $C_6\underline{H}_5$), 4.56 (s, 2H, $C\underline{H}_2$-C_6H_5), 3.69-3.36 (m, 21H, $C_{18}H_{37}$-O-$C\underline{H}_2$-$C\underline{H}$(O-$C_{18}H_{37}$)-$C\underline{H}_2$-O-($C\underline{H}_2$-$C\underline{H}_2$-O)$_4$-Bn), 1.57-1.50 (m, 4H, $C_{17}H_{35}$-$C\underline{H}_2$-O), 1.32-1.19 (m, 64H, CH_3-$C_{16}\underline{H}_{32}$-CH_2-O), 0.88 (t, *J* = 6.7 Hz, 6H, $C\underline{H}_3$-$C_{17}H_{34}$-O). ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.2 (C_5H_5-$\underline{C}$-CH_2), 128.3 ($\underline{C}_6H_6$, 2C), 127.7 ($\underline{C}_6H_6$, 2C), 127.6 ($\underline{C}_6H_6$), 77.8 (CH_2-$\underline{C}H$-CH_2), 73.2 (C_6H_6-$\underline{C}H_2$), 71.6 ($C_{18}H_{37}$-O-$\underline{C}H_2$-CH-CH_2), 71.4 (C_{17}-H_{35}-$\underline{C}H_2$-O- and TEG), 70.8 (C_{17}-H_{35}-$\underline{C}H_2$-O- and TEG, 2C), 70.6 (C_{17}-H_{35}-$\underline{C}H_2$-O- and TEG, 5C), 70.5 (C_{17}-H_{35}-$\underline{C}H_2$-O- and TEG, 2C), 69.4 (CH_2-CH-$\underline{C}H_2$-OTEG), 31.9 (CH_3-CH_2-$\underline{C}H_2$-CH_2, 2C), 30.1 (CH_3-CH_2-CH_2-CH_2-$\underline{C}_{14}H_{28}$, 2C), 29.7 (CH_3-CH_2-CH_2-CH_2-$\underline{C}_{14}H_{28}$, 20C), 29.5 (CH_3-CH_2-CH_2-CH_2-$\underline{C}_{14}H_{28}$, 2C), 29.4 (CH_3-CH_2-CH_2-CH_2-$\underline{C}_{14}H_{28}$, 2C), 26.1 (CH_3-CH_2-CH_2-CH_2-$\underline{C}_{14}H_{28}$, 2C), 22.7 (CH_3-CH_2-$\underline{C}H_2$-CH_2-$C_{14}H_{28}$, 2C), 14.1 ($\underline{C}H_3$-CH_2-CH_2-CH_2-$C_{14}H_{28}$, 2C). IR (neat): 2916, 2849, 1466, 1099 cm^{-1}. HRMS (ESI^+) $C_{54}H_{102}NaO_7$ [$M+Na^+$] calcd. m/z 885.7523, found m/z 885.7545.

1,2-Di-*O*-octadecyl-3-*O*-(12-hydroxy-3,6,9-trioxadodecyl)-*sn*-glycerol S4

Compound **12** (4.64 mmol, 4 g) was dissolved in EtOAc (45 mL) under N_2 atmosphere, and w/w 10% Pd/C (0.23 mmol, 247 mg) was added. The atmosphere was then exchanged with H_2, and the reaction mixture was stirred at 20 °C under H_2. After 20 h, some starting material still remained, and the atmosphere was exchanged with N_2, w/w 10% Pd/C (0.23 mmol, 247 mg) was added, and the atmosphere was exchanged to H_2 again. After 24 h, TLC showed full conversion of **12**, the reaction mixture was filtered through celite, the residue was rinsed with EtOAc and the solvent was removed in vacuo. The crude solid was purified by flash chromatography (heptane/EtOAc 1:1) to afford **S4** as white crystals in 83% yield (2.97 g). Mp.: 46-47 °C. R_f = 0.16 (heptane/EtOAc). $[\propto]_D^{20}$= -0.7 ° (c =1.0 , $CHCl_3$). ^{1}H NMR (300 MHz; $CDCl_3$): δ 3.74-3.36 (m, 25H, glycerol + TEG+ $C_{17}H_{35}$-$C\underline{H}_2$-O), 2.0 (s, 1H, OH), 1.60-1.51 (m, 4H, CH_3-$C_{15}H_{30}$-$C\underline{H}_2$-CH_2-O), 1.36-1.24 (m, 60H, CH_3-$C_{15}\underline{H}_{30}$-CH_2-CH_2), 0.88 (t, *J* = 6.7 Hz, 6H, $C\underline{H}_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 77.8

(CH_2-C<u>H</u>-CH_2), 72.5 ($C_{18}H_{35}$-O-C<u>H</u>$_2$-CH-CH_2), 71.6 ($C_{17}H_{33}$-C<u>H</u>$_2$-O-CH_2-CH-CH_2), 71.4 (TEG), 70.8 (TEG), 70.7 (TEG), 70.6 (3C, TEG), 70.5 (2C, $C_{17}H_{33}$-C<u>H</u>$_2$-O-CH-CH_2 and TEG), 70.3 (C<u>H</u>$_2$-OTEG), 61.7 (C<u>H</u>$_2$-OH), 31.9 (2C, C<u>$_{15}$</u>H_{30}), 30.1 (C<u>$_{15}$</u>H_{30}), 29.7 (14C, C<u>$_{15}$</u>H_{30}), 29.6 (7C, C<u>$_{15}$</u>H_{30}), 29.5 (2C, C<u>$_{15}$</u>H_{30}), 29.3 (2C, C<u>$_{15}$</u>H_{30}), 26.1 (2C, C<u>$_{15}$</u>H_{30}), 22.7 (2C, C<u>H</u>$_2$-CH_3), 14.1 (2C, C<u>H</u>$_3$). IR (neat): 3461 (br.), 2916, 2849, 1467, 1109 cm^{-1}. HRMS (ESI^+) $C_{47}H_{96}NaO_7$ [M+Na^+] calcd. m/z 795.7054, found m/z 795.7055.

1,2-Di-*O*-octadecyl-3-*O*-(12-(methanesulfonyloxy)-3,6,9-trioxadodecyl)-*sn*-glycerol S5

To a solution of **S4** (1.29 mmol, 1 g) and Et_3N (2.33 mmol, 325 µL) in anhydrous CH_2Cl_2 (4 mL) cooled to 0 °C, was added MsCl (6.47 mmol, 500 µL), and the reaction mixture was allowed to reach 20 °C slowly. After stirring for 16 h, **S4** was not fully converted, and MsCl (6.47 mmol, 500 µL) was added at 0 °C, and the reaction mixture was stirred at 20 °C for additional 20 h. Sat. aq. NH_4Cl (20 mL) was added, the organic phase was separated, and the aqueous phase was washed with CH_2Cl_2 (3×20 mL), the combined organic phases were dried over $MgSO_4$, concentrated *in vacuo* and purified by flash chromatography (heptane/EtOAc 3:1 to 1:1) to afford S**5** as a white solid in 80% yield (883 mg). Mp.: 51.0-51.5 °C R_f = 0.26 (heptane/EtOAc 1:1). $[\propto]_D^{20}$= +0.6 ° (c =1.04, $CHCl_3$). ^{1}H NMR (300 MHz; $CDCl_3$): δ 4.40-4.37 (m, 2H, C<u>H</u>$_2$-OMs), 3.78-3.75 (m, 2H, C<u>H</u>$_2$-CH_2-OMs), 3.68-3.40 (m, 21H, $C_{17}H_{33}$-C<u>H</u>$_2$ + O-C<u>H</u>$_2$-C<u>H</u>$_2$- + C<u>H</u>$_2$-C<u>H</u>-C<u>H</u>$_2$), 3.08 (s, 3H, S-C<u>H</u>$_3$), 1.55 (tt, *J* = 6.5, 6.5 Hz, 4H, CH_3-$C_{15}H_{30}$-C<u>H</u>$_2$-CH_2-O), 1.35-1.23 (m, 60H, CH_3-C_{15}<u>H_{30}</u>-CH_2-CH_2), 0.87 (t, *J* = 6.7 Hz, 6H, C<u>H</u>$_3$-$C_{17}H_{34}$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 77.8 (CH_2-C<u>H</u>-CH_2), 71.6 (TEG + glycerol + $C_{17}H_{33}$-C<u>H</u>$_2$), 71.4 (TEG + glycerol + $C_{17}H_{33}$-C<u>H</u>$_2$), 70.8 (TEG + glycerol + $C_{17}H_{33}$-C<u>H</u>$_2$), 70.7 (TEG + glycerol + $C_{17}H_{33}$-C<u>H</u>$_2$), 70.6 (4C, TEG + glycerol + $C_{17}H_{33}$-C<u>H</u>$_2$), 70.5 (2C, TEG + glycerol + $C_{17}H_{33}$-C<u>H</u>$_2$), 69.2 (C<u>H</u>$_2$-CH_2-OMs), 69.0 (CH_2-C<u>H</u>$_2$-OMs), 37.7 (SO_2-C<u>H</u>$_3$), 31.9 (2C, C<u>$_{15}$</u>H_{30}), 30.1 (C<u>$_{15}$</u>H_{30}), 29.7 (14C, C<u>$_{15}$</u>H_{30}), 29.6 (7C, C<u>$_{15}$</u>H_{30}), 29.5 (2C, C<u>$_{15}$</u>H_{30}), 29.3 (2C, C<u>$_{15}$</u>H_{30}), 26.1 (2C, C<u>$_{15}$</u>H_{30}), 22.7 (2C, C<u>H</u>$_2$-CH_3), 14.1 (2C, C<u>H</u>$_3$). IR (neat): 2916, 2849, 1467, 1350, 1173, 1107 cm^{-1}. HRMS (ESI^+) $C_{48}H_{98}NaO_9S$ [M+Na^+] calcd. m/z 873.6829, found m/z 873.6834.

1,2-Di-*O*-octadecyl-3-*O*-(3,6,9-trioxa-12-azapentadec-14-ynyl)-*sn*-glycerol 13

To a solution of S**5** (0.47 mmol, 400 mg) in anhydrous THF (1 mL) under argon, was added Et_3N (1.175 mmol, 164 µL) followed by Bu_4NI (0.235 mmol, 87 mg) and propargyl amine (0.940 mmol, 80 mg). The reaction mixture was stirred at 20 °C for 18 h, then at 70 °C for 3.5 h, after what the crude mixture was concentrated *in vacuo* on silica and **13** was obtained as a yellow amorphous solid in 79% yield (300 mg) after purification by flash chromatography (EtOAc/heptane/Et_3N 66:33:1). R_f = 0.16 (EtOAc/heptane 3:1). $[\propto]_D^{20}$= -0.45 ° (c = 1.32, $CHCl_3$). ^{1}H NMR (300 MHZ; $CDCl_3$): δ 3.89 (t, *J* = 4.74 Hz, 2H, C<u>H</u>$_2$-CH_2-NH), 3.81 (d, *J* = 2.0 Hz, 2H, NH-C<u>H</u>$_2$-CΞCH), 3.71-3.40 (m, 21H, glycerol + TEG+ $C_{17}H_{35}$-C<u>H</u>$_2$-O), 3.19-3.22 (m, 2H, C<u>H</u>$_2$-NH-CH_2-CΞCH), 2.48 (t, *J* = 2.0 Hz, 1H, CΞC<u>H</u>), 1.58-1.51 (m, 4H, 2×CH_3-$C_{15}H_{30}$-CH_2-CH_2-O), 1.35-1.20 (m, 60H, 2×CH_3-

$C_{15}\underline{H}_{30}$-CH_2-CH_2), 0.87 (t, J = 6.64 Hz, 6H, 2×$C\underline{H}_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 82.0 ($\underline{C}$ΞCH), 77.8 (CH2-$\underline{C}$H-CH2), 71.6 (TEG + glycerol + $C_{17}H_{33}$-$\underline{C}$H2), 71.4 (TEG + glycerol + $C_{17}H_{33}$-$\underline{C}H_2$), 71.3 (CΞ$\underline{C}$H), 70.8 (TEG + glycerol + $C_{17}H_{33}$-$\underline{C}H_2$), 70.7 (TEG + glycerol + $C_{17}H_{33}$-$\underline{C}H_2$), 70.6 (3C, TEG + glycerol + $C_{17}H_{33}$-$\underline{C}H_2$), 70.5 (2C, TEG + glycerol + $C_{17}H_{33}$-$\underline{C}H_2$), 70.4 (TEG + glycerol + $C_{17}H_{33}$-$\underline{C}H_2$), 70.3 (TEG + glycerol + $C_{17}H_{33}$-$\underline{C}H_2$), 48.0 ($\underline{C}H_2$-NH-CH_2-CΞCH), 38.2 (CH_2-NH-$\underline{C}H_2$-CΞCH), 31.9 (2C, $C_{15}H_{30}$), 30.1 ($C_{15}H_{30}$), 29.7 (14C, $C_{15}H_{30}$), 29.6 (7C, $C_{15}H_{30}$), 29.5 (2C, $C_{15}H_{30}$), 29.3 (2C, $C_{15}H_{30}$), 26.1 (2C, $C_{15}H_{30}$), 22.6 (2C, 2×$\underline{C}H_2$-CH_3), 14.1 (2C, 2×$\underline{C}H_3$). IR (neat): 3250 (br.), 2915, 2849, 1467, 1106 cm^{-1}. HRMS (ESI$^+$) $C_{50}H_{99}NNaO_6$ [M+Na$^+$] calcd. m/z 832.7370, found m/z 832.7371.

Reaction between 13 and FITC in solution.

Lipid **13** (35 mg, 0.0432 mmol) and FITC (16 mg, 0.0432 mmol) was dissolved in a mixture of CH_2Cl_2 (8 mL) and *t*-BuOH (4 mL) and stirred for 16 hours at 21 °C. The reaction mixture was poured into sat. aq. $NaHCO_3$ (25 mL), extracted with EtOAc (20 mL) and $CHCl_3$ (20 mL), the combined organic phases were dried ($MgSO_4$), filtered, concentrated in vacuo and the resulting yellow oil was purified by flash chromatography (CH_2Cl_2:EtOAc 1:0 → 0:1, then EtOAc:MeOH 4:1) affording the iminothiazolidine (25 mg, 34%). ^{1}H NMR (400 MHZ; $CDCl_3$): δ 7.52 (s, 1H), 7.21 (d, J = 8.2 Hz, 1H), 7.02 (d, J = 8.2 Hz, 1H), 6.71 (s, 2H), 6.66 (d, J = 8.1 Hz, 2H), 6.48 (d, J = 8.1 Hz, 2H), 5.24 (s, 1H), 5.11 (s, 1H), 4.52 (s, 2H), 3.81-3.74 (m, 4H), 3.69-3.41 (m, 21H), 1.70-1.45 (m, 6H, buried under HDO), 1.35-1.19 (m, 60H), 0.88 (t, J = 6.8 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$:CD_3OD 4:1) δ 170.19, 158.00, 153.11, 152.92, 137.01, 130.06, 129.88, 129.67, 129.20, 128.48, 128.06, 127.80, 124.73, 117.52, 112.53, 110.72, 105.69, 102.69, 77.82, 73.72, 71.71, 71.25, 70.76, 70.64, 70.53, 70.46, 70.34, 69.23, 57.77, 46.01, 31.88, 29.97, 29.65, 29.61, 29.46, 29.31, 26.05, 26.02, 22.63, 13.99.

Liposome formulation.

Lipids were dissolved in CH_3Cl/MeOH (9:1) and mixed in the ratio 99:1 POPC/**13**. The solvent was removed under a stream of nitrogen and the films placed under vacuum overnight to remove remaining traces of organic solvent. The obtained films were hydrated in a PBS buffer, at room temperature for 1 h; followed by 5 freeze−thaw cycles and extrusion at room temperature through a 100 nm polycarbonate filter using an Avanti Polar Lipids mini-extruder. The size distribution of the liposomes was analyzed using DLS, before and after the incubation with FITC.

Conjugation experiments with liposomes.

Preformed functionalized liposomes (25 mM, 0.25 mL, 1 equiv.) were mixed with FITC (0.15 mM, 107 μL, 0.5 equiv.) dissolved in PBS, then PBS was added to a final volume of 0.5 mL. The

samples were shaken (not stirred; to avoid foaming) at room temperature and aliquots (40 μL) removed for analysis by analytical HPLC. A linear gradient was used from 70% A (aqueous solution containing 5% MeCN and 0.1% TFA) to 100% B (MeCN containing 0.1% TFA) over 20 min with a flow rate of 1 mL/min. The AUC for the free FITC was compared to the AUC of the phospholipid coupled products at 254 nm to monitor the conjugation efficiency (see copies of chromatograms). Duplicates of the reaction were carried out to ensure reproducibility.

Competition experiment with propargyl amine 2 and ethanolamine in aqueous solvents.

Mixtures of ethanolamine (1 equiv.), propargyl amine 2 (1 equiv.) and FITC (0.5 equiv.) in four different aqueous solutions (0.5 mM FITC) were reacted at 20 °C and samples were removed for HPLC and LC-MS analysis at the indicated times (see figures below).

Solid-phase synthesis of 14.

Amino-terminated ChemMatrix® resin (1.0 g, 0.6 mmol) was washed with CH_2Cl_2 and incubated with Fmoc-protected Rink amide linker (971 mg, 1.8 mmol) in DMF (14 mL) for 2 h. The resin was then washed with DMF, MeOH, and CH_2Cl_2 (3 × 15 mL each). The resin was then treated with piperidine–DMF (1:4, 15 mL, 2 × 20 min), and DBU-piperidine-DMF (2:2:96, 15 mL, 20 min) and washed with DMF, MeOH, and CH_2Cl_2 (3 × 15 mL each). Peptide synthesis was performed with a mixture of Fmoc-aa-OH (1.8 mmol, 3 equiv.), HATU (684 mg, 1.8 mmol, 3 equiv.), and *i*Pr_2NEt (0.63 mL, 3.6 mmol, 6 equiv) in DMF (14 mL), which were preincubated for 10 min before being added to the resin and shaken for a minimum of 2 h. After each coupling step the resin was washed with MeOH, DMF and CH_2Cl_2 (3 × 15 mL each). Fmoc deprotection was achieved with piperidine–DMF (1:4, 15 mL, 2 × 20 min) followed by DBU–piperidine–DMF (2:2:96, 15 mL, 20 min), after each deprotection step the resin was washed using the same procedure as above. This coupling/deprotection sequence was performed 10 times to give the resin-bound decamer **14**.

Synthesis of functionalized decapeptide 15.

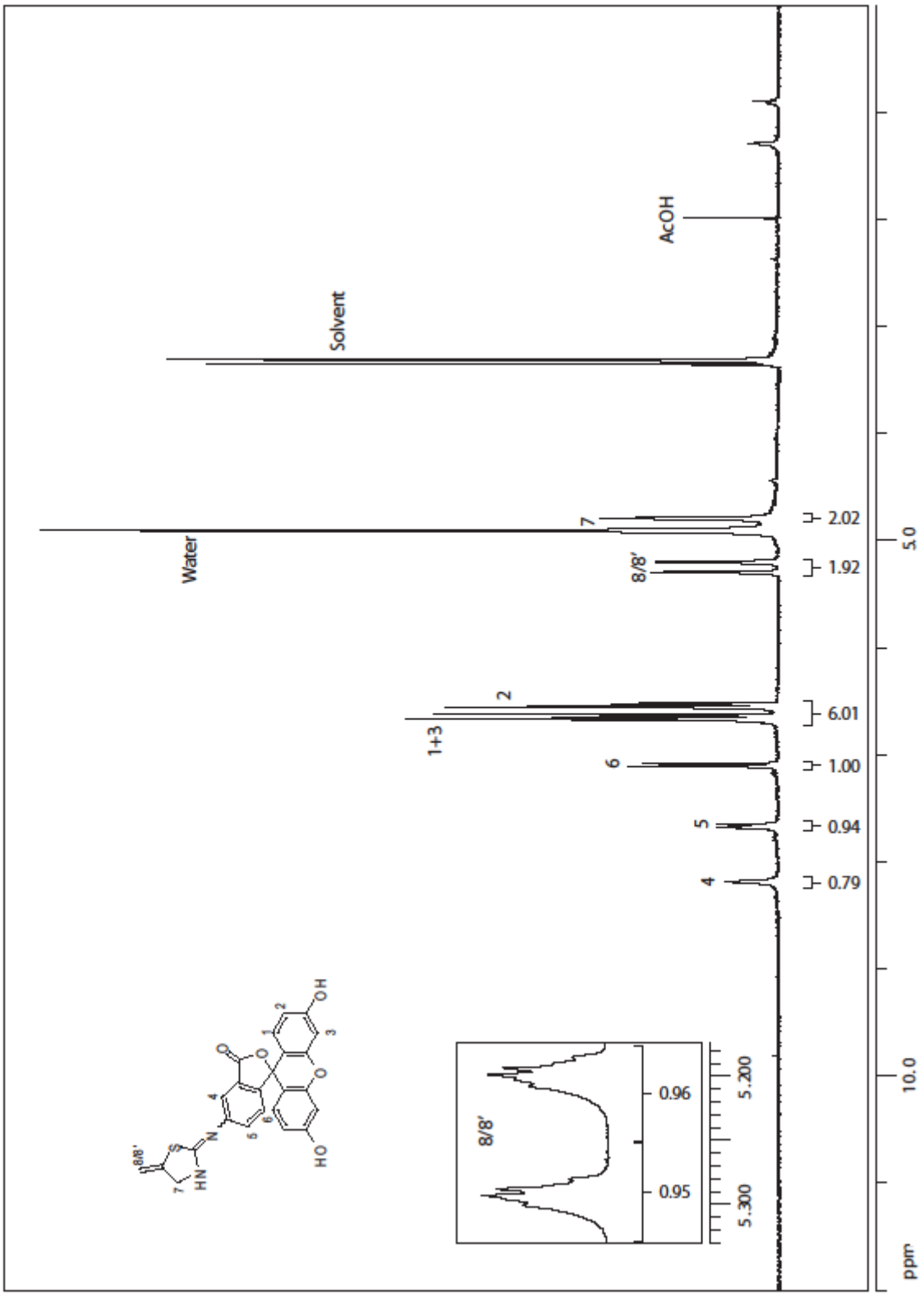
Resin **14** (0.27 mmol) was washed with CH_2Cl_2 (5 mL), and incubated with $BrCH_2COOH$ (500 mg, 3.6 mmol, 12 equiv.) and DIC (614 μL, 4.0 mmol, 13.2 equiv.) in DMF (7 mL) for 30 min. The resin was then washed with DMF, MeOH, and CH_2Cl_2 (3 × 6 mL each) and the coupling and washing repeated. The resin was incubated with propargyl amine (2.5 M in DMF, 7 mL) for 2 h, washed with DMF (3 × 6 mL) and the incubation and wash repeated. Decapeptide **15** was deprotected and cleaved from the support using TFA–ethanedithiol–thioanisole–phenol–H_2O (82.5:2.5:5:5:5, 7 mL) for 2h, filtered and the volatiles removed under a stream of air. The resulting film was dissolved in TFA and treated with Et_2O and the suspension was centrifuged and the supernatant decanted. The crude peptide was dried in vacuo, taken up in MeCN:H_2O 1:1 and

purified by preparative RP-HPLC. Lyophilization of the fractions containing the title compound furnished a white fluffy material [56 mg, 17% (84% per step)].

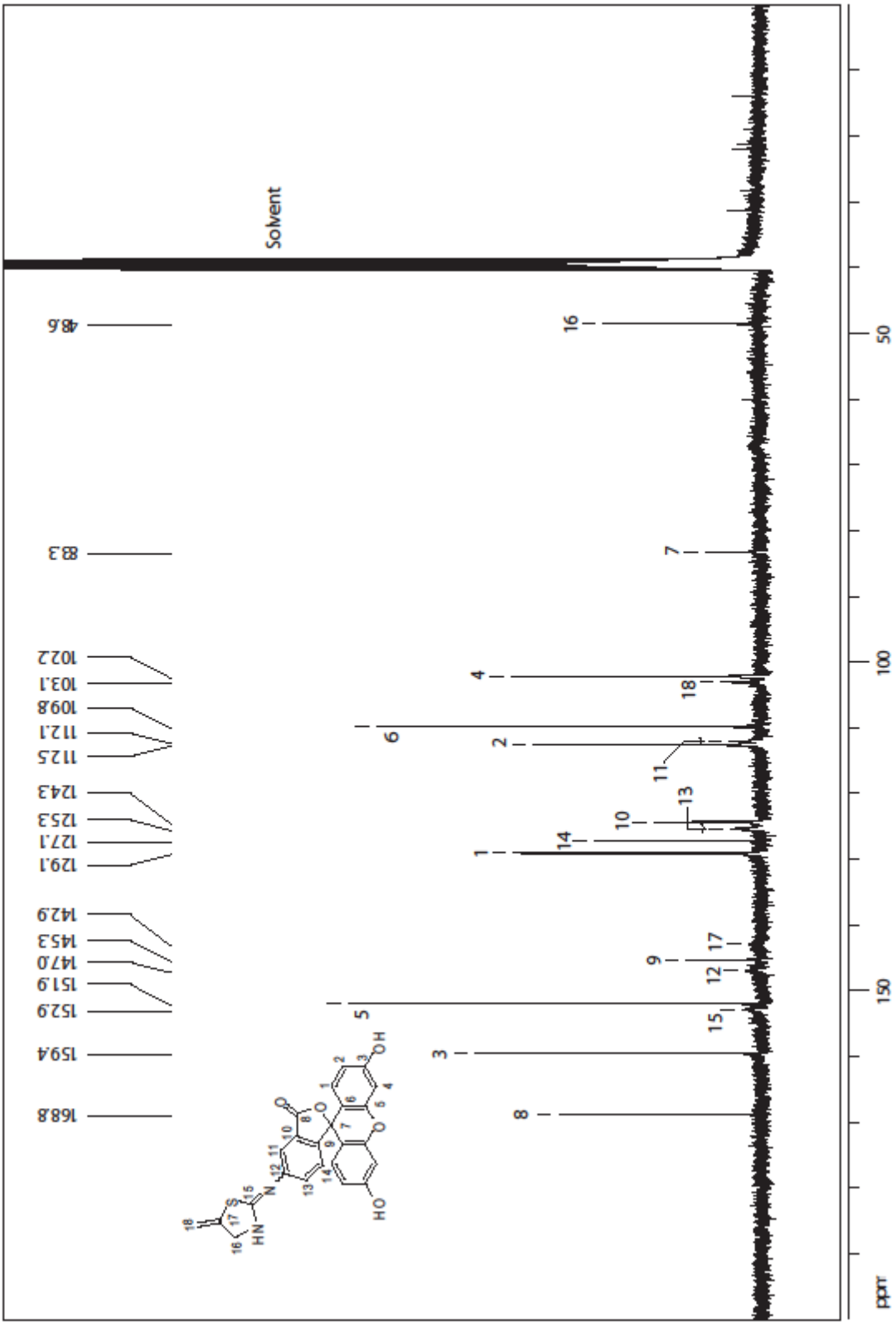
Coupling of FITC and 15 and subsequent trypsin digestion.

Peptide **15** (2.60 mg, 2.12 μmol) and FITC (0.28 mg, 0.35 equiv.) in 2.6 mL PBS buffer (pH 7.4) was shaken for 17 h and analyzed by LC-MS and MALDI-TOF-MS (see figures below). An aliquot of the mixture was treated with trypsin (5% w/w) for 1 h at 37 °C and analyzed by LC-MS.

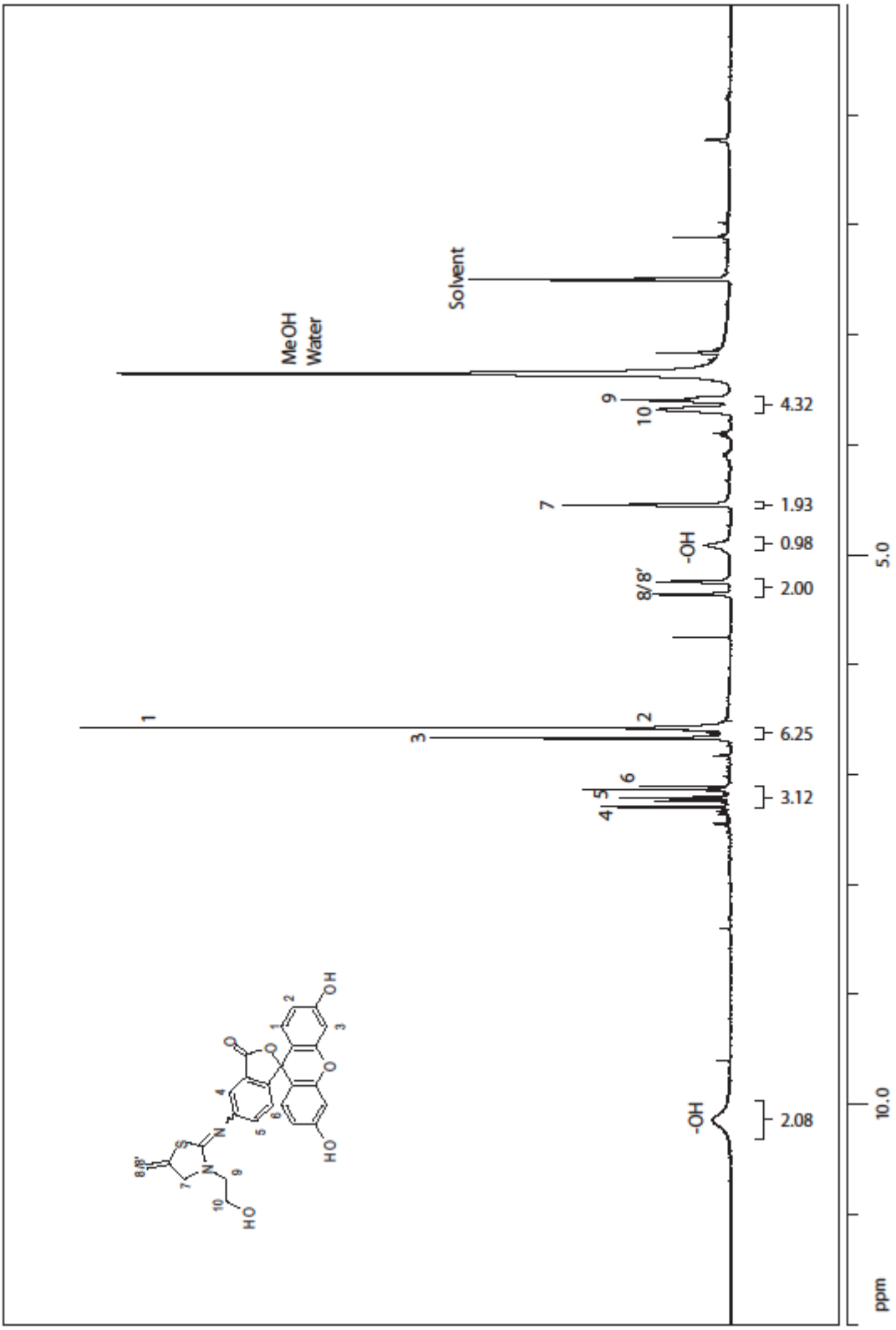
NMR – Compound 4



NMR – Compound 4



NMR – Compound 5



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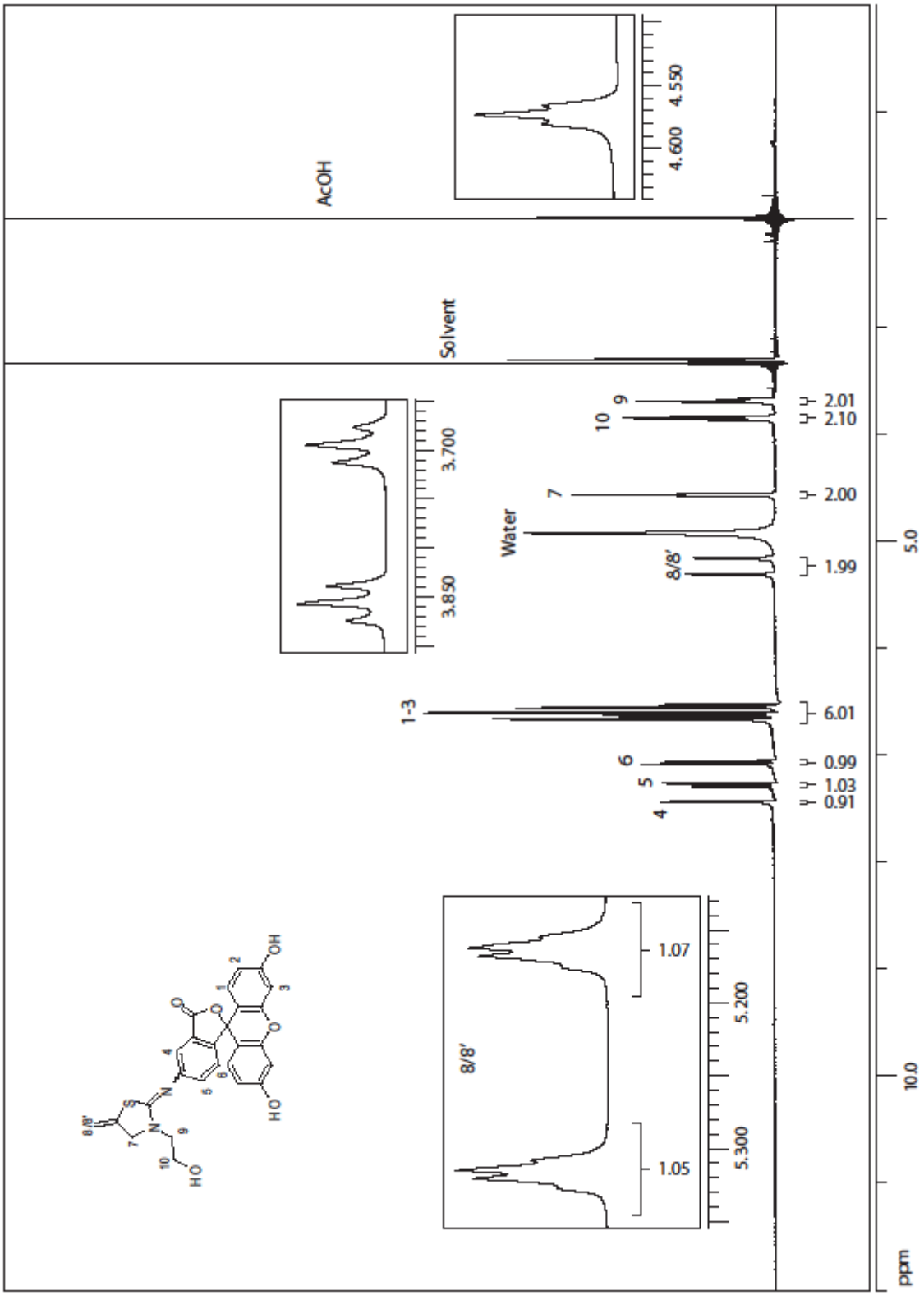

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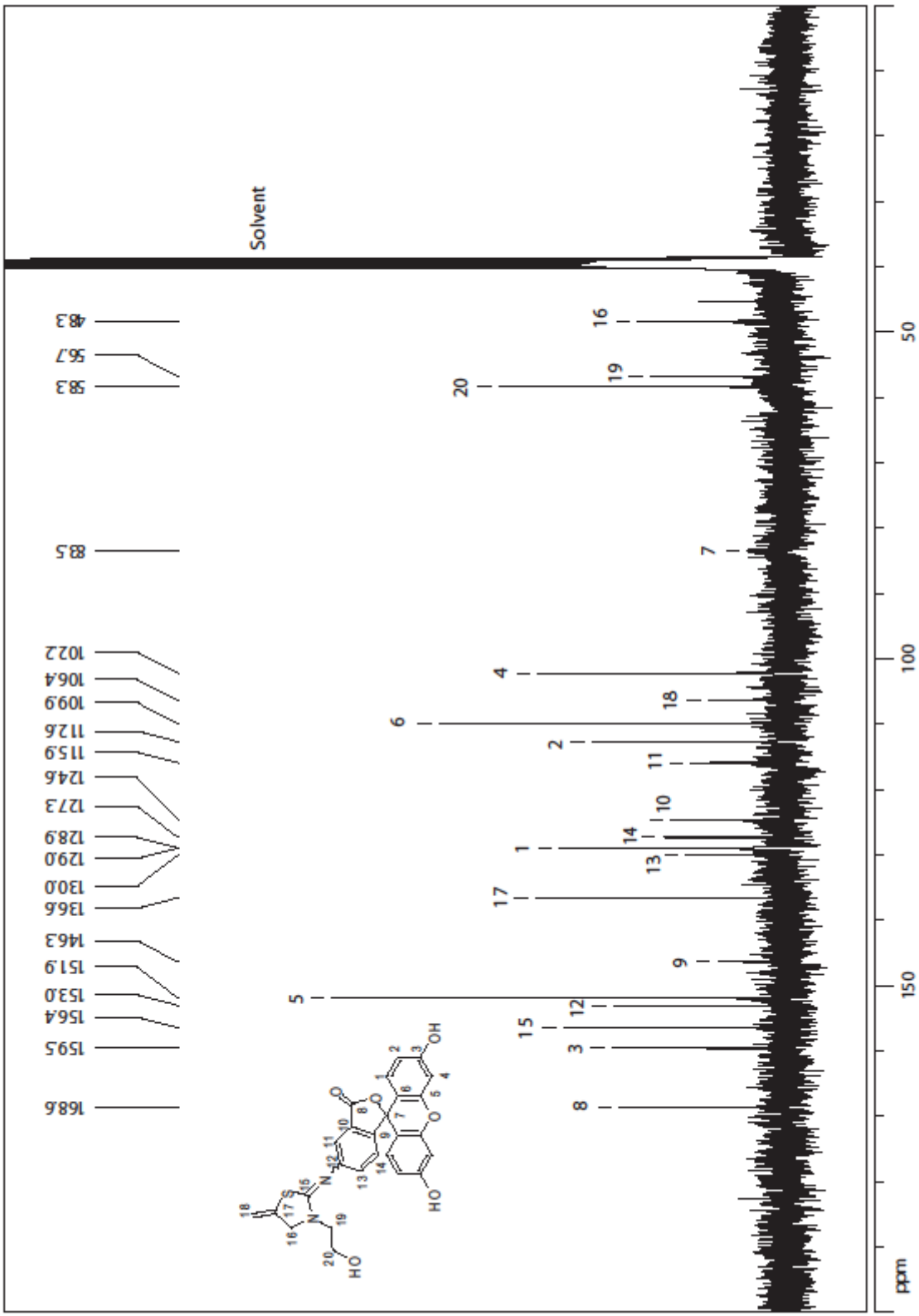
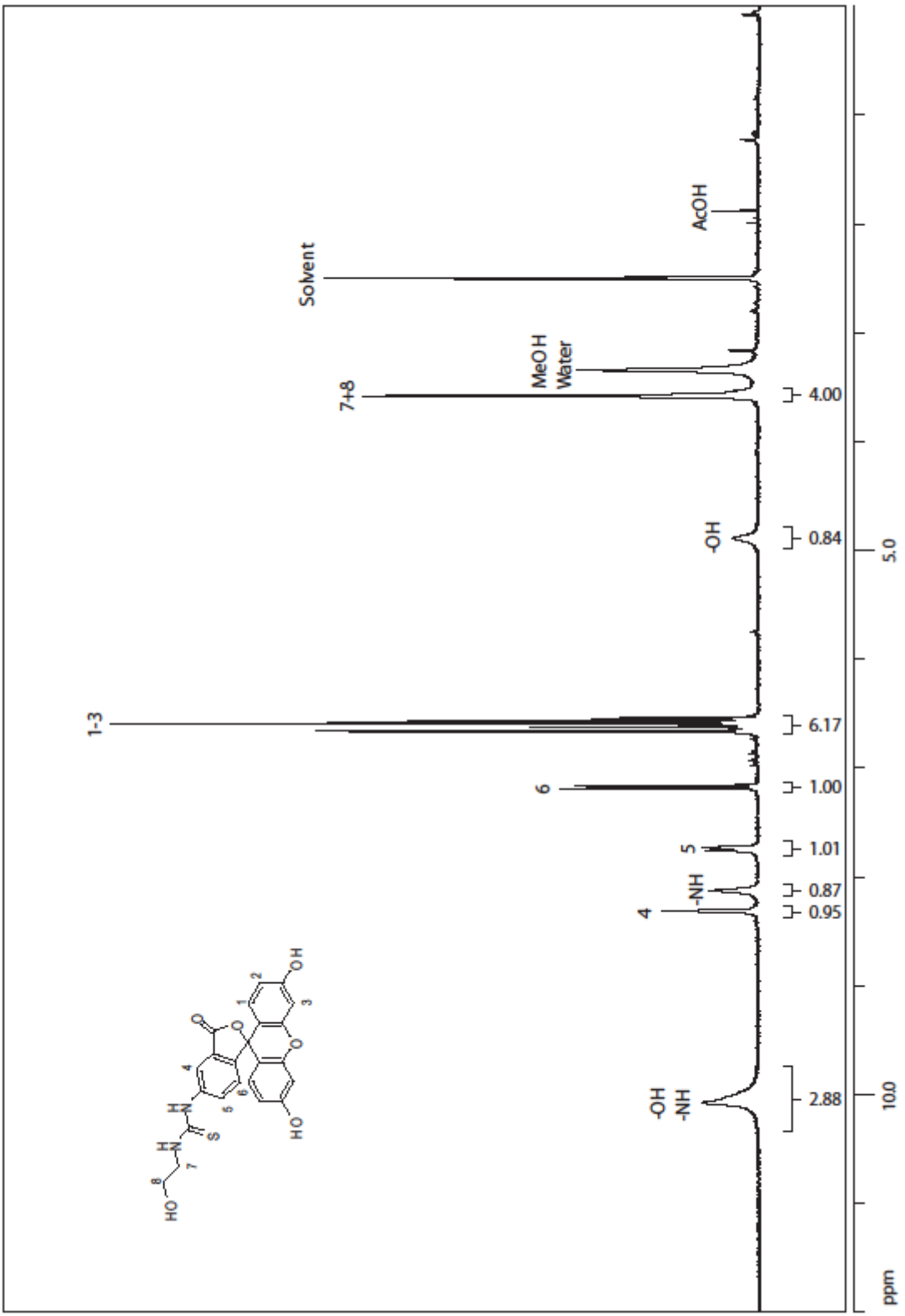


NMR – Compound 6



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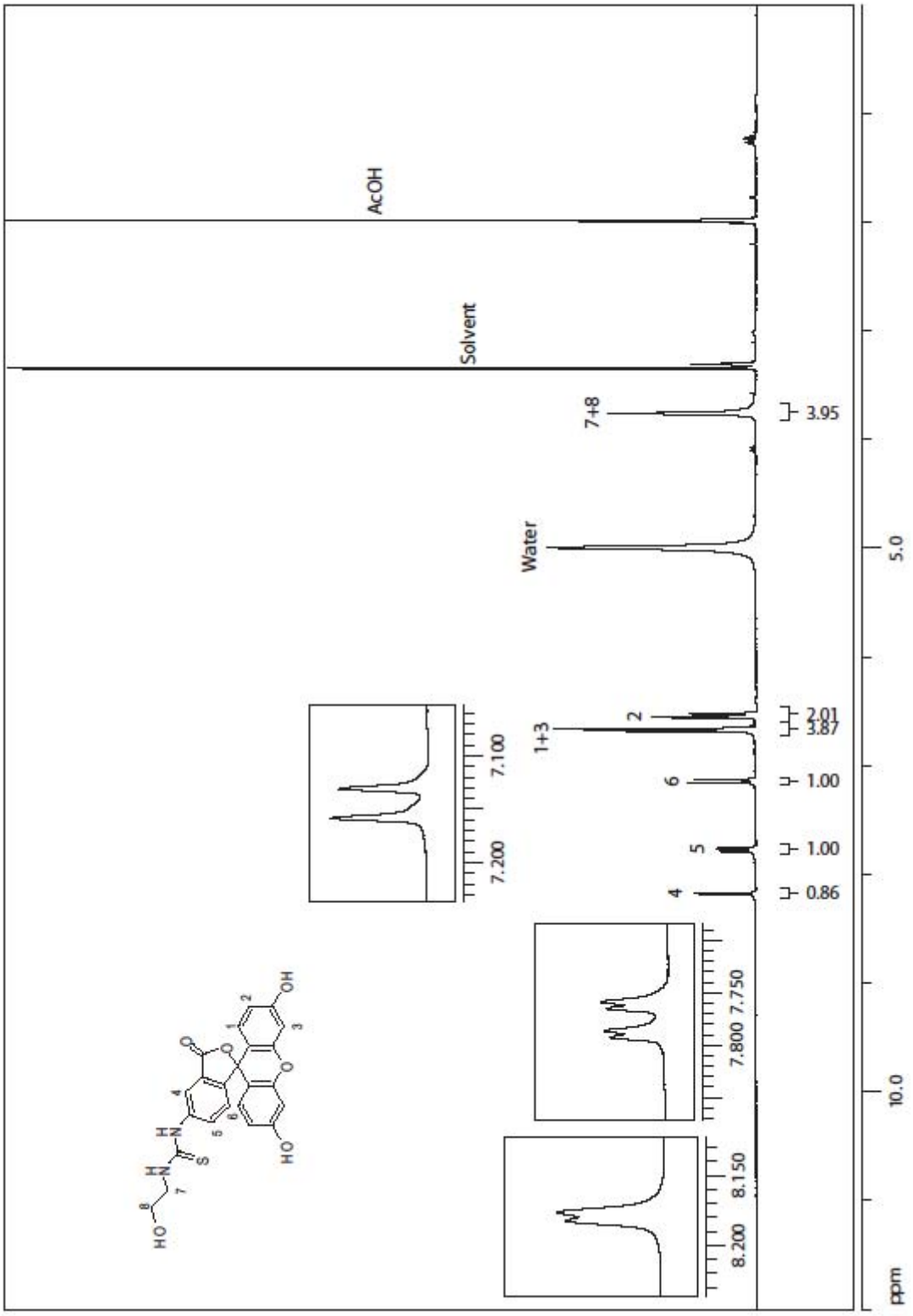

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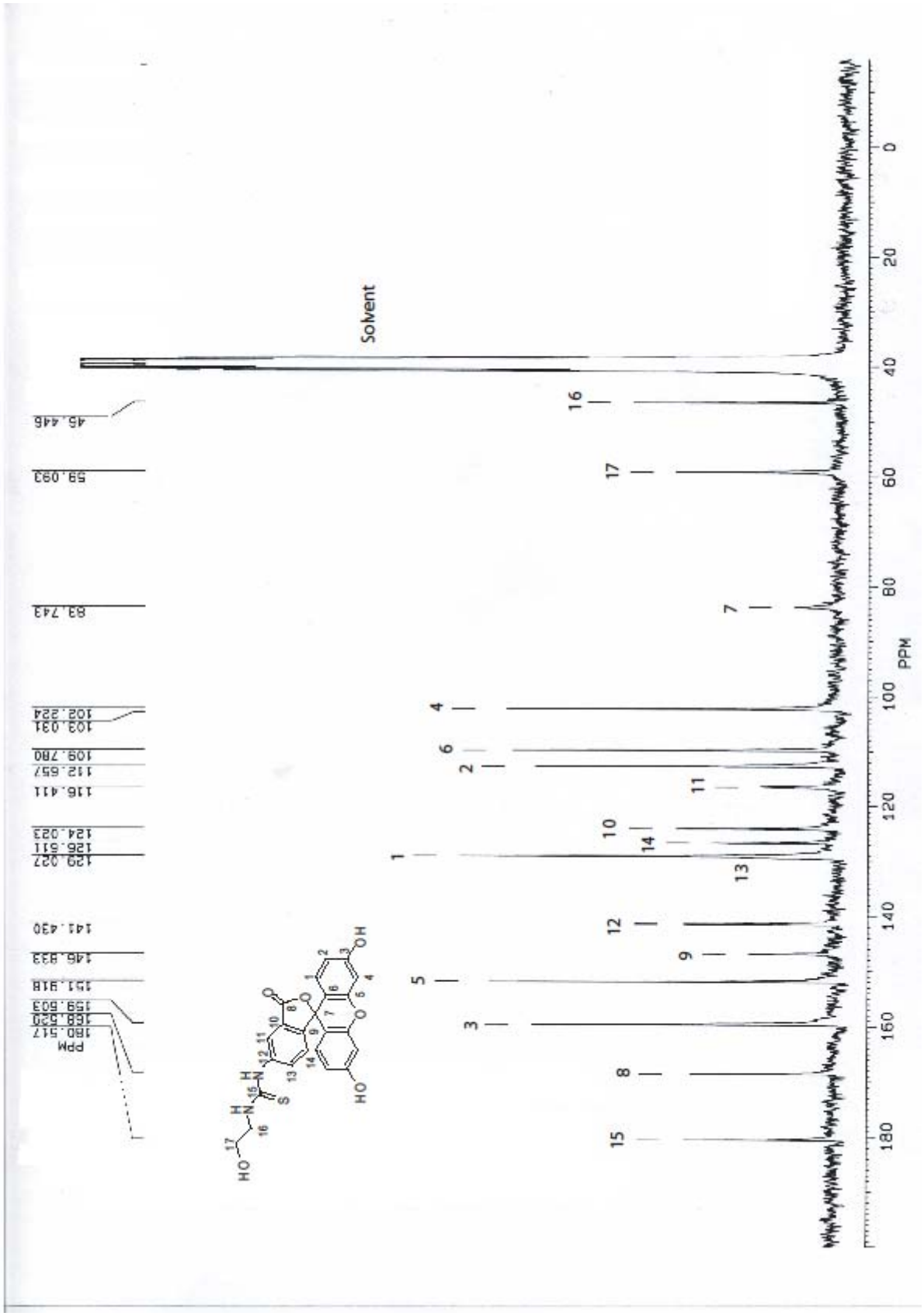
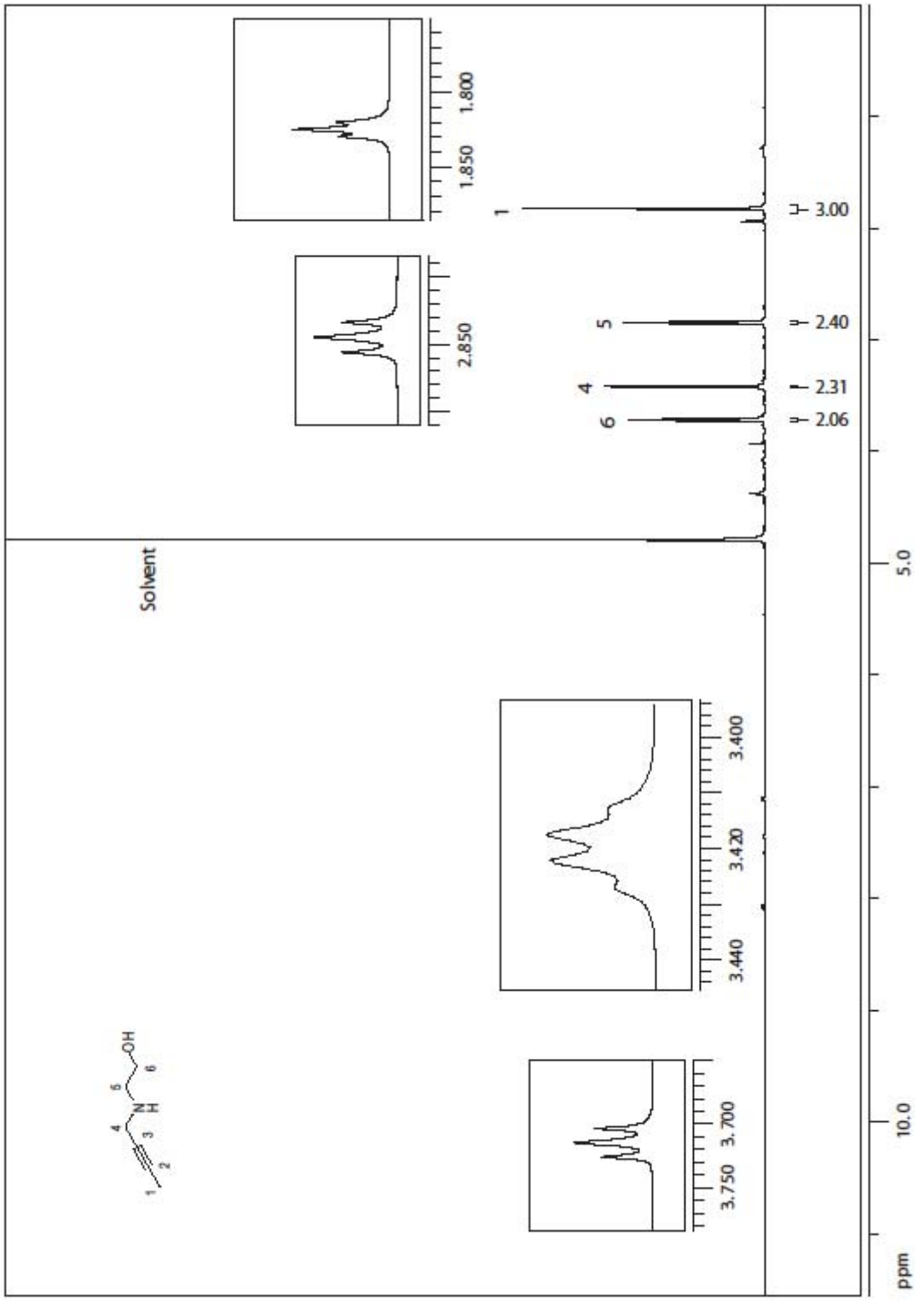
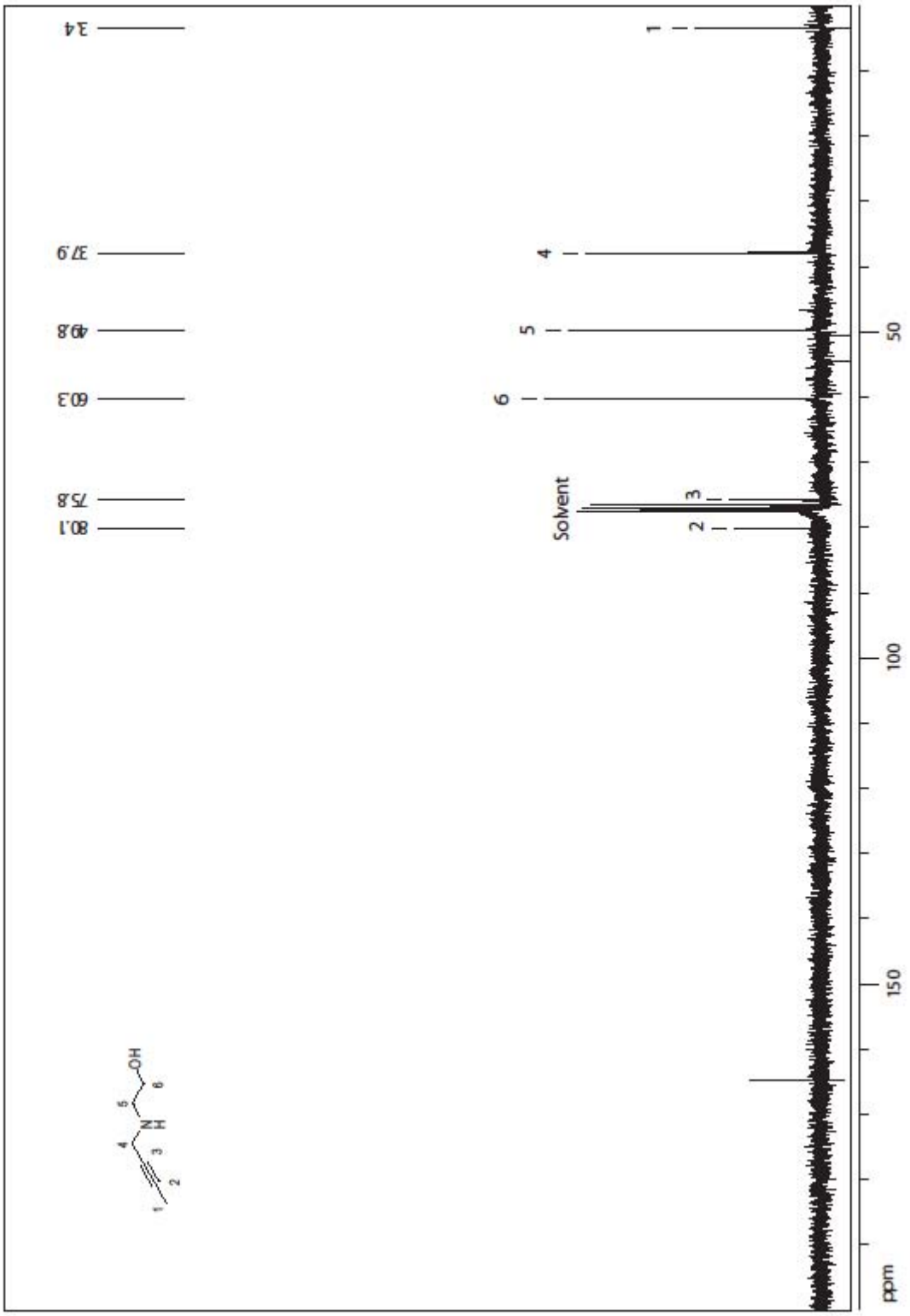


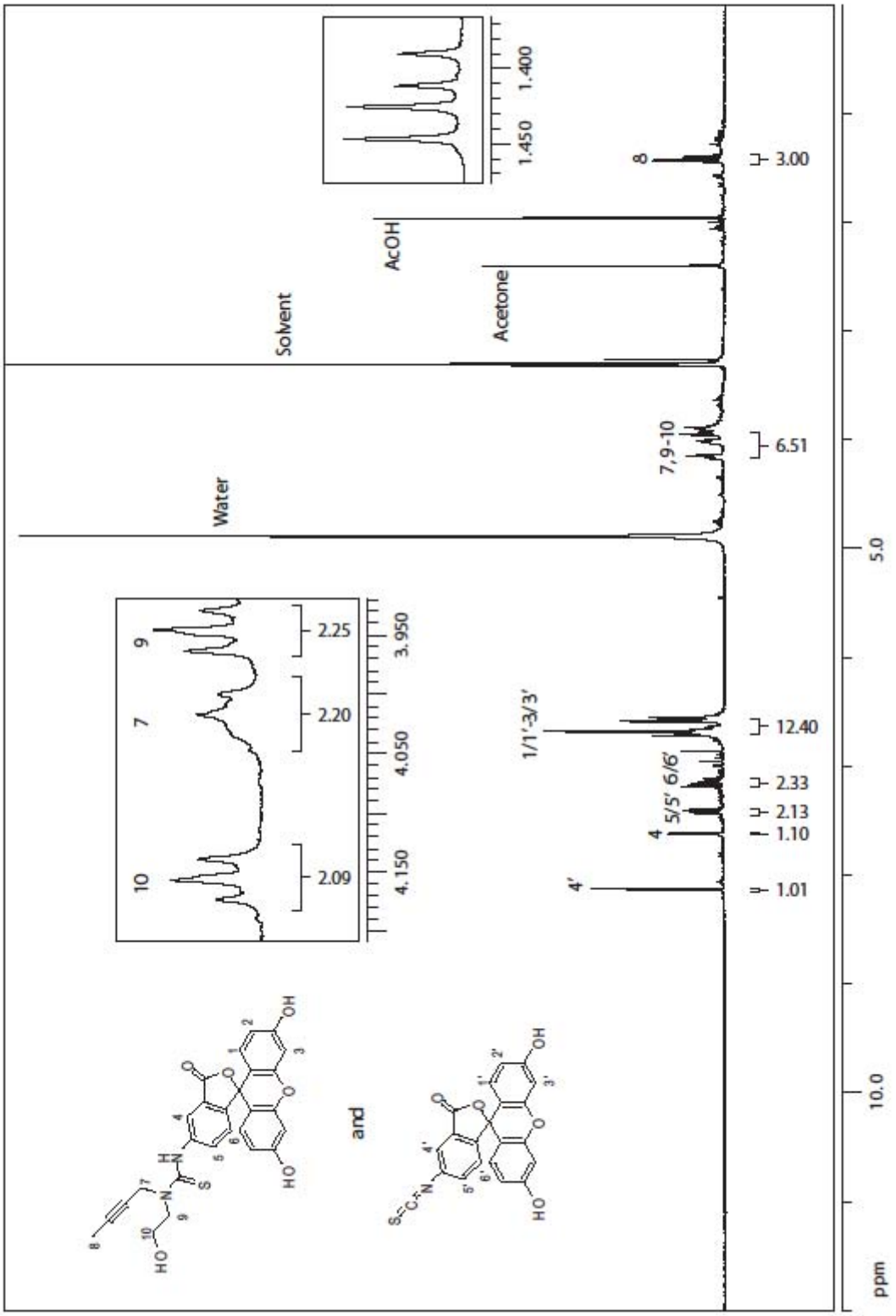
NMR – Compound 7



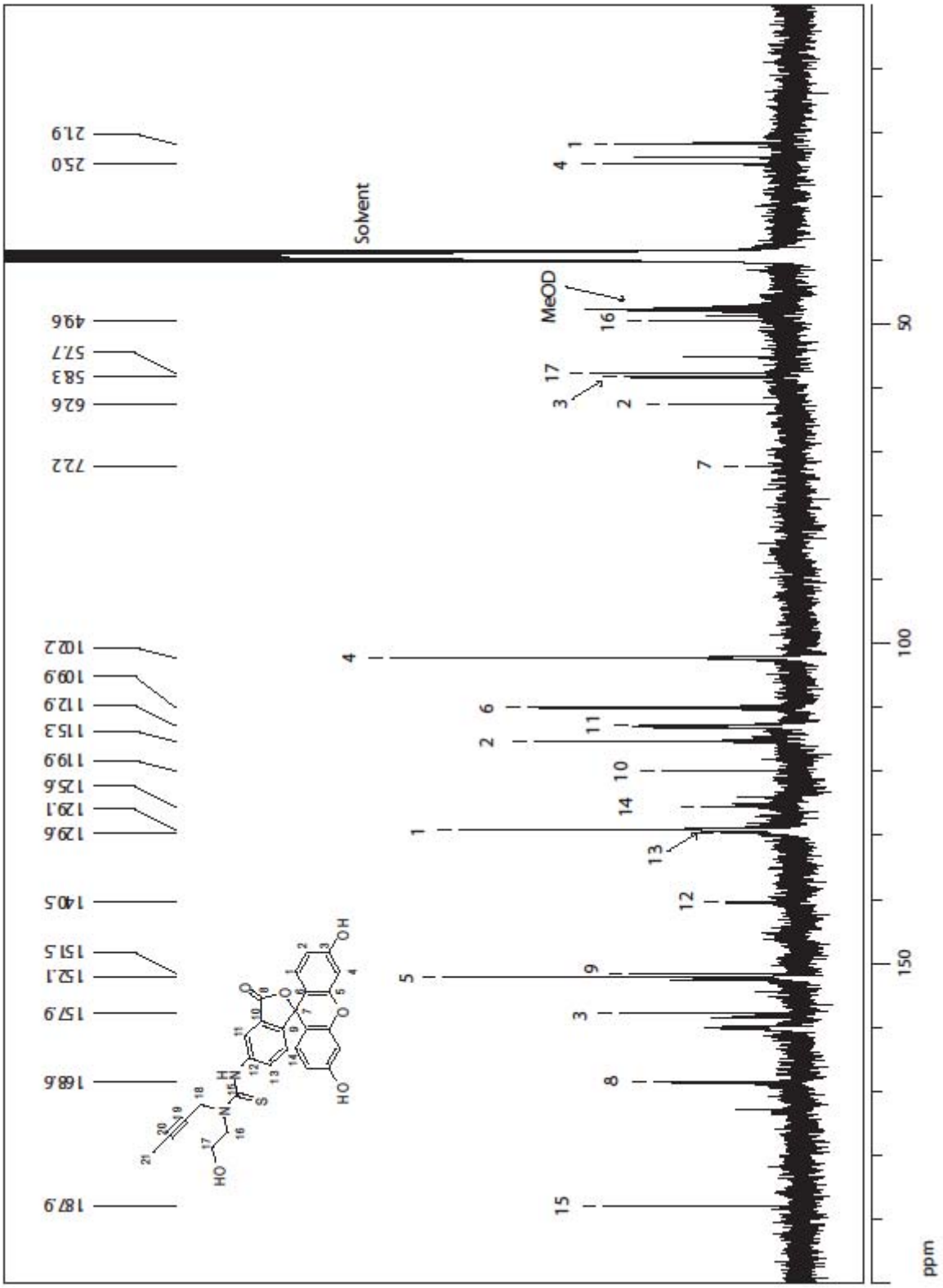
NMR – Compound 7



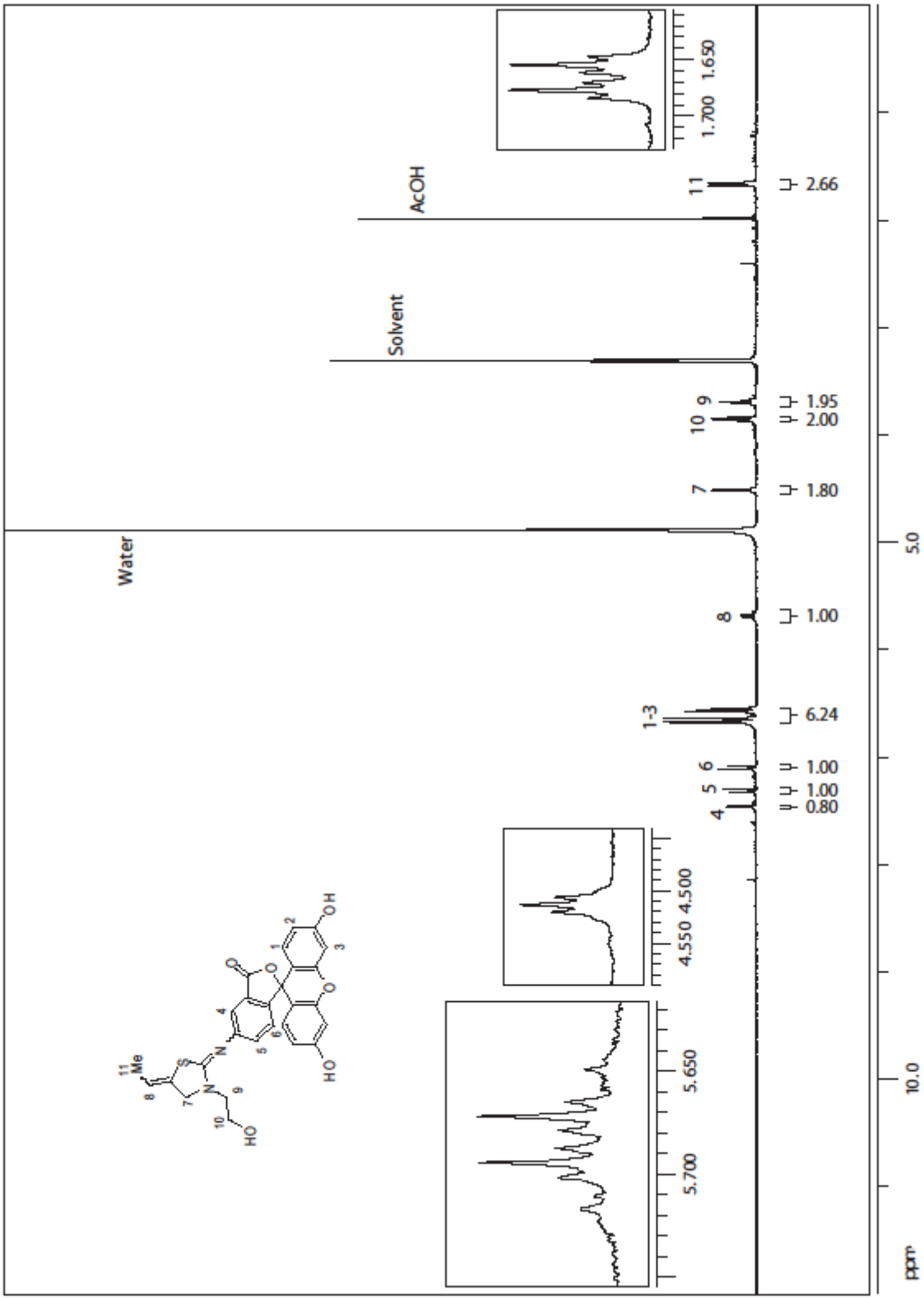
NMR – Compound 8



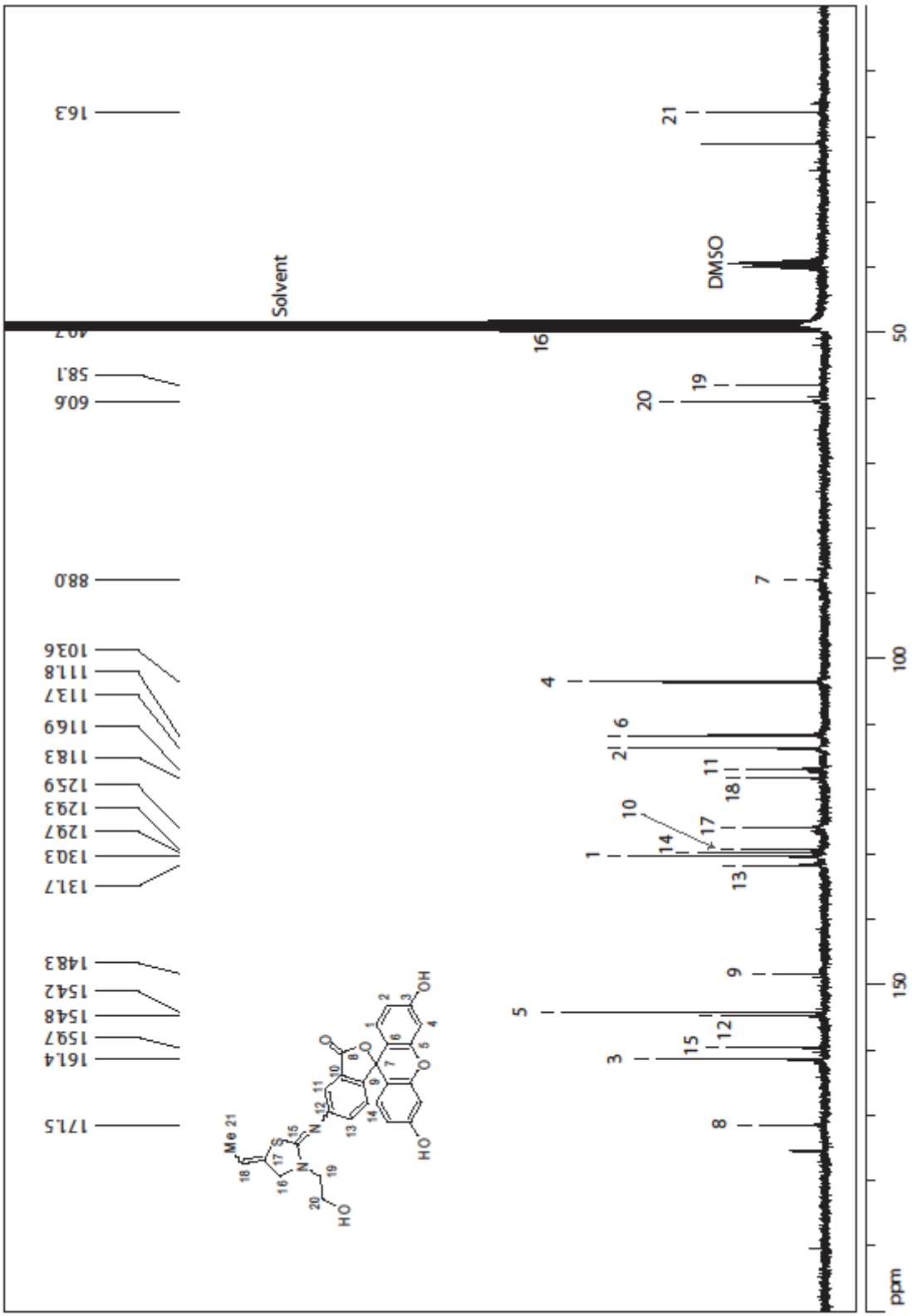
NMR – Compound 8



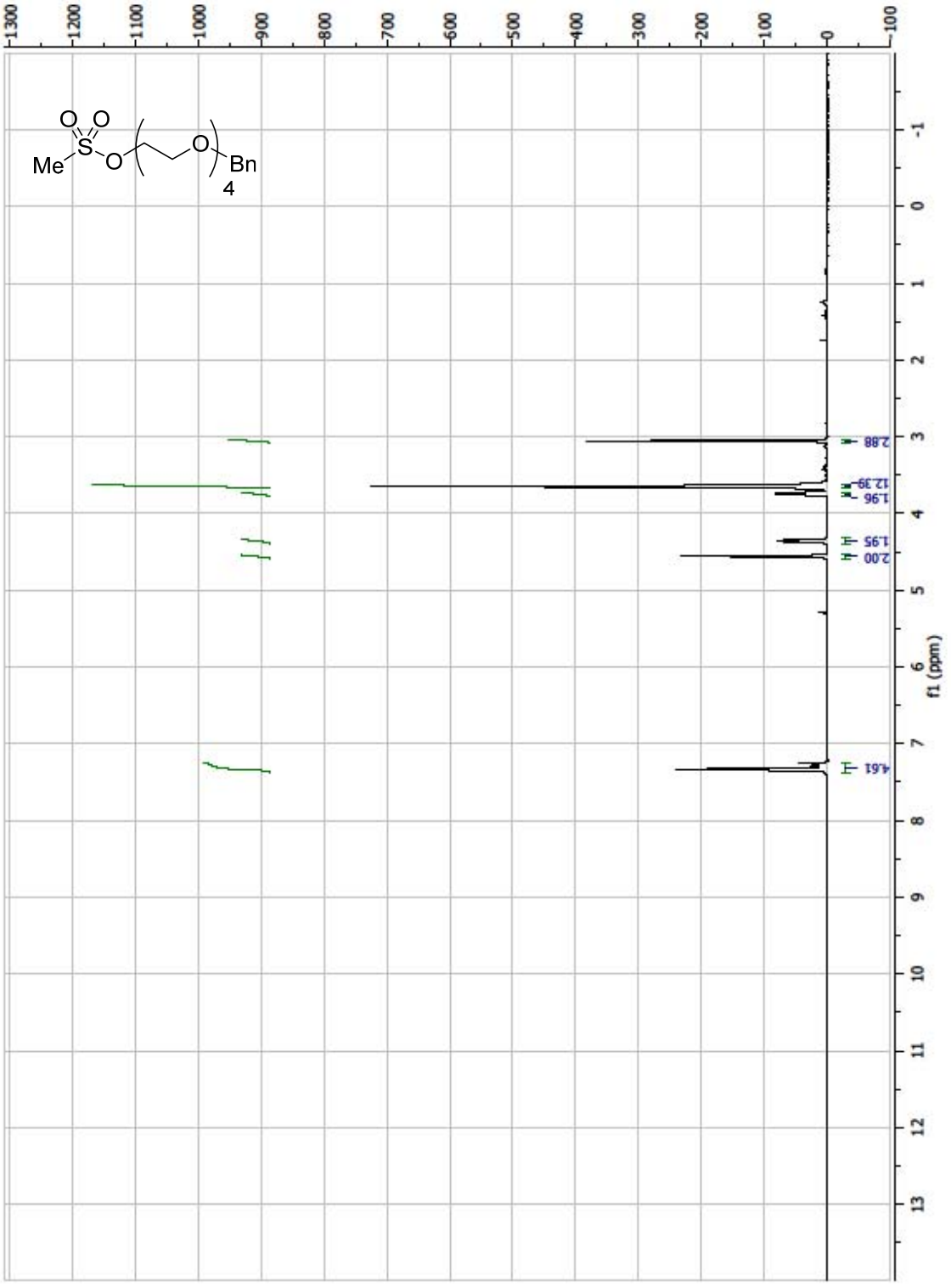
NMR – Compound 9



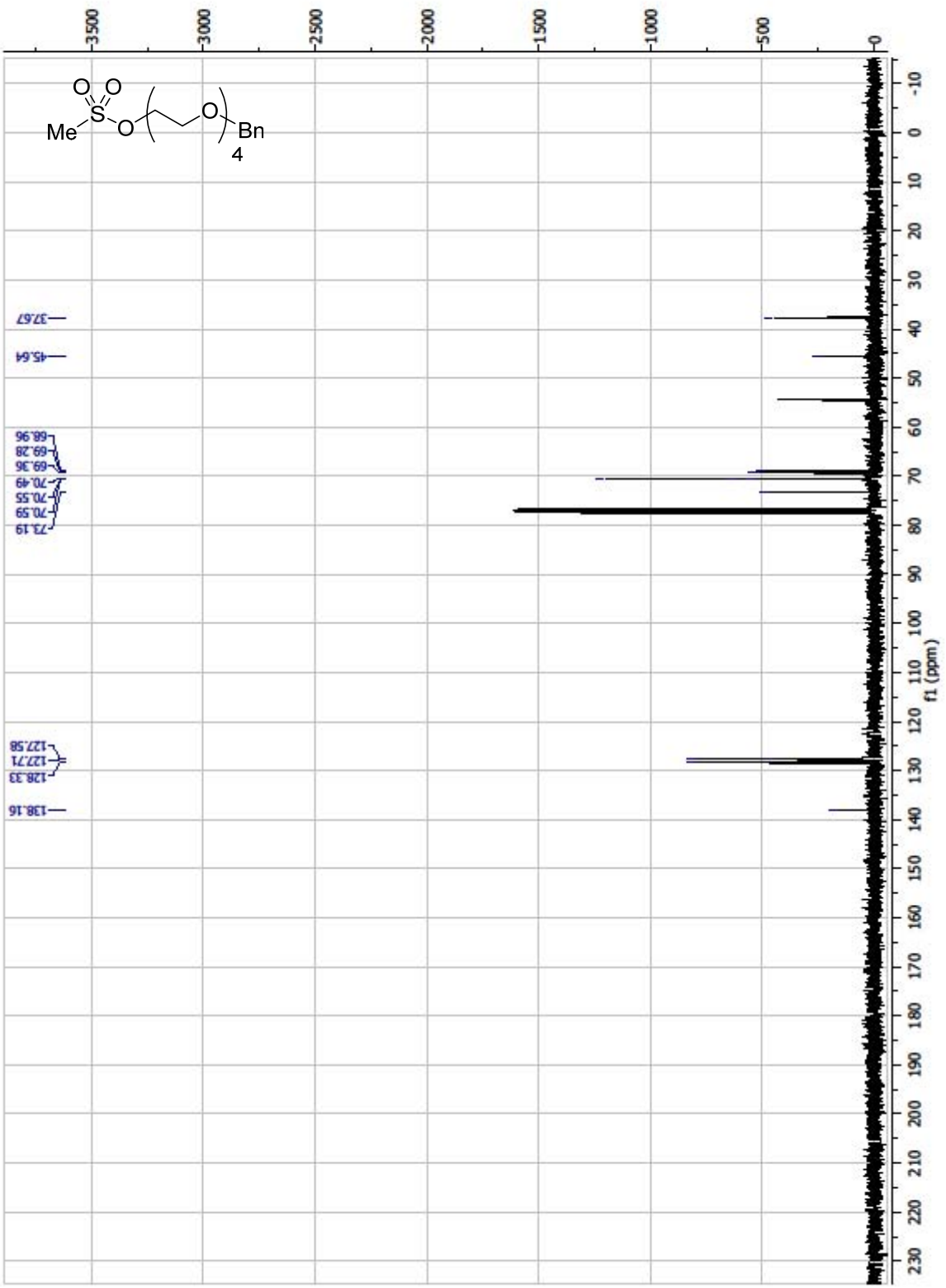
NMR – Compound 9



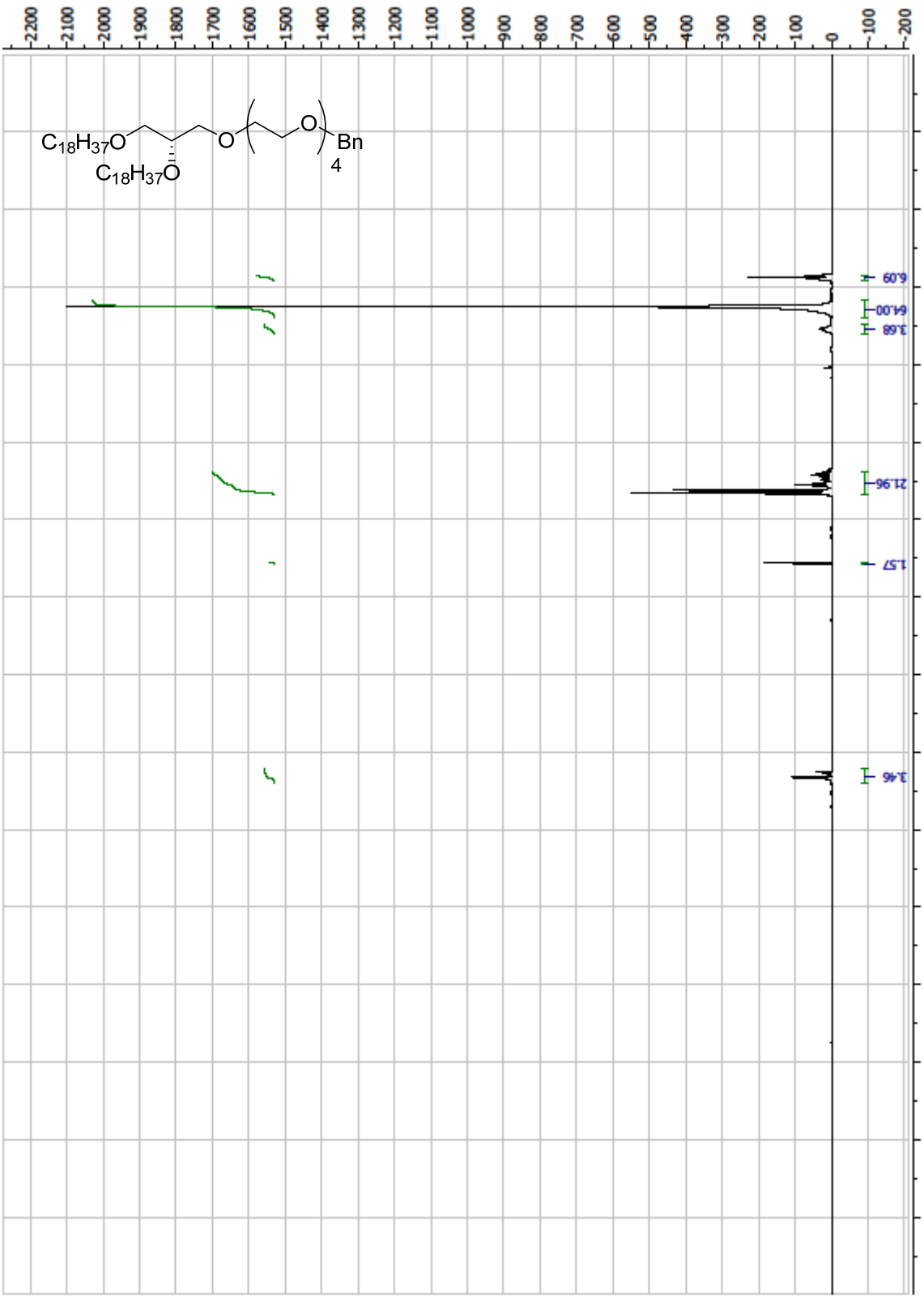
NMR – Compound 11



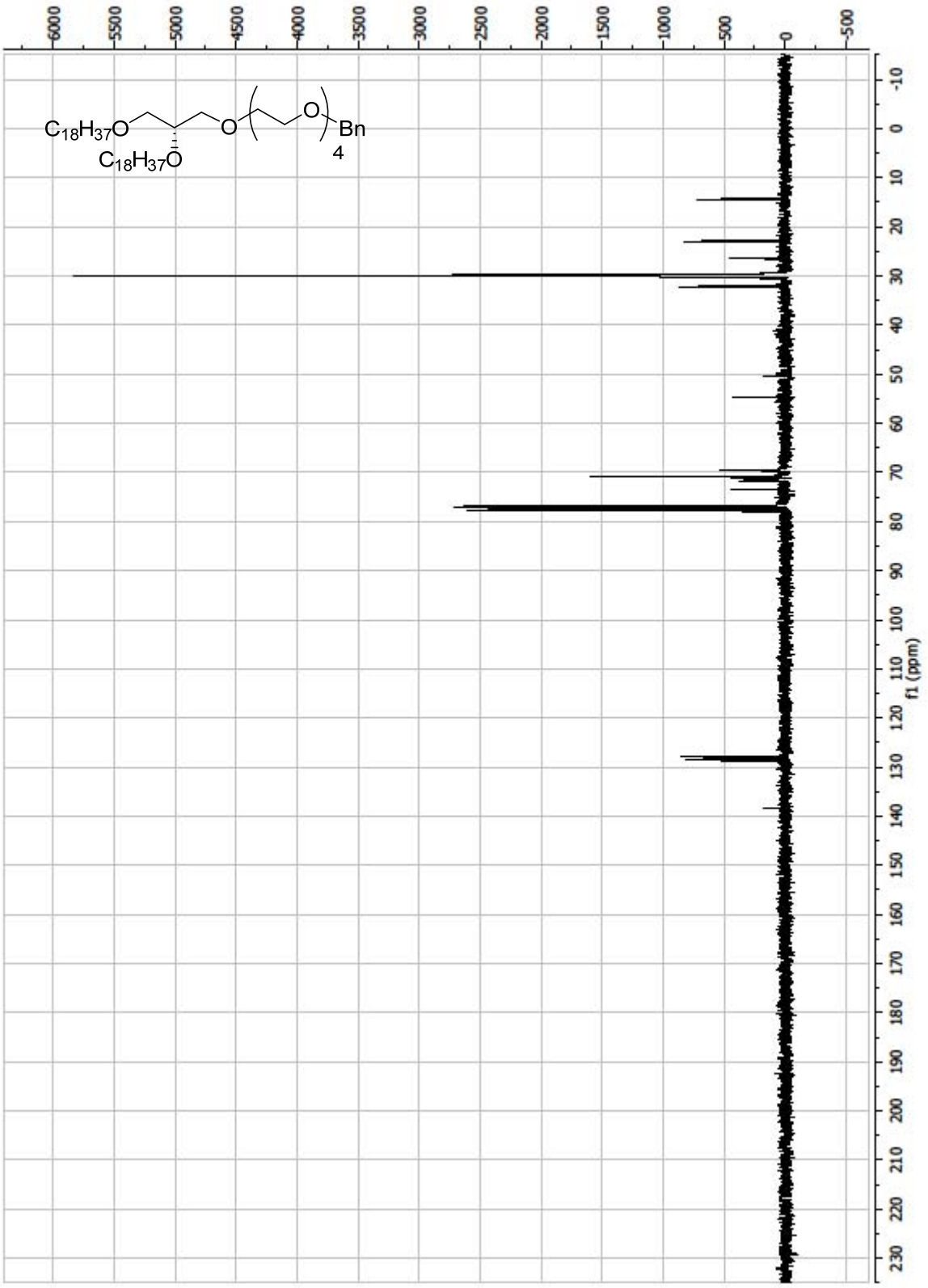
NMR - Compound 11



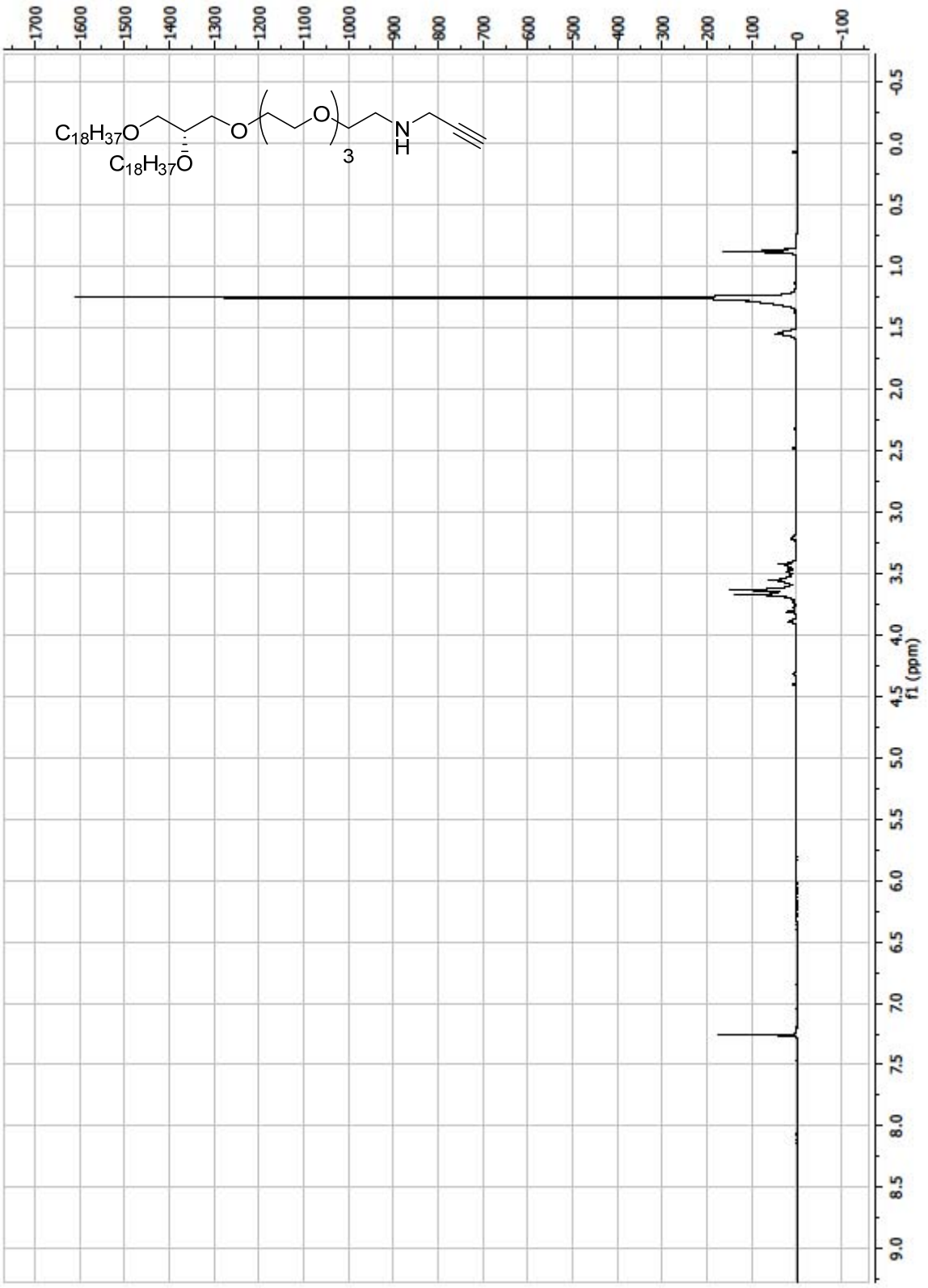
NMR – Compound 12



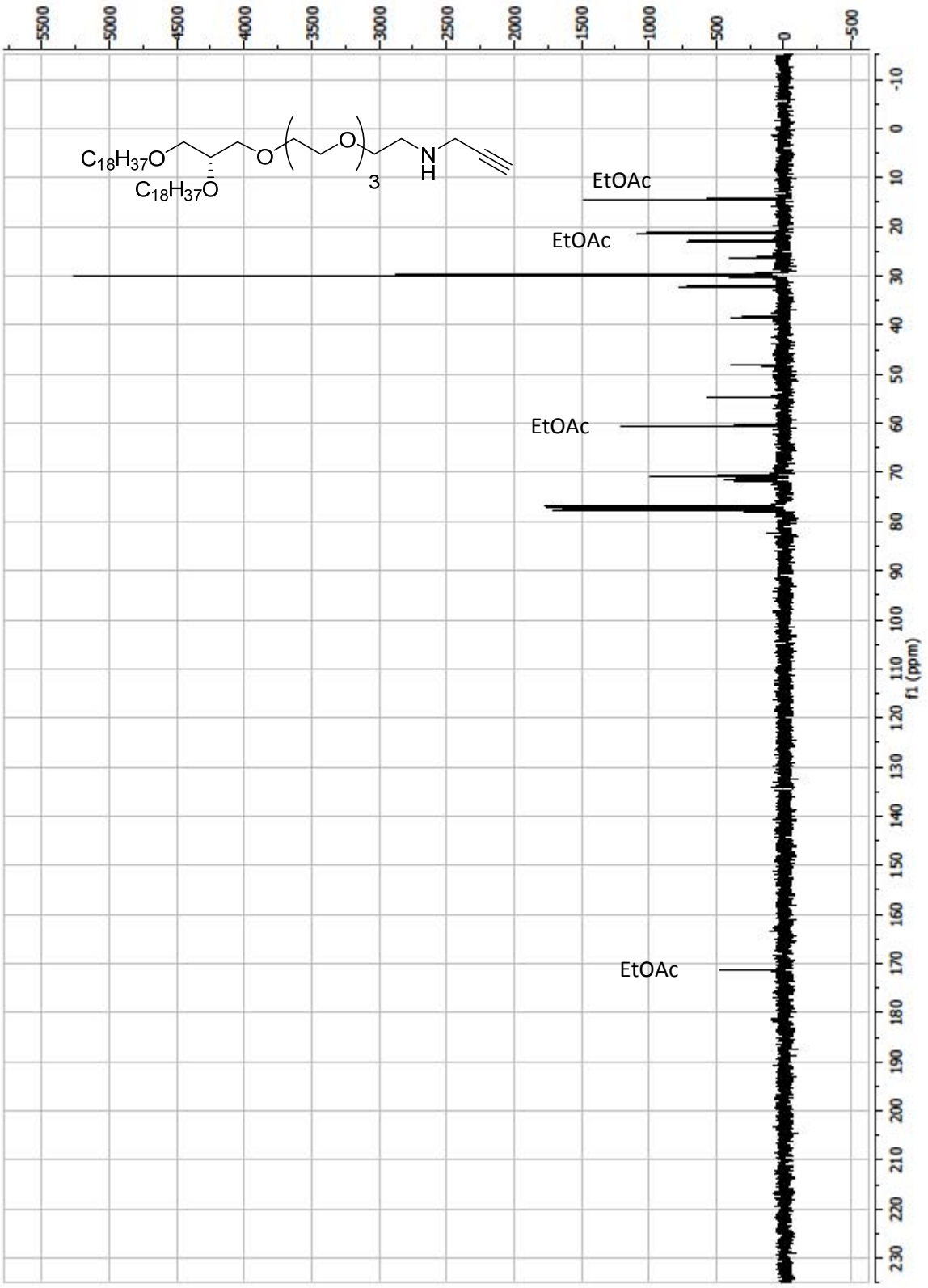
NMR – Compound 12



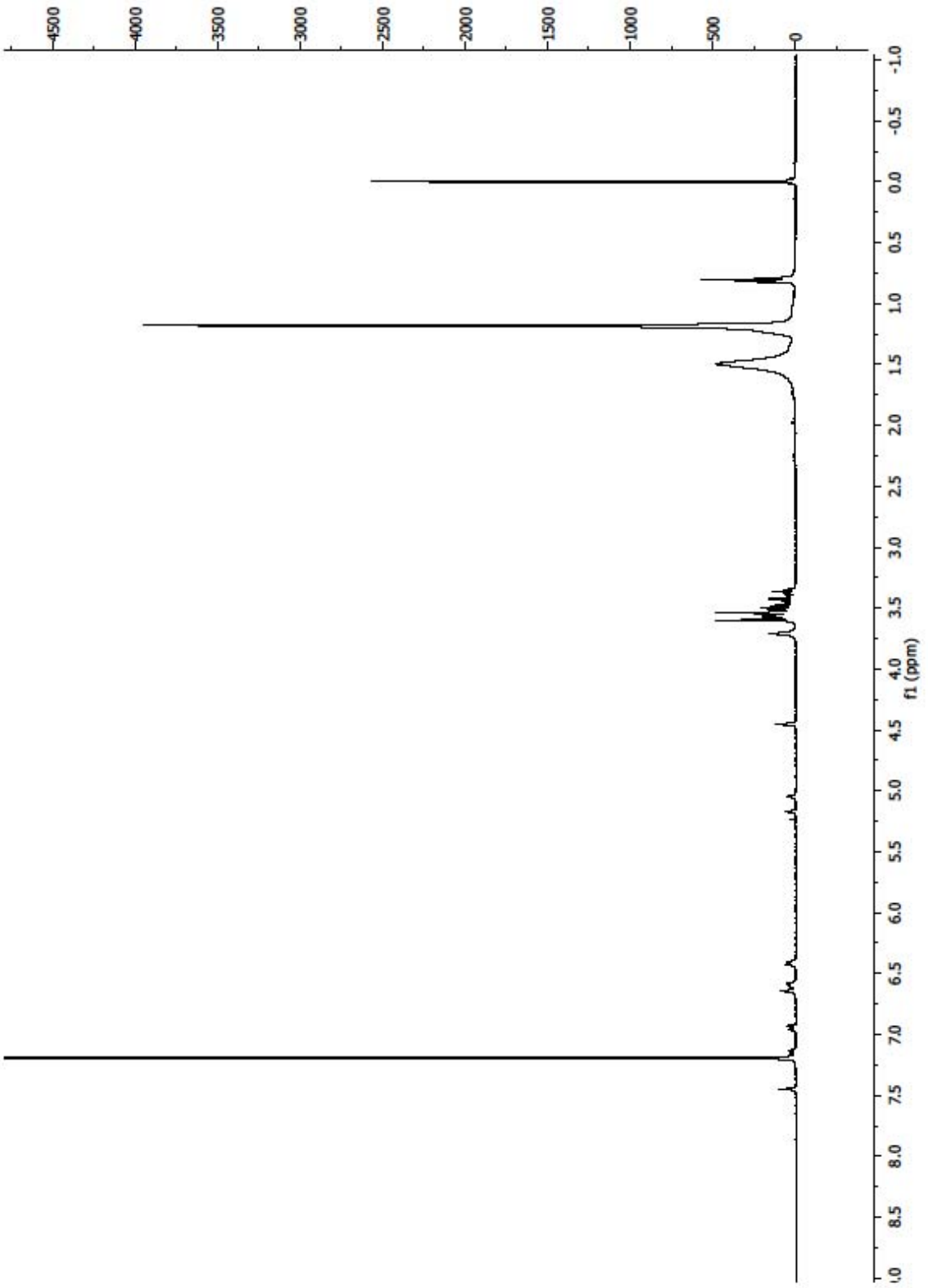
NMR – Compound 13



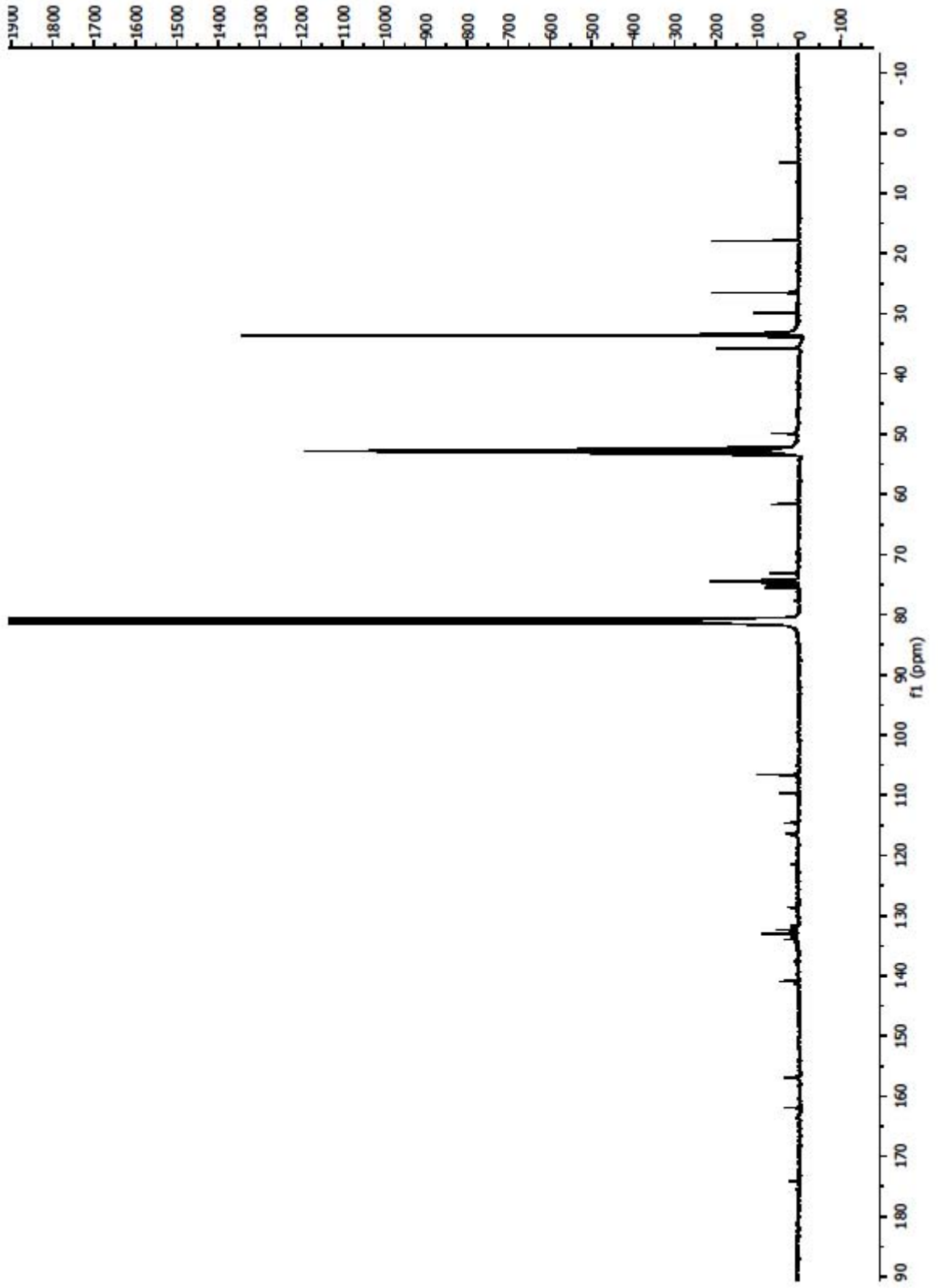
NMR – Compound 13



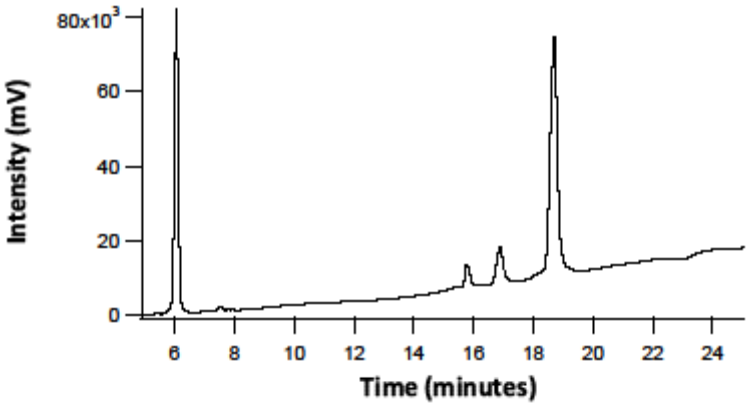
NMR – lipid iminothiazolidine



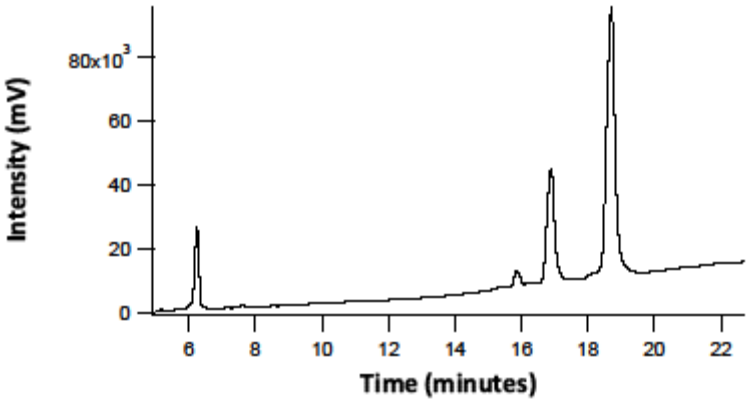
NMR – lipid iminothiazolidine



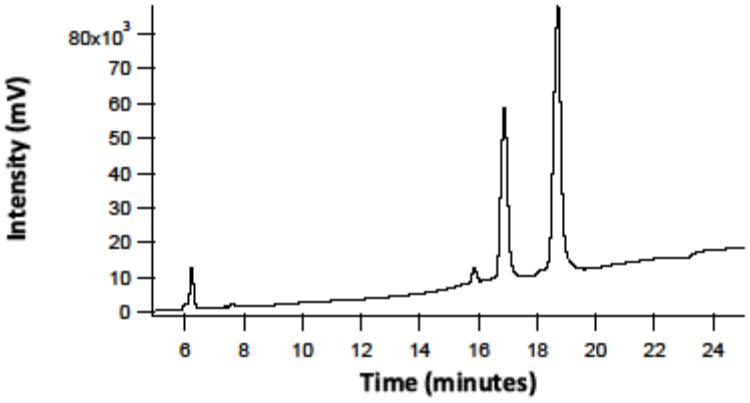
HPLC conjugation reaction on liposomes



5 minutes

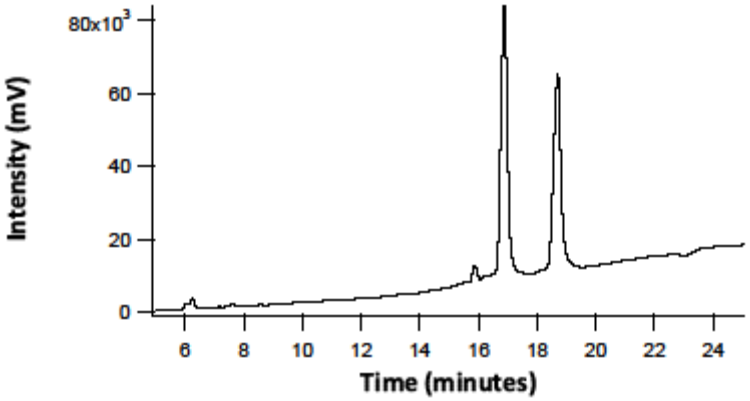


30 minutes

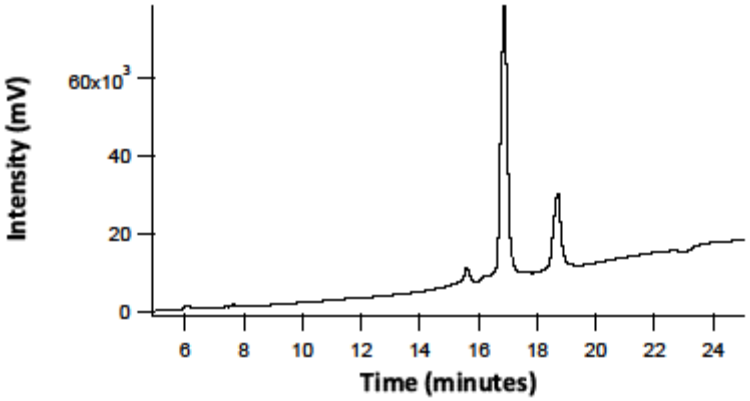


60 minutes

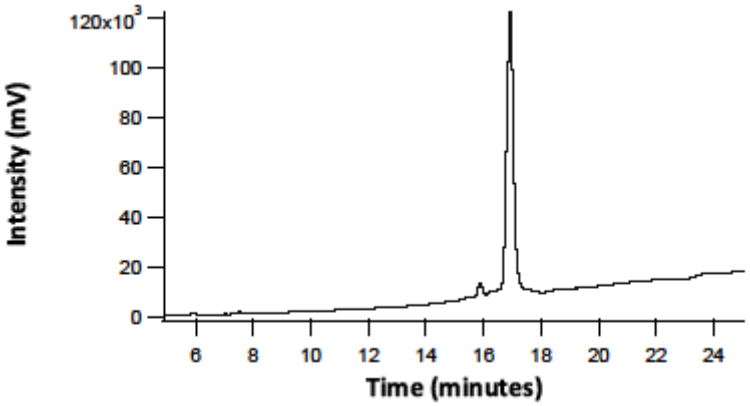
HPLC conjugation reaction on liposomes



120 minutes

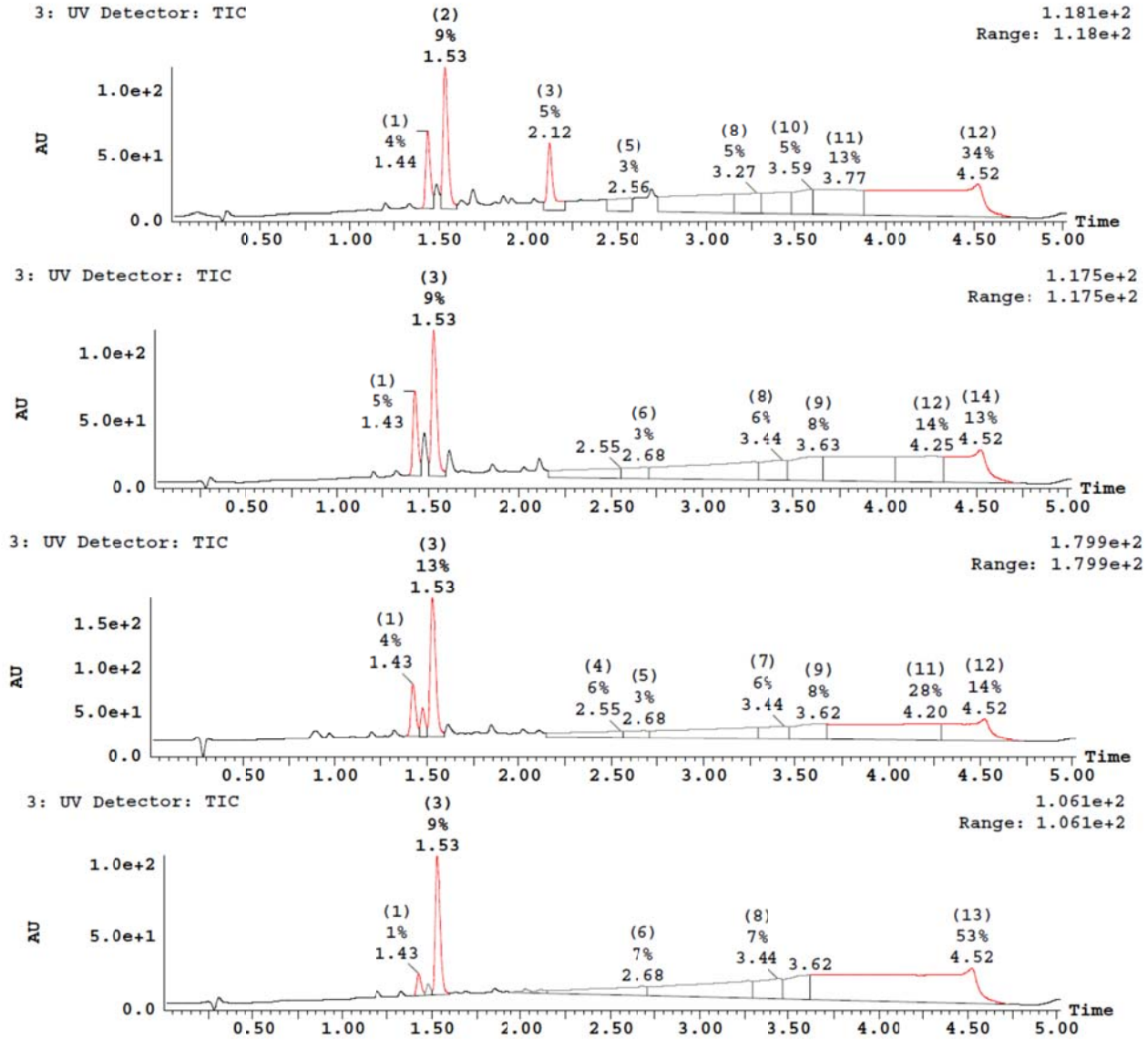


240 minutes

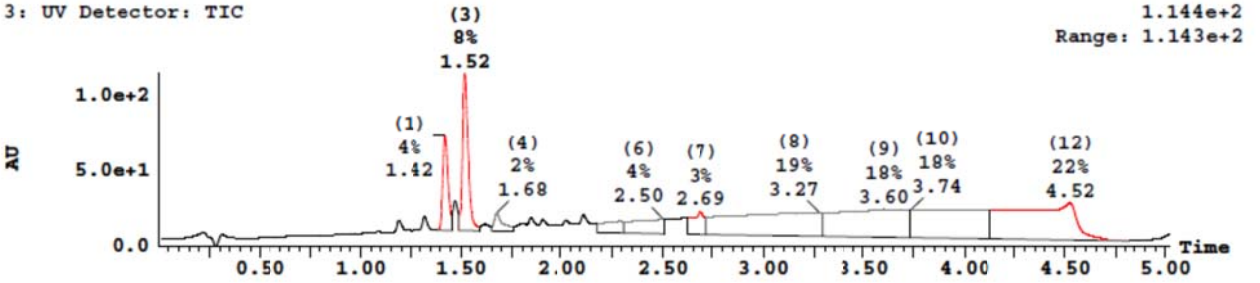


1440 minutes

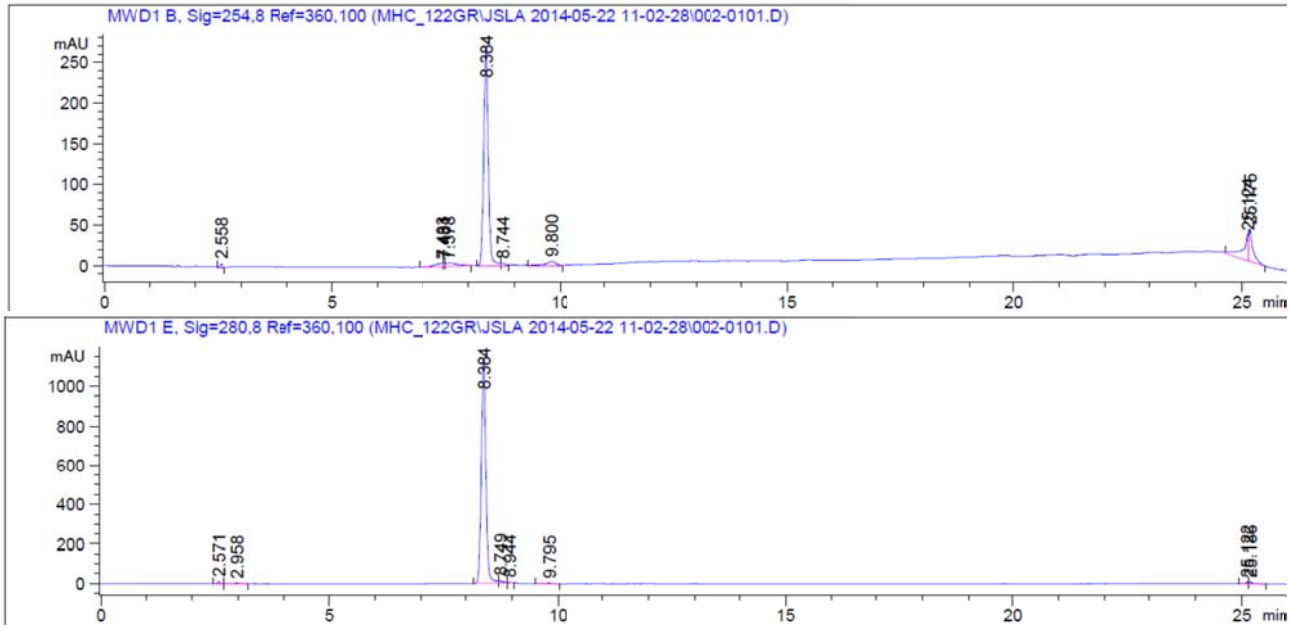
LC-MS analysis data for Table 1.



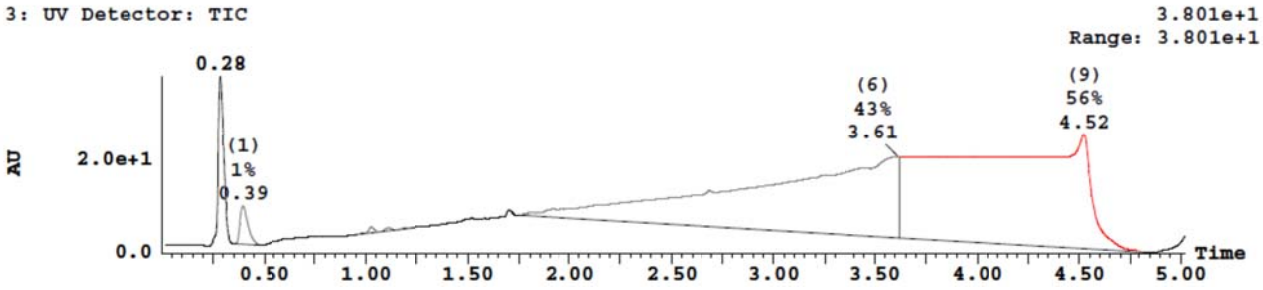
LC-DAD chromatograms for Table 1, entries 1-4. Signal at 1.43 (m/z 451.1, M+H) is thiourea **6**; signal at 1.53 (m/z 489.1, M+H) is thiazolidine **5** and signal at 2.12 is FITC (**3**90.1, M+H). Below the chromatogram for entry 1 after 48 h.



Analysis of functionalized decapeptide 15.

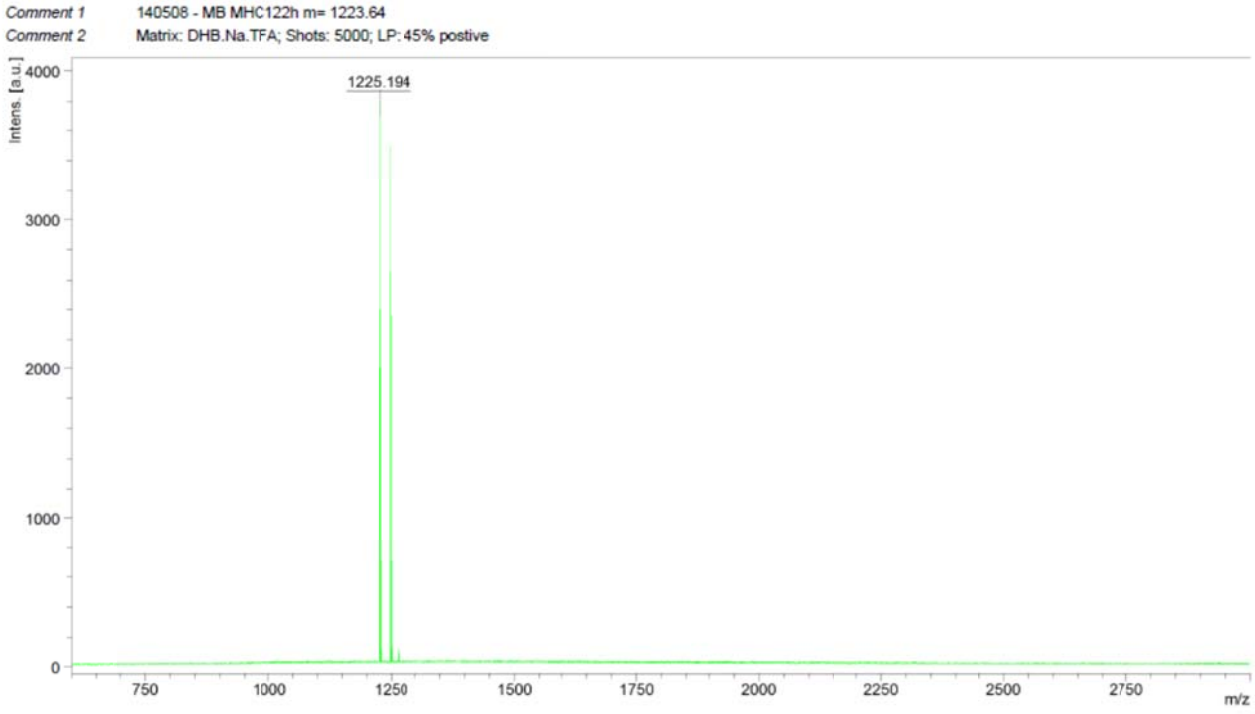


HPLC chromatogram of **15**, with detection at 254 nm (purity >90%) and 280 nm (purity >97%).



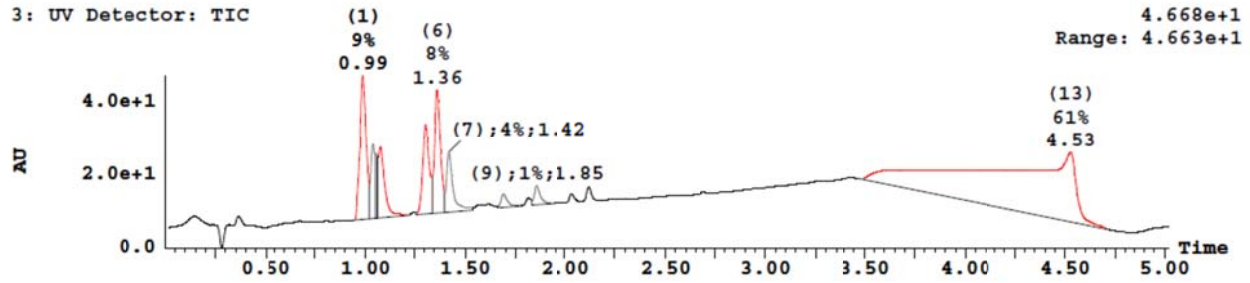
Representative LC-MS chromatogram of **15**: signal at 0.28 (m/z 409.1, M+3H). Signal at 0.39 is an artifact with the same MS profile.

Analysis of functionalized decapeptide 15.

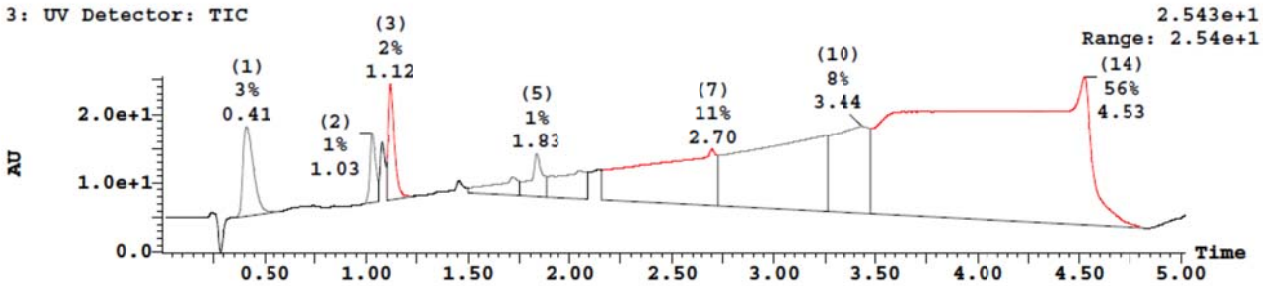


MALDI-TOF-MS of **15**. 1225 is M+H.

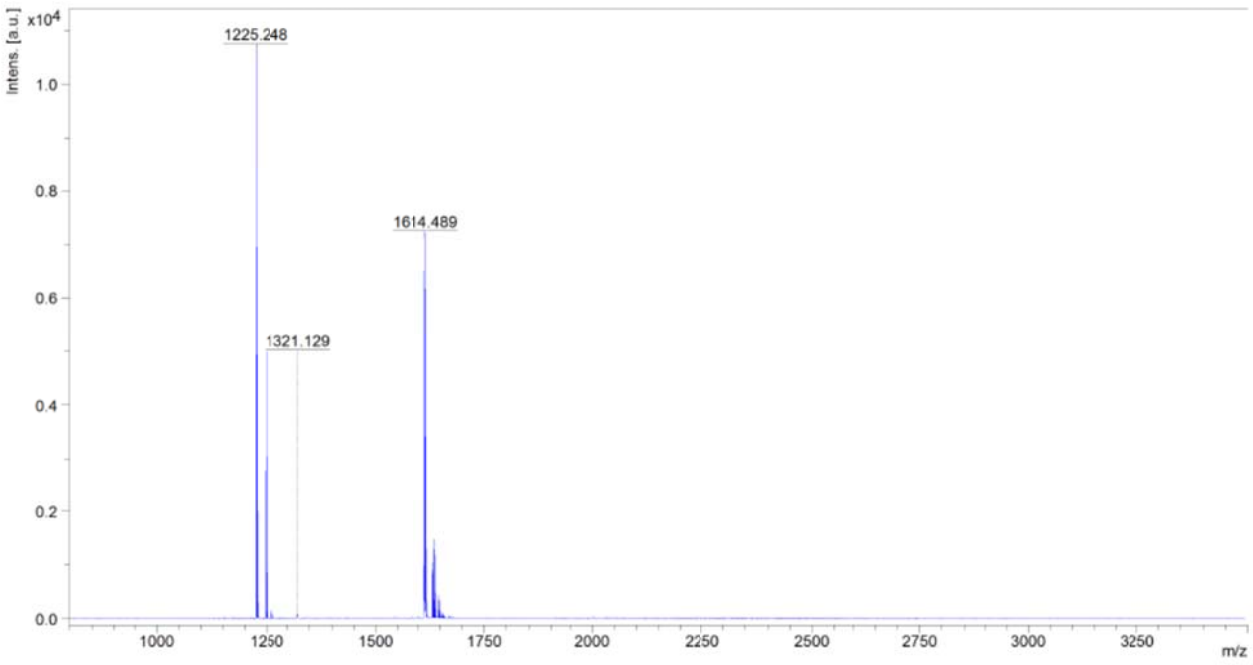
Analysis of reaction between FITC and 15.



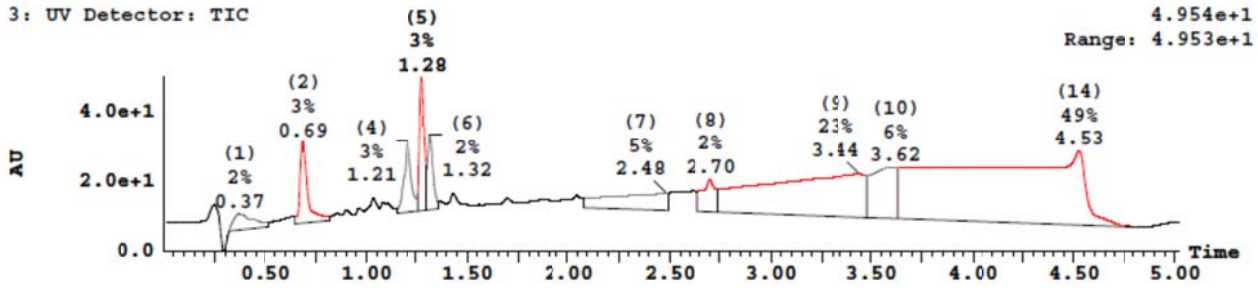
Reaction of **15** with an excess of FITC. Signals at 0.90-1.15 are mono-FITC adducts (m/z 404.4, M+4H) and signals at 1.25-1.45 are di-FITC adducts (m/z 501.6, M+4H).



Reaction of an excess of **15** with FITC. Signal at 0.41 is **15** (m/z 409.1, M+3H). Signals at 0.90-1.15 are mono-FITC adducts (m/z 404.4, M+4H).



MALDI-TOF-MS of conjugation reaction. 1225 is **15** (M+H) and 1614 the mono-FITC adduct (M+H).



Trypsin digest (1 h, 37 °C) of reaction product in PBS. Signal at 0.69 is H-Ty**r**-Tyr-NH_2 (m/z 344.3, M+H). Signals at 1.25-1.45 corresponds to **a** fragment containing FITC and $C_3H_3NHCH_2CO$-Ala-Arg-OH (m/z 365.7 M+2H).

Image showing FITC-conjugated liposomes

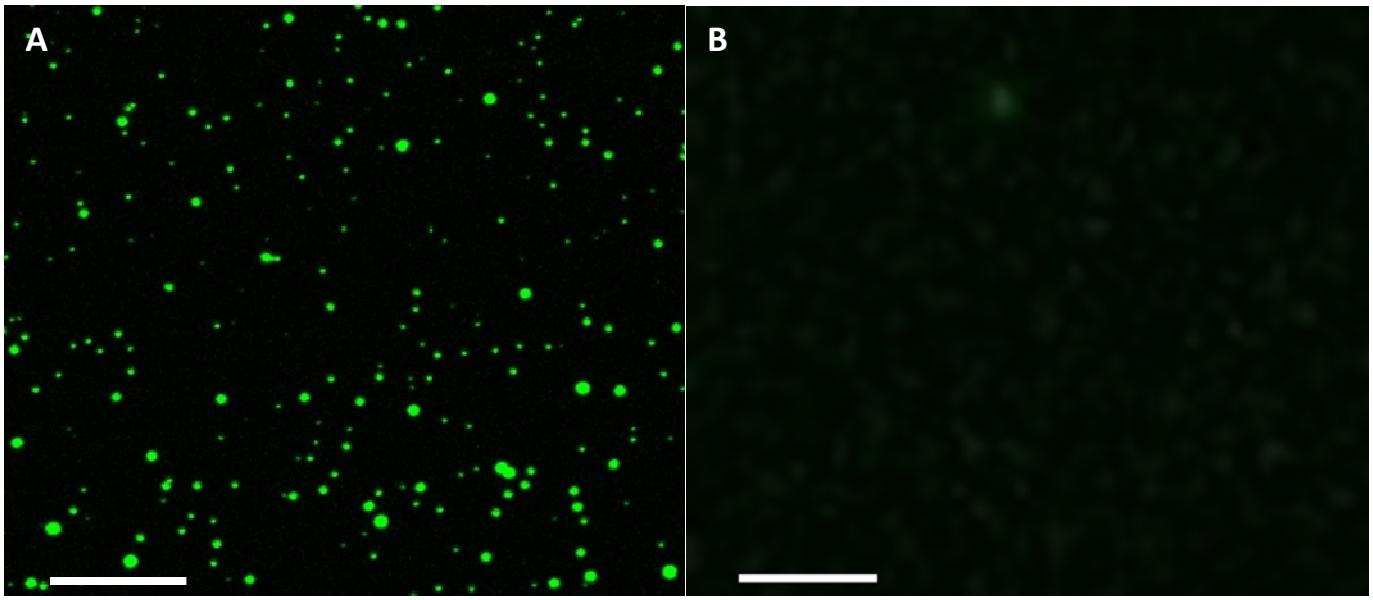


Figure S1. Fluorescent microscopy. A) FITC-functionalized liposomes immobilized on a BSA-coated glass surface, scale bar = 4 μm; B) liposomes formulated from 100% POPC incubated with FITC and dialyzed, scale bar = 2 μm.