Electronic Supplementary Information

Molecular Design of Antimicrobial Conjugated Oligoelectrolytes with Enhanced Selectivity toward Bacterial Cells

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1. General

1.1 Materials and Instruments

Solvent and reagents were purchased from common suppliers unless specified otherwise (e.g. Sigma-Aldrich, Acros Organics, Fisher Scientific, and TCI) and used as received. Dry and inhibitor-free THF and DMF were received from a solvent purification system using packed alumina columns under argon.

Flash chromatography was carried out either on Silicycle SiliaFlash P60 silica gel with pressurized air up to 0.5 bar or on Biotage SNAP C18 columns. For thin layer chromatography (TLC), EMD Millipore Analytical Chromatography TLC Silica gel 60 F254 with aluminum back were used with UV light (254/366 nm) for detection.

^{1}H-NMR (500 MHz and 600 MHz) and ^{13}C-NMR (125 MHz and 151 MHz) were measured on an actively shielded Varian Unity Inova 500 MHz spectrometer or Varian VNMRS 600 MHz. The multiplicity of all signals was described by s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), and m (multiplet). Chemical shifts (δ in ppm) were referenced to the solvent residual peak of $CDCl_3$ (^{1}H-NMR: δ = 7.26; ^{13}C-NMR: δ = 77.0) or DMSO-d_6 (^{1}H-NMR: δ = 2.50; ^{13}C-NMR: δ = 39.52). Mass spectra were collected on Waters Micromass LCT-Premier mass spectrometer or Bruker Microflex LDF MALDI-TOF mass spectrometer.

1.2 Synthesis

COE2-3C-C6 in this study was synthesized according to the previously reported procedure.[1]

General procedure for alkylation of 3,5-dihydroxybenzoate

In a round bottomed flask with a magnetic stir bar, methyl 3,5-dihydroxybenzoate (1.0 eq) and K_2CO_3 (2.5 eq) were suspended in acetone followed by an addition of α,ω-dibromoalkane (10 eq). The reaction flask was equipped with a condenser and heated to reflux under inert atmosphere. After stirring for 2 days, the reaction mixture was allowed to cool down. The mixture was diluted with dichloromethane and washed with water. Combined organic phases were washed with brine, dried over Na_2SO_4, and filtered. Solvents in the resulting solution were removed under reduced pressure. Crude product was then purified by silica gel column chromatography (1:1 DCM/hexane) to yield the desired compound.

Methyl 3,5-bis(4-bromobutoxy)benzoate (1a)

1,4-Dibromobutane was used. The product was obtained as white solid (77%). **^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm)** 7.16 (d, J = 2.5 Hz, 2H), 6.62 (t, J = 2.5 Hz, 1H), 4.01 (t, J = 6 Hz, 4H), 3.90 (s, 3H), 3.47 (t, J = 6.5 Hz, 4H), 2.10 – 2.04 (m, 4H), 1.98 – 1.92 (m, 4H); **^{13}C-NMR (126 MHz, $CDCl_3$): δ (ppm)** 167.19, 160.29, 132.41, 108.16, 106.98, 67.59, 52.65, 33.73, 29.82, 28.19; **HRMS (ESI):** ($[M+Na]^+$) calcd: 460.9763, found: 460.9754.

Methyl 3,5-bis(3-bromopropoxy)benzoate (1b)

1,3-Dibromopropane was used. The product was obtained as white solid (80%). **^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm)** 7.19 (d, *J* = 2.5 Hz, 2H), 6.65 (t, *J* = 2.5 Hz, 1H), 4.12 (t, *J* = 5.5 Hz, 4H), 3.91 (s, 3H), 3.59 (t, *J* = 6 Hz, 4H), 2.53-2.29 (m, 4H); **^{13}C-NMR (126 MHz, $CDCl_3$): δ (ppm)** 166.85, 159.86, 132.24, 108.12, 106.80, 65.77, 52.42, 32.36, 29.94; **HRMS (ESI):** (M^+) calcd: 407.9572, found: 407.9575.

Methyl 3,5-bis(2-bromoethoxy)benzoate (1c)

1,2-Dibromoethane was used. The product was obtained as white solid (31%). **^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm)** 7.21 (d, *J* = 2.5 Hz, 2H), 6.69 (t, *J* = 2.5 Hz, 1H), 4.31 (t, *J* = 6 Hz, 4H), 3.91 (s, 3H), 3.63 (t, *J* = 6 Hz, 4H); **^{13}C-NMR (126 MHz, $CDCl_3$): δ (ppm)** 166.51, 159.33, 132.45, 108.61, 107.37, 68.27, 52.50, 28.97; **HRMS (ESI):** (M^+) calcd: 381.9239, found: 381.9240.

General procedure for the synthesis of 3,5-bis(bromoalkoxy)benzaldehydes (2a-2c)

A flame-dried round bottomed flask was charged with a stir bar, methyl 3,5-bis(bromoalkoxy)benzoate (1 eq), and THF. The solution was cooled down to –78 °C in an acetone-dye ice bath. DIBAL (2.5 eq) was added dropwise to the solution via a syringe. The resulting solution was warmed up to room temperature and stirred overnight. The reaction was worked up using Fieser work-up procedure.[2] The crude product was directly transferred to another round bottom flask equipped with a stir bar without further purification. Activated MnO_2 (15 eq) and DCM were added to the flask. The suspension was stirred overnight at room temperature and then filtered through Celite. The solvent was dried under reduced pressure to obtain the desired product.

3,5-bis(4-bromobutoxy)benzaldehyde (2a)

Methyl 3,5-bis(4-bromobutoxy)benzoate (**1a**) was used. The product was obtained as white solid (89% over two steps). **^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm)** 9.89 (s, 1H), 6.99 (d, *J* = 2 Hz, 2H), 6.68 (t, *J* = 2 Hz, 1H), 4.03 (t, *J* = 6 Hz, 4H), 3.48 (t, *J* = 6.5 Hz, 4H), 2.10-2.04 (m, 4H), 1.99-1.94 (m, 4H) ;**^{13}C-NMR (126 MHz, $CDCl_3$): δ (ppm)** 192.01, 160.65, 138.55, 108.12, 107.85, 67.46, 33.39, 29.53, 27.90; **HRMS (ESI):** ($[M+Na+MeOH]^+$) calcd: 462.9919, found: 462.9913.

3,5-bis(3-bromopropoxy)benzaldehyde (2b)

Methyl 3,5-bis(3-bromopropoxy)benzoate (**1b**) was used. The product was obtained as white solid (90% over two steps). **^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm)** 9.91 (s, 1H), 7.03 (d, *J* = 2 Hz, 2H), 6.72 (t, *J* = 2.5 Hz, 1H), 4.15 (t, *J* = 6 Hz, 4H), 3.59 (t, *J* = 6.5 Hz, 4H), 2.36-2.32 (m, 4H); **^{13}C-NMR (126 MHz, $CDCl_3$): δ (ppm)** 191.91, 160.49, 138.60, 108.20, 108.05, 65.89, 32.28, 29.84; **HRMS (ESI):** ($[M+Na+CH_3OH]^+$) calcd: 434.9606, found: 434.9615.

3,5-bis(2-bromoethoxy)benzaldehyde (2c)

Methyl 3,5-bis(2-bromoethoxy)benzoate was used (**1c**). The product was obtained as white solid (85% over two steps). **^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm)** 9.91 (s, 1H), 7.04 (d, *J* = 2 Hz, 2H), 6.75 (t, *J* = 2 Hz, 1H), 4.33 (t, *J* = 6 Hz, 4H), 3.65 (t, *J* = 6.5 Hz, 4H); **^{13}C-NMR (126 MHz, $CDCl_3$): δ (ppm)** 191.60, 159.97, 138.72, 108.59, 108.48, 68.33, 28.85; **HRMS (ESI):** ($[M]^+$) calcd: 351.9133, found: 351.9124.

General procedure for HWE reactions of 3,5-bis(bromoalkoxy)benzalhydes (2a-2c).

A flame dried round bottomed flask equipped with a stir bar was charged with 3,5-bis(bromoalkoxy)benzaldehyde (1.95 eq), compound **3** (1 eq), and THF under inert atmosphere. The solution was stirred and cooled down to 0 °C in an ice bath. A solution of sodium tert-butoxide (2.2 eq) in dry THF was slowly added to the reaction flask with a syringe. The resulting solution was allowed to warm up to room temperature and was stirred overnight. The reaction mixture was diluted and extracted with DCM, washed with water and brine, dried over Na_2SO_4. Organic solvents were removed under vacuum. The crude product was purified using silica gel column chromatography (2:3 DCM/hexane) to obtain the desire product.

Compound 4a

The product was obtained as pale yellow solid (75%). **^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm)** 7.50 (s, 4H), 7.06 (d, *J* = 16.5 Hz, 2H), 7.01 (d, *J* = 16 Hz, 2H), 6.66 (d, *J* = 2.5 Hz, 4H), 6.37 (t, *J* = 2.5 Hz, 2H), 4.02 (t, *J* = 6 Hz, 8H), 3.50 (t, *J* = 6.5 Hz, 8H), 2.12-2.06 (m, 8H), 1.99-1.94 (m, 8H); **^{13}C-NMR (126 MHz, $CDCl_3$): δ (ppm)** 160.40, 139.51, 136.76, 128.93, 128.70, 127.07, 105.38, 101.11, 67.09, 33.59, 29.63, 28.04; **MS (MALDI-TOF):** calcd: 886.0; found: 886.2.

Compound 4b

The product was obtained as white solid (89%). **^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm)** 7.50 (s, 4H), 7.07 (d, *J* = 16 Hz, 2H), 7.02 (d, *J* = 16.5 Hz, 2H), 6.69 (d, *J* = 2 Hz, 4H), 6.41 (t, *J* = 2 Hz, 2H), 4.13 (t, *J* = 6 Hz, 8H), 3.61 (t, *J* = 6 Hz, 8H), 2.37-2.32 (m, 8H); **^{13}C-NMR (126 MHz, $CDCl_3$): δ (ppm)** 160.23, 139.61, 136.76, 129.06, 128.62, 127.10, 105.59, 101.25, 65.59, 32.51, 30.14; **MS (MALDI-TOF):** calcd: 829.946; found: 830.052.

Compound 4c

Due to very low solubility of the product, the product was purified from crude reaction by vacuum filtration and washed three times with methanol. The product was obtained as white solid (94%). **^{1}H-NMR (500 MHz, DMSO-d_6): δ (ppm)** 7.61 (s, 1H), 7.31 (d, *J* = 16.5 Hz, 2H), 7.20 (d, *J* = 16.5 Hz, 2H), 6.85 (d, *J* = 2.5 Hz, 4H), 6.48 (t, *J* = 2 Hz, 2H), 4.36 (t, *J* = 5 Hz, 8H), 3.81 (t, *J* = 6 Hz, 8H); **MS (MALDI-TOF):** calcd: 773.884; found: 773.969.

General Procedure for Finkelstein Reactions of 4a-4c

In a gas-tight vessel equipped with a magnetic stir bar, **4a**, **4b**, or **4c** (1 eq), NaI (20 eq), and acetone were added under inert atmosphere. The vessel was sealed and heated up to 70 °C (90 ºC for **4c**) with stirring for 2 days. The reaction mixture was diluted with DCM and filtered through a silica plug. Organic solvents were removed under reduced pressure to yield a target molecule.

Compound 5a

Crude product was purified by recrystallization in DCM/MeOH to obtain the compound as pale yellow solid (93%). **^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm)** 7.50 (s, 4H), 7.06 (d, *J* = 16.5 Hz, 2H), 7.01 (d, *J* = 16 Hz, 2H), 6.66 (d, *J* = 2.5 Hz, 4H), 6.36 (t, *J* = 2 Hz, 2H), 4.01 (t, *J* = 6 Hz, 8H), 3.27 (t, *J* = 7 Hz, 8H), 2.08-2.02 (m, 8H), 1.95-1.89 (m, 8H); **^{13}C-NMR (126 MHz, $CDCl_3$): δ (ppm)** 160.40, 139.51, 136.76, 128.94, 128.70, 127.07, 105.38, 101.11, 66.89, 30.33, 30.29, 6.55; **MS (MALDI-TOF):** calcd: 1074.0, found: 1074.3.

Compound 5b

Crude product was purified by recrystallization in DCM/MeOH to obtain the compound as yellow solid (90 %). **^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm)** 7.50 (s, 4H), 7.08 (d, *J* = 16 Hz, 2H), 7.02 (d, *J* = 16 Hz, 2H), 6.69 (d, *J* = 2.5 Hz, 4H), 6.40 (t, *J* = 2 Hz, 2H), 4.07 (t, *J* = 6 Hz, 8H), 3.38 (t, *J* = 7 Hz, 8H), 2.32-2.27 (m, 8H); **^{13}C-NMR (126 MHz, $CDCl_3$): δ (ppm)** 160.22, 139.60, 136.76, 129.07, 128.62, 127.10, 105.60, 101.27, 67.54, 33.12, 2.63; **MS (MALDI-TOF):** calcd: 1017.895, found: 1017.980.

Compound 5c

The product was obtained by vacuum filtration from crude reaction as pale yellow solid (85 %) and was used for quaternization reactions without any further purification.

***N*,*N*-dimethylpentylamine**

To a flame-dried round bottom flask equipped with a magnetic stir bar, dimethylamine (11.1 mL, 2 M in THF) was added under inert atmosphere. The solution was cooled down to –78 °C using dry ice-acetone bath. 1-Iodopentane (0.3 mL, 2.22 mmol) was added to the flask using a syringe. The reaction flask was allowed to warm up to room temperature and kept stirring overnight. The reaction mixture was washed with saturated K_3PO_4 solution, extracted with diethyl ether. Combined organic layers were dried over Na_2SO_4. Solvents were removed under reduced pressure to obtain the product as slightly yellow liquid. The product was used without

further purification. **^{1}H-NMR: δ (ppm) (500 MHz, $CDCl_3$):** 2.21 (t, *J* = 6 Hz, 2H), 2.21 (s, 6H), 1.48 – 1.42 (m, 2H), 1.36 – 1.24 (m, 4H), 0.88 (t, *J* = 7 Hz, 3H); **^{13}C-NMR: δ (ppm) (126 MHz, $CDCl_3$):** 60.11, 45.69, 29.87, 27.64, 22.80, 14.20.

General Procedure for Quaternization Reactions

In a 1 Dr vial equipped with a magnetic stir bar, 20 mg of **5a**, **5b**, or **5c** was added under inert atmosphere. DMF (1 mL) and an amine (10 eq) were then added to the reaction vial. The reaction vial was sealed and kept stirring at 45 °C for 2 days. After cooling down to room temperature, the reaction mixture was precipitated and washed with diethyl ether twice. The precipitate was subjected to reverse phase column chromatography (3:7 MeOH:water) to obtain a pure target molecule. For the ease of handling of the product, the product was dissolved in water and lyophilized.

COE2-3C-C4hexyl

The product was obtained as white solid (60 %). **^{1}H-NMR: δ (ppm) (500 MHz, CD_3OD):** 7.58 (s, 4H), 7.21 (d, *J* = 17 Hz, 2H), 7.14 (d, *J* = 17 Hz, 2H), 6.80 (s, 4H), 6.52 (s, 2H), 4.13 (t, *J* = 6 Hz, 8H), 3.45-3.48 (m, 8H), 3.35-3.37 (m, 8H), 3.14 (s, 23H), 1.99-2.04 (m, 8H), 1.90-1.94 (m, 8H), 1.76-1.80 (m, 8H), 1.34-1.41 (m, 24H), 0.91 (t, *J* = 7 Hz, 12H); **^{13}C-NMR: δ (ppm) (126 MHz, CD_3OD):** 161.64, 141.01, 138.15, 129.91, 129.61, 128.03, 106.57, 102.15, 68.18, 65.42, 64.83, 51.48, 32.41, 27.10, 23.60, 23.53, 20.66, 14.31; **HRMS (ESI):** ($[M-2I]^{2+}$) calcd: 668.3778; found: 668.3784.

COE2-3C-C4pentyl

The product was obtained as white solid (65 %). **^{1}H-NMR: δ (ppm) (600 MHz, DMSO-d_6):** 7.61 (s, 4H), 7.28 (d, *J* = 17 Hz, 2H), 7.20 (d, *J* = 16 Hz, 2H), 6.82 (s, 4H), 6.45 (s, 2H), 4.06 (t, *J* = 6 Hz, 8H), 3.35-3.37 (m, 8H), 3.26-3.27 (m, 8H), 3.04 (s, 23H), 1.82-1.84 (m, 8H), 1.76-1.77 (m, 8H), 1.66-1.69 (m, 8H), 1.32-1.35 (m, 8H), 1.23-1.28 (m, 8H), 0.88 (t, *J* = 6 Hz, 12H); **^{13}C-NMR: δ (ppm) (151 MHz, DMSO-d_6):** 159.80, 139.11, 136.35, 128.54, 128.35, 126.88, 105.18, 100.91, 66.73, 63.00, 62.48, 50.05, 27.90, 25.57, 21.62, 21.40, 18.81, 13.73; **HRMS (ESI):** ($[M-2I]^{2+}$) calcd: 640.3465, found: 640.3456.

COE2-3C-C4butyl

The product was obtained as white solid (75 %). **^{1}H-NMR: δ (ppm) (500 MHz, CD_3OD):** 7.58 (s, 4H), 7.20 (d, *J* = 17 Hz, 2H), 7.13 (d, *J* = 17 Hz, 2H), 6.79 (t, *J* = 2 Hz, 4H), 6.51 (s, 2H), 4.12 (t, *J* = 6 Hz, 8H), 3.44-3.47 (m, 8H), 3.34-3.38 (m, 8H), 1.98-2.04 (m, 8H), 1.88-1.92 (m, 10H), 1.73-1.79 (m, 8H), 1.38-1.44 (m, 10H), 0.99 (t, *J* = 8 Hz, 12H); **^{13}C-NMR: δ (ppm) (151 MHz, DMSO-d_6):** 159.81, 139.13, 136.36, 127.01, 126.80, 105.31, 105.30, 105.06, 101.06, 66.76, 62.84, 62.50, 50.03, 25.58, 23.71, 19.20, 18.82, 13.53; **HRMS (ESI):** ($[M-2I]^{2+}$) calcd: 612.3152, found: 612.3157.

COE2-3C-C4ethyl

The product was obtained as white solid (94 %). **^{1}H-NMR: δ (ppm) (500 MHz, DMSO-d_6):** 7.61 (s, 4H), 7.29 (d, *J* = 16 Hz, 2H), 7.20 (d, *J* = 16 Hz, 2H), 6.82 (s, 4H), 6.46 (s, 2H), 4.06 (t, *J* = 6 Hz, 8H), 3.34-3.39 (m, 15H), 3.02 (s, 23H), 1.81-1.87 (m, 8H), 1.74-1.79 (m, 8H), 1.24 (t, *J* = 7 Hz, 12H); **^{13}C-NMR: δ (ppm) (126 MHz, DMSO-d_6):** 159.81, 139.13, 136.36, 128.56, 128.36, 126.91, 105.19, 100.96, 66.83, 62.00, 58.61, 49.50, 25.62, 18.80, 7.86; **HRMS (ESI):** ($[M-2I]^{2+}$) calcd: 556.2526; found: 556.2526.

COE2-3C-C4

The product was obtained as white solid (94 %). **^{1}H-NMR: δ (ppm) (600 MHz, DMSO-d_6):** 7.60 (s, 4H), 7.25 (dd, *J* = 36.5, 15.9 Hz, 4H), 6.81 (s, 4H), 6.44 (s, 2H), 4.05 (t, *J* = 6.2 Hz, 8H), 3.43 – 3.37 (m, 8H), 3.10 – 3.04 (m, 36H), 1.90 – 1.79 (m, 8H), 1.74 (p, *J* = 6.8 Hz, 8H); **^{13}C-NMR: δ (ppm) (151 MHz, DMSO-d_6):** 160.27, 139.60, 136.82, 129.03, 128.82, 127.36, 109.99, 105.67, 101.43, 67.32, 65.45, 52.74, 52.72, 52.69, 40.43, 40.30, 40.16, 40.02, 39.88, 39.81, 39.74, 39.60, 26.10, 19.70; **HRMS (ESI):** ($[M-2I]^{2+}$) calcd: 528.2213, found: 528.2205.

COE2-3C-C3hexyl

The product was obtained as white solid (62 %). **^{1}H-NMR: δ (ppm) (600 MHz, DMSO-d_6):** 7.63 (s, 4H), 7.27 (dd, *J* = 28.2, 16.5 Hz, 4H), 6.85 (d, *J* = 2.1 Hz, 4H), 6.47 (t, *J* = 2.1 Hz, 2H), 4.11 (t, *J* = 5.9 Hz, 8H), 3.51 – 3.45 (m, 8H), 3.09 (s, 24H), 2.22 – 2.13 (m, 8H), 1.73 – 1.64 (m, 8H), 1.32 – 1.27 (m, 24H), 0.88 (t, *J* = 6.7 Hz, 8H); **^{13}C-NMR: δ (ppm) (151 MHz, DMSO-d_6):** 159.98, 139.76, 136.82, 129.21, 128.73, 127.41, 105.98, 101.61, 65.29, 63.50, 60.86, 50.72, 40.42, 40.28, 40.15, 40.01, 39.87, 39.73, 39.59, 31.12, 25.86, 22.70, 22.34, 22.14, 14.29; **HRMS (ESI):** ($[M-2I]^{2+}$) calcd: 640.3465, found: 640.3469.

COE2-3C-C3butyl

The product was obtained as white solid (72 %). **^{1}H-NMR: δ (ppm) (600 MHz, DMSO-d_6):** 7.63 (s, 4H), 7.27 (dd, *J* = 26.7, 16.3 Hz, 4H), 6.85 (d, *J* = 2.3 Hz, 4H), 6.47 (d, *J* = 1.7 Hz, 4H), 4.11 (t, *J* = 6.0 Hz, 8H), 3.53 – 3.44 (m, 8H), 3.09 (s, 24H), 2.24 – 2.11 (m, 8H), 1.72 – 1.64 (m, 8H), 1.33 (h, *J* = 7.4 Hz, 8H), 0.95 (t, *J* = 7.4 Hz, 12H); **^{13}C-NMR: δ (ppm) (151 MHz, DMSO-d_6):** 159.98, 139.75, 136.83, 129.22, 128.73, 127.43, 105.98, 101.61, 65.31, 63.34, 60.91, 50.72, 40.42, 40.28, 40.14, 40.00, 39.86, 39.72, 39.59, 24.19, 22.71, 19.63, 14.05, 13.98; **HRMS (ESI):** ($[M-2I]^{2+}$) calcd: 584.2839, found: 584.2831.

COE2-3C-C3ethyl

The product was obtained as white solid (80 %). **^{1}H-NMR: δ (ppm) (600 MHz, DMSO-d_6):** 7.63 (s, 4H), 7.28 (dd, *J* = 30.0, 16.4 Hz, 4H), 6.85 (s, 2H), 6.47 (t, *J* = 2.2 Hz, 1H), 4.12 (t, *J* = 6.0 Hz, 8H), 3.52 – 3.44 (m, 8H), 3.42 (q, *J* = 7.2 Hz, 8H), 3.07 (s, 24H), 2.21 – 2.13 (m, 8H), 1.28 (t, *J* = 7.2 Hz, 13H); **^{13}C-NMR: δ (ppm) (151 MHz, DMSO-d_6):** 160.01, 139.75, 136.83, 129.22, 128.73, 127.43, 105.98, 101.58, 65.33, 60.38, 59.11, 50.16, 40.43, 40.29, 40.15, 40.01, 39.87, 39.73, 39.59, 22.68, 8.36; **HRMS (ESI):** ($[M-2I]^{2+}$) calcd: 528.2213, found: 528.2213.

COE2-3C-C3

The product was obtained as white solid (76 %). **^{1}H-NMR: δ (ppm) (600 MHz, DMSO-d_6):** 7.63 (s, 4H), 7.27 (dd, *J* = 30.9, 15.9 Hz, 4H), 6.85 (s, 4H), 6.48 (s, 2H), 4.11 (t, *J* = 6.0 Hz, 8H), 3.56 – 3.50 (m, 8H), 3.14 (s, 36H), 2.20 (dq, *J* = 11.9, 6.0 Hz, 8H); **^{13}C-NMR (151 MHz, DMSO-d_6):** 160.02, 139.75, 136.82, 129.23, 128.72, 127.43, 105.98, 101.56, 65.37, 63.47, 63.45, 63.43, 52.87, 52.84, 52.82, 40.41, 40.27, 40.13, 39.99, 39.85, 39.71, 39.57, 23.09; **HRMS (ESI):** ($[M-2I]^{2+}$) calcd: 500.1900, found: 500.1905.

COE2-3C-C2hexyl

The product was obtained as white solid (55 %). **^{1}H-NMR: δ (ppm) (500 MHz, DMSO-d_6):** 7.64 (s, 4H), 7.34 (d, *J* = 17 Hz, 2H), 7.24 (d, *J* = 17 Hz, 2H), 6.94 (s, 4H), 6.55 (s, 2H), 4.51 (t, *J* = 5 Hz, 8H), 3.78 (t, *J* = 5 Hz, 8H), 3.39-3.42 (m, 8H), 3.16 (s, 22H), 1.72-1.75 (m, 8H), 1.29-1.36 (m, 23H), 0.87 (t, *J* = 7 Hz, 12H); **^{13}C-NMR: δ (ppm) (126 MHz, DMSO-d_6):** 158.72, 139.43, 136.35, 129.02, 128.08, 126.97, 105.92, 101.40, 64.09, 61.88, 61.69, 50.89, 30.70, 25.44, 21.88, 21.82, 13.83; **HRMS (ESI):** ($[M-2I]^{2+}$) calcd: 612.3152, found: 612.3157.

COE2-3C-C2butyl

The product was obtained as white solid (71 %). **^{1}H-NMR: δ (ppm) (500 MHz, DMSO-d_6):** 7.65 (s, 4H), 7.36 (d, *J* = 16 Hz, 2H), 7.25 (d, *J* = 16 Hz, 2H), 6.95 (s, 4H), 6.56 (s, 2H), 4.51

(t, *J* = 5 Hz, 8H), 3.79 (t, *J* = 5 Hz, 8H), 3.41-3.44 (m, 8H), 3.17 (s, 23H), 1.71-1.74 (m, 8H), 1.32-1.36 (m, 8H), 0.94 (t, *J* = 8 Hz, 12H); **^{13}C-NMR: δ (ppm) (126 MHz, DMSO-d_6):** 159.22, 139.96, 136.86, 129.55, 128.59, 127.49, 106.41, 101.95, 64.22, 62.39, 62.23, 51.41, 24.34, 19.72, 14.03; **HRMS (ESI):** ($[M-2I]^{2+}$) calcd: 556.2526, found: 556.2534.

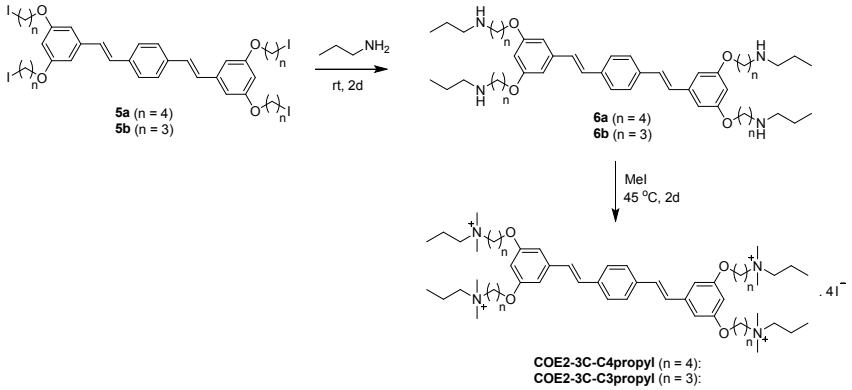
COE2-3C-C2ethyl

The product was obtained as white solid (75 %). **^{1}H-NMR: δ (ppm) (500 MHz, DMSO-d_6):** 7.64 (s, 4H), 7.36 (d, *J* = 16.5 Hz, 2H), 7.24 (d, *J* = 16 Hz, 2H), 6.95 (d, *J* = 2 Hz, 4H), 6.60 (s, 2H), 4.51 (t, *J* = 5 Hz, 8H), 3.79 (t, *J* = 5 Hz, 8H), 3.53-3.50 (m, 8H), 3.15 (s, 24H), 1.30 (t, *J* = 7 Hz, 12H); **^{13}C-NMR: δ (ppm) (126 MHz, DMSO-d_6):** 158.72, 139.37, 136.34, 129.01, 128.04, 126.95, 105.98, 101.41, 61.70, 61.38, 59.70, 50.29, 8.01; **HRMS (ESI):** ($[M-2I]^{2+}$) calcd: 500.1900, found: 500.1896.

COE2-3C-C2

The product was obtained as white solid (81 %). **^{1}H-NMR: δ (ppm) (600 MHz, DMSO-d_6):** 7.64 (s, 4H), 7.36 (d, *J* = 16 Hz, 2H), 7.25 (d, *J* = 16 Hz, 2H), 6.96 (d, *J* = 2 Hz, 4H), 6.60 (t, *J* = 2 Hz, 2H), 4.52 (t, *J* = 5 Hz, 8H), 3.82 (t, *J* = 5 Hz, 8H), 3.23 (s, 36H); **^{13}C-NMR: δ (ppm) (151 MHz, DMSO-d_6):** 158.71, 139.41, 136.36, 129.01, 128.07, 126.96, 106.04, 101.53, 64.87, 64.04, 53.23; **HRMS (ESI):** ($[M-2I]^{2+}$) calcd: 472.1587, found: 472.1596.

General procedure for COE2-3C-Cnpropyl (n = 3 or 4)



In a 1 Dr vial equipped with a magnetic stir bar, **5a** or **5b** was added followed by the addition of propylamine and THF (2:3). The reaction vial was then sealed and kept stirring under inert atmosphere at room temperature. After the solution was stirred for 2 days, the solvent was removed under reduced pressure and the crude reaction was azeotroped with methanol twice. The crude reaction was used without further purification. To the vial, K_2CO_3 (10 eq), iodomethane (20 eq), and DMF was added under inert atmosphere. The resulting suspension

was stirred at 45 °C for 2 days. The crude product was precipitated and washed with diethyl ether, purified by reverse phase column chromatography to obtain a pure product. The product was dissolved in water and lyophilized for the ease of handling.

COE2-3C-C4propyl

The product was obtained as white solid (73 %). **^{1}H-NMR: δ (ppm) (500 MHz, DMSO-d_6):** 7.62 (s, 4H), 7.30 (d, *J* = 17 Hz, 2H), 7.21 (d, *J* = 17 Hz, 2H), 6.83 (s, 4H), 6.45 (t, *J* = 3 Hz, 2H), 4.06 (t, *J* = 7 Hz, 8H), 3.36-3.39 (m, 8H), 3.24-3.27 (m, 8H), 1.82-1.88 (m, 8H), 1.76-1.70 (m, 8H), 1.66-1.75 (m, 8H), 0.89 (t, *J* = 7 Hz, 12H); **^{13}C-NMR: δ (ppm) (126 MHz, DMSO-d_6):** 167.02, 145.12, 142.17, 133.91, 133.67, 132.16, 109.16, 104.67, 68.47, 65.94, 64.09, 50.71, 24.75, 17.59, 13.98, 8.77; **HRMS (ESI):** ($[M-2I]^{2+}$) calcd: 584.2839, found: 584.2826.

COE2-3C-C3propyl

The product was obtained as white solid (77 %). **1H-NMR: δ (ppm) (500 MHz, DMSO-d_6):** 7.63 (s, 4H), 7.30 (d, *J* = 17 Hz, 2H), 7.22 (d, *J* = 17 Hz, 2H), 6.85 (d, *J* = 2 Hz, 4H), 6.47 (t, *J* = 2 Hz, 2H), 4.10 (t, *J* = 6 Hz, 8H), 3.48-3.51 (m, 8H), 3.29-3.31 (m, 8H), 3.09 (s, 22H), 2.15-2.21 (m, 8H), 1.68-1.75 (m, 8H), 0.91 (t, *J* = 7 Hz, 12H); **13C-NMR: δ (ppm) (126 MHz, DMSO-d_6):** 159.50, 139.25, 136.33, 128.72, 128.22, 126.93, 105.50, 101.12, 64.85, 64.41, 60.55, 50.21, 22.22, 15.41, 10.46; **HRMS (ESI):** ($[M-2I]^{2+}$) calcd: 556.2526, found: 556.2536.

2. Minimum Inhibitory Concentration Experiments

MICs of selected COEs were determined by Emery Pharma (Alameda, CA) per Clinical and Laboratory Standards Institute (CLSI) guidelines.[3,4] Bacterial strains tested include two Gram-positive bacteria: *Enterococcus faecium* VRE (EF; 1674620), methicillin-resistant *Staphylococcus aureus* (SA; ATCC 33591); and one Gram-negative bacteria: *Klebsiella pneumoniae* MDR (KP; ATCC BAA- 2473). COEs were dissolved in DMSO and serially diluted in a 96-well plate while maintaining a final DMSO concentration at 1% v/v in every well. For each bacterial strain, bacteria were inoculated in Cation-Adjusted Mueller Hinton broth (CAMHB) from a single colony picked from a Trypticase Soy Agar (TSA) plate. The inocula were diluted and pipetted into the 96-well plate to achieve 5×10^5 cfu mL^{-1}. The highest concentration of COEs tested was 64 μg mL^{-1}. Plates were incubated for 18–20 hours at 37 ºC. The experiments were performed in triplicate for each compound. Gentamicin was used as a positive control in the studies. The results are summarized below:

Table S1. MICs of some COEs against *E. faecium* (EF), *S. aureus* (SA), and *K. pneumoniae* (KP).

n	m	Compound	MIC (μg mL^{-1})		
			EF	SA	KP
2	4	COE2-3C-C2butyl	1	1	4
3	4	COE2-3C-C3butyl	0.25	0.5	4
	6	COE2-3C-C3hexyl	0.25	2	2
4	1	COE2-3C-C4	8	2	32
	2	COE2-3C-C4ethyl	4	1	16
	3	COE2-3C-C4propyl	1	1	8
	4	COE2-3C-C4butyl	0.5	0.5	8
	6	COE2-3C-C4hexyl	0.25	0.5	4
Gentamicin			>64	1	2

3. Mammalian Cell Cytotoxicity

3.1 Determination of IC_{50}

The method for IC_{50} curve-fitting was adapted from the method published by Lambert and Pearson for determining MIC values.[5] The data, plotted as cell viability (from 0 to 1) versus the logarithm of COE concentrations, was fit to a Gompertz model. The resulting fit equation was used to determine the IC_{50} by solving for the COE concentration that resulted in y = 0.5 (i.e. where cell viability was equal to 50% of control). An example of the fitted data (black), model fit (blue line), and calculated IC_{50} (red) is provided below.

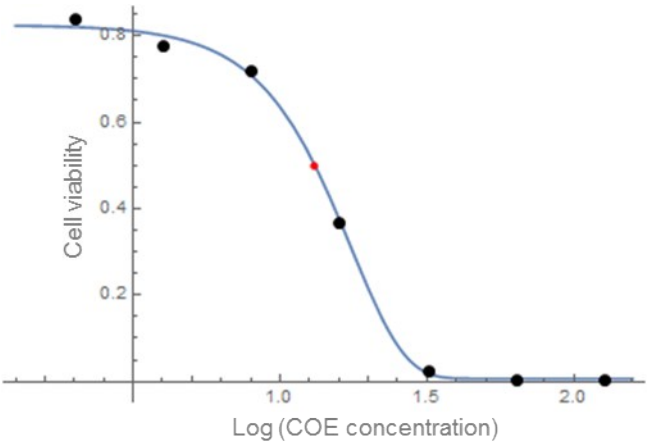
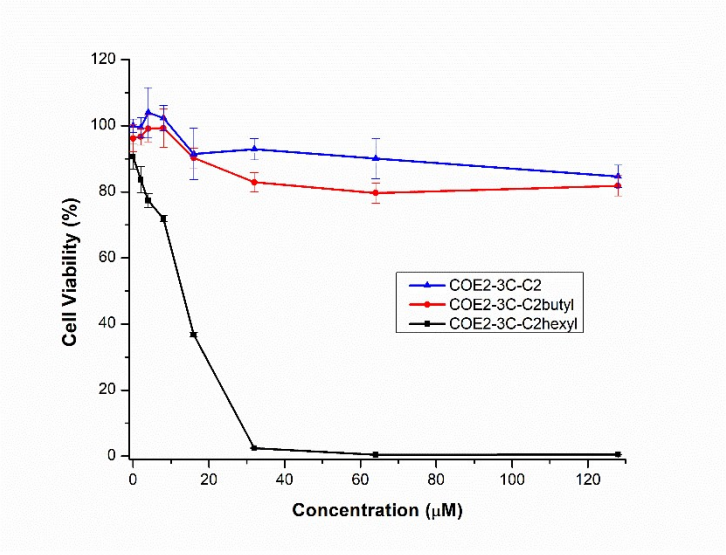
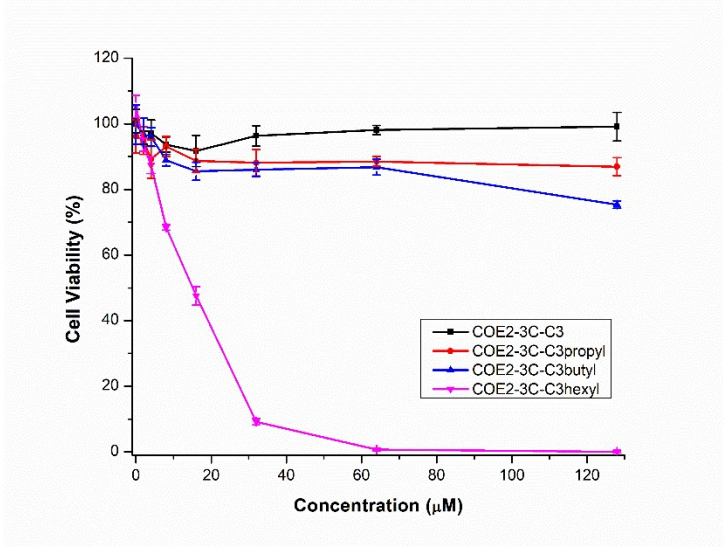
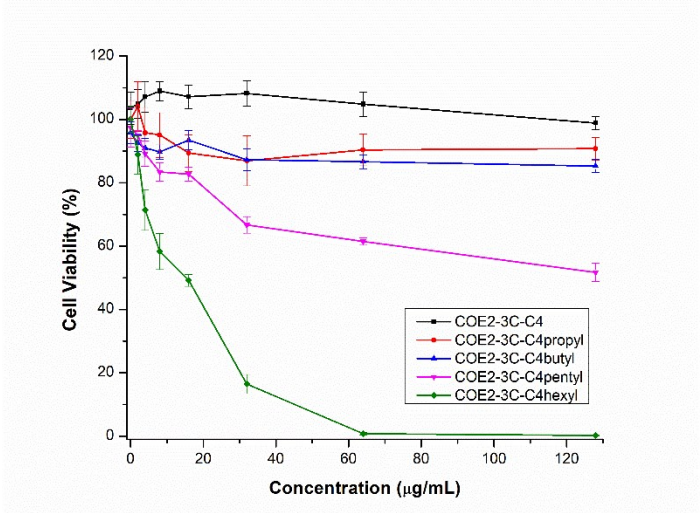


Figure S1. Curve fitting method for IC_{50} determination

3.2 Cell Viability of HepG2







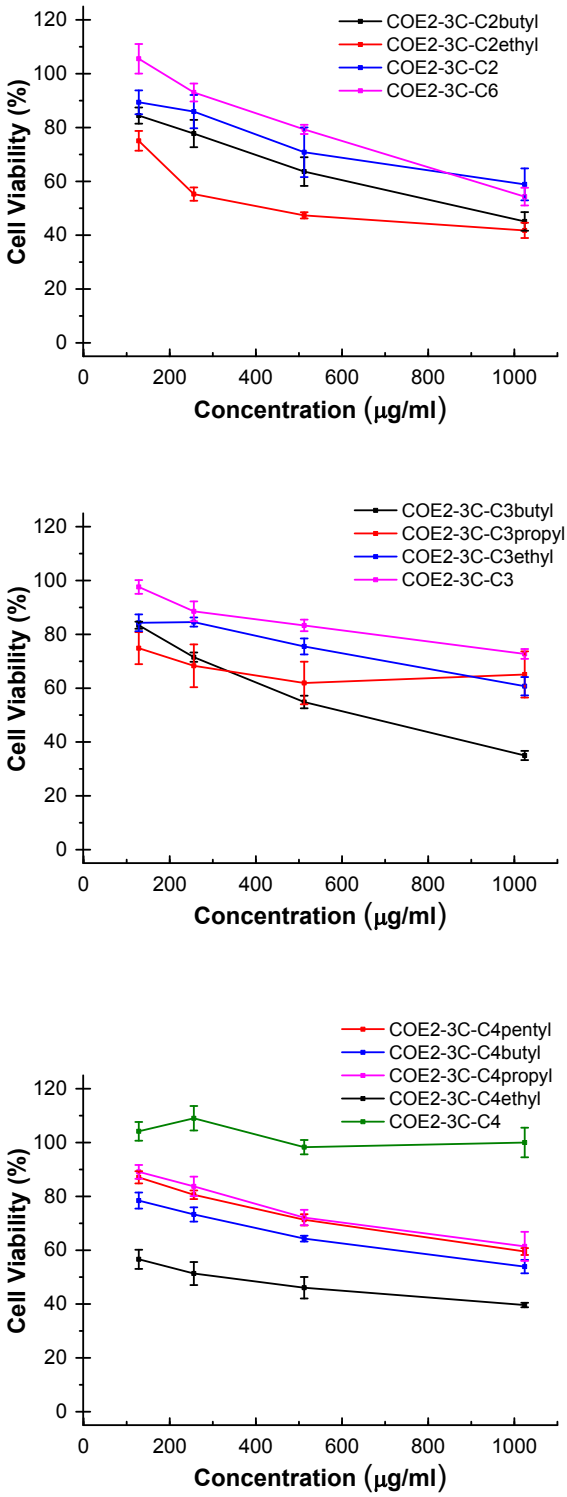


Figure S2. Percent viability of HepG2 after treatments with different concentrations of COEs.

4. Hemolytic Activity

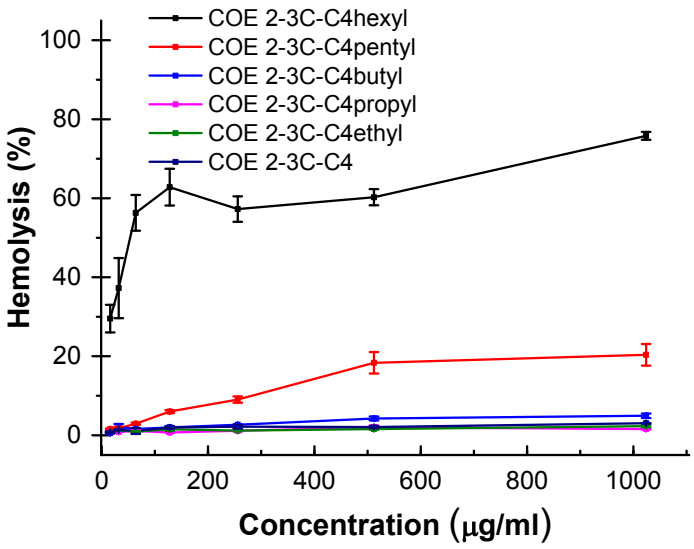


Figure S3. Percent hemolysis of CD-1 mouse red blood cells incubated with COE2-3C-C4 series for 1 h at 37 ºC in PBS

5. Cell Association Experiments

The number of COE molecules associated in each cell was determined with the following formula:

$$\text{Percent associated} \times \frac{1.2 \times 10^{13} \text{ molecules}}{\text{number of cells}}$$

The numbers of *E. coli* K12 and HepG2 cells used in each study were 7.4×10^7 cells and 8.0×10^5 cells, respectively. The number of molecules was calculated from the final concentration and volume of COE solutions in these experiments (200 µL of 20 µM solution). The results are shown as follow:

Table S2. The number of COE molecules associated to each *E. coli* K12 and HepG2 cell at 37 ºC for 2 hours in HBSS. The concentration of COEs used was 20 µM.

n	**m**	**Compound**	**10^5 Molecules associated**	
			***E. coli* K12**	**HepG2**
2	1	COE2-3C-C2	0.16 ± 0.04	45.0 ± 5.0
3	1	COE2-3C-C3	0.10 ± 0.03	32.1 ± 6.9
4	1	COE2-3C-C4	0.10 ± 0.02	21.3 ± 16.7
	2	COE2-3C-C4ethyl	0.34 ± 0.07	38.0 ± 7.7
	3	COE2-3C-C4propyl	0.51 ± 0.05	29.0 ± 7.8
	4	COE2-3C-C4butyl	1.12 ± 0.06	33.3 ± 1.7
	5	COE2-3C-C4pentyl	1.28 ± 0.01	34.2 ± 18.5
	6	COE2-3C-C4hexyl	1.61 ± 0.04	106.2 ± 4.5
6	1	COE2-3C-C6	1.33 ± 0.01	87.0 ± 4.5

6. Time-dependent cell association experiment

A single colony of *E. coli* K12 from an LB agar plate was inoculated in LB medium and cells were harvested during a mid-log phase. The cells were centrifuged at 7000 rpm for 5 minutes to remove LB, washed with HBSS, and resuspended in HBSS to achieve the cell concentration twice of the concentration in 1 OD_{600}. The cell suspension was mixed with a solution of COE2-3C-C4butyl in HBSS to achieve the COE final concentrations of 10 µM, 20 µM, and 27 µM in microcentrifuge tubes. The tubes were incubated in an incubator at 37 ºC. Treated bacterial suspension was sampled out after 5, 15, 30, 60, 90, and 120 minutes of incubation. Supernatants from the sampled suspension was obtained by pelleting cells at 7000 rpm for 4.5 minutes. The amount of COE left in the supernatant was determined by the absorption at 380 nm using Tecan Infinite M200 Plate Reader.

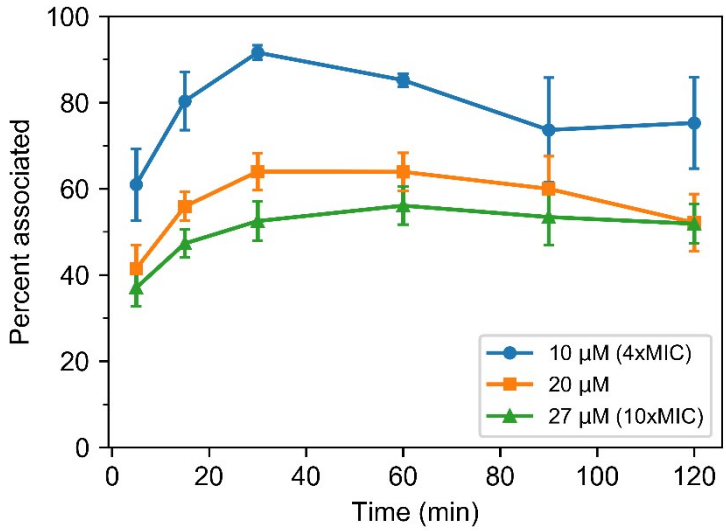
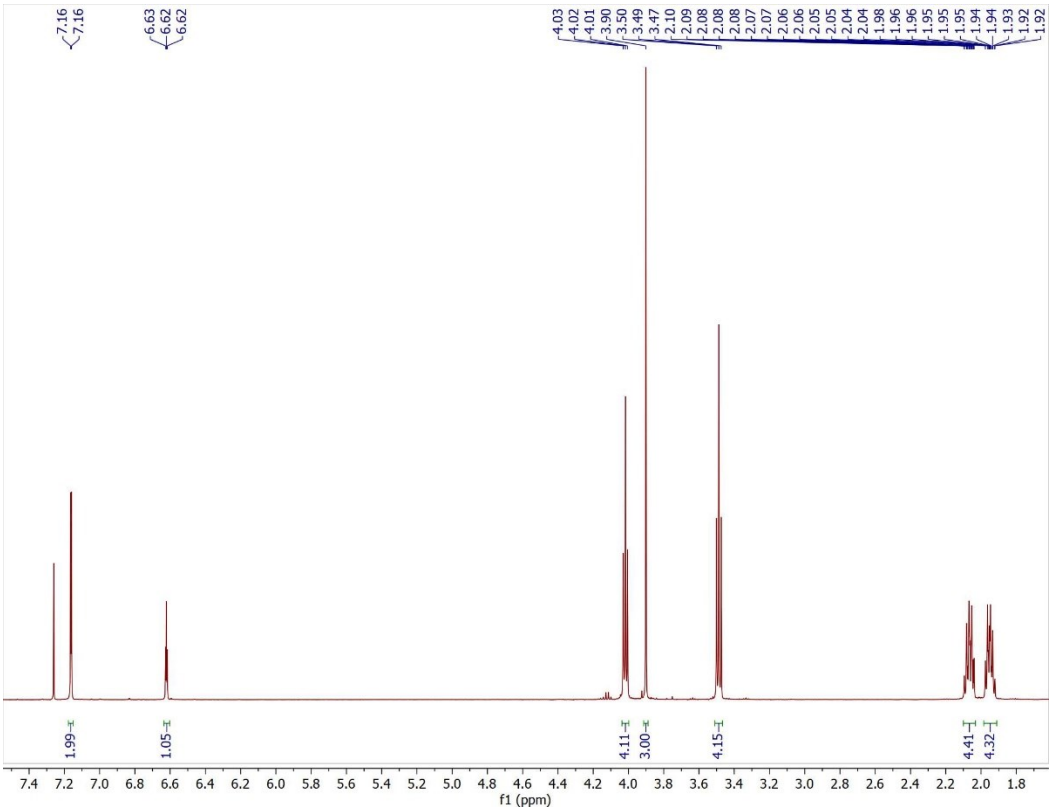
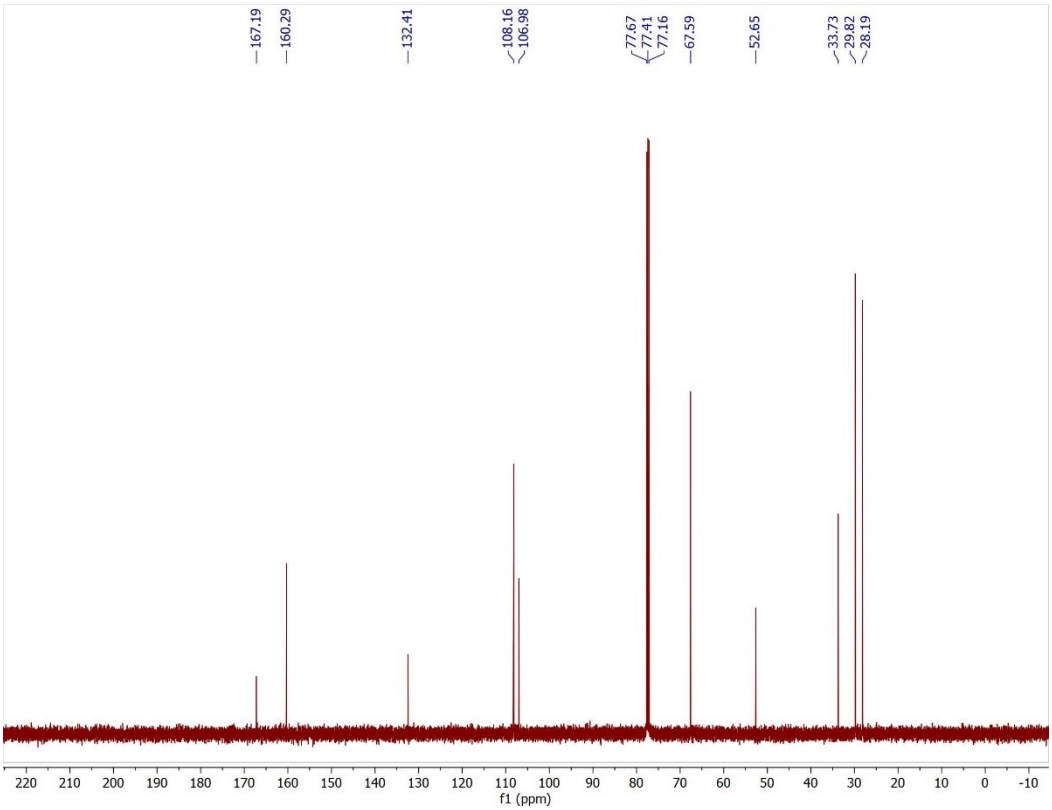


Figure S4. Percent association of COE2-3C-C4butyl to 1 OD_{600} of *E. coli* K12 in HBSS with the initial concentrations of COEs of 10 µM, 20 µM, and 27 µM.

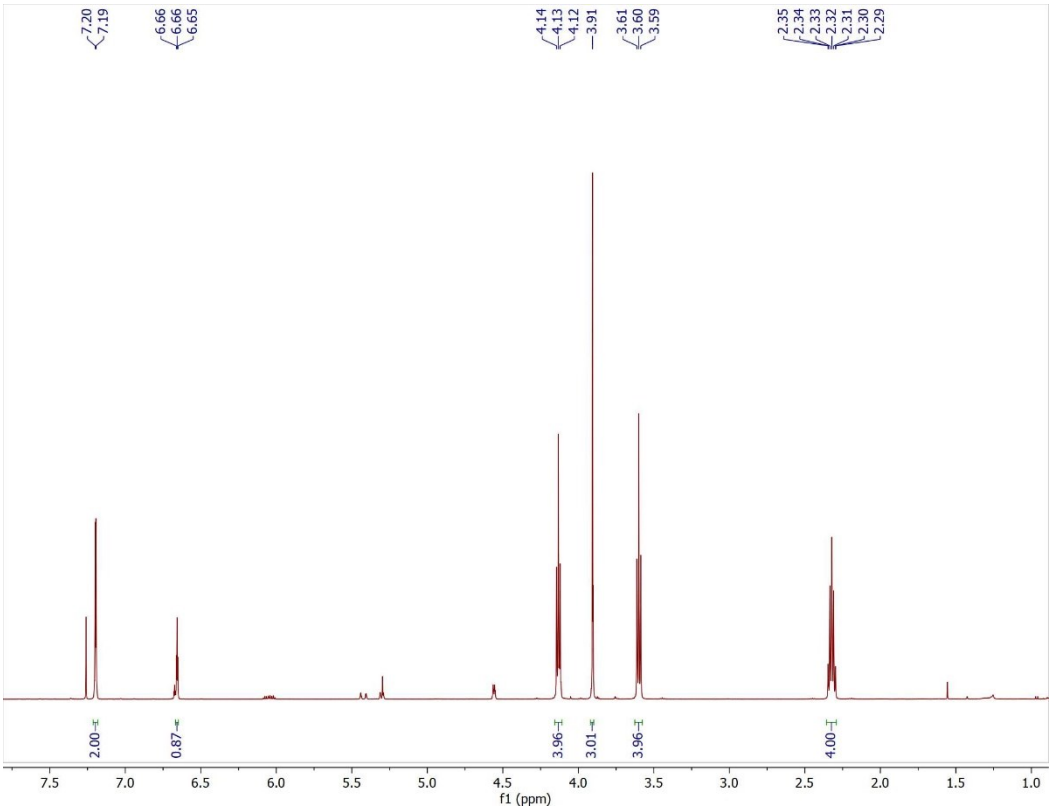
7. NMR Spectra

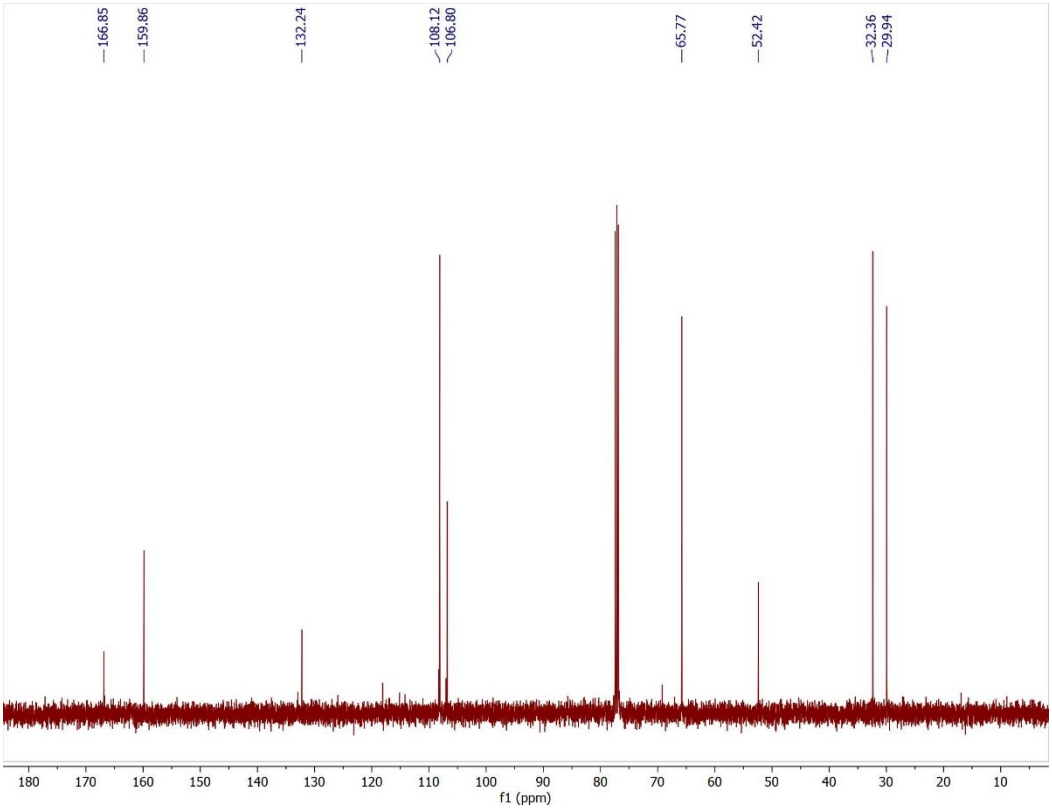
Methyl 3,5-bis(4-bromobutoxy)benzoate (1a)



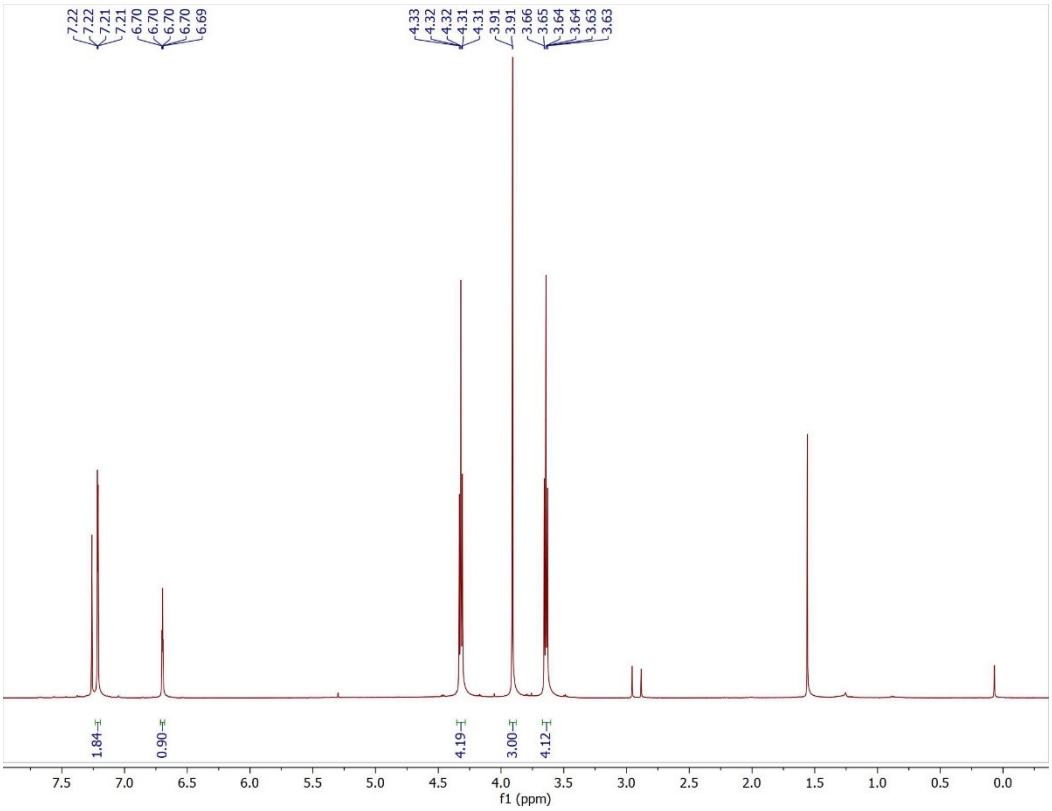


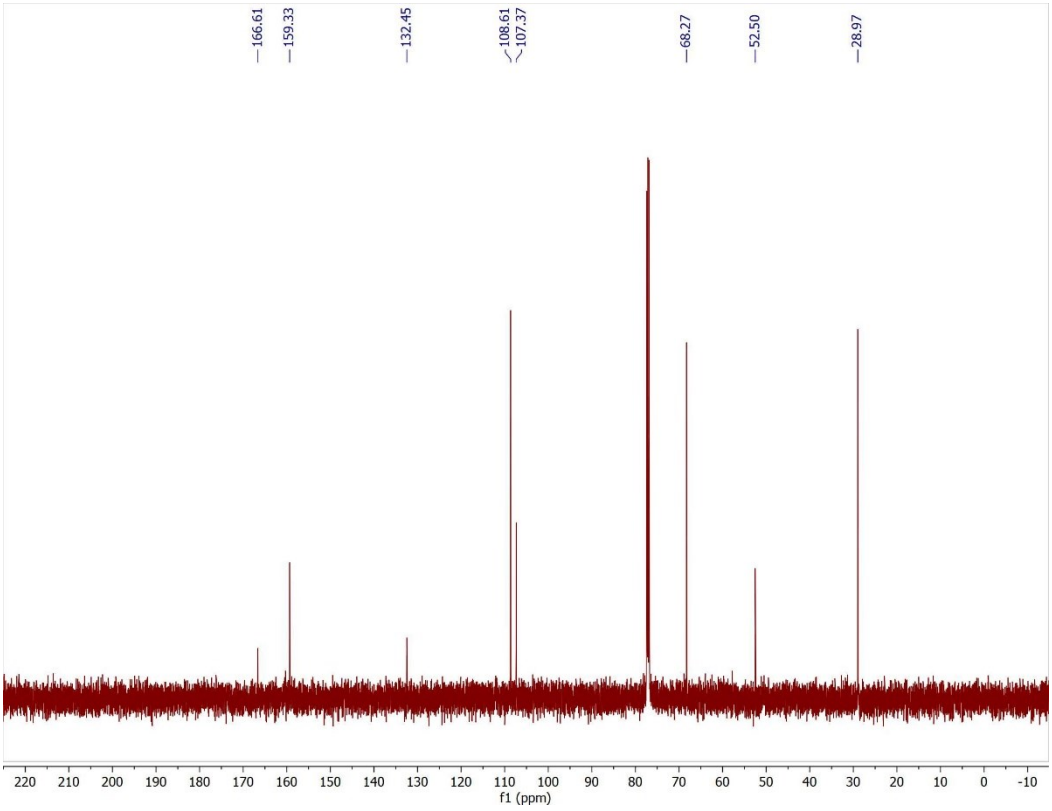
Methyl 3,5-bis(3-bromopropoxy)benzoate (1b)



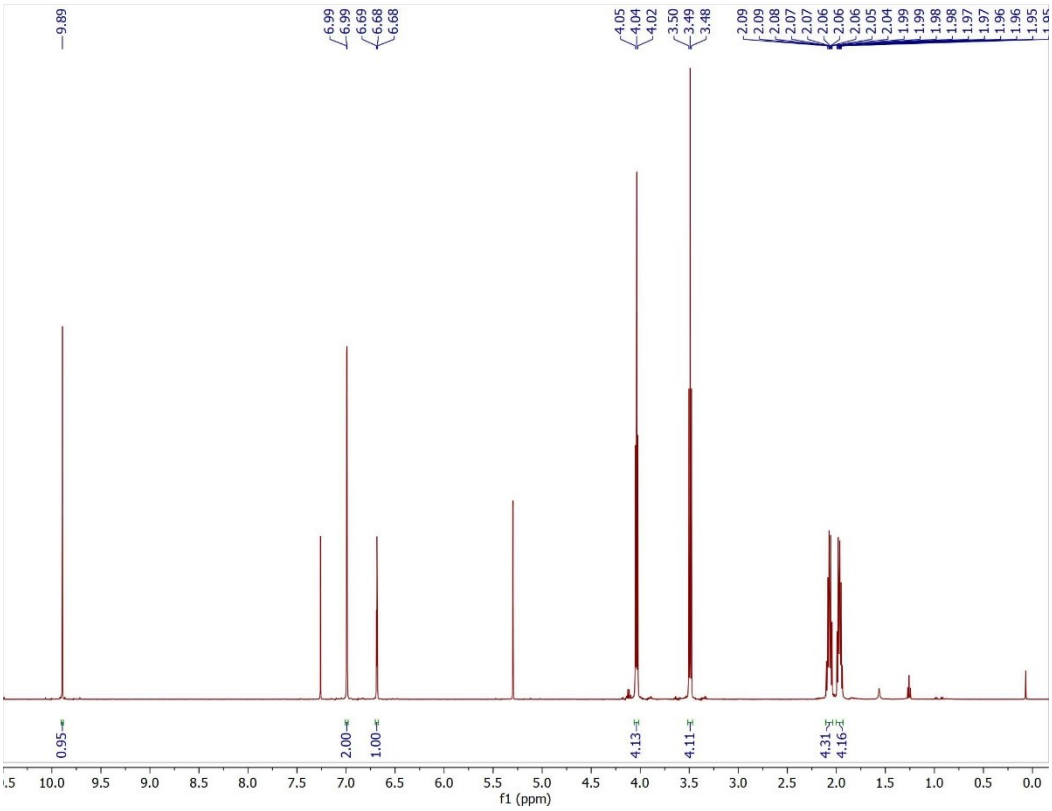


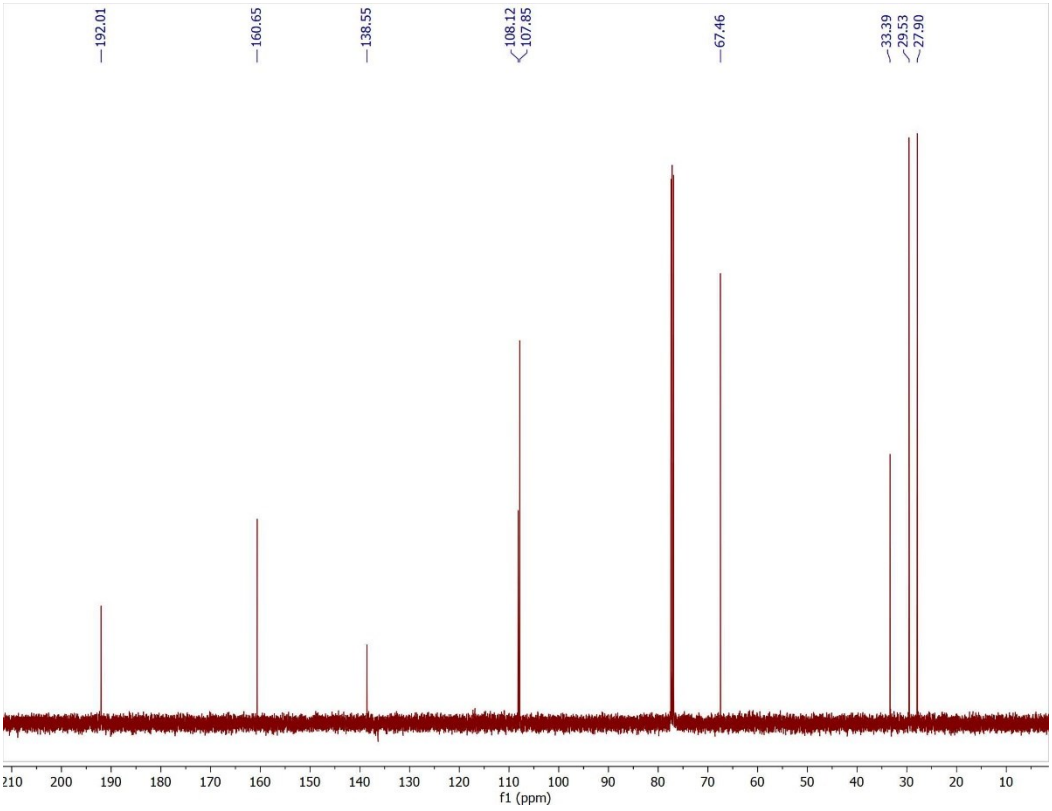
Methyl 3,5-bis(2-bromoethoxy)benzoate (1c)





3,5-Bis(4-bromobutoxy)benzaldehyde (2a)





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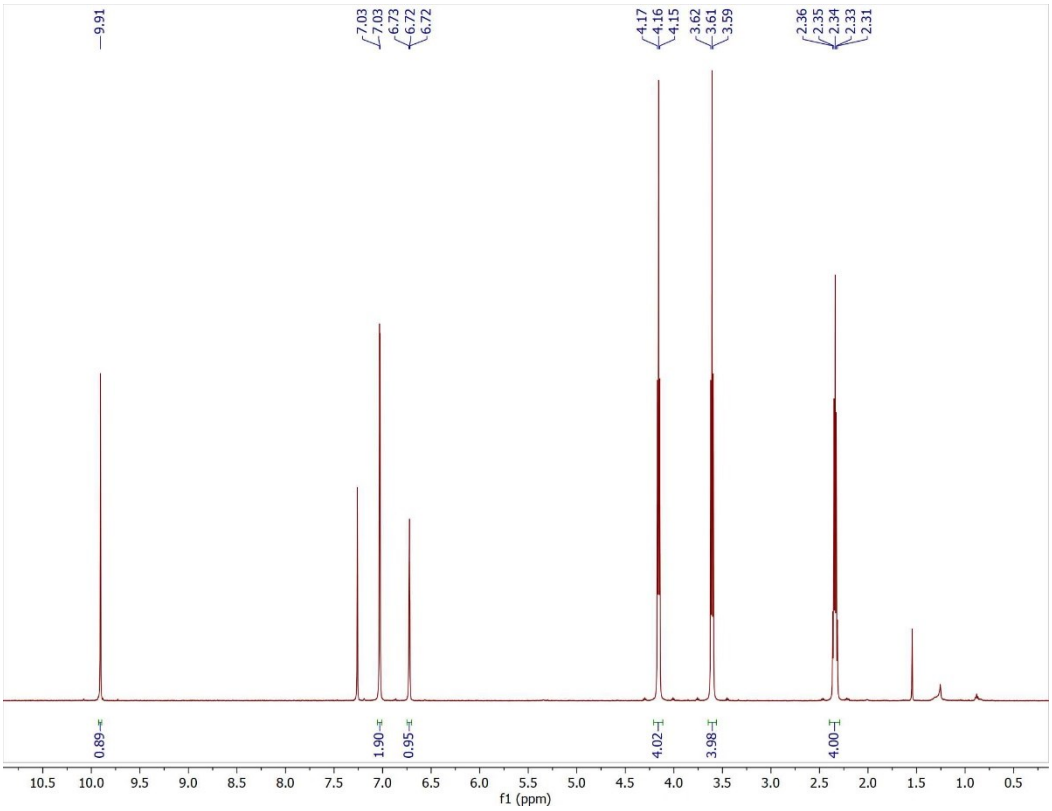

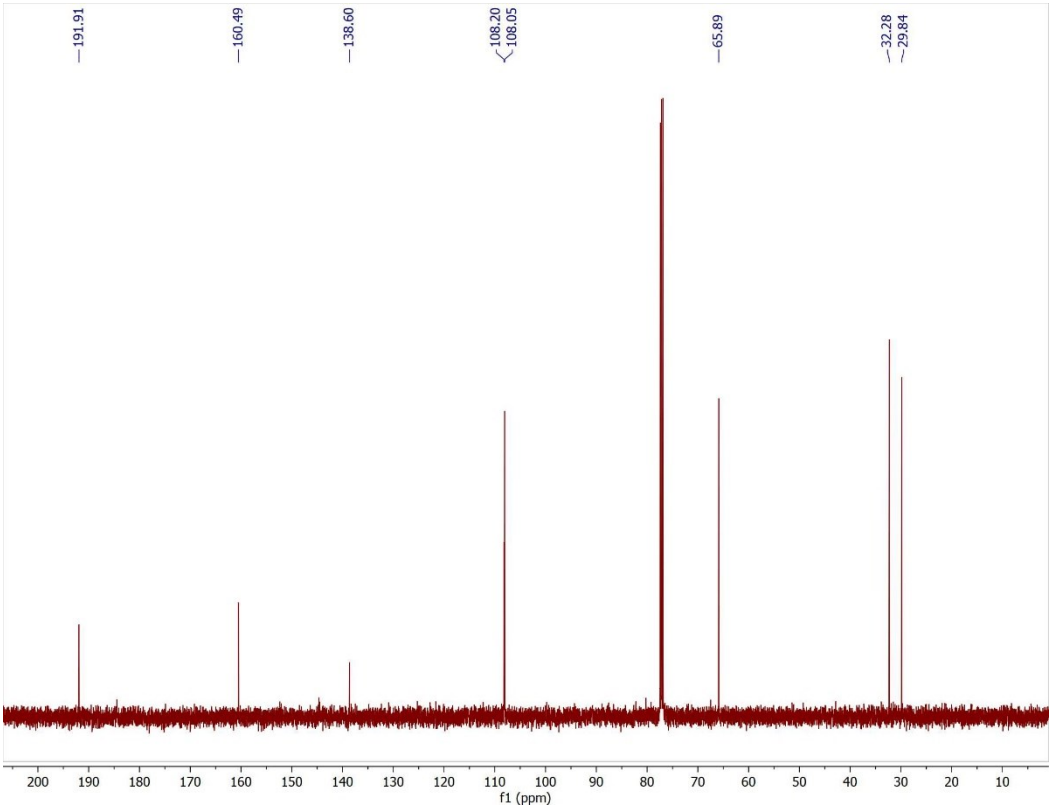

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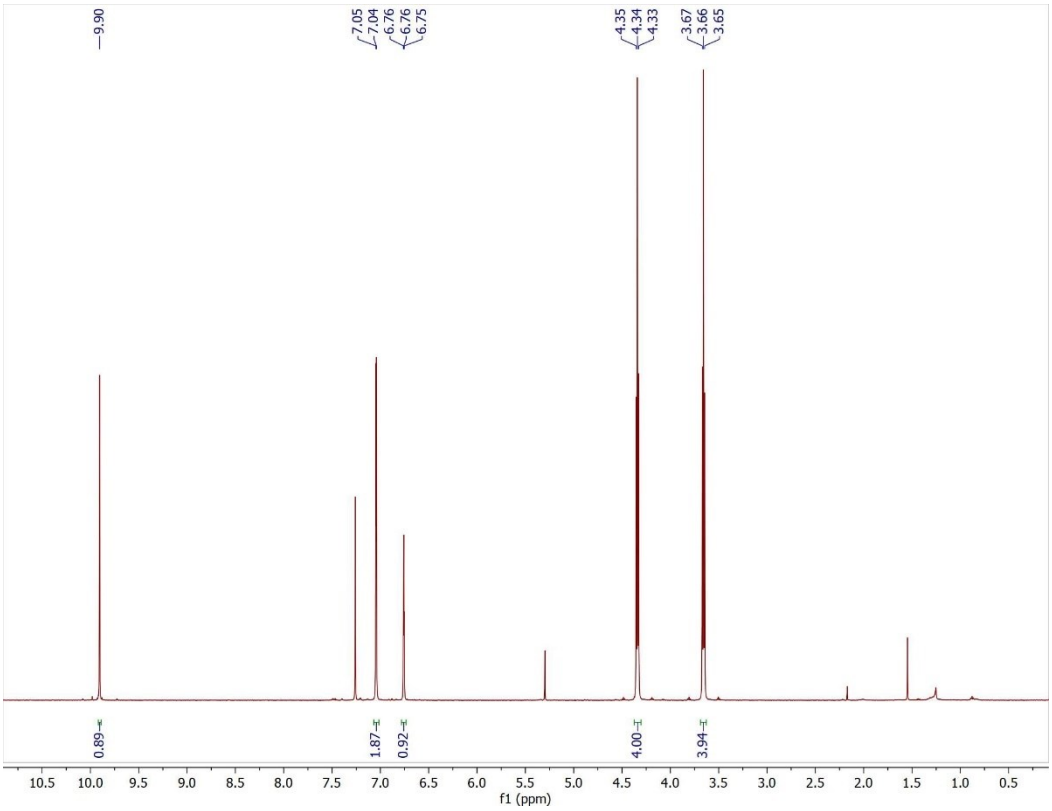
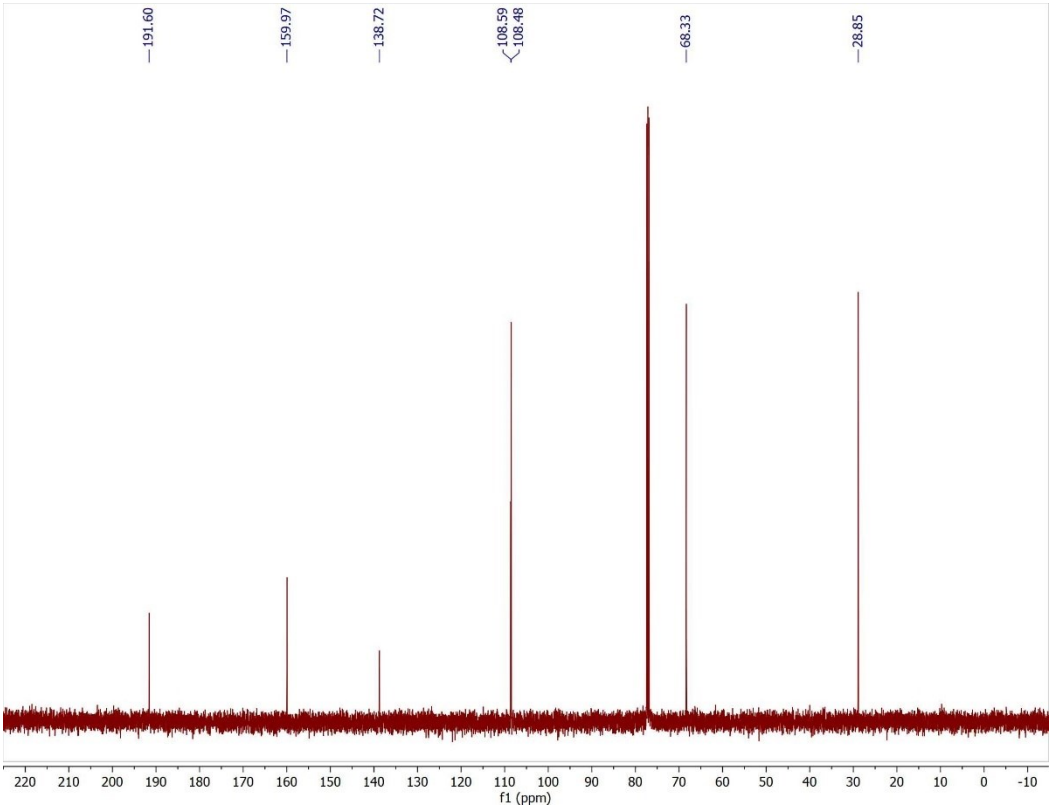
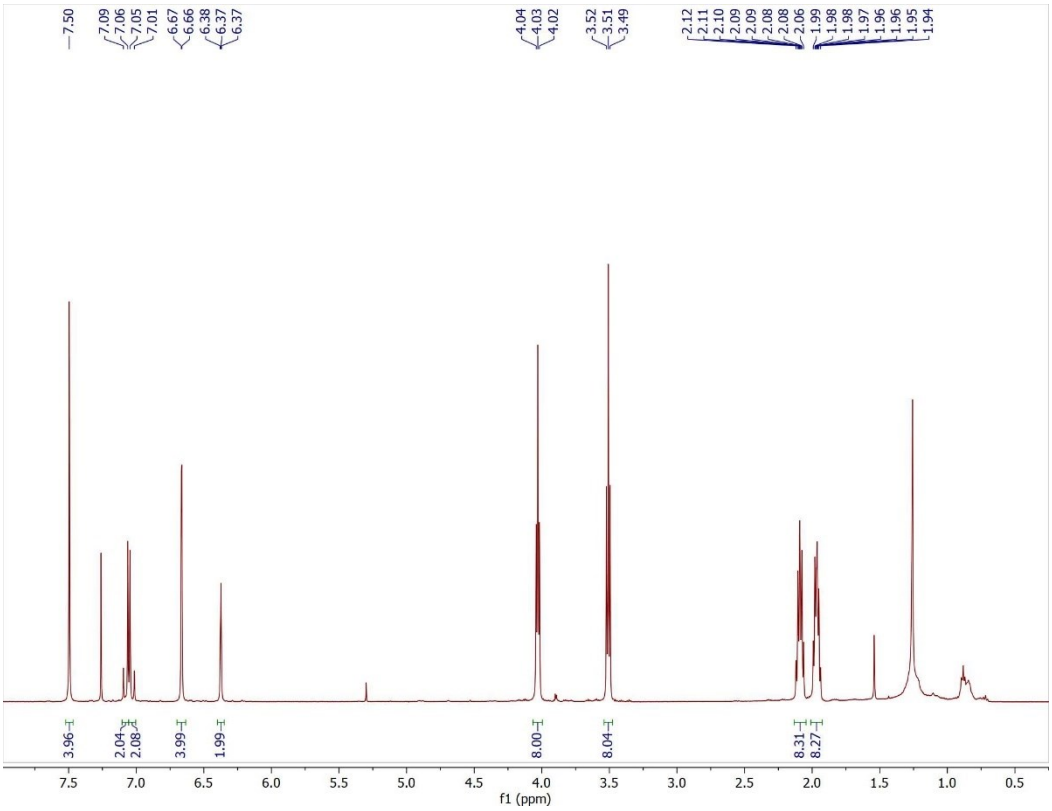




Compound 4a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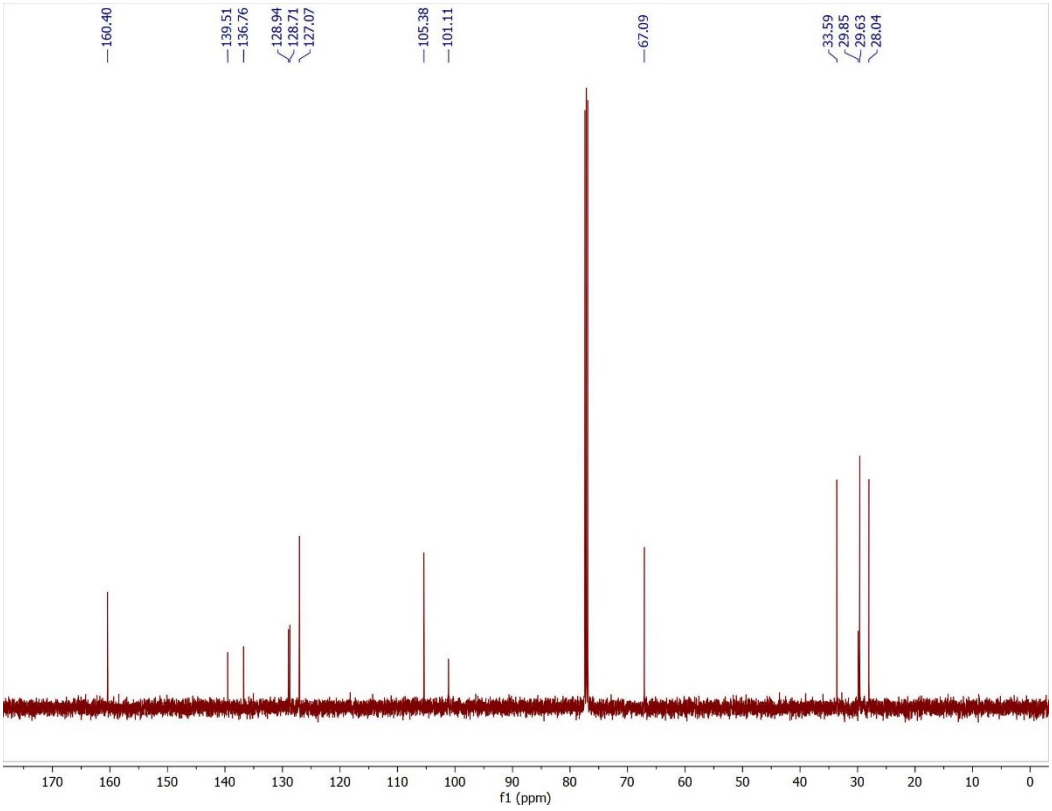
8.27
f1 (ppm)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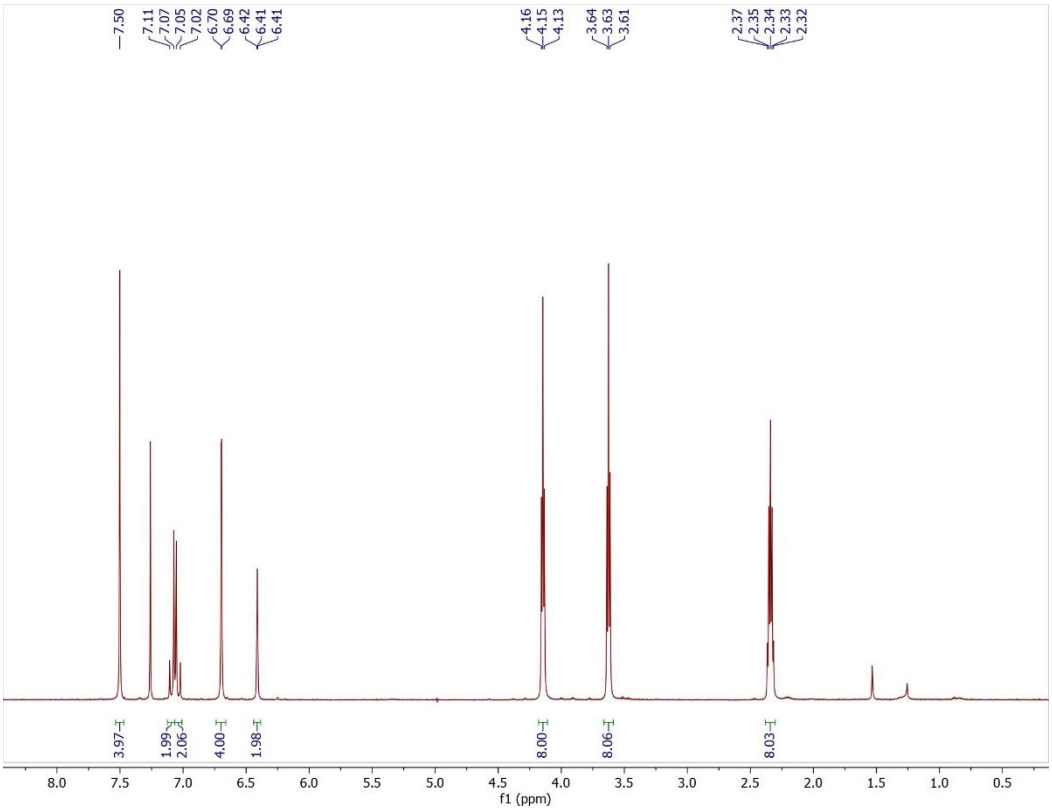
f1 (ppm)

Compound 4b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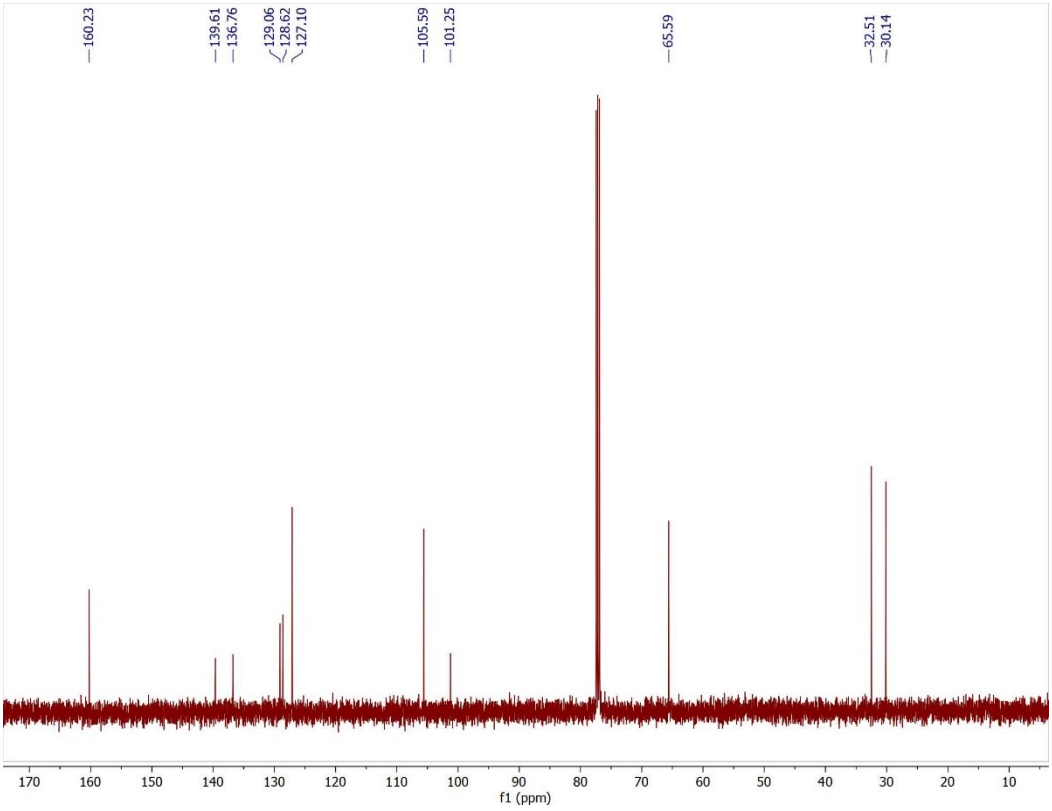
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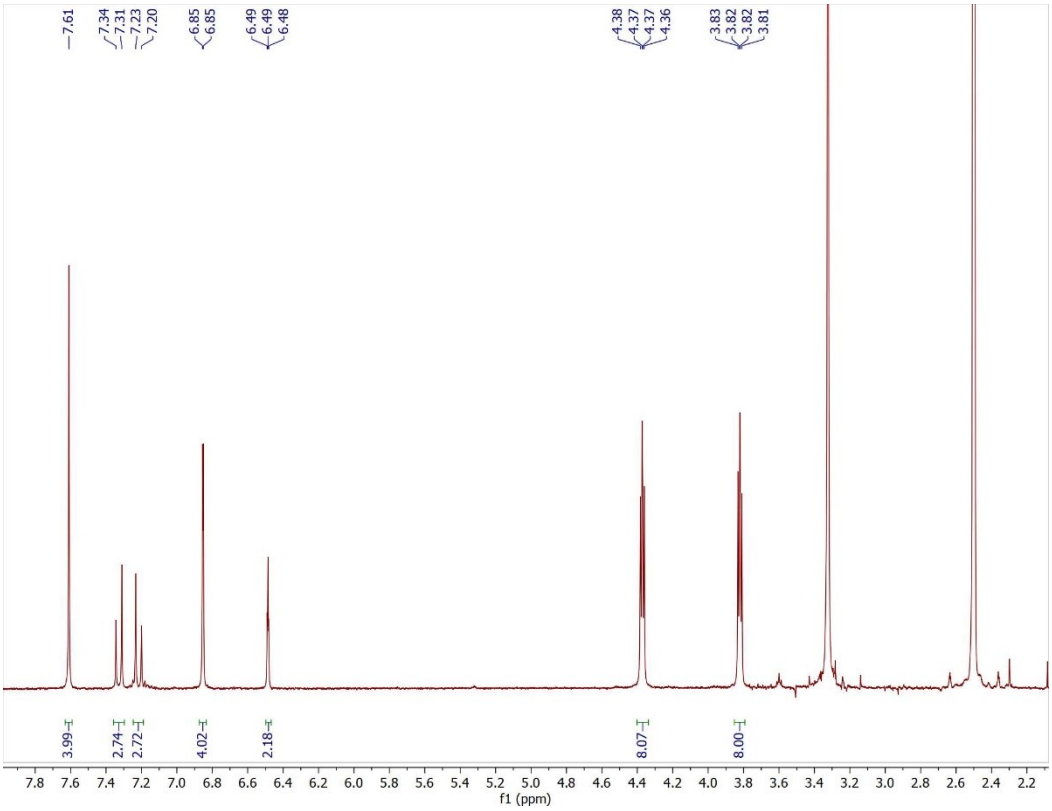
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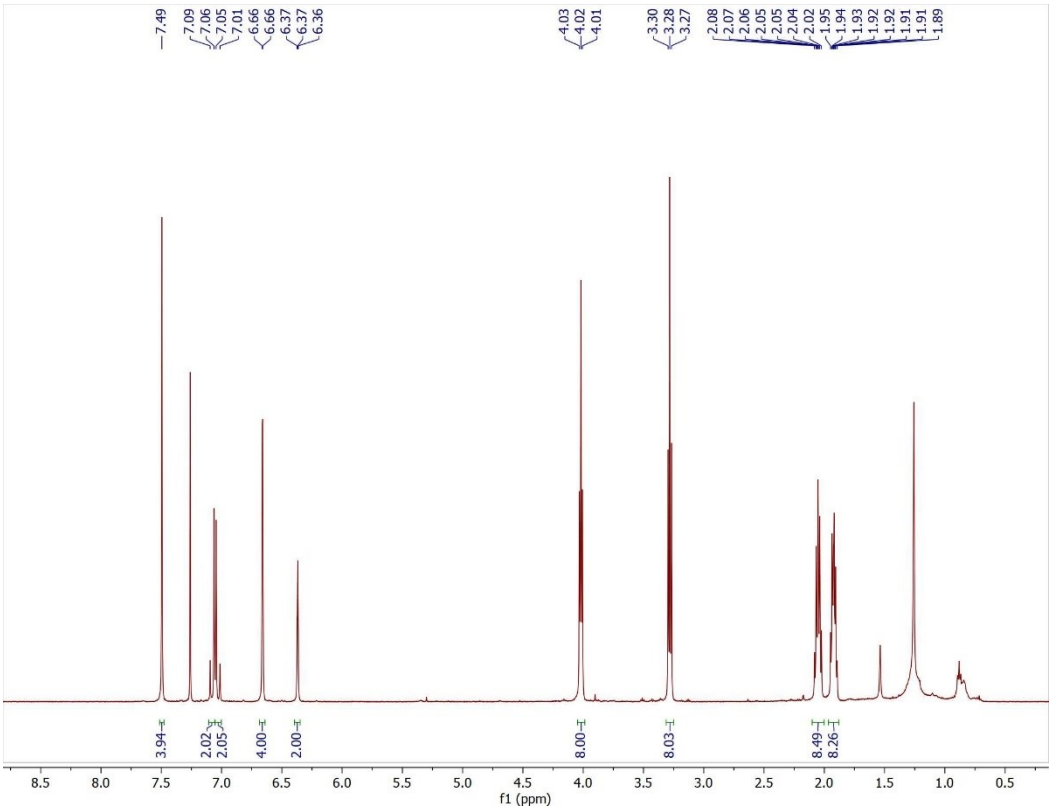
Compound 5a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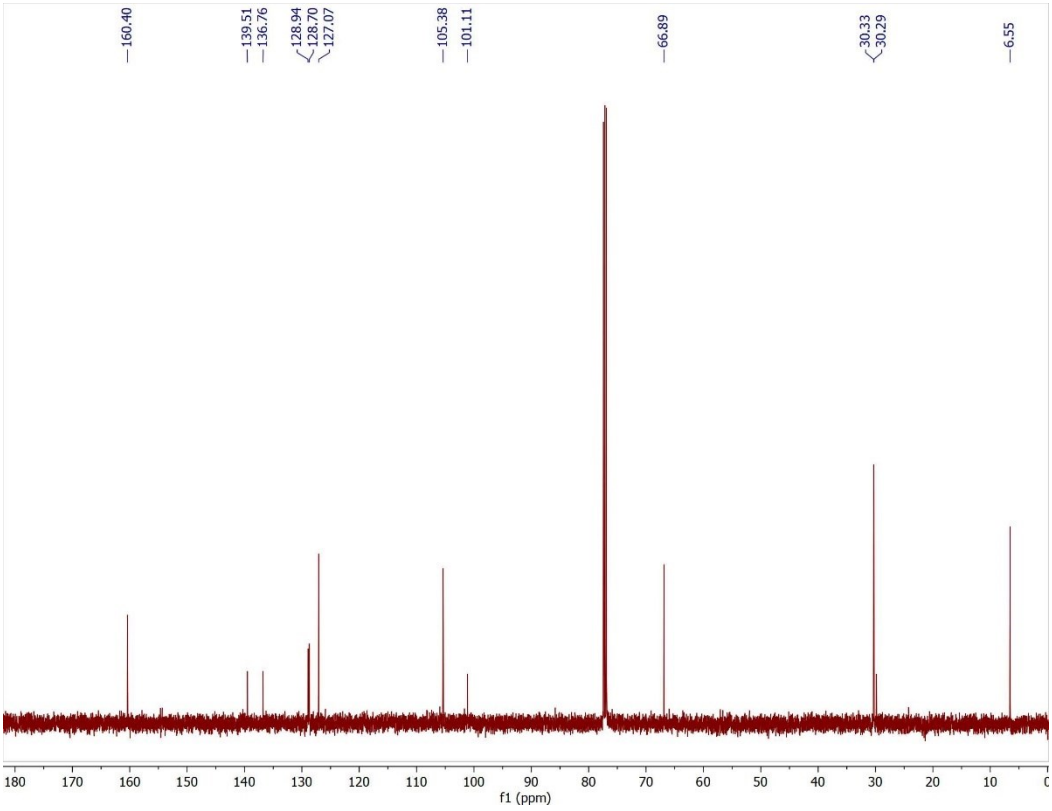
f1 (ppm)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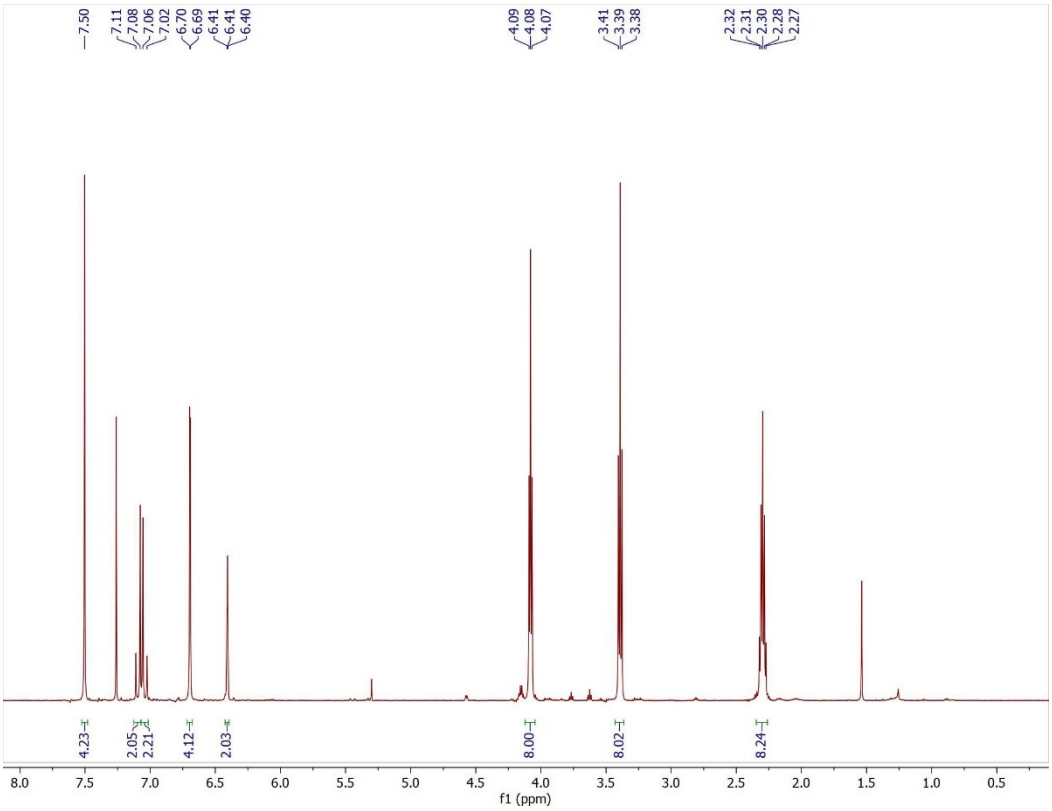
Compound 5b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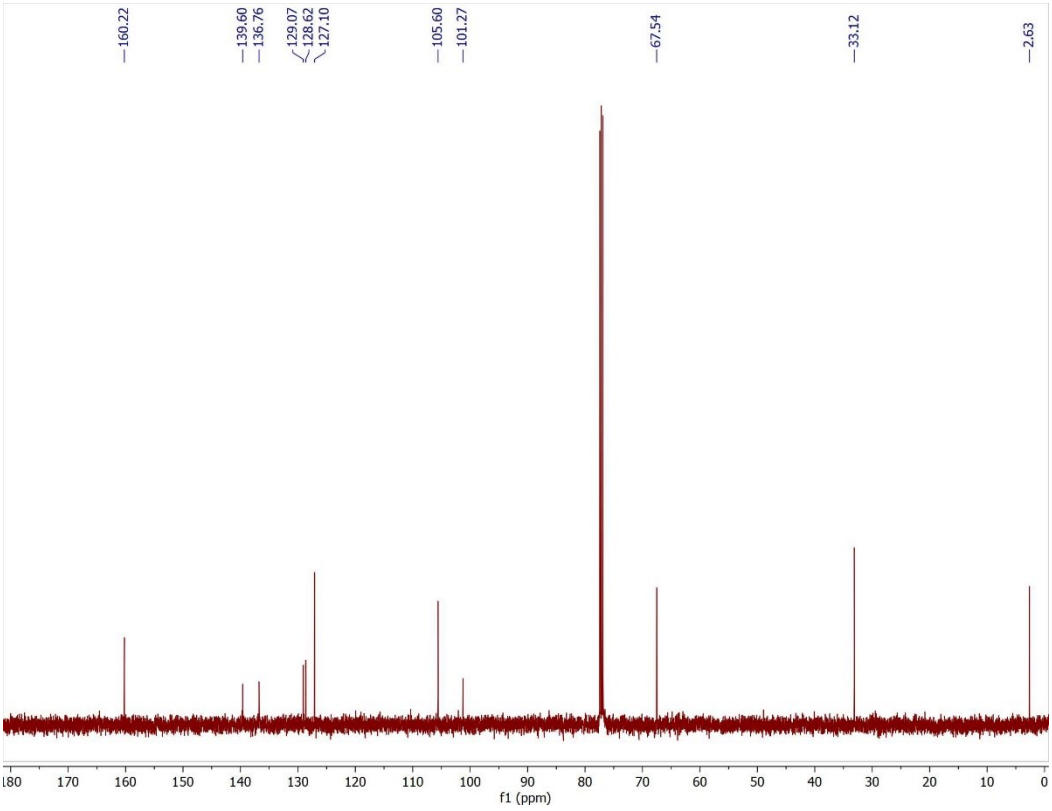
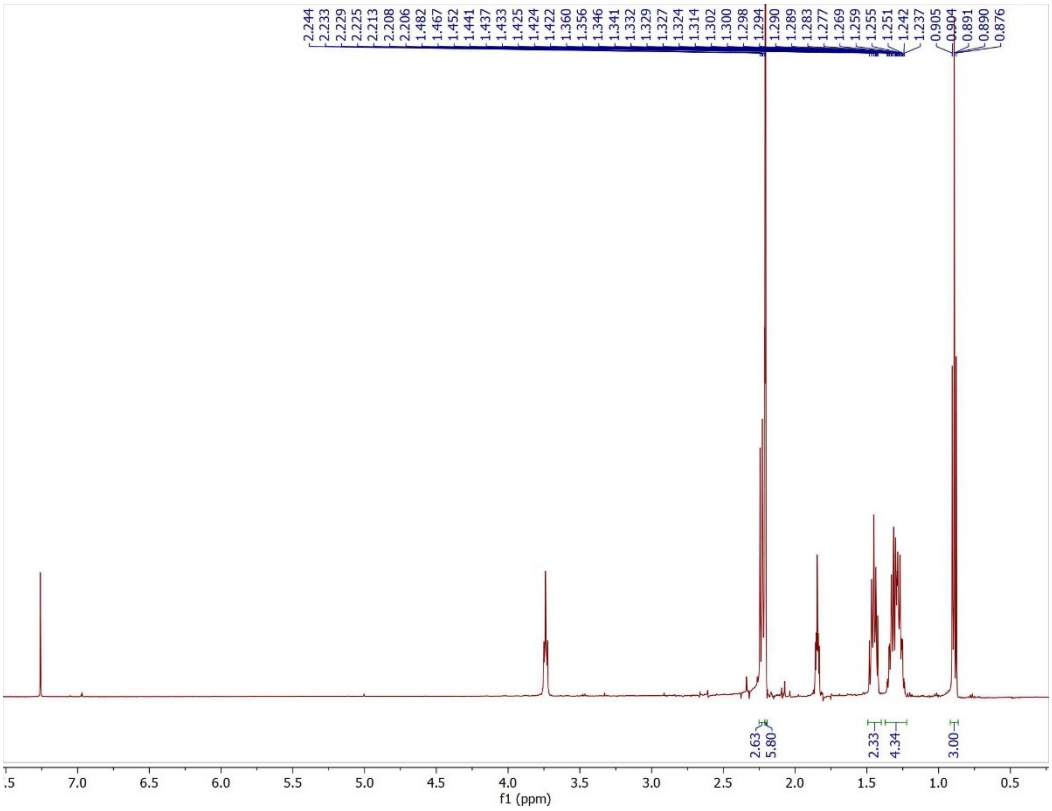
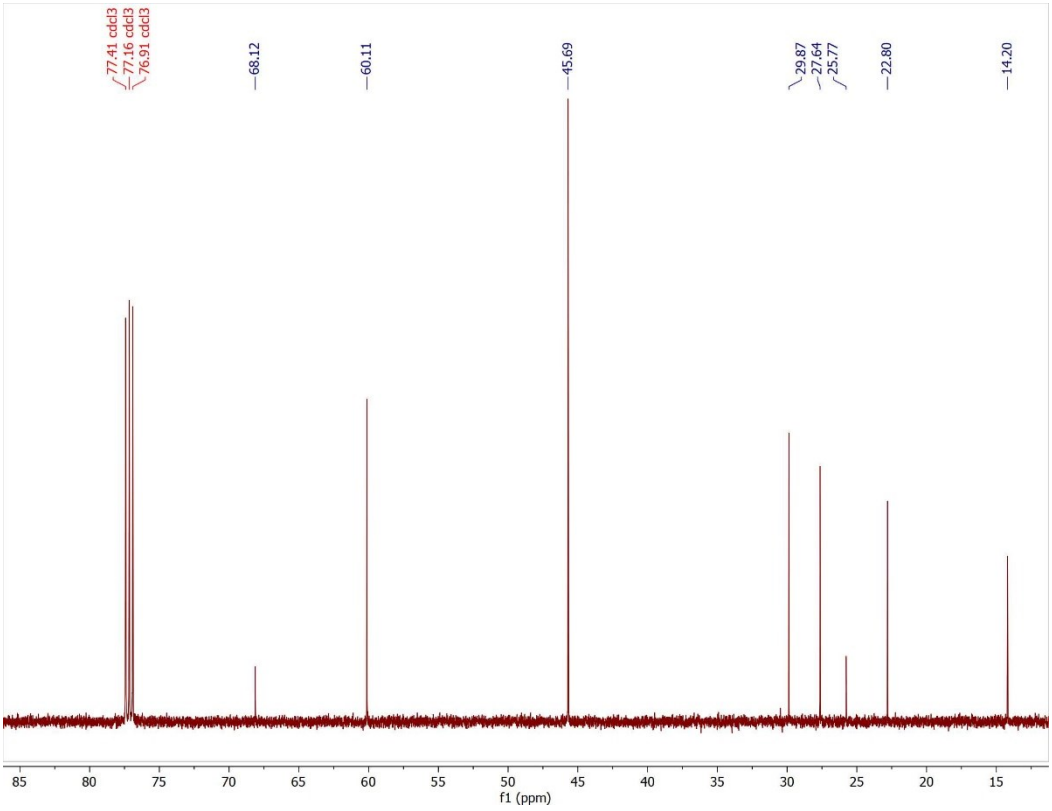
2.27
4.23
2.05
2.21
4.12
2.03
8.00
8.02
8.24
f1 (ppm)

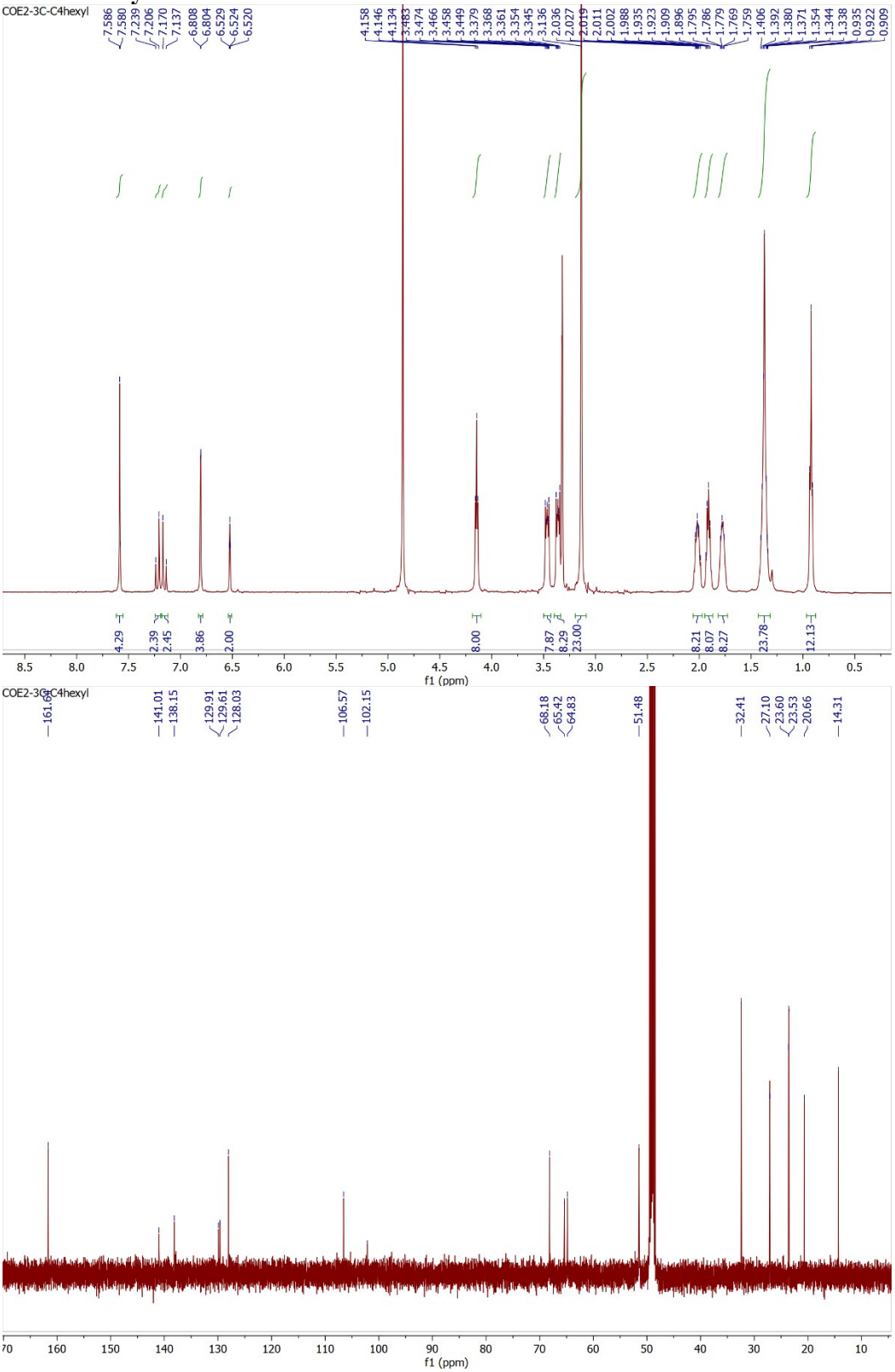

160.22
139.60
136.76
129.07
128.62
127.10
105.60
101.27
67.54
33.12
2.63
f1 (ppm)

***N*,*N*-dimethylpentylamine**

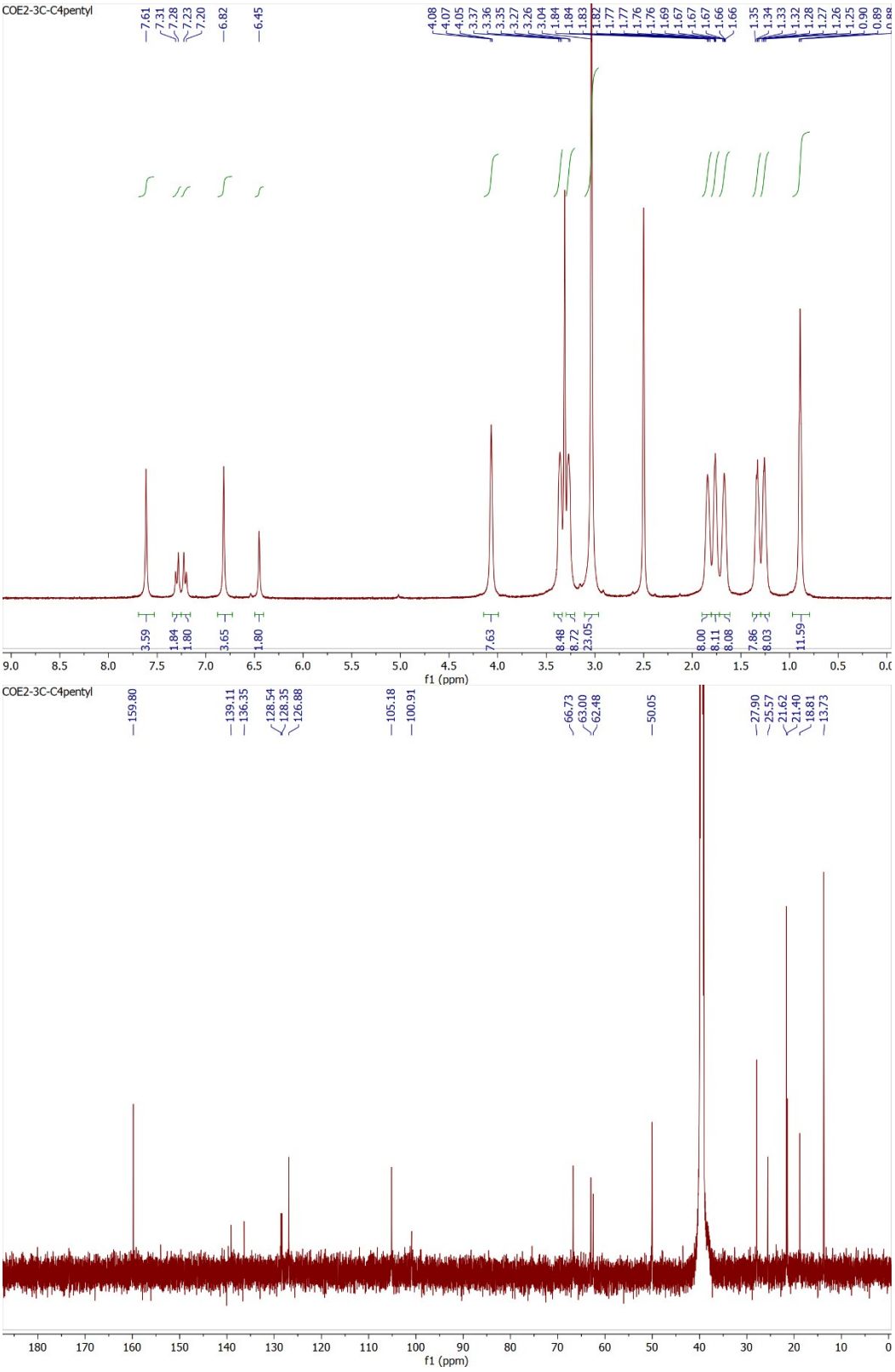




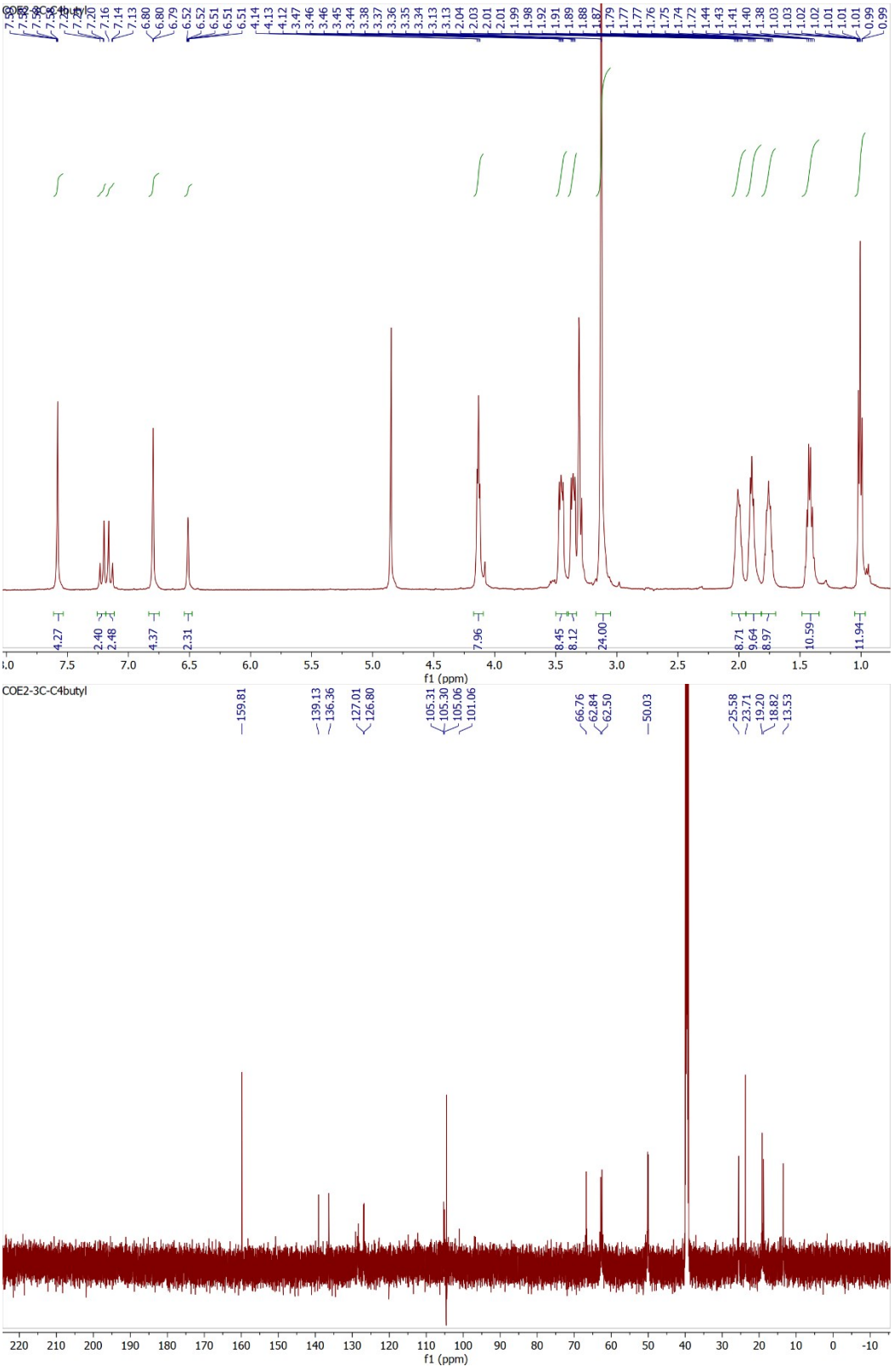
COE2-3C-C4hexyl



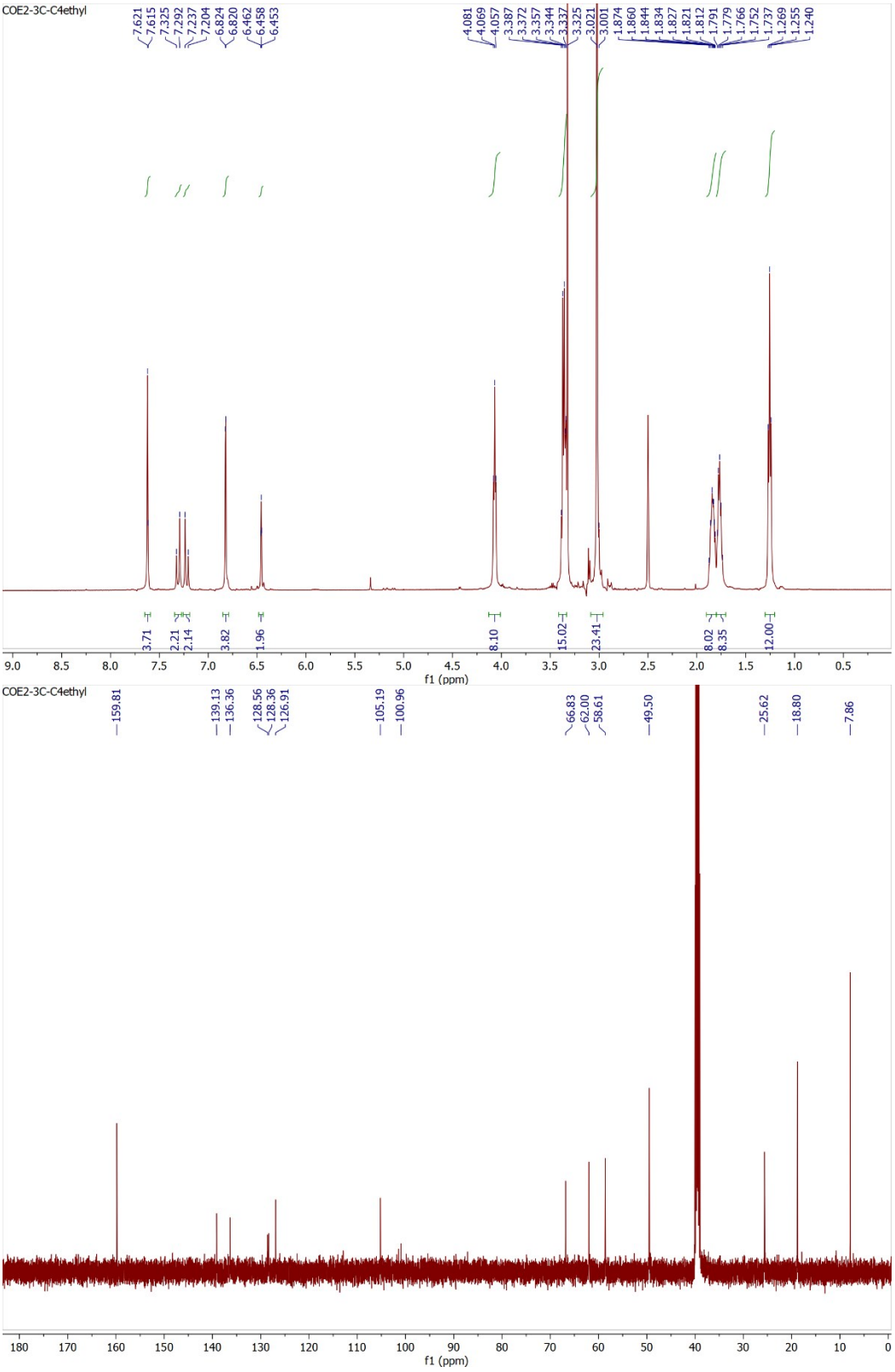
COE2-3C-C4pentyl



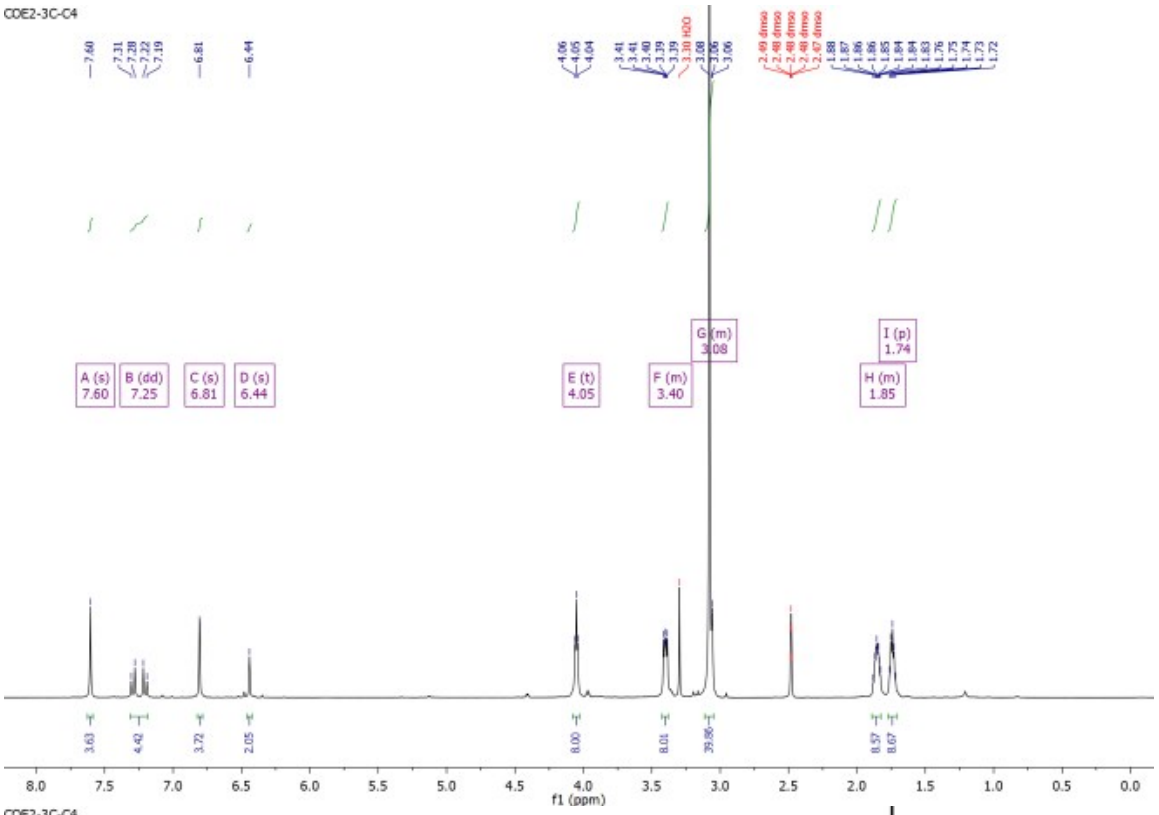
COE2-3C-C4butyl

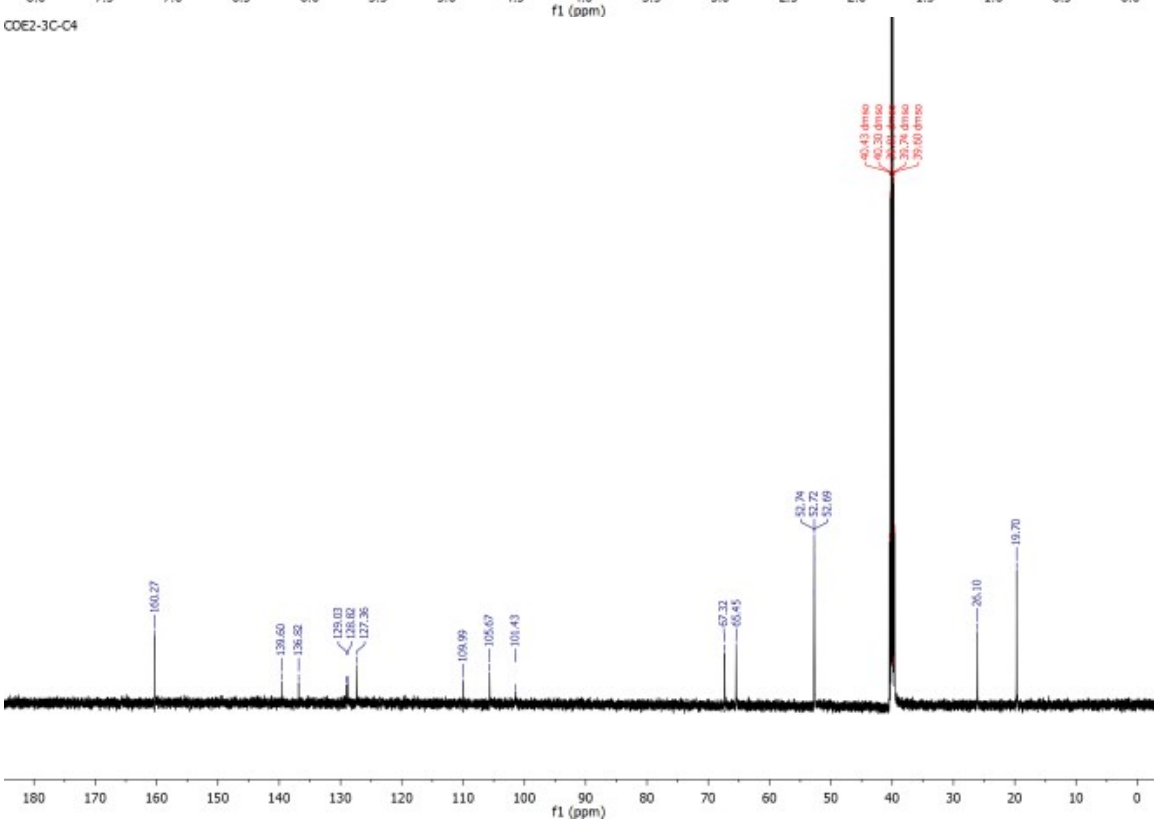


COE2-3C-C4ethyl

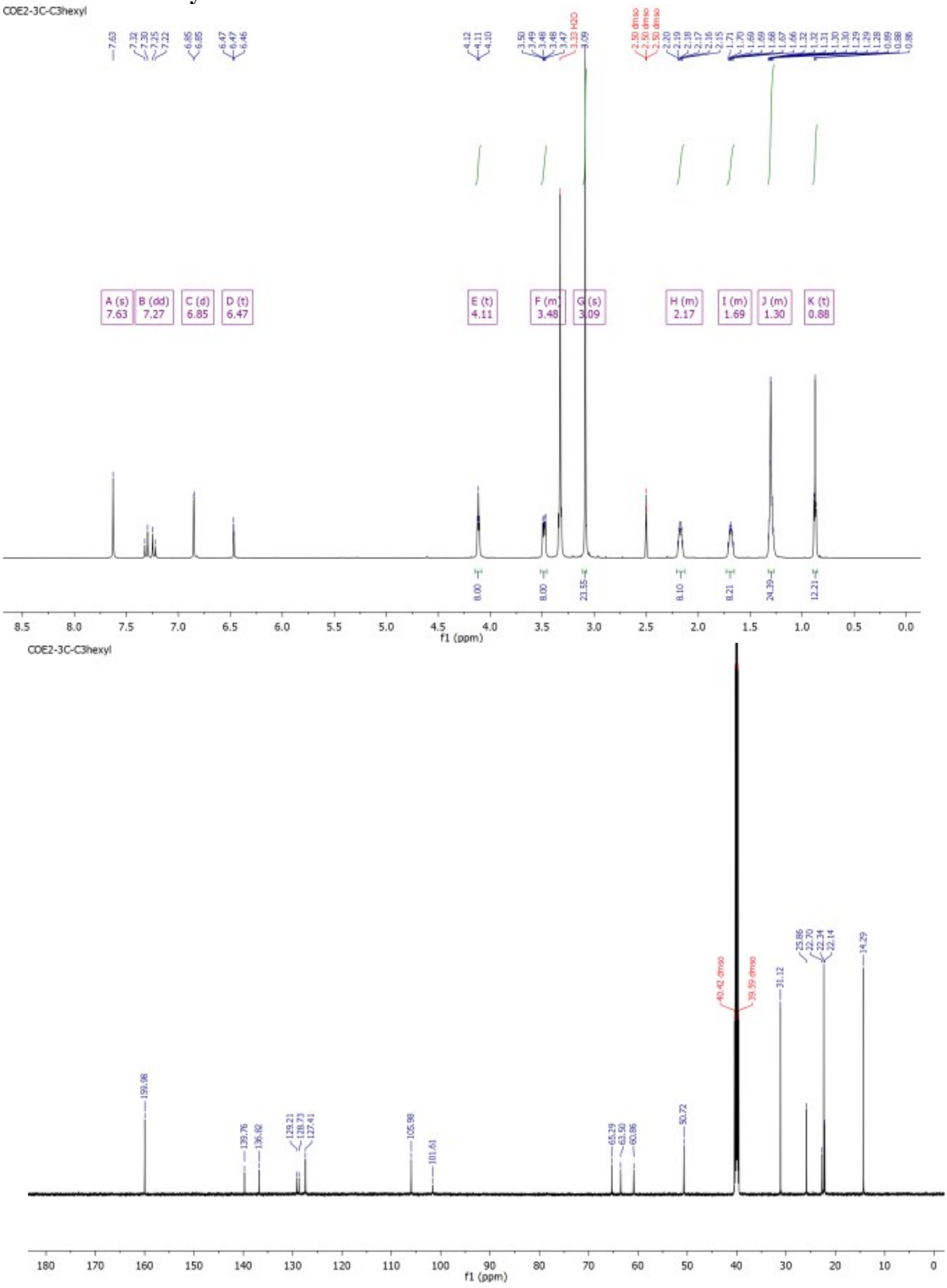


COE2-3C-C4

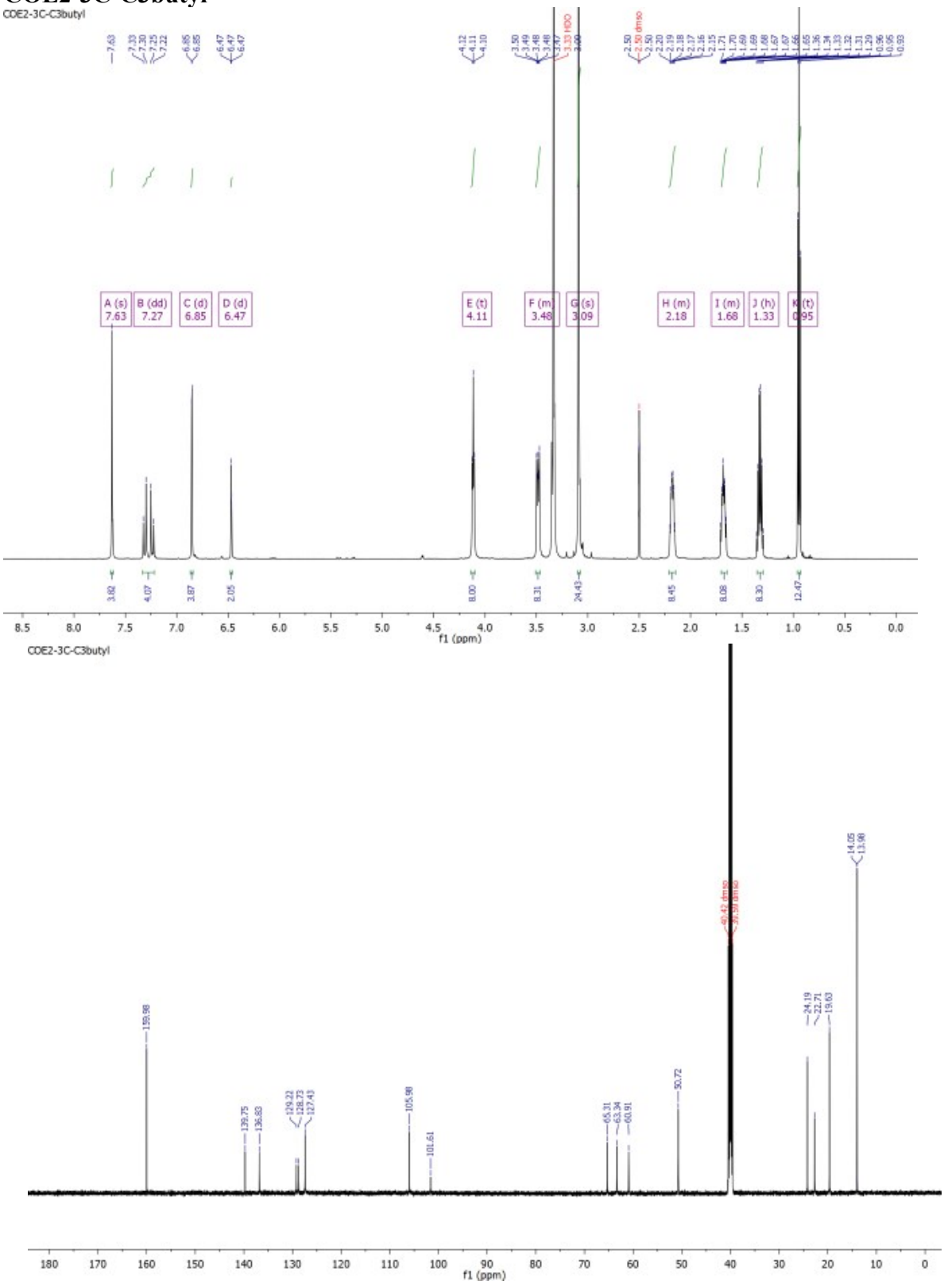

COE2-3C-C4
A (s) 7.60
B (dd) 7.25
C (s) 6.81
D (s) 6.44
E (t) 4.05
F (m) 3.40
G (m) 3.08
H (m) 1.85
I (p) 1.74
f1 (ppm)


COE2-3C-C4
f1 (ppm)

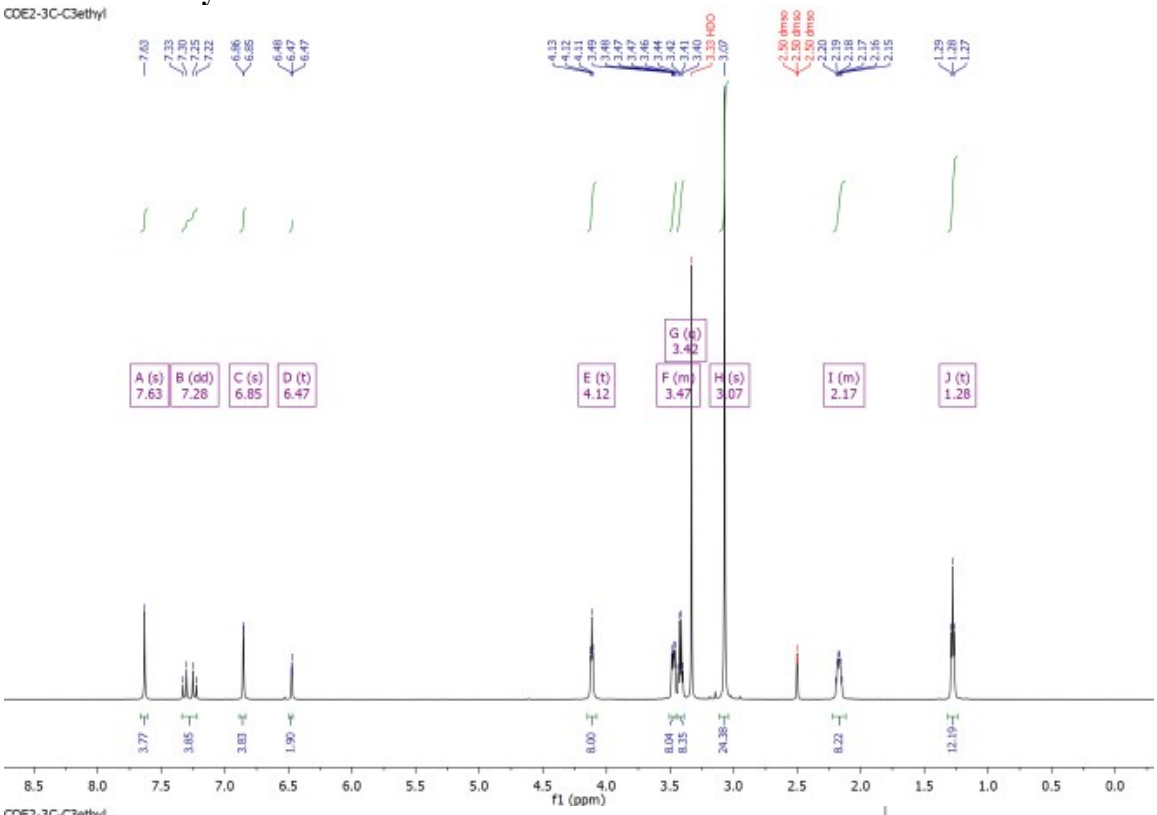
COE2-3C-C3hexyl

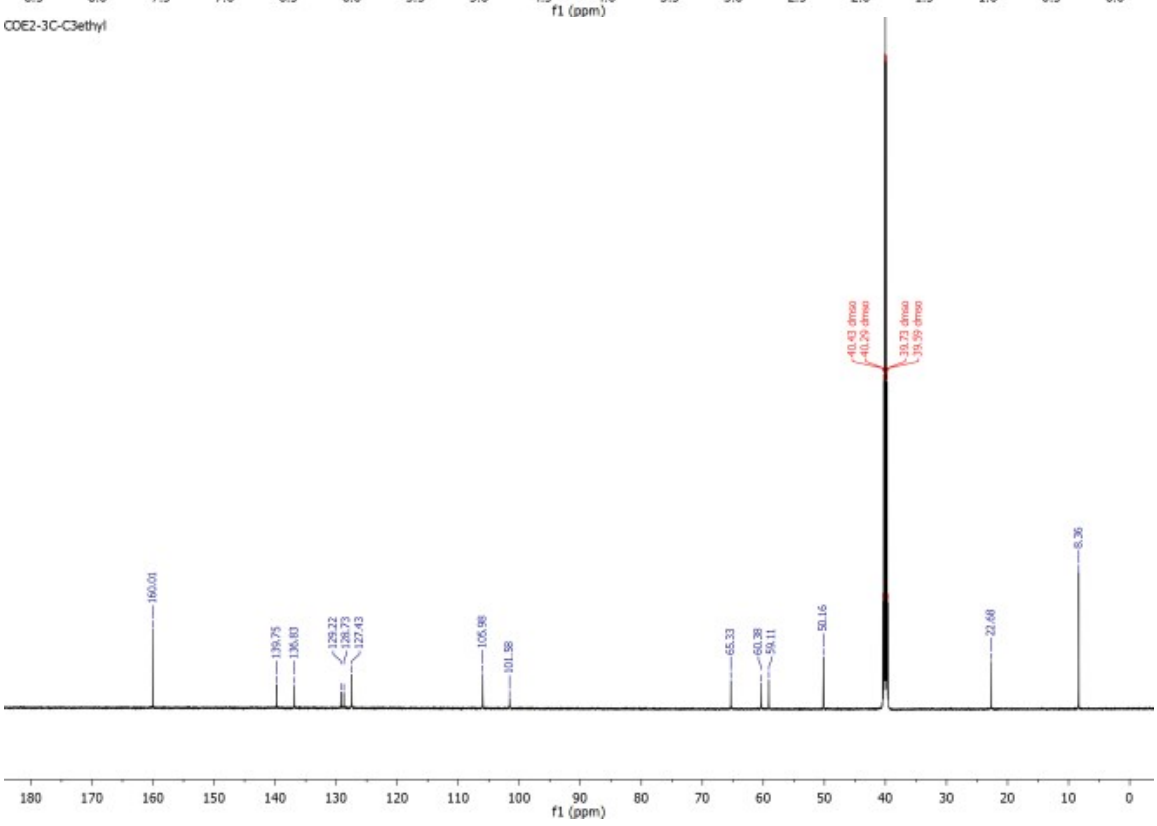


COE2-3C-C3butyl

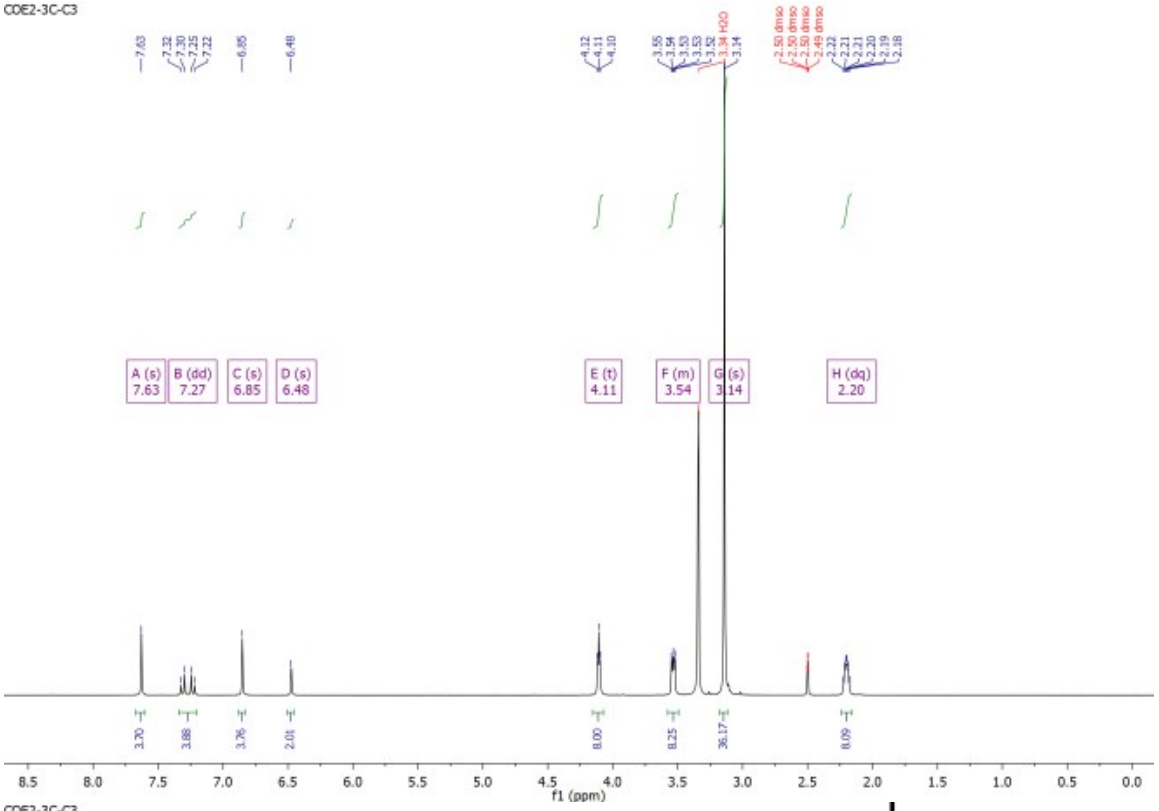


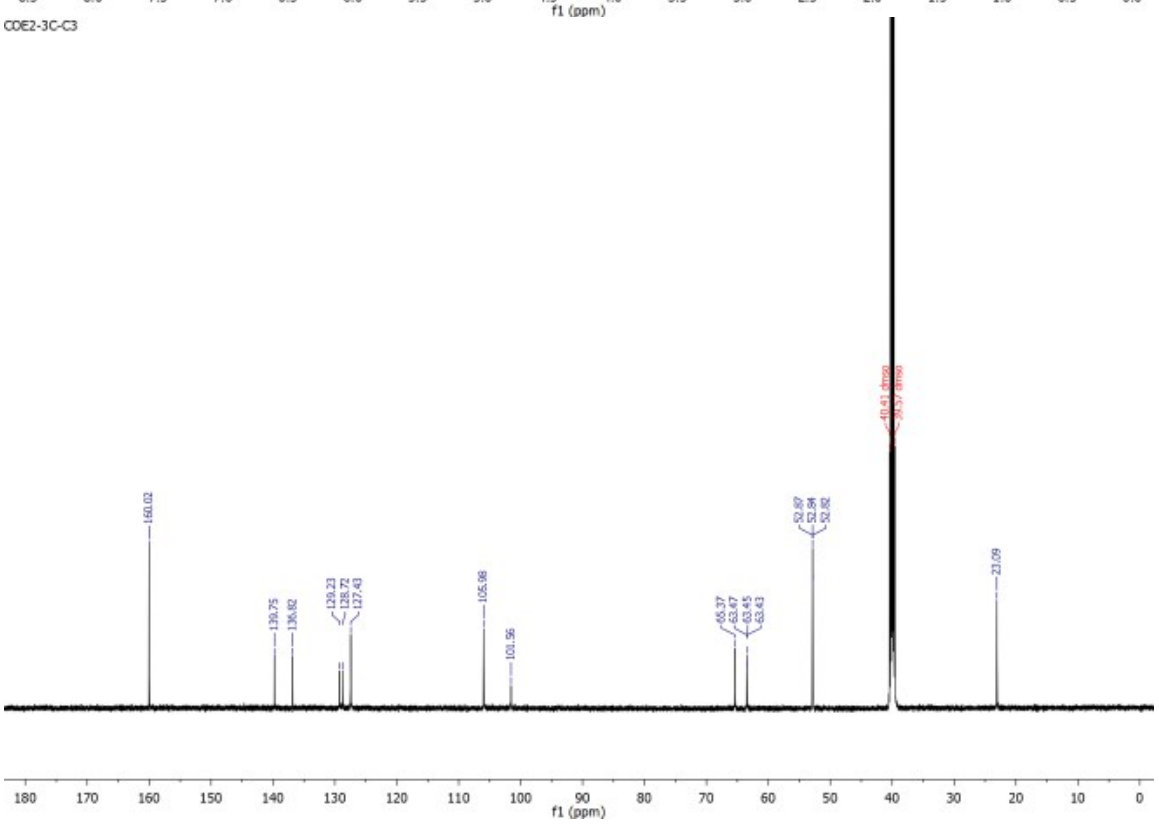
COE2-3C-C3ethyl



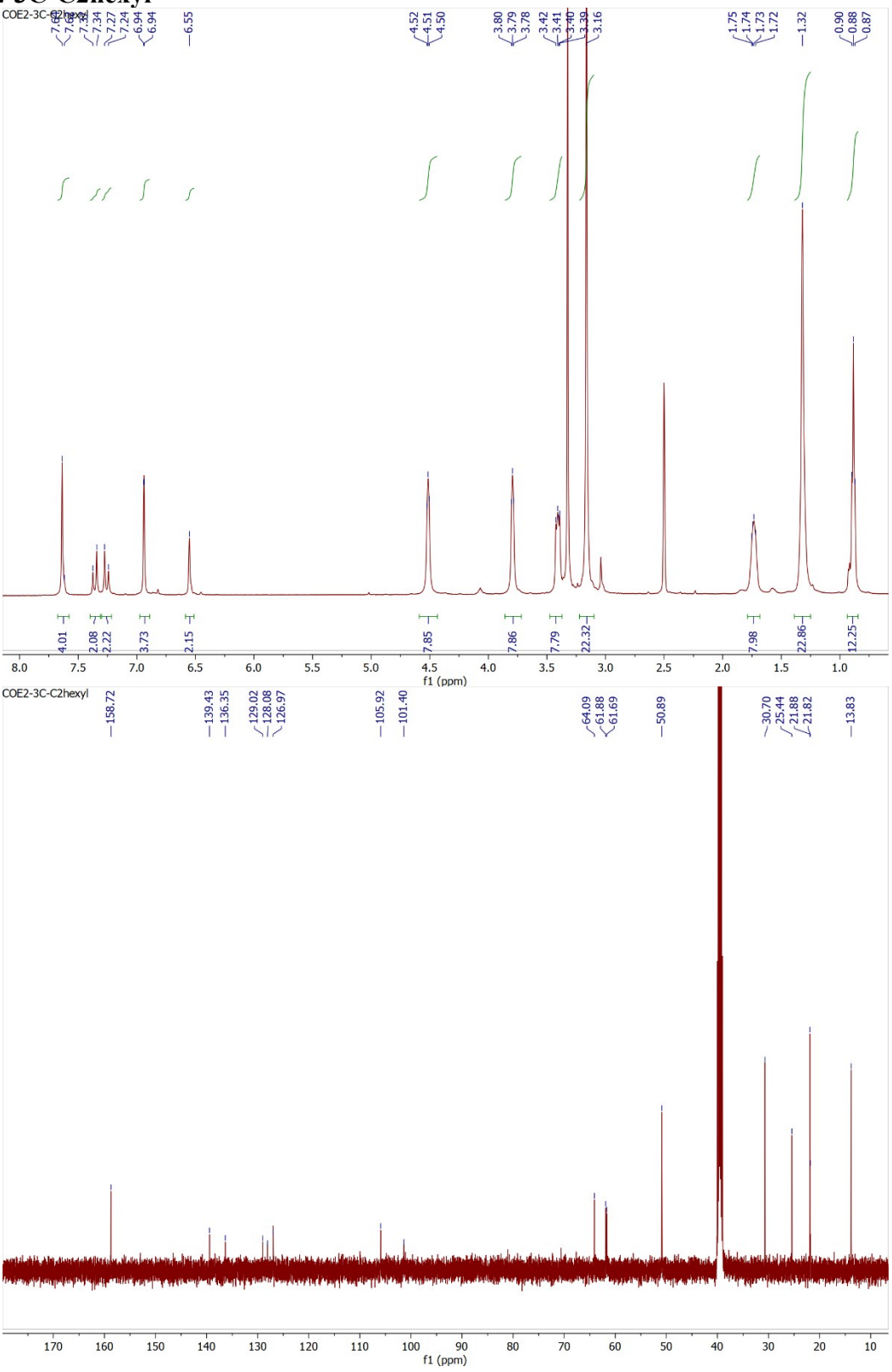


COE2-3C-C3

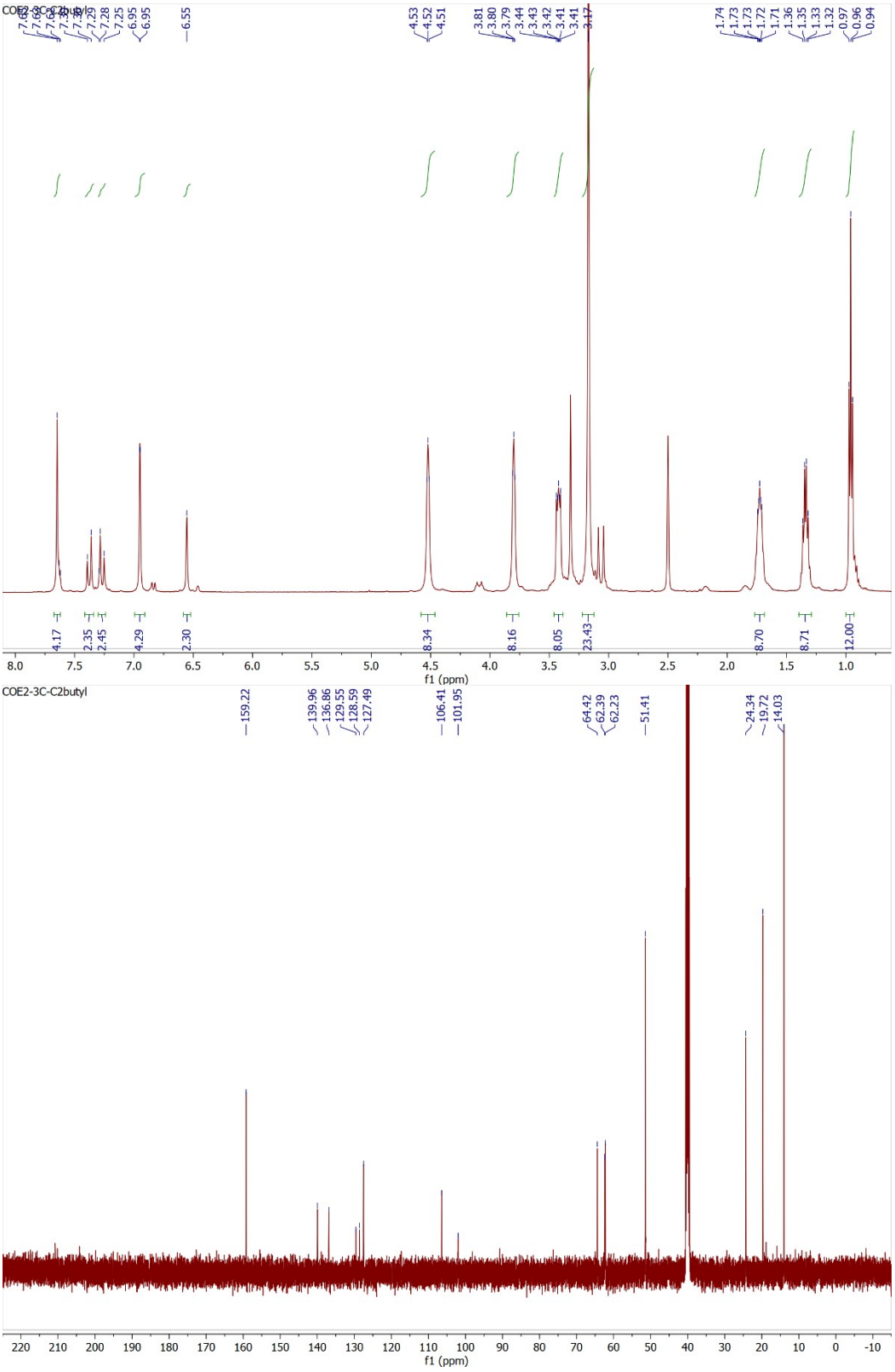

COE2-3C-C3
A (s) 7.63
B (dd) 7.27
C (s) 6.85
D (s) 6.48
E (t) 4.11
F (m) 3.54
G (s) 3.14
H (dq) 2.20
f1 (ppm)


COE2-3C-C3
160.02
139.75
136.82
129.23
128.72
127.43
105.98
101.96
65.37
63.67
63.45
63.43
52.87
52.84
52.82
40.41 dmso
29.57 dmso
23.09
f1 (ppm)

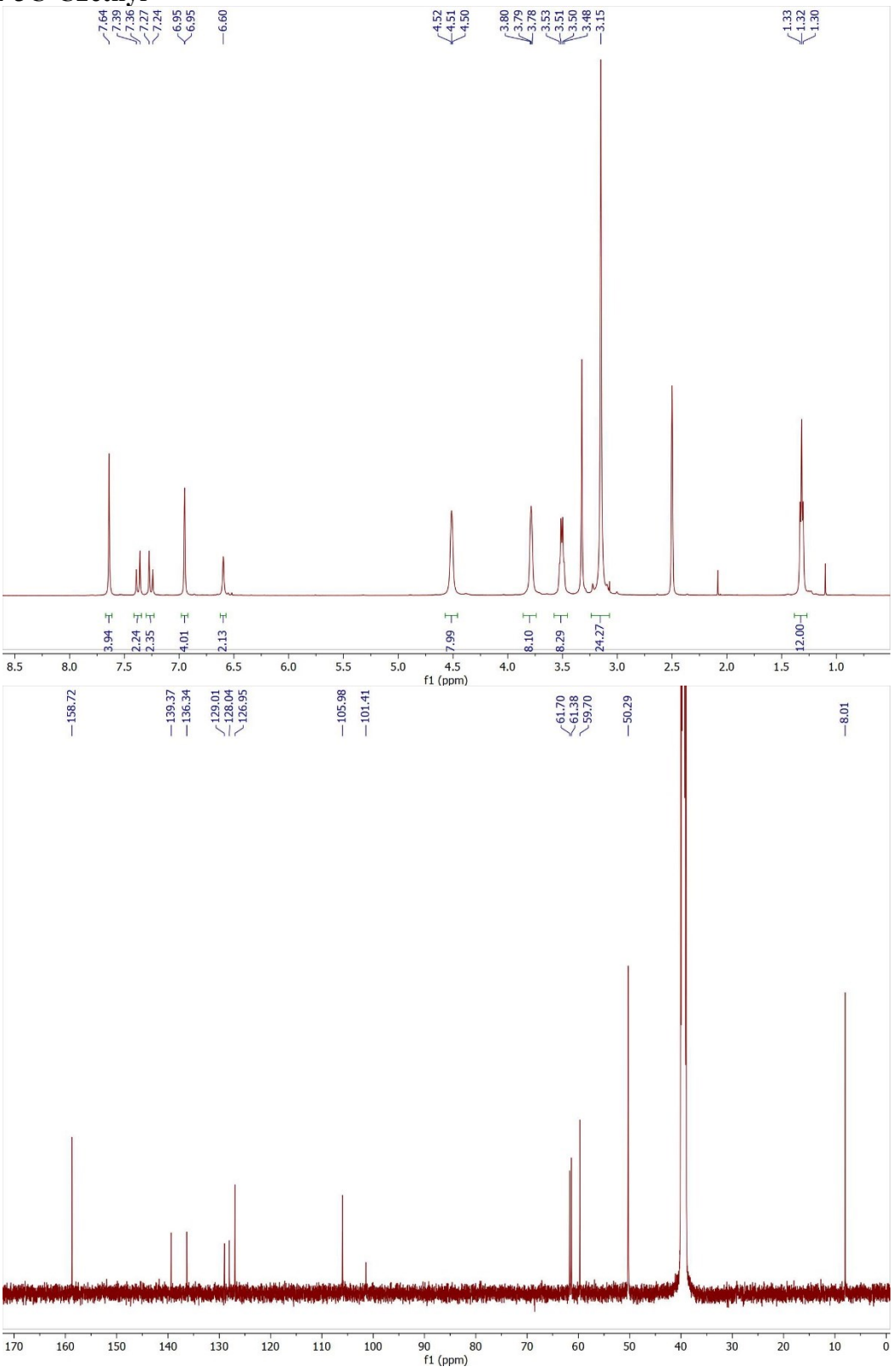
COE2-3C-C2hexyl



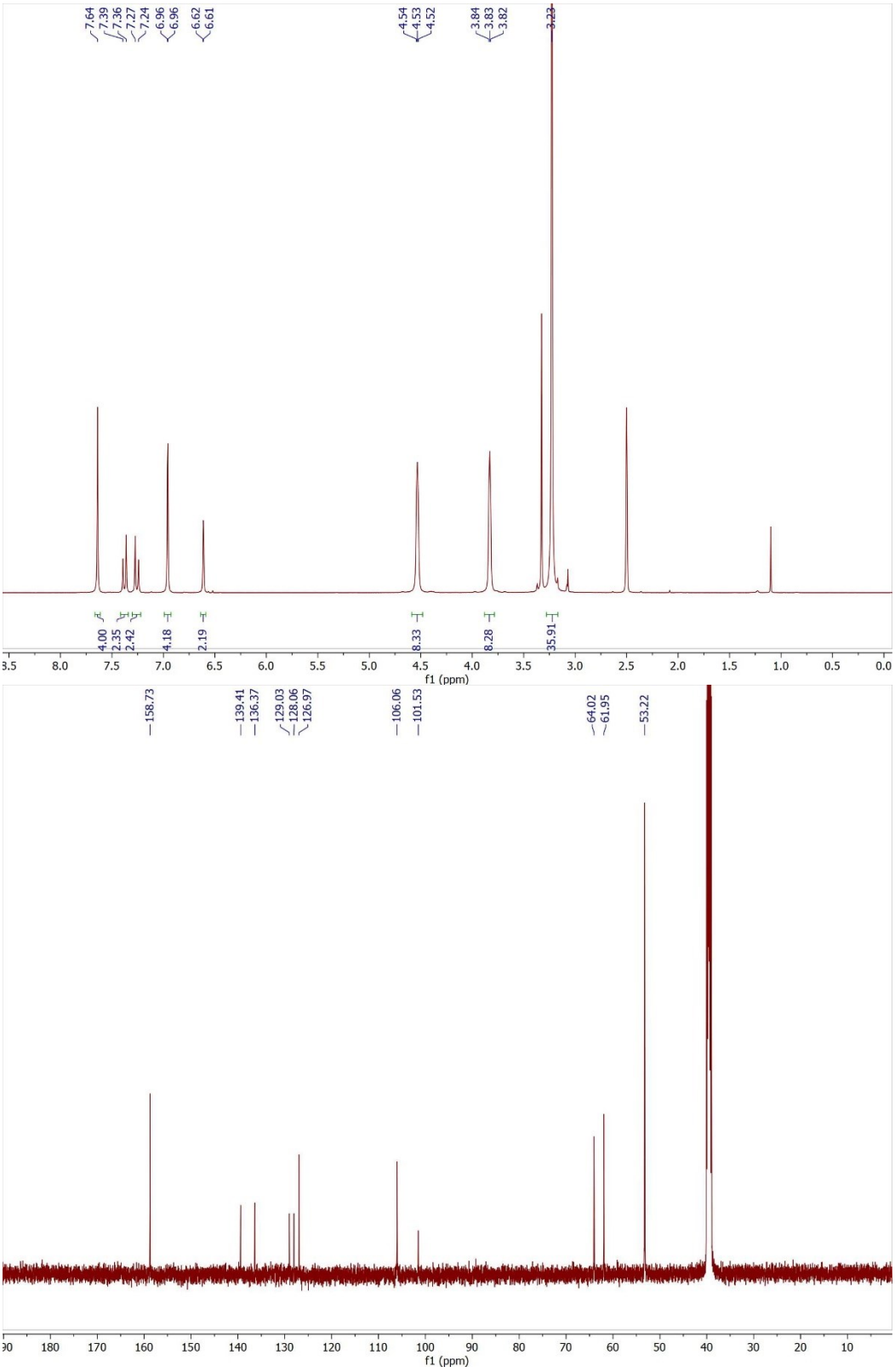
COE2-3C-C2butyl



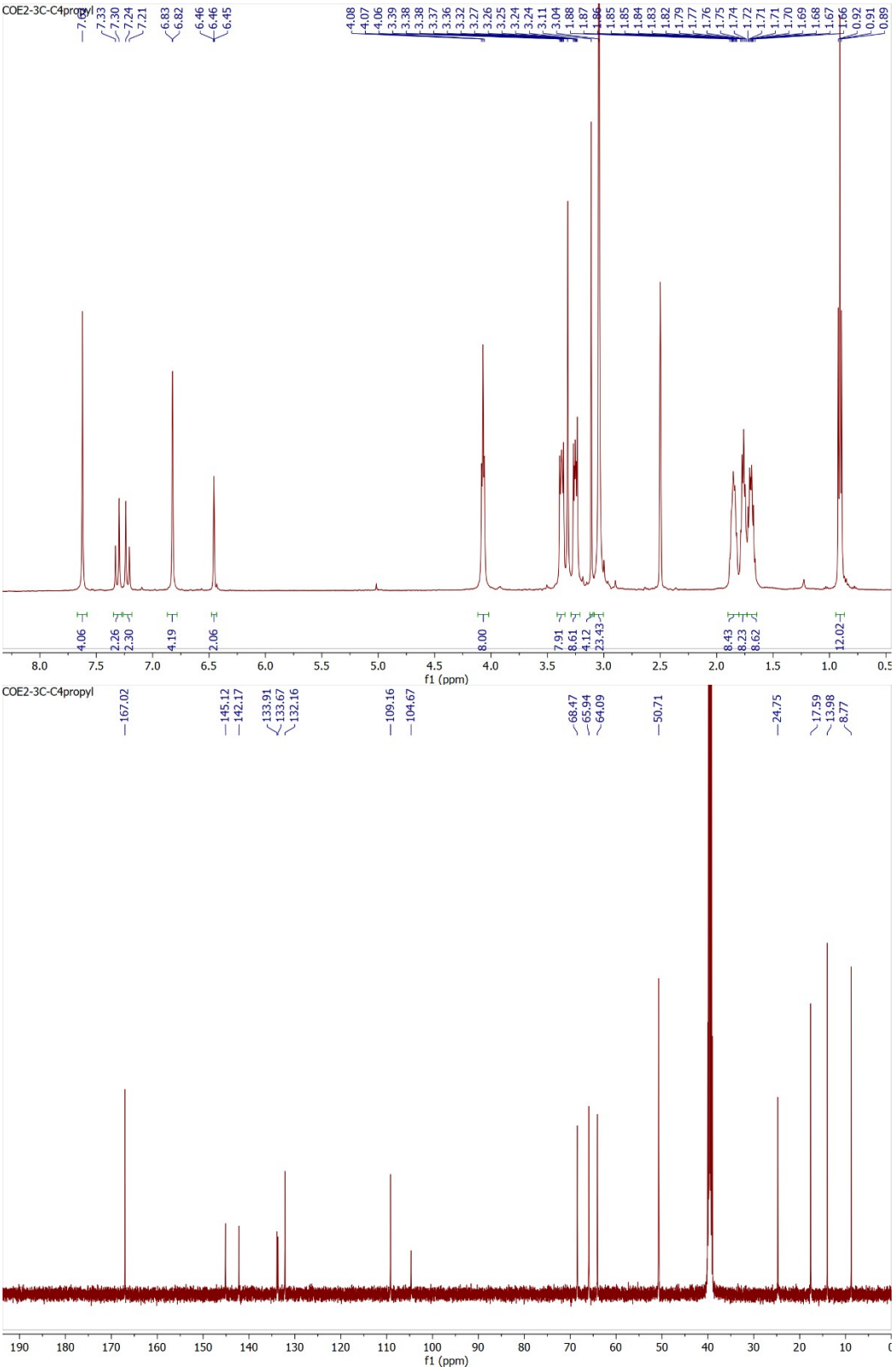
COE2-3C-C2ethyl



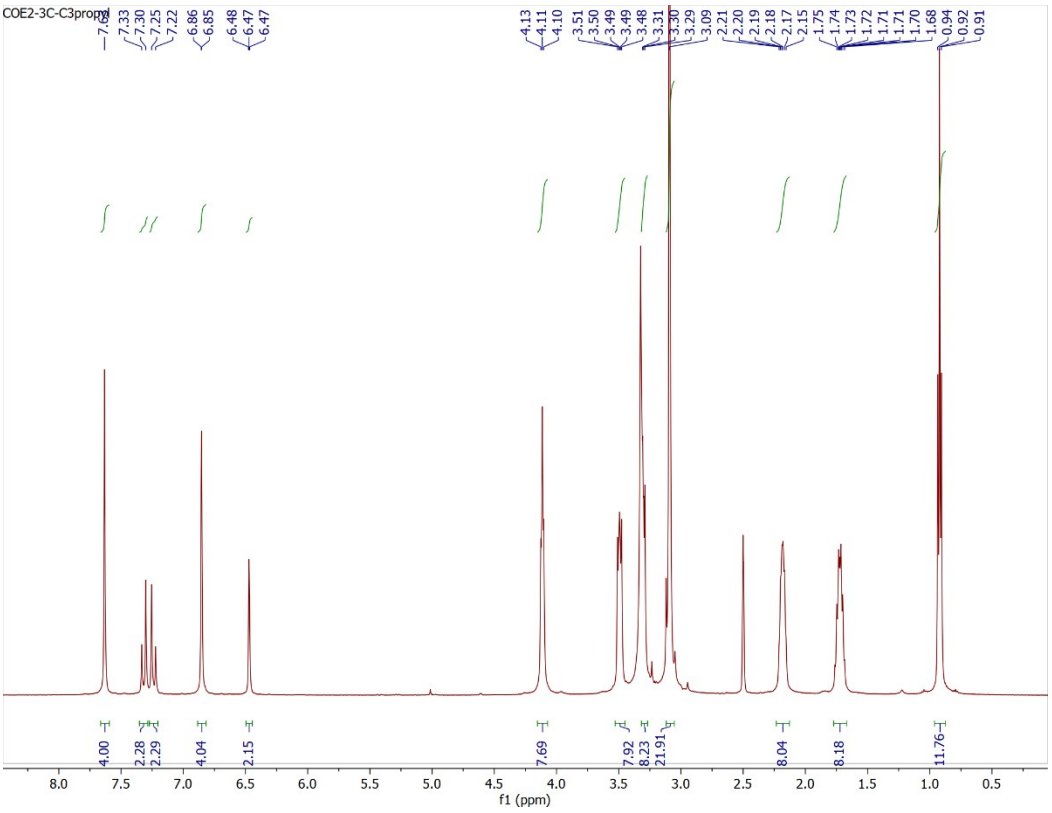
COE2-3C-C2

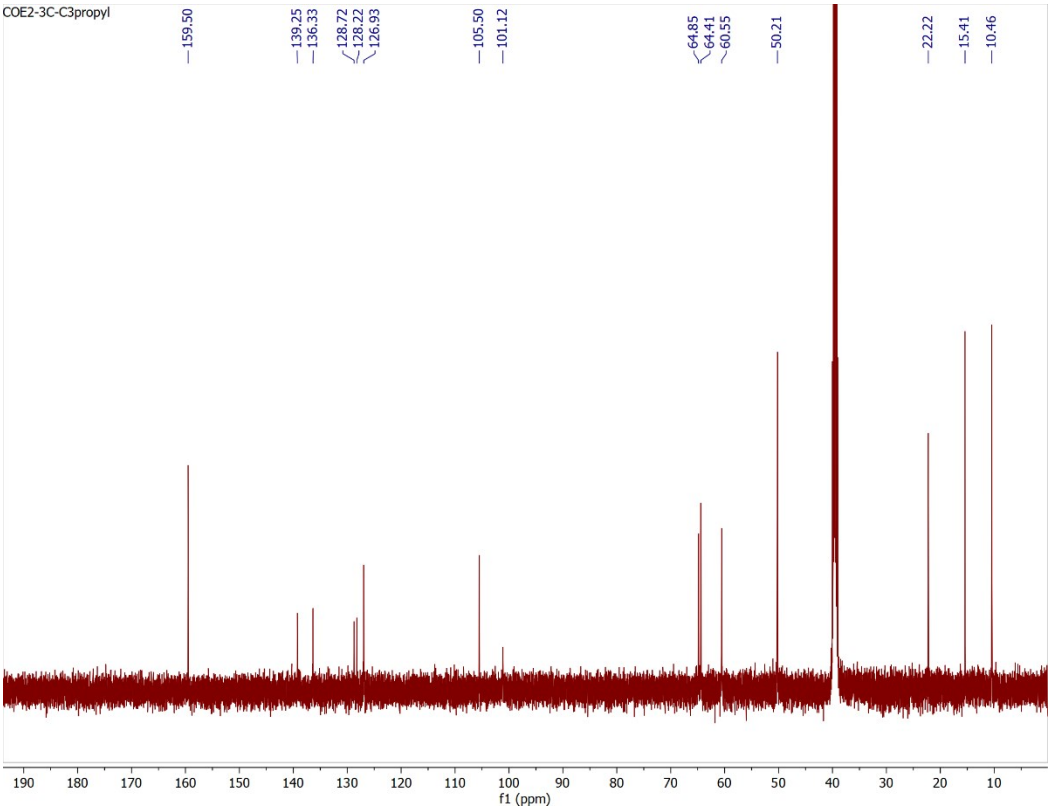

7.64
7.39
7.36
7.27
7.24
6.96
6.96
6.62
6.61
4.54
4.53
4.52
3.84
3.83
3.82
3.23
4.00
2.35
2.42
4.18
2.19
8.33
8.28
35.91
f1 (ppm)
158.73
139.41
136.37
129.03
128.06
126.97
106.06
101.53
64.02
61.95
53.22
f1 (ppm)

COE2-3C-C4propyl



COE2-3C-C3propyl





8. References

1 H. Hou, X. Chen, A. W. Thomas, C. Catania, N. D. Kirchhofer, L. E. Garner, A. Han, G. C. Bazan, *Adv. Mater*., 2013, **25**, 1593-1597.

2 L. F. Fieser, M. Fieser in *Reagents for Organic Synthesis, Vol. 1*, Wiley, New York, 1967, pp. 581–595.

3 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th edition. CLSI standard M07. 2018.

4 CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*. 28th edition. CLSI supplement M100. 2018.

5 R. J. W. Lambert, J. Pearson, *J. Appl. Microbiol*., 2001, **5**, 784–790.