Chemoenzymatic Macrocycle Synthesis Using Resorcylic Acid Lactone Thioesterase Domains

Graham W. Heberlig,[a] Jesse T. C. Brown,[a] Ryan Simard,[a] Monica Wirz,[a] Wei Zhang,[a] Meng Wang,[b] Leah I. Susser,[a] Mark E. Horsman,[a] and Christopher N. Boddy*[a]

[a]*Department of Chemistry and Biomolecular Sciences, Centre for Catalysis Research and Innovation, University of Ottawa, Ottawa, ON, K1N 6N5, Canada*
[b] *Key Laboratory of Systems Microbial Biotechnology, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin, 300308 China*

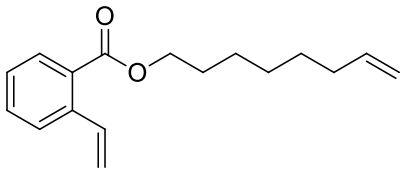
Table of Contents

General Synthetic Protocols

All reagents were purchased from Sigma-Aldrich at the highest available purity and used without further purification. All solvents were purchased from Fisher Scientific. All reactions were conducted using dry solvents under an argon atmosphere unless otherwise noted. NMR spectroscopy, with the exception of LC-SPE-NMR, was performed with a Bruker Avance II, operating at 400 MHz for ^{1}H spectra, and 100 MHz for ^{13}C spectra. HPLC analysis was conducted with an Agilent 1260 Modular system using an Agilent Zorbax C18 100x2.1 mm column. LC-SPE-NMR experiments were conducted using the same Agilent 1260 HPLC system and column with a Prospekt 2 (Spark Holland) solid-phase extraction module using HySphere Resin GP cartridges. Compounds were eluted off the cartridges with *d3*-acetonitrile and analyzed by mass spectroscopy and ^{1}H NMR using a Bruker Avance III, with cryoprobe operating at 600 MHz. High-resolution mass spectroscopy (HRMS) was conducted on a Micromass Q-TOF I for ESI measurements and a Kratos Concept 1S High Resolution Mass Spectrometer for EI measurements (John L. Holmes Mass Spectroscopy Facility).

Synthetic Protocols

Oct-7-en-1-yl 2-vinylbenzoate (S1).



A 15 ml round bottom flask equipped with a stir bar was charged with 7 ml dry DMF, to which was added 200 mg 2-vinylbenzoic acid[1] (1.37 mmol, 1 eq.), 668 mg cesium carbonate (2.05 mmol, 1.5 eq.), and 275 mg 8-bromooctene (1.44 mmol, 1.05 eq.). The reaction was stirred overnight at room temperature. The reaction mixture was diluted with 50 ml water, and extracted 3x10 ml Et_2O. The combined organic extracts were washed sequentially with 25 ml 1/2 sat. NaCl, 3/4 sat. NaCl, and finally brine. The organic layer was dried with Na_2SO_4 and concentrated *in vacuo* and purified by column chromatography 5% EtOAc/Hex. Yielded 283 mg **S1** (80%).

R_f = 0.5 (5:95 EtOAc:Hexanes)

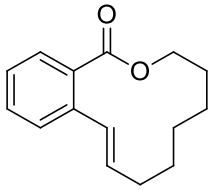
IR (NaCl) ν_{max} = 2945, 2871, 1718, 1286 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 – 7.83 (m, 1H), 7.58 – 7.54 (m, 1H), 7.48 – 7.40 (m, 2H), 7.30 (td, *J* = 7.6, 1.2 Hz, 1H), 5.79 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 1H), 5.63 (dd, *J* = 17.5, 1.3 Hz, 1H), 5.33 (dd, *J* = 11.0, 1.3 Hz, 1H), 4.98 (ddd, *J* = 17.1, 3.7, 1.6 Hz, 1H), 4.92 (ddt, *J* = 10.2, 2.2, 1.2 Hz, 1H), 4.28 (t, *J* = 6.7 Hz, 2H), 2.04 (q, *J* = 6.8 Hz, 2H), 1.79 – 1.70 (m, 2H), 1.50 – 1.22 (m, 8H)

^{13}C NMR (100 MHz, $CDCl_3$) δ 167.53, 139.51, 138.96, 135.97, 131.98, 130.23, 129.03, 127.38, 127.21, 116.33, 114.35, 65.19, 33.68, 28.78, 28.73, 28.65, 25.96.

HRMS (+EI) : calcd for $C_{15}H_{18}O_2$ (M) 230.1307, obsd 230.1302.

(E)-3,4,5,6,7,8-hexahydro-1H-benzo[c][1]oxacyclododecin-1-one (20).



To 192 mL dry toluene was added 200 mg **S1** (0.77 mmol) under argon, to this stirred solution 33 mg of Grubbs 2nd Gen. catalyst (0.04 mmol, 5 mol%) was added. The reaction was stirred at 88˚C for 4 days. At completion the reaction was concentrated and purified by column chromatography (95:5 Hexanes:EtOAc), yielding 79 mg (45%) of the title compound as a colorless oil.

R_f = 0.47 (9:1 hexanes:EtOAc)

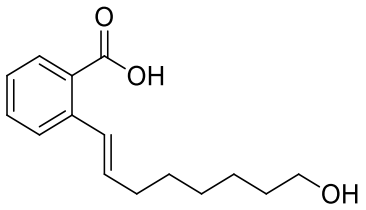
IR (NaCl) ν_{max} = 2924, 2867, 1720, 1288, 1249 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (dd, *J* = 7.7, 1.2 Hz, 1H), 7.41 (td, *J* = 7.5, 1.3 Hz, 1H), 7.32 – 7.24 (m, 2H), 6.69 (d, *J* = 15.8 Hz, 1H), 5.69 (dt, *J* = 15.8, 7.1 Hz, 1H), 4.34 – 4.27 (m, 2H), 2.27 (dt, *J* = 7.2, 3.6 Hz, 2H), 1.76 – 1.68 (m, 2H), 1.64 – 1.41 (m, 8H)

^{13}C NMR (100 MHz, $CDCl_3$) δ 169.53, 139.30, 132.29, 131.52, 131.49, 130.53, 129.79, 127.84, 126.59, 66.09, 30.90, 26.71, 26.21, 25.45, 23.78.

HRMS (+EI) : calcd for $C_{15}H_{18}O_2$ (M) 230.1307, obsd 230.1302.

(E)-2-(8-hydroxyoct-1-en-1-yl)benzoic acid (S3).



In a 10 mL round bottom 79 mg **S2** (0.343 mmol) was dissolved in 2:1 methanol:water (3 mL total) and to this 144 mg of LiOH (3.43 mmol, 10 eq.) was added. Several drops of THF were added until the solution became clear. The reaction was stirred over night at room temperature. At completion the reaction was diluted with water and the pH was adjusted to 2 using 10% HCl. This was extracted 3x 10 mL with EtOAc and the organic fractions were combined, dried over Na_2SO_4, and concentrated *in vacuo*. This yielded 86 mg of seco-acid **S3** (100%) as a clear oil.

R_f = 0.19 (1:1 EtOAc:hexanes)

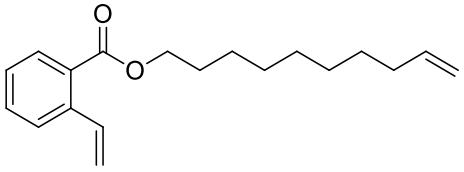
IR (NaCl) ν_{max} = 3859, 2936, 2863, 1692, 1260 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (dd, *J* = 7.9, 1.1 Hz, 1H), 7.53 – 7.49 (m, 1H), 7.46 (td, *J* = 7.6, 1.1 Hz, 1H), 7.29 – 7.24 (m, 1H), 7.19 (d, *J* = 15.8 Hz, 1H), 6.08 (dt, *J* = 15.7, 6.9 Hz, 1H), 3.66 (t, *J* = 6.4 Hz, 2H), 2.26 (qd, *J* = 7.0, 1.4 Hz, 2H), 1.61 – 1.36 (m, 8H)

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.65, 140.63, 133.79, 132.67, 131.05, 129.11, 127.55, 127.18, 126.58, 62.73, 32.61, 28.71, 28.40, 25.29.

HRMS (+EI) : calcd for $C_{15}H_{20}O_3$ (M) 248.1412, obsd 248.1405.

Dec-9-en-1-yl 2-vinylbenzoate (3).

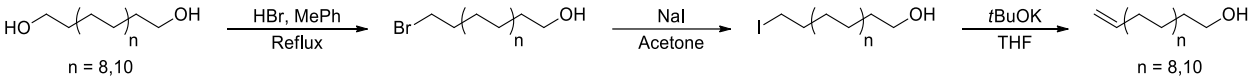


Prepared according to our previously reported conditions.[2] (72%)

R_f = 0.74 (9:1 hexanes:EtOAc).

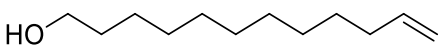
^{1}H NMR (400 MHz, CDCl3) δ 7.86 (dd, *J* = 7.9, 1.3 Hz, 1 H), 7.56 (d, *J* = 7.9 Hz, 1 H), 7.50-7.38 (m, 2 H), 7.30 (ddd, *J* = 7.7, 7.4, 0.9 Hz, 1 H), 5.79 (ddt, *J* = 17.1, 10.3, 6.5 Hz, 1 H), 5.63 (dd, *J* = 17.4, 1.3 Hz, 1 H), 5.33 (dd, *J* = 10.9, 1.3 Hz, 1 H), 4.98 (ddt, *J* = 17.1, 2.0, 1.7 Hz, 1 H), 4.92 (ddt, *J* = 10.3, 1.1, 1.1 Hz, 1 H), 4.29 (t, *J* = 6.8 Hz, 2 H), 2.08-1.97 (m, 2 H), 1.80-1.62 (m, 2 H), 1.48-1.21 (m, 10 H)

^{13}C NMR (100 MHz, CDCl3) δ 167.5, 139.5, 139.1, 136.0, 132.0, 130.2, 129.1, 127.4, 127.2, 116.3, 114.2, 65.2, 33.8, 29.4, 29.2, 29.0, 28.9, 28.7, 26.1.



Scheme S1. General synthetic strategy for the synthesis of the olefin terminated alcohols used to generate **8** and **9** from commercially available diols.

Dodec-11-en-1-ol (S4).

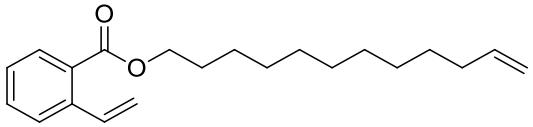


To 5 mL of toluene in a 25 mL round bottom flask was added 300 mg of 1,12-dodecanediol (1.48 mmol), to this was added 268 µL of 48% HBr (2.37 mmol, 1.6 eq.). The mixture was stirred overnight at reflux, at completion the aqueous layer was removed and the organic layer was washed with 1 N NaOH, brine, dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was then dissolved in 8 mL of acetone to which was added 794 mg of NaI (5.3 mmol, 3.5 eq.) followed by 4A molecular sieves. The reaction was stirred at reflux overnight, then diluted with brine and extracted with Et_2O. The combined organic extracts were dried over Na_2SO_4 and concentrated. The iodide was dissolved in 12 mL of anhydrous THF and 364 mg of solid potassium *t*-butoxide (3.24 mmol, 2.2 eq.) was added as a single portion. The reaction was stirred for a further 20 minutes then slowly quenched by the addition of water until the solution became clear. This was further diluted with brine and extracted with EtOAc, the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo* to yield 270 mg **S4** (89%, over three steps) as a clear oil. Characterization data is consistent with reported values.[3]

R_f = 0.5 (7:3 hexanes:EtOAc)

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.79 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 1H), 4.97 (ddd, *J* = 17.1, 3.6, 1.7 Hz, 1H), 4.93 – 4.87 (m, 1H), 3.62 (t, *J* = 6.6 Hz, 2H), 2.08 – 1.97 (m, 2H), 1.60 – 1.49 (m, 2H), 1.25 (m, 14H).

Dodec-11-en-1-yl 2-vinylbenzoate (S5).



2-vinyl benzoic acid (180 mg, 1.22 mmol) was dissolved in 7 mL anhydrous DCM, to this solution was added 270 mg **S4** (1.46 mmol, 1.2 eq.), 350 mg of EDC (1.83 mmol, 1.5 eq.), and 224 mg DMAP (1.83 mmol, 1.5 eq.). The reaction was stirred under argon at room temperature overnight. The reaction was quenched by the addition of sat. NH_4Cl, and the organic layer was removed. The aqueous layer was extracted with DCM, and combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated. The product was purified by column chromatography (98:2 hexanes:EtOAc) yielding 230 mg of **S5** (60%) as a colorless oil.

R_f = 0.55 (9:1 hexanes:EtOAc)

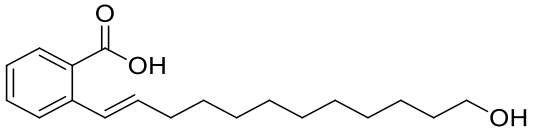
IR (NaCl) ν_{max} = 2925, 2853, 1712, 1260 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (dd, *J* = 7.8, 1.2 Hz, 1H), 7.58 – 7.54 (m, 1H), 7.44 (ddd, *J* = 14.2, 10.2, 8.6 Hz, 2H), 7.30 (td, *J* = 7.6, 1.2 Hz, 1H), 5.79 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 1H), 5.63 (dd, *J* = 17.4, 1.3 Hz, 1H), 5.32 (dd, *J* = 11.0, 1.3 Hz, 1H), 4.97 (ddd, *J* = 17.1, 3.7, 1.6 Hz, 1H), 4.91 (ddt, *J* = 10.2, 2.3, 1.2 Hz, 1H), 4.28 (t, *J* = 6.7 Hz, 2H), 2.06 – 1.98 (m, 2H), 1.78 – 1.69 (m, 2H), 1.46 – 1.21 (m, 14H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 167.5, 139.5, 139.2, 136.0, 132.0, 130.2, 129.1, 127.4, 127.2, 116.3, 114.1, 64.7, 33.8, 29.5, 29.5, 29.3, 29.1, 28.9, 28.6, 26.1, 25.9

HRMS (+EI) : calcd for $C_{21}H_{30}O_2$ (M) 314.2246, obsd 314.2248.

(E)-2-(12-hydroxydodec-1-en-1-yl)benzoic acid (S6).



A 250 ml flame-dried round bottom flask was charged with 160 mL of anhydrous toluene, 205 mg **S5** (0.65 mmol), and 28 mg of Grubbs 2nd Generation catalyst (5 mol%). The reaction was stirred at 80˚C for 24 hours, at which the reaction was concentrated and passed through a plug of silica to remove the catalyst. This crude product was dissolved in a 4:3:1 methanol:THF: water (14.5 mL total volume) solution and 273 mg of LiOH (6.5 mmol, 10 eq.) was added the reaction was stirred at 50˚C overnight. At completion the reaction was acidified to pH=2 with 10% HCl, and then was extracted with EtOAc. The combined organic fractions were combined, dried over Na_2SO_4, and concentrated. To yield 149 mg **S6** (75%, over two steps) as a colorless oil.

R_f = 0.41 (1:1 EtOAc:hexanes)

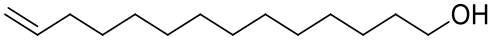
IR (NaCl) ν_{max} = 3861 2925, 2853, 1717 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (dd, *J* = 7.9, 1.2 Hz, 1H), 7.54 (d, *J* = 6.9 Hz, 1H), 7.49 – 7.43 (m, 1H), 7.27 (dd, *J* = 10.8, 4.3 Hz, 1H), 7.22 (d, *J* = 15.7 Hz, 1H), 6.13 (dt, *J* = 15.7, 6.9 Hz, 1H), 3.66 – 3.61 (m, 2H), 2.28 – 2.20 (m, 2H), 1.59 – 1.44 (m, 4H), 1.37 – 1.24 (m, 14H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2, 140.6, 134.4, 132.7, 131.2, 128.6, 127.4, 127.1, 126.5, 63.1, 33.1, 32.6, 29.4, 29.3, 39.3, 29.3, 29.1, 29.0, 25.6

HRMS (+EI) : calcd for $C_{19}H_{28}O_3$ (M) 304.2038, obsd 304.2048.

Tetradec-13-en-1-ol (S7).

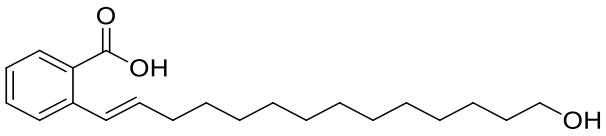


To 6 mL of toluene in a 25 mL round bottom flask was added 200 mg of 1,14-tetradecanediol (0.868 mmol), to this was added 160 µL of 48% HBr (1.40 mmol, 1.6 eq.). The mixture was stirred overnight at reflux, at completion the aqueous layer was removed and the organic layer was washed with 1 N NaOH, brine, dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was then dissolved in 7 mL of acetone to which was added 601 mg of NaI (4.00 mmol, 3.5 eq.) followed by 4A molecular sieves. The reaction was stirred at reflux overnight, then diluted with brine and extracted with Et_2O. The combined organic extracts were dried over Na_2SO_4 and concentrated. The iodide was dissolved in 7 mL of anhydrous THF and 218 mg of solid potassium *t*-butoxide (1.94 mmol, 2.2 eq.) was added as a single portion. The reaction was stirred for a further 20 minutes then slowly quenched by the addition of water until the solution became clear. This was further diluted with brine and extracted with EtOAc, the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo* to yield 270 mg **S7** (71%, over three steps) as a clear oil. Characterization is consistent with previously reported values.[4]

R_f = 0.37 (1:1 hexanes:EtOAc)

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.79 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 1H), 5.01 – 4.93 (m, 1H), 4.91 (ddt, *J* = 10.2, 2.3, 1.2 Hz, 1H), 3.62 (t, *J* = 6.6 Hz, 2H), 2.05 – 1.98 (m, 2H), 1.59-1.49 (m, 2H), 1.35 – 1.23 (m, 20H).

(E)-2-(14-hydroxytetradec-1-en-1-yl)benzoic acid (S8).



2-vinyl benzoic acid (75 mg, 0.51 mmol) was dissolved in 3 mL anhydrous DCM, to this solution was added 130 mg **S7** (0.61 mmol, 1.2 eq.), 147 mg of EDC (0.77 mmol, 1.5 eq.), and 93 mg DMAP (0.77 mmol, 1.5 eq.). The reaction was stirred under argon at room temperature overnight. The reaction was

quenched by the addition of sat. NH_4Cl, and the organic layer was removed. The aqueous layer was extracted with DCM, and combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated. A 250 ml flame-dried round bottom flask was charged with 60 mL of anhydrous toluene, the crude esterification product, and 10 mg of Grubbs 2nd Generation catalyst (5 mol%). The reaction was stirred at 80˚C for 24 hours, at which the reaction was concentrated and passed through a plug of silica to remove the catalyst. This crude product was dissolved in a 4:3:1 methanol:THF: water (5.5 mL total volume) solution and 100 mg of LiOH (2.4 mmol, 10 eq.) was added the reaction was stirred at 50˚C overnight. At completion the reaction was acidified to pH=2 with 10% HCl, and then was extracted with EtOAc. The combined organic fractions were combined, dried over Na_2SO_4, and concentrated. To yield 75 mg **S8** (44%, over three steps) as a colorless oil.

R_f = 0.43 (1:1 EtOAc:hexanes)

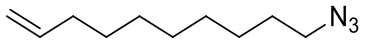
IR (NaCl) ν_{max} = 3460(br), 2930, 2857, 1694, 1261 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (dd, *J* = 7.9, 1.1 Hz, 1H), 7.56 (d, *J* = 7.6 Hz, 1H), 7.51 – 7.45 (m, 1H), 7.31 – 7.26 (m, 1H), 7.24 (d, *J* = 15.6 Hz, 1H), 6.16 (dt, *J* = 15.6, 6.9 Hz, 1H), 3.65 (t, *J* = 6.6 Hz, 2H), 2.26 (td, *J* = 8.1, 1.2 Hz, 2H), 1.61 – 1.45 (m, 4H), 1.38 – 1.26 (m, 16H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 172.28, 140.58, 134.43, 132.69, 131.16, 128.55, 127.43, 127.04, 126.52, 63.10, 33.18, 32.65, 29.53, 29.50, 29.49 (2C), 29.41, 29.36, 29.17, 29.14, 25.69.

HRMS (+EI) : calcd for $C_{21}H_{32}O_3$ (M) 332.2351, obsd 332.2343.

10-azidodec-1-ene (S9).

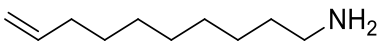


A 50 mL round bottom equipped with a stir bar was charged with 21 mL of anhydrous THF and 2.52 g triphenylphosphine (9.6 mmol, 1.5 eq.) then chilled to -20˚C. To this stirred solution was added 1.94 g diisopropyl azodicarboxylate (9.6 mmol, 1.5 eq.) then stirred for 10 additional minutes after which 1.0 g of 9-decen-1-ol (6.4 mmol) was added dropwise. After stirring for 30 minutes the reaction was warmed to 0˚C and 2.64 g diphenylphosphoryl azide (9.6 mmol, 1.5 eq.) was added dropwise then stirred for 10 minutes. THF was added until a yellow precipitate was formed, the precipitate was removed by filtration and the reaction mixture was concentrated and purifiedby column chromatography eluting with 5% EtOAc in hexanes. Yielded 902 mg **S9** (78%) as a slightly yellow oil. Characterization data is consistent with reported values.[5]

R_f = 0.87 (95:5 Hexanes:EtOAc)

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.79 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 1H), 4.97 (ddd, *J* = 17.1, 3.7, 1.6 Hz, 1H), 4.91 (ddt, *J* = 10.2, 2.3, 1.2 Hz, 1H), 3.24 (t, *J* = 7.0 Hz, 2H), 2.07 – 1.96 (m, 2H), 1.62 – 1.54 (m, 2H), 1.39 – 1.26 (m, 10H).

Dec-9-en-1-amine (S10).

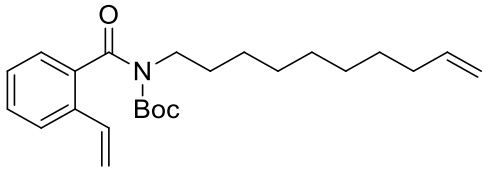


To 50 mL round bottom flask charged with 20 mL of THF and 1 mL water was added 900 mg **S9** (4.97 mmol) and 1.37 g triphenylphosphine (5.22 mmol, 1.05 eq.) at room temperature. The reaction mixture was stirred overnight and quenched with sat. NH_4Cl. The organic layer was removed and the aqueous layer was extracted with THF. The combined organic fractions were washed with brine, over Na_2SO_4, and concentrated. The compounds was purified by column chromatography (80: 25: 0.5 DCM:methanol: NH_4OH) yielding 700 mg **S10** (91%) as a slightly yellow oil. Characterization data is consistent with reported values.[6]

R_f = 0.38 (80: 25: 0.5 DCM:methanol: NH_4OH)

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.79 (ddt, *J* = 17.0, 10.2, 6.7 Hz, 1H), 4.97 (ddd, *J* = 17.1, 3.7, 1.6 Hz, 1H), 4.91 (ddt, *J* = 10.2, 2.3, 1.2 Hz, 1H), 2.67 (t, *J* = 7.0 Hz, 2H), 2.06 – 1.97 (m, 2H), 1.44 – 1.25 (m, 12H).

Tert-butyl dec-9-en-1-yl(2-vinylbenzoyl)carbamate (S11).



2-vinyl benzoic acid (120 mg, 0.81 mmol) was dissolved in 5 mL anhydrous dimethylformamide (DMF), to this solution was added 138 mg **S10** (0.89 mmol, 1.1 eq.), 171 mg of EDC (0.89 mmol, 1.1 eq.), 300 µL Hünig's base (1.70 mmol, 2.1 eq.), and 137 mg HOBt (0.89 mmol, 1.1 eq.). The reaction was stirred under argon at room temperature overnight. The reaction was quenched by the addition of sat. NH_4Cl, and the organic layer was removed. The aqueous layer was extracted with DCM, and combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated. This was dissolved in 6 mL THF to which was added 306 mg of di-*tert*-butyl dicarbonate (1.4 mmol, 2 eq.) and 94 mg DMAP (0.77 mmol, 1.1 eq.). The reaction was stirred at room temperature overnight, concentrated *in vacuo* and purified by column chromatography (8:2 hexanes:acetone) yielding 243 mg **S11** (78%, over 2 steps) as a slightly yellow oil.

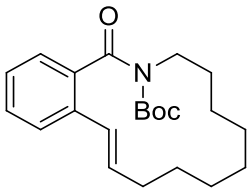
R_f = 0.51 (8:2 hexanes:acetone)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 – 7.51 (m, 1H), 7.37 – 7.31 (m, 1H), 7.26 – 7.21 (m, 1H), 7.17 (dd, *J* = 7.6, 1.1 Hz, 1H), 6.78 (dd, *J* = 17.4, 11.0 Hz, 1H), 5.79 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 1H), 5.68 (dd, *J* = 17.4, 1.0 Hz, 1H), 5.30 (dd, *J* = 11.0, 1.0 Hz, 1H), 4.97 (ddd, *J* = 17.1, 3.7, 1.6 Hz, 1H), 4.91 (ddt, *J* = 10.2, 2.3, 1.2 Hz, 1H), 3.84 – 3.76 (m, 2H), 2.06 – 1.98 (m, 2H), 1.72 – 1.61 (m, 2H), 1.39 – 1.26 (m, 10H), 1.07 (s, 9H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 172.11, 152.87, 139.22, 138.02, 134.89, 133.76, 129.26, 127.27, 126.00, 125.56, 116.55, 114.14, 82.96, 45.04, 33.81, 29.42, 29.28, 29.06, 28.91, 28.59, 27.31, 26.96.

HRMS (ESI+) calc. for $C_{24}H_{35}NO_3Na$ (M+Na) 408.2515, obsd. 408.2532

(E)-tert-butyl 1-oxo-3,4,5,6,7,8,9,10-octahydrobenzo[c][1]azacyclotetradecine-2(1H)-carboxylate (S12)



To 52 mL dry toluene was added 80 mg **S11** (0.21 mmol) under argon, to this stirred solution 9 mg of Grubbs 2nd Gen. catalyst (0.01 mmol, 5 mol%) was added. The reaction was stirred at 80˚C for 24 hours. At completion the reaction was concentrated and purified by column chromatography (90:10 Hexanes:EtOAc), yielding 37 mg (49%) of the title compound as a colorless oil.

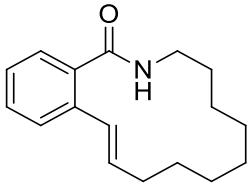
R_f = 0.37 (90:10 Hexanes:EtOAc)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (d, *J* = 7.8 Hz, 1H), 7.30 – 7.25 (m, 1H), 7.17 (td, *J* = 7.5, 1.2 Hz, 1H), 7.07 (dd, *J* = 7.6, 1.0 Hz, 1H), 6.53 (d, *J* = 15.8 Hz, 1H), 5.99 (dt, *J* = 15.8, 6.6 Hz, 1H), 2.23 (dd, *J* = 10.3, 6.4 Hz, 2H), 1.53 – 1.46 (m, 2H), 1.40 – 1.21 (m, 10H), 1.06 (s, 9H)

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.62, 153.21, 138.07, 135.11, 133.30, 128.58, 127.44, 126.19, 125.95, 124.42, 83.08, 44.44, 30.45, 29.71, 27.55, 27.21, 27.14, 26.04, 24.81, 24.04, 23.58.

HRMS (ESI+) calc. for $C_{22}H_{31}NO_3Na$ (M+Na) 380.2202, obsd. 380.2228

(E)-3,4,5,6,7,8,9,10-octahydrobenzo[c][1]azacyclotetradecin-1(2H)-one (23).



To a solution of 9.7 mg **S12** (0.03 mmol) in DCM at 0˚C was added 49 µL TMSOTf (0.27 mmol, 10 eq.). The reaction was stirred at 0˚C until complete as determined by TLC, at completion 38 µL of 2,6-lutidine (0.32 mmol, 12 eq.) was added and the reaction stirred 5 minutes. The reaction was then quenched with the addition of sat. $NaHCO_3$ and was extracted 3x 5 mL with DCM. The combined organic fractions were washed sequentially with $NaHCO_3$, $NaHSO_4$, and brine. Finally the organic fractions were dried with Na_2SO_4, and concentrated giving 6.6 mg **23** (95%) as a slightly yellow solid.

R_f = 0.20 (9:1 Hexanes:EtOAc)

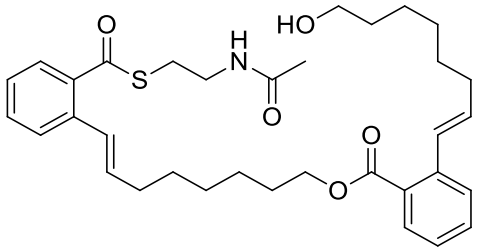
HPLC 10.5 min

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, *J* = 7.8 Hz, 1H), 7.38 – 7.30 (m, 2H), 7.21 (t, *J* = 7.5 Hz, 1H), 6.64 (d, *J* = 15.7 Hz, 1H), 6.02 (dt, *J* = 15.7, 7.0 Hz, 1H), 5.81 (s, 1H), 3.46 (dd, *J* = 10.8, 5.9 Hz, 2H), 2.26 (dd, *J* = 11.4, 6.3 Hz, 2H), 1.61 – 1.26 (m, 12H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 169.96, 135.94, 135.23, 133.75, 129.66, 128.03, 126.87, 125.97, 124.60, 39.45, 30.64, 28.10, 27.04, 26.47, 24.56, 24.35, 24.01.

HRMS (ESI+) calc. for $C_{17}H_{23}NONa$ (M+Na) 280.1677; obsd. 280.1656

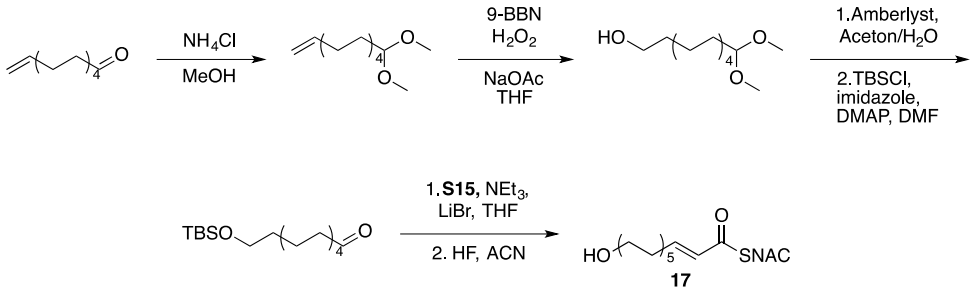
(E)-8-(2-(((2-acetamidoethyl)thio)carbonyl)phenyl)oct-7-en-1-yl 2-((E)-8-hydroxyoct-1-en-1-yl)benzoate (S14).



The title compound was isolated by LC-SPE trapping of the peak at 14.2 min in **Figure S4 D**, NMR characterization of the eluted peak can be found in **Figure S8** and **Figure S9**. Tabulated ^{1}H NMR data for the visible peaks is located below.

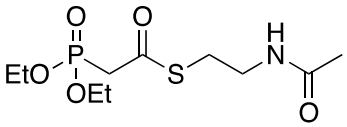
^{1}H NMR (600 MHz, CD_3CN) δ 7.78 (d, *J* = 7.9 Hz, 1H), 7.70 (d, *J* = 7.7 Hz, 1H), 7.64 (d, *J* = 8.1 Hz, 1H), 7.62 (d, *J* = 7.8 Hz, 1H), 7.50 (q, *J* = 6.2 Hz, 2H), 7.38 – 7.29 (m, 2H), 7.05 (d, *J* = 15.9 Hz, 1H), 6.75 (d, *J* = 15.8 Hz, 1H), 6.64 (s, 1H), 6.29 (dt, *J* = 15.6, 6.9 Hz, 1H), 6.23 (dt, *J* = 15.7, 6.9 Hz, 1H), 4.31 (t, *J* = 6.5 Hz, 2H), 3.50 (dd, *J* = 12.0, 6.5 Hz, 2H), 3.42 (q, *J* = 6.4 Hz, 2H), 3.16 (t, *J* = 6.6 Hz, 2H), 1.87 (s, *J* = 2.9 Hz, 3H), 1.81 – 1.75 (m, 2H), 1.54 – 1.44 (m, 10H), 1.40 – 1.29 (m, 11H).

HRMS (ESI+) calc. for $C_{34}H_{45}NO_5SNa$ (M+Na) 602.2916; obsd. 602.2959



Scheme S2. General synthetic strategy for the synthesis of compound **17**.

S-(2-acetamidoethyl) 2-(diethoxyphosphoryl)ethanethioate (S15)

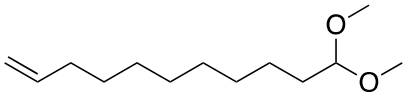


To a solution of 1.22 g diethyl phosphonoacetic acid (6.22 mmol) in DCM at 0˚C was added 1.48 mg EDC (7.74 mmol, 1.3 eq.), 82 mg DMAP (0.672 mmol 0.1 eq.), and 888 mg of **13**. The reaction was stirred overnight at room temperature. Upon completion the reaction was quenched with sat. NH_4Cl. The organic layer was separated and the aqueous layer extracted with DCM. The organic layers were washed with sat. $NaHCO_3$, Brine, dried over $MgSO_4$, and concentrated. The crude product was purified by column chromatography (5:95 MeOH/DCM) yielding 422 mg of **S15** (23%) as a colorless oil. Characterization is consistent with previously reported values.[7]

R_f = 0.17 (5:95 methanol/DCM)

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.15 – 5.99 (s, 1H), 4.15 (q, *J* = 7.1, 4H), 3.45 (dd, *J* = 12.2, 6.1 Hz, 2H), 3.22 (s, 2H), 3.14 – 3.02 (m, 2H), 1.95 (s, 3H), 1.34 (t, *J* = 7.1 Hz, 6H).

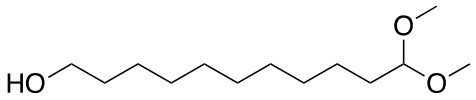
11,11-dimethoxyundec-1-ene (S16)



To a solution of 1.68 g undec-10-enal (10 mmol) in dry methanol was added 50 mg NH_4Cl (0.5 mmol, 0.05 eq.) The solution was heated to reflux for 18 h. The solution was quenched with sat. $NaHCO_3$, extracted with EtOAc, dried over $MgSO_4$, and concentrated to yield 1.97 g **S16** (92%) as a colorless oil. Characterization is consistent with previously reported data.[8]

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.84 – 5.73 (m, 1H), 4.99 – 4.87 (m, 2H), 4.34 (t, *J* = 5.7 Hz, 1H), 3.29 (s, 6H), 2.03 – 1.98 (m, 2H), 1.60 – 1.53 (m, 2H), 1.37 – 1.26 (m, 12H).

11,11-dimethoxyundecan-1-ol (S17)

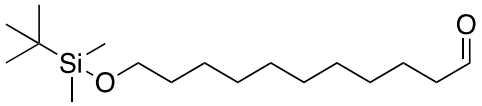


To a solution of 1.15 g **S16** (5.37 mmol) in dry THF was added 11 ml of 9-BBN THF solution (0.5 M, 5.5 mmol, 1.1 eq.). The solution was stirred for 1 h at room temperature followed by the addition of 5.5 ml NaOAc solution (3 M, 16.5 mmol, 3 eq.) and 1 ml of water. The solution was cooled to 0˚C and 2.25 ml of 30% H_2O_2 in water (20 mmol, 4 eq.) was slowly added over 30 minutes and stirred for 16 h. The solution was extracted with Et_2O, dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography (20:80 EtOAc/hexanes) to yield 450 mg **S17** (37%) as a colorless oil. Characterization is consistent with previously reported data.[9]

R_f = 0.1 (20:80 EtOAc/hexanes)

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.34 (t, *J* = 5.7 Hz, 1H), 3.62 (t, *J* = 6.7 Hz, 2H), 3.29 (s, 6H), 1.61 – 1.49 (m, 4H), 1.26 (s, 14H).

11-((*tert*-butyldimethylsilyl)oxy)undecanal (S18)

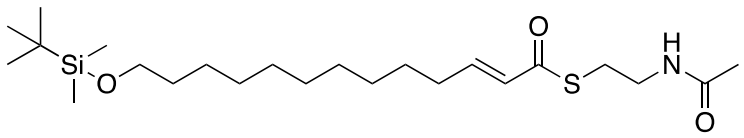


400 mg of Amberlyst cation exchange resin was added to 900 mg of **S17** (3.88 mmol) in an Acetone/water solution (60:1) and stirred overnight. The solution was filtered, dried over $MgSO_4$ and concentrated to yield 720 mg of 11-hydroxyundecanal. The crude product was dissolved in DMF, followed by the addition of 310 mg imidazole (4.55 mmol, 1.2 eq.), 31 mg DMAP (0.254 mmol, 0.05 eq.), and 800 mg TBSCl (5.3 mmol, 1.4 eq.). The solution was stirred for 18 h and quenched with sat. NH_4Cl. The aqueous mixture was extracted with Et_2O, dried over $MgSO_4$ and concentrated. The crude mixture was purified by column chromatography (5:95 EtOAc/hexanes) to yield 900 mg **S18** (77% over 2 steps) as a colorless oil. Characterization is consistent with previously reported data.[10]

R_f = 0.45 (5:95 EtOAc/hexanes)

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.74 (t, *J* = 1.9 Hz, 1H), 3.57 (t, *J* = 6.7 Hz, 2H), 2.40 (td, *J* = 7.3, 1.9 Hz, 2H), 1.60 (m, 2H), 1.48 (m, 2H), 1.26 (m, 12H), 0.89 – 0.85 (s, 9H), 0.10 – -0.06 (s, 6H).

S-(2-acetamidoethyl) (E)-13-((tert-butyldimethylsilyl)oxy)tridec-2-enethioate (S19)



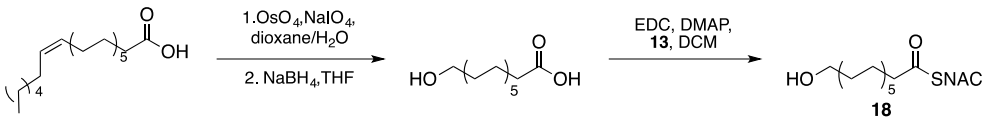
To a solution of 104 mg LiBr (1.2 mmol, 5 eq.) in dry THF was added 144 mg **S15** (0.485 mmol, 2 eq.) under argon, the solution was stirred for 10 minutes at room temperature. 210 µl NEt_3 (1.5 mmol, 6 eq.) was slowly added and the solution was stirred 10 more minutes. 97 mg **S18** (0.323 mmol) was added over 30 minutes and the solution was stirred for 16 h. The solution was quenched with NH_4Cl, extracted with EtOAc, dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography (50:50 EtOAc/hexanes) to yield 109 mg **S19** (76%) as a colorless oil.

R_f = 0.14 (50:50 EtOAc/hexanes)

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.91 (dt, *J* = 15.6, 7.0 Hz, 1H), 6.11 (d, *J* = 15.6 Hz, 1H), 5.91 – 5.77 (m, 1H), 3.57 (t, *J* = 6.6 Hz, 2H), 3.45 (q, *J* = 6.5 Hz, 2H), 3.07 (t, *J* = 6.3 Hz, 2H), 2.18 (q, *J* = 6.6 Hz, 2H), 1.94 (s, 3H), 1.46 (m, 4H), 1.25 (m, 12H), 0.87 (s, 9H), 0.02 (s, 6H).

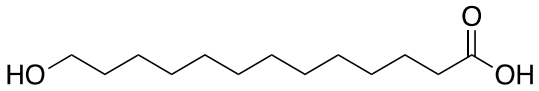
^{13}C NMR (100 MHz, $CDCl_3$) δ 170.26, 146.91, 128.29, 63.35, 39.90, 32.92, 32.31, 29.61, 29.43, 29.38, 29.34, 29.20, 28.29, 27.95, 25.99, 25.83, 23.28, -5.25

HRMS (ESI+) calc. for $C_{23}H_{45}NO_3SSiNa$ (M+Na) 466.2787; obsd. 466.2766



Scheme S3. General synthetic strategy for the synthesis of compound **18**.

13-hydroxytridecanoic acid (S20)



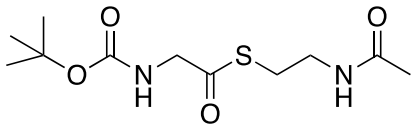
To a solution of 2 g of erucic acid (5.9 mmol) in dioxane/water 3:1, was added 1.4 ml 2,6-lutidine (11.8 mmol, 2 eq.), 53 mg potassium osmate dihydrate (0.59 mmol, 0.1 eq.), and 5 g sodium periodate (23.6 mmol, 4 eq.).The solution was stirred at room temperature for 16 h. The solution was diluted with water, extracted with EtOAc, washed with $NaHCO_3$, Brine, dried over $MgSO_4$ and concentrated. The crude product was dissolved in dry THF and cooled to 0˚C. 70 mg of $NaBH_4$ (1.10 mmol, 0.5 eq.) was added slowly over 15 minutes under argon. The solution was stirred for 3 h at room temperature, at completion 1.0 M HCl was added and the solution was extracted with EtOAc, dried over $MgSO_4$, and concentrated. The crude product was purified by column chromatography (40:60 EtOAc/hexanes) to

yield 1.1 g of pure **S20** (81%) as a grey solid. Characterization is consistent with previously reported data.[11]

R_f = 0.19 (40:60 EtOAc/hexanes)

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.63 (t, *J* = 6.6 Hz, 2H), 2.33 (t, *J* = 7.4 Hz, 2H), 1.55 (m, 4H), 1.25 (m, 16H).

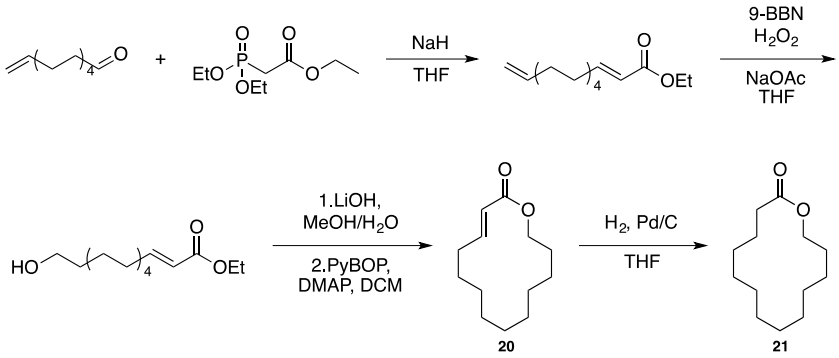
S-(2-acetamidoethyl) 2-((tert-butoxycarbonyl)amino)ethanethioate (S21)



To a solution of 450 mg Boc-Gly-OH (2.83 mmol) in dry DCM at 0˚C was added 755 mg EDC (3.95 mmol, 1.5 eq.) and 29 mg DMAP (0.28 mmol, 0.1 eq.), the solution was then stirred for 10 min. 350 mg **13** (2.93, 1.1 eq.) was added, and the solution was stirred for 22 h at room temperature. The solution was quenched with NH_4Cl, extracted with DCM, washed with Brine, dried over $MgSO_4$, and concentrated. The crude product was purified by column chromatography (40:60 EtOAc/hexanes) to yield 500 mg **20** (68%) as colorless oil. Characterization is consistent with previously reported data.[12]

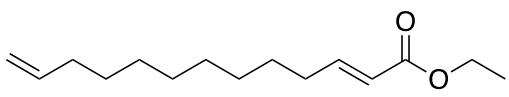
R_f = 0.15 (40:60 EtOAc/hexanes)

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.96 (s, 1H), 5.20 (s, 1H), 4.00 (d, *J* = 6.1 Hz, 2H), 3.40 (q, *J* = 6.2 Hz, 2H), 3.02 (t, *J* = 13.6 Hz, 2H), 1.93 (s, 3H), 1.43 (s, 9H).



Scheme S4. General synthetic strategy for the synthesis of compound **20** and **21**.

Ethyl (E)-trideca-2,12-dienoate (S22)

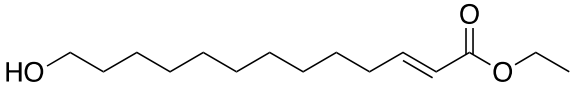


To a solution of 9 g triethyl phosphonacetate (40 mmol, 1.5 eq.) cooled to 0˚C in dry THF, was added 1.9 g NaH (40 mmol 1.5 eq.) slowly over 10 minutes. The solution was stirred 20 minutes followed by the addition of 6 g undec-10-enal (27 mmol) over 3 h. The solution was stirred for 5 h and quenched with NH_4Cl. The aqueous layer was extracted with ether, washed with brine, dryed over $MgSO_4$ and concentrated. The crude product was purified by column chromatography (5:95 EtOAc/hexanes) to yield 6.67 g of **S22** (93%) as a colorless oil. Characterization is consistent with previously reported data.[13]

R_f = 0.5 (5:95 EtOAc/hexanes)

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.94 (dt, *J* = 15.6, 7.0 Hz, 1H), 5.84 – 5.73 (m, 2H), 5.00 – 4.88 (m, 2H), 4.16 (q, *J* = 7.1 Hz, 2H), 2.17 (q, *J* = 7.3 Hz, 2H), 2.02 (q, *J* = 6.8 Hz, 2H), 1.47 – 1.20 (m, 15H).

ethyl (E)-13-hydroxytridec-2-enoate (S23)

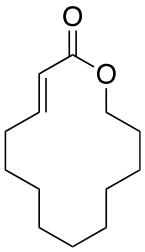


To a solution of 3.04 g **S22** (10.7 mmol) in dry THF was added 20 ml of 9-BBN THF solution (0.5 M, 10.7 mmol, 1.1 eq.). The solution was stirred for 1 h at room temperature followed by the addition of 15 ml NaOAc solution (3 M, 32.1 mmol, 3 eq.) and 2.5 ml of water. The solution was cooled to 0˚C and 7.5 ml of 30% H_2O_2 in water (42.8 mmol, 4 eq.) was slowly added over 30 minutes and stirred for 16 h. The solution was extracted with Et_2O, dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography (50:50 EtOAc/hexanes) to yield 2.0 g **S23** (61%) as a colorless oil. Characterization is consistent with previously reported data.[14]

R_f = 0.45 (50:50 EtOAc/hexanes)

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.94 (dt, *J* = 15.6, 7.0 Hz, 1H), 5.78 (d, *J* = 15.6 Hz, 1H), 4.20 – 4.13 (m, 2H), 3.62 (t, *J* = 6.6 Hz, 2H), 2.16 (q, *J* = 7.2 Hz, 2H), 1.53 (m, 2H), 1.42 (m, 2H), 1.30 – 1.24 (m, 15H).

(E)-oxacyclotetradec-3-en-2-one (S24)

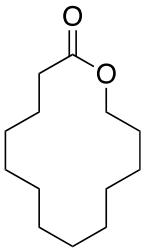


63 mg of **S23** (0.424 mmol) was dissolved in a MeOH/THF/H_2O (4:3:1) mix, followed by 80 mg LiOH (4.2 mmol). The solution was heated to 50˚C and stirred for 12 hours. Upon completion the solution was acidified to pH = 2, extracted with EtOAc, dried over $MgSO_4$ and concentrated. The seco acid was dissolved in dry DCM and added to a solution of 705 g DMAP (5.8 mmol, 30 eq.) and 407 mg PyBOP (0.8 mmol, 4 eq.), over a 10-hour period at room temperature to final concentration 0.004 M. The solution was stirred an additional 2 h, quenched with NH_4Cl, extracted with EtOAc, dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography (3:97 EtOAc/hexanes) to yield 24 mg **S24** (52%) as a colorless oil. Characterization is consistent with previously reported data.[15]

R_f = 0.3 (5:95 EtOAc/hexanes)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05 – 6.92 (dt, *J* = 15.6, 7.0 Hz, 1H), 5.79 (d, *J* = 15.7 Hz, 1H), 4.29 – 4.18 (m, 2H), 2.24 (m, 2H), 1.66 (m, 2H), 1.57 (m, 2H), 1.26 (m, 12H).

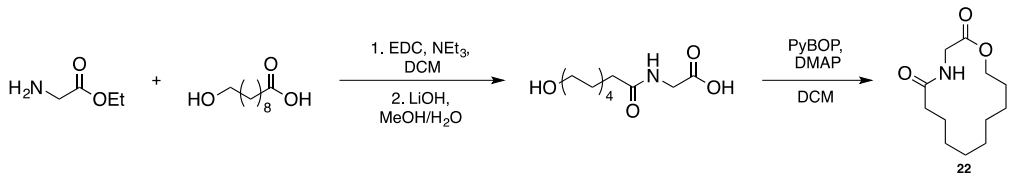
oxacyclotetradecan-2-one (S25)



12 mg **S24** (0.06 mmol) was dissolved in a mixture of THF/MeOH (1:1), containing 5 mg Pd/C catalyst. The flask was stirred for 24 h under 1 atm of H_2. The solution was diluted with THF, filtered through celite and concentrated. The crude product was purified by column chromatography (3:97 EtOAc/hexanes) to yield 9 mg **S25** (75%) as a colorless oil. Characterization is consistent with previously reported data.[16]

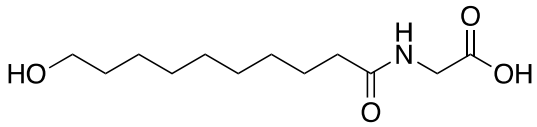
R_f = 0.1 (3:97 EtOAc/hexanes)

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.17 – 4.09 (m, 2H), 2.39 – 2.30 (m, 2H), 1.62 (dd, *J* = 11.7, 6.2 Hz, 4H), 1.42 – 1.21 (m, 16H).



Scheme S4. General synthetic strategy for the synthesis of compound **22**.

(10-hydroxydecanoyl)glycine (S26)



To a solution of 685 mg ethyl glycinate (4.91 mmol, 1.5 eq.) in DCM was added 1.1 g EDC (3.19 mmol, 1.6 eq.) and 1.37 mL NEt_3 (9.8 mmol, 3 eq.), the solution was then stirred for 10 minutes. 600 mg 10-hydroxydecanoic acid (3.19 mmol) was added to the solution at 0˚C and stirred for 14 h at roomtemperature. The solution was quenched with NH_4Cl, extracted with EtOAc, dried over $MgSO_4$, and concentrated. The crude product was dissolved in MeOH/THF/H_2O (6:10:6), followed by the addition of 845 mg LiOH (36.7 mmol, 10 eq.) and subsequently stirred at 60˚C for 4 h. The solution was acidified to pH = 2, extracted with EtOAc, dried over $MgSO_4$, and concentrated. The crude product was purified by column chromatography (80:20 EtOAc) to yield 600 mg **S26** (77%) as a white powder.

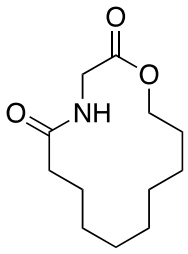
R_f = 0.2 (80:20 EtOAc/hexanes)

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02 (s, 1H), 3.66 (d, *J* = 5.9 Hz, 2H), 3.33 (m, 2H), 2.06 (t, *J* = 7.4 Hz, 2H), 1.50 – 1.30 (m, 4H), 1.20 (m, 10H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 173.02, 171.92, 61.18, 40.99, 35.52, 33.01, 29.46, 29.39, 29.25, 29.07, 25.96, 25.65.

HRMS (ESI+) calc. for $C_{12}H_{23}NO_4Na$ (M+Na) 268.1525; obsd. 268.1536

1-oxa-4-azacyclotetradecane-2,5-dione (24)



100 mg **S26** (0.41 mmol) was dissolved in dry DCM and added to a solution of 705 g DMAP (5.8 mmol, 30 eq.) and 407 mg PyBOP (0.8 mmol, 4 eq.), over a 10-hour period at room temperature to a final concentration of 0.004 M. The solution was stirred an additional 2 h, quenched with NH_4Cl, extracted with EtOAc, dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography (30:70 EtOAc/hexanes) to yield 56 mg **24** (56%) as a white solid.

R_f = 0.3 (30:70 EtOAc/hexanes)

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.92 – 5.76 (s, 1H), 4.22 – 4.15 (m, 2H), 4.08 (d, *J* = 6.3 Hz, 2H), 2.30 – 2.22 (m, 2H), 1.73 – 1.59 (m, 4H), 1.36 (m, 10H).

^{13}C NMR (100 MHz, CD_3OD) δ 172.91, 169.16, 64.57, 41.86, 35.50, 26.76, 26.30, 26.01, 25.85, 25.25, 24.89, 23.16.

HRMS (ESI+) calc. for $C_{12}H_{21}NO_3Na$ (M+Na)250.1419; obsd. 250.1407

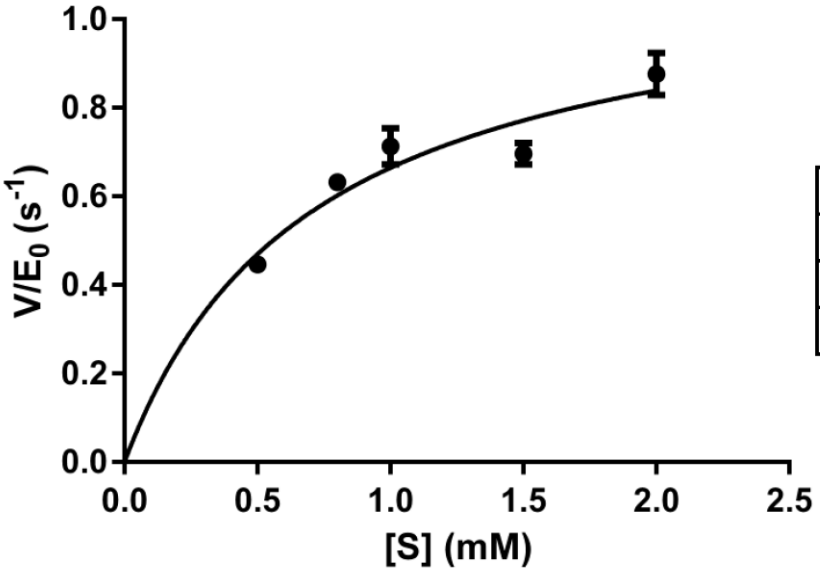
Enzymatic Assays

Rdc TE and Zea TE were expressed from pMW29[1] and pMW14[2] respectively. The proteins were purified to homogeneity according to our previously reported method[1]. The enzymes were stored at -80˚C in a 30% glycerol solution until immediately before use, where they were diluted to 100 µM stock solutions in 50 mM phosphate buffer (pH 7.4). The lyophilized Rdc TE was expressed and purified in an identical manner as the freezer stocks however it was freeze dried prior to the addition of glycerol. The powdered enzyme was reconstituted in 50 mM phosphate buffer (pH 7.4) and diluted to a working concentration of 100 µM in 50 mM phosphate buffer (pH 7.4). All TE assays where HPLC was used to characterize product distribution were carried out with 1.0 mM of the SNAC substrate, 50 mM phosphate buffer at pH 7.4, DMSO up to 10% by volume, and 5 µM thioesterase with the exception of the 10-member substrate (**11**) which used 20 µM of both thioesterases, and the 14-member lactam substrate (**16**) which used 25 µM for the Zea TE assay. Assays were quenched by the addition of an equal volume of 0.5% formic acid in acetonitrile. HPLC analysis was conducted using a gradient from 15% B to 95% B over 18 minutes and held at 95% B for 1 minute (A: Water, B: Acetonitrile) with the exception of 14-membered substrates **17, 18,** and **19**, that used a gradient of 0% B to 100% B over 35 minutes and held at 100% B for 2 minutes (A: Water, B: Acetonitrile).

Macrocyclization kinetics for the Rdc TE with the 14-member substrate (**6**) was conducted in a discontinuous manner in 50 mM phosphate buffer (pH 7.4), substrate concentrations ranging from 0.5 mM to 2.0 mM (from a 50 mM stock solution), 5 µM of Rdc TE, and DMSO up to 10% solution volume. The assays were quenched with an equal volume of 0.5% formic acid in acetonitrile before being analyzed by HPLC. HPLC analysis was conducted in an identical manner as above. Amount of macrocycle produced was determined by comparison to a standard curve of authentic, synthetic macrocycle. Non-linear regression was performed with GraphPad Prism 6.

Activity was assayed discontinuously with reaction aliquots mixed with saturated Ellman's reagent. Rdc TE has been shown to inactivate in real-time assays in the presence of DTNB. 1 uL of saturated DTNB in 50 mM potassium phosphate was mixed with 24 µL of reaction mixture at its endpoint to reach a final 4 % volume as shown in previous assay conditions. 2 µL of this mixture was loaded onto a Nanodrop 2000 podium and recorded at 412 nm. A standard curve using *N*-acetyl cysteamine in assay conditions was used to generate a standard curve (0, 5, 10, 25, 50, 75, 100, 500, 1000 µM in triplicate).

The effect of DMSO concentration on lyophilized Rdc TE activity was assayed on the 100 µL scale with 5 µM enzyme, 2.5 mM *S*-(2-acetamidoethyl) benzothioate[2], 10-50% (v/v) DMSO, and 50 mM potassium phosphate (pH 7.4). Conversion was measured after 10 minutes of reaction.



	Value	Error
R^2	0.846	N/A
k_{cat}	1.14	0.11
K_M	0.71	0.18

Figure S1. Initial reaction rates (s^{-1}) plotted against substrate concentration (mM) for conversion to macrocycle for Rdc TE with substrate **6**. These data were fit with the Michaelis-Menten model.

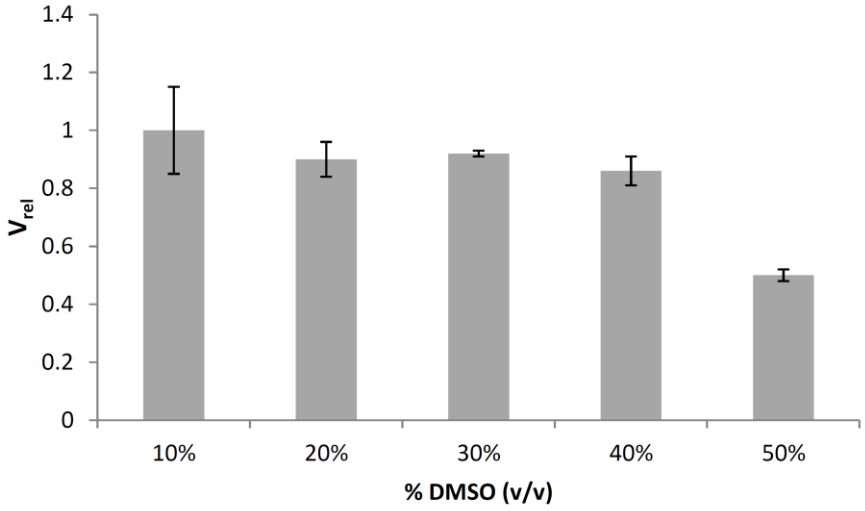


Figure S2. Initial reaction rates for *S*-(2-acetamidoethyl) benzothioate with Rdc TE with varying concentrations of DMSO. Rates are relative to the standard reaction conditions at 10% (v/v) DMSO. Duplicate data for 20% and 30% DMSO, triplicate for all others.

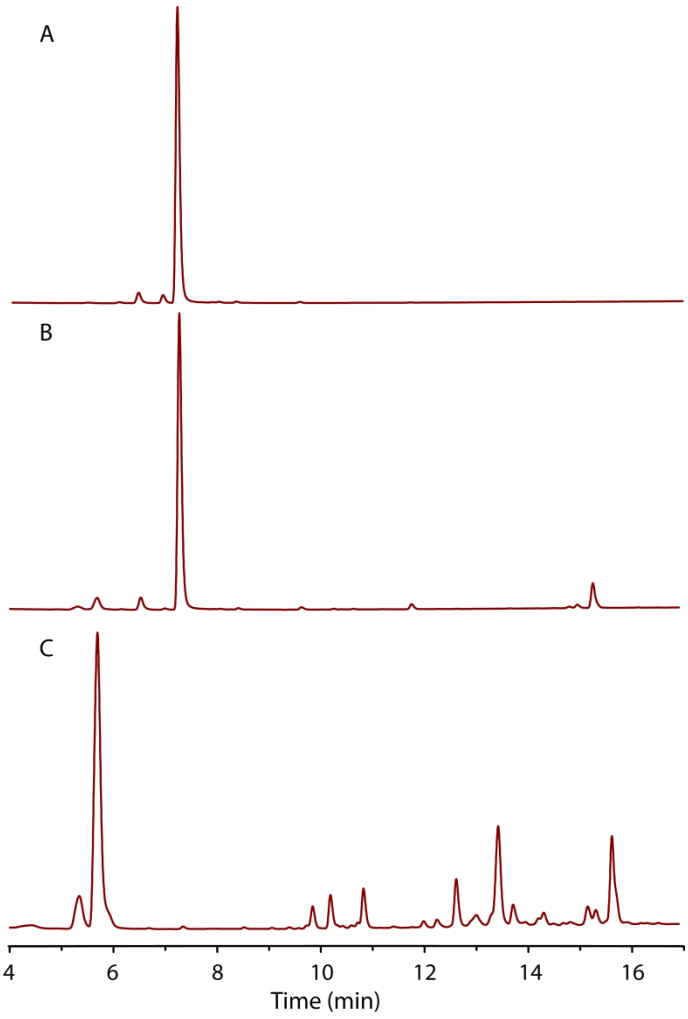


Figure S3. Summary trace for the enzymatic reaction of **11** with Radicicol and Zearalenone TEs. A: No enzyme blank containing 1 mM **11**, 50 mM phosphate buffer (pH 7.4) and 10% v/v DMSO, 12 hours. B: 1 mM **11** incubated with 20 µM Rdc TE, 50 mM phosphate buffer (pH 7.4), 10% v/v DMSO, 12 hours. C: 1 mM **11**, incubated with 20 µM Zea TE, 50 mM phosphate buffer (pH 7.4), 10% v/v DMSO, 12 hours.

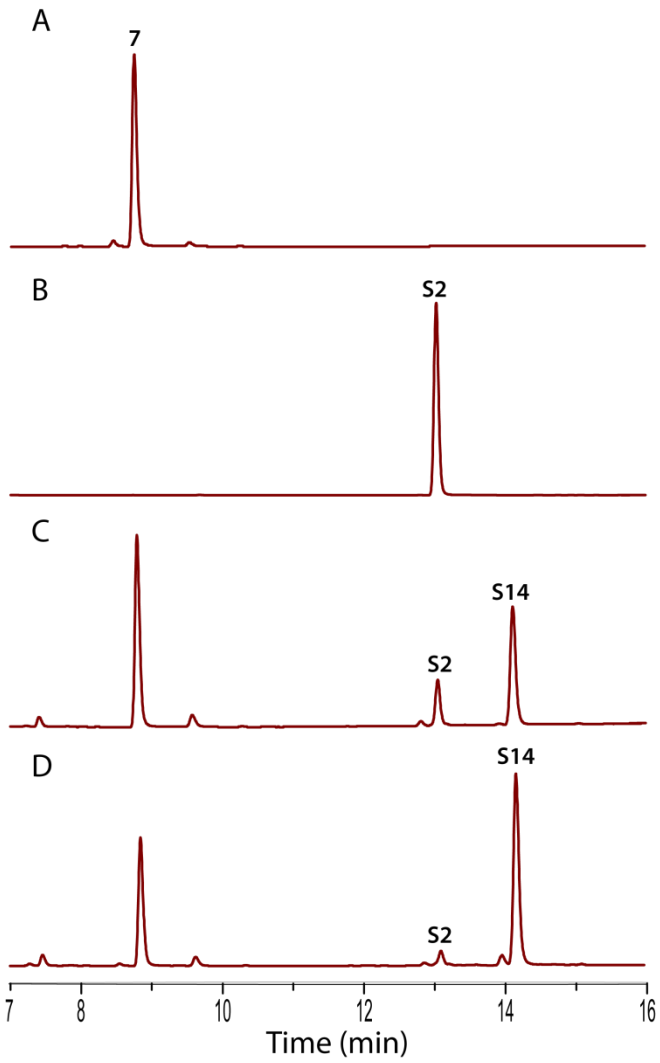


Figure S4. Summary trace for the enzymatic reaction of **7** with Radicicol and Zearalenone TEs. A: No enzyme blank containing 1 mM **7**, 50 mM phosphate buffer (pH 7.4) and 10% v/v DMSO, 2 hours. B: Macrocycle authentic standard **S2**. C: 1 mM **7** incubated with 5 µM Rdc TE, 50 mM phosphate buffer (pH 7.4), 10% v/v DMSO, 2 hours. D: 1 mM **7**, incubated with 5 µM Zea TE, 50 mM phosphate buffer (pH 7.4), 10% v/v DMSO, 2 hours. Peak at 14.2 min is linear-SNAC dimer **S14**

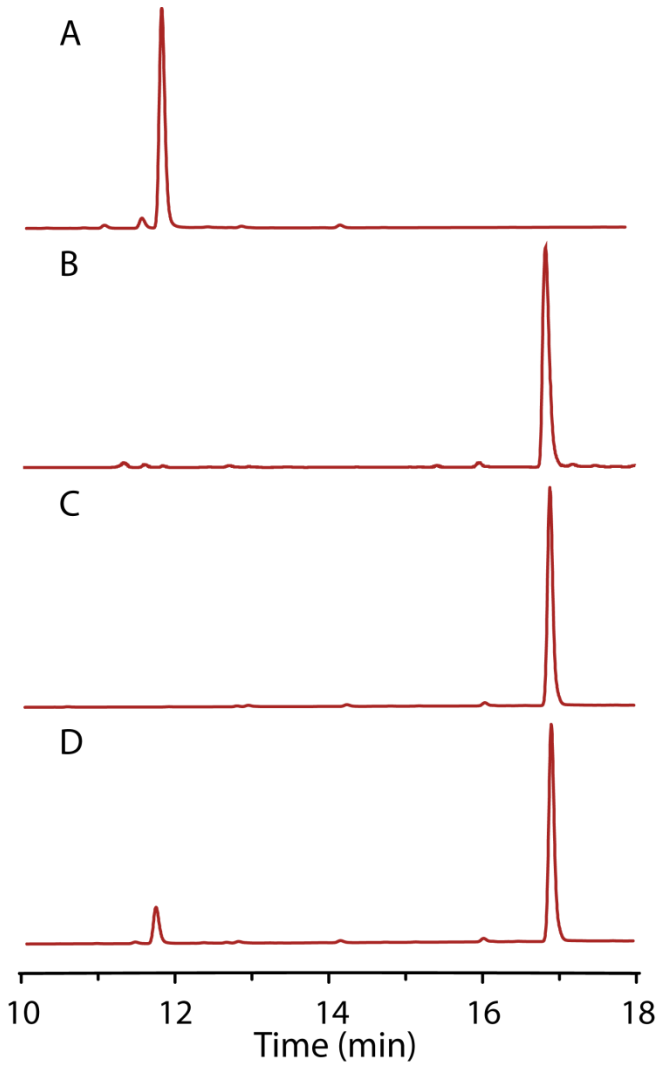


Figure S5. Summary trace for the enzymatic reaction of **8** with Radicicol and Zearalenone TEs. A: No enzyme blank containing 1 mM **8**, 50 mM phosphate buffer (pH 7.4) and 10% v/v DMSO, 2 hours. B: Authentic macrocycle standard. C: 1 mM **8** incubated with 5 µM Rdc TE, 50 mM phosphate buffer (pH 7.4), 10% v/v DMSO, 2 hours. D: 1 mM **8**, incubated with 5 µM Zea TE, 50 mM phosphate buffer (pH 7.4), 10% v/v DMSO, 2 hours.

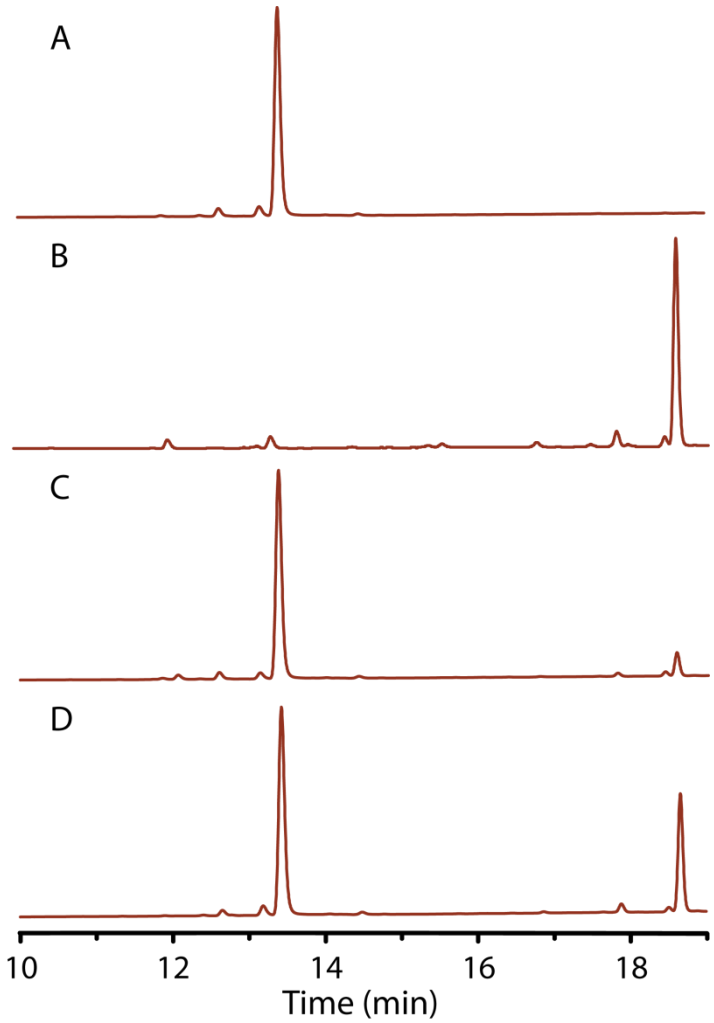


Figure S6. Summary trace for the enzymatic reaction of **9** with Radicicol and Zearalenone TEs. A: No enzyme blank containing 1 mM **9**, 50 mM phosphate buffer (pH 7.4) and 10% v/v DMSO, 2 hours. B: Authentic macrocycle standard. C: 1 mM **9** incubated with 5 µM Rdc TE, 50 mM phosphate buffer (pH 7.4), 10% v/v DMSO, 2 hours. D: 1 mM **9**, incubated with 5 µM Zea TE, 50 mM phosphate buffer (pH 7.4), 10% v/v DMSO, 2 hours.

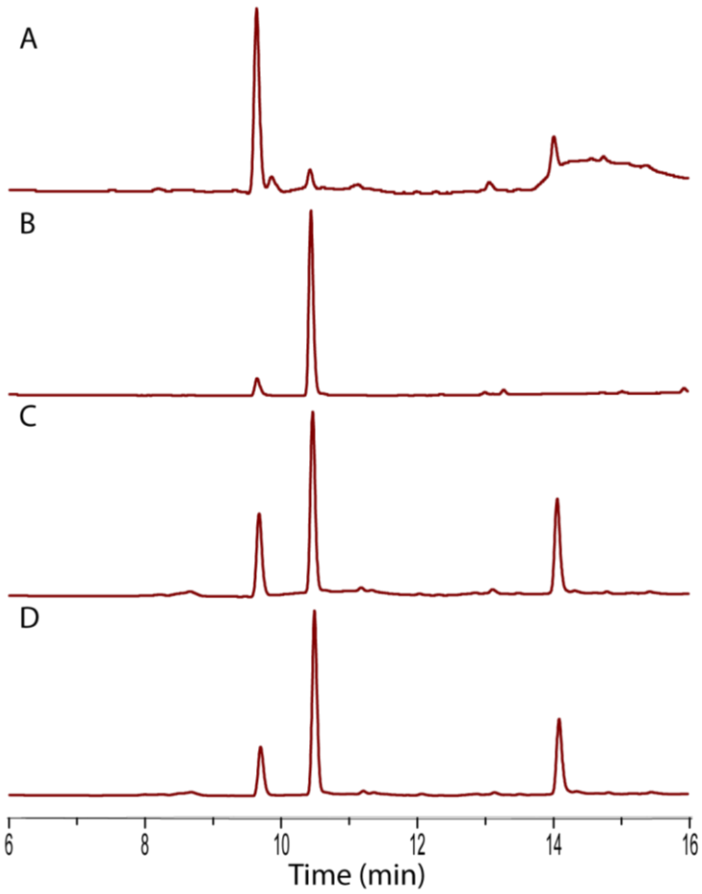


Figure S7. Summary HPLC traces for macrolactam substrate **16** showing production of macrocycle **S13** as well as dimer formation. A: No enzyme blank containing 1 mM **16**, 50 mM phosphate (pH 7.4) and 10% DMSO v/v, 3 h. B: Macrocycle standard **S12**. C: 1 mM **16** incubated with 5 µM Rdc TE, 50 mM phosphate buffer (pH 7.4), 10% v/v DMSO, 3 h. D: 1 mM **16**, incubated with 25 µM Zea TE, 50 mM phosphate buffer (pH 7.4), 10% v/v DMSO, 3 h.

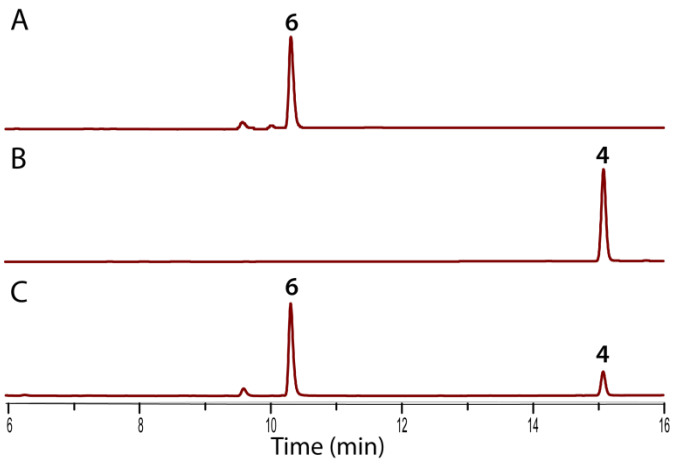


Figure S8. HPLC traces demonstrating that Rdc TE retains activity after lyophylization and prolonged room temperature storage. A. No enzyme blank containing 1 mM **6**, 50 mM phosphate (pH 7.4) and 10% DMSO v/v, 3 h. B: Macrocycle standard **4**. C: 1 mM **6** incubated with lyophilized 5 µM Rdc TE, 50 mM phosphate buffer (pH 7.4), 10% v/v DMSO, 3 h. D: 1 mM **6**, incubated with lyophilized 25 µM Zea TE, 50 mM phosphate buffer (pH 7.4), 10% v/v DMSO, 3 h

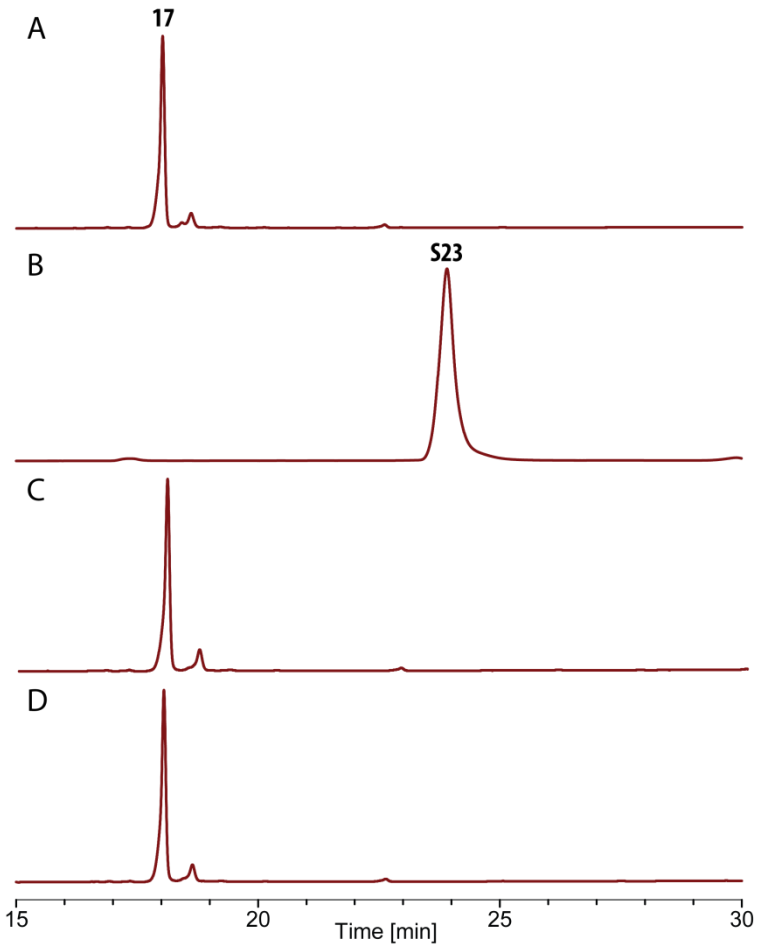


Figure S9. Summary trace for the enzymatic reaction of **17** with Radicicol and Zearalenone TEs. A. No enzyme blank containing 1 mM **17**, 50 mM phosphate (pH 7.4) and 10% DMSO v/v, 16 h. B: Macrocycle standard **S23**. C: 1 mM **17** incubated with 5 µM Rdc TE, 50 mM phosphate buffer (pH 7.4), 10% v/v DMSO, 16 h. D: 1 mM **17**, incubated with 5 µM Zea TE, 50 mM phosphate buffer (pH 7.4), 10% v/v DMSO, 16 h

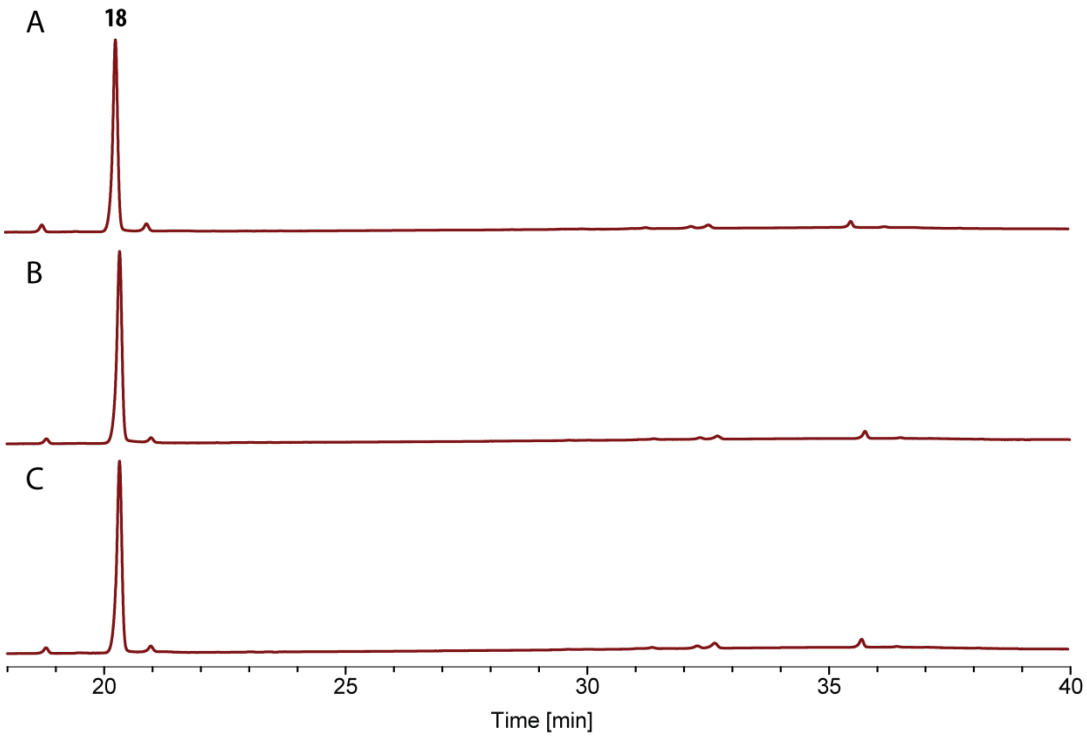


Figure S10. Summary trace for the enzymatic reaction of **18** with Radicicol and Zearalenone TEs. A. No enzyme blank containing 1 mM **18**, 50 mM phosphate (pH 7.4) and 10% DMSO v/v, 16 h. B: 1 mM **18** incubated with 5 µM Rdc TE, 50 mM phosphate buffer (pH 7.4), 10% v/v DMSO, 16 h. C: 1 mM **18**, incubated with 5 µM Zea TE, 50 mM phosphate buffer (pH 7.4), 10% v/v DMSO, 16 h

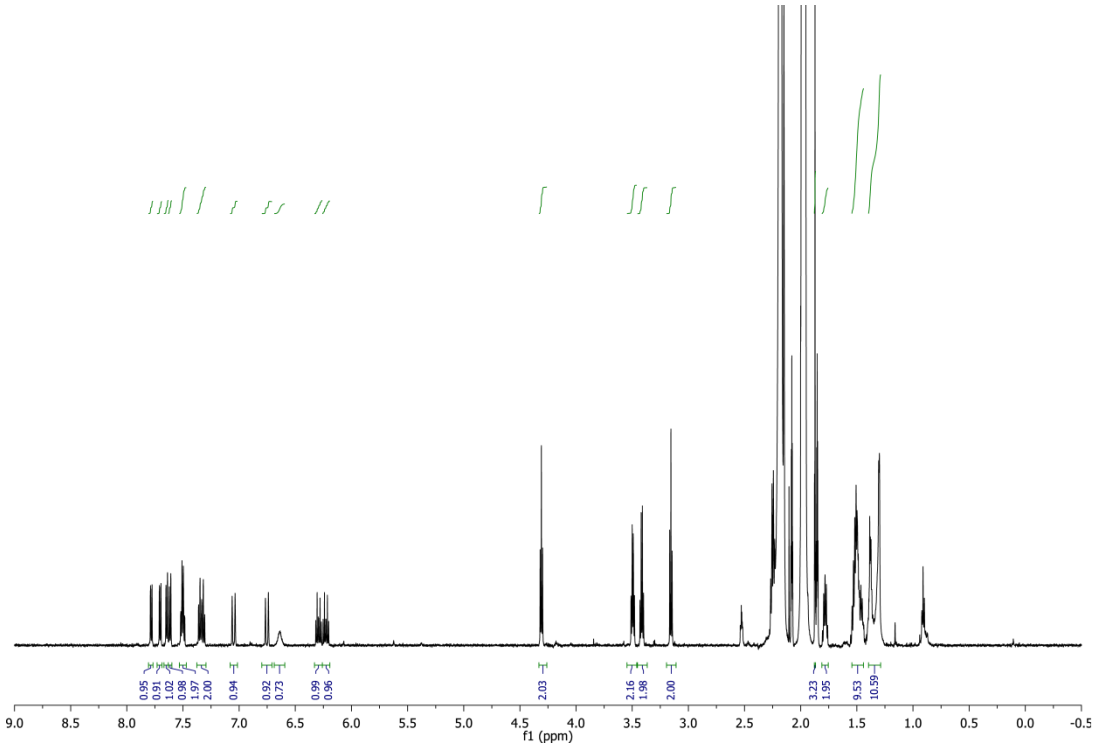


Figure S11. 600 MHz ^{1}H spectra of the enzymatically generated linear SNAc dimer of **7, S14**. The spectra shows characteristic doubling of peaks in the aromatic region as well as characteristic peaks for the -C**H**$_2$C**H**$_2$- methylenes and broad -N**H**- singlet of the SNAc thioester.

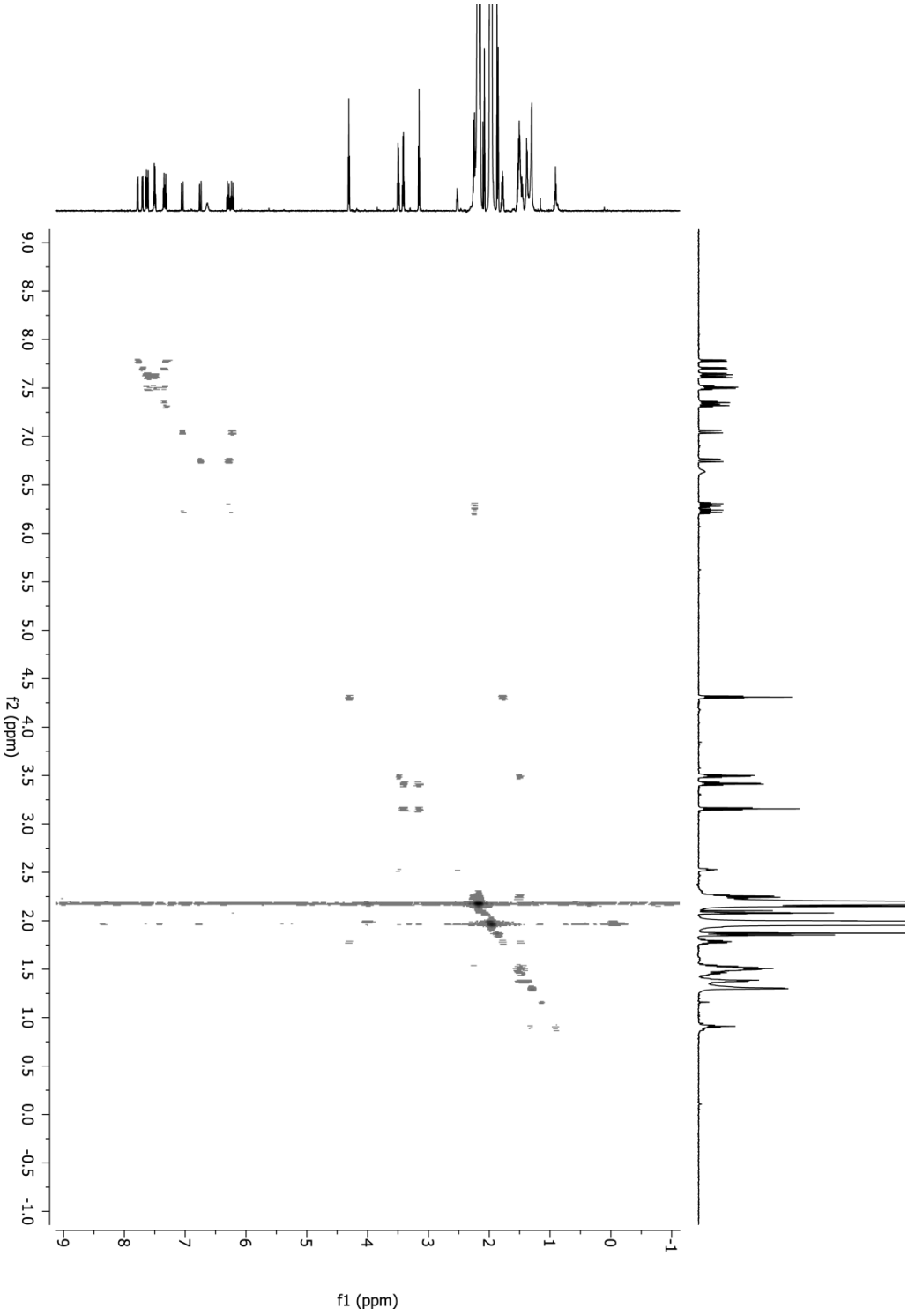
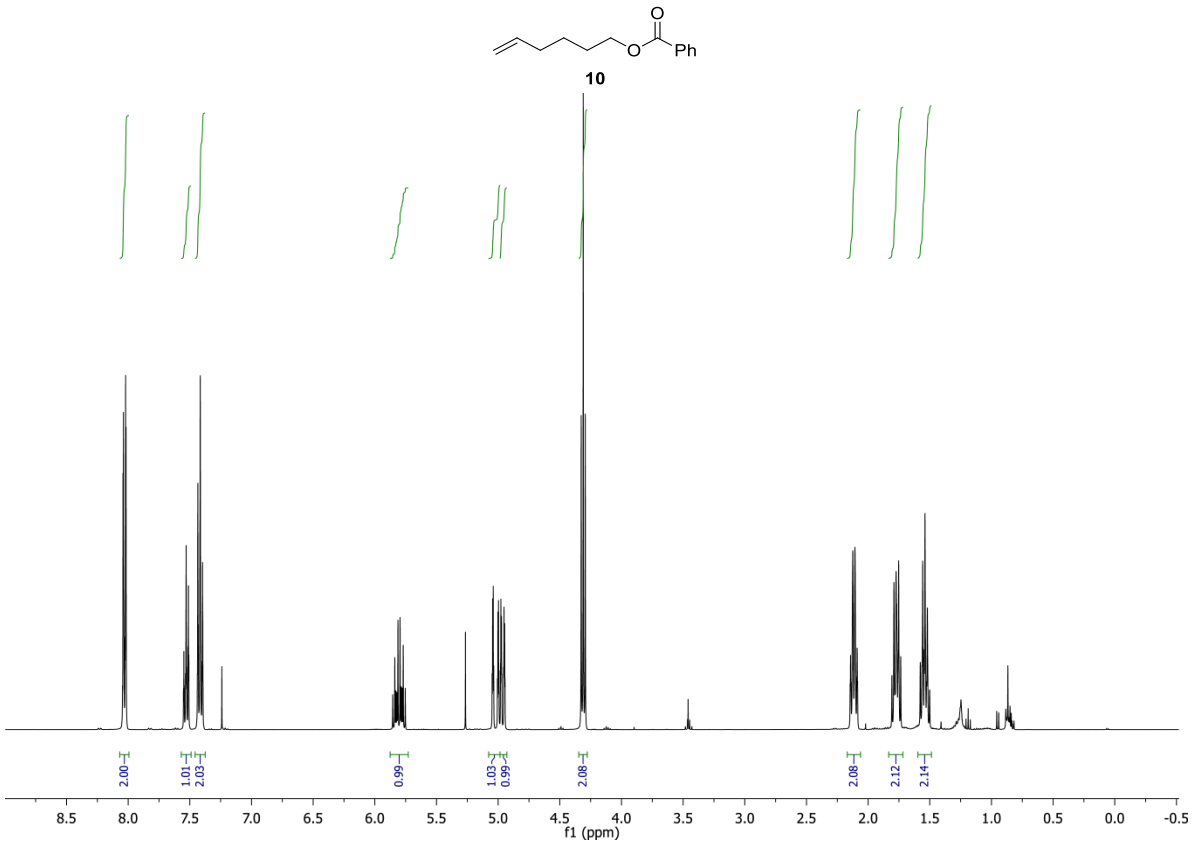
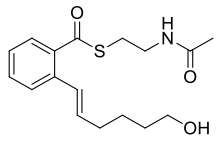


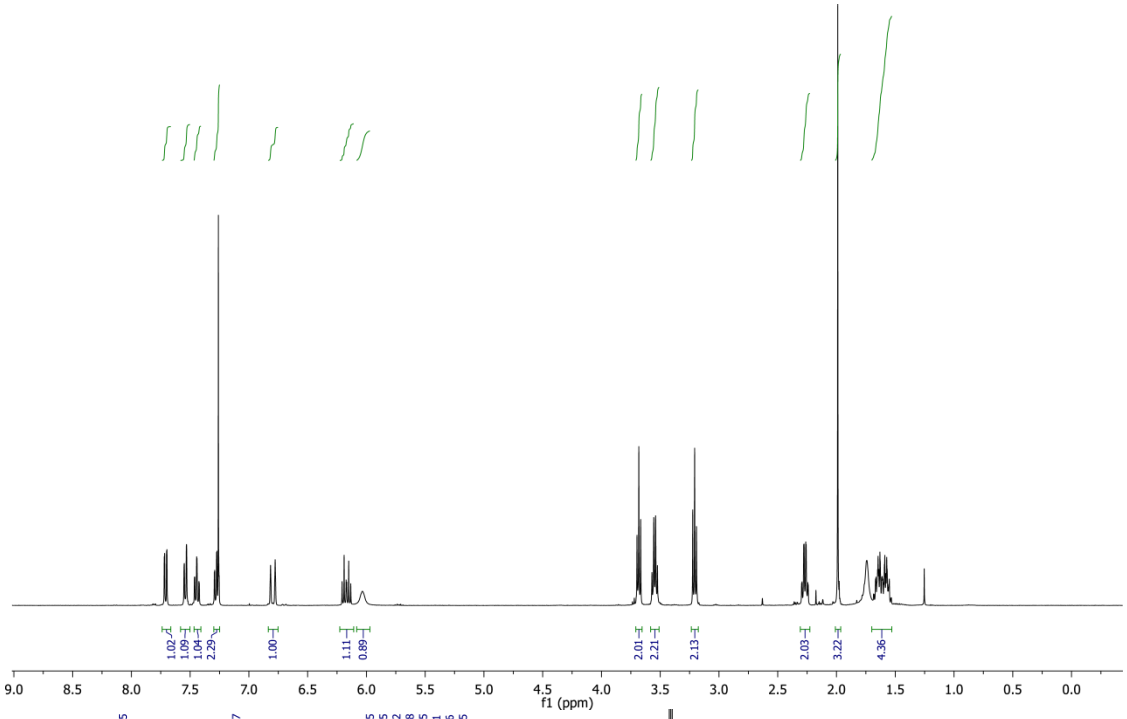
Figure S12. 2D COSY spectra of the enzymatically generated linear SNAc dimer of **7, S14**

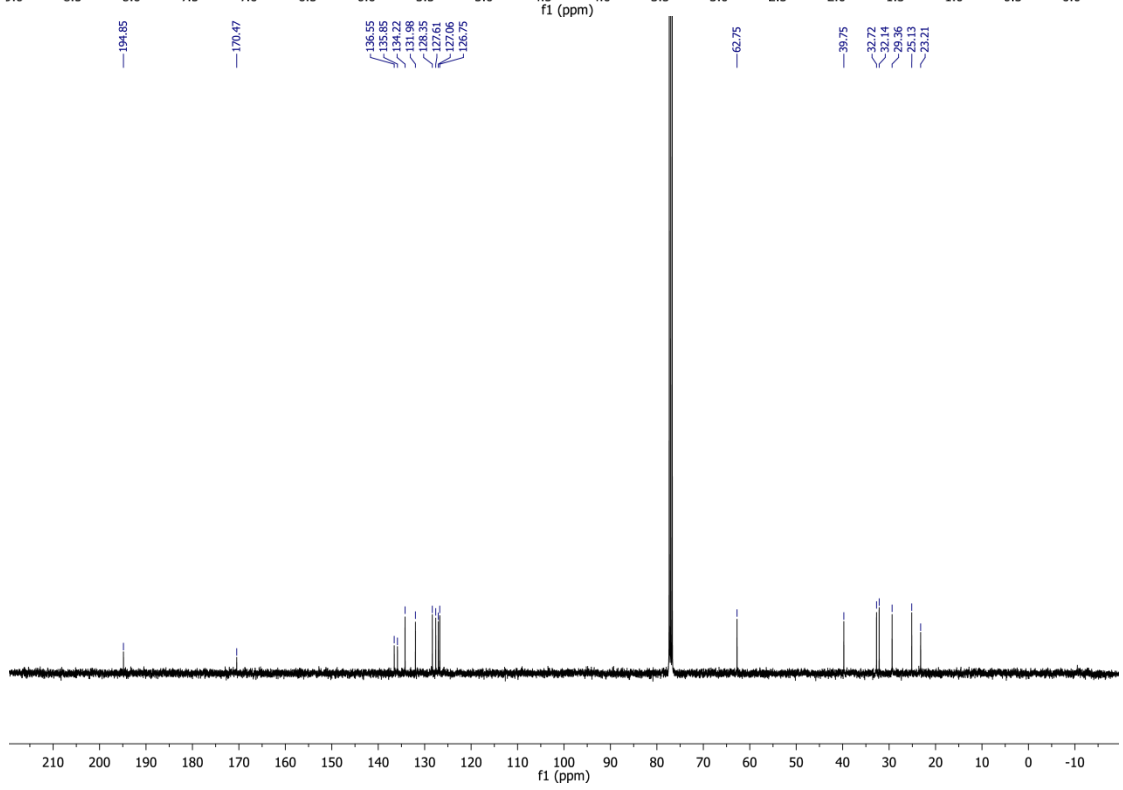
Selected NMR Spectra

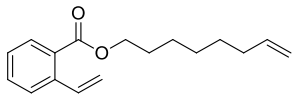



O
S
H
N
O
OH

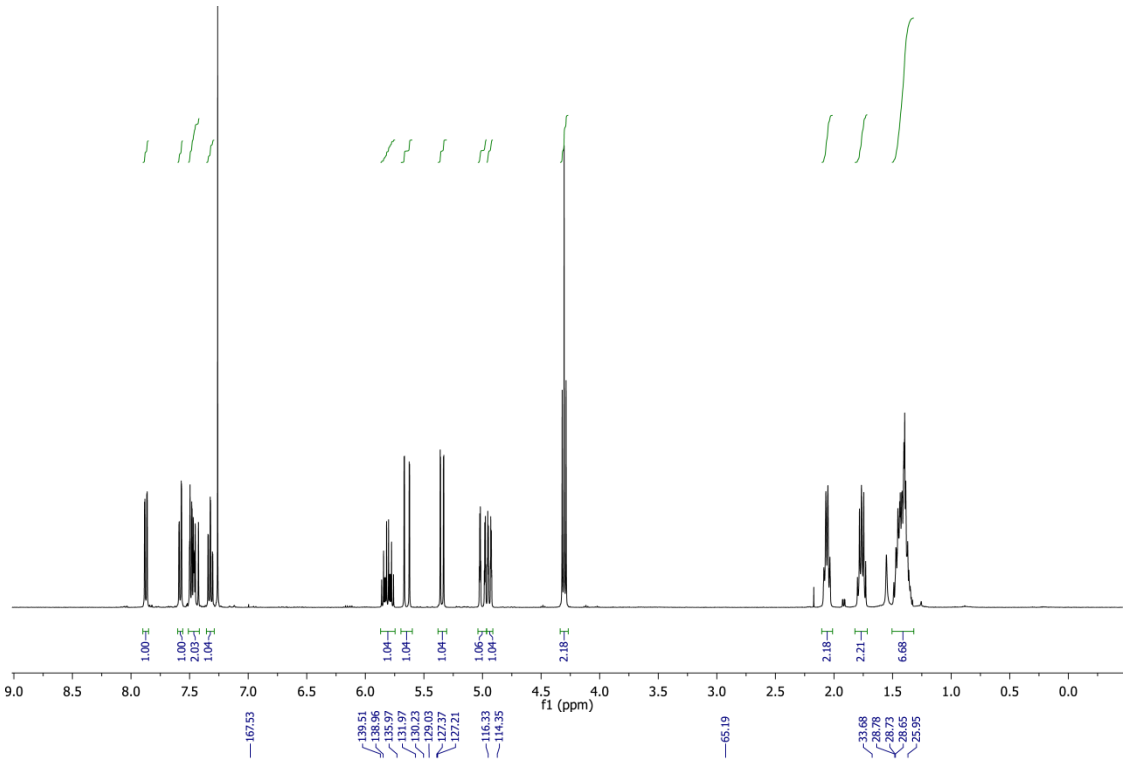
11

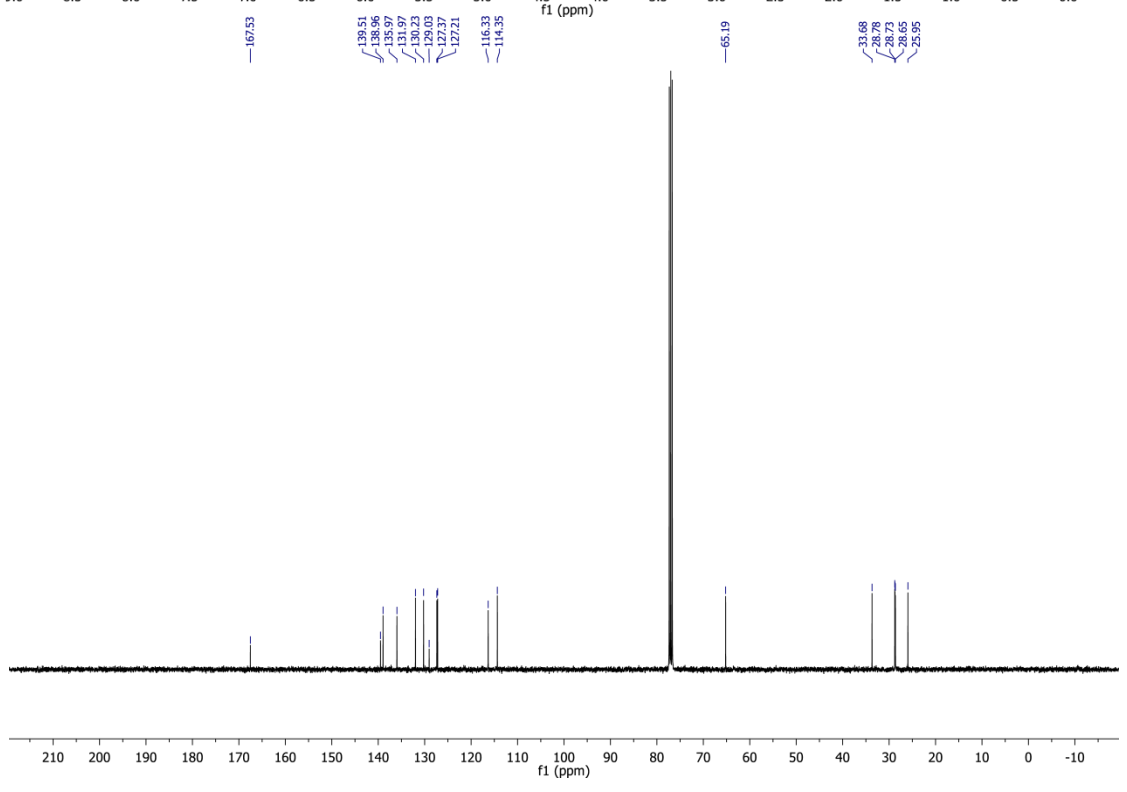

1.02
1.09
1.04
2.29
1.00
1.11
0.89
2.01
2.21
2.13
2.03
3.22
4.36
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

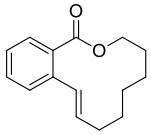

194.85
170.47
136.55
135.85
134.22
131.98
128.35
127.61
127.06
126.75
62.75
39.75
32.72
32.14
29.36
25.13
23.21
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)



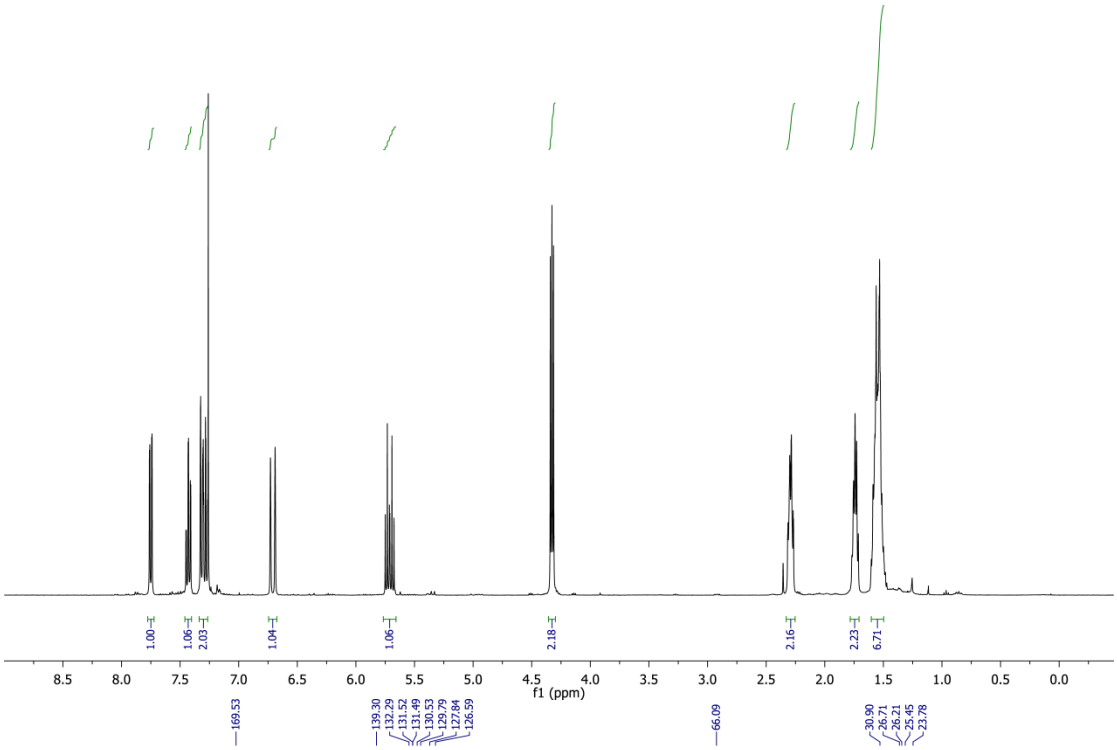
S1

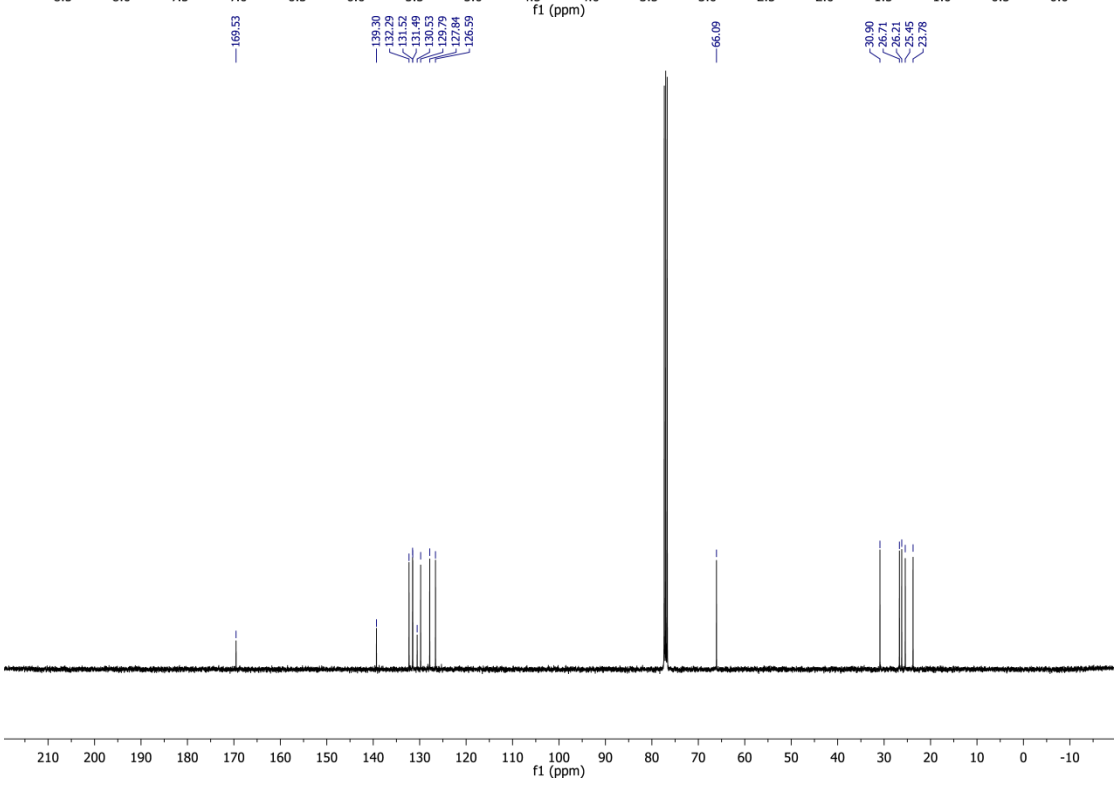

1.00
1.00
2.03
1.04
1.04
1.04
1.04
1.06
1.04
2.18
2.18
2.21
6.68
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

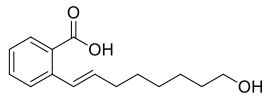

167.53
130.51
138.96
135.97
131.97
130.23
129.03
127.37
127.21
116.33
114.35
65.19
33.68
28.78
28.73
28.65
25.95
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)



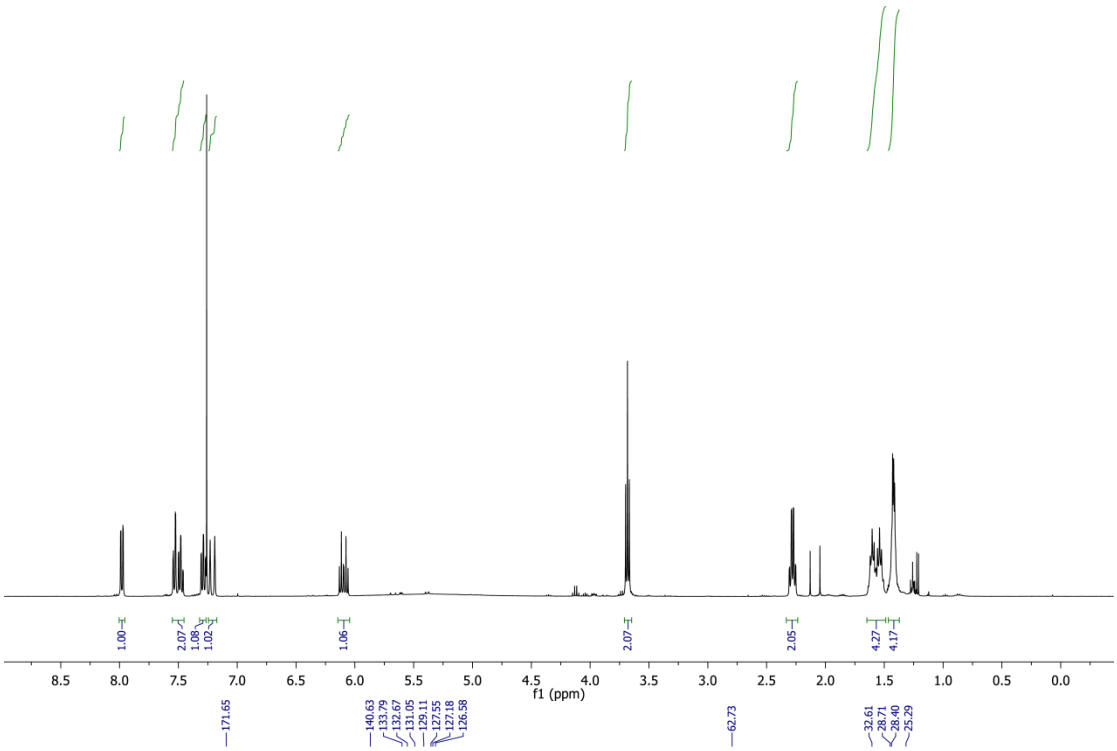
20

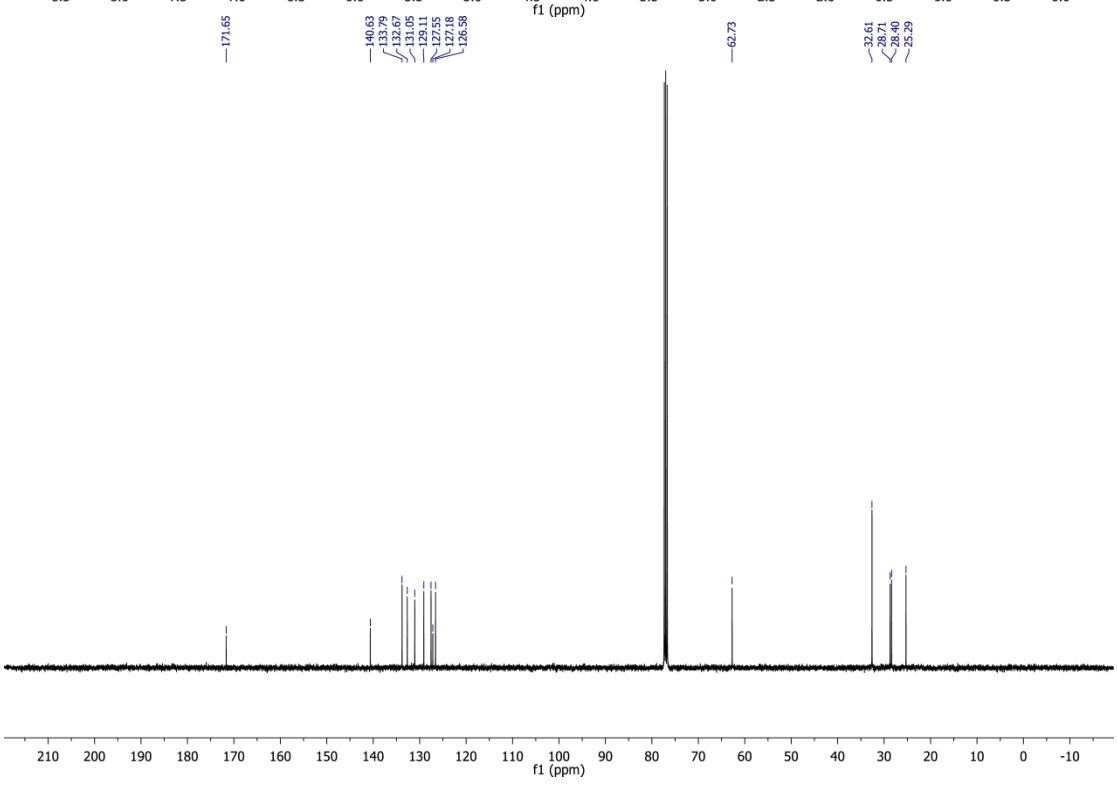

1.00
1.06
2.03
1.04
1.06
2.18
2.16
2.23
6.71
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

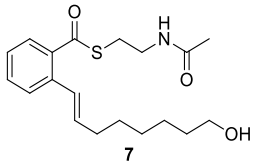

169.53
139.30
132.29
131.52
131.49
130.53
129.79
127.84
126.59
66.09
30.90
26.71
26.21
25.45
23.78
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


O
OH
OH

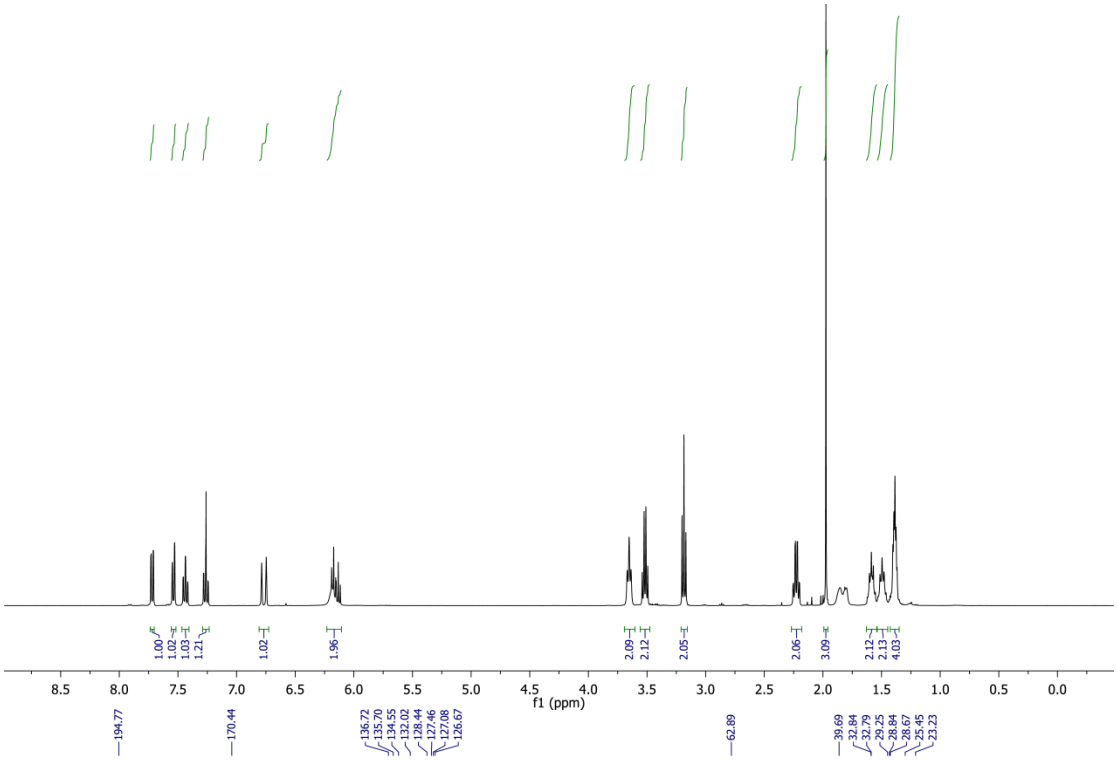
S5

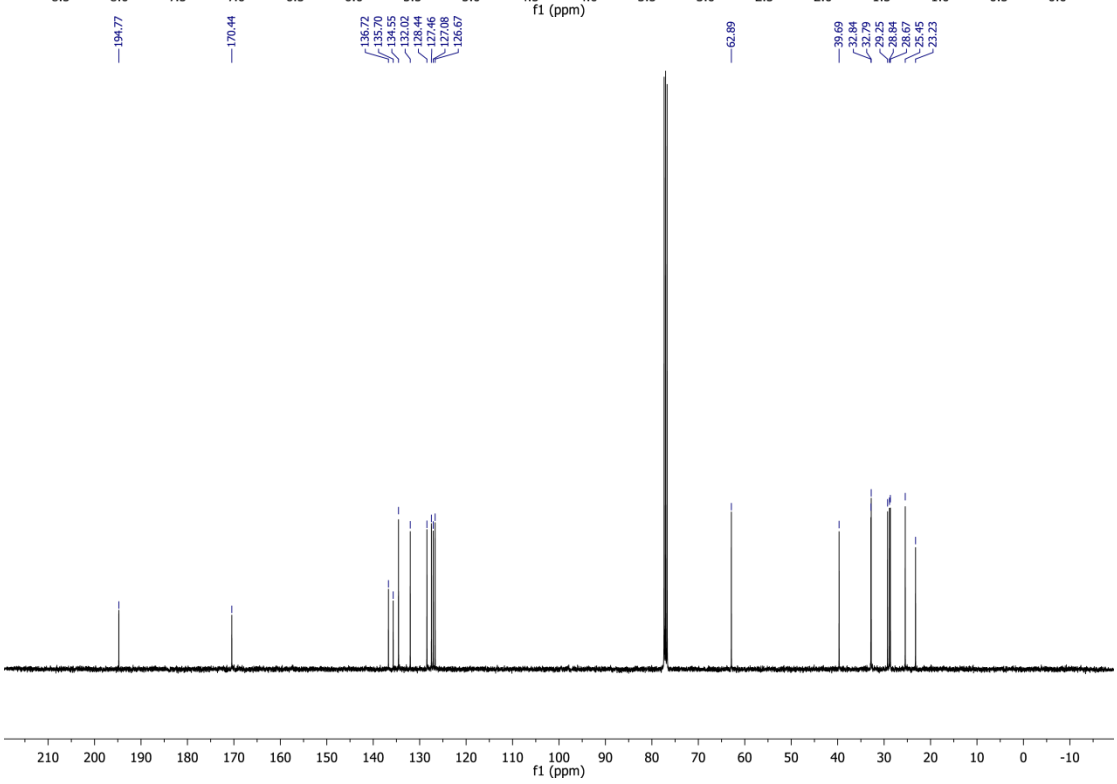

1.00
2.07
1.08
1.02
1.06
2.07
2.05
4.27
4.17
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

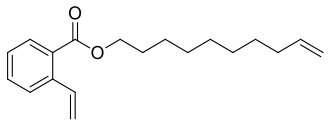

171.65
140.63
133.79
132.67
131.05
129.11
127.55
127.18
126.58
62.73
32.61
28.71
28.40
25.29
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


O
H
N
S
O
OH

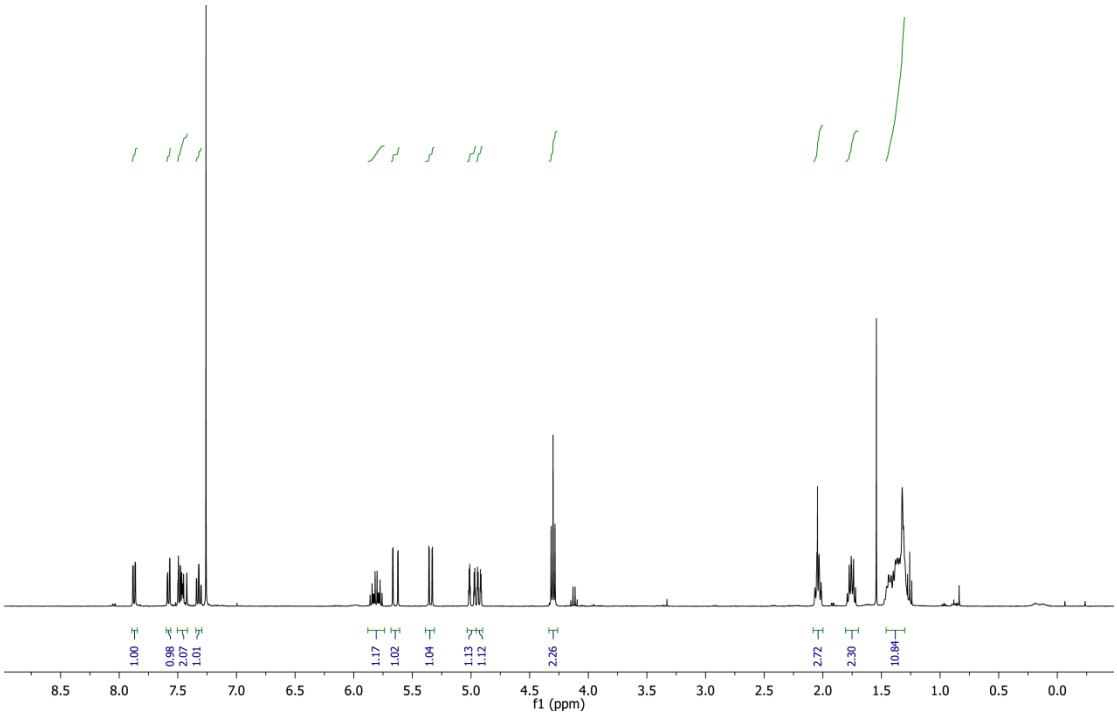
7

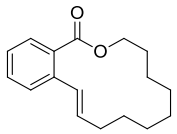

1.00
1.02
1.03
1.21
1.02
1.96
2.09
2.12
2.05
2.06
3.09
2.12
2.13
4.03
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


194.77
170.44
136.72
135.70
134.55
132.02
128.44
127.46
127.08
126.67
62.89
39.69
32.84
32.79
29.25
28.84
28.67
25.45
23.23
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

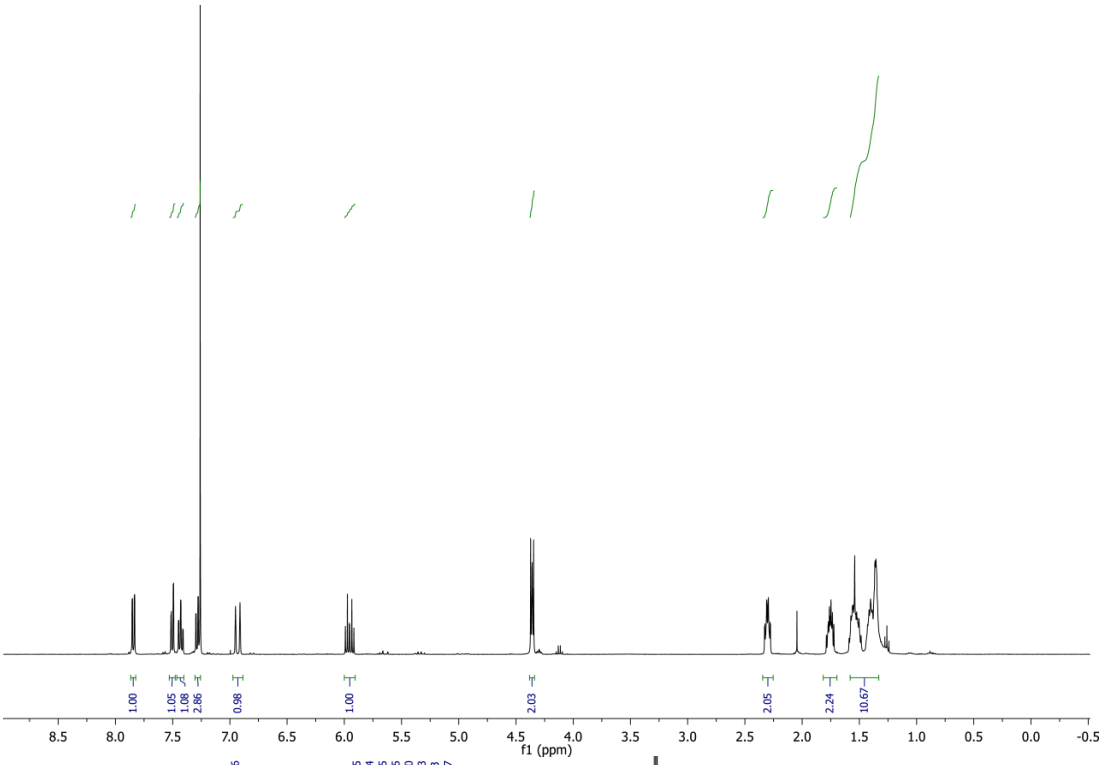


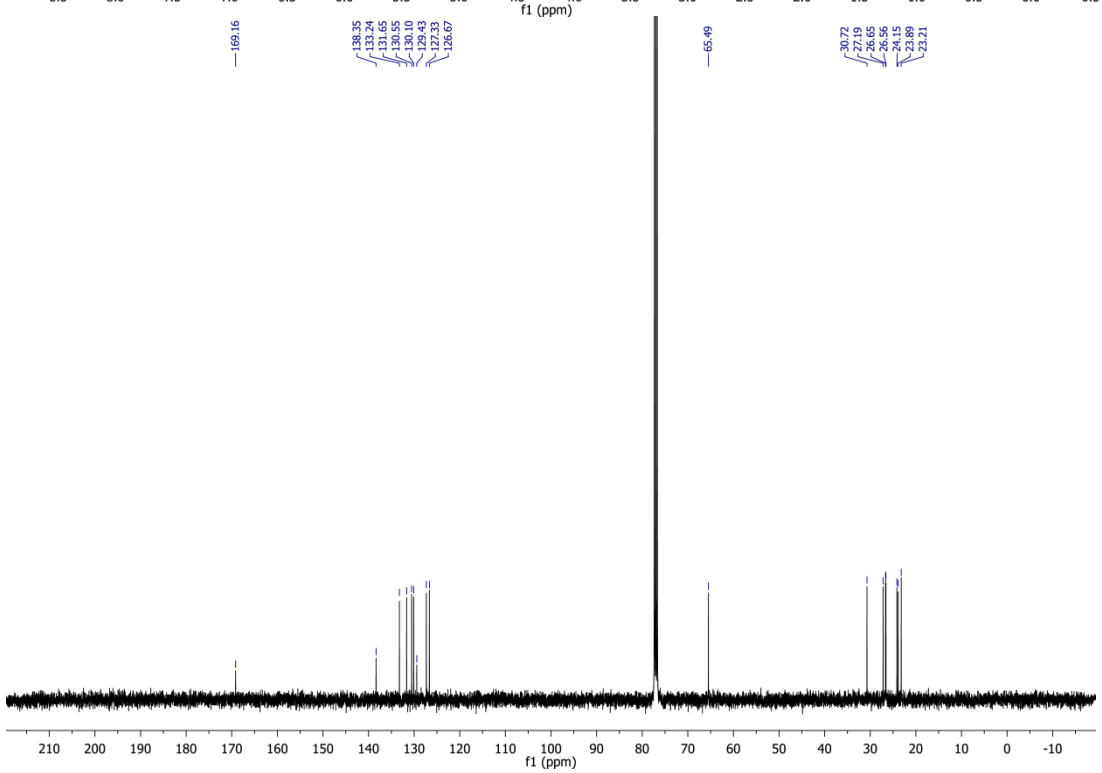
3

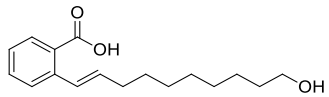

1.00
0.98
2.07
1.01
1.17
1.02
1.04
1.13
1.12
2.26
2.72
2.30
10.84
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)



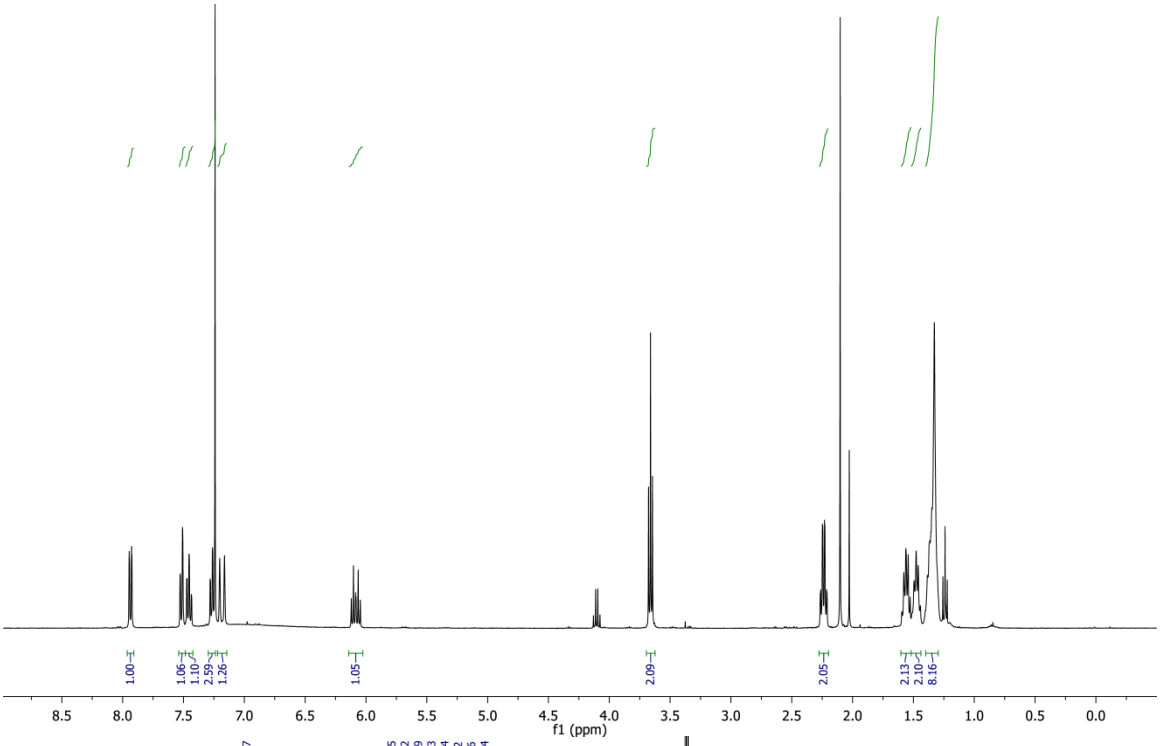
4

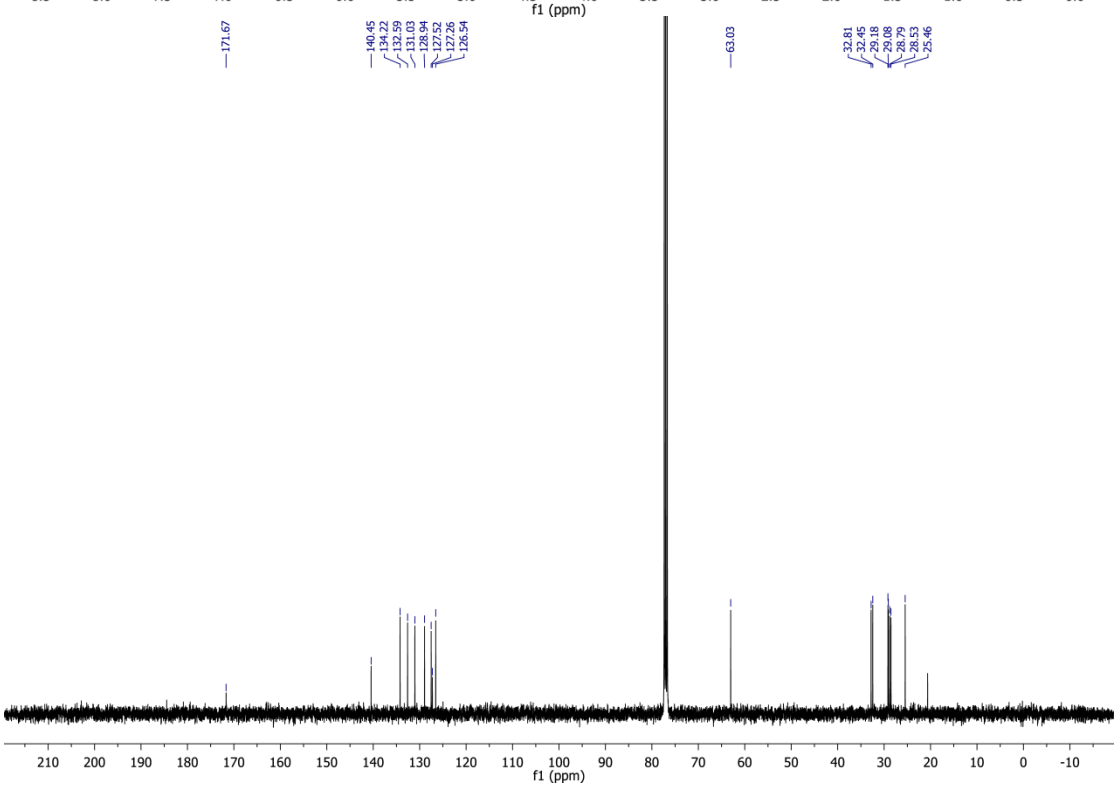

1.00
1.05
1.08
2.86
0.98
1.00
2.03
2.05
2.24
10.67
f1 (ppm)

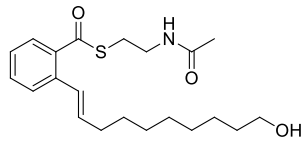

169.16
138.35
133.24
131.65
130.55
130.10
129.43
127.33
126.67
65.49
30.72
27.19
26.65
26.56
24.15
23.89
23.21
f1 (ppm)


O
OH
OH

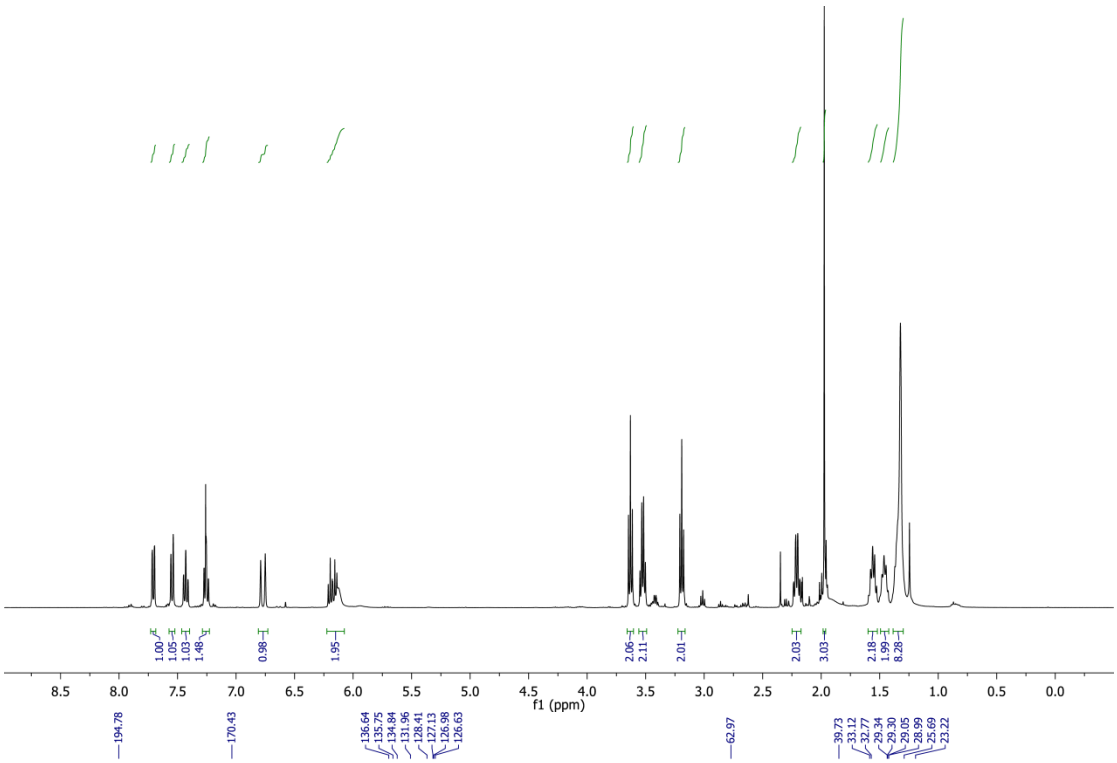
5

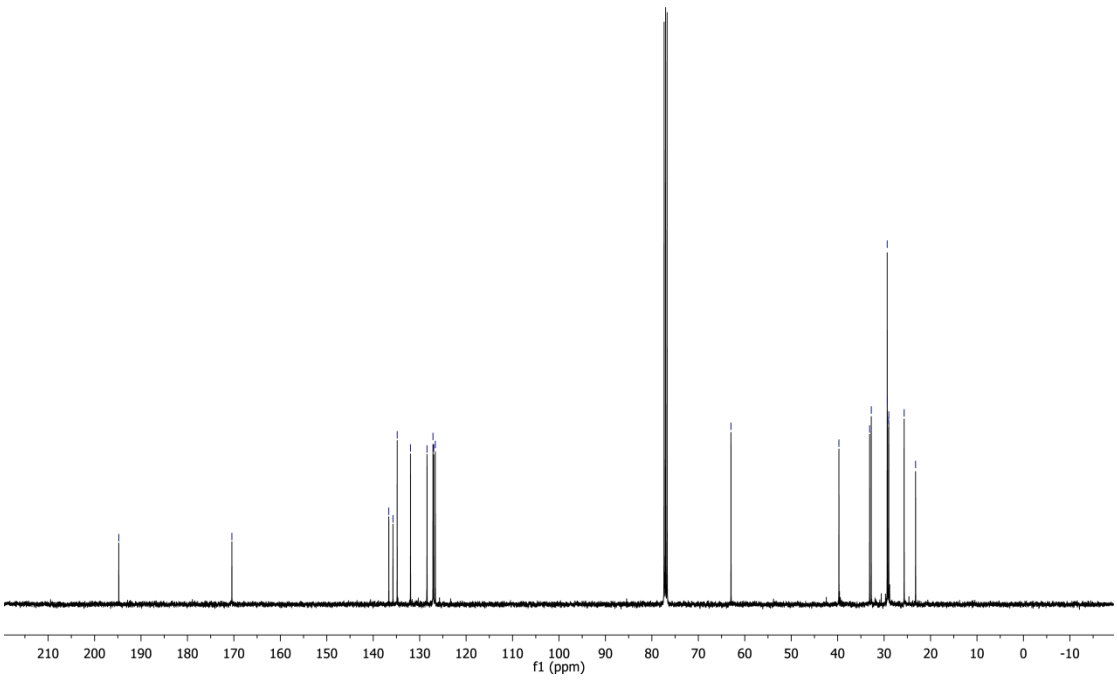

1.00
1.06
1.10
2.59
1.26
1.05
2.09
2.05
2.13
2.10
8.16
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

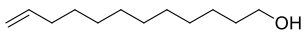

171.67
140.45
134.22
132.59
131.03
128.94
127.52
127.26
126.54
63.03
32.81
32.45
29.18
29.08
28.79
28.53
25.46
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


O
H
N
S
O
OH

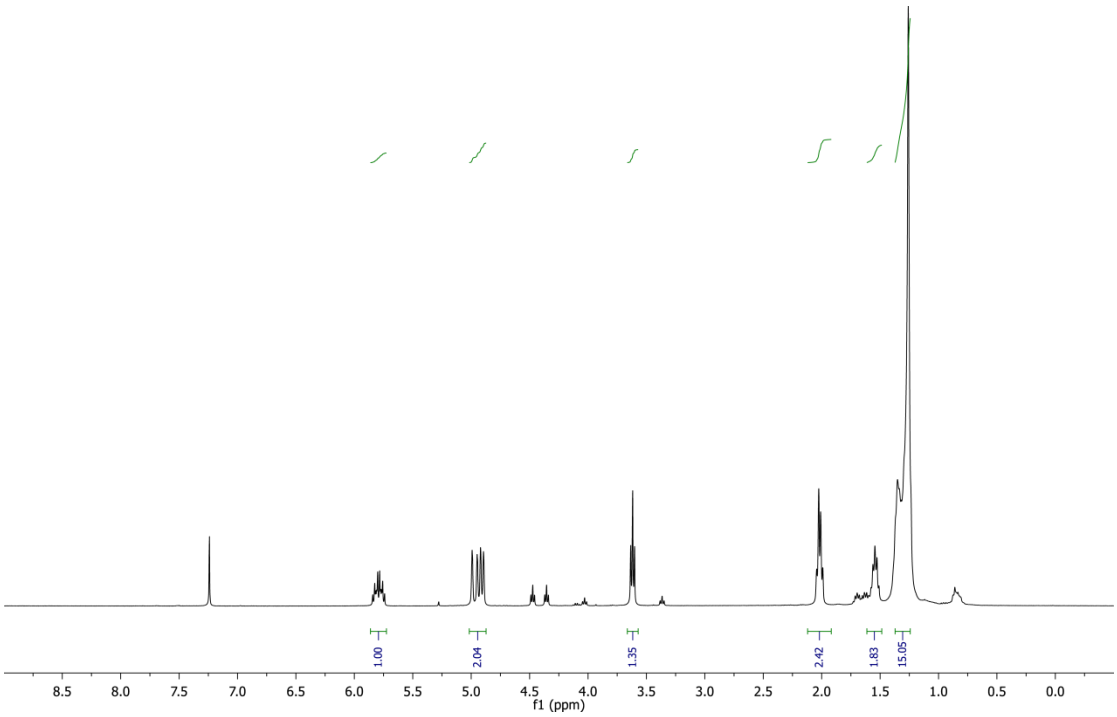
6

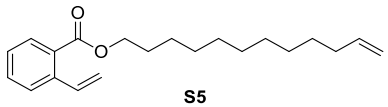

1.00
1.05
1.03
1.48
0.98
1.95
2.06
2.11
2.01
2.03
3.03
2.18
1.99
8.28
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)
194.78
170.43
136.64
135.75
134.84
131.96
128.41
127.13
126.98
126.63
62.97
39.73
33.12
32.77
29.34
29.30
29.05
28.99
25.69
23.22


210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

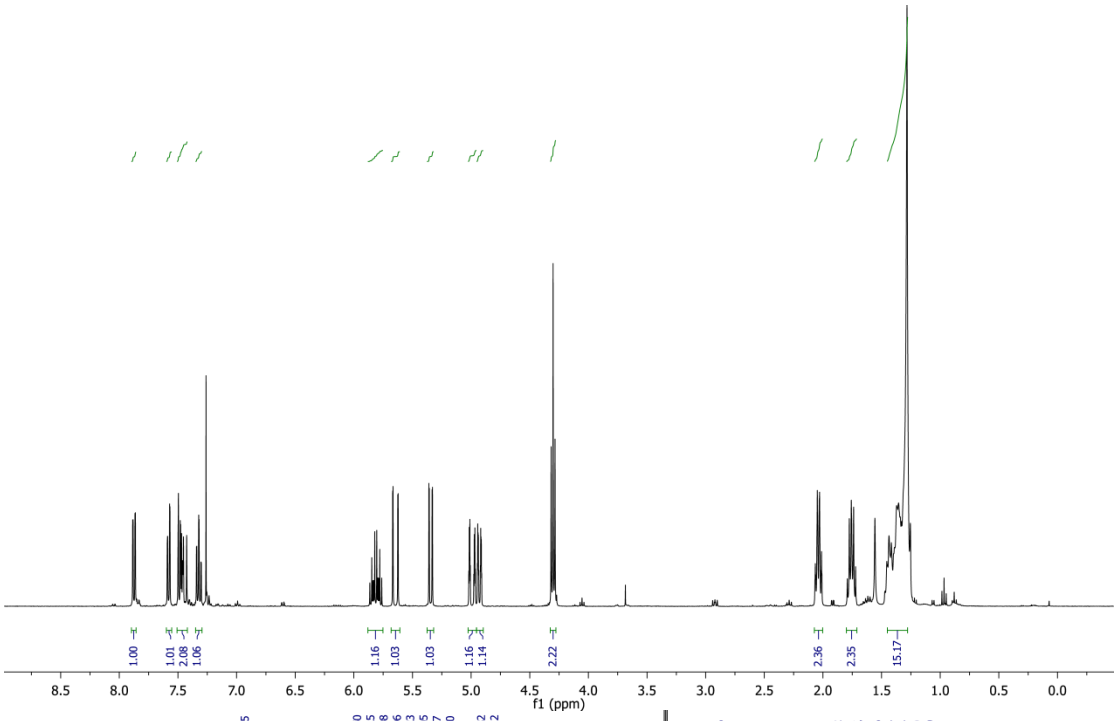

OH

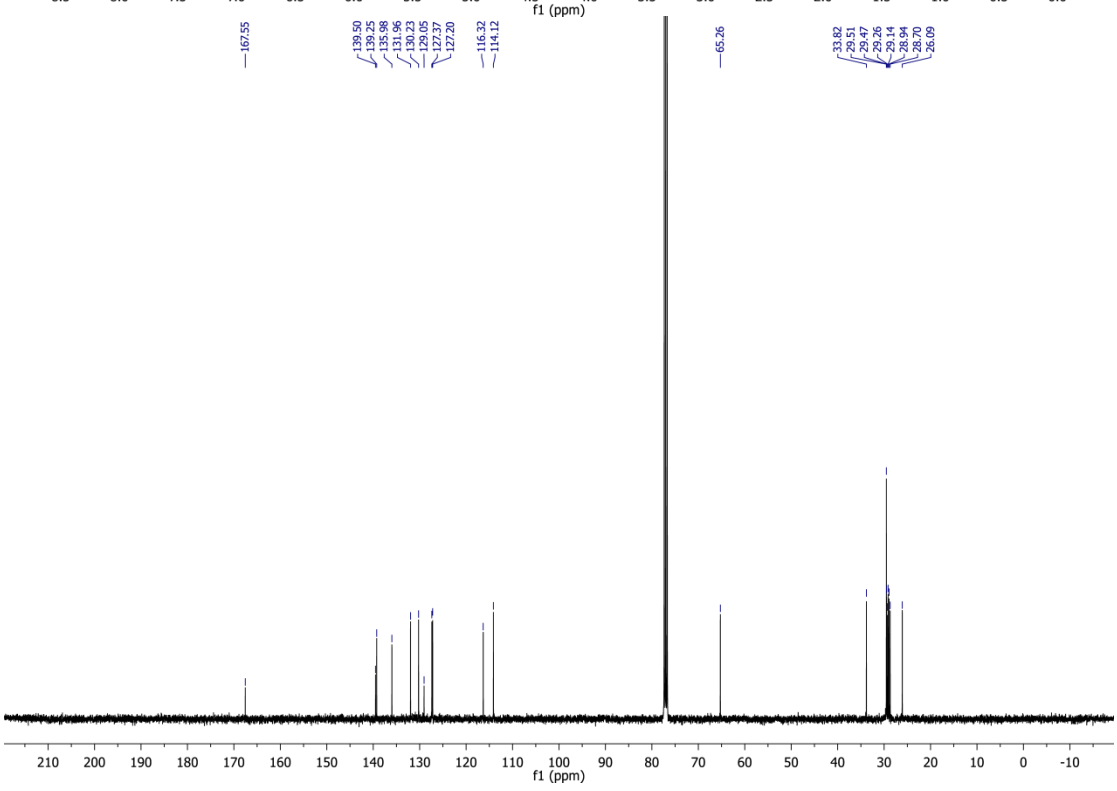
S4

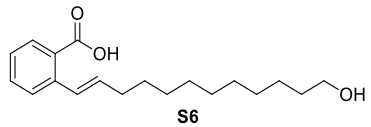

1.00
2.04
1.35
2.42
1.83
15.05
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)



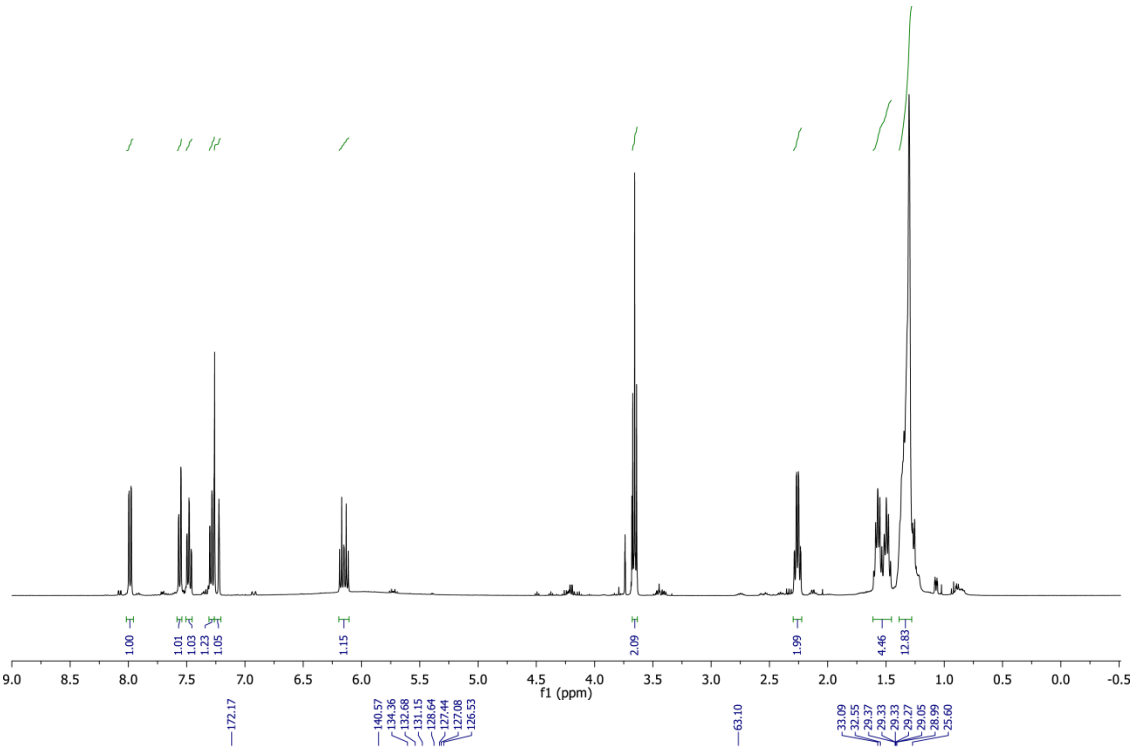
S5

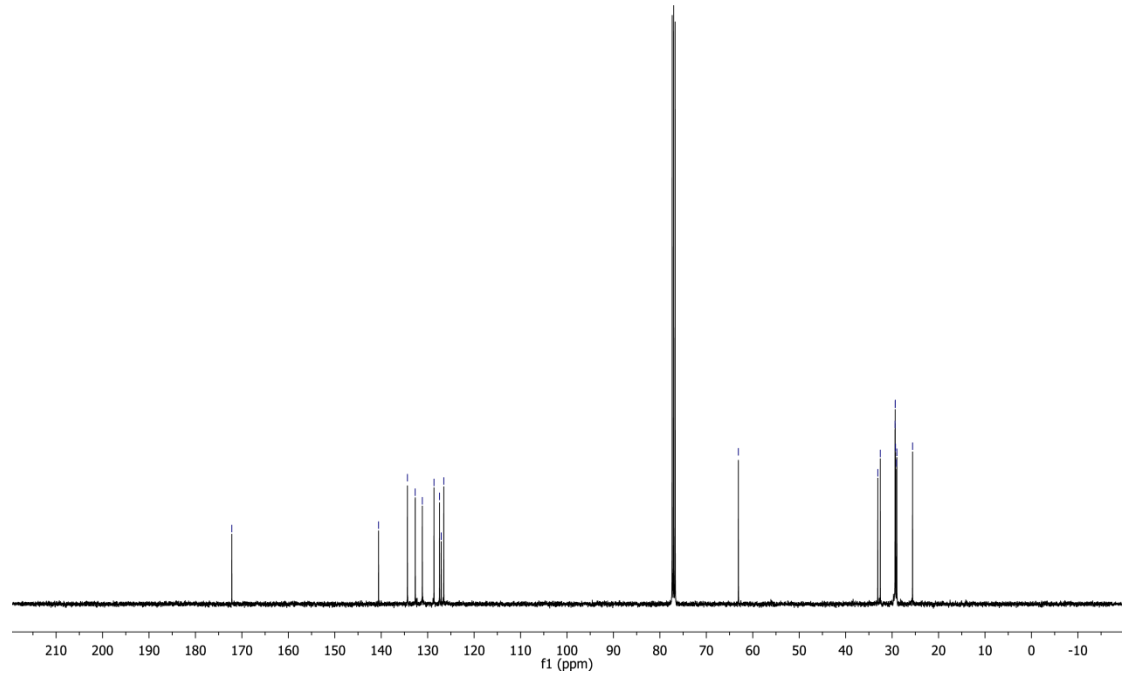

1.00
1.01
2.08
1.06
1.16
1.03
1.03
1.16
1.14
2.22
2.36
2.35
15.17
f1 (ppm)

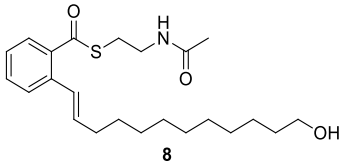

167.55
130.50
130.25
135.98
131.96
130.23
129.05
127.37
127.20
116.32
114.12
65.26
33.82
29.51
29.47
29.26
29.14
28.94
28.70
26.09
f1 (ppm)


O
OH
OH

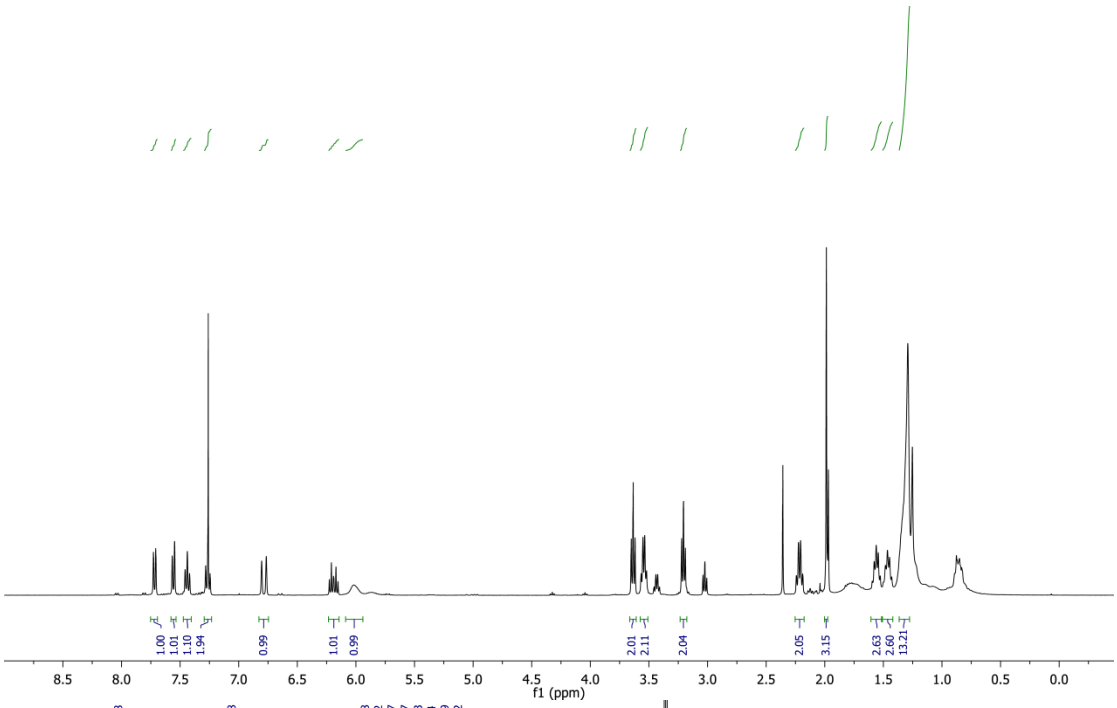
S6

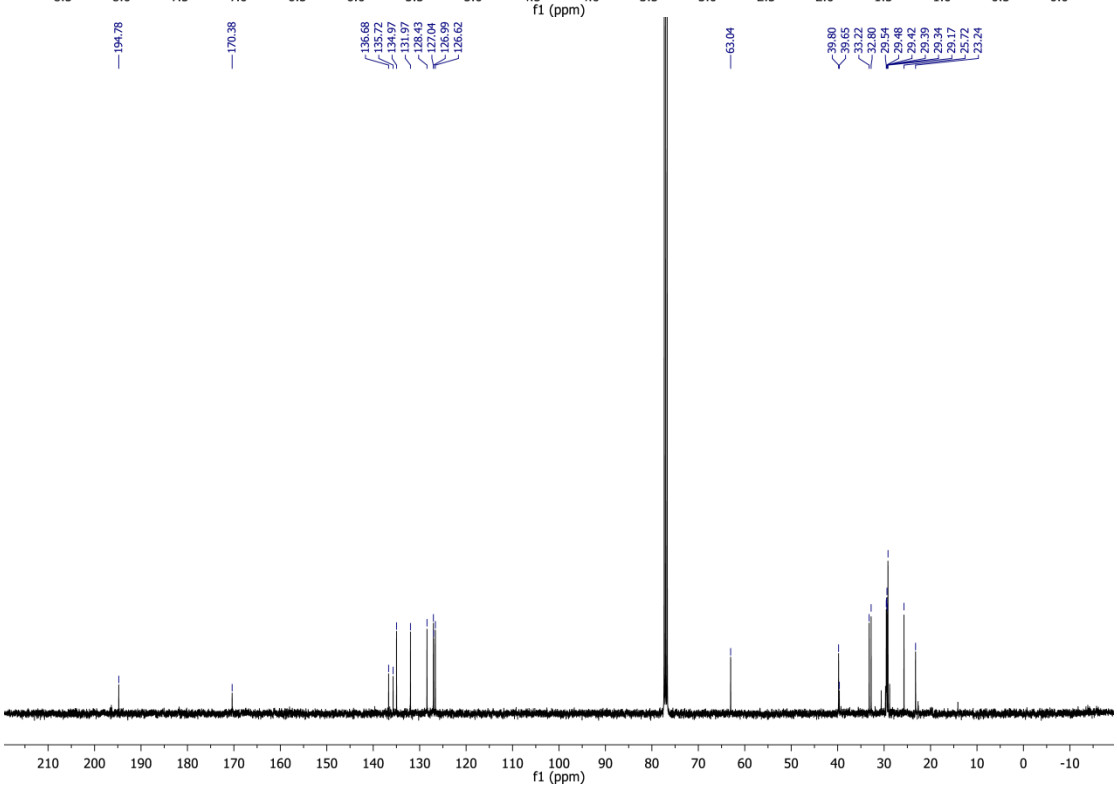

1.00
1.01
1.03
1.23
1.05
1.15
2.09
1.99
4.46
12.83
f1 (ppm)
172.17
140.57
134.36
132.68
131.15
128.64
127.44
127.08
126.53
63.10
33.09
32.55
29.37
29.33
29.27
29.05
28.99
25.60

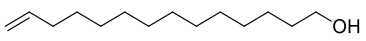

f1 (ppm)


O
H
N
S
O
OH

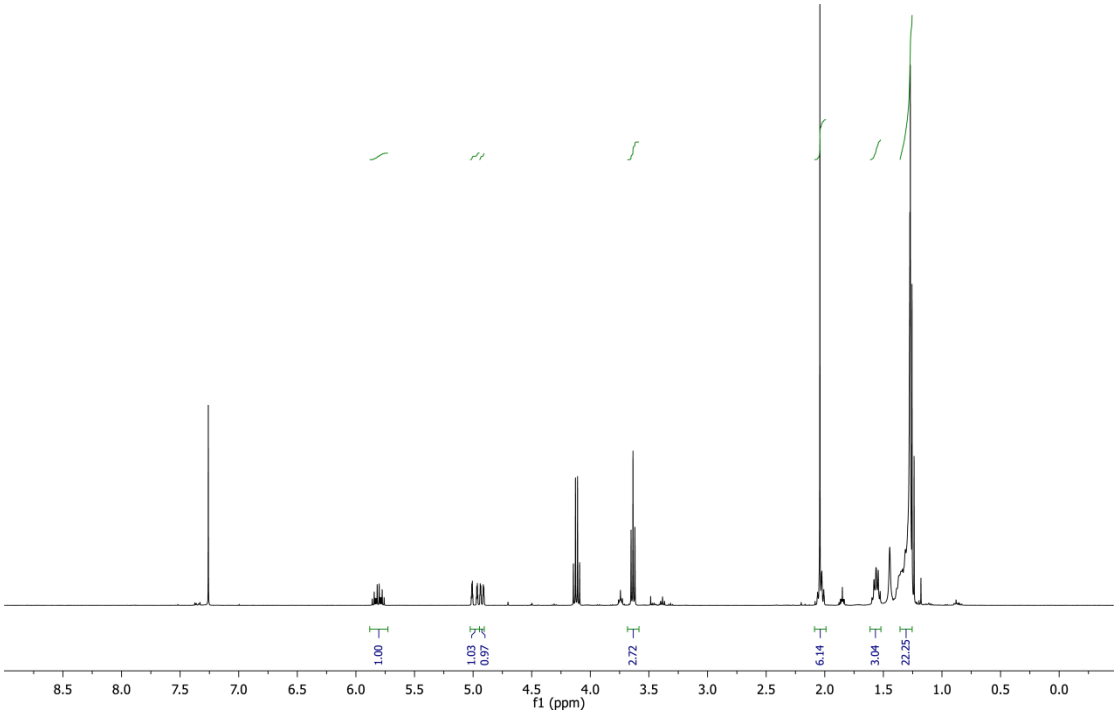
8

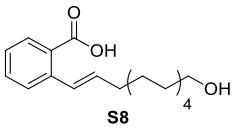

1.00
1.01
1.10
1.94
0.99
1.01
0.99
2.01
2.11
2.04
2.05
3.15
2.63
2.60
13.21
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


194.78
170.38
136.68
135.72
134.97
131.97
128.43
127.04
126.99
126.62
63.04
39.80
39.65
33.22
32.80
29.54
29.48
29.42
29.39
29.34
29.17
25.72
23.24
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

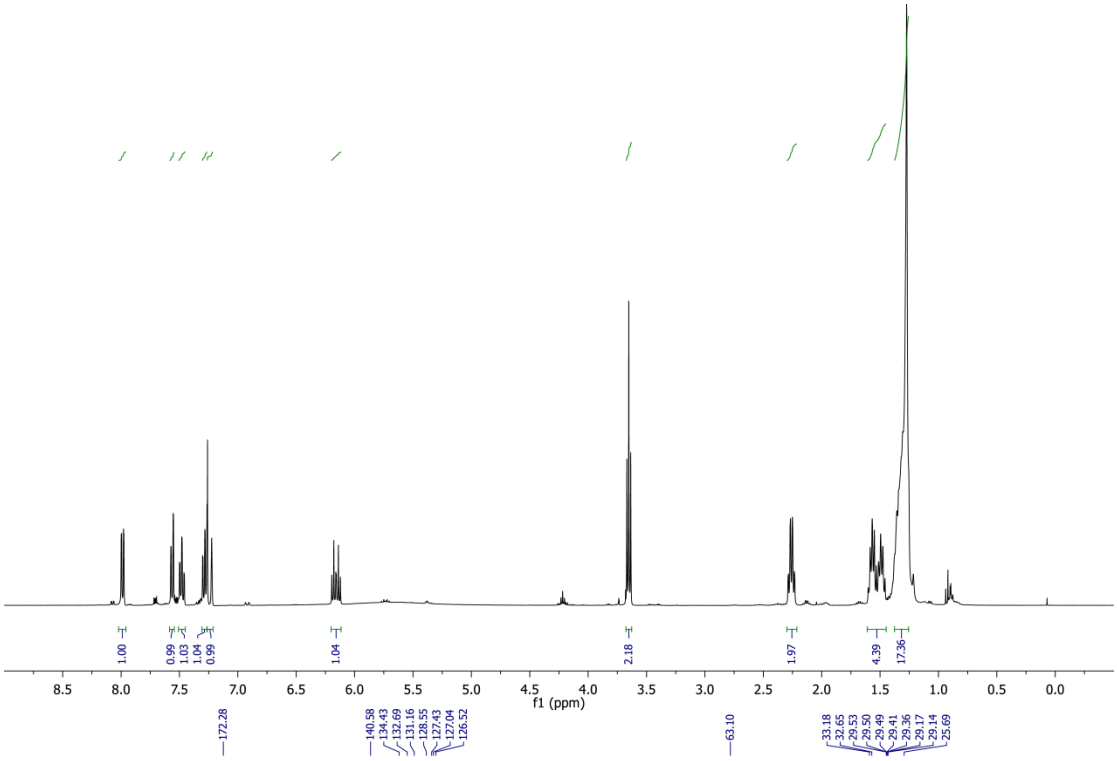

OH

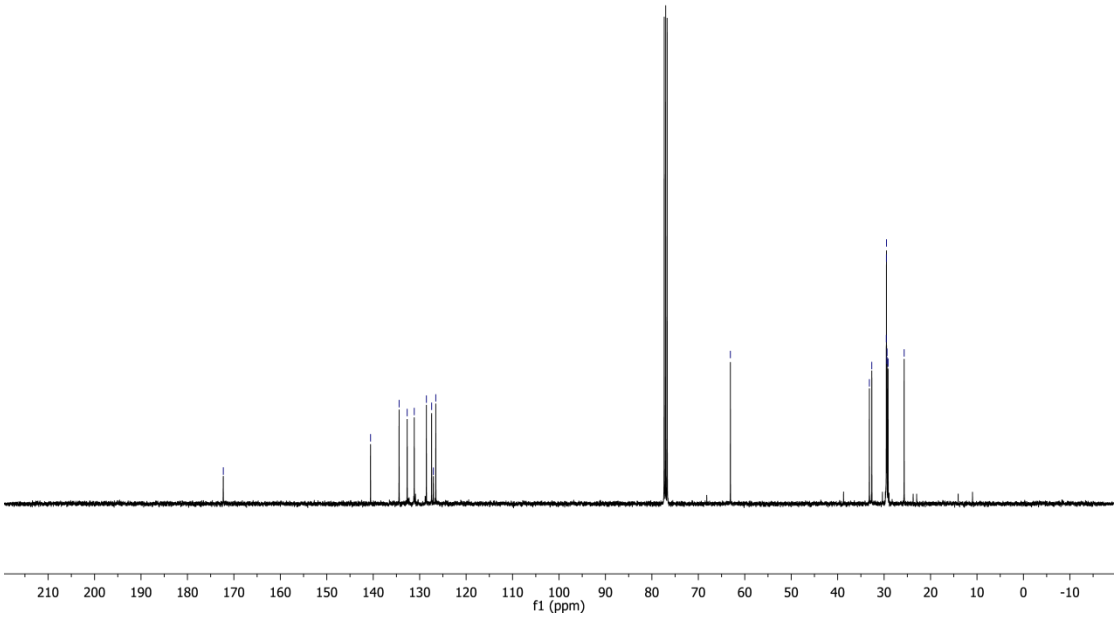
S7

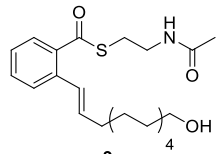

1.00
1.03
0.97
2.72
6.14
3.04
22.25
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


O
OH
OH
4

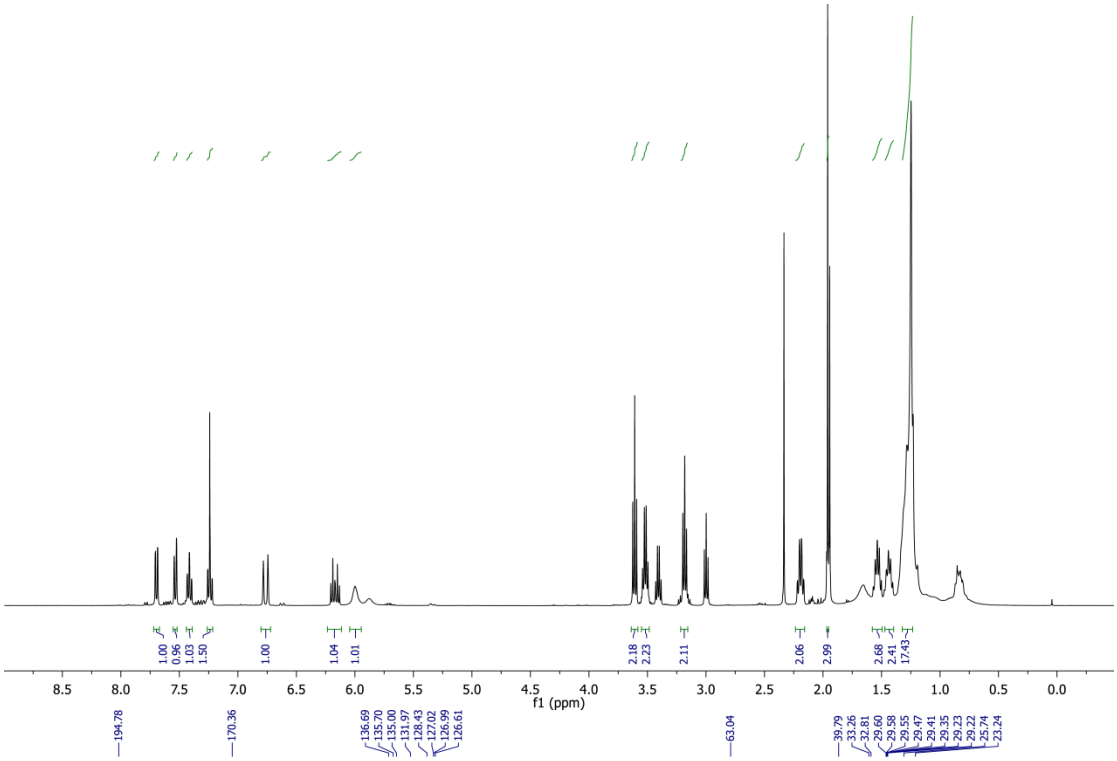
S8

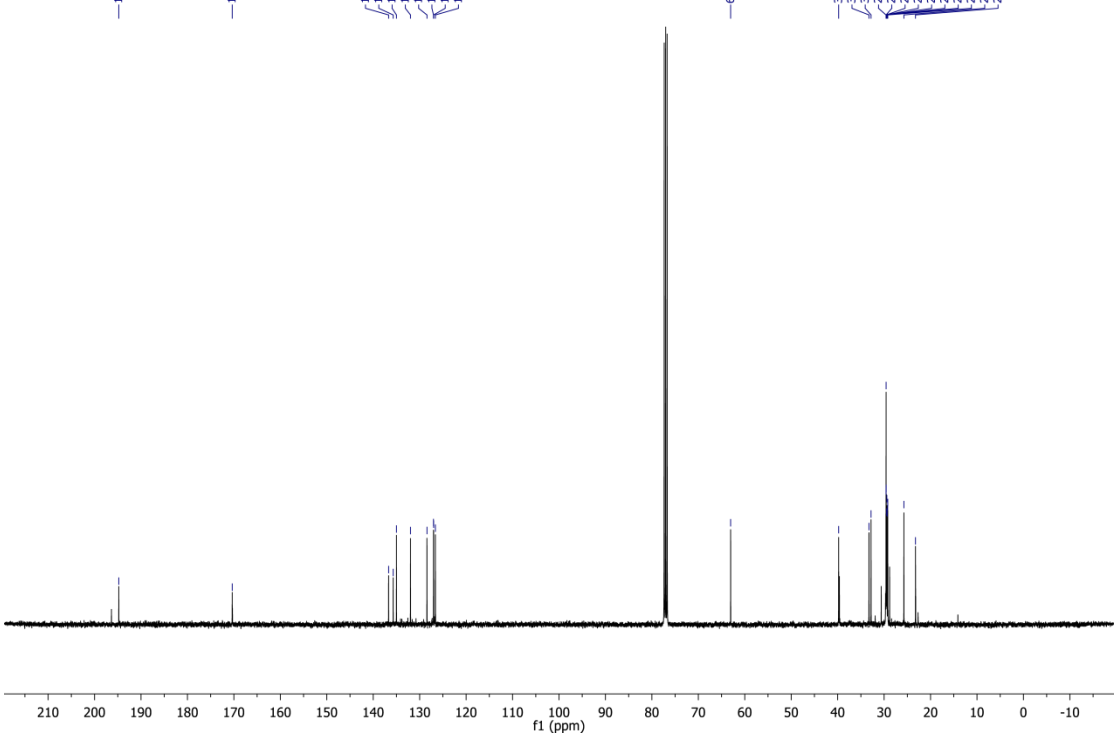

1.00
0.99
1.03
1.04
0.99
1.04
2.18
1.97
4.39
17.36
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)
172.28
140.58
134.43
132.69
131.16
128.55
127.43
127.04
126.52
63.10
33.18
32.65
29.53
29.50
29.49
29.41
29.36
29.17
29.14
25.69

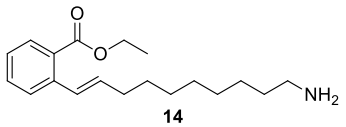

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


O
S
H
N
O
OH
4

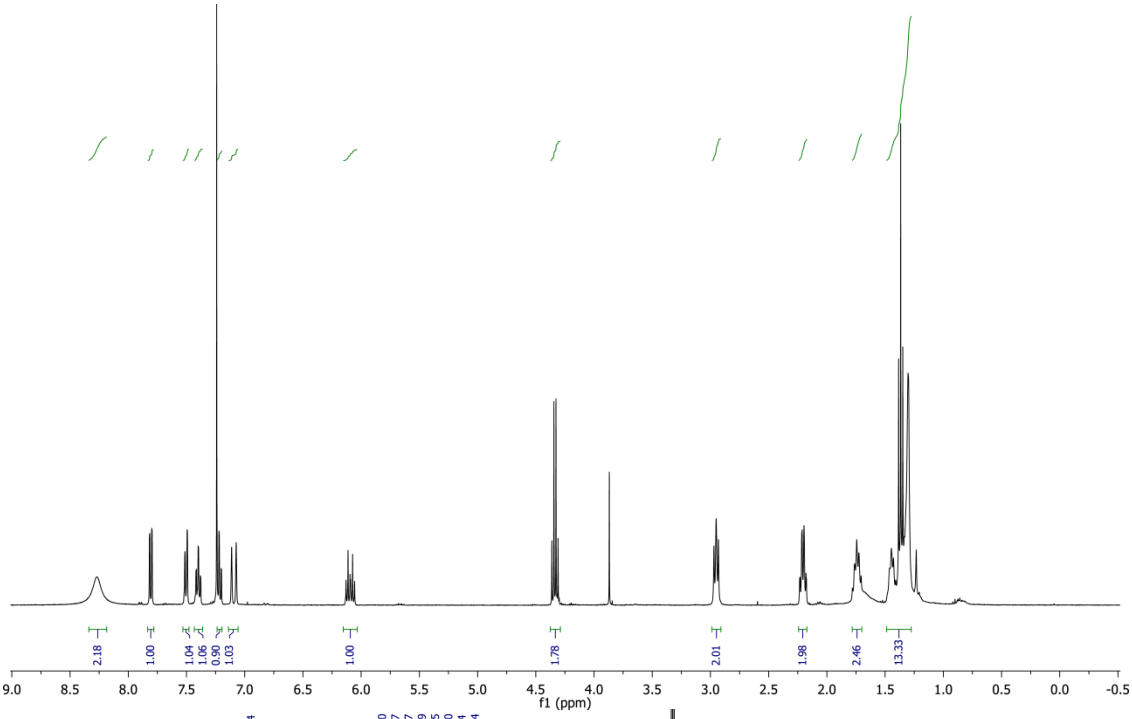
9

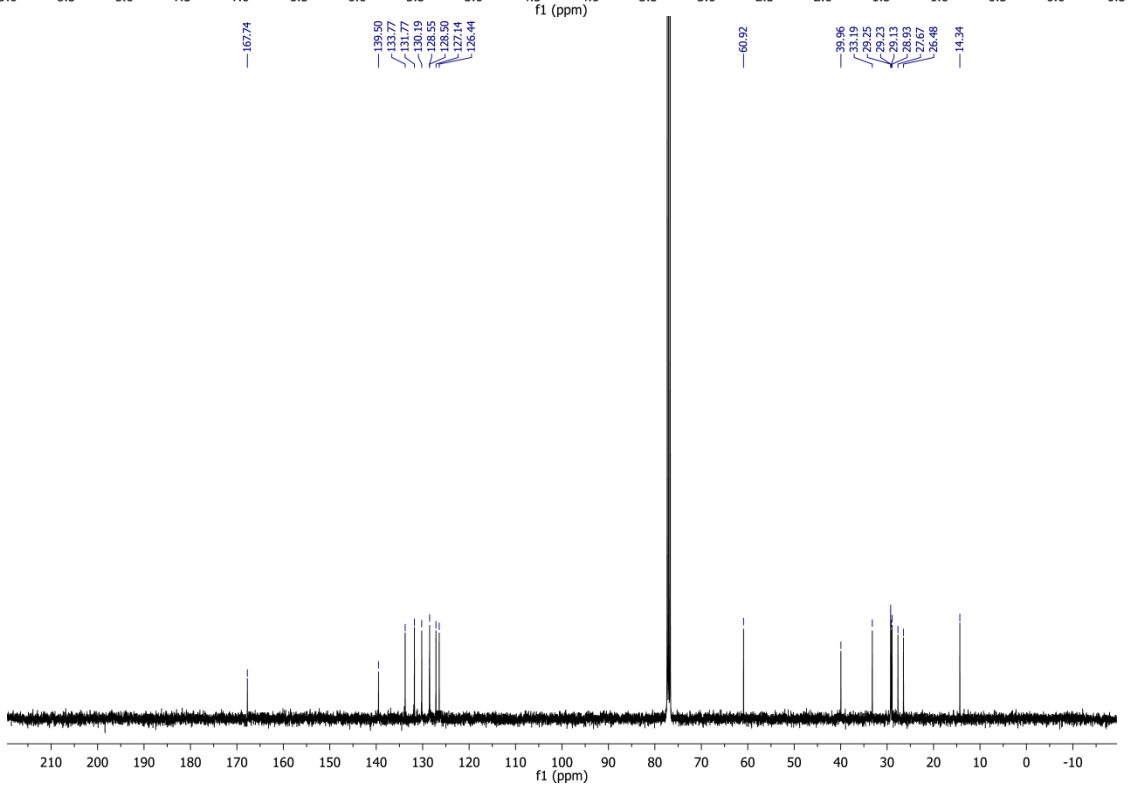

1.00
0.96
1.03
1.50
1.00
1.04
1.01
2.18
2.23
2.11
2.06
2.99
2.68
2.41
17.43
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

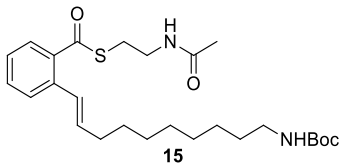

194.78
170.36
136.69
135.70
135.00
131.97
128.43
127.02
126.99
126.61
63.04
39.79
33.26
32.81
29.60
29.58
29.55
29.47
29.41
29.35
29.23
29.22
25.74
23.24
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


O
O
NH2

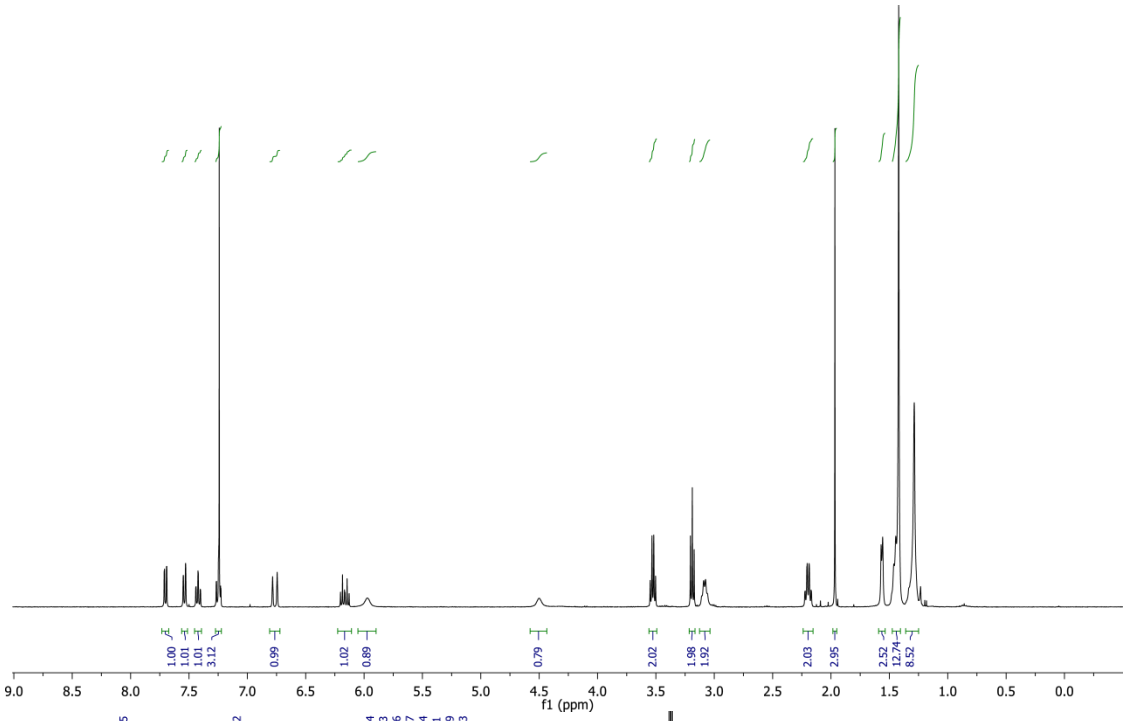
14

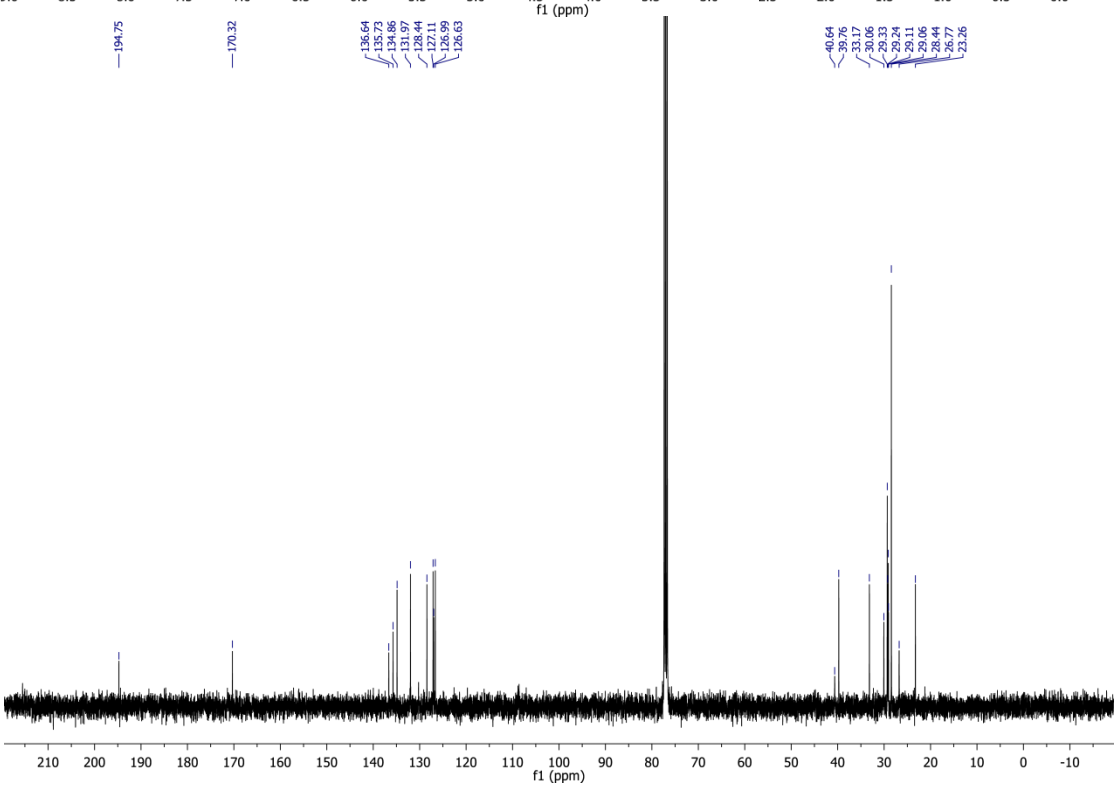

2.18
1.00
1.04
1.06
0.90
1.03
1.00
1.78
2.01
1.98
2.46
13.33
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

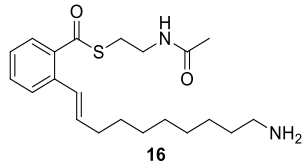

167.74
139.50
133.77
131.77
130.19
128.55
128.50
127.14
126.44
60.92
39.96
33.19
29.25
29.23
29.13
28.93
27.67
26.48
14.34
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


O
H
N
S
O
NHBoc

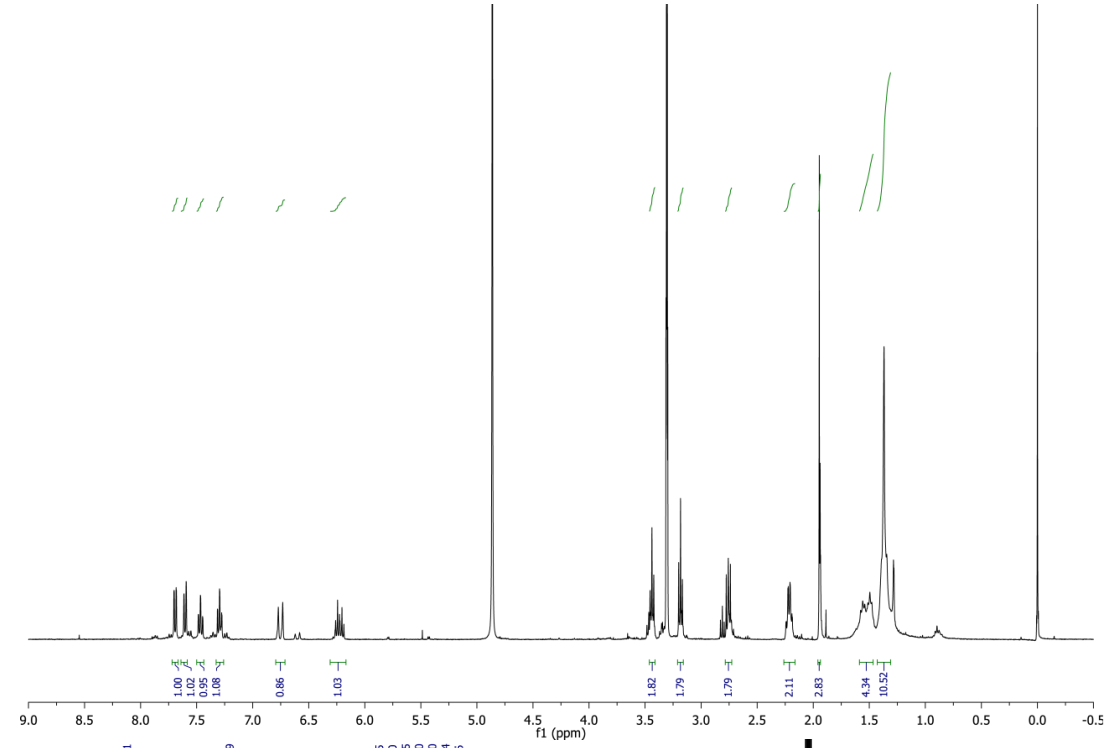
15

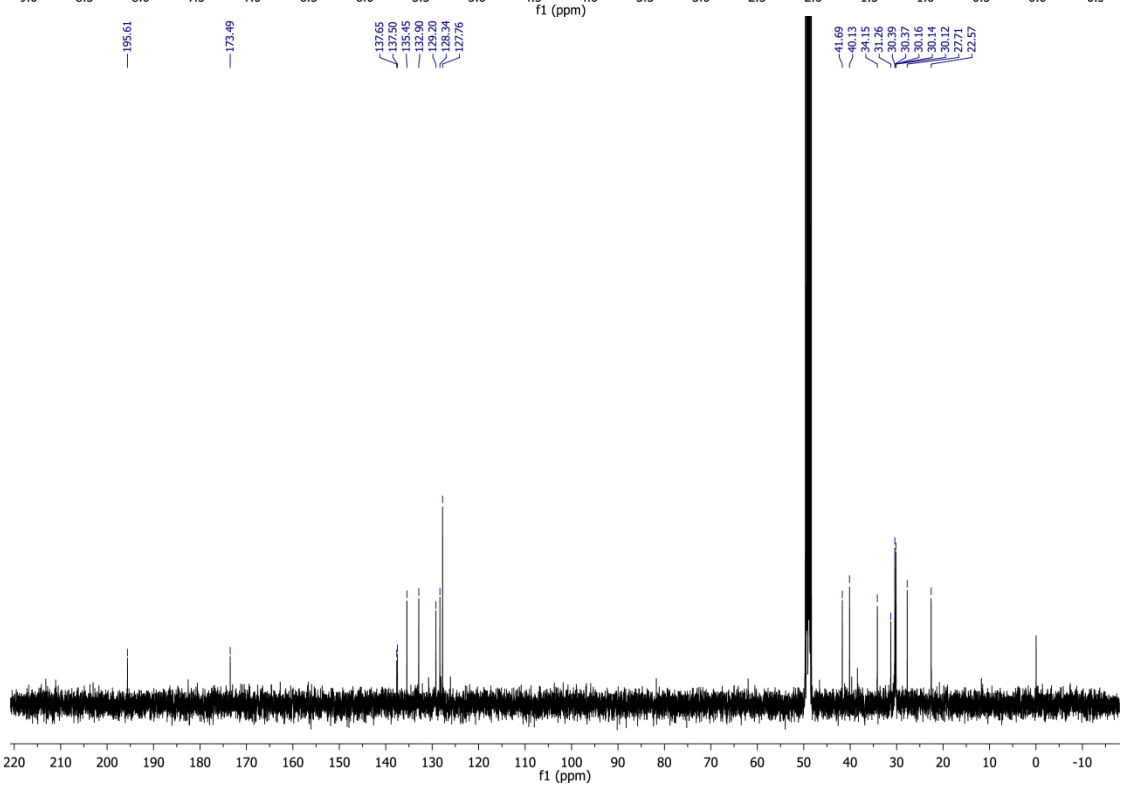

1.00
1.01
1.01
3.12
0.99
1.02
0.89
0.79
2.02
1.98
1.92
2.03
2.95
2.52
12.74
8.52
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

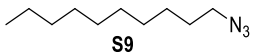

194.75
170.32
136.64
135.73
134.86
131.97
128.44
127.11
126.99
126.63
40.64
39.76
33.17
30.06
29.33
29.24
29.11
29.06
28.44
26.77
23.26
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


O
H
N
S
O
NH2

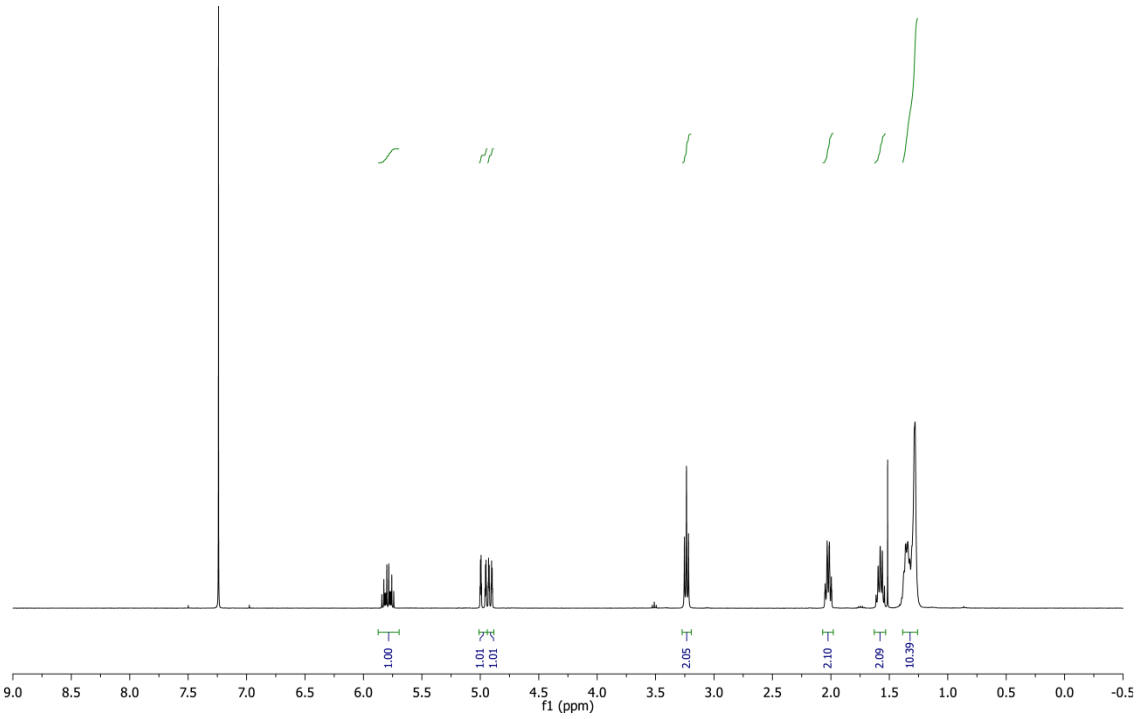
16

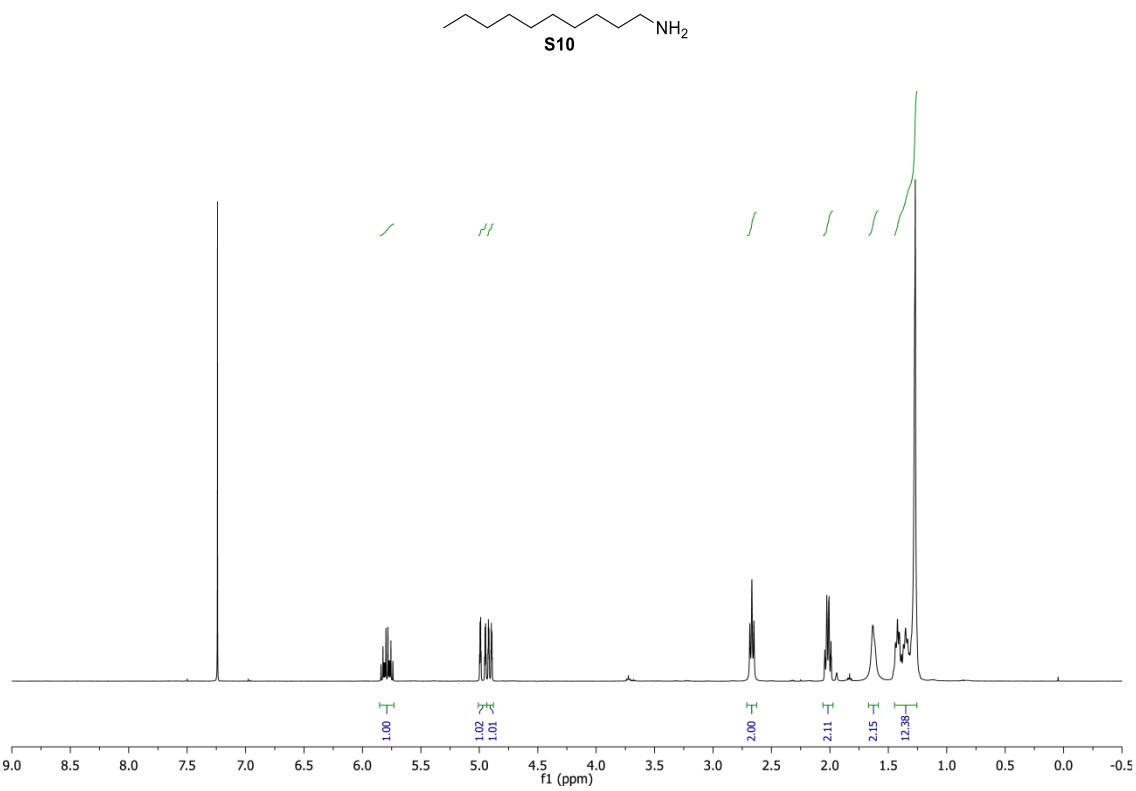

1.00
1.02
0.95
1.08
0.86
1.03
1.82
1.79
1.79
2.11
2.83
4.34
10.52
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

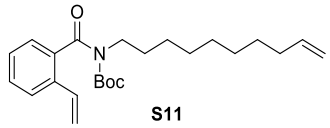

195.61
173.49
137.65
137.50
135.45
132.90
129.20
128.34
127.76
41.69
40.13
34.15
31.26
30.39
30.37
30.16
30.14
30.12
27.71
22.57
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


N3

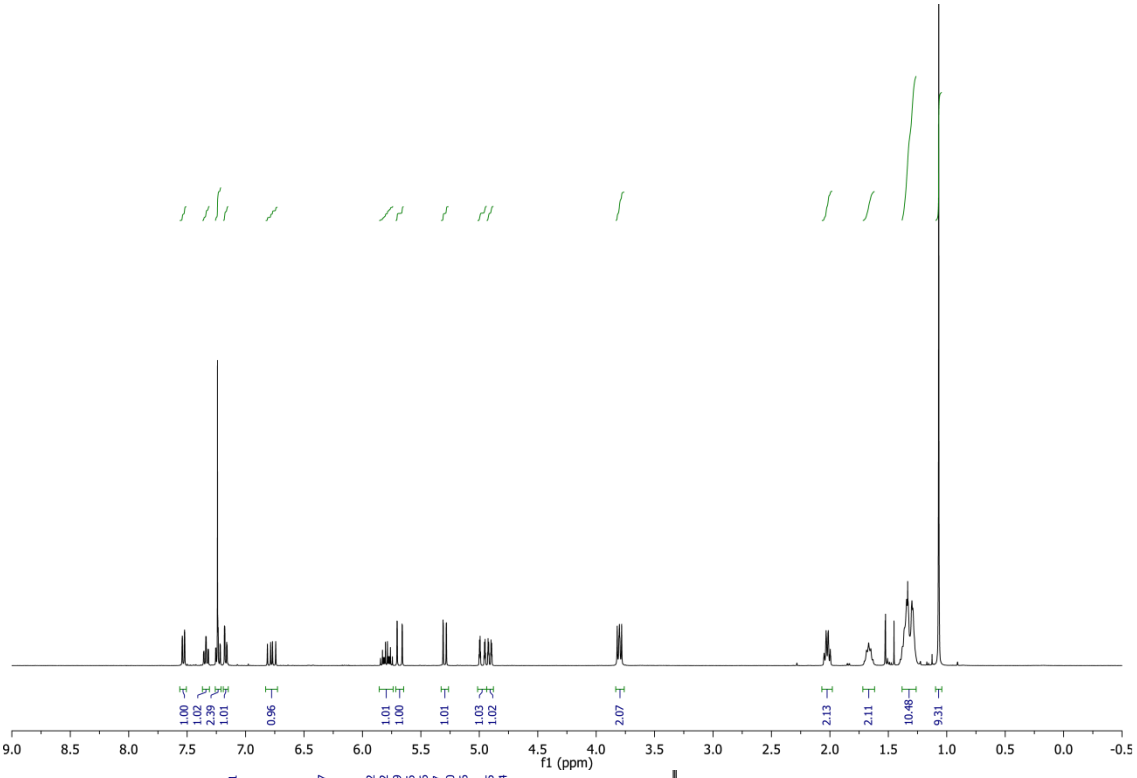
S9

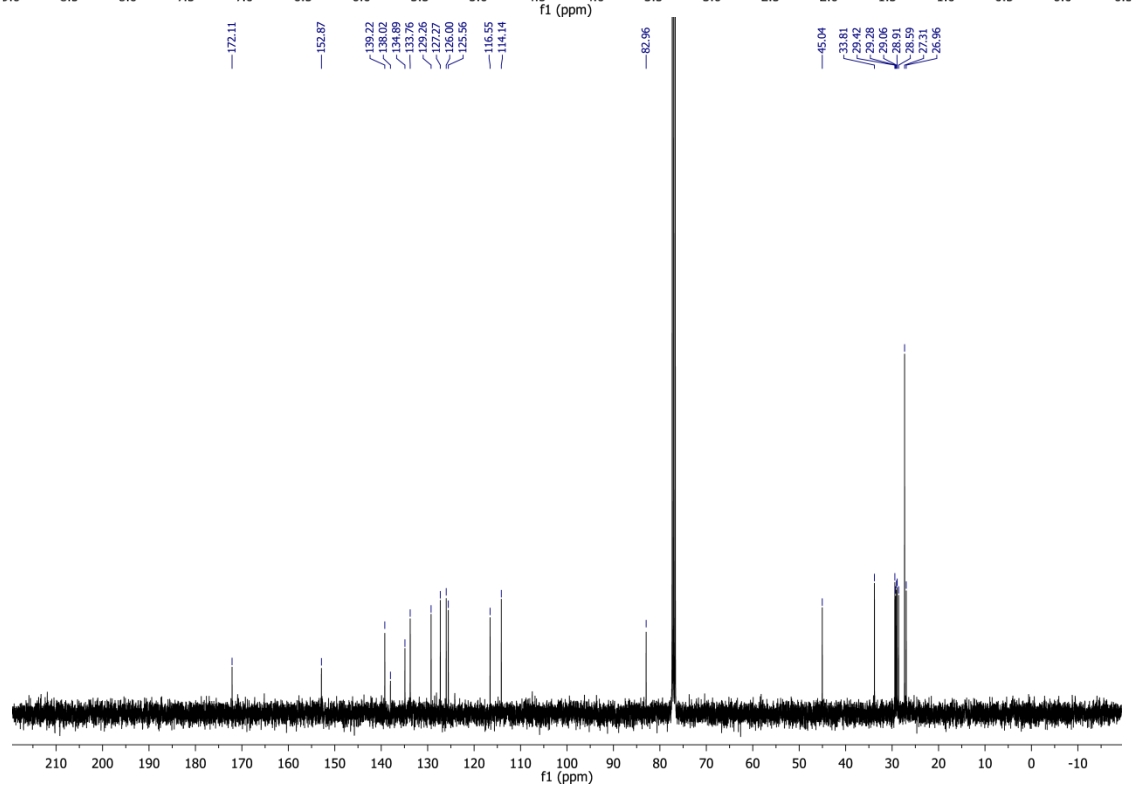

1.00
1.01
1.01
2.05
2.10
2.09
10.39
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

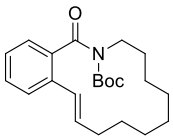

NH2
S10
1.00
1.02
1.01
2.00
2.11
2.15
12.38
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


O
N
Boc

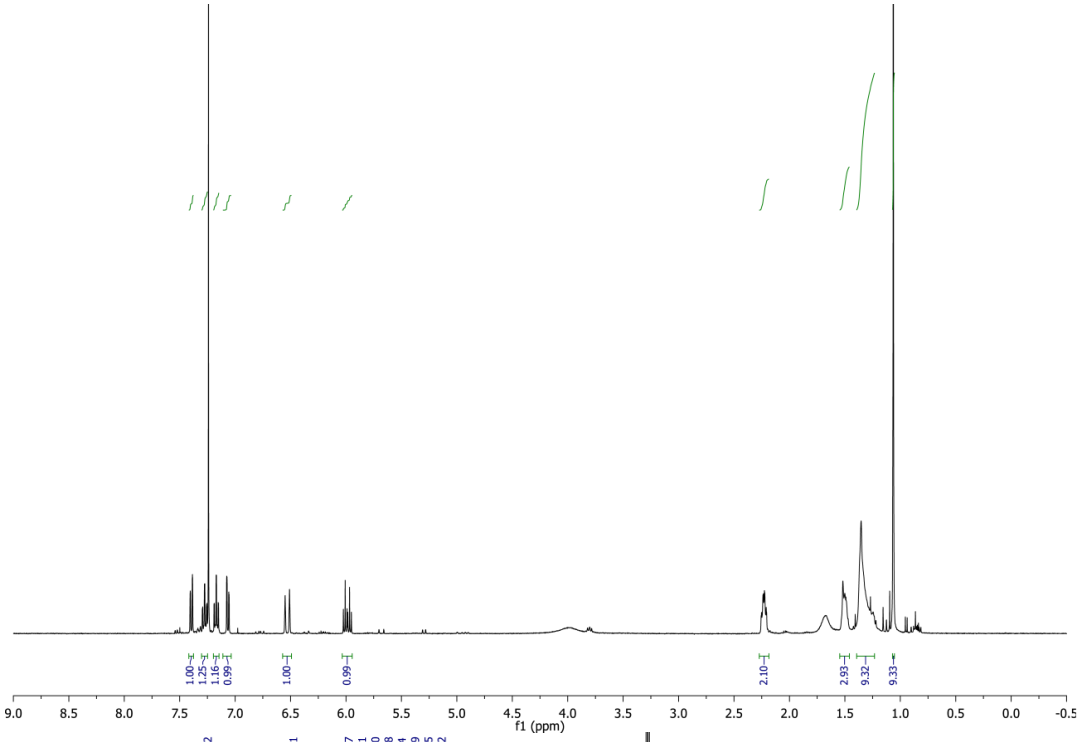
S11

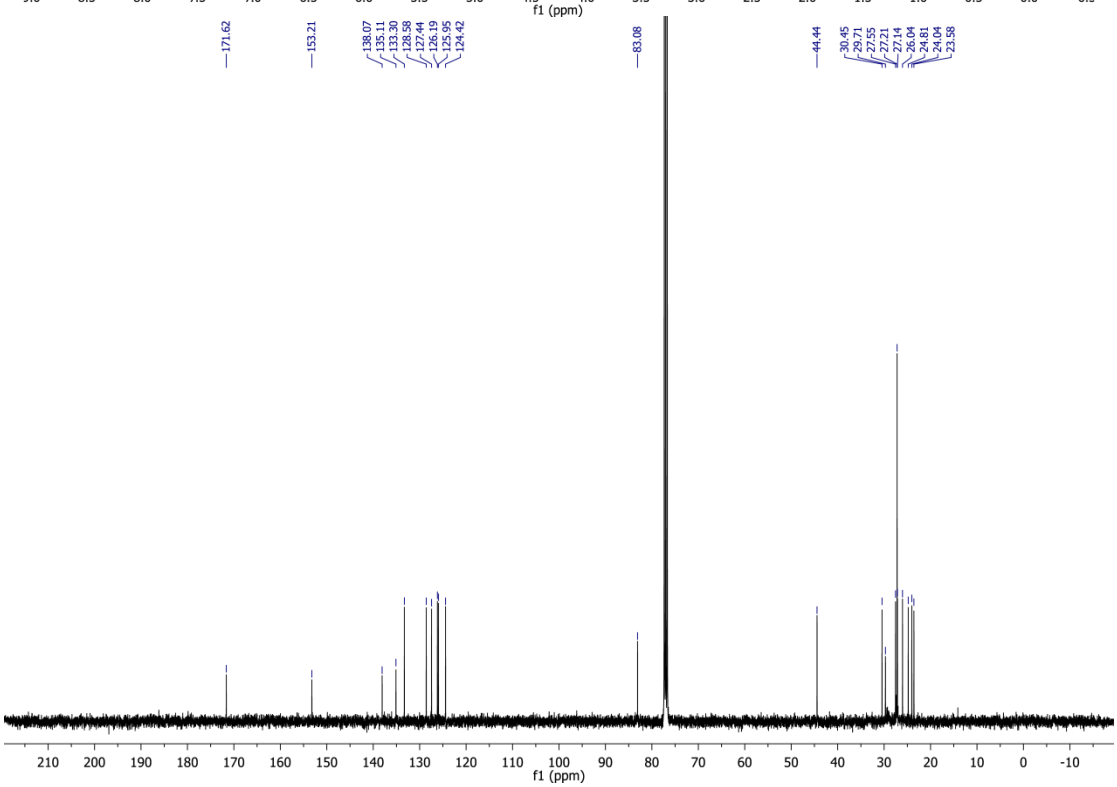

1.00
1.02
2.39
1.01
0.96
1.01
1.00
1.01
1.03
1.02
2.07
2.13
2.11
10.48
9.31
f1 (ppm)

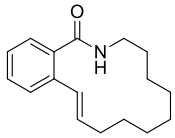

172.11
152.87
139.22
138.02
134.89
133.76
129.26
127.27
126.60
125.56
116.55
114.14
82.96
45.04
33.81
29.42
29.28
29.06
28.91
28.59
27.31
26.96
f1 (ppm)


O
N
Boc

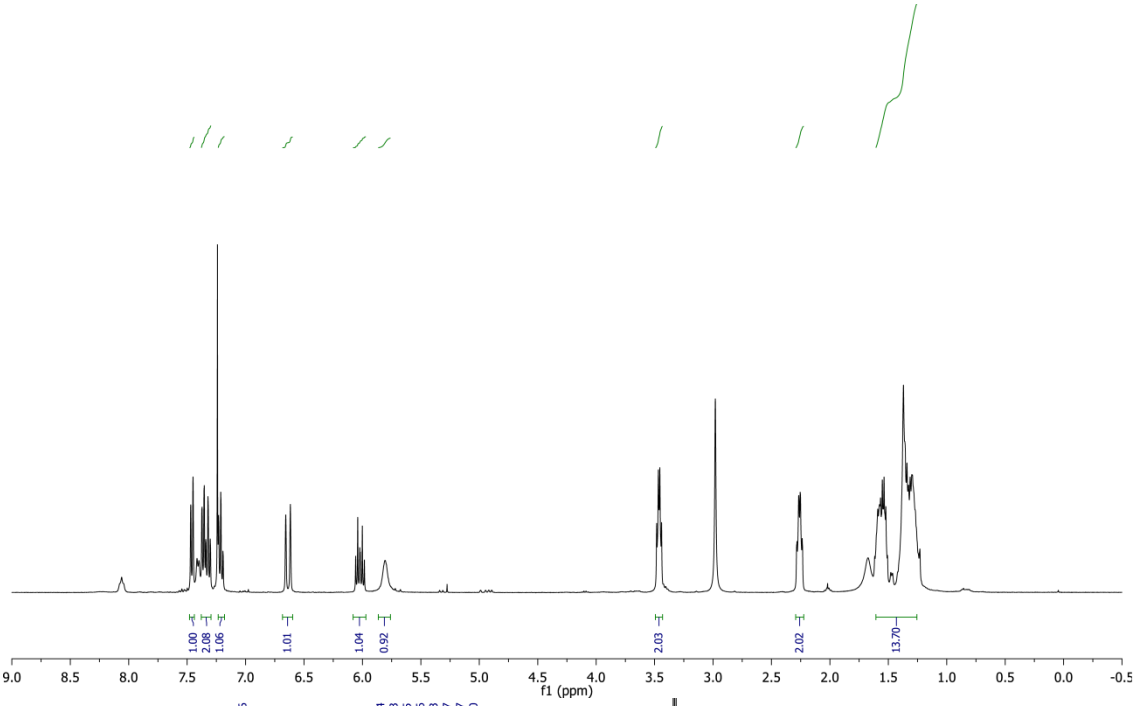
S12

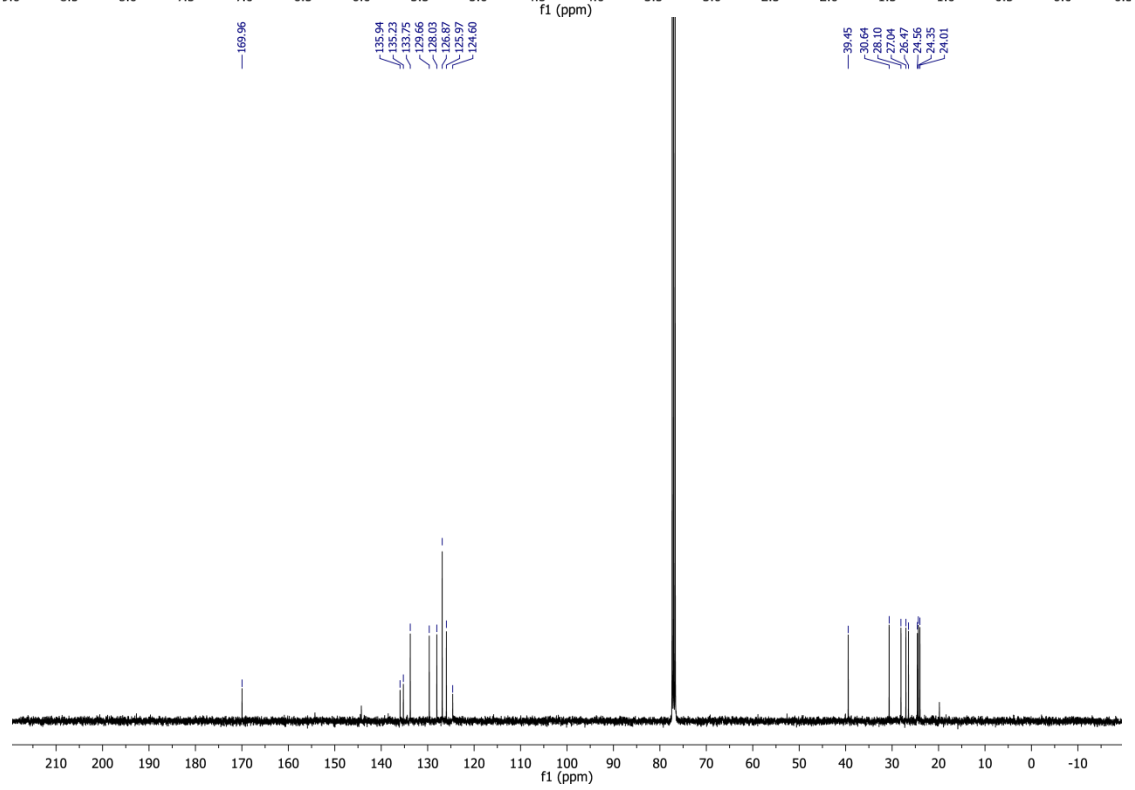

1.00
1.25
1.16
0.99
1.00
0.99
2.10
2.93
9.32
9.33
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

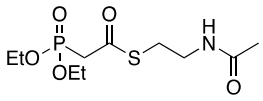

171.62
153.21
138.07
135.11
133.30
128.58
127.44
126.19
125.95
124.42
83.08
44.44
30.45
29.71
27.55
27.21
27.14
26.04
24.81
24.04
23.58
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


O
N
H

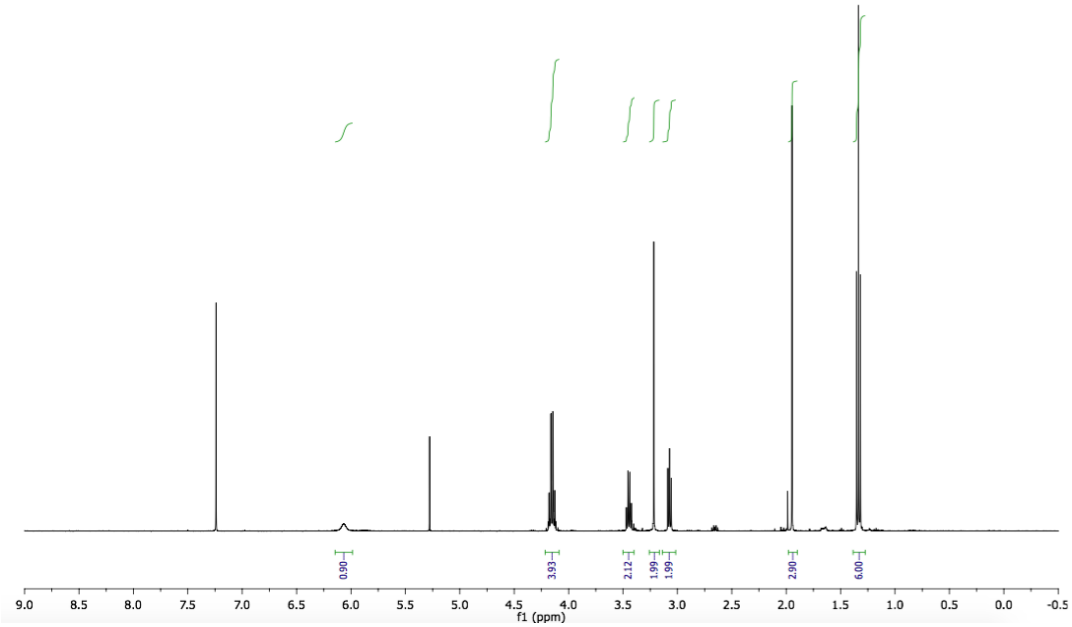
23

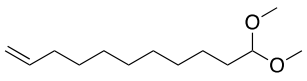

1.00
2.08
1.06
1.01
1.04
0.92
2.03
2.02
13.70
f1 (ppm)


169.96
135.94
135.23
133.75
129.66
128.03
126.87
125.97
124.60
39.45
30.64
28.10
27.04
26.47
24.56
24.35
24.01
f1 (ppm)

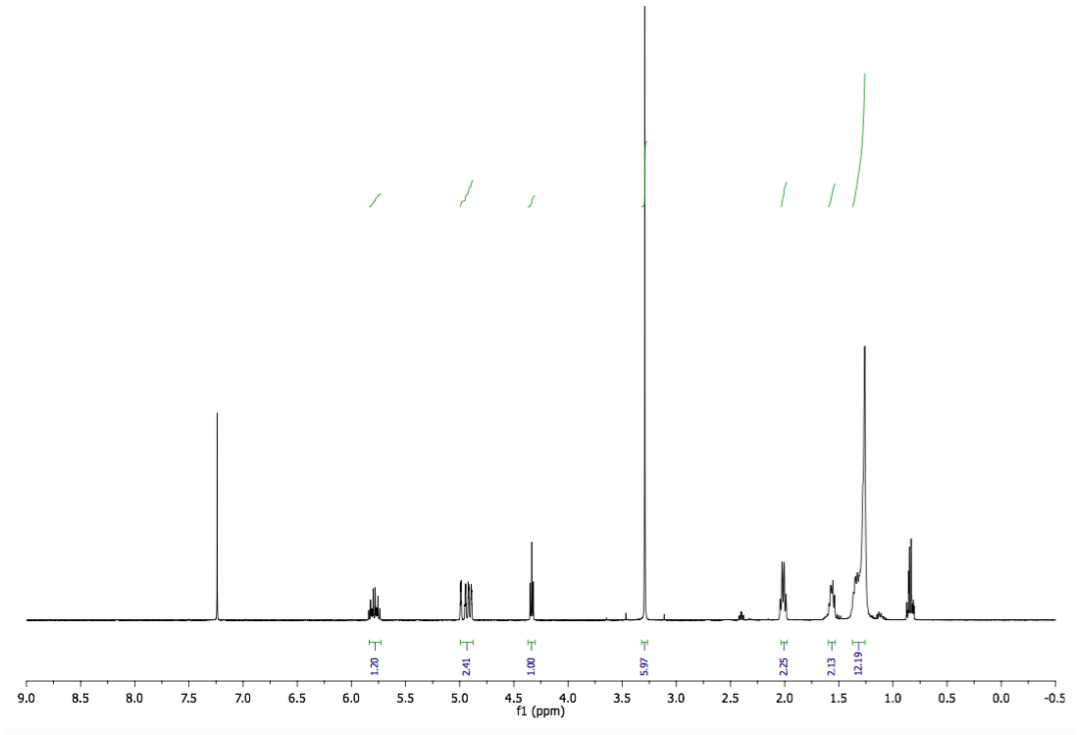


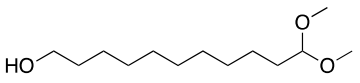
S15


0.90
3.93
2.12
1.99
1.99
2.90
6.00
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

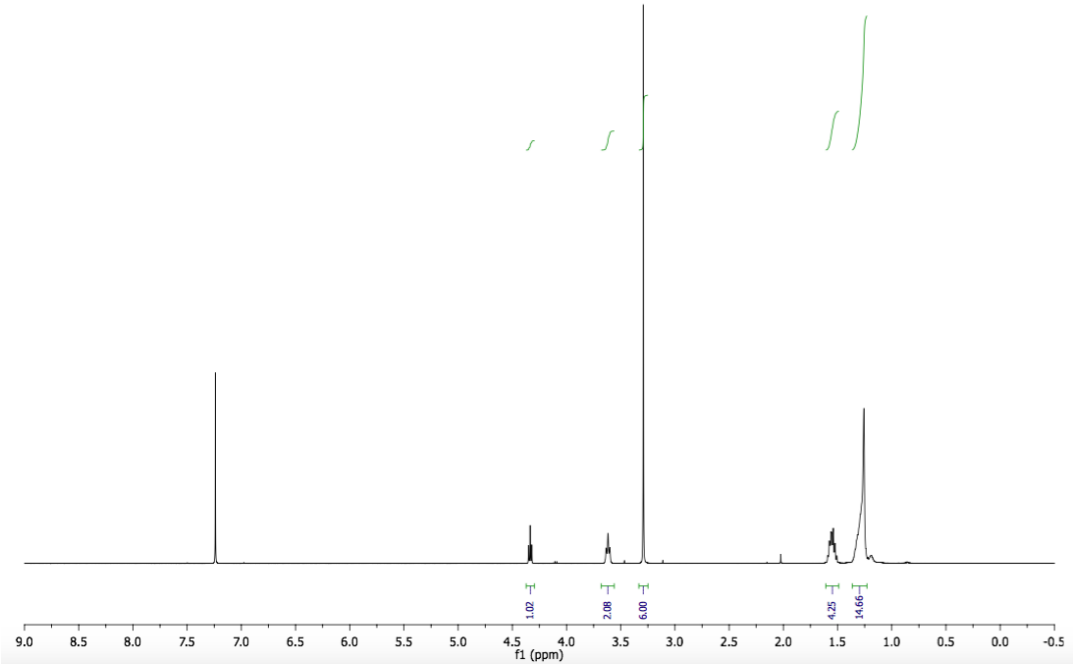


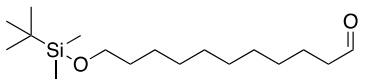
S16


1.20
2.41
1.00
5.97
2.25
2.13
12.19
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

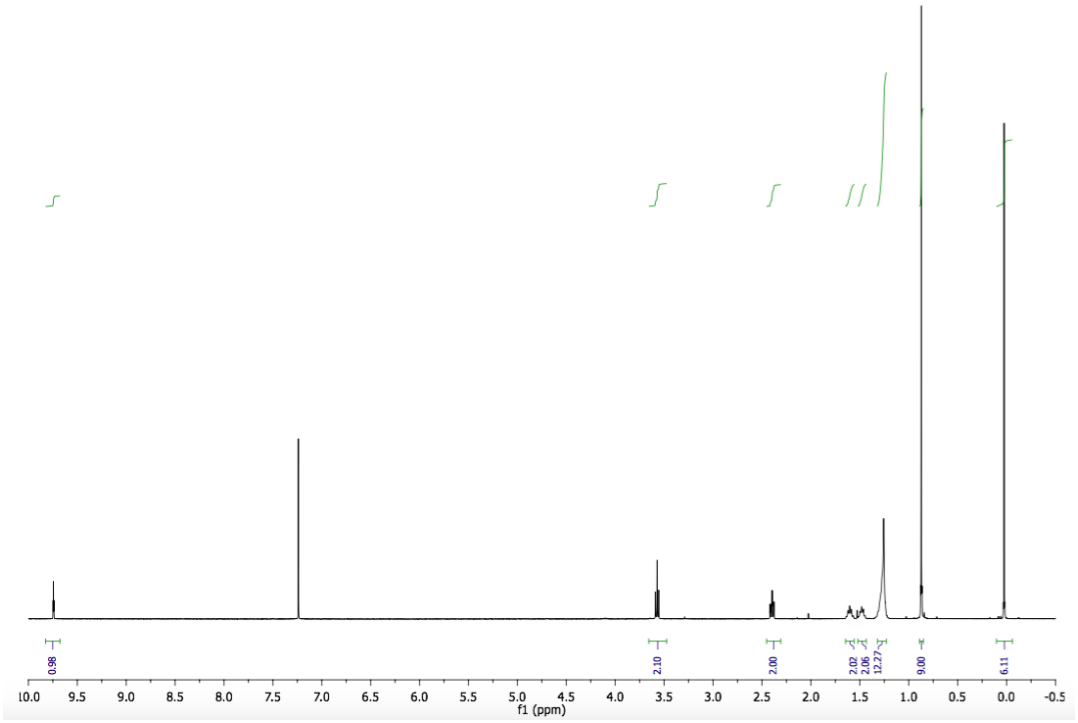

HO
O
O

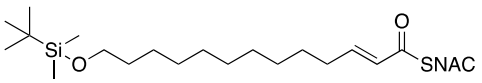
S17


1.02
2.08
6.00
4.25
14.66
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

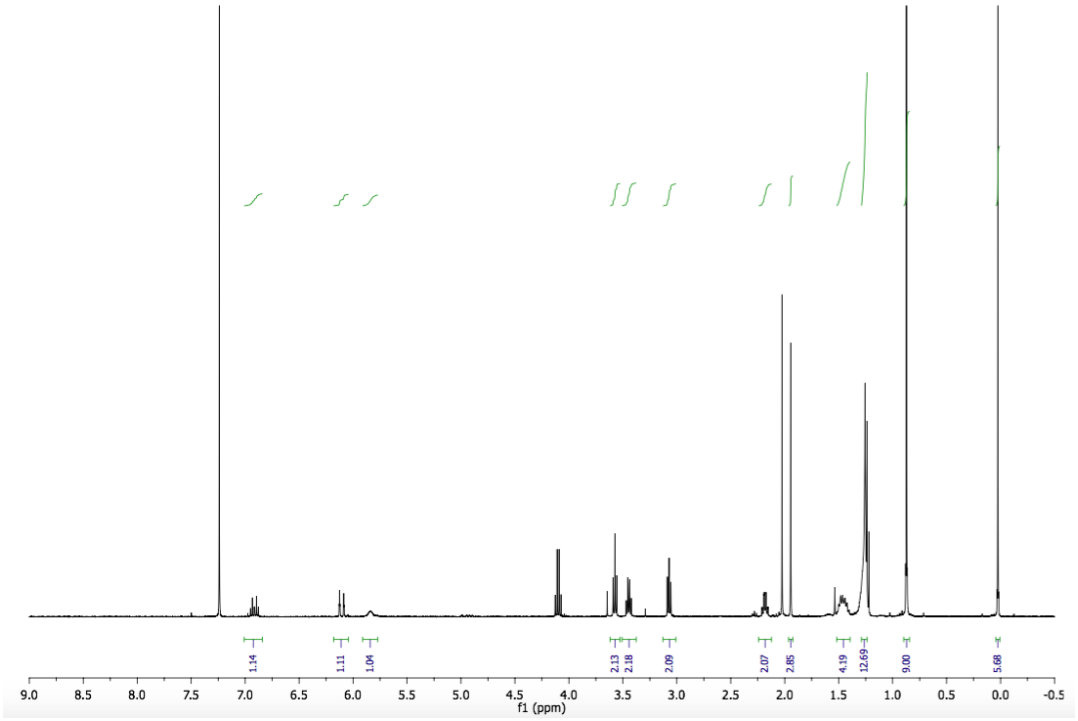

Si
O
O

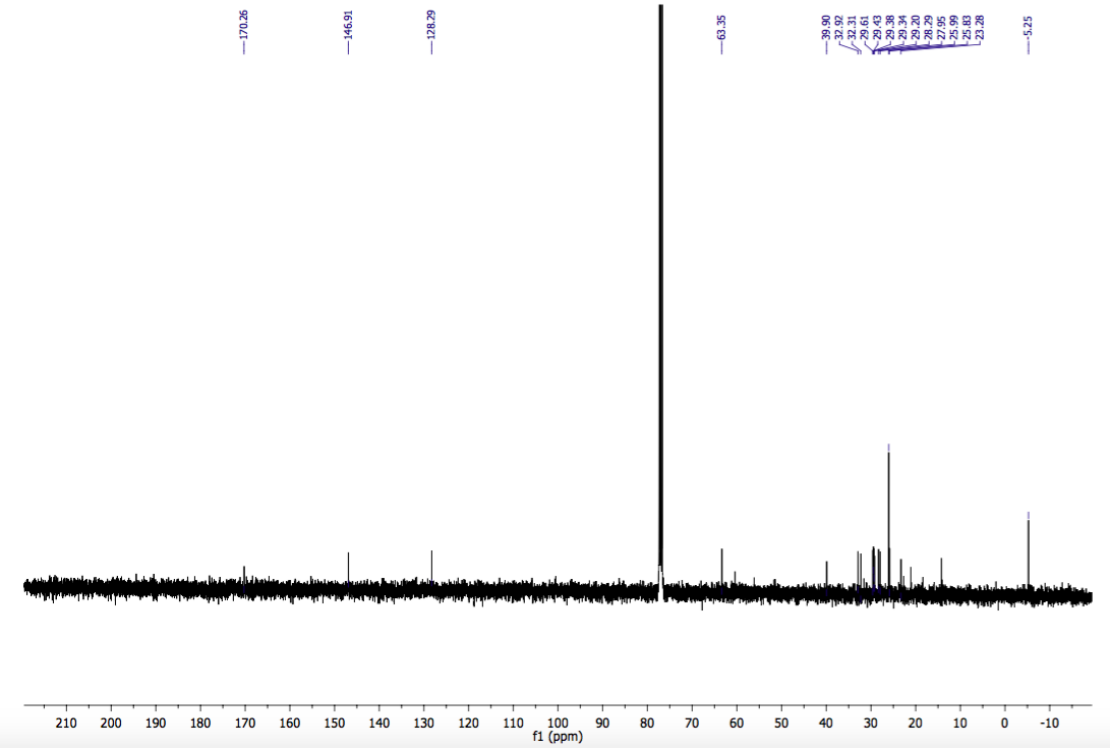
S18

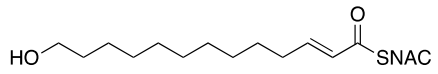

0.98
2.10
2.00
2.02
2.06
12.27
9.00
6.11
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)


O
Si
O
SNAC

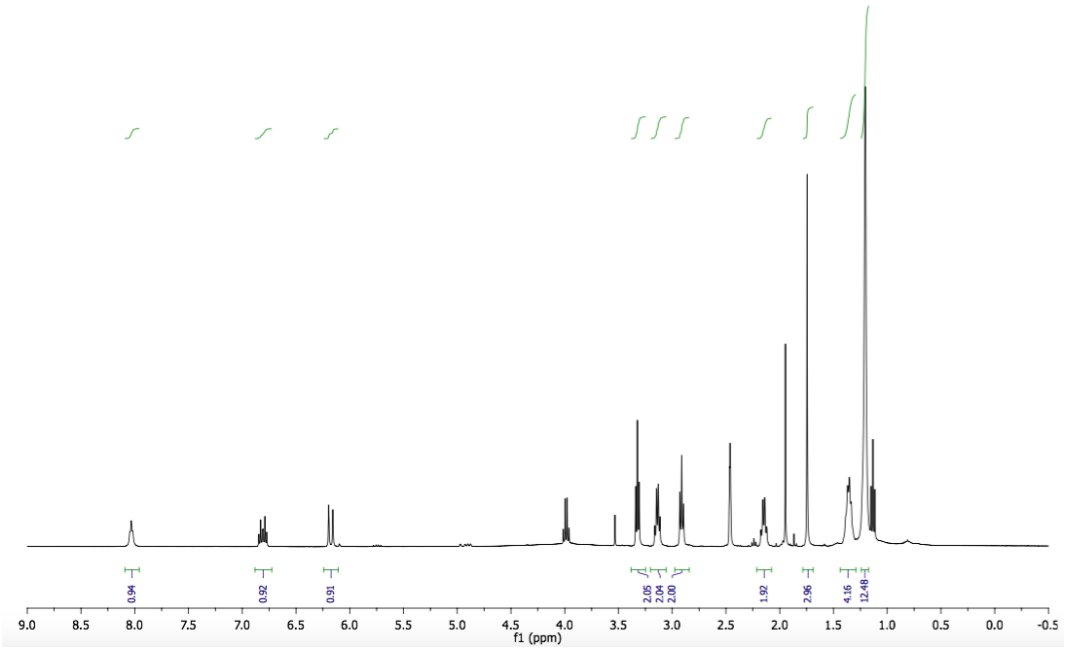
S19

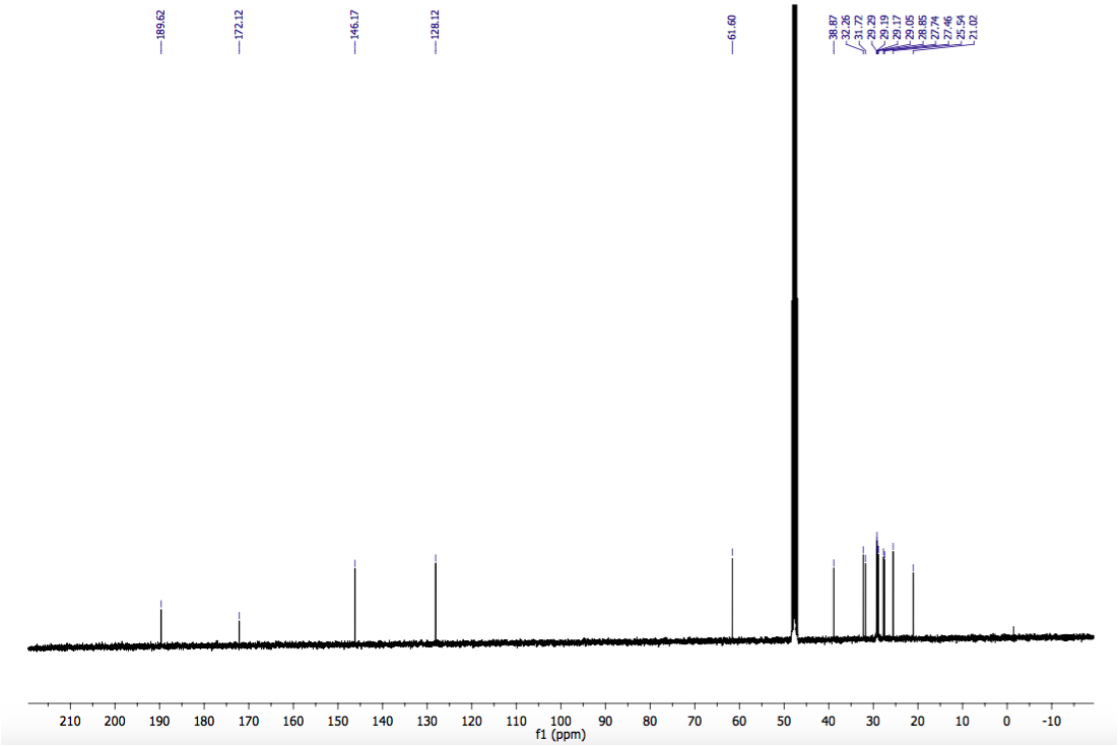

1.14
1.11
1.04
2.13
2.18
2.09
2.07
2.85
4.19
12.69
9.00
5.68
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

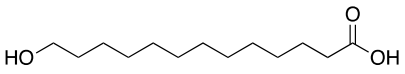

170.26
146.91
128.29
63.35
39.90
32.92
32.31
29.61
29.43
29.38
29.34
29.20
29.09
27.95
25.99
25.83
23.28
5.25
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


HO
O
SNAC

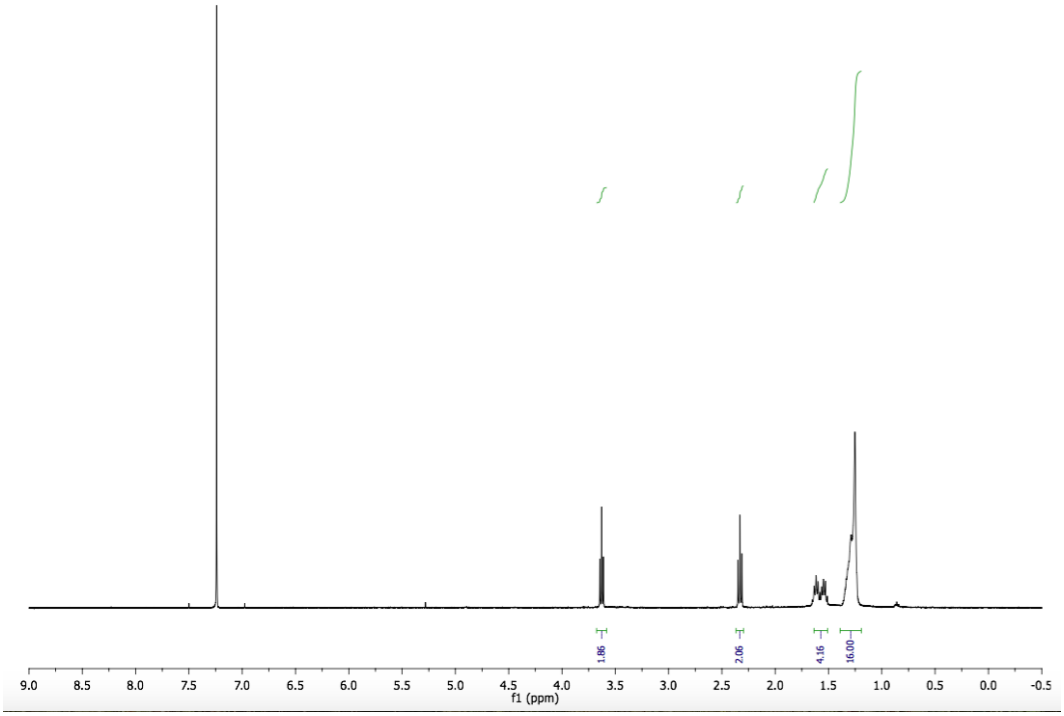
17

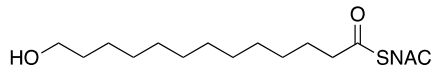

0.94
0.92
0.91
2.05
2.04
2.00
1.92
2.96
4.16
12.48
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


189.62
172.12
146.17
128.12
61.60
38.87
32.26
31.72
29.29
29.19
29.17
29.05
28.85
27.74
27.46
25.54
21.02
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

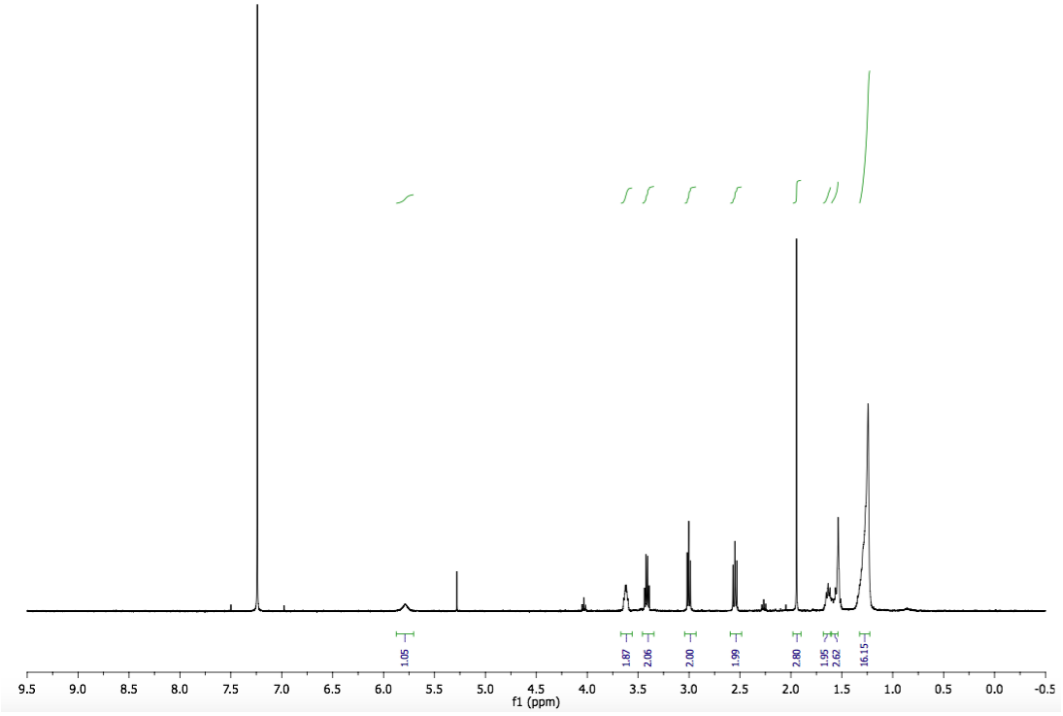

HO
O
OH

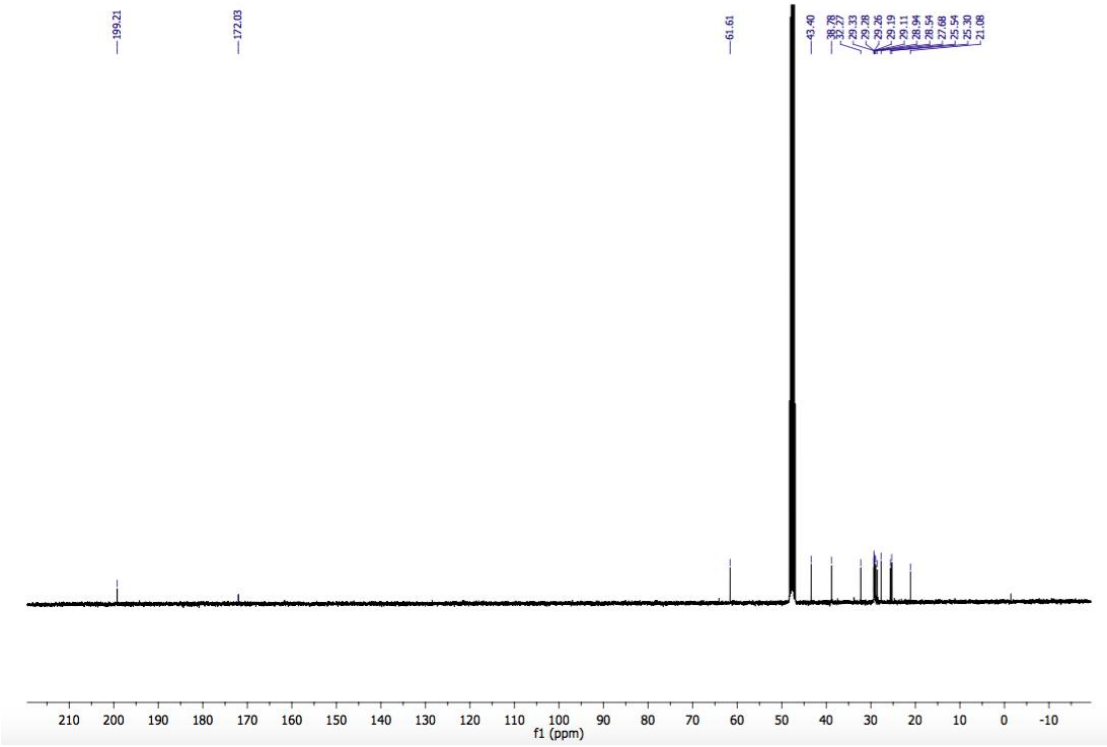
S20

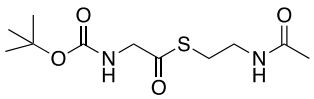

1.86
2.06
4.16
16.00
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)


HO
O
SNAC

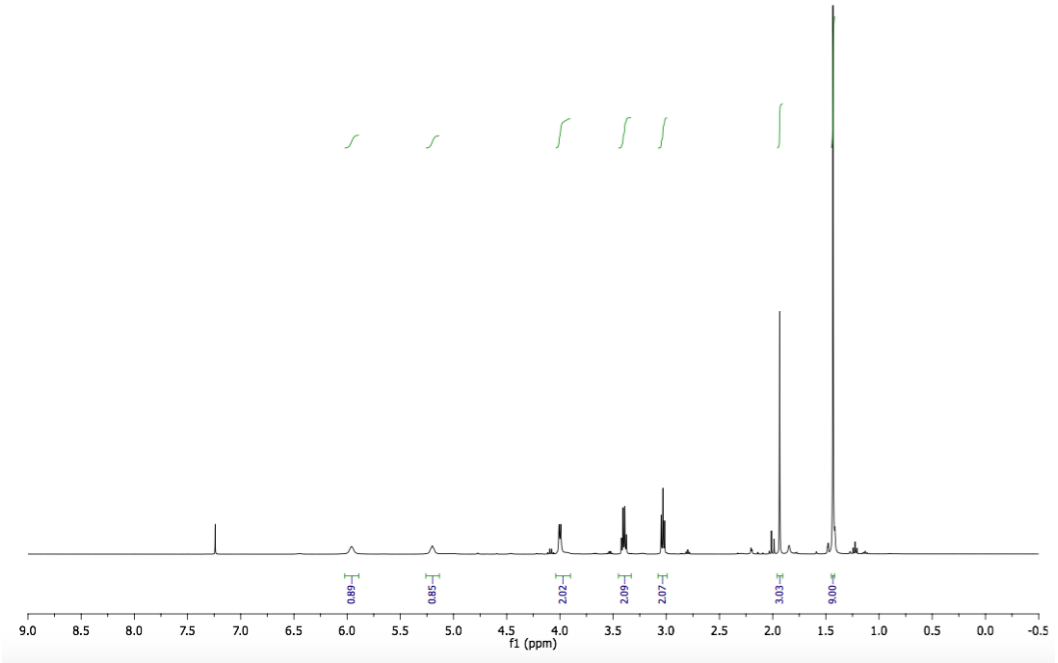
18

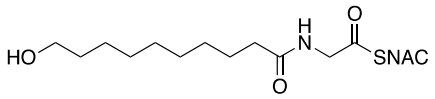

1.05
1.87
2.06
2.00
1.99
2.80
1.95
2.62
16.15
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


199.21
172.03
61.61
43.40
38.78
38.72
38.67
29.33
29.28
29.26
29.19
29.11
28.94
28.54
28.08
25.64
25.30
21.08
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

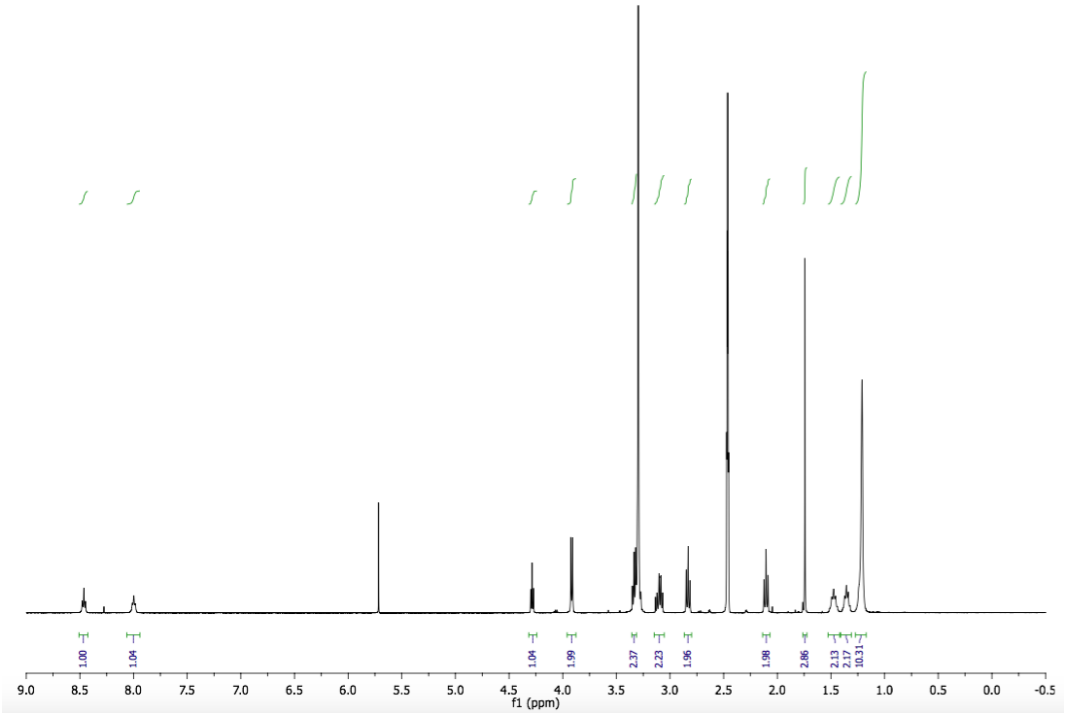


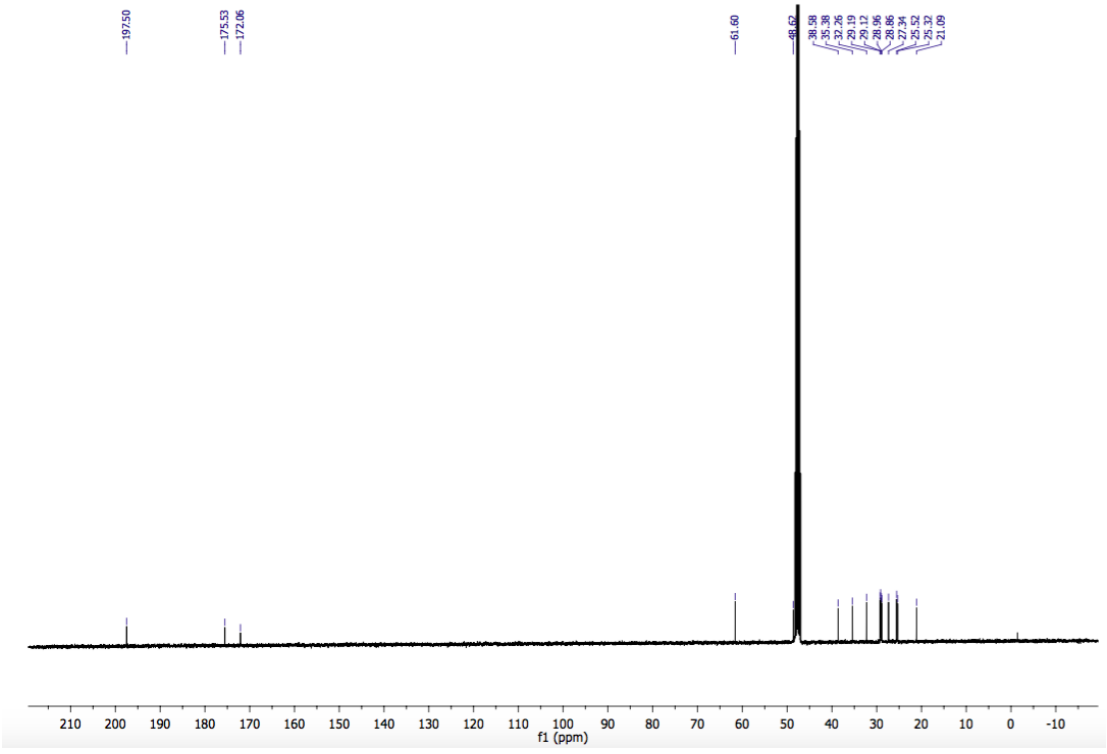
S21

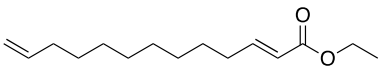

0.89
0.85
2.02
2.09
2.07
3.03
9.00
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


HO
H
N
O
O
SNAC
O

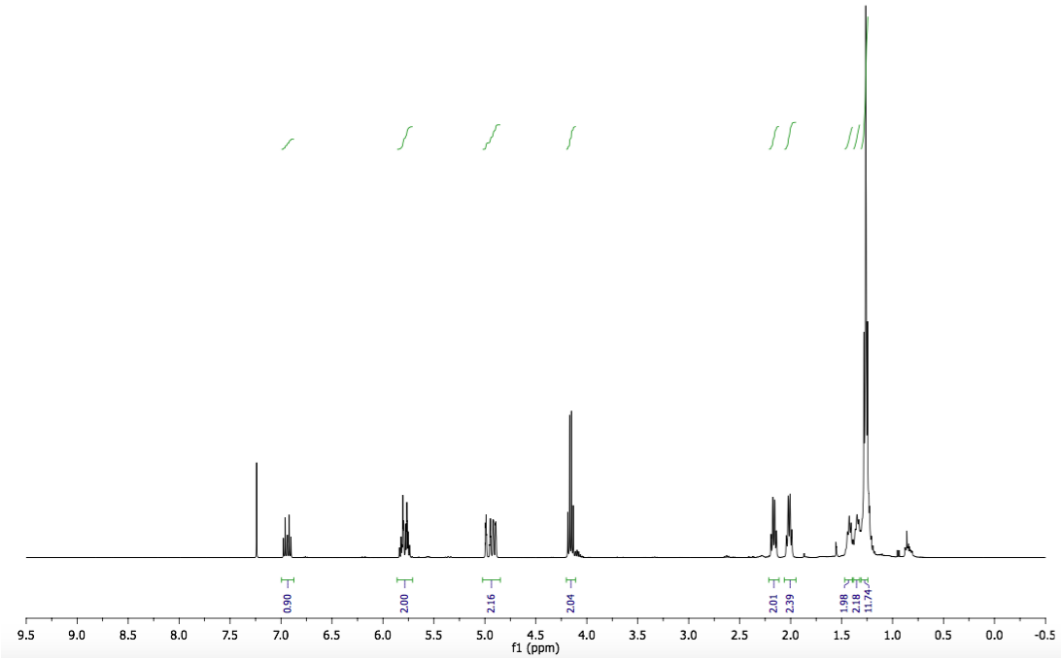
19

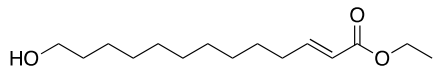

1.00
1.04
1.04
1.99
2.37
2.23
1.96
1.98
2.86
2.13
2.17
10.31
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)


197.50
175.53
172.06
61.60
48.62
38.58
35.38
32.26
29.19
29.12
28.96
28.86
27.34
25.52
25.33
21.09
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

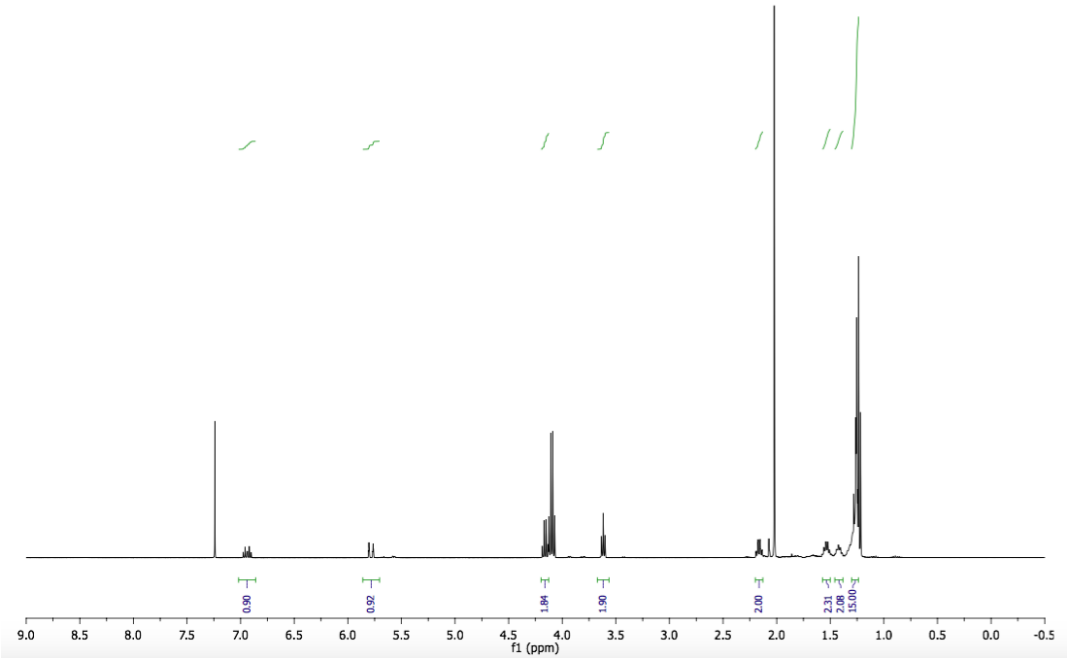


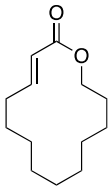
S22


0.90
2.00
2.16
2.04
2.01
2.39
1.98
2.18
11.74
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

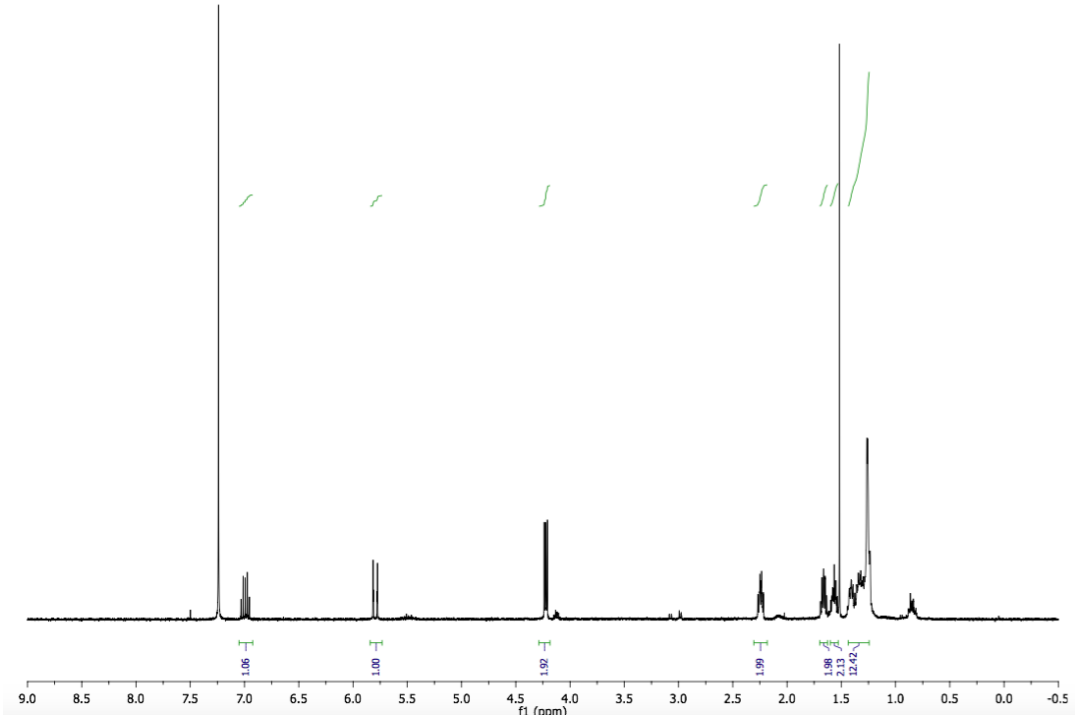

HO
O
O

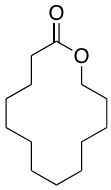
S23


0.90
0.92
1.84
1.90
2.00
2.31
2.08
15.00
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

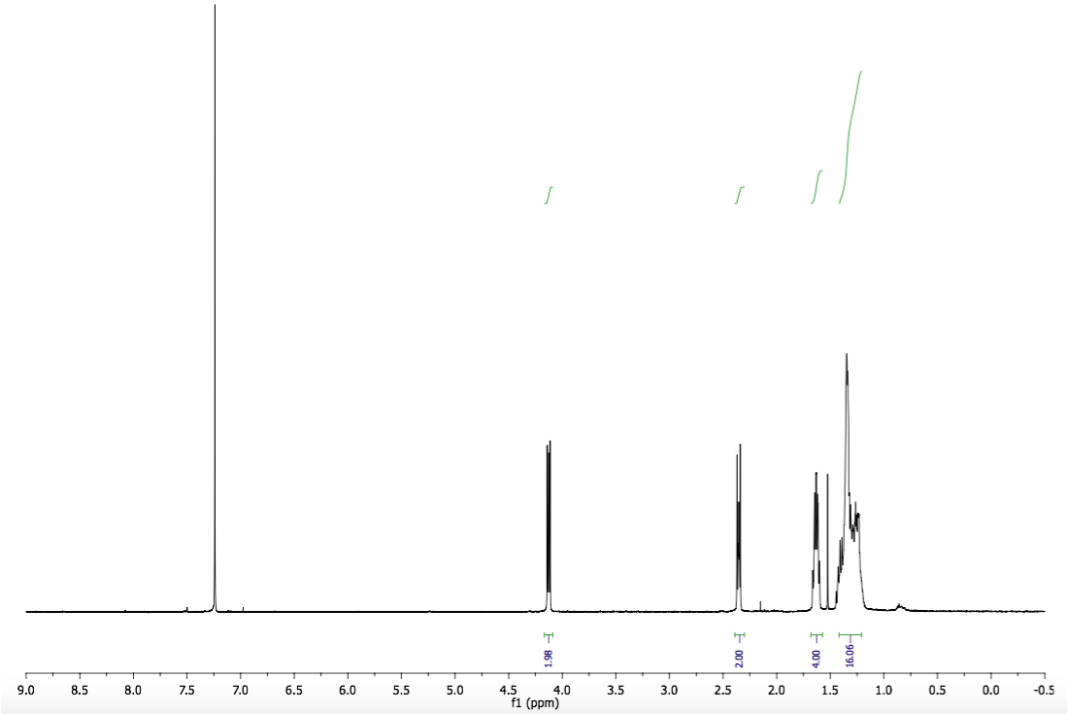

O
O

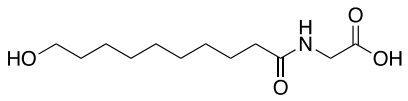
S24


1.06
1.00
1.92
1.99
1.98
2.13
12.42
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

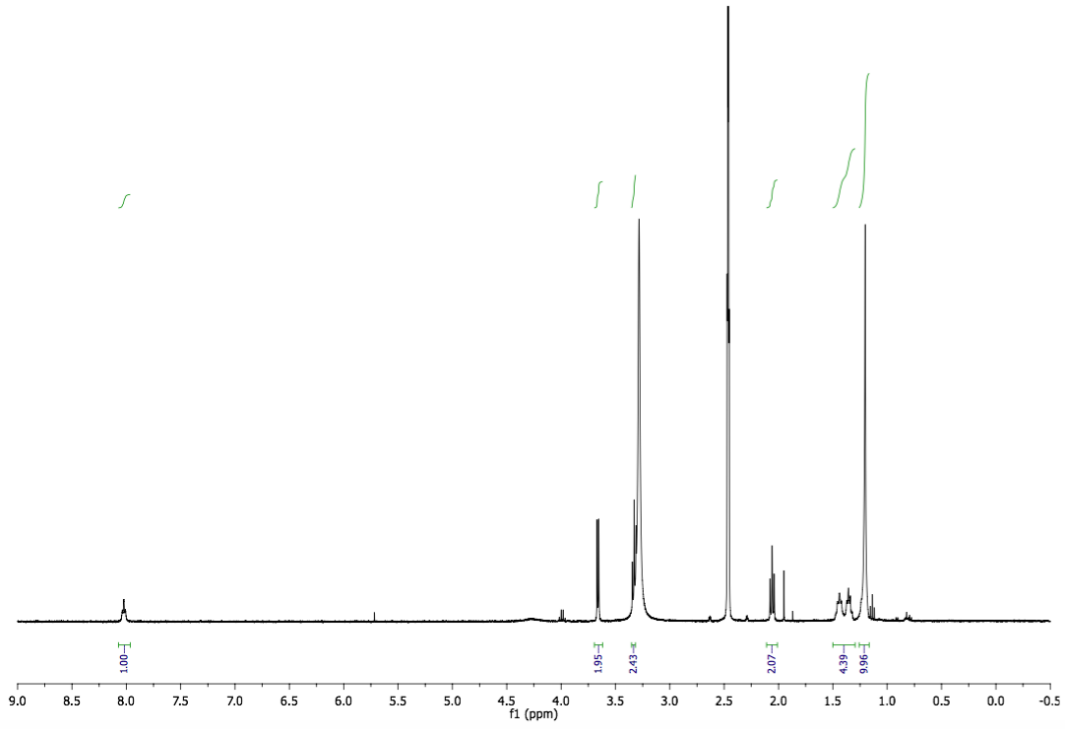

O
O

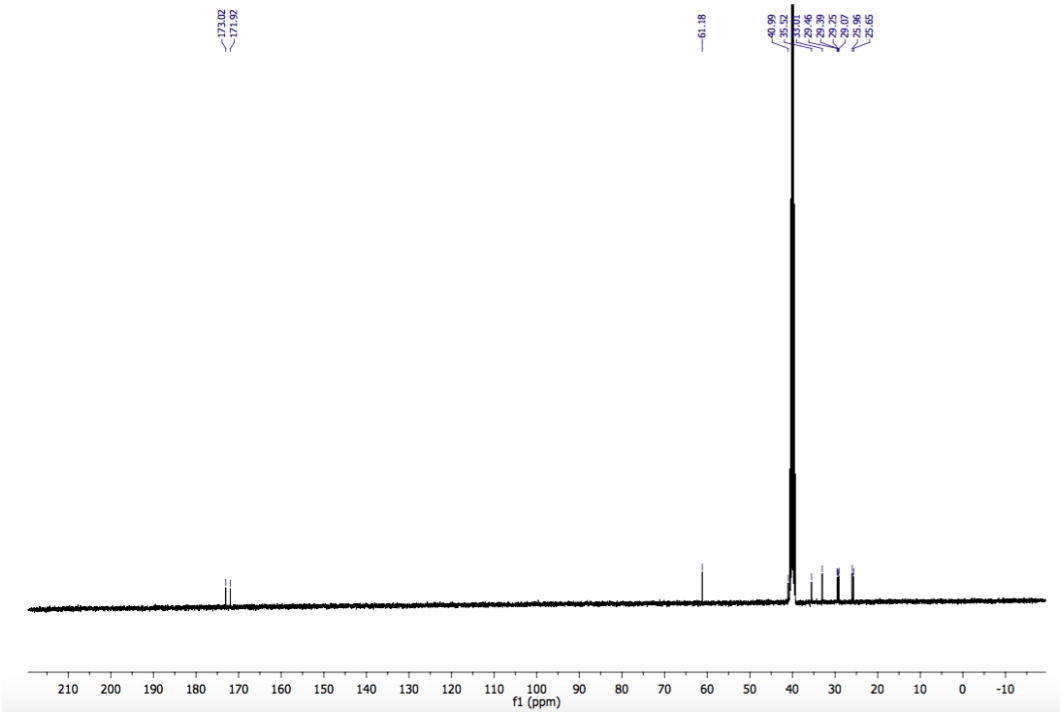
S25

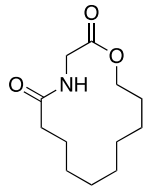

1.98
2.00
4.00
16.06
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)


HO
H
N
O
O
OH
O

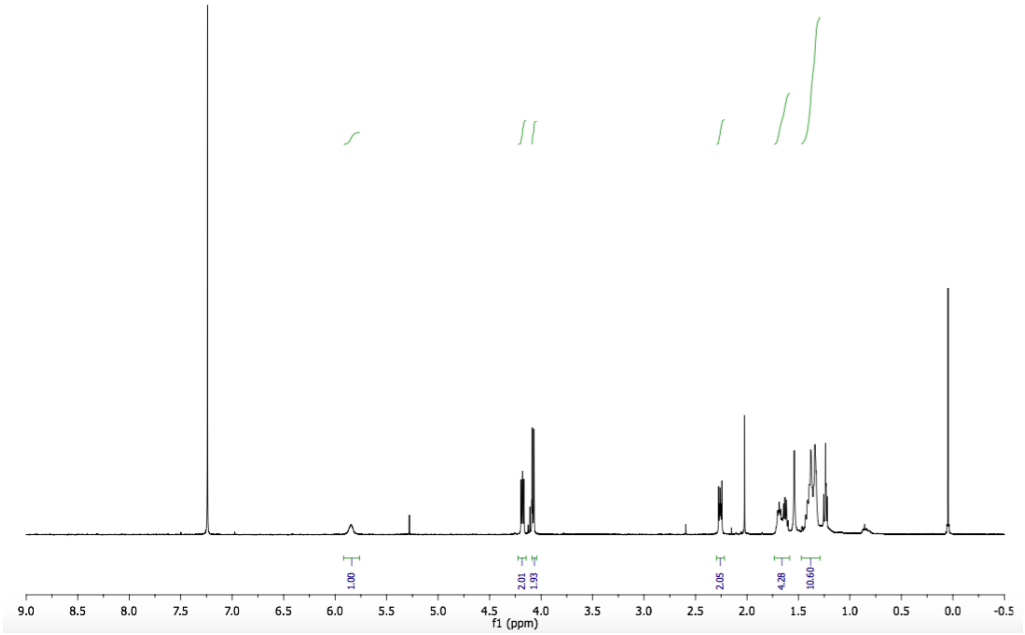
S26

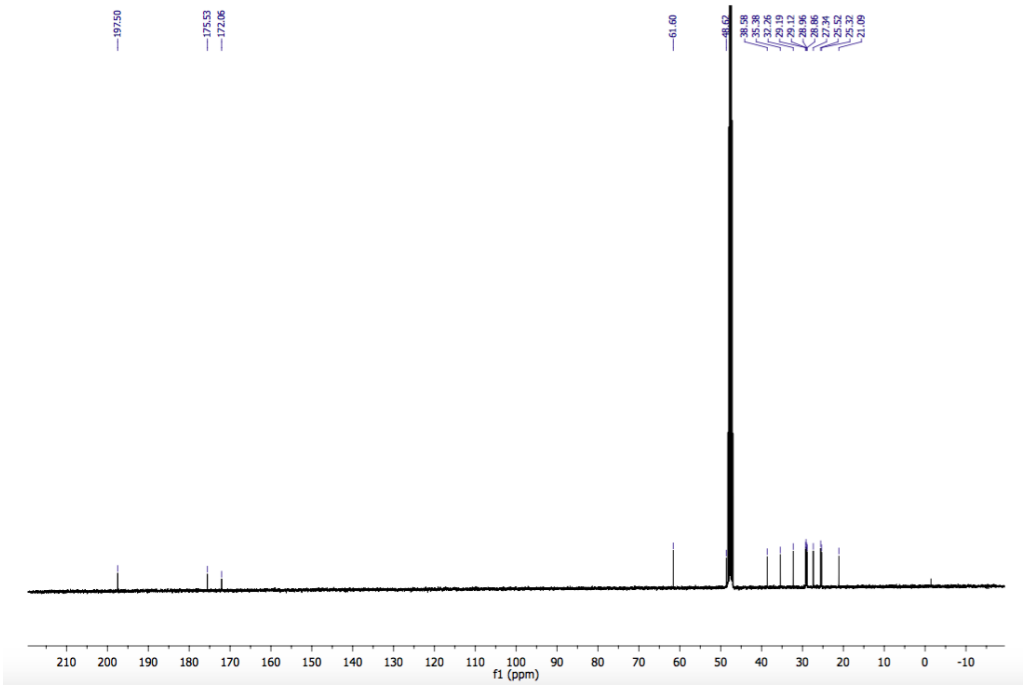

1.00
1.95
2.43
2.07
4.39
9.96
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


173.02
171.92
61.18
40.99
35.52
33.01
29.46
29.39
29.35
29.07
25.96
25.65
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


O
O
O
NH

24


1.00
2.01
1.93
2.05
4.28
10.60
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


197.50
175.53
172.06
61.60
46.62
38.58
35.38
32.26
29.19
29.12
28.96
28.86
27.34
25.52
25.32
21.09
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

Bibliography

1 G. W. Heberlig, M. Wirz, M. Wang and C. N. Boddy, *Org. Lett.*, 2014, **16**, 5858–5861.

2 M. Wang, H. Zhou, M. Wirz, Y. Tang and C. N. Boddy, *Biochemistry*, 2009, **48**, 6288–6290.

3 G. Kumaraswamy, K. Sadaiah and N. Raghu, *Tetrahedron: Asymmetry*, 2012, **23**, 587–593.

4 Y.-S. Hon, Y.-C. Wong, C.-P. Chang and C.-H. Hsieh, *Tetrahedron*, 2007, **63**, 11325–11340.

5 H. Yun, J. Sim, H. An, J. Lee, H. S. Lee, Y. K. Shin, S.-M. Paek and Y.-G. Suh, *Org. Biomol. Chem.*, 2014, **12**, 7127–35.

6 V. N. Tsarev, Y. Morioka, J. Caner, Q. Wang, R. Ushimaru, A. Kudo, H. Naka and S. Saito, *Org. Lett.*, 2015, **17**, 2530–2533.

7 J. M. Winter, D. Cascio, D. Dietrich, M. Sato, K. Watanabe, M. R. Sawaya, J. C. Vederas and Y. Tang, *J. Am. Chem. Soc.*, 2015, **137**, 9885–9893.

8 S. T. Kemme, T. Šmejkal and B. Breit, *Adv. Synth. Catal.*, 2008, **350**, 989–994.

9 B. M. Trost and M. Lautens, *J. Am. Chem. Soc.*, 1987, **109**, 1469–1478.

10 G. Makado, T. Morimoto, Y. Sugimoto, K. Tsutsumi, N. Kagawa and K. Kakiuchi, *Adv. Synth. Catal.*, 2010, **352**, 299–304.

11 J. Jose, G. Pourfallah, D. Merkley, S. Li, L. Bouzidi, A. L. Leao and S. S. Narine, *Polym. Chem.*, 2014, **5**, 3203–3213.

12 M. Wang, P. Opare and C. N. Boddy, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 1413–5.

13 I. G. Molnár and R. Gilmour, *J. Am. Chem. Soc.*, 2016, **138**, 5004–5007.

14 V. D. Kadam and G. Sudhakar, *Tetrahedron*, 2015, **71**, 1058–1067.

15 M. Nagarajan, V. S. Kumar and B. V. Rao, *Tetrahedron*, 1999, **55**, 12349–12360.