Resorcylic acid lactone biosynthesis relies on a stereo-tolerant macrocyclizing thioesterase

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This manuscript uses Fischer–Rosanoff convention (D and L notation) throughout. This absolute stereochemical nomenclature defines descriptors based on the orientation of a substituent on a directional chain, such as a carbohydrate backbone or polyketide chain. Fischer-Rosanoff priorities and descriptors do not change as the polyketide intermediates are elongated and tailored, as can often be the case with the Cahn-Ingold-Prelog (CIP) priority system. The titles of the stereo-enriched substrates (**8** and *ent*-**8**) will have both the CIP IUPAC name followed by the Fischer–Rosanoff name in the supporting information.

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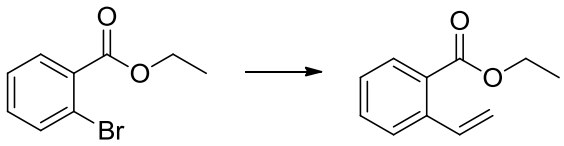
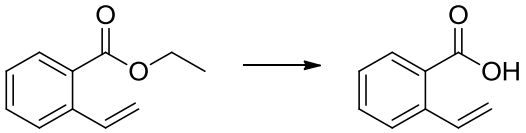
General synthetic protocols

All reagents were purchased from Sigma-Aldrich at highest available purity and used without further purification. Solvents were purchased from Fischer. All reactions were performed with dried solvents except where noted. NMR analysis was performed on a Bruker Avance II, operating at 400MHz for ^{1}H spectra, and 100MHz for ^{13}C spectra. High-resolution mass spectroscopy (HR-MS) was conducted on a Micromass Q-TOF I (John L. Holmes Mass Spectroscopy Facility). HPLC-MS analysis was conducted with a Shimadzu Prominence 20A Modular HPLC using a Hypersil 3µm C18 100mm reverse phase column coupled with an Applied Biosciences API 2000 Triple Quad in positive mode. Preparatory TLC was performed using Merck Millipore 20x20cm silica gel 60 F_{254} plates.

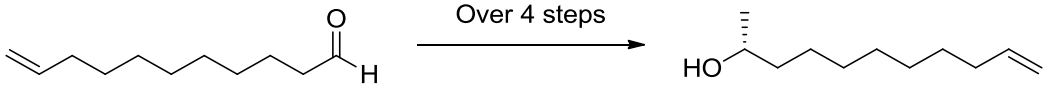
Synthetic Protocols



Ethyl 2-vinylbenzoate (S1). Based on procedure reported by Denmark et al[1]. To a 50 mL round bottom flask is added, under argon, 94 mg JohnPhos (0.15 mmol, 10 mol%), 42 mg palladium (II) bromide (0.15 mmol, 5 mol%), 7.0 mL tetrabutylammonium fluoride (1.0M in THF, 7.0 mmol) , and 545 mg 2,4,6,8-tetramethyl-2,4,6,8-tetravinylcyclotetrasiloxane (1.58 mmol), and the mixture is stirred for 10 minutes. 721 mg ethyl 2-bromobenzoate (3.15 mmol) is then added and the reaction was stirred for 8 hours at 50 ˚C. At completion the reaction was cooled to room temperature and 15 mL of ether was added and the reaction stirred for an additional 15 minutes. The biphasic mixture was passed through a plug of silica gel with 100 mL additional ether, the flow through was concentrated *in vacuo* and the product was purified by column chromatography (5% EtOAc:Hex) yielding 551 mg (>99%) of the product as a slightly yellow oil. Characterization is consistent with values reported in the literature.[1] R_f = 0.35 (silica gel, 9:1 hexanes/EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (dd, *J* = 7.8, 1.4 Hz, 1H), 7.60 – 7.56 (m, 1H), 7.51 – 7.42 (m, 2H), 7.32 (td, *J* = 7.7, 1.2 Hz, 1H), 5.65 (dd, *J* = 17.5, 1.3 Hz, 1H), 5.35 (dd, *J* = 11.0, 1.3 Hz, 1H), 4.37 (q, *J* = 7.1 Hz, 2H), 1.40 (t, *J* = 7.1 Hz, 3H).

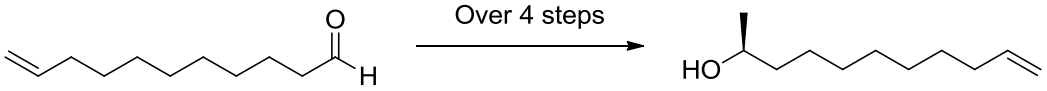


2-vinylbenzoic acid (S2). 550 mg of **S1** was dissolved in 35 mL of methanol to which was added 1.25g of lithium hydroxide hydrate which was previously dissolved in 12 mL of water. The reaction was stirred overnight at room temperature, the reaction was quenched by the addition of 10% HCl to pH 2, and extracted (3x30 mL) with EtOAc, the combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. Yielded 437 mg (95%) of the expected product as a white solid. Characterization is consistent with values reported in the literature.[2] R_f = 0.18 (silica gel, 1:1 hexanes/ EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.64 – 7.51 (m, 3H), 7.41 – 7.33 (m, 1H), 5.68 (dd, *J* = 17.4, 1.3 Hz, 1H), 5.39 (dd, *J* = 11.0, 1.3 Hz, 1H).

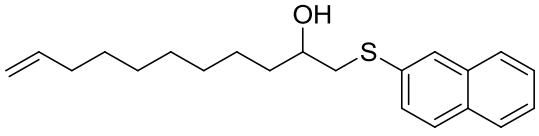


(*R*)-undec-10-en-2-ol (3). In a 100 mL round bottom flask 175 mg (2R,5S)-2-(tert-butyl)-3,5-dimethylimidazolidin-4-one hydrochloride (0.85 mmol, 20 mol%), 270 mg lithium chloride (6.37 mmol), 612 mg copper(II) trifluoroacetate (2.11 mmol), and 1.06g sodium persulfate (4.46 mmol) was combined

in 34 mL acetonitrile and 180 µL water and cooled to 4 ˚C. After stirring for 10 minutes 750 mg of undecylenic aldehyde (4.46 mmol) was added and the mixture stirred for 8 hours. After 4 hours the reaction was supplemented with an additional 0.5 eq of lithium chloride and sodium persulfate. After 8 hours the reaction was cooled to 0 ˚C on ice and 400 mg of sodium borohydride (10.6 mmol) was added and continued stirring for 10 minutes after which the reaction was warmed to room temperature. After reaching room temperature 16.5 mL of aq. KOH in EtOH (25g KOH dissolved in 50 mL water, added to 24 mL EtOH) was added to the reaction mixture and stirred for an additional 30 minutes, generating the intermediate epoxide. Reaction was then diluted with 90 mL water and extracted (3x100 mL) diethyl ether. The combined organic extracts were dried over Na_2SO_4 and carefully concentrated *in vacuo*, flash chromatography (5% EtOAc:Hex) was used to remove bulk impurities. Approximately 600 mg of partially purified epoxide product was dissolved in 35 mL THF and added to a dried 100 mL round bottom and chilled to 0 ˚C on ice. To this 17.83 mL of "Super Hydride" ($LiEt_3BH$) (1.0M in THF, 5eq) was slowly added and the reaction allowed to stir for 1 hour at 0 ˚C. After 1 hour the reaction was quenched by ***slow*** addition of 18 mL of water. This was extracted (3x25 mL) with DCM, the combined organic extracts were combined, dried over Na_2SO_4, and concentrated *in vacuo*. Purified by column chromatography 40% EtOAc:Hex yielding 369 mg (48%) of product as a colorless oil. Characterization is consistent with values reported in the literature.[3] R_f = 0.35 (silica gel, 4:1 hexanes/EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.79 (ddt, *J* = 16.9, 10.1, 6.7 Hz, 1H), 4.97 (ddd, *J* = 17.1, 3.6, 1.6 Hz, 1H), 4.93 – 4.88 (m, 1H), 3.82 – 3.72 (m, 1H), 2.02 (dd, *J* = 14.3, 6.8 Hz, 2H), 1.44 – 1.26 (m, 14H), 1.17 (d, *J* = 6.2 Hz, 3H).

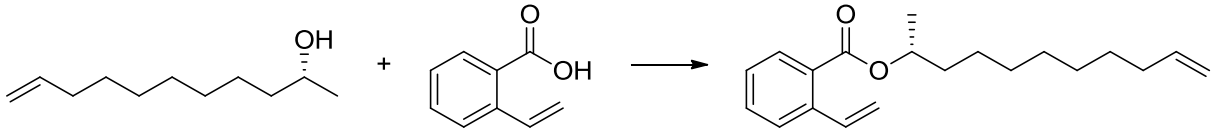


(*S*)-undec-10-en-2-ol (4). As described above for **3**, with the following exception: (2S,5R)-2-(tert-butyl)-3,5-dimethylimidazolidin-4-one hydrochloride is used as the catalyst. Yielded 404 mg (53%) as a colorless oil. Characterization is consistent with values reported in the literature.[3] R_f = 0.35 (silica gel, 4:1 hexanes/EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.79 (ddt, *J* = 16.9, 10.1, 6.7 Hz, 1H), 4.97 (ddd, *J* = 17.1, 3.6, 1.6 Hz, 1H), 4.93 – 4.88 (m, 1H), 3.82 – 3.72 (m, 1H), 2.02 (dd, *J* = 14.3, 6.8 Hz, 2H), 1.44 – 1.26 (m, 14H), 1.17 (d, *J* = 6.2 Hz, 3H).

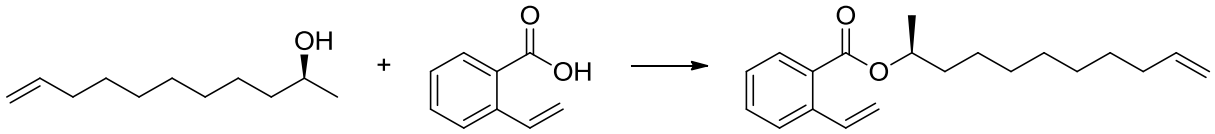


Analysis of enantiopurity. To determine the level of stereo control approximately 5 mg (0.03 mmol) of the partially purified epoxide intermediate was added to 5 mg 2-naphthalenethiol (0.031 mmol) and 3.75 mg triethylamine (0.037 mmol) in 200 µL methanol cooled to 0 ˚C on ice. The resulting mixture was stirred at room temp for 16 hours, the reaction was concentrated *in vacuo* and purified by preparatory TLC (1:4 EtOAc:Hexanes), the UV active band was removed and eluted with EtOAc. Stereochemical

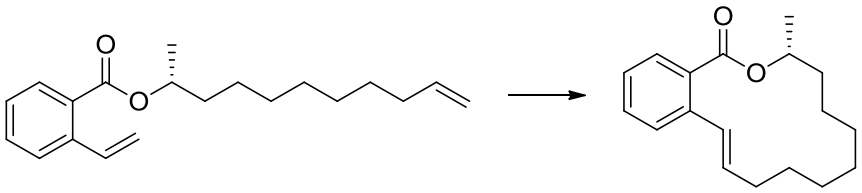
purity was determined by analyzing the UV active band by chiral HPLC, using a Chiracel OD-H column with isocratic mobile phase (5% isopropanol/Hexanes) at 1 mL/min; detection at 220 and 254nm. 94%*ee* for **3** and **4** determined by area under curve. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86 – 7.74 (m, 4H), 7.54 – 7.43 (m, 3H), 5.81 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 1H), 5.05 – 4.90 (m, 2H), 3.80 – 3.67 (m, 1H), 3.27 (dd, *J* = 13.7, 3.4 Hz, 1H), 2.95 (dd, *J* = 13.7, 8.7 Hz, 1H), 2.09 – 1.98 (m, 3H), 1.59 – 1.27 (m, 12H). See **Supplementary Figure 1** for traces.



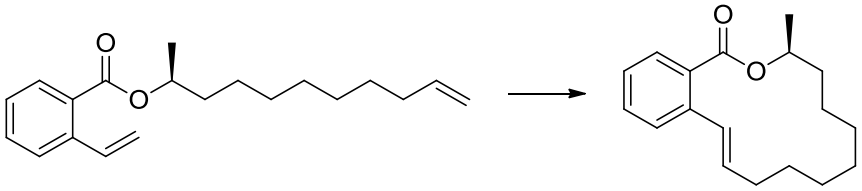
(*R*)-undec-10-en-2-yl 2-vinylbenzoate (5). In a 10 mL round bottom flask charged with 5 mL dry DCM was added 150 mg **3** (0.88 mmol), 196 mg 2-vinylbenzoic acid (1.36 mmol), 253 mg 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (1.32 mmol), and 161 mg 4-(Dimethylamino)pyridine (DMAP) (1.32 mmol). The reaction was stirred under N_2 overnight, the reaction was then quenched by the addition of 5 mL sat. aq. NH_4Cl, extracted (3x15 mL) DCM, the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography 10% EtOAc:Hexanes, yielded 203 mg product as colorless oil (81%). R_f = 0.52 (silica gel, 9:1 Hexanes:EtOAc). IR (NaCl) vmax = 2926, 2855, 1726, 1245, 1126 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, *J* = 7.7 Hz, 1H), 7.56 (d, *J* = 7.3 Hz, 1H), 7.48 – 7.39 (m, 2H), 7.30 (t, *J* = 7.7 Hz, 1H), 5.78 (dq, *J* = 10.2, 6.6 Hz, 1H), 5.62 (d, *J* = 17.4 Hz, 1H), 5.32 (d, *J* = 11.0 Hz, 1H), 5.13 (dd, *J* = 12.8, 6.3 Hz, 1H), 4.96 (d, *J* = 17.1 Hz, 1H), 4.90 (d, *J* = 10.8 Hz, 1H), 2.01 (dd, *J* = 14.0, 7.1 Hz, 2H), 1.77– 1.25 (m, 15H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.12, 139.37, 139.18, 135.97, 131.80, 130.06, 129.54, 127.35, 127.14, 116.20, 114.16, 71.94, 36.03, 33.78, 29.43, 29.37, 29.03, 28.89, 25.46, 20.05. HRMS (+ESI) Calculated for $C_{20}H_{28}O_2Na$ (M) 323.1987, observed 323.1985.



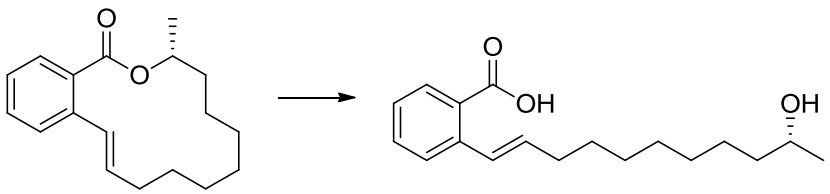
(*S*)-undec-10-en-2-yl 2-vinylbenzoate (*ent*-5). As described above for **5.** Yielded 177 mg product as a colorless oil (71%). %). R_f = 0.52 (silica gel, 9:1 Hexanes:EtOAc). IR (NaCl) vmax = 2926, 2855, 1726, 1245, 1126 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, *J* = 7.7 Hz, 1H), 7.56 (d, *J* = 7.3 Hz, 1H), 7.48 – 7.39 (m, 2H), 7.30 (t, *J* = 7.7 Hz, 1H), 5.78 (dq, *J* = 10.2, 6.6 Hz, 1H), 5.62 (d, *J* = 17.4 Hz, 1H), 5.32 (d, *J* = 11.0 Hz, 1H), 5.13 (dd, *J* = 12.8, 6.3 Hz, 1H), 4.96 (d, *J* = 17.1 Hz, 1H), 4.90 (d, *J* = 10.8 Hz, 1H), 2.01 (dd, *J* = 14.0, 7.1 Hz, 2H), 1.80 – 1.25 (m, 15H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.12, 139.37, 139.18, 135.97, 131.80, 130.06, 129.54, 127.35, 127.14, 116.20, 114.16, 71.94, 36.03, 33.78, 29.43, 29.37, 29.03, 28.89, 25.46, 20.05. HRMS (+ESI) Calculated for $C_{20}H_{28}O_2Na$ 323.1987 M, observed 323.2044.



(*R*,E)-3-methyl-3,4,5,6,7,8,9,10-octahydro-1H-benzo[c]-1-oxacyclotetradecin-1-one (6). 120 mg **5** (0.40 mmol) was dissolved in 100 mL dry toluene and placed in a flame dried 250 mL round bottom flask equipped with a stir bar. 17 mg of Grubbs 2nd Generation catalyst (0.02 mmol, 5mol%) was added with stirring and the reaction was heated to 80 ˚C and continued stirring for 24 hours under an N_2 atmosphere. At completion the reaction was concentrated *in vacuo* and partially purified by column chromatography (silica, 9:1 Hexanes:EtOAc). Yields 203 mg product as colorless oil. As the R_f of the product and the starting material were so similar the product was isolated as a mix with the starting material. This mixture was taken without further purification into the next step. R_f = 0.49 (silica gel, 9:1 Hexanes:EtOAc). ^{1}H NMR (400 MHz, CDCl3) δ 7.80 (dd, *J* = 7.8, 1.3 Hz, 1H), 7.52 – 7.46 (m, 1H), 7.41 (td, *J* = 7.7, 1.2 Hz, 1H), 7.30 – 7.24 (m, 1H), 7.00 (d, *J* = 15.7 Hz, 1H), 5.91 (dt, *J* = 15.6, 7.2 Hz, 1H), 5.22 (dt, *J* = 12.2, 6.1 Hz, 1H), 2.30 (td, *J* = 7.1, 1.3 Hz, 2H), 1.71 – 1.65 (m, 2H), 1.53 (dt, *J* = 10.3, 5.1 Hz, 2H), 1.39 – 1.26 (m, 11H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.83, 138.14, 133.72, 131.40, 130.18, 130.13, 130.04, 127.30, 126.63, 72.37, 34.61, 30.70, 26.90, 26.51, 23.97, 23.83, 21.75, 20.13

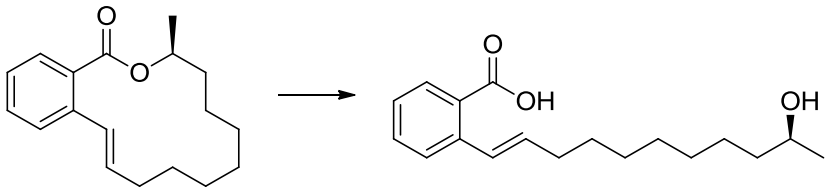


(*S*,E)-3-methyl-3,4,5,6,7,8,9,10-octahydro-1H-benzo[c]-1-oxacyclotetradecin-1-one (*ent*-6). As described above for **6**. Yielded 177 mg of product as a colorless oil. R_f = 0.49 (silica gel, 9:1 Hexanes:EtOAc). ^{1}H NMR (400 MHz, CDCl3) δ 7.80 (dd, *J* = 7.8, 1.3 Hz, 1H), 7.52 – 7.46 (m, 1H), 7.41 (td, *J* = 7.7, 1.2 Hz, 1H), 7.30 – 7.24 (m, 1H), 7.00 (d, *J* = 15.7 Hz, 1H), 5.91 (dt, *J* = 15.6, 7.2 Hz, 1H), 5.22 (dt, *J* = 12.2, 6.1 Hz, 1H), 2.30 (td, *J* = 7.1, 1.3 Hz, 2H), 1.71 – 1.65 (m, 2H), 1.53 (dt, *J* = 10.3, 5.1 Hz, 2H), 1.39 – 1.26 (m, 11H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.83, 138.14, 133.72, 131.40, 130.18, 130.13, 130.04, 127.30, 126.63, 72.37, 34.61, 30.70, 26.90, 26.51, 23.97, 23.83, 21.75, 20.13

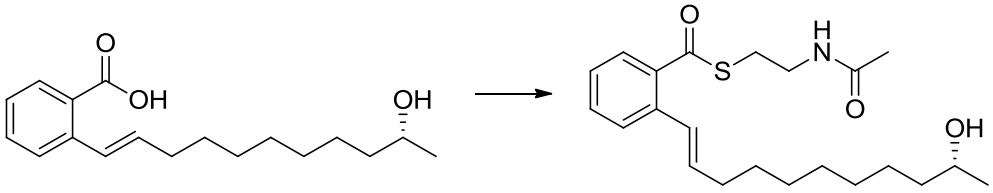


(*R*,E)-2-(10-hydroxyundec-1-en-1-yl)benzoic acid (7). 80 mg of **6** was dissolved in 1 mL of THF and to this was added 176 mg NaOH (15eq.) that was previously dissolved in 1 mL of water forming a biphasic system. Methanol and THF were added drop wise until a homogeneous solution was obtained, this was

refluxed at 76 ˚C for 12 hours. The reaction was quenched by the addition of 1M HCl to a pH of 2. This was extracted (3x10 mL) with EtOAc, the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography 1:3 EtOAc:Hexanes supplemented with a drop of glacial acetic acid per 250 mL of running solvent. Yielded 55 mg product as a colorless oil (47% over two steps). R_f = 0.55 (1:1 EtOAc:Hexanes). IR (NaCl) vmax = 3372 (br), 2920, 2855, 1707, 1249, 1090 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.93 (dd, *J* = 7.8, 1.0 Hz, 1H), 7.51 (d, *J* = 6.5 Hz, 1H), 7.47 – 7.42 (m, 1H), 7.27 (dd, *J* = 11.0, 4.5 Hz, 1H), 7.18 (d, *J* = 15.7 Hz, 1H), 6.06 (dt, *J* = 15.7, 7.0 Hz, 1H), 3.84 (dd, *J* = 11.6, 5.4 Hz, 1H), 2.24 (qd, *J* = 6.9, 1.4 Hz, 2H), 1.50 – 1.30 (m, 12H), 1.19 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.21, 140.41, 134.15, 132.50, 130.94, 129.11, 127.57, 127.37, 126.53, 68.54, 39.01, 32.69, 29.39, 29.01, 28.67, 28.33, 25.35, 23.51. HRMS (-ESI) Calculated for $C_{18}H_{25}O_3$ 289.1804, found 289.1808.

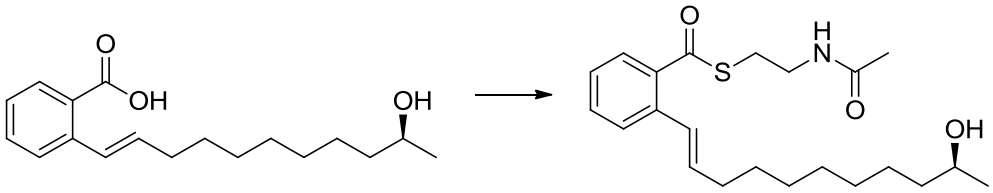


(*S*,E)-2-(10-hydroxyundec-1-en-1-yl)benzoic acid (*ent*-7). Prepared as for **7** starting with 40 mg of **ent-6** (0.147 mmol) and 88 mg of NaOH, dissolved in 700 µL THF. Yielded 26 mg of product as a colorless oil (23% over two steps). R_f = 0.55 (1:1 EtOAc:Hexanes). IR (NaCl) vmax = 3372 (br), 2920, 2855, 1707, 1249, 1090 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.93 (dd, *J* = 7.8, 1.0 Hz, 1H), 7.51 (d, *J* = 6.5 Hz, 1H), 7.47 – 7.42 (m, 1H), 7.27 (dd, *J* = 11.0, 4.5 Hz, 1H), 7.18 (d, *J* = 15.7 Hz, 1H), 6.06 (dt, *J* = 15.7, 7.0 Hz, 1H), 3.84 (dd, *J* = 11.6, 5.4 Hz, 1H), 2.24 (qd, *J* = 6.9, 1.4 Hz, 2H), 1.50 – 1.30 (m, 12H), 1.19 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.21, 140.41, 134.15, 132.50, 130.94, 129.11, 127.57, 127.37, 126.53, 68.54, 39.01, 32.69, 29.39, 29.01, 28.67, 28.33, 25.35, 23.51. HRMS (-ESI) Calculated for $C_{18}H_{25}O_3$ 289.1804, found 289.1813.



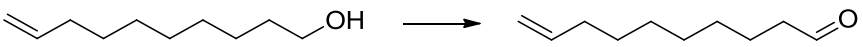
(*R*,E)-S-(2-acetamidoethyl) 2-(10-hydroxyundec-1-en-1-yl)benzothioate (8). D-(E)-S-(2-acetamidoethyl) 2-(10-hydroxyundec-1-en-1-yl)benzothioate. 46 mg of **7** (0.158 mmol) was dissolved in 400 µL DCM in a 2 mL round bottom flask equipped with a stir bar. 24.5 mg *N*-acetylcysteamine (22 µL, 0.206 mmol) and 39.5 mg EDC (0.206 mmol). The reaction was stirred at room temperature for 4 hours. The reaction was quenched with the addition of 0.5 mL of sat. aq. NH_4Cl and extracted with 3x2 mL portions of DCM. The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The compound was purified by preparatory TLC (40% Acetone:Hexanes), the product band was cut and eluted into a clean vial using DCM and acetone. Yielded the expected product as colorless oil, 30 mg (49%). R_f = 0.19, 2:3

Acetone:Hexanes; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (dd, *J* = 7.8, 1.1 Hz, 1H), 7.54 (d, *J* = 7.6 Hz, 1H), 7.43 (dd, *J* = 10.9, 4.3 Hz, 1H), 7.28 – 7.21 (m, 1H), 6.76 (d, *J* = 15.7 Hz, 1H), 6.16 (dt, *J* = 15.7, 7.0 Hz, 1H), 6.06 (s, 1H), 3.77 (dt, *J* = 12.5, 6.2 Hz, 1H), 3.52 (dd, *J* = 12.4, 6.3 Hz, 2H), 3.18 (t, *J* = 6.5 Hz, 2H), 2.24 – 2.15 (m, 2H), 1.97 (s, 3H), 1.48 – 1.28 (m, 12H), 1.17 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.81, 170.38, 136.66, 135.74, 134.85, 131.98, 128.44, 127.15, 126.99, 126.64, 68.17, 39.78, 39.36, 33.12, 29.56, 29.36, 29.30, 29.06, 28.99, 25.70, 23.51, 23.23. Low Resolution Mass Spectroscopy (LRMS) (+ESI) found 392.3 (M); HRMS (+ESI) calculated for $C_{22}H_{33}NO_3SNa$ 414.2079, found 414.2047. HPLC retention time: 20.45min using method described below.



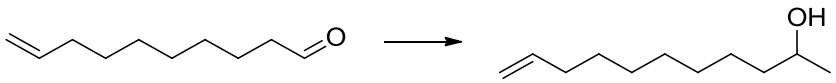
(*S*,E)-S-(2-acetamidoethyl) 2-(10-hydroxyundec-1-en-1-yl)benzothioate (*ent*-8). L-(E)-S-(2-acetamidoethyl) 2-(10-hydroxyundec-1-en-1-yl)benzothioate. Prepared as described above for **8** starting with 15 mg of *ent*-**7** (0.052 mmol) dissolved in 150 µL of DCM and combined with 8 mg *N*-acetylcysteamine (7.1 µL, 0.067 mmol) and 13 mg EDC (0.067 mmol) in a 2 mL pear-shape flask fitted with a stir bar. Yielded product as a colorless oil, 9 mg (44%). R_f = 0.19, 2:3 Acetone:Hexanes; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (dd, *J* = 7.8, 1.1 Hz, 1H), 7.54 (d, *J* = 7.6 Hz, 1H), 7.43 (dd, *J* = 10.9, 4.3 Hz, 1H), 7.28 – 7.21 (m, 1H), 6.76 (d, *J* = 15.7 Hz, 1H), 6.16 (dt, *J* = 15.7, 7.0 Hz, 1H), 6.06 (s, 1H), 3.77 (dt, *J* = 12.5, 6.2 Hz, 1H), 3.52 (dd, *J* = 12.4, 6.3 Hz, 2H), 3.18 (t, *J* = 6.5 Hz, 2H), 2.24 – 2.15 (m, 2H), 1.97 (s, 3H), 1.48 – 1.28 (m, 12H), 1.17 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.81, 170.38, 136.66, 135.74, 134.85, 131.98, 128.44, 127.15, 126.99, 126.64, 68.17, 39.78, 39.36, 33.12, 29.56, 29.36, 29.30, 29.06, 28.99, 25.70, 23.51, 23.23. LRMS (+ESI) found 392.3 HRMS (+ESI) calculated for $C_{22}H_{33}NO_3SNa$ 414.2079, found 414.2101. HPLC retention time: 20.45min using method described below.

Racemic Macrocycle Synthesis

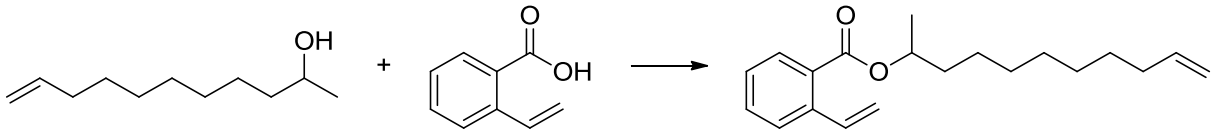


Dec-9-enal (S3). To a dry 100 mL round bottom charged with 35 mL dry DCM was added 1.0g 9-decenol (6.4 mmol) and 3.45g pyridinium chlorochromate (PCC) (16.0 mmol, 2.5eq) and an equal mass of silica gel. The mixture was stirred for 3hours after which the reaction was concentrated *in vacuo* and the resulting black power was applied directly to the top of silica gel column and was purified with 95:5 Hexanes:EtOAc. Yielded 945 mg of the title compound as a colorless oil (96%). Characterization was consistent with reported values in the literature.[4] R_f=0.55 (EtOAc:Hexanes 1:4) ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.76 (t, *J* = 1.9 Hz, 1H), 5.80 (ddt, *J* = 17.0, 10.2, 6.7 Hz, 1H), 4.99 (ddd, *J* = 17.1, 3.6, 1.6 Hz, 1H), 4.96 – 4.91 (m, 1H), 2.42 (td, *J* = 7.4, 1.9 Hz, 2H), 2.04 (dd, *J* = 14.2, 6.9 Hz, 2H), 1.62 (dd, *J* = 14.6, 7.3 Hz, 2H),

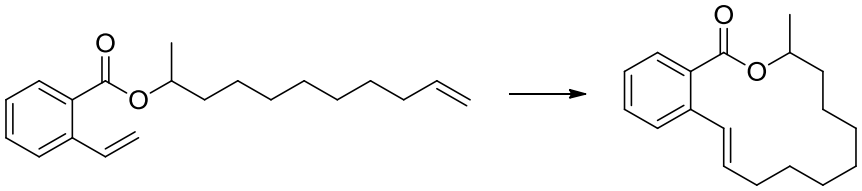
1.41 – 1.30 (m, 8H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 202.90, 139.08, 114.24, 43.91, 33.74, 29.19, 29.11, 28.88, 28.82, 22.06.



Undec-10-en-2-ol (S4). A 50 mL flame dried round bottom was charged with 13 mL dry ether and a stir bar. This was cooled to -78 ˚C (dry ice/acetone) and 2.16 mL of a 1.5M methyllithium (MeLi) solution (3.24 mmol, 1eq) was added and stirred for several minutes. With stirring, 500 mg of **S3** (3.24 mmol) was added dropwise, after full addition the reaction was allowed to slowly warm to room temperature, and was quenched with 10 mL 10% HCl. The reaction mixture was extracted 3x10 mL with ether, the combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*, then the tile compound was purified using column chromatography (2:3 EtOAc:Hexanes) yielding 390 mg product as a colorless oil (77%). Characterization was consistent with **3** and **4**. R_f = 0.35 (silica gel, 4:1 hexanes/EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.79 (ddt, *J* = 16.9, 10.1, 6.7 Hz, 1H), 4.97 (ddd, *J* = 17.1, 3.6, 1.6 Hz, 1H), 4.93 – 4.88 (m, 1H), 3.82 – 3.72 (m, 1H), 2.02 (dd, *J* = 14.3, 6.8 Hz, 2H), 1.44 – 1.26 (m, 14H), 1.17 (d, *J* = 6.2 Hz, 3H).



Undec-10-en-2-yl 2-vinylbenzoate (*racemic***-5)**. As described above for **5**, yielded 175 mg of the title compound as a colorless oil (66%). Characterization was consistent with **5**. R_f = 0.52 (silica gel, 9:1 Hexanes:EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, *J* = 7.7 Hz, 1H), 7.56 (d, *J* = 7.3 Hz, 1H), 7.48 – 7.39 (m, 2H), 7.30 (t, *J* = 7.7 Hz, 1H), 5.78 (dq, *J* = 10.2, 6.6 Hz, 1H), 5.62 (d, *J* = 17.4 Hz, 1H), 5.32 (d, *J* = 11.0 Hz, 1H), 5.13 (dd, *J* = 12.8, 6.3 Hz, 1H), 4.96 (d, *J* = 17.1 Hz, 1H), 4.90 (d, *J* = 10.8 Hz, 1H), 2.01 (dd, *J* = 14.0, 7.1 Hz, 2H), 1.77– 1.25 (m, 15H).



(E)-3-methyl-3,4,5,6,7,8,9,10-octahydro-1H-benzo[c]-1-oxacyclotetradecin-1-one (*racemic***-6).** As described above for **6**. Starting with 160 mg **racemic 5** (0.533 mmol), and 22.6 mg Grubbs 2nd Gen catalyst (0.027, 5mol%) in 133 mL dry toluene. yielded 24 mg product as a colorless oil (17%). Characterization is consistent with **6**. R_f = 0.49 (silica gel, 9:1 Hexanes:EtOAc). ^{1}H NMR (400 MHz, CDCl3) δ 7.80 (dd, *J* = 7.8, 1.3 Hz, 1H), 7.52 – 7.46 (m, 1H), 7.41 (td, *J* = 7.7, 1.2 Hz, 1H), 7.30 – 7.24 (m, 1H), 7.00 (d, *J* = 15.7 Hz, 1H), 5.91 (dt, *J* = 15.6, 7.2 Hz, 1H), 5.22 (dt, *J* = 12.2, 6.1 Hz, 1H), 2.30 (td, *J* = 7.1, 1.3 Hz, 2H), 1.71 – 1.65 (m, 2H), 1.53 (dt, *J* = 10.3, 5.1 Hz, 2H), 1.39 – 1.26 (m, 11H).

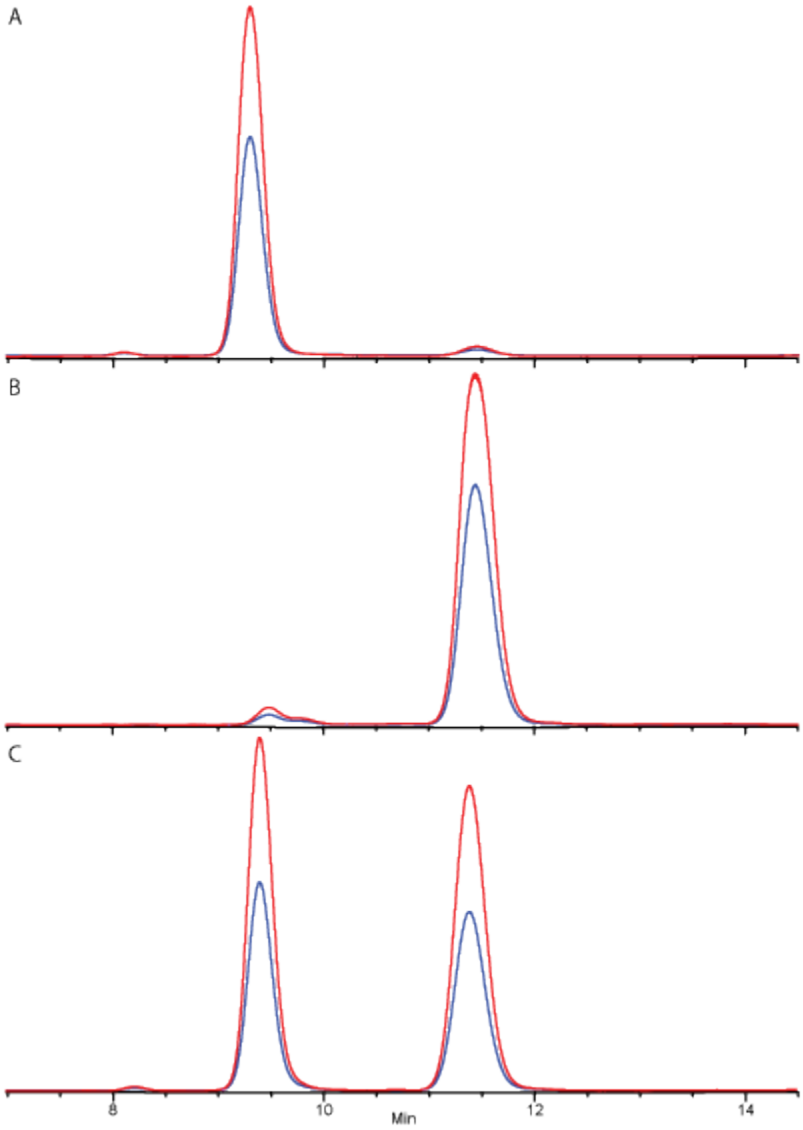


Figure S1. Chiral HPLC traces of **A:** 2-naphthalenethiol derivative of **3; B:** 2-naphthalenethiol derivative of **4**; **C:** Racemic standard. The blue line is the trace for 254 nm and the red is 220 nm.

Enzymatic Assays

Expression and purification of Rad TE.

Rad TE was amplified from pKJ31[5] by PCR (forward primer 5'-TCATACATATGCAGTTGACGCCCATGTT-3'; reverse primer 5'-TACCGGAATTCCGTCCAAAGTGCTCAA-3') and cloned into NdeI, EcoRI linearized pET28b, generating pMW29. pMW29 was confirmed by sequencing (Genome Quebec, Montreal QC).

The Expression vector encoding Rad TE (pMW29) was transformed into chemical competent *E. coli* BL21(DE3) for protein expression. 400 mL of standard Luria-Bertani media supplemented with 50 µg/mL of kanamycin was inoculated with 0.5 % *v*/*v* of a dense overnight culture of *E. coli* BL21(DE3)/pMW29 and the culture was grown at 37 °C to OD600 of 0.5. Protein expression was induced by adding isopropyl thiogalactoside (IPTG) to a final concentration of 0.1 mM. The culture was incubated at 20 °C with shaking at 200 rpm for 12 h.

All protein purification procedures were performed at 4 °C. The cells were harvested by centrifugation at 4000g and resuspended in 10 mL of lysis buffer (100 mM sodium phosphate, 300 mM NaCl, 10% (*v*/*v*) glycerol, 1 mg/mL lysozyme, 1µg/mL pepstatin A, 1µg/mL leupeptin, pH 8.0). The cells were disrupted by sonication on ice and cell debris was removed by centrifugation at 15000 g at 4 °C. After adding imidazole to a final concentration of 10 mM, the cleared lysate was incubated for 1 h with 400 µL of nickel-nitrotriacetic acid (Ni-NTA) resin (QIAGEN, Valenica, CA) and loaded onto a column. The resin was first washed with wash buffer (100 mM Tris, 300 mM NaCl, 20 mM imidazole, pH 8.0) and the protein was eluted with wash buffer supplemented with 250 mM imidazole. The purified protein was exchanged into dialysis buffer (100 mM Tris, 300 mM NaCl, pH 7.43) and concentrated by centrifugation (Amicon 5000 MWCO). The concentrated protein was flash frozen and stored at –78 °C. Protein concentration was determined by the Bradford assay (Bio-rad). Approximately 5.4 mg of purified protein was obtained per L of cell culture.

Figure S2. SDS-PAGE analysis of purified recombinant Rad TE and Zea TE. Expected molecular weights are 47kDa and 45kDa. Left to Right: Fischer Rec-Protein ladder (50kDa reference band) , Rad TE, Zea TE.

Kinetic Analysis of Macrocycle Formation

Discontinuous enzymatic assays were carried out in 50mM phosphate buffer (pH7.4), with 5µM Zea TE (both substrates) and Rad TE for the L-substrate (*ent*-**8**) and 2 µM for Rad TE with the D-substrate (**8**). Substrate concentrations between 0.1mM and 5mM (from 10mM or 50mM stock solutions in DMSO), and DMSO up to 10% solution volume if necessary. Assays were quenched with an equal volume of 0.5% formic acid in acetonitrile before analysis by HPLC. Analysis was performed using a BDS Hypersil C18 100x2.1mm reverse phase column, using a gradient elution of 0 to 100% B over 30 minutes (A: 95% H_2O,

5% MeCN, 0.05% formic acid; B: 95% MeCN, 5% H_2O, 0.05% formic acid). Amount of macrocycle produced was determined by comparison to a standard curve of authentic macrocycle. Non-linear regression analysis was performed in GraphPad Prism 5.

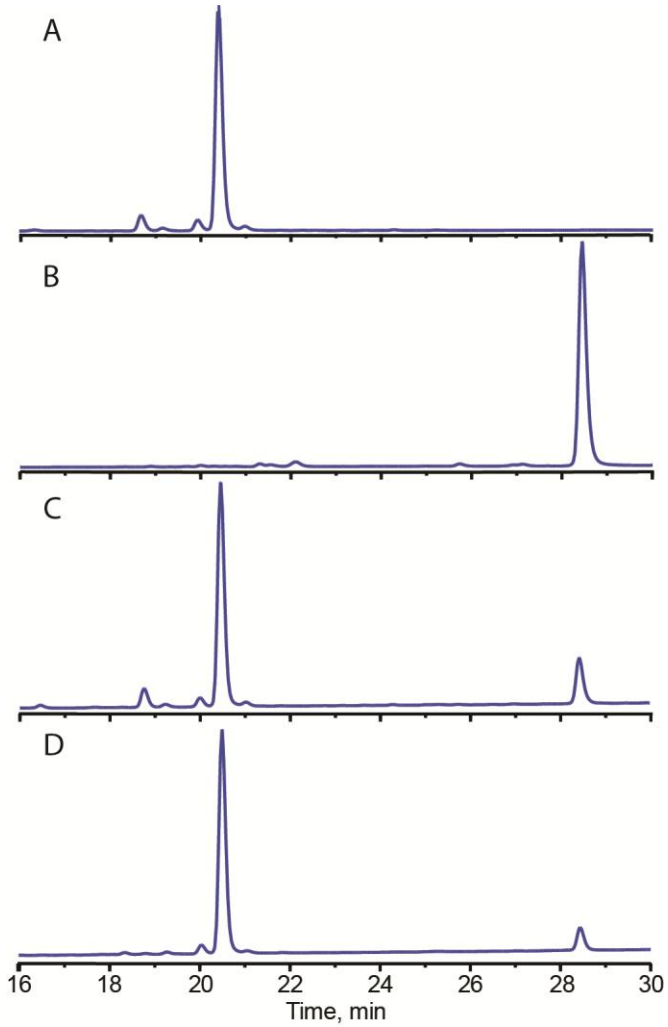
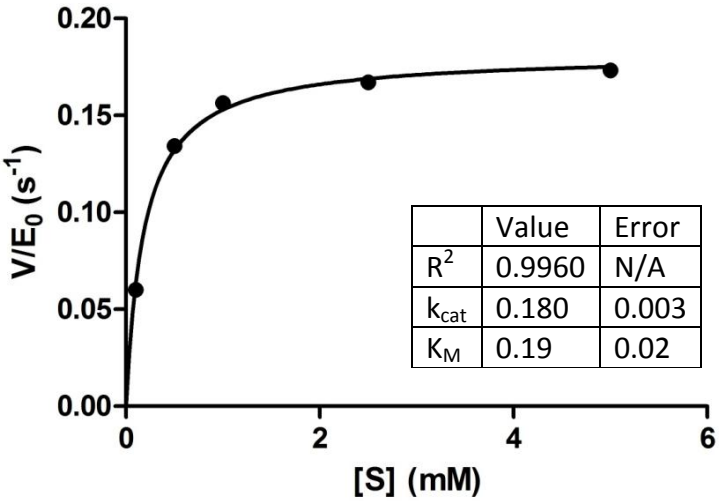


Figure S3. HPLC traces for incubations of **8** and *ent*-**8** with ZeaTE. A, no enzyme blank (5 mM **8**, 50 mM phosphate buffer pH 7.4, 24 h, rt). B, Racemic macrocycle, *racemic*-**6** . C, **8** with Zea TE (3 mM **8**, 5 µM Zea TE, 50 mM phosphate buffer pH 7.4, 0.5 h, rt). D *ent*-**8** with Zea TE (3 mM *ent*-**8**, 5 µM Zea TE, 50 mM phosphate buffer, pH 7.4, 0.5 h, rt). Small peak at 20min is the hydrolysis product.

Fitting of rate data

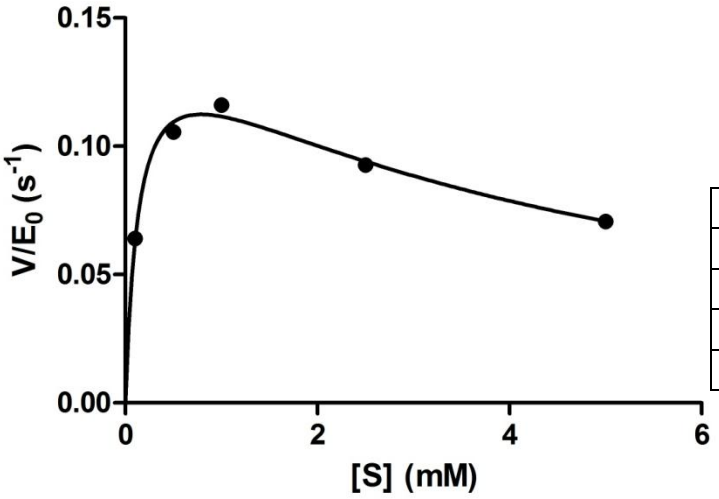
$$v = \frac{V_{Max}}{1 + \frac{K_M}{[S]} + \frac{[S]}{K_i}}$$

Equation S1. Equation, as described by Copeland[6], used to model substrate inhibition of Rad TE by *ent*-**8**, based on allosteric binding of a second molecule of substrate to the enzyme-substrate complex. Used to generate Figure S5.



	Value	Error
R^2	0.9960	N/A
k_{cat}	0.180	0.003
K_M	0.19	0.02

Figure S4. Initial reaction rates (s^{-1}) plotted against substrate concentration (mM) for conversion to macrocycle by Rad TE with **8**. These data was fit to the Michaelis-Menten model.



	Value	Error
R^2	0.9807	N/A
k_{cat}	0.15	0.01
K_M	0.14	0.03
K_I	4.5	0.9

Figure S5. Initial reaction rates (s^{-1}) plotted against substrate concentration (mM) for conversion to macrocycle by Rad TE with *ent*-**8** . These data were fit to Equation S1.

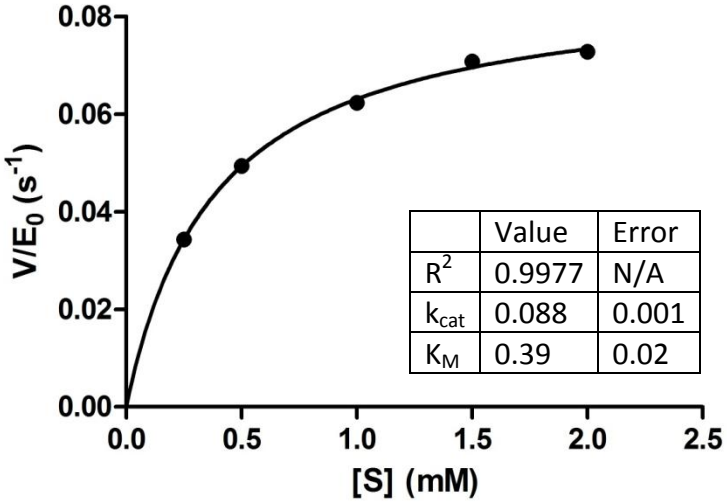


Figure S6. Initial reaction rates (s^{-1}) plotted against substrate concentration (mM) for conversion to macrocycle by Zea TE with **8**. These data were fit to the Michaelis-Menten model.

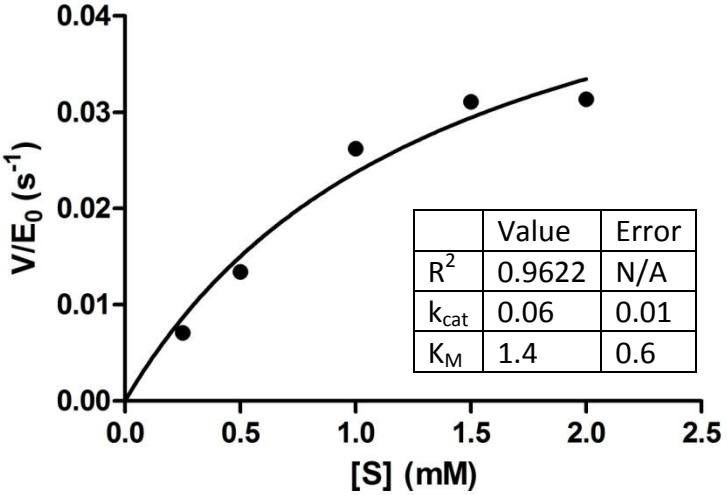
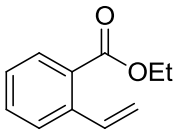
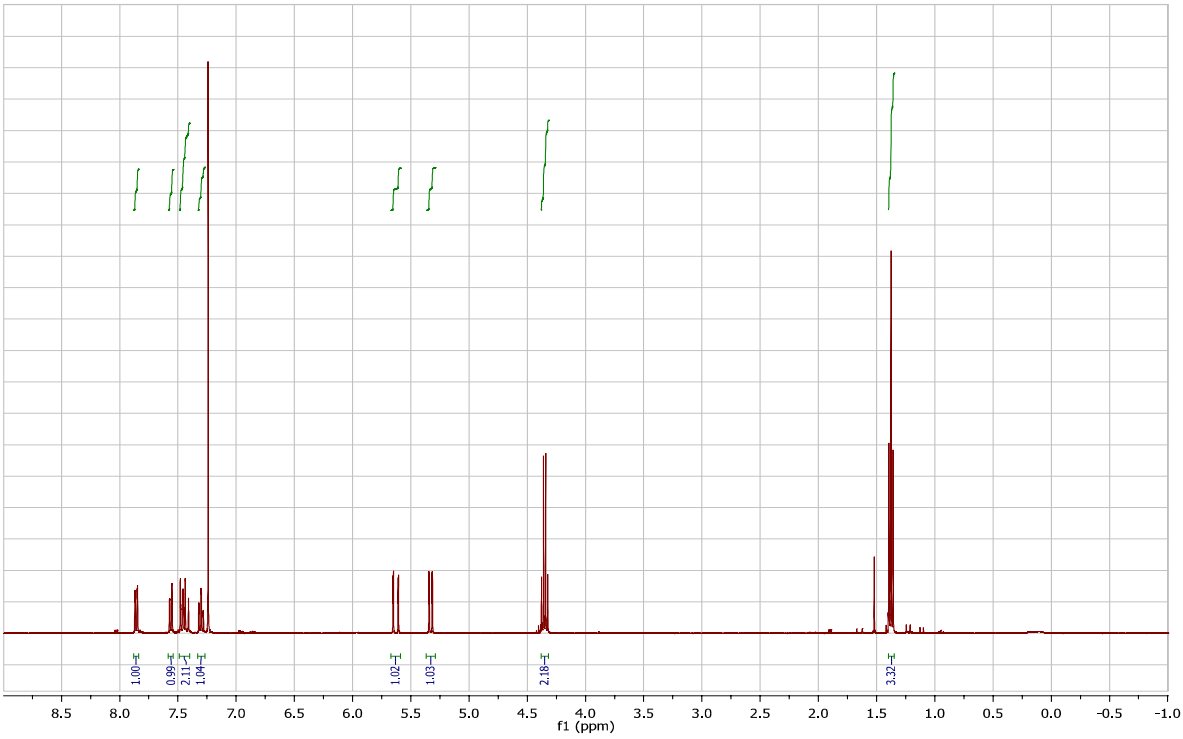


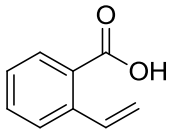
Figure S5. Initial reaction rates (s^{-1}) plotted against substrate concentration (mM) for conversion to macrocycle by ZeaTE with *ent*-**8**. These data were fit to the Michaelis-Menten model.

Selected NMR Spectra

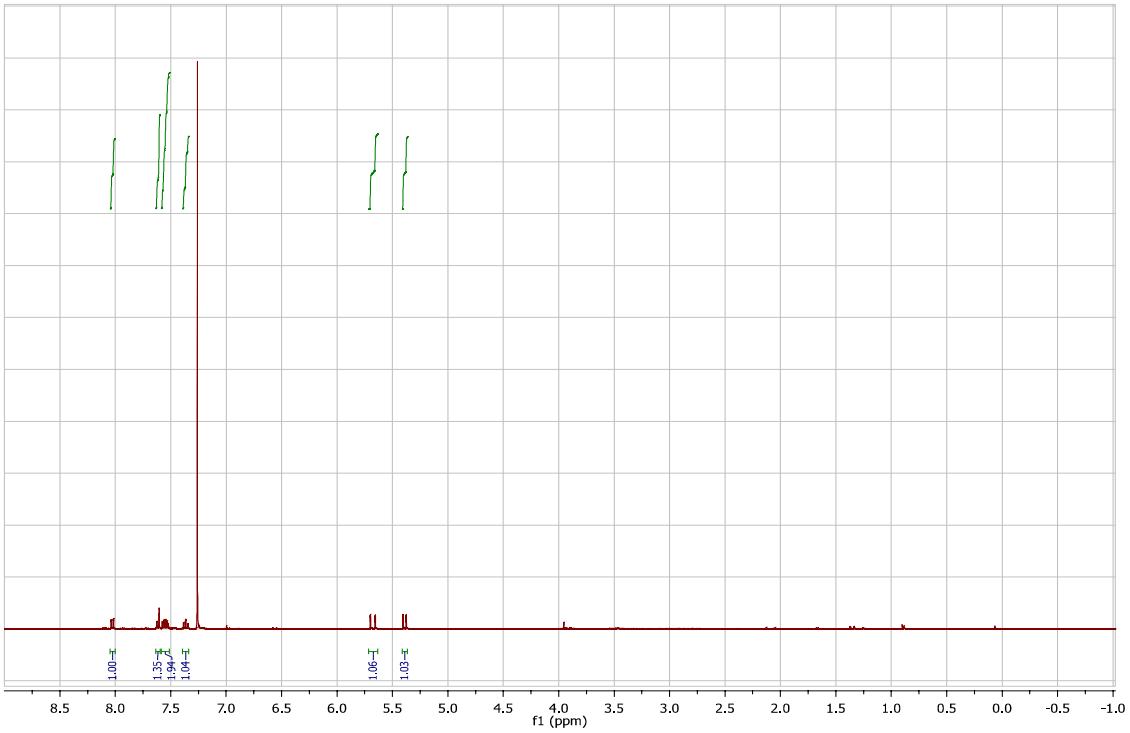


S1




O
OH

S2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5.0
4.5
4.0
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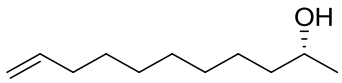
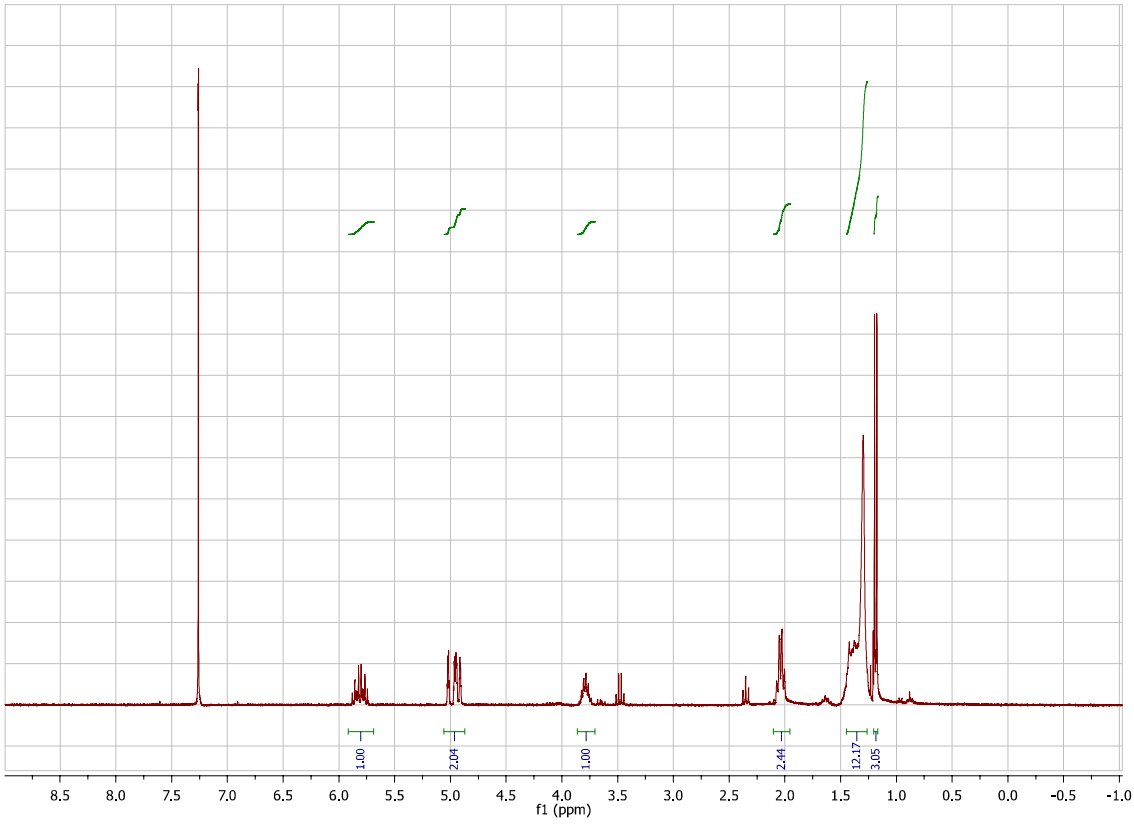
-1.0
f1 (ppm)


OH

3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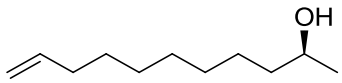
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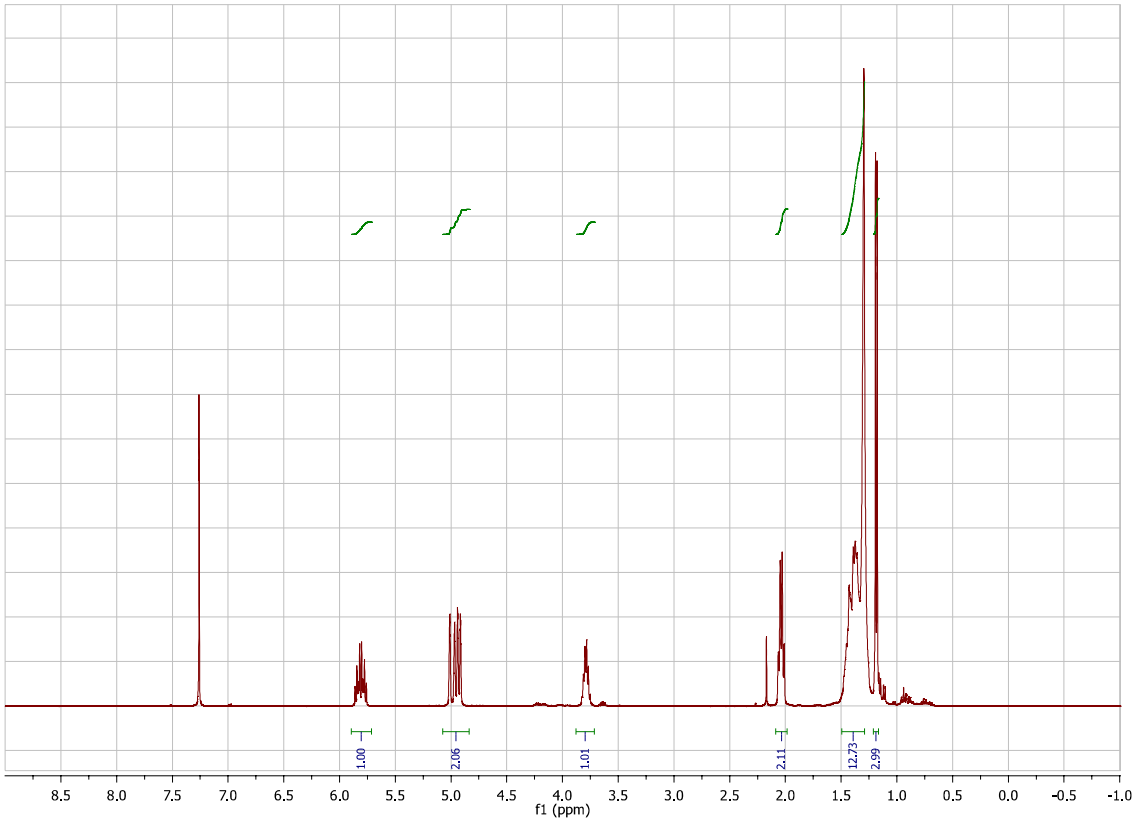
4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5.0
4.5
4.0
3.5
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2.5
2.0
1.5
1.0
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0.0
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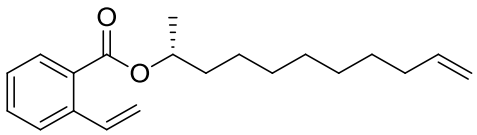
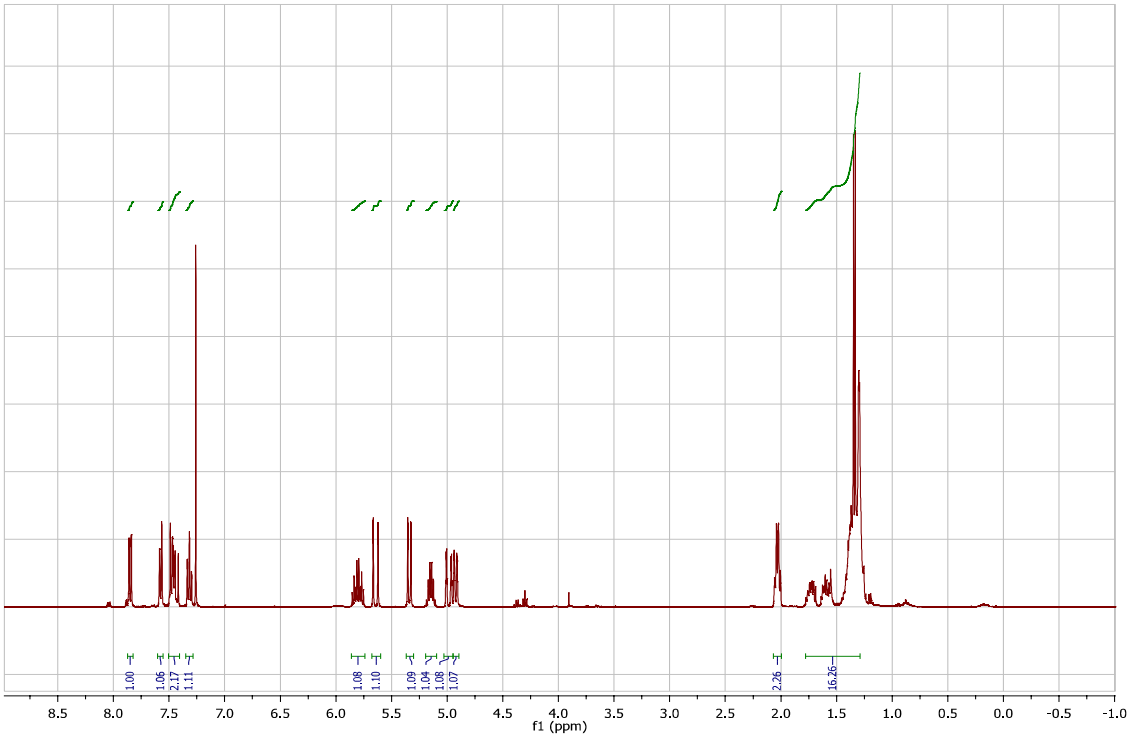
-1.0
f1 (ppm)



5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16.26
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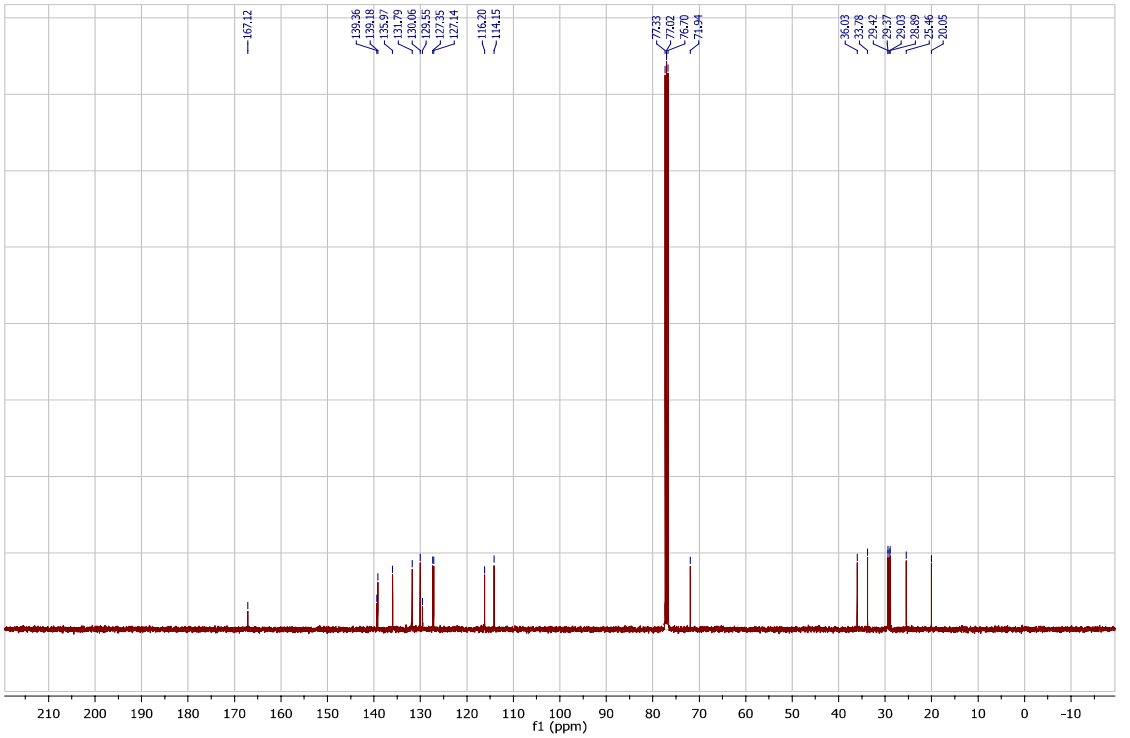
f1 (ppm)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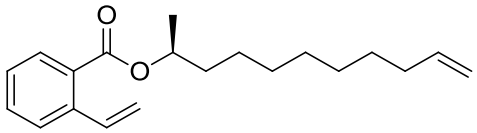
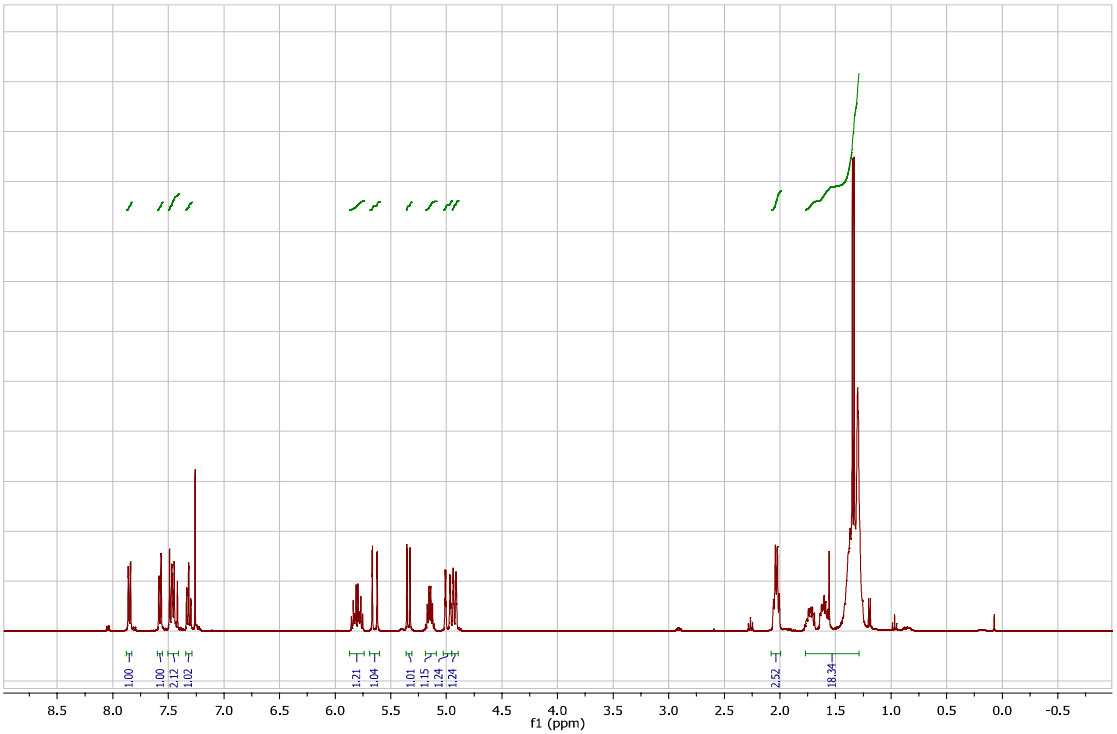
f1 (ppm)



ent-**5**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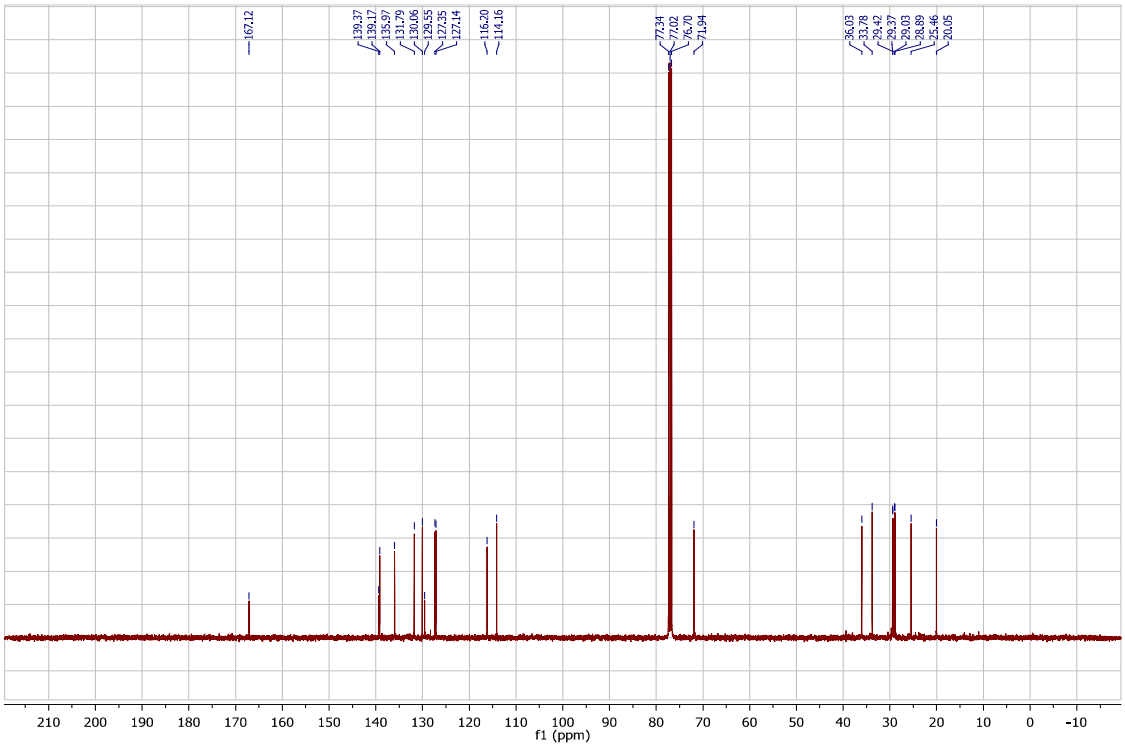
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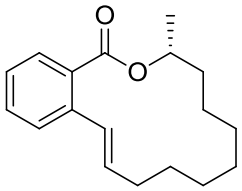
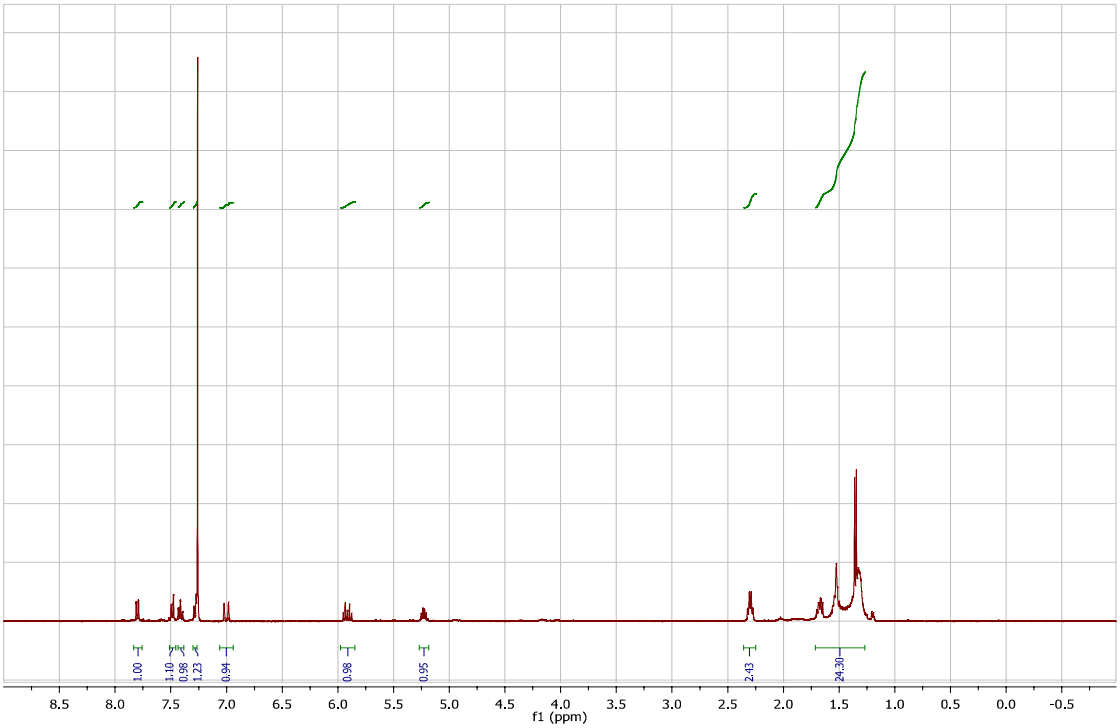
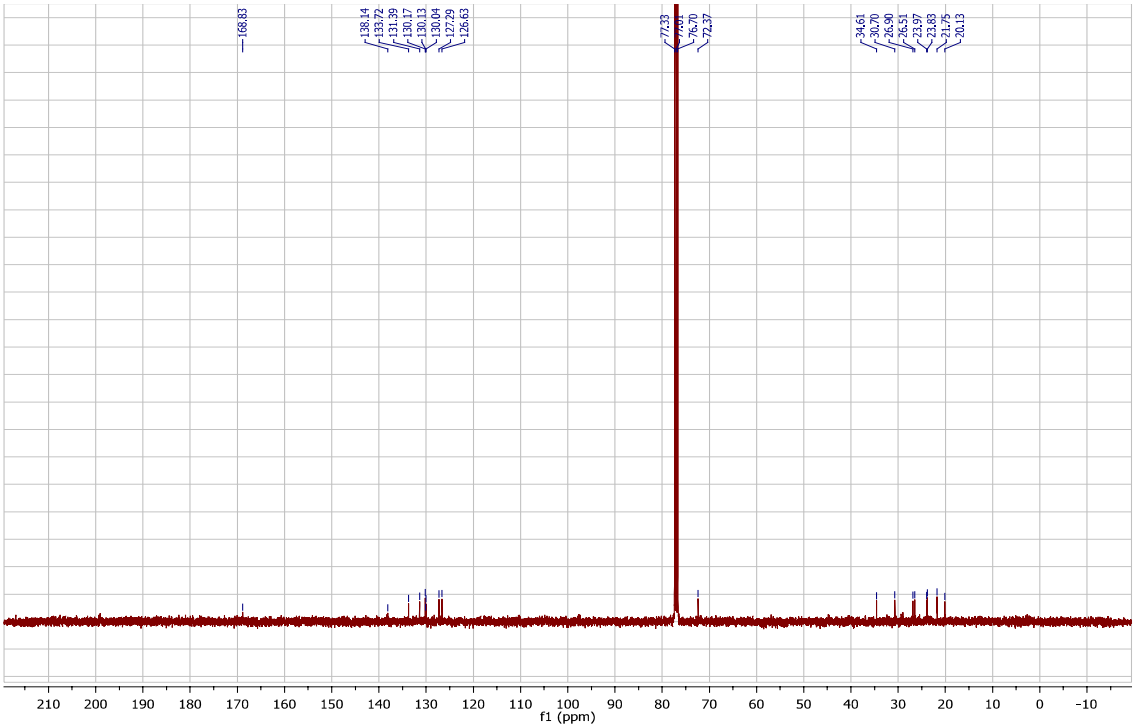
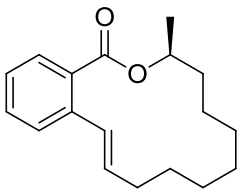
76.70
71.94
36.03
33.78
29.42
29.37
29.03
28.89
25.46
20.05
f1 (ppm)



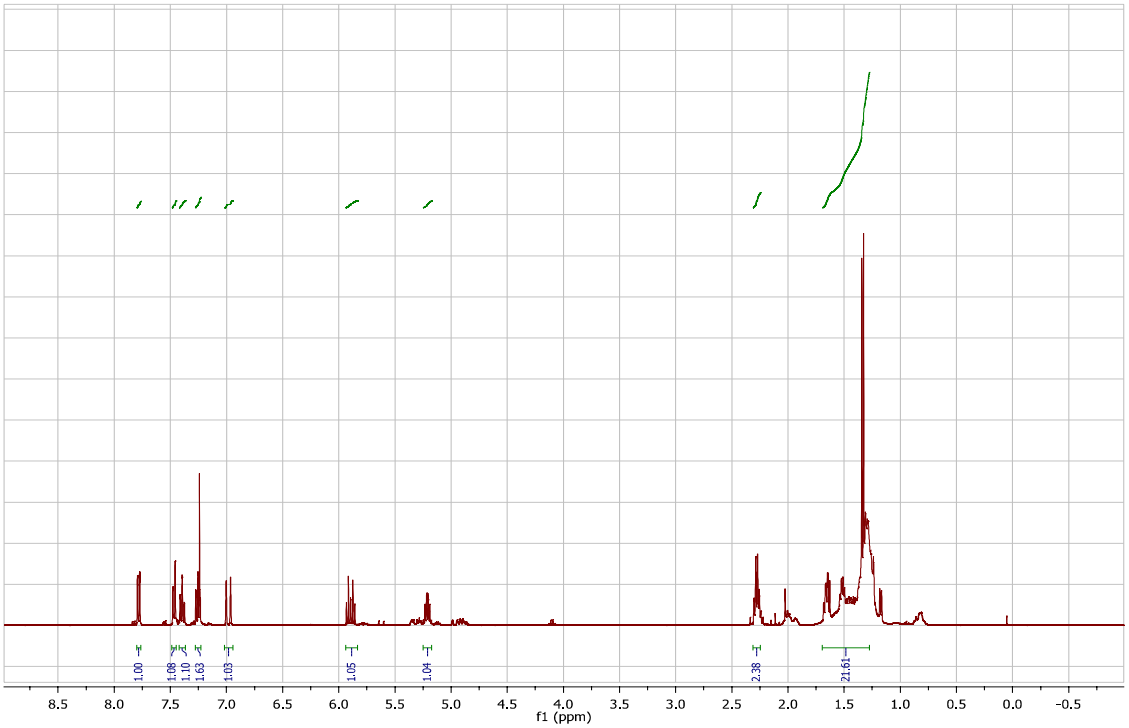
6

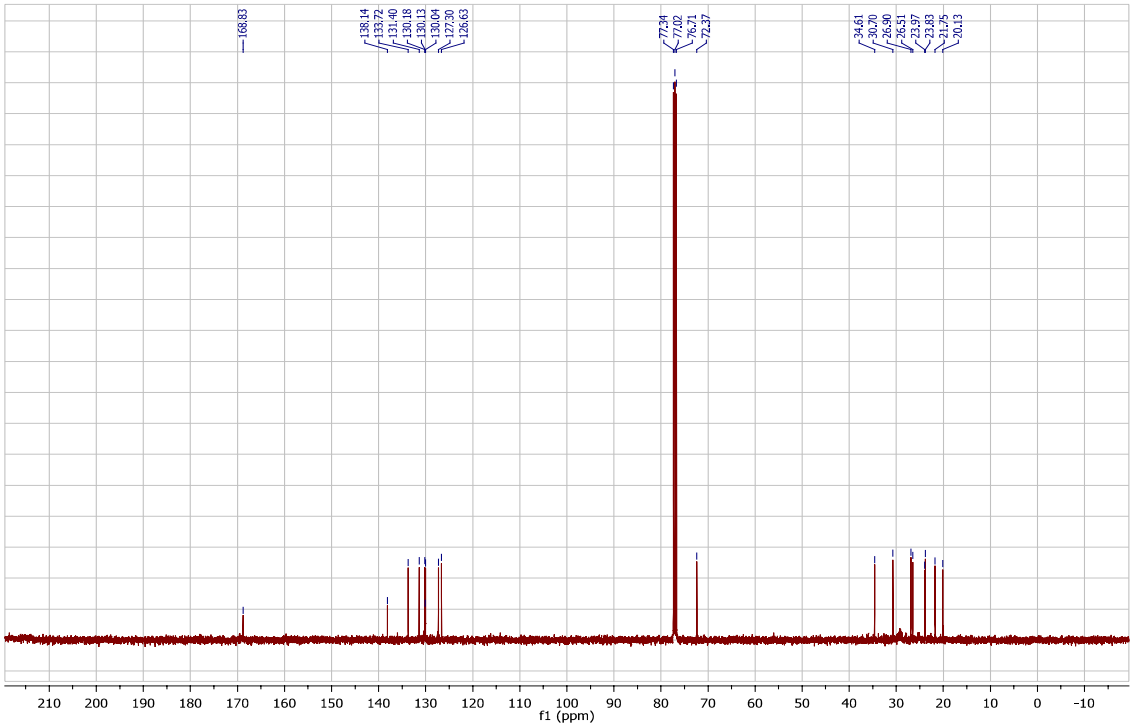

1.00
1.10
0.98
1.23
0.94
0.88
0.95
2.43
24.30
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

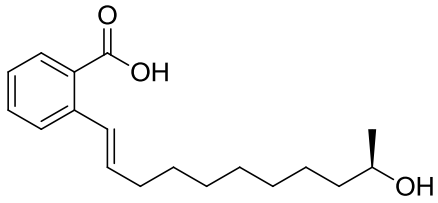

168.83
138.14
133.72
131.39
130.17
130.13
130.04
127.29
126.63
77.33
77.00
76.70
72.37
34.61
30.70
26.90
26.51
23.97
23.83
21.75
20.13
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)



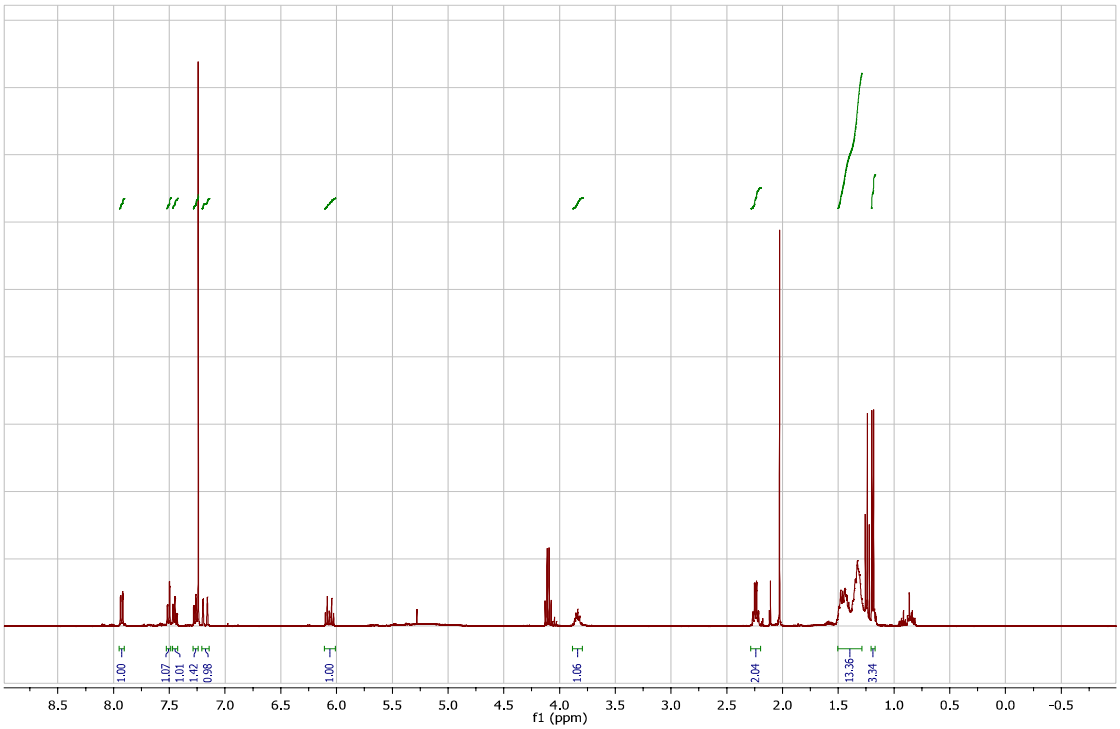
ent-**6**

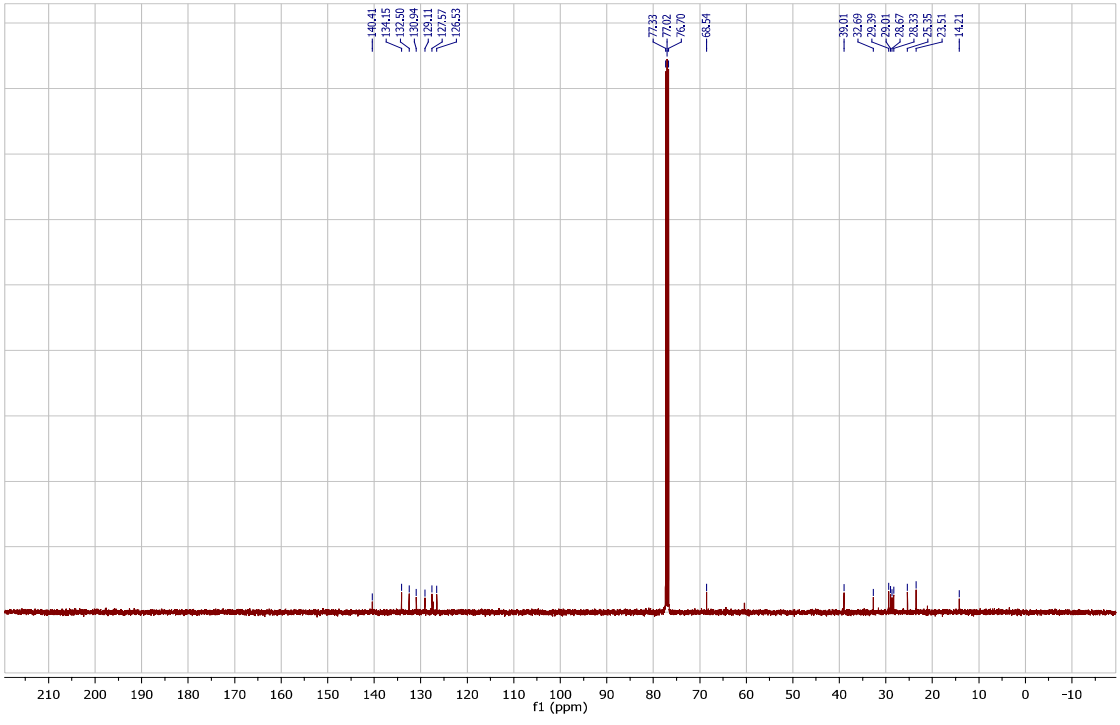

1.00
1.08
1.10
1.63
1.03
1.05
1.04
2.38
21.61
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

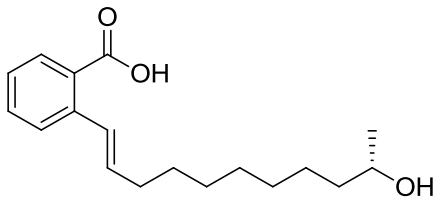

168.83
138.14
133.72
131.40
130.18
130.13
130.04
127.30
126.63
77.34
77.02
76.71
72.37
34.61
30.70
26.90
26.51
23.97
23.83
21.75
20.13
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


O
OH
OH

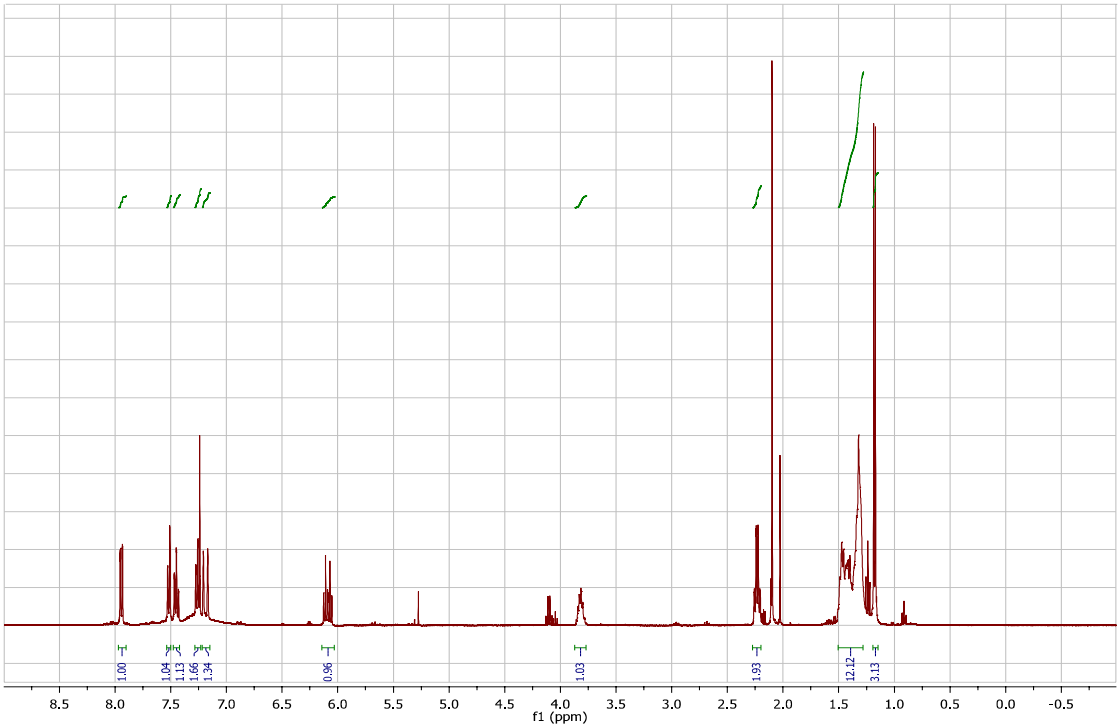
7

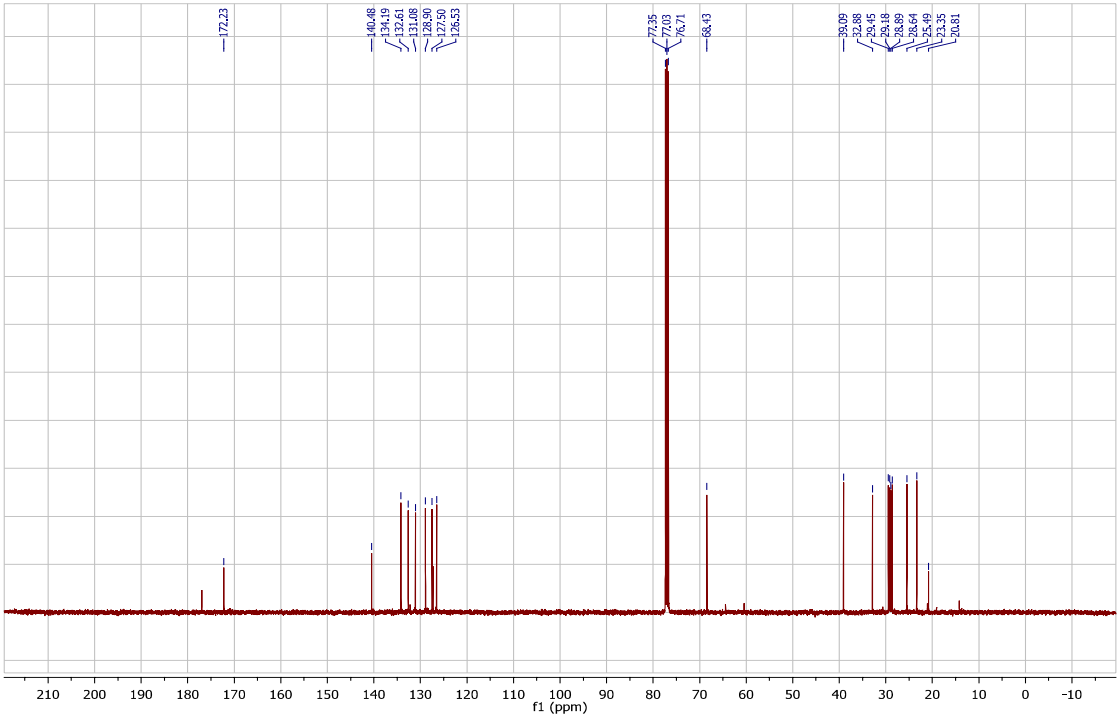

1.00
1.07
1.01
1.42
0.98
1.00
1.06
2.04
13.36
3.34
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

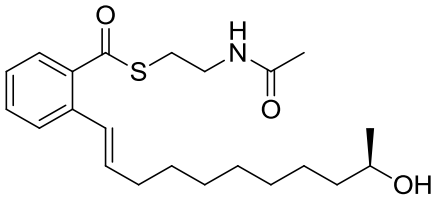

140.41
134.15
132.50
130.94
129.11
127.57
126.53
77.33
77.02
76.70
68.54
39.01
32.69
29.39
29.01
28.67
28.33
25.35
23.51
14.21
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


O
OH
OH

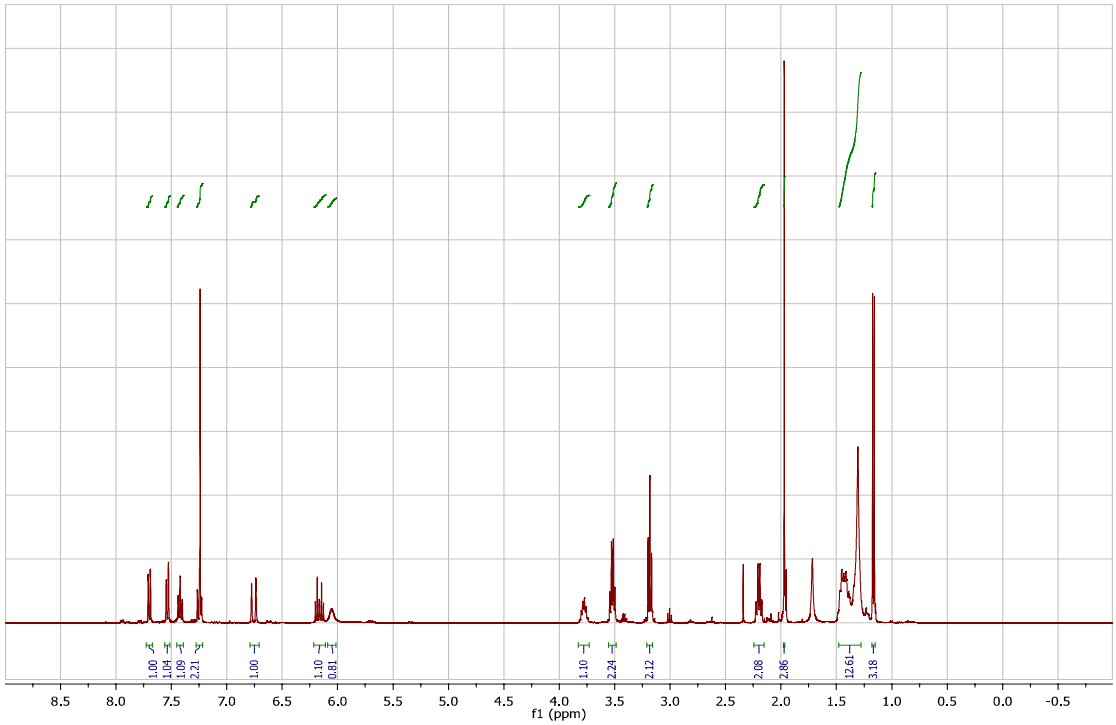
ent-**7**

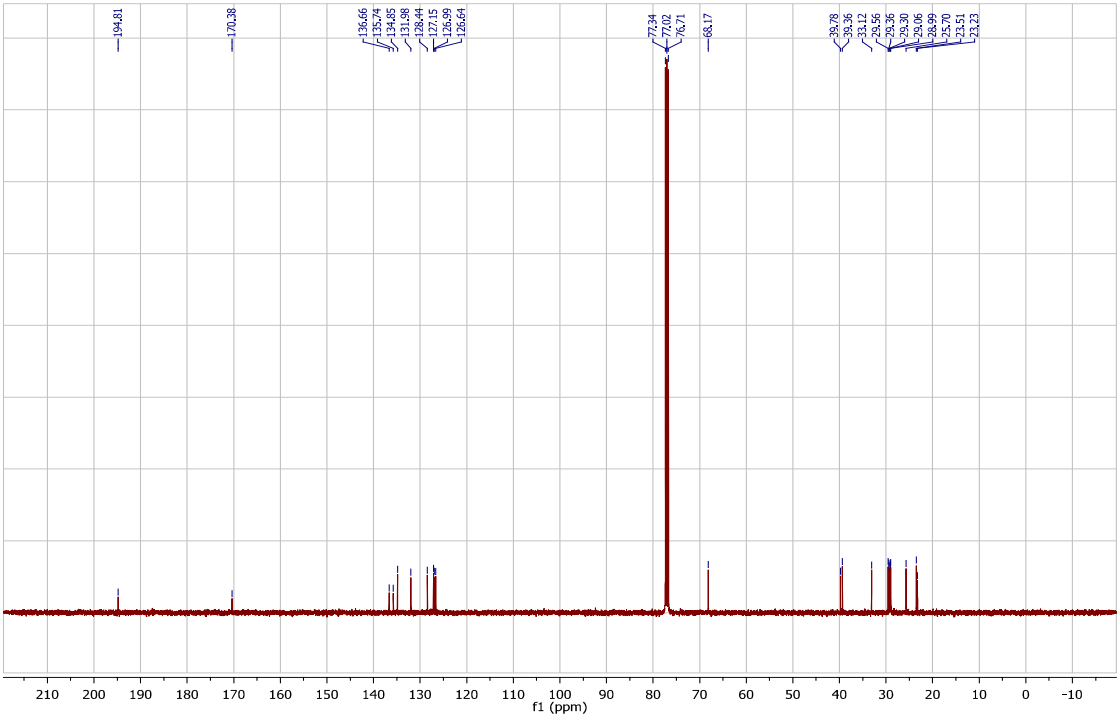

1.00
1.04
1.13
1.66
1.34
0.96
1.03
1.93
12.12
3.13
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

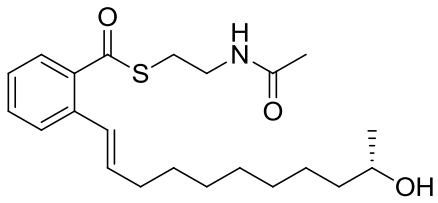

172.23
140.48
134.19
132.61
131.08
128.90
127.50
126.53
77.35
77.03
76.71
68.43
39.09
32.88
29.45
29.18
28.89
28.64
25.49
23.35
20.81
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


O
H
N
S
O
OH

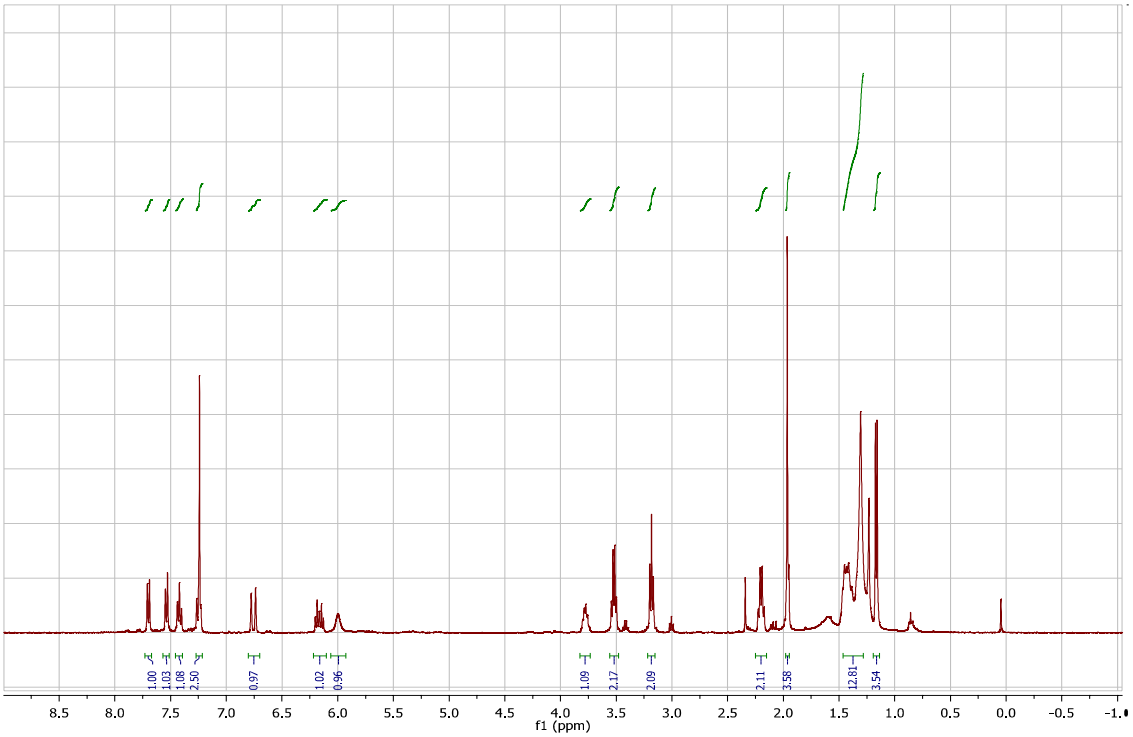
8

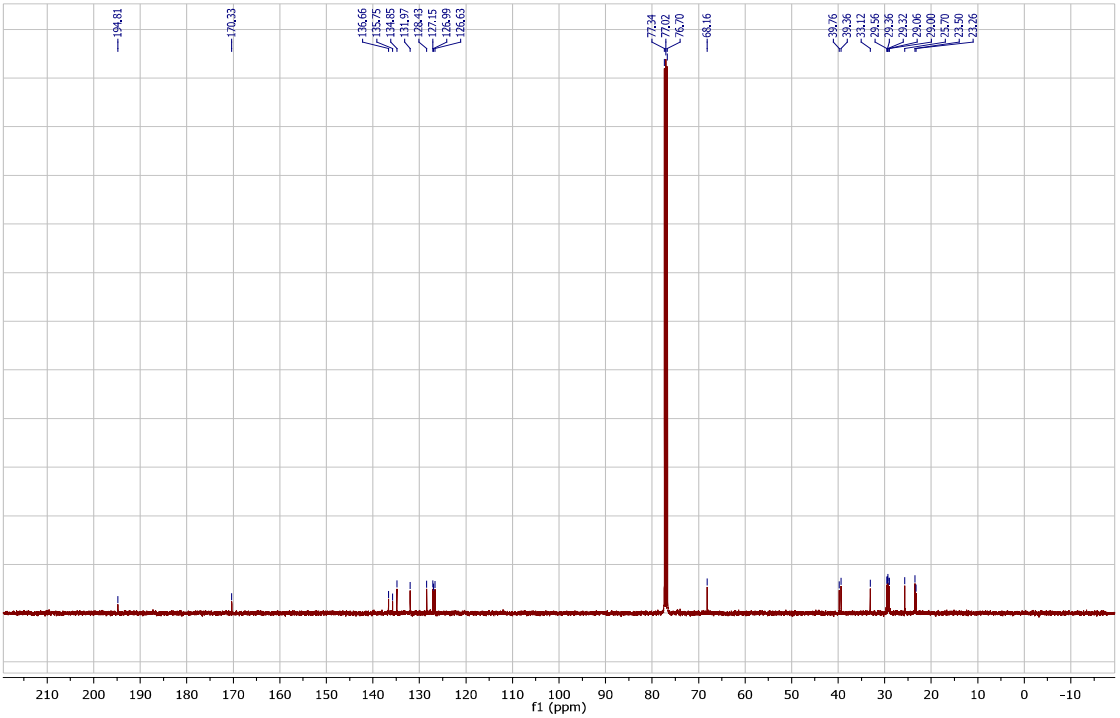

1.00
1.04
1.09
2.21
1.00
1.10
0.81
1.10
2.24
2.12
2.08
2.86
12.61
3.18
f1 (ppm)

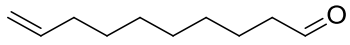

194.81
170.38
136.66
135.74
134.85
131.98
128.44
127.15
126.99
126.64
77.34
77.02
76.71
68.17
39.78
39.36
33.12
29.56
29.36
29.30
29.06
28.99
25.70
23.51
23.23
f1 (ppm)


O
H
N
S
O
OH

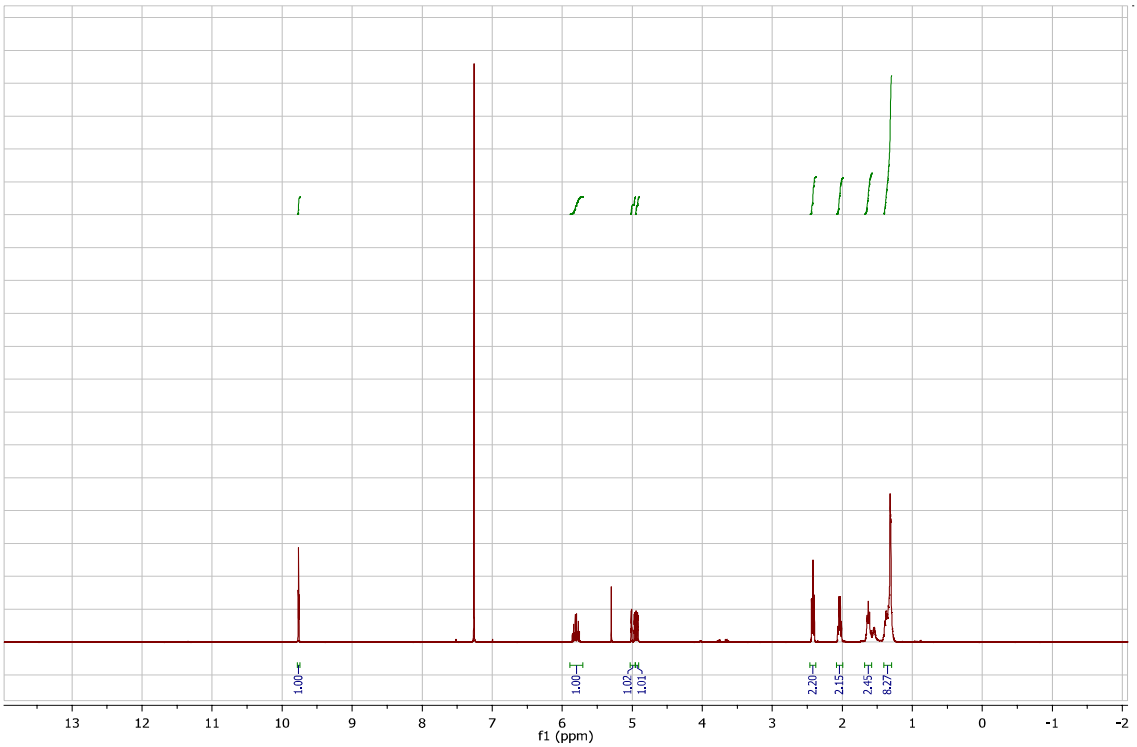
ent-**8**

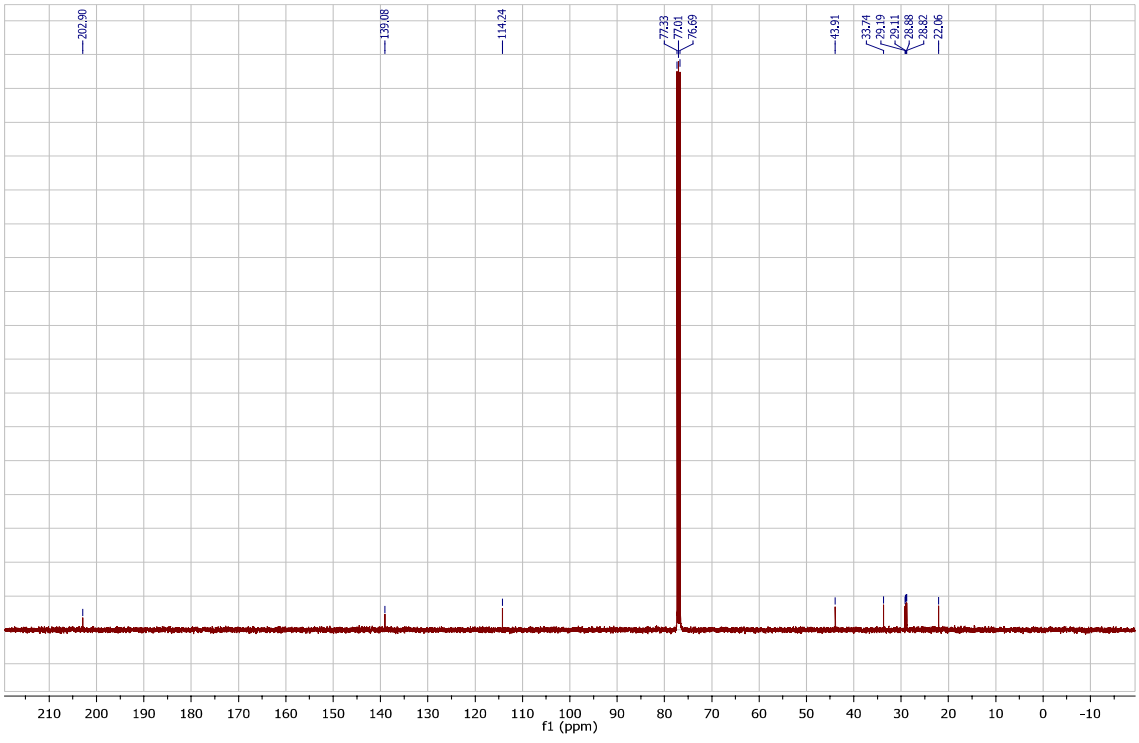

1.00
1.03
1.08
2.50
0.97
1.02
0.96
1.09
2.17
2.09
2.11
3.58
12.81
3.54
f1 (ppm)

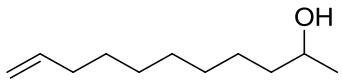

194.81
170.33
136.66
135.75
134.85
131.97
128.43
127.15
126.99
126.63
77.24
77.02
76.70
68.16
39.76
39.36
33.12
29.56
29.36
29.32
29.06
29.00
25.70
23.50
23.26
f1 (ppm)


O

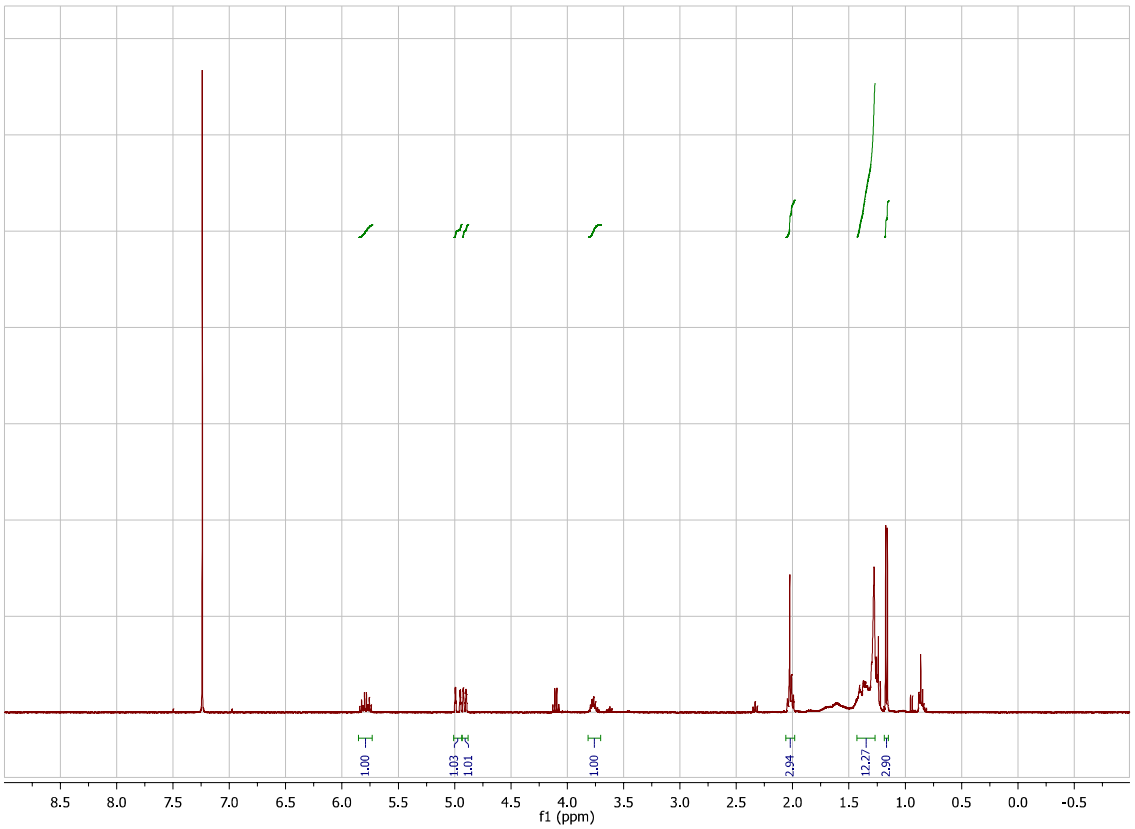
S3

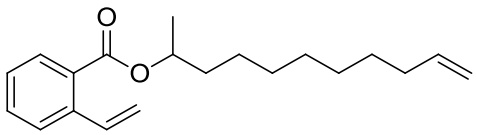

1.00
1.00
1.02
1.01
2.20
2.15
2.45
8.27
13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)


202.90
139.08
114.24
77.33
77.01
76.69
43.91
33.74
29.19
29.11
28.88
28.82
22.06
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)

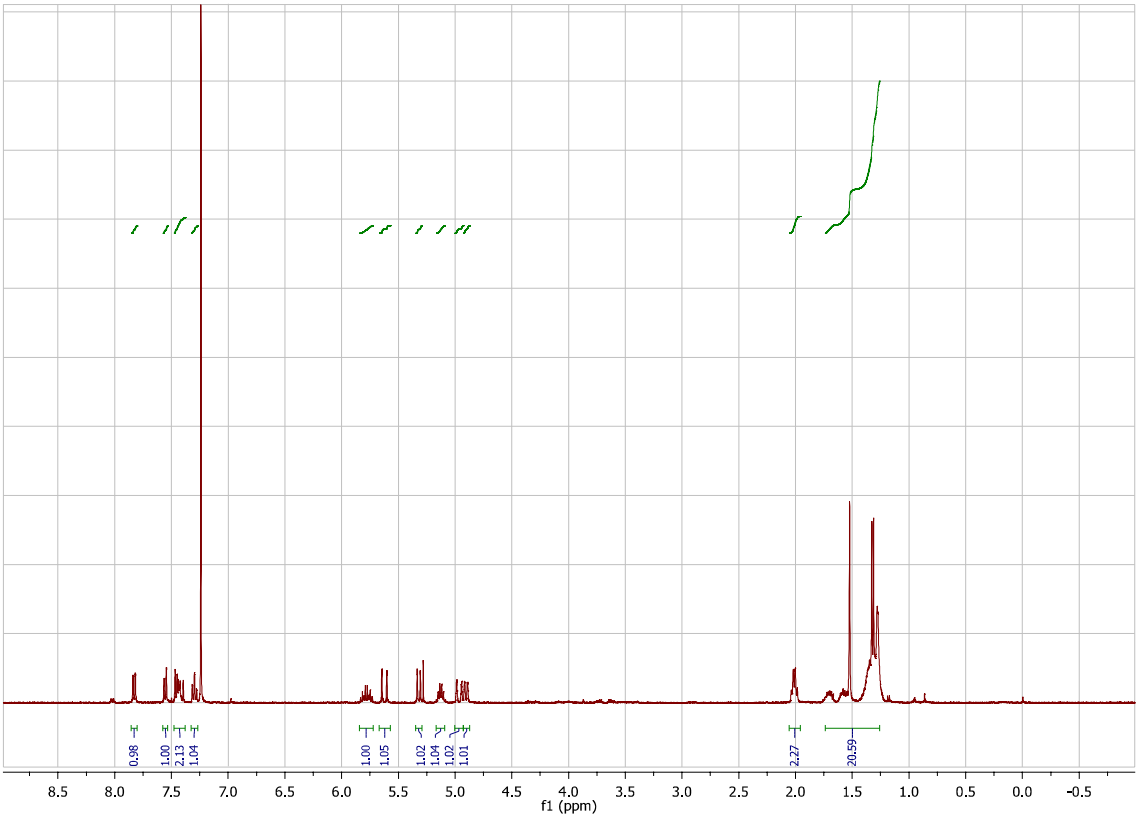

OH

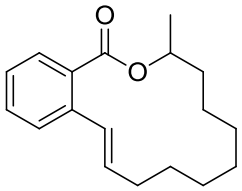
S4


1.00
1.03
1.01
1.00
2.94
12.27
2.90
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)

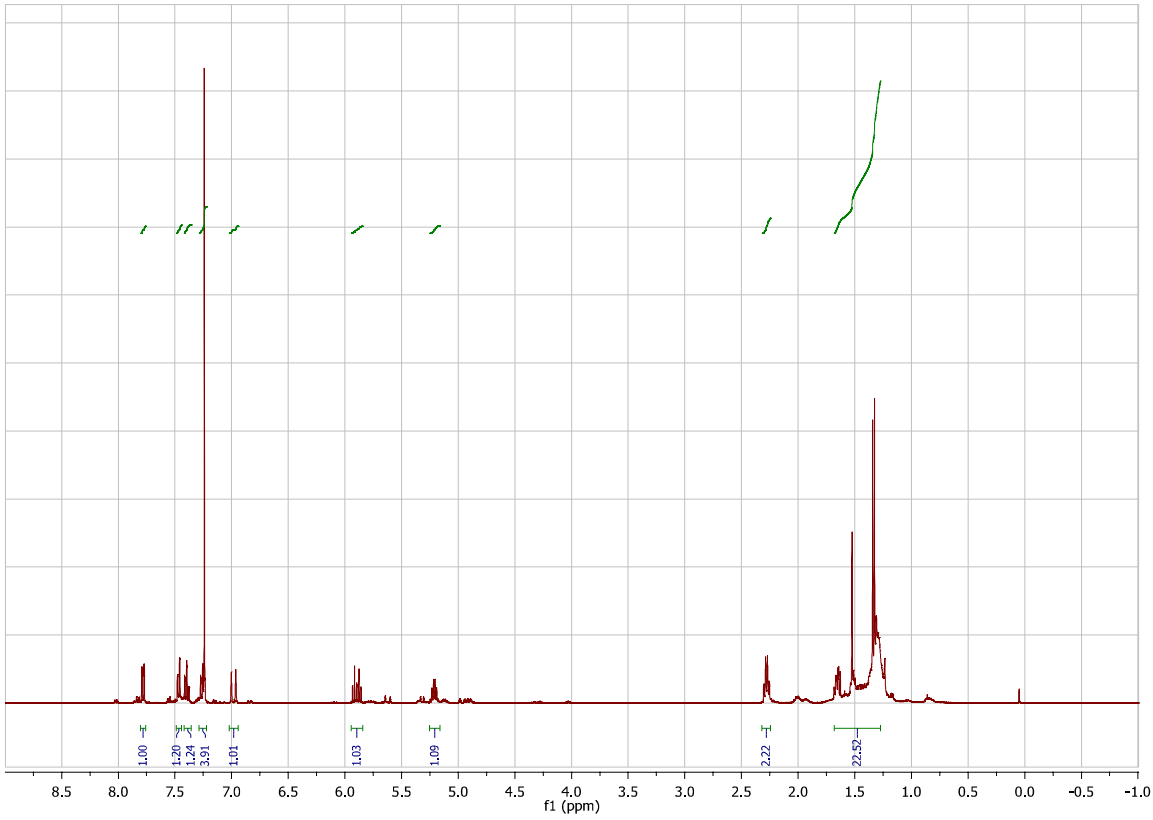


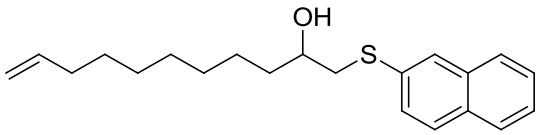
racemic-**5**

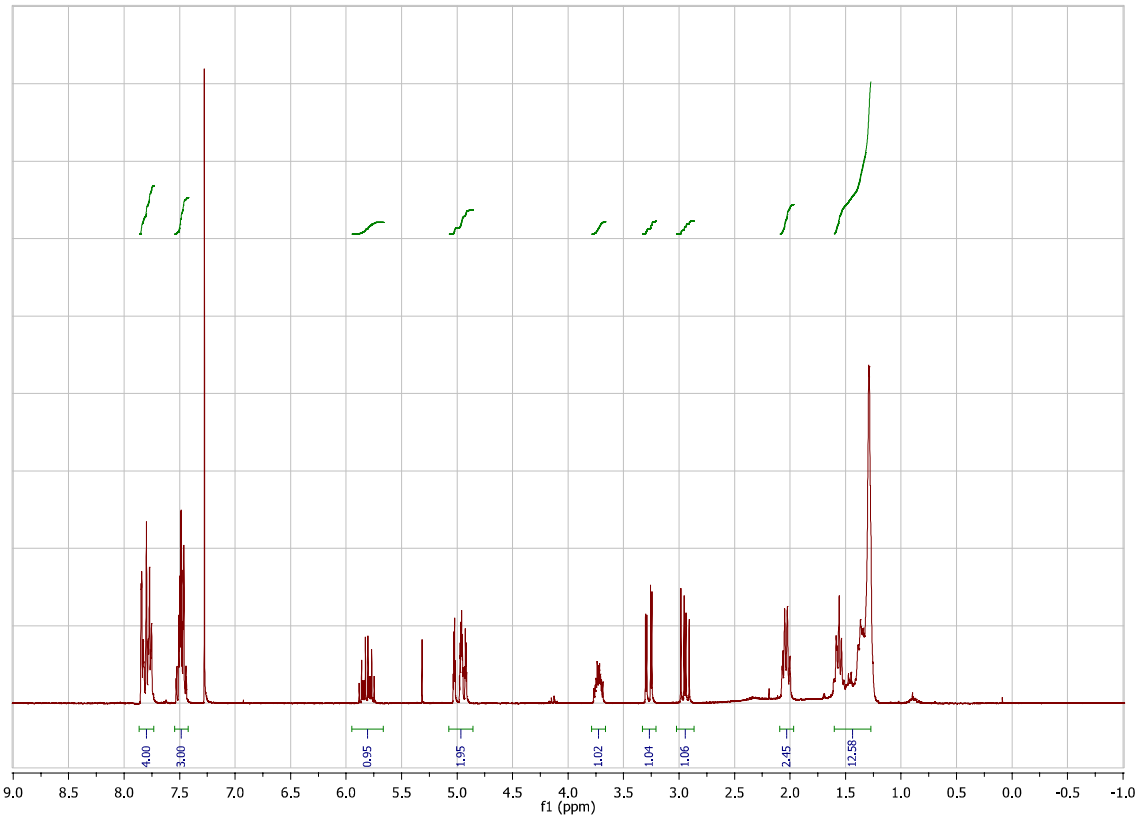

0.98
1.00
2.13
1.04
1.00
1.05
1.02
1.04
1.02
1.01
2.27
20.59
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)


O
O

racemic-**6**


1.00
1.20
1.24
3.91
1.01
1.03
1.09
2.22
22.52
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
-1.0
f1 (ppm)


OH
S


4.00
3.00
0.95
1.95
1.02
1.04
1.06
2.45
12.58
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 -1.0
f1 (ppm)

References

(1) Denmark, S. E.; Butler, C. R. *Org. Lett.* **2006**, *8*, 63.

(2) Matos, M.; Murphy, P. V. *J. Org. Chemsitry* **2007**, *72*, 1803.

(3) Bracher, F.; Krauß, J. *European J. Org. Chem.* **2001**, *2001*, 4701.

(4) Uyanik, M.; Akakura, M.; Ishihara, K. *J. Am. Chem. Soc.* **2009**, *131*, 251.

(5) Zhou, H.; Qiao, K.; Gao, Z.; Vederas, J. C.; Tang, Y. *J. Biol. Chem.* **2010**, *285*, 41412.

(6) Robert A. Copeland. In *Enzymes: A Practical Introduction to Structure, Mechanism, and Data Analysis*; Robert A. Copeland, Ed.; Wiley-VCH, Inc., 2000; Vol. 7, pp. 109–145.