Supplementary Data

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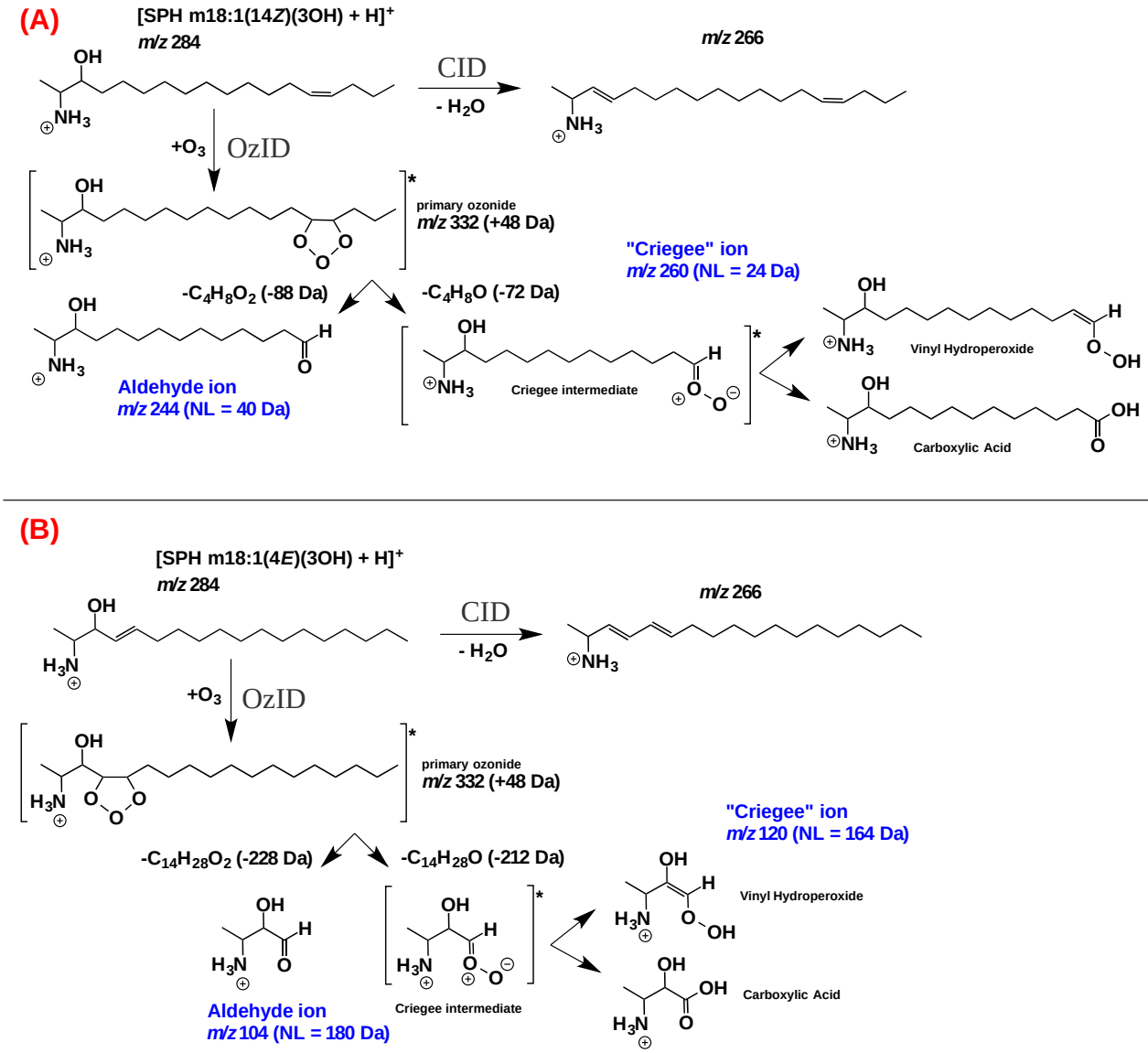
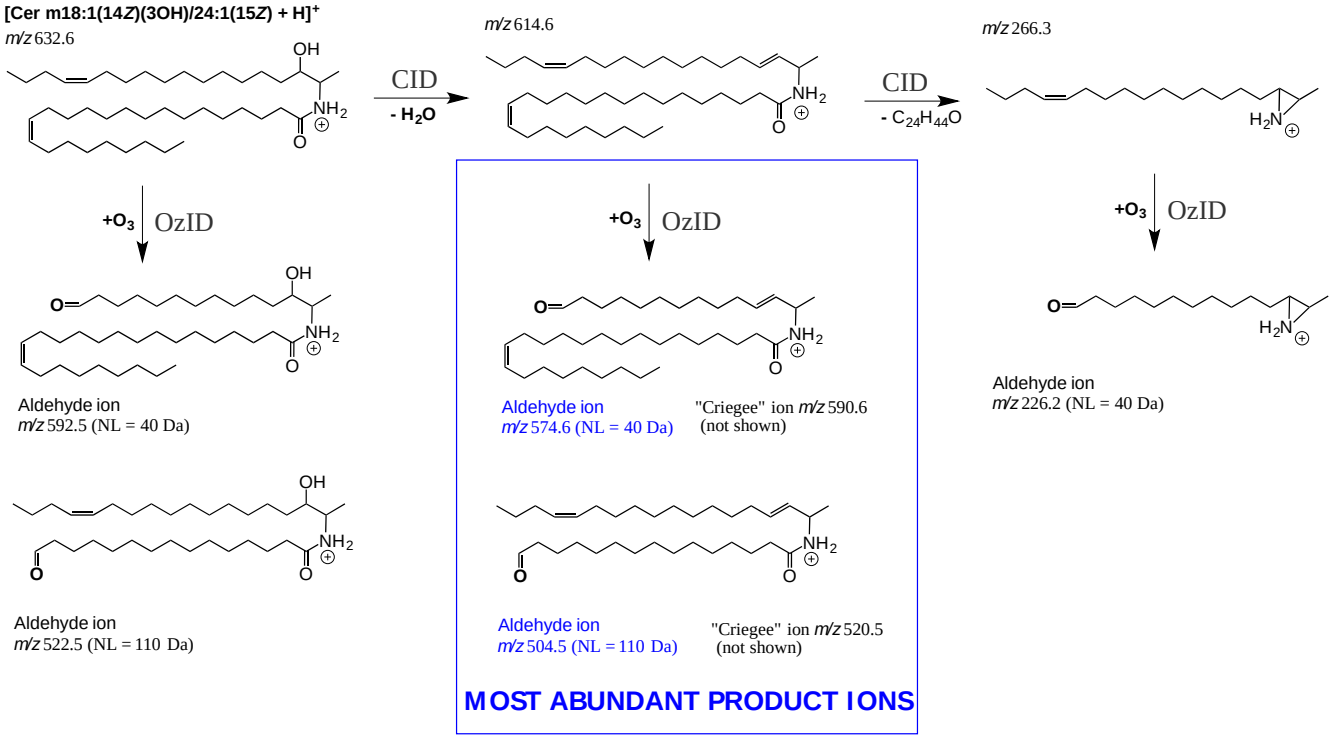


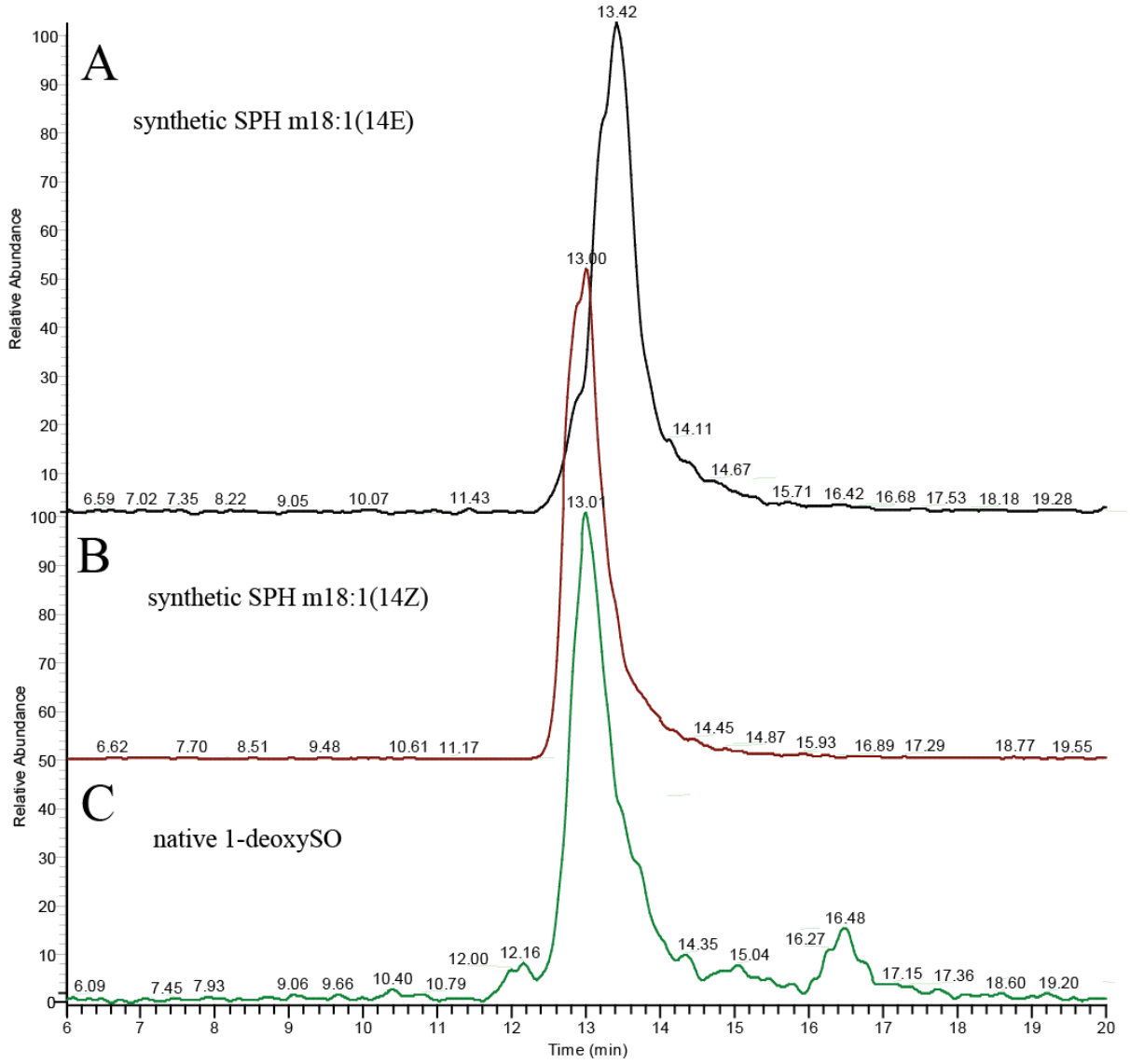
Supplementary Figure 1: Mechanism of ozone-induced dissociation (OzID) on the double-bond positional isomers [SPH m18:1(14*Z*)(3OH) + H]$^+$ (panel A) and [SPH m18:1(4*E*)(3OH) + H]$^+$ (panel B). Mechanisms are based on published proposals and suggest that the "Criegee ions" are most likely to be vinyl hydroperoxide and/or carboxylic acid structures (reference Thomas, M. C., T. W. Mitchell, D. G. Harman, J. M. Deeley, J. R. Nealon, and S. J. Blanksby. 2008. Ozone-induced dissociation: elucidation of double bond position within mass-selected lipid ions. *Anal Chem* 80: 303-311). Collision-induced dissociation processes such as water loss from the precursor ions are also observed under the experimental conditions and the putative structures of the product ions shown in this scheme are based on published mechanisms (reference Murphy, R.C., Royal Society of Chemistry Publishing, *Tandem Mass Spectrometry of Lipids: Molecular Analysis of Complex Lipids*, Chapter 6, pp. 194-232, 2014).



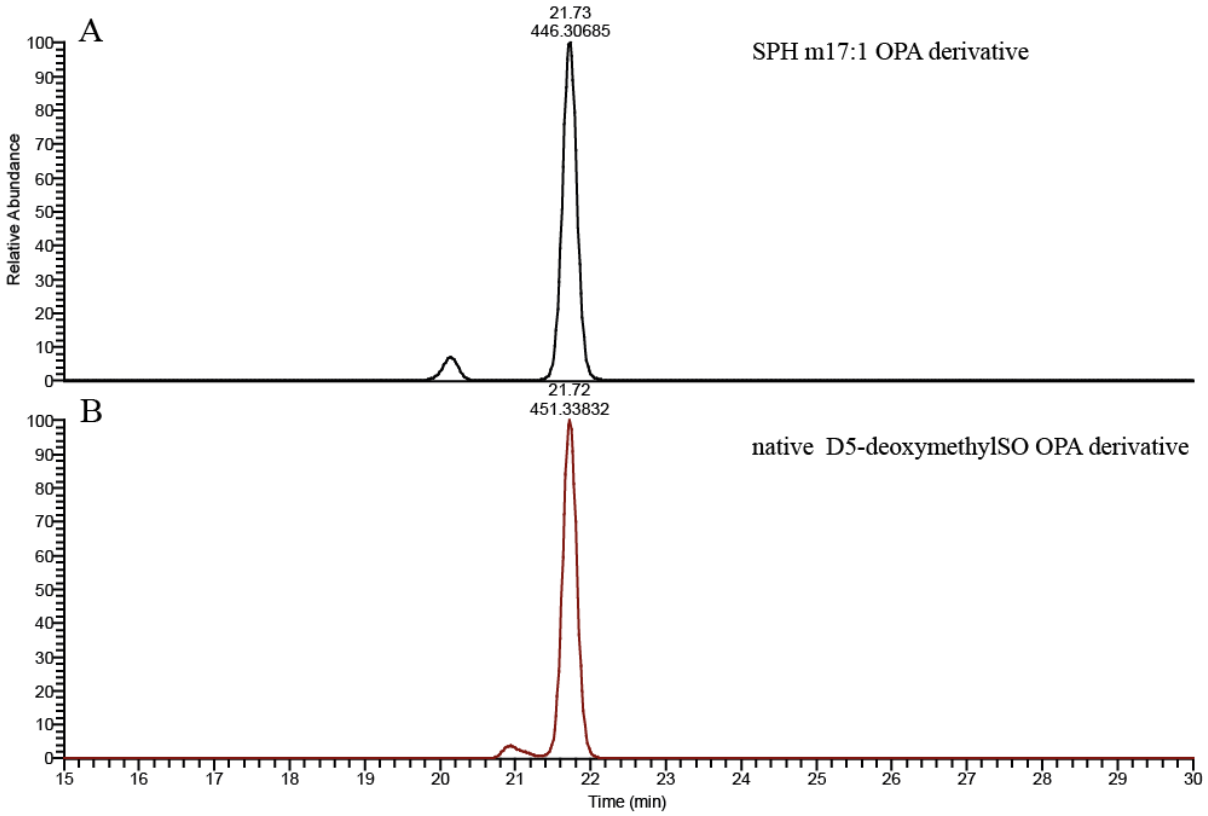
Supplementary Figure 2: Proposed dissociation scheme for [Cer m18:1(14*Z*)(3OH)/24:1(15*Z*) + H]$^+$ trapped in the presence of ozone. Under the reported experimental conditions loss of water by a collision-induced dissociation pathway occurs along with loss of the acyl chain as a ketene. In principle all three ions $[M+H]^+$, $[M+H-H_2O]^+$ and $[M+H-H_2O-C_{24}H_{44}O]^+$ can undergo ozonolysis reactions but the major OzID product ions are the aldehyde ions arising from oxidative cleavage of the carbon-carbon double bonds in the m18:1 backbone and in the 24:1 acyl chain. Criegee ions are also formed but are of minor abundance in these experiments and structures are therefore not indicated. It has previously been noted that the relative abundance of aldehyde and Criegee ions can vary significantly between lipid classes so the low abundance of the Criegee ions in this case is not unusual (reference Thomas, M. C., T. W. Mitchell, D. G. Harman, J. M. Deeley, J. R. Nealon, and S. J. Blanksby. 2008. Ozone-induced dissociation: elucidation of double bond position within mass-selected lipid ions. *Anal Chem* 80: 303-311).

Supplementary Table 1: Neutral losses or gains predicted for OzID product ions arising from ozonolysis of carbon-carbon double bonds at different positions (relative to the methyl terminus) of a mono-unsaturated chain. Neutral losses are based on those previously published (reference: Brown, S.H.J., T.W. Mitchell and S.J. Blanksby. 2011. Analysis of unsaturated lipids by ozone-induced dissociation. *Biochim. Biophys. Acta* 1811: 807-817.) and the mechanisms presented above.

Position of unsaturation		Neutral loss (Da) of OzID product ions relative to the precursor ion $[M + H]^+$			
Relative to methyl end ($n - x$)	Relative to 1-deoxymethyl (Δy)	**Aldehyde**	**Criegee**	**Aldehyde-H_2O**	**Criegee-H_2O**
x =	$y = n - x$, n = 18				
1	**17**	-2	-18	16	0
2	**16**	12	-4	30	14
3	**13**	26	10	44	28
4	**14**	40	24	58	42
5	**13**	54	38	72	56
6	**12**	68	52	86	70
7	**11**	82	66	100	84
8	**10**	96	80	114	98
9	**9**	110	94	128	112
10	**8**	124	108	142	126
11	**7**	138	122	156	140
12	**6**	152	136	170	154
13	**5**	166	150	184	168
14	**4**	180	164	198	182



Supplementary Figure 3: RPLC-MS chromatograms of (A) SPH m18:1(14*E*)(3OH) and (B) SPH m18:1(14*Z*)(3OH) synthetic standards and (C) native 1-deoxySO. The RT of the native 1-deoxySO was identical with that of the *(*14*Z)*-synthetic isomer



Supplementary Figure 4: HEK cells were fed with labelled D5-1-deoxymethylsphinganine (Avanti Polar Lipids, Alabaster, AL). Cells were harvested after 24h and the whole sphingolipid extract was hydrolyzed to get the free sphingoid bases. Extract was treated with o-phthalaldehyde (OPA) to get the OPA derivative of sphingoid bases. Sphingoid bases were separated on a C18 column and detected on an HRAMS Q Exactive. Retention time and m/z of deuterium labelled D5-1-deoxymethylSO were compared to synthetic SPH m17:1(3E). Retention time of native D5-1-deoxymethylSO was 21.73 min (B) and for SPH m17:1(3E) 21.73 min (A). m/z of native D5-1-deoxymethylSO was 451.33832 (calculated 451.34011, < 5 ppm) and for SPH m17:1(3E) 446.30685 (calculated 446.30872, < 5 ppm).

a) General description of materials and methods used during synthetic work

General Considerations

Unless otherwise specified, all the commercially available reagents were purchased from Sigma-Aldrich or Fluka or Acros and used without any further purification. Air and/or moisture sensitive reactions were carried out in well dried glassware under argon atmosphere with dry, freshly distilled solvents using standard syringe-cannula/septa techniques. All the solvents were used after distillation by standard methods. The petroleum ether used throughout had a boiling range of 40–60°C. All corresponding glassware was oven dried and/or carefully dried in the line with a flameless heat gun. Reaction progress was monitored with analytical normal-phase thin layer chromatography (NP TLC). All synthesized compounds were purified to homogeneity as judged by TLC analysis, mass spectrometry (MS) analysis and nuclear magnetic resonance spectroscopy (NMR) analysis.

Chromatography

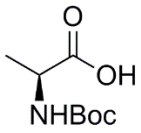
TLC studies were performed on pre-coated silica gel 60-F_{254} on aluminum sheets (Merck KGaA) and spots were detected by UV illumination (254 nm) and/or spraying with either 1.3% ninhydrin solution, ceric ammonium molybdate solution or $KMnO_4$ solution followed by heating. Preparative flash column chromatography was performed manually using glass columns of different size packed with Silica Gel 60M (0.04-0.063 mm) as stationary phase with indicated eluent systems in parenthesis following the description of purification. Solvent ratios for chromatography and R_f values are reported in v/v% ratios.

Spectroscopic Data

The structure of all synthesized compounds was confirmed with ^{1}H NMR, ^{13}C NMR, DEPT and MS analyses. (^{1}H, ^{13}C) NMR spectra of the synthesized compounds were recorded on Bruker Avance -300 and Bruker Avance-500 nuclear magnetic resonance spectrometers (^{1}H at 300 or 500 MHz and ^{13}C at 75 or 126) as a solution in deuterated methanol (MeOD), deuterated chloroform ($CDCl_3$) or deuterated water (D_2O) at 27°C, unless otherwise indicated. ^{1}H NMR spectra are reported as chemical shifts (δ) in parts per million (ppm) as referenced to the residual solvent peak (chloroform: δ 7.27 ppm; methanol: δ 3.31ppm, unless otherwise noted). Peak multiplicities are designed by the following abbreviations: s = singlet; d= doublet; t = triplet; q = quartet; dd = doublet of doublets, ddd = doublet of doublet of doublets, dt = doublet of triplets, qd = quartet of doublets, m = multiplet; br = broad. The coupling constants (*J)* are quoted in hertz (Hz). The reported chemical shifts are tabulated in the following format: δ (ppm) (multiplicity, coupling constant (s), number of protons). ^{13}C NMR chemical shifts are expressed in parts per million (ppm) and referenced to the residual solvent peak (chloroform: δ 77.16 ppm; methanol δ 49.05 ppm, unless otherwise indicated). High-resolution mass spectroscopy (HRMS) experiments were recorded on a Hewlett-Packard GCMS 5995-A mass spectrometer equipped with standard electrospray ionization (ESI) in the positive and negative ion detection modes.

b) Synthesis procedures and analytic data of compounds

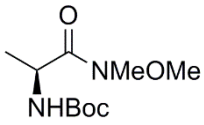
(*tert*-butoxycarbonyl)-L-alanine (1).



1

A solution of di-*tert*-butyl dicarbonate (7.17 g, 35.36 mmol) in dioxane (60 mL) was added dropwise to a stirred solution of *L*-alanine (3.00 g, 33.67 mmol) in aqueous 1M NaOH (60 mL) at -10ºC. After the resulting reaction mixture was stirred for 12h at ambient temperature, the reaction mixture was washed with ethylacetate (2×100 mL) to remove excess of unreacted Boc_2O. The aqueous layer was cooled again at -15ºC and carefully acidified with conc. HCl to pH=2-3. The resulting mixture was subsequently extracted several times with ethylacetate (4×100 mL). The organic layers were combined, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to provide compound **59** as white solid. The product was used in the next step without further purification. Yield: 6.06 g (95.1%). R_f: 0.3 ($CHCl_3$/MeOH 12:1; visualized with 1.3% ninhydrine). ^{1}H NMR ($CDCl_3$, 500 MHz, ppm): δ 1.43-1.48 (m, 12H), 4.32-4.40 (m, 1H), 5.04-5.12 (m, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz, ppm): δ 18.43. 28.43, 49.25, 80.46, 155.62, 177.83. ESI-HRMS: *m/z* calcd for $C_8H_{16}NO_4$ $[M+H]^+$ 190.1079; observed 190.1075.

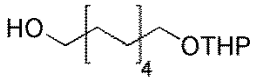
(*S*)-*tert*-butyl-1-(*N*-methoxy-*N*-methylcarbamoyl)ethylcarbamate (2).



2

To a stirred solution of *N*-Boc-L-alanine **59** (3.00 g, 15.85 mmol), *N*,*O*-dimethylhydroxylamine hydrochloride (1.70 g, 17.44 mmol) and *N*-methylmorpholine (3.5 mL, 31.71 mmol, until pH=8-9) in dry DCM (80 mL) at -20°C under argon atmosphere, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI.HCl) (3.34 g, 17.44 mmol) was added portionwise over a period of 30 min. The resulting reaction mixture was allowed to stir for 4h at 0°C (as monitored by TLC analysis for almost complete reaction; Silica gel, EtOAc 100%; $R_{f(adduct)}$= 0.1; $R_{f(product)}$=0.56; visualized with 1.3% ninhydrine), and was then quenched with saturated NH_4Cl solution (150 mL). The layers were separated and the aqueous layer was extracted several times with CH_2Cl_2 (3×100 mL). The organic layers were combined, washed with saturated $NaHCO_3$ solution (100 mL), brine (100 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to afford compound **60** as a white solid. The product was used in the next step without any further purification. Yield: 2.88 g (78.3 %). R_f: 0.42 (cyclohexane/ethylacetate 3:2, visualized with 1.3% ninhydrine). ^{1}H NMR ($CDCl_3$, 500 MHz, ppm): δ 1.31 (d, *J*= 6.9 Hz, 3H), 1.44 (s, 9H), 3.21 (s, 3H), 3.77 (s, 3H), 4.62-4.74 (br. m, 1H), 5.27 (d, *J*= 6.9 Hz, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz, ppm): δ 18.80, 28.50, 32.27, 46.66, 61.75, 79.65, 155.33, 173.80. ESI-HRMS: *m/z* calcd for $C_{10}H_{19}N_2O_4$ $[M-H]^-$ 231.1345; observed 231.1351.

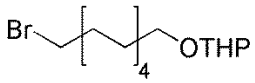
10-(tetrahydro-2H-pyran-2-yloxy)decan-1-ol (3)



3

A stirred solution of 1,10-decanediol (5.5 g, 31.8 mmol) in anhydrous mixture of THF:DCM (2:1, 160 mL) at 0°C under argon atmosphere was treated with a catalytic amount of *p*-toluenesulfonic acid monohydrate (PTSA) (0.48 g, 2.54 mmol, 8 mol%), followed by dropwise addition of dihydropyran (2.9 mL, 31.8 mmol). The resulting reaction mixture was allowed to stir for 1h at the same conditions, gradually warmed to ambient temperature and followed by TLC analysis. After the TLC analysis showed a complete disappearance of the starting compound (~10h) (cyclohexane/ EtOAc 3:2; $R_{f\ (adduct)}$= 0.37; $R_{f\ (product)}$=0.68; visualized with ceric ammonium molybdate solution), the reaction mixture was diluted with diethylether (100 mL) and carefully quenched with saturated aqueous $NaHCO_3$ solution (150 mL). The layers were separated, and the aqueous layer was extracted with diethylether (3x100 mL). The organic layers were combined and subsequently washed with brine (150 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. Flash column chromatography of the crude mixture over silica gel using cyclohexane and ethylacetate as eluents (from 15-20% ethylacetate in cyclohexane) provided compound **3** as colorless oil. Yield: 4.36 g (53%; **note**, the di-protected diol byproduct was isolated in ~14% yield). R_f: 0.68 (cyclohexane/EtOAc 3:2, ceric ammonium molybdate solution). ^{1}H NMR (500 MHz, $CDCl_3$, ppm) δ 4.56 (dd, *J* = 4.3, 2.8 Hz, 1H), 3.85 (ddd, *J* = 11.2, 7.6, 3.2 Hz, 1H), 3.71 (dt, *J* = 9.6, 6.9 Hz, 1H), 3.60 (t, *J* = 6.7 Hz., 2H), 3.51 – 3.45 (m, 1H), 3.36 (dt, *J* = 9.6, 6.7 H, 1Hz), 1.86 – 1.77 (m, 1H), 1.73 – 1.66 (m, 1H), 1.60 – 1.47 (m, 8H), 1.37 – 1.25 (m, 12H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 98.91, 67.77, 63.02, 62.40, 32.86, 30.84, 29.80, 29.62, 29.58, 29.53, 29.49, 26.29, 25.82, 25.57, 19.74. HRMS (ESI^+) *m/z* calcd for $C_{15}H_{30}O_3Na$ $[M+Na]^+$ 281.2093; observed 281.2087.

10-(tetrahydro-2H-pyran-2-yloxy)-1-bromodecane (4)



4

Alcohol **3** (4.0 g, 15.6 mmol) and tetrabromomethane (CBr_4) (5.7 g, 17.16 mmol) were dissolved in anhydrous dichloromethane (70 mL) under argon atmosphere, and the resulting mixture was cooled to 0°C. To this mixture, a solution of triphenyl phosphine (PPh_3) (4.7 g, 17.94 mmol) in anhydrous dichloromethane (20 mL) was added dropwise over a period of 20 min. After the resulting reaction mixture was allowed to stir for 2h at ambient temperature (as judged by TLC analysis for almost a complete reaction, Pet. ether/ EtOAc 4:1; $R_{f\ (adduct)}$= 0.47; $R_{f\ (product)}$=0.73; visualized with ceric ammonium molybdate solution), the solvent was removed under reduced pressure. The resultant residue was taken up in petroleum ether (200 mL) and the mixture was vigorously stirred for 30 min, during while a white precipitate was formed. The formed white precipitate of triphenylphosphonium oxide was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo*. The process was repeated until clear oil was obtained. This crude product was subsequently purified by flash column chromatography over silica gel using petroleum ether and ethylacetate as eluents (from 0-7% ethylacetate in petroleum ether) to afford compound **4** as colorless oil. Yield: 3.9 g (78 %). R_f: 0.56 (petroleum ether /EtOAc 9:1, ceric ammonium molybdate solution). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.58 (dd, *J* = 4.4, 2.8 Hz, 1H), 3.88 (ddd, *J* = 11.1, 7.0, 3.9 Hz, 1H), 3.74 (dt, *J* = 9.6, 6.9

Hz, 1H), 3.50 (ddd, *J* = 8.1, 6.5, 4.6 Hz, 1H), 3.46 – 3.34 (m, 3H), 1.92 – 1.78 (m, 3H), 1.79 – 1.66 (m, 1H), 1.63 – 1.49 (m, 6H), 1.46 – 1.25 (m, 12H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 99.01, 67.82, 62.52, 34.22, 32.97, 30.93, 29.88, 29.60, 29.57, 29.51, 28.88, 28.30, 26.36, 25.65, 19.86. HRMS (ESI^+) *m/z* calcd for $C_{15}H_{30}O_2Br$ $[M+H]^+$ 321.1429; observed 321.1381.

2-(pentadec-11-ynyloxy)-tetrahydro-2H-pyran (5)

To a stirred solution of 1-pentyne (2.3 mL, 23.2 mmol) in anhydrous toluene (80 mL) at -78°C under an argon atmosphere was added a solution of *t*-BuLi (12.3 mL, 20.9 mmol, 1.7M in pentane) dropwise over a period of 30 min (note; the rate of addition was adjusted so as to keep the internal temperature below -65°C). The resulting mixture was allowed to stir for 90 min at the same conditions, and subsequently treated with *N*,*N*'-dimethylpropyleneurea (DMPU) (6.3 ml, 52.25 mmol), followed by dropwise addition of a solution of compound 4 (3.7 g, 11.6 mmol, in 50 mL of anhydrous THF) over a period of 20 min. After the resulting reaction mixture was allowed to stir at -45°C for 1 h, gradually warmed to ambient temperature and stirred for additional 6 h (as monitored by TLC analysis, Pet. ether / EtOAc 9:1; $R_{f(adduct)}$= 0.56; $R_{f(product)}$=0.67; visualized with ceric ammonium molybdate solution), the reaction was cooled again to 0°C and carefully quenched with an ice-cold NH_4Cl solution (150 mL). The resulting mixture was allowed to warm gradually to ambient temperature and subsequently extracted with diethylether (3 × 100 mL). The organic layers were combined, sequentially washed with saturated $NaHCO_3$ solution (100 mL) and brine (150 mL), dried over anhydrous Na_2SO_4, and filtered. Removing of solvents under reduced pressure provided a brown residue which was immediately purified by flash column chromatography over silica gel using petroleum ether and ethylacetate as eluents (from 0-5% ethylacetate in petroleum ether) to afford compound 5 as colorless oil. Yield: 2.4 g (67 %). R_f: 0.52 (Pet. ether / EtOAc 95:5, visualized with ceric ammonium molybdate solution). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.57 (t, *J* = 3.5 Hz, 1H), 3.87 (ddd, *J* = 11.2, 7.5, 3.4 Hz, 1H), 3.73 (dt, *J* = 9.6, 6.9 Hz, 1H), 3.56 – 3.45 (m, 1H), 3.38 (dt, *J* = 9.6, 6.7 Hz, 1H), 2.13 (dtt, *J* = 9.4, 4.6, 2.3 Hz, 4H), 1.88 – 1.67 (m, 2H), 1.65 – 1.41 (m, 10H), 1.42 – 1.24 (m, 12H), 0.97 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 98.97, 80.51, 80.15, 67.82, 62.46, 30.92, 29.89, 29.67, 29.61, 29.31, 29.27, 28.98, 26.37, 25.65, 22.69, 20.90, 19.83, 18.88, 13.60.HRMS (ESI^+) *m/z* calcd for $C_{20}H_{36}O_2Na$ $[M+Na]^+$ 331.2613; observed 331.2603.

2-((Z)-pentadec-11-enyloxy)-tetrahydro-2H-pyran (6)

A stirred solution of compound 5 (2.2 g, 7.15 mmol) in a mixture of ethylacetate: DMF (95:5, 140 mL) at 0°C under an argon atmosphere was treated with ethylenediamine (EDA) (0.43 mL, 6.4 mmol), followed by a catalytic amount of Lindlar catalyst (100 mg). The reaction vessel was evacuated with the help of a pump and then backfilled with hydrogen gas via a hydrogen balloon with positive pressure. The resulting heterogeneous reaction mixture was allowed to stir for 6 h at the same conditions (as monitored by TLC analysis; Pet. ether/ EtOAc 95:5; $R_{f(adduct)}$= 0.52; $R_{f(product)}$=0.56; visualized with ceric ammonium molybdate solution), and subsequently filtered through a pad of Celite, which was rinsed several times with ethylacetate (3 × 50 mL). The filtrates were combined and concentrated under reduced pressure to afford an oily residue which was immediately chromatographed over silica gel using

petroleum ether and ethylacetate as eluents (from 0-5% ethylacetate in petroleum ether) to yield compound 6 as colorless oil (note; two times purification are suggested for more isomerically pure compound).

(Z)-pentadec-11-en-1-ol (7)

A stirred solution of compound 6 (1.85 g, 6 mmol) in absolute ethanol (30 mL) at ambient temperature was treated with a catalytic amount of pyridinium *p*-toluenesulfonate (PPTS) (0.15 g, 0.6 mmol, 10 mol%), and the resulting reaction mixture was allowed to heat at 62°C. After being stirred for 2 h at the same conditions (as monitored by TLC analysis for almost a complete deprotection, Silica gel; Pet. ether / EtOAc 9:1; $R_{f(adduct)}$= 0.67; $R_{f(product)}$=0.44; visualized with ceric ammonium molybdate solution), the reaction mixture was concentrated under reduced pressure. The resultant residue was partitioned between diethylether (100 mL) and saturated $NaHCO_3$ solution (80 mL), and the layers were separated. The aqueous layer was extracted several times with diethylether (3 × 80 mL), and the combined organic layers were washed with brine (150 mL), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. Flash column chromatography of the resultant residue over silica gel using cyclohexane and ethylacetate as eluents (from 10-15% ethylacetate in cyclohexane) afforded compound 7 as colorless oil. Yield: 0.92 g (68 %). R_f: 0.55 (Pet. ether/ EtOAc 4:1, visualized with ceric ammonium molybdate solution). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.36 (ddd, *J* = 5.7, 3.5, 2.2 Hz, 2H), 3.64 (t, *J* = 6.6 Hz, 2H), 2.08 – 1.97 (m, 4H), 1.62 – 1.51 (m, 2H), 1.41 – 1.26 (m, 16H), 0.91 (t, *J* = 7.3 Hz, 3H).^{13}C NMR (126 MHz, $CDCl_3$) δ 130.23, 129.78, 63.21, 32.94, 29.90, 29.74, 29.69, 29.66, 29.57, 29.43, 27.35, 25.88, 23.03, 13.94.HRMS (ESI^+) *m/z* calcd for $C_{15}H_{30}ONa$ $[M + Na]^+$ 249.2194; found 249.2187.

(Z)-15-bromopentadec-4-ene (8)

A stirred solution of alcohol 7 (0.8 g, 3.62 mmol) and CBr_4 (1.3 g, 4.0 mmol) in anhydrous dichloromethane (20 mL) at 0°C under an argon atmosphere was treated with a solution of triphenyl phosphine (1.1 g, 4.17 mmol) in anhydrous dichloromethane (5 mL) over a period of 20 min. After the resulting reaction mixture was allowed to stir for 1 h at 0°C, gradually warmed to ambient temperature, and stirred for additional 3 h (as monitored by TLC analysis; Pet. ether / EtOAc 95:5; $R_{f(adduct)}$= 0.39; $R_{f(product)}$=0.62; visualized with $KMnO_4$ solution), the solvent was removed under reduced pressure. Petroleum ether (100 mL) was subsequently added to the resultant oily residue and the mixture was stirred for 30 min at ambient temperature, during while a white precipitate was formed. The mixture was filtered through a pad of Celite to remove the formed triphenylphosphonium oxide, and the filtrate was concentrated *in vacuo*. This process was repeated (at least three times) until no further precipitate was formed. Purification of the obtained oily residue by flash column chromatography over silica gel using petroleum ether and ethylacetate as eluents (from 0-4% ethylacetate in petroleum ether) yielded compound 8 as colorless oil. Yield: 0.65 g (62 %). R_f: 0.62 (Pet. ether /EtOAc 95:5, visualized with $KMnO_4$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.37 (ddd, *J* = 5.6, 3.5, 1.9 Hz, 2H), 3.42 (t, *J* = 6.9 Hz, 2H), 2.01 (dt, *J* = 7.2, 4.1 Hz, 4H), 1.93 – 1.80 (m, 2H), 1.48 – 1.25 (m, 16H), 0.91 (t, *J* = 7.3 Hz, 3H).^{13}C NMR (126 MHz, $CDCl_3$) δ 130.22, 129.81, 34.22, 32.99, 29.90, 29.64, 29.57, 29.42, 28.91, 28.32, 27.35, 23.04, 13.97.HRMS (ESI^+) *m/z* calcd for $C_{15}H_{30}Br$ $[M +H]^+$ 289.1531; observed 289.1491. In order to prevent Z/E isomerization, this compound must be stored under argon atmosphere at -70°C.

2S,14Z)-tert-butyl -3-oxooctadec-14-en-2-ylcarbamate (10)

The Grignard reagent of (*Z*)-15-bromopentadec-4-ene (9) was synthesized as follows; A mixture of magnesium turnings (0.23 g, 11.25 mmol) in anhydrous Et_2O (10 mL) under an argon atmosphere was treated with 4 drops of 1,2-dibromoethane (in order to activate the magnesium), followed by a solution of (*Z*)-15-bromopentadec-4-ene (0.6 g, 2.25 mmol, in 5 mL of anhydrous Et_2O). The reaction mixture was allowed to stir for 90 min at 35°C to yield a transparent Grignard solution 9 which was immediately used in the next step.

Compound 10 was synthesized as follows: To a stirred solution of Weinreb amide derivative 2 (0.17 g, 0.75 mmol),which was prepared from L-alanine in two steps, in anhydrous mixture of DCM:Et_2O (2:1; 7.5 mL) at -20°C under an argon atmosphere was added dropwise a solution of methylmagnesium bromide (0.25 mL, 0.67 mmol, 3M in diethylether). The resulting mixture was stirred for 20 min at the same conditions, before a freshly prepared Grignard solution 9 was added dropwise within 10 min. After being stirred for 2 h at 35°C (as monitored by TLC analysis; Pet. ether/ EtOAc 7:3; $R_{f(adduct)}$= 0.24; $R_{f(product)}$=0.79; visualized with ceric ammonium molybdate solution), the reaction mixture was cooled again to 0°C and successfully quenched with an ice-cold 1M HCl solution (40 mL). The resulting mixture was subsequently extracted with diethylether (3 × 50 mL), and the combined organic phases were washed with saturated $NaHCO_3$ solution (80 mL) and brine (80 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The resultant residue was purified by flash column chromatography over silica gel using cylcohexane and ethylacetate as eluents (from 5-10% ethylacetate in cyclohexane) to provide ketone derivative 10 as pale yellow oil. Yield: 0.22 g (78 %). R_f: 0.57 (Pet. ether /EtOAc 9:1, visualized with 1.3% ninhydrine). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.36 (ddd, *J* = 5.8, 3.7, 1.5 Hz, 2H), 5.29 (d, *J* = 6.1 Hz, 1H), 4.35 – 4.27 (m, 1H), 2.49 (qd, *J* = 17.0, 8.6 Hz, 2H), 2.05 – 1.96 (m, 4H), 1.63 – 1.56 (m, 2H), 1.44 (s, 9H), 1.40 – 1.24 (m, 18H), 0.90 (t, *J* = 7.4 Hz, 3H).^{13}C NMR (126 MHz, $CDCl_3$) δ 209.92, 155.31, 130.23, 129.78, 79.79, 55.15, 39.34, 29.89, 29.69, 29.65, 29.57, 29.50, 29.43, 29.34, 28.48, 27.35, 23.71, 23.03, 18.10, 13.96. HRMS (ESI^+) *m/z* calcd for $C_{23}H_{43}NO_3Na$ $[M+Na]^+$ 404.3141; observed 404.3127.

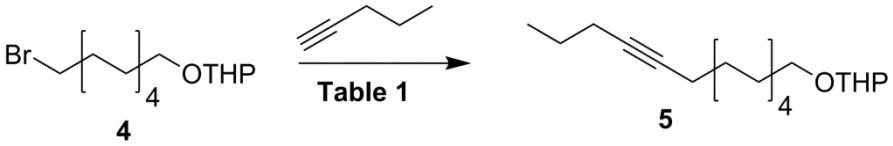
(2S,3R,14Z)-tert-butyl -3-hydroxyoctadec-14-en-2-ylcarbamate (11)

Lithium tri-(*tert*-butoxy)-aluminum hydride (TBLAH) (0.12 g, 0.5 mmol) was added in portions to a stirred solution of ketone 10 (0.11 g, 0.29 mmol) in anhydrous ethanol (2 mL) at -78°C under an argon atmosphere; the addition rate was adjusted so as to keep the internal temperature below -65°C and it took 20 min to complete. The resulting reaction mixture was allowed to stir for 40 min at -78°C (as monitored by TLC analysis; Pet. ether /EtOAc 4:1; $R_{f(adduct)}$ = 0.67; $R_{f(product)}$ = 0.5; visualized with 1.3% ninhydrine), and subsequently quenched with an ice-cold 1M HCl solution (60 mL); the quenching rate was adjusted so as to keep the internal temperature below -55°C. After being stirred for 1 h at ambient temperature, the resulting mixture was diluted with ethylacetate (80 mL) and the layers were separated. The aqueous layer was extracted with ethylacetate (3 × 50 mL), and the combined organic layers were washed with saturated $NaHCO_3$ solution (100 mL) and brine (100 mL), dried over anhydrous Na_2SO_4, filtered and concentrated. Purification of the obtained crude mixture by flash column chromatography over silica gel using cyclohexane and ethylacetate as eluents (from 10 -15 % ethylacetate in cyclohexane) provided the desired

compound 9 as a white waxy semisolid. Yield: 70 mg (63 %, note, 20% of starting material was recovered). R_f: 0.5 (Pet. ether/EtOAc 4:1, visualized with 1.3% ninhydrine). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.36 (ddd, *J* = 5.8, 4.1, 2.2 Hz, 2H), 4.78 (d, *J* = 4.0 Hz, 1H), 3.74 – 3.59 (m, 2H), 2.06 – 1.98 (m, 4H), 1.45 (s, 9H), 1.40 – 1.24 (m, 18H), 1.08 (d, *J* = 6.8 Hz, 3H), 0.91 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$)δ 156.01, 130.25, 129.76, 79.56, 74.61, 50.72, 33.60, 29.91, 29.81, 29.75, 29.71, 29.70, 29.68, 29.45, 29.43, 28.55, 27.36, 26.19, 23.03, 14.48, 13.95. HRMS (ESI^-) *m/z* calcd for $C_{23}H_{44}NO_3$ $[M-H]^-$ 382.3321; observed 382.3327.

c) Tables.

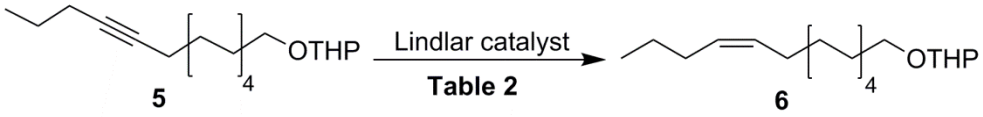
Supplementary Table 2 Optimization studies for the coupling reaction of 4 with lithiated 1-pentyne.



entry	1-pentyne (equiv)	Li-reagent (equiv)	additive (equiv)	reaction conditions	Yield[b]
1[a]	1.0	n-BuLi (1.0)	HMPA (1.3)	THF, 0C-rt, 4h	nd
2	1.0	*tert*-BuLi (1.0)	HMPA (1.3)	THF, 0C-rt, 4h	11%
3	2.0	*n*-BuLi (2.1)	DMPU (2.5)	Toluene, -78°C-0°C, 4h	28%
4	2.3	*tert*-BuLi (2.5)	DMPU (4.5)	Toluene, -78°C-0°C, 6h	41%
5[c]	2.3	*tert*-BuLi (2.5)	DMPU (9.25)	Toluene, -78°C-rt, 9h	67%

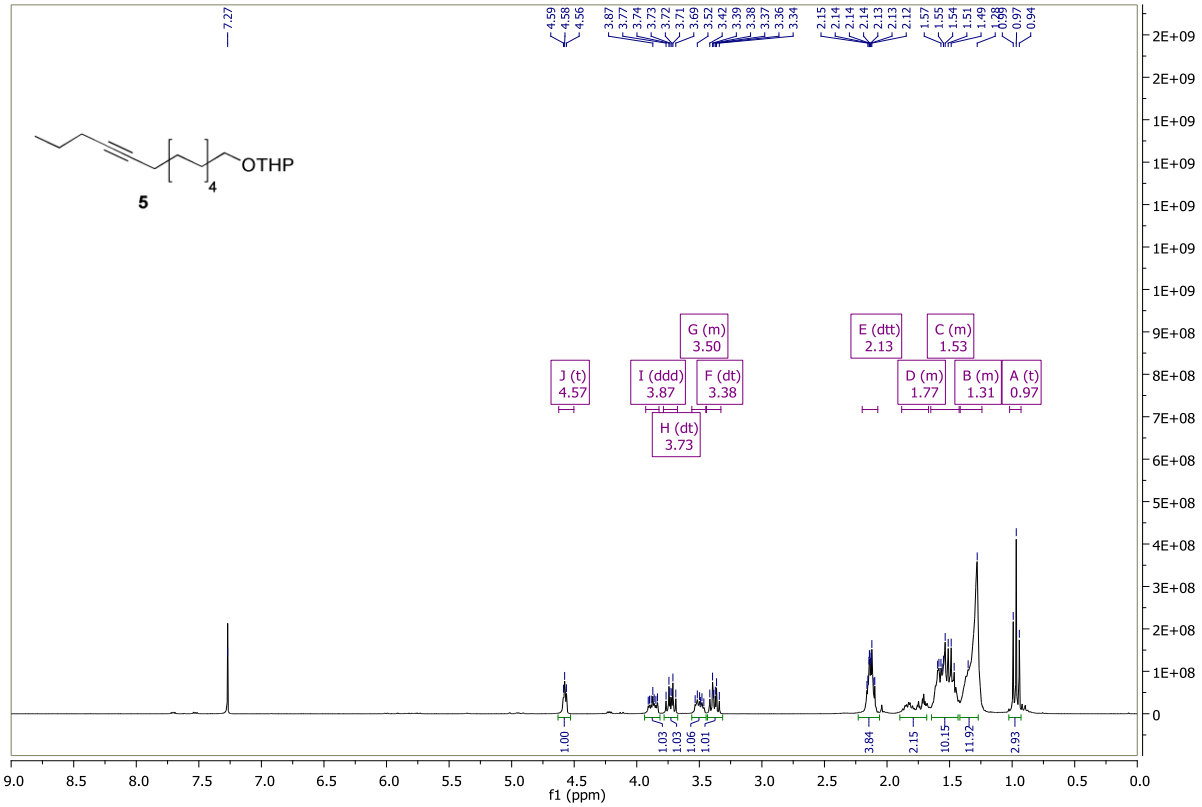
(a) Starting material was decomposed.
(b) Yield was determined after flash chromatography.
(c) Traces of moisture in the reaction media dramatically decrease the yield of the coupling product.

Supplementary Table 3: Optimization studies of the stereoselective reduction of 5 with Lindlar catalyst



entry	additive (equiv)	reaction conditions	Yield[a] (Z-isomer: by-products)[d]
1	---	EtOAc, r.t, 16h	79% (4:1)
2	quinolone (1.5)	Hexane, 0°C, 9h	complex mixture
3[b]	EDA (0.5)	DMF, rt, 12h	39% (15:1)
4[c]	EDA (1.5)	EtOAc, 0°C, 6h	57% (only Z-isomer)
5[b,c]	EDA (0.9)	EtOAc, 5% DMF, 0°C, 6h	86% (only Z-isomer)

(d) Yield was determined after column chromatography.
(e) Catalyst added portionwise over 3h.
(f) Reaction run in 0.05M solution
(g) Results based on NMR analysis.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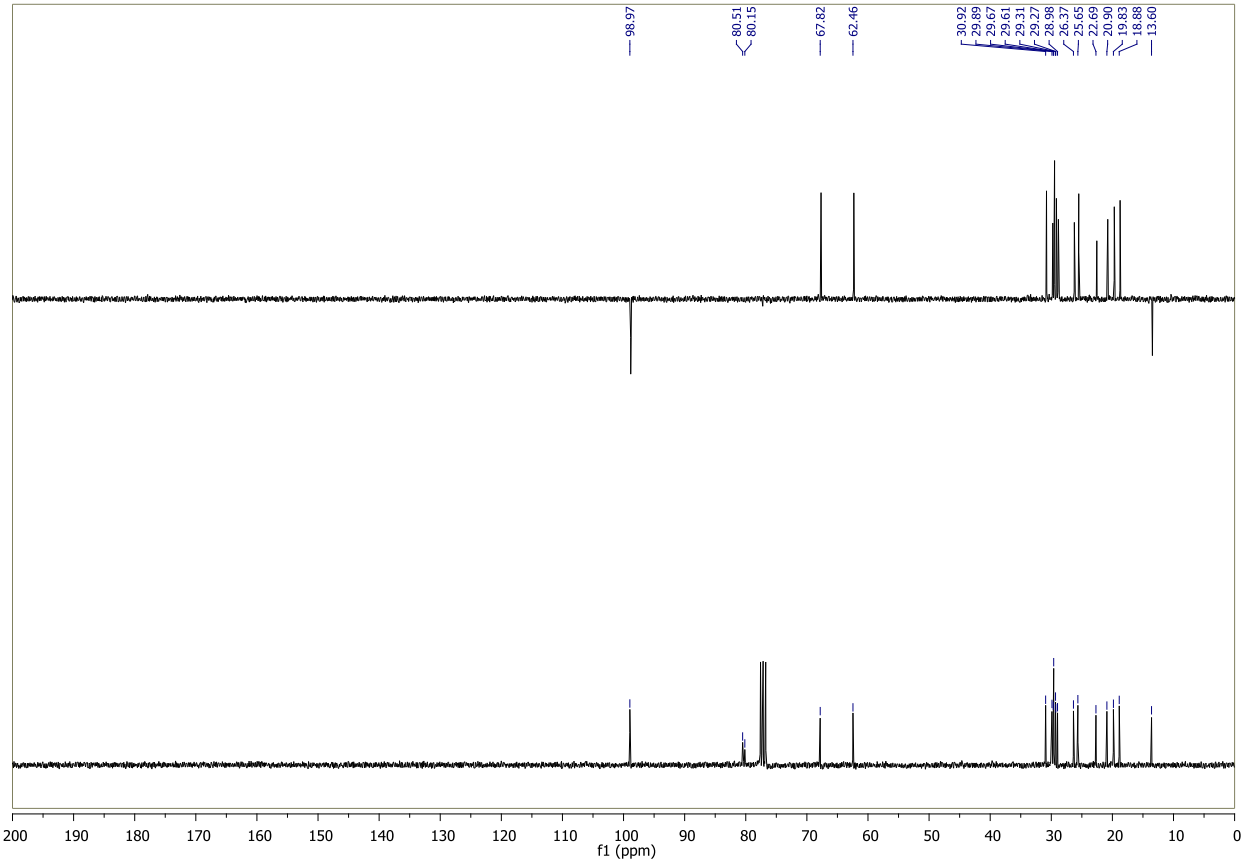
4
5
J (t) 4.57
I (ddd) 3.87
H (dt) 3.73
F (dt) 3.38
G (m) 3.50
E (dtt) 2.13
D (m) 1.77
B (m) 1.31
C (m) 1.53
A (t) 0.97
f1 (ppm)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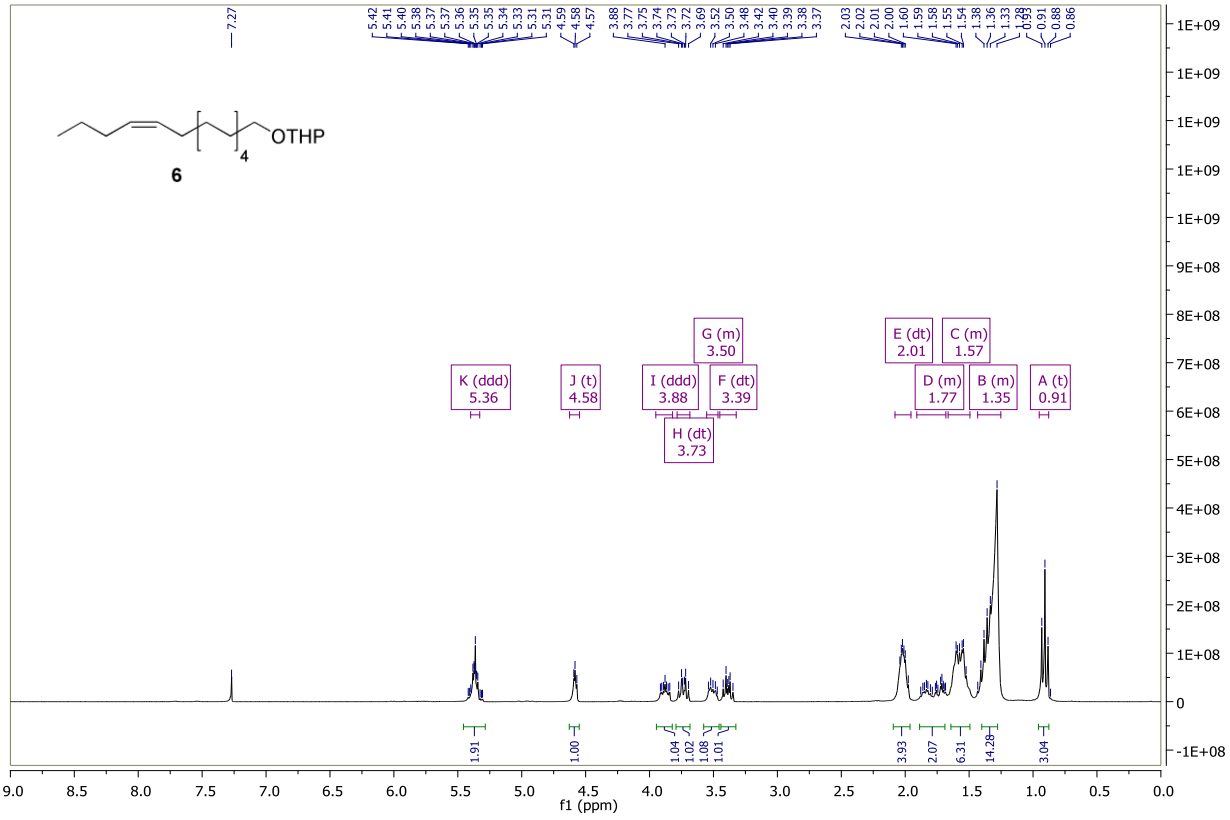
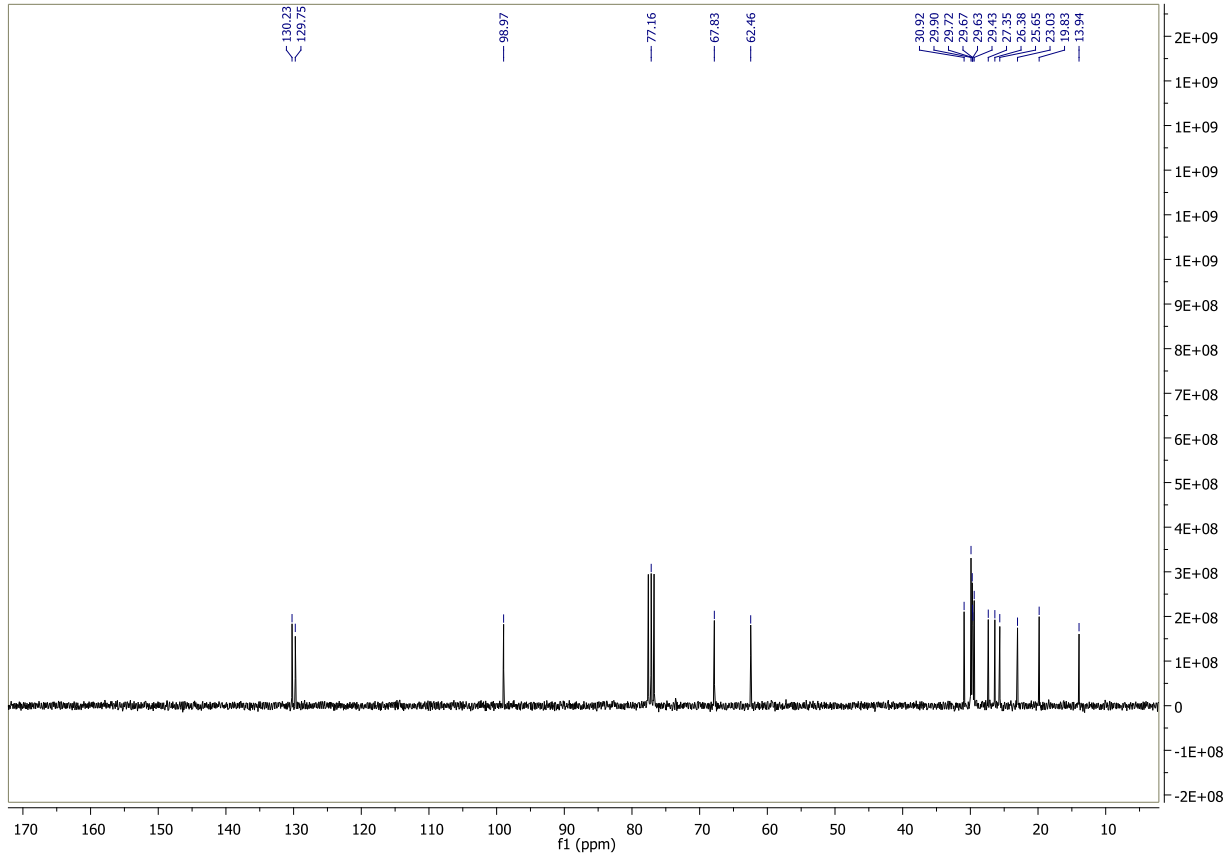

OTHP
4
6
K (ddd) 5.36
J (t) 4.58
I (ddd) 3.88
H (dt) 3.73
F (dt) 3.39
G (m) 3.50
E (dt) 2.01
D (m) 1.77
C (m) 1.57
B (m) 1.35
A (t) 0.91
f1 (ppm)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129.75
98.97
77.16
67.83
62.46
30.92
29.90
29.72
29.67
29.63
29.43
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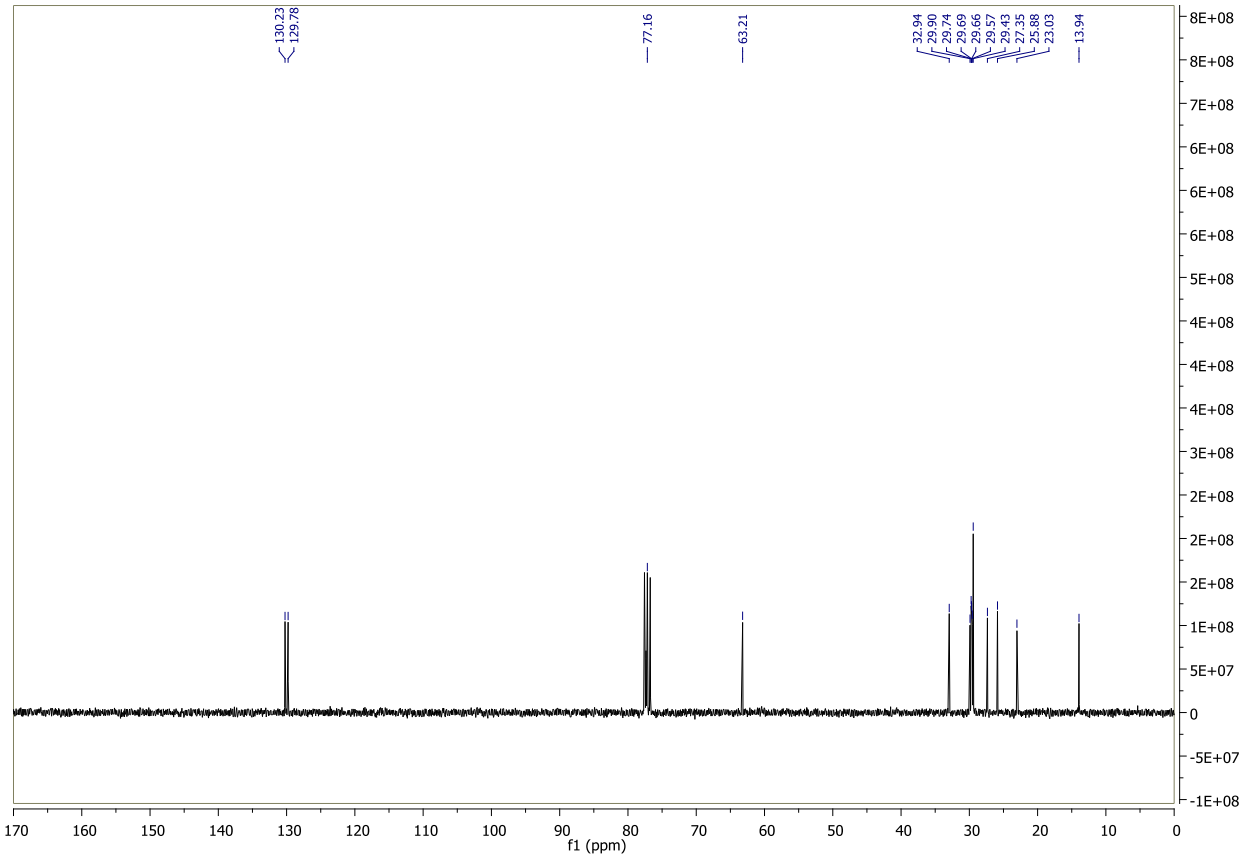
25.65
23.03
19.83
13.94
f1 (ppm)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129.78
77.16
63.21
32.94
29.90
29.74
29.69
29.66
29.57
29.43
27.35
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23.03
13.94
8E+08
8E+08
7E+08
6E+08
6E+08
6E+08
5E+08
4E+08
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
-5E+07
-1E+08
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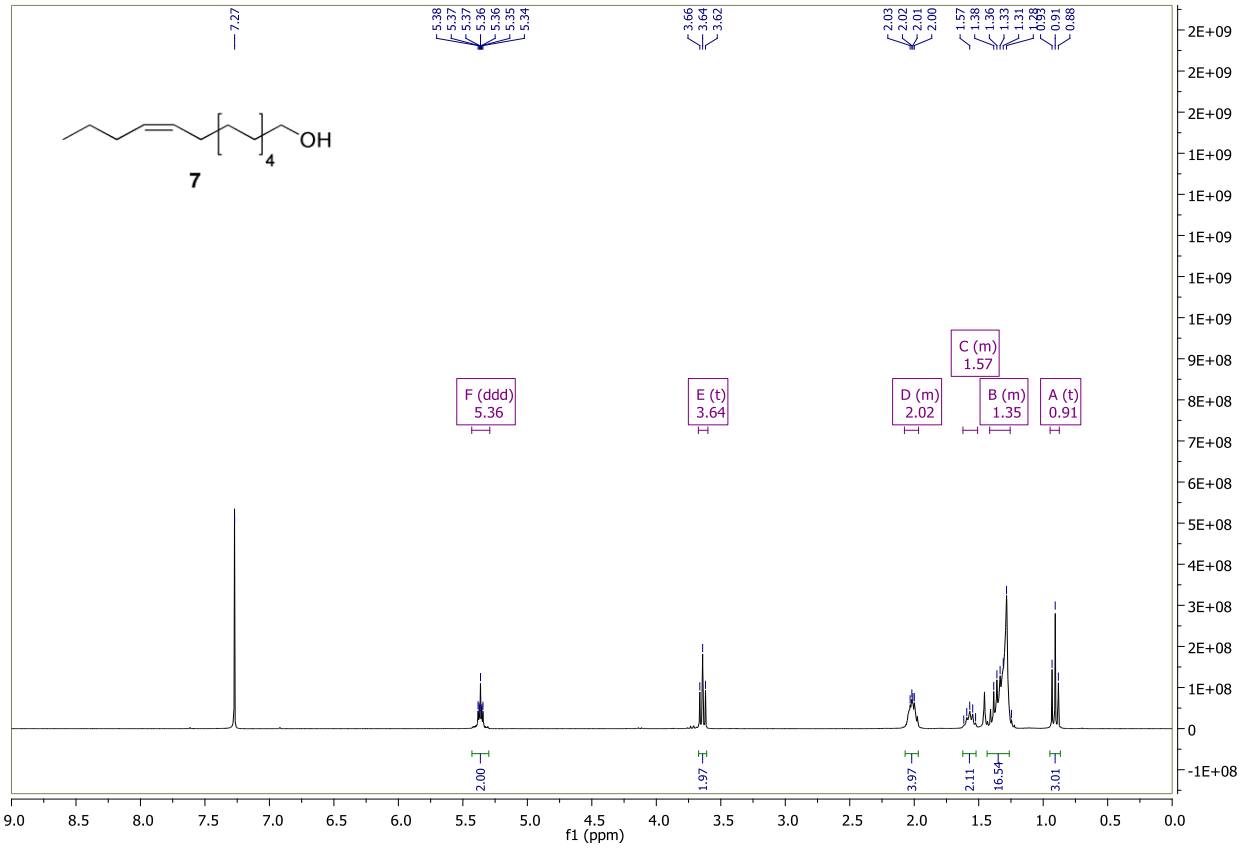
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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5.37
5.37
5.36
5.36
5.35
5.34
3.66
3.64
3.62
2.03
2.02
2.01
2.00
1.57
1.38
1.36
1.33
1.31
1.28
0.93
0.91
0.88
OH
4
7
F (ddd)
5.36
E (t)
3.64
D (m)
2.02
C (m)
1.57
B (m)
1.35
A (t)
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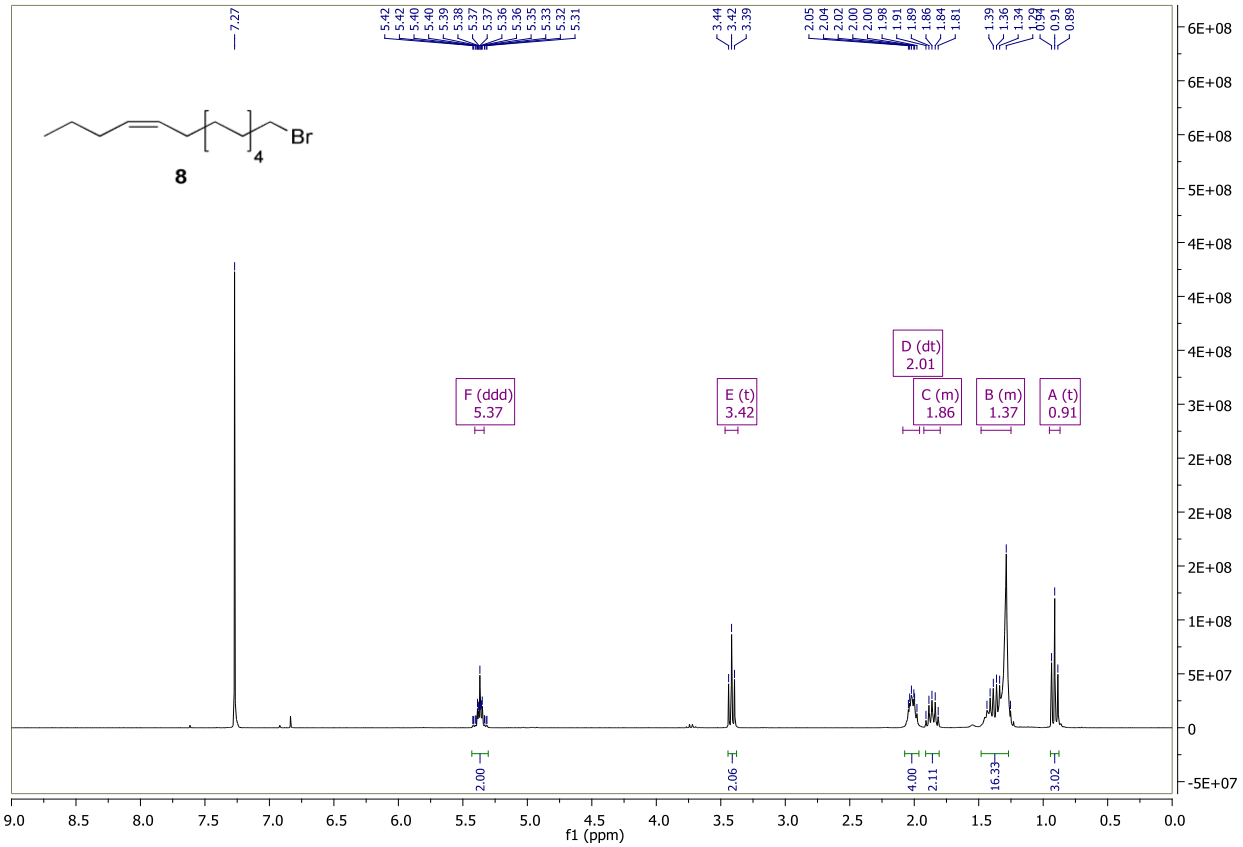
2.00
1.97
3.97
2.11
16.54
3.01
f1 (ppm)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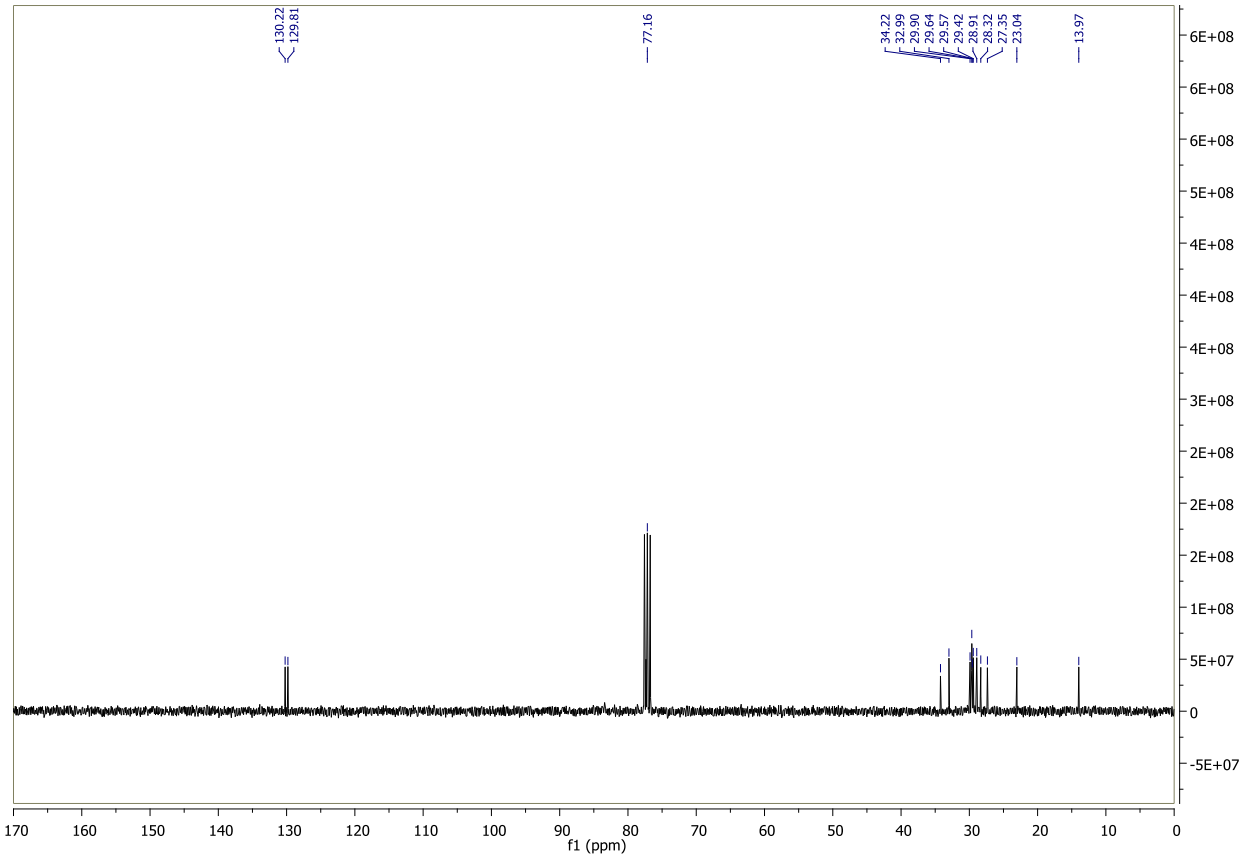
5.33
5.32
5.31
3.44
3.42
3.39
2.05
2.04
2.02
2.00
2.00
1.98
1.91
1.89
1.86
1.84
1.81
1.39
1.36
1.34
0.94
0.91
0.89
Br
4
8
F (ddd)
5.37
E (t)
3.42
D (dt)
2.01
C (m)
1.86
B (m)
1.37
A (t)
0.91
2.00
2.06
4.00
2.11
16.33
3.02
6E+08
6E+08
6E+08
5E+08
4E+08
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
-5E+07
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)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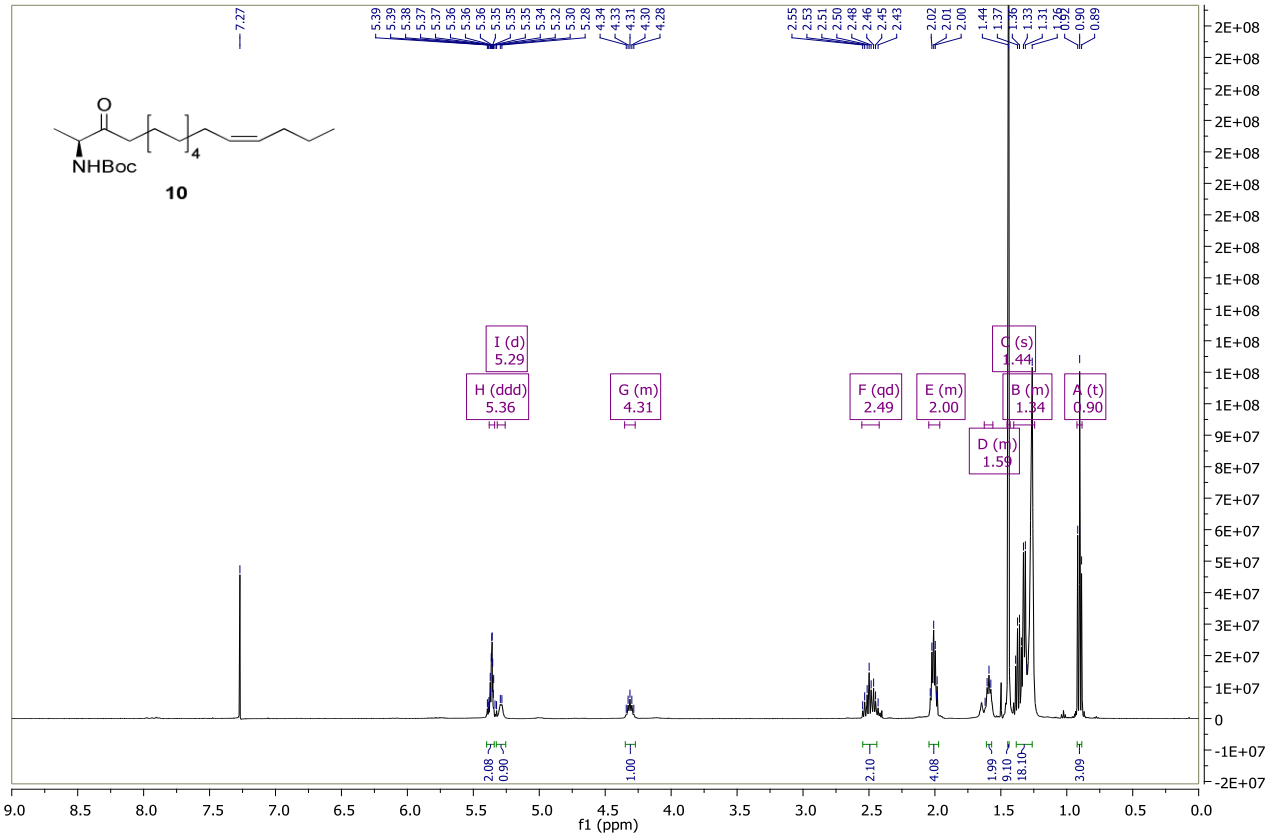
77.16
34.22
32.99
29.90
29.64
29.57
29.42
28.91
28.32
27.35
23.04
13.97
6E+08
6E+08
6E+08
5E+08
4E+08
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
-5E+07
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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G (m) 4.31
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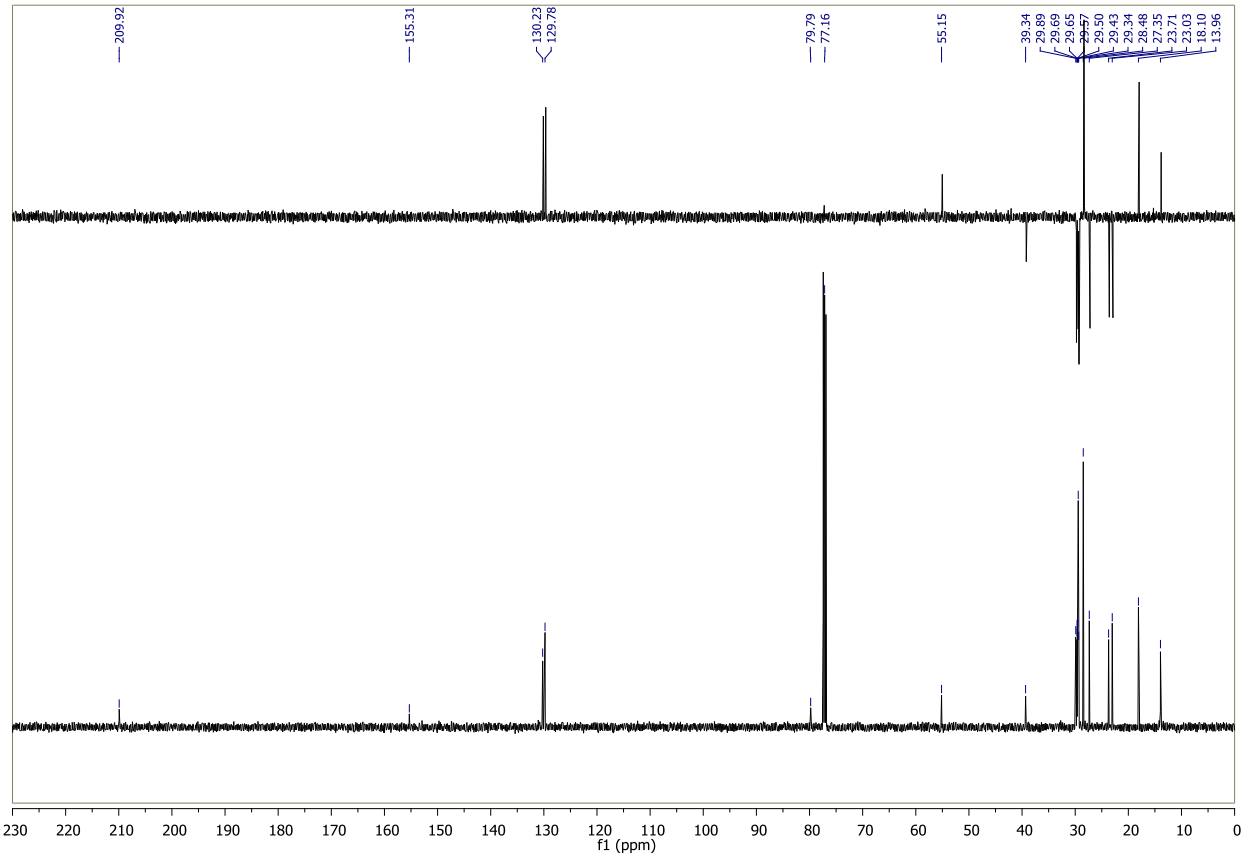
E (m) 2.00
D (m) 1.59
C (s) 1.44
B (m) 1.34
A (t) 0.90
f1 (ppm)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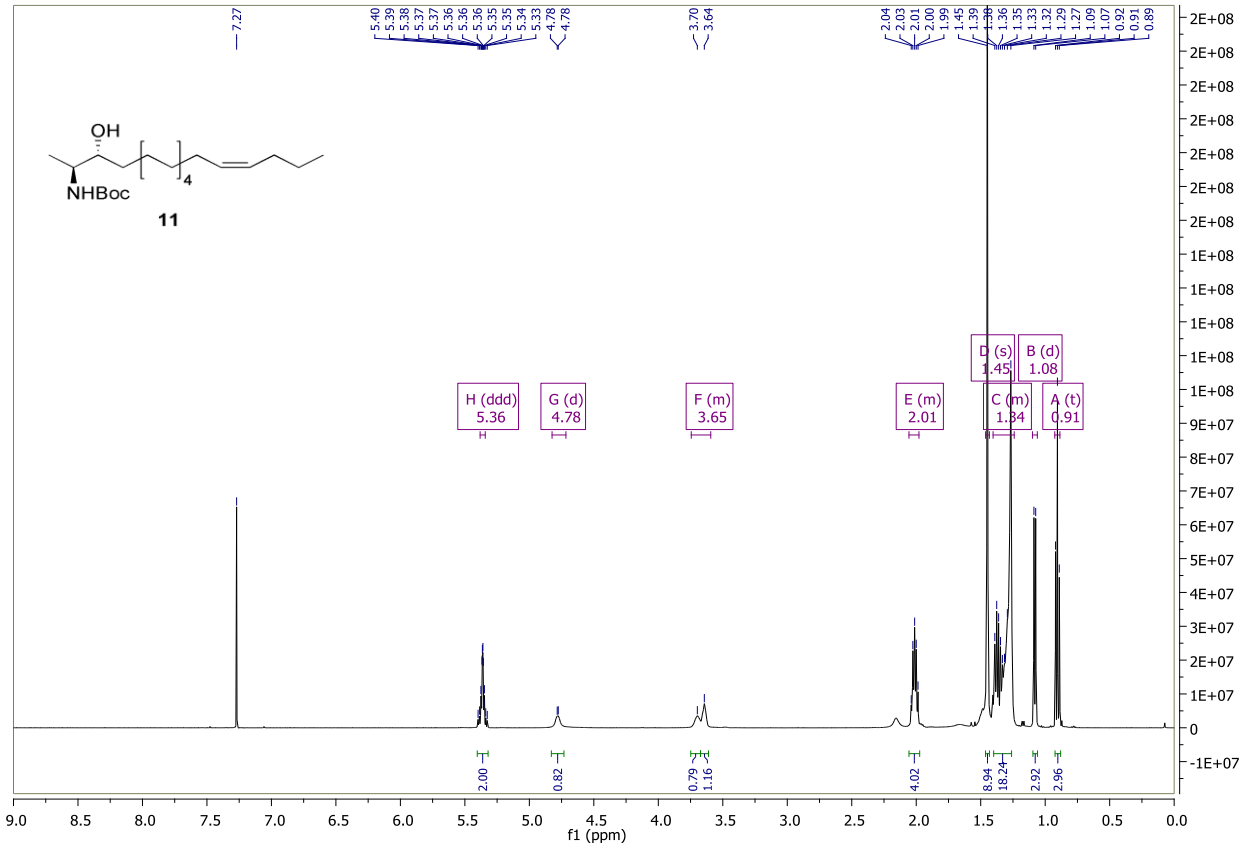
129.78
79.79
77.16
55.15
39.34
f1 (ppm)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NHBoc
4
11
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G (d) 4.78
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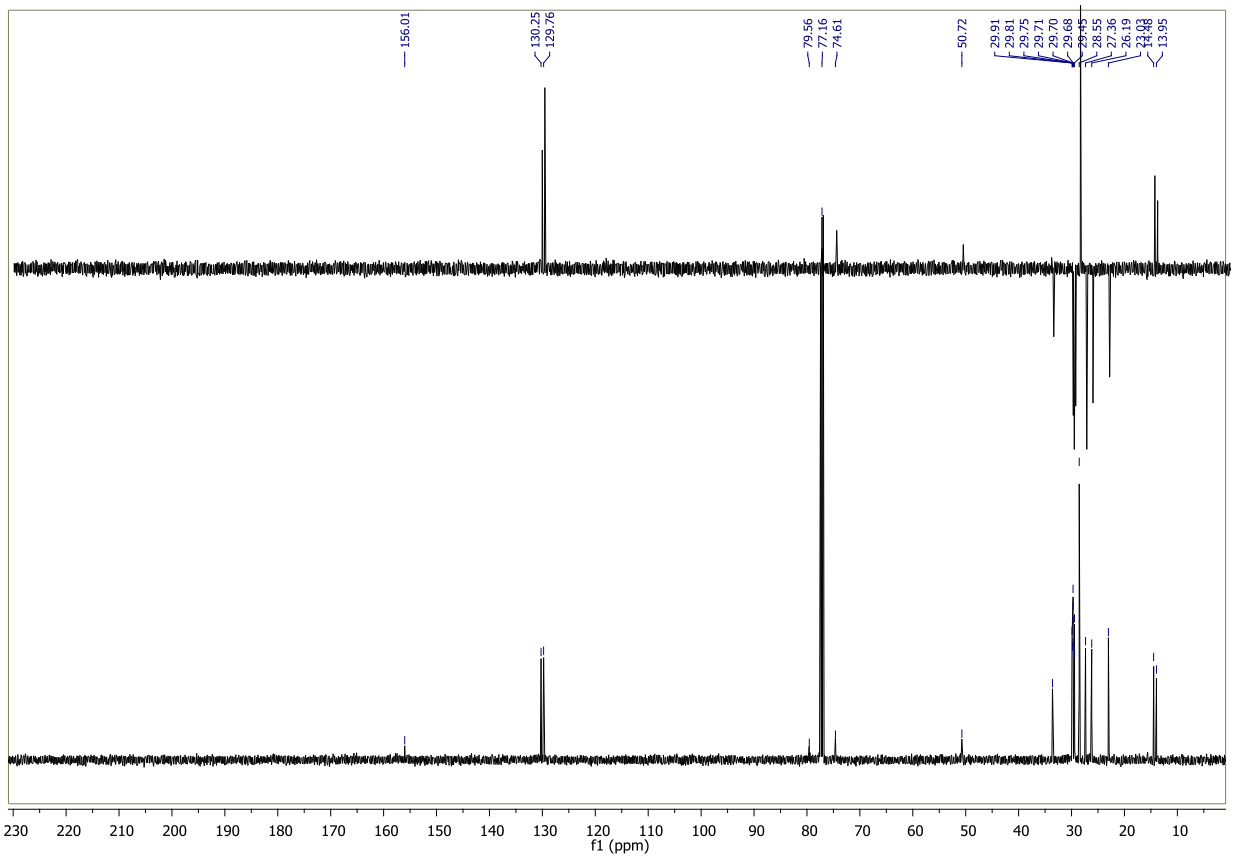
D (s) 1.45
C (m) 1.34
B (d) 1.08
A (t) 0.91
f1 (ppm)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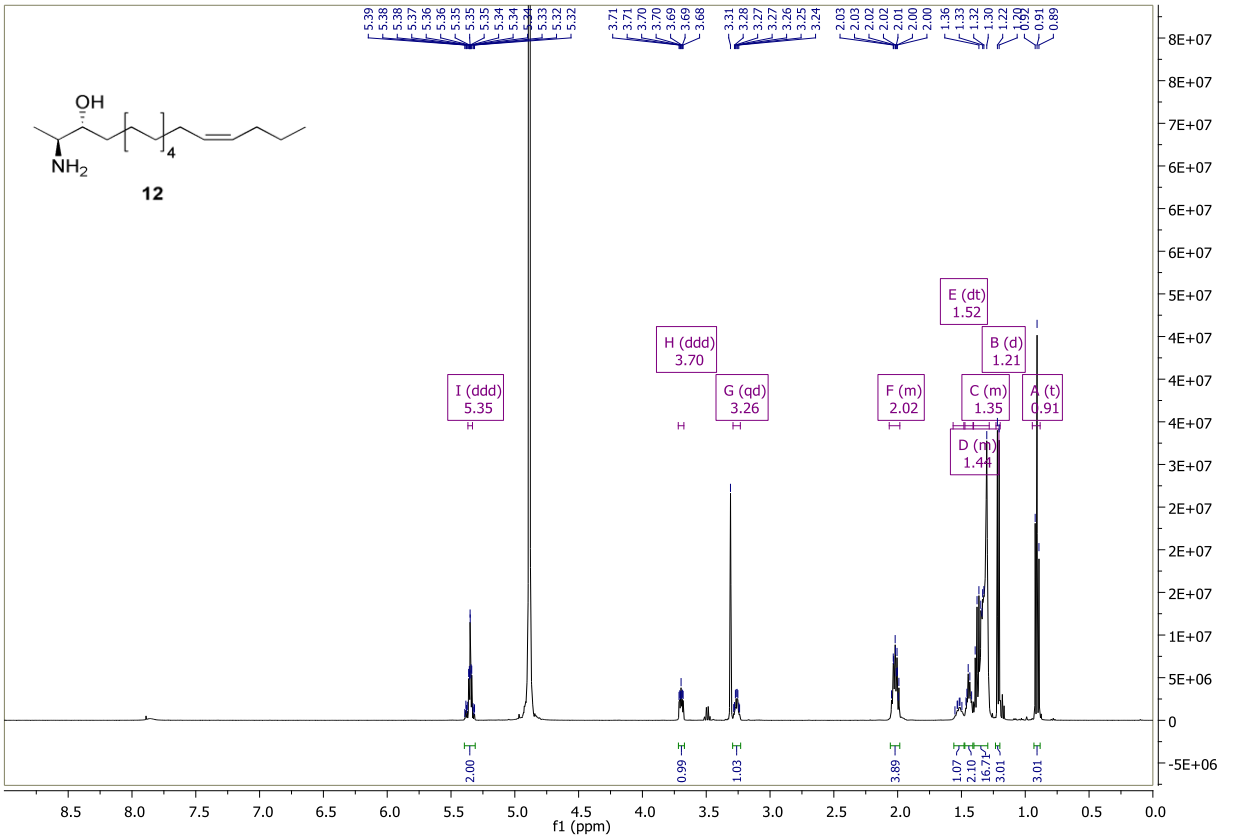
77.16
74.61
50.72
29.91
29.81
29.75
29.71
29.70
29.68
29.45
28.55
27.36
26.19
13.95
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)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NH2
4
12
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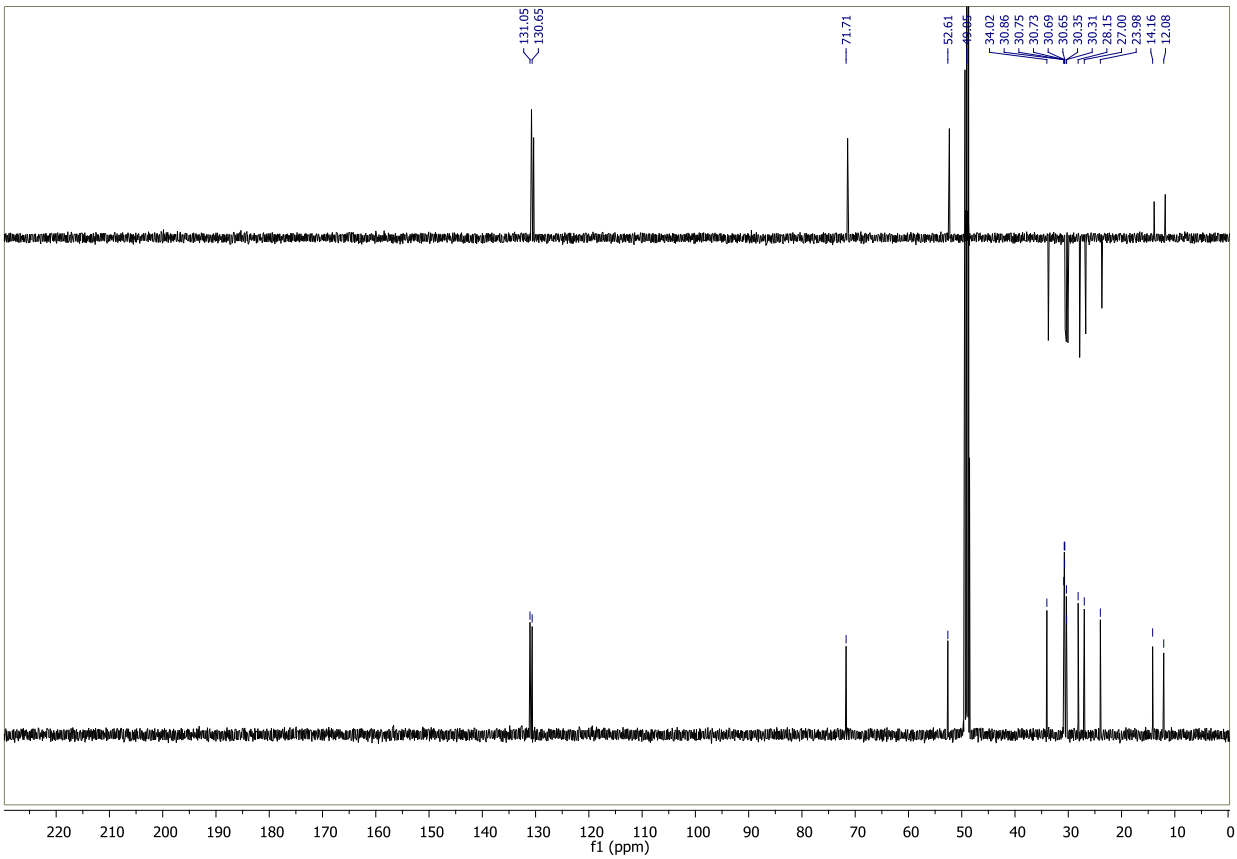
H (ddd) 3.70
G (qd) 3.26
F (m) 2.02
E (dt) 1.52
B (d) 1.21
C (m) 1.35
A (t) 0.91
D (m) 1.44
f1 (ppm)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30.35
30.31
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27.00
23.98
14.16
12.08
f1 (ppm)