Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Expeditious synthesis of polyacetylenic water hemlock toxins and their effects on the major $GABA_A$ receptor isoform

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1 Contents

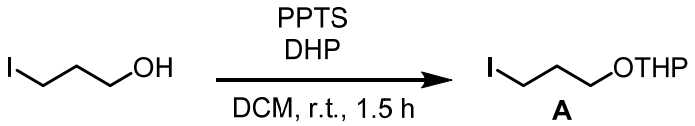
2 General information

Unless otherwise stated, all glassware was oven dried before use and all reactions were carried out under an Argon atmosphere using standard Schlenk-techniques. Dry solvents were purchased from Acros Organics or Sigma-Aldrich and used without further purification. All reagents were purchased from commercial sources and were used without further purification unless otherwise stated. Reaction progress was monitored by thin layer chromatography (TLC) performed on aluminum plates coated with Kieselgel F254 with 0.2 mm thickness. Visualization was achieved by ultraviolet light (254 nm) or by staining with potassium permanganate. Flash column chromatography was performed using silica gel 60 (230-400 mesh, Merck ans co.). Neat infra-red spectra were recorded using a Perkin-Elmer Spectrum 100 FT-IR spectrometer. Mass spectra were obtained using a Finnigan MAT 8200 (70 eV), an Agilent 5973 (70 eV), using electrospray ionization (ESI) or electron impact ionization (EI). All ^{1}H NMR, ^{13}C NMR NMR were recorded on a BrukerAV-400 or AV-600 spectrometer in Chloroform-d_1. Chemical shifts are given in parts per million (ppm), referenced to tetramethylsilane using the solvent peak as internal standard ($CDCl_3$: ^{1}H = 7.26 ppm, ^{13}C = 77.16 ppm). Coupling constants were quoted in Hz. ^{1}H-NMR splitting patterns were designated as singlet (s), doublet (d), triplet (t), quartet (q), pentet (p), sextet (se), septet (sep), octet (o) or combinations thereof. Splitting patterns that could not be interpreted were designated as multiplet (m).

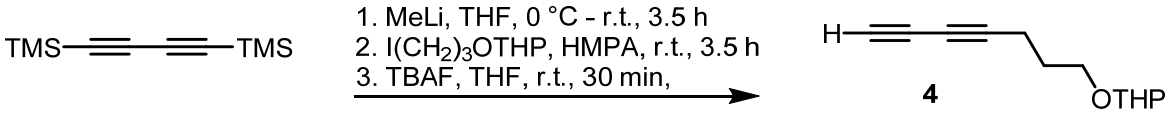
3 Synthesis of (*S*) and Racemic Virol A (1)

3.1 (S)-Virol A (1)

3.1.1 2-(hepta-4, 6-diyn-1-yloxy) tetrahydro-2H-pyran (4)



3-Iodopropan-1-ol (1.0 equiv., 0.48 mL, 5.0 mmol), pyridinium p-toluenesulfonate (PPTS) (0.15 equiv., 188.0 mg, 0.75 mmol) and 3, 4-Dihydro-2H-pyrane (DHP) (1.5 equiv., 0.68 mL, 7.5 mmol) were dissolved in dry DCM (10 mL) in a 25 mL round bottom flask. After purging with Argon, the mixture was stirred for 1.5 h at room temperature while covered with aluminium foil. The mixture was concentrated under reduced pressure and loaded directly on a column (silica gel, 0-3 % Et_2O in heptane) to give the product **A** in 88% yield. The procedure was adapted[1] and the spectral data is in accordance with the literature[2]. **^{1}H NMR (400 MHz, $CDCl_3$):** δ = 4.62-4.59 (m, 1H), 3.91-3.84 (m, 1H), 3.81 (dt, *J* = 10.0, 5.9 Hz, 1H), 3.56-3.49 (m, 1H), 3.54 (dt, *J* = 10.0, 5.9 Hz, 1H), 3.30 (td, *J* = 6.9, 1.1 Hz, 2H), 2.13-2.06 (m, 2H), 1.86-1.76 (m, 1H), 1.76-1.66 (m, 1H), 1.62-1.48 (m, 4H) ppm.

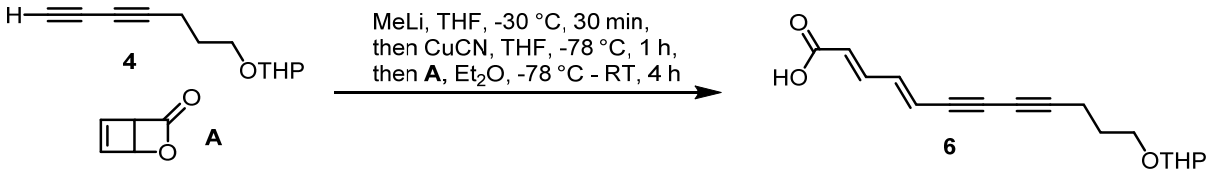


1, 4-Bis-(trimethylsilyl)-1, 3-budadiyne (1.0 equiv., 5.15 g, 5.3 mL, 26.5 mmol) was dissolved in dry THF (50 mL) in a flame-dried Schlenk tube. MeLi (1.6 M in ether, 1.0 equiv., 16.6 mL, 26.5 mmol) was added dropwise at 0 °C and stirred for 3.5 hours at room temperature. Iodide **A** (1.0 equiv., 7.16 g, 26.5 mmol), dissolved in hexamethylphosphoramide (HMPA) (2.0 equiv., 9.2 mL, 53 mmol), was added dropwise and stirred overnight at room temperature. The mixture was quenched with brine (50 mL) and extracted with DCM (2 x 100 mL). The combined organic layers were dried with $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was redissolved in dry THF (50 mL), and tetra-n-butylammonium fluoride (TBAF) (1 M in THF, 3.0 equiv., 79.5 mL, 79.5 mmol) was added slowly at room temperature and stirred for 30 min. After removal of the solvent *in vacuo*, the crude product was dissolved in DCM (100 mL) and washed with water (100 mL). After extracting the aqueous layer with DCM (100 mL), the combined organic layers were dried with $MgSO_4$ and concentrated under reduced pressure. Column chromatography (silica gel, 0-3% EtOAc in heptane) afforded 3.67 g (72%) of the product **4** as a red oil. The spectral data

[1] Gorske, B. C.; Mbofana, C. T.; Miller, S. J. *Org. Lett.*, **2009**, *11 (19),* 4318–4321.
[2] Miura, K.; Fujisawa, N.; Saito, H.; Wang, D.; Hosomi, A. *Org. Lett.*, **2001**, *3 (16),* 2591–2594.

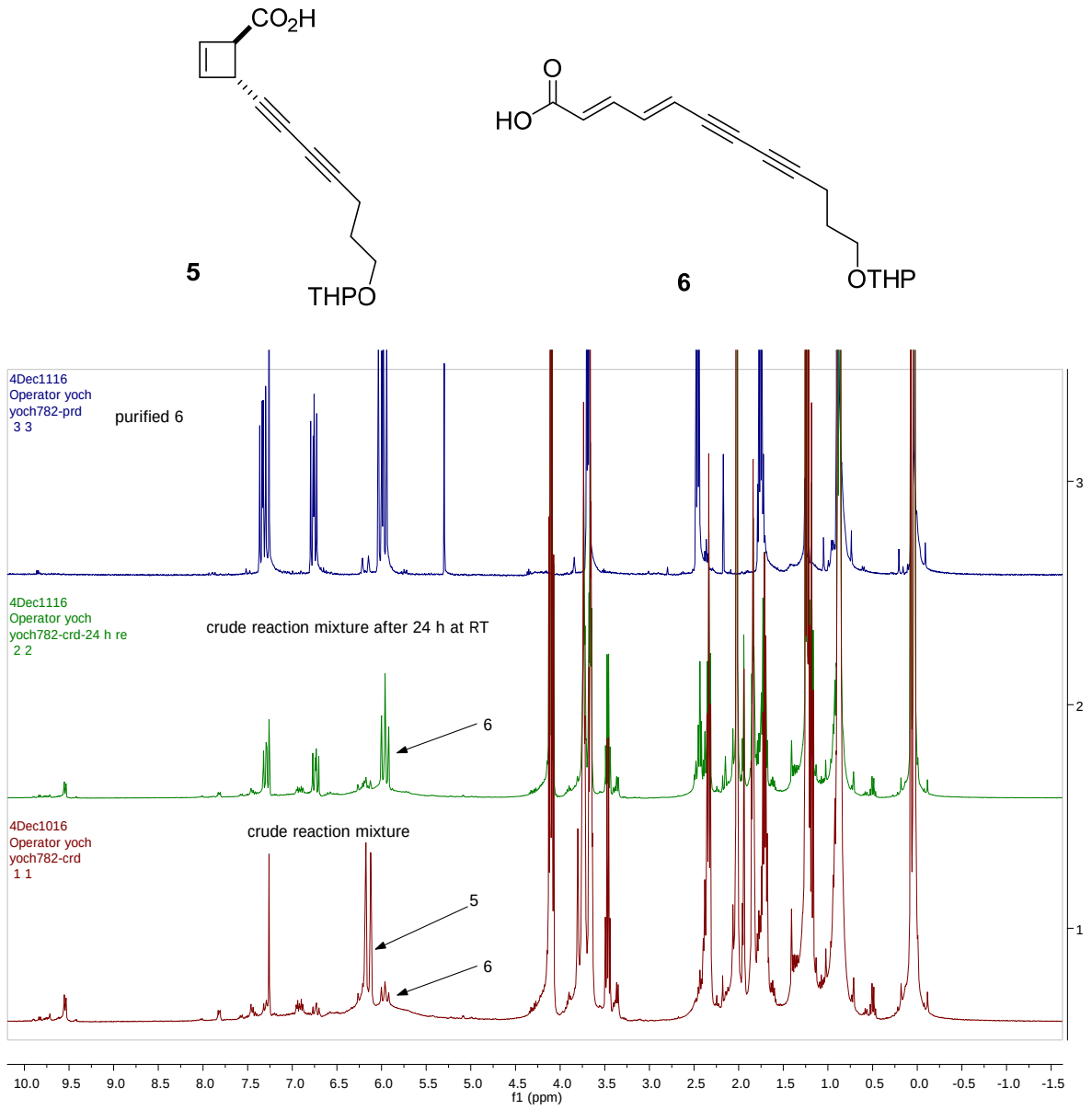
is in accordance with the literature[3]. **^{1}H NMR (600 MHz, $CDCl_3$):** δ = 4.61-4.57 (m, 1H), 3.91-3.78 (m, 2H), 3.56-3.44 (m, 2H), 3.40 (bt, *J* = 7.0 Hz, 2H), 1.95 (t, *J* = 1.2 Hz, 1H), 1.87-1.79 (m, 3H), 1.75-1.62 (m, 1H), 1.61-1.48 (m, 4H) ppm.

3.1.2 (2*E*, 4*E*)-12-((tetrahydro-2H-pyran-2-yl) oxy) dodeca-2, 4-dien-6, 8-diynoic acid (6)



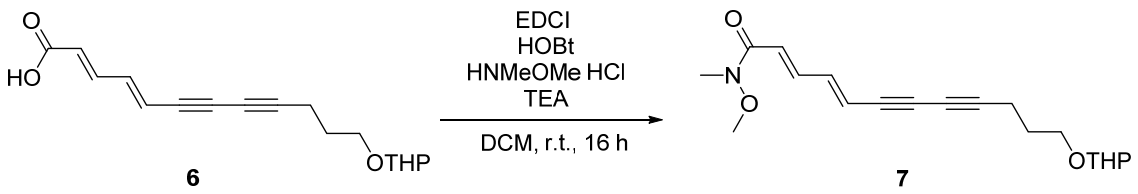
Diyne **4** (2.0 equiv., 700 mg, 3.64 mmol) was dissolved in dry THF (4.0 mL) in a flame-dried Schlenk tube and cooled to -40 °C. MeLi (1.6 M in ether, 2.0 equiv., 2.4 mL, 3.64 mmol) was added and the mixture was stirred for 30 minutes, before added via canula to a solution of CuCN (1.0 equiv., 163.0 mg, 1.82 mmol) dry THF (4.0 mL) at -78 °C, which was stirred for 1 h at -78 °C. Lactone **3** (0.1 M in ether, 1.0 equiv., 18.2 mL, 1.82 mmol) was added dropwise and the mixture was allowed to slowly warm up to room temperature. The slurry was quenched after warming to room temperature with water (80 mL), acidified to pH 2 with 1 M HCl and extracted with DCM (2 x 100 mL). The combined organic layers were dried with $MgSO_4$ and concentrated under reduced pressure. The crude mixture was allowed to stay at room temperature for 1 day. Purification by column chromatography (silica gel, EtOAc/heptane/HOAc = 20:77:3) afforded 429 mg (82%) of product **6** as white solid. **^{1}H NMR (600 MHz, $CDCl_3$):** δ = 7.23 (dd, *J* = 15.3, 11.6 Hz, 1H), 6.76 (dd, *J* = 15.4, 11.5 Hz, 1H), 6.01 (d, *J* = 15.5 Hz, 1H), 5.97 (d, *J* = 15.3 Hz, 1H), 4.60 (m, 1H), 3.89-3.79 (m, 2H), 3.54-3.50 (m, 1H), 3.48 (dt, *J* = 9.9, 6.1 Hz, 1H), 2.50 (bt, *J* = 7.0 Hz, 2H), 1.88-1.78 (m, 3H), 1.74-1.68 (m, 1H), 1.62-1.49 (m, 4H) ppm. **^{13}C-NMR (150 MHz, $CDCl_3$):** δ = 169.7, 144.9, 140.9, 122.4, 119.7, 99.0, 88.0, 81.7, 73.4, 65.8, 65.4, 62.4, 30.8, 28.5, 25.6, 19.7, 16.9 ppm. **FTIR ($CHCl_3$, cm^{-1}):** 2950, 2925, 2854, 1673, 1618, 1344, 1275, 1263, 1138, 1075, 1034, 1001, 764, 751, 528. **HRMS (ESI) (m/z):** calculated for $[M - H^+]^-$ $(C_{17}H_{19}O_4)^-$: 287.1289, found: 287.1281.

[3] Uwai, K.; Oshima, Y. *Tetrahedron* **1999**, *55*, 9469-9480;



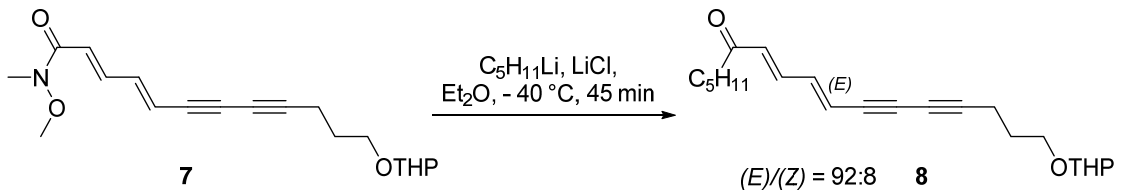
As can be seen from the crude ^{1}H NMR, the ring opened product **6** was formed already after the work up, despite **5** was the major product. After 24 h at RT, compound **6** was almost the major product can been seen on crude ^{1}H NMR. Due to the meta-stability of **5** and also the close Rf to **6**, we are not able to isolate the clean **5**.

3.1.3 (2*E*, 4*E*)-*N*-Methoxy-*N*-methyl-12-((tetrahydro-2H-pyran-2-yl) oxy) dodeca-2, 4-dien-6, 8-diynamide (7)



Carboxylic acid **6** (1.0 equiv., 270 mg, 0.936 mmol) was dissolved in dry DCM (5 mL) in a flame-dried Schlenk tube. N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDCI) (1.2 equiv., 215 mg, 1.12 mmol) and 1-Hydroxybenzotriazole hydrate (HOBt) (88% w/w, 1.2 equiv., 172 mg, 1.12 mmol) was added at room temperature and the mixture stirred for 30 min. After addition of *N, O*-Dimethylhydroxylamine hydrochloride (1.5 equiv., 137 mg, 1.40 mmol) and triethylamine (TEA) (2.0 equiv., 0.26 mL, 1.87 mmol), the mixture was kept stirring for 12 hours and quenched with sat. $NaHCO_3$ (aq.) (15 mL). The mixture was extracted with DCM (2 x 15 mL), the combined organic layers were dried with $MgSO_4$ and concentrated under reduced pressure. Purification by column chromatography (silica gel, 50% EtOAc in heptane) afforded 245 mg (79%) of the Weinreb amide **7** as a yellow oil. **^{1}H NMR (600 MHz, $CDCl_3$):** δ = 7.31 (dd, *J* = 15.1, 11.4 Hz, 1H), 6.80 (dd, *J* = 15.5, 11.5 Hz, 1H), 6.55 (d, *J* = 15.2 Hz, 1H), 5.96 (d, *J* = 15.5 Hz, 1H), 4.61-4.58 (m, 1H), 3.89-3.79 (m, 2H), 3.71 (s, 3H), 3.54-3.50 (m, 1H), 3.48 (dt, *J* = 9.9, 6.1 Hz, 1H), 3.26 (s, 3H), 2.49 (bt, *J* = 7.0 Hz, 2H), 1.88-1.78 (m, 3H), 1.74-1.68 (m, 1H), 1.61-1.49 (m, 4H) ppm. **^{13}C-NMR (150 MHz, $CDCl_3$):** δ = 166.5, 142.0, 141.6, 121.8, 117.7, 99.0, 87.2, 80.5, 73.8, 65.8, 65.5, 62.4, 62.1, 32.6, 30.8, 28.6, 25.6, 19.7, 16.9 ppm. **FTIR ($CHCl_3$, cm^{-1}):** 2938, 2871, 2225, 1651, 1607, 1462, 1440, 1416, 1380, 1261, 1202, 1179, 1136, 1120, 1075, 1034, 996, 869, 816. **HRMS (ESI) (m/z):** calculated for $[M + Na^+]^+$ $(C_{19}H_{25}NNaO_4)^+$: 354.1679, found: 354.1676.

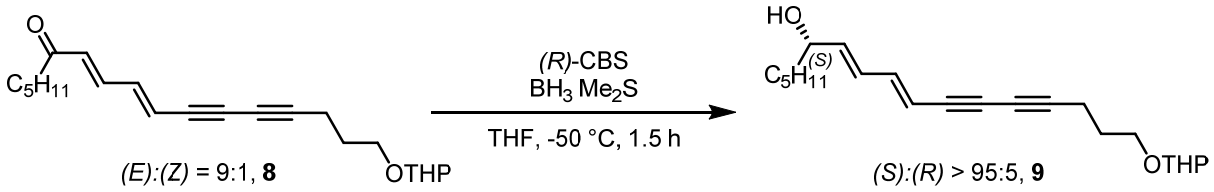
3.1.4 (7*E*, 9*E*)-17-((tetrahydro-2H-pyran-2-yl) oxy) heptadeca-7, 9-dien-11, 13-diyn-6-one (8)



A flame-dried Schlenk flask was charged with 1-Iodopentane (3.0 equiv., 26 µL, 0.2 mmol) and dry Et_2O (1 mL). After cooling to -78 °C, t-BuLi (1.7 M in pentane, 6.0 equiv., 0.24 mL, 0.4 mmol) was added dropwise, and the mixture was stirred for 1 h at -78 °C. A separate flame-dried Schlenk was charged with Weinreb amide **7** (1.0 equiv., 22.1 mg, 0.067 mmol), LiCl (5.0 equiv., 14.1 mg, 0.333 mmol) and dry Et_2O (0.5 mL) and cooled to -40 °C. The Pentyllithium solution was added via canula and the mixture was stirred for 45 minutes at -40 °C. The mixture was quenched with brine and water (5 mL, 1:1) and extracted with DCM (2 x 10 mL). The combined organic layers were dried with $MgSO_4$ and concentrated *in vacuo*. Purification by prepTLC (aluminium oxide, 30% EtOAc in heptane) afforded 15.2 mg (67%) of desired ketone **8** as a yellow oil, containing 8% of isomerization product. **^{1}H-NMR (600 MHz, $CDCl_3$):** δ = 7.12 (dd, *J* = 15.5, 11.3 Hz, 1H), 6.74 (dd, *J* = 15.4, 11.3 Hz, 1H), 6.23 (bd, *J* = 15.4 Hz, 1H), 6.00 (d, *J* = 15.5 Hz, 1H), 4.61-4.58 (m, 1H), 3.88-3.78 (m, 2H), 3.54-3.45 (m, 2H), 2.55 (t, J = 7.44 Hz, 2 H), 2.47 (bt, J = 6.84 Hz, 2H), 1.89-1.78 (m, 3 H), 1.74-1.68 (m, 1H), 1.65-1.48 (m,

6H), 1.36-1.24 (m, 4H), 0.89 (t, *J* = 7.1 Hz, 3H) ppm. **^{13}C-NMR (150 MHz, $CDCl_3$):** δ = 200.5, 141.9, 140.2, 131.6, 119.0, 99.0, 87.8, 81.2, 73.7, 65.8, 65.4, 62.4, 41.3, 31.6, 30.8, 28.5, 25.6, 24.0, 22.6, 19.6, 16.9, 14.1 ppm.

3.1.5 (6*S,* 7*E,* 9*E*)-17-((tetrahydro-2H-pyran-2-yl)oxy)heptadeca-7, 9-dien-11, 13-diyn-6-ol (9)



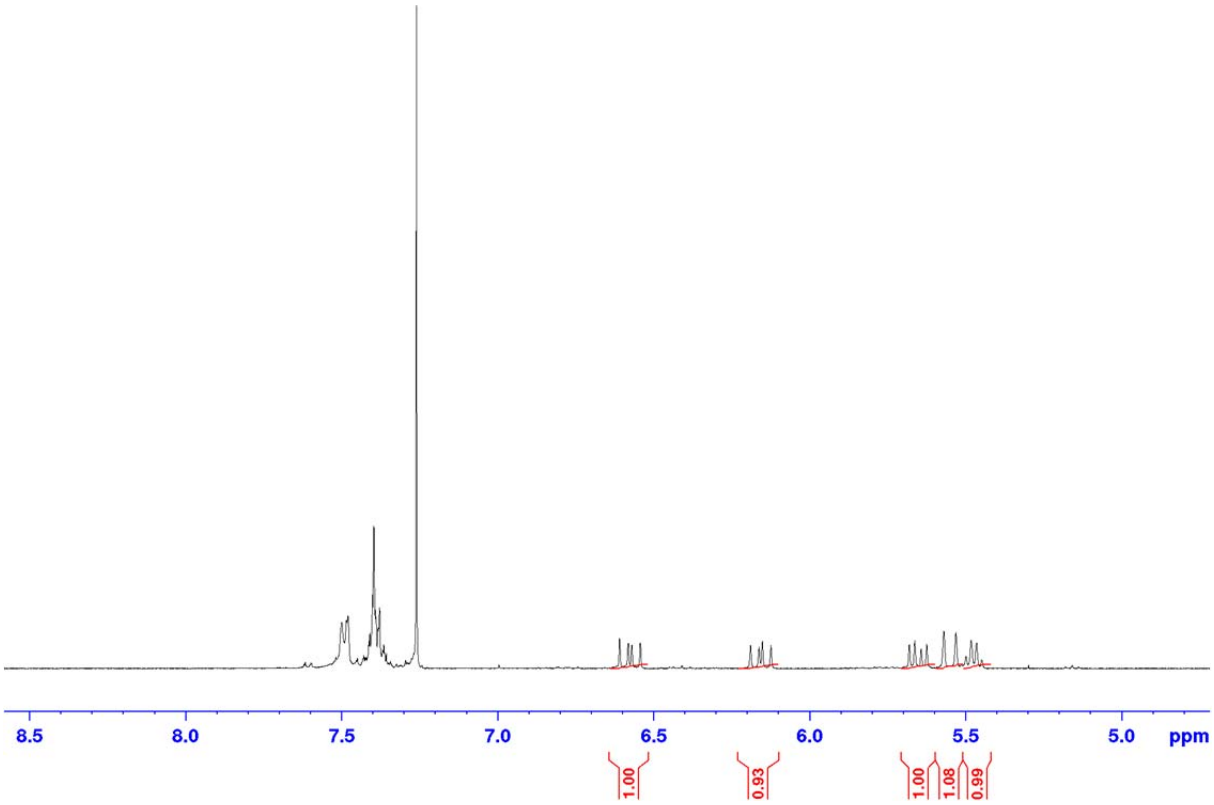
A flame-dry Schlenk tube was charged with a solution of *(R)-(+)*-2-Methyl-CBS-oxazaborolidine [(*R*)-CBS] (2.0 equiv., 1 M in THF, 76 µL, 0.076 mmol), then a solution of ketone **8** (*(E):(Z)* = 9:1, 1.0 equiv., 13.0 mg, 0.038 mmol) in dry THF (0.5 mL) was added. The mixture stirred at -50 °C for 10 min. Then BH_3-Me_2S complex (2.0 equiv., 7 µL, 0.076 mmol) was added. The mixture was stirred vigorously at -50 °C for 1.5 hours. After quenching with Sat. NH_4Cl (aq.) (1 mL), the aqueous layer was extracted with EtOAc (2 x 4 mL), the combined organic layers were dried with $MgSO_4$ and filtered. Concentration under reduced pressure and column chromatography (silica gel, 50% EtOAc in heptane) afforded 10.0 mg (76%) of the desired product **9** as a colourless oil. The spectral data is in accordance with the literature[4]. **^{1}H NMR (400 MHz, $CDCl_3$):** δ = 6.67 (dd, *J* = 15.6, 10.9 Hz, 1H), 6.29 (dd, *J* = 15.3, 10.9 Hz, 1H), 5.84 (dd, *J* = 15.2, 6.4 Hz, 1H), 5.61 (d, *J* = 15.4 Hz, 1H), 4.61-4.58 (m, 1H), 4.21-4.14 (m, 1H), 3.90-3.78 (m, 2H), 3.55-3.43 (m, 2H), 2.47 (bt, *J* = 7.0 Hz, 2H), 1.90-1.65 (m, 4H), 1.64-1.19 (m, 13H), 0.89 (t, *J* = 6.8 Hz, 3H) ppm. Specific rotation is in accordance with the literature[5]: $[\alpha]_D$ = + 9.6 (c = 0.5, $CHCl_3$), $[\alpha]_D$ (Lit.) = + 10.8 (c = 1.1, $CHCl_3$);

Determination of enantiomeric excess using Mosher ester method:

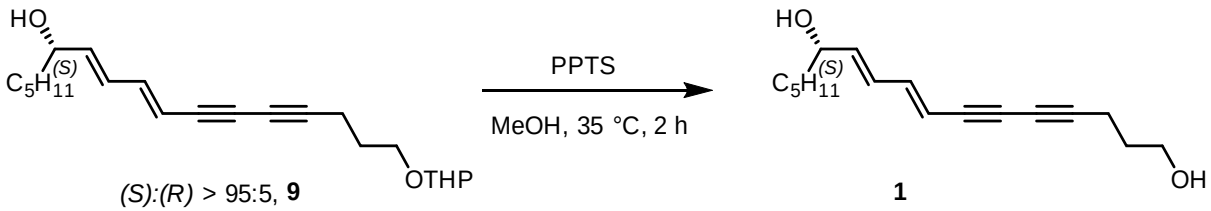
A dry schlenk tube was charged with *(R)*-α-Methoxy-α-(trifluoromethyl)phenylacetic acid (9.9 mg, 41.8 µmol) and dissolved in dry pentane (1.5 mL). $(COCl)_2$ (697 mmol, 59 µL) was added, followed by addition of dry DMF (1 drop). The mixture was stirred for 30 minutes at room temperature and filtered through a plug of cotton. Removal of the solvent at 100 mbar (40 °C waterbath) afforded the acid chloride, which was redissolved in $CDCl_3$ (0.7 mL). After addition of triethylamine (5.8 µL, 42 µmol), the solution was added to 3.6 mg of crude alcohol **9**. Addition of DMAP (8.5 mg, 0.7 µmol) led to completion of the ester formation within 15 minutes. NMR data of olefinic signals of Mosher ester: **^{1}H NMR (400 MHz, $CDCl_3$):**

[4] Uwai, K.; Oshima, Y. *Tetrahedron* **1999**, *55*, 9469-9480
[5] Uwai, K.; Oshima, Y. *Tetrahedron* **1999**, *55*, 9469-9480

δ = 6.56 (dd, *J* = 15.6, 10.9 Hz, 1H), 6.14 (dd, *J* = 15.2, 10.9 Hz, 1H), 5.64 (dd, *J* = 15.2, 7.1 Hz, 1H), 5.54 (d, *J* = 15.6 Hz, 1H) ppm.



3.1.6 Virol A (1)



(S):(R) > 95:5, **9** **1**

THP-protected alcohol (1.0 equiv., 8.7 mg, 25 µmol) and PPTS (1.0 equiv., 6.4 mg, 25 µmol) were dissolved in MeOH (1 mL) in a glass vial and stirred at 35 °C for 2 h. After removing most of the methanol under reduced pressure, the mixture was diluted with ether (5 mL) and washed with NH_4Cl sat. (5 mL). The organic layer was dried with $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (silica prewashed with 5% Et_3N in DCM, elute remaining starting material (2.1 mg) with DCM, elute product with EtOAc) affords 4.7 mg (72%) of Virol A **(1)** as a colourless oil. The spectral data is in accordance with the literature[6]. **^{1}H NMR (600 MHz, $CDCl_3$):** δ = 6.68 (dd, *J* = 15.6, 10.9 Hz,

[6] Uwai, K.; Oshima, Y. *Tetrahedron* **1999**, *55*, 9469-9480.

1H), 6.27 (dd, *J* = 15.2, 11.0 Hz, 1H), 5.84 (dd, *J* = 15.2, 6.3 Hz, 1H), 5.60 (d, *J* = 15.6 Hz, 1H), 4.20-4.14 (m, 1H), 3.78-3.74 (m, 2H), 2.48 (t, *J* = 6.8 Hz, 2 H), 1.80 (app. pent, *J* = 6.5 Hz, 2H), 1.56 (s, 2H), 1.55-1.48 (m, 2H), 1.39-1.26 (m, 6H), 0.88 (t, *J* = 6.9 Hz, 3H) ppm. **^{13}C-NMR (150 MHz, $CDCl_3$):** δ = 144.0, 140.2, 129.1, 110.3, 84.8, 76.9, 74.7, 72.4, 65.9, 61.6, 37.3, 31.8, 31.0, 25.1, 22.7, 16.3, 14.2 ppm. **FTIR ($CHCl_3$, cm^{-1}):** 3343, 3319, 2954, 2929, 2859, 1463, 1426, 1348, 1292, 1219, 1054, 984, 772. **HRMS (ESI) (m/z):** calculated for $[M + Na^+]^+$ $(C_{17}H_{24}ONa)^+$: 283.1669, found: 283.1665. $[\alpha]_D$ = + 12.5 (c = 0.25, MeOH), $[\alpha]_D$ (Lit.) = + 15.4 (c = 1.1, MeOH)[7]

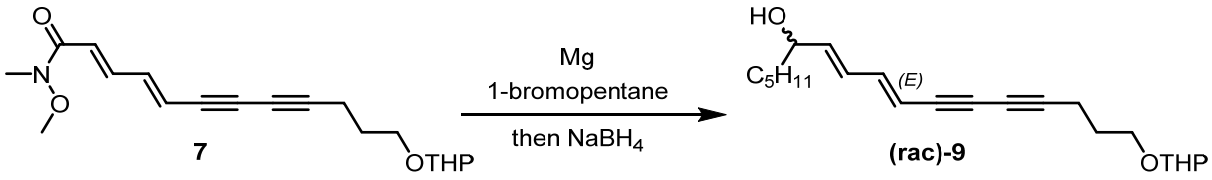
Comparison of spectra of synthetic Virol A (1) with that of the isolated Virol A (1)

^{1}H-NMR (500 M Hz, $CDCl_3$) (isolated)[7]	^{1}H-NMR (600 M Hz, $CDCl_3$) (synthetic)	^{13}C-NMR (125 M Hz, $CDCl_3$) (isolated)[7]	^{13}C-NMR (150 M Hz, $CDCl_3$) (synthetic)
6.68 (dd, *J* = 15.5, 11.3 Hz, 1H)	6.68 (dd, *J* = 15.6, 10.9 Hz, 1H)	143.8	144.0
6.27 (dd, *J* = 15.3, 11.3 Hz, 1H)	6.27 (dd, *J* = 15.2, 11.0 Hz, 1H)	140.1	140.2
5.84 (dd, *J* = 15.3, 6.3 Hz, 1H)	5.84 (dd, *J* = 15.2, 6.3 Hz, 1H)	129.0	129.1
5.61 (d, *J* = 15.5 Hz, 1H)	5.60 (d, *J* = 15.6 Hz, 1H)	110.5	110.3
4.17 (m, 1H)	4.20-4.14 (m, 1H)	84.7	84.8
3.67 (t, *J* = 6.1 Hz, 2H)	3.78-3.74 (m, 2H)	77.4	76.9
2.48 (t, *J* = 7.0 Hz, 2H)	2.48 (t, *J* = 6.8 Hz, 2H)	72.5	74.7
1.81 (m, 2H)	1.80 (app. pent, *J* = 6.5 Hz, 2H)	72.3	72.4
	1.56 (s, 2H, OH)	65.8	65.9
1.53 (m, 2H)	1.55-1.48 (m, 2H)	61.4	61.6
1.38 (m, 1H)	1.39-1.26 (m, 6H)	37.2	37.3
1.29 (m, 1H)		31.7	31.8
1.29 (m, 2H)		30.9	31.0
1.29 (m, 2H)		25.0	25.1
0.89 (t, *J* = 6.8 Hz, 3H)	0.88 (t, *J* = 6.9 Hz, 3H)	22.6	22.7
		16.2	16.3
		14.0	14.2

[7] Ohta, T.; Uwai, K.; Kikuchi, R.; Nozoe, S.; Oshima, Y.; Sasaki, K.; Yoshizaki, F. *Tetrahedron*, **1999**, *55*, 12087-12098.

3.2 racemic Virol A (1)

3.2.1 (7*E,* 9*E*)-17-((tetrahydro-2H-pyran-2-yl)oxy)heptadeca-7, 9-dien-11, 13-diyn-6-ol ((rac)-9)



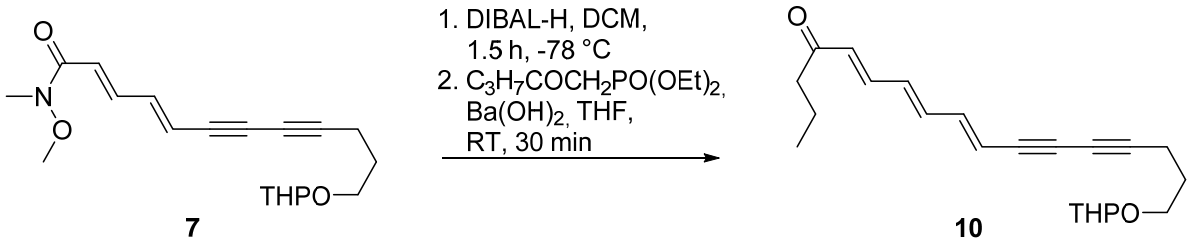
(rac-9): Mg powder (20 equiv., 54.3 mg, 2.23 mmol) was covered in a flame-dried Schlenk tube with dry Et_2O (0.2 mL). Dropwise addition of 1-bromopentane (10 equiv., 138 µL, 1.12 mmol), dissolved in 0.5 mL Et_2O), at room temperature led to exothermic reaction. After subsiding, dry Et_2O (0.5 mL) was added and the mixture was kept at reflux for 15 min. The metallic solution was added to a solution of weinreb amide **7** (1 equiv., 37 mg, 0.112 mmol) in dry Et_2O (0.5 mL) at 0 °C and kept stirring for 15 min followed by 30 minutes at room temperature. The paste-like residue was suspended in NH_4Cl sat. (10 mL) and extracted with DCM (2 x 10 mL). The combined organic layers were dried with $MgSO_4$ and concentrated *in vacuo*. The crude ketone was redissolved in ethanol (abs., 2 mL) in a glass vial. $NaBH_4$ (10 eq, 42.4 mg, 1.12 mmol) was added and the mixture was stirred for 2 h at room temperature. The mixture was quenched with NH_4Cl sat. (5 mL) and extracted with DCM (2 x 10 mL). The combined organic layers were dried with $MgSO_4$ and concentrated *in vacuo*. Purification by preparative TLC (Alox, 40% EtOAc in heptane) afforded 8.7 mg (23%) of racemic alcohol **rac-9** as a colourless oil.

Racemic Virol A was prepared from **(rac)-9** according to the procedure described for (*S*)-Virol A.

4 Synthesis of (*R*) and Racemic Cicutoxin (2)

4.1 *(R)*-Cicutoxin (2)

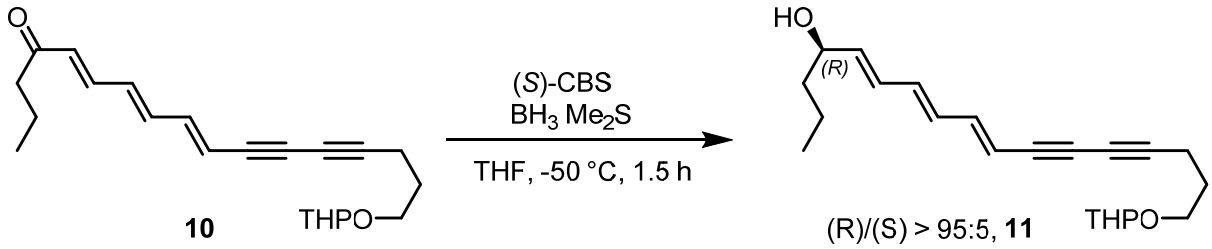
4.1.1 (5*E*, 7*E*, 9*E*)-17-((tetrahydro-2H-pyran-2-yl) oxy) heptadeca-5, 7, 9-trien-11, 13-diyn-4-one (10)



7 **10**

The following reaction was performed in the shadows, since light may cause double bond isomerization of the following intermediates: A flame-dried Schlenk was charged with Weinreb amide **7** (1.0 equiv., 38.3 mg, 0.116 mmol) and dry DCM (1 mL). After cooling to -78 °C, a solution of diisobutylaluminum hydride (DIBAL-H) in DCM (2.0 equiv., 1 M in DCM, 0.23 mL, 0.23 mmol) was added and the mixture was stirred for 1.5 h at -78 °C. After quenching with EtOAc (0.5 mL), the solution was allowed to warm up to room temperature. Na-K-tartrate sat. (10 mL) and water (5 mL) was added, and the aqueous layer was extracted with DCM (2 x 20 mL). The combined organic layers were dried with $MgSO_4$, filtered and concentrated to give the crude aldehyde as yellow oil. A flame-dried Schlenk tube was charged with phosphonate (2.0 equiv., 51.6 mg, 0.232 mmol) in dry THF (0.5 mL). Anhydrous barium hydroxide (4.0 equiv., 79.5 mg, 0.464 mmol) was added and the mixture was stirred for 30 minutes at room temperature. A solution of crude aldehyde in THF (1 mL) and water (25 µL) was added dropwise and stirred for 30 minutes at room temperature. Water (10 mL) was added and the aqueous layer was extracted with DCM (3 x 10 mL). The combined organic layers were dried with $MgSO_4$, filtered and concentrated. Purification by column chromatography (silica gel prewashed with Et_3N/EtOAc/heptane 5:20:80, elution with 20% EtOAc in heptane) afforded 20.6 mg (52%) of product **10** as a yellow oil and single isomer. **^{1}H-NMR (600 MHz, $CDCl_3$):** δ = 7.15 (dd, *J* = 15.5, 11.3 Hz, 1H), 6.74 (dd, *J* = 15.4, 11.3 Hz, 1H), 6.60 (dd, *J* = 14.8, 11.4 Hz, 1H), 6.40 (dd, *J* = 14.8, 11.3 Hz, 1H), 6.23 (bd, *J* = 15.4 Hz, 1H), 5.79 (d, *J* = 15.4 Hz, 1H), 4.60-4.58 (m, 1H), 3.88-3.79 (m, 2H), 3.54-3.44 (m, 2H), 2.53 (t, *J* = 7.4 Hz, 2H), 2.47 (bt, *J* = 7.1 Hz, 2H), 1.87-1.78 (m, 3 H), 1.74-1.68 (m, 1H), 1.65 (aqq. hex, *J* = 7.4 Hz, 2H), 1.60-1.49 (m, 4H), 1.25 (bs, 2H, OH), 0.94 (t, *J* = 7.4 Hz, 3H) ppm. **^{13}C-NMR (150 MHz, $CDCl_3$):** δ = 200.5, 143.1, 141.1, 139.6, 133.4, 131.1, 114.5, 99.0, 87.2, 80.3, 74.4, 65.8, 65.7, 62.4, 43.0, 30.8, 28.6, 25.6, 19.6, 17.9, 16.9, 14.0 ppm. **FTIR ($CHCl_3$, cm^{-1}):** 2932, 2873, 1682, 1661, 1602, 1563, 1366, 1261, 1201, 1183, 1156, 1136, 1121, 1062, 1035, 1020, 1001. **HRMS (ESI) (m/z):** calculated for $[M + Na^+]^+$ $(C_{22}H_{28}O_3Na)^+$: 363.1931, found: 363.1931.

4.1.2 (4*R*, 5*E*, 7*E*, 9*E*)-17-((tetrahydro-2H-pyran-2-yl) oxy) heptadeca-5, 7, 9-trien-11, 13-diyn-4-ol (11)

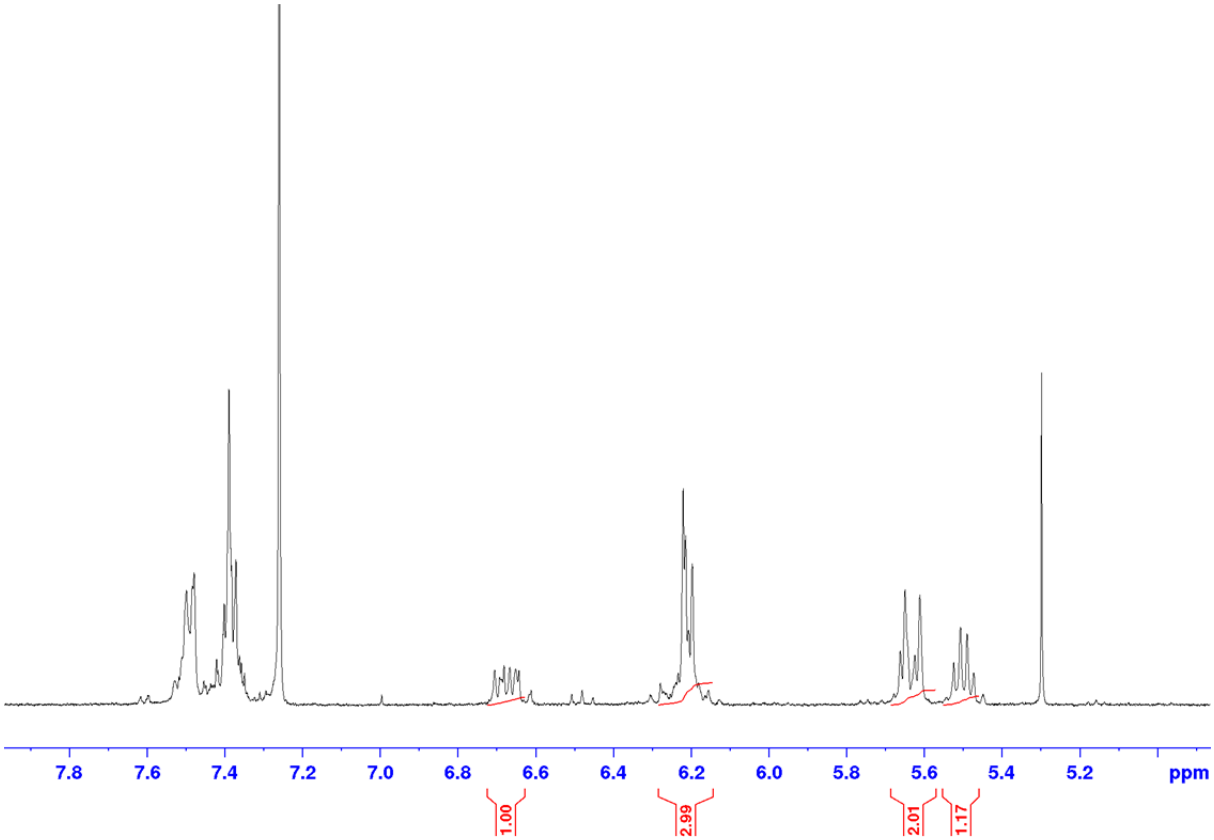


A flame-dry Schlenk tube was charged with a solution of *(S)*-2-Methyl-CBS-oxazaborolidine [(*S*)-CBS] (2.0 equiv., 21.2 mg, 0.076 mmol), then a solution of ketone **10** (1.0 equiv., 13.0 mg, 0.038 mmol) in dry THF (0.5 mL) was added. The mixture stirred at -50 °C for 10 min. Then BH_3-Me_2S complex (2.0 equiv., 7.0 µL, 0.076 mmol) was added. The mixture was stirred vigorously at -50 °C for 1.5 hours. After quenching with Sat. NH_4Cl (aq.) (1 mL), the aqueous layer was extracted with EtOAc (2 x 4 mL), the combined organic layers were dried with $MgSO_4$ and filtered. Concentration under reduced pressure and column chromatography (silica gel, 50% EtOAc in heptane) afforded 10.0 mg (76%) of the desired product **11** as a colourless oil. **^{1}H-NMR (600 MHz, $CDCl_3$):** δ = 6.70 (dd, *J* = 15.4, 10.6 Hz, 1H), 6.33-6.21 (m, 3H), 5.81 (dd, *J* = 15.2, 6.5 Hz, 1H), 5.60 (d, *J* = 15.4 Hz, 1H), 4.60-4.58 (m, 1H), 4.22-4.16 (m, 1H), 3.88-3.79 (m, 2H), 3.54-3.45 (m, 2H), 2.47 (btd, *J* = 7.1, 3.1 Hz, 2 H), 1.87-1.78 (m, 3H), 1.74-1.67 (m, 1H), 1.61-1.49 (m, 6H), 1.47 (d, *J* = 4.2 Hz, OH), 1.46-1.31 (m, 2H), 0.93 (t, *J* = 7.3 Hz, 3H) ppm. **^{13}C-NMR (150 MHz, $CDCl_3$):** δ = 144.3, 139.3, 135.4, 131.7, 129.9, 110.2, 99.0, 85.7, 77.9, 75.0, 72.4, 65.9, 65.8, 62.4, 39.5, 30.8, 28.7, 25.6, 19.7, 18.7, 16.8, 14.1 ppm. **FTIR ($CHCl_3$, cm^{-1}):** 3422, 2954, 2930, 2871, 1455, 1441, 1354, 1287, 1200, 1157, 1136, 1121, 1073, 1063, 1034, 1022, 995. **HRMS (ESI) (m/z):** calculated for $[M + Na^+]^+$ $(C_{22}H_{30}O_3Na)^+$: 365.2087, found: 365.2096.

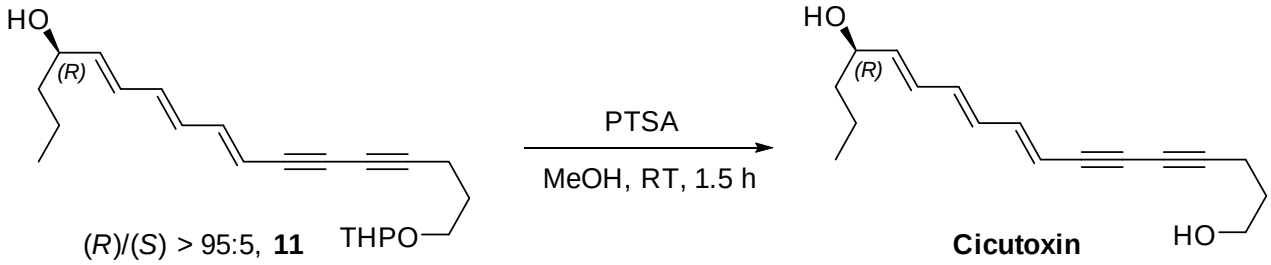
Determination of enantiomeric excess using Mosher ester method:

A dry schlenk tube was charged with *(S)*-α-Methoxy-α-(trifluoromethyl)phenylacetic acid (5.0 equiv., 12.0 mg, 51.1 µmol) and dissolved in dry pentane (1.5 mL). $(COCl)_2$ (25 equiv., 22 µL, 0.255 mmol) was added, followed by addition of dry DMF (1 drop). The mixture was stirred for 30 minutes at room temperature and filtered through a plug of cotton. Removal of the solvent at 100 mbar (40 °C waterbath) afforded the acid chloride. Then the above alcohol **11** (1.0 equiv., 3.5 mg, 10.2 µmol) which was dissolved in DCM (0.7 mL) was added to the acid chloride, followed by the addition of DMAP (1.0 equiv., 1.2 mg, 10.2 µmol) and triethylamine (6.0 equiv., 8.6 µL, 61.3 µmol). The mixture stirred vigorously at room temperature for 30 min leading to the complete consumption of alcohol **11**. Then the solvent was removed, flash column chromatography (silica gel, 20% EtOAc in heptane) gave the mosher ester (5.0 mg, 88%) as a colorless oil. (NMR data of olefinic signals of Mosher

ester: **^{1}H NMR (400 MHz, $CDCl_3$):** δ = 6.71-6.64 (m, 1H), 6.28-6.16 (m, 3H), 5.66-5.61 (m, 2H), 5.50 (dd, *J* = 13.7, 7.0 Hz, 1H) ppm.



4.1.3 (R)-Cicutoxin (2)



To a solution of alcohol **11** (1.0 equiv., 7.0 mg, 20.4 µmol) in MeOH (2.0 mL) in a glass vial covered by aluminium foil was added p-toluenesulfonic acid (PTSA) (0.5 equiv., 1.8 mg, 10.2 µmol) and the mixture was stirred for 1.5 hours at RT. The crude mixture was quenched by sat. $NaHCO_3$ (aq.) (1.0 mL), after concentrating, the water phase was extracted by EtOAc (3 x 10 mL), the combined organic phase was washed by brine, dried over $MgSO_4$, filtered and concentrated. Purification by column chromatography (silica gel prewashed with Et_3N/EtOAc/heptane 5:20:80, elution with 20% EtOAc in heptane) afforded 2.8 mg (53%) Cicutoxin as a colourless oil. **^{1}H-NMR (600 MHz, $CDCl_3$):** δ = 6.70 (dd, *J* = 15.4, 10.7 Hz, 1H),

6.37-6.21 (m, 3H), 5.82 (dd, *J* = 15.2, 6.6 Hz, 1H), 5.60 (d, *J* = 15.4 Hz, 1H), 4.22-4.16 (m, 1H), 3.76 (bq, *J* = 5.8 Hz, 2H), 2.49 (bt, *J* = 7.0 Hz, 2H), 1.81 (app. pent., *J* = 6.5 Hz, 2 H), 1.60-1.48 (m, 2H), 1.46 (d, *J* = 4.2 Hz, OH), 1.45-1.33 (m, 3H), 0.93 (t, *J* = 7.3 Hz, 3H) ppm. **^{13}C-NMR (150 MHz, $CDCl_3$):** δ = 144.4, 139.4, 135.4, 131.7, 129.9, 110.1, 85.3, 77.7, 75.2, 72.4, 66.0, 62.4, 39.5, 31.1, 18.7, 16.4, 14.1 ppm. **FTIR ($CHCl_3$, cm^{-1}):** 3348, 2956, 2930, 2871, 1061, 995, 751. **HRMS (ESI) (m/z):** calculated for $[M + Na^+]^+$ $(C_{17}H_{22}O_2Na)^+$: 281.1512, found: 281.1512. $[\alpha]_D$ = -17.1 (c = 0.17, MeOH), $[\alpha]_D$ (Lit.) = - 14.9 (c = 1.12, MeOH)[8]

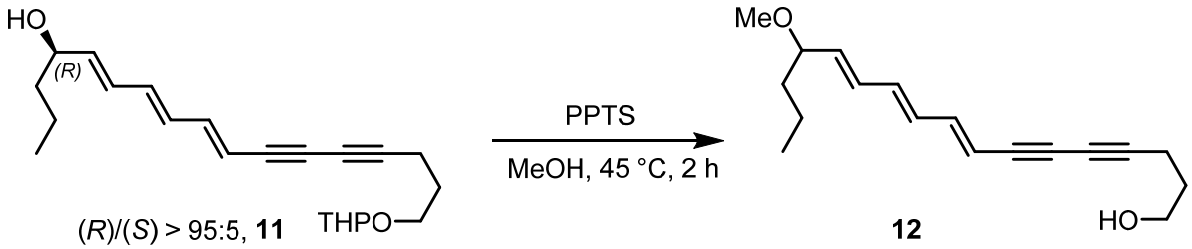
Comparison of spectra of synthetic Cicutoxin (2) with that of the isolated Cicutoxin (2)

^{1}H NMR (500 MHz, $CDCl_3$) (isolated)[8]	^{1}H NMR (600 MHz, $CDCl_3$) (synthetic)	^{13}C NMR (125 MHz, $CDCl_3$) (isolated)[8]	^{13}C NMR (150 MHz, $CDCl_3$) (synthetic)
6.69 (dd, *J* = 15.5, 10.5 Hz, 1H)	6.70 (dd, *J* = 15.4, 10.7 Hz, 1H)	144.4	144.4
6.24 (dd, *J* = 15.0, 10.5 Hz, 2H)	6.37-6.21 (m, 3H)	139.3	139.4
6.32 (dt, *J* = 15.5, 10.5 Hz, 1H)		135.4	135.4
5.82 (dd, *J* = 15.5, 7.0 Hz, 1H)	5.82 (dd, *J* = 15.2, 6.6 Hz, 1H)	131.6	131.7
5.61 (d, *J* = 15.5 Hz, 1H)	5.60 (d, *J* = 15.4 Hz, 1H)	129.8	129.9
4.19 (m, 1H)	4.22-4.16 (m, 1H)	110.0	110.1
3.76 (t, *J* = 6.0 Hz, 2H)	3.76 (bq, *J* = 5.8 Hz, 2H)	85.3	85.3
2.48 (dt, *J* = 7.0, 1.0 Hz, 2H)	2.49 (bt, *J* = 7.0 Hz, 2 H)	77.7	77.7
1.79 (m, 2H)	1.81 (app. pent., *J* = 6.5 Hz, 2 H)	75.1	75.2
1.54 (m, 2H)	1.60-1.48 (m, 2H)	72.3	72.4
	1.46 (d, *J* = 4.2 Hz, OH)	65.9	66.0
1.39 (m, 2H)	1.45-1.33 (m, 3H (2H +OH))	61.4	62.4
0.92 (m, *J* = 7.0 Hz, 3H),	0.93 (t, *J* = 7.3 Hz, 3H)	39.4	39.5
		31.0	31.1
		18.7	18.7
		16.3	16.4
		14.0	14.1

[8] T. Ohta, K. Uwai, R. Kikuchi, S. Nozoe, Y. Oshima, K. Sasaki, F. Yoshizaki, *Tetrahedron,* **1999**, *55*, 12087–12098.

4.2 14-O-Me-Cicutoxin (12)

4.2.1 (8*E*,10*E*,12*E*)-14-methoxyheptadeca-8,10,12-trien-4,6-diyn-1-ol (12)



To a solution of alcohol **11** (1.0 equiv., 2.1 mg, 6.1 µmol) in MeOH (0.5 mL) in a glass vial covered by aluminium foil was added pyridinium p-toluenesulfonate (PPTS) (1.0 equiv., 1.5 mg, 6.1 µmol) and the mixture was stirred for 2 hours at 45 °C. The crude mixture was quenched by sat. $NaHCO_3$ (aq.) (1.0 mL), after concentrating, the water phase was extracted by EtOAc (3 x 10 mL), the combined organic phase was washed by brine, dried over $MgSO_4$, filtered and concentrated. Purification by column chromatography (silica gel prewashed with Et_3N/EtOAc/heptane 5:20:80, elution with 20% EtOAc in heptane) afforded **12** 1.0 mg (60%) as a colourless oil.

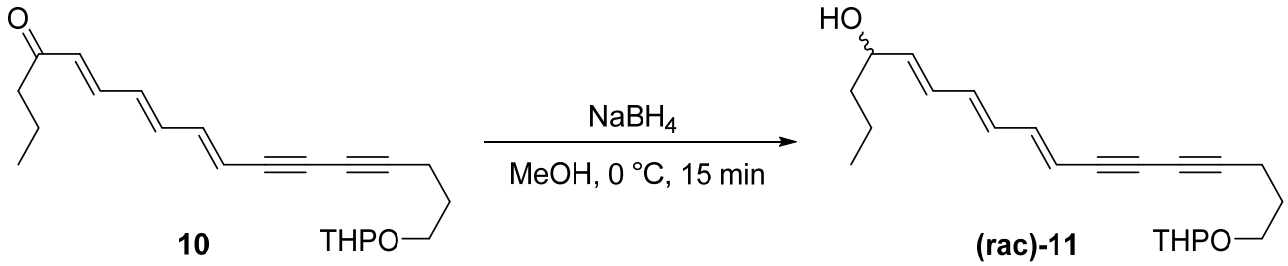
^{1}H-NMR (600 MHz, $CDCl_3$): δ = 6.71 (dd, *J* = 15.4, 10.8 Hz, 1H), 6.33 (dd, *J* = 14.8, 10.6 Hz, 1H), 6.24 (dd, *J* = 14.8, 11.0 Hz, 1H), 6.20 (dd, *J* = 15.2, 10.7 Hz, 1H), 5.65 (dd, *J* = 15.2, 7.9 Hz, 1H), 5.61 (d, *J* = 15.4 Hz, 1H), 3.78-3.74 (bq, *J* = 5.8 Hz, 2H), 3.59 (bq, *J* = 7.0 Hz, 1H), 3.26 (s, 3H), 2.49 (td, *J* = 7.0, 0.7 Hz, 2H), 1.81 (app. pent., *J* = 6.5 Hz, 2H), 1.62-1.55 (m, 1H), 1.48-1.41 (m, 1H), 1.40-1.29 (m, 3H), 0.90 (t, *J* = 7.3 Hz, 3H) ppm. **^{13}C-NMR (150 MHz, $CDCl_3$):** δ = 144.5, 137.6, 135.4, 131.8, 131.6, 110.0, 85.3, 81.9, 77.7, 75.2, 66.0, 61.6, 56.5, 37.8, 31.1, 18.7, 16.4, 14.2 ppm. **FTIR ($CHCl_3$, cm^{-1}):** 3366, 2958, 2930, 2871, 1111, 1084, 996, 764, 750. **HRMS (ESI) (m/z):** calculated for $[M + Na]^+$ $(C_{18}H_{24}O_2Na)^+$: 295.1669, found: 295.1662.

Although the compound **(12)** was claimed in the literature[9], the reported characterization data (^{13}C-NMR was not provided, HRMS was not in agreement with the structure and ^{1}H-NMR was not in full agreement with our isolated product.) is not consistent with our product.

[9] K. Uwai, K. Ohashi, Y. Takata, T. Ohta, T. Tadano, K. Kisara, K. Shibusawa, R. Sakakibara, Y. Oshima, *J. Med. Chem.*, **2000**, *43*, 4508–4515.

4.3 Racemic Cicutoxin (2)

4.3.1 (5*E*, 7*E*, 9*E*)-17-((tetrahydro-2H-pyran-2-yl) oxy) heptadeca-5, 7, 9-trien-11, 13-diyn-4-ol ((rac)-11)



10 **(rac)-11**

To a solution of ketone **10** (1.0 equiv., 17.0 mg, 50 µmol) in MeOH (1 mL) in a glass vial, covered with aluminium foil, was added $NaBH_4$ (1.5 equiv., 2.8 mg, 0.26 mmol) and the mixture was stirred for 15 minutes at 0 °C. Then the mixture was carefully quenched by addition of sat. NH_4Cl (aq.) (2 mL), and the aqueous layer was extracted with EtOAc (3 x 5 mL). The combined organic layers were washed by brine and dried with $MgSO_4$, filtered and concentrated. Purification by column chromatography (silica gel prewashed with Et_3N/EtOAc/heptane 5:20:80, elution with 30% EtOAc in heptane) afforded **(rac)-11** (14.2 mg, 83%) as a colourless oil.

(rac)-Cicutoxin: Racemic Cicutoxin was prepared according to the procedure described for (*R*)-Cicutoxin.

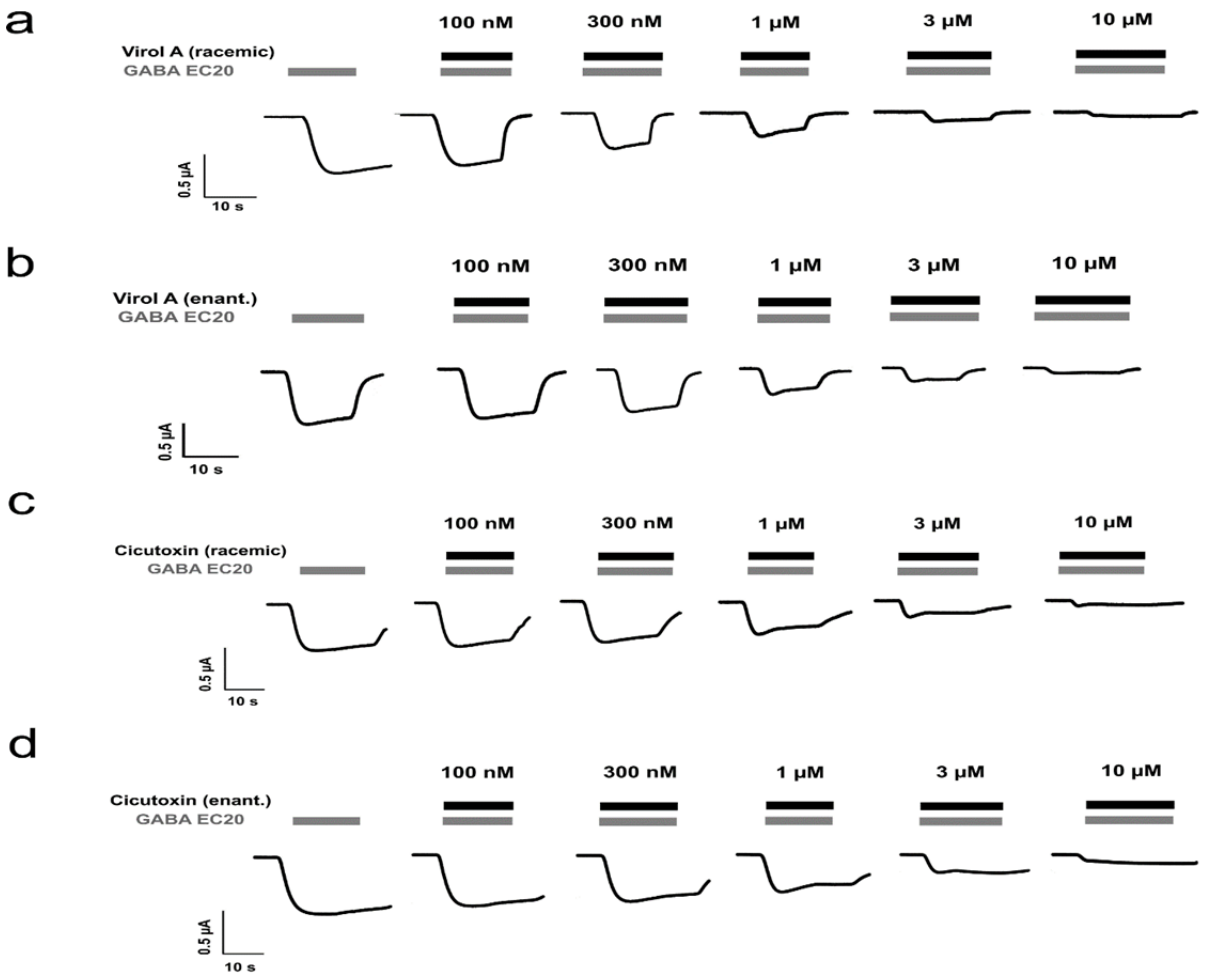
5 Electrophysiology details

Two electrode voltage clamp (TEV) in *Xenopus laevis* oocytes

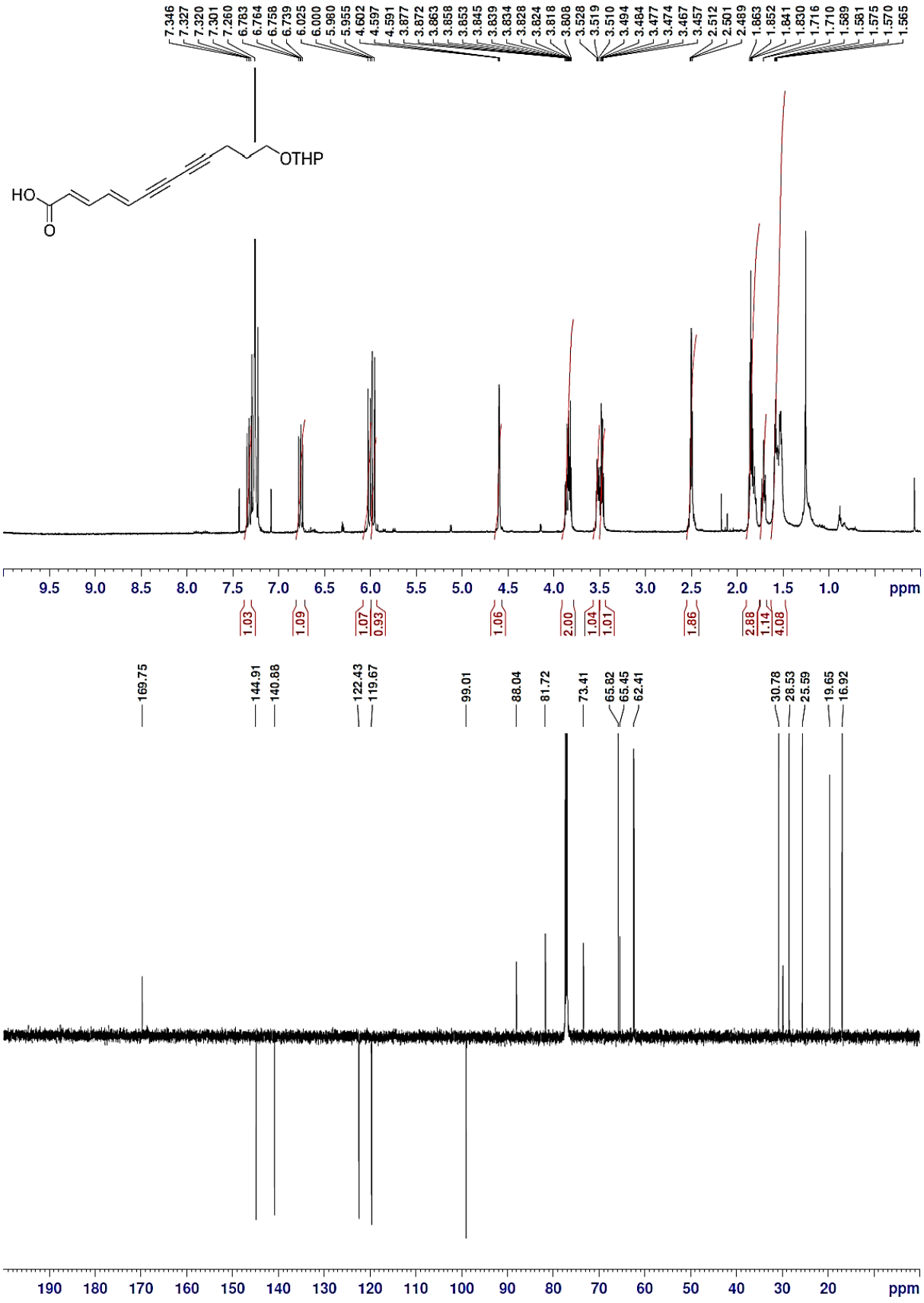
Electrophysiological recordings were performed utilizing the α1, β3 and γ2S subunits and applying test compounds with a concentration of GABA that was titrated to trigger ~20% of the respective maximum GABA-elicited current of the individual oocyte (EC20) and analysed as described in: X. Simeone, D. C. B. Siebert, K. Bampali, Z. Varagic, M. Treven, S. Rehman, J. Pyszkowski, R. Holzinger, F. Steudle, P. Stolze, M. D. Mihovilovic, M. Schnürch, M. Ernst, *Sci. Rep.* 2017, *7*, 5674.

Data are given as mean ± SEM from four oocytes of two oocyte batches.

Sample traces of electrophysiological recordings obtained by racemic and enantioenrinched Virol A (**1**) and Cicutoxin (**2**)



NMR spectra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4.597
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3.877
3.872
3.863
3.858
3.853
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3.839
3.834
3.828
3.824
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3.519
3.510
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1.09
1.07
0.93
1.06
2.00
1.04
1.01
1.86
2.68
1.14
4.08
169.75
144.91
140.88
122.43
119.67
99.01
88.04
81.72
73.41
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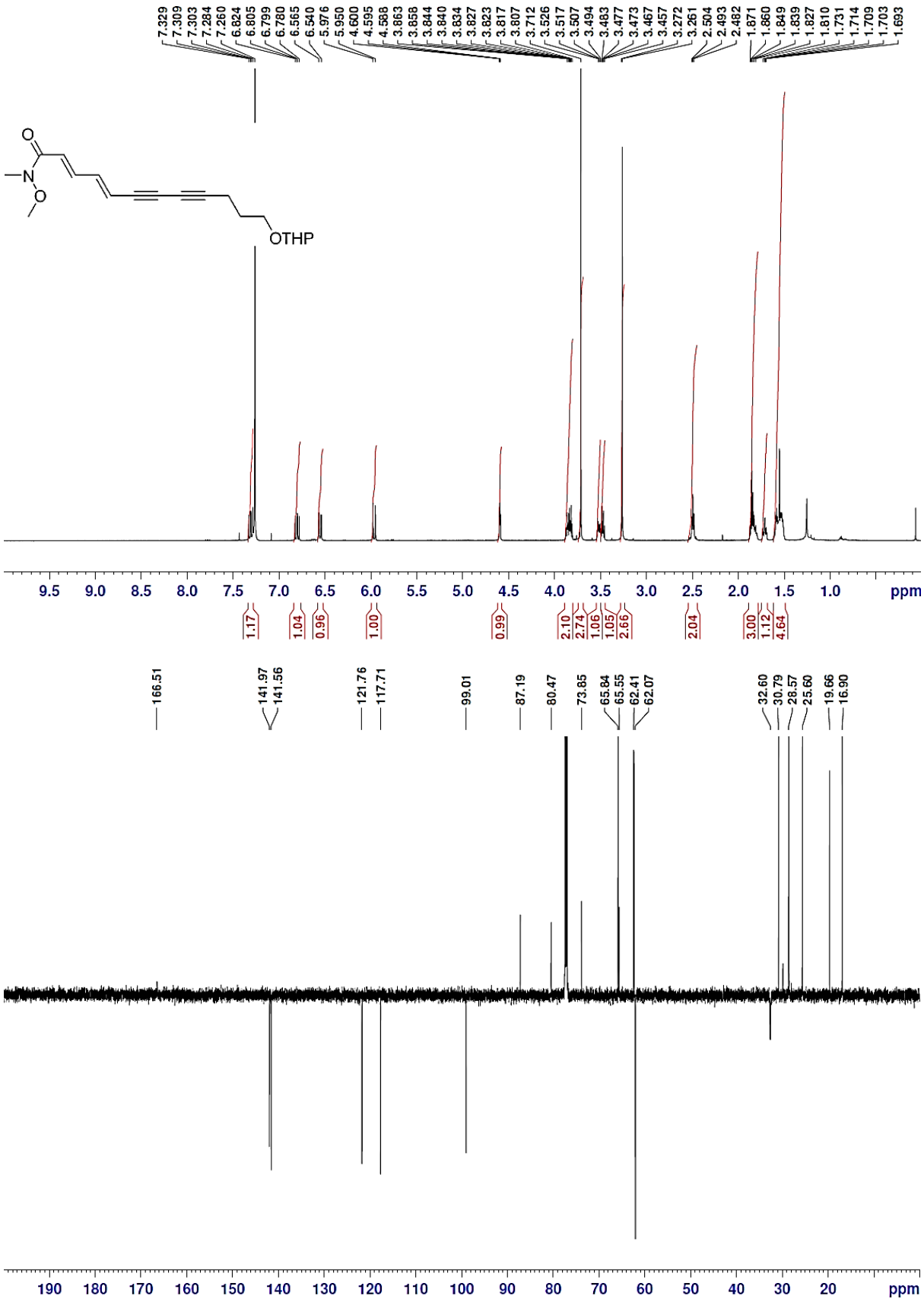
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1.04
0.96
1.00
0.99
2.10
2.74
1.06
1.05
2.66
2.04
3.00
1.12
4.64
166.51
141.97
141.56
121.76
117.71
99.01
87.19
80.47
73.85
65.84
65.55
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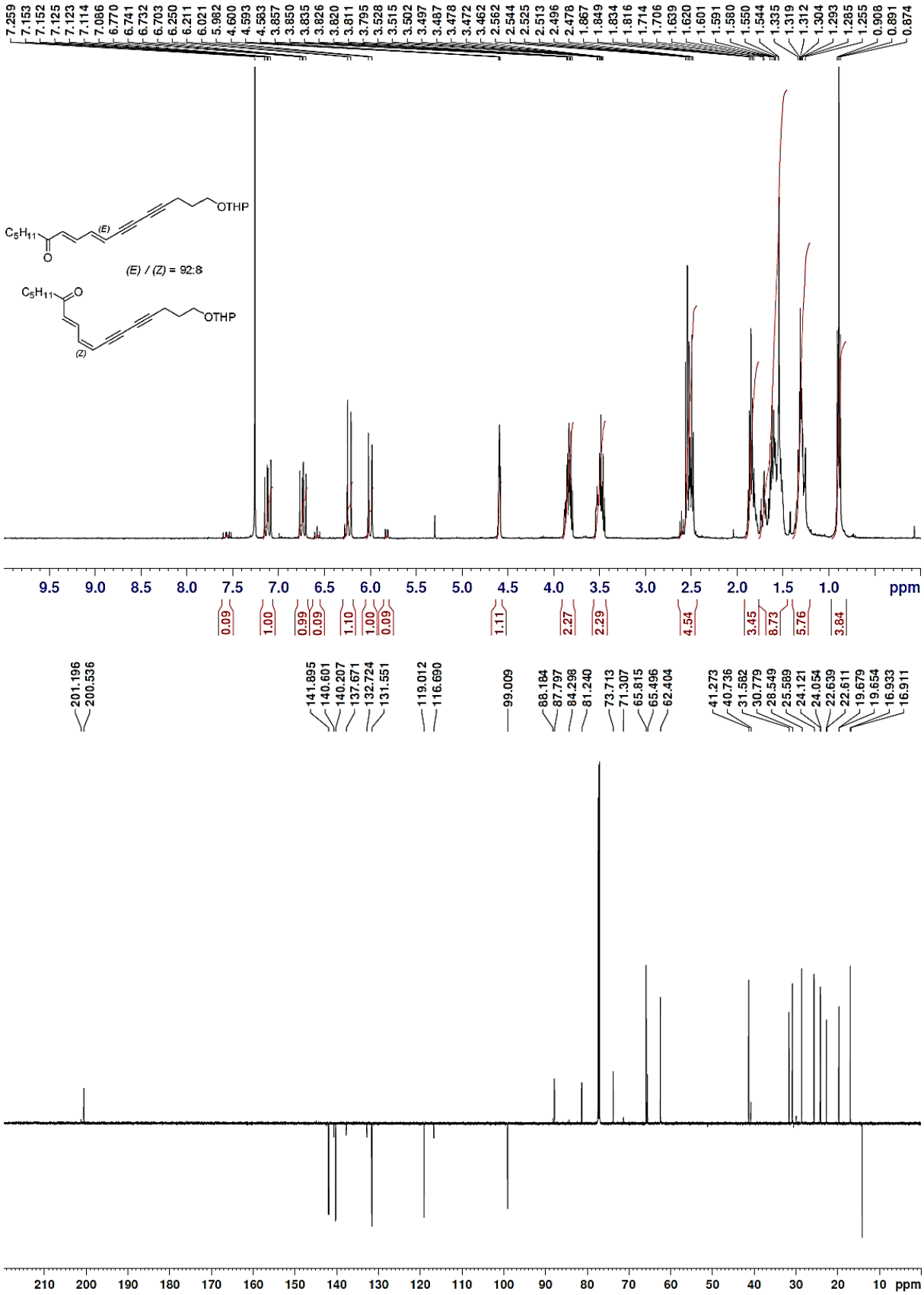
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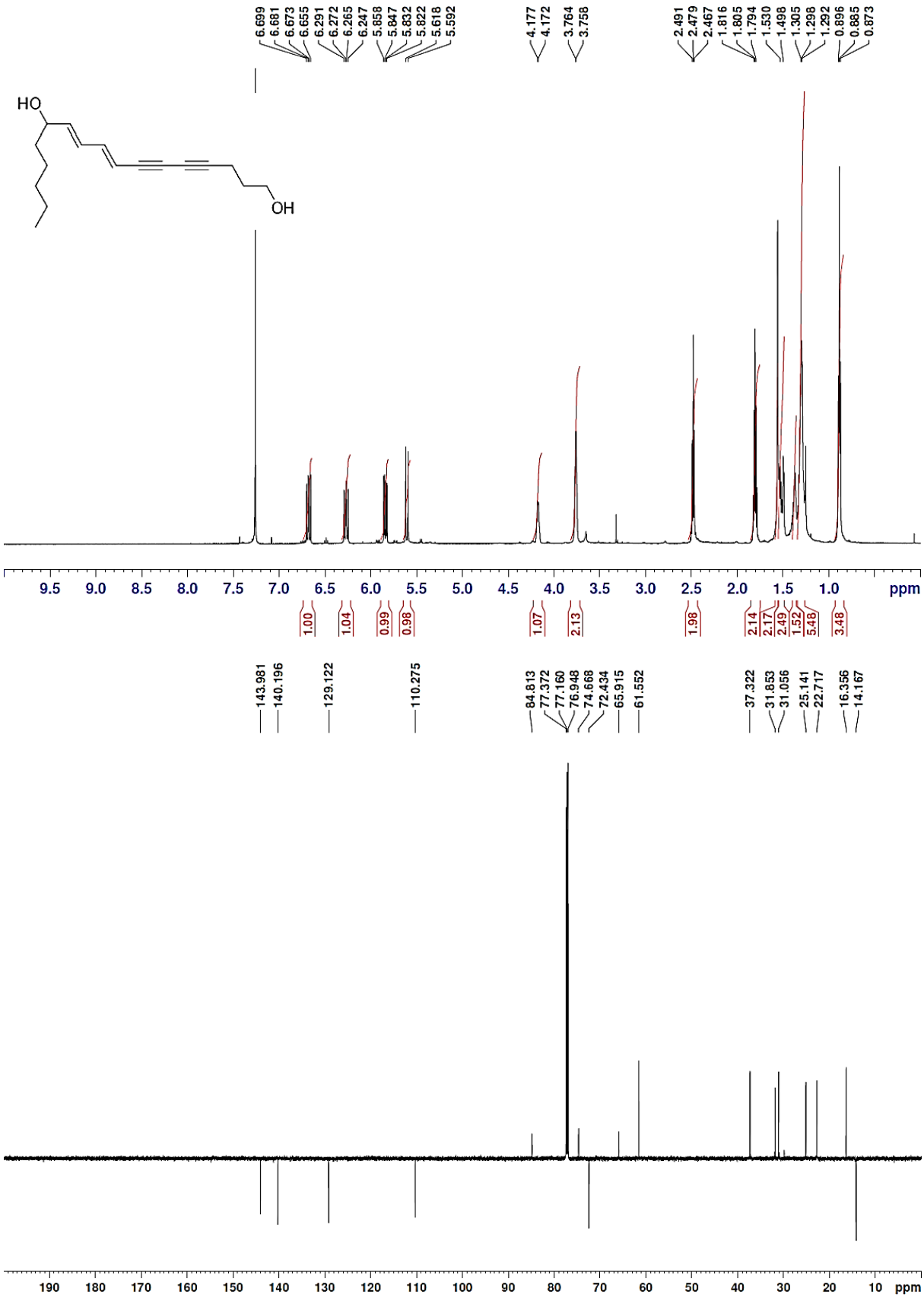
6.732
6.703
6.250
6.211
6.021
5.982
4.600
4.593
4.583
3.857
3.850
3.835
3.826
3.820
3.811
3.795
3.528
3.515
3.502
3.497
3.487
3.478
3.472
3.462
2.562
2.544
2.525
2.513
2.496
2.478
1.867
1.849
1.834
1.816
1.714
1.706
1.639
1.620
1.601
1.591
1.580
1.550
1.544
1.335
1.319
1.312
1.304
1.293
1.285
1.255
0.908
0.891
0.874
OTHP
C5H11
(E)
O
(E) / (Z) = 92:8
C5H11
O
OTHP
(Z)
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
ppm
0.09
1.00
0.99
0.09
1.10
1.00
0.09
1.11
2.27
2.29
4.54
3.45
8.73
5.76
3.84
201.196
200.536
141.895
140.601
140.207
137.671
132.724
131.551
119.012
116.690
99.009
88.184
87.797
84.298
81.240
73.713
71.307
65.815
65.496
62.404
41.273
40.736
31.582
30.779
28.549
25.589
24.121
24.054
22.639
22.611
19.679
19.654
16.933
16.911
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm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5.618
5.592
4.177
4.172
3.764
3.758
2.491
2.479
2.467
1.816
1.805
1.794
1.530
1.498
1.305
1.298
1.292
0.896
0.885
0.873
HO
OH
ppm
1.00
1.04
0.99
0.98
1.07
2.13
1.98
2.14
2.17
2.49
1.52
5.48
3.48
143.981
140.196
129.122
110.275
84.813
77.372
77.160
76.948
74.668
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65.915
61.552
37.322
31.853
31.056
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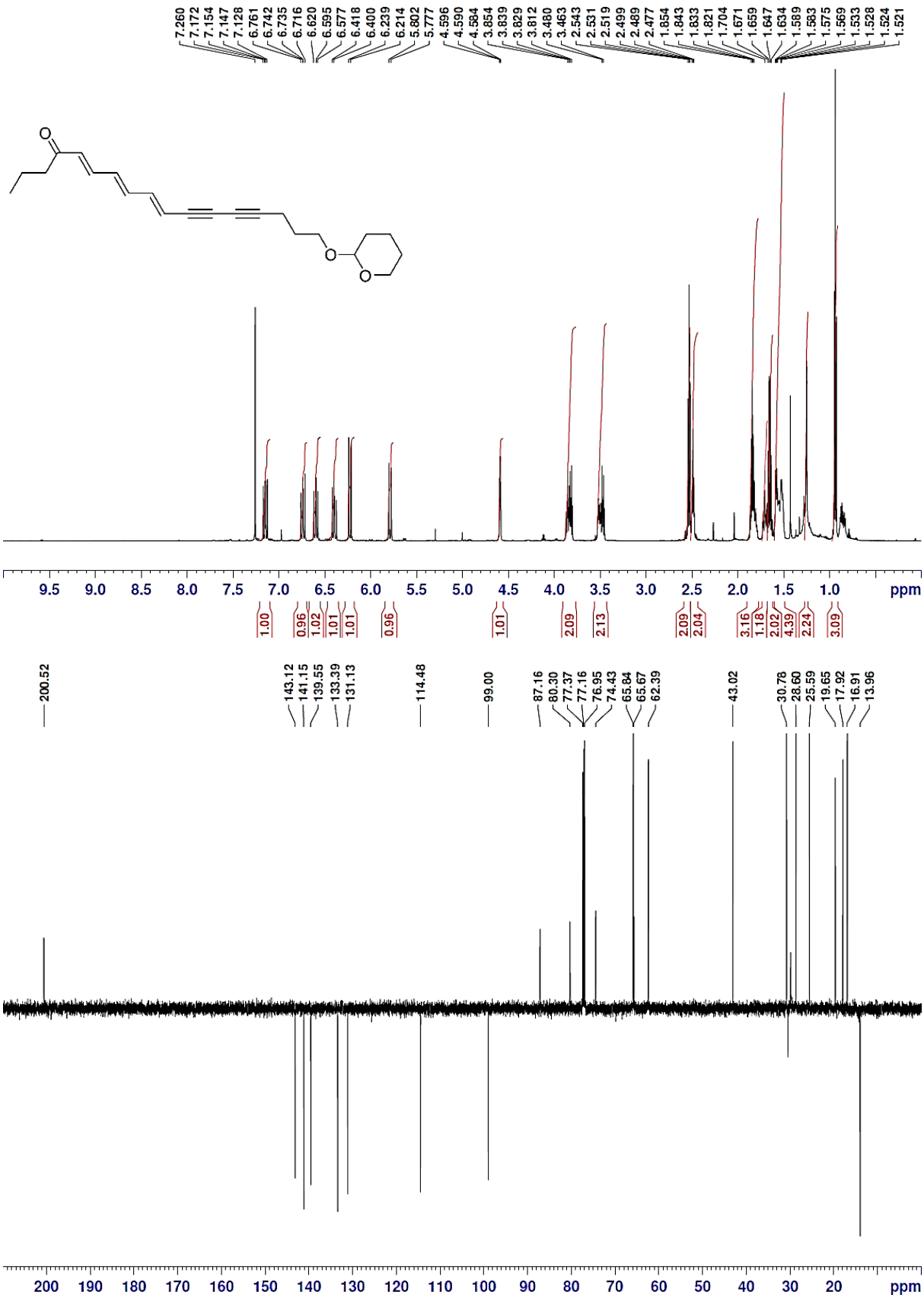
14.167
ppm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6.735
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6.620
6.595
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6.418
6.400
6.239
6.214
5.802
5.777
4.596
4.590
4.584
3.854
3.839
3.829
3.812
3.480
3.463
2.543
2.531
2.519
2.499
2.489
2.477
1.854
1.843
1.833
1.821
1.704
1.671
1.659
1.647
1.634
1.589
1.583
1.575
1.569
1.533
1.528
1.524
1.521
O
O
O
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 ppm
1.00 0.96 1.02 1.01 1.01 0.96 1.01 2.09 2.13 2.09 2.04 3.16 1.18 2.02 4.39 2.24 3.09
200.52
143.12
141.15
139.55
133.39
131.13
114.48
99.00
87.16
80.30
77.37
77.16
76.95
74.43
65.84
65.67
62.39
43.02
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25.59
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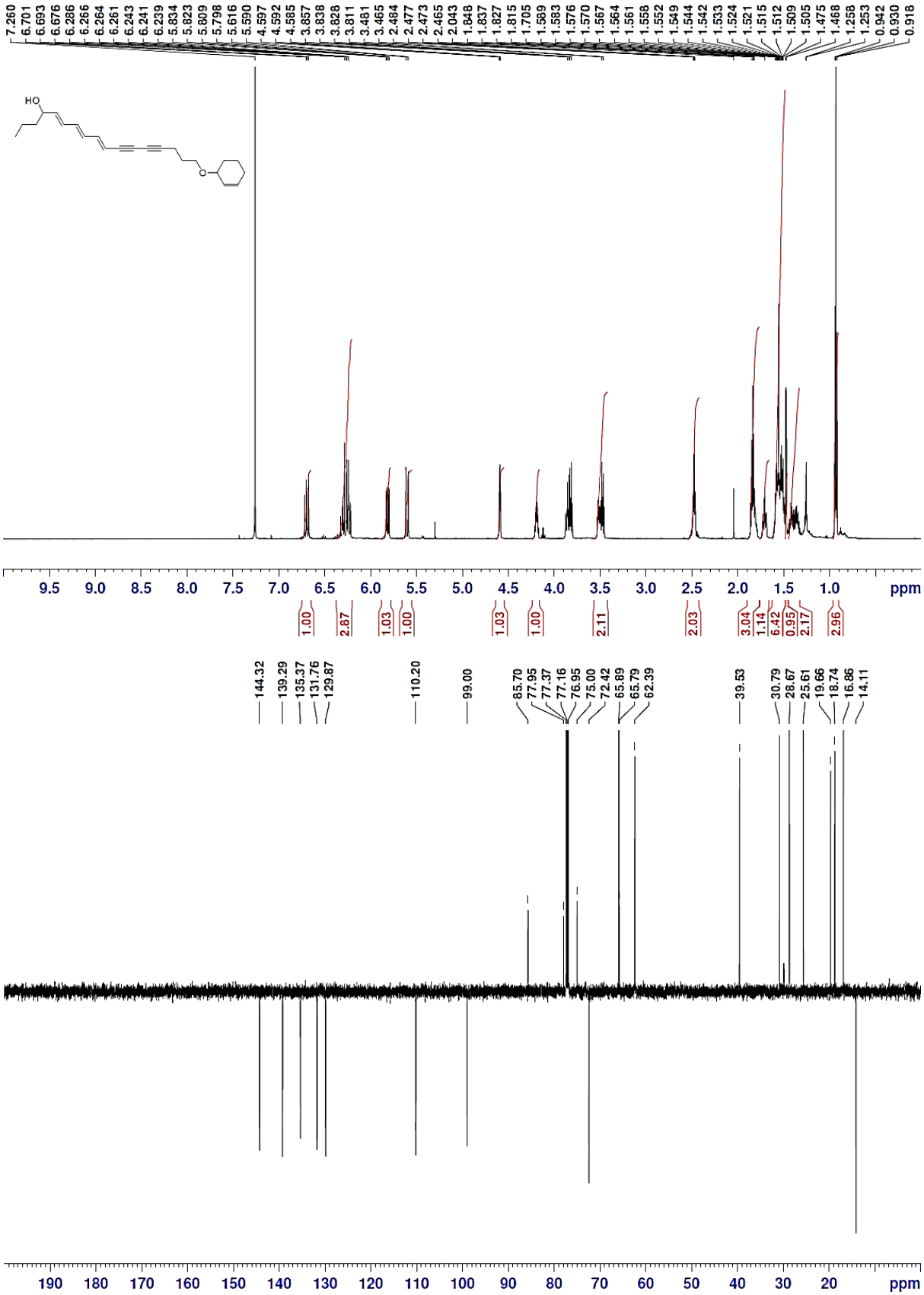
17.92
16.91
13.96
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm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6.266
6.264
6.261
6.243
6.241
6.239
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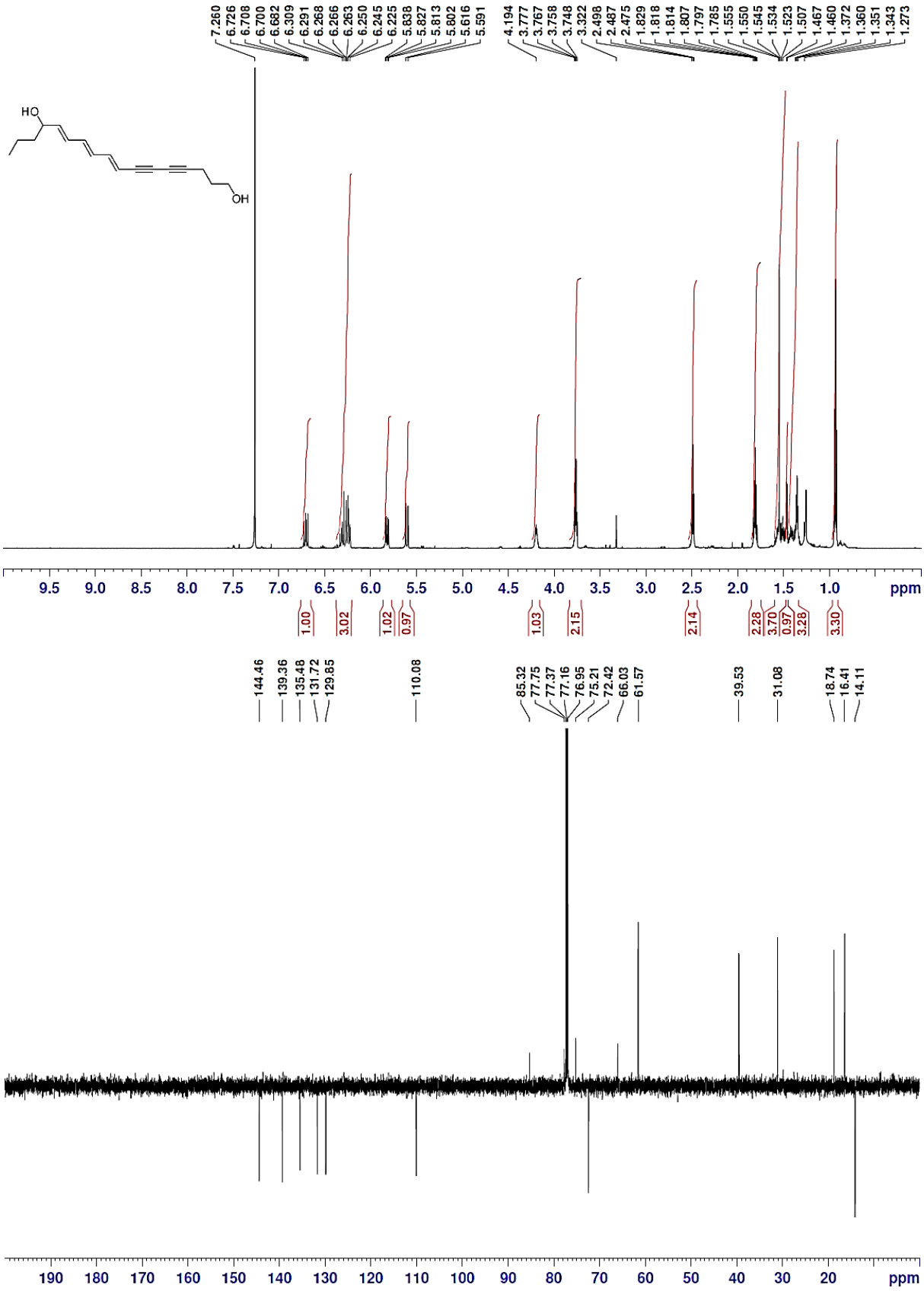
5.823
5.809
5.798
5.616
5.590
4.597
4.592
4.585
3.857
3.838
3.828
3.811
3.481
3.465
2.484
2.477
2.473
2.465
2.043
1.848
1.837
1.827
1.815
1.705
1.589
1.583
1.576
1.570
1.567
1.564
1.561
1.558
1.552
1.549
1.544
1.542
1.533
1.524
1.521
1.515
1.512
1.509
1.505
1.475
1.468
1.258
1.253
0.942
0.930
0.918
HO
O
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 ppm
1.00
2.87
1.03
1.00
1.03
1.00
2.11
2.03
3.04
1.14
6.42
0.95
2.17
2.96
144.32
139.29
135.37
131.76
129.87
110.20
99.00
85.70
77.95
77.37
77.16
76.95
75.00
72.42
65.89
65.79
62.39
39.53
30.79
28.67
25.61
19.66
18.74
16.86
14.11
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm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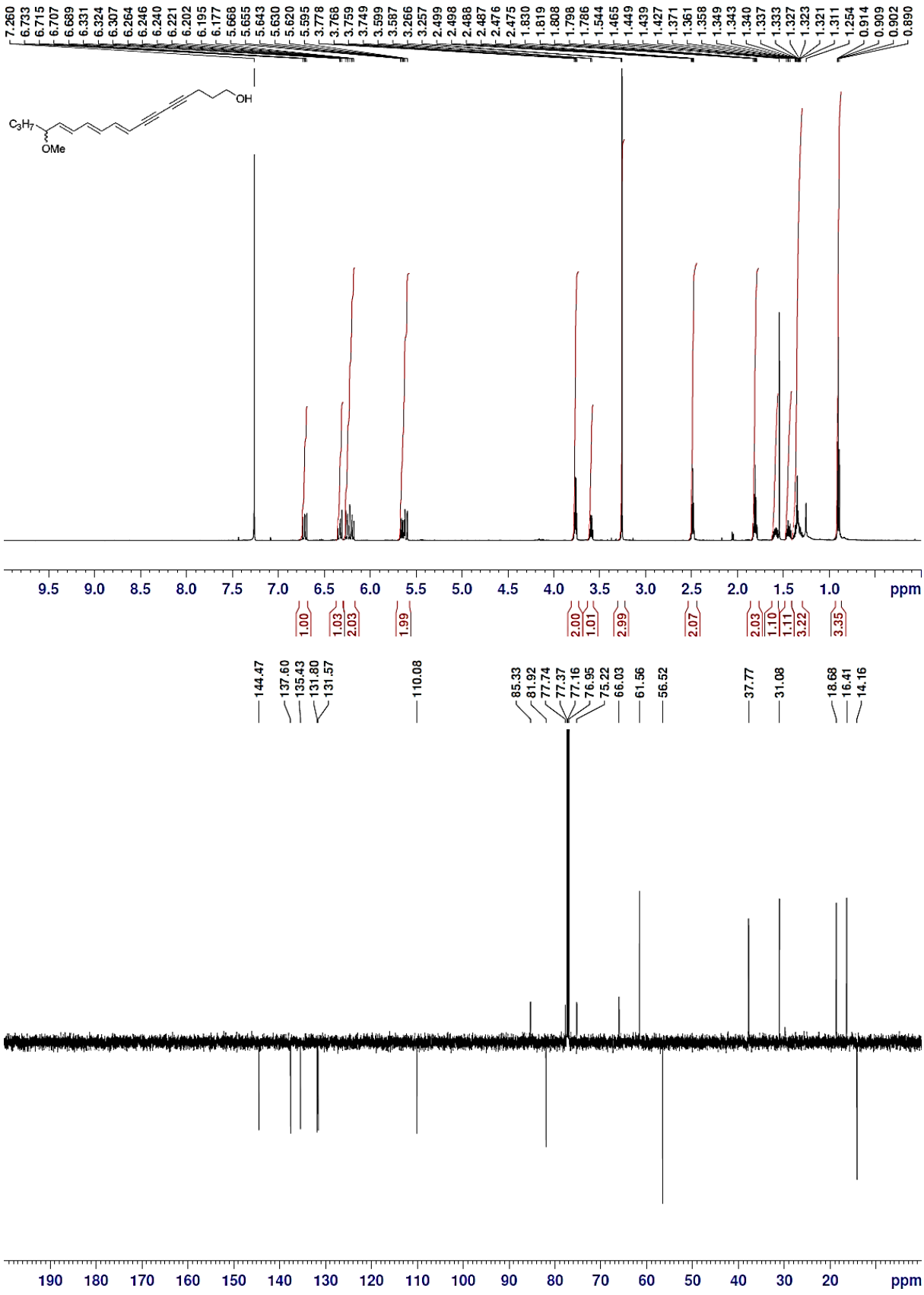
6.268
6.266
6.263
6.250
6.245
6.225
5.838
5.827
5.813
5.802
5.616
5.591
4.194
3.777
3.767
3.758
3.748
3.322
2.498
2.487
2.475
1.829
1.818
1.814
1.807
1.797
1.785
1.555
1.550
1.545
1.534
1.523
1.507
1.467
1.460
1.372
1.360
1.351
1.343
1.273
HO
OH
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
1.00
3.02
1.02
0.97
1.03
2.15
2.14
2.28
3.70
0.97
3.28
3.30
144.46
139.36
135.48
131.72
129.85
110.08
85.32
77.75
77.37
77.16
76.95
75.21
72.42
66.03
61.57
39.53
31.08
18.74
16.41
14.11
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm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6.715
6.707
6.689
6.331
6.324
6.307
6.264
6.246
6.240
6.221
6.202
6.195
6.177
5.668
5.655
5.643
5.630
5.620
5.595
3.778
3.768
3.759
3.749
3.599
3.587
3.266
3.257
2.499
2.498
2.488
2.487
2.476
2.475
1.830
1.819
1.808
1.798
1.786
1.544
1.465
1.449
1.439
1.427
1.371
1.361
1.358
1.349
1.343
1.340
1.337
1.333
1.327
1.323
1.321
1.311
1.254
0.914
0.909
0.902
0.890
OH
C3H7
OMe
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 ppm
1.00 1.03 2.03 1.99 2.00 1.01 2.99 2.07 2.03 1.10 1.11 3.22 3.35
144.47
137.60
135.43
131.80
131.57
110.08
85.33
81.92
77.74
77.37
77.16
76.95
75.22
66.03
61.56
56.52
37.77
31.08
18.68
16.41
14.16
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm