# Identification of a thioesterase bottleneck in the pikromycin pathway through full-module processing of unnatural pentaketides

**AUTHOR INFORMATION**

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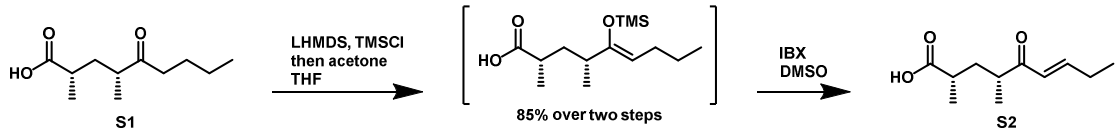
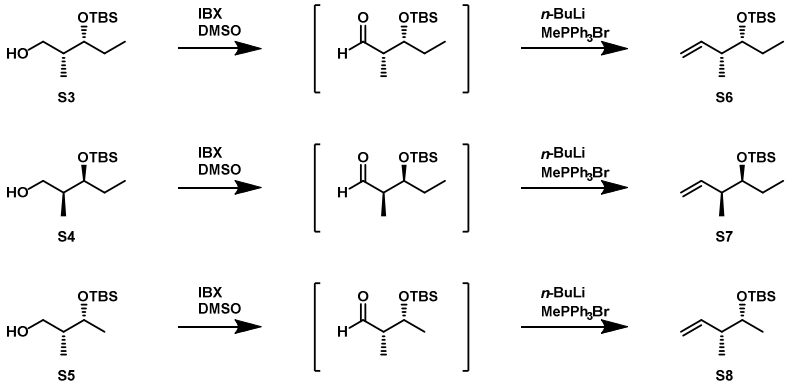
**Chemistry**: Reactions were performed in evacuated (<0.05 torr) flame dried glassware backfilled with dry $N_2$ and run under a positive pressure of dry $N_2$ provided by a mineral oil bubbler unless stated otherwise (open flask). Reactions at elevated temperatures were controlled by IKA RET Control Visc (model RS 232 C), room temperature (RT) reactions were conducted at ~23 °C, reactions run cooler than room temperature were performed in ice (0 °C) or dry ice/acetone (-78 °C) baths. Analytical thin-layer chromatography (TLC) was performed with EMD 60 $F_{254}$ pre-coated glass plates (0.25 mm) and visualized using a combination of UV, *p*-anisaldehyde, $KMnO_4$, and bromocresol green stains. Flash column chromatography was performed using EMD 60 Gerduran® (particle size 0.04-0.063) silica gel. Commercial purification system MBraun-MB-SPS # 08-113 provided all dry solvents unless stated otherwise (technical grade). NMR spectra were recorded on a Varian 600 MHz spectrometer. $^1$H NMR spectra were recorded relative to residual solvent peak ($CDCl_3$ $\delta_H$ 7.26 ppm, $D_6$-DMSO $\delta_H$ 2.50 ppm, $D_6$-acetone $\delta_c$ 2.05 ppm) and reported as follows: chemical shift (ppm), multiplicity, coupling constant (Hz), and integration. Multiplicity abbreviations are as follows: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, h = hextet, ovlp = overlap, br = broad signal. $^{13}$C NMR spectra were recorded relative to residual solvent peaks ($CDCl_3$ $\delta_C$ 77.0 ppm, $D_6$-DMSO $\delta_c$ 39.5 ppm, $D_6$-acetone $\delta_c$ 29.8 ppm). High resolution mass spectrometry was performed on an Agilent quadrapole time-of-flight spectrometer (Q-TOF 6500 series) by electrospray ionization (ESI).




**S2** Adapted from literature procedure**:**[1] To a 500 mL flask containing **S1**[1] (9.0 g, 44.8 mmol, 1 equiv) in THF (30 mL) at -78 °C was added TMSCl (freshly distilled, Sigma, 22.8 mL, 19.5 g, 179.3 mmol, 4 equiv) down the side of the flask. A second flask was charged with LHMDS solution (Sigma, 1 M in THF, 179.3 mL, 179.3 mmol, 4 equiv) and cooled to -78 °C. LHMDS solution was added dropwise to the **S1** solution via by cannula then stirred at -78 °C for 30 min, followed by dropwise addition of acetone (11.7 g, 14.8 mL, 201.6 mmol, 4.5 equiv) with 10 min additional stirring. The solution was allowed to warm to RT and concentrated. The crude solid was suspended in hexanes, filtered through a fine fritted glass funnel, and the resulting solid was then rinsed with hexanes (2x). The filtrate was poured through a sodium sulfate plug, which was then rinsed with hexanes (2x) and subsequent concentration gave the crude trimethylsilyl enol ether of **S1** (contaminating (isopropenyloxy)trimethylsilane was mostly removed under subsequent high vacuum).
IBX[2] (0.4 M in technical grade DMSO, 224 mL, 89.6 mmol, 2.0 equiv) was added to the crude silyl enol ether and stirred for 12 h (the solution turns yellow and a white precipitate forms) in an open flask. The reaction was diluted with $H_2O$ (2 volumes) and extracted with $Et_2O$:hexanes (4:1, 3x). Combined organic extracts were washed with brine (1x), filtered through a sodium sulfate plug, which was then rinsed with $Et_2O$ (2x). Concentration and flash chromatography: AcOH/EtOAc/Hexanes (0:10:90) to AcOH/EtOAc/Hexanes (1:10:89) gave **S2** as a pale yellow oil (7.6 g, 38.1 mmol, 85% yield). Matched previously reported spectral data.[1]



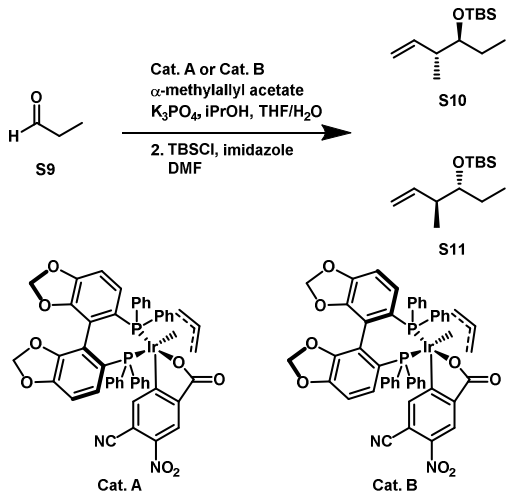

General two-step oxidation/olefination procedure[1] for **S6-S8** from Evans aldol products **S3-S5**.[3-5]
A flask containing $MePPh_3Br$ (AK, 1.10 equiv) was placed in an oil bath, and heated to 110 °C under high vacuum for 4 h. The flask was cooled to RT and backfilled with $N_2$, THF (0.2 M) and cooled to 0 °C. *n*-BuLi (Sigma, 1.10 equiv) was added dropwise (solution turns from colorless to red and solid $MePPh_3Br$ dissolves completely) and the reaction was allowed to warm to RT and stirred for a minimum 1 h.
To an open flask was added **S3, S4, or S5** (1 equiv), technical grade DMSO (80 mL, 0.25 M) and IBX (1.50 equiv) in a single portion. The reaction was monitored by TLC, and after complete consumption of starting material (~4 h) $Et_2O$ was added. The reaction was quenched with cold sodium thiosulfate (sat. aq. solution), and stirred for 30 min, the aqueous layer was separated and the organic layer was washed with saturated thiosulfate (sat. aq. solution, 2x), brine, filtered through a sodium sulfate plug, rinsed with $Et_2O$ (2x) and concentrated. The crude aldehyde was dissolved in THF (20 mL) and used immediately.
Both flasks were cooled to -78 °C and the crude aldehyde was added via cannula to the prepared ylide. The solution was stirred at -78 °C for 30 min, warmed to RT and stirred for an additional 30 min. The reaction was quenched with $NH_4Cl$ (half sat. aq. solution) and extracted with pentane (3x), filtered through a sodium sulfate plug, rinsed with pentane (2x) and carefully concentrated (rotovap bath cooled to 0°C) to give the crude alkene product. Flash chromatography: pentane afforded **S6, S7,** or **S8** as a clear oil.

Note: **S6, S7,** and **S8** are volatile under high vacuum.

**S6:** 87% at 18 millimole scale. Matched previously reported spectral data.[1]

**S7:** 88% at 6.5 millimole scale.
**$^1$H NMR** ($CDCl_3$, 599 MHz) δ 5.84 (ddd, *J* = 17.5, 10.4, 7.3 Hz, 1H), 5.07 – 4.91 (m, 2H), 3.46 (q, *J* = 5.5 Hz, 1H), 2.31 (h, *J* = 6.7 Hz, 1H), 1.49-1.36 (m, 2H), 0.97 (d, *J* = 6.8 Hz, 3H), 0.90 (s, 9H), 0.86 (t, *J* = 7.4 Hz, 3H), 0.04 (s, 6H).
**$^{13}$C NMR** ($CDCl_3$, 151 MHz): δ 141.8, 113.6, 77.00, 42.3, 26.5, 25.9, 18.2, 15.0, 9.5, -4.3, -4.4.
**HRMS**: Calculated $[M\text{-}CH_3]^+$ 213.1669, found 213.1678.

**S8:** 82% yield at 10 millimole scale.
**$^1$H NMR** ($CDCl_3$, 599 MHz) δ 5.80 (ddd, *J* = 17.7, 10.5, 7.5 Hz, 1H), 5.03 – 4.95 (m, 2H), 3.63 (p, *J* = 6.1 Hz, 1H), 2.16 (h, *J* = 6.6 Hz, 1H), 1.07 (d, *J* = 6.2 Hz, 3H), 0.98 (d, *J* = 6.8 Hz, 3H), 0.89 (s, 9H), 0.04 (d, *J* = 2.9 Hz, 6H).
**$^{13}$C NMR** ($CDCl_3$, 151 MHz): δ 141.6, 113.9, 71.9, 45.5, 25.9, 21.0, 18.1, 15.4, -4.4, -4.8.
**HRMS:** Calculated $[M\text{-}CH_3]^+$ 199.1513, found 199.1526.



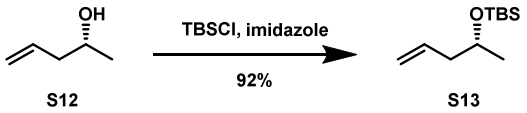

General two-step crotylation[6]/silylation procedure for **S10** and **S11**.

An oven dried pressure tube was charged with catalyst **A or B**[10] (5 mol %), α-methylallyl acetate (TCI, 2 equiv), $K_3PO_4$ (EMD, 0.5 equiv), iPrOH (EMD, 2 equiv), $H_2O$ (5 equiv), propionaldehyde (**S9**) (Sigma, distilled neat, 1 equiv), THF (2 M) under a stream of nitrogen. The tube was sealed and placed in a 60 °C oil bath and stirred for 48 hours. After cooling to RT, *n*-pentane was added (5 volumes relative to THF) and the heterogeneous mixture was filtered through a plug of sodium sulfate, then rinsed with *n*-pentane (2x). (Crude catalyst was subsequently recovered by rinsing 3x with $CH_2Cl_2$.)[7] Pentane carefully removed (rotovap bath cooled to 0°C).
To the crude crotylation product was added DMF (0.5 M) followed by imidazole (Fisher, 5 equiv) and tertbutyldimethylsilyl chloride (Oakwood, 5 equiv). The solution was warmed to 60 °C and stirred for 12 hours. After cooling to RT, the solution was diluted with $H_2O$ and extracted with *n*-pentane (2x), filtered through a plug of sodium sulfate, then rinsed with *n*-pentane (2x). Careful concentration (rotovap bath cooled to 0 °C) and flash chromatography: pentane yields **S10** or **S11** as a clear oil.

Note: **S10** and **S11** are volatile under high vacuum, as are intermediate alcohols.

**S10:** 64% over two steps at 38 millmole scale (using catalyst **B,** from (*R*)-SEGPHOS).
(**dr** >**20:1***;* only one diastereomer is observed by **$^1$H NMR**)
(only one diastereomer is observed by crude **$^1$H NMR** after cross-metathesis with **S2,** suggesting **er >15:1**)
**$^1$H NMR** ($CDCl_3$, 599 MHz) δ 5.84 – 5.75 (m, 1H), 5.03 – 4.96 (m, 2H), 3.46 (td, *J* = 6.1, 4.0 Hz, 1H), 2.39 – 2.23 (m, 1H), 1.45 – 1.37 (m, 1H), 0.98 (d, *J* = 6.9 Hz, 3H), 0.89 (s, 9H), 0.84 (t, J = 7.5 Hz, 1H), 0.04 (s, 6H).
**$^{13}$C NMR** ($CDCl_3$, 151 MHz): δ 141.1, 114.1, 77.2, 42.7, 26.4, 25.9, 18.2, 15.5, 10.1, -4.3, -4.5.
**HRMS**: Calculated $[M\text{-}CH_3]^+$ 213.1669, found 213.1679.

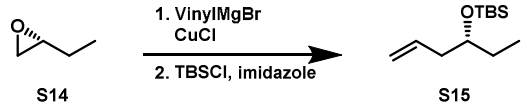
**S11:** 53% over two steps at 19 millimole scale. (Using catalyst **A**, from (*S*)-SEGPHOS).
(**dr** >**20:1***;* only one diastereomer is observed by **$^1$H NMR**)
(only one diastereomer is observed by crude **$^1$H NMR** after cross-metathesis with **S2,** suggesting **er >15:1**)
**$^1$H NMR** ($CDCl_3$, 599 MHz) δ 5.84 – 5.74 (m, 1H), 5.02 – 4.95 (m, 2H), 3.46 (td, J = 6.0, 4.2 Hz, 1H), 2.43 – 2.26 (m, 1H), 1.43 – 1.37 (m, 1H), 0.98 (d, J = 6.9 Hz, 1H), 0.89 (s, 9H), 0.84 (t, J = 7.5 Hz, 1H), 0.04 (s, 6H).
**$^{13}$C NMR** ($CDCl_3$, 151 MHz): δ 141.1, 114.1, 77.2, 42.7, 26.4, 25.9, 18.2, 15.6, 10.1, -4.3, -4.5.
**HRMS**: Calculated $[M\text{-}CH_3]^+$ 213.1669, found 213.1681.




**S13:** A 50-mL flask was charged with (*R*)-4-penten-2-ol (**S12**, Sigma, 1.0 g, 11.6 mmol, 1 equiv), DMF (11.6 mL, 1M), followed by imidazole (Fisher, 0.9 g, 14.0 mmol, 1.2 equiv) and tertbutyldimethylsilyl chloride (Oakwood, 4.2 g, 27.9 mmol, 1.1 equiv). The reaction was stirred for 12 h at RT before the solution was loaded onto a flash column: pentane to yield **S13** (2.1 g, 10.7 mmol, 92% yield) as a colorless oil.

Note: **S13** is volatile under high vacuum.

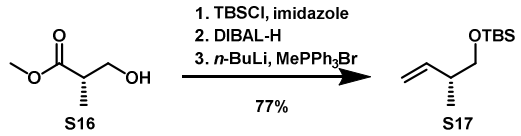
**$^1$H NMR** ($CDCl_3$, 599 MHz) δ 5.81 (ddt, *J* = 17.3, 10.2, 7.2 Hz, 1H), 5.06 – 4.99 (m, 2H), 3.84 (h, *J* = 6.1 Hz, 1H), 2.30 – 2.09 (m, 2H), 1.13 (d, *J* = 6.1 Hz, 3H), 0.89 (s, 9H), 0.05 (s, 3H), 0.05 (s, 3H).
**$^{13}$C NMR** ($CDCl_3$, 151 MHz) δ 135.6, 116.5, 68.4, 44.3, 25.8, 23.38, 18.14, -4.5, -4.7.
**HRMS**: Calculated $[M\text{-}CH_3]^+$ 185.1356, found 185.1363.




**S15** adapted from literature procedure:[8] To a 250 mL flask was added CuCl (Sigma, 0.6 g, 5.4 mmol, 20 mol %), vinylmagnesium bromide (Sigma, 1 M in THF, 55.5 mL, 55.5 mmol, 2 equiv), then cooled to -10 °C. (*R*)-1,2-epoxybutane (**S14**, Sigma, 2.0 g in 8 mL THF, 27.7 mmol, 1 equiv) was added via syringe drive over the course of 1 h. After complete addition, the solution was allowed to warm to 0 °C followed by the addition of solid imidazole (Fisher, 4.0 g, 58.2 mmol, 2.1 equiv) and tertbutyldimethylsilyl chloride (Oakwood, 8.8 g, 58.2 mmol, 2.1 equiv). The flask was fitted with a reflux condenser and heated to 70 °C for 18 h. After cooling to RT the solution was diluted with *n*-pentane, washed with $H_2O$ (2x), sodium thiosulfate (sat. aq. solution), and filtered through a sodium sulfate plug then rinsed with *n*-pentane (2x). Careful concentration (rotovap bath cooled to 0°C) and flash chromatography: pentane yields **S15 (**5.2 g, 24.1 mmol, 87% over two steps) as a colorless oil.

Note: **S15** is volatile under high vacuum.

**$^1$H NMR** ($CDCl_3$, 599 MHz) δ 5.81 (ddt, *J* = 17.3, 10.1, 7.2 Hz, 1H), 5.08 – 4.98 (m, 1H), 3.63 (p, *J* = 5.8 Hz, 1H), 2.26 – 2.15 (m, 2H), 1.54 – 1.38 (m, 2H), 0.89 (s, 9H), 0.87 (ovlp t, *J* = 8.0 Hz, 3H), 0.05 (s, 6H).
**$^{13}$C NMR** ($CDCl_3$, 151 MHz): δ 135.5, 116.4, 73.1, 41.4, 29.4, 25.9, 18.1, 9.6, -4.5, -4.6.
**HRMS**: Calculated $[M\text{-}CH_3]^+$ 199.1513, found 199.1521.




**S17:** An open 250-mL flask was charged with (*S*)-Roche ester (**S16**, TCI, 3.0 g, 25.4 mmol, 1.0 equiv), imidazole (Fisher, 1.9 g, 27.9 mmol, 1.1 equiv), technical grade $CH_2Cl_2$, (51 mL, 0.5M) and cooled to 0 °C. Tertbutyldimethylsilyl chloride (Oakwood, 4.2 g, 27.9 mmol, 1.1 equiv) was added in 5 portions. The resulting solution was stirred for 1 h at 0 °C and became cloudy with white precipitate. $NH_4Cl$ (half sat. aq. solution) was added until the precipitate was completely dissolved. The organic layer was separated and the aqueous layer extracted with $CH_2Cl_2$ (2x). The combined organic extracts were washed with brine and filtered through a sodium sulfate plug, which was subsequently rinsed with $CH_2Cl_2$ (2x). Concentration and subsequent high vacuum yielded colorless oil that was carried onto the next step without further purification.

The following two steps were performed concurrently:

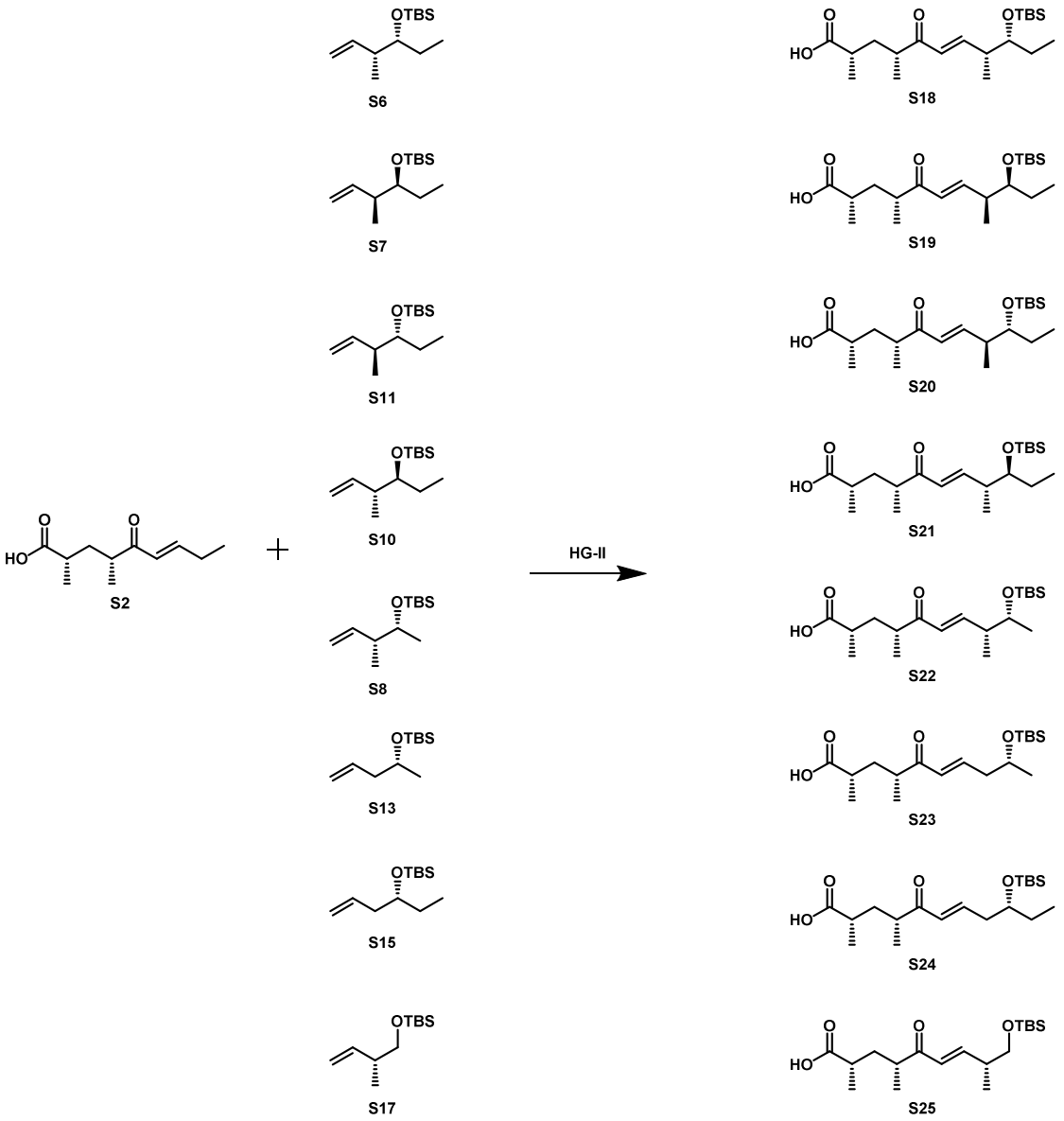
To a 250-mL flask containing $MePPh_3Br$ (AK, 10.0 g, 27.9 mmol, 1.1 equiv) was placed in an oil bath, and heated to 110 °C under high vacuum for 4 h. The flask was cooled to RT and backfilled with $N_2$, THF (128 mL, 0.2M) and cooled to 0 °C. *n*-BuLi (Sigma, 2.48M, 11.3 mL, 1.10 equiv) was added dropwise (solution turns colorless to red and solid $MePPh_3Br$ dissolves completely) and the reaction was allowed to warm to RT and stirred for a minimum 1 h.

To a 250-mL flask containing crude silyl ether (~25.4 mmol, 1 equiv) was added $CH_2Cl_2$ (51 mL, 0.5 M) and cooled to -78 °C. DIBAL-H (Sigma, 3.8 g, 4.8 mL, 26.7 mmol, 1.05 equiv) was added slowly down the side of the flask and stirred for 1 h at -78 °C. Methanol (30 mL) was added slowly and the solution was stirred for 15 min at -78 °C. The reaction was decanted into of vigorously stirring $CH_2Cl_2$ (50 mL) at RT, layered with Na/K tartrate (sat. aq. solution, 100 mL) and stirred until the layers became clear. The organic layer was separated and the aqueous layer was extracted with $CH_2Cl_2$ (2x). The combined organic extracts were washed with brine and filtered through a sodium sulfate plug, which was then rinsed with $CH_2Cl_2$ (2x). Concentration and subsequent high vacuum yielded crude aldehyde which was dissolved in THF (10 mL) and used immediately.

Both flasks were cooled to -78 °C and crude aldehyde was added by cannula to the prepared ylide. The solution was stirred at -78 °C for 30 min, warmed to RT and stirred for an additional 30 min. The reaction was quenched with $NH_4Cl$ (sat. aq. solution, 50 mL) and extracted with pentane (3x), filtered through a sodium sulfate plug, rinsed with pentane (2x) and carefully concentrated to give the crude alkene product **S17**. Flash chromatography: pentane afforded **S17** as a clear oil (3.9 g, 19.5 mmol, 77% over three steps.)

Note: **S17** is volatile under high vacuum.

**$^1$H NMR** ($CDCl_3$, 599 MHz) δ 5.77 (ddd, *J* = 17.3, 10.4, 6.9 Hz, 1H), 5.07 – 4.96 (m, 2H), 3.51 (dd, *J* = 9.8, 6.2 Hz, 1H), 3.41 (dd, *J* = 9.7, 7.0 Hz, 1H), 2.37 – 2.27 (m, 1H), 1.00 (d, *J* = 6.8 Hz, 3H), 0.89 (s, 9H). 0.04 (s, 6H).
**$^{13}$C NMR** ($CDCl_3$, 151 MHz) δ 141.4, 113.9, 67.9, 40.3, 25.9, 18.3, 16.0, -5.3, -5.4.
**HRMS**: Calculated $[M\text{-}CH_3]^+$ 185.1356, found 185.1366.




**S18-25:** General cross metathesis of **S2** and silyl ethers **S6-S8, S10, S11, S13, S15, S17**.
A 10 mL recovery flask was charged with **S2** (1 equiv), **silyl-ether** (1.5 equiv) and Hoveyda-Grubbs 2nd generation (Sigma, 24 mg, 0.04 mmol, 3 mol%) under a stream of $N_2$. An 18-gauge needle was placed into the septum, venting to the atmosphere (in addition to positive pressure of $N_2$) and the flask was heated to 60 °C for 12 h. Flash chromatography: AcOH/EtOAc/hexanes (1:10:89) to yield **S18-S25**.

Note: Typically, Hoveyda-Grubbs 2nd generation catalyst had decomposed completely (TLC) after 12 h, though it remained detectable by TLC on occasion. Raising the temperature to 80 °C for an additional 2 h resulted in complete catalyst decomposition before flash chromatography.

**S18:** 81% yield at a 1.26 mmol scale. Matched previously reported spectral data.[1]

**S19:** 78% yield at a 1.26 millimole scale.

**$^1$H NMR** ($CDCl_3$, 599 MHz) δ 6.94 (dd, J = 15.9, 7.4 Hz, 1H), 6.14 (dd, J = 15.9, 1.0 Hz, 1H), 3.59-3.53 (m, 1H), 2.87 (h, *J* = 6.9 Hz, 1H), 2.58-2.44 (m, 2H), 2.17 – 2.08 (m, 2H), 1.52-1.44 (m, 1H), 1.19 (d, *J* = 7.0 Hz, 3H), 1.12 (d, *J* = 6.9 Hz, 3H), 1.03 (d, *J* = 6.8 Hz, 3H), 0.88 (s, 9H), 0.86 (ovlp t, *J* = 7.0Hz, 3H), 0.04 (s, 3H), 0.03 (s, 3H).
**$^{13}$C NMR** ($CDCl_3$, 151 MHz) δ 202.9, 181.8, 150.8, 127.9, 76.4, 41.6, 41.3, 37.0, 36.2, 26.8, 25.8, 18.1, 17.5, 16.7, 14.3, 9.6, -4.4, -4.5.
**HRMS**: Calculated $[M+H]^+$ 371.2612, found 371.2614.

**S20:** 77% yield at a 1.26 millimole scale.
**$^1$H NMR** ($CDCl_3$, 599 MHz) δ 6.90 (dd, *J* = 15.9, 7.9 Hz, 1H), 6.14 (d, *J* = 15.9 Hz, 1H), 3.56 – 3.52 (m, *J* = 1H), 2.87 (h, *J* = 7.2 Hz, 1H), 2.56 – 2.43 (m, 2 H), 2.12 (ddd, 14.4, 7.8, 6 Hz, 1H), 1.50 – 1.33 (m, 2H), 1.19 (d, *J* = 7.0 Hz, 3H), 1.12 (d, *J* = 6.9 Hz, 3H), 1.05 (d, *J* = 6.8 Hz, 3H), 0.88 (s, 9H), 0.85 (t, *J* = 7.4 Hz, 3H), 0.04 (s, 3H), 0.03 (s, 3H).
**$^{13}$C NMR** ($CDCl_3$, 151 MHz) δ 203.0, 182.1, 150.2, 128.3, 76.6, 41.5, 41.2, 37.9, 36.2, 27.1, 25.9, 25.8, 18.1, 17.4, 16.7, 15.4, 9.6, -4.3, -4.6.
**HRMS**: Calculated $[M+H]^+$ 371.2612, found 371.2612.

**S21:** 82% yield at a 1.26 millimole scale.
**$^1$H NMR** ($CDCl_3$, 599 MHz) δ 6.91 (dd, *J* = 16.0, 7.9 Hz, 1H), 6.14 (d, *J* = 15.9 Hz, 1H), 3.58 - 3.51 (m, 1H), 2.87 (h, *J* = 7.2 Hz, 1H), 2.58 – 2.44 (m, 2H), 2.13 (ddd, 14.4, 7.8, 6 Hz, 1H), 1.52 – 1.35 (m, 2H), 1.20 (d, *J* = 7.0 Hz, 3H), 1.13 (d, *J* = 7.0 Hz, 3H), 1.06 (d, *J* = 6.8 Hz, 3H), 0.89 (s, 9H), 0.86 (t, *J* = 7.4 Hz, 3H), 0.05 (s, 3H), 0.04 (s, 3H).
**$^{13}$C NMR** ($CDCl_3$, 151 MHz) δ 203.0, 181.7, 150.2, 128.3, 76.6, 41.5, 41.2, 36.9, 36.2, 27.1, 25.8, 18.1, 17.5, 16.7, 15.4, 9.6, -4.3, -4.6.
**HRMS**: Calculated $[M+H]^+$ 371.2612, found 371.2609.

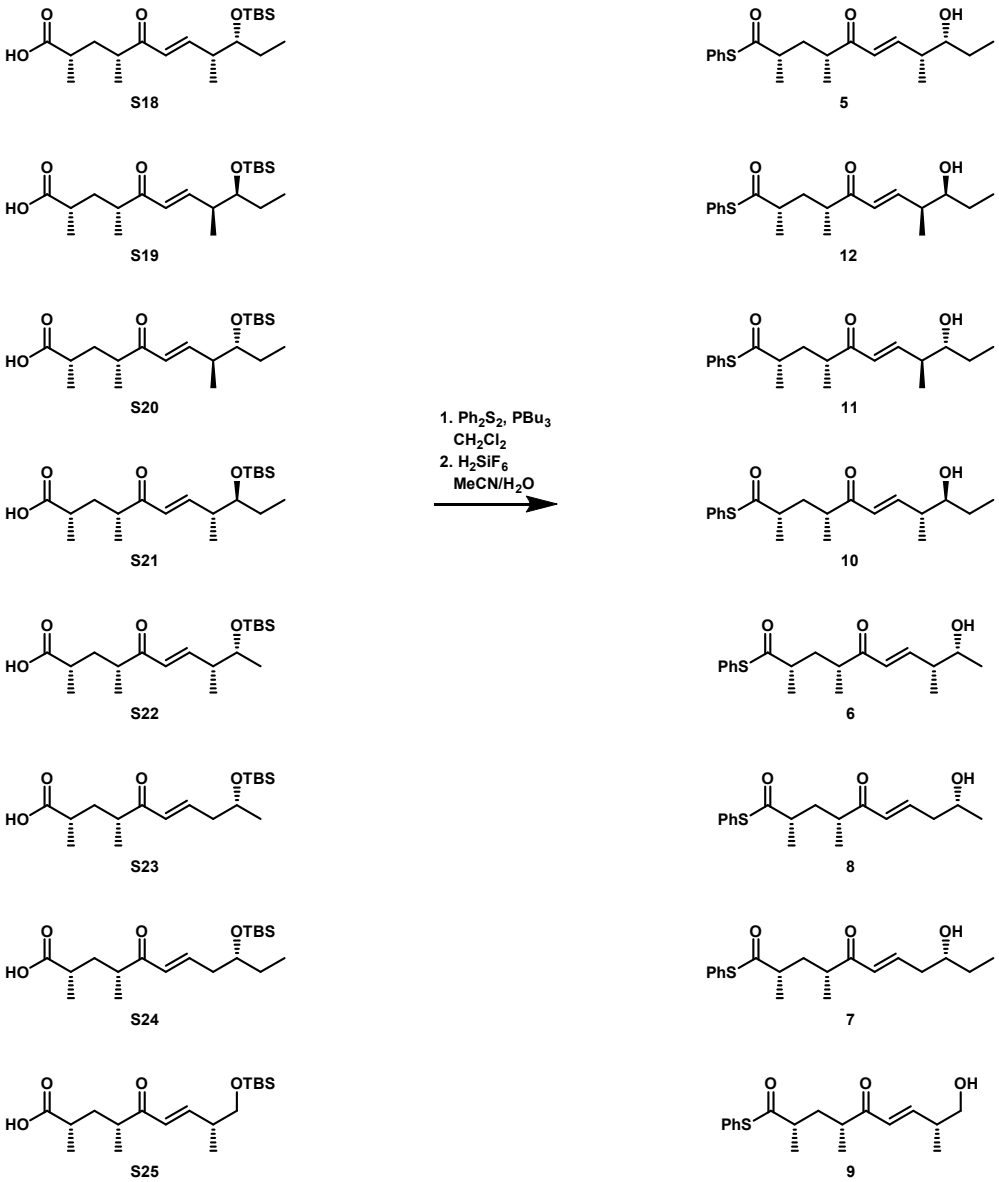
**S22:** 66% yield at a 1.26 millimole scale.
**$^1$H NMR** ($CDCl_3$, 599 MHz) δ 6.91 (dd, *J* = 15.9, 7.7 Hz, 1H), 6.14 (d, *J* = 15.9 Hz, 1H), 3.76 (p, *J* = 6.0 Hz, 1H), 2.87 (h, *J* = 7.2 Hz, 1H), 2.53 (h, *J* = 7.8 Hz, 1H), 2.35 (h, *J* = 6.6 Hz, 1H), 2.12 (ddd, *J* = 14.3, 8.2, 6.5 Hz, 1H), 1.41 (ddd, *J* = 13.8, 7.2, 6.5 Hz, 1H), 1.20 (d, *J* = 7.0 Hz, 3H), 1.12 (d, *J* = 6.9 Hz, 3H), 1.08 (d, *J* = 6.2 Hz, 3H), 1.04 (d, *J* = 6.8 Hz, 3H), 0.88 (s, 9H), 0.04 (s, *J* = 3H), 0.03 (s, *J* = 3H).
**$^{13}$C NMR** ($CDCl_3$, 151 MHz) 203.9, 182.0, 150.4, 128.2, 71.3, 44.5, 41.2, 37.0, 36.3, 25.8, 21.0, 18.0, 17.4, 16.6, 14.8, -4.4, -4.9.

**S23:** 77% yield at a 1.26 millimole scale.
**$^1$H NMR** ($CDCl_3$, 599 MHz) δ 6.90 (dt, *J* = 15.2, 7.4 Hz, 1H), 6.18 (d, *J* = 15.7 Hz, 1H), 3.95 (h, *J* = 15.7 Hz, 1H), 2.85 (h, *J* = 7.2 Hz, 1H), 2.57 - 2.49 (m, 1H), 2.38 – 2.30 (m, 1H), 2.12 (ddd, *J* = 14.4, 7.8, 6 Hz, 1H), 1.41 (ddd, *J* = 13.9, 7.6, 6.2 Hz, 11H), 1.20 (d, *J* = 7.0 Hz, 3H), 1.16 (d, *J* = 6.1 Hz, 3H), 1.12 (d, *J* = 6.9 Hz, 3H), 0.87 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H).
**$^{13}$C NMR** ($CDCl_3$, 151 MHz) δ 202.7, 181.9, 144.7, 130.4, 77.19, 67.6, 42.7, 41.3, 37.0, 36.2, 25.8, 23.8, 18.0, 17.5, 16.6, -4.5, -4.8.
**HRMS**: Calculated $[M+H]^+$ 357.2456, found 357.2457.

**S24**: 77% yield at a 1.26 millimole scale.
**$^1$H NMR** ($CDCl_3$, 599 MHz) δ 6.91 (dt, *J* = 15.3, 7.5 Hz, 1H), 6.18 (dt, *J* = 15.8, 1.2 Hz, 1H), 3.73 (p, *J* = 5.8 Hz, 1H), 2.85 (h, *J* = 7.8 Hz, 1H), 2.59 – 2.45 (m, 1H), 2.41 (m, 2H), 2.12 (ddd, *J* = 14.4, 7.4, 5.3 Hz, 4H), 1.51 –

1.44 (m, 1H), 1.41 (ddd, *J* = 13.9, 7.7, 6.2 Hz, 1H), 1.20 (d, *J* = 7.0 Hz, 3H), 1.12 (d, *J* = 6.9 Hz, 3H), 0.88 (ovlp t, *J* = 7.4 Hz, 3H), 0.88 (s, 9H), 0.04 (s, 3H), 0.03 (s, 3H).
**$^{13}$C NMR** ($CDCl_3$, 151 MHz) δ 202.6, 181.9, 144.5, 130.4, 72.4, 41.3, 40.0, 37.0, 36.2, 29.9, 25.8, 18.0, 17.5, 16.6, 9.5, -4.5, -4.5.
**HRMS**: Calculated $[M+H]^+$ 357.2456, found 357.2452.

**S25:** 51% yield at a 1.26 millimole scale.
**$^1$H NMR** ($CDCl_3$, 599 MHz) δ 6.86 (dd, *J* = 15.9, 7.3 Hz, 1H), 6.18 (dd, *J* = 15.9, 1.2 Hz, 1H), 3.54 (d, *J* = 6.4 Hz, 1H), 2.86 (h, *J* = 7.2 Hz, 1H), 2.60 – 2.47 (m, 1H), 2.12 (ddd, *J* = 13.8, 7.8, 6 Hz, 1H), 1.41 (ddd, *J* = 13.9, 7.7, 6.1 Hz, 1H), 1.20 (d, *J* = 7.0 Hz, 3H), 1.12 (d, *J* = 6.9 Hz, 3H), 1.06 (d, *J* = 6.8 Hz, 3H), 0.88 (s, 9H), 0.04 (s, 3H), 0.03 (s, 3H).
**$^{13}$C NMR** ($CDCl_3$, 151 MHz) δ 203.0, 182.2, 150.2, 128.0, 66.9, 41.4, 39.4, 37.0, 36.2, 25.8, 18.2, 17.5, 16.6, 15.6, -5.4, -5.4
**HRMS**: Calculated $[M+H]^+$ 343.2299, found 343.2301.



**5-12:** General thioesterification and TBS deprotection of **S18-S25.**

A round bottom flask was charged with seco-acid (1 equiv), $Ph_2S_2$ (Sigma, 1.1 equiv), and $CH_2Cl_2$ (0.1 M) and cooled to -78 °C. $PBu_3$ (Sigma, distilled neat, 1.3 equiv) was added dropwise and the reaction was stirred for 45 min at -78 °C before being removed from the cooling bath and immediate quench with a saturated aq. $CuSO_4$ solution and warming to RT. The organic layer was separated, and the aq. layer was extracted further with $CH_2Cl_2$ (2x), $CH_2Cl_2$ layers combined and filtered through a sodium sulfate plug, rinsed with $CH_2Cl_2$ (2x) and concentrated. Flash chromatography (silica topped with 1:1 $SiO_2$:$CuSO_4$): EtOAc/Hexanes (2:98 to 4:96) afforded crude thioesters as a clear oils, which were used immediately in the subsequent step.

An open polyethylene bottle was charged with crude thioester, MeCN (1 M) and cooled to 0 °C. $H_2SiF_6$[9,10] (Fisher, 25% in $H_2O$, 0.8 equiv) was added and the reaction was stirred at 0 °C until complete by TLC. The reaction was monitored by TLC and upon completion (~2-4 h) it was diluted with $CH_2Cl_2$ (10 mL) and carefully quenched with sodium bicarbonate (sat. aq. solution). The aqueous layer was extracted with $CH_2Cl_2$ (2x). Filtration through a sodium sulfate plug then rinsed with $CH_2Cl_2$ (2x) and concentrated. Flash chromatography: EtOAc/hexanes (15:85) gave **5-12** as colorless oils.

**S5:** 90% at a 4 millimole scale. Matched previously reported spectral data.[1]

**S12:** 91% at a 1.92 millimole scale.
**$^1$H NMR** (599 MHz; $D_6$-acetone): δ 7.46-7.43 (m, 5H), 6.93 (dd, *J* = 15.9, 8.0 Hz, 1H), 6.22 (dd, *J* = 15.9, 1.0 Hz, 1H), 3.68 (d, *J* = 5.7 Hz, 1H), 3.45 (dtt, *J* = 11.5, 5.8, 2.9 Hz, 1H), 2.98 (h, *J* = 6.9 Hz, 1H), 2.84 (h, *J* = 6.9 Hz, 1H), 2.45-2.40 (m, 1H), 2.16 (dt, *J* = 14.0, 7.1 Hz, 1H), 1.51 (dqd, *J* = 14.1, 7.2, 3.6 Hz, 1H), 1.43 (dt, *J* = 13.7, 6.9 Hz, 1H), 1.39-1.31 (m, 1H), 1.22 (d, *J* = 6.9 Hz, 3H), 1.13 (d, *J* = 6.9 Hz, 3H), 1.09 (d, *J* = 6.8 Hz, 3H), 0.93 (t, *J* = 7.4 Hz, 3H).
**$^{13}$C NMR** (151 MHz; $D_6$-acetone): δ 202.4, 200.8, 151.1, 135.3, 130.1, 130.0, 128.9, 128.8, 76.0, 46.8, 43.7, 41.6, 37.7, 28.4, 18.4, 17.2, 14.9, 10.7.
**HRMS**: Calculated $[M+H]^+$ 349.1832, found 349.1835.

**S11**: 77% at a 1.62 millimole scale.
**$^1$H NMR** (599 MHz; $D_6$-acetone): δ 7.43-7.39 (m, 5H), 6.93 (dd, *J* = 15.9, 8.3 Hz, 1H), 6.17 (dd, *J* = 15.9, 0.9 Hz, 1H), 3.63 (d, *J* = 5.5 Hz, 1H), 3.42 (dq, *J* = 8.9, 4.5 Hz, 1H), 2.95 (hex, *J* = 6.9 Hz, 1H), 2.80 (dq, *J* = 14.3, 7.0 Hz, 1H), 2.43-2.38 (m, 1H), 2.13 (dt, *J* = 14.0, 7.1 Hz, 1H), 2.01 (dt, *J* = 4.4, 2.2 Hz, 1H), 1.47-1.32 (m, 1H), 1.19 (d, *J* = 6.9 Hz, 3H), 1.09 (d, *J* = 6.9 Hz, 3H), 1.07 (d, *J* = 6.9 Hz, 3H), 0.89 (t, *J* = 7.4 Hz, 3H).
**$^{13}$C NMR** (151 MHz; $D_6$-acetone): δ 202.3, 200.8, 150.3, 135.3, 130.1, 130.0, 129.6, 128.8, 76.3, 46.8, 43.3, 41.5, 37.7, 28.6, 18.3, 17.3, 16.7, 10.6
**HRMS**: Calculated $[M+H]^+$ 349.1832, found 349.1829.

**S10:** 78% at a 1.76 millimole scale.
**$^1$H NMR** (599 MHz; $D_6$-acetone): δ 7.46-7.44 (m, 5H), 6.97 (dd, *J* = 15.9, 8.4 Hz, 1H), 6.20 (dd, *J* = 16.0, 0.9 Hz, 1H), 3.70-3.69 (m, 1H), 3.48-3.44 (m, 1H), 3.00 (h, *J* = 6.9 Hz, 1H), 2.87-2.81 (m, 1H), 2.47-2.41 (m, 1H), 2.16 (ddd, *J* = 14.0, 7.6, 6.6 Hz, 1H), 1.50-1.35 (m, 3H), 1.22 (d, *J* = 6.9 Hz, 3H), 1.13 (d, *J* = 6.9 Hz, 3H), 1.11 (d, *J* = 6.9 Hz, 3H), 0.93 (t, *J* = 7.4 Hz, 3H).

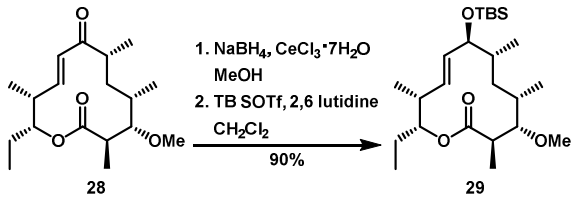
**$^{13}$C NMR** (151 MHz; $D_6$-acetone): δ 202.4, 200.7, 150.3, 135.3, 130.1, 129.9, 129.6, 128.8, 76.3, 46.8, 43.4, 41.4, 37.8, 28.6, 18.3, 17.2, 16.7, 10.6.
**HRMS**: Calculated $[M+H]^+$ 349.1832, found 349.1851.

**S6:** 75 % at a 2.19 millimole scale.
**$^1$H NMR** (599 MHz; $D_6$-acetone): δ 7.47-7.44 (m, 5H), 6.94 (dd, *J* = 15.9, 7.9 Hz, 1H), 6.22 (dd, *J* = 15.9, 1.2 Hz, 1H), 3.75-3.70 (m, 2H), 2.99 (sextet, *J* = 6.9 Hz, 1H), 2.84 (dq, *J* = 14.3, 7.0 Hz, 1H), 2.41-2.37 (m, 1H), 2.16 (ddd, *J* = 13.8, 7.8, 6.7 Hz, 1H), 1.44 (dt, *J* = 13.7, 6.9 Hz, 1H), 1.22 (d, *J* = 6.9 Hz, 3H), 1.13 (d, *J* = 6.9 Hz, 3H), 1.11-1.08 (m, 6H).
**$^{13}$C NMR** (151 MHz; $D_6$-acetone): δ 202.4, 200.7, 150.6, 135.3, 130.1, 130.0, 129.2, 128.8, 70.5, 46.7, 45.0, 41.5, 37.7, 21.1, 18.3, 17.2, 15.2.
**HRMS**: Calculated $[M+Na]^+$ 357.1495, found 357.1497.

**S8:** 72% at a 2.21 millimole scale.
**$^1$H NMR** (599 MHz; $D_6$-acetone): δ 7.46-7.43 (m, 5H), 6.96 (dt, *J* = 15.8, 7.4 Hz, 1H), 6.24 (dt, *J* = 15.8, 1.4 Hz, 1H), 3.94-3.88 (m, 1H), 3.76 (t, *J* = 5.2 Hz, 1H), 3.00-2.94 (m, 1H), 2.87-2.81 (m, 1H), 2.37 (ovlp m, 2H), 2.16 (ddd, *J* = 13.9, 7.9, 6.6 Hz, 1H), 1.43 (ddd, *J* = 13.7, 7.1, 6.7 Hz, 1H), 1.22 (d, *J* = 6.9 Hz, 3H), 1.16 (d, *J* = 6.2 Hz, 3H), 1.13 (d, *J* = 6.9 Hz, 3H).
**$^{13}$C NMR** (151 MHz; $D_6$-acetone): δ 201.3, 199.9, 144.3, 134.5, 130.4, 129.2, 129.1, 127.9, 65.9, 45.9, 42.1, 40.7, 36.7, 22.9, 17.5, 16.3.
**HRMS**: Calculated $[M+Na]^+$ 343.1338, found 343.1341.

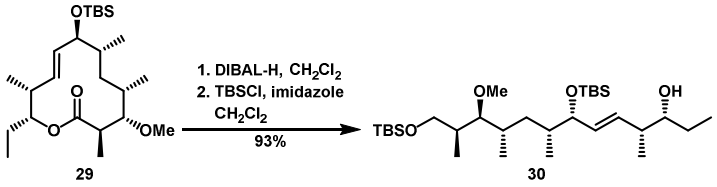
**S7:** 72% at a 2.19 millimole scale.
**$^1$H NMR** (599 MHz; $D_6$-acetone): δ 7.46-7.43 (m, 5H), 6.98 (dt, *J* = 15.8, 7.4 Hz, 1H), 6.25 (dt, *J* = 15.8, 1.4 Hz, 1H), 3.73 (d, *J* = 5.2 Hz, 1H), 3.66-3.62 (m, 1H), 3.00-2.94 (m, 1H), 2.87-2.81 (m, 1H), 2.44-2.38 (m, 1H), 2.36-2.30 (m, 1H), 2.15 (ddd, *J* = 13.8, 7.9, 6.7 Hz, 1H), 1.52-1.47 (m, 3H), 1.22 (d, *J* = 6.9 Hz, 3H), 1.13 (d, *J* = 6.9 Hz, 3H), 0.93 (t, *J* = 7.4 Hz, 3H).
**$^{13}$C NMR** (151 MHz; $D_6$-acetone): δ 202.2, 200.8, 145.5, 135.4, 131.2, 130.1, 130.0, 128.8, 72.0, 46.7, 41.5, 40.9, 37.6, 30.8, 18.3, 17.2, 10.3.
**HRMS**: Calculated $[M+Na]^+$ 357.1495, found 357.1495.

**S9**: 75% at a 1.64 millimole scale.
**$^1$H NMR** (599 MHz; $D_6$-acetone): δ 7.47-7.43 (m, 5H), 6.90 (dd, *J* = 15.9, 7.3 Hz, 1H), 6.24 (dd, *J* = 15.9, 1.3 Hz, 1H), 3.77 (t, *J* = 5.6 Hz, 1H), 3.54-3.51 (m, 2H), 2.97 (q, *J* = 6.9 Hz, 1H), 2.86-2.82 (m, 1H), 2.54-2.49 (m, 1H), 2.16 (ddd, *J* = 13.8, 7.9, 6.6 Hz, 1H), 1.43 (ddd, *J* = 13.8, 7.2, 6.6 Hz, 1H), 1.22 (d, *J* = 6.9 Hz, 3H), 1.13 (d, *J* = 6.9 Hz, 3H), 1.07 (d, *J* = 6.8 Hz, 3H).
**$^{13}$C NMR** (151 MHz; $D_6$-acetone): δ 202.4, 200.8, 150.7, 135.3, 130.1, 130.0, 128.9, 66.7, 46.7, 41.7, 40.4, 37.7, 18.3, 17.2, 16.0.
**HRMS**: Calculated $[M+Na]^+$ 343.1338, found 343.1337.




**29:** An open 100-mL flask was charged with **28**[11] (1.2 g, 3.6 mmol, 1.0 equiv), $CeCl_3 \bullet 7H_2O$ (Fisher, 1.3 g, 3.6 mmol, 1.0 equiv), technical grade MeOH (36 mL, 0.1 M), stirred at RT until dissolved and subsequently cooled to -78 °C. $NaBH_4$ (Fisher, 0.14 g, 3.6 mmol, 1 equiv) was added in a single portion. The resulting solution was stirred for 10 min at -78 °C and decanted into HCl (aq. 1 M), the organic layer was separated and the aqueous layer extracted with $CH_2Cl_2$ (2x). The combined organic extracts were washed with brine and filtered through a sodium sulfate plug, which was subsequently rinsed with $CH_2Cl_2$ (2x). Concentration and subsequent high vacuum yielded a colorless oil that was carried onto the next step without further purification.
A 100-mL flask was charged with the crude allylic-alcohol, $CH_2Cl_2$ (36 mL, 0.1 M) and cooled to -78 °C. 2,6-lutidine (Sigma, 0.5 g, 0.5 mL, 4.7 mmol, 1.3 equiv) was added followed by dropwise addition of TBSOTf (Sigma, 1.1 g, 1.0 mL, 4.32 mmol, 1.2 equiv) and the resulting solution was stirred -78 °C for 1 h before $NH_4Cl$ (sat. aq. solution) quench. The organic layer was separated and the aqueous layer extracted with $CH_2Cl_2$ (2x). The combined organic extracts were washed with brine and filtered through a sodium sulfate plug, which was subsequently rinsed with $CH_2Cl_2$ (2x). Flash chromatography: EtOAc/hexanes (5:95) gave **29** as a colorless oil (1.4 g, 3.2 mmol, 90% over two steps).

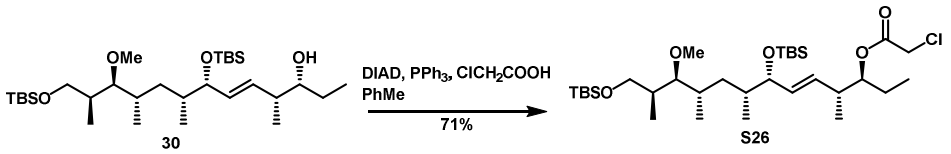
**$^1$H NMR** (599 MHz; $CDCl_3$): δ 5.69-5.66 (m, 1H), 5.39 (dt, *J* = 15.4, 2.3 Hz, 1H), 5.00 (ddd, *J* = 8.8, 5.4, 3.2 Hz, 1H), 4.06 (s, 1H), 3.49 (s, 3H), 3.12 (dd, *J* = 10.3, 1.7 Hz, 1H), 2.61-2.55 (m, 1H), 2.51-2.48 (m, 1H), 2.02-1.96 (m, 1H), 1.89-1.85 (m, 1H), 1.68-1.63 (m, 1H), 1.57-1.51 (m, 1H), 1.35-1.30 (m, 1H), 1.25 (d, *J* = 6.8 Hz, 3H), 1.04 (ovlp m, 6H), 1.01-0.96 (m, 1H), 0.93-0.90 (m, 15H), 0.03 (s, 3H), -0.01 (s, 3H).
**$^{13}$C NMR** (151 MHz; $CDCl_3$): δ 175.2, 131.3, 127.5, 89.1, 77.3, 75.5, 62.7, 43.5, 37.6, 35.7, 32.6, 32.5, 26.0, 24.6, 20.5, 18.3, 17.7, 16.3, 10.3, 10.2, -4.9, -5.2.
**HRMS**: Calculated $[M+Na]^+$ 449.3058, found 449.3056




**30:** A 100-mL flask was charged with **29** (1.5 g, 3.5 mmol, 1.0 equiv), $CH_2Cl_2$ (35 mL, 0.1 M) and cooled to -78 °C. DIBAL-H (Sigma, 2.2 g, 15.2 g, 4.4 equiv) was added dropwise, and the solution was stirred at -78 °C for 5 min, warmed to 0 °C for ~ 1 min, and then recooled to -78 °C. The reaction was partially quenched by dropwise addition of MeOH (5 mL) followed by an additional 15 min at -78 °C, followed by Na/K tartrate (sat. aq. solution) and warming to RT. The biphasic solution was stirred until the layers became clear. The organic layer was separated and the aqueous layer extracted with $CH_2Cl_2$ (2x). The combined organic extracts were washed with brine and filtered through a sodium sulfate plug, which was subsequently rinsed with $CH_2Cl_2$ (2x). Concentration and subsequent high vacuum yielded a colorless oil that was carried onto the next step without further purification.

An open 100-mL flask was charged with the crude diol and $CH_2Cl_2$ (7 mL, 0.5 M), and cooled to 0 °C. Imidazole (Fisher, 0.3 g, 4.2 mmol, 1.2 equiv) was added followed by TBSCl (Oakwood, 0.6 g, 3.8 mmol, 1.1 equiv), and the resulting solution was stirred for 1 h at 0 °C before $NH_4Cl$ (sat. aq. solution) quench. The organic layer was separated and the aqueous layer extracted with $CH_2Cl_2$ (2x). The combined organic extracts were washed with brine and filtered through a sodium sulfate plug, which was subsequently rinsed with $CH_2Cl_2$ (2x). Flash chromatography: EtOAc/hexanes (5:95) gave **30** as colorless oil (1.7 g, 3.1 mmol, 93% over two steps).

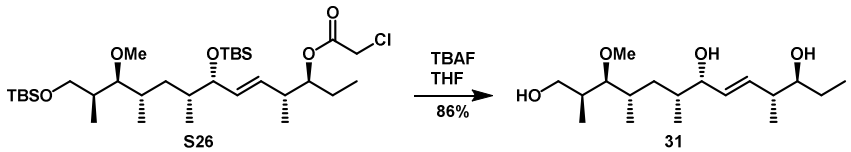
**$^1$H NMR** (599 MHz; $CDCl_3$): δ 5.46-5.44 (m, 2H), 3.88-3.84 (m, 1H), 3.52-3.48 (m, 1H), 3.43-3.34 (ovlp m, 4H), 3.00 (dd, J = 7.7, 2.2 Hz, 1H), 2.28-2.21 (m, 1H), 1.86-1.79 (m, 2H), 1.76-1.70 (m, 1H), 1.65-1.59 (m, 1H), 1.58-1.49 (m, 1H), 1.40-1.43 (m, 1H), 1.00 (d, J = 6.9 Hz, 3H), 0.96 (t, J = 7.4 Hz, 3H), 0.90-0.86 (ovlp m, J = 7.6 Hz, 21H), 0.82 (d, J = 6.7 Hz, 3H), 0.78 (d, J = 6.9 Hz, 3H), 0.03 (ovlp m, 9H), -0.01 (s. 3H).
**$^{13}$C NMR** (151 MHz; $CDCl_3$): δ 133.4, 132.9, 85.1, 77.8, 76.5, 65.9, 60.3, 42.1, 37.4, 37.3, 36.9, 33.6, 26.9, 25.9, 18.2, 18.2, 17.5, 16.5, 14.4, 10.52, 10.36, -3.9, -4.8, -5.3, -5.4
**HRMS**: Calculated $[M-(HOTBS)+H]^+$ 413.3445, found 413.3440.



30 S26

**S26**: A 100-mL round bottom flask was charged with **30** (0.9 g, 1.7 mmol, 1.0 equiv), $PPh_3$ (AK, 2.2 g, 8.5 mmol, 5.0 equiv), chloroacetic acid (Sigma, 0.8 g, 8.5 mmol, 5.0 equiv), PhMe (17 mL, 0.1 M), and then cooled to 0 °C. DIAD (Sigma, 1.8 g, 1.7 mL, 8.5 mmol, 5.0 equiv) was added dropwise over 10 min, the resulting solution was stirred at 0 °C for 10 min and then RT for 12 h. The solution was concentrated and purified by flash chromatography: $Et_2O$/hexanes (1:99 to 4:96) gave **S26** as colorless oil (0.8 g, 1.2 mmol, 71%).

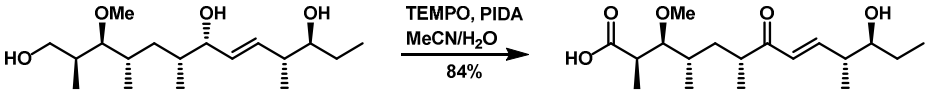
**$^1$H NMR** (599 MHz; $CDCl_3$): δ 5.52-5.46 (m, 1H), 5.43-5.38 (m, 1H), 4.84 (q, *J* = 5.6 Hz, 1H), 4.04 (s, 2H), 3.95 (dd, *J* = 6.5, 3.5 Hz, 1H), 3.51 (t, *J* = 8.8 Hz, 1H), 3.42 (dd, *J* = 9.6, 6.0 Hz, 1H), 3.37 (s, 3H), 3.03 (dd, *J* = 7.0, 2.7 Hz, 1H), 2.45-2.42 (m, 1H), 1.93-1.87 (m, 1H), 1.86-1.80 (m, 1H), 1.73-1.68 (m, 1H), 1.68-1.62 (m, 1H), 1.60-1.54 (m, 2H), 1.01 (d, *J* = 6.9 Hz, 3H), 0.89 (d, *J* = 7.7 Hz, 18H), 0.87-0.84 (m, 6H), 0.81 (d, *J* = 6.9 Hz, 3H), 0.04 (s, 6H), 0.03 (s, 3H), -0.02 (s, 3H).
**$^{13}$C NMR** (151 MHz; $CDCl_3$): δ 167.1, 133.9, 130.6, 84.8, 80.9, 76.5, 66.2, 60.1, 41.0, 39.9, 37.4, 36.6, 33.0, 25.9, 25.9, 24.6, 18.2, 18.2, 16.8, 16.7, 16.1, 10.8, 9.7, -3.9, -4.8, -5.3, -5.4.
**HRMS**: Calculated $[M+Na]^+$ 643.3951, found 643.3949.



S26 31

**31**: A 100-mL round bottom flask was charged with **S26** (1.2 g, 1.9 mmol, 1.0 equiv), THF (9 mL, 0.5 M), and cooled to 0 °C. TBAF (Sigma, 1 M in THF, 27.9 mL, 15.0 equiv) was added dropwise over 30 min, the resulting solution was warmed to RT and stirred for 48 h. The reaction was mostly complete after 48 h (TLC), and quenched with $CaCO_3$ (5.6 g, 55.8 mmol, 30.0 equiv) and $H_2O$/EtoAC with 5 min additional stirring. The organic layer was separated and the aqueous layer extracted with EtoAc (2x). The combined organic extracts

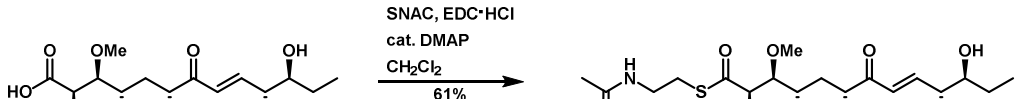
were washed with brine and filtered through a sodium sulfate plug, which was subsequently rinsed with EtOAc (2x). Flash chromatography: acetone/hexanes (30:70) gave **31** as colorless oil (0.5 g, 1.6 mmol, 86%).
**$^1$H NMR** (599 MHz; $D_6$-DMSO): δ 5.52-5.46 (m, 1H), 5.40-5.34 (m, 1H), 4.42 (t, *J* = 5.1 Hz, 1H), 4.34 (d, *J* = 4.6 Hz, 1H), 4.24 (d, *J* = 5.3 Hz, 1H), 3.78 (q, *J* = 4.9 Hz, 1H), 3.34-3.31 (m, 1H), 3.30 (s, 3H), 3.26-3.19 (m, 2H), 2.96 (dd, *J* = 6.2, 3.4 Hz, 1H), 2.19-2.12 (m, 1H), 1.85-1.77 (m, 1H), 1.74-1.63 (m, 2H), 1.58-1.51 (m, 1H), 1.36-1.21 (m, 2H), 0.95 (d, *J* = 6.9 Hz, 3H), 0.84 (t, *J* = 7.4 Hz, 3H), 0.82-0.80 (ovlp m, 6H), 0.77 (d, *J* = 6.8 Hz, 3H).
**$^{13}$C NMR** (151 MHz; $D_6$-DMSO): δ 132.7, 131.7, 84.8, 75.1, 73.6, 64.3, 59.6, 41.5, 37.2, 36.3, 36.1, 32.5, 26.7, 17.0, 16.6, 15.8, 11.5, 10.5.
**HRMS**: Calculated $[M+Na]^+$ 339.2506, found 339.2508.



31 32

**32**: A 9-dram vial was charged with **31** (0.4 g, 1.4 mmol, 1.0 equiv), $MeCN/H_2O$ (14 mL, 0.1 M) and cooled to 4 °C. TEMPO (Sigma, 0.2 g, 1.4 mmol, 1.0 equiv) and PIDA (AK scientific, 1.8 g, 5.6 mmol, 4.0 equiv) were added in single portions and the resulting solution was stirred vigorously at 4 °C. The reaction was monitored by $^1$H NMR spectroscopy after 12 h (small aliquot added to excess MeOH, concentrated, and dissolved in $CDCl_3$) for loss of the intermediate aldehyde. After 16 h, the reaction was decanted into MeOH and concentrated. Flash chromatography: EtOAc/hexanes (50:50) to AcOH/EtOAc/hexanes (1:50:49) gave **32** as a colorless oil (0.4 g, 1.2 mmol, 84%).

**$^1$H NMR** (599 MHz; $CDCl_3$): δ 6.97 (dd, *J* = 15.9, 7.6 Hz, 1H), 6.23 (d, *J* = 15.9 Hz, 1H), 3.46 (dt, *J* = 8.6, 4.4 Hz, 1H), 3.42 (s, 3H), 3.28 (dd, *J* = 7.1, 4.5 Hz, 1H), 2.87-2.81 (m, 1H), 2.57 (p, *J* = 7.0 Hz, 1H), 2.42 (h, *J* = 6.4 Hz, 1H), 1.93 (ddd, *J* = 13.7, 9.4, 4.1 Hz, 1H), 1.87-1.70 (m, 2H), 1.59-1.53 (m, 2H), 1.49-1.42 (m, 1H), 1.17 (d, *J* = 6.9 Hz, 3H), 1.11-1.09 (m, 6H), 0.97-0.95 (m, 6H).
**$^{13}$C NMR** (151 MHz; $CDCl_3$): δ 203.9, 178.9, 148.2, 128.2, 86.9, 76.9, 61.0, 42.5, 42.0, 41.6, 35.1, 34.3, 27.8, 17.8, 17.0, 16.0, 12.9, 10.1.
**HRMS**: Calculated $[M+Na]^+$ 351.2142, found 351.2140.



32 27

**27**: A 50 mL round bottom flask was charged with **32** (0.4 g, 1.3 mmol, 1.0 equiv), EDC•HCl (Chem-impex, 0.4 g, 1.9 mmol, 1.5 equiv), HOBT (Sigma, 0.2 g, 1.5 mmol, 1.5 equiv), and the flask was cooled to 0 °C. DMF (13 mL, 0.1 M) was added and the resulting solution was stirred at 0 °C for 30 min, followed by HSNAC (0.2 g, 1.5 mmol, 1.2 equiv), stirred at 0 °C for an additional 10 min before DMAP addition (cat., ~1 mg). The solution was stirred at 0 °C for an additional 10 min before warming to RT and 24 h additional stirring. The reaction was diluted with EtOAc, washed with $H_2O$. The organic layer was separated and the aqueous layer extracted 2x with EtoAc. The combined organic extracts were washed with brine and filtered through a sodium sulfate plug, which was subsequently rinsed 2x with EtOAC. Flash chromatography [column topped with ($SiO_2$:$CuSO_4$)]: EtOAc gave **27** as a colorless oil (0.3 g, 0.8 mmol, 61%).

**$^1$H NMR** (599 MHz; $D_6$-acetone): δ 7.34 (br s, 1H), 6.96 (dd, *J* = 15.9, 8.4 Hz, 1H), 6.22 (dd, *J* = 15.9, 0.8 Hz, 1H), 3.77 (d, *J* = 5.3 Hz, 1H), 3.46 (dq, *J* = 8.6, 4.3 Hz, 1H), 3.36 (s, 3H), 3.35-3.26 (m, 3H), 3.06-2.92 (m, 3H), 2.97-2.83 (m, 2H), 2.47-2.41 (m, 1H), 1.91 (ddd, *J* = 13.6, 9.6, 4.0 Hz, 1H), 1.85 (s, 3H), 1.63-1.57 (m, 1H), 1.49-1.35 (m, 2H), 1.17 (d, *J* = 6.9 Hz, 3H), 1.20-1.15 (ovlp m, 1H), 1.11 (d, *J* = 6.9 Hz, 3H), 1.07 (d, *J* = 6.9 Hz, 3H), 0.95-0.92 (ovlp m, 6H).
**$^{13}$C NMR** (151 MHz; $D_6$-acetone): δ 203.9, 202.4, 170.0, 150.1, 130.0, 87.5, 76.3, 61.0, 51.8, 43.4, 41.6, 39.5, 36.1, 35.0, 29.2, 28.7, 22.8, 18.7, 17.3, 16.8, 13.7, 10.6.
**HRMS**: Calculated $[M+H]^+$ 430.2622, 430.2628.

**PKS and TE Protein Expression**

All $H_2O$ was obtained from a Millipore Milli-Q system (serial P3MNO3809A) using Millipore Q-Gard 2/Quantum Ex Ultrapure organex cartridges. LB broth Miller was obtained from EMD and autoclaved before use. Glycerol was obtained from EMD, HEPES was obtained from Calbiochem (Omnipur grade), isopropyl-β-D-thiogalactopyranoside (IPTG) was obtained from Gold Biotechnology. Kanamycin Sulfate (Kan) was obtained from Amresco. ACS grade imidazole and NaCl were obtained from Fisher Scientific. pH was determined on a Symphony SB70P pH meter (serial SN005695) calibrated according to manufacturer's specifications. Ni-NTA agarose was purchased from Qiagen and pre-equilibrated with five column volumes of lysis buffer. PD-10 columns were purchased from GE and pre-equilibrated with five column volumes of storage buffer. Cells were lysed using a model 705 Sonic Dismembrator purchased from Fisher Scientific. Optical density ($OD_{600}$) was determined using an Eppendorf Biophotometer.

Bap1[12] cells bearing plasmids for expression of respective PKS modules were taken from glycerol cell stocks stored at -80 °C and grown in LB (10mL) with Kan (50 mg/L), and grown overnight at 37°C. The following morning, LB (1L) containing Kan (50 mg/L) was inoculated with the entire overnight culture, and shaken at 37°C until they reached an $OD_{600}$ of 0.6-0.7 at which point they were removed and allowed to cool to RT, then to 20 °C. When an $OD_{600}$ of 0.8 was reached, the cultures were induced with IPTG (300μM) and shaken at 180RPM at 20 °C for 18 hours. Cells were pelleted at 5000g (4 °C) for 10 minutes.

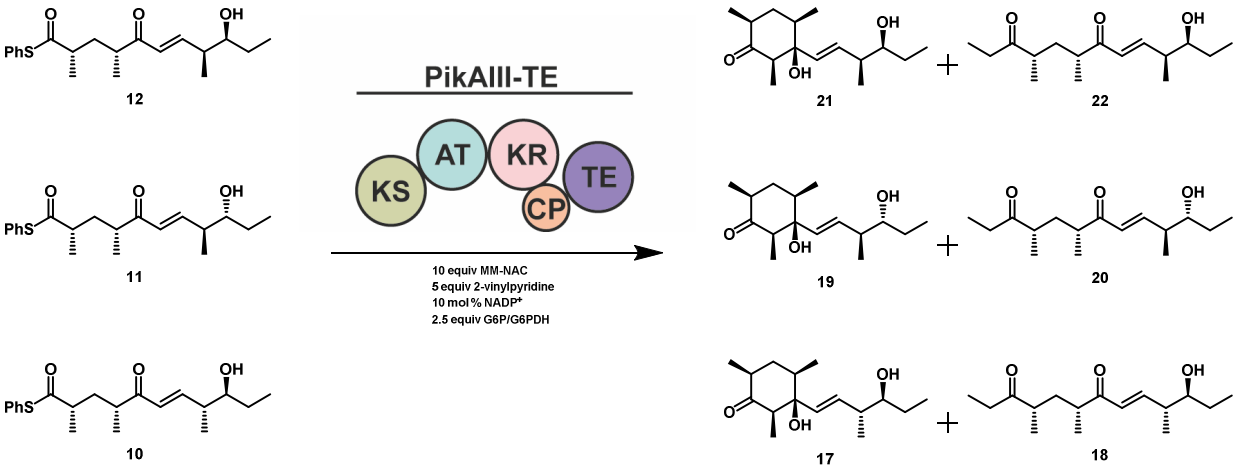
**PKS Crude Cell Lysate Preparation**

Frozen cells were resuspended in 5 mL of storage buffer [HEPES (50 mM), NaCl (150 mM), EDTA (1 mM), glycerol (20% v/v), pH 7.2] per gram of cells via vortex. Cells were lysed by addition of 1 mg/ml lysozyme immediately before sonication in a brine/ice at 70% power 100 x 5s with 15s rest periods. Cellular debris was pelleted in a precooled (4 °C) centrifuge at 65,000g for 10 min. Crude cell lysate was either used immediately or flash frozen in $N_2$ and thawed on ice without discernible loss in activity. Protein concentration was crudely normalized to that of purified protein though densitometry, and used without further manipulation.

**PKS Protein Purification**

The following steps were conducted in <2 hours for maximum and reproducible enzymatic activity. Cells were suspended in 5 mL of lysis buffer [HEPES (50 mM), NaCl (300 mM), imidazole (10mM), glycerol (10% v/v), pH 8.0] per gram of pelleted culture broth via vortex. Cells were lysed by addition of 1 mg/ml lysozyme immediately before sonication in a brine/ice at 70% power 100 x 5s with 15s rest periods. Cellular debris was pelleted in a precooled (4°C) centrifuge at 40,000 g for 10 min, and the supernatant was applied to 4 mL of Ni-NTA resin and allowed to drip through. 15 mL of wash buffer [HEPES (50 mM), NaCl (300 mM), imidazole (30mM), glycerol (10% v/v), pH 8.0] was added, the column was gently pressurized with a syringe, and the enzyme of interest was eluted with 15 mL of elution buffer [HEPES (50 mM), NaCl (300 mM), imidazole (300mM), glycerol (10% v/v), pH 8.0] with gentle syringe pressure. Protein containing fractions were determined via their absorption at 280 nm. Buffer exchange was performed using a PD-10 column, and

protein containing fractions were determined via absorption at 280 nm and pooled, aliquoted, flash frozen in liquid $N_2$, and stored at -80 °C.




Incubation of **10-12** with PikAIII-TE:

Reaction conditions: sodium phosphate buffer (50 mM, 2.5% v/v glycerol, 50 mL total, pH = 7.2), pentaketide **10–12** (70 mg, 0.2 mmol, 4 mM) MM-NAC (10 equiv, 40 mM), $NADP^+$ (0.1 equiv, 0.4 mM), glucose-6-phosphate (2.5 equiv, 10 mM), glucose-6-phosphate dehydrogenase (2 units/mL), 2-vinylpyridine (20 mM), cell free PikAIII-TE (crude cell estimated conc. ~15 µM, 4 µM in reaction, 0.1 mol %), 8 hours, stationary, RT.

Workup and purification: quenched with acetone (2x volume, 100mL), placed in a -20 °C freezer for 1 h and filtered through a celite plug. Remaining insoluble material was suspended in acetone and this solution was used to rinse the celite plug. Acetone was removed through rotary evaporation and the aqueous layer was saturated with NaCl and extracted EtOAc (3x). Combined organic layers were washed with brine and filtered through a sodium sulfate plug was performed then rinsed with EtOAc (2x) and concentrated. Flash chromatography: acetone/hexanes (8:92) afforded compounds **17-21**.

**21** from pentaketide **12** (2.5 mg, 0.009 mmol, 4% yield).
**$^1$H NMR** (599 MHz; $CDCl_3$): δ 5.57 – 5.51 (m, 1H), 5.39 (d, *J* = 15.7 Hz, 1H), 3.41 (ddd, *J* = 8.7, 5.3, 3.6 Hz, 1H), 2.55 -2.51 (m, 2H), 2.34 (h, *J* = 6.8 Hz, 1H), 2.09-2.03 (m, 1H), 1.91 (ddd, *J* = 13.3, 5.7, 4.1 Hz, 1H), 1.60-1.53 (m, 3H), 1.42-1.33 (m, 3H), 1.05 (d, *J* = 6.8 Hz, 3H), 1.03 (d, *J* = 6.4 Hz, 3H), 0.97 (t, *J* = 7.4 Hz, 3H), 0.94 (d, *J* = 6.8 Hz, 3H), 0.87 (d, *J* = 6.8 Hz, 3H).
**$^{13}$C NMR** (151 MHz; $CDCl_3$): δ 211.9, 135.0, 131.7, 81.5, 76.7, 52.1, 44.6, 42.2, 39.8, 39.6, 27.2, 15.6, 15.0, 14.4, 10.2, 7.9.
**HRMS**: Calculated $[M+Na]^+$ 291.1931, found 291.1931.

**22** from pentaketide **12** (2.5 mg, 0.009 mmol, 4% yield).
**$^1$H NMR** (599 MHz; $CDCl_3$): δ 6.87 (dd, *J* = 15.9, 8.1 Hz, 1H), 6.18 (dd, *J* = 15.9, 0.8 Hz, 1H), 3.57-3.52 (m, 1H), 2.77 (h, *J* = 6.9 Hz, 1H), 2.60 (dq, *J* = 14.0, 7.0 Hz, 1H), 2.55-2.42 (m, 3H), 2.15-2.10 (m, 1H), 1.90 (s, 1H), 1.55 (dqd, *J* = 14.3, 7.2, 3.9 Hz, 1H), 1.47-1.39 (m, 1H), 1.24 (dt, *J* = 13.8, 6.7 Hz, 2H), 1.10 (d, *J* = 6.8 Hz, 3H), 1.08 (d, *J* = 6.9 Hz, 3H), 1.07-1.03 (ovlp m, 6H), 0.98 (t, *J* = 7.4 Hz, 3H).
**$^{13}$C NMR** (151 MHz; $CDCl_3$): δ 215.0, 203.2, 149.9, 128.4, 75.9, 43.7, 42.5, 41.4, 36.1, 34.5, 27.3, 17.2, 16.7, 13.9, 10.4, 7.7

**HRMS**: Calculated $[M+Na]^+$ 291.1931, found 291.1932.

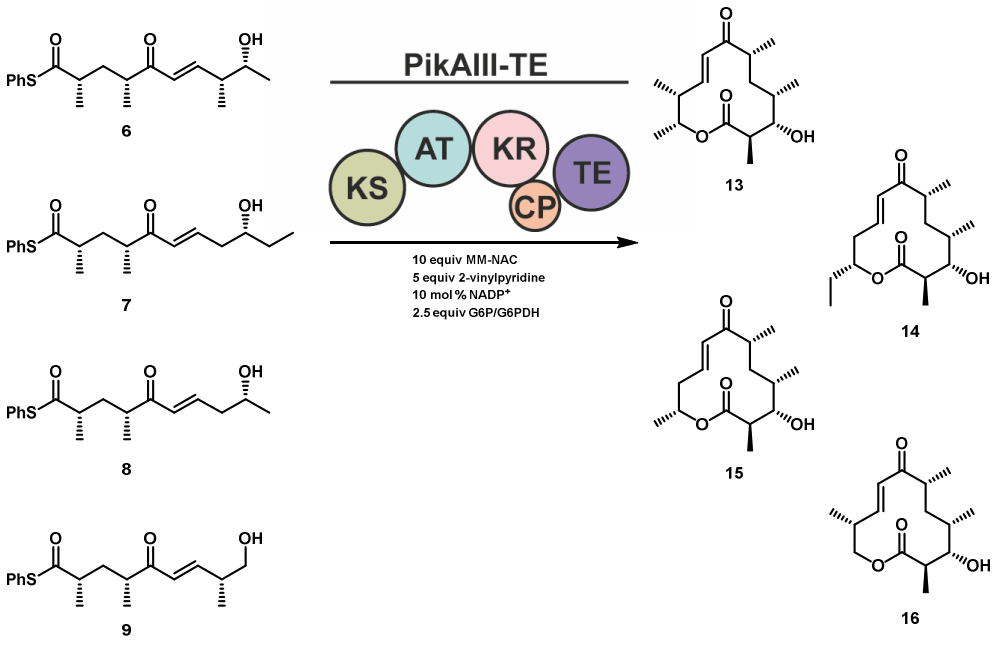
**19** from pentaketide **11** (2 mg, 0.007 mmol, 3.5% yield).
**$^1$H NMR** (599 MHz; $CDCl_3$): δ 5.57 – 5.51 (m, 1H), 5.42 (d, *J* = 15.7 Hz, 1H), 3.37 (dt, *J* = 7.9, 4.2 Hz, 1H), 2.55-2.48 (m, 2H), 2.30 (dq, *J* = 14.2, 7.0 Hz, 1H), 2.06 (dqd, *J* = 12.7, 6.5, 4.1 Hz, 1H), 1.91 (ddd, *J* = 13.3, 5.8, 4.0 Hz, 1H), 1.60-1.53 (m, 2H), 1.44-1.37 (m, 2H), 1.06 (d, *J* = 6.9 Hz, 3H), 1.03 (d, *J* = 6.4 Hz, 3H), 0.97 (t, *J* = 7.4 Hz, 3H), 0.94 (d, *J* = 6.8 Hz, 3H), 0.89 (d, *J* = 6.8 Hz, 3H).
**$^{13}$C NMR** (151 MHz; $CDCl_3$): δ 211.8, 136.1, 130.8, 81.5, 76.5, 52.0, 44.6, 42.4, 39.67, 39.52, 27.4, 17.3, 15.1, 14.4, 10.0, 7.9.
**HRMS**: Calculated $[M+Na]^+$ 291.1931, found 291.1930.

**20** from pentaketide **11** (2 mg, 0.007 mmol, 3.5% yield).
**$^1$H NMR** (599 MHz; $CDCl_3$): δ 6.83 (dd, *J* = 15.9, 8.7 Hz, 1H), 6.16 (d, *J* = 15.9 Hz, 1H), 3.48 (dt, *J* = 8.5, 4.4 Hz, 1H), 2.77 (h, *J* = 6.9 Hz, 1H), 2.62 (dq, *J* = 13.9, 7.0 Hz, 1H), 2.56-2.50 (m, *J* = 17.8, 7.3 Hz, 1H), 2.47-2.40 (m, 2H), 2.15-2.10 (m, 1H), 1.62-1.53 (m, 1H), 1.46 (tt, *J* = 14.7, 7.4 Hz, 1H), 1.25-1.20 (m, 2H), 1.12 (d, *J* = 6.8 Hz, 3H), 1.08 (d, *J* = 6.9 Hz, 3H), 1.06-1.03 (m, 6H), 0.98 (t, *J* = 7.4 Hz, 3H).
**$^{13}$C NMR** (151 MHz; $CDCl_3$): δ 215.2, 203.2, 149.1, 129.3, 76.3, 43.8, 42.7, 41.3, 36.3, 34.6, 27.7, 17.4, 16.6, 16.4, 10.1, 7.8.
**HRMS**: Calculated $[M+Na]^+$ 291.1931, found 291.1930.

**17** from pentaketide **10** (1 mg, 0.004 mmol, 2% yield) .
**$^1$H NMR** (599 MHz; $CDCl_3$): δ 5.57 – 5.51 (m, 1H), 5.41 (d, *J* = 15.7 Hz, 1H), 3.37 (dt, *J* = 8.2, 4.3 Hz, 1H), 2.54-2.48 (m, 2H), 2.33-2.27 (m, 1H), 2.04 (dqd, *J* = 12.6, 6.4, 4.1 Hz, 1H), 1.91 (ddd, *J* = 13.3, 5.9, 4.0 Hz, 1H), 1.59-1.51 (m, 3H), 1.43-1.37 (m, 2H), 1.06 (d, *J* = 6.9 Hz, 3H), 1.02 (d, *J* = 6.4 Hz, 3H), 0.97-0.95 (ovlp m, 6H), 0.87 (d, *J* = 6.8 Hz, 3H).
**$^{13}$C NMR** (151 MHz; CDCl3): δ 211.9, 136.0, 130.8, 81.5, 76.6, 52.1, 44.6, 42.3, 39.67, 39.61, 27.5, 17.3, 14.9, 14.4, 10.1, 8.1.
**HRMS**: Calculated $[M+Na]^+$ 291.1931, found 291.1931.

**18** from pentaketide **10** (1 mg, 0.004 mmol, 2% yield).
**$^1$H NMR** (599 MHz; $CDCl_3$): δ 6.91 (dd, *J* = 15.9, 8.0 Hz, 1H), 6.18 (d, *J* = 15.9 Hz, 1H), 3.48-3.47 (m, 1H), 2.80-2.75 (m, 1H), 2.60-2.37 (ovlp m, 4H), 2.13-2.09 (m, 1H), 1.84-1.80 (m, 1H), 1.59-1.52 (m, 1H), 1.47-1.40 (m, 1H), 1.26-1.22 (m, 1H), 1.11 (d, *J* = 6.9 Hz, 3H), 1.07 (d, *J* = 6.9 Hz, 3H), 1.06-1.02 (m, 6H), 0.97 (t, *J* = 7.4 Hz, 3H).
**$^{13}$C NMR** (151 MHz; $CDCl_3$): δ 214.9, 203.2, 149.0, 129.1, 76.3, 43.7, 42.3, 41.4, 36.0, 34.3, 27.6, 17.08, 16.94, 15.9, 10.0, 7.8.
**HRMS**: Calculated $[M+Na]^+$ 291.1931, found 291.1932.

Incubation of **6-9** with PikAIII-TE




Reaction conditions: sodium phosphate buffer (50 mM, 2.5% v/v glycerol, 50 mL total, pH = 7.2), pentaketide **6-9** (70 mg, 0.2 mmol, 4 mM) MM-NAC (10 equiv, 40 mM), $NADP^+$ (0.1 equiv, 0.4 mM), glucose-6-phosphate (2.5 equiv, 10 mM), glucose-6-phosphate dehydrogenase (2 units/mL), 2-vinylpyridine (20 mM), cell free PikAIII-TE (Crude cell estimated conc. ~15 µM, 4 µM in reaction, 0.1 mol %), 8 hours, stationary, RT.

Workup and purification: Quenched with acetone (2x volume, 100mL), placed in a -20 °C freezer for 1 h and filtered through a celite plug. Remaining insoluble material was suspended in acetone and this solution was used to rinse the celite plug. Acetone was removed through rotary evaporation and the aqueous layer was saturated with NaCl and extracted EtOAc (3x). Combined organic layers were washed with brine and filtered through a sodium sulfate plug was performed then rinsed with EtOAc (2x) and concentrated. Flash chromatography: acetone/hexanes (8:92) afforded compounds **13–16**.

**13** from pentaketide **6** (32 mg, 0.11 mmol, 56% yield).
**$^1$H NMR** (599 MHz; $CDCl_3$): δ 6.73 (dd, *J* = 15.8, 5.5 Hz, 1H), 6.43 (dd, *J* = 15.8, 1.1 Hz, 1H), 5.15 (qd, *J* = 6.5, 2.3 Hz, 1H), 3.52 (dd, *J* = 10.3, 5.1 Hz, 1H), 2.60-2.49 (m, 3H), 1.67-1.61 (m, 2H), 1.33 (td, *J* = 13.1, 3.7 Hz, 1H), 1.27 (d, *J* = 6.7 Hz, 6H), 1.23 (ovlp m, 1H), 1.21 (d, *J* = 7.0 Hz, 3H), 1.14 (d, *J* = 6.9 Hz, 3H), 0.99 (d, *J* = 6.7 Hz, 3H).
**$^{13}$C NMR** (151 MHz; $CDCl_3$): δ 205.0, 174.4, 146.8, 125.8, 78.4, 68.9, 45.0, 43.3, 39.5, 33.4, 33.1, 17.7, 17.6, 17.4, 16.0, 9.4.
**HRMS**: Calculated $[M\text{-}H_2O+H]^+$ 265.1798, found 265.1798.

**14** from pentaketide **7** (22 mg, 0.078 mmol, 39% yield).
**$^1$H NMR** (599 MHz; $CDCl_3$): δ 6.59 – 6.52 (m, 1H), 6.41 (d, *J* = 15.3 Hz, 1H), 4.97-4.93 (m, 1H), 3.55 (dd, *J* = 10.3, 3.9 Hz, 1H), 2.66-2.60 (m, 1H), 2.53-2.44 (m, 2H), 2.22-2.15 (m, 1H), 1.69-1.57 (m, 3H), 1.51 (d, *J* = 5.0 Hz, 1H), 1.32-1.29 (m, 4H), 1.18 (d, *J* = 7.0 Hz, 3H), 0.99 (d, *J* = 6.1 Hz, 3H), 0.92 (t, *J* = 7.4 Hz, 3H).

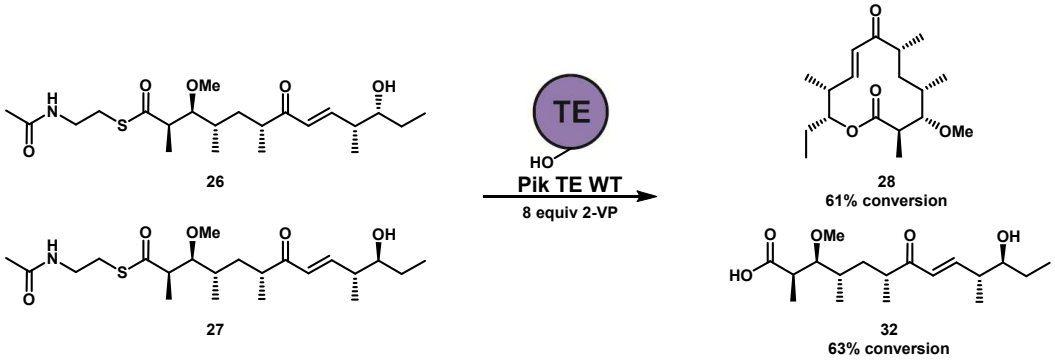
**$^{13}$C NMR** (151 MHz; $CDCl_3$): δ 204.1, 174.7, 140.5, 129.6, 78.2, 71.8, 44.9, 43.5, 38.2, 33.3, 33.0, 28.2, 17.6, 17.4, 16.4, 9.9.
**HRMS**: Calculated $[M\text{-}H_2O+H]^+$ 265.1798, found 265.1799.

**15** from pentaketide **8** (9 mg, 0.034 mmol, 17% yield).
**$^1$H NMR** (599 MHz; $CDCl_3$): δ 6.59 – 6.52 (m, 1H), 6.45 (d, *J* = 15.3 Hz, 1H), 5.12 (dqd, *J* = 11.7, 6.0, 2.3 Hz, 1H), 3.57-3.55 (m, 1H), 2.63 (dq, *J* = 10.4, 6.8 Hz, 1H), 2.55-2.50 (m, 1H), 2.47-2.44 (m, 1H), 2.25 (q, *J* = 11.9 Hz, 1H), 1.70 (t, *J* = 12.9 Hz, 1H), 1.51-1.50 (m, 1H), 1.38-1.29 (ovlp m, 7H), 1.21 (d, *J* = 7.0 Hz, 3H), 1.01 (d, *J* = 6.5 Hz, 3H).
**$^{13}$C NMR** (151 MHz; $CDCl_3$): δ 204.0, 174.3, 140.2, 129.8, 78.3, 67.2, 44.9, 43.4, 39.9, 33.3, 32.9, 20.6, 17.6, 17.3, 16.1.
**HRMS**: Calculated $[M\text{-}H_2O+H]^+$ 251.1642, found 251.1641.

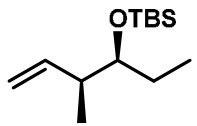
**16** from pentaketide **9** (5 mg, 0.019 mmol, 9% yield).
**$^1$H NMR** (599 MHz; $CDCl_3$): δ 6.70 - 6.65 (m, 1H), 6.45 (d, *J* = 15.8 Hz, 1H), 4.94 (dd, *J* = 11.0, 2.8 Hz, 1H), 3.58-3.53 (m, *J* = 3.5 Hz, 2H), 2.76-2.74 (m, 1H), 2.64 (dq, *J* = 10.4, 6.8 Hz, 1H), 2.55-2.49 (m, *J* = 2.8 Hz, 1H), 1.66 (t, *J* = 12.3 Hz, 1H), 1.50-1.49 (m, 1H), 1.36-1.29 (ovlp m, 4H), 1.25 (d, *J* = 6.9 Hz, 3H), 1.22 (d, *J* = 7.0 Hz, 3H), 1.01 (d, *J* = 6.2 Hz, 3H).
**$^{13}$C NMR** (151 MHz; $CDCl_3$): δ 204.7, 174.6, 145.1, 126.6, 78.3, 64.5, 44.8, 43.2, 35.5, 33.27, 33.15, 17.7, 17.4, 16.0, 14.6.
**HRMS**: Calculated $[M\text{-}H_2O+H]^+$ 251.1642, found 251.1644.

Incubation of **26** and **27** with Pik TE:

Analytical enzymatic reactions were performed at a volume of 50 µL and quenched with MeOH (3 volumes, 150 µL), clarified by centrifugation (17,000 x g, 15 min, 4 ˚C) and analyzed for macrolactone production. 2-vinylpyridine (Sigma) was employed as a thiol scavenger.




Reaction conditions: sodium phosphate buffer (400 mM, pH = 7.2), hexaketide **26** or **27** (1mM), 2-vinylpyridine (8 mM), purified Pik TE (10 µM in reaction, 1 mol %), 4 h, stationary, RT.


OTBS

S7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6.0
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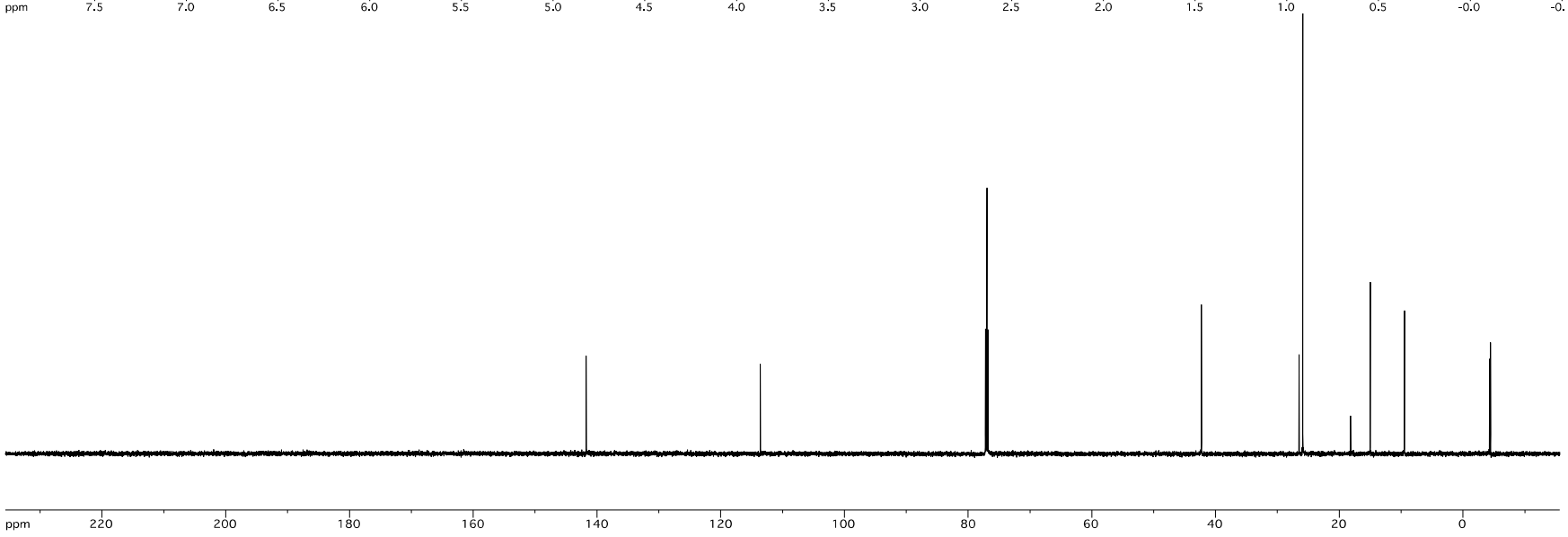
0.5
-0.0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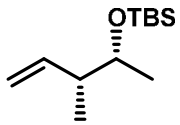
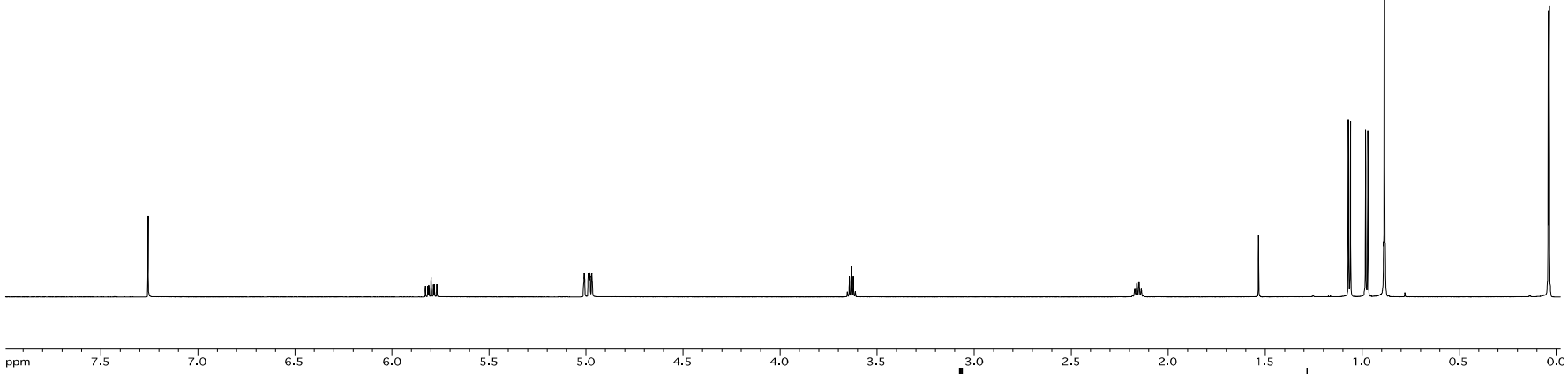

OTBS

**S8**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1.0
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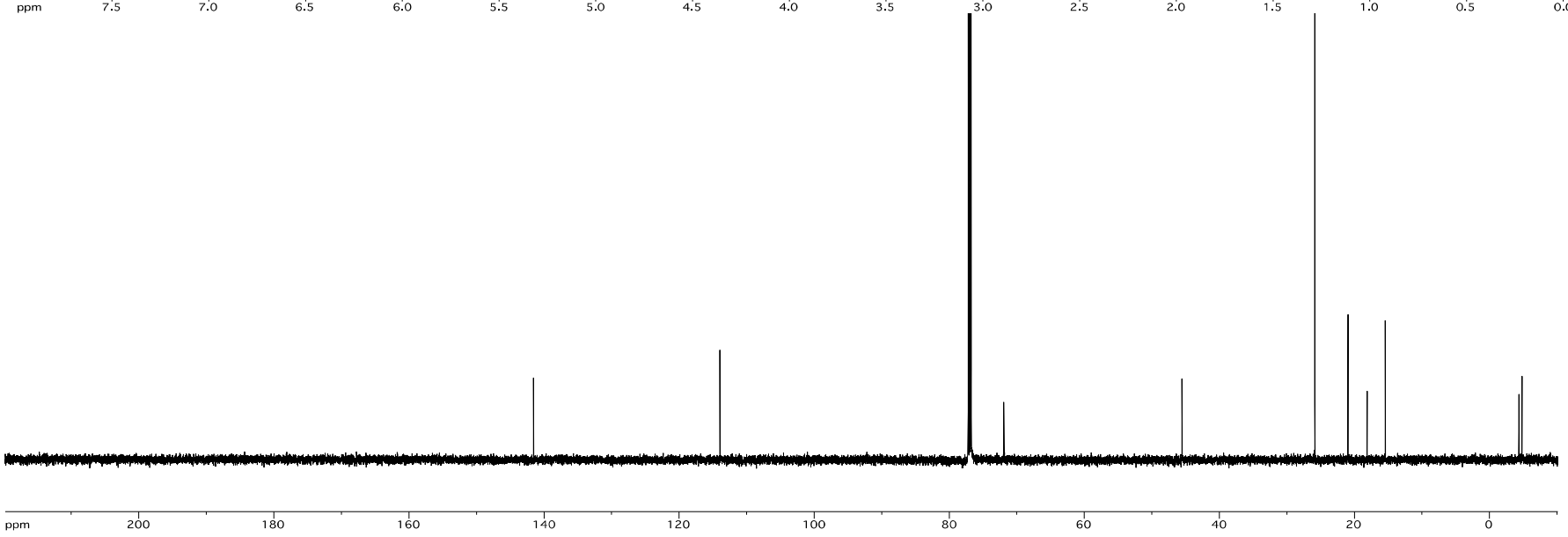
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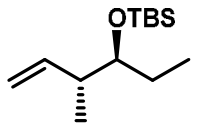
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S10


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2.0
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1.0
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ppm
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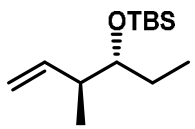
OTBS

S11


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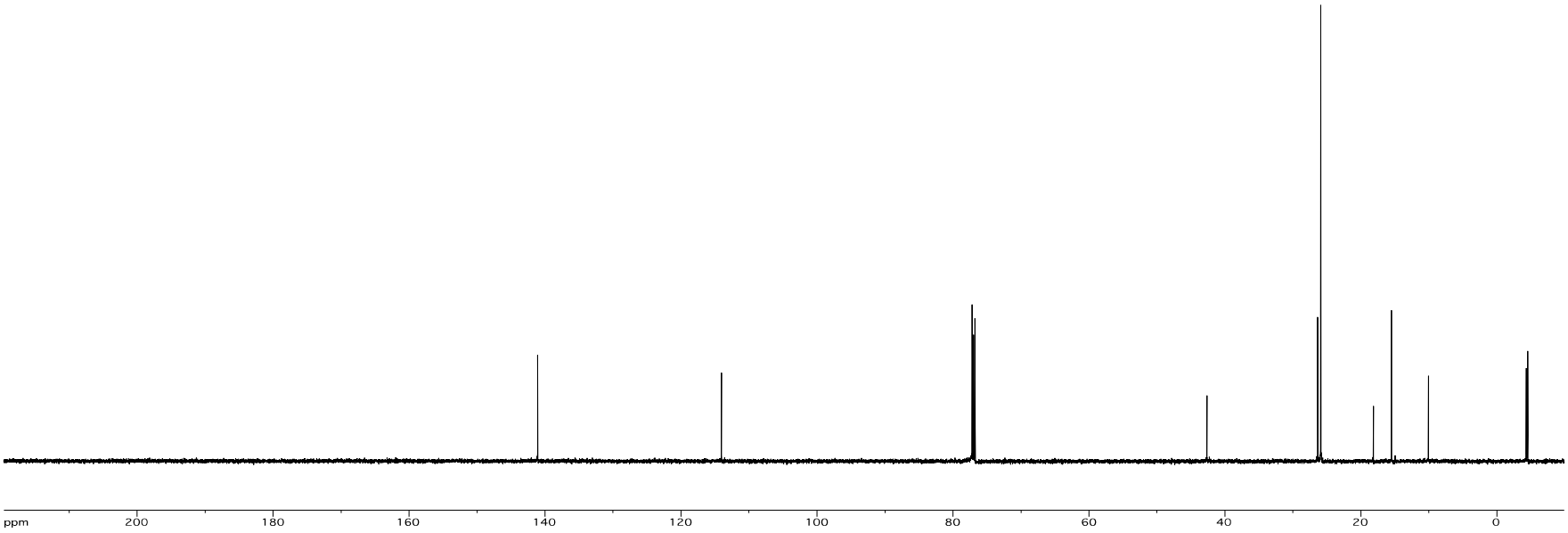
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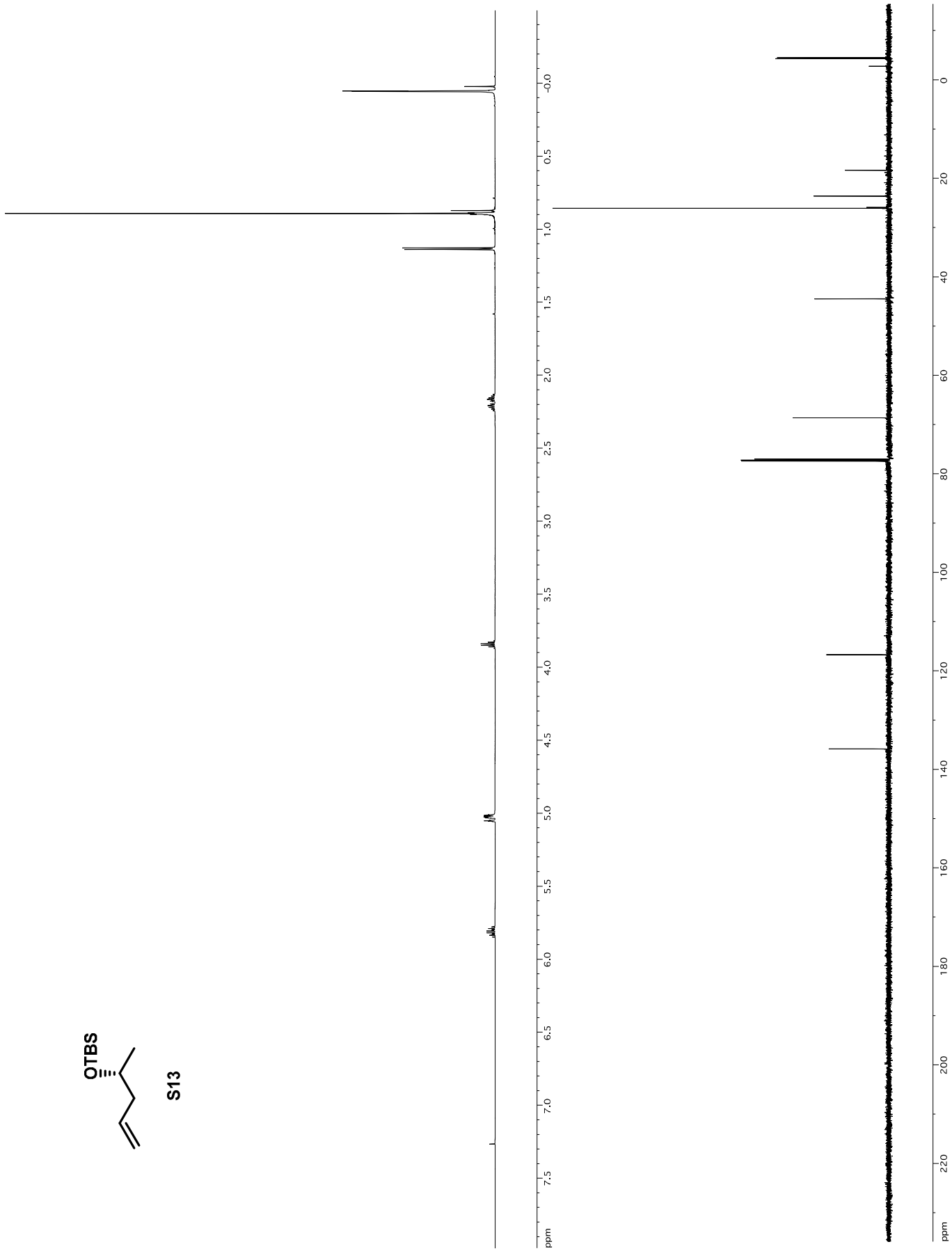
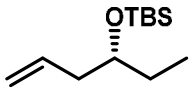
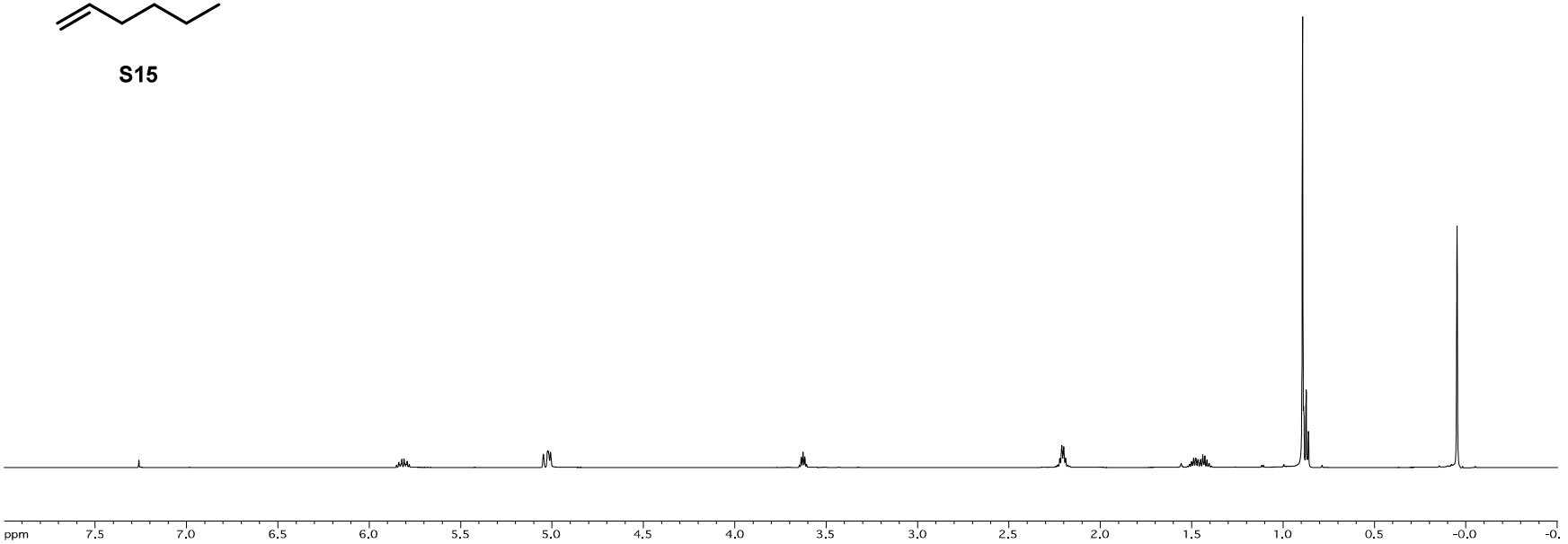

OTBS
S13
ppm
ppm


OTBS

**S15**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5.5
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2.0
1.5
1.0
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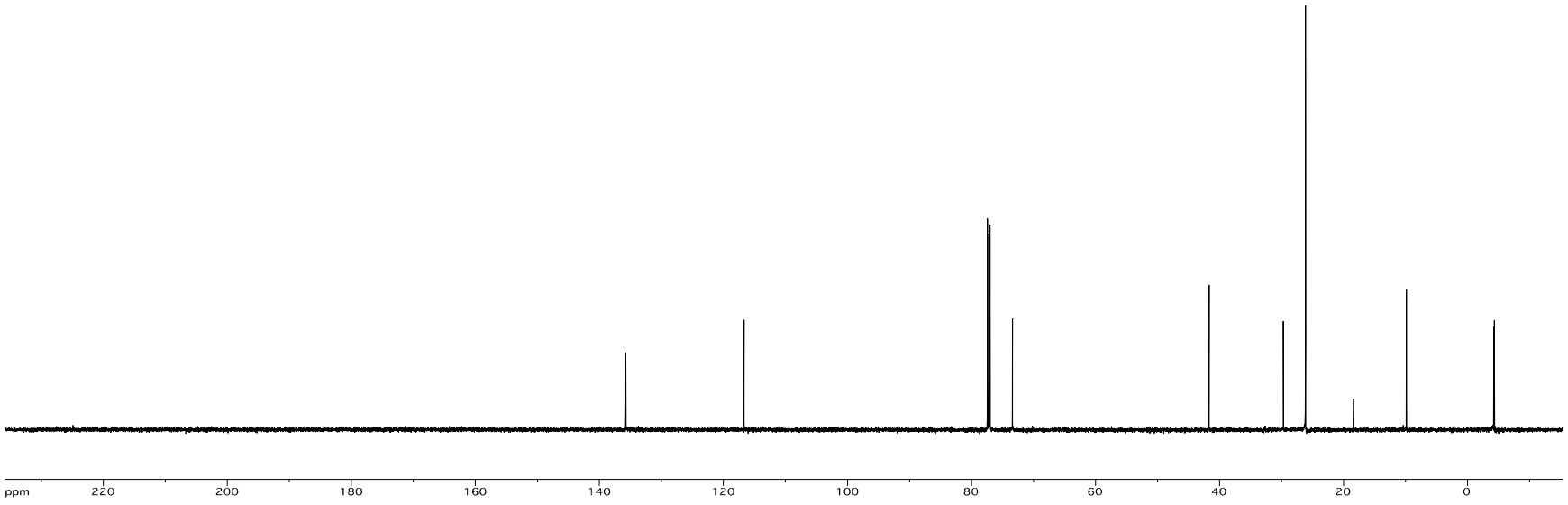
-0.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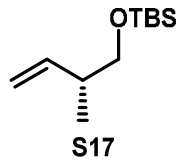
OTBS

S17


ppm
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6.0
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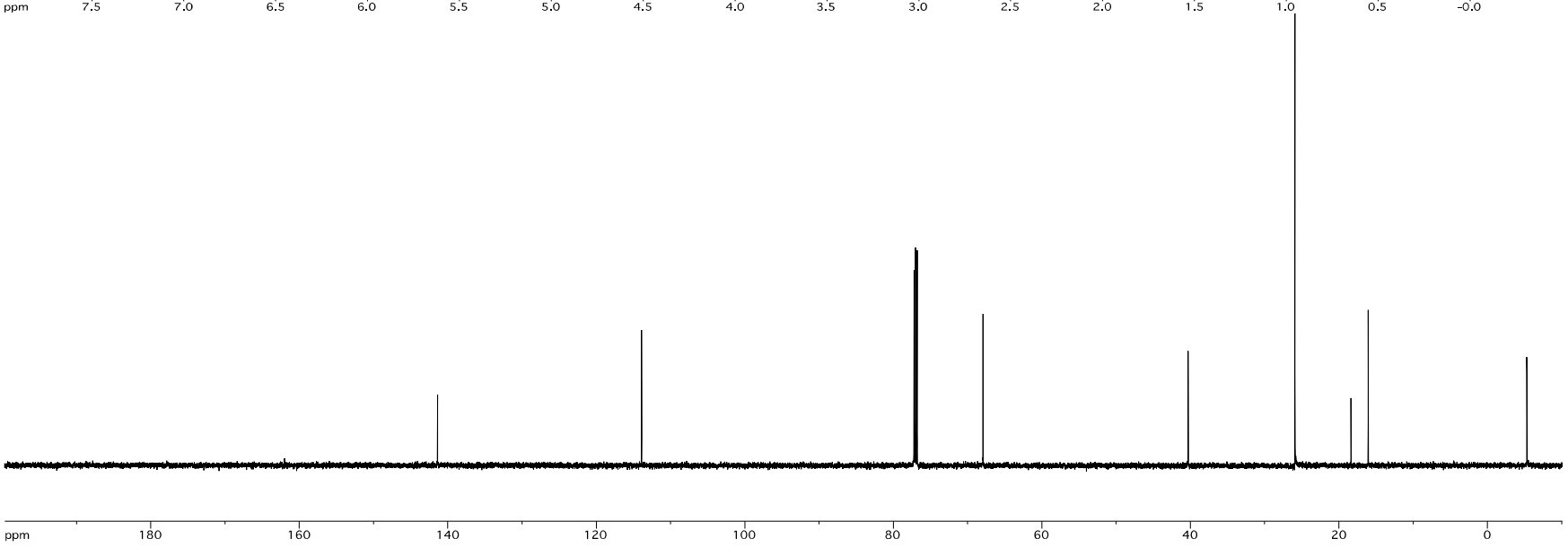
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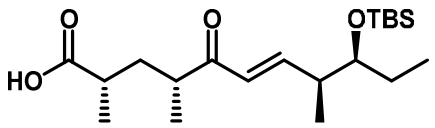
O
O
OTBS
HO

S19


ppm
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6.0
5.5
5.0
4.5
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3.5
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2.0
1.5
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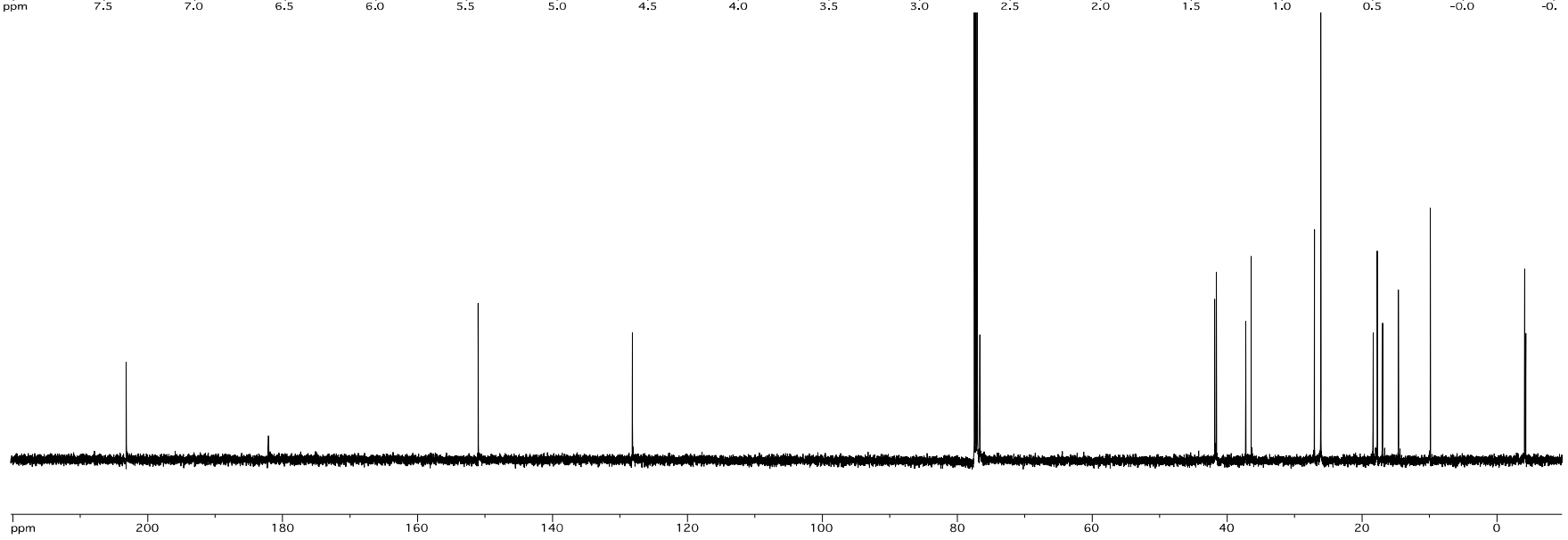
-0.0
-0.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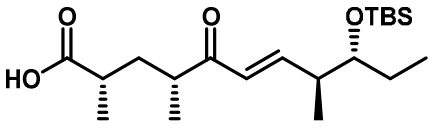
O
O
OTBS
HO

S20


ppm
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6.0
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0.5
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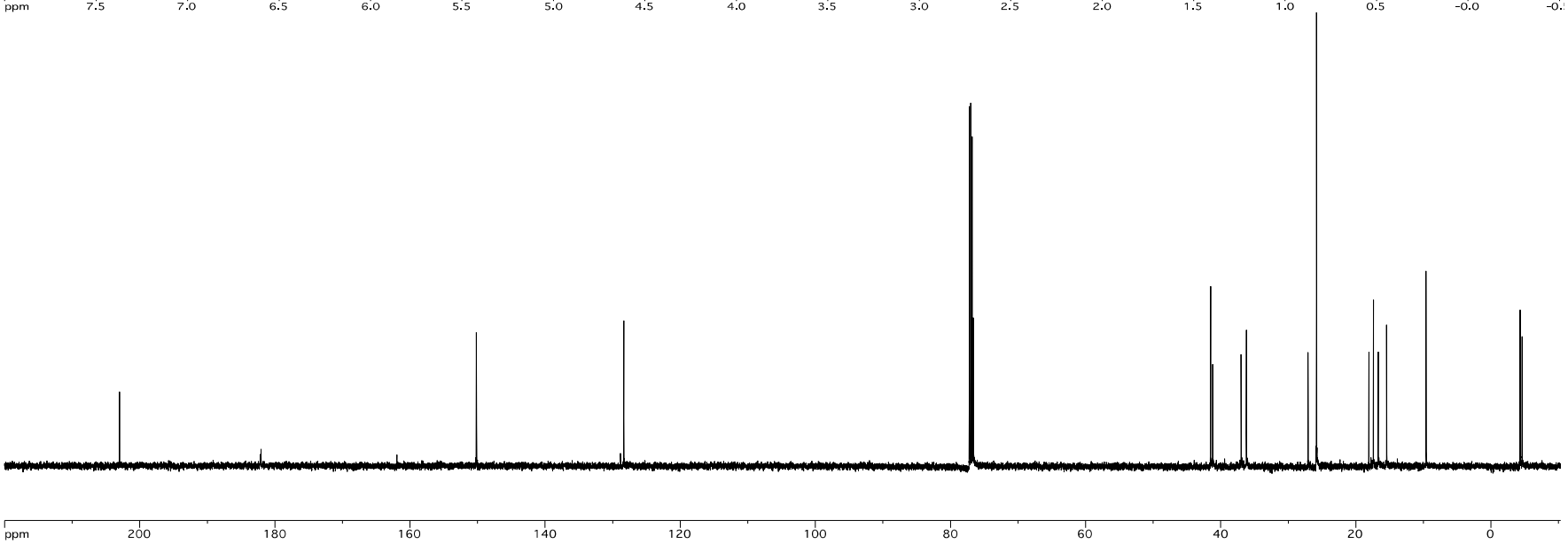
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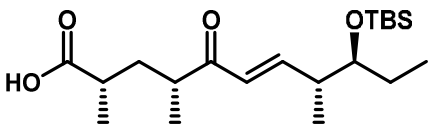
O
O
OTBS
HO

S21


ppm
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6.0
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0.5
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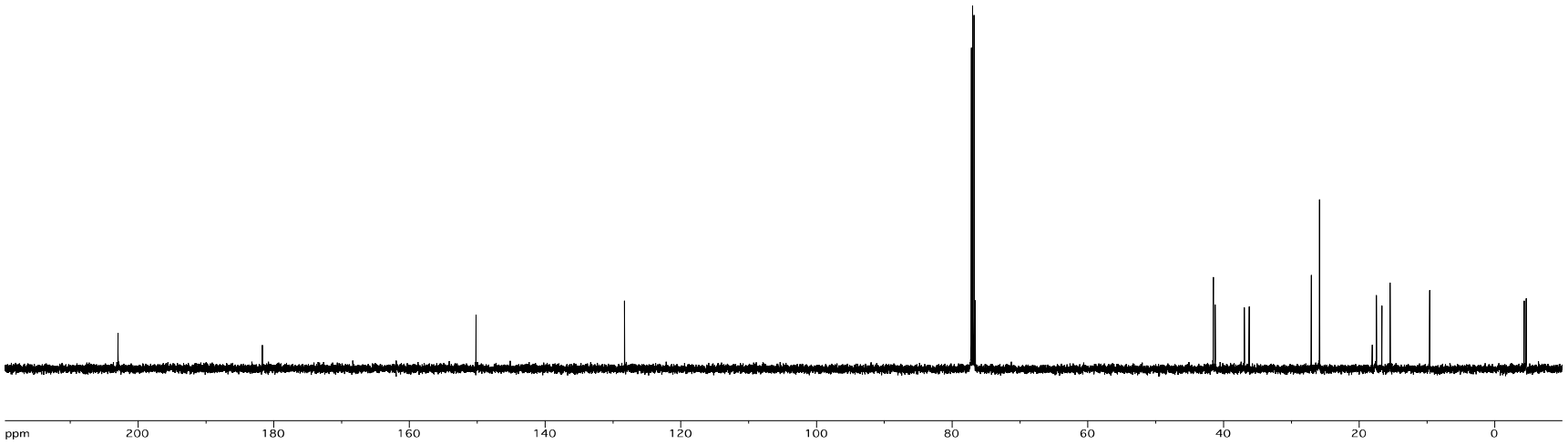
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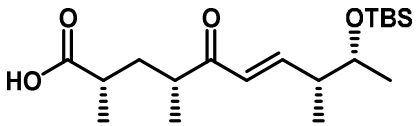
O
O
OTBS
HO

S22


ppm
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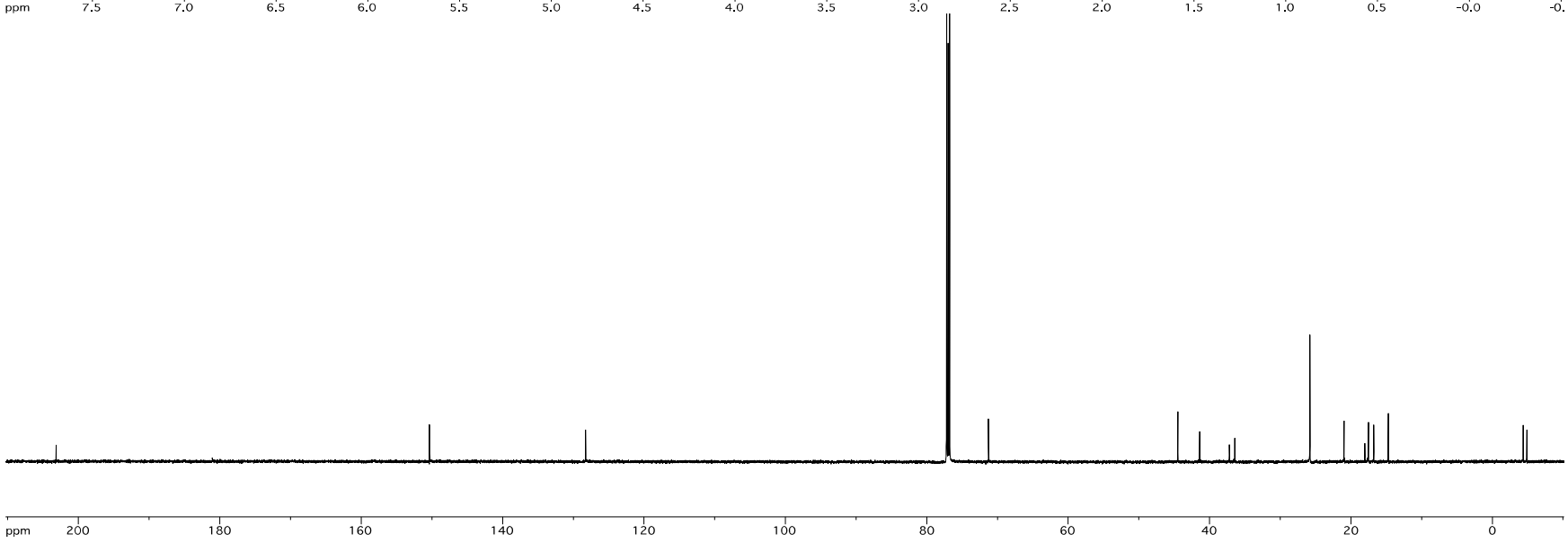
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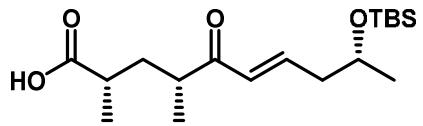
O
O
OTBS
HO

S23


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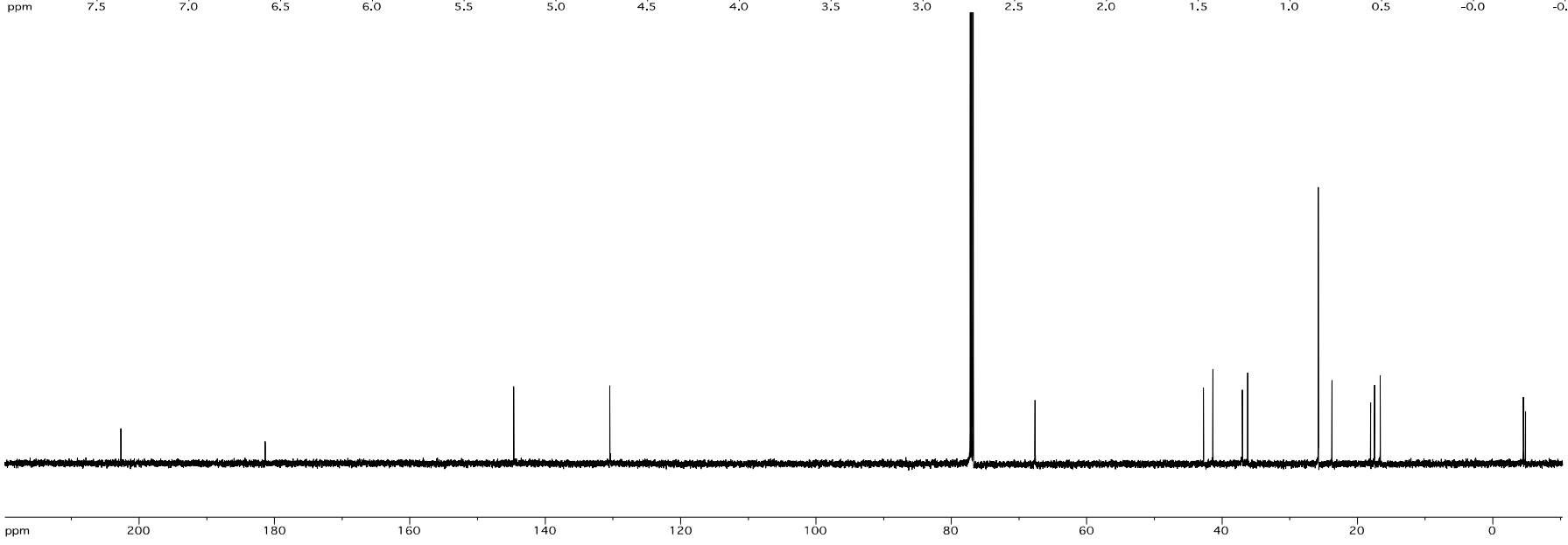
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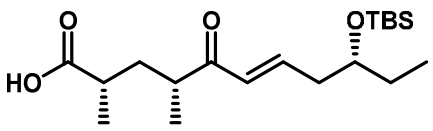
O
O
OTBS
HO

**S24**


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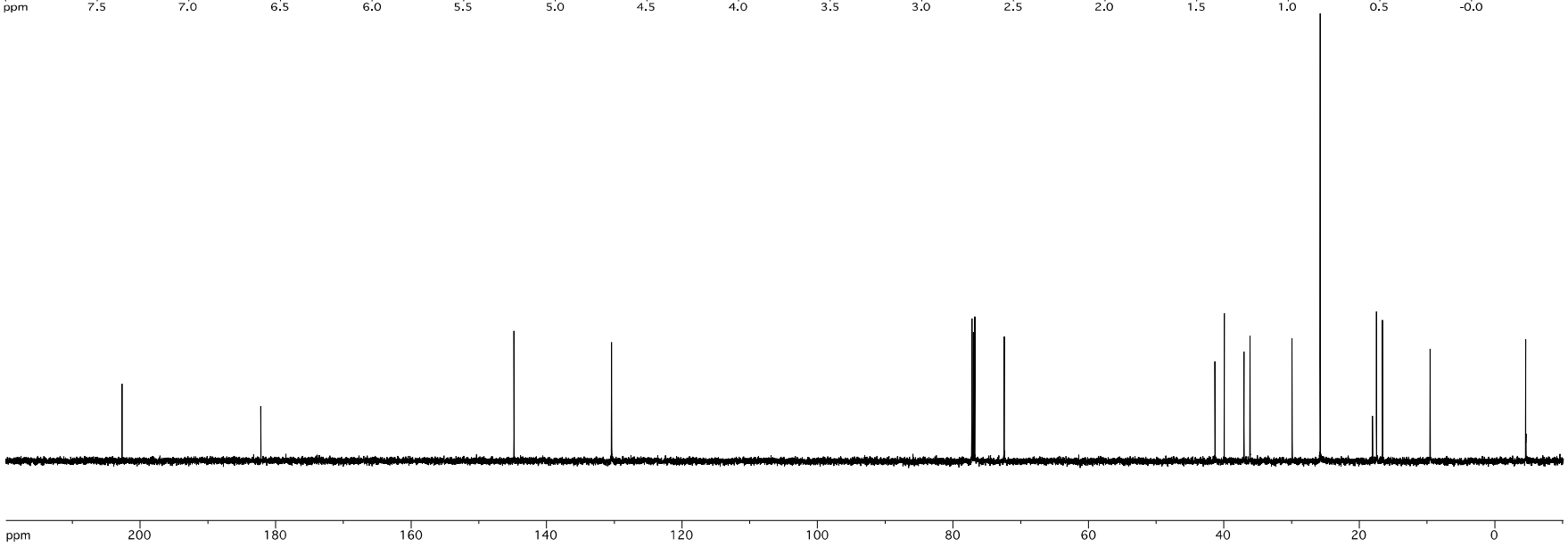
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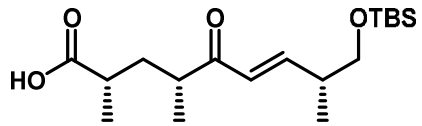
O
O
OTBS
HO

S25


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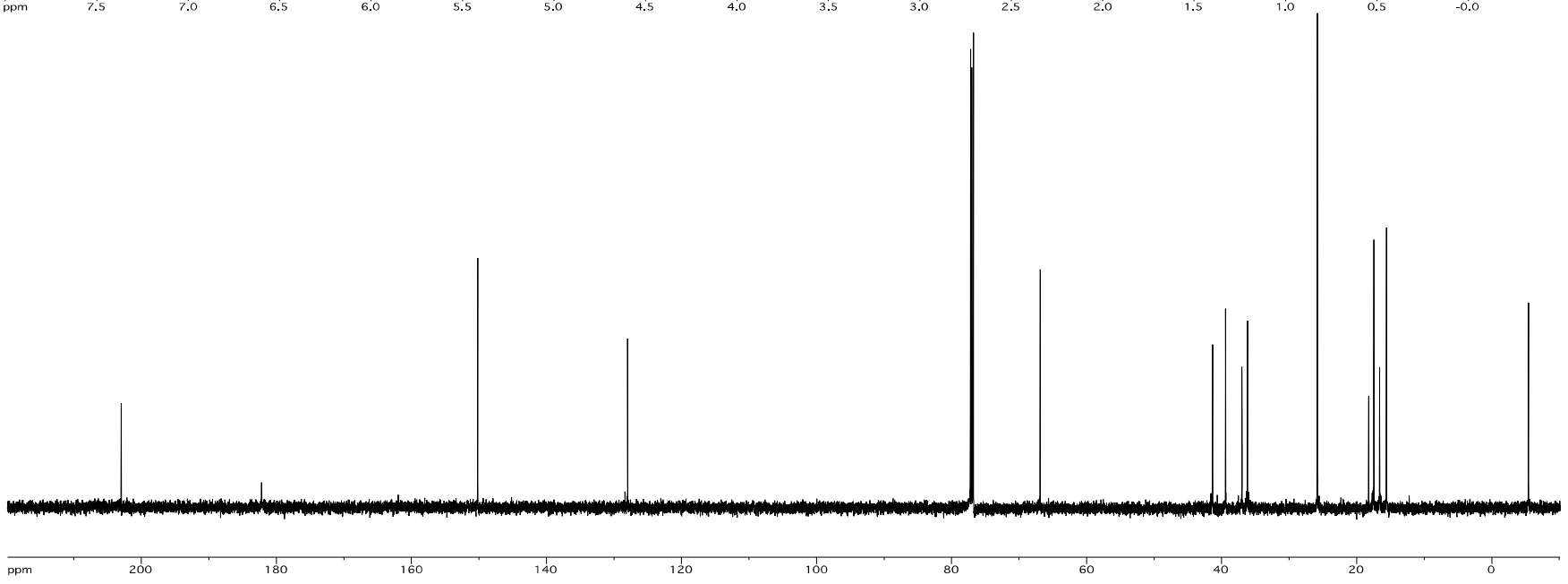
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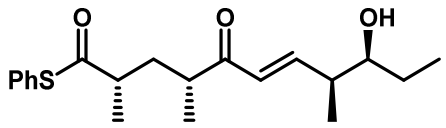
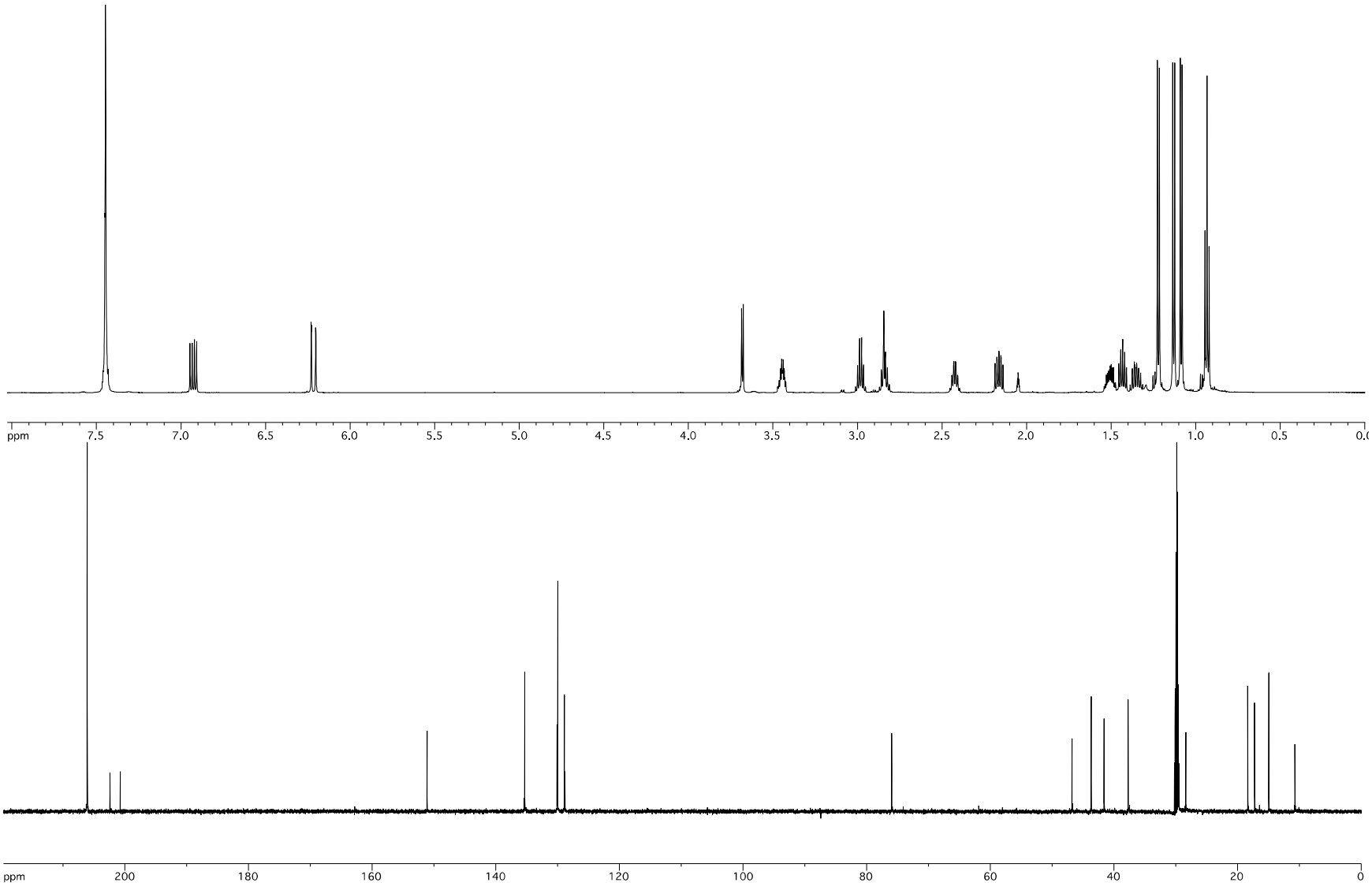

O
O
OH
PhS

12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1.0
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ppm
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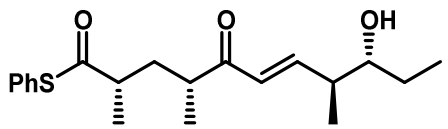
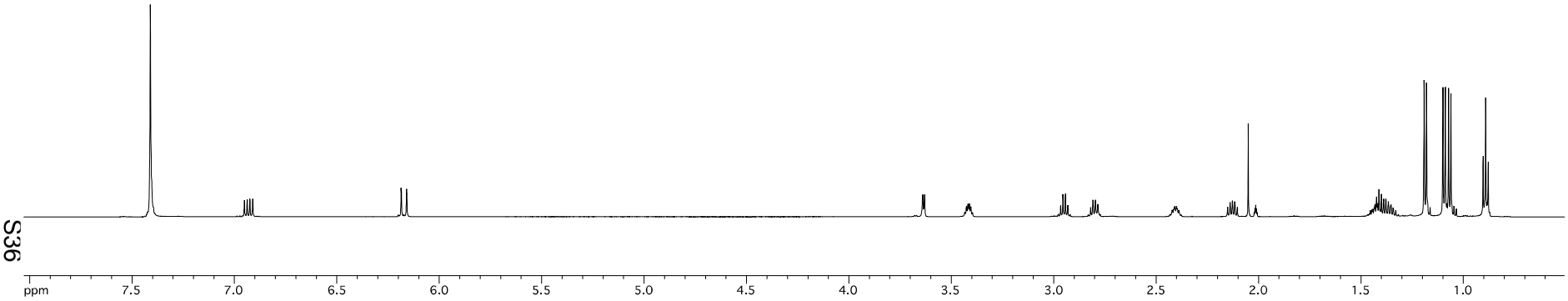

O
O
OH
PhS

11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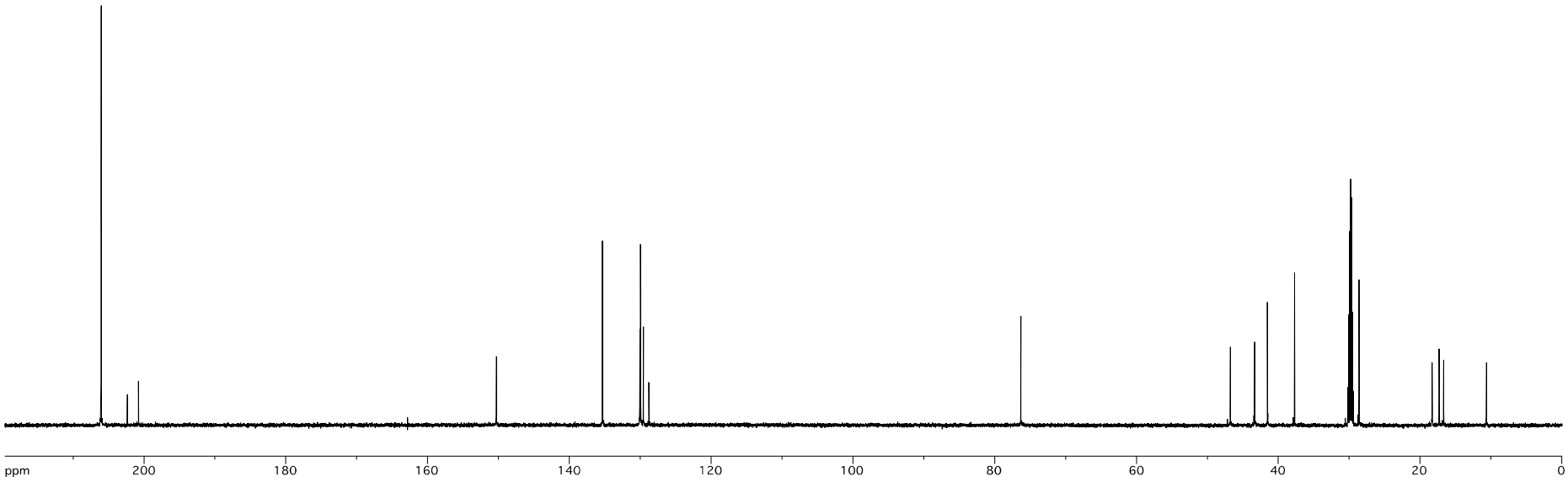
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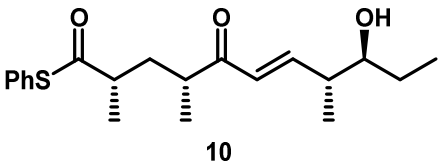
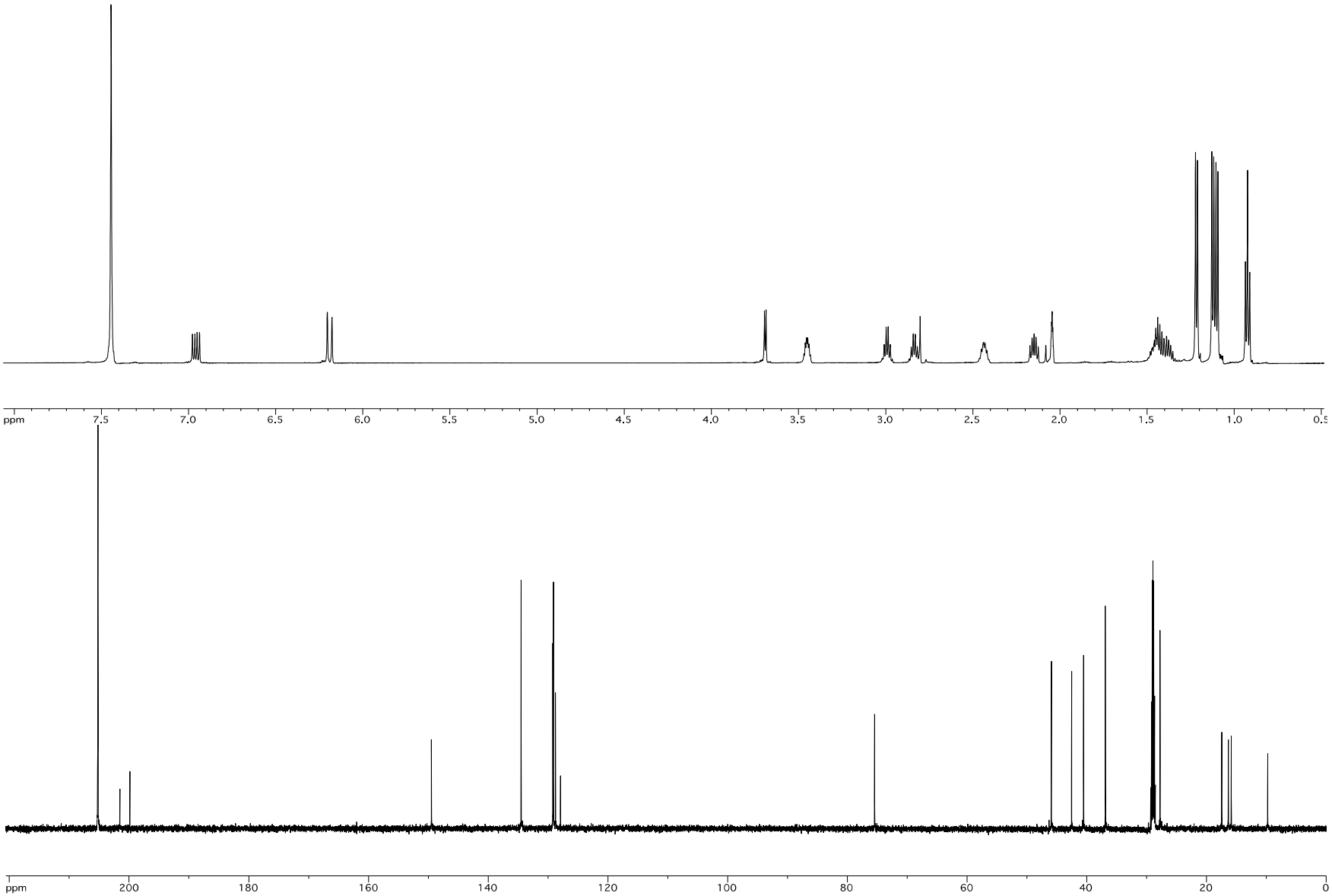

O
O
OH
PhS

10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3.0
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1.0
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ppm
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20
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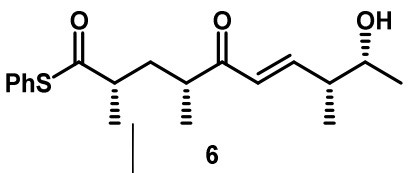
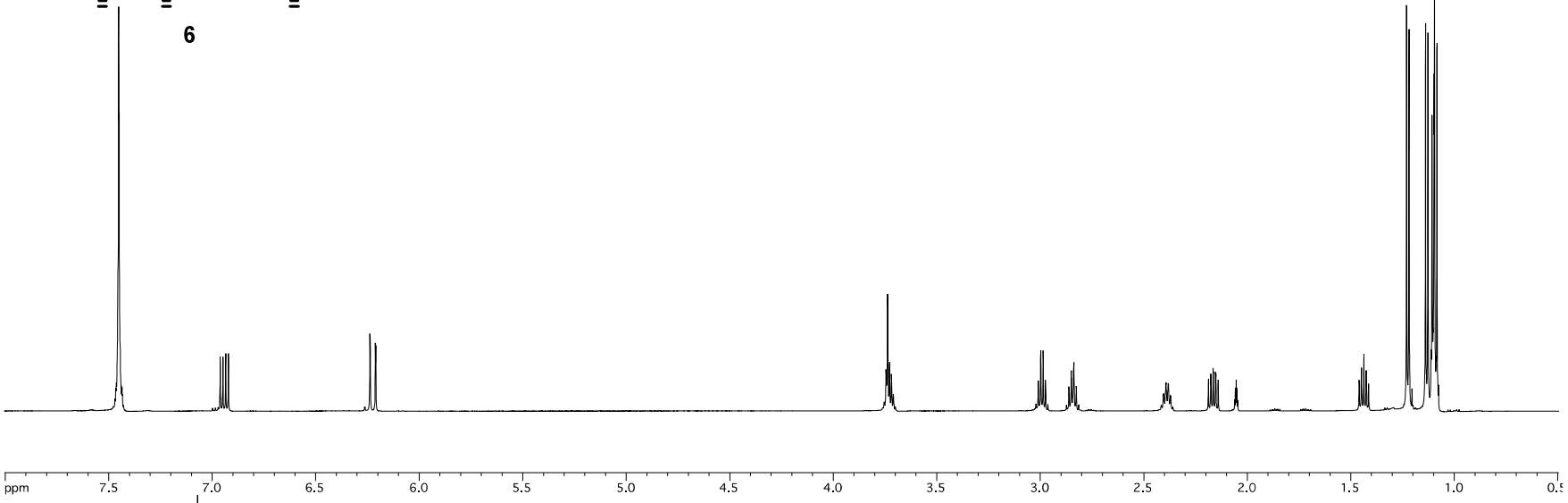

O
O
OH
PhS

6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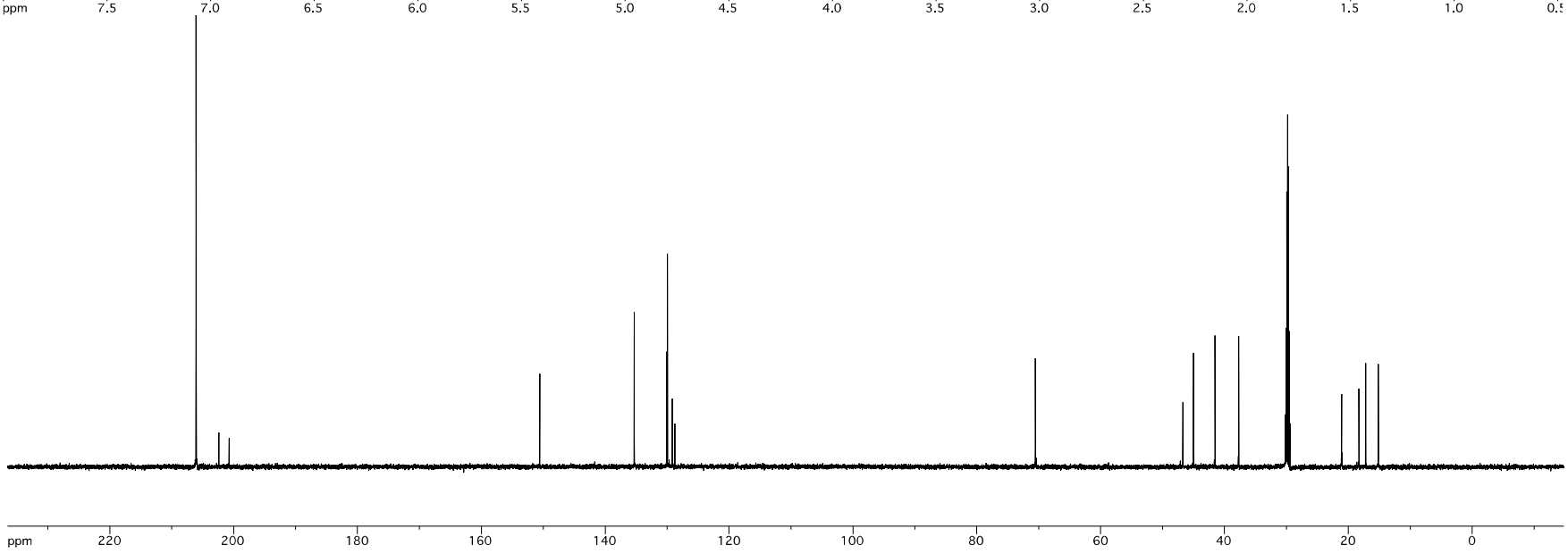
1.0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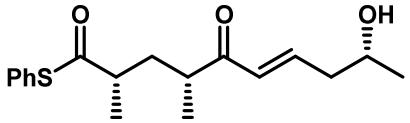
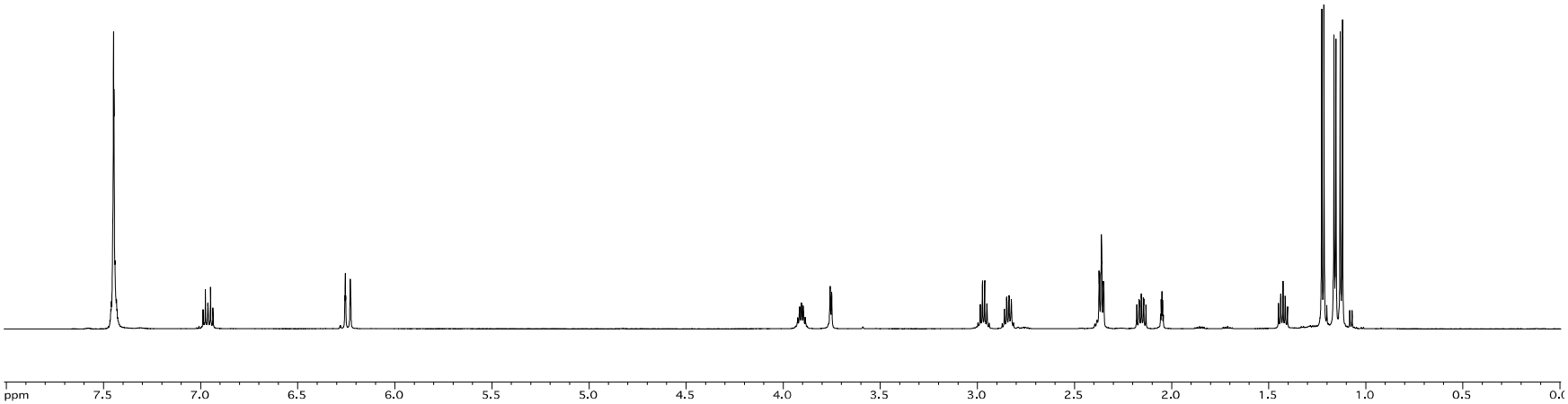

O
O
OH
PhS

8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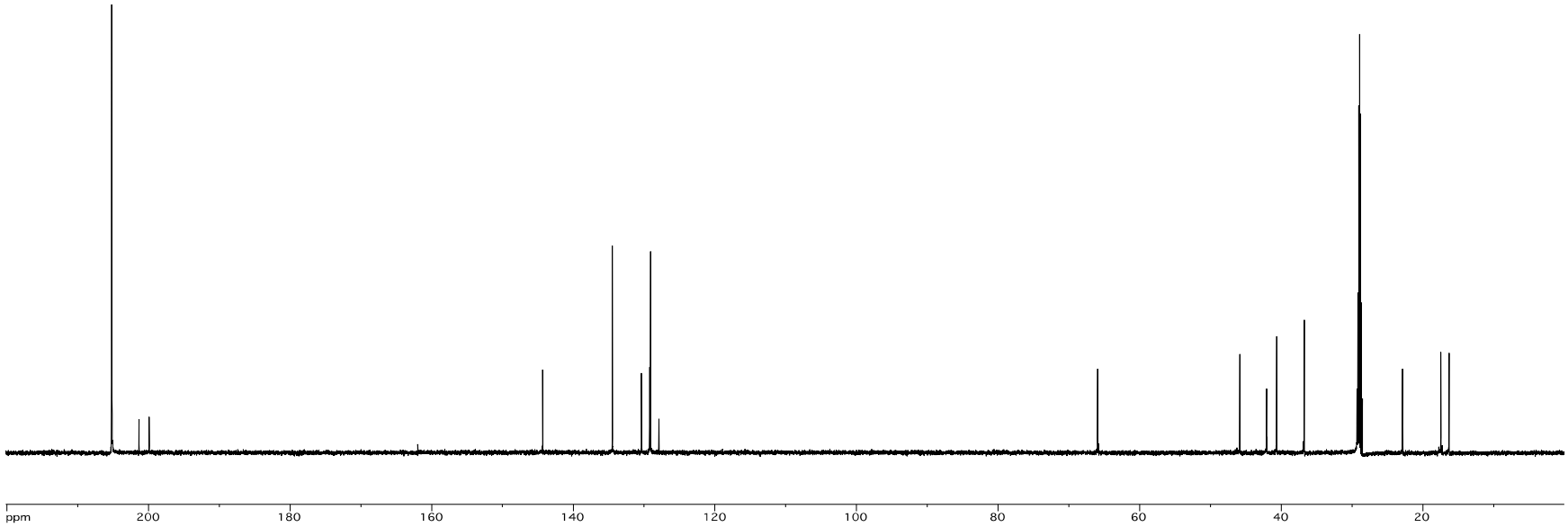
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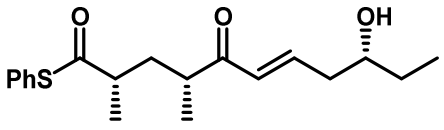
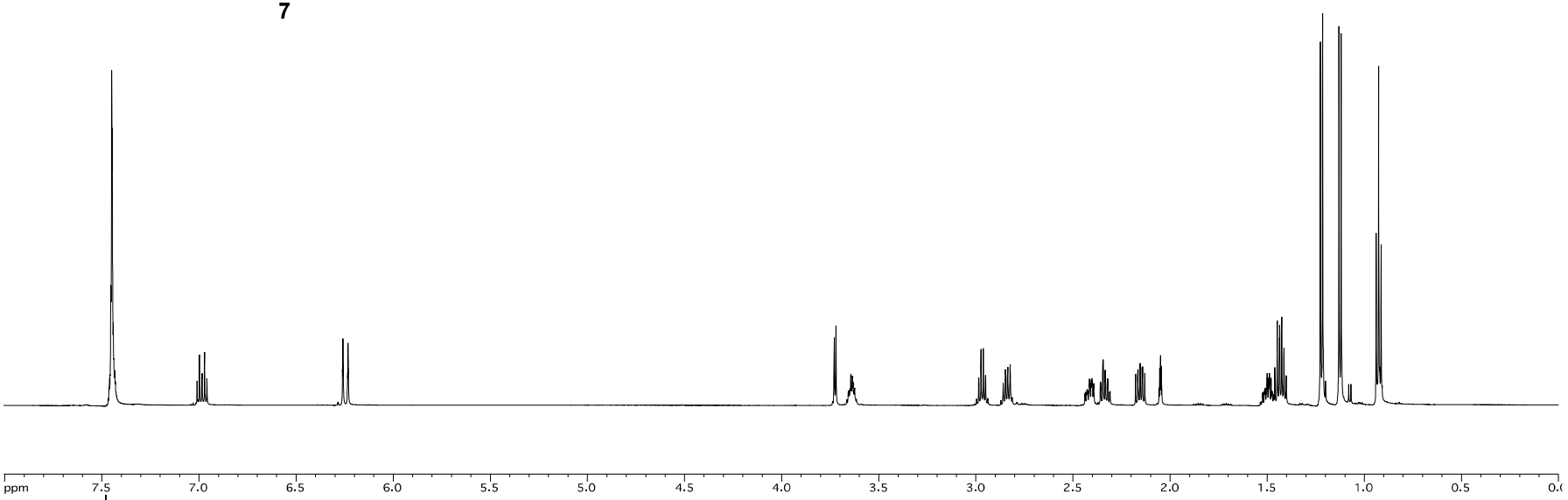

O
O
OH
PhS

7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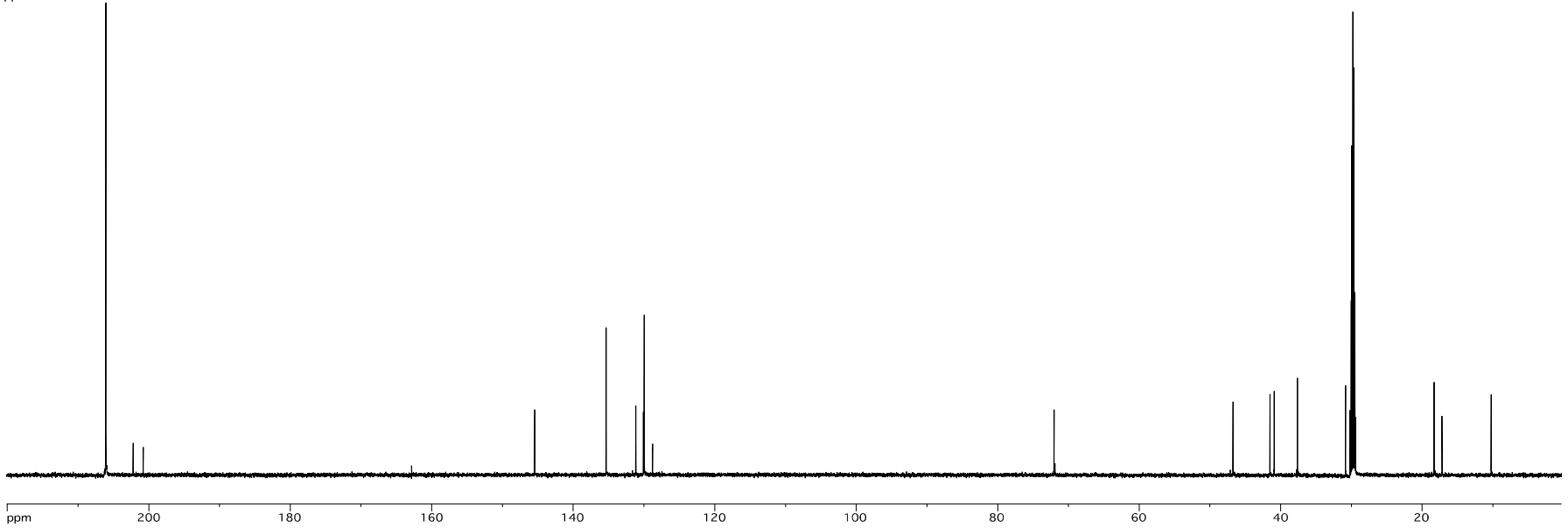
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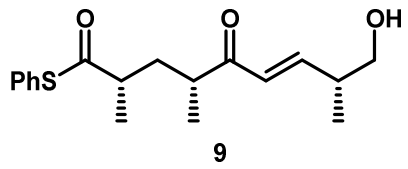
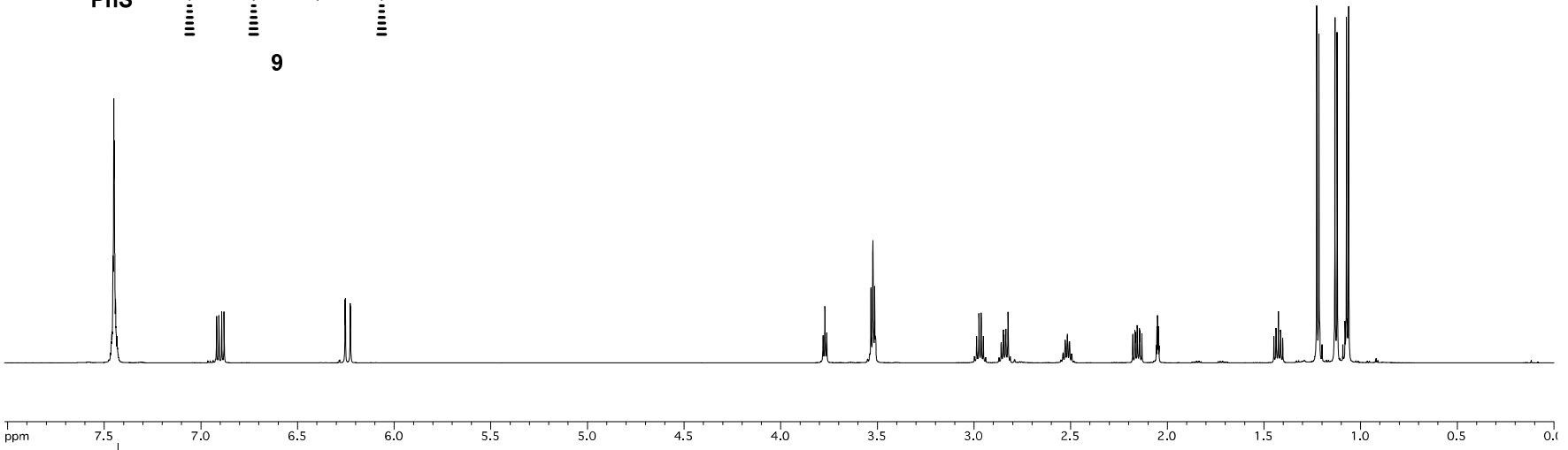

O
O
OH
PhS

9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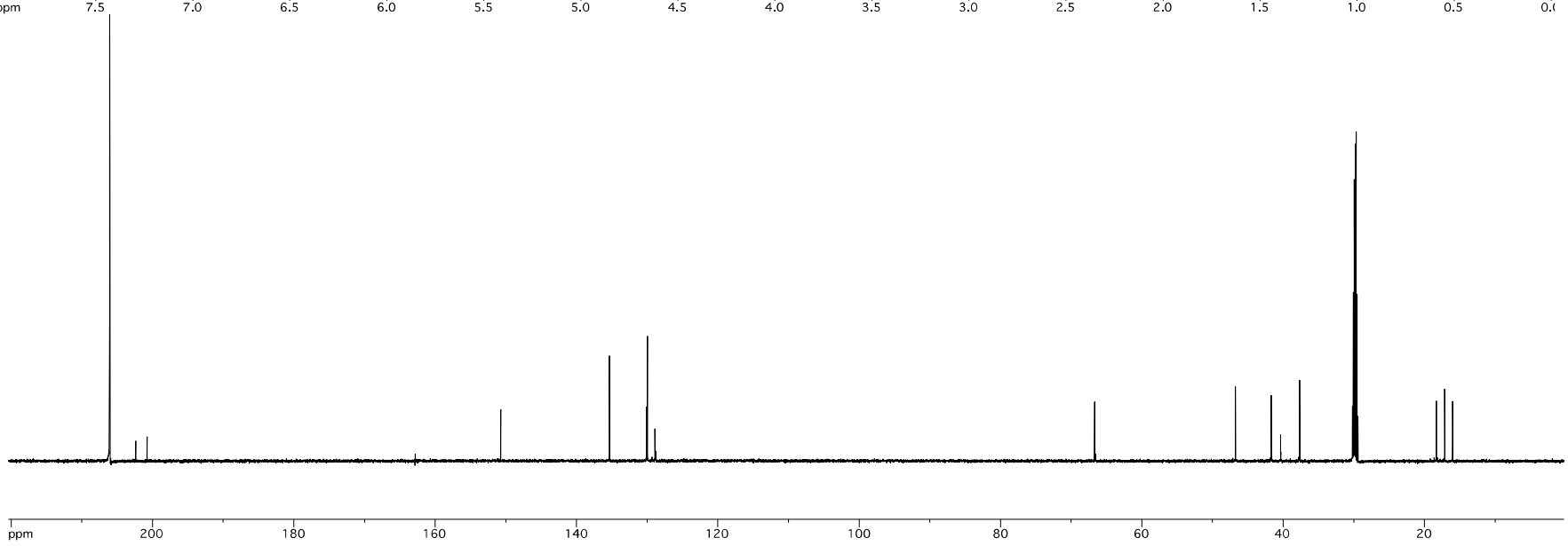
1.0
0.5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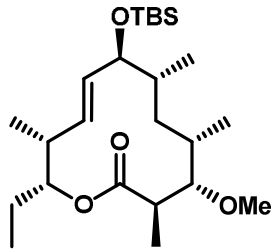
OTBS
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O
OMe

29


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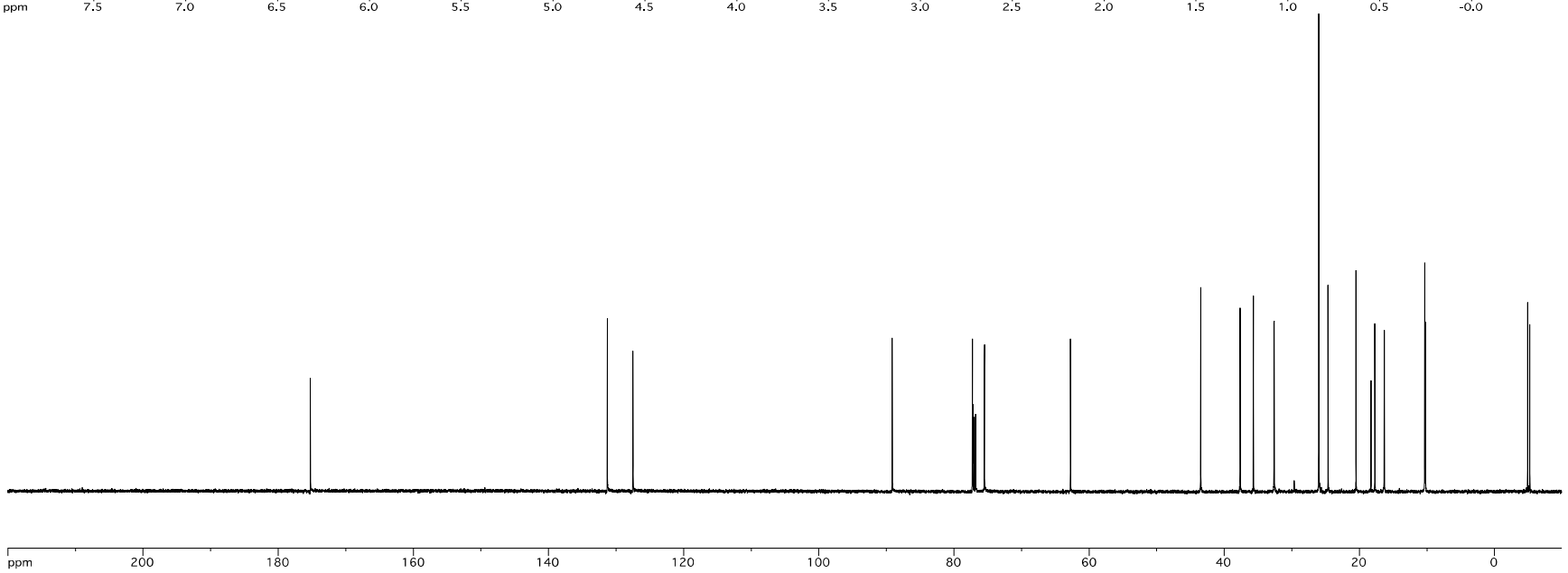
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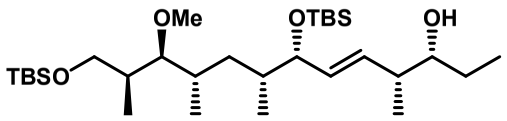
OMe
OTBS
OH
TBSO

30


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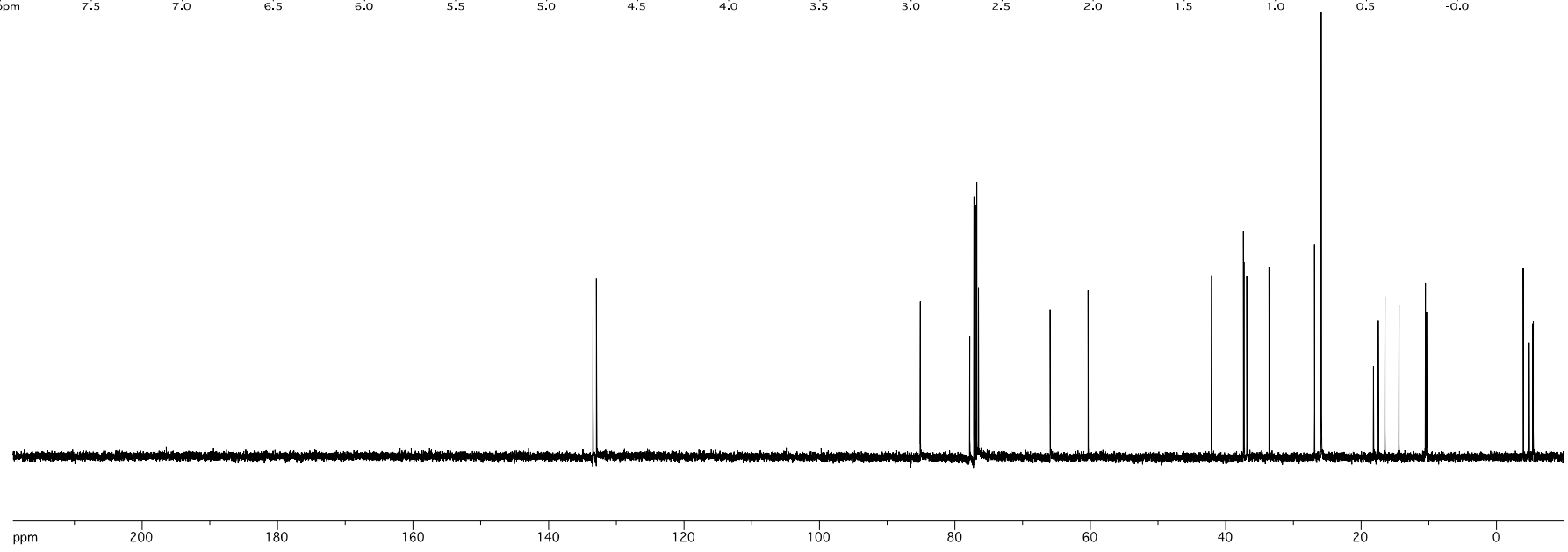
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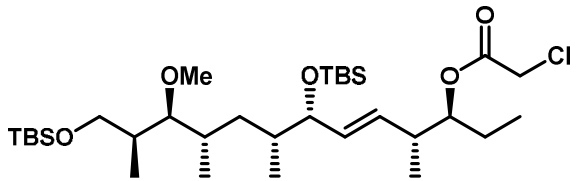
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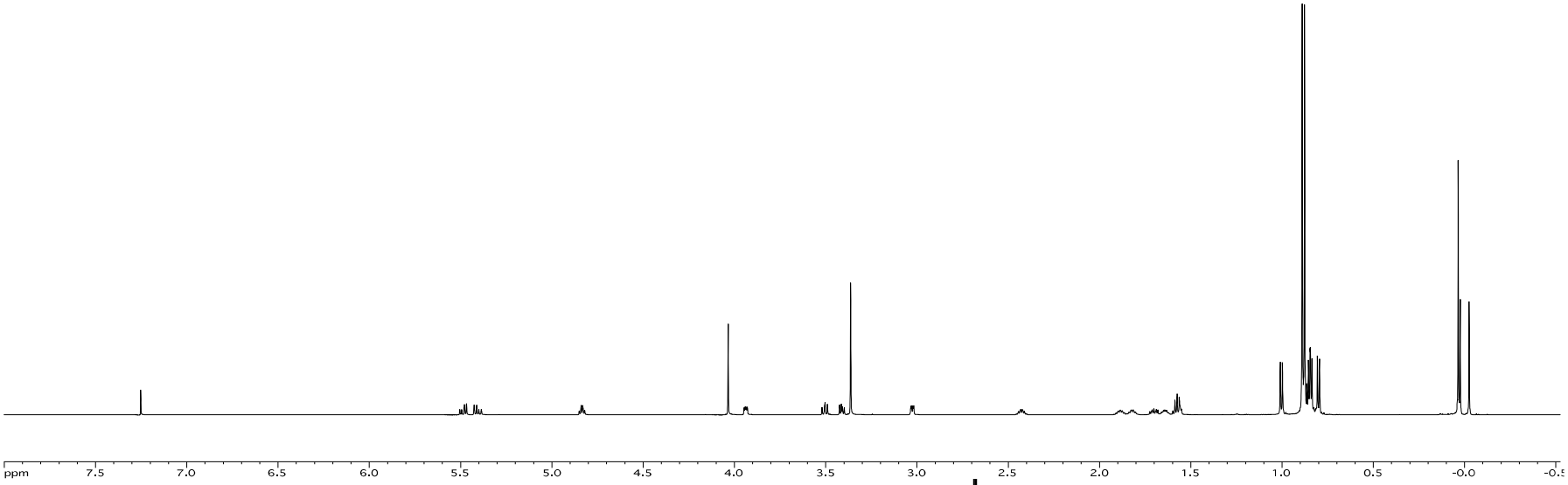
OTBS
O
O
Cl
TBSO

S26

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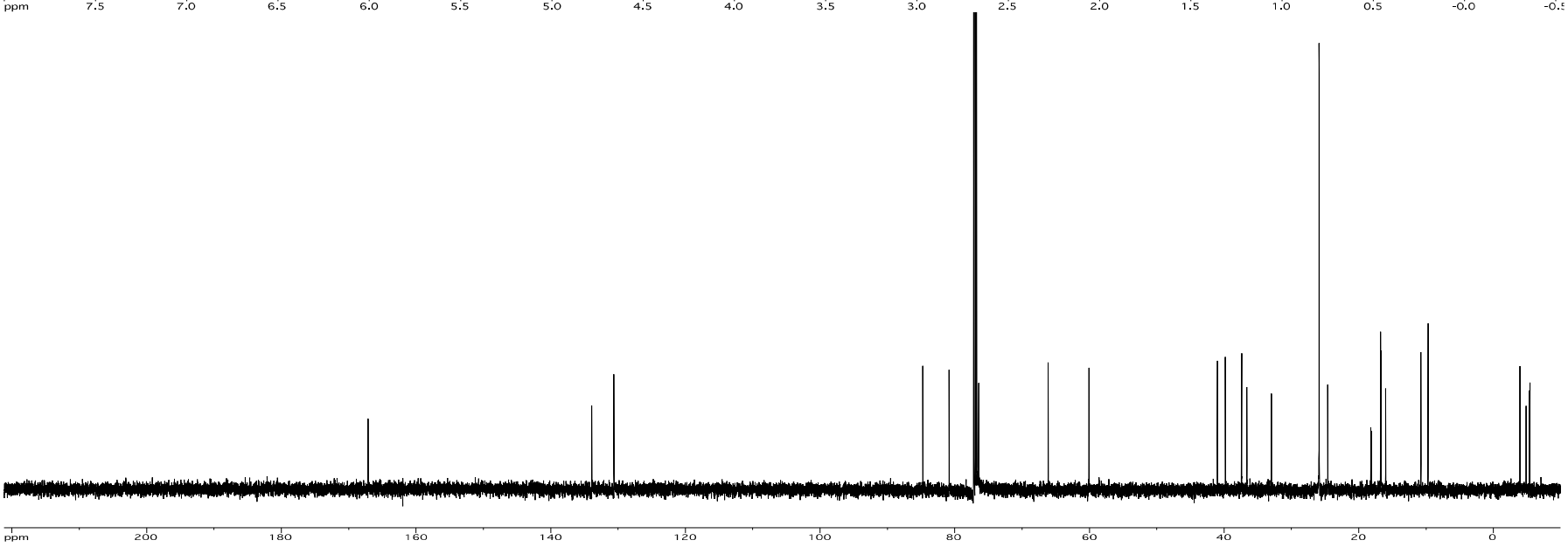
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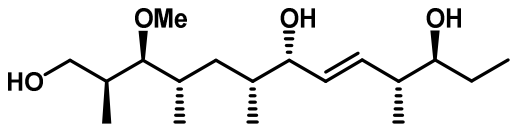
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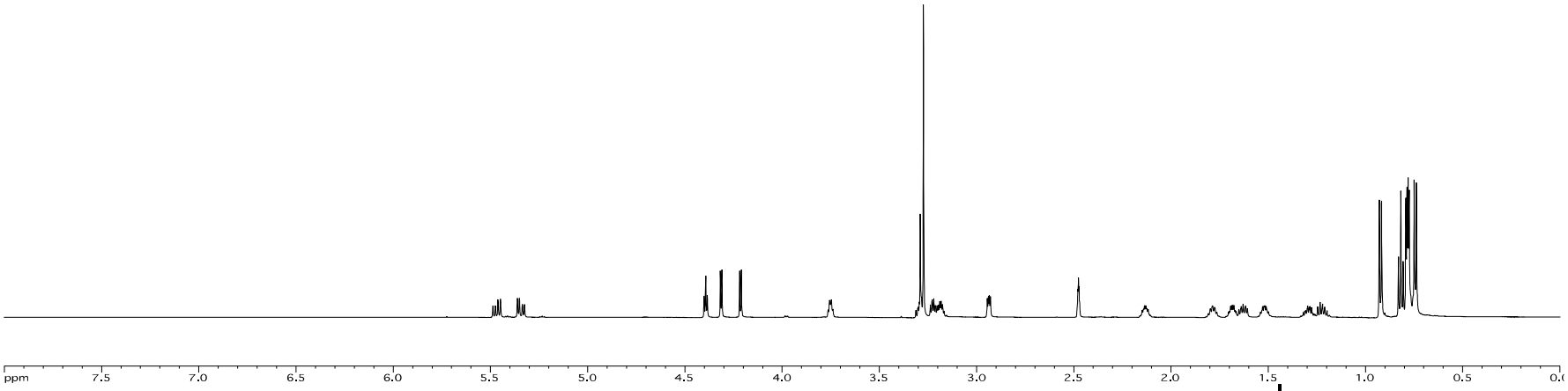
OH
HO

31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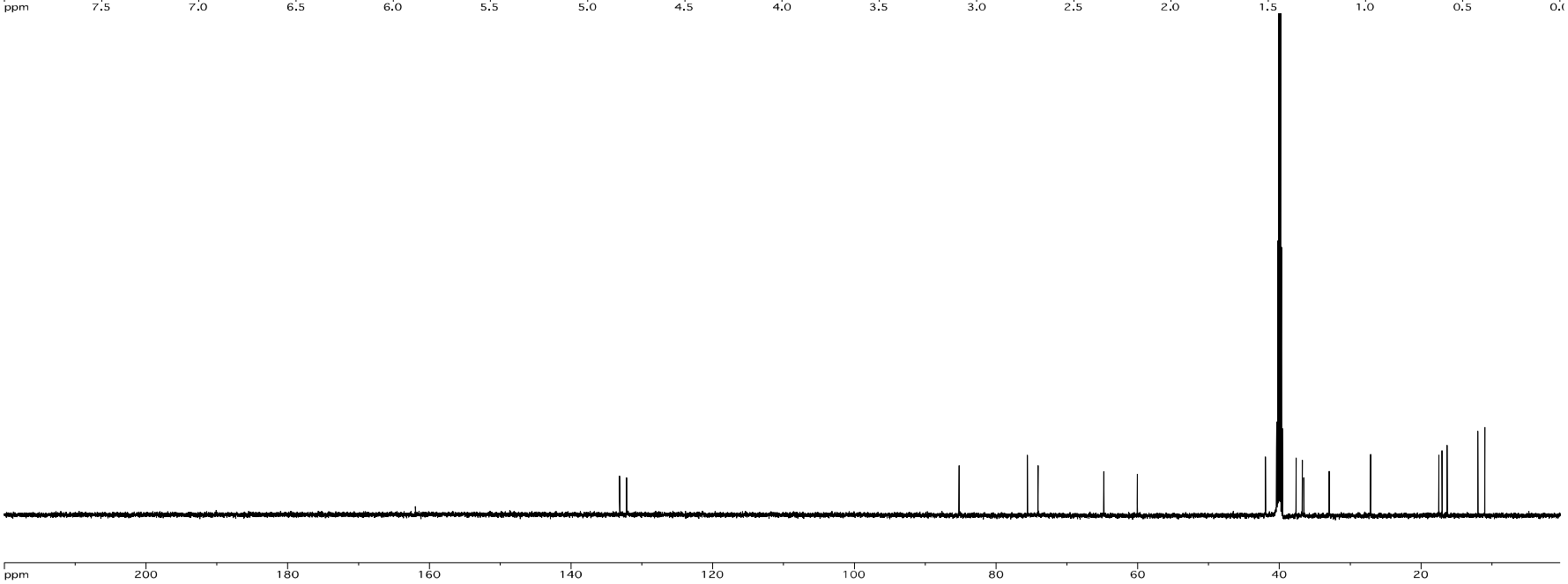
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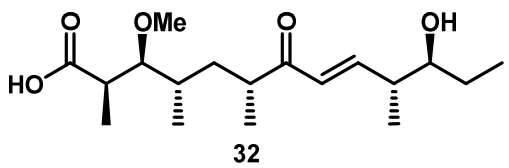
O
OMe
O
OH
HO

32


ppm
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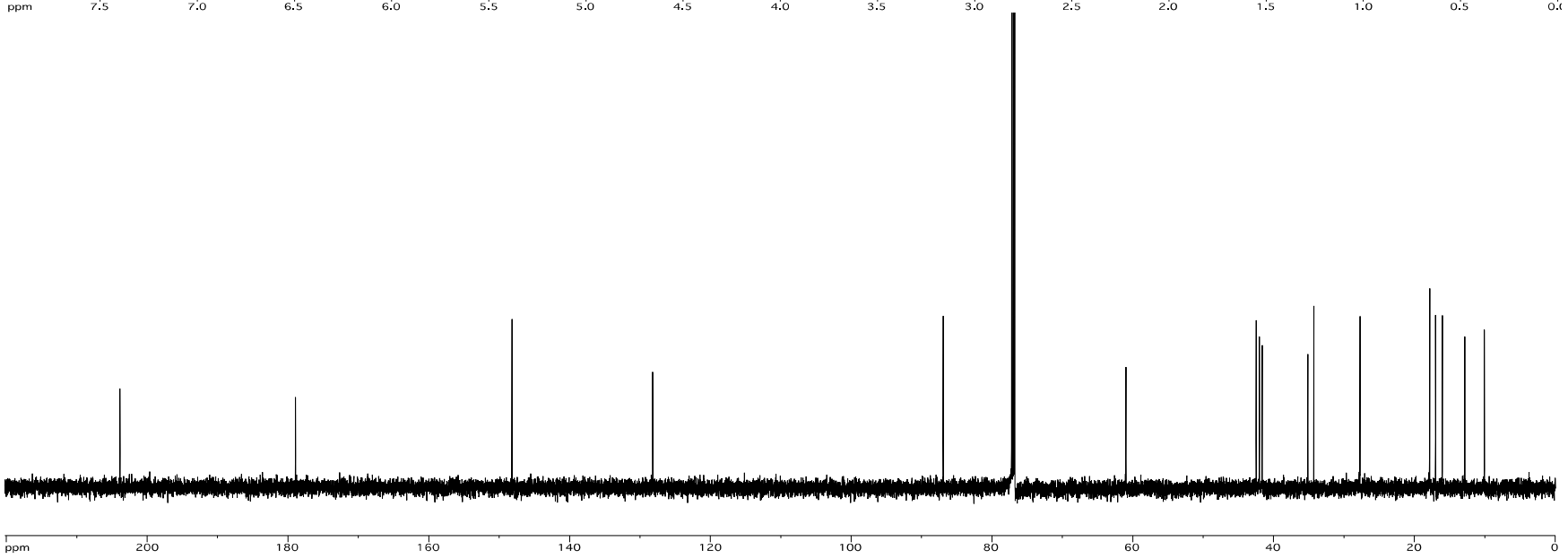
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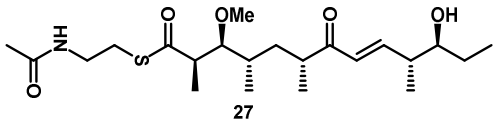
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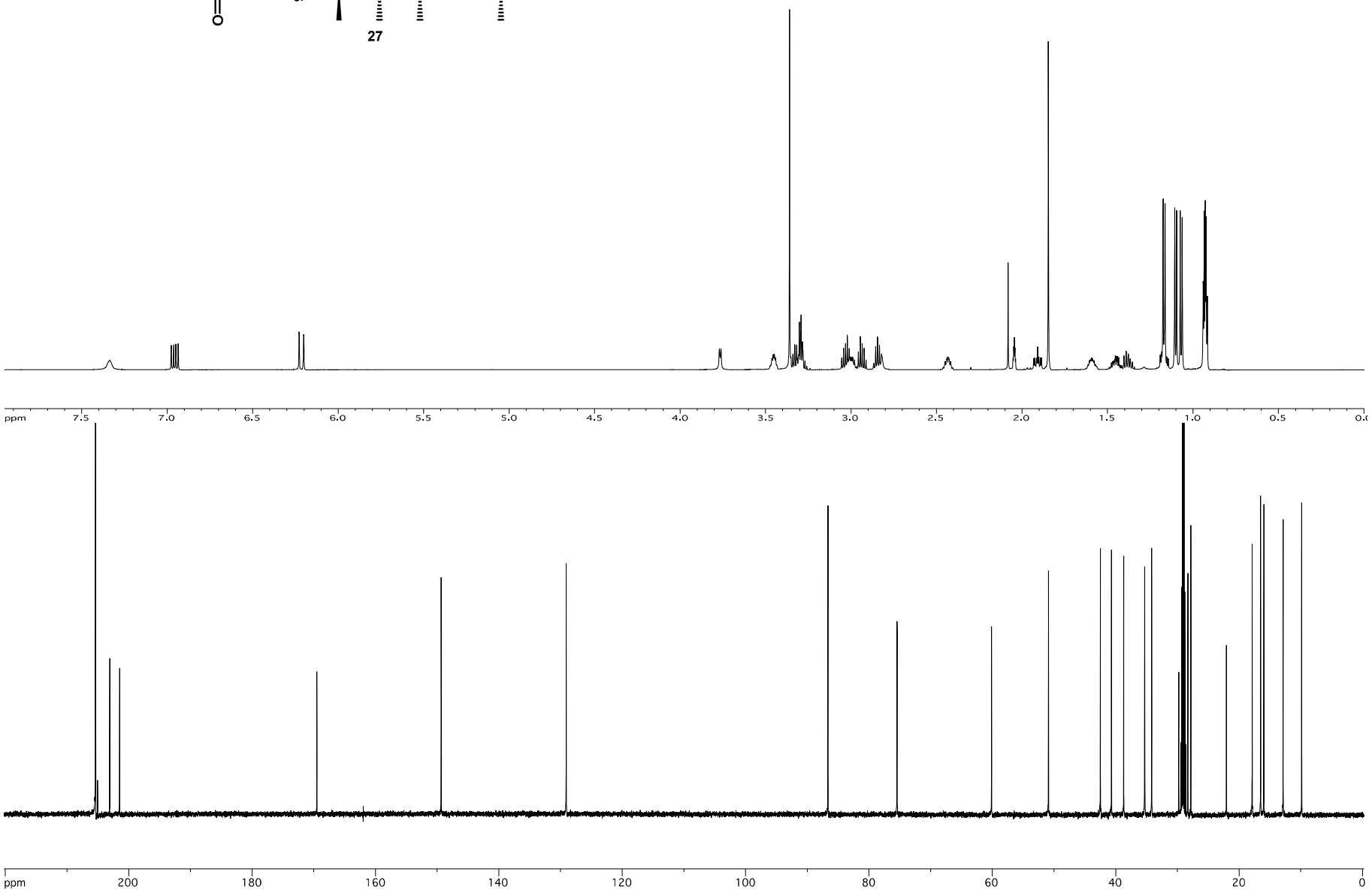
OMe
O
OH
NH
O

27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ppm
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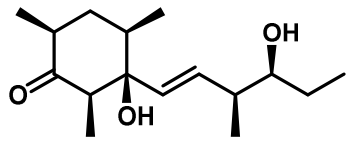
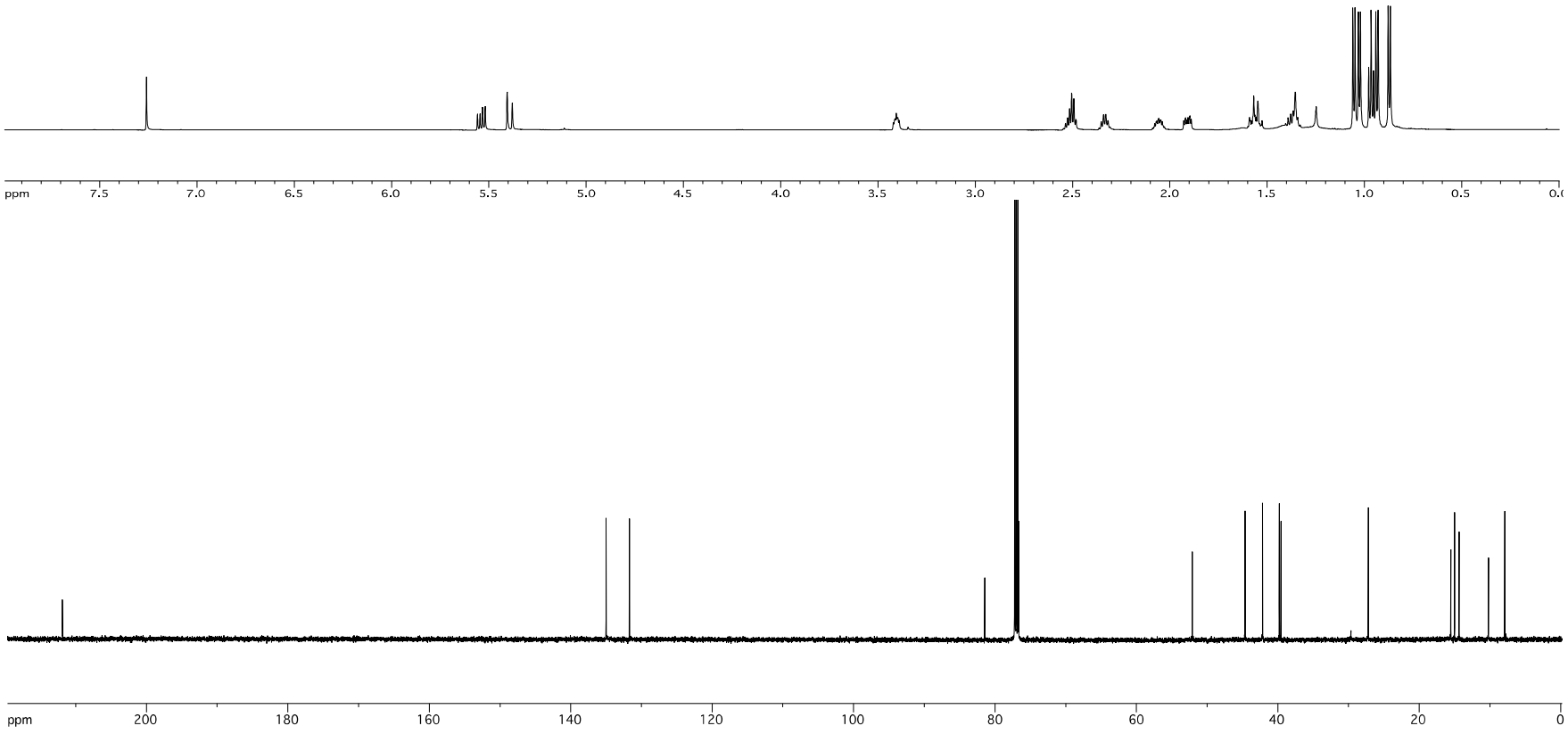

OH
O
OH

**21**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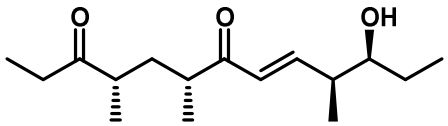
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OH

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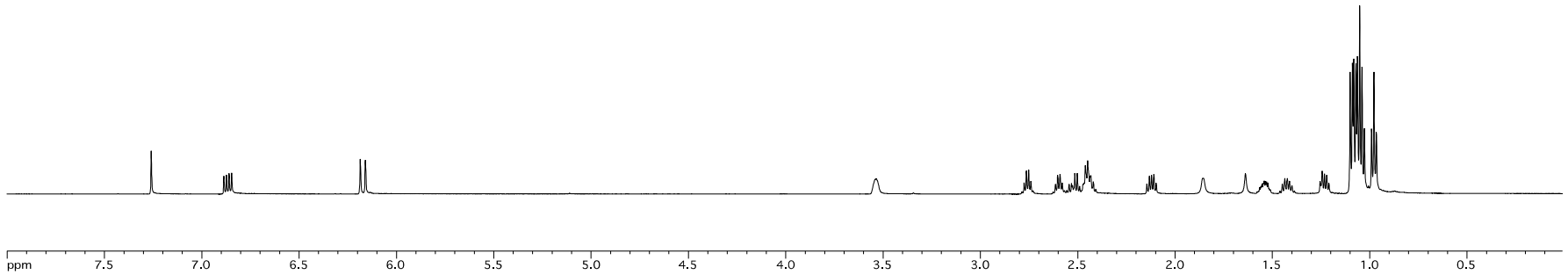
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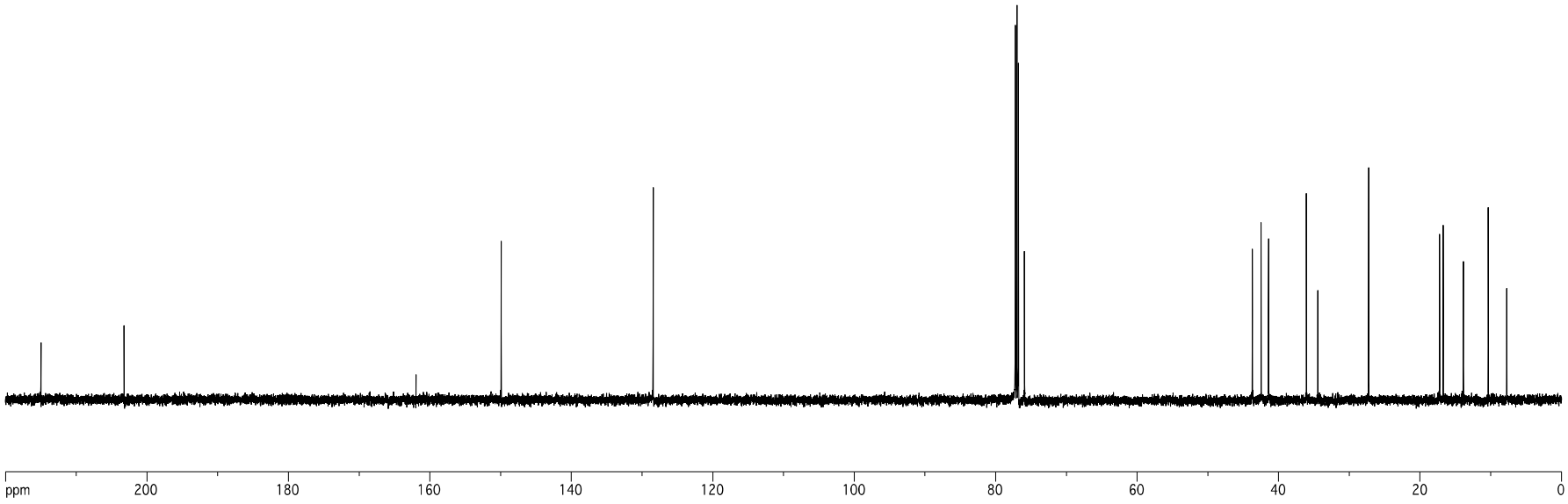
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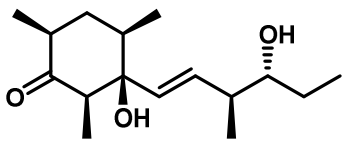
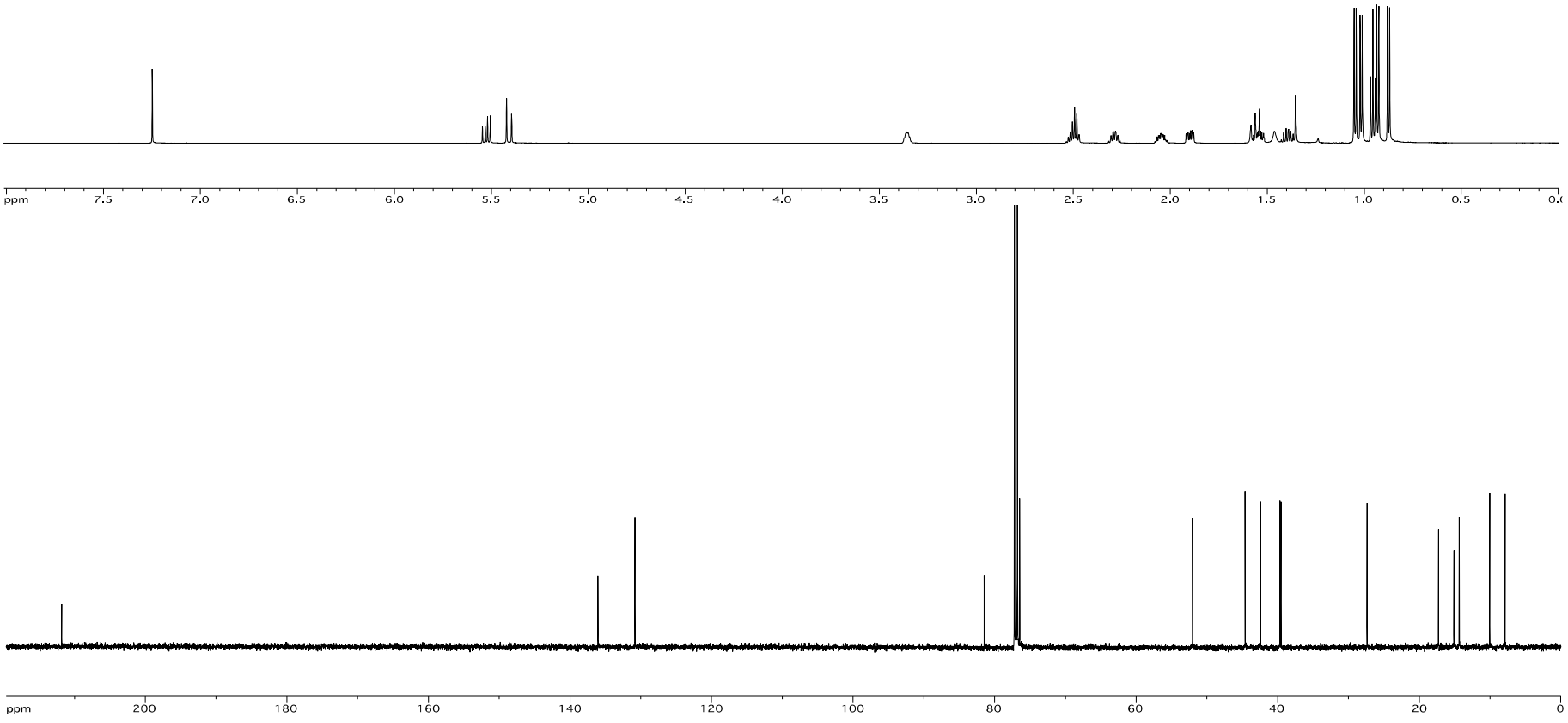

OH
O
OH

**19**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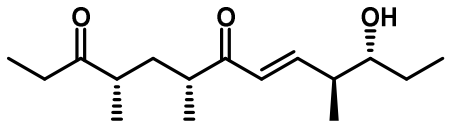
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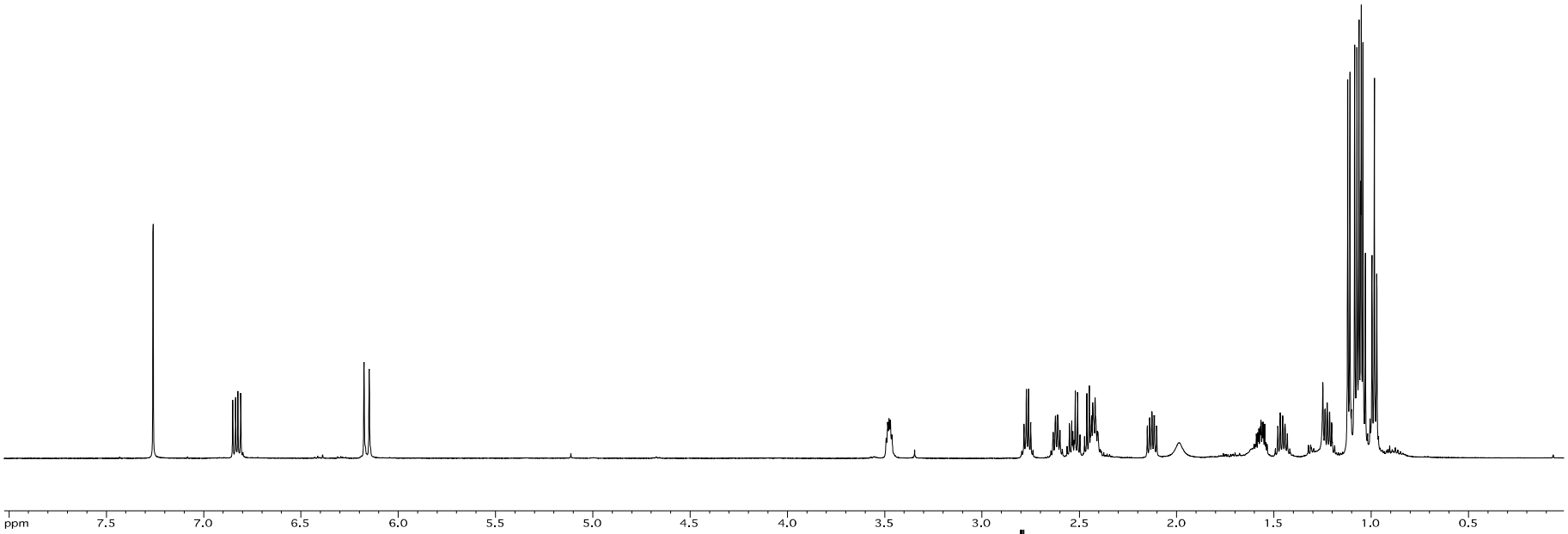
**20**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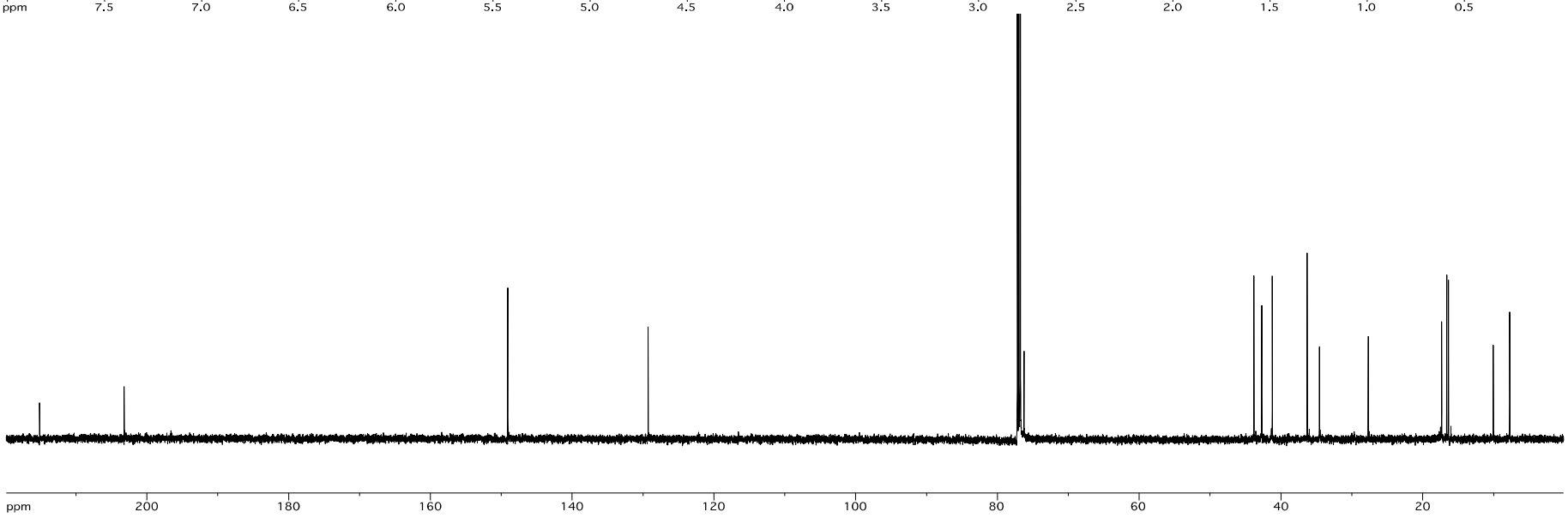
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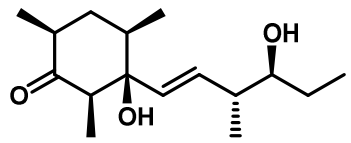
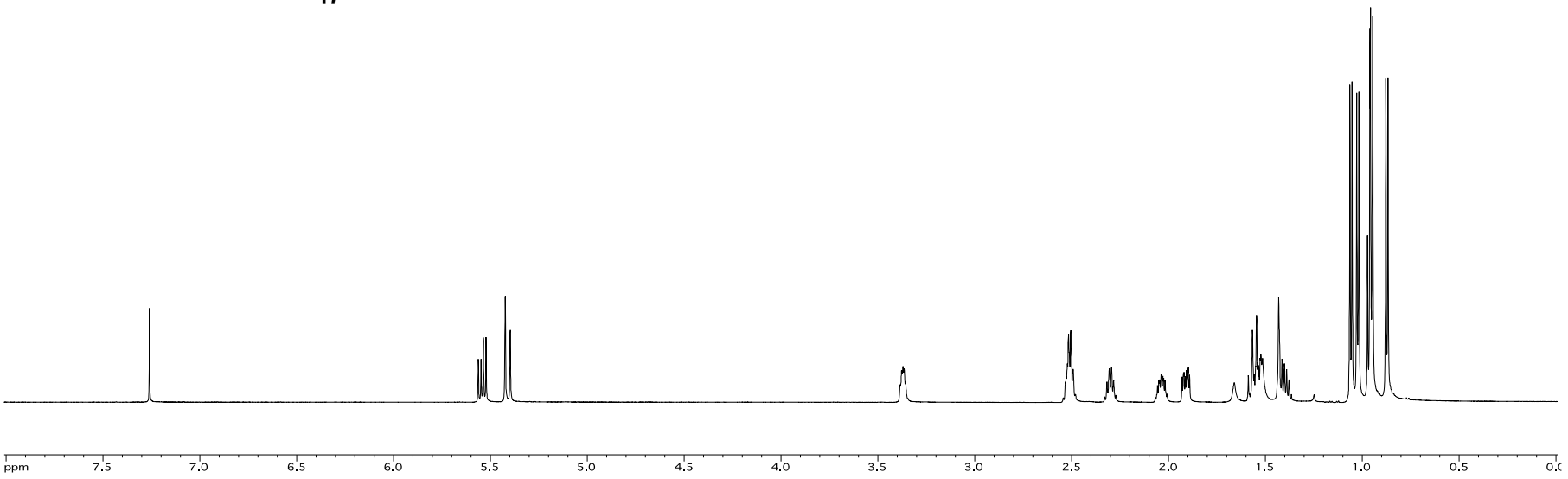

OH
O
OH

17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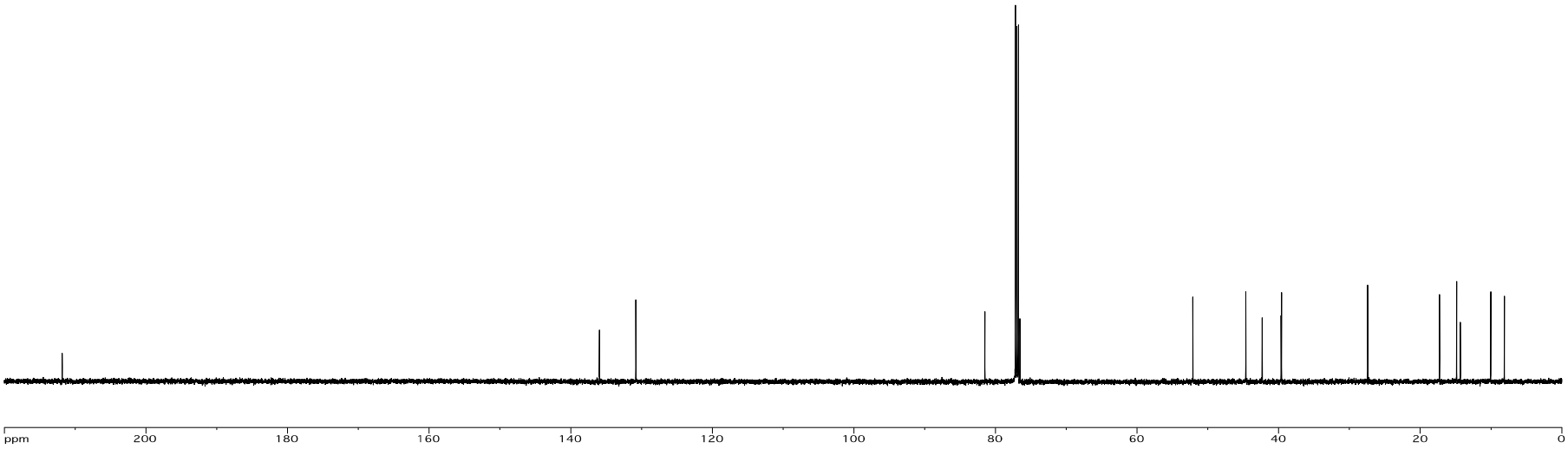
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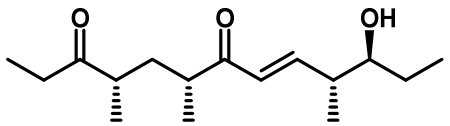
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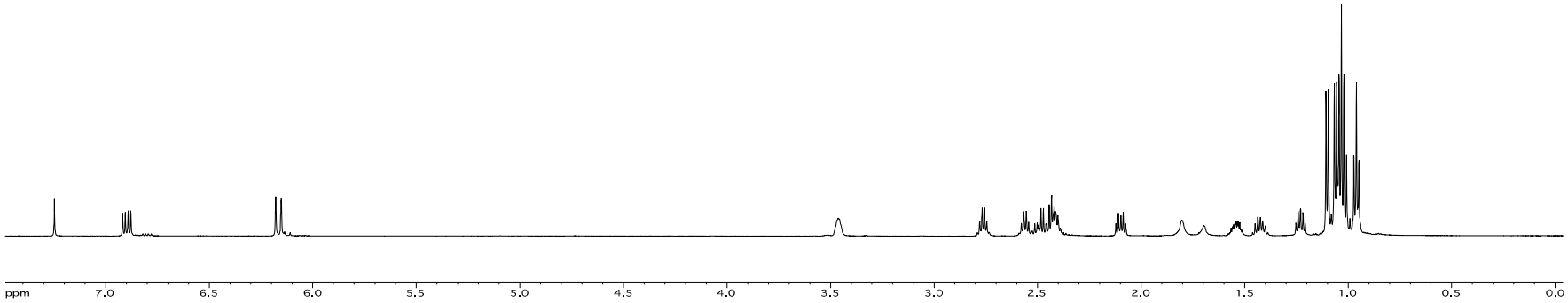
OH

**18**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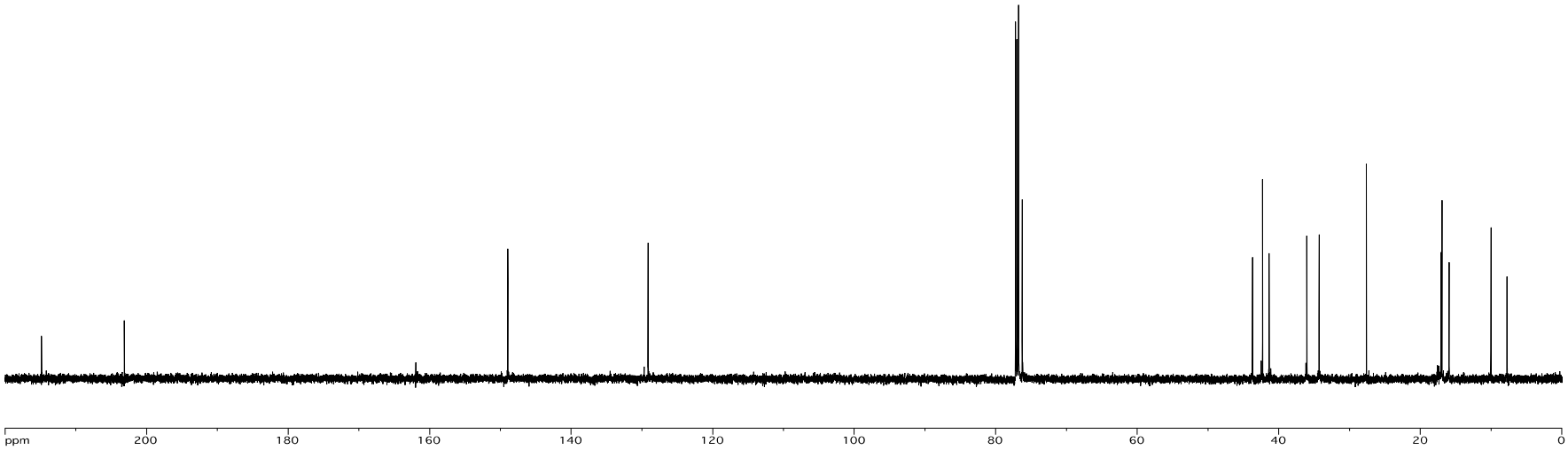
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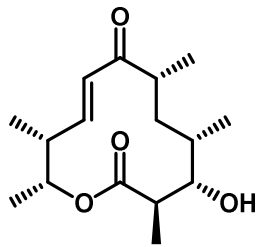
O
O
O
OH

13


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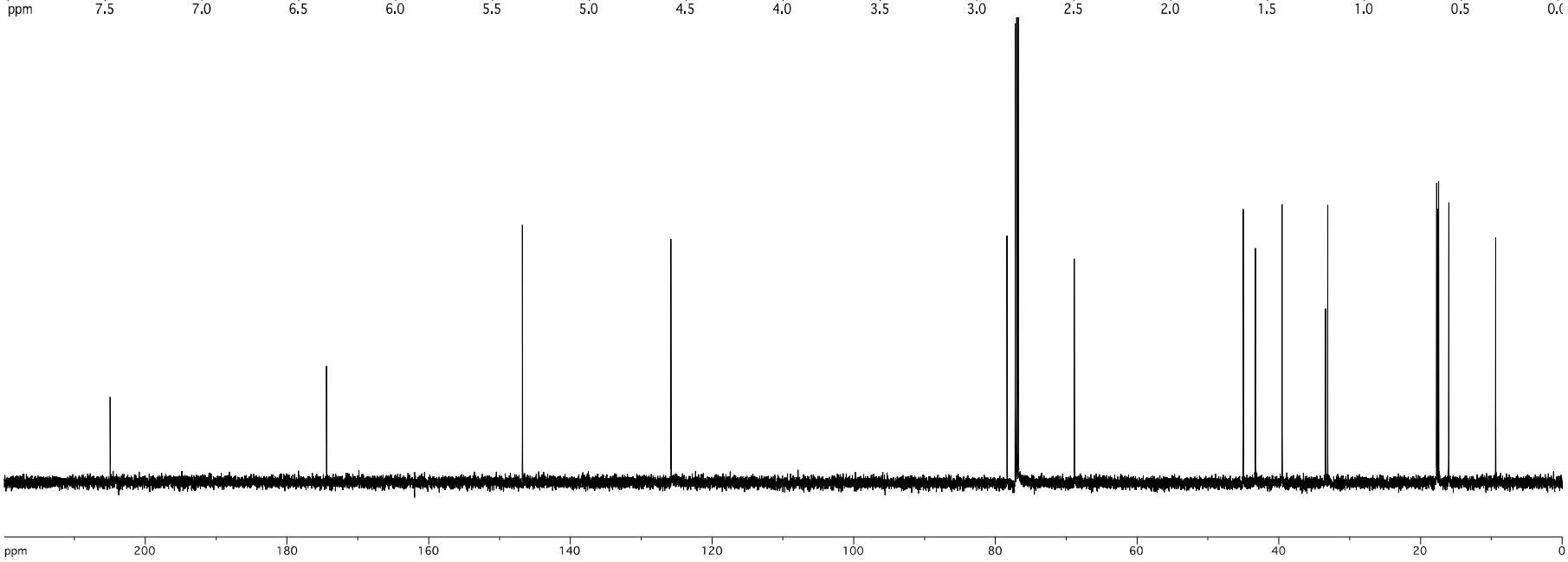
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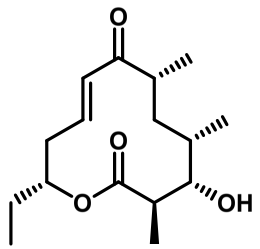
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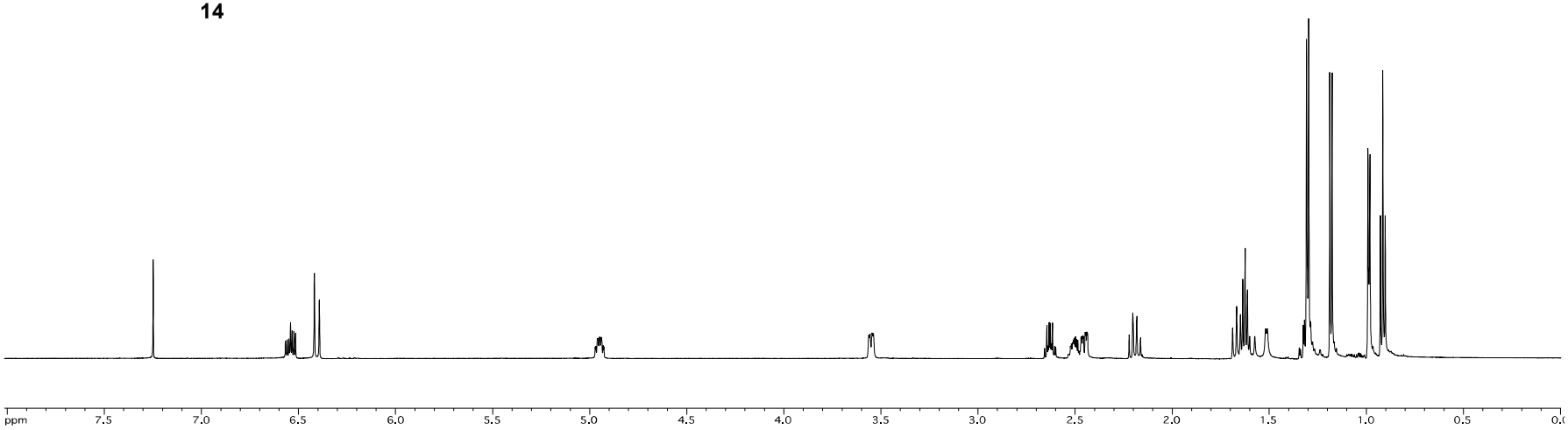
O
O
OH

14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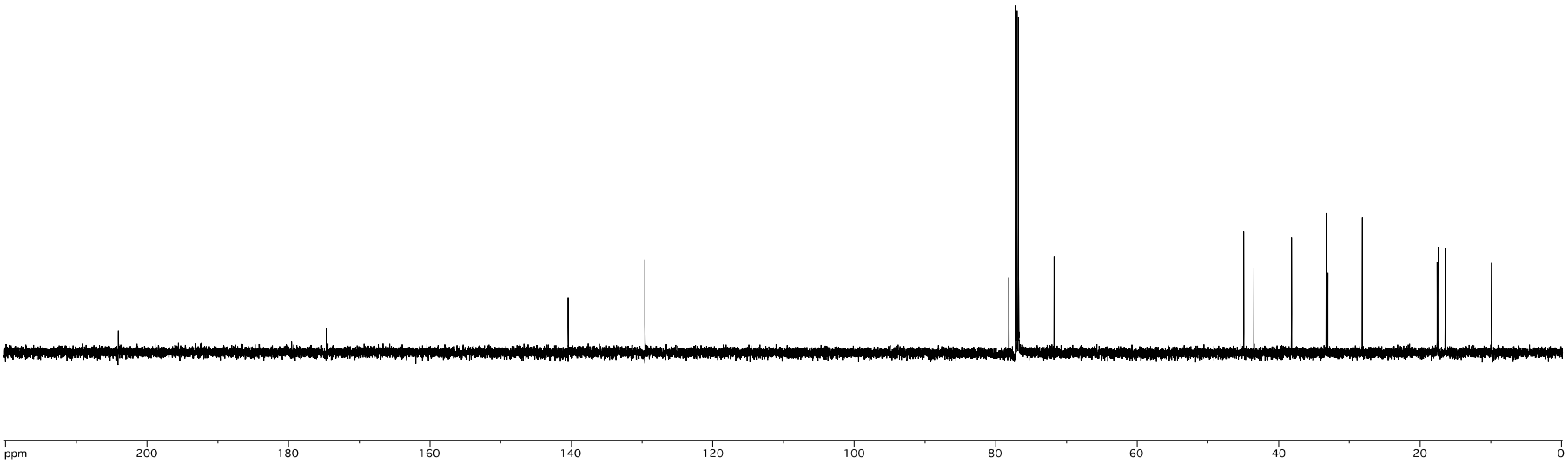
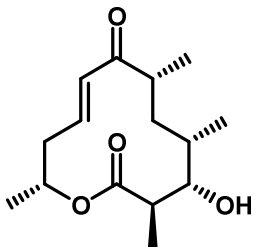
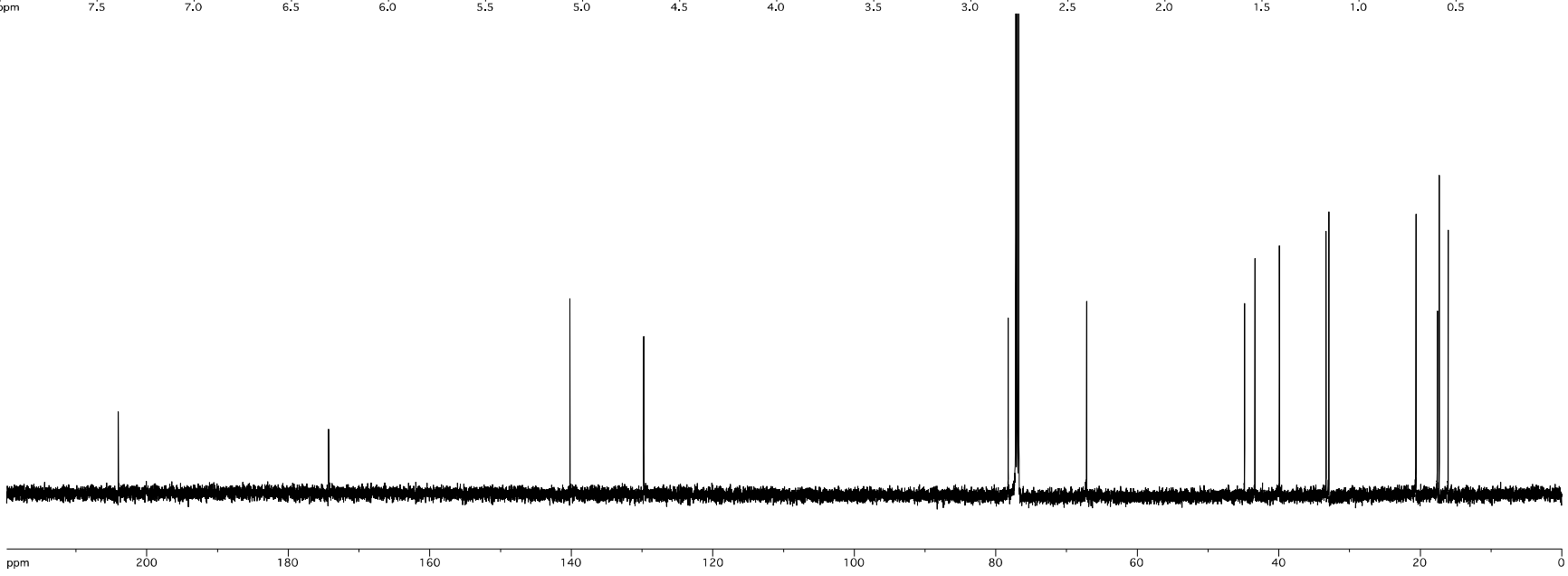
3.0
2.5
2.0
1.5
1.0
0.5

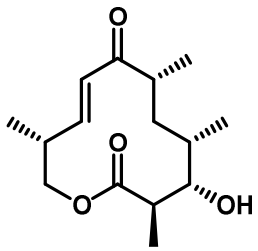

ppm
200
180
160
140
120
100
80
60
40
20
0


O
O
O
OH

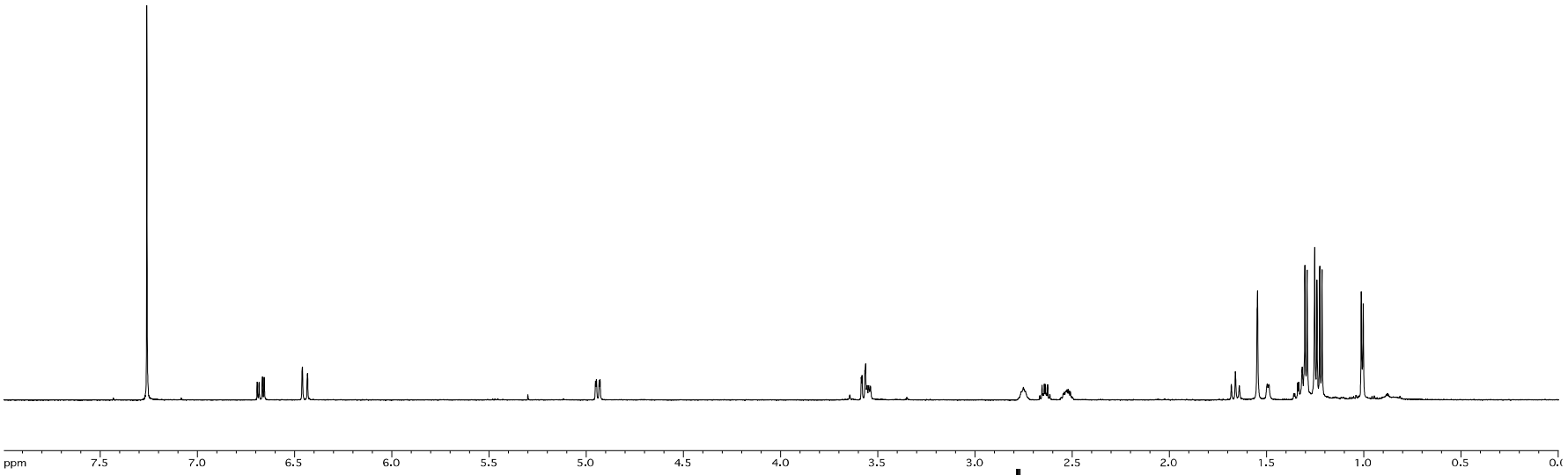
15

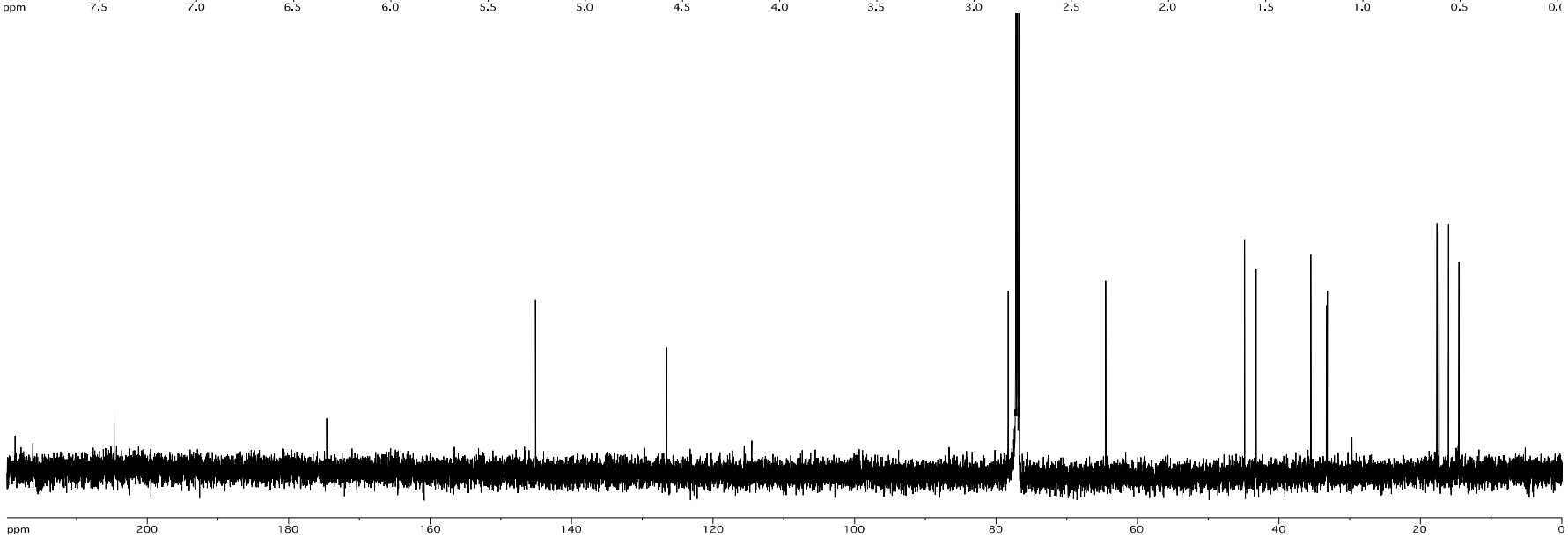

ppm
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

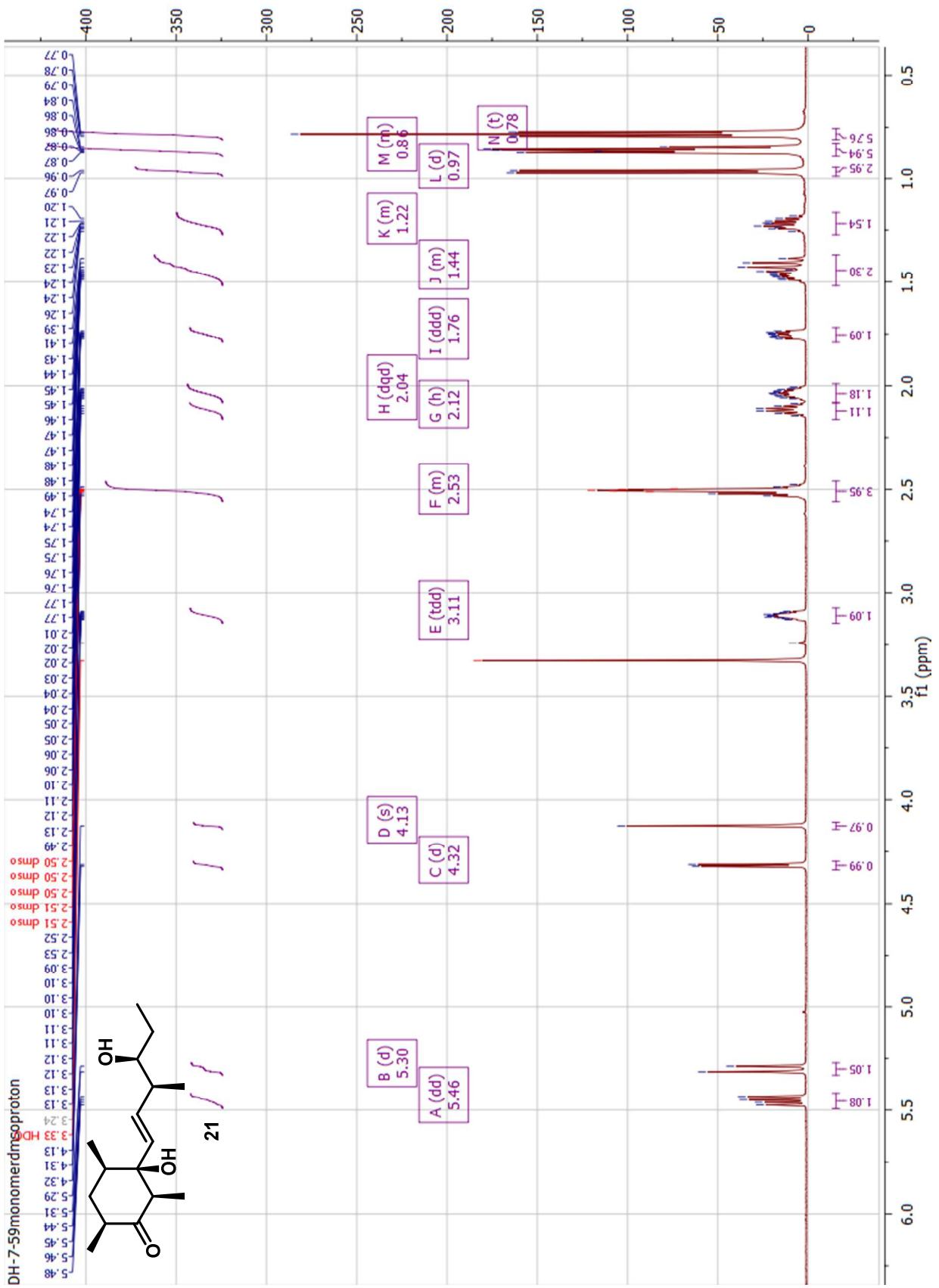

ppm
200
180
160
140
120
100
80
60
40
20
0

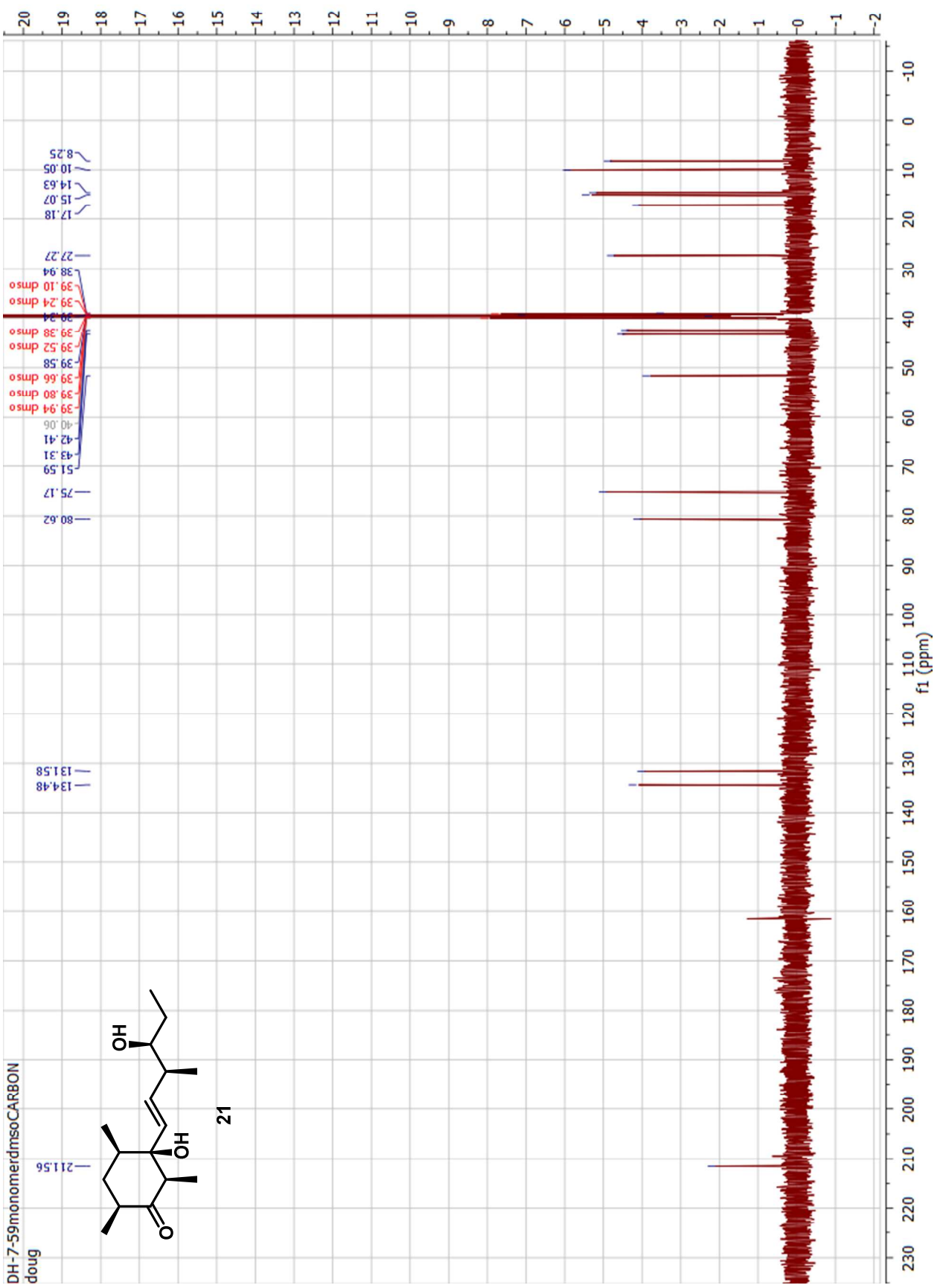

O
O
O
OH

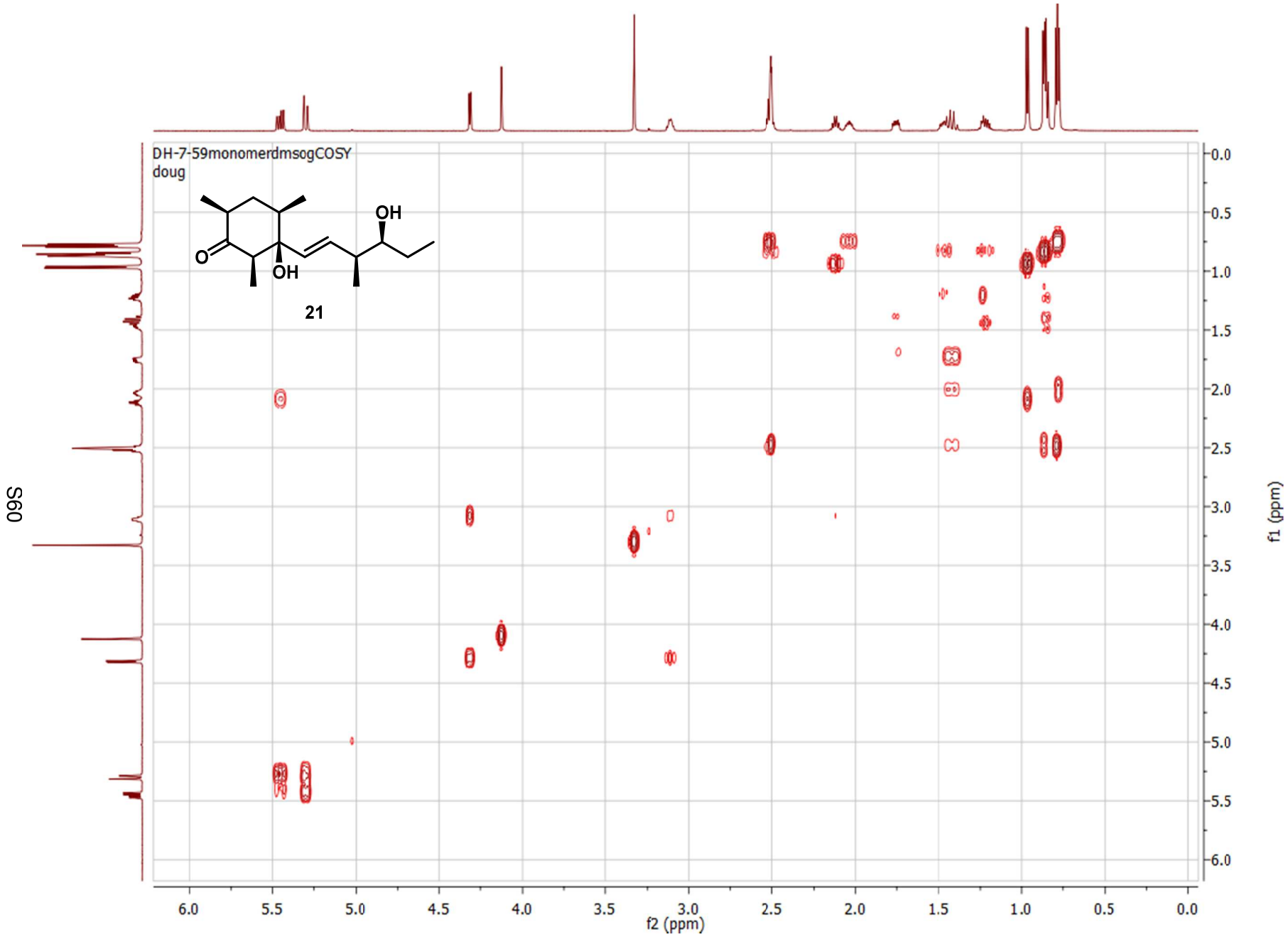
16

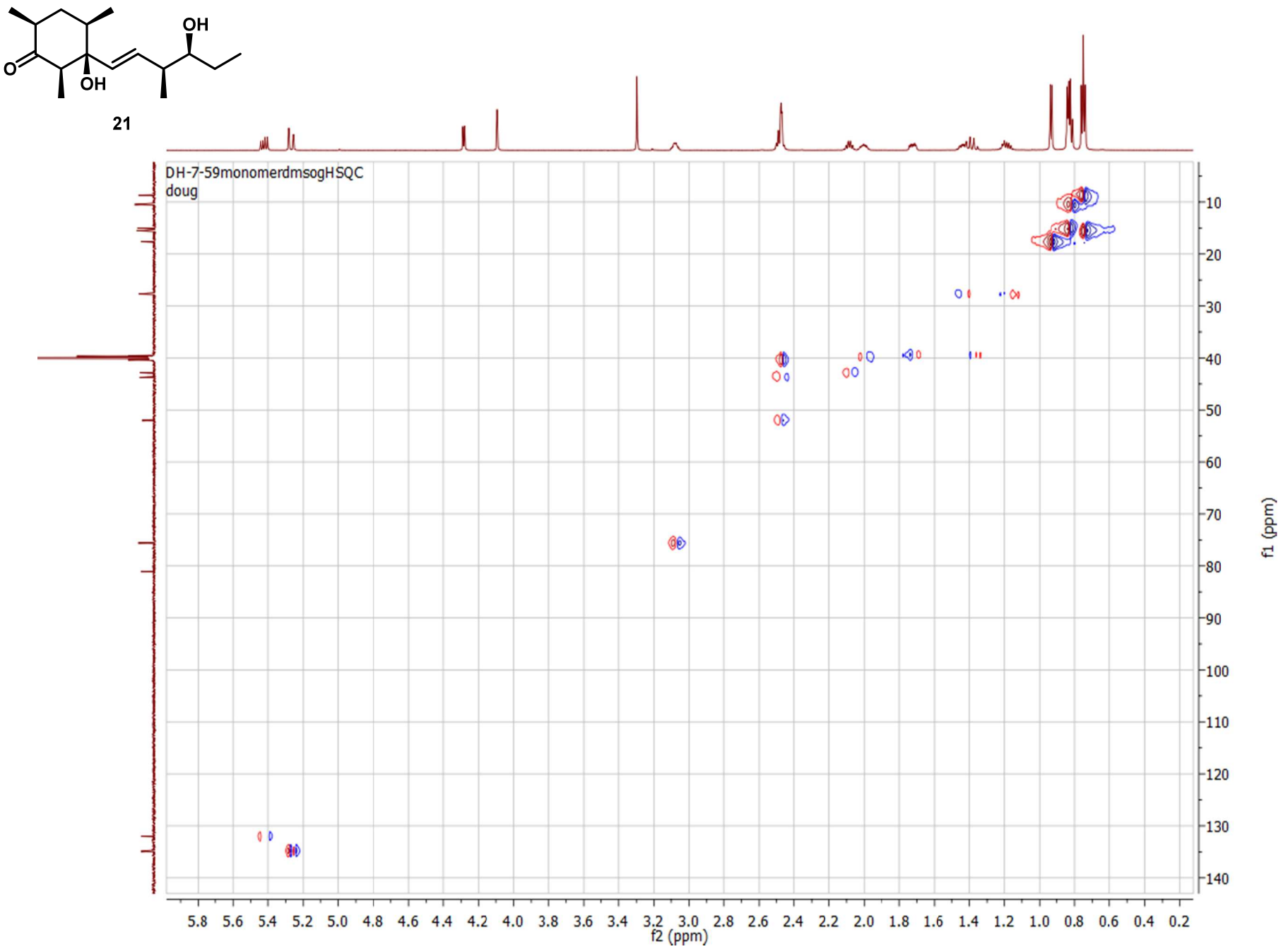

ppm
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

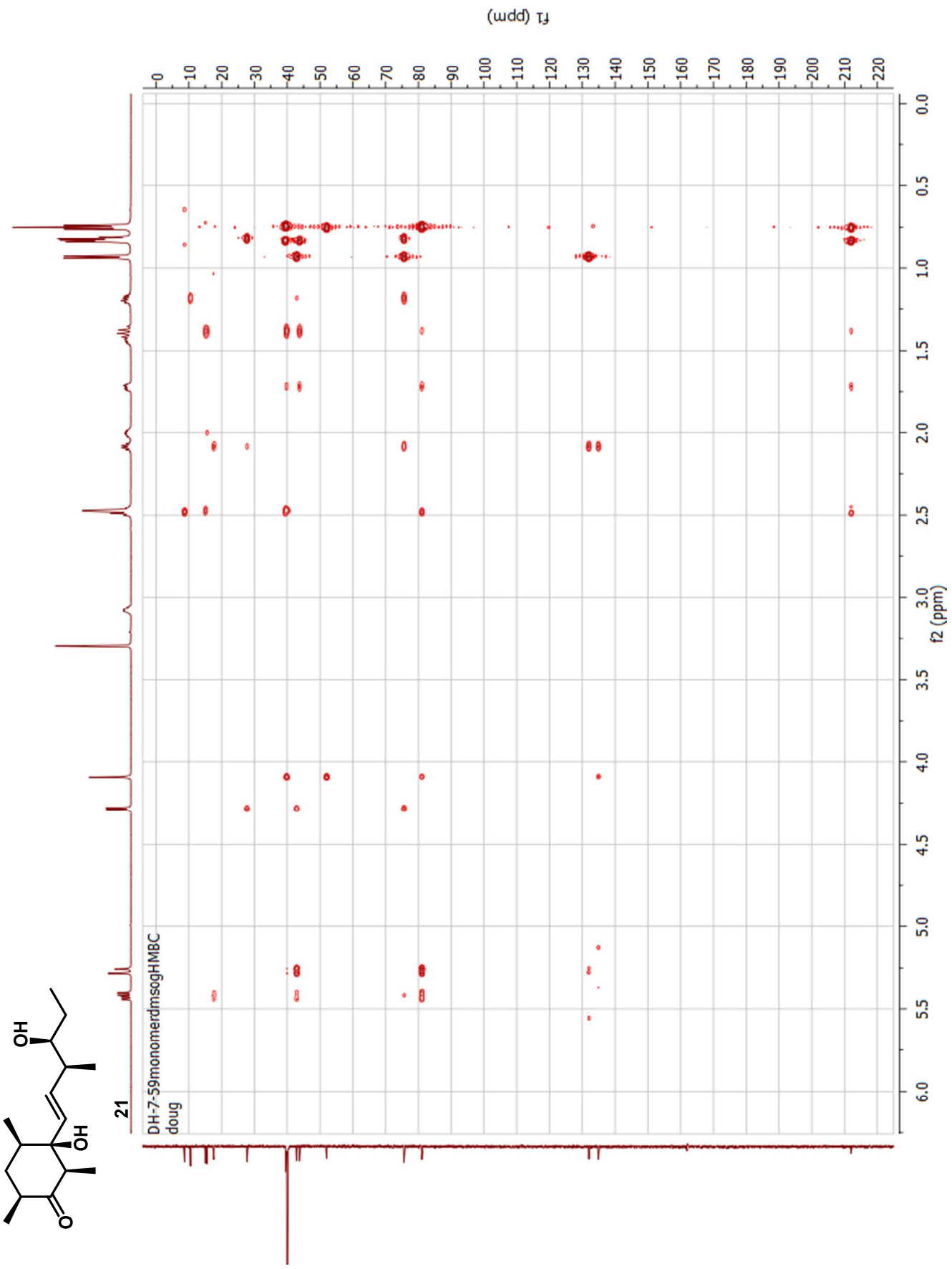

ppm
200
180
160
140
120
100
80
60
40
20
0

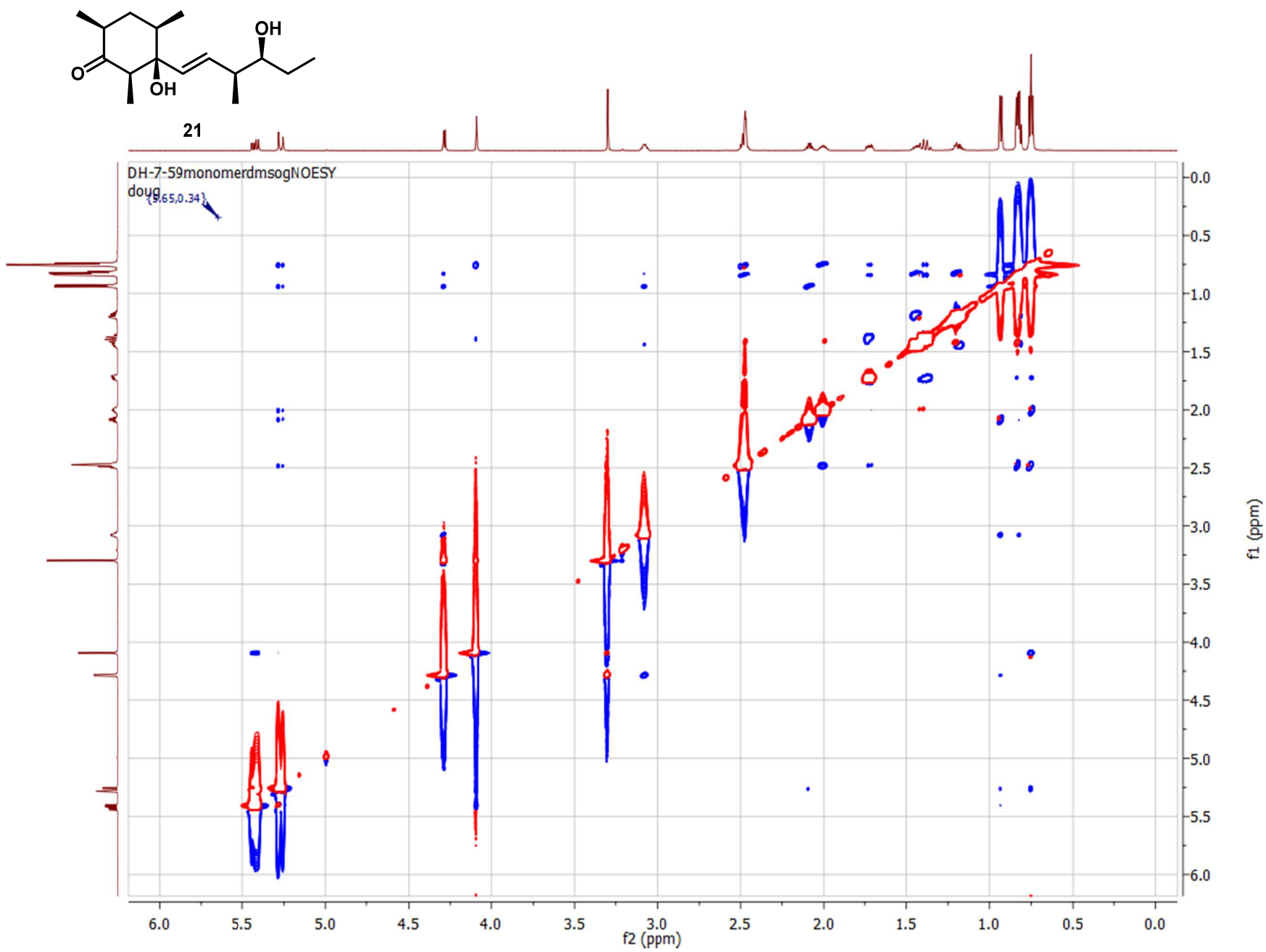

DH-7-59monomerdmsoproton
21
A (dd) 5.46
B (d) 5.30
C (d) 4.32
D (s) 4.13
E (tdd) 3.11
F (m) 2.53
G (h) 2.12
H (dqd) 2.04
I (ddd) 1.76
J (m) 1.44
K (m) 1.22
L (d) 0.97
M (m) 0.86
N (t) 0.78
f1 (ppm)


DH-7-59monomerdmsoCARBON
doug
211.56
134.48
131.58
80.62
75.17
51.59
43.31
42.41
40.06
39.94 dmso
39.80 dmso
39.66 dmso
39.58
39.52 dmso
39.38 dmso
39.34
39.24 dmso
39.10 dmso
38.94
27.27
17.18
15.07
14.63
10.05
8.25
OH
OH
O
21
f1 (ppm)

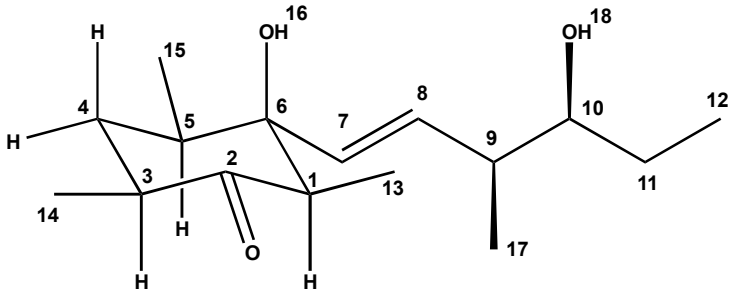

DH-7-59monomerdmsogCOSY
doug
OH
O
OH
21
f2 (ppm)
f1 (ppm)


OH
O
OH
21
DH-7-59monomerdmsogHSQC
doug
f2 (ppm)
f1 (ppm)

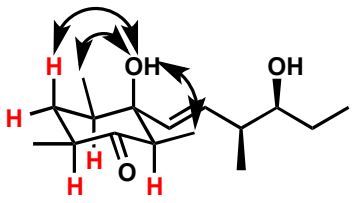

OH
OH
O
21
DH-7-59monomerdmsogHMBC
doug
f1 (ppm)
f2 (ppm)


OH
O
OH
21
DH-7-59monomerdmsogNOESY
doug
{9.65,0.34}
f2 (ppm)
f1 (ppm)

| C/H | H | mult | C | COSY | HMBC | NOE |
|---|---|---|---|---|---|---|
| 1 | 2.53 | ovlp m | 51.6 | 13 | 2,6,13,16 | 3,5,7,13 |
| 2 | | | 211.6 | | | |
| 3 | 2.53 | ovlp m | 43.3 | 4β,14 | 2,4,6,14 | 1,4α,5,14 |
| 4α | 1.76 | ddd | 38.9 | 3,4β,5 | 2,3,5,6 | 3,4β,5,14,15 |
| 4β | 1.42 | ovlp m | 38.9 | 3,4α,5 | 3,5,6,15 | 4α,14,15,16 |
| 5 | 2.04 | m | 39.3 | 4α,4β,15 | 4,15 | 1,3,4α,7,15 |
| 6 | | | 80.6 | | | |
| 7 | 5.3 | d | 134.5 | 8 | 1,5,6,9 | 1,5,9,13,15,16,17 |
| 8 | 5.46 | dd | 131.6 | 7, 9 | 6,10,17 | 9,10,13,15,16,17 |
| 9 | 2.12 | h | 41.4 | 8, 10,17 | 7,8,11 | 7,10,17 |
| 10 | 3.11 | m | 75.2 | 9,11α,11β,18 | 8,9,12,17 | 8,11α,11β,12,17 |
| 11α | 1.47 | m | 27.3 | 10,11β,12 | 9,10,12 | 10,11β,12 |
| 11β | 1.22 | m | 27.3 | 10,11α,12 | 9,10,12 | 11α,12,17 |
| 12 | 0.86 | t | 10 | 11 | 10,11 | 10,11α,11β,18 |
| 13 | 0.78 | ovlp m | 8.2 | 1 | 1,2 | 1,7,8,16 |
| 14 | 0.86 | ovlp m | 14.6 | 3 | 3,4 | 1,3,4α,4β |
| 15 | 0.78 | ovlp m | 15.1 | 5 | 5 | 4α,4β,5,7,8,16 |
| 16 | 4.13 | s | | | 1,5,6,7 | 4β,7,13,15 |
| 17 | 0.97 | d | 17.2 | 9 | 9 | 7,8,9,10,11α,11β,18 |
| 18 | 4.32 | d | | 10 | 9,10,11 | 9,11β,12,17 |

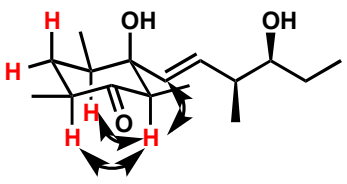



Full NMR data sets were acquired in $CDCl_3$, $CD_3OD$, $D_6$-acetone, and $D_6$-DMSO, with the latter providing superior results. Nevertheless, $^1H$ spectra in $D_6$-DMSO had overlapping signals for protons at positions 1 and 3 (along with residual solvent), and methyls 13 and 15, complicating analysis (pacificanone numbering).




Stereochemistry in question is at C1 and C6. Key nOe correlations are observed between hydroxyl 16, and protons at positions 4β, 8, 13 and 15. These correlations suggest an axial position for hydoxyl 16, as observed in pacificanone A and B, and an equitorial position for methyl 13 as observed in pacificanone B.[13]

Further support for this conformation involves nOe correlations between the proton at position 1 and protons at positions 3, 5, and 7.



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