Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Conformation-Guided Analogue Design Identifies Potential Antimalarial Compounds through Inhibition of Mitochondrial Respiration

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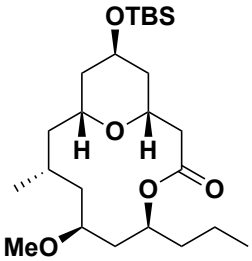
Supporting Information

General Experimental Information

Unless otherwise noted, all materials were used as received from a commercial supplier without further purification. All anhydrous reactions were performed using oven-dried or flame-dried glassware, cooled under vacuum and purged with argon gas. Dichloromethane (DCM), tetrahydrofuran (THF), toluene and diethyl ether (Et_2O) were filtered through activated alumina under nitrogen. Triethylamine (Et_3N) was distilled over CaH_2 and stored over KOH pellets. 4Å molecular sieves were oven-dried overnight and cooled under high vacuum prior to use. All reactions were monitored by Silicycle thin layer chromatography (TLC) plates (Extra Hard Layer, 60Å, glass back) and analyzed with 254 nm UV light and/or anisaldehyde treatment. Silica gel for column chromatography was purchased from Silicycle (SiliaFlash® P60, 230 – 400 mesh). Unless otherwise noted, all ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ using either a Varian Inova 500 spectrometer operating at 499.86 MHz for ^1H and 125.69 MHz for ^{13}C, or Varian VNMRS 600 operating 599.87 MHz for ^1H and 150.84 MHz for ^{13}C. Chemical shifts (δ) were reported in ppm relative to the residual CHCl3 as an internal reference (^1H: 7.26 ppm, ^{13}C: 77.23 ppm). Coupling constants (*J*) were reported in Hertz (Hz). Peak multiplicity is indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), x (septet), h (heptet), b (broad) and m (multiplet). Mass spectra (FAB) were obtained at the Department of Chemistry and Biochemistry, University of Notre Dame using either a JEOL AX505HA or JEOL JMS-GCmate mass spectrometer. LCMS analysis was performed on a Waters 2695 Separations Module integrated 3100 Mass Detector system employing positive ion ESI mode, equipped with a Waters 996 Photodiode Array Detector, using a Waters XBridge BEH C18 2.5 µm column (3.0 × 30 mm), DAD detected at 254 nm, at 50°C. The operation software is MassLynx Software License V4.1 (Waters, Milford, MA, USA). Semiprep-HPLC separation was performed on a Waters 1525 Binary pump equipped with a Waters 2998 Photodiode Array Detector, Waters 2707 Autosampler, and Water Fraction Collector III utilizing Empower 3 Chromatography Manager software (Waters, Milford, MA, USA).

Experimental Methods

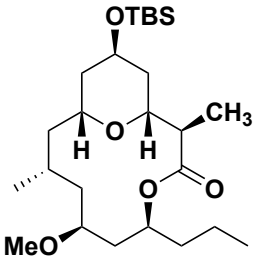
TBS-Protected Macrolide



To a solution of macrolide **3** (40 mg, 0.12 mmol) in CH_2Cl_2 (3 mL) at -78°C was added 2,6-lutidine (28.4 µL, 0.244 mmol) and TBSOTf (33 µL, 0.18 mmol) sequentially. The reaction mixture was stirred for 2 h at -78°C and then quenched with saturated aqueous

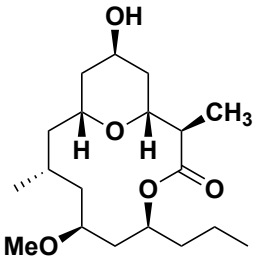
NH_4Cl. After warming to room temperature, the layers were separated and the aqueous layer further extracted with CH_2Cl_2 (4 × 10 mL). The combined organic layers were dried with $MgSO_4$, filtered, and concentrated *in vacuo*. Purification by column chromatography (95:5 Hexanes:EtOAc) gave the TBS-protected macrolide (50 mg, 93% yield) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.13 (td, *J* = 9.4, 4.8 Hz, 1H), 4.17 – 4.09 (m, 2H), 3.69 – 3.61 (m, 1H), 3.61 – 3.53 (m, 1H), 3.31 (s, 3H), 2.55 (dd, *J* = 14.2, 4.6 Hz, 1H), 2.30 (dd, *J* = 14.2, 10.8 Hz, 1H), 1.95 – 1.86 (m, 1H), 1.79 – 1.69 (m, 1H), 1.60 – 1.55 (m, 1H), 1.53 (d, *J* = 2.6 Hz, 1H), 1.52 (d, *J* = 2.2 Hz, 2H), 1.43 – 1.24 (m, 8H), 1.18 (dd, *J* = 14.9, 2.1 Hz, 1H), 1.12 (ddd, *J* = 12.9, 10.7, 2.2 Hz, 1H), 0.97 (d, *J* = 6.8 Hz, 3H), 0.91 (t, *J* = 5.4 Hz, 3H), 0.90 – 0.89 (m, 9H), 0.04 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.57, 75.97, 74.83, 73.52, 69.36, 65.54, 56.45, 44.46, 42.91, 42.54, 40.33, 40.30, 39.55, 37.18, 31.30, 30.57, 26.09, 25.75, 19.25, 18.35, 14.16, -4.59, -4.63. HRMS-FAB: $(M+H)^+$ = 443.3193 calculated for $C_{24}H_{47}O_5Si$, experimental: 443.3191.

TBS-Protected (*R*)-2-Methyl Macrolide



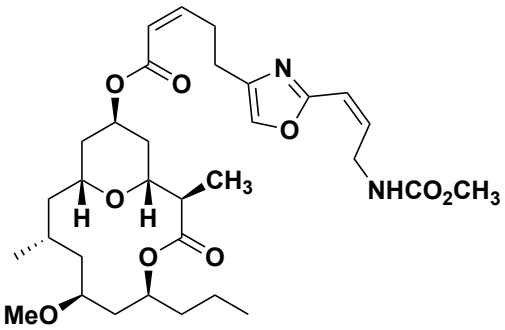
To a solution of TBS-protected macrolide (11.5 mg, 0.0259 mmol) in THF (0.5 mL) at -78°C was added 65 µL of NaHMDS (2.0 M in THF, 0.13 mmol). The solution was stirred at -78°C for 2 h, then MeI (10 µL, 0.16 mmol) was added and the mixture warmed to 0°C. After 1 h of further stirring, full conversion was observed and the reaction was quenched with 1 mL of H_2O. 3 mL each of saturated aqueous $NaHCO_3$ and EtOAc were added and the organic layer was separated. The aqueous layer was further extracted with EtOAc (2 × 2 mL), and the combined organic layers were washed with brine (3 mL), then dried with $MgSO_4$, filtered, and concentrated *in vacuo*. Purification by column chromatography (95:5 Hexanes:EtOAc) gave the methylated macrolide (10 mg, 84% yield) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.98 (s, 1H), 4.20 – 4.12 (m, 1H), 3.69 (ddd, *J* = 11.6, 9.7, 2.0 Hz, 1H), 3.61 (dt, *J* = 9.8, 6.8 Hz, 1H), 3.54 (dddd, *J* = 10.8, 9.4, 2.5, 1.3 Hz, 1H), 3.32 (s, 3H), 2.41 (dq, *J* = 9.8, 7.0 Hz, 1H), 2.09 – 1.98 (m, 1H), 1.89 – 1.78 (m, 1H), 1.73 – 1.65 (m, 1H), 1.60 – 1.47 (m, 3H), 1.41 (dd, *J* = 7.3, 2.8 Hz, 2H), 1.39 – 1.37 (m, 1H), 1.37 – 1.22 (m, 7H), 1.18 – 1.12 (m, 1H), 1.12 – 1.06 (m, 4H), 0.96 (d, *J* = 6.9 Hz, 3H), 0.92 (t, 3H), 0.90 (m, 9H), 0.04 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.02, 75.76, 75.22, 74.42, 65.14, 56.13, 47.91, 44.04, 41.95, 40.12, 39.78, 37.49, 36.71, 30.51, 25.79, 25.17, 19.10, 18.07, 13.89, 13.86, -4.83, -4.84. HRMS-FAB: $(M+Na)^+$ = 479.3169 calculated for $C_{25}H_{48}NaO_5Si$, experimental: 479.3163.

(*R*)-2-Methyl Neopeltolide Macrolide (**4**)



To a solution of macrolide (9.0 mg, 0.020 mmol) in THF (0.7 mL) was added TBAF (1.0 M in THF, 98 µL, 0.098 mmol). After 2 h, additional TBAF (98 µL) was added, and this procedure was repeated to a total reaction time of 6 h. The reaction mixture was quenched with 2 mL saturated aqueous NH_4Cl, then extracted with EtOAc (3 × 3 mL). The organic layers were combined, dried with $MgSO_4$, filtered, and concentrated *in vacuo*. Purification by column chromatography (90:10 to 70:30 Hexanes:EtOAc) gave the macrolide alcohol **3.58** (5.2 mg, 77%) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.06 (s, 1H), 4.29 – 4.23 (m, 1H), 3.76 (ddd, *J* = 11.8, 10.0, 2.1 Hz, 1H), 3.64 (td, *J* = 9.8, 3.1 Hz, 1H), 3.58 – 3.50 (m, 1H), 3.32 (s, 3H), 2.45 (dq, *J* = 9.9, 7.0 Hz, 1H), 2.01 – 1.91 (m, 1H), 1.84 – 1.71 (m, 2H), 1.61 – 1.47 (m, 5H), 1.42 (m, 3H), 1.38 – 1.28 (m, 3H), 1.22 – 1.17 (m, 1H), 1.15 – 1.04 (m, 4H), 0.97 (d, *J* = 6.8 Hz, 3H), 0.91 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.95, 75.94, 75.18, 74.81, 73.44, 65.04, 56.35, 48.26, 44.24, 42.23, 40.17, 39.71, 37.04, 36.75, 31.11, 29.91, 25.54, 19.23, 14.10. HRMS-FAB: $(M+Na)^+$ = 365.2304 calculated for $C_{19}H_{34}NaO_5$, experimental: 365.2295. $[\alpha]^{20}_D$ = +20.5° (*c* = 0.50 in $CHCl_3$).

(*R*)-2-Methyl Neopeltolide (**6**)

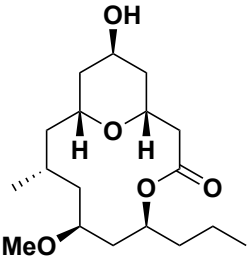


To a solution of **4** (6.0 mg, 18 µmol) and bis(2,2,2-trifluoroethyl)phosphonoacetic acid (11 mg, 36 µmol) in CH_2Cl_2 (2.5 mL) was added HOBt·H_2O (1.0 mg, 7.0 µmol) and EDCI·HCl (33.6 mg, 180 µmol). After 20 min, the mixture was filtered through a short plug of silica gel, eluting with 50 mL EtOAc. The eluant was concentrated and the residue was used immediately without further purification.

To a cooled (-78°C) solution of 18-crown-6 ether (24 mg, 90 µmol) and the unpurified phosphonate in THF (0.5 mL) was added potassium bis(trimethylsilyl)amide (0.5M in toluene, 45 µL, 22 µmol). After 1 h the reaction mixture was cooled to -85°C and a solution of aldehyde **7** (9.0 mg, 36 µmol) in THF (0.5 mL) was added dropwise by cannula. The reaction mixture was stirred for 3 h at -85°C and then quenched by the addition of saturated

aqueous NH_4Cl (5 mL). The aqueous layer was extracted with Et_2O (4 x 5 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Preparative TLC (50:50 Hexanes:EtOAc) afforded 6.0 mg (57% yield over 2 steps) of **8**, judged to be a 4:1 mixture of *Z*:*E* isomers by ^{1}H NMR analysis. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 (d, *J* = 1.2 Hz, 1H), 6.40 – 6.22 (m, 2H), 6.17 – 6.03 (m, 1H), 5.88 (dt, *J* = 11.5, 1.7 Hz, 1H), 5.27 – 5.16 (m, 1H), 4.97 (s, 1H), 4.32 (t, *J* = 6.5 Hz, 2H), 3.77 – 3.58 (m, 4H), 3.58 – 3.42 (m, 2H), 3.32 (s, 3H), 3.04 (qd, *J* = 7.4, 1.8 Hz, 2H), 2.72 (td, *J* = 7.5, 1.2 Hz, 2H), 2.45 (dq, *J* = 9.7, 7.0 Hz, 1H), 2.02 (t, *J* = 13.0 Hz, 1H), 1.92 (d, *J* = 13.6 Hz, 1H), 1.84 (h, *J* = 8.8 Hz, 1H), 1.70 – 1.63 (m, 1H), 1.55 – 1.47 (m, 3H), 1.46 – 1.29 (m, 7H), 1.29 – 1.14 (m, 5H), 1.14 – 1.04 (m, 4H), 0.97 (t, *J* = 7.3 Hz, 3H), 0.92 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.88, 160.22, 165.62, 157.36, 149.34, 141.38, 136.48, 134.07, 120.93, 116.89, 75.92, 75.31, 67.62, 66.05, 56.35, 48.13, 44.08, 42.13, 39.86, 39.55, 36.92, 36.54, 33.90, 30.67, 29.91, 27.87, 25.91, 25.35, 19.33, 15.48, 14.08, 13.96. HRMS-FAB: $(M+Na)^+$ = 627.3252 calculated for $C_{32}H_{48}N_2NaO_9$, experimental: 627.3282. $[\alpha]^{20}_D$ = +22.0° (*c* = 0.20 in $CHCl_3$).

Conformational Analysis



^{1}H signals of neopeltolide macrolide **3** in $CDCl_3$. Original data is from Kartika *et al*, *Org. Lett*., **2008**, *10*, 5047. Specific coupling constants used for DISCON calculations are found in Figure 4.

Proton	Chemical Shift (ppm) and Splitting	*J* (Hz)
2proR	2.56, dd	14.4, 4.2
2proS	2.32, dd	15.0, 11.4
3	4.2, dddd	11.4, 11.4, 4.8, 2.4
4proR	1.67, m	-
4proS	1.5, m	-
5	4.25, b	-
6proR	1.5, m	-
6proS	1.67, m	-
7	3.69, ddd	11.4, 9.0, 2.4
8proR	1.21, dd	15.6, 1.8
8proS	1.39, ddd	15.0, 9.0, 5.4
9	1.5, m	-
10proR	1.58, ddd	13.2, 11.4, 2.4

10proS	1.12, ddd	13.2, 10.8, 2.4
11	3.6, dddd	10.8, 9.6, 2.4, 1.2
12proR	1.35, m	-
12proS	1.83, ddd	15.0, 10.8, 1.8
13	5.2, dddd	9.6, 9.6, 4.8, 0.6

Conformational Searches

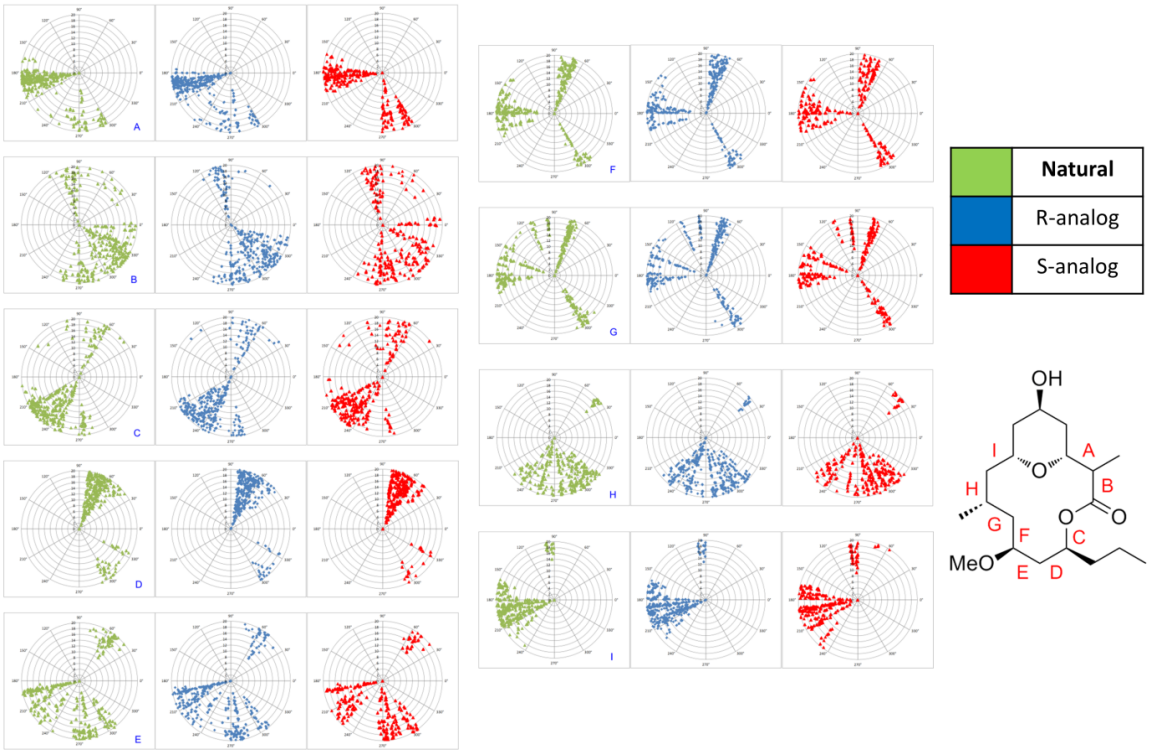
Conformer libraries were generated using the Batchmin package and the MM3 force field as implemented in MacroModel 9.0. Solvation was simulated using the GB/SA continuum model for water. Conformational searches were performed with the MCMM torsional sampling, typically using 50,000 Monte Carlo steps per run. Structures within a 20 kJ/mol window were saved and minimized for complete convergence using the Polak-Ribiere conjugate gradient method (5000 steps, convergence threshold of 0.05).

Polar Maps

Backbone dihedral angles were extracted from the conformers using Maestro 9.2 and converted to 0-360° scale before being plotted onto polar coordinate maps using Microsoft Excel 2007 with the Polar Plot2[1] add-in.

Comparison of C2-methyl substituted analogue conformational searches

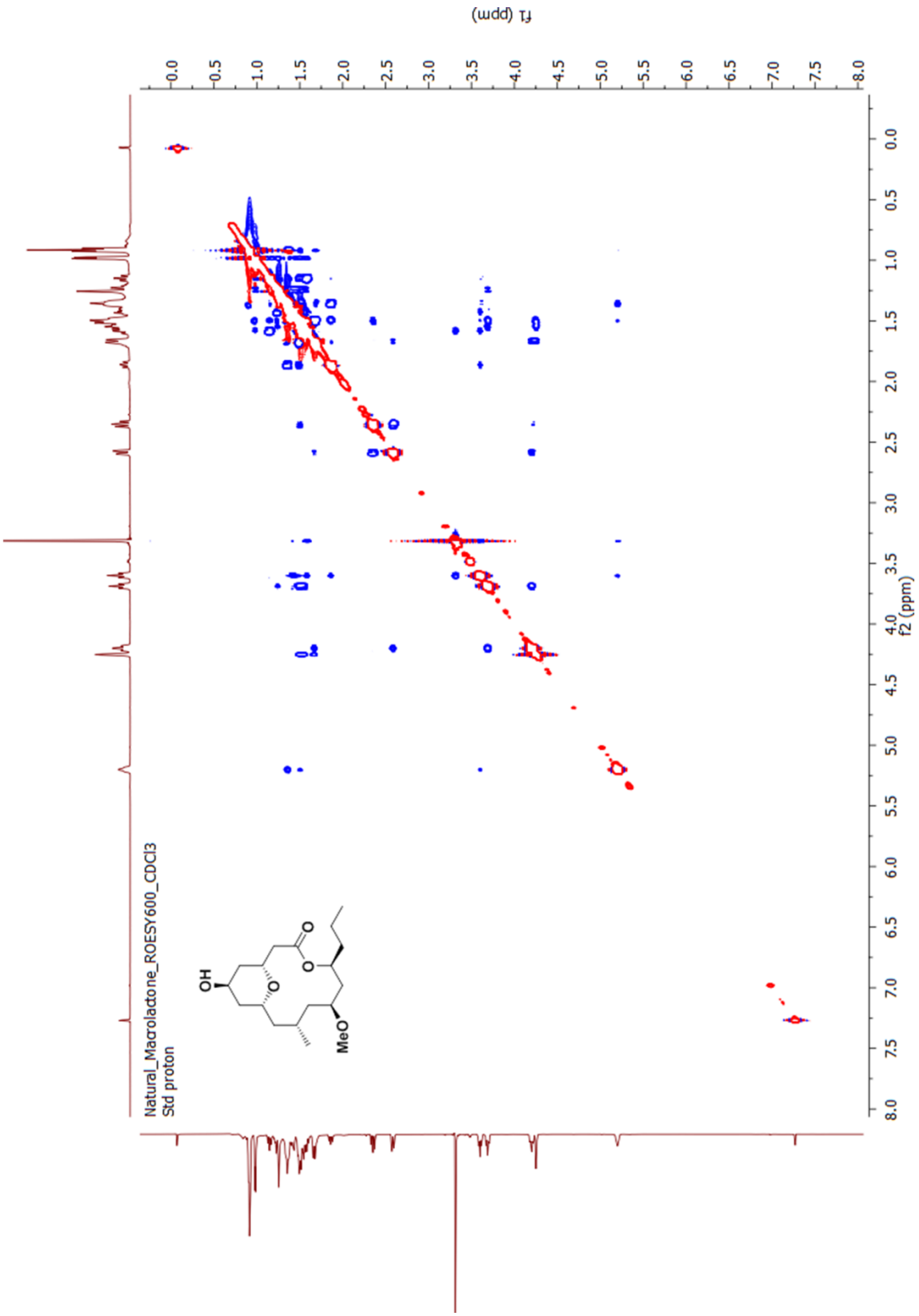
[1] http://www.andypope.info/charts/polarplot3.htm

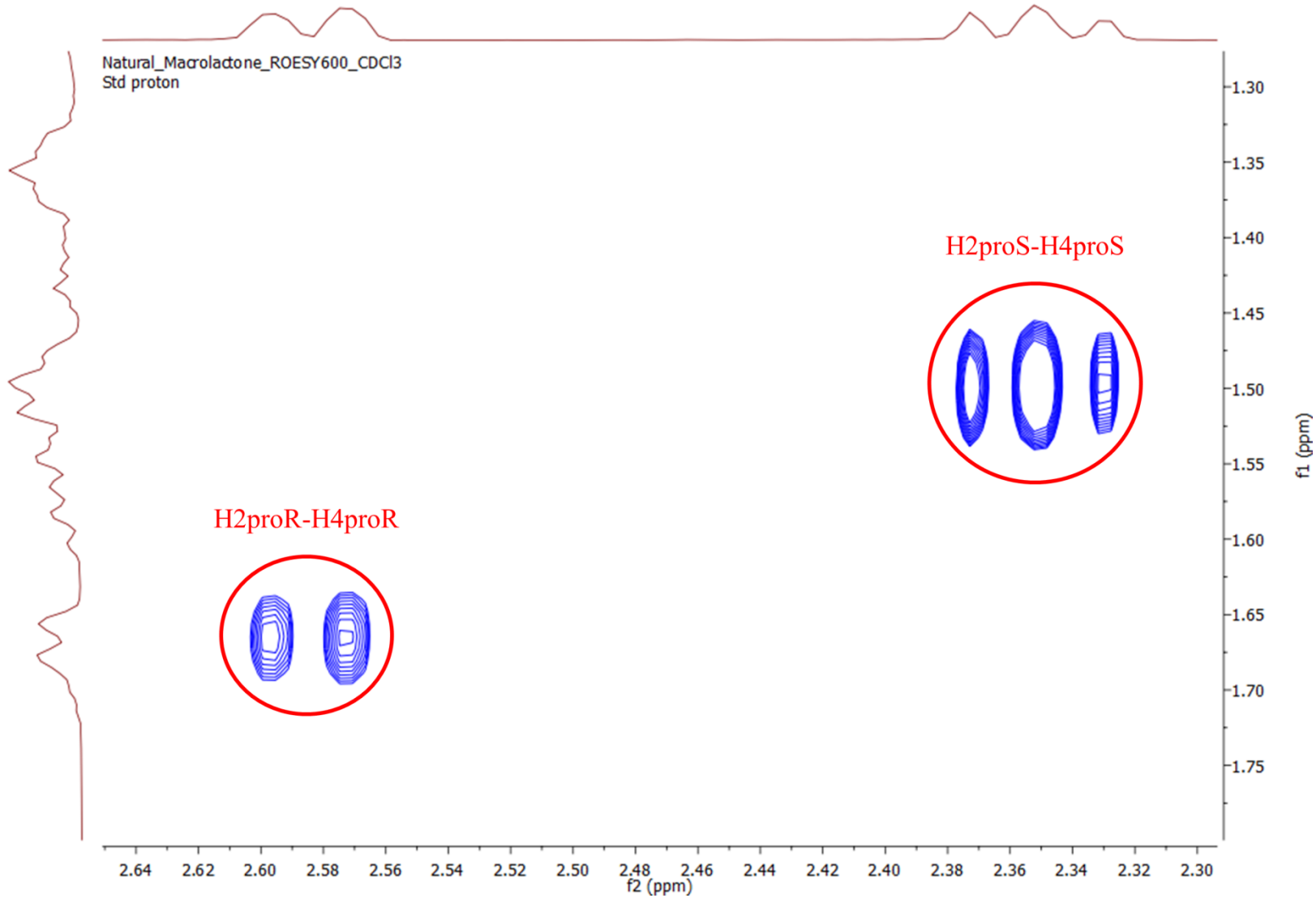


DISCON

DISCON is a Windows/Mac/Linux application developed by the Amos Smith laboratory for calculating the distribution of solution conformations of a flexible organic molecule by using NOE cross-peak volumes, coupling constants, and a pre-generated library of conformers. The program uses a similar approach to the NAMFIS methodology with the additional steps of using NMR variables to initially cluster compounds and the use of a genetic algorithm to assist with deconvolution, which are beneficial in identifying representative structures while avoiding overfitting. Additional information on DISCON, including software binaries, test files and program documentation, may be found at http://discon.sourceforge.net and in selected publications from the Smith lab[2].

[2] Smith, A. B.; Atasoylu, O.; Risatti, C.; Bennett, C. S.; Liu, J.; Cheng, H.; TenDyke, K.; Xu, Q. *J. Am. Chem. Soc*. **2011**, *133*, 14042-14053.


Natural_Macrolactone_ROESY600_CDCl3
Std proton
OH
MeO
O
O
O
f2 (ppm)
f1 (ppm)


Natural_Macrolactone_ROESY600_CDCl3
Std proton
H2proS-H4proS
H2proR-H4proR
1.30
1.35
1.40
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1.50
1.55
1.60
1.65
1.70
1.75
f1 (ppm)
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2.62
2.60
2.58
2.56
2.54
2.52
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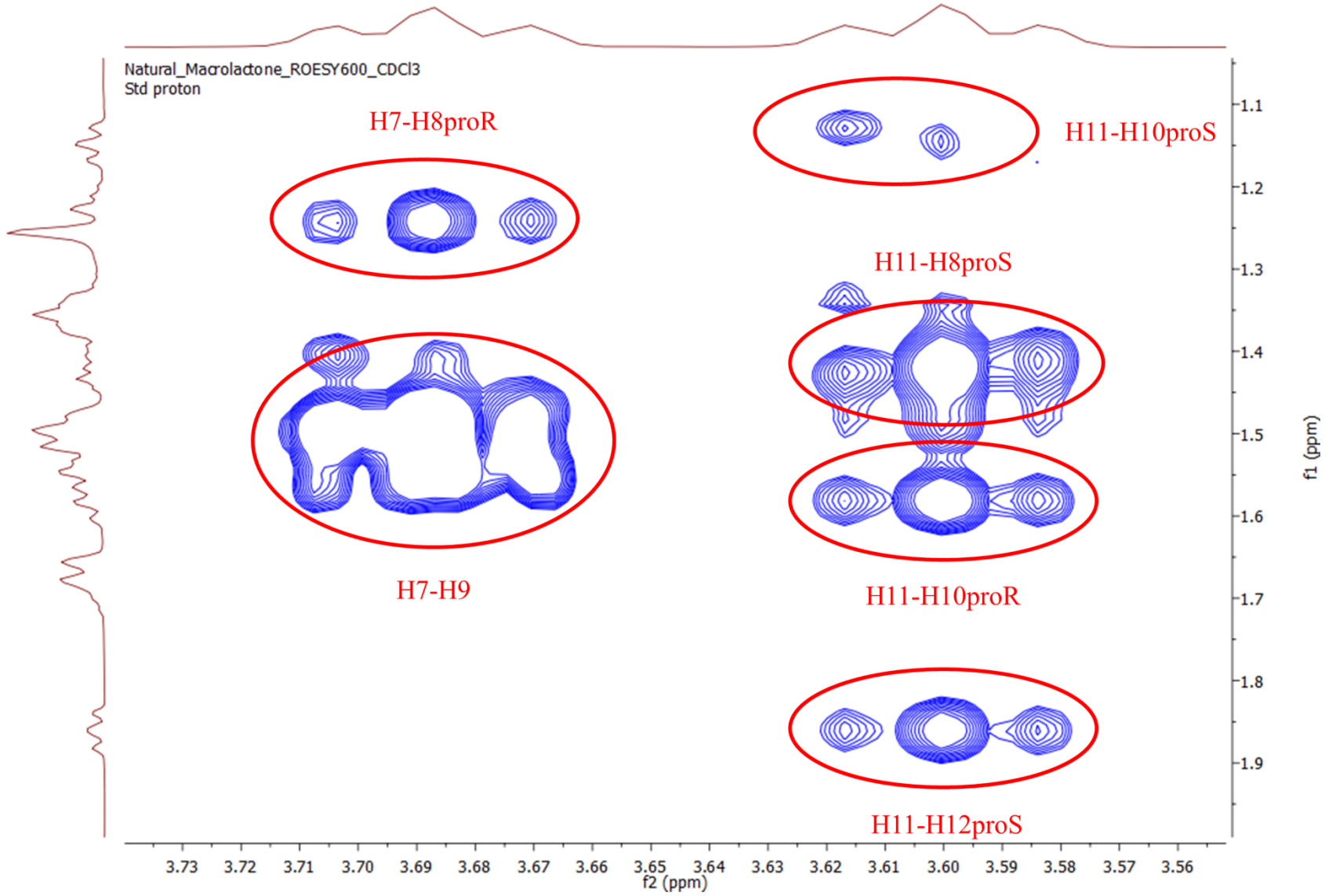
2.32
2.30
f2 (ppm)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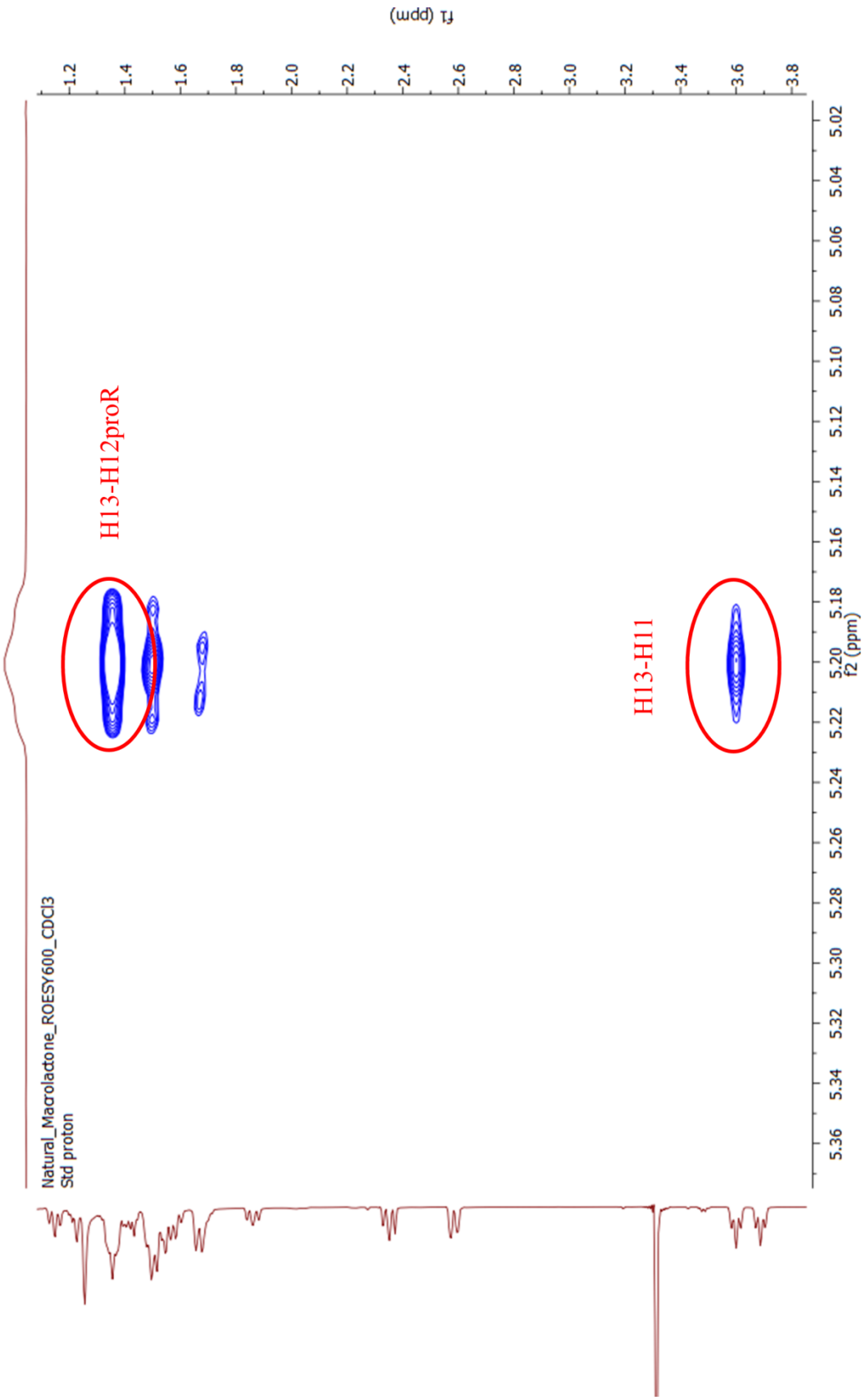
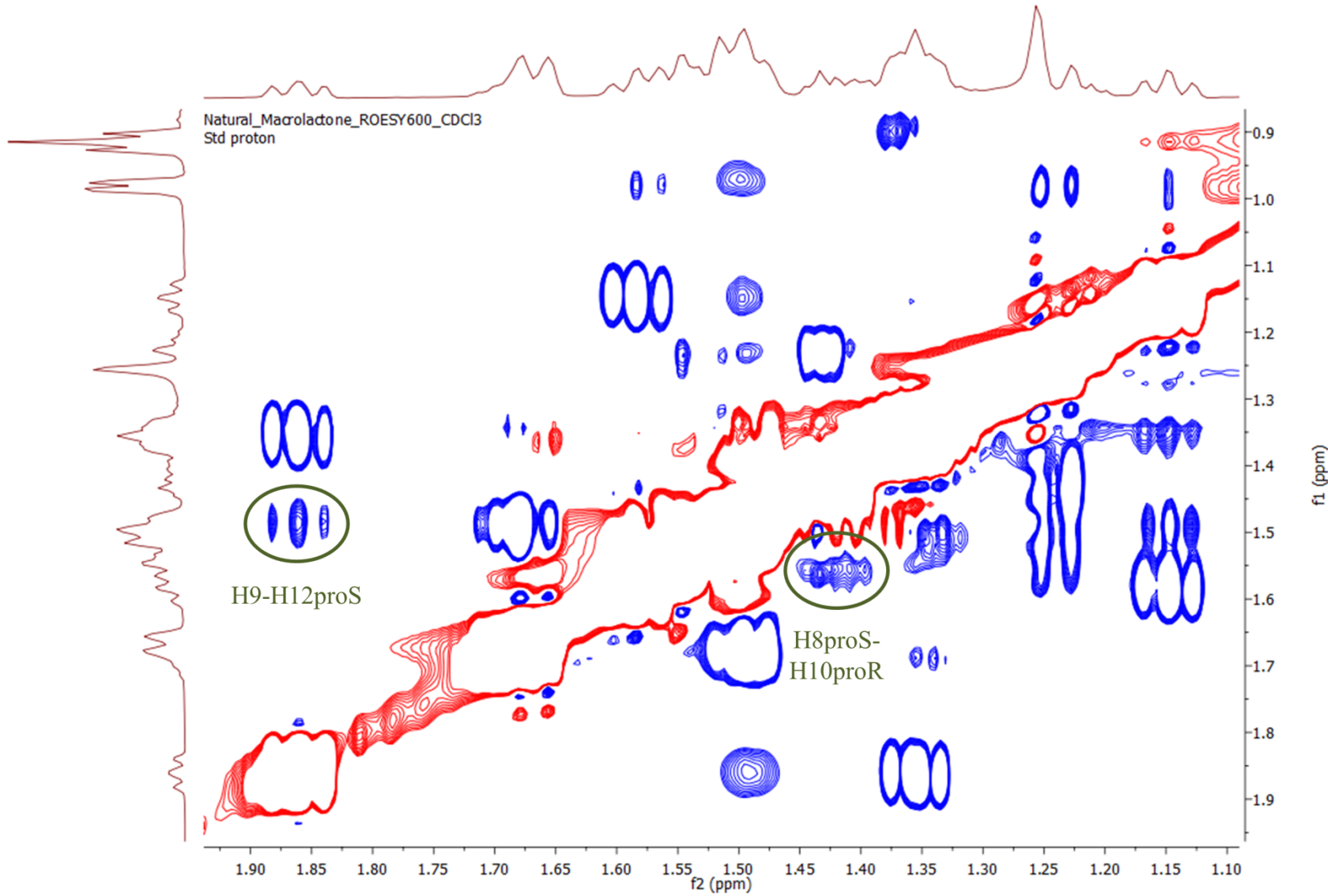
H11-H10proS
H11-H8proS
H7-H9
H11-H10proR
H11-H12proS
3.73 3.72 3.71 3.70 3.69 3.68 3.67 3.66 3.65 3.64 3.63 3.62 3.61 3.60 3.59 3.58 3.57 3.56
f2 (ppm)
1.1 1.2 1.3 1.4 1.5 1.6 1.7 1.8 1.9
f1 (ppm)


H13-H12proR
H13-H11
Natural_Macrolactone_ROESY600_CDCl3
Std proton
f1 (ppm)
f2 (ppm)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Std proton
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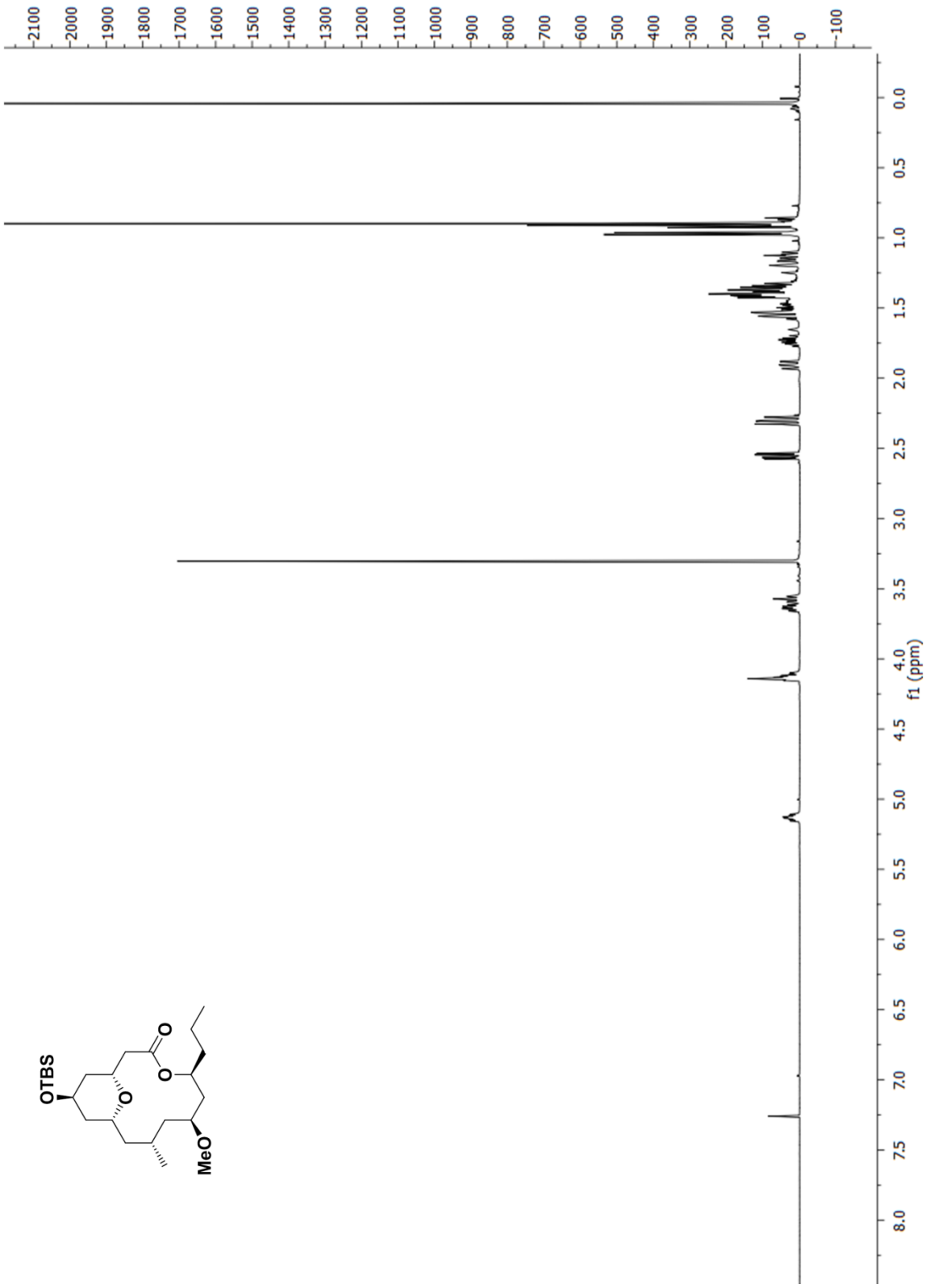
H8proS-
H10proR
f2 (ppm)
f1 (ppm)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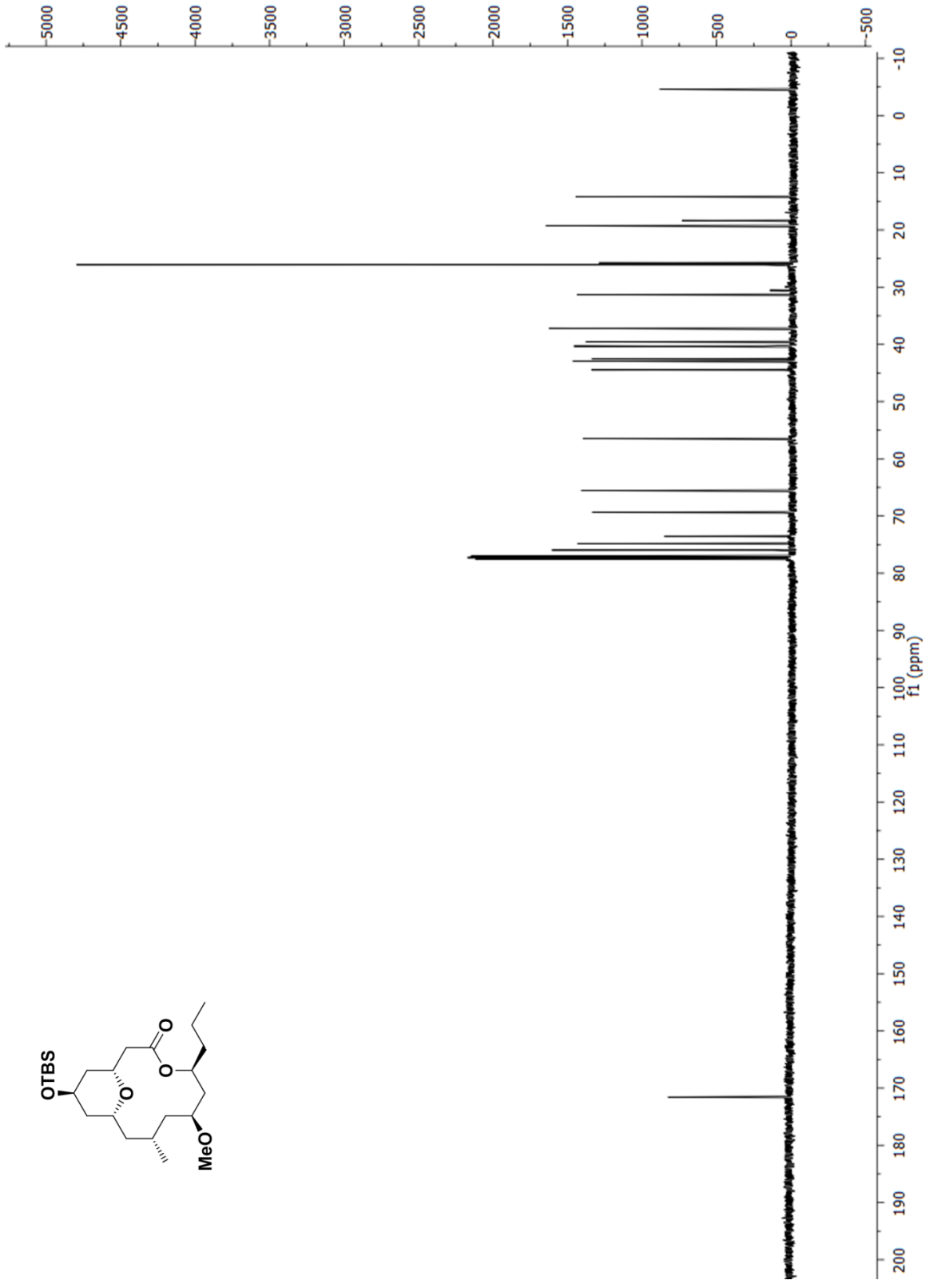
O
O
MeO
f1 (ppm)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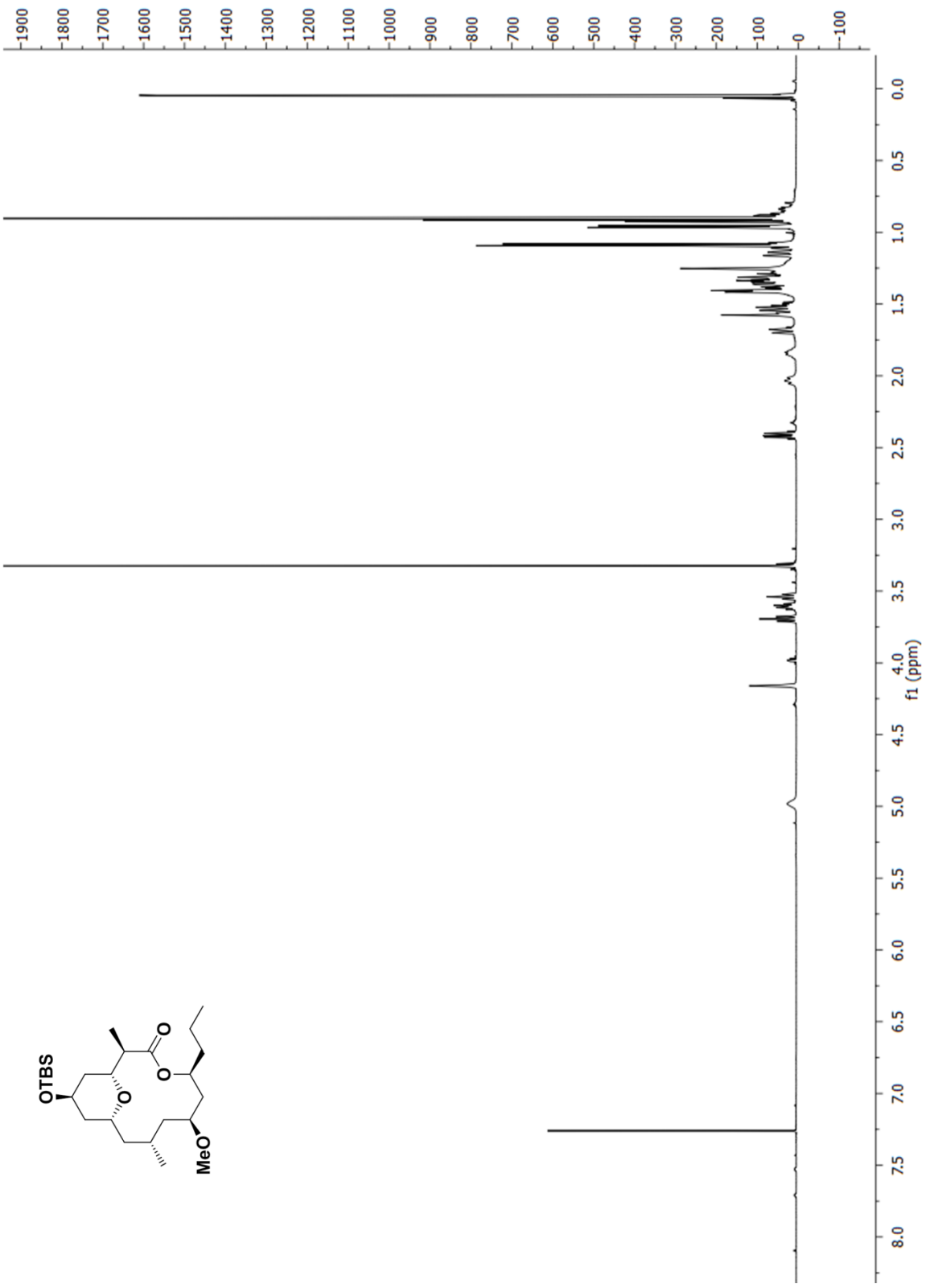
MeO
O
O
O
f1 (ppm)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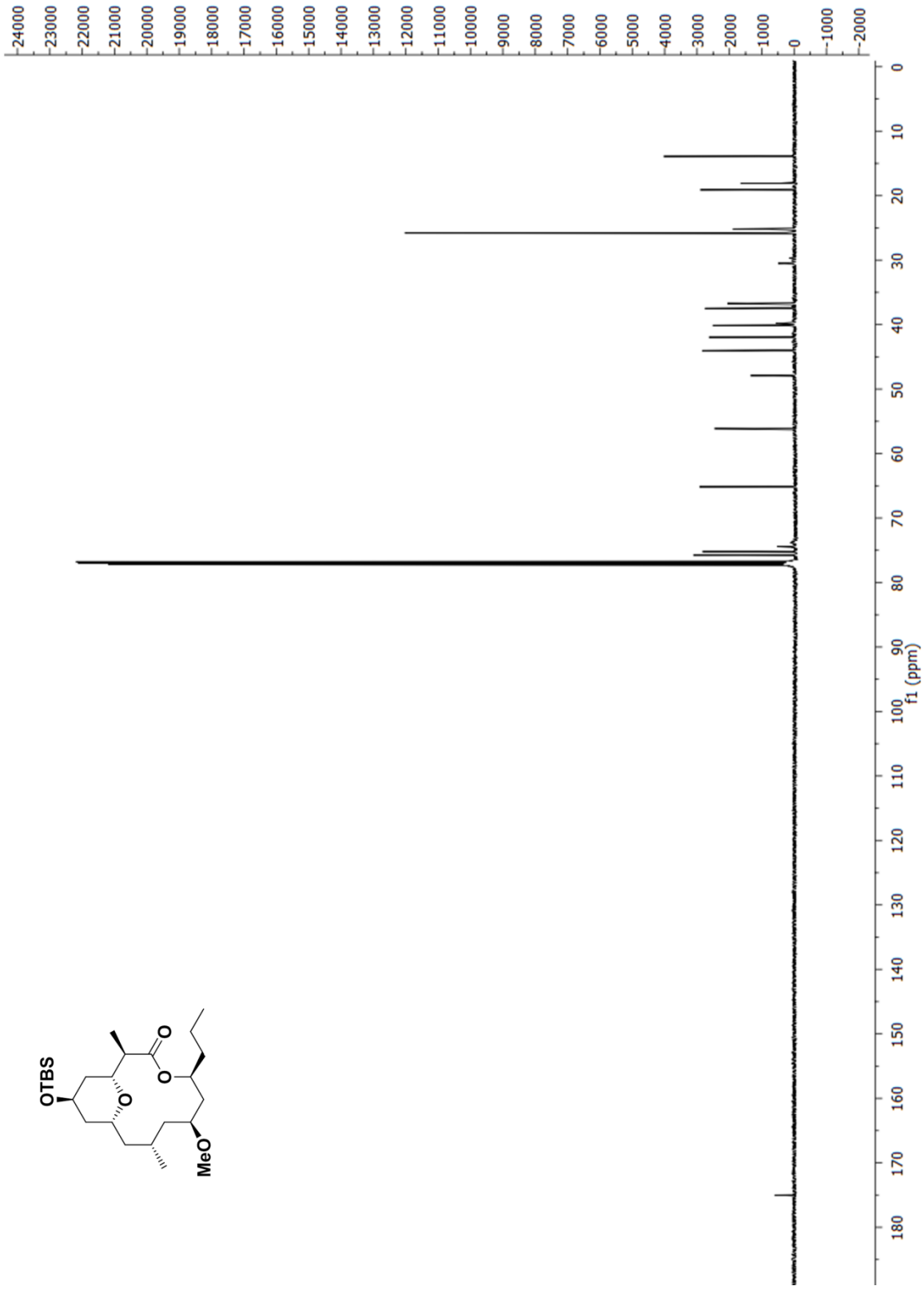
MeO
f1 (ppm)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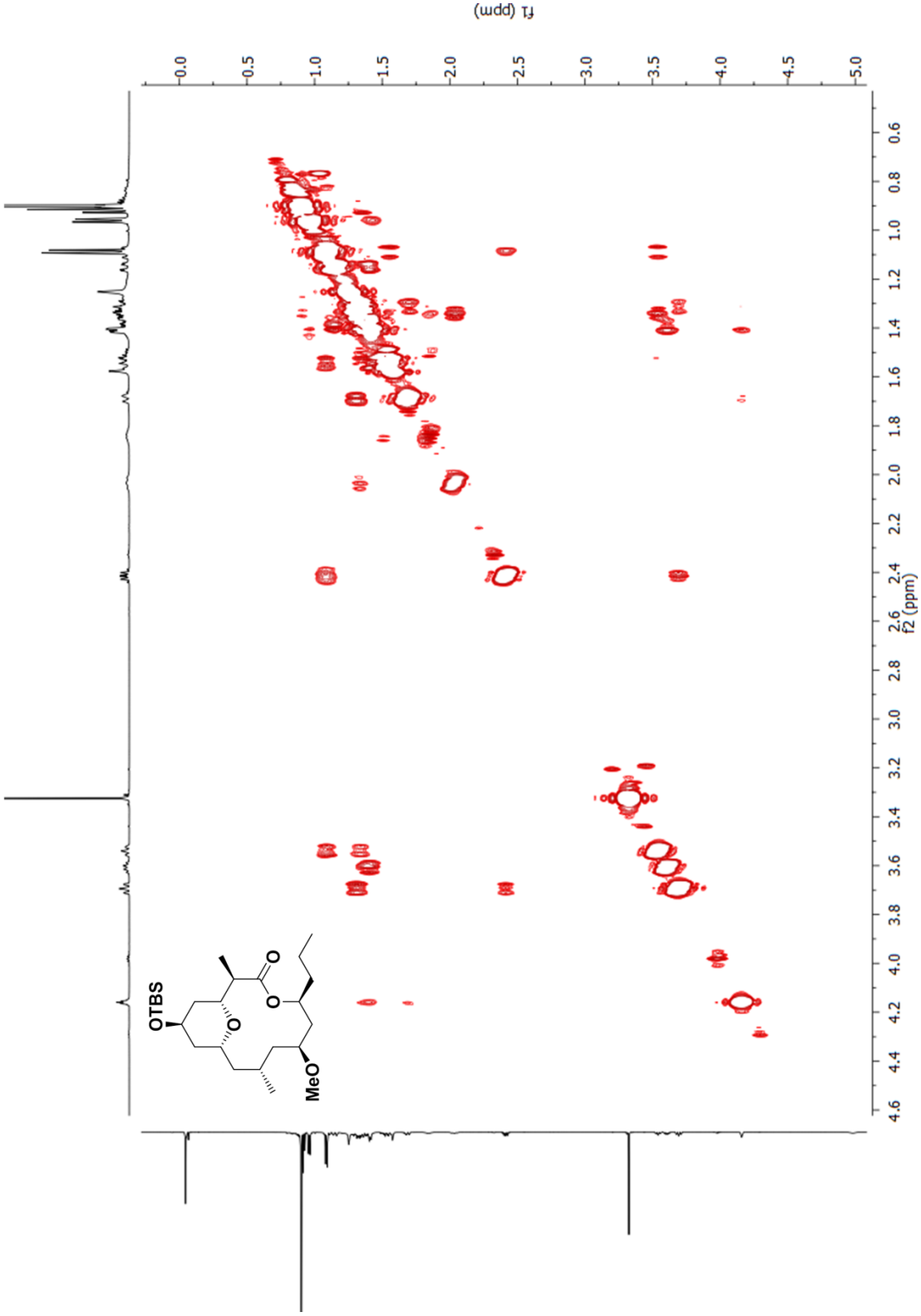
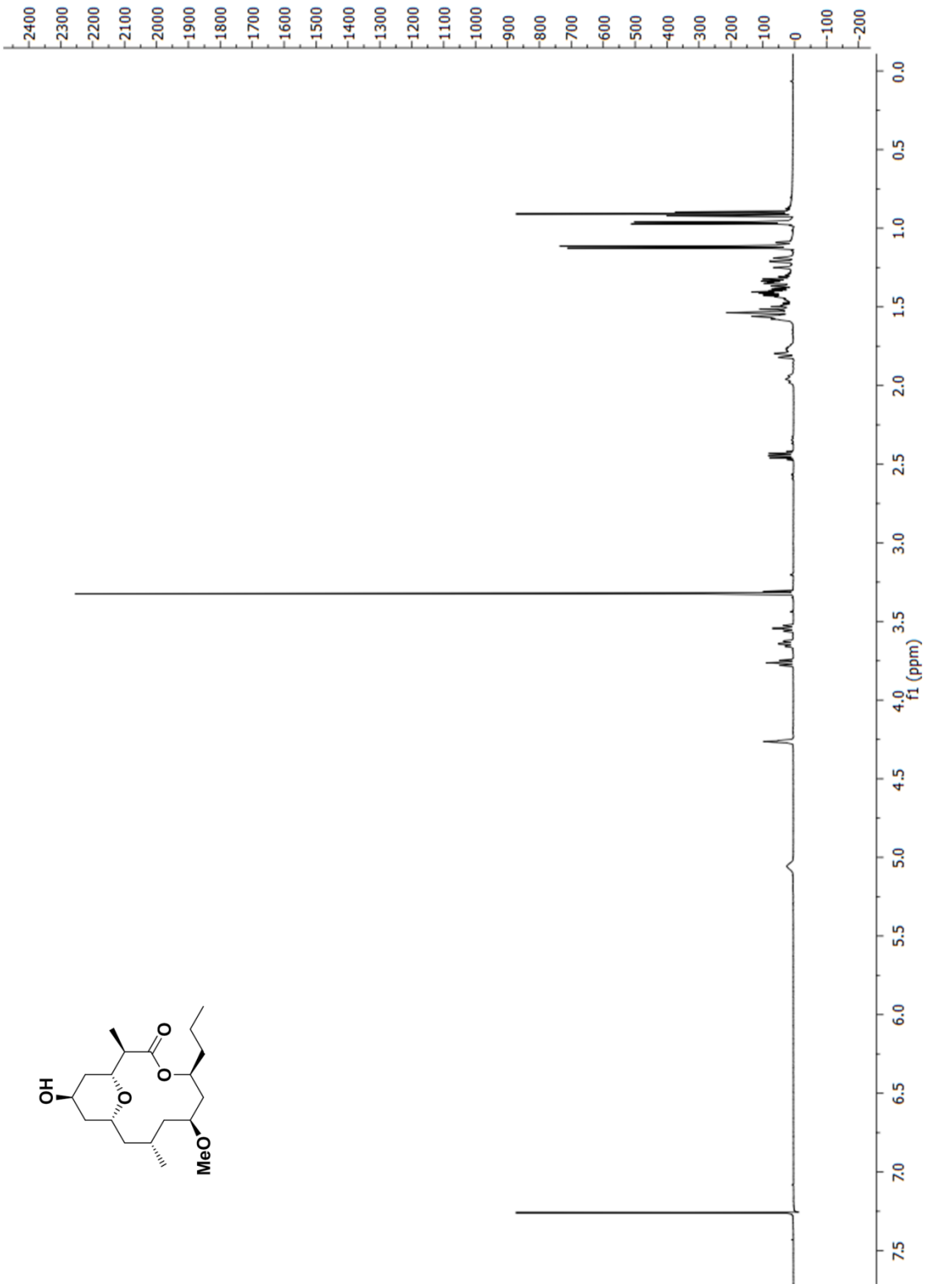
O
O
MeO
f1 (ppm)


f1 (ppm)
f2 (ppm)
OTBS
MeO
O
O
O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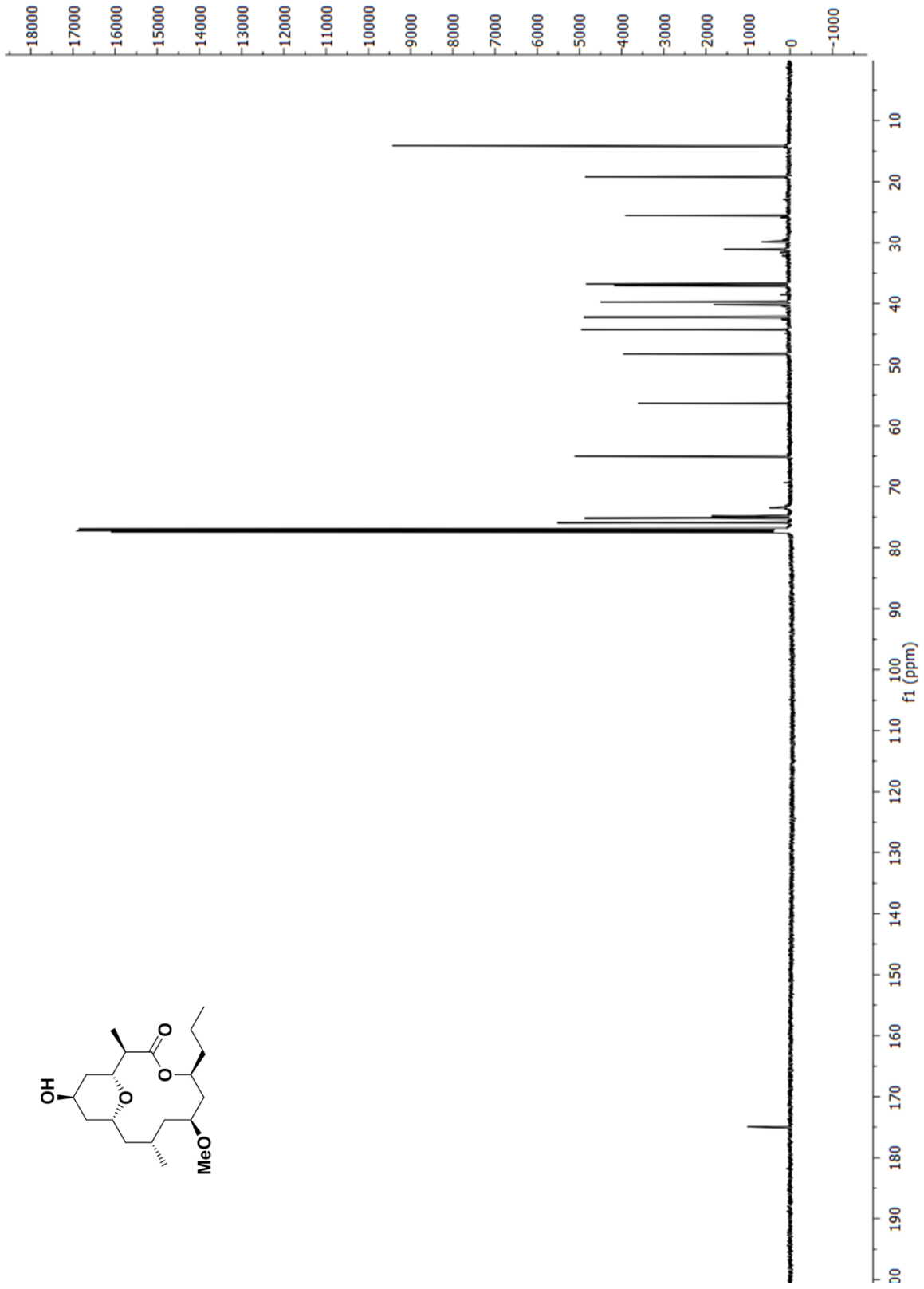
O
O
O
MeO
f1 (ppm)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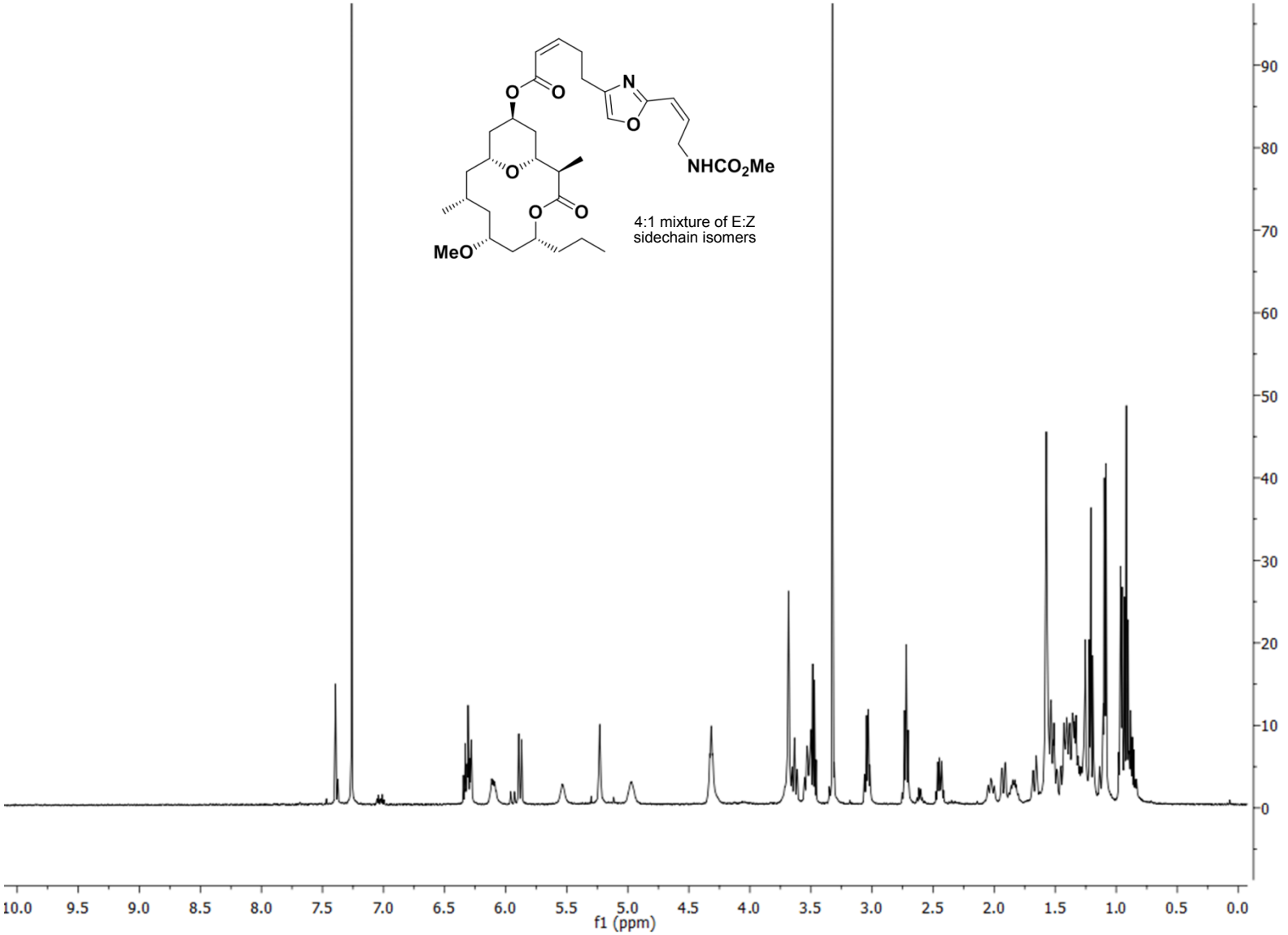
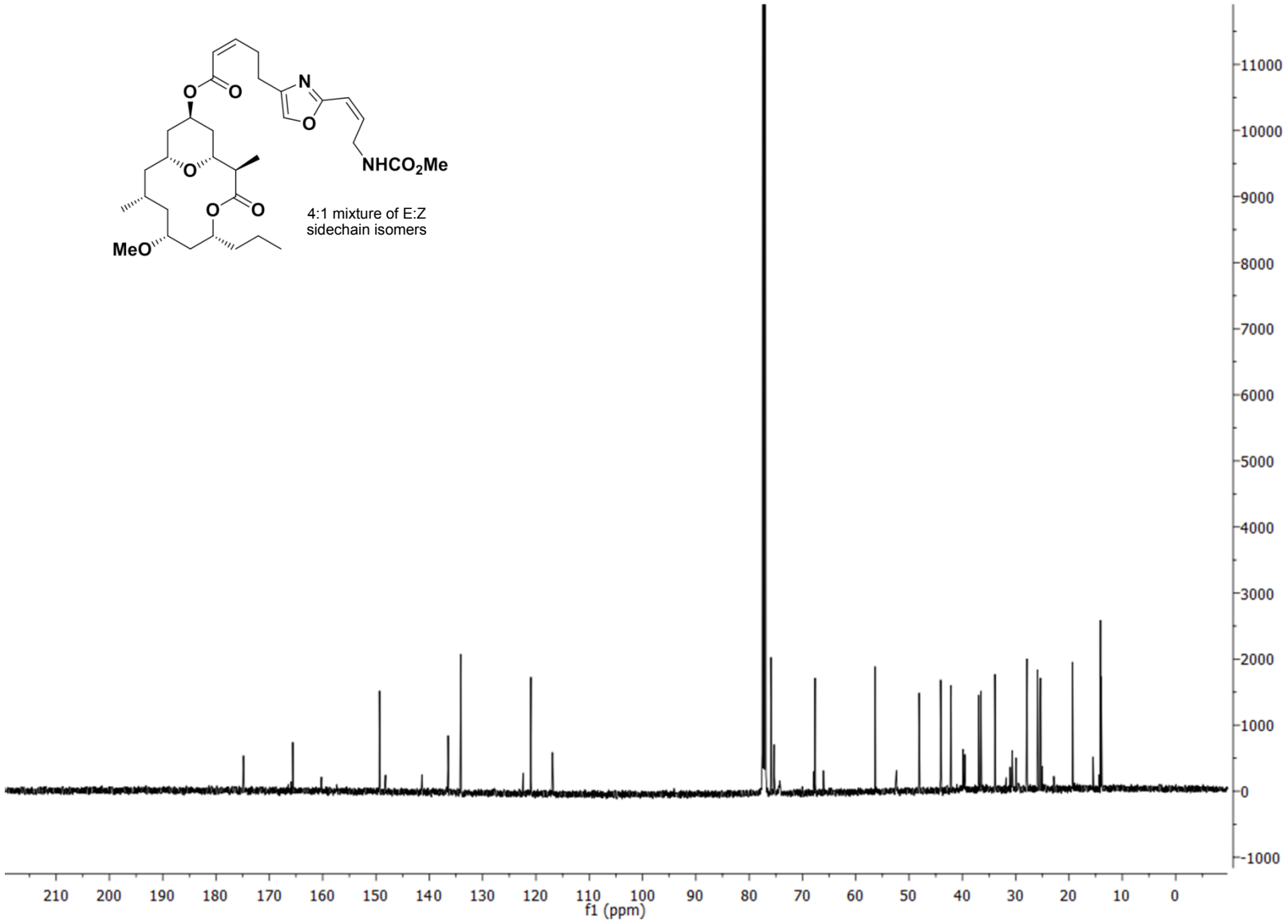
O
O
MeO
f1 (ppm)


NHCO2Me
MeO
4:1 mixture of E:Z
sidechain isomers
f1 (ppm)


O
O
N
O
O
NHCO2Me
O
O
O
MeO
4:1 mixture of E:Z
sidechain isomers
11000
10000
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8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)