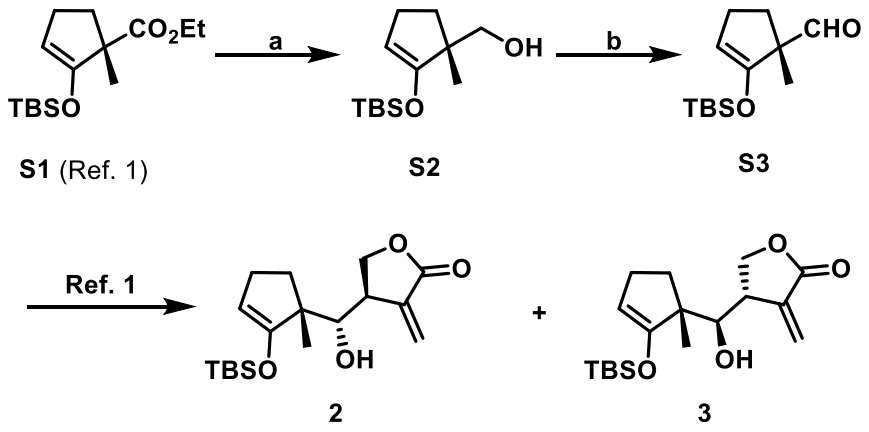
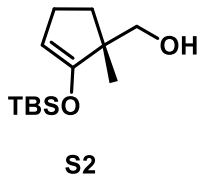
Table of Contents for Supporting Information

I. Synthesis of Intermediates 2 and 3:

Second generation route to **2** and **3**, which have been previously reported by our lab.[1]



Scheme S1. Synthesis of intermediates **2** and **3**. (a) 2 equiv. DIBAL-H, THF, –78 °C, 4 h 96%. (b) 1:1 DMSO:DCM, DCC, H_3PO_4, RT, 16 h, 91%.



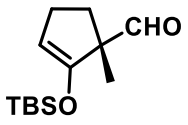
S2

(2-((tert-butyldimethylsilyl)oxy)-1-methylcyclopent-2-en-1-yl)methanol (**S2**)**. S1** (0.75 g, 2.6 mmol) was dissolved in THF (20 mL) and cooled to –78 °C. Then, DIBAL-H (5.2 mL, 5.2 mmol) was added dropwise at the same temperature and allowed to stir for 4 h. The reaction was quenched at –78 °C with H_2O (0.5 mL) and stirred for 5 min. Then, aqueous NaOH (0.5 M, 0.5 mL) was added followed by addition of H_2O (2 mL). The reaction mixture was allowed to warm to RT and then the precipitate was filtered. The resulting solution was concentrated *in vacuo* and SiO_2 purified with EtOAc (0-50%) in hexanes to give **S2** (0.61 g, 96% yield).

^{1}H NMR (500 MHz, $CDCl_3$): δ 4.60 (t, *J* = 2.4 Hz, 1H), 3.49 (dd, *J* = 10.5, 5.7 Hz, 1H), 3.39 (dd, *J* = 10.5, 6.6 Hz, 1H), 2.22 – 2.16 (m, 2H), 1.93 – 1.86 (m, 1H), 1.68 – 1.64 (m, 1H), 1.63 – 1.56 (m, 1H), 1.04 (s, 3H), 0.93 (s, 9H), 0.19 (s, 3H), 0.17 (s, 3H). Impurities: 5.33 (DCM), 1.56 (H_2O).

^{13}C NMR (125 MHz, $CDCl_3$): δ 157.4, 100.9, 69.3, 48.6, 32.2, 25.6 (3C, CH_3), 25.4, 21.0, 18.0, -4.7, -5.2.

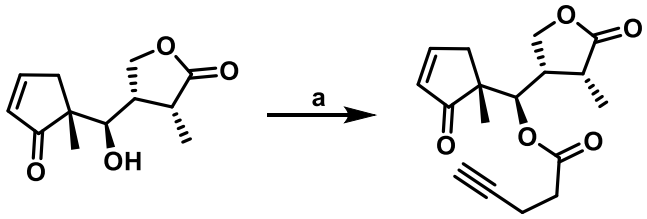
HRMS-ESI$^+$ (m/z): calc'd $[M+H]^+$ for $C_{13}H_{26}O_2Si$ 243.1775 , found 243.1170.



S3

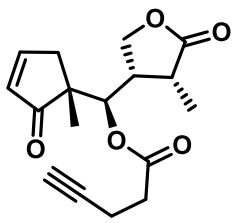
2-((tert-butyldimethylsilyl)oxy)-1-methylcyclopent-2-ene-1-carbaldehyde (**S3**). **S2** (0.43 g, 1.8 mmol) was dissolved in a solution of DCM (3 mL) and DMSO (3 mL). Then, DCC (1.1 g, 5.3 mmol) was added followed by addition of H_3PO_4 (0.1 mL, 0.9 mmol). The reaction mixture was stirred for 16 h at RT. The reaction was quenched with H_2O (20 mL) and extracted with Et_2O (3X, 20 mL). The combined organic layer was washed with H_2O (10 mL), then brine (10 mL), and then concentrated *in vacuo*. The crude product was SiO_2 purified with EtOAc (0-10%) in hexanes to give **S3** (0.40 g, 91% yield). This compound has been characterized previously.[1]

II. Synthesis of S4



17 **S4**

Scheme S2. Esterification of **17**. (a) DCC, 4-DMAP, 4-pentynoic acid, 40 °C, 16 h, 32%.



S4

(R)-((R)-1-methyl-2-oxocyclopent-3-en-1-yl)((3S,4R)-4-methyl-5-oxotetrahydrofuran-3-yl)methyl pent-4-ynoate (**S4**). Compound **17** (15 mg, 0.065 mmol) was dissolved in DCM (3 mL). Then, 4-pentyonic acid (19 mg, 0.194 mmol) and 4-DMAP (24 mg, 0.194 mmol) were added, followed by addition of EDCI (37 mg, 0.194 mmol). The reaction was heated to 40 °C for 16 h. The reaction was quenced with H_2O (5 mL), extracted with DCM (3X, 10 mL), and the organic layer was concentrated *in vacuo*. The crude product was SiO_2 purified with EtOAc (0-60%) in hexanes to give **S4** (6 mg) in 32% yield.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.78 – 7.71 (m, 1H), 6.26 – 6.12 (m, 1H), 5.38 (d, *J* = 10.2 Hz, 1H), 4.02 – 3.88 (m, 2H), 3.03 – 2.92 (m, 1H), 2.86 – 2.76 (m, 1H), 2.71 – 2.45 (m, 5H), 2.46 – 2.31 (m, 1H), 2.00 (t, *J* = 2.5 Hz, 1H), 1.18 (d, *J* = 7.5 Hz, 3H), 1.15 (s, 3H). Impurity: 1.56 (H_2O).

^{13}C NMR (125 MHz, $CDCl_3$): δ 210.1, 178.8, 171.0, 163.1, 132.9, 82.2, 73.9, 69.7, 67.8, 50.3, 42.3, 39.9, 37.4, 33.7, 23.1, 14.7, 10.6. Impurity: 29.9 (grease).

HRMS-ESI$^+$ (m/z): calc'd $[M+H]^+$ for $C_{17}H_{20}O_5$ 305.1384, found 305.1374.

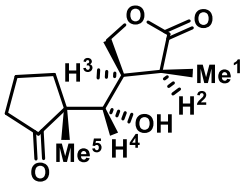
III. NOESY Spectral Data for Determination of Stereochemistry

The stereochemistry assignments for newly synthesized compounds were made using NOESY with a Bruker Avance 500 MHz NMR. All samples were run at RT. All protons were unambiguously assigned prior to assigning stereochemistry based on NOE correlations.

NOESY spectral data is below. Diagnostic NOE signals surrounded by a red box indicate the shown NOE correlation(s) on the compound structure. All NOE signals are normalized to the respective excitation signal to obtain percent NOE.

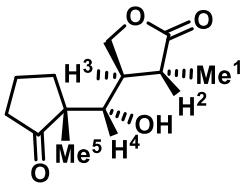
All protons found to have diagnostic NOE correlations were predicted to be within a distance of 4 Å based on computationally minimized structures. All ab initio minimizations were completed using Jaguar (version 8.0) within Maestro (Schrödinger, Inc., version 10.2.010). All ab initio equilibrium conformer minimizations were calculated using the B3LYP method in the gas phase (maximum iterations set to 48).

NOESY Spectral Data for Compounds **9**-**10**, **11**, **15**-**16**:



9

Proton Correlation	% NOE
H^1, H^4	3
H^1, H^2	1
H^2, H^3	2
H^4, H^5	1



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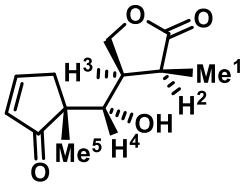
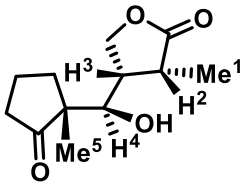
Proton Correlation	% NOE
H^2, H^4	1
H^1, H^2	2
H^1, H^3	1
H^4, H^5	2



11

Proton Correlation	% NOE
H^1, H^4	2
H^1, H^2	1
H^2, H^3	N/A*
H^4, H^5	1

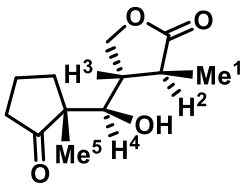
*Significant overlap in signals



15

Proton Correlation	% NOE
H^1, H^4	3
H^1, H^2	2
H^2, H^3	N/A*

*Significant overlap in signals



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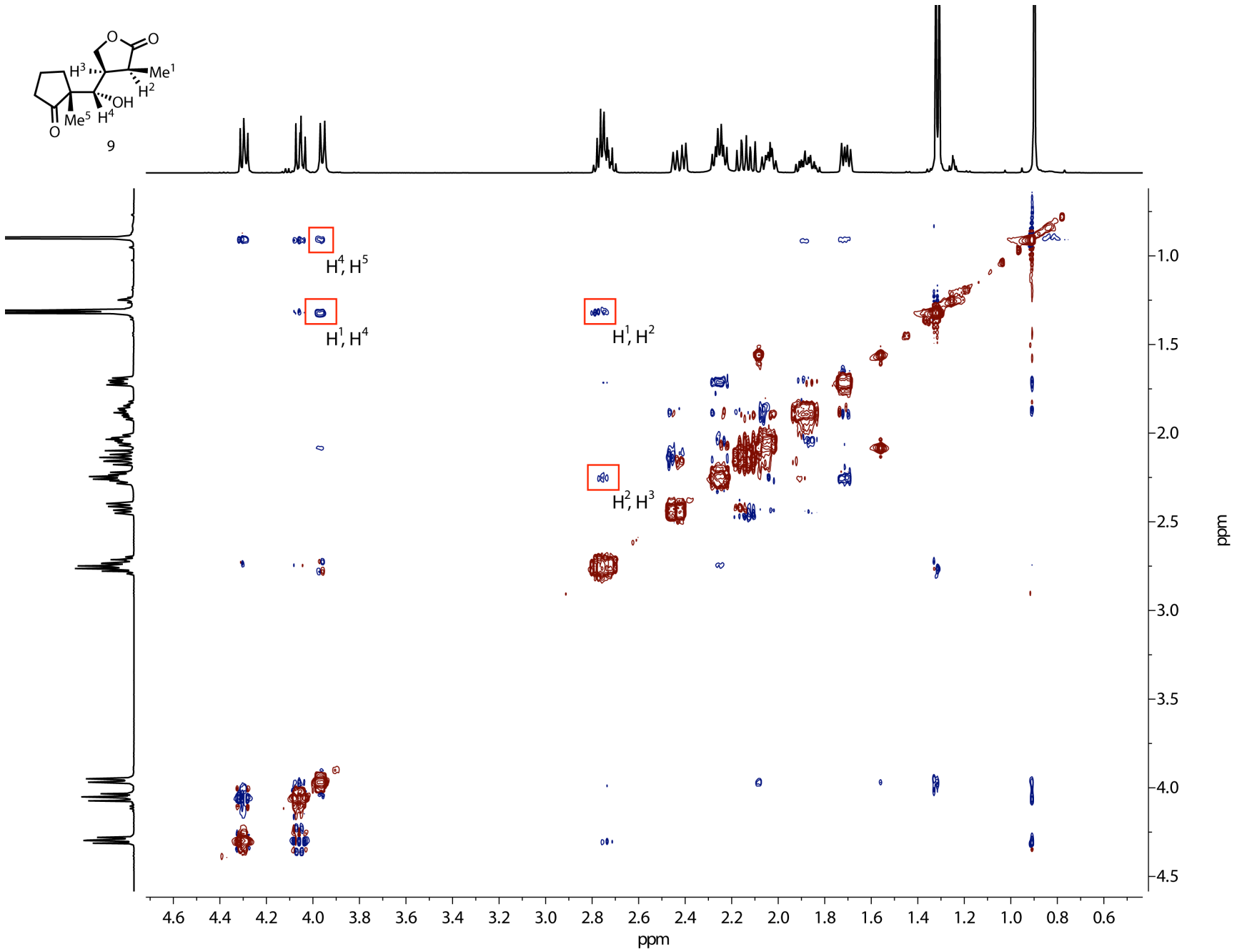
Proton Correlation	% NOE
H^2, H^4	1
H^1, H^2	2
H^1, H^3	1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H3
Me1
H2
OH
Me5
H4
O
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H4, H5
H1, H4
H1, H2
H2, H3
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4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
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1.4
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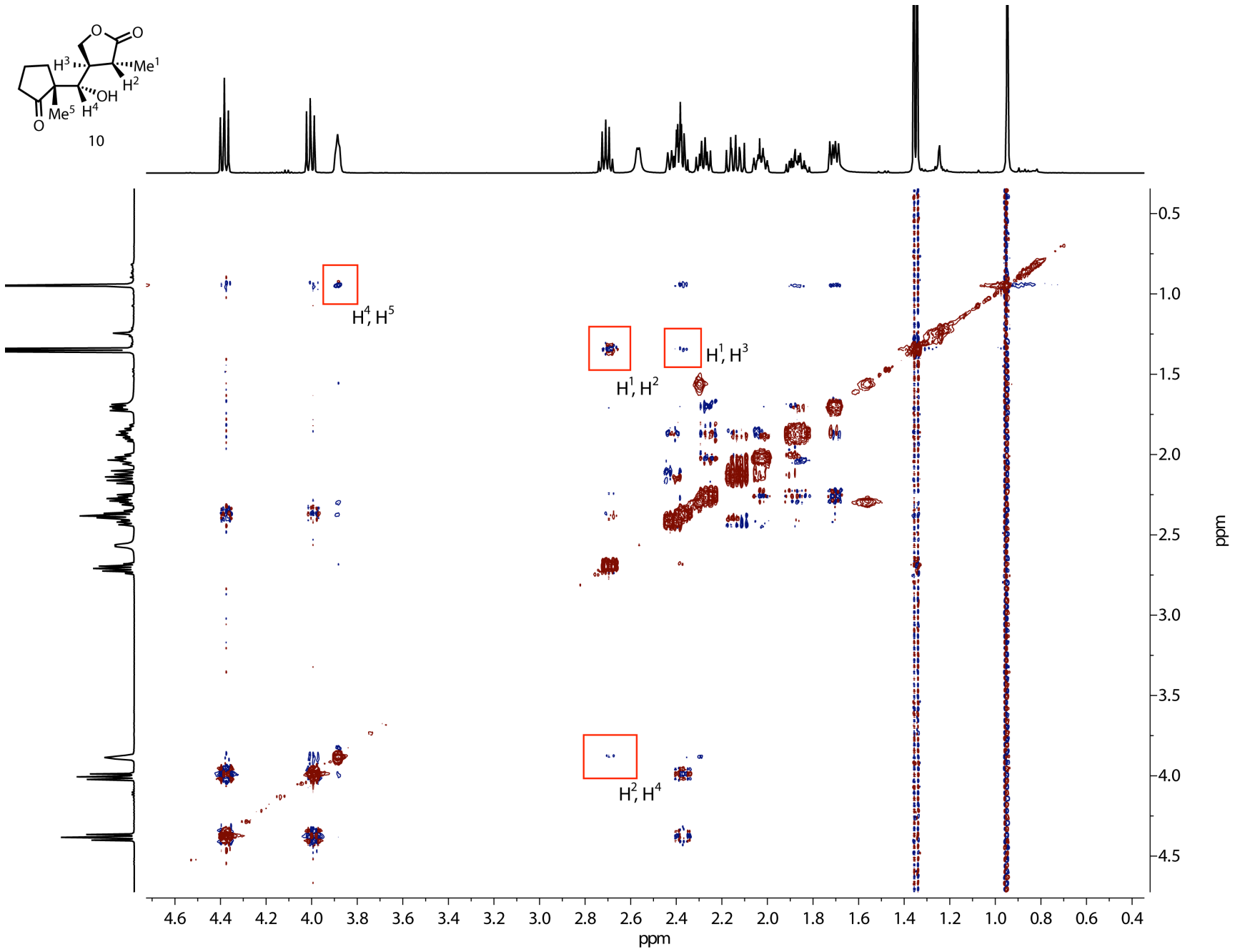
3.5
4.0
4.5
ppm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O
H3
Me1
H2
OH
Me5
H4
O
10
H4, H5
H1, H2
H1, H3
H2, H4
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4.4
4.2
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3.6
3.4
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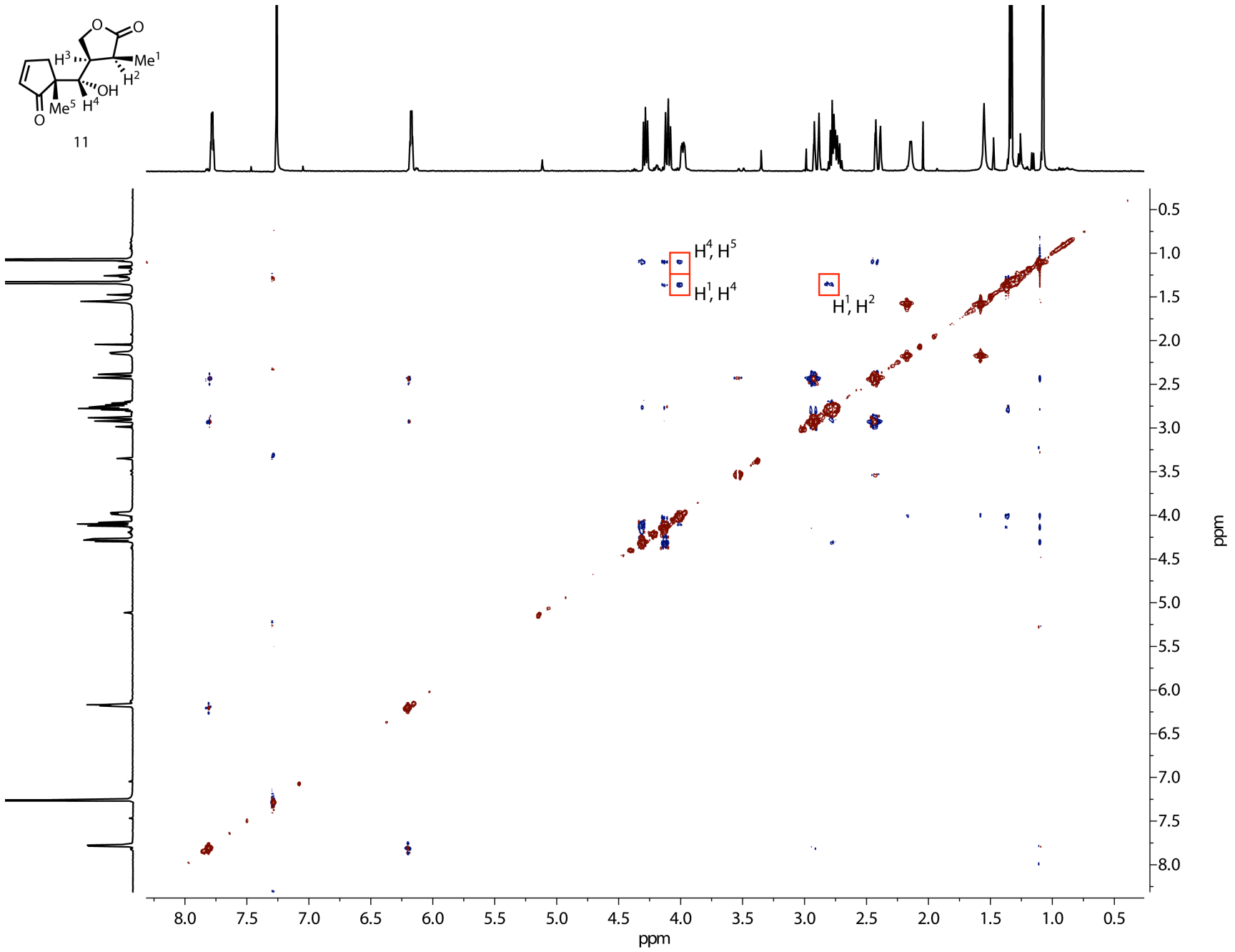
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O
H3
Me1
H2
OH
Me5
H4
O
11
H4, H5
H1, H4
H1, H2
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7.0
6.5
6.0
5.5
5.0
4.5
4.0
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2.5
2.0
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7.5
8.0
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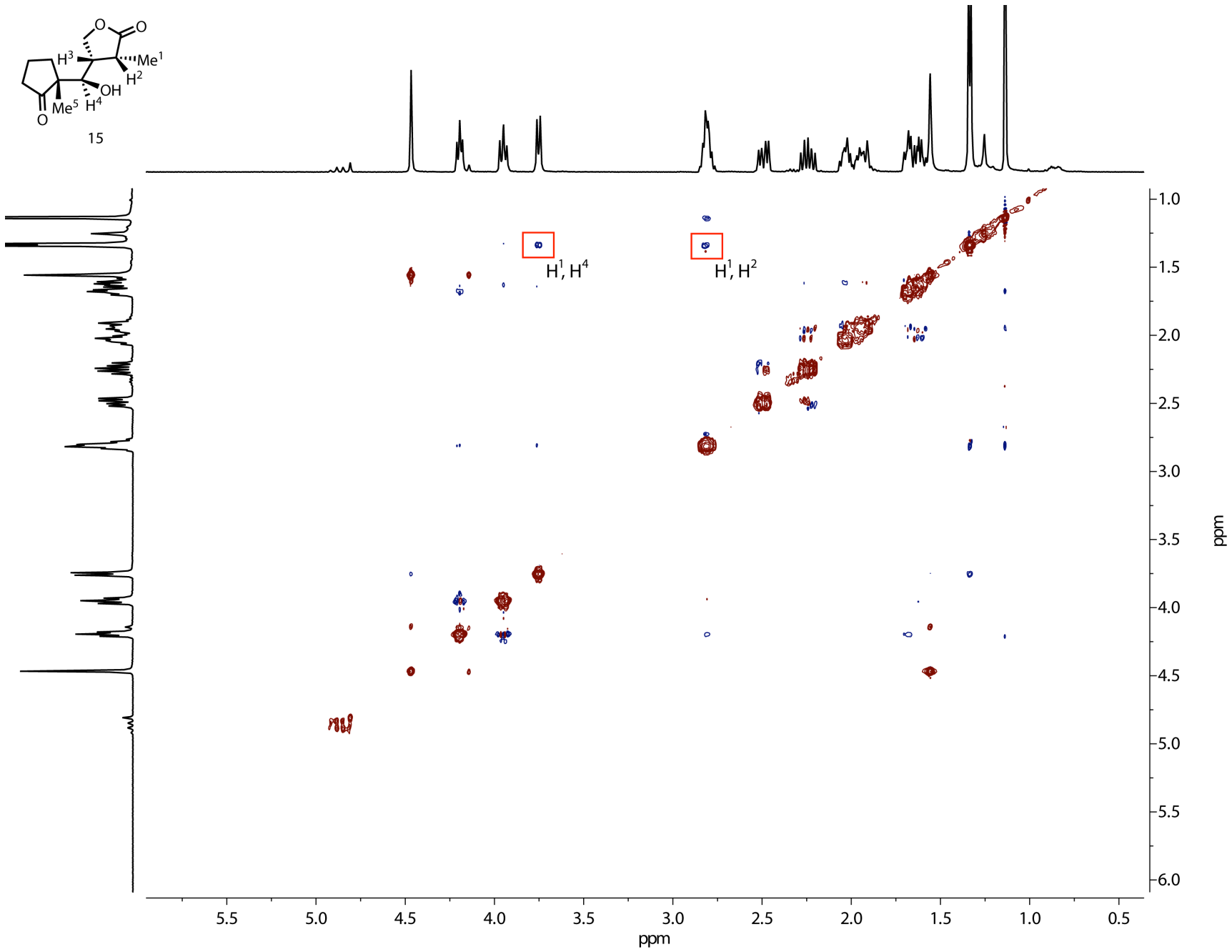
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H3
Me1
H2
OH
Me5
H4
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H1, H4
H1, H2
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4.0
3.5
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2.5
2.0
1.5
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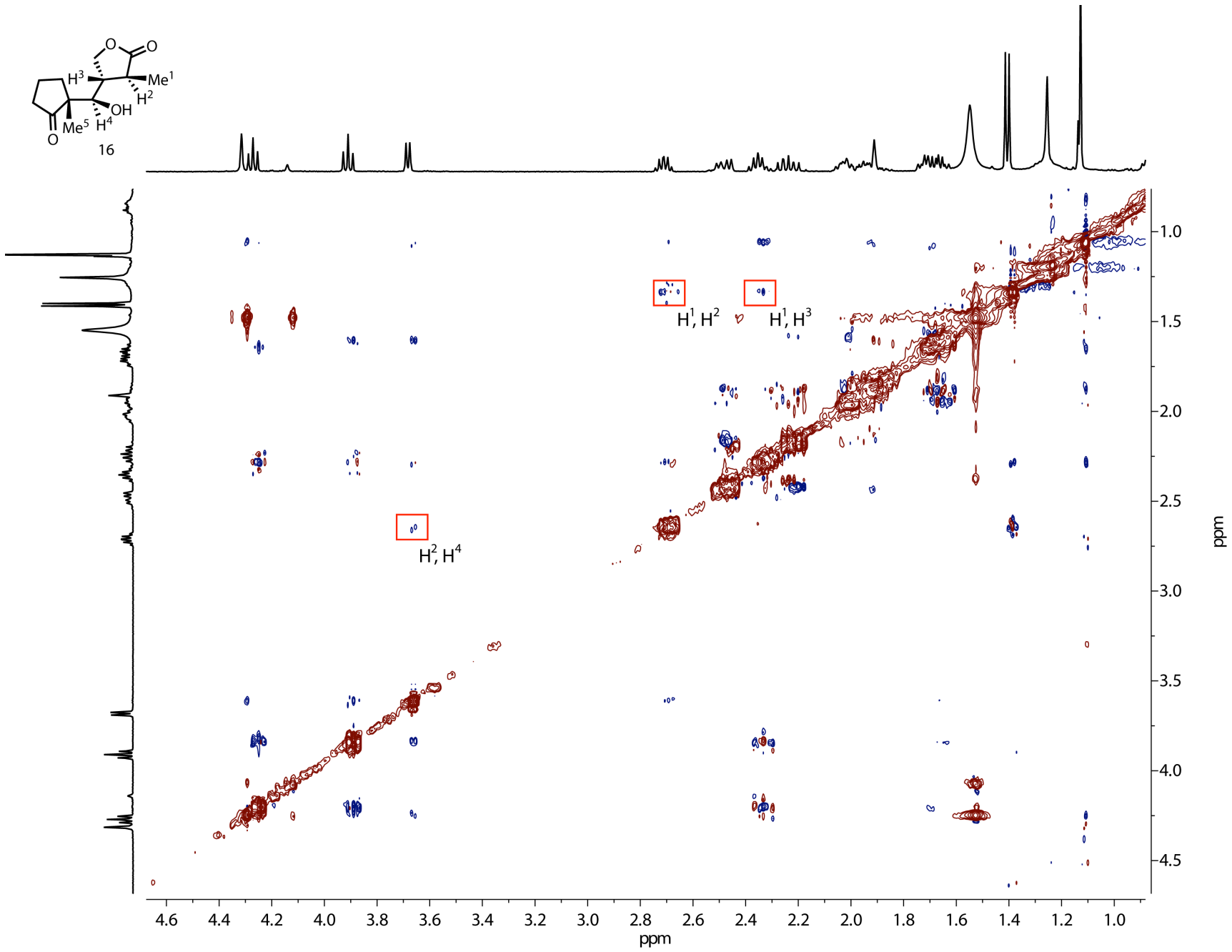
ppm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H2
OH
Me5
H4
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H2, H4
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4.2
4.0
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2.8
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2.4
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3.5
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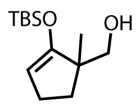
ppm

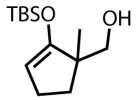
IV. ^{1}H and ^{13}C NMR Spectral Data

^{1}H NMR ($CDCl_3$)

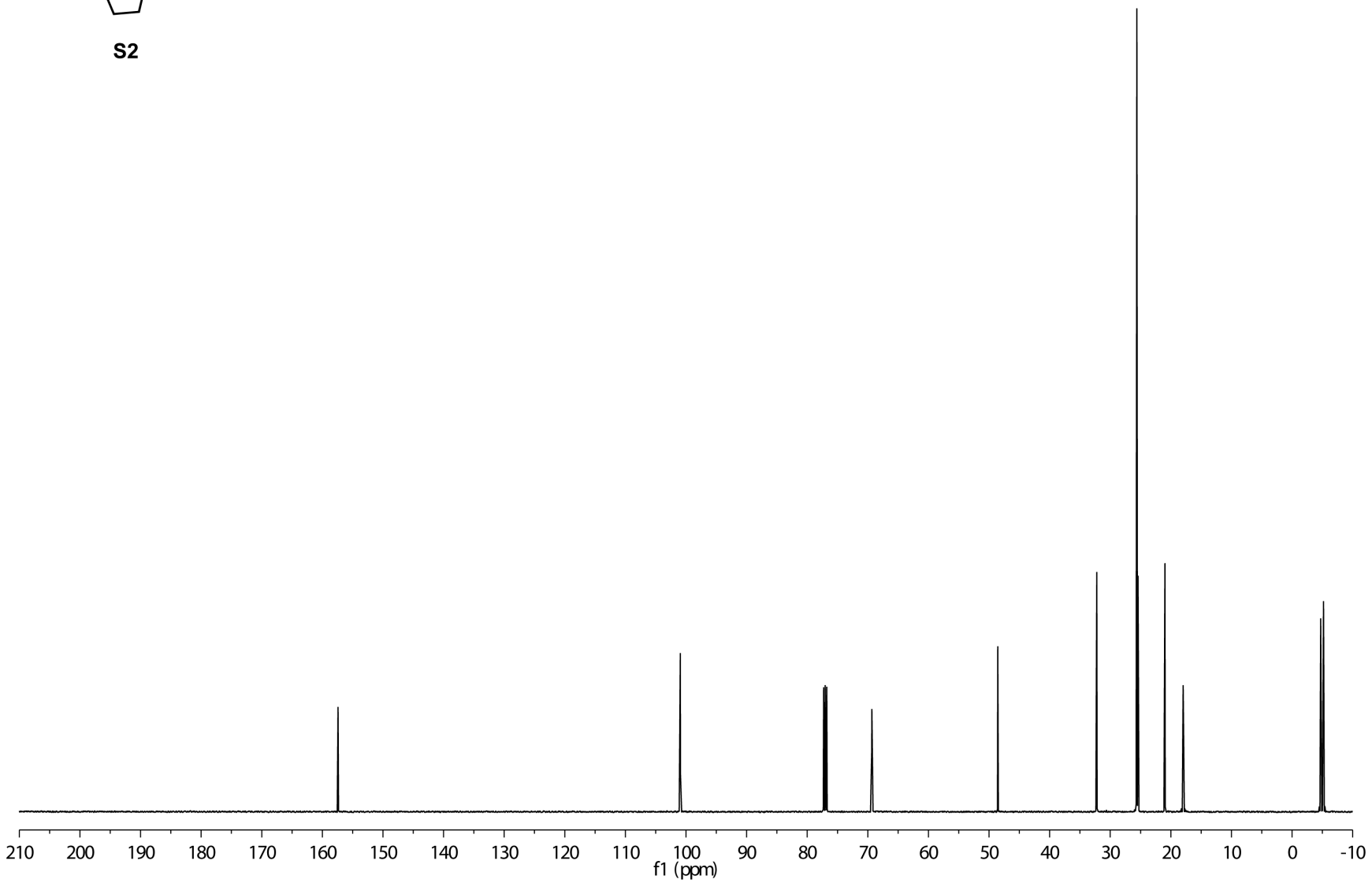


S2

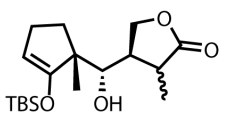
^{13}C NMR ($CDCl_3$)



S2

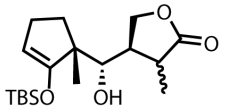


^{1}H NMR ($CDCl_3$)



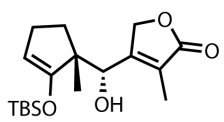
4 (cis):**5** (trans)
3:2 dr

^{13}C NMR ($CDCl_3$)



4 (cis):**5** (trans)
3:2 dr

^{1}H NMR ($CDCl_3$)



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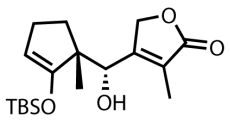

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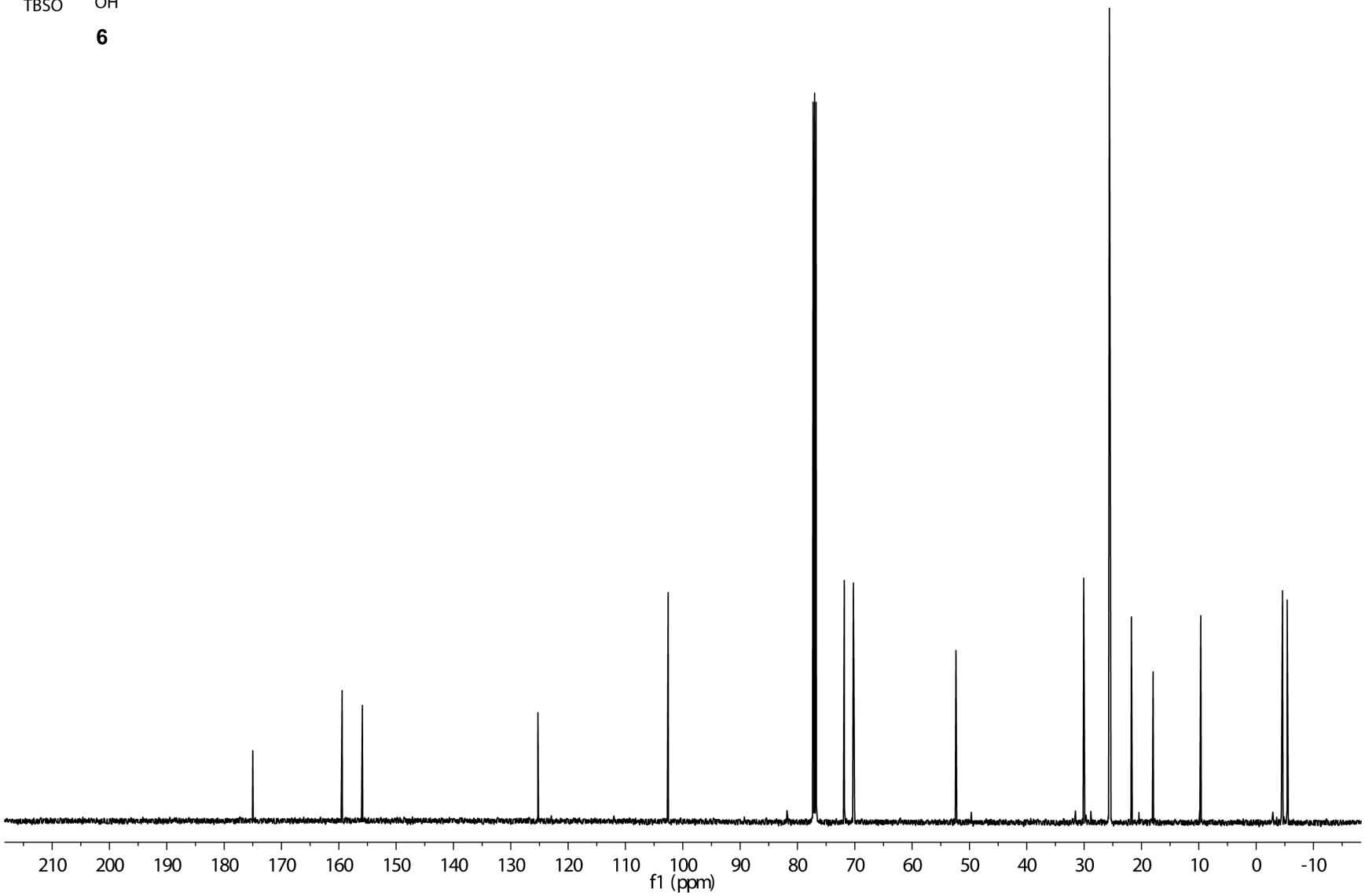

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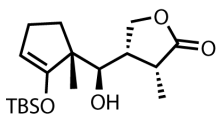

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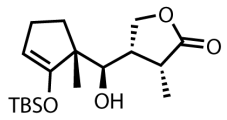

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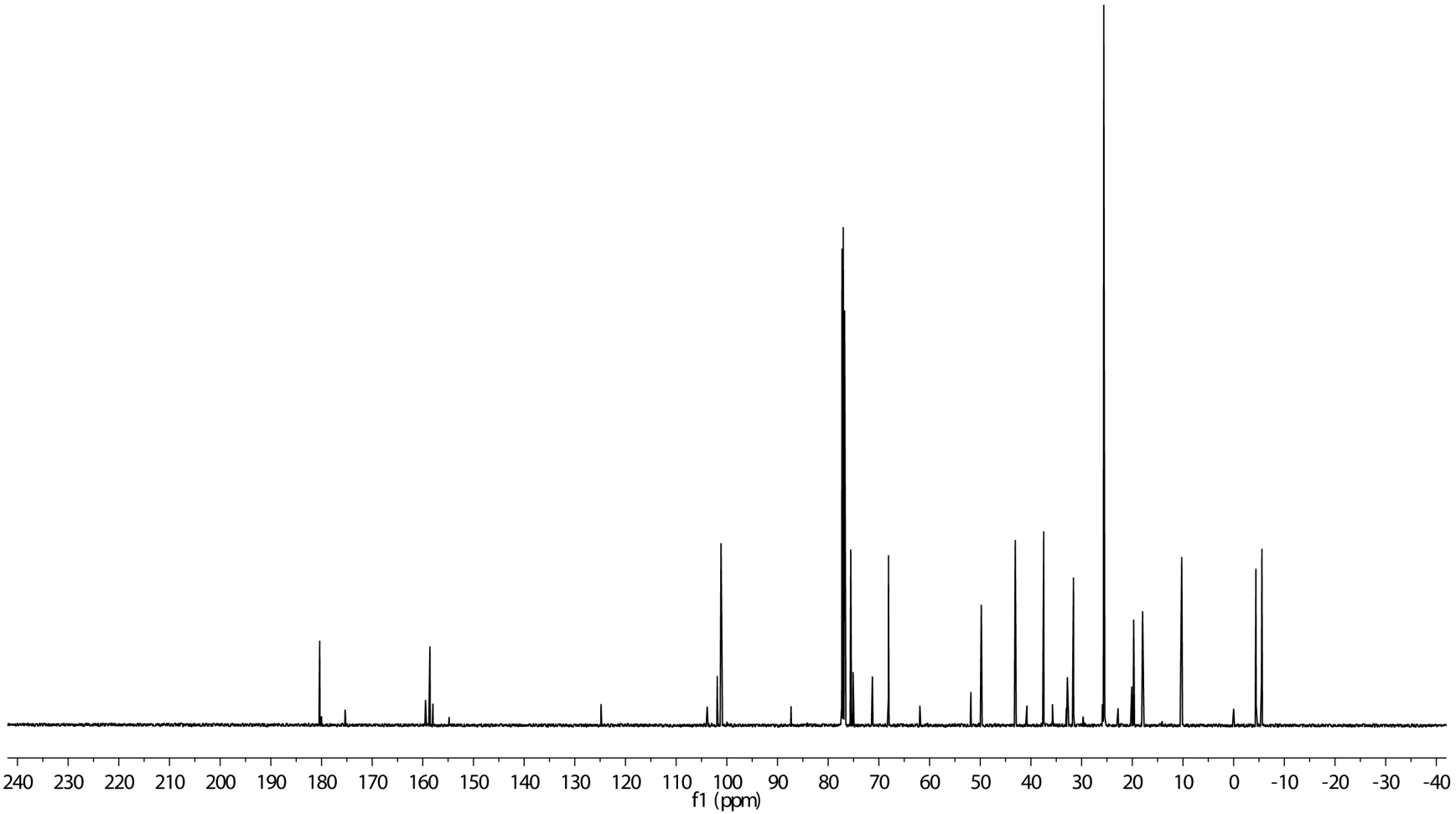
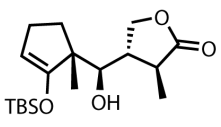


^{1}H NMR ($CDCl_3$)



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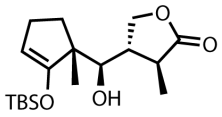
^{13}C NMR ($CDCl_3$)



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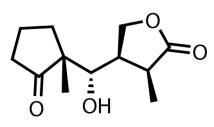

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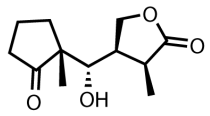

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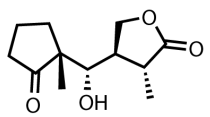

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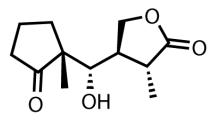

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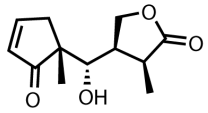
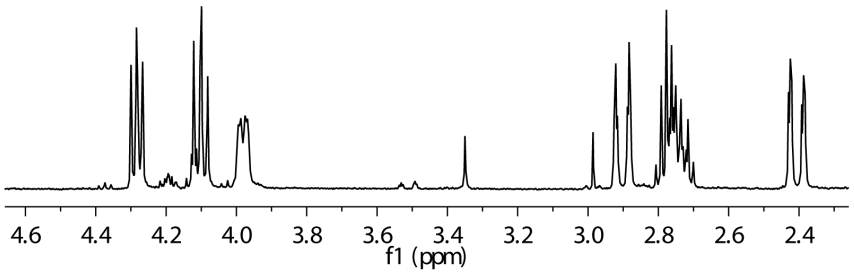
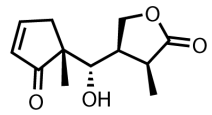
^{1}H NMR ($CDCl_3$)



11

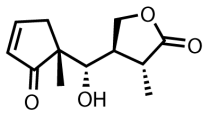


^{13}C NMR ($CDCl_3$)

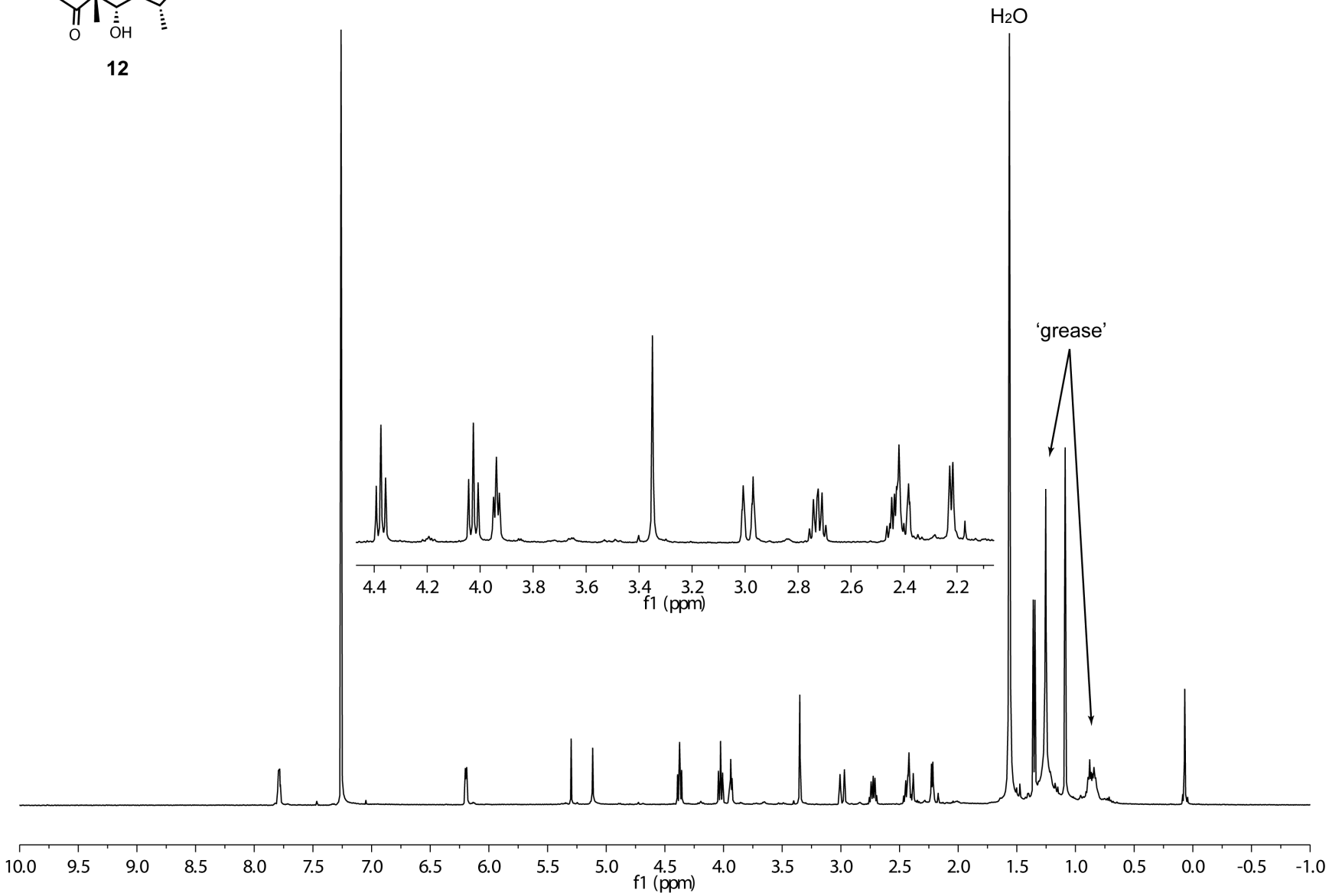


11

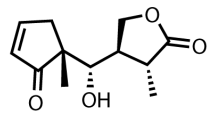
^{1}H NMR ($CDCl_3$)



12

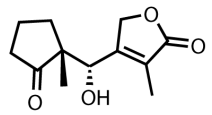


^{13}C NMR ($CDCl_3$)

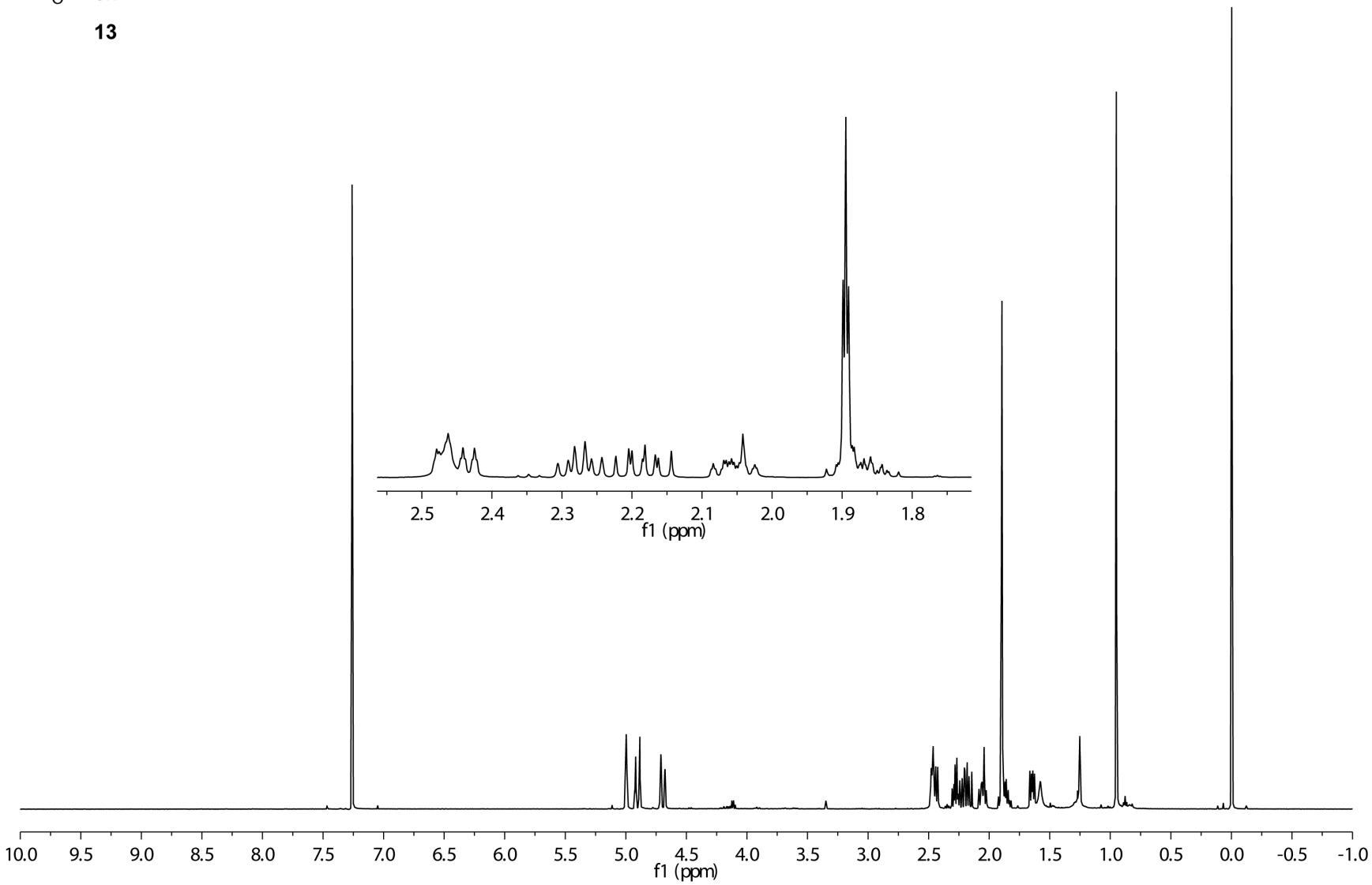


12

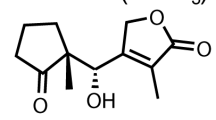
^{1}H NMR ($CDCl_3$)



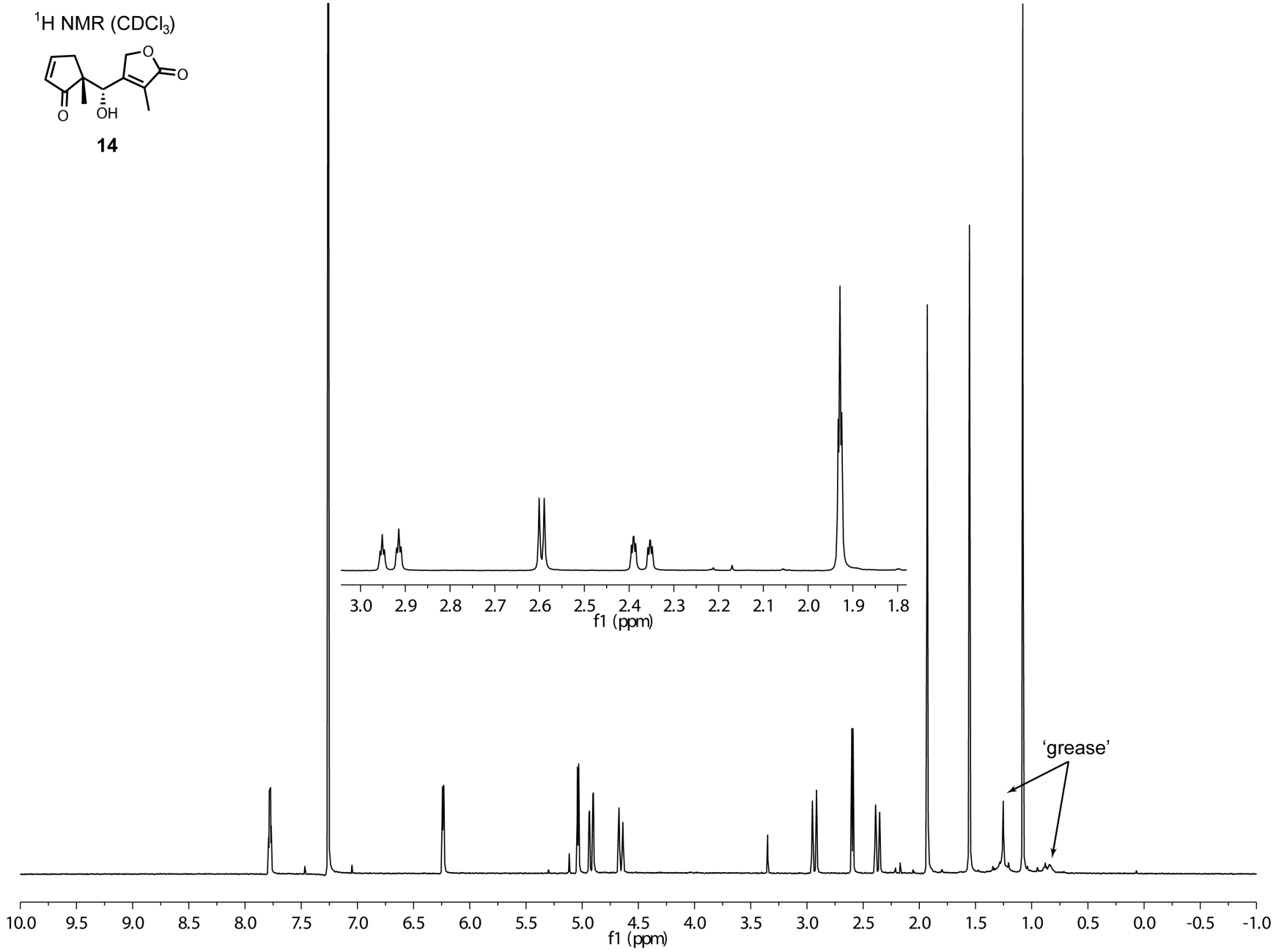
13



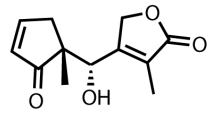
^{13}C NMR ($CDCl_3$)



13

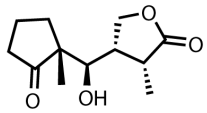

^{1}H NMR ($CDCl_3$)
O
O
OH
14
3.0 2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2 2.1 2.0 1.9 1.8
f1 (ppm)
'grease'
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

^{13}C NMR ($CDCl_3$)



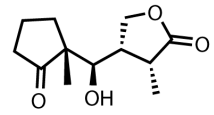
14

^{1}H NMR ($CDCl_3$)

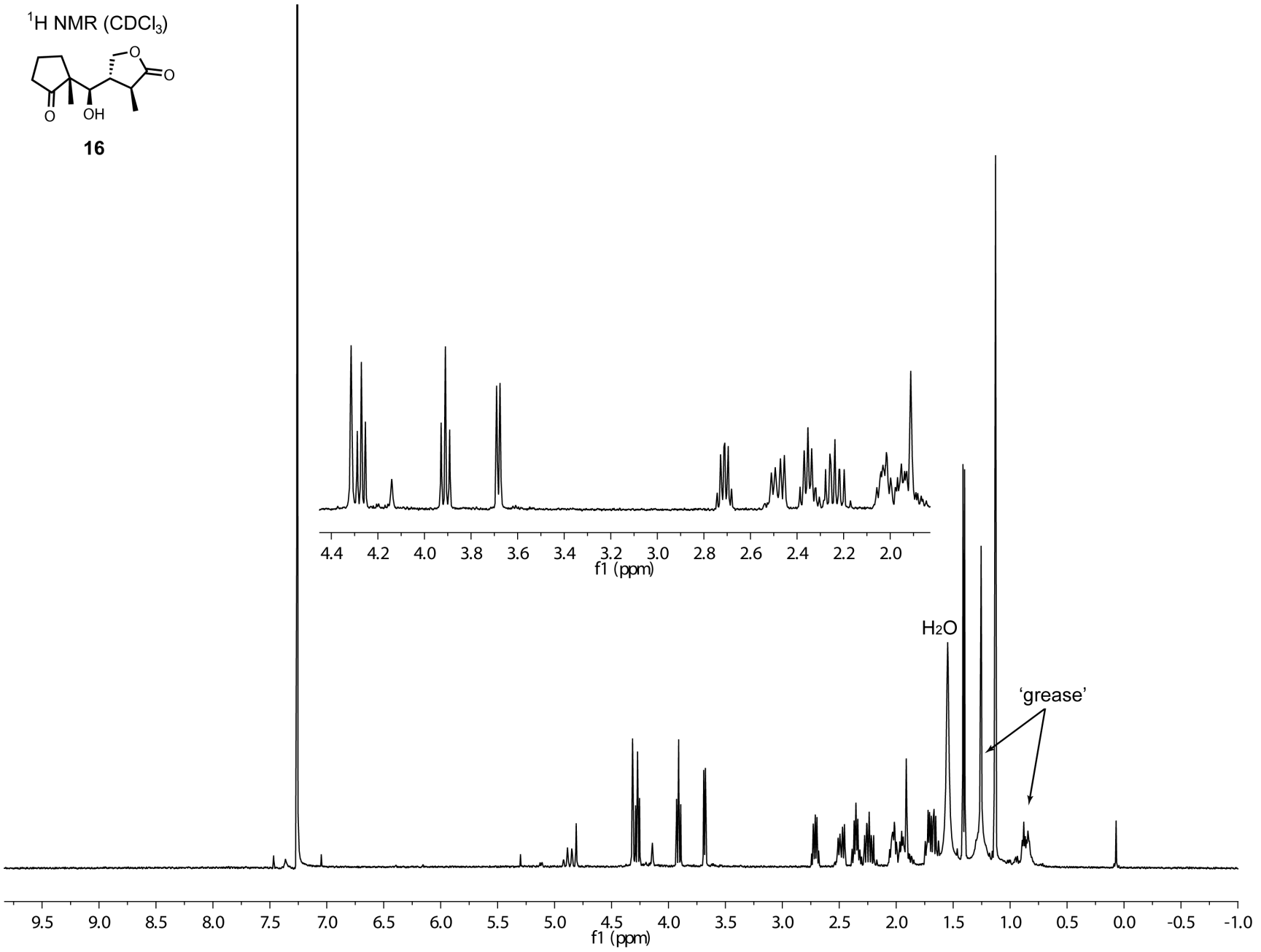


15

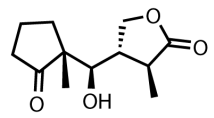
^{13}C NMR ($CDCl_3$)



15

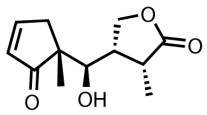

^{1}H NMR ($CDCl_3$)
O
O
O
OH
16
4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0
f1 (ppm)
H_2O
'grease'
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

^{13}C NMR ($CDCl_3$)



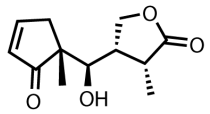
16

^{1}H NMR ($CDCl_3$)



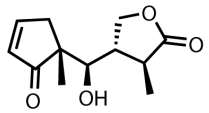
17

^{13}C NMR ($CDCl_3$)



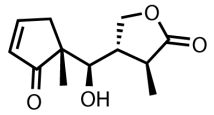
17

^{1}H NMR ($CDCl_3$)

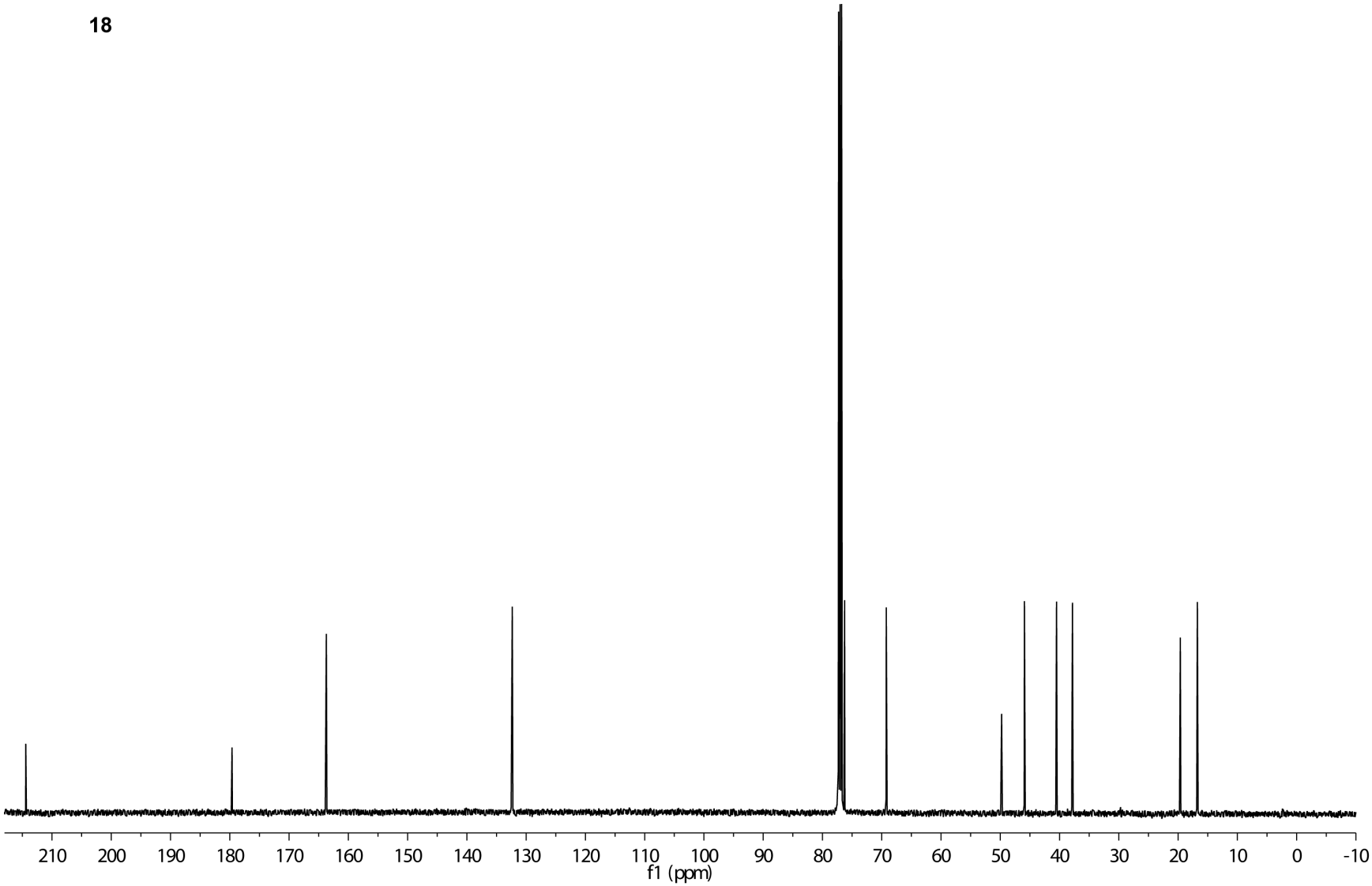


18

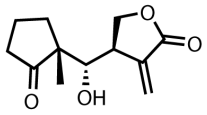
^{13}C NMR ($CDCl_3$)



18

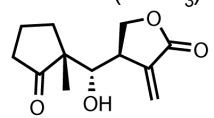


^{1}H NMR ($CDCl_3$)



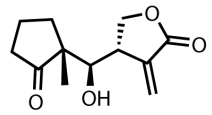
19

^{13}C NMR ($CDCl_3$)

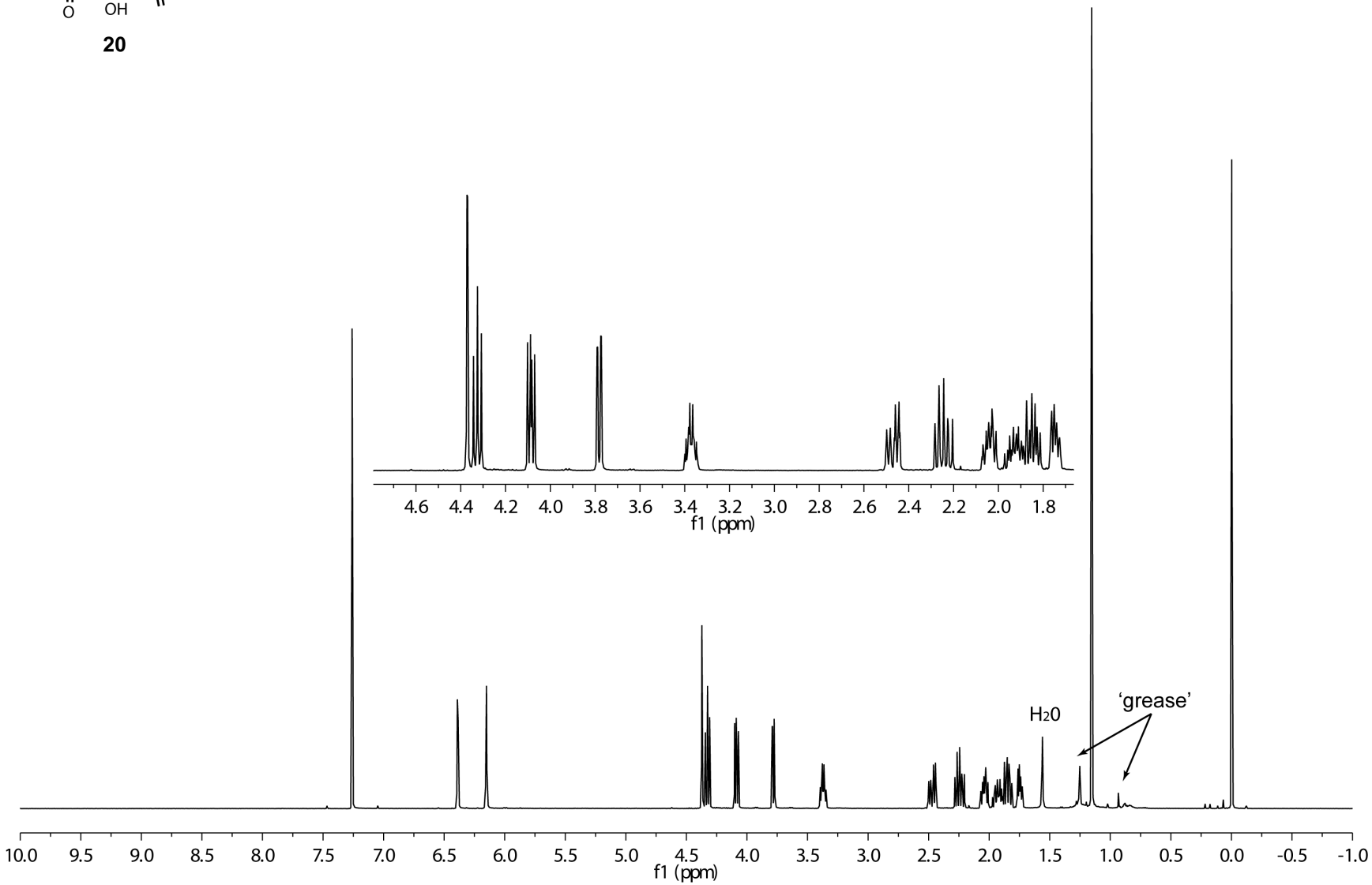


19

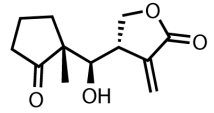
^{1}H NMR ($CDCl_3$)



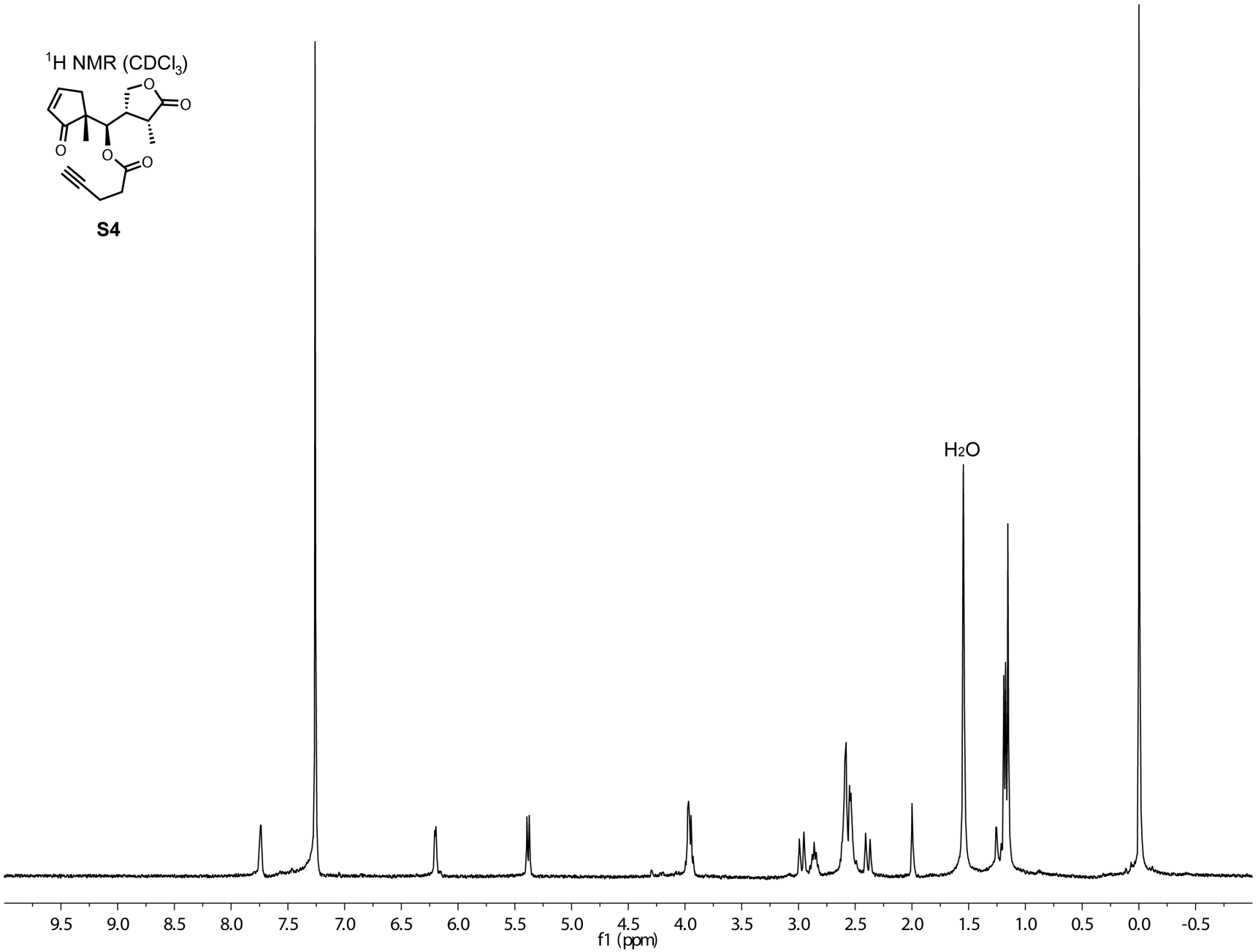
20



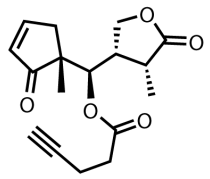
^{13}C NMR ($CDCl_3$)



20


^{1}H NMR ($CDCl_3$)
S4
H_2O
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

^{13}C NMR ($CDCl_3$)



S4

IV. HPLC Purity Analysis of Synthesized Compounds

General Protocol for HPLC Analysis of Synthesized Compounds. DMSO stock solutions of newly synthesized molecules were dissolved in methanol and distilled and deionized water (ddH_2O) and analyzed on an Agilent 1200 series instrument equipped with a diode array detector and Zorbax SB-C18 column (4.6 x 150 mm, 5 µm, Agilent Technologies). The analysis method (1 mL/min flow rate) starts with an isocratic eluent system of 10% MeCN in ddH_2O from 0-2 minutes followed by a linear gradient of 10% to 85% MeCN in ddH_2O from 2-24 minutes, followed by 85% to 95% MeCN in ddH_2O from 24-26 minutes, and finally an isocratic eluent system of 95% MeCN in ddH_2O from 26-30 minutes. No TFA was added to the eluent solvents. Wavelength monitored = 215 nm.

Preparation of Stock Solutions. Compound stock solutions were prepared in DMSO (40 mM to 100 mM concentrations) and stored at -20 °C when not in use. Compound purities were assessed frequently by analytical reverse-phase HPLC analysis and fresh solutions were prepared as needed.

Table S1. Compound Purity by HPLC. All compounds were tested for purity by HPLC with the exception of **9**, **10**, **15**, and **16** due to their lack of UV absorbance. These compounds were determined to be ≥ 95% pure by ^{1}NMR analysis.

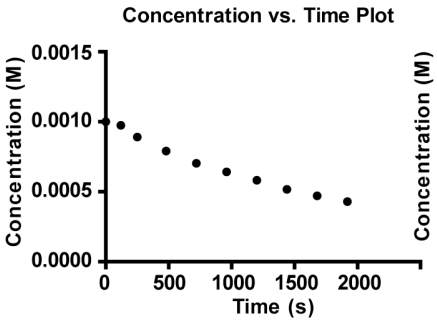
Compound	R_T (min)	HPLC Purity (%)
11	9.0	96
12	9.1	>99
13	11.4	>99
14	9.9	>99
17	7.7	>99
18	8.0	>99
19	9.6	>99
20	9.7	>99
S4	15.7	>99

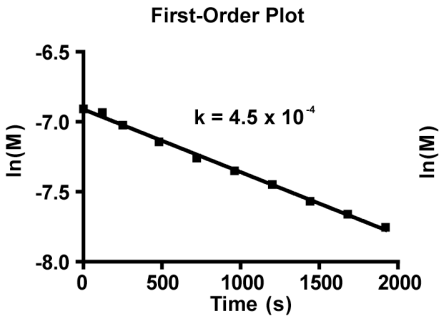
V. Kinetic Studies with *N*-Ac Cysteine

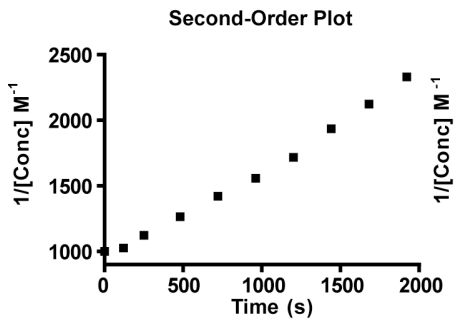
All Concentrations measured refer to unreacted starting material (**11**, **19**, or **20**).

Kinetic Plots for Compound 11 (Endocyclic Enone Only):

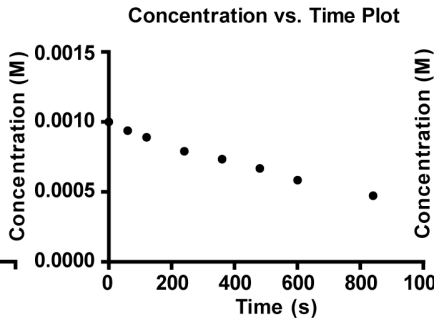
1 equiv *N*-Ac Cysteine

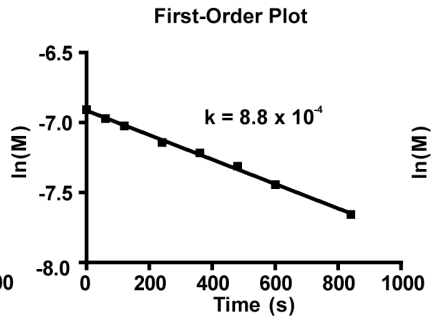


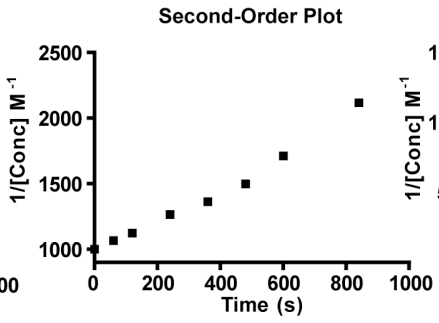




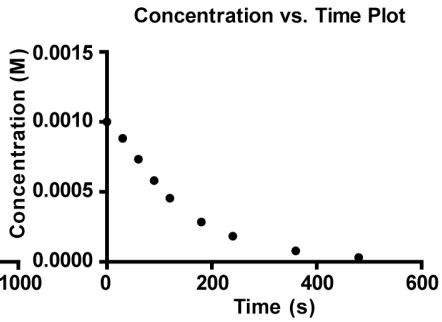
2 equiv *N*-Ac Cysteine

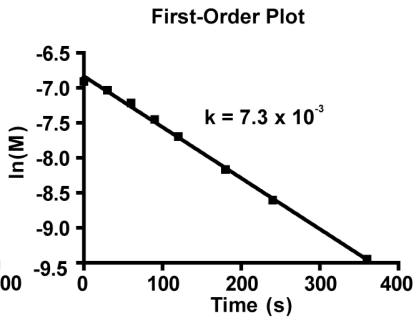


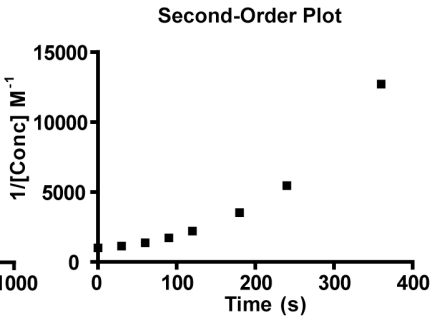




10 equiv *N*-Ac Cysteine

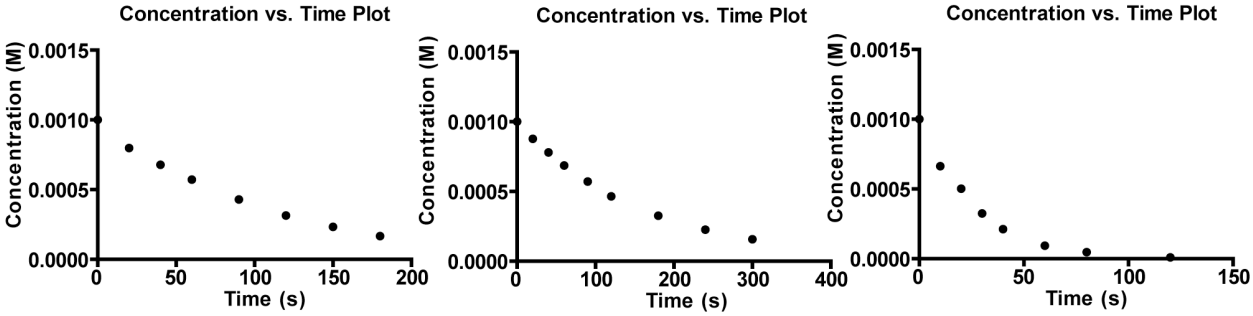


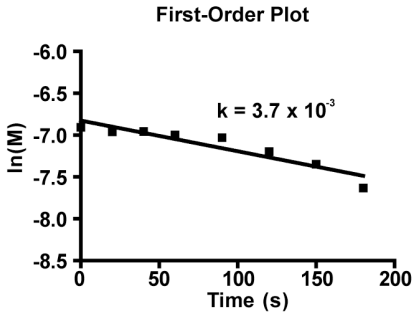


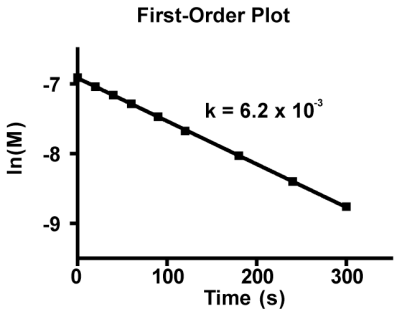


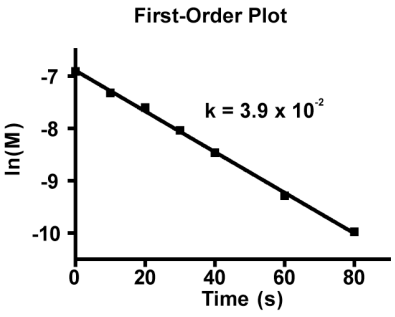
Kinetic Plots for Compound 19 (Exocyclic Methylene Only):

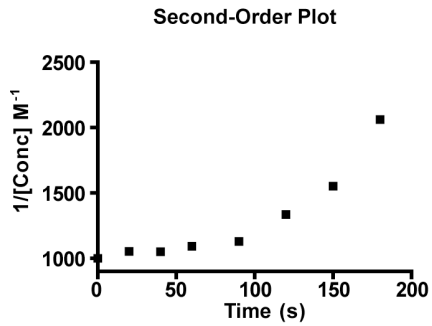
1 equiv *N*-Ac Cysteine	2 equiv *N*-Ac Cysteine	10 equiv *N*-Ac Cysteine

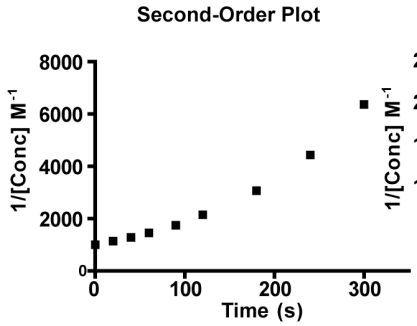


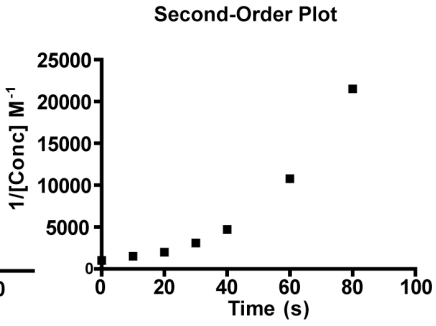






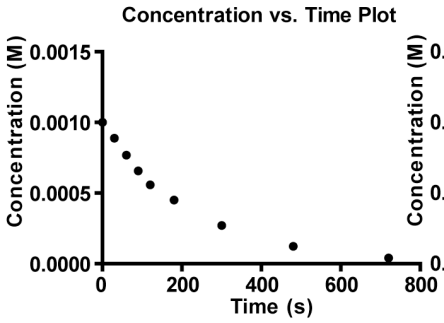


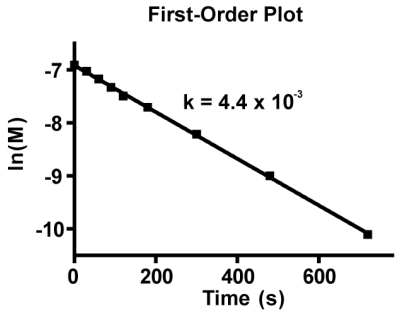


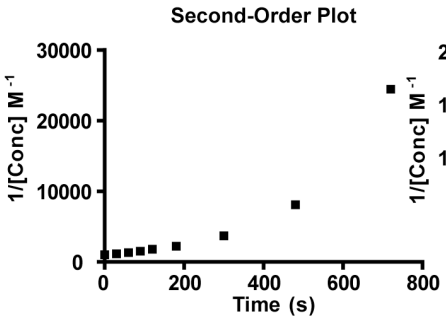


Kinetic Plots for Compound 20 (Exocyclic Methylene Only):

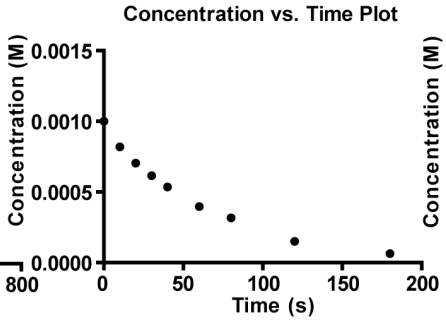
1 equiv *N*-Ac Cysteine

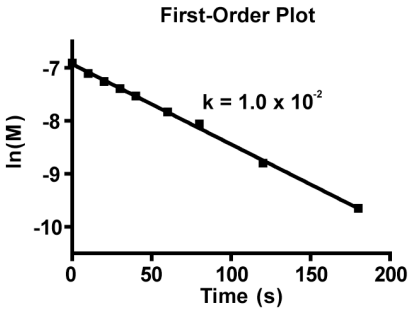


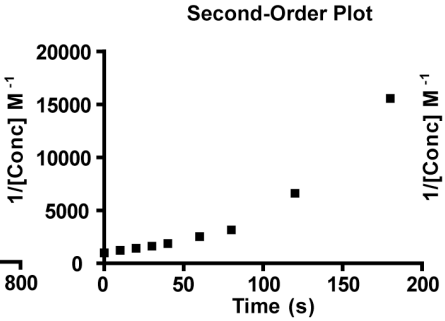




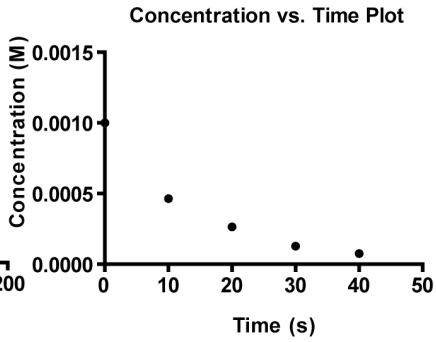
2 equiv *N*-Ac Cysteine

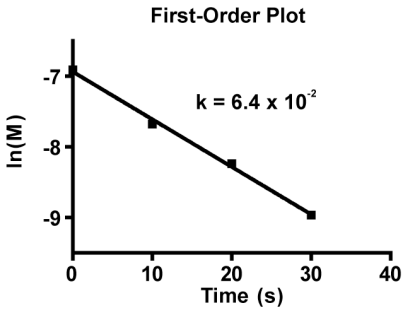


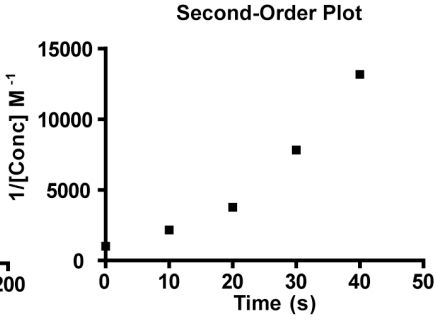




10 equiv *N*-Ac Cysteine







VI. Cell Viability for A549 NF-κB Luciferase Assays

Table S2. Alamar Blue cytotoxicity analysis of A549 NF-κB-luc cells treated with compounds at varying concentrations. All compounds maintained ≥ 80% cell viability throughout the assay with the exception of derivatives **19** and **20** at 50 μM (cell viabilities at 50 μM were 67 ± 6% and 70 ± 6%, respectively). All wells were normalized to non-treated (0.5% v/v DMSO) control wells.

	Cell Viability ± S.D. (%)				
Compound	250 μM	100 μM	50 μM	20 μM	10 μM
9	94 ± 1	113 ± 25	-	-	-
10	96 ± 5	99 ± 8	-	-	-
11	88 ± 3	96 ± 3	102 ± 2	-	-
12	109 ± 5	111 ± 14	105 ± 6	-	-
13	103 ± 5	102 ± 5	-	-	-
14	89 ± 6	98 ± 8	-	-	-
15	99 ± 1	95 ± 11	-	-	-
16	103 ± 4	102 ± 3	-	-	-
17	104 ± 7	96 ± 11	95 ± 3	-	-
18	97 ± 5	93 ± 17	96 ± 8	-	-
19	-	-	67 ± 6	86 ± 4	90 ± 5
20	-	-	70 ± 6	87 ± 14	93 ± 6
S4	80 ± 3	84 ± 2	97 ± 3	-	-

Reference:

1. Widen, J. C.; Kempema, A. M.; Villalta, P. W.; Harki, D. A., Targeting NF-κB p65 with a Helenalin Inspired Bis-electrophile. *ACS Chem Biol* **2017,** *12* (1), 102-113.