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Synthesis of natural phaeosphaeride A and semi-natural phaeosphaeride B derivatives

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General Remarks. Natural phaeosphaeride A was provided to us by Dr. Alexander Berestetskiy (All-Russian Institute of Plant Protection, Saint-Petersburg, Russian Federation). ^{1}H NMR spectra were acquired on a Bruker AVANCE III 400 MHz NMR spectrometer in $CDCl_3$. *J*-values are given in Hz. Multiplicities are given as: s (singlet), d (doublet), t (triplet) and m (multiplet). 2-D COSY, HMQC, HMBC and ROESY experiments were carried out to aid assignment and establish the relative stereochemistry where appropriate. Optical rotations were acquired on an Optical Activity Polaar 3005 Polarimeter using a 2.5 cm cell with a Na 589 nm filter and the concentration of samples was denoted as *c*. Mass spectra data were acquired on a Thermo Scientific TSQ Quantum Access Max Mass spectrometer. High-resolution mass spectra (HRMS) were acquired on a LTQ Orbitrap Velos spectrometer. FTIR spectra were acquired on a Shimadzu IR Affinity-1 spectrometer. Organic solvents used were dried by standard methods when necessary. Commercially available reagents were used without further purification. All reactions were monitored by TLC with EMD/Merck KGaA silica gel coated plates, with visualization by UV light and by charring with 0.1% ninhydrin in EtOH. Column chromatography was performed using Merck 60 Å 70-230 mesh silica gel. The synthesis of compounds **1**, **2** was described in our paper [V. V. Abzianidze, D. S. Prokofieva, L. A. Chisty, K. P. Bolshakova, A. O. Berestetskiy, T. L. Panikorovskii, A. S. Bogachenkov and A. A. Holder, *Bioorg. Med. Chem. Lett.*, 2015, **25**, 5566]. Compounds **3-9** are new.

(2S,3R,4R)-3-Hydroxy-6-methoxy-3-methyl-7-methylidene-2-pentyl-4-(piperidin-1-yl)-3,4,6,7-tetrahydropyrano[2,3-c]pyrrol-5(2H)-one **4**: 0.246 mmol scale, 43 mg, yellow oil, 48% yield. R_f 0.75 (DCM:methanol, 10:1 v/v). $[\alpha]_D^{20.0}$ = -157.75 (c 0.4, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.38 (s, 1H), 5.00 (dd, *J* = 16.0, 1.4 Hz, 2H), 3.93 (s, 3H), 3.59 (d, *J* = 9.9 Hz, 1H), 2.95 (s, 1H), 2.92-2.13 (m, 4H), 1.98-

1.91 (m, 1H), 1.85 – 1.30 (m, 15H), 1.03 (s, 3H), 0.91 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.13 (s), 158.15 (s), 137.01 (s), 101.55 (s), 91.33 (s), 83.48 (s), 68.10 (s), 64.41 (s), 63.36 (s), 31.77 (s), 29.69 (s), 28.38 (s), 26.89 (s), 26.48 (s), 23.83 (s), 22.55 (s), 20.02 (s), 14.05 (s). IR (KBr) 2953, 2930, 2856, 1723, 1636, 1438, 1153, 1084 cm^{-1}. HRMS $[M + H]^+$, calcd. for $C_{20}H_{33}N_2O_4$ 365.243484, found 365.24317.

Synthesis of semi-natural phaeosphaeride B. Natural PPA (90 mg, 0.303 mmol) was dissolved in TFA (3 ml), and the mixture was stirred for 1 h at room temperature. After concentration under vacuum, the crude product was dissolved in THF (3 ml). To this solution was added a half saturated aqueous solution of $NaHCO_3$ (2 ml). The reaction mixture was stirred for 30 min at room temperature, and then diluted with brine (30 ml). The mixture was extracted with Et_2O (40 ml × 2), and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by column chromatography on silica gel (DCM:methanol, 80:1 v/v).

Semi-natural phaeosphaeride B: 0.319 mmol scale, 40 mg, colorless oil, 42% yield. Rf 0.50 (DCM:methanol,10:1). $[\alpha]_D^{20.0}$ = -150.75 (c 0.5, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.06 (dd, *J* = 24.2, 1.3 Hz, 2H), 4.14 (d, *J* = 4.0 Hz, 1H), 4.04 – 3.97 (m, 1H), 3.92 (s, 1H), 3.91 (s, 3H), 3.24 (s, 1H), 1.98-1.87 (m, 1H), 1.72-1.55 (m, 2H), 1.50 – 1.28 (m, 5H), 1.07 (s, 3H), 0.91 (t, *J* = 6.6 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.30 (s), 158.09 (s), 136.35 (s), 104.09 (s), 92.63 (s), 81.79 (s), 69.96 (s), 64.51 (s), 64.16 (s), 31.61 (s), 27.45 (s), 26.10 (s), 22.52 (s), 17.92 (s), 14.03 (s). IR (KBr) 3396, 2956, 2929, 2859, 1715, 1636, 1451, 1012 cm^{-1}. HRMS $[M + H]^+$, calcd. for $C_{15}H_{24}NO_5$ 298.164899, found 298.16473.

Synthesis of **9**. Phaeosphaeride B (0.128 mmol, 1 equiv.) was stirred in dichloromethane (3 ml) on ice with TEA (0.575 mmol, 4.5 equiv.) and a catalytic amount of (dimethylamino)pyridine, then chloroacetyl chloride (0.511 mmol, 4 equiv.) was added dropwise. After 3 h, the reaction mixture was diluted with DCM (30 ml), washed with $NaHCO_3$ saturated followed by a brine solution. After extraction, the organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*, and the crude product was purified by column chromatography on silica gel (DCM:methanol, 200:1 v/v).

(2S,3S,4R)-3-Hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-c]pyrrol-4-yl chloroacetate **9**: 0.128 mmol scale, 30 mg, colorless oil, 63% yield. R_f 0.70 (DCM:methanol, 20:1). $[\alpha]_D^{20.0}$ = -151.50 (c 0.4, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.52 (s, 1H), 5.07 (dd, *J* = 21.2, 1.7 Hz, 2H), 4.14 (s, 2H), 4.07 – 4.01 (m, 1H), 3.91 (s, 3H), 2.44 (s, 1H), 1.97 – 1.81 (m, 1H), 1.75 – 1.55 (m, 2H), 1.51 – 1.29 (m, 5H), 1.19 (s, 3H), 0.91 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.62 (s), 164.46 (s), 159.67 (s), 136.06 (s), 100.09 (s), 92.78 (s), 81.73 (s), 70.73 (s), 68.08 (s), 64.59 (s), 40.93 (s), 31.58 (s),

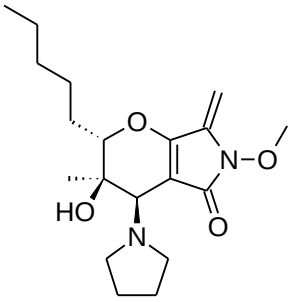
29.69 (s), 27.37 (s), 25.89 (s), 22.50 (s), 19.33 (s), 14.01 (s). IR (KBr) 3422, 2957, 2929, 2858, 1768, 1723, 1637, 1453, 1194, 1166, 987 cm^{-1}. HRMS $[M + H]^+$, calcd. for $C_{17}H_{25}ClNO_6$ 374.136492, found 374.13602.

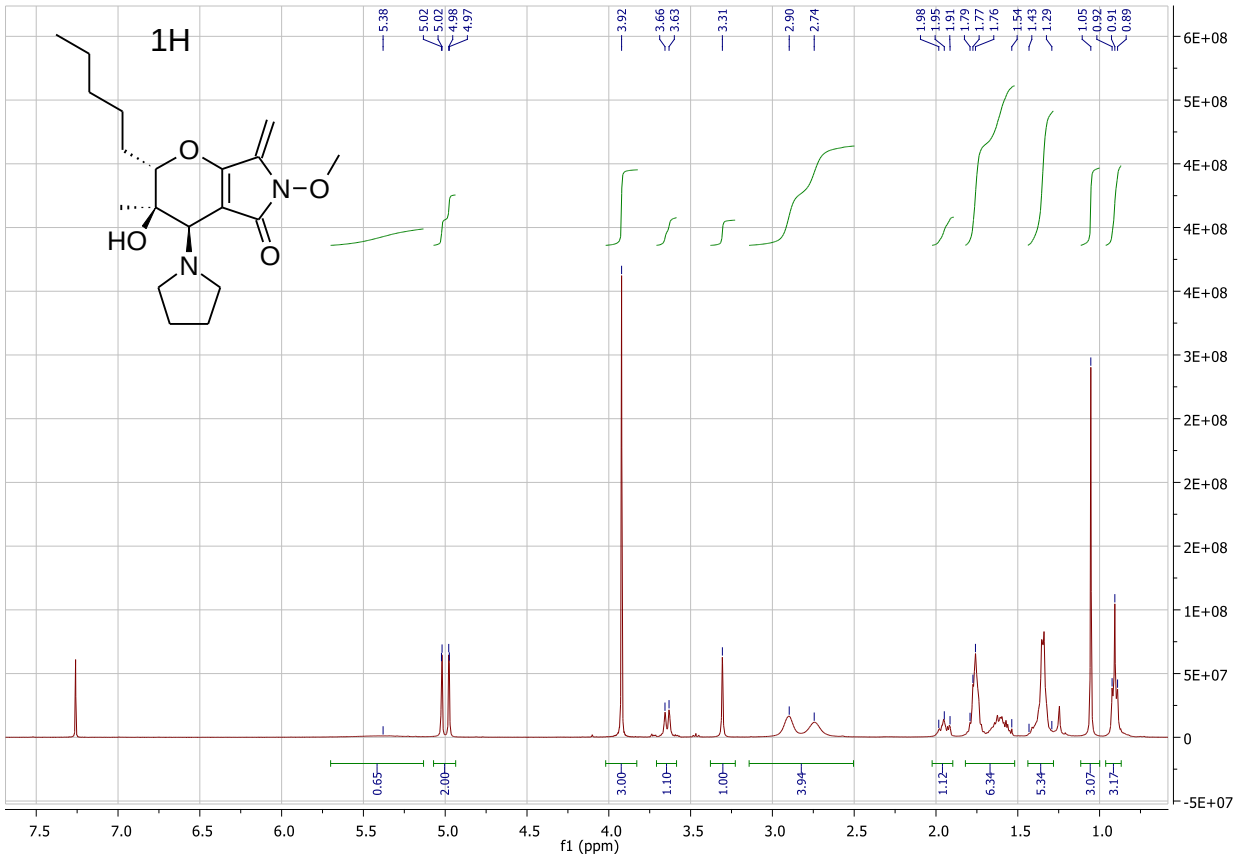
Synthesis of **4** *via* **9**. Piperidine (2 mmol, 20 equiv.) was added to a stirred solution of **9** (0.1 mmol, 1 equiv.) in anhydrous THF (2 ml) and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was concentrated under reduced pressure. The crude material was chromatographed on silica gel (DCM:methanol, 100:1 v/v).

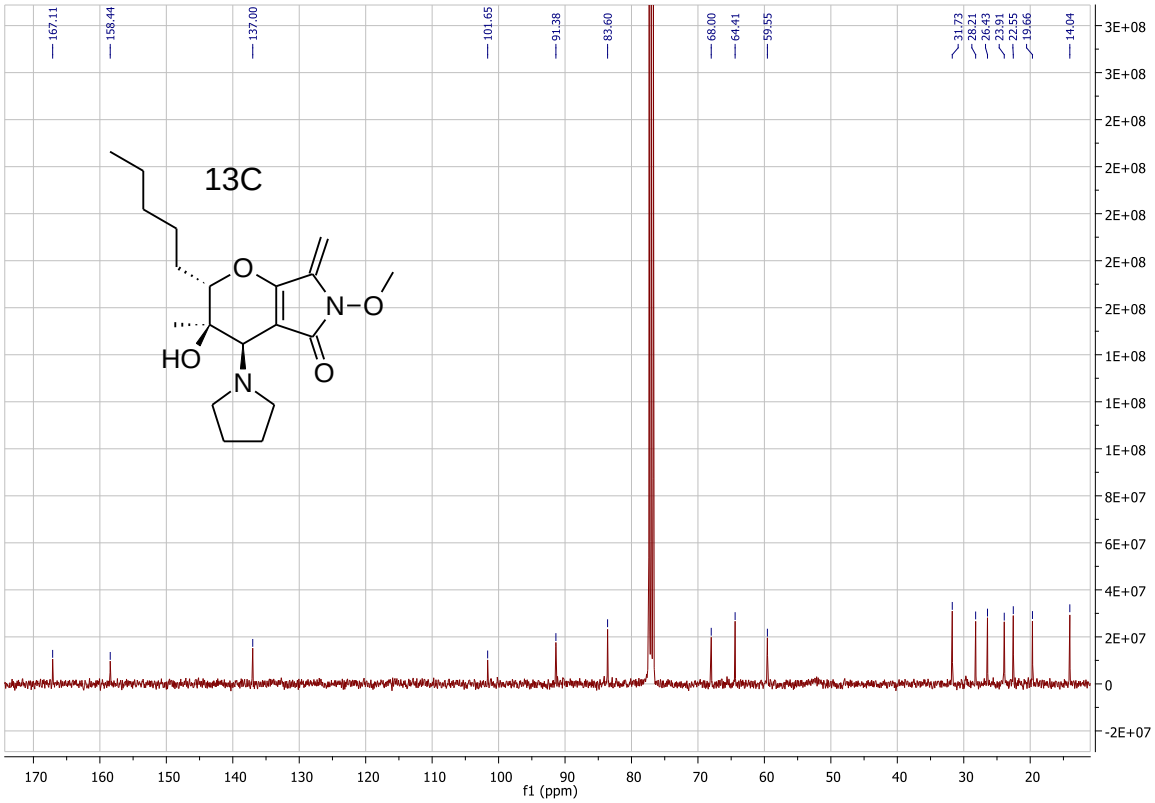
(2S,3R,4R)-3-Hydroxy-6-methoxy-3-methyl-7-methylidene-2-pentyl-4-(piperidin-1-yl)-3,4,6,7-tetrahydropyrano[2,3-c]pyrrol-5(2H)-one **4**: 0.179 mmol scale, 28 mg, yellow oil, 43% yield. R_f 0.75 (DCM:methanol, 10:1 v/v). $[\alpha]_D^{20.0}$ = -159.60 (c 0.4, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.31 (s, 1H), 5.01 (dd, *J* = 16.0, 1.4 Hz, 2H), 3.95 (s, 3H), 3.61 (d, *J* = 9.9 Hz, 1H), 2.97 (s, 1H), 2.91 (m, 2H), 2.05-1.96 (m, 1H), 1.83 – 1.15 (m, 16H), 1.05 (s, 3H), 0.93 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.13 (s), 158.15 (s), 137.01 (s), 101.55 (s), 91.33 (s), 83.48 (s), 68.10 (s), 64.41 (s), 63.36 (s), 31.77 (s), 29.69 (s), 28.38 (s), 26.89 (s), 26.48 (s), 23.83 (s), 22.55 (s), 20.02 (s), 14.05 (s).

NMR spectra

(2*S*,3*R*,4*R*)-3-Hydroxy-6-methoxy-3-methyl-7-methylidene-2-pentyl-4-(pyrrolidin-1-yl)-3,4,6,7-tetrahydropyrano[2,3-*c*]pyrrol-5(2*H*)-one 3



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91.38
83.60
68.00
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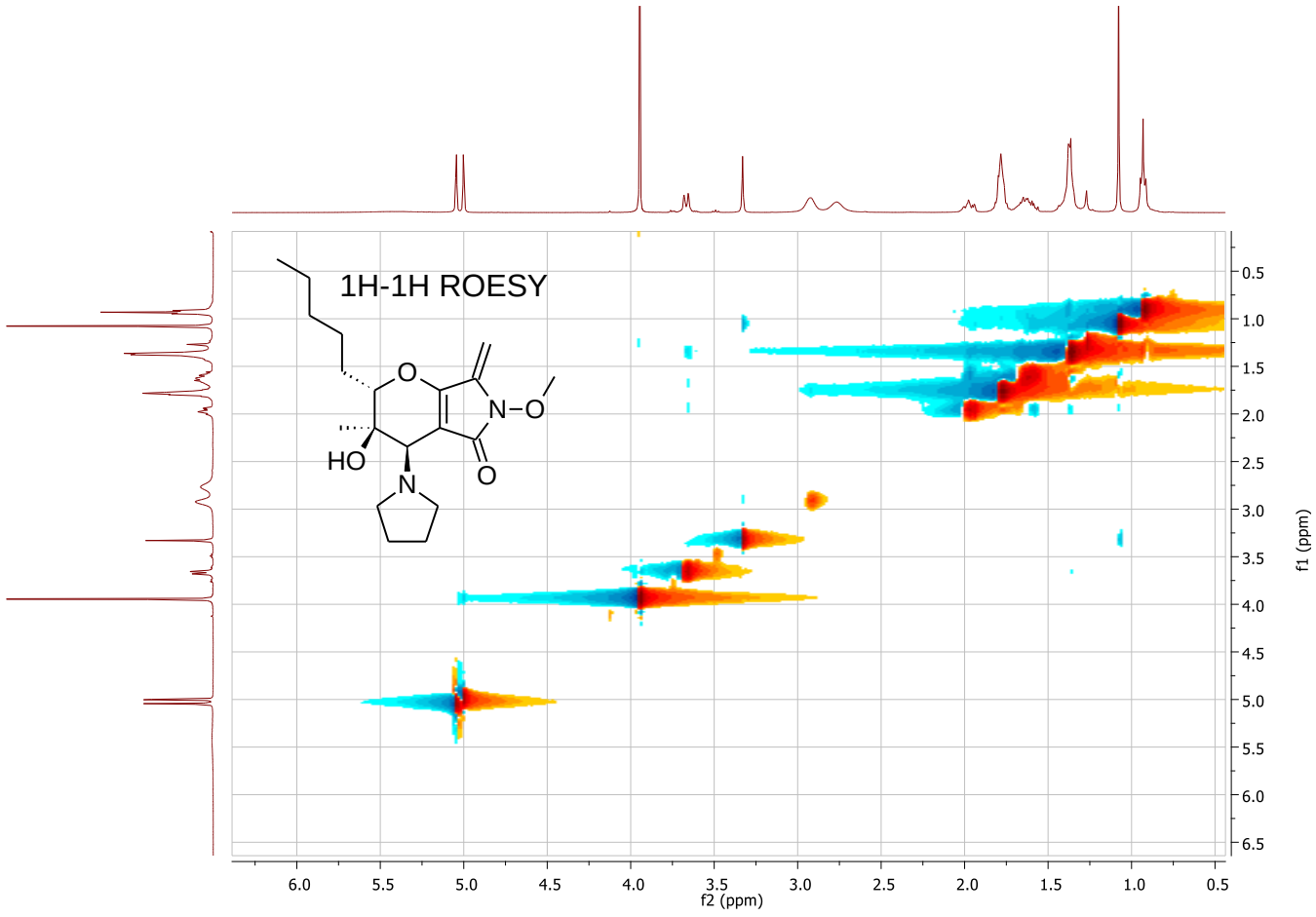
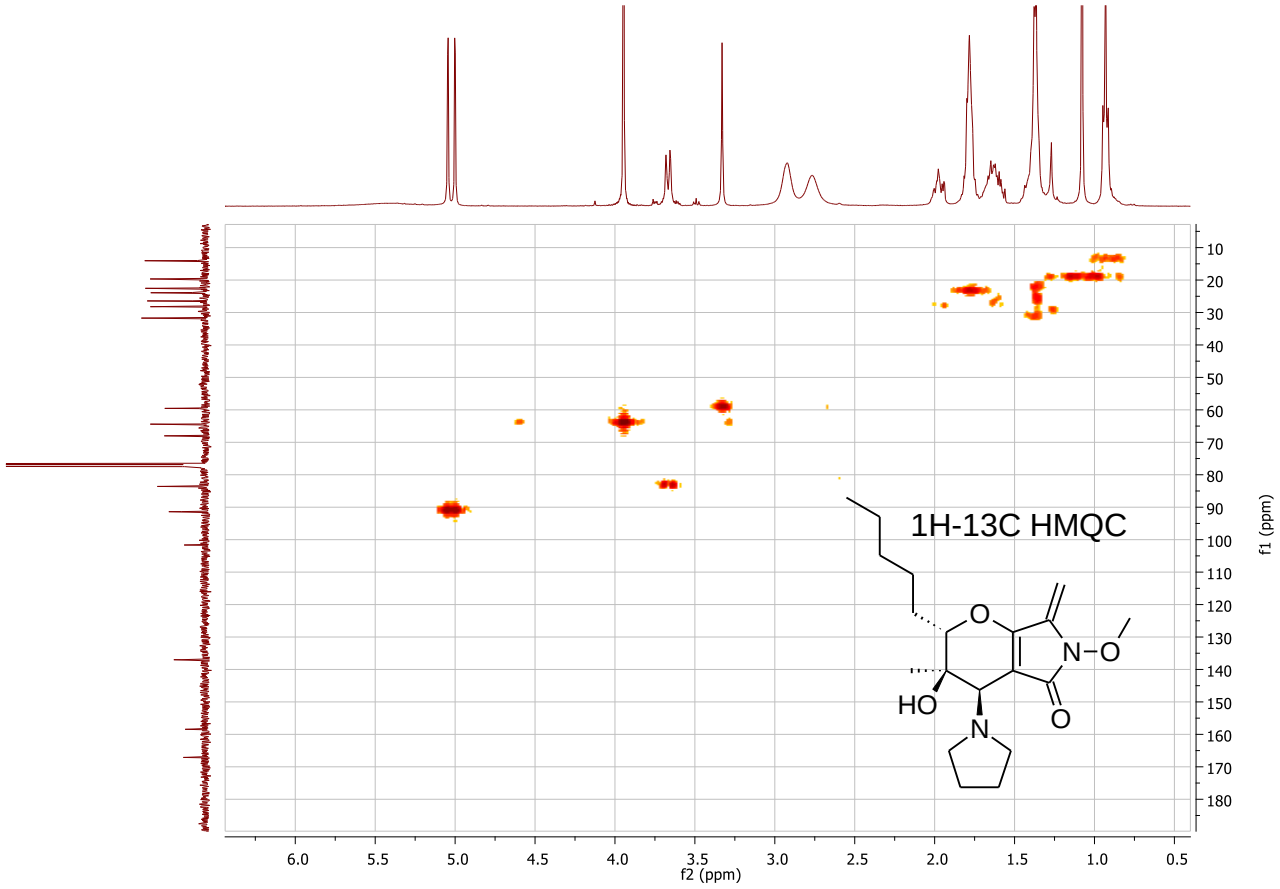
14.04
HO
N
O
N–O
f1 (ppm)


1H-1H ROESY
HO
N
O
N–O
f2 (ppm)
f1 (ppm)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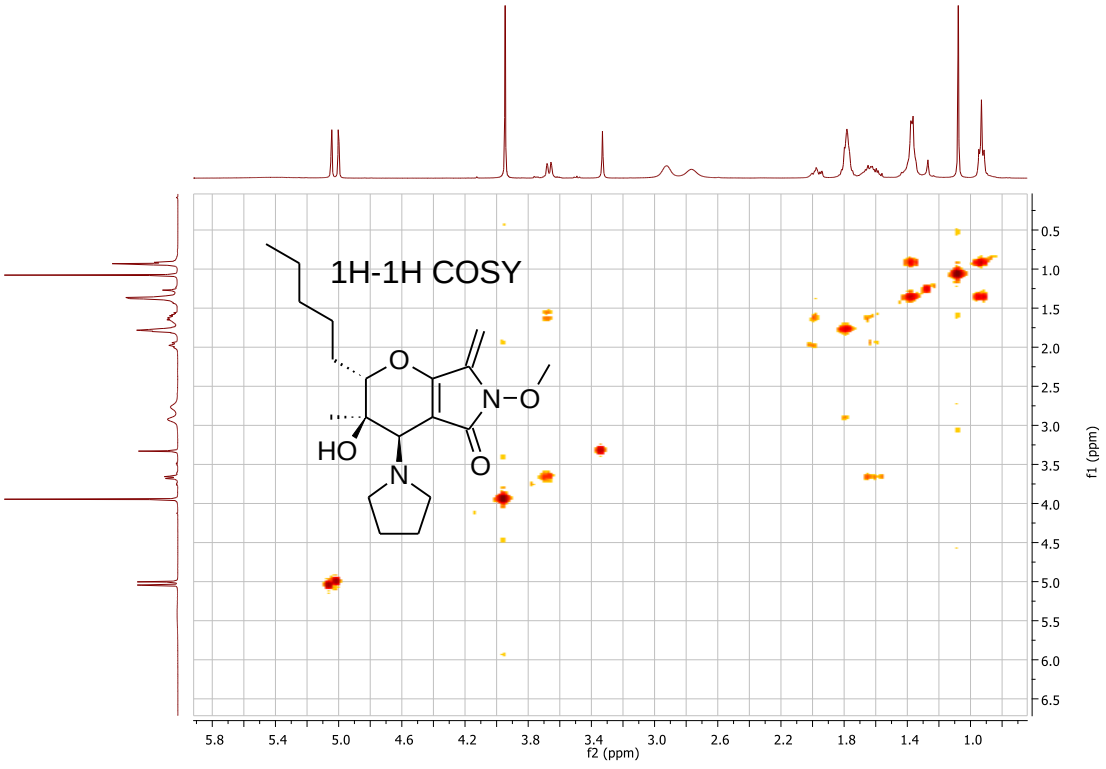
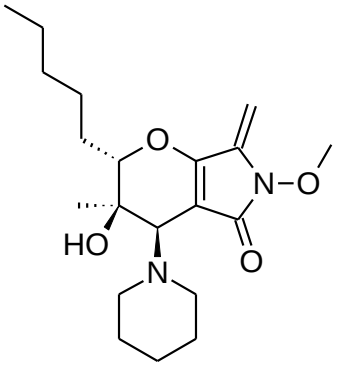
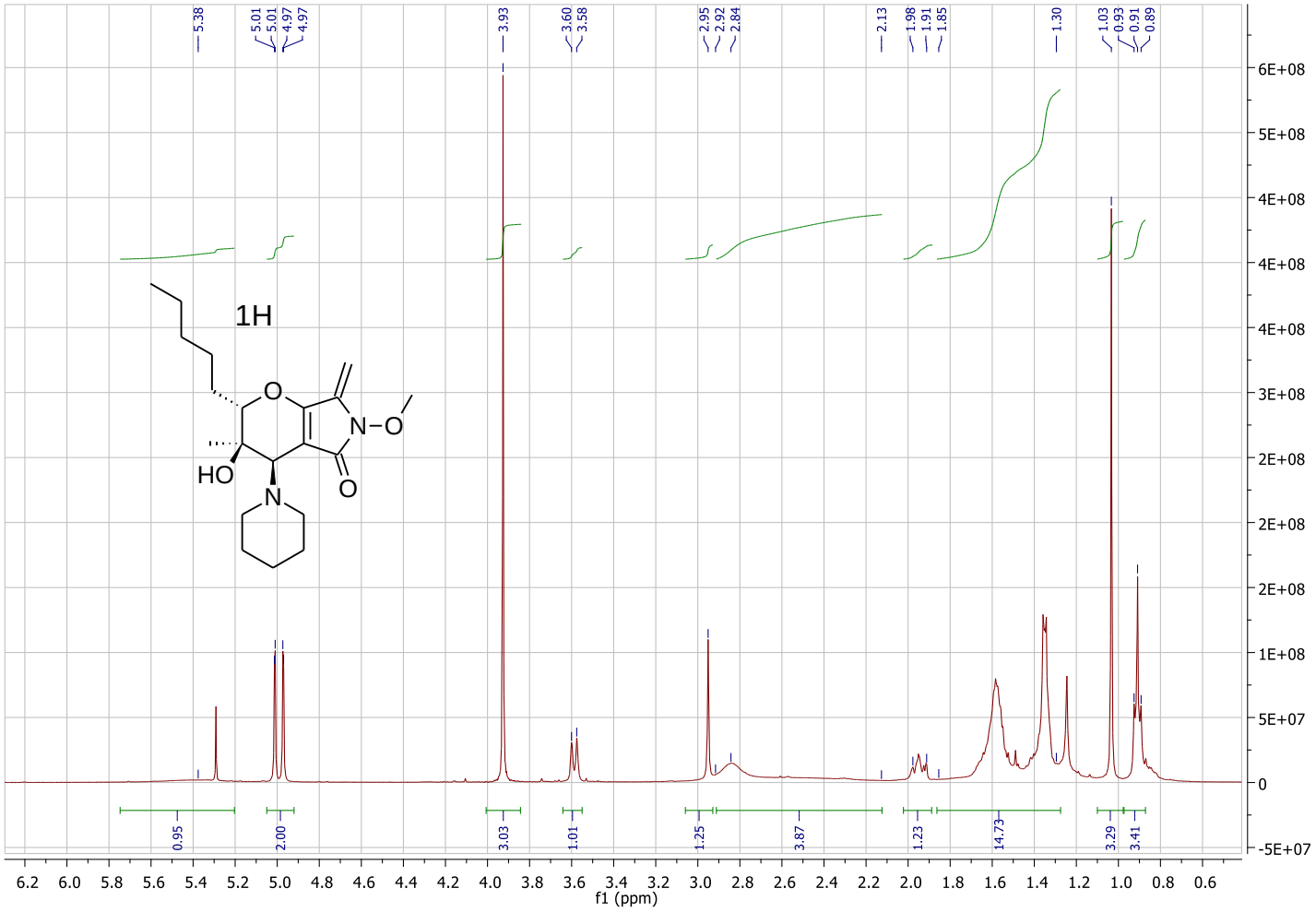
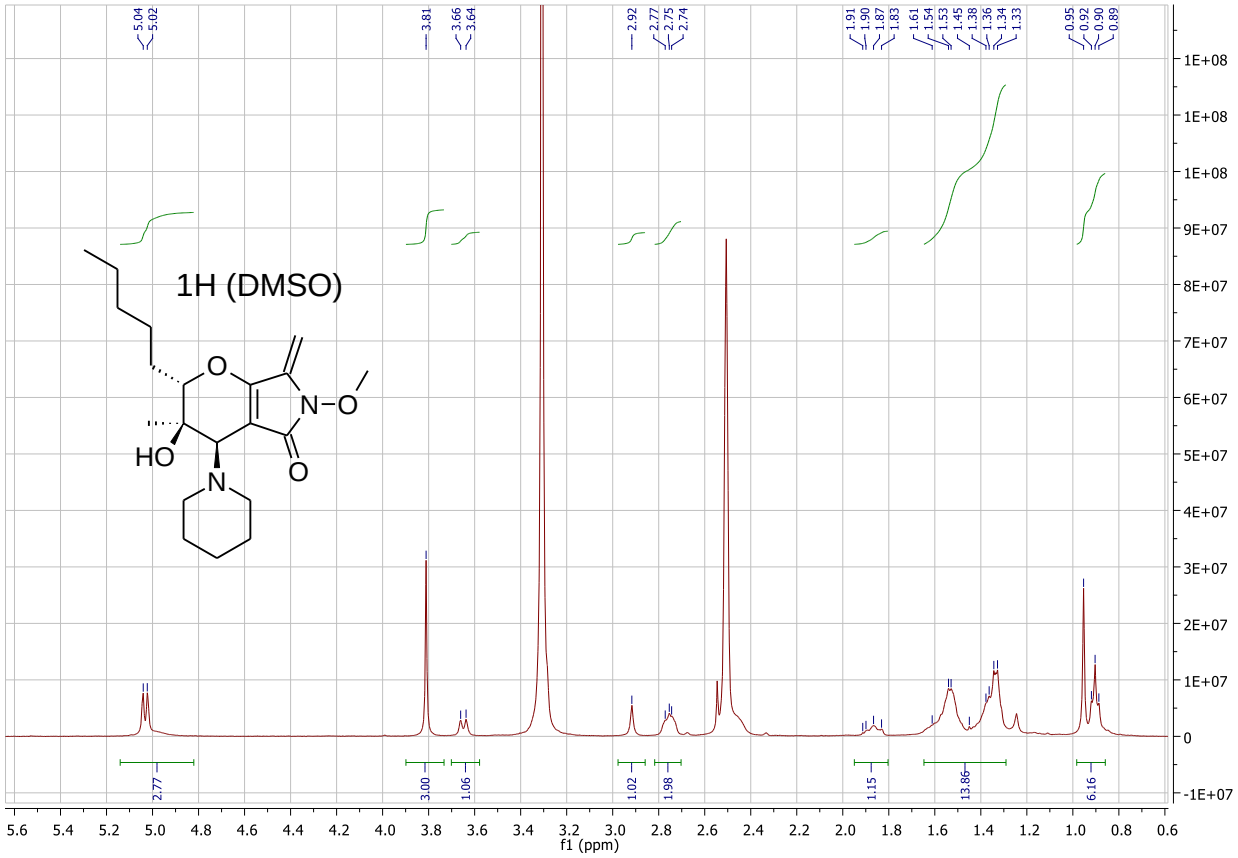
HO
O
N
N–O
O
f2 (ppm)
f1 (ppm)

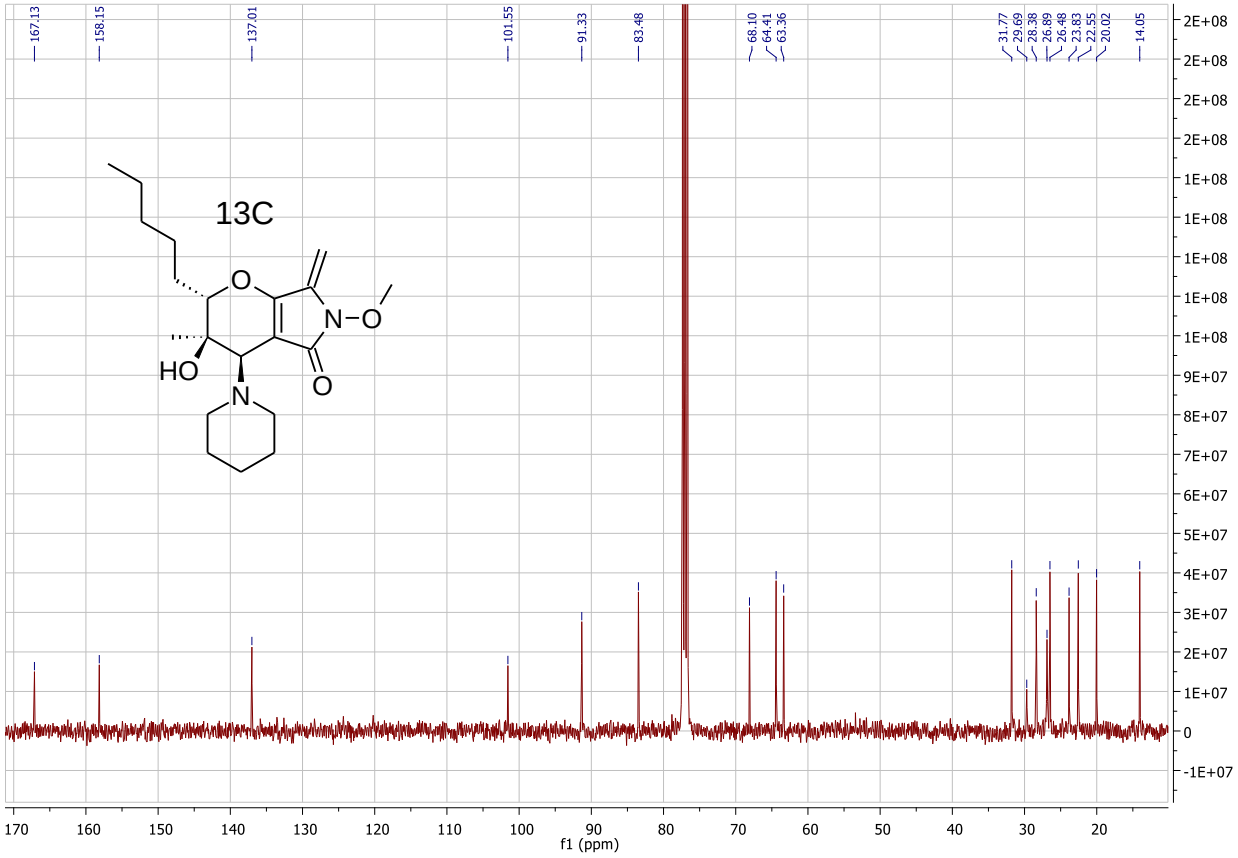

1H-1H COSY
HO
O
N
N–O
O
f2 (ppm)
f1 (ppm)

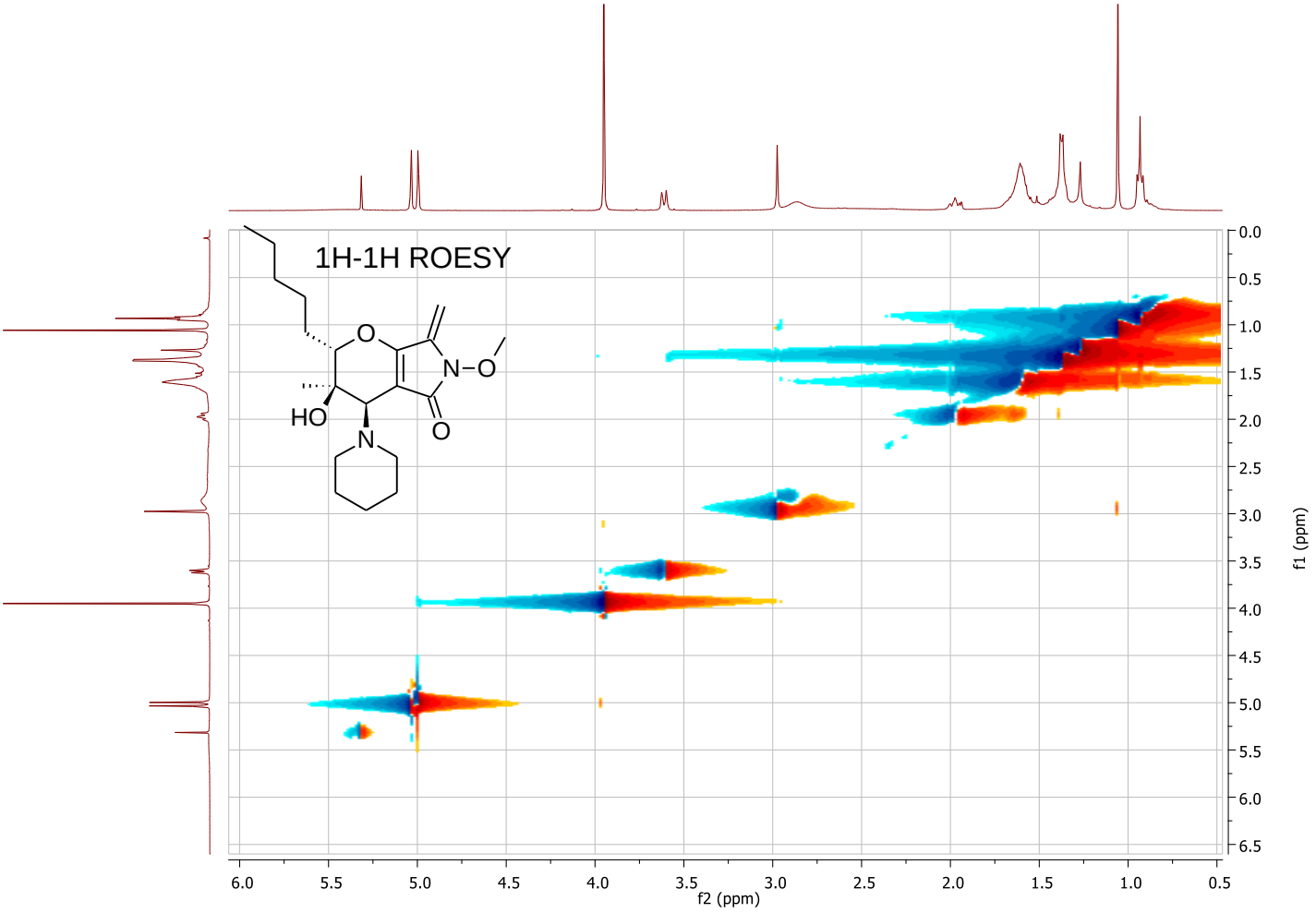
(2*S*,3*R*,4*R*)-3-Hydroxy-6-methoxy-3-methyl-7-methylidene-2-pentyl-4-(piperidin-1-yl)-3,4,6,7-tetrahydropyrano[2,3-*c*]pyrrol-5(2*H*)-one 4



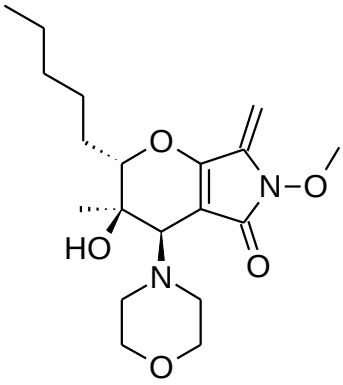


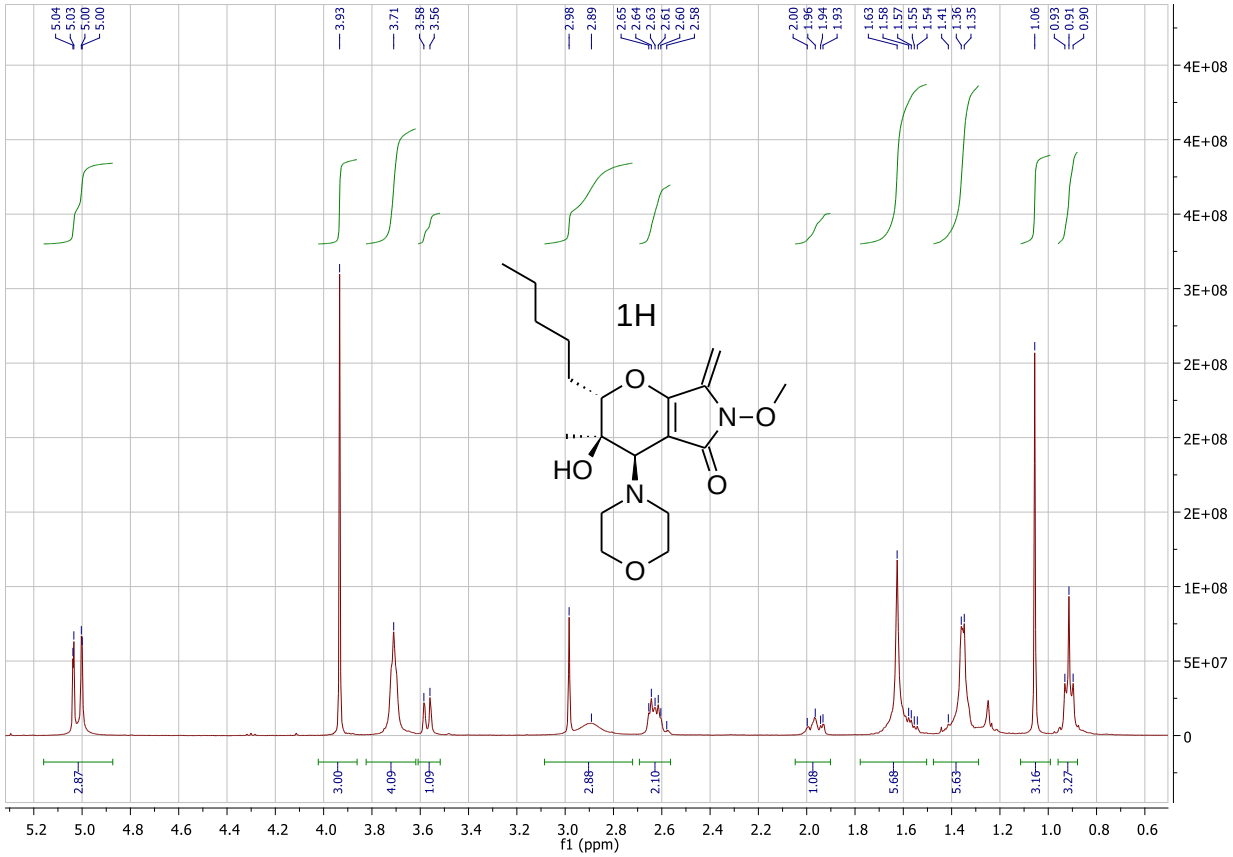

1H (DMSO)
f1 (ppm)

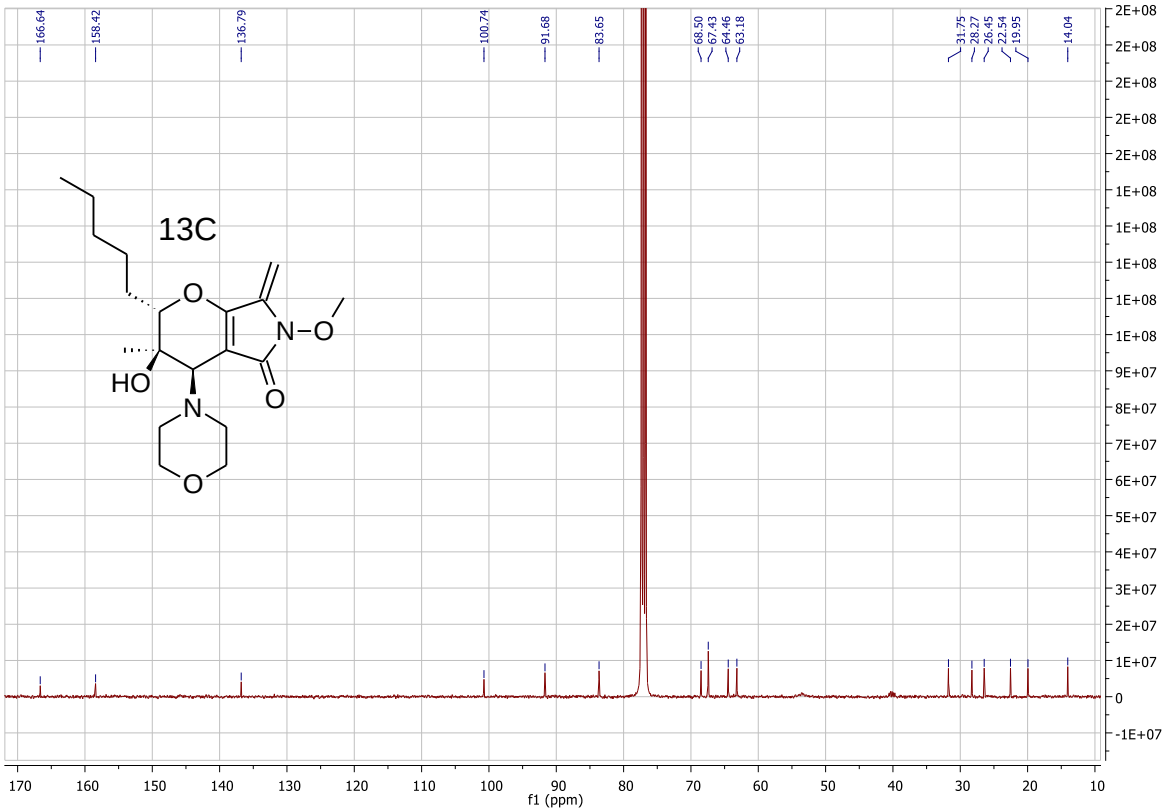

13C
f1 (ppm)


1H-1H ROESY
HO
N
O
N–O
O
f2 (ppm)
f1 (ppm)
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
6.5

(2*S*,3*R*,4*R*)-3-Hydroxy-6-methoxy-3-methyl-7-methylidene-4-(morpholin-4-yl)-2-pentyl-3,4,6,7-tetrahydropyrano[2,3-*c*]pyrrol-5(2*H*)-one 5






13C
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136.79
100.74
91.68
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68.50
67.43
64.46
63.18
31.75
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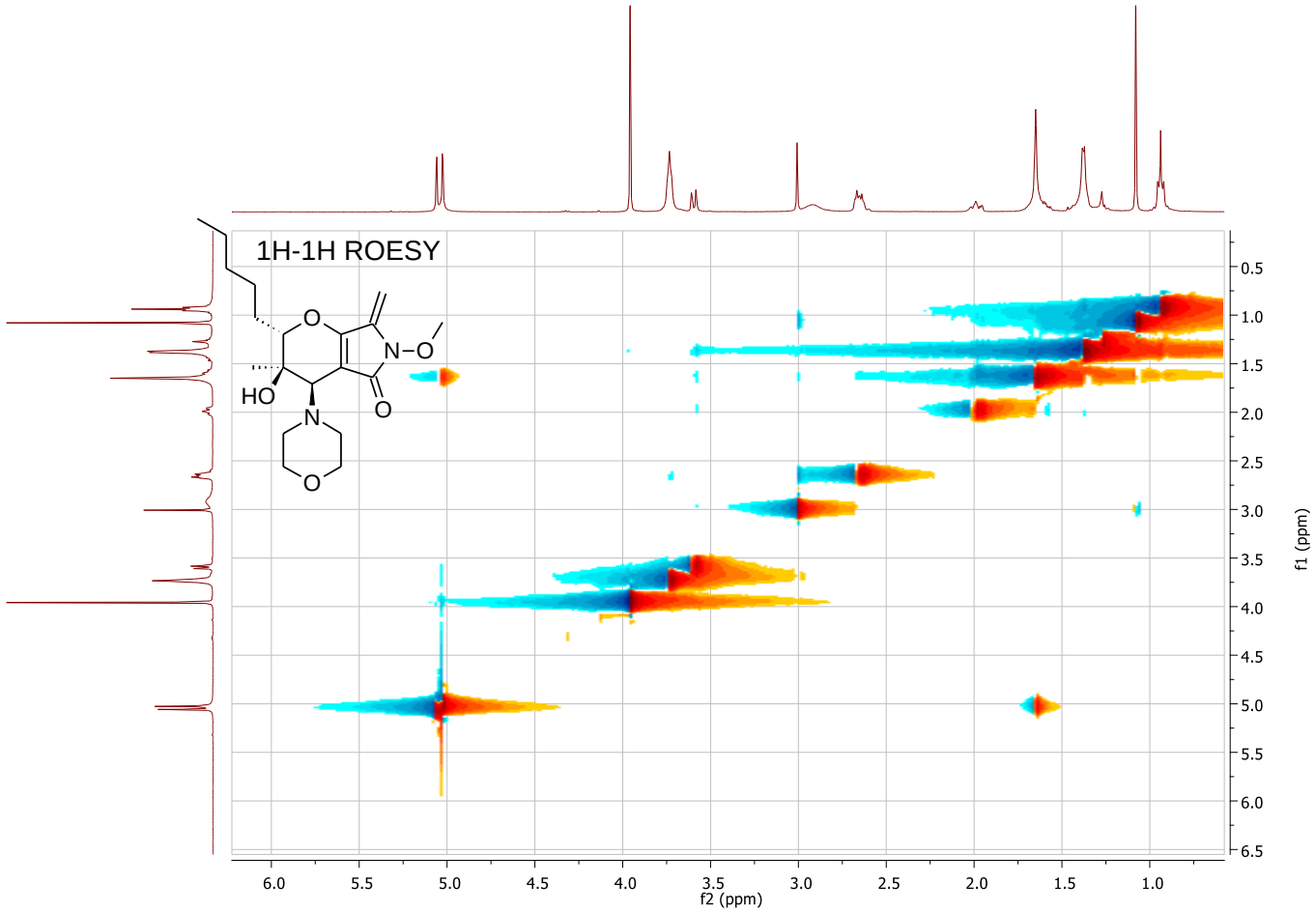
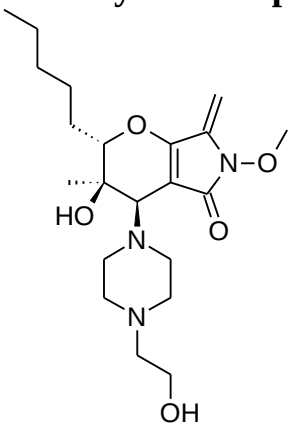
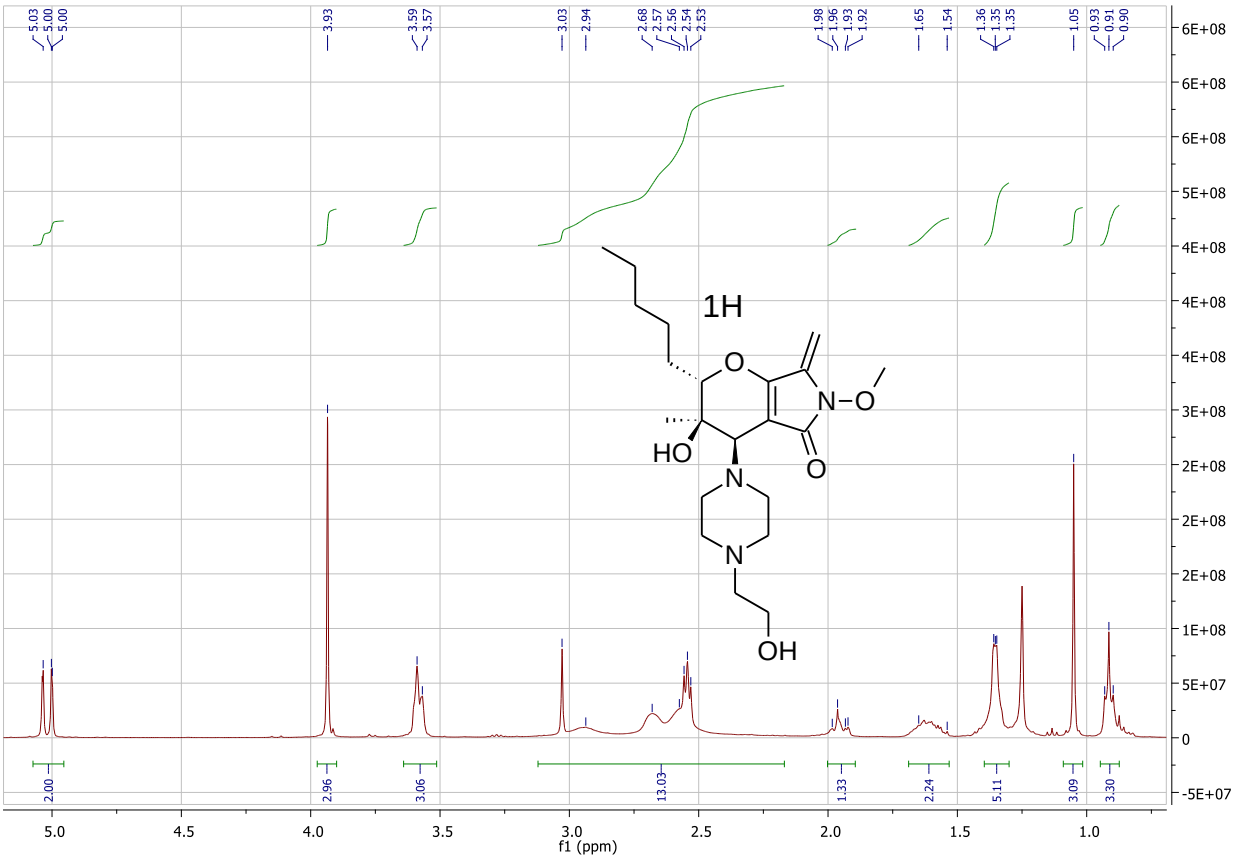
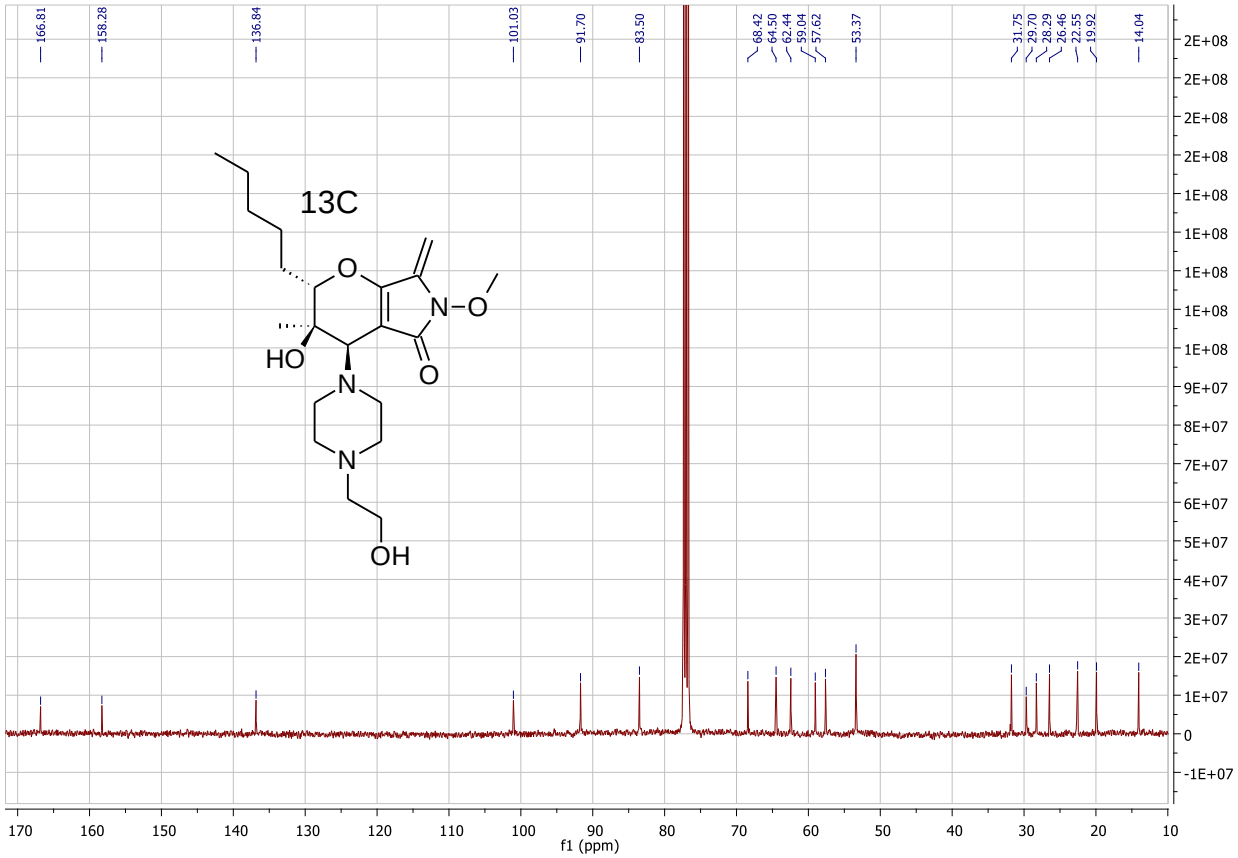
26.45
22.54
19.95
14.04
f1 (ppm)


1H-1H ROESY
f2 (ppm)
f1 (ppm)

(2*S*,3*R*,4*R*)-3-Hydroxy-4-[4-(2-hydroxyethyl)piperazin-1-yl]-6-methoxy-3-methyl-7-methylidene-2-pentyl-3,4,6,7-tetrahydropyrano[2,3-*c*]pyrrol-5(2*H*)-one 6



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91.70
83.50
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64.50
62.44
59.04
57.62
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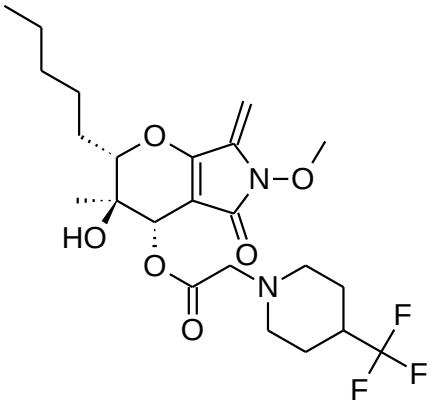
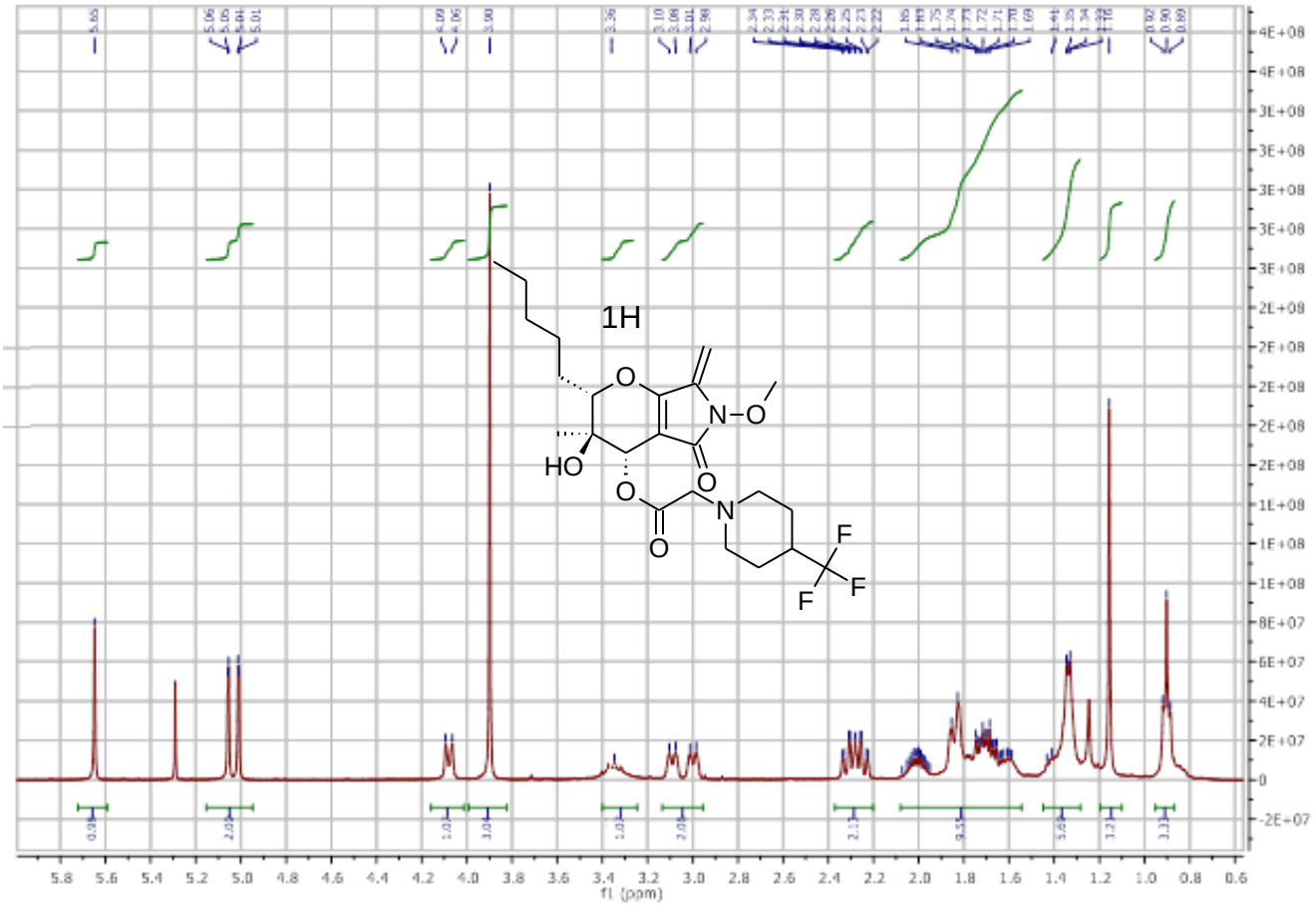
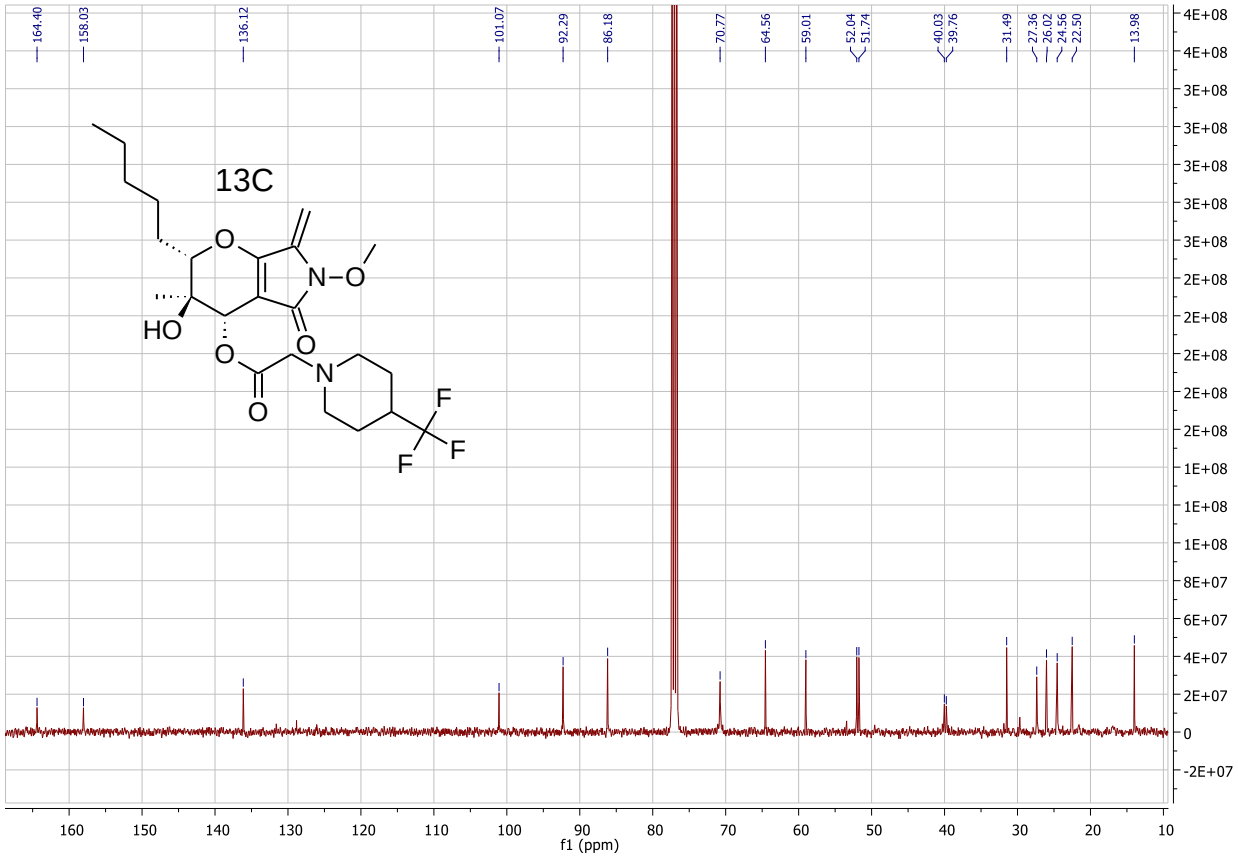
29.70
28.29
26.46
22.55
19.92
14.04
13C
O
N–O
HO
O
N
N
OH
2E+08
2E+08
2E+08
2E+08
1E+08
1E+08
1E+08
1E+08
1E+08
1E+08
9E+07
8E+07
7E+07
6E+07
5E+07
4E+07
3E+07
2E+07
1E+07
0
-1E+07
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

(2*S*,3*S*,4*S*)-3-Hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl [4-(trifluoromethyl)piperidin-1-yl]acetate 7



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101.07
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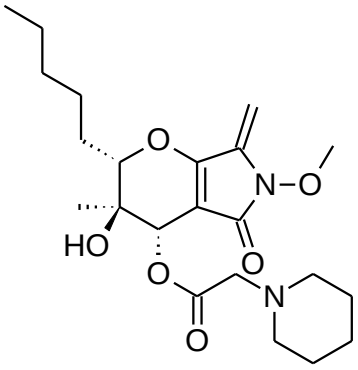
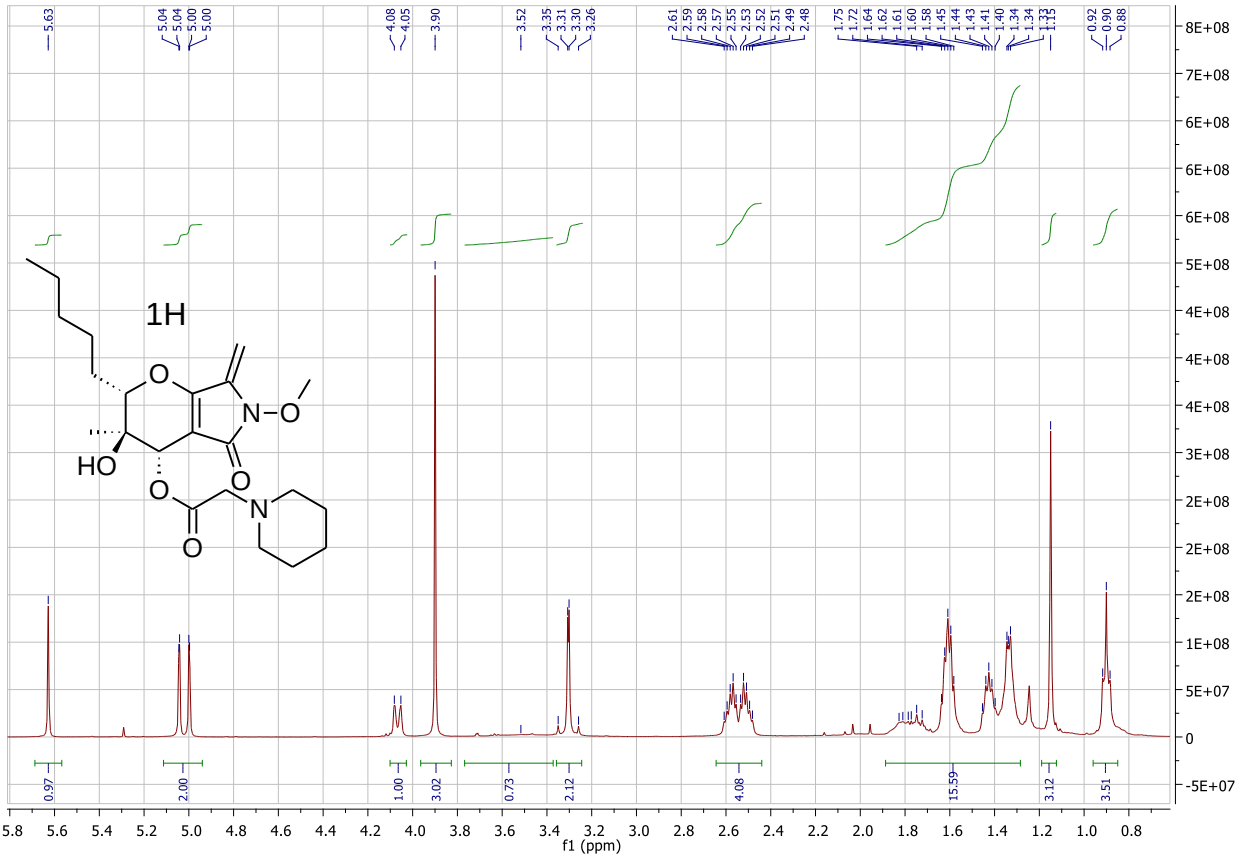
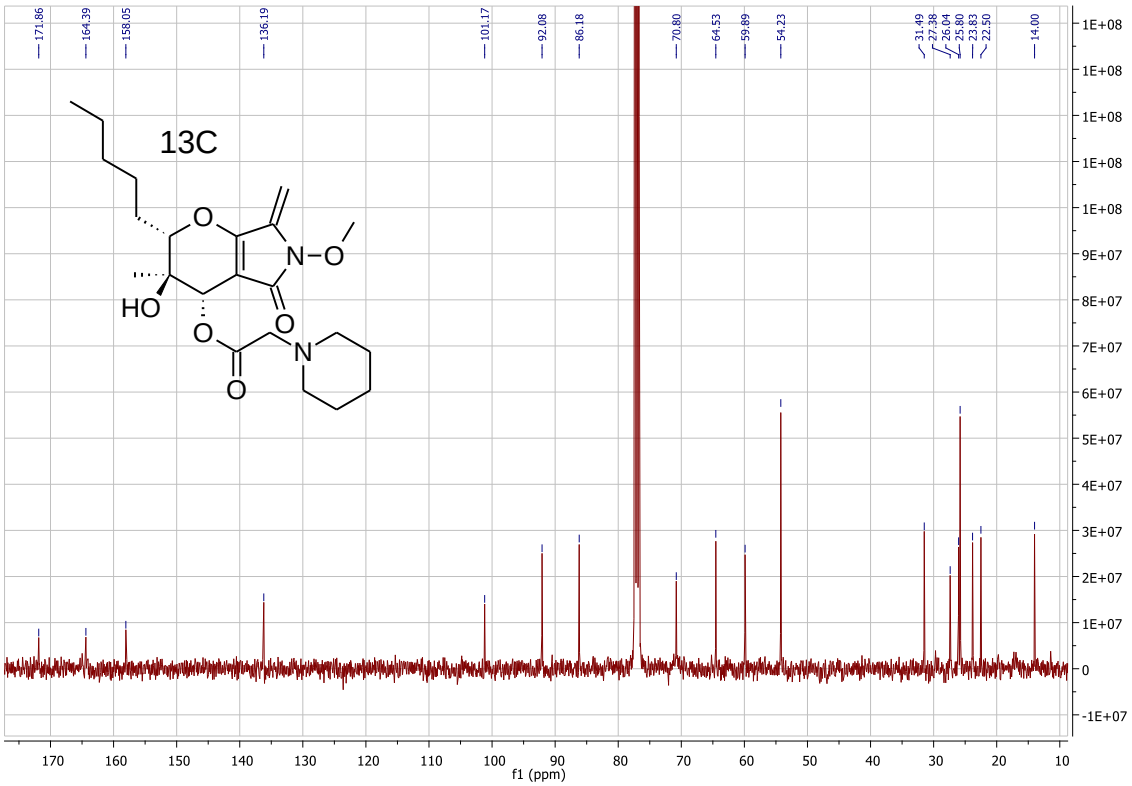
86.18
70.77
64.56
59.01
52.04
51.74
40.03
39.76
31.49
27.36
26.02
24.56
22.50
13.98
13C
HO
O
N–O
O
O
O
N
F
F
F
4E+08
3E+08
2E+08
1E+08
8E+07
6E+07
4E+07
2E+07
0
-2E+07
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

(2*S*,3*S*,4*S*)-3-Hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl piperidin-1-ylacetate 8






13C
171.86
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158.05
136.19
101.17
92.08
86.18
70.80
64.53
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54.23
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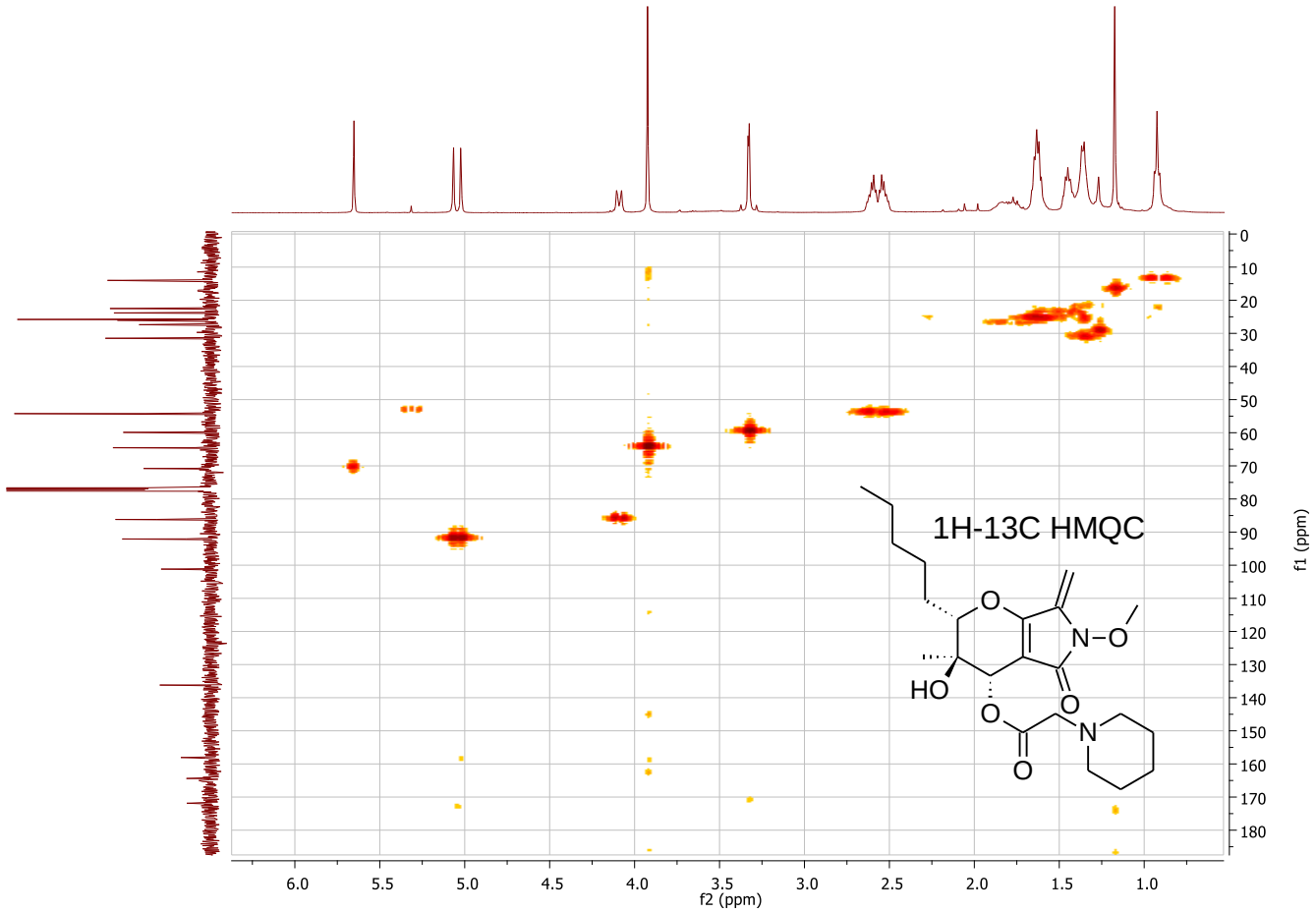
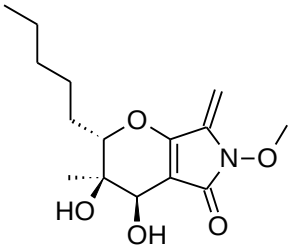
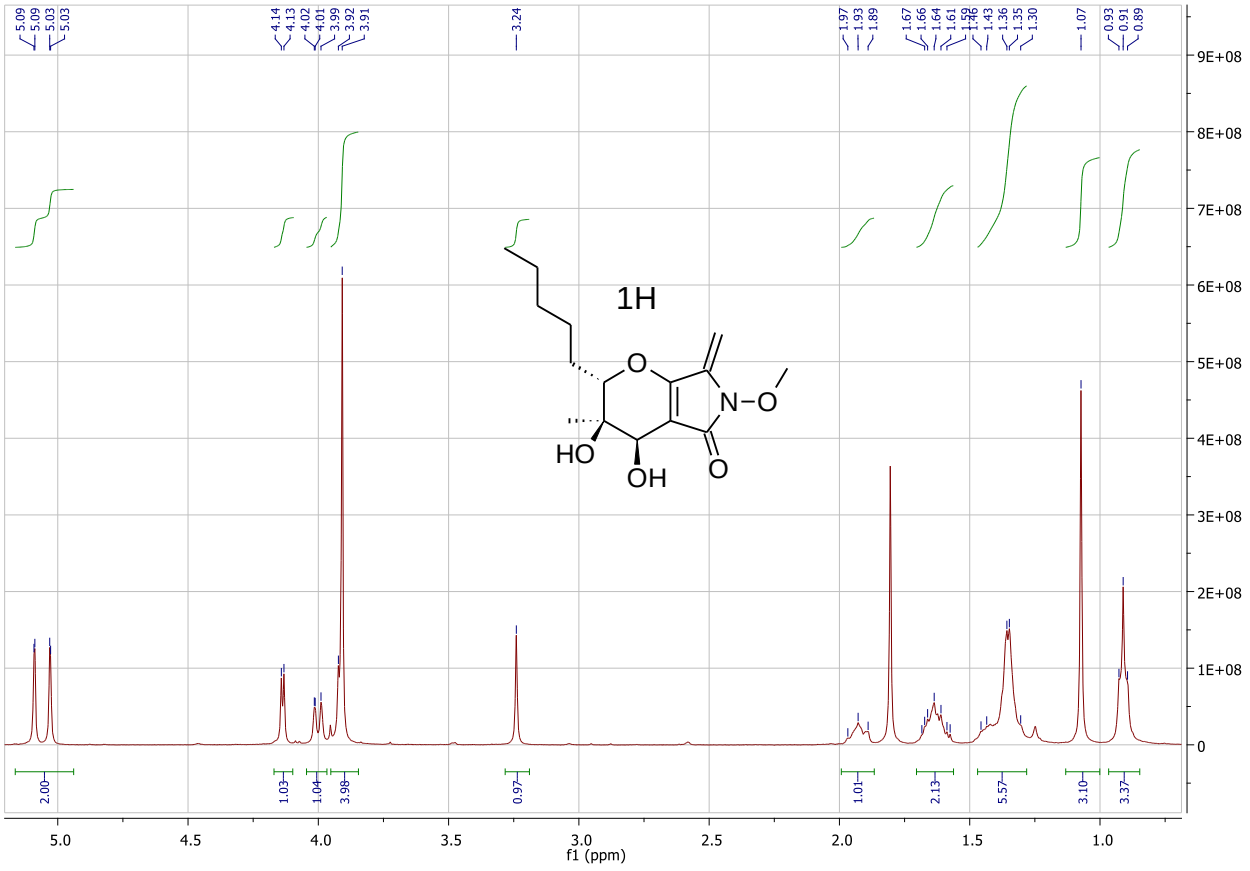
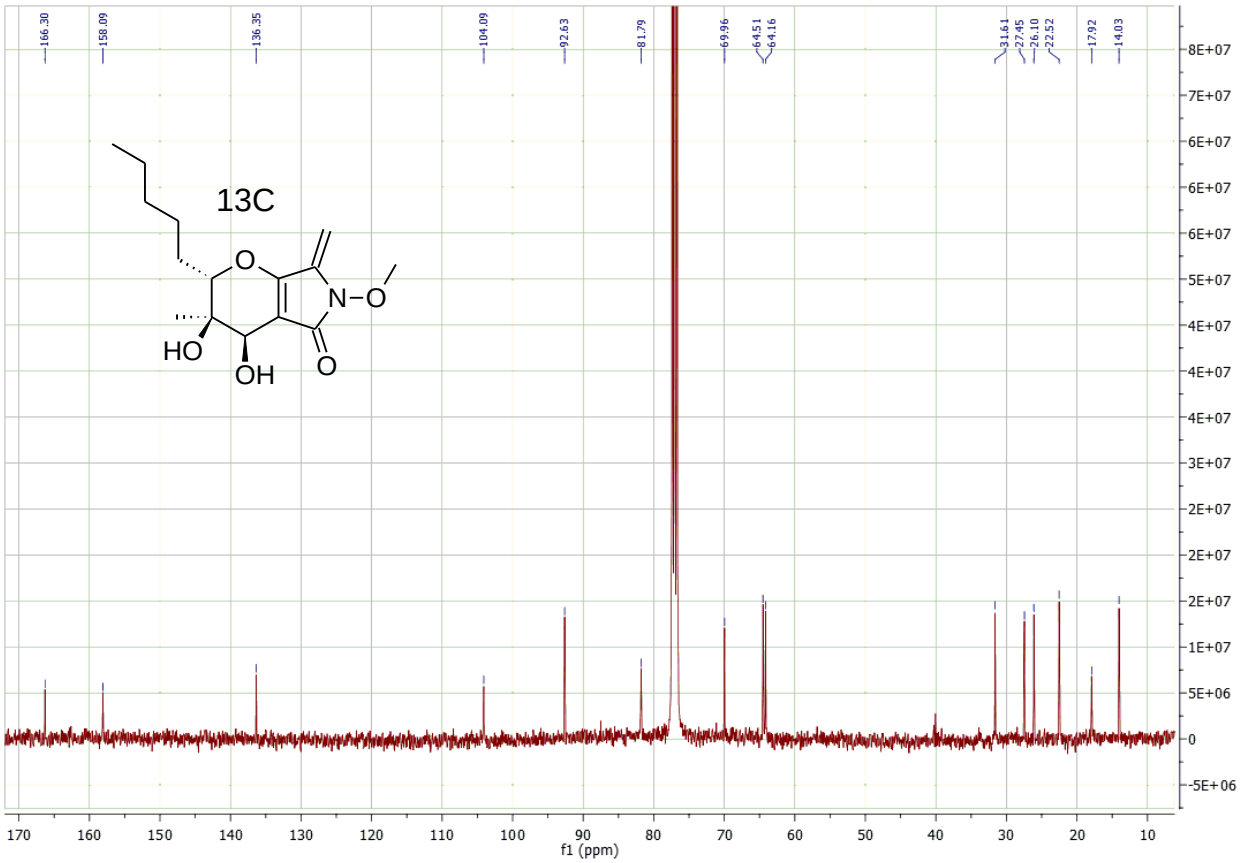
27.38
26.04
25.80
23.83
22.50
14.00
f1 (ppm)


1H-13C HMQC
f2 (ppm)
f1 (ppm)

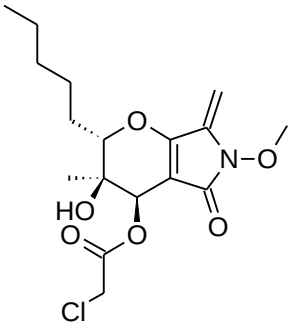
Phaeosphaeride B

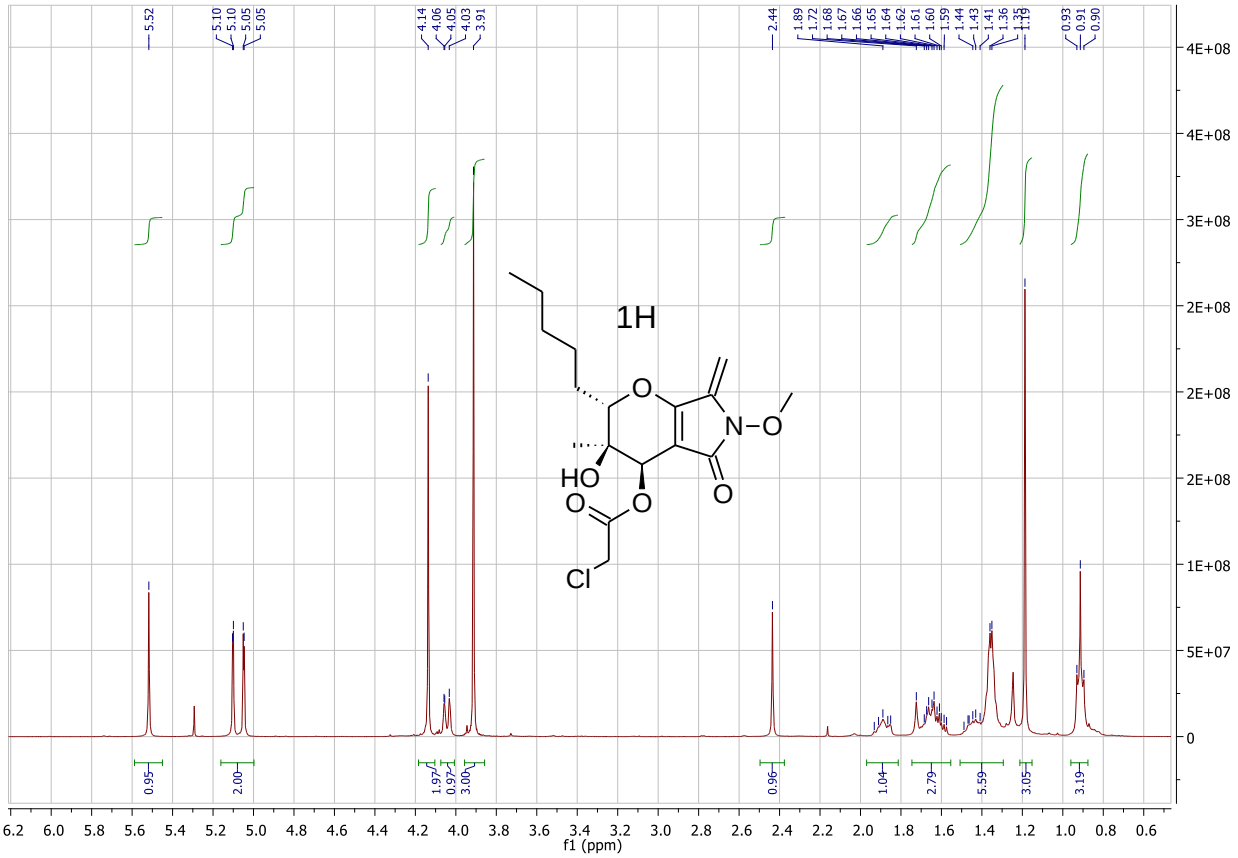


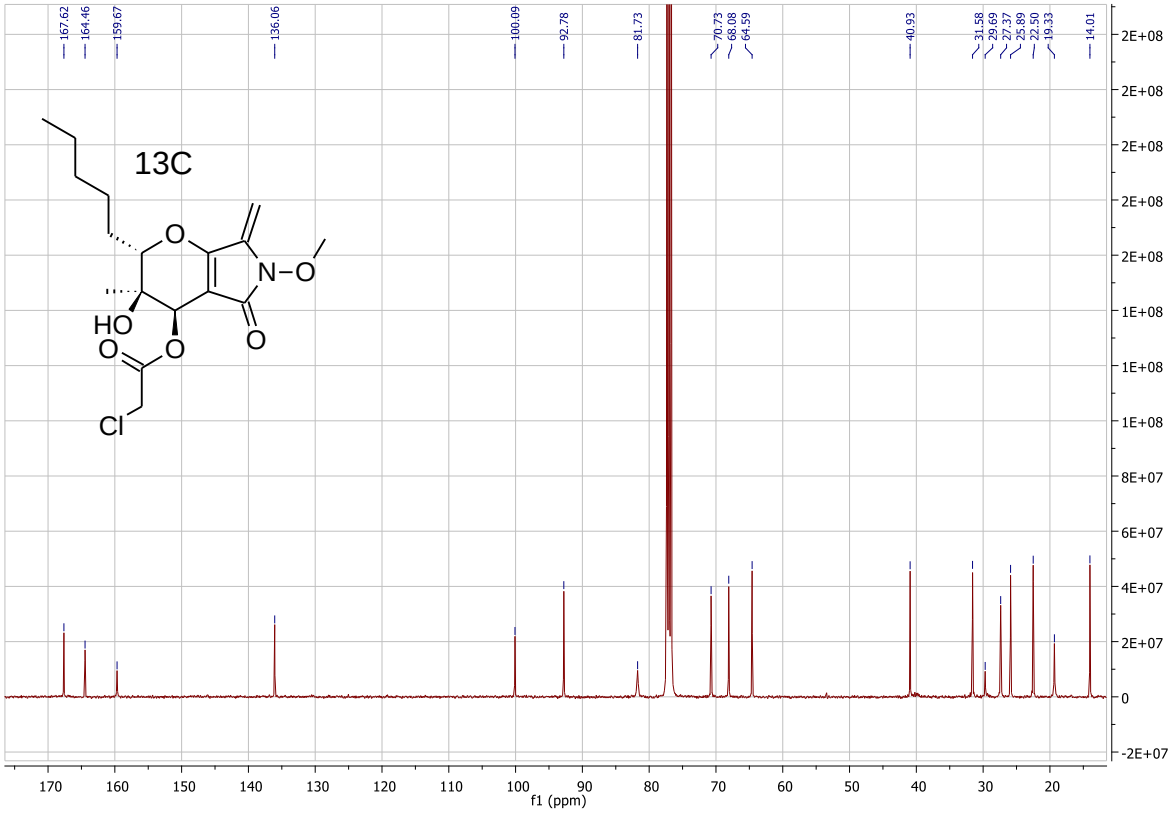


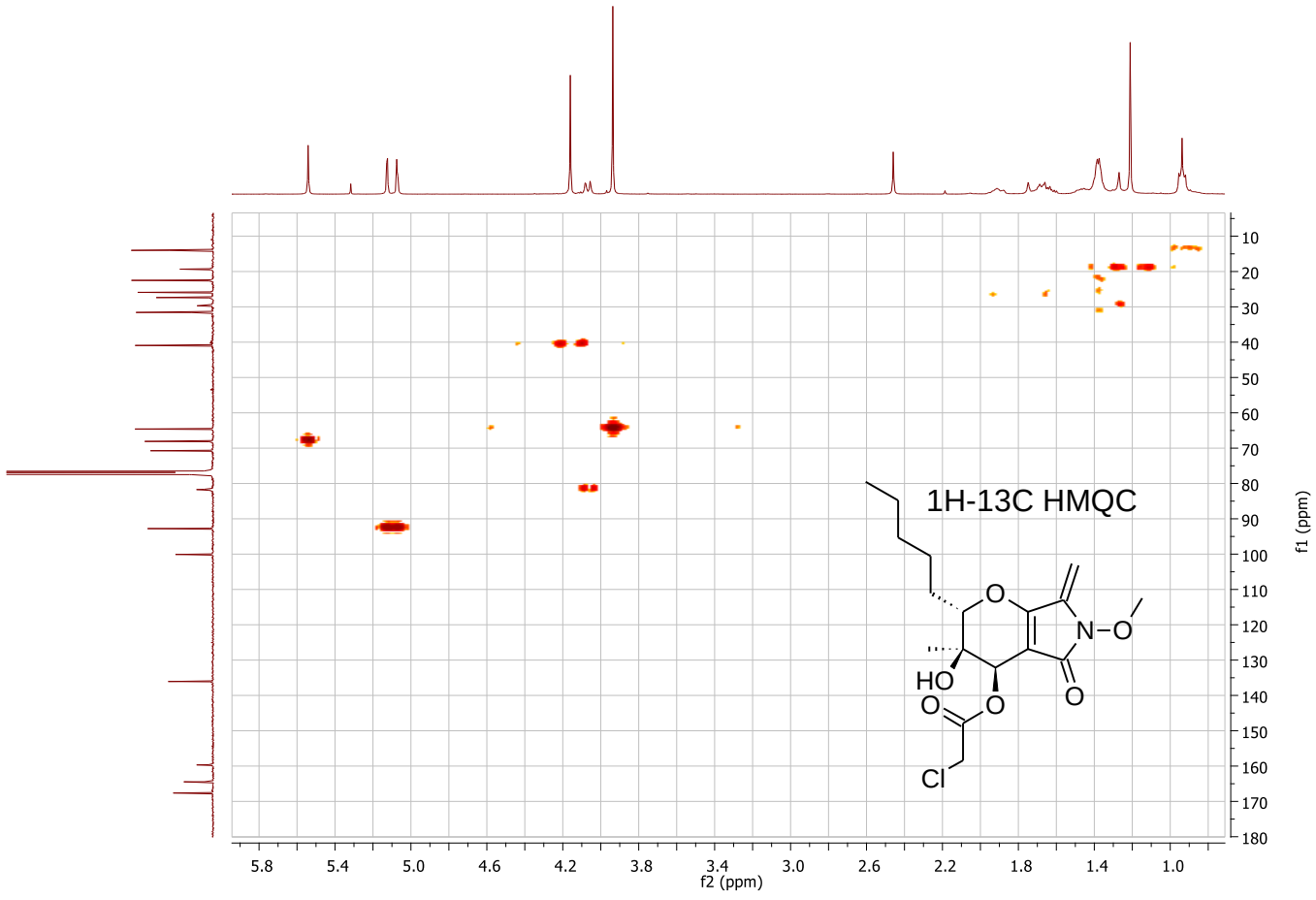

166.30
158.09
136.35
104.09
92.63
81.79
69.96
64.51
64.16
31.61
27.45
26.10
22.52
17.92
14.03
13C
O
N–O
HO
OH
O
8E+07
7E+07
6E+07
6E+07
6E+07
5E+07
4E+07
4E+07
4E+07
3E+07
2E+07
2E+07
2E+07
1E+07
5E+06
0
-5E+06
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

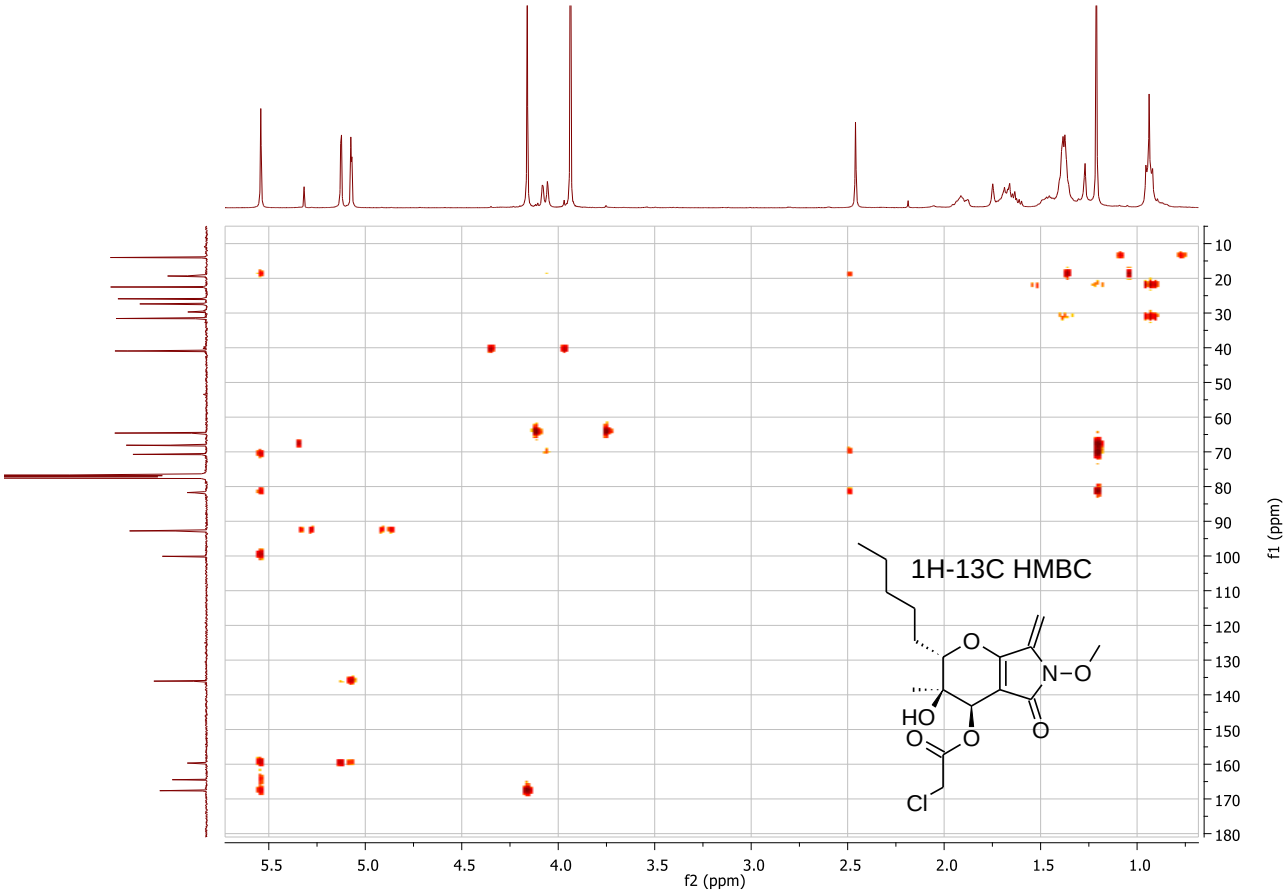
(2*S*,3*S*,4*R*)-3-Hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl chloroacetate 9

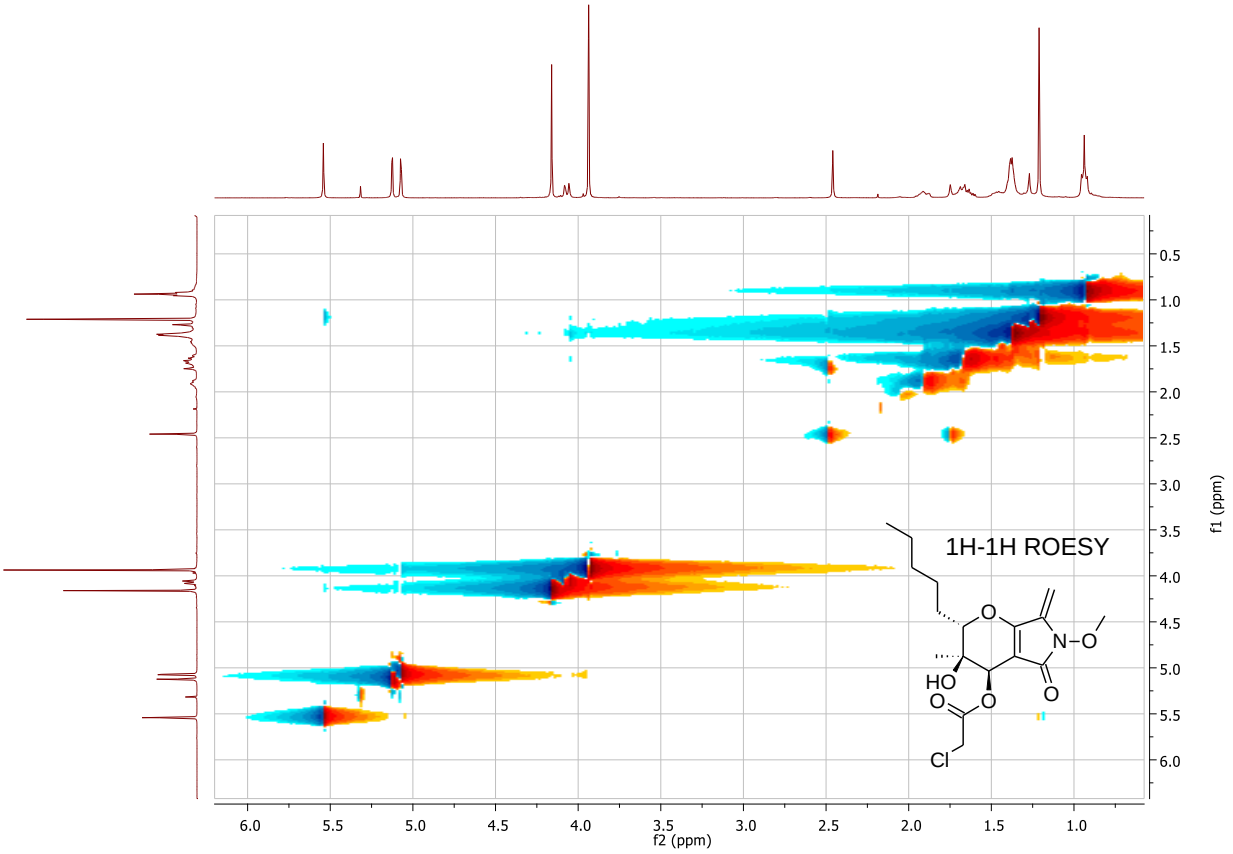





13C
167.62
164.46
159.67
136.06
100.09
92.78
81.73
70.73
68.08
64.59
40.93
31.58
29.69
27.37
25.89
22.50
19.33
14.01
f1 (ppm)


1H-13C HMQC
f2 (ppm)
f1 (ppm)


1H-13C HMBC
f2 (ppm)
f1 (ppm)
HO
O
O
O
N–O
O
Cl


1H-1H ROESY
f2 (ppm)
f1 (ppm)
HO
O
O
O
N–O
O
Cl