frontiers

Supplementary Material

Identification of Ellagic Acid Rhamnoside as a Bioactive Component of a Complex Botanical Extract with Anti-biofilm Activity

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1 Supplementary Data

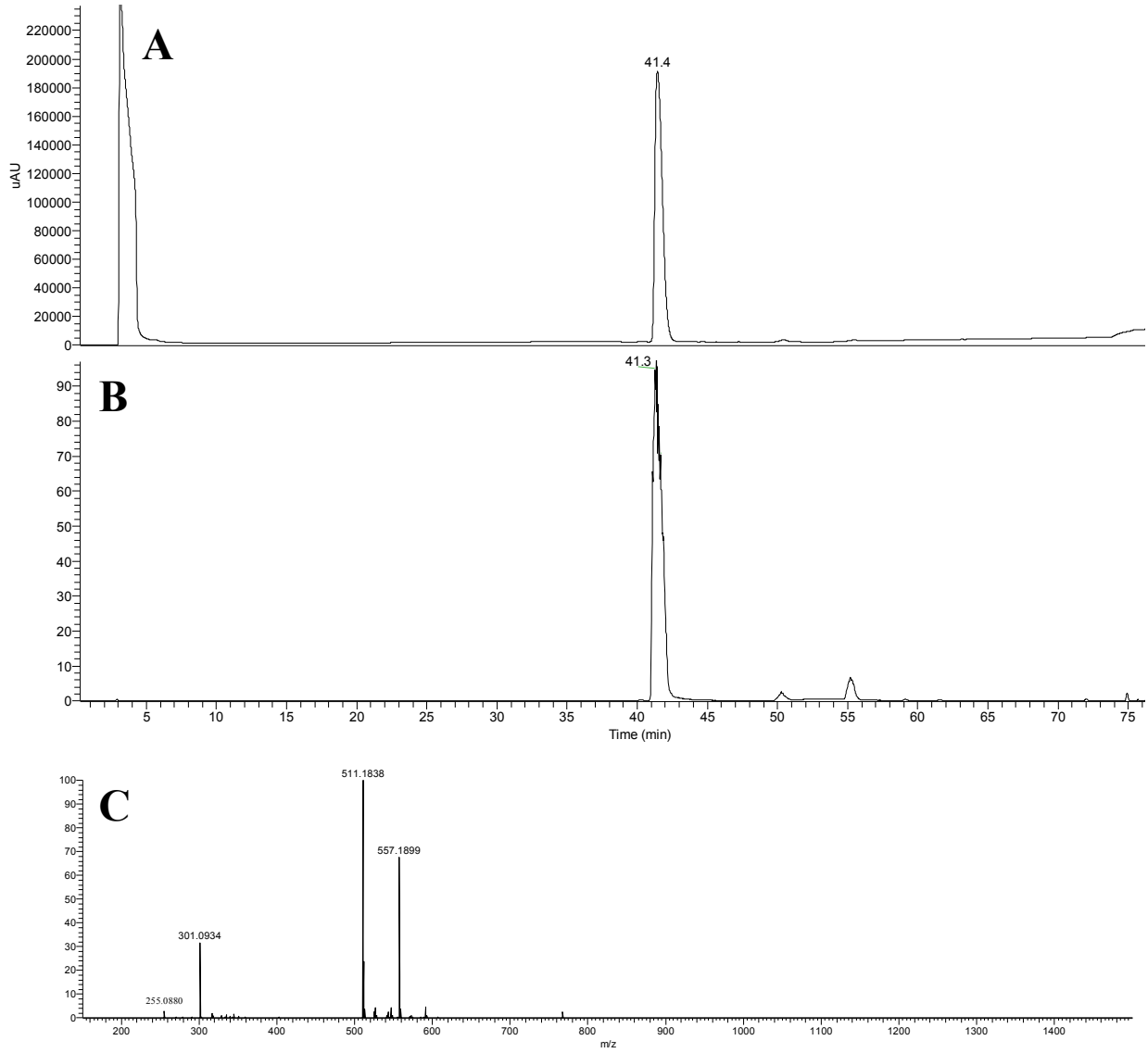


Figure S1: FT-MS ESI negative spectra of (**A**) ellagic acid rhamnoside eluting at 63.3 min, (**B**) ellagic acid xyloside eluting at 62.5 min, and (**C**) ellagic acid xyloside 63.7 min in extract 220D-F2.

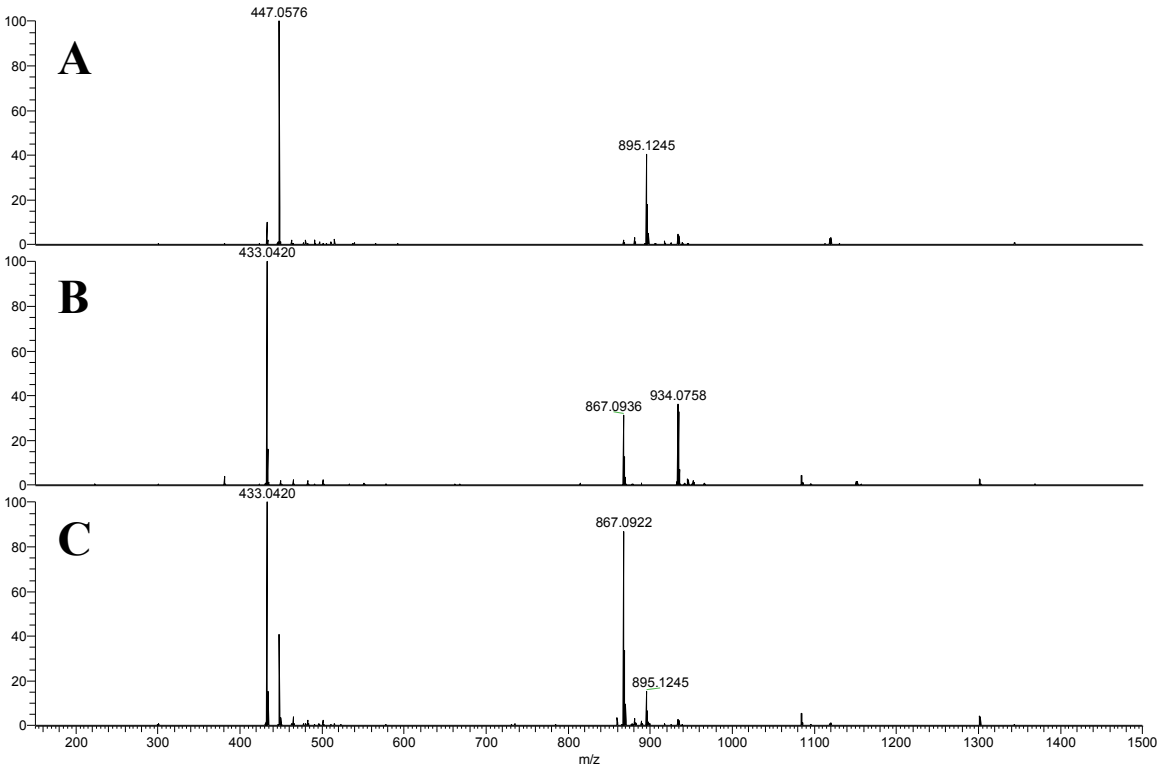


Figure S2: Catechol rhamnoside (**A**) UV-Vis chromatogram 210-500 nm total spectrum, (**B**) base peak FT-MS chromatogram, and (**C**) mass spectra of peak at 41.3 min showing $[M-H]^-$ at *m/z* 255.0880, $[M+formate-H]^-$, $[2M-H]^-$, and $[2M+formate-H]^-$ ions.

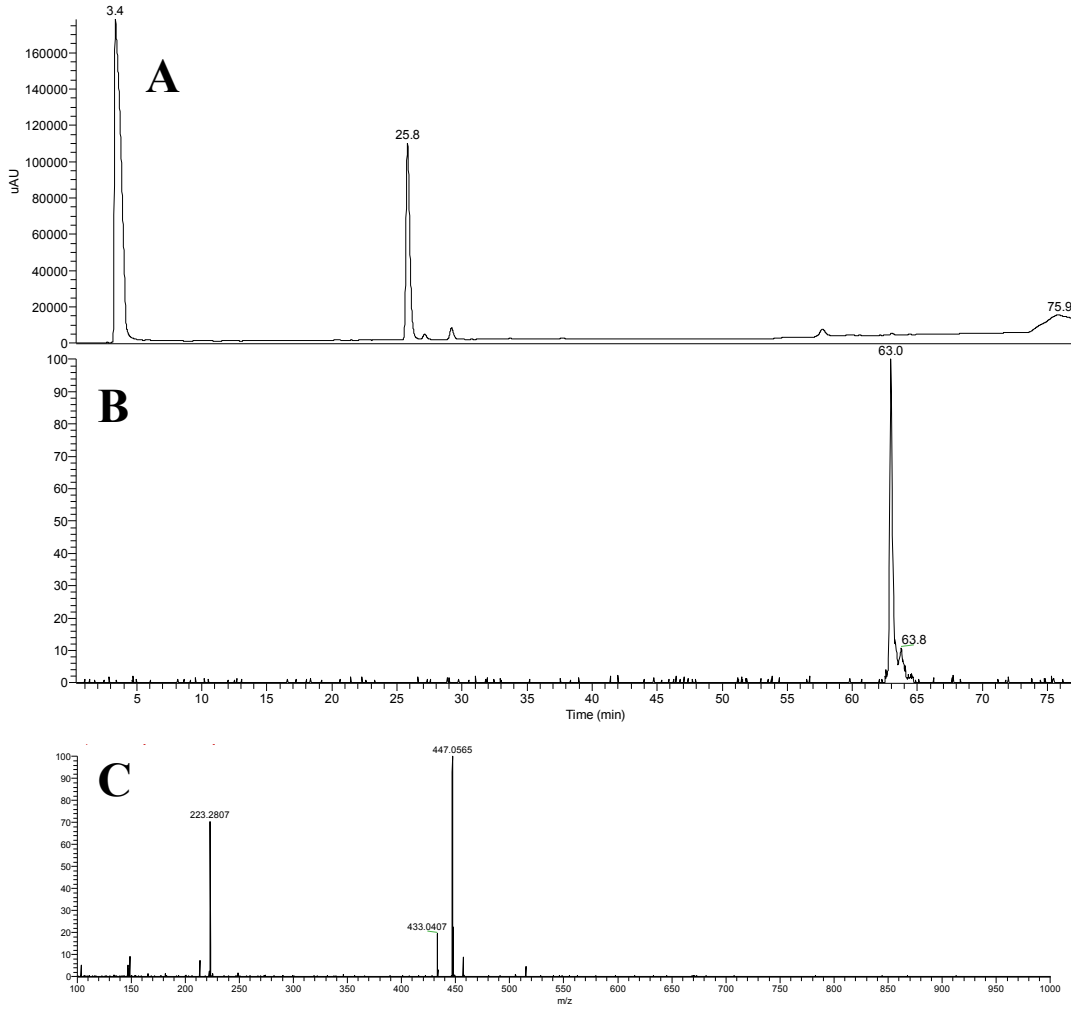


Figure S3: Ellagic acid rhamnoside (**A**) UV-Vis chromatogram 210-500 nm total spectrum, (**B**) FT-MS chromatogram showing *m/z* 447-448, and mass spectra.

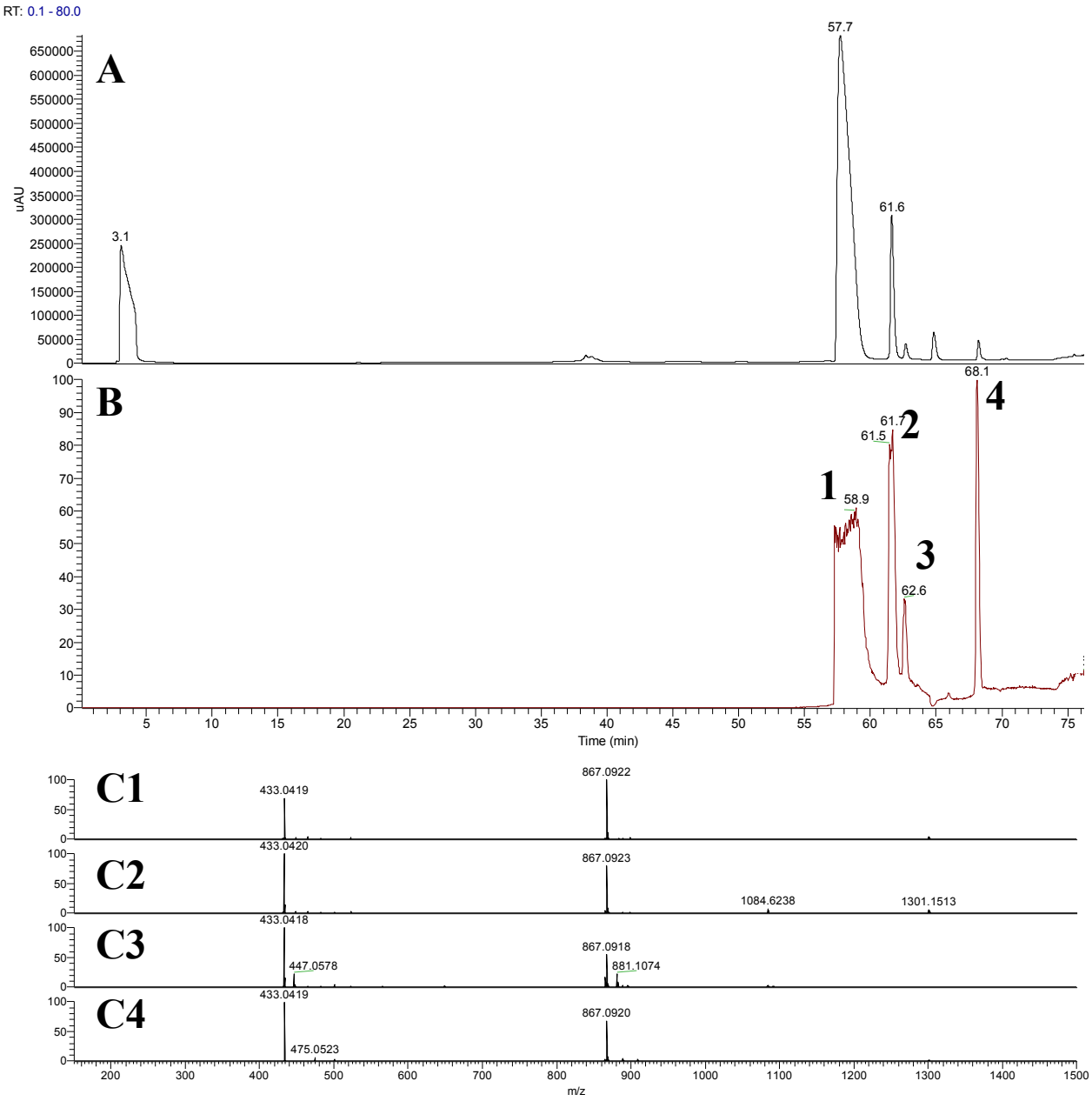


Figure S4: Ellagic acid xyloside (**A**) UV-Vis chromatogram, (**B**) FT-MS chromatogram showing *m/z* 433-434, and (**C**) mass spectra of peaks **1**, **2**, **3**, **4**.

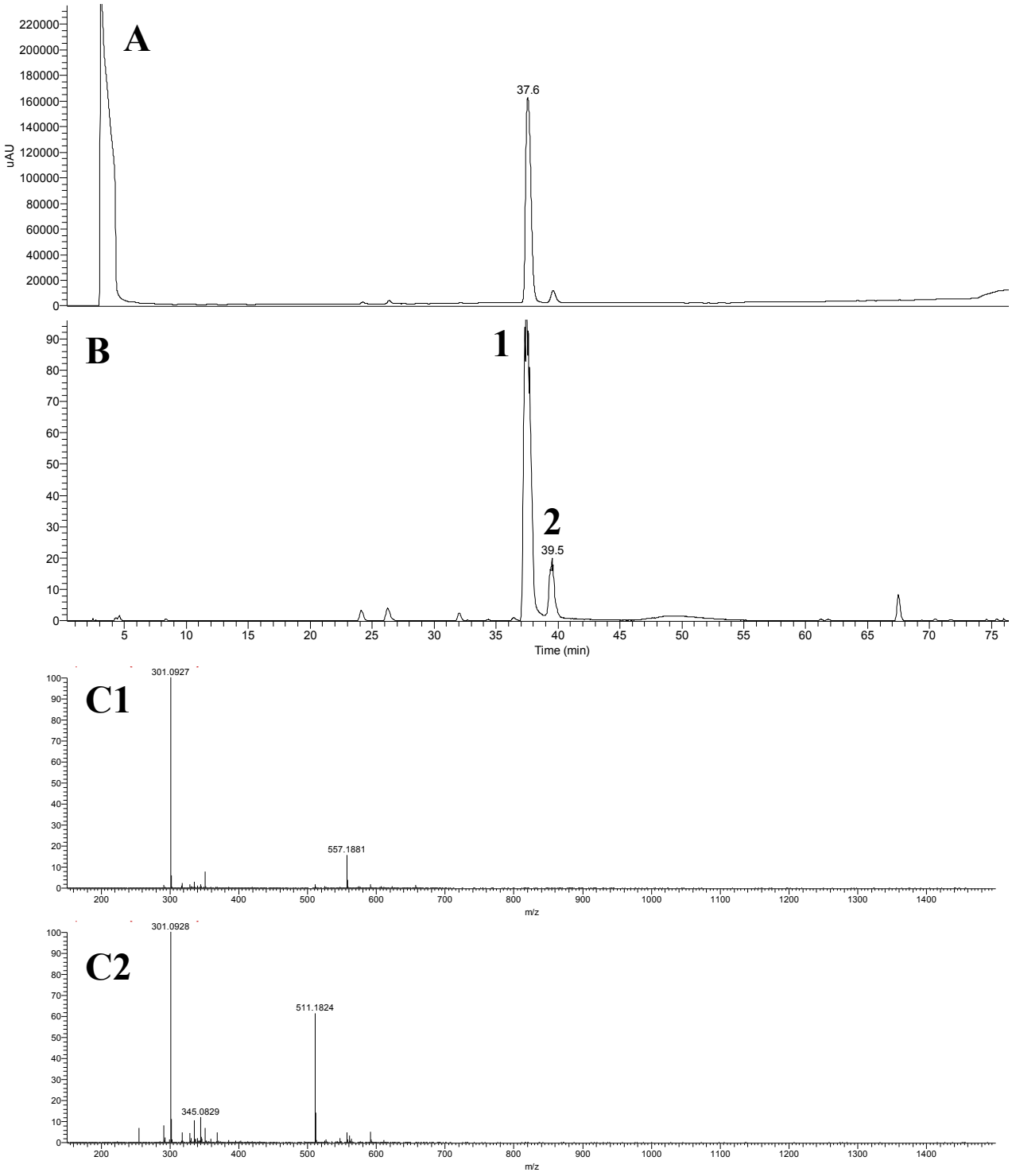


Figure S5: Phenyl glycoside (**A**) UV-Vis chromatogram 210-500 nm total spectrum, (**B**) base peak FT-MS chromatogram, (**C1**) mass spectra of peak at 37.3 min showing $[M+formate-H]^-$ and $[2M+formate-H]^-$ ions at *m/z* 301.0927and 557.1881, respectively, and (**C2**) mass spectra of peak at 39.5 min showing $[M+formate-H]^-$ at *m/z* 301.0928 and $[2M-H]^-$ at *m/z* 511.1824.

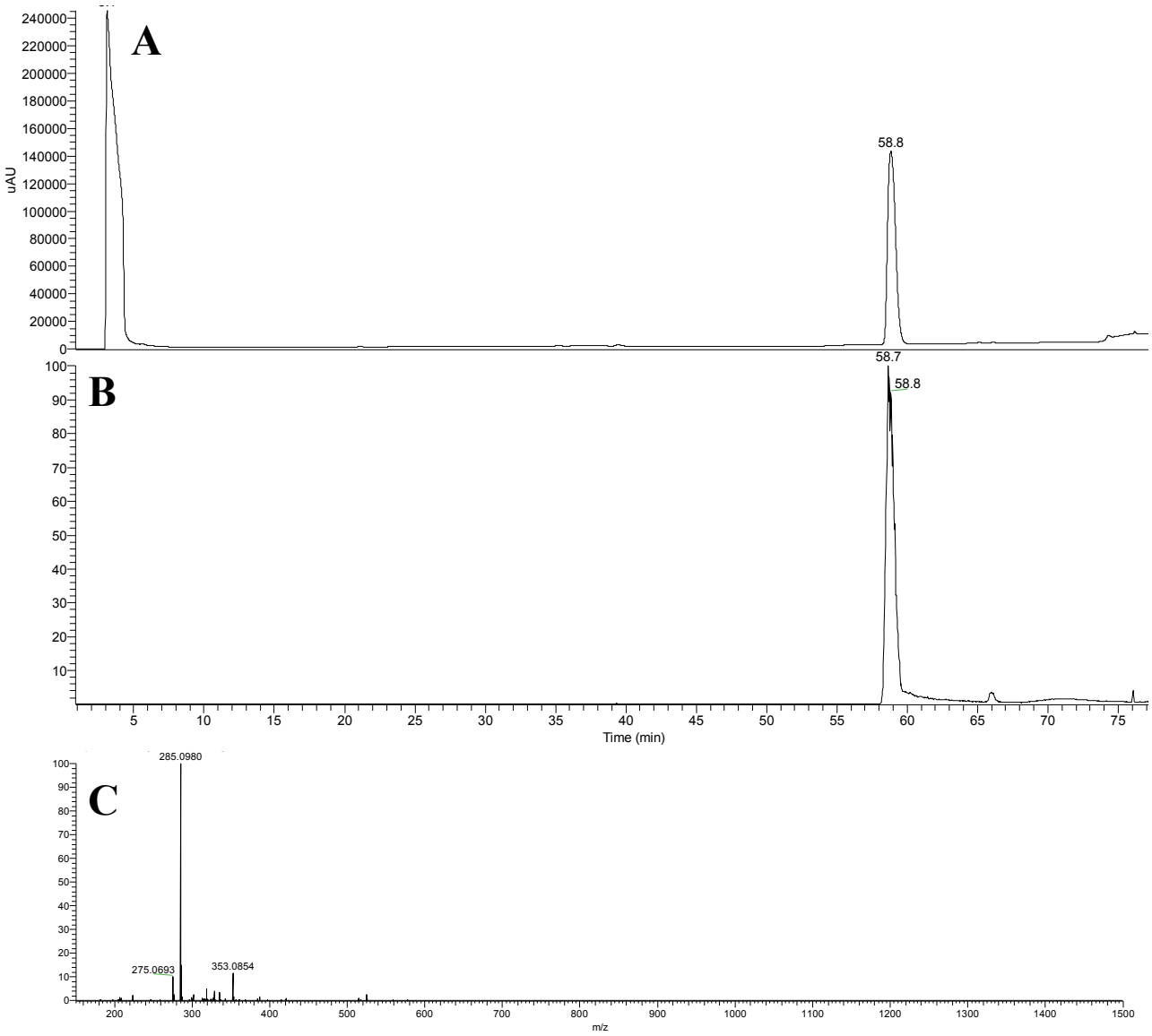


Figure S6: Phenyl rhamnoside (**A**) UV-Vis chromatogram 210-500 nm total spectrum, (**B**) base peak FT-MS chromatogram, and (**C**) mass spectra of peak at 58.7 min showing, $[M+formate-H]^-$ at *m/z* 285.0980.

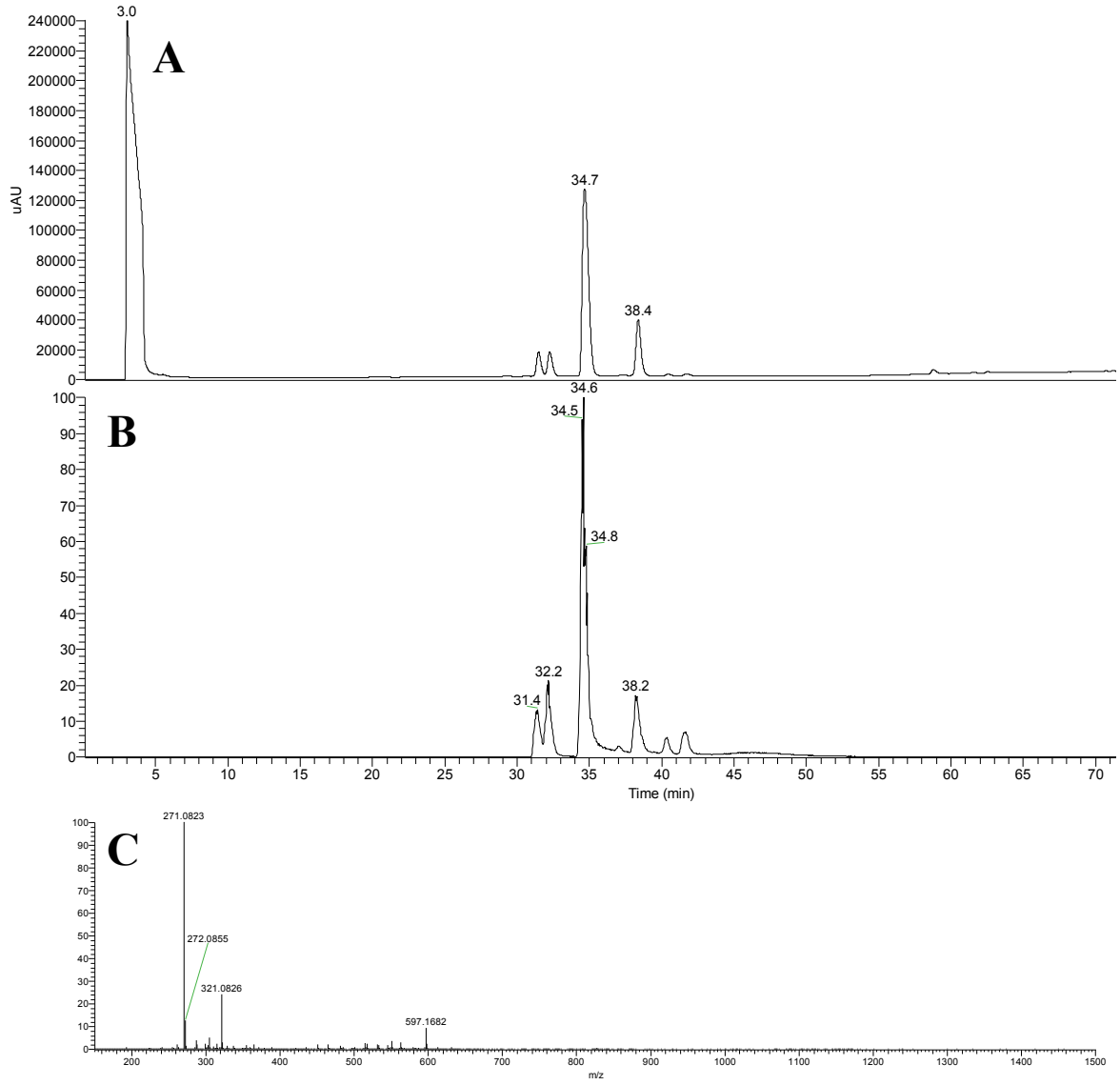
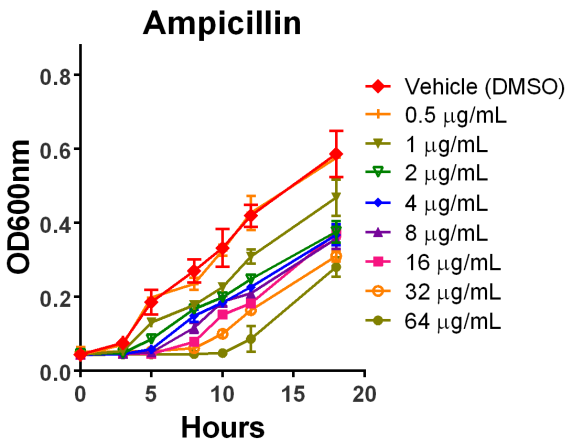
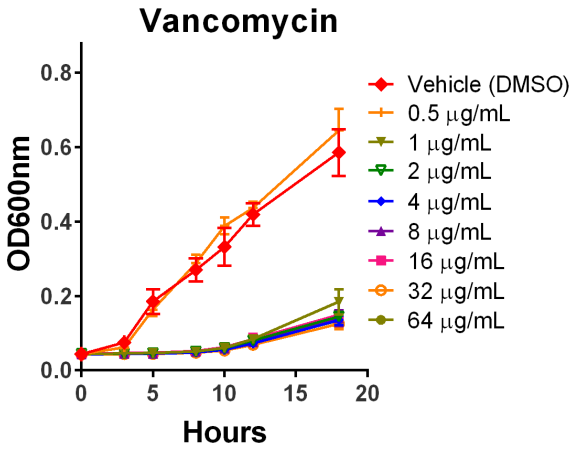
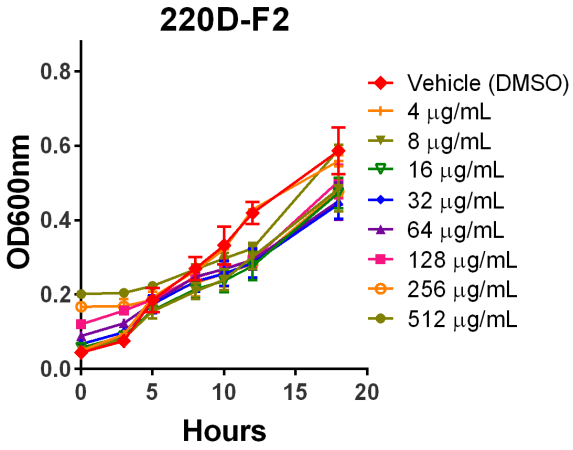
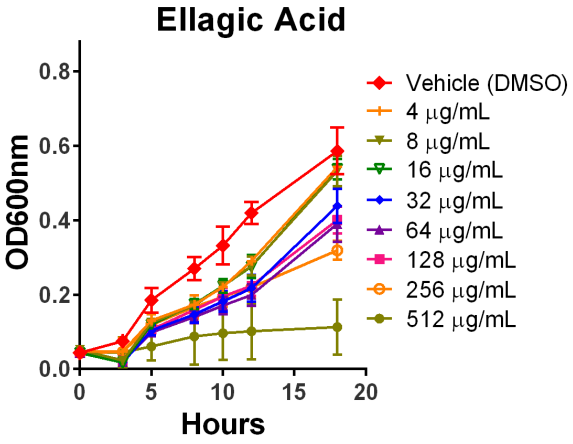


Figure S7: Phenyl xyloside (**A**) UV-Vis chromatogram 210-500 nm total spectrum, (**B**) base peak FT-MS chromatogram, and (**C**) mass spectra of peak at 34.6 min showing, $[M+formate-H]^-$ at *m/z* 271.0823.


Ampicillin
OD600nm
Hours
Vehicle (DMSO)
0.5 μg/mL
1 μg/mL
2 μg/mL
4 μg/mL
8 μg/mL
16 μg/mL
32 μg/mL
64 μg/mL


Vancomycin
OD600nm
Hours
Vehicle (DMSO)
0.5 μg/mL
1 μg/mL
2 μg/mL
4 μg/mL
8 μg/mL
16 μg/mL
32 μg/mL
64 μg/mL


220D-F2
OD600nm
Hours
Vehicle (DMSO)
4 μg/mL
8 μg/mL
16 μg/mL
32 μg/mL
64 μg/mL
128 μg/mL
256 μg/mL
512 μg/mL


Ellagic Acid
OD600nm
Hours
Vehicle (DMSO)
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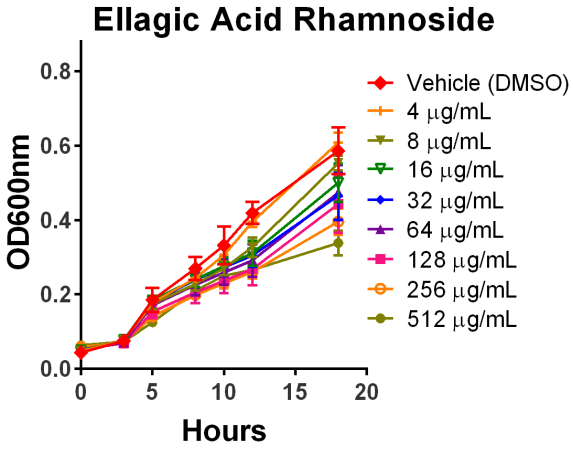
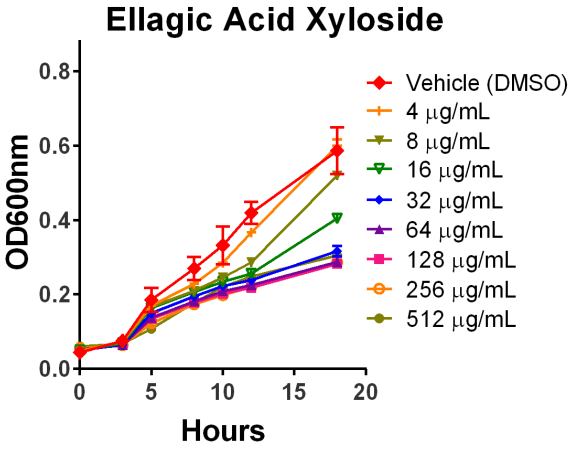
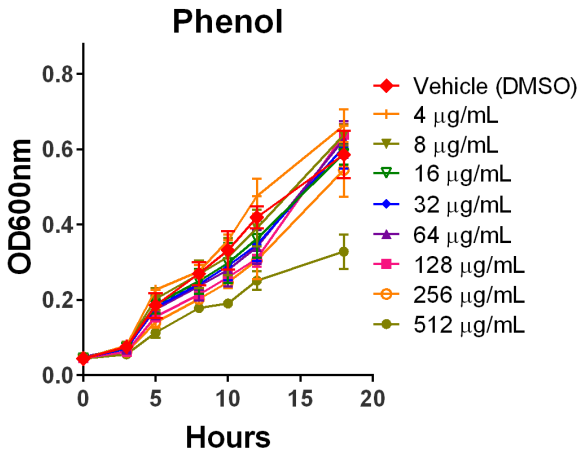
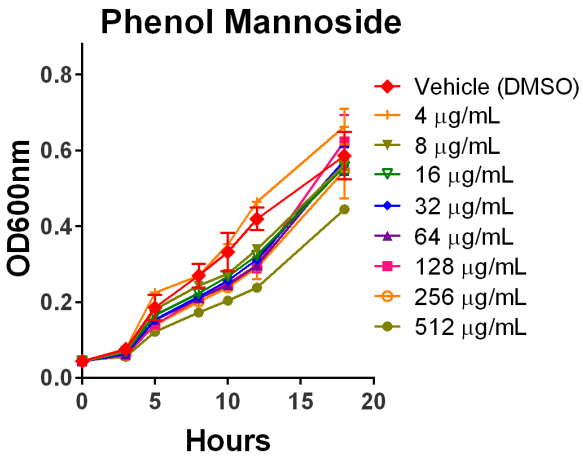
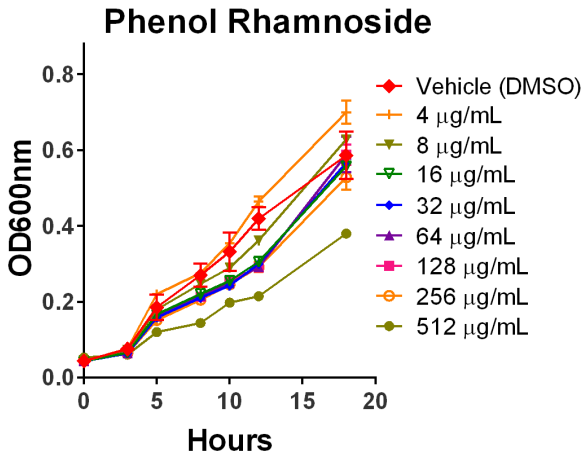
8 μg/mL
16 μg/mL
32 μg/mL
64 μg/mL
128 μg/mL
256 μg/mL
512 μg/mL

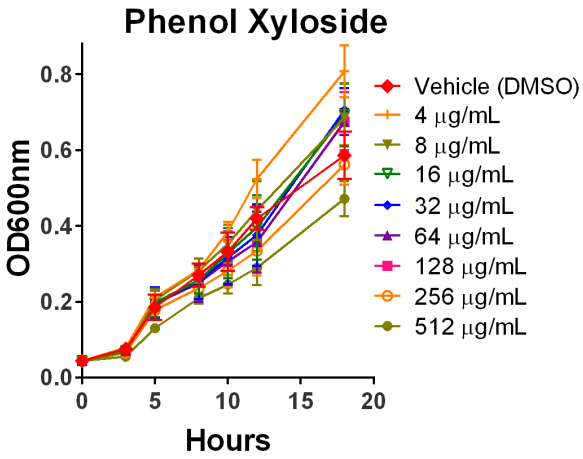

Ellagic Acid Rhamnoside
OD600nm
Hours
Vehicle (DMSO)
4 μg/mL
8 μg/mL
16 μg/mL
32 μg/mL
64 μg/mL
128 μg/mL
256 μg/mL
512 μg/mL


Ellagic Acid Xyloside
OD600nm
Hours
Vehicle (DMSO)
4 μg/mL
8 μg/mL
16 μg/mL
32 μg/mL
64 μg/mL
128 μg/mL
256 μg/mL
512 μg/mL


Phenol
OD600nm
Hours
0.0
0.2
0.4
0.6
0.8
0
5
10
15
20
Vehicle (DMSO)
4 μg/mL
8 μg/mL
16 μg/mL
32 μg/mL
64 μg/mL
128 μg/mL
256 μg/mL
512 μg/mL


Phenol Mannoside
OD600nm
Hours
0.0
0.2
0.4
0.6
0.8
0
5
10
15
20
Vehicle (DMSO)
4 μg/mL
8 μg/mL
16 μg/mL
32 μg/mL
64 μg/mL
128 μg/mL
256 μg/mL
512 μg/mL


Phenol Rhamnoside
OD600nm
Hours
0.0
0.2
0.4
0.6
0.8
0
5
10
15
20
Vehicle (DMSO)
4 μg/mL
8 μg/mL
16 μg/mL
32 μg/mL
64 μg/mL
128 μg/mL
256 μg/mL
512 μg/mL


Phenol Xyloside
OD600nm
Hours
0.0
0.2
0.4
0.6
0.8
0
5
10
15
20
Vehicle (DMSO)
4 μg/mL
8 μg/mL
16 μg/mL
32 μg/mL
64 μg/mL
128 μg/mL
256 μg/mL
512 μg/mL

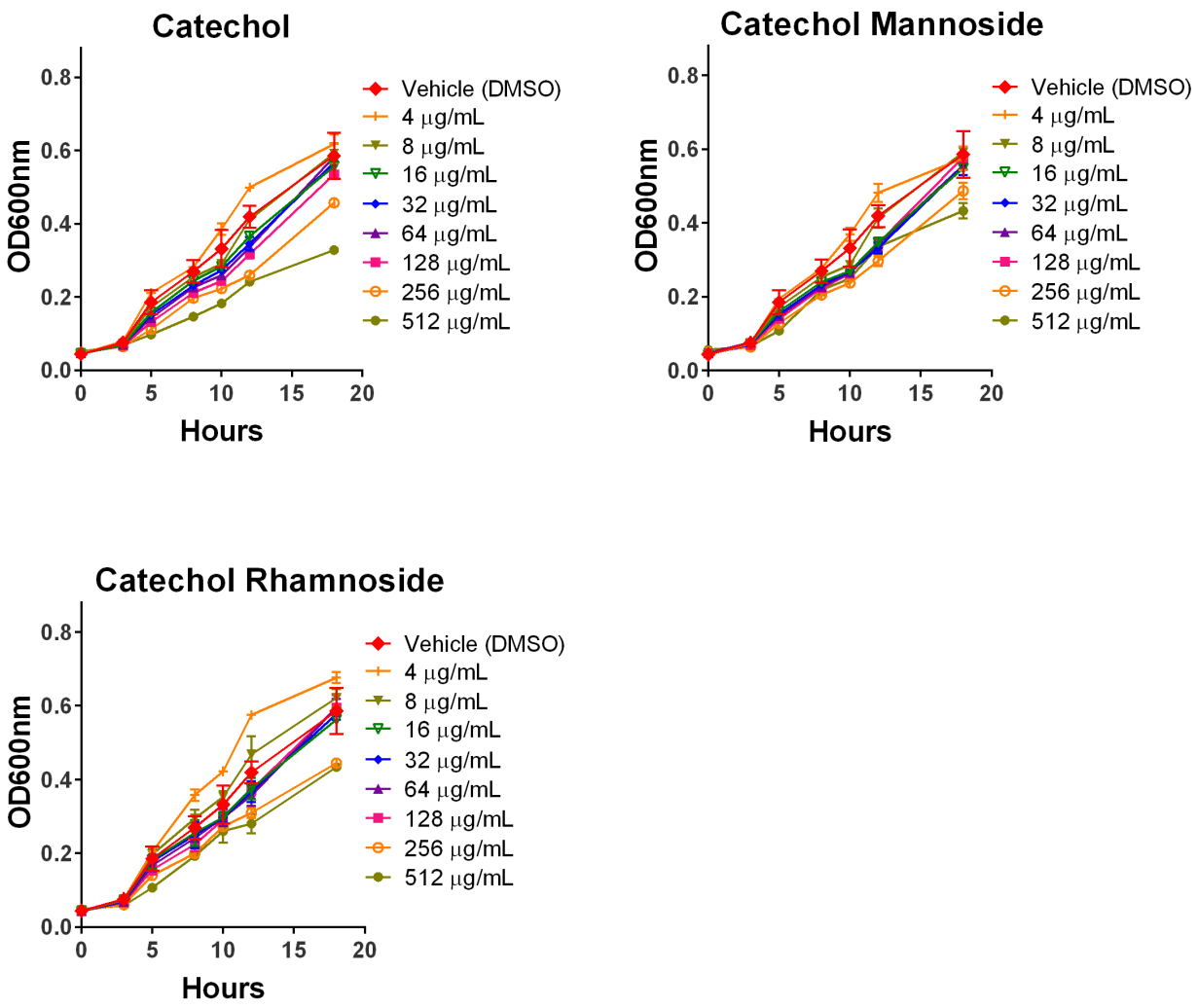


Figure S8: Growth curves for controls, 220D-F2 and synthesized analogs.

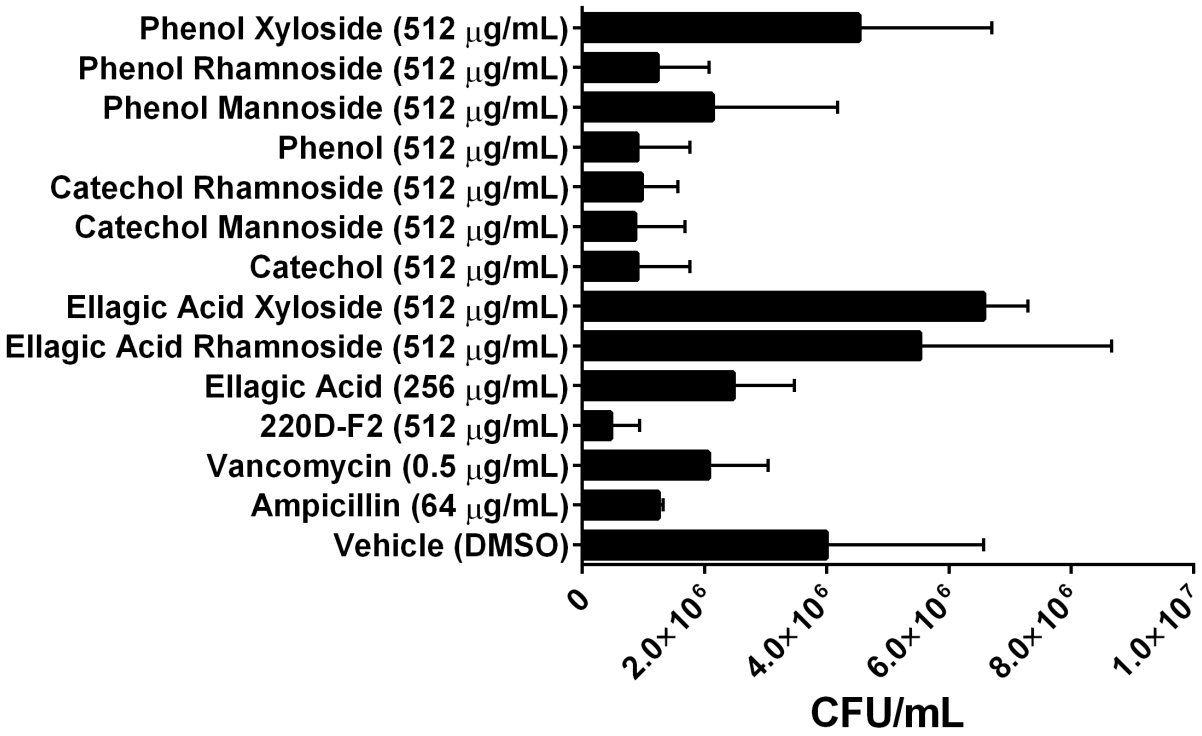
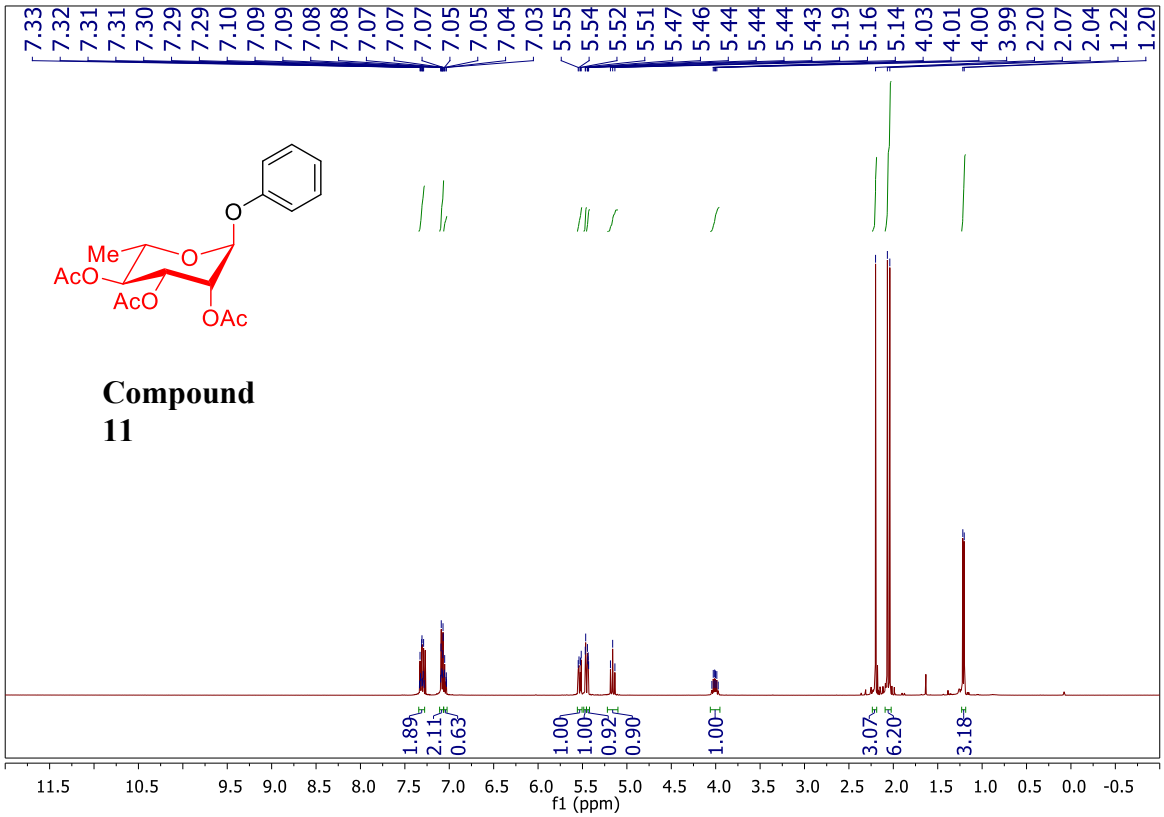
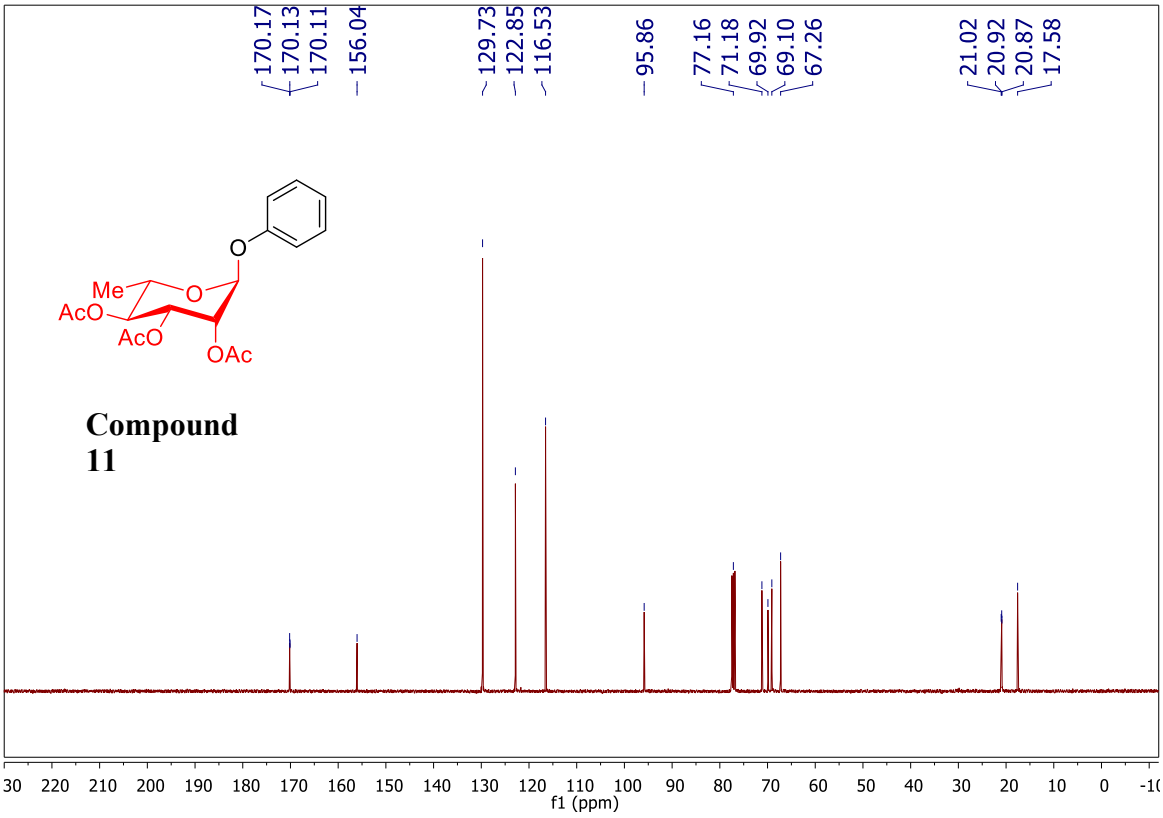
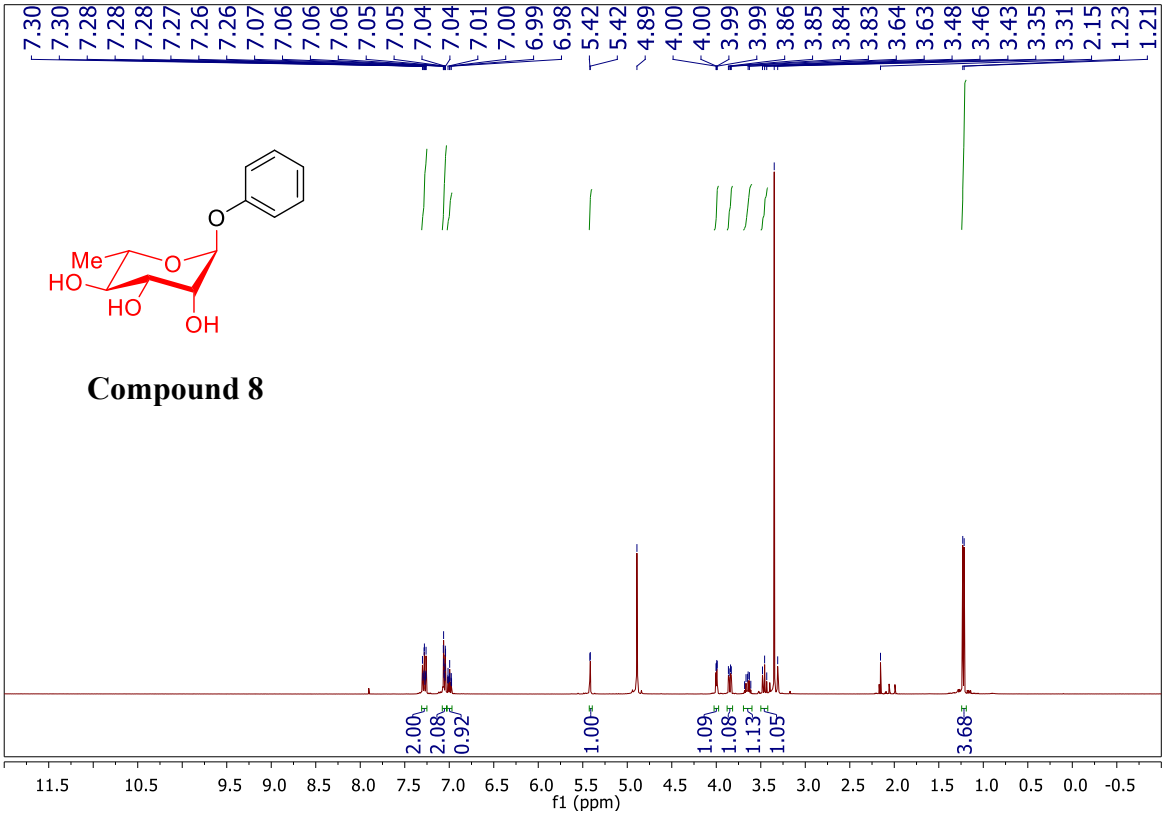


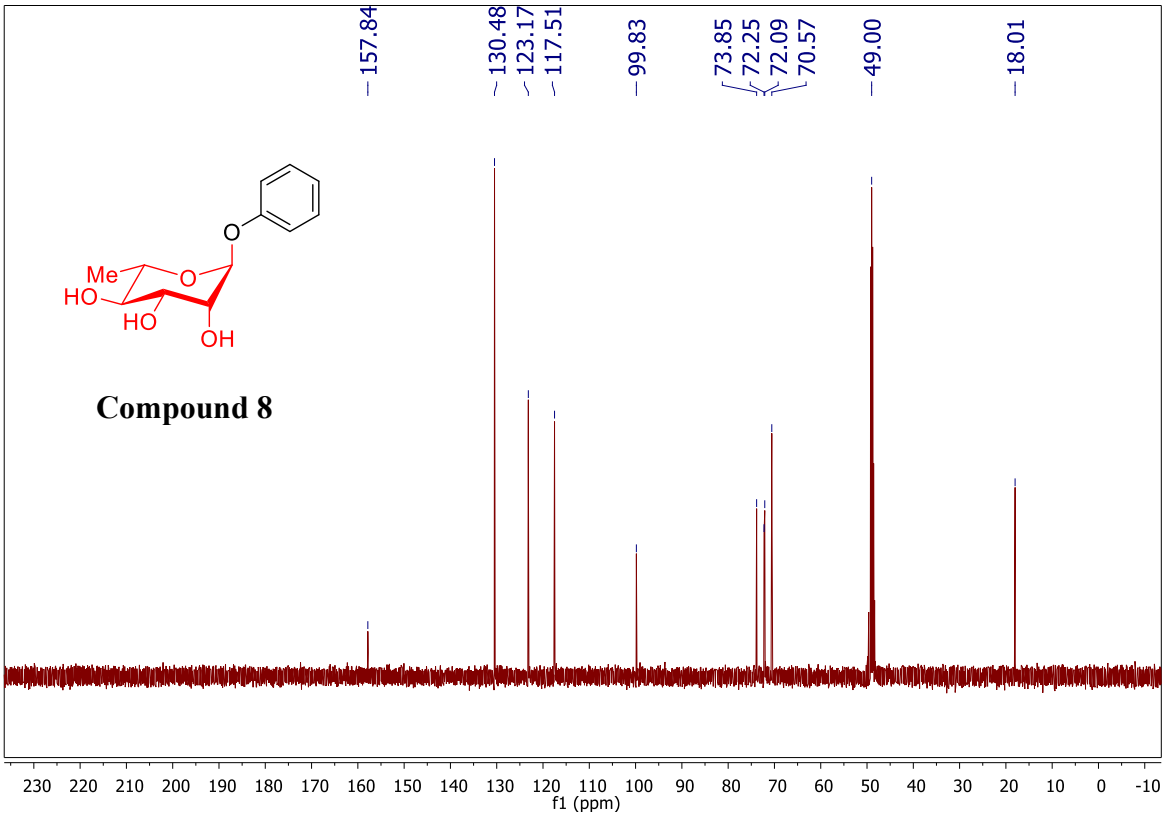
Figure S9: Total CFU/mL following 18 h incubation. None of the above represented concentrations exhibited a statistically significant (P<0.05) difference from the vehicle (DMSO) control.

NMR Spectra

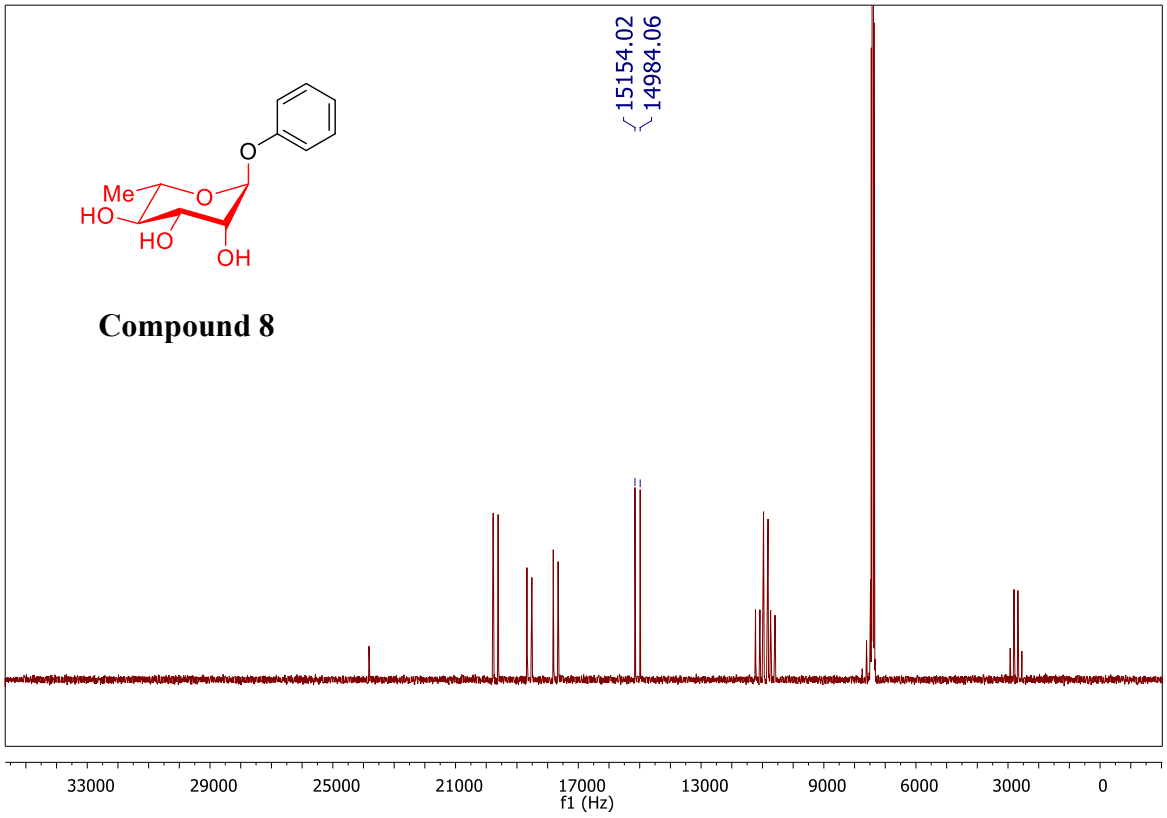

7.33 7.32 7.31 7.31 7.30 7.29 7.29 7.10 7.09 7.09 7.08 7.08 7.07 7.07 7.07 7.05 7.05 7.04 7.03
5.55 5.54 5.52 5.51 5.47 5.46 5.44 5.44 5.44 5.43 5.19 5.16 5.14 4.03 4.01 4.00 3.99 2.20 2.07 2.04 1.22 1.20
Me
AcO
AcO
O
O
OAc
Compound 11
1.89 2.11 0.63 1.00 1.00 0.92 0.90 1.00 3.07 6.20 3.18
11.5 10.5 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)


170.17
170.13
170.11
156.04
129.73
122.85
116.53
95.86
77.16
71.18
69.92
69.10
67.26
21.02
20.92
20.87
17.58
Me
AcO
AcO
OAc
O
Compound
11
f1 (ppm)

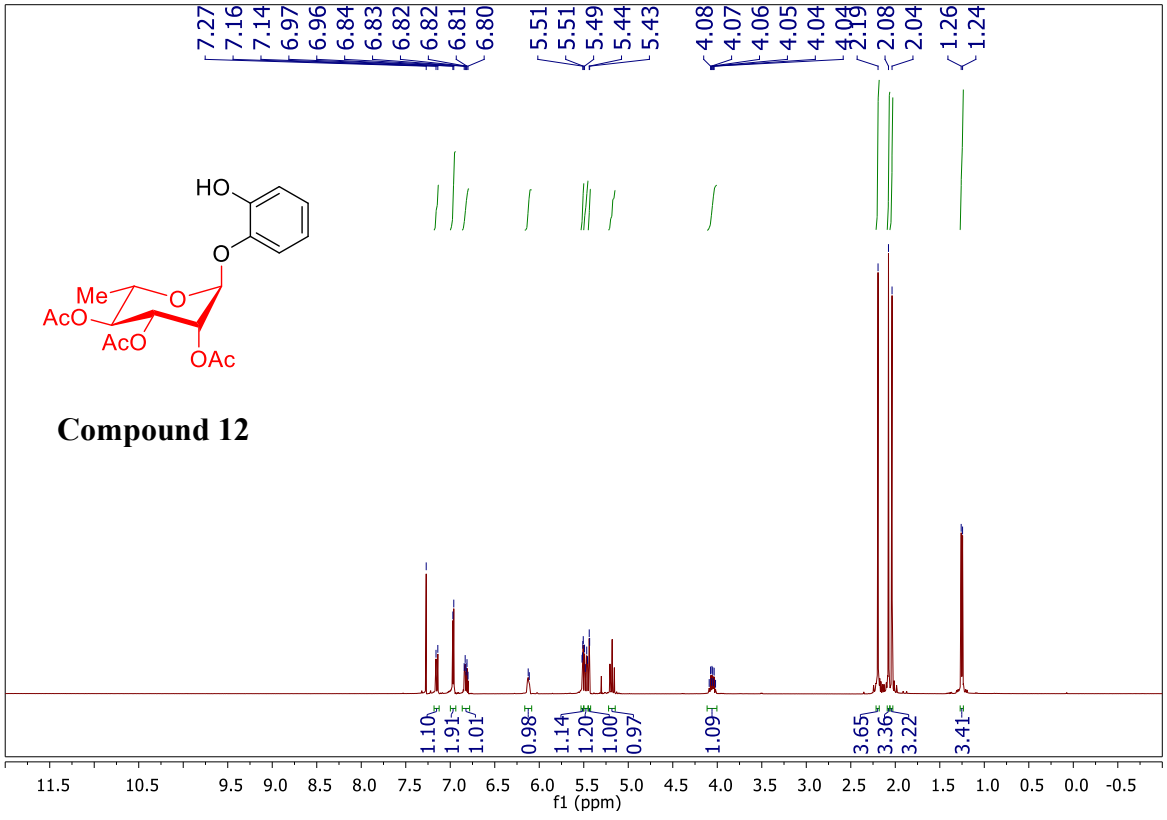

7.30
7.30
7.28
7.28
7.28
7.27
7.26
7.26
7.07
7.06
7.06
7.06
7.05
7.05
7.04
7.04
7.01
7.00
6.99
6.98
5.42
5.42
4.89
4.00
4.00
3.99
3.99
3.86
3.85
3.84
3.83
3.64
3.63
3.48
3.46
3.43
3.35
3.31
2.15
1.23
1.21
Me
HO
HO
OH
O
Compound 8
2.00
2.08
0.92
1.00
1.09
1.08
1.13
1.05
3.68
f1 (ppm)



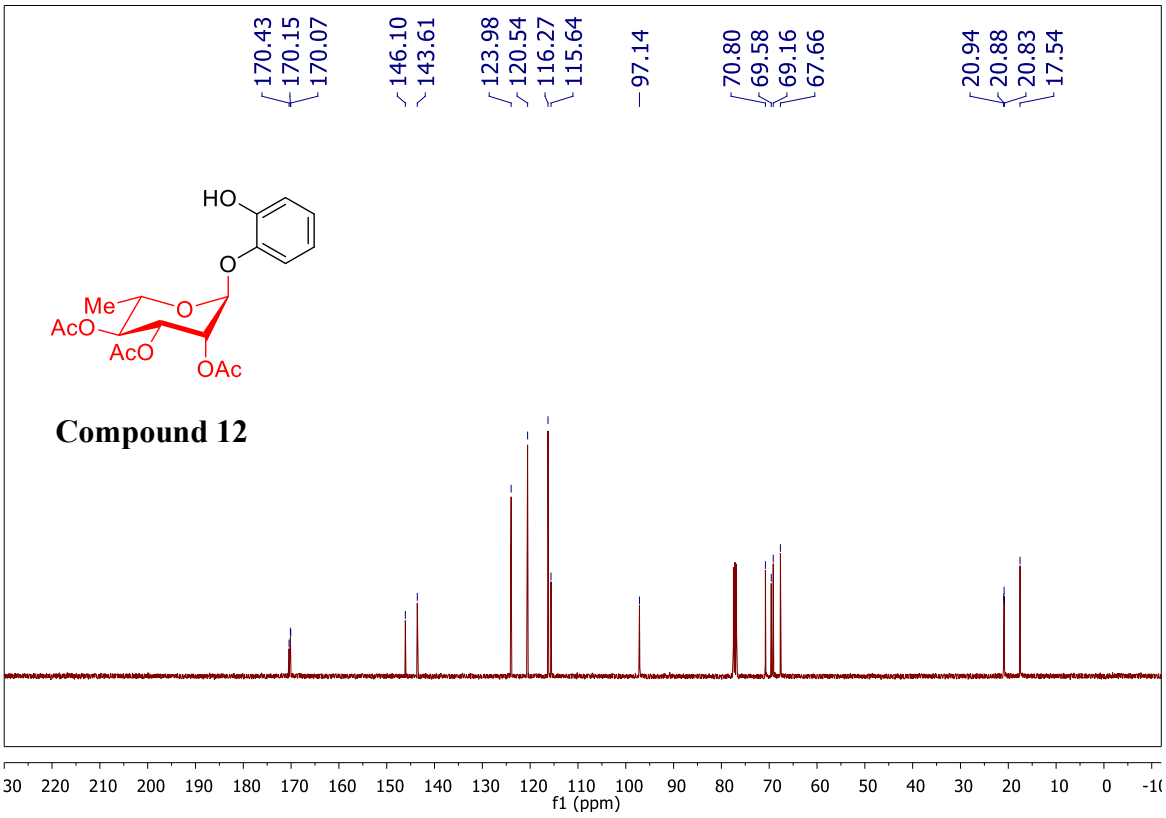
^{13}C-^{1}H coupled NMR (anomeric $^{1}J_{CH}$ = 169.96 Hz)

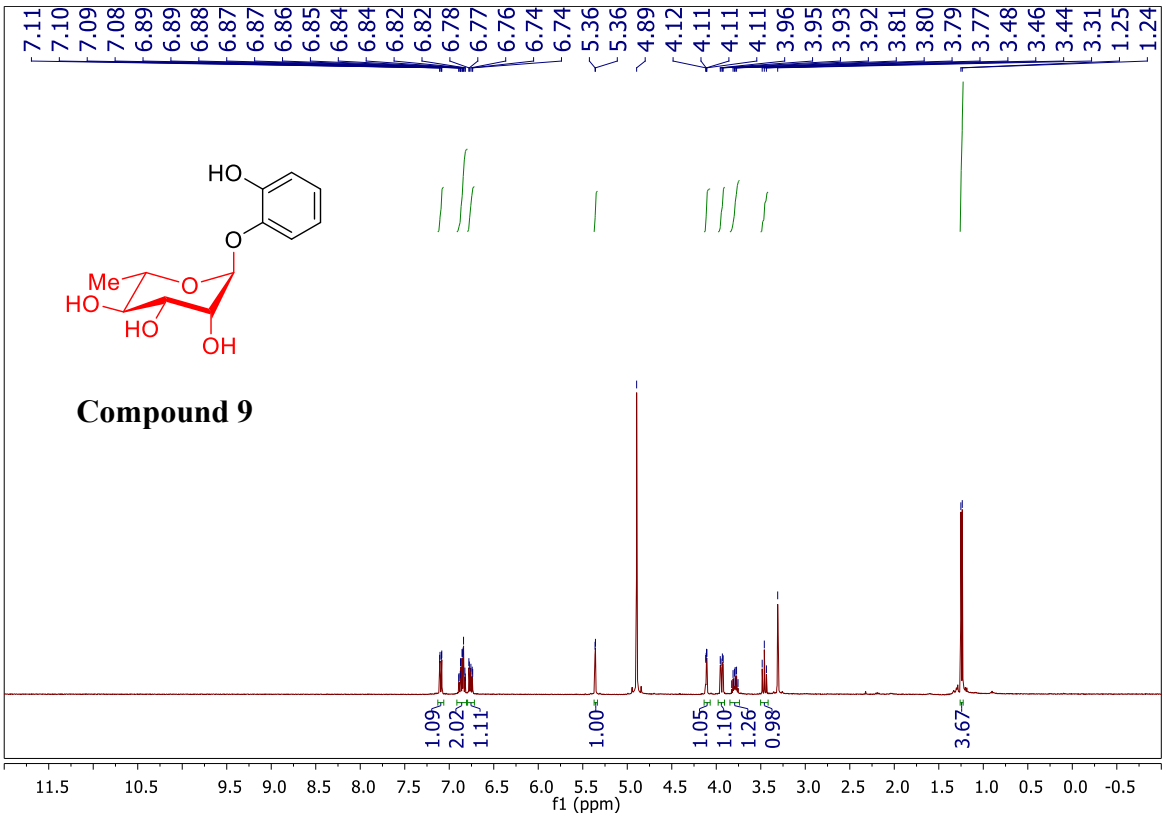


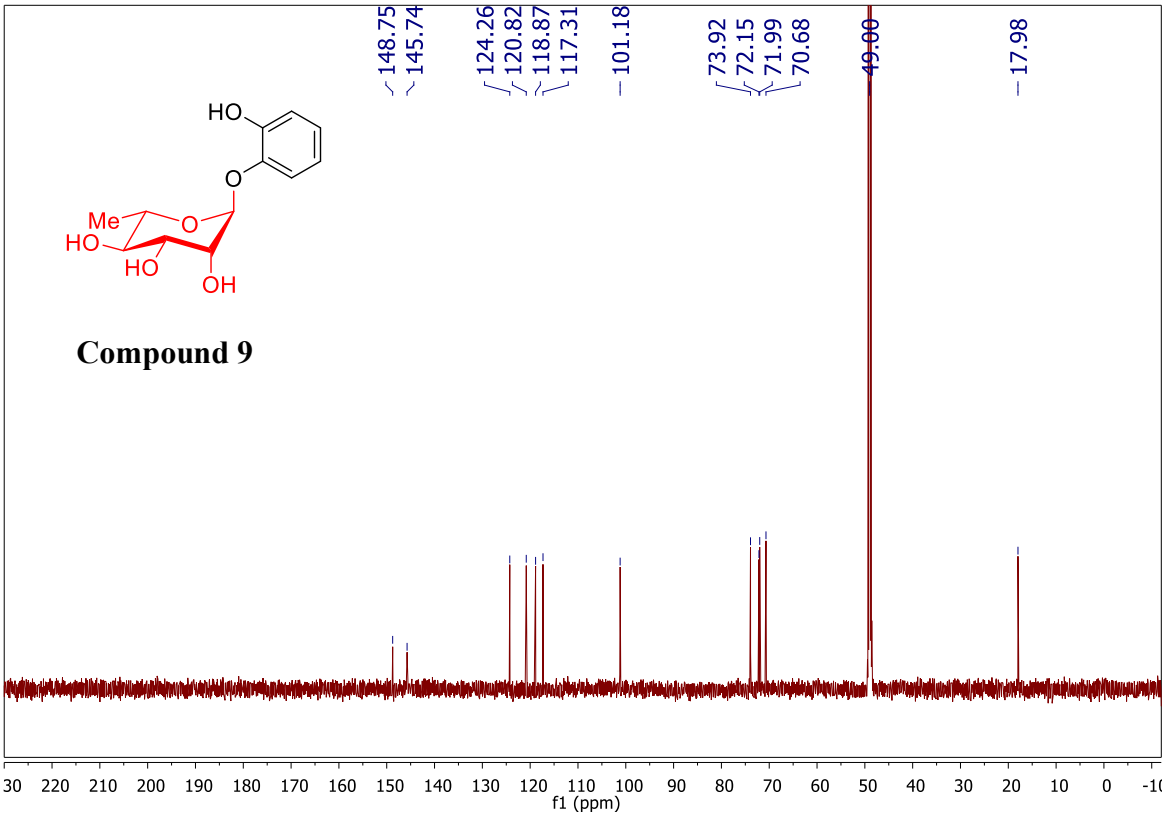
Compound 8

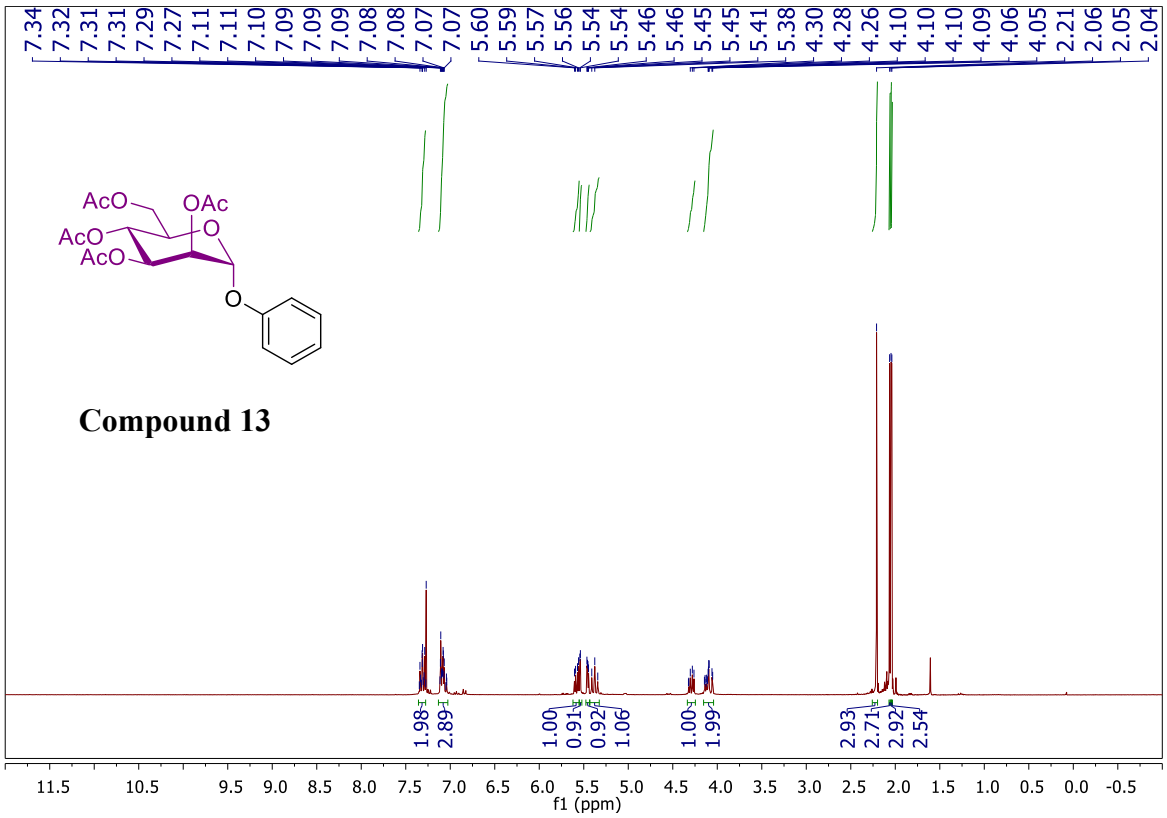


Compound 12

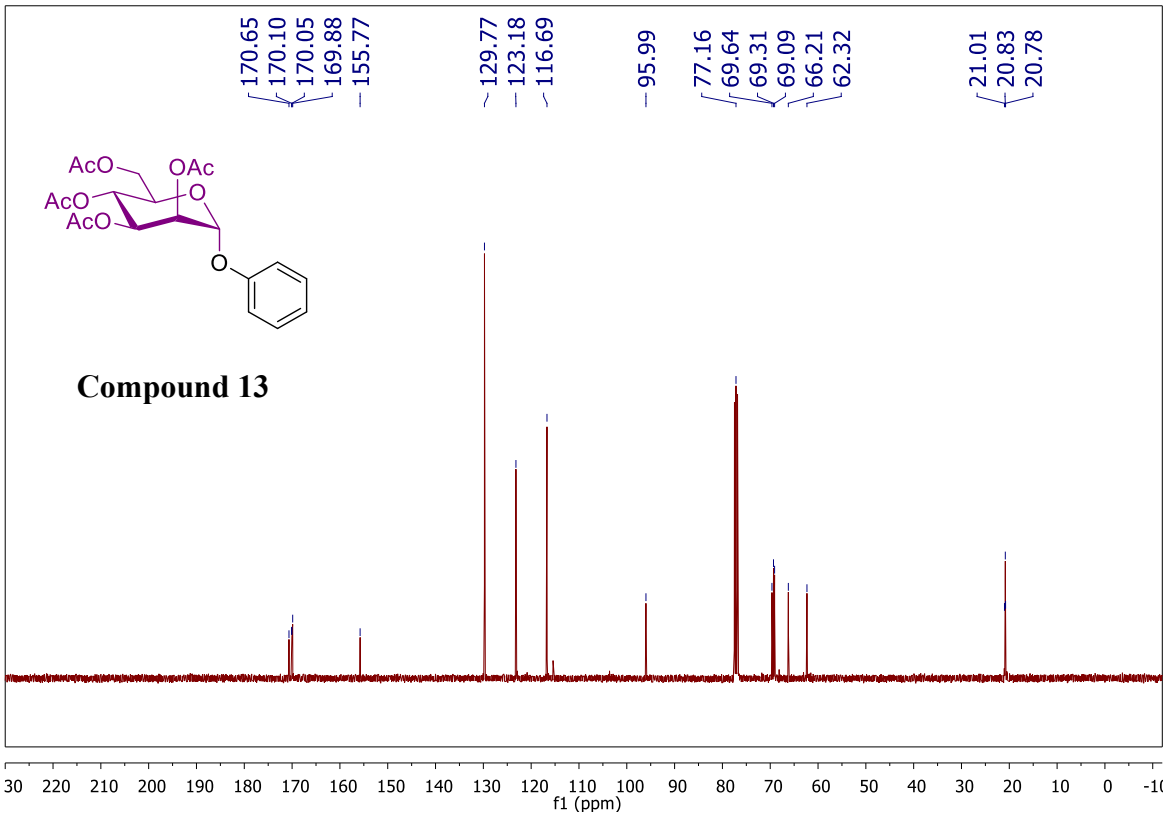

170.43
170.15
170.07
146.10
143.61
123.98
120.54
116.27
115.64
97.14
70.80
69.58
69.16
67.66
20.94
20.88
20.83
17.54
HO
O
Me
AcO
O
AcO
OAc
Compound 12
30 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


7.11
7.10
7.09
7.08
6.89
6.89
6.88
6.87
6.87
6.86
6.85
6.84
6.84
6.82
6.82
6.78
6.77
6.76
6.74
6.74
5.36
5.36
4.89
4.12
4.11
4.11
4.11
3.96
3.95
3.93
3.92
3.81
3.80
3.79
3.77
3.48
3.46
3.44
3.31
1.25
1.24
HO
O
Me
HO
HO
OH
Compound 9
1.09
2.02
1.11
1.00
1.05
1.10
1.26
0.98
3.67
11.5 10.5 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)

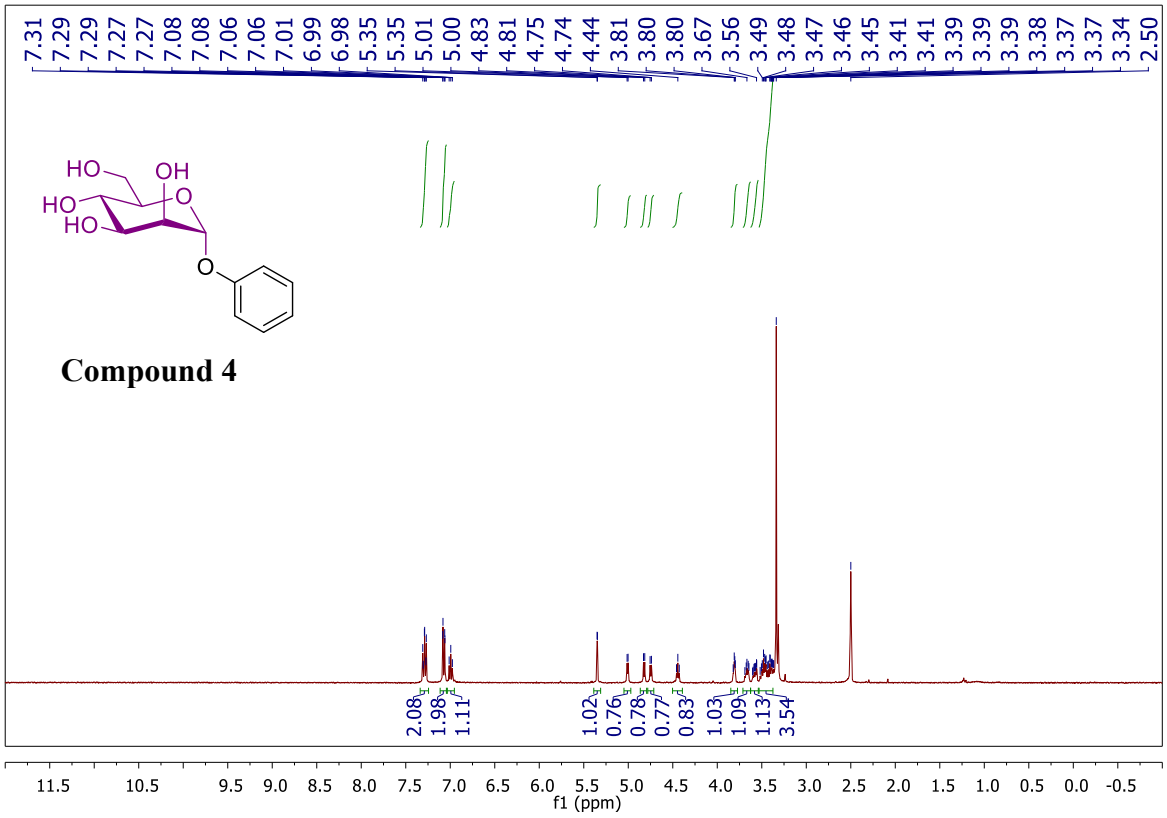

148.75
145.74
124.26
120.82
118.87
117.31
101.18
73.92
72.15
71.99
70.68
49.00
17.98
HO
O
Me
O
HO
HO
OH
Compound 9
30 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)



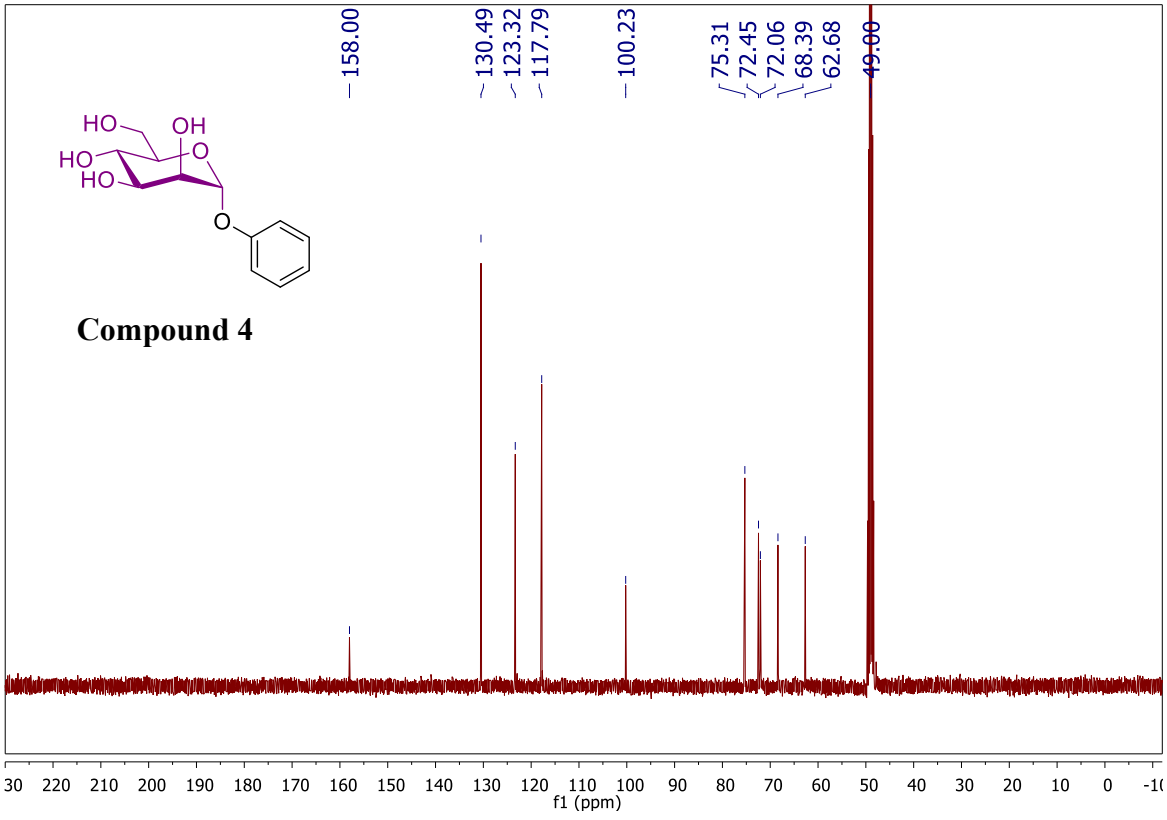
Compound 13



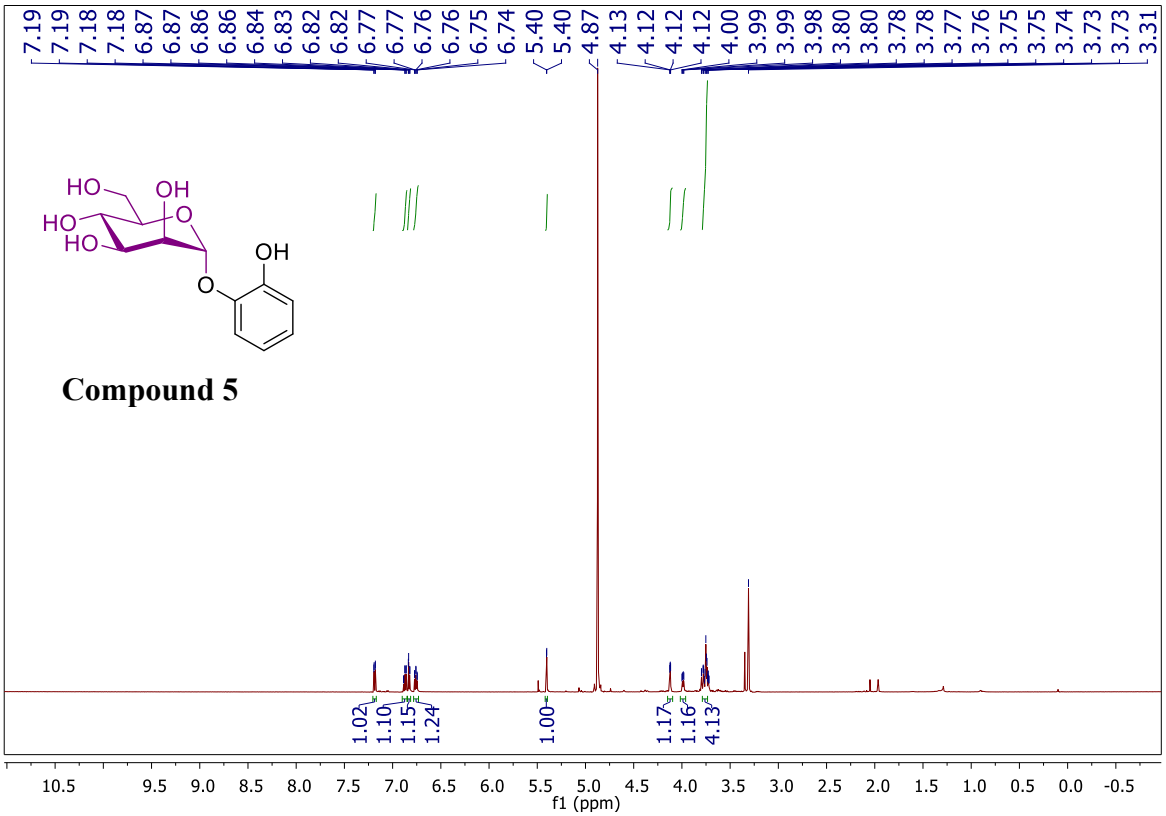
Compound 13



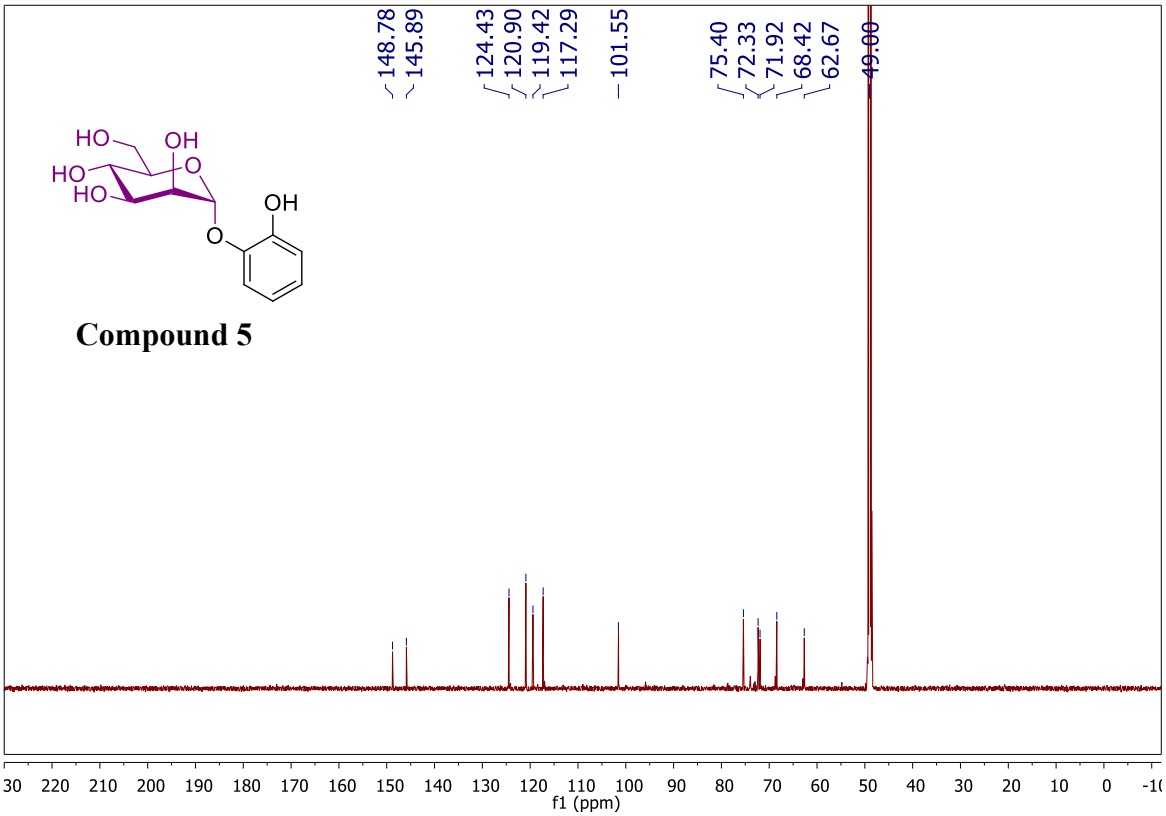
Compound 4



Compound 4

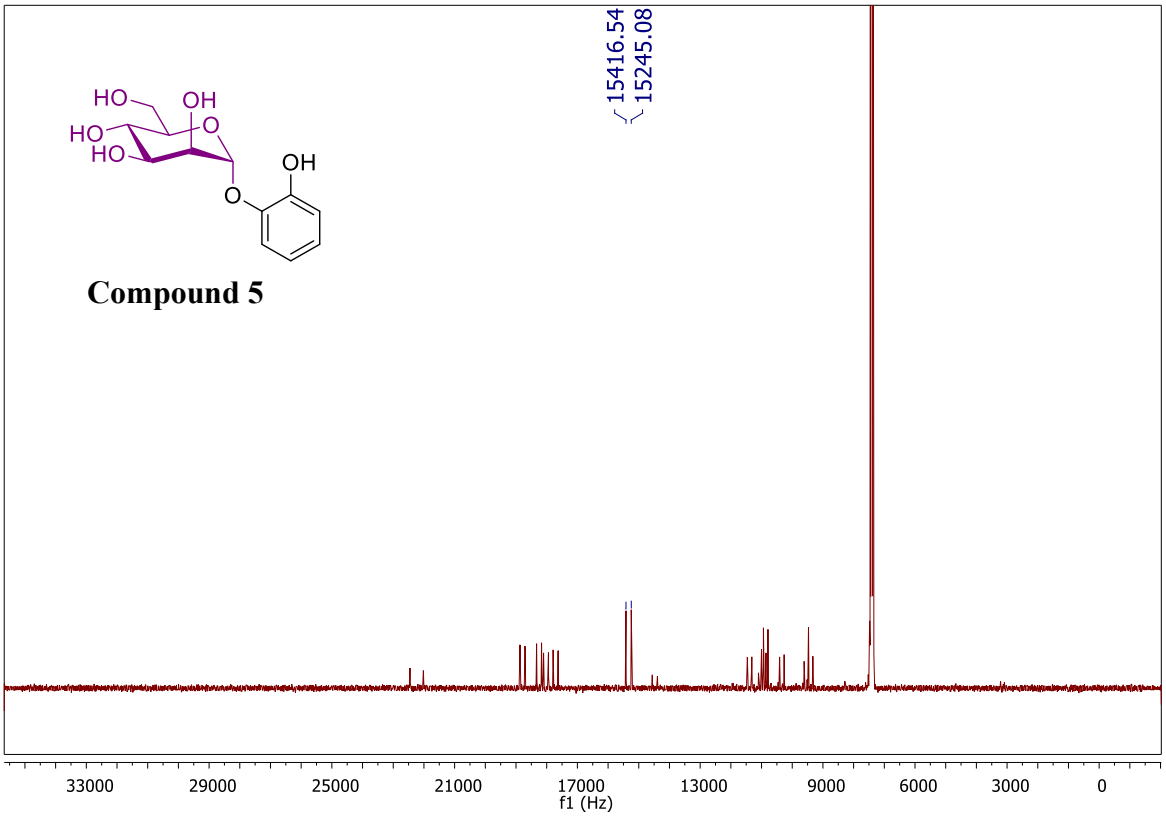


Compound 5

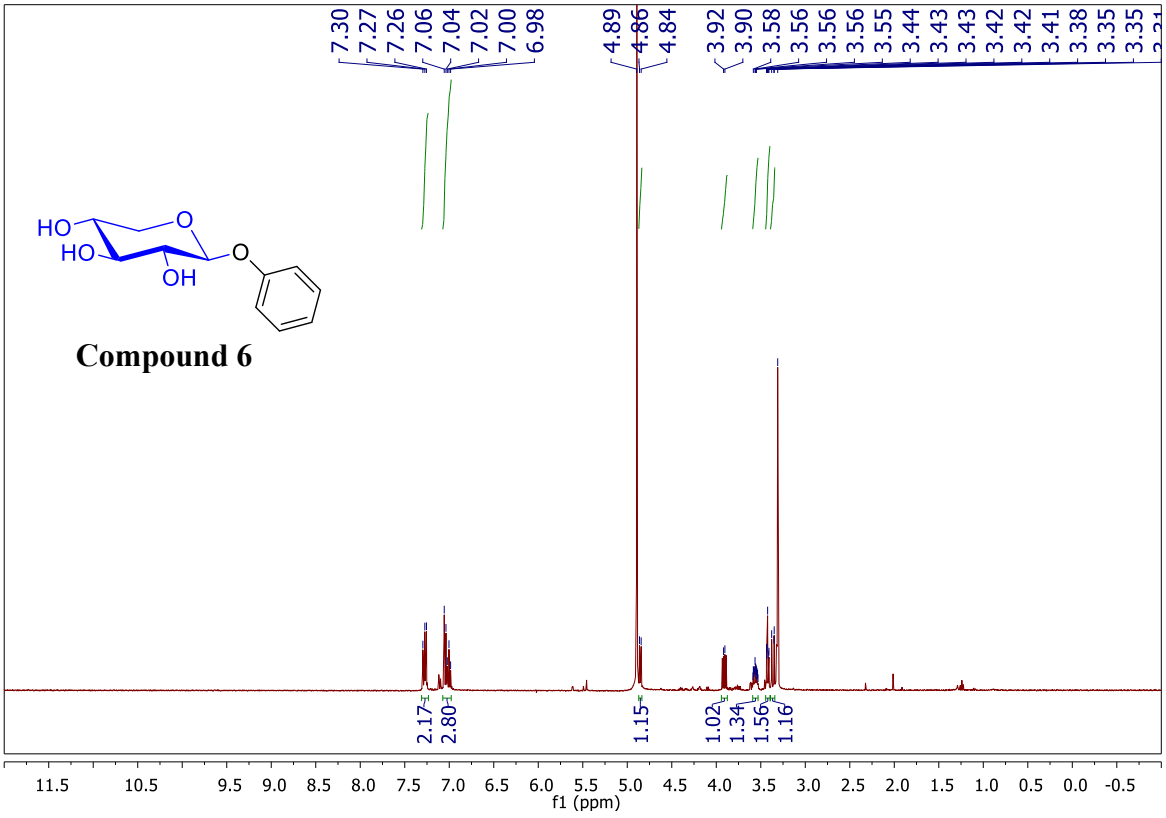


Compound 5

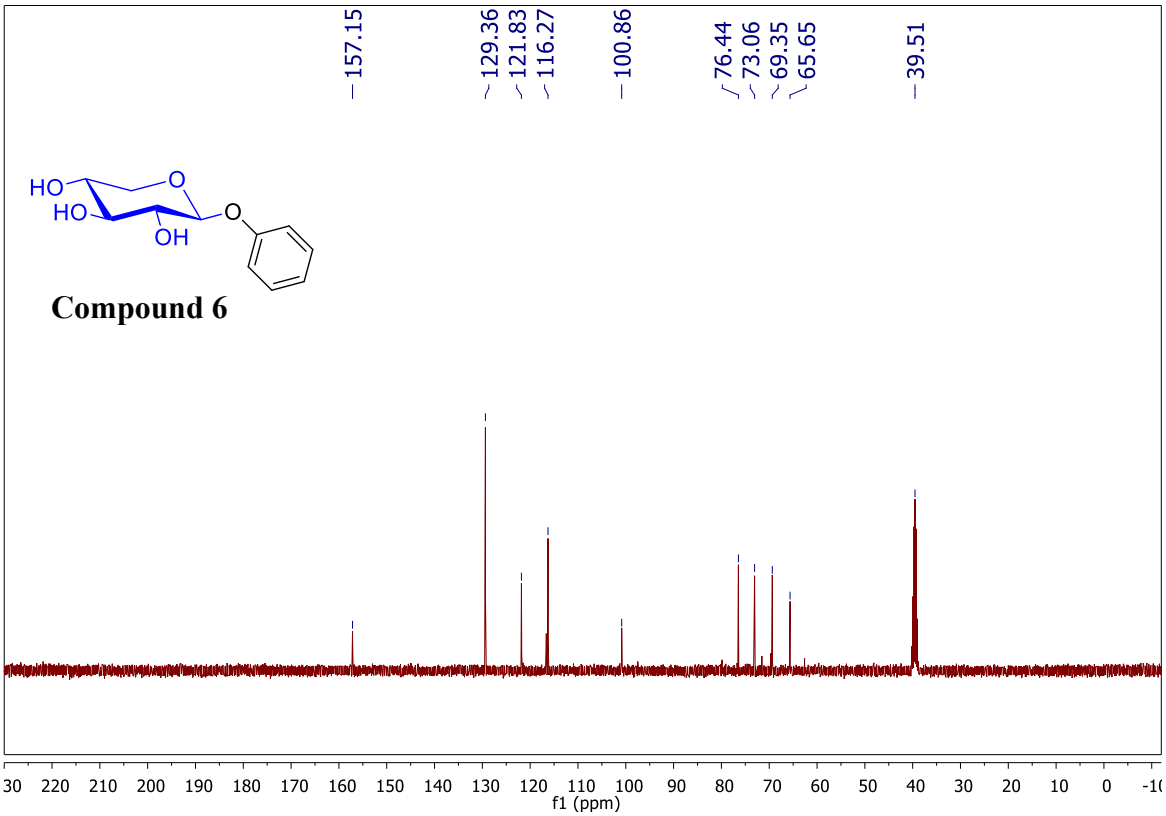
^{13}C-^{1}H coupled NMR (anomeric $^{1}J_{CH}$ = 171.46 Hz)

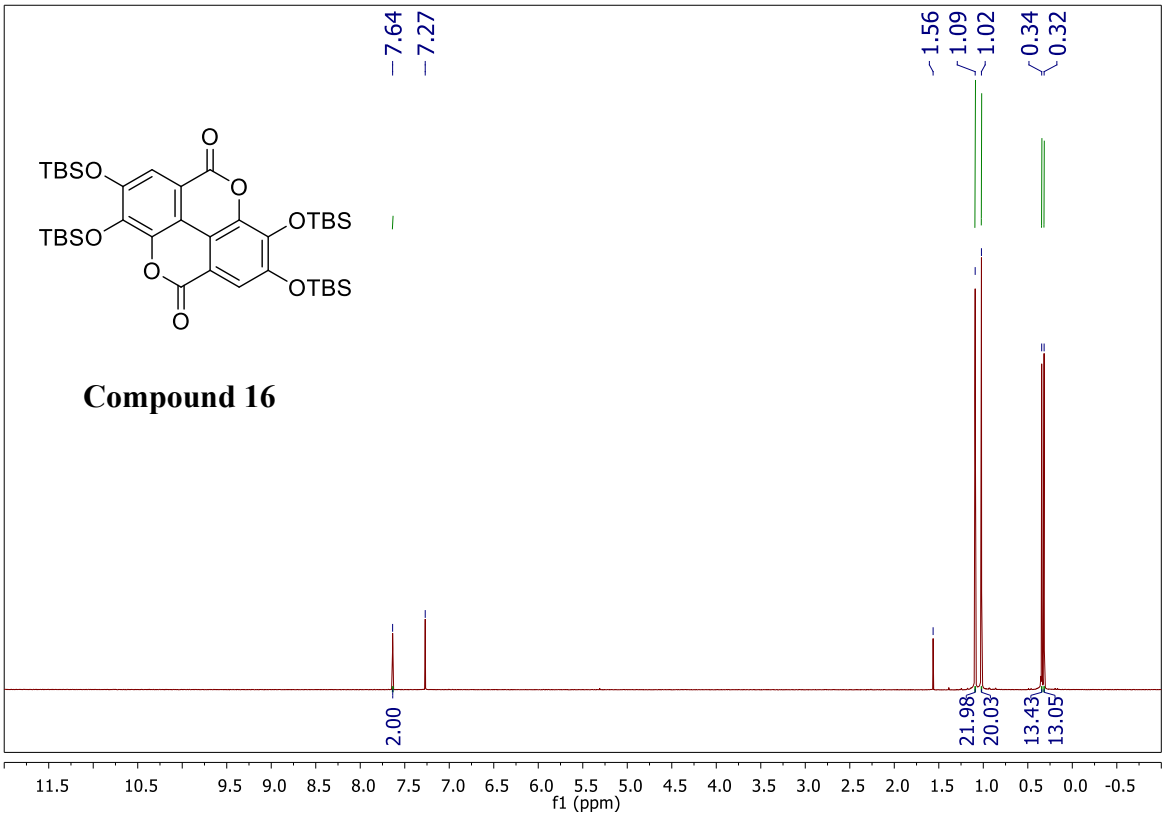


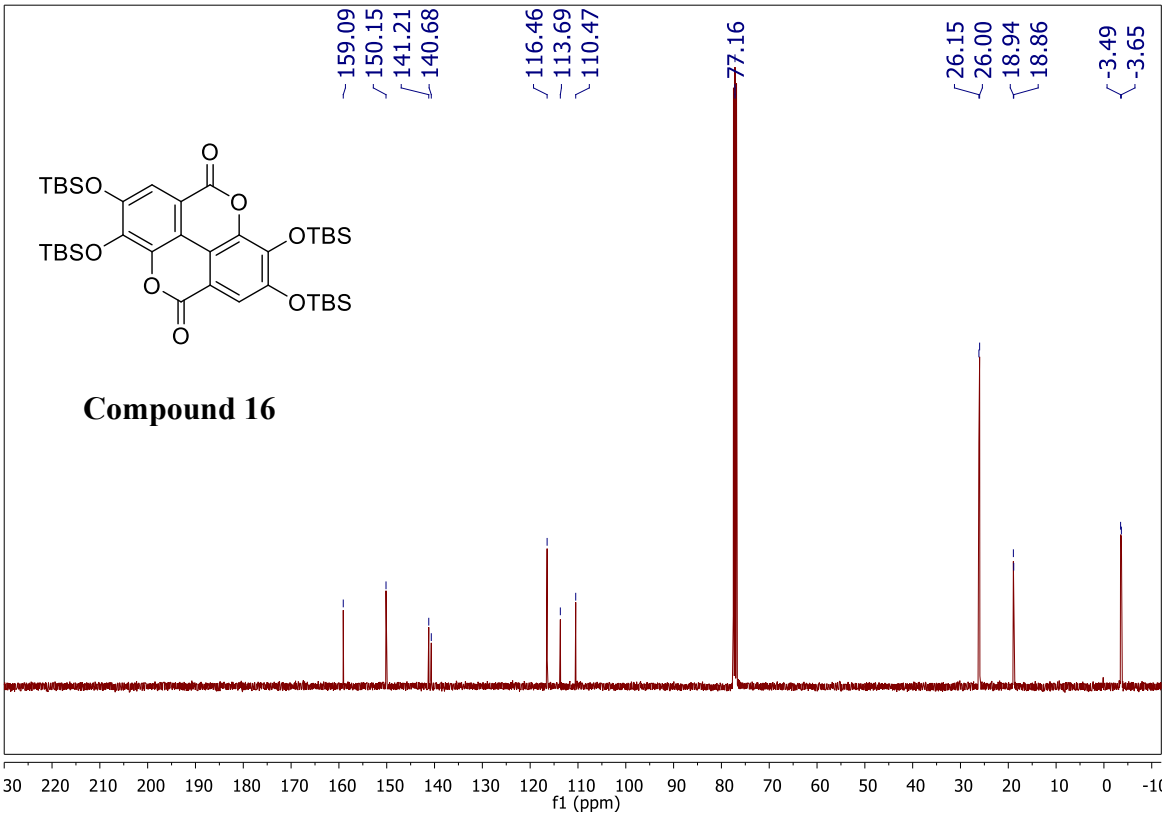
Compound 5

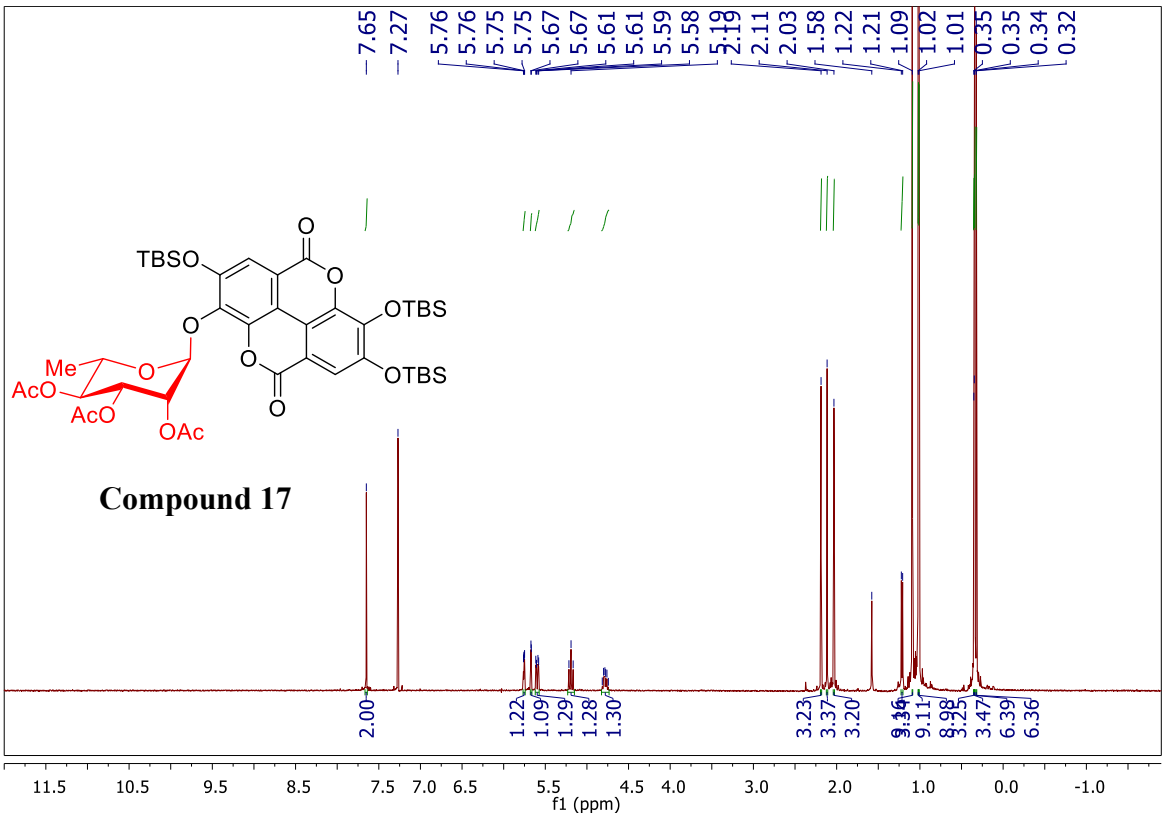


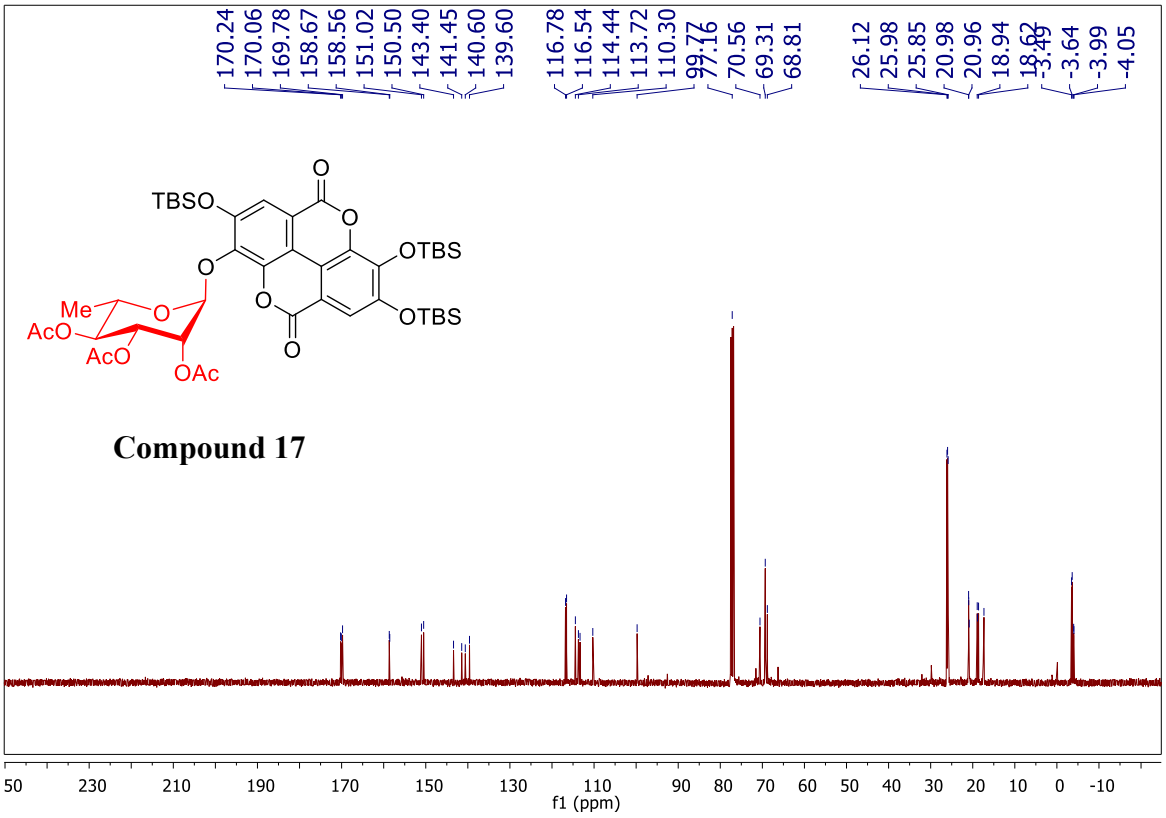
Compound 6


157.15
129.36
121.83
116.27
100.86
76.44
73.06
69.35
65.65
39.51
HO
HO
OH
O
O
Compound 6
30 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

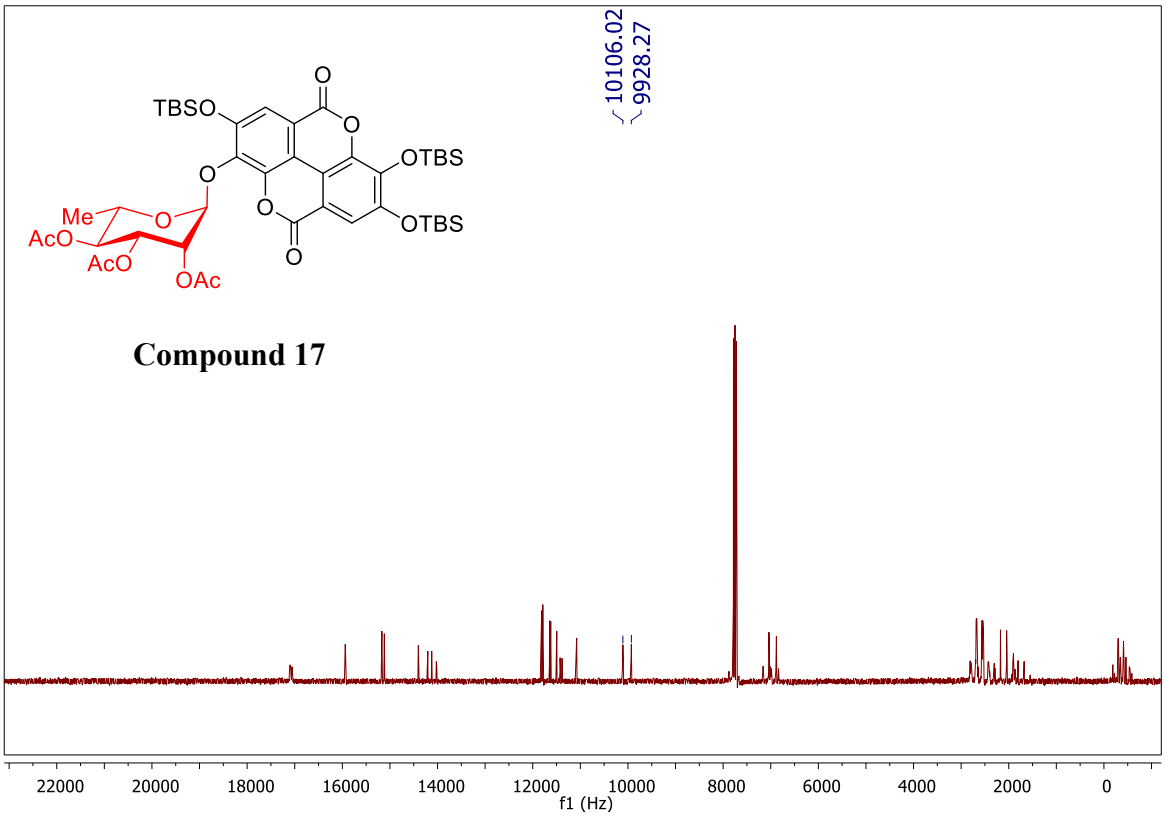

7.64
7.27
1.56
1.09
1.02
0.34
0.32
TBSO
TBSO
OTBS
OTBS
O
O
O
O
Compound 16
2.00
21.98
20.03
13.43
13.05
11.5 10.5 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

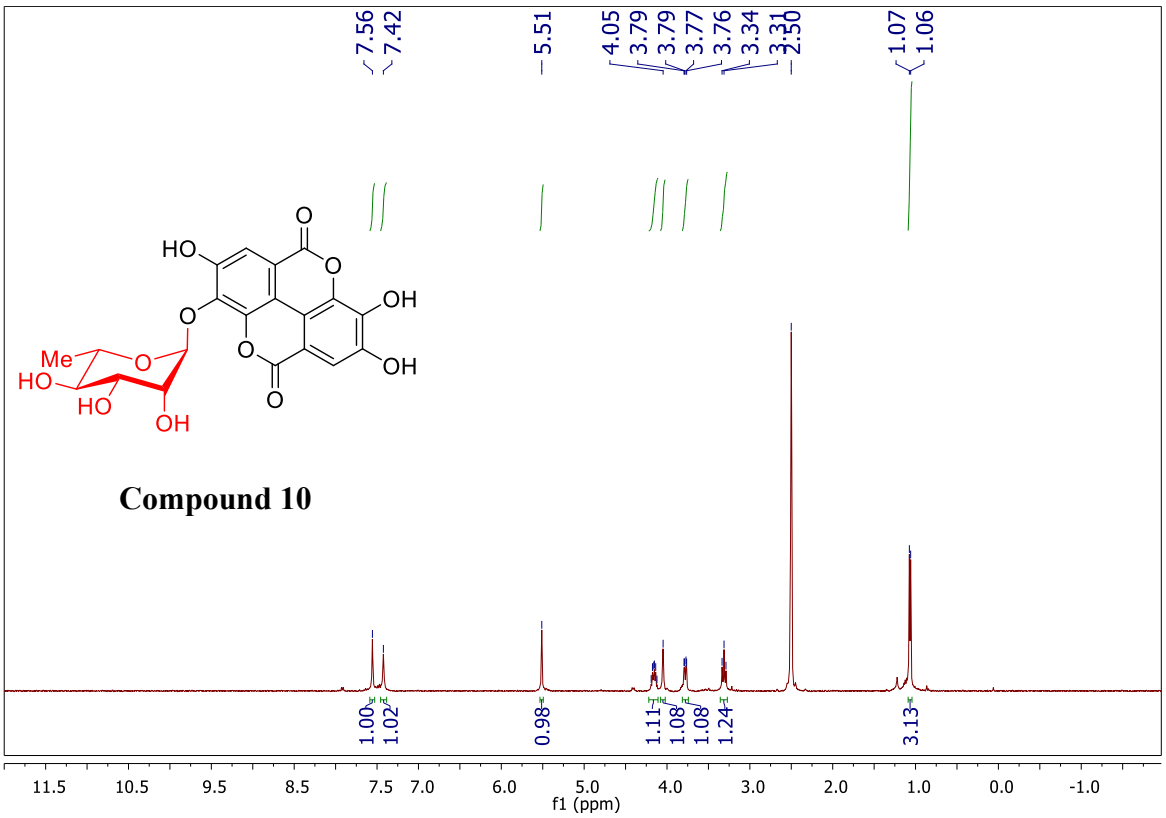

159.09
150.15
141.21
140.68
116.46
113.69
110.47
77.16
26.15
26.00
18.94
18.86
-3.49
-3.65
TBSO
TBSO
OTBS
OTBS
O
O
O
O
O
Compound 16
f1 (ppm)


TBSO
OTBS
OTBS
Me
AcO
AcO
OAc
Compound 17
f1 (ppm)

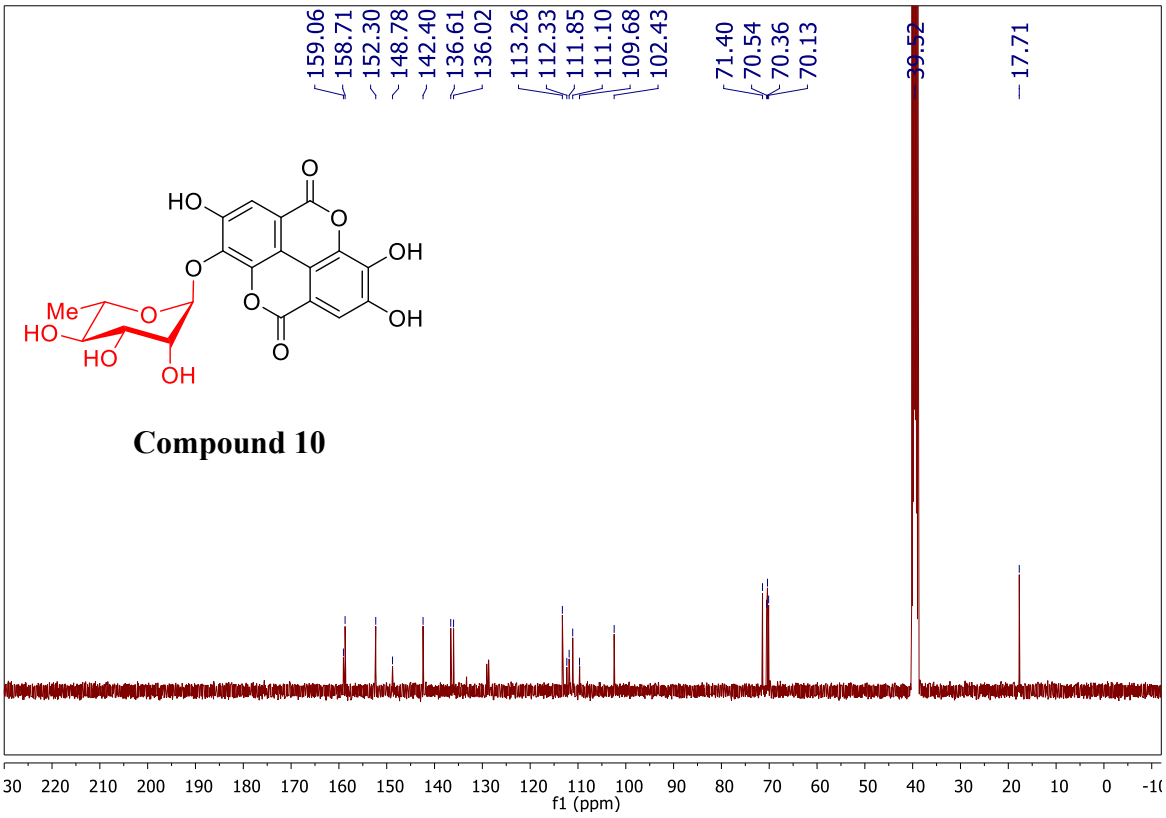

TBSO
OTBS
OTBS
Me
AcO
AcO
OAc
Compound 17
f1 (ppm)

^{13}C-^{1}H coupled NMR (anomeric $^{1}J_{CH}$ = 177.75 Hz)

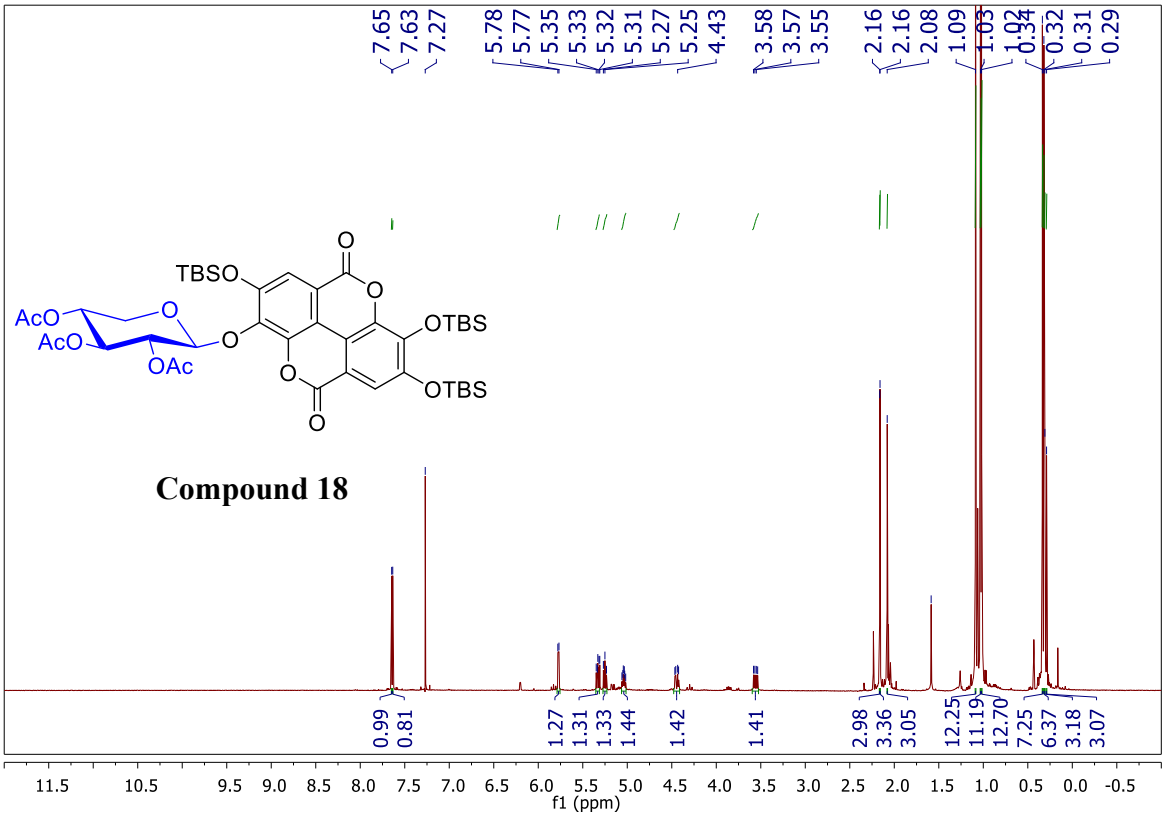


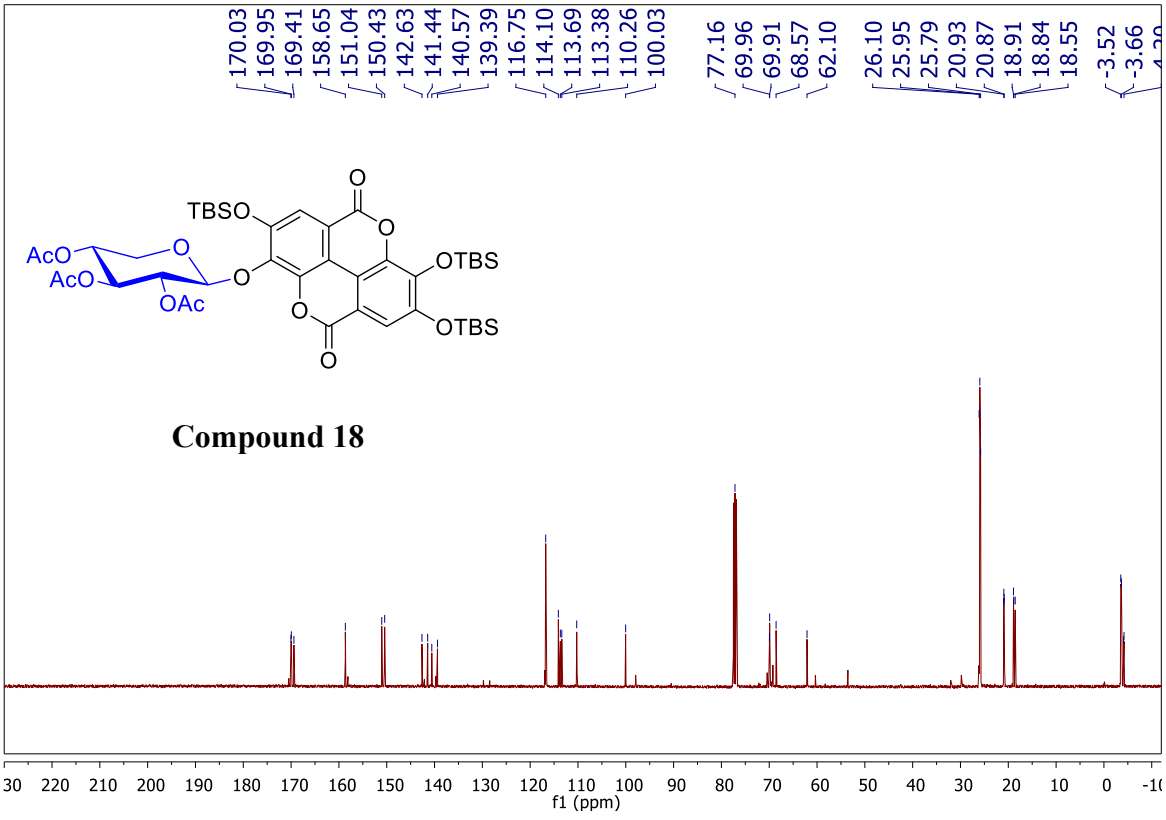


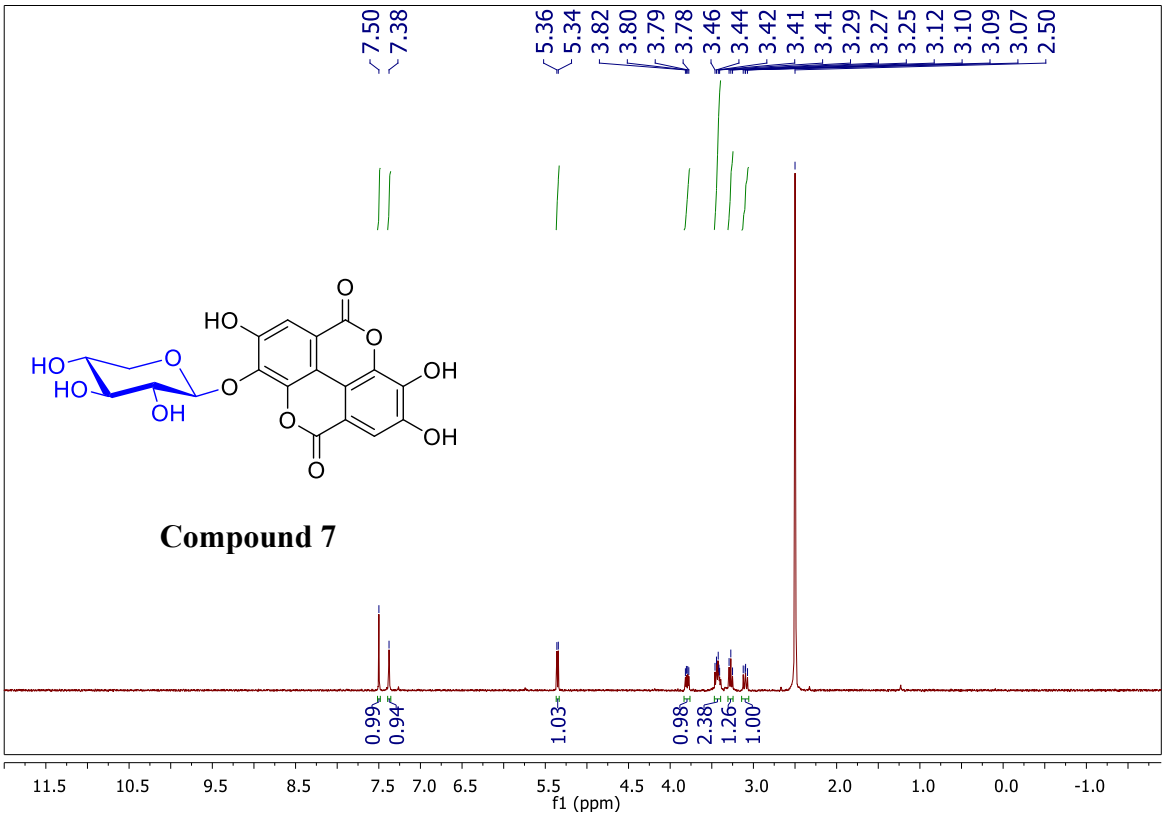
Compound 10



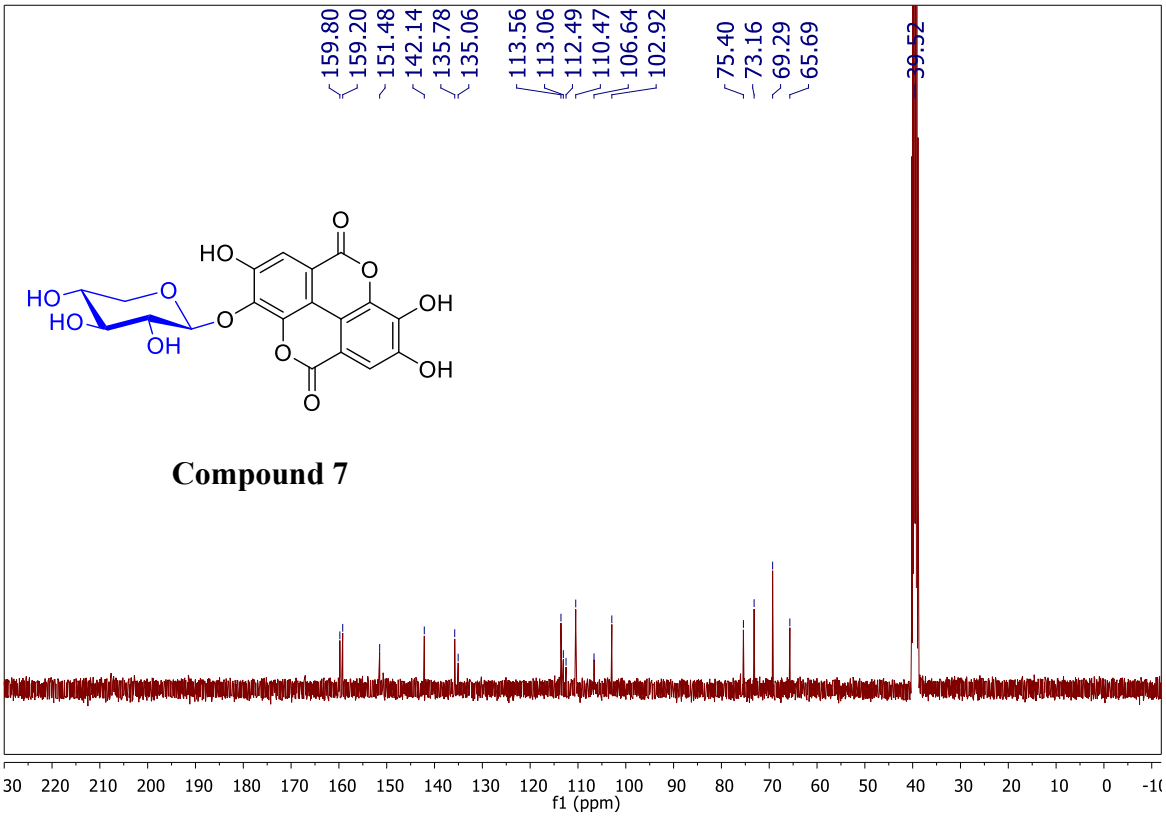
Compound 10


7.65
7.63
7.27
5.78
5.77
5.35
5.33
5.32
5.31
5.27
5.25
4.43
3.58
3.57
3.55
2.16
2.16
2.08
1.09
1.03
0.34
0.32
0.31
0.29
TBSO
AcO
AcO
OAc
OTBS
OTBS
Compound 18
0.99
0.81
1.27
1.31
1.33
1.44
1.42
1.41
2.98
3.36
3.05
12.25
11.19
12.70
7.25
6.37
3.18
3.07
f1 (ppm)


170.03
169.95
169.41
158.65
151.04
150.43
142.63
141.44
140.57
139.39
116.75
114.10
113.69
113.38
110.26
100.03
77.16
69.96
69.91
68.57
62.10
26.10
25.95
25.79
20.93
20.87
18.91
18.84
18.55
-3.52
-3.66
TBSO
AcO
AcO
OAc
OTBS
OTBS
Compound 18
f1 (ppm)



Compound 7



Compound 7