Directing Drugs to Bugs: Antibiotic-Carbohydrate Conjugates targeting Biofilm-associated Lectins of *Pseudomonas aeruginosa*

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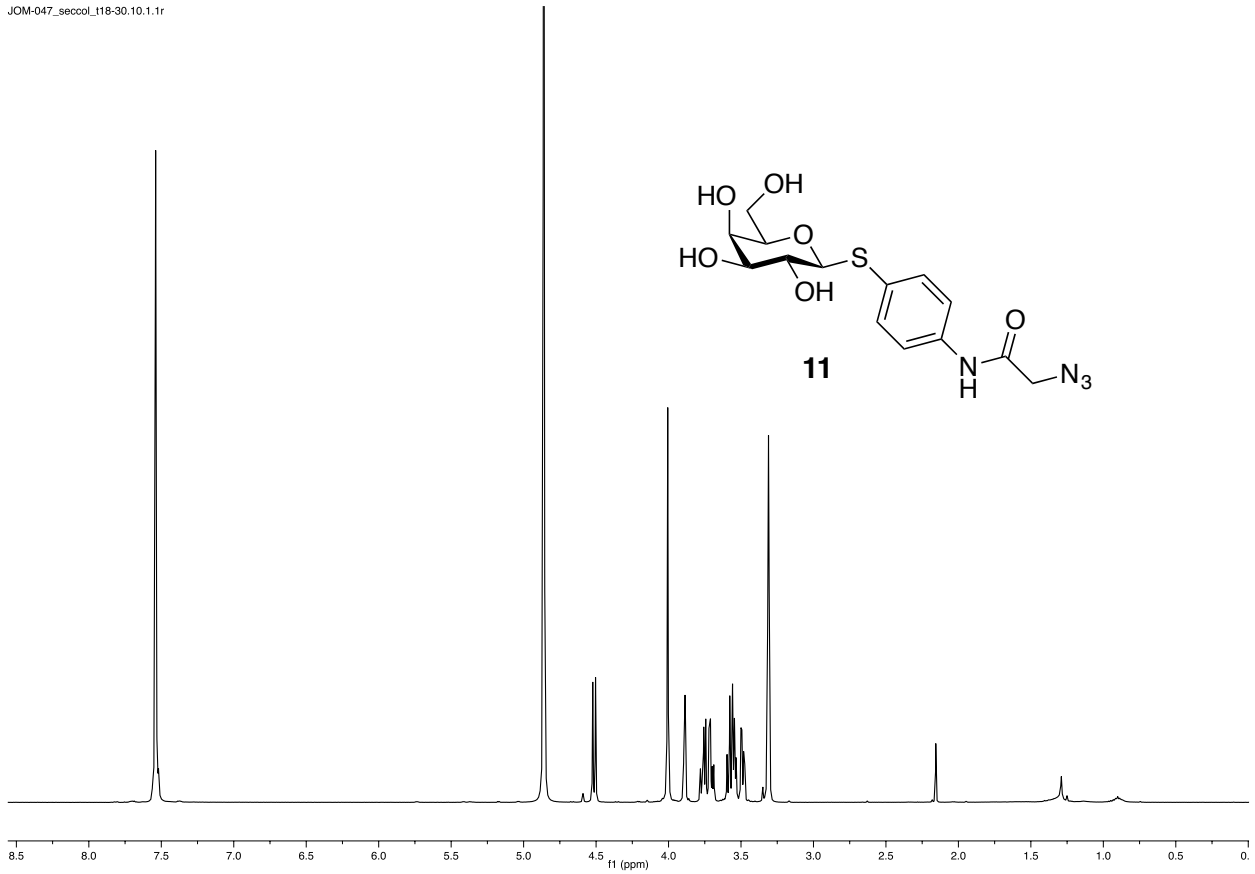
Table of contents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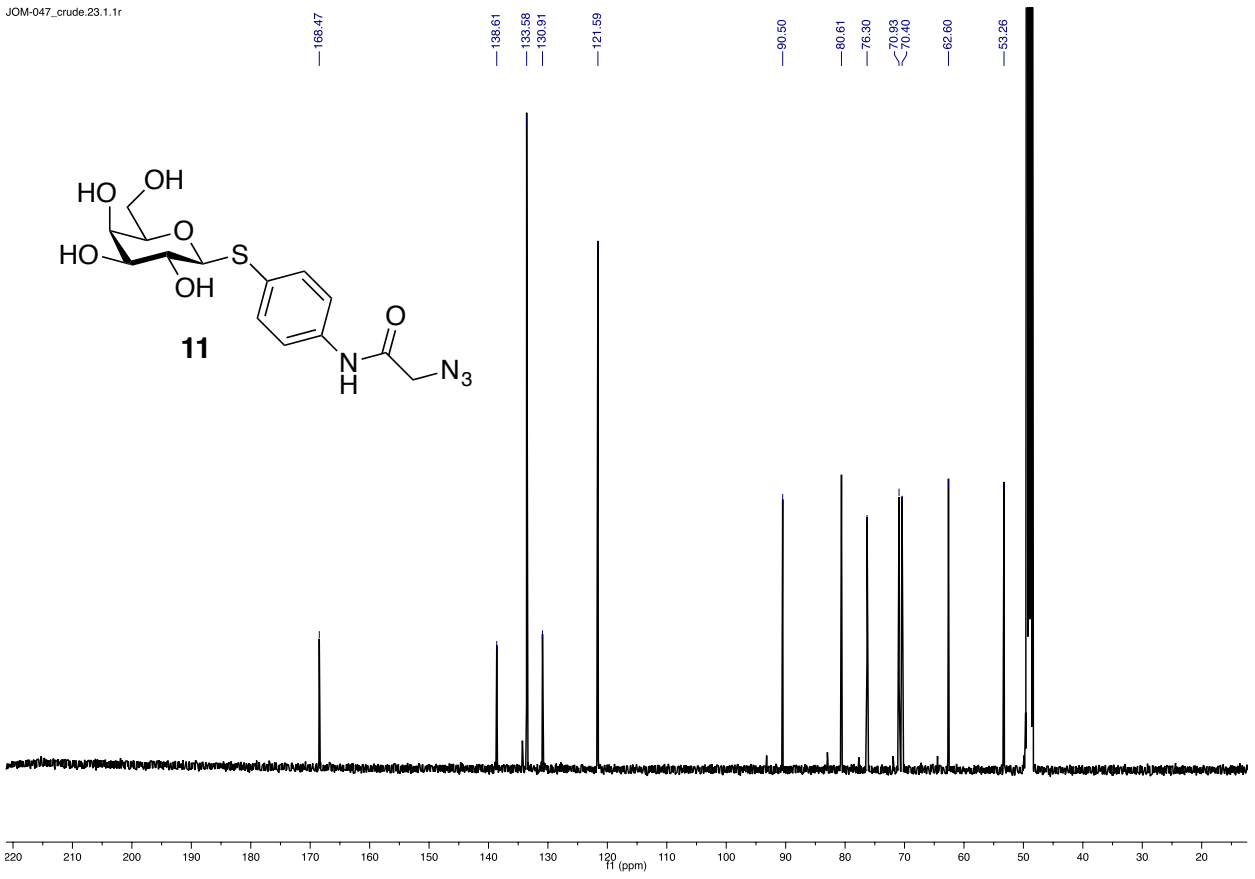
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70.40
62.60
53.26
HO
OH
O
HO
S
OH
O
N
H
N3
11
220
210
200
190
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150
140
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120
110
100
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20
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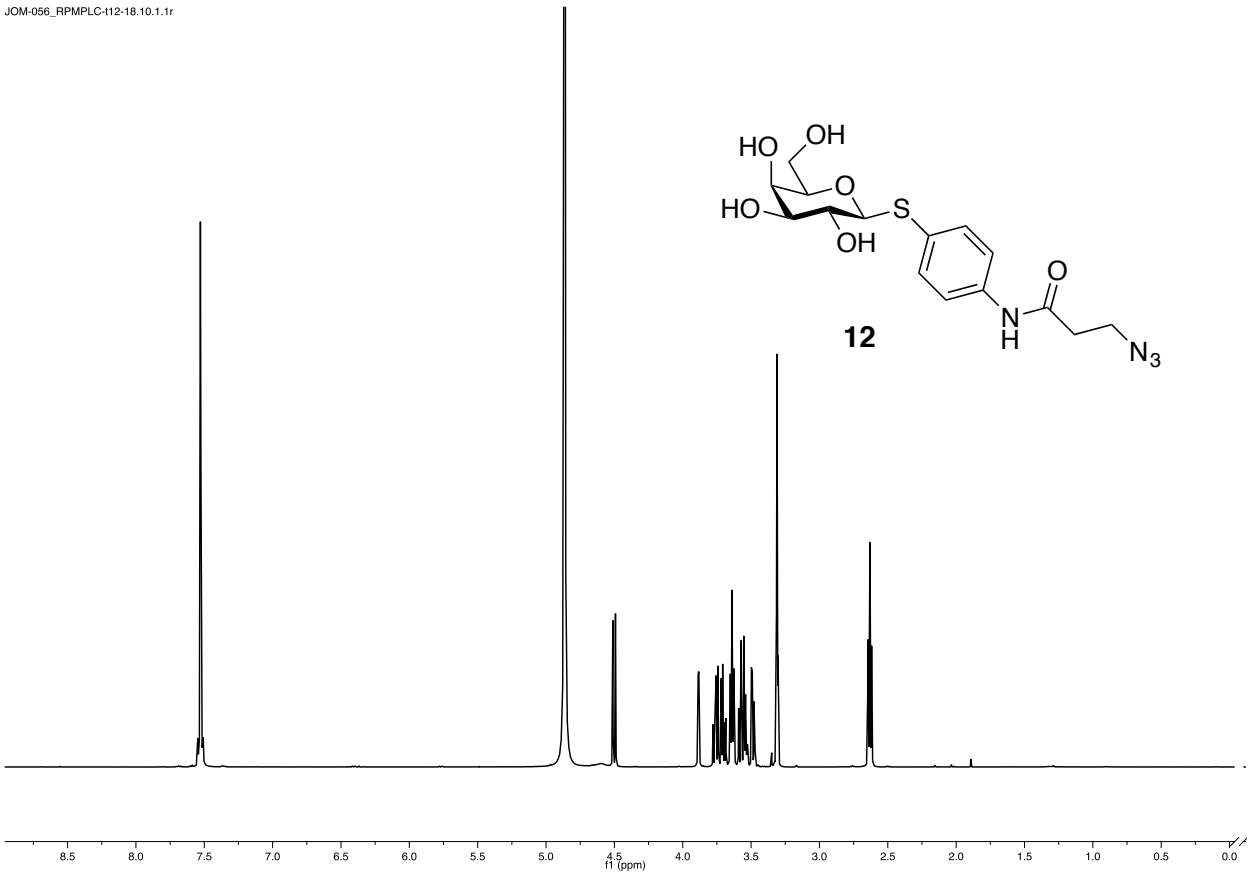
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H
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6.5
6.0
5.5
5.0
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2.5
2.0
1.5
1.0
0.5
0.0
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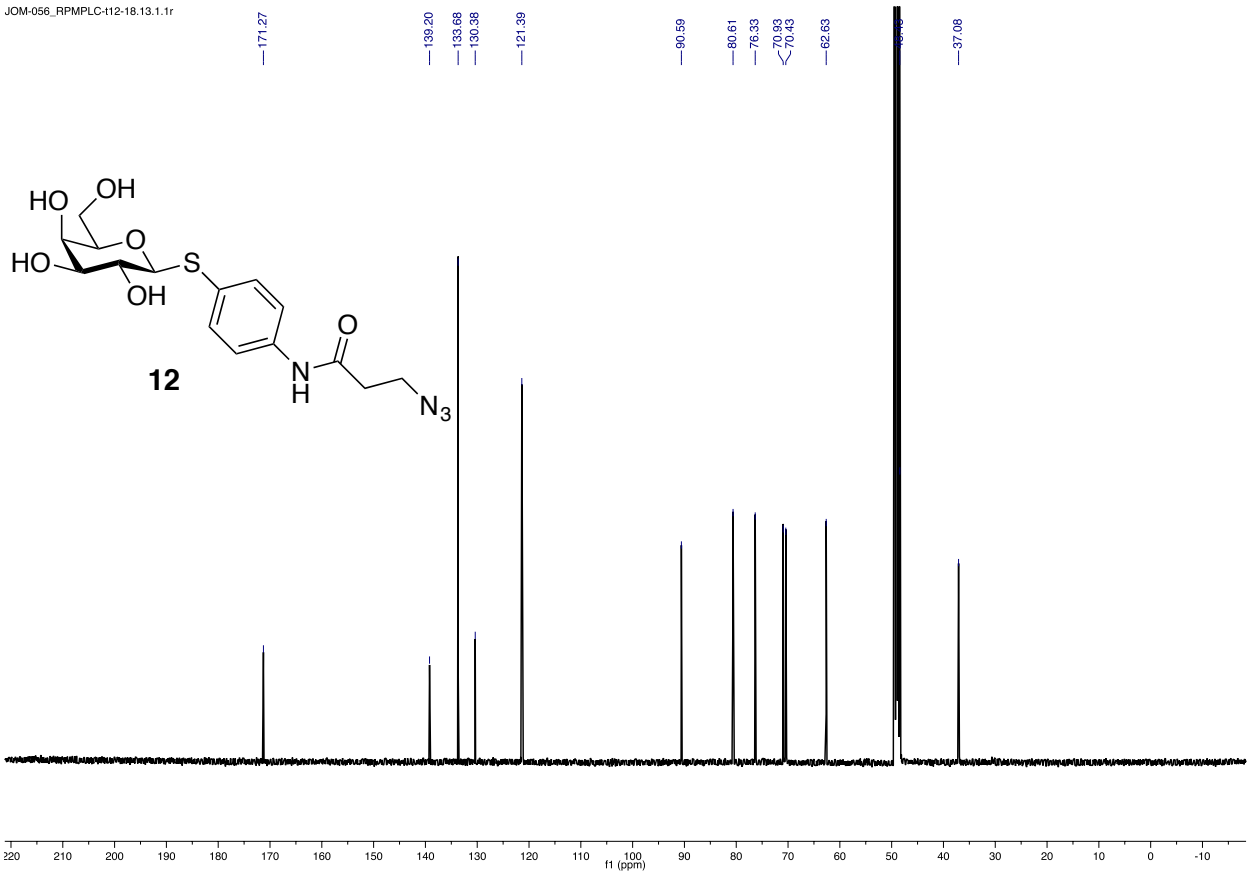
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70.93
70.43
62.63
37.08
HO
OH
O
HO
S
OH
O
N
H
N3
12
220
210
200
190
180
170
160
150
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110
100
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80
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40
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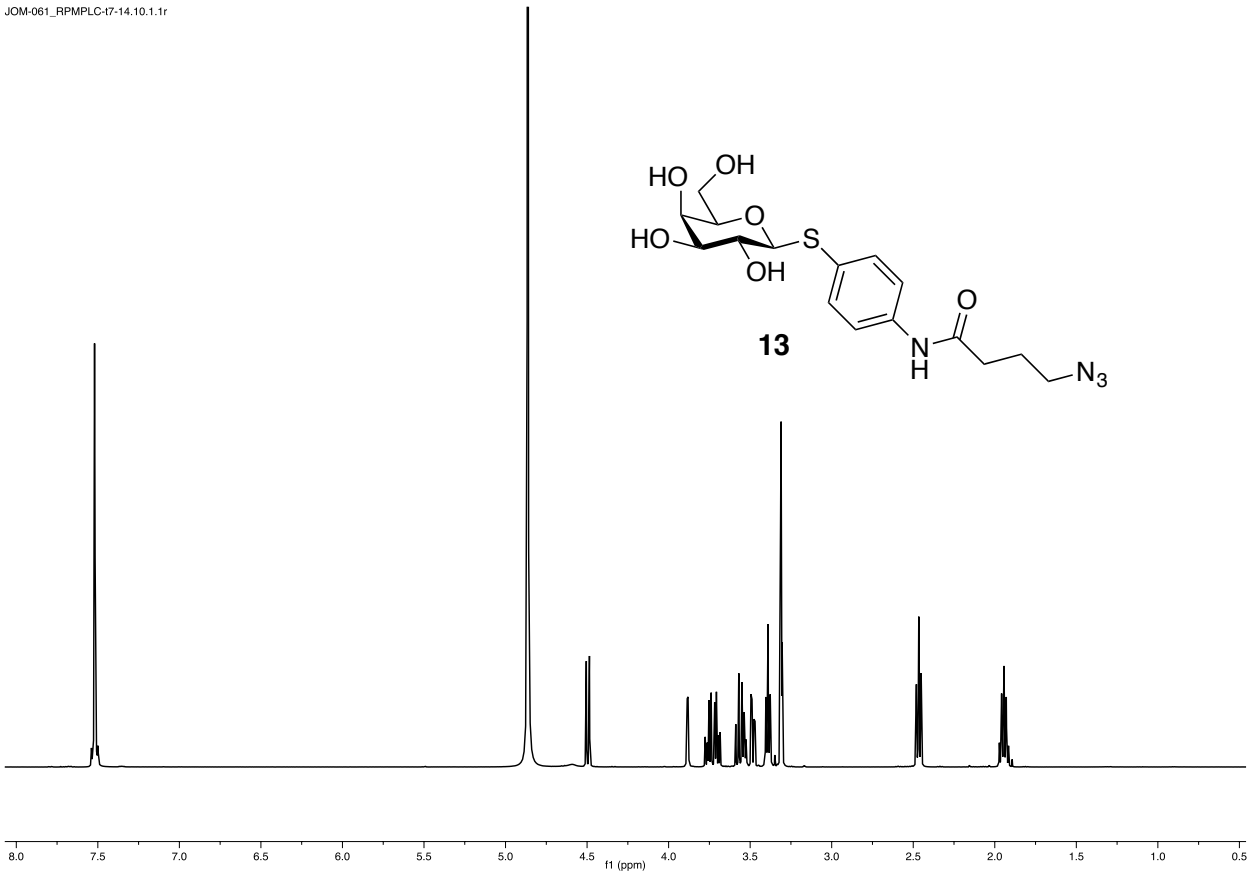
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O
N
H
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6.5
6.0
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5.0
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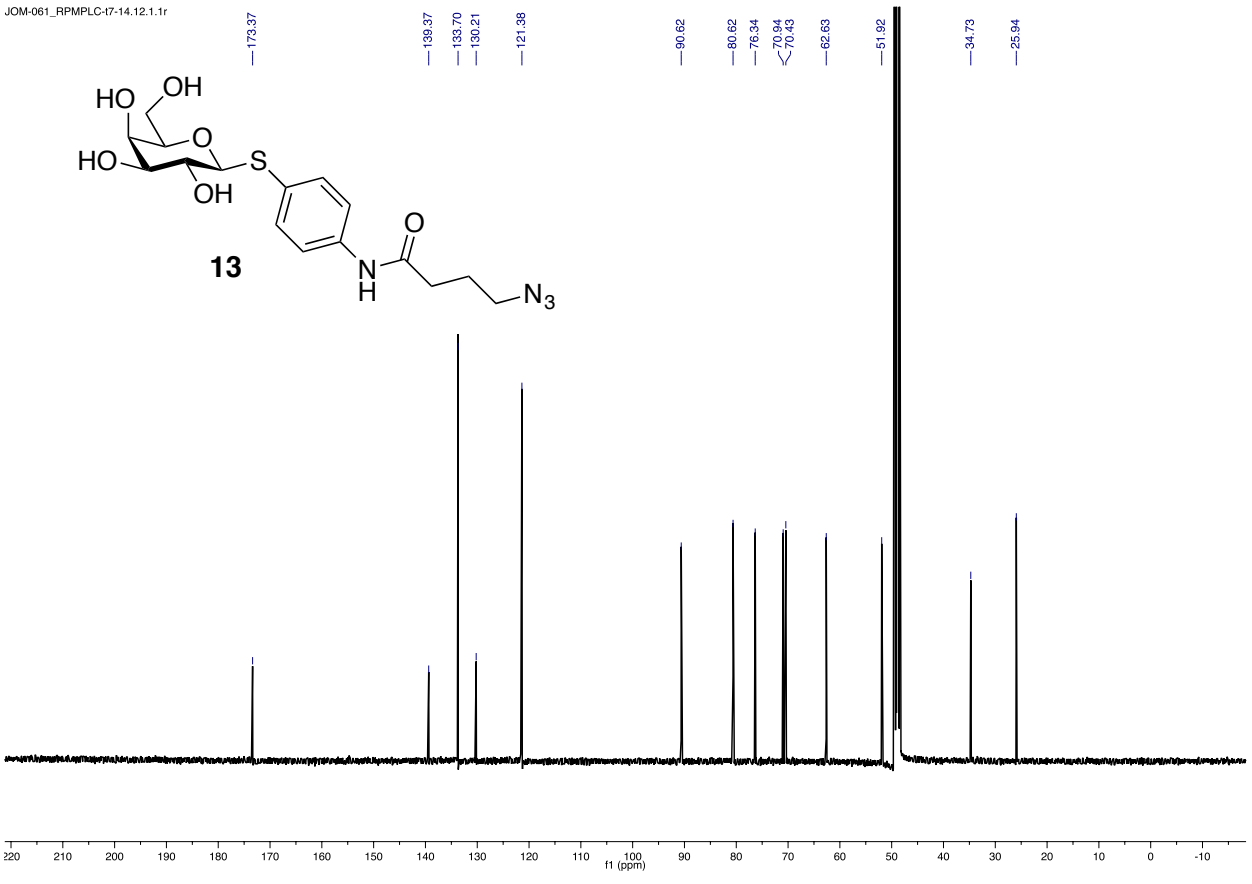
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)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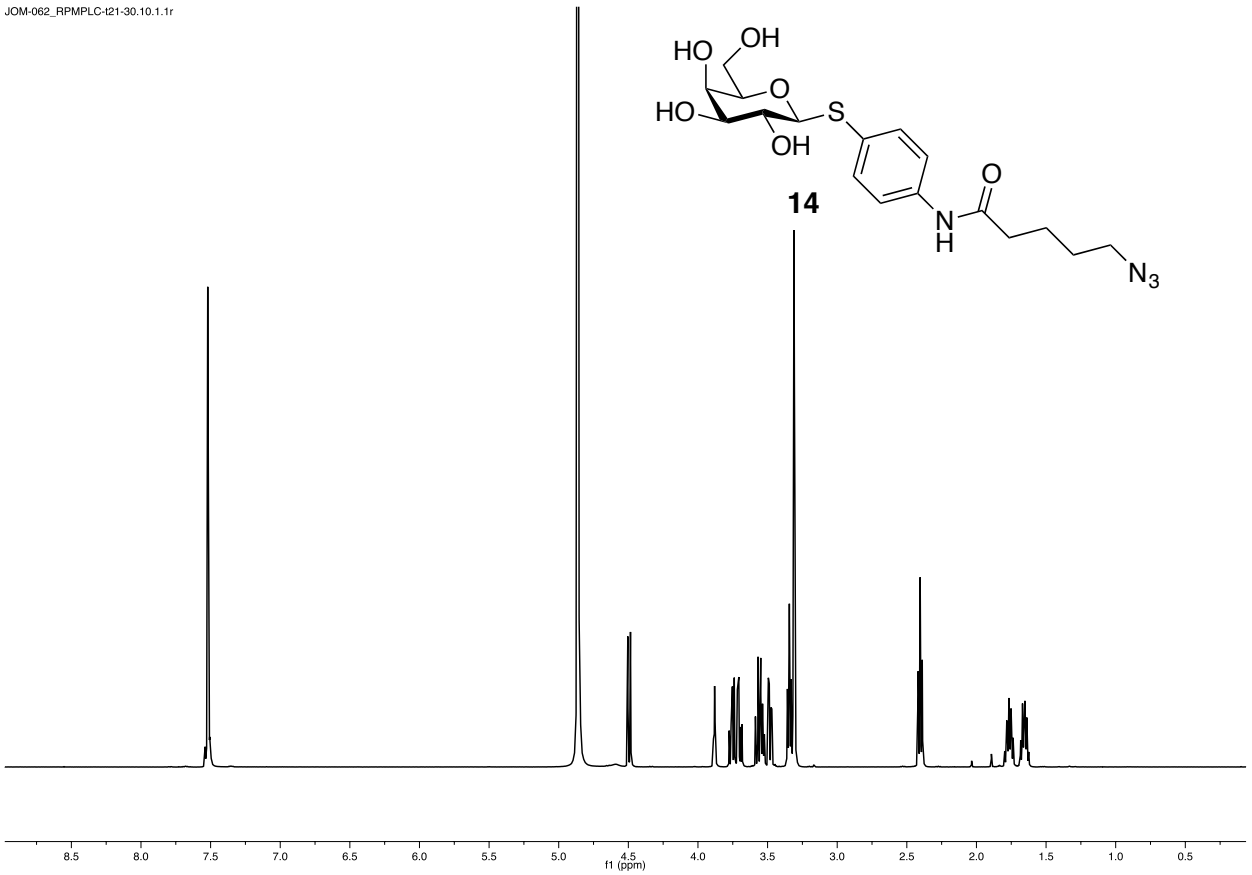
70.94
70.43
62.63
51.92
34.73
25.94
HO
OH
O
HO
S
OH
O
N
H
N3
13
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)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O
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H
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7.0
6.5
6.0
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4.0
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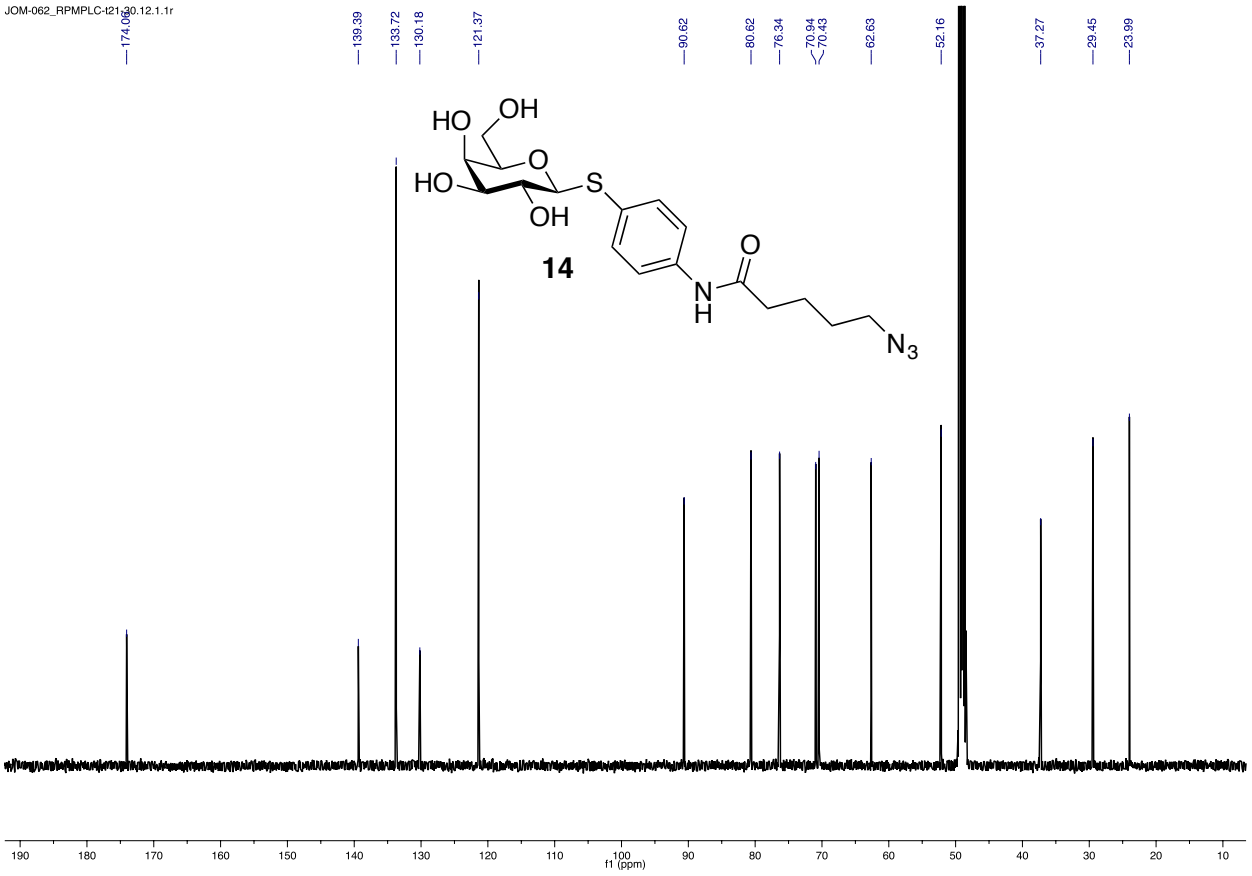
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)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121.37
90.62
80.62
76.34
70.94
70.43
62.63
52.16
37.27
29.45
23.99
HO
OH
O
HO
S
OH
14
O
N
H
N3
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

JOM-048_t13-22

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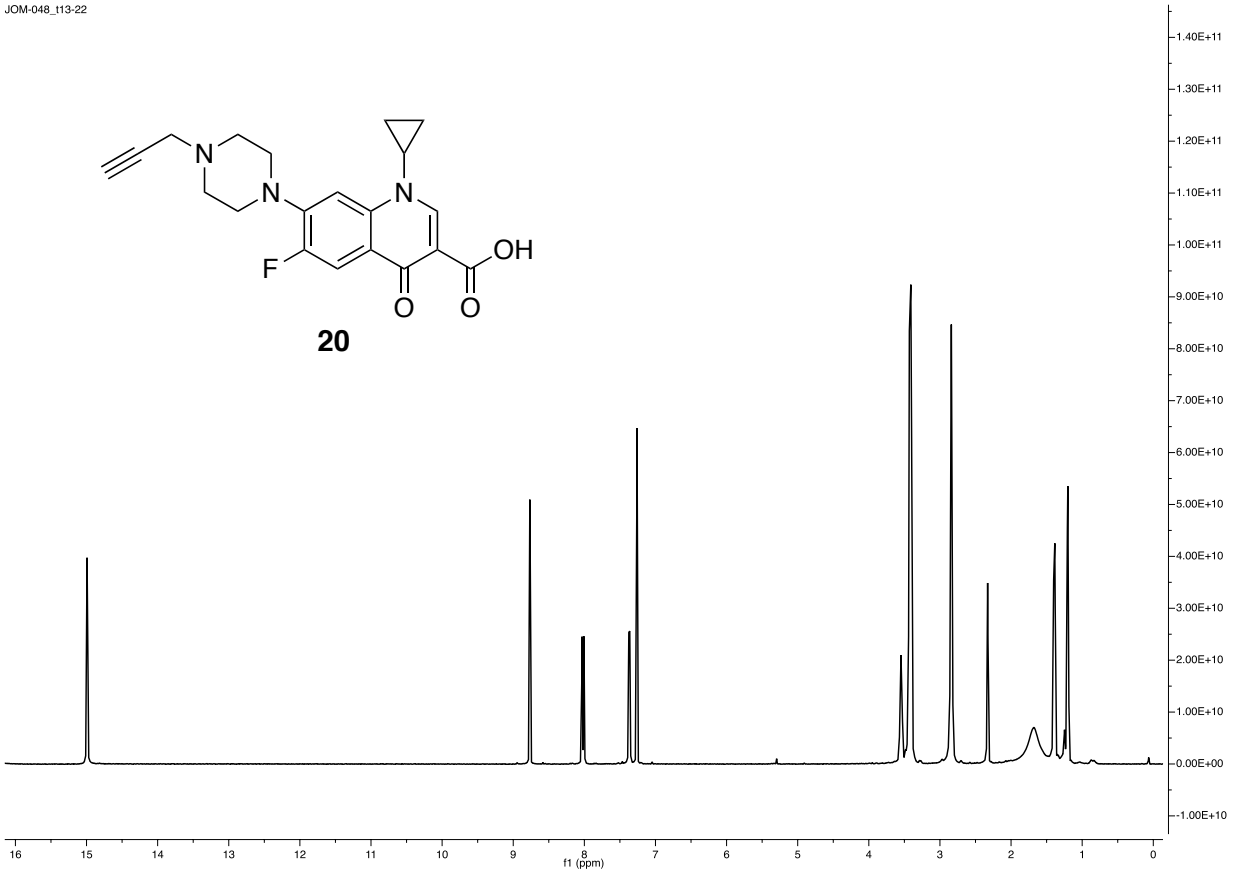

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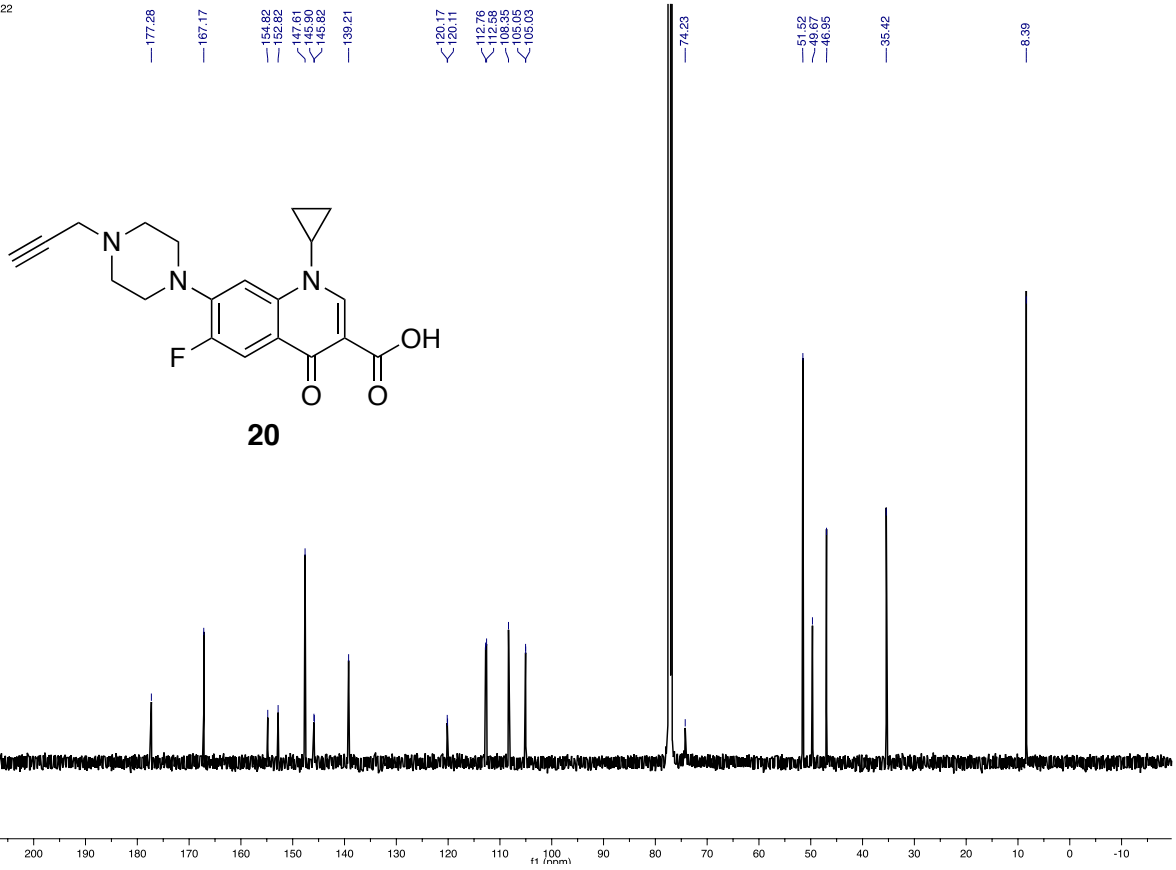

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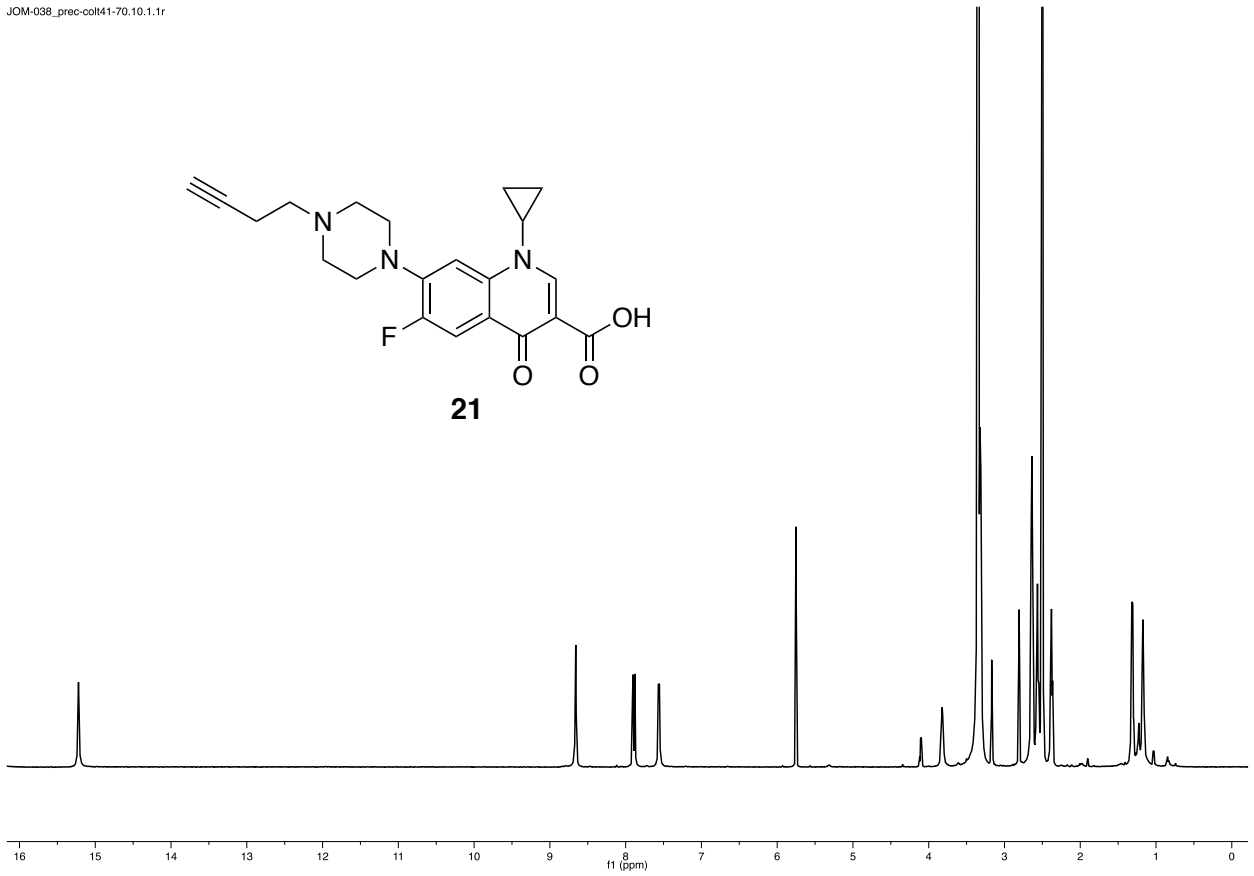
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N
F
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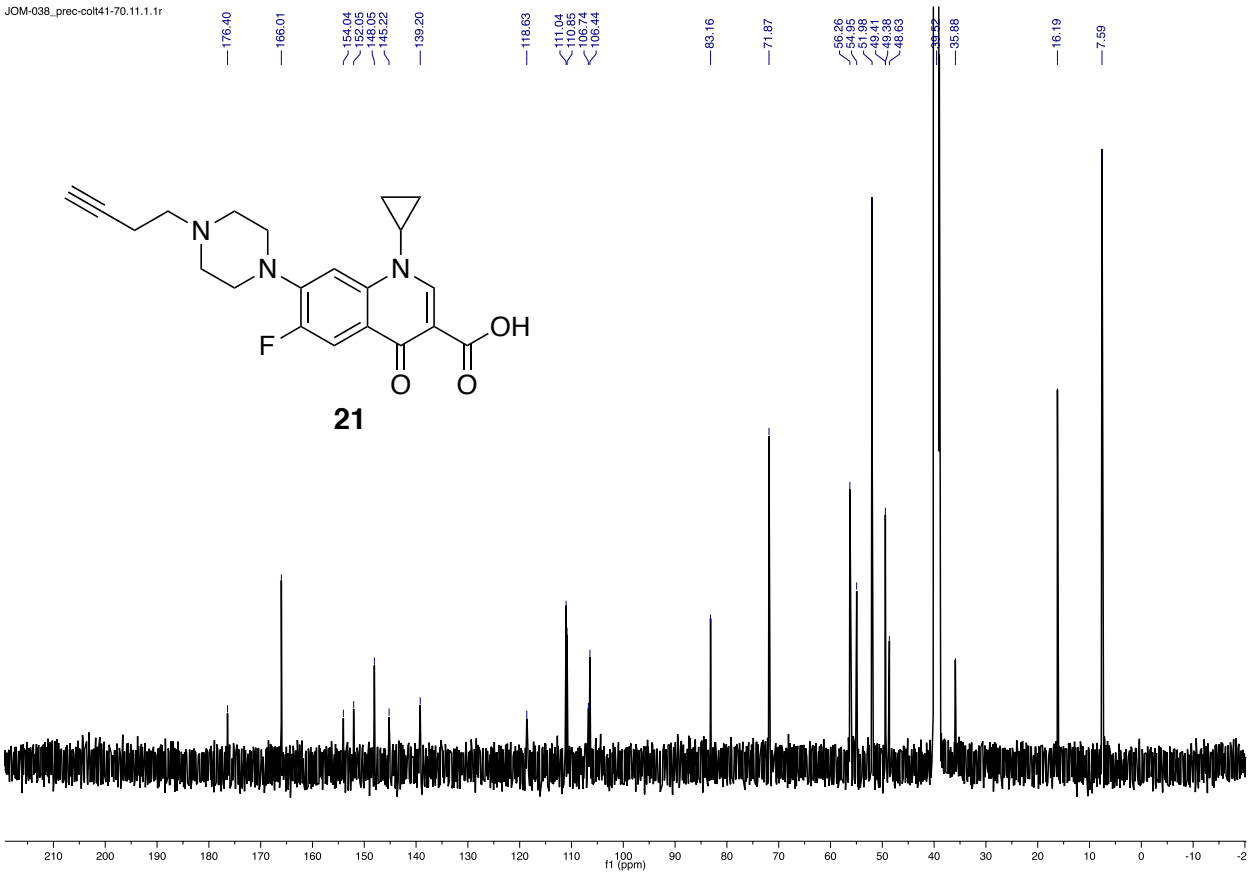
O
O
21
f1 (ppm)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106.44
83.16
71.87
56.26
54.95
51.98
49.41
49.38
48.63
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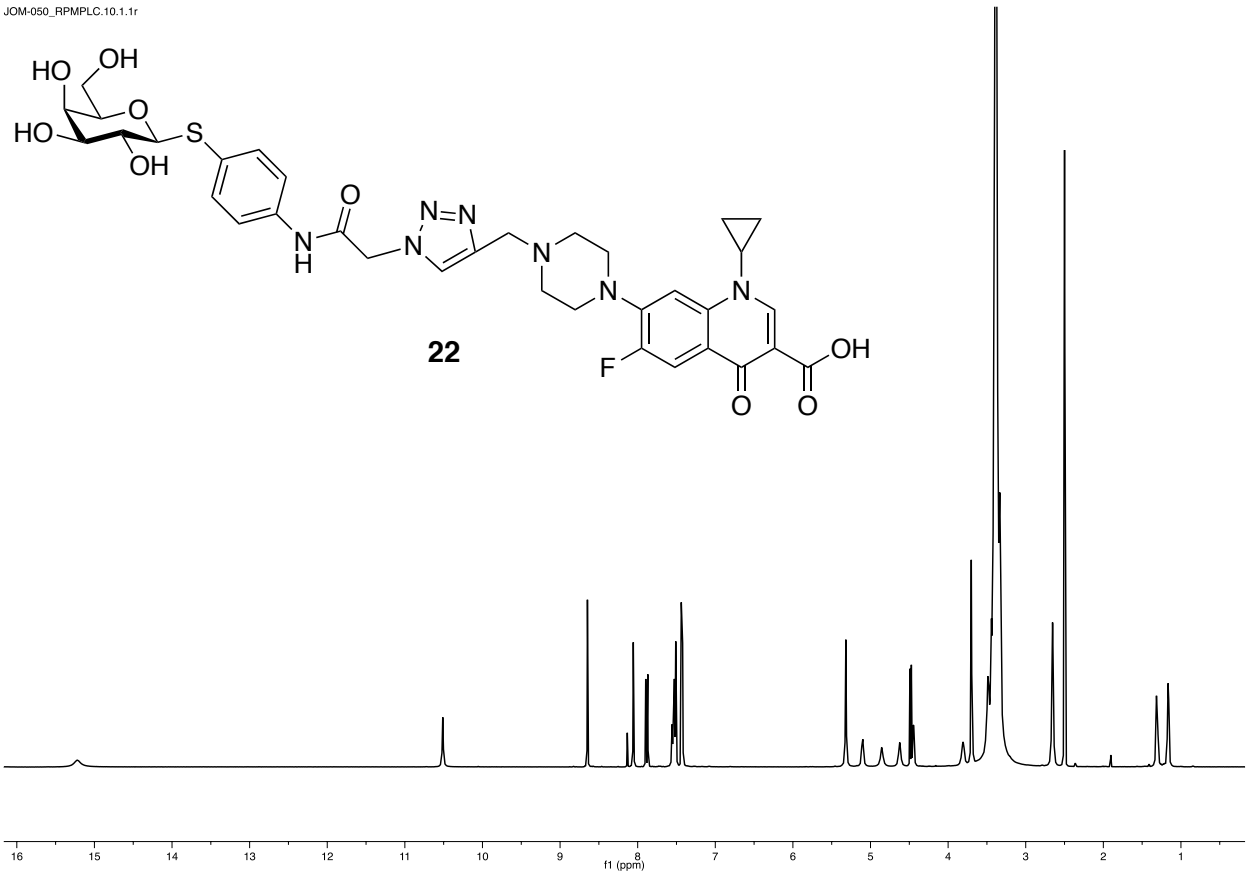
35.88
16.19
7.59
N
N
N
F
OH
O
O
21
f1 (ppm)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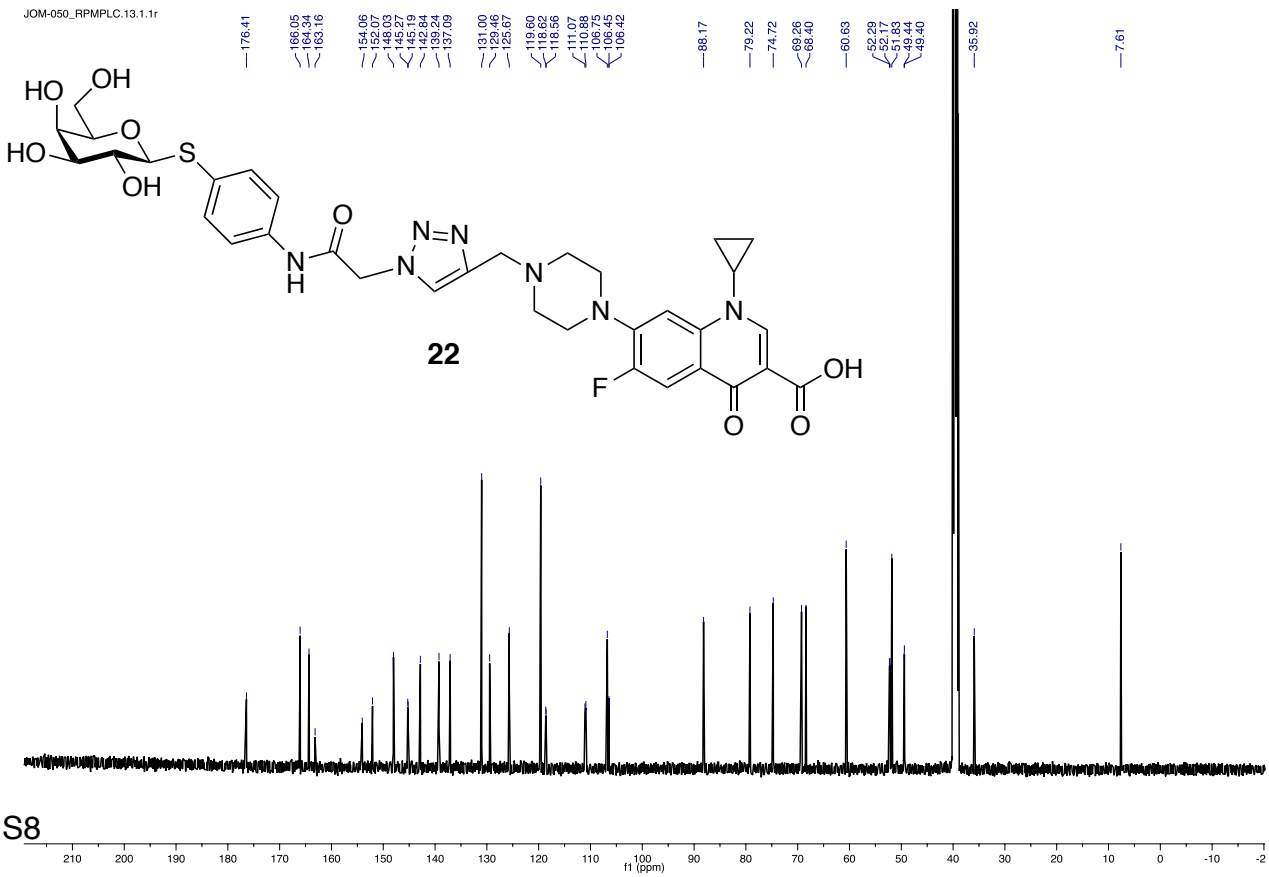
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49.44
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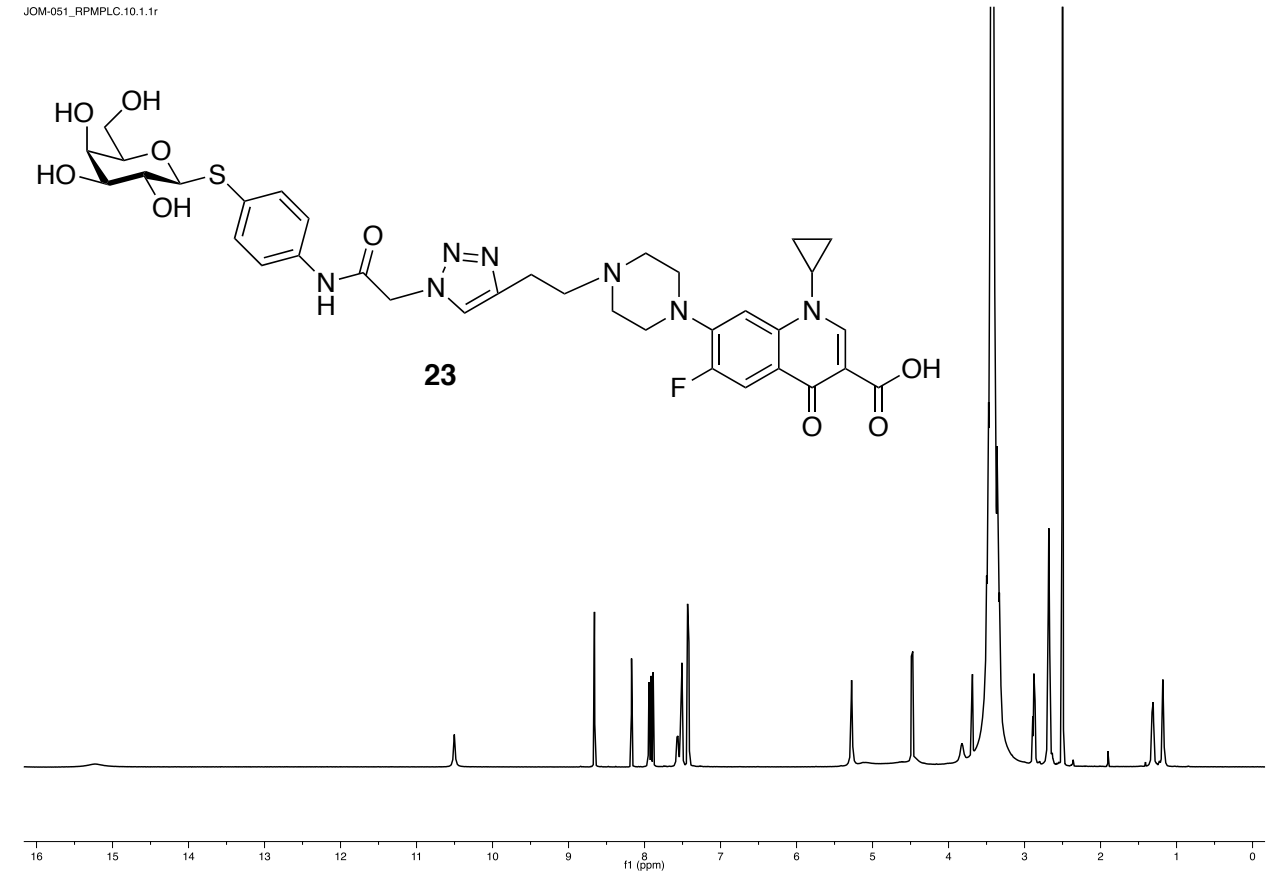
22
f1 (ppm)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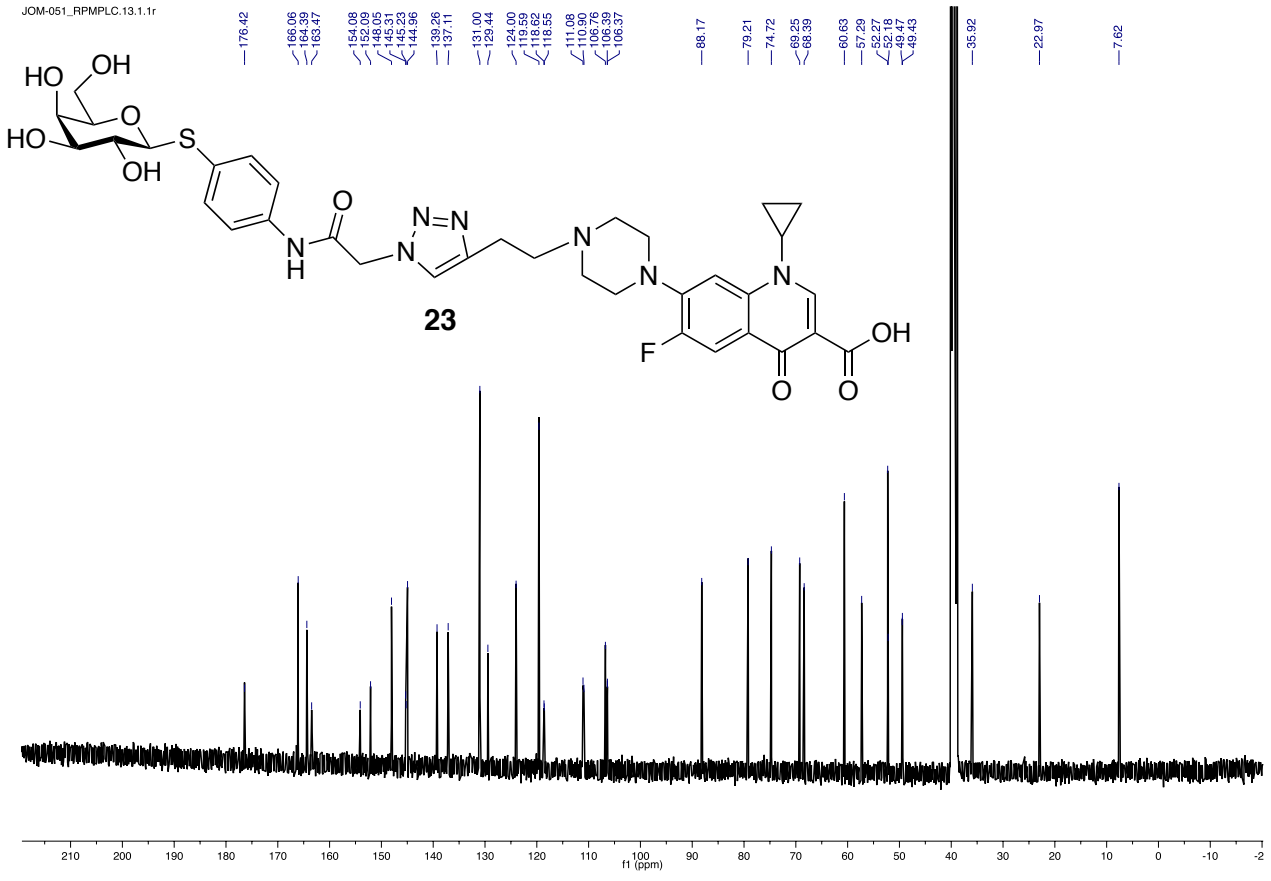
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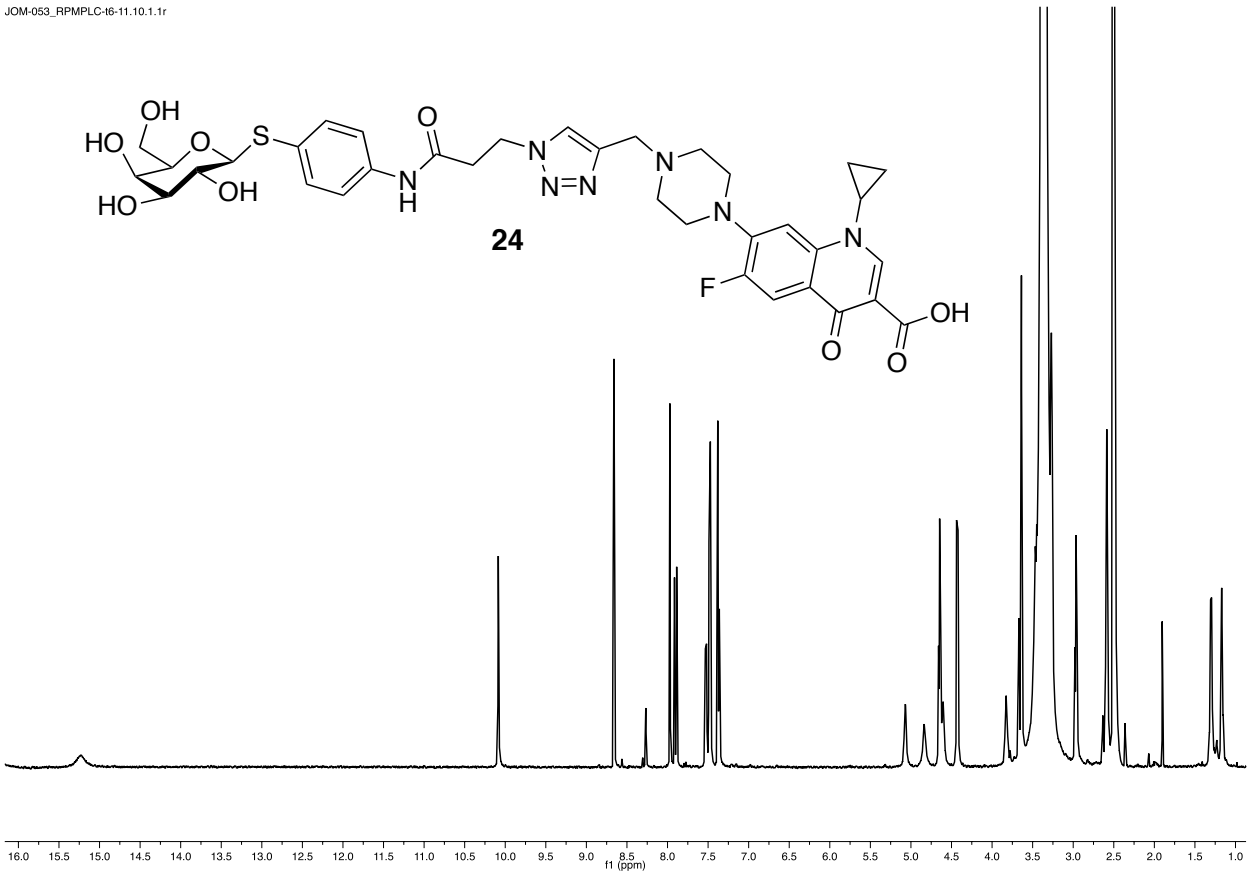
7.62
23
f1 (ppm)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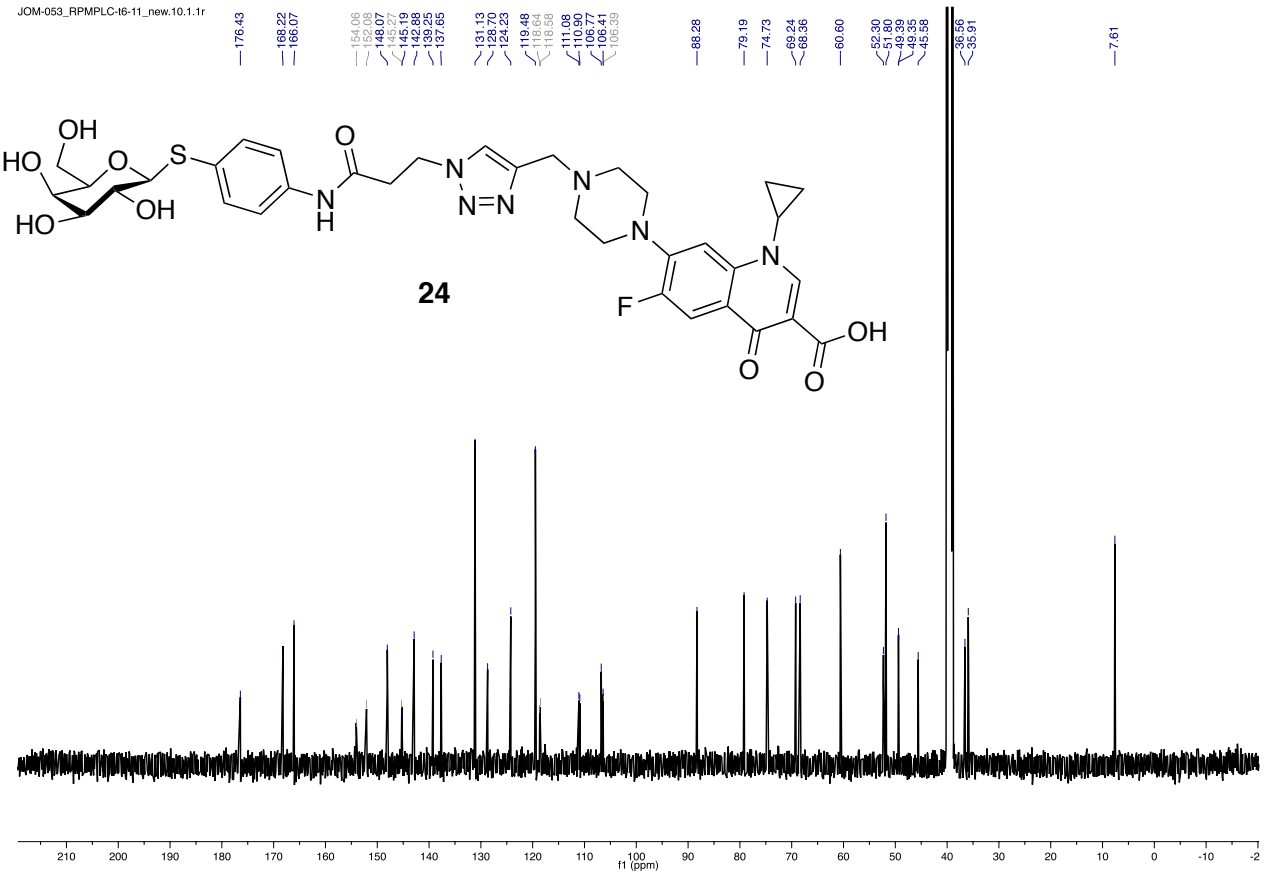
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139.25
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131.13
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124.23
119.48
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111.08
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106.41
106.39
88.28
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74.73
69.24
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24
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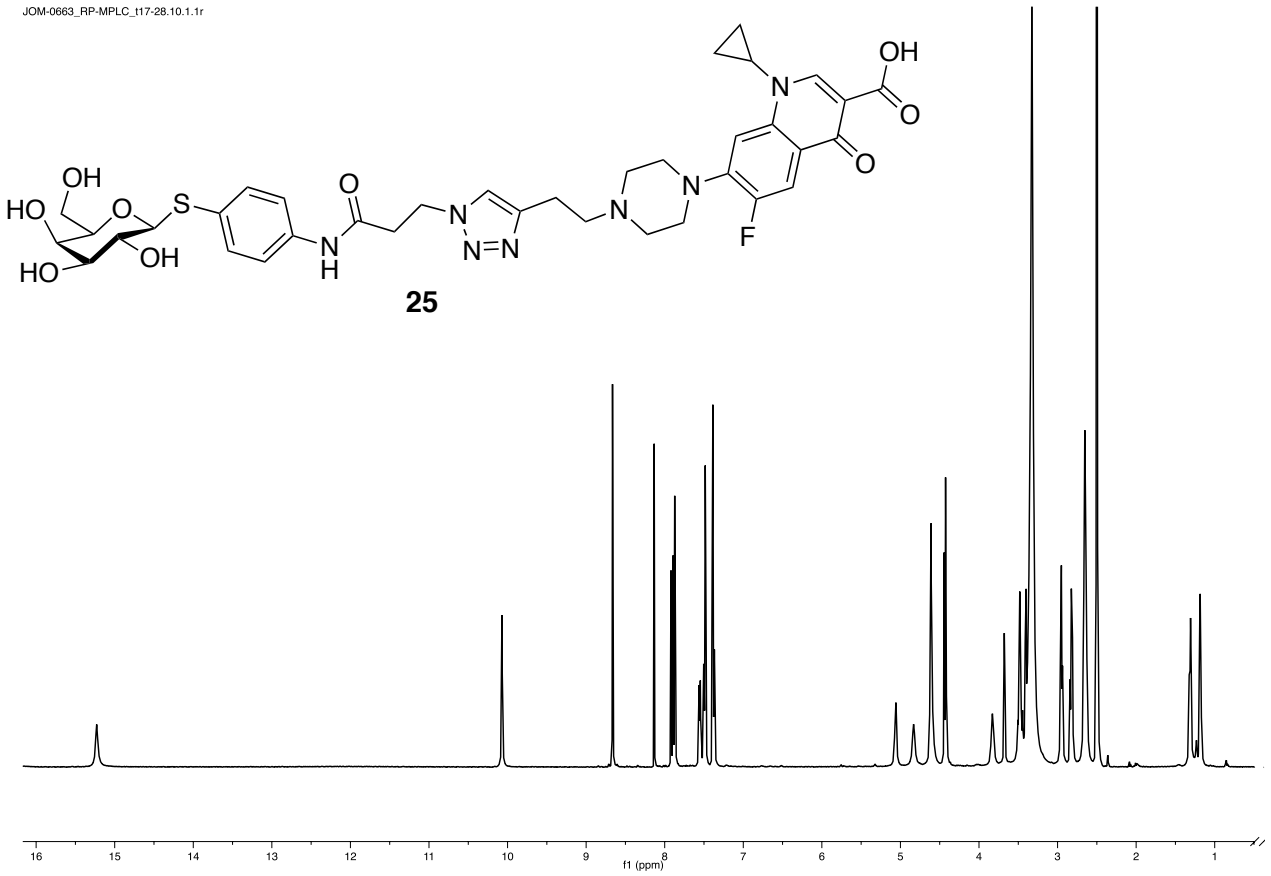
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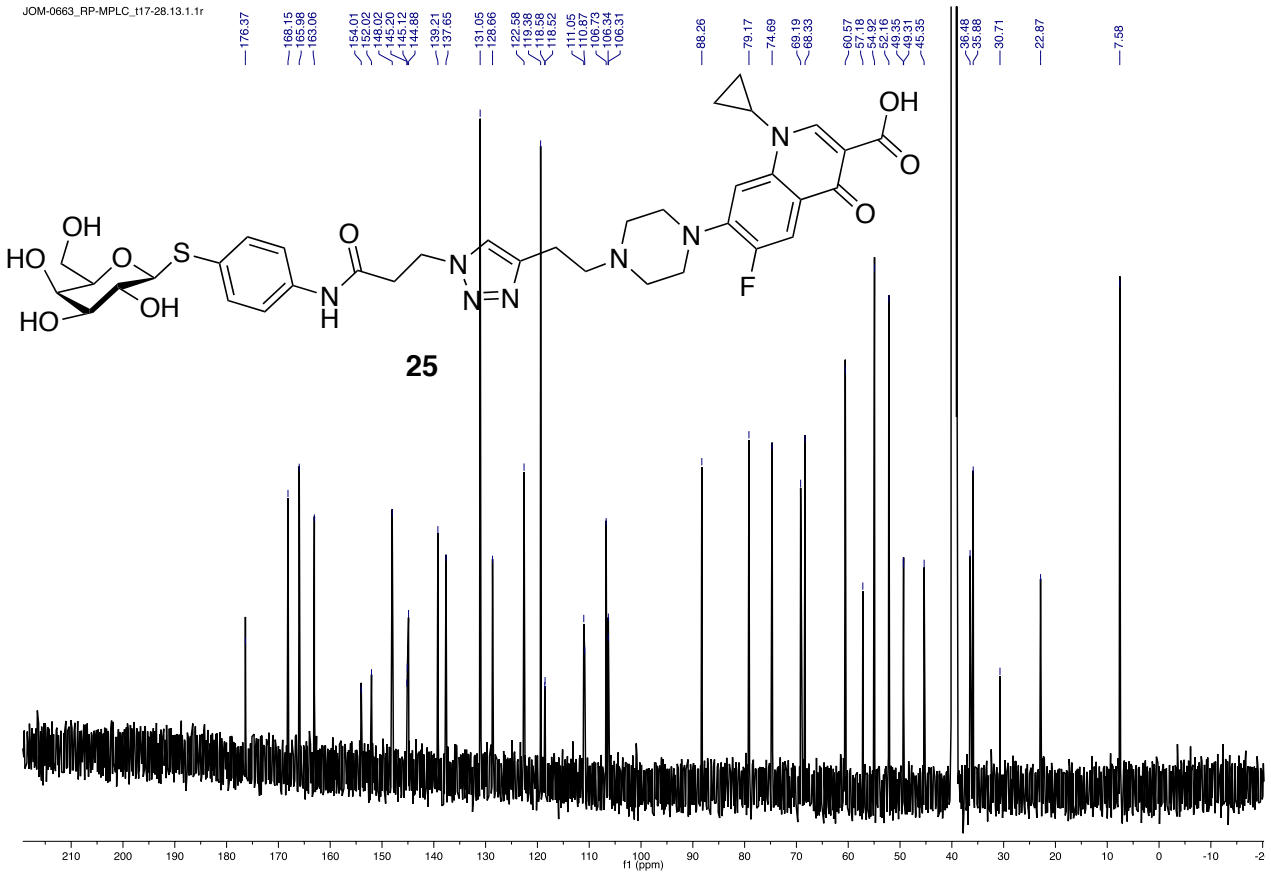
HO
N
H
O
N
N=N
N
N
OH
O
N
O
F
f1 (ppm)

25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137.65
131.05
128.66
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OH
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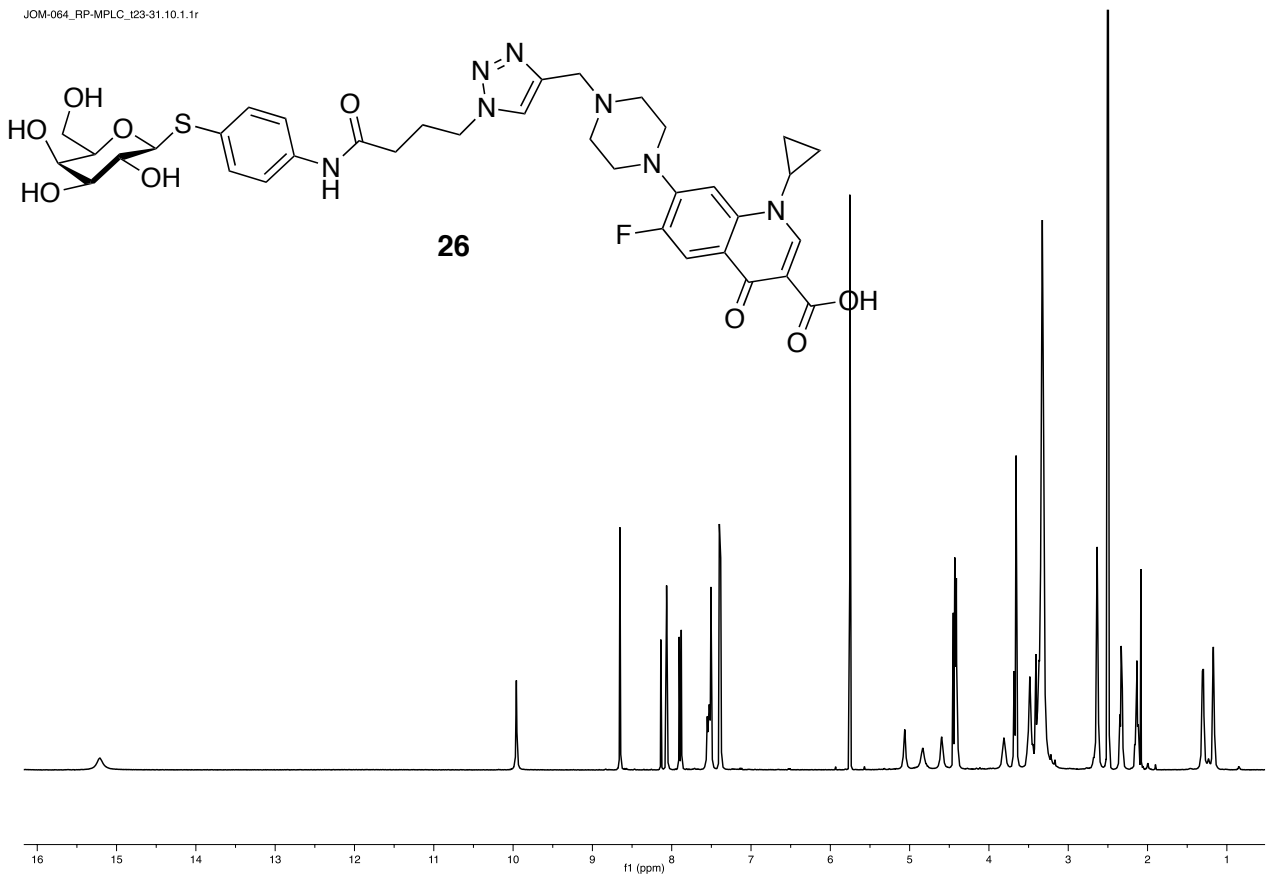
O
F
f1 (ppm)

25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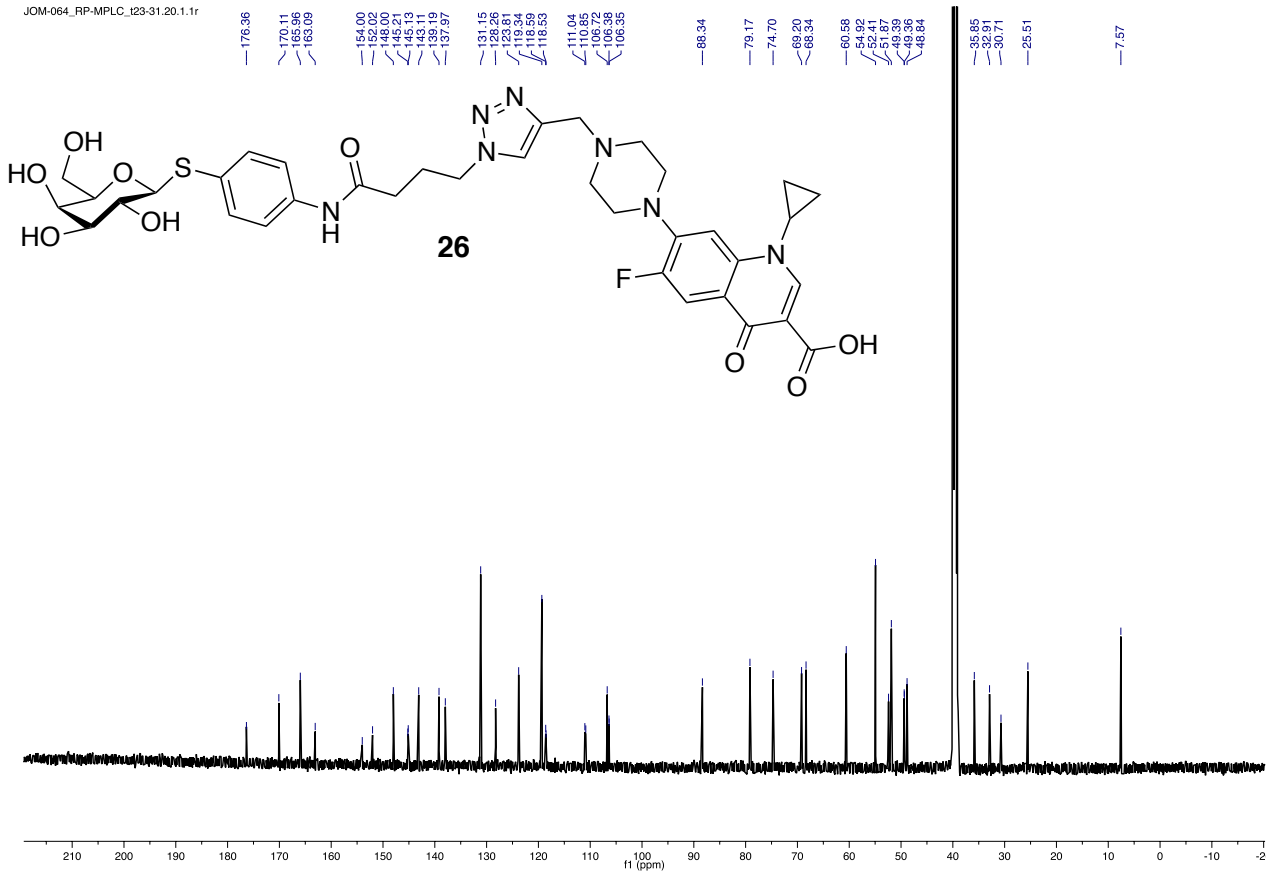
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145.13
143.11
139.19
137.97
131.15
128.26
123.61
119.34
118.59
118.53
111.04
110.85
106.72
106.38
106.35
88.34
79.17
74.70
69.20
68.34
60.58
54.92
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48.84
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32.91
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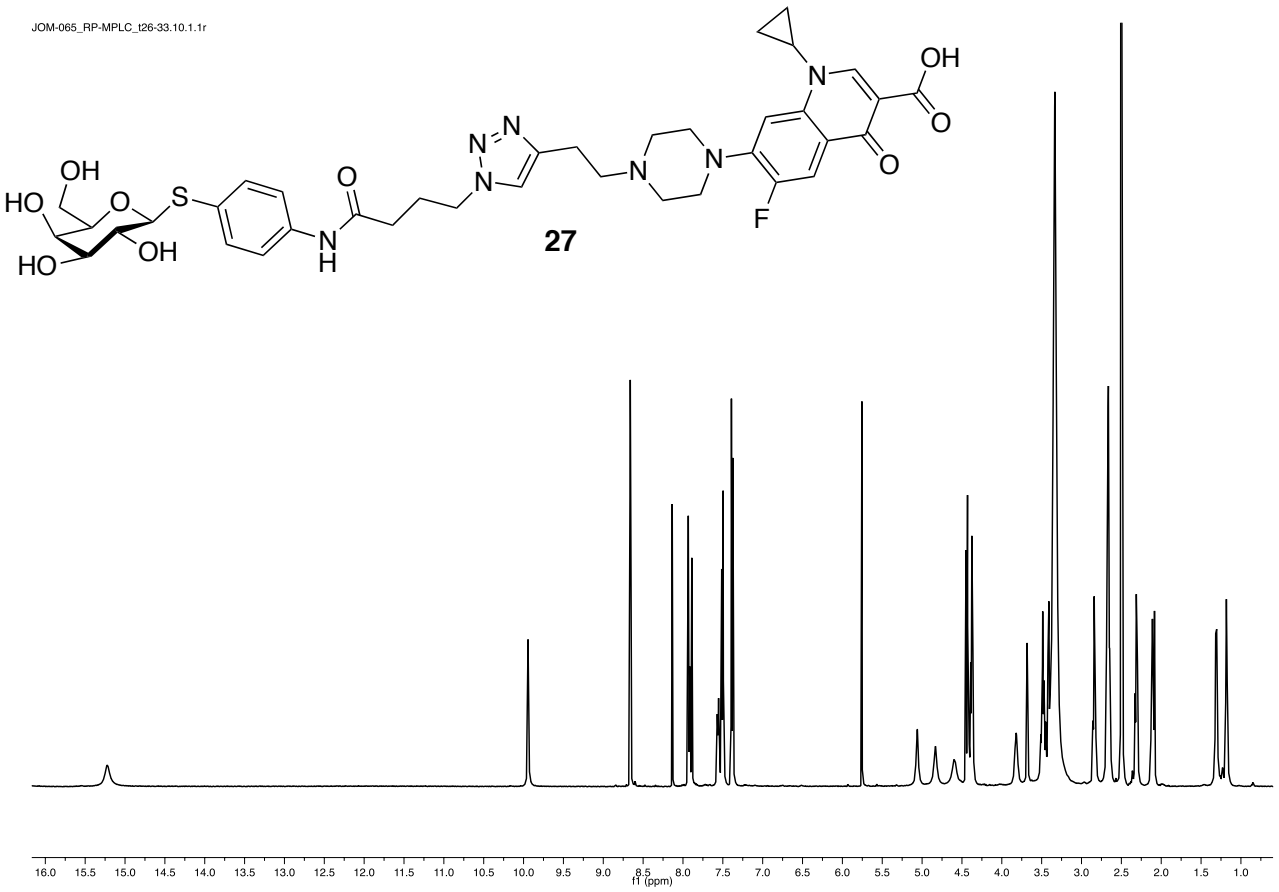
25.51
7.57
26
f1 (ppm)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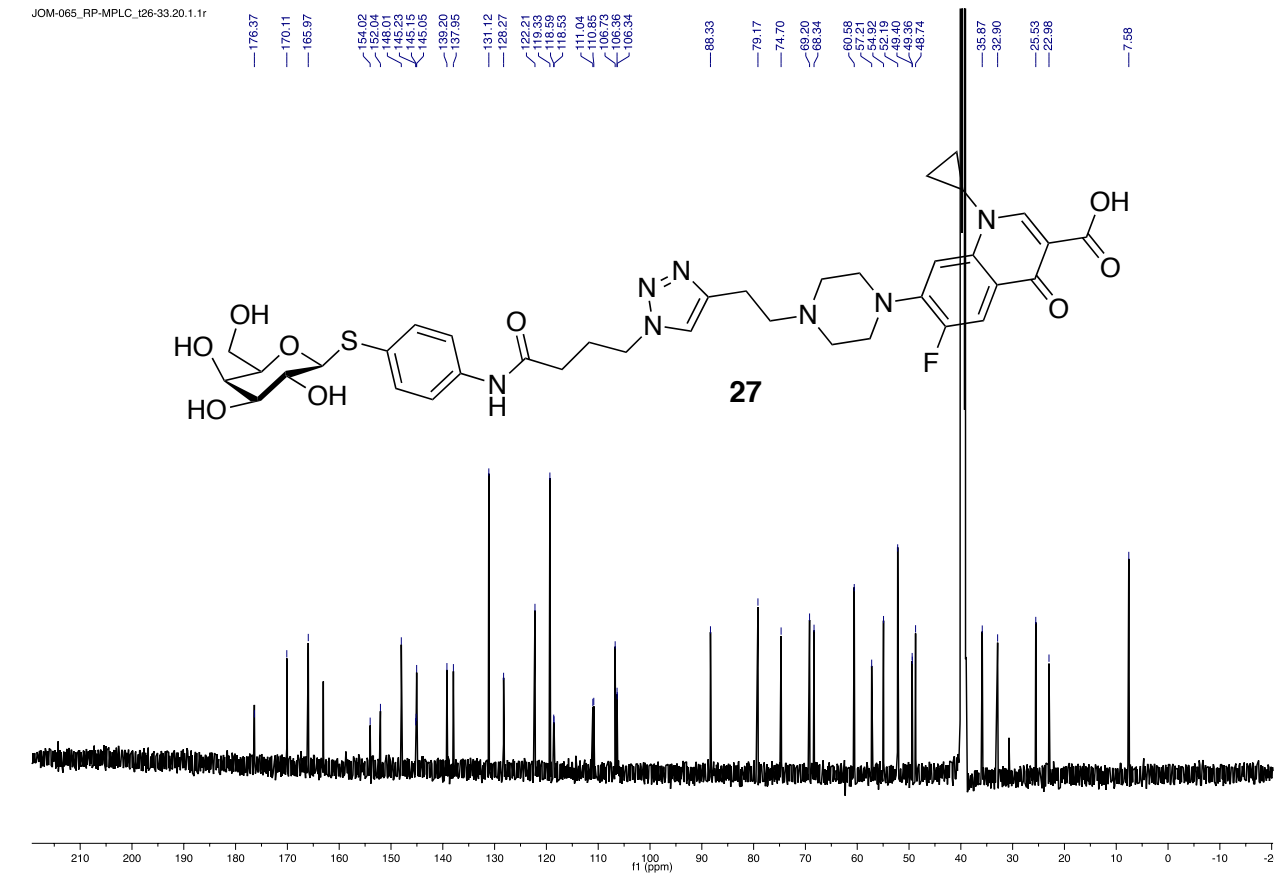
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106.34
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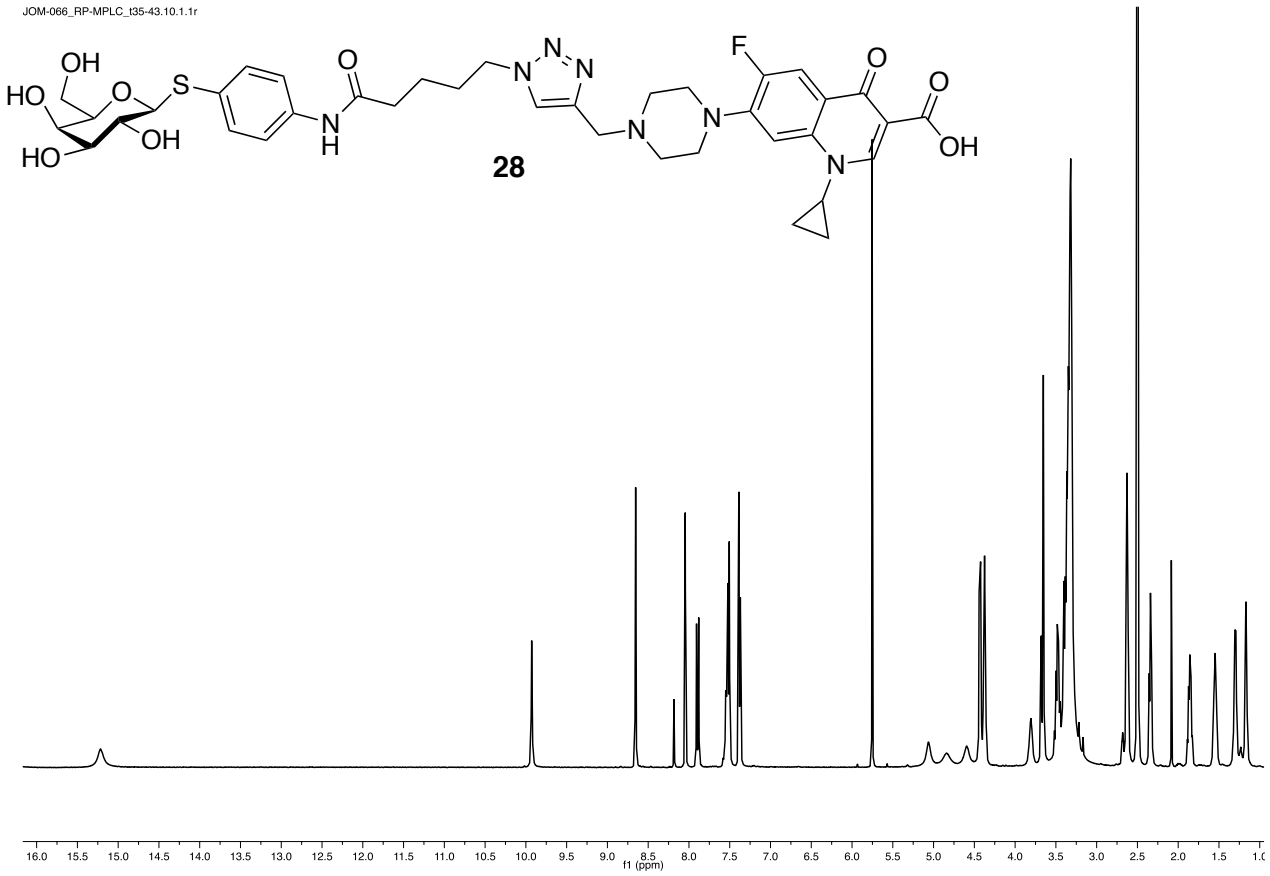
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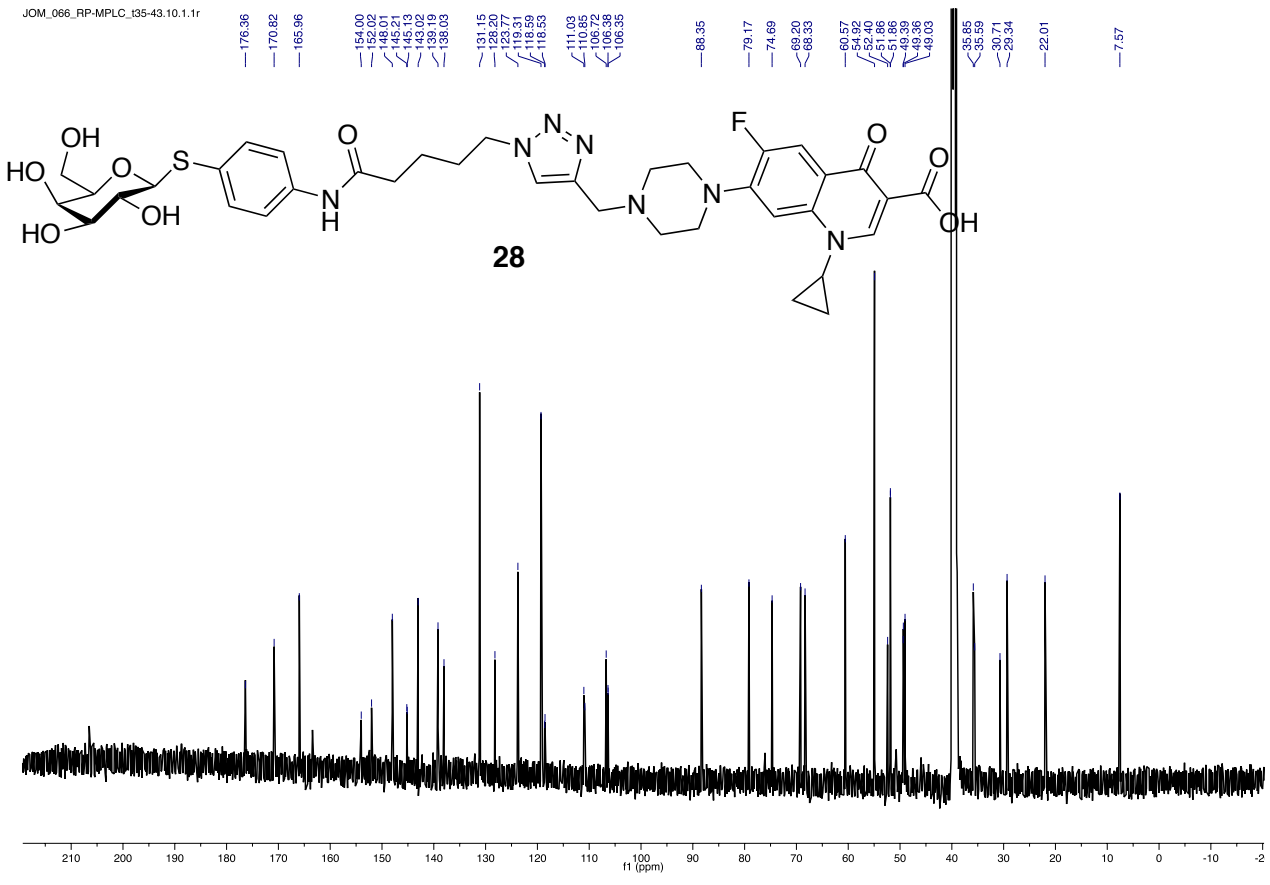
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143.02
139.19
138.03
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128.20
123.77
119.31
118.59
118.53
111.03
110.85
106.72
106.38
106.35
88.35
79.17
74.69
69.20
68.33
60.57
54.92
52.40
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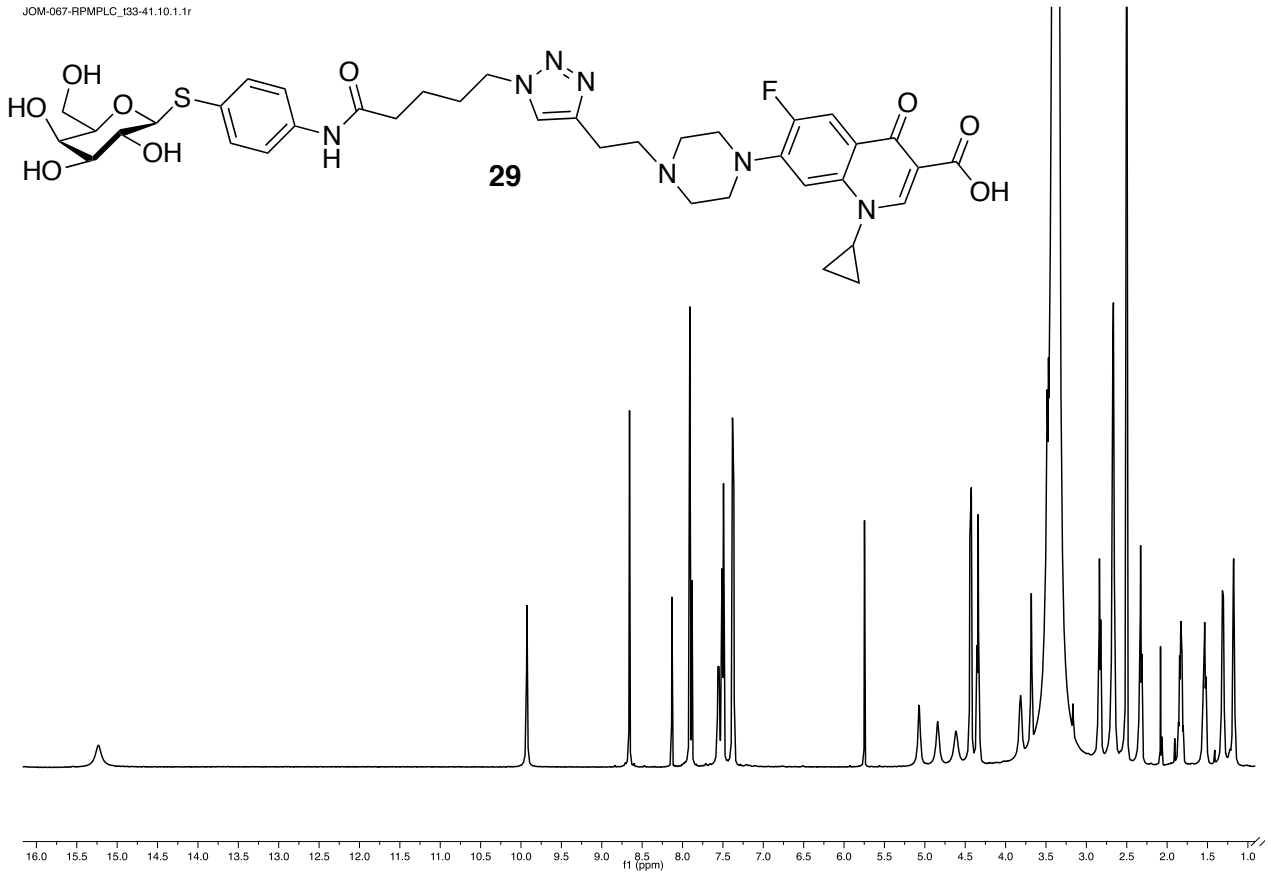
29.34
22.01
7.57
28
f1 (ppm)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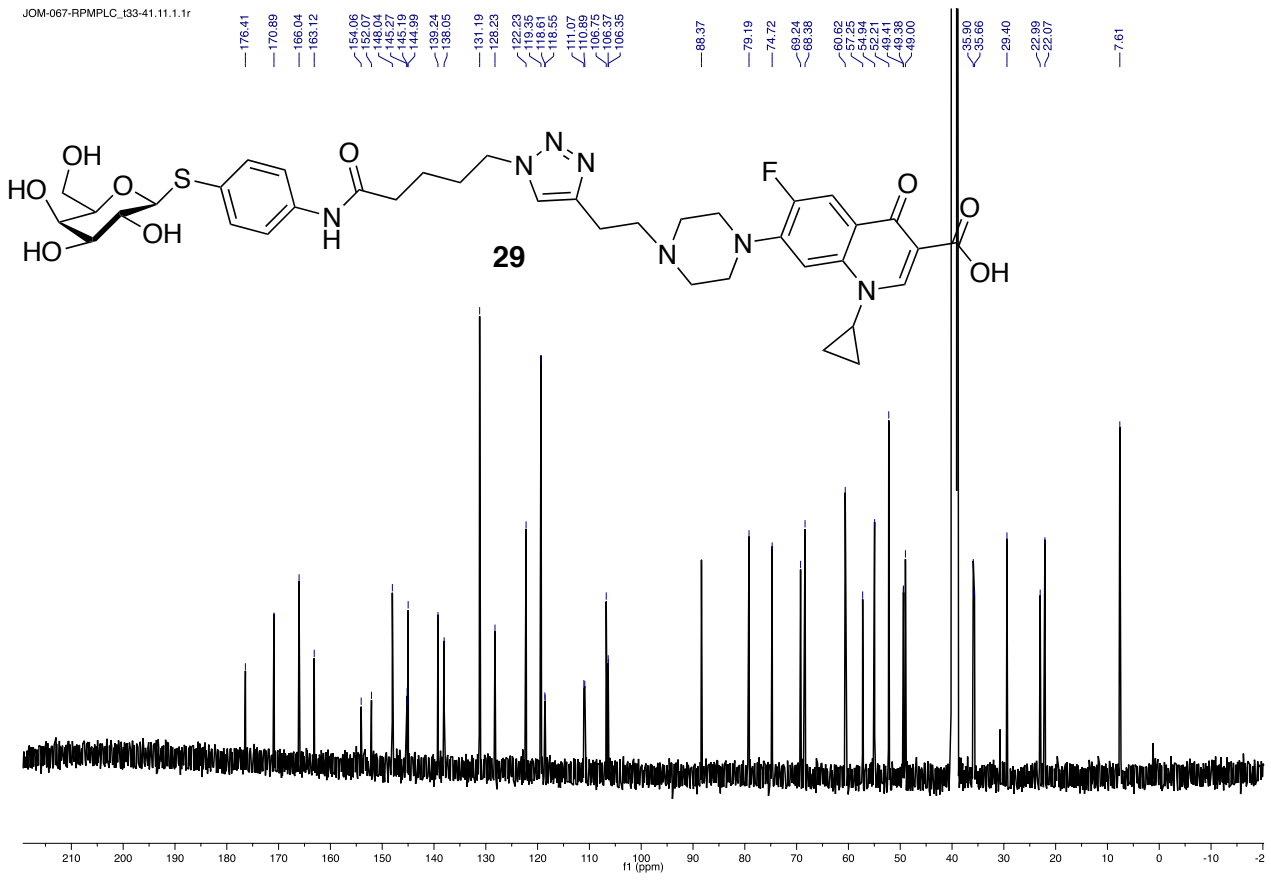
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144.99
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131.19
128.23
122.23
119.35
118.61
118.55
111.07
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106.35
88.37
79.19
74.72
69.24
68.38
60.62
57.25
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22.99
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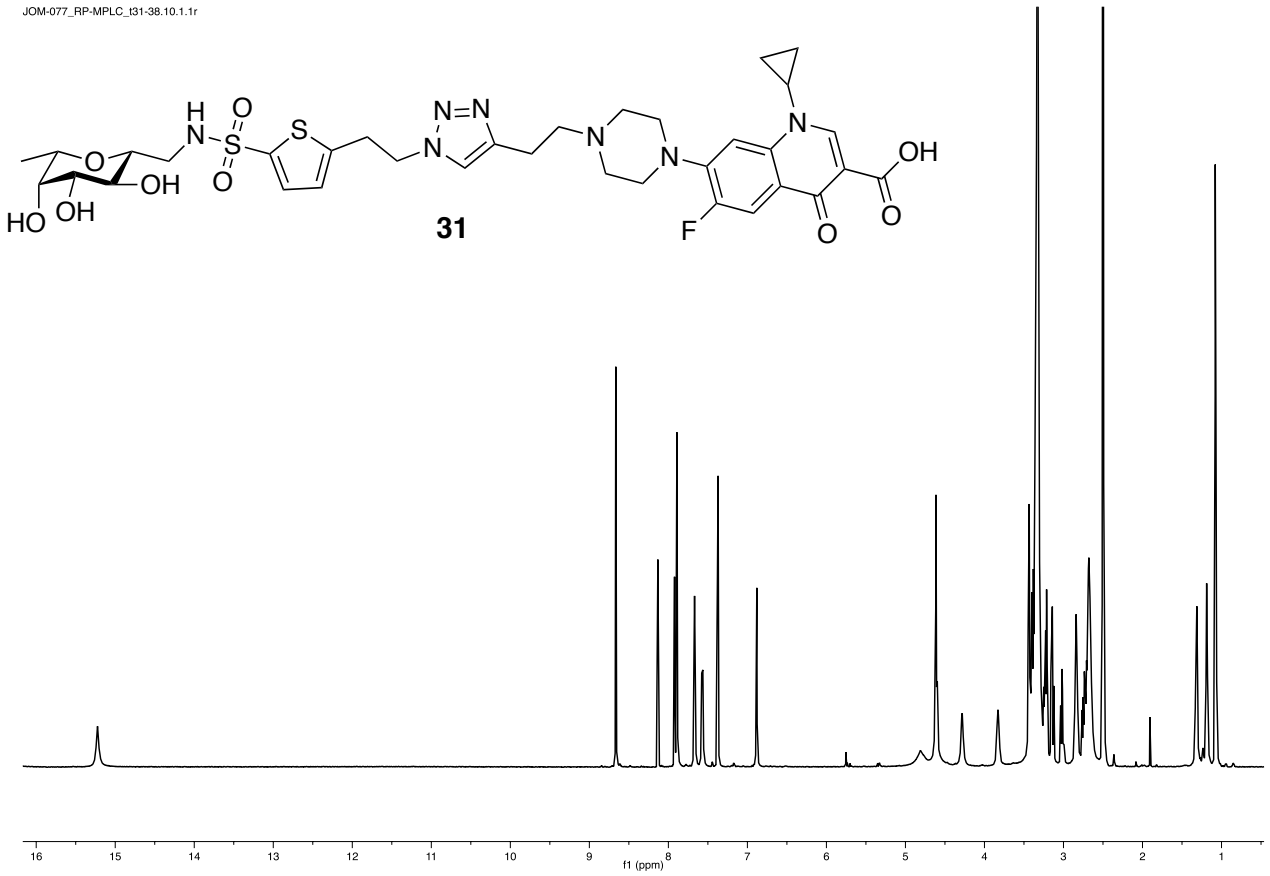
29
f1 (ppm)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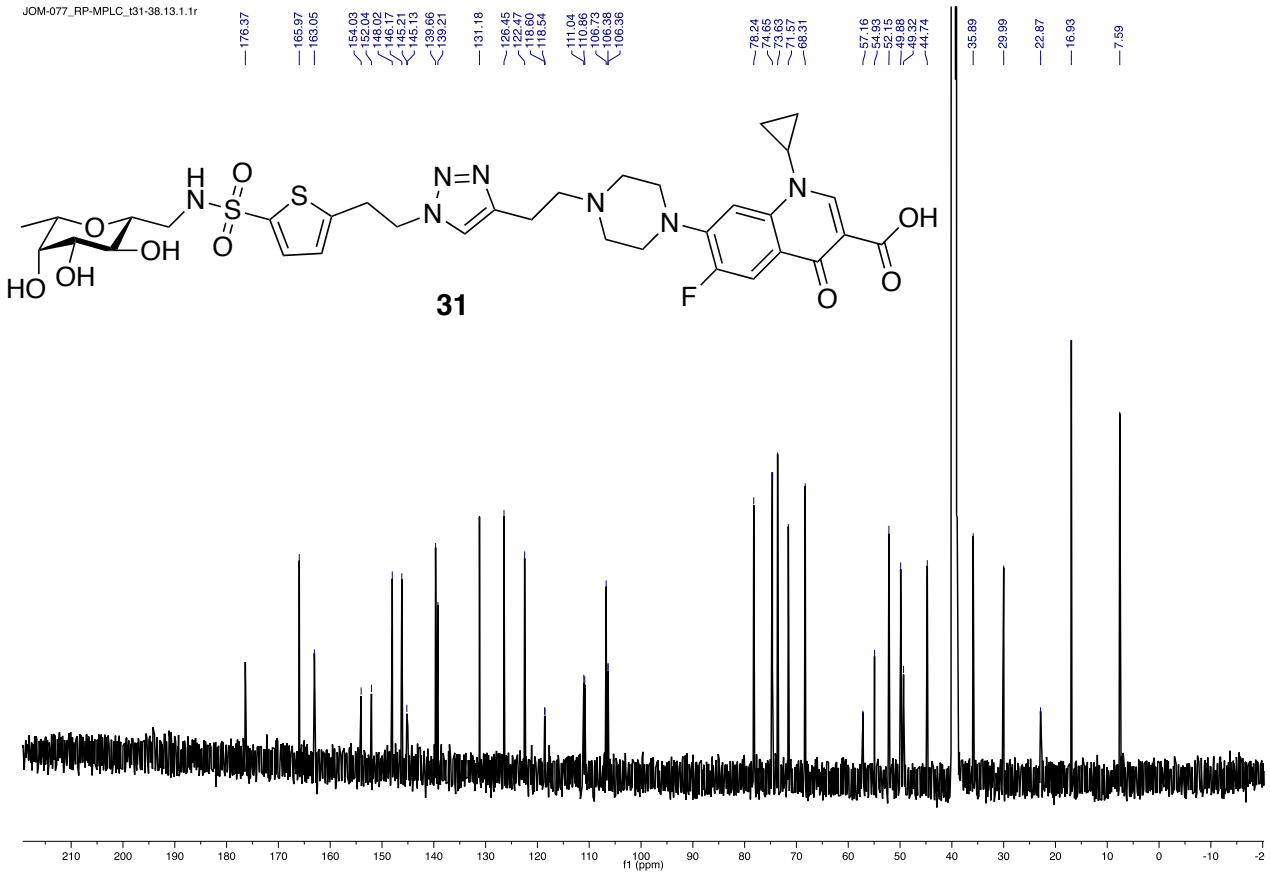
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139.21
131.18
126.45
122.47
118.60
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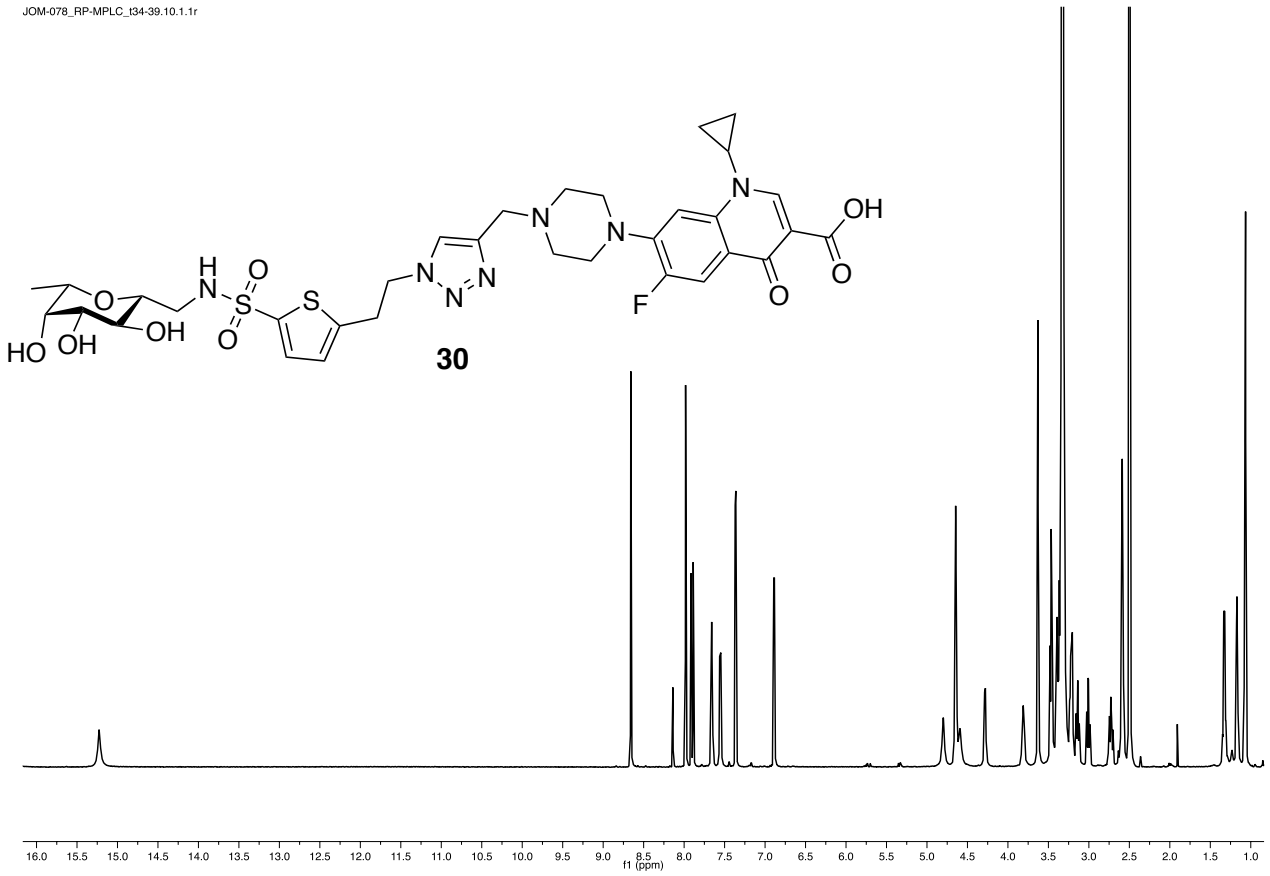
35.89
29.99
22.87
16.93
7.59
31
f1 (ppm)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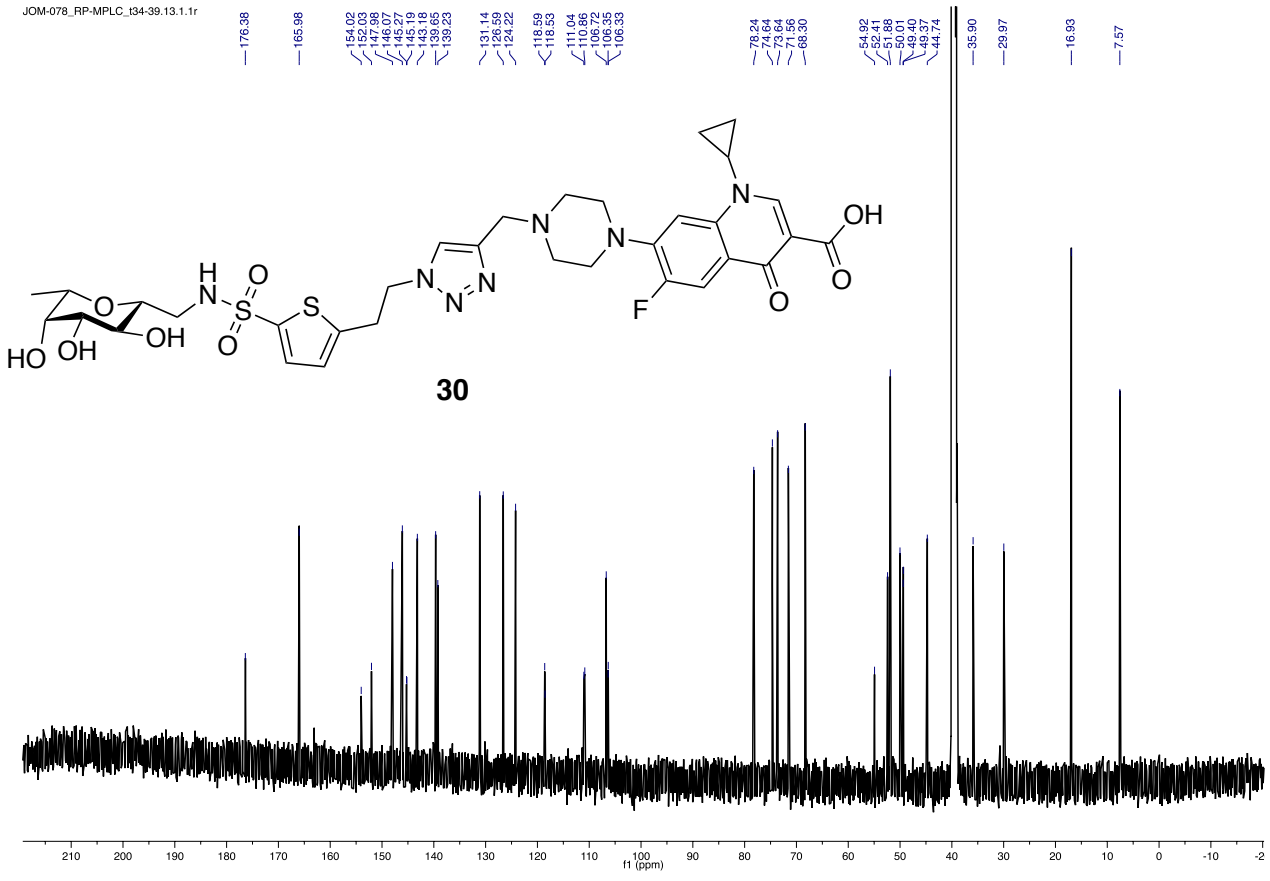
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139.23
131.14
126.59
124.22
118.59
118.53
111.04
110.86
106.72
106.35
106.33
78.24
74.64
73.64
71.56
68.30
54.92
52.41
51.88
50.01
49.40
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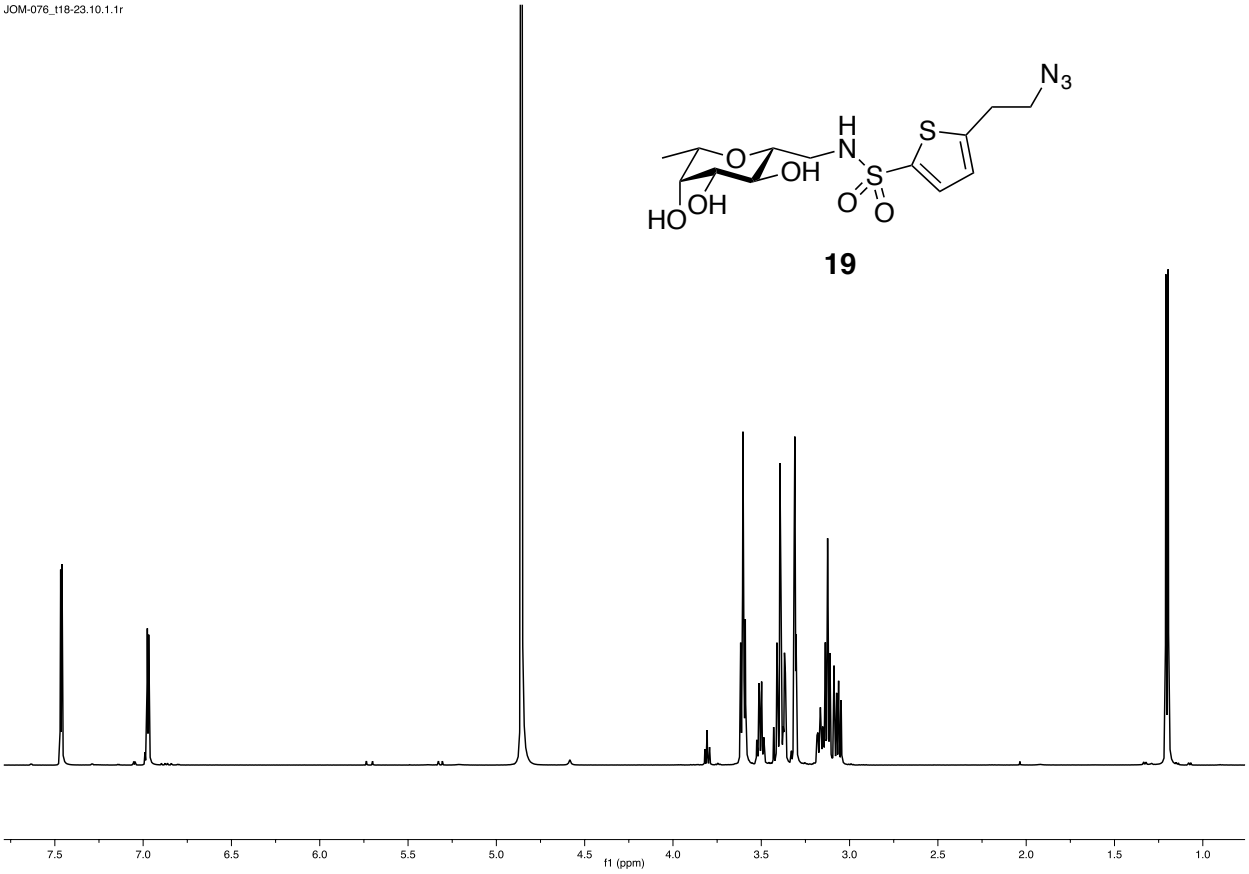
16.93
7.57
30
f1 (ppm)

JOM-076_t18-23.10.1.1r

19



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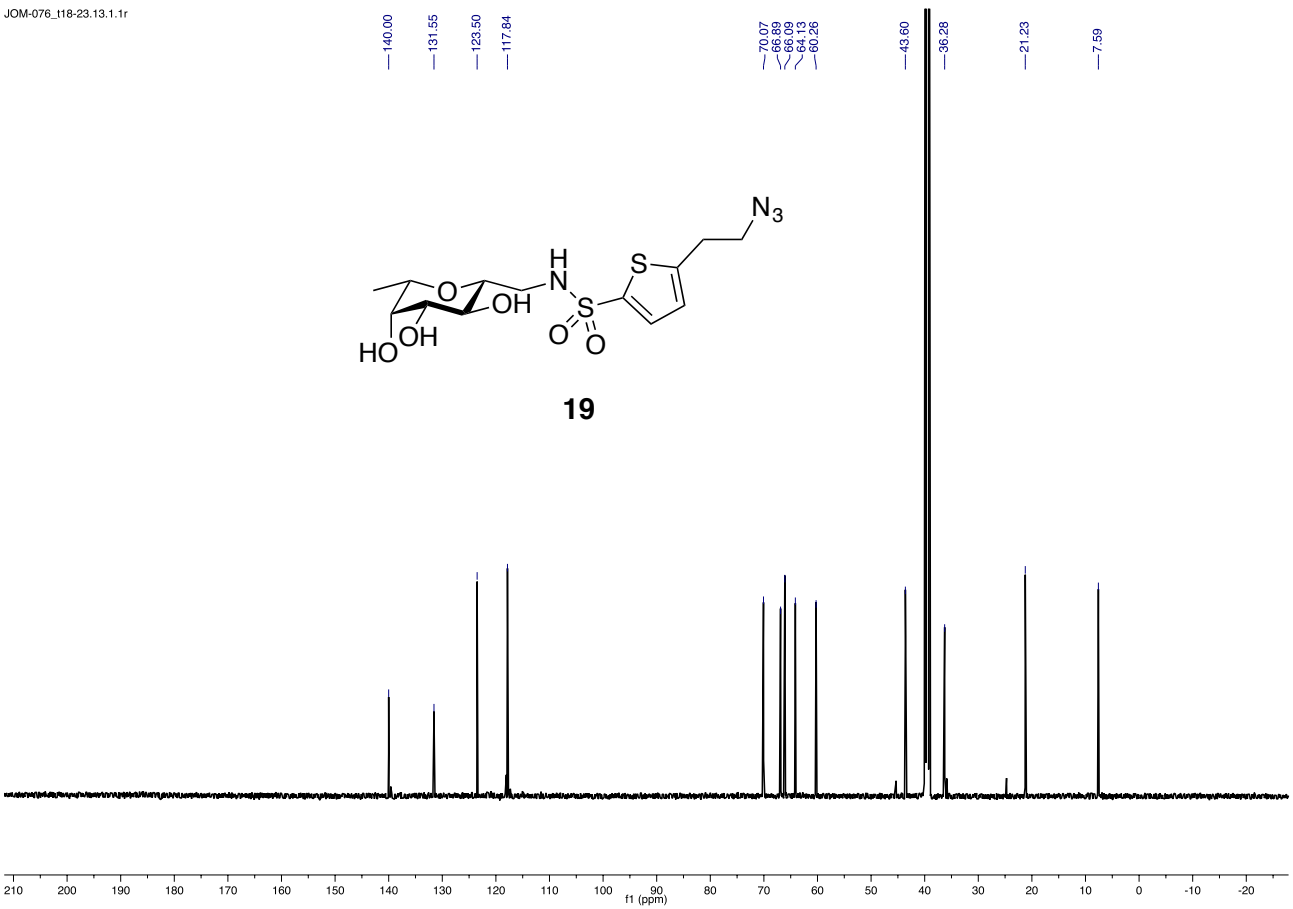

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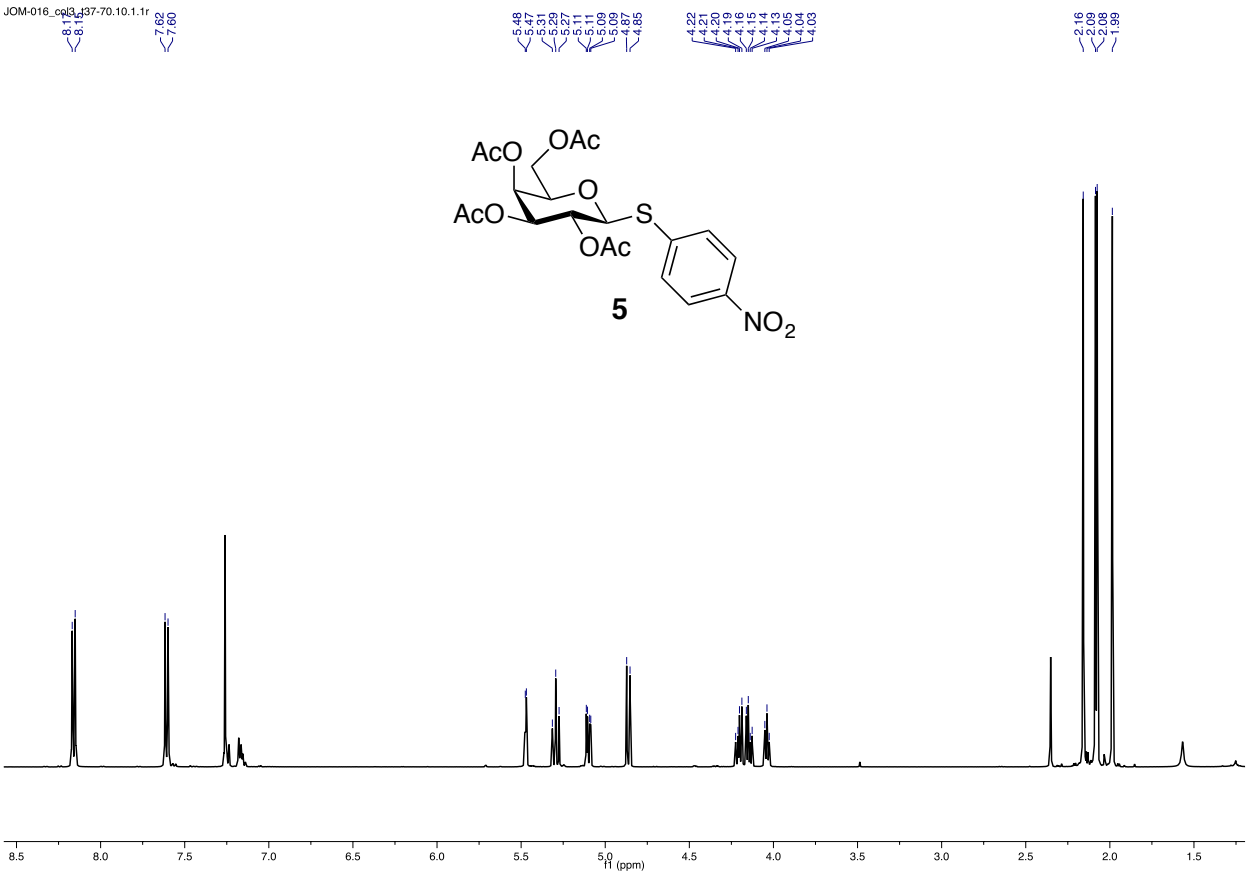
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8.15
7.62
7.60
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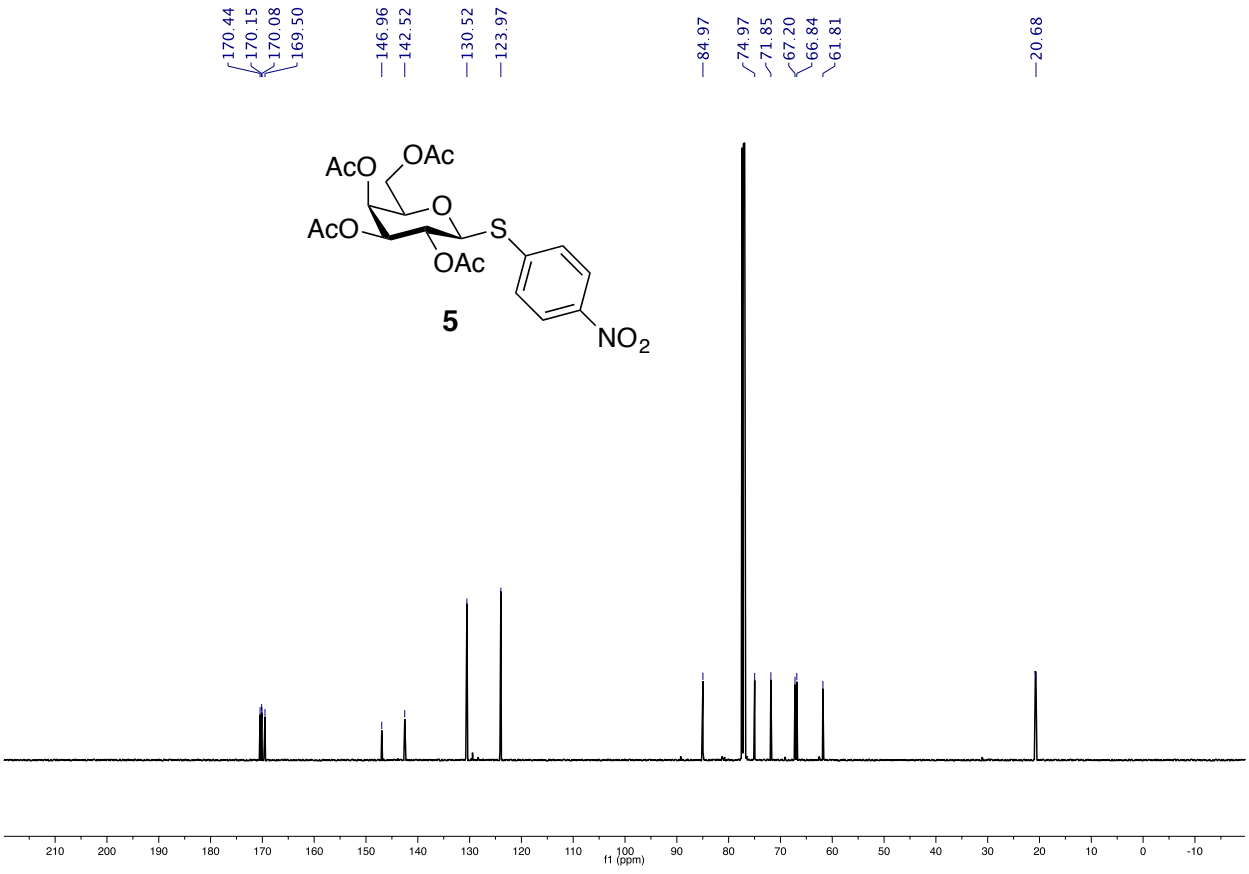
5.47
5.31
5.29
5.27
5.11
5.11
5.09
5.09
4.87
4.85
4.22
4.21
4.20
4.19
4.16
4.15
4.14
4.13
4.05
4.04
4.03
2.16
2.09
2.08
1.99
AcO
OAc
O
AcO
S
OAc
5
NO2
f1 (ppm)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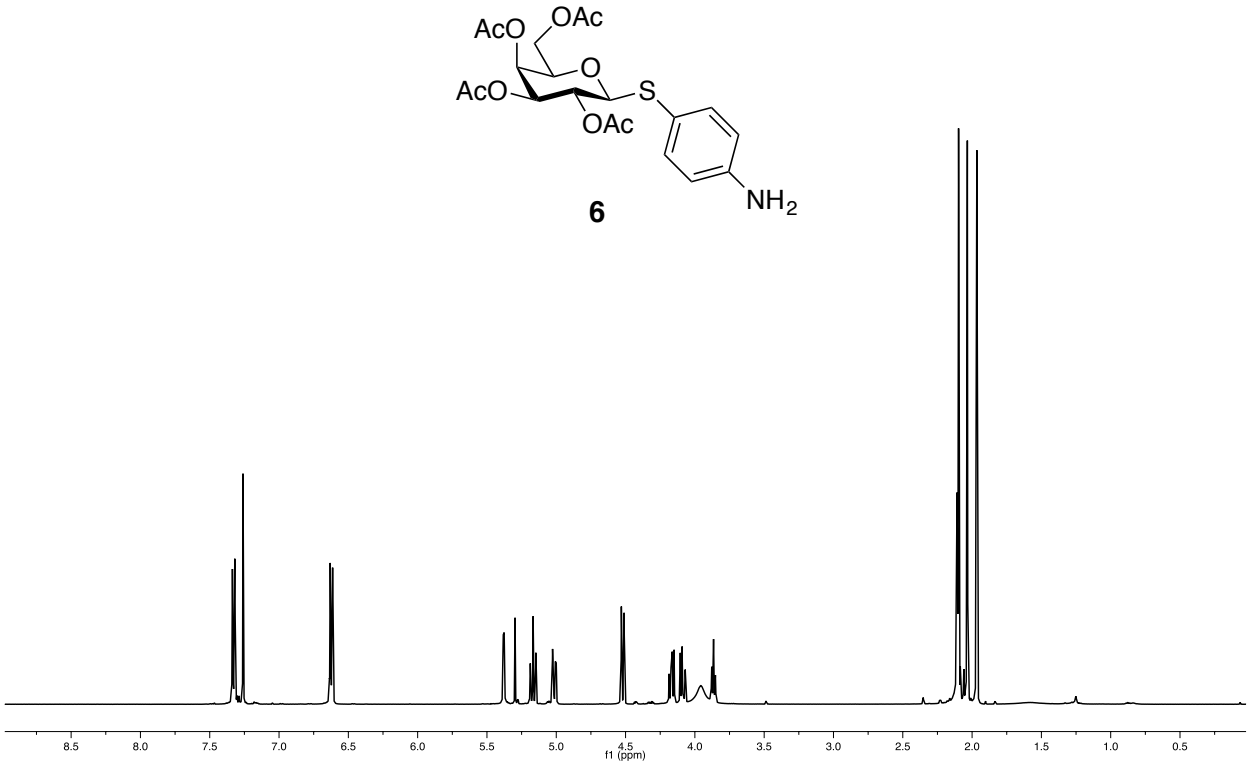
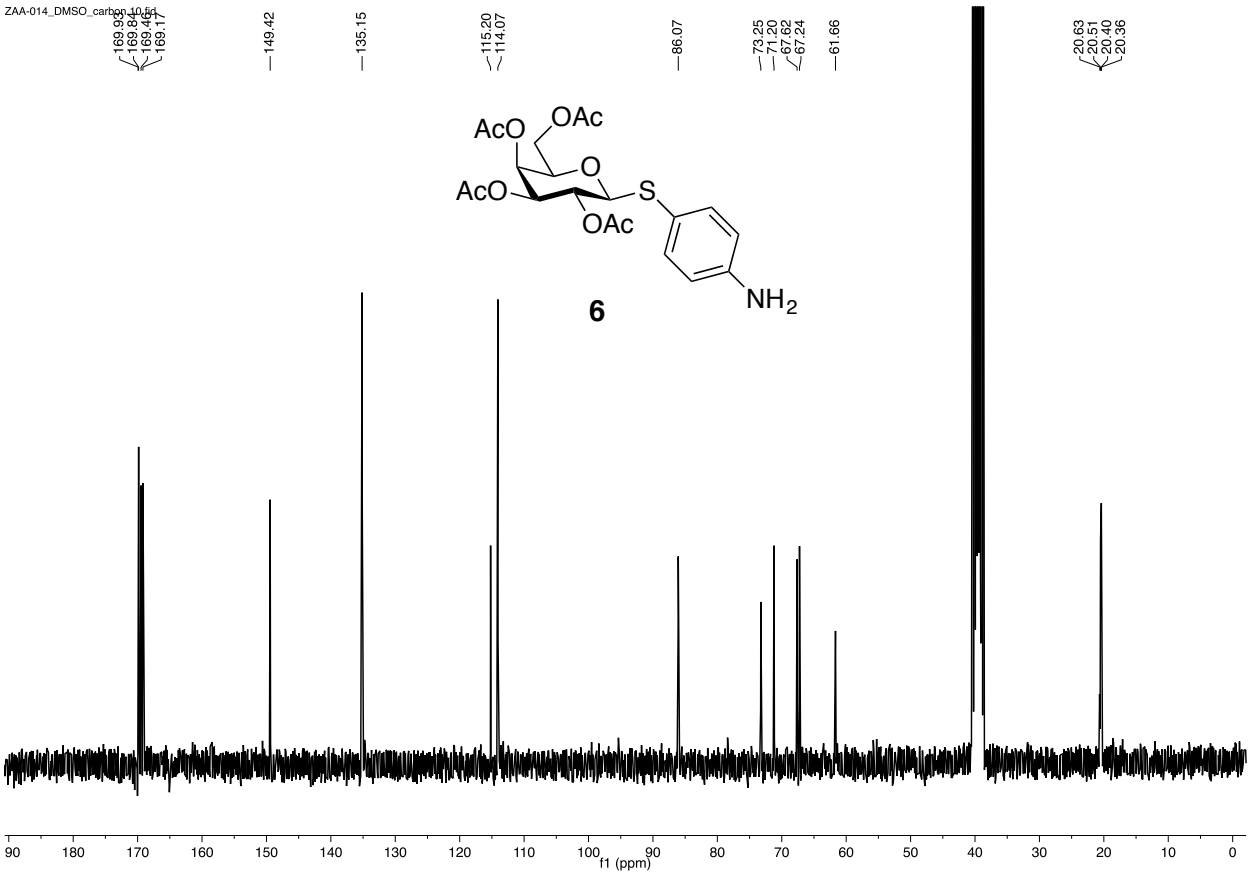
170.08
169.50
146.96
142.52
130.52
123.97
84.97
74.97
71.85
67.20
66.84
61.81
20.68
AcO
OAc
O
AcO
S
OAc
5
NO2
f1 (ppm)

JOM-019.10.1.1r



ZAA-014_DMSO_carbon_10.fid



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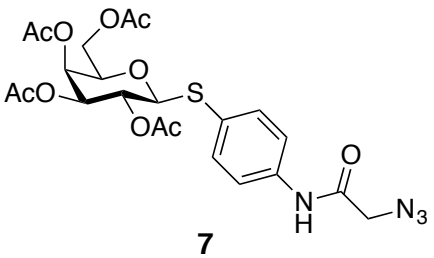

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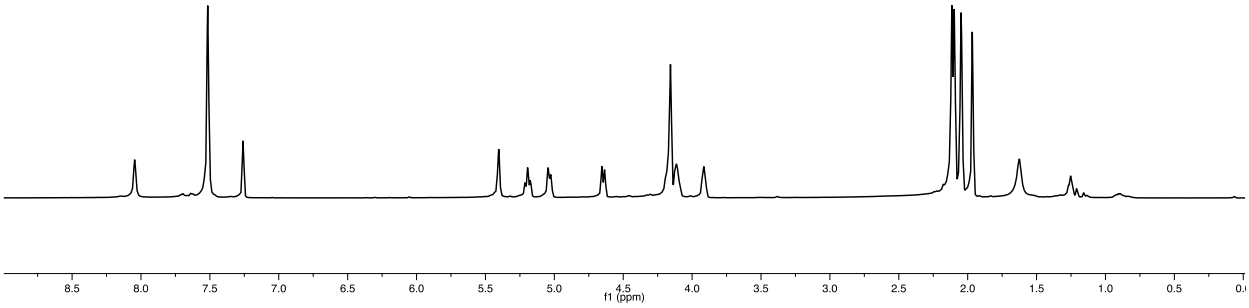

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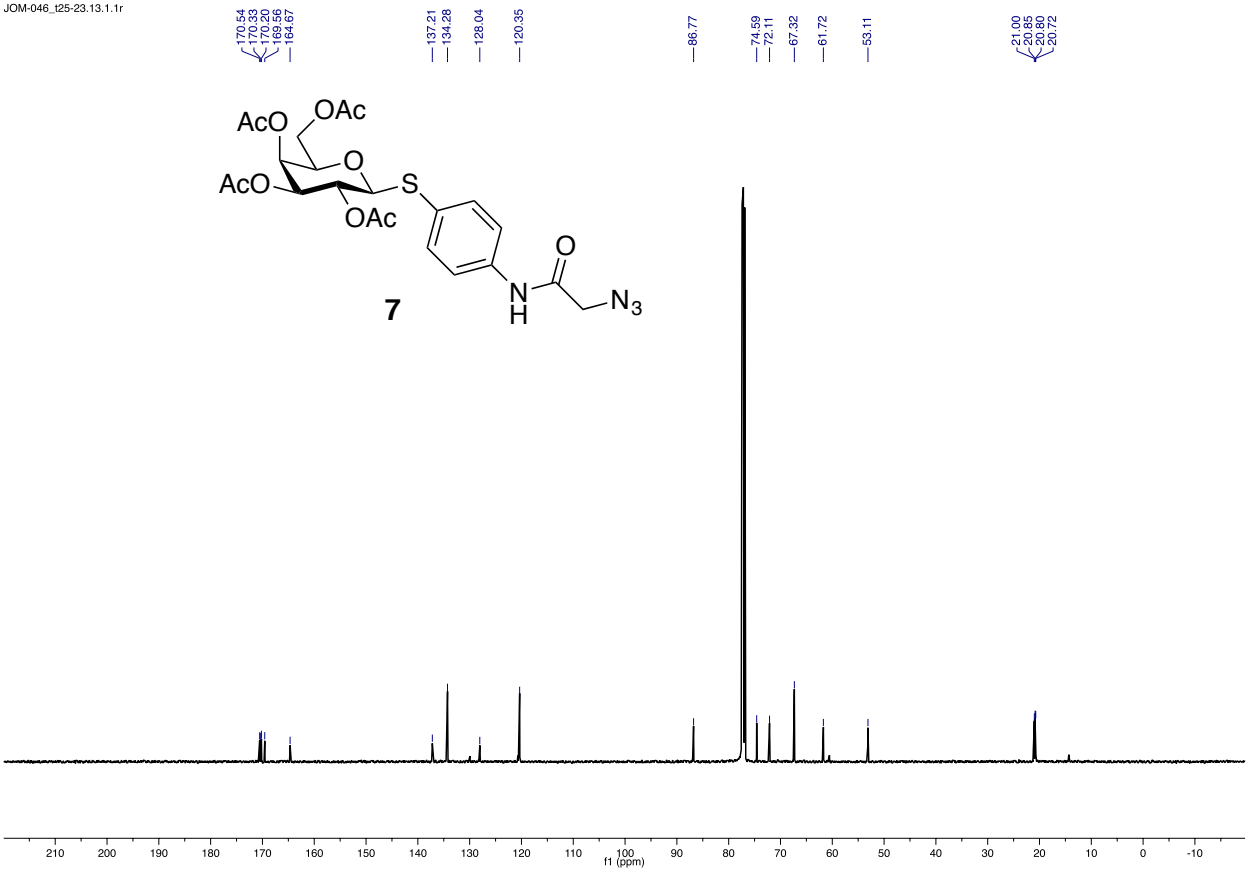

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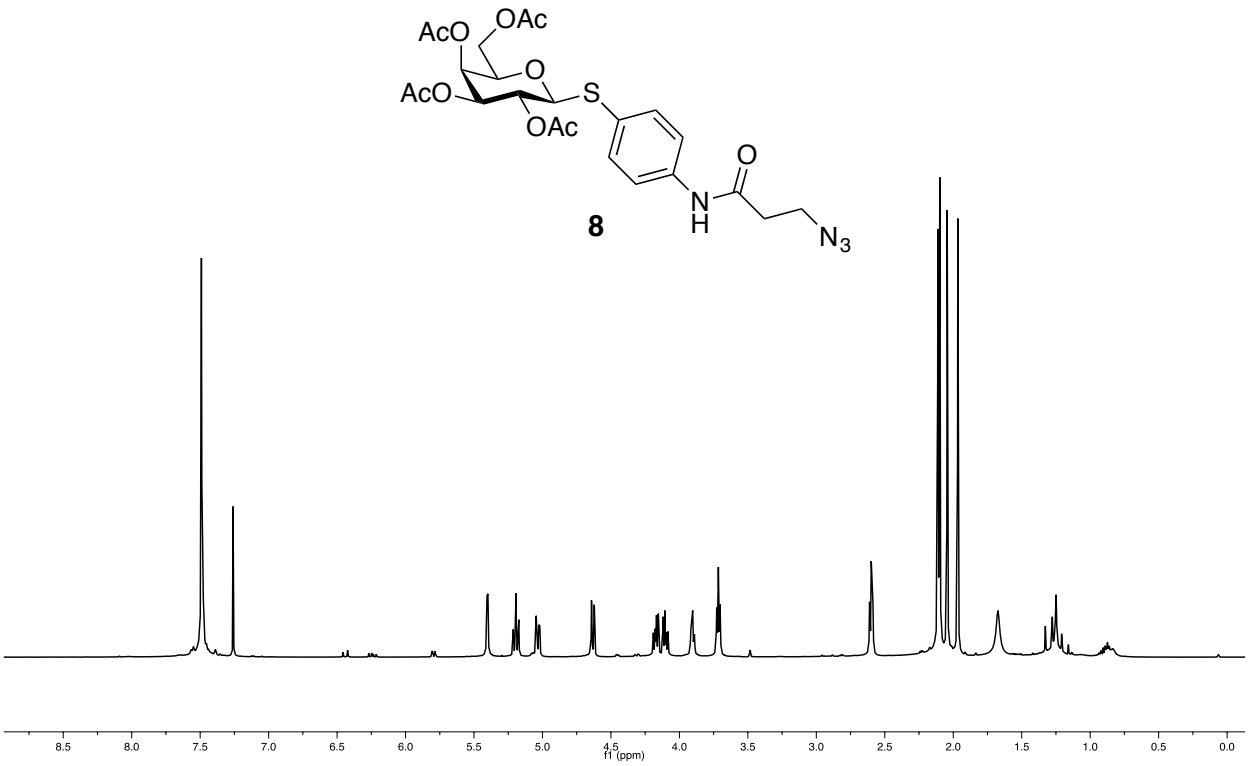

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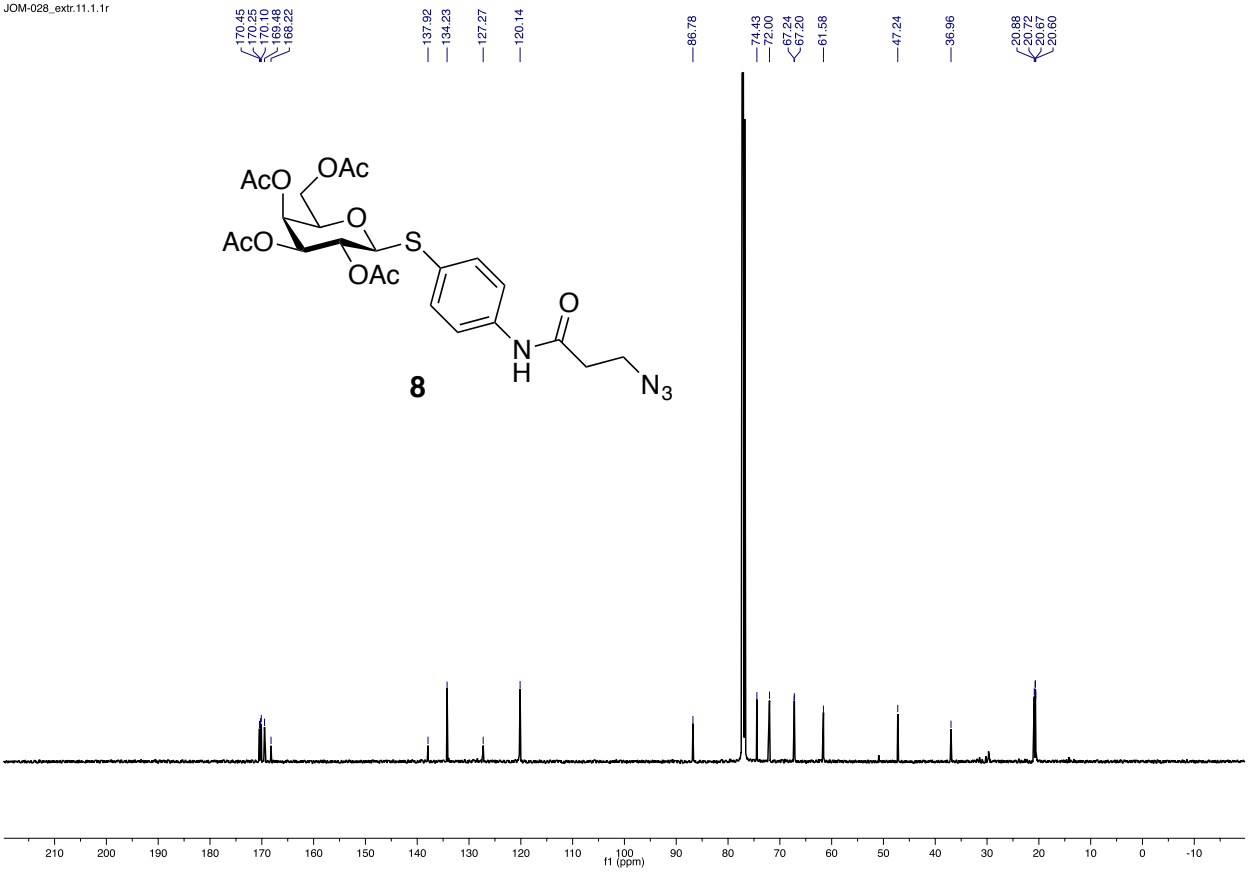
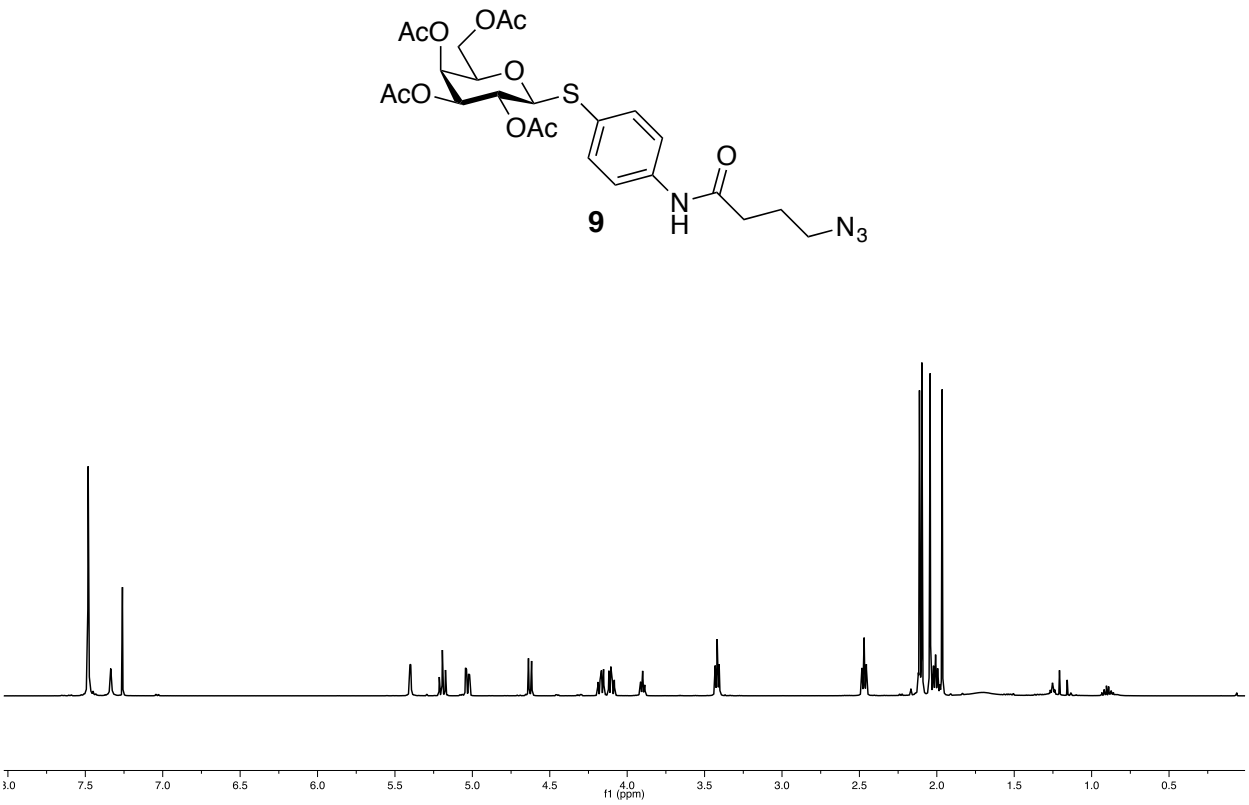


JOM-059_t20-26.10.1.1r



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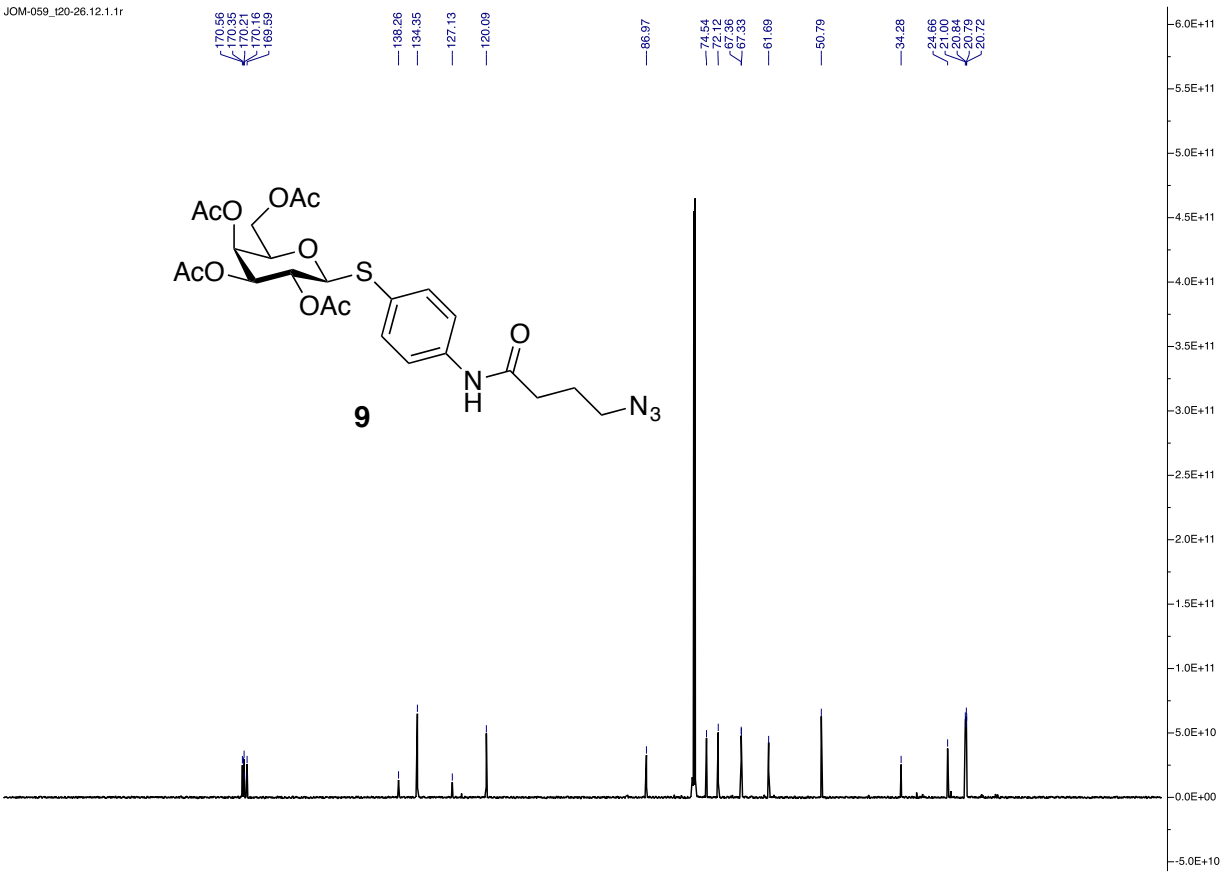

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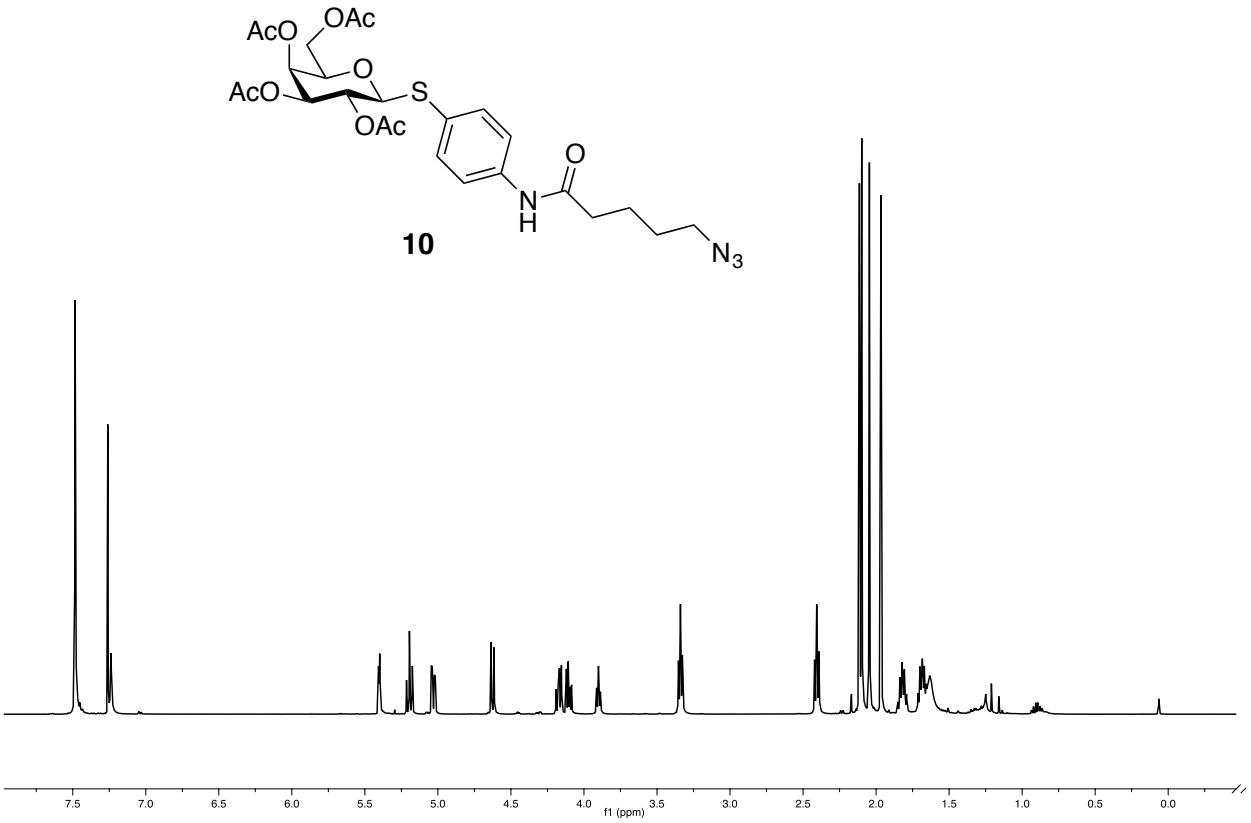

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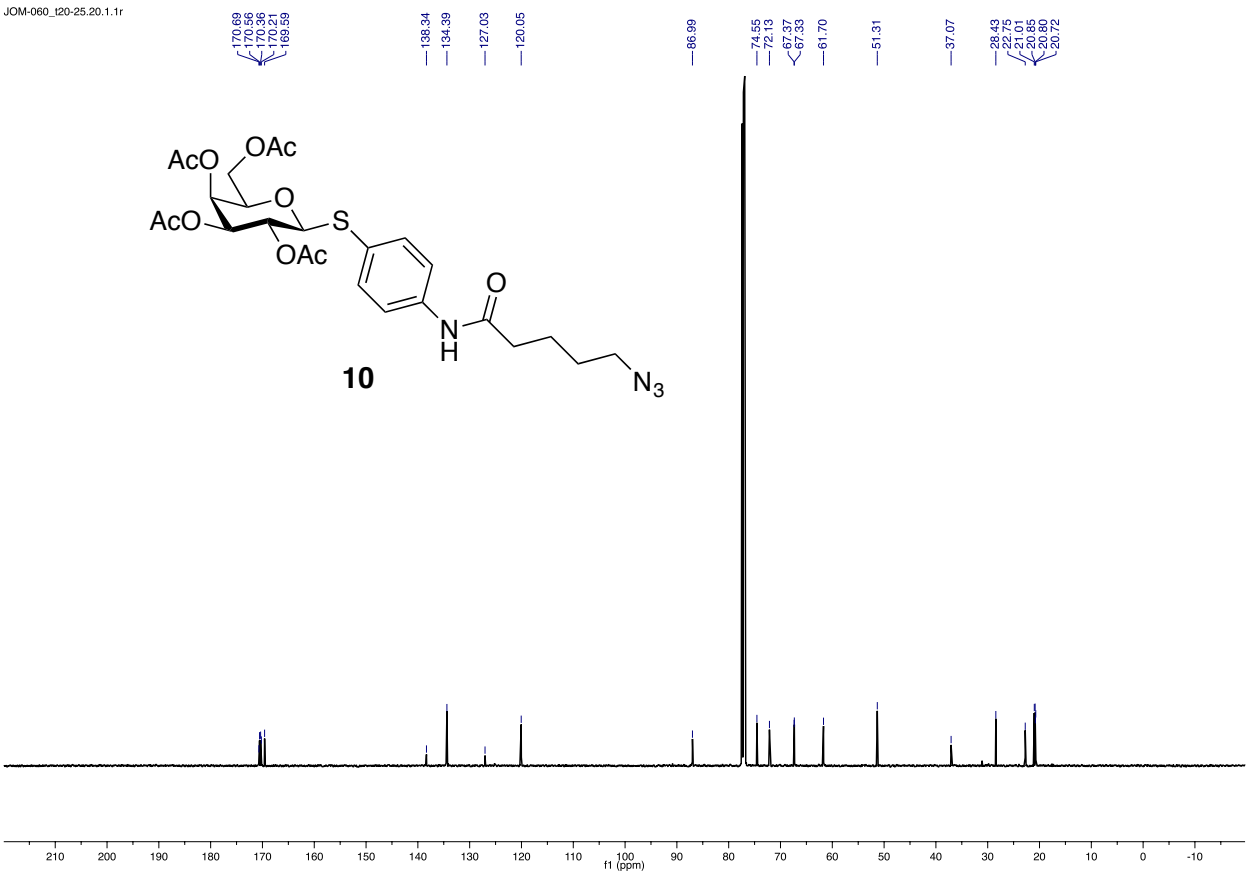
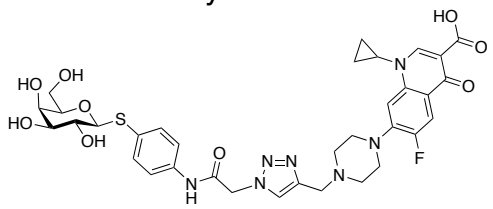


Figure S1: representative Gels for each compound in gyrase-catalyzed DNA supercoiling inhibition assays.



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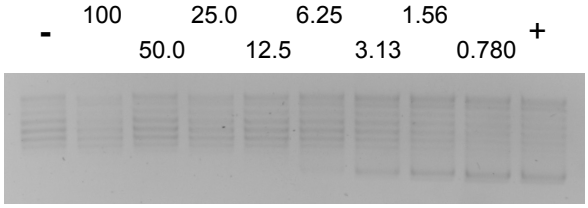
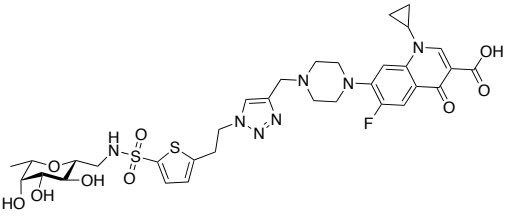


[µM]



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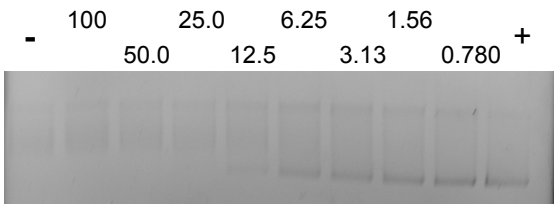

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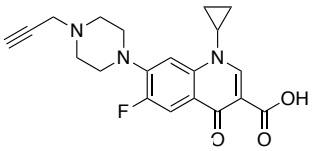

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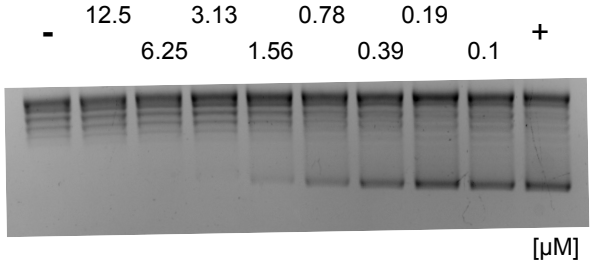

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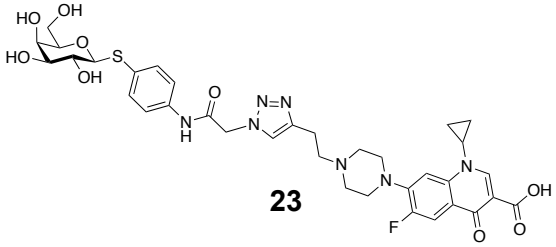

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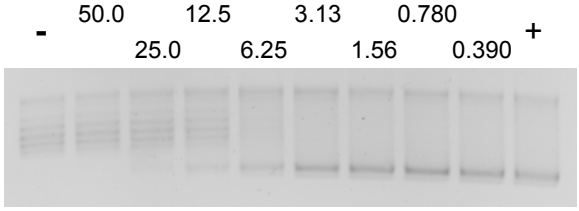

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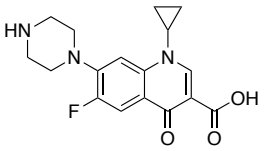

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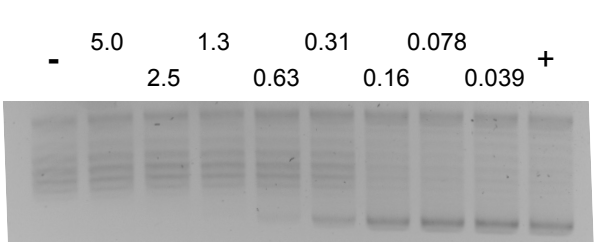
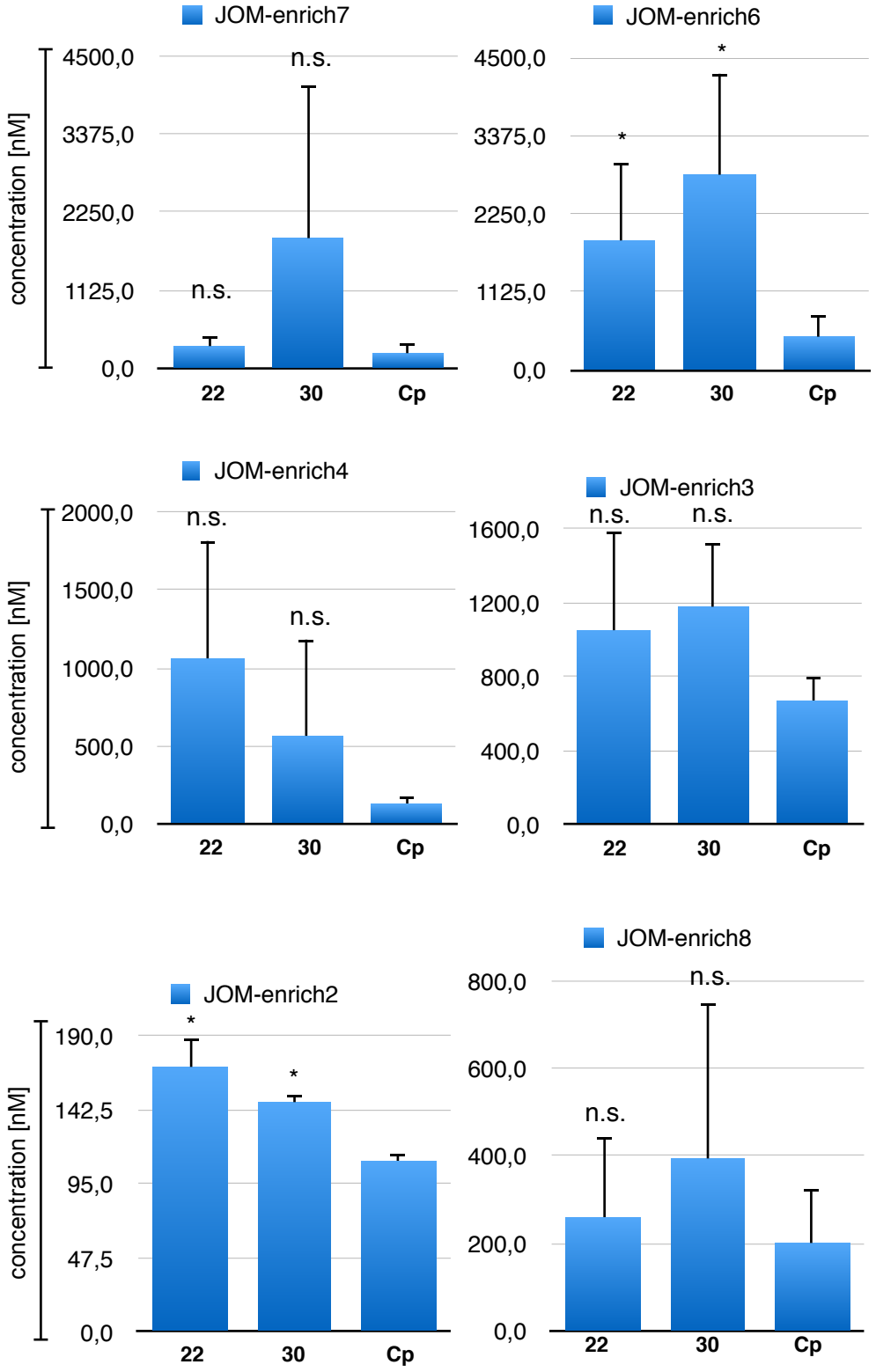


[µM]

Figure S2: *P. aeruginosa* PAO1 biofilm accumulation raw data.



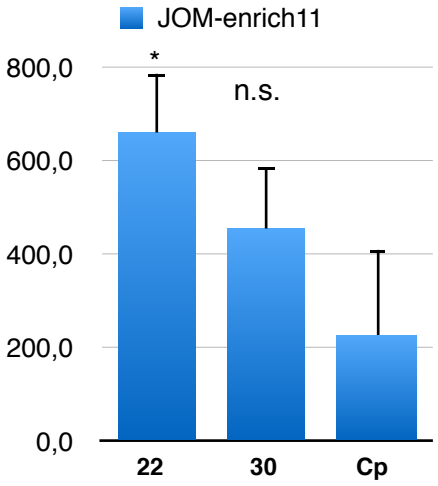


Table S1: Lectin inhibition, calculated as K_i [μM] from IC_{50} according to Huang et al. [1] . N.a. = not applicable. Data is comparable to experimental K_i-values, determined by ITC. [2]

LecA				
compound	**n**	**m**	**K_i ± s.d. [μM]**	**IC50 ± s.d. [μM]**
11	1	LecA-probes	4.16 ± 2.99	31.7 ± 11
12	2		3.96 ± 2.33	30.9 ± 8.7
13	3		4.01 ± 2.22	31.1 ± 8.3
14	4		3.67 ± 2.54	29.9 ± 9.5
22	1	0	3.82 ± 2.13	30.4 ± 8.0
23	1	1	1.48 ± 1.47	21.6 ± 5.5
24	2	0	4.29 ± 0.87	32.2 ± 3.3
25	2	1	3.19 ± 0.48	28.0 ± 1.8
26	3	0	3.00 ± 1.08	27.3 ± 4.0
27	3	1	3.53 ± 0.99	29.3 ± 3.7
28	4	0	3.27 ± 2.17	28.3 ± 8.1
29	4	1	2.70 ± 0.64	26.2 ± 2.4
Me-α-D-Gal			9.76 ± 3.53	71.7 ± 16
pNP-β-D-Gal		controls	14.8 ± 4.25	52.7 ± 13

$LecB_{PAO1}$			
compound	**m**	**K_i ± s.d. [μM]**	**IC50 ± s.d. [μM]**
19	LecB-probe	1.52 ± 0.72	3.91 ± 1.6
30	0	0.82 ± 0.55	2.37 ± 1.2
31	1	0.89 ± 0.39	2.53 ± 0.87
Me-α-D-Man		75.11 ± 9.79	166 ± 22
L-Fuc	controls	0.93 ± 0.78	2.63 ± 1.7
Me-α-L-Fuc		n.a.	0.534 ± 0.07
$LecB_{PA14}$			
19	LecB-probe	0.36 ± 0.04	1.87 ± 0.21
30	0	0.18 ± 0.01	2.24 ± 0.23
31	1	0.44 ± 0.05	1.00 ± 0.06
Me-α-D-Man		21.21 ± 2.08	101 ± 10
L-Fuc	controls	0.49 ± 0.07	2.46 ± 0.33
Me-α-L-Fuc		0.14 ± 0.02	0.79 ± 0.11

Table S2: Antibiotic susceptibility assay data in molar concentration [μM].

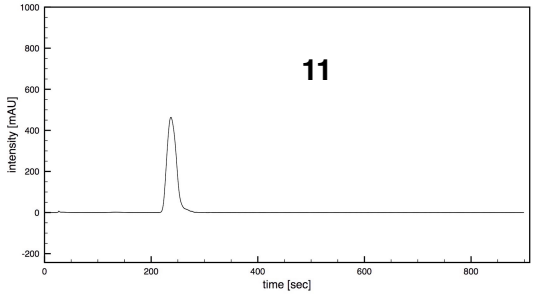
compound	target: LecA							target: LecB		references	
	22	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**20**	**3**
molecular mass [g/mol]	739.8	753.8	753.8	767.8	767.8	781.9	781.9	795.9	761.8	775.9	369.4	331.3
linker length n/m	1/0	1/1	2/0	2/1	3/0	3/1	4/0	4/1	-/0	-/1	0	-
test organism	**MIC [μM]**											
E. coli K12 MG1655	2.7	11 - 21	2.7	21	1.3 - 2.6	20	2.6 - 5.11	20	11 - 21	21	n.d.	< 0.37
E. coli DSM 1116	2.7 - 5.4	22	2.7 - 5.3	42	2.6 - 42	5.1 - 41	5.11 - 41	5 - 40	21 - 42	41	n.d.	< 0.37
S. carnosus [a]	43	85	42	> 83	21	82	10	≥ 80	> 84	> 82	n.d.	< 0.37
P. aeruginosa PA14 wt	22	≥ 85	11 - 21	> 83	10 - 21	> 82	41	> 80	84	> 82	5.4 - 10.8	0.075 - 0.3
P. aeruginosa PA14 wt + 1 μg/mL PMBN	5.4 - 22	21 - 85	11 - 21	42 - 83	5.2	41 - 82	2.6 - 10	40 - 80	84	82	0.068 - 1.4	0.075
P. aeruginosa PA14 *ΔlecA*	22 - 44	≥ 85	11 - 21	> 83	10 - 21	> 82	41	> 80	≥ 84	> 82	11 - 22	0.15-0.24
P. aeruginosa PA14 *ΔlecB*	22 - 44	≥ 85	11 - 42	> 83	10 - 21	> 82	41 - 82	> 80	84	> 82	11	0.15-0.24
P. aeruginosa PAO1 wt	22 - 44	> 85	21	> 83	21 - 42	> 82	41 - 82	> 80	≥ 84	> 82	11 - 22	0.075 - 0.24
P. aeruginosa PAO1 wt + 1 μg/mLPMBN	5.4 - 11	42 - 85	5.3 - 11	42 - 83	5.2 - 10	41 - 82	10 - 20	40 - 80	42 - 84	≥ 82	2.7 - 5.4	0.075-0.15

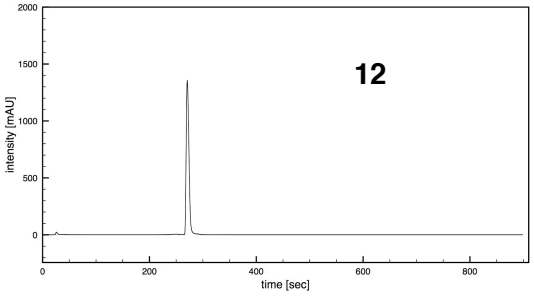
n.d. = not determined, [a] DSM 20501

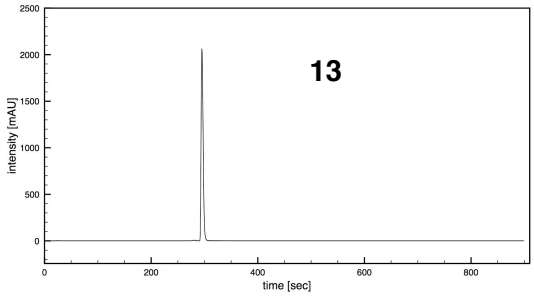
compound	SMILE	IC_{50} LecA ± s.d. [μM]	IC_{50} $LecB_{PAO1}$ ± s.d. [μM]	IC_{50} $LecB_{PA14}$ ± s.d. [μM]
11	O[C@H]1[C@@H](O)[C@@H](CO)O[C@@H](SC2=CC=C(NC(CN=[N+]=[N-])=O)C=C2)[C@@H]1O	31.7 ± 11	n.a.	n.a.
12	O[C@H]1[C@@H](O)[C@@H](CO)O[C@@H](SC2=CC=C(NC(CCN=[N+]=[N-])=O)C=C2)[C@@H]1O	30.9 ± 8.7	n.a.	n.a.
13	O[C@H]1[C@@H](O)[C@@H](CO)O[C@@H](SC2=CC=C(NC(CCCN=[N+]=[N-])=O)C=C2)[C@@H]1O	31.1 ± 8.3	n.a.	n.a.
14	O[C@H]1[C@@H](O)[C@@H](CO)O[C@@H](SC2=CC=C(NC(CCCCN=[N+]=[N-])=O)C=C2)[C@@H]1O	29.9 ± 9.5	n.a.	n.a.
19	C[C@H]1[C@@H](O)[C@@H](O)[C@H](O)[C@@H](CNS(C2=CC=C(CCN=[N+]=[N-])S2)(=O)=O)O1	n.a.	3.91 ± 1.6	1.87 ± 0.21
20	O=C(O)C1=CN(C2=CC(N3CCN(CC#C)CC3)=C(F)C=C2C1=O)C4CC4	n.a.	n.a.	n.a.
21	O=C(O)C1=CN(C2=CC(N3CCN(CCC#C)CC3)=C(F)C=C2C1=O)C4CC4	n.a.	n.a.	n.a.
22	O=C(O)C1=CN(C2=CC(N3CCN(CC4=CN(CC(NC(C=C5)=CC=C5S[C@H]6[C@H](O)[C@@H](O)[C@@H](O)[C@@H](CO)O6)=O)N=N4)CC3)=C(F)C=C2C1=O)C7CC7	30.4 ± 8.0	n.a.	n.a.
23	O[C@H]1[C@@H](O)[C@@H](CO)O[C@@H](SC2=CC=C(NC(CN3N=NC(CCN(CC4)CCN4C5=C(F)C=C6C(N(C7CC7)C=C(C6=O)C(O)=O)=C5)=C3)=O)C=C2)[C@@H]1O	21.6 ± 5.5	n.a.	n.a.
24	O=C(O)C1=CN(C2=CC(N3CCN(CC4=CN(CCC(NC(C=C5)=CC=C5S[C@H]6[C@H](O)[C@@H](O)[C@@H](O)[C@@H](CO)O6)=O)N=N4)CC3)=C(F)C=C2C1=O)C7CC7	32.2 ± 3.3	n.a.	n.a.
25	O[C@H]1[C@@H](O)[C@@H](CO)O[C@@H](SC2=CC=C(NC(CCN3N=NC(CCN(CC4)CCN4C5=C(F)C=C6C(N(C7CC7)C=C(C6=O)C(O)=O)=C5)=C3)=O)C=C2)[C@@H]1O	28.0 ± 1.8	n.a.	n.a.
26	O=C(O)C1=CN(C2=CC(N3CCN(CC4=CN(CCCC(NC(C=C5)=CC=C5S[C@H]6[C@H](O)[C@@H](O)[C@@H](O)[C@@H](CO)O6)=O)N=N4)CC3)=C(F)C=C2C1=O)C7CC7	27.3 ± 4.0	n.a.	n.a.
27	O[C@H]1[C@@H](O)[C@@H](CO)O[C@@H](SC2=CC=C(NC(CCCN3N=NC(CCN(CC4)CCN4C5=C(F)C=C6C(N(C7CC7)C=C(C6=O)C(O)=O)=C5)=C3)=O)C=C2)[C@@H]1O	29.3 ± 3.7	n.a.	n.a.
28	O[C@H]1[C@@H](O)[C@@H](CO)O[C@@H](SC2=CC=C(NC(CCCCN3N=NC(CN(CC4)CCN4C5=C(F)C=C6C(N(C7CC7)C=C(C6=O)C(O)=O)=C5)=C3)=O)C=C2)[C@@H]1O	28.3 ± 8.1	n.a.	n.a.
29	O[C@H]1[C@@H](O)[C@@H](CO)O[C@@H](SC2=CC=C(NC(CCCCN3N=NC(CCN(CC4)CCN4C5=C(F)C=C6C(N(C7CC7)C=C(C6=O)C(O)=O)=C5)=C3)=O)C=C2)[C@@H]1O	26.2 ± 2.4	n.a.	n.a.
30	C[C@H]1[C@@H](O)[C@@H](O)[C@H](O)[C@@H](CNS(C2=CC=C(CCN3N=NC(CN(CC4)CCN4C5=C(F)C=C6C(N(C7CC7)C=C(C6=O)C(O)=O)=C5)=C3)S2)(=O)=O)O1	n.a.	2.37 ± 1.2	2.24 ± 0.23
31	C[C@H]1[C@@H](O)[C@@H](O)[C@H](O)[C@@H](CNS(C2=CC=C(CCN3N=NC(CCN(CC4)CCN4C5=C(F)C=C6C(N(C7CC7)C=C(C6=O)C(O)=O)=C5)=C3)S2)(=O)=O)O1	n.a.	2.53 ± 0.87	1.00 ± 0.06
5	O=C(O[C@H]1[C@@H](OC(C)=O)[C@@H](COC(C)=O)O[C@@H](SC2=CC=C([N+]([O-])=O)C=C2)[C@@H]1OC(C)=O)C	n.a.	n.a.	n.a.
6	NC(C=C1)=CC=C1S[C@H]2[C@H](OC(C)=O)[C@@H](OC(C)=O)[C@@H](OC(C)=O)[C@@H](COC(C)=O)O2	n.a.	n.a.	n.a.
7	O=C(CN=[N+]=[N-])NC(C=C1)=CC=C1S[C@H]2[C@H](OC(C)=O)[C@@H](OC(C)=O)[C@@H](OC(C)=O)[C@@H](COC(C)=O)O2	n.a.	n.a.	n.a.
8	O=C(CCN=[N+]=[N-])NC(C=C1)=CC=C1S[C@H]2[C@H](OC(C)=O)[C@@H](OC(C)=O)[C@@H](OC(C)=O)[C@@H](COC(C)=O)O2	n.a.	n.a.	n.a.
9	O=C(CCCN=[N+]=[N-])NC(C=C1)=CC=C1S[C@H]2[C@H](OC(C)=O)[C@@H](OC(C)=O)[C@@H](OC(C)=O)[C@@H](COC(C)=O)O2	n.a.	n.a.	n.a.
10	O=C(CCCCN=[N+]=[N-])NC(C=C1)=CC=C1S[C@H]2[C@H](OC(C)=O)[C@@H](OC(C)=O)[C@@H](OC(C)=O)[C@@H](COC(C)=O)O2	n.a.	n.a.	n.a.
16	BrCCC1=CC=C(S(=O)(Cl)=O)S1	n.a.	n.a.	n.a.

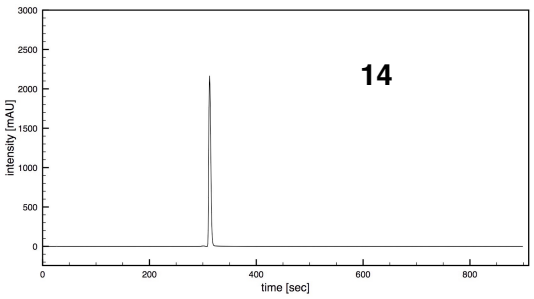
Table S3**:** key compounds and intermediates as smiles (n.a. = not applicable).

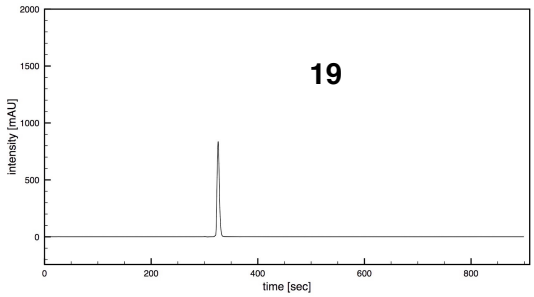
Figure S3**:** Purity of key compounds by HPLC-UV.

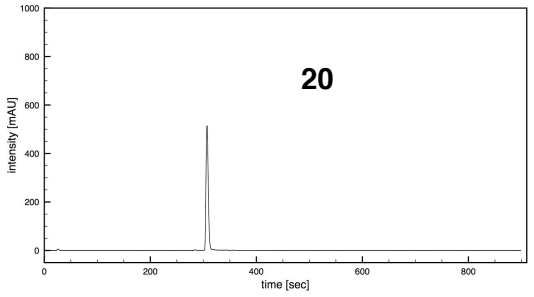


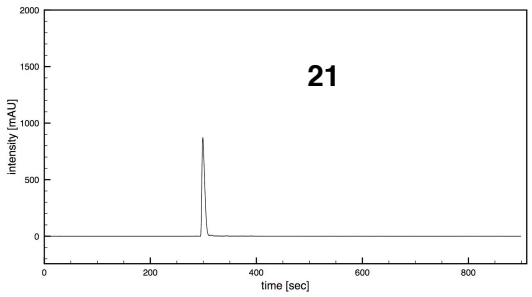


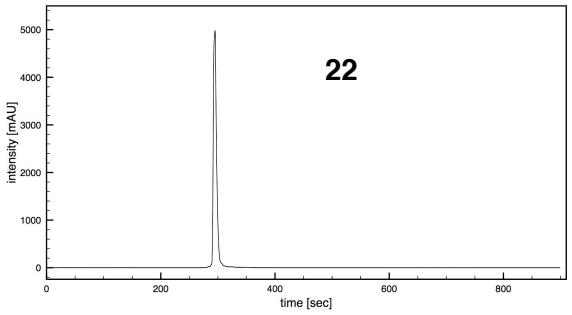


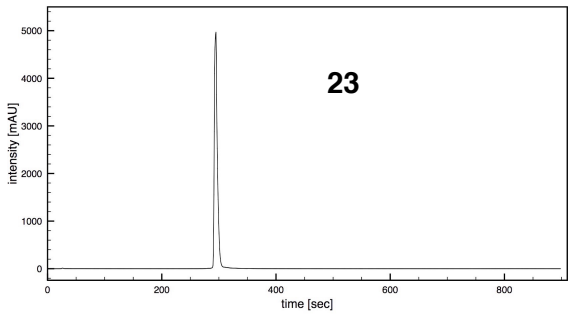


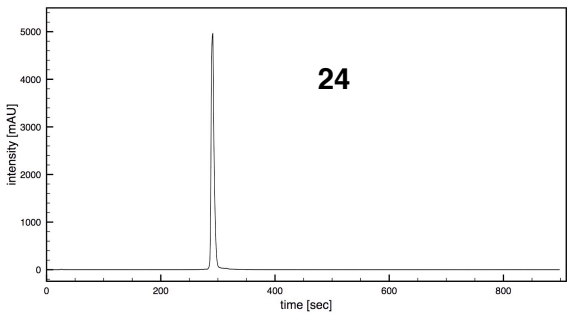


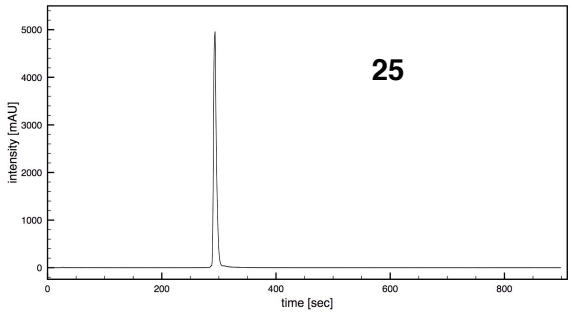








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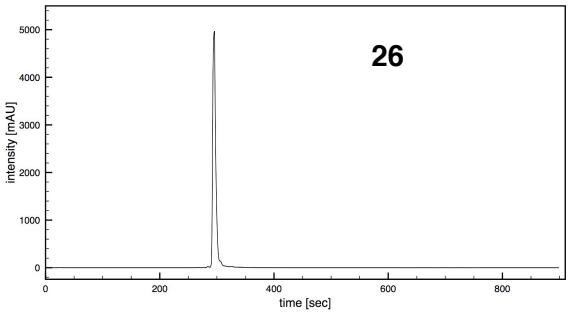
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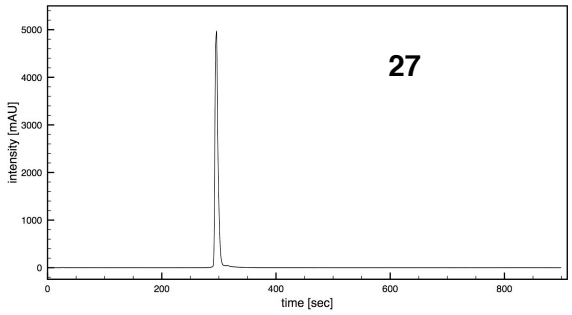
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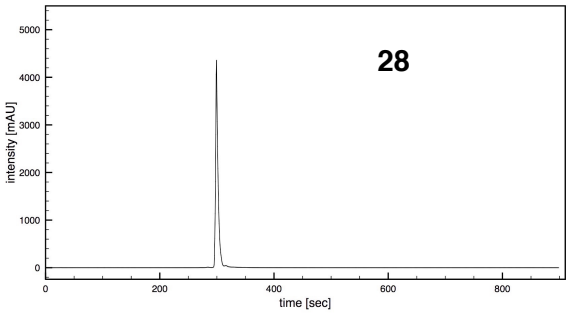
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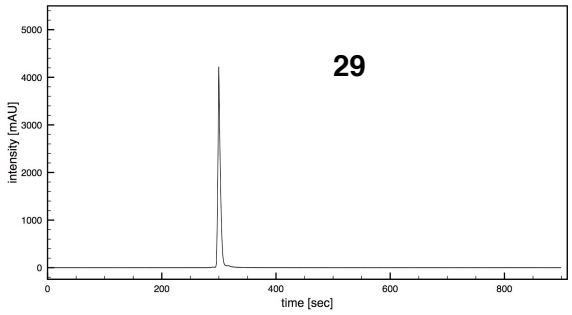
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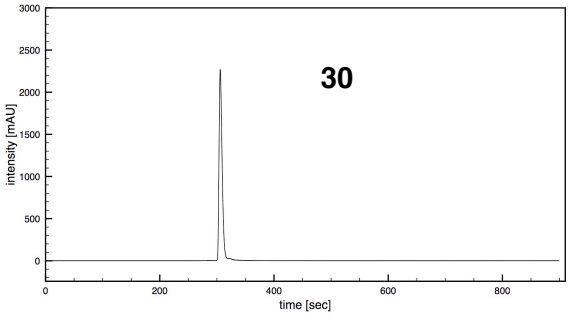
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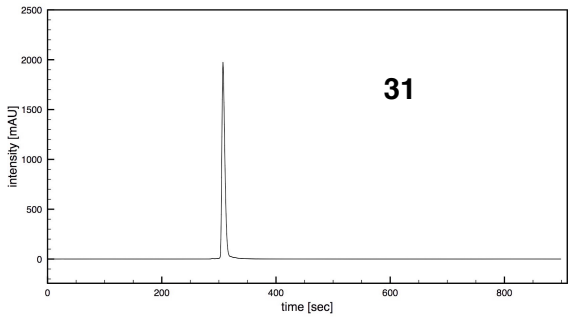
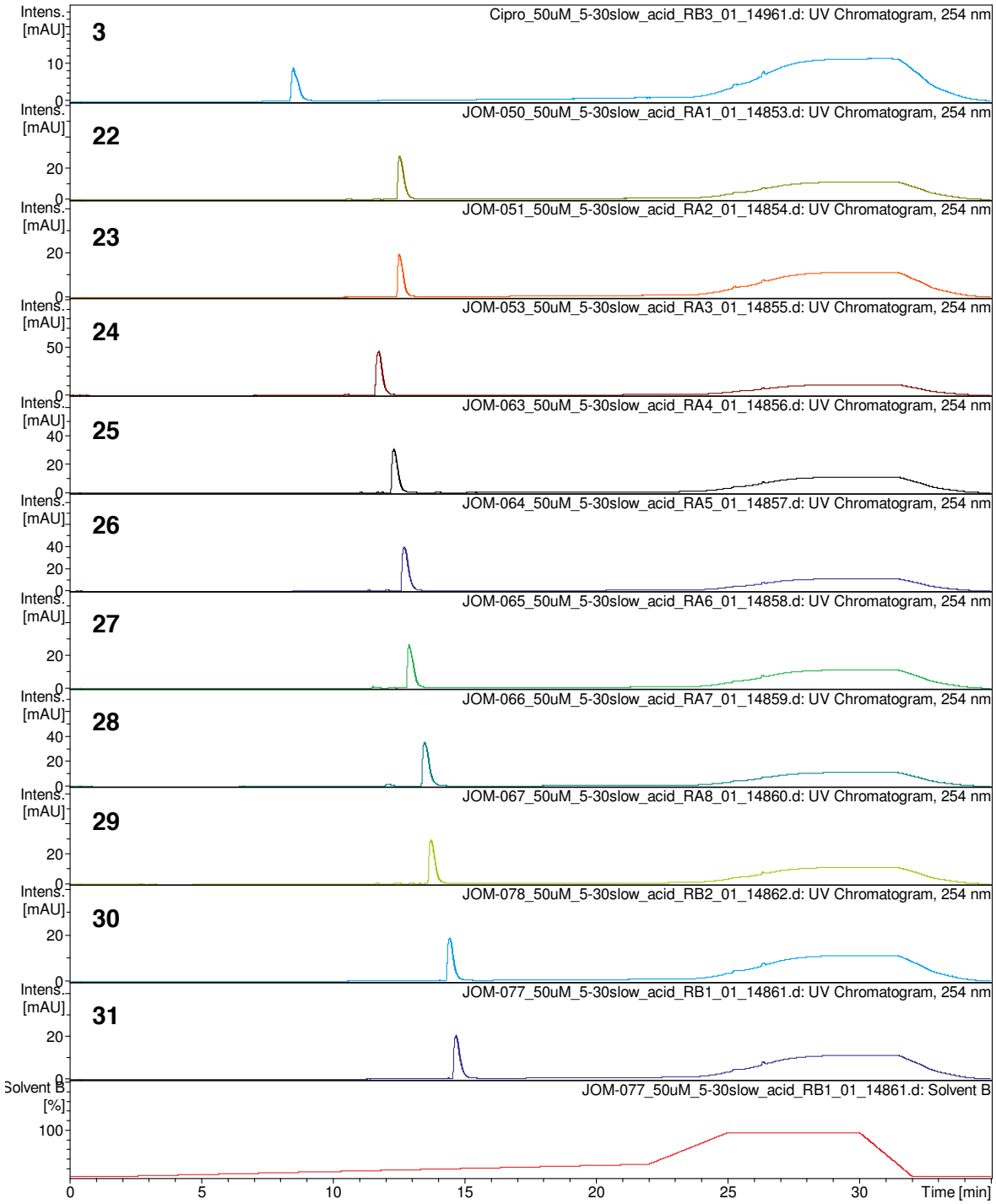
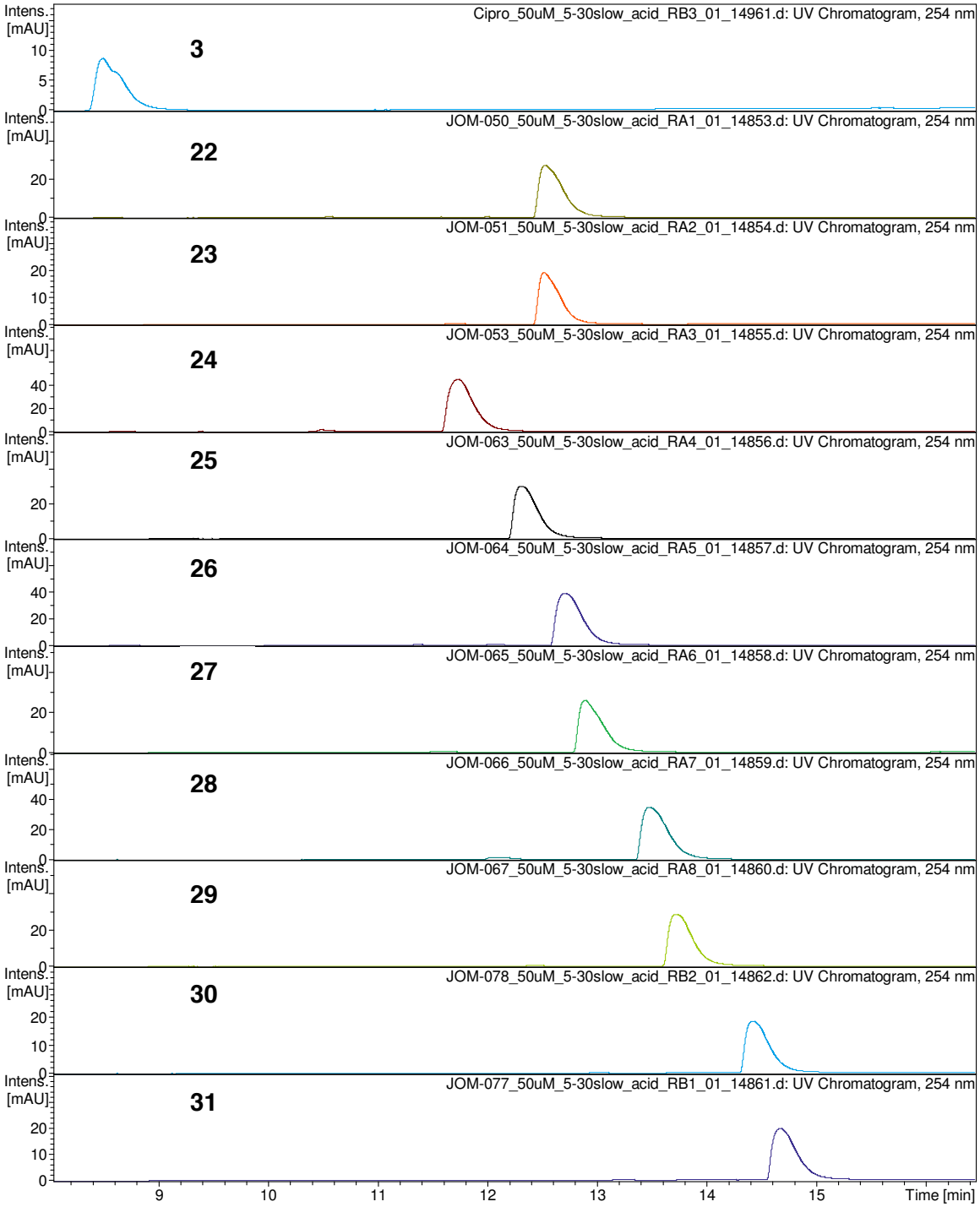

31
intensity [mAU]
time [sec]

Table S4: Retention times measured by reversed-phase HPLC with a H_2O/MeCN system, using three gradients a-c: a. 5-55% B over 20 min; b. 5-30% B over 20 min; c. 2-25% B over 20 min. Eluent A: H_2O (0.1% formic acid), eluent B: MeCN (0.1% formic acid).

compound	**target**	**m**	**n**	**gradient**		
				a	b	c
				retention time t_R [min]		
22	LecA	0	1	9.0	12.5	15.7
23		1	1	9.0	12.9	15.7
24		0	2	8.6	11.7	14.9
25		1	2	8.9	12.3	15.5
26		0	3	9.1	12.7	15.9
27		1	3	9.2	12.9	16.1
28		0	4	9.5	13.5	16.7
29		1	4	9.6	13.7	17.0
30	LecB	0	-	10.0	14.4	17.7
31		1	-	10.2	14.7	18.0
3		-	-	7.0	8.5	11.6

Figure S4: representative chromatogram of conjugates **22** - **31** and ciprofloxacin (**3**) from slow gradient b (see table S4) HPLC runs for lipophilicity comparison. Top: full UV-chromatogram and gradient for eluent B, bottom: UV-chromatogram from t = 8.2 - 16.4 min.





References

1. Huang, X. Fluorescence polarization competition assay: the range of resolvable inhibitor potency is limited by the affinity of the fluorescent ligand. *J Biomol. Screen.* **2003**, *8*, 34-38.
2. Sommer, R.; Wagner, S.; Varrot, A.; Nycholat, C. M.; Khaledi, A.; Häussler, S.; Paulson, J. C.; Imberty, A.; Titz, A. The virulence factor LecB varies in clinical isolates: consequences for ligand binding and drug discovery. *Chem. Sci.* **2016**, *7*, 4990–5001.