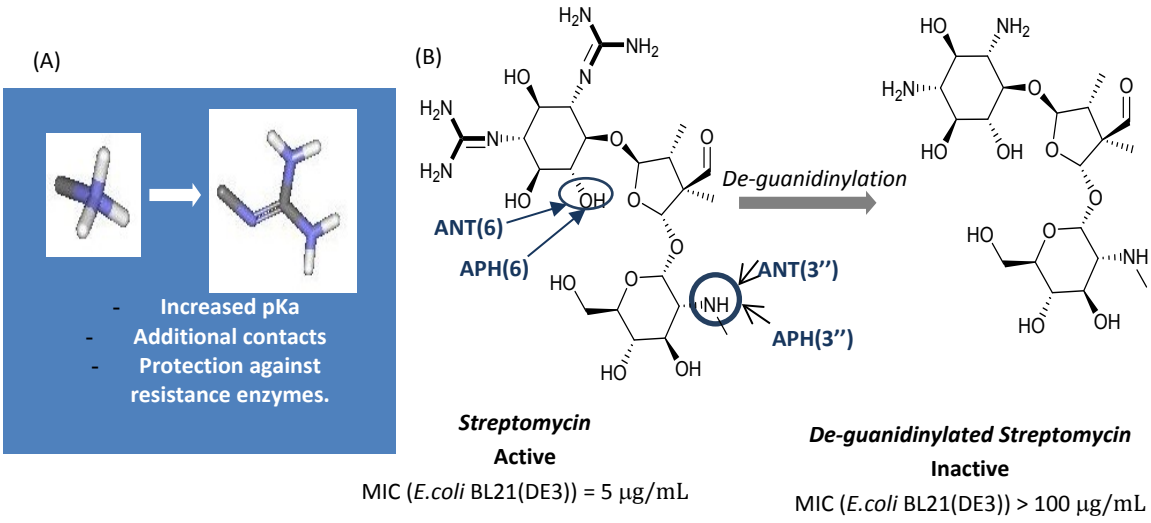
Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

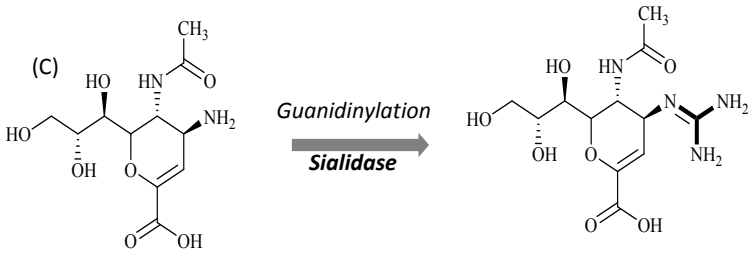
Selective modification of the 3''-amino group of Kanamycin prevents significant loss of activity in resistant bacterial strains

Andrés G. Santana,[a] Sandra G. Zárate,[a] Juan Luis Asensio,[a] Julia Revuelta,[a] * Agatha Bastida[a]*

[a] CSIC. Department of Bioorganic Chemistry, c/ Juan de la Cierva, 3. 28006-Madrid. Spain. Fax: (+) 34-915 644 853

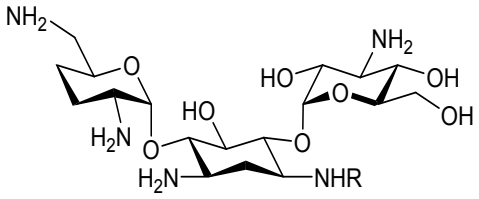
Figure S1.- (A) Schematic representation of some of the potential benefits promoted by *N*-guanidinylation in RNA- and protein-binding polycationic molecules. (B) Example of contribution of guanidine groups to biological activity of RNA ligands: antibiotic streptomycin de-guanidinylation produces a total loss of antibiotic activity. Streptomycin modifying resistance enzymes are highlighted in blue. (C) Example of effect of amine substitution by a guanidine: Increase of potency against a viral sialidase (Zanamivir®)

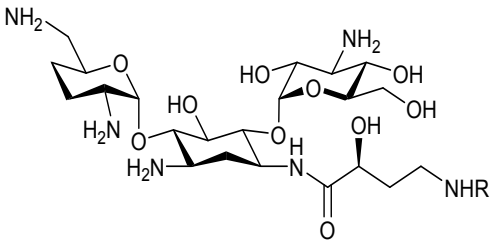




		Zanamivir®
K_i (μM)	4 x 10^{-2}	3 x 10^{-3}

Figure S2. Effect of *N*-1 modification of 4,6-disubstituted-2-deoxystreptamine aminoglycosides**.**





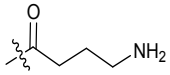
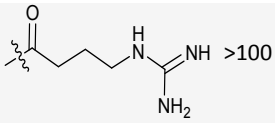
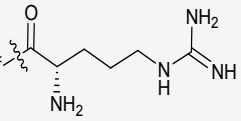
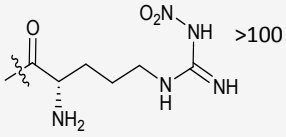
	MIC (μg/mL)[a]	[a]Strain: *S. aureus*		MIC (μg/mL) [a]
R= H (Dibekacin)	50		R= H (Arbekacin)	1-2
R= 	>100		R= 	1-2
R= 	>100			
R= 	>100			
R= 	50			
R= 	>100			

Figure S3. (a) Selected signals of hetero-nuclear HSQC experiments in DMSO-d6 of kanamycin A **1** (top) and of compound **13** (bottom), corresponding to the position N-6 . For both compounds similar chemical shifts of C-13 have been observed. However, a clear shift is observed in the corresponding proton signals of these positions, moving from 2.7 and 3.0 ppm for compound **1** (in red) to 3.41 and 3.67 for compound **13** (in blue). Structurally relevant carbon signals (position 6') are shown on the left and have been linked to these ^{13}C HSQC signals through dashed lines. The corresponding 1H-signals that correlate with these carbons have been marked with arrows. These data are in consonance with a regioselective guanidinylation of position N-6'. (b) Sectors of hetero-nuclear HSQC experiments in D_2O of kanamycin A **1** (top) and 3-azido-kanamycin A **14** (bottom). In comparison to the HSQC spectrum of kanamycin A **1**, the corresponding *J*(C3-H) coupling for compound **14** shows a remarkable shift to lower field proving the regioselective introduction of the azide moiety at this position.

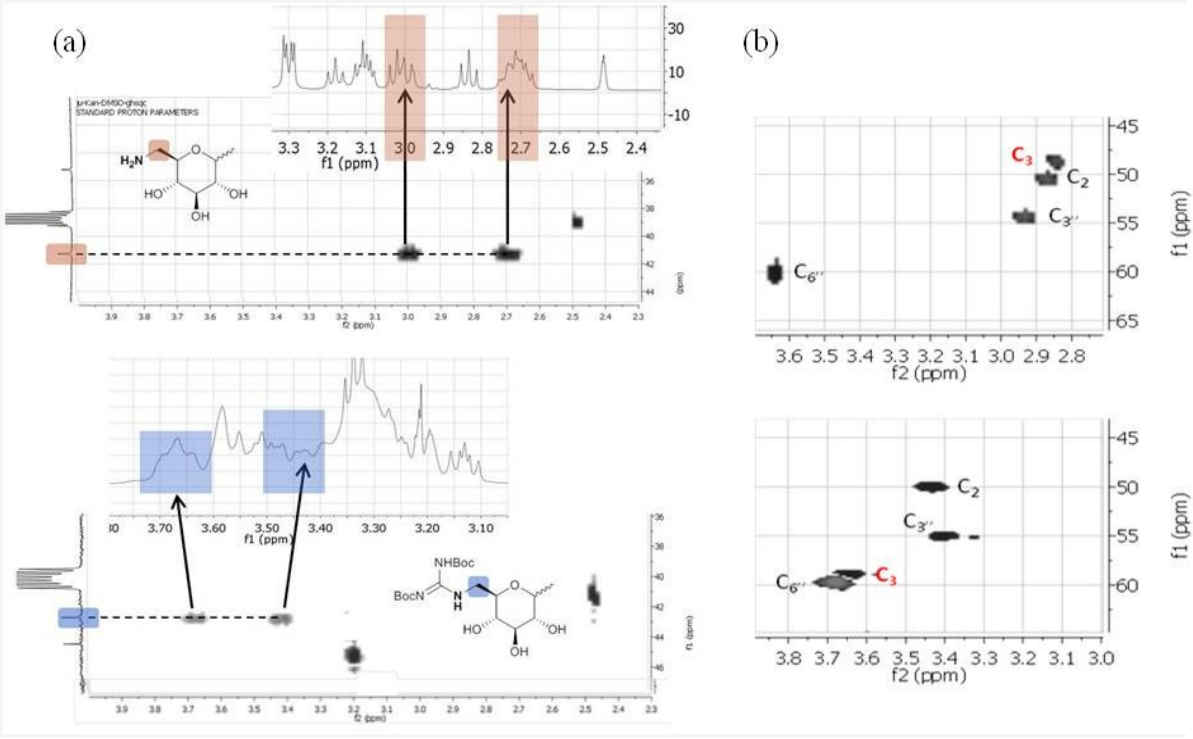
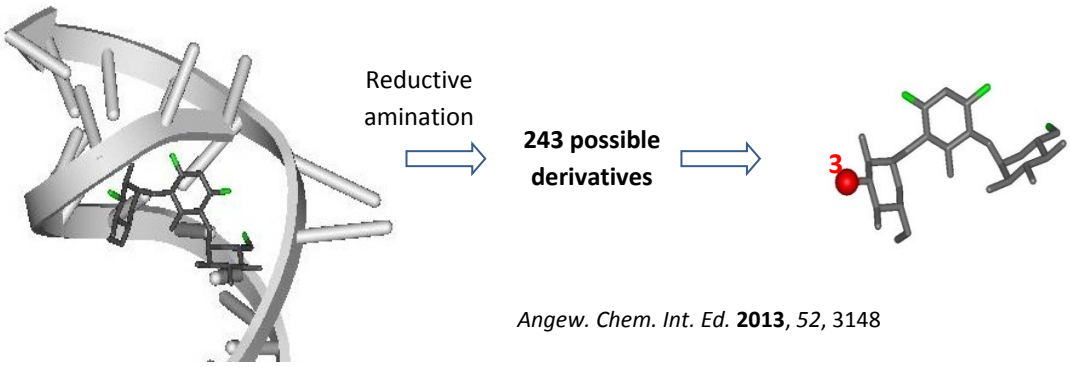


Figure S4. Schematic representation of the combinatorial approach developed by our group for the determination the rRNA preferences for punctual kanamycin amine modification. Interestingly, the N-3'' position in the aminoglycoside constitutes the most common *N*-modificated position found in natural aminolgycosides.

a



Natural aminoglycosides

b

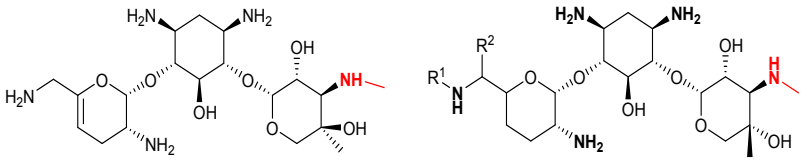


Figure S5. Left: Schematic representation of natural kanamycins A and B aminoglycosides. Interaction between position N-3'' and G 1405 residue from the rRNA is shown[15]. Right: Schematic representation of several compounds with structural modifications on N-3'' and O-2'' (deamination, substitution of the amino function by a hydroxyl group and displacement of the amino function to O-2'' position respectively). The effect of these modifications in the interaction with G1405 residue and in biological activity is indicated.

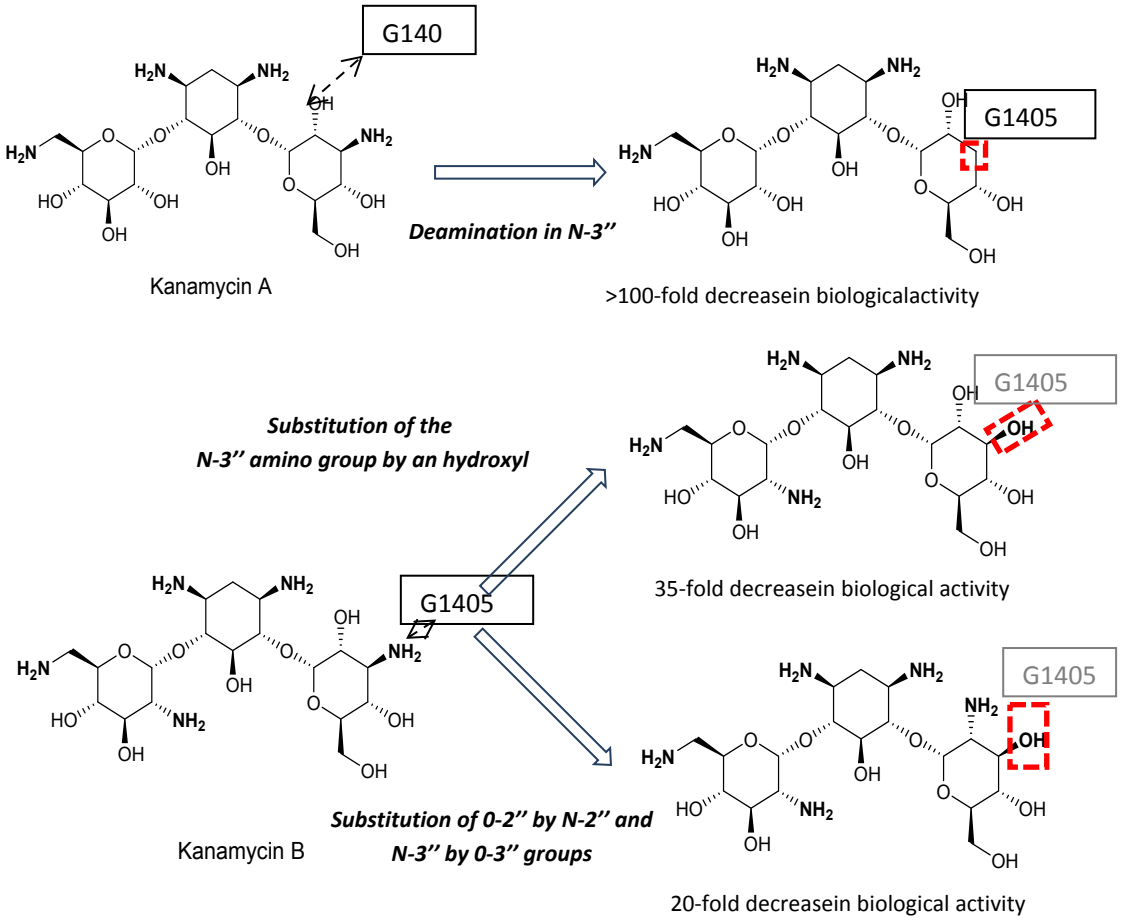
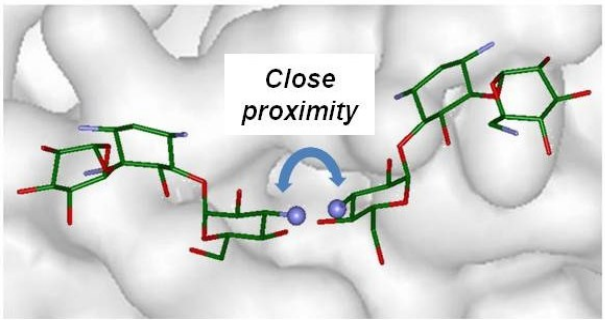


Table S1.- Tm values of the enzymes ANT(4'), AAC(6') APH(3') in presence of **1** and with compound **7**.

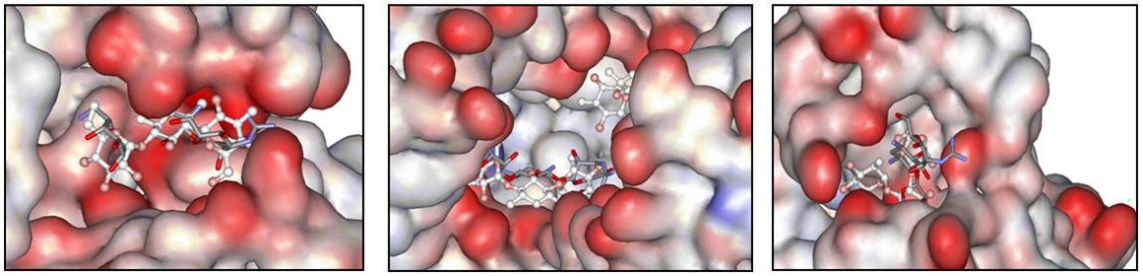
	T_m (°C)		
Enzymes	**Control**	**Compounds**	
	buffer	**1**	**7**
ANT(4')	56±0.1	60.5±0.1	62±0.07
APH(3')	49±0.1	62±0.12	55.5±0.2
AAC(6')	42±0.2	46±0.15	47±0.15

Figure S6. (a) X-ray structure of ANT(4') enzyme with two kanamycin A molecules. The close proximity between the two sugar units and specifically of the amine structural motifs at N-3''-position (blue) prevents an optimal fit of the guanidine-ligands to the receptor binding pocket. (b) Docking studies support the idea that the incorporation of N-3'' modifications disrupts the proper orientation of the aminoglycoside in the active site of the APH(3') enzyme, (c) the ANT(4') enzyme and (d) the AAC(6') enzyme.

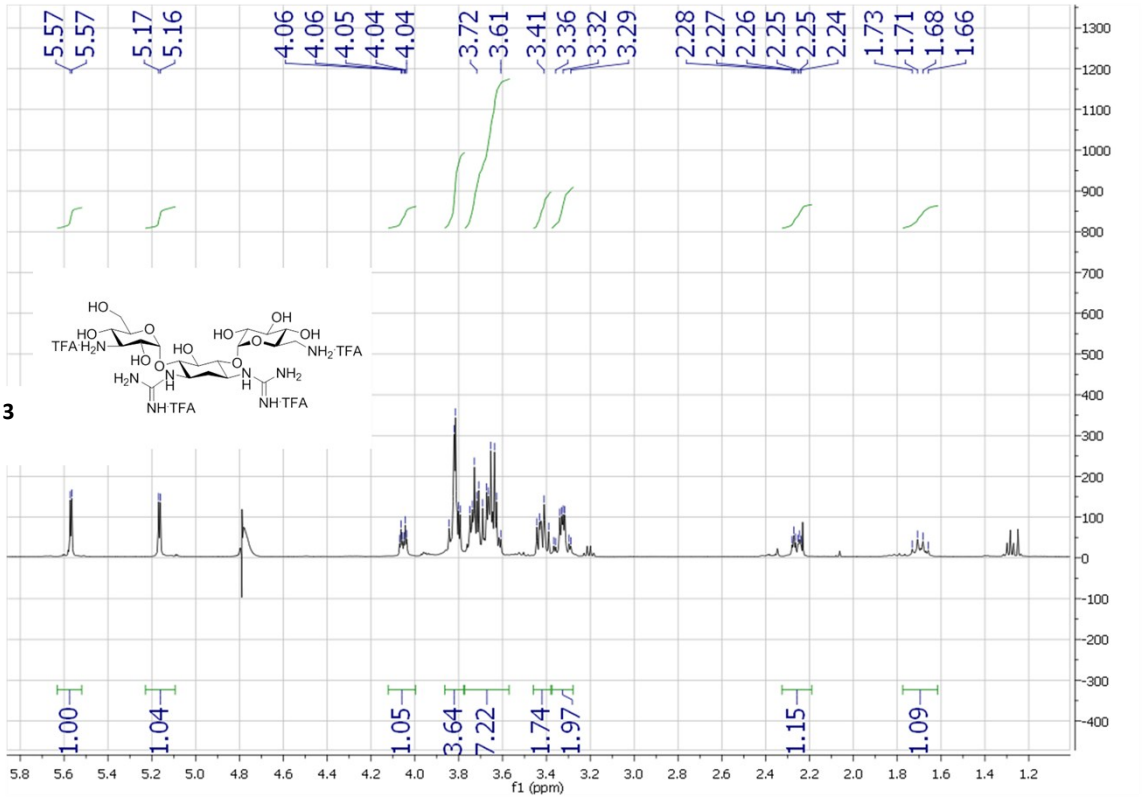
(a)

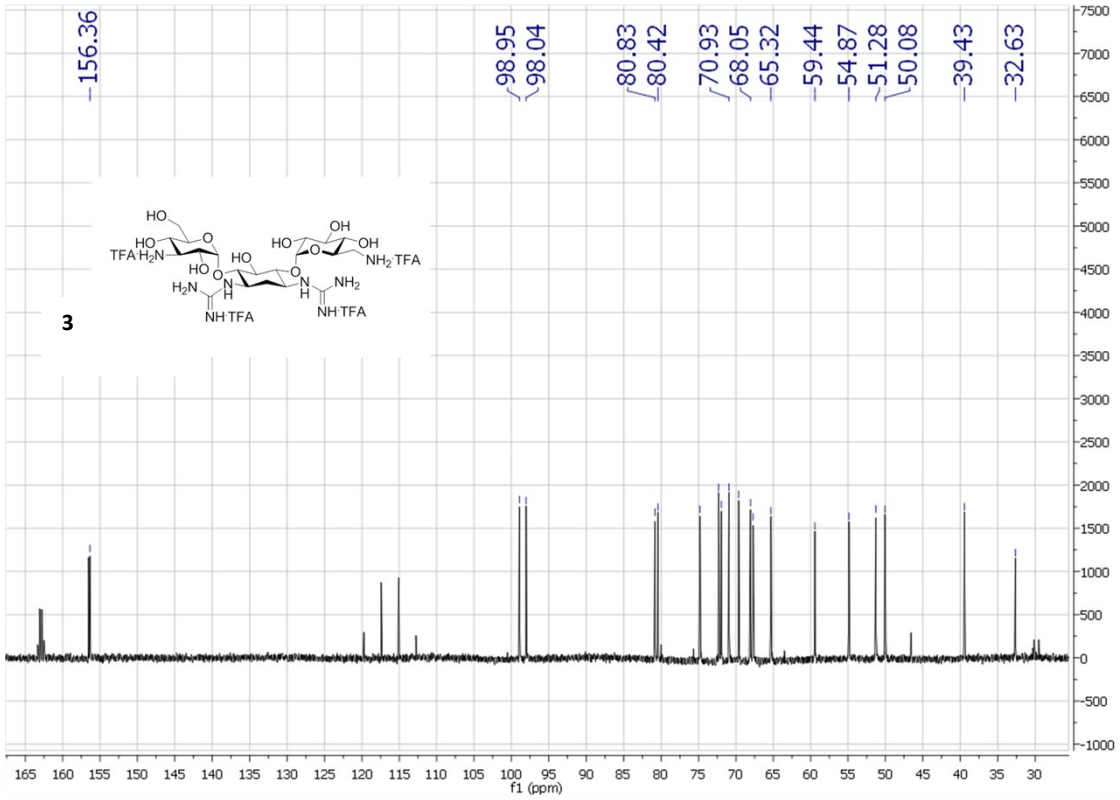


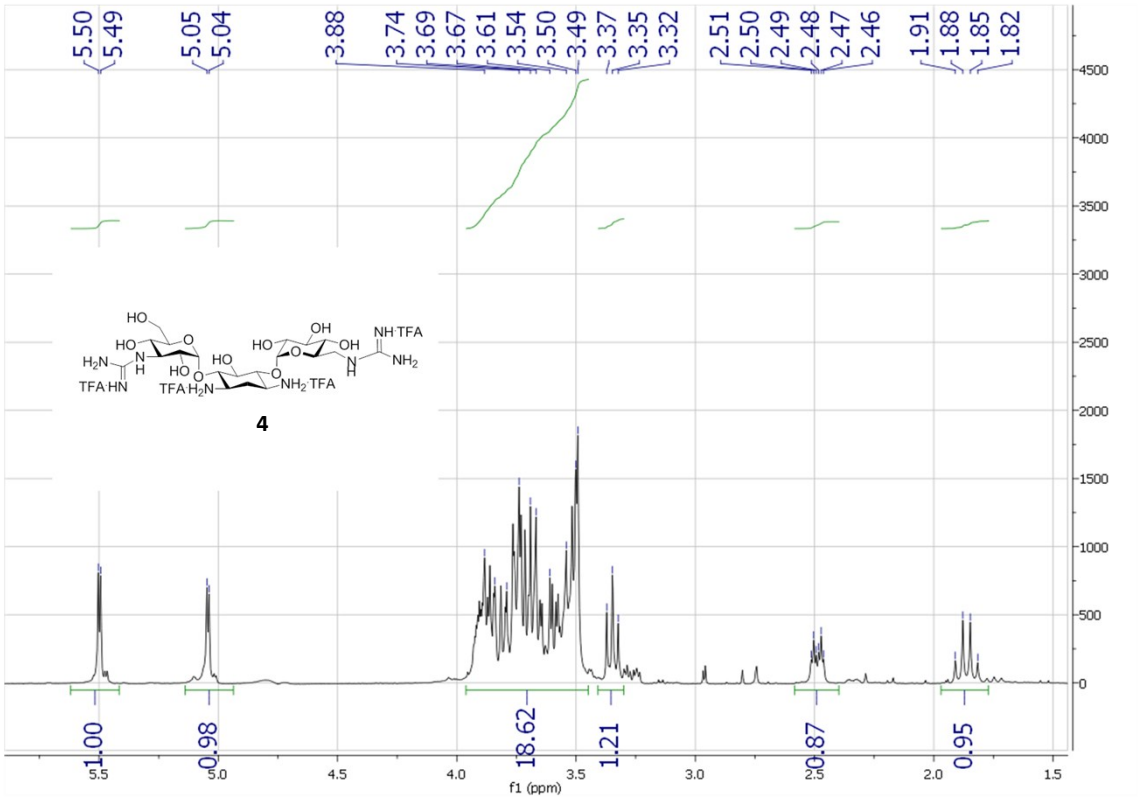
(b) (c) (d)



^{1}H and ^{13}C NMR spectrums of the compounds **3**-**7**, **10**, **11**, **13**, **14**, **16** and **17.**



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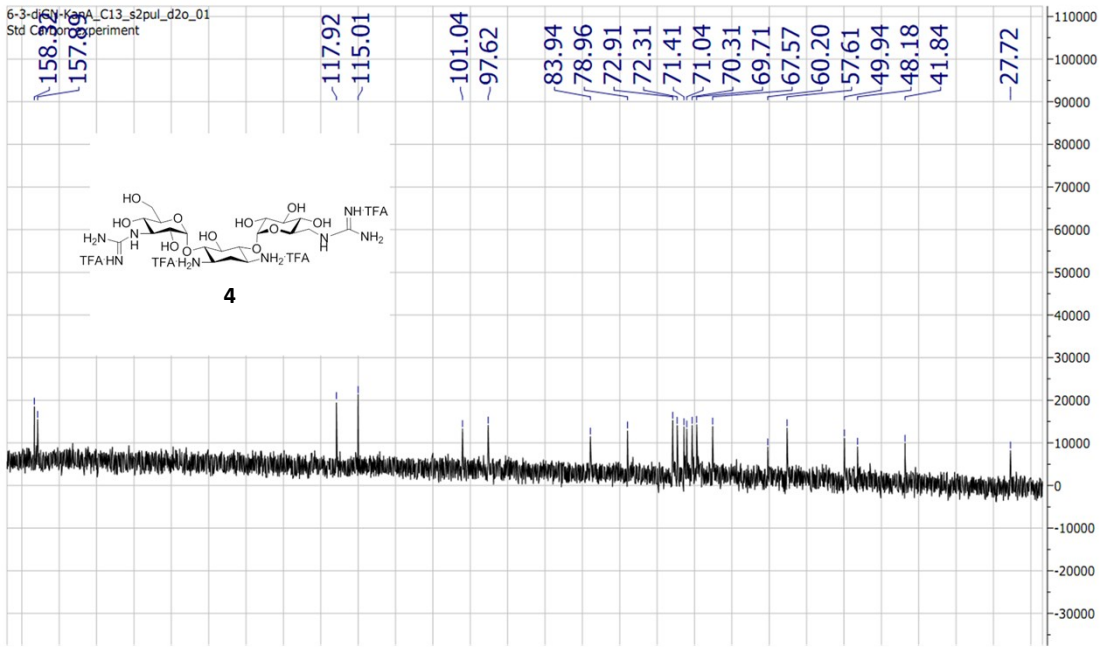
5.49
5.05
5.04
3.88
3.74
3.69
3.67
3.61
3.54
3.50
3.49
3.37
3.35
3.32
2.51
2.50
2.49
2.48
2.47
2.46
1.91
1.88
1.85
1.82
4
1.00
0.98
18.62
1.21
0.87
0.95
f1 (ppm)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Std Carbon Experiment
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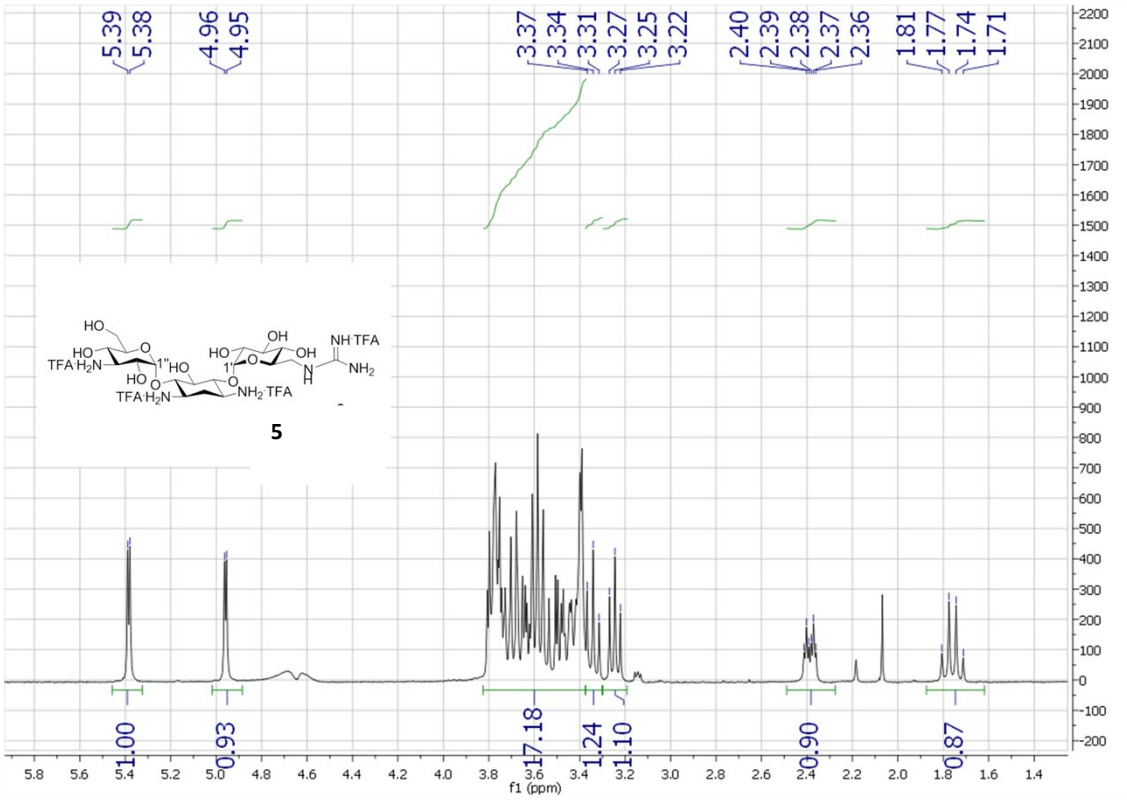
157.89
117.92
115.01
101.04
97.62
83.94
78.96
72.91
72.31
71.41
71.04
70.31
69.71
67.57
60.20
57.61
49.94
48.18
41.84
27.72
4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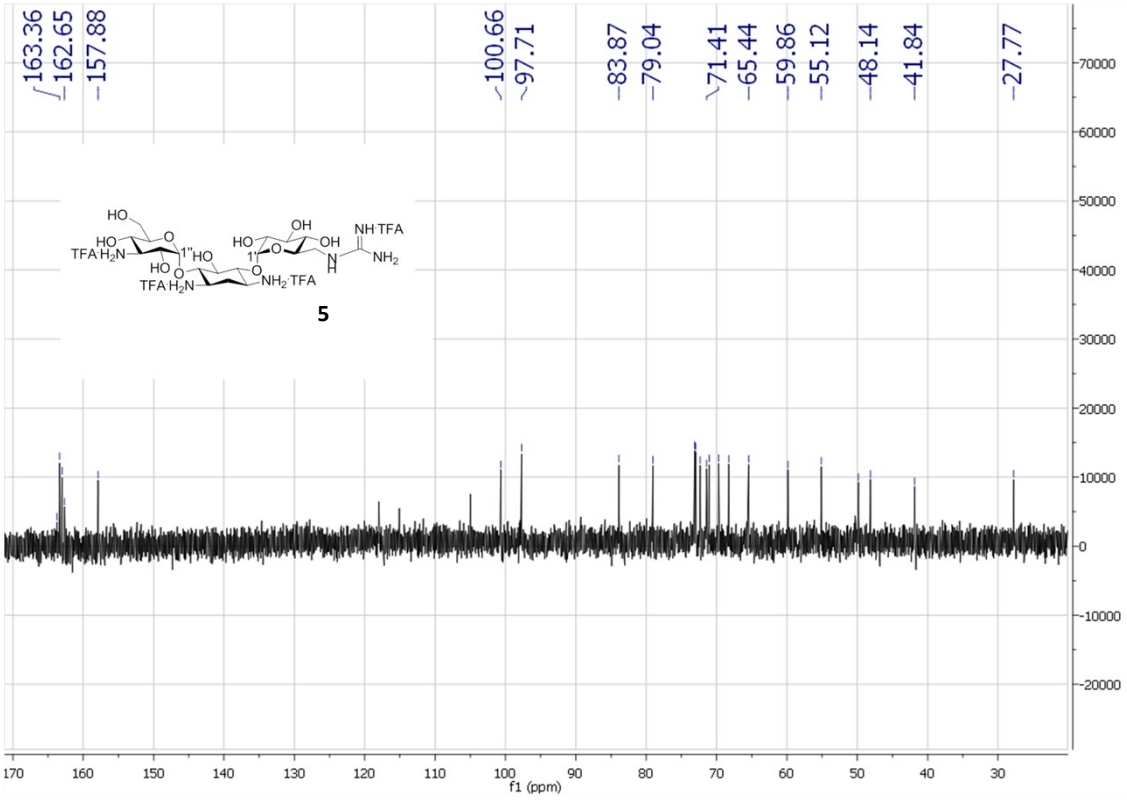
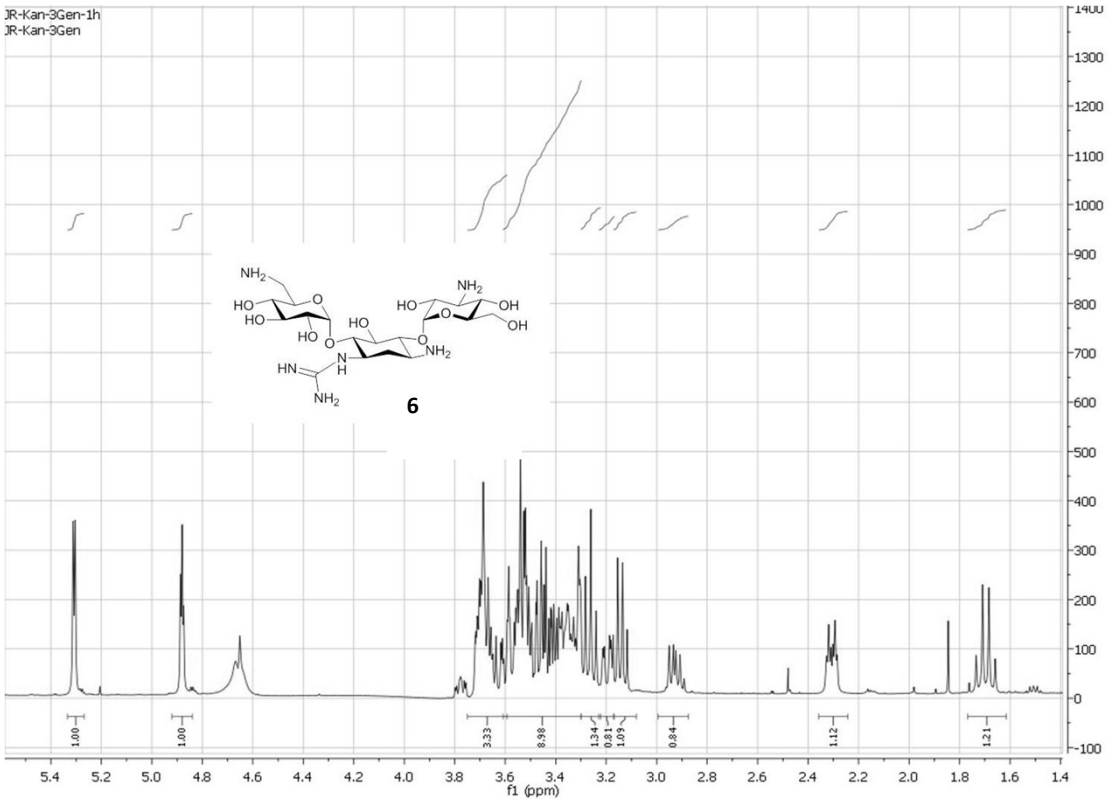
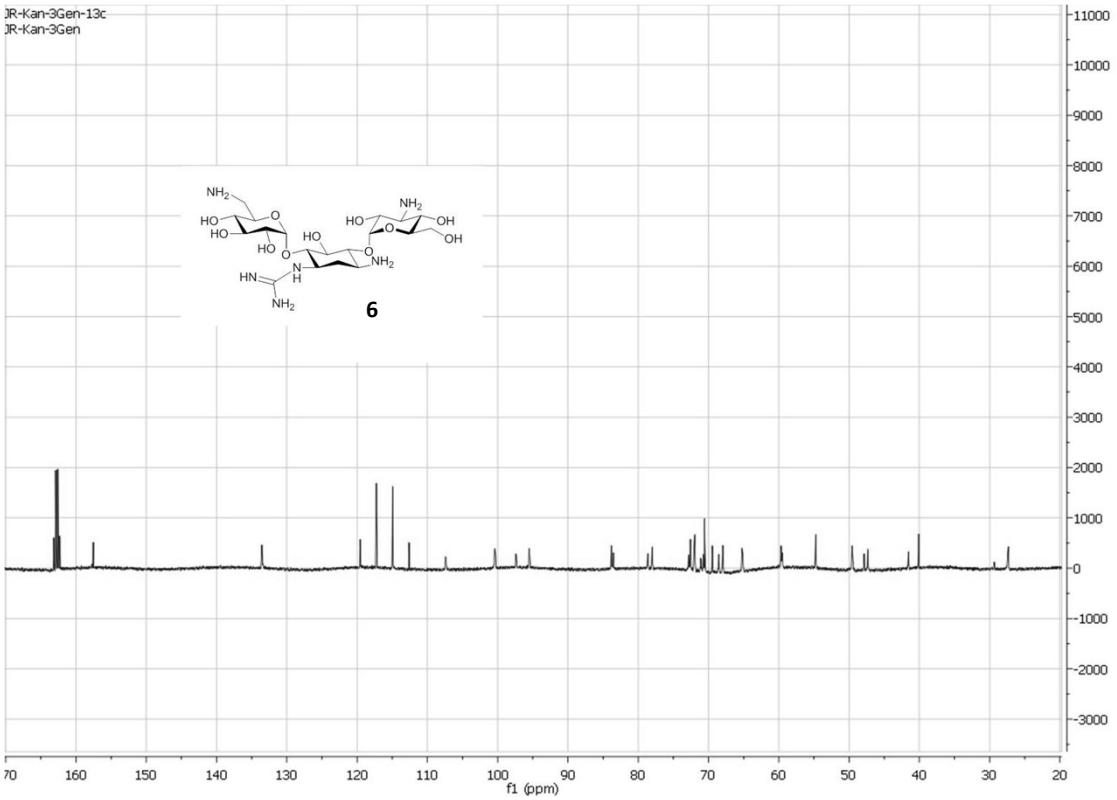
5.38
4.96
4.95
3.37
3.34
3.31
3.27
3.25
3.22
2.40
2.39
2.38
2.37
2.36
1.81
1.77
1.74
1.71
1.00
0.93
17.18
1.24
1.10
0.90
0.87
f1 (ppm)

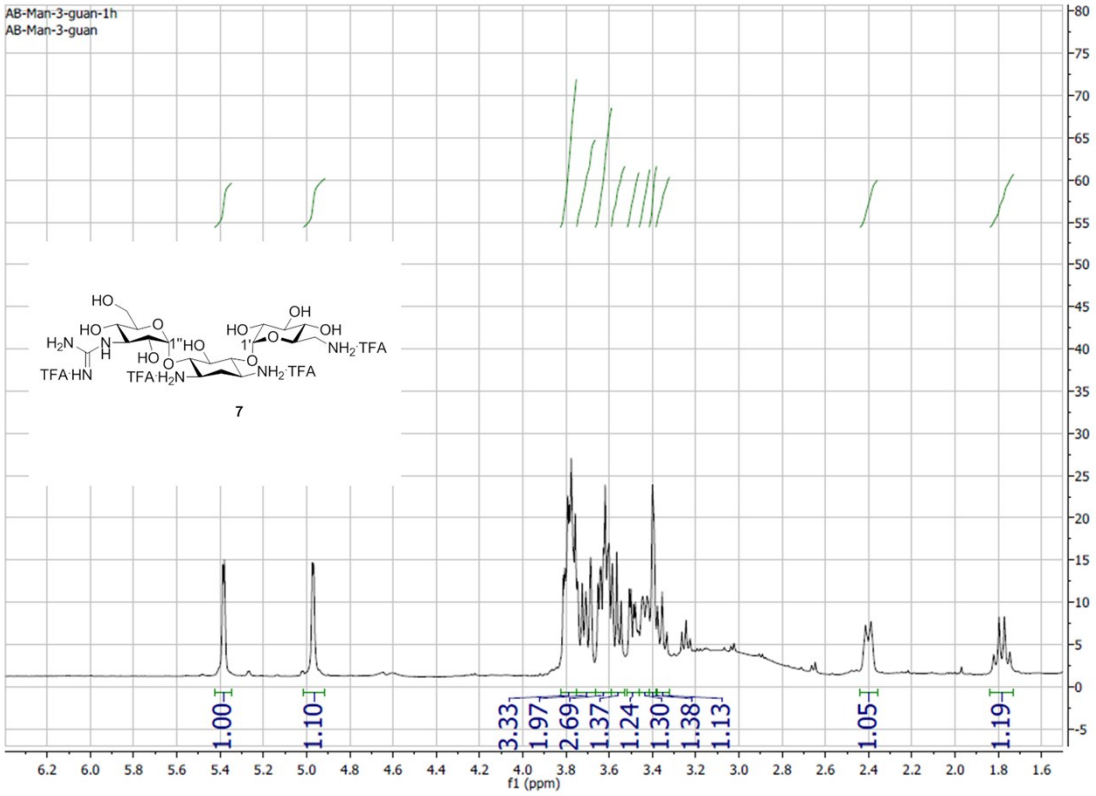
5


163.36
162.65
157.88
100.66
97.71
83.87
79.04
71.41
65.44
59.86
55.12
48.14
41.84
27.77
f1 (ppm)

5


JR-Kan-3Gen-1h
JR-Kan-3Gen
6
f1 (ppm)


JR-Kan-3Gen-13c
JR-Kan-3Gen
6
f1 (ppm)


AB-Man-3-guan-1h
AB-Man-3-guan
7
1.00
1.10
3.33
1.97
2.69
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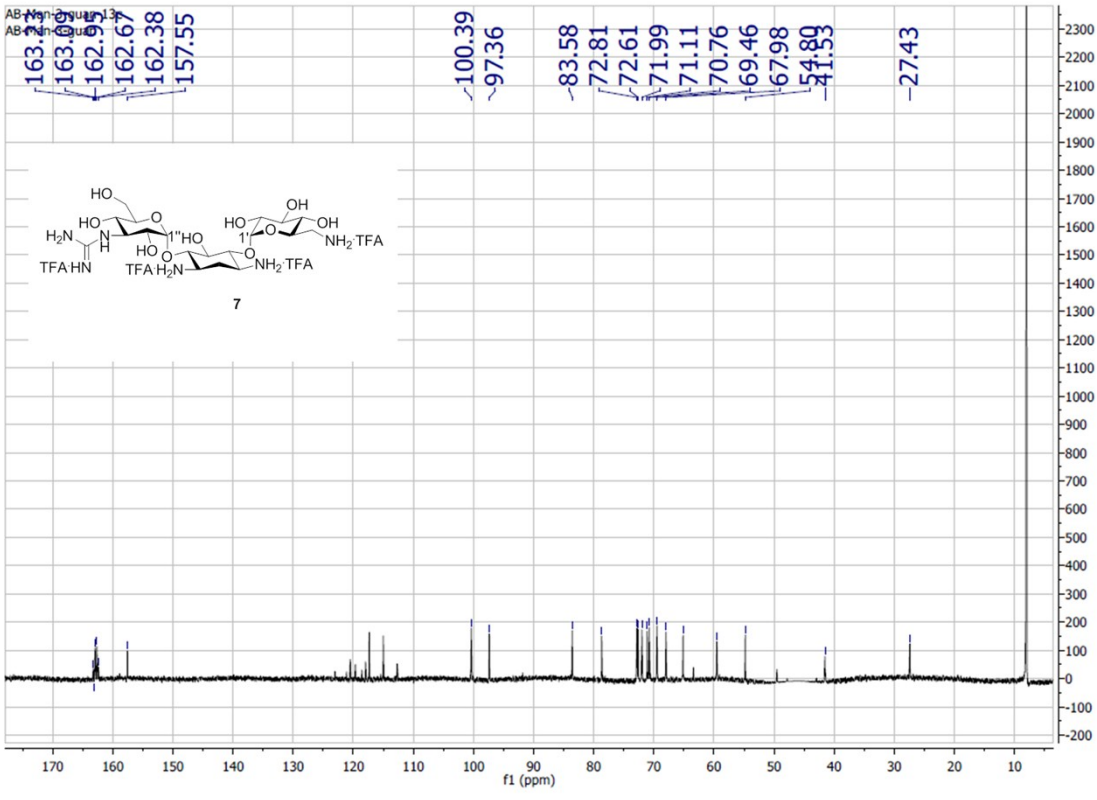
1.30
1.38
1.13
1.05
1.19
f1 (ppm)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AB-Man-3-guan
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163.05
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162.67
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83.58
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54.80
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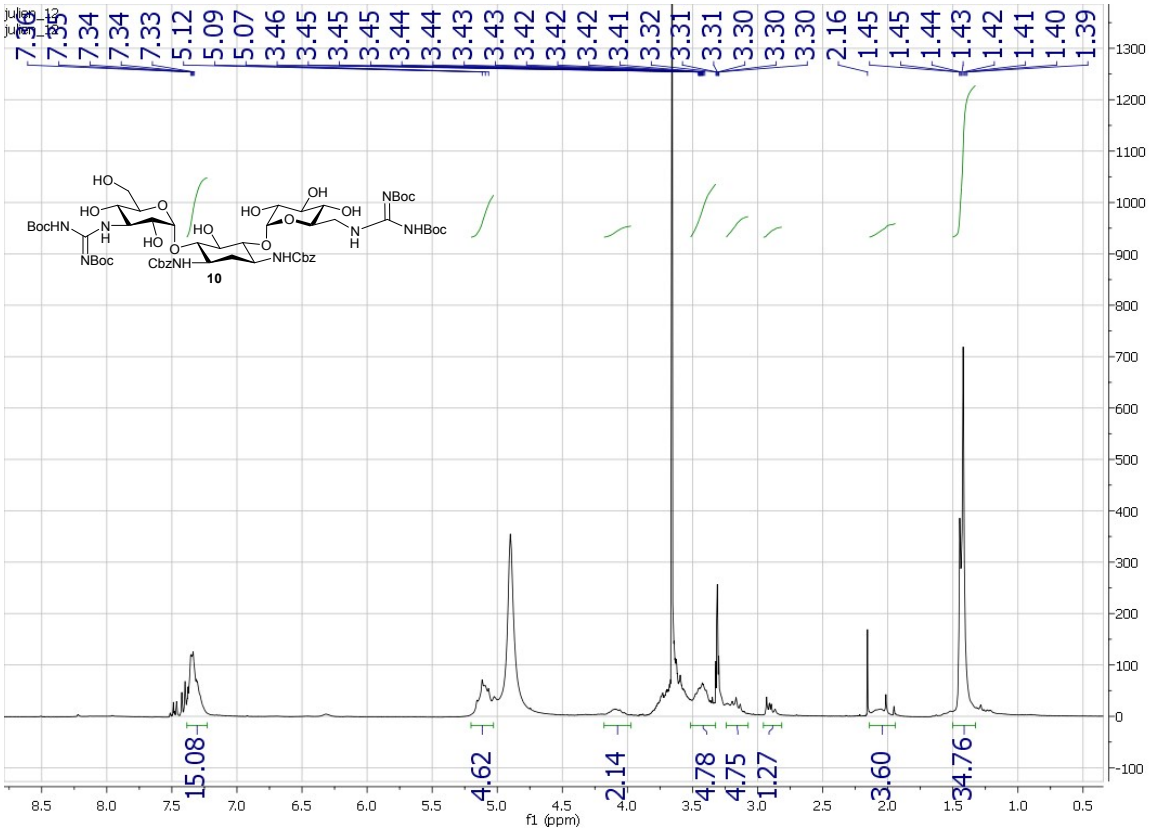
27.43
7
f1 (ppm)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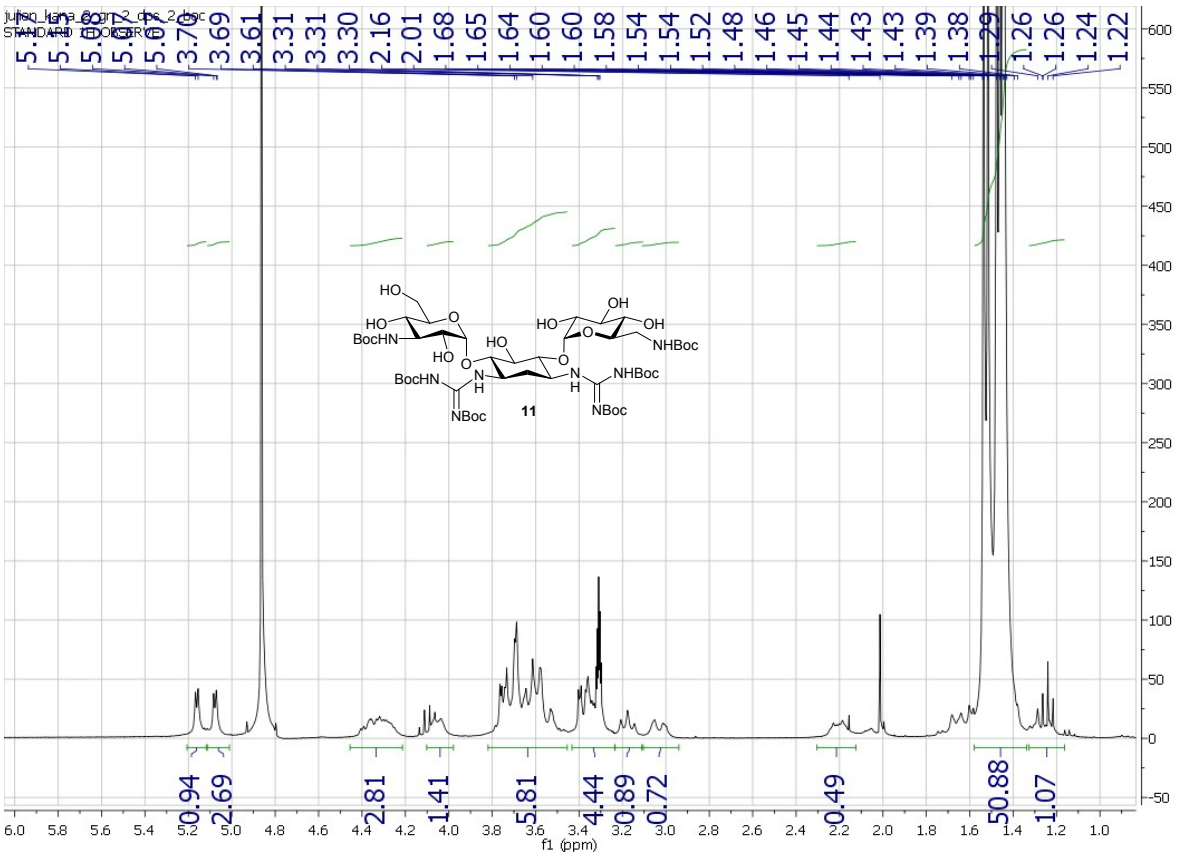
15.08 4.62 2.14 4.78 4.75 1.27 3.60 34.76
f1 (ppm)
10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0.94 2.69 2.81 1.41 5.81 4.44 0.89 0.72 0.49 50.88 1.07
f1 (ppm)
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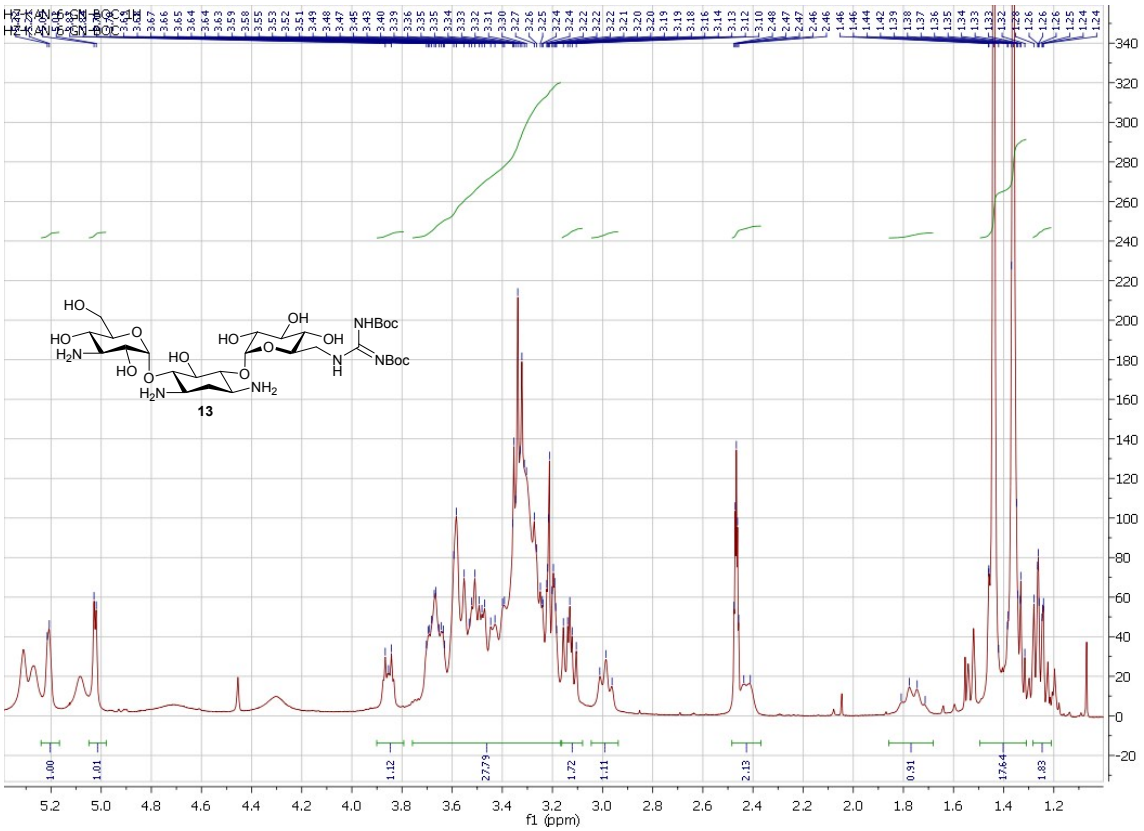
HZ-KAN-6-GN-BOC
HZ-KAN-6-GN-BOC
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HO
H2N
O
HO
O
HO
H2N
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HO
O
OH
NHBoc
N
H
NBoc
13
1.00
1.01
1.12
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1.11
2.13
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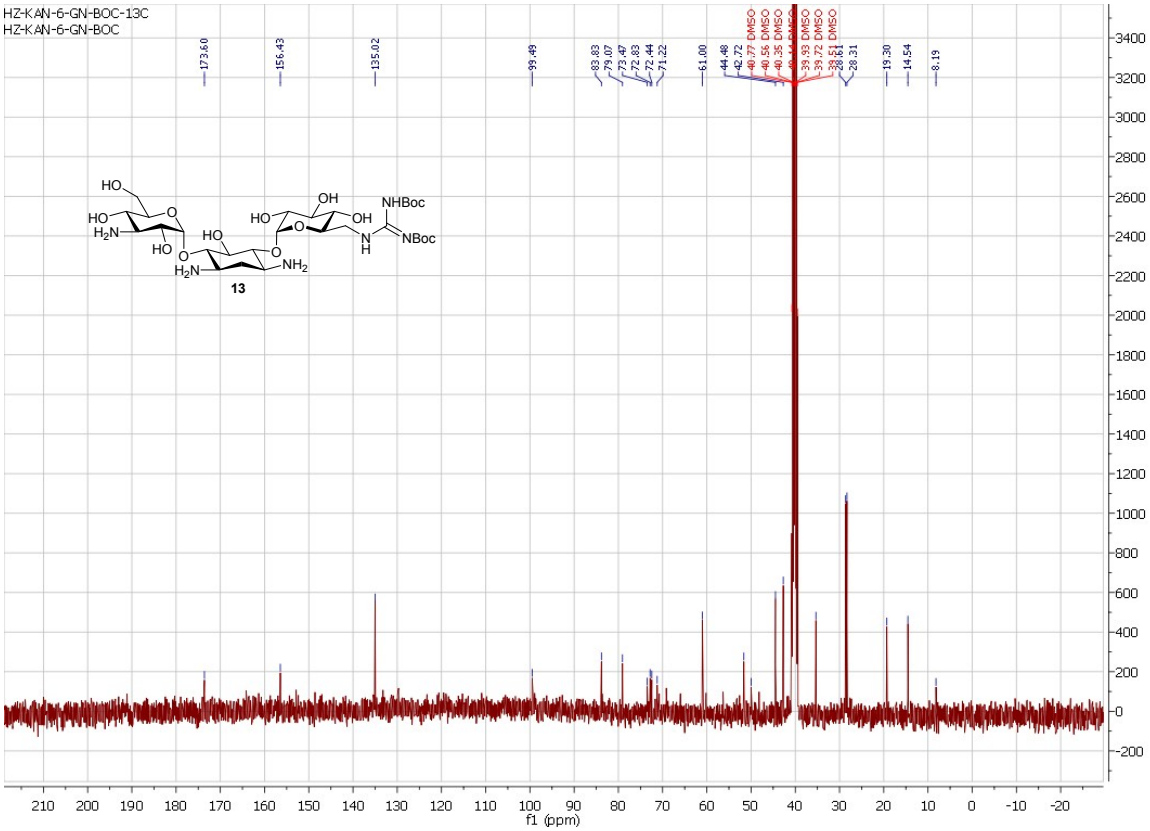
17.64
1.83
f1 (ppm)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HZ-KAN-6-GN-BOC
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156.43
135.02
99.49
83.83
79.07
73.47
72.83
72.44
71.22
61.00
44.48
42.72
40.77 DMSO
40.56 DMSO
40.35 DMSO
39.93 DMSO
39.72 DMSO
39.51 DMSO
28.31
19.30
14.54
8.19
HO
HO
H2N
O
HO
O
HO
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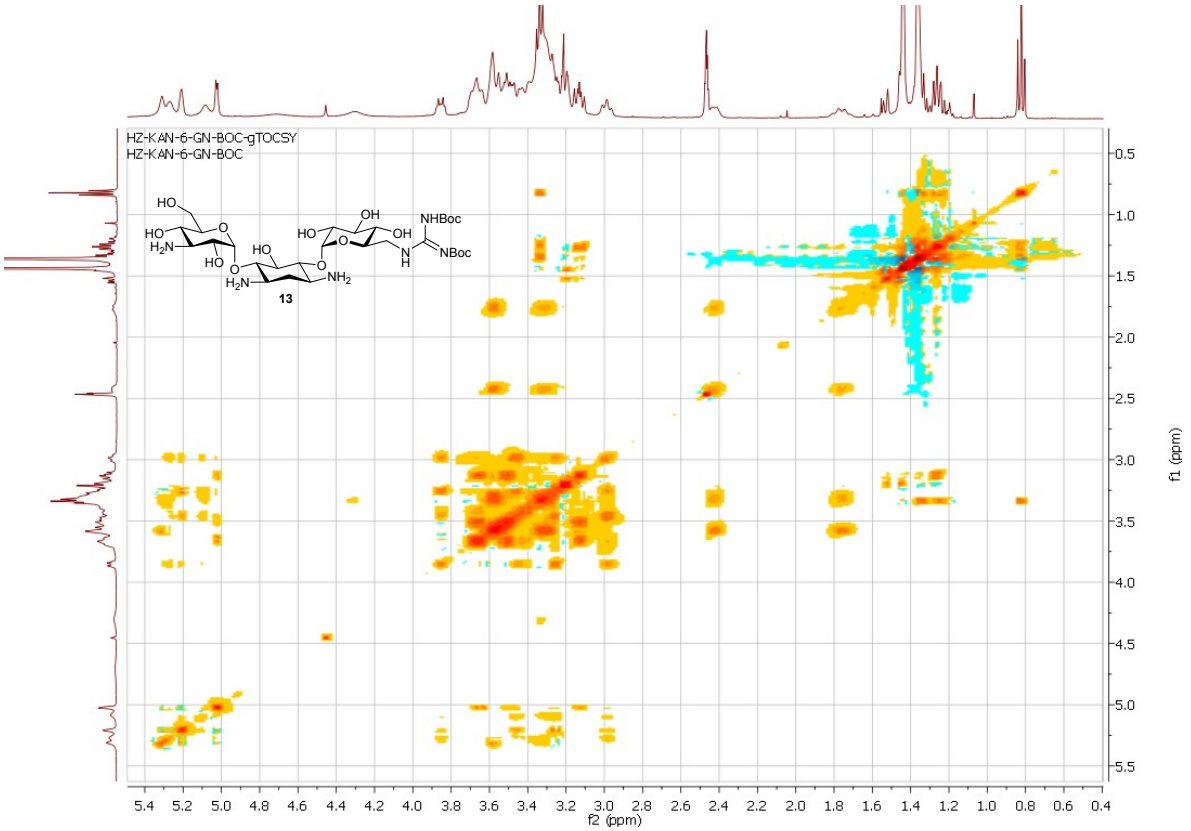
OH
NHBoc
N
H
NBoc
13
f1 (ppm)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HZ-KAN-6-GN-BOC
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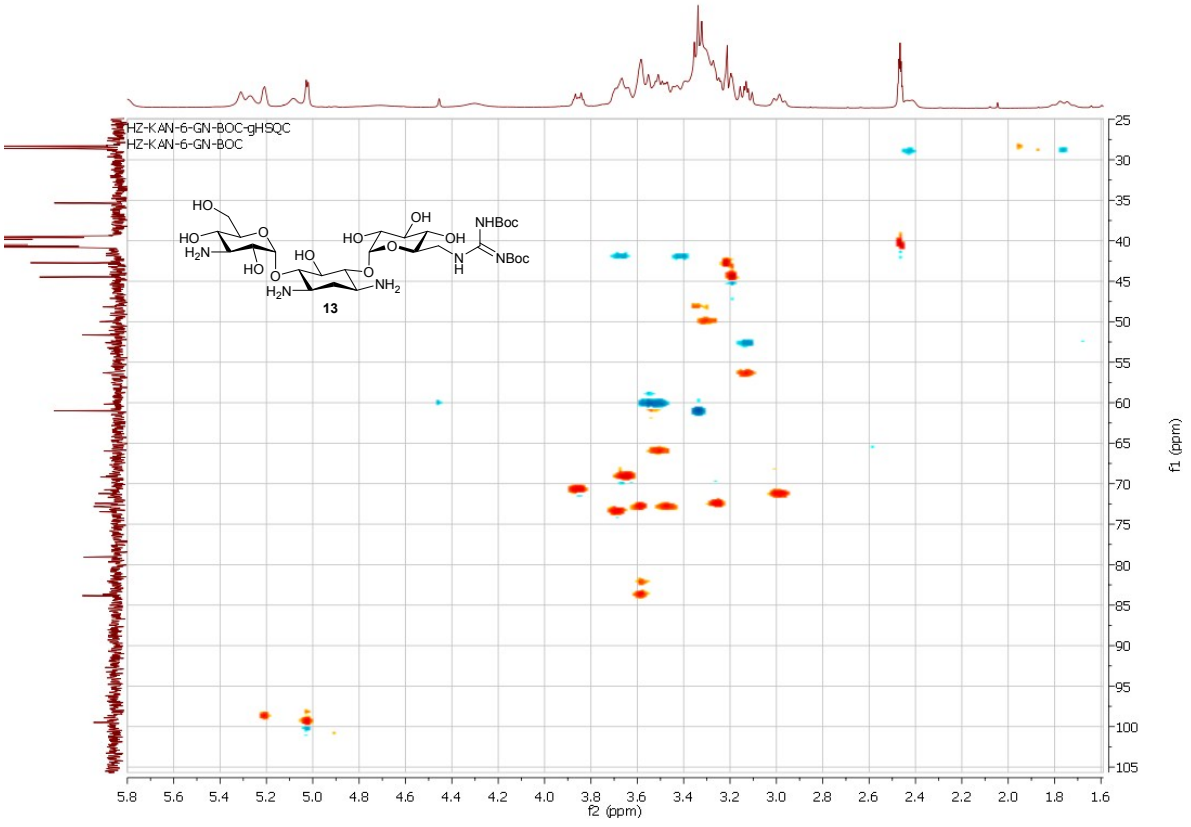
f2 (ppm)
f1 (ppm)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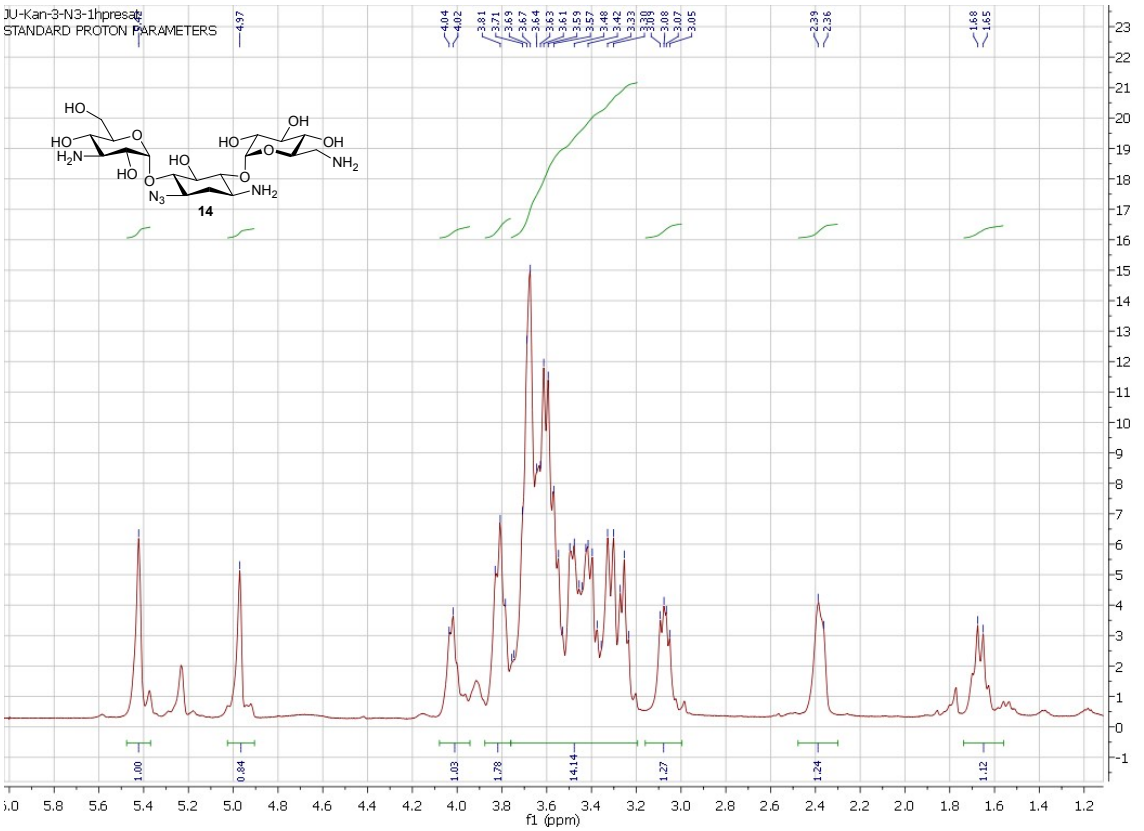
f1 (ppm)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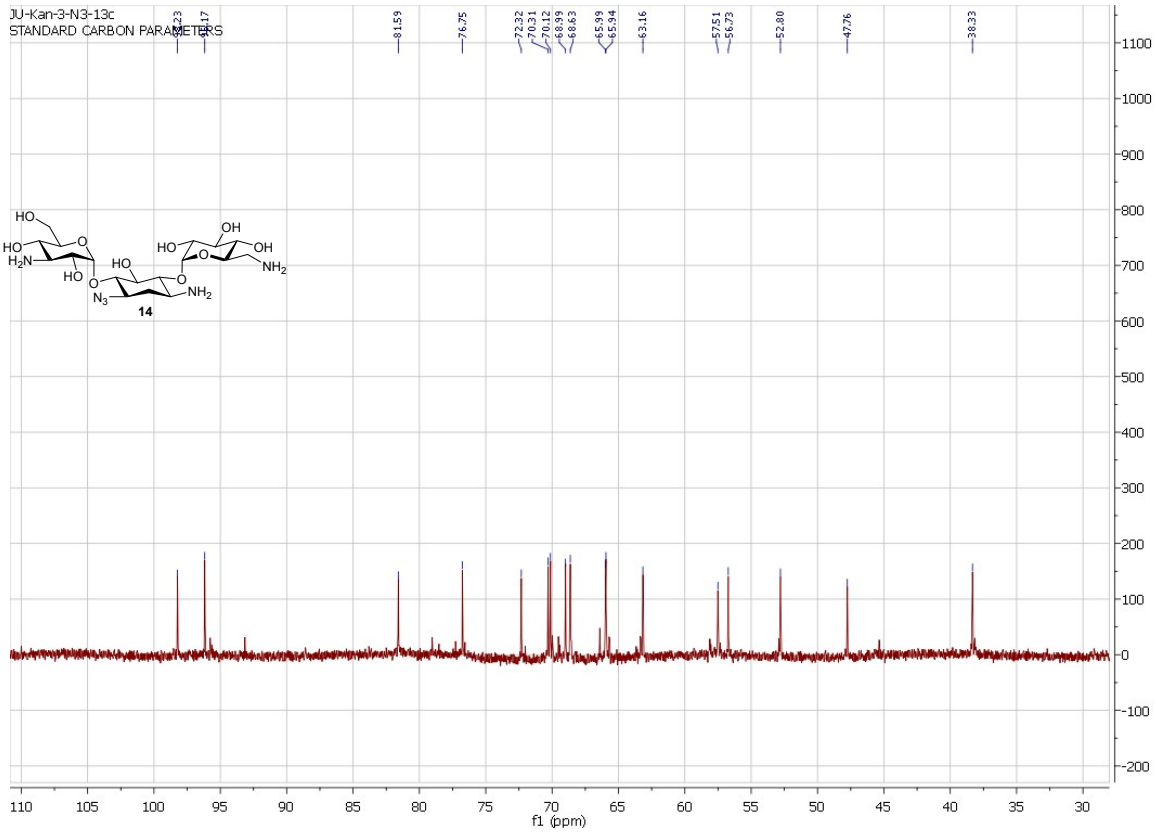
STANDARD PROTON PARAMETERS
14
f1 (ppm)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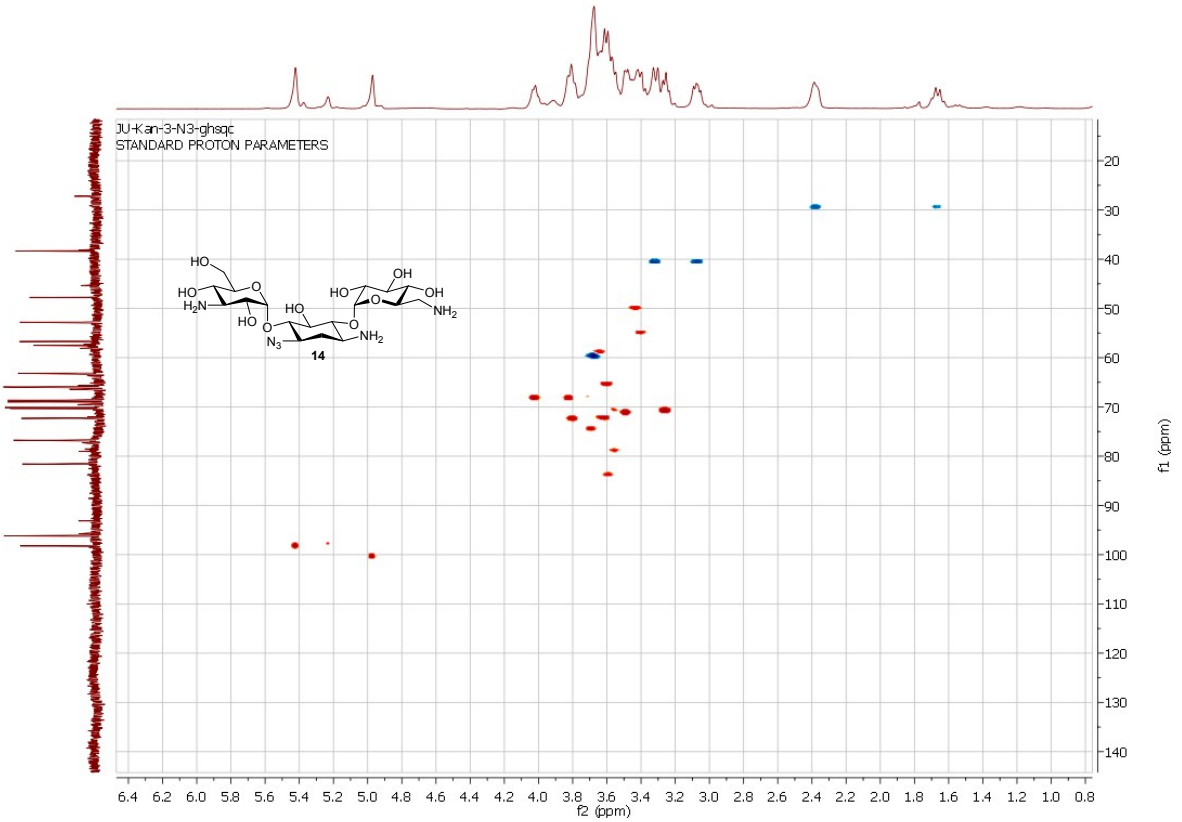
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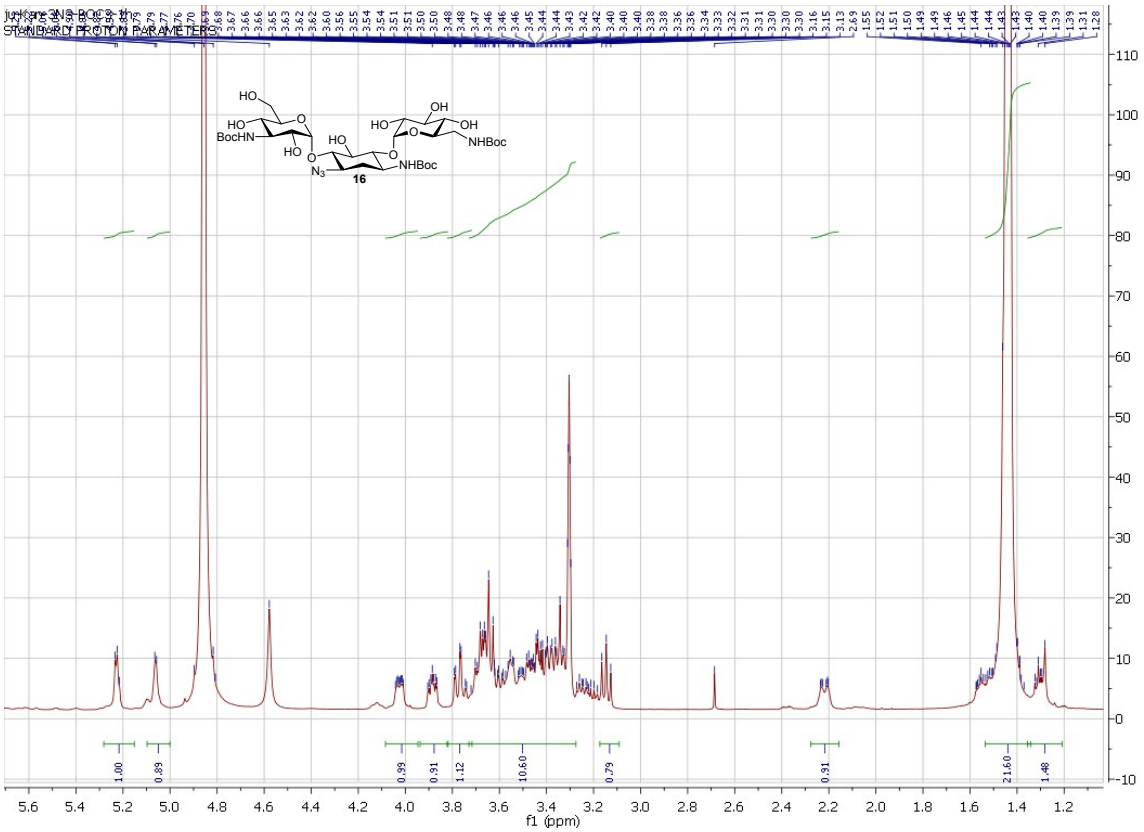
f2 (ppm)
f1 (ppm)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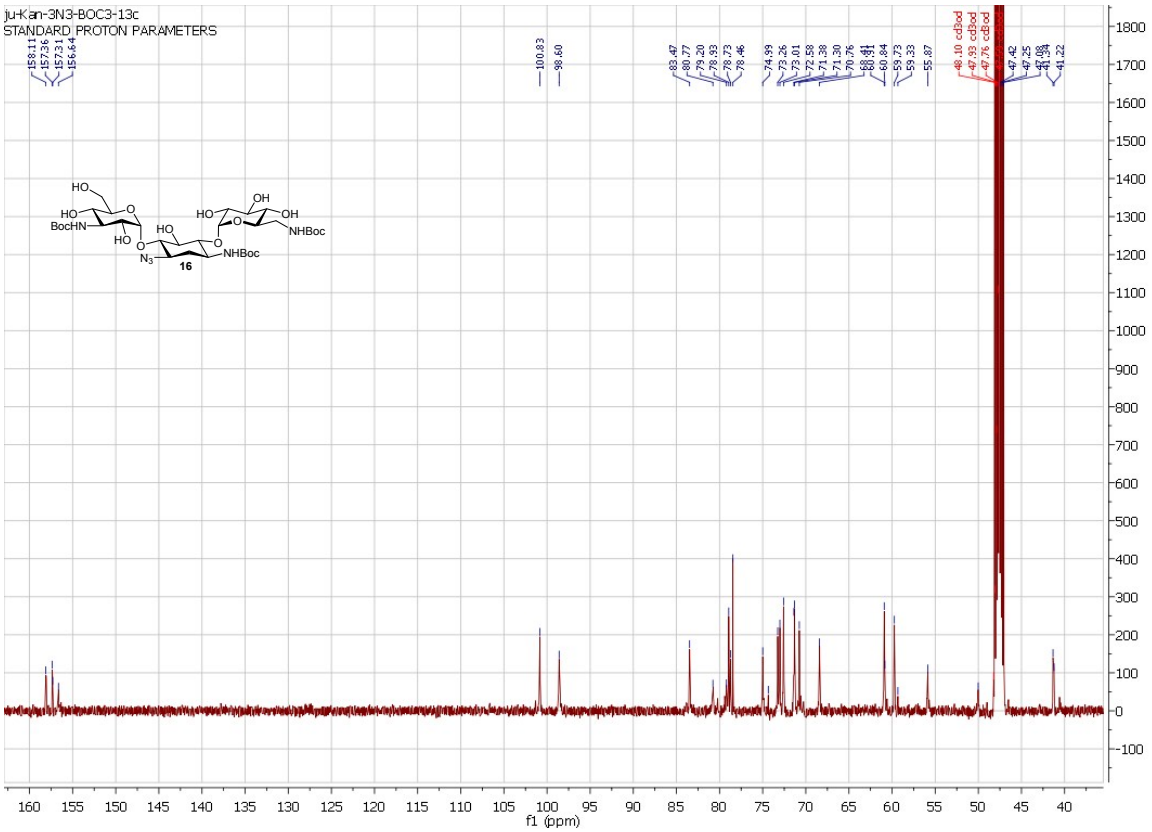
ju-Kan-3N3-BOC3-13c
STANDARD PROTON PARAMETERS
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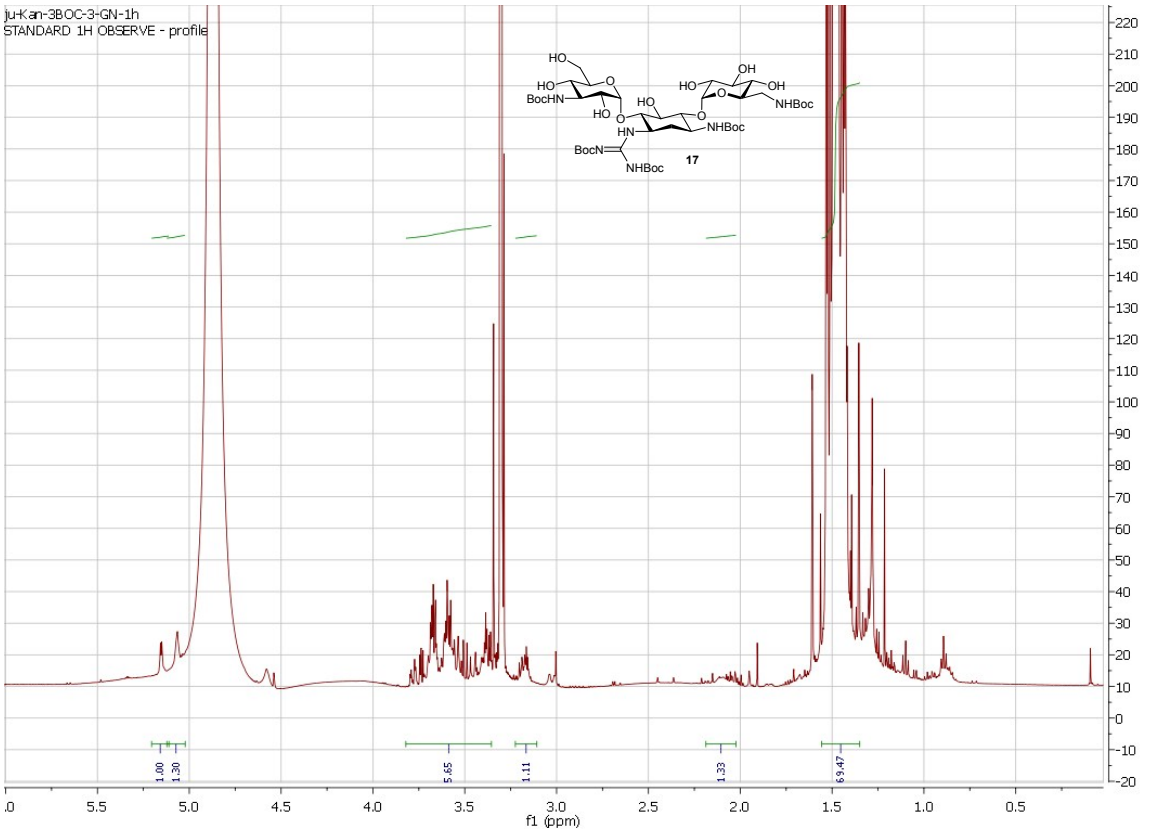
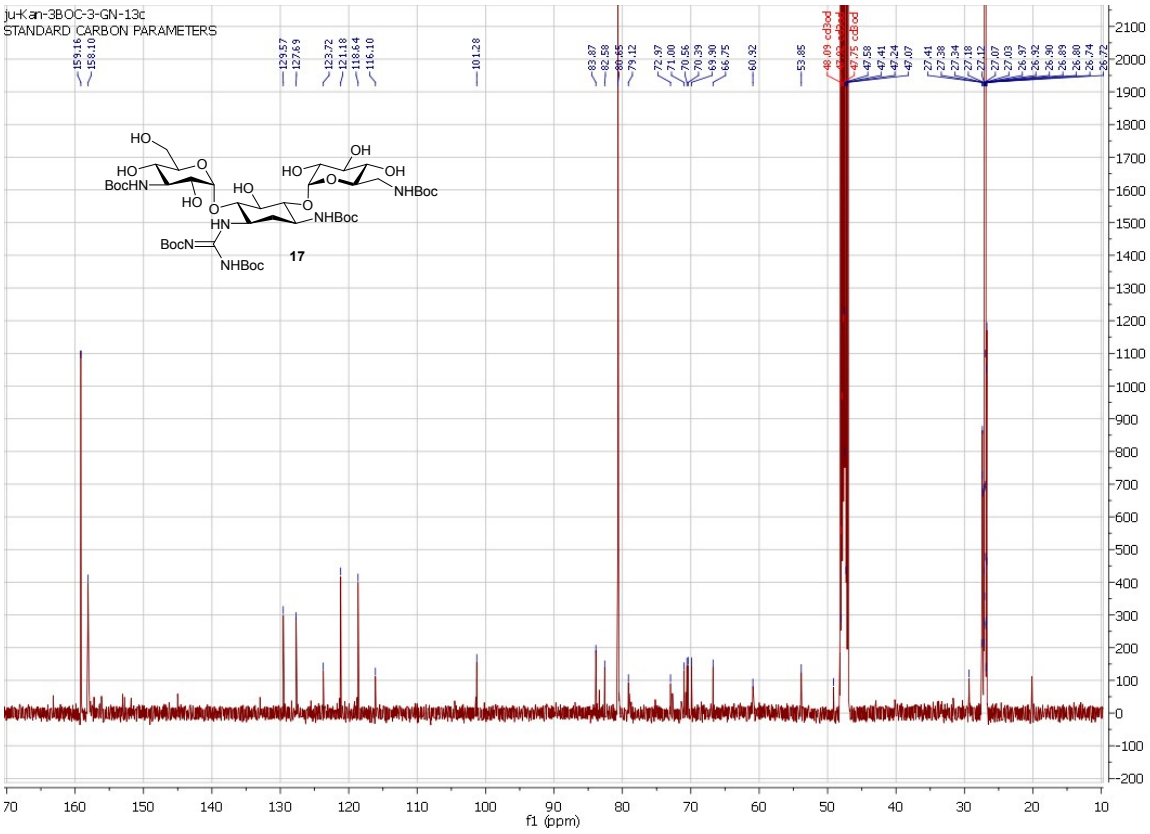
157.36
157.31
156.64
100.83
98.60
83.47
80.77
79.20
78.93
78.73
78.46
74.99
73.26
73.01
72.58
71.38
71.30
70.76
68.41
68.11
60.84
59.73
59.33
55.87
48.10 cd3od
47.93 cd3od
47.76 cd3od
47.42
47.25
41.22
16
f1 (ppm)


ju-Kan-3BOC-3-GN-1h
STANDARD 1H OBSERVE - profile
17
1.00
1.30
5.65
1.11
1.33
6.9.47
f1 (ppm)


ju-Kan-3BOC-3-GN-13c
STANDARD CARBON PARAMETERS
159.16
158.10
129.57
127.69
123.72
121.18
118.64
116.10
101.28
83.87
82.58
80.65
79.12
72.97
71.00
70.56
70.39
69.90
66.75
60.92
53.85
48.09 cd3od
47.75 cd3od
47.58
47.41
47.24
47.07
27.41
27.38
27.34
27.18
27.12
27.07
27.03
26.97
26.92
26.90
26.89
26.80
26.74
26.72
HO
HO
BocHN
HO
O
HO
O
HN
BocN
NHBoc
HO
O
NHBoc
HO
OH
HO
O
OH
NHBoc
17
f1 (ppm)

Purity determination of compound **7** by NMR tritation, using DMSO as internal standard.