

Synthesis and Immunological Studies of Linear Oligosaccharides of β -Glucan as Antigens for Anti-Fungal Vaccine Development

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I. Calculated Antibody Titers of Various ELISA Experiments

Table S-1: Antibody titers induced by conjugate 1

Mouse #	1	2	3	4	5	6	Average	SD
kappa	26	6673	23277	1166	22543	49127	17135	18696
IgG1	5569	3593	285	0	13220	58324	13498	22482
IgG2a	0	0	0	0	0	0	0	0
IgG2b	0	0	20251	0	405	12762	5570	8798
IgG3	66	6528	0	0	0	57061	10609	22905
IgM	5473	41	8	5211	7342	102187	20044	40355

Table S-2: Antibody titers induced by conjugate 2

Mouse #	1	2	3	4	5	6	Average	SD
kappa	3717	57177	114125	65939	55022	47983	57327	35403
IgG1	125	70980	172826	140541	78955	49184	85435	62477
IgG2a	0	0	0	0	0	0	0	0
IgG2b	51	26578	68697	253	102	2	15947	27928
IgG3	1	40975	27647	1	2	12	11440	18210
IgM	2162	38087	45483	32135	8436	73711	33336	26052

Table S-3: Antibody titers induced by conjugate 3

Mouse #	1	2	3	4	Average	SD
kappa	63764	1496	67279	67455	49998	32380
IgG1	53163	2	95510	109124	64450	49131
IgG2a	0	0	0	0	0	0
IgG2b	8271	0	61492	34461	26056	27818
IgG3	58109	103	37301	27645	30789	24085
IgM	35849	2135	46170	42884	31759	20214

Table S-4: Antibody titers induced by conjugate 4

Mouse #	1	2	3	4	5	6	Average	SD
kappa	13328	53453	12192	31169	24779	85058	36663	28067
IgG1	25459	37160	3563	40853	34196	173046	52380	60602
IgG2a	0	0	0	0	0	0	0	0
IgG2b	99	30972	2251	3866	7294	50711	15866	20464
IgG3	19	23746	0	40791	37	80927	24253	32396
IgM	24949	50422	27435	3647	12219	61883	30093	22254

SD: standard deviation

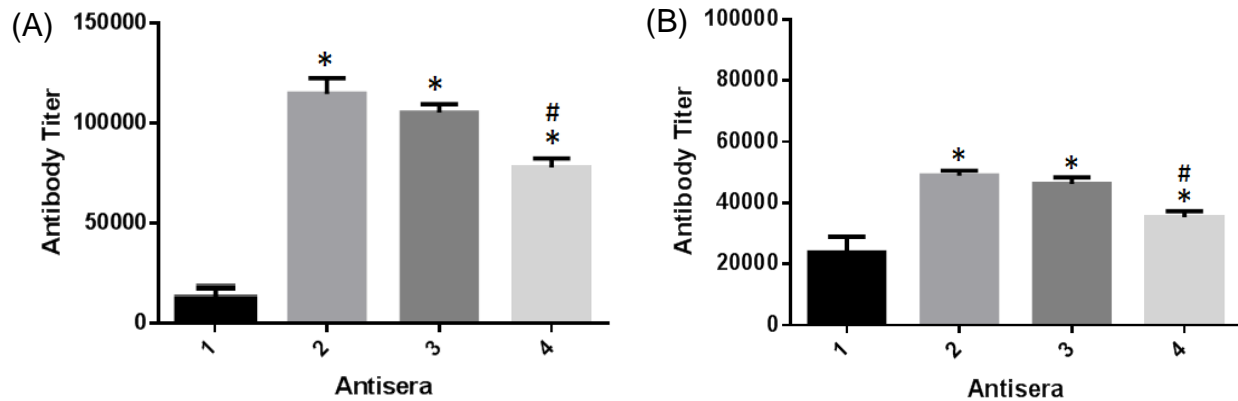
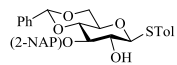
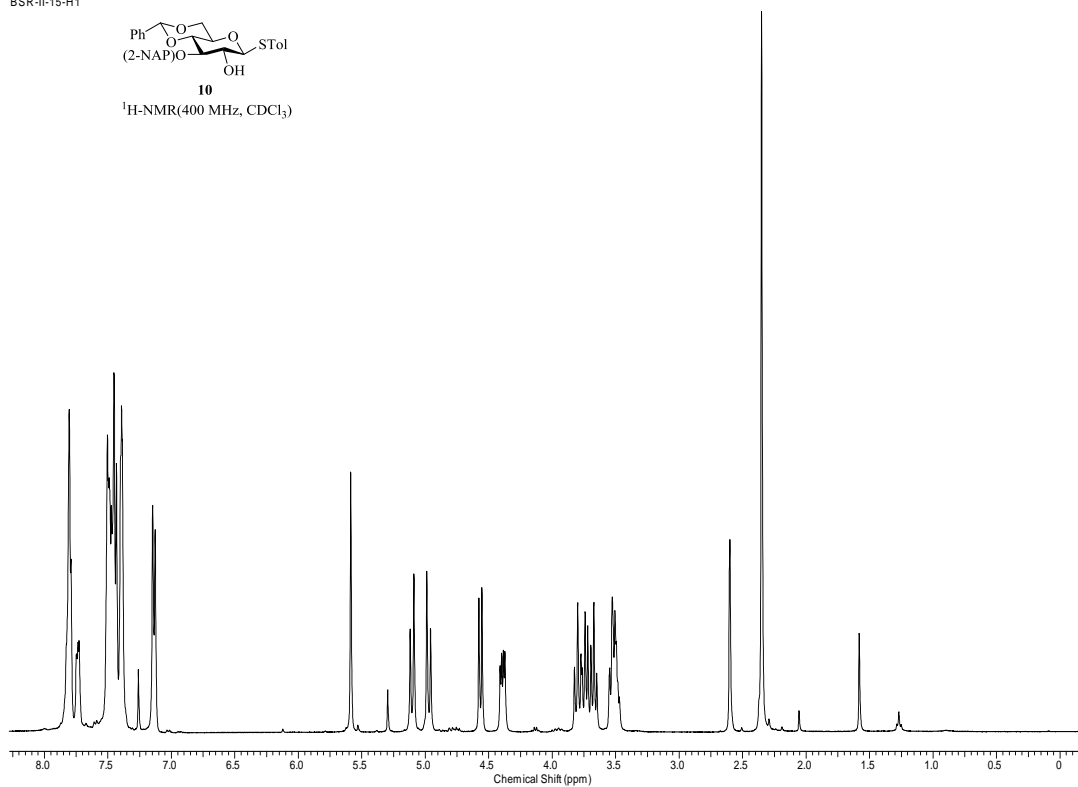


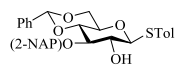
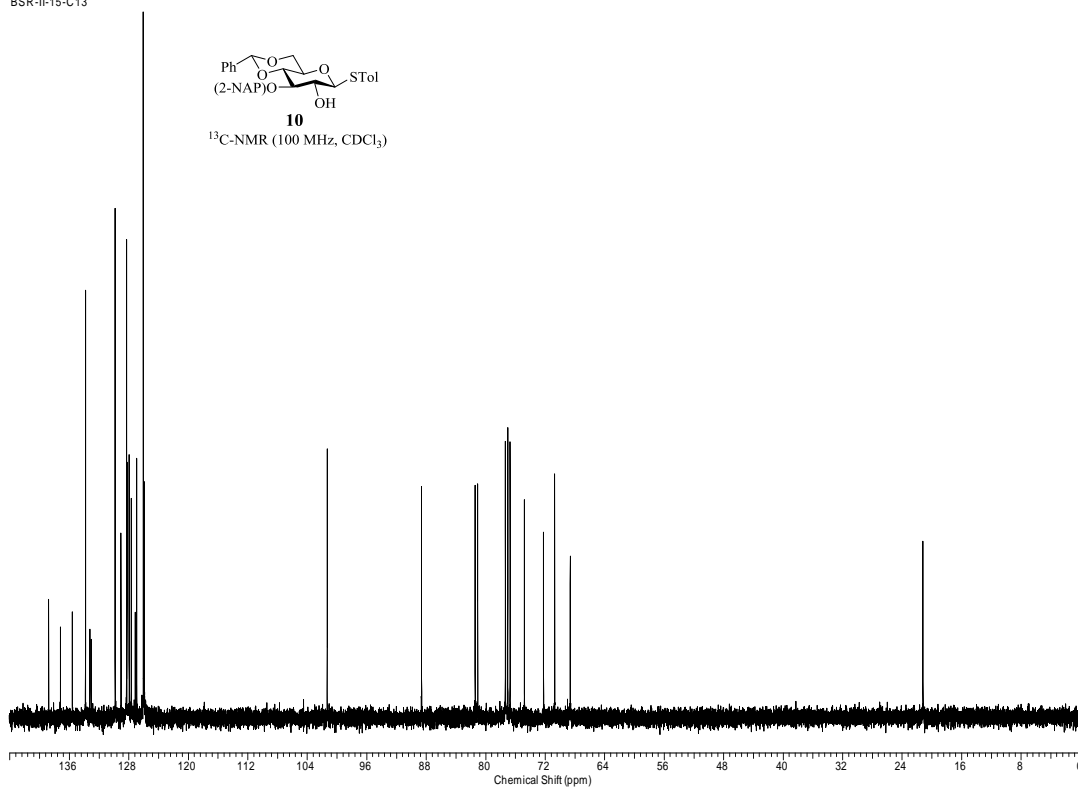
Figure S-1. Comparison of the average antibody titers of corresponding antigen-specific (A) total IgG antibodies and (B) total IgM antibodies in the day 48 pooled antisera of mice immunized with conjugates 1-4, respectively. The error bar is the standard deviation of three parallel experiments. * $P < 0.01$ as compared to 1; # $P < 0.05$ as compared to 2.

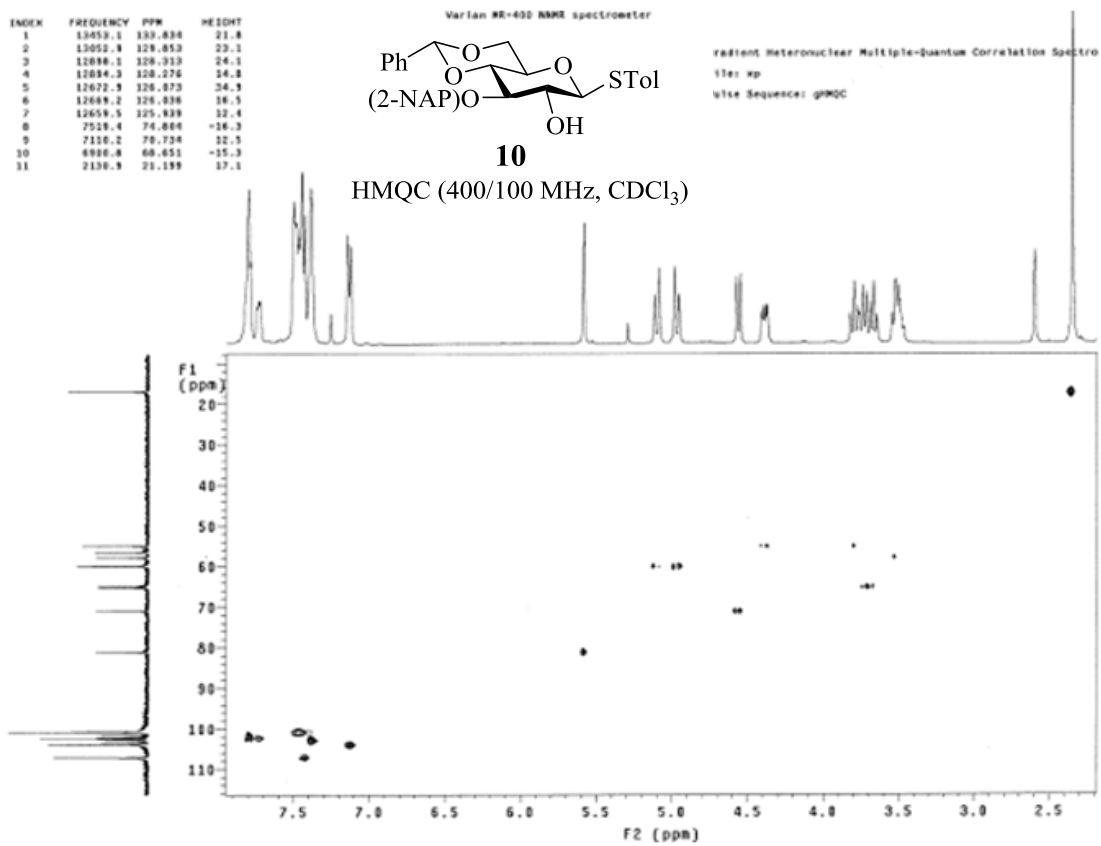
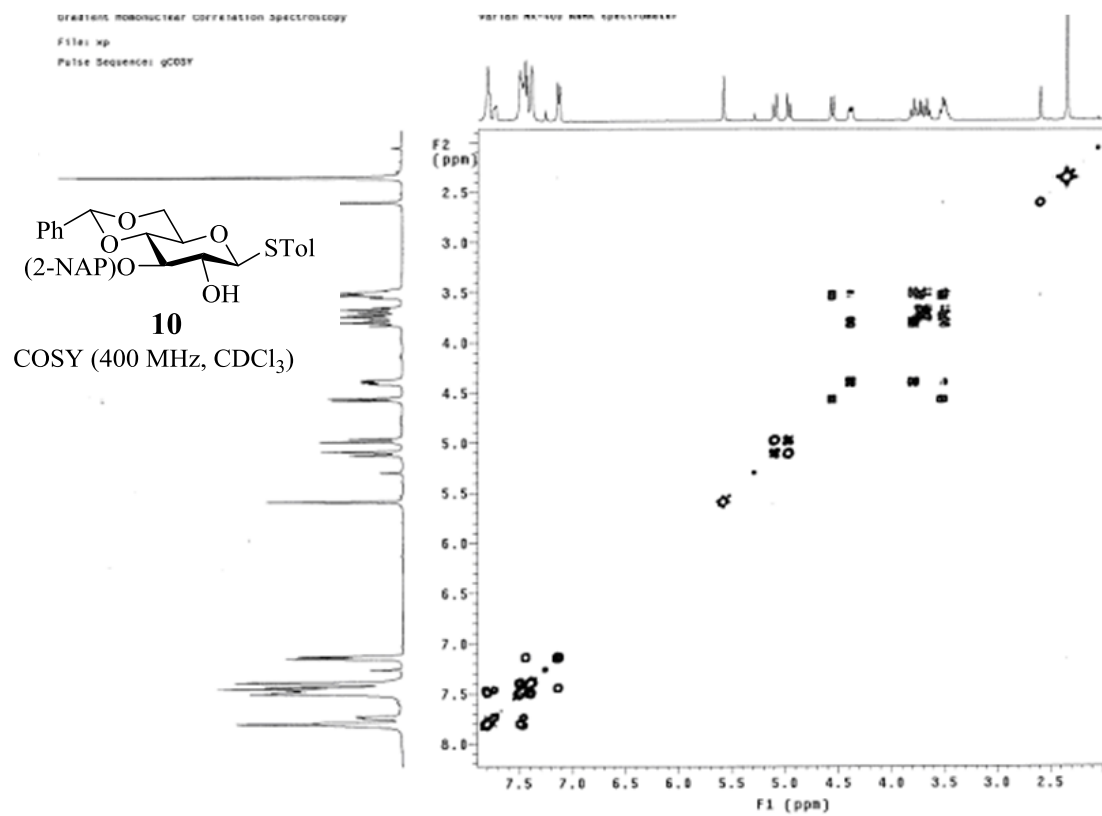
II. NMR and MS Spectra of Synthesized Compounds.

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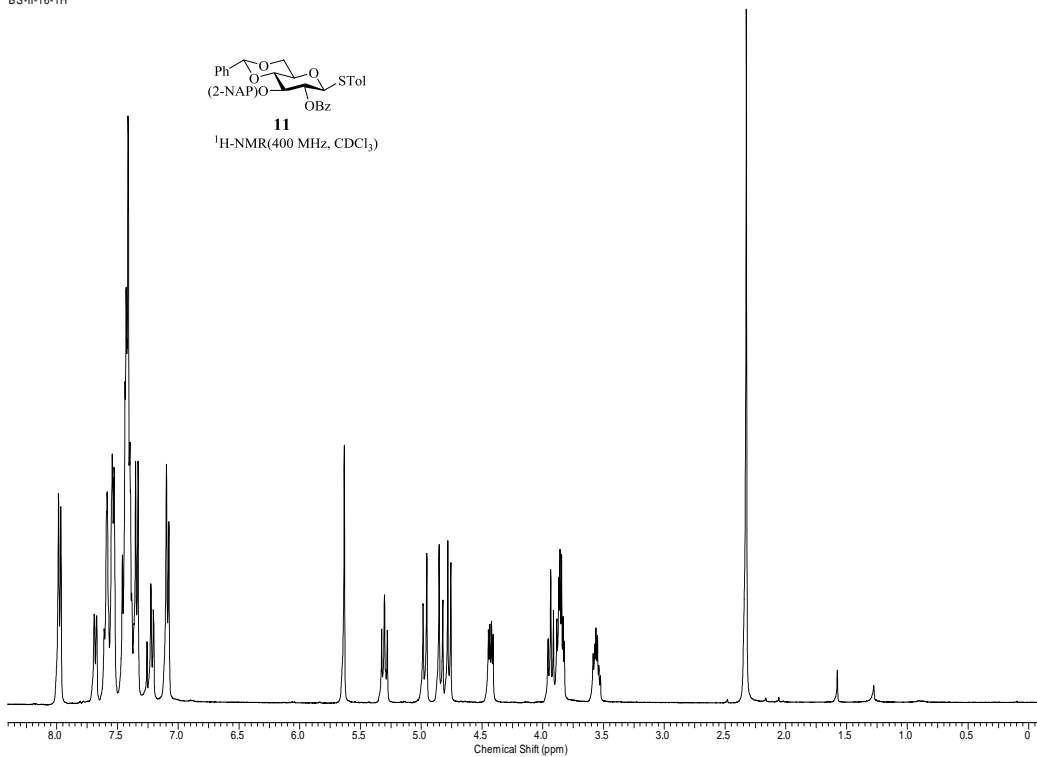
**10** $^1\text{H-NMR}$ (400 MHz, CDCl_3)

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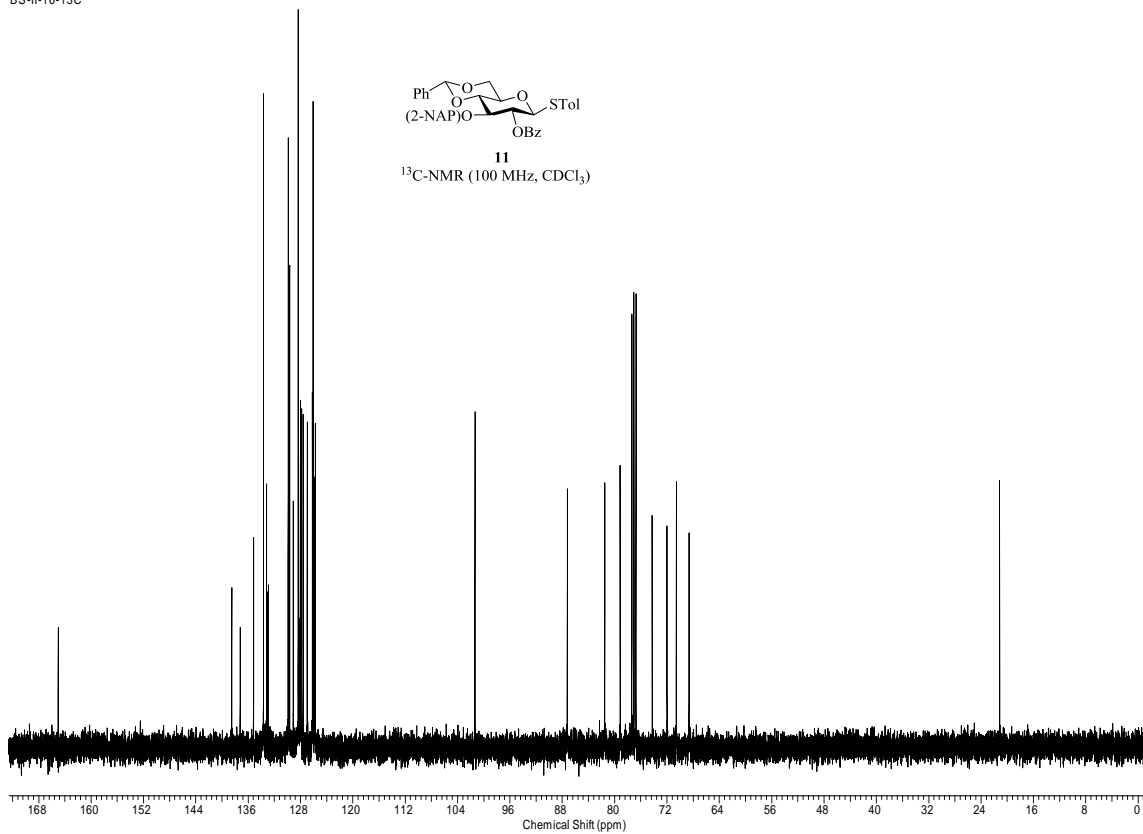
**10** $^{13}\text{C-NMR}$ (100 MHz, CDCl_3)



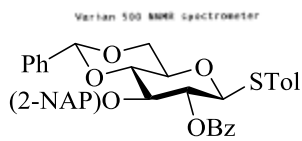
BS-II-16-1H



BS-II-16-13C

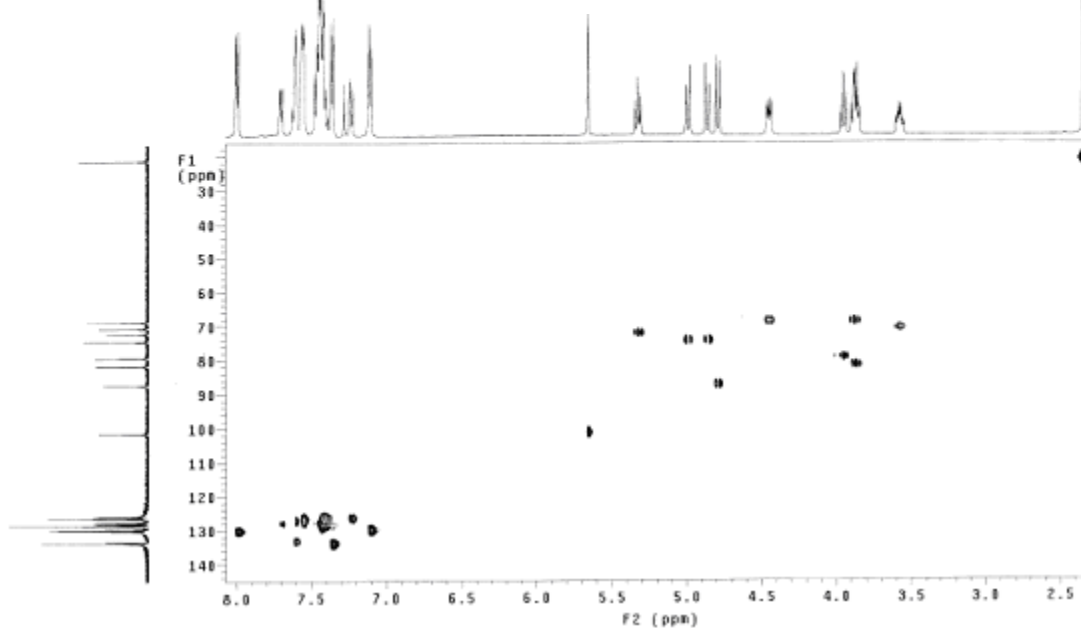


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1	16790.6	133.628	24.6
2	16317.0	128.507	20.8
3	16289.6	128.688	22.0
4	16124.6	128.374	34.7
5	16084.6	128.055	12.6
6	16029.0	127.615	12.4
7	15950.9	128.194	12.8
8	15838.8	128.100	22.0
9	8961.3	78.148	12.5
10	8330.1	74.283	-15.0
11	8422.0	68.646	-14.0
12	2861.8	21.192	13.2



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11

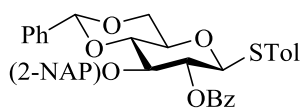
HMQC (400/100 MHz, CDCl₃)

Gradient Heteronuclear Correlation Spectroscopy

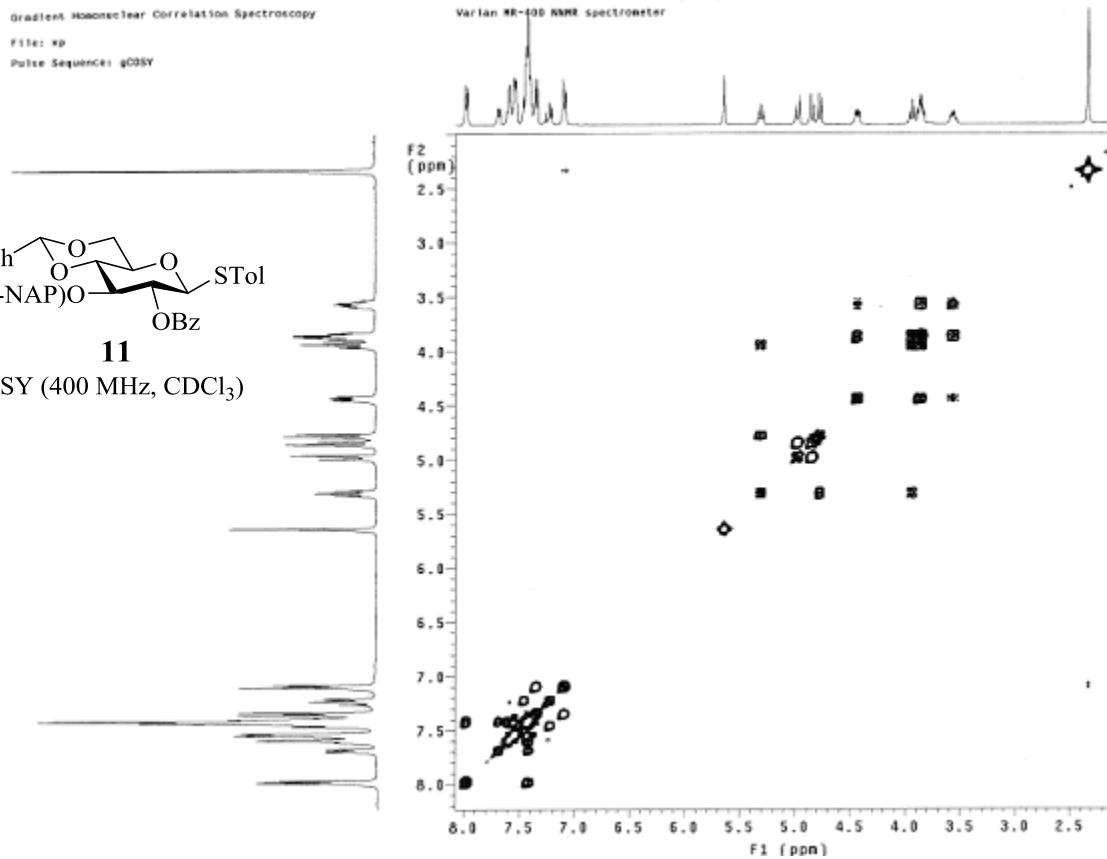
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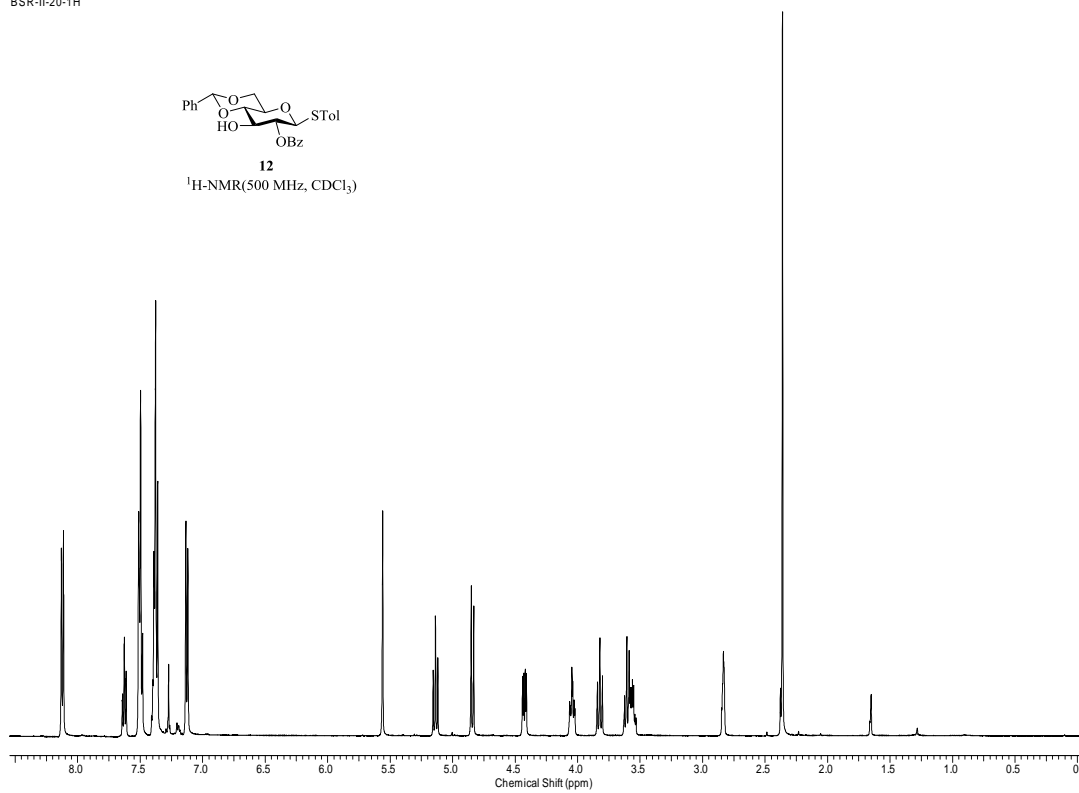
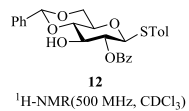
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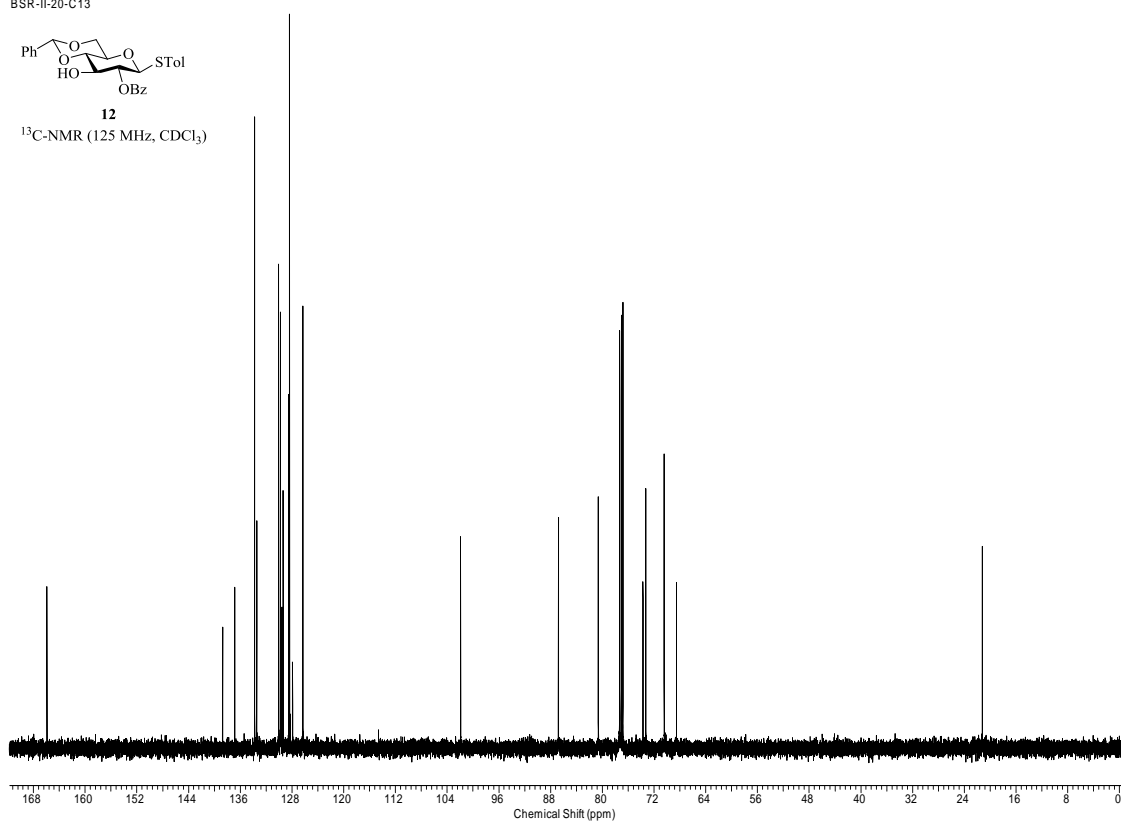
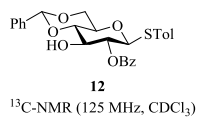
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COSY (400 MHz, CDCl₃)

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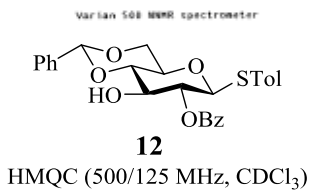


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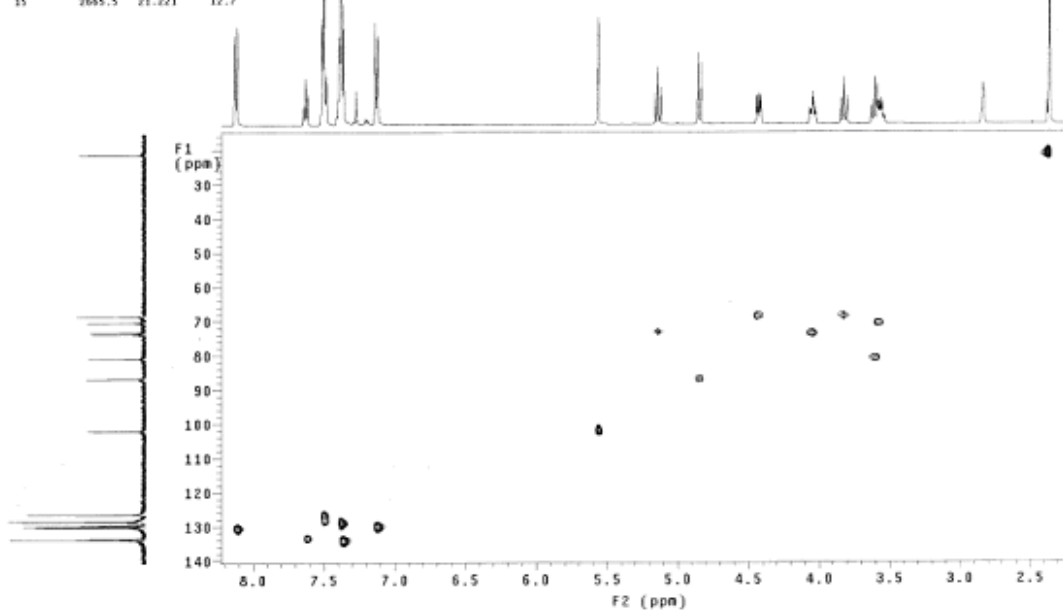


S8

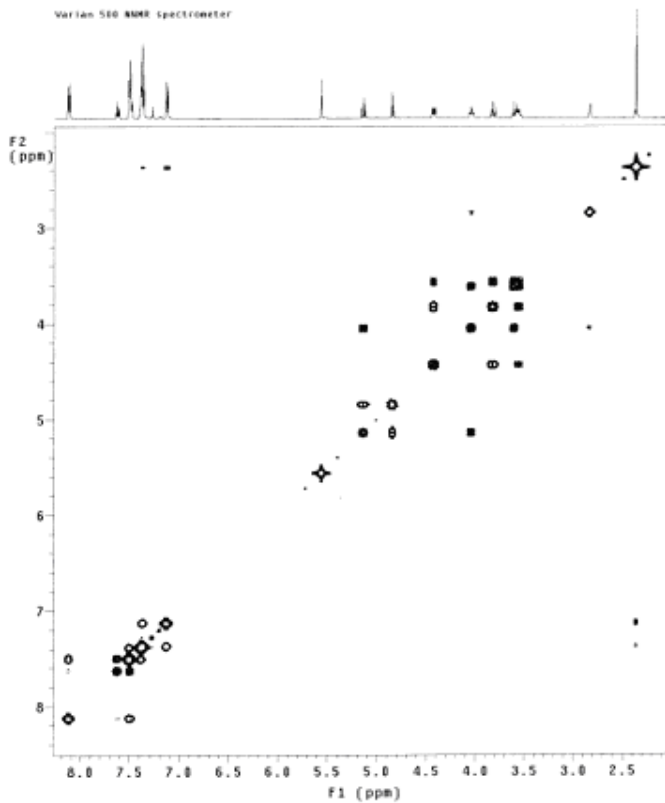
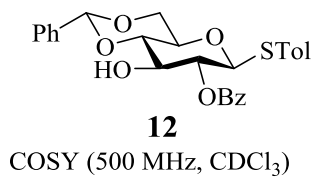
INDEX	FREQUENCY	PPM	HEIGHT
1	16084.1	133.785	33.7
2	16764.5	133.470	13.8
3	16337.3	128.074	25.8
4	16389.7	128.778	28.2
5	16249.6	128.370	15.2
6	16140.2	128.580	28.7
7	16126.7	128.392	24.6
8	15868.1	124.333	27.2
9	12882.4	101.826	19.4
10	10982.5	86.880	13.6
11	10127.4	89.828	13.1
12	9282.4	73.286	12.8
13	8845.3	78.422	13.4
14	8037.8	68.528	-15.8
15	2665.5	21.221	12.7



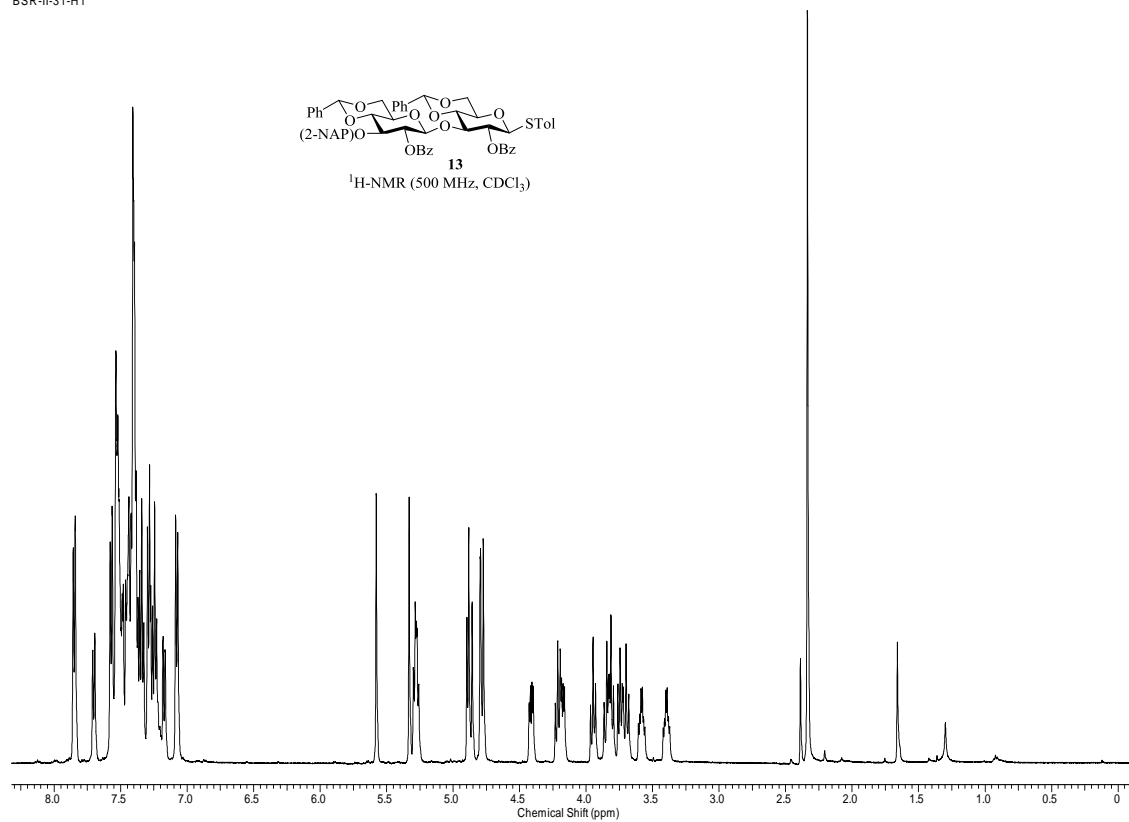
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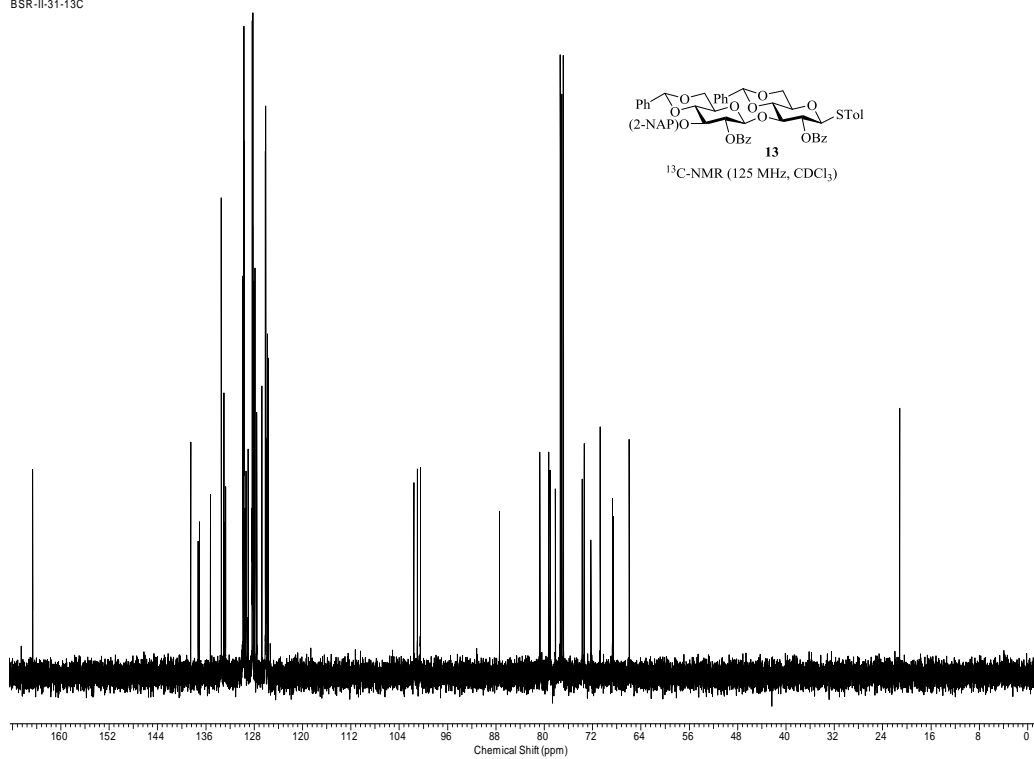
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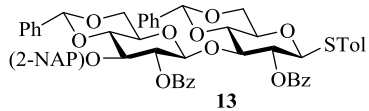


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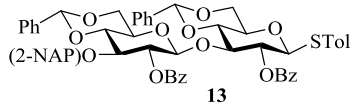
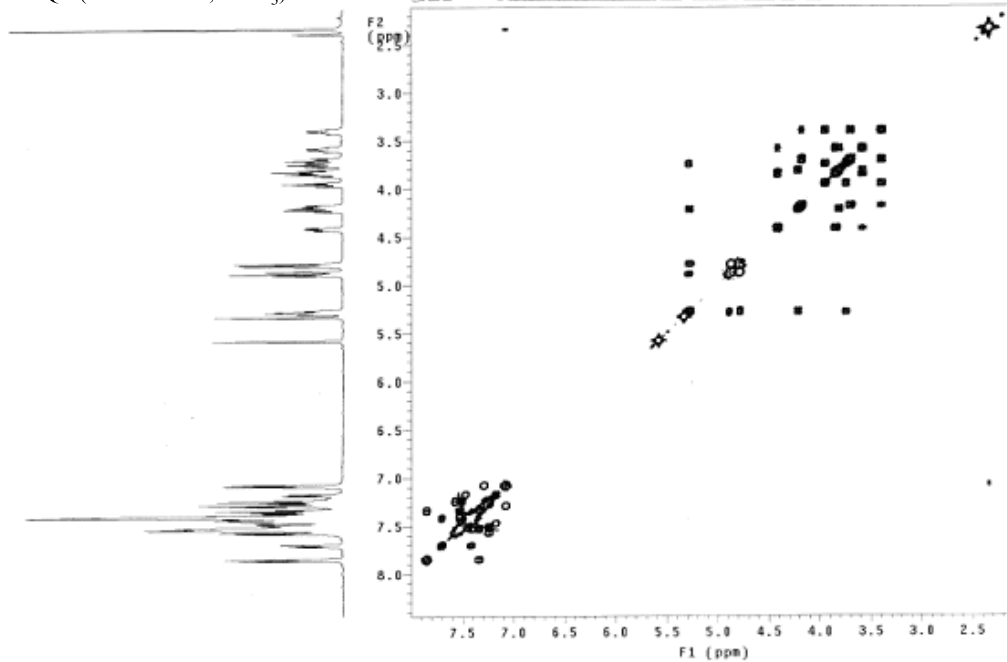


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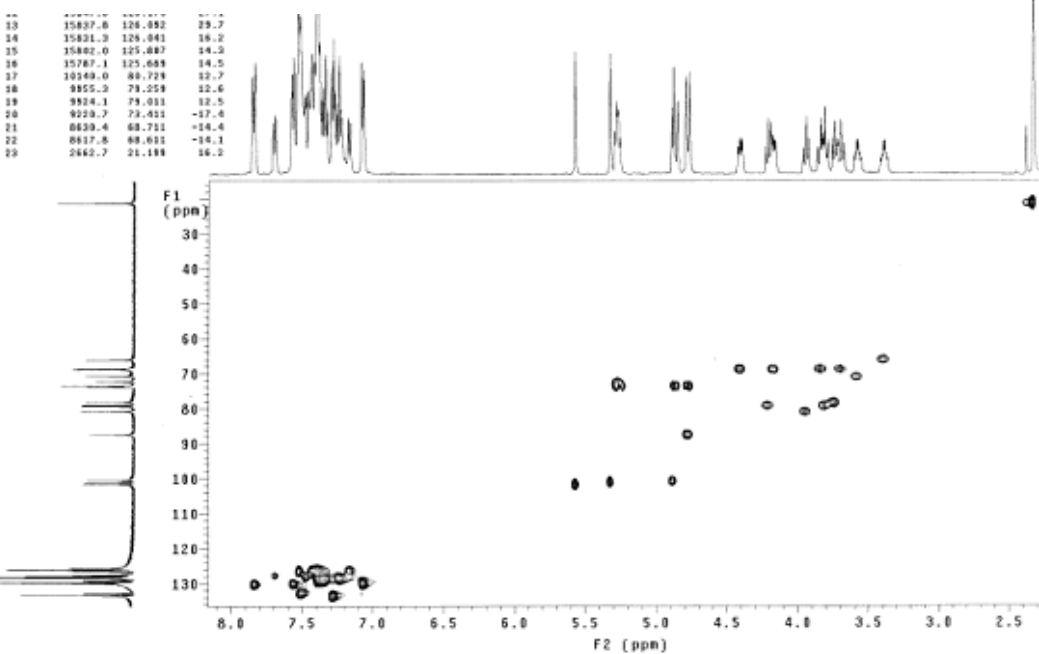




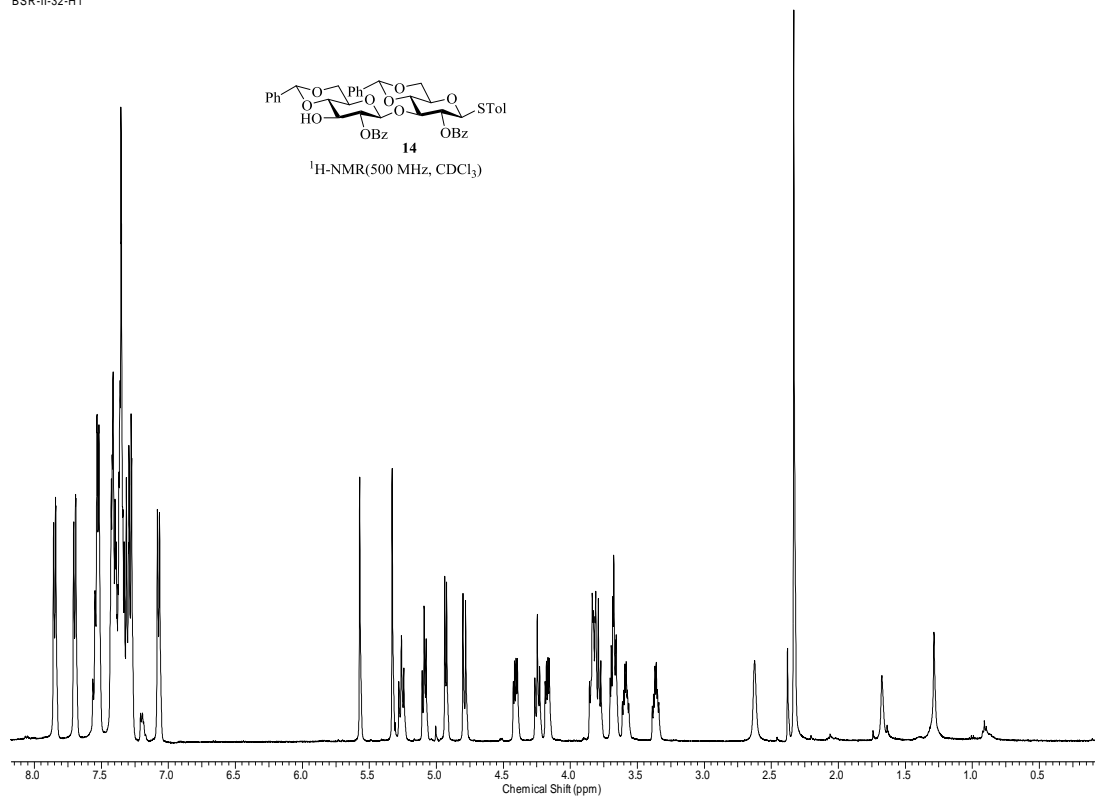
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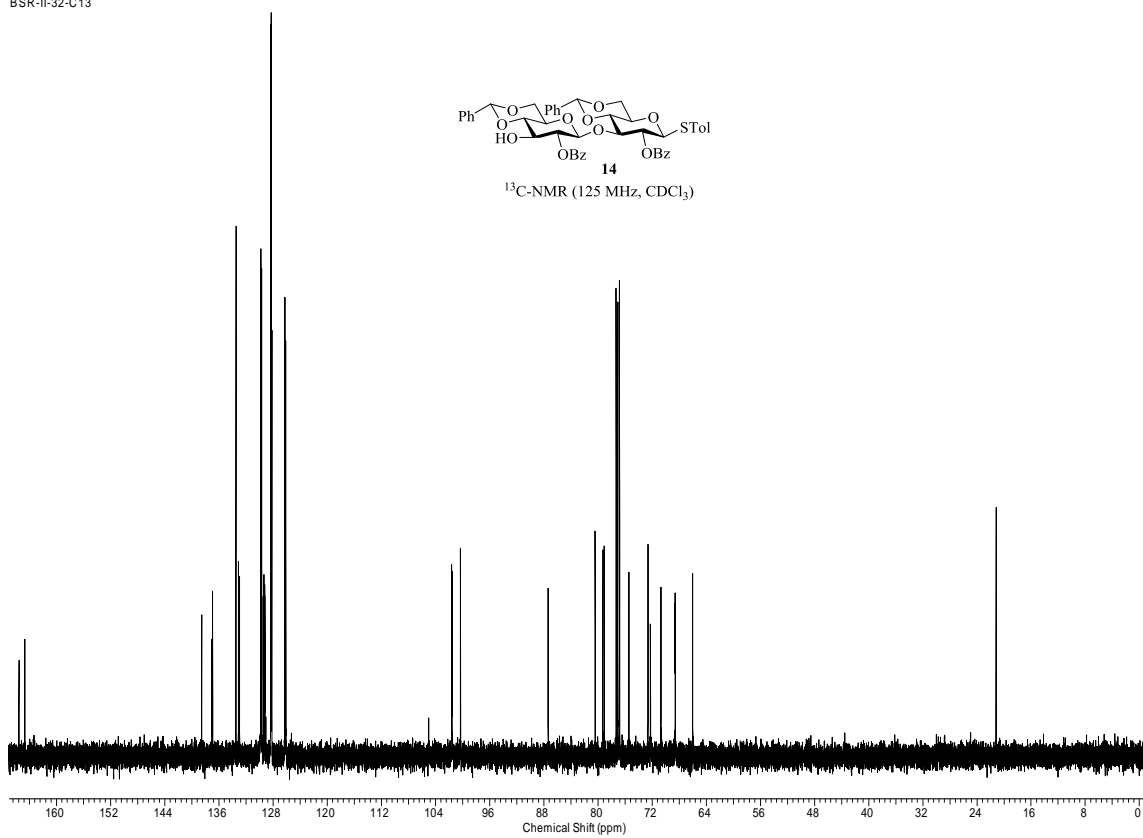
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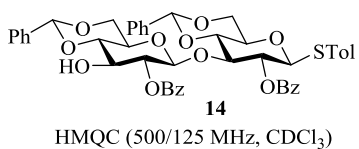
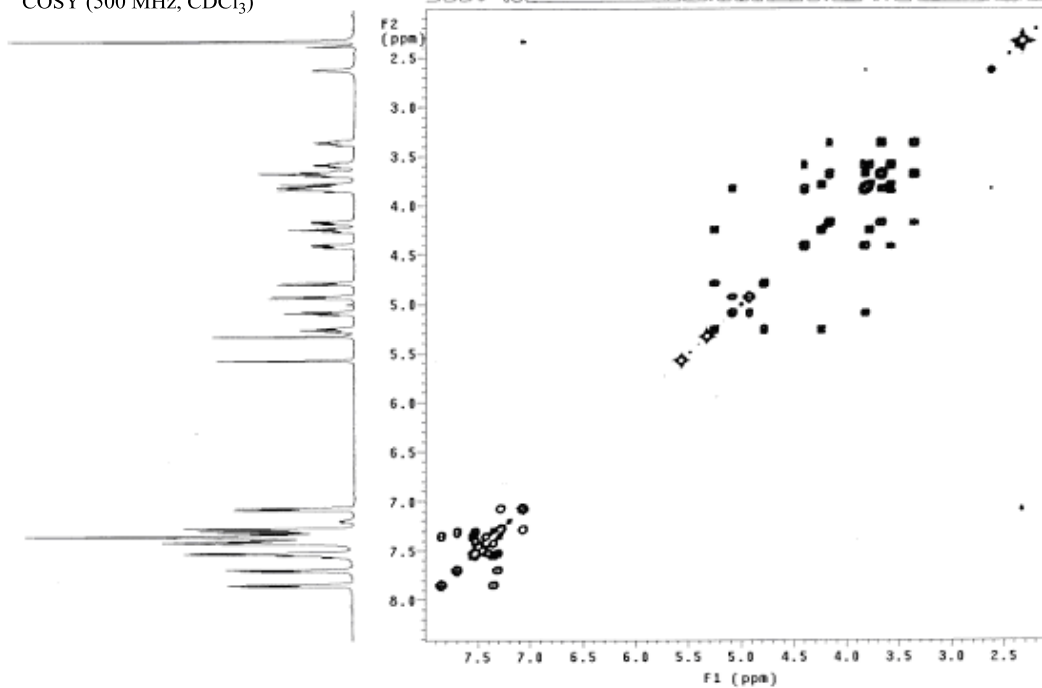
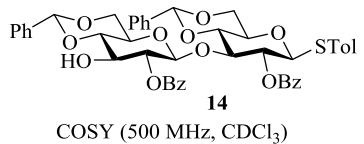


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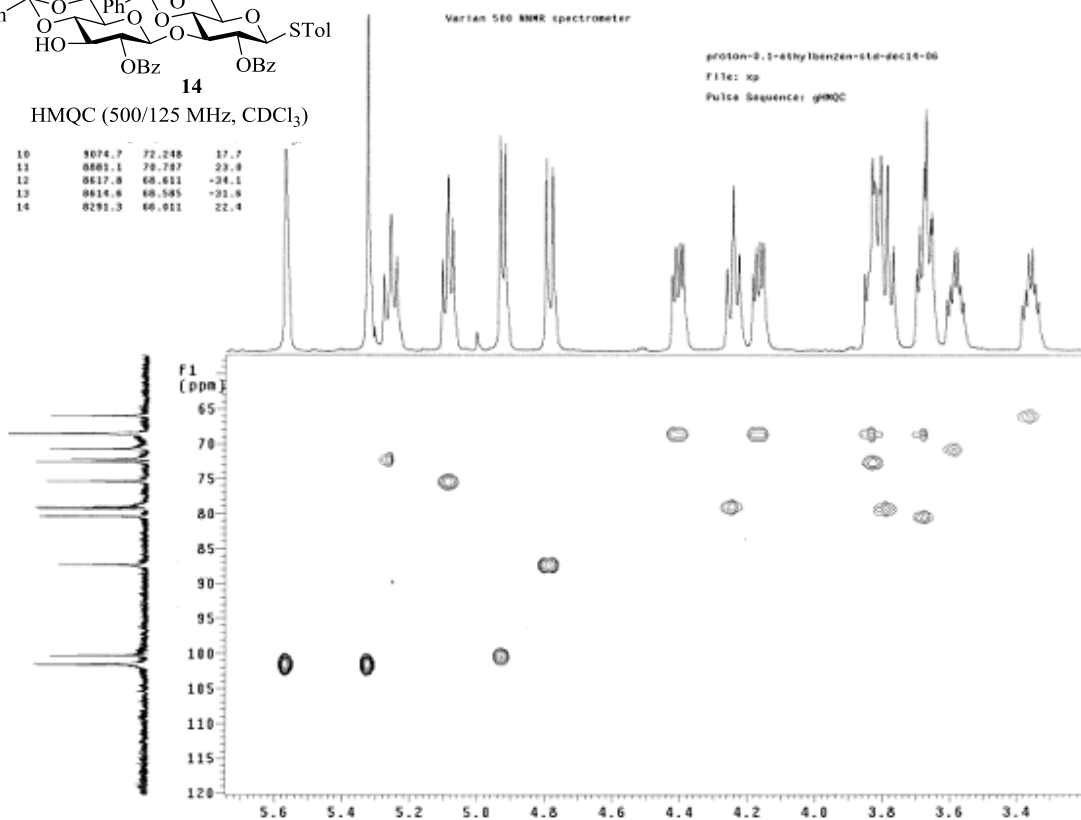


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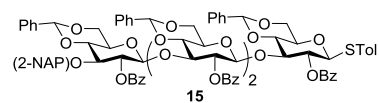
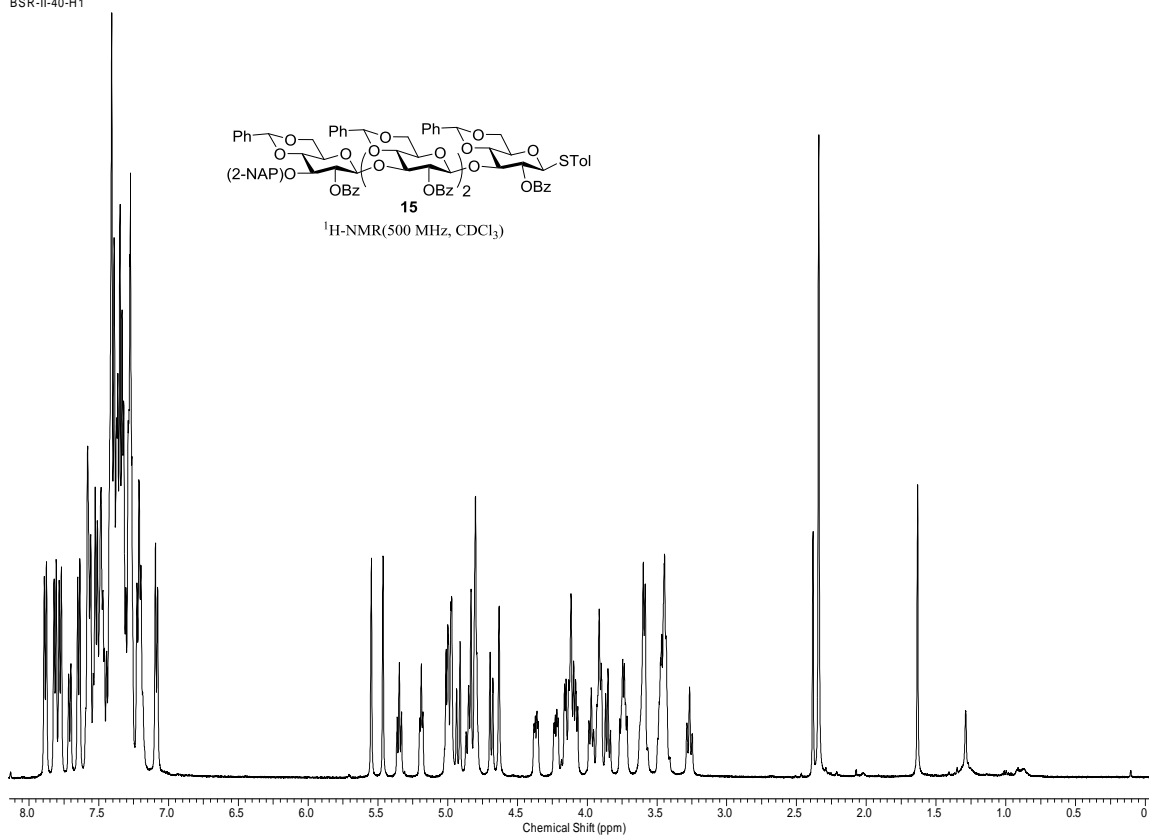




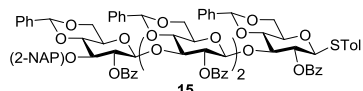
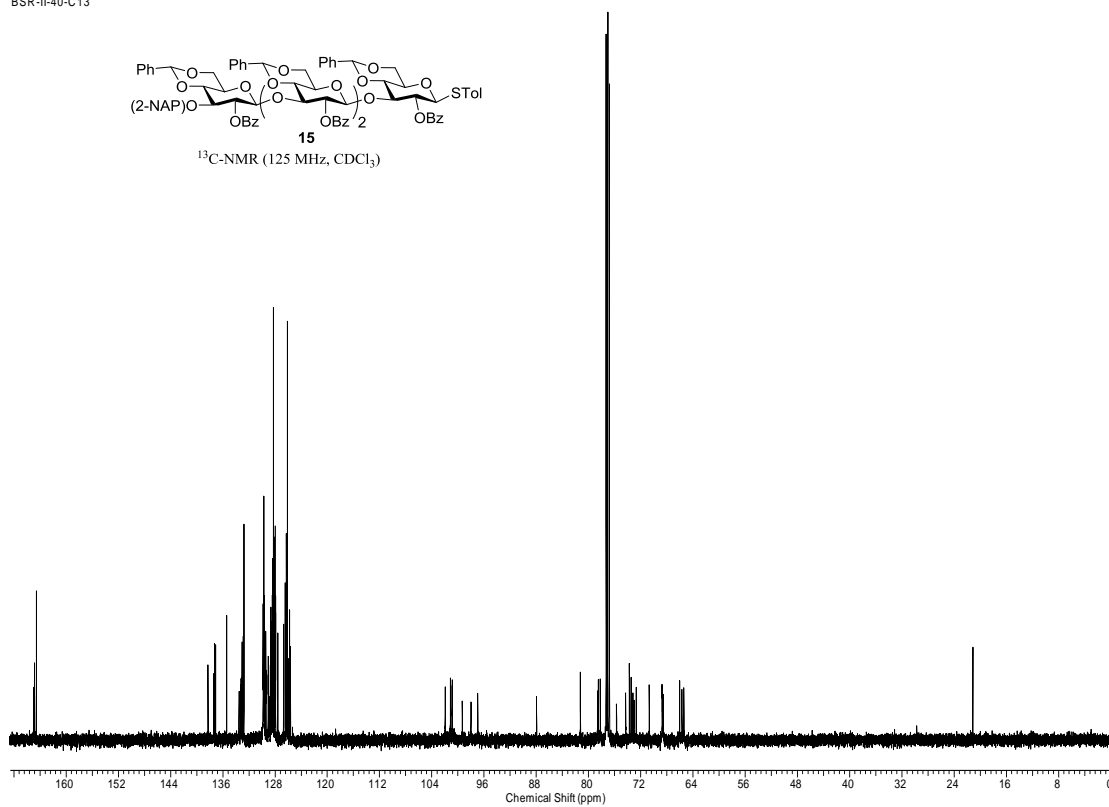
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14	8293.3	66.911	22.4

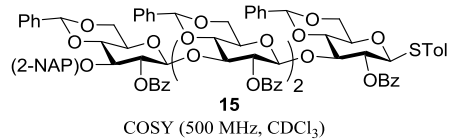
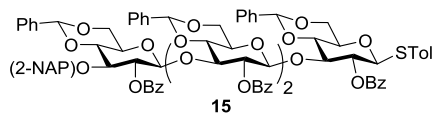
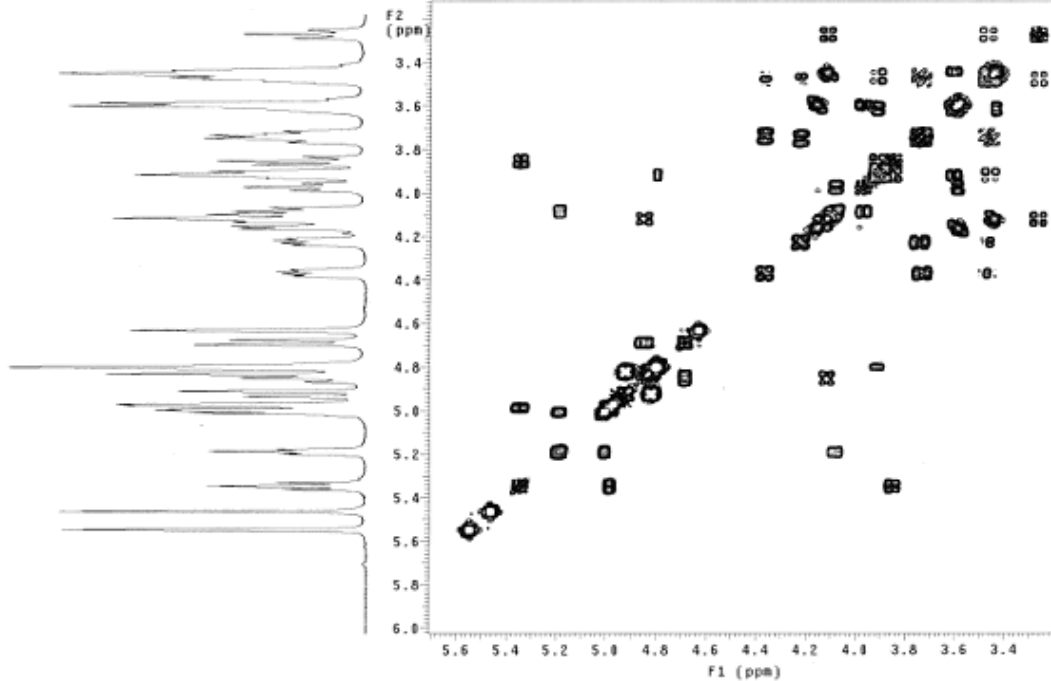
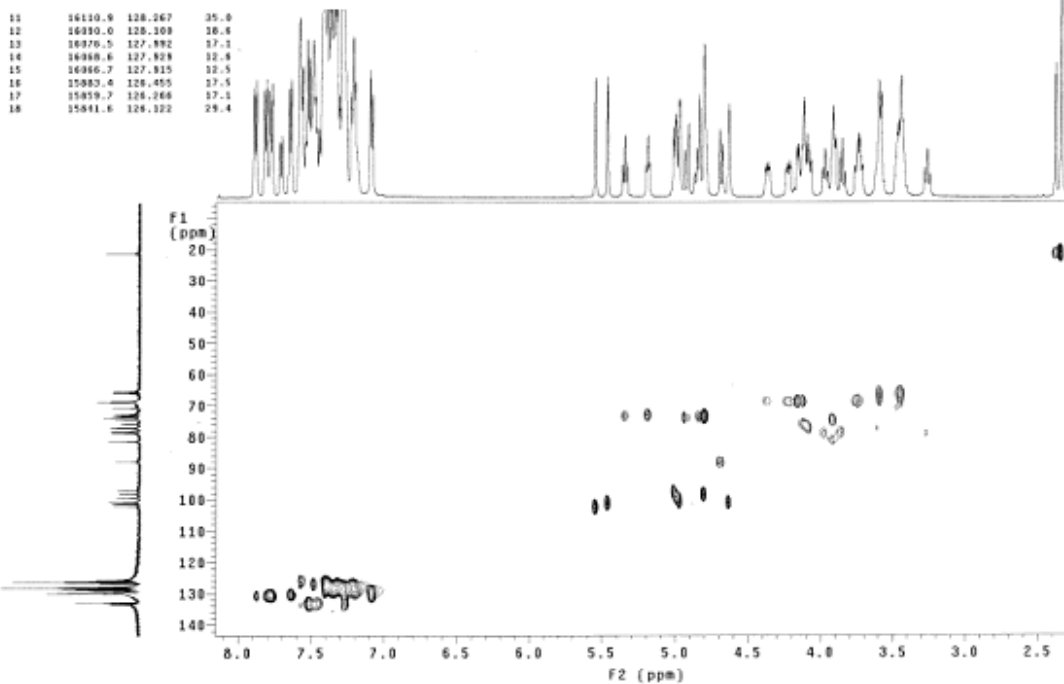


BSR-II-40-H1

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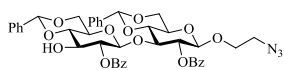
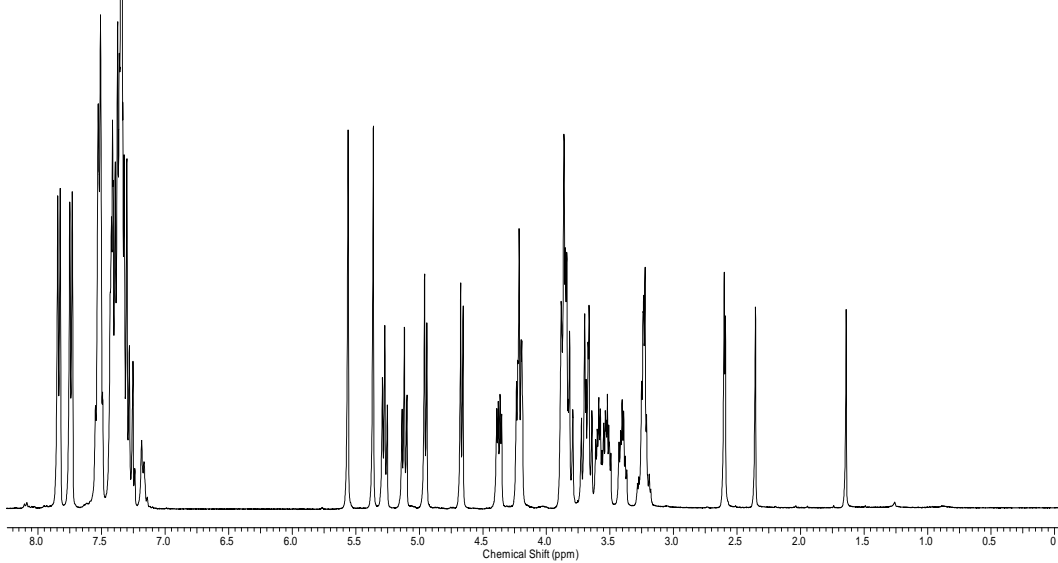
BSR-II-40-C13

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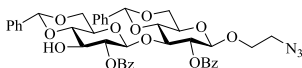
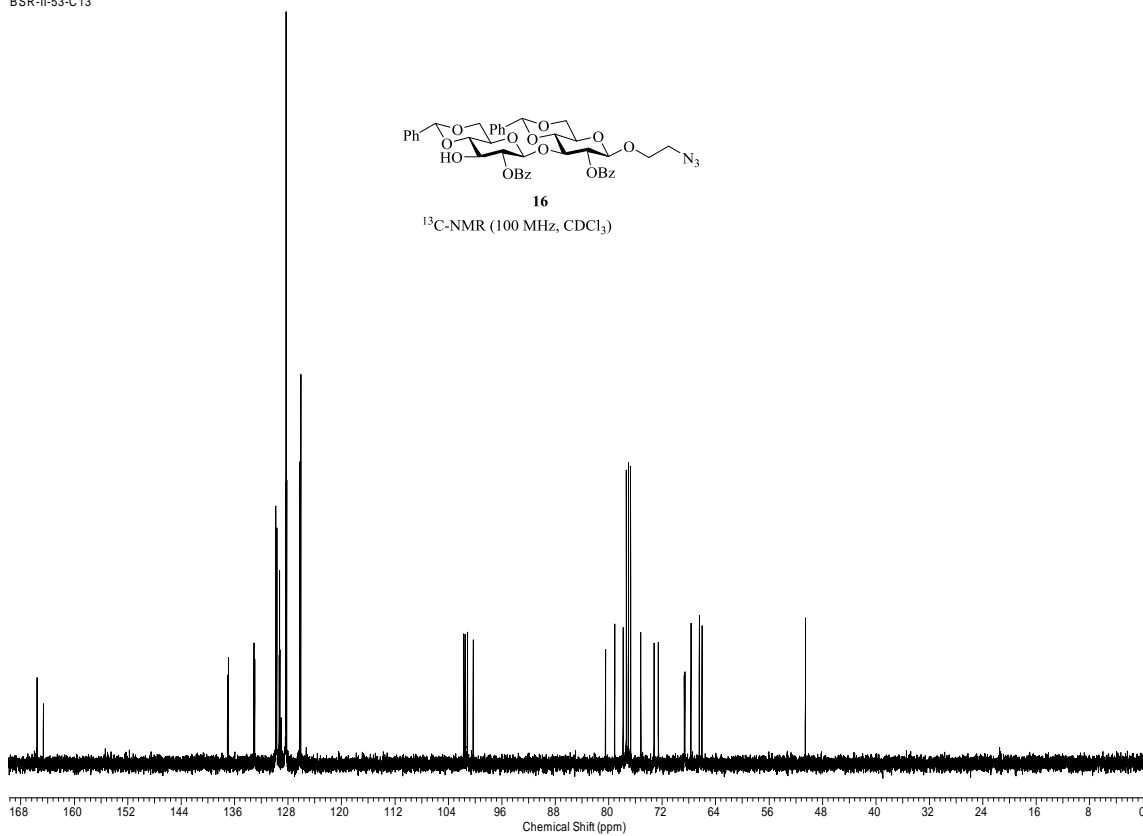
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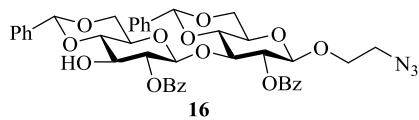
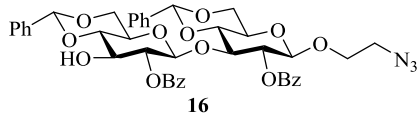
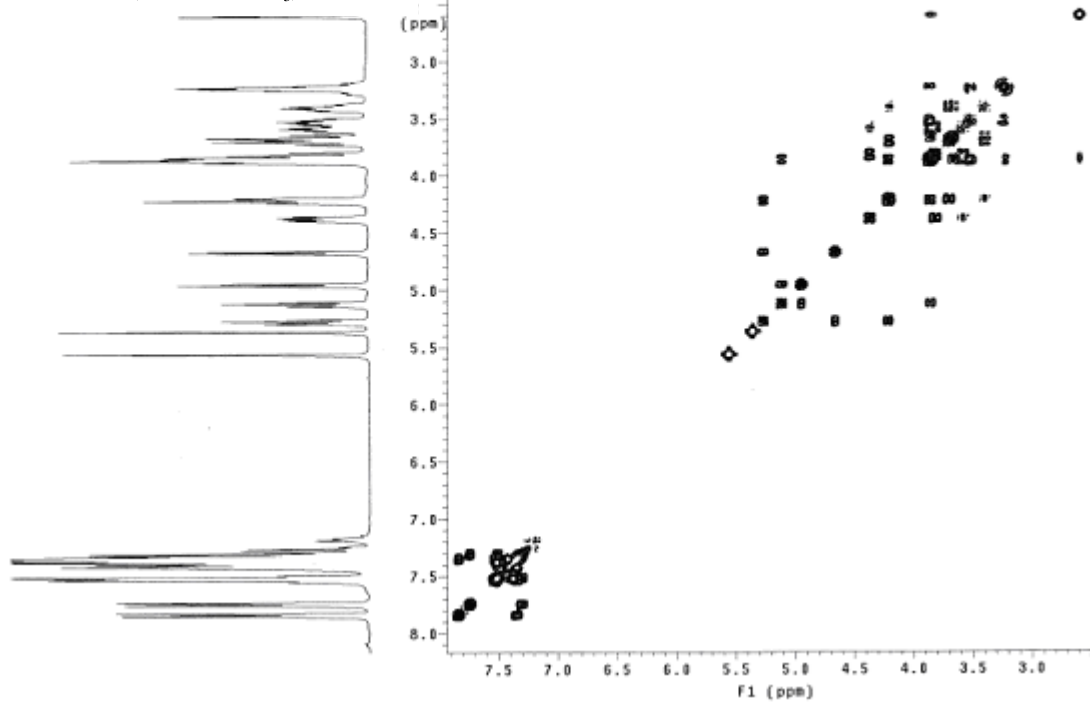
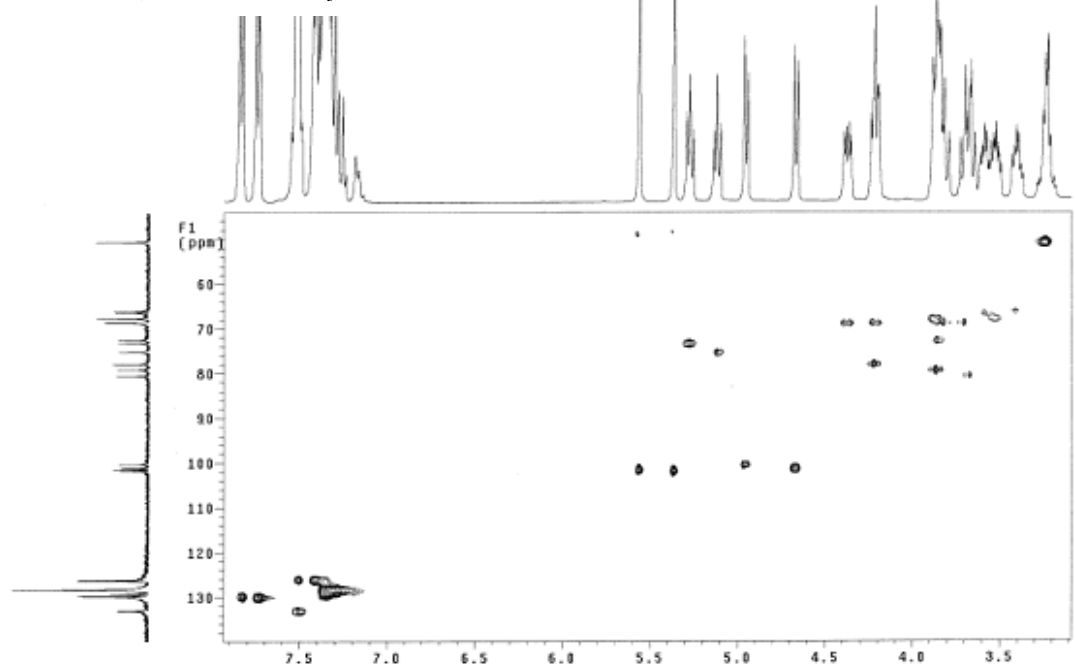
S15

BSR-II-53-H1

**16** $^1\text{H-NMR}$ (400 MHz, CDCl_3)

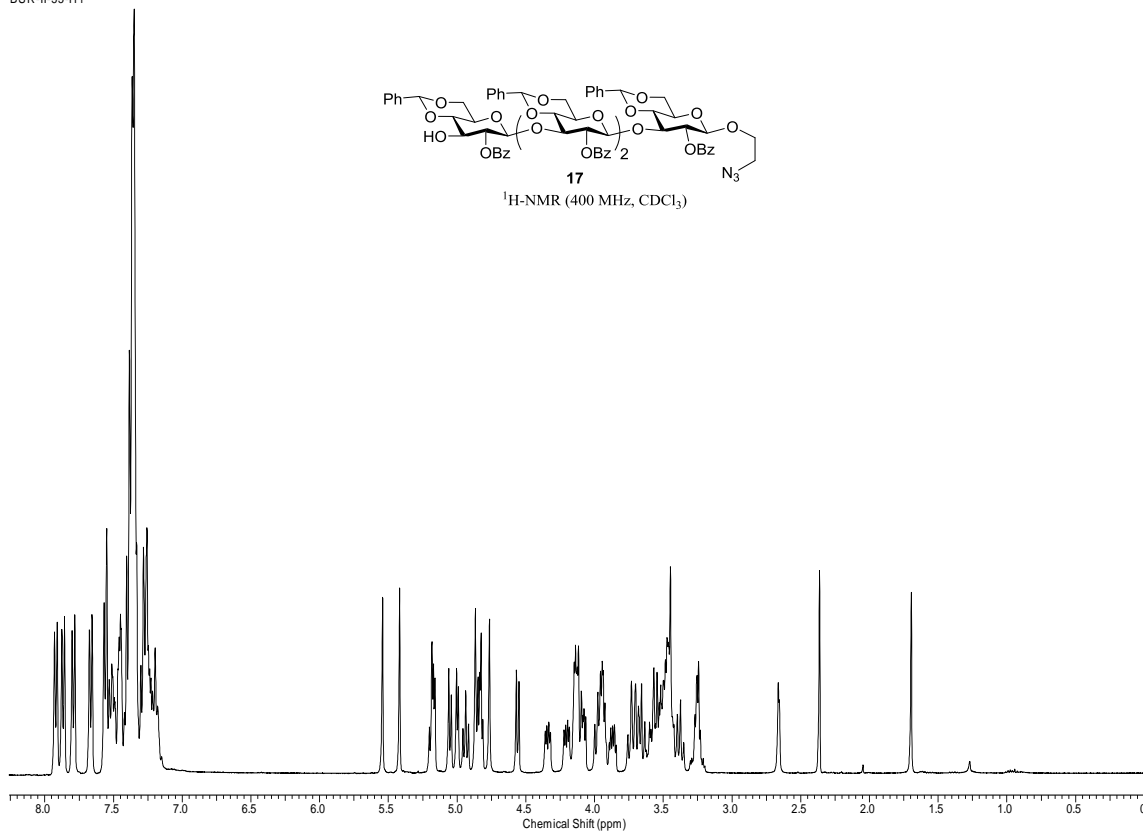
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**16** $^{13}\text{C-NMR}$ (100 MHz, CDCl_3)

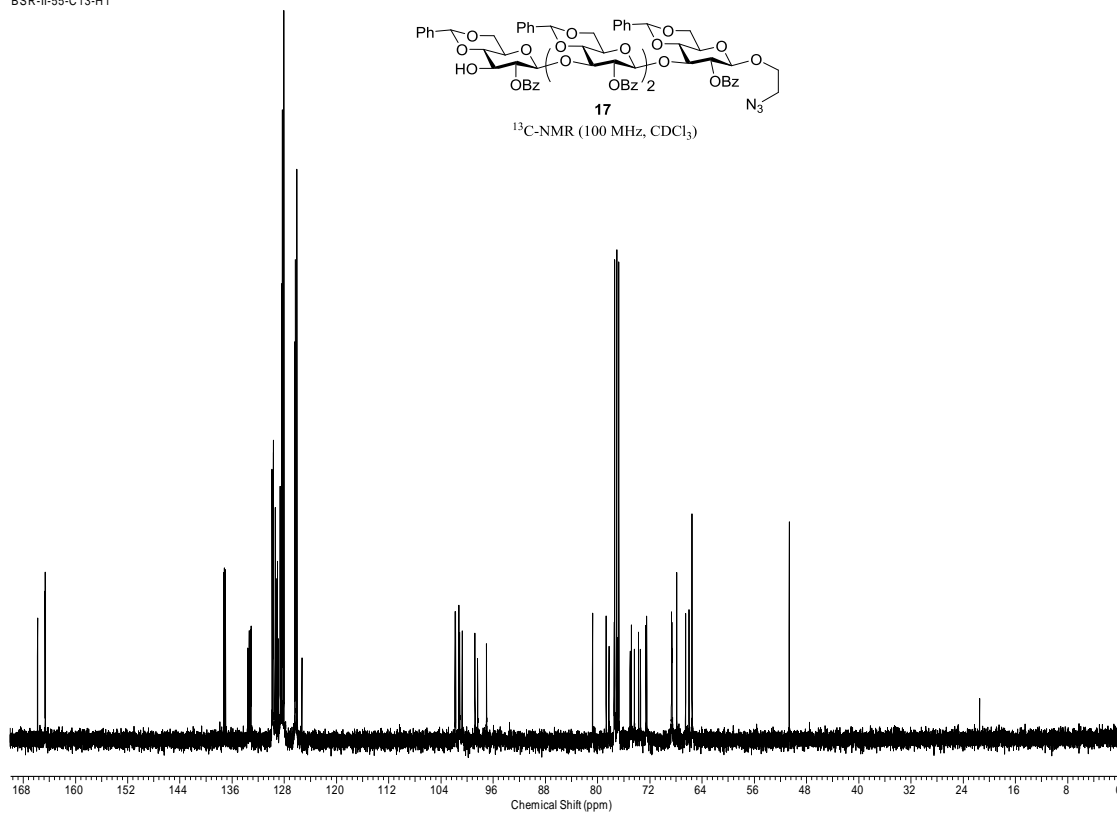
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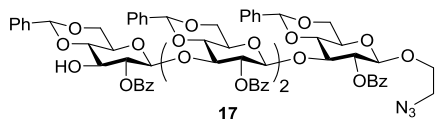
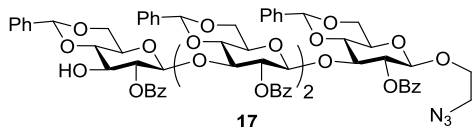
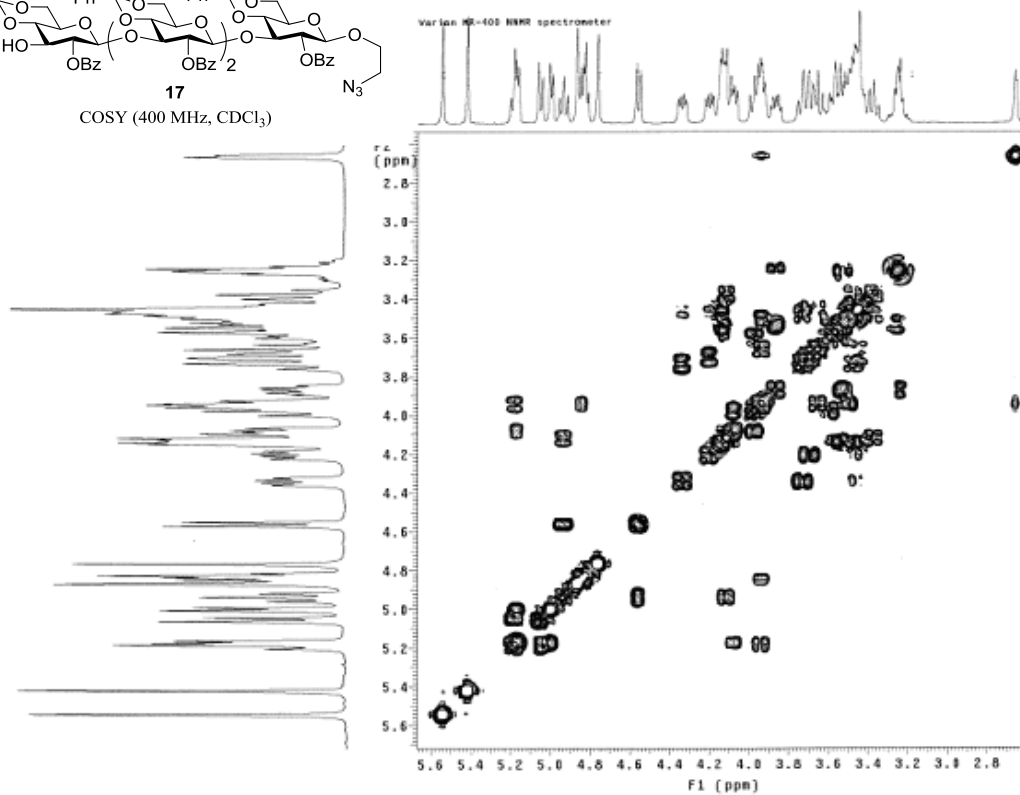
S17

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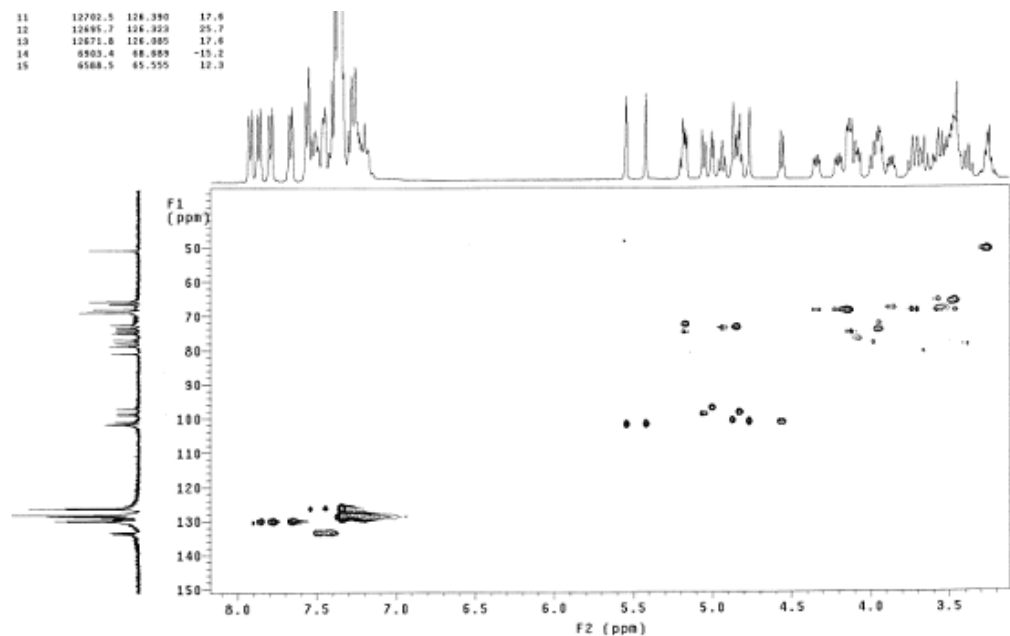


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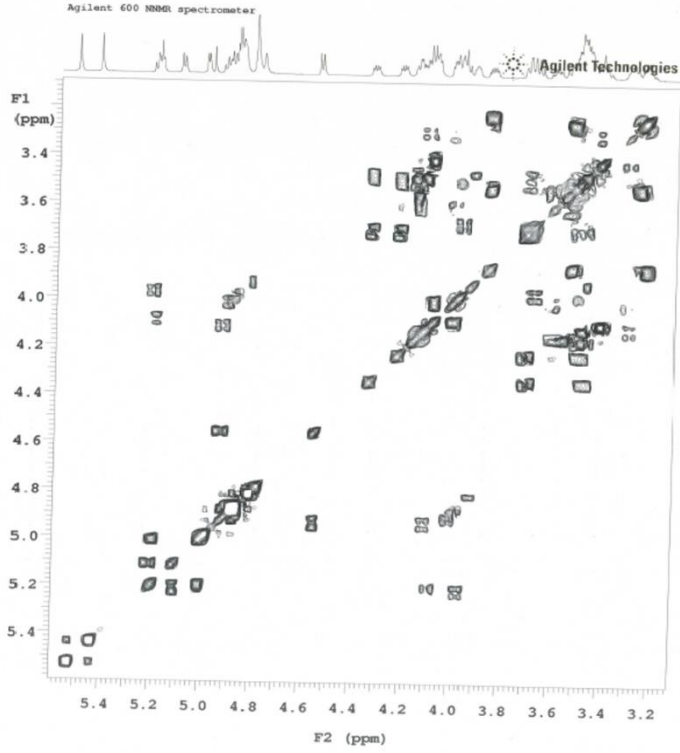


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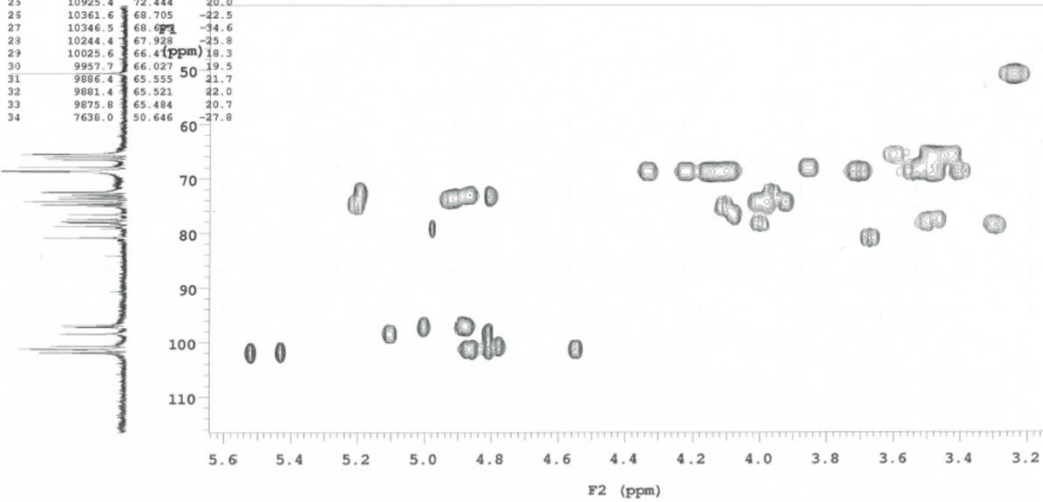
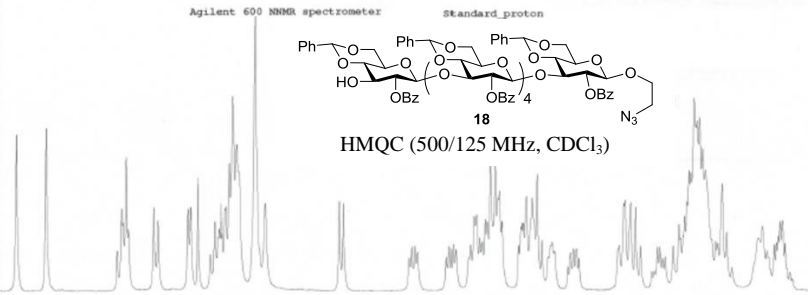
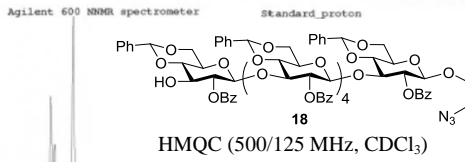
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15	6588.5	65.555	12.3



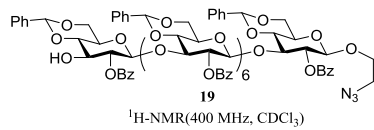
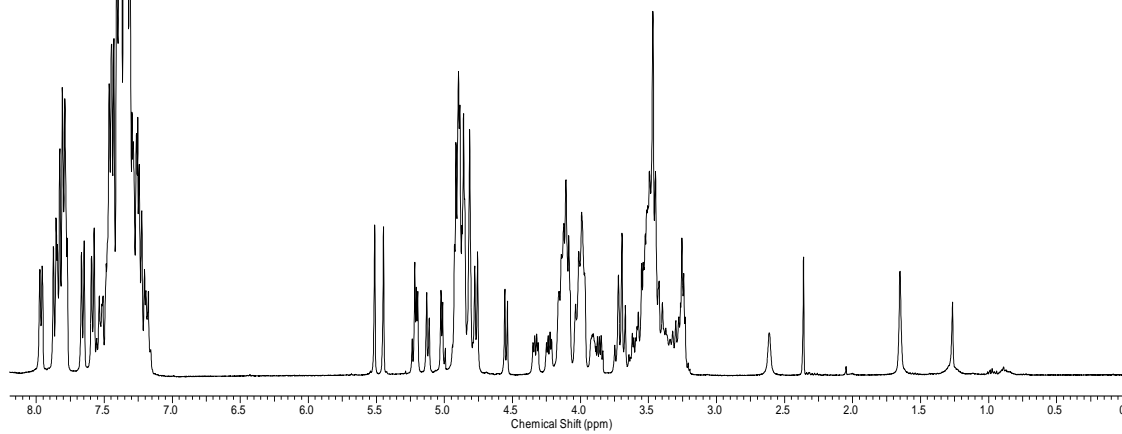
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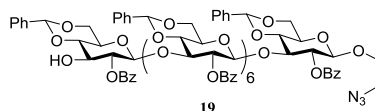
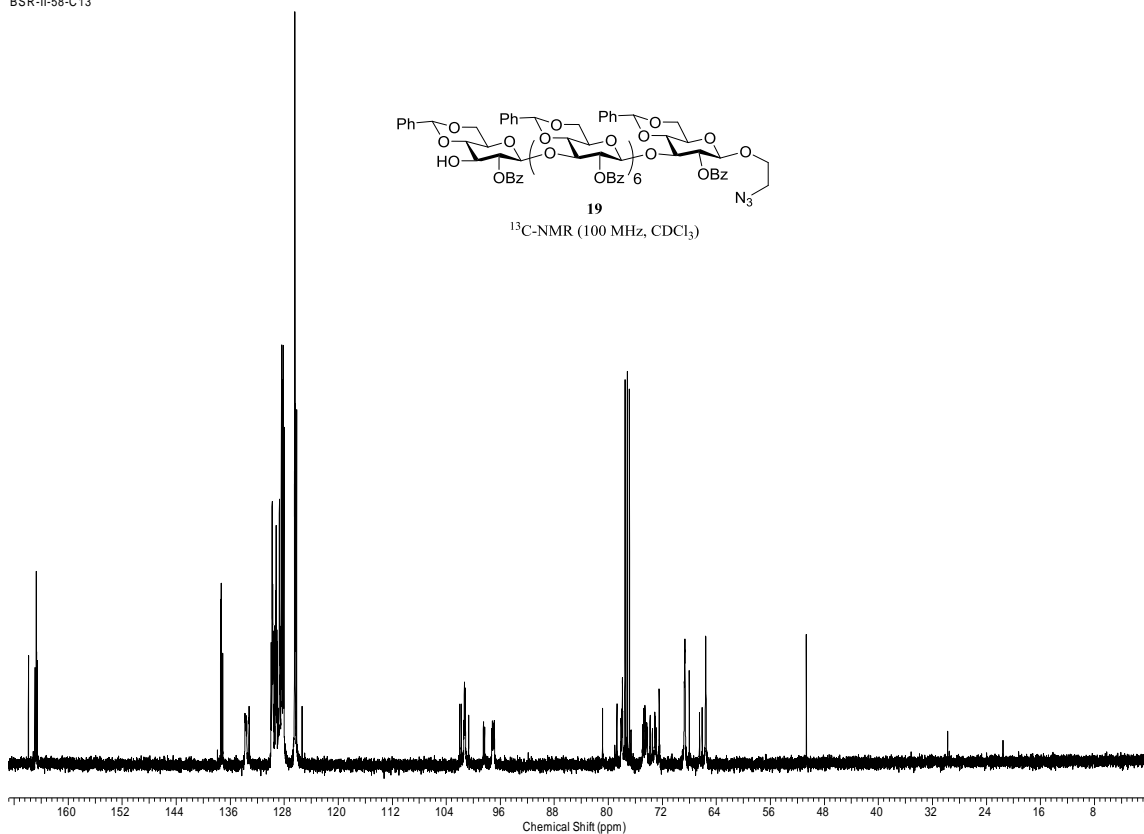
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5	15264.6	101.216	22.3
6	15181.6	100.666	18.3
7	14845.6	98.438	14.5
8	14639.9	98.400	14.3
9	14644.2	97.102	15.1
10	14607.1	96.857	15.5
11	12191.0	80.836	19.7
12	11870.6	78.712	16.4
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15	11745.5	77.882	15.8
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17	11554.8	76.617	13.8
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19	11196.3	74.240	24.5
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24	10938.3	72.529	20.2
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26	10361.6	68.705	-22.5
27	10346.5	68.671	-34.6
28	10244.4	67.925	-25.8
29	10025.6	66.411	18.3
30	9957.7	66.027	19.5
31	9886.4	65.555	21.7
32	9861.4	65.521	22.0
33	9875.8	65.484	20.7
34	7638.0	50.646	-27.8

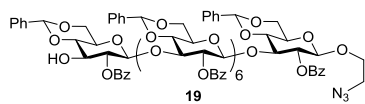
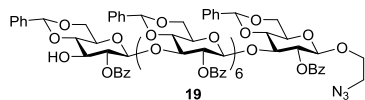
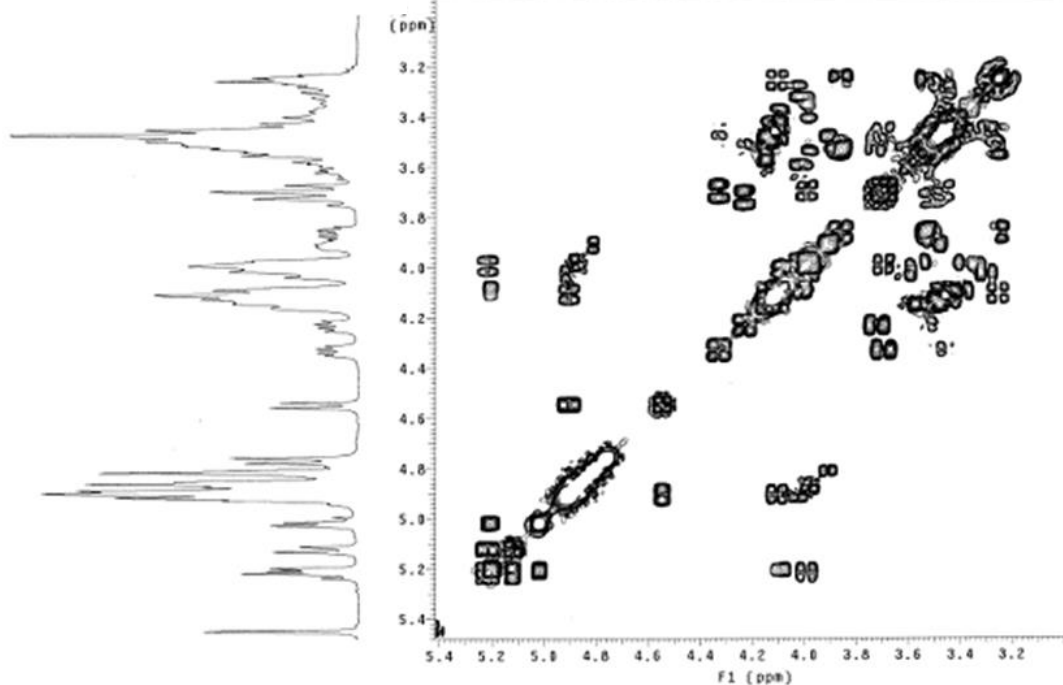
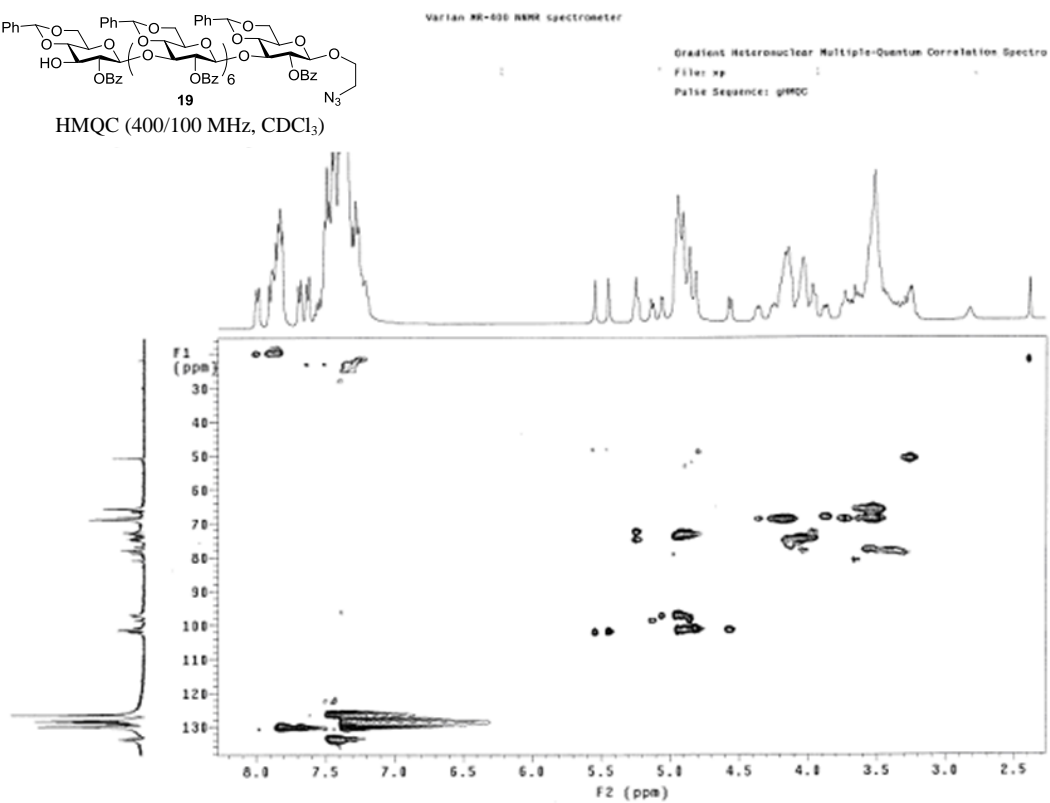


BSR-II-58-1H3

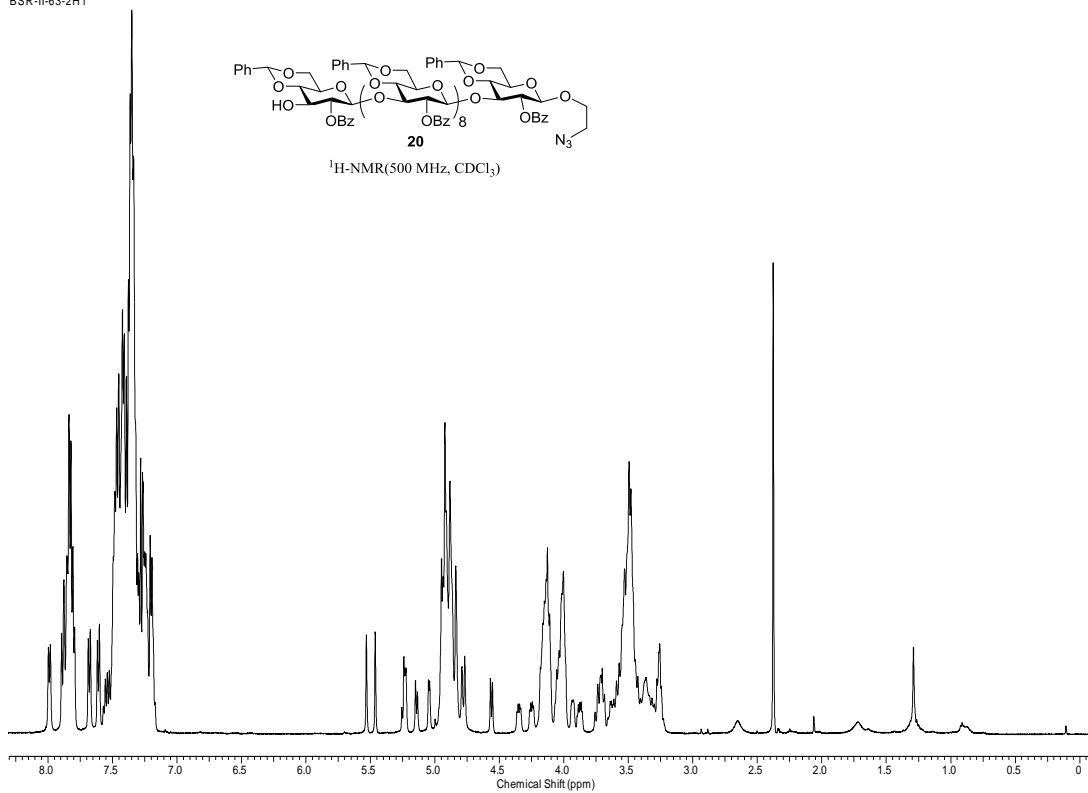
¹H-NMR(400 MHz, CDCl₃)

BSR-II-58-C13

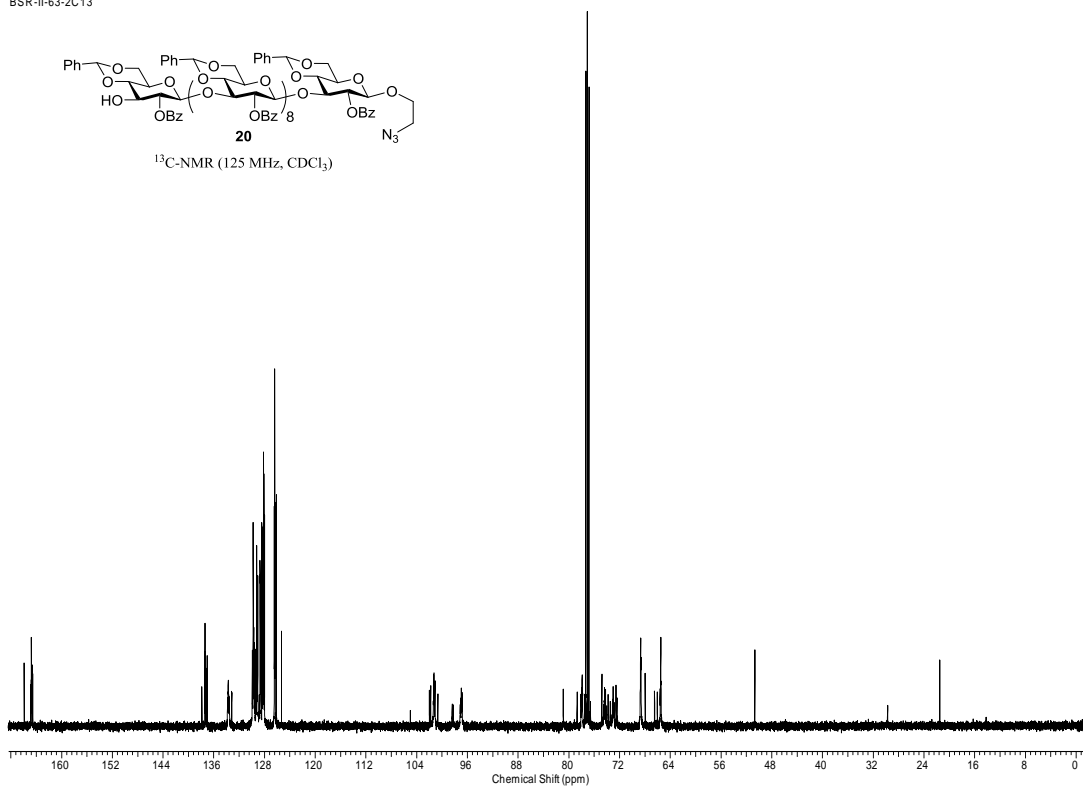
¹³C-NMR (100 MHz, CDCl₃)

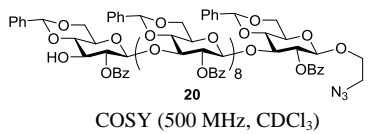
COSY (400 MHz, CDCl₃)HMQC (400/100 MHz, CDCl₃)

BSR-II-63-2H1

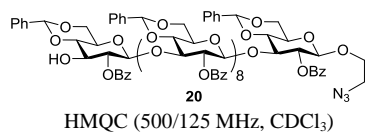
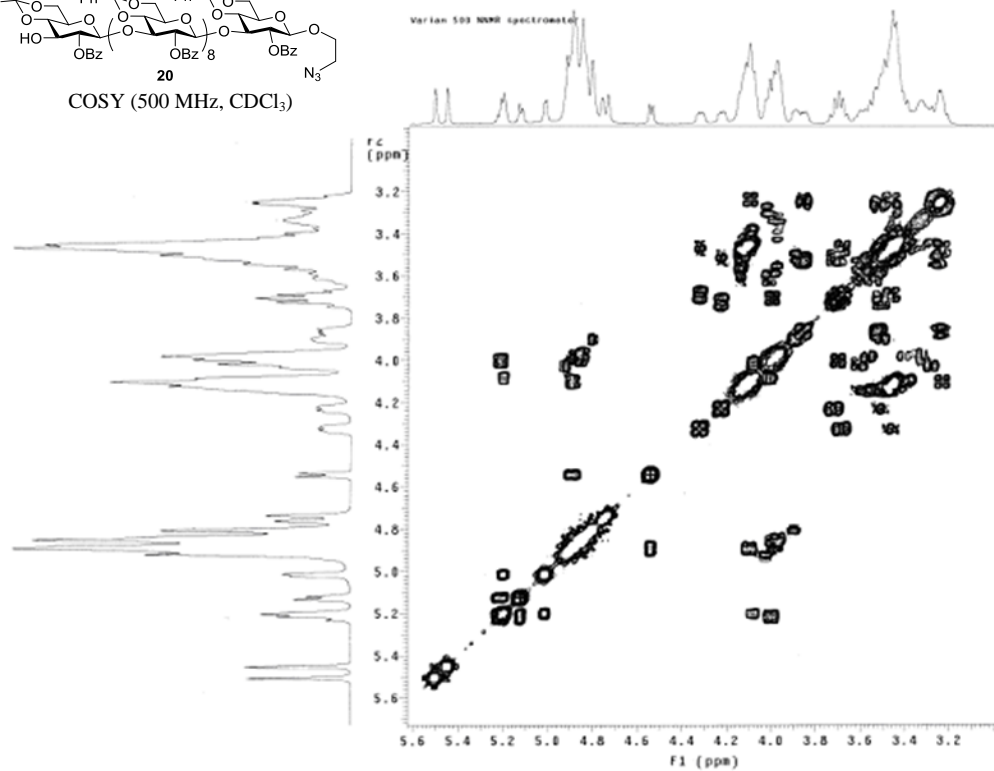


BSR-II-63-2C13

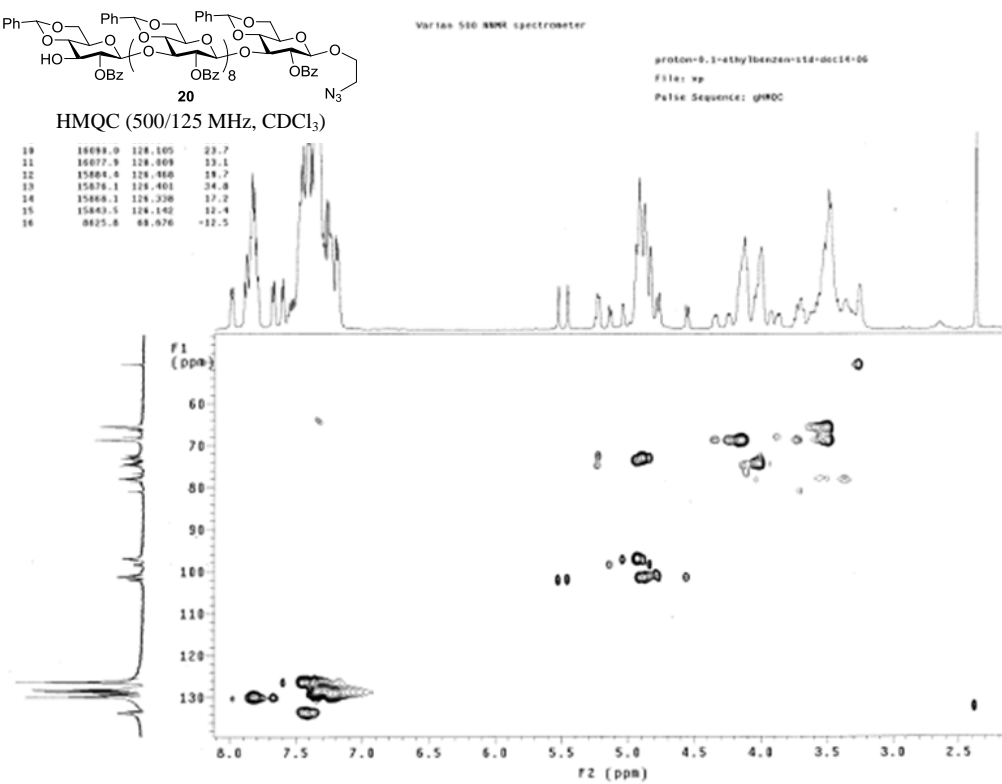




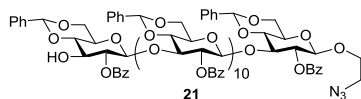
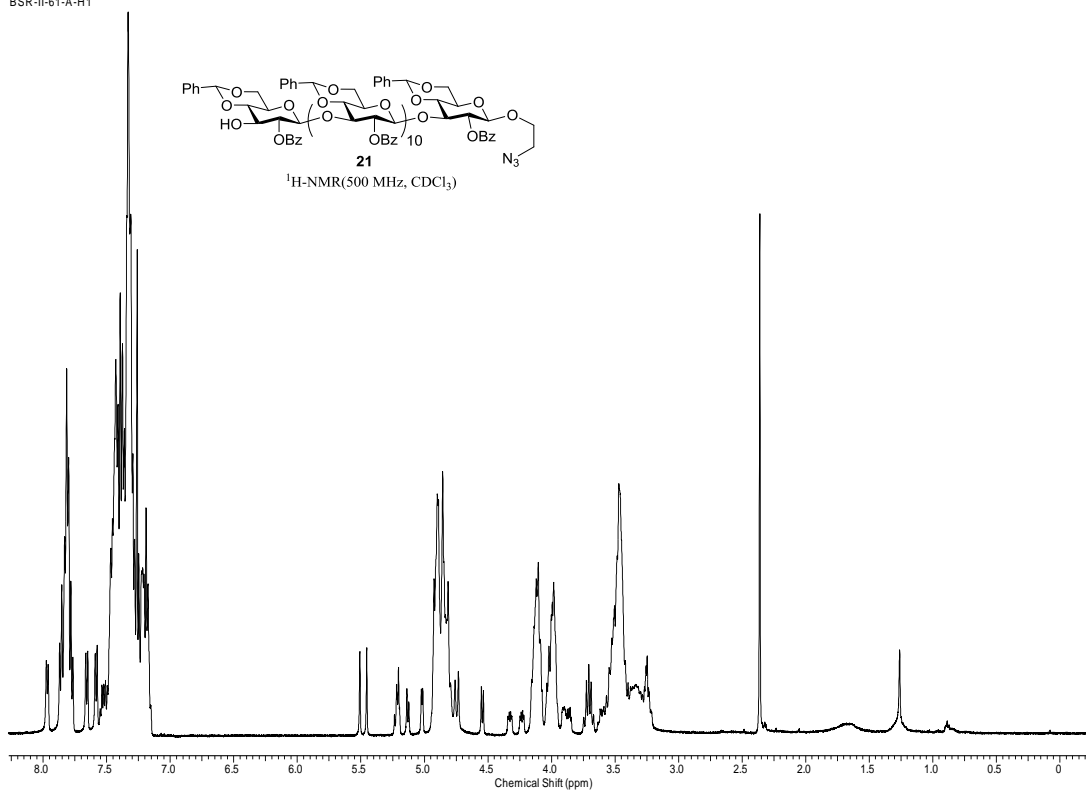
COSY (500 MHz, CDCl₃)



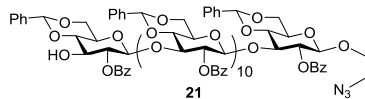
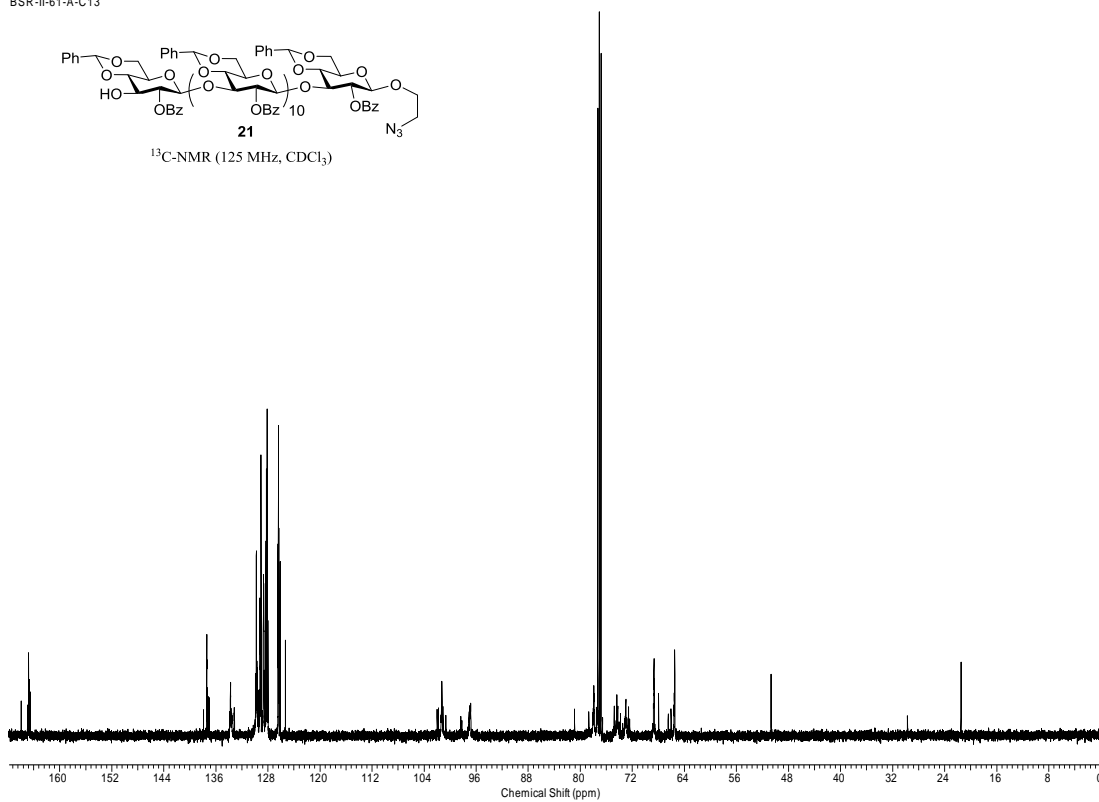
HMQC (500/125 MHz, CDCl₃)

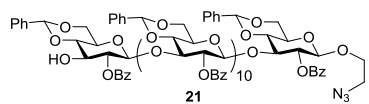
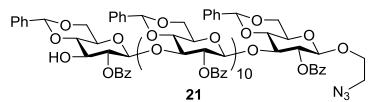
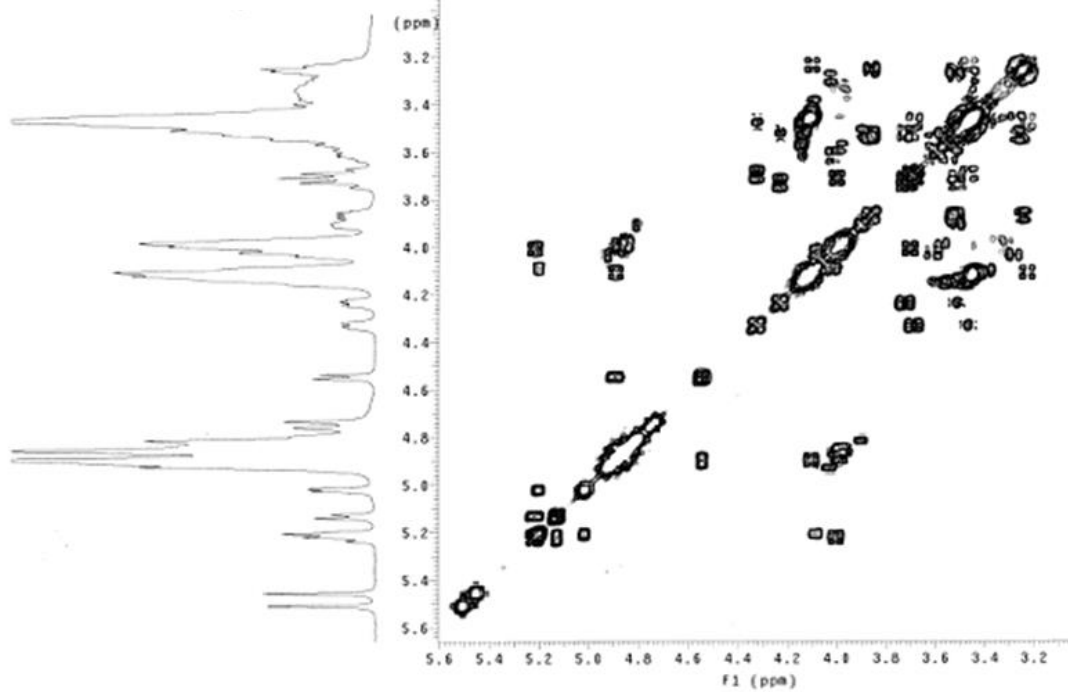


BSR-II-61-A-H1

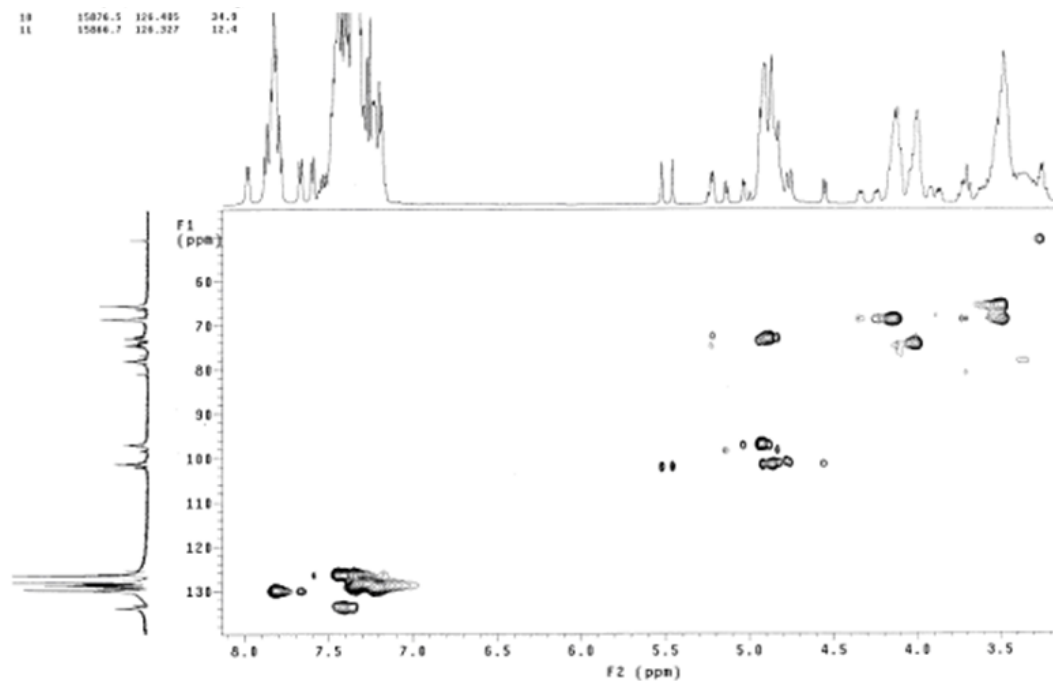
¹H-NMR (500 MHz, CDCl₃)

BSR-II-61-A-C13

¹³C-NMR (125 MHz, CDCl₃)

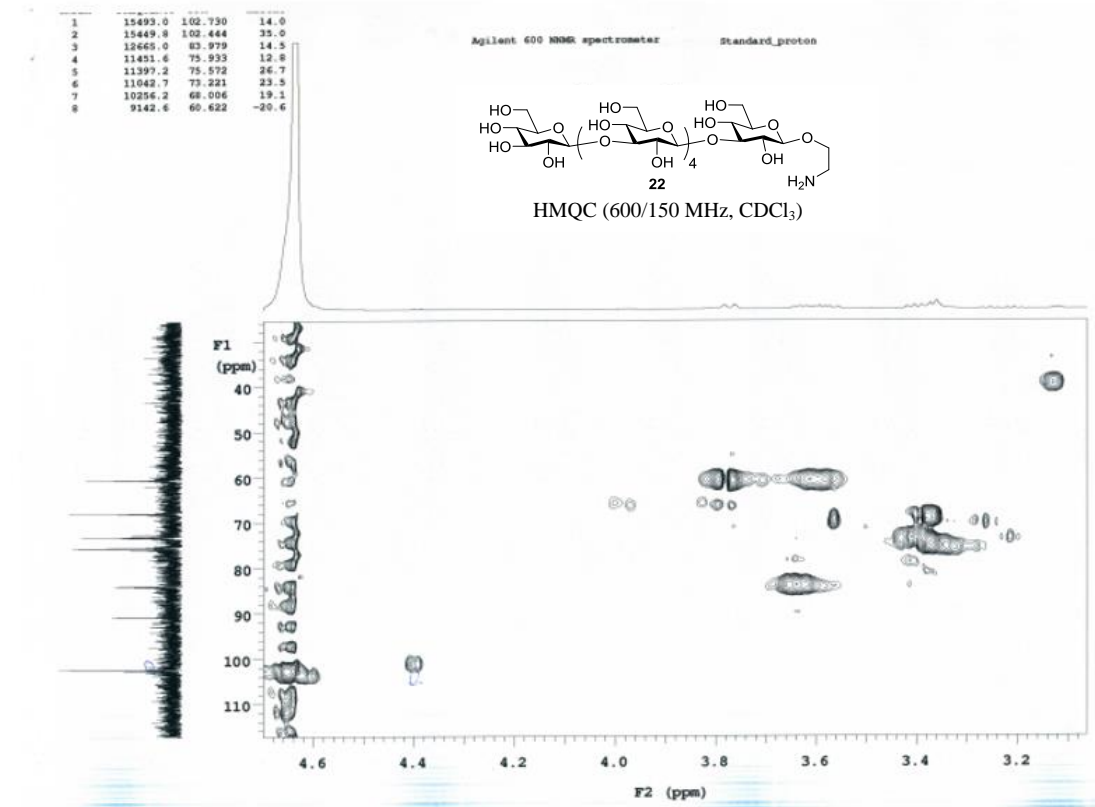
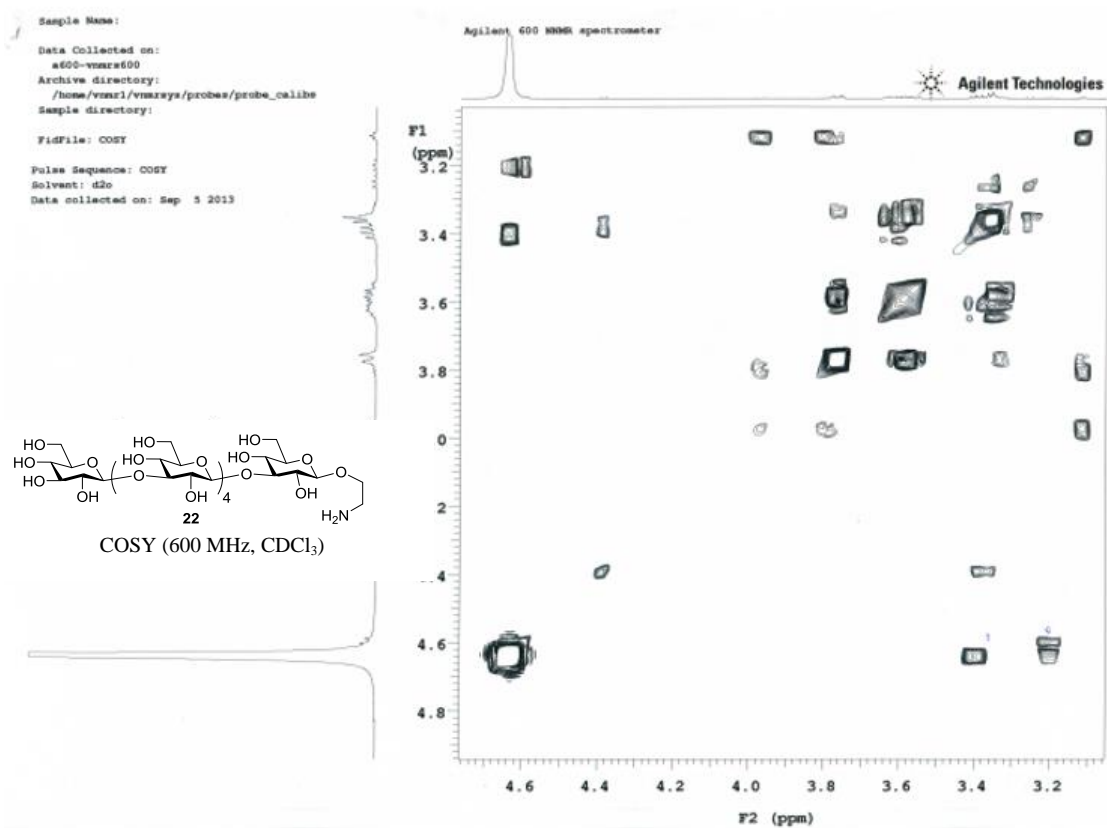
COSY (500 MHz, CDCl₃)HMQC (500/125 MHz, CDCl₃)

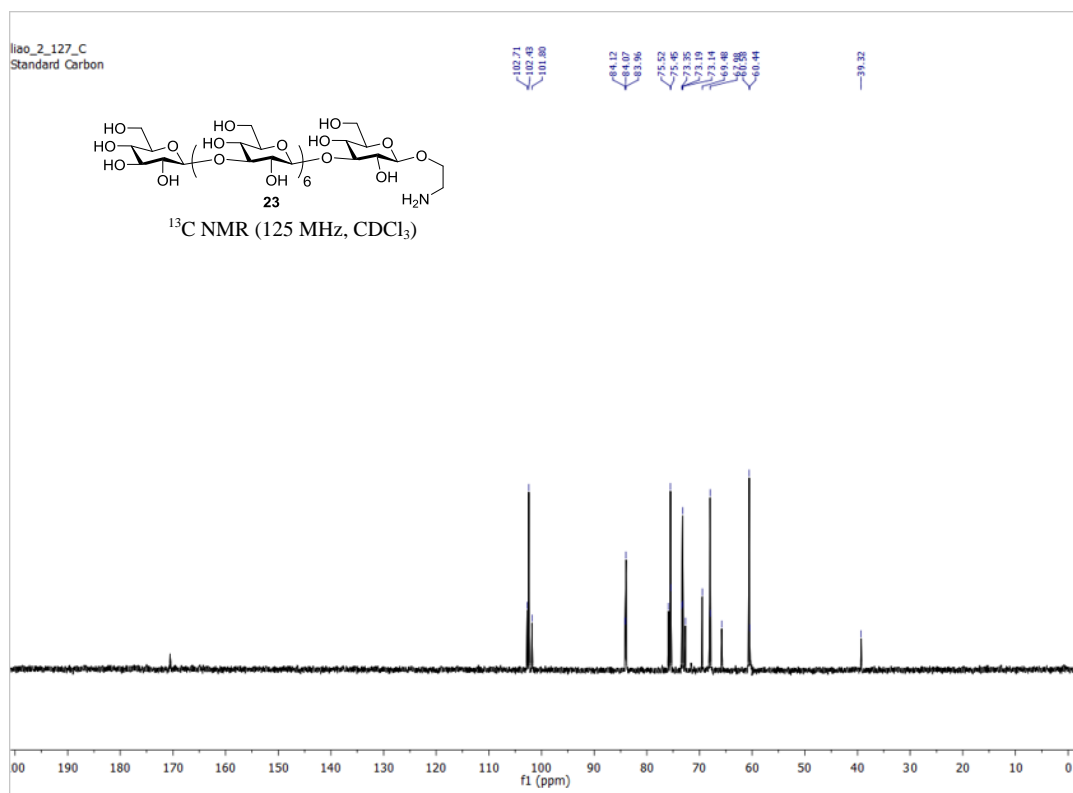
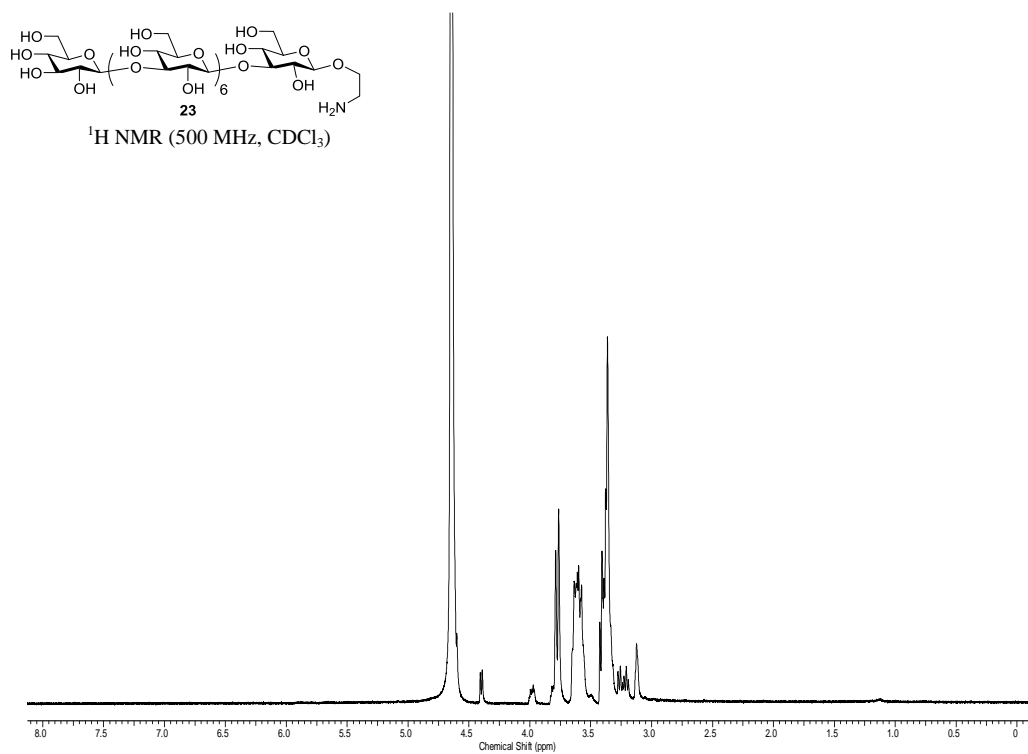
10	15876.5	326.485	34.9
11	15866.7	329.327	12.4

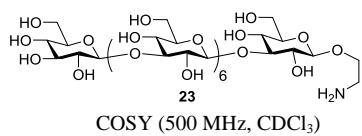
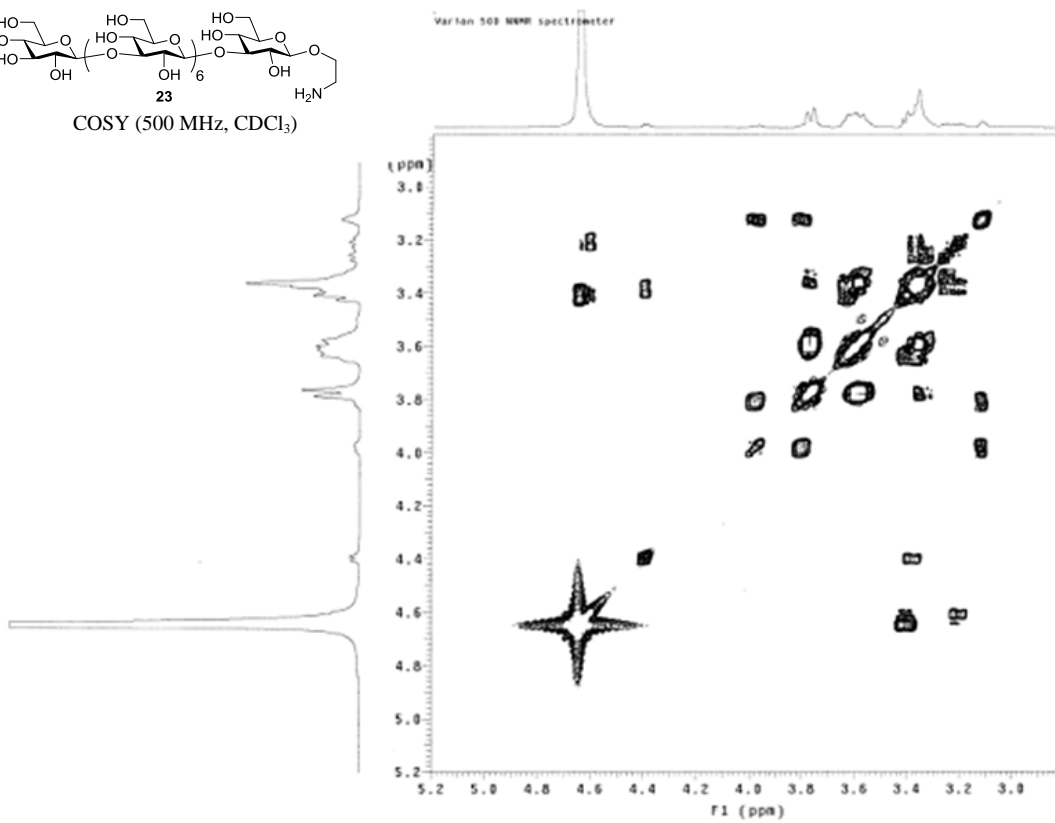


Varian 500 NMR spectrometer

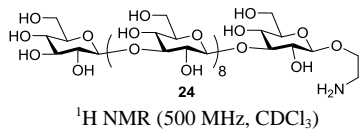
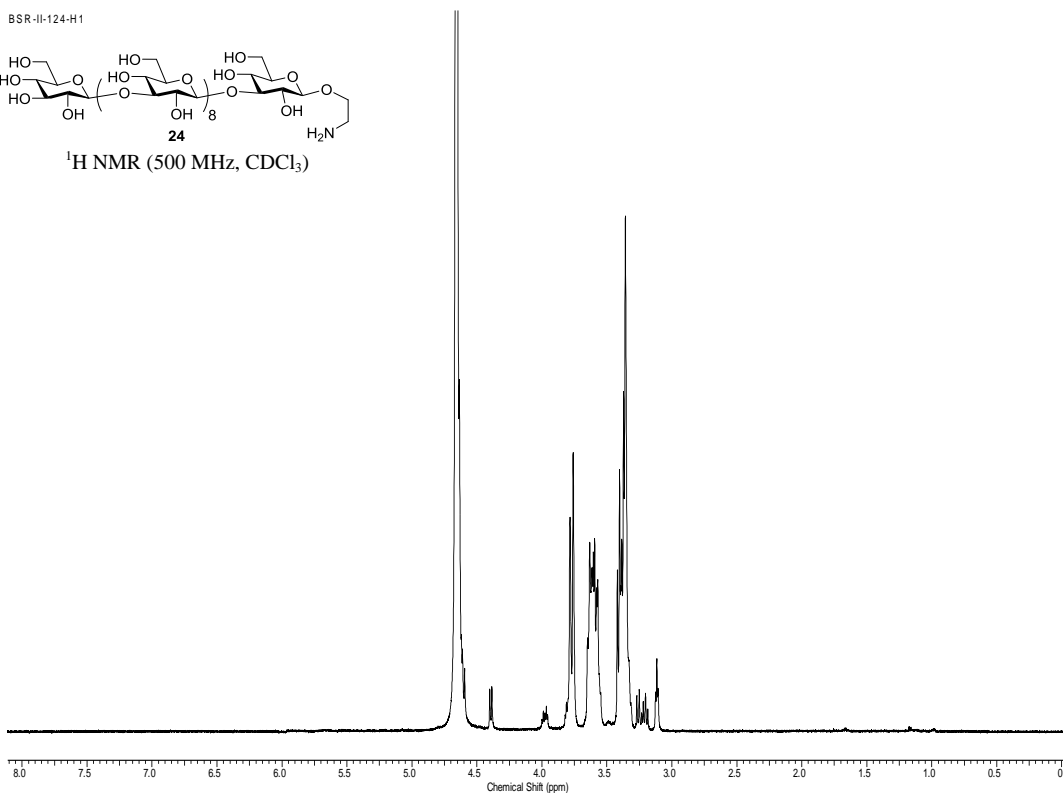
proton-0.1-ethylbenzen-std-dec10-06
 file: xp
 Pulse Sequence: ghmoc





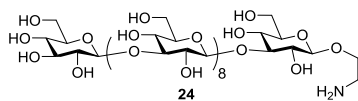
COSY (500 MHz, CDCl₃)

BSR-II-124-H1

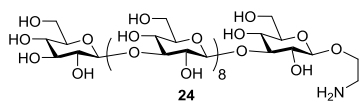
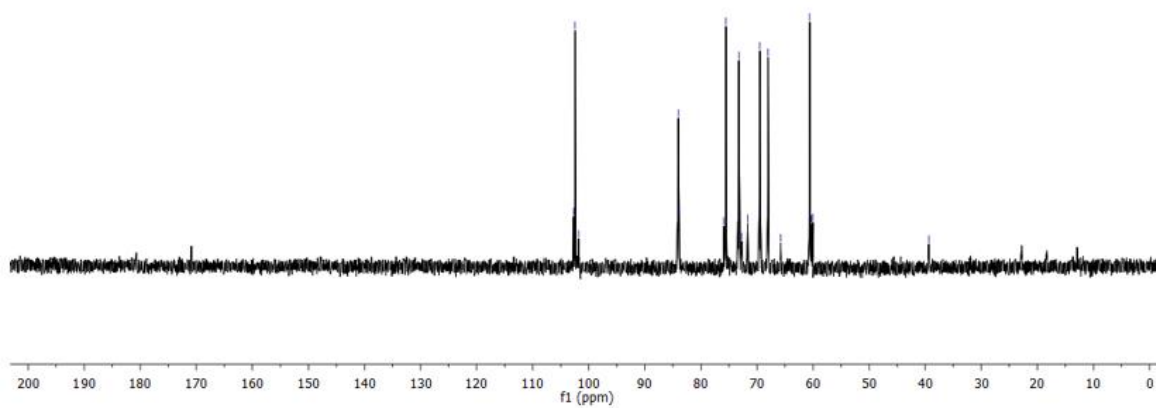
¹H NMR (500 MHz, CDCl₃)

liao_2_124_C
Standard Carbon

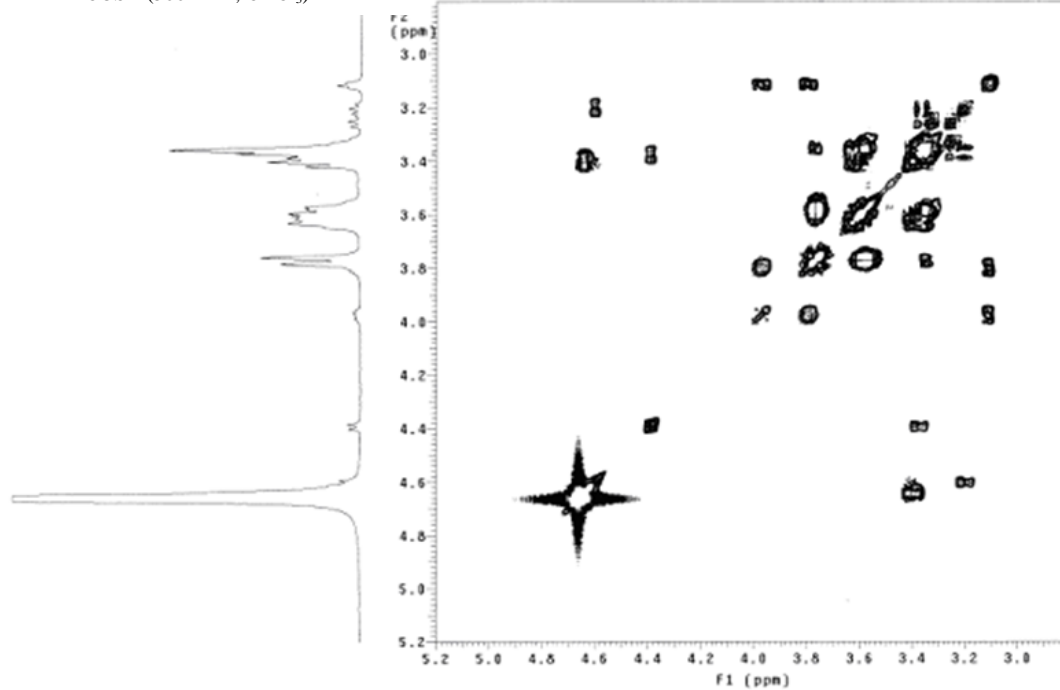
102.73
102.45
101.82
84.13
84.01
75.55
73.38
72.52
71.64
69.50
69.37
68.82
60.30
60.03
39.36



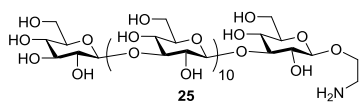
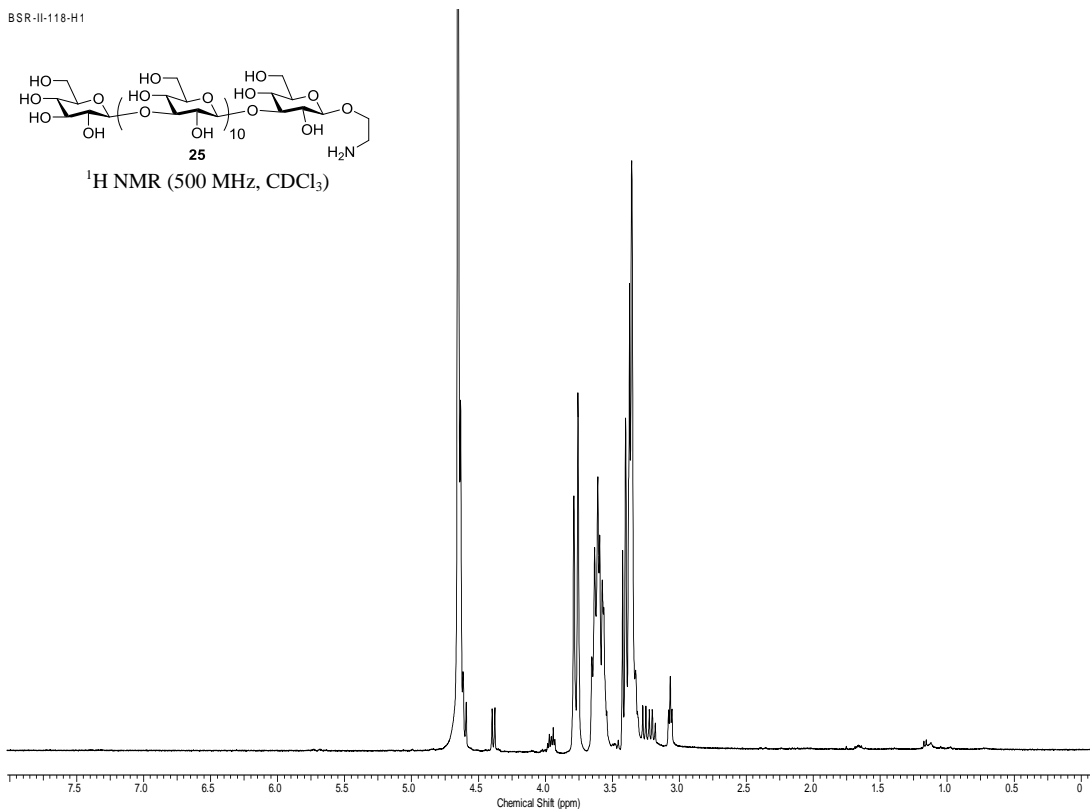
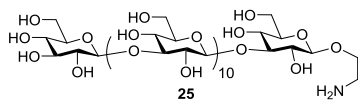
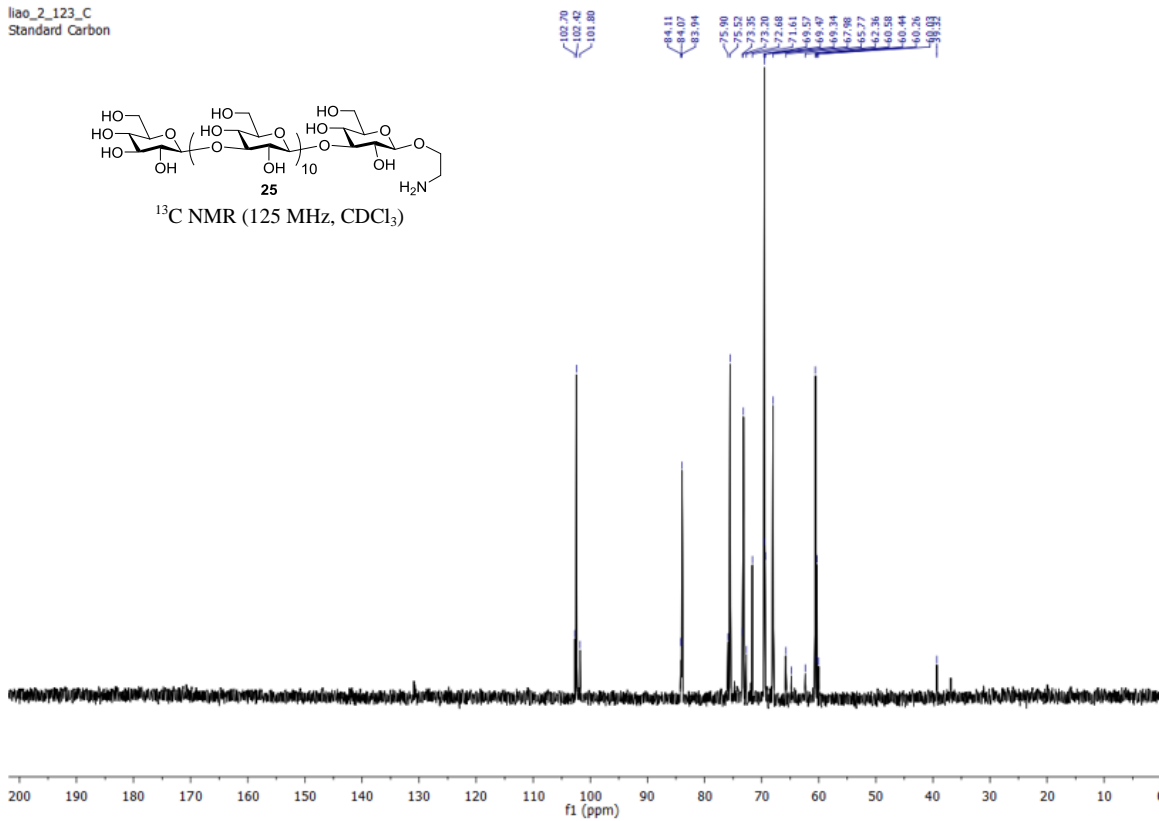
^{13}C NMR (125 MHz, CDCl_3)

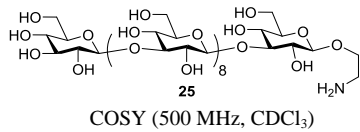
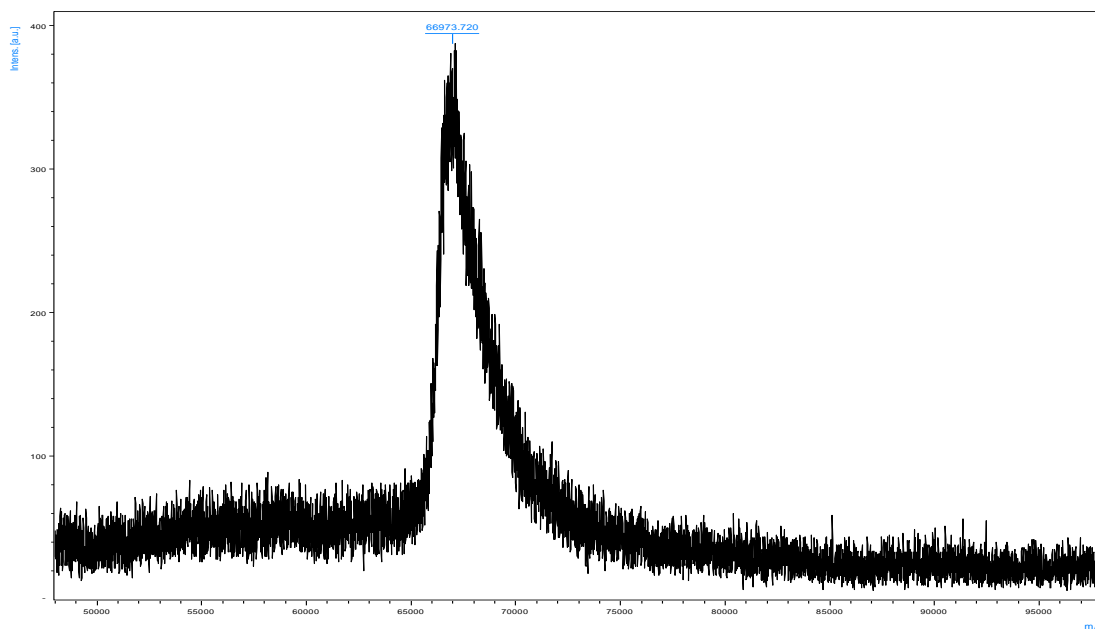
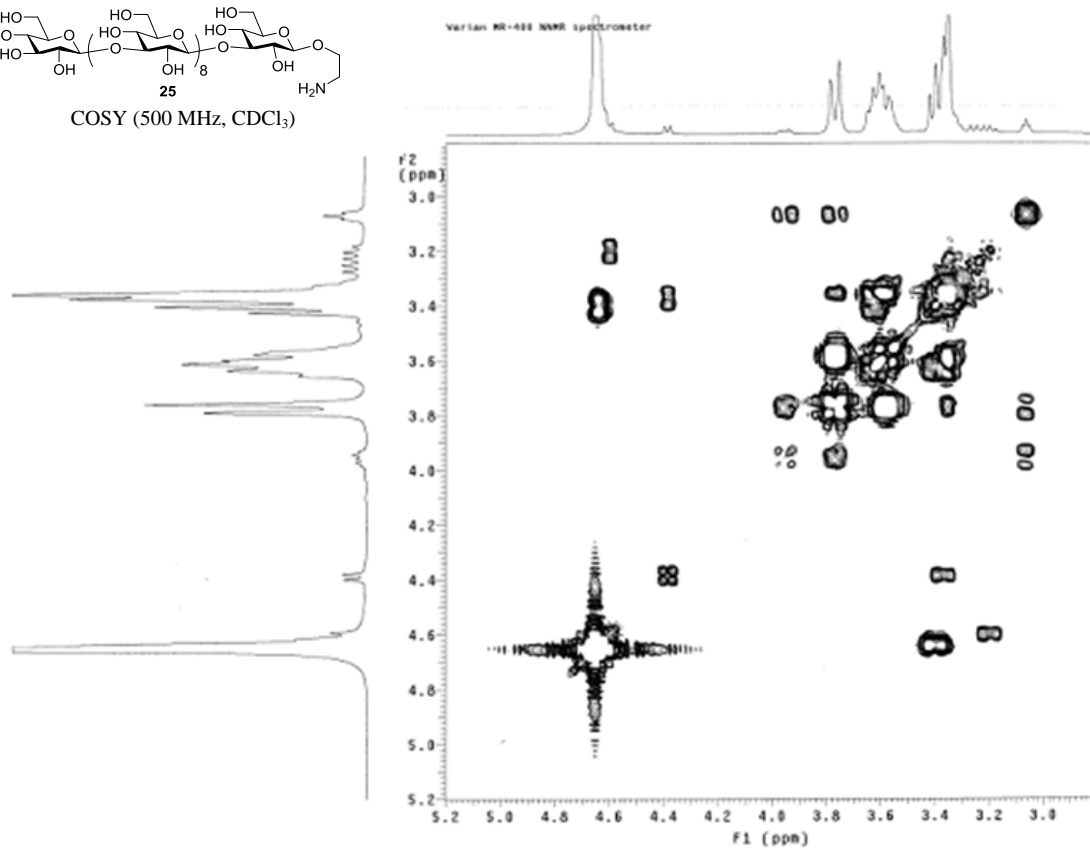


COSY (500 MHz, CDCl_3)

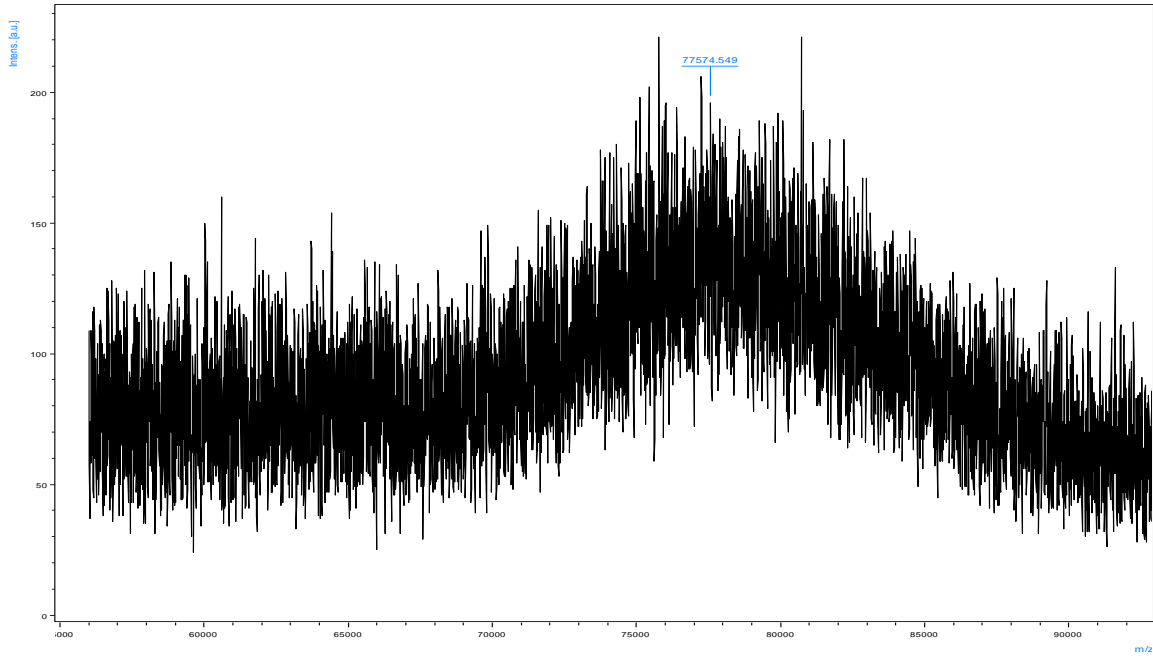


BSR-II-118-H1

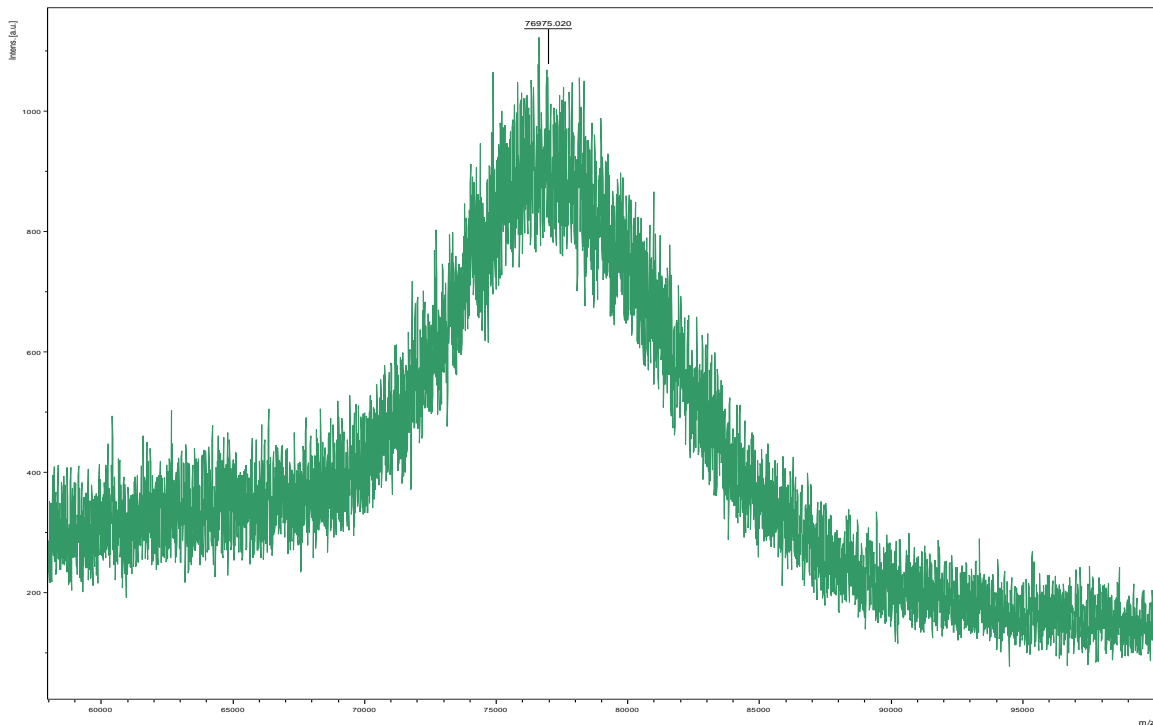
 $^1\text{H NMR}$ (500 MHz, CDCl_3)liao_2_123_C
Standard Carbon $^{13}\text{C NMR}$ (125 MHz, CDCl_3)

COSY (500 MHz, CDCl_3)

MALDI TOF MS spectrum of HSA protein

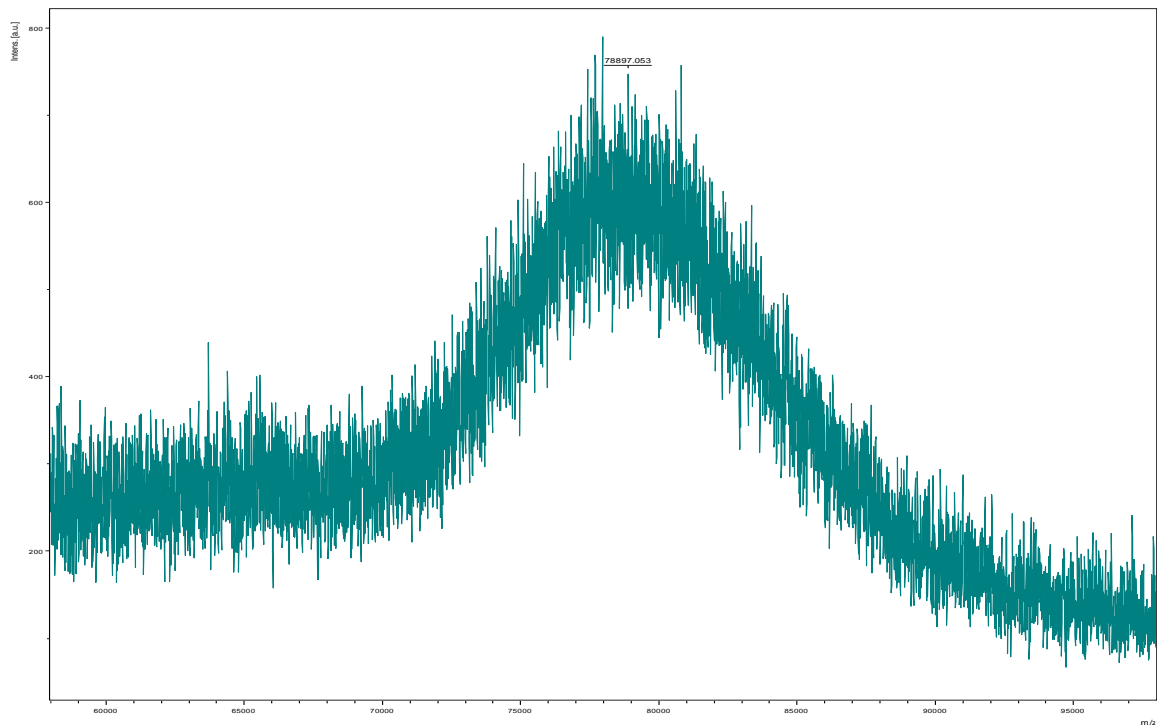


MALDI TOF MS spectrum of HSA-hexasaccharide conjugate **5**

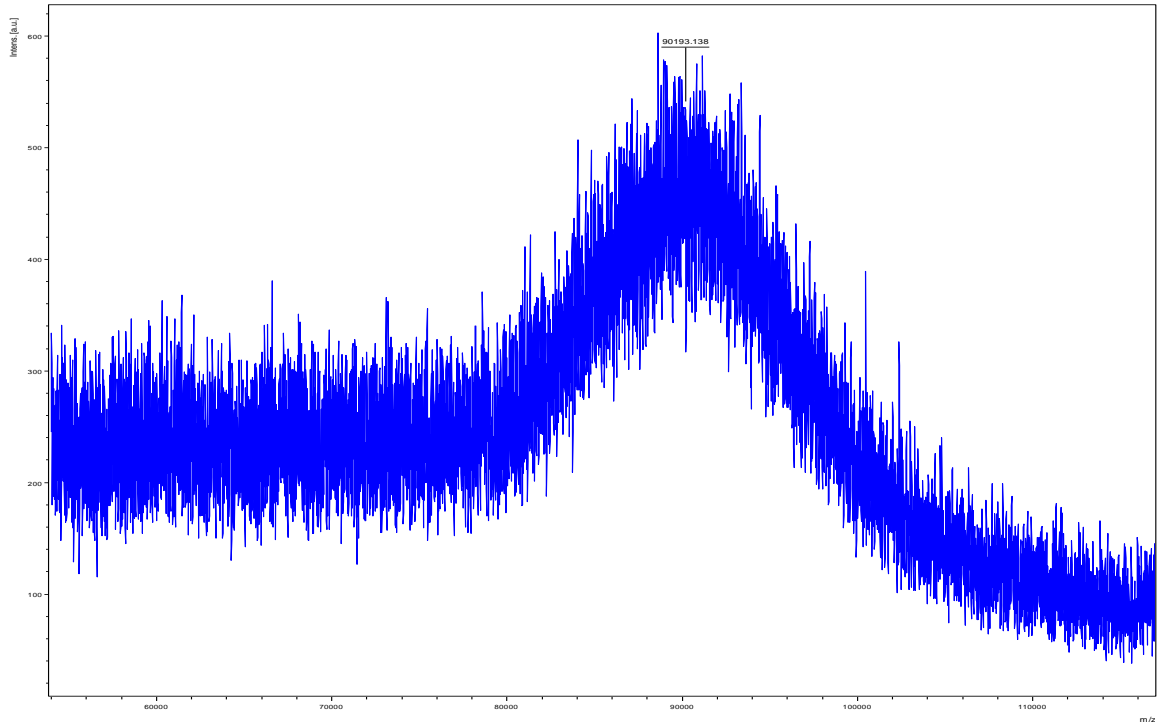


MALDI TOF MS spectrum of HSA-octasaccharide conjugate **6**

S35



MALDI TOF MS spectrum of HSA-decasaccharide conjugate **7**



MALDI TOF MS spectrum of HSA-dodecasaccharide conjugate **8**