Supporting Information

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Materials and general synthetic methods for Man_9 preparation:
All synthetic oligos were purchased from Integrated DNA Technologies. A complete list of oligos and primers for SELMA is in Table 1. Vent polymerase, Vent(exo) polymerase, Bst polymerase, T4 polynucleotide kinase, Exonuclease I, Taq polymerase and hydrophilic streptavidin magnetic beads were purchased from New England Biolabs. Centrisep desalting columns were purchased from Princeton Separations. Sephadex G-50 superfine resin was purchased from GE Healthcare. Antibody 2G12 was purchased from Polymun Scientific. Protein A Dynabeads and a TOPO-TA cloning kit were purchased from Invitrogen. ATP (γ-32P) was purchased from Perkin Elmer.

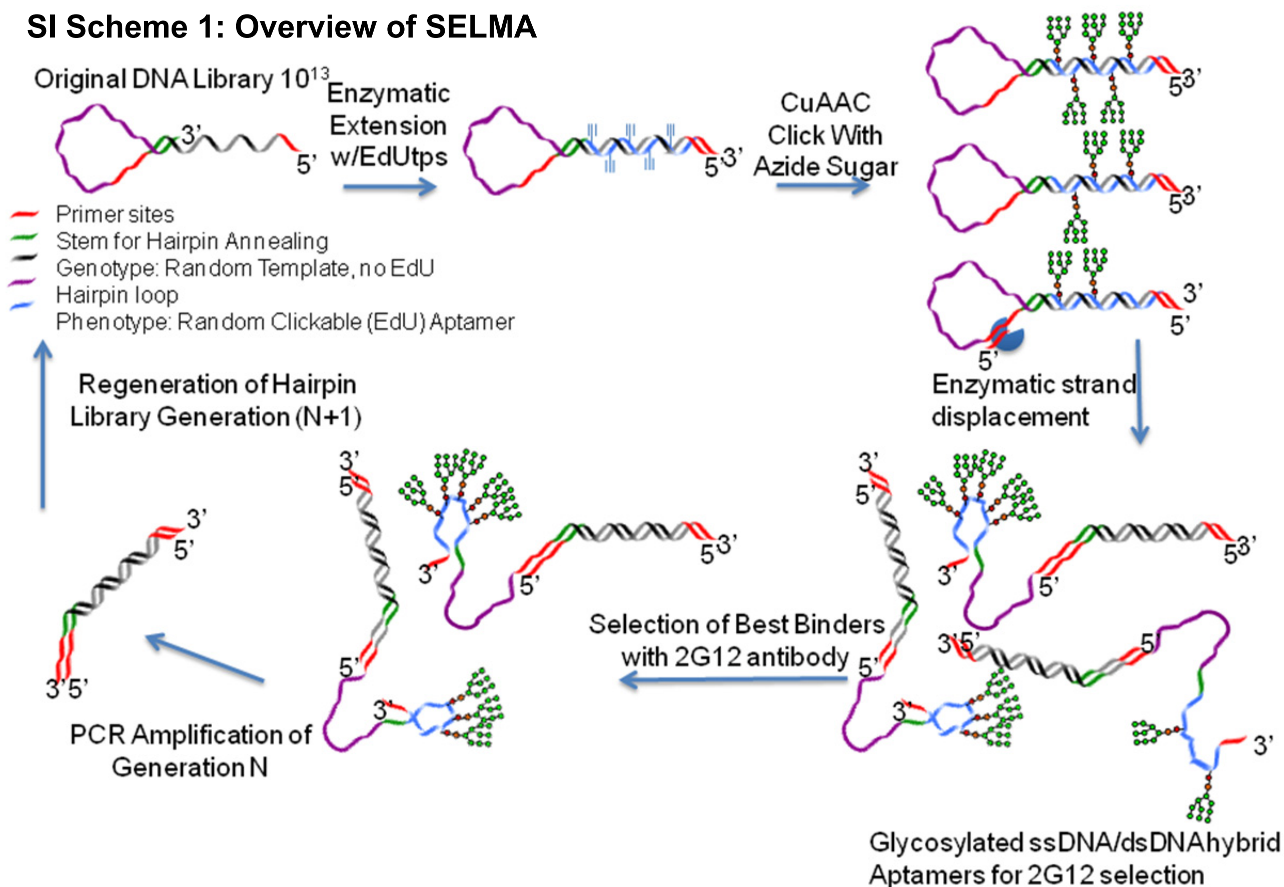
All synthesis reagents were purchased from Sigma-Aldrich, Acros Organics, Fluka, Alfa Aesar, or Strem, and used without further purification unless otherwise noted. Toluene, THF, and DCM were dried by passage through activated alumina columns and stored under argon gas. DriSolv Acetonitrile and Methanol were purchased from EMD. Glassware was flame dried or dried in a 150° C oven. Silicycle Siliaflash® P60 silica was used for column chromatography. All ^{1}H and ^{13}C NMR spectra were obtained on either a Varian 400MR or a Varian iNova 400 instrument. Spectra acquired in $CDCl_3$ were internally referenced to TMS or residual $CHCl_3$. Spectra acquired in D_2O were internally or externally referenced to sodium 3-(trimethylsilyl) propanesulfonate. Chemical shifts are reported in parts per million (ppm), and coupling constants are reported in Hz. Coupling is referred to with the following abbreviations (d = doublet, t = triplet, q = quartet, m = multiplet, app = apparent, and br = broad). For NMR spectra in which large numbers of resonances are unresolved, only the clearly-resolved “selected signals” are listed in text-format listing of data. LC/MS analysis was performed on a Waters Acquity UPLC chromatograph with a reverse phase C18 or C8 column, and a Waters Photodiode Array and Micromass ZQ4000 mass detectors. Infrared spectra were obtained using a Varian 640-IR spectrometer with a ZnSe ATR.

SI Table 1: Oligos for Man_9SELMA

Original 7A Library	5’- CCT GTT GTT CCG CTG TCT CCT TNN NNN NNN NNN NNN NNN NNN NNN NNC CCG TAC CCG TAT TGG TGG CAA GGA TGA CAA GG -3’ Where N= 7%A, 31%C, 31%G, 31%T (Random Region)
Original 15A Library	5’- CCT GTT GTT CCG CTG TCT CCT TNN NNN NNN NNN NNN NNN NNN NNN NNC CCG TAC CCG TAT TGG TGG CAA GGA TGA CAA GG -3’ Where N= 15%A, 28%C, 28%G, 28%T (Random Region)
Regeneration Primer	5’ biotin/CCC GTA CCC GAT AAT AAA ATA AAA ATA TAA AAT ATA AAA TCC TTG TCA TCC TTG CCA CCA -3’
Forward Primer	5’- CCT TGT CAT CCT TGC CCA CCA - 3’
Reverse Primer	5’- CCT GTT GTT CCG CTG TCT CCT T - 3’
Biotin Forward Primer	5’- biotin/CCT TGT CAT CCT TGC CCA CCA - 3’
Biotin Reverse Primer	5’- biotin/CCT GTT GTT CCG CTG TCT CCT T - 3’

SELMA was performed according to our published procedure.[1]

SI Scheme 1: Overview of SELMA



SI Table 2a*: Conditions and recovery for the 7A selections

Round	PCR Cycles	2g12 nm	Counter selection
1	24	50	No
2	20	50	Yes
3	21	50	No
4	12	50	Yes
5	14	10	No
6	13	5	Yes
7	17	1.4	No

SI Table 2b*: Conditions and recovery for the 7A selections

Round	PCR Cycles	2g12 nm	Counter selection
1	15	50	No
2	18	50	Yes
3	20	50	No
4	15	50	Yes
5	18	10	No
6	15	5	Yes
7	17	1.4	No

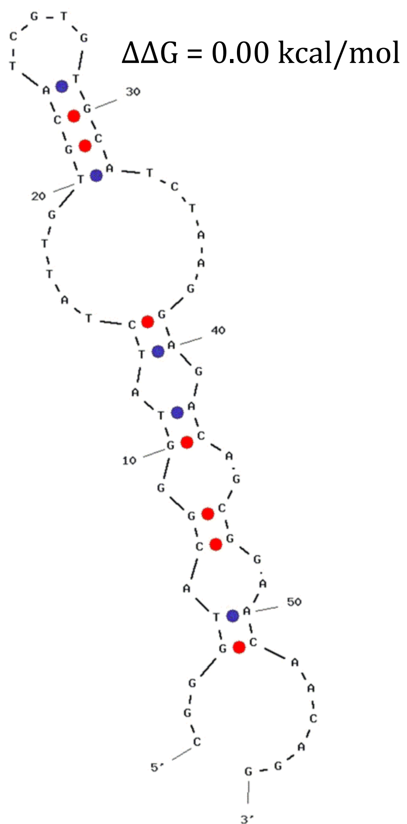
*SI Table 2 Notes: As with our previous selection, the enrichment of the library was monitored by the PCR cycles required to recover the selected binding clones.

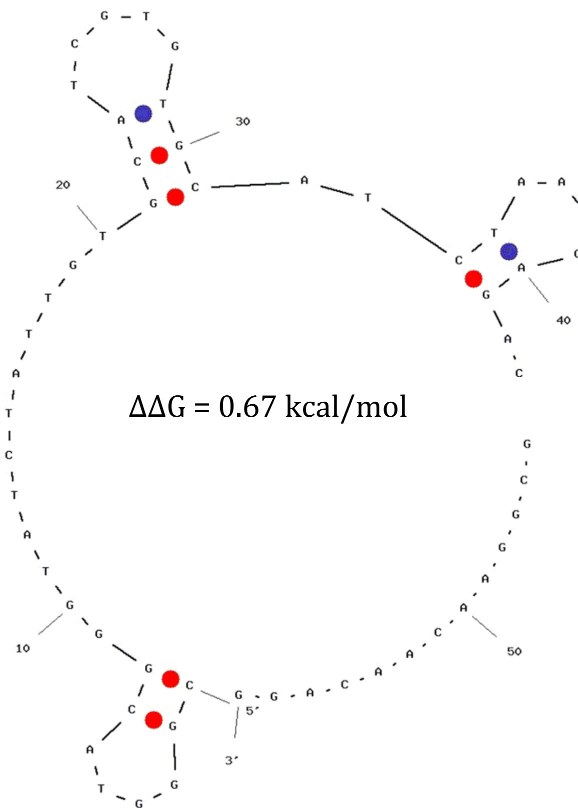
SI Table 3: Sequences obtained from Man$_9$SELMA

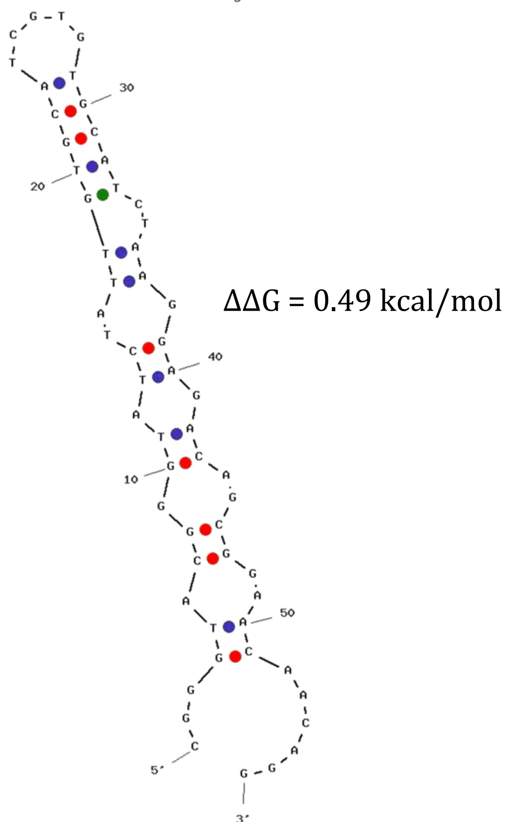
CLUSTAL W (1.81) multiple sequence alignment is courtesy of Biology Workbench.
Each red S represents a glycosylated EdU base in the binding clone. Highlighted clones were chosen for filter binding studies.

Clone Identifier(s)	Sequence Repeats	Random Region Sequence	Number of Click Sites
7A8-1	1	SASCSASSGSG-CASC-GSGSGCASCS--------	11
7A8-2	8	--SSSASSGSC-GASS-GSGSCGASCAAS------	11
7A8-4	16	------ASGCGAGASCSASGSCGASGCGSG-----	7
7A8-5	1	-------AGCC-GASC-GSGSAGGSCGCAGGSAC-	5
7A8-8	1	---SSCAGGCG-CASC-GSGSGCGSCCGSC-----	7
7A8-9	3	-------AGCC-GASC-GSGSCGGSCGSAGGSAC-	6
7A8-14	1	--AASGGSGSG-CASC-ASGSGCASCACS------	8
15A8-A	1	---AGAGSAGGSAGCAAASAAACACGAG-------	3
15A8-B	1	GSCAGASGCGAGASC-ASGSCGASGC--------	6
15A8-E	1	--SSSASSGSA-GASC-GSGSCGASCAAS------	11
15A8-F	11	---CCCGSGCG-CASCSASGSGCGSCACS------	7
15A8-H	1	--SSSASSGSC-GASS-GSGSCGASCAAS------	12
15A8-J	1	-----SGAACCCAGSCACGGAACASAGASC-----	4
15A8-L	3	--SCCSASGCC-GASC-ASGSCGGSCASS------	9
M1-14	1	-----CASGSG-CASCSASGSACASCSASGG----	9
M1-16	1	--SSGCASGCG-AASCSASGSGAGSCS--------	9
M1-CC	1	--SCCSASGCG-CACC-GSGSAAGSCSAS------	8
M3-4	21	-----CASGSG-CASCSASGSGCASCSASGG----	9
M3-30	1	-ASCAAAAGSAAGASSAGGACCCACA---------	4
M3-33	1	-----CASGSG-CASCSASGSGCGSCSASGG----	9
M3-43	1	------GSGCGAGASCSASGSCGASGCGSGA----	7
M3-46	1	-----CACGSG-CASCSASGSGCASCSGSGG----	9
M3-47	3	--------CCG-CASC-GSGSGCGSGCASGGAGSS	7
M3-L	1	------GSACGAGASCSASGSCGASGCGSGA----	7
M3-N	2	---ACCGSGCG-CASCSASGSGCGSCACS------	7
M3-S	1	-SCCACAAAACCAASSACCCGCCACC---------	3
M3-CC	1	ACCSSGCG-CASCSASGSGCGSCACS------	8
M3-HH	2	--SCCSASGCG-CASC-GSGSAAGSCSAS------	9
9b-B	1	--CACCGSGSG--ASGCGSGCGAGSCSGS------	7
9b-C	2	--CACCGSGCG--ASGCGSGCGAGSCSGS------	6
9b-H	1	-GSCCSASGC--GASC-GSGSCGGSCASS------	9

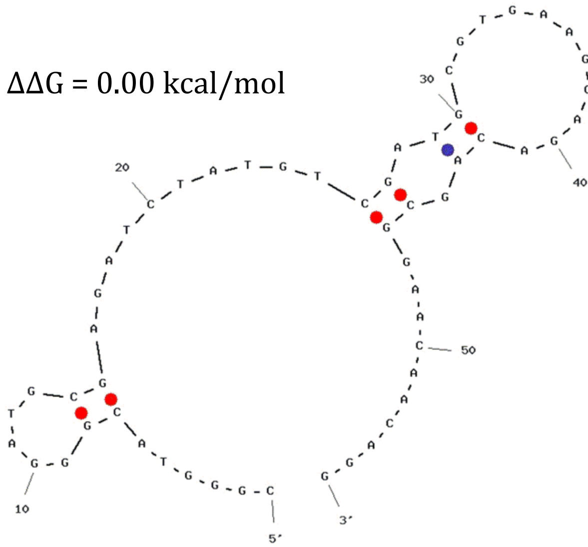
SI Figure 1: Mfold Predictions of Lowest-Energy Structures for Selected Clones

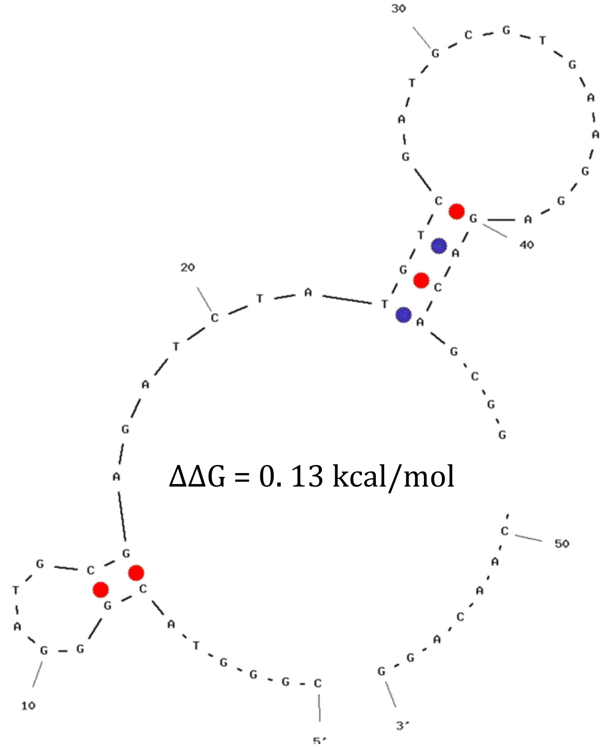


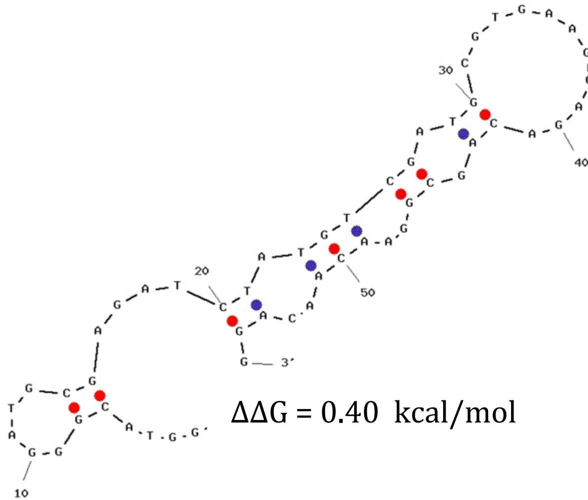




7A-8-1







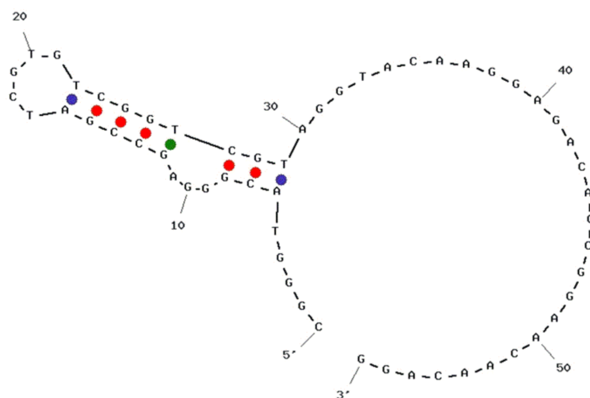
7A-8-4

ΔΔG = 0.00 kcal/mol

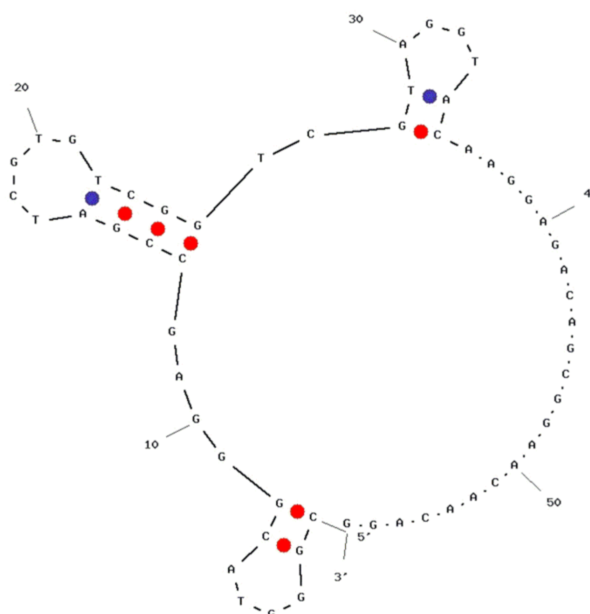
ΔΔG = 0.89 kcal/mol

ΔΔG = 0.21 kcal/mol

7A-8-8

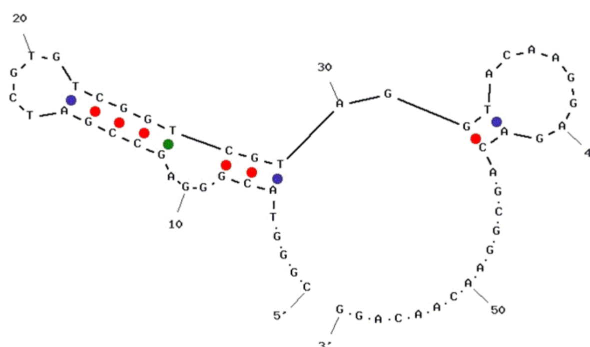


ΔΔG = 0.00 kcal/mol



ΔΔG = 0.14 kcal/mol

7A-8-9



ΔΔG = 0.37 kcal/mol

$\Delta\Delta G$ = 0.00 kcal/mol

$\Delta\Delta G$ = 0.67 kcal/mol

$\Delta\Delta G$ = 0.27 kcal/mol

15A-8-B

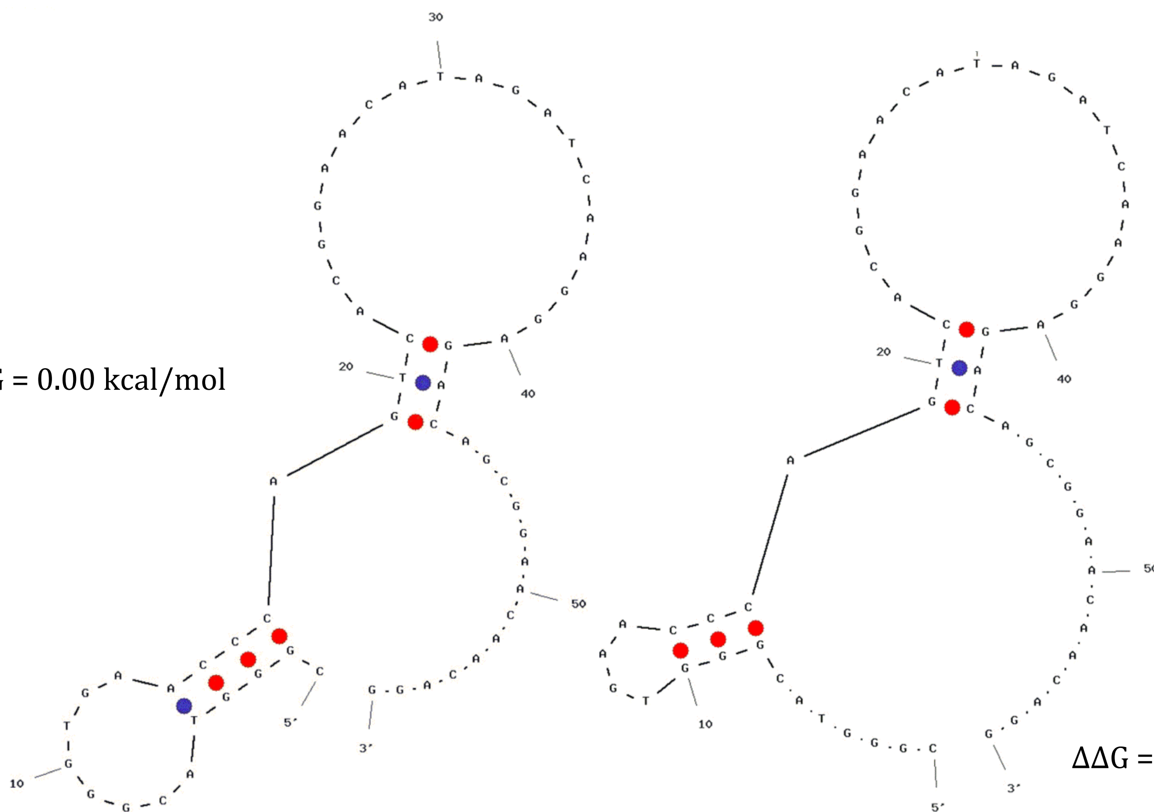
$\Delta\Delta G$ = 0.00 kcal/mol

$\Delta\Delta G$ = 1.60 kcal/mol

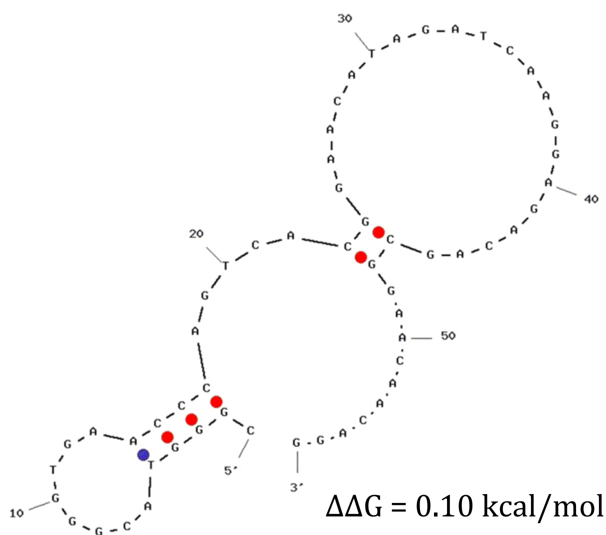
$\Delta\Delta G$ = 1.17 kcal/mol

15A-8-F

$\Delta\Delta G$ = 0.00 kcal/mol



$\Delta\Delta G$ = 0.32 kcal/mol



$\Delta\Delta G$ = 0.10 kcal/mol

15A-8-J

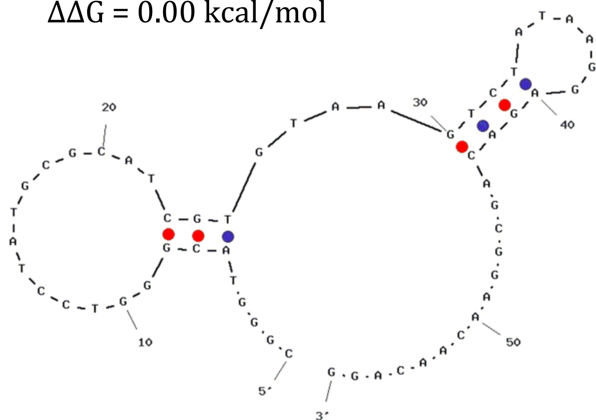
ΔΔG = 0.00 kcal/mol

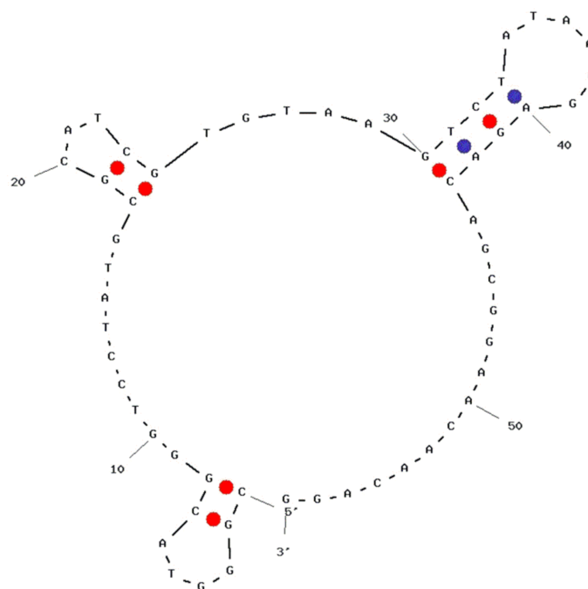
ΔΔG = 0.05 kcal/mol

M3-30

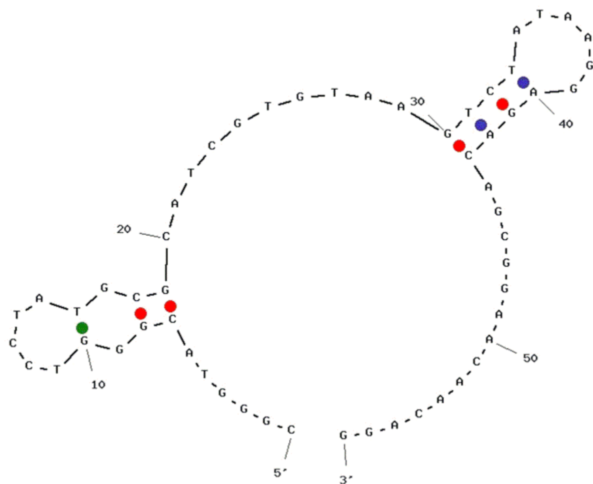
ΔΔG = 0.36 kcal/mol

ΔΔG = 0.00 kcal/mol



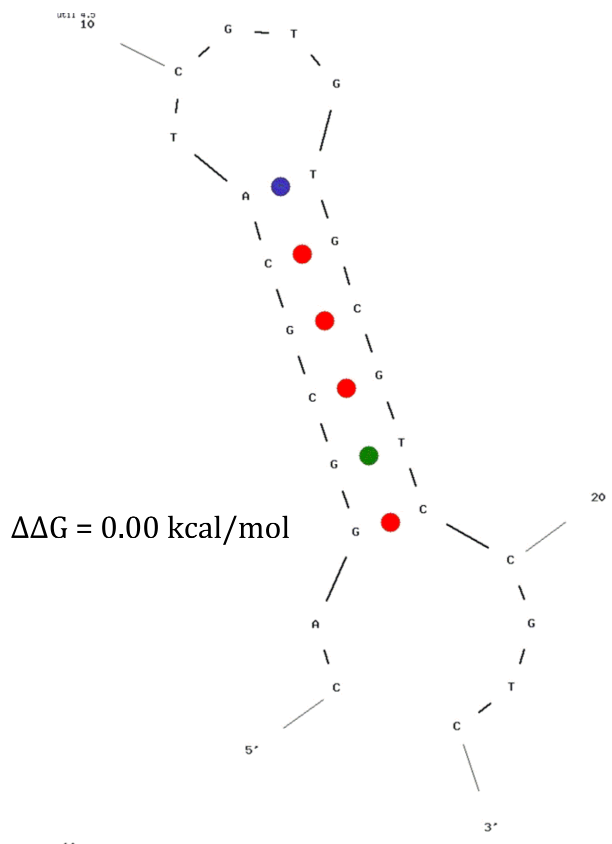


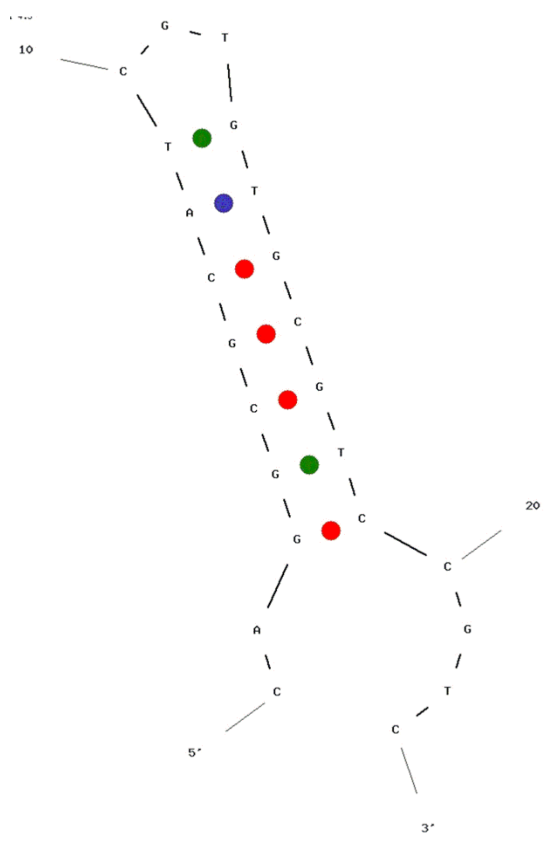
ΔΔG = 0.76 kcal/mol



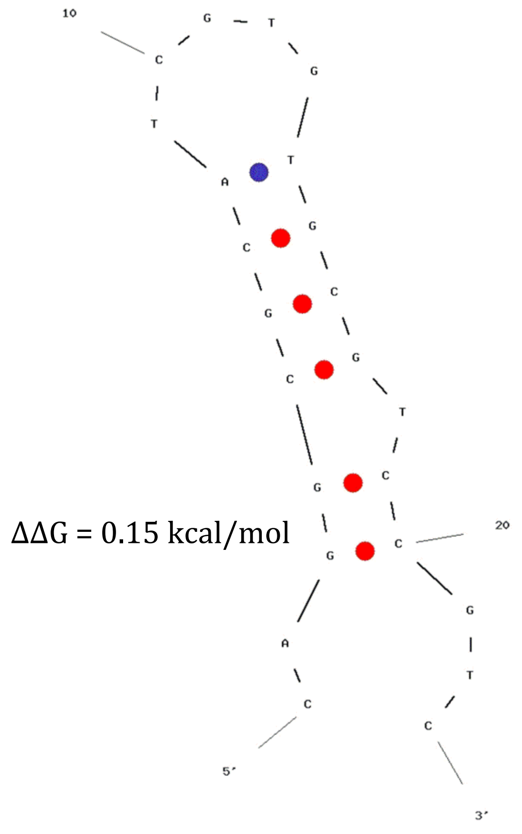
ΔΔG = 0.62 kcal/mol

M3-HH





ΔΔG = 1.03 kcal/mol



7A-8-8-T2

Preparation of glycosylated oligos for filter binding

Polymerase extension with EdU against template oligos to prepare alkynyl oligo

SI Table 4: Template oligos purchased for synthesis of filter binding oligos

Oligo Name	Sequence
Stem Primer	5'- CGGGTACGGG -3'
7A8-4	5'- CCTGTTGTTCCGCTGTCTCCTTCACGCATCGACATAGATCTCGCATCCCGTACCCG -3'
7A8-9	5'- CCTGTTGTTCCGCTGTCTCCTTGTACCTACGACCGACACGATCGGCTCCCGTACCCG -3'
15A8-B	5'- CCTGTTGTTCCGCTGTCTCCTTGCATCGACATGATCTCGCATCTGACCCCGTACCCG -3'
15A8-F	5'- CCTGTTGTTCCGCTGTCTCCTTAGTGACGCACATAGATGCGCACGGGCCCGTACCCG -3'
M3-HH	5'- CCTGTTGTTCCGCTGTCTCCTTATAGACTTACACGATGCGCATAGGACCCGTACCCG -3'
7A8-1	5'- CCTGTTGTTCCGCTGTCTCCTTAGATGCACACGATGCACAATAGATACCCGTACCCG -3'
7A8-8	5'- CCTGTTGTTCCGCTGTCTCCTTGACGGACGCACACGATGCGCCTGAACCCGTACCCG -3'
15A8-J	5'- CCTGTTGTTCCGCTGTCTCCTTGATCTATGTTCCGTGACTGGGTTCACCCGTACCCG -3'
M3-30	5'- CCTGTTGTTCCGCTGTCTCCTTTGTGGGTCCTAATCTTACTTTTGATCCCGTACCCG -3'

The following procedure is an optimized version of our previously reported method,[1] affording very clean material which gives highly reproducible data in the filter binding assay. Oligos complementary to the desired glyco-DNA sequence (see **SI Table 4**) were purchased from IDT with standard desalting purification, no HPLC or PAGE purification ordered. As shown below, the stem primer was annealed and BST polymerase extension using EdUTP, dCTP, dATP and dGTP resulted in the ethynyl-containing dsDNA:

Underlined regions indicate primer annealing site

5′ *CGGGTACGGG*

3′ *GCCCATGCCC***AAGTCCGCGTAGCACACGCAGGCAG**TTCCTCTGTCGCCTTGTTGTCC 5′

BST Polymerase extension reaction		
Reagent	V(μL)	Final
Millipore Water	25	
Thermopol Buffer(10X)	5	1X
7A8-8 (10 μM)	8	1.6 μM
Stem Primer (10 μM)	10	2.0 μM
EdUTP, dATP, dGTP and dCTP (10 mM each)	1	200 μM
BST (8U/μL)	1	
Total Reaction	50	

60°C for 2 min

40°C for 2 min

Repeat 3 times to produce dsDNA (U = EdU):

5′ *CGGGTACGGG***UUCAGGCGCAUCGUGUGCGUCCGUC**AAGGAGACAGCGGAACAACAGG 3′

3′ *GCCCATGCCC***AAGTCCGCGTAGCACACGCAGGCAG**TTCCTCTGTCGCCTTGTTGTCC 5′

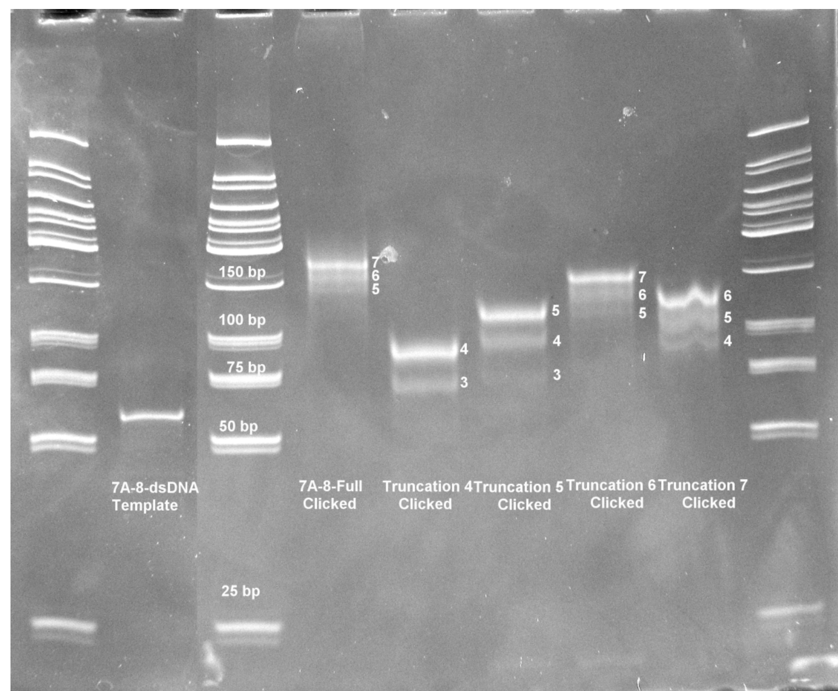
Click Reaction

Use of Ar atmosphere was found to promote more complete click reactions. Solutions were prepared according to volumes and concentrations in the table below.

$CuSO_4$ and THPTA was premixed in a 500-uL eppendorf tube. Working on a schlenk line, the tube was placed into a pointed bottom flask and the mixture was degased by cycling between reduced pressure and positive Ar pressure. Under Ar, the dsDNA-alkyne oligo and sugar azide was added to the reaction mixture. The reaction was immediately degassed as before. The solution of sodium ascorbate was then added under Ar and the final reaction was degassed under reduced pressure. After 2 hours of reaction under argon, the mixture was quenched by addition of excess (5eq, 12.5μL) THPTA ligand. The oligo was desalted by passing through a spin column of packed Sephadex G-50, and the extent of reaction was visualized on a 10% PAGE gel (**SI Image 3**).

Click Reaction of Clone 7A-8-Full		
Reagent	V(μL)	Final
BST extension reaction	42	
THPTA (10 mM)	2.5	0.5 mM
$CuSO_4$ (10 mM)	2.5	0.5 mM
Man_9Azide (35 mM)	2	1.4 mM
Sodium Ascorbate (250 mM)	1	5 mM
Total Reaction	50	

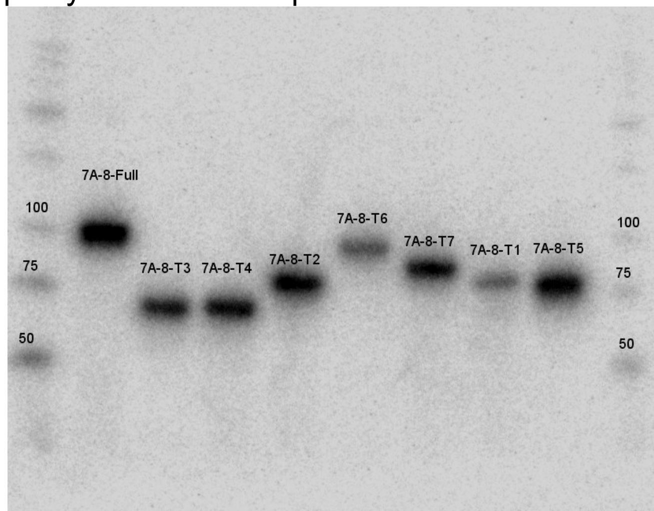
5′ *CGGGTACGGG***SSCAGGCGCASCGSGSGCGSCCGSC**AAGGAGACAGCGGAACAACAGG 3′
3′ *GCCCATGCCC***AAGTCCGCGTAGCACACGCAGGCAG**TTCCTCTGTCGCCTTGTTGTCC 5′



SI Image 3: 10 % acrylamide gel stained with ethidium bromide. NEB low MW ladder can be seen in lanes 1,3, and 9. Lane 2 is the dsDNA 7A8-8 product of BST polymerase extension using EdU containing DNTPS. Lane 4 is the glycosylated 7A8-8. As can be seen, the majority of the product contains 7 glycosylations, and this is further purified by preparative PAGE. The sequences of the oligos used for the truncations are found in **SI Table 6**.

Purification of fully-glycosylated ssDNA

The oligo was denatured in 8M urea, then loaded onto a 12% preparative denaturing urea PAGE. The click-glycosylated ssDNA was visualized using acridine orange stain under UV light. With minimal UV exposure, the fully glycosylated oligo band was excised from the gel and crushed in 100ul of 0.5XTBE buffer and shaken at 37°C. Following overnight incubation, the supernatant was desalted as before. The desalted modified DNA was then radioactively labeled by 5'-phosphorylation using T4 polynucleotide kinase and ATP (γ-^{32}P) according to manufacturer's instructions. The purity of the labeled product can be seen in **SI Image 4**.



SI Image 4: 6% urea gel of glycosylated and labeled oligos purified via preparative denaturing PAGE. Gel exposed to phosphorimaging screen
Lane 1: Labeled NEB Low MW ladder
Lane 2: 7 click 7A-8-full
Lane 3: 4 click 7A-8 truncation 3
Lane 4: 4 click 7A-8 truncation 4
Lane 5: 5 click 7A-8 truncation 2
Lane 6: 7 click 7A-8 truncation 6
Lane 7: 6 click 7A-8 truncation 7
Lane 8: 5 click 7A-8 truncation 1
Lane 9: 5 click 7A-8 truncation 5
Lane 10: Labeled NEB Low MW ladder

Filter Binding Assay

The Binding Buffer was prepared as 20mM Tris pH 7.5, 150 mM NaCl, 4 mM $MgSO_4$, and with or without 50ug/mL BSA. The use of BSA and omission of detergents was found to be critical for reproducible results.[1]

For the typical binding study, 10-100 fmol of the ^{32}P labeled glycosylated DNA was diluted into 35 μL of Binding Buffer + BSA and heated to 65 ^{o}C for 5 minutes, then cooled to RT to promote proper folding. 2G12 was serially diluted in Binding Buffer + BSA from 87.3 μM stock solution (Polymun Scientific). For every clone studied, 50 μL aliquots of each 2G12 dilution were pipetted into low retention 0.5 mL tubes (USA Scientific). 5μl of the labeled and folded glycosylated DNA was then added to each and incubated at 20°C for 8 hours.

Two hours prior to filtration, the filters were activated for the assay. Nitrocellulose was cut to size and incubated in 0.4M NaOH for 10 minutes, then washed thoroughly with water, and incubated for at least an hour in the Binding Buffer without BSA. Similarly, the PVDF membrane was soaked in MeOH for 2 minutes, then washed thoroughly with water, then incubated for at least an hour in the Binding Buffer without BSA. The sandwich filtration apparatus was assembled at the time of the assay. 50ul of each 2G12-glycoDNA sample was added to the appropriate well and vacuum was applied to filter the aliquots. Each well was then washed with 200ul of freshly prepared binding buffer with BSA. The membranes were then dried under vacuum, wrapped in cellophane, and exposed to a phosphorimaging plate. The plate was imaged after an appropriate length of time for adequate exposure. Initial full length clones were studied in duplicate data, whereas clone 7A8-8 and all truncations/mutations were studied in at least triplicate.

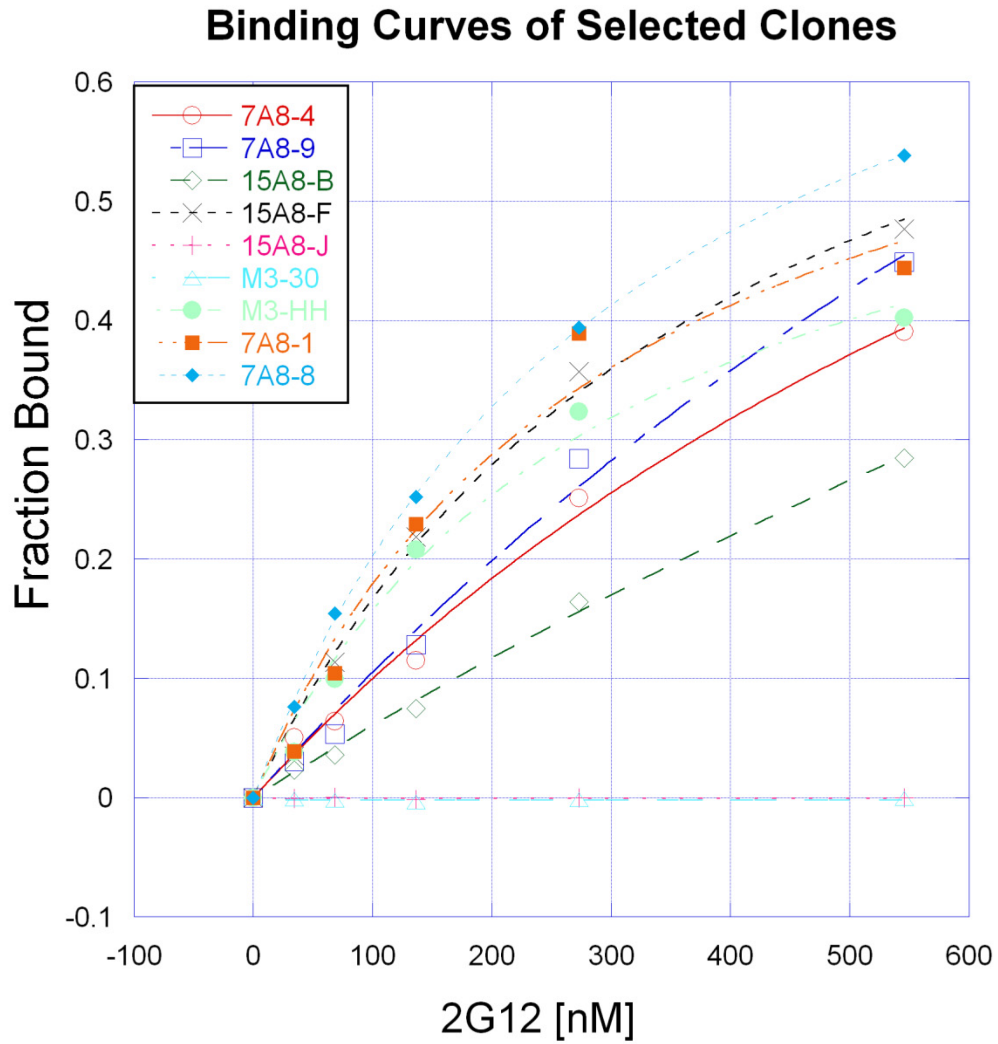
SI Table 6: Purchased oligos from IDT for synthesis of truncations and mutations

Oligo Name	Sequence
Stem Primer	5'-CGGGTACGGG-3'
Primer 2	5'-CAGGCGCA-3'
Primer 3	5'-CGGGTACGGGTT-3'
7A8-8-Full	5'-CCTGTTGTTCCGCTGTCTCCTTGACGGACGCACACGATGCGCCTGAACCCGTACCCG-3'
7A8-8-Trunc A	5'-GACGGACGCACACGATGCGCCTGAACCCGTACCCG-3'
7A8-8-Trunc B	5'-CGGACGCACACGATGCGCCTGAACCCGTACCCG-3'
7A8-8-1del	5'-GACGGACGCACACGGTGCGCCTG-3'
7A8-8-2del	5'-GACGGACGCACGCGATGCGCCTG-3'
7A8-8-3del	5'-GACGGACGCGCACGATGCGCCTG-3'
7A8-8-4del	5'-GACGGGCGCACACGATGCGCCTG-3'
7A8-8-5del	5'-GGCGGACGCACACGATGCGCCTG-3'

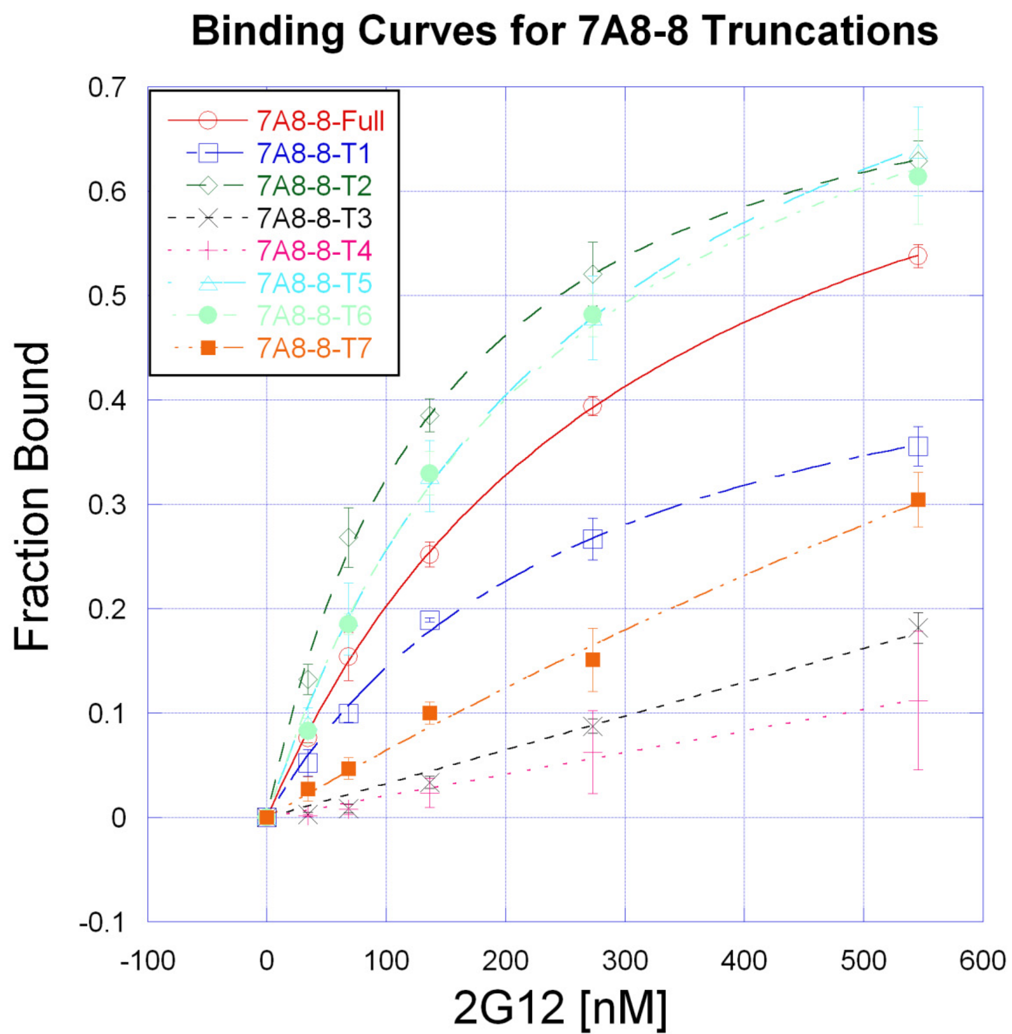
SI Table 7: Primer/Template combinations for synthesis of truncations and mutations

Clone Product	Primer	Template
7A8-8-T1	Primer 3	7A8-8-Full
7A8-8-T2	Primer 3	7A8-8-Trunc A
7A8-8-T3	Primer 3	7A8-8-Trunc B
7A8-8-T4	Primer 2	7A8-8-Trunc B
7A8-8-T5	Primer 2	7A8-8-Trunc A
7A8-8-T6	Stem Primer	7A8-8-Trunc A
7A8-8-T7	Stem Primer	7A8-8-Trunc B
7A8-8-T2-1del	Primer 2	7A8-8-1del
7A8-8-T2-2del	Primer 2	7A8-8-2del
7A8-8-T2-3del	Primer 2	7A8-8-3del
7A8-8-T2-4del	Primer 2	7A8-8-4del
7A8-8-T2-5del	Primer 2	7A8-8-5del

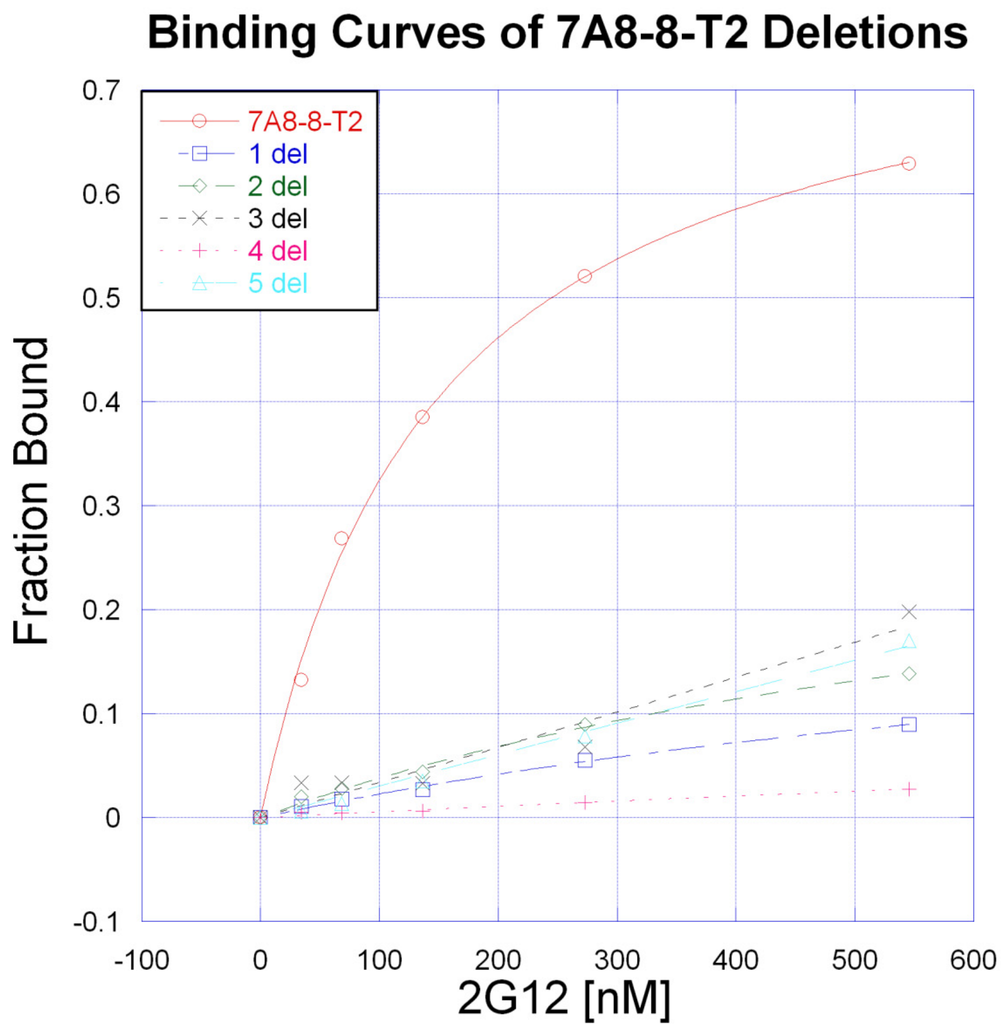
SI Figure 2: Binding Curves of Selected Clones



SI Figure 3: Binding Curves of 7A8-8 Truncations



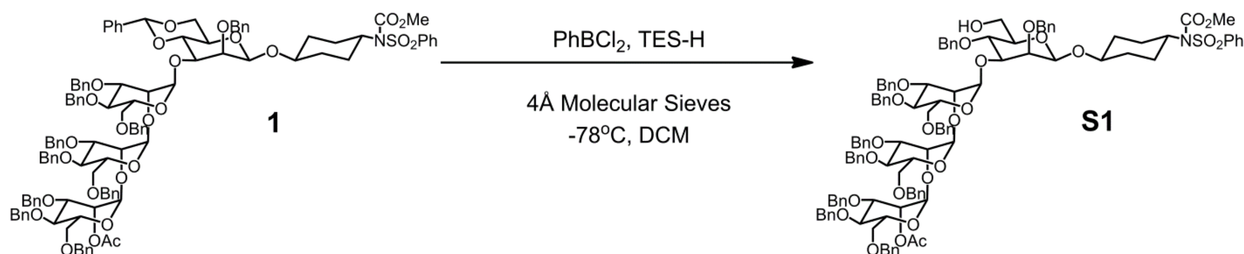
SI Figure 4: Binding Curves of 7A8-8-T2 Deletions



Synthesis of Man9 azide (4)

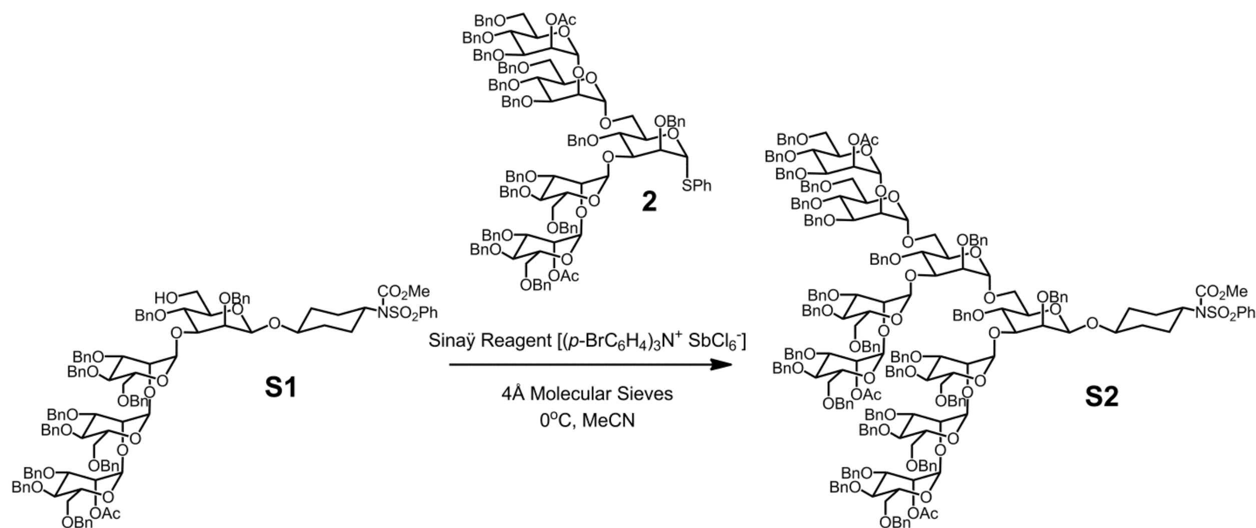
1[2] and **2**[3] were prepared according to literature procedures.

Benzylidine ring opening of cyclohexyl linked tetrasaccharide **(S1)**



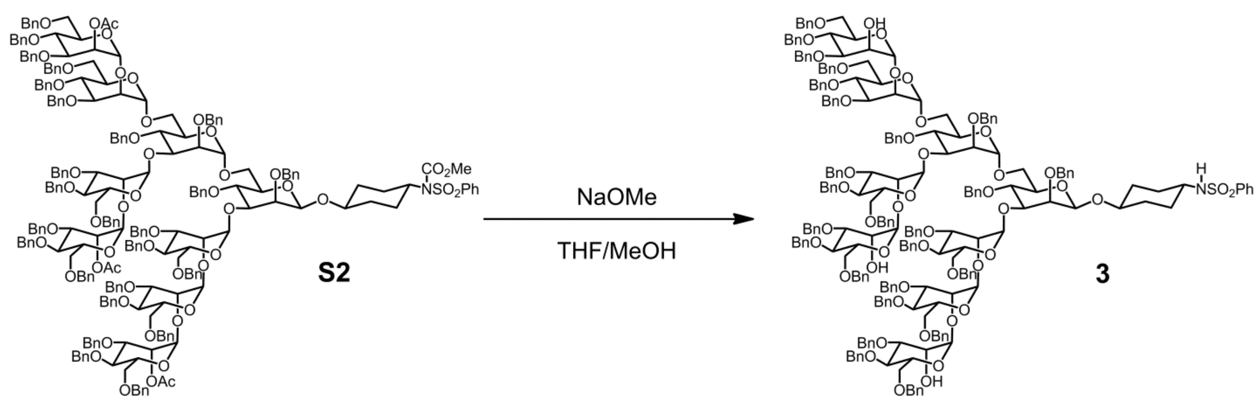
1.292 g (0.648 mmol, 1eq) **1** in a 50 ml flask was dissolved in 7.6 mL of DCM. Freshly flame-dried 4Å powdered molecular sieves were added, and this was allowed to stir for 30 minutes. The flask was cooled to -78° C, and 0.311 mL (1.944 mmol, 3 eq) triethylsilane was added, followed by dropwise addition of 0.286 mL (2.205 mmol, 3.4 eq) $PhBCl_2$. This was allowed to react for 15 minutes, and 1.5 mL (11 mmol, 17 eq) of triethylamine was added dropwise, followed by dropwise addition of 1.5 mL (37.6 mmol, 58 eq) methanol. The reaction was filtered through celite, washed with saturated $NaHCO_3$ solution, dried with $MgSO_4$, filtered, and concentrated in vacuo. Purification by flash chromatography with 40% ethyl acetate in hexanes gave 1.034 g (0.518 mmol, yield 80%) product **S1** as a white foam.. ^{1}H NMR (400 MHz, $CDCl_3$):7.91 (d, 2H, *J*= 8 Hz), 7.62 (t, 1H, *J*=7.43 Hz), 7.52 (t, 2H, *J*=7.63 Hz), 7.36 (d, 2H, *J*=7.6 Hz), 7.32-7.08 (multiple signals, 53H + residual $CDCl_3$), 5.54 (dd, 1H, *J*=2.7 Hz, *J*=1.7 Hz), 5.25(s, 1H), 5.16 (d, 1H, *J*=1.18), 5.07 (d, 1H, *J*=1.18), 4.94 (d, 1H, *J*=12.5), 4.827 (d, 1H, *J*=10.6 Hz), 4.820 (d, 1H, *J*=11.3 Hz), 4.73 (d, 1H, *J*=11.6 Hz), 4.69 (d, 1H, *J*=12.9 Hz), 4.66 (d, 1H, *J*=10.8 Hz), 4.58 (d, 1H, *J*=2.55 Hz), 4.55-4.31 (multiple signals, 15H), 4.23 (d, 1H, *J*=12.1 Hz), 4.09 (s, 1H), 4.03-3.76(multiple signals, 11H), 3.66 (s, 3H), 3.75-3.40(multiple signals, 11H), 3.12 (m, 1H), 2.30-2.16 (m, 2H), 2.12 (s, 3H), 2.12-2.05 (m, 1H), 1.98 (t, 1H, *J*=6.85 Hz), 1.93-1.85 (m, 1H), 1.84-1.76 (m, 2H), 1.54-1.41(m, 1H), 1.32-1.19 (m, 1H), 0.91-0.80 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, selected signals):170.1, 152.4, 140.1, 138.7, 138.5, 138.49, 138.47, 138.38, 138.36, 138.31, 138.02, 137.98, 137.94, 137.85, 133.3, 128.7, 128.4,128.3, 128.0, 127.8, 127.3, 127.1, 100.9, 100.6, 99.4, 99.3, 81.3, 79.1, 78.9, 78.2, 78.1, 76.1, 75.5, 75.4, 72.4, 72.3, 72.1,71.9,71.8, 69.5, 69.1, 68.6, 68.5, 62.2, 58.3, 53.4, 33.1, 31.3, 28.3, 21.1. IR (cm^{-1}): 3423, 3027, 2941, 2860, 1736, 1452, 1362, 1267, 1228, 1053, 737, 698. MS (ESI+): calcd. for $C_{117}H_{128}NO_{26}S^+$ [M + H$^+$] 1994.8445, found 1994.8464.

Fully protected cyclohexyl linked nonasaccharide **(S2)**



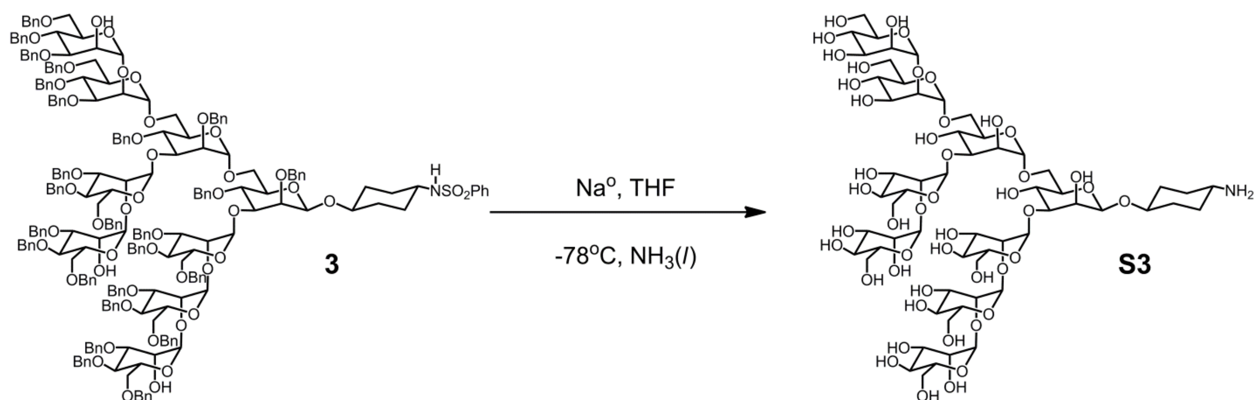
600.3 mg (0.300 mmol, 1 eq) of **S1** and 1092.7 mg (0.480 mmol, 1.6 eq) of **2** in a 50 mL flask were dissolved in toluene and cooled to -78 °C. Vacuum was applied and the cooling bath was removed and allowed to warm to room temperature as the toluene evaporated. This procedure was repeated twice. The dry residue was redissolved in 12 mL of acetonitrile, freshly flame-dried 4Å molecular sieves were added, and this was allowed to stir for 1 hour. The flask was then wrapped in foil, cooled to 0° C, and 305mg (0.374 mmol, 1.25 eq) Sinaÿ reagent, $[(p\text{-}BrC_6H_4)_3N^+ SbCl_6^-,]$ was added. This was allowed to react at 0° C for 30 minutes, at which time an additional 305 mg (0.374 mmol, 1.25 eq) $[(p\text{-}BrC_6H_4)_3N^+ SbCl_6^-]$ was added. This was allowed to react at 0° C for 30 minutes, then at room temperature for 30 minutes. After this time, 1 mL triethylamine was added, and the reaction was filtered through celite and concentrated in vacuo. The crude residue was purified by flash chromatography with 1:3:1 ethyl acetate / hexanes / DCM to give 1015 mg (0.245 mmol, yield 81%,) **S2,** as an off white foam. ^1H NMR (400 MHz, $CDCl_3$): 7.87 (d, 2H, *J*=7.8 Hz), 7.56 (t, 1H, *J*=7.43 Hz), 7.47 (t, 2H, *J*=7.63 Hz), 7.40-6.94 (multiple signals, 125H + residual $CDCl_3$), 5.55 (s, 1H), 5.52 (s, 1H), 5.50 (s, 1H), 5.26 (s, 1H), 5.21 (s, 1H), 5.15 (s, 1H), 5.10 (s, 2H), 5.05 (s, 1H), 4.98 (s, 1H), 4.92 (s, 1H), 4.91(d, 1H, *J*=12.5 Hz), 4.87-3.25 (multiple signals, 99H), 3.50 (s, 3H), 3.16-3.08 (m, 2H), 2.20-2.11 (m, 2H), 2.11 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 1.99-1.89 (m, 1H), 1.87-1.77 (m, 1H), 1.76-1.62 (m, 2H), 1.37-1.07 (m, 3H), 0.91-0.76 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, selected signals): 170.1, 170.0, 152.3, 140.2, 138.7, 133.2, 128.7, 128.4, 126.4, 101.2, 100.5, 100.2, 99.7, 99.5, 99.1, 99.0, 97.6, 81.8, 79.7, 76.0, 71.1, 70.8, 69.5, 58.3, 53.3, 33.1, 31.5, 28.3, 21.1. IR (cm^{-1}): 3030, 2925, 2869, 1740, 1496, 1453, 1362, 1234, 1052, 735, 697. MS (MALDI): calcd. for $C_{249}H_{265}NNaO_{53}S$ $[M+Na]^+$ 4171.769, found 4171.870.

Partially deprotected cyclohexyl linked nonasaccharide **(3)**



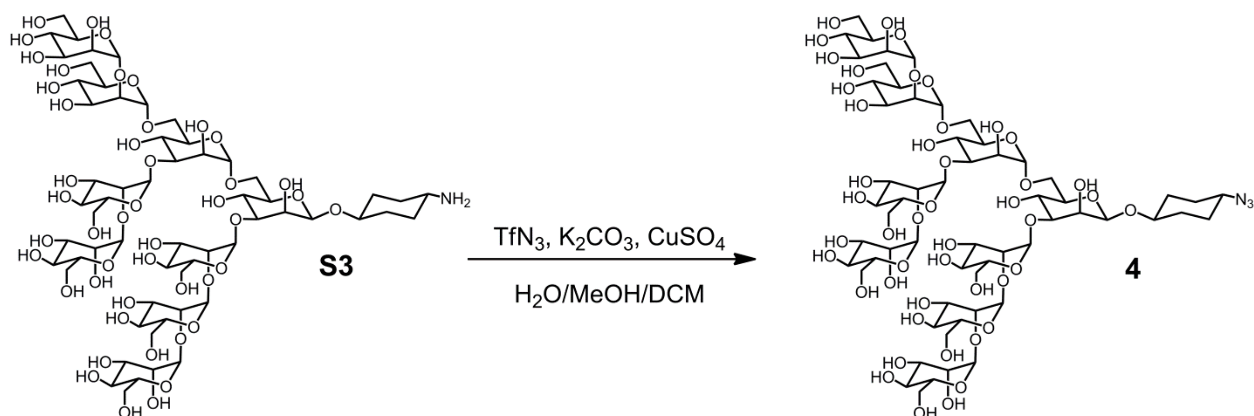
995.0 mg (0.240 mmol 1 eq) of **S2** was dissolved in 14 ml THF and 7 ml MeOH in a 50 ml flask. 0.275 ml (1.20 mmol, 5 eq) of a 25% NaOMe solution in MeOH was added. The reaction was left stirring at RT under N_2 for 13 hrs until reaction was completed based on LCMS and TLC monitoring. Upon completion, Amberlite IR 120 H+ resin was used to quench the reaction to pH~7. Care was taken to ensure the pH did not drop below 5. The Amberlite resin was filtered from the reaction and the filtrate was condensed in vacuo. The crude orange residue was purified by flash chromatography with 1:2:1 ethyl acetate / hexanes / DCM to give 774 mg (0.195 mmol, yield 81%) **3,** as a white foamy solid. ^{1}H NMR (400 MHz, $CDCl_3$): 7.78 (d, 2H, *J*=7.8Hz), 7.47 (t, 1H, *J*=7.63 Hz), 7.39 (t, 2H, *J*=7.24 Hz), 7.40-7.02 (multiple signals, 124H + residual $CDCl_3$), 7.00 (t, 1H, *J*=7.43 Hz), 5.29 (s, 1H), 5,17 (s, 3H), 5.14 (s, 1H), 5.04 (s, 1H), 5.03, (s, 1H), 4.97 (s, 1H), 4.87 (d, 1H, *J*=12.5Hz), 4.84-4.76 (multiple signals, 8H), 4.74 (s, 1H), 4.70-4.32 (multiple signals, 37H), 4.28 (d, 1H, *J*=12.2Hz), 4.25 (d, 1H, *J*=11.4Hz), 4.19 (d, 1H, *J*=12.1Hz), 4.15-3.34 (multiple signals, 53H), 3.26-3.12 (m, 2H), 23.01-2.90 (m, 2H), 2.36 (dd, 2H, *J*=7.8Hz, *J*=2.0Hz), 1.97-1.87 (m, 1H), 1.72-1.62 (m, 1H), 1.52 (d, 2H, *J*=9.4Hz), 1.24-1.13 (m, 2H), 1.07-0.96 (m, 2H), 0.91-0.75 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, selected signals): 141.6, 137.8, 132.1, 128.8, 128.5, 127.9, 127.1, 126.8, 126.7, 101.4, 101.1, 101.0, 100.8, 100.6, 100.3, 99.4, 96.1, 80.0, 79.9, 78.7, 75.6, 73.7, 70.7, 68.2, 51.6, 32.3, 31.2, 30.8, 30.3, 29.7. IR (cm^{-1}): 3562, 3062, 3030, 2928, 2866, 1880, 1815, 1496, 1453, 1361, 1209, 1050, 909, 734, 696. MS (MALDI): calcd. for $C_{241}H_{257}NNaO_{48}S$ $[M+Na]^+$ 3987.732, found 3987.807.

Fully deprotected cyclohexyl linked nonasaccaride amine **(S3)**



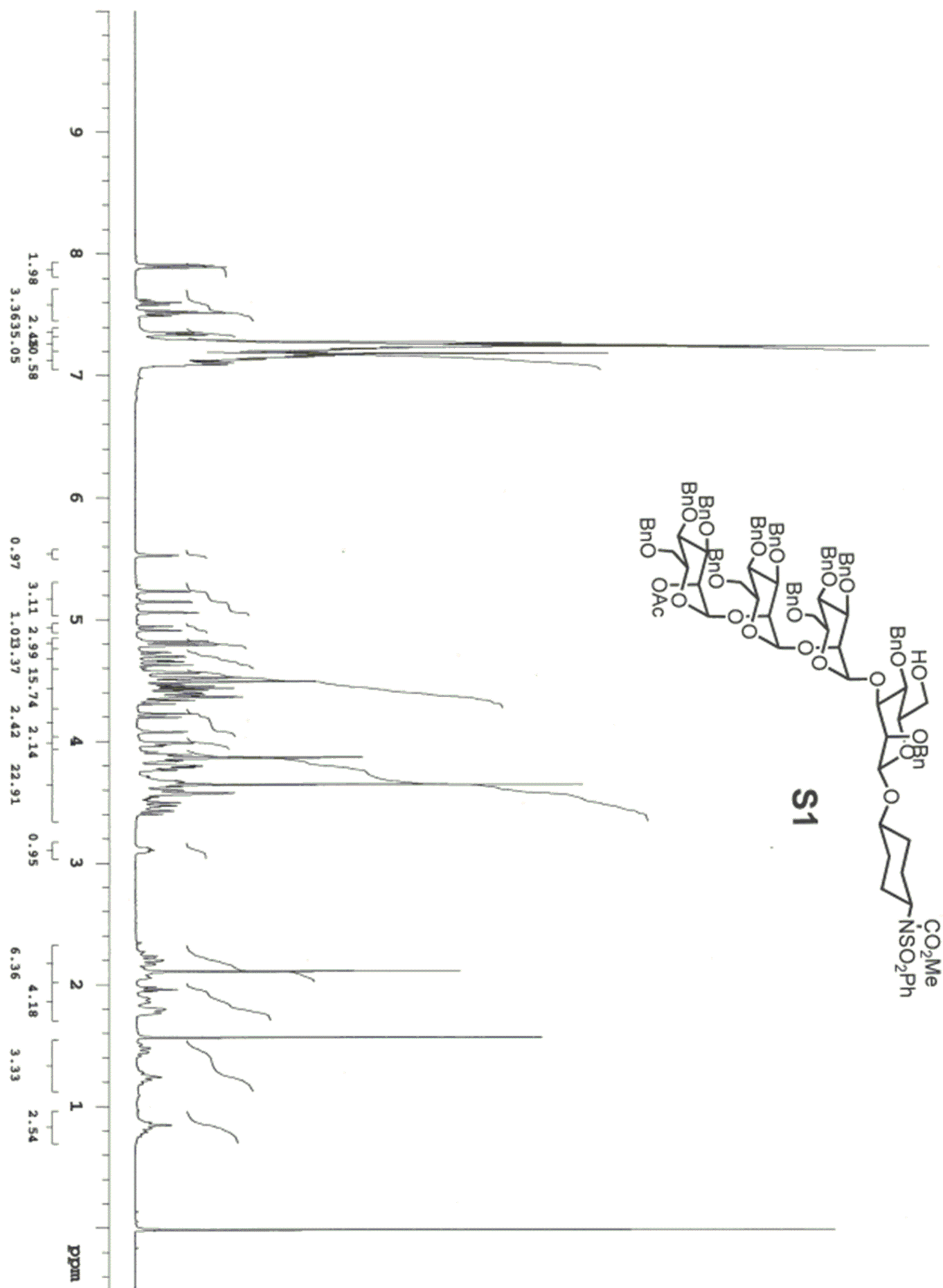
Along with a stream of N_2, ammonia gas was condensed against a -78° C coldfinger into a -78° C-cooled 500 mL 3-necked flask until 200ml had accumulated. 1.34 g (58.5 mmol) Na° was then added, and the resulting blue solution was left to stir for 1 hr. 774 mg (0.195 mmol) of **3** in 3 mL of THF was then added. The reaction progress was monitored by direct infusion ESI MS.. After 5 hrs, 4.3 g (80.47 mmol) NH_4Cl was added to quench the reaction. At this time, the cooling bath was removed and the ammonia was blown off under a stream of N_2. The crude white solids were dissolved in water and desalted by passage through a Biogel P-2 size exclusion column to give 432 mg of crude compound **S3** (>100% yield). ^{1}H NMR (400 MHz, D_2O): 5.42 (s, 1H), 5.35 (s, 1H), 5.32 (s, 1H), 5.16 (s, 1H), 5.05 (s, 3H), 4.87 (s, 1H), 4.81 (s, 1H), 4.17 (s, 1H), 4.13-3.60 (multiple signals), 3.57-3.51 (m, 1H), 3.25-3.15 (m, 1H), 2.18-2.05 (m, 4H), 1.55-1.33 (m,4H). ^{13}C NMR (100 MHz, D_2O, selected signals):105.2, 105.1, 103.6, 103.5, 102.5, 101.2, 100.9, 84.0, 82.1, 79.3, 76.9, 75.6, 74.0, 73.6, 72.3, 69.7, 64.0, 52.0, 46.2, 33.2, 32.0, 31.0, 30.9 IR (cm^{-1}): 3265, 2916, 1584, 1348, 1242, 1207, 1124, 1023, 967, 807. MS (ESI+): calcd. for $C_{60}H_{104}NO_{46}$ $[M+H]^+$ 1574.5829, found 1574.5752.

Cyclohexyl linked nonasaccaride azide **(4, Man_9Azide)**

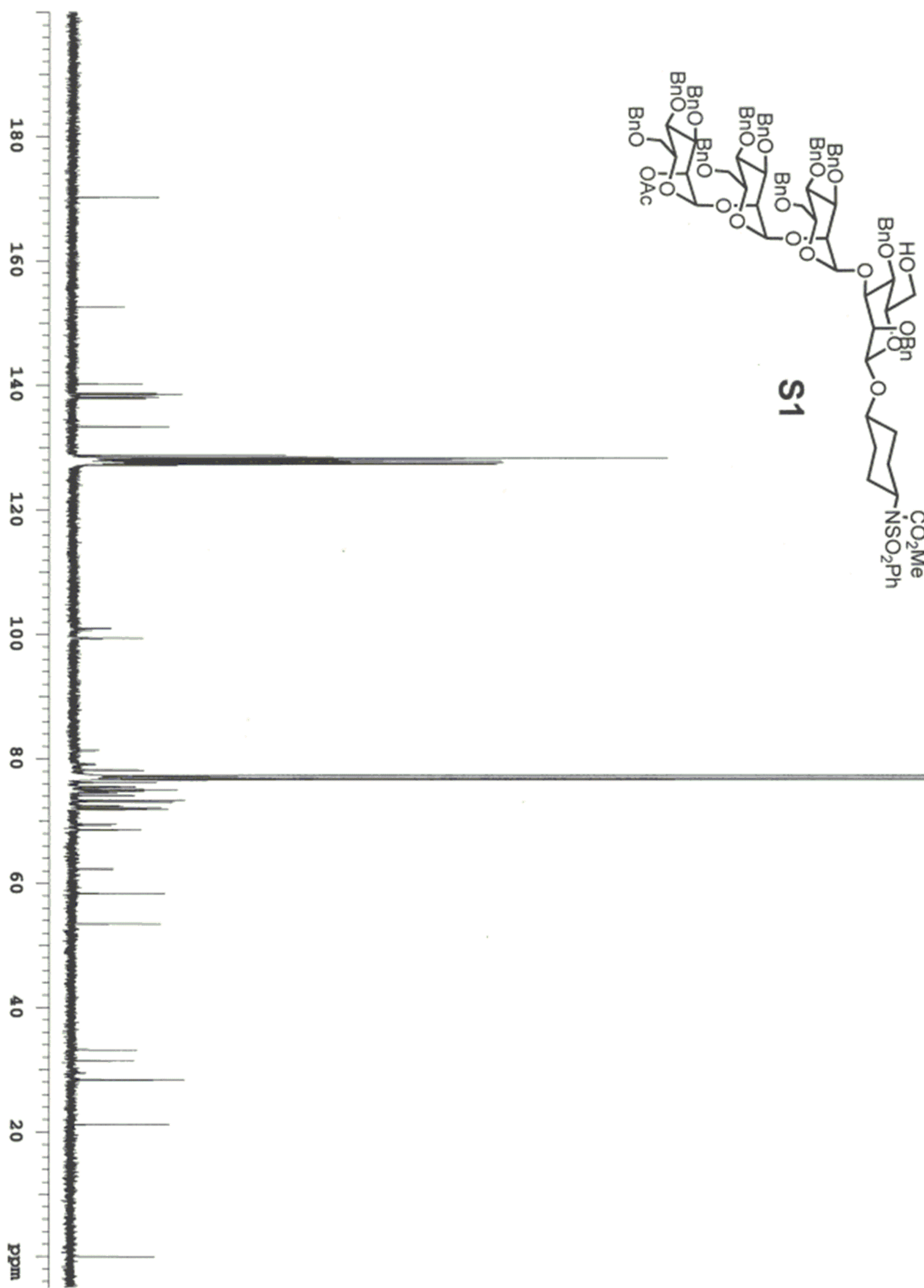


Into a 25 mL flask containing 145 mg (0.0921 mmol, 1 eq) of crude amine **S3** was added 2.4 mL water, 92 uL (0.00921 mmol, 0.1 eq) of 0.1M aqueous $CuSO_4$ and 38 mg (0.276 mmol, 3eq) of K_2CO_3. 4.8 mL of MeOH was added, followed by the addition of 1.3 mL (0.276 mmol, 3.0 eq) of freshly prepared 0.2M TfN_3 in DCM.[4] The resulting homogeneous reaction was left to stir at RT for 1 hr until complete conversion was observed by direct infusion ESI-MS. The reaction was quenched with 100 mg (1.190 mmol, 13 eq) solid $NaHCO_3$ and concentrated *in vacuo*. The residue was desalted on a Biogel P-2 size exclusion column. Reversed phase HPLC purification (Column: Waters Xbridge Prep, C_{18}, 5μm, OBD, 19x250mm, 156 Å pore diameter. Method: 17mL/min flow rate. A= H_2O, B= 30% MeOH in H_2O. 98.1% A to 60% A over 8 min, then 60% to 5% A over 22 minutes. Desired product eluted at 18.5 minutes.) providing 88 mg (0.0553 mmol, 60%) of Man-9-Azide(**4**) as a glassy solid. ^{1}H NMR (400 MHz, D_2O): 5.42 (s,1H), 5.34 (s, 1H), 5.31 (s,1H), 5.16 (s, 1H), 5.05 (s, 3H), 4.87 (s, 1H), 4.80 (s, 1H), 4.16 (s, 1H), 4.13-3.60 (complex region), 3.57-3.47 (m, 2H), 2.10-1.97 (m, 4H), 1.53-1.35 (m, 4H).^{13}C NMR (100 MHz, D_2O, selected signals):105.2, 105.2, 105.1, 103.6, 103.5, 102.4, 100.9, 84.0, 81.9, 79.2, 76.9, 75.6, 73.7, 72.3, 61.7, 32.9, 31.1 IR (cm^{-1}): 3306, 2927, 2099, 1676, 1368, 1126, 1046, 812. MS (ESI+): calcd. for $C_{60}H_{101}N_3NaO_{46}$ $[M+Na]^+$ 1622.5554, found 1622.5885.

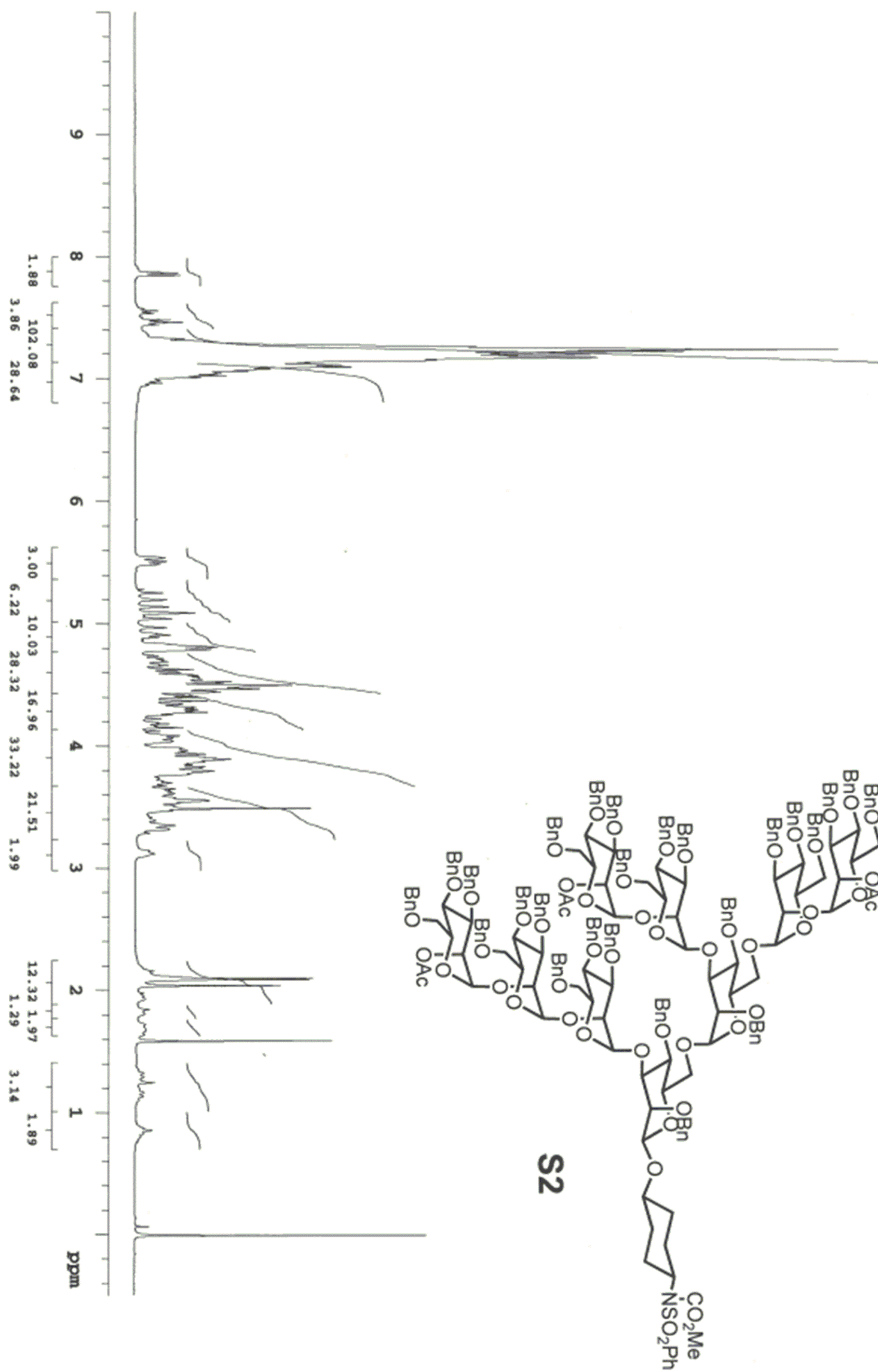
^{1}H NMR Spectrum of **S1** (400 MHz, $CDCl_3$)



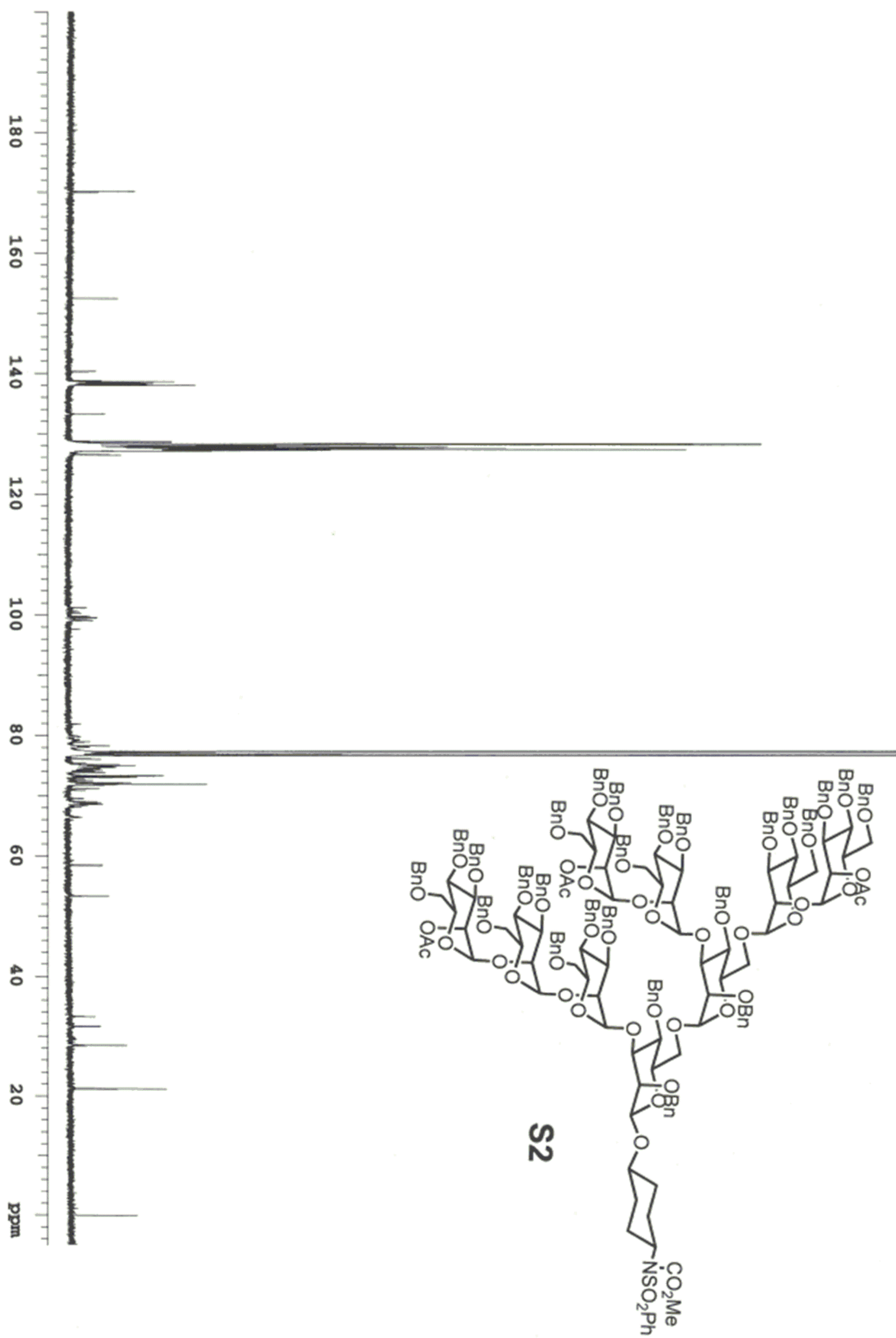
^{13}C NMR Spectrum of **S1** (100 MHz, $CDCl_3$)



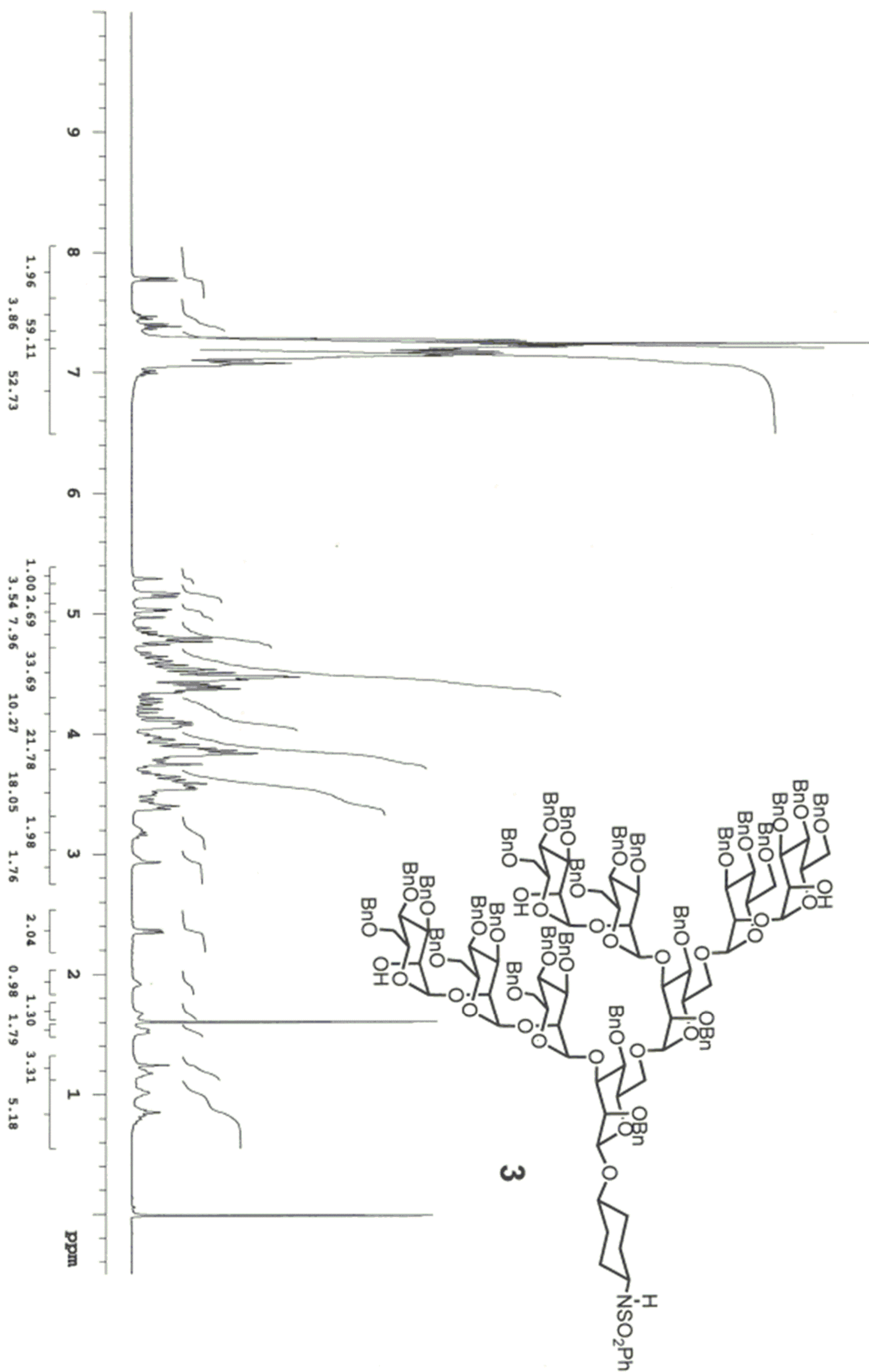
^{1}H NMR Spectrum of **S2** (400 MHz, $CDCl_3$)



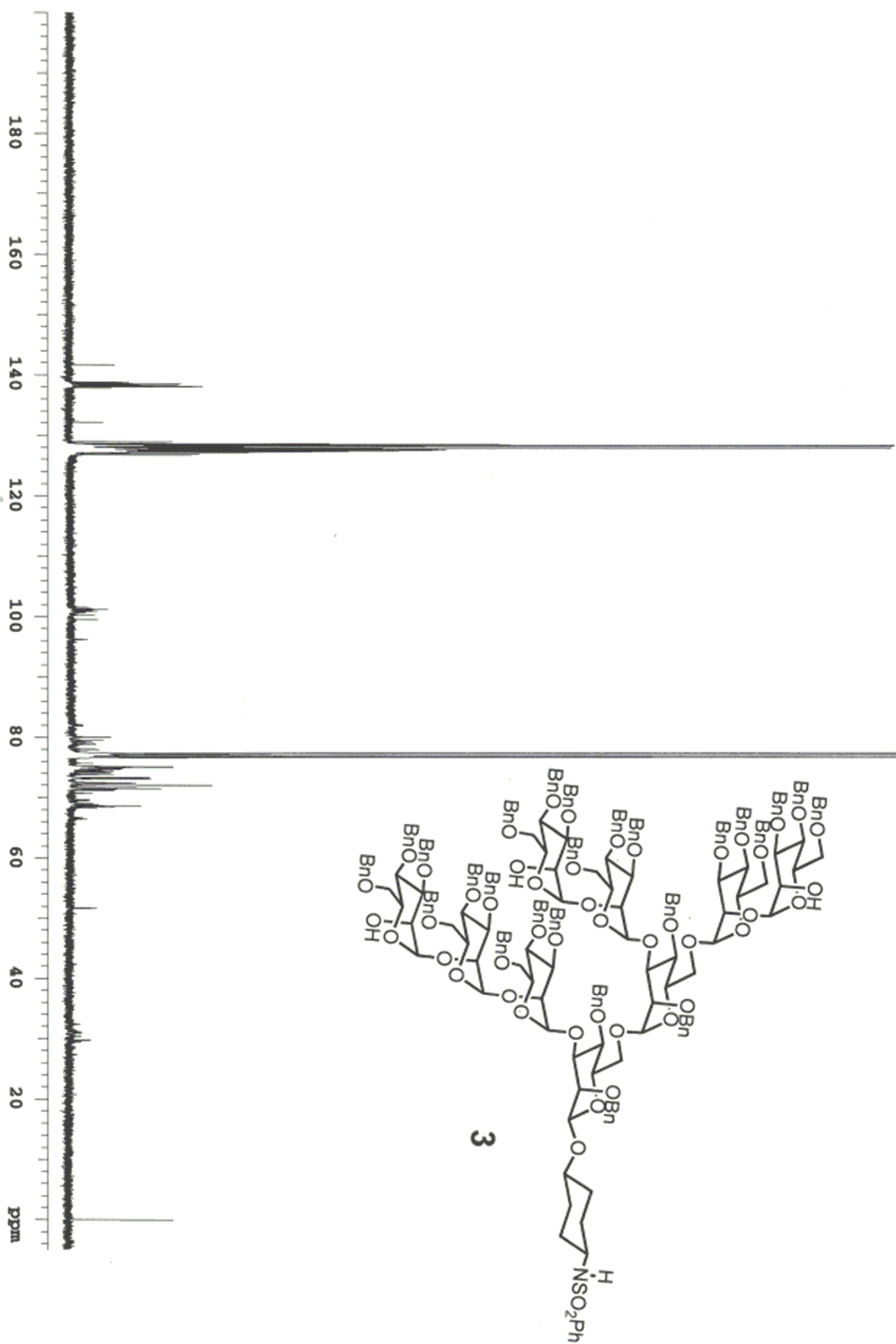
^{13}C NMR Spectrum of **S2** (100 MHz, $CDCl_3$)



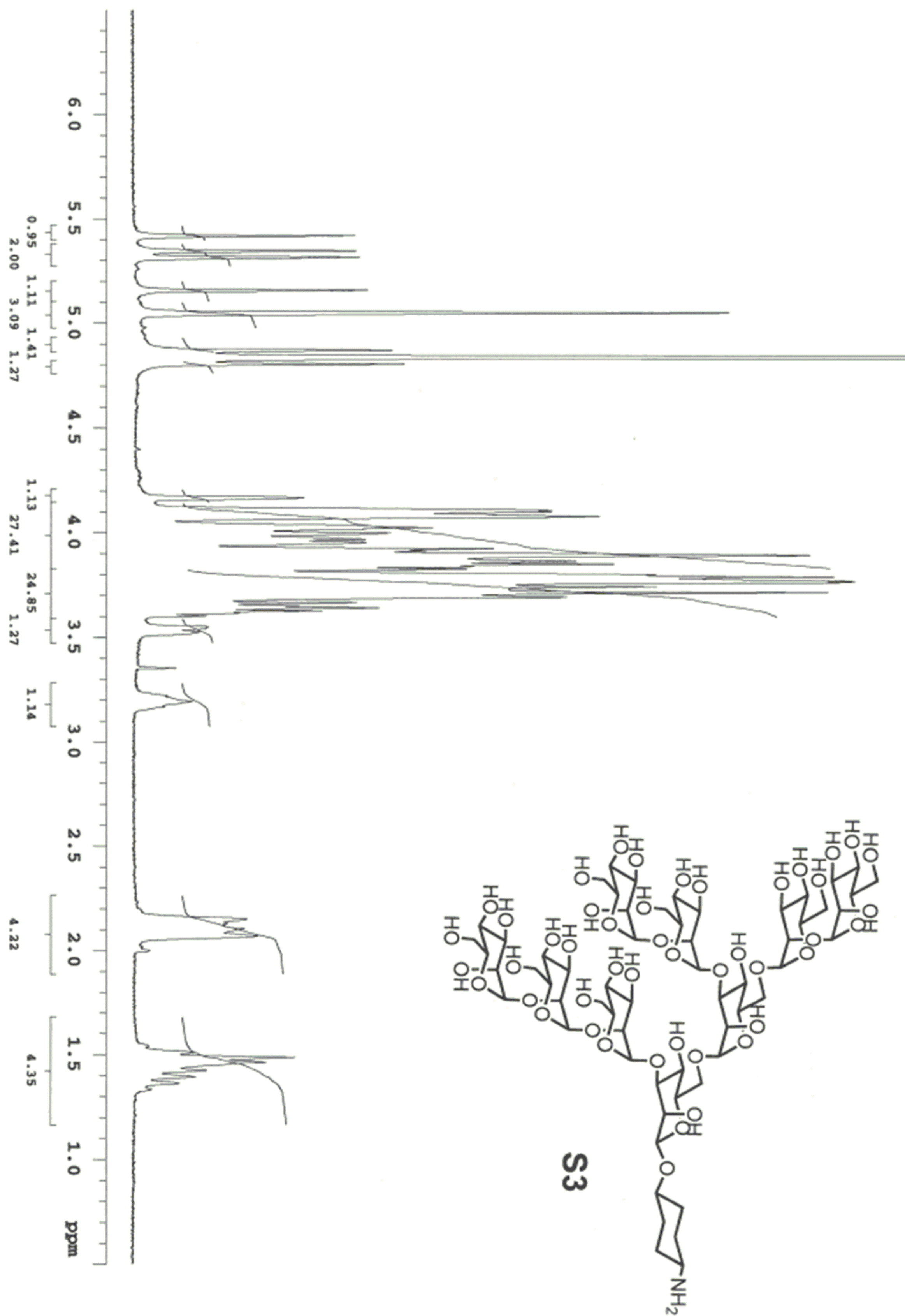
^{1}H NMR Spectrum of **3** (400 MHz, $CDCl_3$)



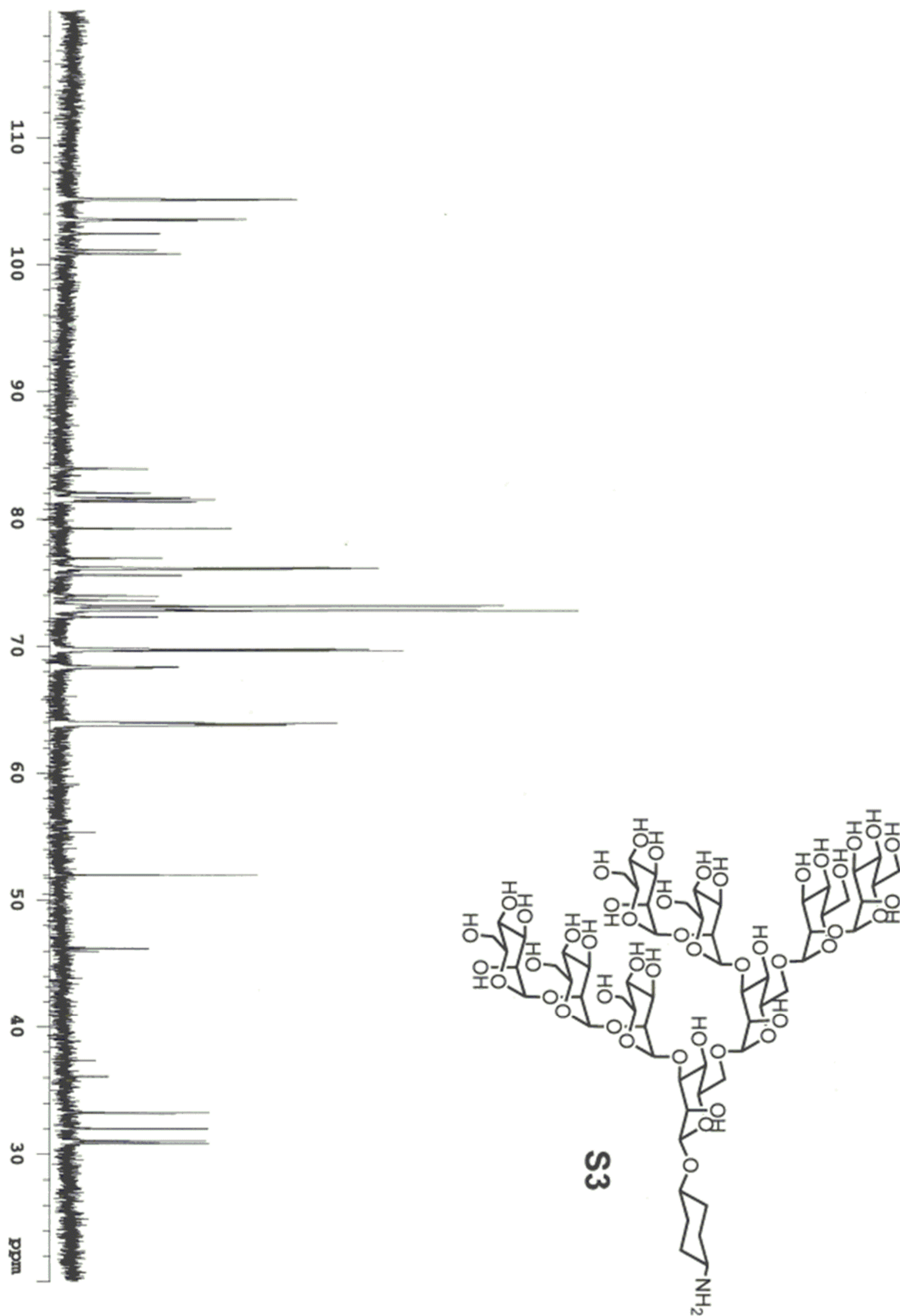
^{13}C NMR Spectrum of **3** (100 MHz, $CDCl_3$)



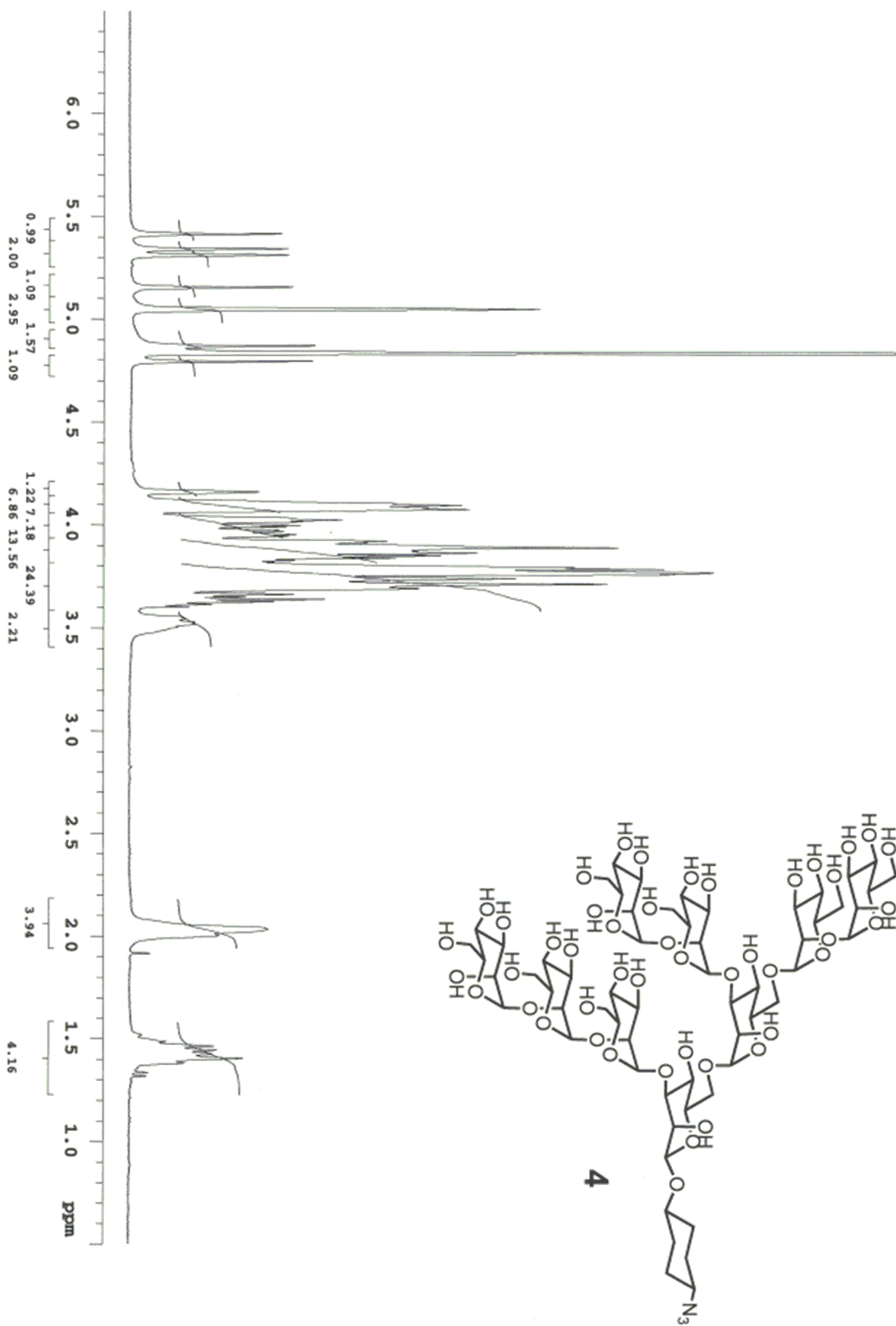
^1H NMR Spectrum of **S3** (400 MHz, D_2O)



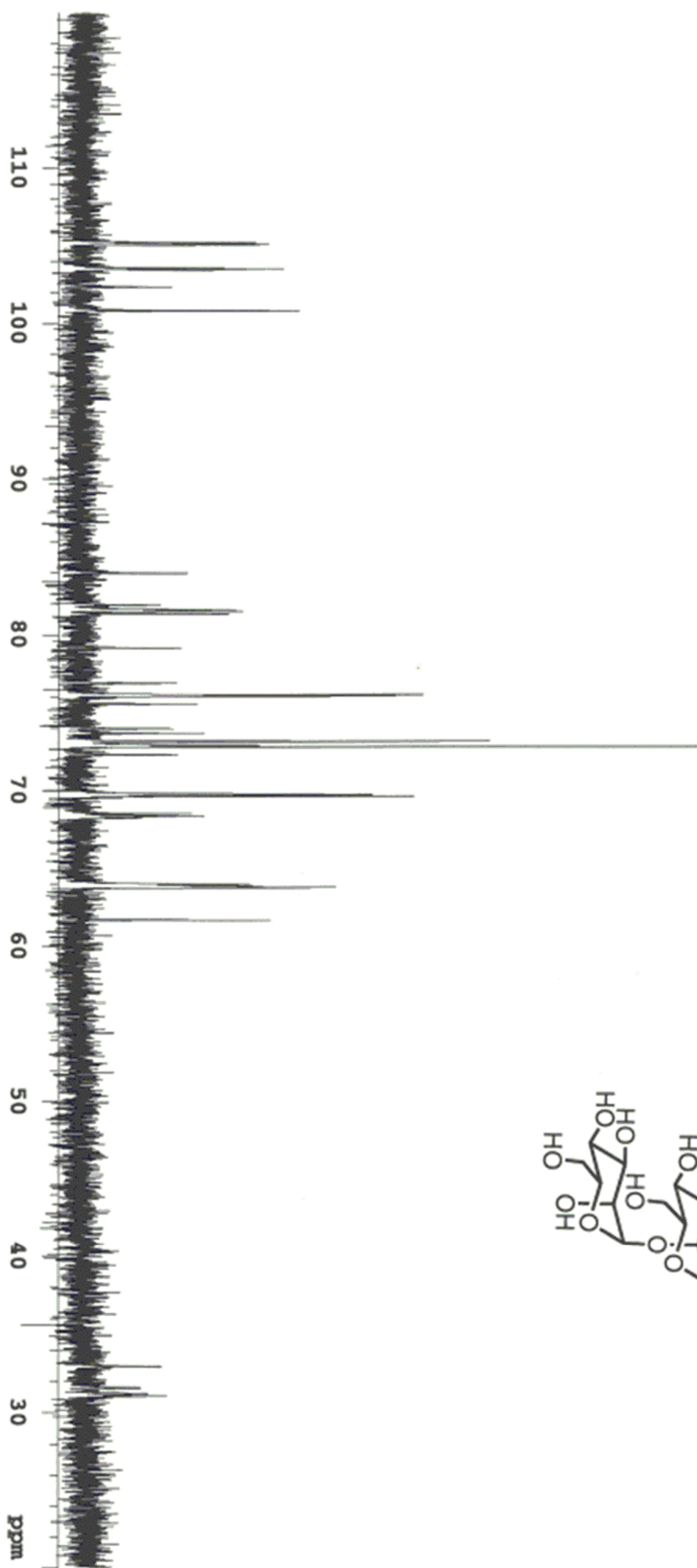
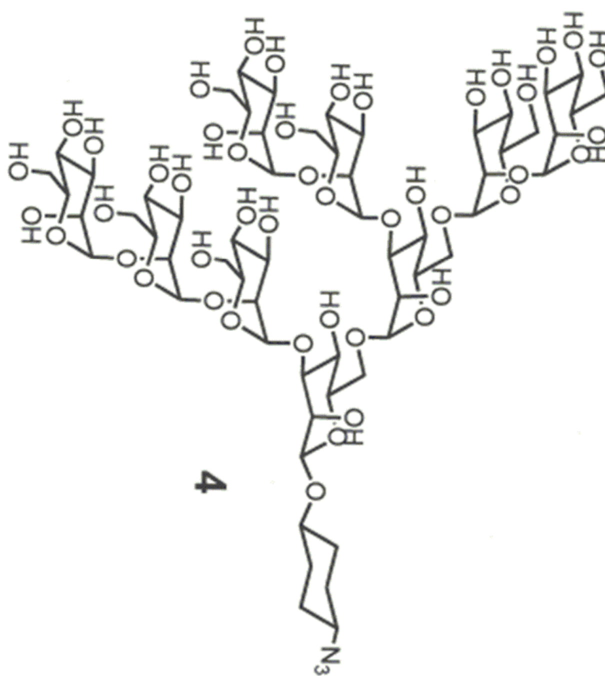
^{13}C NMR Spectrum of **S3** (100 MHz, D_2O)



^{1}H NMR Spectrum of **4 (Man$_9$Azide)** (400 MHz,D_2O)



^{13}C NMR Spectrum of **4 (Man$_9$Azide)** (100 MHz, D_2O)

References

(1) Stockley, P.G. Filter Binding Assays. from *DNA-Protein Interactions : Principles and Protocols;* Methods in Molecular Biology Series. **2001**, *148*, 1-11.

(2) MacPherson, I. S.; Temme, J. S.; Habeshian, S.; Felczak, K.; Pankiewicz, K.; Hedstrom, L.; Krauss, I. J. *Angew. Chem.-Int. Edit.* **2011**, *50*, 11238-11242.

(3) Geng, X.; Dudkin, V. Y.; Mandal, M.; Danishefsky, S. J. *Angew. Chem. Int. Ed.* **2004**, *43*, 2562-2565.

(4) Nyffeler, P. T.; Liang, C.-H.; Koeller, K. M.; Wong, C.-H. *J. Am. Chem. Soc.* **2002**, *124*, 10773-10778.