Supplemental Information

for

Activity-Based Protein Profiling of Retaining α-Amylases in Complex Biological Samples

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1. Supplemental Results and Discussion

From the medical perspective, it would be very attractive to measure α-amylase in human samples that allow to discriminate between disease and healthy patients. For instance, serum amylase level is often used as a biomarker of acute pancreatitis.[1-3] The normal range of serum amylase level for adults is usually between 15 international units (IU)/L to 110 IU/L (may vary between laboratories).[4] However, patients with acute pancreatitis present elevated α-amylase level in plasma, which can rise up to 4 to 6 times the upper limit of normal range. Here, we explored a potential application of our ABP in biomedical sector by analysing the detection of α-amylase in human plasma.

To determine the sensitivity of detection, we first explored the amount of enzyme detectable with the Cy5 ABP **1c** by spiking recombinant human salivary amylase at varying concentrations (0–1 unit) into 25 µL of normal human plasma (Figure S6, set C), followed by Concanavalin A (ConA) beads purification to clean the sample. Then the purified glycoproteins were labeled with 50 µM ABP **1c** at pH 5.0 for 4 h. Unfortunately, recombinant human salivary amylase can only be detected at the maximum concentration applied here (1 unit/25 µL plasma), which is much higher than the upper limit of amylase level in normal (0.003 unit/25 µL) or pancreatitis (0.017 unit/25 µL) plasma. Besides, all other purified glycoproteins in plasma were also labeled under this condition, which can further decrease the sensitivity of detection. In addition, two positive control experiments were also included by first incubating pure enzyme with 50 µM ABP **1c** at pH 5.0 for 4 h, with (Figure S6, set B) or without (Figure S6, set A) adding the final reaction mixture into ConA purified plasma. The detection limit of pure recombinant amylase with ABP **1c** (no plasma control, set A) is about 0.01 unit, which is just above the level of 0.017 unit/25 µL plasma of acute pancreatitis patients, but still too insensitive to the level in normal person.

In conclusion, the nonspecific labeling of all other purified glycoproteins together with the low probe potency points to a detection limit of 1 unit/25 µL plasma, meaning that these biochemical probes are not optimal for the detection of α-amylase in human plasma of patient suffering from pancreatitis.

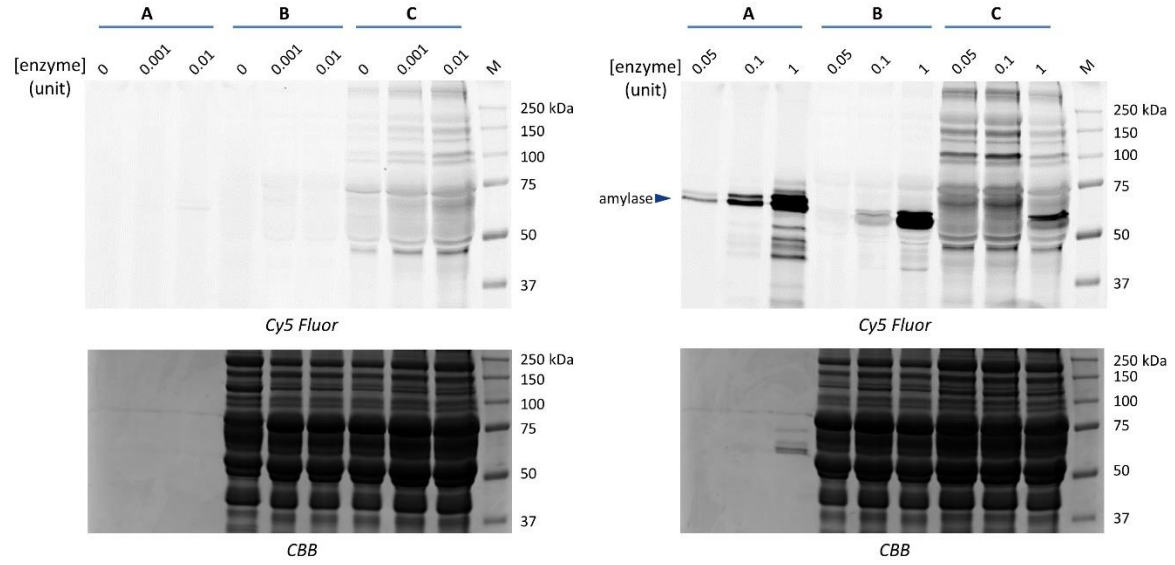


Figure S1. Determine the detection limit of α-amylase in human plasma with ABP **1c**. Set **A**: no plasma control, recombinant human saliva α-amylase (0–1 unit) was incubated with 50 µM ABP **1c** at pH 5.0 for 4 h; Set **B**: recombinant human saliva α-amylase (0–1 unit) was incubated with 50 µM ABP **1c** at pH 5.0 for 4 h, then the reaction mixture was added to ConA purified plasma; Set **C**: recombinant human saliva α-amylase (0–1 unit) was first spiked into 25 µL normal human plasma, followed by ConA purification. The purified glycoproteins were then incubated with 50 µM ABP **1c** at pH 5.0 for 4 h.

2. Supplemental Figures and Tables

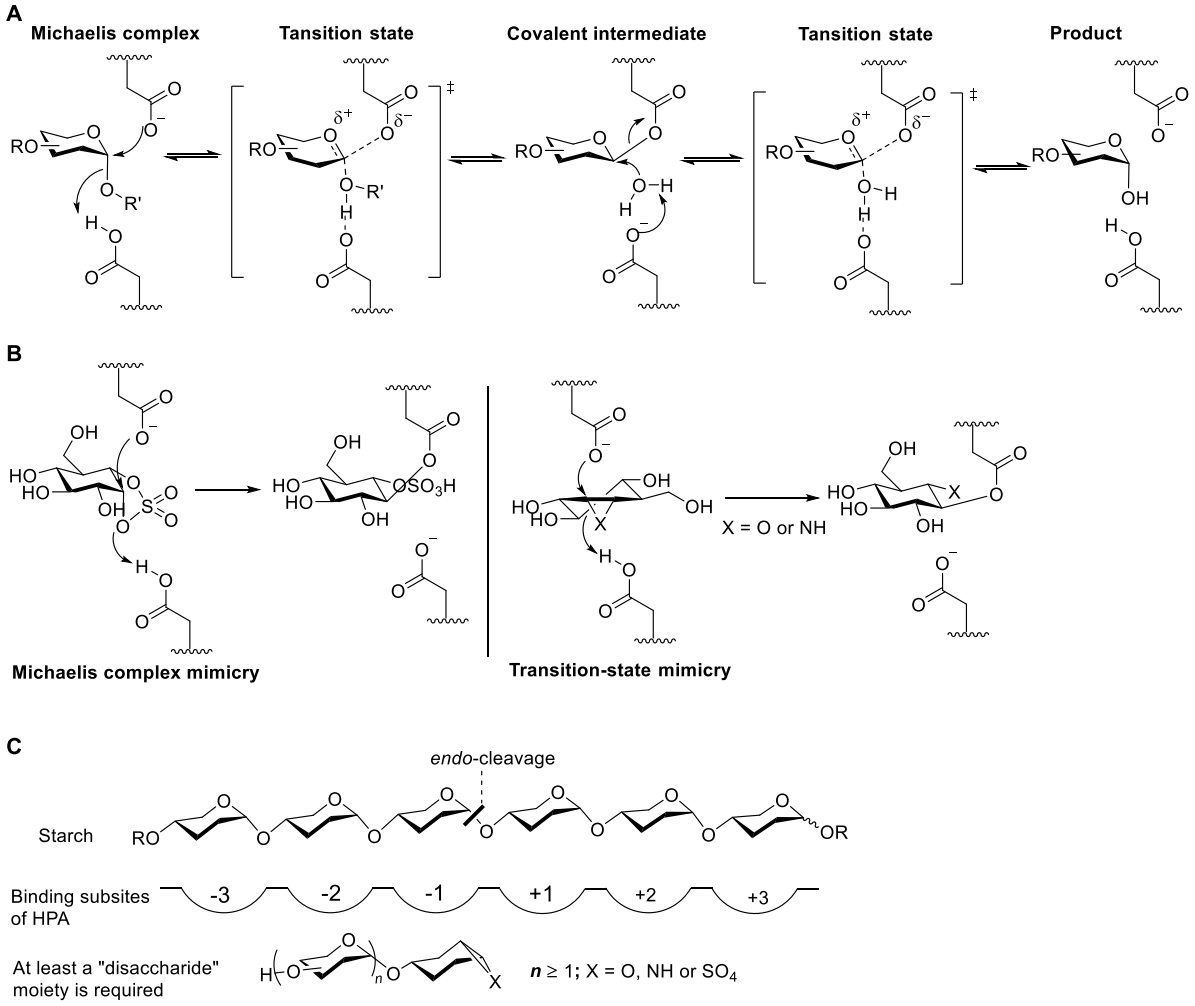


Figure S2. A) General Koshland double displacement mechanism employed by retaining α-glycosidases; B) Covalent irreversible inactivation of α-glucosidases by mechanism-based inhibitors; C) The binding subsites in the active site of HPA and the expected cleavage point for a normal starch substrate indicate the structure of the required inhibitors and ABPs.

In our initial studies on human saliva, we did labeling of untreated saliva, saliva supernatant and saliva pellet with ABP **1c**. For labeling, untreated saliva (24 µL per sample), saliva supernatant (24 µL per sample), or saliva pellet (55 µg total protein per sample, diluted with assay buffer pH 7.0 to a final 24 µL volume) was incubated with 6 µL of ABP **1c** at intended concentrations in assay buffer pH 7.0 at 37 °C for 1 h. As is shown in Figure S2, the detection limit of α-amylase in all these three samples is very similar (10 µM of **1c**) although slightly stronger labeling was observed in untreated saliva and saliva supernatant. However, the protein concentration of human saliva varies among different individuals, to facilitate the quantification of the protein amount needed, and to study the optimal labeling pH, the concentrated saliva pellet (16.9 µg/µL) was considered for further studies.

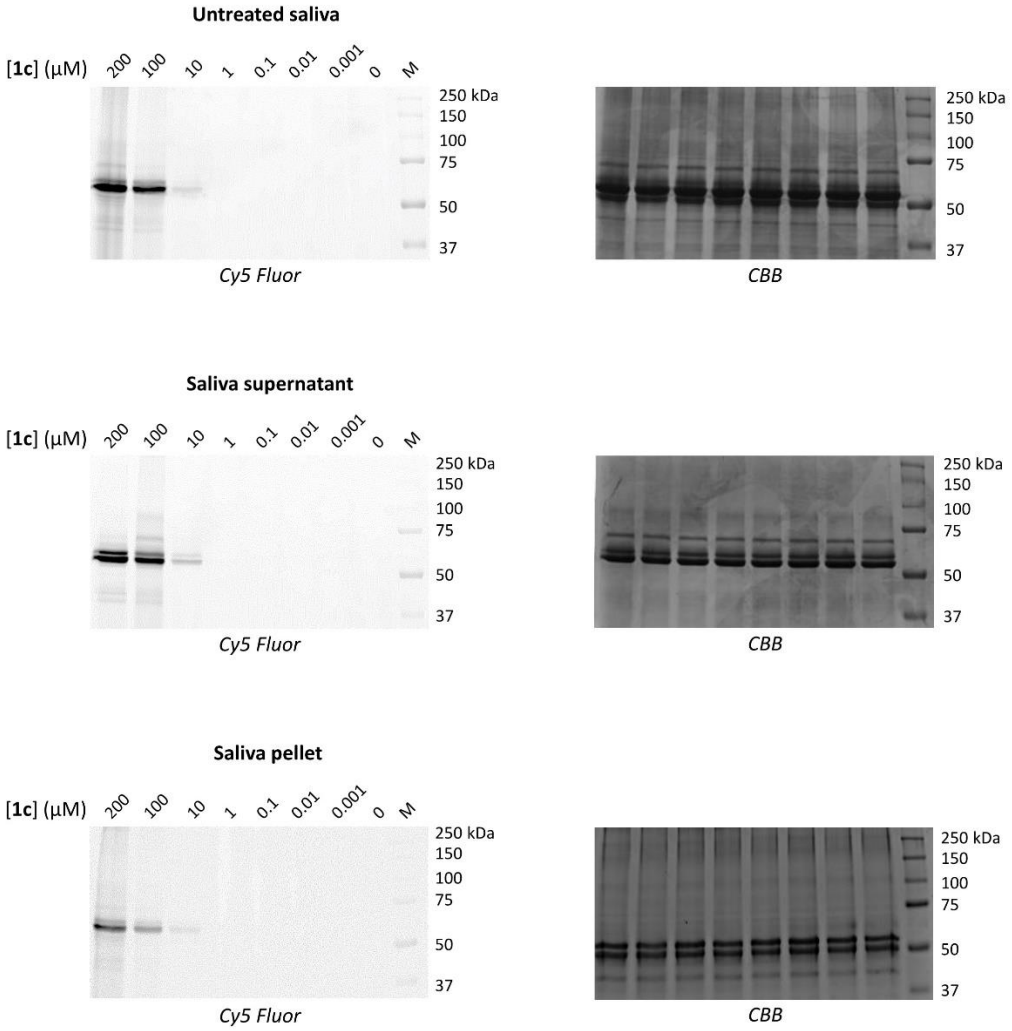


Figure S3. Fluorescent labeling of untreated human saliva (24 µL), saliva supernatant (24 µL) and saliva pellet (55 µg total protein) with different concentrations of ABP **1c** (1 h, 37 °C, pH 7.0).

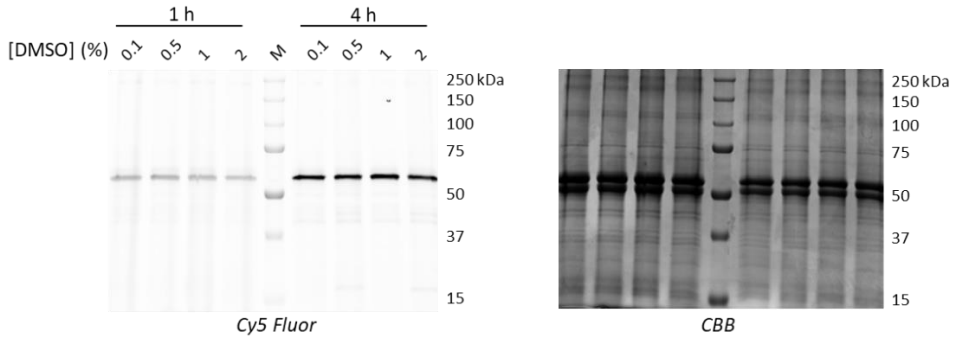


Figure S4. Fluorescent labeling of concentrated human saliva (55 µg total protein) with ABP **1c** (10 µM, 37 °C, pH 5.0, 1 h or 4 h) in the presence of varying concentrations of DMSO (v/v).

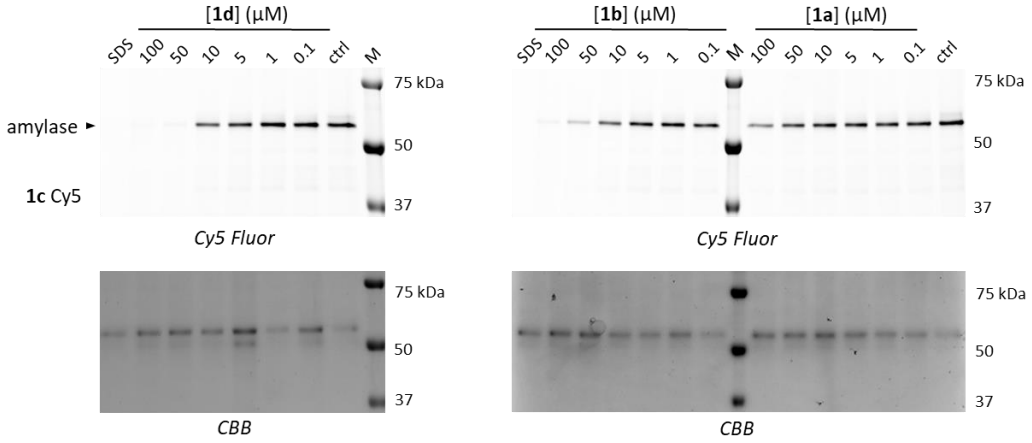


Figure S5. Competitive assay of human saliva. The sample (5 µg/10 µL of total protein) was pre-incubated with **1a**, **1b** or **1d** at different concentrations (4 h, 37 °C, pH 5.0), followed by labeling with 10 µM of ABP **1c** (2 h, 37 °C, pH 5.0).

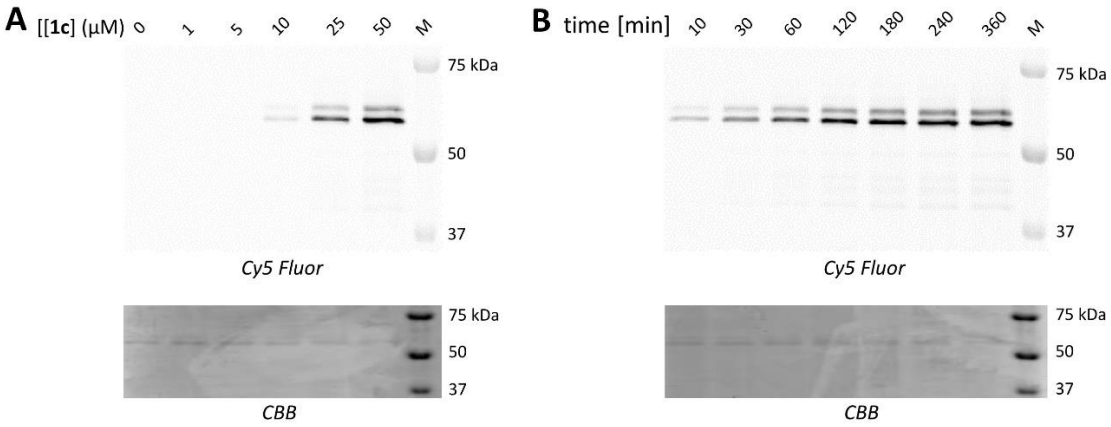


Figure S6. A) Concentration-dependent labeling of recombinant human saliva α-amylase (0.2 unit) with ABP **1c** under optimal pH 5.0 after incubation 4 h at 37 °C; B) Time-dependent labeling of recombinant human saliva α-amylase with 50 µM of ABP **1c** at pH 5.0 and 37 °C.

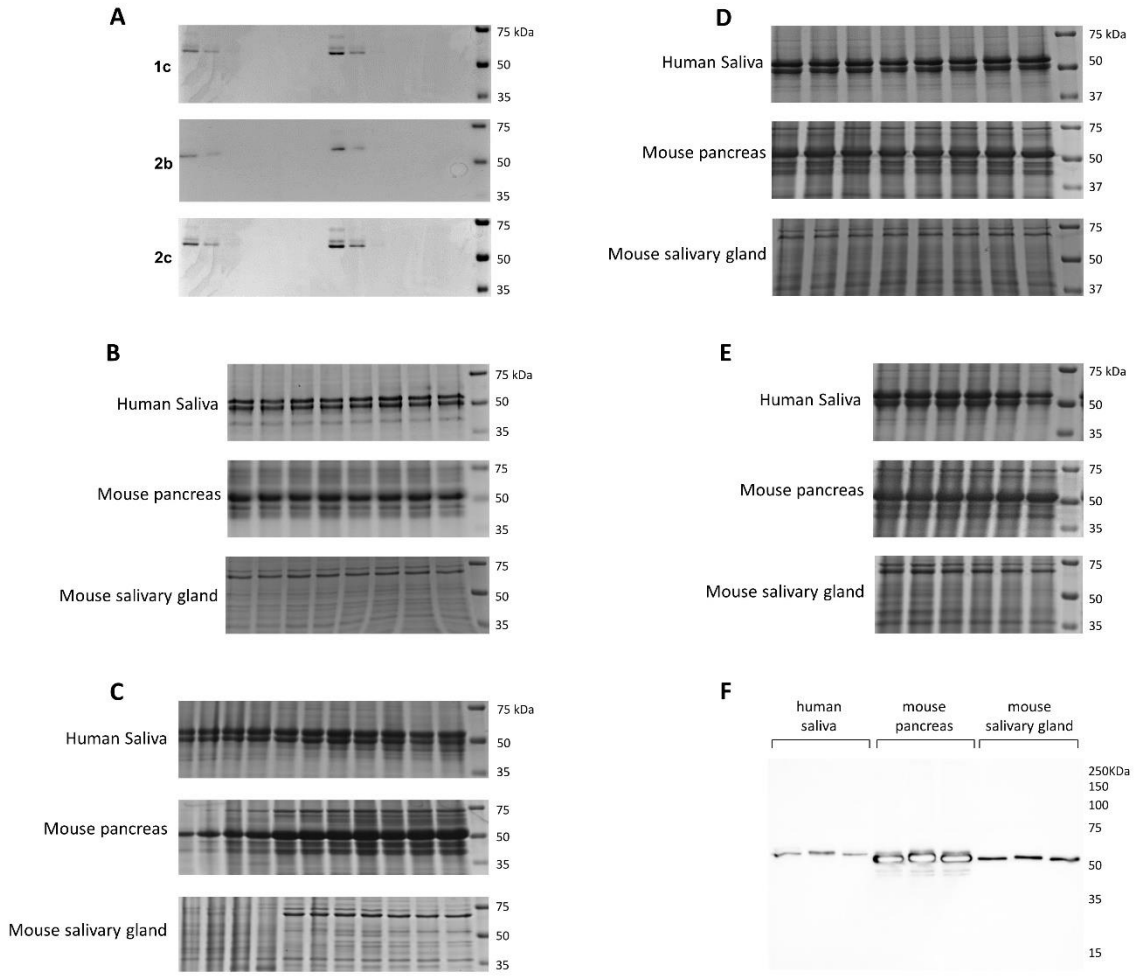


Figure S7. Coomassie Brilliant Blue staining and full-length image of western blot of gels presented in main text Figure 4.

Table S1. Data collection and refinement statistics (molecular replacement)

	Taka-amylase	Taka-amylase + **1a**	Taka-amylase + **2a**	Taka-amylase + **3a**	Taka-amylase + **1b**
PDB ID	6YQ7	6YQ9	6YQA	6YQB	6YQC
Data collection					
Space group	$P12_11$	$P12_11$	$P12_11$	$P12_11$	$P12_11$
Cell dimensions					
a, *b*, *c* (Å)	65.2, 103.1, 75.2	65.2, 103.1, 75.4	65.7, 103.3, 75.5	65.6, 102.9, 75.6	64.8, 99.0, 75.5
α, β, γ (°)	90.0, 103.6, 90.0	90.0, 103.6, 90.0	90.0, 103.8, 90.0	90.0, 103.8, 90.0	90.0, 103.6, 90.0
Resolution (Å)	59.65-1.58 (1.61-1.58)	103.10-1.55 (1.58-1.55)	103.32-1.67 (1.70-1.67)	59.78 -1.50 (1.53-1.50)	73.33-1.35 (1.37-1.35)
$CC_{1/2}$	0.99 (0.73)	0.99 (0.87)	0.99 (0.72)	0.99 (0.92)	0.99 (0.76)
I / σI	5.7 (1.7)	11.8 (2.1)	9.6 (1.1)	10.3 (1.6)	7.5 (1.0)
Completeness (%)	98.4 (97.0)	99.9 (99.8)	100 (100)	98.7 (97.0)	95.5 (73.0)
Redundancy	4.2 (4.2)	4.0 (3.8)	4.2 (4.2)	6.8 (6.8)	3.9 (2.7)
Refinement					
Resolution (Å)	1.58	1.55	1.67	1.50	1.35
No. reflections	129,847	139,928	113,215	153,221	192,746
R_{work} / R_{free}	0.16/0.19	0.15/0.17	0.17/0.20	0.16 / 0.19	0.18/0.20
No. atoms					
Protein	7,441	7,480	7,404	7,464	7,468
Ligand/ion	40/2	90/2	105/2	106/2	151/2
Water	518	815	519	785	687
B-factors					
Protein	21	18	28	22	18
Ligand/ion	36/16	23/13	34/23	27/17	22/14
Water	28	28	34	31	28
R.m.s. deviations					
Bond lengths (Å)	0.01	0.01	0.01	0.01	0.01
Bond angles (°)	1.7	1.8	1.7	1.7	1.7
*ligands in the uncomplexed structure are derived from ethylene glycol/Calcium					

Table S4. α-Amylases previously identified in the starch secretomes of Aspergillus nidulans.

Protein (name)	**CBM**	**Protein family**	**Uniprot**	**Molecular mass (kDa)***
α-amylase (AmyA)		GH13_1	G5EB45	52.6
α-amylase (AmyB)	CBM20	GH13_1	G5EAT0	66.7
α-amylase (AmyF)		GH13_1	Q5B7U2	48.3
*The mass is of the mature protein lacking the predicted signal peptide using Signal P (http://www.cbs.dtu.dk/services/SignalP/)				

3. Biochemical Experiments

Materials

Recombinant human saliva α-amylase (Type XIII-A) was obtained as a lyophilized power from Sigma-Aldrich (Product Number: A1031). Taka-amylase was purchased as a solid power from Sigma-Aldrich (Product Number: 86247). Anti-Pancreatic alpha amylase antibody was purchased as a liquid from Abcam (Product Number: ab199132). Concanavalin A beads was purchased from Sigma-Aldrich (Product Number: C9017). Mouse tissue were isolated according to guidelines approved by the ethical committee of Leiden University (DEC#13191). All the tissue lysates were prepared in potassium phosphate lysis buffer(25 mM in pH 6.5, supplemented with 0.1% (v/v) Triton X-100 and protease inhibitor 1x cocktail (Roche)) via homogenization with silent crusher S equipped with Typ 7 F/S head (30 rpm x 1000, 3 × 7 sec) on ice and lysate concentration was determined with Bicinchoninic acid (BCA) Protein Assay Kit (PierceTM).[5] The protein fractions were stored in small aliquots at -80 °C until use. BCA protein assay kit was acquired from Thermo Fisher Scientific. Trypsin was commercially available from Promega. Dynabeads™ MyOne™ Streptavidin T1 was obtained from Invitrogen. Dithiothreitol (DTT) was obtained from Biochemica and Iodoacetamide (IAA) was obtained from Sigma Aldrich. All other chemicals were obtained from standard commercial sources.

Preparation of human saliva sample

Unstimulated whole saliva sample (20 mL) from a normal subject was expectorated into a 50-mL polypropylene tube. The tube was centrifuged at 4 °C for 10 min at 4000 rpm. Supernatant was collected into a new, ice cold 50-mL tube and pellets were resuspended into 1 mL ice-cold KPi lysis buffer (25 mM phosphate buffer pH 6.5 supplemented with 0.1% (v/v) Triton X-100 and protease inhibitor 1x cocktail (Roche)). To homogenize, the suspension was sonicated for 60 seconds at 60% amplitude strength on ice. Obtained lysates were stored in aliquots at -20 °C. Protein concentration of lysate was determined by using the BCA method.[5]

Purification of Taka-amylase

To purify Taka-amylase for crystallization, 10 g of powder was dissolved in 200 mL of buffer A (20 mM Tris pH 7.5, 5 mM $CaCl_2$). This suspension was concentrated and washed extensively with buffer A using 10 kDa cutoff Vivaspin centrifugal filter columns (Sigma-Aldrich). Concentrated protein was further purified using a gel-filtration column (HiLoad 16/600 Superdex 75 pg; GE Healthcare), which had been pre-equilibrated with buffer B (20 mM Tris pH 7.5, 5 mM $CaCl_2$, 200 mM NaCl). Peak fractions were pooled, concentrated and washed with buffer A. Protein stocks were diluted to 30 mg/ml and flash frozen in liquid nitrogen.

Inhibition Kinetics

To assess the time dependent inactivation of the synthesized inhibitors samples, Taka-amylase (60 µM) was incubated with 2 mM of either **1a**, **1b**, **2a**, **3a** or buffer alone in assay buffer (20 mM MES, 20 mM NaCl, 5 mM $CaCl_2$ pH 5.0) at 30 °C. Aliquots of these inactivation mixtures were removed at time intervals and diluted 100-fold into assay cells containing assay buffer and 2-chloro-4-nitrophenyl α-maltotrioside (5 mM) pre-incubated at 30 °C, and absorbance change was monitored at 400 nm. Measured residual rates as a function of time for each inactivator were fit to an equation describing first order decay with offset in OriginPro 2019 (OriginLab).

Crystallization of Taka-amylase

Taka-amylase at 30 mg/mL was tested against a range of commercial crystallization screens. Large split crystals were found in 0.1 M sodium citrate pH 5.6, 20% (v/v) 2-Propanol, 20% (w/v) PEG 4,000, a condition that was used for further optimization. The optimized crystals were grown in MRC maxi 48-well plates (Swissci) using the

sitting-drop vapor-diffusion method at 20 °C using a well-solution composed of: 0.1 M sodium citrate pH 5.6, 18% (v/v) 2-propanol. Droplets contained a mixture of 500 nL protein stock and 500 nL of well-solution. Crystals appeared within one week. Crystals were soaked in 0.1 M sodium citrate pH 5.6, 25 % (v/v) ethylene glycol, 20 % (w/v) PEG 4,000 prior to flash cooling in liquid nitrogen. Inhibitor complexes for **1a**, **1b**, **2a** and **3a** were obtained by soaking crystals in well solution containing 10 mM of the inhibitor for several days (3 days for **1a** and **1b** and 1 month for **2a** and **3a**) before flash cooling in liquid nitrogen.

All diffraction data were collected at Diamond beamlines (un-liganded and **1a** complex: I03, complexes with **1b**, **2a** and **3a**: I04), and data were processed with the CCP4i2 suite.[6] All structures of Taka-amylase were solved by molecular replacement using the PDB structure 7TAA as the search model in Phaser.[7,8] Refinement was performed using cycles of maximum-likelihood refinement using REFMAC5[9] were interspersed with manual corrections of the models using COOT.[10] Structural figures were drawn with PyMol (DeLano Scientific LLC, http://pymol.sourceforge.net/).

Labeling and SDS-PAGE of recombinant human saliva α-amylase

To prepare for labeling, recombinant human saliva α-amylase stock (1 unit/µL) was diluted with assay buffer (150 mM McIlvaine buffer supplemented with 10 mM $CaCl_2$ and 10 mM NaCl, pH 7.0, if not otherwise specified) to final concentrations of 1, 0.5, 0.1, 0.05, 0.01, 0.001, 0.0001 unit in 10 µL volume. ABPs **1c**, **2b** or **2c** stock (10 mM in DMSO) was diluted with assay buffer (2% (v/v) DMSO) to 3.5x of intended assay concentrations. For labeling, 10 µL enzyme dilution was incubated with 4 µL ABP (**1c**, **2b** or **2c**) dilution at 37 °C for 1 h. To determine the detection limit, 0.2 units protein in 10 µL assay buffer (pH 5.0) was incubated with 4 µL ABP **1c** at varying concentration at pH 5.0 for 4 h, at 37 °C. The time-dependent labeling kinetics were assessed by incubating 0.2 unit protein in 10 µL assay buffer (pH 5.0) with 4 µL of 175 µM ABP **1c** dissolved in assay buffer pH 5.0 at 37 °C for 10, 30, 60, 120, 180, 240 or 360 min. Samples were then denatured with 4 µL sample buffer (5x Laemmli buffer, containing 50% (v/v) 1M Tris-HCl pH 6.8, 50% (v/v) glycerol, 10% (w/v) Dithiothreitol (DTT), 10% (w/v) sodium dodecyl sulphate (SDS), 0.01% bromophenol blue) and heated at 98 °C for 5 minutes. Proteins were resolved by electrophoresis in sodium dodecylsulfate (SDS-PAGE) 10% polyacrylamide gels, running at a constant of 90V for 30 minutes followed by 140V for approximately 60 minutes. Wet slab gels were scanned on fluorescence using a Typhoon FLA9500 Imager (GE Healthcare) using λ_{EX} 635 nm; λ_{EM} > 665 nm and images were processed using ImageLab 5.2.1 (BioRad). Gels were subsequently extensively stained with Coomassie Brilliant Blue solution for assessing total protein amounts in each lane of sample.

Labeling of untreated human saliva, saliva supernatant and saliva pellet

To prepare for labeling, concentrated human saliva pellet (16.9 µg/µL in KPi buffer) was diluted with assay buffer pH 7.0 (2% (v/v) DMSO) to final concentration of 55 µg in 24 µL volume. Untreated saliva and saliva supernatant were thaw on ice and 24 µL of each was directly used for labeling. ABPs **1c** (10 mM in DMSO) was diluted with assay buffer pH 7.0 (2% (v/v) DMSO) to 5x of intended assay concentrations. For labeling, 6 µL of ABP **1c** dilution was incubated with 24 µL enzyme dilution at 37 °C for 1 h. Samples were then denatured with 8 µL sample buffer (5x Laemmli buffer, containing 50% (v/v) 1M Tris-HCl pH 6.8, 50% (v/v) glycerol, 10% (w/v) Dithiothreitol (DTT), 10% (w/v) sodium dodecyl sulphate (SDS), 0.01% bromophenol blue) and heated at 98 °C for 5 minutes, and subjected to SDS-PAGE and fluorescence scan as described above.

Labeling of lysates and SDS-PAGE analysis

Human saliva sample (55 µg total protein per sample), mouse pancreas lysates (55 µg total protein per sample), or mouse salivary gland lysates (55 µg total protein per sample) were diluted in assay buffer (pH 7.0, if not

otherwise specified) and incubated with ABP **1c** at intended concentrations (2% (v/v) DMSO) at 37 °C for the intended time periods. Influence of pH on ABP labeling was analyzed using 55 µg total protein incubated with 25 µM **1c** (for human saliva and mouse pancreas) or 50 µM **1c** (for mouse salivary gland), dissolved in assay buffer (2% (v/v) DMSO), pH 3.0-8.0, for 4 h at 37 °C. To determine the detection limit under optimal pH, 55 µg protein was labelled with 0.01-50 µM ABP **1c** in assay buffer pH 5.0 (2% (v/v) DMSO) at 37 °C for the intended time periods. The time-dependent labeling kinetics were assessed by incubating 55 µg protein with 10 µM **1c** (for human saliva and mouse pancreas) or 50 µM **1c** (for mouse salivary gland), dissolved in assay buffer pH 5.0 (2% (v/v) DMSO) at 37 °C for 0.25, 0.5, 1, 2, 3 or 4 h. Samples were then denatured with sample buffer (5x Laemmli buffer, containing 50% (v/v) 1M Tris-HCl pH 6.8, 50% (v/v) glycerol, 10% (w/v) Dithiothreitol (DTT), 10% (w/v) sodium dodecyl sulphate (SDS), 0.01% bromophenol blue) and heated at 98 °C for 5 minutes and subjected to SDS-PAGE and fluorescence scan as described above.

DMSO control experiment

To prepare for labeling, assay buffer pH 5.0 was supplemented with varying concentrations of DMSO (0%, 0.5%, 1% and 2% (v/v)). Concentrated human saliva (16.9 µg/µL in KPi buffer) was diluted with assay buffer containing 0–2% (v/v) DMSO to a final concentration of 55 µg protein in 24 µL volume. ABPs **1c** (10 mM in DMSO) was diluted with assay buffer containing 0–2% (v/v) DMSO to 5x of intended assay concentrations. For labeling, 6 µL of ABP **1c** dilution was incubated with 24 µL enzyme dilution at 37 °C for 1 h. Samples were then denatured with 8 µL Laemmli (5x) sample buffer and heated at 98 °C for 5 minutes, and subjected to SDS-PAGE and fluorescence scan as described above.

Labeling of *A. nidulans* secretome and SDS-PAGE analysis

A. nidulans strain FGSC A4 was grown in minimal medium containing 1 % (w/v) wheat starch (Sigma-Aldrich, S5127) as described previously.[11] Secretome samples were removed daily over a period of five days, flash frozen in liquid nitrogen and stored at -20 ˚C until use in labeling experiments. Secretome samples (12 µL) were diluted with 150 mM McIlvaine buffer of appropriate pH to a final volume of 15 µL, before addition of 3 µL ABP **1c** to a final concentration of 100 µM. Labeling reactions were carried out for 1 h at 37 °C with shaking at 400 rpm, then stopped by addition of 6 µL Laemmli (4X) sample buffer and boiling at 95°C for 5 minutes. 10 µL of each reaction was separated on a 10% SDS-PAGE gel prior to imaging using the Cy5 laser/filter settings on a Typhoon 5 scanner (GE Healthcare). PageRuler Plus Prestained protein ladder (Thermo Fisher Scientific) was used as marker.

Competitive ABPP experiments

Concentrated human saliva (5 µg total protein), mouse pancreas (5 µg total protein) and mouse salivary gland (25 µg total protein) were diluted with assay buffer (pH 5.0, 2% (v/v) DMSO) to a final 10 µL volume, pre-incubated with 2.5 µL **1d** (10 µM for human saliva and 50 µM for mouse pancreas and mouse salivary gland) at 37 °C for 4 h. Then, 2.5 µL of ABP **1c** was added to a final concentration of 10 µM (for human saliva and mouse pancreas) or 50 µM (for mouse salivary gland). The labeling reaction was incubated at 37 °C for 2 h. Additionally a positive control was performed; the protein was pre-incubated with 2.5 µL assay buffer (pH 5.0, 2% (v/v) DMSO) for 4 h at 37 °C, and subsequently labelled with ABP **1c** with the concentration described above. A negative control was also performed. 2.5 µL SDS (10 % (w/v)) was added to the 10 µL protein, boiling at 98 °C for 5 min, and incubated with ABP **1c** at 37 °C for 2 h. Samples were then denatured with 4 µL Laemmli (5x) sample buffer and heated at 98 °C for 5 minutes. Proteins were resolved by electrophoresis in 10% SDS-PAGE gels, running at a constant of 90V for 30 minutes followed by 140V for approximately 60 minutes. Wet slab gels

were scanned on fluorescence using a Typhoon FLA9500 Imager (GE Healthcare) using λ_{EX} 635 nm; λ_{EM} > 665 nm and images were processed using ImageLab 5.2.1 (BioRad).

Pre-incubation of secretomes with inhibitors was carried out in 18 µL reactions which contained, 12 µL of secretome, 3 µL McIlvaine buffer pH 5.0 and 3 µL of inhibitor at varying concentrations. After pre-incubation for 30 min at 37°C, ABP **1c** was added to a final concentration of 67.5 µM. This labeling reaction was incubated at 37°C for 1 h with shaking at 400 rpm, then stopped by addition of 5 µL Laemmli (4X) sample buffer and boiling at 95°C for 5 minutes. 10 µL of each reaction was separated on a 10% SDS-PAGE gel prior to imaging using the Cy5 laser/filter settings on a Typhoon 5 scanner (GE Healthcare). PageRuler Plus Prestained protein ladder (Thermo Fisher Scientific) was used as marker.

Western blotting

Proteins resolved by SDS–PAGE were transferred to a PVDF membrane using a Trans-Blot Turbo system (Bio-Rad). Membrane was blocked in 10 mL of 5% (w/v) powder milk in TBST (50 mM Tris (pH 7.5), 150 mM NaCl, 0.1% Tween 20) for 1 h at room temperature, then incubated with rabbit polyclonal anti-Pancreatic alpha amylase (Abcam; ab199132) at 1:2,000 dilution in TBST (5% (w/v) powder milk) at 4 °C overnight. Membrane was washed 3× with TBST and blocked in 5% (w/v) powder milk in TBST for 30 min at room temperature, then incubated with HRP-conjugated mouse anti-rabbit IgG at 1:5,000 dilution in 5% (w/v) powder milk in TBST for 1 h at room temperature. Membrane was washed again 3× with TBST, 3× with TBS and 2× with Demi-H_2O and blots visualized using Amersham prime ECL reagent (GE Healthcare) and recorded using a Bio-Rad ChemiDoc system.

Detection sensitivity of ABP 1c toward α-amylase in human plasma

To prepare for labeling, recombinant human saliva α-amylase stock (1 unit/µL) was diluted with assay buffer pH 5.0 to final concentrations of 0, 0.0005, 0.005, 0.025, 0.05, 0.5 unit/µL. ABPs **1c** stock (10 mM in DMSO) was diluted with assay buffer pH 5.0 to two different concentrations: 200 µM and 50 µM.

Pre-wash of Concanavalin A (ConA) beads: 940 µL of ConA bead slurry was put in a 15 mL centrifuge tube and washed with 4700 µL of ConA buffer (0.1 M NaOAc, 0.1 M NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mM $MnCl_2$, pH 6.0. Keep on ice all the time when using). Centrifuging at 4 °C for 1 min and the supernatant (4700 µL) was carefully pipette out. After washing 4x more, 564 µL of ConA buffer was added to the bead slurry and 100 µL of the bead slurry was pipette into a series of 1.5 mL Eppendorf tubes.

For labeling:

Set **A**: 2 µL of enzyme dilution + 22 µL of assay buffer + 8 µL of 200 µM ABP **1c** dilution was incubated at 37 °C for 4 h. Samples were then denatured with 8 µL Laemmli (5x) sample buffer and heated at 98 °C for 5 minutes, and subjected to SDS-PAGE as described above.

Set **B**: 2 µL of enzyme dilution + 22 µL of assay buffer + 8 µL of 200 µM ABP **1c** dilution was incubated at 37 °C for 4 h. During this period, 25 µL of human plasma was added to each of the Eppendorf tubes that contain 100 µL pre-washed ConA bead slurry, binding at 4 °C on a roller for 1 h. After binding, the beads were washed with ConA buffer (5 x 1000 µL) and all the supernatant was carefully pipette out upon the fifth wash. Samples were then denatured with 8 µL Laemmli (5x) sample buffer, vortexed slightly and the final 40 µL mixture was immediately added to the ConA purified plasma and heated at 98 °C for 5 minutes. After short spin down, the supernatants were collected in new Eppendorf tubes and subjected to SDS-PAGE as described above.

Set **C**: 2 µL of enzyme dilution was spiked into 25 µL of human plasma, which was added to each of the Eppendorf tubes that contain 100 µL pre-washed ConA bead slurry, incubating at 4 °C on a roller for 1 h. After washing, 32 µL of 50 µM ABP **1c** dilution was immediately added to the ConA purified plasma and the mixture was incubated at 37 °C for 4 h. Samples were then denatured with 8 µL Laemmli (5x) sample buffer and heated at 98 °C for 5 minutes. After short spin down, the supernatants were collected in new Eppendorf tubes and subjected to SDS-PAGE as described above.

Pull-down and LC-MS/MS analysis

Proteomics experiment was performed with human saliva in triplicate for the DMSO control, **1b**-inhibited control and **1d** pulldown. Human saliva sample (300 µg total protein) was incubated with either 0.5% (v/v) DMSO or ABP **1d** (5 µM), or firstly with 25 µM inhibitor **1b** followed by 5 µM of ABP **1d**, each step taking 4 h at 37 °C, in a total volume of 200 µL McIlvaine buffer pH 5.0 supplemented with 10 mM $CaCl_2$ and 10 mM NaCl, followed by denaturation by addition of 10% (w/v) SDS 20 µL and heating for 5 minutes at 98 °C. The solutions were centrifuged at 10,000 rpm for 10 min at room temperature and clear supernatants were transferred carefully into new 2-mL Eppendorf tubes, followed by methanol-chloroform precipitation: for each sample, 800 µL methanol was added into the 220 µL protein solution followed by 200 µL chloroform and vortexed vigorously upon each addition. Then 600 µL Milli-Q was added and the suspension was vortexed vigorously prior to centrifuging at 10,000 rpm for 10 minutes at room temperature. The aqueous top layer was removed carefully and 600 µL methanol was added, then the sample was vortexed slightly and centrifuged as above. The supernatant was then removed, and the pellet was left to air-day for 5 min.

The protein pellet was resuspended in 100 µL PBS buffer with 2% (w/v) SDS, vortexed vigorously and heated to 45 ^{o}C for 20 minutes with shaking at 600 rpm. 14 µL of 50 mM DTT was added and the samples were incubated at 65 ^{o}C for 30 minutes to reduce disulfide bonds, then 200 µL PBS buffer was added and the suspensions were sonicated for 30 seconds at 20% amplitude at 0 ^{o}C. After which, 14 µL of freshly-prepared 150 mM iodoacetamide (IAA) was added and the samples were incubated in the dark for 30 minutes at room temperature. Then the excessive IAA was quenched by adding 7 µL of 50 mM DTT, incubating for 30 minutes at room temperature. The 335 µL protein suspensions were stepwise diluted with PBS buffer (200 µL/1000 µL/500 µL, vortex vigorously upon each addition) to a final 2-mL volume. Then the resulting 2-mL suspensions were centrifuged at 10,000 rpm for 10 minutes at room temperature and supernatants were transferred carefully into new 2-mL Eppendorf tubes.

The samples were then ready for pull-down with prewashed Dynabeads™ MyOne™ Streptavidin T1 (30 µL) as published previously.[12] After pull-down, all samples were used for on-bead digestion. The samples were treated with 100 µL on-bead digestion buffer (50 mM NH_4HCO_3 (pH 8), 2% ACN, 1 mM $CaCl_2$, 2.5 ng/µL of trypsin) and incubated overnight at 37 °C. The supernatants, containing tryptic-digested peptides were then transferred to new 2-mL low-binding tubes and desalted using StageTips. Consequently, the acetonitrile was evaporated using a SpeedVac at 45 °C followed by addition of 50 µL of LC-MS sample solution (97:3:0.1, H_2O:acetonitrile:formic acid) for LC-MS analysis. All peptide samples were analyzed with a two-hour gradient of 5% to 25% acetonitrile on nano-LC, hyphenated to an LTQ-Orbitrap and identified using the Mascot protein search engine.[13] Raw data was calculated using MaxQuant against UniProt of human saliva proteome database to obtain an identification list of found proteins. The abundance of the protein hits was quantified as previously described,[14] in unsupervised mode using the default settings of the PLGS (Waters) and IsoQuant software. Raw and quantified data can be found in Table S2 as a separate excel file.

Fungal Secretome Proteomics

Proteomic analysis was performed in triplicate for the DMSO control, **1d** pulldown and total secretome. *A. nidulans* day 5 wheat secretome was concentrated 12.5-fold using a Vivaspin centrifugal concentrator (3 kDa cut-off). Concentrated secretome (40 µl) was buffered with 30 mM McIlvane buffer pH 5.0 and transferred to a Lo-bind 0.5 ml tube (Eppendorf). The buffered secretome was then treated with either 300 µM **1d** or DMSO control. Reactions were incubated 4 hours at 30 °C with shaking at 450 rpm. Labeling was terminated by the addition of DTT and SDS to a final concentration of 4 mM and 0.2 % (v/v) respectively, and heating to 95 °C for 5 minutes. The denatured proteins were then cooled to 25 °C, IAA was added to a final concentration of 24 mM then samples were incubated in the dark for 30 minutes at 25°C. IAA, DTT, SDS, buffer and excess probe were removed by acetone precipitation; 4 volume equivalents of acetone (-20 °C) was added to the sample and the mixture was vortexed. The sample was then placed at -20 °C for one hour, then proteins were pelleted by centrifugation at 14,000 x *g* for 1 minute. The supernatant was removed, then the pellet was washed with the same volume of -20 °C acetone then centrifuged again at 14,000 x *g* for 1 minute. The supernatant was then removed, and the pellet was left to briefly air dry.

Samples were then resuspended 50 µl of 4 M urea, then diluted with 450 µL of 0.05% SDS in phosphate buffered saline. Strep Mag Sepharose beads (GE healthcare; 20 µL; washed twice with phosphate buffered saline (PBS) were added to each sample, followed by incubation at 25 °C with constant agitation to prevent bead settling for 3 hours. Beads were collected using a magnetic rack and the supernatant was discarded. Beads were washed with 1 mL of 2 % SDS at 25 °C then with 1 mL of 2 % SDS at 65 °C to eliminate non-specific binding, followed by 1 ml of 2 M urea for 5 minutes to remove SDS and then 1 mL of water two times to remove residual SDS and urea. The washed beads were then resuspended in 20 µL of 0.05 M triethylamonium bicarbonate and 1 µL of 0.5 µg/µL trypsin (Promega V5280) in 50 mM acetic acid was added. The on-bead digest was incubated overnight at 37 °C. Following the tryptic digest, the beads were immobilized and released peptides were removed. The total secretome samples were treated as above, except the acetone pellet was resuspended directly in 0.05 M triethylamonium bicarbonate and digested with trypsin in solution.

The samples were desalted via a C18 ZipTip then resolubilised with aqueous 0.1% TFA, before injection onto a 50 cm EasyNano C18 PepMap column. Peptides were eluted at a flow rate of 300 nl/min over 1 hour acquisitions onto an Orbitrap Fusion Tribrid mass spectrometer operated in DDA mode. Tandem mass spectra were searched using both Mascot and X!Tandem search programs against the *Emericella nidulans* strain FGSC A4 subset of the Uniprot database and typical contaminants. Carboxymethylation of Cysteine was set as a fixed modification and oxidation of Met was set as variable. Resulting spectral matches were combined in Scaffold and filtered to require a global protein and PSM false discovery rate of 1%, and a minimum of two unique peptides per protein group identification. Label-free quantification was performed using Progenesis QI (Waters). Following chromatographic alignment, peaks were integrated and assigned. Protein abundance was estimated using the integrated intensity of non-conflicting peptides. Results of this analysis for all identified proteins can be found in Table S3 as a separate excel file.

4. Chemical Synthesis

4.1 General experimental details

All reagents were of experimental grade and were used without further purification unless stated otherwise. Dichloromethane (DCM) and tetrahydrofuran (THF) were stored over 3 Å molecular sieves and *N,N*-dimethylformamide (DMF) was stored over 4 Å molecular sieves, which were dried *in vacuo* before use. All reactions were performed under an Argon or N_2 atmosphere unless stated otherwise. Reactions were monitored by analytical thin layer chromatography (TLC) using Merck aluminum sheets pre-coated with silica gel 60 with detection by UV-absorption (254 nm) and by spraying with a solution of $(NH_4)_6Mo_7O_{24}\cdot H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4\cdot H_2O$ (10 g/mL) in 10% sulfuric acid followed by charring at ~150 °C or by spraying with an aqueous solution of $KMnO_4$ (7%) and K_2CO_3 (2%) followed by charring at ~150 °C. Column chromatography was performed manually using Screening Device b.v. silica gel 60 (0.04-0.063 mm) in the indicated solvents. LC-MS analysis was performed on a LCQ Advantage Max (Thermo Finnigan) ion-trap spectrometer (ESI^+) coupled to a Surveyor HPLC system (Thermo Finnigan) equipped with a C18 column (Gemini, 4.6 mm x 50 mm, 5 µM particle size, Phenomenex). The applied buffers were H_2O, acetonitrile (MeCN) and 1% aqueous trifluoroacetic acid (TFA). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker AV-400 (400/101 MHz), Bruker AV-500 (500/126 MHz), and Bruker AV-850 (850/214 MHz) spectrometers in the given solvent. Chemical shifts (δ) are given in ppm relative to tetramethylsilane (TMS) as internal standard (^{1}H NMR in $CDCl_3$) or the residual signal of the deuterated solvent. Coupling constants (*J*) are given in Hz. All given ^{13}C-NMR spectra are proton decoupled. The following abbreviations are used to describe peak patterns when appropriate: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), Ar (aromatic), C_q (quarternary carbon). 2D NMR experiments (COSY, HSQC, HMBC-ipv-GATED) were carried out to assign protons and carbons of the new structures. High-resolution mass spectrometry (HRMS) analysis was performed with a LTQ Orbitrap mass spectrometer (Thermo Finnigan), equipped with an electronspray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10 mL/min, capillary temperature 250 °C) with resolution R = 60000 at m/z 400 (mass range m/z = 150 – 2000) and dioctyl phthalate (m/z = 391.28428) as a "lock mass". The high-resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan).

4.2 Experimental Procedures and Characterization Data of Products

Known compounds **4a**[15], **5**[16], **S1**[15], **S2**[16], Cy5-OSu[17], biotin-OSu[18] were synthesized following procedures previously described and their spectroscopic data are in agreement with those previously reported.

Standard procedure A: glycosylation with imidate donor (4a)

The donor (1.5 equiv.) was co-evaporated with toluene (3 x) and dissolved in dry DCM (0.05 M) under nitrogen and stirred over fresh flame-dried molecular sieves 3 Å, after which DMF (16 equiv. of donor) was added to the solution. The solution was cooled to -20 °C and TfOH (1.5 equiv.) was added. After 1 h, the pre-activation was complete as indicated by TLC-analysis. Acceptor (1.0 equiv.) was added to the solution and the mixture was placed in an ice bath. The reaction was stirred at 0 °C until TLC-analysis showed complete conversion of the acceptor. The reaction was quenched with Et_3N, filtered and concentrated in vacuo. The products were purified by size exclusion (eluent MeOH/DCM: 1/1) and silica gel column chromatography (See experimental description below for eluent system). *Note: for the glycosylation of donor **4a** with acceptor **5**, the reaction was kept at -20 °C for 24 h then warmed to 0 °C and stirred until TLC-analysis showed complete conversion of the acceptor.*

Standard procedure B: glycosylation with thio-donors (4b and 4c)

The donor (1.5 equiv.) was co-evaporated with toluene (3 x) and dissolved in dry DCM (0.05 M) under nitrogen

and stirred over fresh flame-dried molecular sieves 3 Å, after which DMF (16 equiv. of donor) was added to the solution. The solution was cooled to -20 °C, NIS (1.5 equiv.) and TfOH (1.5 equiv.) were added successively. The mixture was pre-activated at -20 °C for 2 h. Then acceptor (1.0 equiv.) was added to the solution and the mixture was placed in an ice bath. The reaction was stirred at 0 °C until TLC-analysis showed complete conversion of the acceptor. The reaction mixture was diluted with DCM and was quenched with sat. aq. $Na_2S_2O_3$. The organic phase was washed with water and brine, dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The products were purified by size exclusion (eluent MeOH/DCM: 1/1) and silica gel column chromatography (See experimental description below for eluent system).

General procedure C: Deprotection of PMB protecting group

The starting material (1.0 equiv.) was dissolved in DCM/H_2O (19/1, 0.05 M). DDQ (1.2 equiv.) was added to the mixture. The reaction was stirred at rt until TLC-analysis indicated full consumption of the starting material (± 4 h). Then the mixture was diluted with DCM and washed with sat. aq. $NaHCO_3$ (2 x), water (1 x) and brine, dried with anhydrous Na_2SO_4, filtered and concentrated in vacuo. The product was purified by silica gel column chromatography (See experimental description below for eluent system).

General procedure D: Epoxidation with *m*CPBA

The starting material (1.0 equiv.) was dissolved in dry DCM (0.05 M) and cooled 0 °C, *m*CPBA (6.0 equiv.) was added and the reaction was stirred at rt overnight. The mixture was diluted with DCM and washed with sat. aq. $Na_2S_2O_3$ (1 x), sat. aq. $NaHCO_3$ (1 x), water and brine successively, dried with anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude was directly used for next step (silylation) without further purification.

General procedure E: Protection with TBS group

The starting material (1.0 equiv.) was dissolved in dry DCM (0.1 M) and cooled 0 °C, imidazole (3.0 equiv.), TBSCl (2.0 equiv.) and DMAP (0.1 equiv.) were added successively and the reaction was stirred at rt overnight. The mixture was diluted with DCM, washed with water (3 x) and brine, dried with anhydrous Na_2SO_4, filtered and concentrated in vacuo. The product was purified by silica gel column chromatography (See experimental description below for eluent system).

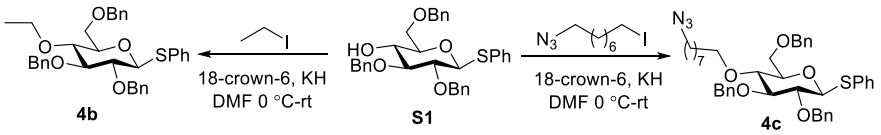
General procedure F: Deprotection of TBS group

The starting material (1.0 equiv.) was dissolved in dry THF (0.1 M), TBAF (1 M in THF, 6.0 equiv.) was added. The reaction was stirred at rt until TLC-analysis indicated full consumption of the starting material (± 2h). The reaction was quenched sat. aq. NH_4Cl, extracted with DCM (2 x), the combined organic layers were washed with sat. aq. $NaHCO_3$, water and brine, dried with anhydrous Na_2SO_4, filtered and concentrated in vacuo. The product was purified by silica gel column chromatography (See experimental description below for eluent system).

General procedure G: Protection with Bn group

The starting material (1.0 equiv.) was dissolved in dry DMF (0.1 M) and cooled to 0 °C. To the mixture was added NaH (60% in mineral, 2.0 equiv.) and stirred at 0 °C for 30 min. Then BnBr (1.5 equiv.) and TBAI (0.05 equiv.) were added successively and the mixture was warmed to rt and stirred overnight. The reaction was quenched with H_2O at 0 °C, extracted with EtOAc (2 x), the combined organic layers were washed with water (2 x) and brine, dried with anhydrous Na_2SO_4, filtered and concentrated in vacuo. The product was purified by silica gel column chromatography (See experimental description below for eluent system).

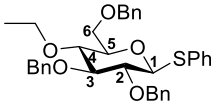
Scheme S1. Preparation of donor **4b** and **4c**



For **4b**: KH (30 wt%, 369 mg, 2.76 mmol) was dispersed in dry DMF (18 mL), purged with N_2 and cooled to 0 °C. Compound **S1** (1 g, 1.85 mmol) was added to the mixture in several portions over a period of 15 min, then the reaction was warmed to rt and stirred for 30 min. After which the mixture was cooled to 0 °C again, 18-crown-6 (97 mg, 0.37 mmol) and iodoethane (0.44 mL, 5.5 mmol) were added. After stirring at rt overnight, the reaction was carefully quenched with H_2O at 0 °C, diluted with water (80 mL), extracted with EtOAc (2 x 100 mL), the combined organic layers were washed with water (2 x 150 mL), brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The product was purified by flash column chromatography (pentane/EtOAc, 20:1→12:1) affording compound **4b** (906 mg, 86%) as a white solid.

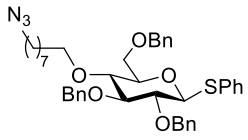
For **4c**: Starting from compound **S1** (1.5 g, 2.76 mmol) and 1-azido-8-iodooctane (1.17 g, 4.15 mmol), the reaction was carried out following the same procedure described for **4b**. The product was purified by flash column chromatography (pentane/EtOAc, 20:1→15:1) affording compound **4c** (1.27 g, 66%) as a yellow oil.

(2S,3R,4S,5R,6R)-3,4-bis(benzyloxy)-6-((benzyloxy)methyl)-5-ethoxy-2-(phenylthio)tetrahydro-2H-pyran (**4b**)



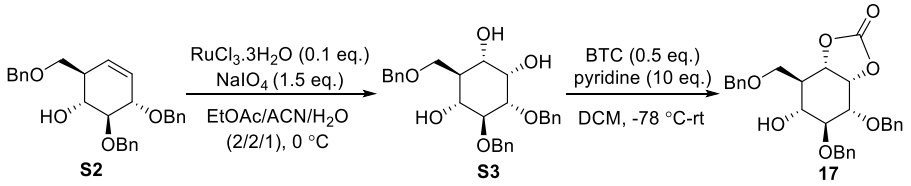
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 – 7.53 (m, 2H, C*H* Ar), 7.41 – 7.24 (m, 15H, C*H* Ar), 7.23 – 7.17 (m, 3H, C*H* Ar), 4.90 – 4.81 (m, 3H, CH*H* Bn), 4.71 (d, *J* = 10.3 Hz, 1H, CH*H* Bn), 4.65 (d, *J* = 9.6 Hz, 1H, H1), 4.56 (dd, *J* = 29.6, 11.9 Hz, 2H, CH*H* Bn), 3.86 – 3.76 (m, 2H, 6a/CH_3CH*H*O), 3.75 – 3.69 (m, 1H, 6b), 3.60 (m, 2H, H4/CH_3CH*H*O), 3.49 – 3.41 (m, 3H, H2/H3/H5), 1.13 (t, *J* = 7.0 Hz, 3H, C*H*$_3$$CH_2O$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.5, 138.4, 138.2, 133.9 (4C_q Ar), 132.0, 129.0, 128.6, 128.5, 128.4, 128.3, 128.0, 127.8, 127.7, 127.6, 127.5 (20*C*H Ar), 87.5 (C1), 86.7 (C4), 80.7 (C2), 79.3 (C3), 78.1 (C5), 75.9 (Bn), 75.5 (Bn), 73.5 (Bn), 69.1 (C6), 68.5 (O*CH*$_2$$CH_3$), 15.9 (*C*H$_3$). HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{35}H_{38}O_5SNa$ 593.2332, found 593.2330.

(2R,3R,4S,5R,6S)-3-((8-azidooctyl)oxy)-4,5-bis(benzyloxy)-2-((benzyloxy)methyl)-6-(phenylthio)tetrahydro-2H-pyran (**4c**)



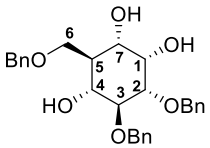
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (dd, *J* = 6.6, 3.0 Hz, 2H, C*H* Ar), 7.44 – 7.11 (m, 18H, C*H* Ar), 4.90 – 4.78 (m, 3H, CH*H* Bn), 4.70 (d, *J* = 10.3 Hz, 1H, CH*H* Bn), 4.65 (d, *J* = 9.6 Hz, 1H, H1), 4.56 (dd, *J* = 29.6, 11.9 Hz, 2H, CH*H* Bn), 3.82 – 3.66 (m, 3H, 6a/ 6b/ O*CH*$H(CH_2)_7N_3$), 3.61 (t, *J* = 8.4 Hz, 1H, H4), 3.55 – 3.37 (m, 4H, O*CH*$H(CH_2)_7N_3$/H2/H3/H5), 3.22 (t, *J* = 7.0 Hz, 2H, CH_2N_3), 1.66 – 1.40 (m, 4H, 2CH_2 linker), 1.39 – 1.12 (m, 8H, 4CH_2 linker). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.6, 138.5, 138.2, 134.0 (4C_q Ar), 132.0, 129.0, 128.5, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5 (20*C*H Ar), 87.5 (C1), 86.8 (C4), 80.8 (C2), 79.4 (C3), 78.1 (C5), 75.9 (Bn), 75.5 (Bn), 73.5 (Bn), 73.3 (O*C*$H_2(CH_2)_7N_3$), 69.2 (C6), 51.5 (*C*H_2N_3), 30.4, 29.5, 29.2, 28.9, 26.8, 26.2 (6*C*H_2 linker). HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{41}H_{49}N_3O_5SNa$ 718.3285, found 718.3281.

Scheme S2. Preparation of acceptor **17**



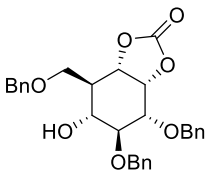
Cyclohexane **S2** (1.94 g, 4.50 mmol) was dissolved in EtOAc (25 mL) and MeCN (25 mL) and cooled to 0 °C. A solution of $RuCl_3.3H_2O$ (94 mg, 0.45 mmol) and $NaIO_4$ (1.45 g, 6.78 mmol) in H_2O (9 mL) was added and the mixture was stirred at 0 °C vigorously for 2 h. The reaction was quenched by addition of sat. aq. $Na_2S_2O_3$ (10 mL) and the mixture was stirred for 15 min. Then the mixture was diluted with H_2O (100 mL) and extracted with EtOAc (3 x 80 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The crude was purified by flash column chromatography (pentane/EtOAc, 5:1 → 2:1) affording compound **S3** (750 mg, 36%) as a clean oil. Compound **S3** (565 mg, 1.21 mmol) was dissolved in dry DCM (5 mL) and pyridine (1 mL, 12.1 mmol) was added. The mixture was purged with N_2 and cooled to -78 °C. A solution of triphosgene (180 mg, 0.61 mmol) in dry DCM (2 mL) was added to the mixture at -78 °C. Then the reaction was stirred at rt for 1 h and treated with sat. aq. NH_4Cl (60 mL), extracted with DCM (2 x 80 mL). The combined organic layers were washed successively with water, sat. aq. $NaHCO_3$, water and brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The crude was purified by flash column chromatography (pentane/acetone, 11:1 → 7:1) affording compound **17** (465 mg, 78%) as a yellow oil.

(1S,2S,3S,4R,5S,6S)-5,6-bis(benzyloxy)-3-((benzyloxy)methyl)cyclohexane-1,2,4-triol (**S3**)



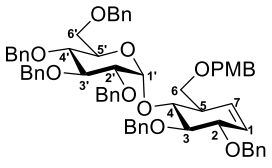
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49 – 7.11 (m, 15H, C*H* Ar), 4.99 (d, *J* = 11.4 Hz, 1H, CH*H* Bn), 4.75 – 4.62 (m, 3H, CH*H* Bn), 4.56 – 4.45 (m, 2H, CH*H* Bn), 4.13 (t, *J* = 2.7 Hz, 1H, H1), 3.88 (ddd, *J* = 9.2, 4.0, 1.2 Hz, 1H, H6a), 3.80 – 3.67 (m, 2H, H3/H6b), 3.53 (dt, *J* = 10.8, 1.7 Hz, 1H, H7), 3.42 – 3.29 (m, 2H, H2/H4), 2.89 – 2.63 (br s, 2H, 2OH), 2.16 (tdd, *J* = 10.5, 6.1, 3.9 Hz, 1H, H5). ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.8, 137.9, 137.9 ($3C_q$ Ar), 128.6, 128.6, 128.5, 128.0, 128.0, 127.9, 127.9, 127.7 (15*C*H Ar), 82.4 (C3), 79.9 (C2), 75.5 (Bn), 73.7 (Bn), 72.1 (Bn), 70.3 (C4), 70.2 (C1), 70.17 (C7), 70.0 (C6), 42.5 (C5). HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{28}H_{32}O_6Na$ 487.2091, found 487.2091.

(3aS,4R,5S,6R,7R,7aS)-4,5-bis(benzyloxy)-7-((benzyloxy)methyl)-6hydroxyhexahydrobenzo[d][1,3]dioxol-2-one (**17**)



^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.20 (m, 15H, C*H* Ar), 4.82 – 4.72 (m, 2H, H1/H7), 4.70 – 4.54 (m, 4H, CH*H* Bn), 4.52 (s, 2H, CH*H* Bn), 3.89 – 3.78 (m, 2H, H6a/H2), 3.73 – 3.64 (m, 2H, H3/H4), 3.61 (dd, *J* = 9.5, 3.9 Hz, 1H, H6b), 2.78 (s, 1H, OH), 2.44 – 2.30 (m, 1H, H5). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.6 (C=O), 137.7, 137.7, 137.1 ($3C_q$ Ar), 128.7, 128.6, 128.6, 128.3, 128.1, 128.0, 128.0, 127.8, 127.8 (15*C*H Ar), 81.3 (C3), 76.0 (C2), 75.0 (C1), 74.4 (C7), 73.6 (Bn), 73.4 (Bn), 73.2 (Bn), 69.6 (C4), 66.7 (C6), 43.2 (C5). HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{29}H_{30}O_7Na$ 513.1884, found 513.1887.

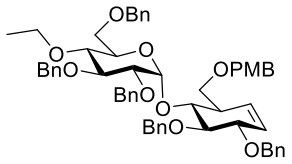
(2R,3R,4S,5R,6S)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)-6-(((1R,2R,5S,6R)-5,6-bis(benzyloxy)-2-(((4-methoxybenzyl)oxy)methyl)cyclohex-3-en-1-yl)oxy)tetrahydro-2H-pyran (**6a**)



Starting from compound **5** (460 mg, 1.0 mmol), using donor **4a** (1.06 g, 1.5 mmol) and following **Standard procedure A**. The crude was divided into two portions and purified by size exclusion (DCM/MeOH = 1/1) giving product as a single isomer (970 mg, α/β > 20/1). Then the compound was further purified by flash column chromatography (pentane/EtOAc 13:1→9:1) to obtain compound **6a** (951 mg, 96%) as a clean oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.03 (m, 32H, C*H* Ar), 6.83 – 6.78 (m, 2H, C*H* Ar), 5.74 (tt, *J* = 4.8, 2.4 Hz, 2H), 5.63 (dt, *J* = 10.1, 2.1 Hz, 1H), 5.00 (d, *J* = 12.1 Hz, 1H), 4.95 – 4.87 (m, 2H), 4.86 – 4.74 (m, 3H), 4.72 – 4.22 (m, 12H), 4.10 (t, *J* = 9.1 Hz, 1H), 4.03 – 3.86 (m, 2H), 3.79 (d, *J* = 16.4 Hz, 5H), 3.71 – 3.65 (m, 1H), 3.65 – 3.58 (m, 2H), 3.56 – 3.45 (m, 3H), 2.67 (ddt, *J* = 8.2, 5.3, 2.7 Hz, 1H, H5). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.2, 139.3,

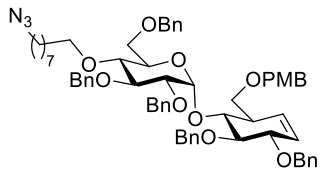
139.0, 138.6, 138.4, 138.2, 138.0, 130.5 (8C_q Ar), 129.8 (C7), 129.2, 129.0, 128.4, 128.4, 128.4, 128.3, 128.3, 128.3, 128.2, 128.1, 128.0, 128.0, 127.9, 127.9, 127.9, 127.8, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 127.3, 127.1, 127.0, 126.8 (32*C*H Ar), 126.6 (C1), 113.9, 113.8 (2*C*H Ar), 97.0 (C1'), 85.0, 82.0, 80.8, 79.6, 77.9, 75.6 (Bn), 75.1 (Bn), 74.1 (Bn), 73.6 (Bn), 73.2, 72.8 (Bn), 72.5 (Bn), 71.8 (Bn), 70.9, 69.6 (C6), 68.4 (C6'), 55.4 (*C*H_3 PMB), 43.9 (C5). HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{63}H_{66}O_{10}Na$ 1005.4548, found 1005.4550.

(2S,3R,4S,5R,6R)-3,4-bis(benzyloxy)-6-((benzyloxy)methyl)-2-(((1R,2R,5S,6R)-5,6-bis(benzyloxy)-2-(((4-methoxybenzyl)oxy)methyl)cyclohex-3-en-1-yl)oxy)-5-ethoxytetrahydro-2H-pyran (**6b**)



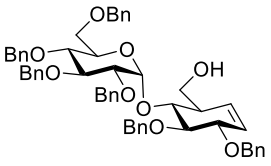
Starting from compound **5** (460 mg, 1.0 mmol), using donor **4b** (856 mg, 1.5 mmol) and following **Standard procedure B**. The crude was divided into two portions and purified by size exclusion (DCM/MeOH = 1/1) giving product as a single isomer (845 mg, α/β > 20/1). Then the compound was further purified by flash column chromatography (pentane/EtOAc 13:1→9:1) to obtain compound **6b** (810 mg, 88%) as a clean oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.06 (m, 27H, C*H* Ar), 6.86 (d, *J* = 8.3 Hz, 2H, C*H* Ar), 5.78 – 5.67 (m, 2H, H1/H1'), 5.66 – 5.60 (m, 1H, H7), 4.98 (d, *J* = 12.0 Hz, 1H, CH*H* Bn), 4.92 – 4.84 (m, 2H, CH*H* Bn), 4.77 (d, *J* = 10.9 Hz, 1H, CH*H* Bn), 4.59 (td, *J* = 12.7, 12.1, 10.5 Hz, 3H, CH*H* Bn), 4.49 – 4.28 (m, 5H, CH*H* Bn), 4.28 – 4.22 (m, 1H, H2), 4.08 (t, *J* = 9.1 Hz, 1H, H4), 3.94 – 3.85 (m, 2H, H3/H3'), 3.78 (s, 5H, 1CH*H* Et/C*H*$_3$ PMB/H5'), 3.66 – 3.39 (m, 7H, H6ab/H6'ab/H4'/H2'/1CH*H* Et), 2.67 (dq, *J* = 6.8, 3.5 Hz, 1H, H5), 1.07 (q, *J* = 6.7 Hz, 3H, CH_3 Et). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.2, 139.4, 139.0, 138.4, 138.3, 138.1, 130.5 (7C_q Ar), 129.8 (C7), 129.1, 128.4, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.18, 128.0, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.5, 127.5, 127.1, 126.8 (27*C*H Ar), 126.6 (C1), 113.8 (2*C*H Ar), 97.1 (C1'), 85.0 (C3), 81.9 (C3'), 80.8 (C2), 79.4, 77.8, 75.6 (Bn), 74.1 (Bn), 73.6 (Bn), 73.4 (C4), 72.9 (Bn), 72.5 (Bn), 71.8 (Bn), 71.0 (C5'), 69.7 (C6), 68.4 (*C*H_2 Et), 68.3 (C6'), 55.4 (*C*H_3 PMB), 43.9 (C5), 16.0 (*C*H_3 Et). HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{58}H_{64}O_{10}Na$ 943.4392, found 943.4385.

(2R,3R,4S,5R,6S)-3-((8-azidooctyl)oxy)-4,5-bis(benzyloxy)-2-((benzyloxy)methyl)-6-(((1R,2R,5S,6R)-5,6-bis(benzyloxy)-2-(((4-methoxybenzyl)oxy)methyl)cyclohex-3-en-1-yl)oxy)tetrahydro-2H-pyran (**6c**)



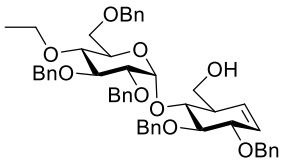
Starting from compound **5** (560 mg, 1.2 mmol), using donor **4c** (1.27 g, 1.8 mmol) and following **Standard procedure B**. The crude was divided into two portions and purified by size exclusion (DCM/MeOH = 1/1) giving product as a single isomer (1.21 g, α/β > 20/1). Then the compound was further purified by flash column chromatography (pentane/EtOAc 13:1→9:1) to obtain compound **6c** (1.1 g, 87%) as a clean oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.02 (m, 27H, C*H* Ar), 6.90 – 6.81 (m, 2H, C*H* Ar), 5.77 – 5.68 (m, 2H, H1/H1'), 5.63 (dt, *J* = 10.1, 2.1 Hz, 1H, H7), 4.98 (d, *J* = 12.0 Hz, 1H, CH*H* Bn), 4.94 – 4.82 (m, 2H, CH*H* Bn), 4.75 (d, *J* = 10.9 Hz, 1H, CH*H* Bn), 4.65 – 4.28 (m, 8H, CH*H* Bn), 4.25 (dt, *J* = 7.4, 2.4 Hz, 1H, H2), 4.08 (t, *J* = 9.1 Hz, 1H, H4)), 3.95 – 3.83 (m, 2H, H3/H3'), 3.81 – 3.67 (m, 5H, 1C*H*$_2$O linker/C*H*$_3$ PMB/H5'), 3.66 – 3.29 (m, 7H, H6ab/H6'ab/H4'/H2'/1C*H*$_2$O linker), 3.20 (t, *J* = 7.0 Hz, 2H, C*H*$_2$N$_3$), 2.67 (dp, *J* = 8.3, 2.8 Hz, 1H, H5), 1.54 (p, *J* = 7.0 Hz, 2H, 2CH*H* linker), 1.43 (h, *J* = 7.8, 6.9 Hz, 2H, 2CH*H* linker), 1.26 (ddd, *J* = 18.1, 12.9, 5.5 Hz, 8H, 8CH*H* linker). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.2, 139.3, 139.1, 138.4, 138.2, 138.1, 130.5 (7C_q Ar), 129.8 (C7), 129.1, 128.4, 128.4, 128.4, 128.3, 128.2, 128.2, 128.1, 128.0, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.5, 127.5, 127.1, 126.8 (27*C*H Ar), 126.6 (C1), 113.8 (2*C*H Ar), 97.1 (C1'), 85.0 (C3), 81.9 (C3'), 80.8 (C2), 79.4, 77.8, 75.6 (Bn), 74.1 (Bn), 73.6 (Bn), 73.3 (C4), 73.2 (*C*H$_2$O linker), 72.9 (Bn), 72.5 (Bn), 71.8 (Bn), 71.0 (C5'), 69.6 (C6), 68.4 (C6'), 55.4 (*C*H_3 PMB), 51.5 (*C*H$_2$N$_3$), 43.9 (C5), 30.5, 29.5, 29.2, 28.9, 26.8, 26.2 (6*C*H$_2$ linker). HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{64}H_{75}N_3O_{10}Na$ 1068.5345, found 1068.5342.

((1R,4S,5R,6R)-4,5-bis(benzyloxy)-6-(((2S,3R,4S,5R,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)cyclohex-2-en-1-yl)methanol (**7a**)



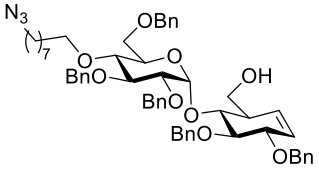
Starting from compound **6a** (1.04 g, 1.06 mmol), and following **General procedure C**, the reaction was purified by flash column chromatography (pentane/EtOAc 5:1→2:1) to obtain compound **6a** (726 mg, 80%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.12 (m, 26H, C*H* Ar), 7.11 – 6.98 (m, 4H, C*H* Ar), 5.86 – 5.75 (m, 2H, H1/H1'), 5.65 (dt, *J* = 10.1, 2.1 Hz, 1H, H7), 5.05 (d, *J* = 12.2 Hz, 1H, CH*H* Bn), 4.88 (dd, *J* = 11.6, 8.1 Hz, 2H, CH*H* Bn), 4.75 (dd, *J* = 10.9, 3.1 Hz, 2H, CH*H* Bn), 4.65 – 4.43 (m, 6H, CH*H* Bn), 4.38 (d, *J* = 10.8 Hz, 1H, CH*H* Bn), 4.27 (ddt, *J* = 7.3, 3.8, 1.9 Hz, 1H, H2), 4.18 (t, *J* = 9.2 Hz, 1H, H4), 4.03 – 3.87 (m, 4H, H3/H3'/H5'/H6a), 3.71 – 3.61 (m, 2H, H6'a/H6b), 3.47 (dd, *J* = 9.8, 4.0 Hz, 1H, H2'), 3.40 (dd, *J* = 9.8, 7.1 Hz, 1H, H6'b), 3.29 (dd, *J* = 10.2, 8.7 Hz, 1H, H4'), 3.10 (br s, 1H, OH), 2.57 (dp, *J* = 8.8, 3.0 Hz, 1H, H5). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.4, 138.7, 138.3, 138.1, 137.8, 137.3 (6C_q, Ar), 129.8 (C7), 128.6, 128.6, 128.5, 128.5, 128.3, 128.3, 128.3, 128.3, 128.1, 128.1, 128.0, 127.9, 127.7, 127.7, 127.6, 127.5 (C1), 127.0, 126.5, 97.3 (C1'), 84.5 (C3), 82.0 (C3'), 80.9 (C2), 79.2 (C2'), 78.4 (C4'), 75.6 (Bn), 75.2 (Bn), 73.8 (Bn), 73.6 (Bn), 73.1(C4), 73.0 (Bn), 71.7 (Bn), 71.5 (C5'), 69.1 (C6'), 61.9 (C6), 45.8 (C5). HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{55}H_{58}O_9Na$ 885.3973, found 885.3971.

((1R,4S,5R,6R)-4,5-bis(benzyloxy)-6-(((2S,3R,4S,5R,6R)-3,4-bis(benzyloxy)-6-((benzyloxy)methyl)-5-ethoxytetrahydro-2H-pyran-2-yl)oxy)cyclohex-2-en-1-yl)methanol (**7b**)



Starting from compound **6b** (780 mg, 0.85 mmol), and following **General procedure C**, the reaction was purified by flash column chromatography (pentane/EtOAc 7:1→3:1) to obtain compound **7b** (500 mg, 74%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45 – 7.02 (m, 25H, C*H* Ar), 5.81 (dt, *J* = 10.2, 2.4 Hz, 1H, H1), 5.75 (d, *J* = 4.0 Hz, 1H, H1'), 5.65 (dt, *J* = 10.2, 2.1 Hz, 1H, H7), 5.02 (d, *J* = 12.1 Hz, 1H, CH*H* Bn), 4.94 – 4.78 (m, 2H, CH*H* Bn), 4.73 (d, *J* = 10.9 Hz, 1H, CH*H* Bn), 4.63 – 4.58 (m, 3H, CH*H* Bn), 4.54 (d, *J* = 11.5 Hz, 1H, CH*H* Bn), 4.46 (s, 2H, CH*H* Bn), 4.26 (ddt, *J* = 7.3, 3.1, 2.0 Hz, 1H, H2), 4.16 (dd, *J* = 9.6, 8.7 Hz, 1H, H4), 4.02 – 3.81 (m, 4H, H6a/H3/H5'/H3'), 3.76 (dq, *J* = 9.2, 7.0 Hz, 1H, 1CH*H* Et), 3.67 (td, *J* = 11.1, 10.5, 2.3 Hz, 2H, H6'a/H6b), 3.50 (dd, *J* = 9.9, 6.9 Hz, 1H, H6'b), 3.45 – 3.36 (m, 2H, H2'/1CH*H* Et), 3.10 (dd, *J* = 10.1, 8.7 Hz, 1H, H4'), 3.02 (t, *J* = 6.3 Hz, 1H, OH), 2.58 (dh, *J* = 8.8, 2.9 Hz, 1H, H5), 1.04 (t, *J* = 7.0 Hz, 3H, CH_3 Et). ^{13}C NMR (126 MHz, $CDCl_3$) δ 139.5, 138.8, 138.3, 138.2, 137.4 (5C_q Ar), 129.8 (C7), 128.6, 128.5, 128.4, 128.3, 128.2, 128.2, 128.1, 128.0, 127.9, 127.7, 127.7, 127.6, 127.6, 127.5 (24*C*H Ar), 127.0 (C1), 126.6 (*C*H Ar), 97.4 (C1'), 84.5 (C3), 81.8 (C3'), 80.8 (C2), 79.1 (C2'), 78.8 (C4'), 75.6 (Bn), 73.9 (Bn), 73.7 (Bn), 73.3 (C4), 73.1 (Bn), 71.7 (Bn), 71.6 (C5'), 69.3 (C6'), 68.6 (*C*H_2 Et), 62.1 (C6), 45.8 (C5), 15.8 (*C*H_3 Et). HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{50}H_{56}O_9Na$ 823.3817, found 823.3821.

((1R,4S,5R,6R)-6-(((2S,3R,4S,5R,6R)-5-((8-azidooctyl)oxy)-3,4-bis(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)-4,5-bis(benzyloxy)cyclohex-2-en-1-yl)methanol (**7c**)

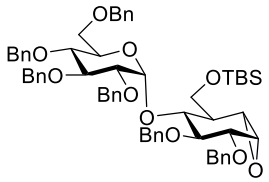


Starting from compound **6c** (1.1 g, 1.05 mmol) and following **General procedure C**, The reaction was purified by flash column chromatography (pentane/EtOAc 7:1→3:1) to obtain compound **7c** (696 mg, 71%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.11 (m, 23H, C*H* Ar), 7.10 – 7.02 (m, 2H, C*H* Ar), 5.81 (dt, *J* = 10.2, 2.4 Hz, 1H, H1), 5.76 (d, *J* = 4.0 Hz, 1H, H1'), 5.65 (dt, *J* = 10.1, 2.1 Hz, 1H, H7), 5.03 (d, *J* = 12.1 Hz, 1H, CH*H* Bn), 4.92 – 4.81 (m, 2H, CH*H* Bn), 4.71 (d, *J* = 10.9 Hz, 1H, CH*H* Bn), 4.64 – 4.50 (m, 4H, CH*H* Bn), 4.46 (s, 2H, CH*H* Bn), 4.26 (ddt, *J* = 7.3, 3.4, 2.0 Hz, 1H, H2), 4.17 (t, *J* = 9.1 Hz, 1H, H4), 4.01

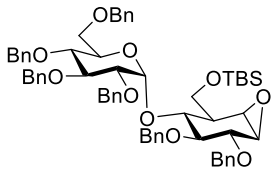
– 3.81 (m, 4H, H6a/H3/H5'/H3'), 3.69 (tdd, *J* = 10.4, 7.6, 2.6 Hz, 3H,1C*H*$_2$O linker/H6'a/H6b), 3.50 (dd, *J* = 9.9, 6.8 Hz, 1H, H6'b), 3.43 (dd, *J* = 9.8, 4.0 Hz, 1H, H2'), 3.31 (dt, *J* = 9.0, 6.8 Hz, 1H, 1C*H*$_2$O linker), 3.21 (t, *J* = 6.9 Hz, 2H, CH_2N_3), 3.10 (dd, *J* = 10.1, 8.7 Hz, 1H, H4'), 2.58 (dh, *J* = 8.7, 2.8 Hz, 1H, H5), 1.54 (p, *J* = 7.0 Hz, 2H, 2CH*H* linker), 1.40 (dq, *J* = 13.4, 6.7 Hz, 2H, 2CH*H* linker), 1.33 – 1.05 (m, 8H, 8CH*H* linker). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.3, 138.7, 138.2, 138.1, 137.3 (5C_q Ar), 129.7 (C7), 128.5, 128.4, 128.3, 128.2, 128.1, 128.1, 127.9, 127.9, 127.7, 127.6, 127.6, 127.5, 127.5, 127.4 (24*C*H Ar), 126.9 (C1), 126.4 (C*H* Ar), 97.3 (C1'), 84.4 (C3), 81.7 (C3'), 80.7 (C2), 79.0 (C2'), 78.8 (C4'), 75.4 (Bn), 73.8 (Bn), 73.6 (Bn), 73.3 (*C*H$_2$O linker), 73.2 (C4), 72.9 (Bn), 71.6 (Bn), 71.5 (C5'), 69.2 (C6'), 62.0 (C6), 51.4 (*C*H_2N_3), 45.7 (C5), 30.2, 29.3, 29.0, 28.8, 26.7, 26.0 (6*C*H$_2$ linker). HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{56}H_{67}N_3O_9Na$ 948.4770, found 948.4769.

(((1S,2S,3R,4S,5R,6S)-4,5-bis(benzyloxy)-3-(((2S,3R,4S,5R,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)-7-oxabicyclo[4.1.0]heptan-2-yl)methoxy)(tert-butyl)dimethylsilane (**8a**) and (((1R,2S,3R,4S,5R,6R)-4,5-bis(benzyloxy)-3-(((2S,3R,4S,5R,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)-7-oxabicyclo[4.1.0]heptan-2-yl)methoxy)(tert-butyl)dimethylsilane (**9a**)

Starting from compound **7a** (700 mg, 0.8 mmol) and following **General procedure D and E**, the product was purified by flash column chromatography (pentane/Et_2O 13:1→9:1) to obtain compound **8a** (160 mg, 20%) as a clean oil and compound **9a** (467 mg, 58%) as a clean oil.

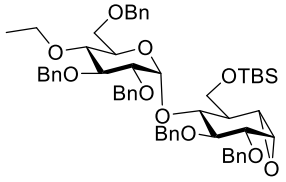


8a: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 – 7.10 (m, 30H, C*H* Ar), 5.34 (d, *J* = 3.6 Hz, 1H, H1'), 4.96 (dd, *J* = 27.9, 11.2 Hz, 2H, CH*H* Bn), 4.89 – 4.79 (m, 3H, CH*H* Bn), 4.78 – 4.67 (m, 2H, CH*H* Bn), 4.59 (dd, *J* = 17.7, 12.2 Hz, 2H, CH*H* Bn), 4.51 – 4.43 (m, 3H, CH*H* Bn), 4.02 (t, *J* = 9.4 Hz, 1H, H3'), 3.95 (t, *J* = 9.1 Hz, 1H, H3), 3.88 (dt, *J* = 9.8, 3.0 Hz, 1H, H5'), 3.85 – 3.75 (m, 3H, H2/H4/H6a), 3.75 – 3.70 (m, 2H, H6b/H6'a), 3.66 (dd, *J* = 10.0, 9.0 Hz, 1H, H4'), 3.58 (dd, *J* = 10.4, 2.0 Hz, 1H, H6'b), 3.48 (dd, *J* = 9.9, 3.6 Hz, 1H, H2'), 3.31 (d, *J* = 4.2 Hz, 1H, epoxide), 3.08 (d, *J* = 4.2 Hz, 1H, epoxide), 2.39 (dq, *J* = 8.4, 4.8, 4.1 Hz, 1H, H5), 0.87 (s, 9H, (C*H*$_3$)$_3$CSi), 0.02 (d, *J* = 5.2 Hz, 6H, 2C*H*$_3$Si). ^{13}C NMR (126 MHz, $CDCl_3$) δ 139.6, 138.8, 138.5, 138.4, 138.4, 138.0 (6C_q Ar), 128.5, 128.5, 128.3, 128.2, 128.1, 128.1, 127.9, 127.9, 127.8, 127.7, 127.6, 127.5, 127.0, 127.0 (30*C*H Ar), 98.7 (C1'), 81.9 (C3'), 80.9 (C3), 79.6 (C2'), 79.0 (C2), 78.6 (C4), 78.0 (C4'), 75.7 (Bn), 75.1 (Bn), 74.7 (Bn), 73.7 (Bn), 73.1 (Bn), 72.9 (Bn), 71.1 (C5'), 68.6 (C6'), 63.0 (C6), 55.7 (epoxide), 55.2 (epoxide), 44.1 (C5), 26.0 ((*C*H$_3$)$_3$CSi), -5.2 (*C*H$_3$Si), -5.4 (*C*H$_3$Si). HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{61}H_{72}O_{10}SiNa$ 1015.4787, found 1015.4792.

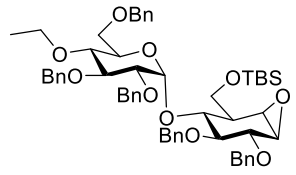


9a:^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 – 6.83 (m, 30H, C*H* Ar), 5.70 (d, *J* = 3.6 Hz, 1H, H1'), 4.93 – 4.67 (m, 6H, CH*H* Bn), 4.64 – 4.54 (m, 2H, CH*H* Bn), 4.49 – 4.39 (m, 4H, CH*H* Bn), 4.15 (dd, *J* = 9.1, 4.3 Hz, 1H, H6a), 3.94 (dd, *J* = 9.9, 8.7 Hz, 1H, H3'), 3.90 – 3.85 (m, 1H, H3), 3.76 (dt, *J* = 10.1, 2.6 Hz, 1H, H5'), 3.74 – 3.59 (m, 6H, H6'a/H6'b/H6b/H2/H4/H4'), 3.53 (dt, *J* = 4.1, 1.0 Hz, 1H, epoxide), 3.45 (dd, *J* = 9.9, 3.7 Hz, 1H, H2'), 3.22 (d, *J* = 3.8 Hz, 1H, epoxide), 2.36 (dtd, *J* = 10.5, 5.4, 2.5 Hz, 1H, H5), 0.87 (s, 9H, (C*H*$_3$)$_3$CSi), 0.04 (d, *J* = 2.1 Hz, 6H, 2C*H*$_3$Si). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.1, 138.8, 138.7, 138.2, 138.0, 137.6 (6C_q Ar), 128.6, 128.5, 128.4, 128.3, 128.3, 128.1, 128.1, 128.0, 128.0, 127.8, 127.7, 127.6, 127.6, 127.5, 127.1, 126.5 (30*C*H Ar), 97.0 (C1'), 85.0, 81.9 (C3'), 80.3 (C3), 79.3 (C2'), 77.7 (C4'), 75.7 (Bn), 74.8 (Bn), 73.8 (Bn), 73.7 (Bn), 73.0 (Bn), 72.9 (Bn), 71.5 (C5'), 70.0, 68.4 (C6'), 62.8 (C6), 55.0 (epoxide), 53.8 (epoxide), 43.8 (C5), 26.1 ((*C*H$_3$)$_3$CSi), -5.3 (*C*H$_3$Si), -5.4 (*C*H$_3$Si). HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{61}H_{72}O_{10}SiNa$ 1015.4787, found 1015.4789.

(((1S,2S,3R,4S,5R,6S)-4,5-bis(benzyloxy)-3-(((2S,3R,4S,5R,6R)-3,4-bis(benzyloxy)-6-((benzyloxy)methyl)-5-ethoxytetrahydro-2H-pyran-2-yl)oxy)-7-oxabicyclo[4.1.0]heptan-2-yl)methoxy)(tert-butyl)dimethylsilane (**8b**) and (((1R,2S,3R,4S,5R,6R)-4,5-bis(benzyloxy)-3-(((2S,3R,4S,5R,6R)-3,4-bis(benzyloxy)-6-((benzyloxy)methyl)-5-ethoxytetrahydro-2H-pyran-2-yl)oxy)-7-oxabicyclo[4.1.0]heptan-2-yl)methoxy)(tert-butyl)dimethylsilane (**9b**)
Starting from compound **7b** (447 mg, 0.56 mmol) and following **General procedure D and E**, the product was purified by flash column chromatography (pentane/Et_2O 11:1→5:1) to obtain compound **8b** (108 mg, 21%) as a light yellow oil and compound **9b** (338 mg, 65%) as a light yellow oil.

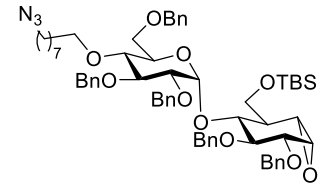


8b:^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 – 7.01 (m, 25H, C*H* Ar), 5.34 (d, *J* = 3.6 Hz, 1H, H1'), 5.02 (d, *J* = 11.6 Hz, 1H, CH*H* Bn), 4.94 – 4.81 (m, 3H, CH*H* Bn), 4.80 – 4.70 (m, 2H, CH*H* Bn), 4.67 (d, *J* = 12.0 Hz, 1H, CH*H* Bn), 4.60 (d, *J* = 12.3 Hz, 1H, CH*H* Bn), 4.49 (dd, *J* = 14.5, 12.1 Hz, 2H, CH*H* Bn), 4.01 – 3.91 (m, 2H), 3.88 – 3.65 (m, 7H), 3.61 (dd, *J* = 10.3, 2.1 Hz, 1H), 3.57 – 3.41 (m, 3H), 3.36 – 3.31 (m, 1H, H1), 3.10 (dd, *J* = 4.3, 0.8 Hz, 1H, H7), 2.43 (dt, *J* = 7.1, 3.4 Hz, 1H, H5), 1.11 (t, *J* = 7.0 Hz, 3H, C*H*$_3$ Et), 0.90 (s, 9H, *t*Bu TBS), 0.05 (d, *J* = 4.2 Hz, 6H, 2C*H*$_3$ TBS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.6, 138.9, 138.4, 138.4, 138.0 (5C_q Ar), 128.5, 128.4, 128.3, 128.2, 128.1, 127.8, 127.8, 127.6, 127.6, 127.4, 127.0, 127.0 (25*C*H Ar), 98.7 (C1'), 81.8, 80.9, 79.4, 79.0, 78.5, 78.0, 75.7 (Bn), 74.8 (Bn), 73.7 (Bn), 73.1 (Bn), 72.9 (Bn), 71.21 (C5'), 68.6 (C6'), 68.5 (CH_2 Et), 63.0 (C6), 55.7 (C7), 55.2 (C1), 44.1 (C5), 26.0 (*t*Bu TBS), 15.9 (*C*H$_3$ Et), -5.2 (*C*H$_3$ TBS), -5.4 (*C*H$_3$ TBS). HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{56}H_{70}O_{10}SiNa$ 953.4631, found 953.4635.



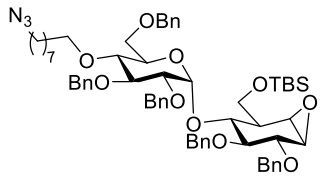
9b:^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 – 6.93 (m, 25H, C*H* Ar), 5.67 (d, *J* = 3.6 Hz, 1H, H1'), 4.98 – 4.36 (m, 10H, CH*H* Bn), 4.14 (dd, *J* = 9.2, 4.3 Hz, 1H, H6a), 3.90 – 3.82 (m, 2H), 3.80 – 3.56 (m, 7H), 3.52 (dt, *J* = 3.8, 1.2 Hz, 1H, epoxide), 3.49 – 3.35 (m, 3H), 3.21 (d, *J* = 3.8 Hz, 1H, epoxide), 2.35 (tdd, *J* = 9.4, 4.3, 1.5 Hz, 1H, H5), 1.03 (t, *J* = 7.0 Hz, 3H, CH_3 Et), 0.91 (s, 9H, *t*Bu TBS), 0.07 (d, *J* = 1.9 Hz, 6H, 2C*H*$_3$ TBS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.1, 139.0, 138.3, 138.1, 137.6 (5C_q Ar), 128.6, 128.4, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.6, 127.6, 127.4, 127.1, 126.5 (25*C*H Ar), 97.0 (C1'), 85.0, 81.8, 80.3, 79.2, 77.7, 75.6 (Bn), 73.8 (Bn), 73.7 (Bn), 73.1 (Bn), 72.8 (Bn), 71.6, 70.0, 68.4 (C6'), 68.2 (CH_2 Et), 62.8 (C6), 55.0 (epoxide), 53.7 (epoxide), 43.7 (C5), 26.1 (*t*Bu TBS), 15.9 (*C*H$_3$ Et), -5.3 (*C*H$_3$ TBS), -5.4 (*C*H$_3$ TBS). HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{56}H_{70}O_{10}SiNa$ 953.4631, found 953.4632.

(((1S,2S,3R,4S,5R,6S)-3-(((2S,3R,4S,5R,6R)-5-((8-azidooctyl)oxy)-3,4-bis(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)-4,5-bis(benzyloxy)-7-oxabicyclo[4.1.0]heptan-2-yl)methoxy)(tert-butyl)dimethylsilane (**8c**) and (((1R,2S,3R,4S,5R,6R)-3-(((2S,3R,4S,5R,6R)-5-((8-azidooctyl)oxy)-3,4-bis(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)-4,5-bis(benzyloxy)-7-oxabicyclo[4.1.0]heptan-2-yl)methoxy)(tert-butyl)dimethylsilane (**9c**)
Starting from compound **7c** (666 mg, 0.7 mmol) and following **General procedure D and E**, the product was purified by flash column chromatography (pentane/Et_2O 11:1→7:1) to obtain compound **8c** (165 mg, 22%) as a yellow oil and compound **9c** (478 mg, 63%)as a yellow oil.



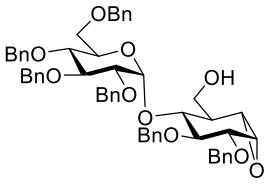
8c:^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.13 (m, 25H, C*H* Ar), 5.33 (d, *J* = 3.6 Hz, 1H, H1'), 5.12 – 4.34 (m, 10H, CH*H* Bn), 4.02 – 3.89 (m, 2H), 3.86 – 3.65 (m, 7H), 3.60 (dd, *J* = 10.3, 2.1 Hz, 1H), 3.50 – 3.37 (m, 3H), 3.36 – 3.31 (m, 1H, epoxide), 3.28 (t, *J* = 6.9 Hz, 2H, C*H*$_2$N$_3$), 3.11 (dd, *J* = 4.2, 0.9 Hz, 1H, epoxide), 2.43 (dt, *J* = 6.7, 3.4 Hz, 1H, H5), 1.69 – 1.55 (m, 2H, 2CH*H* linker), 1.48 (dt, *J* = 8.7, 2.8 Hz,

2H, 2CH*H* linker), 1.39 – 1.24 (m, 8H, 8CH*H* linker), 0.90 (s, 9H, *t*Bu TBS), 0.09 – 0.03 (m, 6H, 2C*H*$_3$ TBS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.6, 138.9, 138.4, 138.4, 138.0 (5C_q Ar), 128.4, 128.4, 128.3, 128.2, 128.1, 128.0, 128.0, 127.8, 127.7, 127.6, 127.4, 127.0 (25*C*H Ar), 98.7 (C1'), 81.8, 80.9, 79.4, 79.0, 78.6, 78.0, 75.6 (Bn), 74.8 (Bn), 73.7 (Bn), 73.2 (*C*H$_2$O linker), 73.1 (Bn), 72.9 (Bn), 71.2, 68.7 (C6'), 63.0 (C6), 55.7 (epoxide), 55.2 (epoxide), 51.6 (*C*H$_2$N$_3$), 44.1 (C5), 30.5, 29.5, 29.2, 28.9, 26.8, 26.2 (6*C*H$_2$ linker), 26.0 (*t*Bu TBS), -5.2 (*C*H$_3$ TBS), -5.4 (*C*H$_3$ TBS). HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{62}H_{81}N_3O_{10}SiNa$ 1078.5583, found 1078.5581.



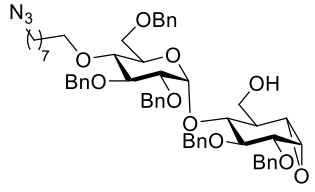
9c:^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 – 6.88 (m, 25H, C*H* Ar), 5.69 (d, *J* = 3.7 Hz, 1H, H1'), 5.00 – 4.28 (m, 10H, CH*H* Bn), 4.15 (dd, *J* = 9.1, 4.3 Hz, 1H, H6a), 3.90 – 3.80 (m, 2H), 3.78 – 3.56 (m, 7H), 3.56 – 3.50 (m, 1H, epoxide), 3.46 – 3.29 (m, 3H), 3.25 – 3.17 (m, 3H, 1H-epoxide/C*H*$_2$N$_3$), 2.36 (tdd, *J* = 9.4, 4.4, 2.4 Hz, 1H, H5), 1.55 (dq, *J* = 8.3, 6.8 Hz, 2H, 2CH*H* linker), 1.46 – 1.33 (m, 2H, 2CH*H* linker), 1.32 – 1.08 (m, 8H, 8CH*H* linker), 0.91 (s, 9H, *t*Bu TBS), 0.08 (d, *J* = 2.3 Hz, 6H, 2C*H*$_3$ TBS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.1, 138.9, 138.2, 138.0, 137.6 (5C_q Ar), 128.5, 128.4, 128.3, 128.2, 128.2, 128.0, 128.0, 127.8, 127.7, 127.6, 127.5, 127.4, 127.0, 126.4 (25*C*H Ar), 96.9 (C1'), 85.1, 81.8, 80.2, 79.1, 77.7, 75.5 (Bn), 73.7 (Bn), 73.6 (Bn), 73.0 (Bn), 72.9 (*C*H$_2$O linker), 72.7 (Bn), 71.6, 69.7, 68.4 (C6'), 62.8 (C6), 54.9 (epoxide), 53.7 (epoxide), 51.5 (*C*H$_2$N$_3$), 43.7 (C5), 30.5, 29.5, 29.2, 28.9, 26.8, 26.2 (6*C*H$_2$ linker), 26.1 (*t*Bu TBS), -5.3 (*C*H$_3$ TBS), -5.4 (*C*H$_3$ TBS). HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{62}H_{81}N_3O_{10}SiNa$ 1078.5583, found 1078.5581.

((1S,2S,3R,4S,5R,6S)-4,5-bis(benzyloxy)-3-(((2S,3R,4S,5R,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)-7-oxabicyclo[4.1.0]heptan-2-yl)methanol (**10a**)



Starting from compound **8a** (100 mg, 0.1 mmol) and following **General procedure F**, the product was purified by flash column chromatography (pentane/EtOAc 4:1→2:1) to obtain compound **10a** (80 mg, 90%) as a clean oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 – 6.99 (m, 30H, C*H* Ar), 5.58 (d, *J* = 4.0 Hz, 1H, H1'), 4.97 – 4.83 (m, 3H, CH*H* Bn), 4.76 – 4.63 (m, 4H, CH*H* Bn), 4.58 (d, *J* = 12.3 Hz, 1H, CH*H* Bn), 4.55 – 4.45 (m, 2H, CH*H* Bn), 4.39 (dd, *J* = 11.5, 7.7 Hz, 2H, CH*H* Bn), 4.01 – 3.75 (m, 7H, H3'/H5'/H6a/H6b/H2/H3/H4), 3.66 (dd, *J* = 9.9, 1.7 Hz, 1H, H6'a), 3.47 – 3.31 (m, 3H, H2'/H6'b/epoxide), 3.29 – 3.08 (m, 2H, H4'/epoxide), 2.34 – 2.25 (m, 1H, H5). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.5, 138.5, 138.2, 138.1, 137.7, 137.1 (6C_q Ar), 128.7, 128.6, 128.5, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 127.8, 127.7, 127.5, 127.0, 126.5 (30*C*H Ar), 97.8 (C1'), 81.8, 81.4, 79.8, 79.0 (C2'), 78.5 (C4'), 75.6 (Bn), 75.1 (Bn), 74.6 (Bn), 74.5, 73.7 (Bn), 73.2 (Bn), 72.8 (Bn), 71.5 (C5'), 69.5 (C6'), 61.6 (C6), 55.5 (epoxide), 54.9 (epoxide), 44.2 (C5). HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{55}H_{58}O_{10}Na$ 901.3922, found 901.3920.

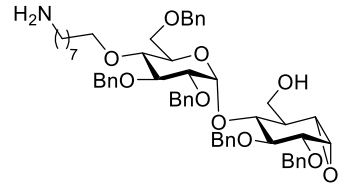
((1S,2S,3R,4S,5R,6S)-3-(((2S,3R,4S,5R,6R)-5-((8-azidooctyl)oxy)-3,4-bis(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)-4,5-bis(benzyloxy)-7-oxabicyclo[4.1.0]heptan-2-yl)methanol (**10b**)



Starting from compound **8c** (150 mg, 0.14 mmol) and following **General procedure F**, the product was purified by flash column chromatography (pentane/EtOAc 3:1→1:1) to obtain compound **10c** (117 mg, 87%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.00 (m, 25H, C*H* Ar), 5.53 (d, *J* = 3.9 Hz, 1H, H1'), 4.91 (s, 2H, CH*H* Bn), 4.82 (d, *J* = 10.9 Hz, 1H, CH*H* Bn), 4.71 (d, *J* = 7.7 Hz, 3H, CH*H* Bn), 4.64 – 4.53 (m, 2H, CH*H* Bn), 4.50 (d, *J* = 12.1 Hz, 1H, CH*H* Bn), 4.39 (d, *J* = 12.1 Hz, 1H, CH*H* Bn), 3.97 – 3.76 (m, 7H, H3'/H5'/H6a/H6b/H2/H3/H4), 3.76 – 3.66 (m, 2H,

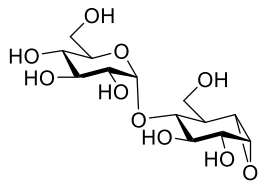
H6'a/1CH*H*O linker), 3.49 (dd, *J* = 9.8, 7.6 Hz, 1H, H6'b), 3.43 – 3.26 (m, 3H, H2'/1H-epoxide/1CH*H*O linker), 3.26 – 3.19 (m, 3H, 1H-epoxide/C*H*$_2$N$_3$), 3.03 (dd, *J* = 10.1, 8.5 Hz, 1H, H4'), 2.31 (ddd, *J* = 7.7, 4.4, 2.6 Hz, 1H, H5), 1.56 (p, *J* = 7.0 Hz, 2H, 2CH*H* linker), 1.41 (p, *J* = 6.9, 6.5 Hz, 2H, 2CH*H* linker), 1.36 – 1.11 (m, 8H, 8CH*H* linker). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.5, 138.6, 138.2, 138.1, 137.2 (5C$_q$ Ar), 128.6, 128.4, 128.4, 128.2, 128.2, 128.2, 128.1, 128.1, 127.8, 127.8, 127.6, 127.5, 127.4, 126.9, 126.5 (25*C*H Ar), 97.9 (C1'), 81.5, 81.4, 79.7, 79.0 (C4'), 78.9 (C2'), 75.4 (Bn), 74.9, 74.6 (Bn), 73.7 (Bn), 73.3 (*C*H$_2$O linker), 73.2 (Bn), 72.7 (Bn), 71.7, 69.7 (C6'), 61.6 (C6), 55.4 (epoxide), 54.8 (epoxide), 51.5 (*C*H$_2$N$_3$), 44.2 (C5), 30.3, 29.4, 29.1, 28.9, 26.7, 26.0 (6*C*H$_2$ linker). HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{56}H_{67}N_3O_{10}Na$ 964.4719, found 964.4717.

((1S,2S,3R,4S,5R,6S)-3-(((2S,3R,4S,5R,6R)-5-((8-aminooctyl)oxy)-3,4-bis(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)-4,5-bis(benzyloxy)-7-oxabicyclo[4.1.0]heptan-2-yl)methanol (**11**)



Compound **10b** (110 mg, 0.12mmol) was dissolved in MeCN (2.4 mL), polymer-bound triphenylphosphine (3 mmol/g loading, 100mg, 0.3mmol) and H_2O (21 uL, 1.2 mmol) were added and the mixture was stirred overnight at 70 °C. TLC indicated total consumption of the starting material, and additional H_2O (500 µL) was added and the mixture was stirred for 5 h at 70 °C. The mixture was cooled to rt, filtered, washed with MeCN (3 x 5 mL), the combined filtrate was diluted with MeCN (50 mL), dried over Na_2SO_4, filtered and concentrated in vacuo. The crude was purified by flash column chromatography (DCM/MeOH, 19:1→15:1) affording compound **11** (84 mg, 79%) as a clean oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 6.99 (m, 25H, C*H* Ar), 5.55 (d, *J* = 3.9 Hz, 1H, H1'), 4.90 (s, 2H, CH*H* Bn), 4.82 (d, *J* = 11.0 Hz, 1H, CH*H* Bn), 4.69 (q, *J* = 6.3, 5.6 Hz, 3H, CH*H* Bn), 4.57 (s, 2H, CH*H* Bn), 4.49 (d, *J* = 12.2 Hz, 1H, CH*H* Bn), 4.39 (d, *J* = 12.1 Hz, 1H, CH*H* Bn), 3.85 (ddt, *J* = 22.1, 12.8, 9.4 Hz, 7H, H3'/H5'/H6a/H6b/H2/H3/H4), 3.75 – 3.65 (m, 2H, H6'a/1CH*H*O linker), 3.54 – 3.43 (m, 1H, H6'b), 3.40 – 3.24 (m, 3H, H2'/1H-epoxide/1CH*H*O linker), 3.22 (d, *J* = 4.1 Hz, 1H, 1H-epoxide), 2.86 (t, *J* = 7.7 Hz, 2H, C*H*$_2$NH$_2$), 2.31 (dt, *J* = 7.7, 3.4 Hz, 1H, H5), 1.66 (dq, *J* = 15.4, 7.7, 7.0 Hz, 2H, 2CH*H* linker), 1.45 – 1.35 (m, 2H, 2CH*H* linker), 1.36 – 1.10 (m, 8H, 8CH*H* linker). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.5, 138.7, 138.2, 138.1, 137.3 (5C$_q$ Ar), 128.6, 128.5, 128.4, 128.4, 128.2, 128.2, 128.2, 128.1, 127.9, 127.8, 127.7, 127.5, 127.5, 127.0, 126.5 (25*C*H Ar), 97.9 (C1'), 81.6, 81.5, 79.8, 79.0 (C4'), 78.9 (C2'), 75.4 (Bn), 74.8, 74.6 (Bn), 73.8 (Bn), 73.4 (*C*H$_2$O linker), 73.2, 72.8, 71.7, 69.7 (C6'), 61.7 (C6), 55.4 (epoxide), 54.9 (epoxide), 44.3 (C5), 40.4 (*C*H$_2$NH$_2$), 30.3, 29.4, 29.0, 28.8, 26.6, 26.1 (6*C*H$_2$ linker). HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{56}H_{70}NO_{10}$ 916.4994, found 916.4993.

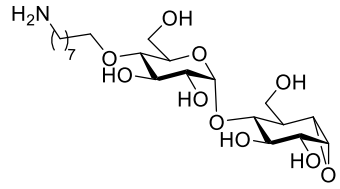
(2S,3R,4S,5S,6R)-2-(((1S,2S,3R,4R,5R,6R)-4,5-dihydroxy-2-(hydroxymethyl)-7-oxabicyclo[4.1.0] heptan-3-yl)oxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol (**1a**)



Compound **10a** (21 mg, 23.8 µmol) was dissolved in a mixture of MeOH/H_2O/dioxane (2/1/2, 1 mL) under Argon and Pd(OH)$_2$/C (20 wt%, 25 mg, 35.8 µmol) was added. While stirring vigorously, the mixture was flushed with a H_2 balloon. After stirring for 3 h under H_2 atmosphere, the mixture was filtered over a small celite pad and evaporate to afford the product in high purity as a white solid (7.8 mg, 100%) after lyophilization. ^{1}H NMR (400 MHz, MeOD) δ 5.02 (d, *J* = 3.9 Hz, 1H, H1'), 3.90 (dd, *J* = 11.1, 3.0 Hz, 1H, H6a), 3.85 – 3.76 (m, 3H, H6b/H6'a/H4'), 3.74 – 3.66 (m, 2H, H6'b/H2), 3.66 – 3.54 (m, 2H, H3/H3'), 3.44 (dd, *J* = 9.7, 3.9 Hz, 1H, H2'), 3.41 – 3.33 (m, 1H, H4), 3.30 – 3.25 (m, 2H, epoxide/H5'), 3.21 (d, *J* = 4.0 Hz, 1H, epoxide), 2.05 (ddd, *J* = 9.0, 5.6, 3.0 Hz, 1H, H5). ^{13}C NMR (101 MHz, MeOD) δ 101.06 (C1'), 80.84 (C4), 72.86 (C3'), 72.72 (C3), 72.32 (C2), 71.82 (C2'), 70.53 (C4'), 69.28 (C5'), 60.51 (C6'), 59.46 (C6), 55.78 (epoxide), 53.39 (epoxide), 43.07 (C5).

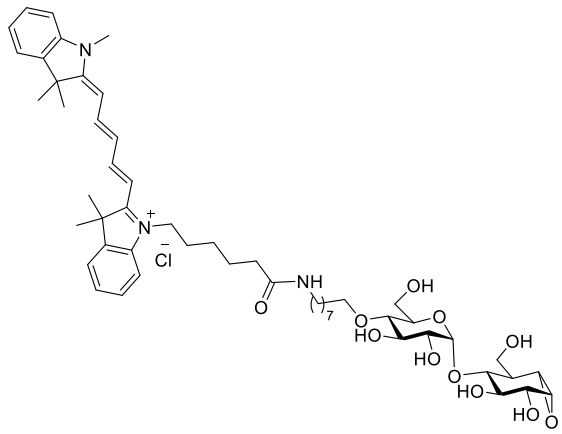
HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{13}H_{22}O_{10}Na$ 361.1105, found 361.1102.

(1R,2R,3R,4R,5S,6S)-4-(((2S,3R,4R,5S,6R)-5-((8-aminooctyl)oxy)-3,4-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-5-(hydroxymethyl)-7-oxabicyclo[4.1.0]heptane-2,3-diol (**1b**)



Compound **11** (30 mg, 32.7 µmol) was dissolved in a mixture of *t*BuOH/H_2O/dioxane (1/2/1, 1.6 mL) under Argon, then HOAc (19 µL, 327 µmol) and $Pd(OH)_2/C$ (20 wt%, 34.5 mg, 49 µmol) was added. While stirring vigorously, the mixture was flushed with a H_2 balloon. After stirring for 6.5 h under H_2 atmosphere, the mixture was filtered over a small celite pad. The filtrate was concentrated and purified by semi-preparative reversed phase HPLC (linear gradient. Solution used: A: 50 mM NH_4HCO_3 in H_2O, B: MeCN). The product was obtained as a white solid (13.7 mg, 91%) after lyophilization. ^{1}H NMR (850 MHz, D_2O) δ 5.11 (d, *J* = 4.0 Hz, 1H, H1'), 3.92 (dd, *J* = 8.9, 1.8 Hz, 1H, H2), 3.87 (dd, *J* = 11.4, 2.9 Hz, 1H, H6a), 3.82 – 3.77 (m, 2H, 1CH*H*O linker/H6'a), 3.76 – 3.67 (m, 4H, H3'/H5'/H6b/H6'b), 3.65 – 3.59 (m, 2H, H3/1CH*H*O linker), 3.54 (ddd, *J* = 9.9, 4.5 Hz, 1H, H2'), 3.46 – 3.42 (m, 2H, H4/1H epoxide), 3.32 (d, *J* = 4.2 Hz, 1H, epoxide), 3.24 (t, *J* = 9.6 Hz, 1H, H4'), 2.94 (t, *J* = 7.6 Hz, 2H, C*H*$_2$$NH_2$), 2.19 (ddd, *J* = 9.0, 5.7, 2.9 Hz, 1H, H5), 1.64 – 1.58 (m, 2H, 2CH*H* linker), 1.58 – 1.51 (m, 2H, 2CH*H* linker), 1.35 – 1.26 (m, 8H, 8CH*H* linker). ^{13}C NMR (214 MHz, D_2O) δ 100.6 (C1'), 79.9 (C4), 77.6 (C4'), 73.4 (C3), 73.4 (*C*H_2O linker), 72.9 (C3'), 71.8 (C2'), 71.6 (C5'), 70.6 (C2), 60.3 (C6), 60.2 (C6'), 57.3 (epoxide), 55.2 (epoxide), 42.8 (C5), 39.5 (*C*$H_2$$NH_2$), 29.2, 28.2, 28.1, 26.7, 25.5, 25.1 (6*C*H_2 linker). HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{21}H_{40}NO_{10}$ 466.2647, found 466.2644.

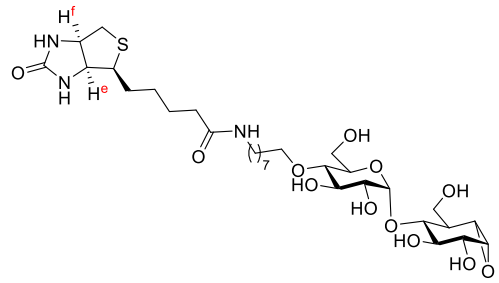
1-(6-((8-(((2R,3S,4R,5R,6S)-6-(((1S,2S,3R,4R,5R,6R)-4,5-dihydroxy-2-(hydroxymethyl)-7-oxabicyclo[4.1.0]heptan-3-yl)oxy)-4,5-dihydroxy-2-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)oxy)octyl)amino)-6-oxohexyl)-3,3-dimethyl-2-((1E,3E)-5-((E)-1,3,3-trimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-3H-indol-1-ium chloride (**1c**)



Compound **1b** (5.43 mg, 11.7 µmol) was dissolved in dry DMF (0.5 mL), then DIPEA (6 µL, 34 µmol) and Cy5-OSu (7.9 mg, 13 µmol) were added and the mixture was stirred at rt for 40 h. Full conversion was observed by LC-MS and the product was purified by semi-preparative reversed phase HPLC (linear gradient. Solution used: A: 50 mM NH_4HCO_3 in H_2O, B: MeCN). The fractions were concentrated under reduced pressure, co-evaporated with Milli-Q/MeCN (1/1, 3 x), dissolved in Milli-Q/^{t}BuOH (1/1) again and lyophilized to obtain the title compound (3.74 mg, 33%) as a blue solid. ^{1}H NMR (500 MHz, MeOD) δ 8.30 – 8.19 (m, 2H), 7.49 (d, *J* = 7.4 Hz, 2H), 7.41 (tdd, *J* = 7.7, 3.8, 1.2 Hz, 2H), 7.33 – 7.23 (m, 4H), 6.63 (t, *J* = 12.4 Hz, 1H), 6.28 (dd, *J* = 13.7, 5.9 Hz, 2H), 5.00 (d, *J* = 3.9 Hz, 1H, H1'), 4.11 (t, *J* = 7.4 Hz, 2H, C*H*$_2$$N^+$), 3.92 – 3.83 (m, 2H, H6a/1CH*H*O linker), 3.82 – 3.72 (m, 3H, H2/H6b/H6'a), 3.72 – 3.65 (m, 3H, H3'/H5'/H6'b), 3.63 (s, 3H, C*H*$_3$N), 3.62 – 3.52 (m, 2H, H3/1CH*H*O linker), 3.43 (dd, *J* = 9.7, 3.9 Hz, 1H, H2'), 3.36 – 3.33 (m, 1H, H4), 3.27 (dd, *J* = 4.0, 1.8 Hz, 1H, 1H epoxide), 3.22 – 3.18 (m, 1H, 1H epoxide), 3.19 – 3.07 (m, 3H, H4'/C*H*$_2$NHC=O), 2.20 (t, *J* = 7.3 Hz, 2H2H, C*H*$_2$C=O), 2.04 (ddt, *J* = 11.5, 6.0, 2.6 Hz, 1H, H5), 1.83 (dt, *J* = 15.0, 7.4 Hz, 2H, 2CH*H* linker), 1.73 (s, 14H, 4C*H*$_3$/2CH*H* linker), 1.63 – 1.50 (m, 2H, 2CH*H* linker), 1.50 – 1.41 (m, 4H, 4CH*H* linker), 1.32 (dq, *J* = 5.6, 2.9, 2.3 Hz, 8H, 8CH*H* linker). ^{13}C NMR (126 MHz, MeOD) δ 175.7, 175.4, 174.7, 155.5, 155.5, 144.3, 143.6, 142.7, 142.5, 129.7, 129.7, 126.7, 126.2, 126.2, 123.4, 123.3, 112.1, 111.8, 104.4, 104.3, 103.2 (C1'), 83.1 (C4), 79.2 (C4'), 75.3 (C3'), 74.9 (C3), 74.1 (C2'), 74.0 (*C*H_2O linker), 73.7 (C5'), 72.7 (C2), 62.3 (C6'), 61.6 (C6), 58.0 (epoxide),

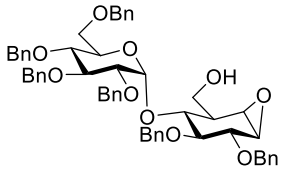
55.5 (epoxide), 50.6 (C_q), 50.5 (C_q), 45.2 (C5), 44.8 (*C*H_2N^+), 40.4 (*C*H_2NHC=O), 36.7 (*C*H_2C=O), 31.5 (*C*H_3N), 31.4, 30.5, 30.4, 30.3, 28.2 (5*C*H_2 linker), 28.0 (2*C*H_3), 27.9 (1*C*H_2 linker), 27.8 (2*C*H_3), 27.4, 27.2, 26.6 (3*C*H_2 linker). HRMS (ESI) m/z: $[M]^+$ calc for $C_{53}H_{76}N_3O_{11}$ 930.5474, found 930.5475.

N-(8-(((2R,3S,4R,5R,6S)-6-(((1S,2S,3R,4R,5R,6R)-4,5-dihydroxy-2-(hydroxymethyl)-7-oxabicyclo[4.1.0]heptan-3-yl)oxy)-4,5-dihydroxy-2-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)oxy)octyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (**1d**)



Compound **1b** (5.7 mg, 12.2 µmol) was dissolved in dry DMF (0.5 mL), then DIPEA (4.3 µL, 24.4 µmol) and biotin-OSu (4.6 mg, 13.5 µmol) were added and the mixture was stirred at rt for 22 h. Full conversion was observed by LC-MS and the product was purified by semi-preparative reversed phase HPLC (linear gradient. Solution used: A: 50 mM NH_4HCO_3 in H_2O, B: MeCN). The fractions were concentrated under reduced pressure, co-evaporated with Milli-Q/MeCN (1/1, 3 x), dissolved in Milli-Q/^{t}BuOH (1/1) again and lyophilized to obtain the title compound (1.42 mg, 16%) as a white solid. ^{1}H NMR (850 MHz, MeOD) δ 5.00 (d, *J* = 3.9 Hz, 1H, H1'), 4.51 – 4.48 (m, 1H, Hf), 4.31 (dd, *J* = 7.9, 4.5 Hz, 1H, He), 3.90 – 3.85 (m, 2H, H6a/1CH*H*O linker), 3.82 – 3.75 (m, 3H, H2/H6b/H6'a), 3.72 – 3.65 (m, 3H, H3'/H5'/H6'b), 3.61 – 3.55 (m, 2H, H3/1CH*H*O linker), 3.43 (dd, *J* = 9.7, 3.9 Hz, 1H, H2'), 3.33 (d, *J* = 9.6 Hz, 1H, H4), 3.27 (dd, *J* = 4.1, 1.8 Hz, 1H, 1H epoxide), 3.23 – 3.19 (m, 2H, 1H epoxide/C*H*S), 3.18 – 3.13 (m, 3H, H4'/C*H*$_2$NHC=O), 2.93 (dd, *J* = 12.8, 5.0 Hz, 1H, Ha C*H*$_2$-S), 2.71 (d, *J* = 12.7 Hz, 1H, Hb C*H*$_2$-S), 2.23 – 2.14 (m, 2H, C*H*$_2$C=O), 2.05 (ddd, *J* = 9.2, 5.8, 3.0 Hz, 1H, H5), 1.78 – 1.53 (m, 6H, 6CH*H* linker), 1.50 (t, *J* = 7.0 Hz, 2H, 2CH*H* linker), 1.45 (q, *J* = 7.6 Hz, 2H, 2CH*H* linker), 1.41 – 1.30 (m, 8H, 8CH*H* linker). ^{13}C NMR (214 MHz, MeOD) δ 176.0, 166.1, 103.2 (C1'), 83.0 (C4), 79.3 (C4'), 75.3 (C3'), 74.9 (C3), 74.1 (C2'), 74.0 (*C*H_2O linker), 73.7 (C5'), 72.7 (C2), 63.4 (Ce), 62.3 (C6'), 61.7 (C6), 61.6 (Cf), 57.9 (epoxide), 57.0 (*C*HS), 55.5 (epoxide), 45.2 (C5), 41.1 (*C*H_2S), 40.4 (*C*H_2NHC=O), 36.9 (*C*H_2C=O), 31.4, 30.5, 30.4, 30.3, 29.8, 29.5, 27.9, 27.1, 27.0 (9*C*H_2 linker). HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{31}H_{54}N_3O_{12}S$ 692.3422, found 692.3412.

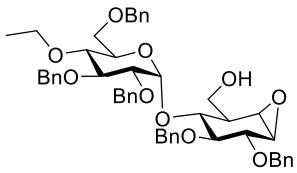
((1R,2S,3R,4S,5R,6R)-4,5-bis(benzyloxy)-3-(((2S,3R,4S,5R,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)-7-oxabicyclo[4.1.0]heptan-2-yl)methanol (**12a**)



Starting from compound **9a** (322 mg, 0.32 mmol) and following **General procedure F**, the product was purified by flash column chromatography (pentane/EtOAc 4:1→2:1) to obtain compound **12a** (260 mg, 91%) as a clean oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47 – 6.91 (m, 30H, C*H* Ar), 5.66 (d, *J* = 3.9 Hz, 1H, H1'), 4.87 (t, *J* = 11.5 Hz, 2H, CH*H* Bn), 4.75 (ddd, *J* = 26.1, 19.7, 11.7 Hz, 4H, CH*H* Bn), 4.61 – 4.55 (m, 2H, CH*H* Bn), 4.55 – 4.38 (m, 4H, CH*H* Bn), 4.03 (dd, *J* = 11.3, 5.0 Hz, 1H, H6a), 4.01 – 3.85 (m, 5H, H6b/H3/H3'/H5'), 3.73 – 3.63 (m, 2H, H6'a), 3.58 (dd, *J* = 10.3, 5.0 Hz, 1H, H6'b), 3.50 – 3.41 (m, 2H, H2'), 3.39 (dd, *J* = 4.1, 1.7 Hz, 1H, epoxide), 3.16 (d, *J* = 3.8 Hz, 1H, epoxide), 2.28 – 2.20 (m, 1H, H5). ^{13}C NMR (126 MHz, $CDCl_3$) δ 139.2, 138.8, 138.2, 138.2, 137.8, 137.6 (6C_q Ar), 128.6, 128.6, 128.5, 128.5, 128.3, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 127.9, 127.7, 127.7, 127.5, 127.1, 126.5 (30*C*H Ar), 97.4 (C1'), 84.8, 81.9, 80.2 (C3'), 79.3 (C2'), 78.1, 75.5 (Bn), 75.1 (Bn), 73.8 (Bn), 73.7 (Bn), 73.2 (Bn), 72.9 (Bn), 71.5 (C5'), 70.3, 68.9 (C6'), 61.9 (C6), 56.5 (epoxide), 52.5 (epoxide), 43.3 (C5). HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{55}H_{58}O_{10}Na$ 901.3922, found 901.3921.

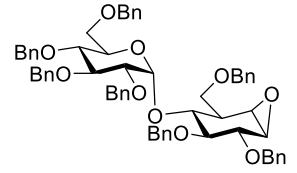
((1R,2S,3R,4S,5R,6R)-4,5-bis(benzyloxy)-3-(((2S,3R,4S,5R,6R)-3,4-bis(benzyloxy)-6-((benzyloxy)methyl)-5-

ethoxytetrahydro-2H-pyran-2-yl)oxy)-7-oxabicyclo[4.1.0]heptan-2-yl)methanol (**12b**)



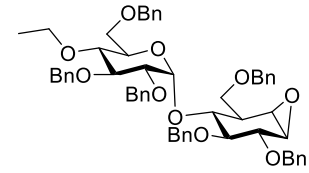
Starting from compound **9b** (338 mg, 0.36 mmol) and following **General procedure F**, the product was purified by flash column chromatography (pentane/EtOAc 3:1→2:1) to obtain compound **12b** (261 mg, 88%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 6.99 (m, 25H, C*H* Ar), 5.66 (d, *J* = 3.9 Hz, 1H, H1'), 4.95 – 4.35 (m, 10H, CH*H* Bn), 4.08 – 3.91 (m, 3H), 3.91 – 3.64 (m, 6H), 3.59 (dd, *J* = 10.3, 4.9 Hz, 1H, H6'b), 3.46 – 3.37 (m, 3H, H2'/1CH*H* Et/1H-epoxide), 3.26 (dd, *J* = 10.1, 8.8 Hz, 1H), 3.17 (d, *J* = 3.8 Hz, 1H, 1H-epoxide), 2.31 – 2.20 (m, 1H, H5), 1.04 (t, *J* = 7.0 Hz, 3H, C*H*$_3$ Et). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.2, 138.8, 138.2, 137.8, 137.5 (5C_q Ar), 128.6, 128.5, 128.4, 128.3, 128.2, 128.2, 128.1, 128.1, 127.9, 127.9, 127.7, 127.6, 127.5, 127.1, 126.5 (25*C*H Ar), 97.4 (C1'), 84.9, 81.8, 80.1, 79.1, 78.3, 75.5 (Bn), 73.8 (Bn), 73.7 (Bn), 73.2 (Bn), 72.9 (Bn), 71.6, 70.0, 68.8 (C6'), 68.5 (CH_2 Et), 62.0 (C6), 56.5 (epoxide), 52.5 (epoxide), 43.3 (C5), 15.8 (CH_3 Et). HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{50}H_{56}O_{10}Na$ 839.3766, found 839.3771.

(1R,2R,3S,4R,5S,6R)-2,3-bis(benzyloxy)-5-((benzyloxy)methyl)-4-(((2S,3R,4S,5R,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)-7-oxabicyclo[4.1.0]heptane (**13a**)



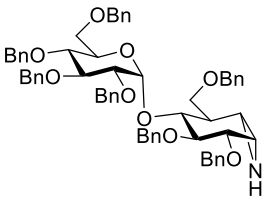
Starting from compound **12a** (260 mg, 0.3 mmol) and following **General procedure G**, the product was purified by flash column chromatography (pentane/EtOAc 11:1→7:1) to obtain compound **13a** (234 mg, 81%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 6.96 (m, 35H, C*H* Ar), 5.71 (d, *J* = 3.7 Hz, 1H, H1'), 4.88 (dd, *J* = 13.8, 11.4 Hz, 2H, CH*H* Bn), 4.82 – 4.66 (m, 4H, CH*H* Bn), 4.61 – 4.31 (m, 8H, CH*H* Bn), 3.94 – 3.79 (m, 4H), 3.73 – 3.58 (m, 4H), 3.55 (dd, *J* = 10.6, 2.8 Hz, 1H, H6'a), 3.51 – 3.40 (m, 3H, H6'b/H2'/epoxide), 3.19 (d, *J* = 3.8 Hz, 1H, epoxide), 2.43 (dddd, *J* = 9.5, 7.7, 3.8, 1.6 Hz, 1H, H5). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.0, 138.87, 138.6, 138.3, 138.1, 138.0, 137.5 (7C_q Ar), 128.6, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 127.9, 127.9, 127.8, 127.7, 127.7, 127.6, 127.5, 127.5, 127.1, 126.5 (35*C*H Ar), 96.9 (C1'), 85.2, 82.0, 80.2 (C3'), 79.3 (C2'), 77.7, 75.6 (Bn), 75.1 (Bn), 73.8 (Bn), 73.6 (Bn), 73.1 (Bn), 73.0 (Bn), 72.8 (Bn), 71.1, 69.5, 69.1 (C6), 68.2 (C6'), 55.7 (epoxide), 53.2 (epoxide), 41.7 (C5). HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{62}H_{64}O_{10}Na$ 991.4392, found 991.4394.

(1R,2R,3S,4R,5S,6R)-2,3-bis(benzyloxy)-5-((benzyloxy)methyl)-4-(((2S,3R,4S,5R,6R)-3,4-bis(benzyloxy)-6-((benzyloxy)methyl)-5-ethoxytetrahydro-2H-pyran-2-yl)oxy)-7-oxabicyclo[4.1.0]heptane (**13b**)



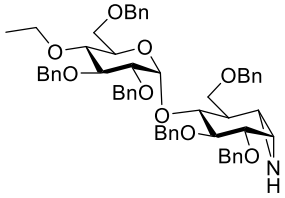
Starting from compound **12b** (255 mg, 0.3 mmol) and following **General procedure G**, the product was purified by flash column chromatography (pentane/EtOAc 11:1→7:1) to obtain compound **13b** (213 mg, 75%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 6.98 (m, 30H, C*H* Ar), 5.67 (d, *J* = 3.7 Hz, 1H, H1'), 4.92 – 4.66 (m, 5H, CH*H* Bn), 4.66 – 4.26 (m, 7H, CH*H* Bn), 3.92 – 3.58 (m, 8H), 3.54 – 3.36 (m, 6H), 3.19 (d, *J* = 3.8 Hz, 1H, 1H-epoxide), 2.44 (dddd, *J* = 9.6, 7.9, 3.8, 1.6 Hz, 1H, H5), 1.06 (t, *J* = 7.0 Hz, 3H, C*H*$_3$ Et). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.1, 139.0, 138.3, 138.2, 138.0, 137.6 (7C_q Ar), 128.6, 128.5, 128.4, 128.4, 128.3, 128.2, 128.2, 128.1, 128.0, 127.9, 127.7, 127.7, 127.7, 127.6, 127.5, 127.5, 127.1, 126.5 (35*C*H Ar), 97.0 (C1'), 85.1, 81.9, 80.2, 79.2, 77.8, 75.5 (Bn), 73.7 (Bn), 73.6 (Bn), 73.2 (Bn), 73.1 (Bn), 72.8 (Bn), 71.3, 69.8, 69.2 (C6), 68.4 (CH_2 Et), 68.2 (C6'), 55.6 (epoxide), 53.3 (epoxide), 41.7 (C5), 16.0 (*C*H_3 Et). HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{57}H_{62}O_{10}Na$ 929.4235, found 929.4234.

(1S,2S,3S,4R,5R,6S)-2,3-bis(benzyloxy)-5-((benzyloxy)methyl)-4-(((2S,3R,4S,5R,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)-7-azabicyclo[4.1.0]heptane (**14a**)



Compound **13a** (234 mg, 0.24 mmol) was dissolved in dry DMF (2.4 mL) and purged with N_2. Then NaN_3 (158 mg, 2.4 mmol) and $LiClO_4$ (514 mg, 4.8 mmol) were added and the reaction was stirred at 100 ^{0}C for 18 h under inert atmosphere. The reaction mixture was diluted with H_2O (50 mL) and extracted with EtOAc (2 x 40 mL), the combined organic layers were washed with water (2 x 50 mL) and brine, dried with anhydrous Na_2SO_4, filtered and concentrated in vacuo to afford a mixture of azido-alcohols (210 mg). The crude (0.2 mmol) was dissolved in dry MeCN (2 mL) and polymer-bound PPh_3 (1.6 mmol/g loading, 260 mg, 0.4 mmol) was added to the solution. The reaction was stirred at 60 ^{0}C for 16 h under inert atmosphere. Then the beads were removed by filtration, the organic solvent was concentrated in vacuo and purification by silica gel column chromatography (pentane/acetone 7:1→4:1) to obtain **14a** (96 mg, 48%) as a clean oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 – 6.97 (m, 35H, C*H* Ar), 5.48 (s, 1H, H1'), 4.94 – 4.73 (m, 5H, CH*H* Bn), 4.69 (s, 2H, CH*H* Bn), 4.60 – 4.31 (m, 7H, CH*H* Bn), 3.97 (t, *J* = 9.3 Hz, 1H, H3'), 3.93 – 3.72 (m, 3H, H3/H2/H6a), 3.61 (dddd, *J* = 14.3, 10.2, 6.3, 3.3 Hz, 5H, H6b/H4/H4'/H5'/H6'a), 3.49 – 3.41 (m, 2H, H6'b/H2'), 2.49 (dd, *J* = 6.3, 2.8 Hz, 1H, aziridine), 2.37 (d, *J* = 7.2 Hz, 2H, H5), 2.30 (d, *J* = 6.2 Hz, 1H, aziridine). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.9, 138.6, 138.4, 138.3, 138.0 (7C_q Ar), 128.8, 128.5, 128.5, 128.5, 128.4, 128.4, 128.3, 128.25, 128.2, 128.2, 128.1, 128.0, 128.0, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 127.5, 127.4, 127.1, 127.0, 126.8 (35*C*H Ar), 82.0, 82.0, 79.5, 79.5, 77.8, 77.8, 75.7 (Bn), 75.1 (Bn), 73.7 (Bn), 73.6 (Bn), 73.0 (Bn), 72.8 (Bn), 72.2 (Bn, assigned by HSQC), 70.9, 70.7 (C6), 68.4 (C6'), 43.4 (C5, assigned by HSQC), 33.8 (*C*H aziridine, assigned by HSQC), 33.1 (*C*H aziridine, assigned by HSQC). HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{62}H_{65}NO_9Na$ 990.4552, found 990.4548.

(1S,2S,3S,4R,5R,6S)-2,3-bis(benzyloxy)-5-((benzyloxy)methyl)-4-(((2S,3R,4S,5R,6R)-3,4-bis(benzyloxy)-6-((benzyloxy)methyl)-5-ethoxytetrahydro-2H-pyran-2-yl)oxy)-7-azabicyclo[4.1.0]heptane (**14b**)



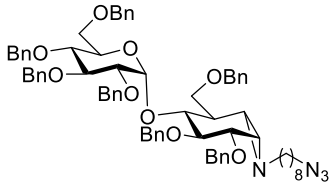
compound **13b** (210 mg, 0.23 mmol) was dissolved in dry DMF (2.4 mL) and purged with N_2. Then NaN_3 (151 mg, 2.3 mmol) and $LiClO_4$ (493 mg, 4.6 mmol) were added and the reaction was stirred at 100 ^{0}C for 18 h under inert atmosphere. The reaction mixture was diluted with H_2O (50 mL) and extracted with EtOAc (2 x 40 mL), the combined organic layers were washed with water (2 x 50 mL) and brine, dried with anhydrous Na_2SO_4, filtered and concentrated in vacuo to afford a mixture of azido-alcohols (204 mg). Starting from azido-alcohols mixture (0.21 mmol), the reaction was carried out following the same procedure described for **14a**. The product was purified by flash column chromatography (pentane/EtOAc 7:1→1:1) to obtain **14b** (81 mg, 42%) as a clean oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.02 (m, 30H, C*H* Ar), 5.48 – 5.36 (m, 1H, H1'), 4.90 – 4.35 (m, 12H, CH*H* Bn), 3.94 – 3.68 (m, 5H), 3.67 – 3.54 (m, 3H), 3.50 – 3.35 (m, 5H), 2.48 (dd, *J* = 6.3, 2.6 Hz, 1H, 1H-aziridine), 2.38 (q, *J* = 6.0, 5.5 Hz, 1H, H5), 2.30 (dd, *J* = 6.1, 1.1 Hz, 1H, 1H-aziridine), 1.05 (t, *J* = 7.0 Hz, 3H, C*H*$_3$ Et). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.4, 138.9, 138.7, 138.4, 138.4, 138.0 (6C_q Ar), 128.5, 128.4, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 127.7, 127.7, 127.6, 127.6, 127.6, 127.4, 127.0, 126.8 (30*C*H Ar), 97.4 (C1'), 81.8, 81.8 79.4, 79.4, 77.8, 77.8, 75.6 (Bn), 74.1 (Bn), 73.6 (Bn), 73.0 (Bn), 72.9 (Bn), 72.1 (Bn), 71.1, 70.8 (C6), 68.4 (*C*H$_2$ Et), 68.4 (C6'), 42.8 (C5, assigned by HSQC), 33.0 (2*C*H aziridine), 15.9 (*C*H$_3$ Et). HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{57}H_{63}NO_9Na$ 928.4395, found 928.4391.

Synthesis of 1-azido-8-trifluoromethylsulfonyloctane

To a dry DCM (0.1 M, 5.8 mL) was added 8-azidooctan-1-ol (100 mg, 0.58 mmol) and pyridine (57 µL, 0.70 mmol) and the mixture was cooled to -20 ^{0}C. Triflic anhydride (118 µL, 0.70 mmol) was added and the mixture was stirred for 15 min. Then the mixture was diluted with DCM (50 mL), washed with cold water (3 x 30 mL). The organic layer was dried with anhydrous Na_2SO_4, filtered and concentrated in vacuo at rt. The crude was used

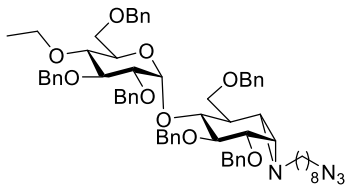
directly for the alkylation of the aziridine.

(1S,2S,3S,4R,5R,6S)-7-(8-azidooctyl)-2,3-bis(benzyloxy)-5-((benzyloxy)methyl)-4-(((2S,3R,4S,5R,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)-7-azabicyclo[4.1.0]heptane (**15a**)



Aziridine **14a** (70 mg, 72 µmol) was dissolved in dry $CHCl_3$ (0.8 mL) and cooled 0 ^{0}C. Then DIPEA (15 µL, 87 µmol) and freshly-made 8-azidooctyl trifluoromethanesulfonate (1 M in $CHCl_3$, 216 µL) were added and the mixture was stirred at rt for 2 h until TLC-analysis showed complete conversion of **14a**. Then another portion of DIPEA (38 µL, 216 µmol) and MeOH (220 µL) were added and stirred at rt overnight. The reaction was diluted with EtOAc (60 mL), washed with sat. aq. $NaHCO_3$ (30 mL), water and brine, dried with anhydrous Na_2SO_4, filtered and concentrated in vacuo. The product was purified by silica gel column chromatography (pentane/acetone 20:1→18:1) to obtain **16a** (72 mg, 90%) as a clean oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 – 6.96 (m, 35H, C*H* Ar), 5.56 (d, *J* = 3.6 Hz, 1H, H1'), 4.97 – 4.85 (m, 3H, CH*H* Bn), 4.78 (t, *J* = 10.8 Hz, 2H, CH*H* Bn), 4.74 – 4.64 (m, 2H, CH*H* Bn), 4.59 – 4.28 (m, 7H, CH*H* Bn), 3.98 (t, *J* = 9.3 Hz, 1H, H3'), 3.88 – 3.72 (m, 3H, H3/H2/H6a), 3.67 – 3.52 (m, 5H, H6b/H4/H4'/H5'/H6'a), 3.44 (dt, *J* = 10.4, 2.8 Hz, 2H, H6'b/H2'), 3.23 (t, *J* = 7.0 Hz, 2H, C*H*$_2$N$_3$), 2.31 (td, *J* = 7.0, 6.6, 3.3 Hz, 2H, H5/Ha C*H*$_2$-N aziridine), 2.07 (dt, *J* = 10.5, 6.9 Hz, 1H, Hb C*H*$_2$-N aziridine), 1.80 (dd, *J* = 6.5, 3.0 Hz, 1H, H1), 1.56 (dq, *J* = 14.9, 7.6, 6.9 Hz, 5H, H7/4CH*H* linker), 1.39 – 1.20 (m, 8H, 8CH*H* linker). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.8, 139.0, 138.9, 138.6, 138.5, 138.3, 138.0 (7C$_q$ Ar), 128.4, 128.4, 128.3, 128.2, 128.2, 128.1, 128.0, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 126.8, 126.6 (35*C*H Ar), 97.7 (C1'), 83.1 (C3), 82.0 (C3'), 80.3 (C2), 79.5 (C2'), 77.9 (C4'), 75.9 (C4), 75.6 (Bn), 75.1 (Bn), 74.5 (Bn), 73.6 (Bn), 72.9 (Bn), 72.9 (Bn), 72.2 (Bn), 70.9 (C5'), 70.8 (C6), 68.3 (C6'), 61.2 (*C*H$_2$-N aziridine), 51.6 (*C*H$_2$N$_3$), 43.4 (C5), 42.1 (C1), 41.6 (C7), 29.9, 29.6, 29.2, 28.9, 27.4, 26.8 (6*C*H$_2$ linker). HRMS (ESI) m/z: [M+H]$^+$ calc for $C_{70}H_{81}N_4O_9$ 1121.5998, found 1121.5994.

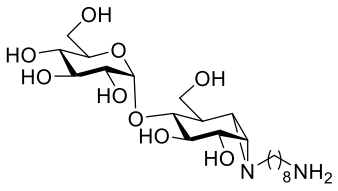
(1S,2S,3S,4R,5R,6S)-7-(8-azidooctyl)-2,3-bis(benzyloxy)-5-((benzyloxy)methyl)-4-(((2S,3R,4S,5R,6R)-3,4-bis(benzyloxy)-6-((benzyloxy)methyl)-5-ethoxytetrahydro-2H-pyran-2-yl)oxy)-7-azabicyclo[4.1.0]heptane (**15b**)



Starting from aziridine **14b** (63 mg, 0.07 mmol), the reaction was carried out following the same procedure described for **15a**. The product was purified by flash column chromatography (pentane/EtOAc 13:1→11:1) to obtain **15b** (65 mg, 88%) as a clean oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 – 6.95 (m, 30H, C*H* Ar), 5.51 (d, *J* = 3.6 Hz, 1H, H1'), 4.89 (d, *J* = 1.4 Hz, 2H, CH*H* Bn), 4.84 (d, *J* = 10.8 Hz, 1H, CH*H* Bn), 4.79 – 4.62 (m, 3H, CH*H* Bn), 4.57 (d, *J* = 12.1 Hz, 1H, CH*H* Bn), 4.52 – 4.43 (m, 3H, CH*H* Bn), 4.43 – 4.32 (m, 2H, CH*H* Bn), 3.92 – 3.68 (m, 5H), 3.67 – 3.58 (m, 3H), 3.53 (dd, *J* = 10.5, 3.1 Hz, 1H), 3.48 – 3.34 (m, 4H), 3.23 (t, *J* = 7.0 Hz, 2H, C*H*$_2$N$_3$), 2.36 – 2.25 (m, 2H, H5/Ha C*H*$_2$-N aziridine), 2.09 (dt, *J* = 11.5, 7.4 Hz, 1H, Hb C*H*$_2$-N aziridine), 1.80 (dd, *J* = 6.5, 3.0 Hz, 1H, H1), 1.62 – 1.48 (m, 5H, H7/4CH*H* linker), 1.38 – 1.24 (m, 8H, 8CH*H* linker), 1.05 (t, *J* = 7.0 Hz, 3H, C*H*$_3$ Et). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.8, 139.0, 139.0, 138.5, 138.4, 138.0 (6C$_q$ Ar), 128.4, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.7, 127.6, 127.5, 127.5, 127.4, 127.3, 126.7, 126.6 (30*C*H Ar), 97.8 (C1'), 83.0, 81.8, 80.3, 79.4, 77.9, 76.4, 75.5 (Bn), 74.4 (Bn), 73.6 (Bn), 72.9 (Bn), 72.9 (Bn), 72.2 (Bn), 71.0, 71.0 (C6), 68.4 (*C*H$_2$ Et), 68.3 (C6'), 61.2 (*C*H$_2$-N aziridine), 51.6 (*C*H$_2$N$_3$), 43.3 (C5), 42.1 (C1), 41.6 (C7), 29.9, 29.6, 29.2, 28.9, 27.4, 26.8 (6*C*H$_2$ linker), 15.9 (*C*H$_3$ Et). HRMS (ESI) m/z: [M+H]$^+$ calc for $C_{65}H_{79}N_4O_9$ 1059.5842, found 1059.5838.

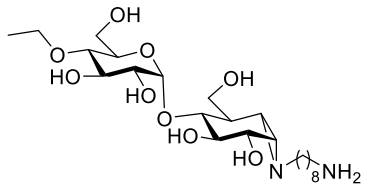
(2S,3R,4S,5S,6R)-2-(((1S,2R,3R,4R,5S,6S)-7-(8-aminooctyl)-4,5-dihydroxy-2-(hydroxymethyl)-7-

azabicyclo[4.1.0]heptan-3-yl)oxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol (**2a**)



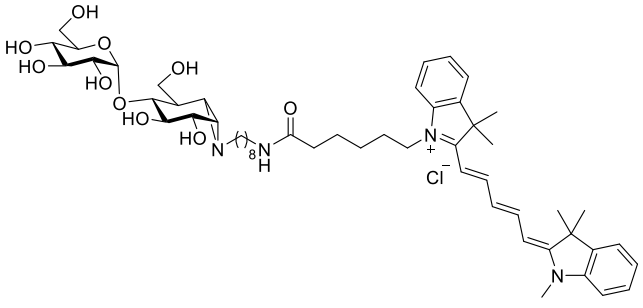
Ammonium (3 mL) was condensed in a dry flask at -60 ^{0}C, and sodium (34 mg, 1.4 mmol) was added. The resulting deep-blue solution was stirred for 15 min to dissolve all sodium. Aziridine **15a** (23 mg, 20 µmol) and *t*BuOH (20 µL, 200 µmol) were taken up in dry THF (1 mL) and slowly added to the reaction mixture. After stirring for 1 h, the reaction was quenched with H_2O. The mixture was slowly warmed to rt and evaporated. The crude was dissolved in H_2O and eluted over a column packed with Amberlite CG-50 (MH_4^+) with 0.5 M NH_4OH as eluent, concentrated in vacuo, affording the title compound (7.51 mg, 79%) as a light-yellow solid after lyophilization. ^{1}H NMR (400 MHz, D_2O) δ 5.11 (d, *J* = 4.0 Hz, 1H, H1'), 3.94 – 3.64 (m, 7H), 3.60 – 3.47 (m, 2H), 3.35 (dt, *J* = 26.7, 9.7 Hz, 2H), 2.94 (t, *J* = 7.5 Hz, 2H, C*H*$_2$NH$_2$), 2.27 (t, *J* = 7.5 Hz, 2H, C*H*$_2$-N aziridine), 2.11 – 1.93 (m, 2H, H5/H1), 1.82 (d, *J* = 6.6 Hz, 1H, H7), 1.66 – 1.57 (m, 2H, 2CH*H* linker), 1.51 (s, 2H, 2CH*H* linker), 1.36 – 1.22 (m, 8H, 8CH*H* linker). ^{13}C NMR (101 MHz, D_2O) δ 101.0 (C1'), 81.3, 74.9, 73.3, 72.9, 72.1, 71.3, 69.7, 61.9 (C6'), 60.8 (C6), 60.4 (*C*H$_2$-N aziridine), 44.5 (C1), 43.6 (C5), 41.1 (C7), 39.9 (*C*H$_2$NH$_2$), 29.0, 28.8, 28.4, 27.2, 26.7, 25.9 (6*C*H$_2$ linker). HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{21}H_{41}N_2O_9$ 465.2807, found 465.2806.

(1S,2S,3R,4R,5R,6S)-7-(8-aminooctyl)-4-(((2S,3R,4R,5S,6R)-5-ethoxy-3,4-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-5-(hydroxymethyl)-7-azabicyclo[4.1.0]heptane-2,3-diol (**16**)



Ammonium (8 mL) was condensed in a dry flask at -60 ^{0}C, and sodium (94 mg, 4.1 mmol) was added. The resulting deep-blue solution was stirred for 15 min to dissolve all sodium. Aziridine **15b** (62 mg, 59 µmol) and *t*BuOH (56 µL, 0.59 mmol) were taken up in dry THF (1.5 mL) and slowly added to the reaction mixture. After stirring for 1 h, the reaction was quenched with H_2O. The mixture was slowly warmed to rt and evaporated. The crude was dissolved in H_2O and eluted over a column packed with Amberlite CG-50 (MH_4^+) with 0.5 M NH_4OH as eluent, concentrated in vacuo, affording the title compound (27 mg, 94%) as a white solid after lyophilization. ^{1}H NMR (400 MHz, D_2O) δ 5.09 (d, *J* = 4.0 Hz, 1H, H1'), 3.89 – 3.64 (m, 9H), 3.61 – 3.48 (m, 2H), 3.34 – 3.21 (m, 2H), 2.93 (dt, *J* = 15.2, 7.2 Hz, 2H, C*H*$_2$NH$_2$), 2.26 (t, *J* = 7.3 Hz, 2H, C*H*$_2$-N aziridine), 2.06 – 1.94 (m, 2H, H1/H5), 1.81 (d, *J* = 6.6 Hz, 1H, H7), 1.66 – 1.41 (m, 4H, 4CH*H* linker), 1.29 (s, 8H, 8CH*H* linker), 1.17 (t, *J* = 7.0 Hz, 3H, C*H*$_3$ Et). ^{13}C NMR (101 MHz, D_2O) δ 100.5 (C1'), 80.9, 77.5, 74.6, 72.8, 71.7, 71.6, 70.9, 68.9 (*C*H$_2$ Et), 61.5 (C6), 60.1 (C6'), 60.0 (*C*H$_2$-N aziridine), 44.2 (C1), 43.1 (C5), 40.7 (C7), 39.6 (*C*H$_2$NH$_2$), 28.6, 28.4, 28.1, 27.2, 26.4, 25.5 (6*C*H$_2$ linker), 14.7 (*C*H$_3$ Et). HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{23}H_{45}N_2O_9$ 493.3120, found 493.3120.

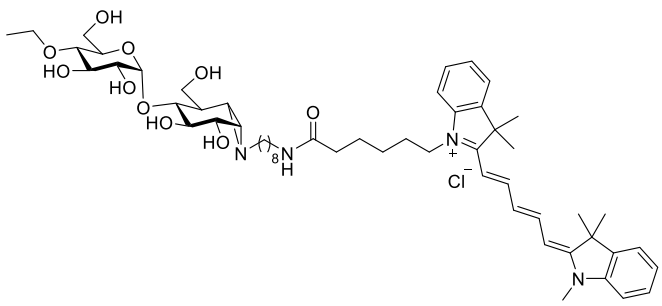
1-(6-((8-((1S,2S,3R,4R,5R,6S)-2,3-dihydroxy-5-(hydroxymethyl)-4-(((2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-7-azabicyclo[4.1.0]heptan-7-yl)octyl)amino)-6-oxohexyl)-3,3-dimethyl-2-((1E,3E)-5-((E)-1,3,3-trimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-3H-indol-1-ium chloride (**2b**)



Compound **2a** (6.95 mg, 15 µmol) was dissolved in dry DMF (0.5 mL), then DIPEA (5.7 µL, 33 µmol) and Cy5-OSu (10 mg, 16.5 µmol) were added and the mixture was stirred at rt for 16 h. Full conversion was observed by LC-MS and the product was purified by semi-preparative reversed phase HPLC (linear gradient. Solution used: A: 50 mM NH_4HCO_3 in H_2O, B: MeCN). The

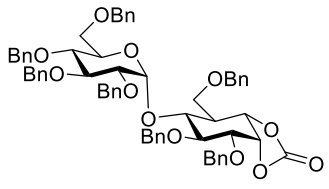
fractions were concentrated under reduced pressure, co-evaporated with Milli-Q/MeCN (1/1, 3 x), dissolved in Milli-Q/*t*BuOH (1/1) again and lyophilized to obtain the title compound (1.36 mg, 10%) as a blue solid. ^{1}H NMR (600 MHz, MeOD) δ 8.29 – 8.20 (m, 2H), 7.50 (dt, *J* = 7.4, 1.4 Hz, 2H), 7.42 (tdd, *J* = 7.6, 4.9, 1.2 Hz, 2H), 7.33 – 7.23 (m, 4H), 6.63 (t, *J* = 12.5 Hz, 1H), 6.28 (dd, *J* = 13.7, 7.4 Hz, 2H), 4.98 (d, *J* = 3.9 Hz, 1H, H1'), 4.11 (t, *J* = 7.4 Hz, 2H, C*H*$_2$N$^+$), 3.88 (dd, *J* = 11.0, 3.1 Hz, 1H, H6a), 3.85 – 3.78 (m, 1H, H6a'), 3.76 – 3.66 (m, 4H, H2/H5'/H6b/H6'b), 3.62 (d, *J* = 15.3 Hz, 4H, C*H*$_3$N/H3'), 3.55 (dd, *J* = 9.9, 8.8 Hz, 1H, H3), 3.45 – 3.40 (m, 1H, H2'), 3.27 (d, *J* = 9.2 Hz, 1H, H4'), 3.21 – 3.15 (m, 1H, H4), 3.12 (t, *J* = 7.2 Hz, 2H, C*H*$_2$NHC=O), 2.31 (dt, *J* = 11.6, 7.1 Hz, 1H, Ha C*H*$_2$-N aziridine), 2.20 (t, *J* = 7.3 Hz, 2H, C*H*$_2$C=O), 2.13 (t, *J* = 5.8 Hz, 1H, Hb C*H*$_2$-N aziridine), 1.95 – 1.88 (m, 1H, H5), 1.87 – 1.79 (m, 3H, H1/2CH*H* linker), 1.73 (s, 15H, H7/2CH*H* linker/4C*H*$_3$), 1.56 (q, *J* = 7.5 Hz, 2H, 2CH*H* linker), 1.50 – 1.42 (m, 4H, 4CH*H* linker), 1.36 – 1.27 (m, 8H, 8CH*H* linker). ^{13}C NMR (214 MHz, MeOD) δ 175.7, 175.4, 174.7, 155.5, 155.5, 144.3, 143.6, 142.7, 142.5, 129.8, 129.8, 126.6, 126.3, 126.3, 123.4, 123.3, 112.1, 111.9, 104.4, 104.3, 103.2 (C1'), 84.1 (C4), 75.9 (C3), 75.0 (C3'), 74.4, 74.0 (C2'), 72.7, 71.4 (C4'), 62.9 (C6), 62.7 (C6'), 62.2 (*C*H$_2$-N aziridine), 50.6 (C$_q$), 50.5 (C$_q$), 45.9 (C1), 45.8 (C5), 44.8(*C*H$_2$N$^+$), 42.1 (C7), 40.4 (*C*H$_2$NHC=O), 36.7 (*C*H$_2$NHC=O), 31.5 (*C*H$_3$N), 30.7, 30.5, 30.4, 30.3, 28.4, 28.2, 28.0 (7*C*H$_2$ linker), 27.9 (2*C*H$_3$), 27.8 (2*C*H$_3$), 27.4, 26.6 (2*C*H$_2$ linker). HRMS (ESI) m/z: [M]$^+$ calc for $C_{53}H_{77}N_4O_{10}$ 929.5634, found 929.5627.

1-(6-((8-((1S,2R,3R,4R,5S,6S)-3-(((2S,3R,4R,5S,6R)-5-ethoxy-3,4-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-4,5-dihydroxy-2-(hydroxymethyl)-7-azabicyclo[4.1.0]heptan-7-yl)octyl)amino)-6-oxohexyl)-3,3-dimethyl-2-((1E,3E)-5-((E)-1,3,3-trimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-3H-indol-1-ium chloride (**2c**)



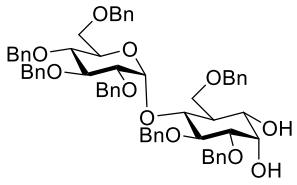
Compound **16** (7.03 mg, 14 µmol) was dissolved in dry DMF (0.5 mL), then DIPEA (5.5 µL, 31 µmol) and Cy5-OSu (10.5 mg, 17 µmol) were added and the mixture was stirred at rt overnight. Full conversion was observed by LC-MS and the product was purified by semi-preparative reversed phase HPLC (linear gradient. Solution used: A: 50 mM NH_4HCO_3 in H_2O, B: MeCN). The fractions were concentrated under reduced pressure, co-evaporated with Milli-Q/MeCN (1/1, 3 x), dissolved in Milli-Q/*t*BuOH (1/1) again and lyophilized to obtain the title compound (2.45 mg, 17%) as a blue solid. ^{1}H NMR (850 MHz, MeOD) δ 8.30 – 8.21 (m, 2H), 7.50 (dt, *J* = 7.3, 1.5 Hz, 2H), 7.44 – 7.39 (m, 2H), 7.32 – 7.25 (m, 4H), 6.63 (t, *J* = 12.4 Hz, 1H), 6.28 (dd, *J* = 13.7, 11.2 Hz, 2H), 4.97 (d, *J* = 3.9 Hz, 1H, H1'), 4.11 (t, *J* = 7.5 Hz, 2H, C*H*$_2$N$^+$), 3.92 – 3.84 (m, 2H, H6a/Ha OC*H*$_2$CH$_3$), 3.77 (dd, *J* = 11.7, 2.1 Hz, 1H, H6'a), 3.74 – 3.61 (m, 9H H2/H3'/H5'/H6b/H6'b/ Hb OC*H*$_2$CH$_3$/C*H*$_3$N), 3.56 – 3.52 (m, 1H, H3), 3.47 – 3.40 (m, 1H, H2'), 3.20 – 3.09 (m, 4H, H4/H4'/C*H*$_2$NHC=O), 2.31 (ddt, *J* = 14.7, 7.7, 3.6 Hz, 1H, Ha C*H*$_2$-N aziridine), 2.20 (t, *J* = 7.2 Hz, 2H, C*H*$_2$C=O), 2.15 (ddd, *J* = 11.7, 8.7, 7.2 Hz, 1H, Hb C*H*$_2$-N aziridine), 1.90 (ddd, *J* = 9.9, 6.7, 3.1 Hz, 1H, H5), 1.87 – 1.75 (m, 3H, H1/2CH*H* linker), 1.73 (s, 15H, H7/2CH*H* linker/4C*H*$_3$), 1.64 – 1.52 (m, 2H, 2CH*H* linker), 1.46 (dq, *J* = 14.2, 6.9, 6.4 Hz, 4H, 4CH*H* linker), 1.39 – 1.27 (m, 8H, 8CH*H* linker), 1.20 – 1.15 (m, 3H, C*H*$_3$ Et). ^{13}C NMR (214 MHz, MeOD) δ 175.7, 175.4, 174.7, 155.5, 155.5, 144.3, 143.6, 142.7, 142.5, 129.78, 129.8, 126.7, 126.3, 126.3, 123.4, 123.3, 112.1, 111.9, 104.4, 104.3, 103.1 (C1'), 84.1, 79.2, 75.8 (C3), 75.2, 74.1 (C2'), 73.6, 72.7, 69.3 (*C*H$_2$ Et), 62.9 (C6), 62.1 (C6'), 62.1 (*C*H$_2$-N aziridine), 50.6 (C$_q$), 50.5 (C$_q$), 45.9 (C1), 45.8 (C5), 44.8 (*C*H$_2$N$^+$), 42.2 (C7), 40.4 ((*C*H$_2$NHC=O)), 36.7 (*C*H$_2$C=O), 31.51 (*C*H$_3$N), 30.7, 30.4, 30.4, 30.3, 28.4, 28.2, 28.0 (7*C*H$_2$ linker), 28.0 (2*C*H$_3$), 27.8 (2*C*H$_3$), 27.4, 26.6 (2*C*H$_2$ linker), 16.0 (*C*H$_3$ Et). HRMS (ESI) m/z: [M]$^+$ calc for $C_{55}H_{81}N_4O_{10}$ 957.5947, found 957.5945.

(3aS,4R,5S,6R,7R,7aS)-4,5-bis(benzyloxy)-7-((benzyloxy)methyl)-6-(((2S,3R,4S,5R,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)hexahydrobenzo[d][1,3]dioxol-2-one (**18**)



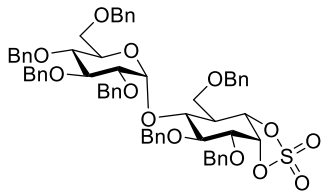
Starting from compound **17** (98 mg, 0.2 mmol), using donor **4a** (213.5 mg, 0.3 mmol) and following **Standard procedure A**. The reaction was purified by size exclusion (DCM/MeOH = 1/1) giving product as a mixture of two isomers (130 mg, α/β = 13/1). Then the mixture was further purified by flash column chromatography (pentane/acetone 13:1 → 9:1) to obtain compound **18** (115 g, 57%) as a clean oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 – 7.03 (m, 35H, C*H* Ar), 5.14 (d, *J* = 3.5 Hz, 1H, H1'), 5.03 (t, *J* = 9.0 Hz, 1H, H7), 4.92 (d, *J* = 10.9 Hz, 1H, CH*H* Bn), 4.82 (dd, *J* = 10.9, 6.3 Hz, 2H, CH*H* Bn), 4.79 – 4.72 (m, 2H, 1CH*H* Bn/H1), 4.61 (s, 2H, CH*H* Bn), 4.55 (d, *J* = 11.9 Hz, 1H, CH*H* Bn), 4.44 (td, *J* = 21.8, 20.8, 12.0 Hz, 4H, CH*H* Bn), 4.25 (d, *J* = 12.2 Hz, 2H, CH*H* Bn), 4.17 (d, *J* = 11.5 Hz, 1H, CH*H* Bn), 3.99 – 3.83 (m, 5H, H3'/H3/H4/H4/H6a), 3.76 (dt, *J* = 10.1, 2.4 Hz, 1H, H5'), 3.73 – 3.66 (m, 1H, H4'), 3.62 (dd, *J* = 9.7, 2.4 Hz, 1H, H6b), 3.57 (dd, *J* = 9.8, 3.5 Hz, 1H, H2'), 3.42 (dd, *J* = 10.9, 2.8 Hz, 1H, H6'a), 3.29 (dd, *J* = 10.9, 1.9 Hz, 1H, H6'b), 2.80 (ddd, *J* = 11.9, 9.4, 2.3 Hz, 1H, H5). ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.8 (C=O), 138.8, 138.6, 138.4, 138.1, 138.0, 137.2, 137.1 (7C_q Ar), 128.7, 128.6, 128.5, 128.4, 128.4, 128.3, 128.2, 128.1, 128.1, 128.0, 128.0, 128.0, 127.9, 127.8, 127.7, 127.6, 127.6, 127.3 (35*C*H Ar), 95.1 (C1'), 82.3, 81.0, 79.8 (C2'), 77.7 (C4'), 75.7 (Bn), 75.1 (Bn), 74.1, 74.2 (C1), 73.9 (C7), 73.8 (Bn), 73.5 (Bn), 73.3 (Bn), 73.0 (Bn), 71.9, 71.6 (Bn), 71.0 (C5'), 68.1 (C6'), 65.6 (C6), 41.3 (C5). HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{63}H_{64}O_{12}Na$ 1035.4290, found 1035.4286.

(1S,2S,3S,4S,5R,6S)-3,4-bis(benzyloxy)-6-((benzyloxy)methyl)-5-(((2S,3R,4S,5R,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)cyclohexane-1,2-diol (**19**)



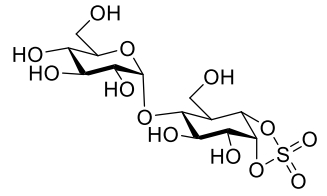
Compound **18** (90 mg, 0.09 mmol) was dissolved in DCM/MeOH (1/1, 2 mL), then 30 wt% NaOMe in MeOH solution (20 µL, 0.11 mmol) was added and the reaction was stirred at rt for 2 h. After which the reaction was diluted with DCM (2 mL) and neutralized with washed Amberlite IR-120 H^+ resin until pH ≈ 7, filtered, washed with DCM (3 x 3 mL) and concentrated in vacuo. The product was purified by flash column chromatography (pentane/acetone 7:1 → 3:1) to obtain compound **19** (81.5 g, 93%) as a clean oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.04 (m, 35H, C*H* Ar), 5.81 (d, *J* = 3.7 Hz, 1H, H1'), 4.99 (d, *J* = 11.9 Hz, 1H, CH*H* Bn), 4.89 (d, *J* = 10.9 Hz, 1H, CH*H* Bn), 4.83 – 4.72 (m, 3H, CH*H* Bn), 4.65 – 4.56 (m, 3H, CH*H* Bn), 4.56 – 4.48 (m, 2H, CH*H* Bn), 4.47 – 4.36 (m, 3H, CH*H* Bn), 4.27 (d, *J* = 12.2 Hz, 1H, CH*H* Bn), 4.15 (t, *J* = 2.8 Hz, 1H), 4.05 (t, *J* = 9.0 Hz, 1H), 3.97 (dd, *J* = 9.9, 8.8 Hz, 1H), 3.91 – 3.68 (m, 5H), 3.65 (dd, *J* = 10.1, 8.8 Hz, 1H), 3.54 – 3.41 (m, 3H), 3.38 (dd, *J* = 10.7, 1.9 Hz, 1H), 2.49 (s, 3H), 2.30 (tt, *J* = 10.6, 3.8 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.0, 138.9, 138.6, 138.2, 138.1, 138.0, 137.8 (7C_q Ar), 128.6, 128.4, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.8, 127.7, 127.6, 127.6, 127.3, 127.1, 126.5 (35*C*H Ar), 96.4 (C1'), 82.8, 82.1, 80.5, 79.5, 77.8, 75.6 (Bn), 75.0 (Bn), 74.0 (Bn), 73.6 (Bn), 73.4 (Bn), 72.9 (Bn), 72.5 (Bn), 71.0, 69.8, 69.0, 68.3 (C6'), 67.6 (C6), 42.9 (C5). HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{62}H_{66}O_{11}Na$ 1009.4497, found 1009.4495.

(3aR,4R,5S,6R,7R,7aS)-4,5-bis(benzyloxy)-7-((benzyloxy)methyl)-6-(((2S,3R,4S,5R,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)hexahydrobenzo[d][1,3,2]dioxathiole 2,2-dioxide (**20**)



Compound **19** (81.5 mg, 0.083 mmol) was dissolved in dry DCM (1.5 mL) and purged with N_2. The mixture was cooled to 0 ^{0}C, then TEA (92 µL, 0.66 mmol) and thionyl chloride (24 µL, 0.33 mmol) were added successively. After stirring at 0 ^{0}C for 15 min, the reaction was diluted with DCM (50 mL), washed with sat. aq. $NaHCO_3$ (30 mL), H_2O (2 x 30 mL) and brine, dried over Na_2SO_4, filtered and concentrated in vacuo to give the crude sulfite as a pale-yellow oil. Then the crude was dissolved in EtOAc (1 mL) and MeCN (1 mL) and cooled to 0 ^{0}C. A solution of $RuCl_3.3H_2O$ (1.7 mg, 0.008 mmol) and $NaIO_4$ (35 mg, 0.16 mmol) in H_2O (0.4 mL) was added and the mixture was stirred at 0 ^{0}C vigorously for 2 h. The reaction was quenched by addition of sat. aq. $Na_2S_2O_3$ (1 mL) and the mixture was stirred for 15 min. Then the mixture was diluted with H_2O (30 mL) and extracted with EtOAc (2 x 40 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The crude was purified by flash column chromatography (pentane/acetone, 13:1 → 11:1) affording compound **20** (50 mg, 58%) as a clean oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 – 6.99 (m, 35H, C*H* Ar), 5.28 (d, *J* = 3.7 Hz, 1H, H1'), 5.22 (dd, *J* = 9.8, 6.5 Hz, 1H, H7), 4.99 (dd, *J* = 6.9, 3.4 Hz, 1H, H1), 4.90 (d, *J* = 10.4 Hz, 1H, CH*H* Bn), 4.81 (dd, *J* = 10.9, 4.0 Hz, 2H, CH*H* Bn), 4.76 – 4.58 (m, 3H, CH*H* Bn), 4.56 – 4.20 (m, 8H, CH*H* Bn), 3.99 – 3.85 (m, 5H, H3'/H3/H4/H4/H6a), 3.78 – 3.63 (m, 2H, H5'/H4'), 3.62 – 3.51 (m, 2H, H6b/H2'), 3.49 – 3.40 (m, 1H, H6'a), 3.31 (d, *J* = 10.8 Hz, 1H, H6'b), 2.87 (t, *J* = 10.1 Hz, 1H, H5). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.7, 138.5, 138.2, 137.9, 137.8, 137.3, 137.2 (7C_q Ar), 128.7, 128.6, 128.6, 128.5, 128.4, 128.4, 128.3, 128.1, 128.0, 128.0, 127.9, 127.8, 127.8, 127.7, 127.3 (35*C*H Ar), 95.8 (C1'), 82.1, 81.1, 79.7 (C2'), 79.4 (C1), 78.3 (C7), 77.7 (C4'), 75.7 (Bn), 75.1 (Bn), 74.3, 73.7 (Bn), 73.6 (Bn), 73.5 (Bn), 73.0 (Bn), 72.4 (Bn), 71.2, 71.1 (C5'), 68.1 (C6'), 64.8 (C6), 41.8 (C5). HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{62}H_{64}O_{13}SNa$ 1071.3960, found 1071.3965.

(3aR,4R,5R,6R,7R,7aS)-4,5-dihydroxy-7-(hydroxymethyl)-6-(((2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)hexahydrobenzo[d][1,3,2]dioxathiole 2,2-dioxide (**3a**)



Compound **20** (20 mg, 0.02 mmol) was dissolved in a mixture of MeOH/H_2O/dioxane (2/1/2, 2 mL) under Argon and $Pd(OH)_2$/C (20 wt%, 20 mg, 0.03 mmol) was added. While stirring vigorously, the mixture was flushed with a H_2 balloon. After stirring for 3 h under H_2 atmosphere, the mixture was filtered over Celite and evaporate to afford the product **3a** in high purity as a white solid (7.1 mg, 89%) after lyophilization. ^{1}H NMR (400 MHz, D_2O) δ 5.47 (dd, *J* = 4.6, 3.4 Hz, 1H, H1), 5.35 – 5.23 (m, 2H, H1'/H7), 4.02 – 3.87 (m, 3H, H2/H3/H6a), 3.86 – 3.55 (m, 7H), 3.40 (t, *J* = 9.2 Hz, 1H), 2.43 – 2.32 (m, 1H, H5). ^{13}C NMR (101 MHz, D_2O) δ 100.3 (C1'), 84.4 (C1), 82.1 (C7), 76.2, 73.2, 72.9, 72.8, 71.7, 69.2, 68.0, 60.4 (C6'), 56.5 (C6), 43.7 (C5). HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{13}H_{22}O_{13}SNa$ 441.0673, found 441.0670.

5. References

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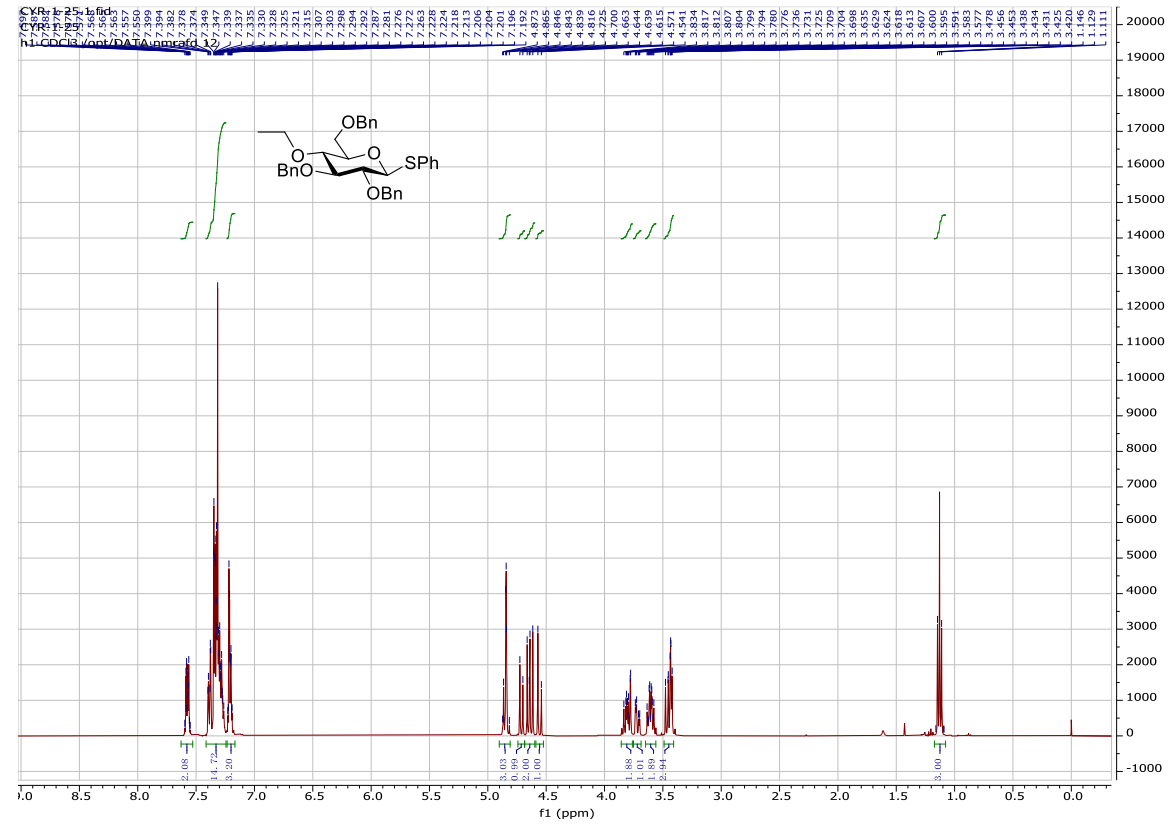
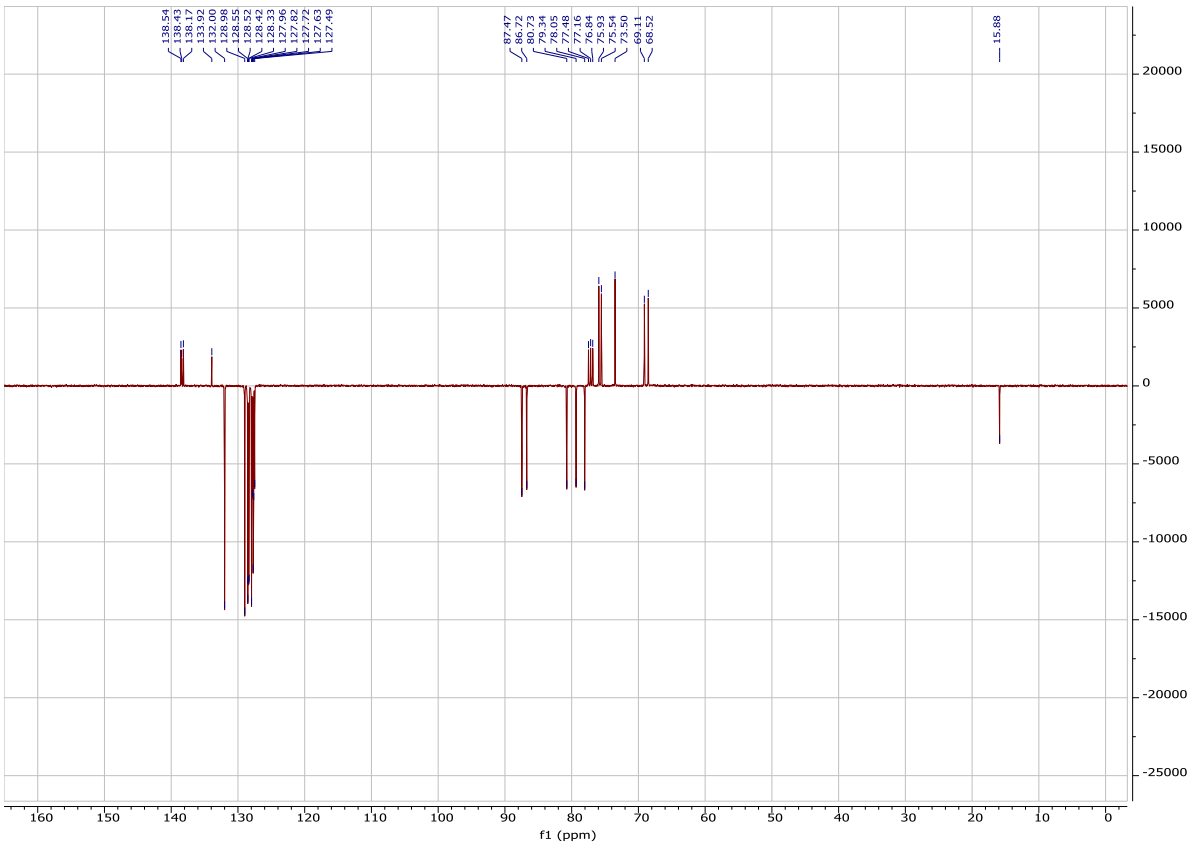
6. NMR spectra

^{1}H and ^{13}C of **4b** in $CDCl_3$





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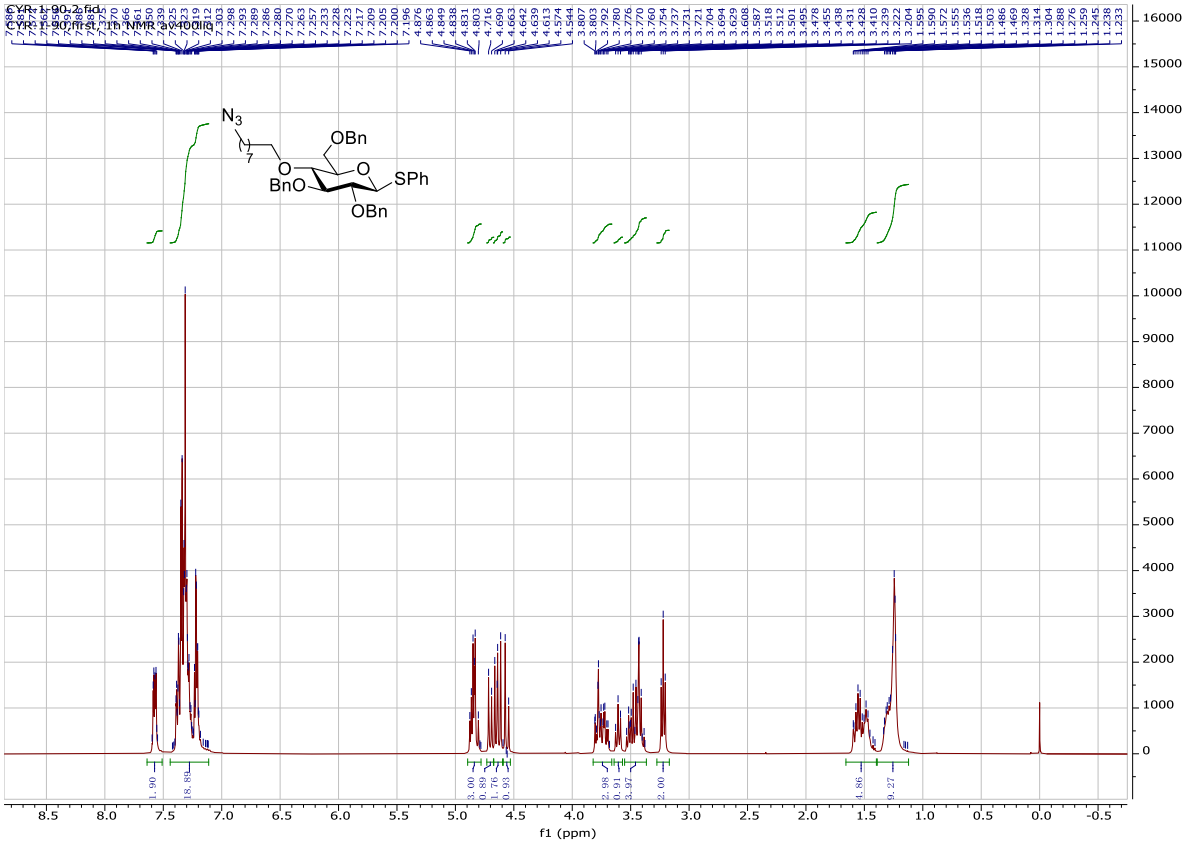

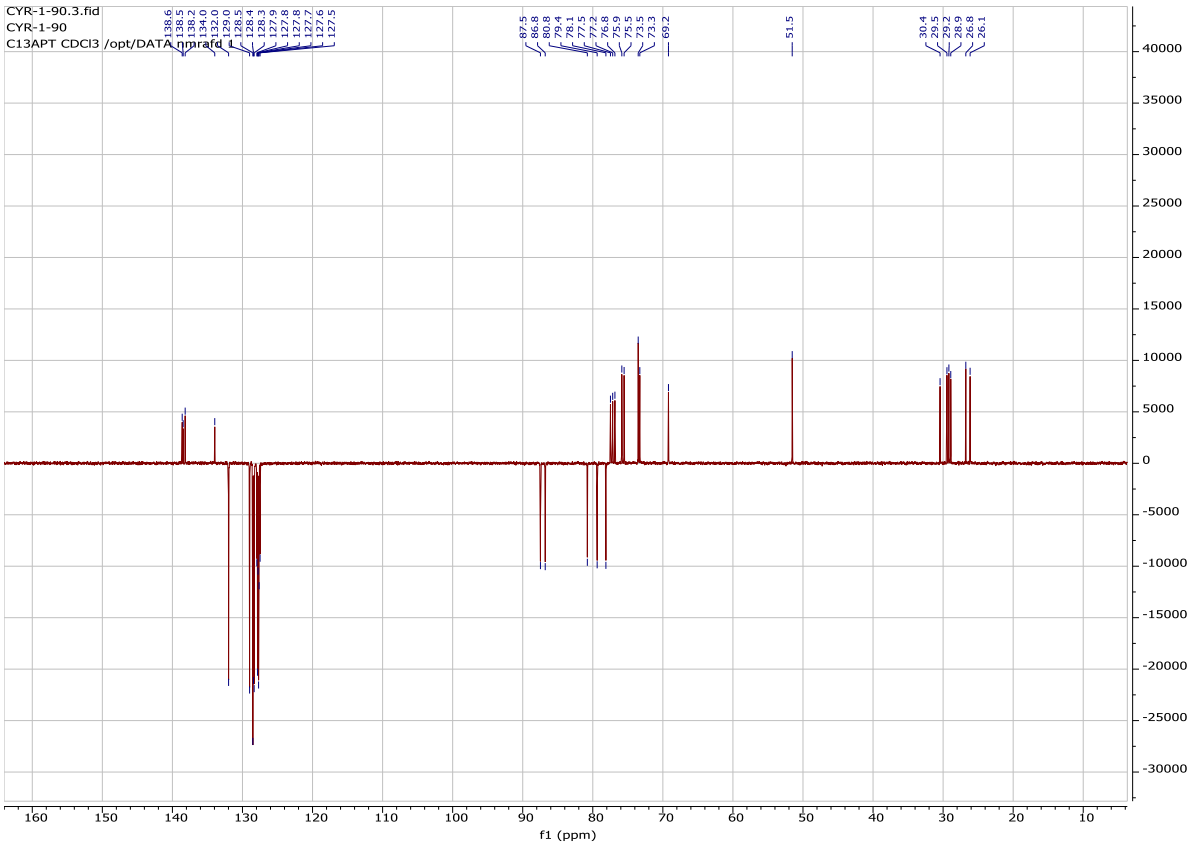

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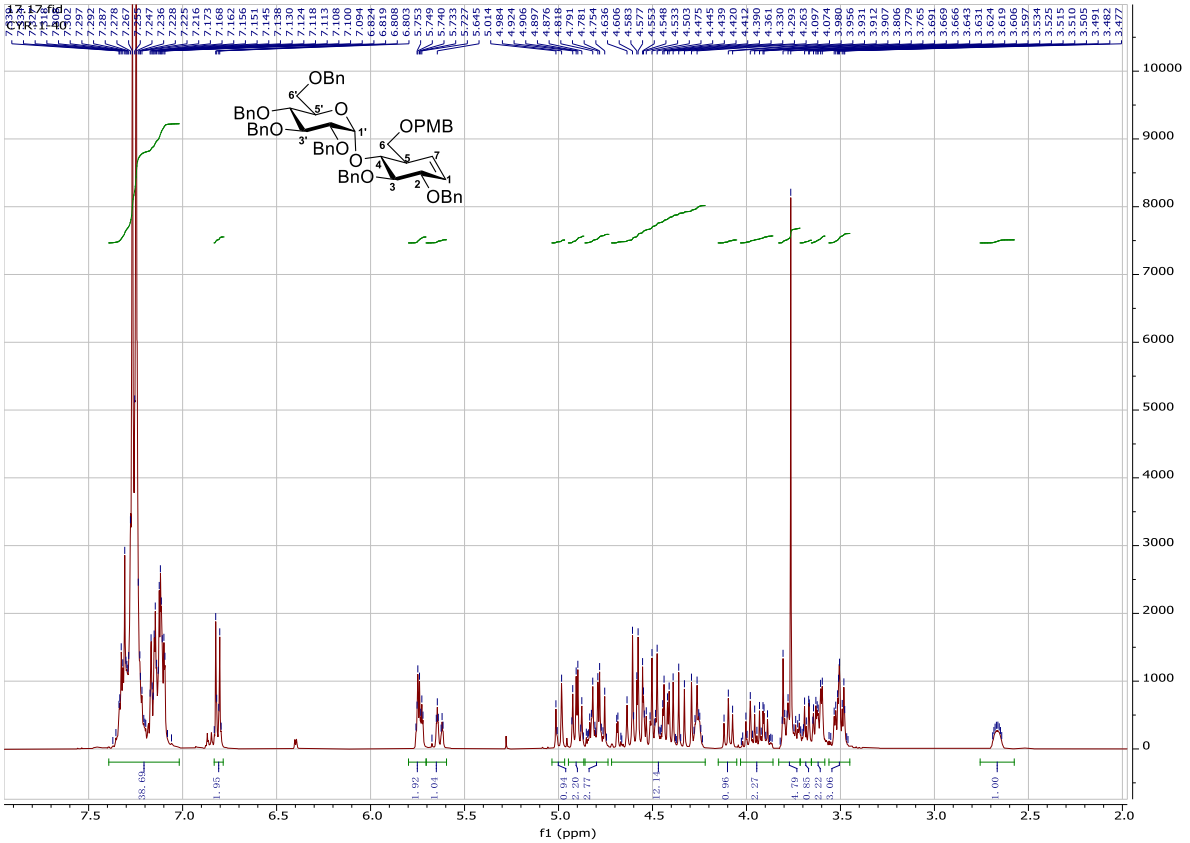

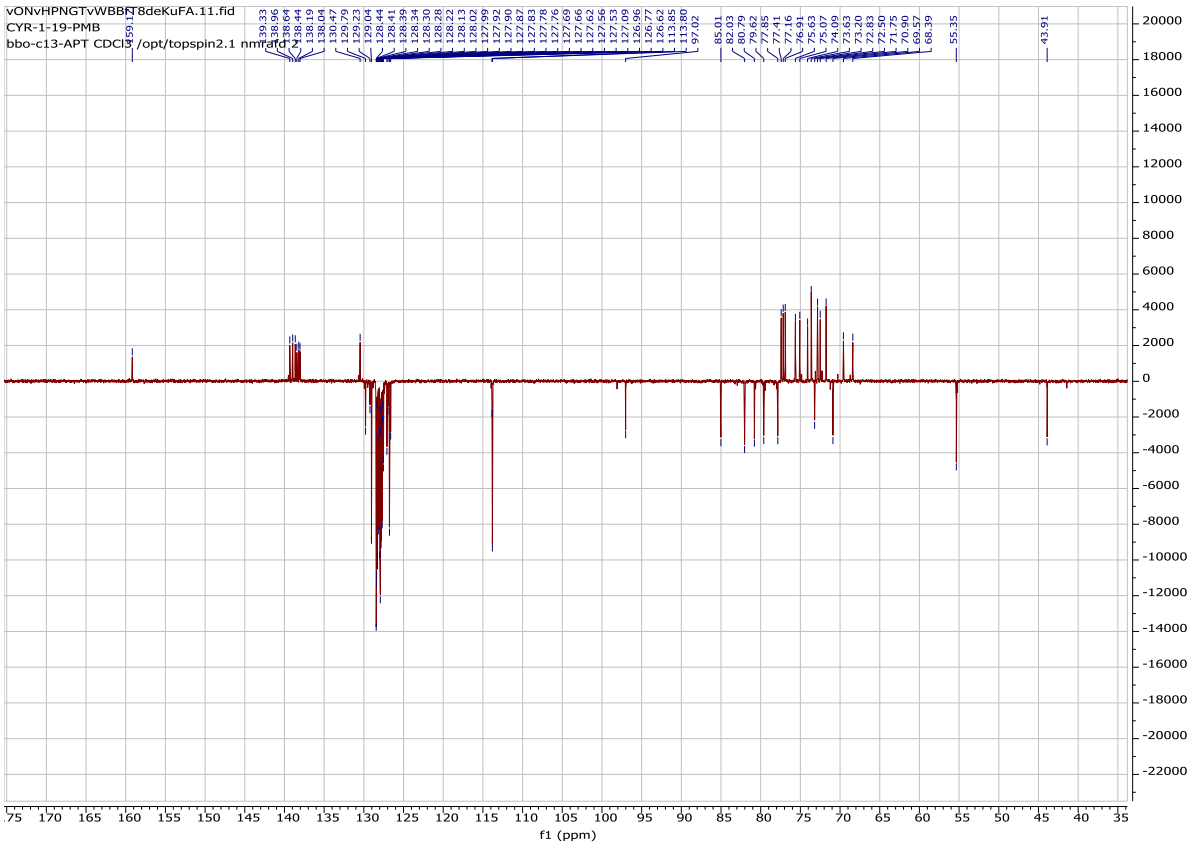

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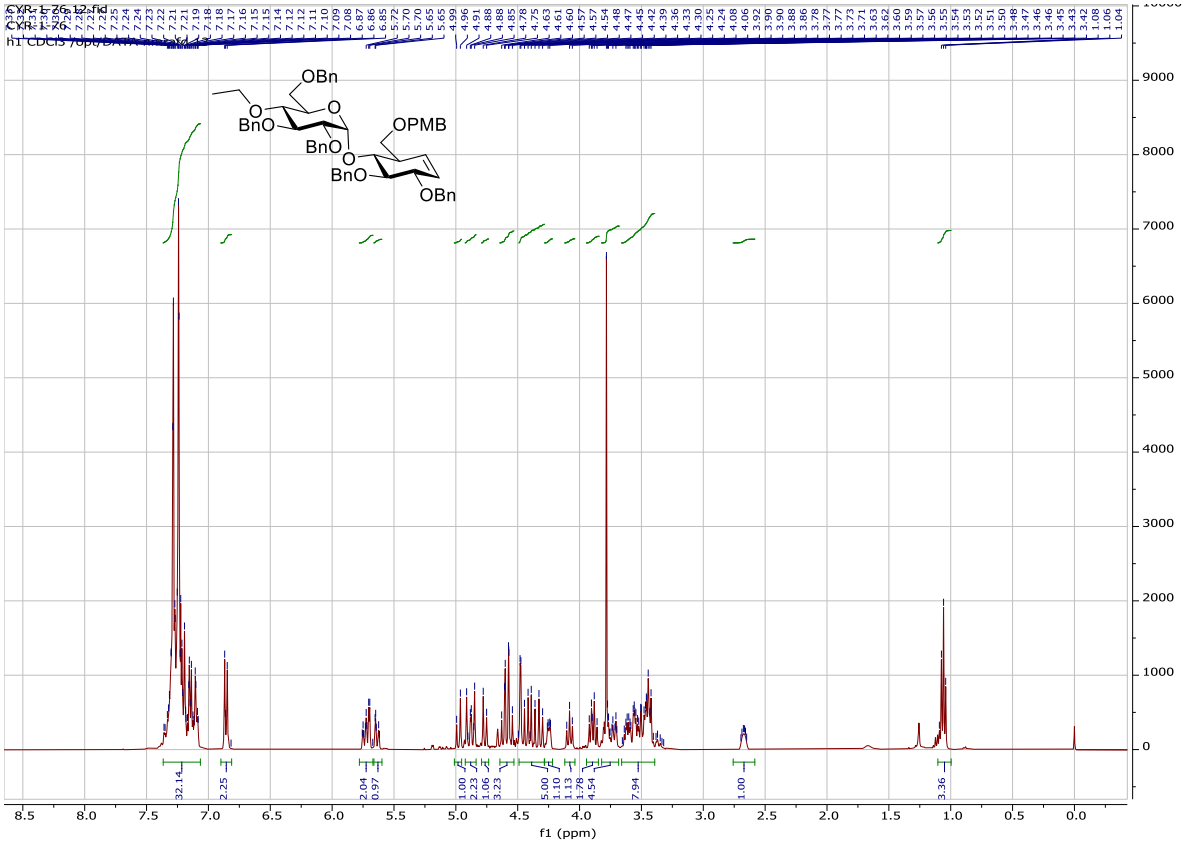

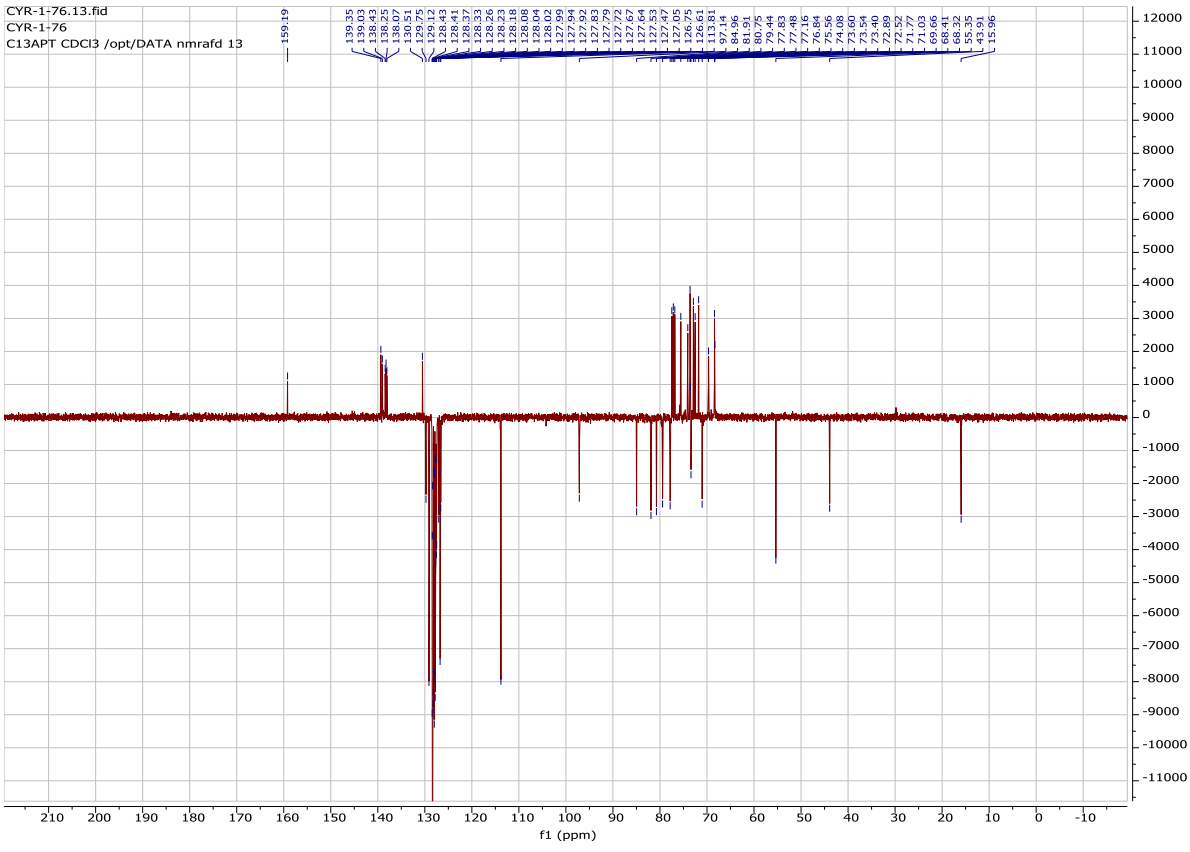

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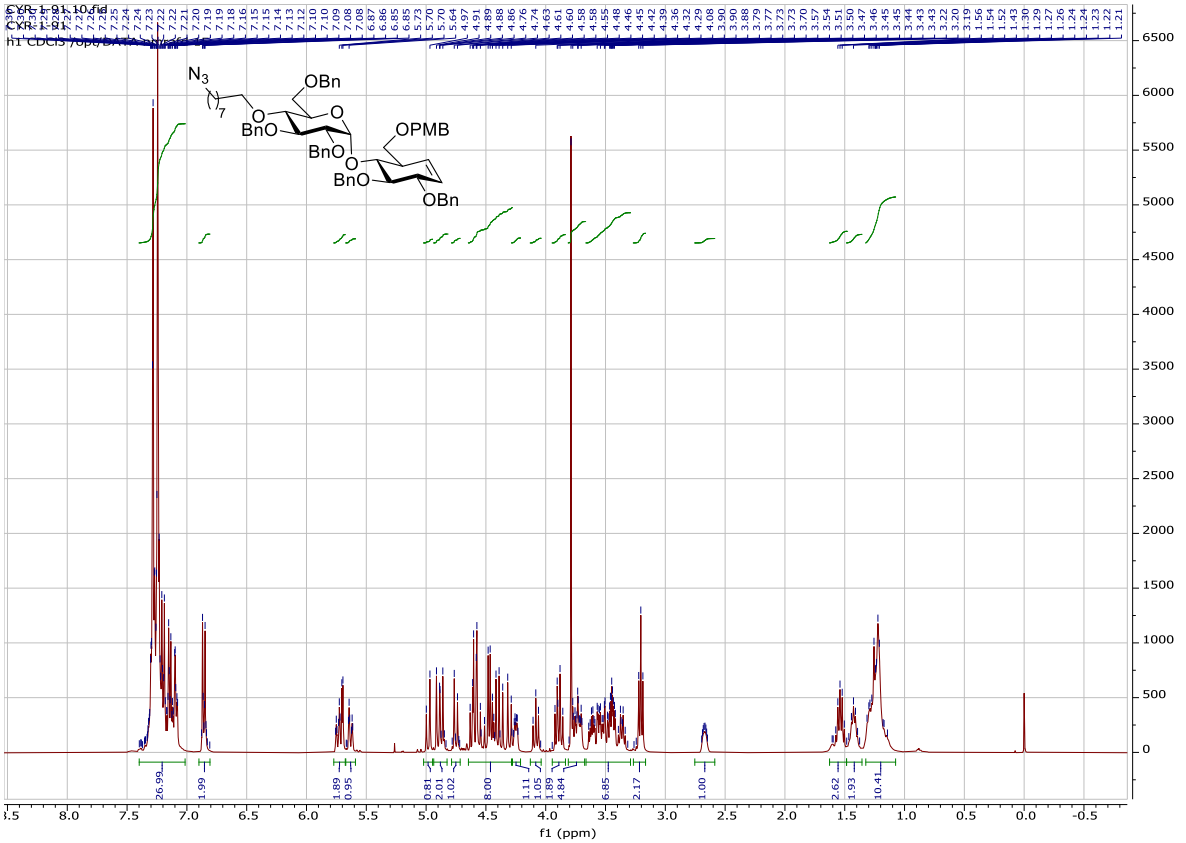

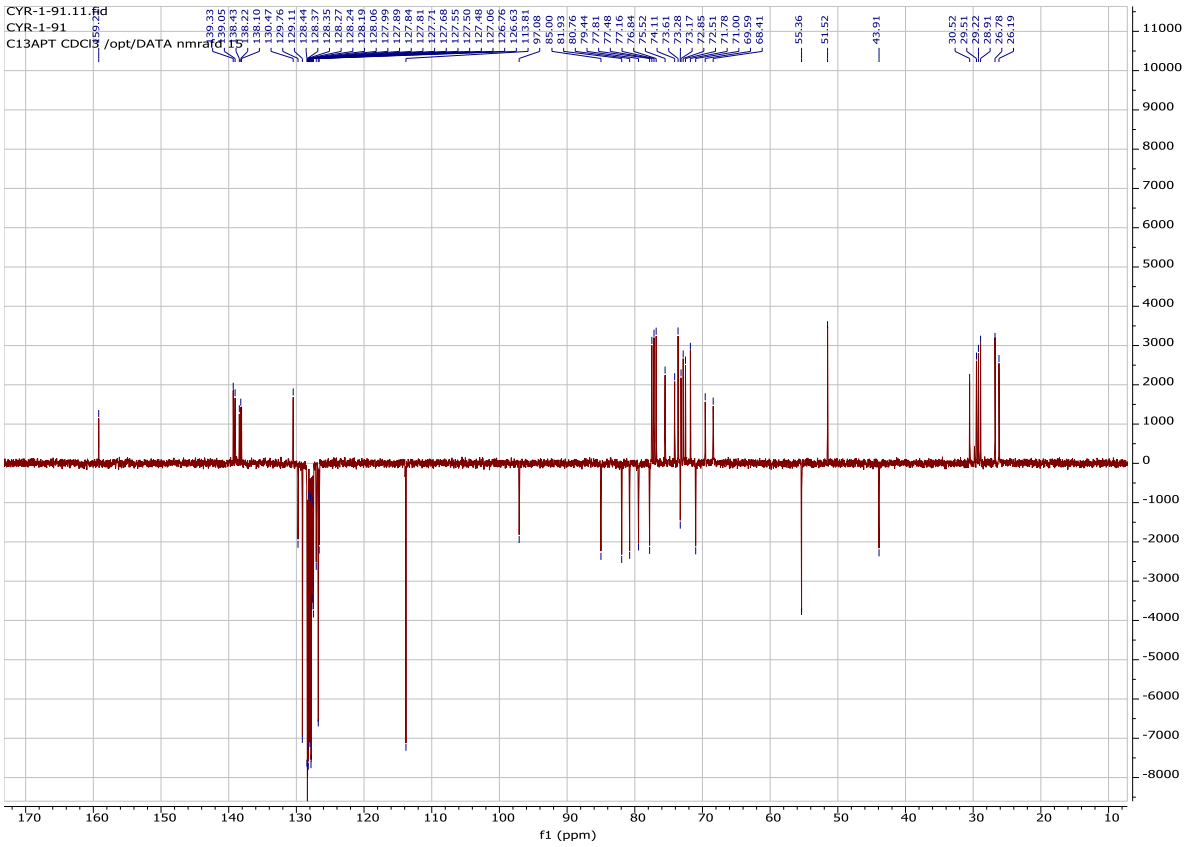

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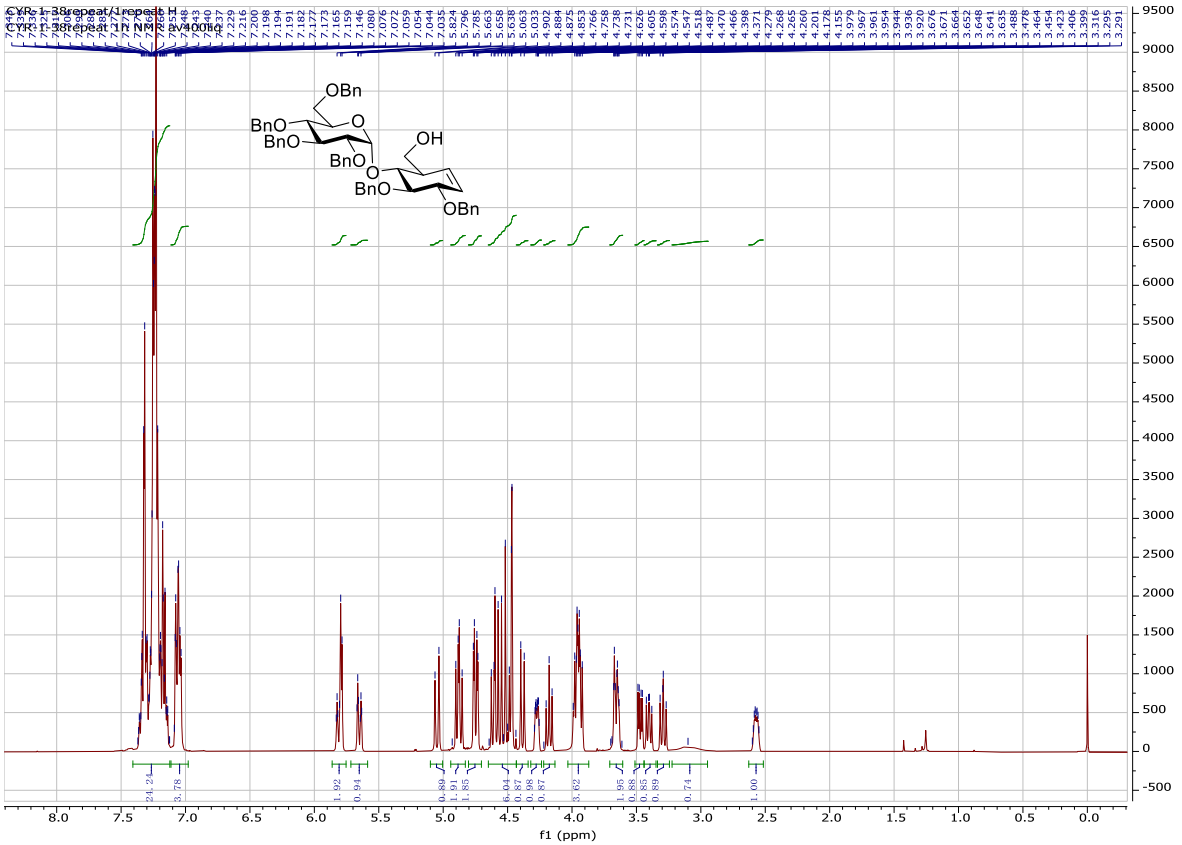

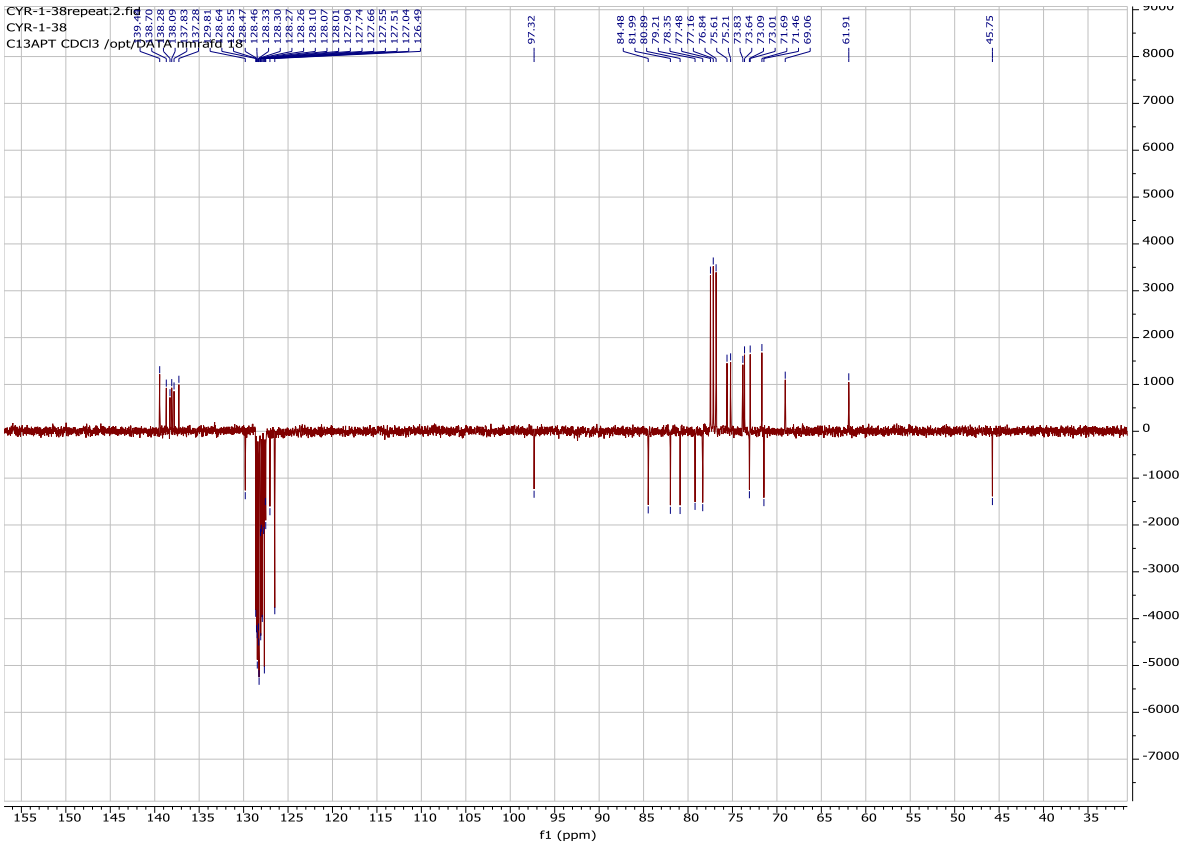

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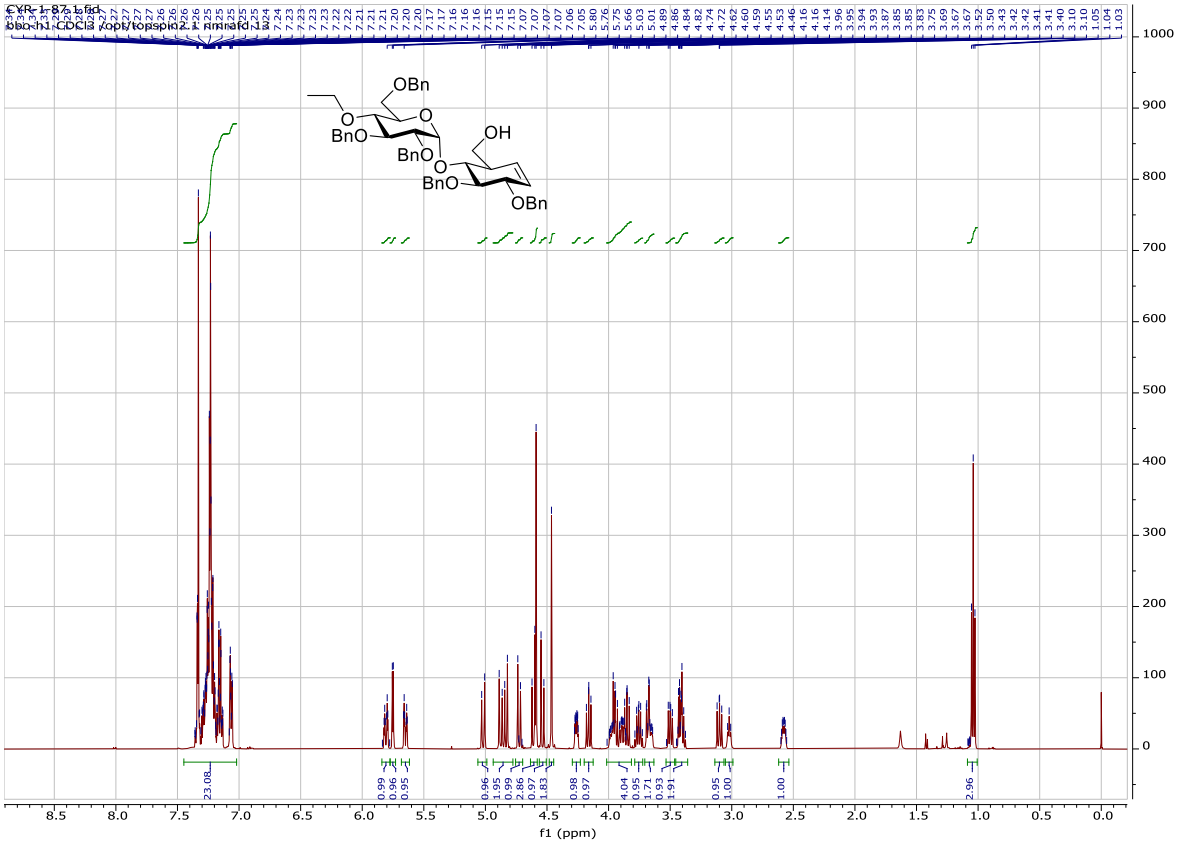

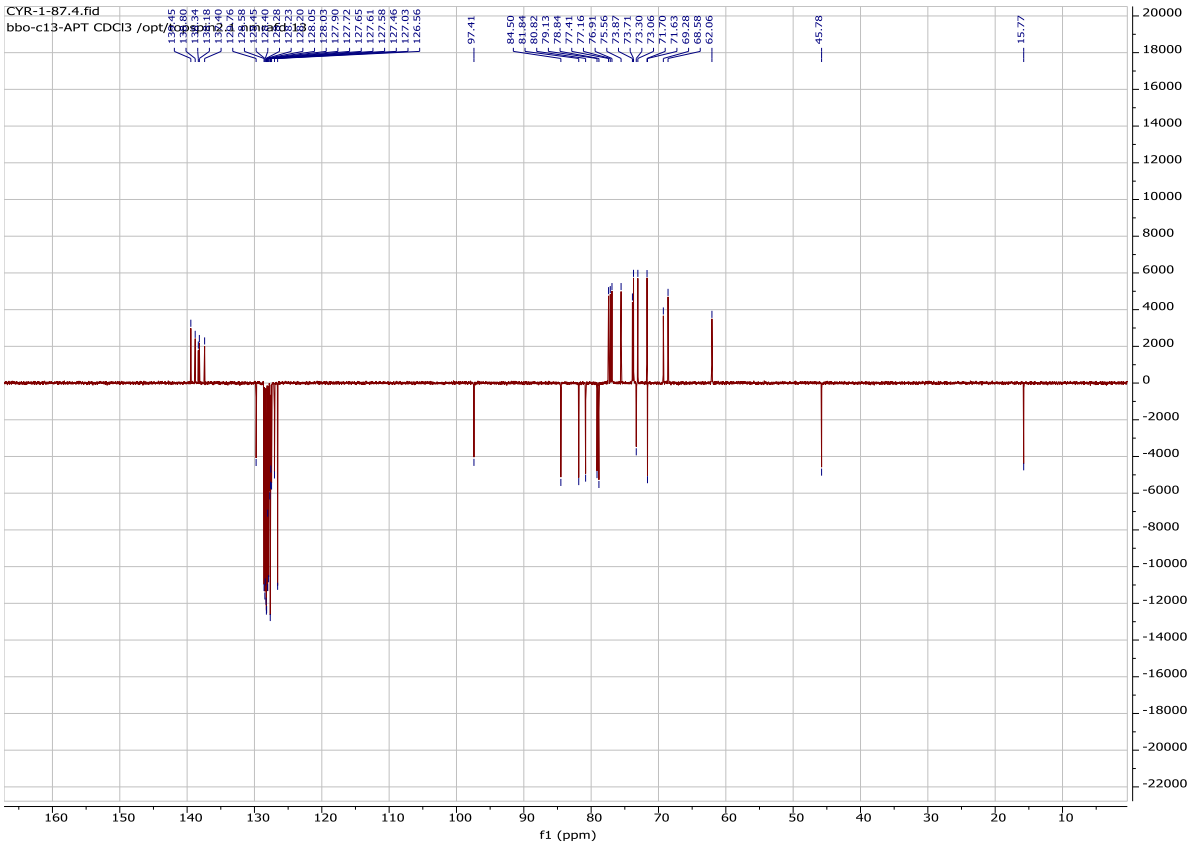

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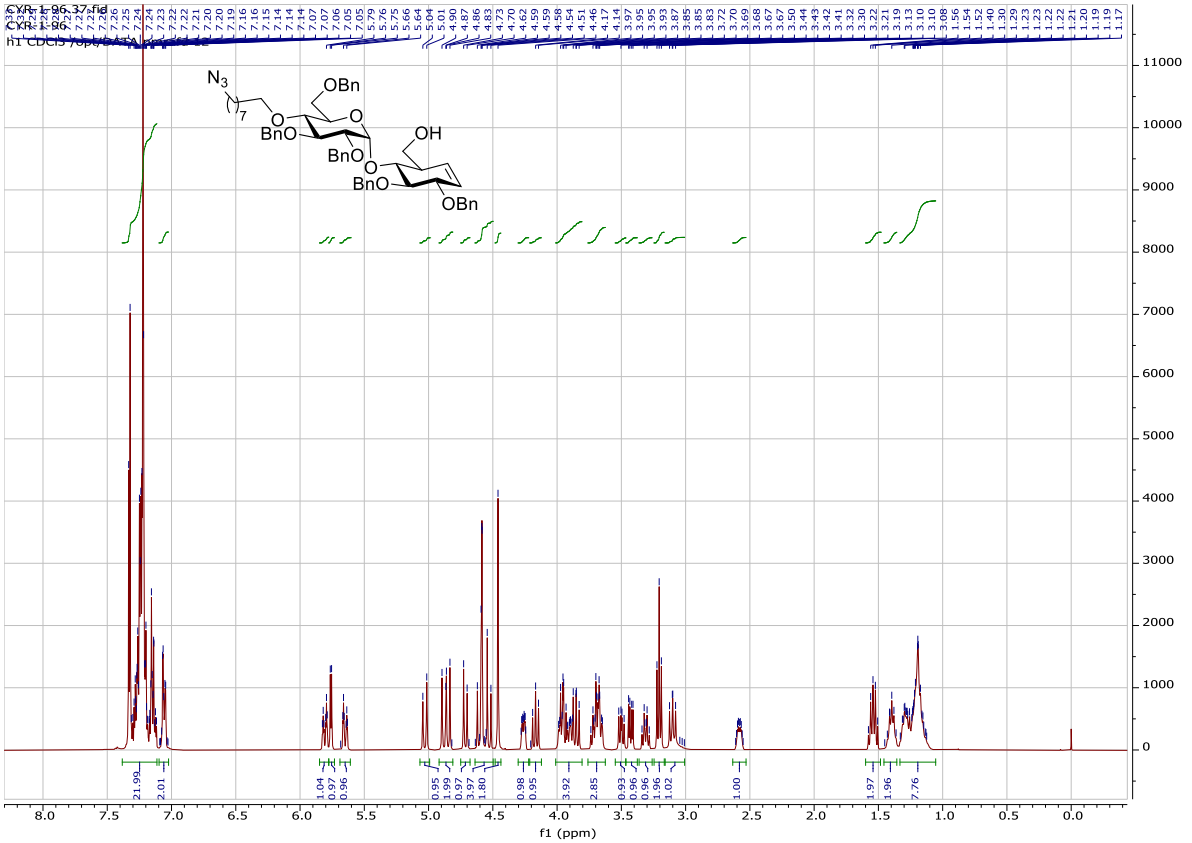

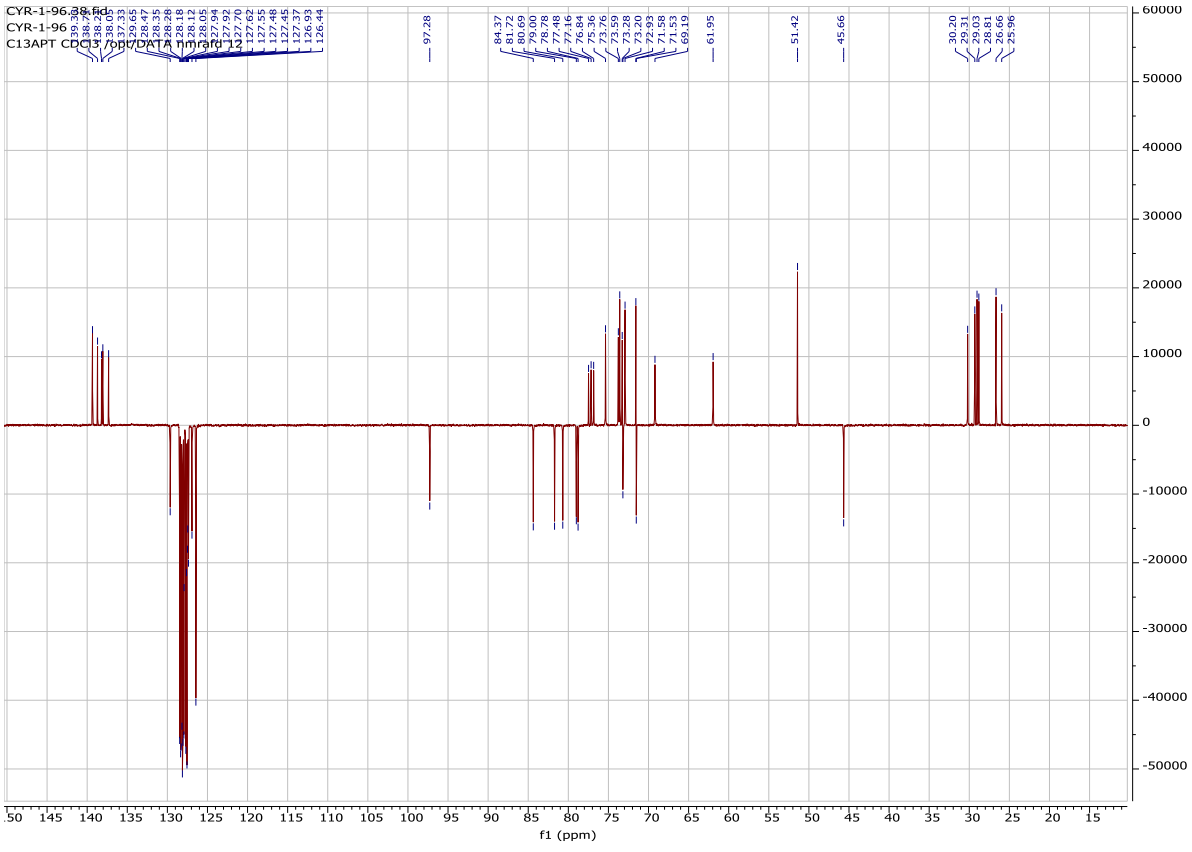

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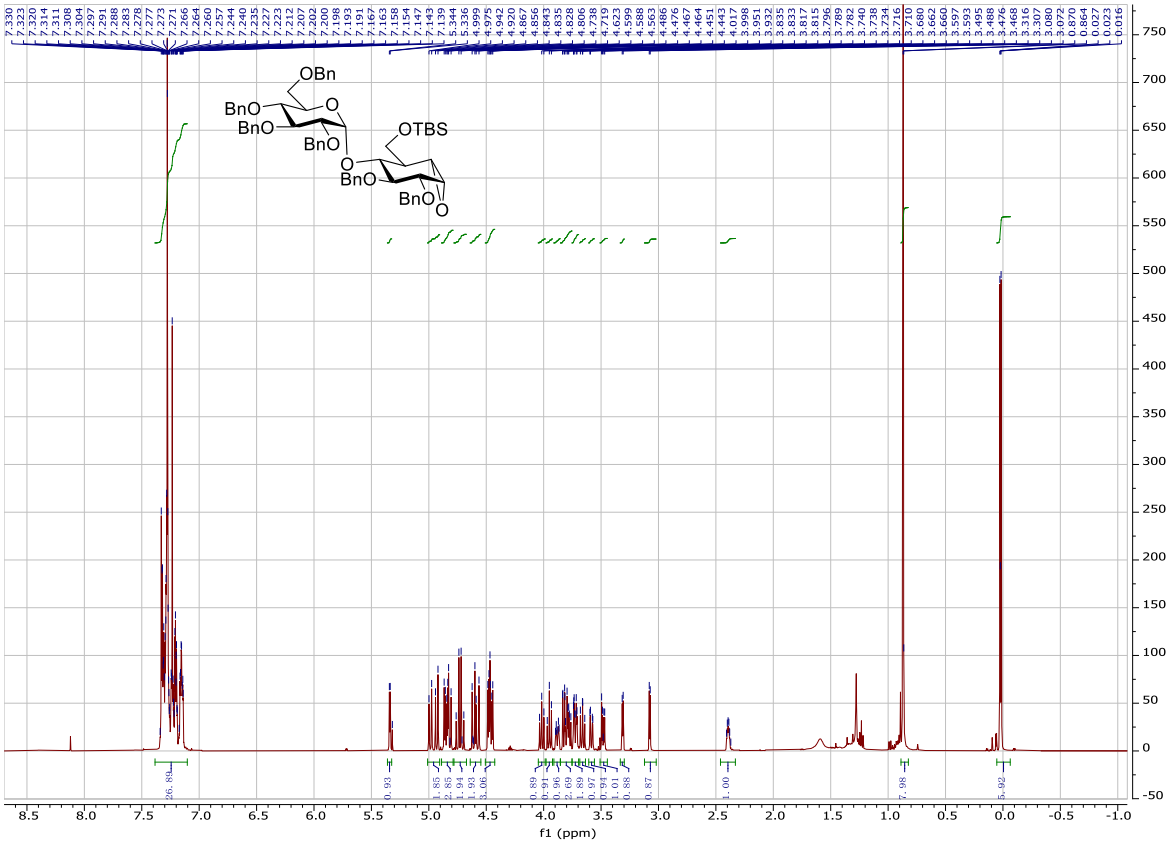

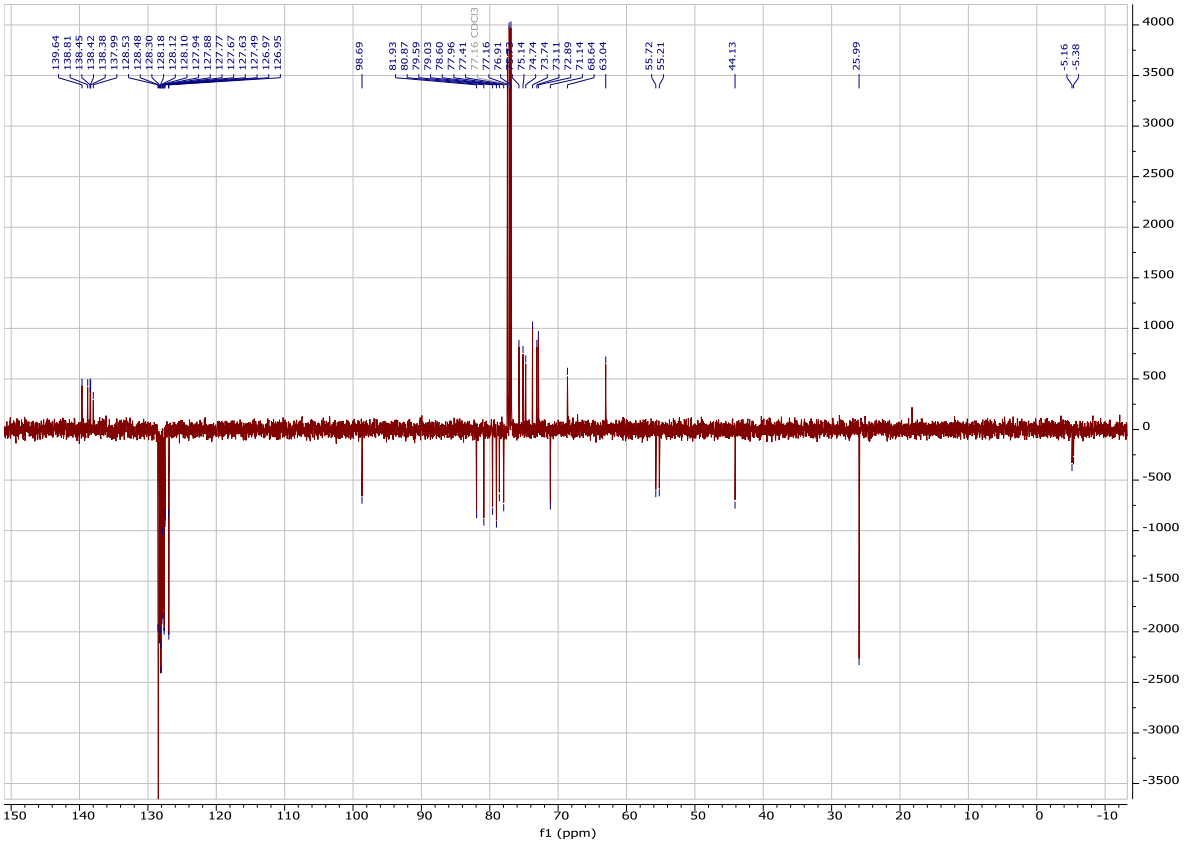

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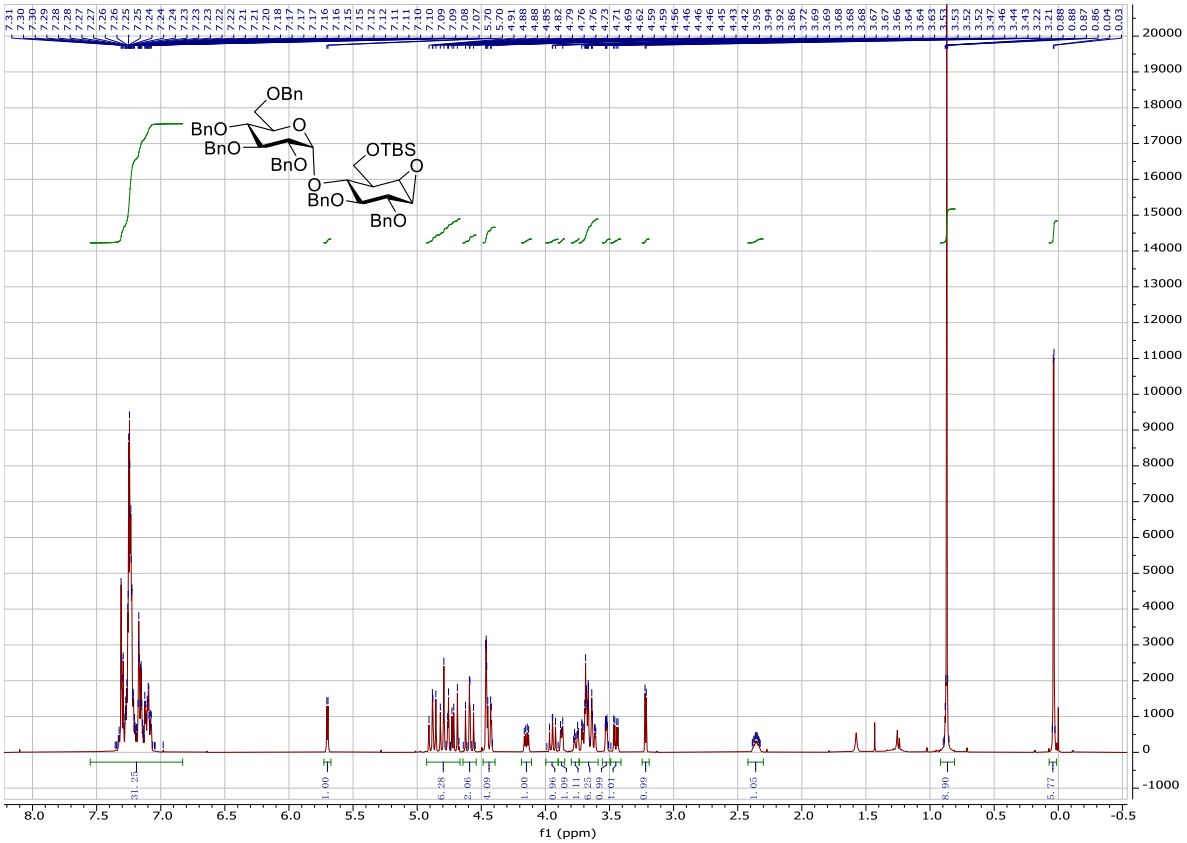

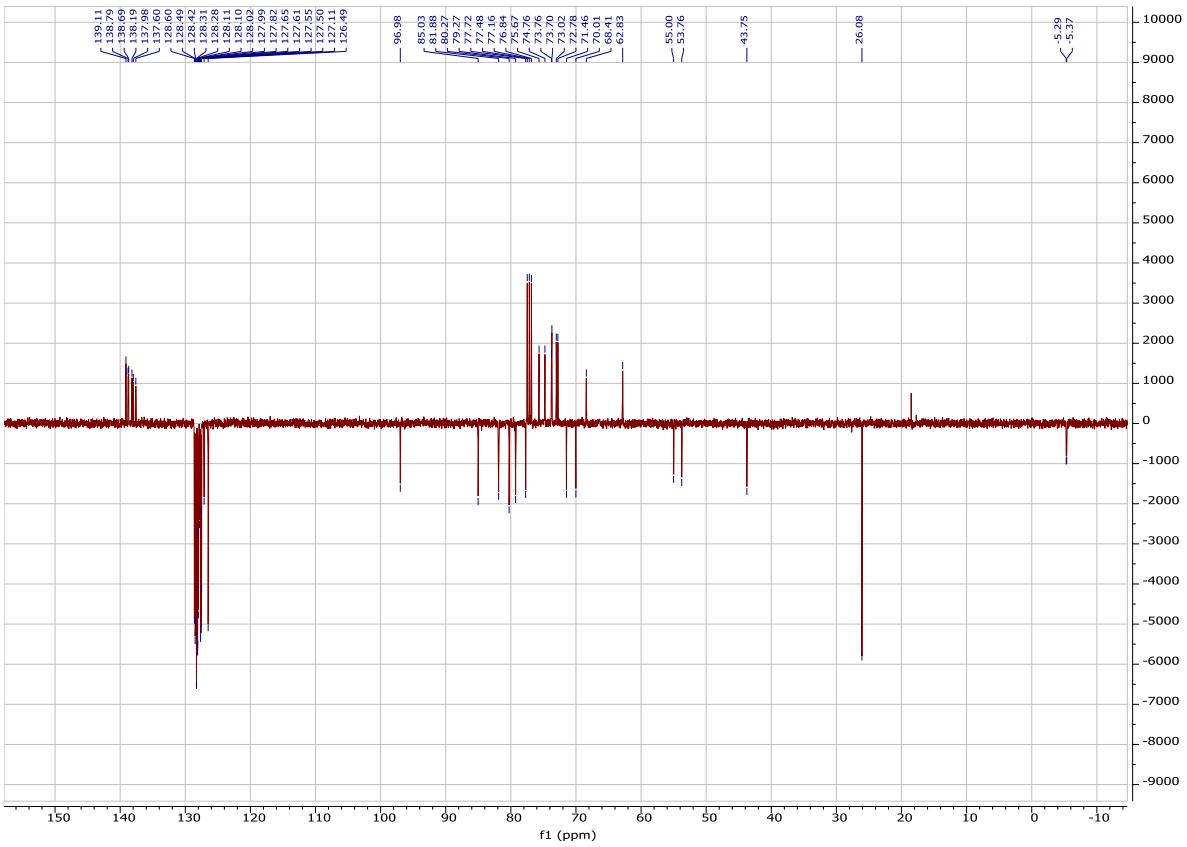

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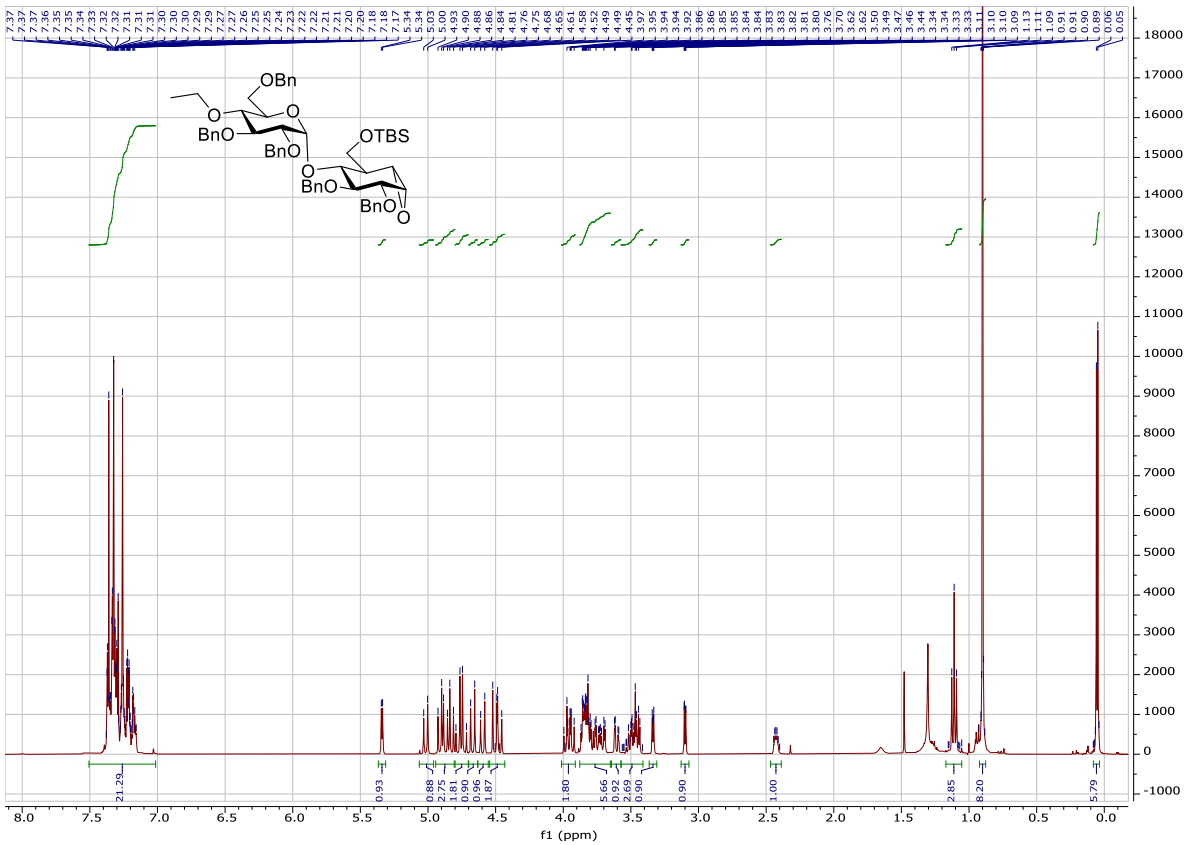

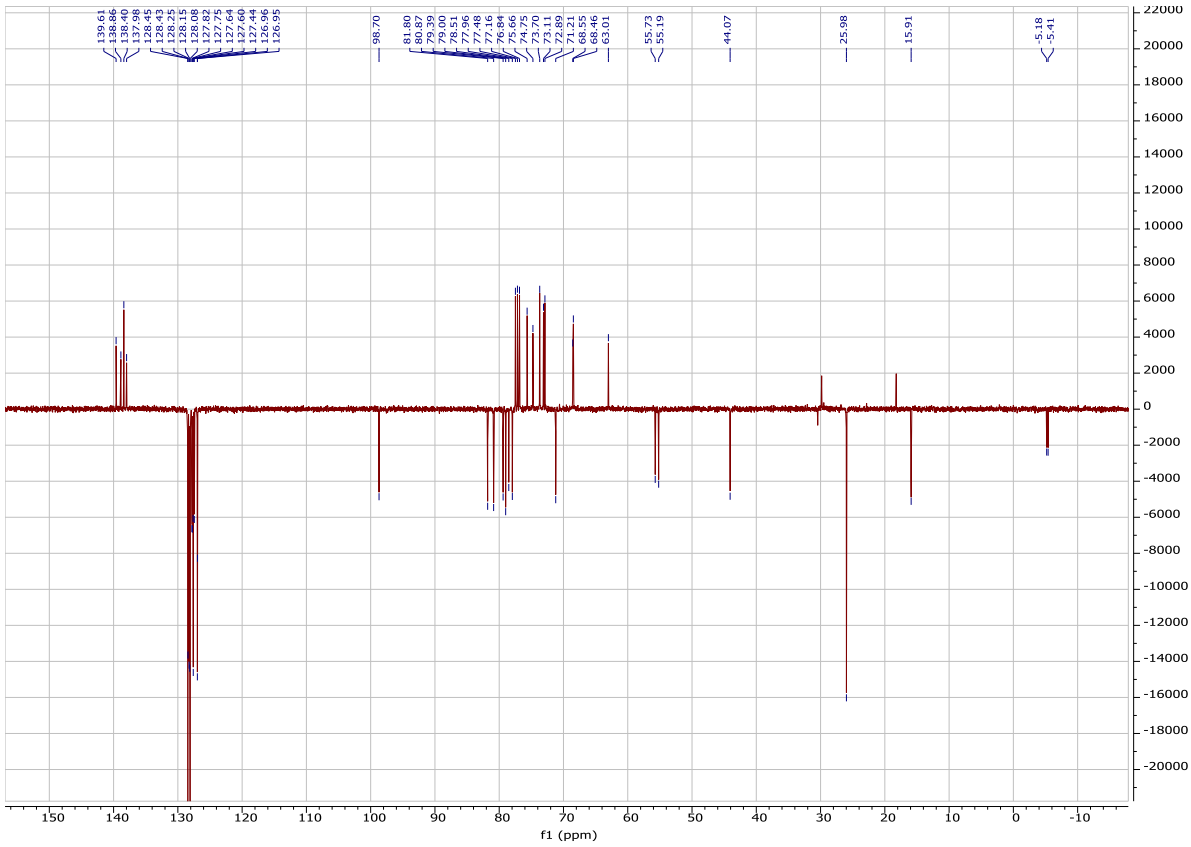

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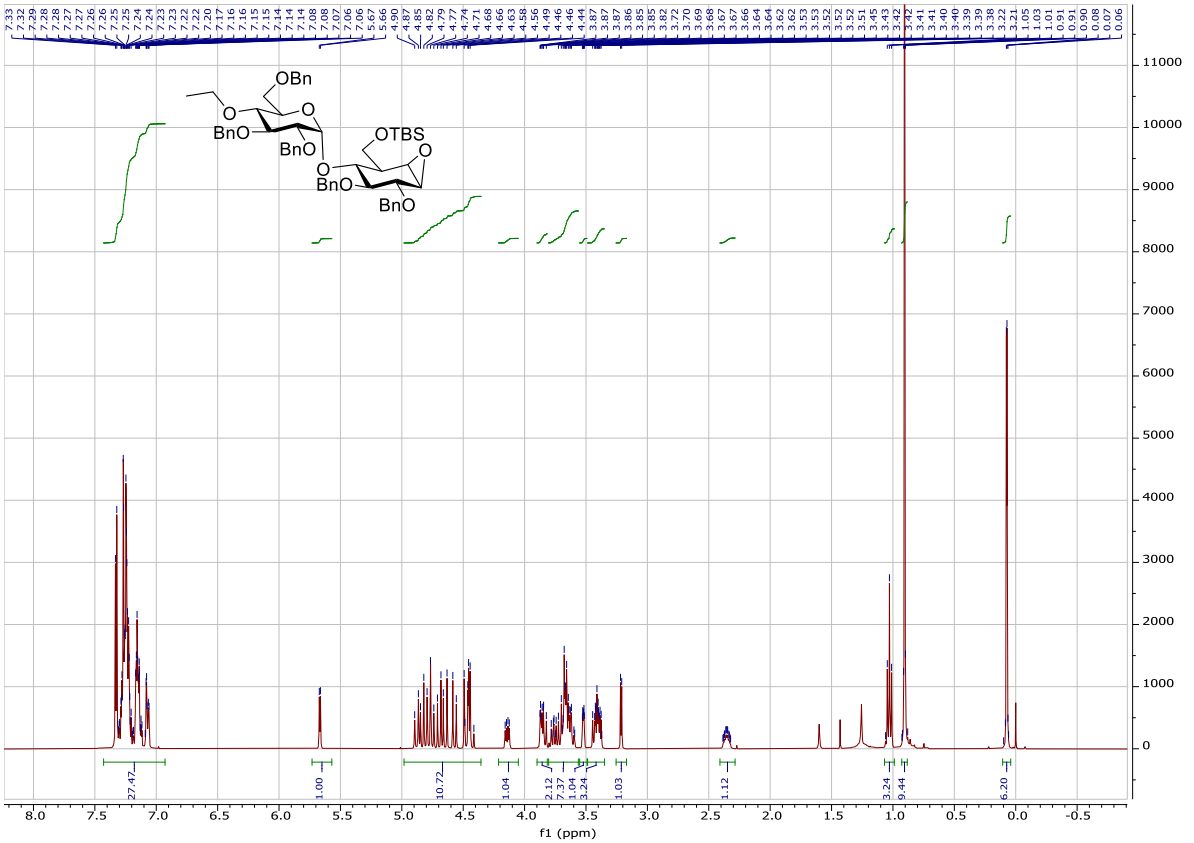

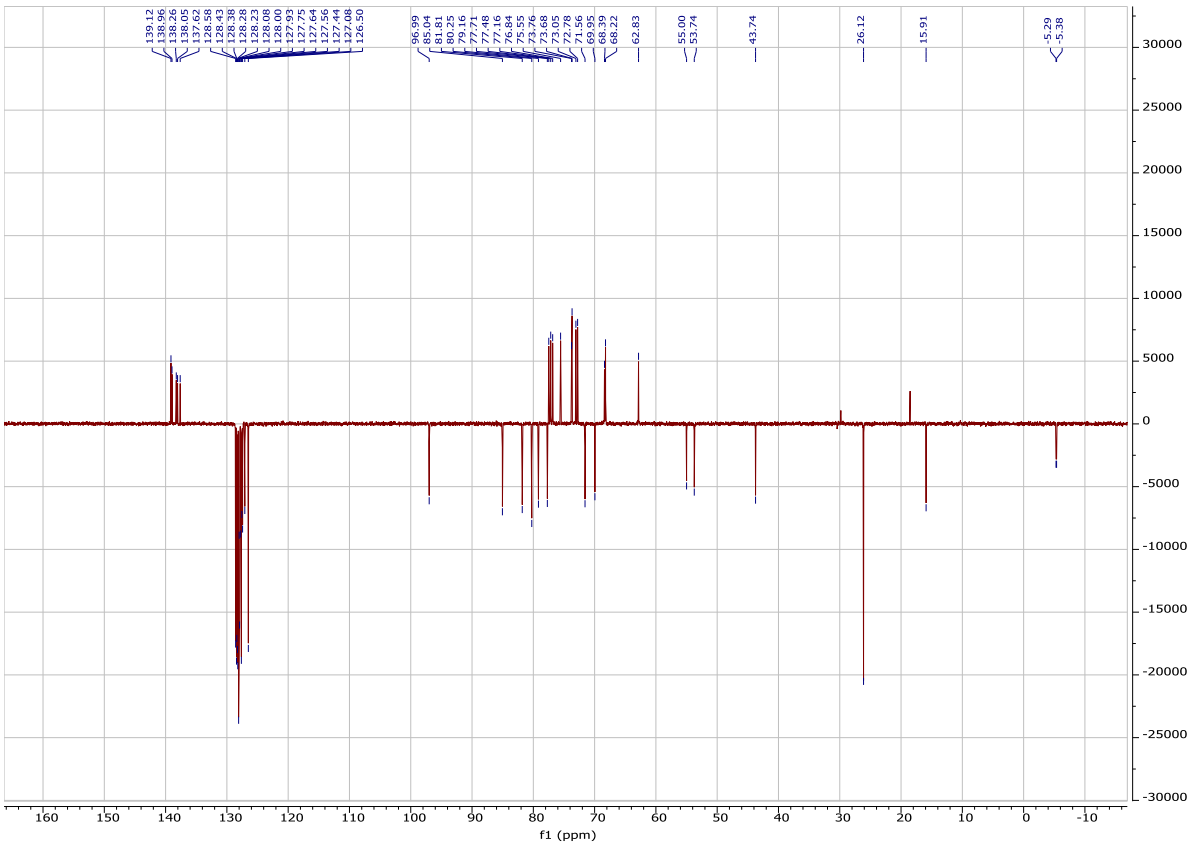

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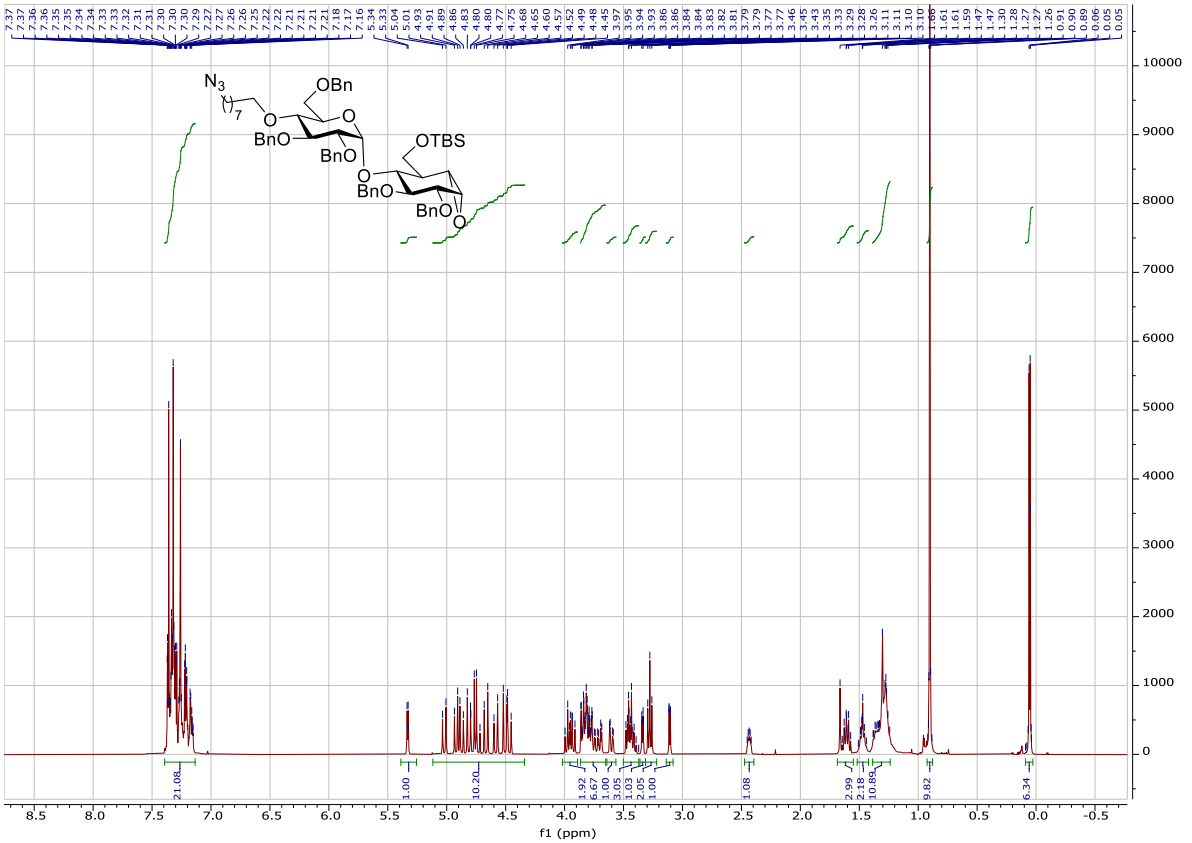

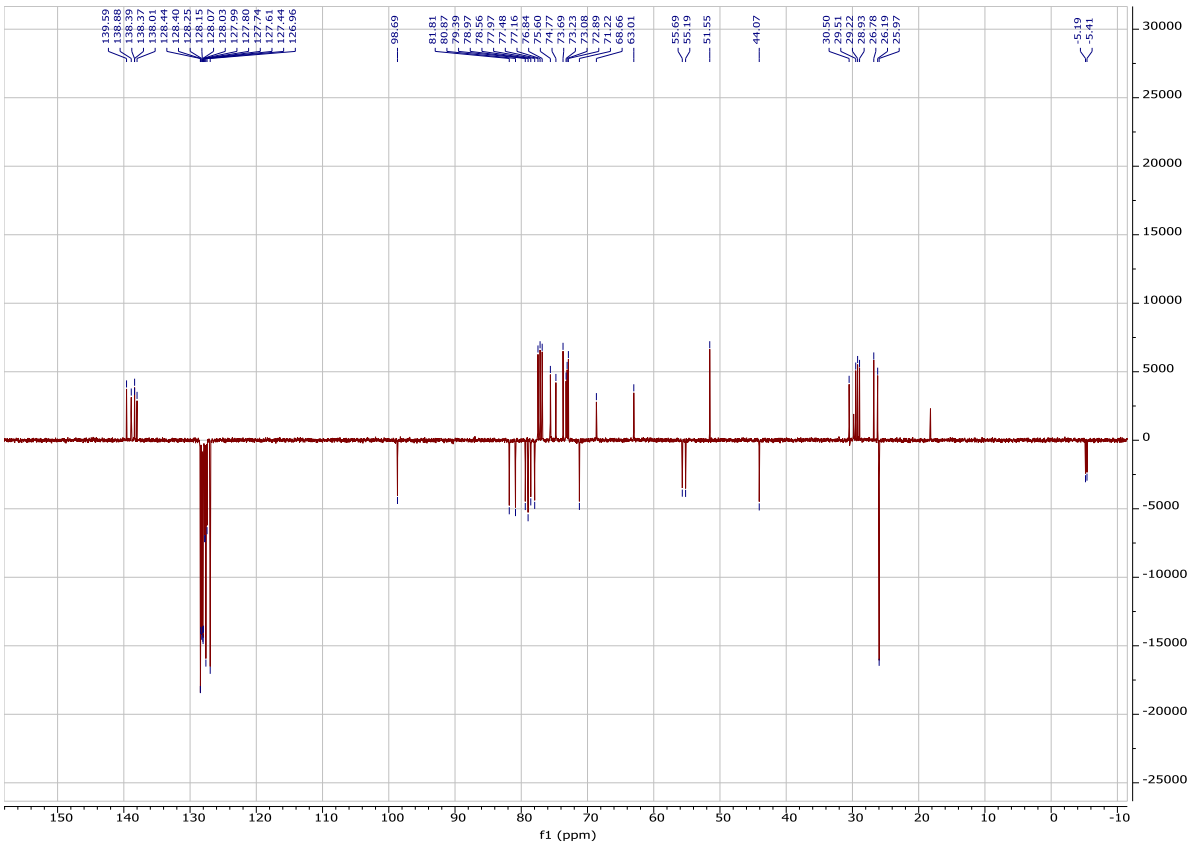

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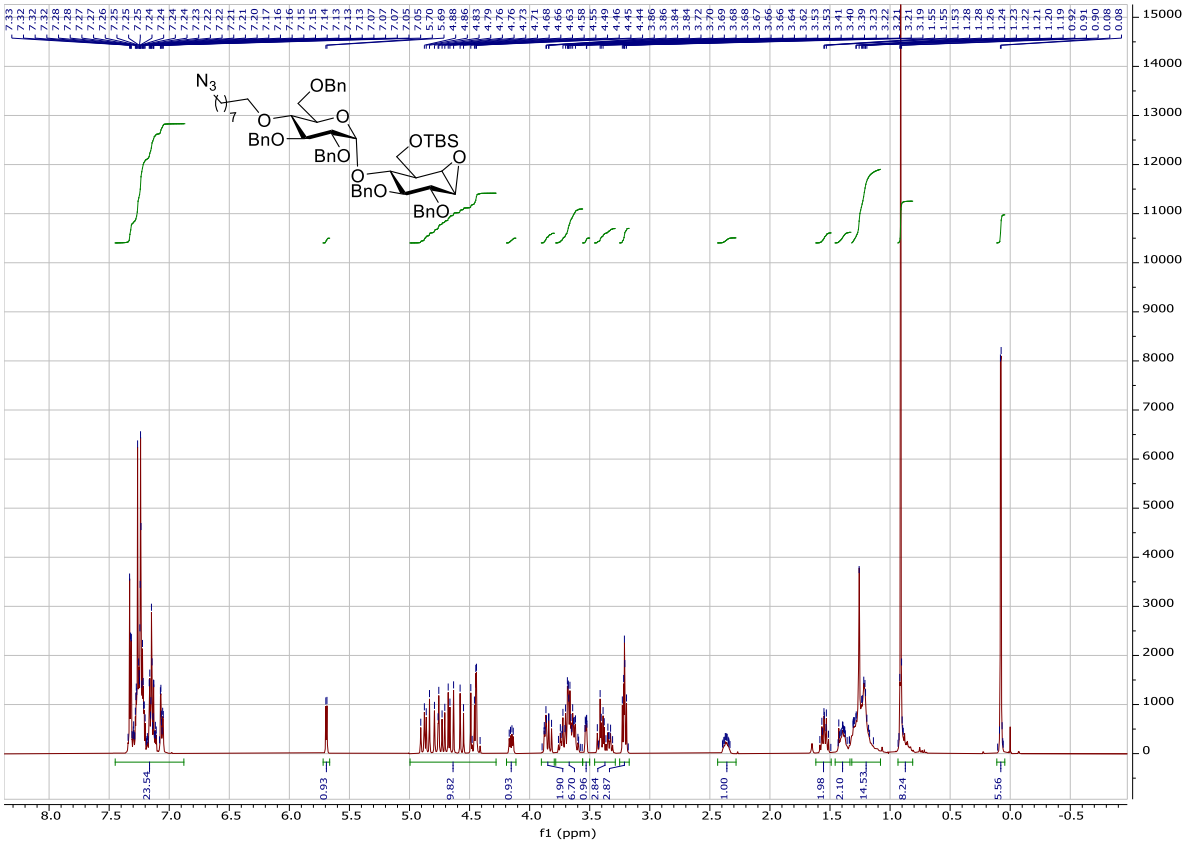

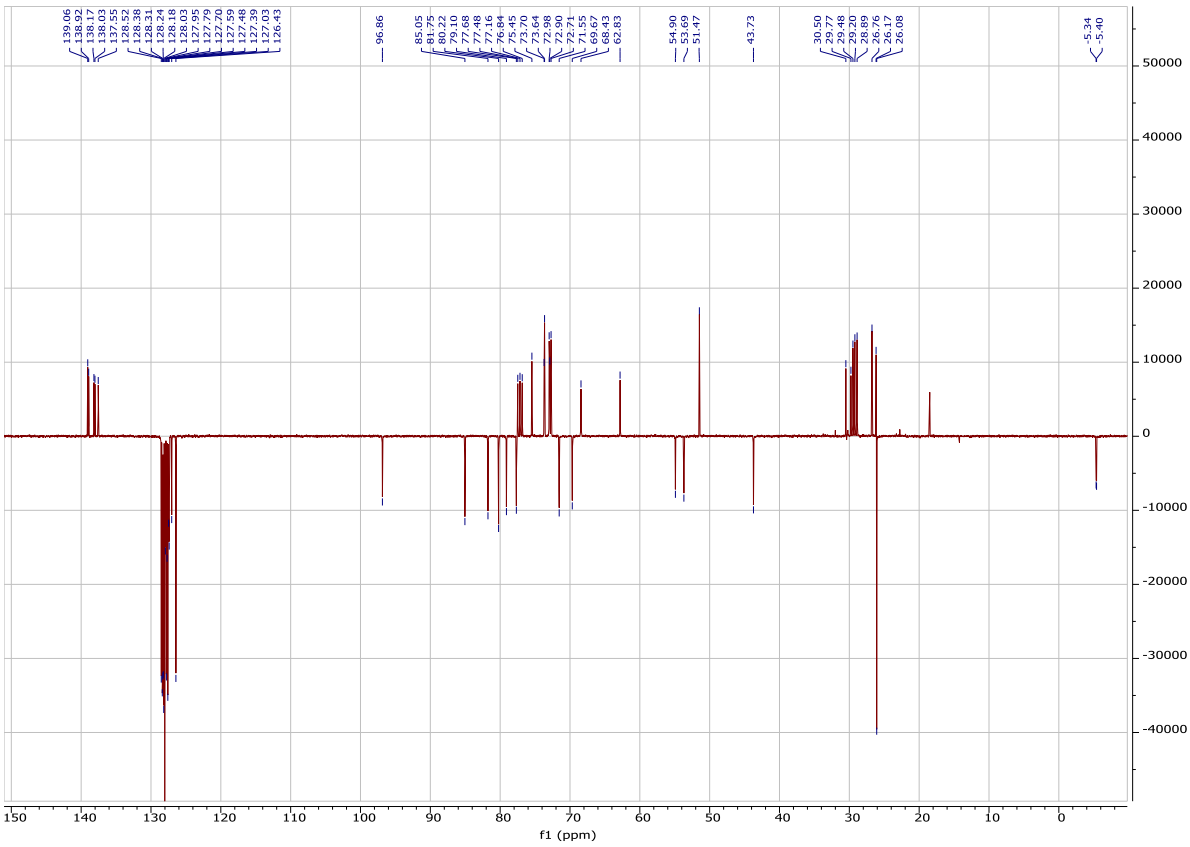

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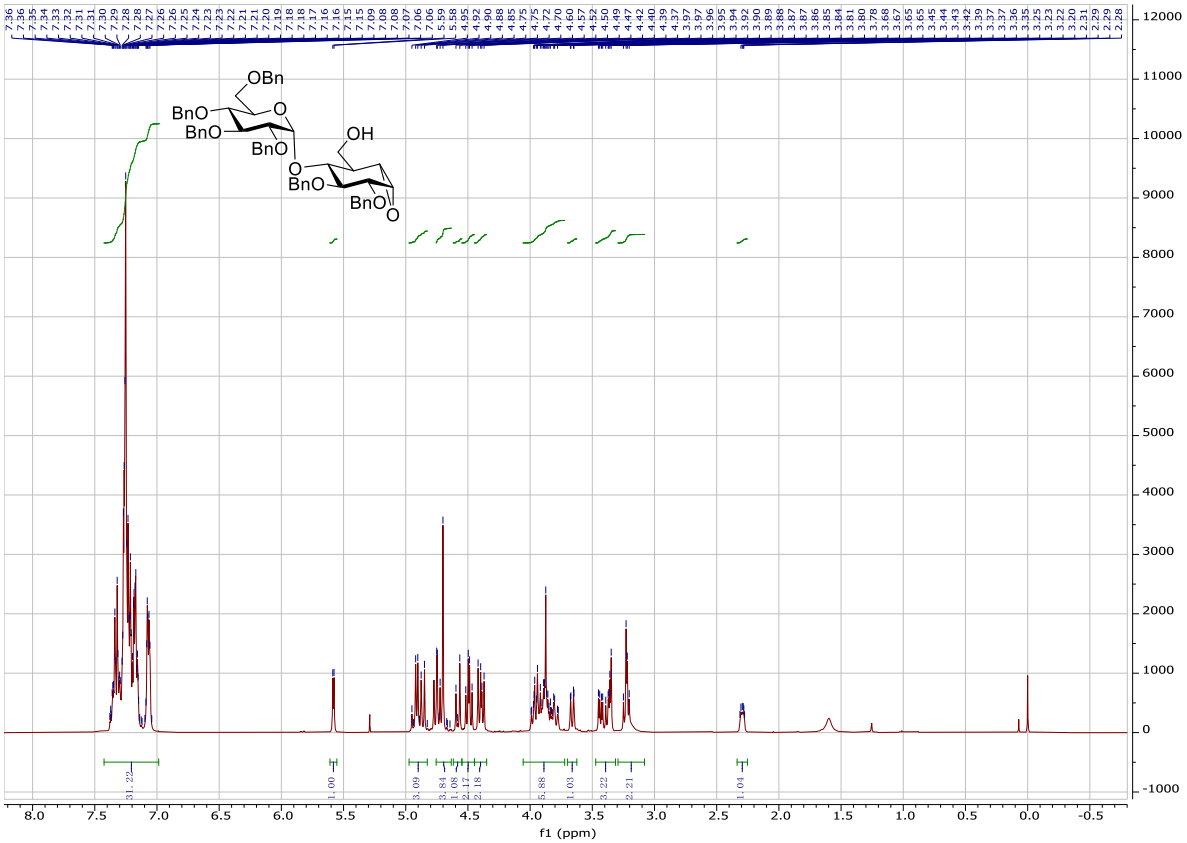

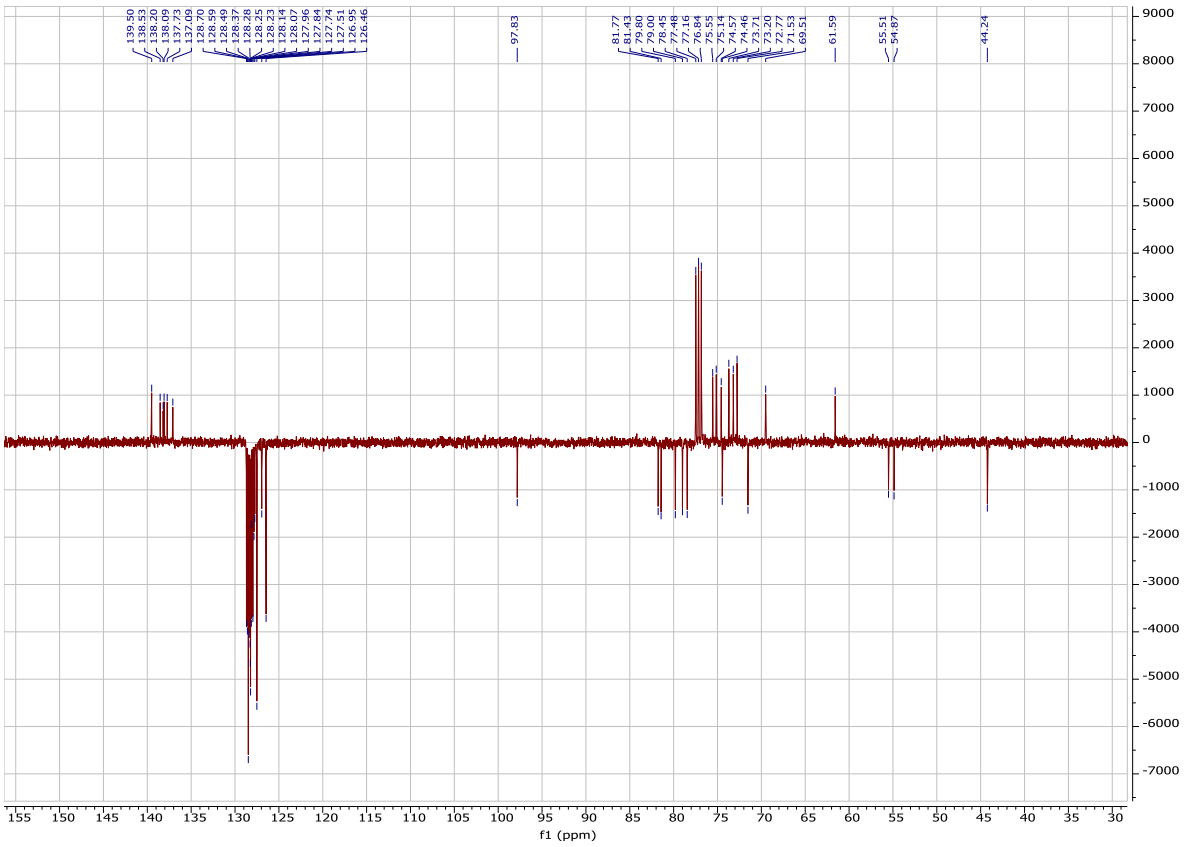

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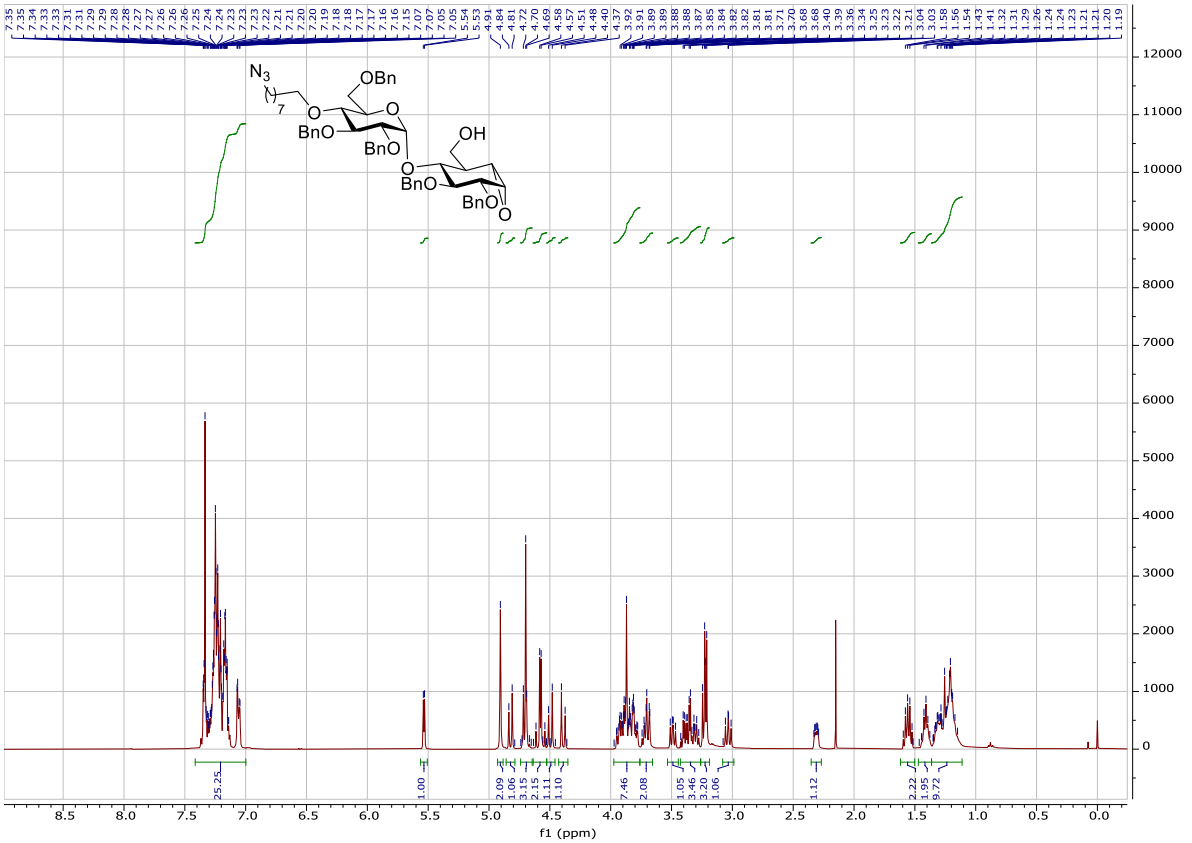

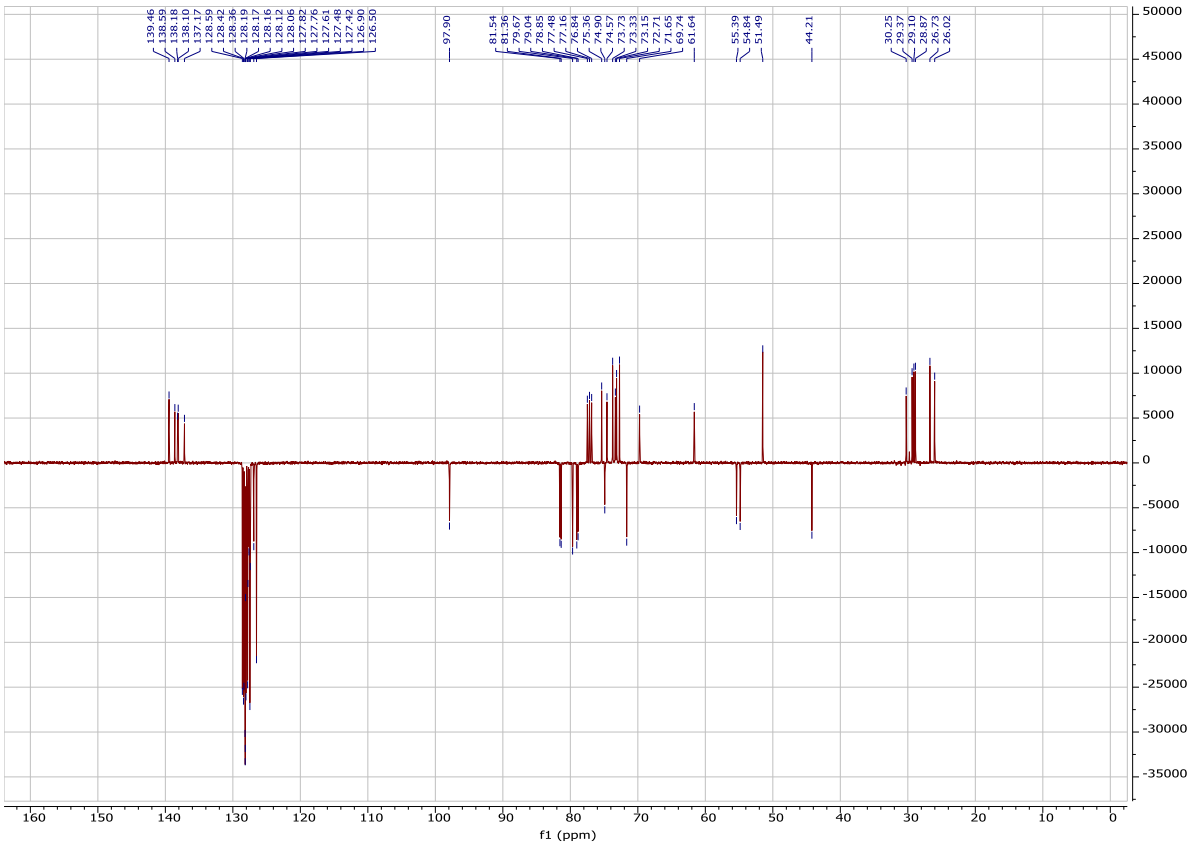

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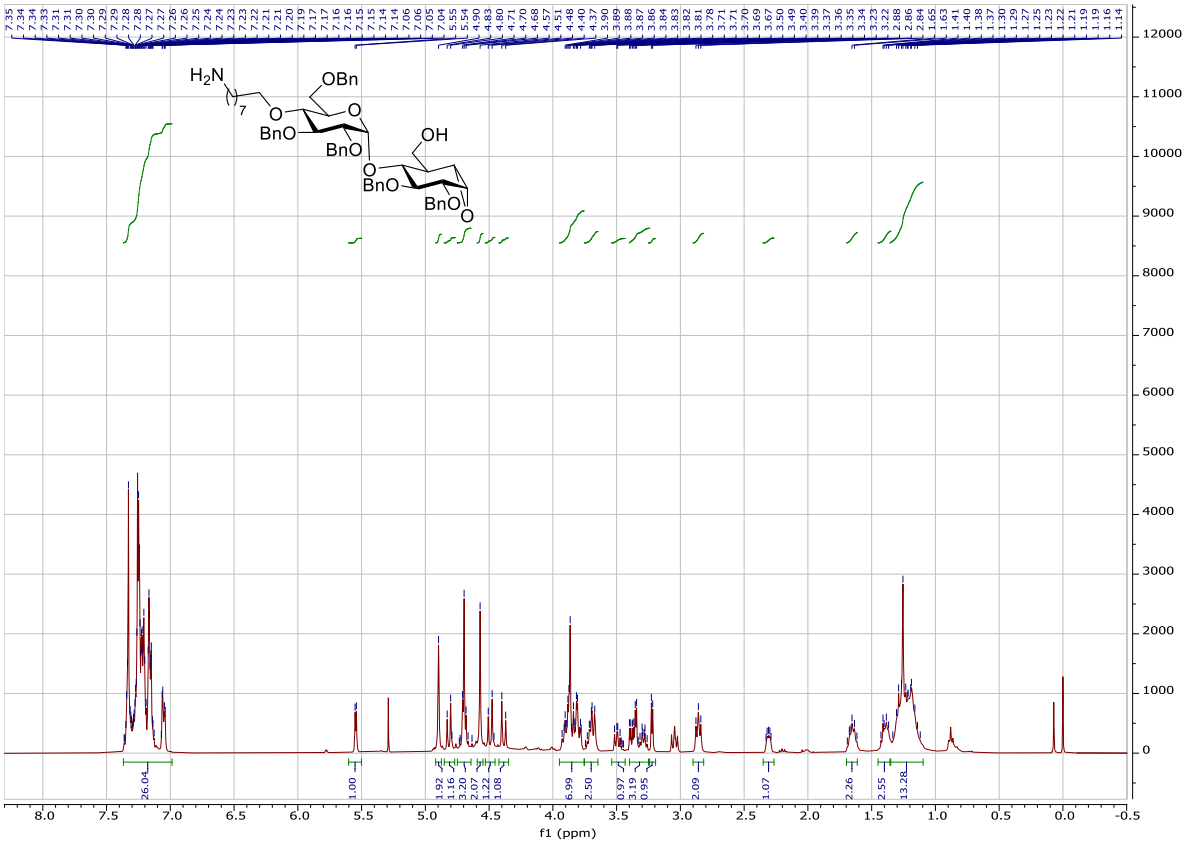
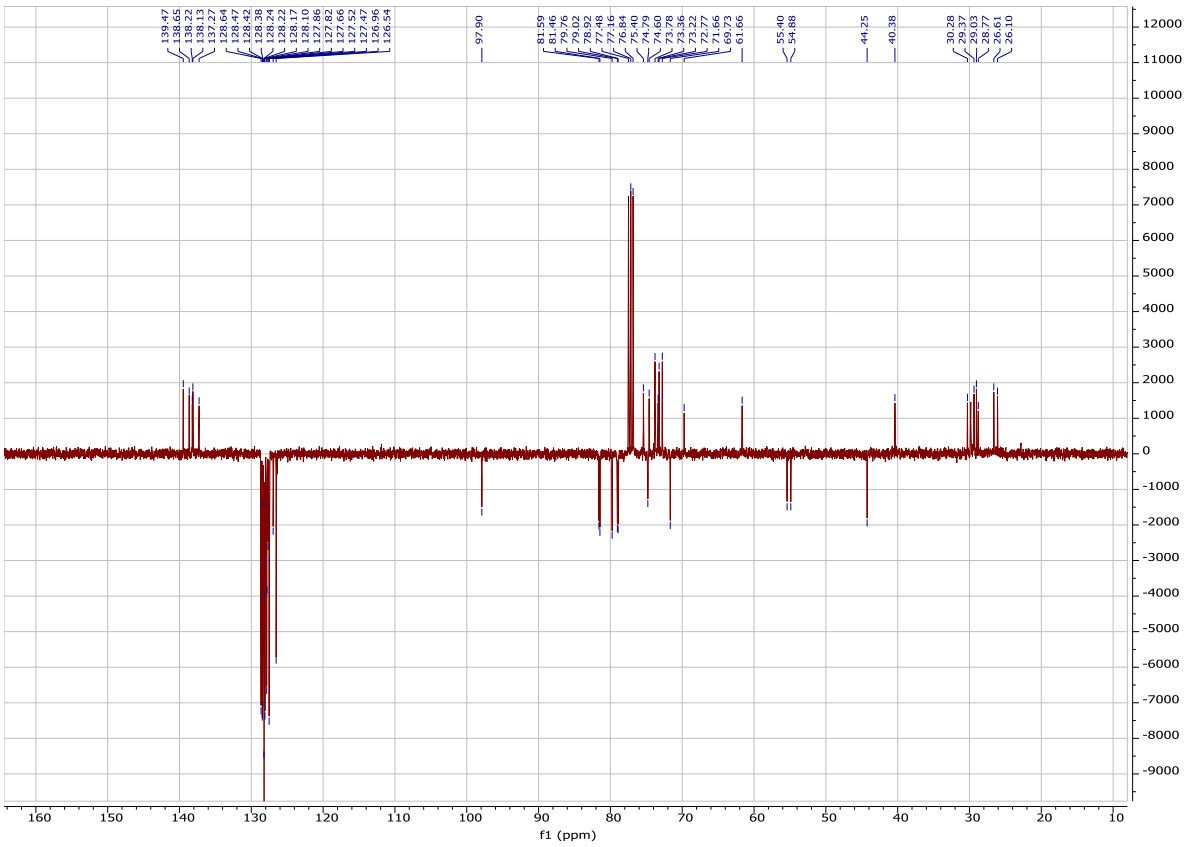
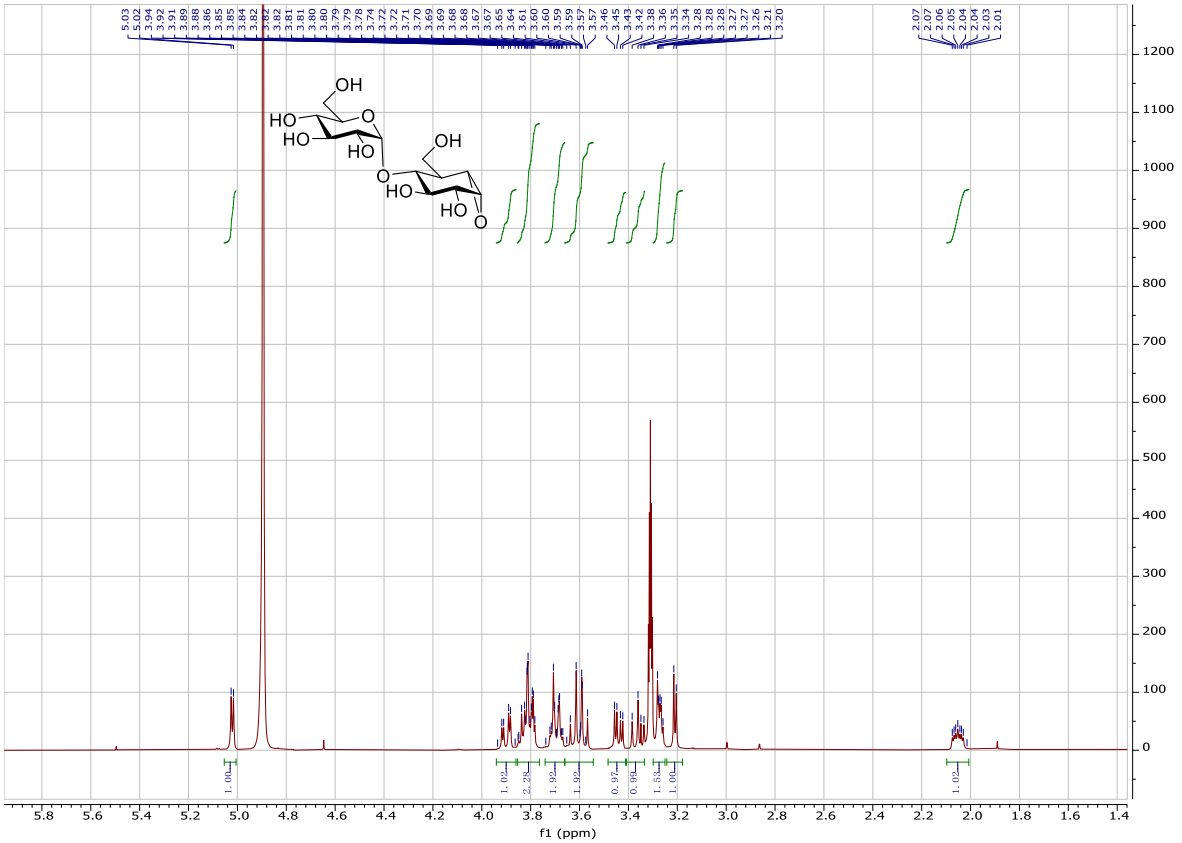
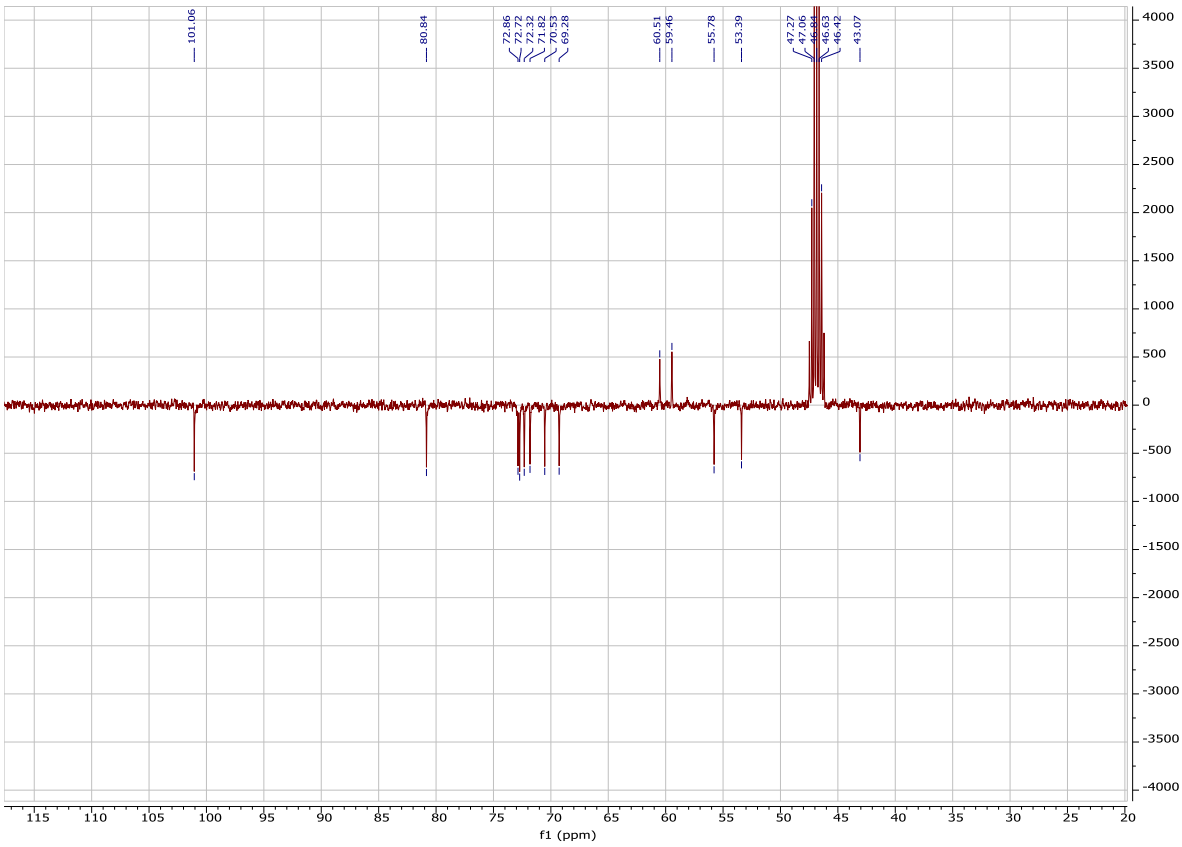




^{1}H and ^{13}C of **1a** in MeOD





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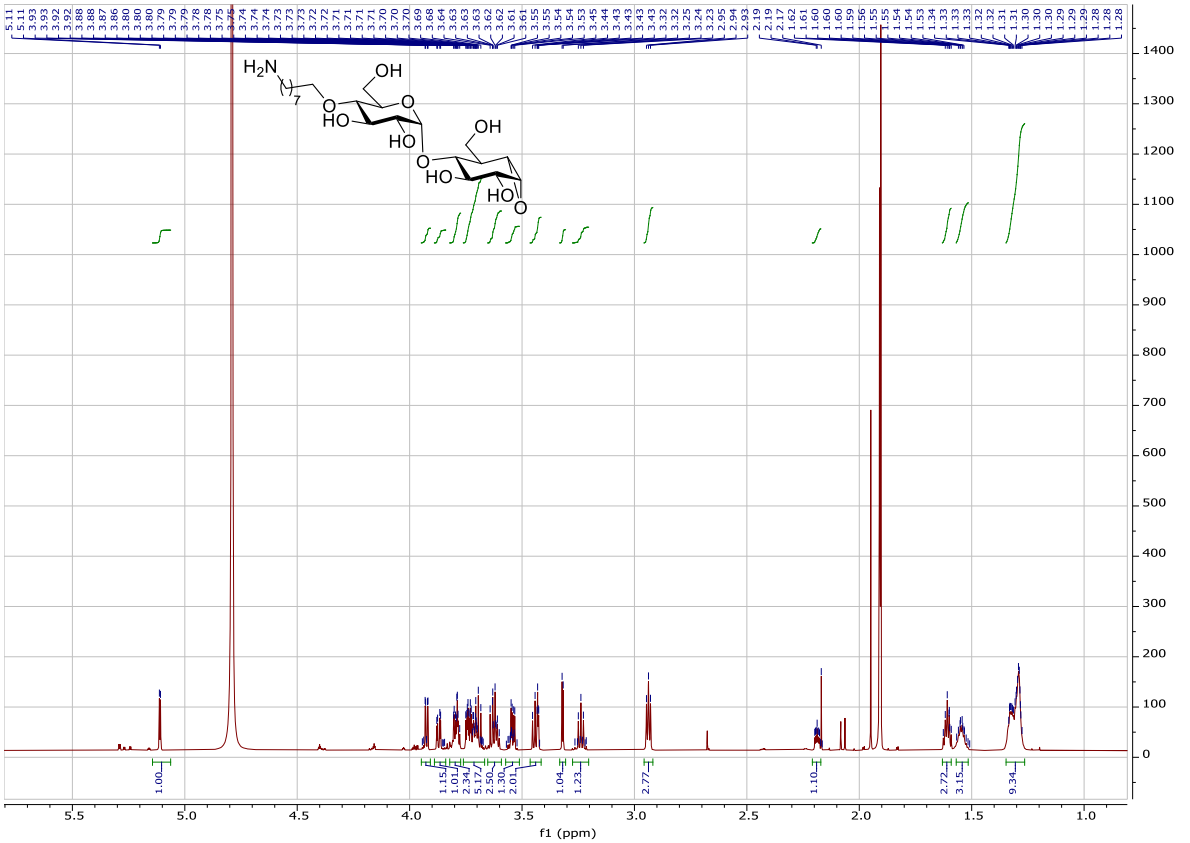

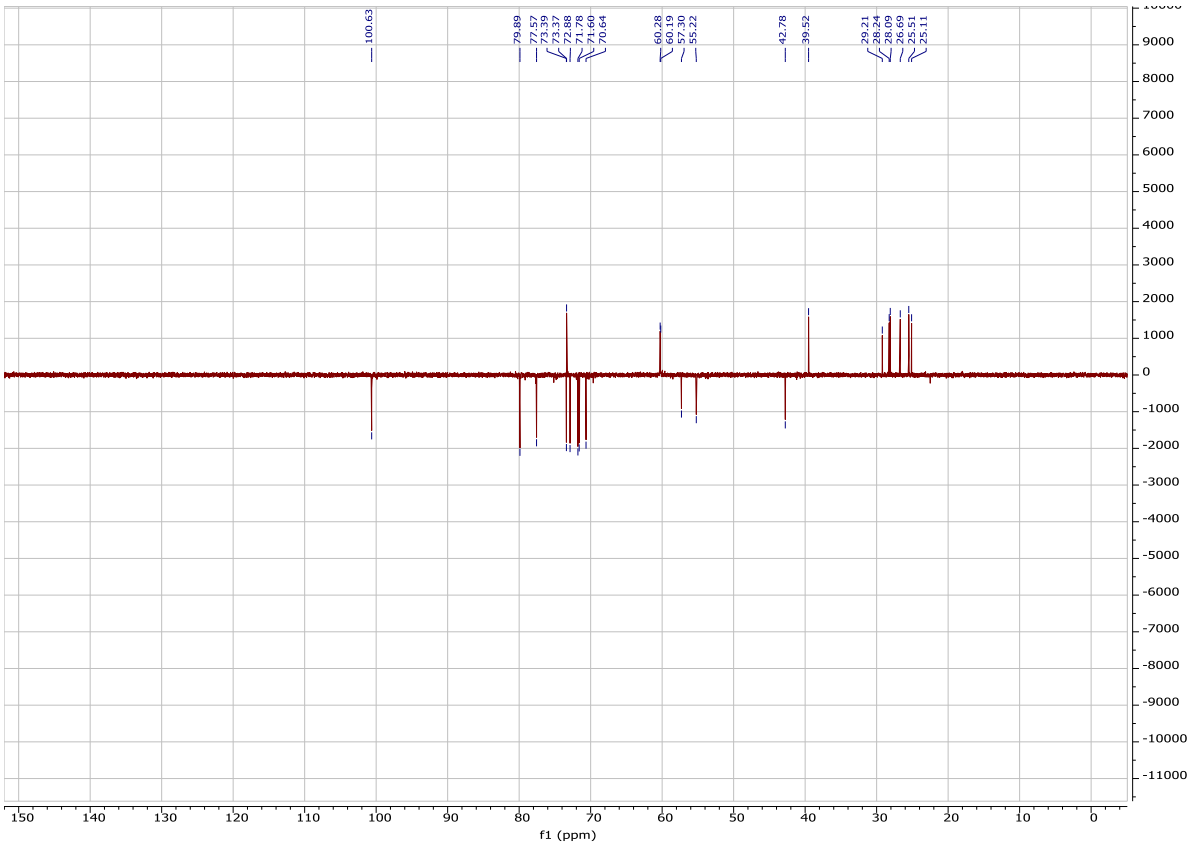

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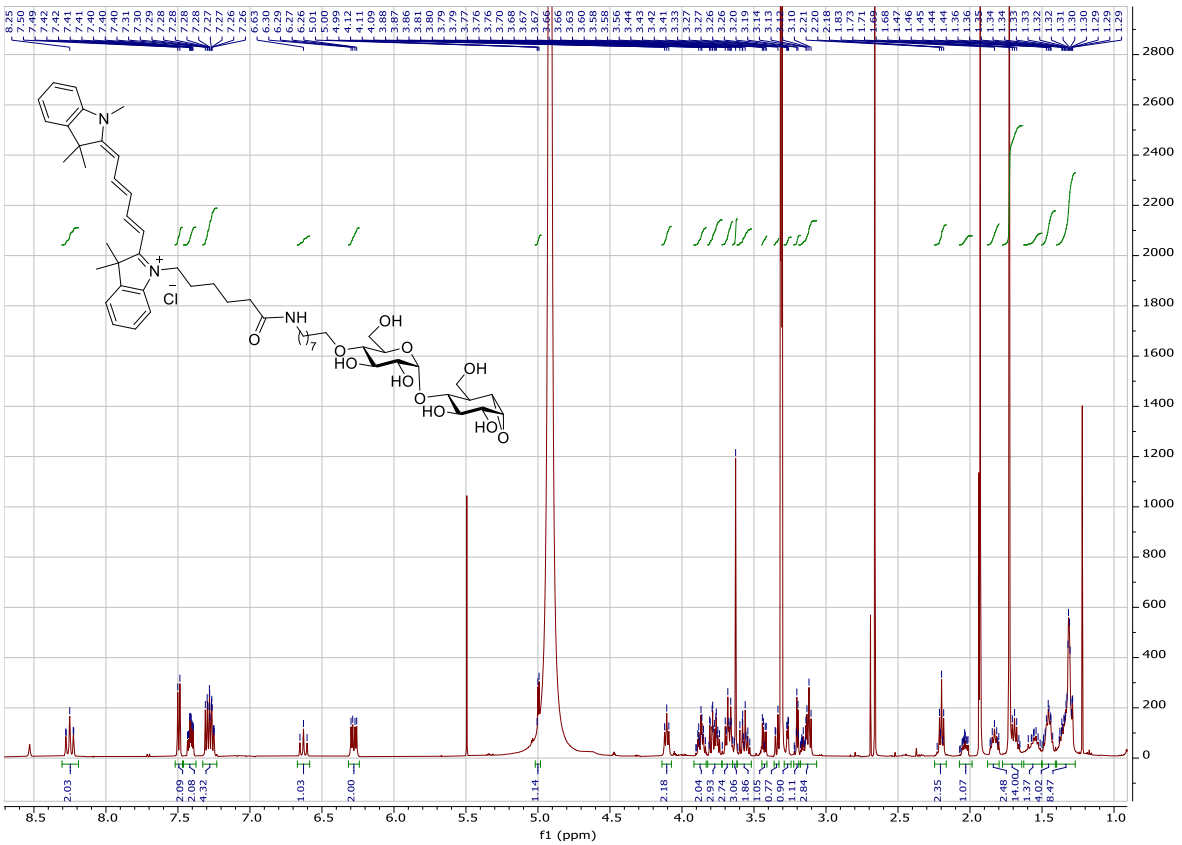

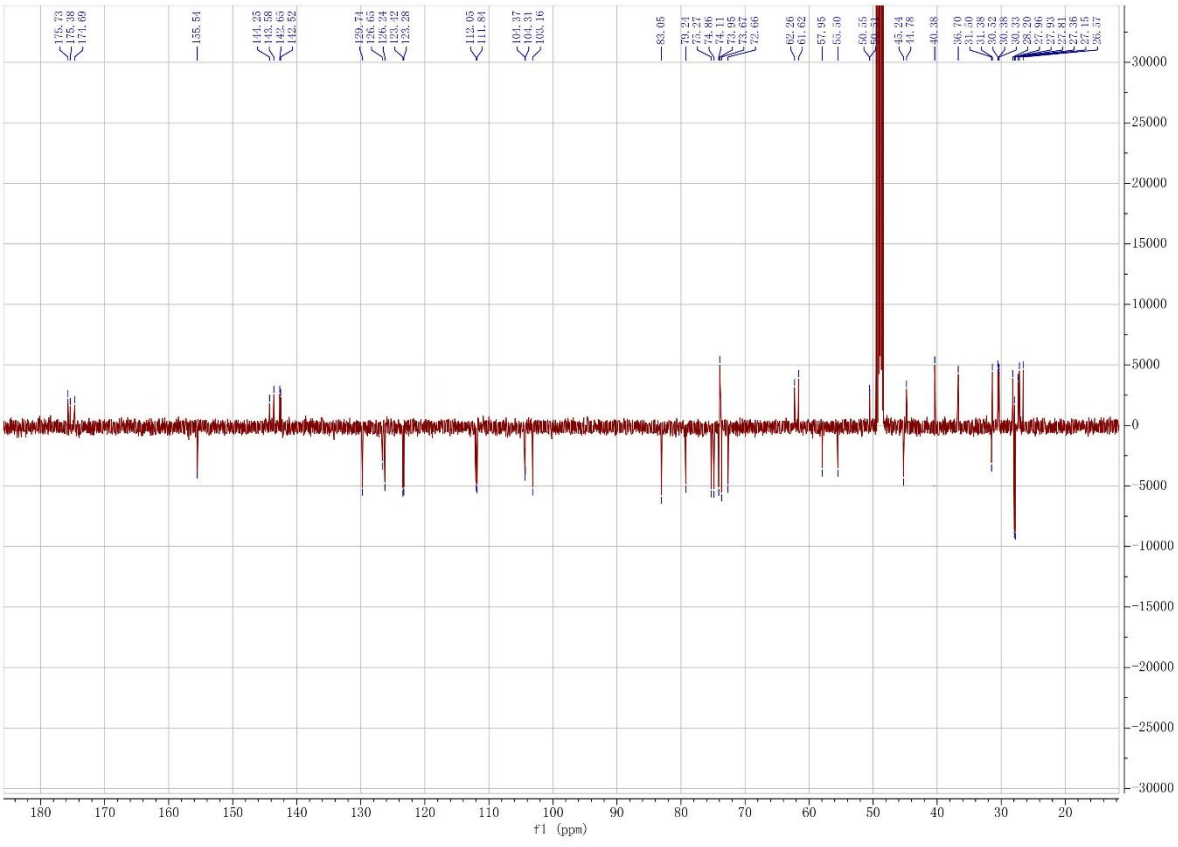

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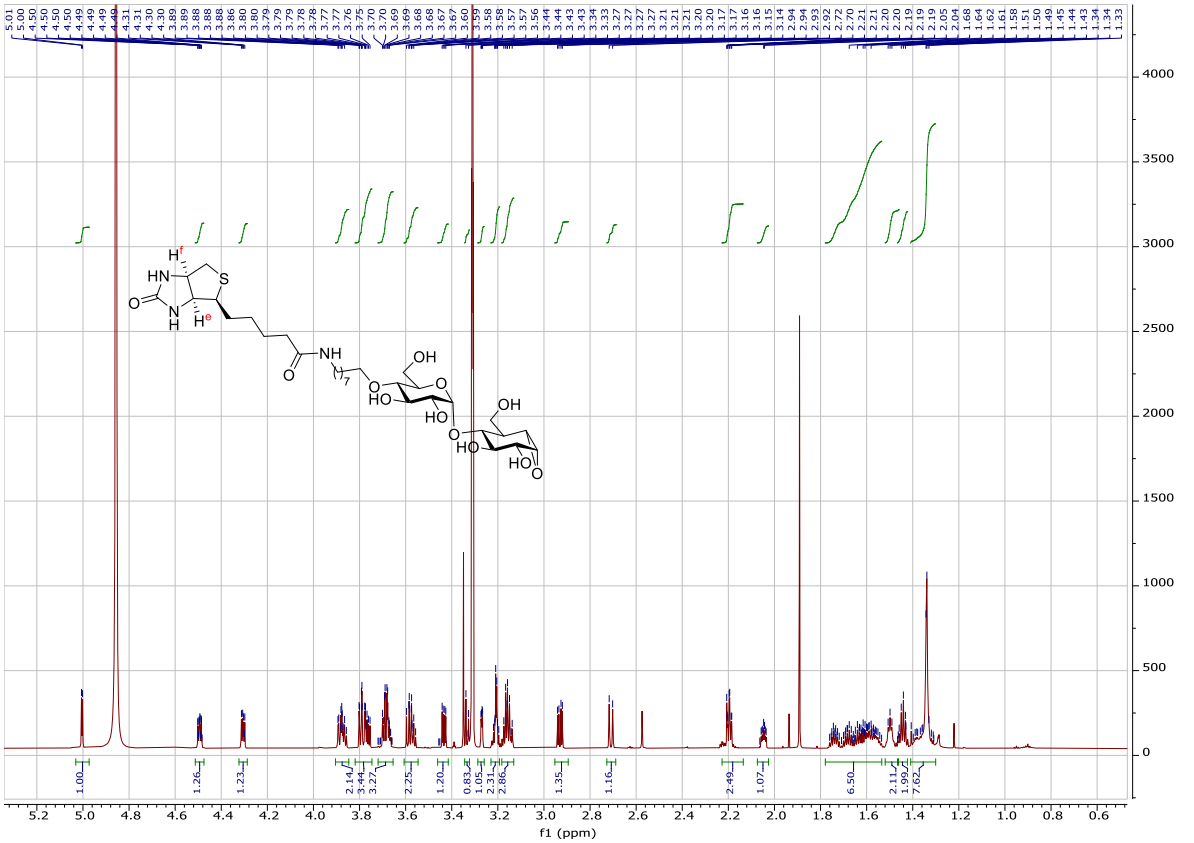
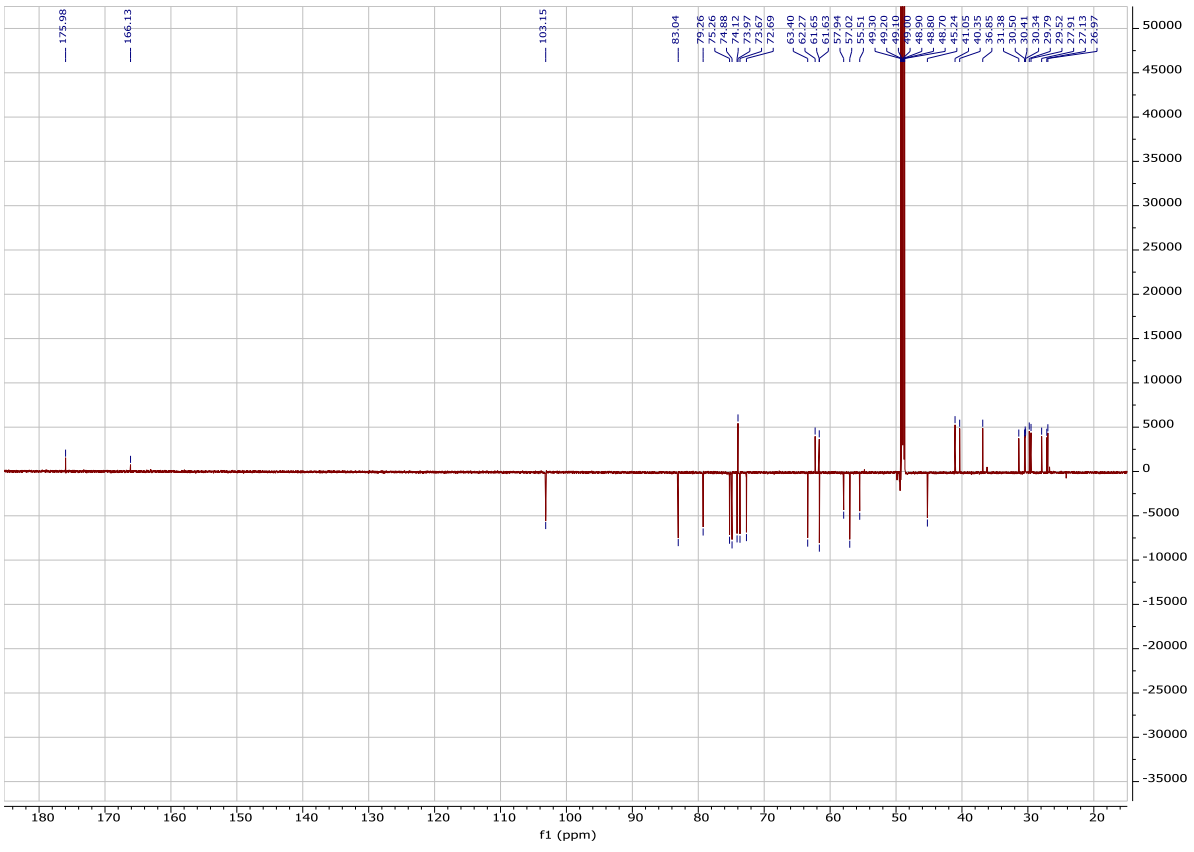
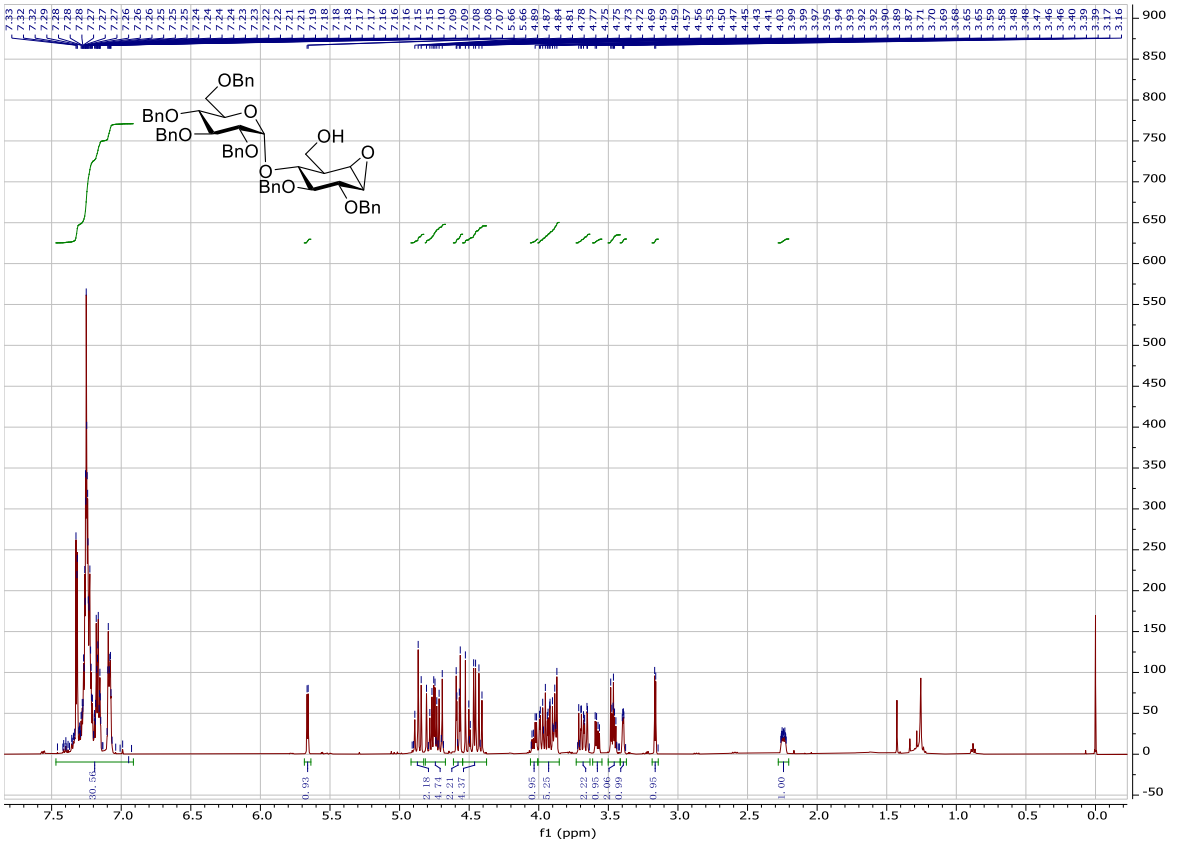
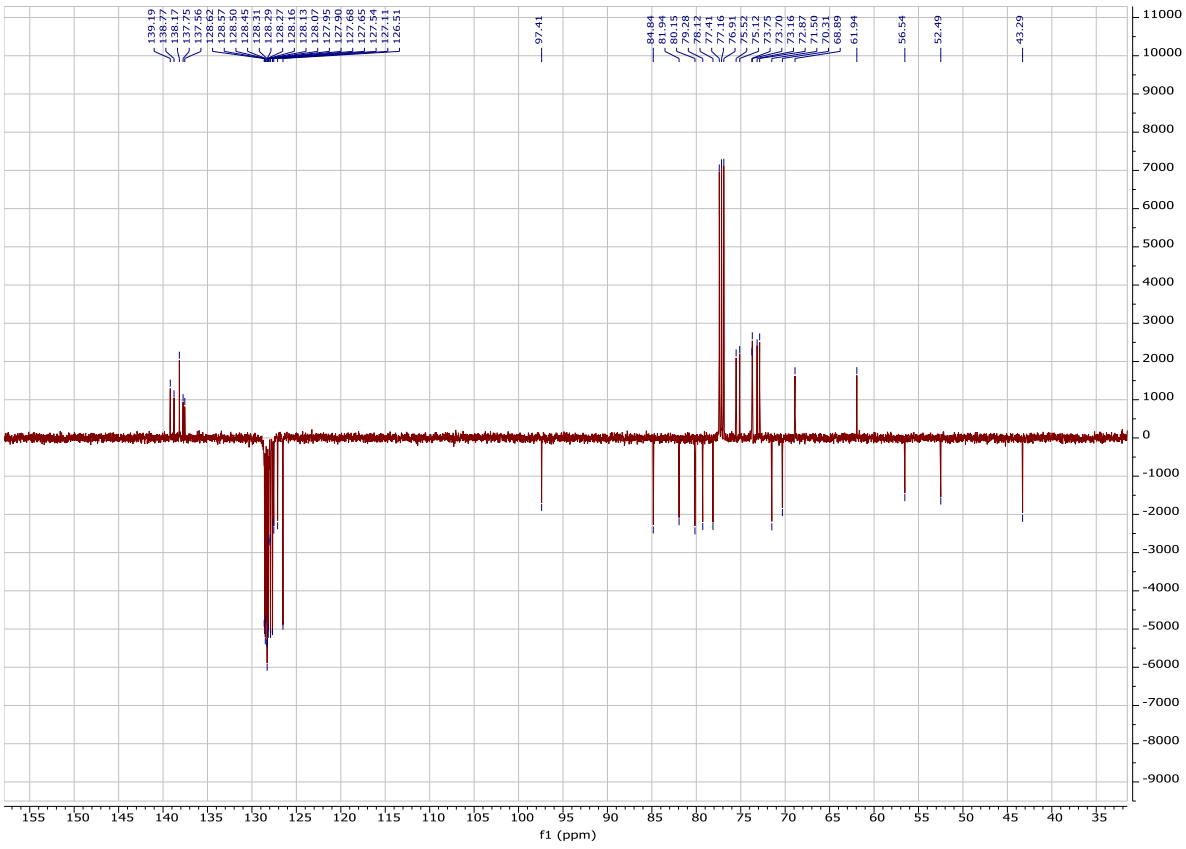




^{1}H and ^{13}C of **12a** in $CDCl_3$





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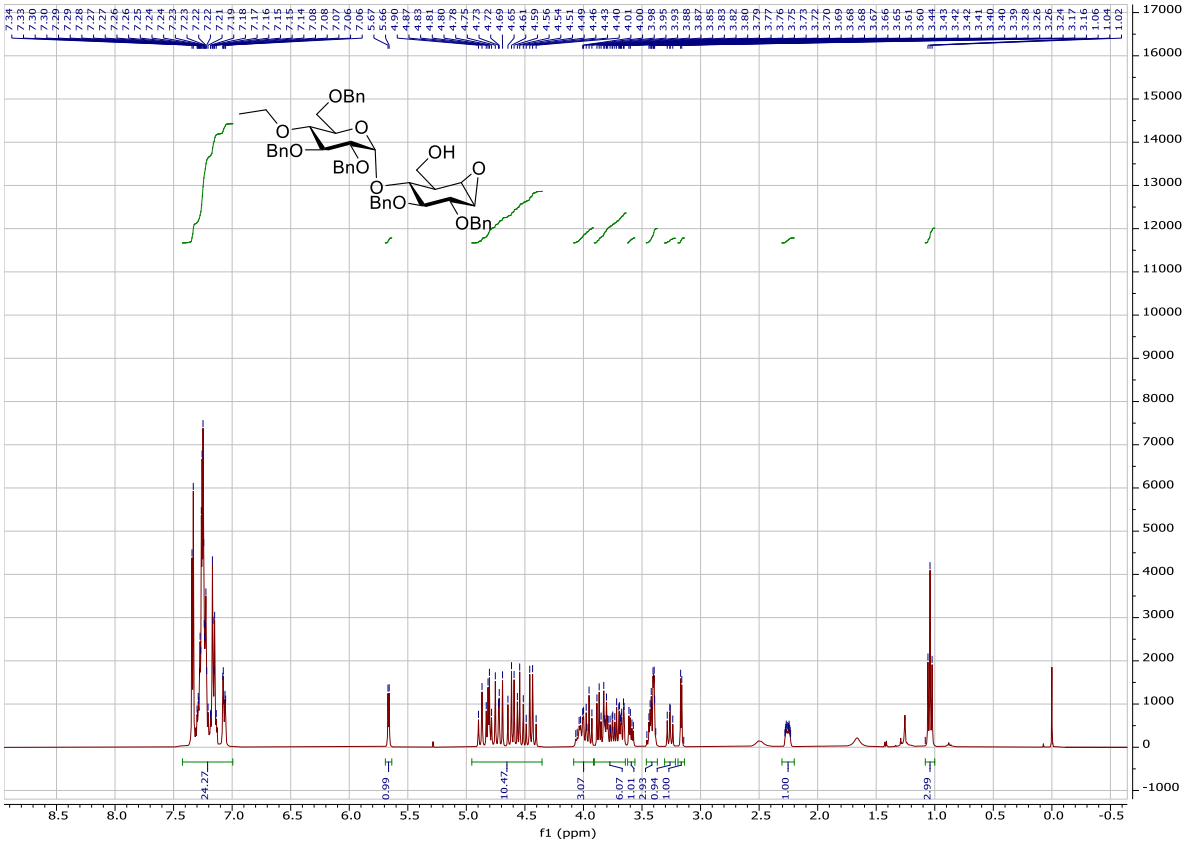
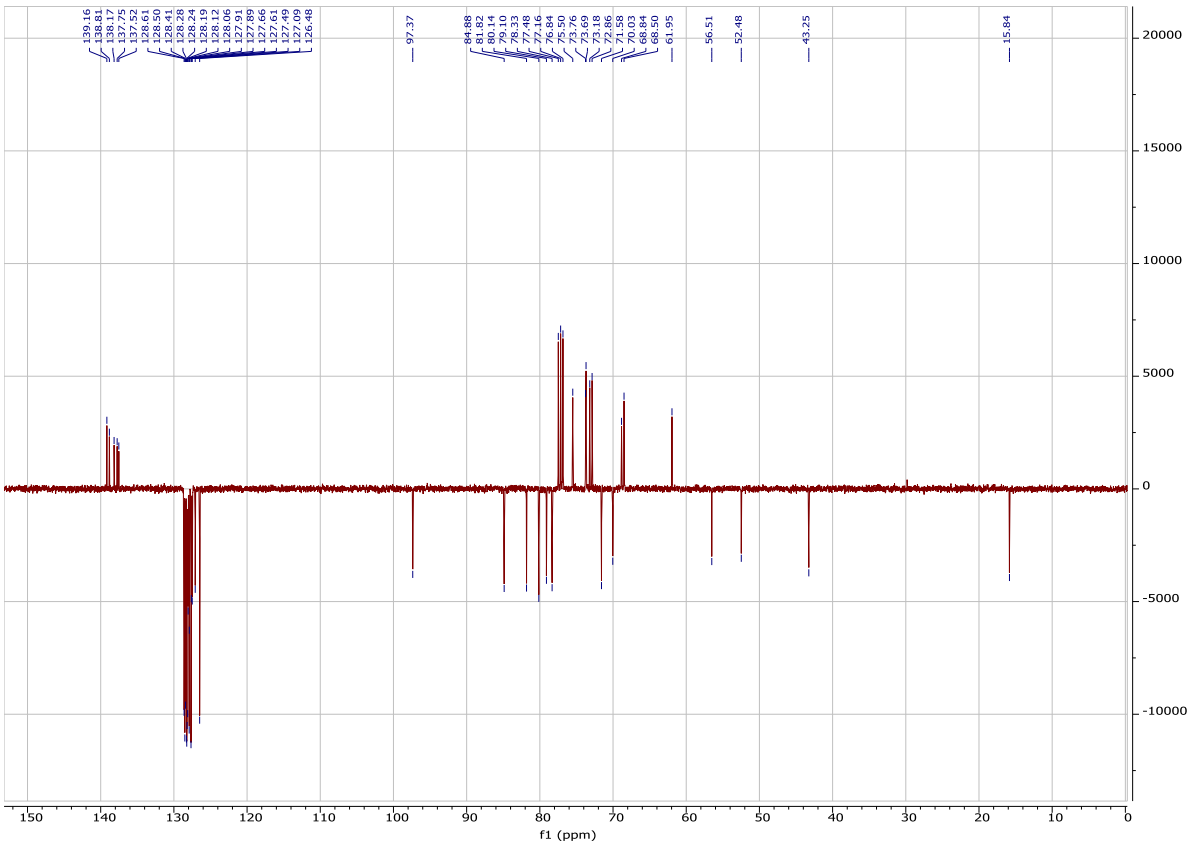
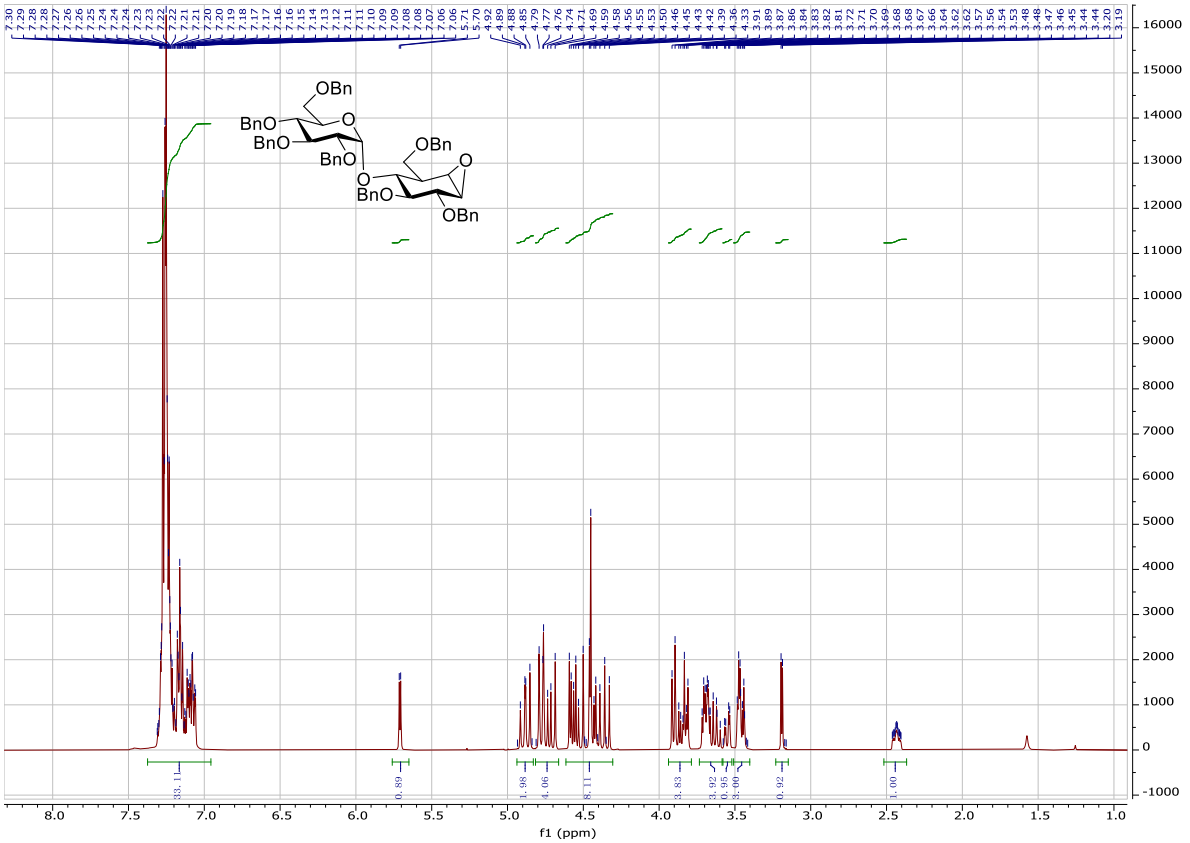
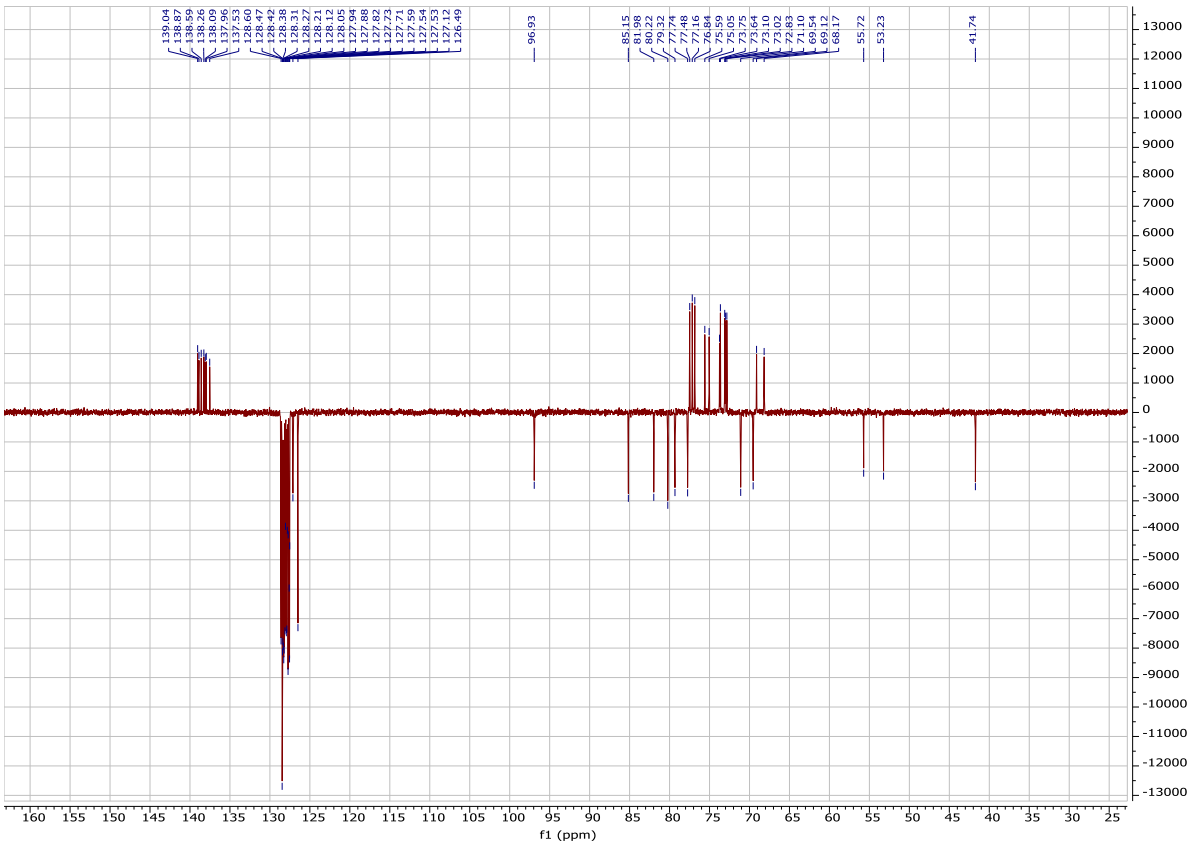


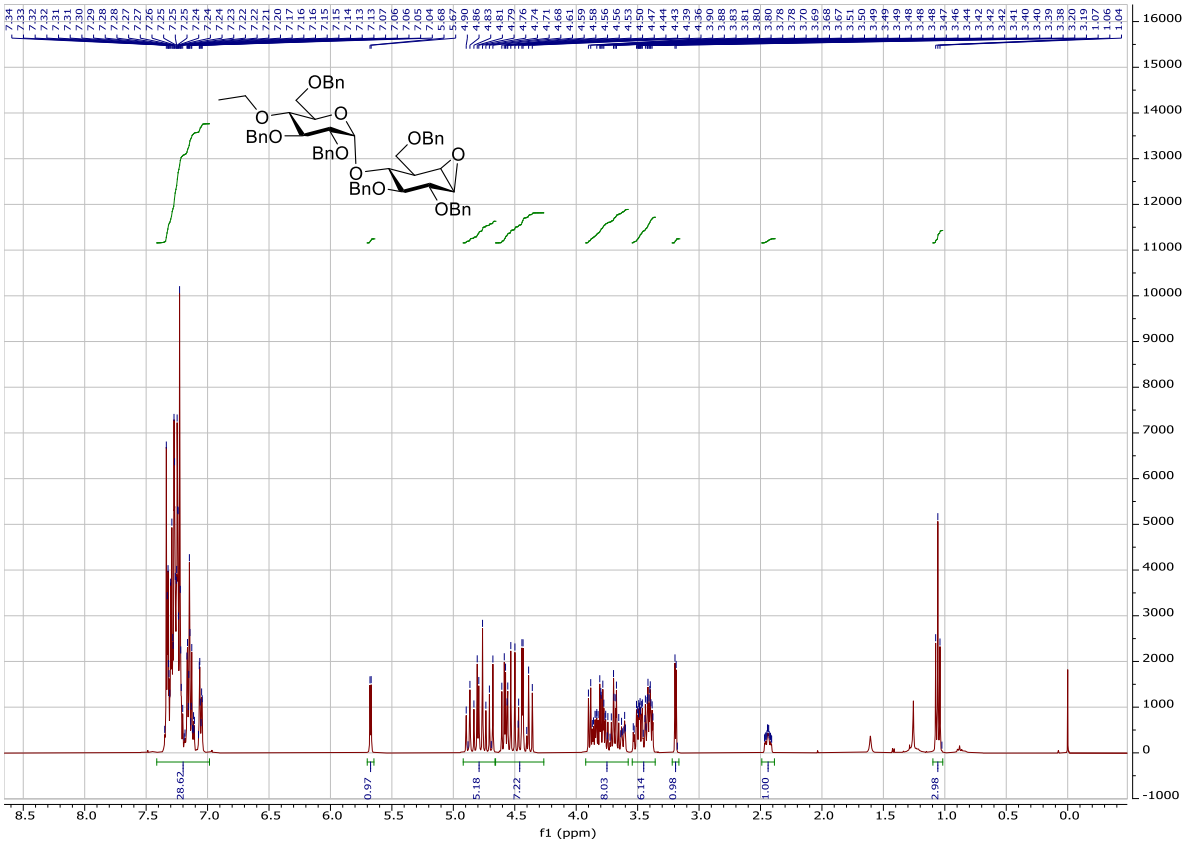


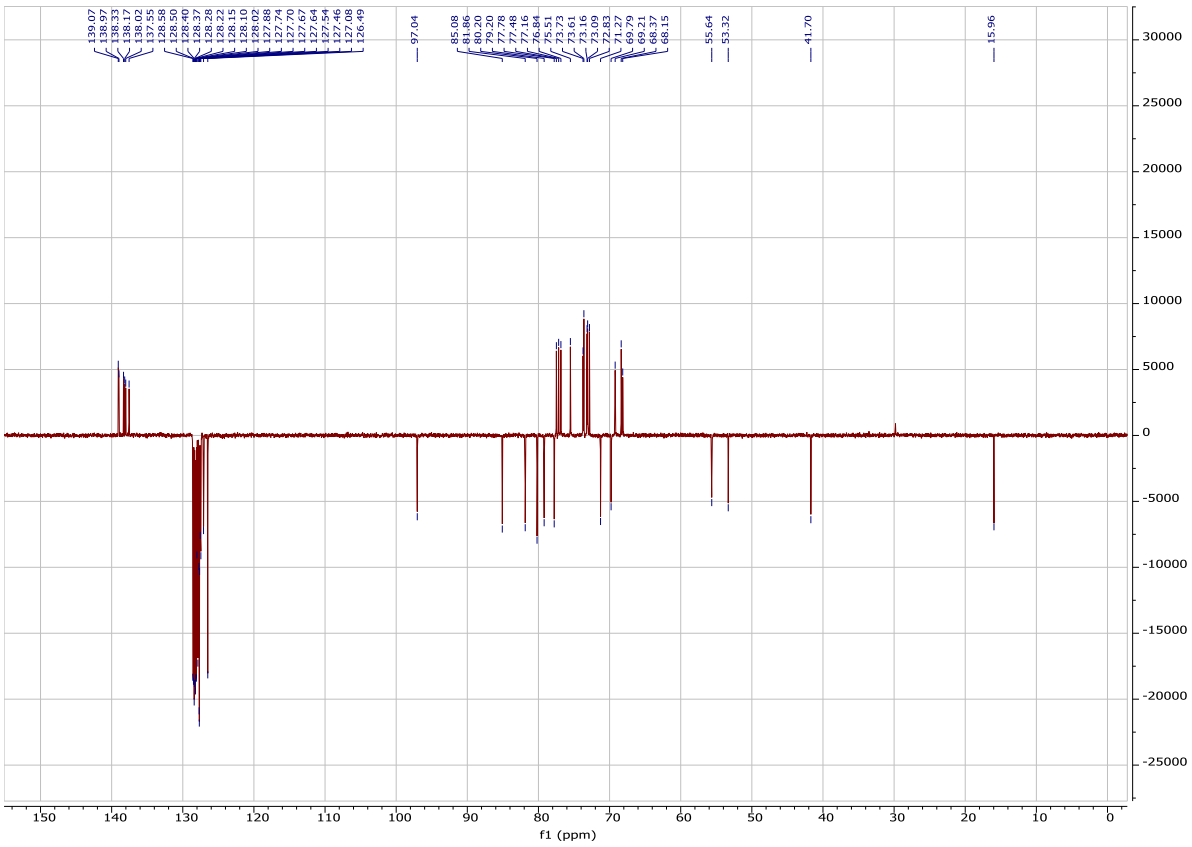
^{1}H and ^{13}C of **13a** in $CDCl_3$



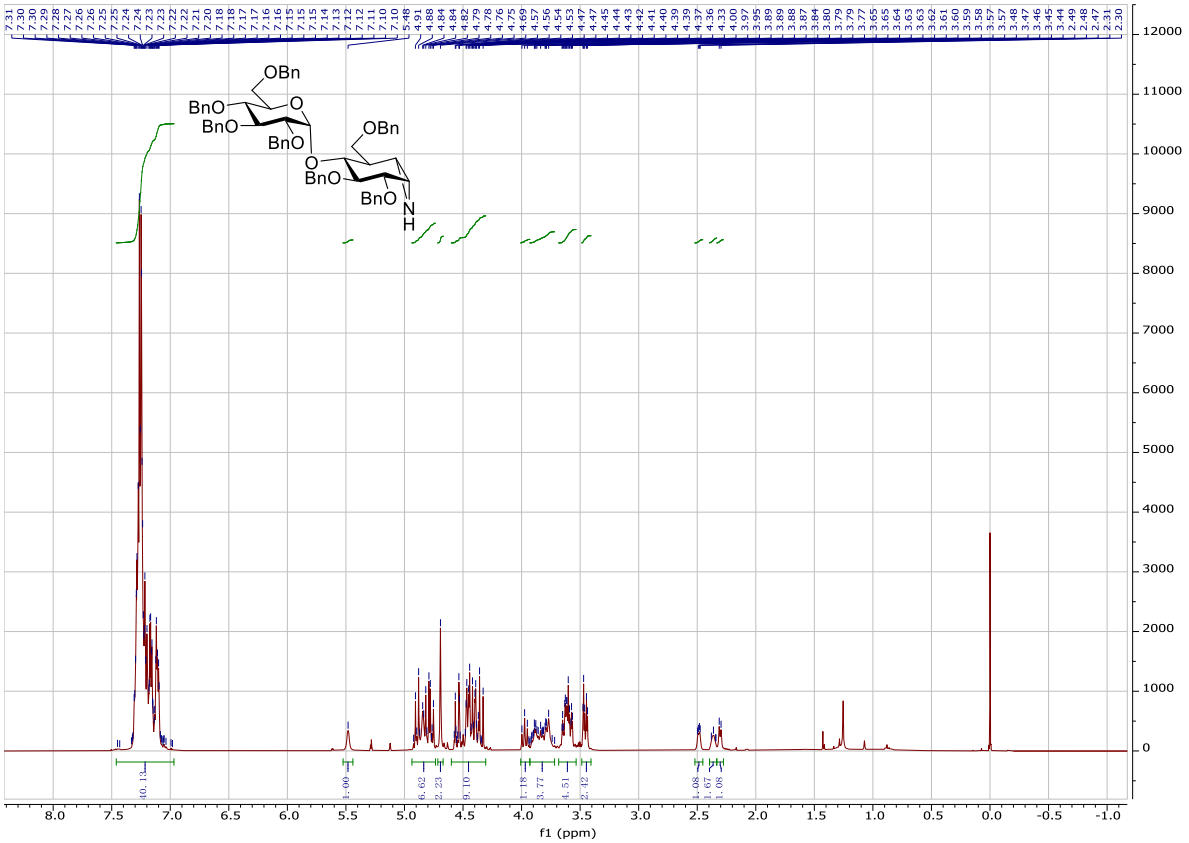


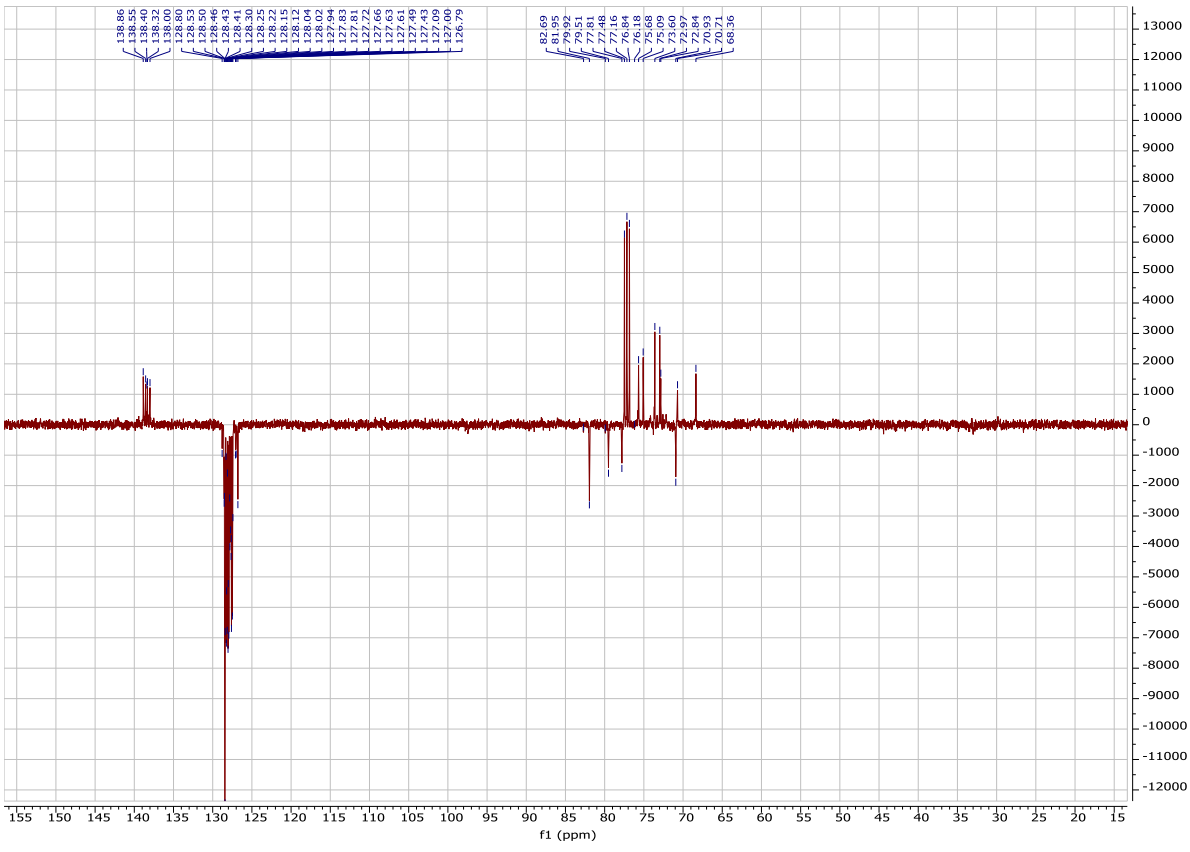
^{1}H and ^{13}C of **13b** in $CDCl_3$



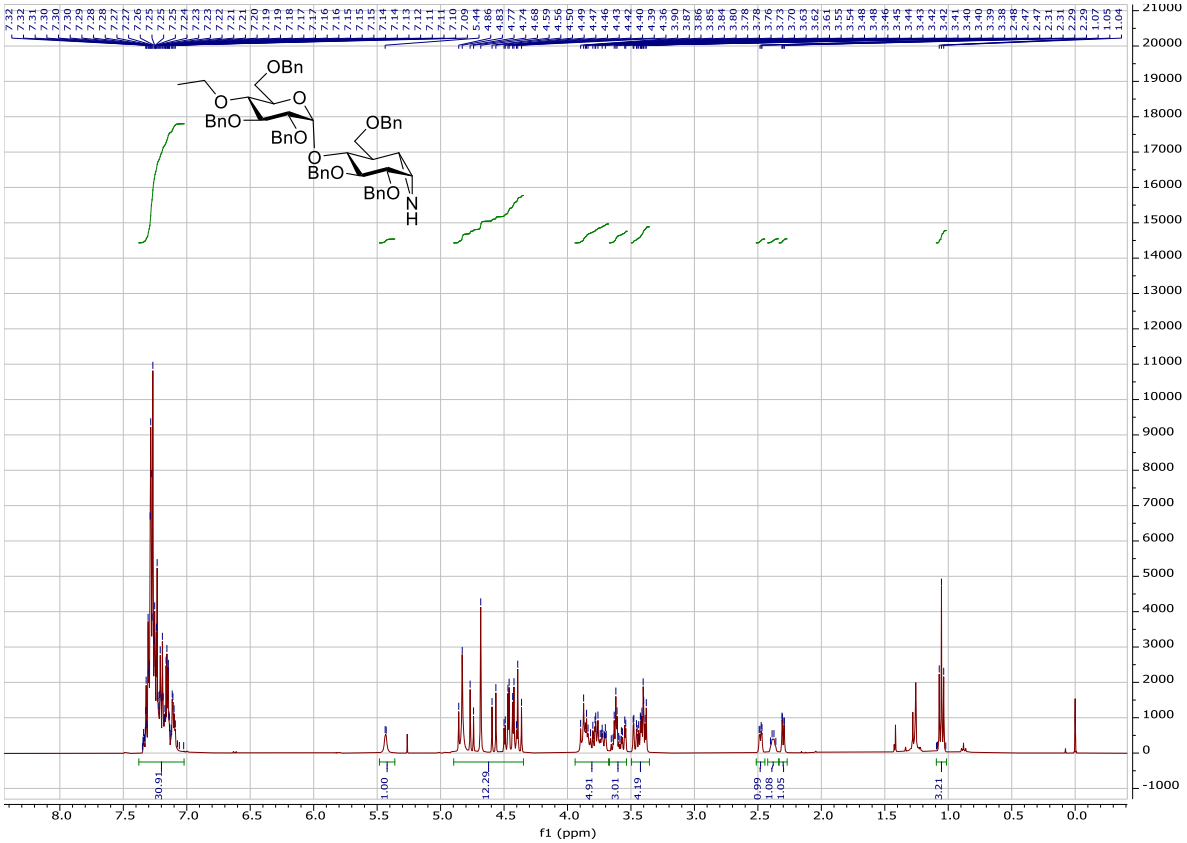


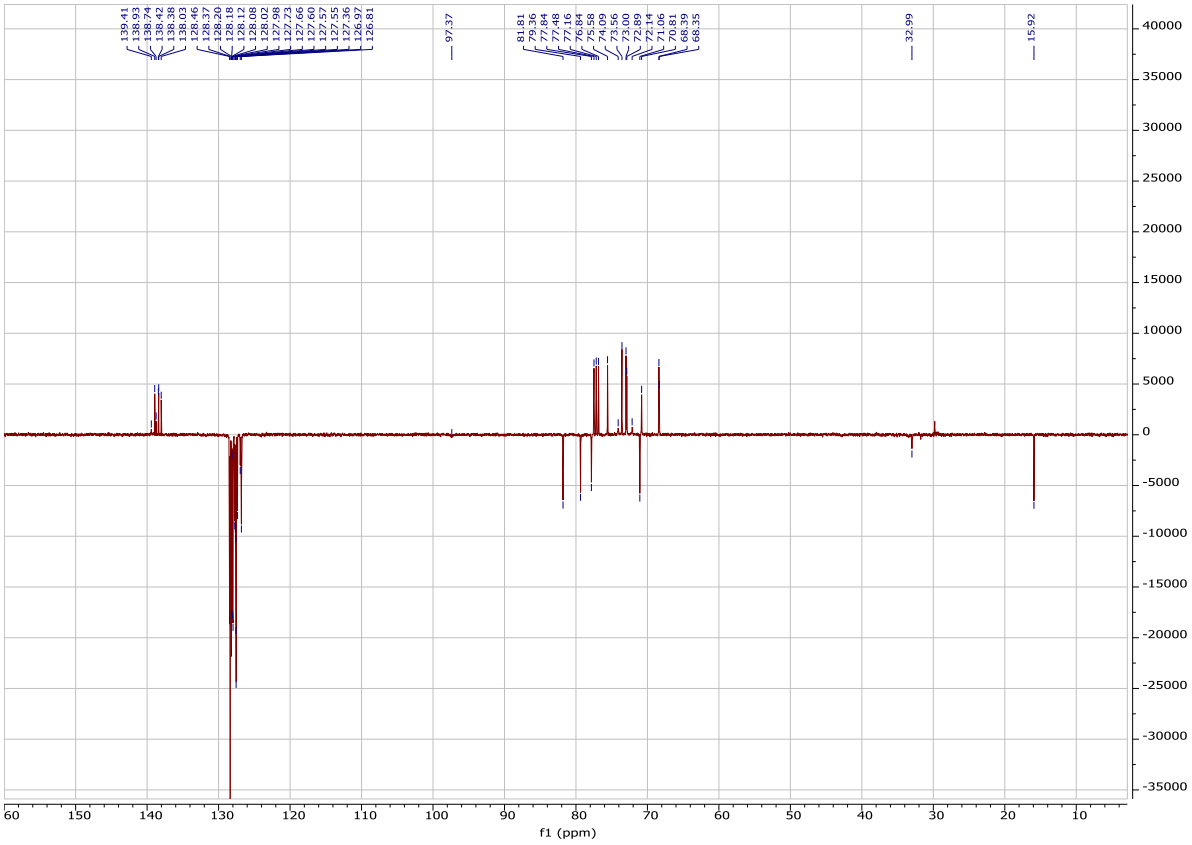
^{1}H and ^{13}C of **14a** in $CDCl_3$



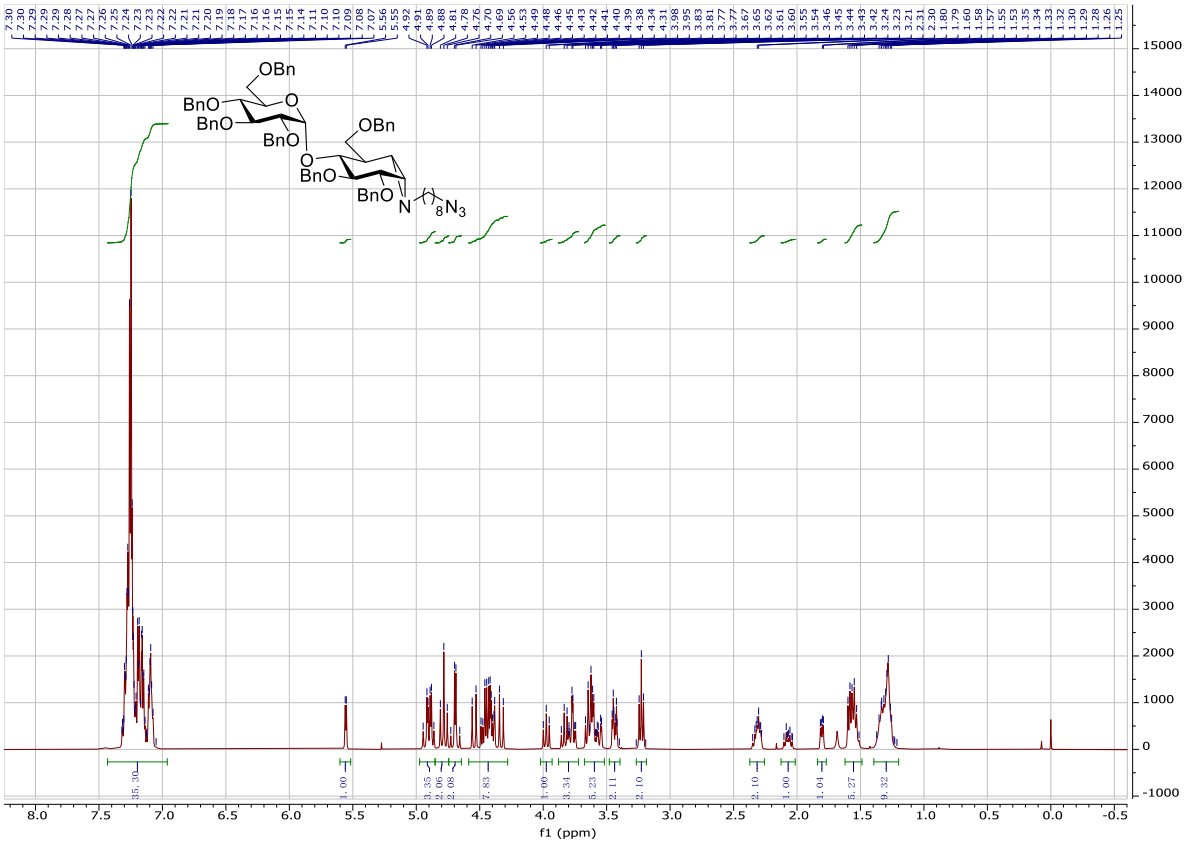


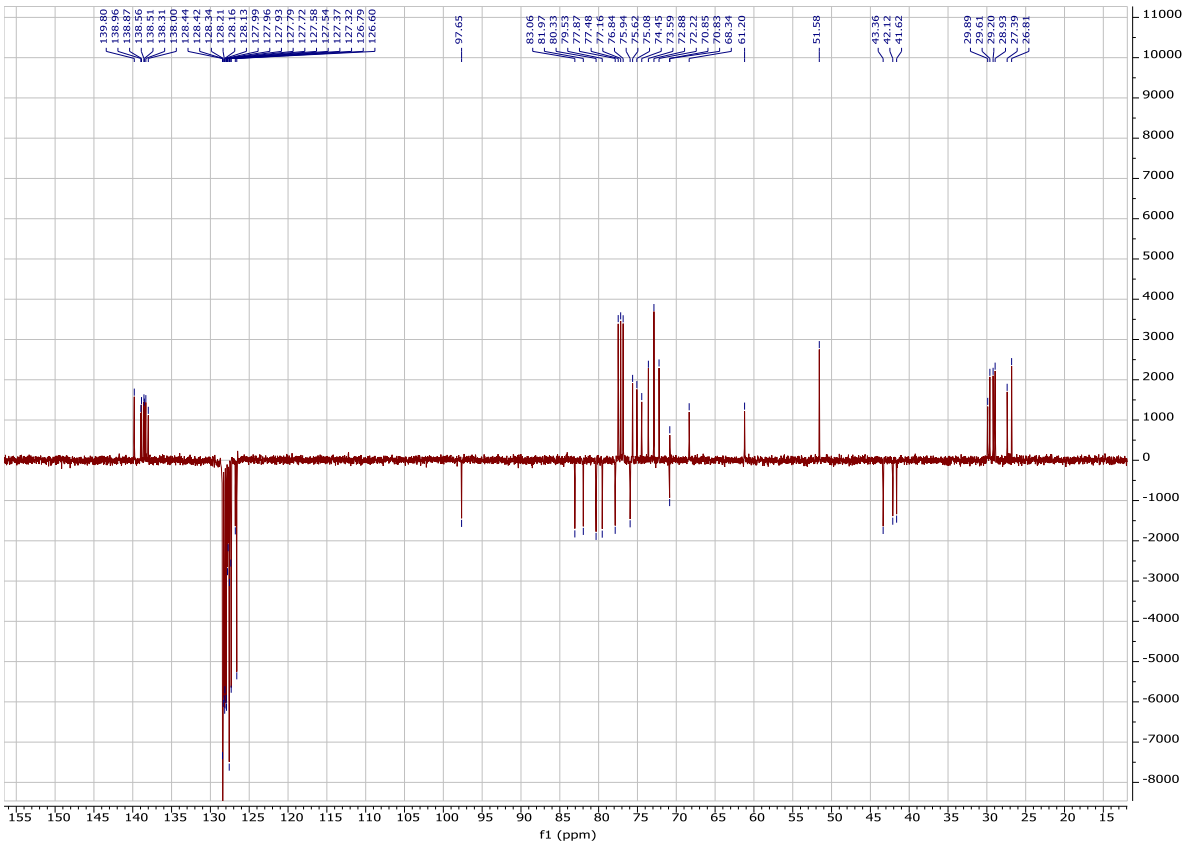
^{1}H and ^{13}C of **14b** in $CDCl_3$



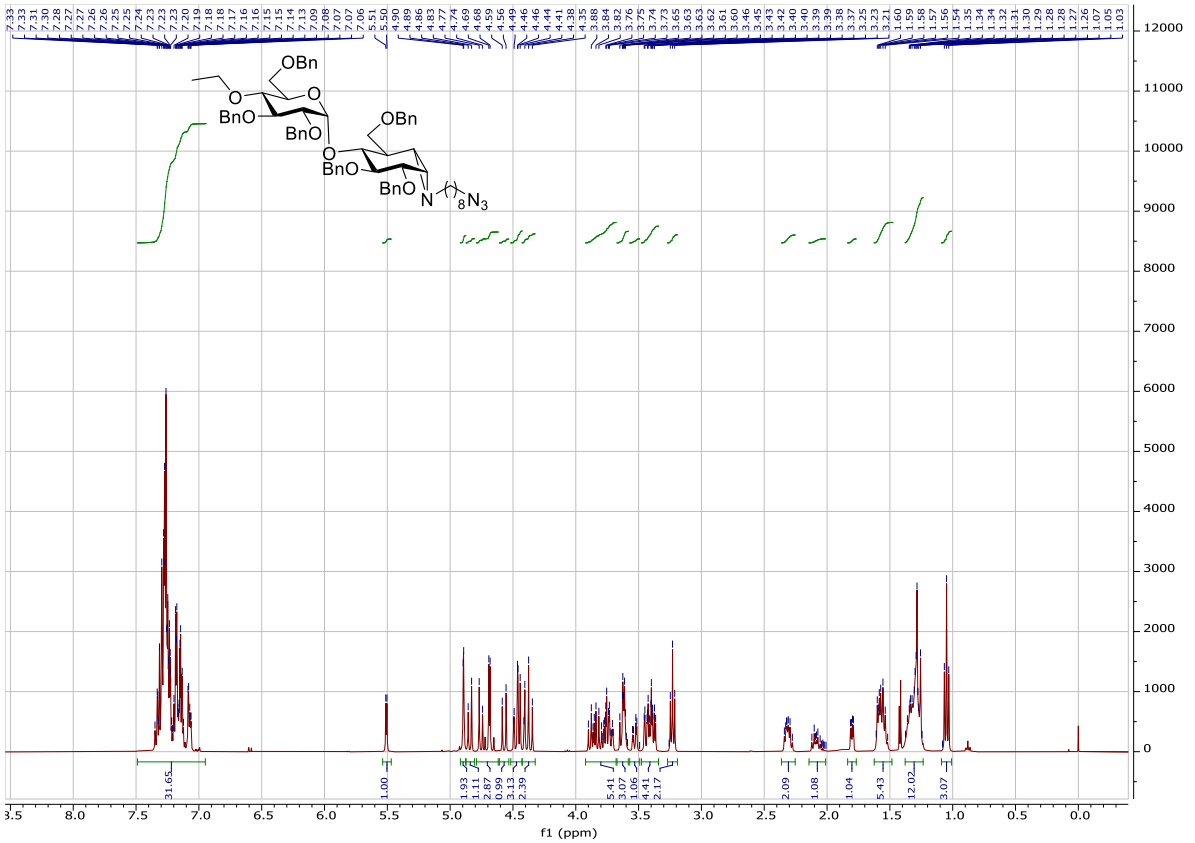


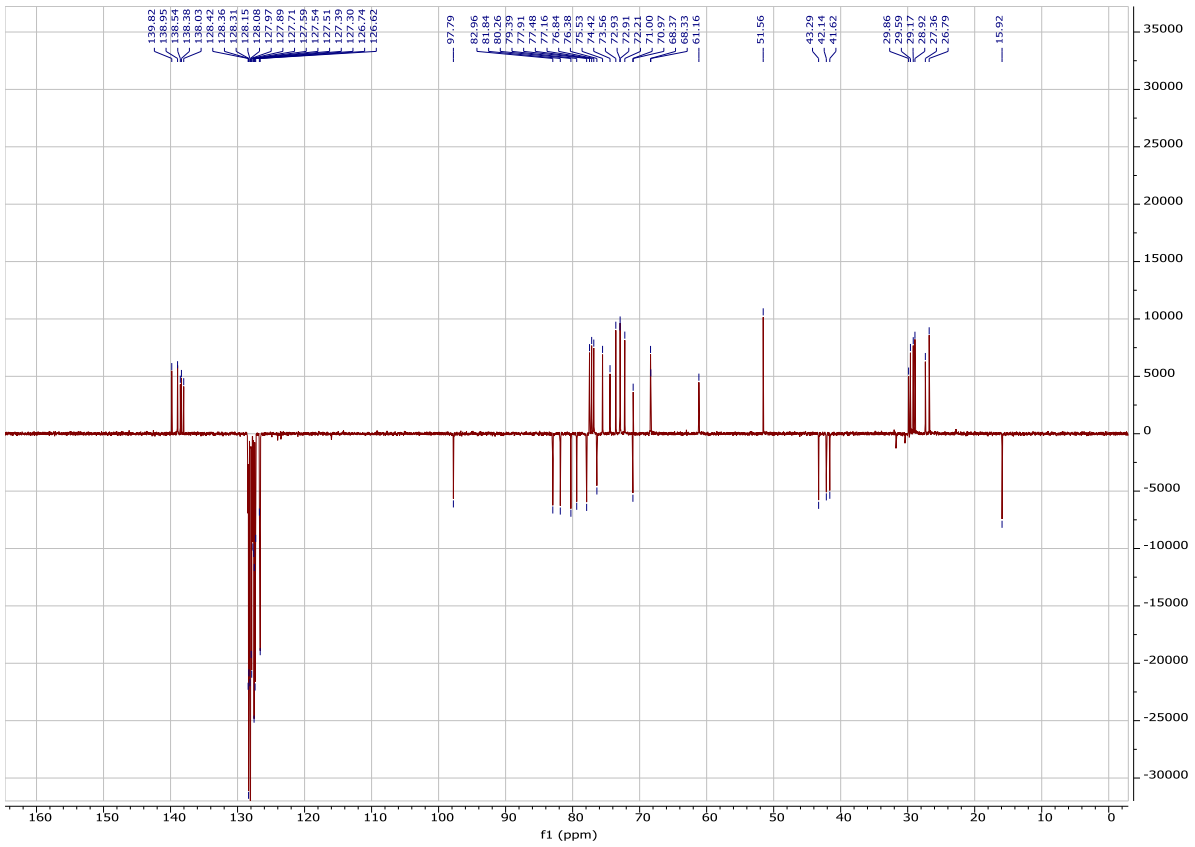
^{1}H and ^{13}C of **15a** in $CDCl_3$



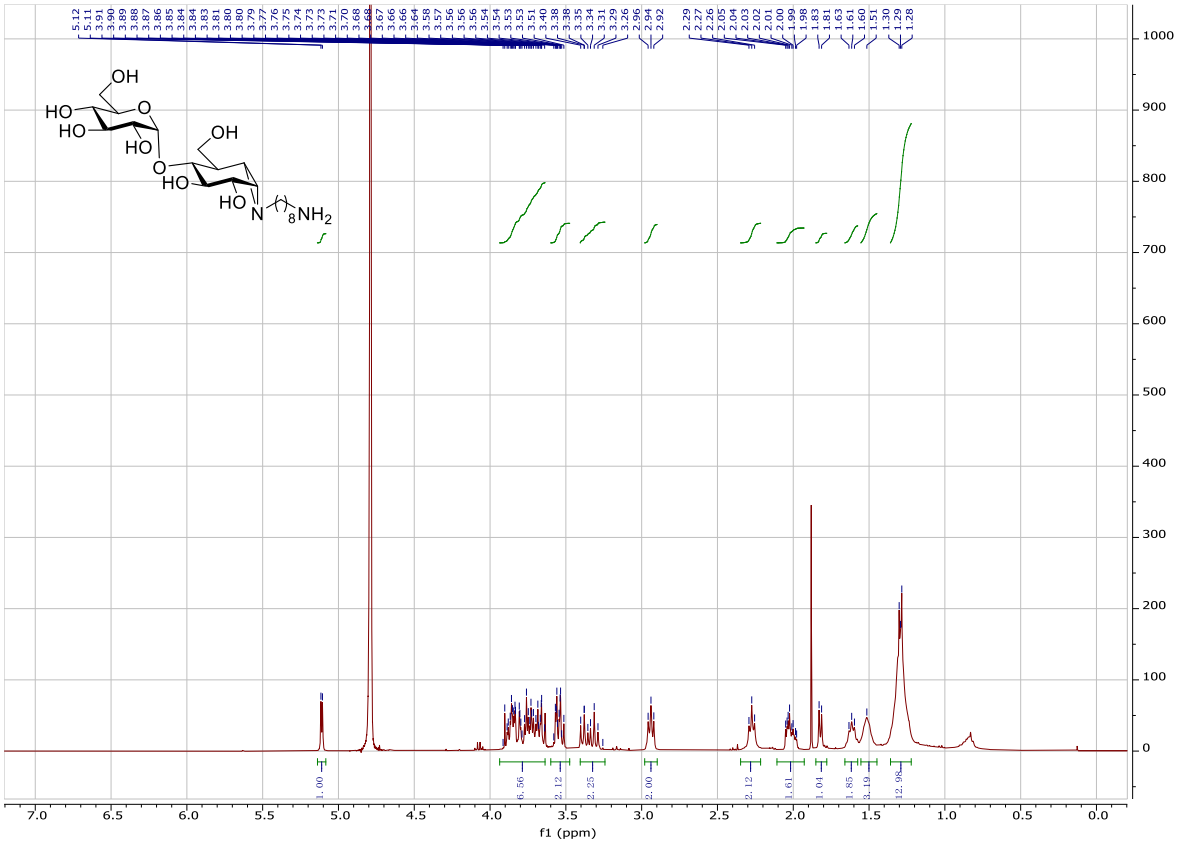


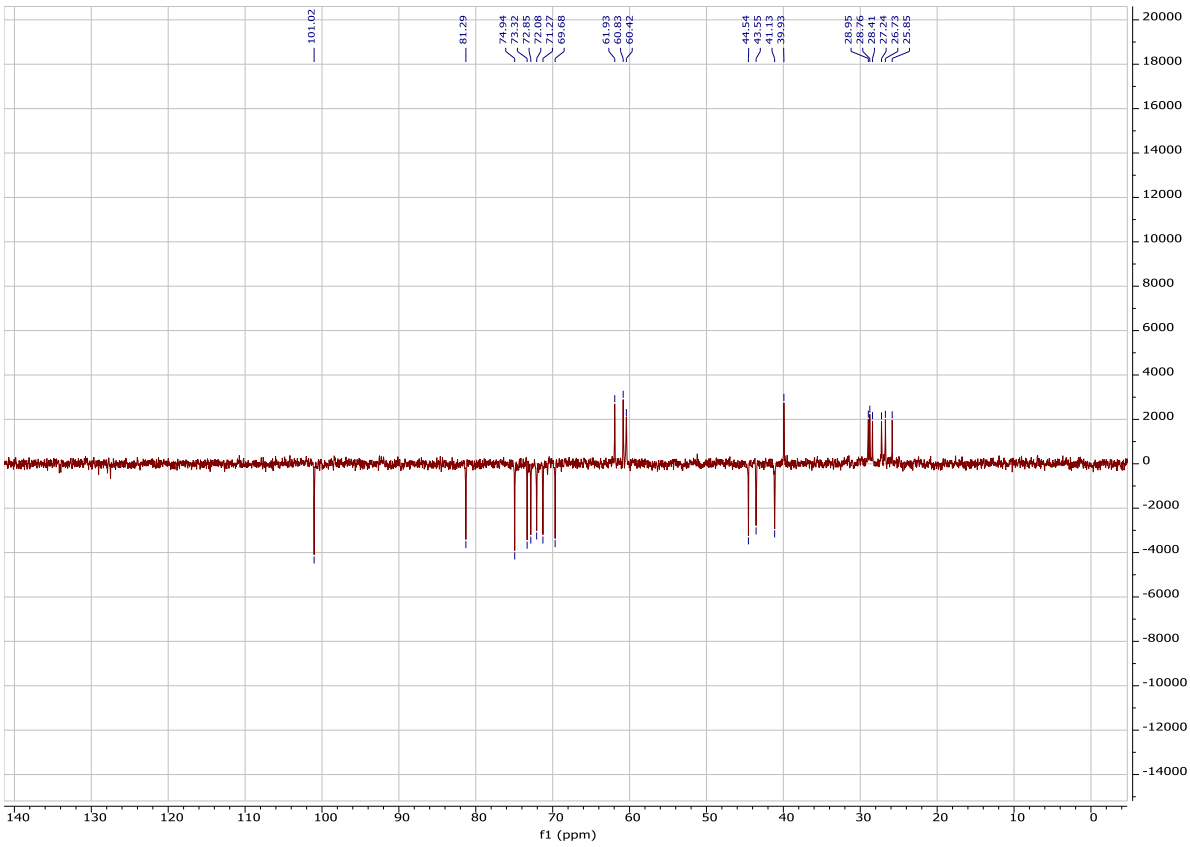
^{1}H and ^{13}C of **15b** in $CDCl_3$



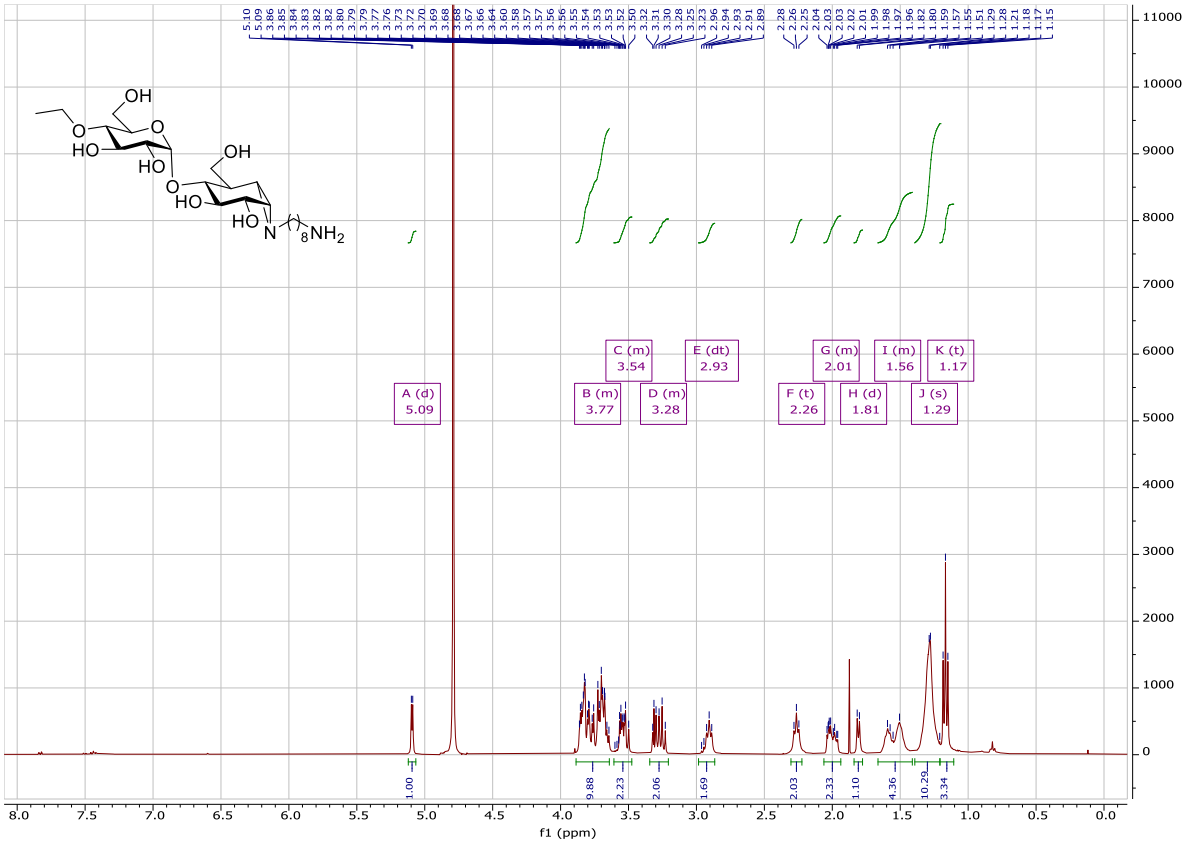


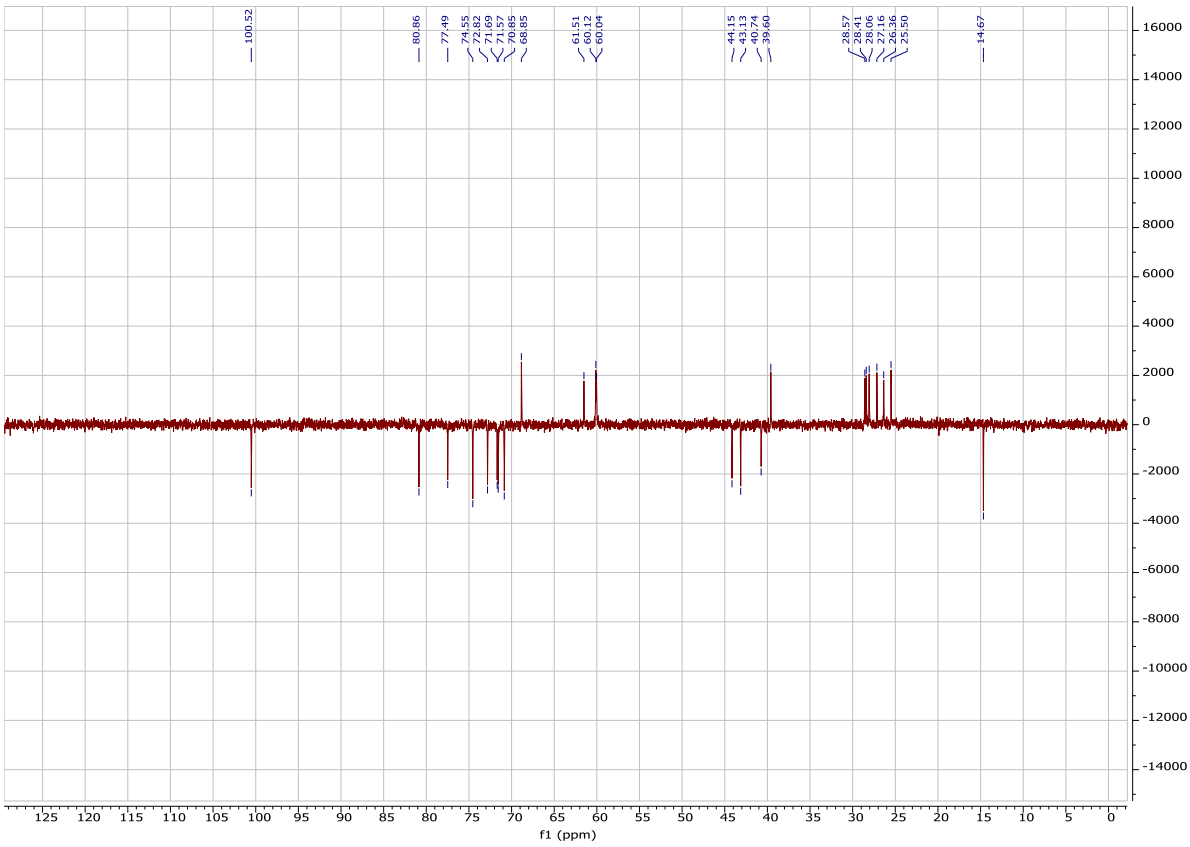
^{1}H and ^{13}C of **2a** in D_2O



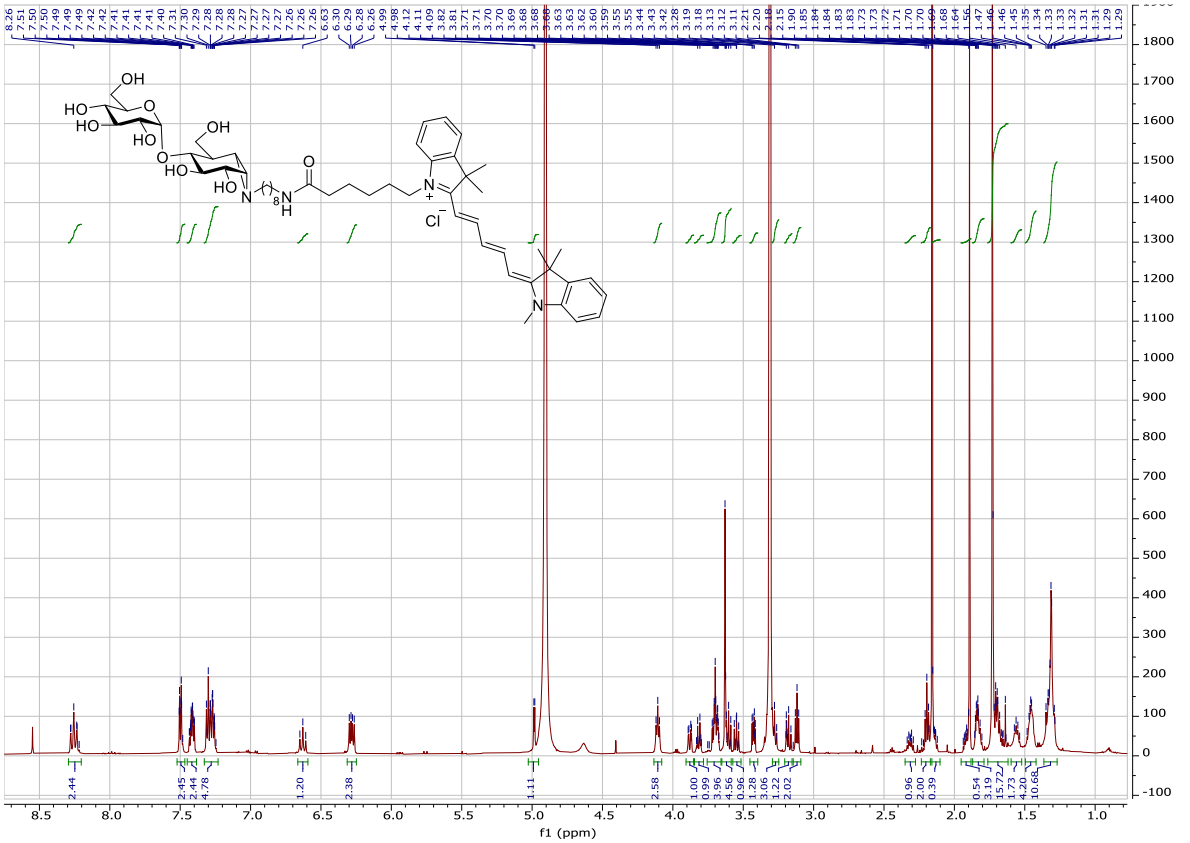


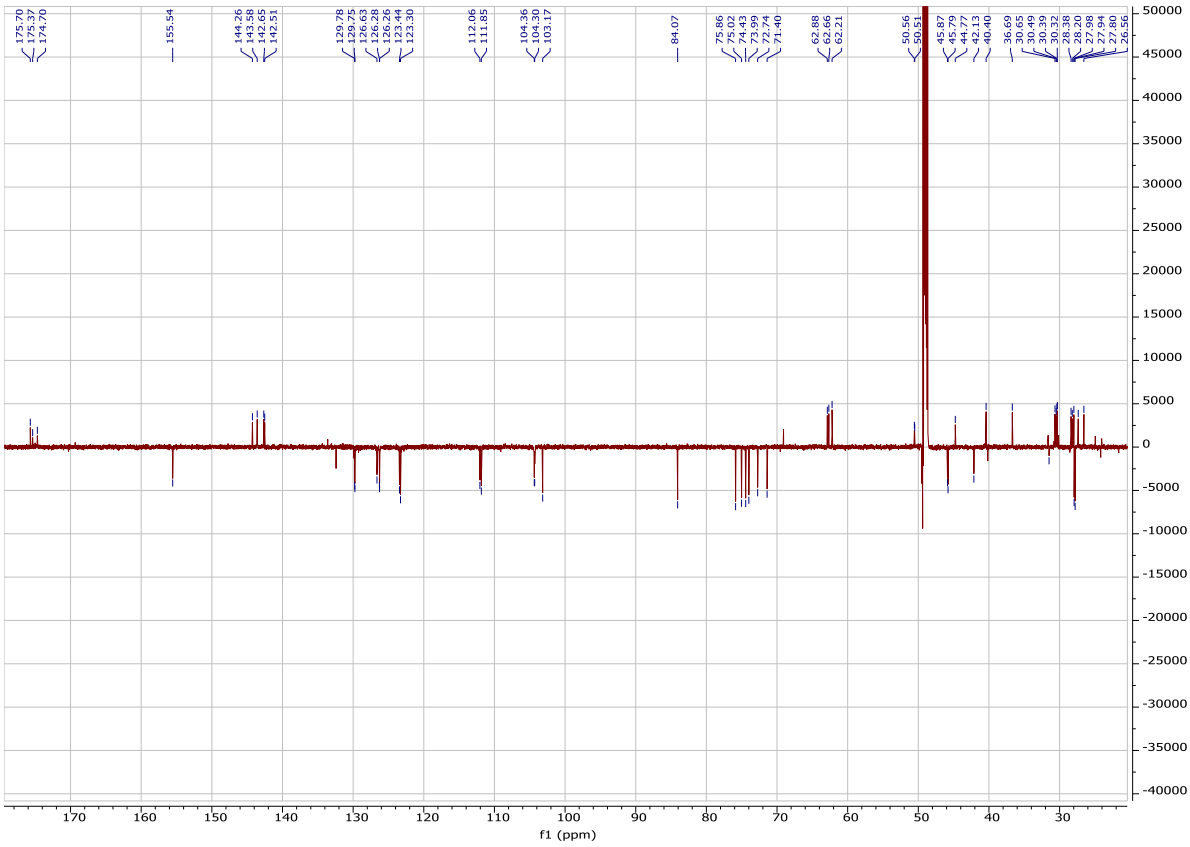
^{1}H and ^{13}C of **16** in D_2O



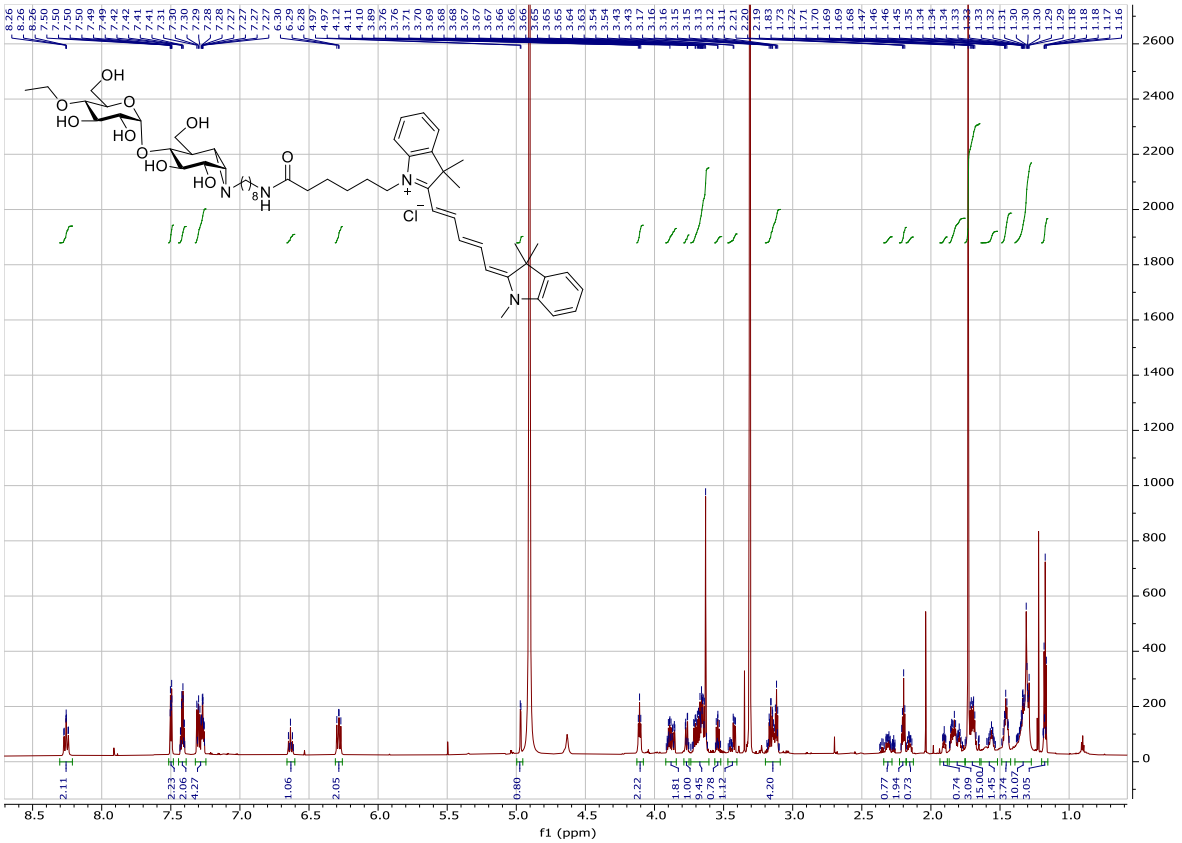


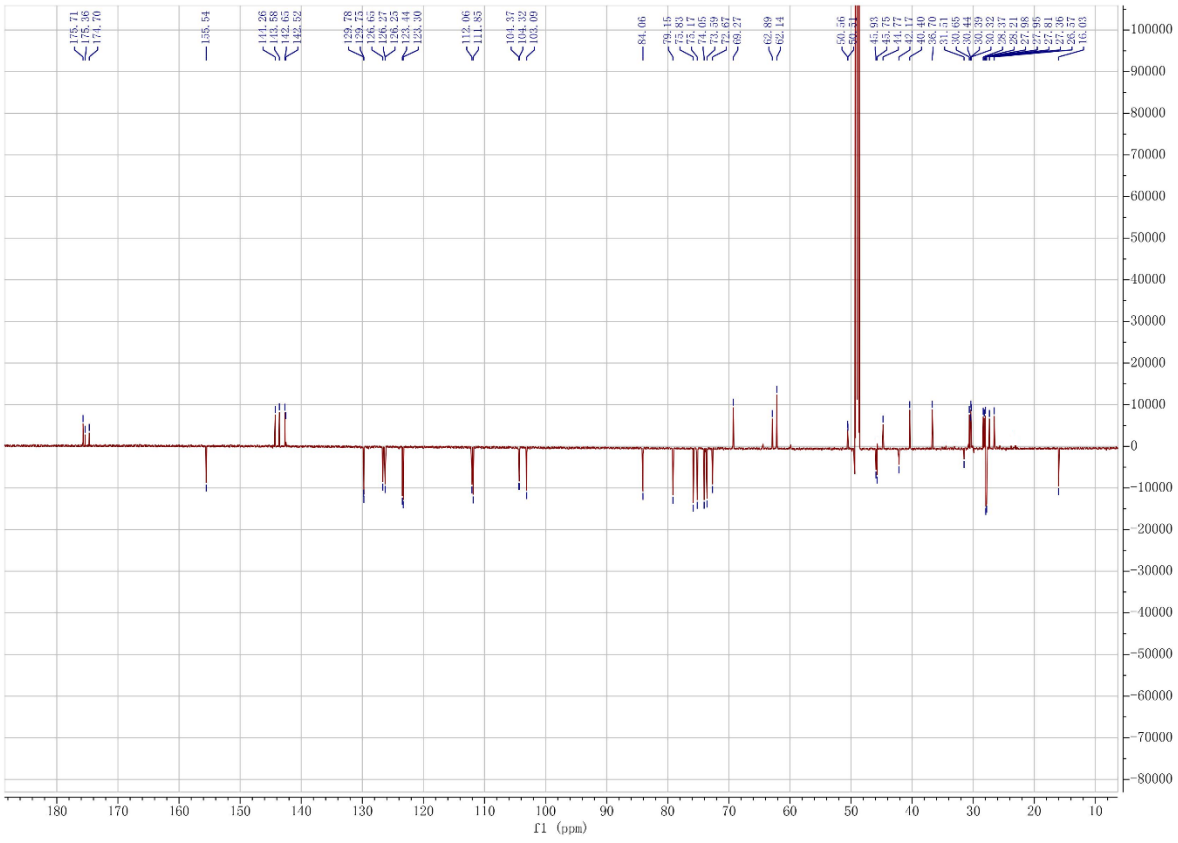
^{1}H and ^{13}C of **2b** in MeOD



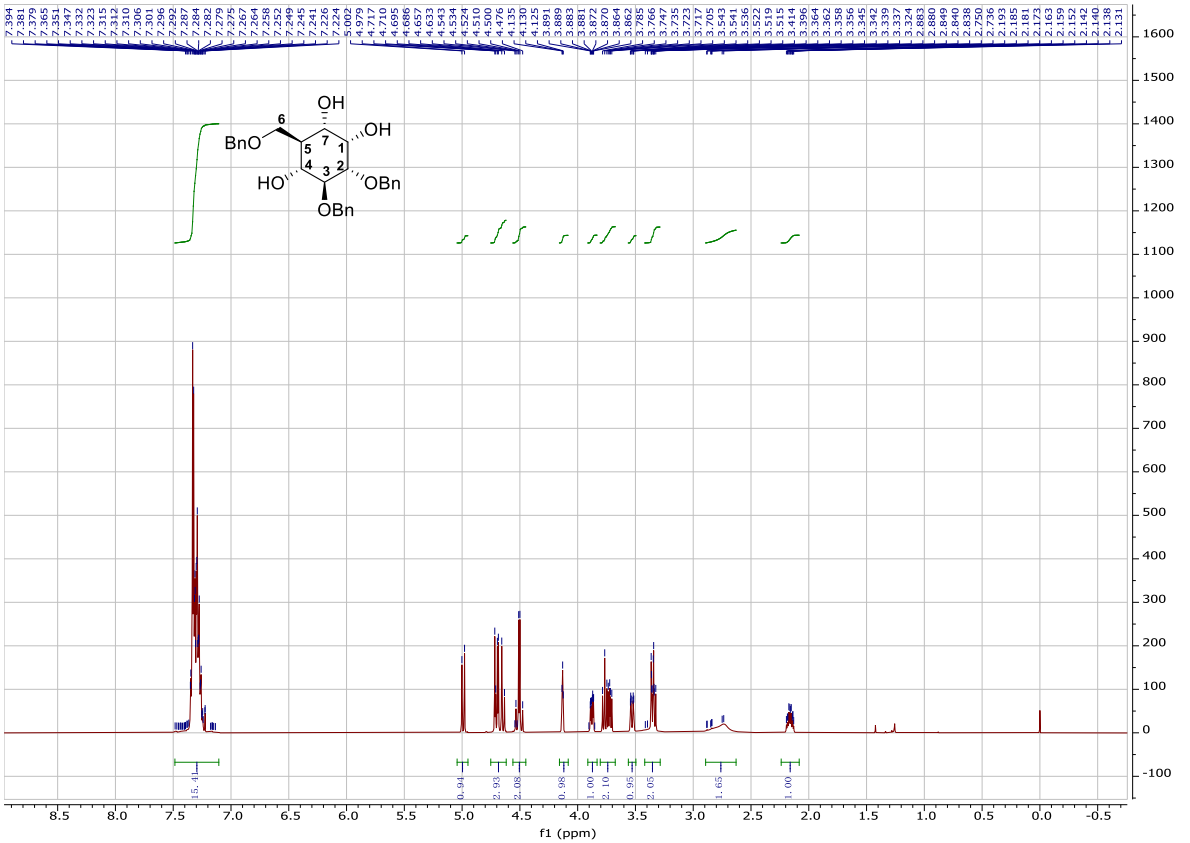


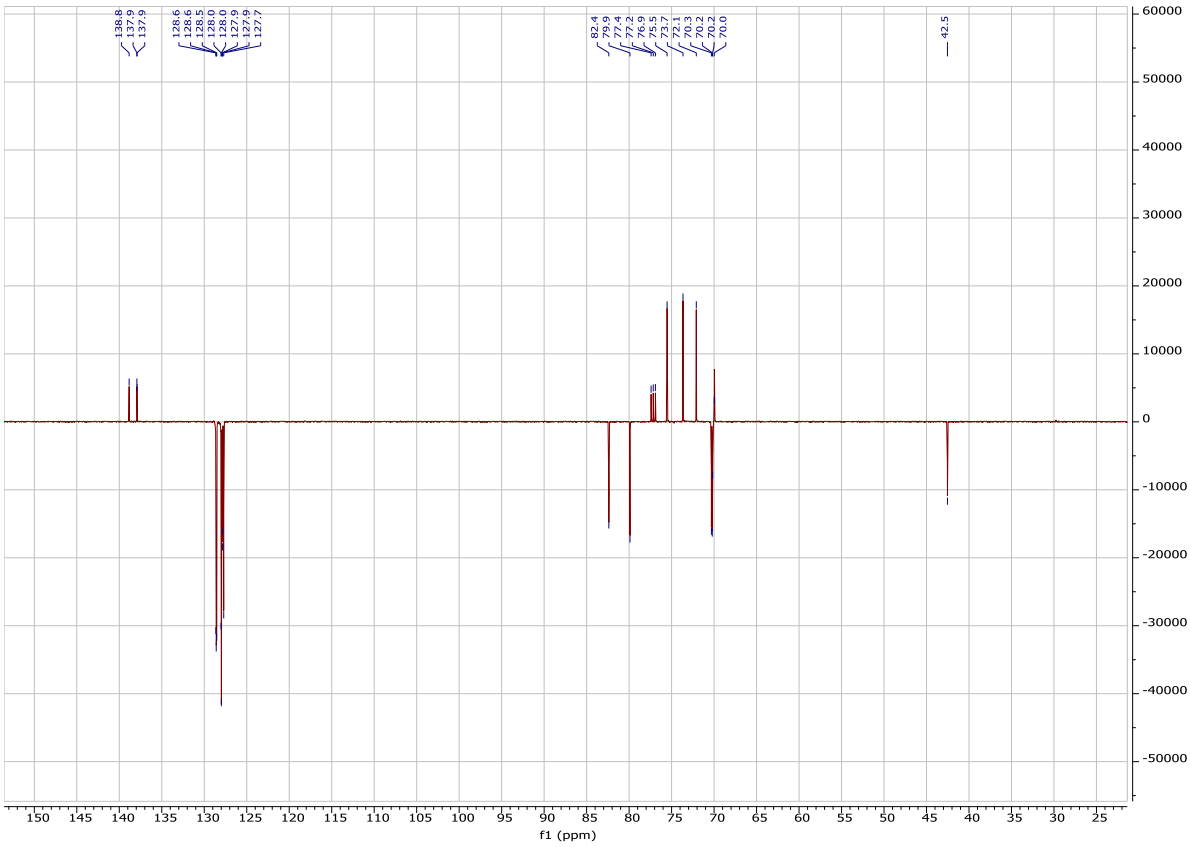
^{1}H and ^{13}C of **2c** in MeOD



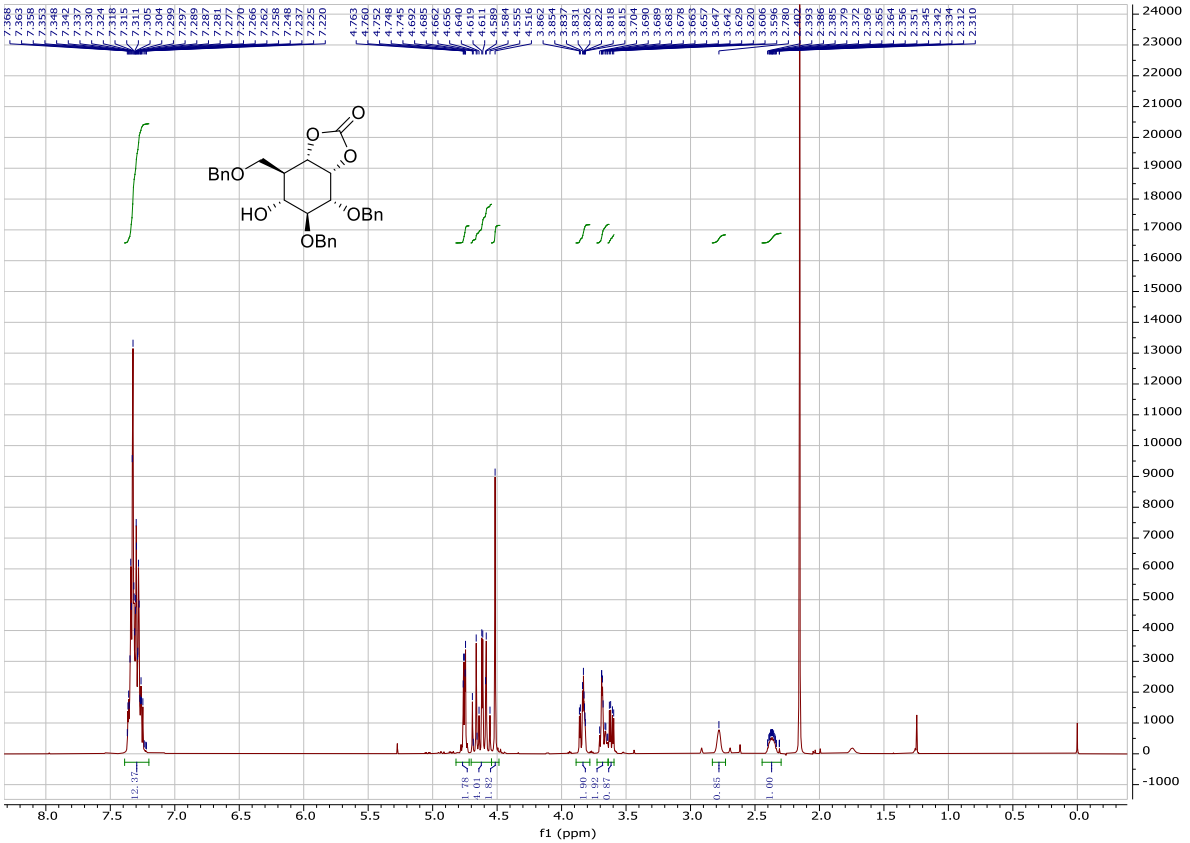


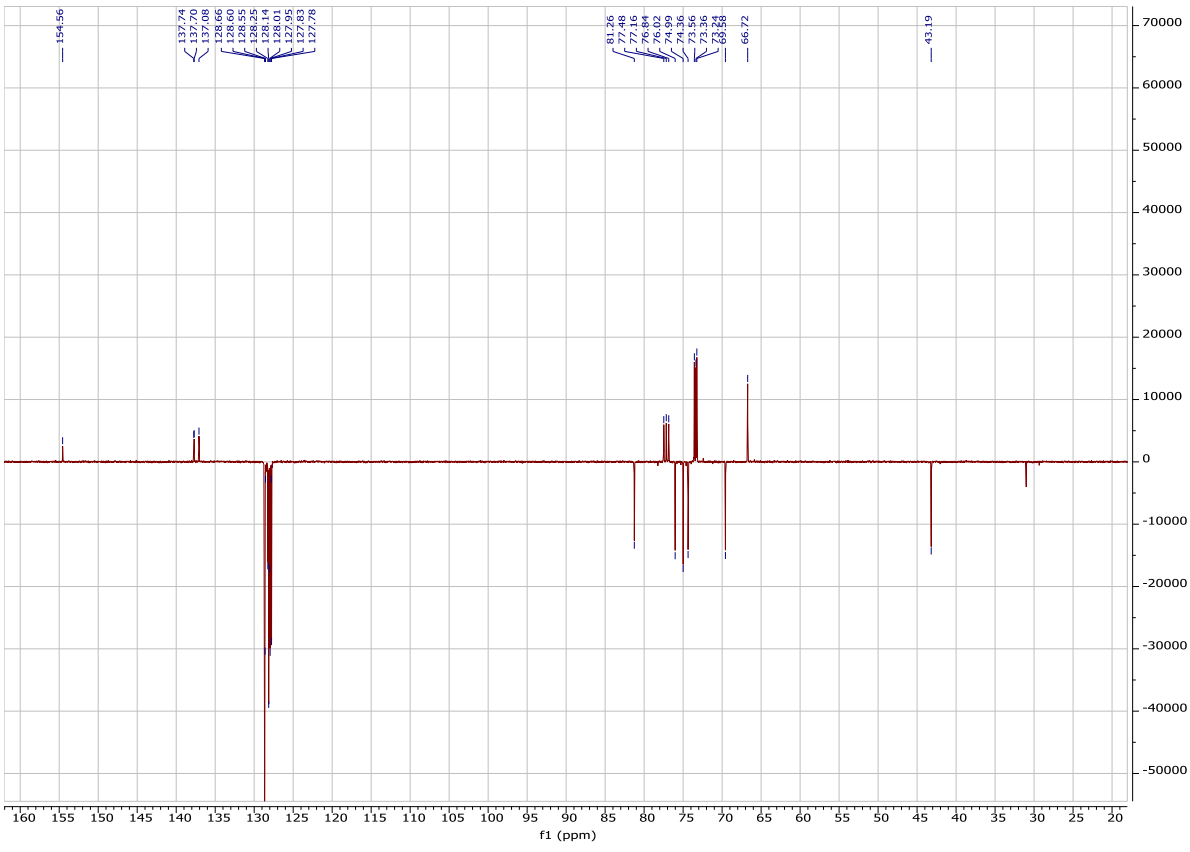
^{1}H and ^{13}C of **S3** in $CDCl_3$



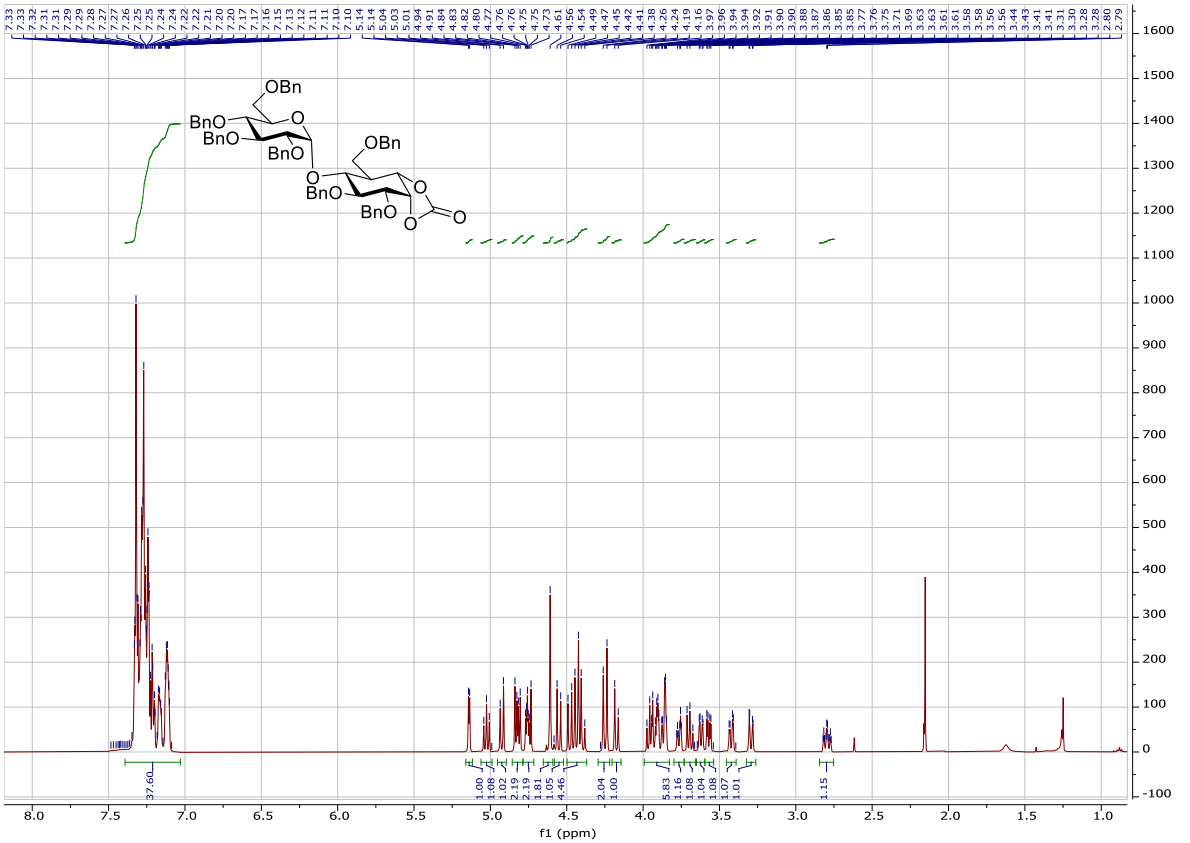


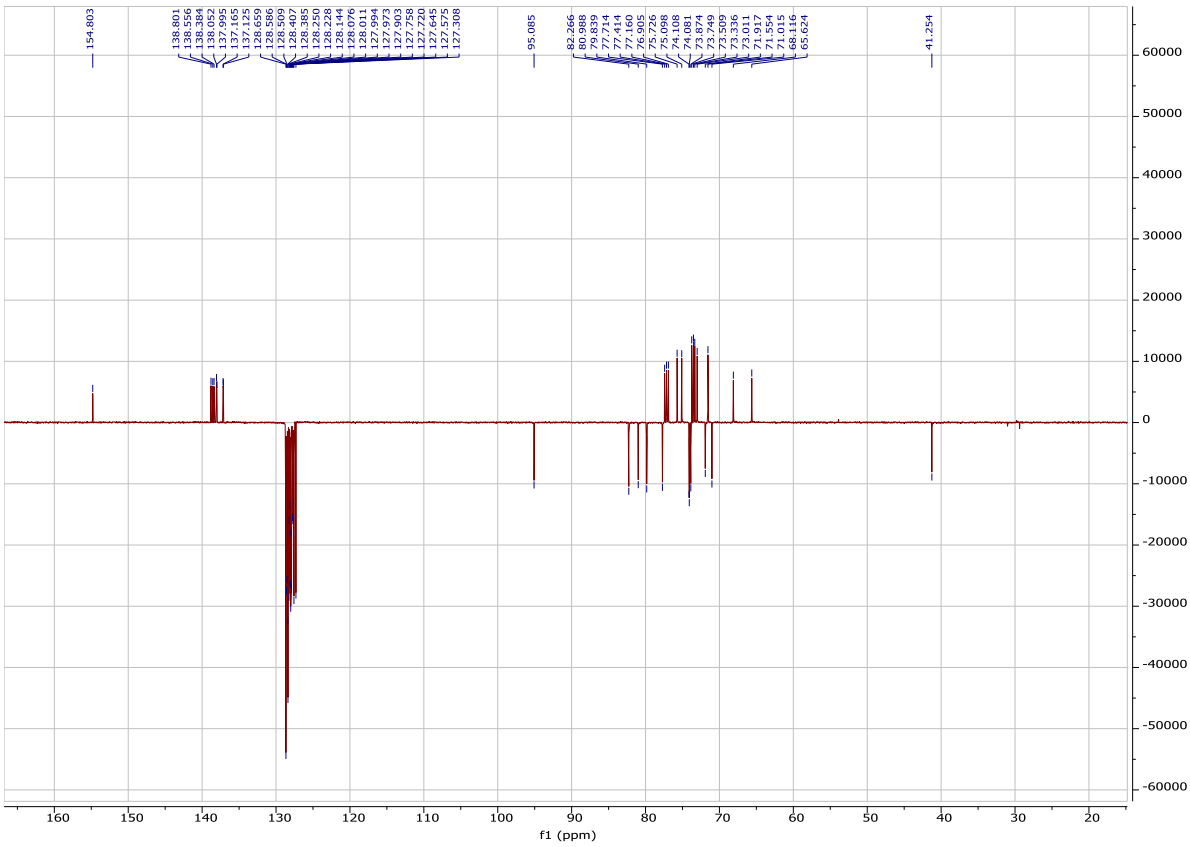
^{1}H and ^{13}C of **17** in $CDCl_3$



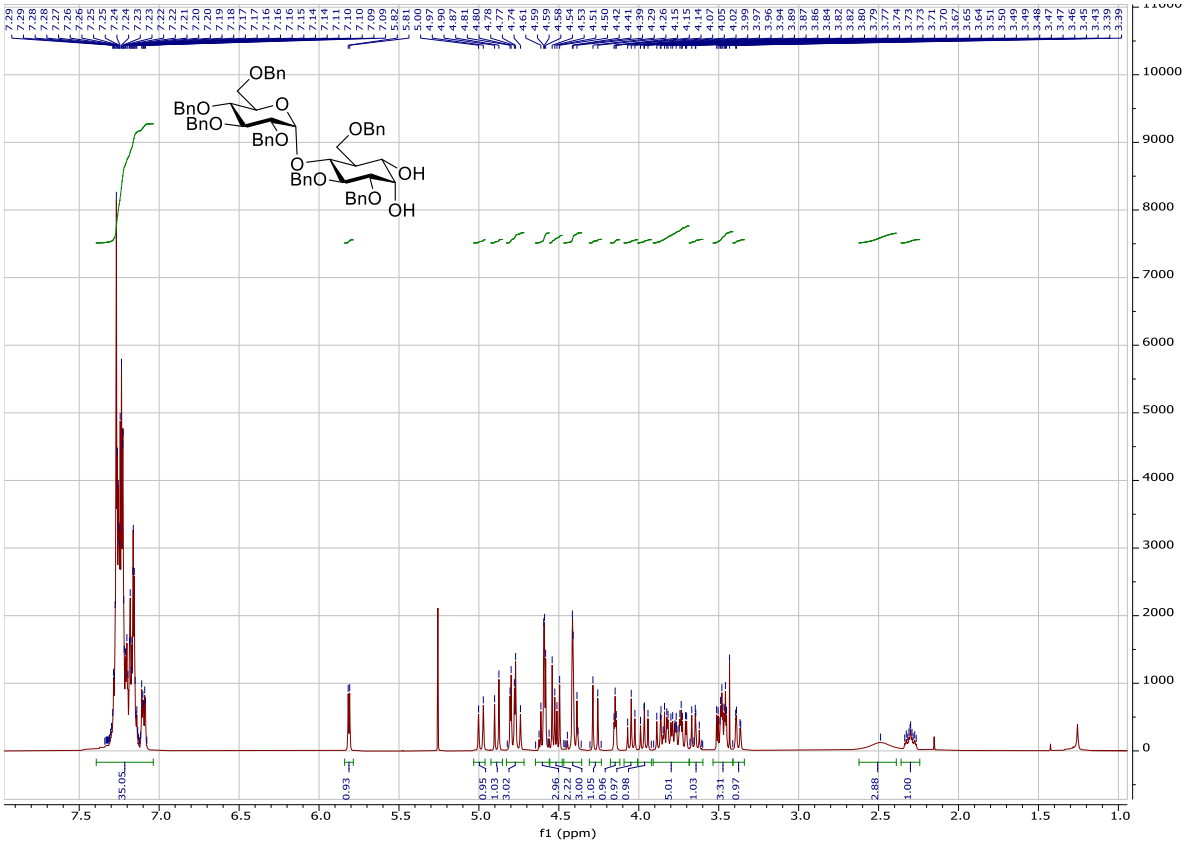


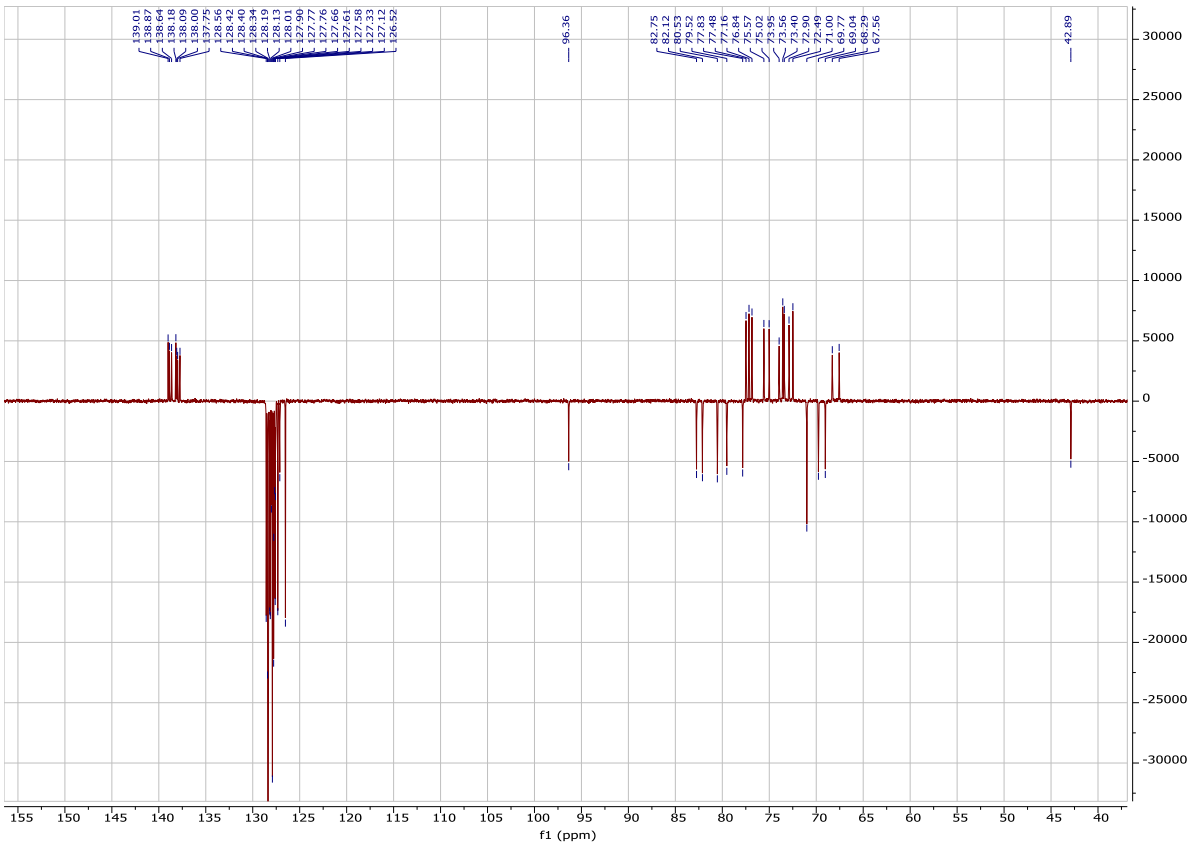
^{1}H and ^{13}C of **18** in $CDCl_3$



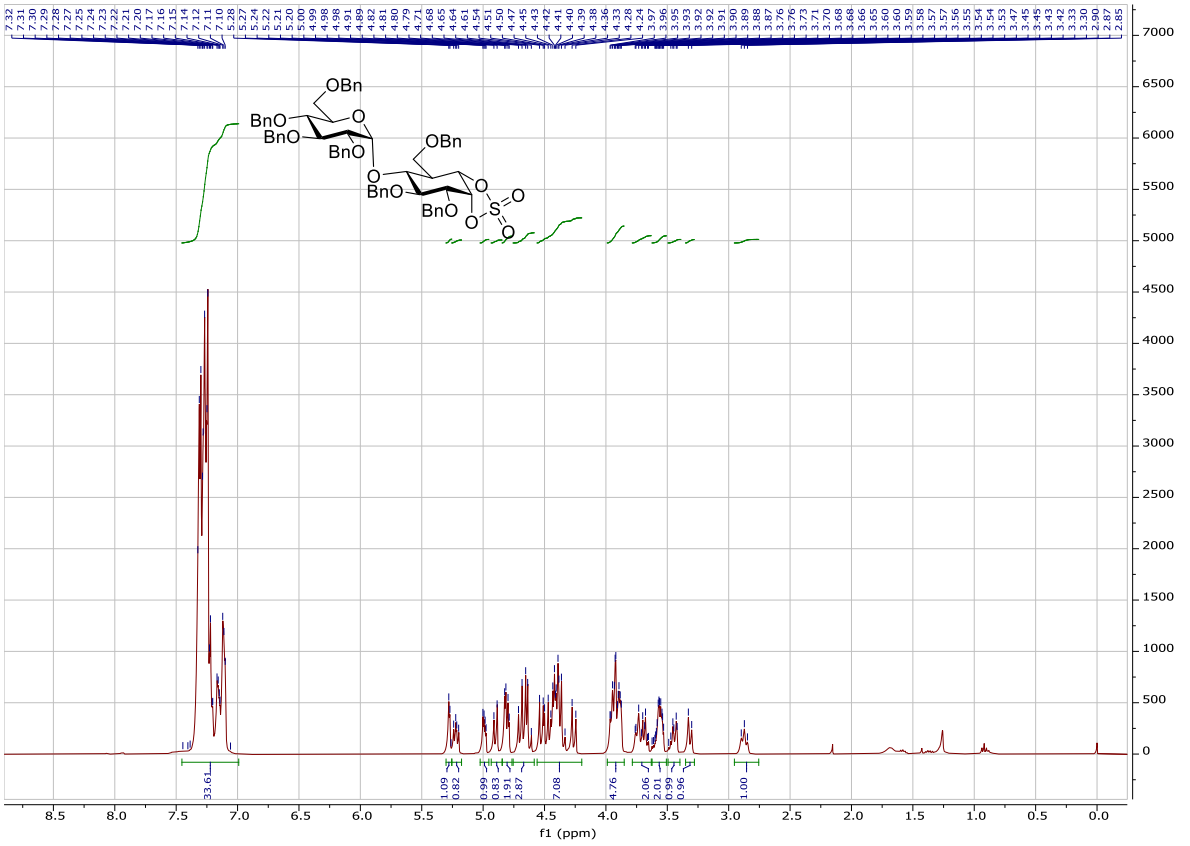


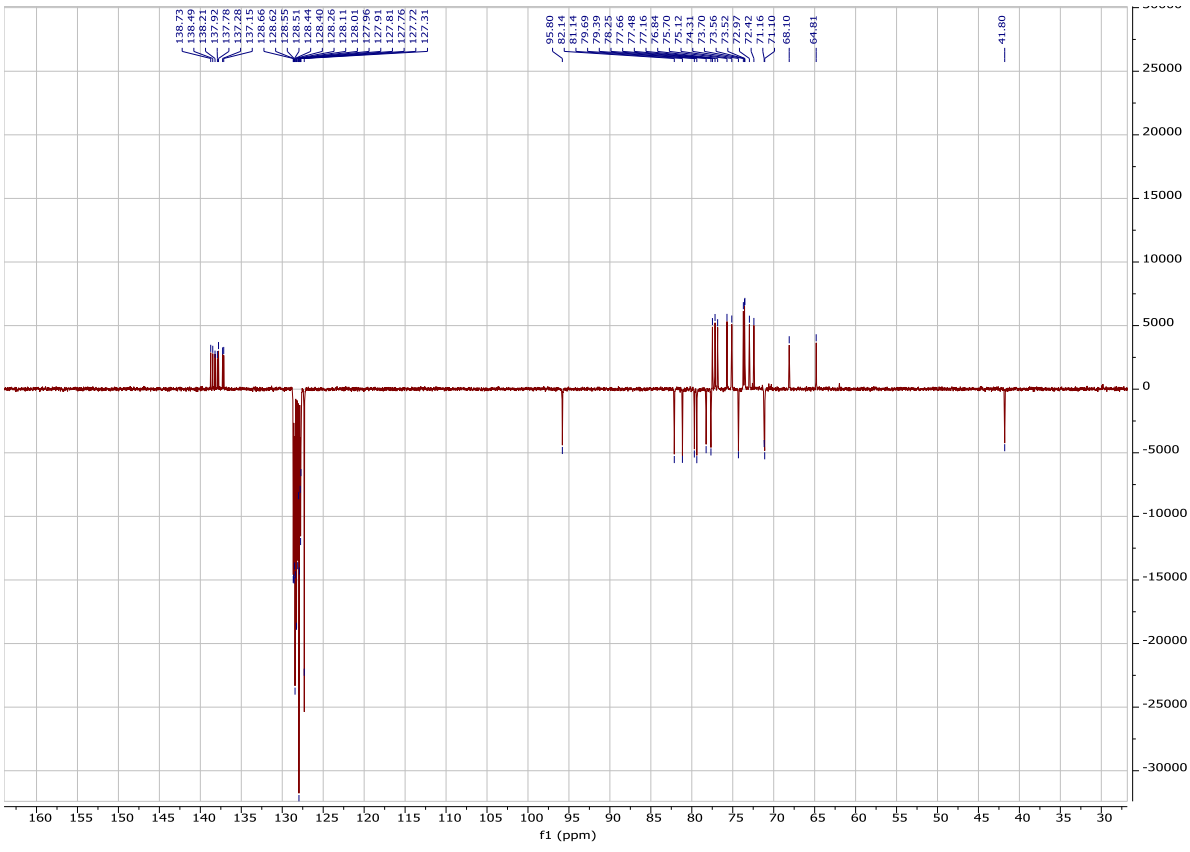
^{1}H and ^{13}C of **19** in $CDCl_3$





^{1}H and ^{13}C of **20** in $CDCl_3$





^{1}H and ^{13}C of **3a** in D_2O