Synthesis

Reviews and Full Papers in Chemical Synthesis

Supporting Information

for DOI: 10.1055/a-2152-0255

Georg Thieme Verlag KG, Rüdigerstraße 14, 70469 Stuttgart, Germany

Thieme

Supporting Information

Synthesis and Immunological Evaluation of *Escherichia Coli* O1-Derived Oligosaccharide-Protein Conjugates toward Avian Pathogenic *Escherichia Coli* O1 Vaccine Development

Katsunori Seki, Takumi Makikawa, Kazunobu Toshima, Daisuke Takahashi

Index

MALDI-TOF MS analysis of glycoconjugates

The sample (1.0 μL) was mixed with a matrix solution (1.0 μL) of sinapinic acid (15 mg/mL in 50:50 H_2O/MeCN containing 0.1% TFA). Analyses by MALDI-TOF MS were performed in the positive ion mode.

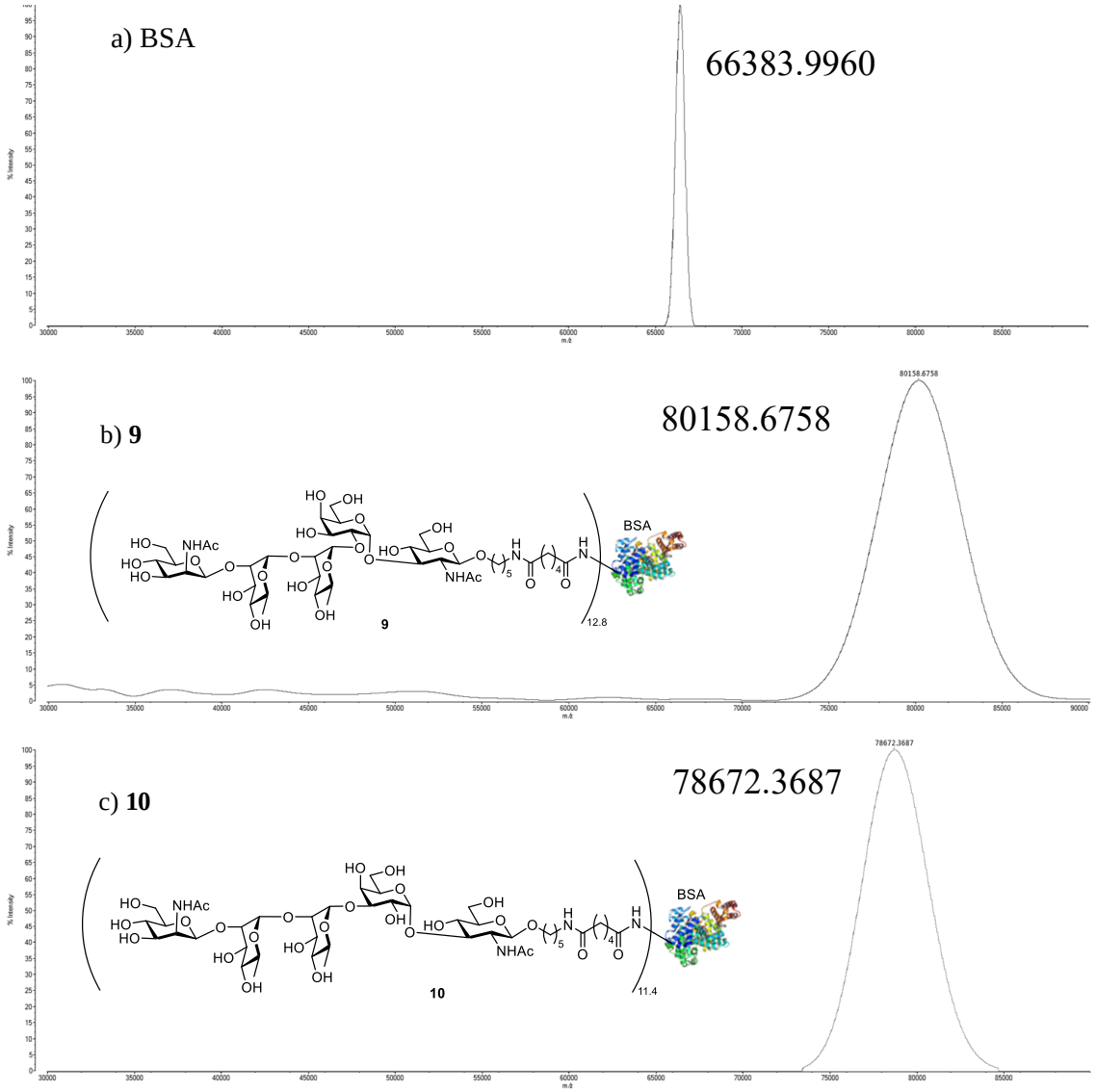
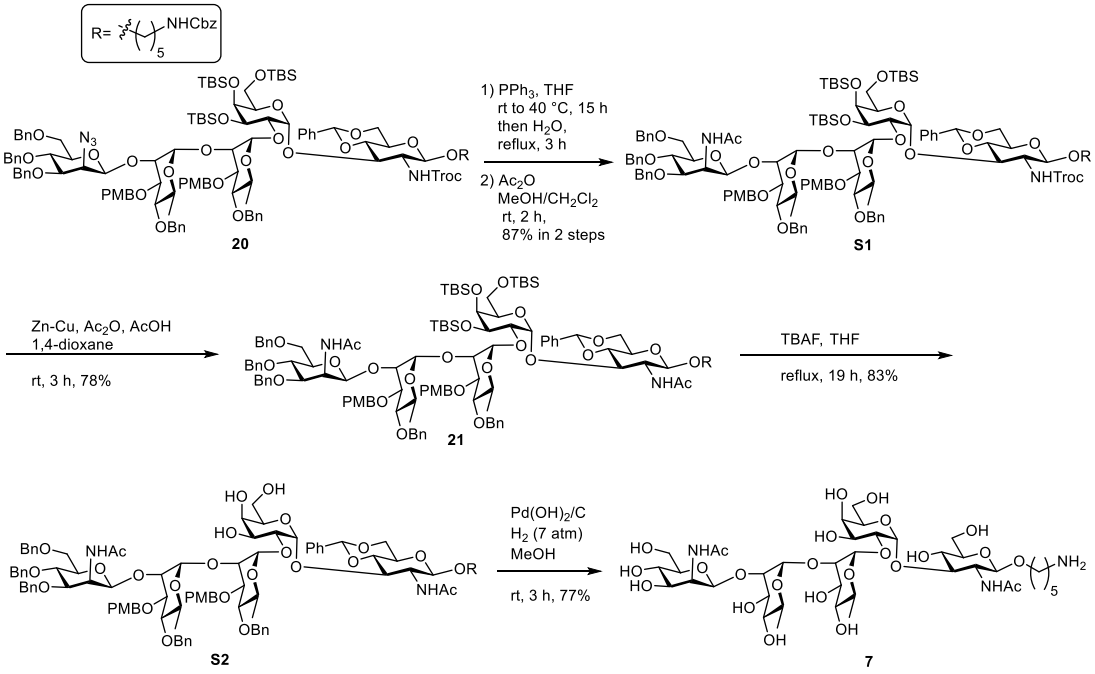
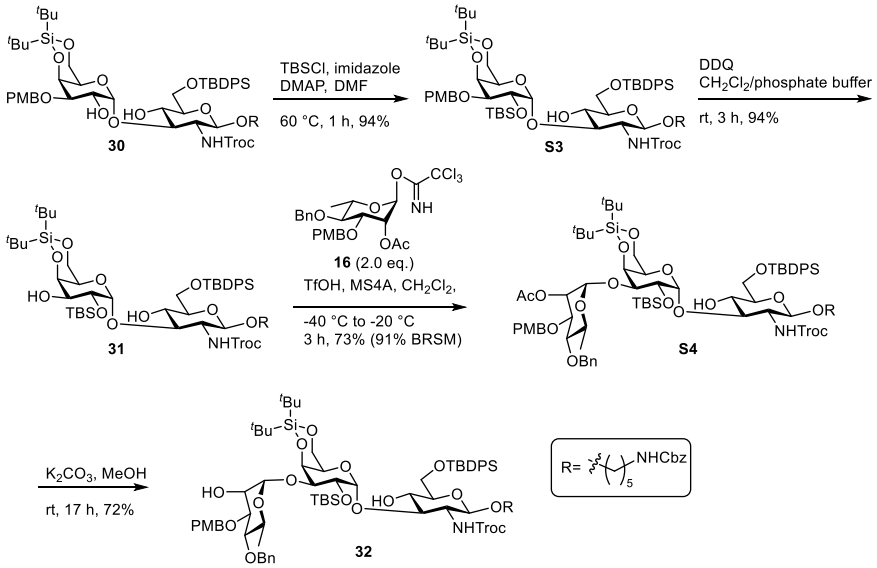


Figure S1 MALDI-TOF MS analysis of a) BSA, b) conjugate **9**, and c) conjugate **10**

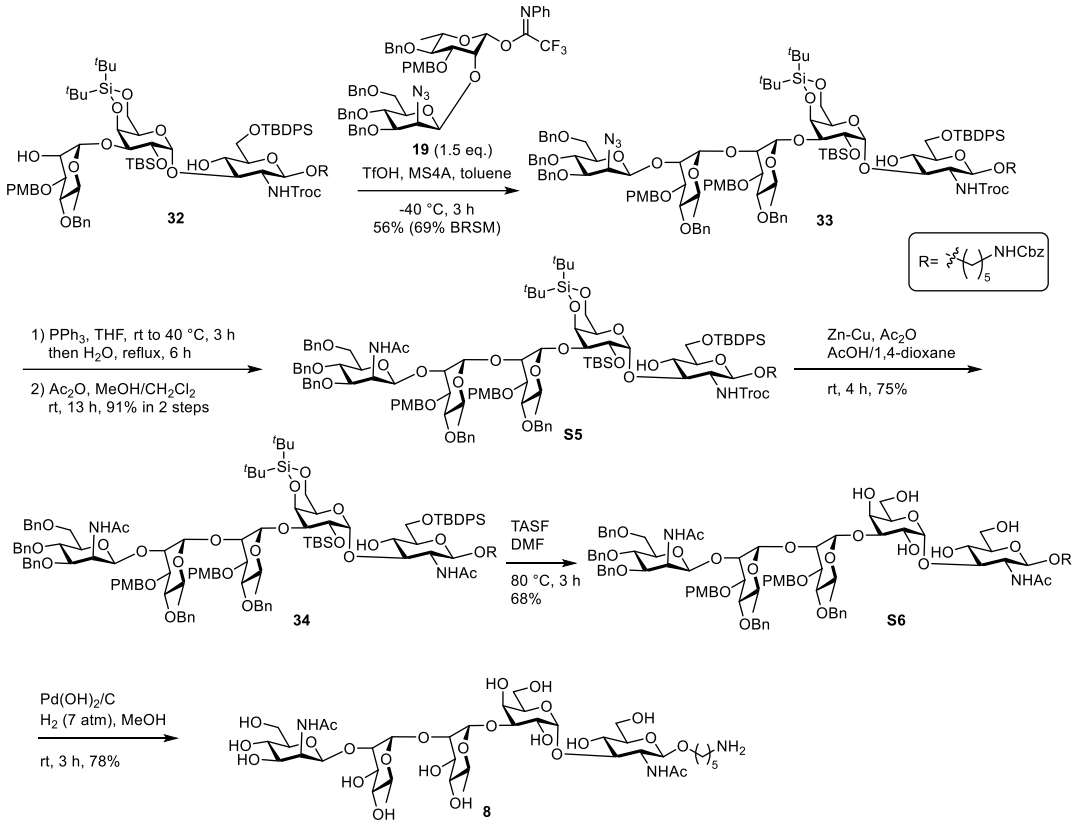
Synthetic schemes of **7**, **32**, and **8**



Scheme S1 Synthesis of pentasaccharide **7**



Scheme S2 Synthesis of trisaccharide **32**



Scheme S3 Synthesis of pentasaccharide **8**

Materials and experimental methods for immunological assays

Bacteria culture and LPS extraction

Escherichia coli (*E. coli*) ATCC®11775™ was purchased from the American type culture collection (ATCC). *E. coli* LPS was extracted with hot phenol-water method.[1] *E. coli* ATCC®11775™ was grown in Luria Broth (LB) medium (5 mL) at 37 °C for 18 h. After centrifugation, the supernatant was removed. The resulting pellet was resuspended in Tris-HCl buffer (pH 6.8, 0.1 M, 2 mL), and then suspended bacteria was boiled in a water bath for 15 min. To the resulting bacteria suspension were added DNase I (50 units) and RNase A (0.5 mg) at room temperature, and then the resulting mixture was incubated at 37 °C for 30 min. Next, to the suspension was added proteinase K (1.0 mg), and then the resulting mixture was incubated at 59 °C overnight. To the resulting mixture was added ice-cold tris-saturated phenol (2 mL), and the resulting mixture was vortexed for 10 seconds. And then, the mixture was incubated at 65 °C for 15 min. After incubating cool to room temperature, Et_2O (50 mL) was added to the resulting mixture, and the mixture was vortexed for 10 sec. After centrifugation, the sample was extracted again under the same conditions. After centrifugation, the aqueous phase was collected and dialyzed against 3 changes of deionized water (1 L) and lyophilized to get the purified LPS.

Preparation of APEC O1 immune chicken serum and non-immune chicken serum

Animal experiments were approved by the Institutional Animal Care and Use Committee of Shokukanken Inc. (Approval Number: AW20Jan005H). Specific-pathogen-free (SPF) chicken eggs were purchased from VALO BioMedia GmbH, and the eggs were hatched. A 10-day-old SPF chicken was orally immunized with 10^6 CFU of APEC O1 strain (ATCC®11775™). The chicken was boosted using the same strain with the same dose on 14 days after the initial immunization. The serum was collected on 28 days after the initial immunization, and serum IgY antibodies were tested with enzyme-linked immunosorbent assay (ELISA).

Non-immune chicken serum was prepared by a 38-day-old SPF chicken, which was not immunized with APEC O1 strain.

Preparation of **11** immune chicken serum and non-immune chicken serum

Animal experiments were approved by the Institutional Animal Care and Use Committee of Shokukanken Inc. (Approval Number: 217061N). SPF chicken eggs were purchased from VALO BioMedia GmbH, and the eggs were hatched. A 10-day-old SPF chicken was orally immunized with **11** (105 μg) in PBS (150 μL) and Freund's Complete adjuvant (150 μL) purchased from Fujifilm wako pure chemical corporation. The chicken was boosted using **11** (105 μg) in PBS (150 μL) and Freund's Incomplete adjuvant (150 μL) purchased from Fujifilm wako pure

chemical corporation on 14 and 28 days after the initial immunization. The serum was collected on 0, 14, 28, and 35 days after the initial immunization, and serum IgY antibodies were tested with enzyme-linked immunosorbent assay (ELISA).

Non-immune chicken serum was prepared as follows. A 10-day-old SPF chicken was orally immunized with PBS (300 μL). The chicken was additionally immunized using PBS (300 μL) on 14 and 28 days after the initial immunization. The serum was collected on 0, 14, 28, and 35 days after the initial immunization,

ELISA experiments

ELISA plates were treated with a solution of each glycoconjugate **4**, **9**, **10**, BSA, and APEC O1 LPS (100 μL/well, 10 μg/mL) in phosphate-buffered saline (PBS) (pH 7.4, 10 mM) at 37 °C for 1 h. After coating, the wells were emptied and filled with 200 μL of PBS-containing 5% glucose and 2.5% defatted milk, and the plates were incubated at 37 °C for 1 h. And then, the wells were washed with PBS buffer that contained 0.05% Tween-20 (PBST) three times. Each chicken serum with serial dilutions from 1:16000 in PBS-containing 1% BSA (100 μL/well) was added to the coated plates, which were incubated at 4 °C overnight. After being washed with PBST (×3) and PBS (×3), the plates were incubated at 37 °C for 1 h with a 1:5000 diluted solution of horseradish peroxidase (HRP)-linked goat anti-chicken IgY antibody (Southern Biotech. Associates). Again, the plates were washed six times and developed with *o*-phenylene diamine solution (0.4 mg/mL in 0.1% H_2O_2-containing citrate-phosphate buffer, 100 μL) for 30 min at room temperature. After quenching with 5 N sulfuric acid (100 μL), the absorbance at 490 nm was determined by a microplate reader. The optical density (OD) values were absorbance of wells coated with glycoconjugate **4**, **9**, and **10** deducting the background absorbance, which was obtained with wells coated with BSA.

Analysis of the carbohydrate loadings of glycoconjugate **11**

The anthrone–sulfuric acid assay[2] was applied for the determination of carbohydrate loading. Anthrone in sulfuric acid (0.1%, 150 μL) was added to each well of the microplate containing 50 μL of glycoconjugate **4** or **11** in PBS. And then, plates were placed at 4 °C for 10 min. Subsequently, plates were incubated at 100 °C for 20 min. After heating, the reaction conditions were optimized by performing a cooling step treatment at room temperature for 20 min before measuring absorbance at 620 nm triplicate in a microplate reader (SpectraMax i3, Molecular Devices). The colorimetric response was compared to a standard curve based on **4**, and total carbohydrate content of **11** was expressed as the carbohydrate concentration of **4**.

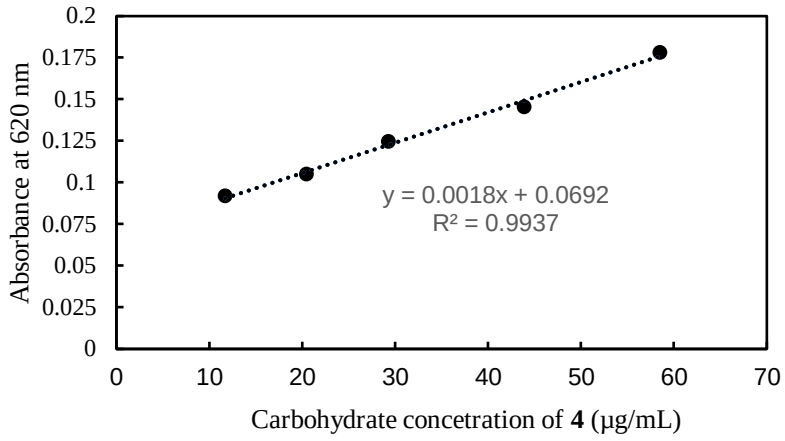


Figure S2 Relationship between carbohydrate concentration of BSA-conjugate **4** and absorbance by anthrone–sulfuric acid assay

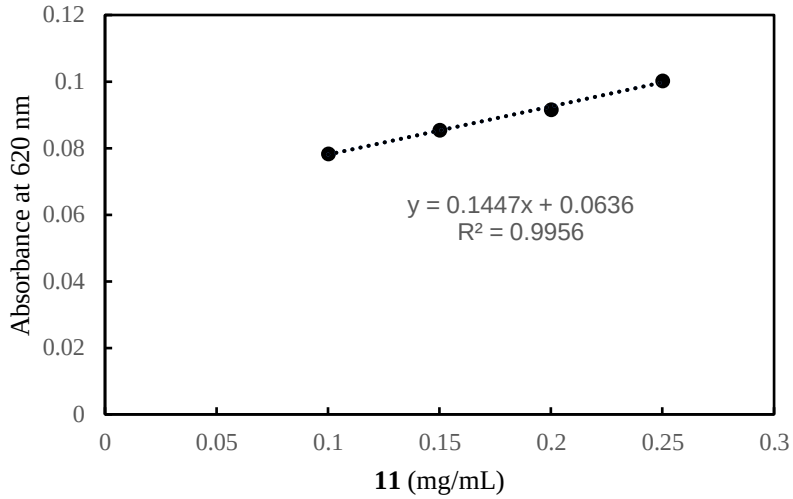


Figure S3 Relationship between KLH-conjugate **11** and absorbance by anthrone–sulfuric acid assay

References

(1) Davis, M. R.; Goldberg, J. B. *J. Vis. Exp.* **2012**, *63*, e3916.

(2) Leyva, A.; Quintana, A.; Sánchez, M.; Rodríguez, E. N.; Cremata, J.; Sánchez, J. C. *Biol. J. Int. Assoc. Biol. Stand.* **2008**, *36*, 134.

NMR spectrum charts

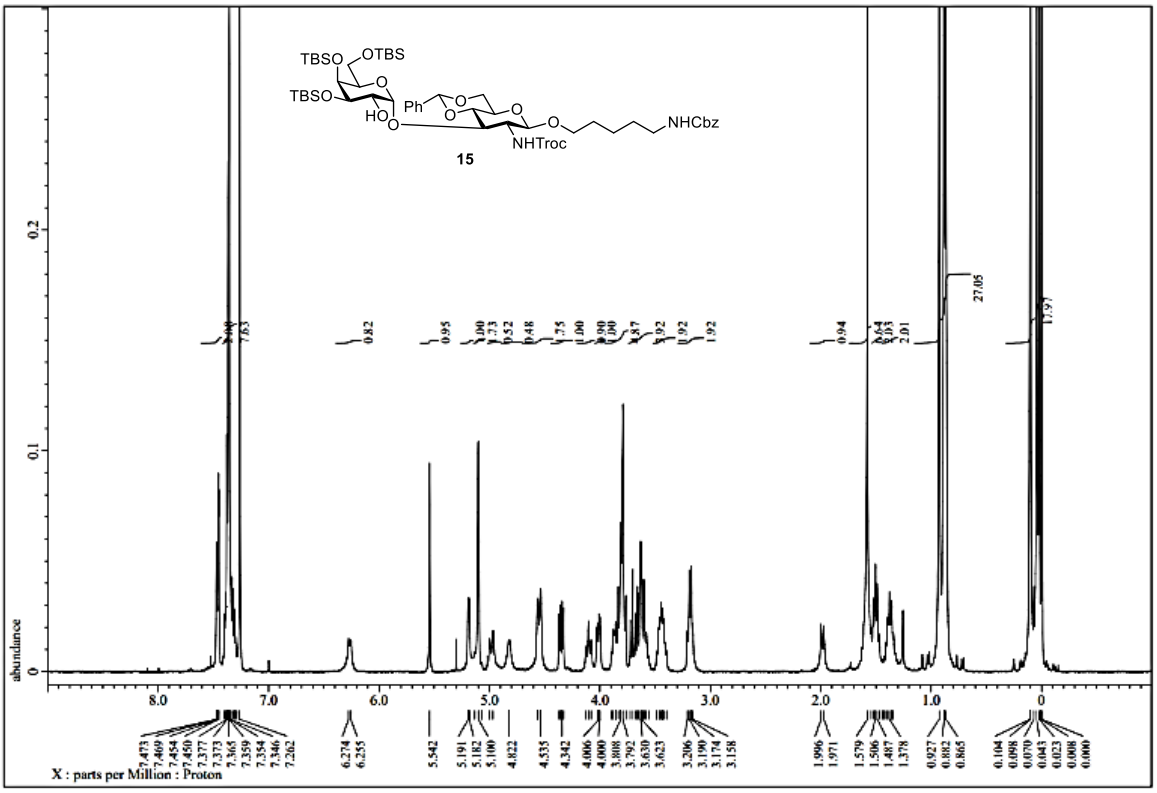


Figure S4 ^{1}H NMR spectrum of compound **15**

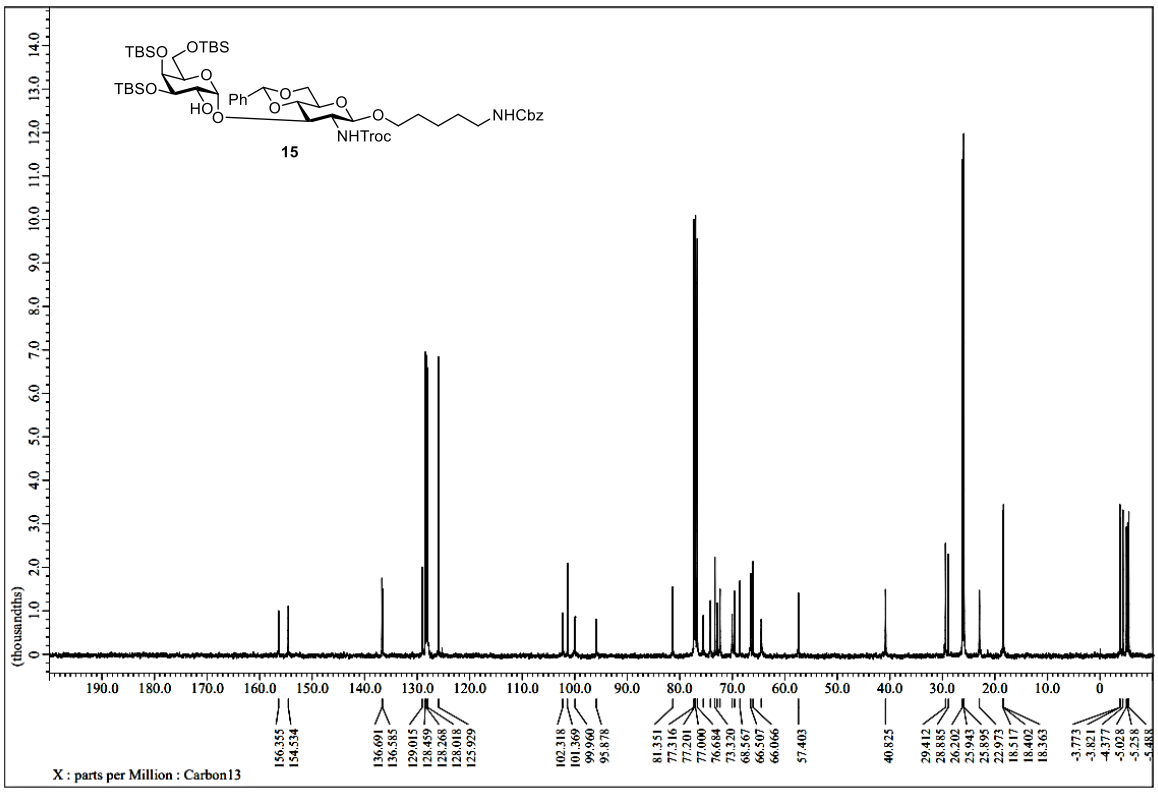


Figure S5 ^{13}C NMR spectrum of compound **15**

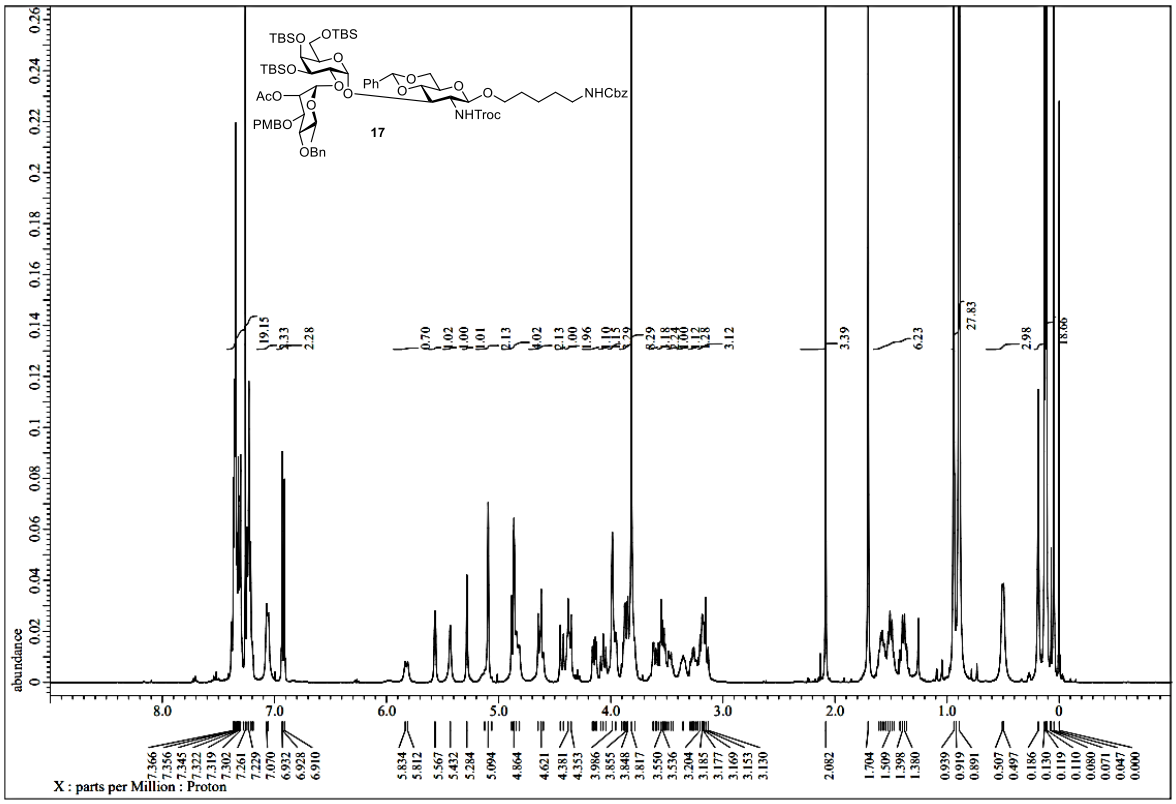


Figure S6 ^{1}H NMR spectrum of compound **17**

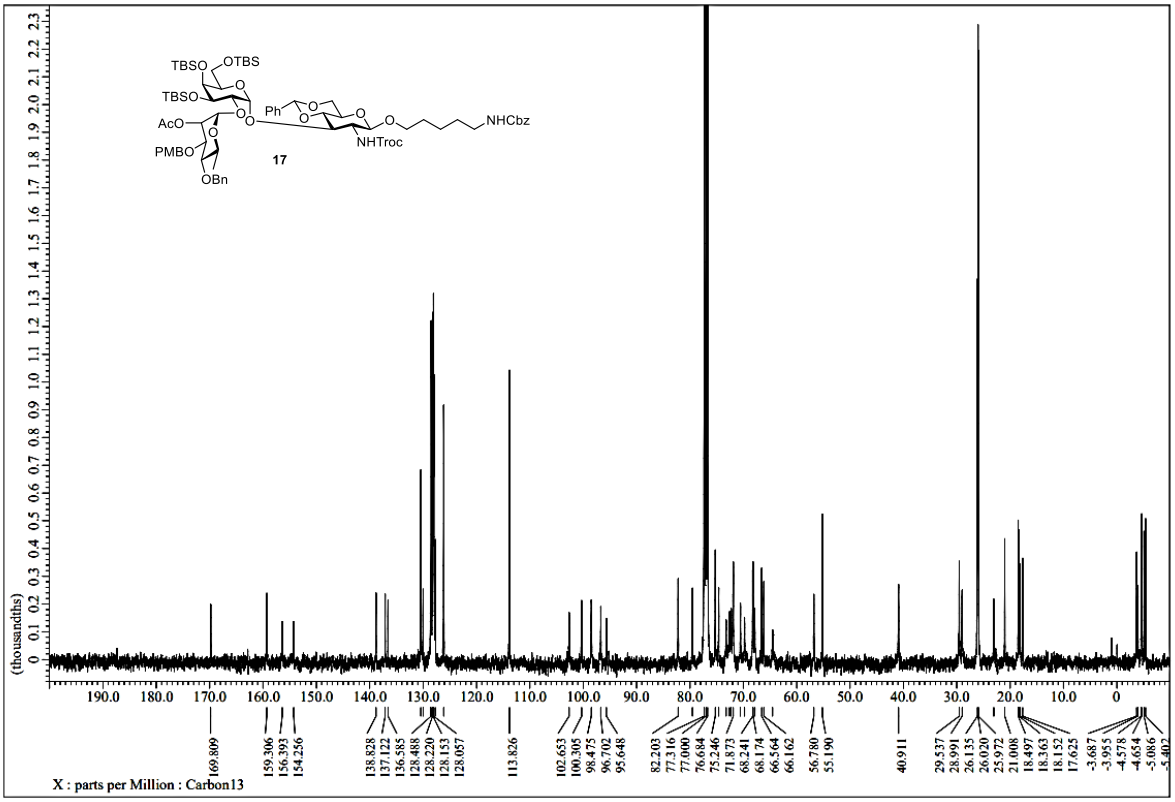


Figure S7 ^{13}C NMR spectrum of compound **17**

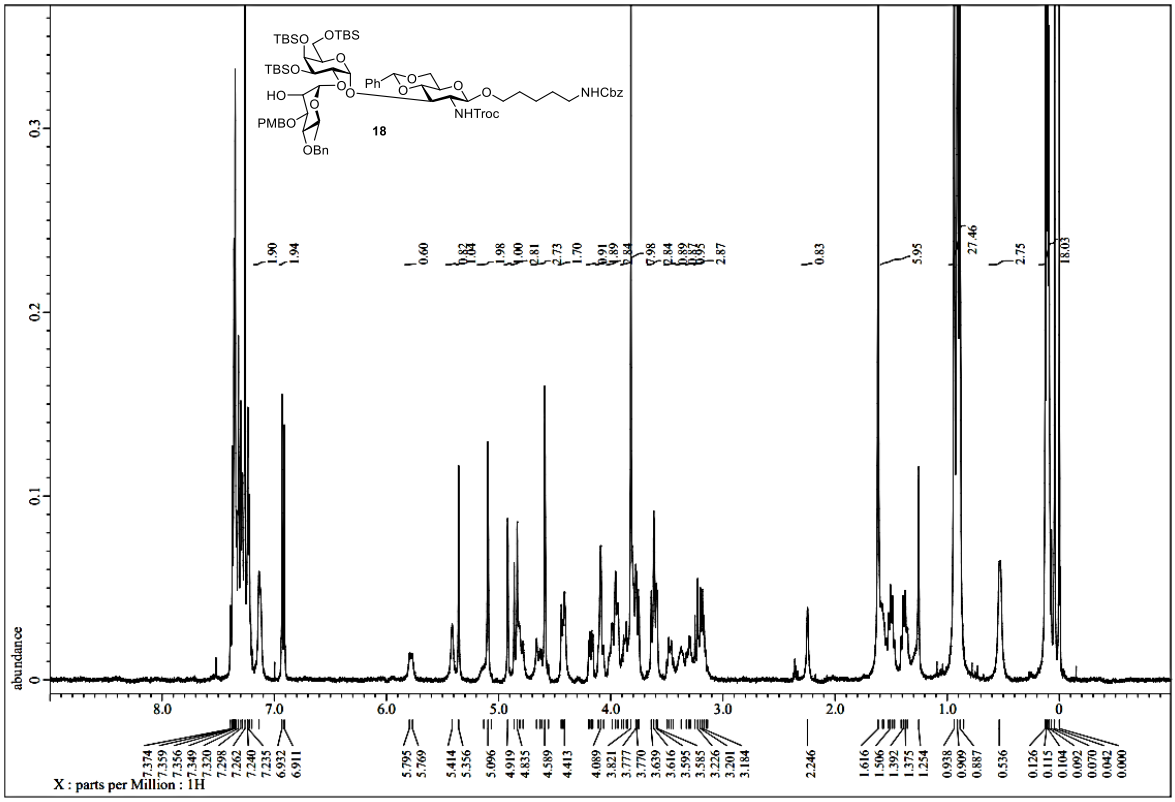


Figure S8 ^{1}H NMR spectrum of compound **18**

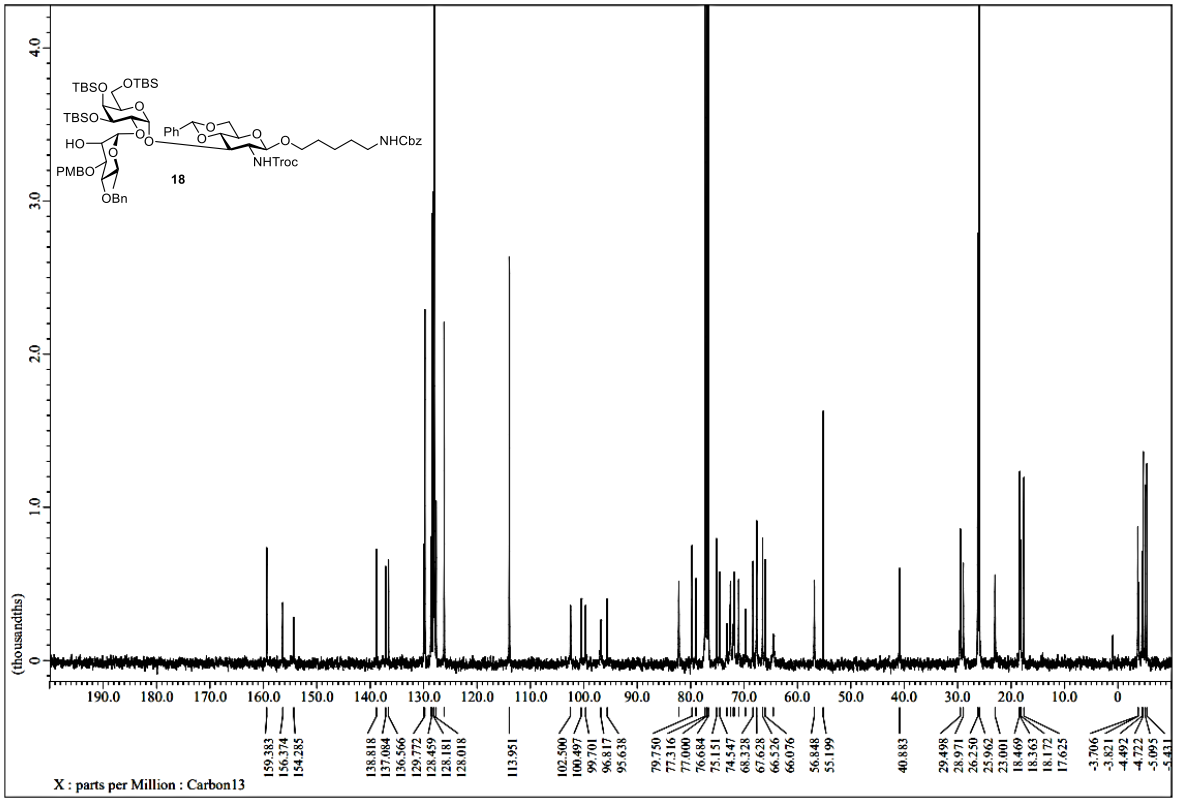


Figure S9 ^{13}C NMR spectrum of compound **18**

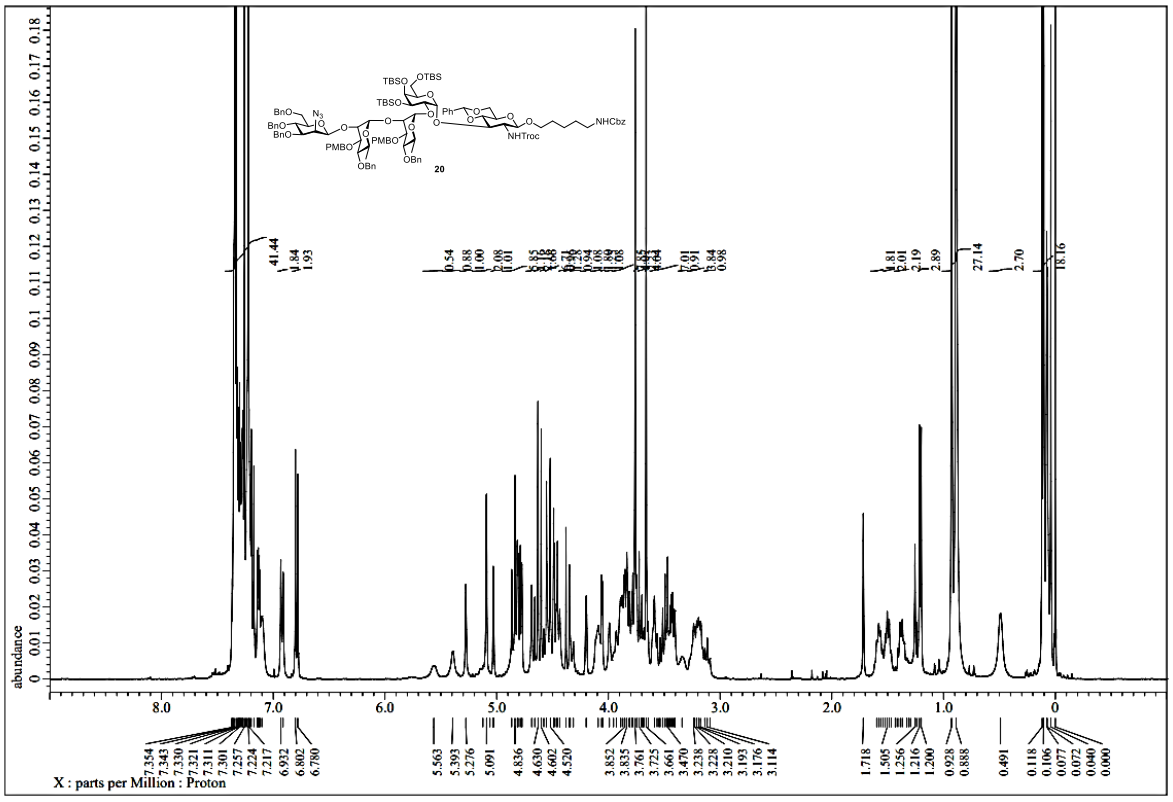


Figure S10 ^{1}H NMR spectrum of compound **20**

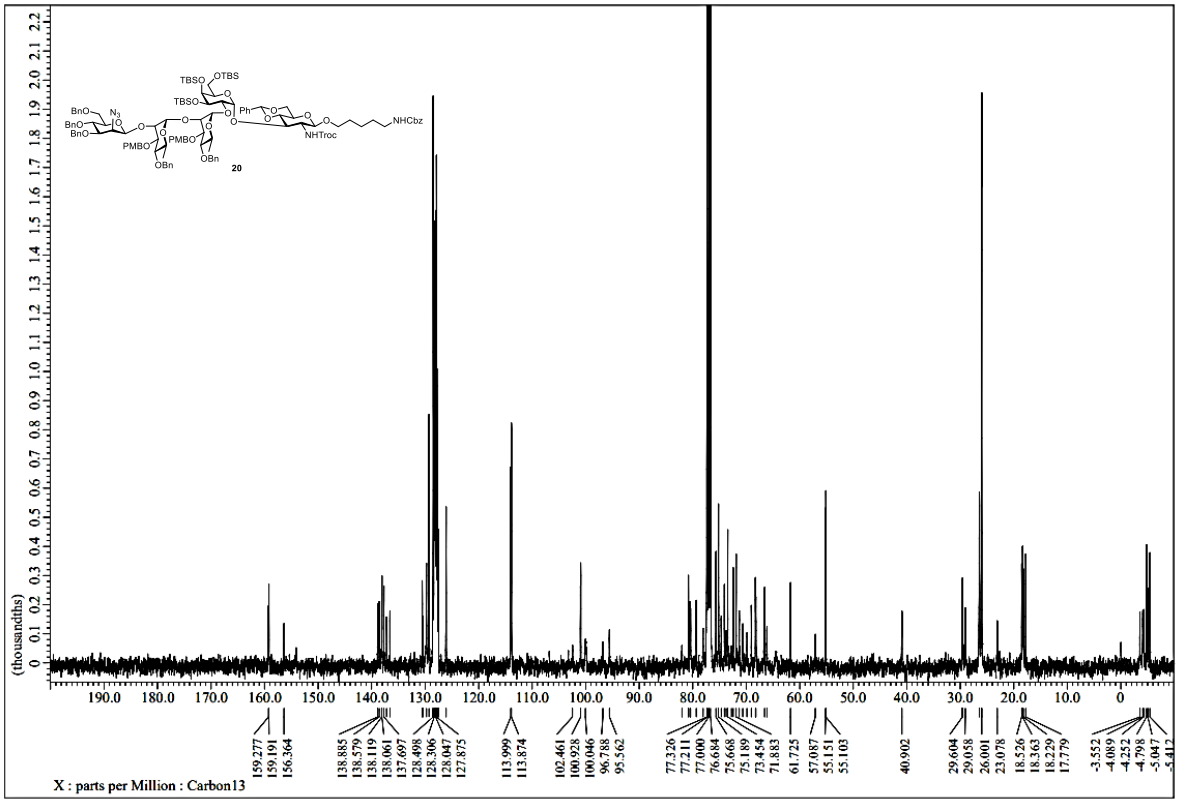


Figure S11 ^{13}C NMR spectrum of compound **20**

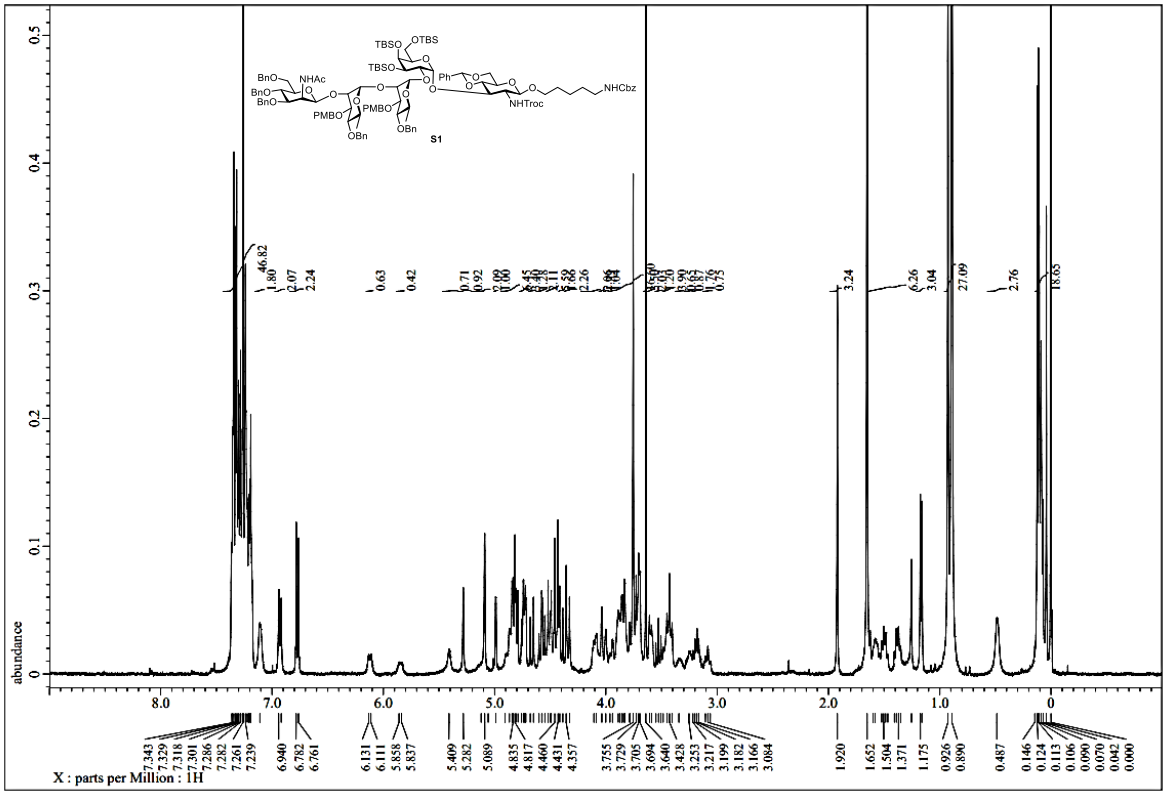


Figure S12 ^{1}H NMR spectrum of compound **S1**

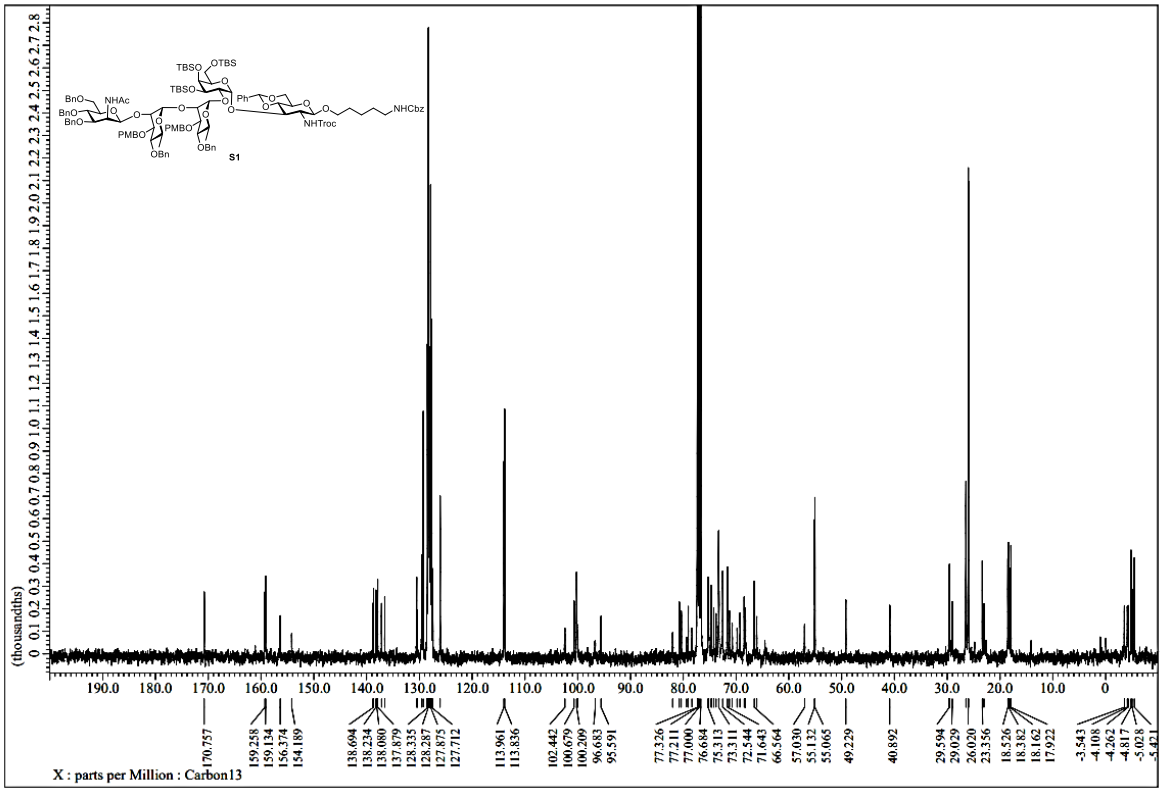


Figure S13 ^{13}C NMR spectrum of compound **S1**

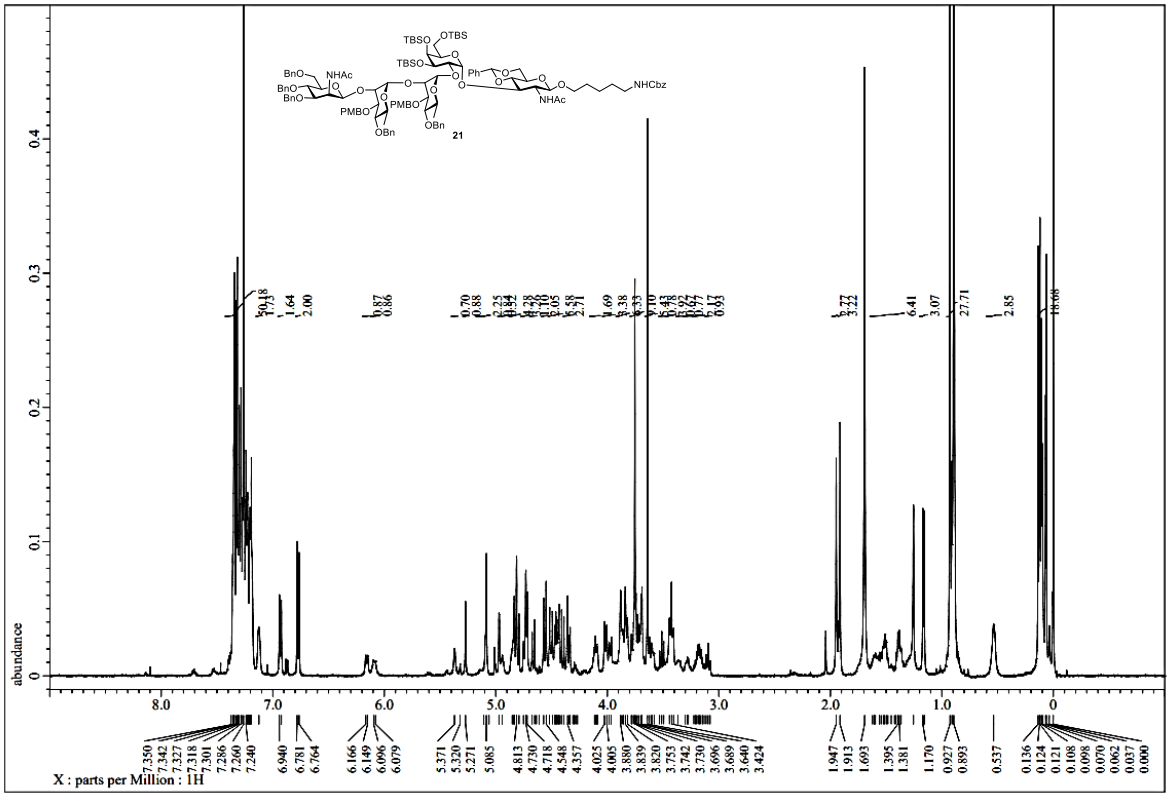


Figure S14 ^{1}H NMR spectrum of compound **21**

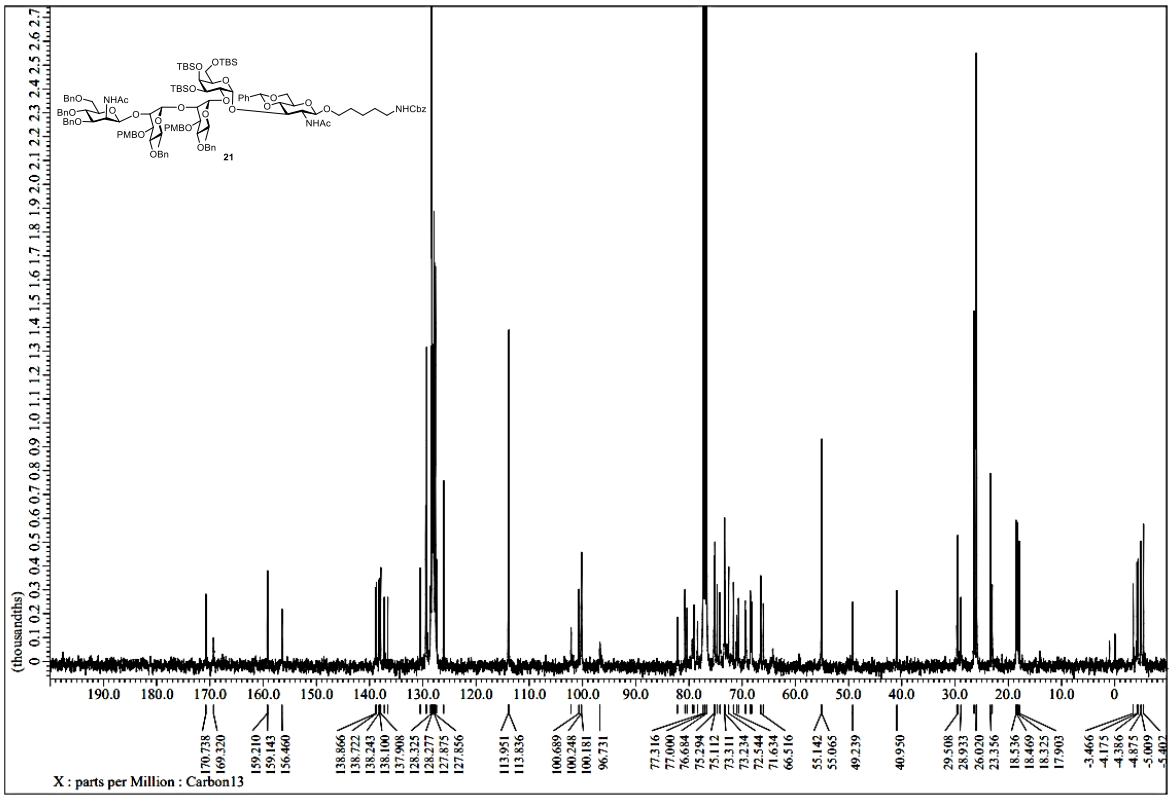


Figure S15 ^{13}C NMR spectrum of compound **21**

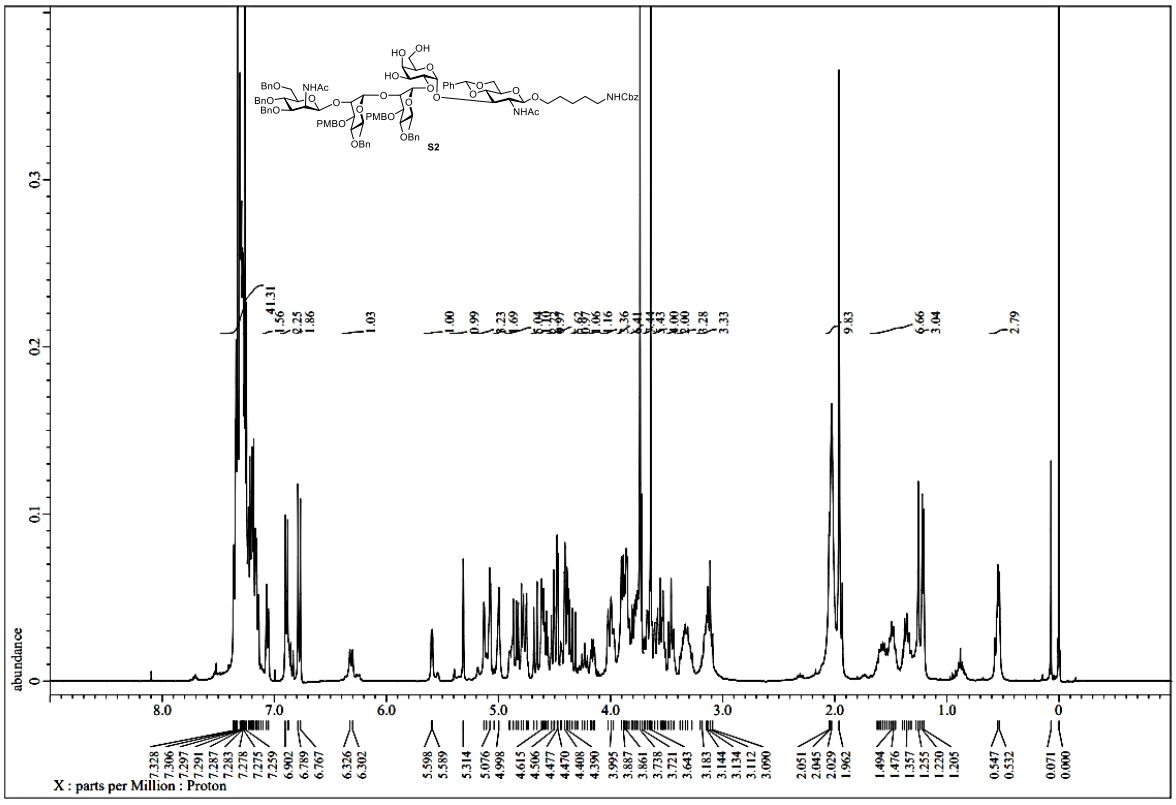


Figure S16 ^{1}H NMR spectrum of compound **S2**

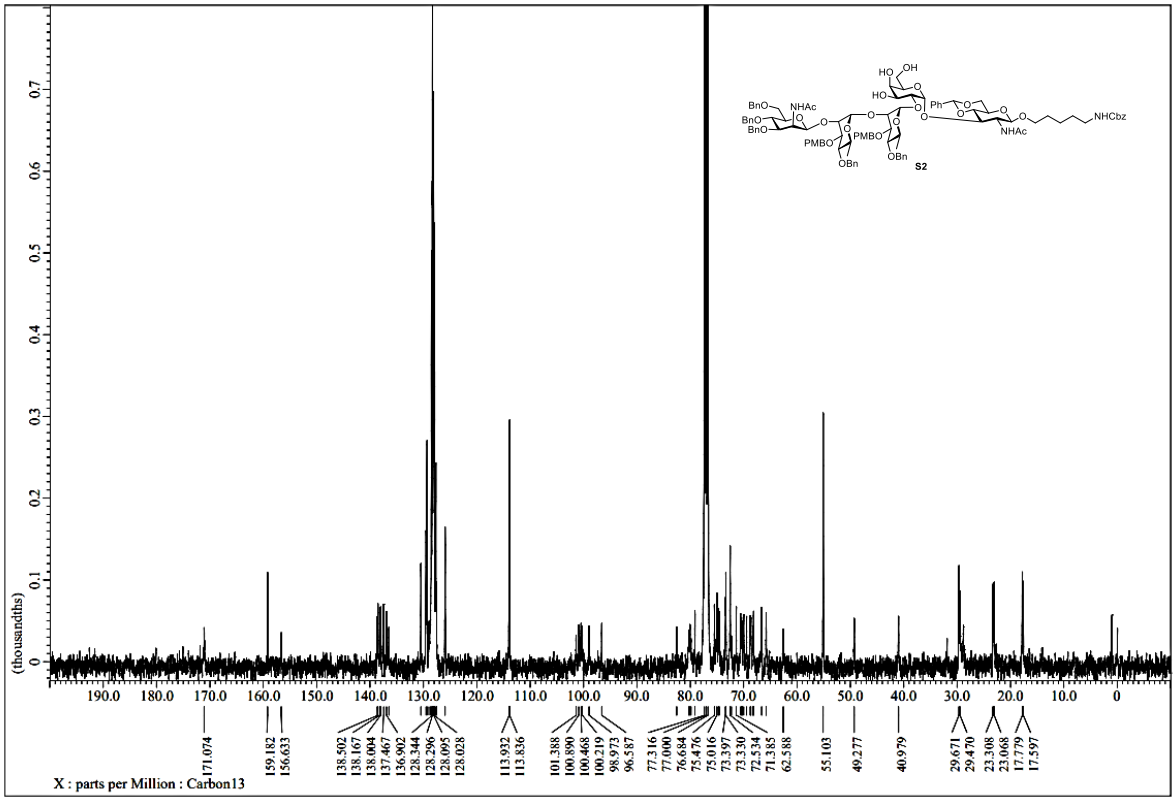


Figure S17 ^{13}C NMR spectrum of compound **S2**

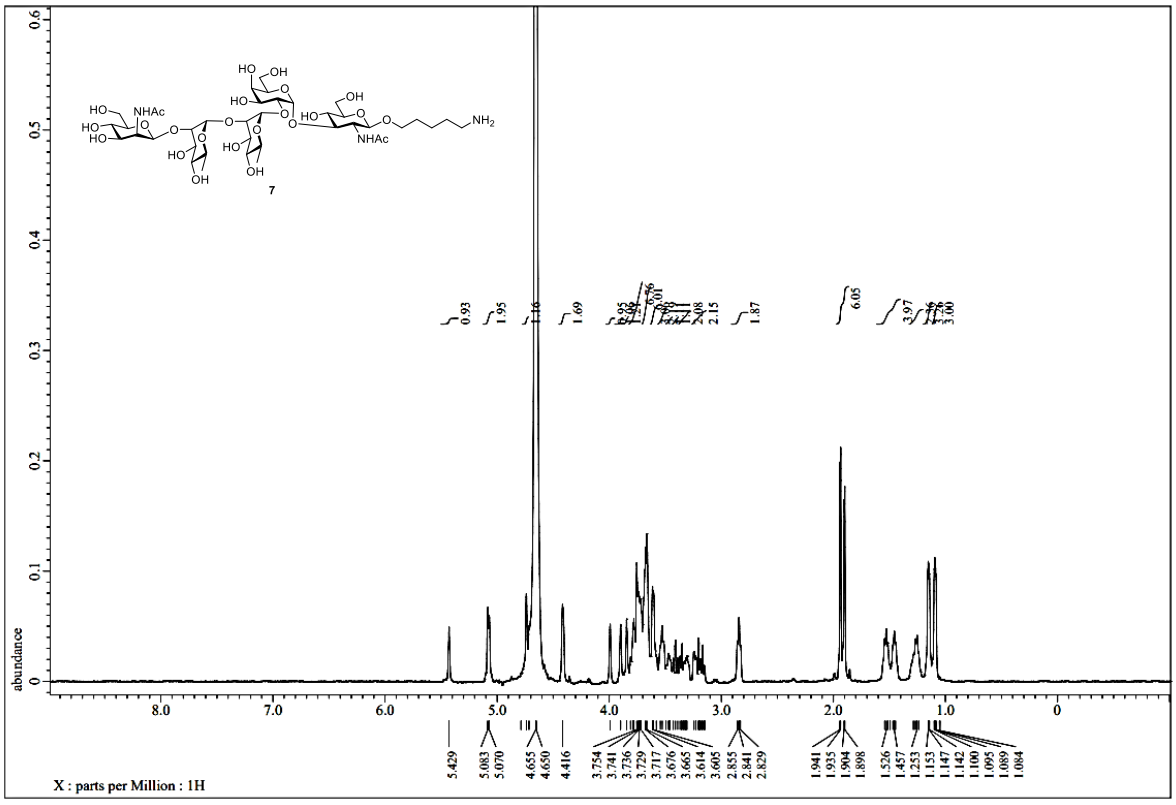


Figure S18 ^{1}H NMR spectrum of compound **7**

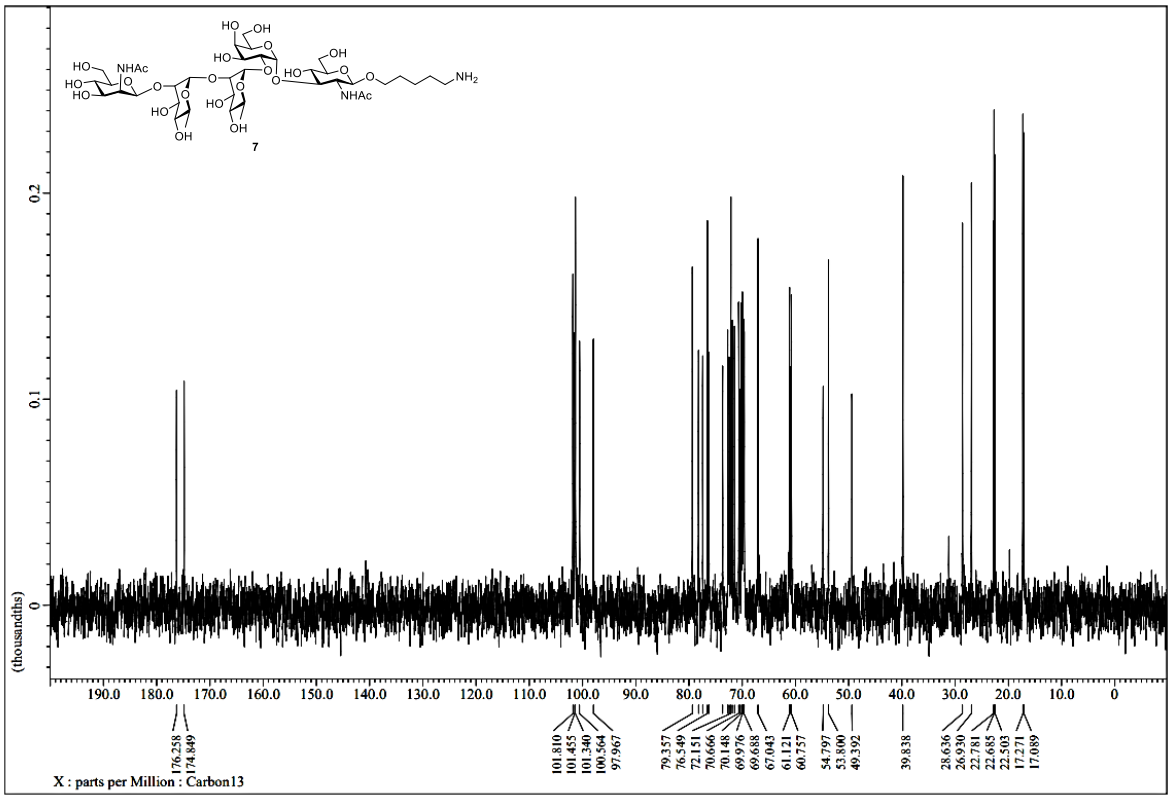


Figure S19 ^{13}C NMR spectrum of compound **7**

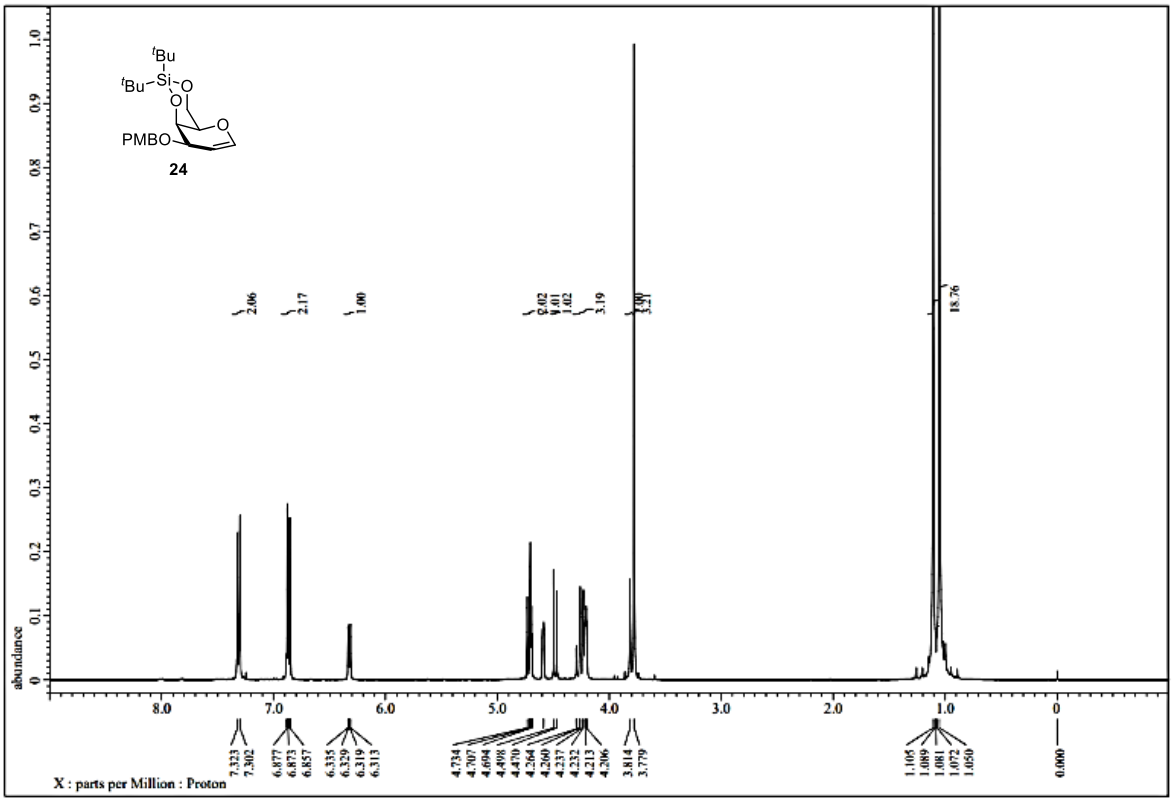


Figure S20 ^{1}H NMR spectrum of compound **24**

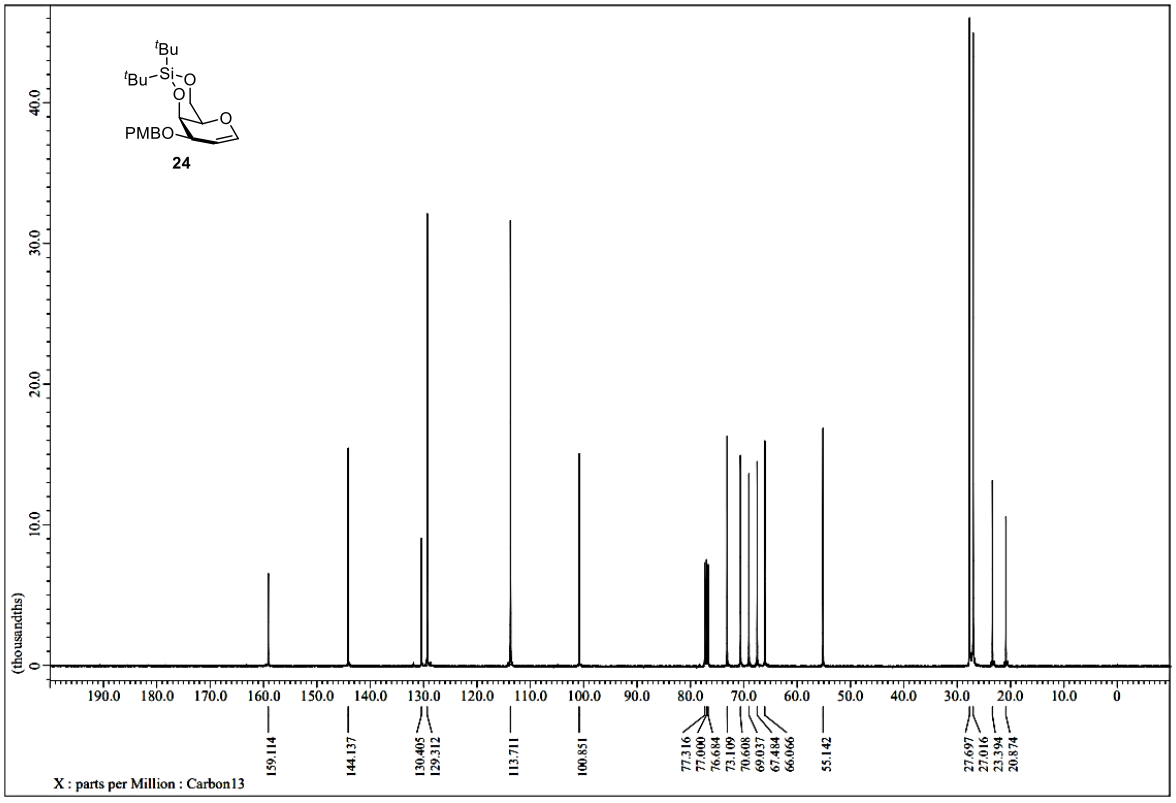


Figure S21 ^{13}C NMR spectrum of compound **24**

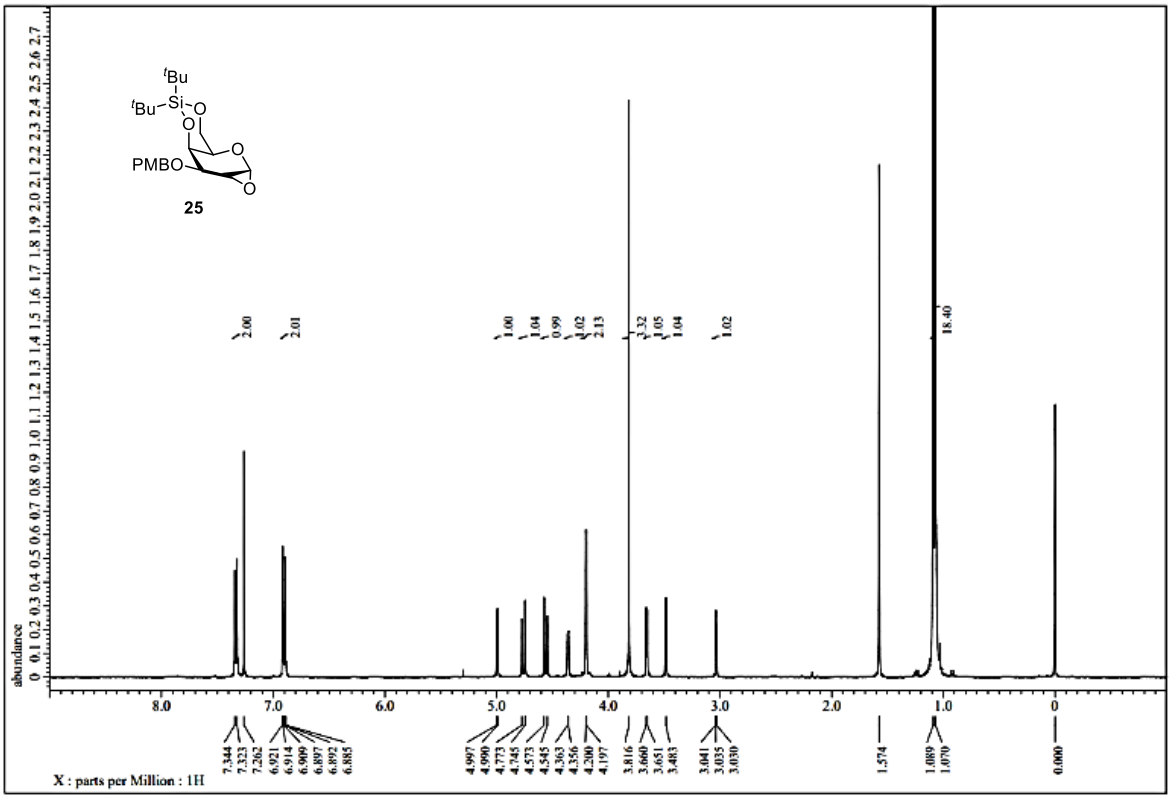


Figure S22 ^{1}H NMR spectrum of compound **25**

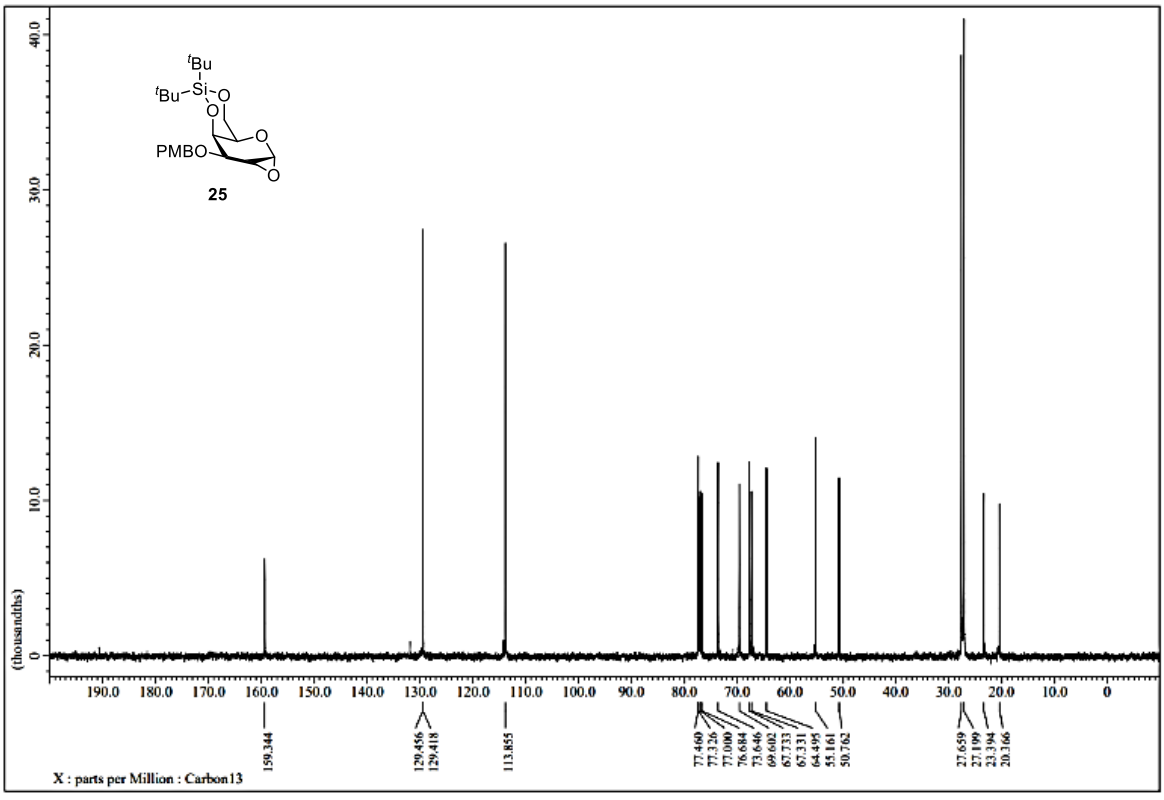


Figure S23 ^{13}C NMR spectrum of compound **25**

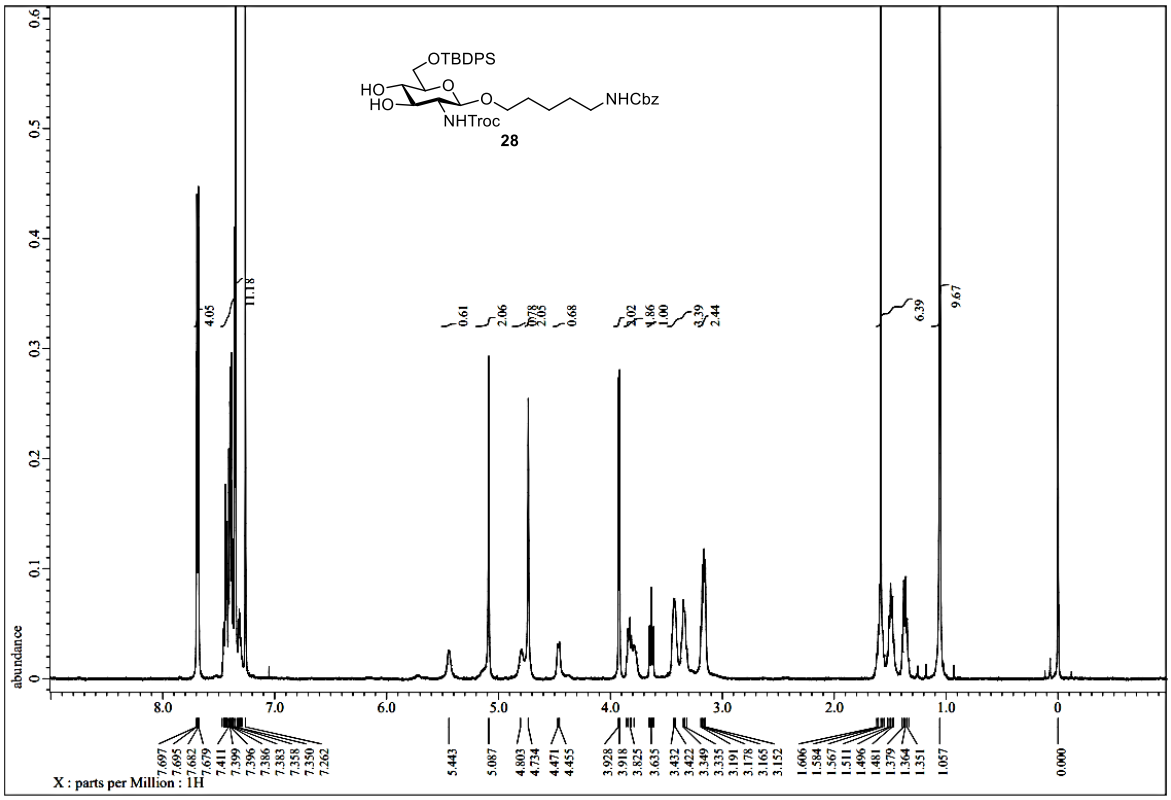


Figure S24 ^{1}H NMR spectrum of compound **28**

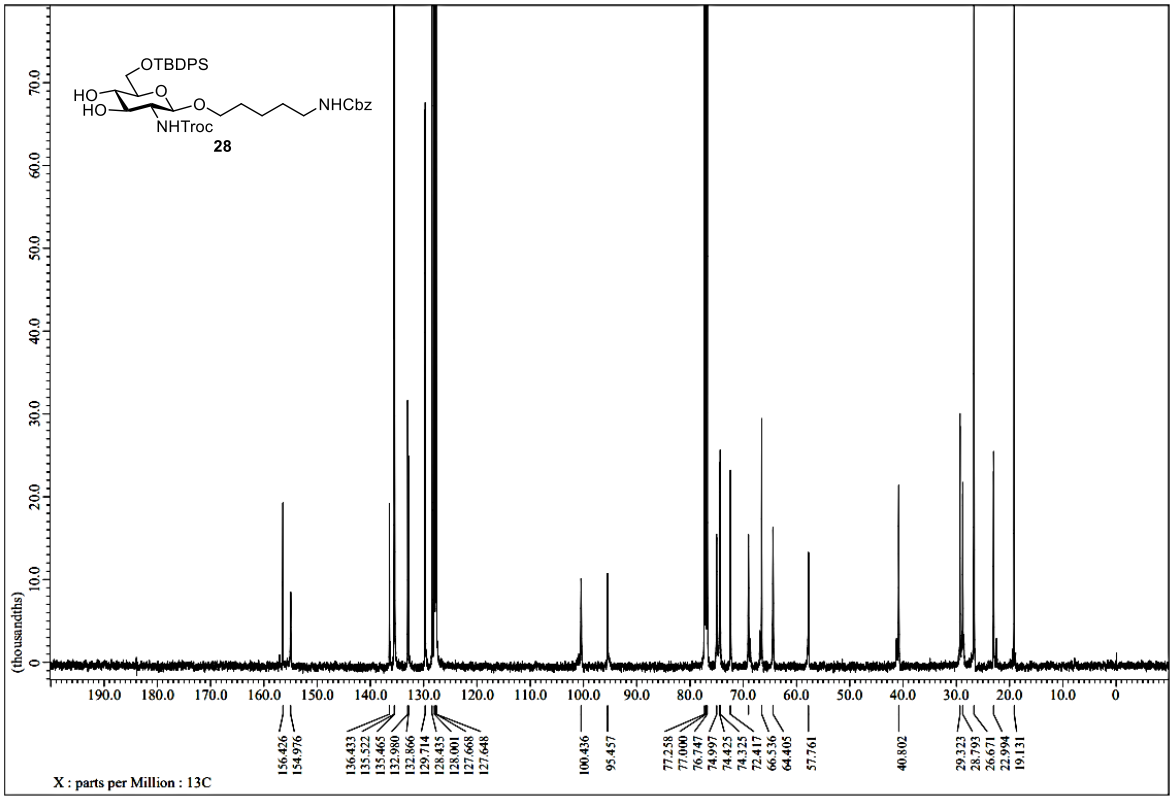


Figure S25 ^{13}C NMR spectrum of compound **28**

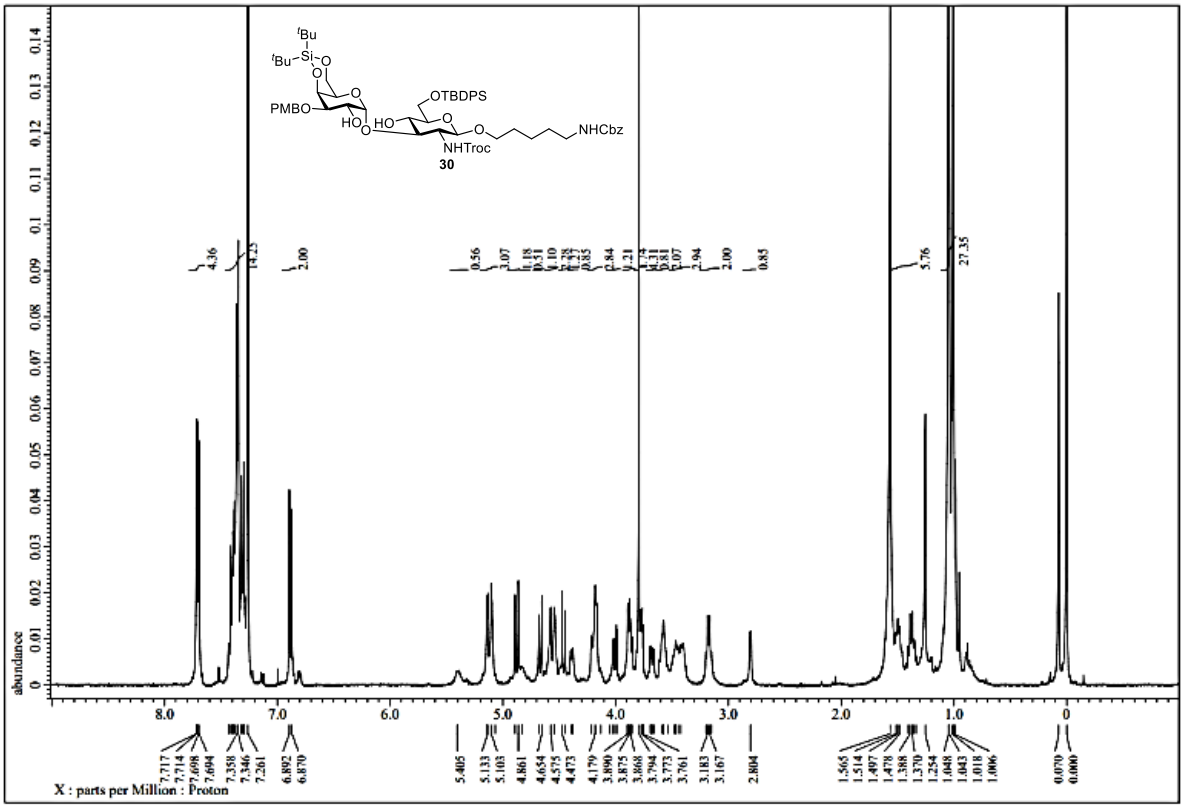


Figure S26 ^{1}H NMR spectrum of compound **30**

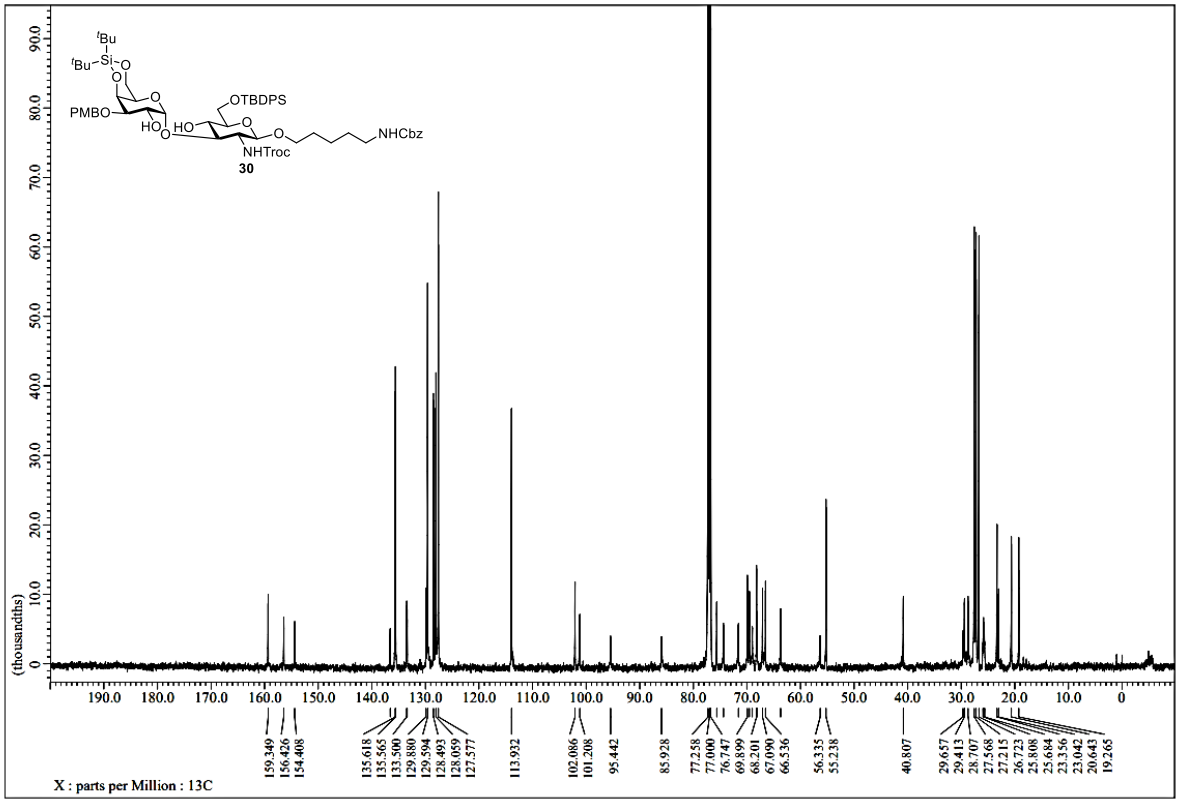


Figure S27 ^{13}C NMR spectrum of compound **30**

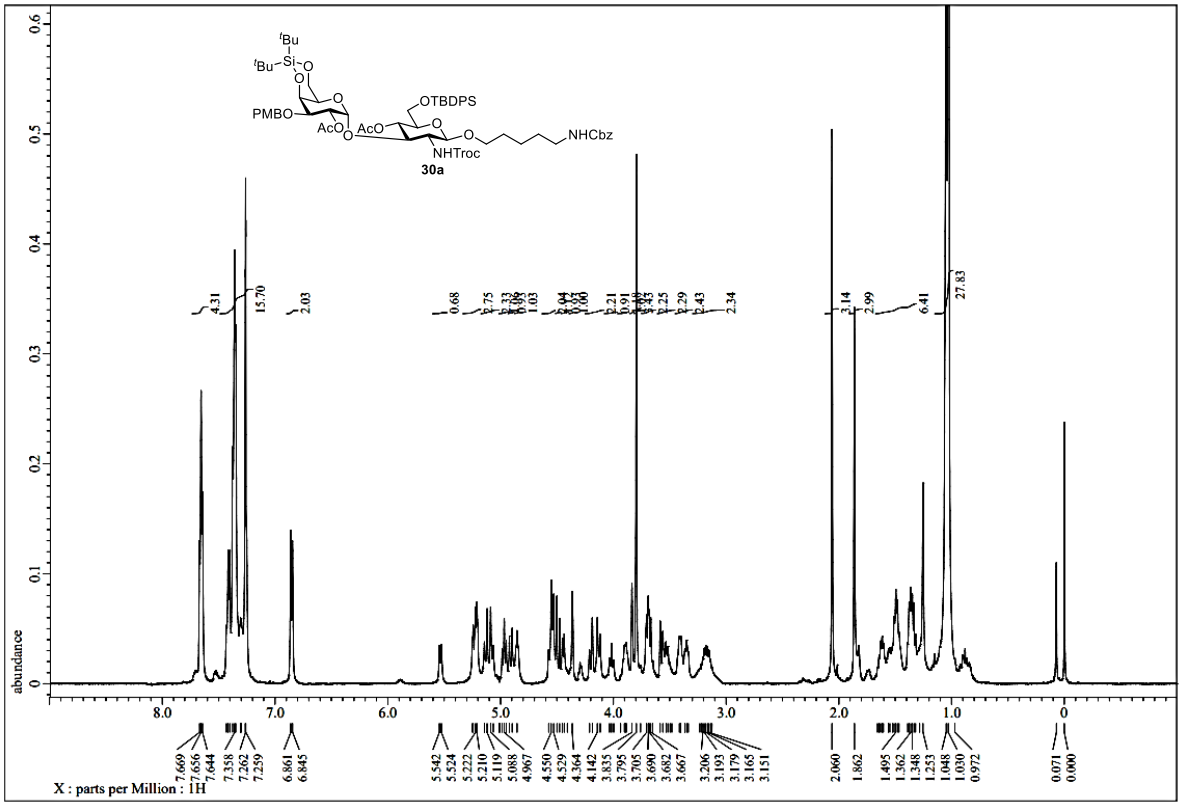


Figure S28 ^{1}H NMR spectrum of compound **30a**

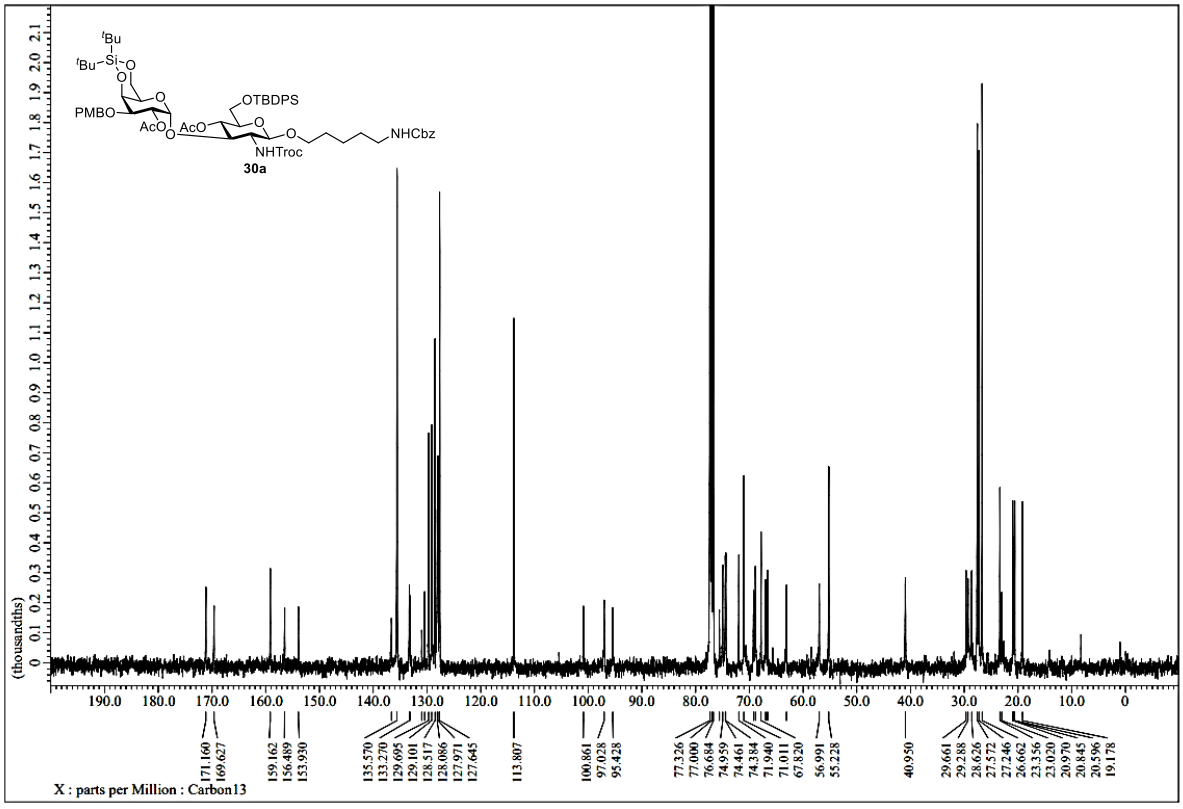


Figure S29 ^{13}C NMR spectrum of compound **30a**

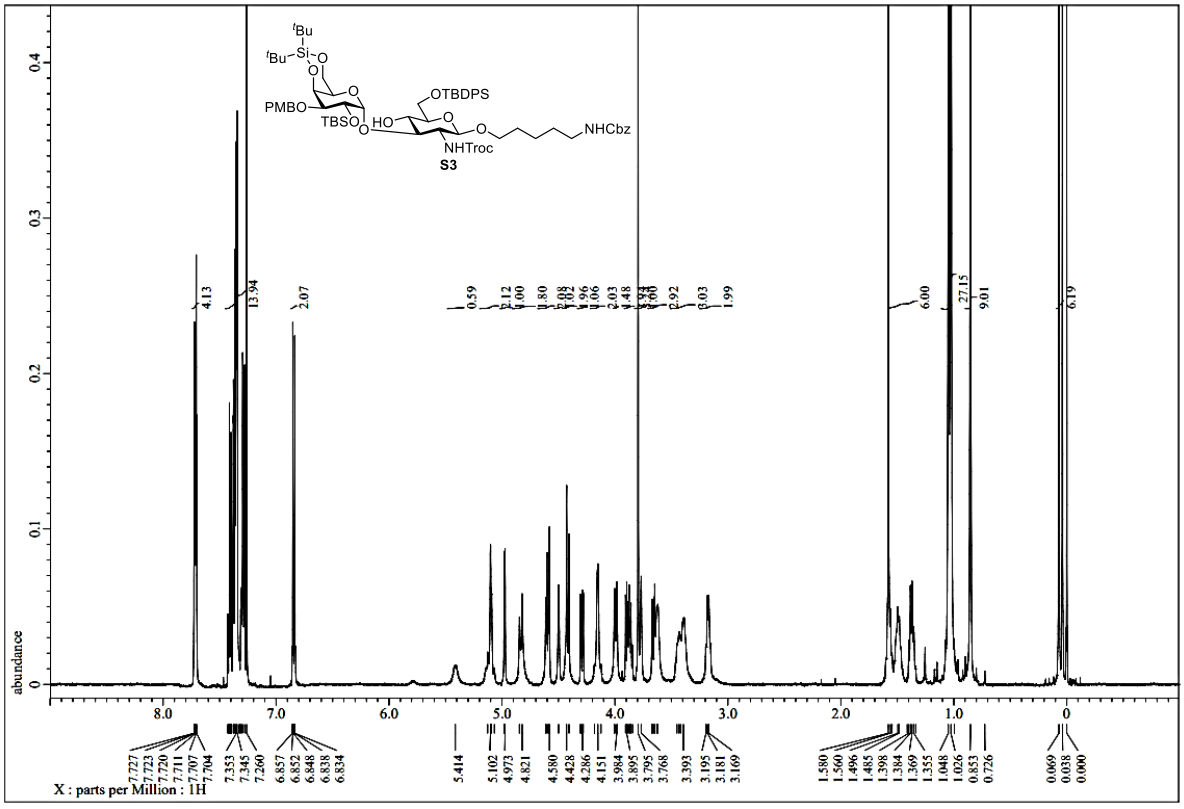


Figure S30 ^{1}H NMR spectrum of compound **S3**

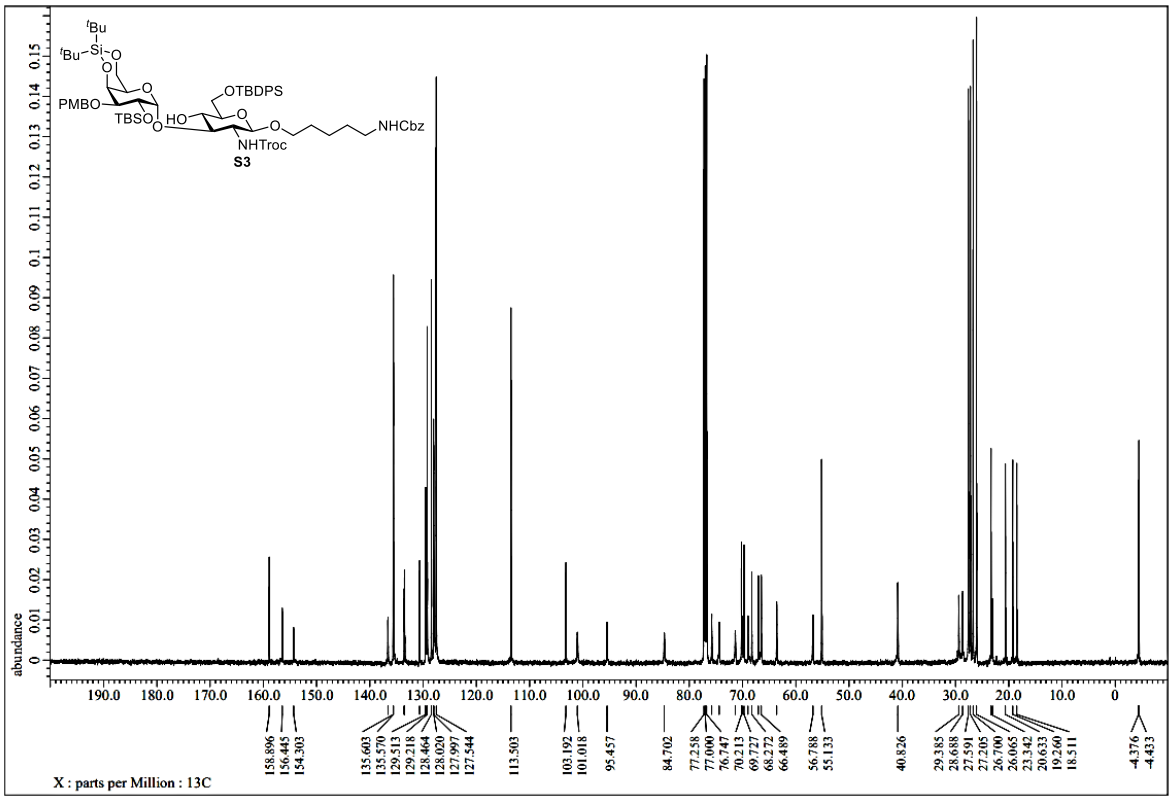


Figure S31 ^{13}C NMR spectrum of compound **S3**

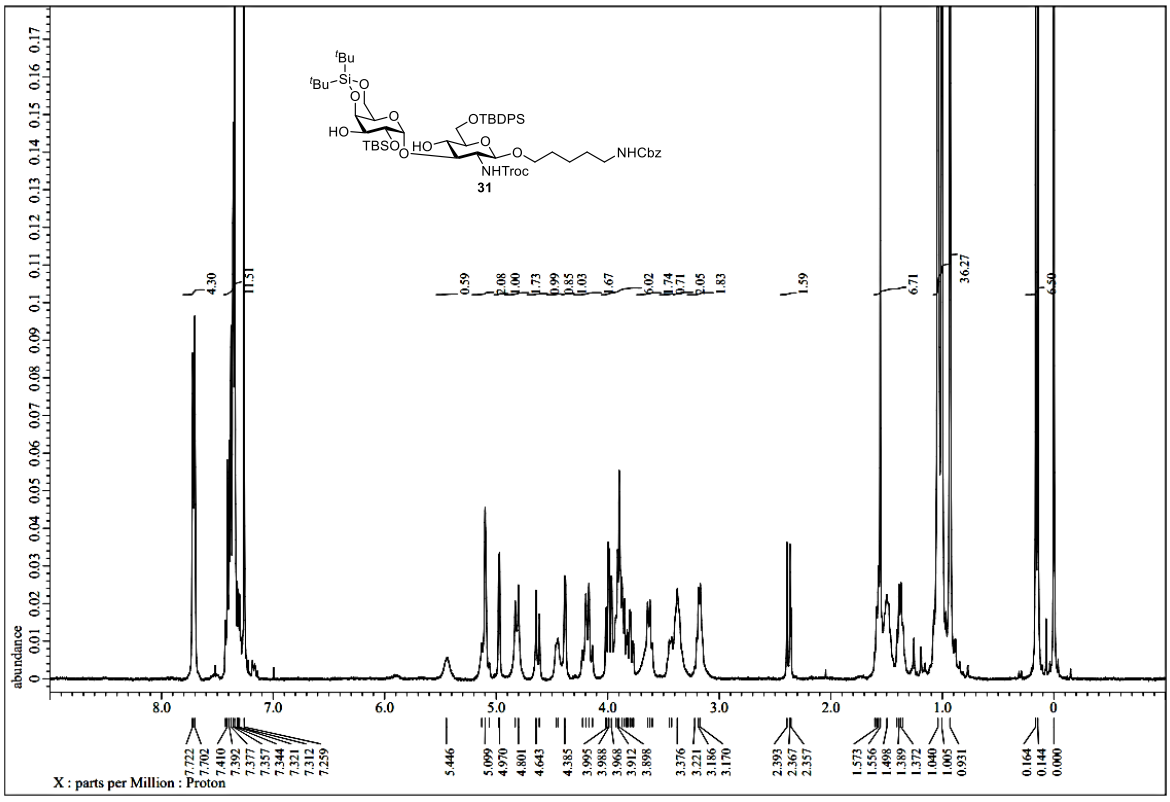


Figure S32 ^{1}H NMR spectrum of compound **31**

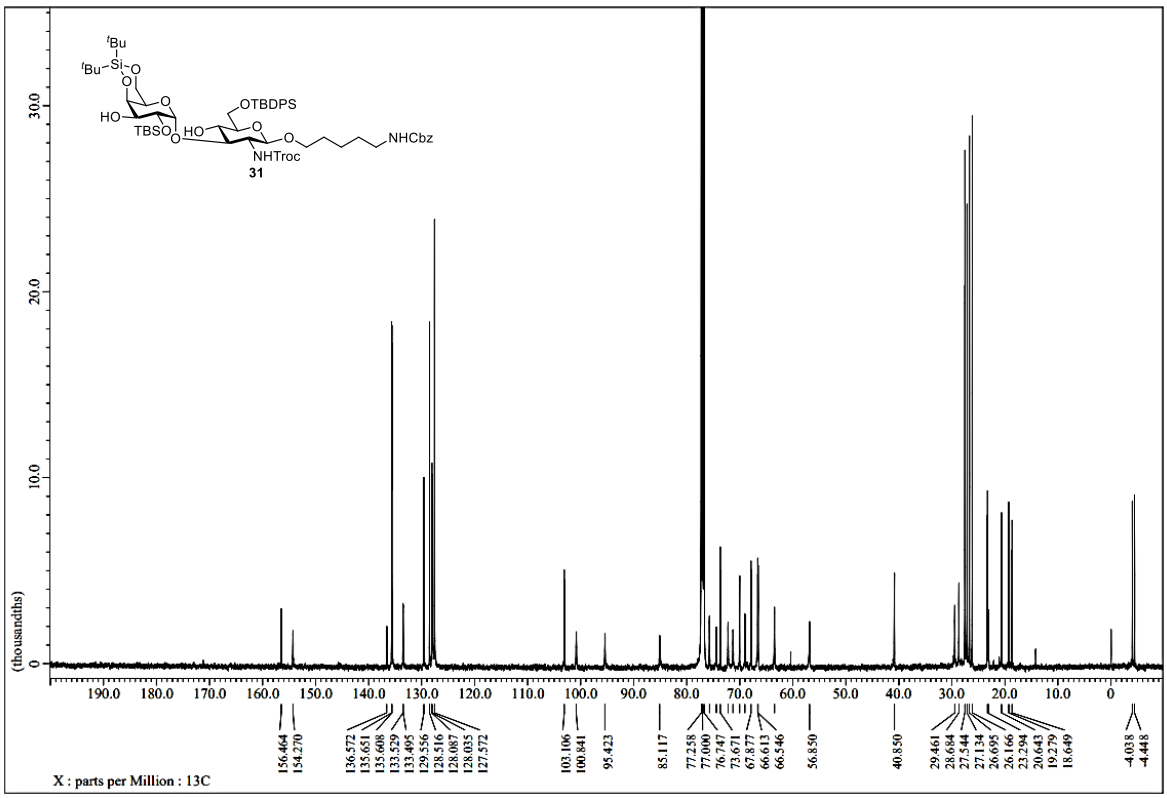


Figure S33 ^{13}C NMR spectrum of compound **31**

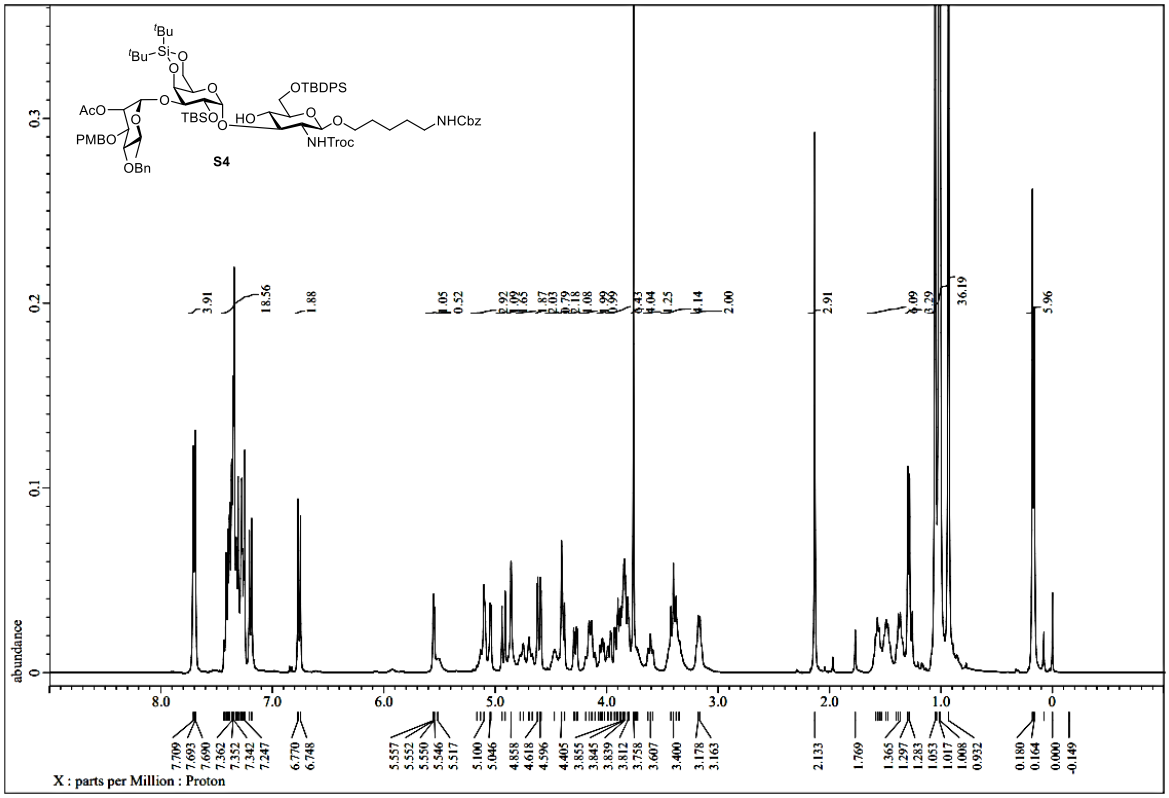


Figure S34 ^{1}H NMR spectrum of compound **S4**

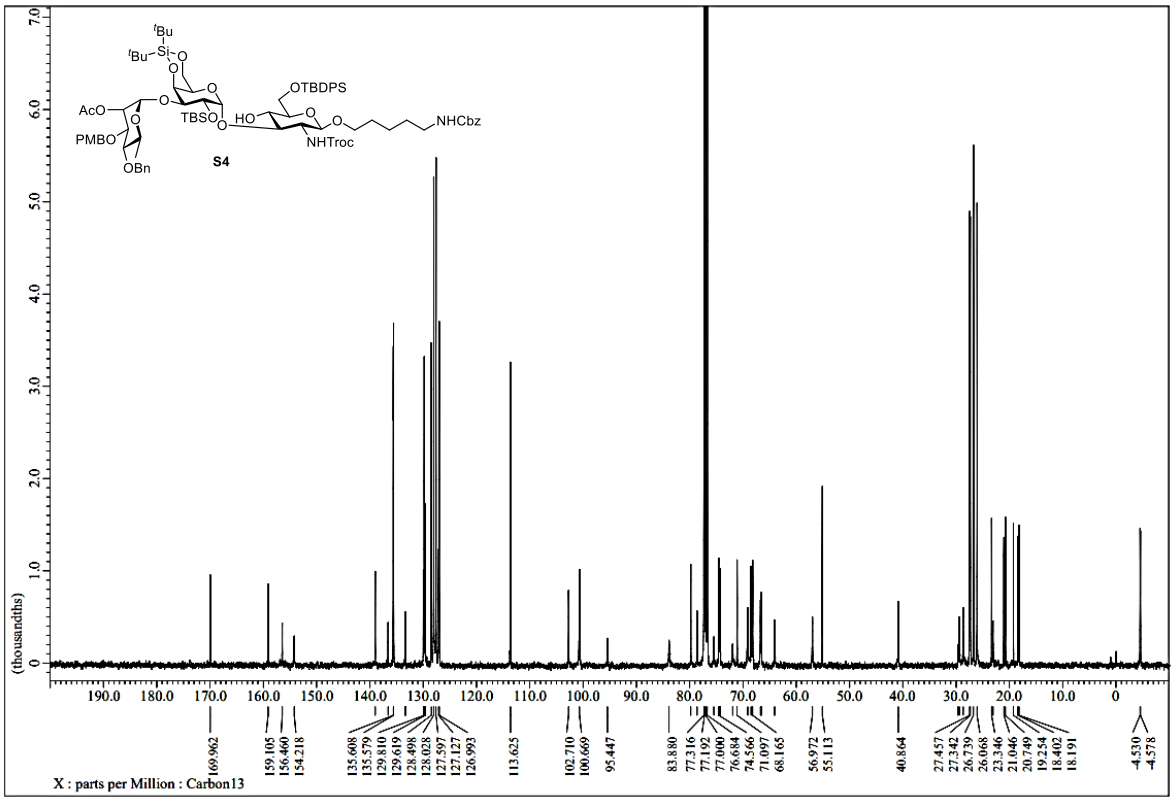


Figure S35 ^{13}C NMR spectrum of compound **S4**

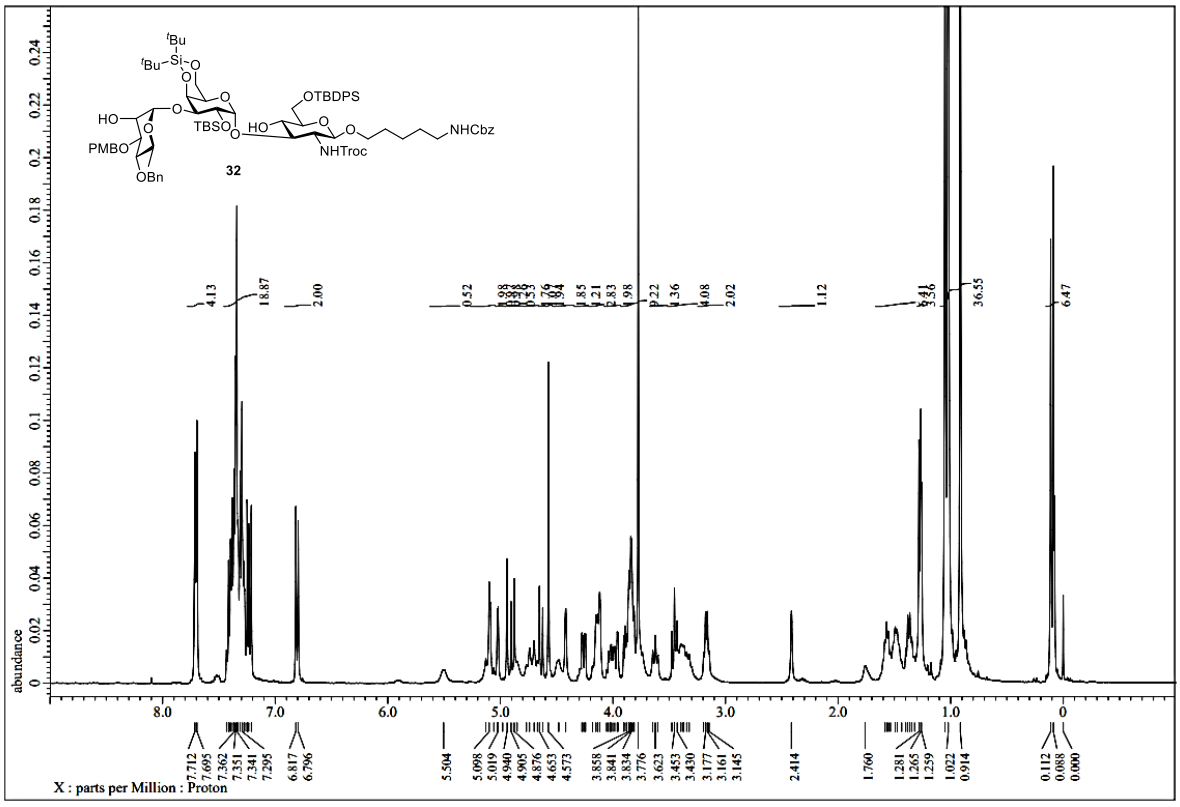


Figure S36 ^{1}H NMR spectrum of compound **32**

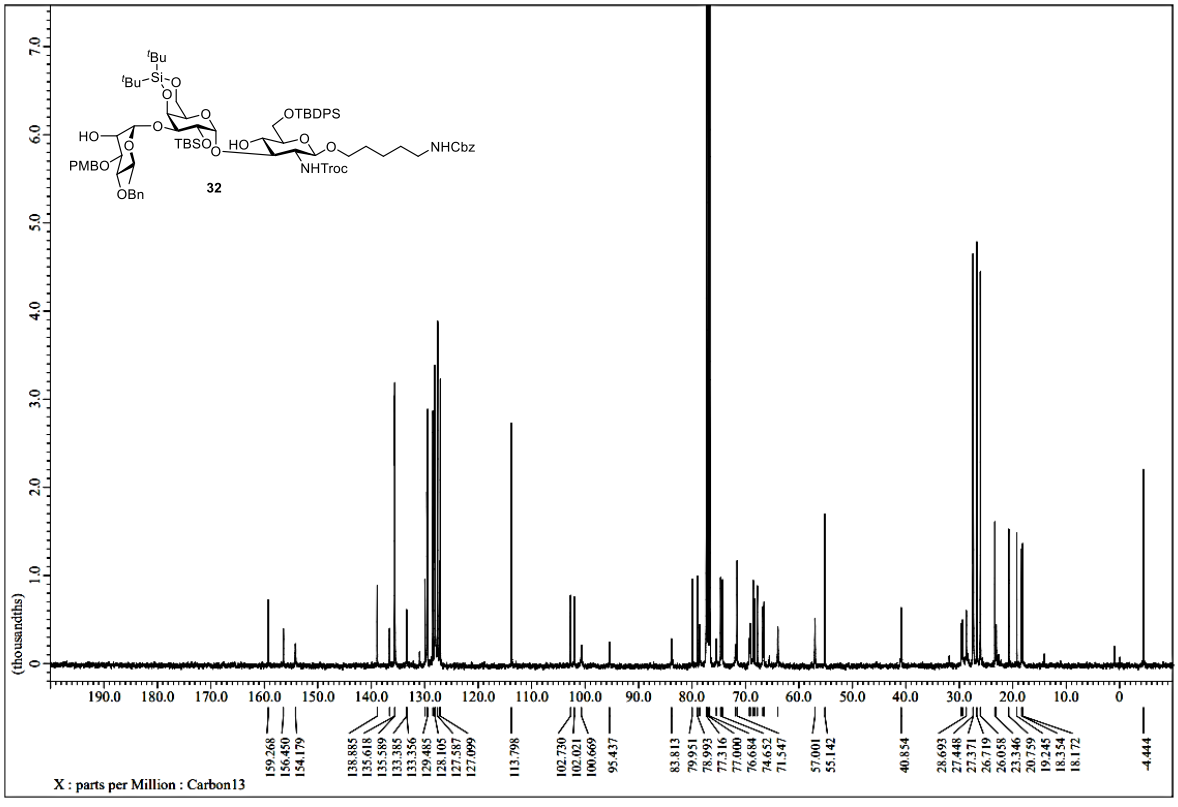


Figure S37 ^{13}C NMR spectrum of compound **32**

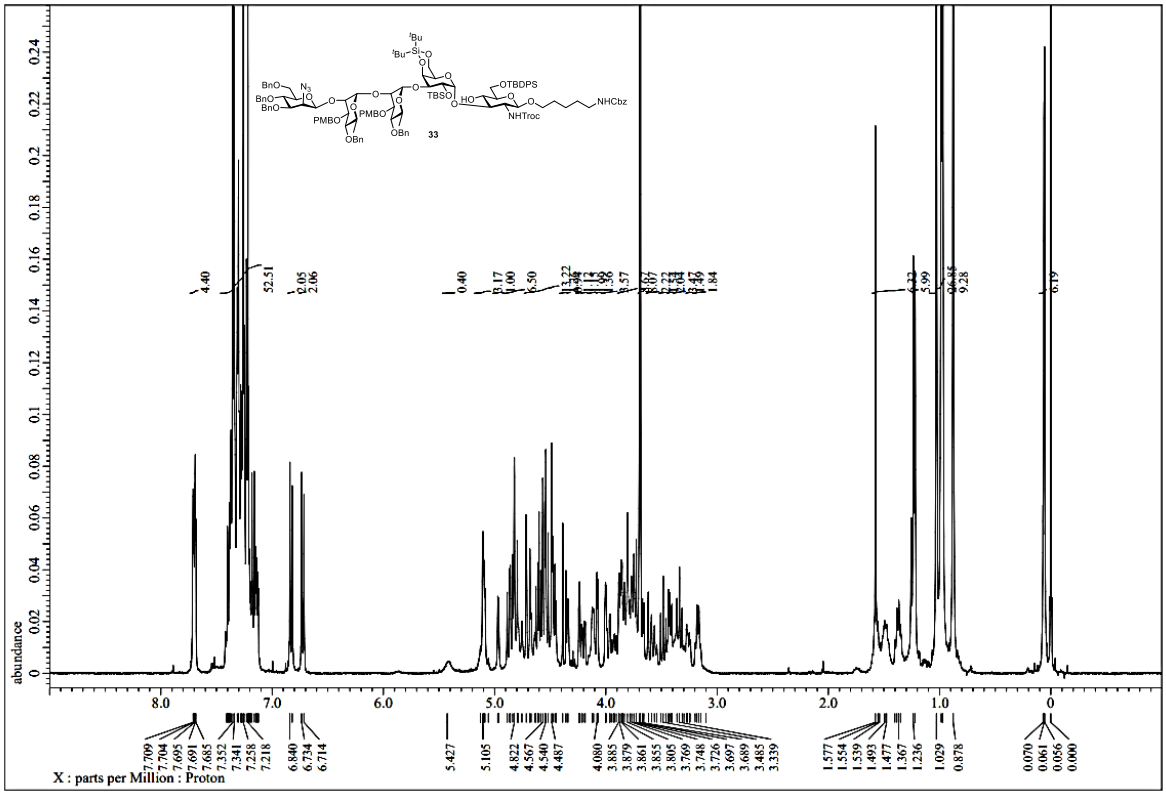


Figure S38 ^{1}H NMR spectrum of compound **33**

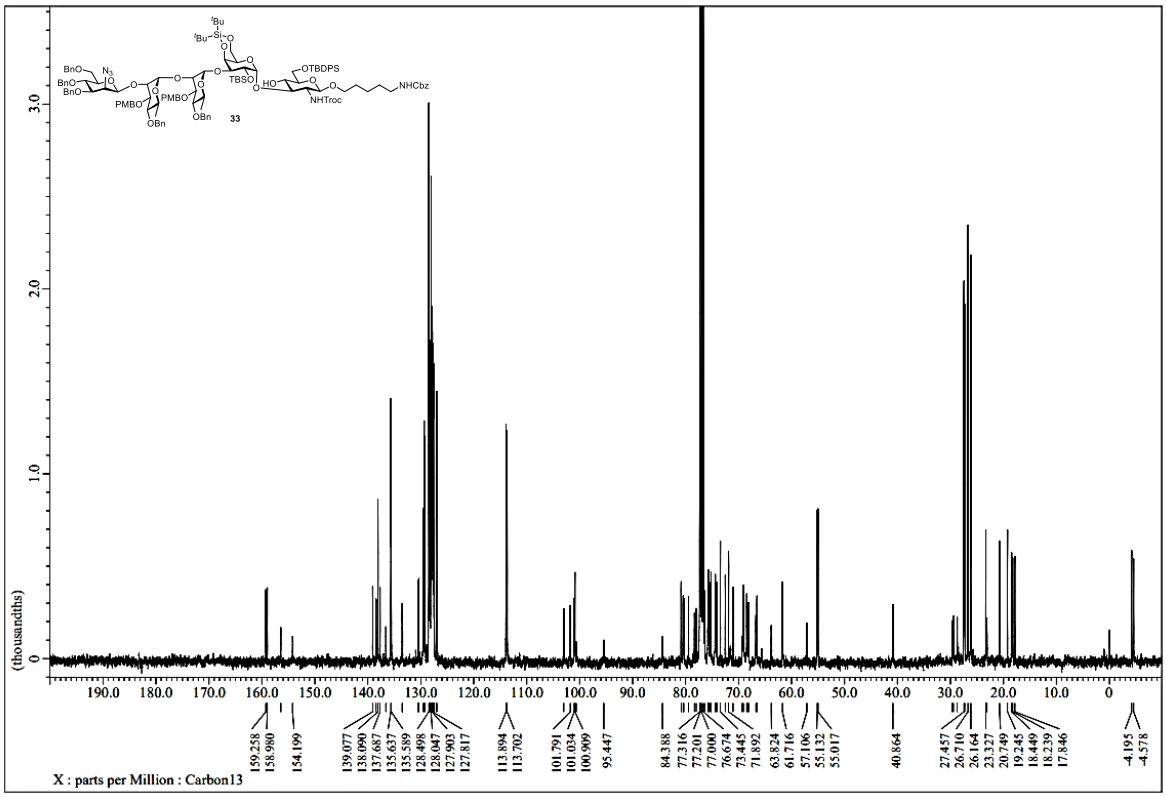


Figure S39 ^{13}C NMR spectrum of compound **33**

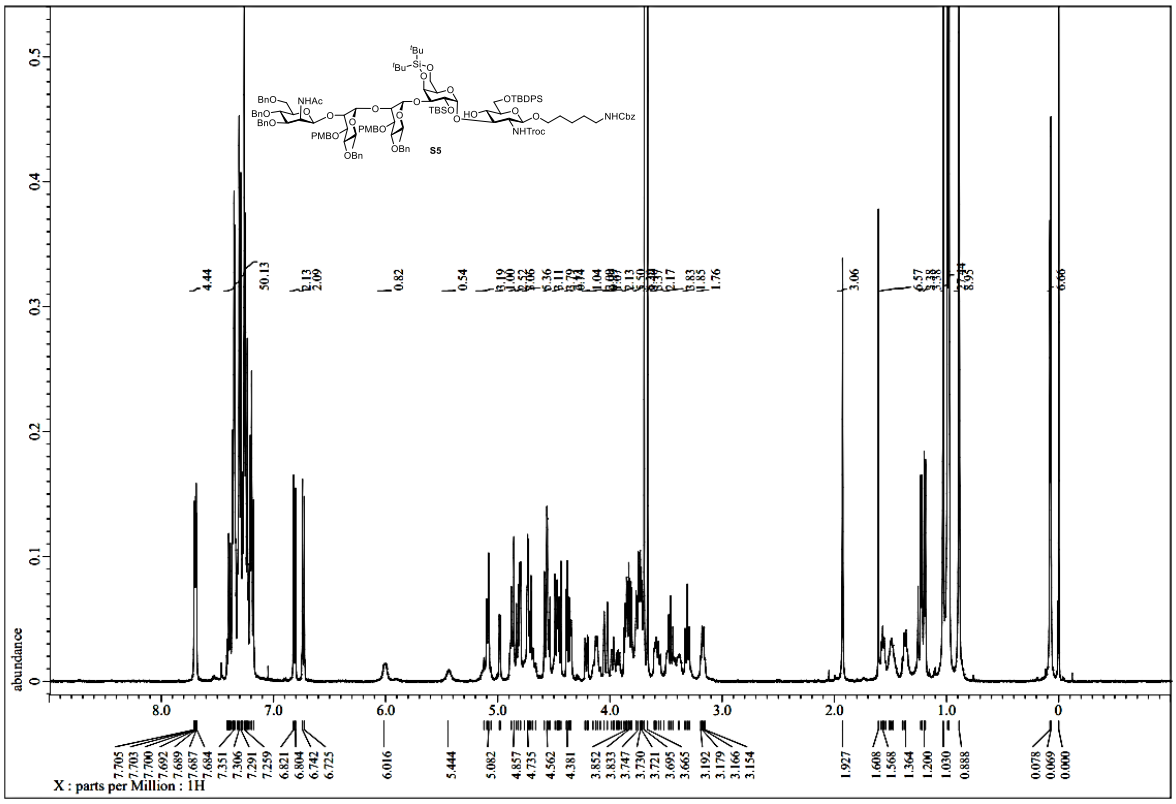


Figure S40 ^{1}H NMR spectrum of compound **S5**

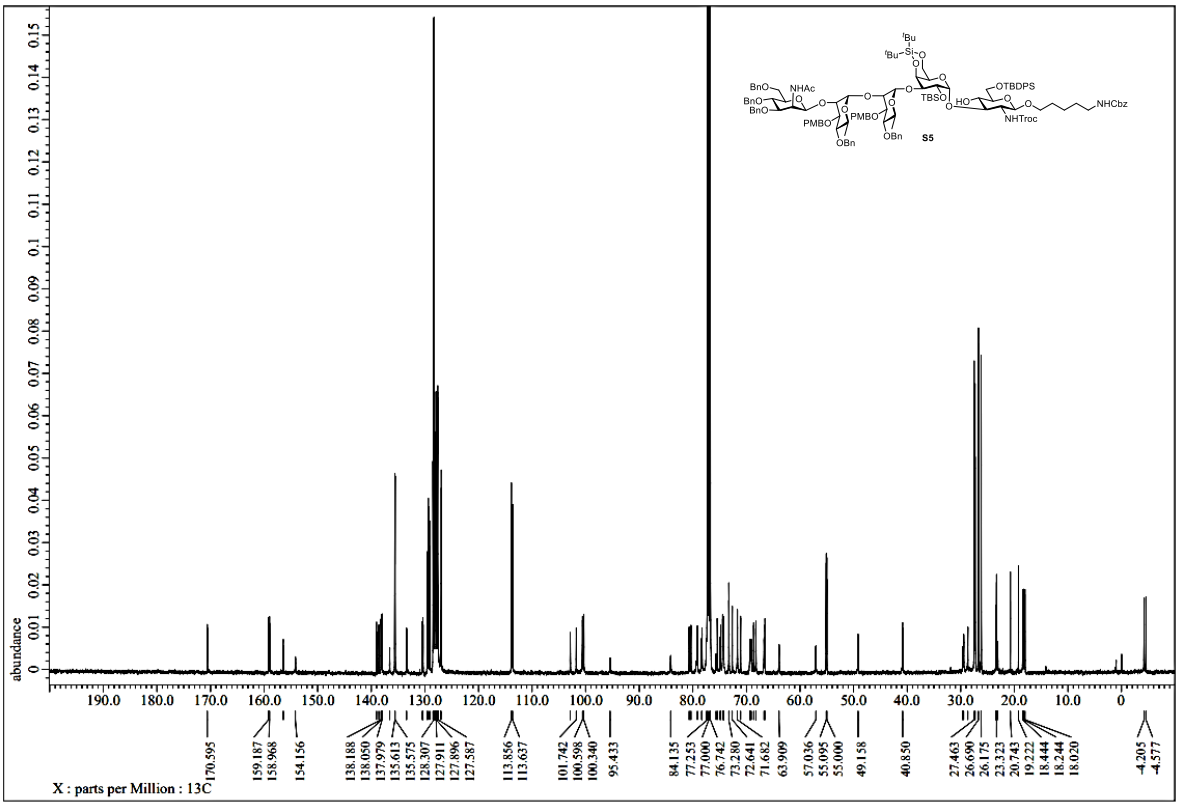


Figure S41 ^{13}C NMR spectrum of compound **S5**

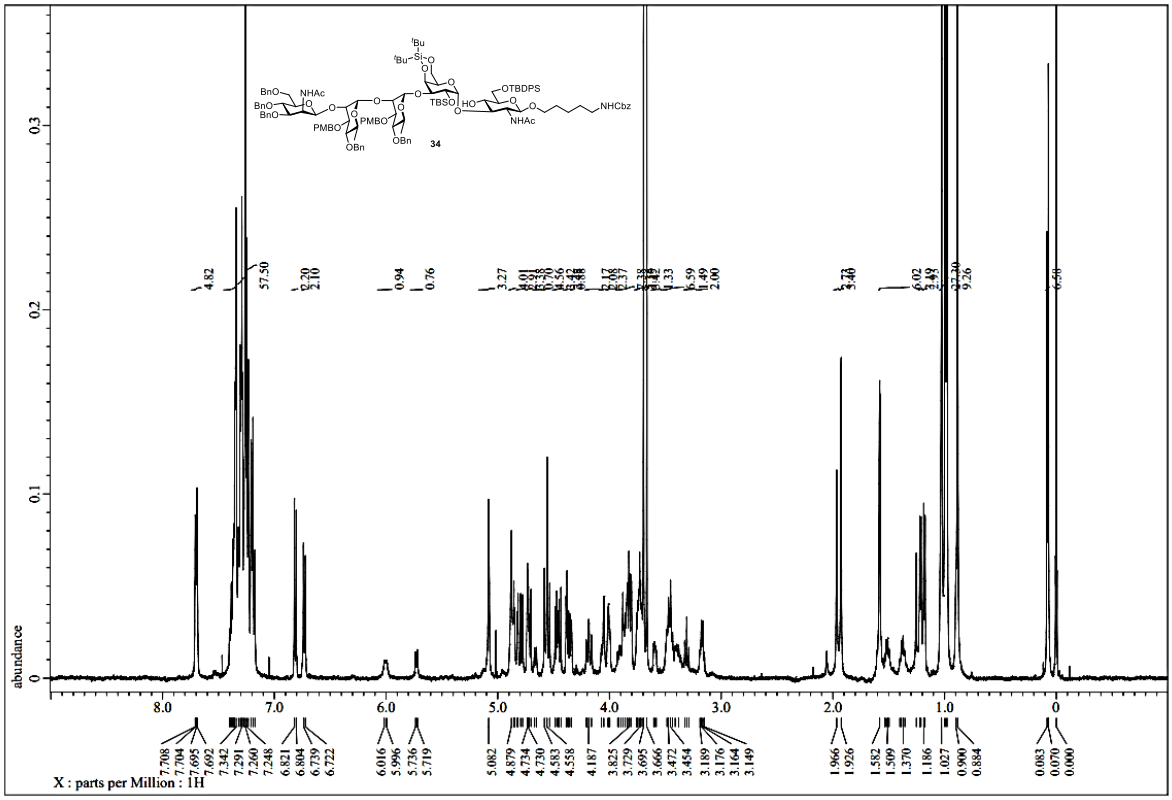


Figure S42 ^{1}H NMR spectrum of compound **34**

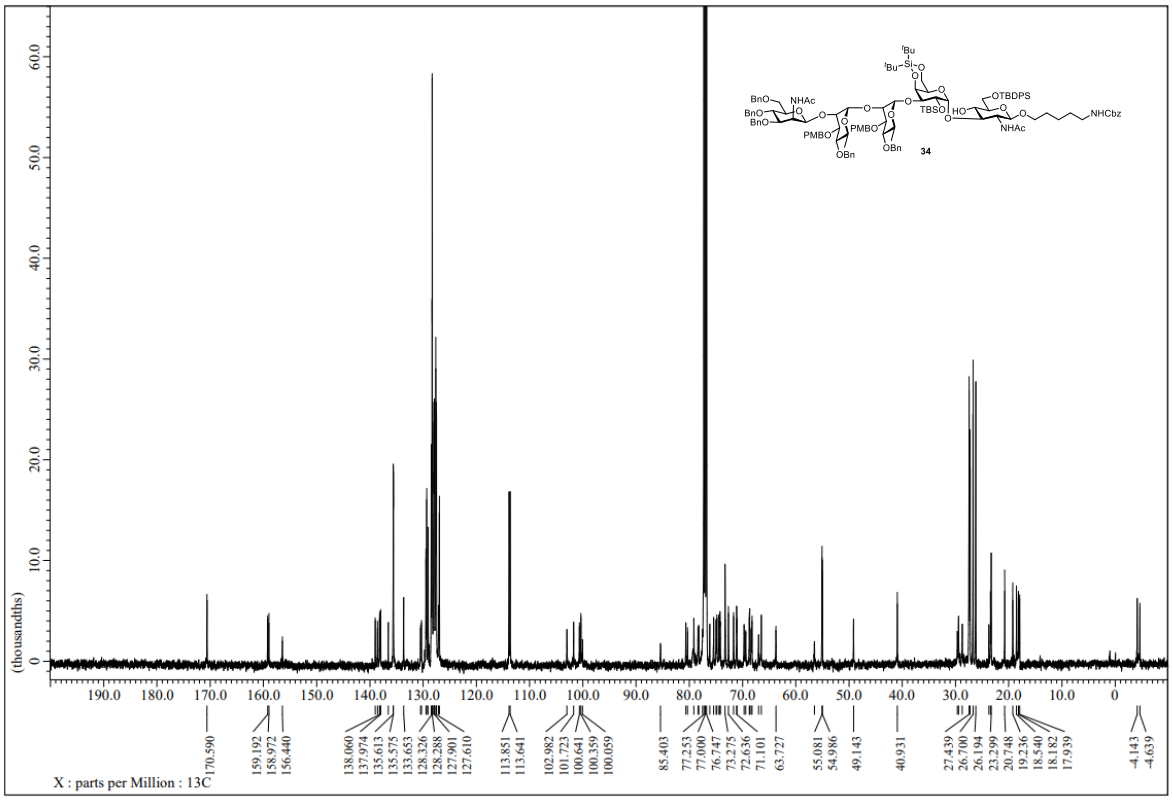


Figure S43 ^{13}C NMR spectrum of compound **34**

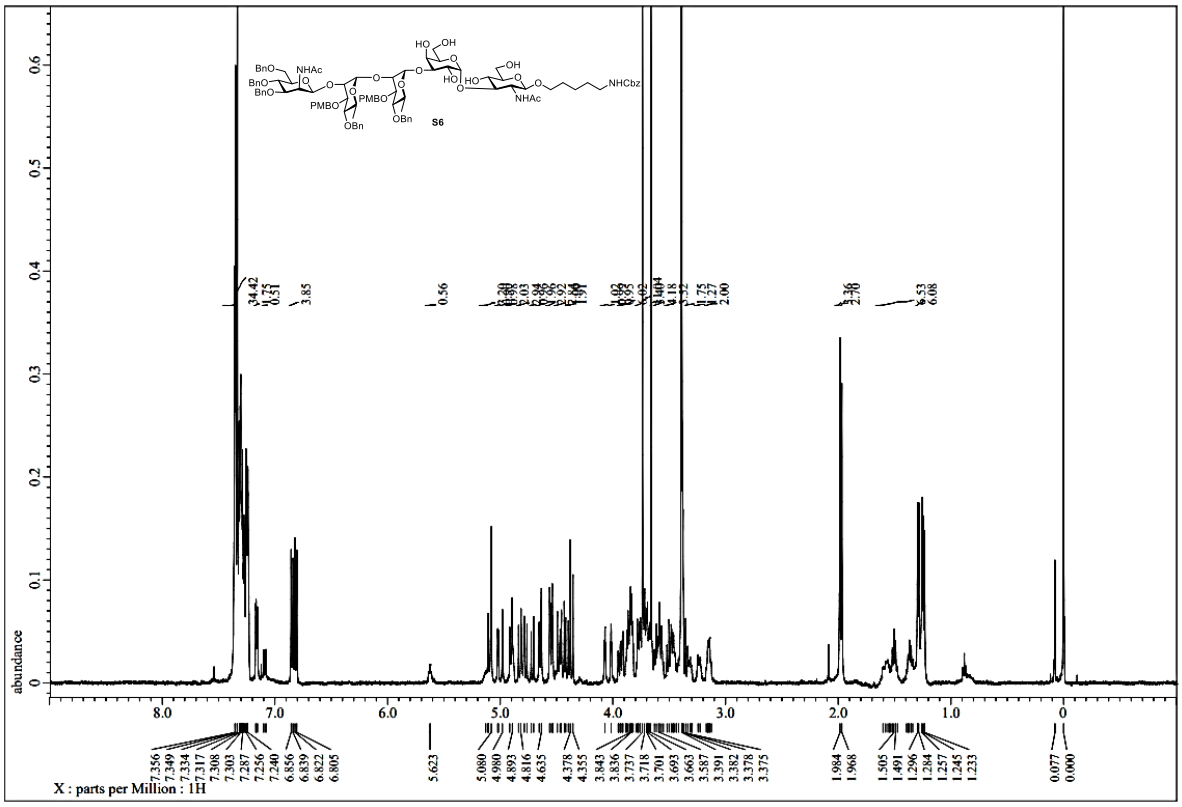


Figure S44 ^{1}H NMR spectrum of compound **S6**

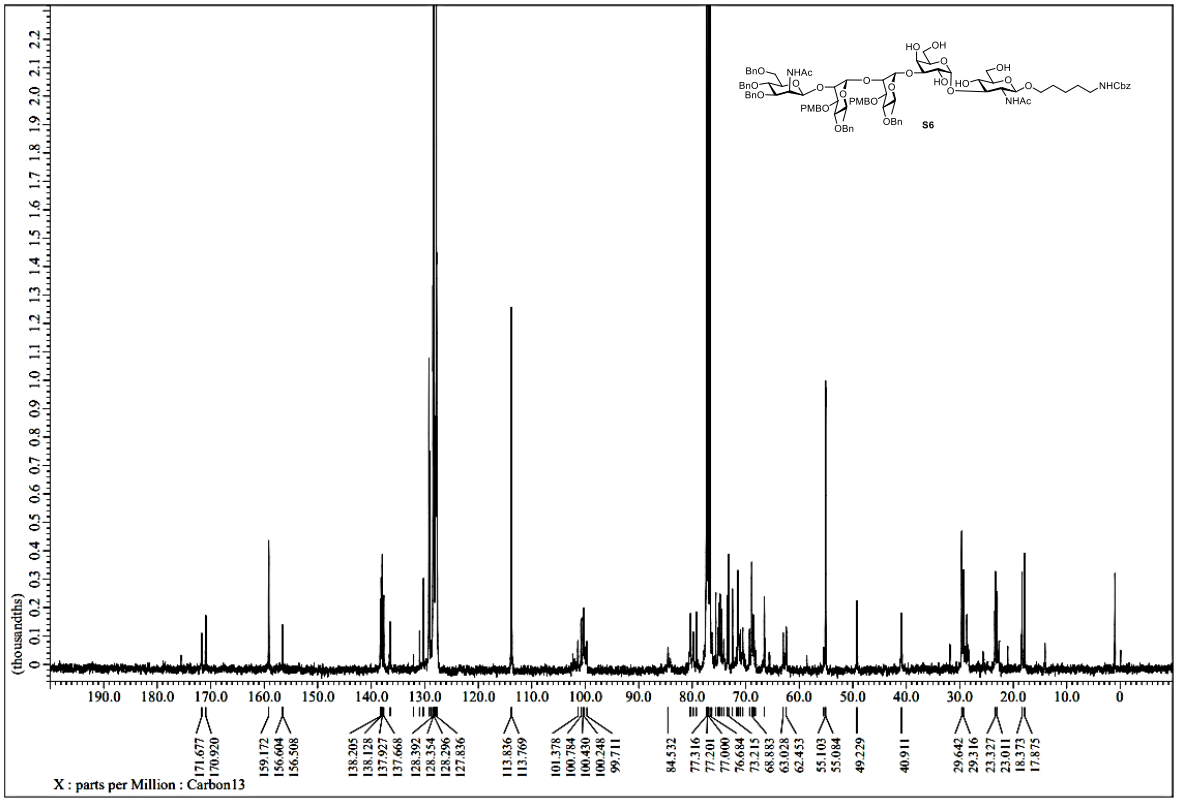


Figure S45 ^{13}C NMR spectrum of compound **S6**

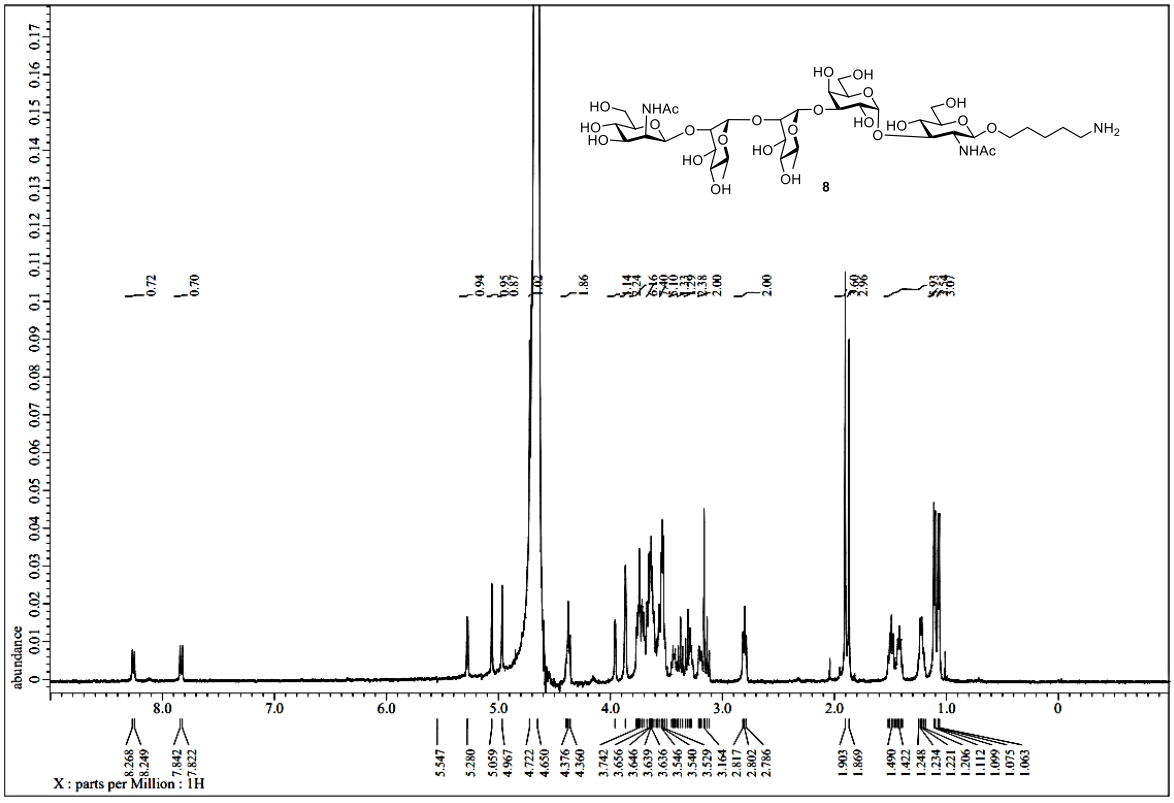


Figure S46 ^{1}H NMR spectrum of compound **8**

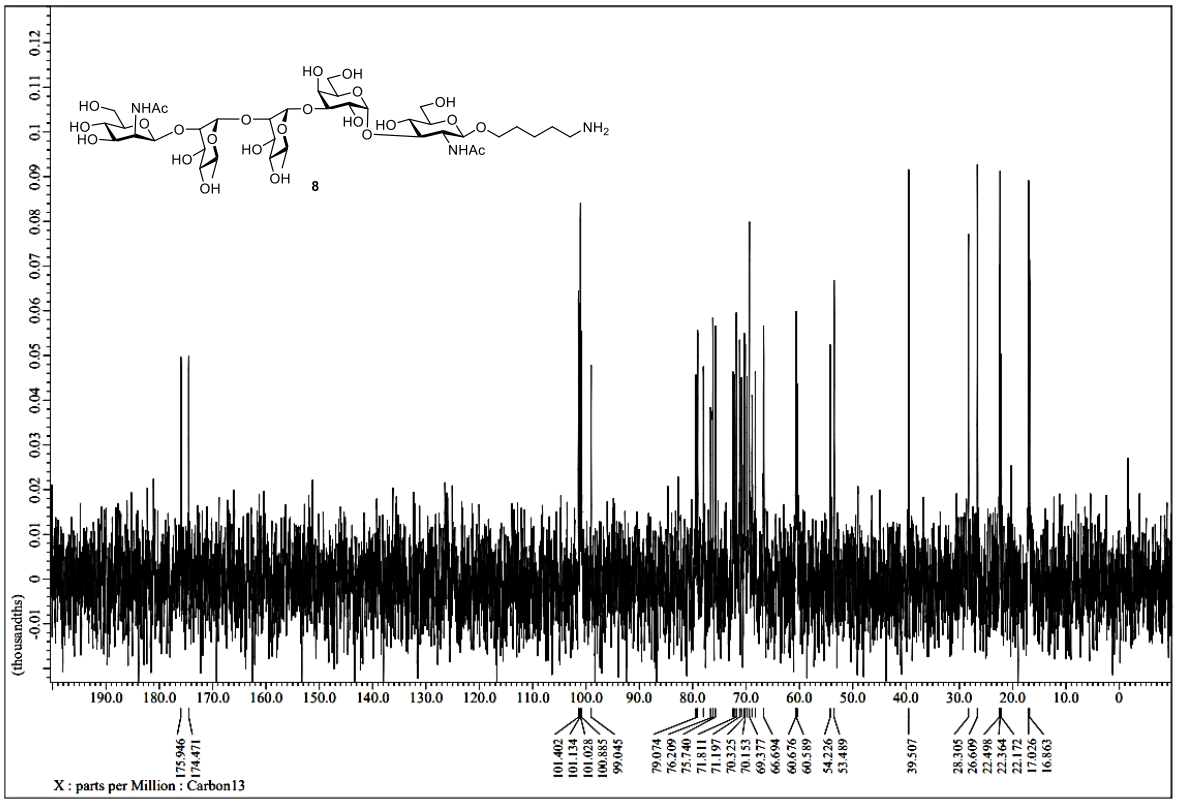


Figure S47 ^{13}C NMR spectrum of compound **8**