Electronic Supplementary Material (ESI) for RSC Chemical Biology.

Supporting Information

Development of lacto-series ganglioside fluorescent probe using late-stage sialylation and behavior analysis with single molecule imaging

Maina Takahashi,[a,b] Naoko Komura,*[c] Yukako Yoshida,[b] Eriko Yamaguchi,[c] Ami Hasegawa,[b] Hide-Nori Tanaka,[a,b,c] Akihiro Imamura,[a,b,c] Hideharu Ishida,[a,b,c,d] Kenichi G. N. Suzuki*[a,b,c] and Hiromune Ando*[a,b,c]

[a] The United Graduate School of Agricultural Science, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan.

[b] Department of Applied Bioorganic Chemistry, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan.

[c] Institute for Glyco-core Research (iGCORE), Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan. Email: komura@gifu-u.ac.jp, hando@gifu-u.ac.jp, kgsuzuki@gifu-u.ac.jp

[d] Center for Highly Advanced Integration of Nano and Life Sciences (G-CHAIN), Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan

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1. Materials and methods

1.1. Chemicals

All chemicals were purchased from commercial suppliers and used without further purification. Molecular sieves were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan) and predried at 300 °C for 2 h in a muffle furnace and then dried in a flask at 300 °C for 2 h in vacuo prior to use. Drierite was purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan) and predried at 230 °C for 2 h in a muffle furnace and then exposed to high vacuum. Dry solvents for reaction media (CH_2Cl_2, toluene, THF, MeCN, DMF, MeOH and pyridine) were purchased from Kanto Chemical Co. Inc. (Tokyo, Japan) and used without purification. Zn nanopowder (<50 nm particle size) were purchased from Sigma–Aldrich. ATTO594 *N*-succinimidyl ester was purchased from ATTO-Tec.

1.2. TLC analysis

TLC analyses were performed on Merck TLC plates (silica gel $60F_{254}$ on glass plate). Compound detection was either by exposure to UV light (253.6 nm) or by soaking in H_2SO_4 solution (10% in EtOH) or phosphomolybdic acid solution (20% in EtOH) followed by heating.

1.3. Chromatographic purification

Silica gel column chromatography separations were performed with a flash column chromatography system. Silica gel (80 mesh and 300 mesh; Fuji Silysia Co. (Aichi, Japan)) was used for flash column chromatography. The quantity of silica gel was typically 100 to 200 times the weight of the crude sample. Sephadex LH-20 purchased from Cytiva (Marlborough, USA) was used for size-exclusion chromatography. Solvent systems for chromatography are specified as *v*/*v* ratios.

1.4. Structural analysis and acquisition of physical data

^1H and ^{13}C NMR spectra were recorded on Avance III 500 and Avance III 800 spectrometers (Bruker, Billerica, MA, USA). Chemical shifts are expressed in ppm (δ) relative to Me_4Si signal (0.00 ppm), MeCN in CD_3CN (1.96 ppm) or $MeNO_2$ in CD_3NO_2 (4.34 ppm). Chemical shifts in the ^{13}C NMR spectra are expressed in ppm (δ) relative to the Me_4Si signal (0.00 ppm), $CDCl_3$ (77.36 ppm), CD_3OD (49.86 ppm), CD_3CN (1.79 ppm) or CD_3NO_2 (62.9 ppm). The sugar units are numbered using letters from *a* to *e*; Glc (*a*), reducing terminal Gal (*b*), GlcN (*c*), non-reducing terminal Gal (*d*) and Neu (*e*). Structural assignments were made with additional information from 2D NMR (^1H–^1H COSY, HMBC and HMQC).

High-resolution mass spectrometry (ESI-TOF MS) data were obtained with a mass spectrometer (micrOTOF, Bruker). Optical rotations were measured with a high-sensitivity polarimeter (SEPA-300 and SEPA-500, Horiba (Kyoto, Japan)). $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}.

1.5. General procedure for chemical reaction

All operations were carried out in a fume hood. All reactions were carried out under a positive pressure of argon unless otherwise noted. Evaporations and concentrations were carried out *in vacuo*. Microwave assisted reactions were carried out using an Initiator+ Eight microwave synthesizer (Biotage Japan, Tokyo, Japan) in sealed reaction vials (0.5-2 mL or 2-5 mL vial) under magnetic stirring (600 rpm) at 40 °C without pressure.

1.6. Pretreatment for glycosidation reaction (except sialylation)

A glycosyl donor and a glycosyl acceptor were mixed in a pear-shaped flask, and then residual water was azeotropically removed with dry toluene. The mixture was exposed to high vacuum for 3 h. Molecular sieves (100 mg was used for 1 mL of reaction solvent) were predried at 300 °C for 2 h in a muffle furnace. The predried molecular sieves were added to a two-necked, round-bottomed flask, and the flask was heated at 300 °C for 2 h *in vacuo*.

1.7. General procedure for glycosidation (except sialylation)

In a pear-shaped flask, a glycosyl donor and a glycosyl acceptor were dissolved in the reaction solvent, and the mixture was then transferred to a two-necked flask containing predried molecular sieves *via* cannula. After stirring for 1 h at −20 °C ~ 0 °C, promoters were added to the mixture at the same temperature. The progress of the

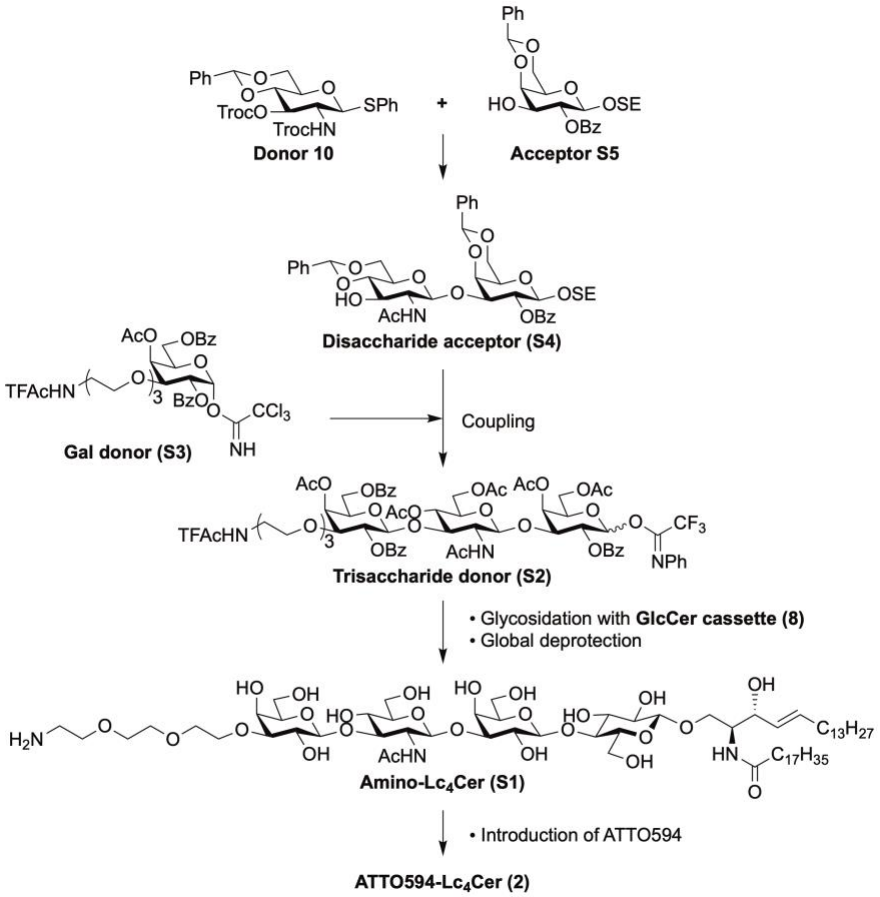
reaction was monitored by TLC analysis and/or mass spectrometry (MALDI-TOF performed with Autoflex, Bruker; matrix: CHCA). Then, the reaction mixture was quenched, filtered, subjected to an aqueous work up and then concentrated. The resulting residue was purified by size-exclusion chromatography or silica gel column chromatography. Isolated yields of the coupled products are reported.

1.8. General procedure for sialylation

In a two-necked round-bottomed flask, a sialyl donor and glycosyl acceptor were dissolved in the reaction solvent. Promoters were added to the mixture at the −70 °C. The progress of the reaction was monitored by TLC analysis and/or mass spectrometry (MALDI-TOF performed with Autoflex, Bruker; matrix: CHCA). Then, the reaction mixture was quenched, subjected to an aqueous work up and then concentrated. The resulting residue was purified by size-exclusion chromatography and silica gel column chromatography. Isolated yields of the coupled products are reported.

2. Synthesis of ATTO594-Lc$_4$Cer 2

2.1. Outline of fluorescent Lc$_4$Cer

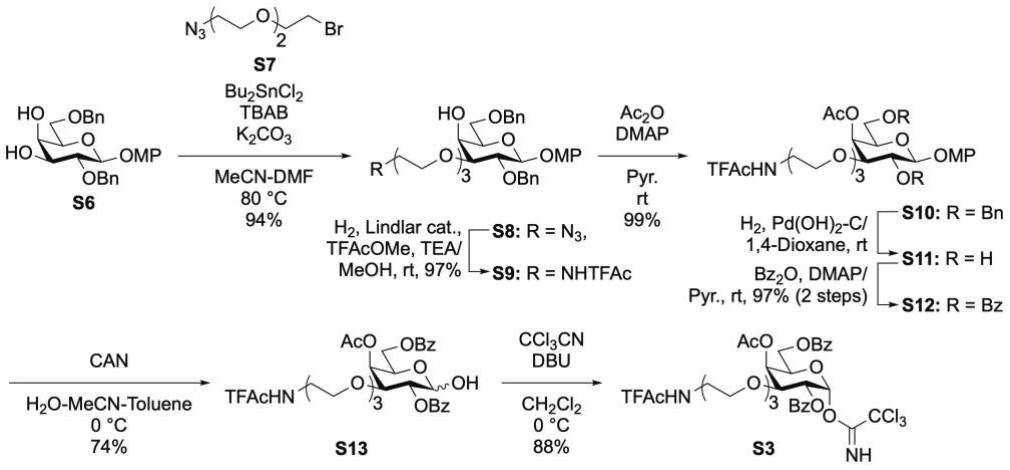


Scheme S1. Outline of fluorescent Lc$_4$Cer

2.2. Synthesis of Gal donor S3

The known galactoside derivative **S6**[1] was regioselectively linked with a spacer **S7**[2] at the C3 position *via* a stannyl acetal intermediate[3] to give **S8** in 94% yield. The azide group at the spacer moiety was then transformed into trifluoroacetamide, yielding **S9**. The C4 hydroxyl group was acetylated to give **S10**. The benzyl groups at the 2- and

6-OHs of **S10** were replaced with benzoyl groups over 2 steps, enabling β-linkage formation by neighboring group participation. Then, the anomeric hydroxyl group of **S12** was deprotected, and subsequent trichloroacetimidoylation yielded Gal donor **S3**



Scheme S2. Synthesis of Gal donor S3

***p*-Methoxyphenyl 3-*O*-{2-[2-(2-azidoethoxy)ethoxy]ethyl}-2,6-di-*O*-benzyl-β-D-galactopyranoside (S8)**. To a solution of **S6**[1] (3.00 g, 6.43 mmol) and spacer **S7**[2] in MeCN/DMF (58.5 mL/5.9 mL) were added TBAB (207 mg, 0.643 mmol), K_2CO_3 (1.33 g, 9.65 mmol), and *n*-Bu_2SnCl_2 (195 mg, 0.643 mmol) at room temperature.[3] After stirring for 67 h at 80 °C as the reaction was monitored by TLC (toluene/acetone = 3:1), the mixture was diluted with EtOAc, and washed with 10% KF aq and brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel using toluene/acetone (9:1) as the eluent to give **S8** (3.77 g, 94%): $[\alpha]_D$ −6.3 (c 2.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39–7.25 (m, 10 H, Ar), 7.06–7.02 (m, 2 H, Ar), 6.81–6.77 (m, 2 H, Ar), 4.97 (d, 1 H, J_{gem} = 11.0 Hz, PhCH_2), 4.83 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H-1), 4.82 (d, 1 H, PhCH_2), 4.60 (d, 1 H, J_{gem} = 11.5 Hz, PhCH_2), 4.56 (d, 1 H, PhCH_2), 4.10 (s, 1 H, H-4), 3.88–3.60 (m, 17 H, H-2, H-5, H-6a, H-6b, OCH_3, 5 CH_2), 3.46 (dd, 1 H, $J_{3,4}$ = 3.5 Hz, $J_{2,3}$ = 9.5 Hz, H-3), 3.36 (m, 2 H, CH_2), 3.12 (s, 1 H, OH-4); ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.2, 151.6, 138.7, 138.2, 128.4, 128.3, 128.0, 127.7, 127.7, 127.6, 118.6, 114.5, 102.8, 81.9, 78.5, 75.2, 73.7, 70.8, 70.6, 70.6, 70.0, 69.5, 69.1, 66.0, 55.6, 50.6; HRMS (ESI) *m/z*: found $[M+Na]^+$ 646.2735, $C_{33}H_{41}N_3O_9$ calcd for $[M+Na]^+$ 646.2735.

***p*-Methoxyphenyl 2,6-di-*O*-benzyl-3-*O*-{2-[2-(2-trifluoroacetamidoethoxy)ethoxy]ethyl}-β-D-galactopyranoside (S9)**. To a solution of **S8** (297 mg, 0.477 mmol) in MeOH (24 mL) were added triethylamine (664 μL, 4.77 mmol), TFAcOMe (240 μL, 2.39 mmol), and Lindlar catalyst [5 wt % $Pd/CaCO_3$ poisoned with lead] (101 mg) at room temperature. After stirring for 6.5 h at room temperature under H_2 atmosphere as the reaction was monitored by TLC (*n*-hexane/acetone = 2:1, developed twice), the solution was filtered through a pad of Celite, and the pad was washed with $CHCl_3$ and MeOH. The combined filtrate and washings were concentrated. The residue was purified by column chromatography on silica gel using *n*-hexane/acetone (2:1) as the eluent to give **S9** (322 mg, 97%): $[\alpha]_D$ −13.3 (c 2.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65 (s, 1 H, NH), 7.39–7.26 (m, 10 H, Ar), 7.05–7.02 (m, 2 H, Ar), 6.81–6.78 (m, 2 H, Ar), 4.97 (d, 1 H, J_{gem} = 11.5 Hz, PhCH_2), 4.82 (d, 1 H, $J_{1,2}$ = 7.5 Hz, H-1), 4.81 (d, 1 H, PhCH_2), 4.58 (d, 1 H, J_{gem} = 11.5 Hz, PhCH_2), 4.54 (d, 1 H, PhCH_2), 4.12 (d, 1 H, $J_{3,4}$ = 3.0 Hz, H-4), 3.84–3.42 (m, 21 H, H-2, H-3, H-5, H-6a, H-6b, OCH_3, 6 CH_2, OH-4); ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.5 (q, $^2J_{C,F}$ = 37.5 Hz, *C*(O)CF_3), 155.3, 151.6, 138.7, 138.0, 128.4, 128.3, 128.0, 127.7, 127.6, 118.7, 115.9 (q, $^1J_{C,F}$ = 286.3 Hz, C(O)*C*F_3), 114.5, 102.8, 81.8, 78.2, 77.6, 75.2, 73.7, 73.4, 70.3, 70.1, 70.0, 69.3, 69.0, 68.6, 65.8, 55.6, 39.8; HRMS (ESI) *m/z*: found $[M+Na]^+$ 716.2653, $C_{35}H_{42}F_3NO_{10}$ calcd for $[M+Na]^+$ 716.2653.

***p*-Methoxyphenyl 4-*O*-acetyl-2,6-di-*O*-benzyl-3-*O*-{2-[2-(2-trifluoroacetamidoethoxy)ethoxy]ethyl}-β-D-galactopyranoside (S10)**. To a solution of **S9** (4.30 g, 6.20 mmol) in pyridine (62.0 mL) were added Ac_2O (2.3 mL, 24.8 mmol) and DMAP (37.9 mg, 310 μmol) at 0 °C. After stirring for 5.5 h at room temperature as the reaction was monitored by TLC (*n*-hexane/acetone = 1:1), MeOH was added to the reaction mixture at 0 °C and the solution was co-evaporated with toluene. The mixture was diluted with EtOAc, and washed with 2 M HCl, H_2O, satd aq $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel using *n*-hexane/EtOAc (2:1 to 1:1) as the eluent to give **S10** (4.53 g, 99%): $[\alpha]_D$ −12.1 (c 1.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37–7.25 (m, 11 H, NH, Ar), 7.05–7.01 (m, 2 H, Ar), 6.81–6.78 (m, 2 H, Ar), 5.54 (d, 1 H, $J_{3,4}$ = 3.5 Hz, H-4), 4.96 (d, 1 H, J_{gem} = 11.0 Hz, PhCH_2), 4.87 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H-1), 4.83 (d, 1 H, PhCH_2), 4.54 (d, 1 H, J_{gem} = 12.0 Hz, PhCH_2), 4.46 (d, 1 H, PhCH_2), 3.89–3.46 (m, 20 H, H-2, H-3, H-5, H-6a, H-6b, OCH_3, 6 CH_2), 2.11 (s, 3 H, Ac); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4, 157.1 (q, $^2J_{C,F}$ = 37.5 Hz, *C*(O)CF_3), 155.4, 151.5, 138.5, 137.6, 128.4, 128.3, 127.9, 127.9, 127.8, 127.6, 118.4, 115.9 (q, $^1J_{C,F}$ = 286.1 Hz, C(O)*C*F_3), 114.5, 102.9, 80.6, 78.6, 75.3, 73.7, 72.5, 70.9, 70.4, 70.4, 70.1, 68.7, 68.3, 67.2, 55.6, 39.7, 20.9; HRMS (ESI) *m/z*: found $[M+Na]^+$ 758.2759, $C_{37}H_{44}F_3NO_{11}$ calcd for $[M+Na]^+$ 758.2759.

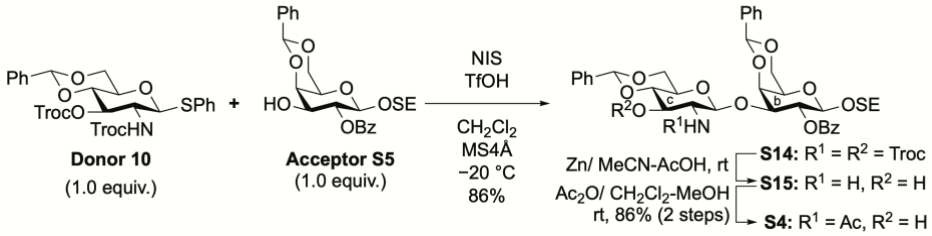
***p*-Methoxyphenyl 4-*O*-acetyl-2,6-di-*O*-benzoyl-3-*O*-{2-[2-(2-trifluoroacetamidoethoxy)ethoxy]ethyl}-β-D-galactopyranoside (S12)**. To a solution of **S10** (2.05 g, 2.79 mmol) in 1,4-dioxane (54.4 mL) was added palladium hydroxide [20 wt % Pd (dry basis) on carbon, wet] (764 mg) at room temperature. After stirring for 3.5 h at room temperature under H_2 atmosphere as the reaction was monitored by TLC (*n*-hexane/acetone = 1:1), the mixture was filtered through a pad of Celite, and the pad was washed with $CHCl_3$. The combined filtrate and washings were concentrated. The resulting residue (**S11**) was exposed to high vacuum for 6 h and then dissolved in pyridine (54.4 mL). To the solution were added Bz_2O (6.15 g, 27.2 mmol) and DMAP (33.2 mg, 272 μmol) at 0 °C. After stirring for 6.5 h at room temperature as the reaction was monitored by TLC (*n*-hexane/acetone = 1:1), MeOH was added to the reaction mixture at 0 °C and the solution was co-evaporated with toluene. The mixture was diluted with $CHCl_3$, and washed with 2 M HCl, H_2O, satd aq $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel using *n*-hexane/EtOAc (4:1 to 3:2) as the eluent to give **S12** (2.06 g, 97%, two steps): $[\alpha]_D$ +2.5 (c 1.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07–8.05 (m, 4 H, Ar), 7.63–7.57 (m, 2 H, Ar), 7.49–7.44 (m, 4 H, Ar), 7.16 (s, 1 H, NH), 6.92–6.89 (m, 2 H, Ar), 6.66–6.63 (m, 2 H, Ar), 5.67 (d, 1 H, $J_{3,4}$ = 3.0 Hz, H-4), 5.63 (dd, 1 H, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 5.02 (d, 1 H, H-1), 4.54 (dd, 1 H, $J_{5,6a}$ = 7.5 Hz, J_{gem} = 11.5 Hz, H-6a), 4.48 (dd, 1 H, $J_{5,6b}$ = 7.5 Hz, H-6b), 4.13 (t, 1 H, H-5), 3.82 (dd, 1 H, H-3), 3.79–3.30 (m, 15 H, OCH_3, 6 CH_2), 2.24 (s, 3 H, Ac); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.5, 166.1, 165.2, 157.3 (q, $^2J_{C,F}$ = 37.5 Hz, *C*(O)CF_3), 155.6, 151.3, 133.4, 133.2, 129.8, 129.8, 129.7, 129.5, 128.5, 128.5, 118.8, 115.9 (q, $^1J_{C,F}$ = 275.3 Hz, C(O)*C*F_3), 114.4, 101.1, 79.0, 71.3, 71.3, 70.7, 70.6, 70.5, 70.2, 68.5, 66.7, 62.4, 55.6, 39.6, 20.9; HRMS (ESI) *m/z*: found $[M+Na]^+$ 786.2344, $C_{37}H_{40}F_3NO_{13}$ calcd for $[M+Na]^+$ 786.2344.

4-*O*-Acetyl-2,6-di-*O*-benzoyl-3-*O*-{2-[2-(2-trifluoroacetamidoethoxy)ethoxy]ethyl}-D-galactopyranose (S13). To a solution of **S12** (501 mg, 655 μmol) in MeCN/toluene/H_2O (5.6 mL/4.7 mL/2.8 mL) was added CAN (3.59 g, 6.55 mmol) at 0 °C. After stirring for 1.5 h at 0 °C as the reaction was monitored by TLC (toluene/EtOAc = 3:2, developed three times), the reaction mixture was diluted with EtOAc, and washed with H_2O, satd aq $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel using *n*-hexane/EtOAc (5:4 to 1:1) as the eluent to give **S13** (318 mg, 74%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09–8.04 (m, 10 H, Ar), 7.24 (s, 1 H, NH), 5.70 (d, 1 H, $J_{3,4}$ = 3.5 Hz, H-4$^\alpha$), 5.65 (m, 1 H, H-4$^\beta$), 5.63 (t, 1 H, $J_{1,2}$ = $J_{1,OH}$ = 3.5 Hz, H-1$^\alpha$), 5.33 (dd, 1 H, $J_{2,3}$ = 10.5 Hz, H-2$^\alpha$), 5.22 (dd, 1 H, $J_{1,2}$ = 8.5 Hz, $J_{2,3}$ = 10.0 Hz, H-2$^\beta$), 4.80 (t, 1 H, H-1$^\beta$), 4.57 (t, 1 H, $J_{5,6a}$ = $J_{5,6b}$ = 6.5 Hz, H-5$^\alpha$), 4.53 (dd, 1 H, $J_{5,6a}$ = 6.5 Hz, J_{gem} = 11.5 Hz, H-6a$^\beta$), 4.48 (dd, 1 H, J_{gem} = 11.0 Hz, H-6a$^\alpha$), 4.39 (dd, 1 H, $J_{5,6b}$ = 6.5 Hz, H-6b$^\beta$), 4.33 (dd, 1 H, H-6b$^\alpha$), 4.17 (dd, 1 H, H-3$^\alpha$), 4.07 (t, 1 H, H-5$^\beta$), 3.85–3.35 (m, 14 H, OH-1$^\beta$, H-3$^\beta$, 6 CH_2), 3.05 (s, 1 H, OH-1$^\alpha$), 2.20–2.18 (s, 3 H, Ac); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4, 166.2, 166.0, 157.2 (q, $^2J_{C,F}$ = 36.9 Hz, *C*(O)CF_3), 133.3, 133.3, 129.8, 129.8, 129.6, 128.5, 128.5, 115.9 (q, $^1J_{C,F}$ = 285.7 Hz, C(O)*C*F_3), 96.2, 91.0, 74.2, 70.9, 70.7, 70.5, 70.3, 70.2, 70.1, 68.7, 68.5, 68.0, 66.9, 66.8, 62.6, 39.7, 39.6, 20.8; HRMS (ESI) *m/z*: found $[M+Na]^+$ 680.1925, $C_{30}H_{34}F_3NO_{12}$ calcd for $[M+Na]^+$ 680.1925.

4-*O*-Acetyl-2,6-di-*O*-benzoyl-3-*O*-{2-[2-(2-trifluoroacetamidoethoxy)ethoxy]ethyl}-α-D-galactopyranosyl trichloroacetimidate (S3). To a solution of **S13** (105 mg, 160 μmol) in CH_2Cl_2 (3.2 mL) were added CCl_3CN (320 μL, 3.19 mmol) and DBU (28.7 μL, 192 μmol) at 0 °C. After stirring for 3 h at 0 °C as the reaction was monitored by TLC (toluene/EtOAc = 1:1), the solution was concentrated. The residue was purified by column chromatography on silica gel using toluene/EtOAc (5:2) as the eluent to give **S3** (112 mg, 88%): $[\alpha]_D$ +71.6 (c 1.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.56 (s, 1 H, C(=NH)CCl_3), 8.03–7.99 (m, 4 H, Ar), 7.59–7.56 (m, 2 H, Ar), 7.45–7.41 (m, 4 H, Ar), 7.15 (s, 1 H, NHTFAc), 6.68 (d, 1 H, $J_{1,2}$ = 3.5 Hz, H-1), 5.78 (d, 1 H, $J_{3,4}$ = 3.0 Hz, H-4), 5.58 (dd, 1 H, $J_{2,3}$ = 10.0 Hz, H-2), 4.56 (t, 1 H, $J_{5,6a}$ = $J_{5,6b}$ = 6.5 Hz, H-5), 4.46 (dd, 1 H, J_{gem} = 11.5 Hz, H-6a), 4.39 (dd, 1 H, H-6b), 4.18 (dd, 1 H, H-3), 3.88–3.38 (m, 12 H, 6 CH_2), 2.21 (s, 3 H, Ac); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3, 166.1, 165.5, 160.5, 157.2 (q, $^2J_{C,F}$ = 37.5 Hz, *C*(O)CF_3), 133.4, 133.3, 129.7, 129.5, 129.4, 128.5, 128.4, 115.9 (q, $^1J_{C,F}$ = 286.3 Hz, C(O)*C*F_3), 93.9, 90.9, 74.8, 70.7, 70.5, 70.4, 70.2, 69.7, 69.1, 68.6, 67.3, 62.4, 39.6, 20.8; HRMS (ESI) *m/z*: found $[M+Na]^+$ 823.1021, $C_{32}H_{34}Cl_3F_3N_2O_{12}$ calcd for $[M+Na]^+$ 823.1022.

2.3. Synthesis of disaccharide acceptor S4

The coupling reaction of known GlcN donor **10**[4] and Gal acceptor **S5**[4] was carried out in the presence of NIS, TfOH, and 4 Å molecular sieves at −20 °C, giving disaccharide **S14** in 86% yield. Then, the Troc groups at C2-NH_2 and C3-OH were chemoselectively removed using Zn and AcOH, and subsequent acetylation yielded disaccharide acceptor **S4**.



Scheme S3. Synthesis of disaccharide acceptor S4

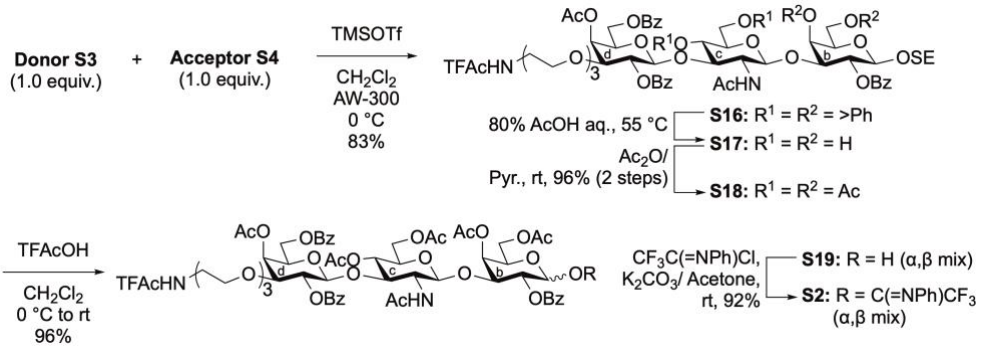
2-(Trimethylsilyl)ethyl [4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbamoyl)-3-*O*-(2,2,2-trichloroethoxycarbonyl)-β-D-glucopyranosyl]-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside (S14). MS 4Å (424 mg) and NIS (35.8 mg, 159 μmol) were added to a solution of donor **10**[4] (75.3 mg, 106 μmol) and acceptor **S5**[4] (50.0 mg, 106 μmol) in CH_2Cl_2 (4.2 mL) at room temperature. After stirring for 1 h at −20 °C, TfOH (1.4 μL, 11 μmol) was added to the mixture at −20 °C. The reaction mixture was stirred for 20 min at −20 °C as the reaction was monitored by TLC (*n*-hexane/EtOAc = 3:2). The reaction mixture was quenched with triethylamine and filtered through a pad of Celite, and the pad was washed with $CHCl_3$. The combined filtrate and washings were washed with satd aq $Na_2S_2O_3$ and brine, dried over Na_2SO_4, and concentrated. The resulting residue was purified by column chromatography on silica gel, using toluene/EtOAc (10:1) as the eluent, to give **S14** (98.6 mg, 86%): $[\alpha]_D$ −5.8 (c 1.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08–7.30 (m, 15 H, 3 Ar), 5.62 (dd, 1 H, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 10.5 Hz, H-2^b), 5.58 (s, 1 H, >C*H*Ph), 5.39 (s, 1 H, >C*H*Ph), 5.24–5.18 (m, 3 H, H-1^c, H-3^c, NH), 4.64 (m, 2 H, CH_2CCl_3), 4.56 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H-1^b), 4.50 (d, 1 H, J_{gem} = 12.0 Hz, CH_2CCl_3), 4.36–4.34 (m, 2 H, H-4^b, H-6a^b), 4.31 (dd, 1 H, $J_{5,6a}$ = 4.8 Hz, J_{gem} = 10.3 Hz, H-6a^c), 4.14 (dd, 1 H, $J_{3,4}$ = 3.0 Hz, H-3^b), 4.09 (d, 1 H, J_{gem} = 11.5 Hz, H-6b^c), 4.00–3.98 (m, 1 H, $SiCH_2CH_2$), 3.76 (d, 1 H, CH_2CCl_3), 3.64–3.48 (m, 6 H, H-2^c, H-4^c, H-5^c, H-5^b, H-6b^b, $SiCH_2CH_2$), 0.84–0.79 (m, 2 H, $SiCH_2CH_2$), −0.10 (s, 9 H, $SiMe_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.4, 154.0, 153.8, 137.7, 136.9, 133.5, 130.4, 130.2, 129.8, 129.5, 128.8, 128.8, 128.5, 126.8, 126.4, 101.7, 101.4, 100.2, 95.7, 94.7, 78.8, 78.3, 75.7, 74.3, 70.0, 69.4, 68.6, 67.4, 66.8, 66.2, 57.3, 18.3, −1.2; HRMS (ESI) *m/z*: found $[M+Na]^+$ 1092.0890, $C_{44}H_{49}Cl_6NO_{15}Si$ calcd for $[M+Na]^+$ 1092.0895.

2-(Trimethylsilyl)ethyl (2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside (S4). To a solution of **S14** (100 mg, 93.2 μmol) in MeCN/AcOH (3.0

mL/750 µL) was added Zn nanopowder (790 mg, 12.1 mmol) at room temperature. After stirring for 2 h at room temperature as the reaction was monitored by TLC ($CHCl_3$/MeOH = 20:1), the reaction mixture was filtered through a pad of Celite, and the pad was washed with THF and EtOAc. The combined filtrate and washings were diluted with EtOAc, and washed with satd aq $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The residue was exposed to high vacuum for 12 h. Next, to a solution of the resulting residue (**S15**) in CH_2Cl_2/MeOH (2.5 mL/1.2 mL) was added Ac_2O (27 µL, 280 µmol) at room temperature. After stirring for 3.5 h at room temperature, as the reaction was monitored by TLC ($CHCl_3$/MeOH = 20:1), the reaction mixture was quenched with EtOH and concentrated. The residue was purified by column chromatography on silica gel, using toluene/EtOAc (2:1) as the eluent, to give **S4** (60.8 mg, 86%, two steps): $[\alpha]_D$ −19.4 (c 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06–7.32 (m, 15 H, 3 Ar), 5.94 (d, 1 H, $J_{NH,2}$ = 3.3 Hz, NH), 5.61–5.57 (m, 2 H, H-2^b, >C*H*Ph), 5.53 (s, 1 H, >C*H*Ph), 5.44 (d, 1 H, $J_{OH,3}$ = 2.0 Hz, OH), 4.65 (d, 1 H, $J_{1,2}$ = 8.5 Hz, H-1^b), 4.64 (d, 1 H, $J_{1,2}$ = 8.5 Hz, H-1^c), 4.38 (dd, 1 H, $J_{5,6a}$ = 1.5 Hz, J_{gem} = 12.5 Hz, H-6a^b), 4.32–4.29 (m, 2 H, H-4^b, H-6a^c), 4.12 (dd, 1 H, $J_{5,6b}$ = 1.3 Hz, H-6b^b), 4.07–4.01 (m, 2 H, H-3^b, $SiCH_2CH_2$), 3.85 (dd, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, H-3^c), 3.76 (t, 1 H, $J_{5,6b}$ = J_{gem} = 10.5 Hz, H-6b^c), 3.59–3.54 (m, 3 H, H-5^b, H-4^c, $SiCH_2CH_2$), 3.42–3.36 (m, 2 H, H-2^c, H-5^c), 1.81 (s, 3 H, Ac), 0.92–0.81 (m, 2 H, $SiCH_2CH_2$), −0.09 (s, 9 H, $SiMe_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.1, 166.4, 138.0, 137.3, 134.0, 130.1, 129.8, 129.4, 129.0, 128.5, 128.5, 126.8, 126.6, 102.1, 101.6, 101.4, 100.8, 81.7, 76.5, 72.0, 71.2, 69.2, 68.8, 67.3, 66.8, 66.7, 60.0, 30.0, 23.2, 23.0, 18.3, 14.4, −1.1; HRMS (ESI) *m*/*z*: found $[M+Na]^+$ 786.2916, $C_{40}H_{49}NO_{12}Si$ calcd for $[M+Na]^+$ 786.2916.

2.4. Synthesis of trisaccharide donor S2

We carried out the glycosylation of disaccharide acceptor **S4** with Gal donor **S3**, producing a trisaccharide derivative **S16** in 83%. Then, **S16** was converted into trisaccharyl donor *via* four-step manipulation of the protecting groups to afford **S2** efficiently.



Scheme S4. Synthesis of trisaccharide donor S2

2-(Trimethylsilyl)ethyl (4-*O*-acetyl-2,6-di-*O*-benzoyl-3-*O*-{2-[2-(2-trifluoroacetamidoethoxy)ethoxy]ethyl}-β-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside (S16). Molecular sieves AW-300 (260 mg) were added to a solution of donor **S3** (52.2 mg, 65.1 µmol) and acceptor **S4** (49.7 mg, 65.1 µmol) in CH_2Cl_2 (2.6 mL) at room temperature. After stirring for 1 h at 0 °C, TMSOTf (1.2 µL, 6.5 µmmol) was added to the mixture at 0 °C. The reaction mixture was stirred for 45 min at 0 °C as the reaction was monitored by TLC ($CHCl_3$/MeOH = 20:1). The reaction mixture was quenched with triethylamine and filtered through a pad of Celite, and the pad was washed with $CHCl_3$. The combined filtrate and washings were washed with satd $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The resulting residue was purified by gel filtration column chromatography on Sephadex LH-20, using $CHCl_3$/MeOH (1:1) as the eluent, to give **S16** (76.3 mg, 83%): $[\alpha]_D$ +5.9 (c 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96–7.23 (m, 26 H, 5 Ar, NHTFAc), 5.50–5.46 (m, 4 H, 2 >C*H*Ph, H-2^b, H-4^d), 5.43 (d, 1 H, $J_{NH,2}$ = 6.7 Hz, N*H*Ac), 5.27 (dd, 1 H, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 10.0 Hz, H-2^d), 5.25 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H-1^c), 4.73 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, H-3^c), 4.59 (d, 1 H , H-1^d), 4.52 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H-1^b), 4.33 (d, 1 H, $J_{5,6}$ = 12.1 Hz, H-6a^b), 4.30–4.16 (m, 4 H, H-4^b, H-5^c, CH_2), 4.08 (d, 1 H, H-6b^b), 3.99–3.92 (m, 2 H, $SiCH_2CH_2$, H-3^b), 3.73 (t, 1 H, $J_{5,6a}$ = J_{gem} = 10.2 Hz, H-6a^d), 3.70–3.61 (m, 3 H, H-4^c, H-6a^c, H-5^d), 3.57 (dd, 1 H, $J_{3,4}$ = 3.4 Hz, H-3^d), 3.55–3.18 (m, 14 H, H-5^b, H-6b^c, H-6b^d, $SiCH_2CH_2$, 5

CH_2), 2.89 (d, 1 H, $J_{2,3}$ = 8.6 Hz, H-2^c), 2.11 (s, 3 H, Ac), 0.89–0.73 (m, 5 H, SiCH_2CH$_2$, Ac), −0.13 (s, 9 H, SiMe$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.1, 170.7, 166.0, 165.4, 157.5 (q, $^2J_{C,F}$ = 37.0 Hz, *C*(O)CF$_3$), 138.1, 133.7, 133.5, 133.3, 130.2, 130.1, 130.0, 129.9, 129.8, 129.4, 129.3, 128.9, 128.7, 128.6, 128.5, 128.4, 126.8, 126.6, 126.4, 116.2 (q, $^1J_{C,F}$ = 286.0 Hz, C(O)*C*F$_3$), 101.7, 101.5, 101.4, 101.1, 100.4, 80.8, 79.1, 78.0, 77.6, 77.4, 77.1, 77.0, 76.7, 72.0, 71.0, 70.9 70.8, 70.6, 70.5, 70.4, 69.2, 69.0, 68.7, 67.1, 66.8, 66.6, 65.9, 61.6, 59.1, 39.8, 30.0, 22.4, 21.1, 18.2, −1.3; HRMS (ESI) *m*/*z*: found [M+Na]$^+$ 1425.4842, $C_{70}H_{81}F_3N_2O_{23}Si$ calcd for [M+Na]$^+$ 1425.4844.

2-(Trimethylsilyl)ethyl (4-*O*-acetyl-2,6-di-*O*-benzoyl-3-*O*-{2-[2-(2-trifluoroacetamidoethoxy)ethoxy]ethyl}-β-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-galactopyranoside (S18). **S16** (91.6 mg, 65.3 μmol) was dissolved in AcOH/H_2O (2.1 mL/0.5 mL) at room temperature. After stirring for 6 h at 55 °C, as the reaction was monitored by TLC ($CHCl_3$/MeOH = 15:1), the solution was co-evaporated with toluene. The residue was exposed to high vacuum for 1 h. Next, to a solution of the resulting residue (**S17**) in pyridine (2.9 mL) was added Ac_2O (734 μL, 7.76 mmol) at room temperature. After stirring for 37 h at room temperature, as the reaction was monitored by TLC ($CHCl_3$/MeOH = 15:1, 30:1), the mixture was quenched with MeOH at 0 °C and co-evaporated with toluene. The mixture was diluted with EtOAc and washed with 2 M HCl, H_2O, satd aq $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel, using $CHCl_3$/*n*-hexane/MeOH (150:90:1 to 150:70:1) as the eluent, to give **S18** (91.1 mg, 96%, two steps): $[\alpha]_D$ +14.4 (c 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03–7.43 (m, 15 H, 3 Ar), 7.18 (s, 1 H, NHTFAc), 5.52 (d, 1 H, $J_{3,4}$ = 3.0 Hz, H-4^b), 5.41 (d, 1 H, $J_{3,4}$ = 3.5 Hz, H-4^d), 5.32 (dd, 1 H, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 10.0 Hz, H-2^d), 5.14–5.10 (m, 3 H, N*H*Ac, H-1^c, H-2^b), 4.91 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4^c), 4.58 (t, 1 H, $J_{2,3}$ = 9.5 Hz, H-3^c), 4.49 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H-1^d), 4.45 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H-1^b), 4.42 (dd, 1 H, $J_{5,6a}$ = 6.5 Hz, J_{gem} = 11.5 Hz, H-6a^b), 4.26 (dd, 1 H, H-6b^b), 4.21 (dd, 1 H, $J_{5,6a}$ = 2.5 Hz, J_{gem} = 12.0 Hz, H-6a^d), 4.15 (dd, 1 H, J_{vic} = 5.5 Hz, J_{gem} = 11.5 Hz, CH$_2$), 4.06 (dd, 1 H, $J_{5,6b}$ = 7.5 Hz, J_{gem} = 11.5 Hz, H-6b^d), 4.01 (dd, 1 H, J_{vic} = 4.5 Hz, J_{gem} = 12.5 Hz, CH$_2$), 3.95–3.81 (m, 4 H, H-5^b, H-3^d, H-5^d, SiCH$_2$CH_2), 3.71–3.64 (m, 2 H, H-3^b, H-6a^c), 3.53–3.33 (m, 11 H, H-5^c, H-6b^c, 4 CH$_2$, SiCH$_2$CH_2), 3.32–3.21 (m, 2 H, CH$_2$), 2.66 (m, 1 H, H-2^c), 2.16–2.03 (5 s, 15 H, 5 Ac), 1.12 (s, 3 H, Ac), 0.94–0.75 (m, 2 H, SiCH_2CH$_2$), −0.13 (s, 9 H, SiMe$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0, 170.7, 170.5, 170.3, 169.5, 169.3, 165.9, 165.0, 164.7, 157.1 (q, $^2J_{C,F}$ = 36.7 Hz, *C*(O)CF$_3$), 133.5, 133.3, 133.2, 130.8, 129.7, 129.7, 129.7, 129.6, 129.4, 129.3, 128.6, 128.4, 128.3, 128.1, 115.8 (q, $^1J_{C,F}$ = 286.1 Hz, C(O)*C*F$_3$), 101.0, 100.7, 98.9, 78.5, 77.8, 76.0, 72.0, 71.5, 71.3, 71.0, 70.6, 70.3, 70.2, 70.0, 69.4, 68.9, 68.4, 67.4, 66.3, 62.3, 61.7, 59.0, 39.5, 22.5, 20.8, 20.8, 20.7, 20.7, 17.8, −1.7; HRMS (ESI) *m*/*z*: found [M+Na]$^+$ 1417.4637, $C_{64}H_{81}F_3N_2O_{27}Si$ calcd for [M+Na]$^+$ 1417.4640.

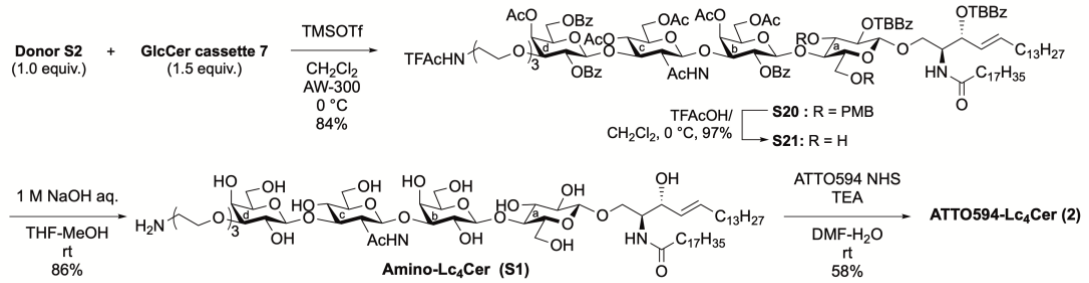
(4-*O*-Acetyl-2,6-di-*O*-benzoyl-3-*O*-{2-[2-(2-trifluoroacetamidoethoxy)ethoxy]ethyl}-β-D-galactopyranosyl)-(1→4)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-4,6-di-*O*-acetyl-2-*O*-benzoyl-D-galactopyranose (S19). To a solution of **S18** (47.9 mg, 34.3 μmol) in CH_2Cl_2 (3.4 mL) was added TFAcOH (1.1 mL) at 0 °C. After stirring for 5 h at room temperature, as the reaction was monitored by TLC ($CHCl_3$/MeOH = 20:1), satd aq $NaHCO_3$ was added to the reaction mixture at 0 °C. The mixture was diluted with $CHCl_3$ and washed with satd aq $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel, using $CHCl_3$/*n*-hexane/MeOH (150:20:1 to 150:0:1) as the eluent, to give **S19** (42.7 mg, 96%): α-isomer; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05–7.40 (m, 15 H, 3 Ar), 7.20 (s, 1 H, NHTFAc), 5.54–5.49 (m, 3 H, H-1^b, H-4^b, H-4^d), 5.30 (dd, 1 H, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 10.5 Hz, H-2^b), 5.21 (d, 1 H, $J_{1,2}$ = 8.2 Hz, H-1^c), 5.15–5.11 (m, 2 H, N*H*Ac, H-2^d), 4.94 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4^c), 4.64 (t, 1 H, $J_{2,3}$ = 9.7 Hz, H-3^c), 4.49 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H-1^d), 4.46–4.38 (m, 2 H, H-6a^b, H-6a^d), 4.31–4.13 (m, 5 H, H-3^b, H-6b^b, H-6b^d, CH$_2$), 4.03–3.91 (m, 3 H, H-5^b, H-6a^c, H-5^d), 3.72–3.64 (m, 3 H, H-3^d, CH$_2$), 3.57–3.51 (m, 1 H, H-5^c), 5.48–3.20 (m, 9 H, H-6b^c, 4 CH$_2$), 3.18 (s, 1 H, OH), 2.70–2.65 (m, 1 H, H-2^c), 2.17–2.05 (5 s, 15 H, 5 Ac), 1.10 (s, 3 H, Ac); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.2, 171.0, 170.7, 169.7, 166.4, 165.1, 157.5 (q, $^2J_{C,F}$ = 40.8 Hz, *C*(O)CF$_3$), 133.9, 133.8, 130.3, 130.1, 129.8, 129.7, 129.5, 129.0, 128.9, 116.2 (q, $^1J_{C,F}$ = 286.8 Hz, C(O)*C*F$_3$), 101.4, 99.3, 91.0, 79.0, 77.4, 77.1, 73.4, 72.5, 71.9, 71.1, 71.0, 70.7, 70.4, 70.3, 69.4, 68.8, 68.5, 67.5, 66.7, 63.0, 62.1, 59.5, 39.9, 39.0, 30.7, 30.0, 29.2, 24.1, 23.3, 23.0, 21.2, 21.2, 21.1, 21.1, 14.4, 11.3; HRMS (ESI) *m*/*z*: found [M+Na]$^+$ 1317.3933, $C_{59}H_{69}F_3N_2O_{27}$ calcd for [M+Na]$^+$ 1317.3932.

(4-*O*-Acetyl-2,6-di-*O*-benzoyl-3-*O*-{2-[2-(2-trifluoroacetamidoethoxy)ethoxy]ethyl}-β-D-galactopyranosyl)-

(1→4)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-4,6-di-*O*-acetyl-2-*O*-benzoyl-D-galactopyranosyl 2,2,2-trifluoro-*N*-phenylacetimidate (S2). To a solution of **S19** (41.5 mg, 32.0 μmol) in acetone (3.2 mL) were added $CF_3C(=NPh)Cl$ (10 μL, 64.0 μmol) and K_2CO_3 (22.1 mg, 160 μmol) at 0 °C. After stirring for 1.5 h at room temperature, as the reaction was monitored by TLC ($CHCl_3$/MeOH = 20:1), the solution was filtered through a pad of Celite, and the pad was washed with $CHCl_3$. The combined filtrate and washings were concentrated. The residue was purified by column chromatography on silica gel, using *n*-hexane/EtOAc (2:1 to 1:3) as the eluent, to give **S2** (43.0 mg, 92%, α/β = 1/0.45): α-isomer; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05–7.30 (m, 15 H, 3 Ar), 7.19 (s, 1 H, NHTFAc), 7.12–6.37 (m, 5 H, Ar), 5.58 (d, 1 H, $J_{1,2}$ = 2.5 Hz, H-1^b), 5.55–5.47 (m, 2 H, H-4^b, H-4^d), 5.25 (d, 1 H, $J_{1,2}$ = 8.2 Hz, H-1^c), 5.19–5.12 (m, 2 H, H-2^d, N*H*Ac), 4.96 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4^c), 4.64 (t, 1 H, $J_{2,3}$ = 9.7 Hz, H-3^c), 4.49 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H-1^d), 4.46–3.21 (m, 24 H, H-2^b, H-3^b, H-5^b, H-6a^b, H-6b^b, H-5^c, H-6a^c, H-6b^c, H-3^d, H-5^d, H-6a^d, H-6b^d, 6 CH_2), 2.71–2.66 (m, 1 H, H-2^c), 2.18 (s, 3 H, Ac), 2.10 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 2.08 (s, 3 H, Ac) , 2.06 (s, 3 H, Ac), 1.11 (s, 3 H, Ac); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.3, 171.2, 171.2, 170.8, 170.7, 169.7, 169.6, 166.4, 165.1, 157.5 (q, $^2J_{C,F}$ = 36.6 Hz, *C*(O)CF_3), 143.2, 134.2, 134.0, 133.9, 133.8, 130.3, 130.2, 130.1, 130.1, 129.8, 129.7, 129.1, 129.0, 129.0, 128.9, 128.9, 124.6, 119.3, 116.2 (q, $^1J_{C,F}$ = 286.0 Hz, C(O)*C*F_3), 101.5, 99.4, 99.2, 79.0, 76.7, 76.5, 73.8, 72.5, 72.1, 71.0, 70.7, 70.4, 70.2, 69.3, 69.2, 68.8, 68.6, 66.7, 62.5, 62.4, 62.1, 62.1, 59.5, 59.4, 39.9, 23.0, 22.9, 21.2, 21.2, 21.1, 21.1, 21.0; HRMS (ESI) *m*/*z*: found $[M+Na]^+$ 1488.4232, $C_{67}H_{73}F_6N_3O_{27}$ calcd for $[M+Na]^+$ 1488.4228.

2.5. Synthesis of ATTO594-Lc$_4$Cer 2

Next, trisaccharide donor **S2** was successfully glycosidated with the C4 hydroxyl group of GlcCer cassette **7**[5,6] through β-glycosidic linkage, thus giving **S20** in 84% yield. Then, the PMB groups at C3 and C6-OH of Glc were removed to give **S21** in 97% yield. Finally, **S21** underwent global deprotection and dye conjugation at an amine group of the terminal Gal residue to furnish ATTO594-Lc$_4$Cer **2**.



Scheme S5. Synthesis of ATTO594-Lc$_4$Cer 2

(4-*O*-Acetyl-2,6-di-*O*-benzoyl-3-*O*-{2-[2-(2-trifluoroacetamidoethoxy)ethoxy]ethyl}-β-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-(4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-(2-*O*-*p*-*tert*-butylbenzoyl-3,6-di-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-*p*-*tert*-butylbenzoyl-2-octadecanamido-4-octadecene-1,3-diol (S20). Molecular sieves AW-300 (118 mg) were added to a solution of donor **S2** (43.2 mg, 29.5 μmol) and acceptor **7**[5,6] (57.1 mg, 44.3 μmol) in CH_2Cl_2 (1.2 mL) at room temperature. After stirring for 1 h at 0 °C, TMSOTf (1.1 μL, 5.9 μmol) was added to the mixture at 0 °C. The reaction mixture was stirred for 1 h at 0 °C as the reaction was monitored by TLC (toluene/EtOAc = 1:2). The reaction mixture was quenched with triethylamine and filtered through a pad of Celite, and the pad was washed with $CHCl_3$. The combined filtrate and washings were washed with brine, dried over Na_2SO_4, and concentrated. The resulting residue was purified by column chromatography on silica gel, using $CHCl_3$/*n*-hexane/MeOH (150:90:1) as the eluent, to give **S20** (63.9 mg, 84%): $[\alpha]_D$ +23.4 (c 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04 (d, 2 H, Ar), 7.95 (d, 2 H, Ar), 7.88 (d, 2 H, Ar), 7.86–7.82 (m, 3 H, Ar), 7.61 (m, 3 H, Ar), 7.59–7.40 (m, 11 H, Ar), 7.21 (d, 2 H, Ar), 7.15 (s, 1 H, NHTFAc), 7.08 (d, 2 H, Ar), 6.98 (d, 2 H, Ar), 6.63 (d, 2 H, Ar), 5.75 (m, 1 H, H-5^{Cer}), 5.64 (d, 1 H, $J_{NH,2}$ = 9.5 Hz, NH^{Cer}), 5.48 (d, 1 H, $J_{3,4}$ = 4.2 Hz, H-4^b), 5.44 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 7.2 Hz, H-3^{Cer}), 5.40–5.36 (m, 2 H, H-4^d, H-4^{Cer}), 5.24 (dd, 1 H, $J_{1,2}$ = 8.1 Hz, $J_{2,3}$ = 10.1 Hz, H-2^d), 5.14–5.07 (m, 3 H, H-

2^{a}, H-2^{b}, N*H*Ac), 5.04 (d, 1 H, $J_{1,2}$ = 8.1 Hz, H-1^{c}), 4.91 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4^{c}), 4.75 (d, 1 H, J_{gem} = 11.1 Hz, PhC*H*$_2$), 4.61–4.54 (m, 4 H, H-3^{c}, H-1^{d}, H-6a^{d}, PhC*H*$_2$), 4.43 (d, 1 H, $J_{1,2}$ = 8.2 Hz, H-1^{b}), 4.40 (t, 1 H, J_{gem} = $J_{1a,2}$ = 4.6 Hz, H-1a^{Cer}), 4.35–4.26 (m, 4 H, H-1^{a}, H-2^{Cer}, PhC*H*$_2$), 4.10–3.99 (m, 4 H, H-4^{a}, H-5^{b}, H-6b^{d}, C*H*$_2$), 3.93 (dd, 1 H, $J_{5,6a}$ = 2.9 Hz, J_{gem} = 9.8 Hz, H-6a^{b}), 3.91–3.84 (m, 2 H, H-5^{d}, H-1b^{Cer}), 3.81 (s, 3 H, OCH_3), 3,71 (s, 3 H, OCH_3), 3.70–3.61 (m, 6 H, H-3^{a}, H-3^{b}, H-5^{c}, H-3^{d}, CH_2), 3.48–3.20 (m, 14 H, H-6a^{a}, H-6b^{a}, H-6b^{b}, H-6a^{c}, H-6b^{c}, 9 C*H*$_2$), 3.14 (q, 1 H, H-5^{a}), 2.62 (m, 1 H, $J_{1,2}$ = 7.5 Hz, $J_{2,3}$ = 9.9 Hz, H-2^{c}), 2.17–2.00 (5 s, 15 H, 5 Ac), 1.93 (m, 2 H, H-6a^{Cer}, H-6b^{Cer}), 1.72–1.68 (m, 2 H, $COCH_2^{Cer}$), 1.33–1.20 (m, 72 H, 2 *t*-Bu, 27 CH_2^{Cer}), 1.05 (s, 3 H, Ac), 0.89–0.86 (m, 6 H, 2 CH_3^{Cer}); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.1, 171.1, 170.9, 170.7, 169.7, 169.5, 166.4, 165.2, 159.7, 159.2, 157.5 (q, $^{2}J_{C,F}$ = 36.9 Hz, *C*(O)CF_3), 156.7, 137.4, 133.9, 133.8, 130.9, 130.5, 130.3, 130.1, 130.0, 129.9, 129.8, 129.8, 129.8, 129.7, 129.0, 128.9, 128.8, 127.9, 127.2, 125.7, 125.6, 125.2, 116.2 (q, $^{1}J_{C,F}$ = 286.0 Hz, C(O)*C*F_3), 114.2, 113.7, 101.5, 100.1, 99.3, 79.0, 74.5, 74.0, 73.6, 71.8, 71.6, 71.0, 70.7, 70.4, 68.8, 66.8, 62.0, 59.4, 55.7, 55.5, 39.9, 36.7, 35.4, 35.4, 32.6, 32.2, 31.4, 31.4, 30.0, 30.0, 30.0, 29.9, 29.8, 29.8, 29.7, 29.6, 29.5, 29.3, 25.9, 23.0, 22.8, 21.2, 21.1, 21.1, 21.0, 14.4; HRMS (ESI) *m*/*z*: found $[M+Na]^+$ 2578.2717, $C_{139}H_{188}F_3N_3O_{38}$ calcd for $[M+Na]^+$ 2587.2715.

(4-*O*-Acetyl-2,6-di-*O*-benzoyl-3-*O*-{2-[2-(2-trifluoroacetamidoethoxy)ethoxy]ethyl}-β-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-(4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-(2-*O*-*p*-*tert*-butylbenzoyl-β-D-glucopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-*p*-*tert*-butylbenzoyl-2-octadecanamido-4-octadecene-1,3-diol (S21). To a solution of **S20** (63.4 mg, 24.7 μmol) in CH_2Cl_2 (1.1 mL) was added TFAcOH (550 μL) at 0 °C. After stirring for 15 min at 0 °C, as the reaction was monitored by TLC ($CHCl_3$/MeOH = 20:1), satd aq $NaHCO_3$ was added to the reaction mixture. The mixture was diluted with $CHCl_3$ and washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel, using $CHCl_3$/*n*-hexane/MeOH (150:90:1 to 150:60:1) as the eluent, to give **S21** (55.7 mg, 97%): $[\alpha]_D$ +15.3 (c 1.0, $CHCl_3$/MeOH = 1:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96 (d, 2 H, Ar), 7.92 (d, 2 H, Ar), 7.88–7.86 (m, 4 H, Ar), 7.80 (d, 2 H, Ar), 7.57–7.52 (m, 3 H, Ar), 7.41–7.35 (m, 8 H, Ar), 7.31 (d, 2 H, Ar), 7.16 (s, 1 H, NHTFAc), 5.83–5.77 (m, 1 H, H-5^{Cer}), 5.69 (d, 1 H, $J_{1,2}$ = 10.0 Hz, NH^{Cer}), 5.49–5.45 (m, 2 H, H-4^{b}, H-3^{Cer}), 5.36–5.28 (m, 3 H, H-2^{d}, H-4^{d}, H-4^{Cer}), 5.07–5.02 (m, 3 H, H-1^{c}, H-2^{b}, N*H*Ac), 4.98 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 8.5 Hz, H-2^{a}), 4.82 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4^{c}), 4.59 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H-1^{d}), 4.47 (t, 1 H, $J_{2,3}$ = 9.5 Hz, H-3^{c}), 4.37–4.28 (m, 4 H, H-1^{a}, H-1^{b}, H-6a^{d}, H-2^{Cer}), 4.19 (dd, 1 H, $J_{5,6b}$ = 7.0 Hz, J_{gem} = 11.0 Hz, H-6b^{Cer}), 4.13–3.27 (m, 24 H, H-3^{a}, H-4^{a}, H-5^{a}, H-6a^{a}, H-6b^{a}, H-3^{b}, H-5^{b}, H-6a^{b}, H-6b^{b}, H-5^{c}, H-6a^{c}, H-6b^{c}, H-3^{d}, H-5^{d}, H-1a^{Cer}, H-1b^{Cer}, 2 OH, 3 CH_2), 3.24–3.20 (m, 1 H, C*H*$_2$), 3.17–3.13 (m, 1 H, C*H*$_2$), 3.07–3.03 (m, 1 H, C*H*$_2$), 2.96–2.94 (m, 1 H, C*H*$_2$), 2.83–2.80 (m, 1 H, C*H*$_2$), 2.75–2.74 (m, 1 H, C*H*$_2$), 2.61–2.56 (m, 1 H, H-2^{c}), 2.09–1.79 (m, 19 H, 5 Ac, $COCH_2^{Cer}$, H-6a^{Cer}, H-6b^{Cer}), 1.38–1.14 (m, 70 H, CO $CH_2CH_2^{Cer}$, 25 CH_2^{Cer}, 2 *t*-Bu), 1.00 (s, 3 H, Ac), 0.82–0.79 (m, 6 H, 2 CH_3^{Cer}); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.0, 171.2, 171.1, 171.1, 170.7, 169.8, 169.7, 166.3, 166.1, 165.3, 165.1, 157.5 (q, $^{2}J_{C,F}$ = 36.4 Hz, *C*(O)CF_3), 157.3, 157.2, 138.8, 133.9, 133.8, 130.3, 130.1, 130.1, 129.9, 129.8, 129.7, 129.7, 129.2, 129.0, 128.8, 128.8, 127.5, 127.4, 125.7, 125.2, 116.2 (q, $^{1}J_{C,F}$ = 285.9 Hz, C(O)*C*F_3), 102.2, 101.4, 99.9, 99.4, 80.8, 78.9, 76.4, 74.2, 73.8, 72.7, 72.5, 72.4, 72.1, 71.0, 70.9, 70.8, 70.7, 70.7, 70.4, 69.5, 69.1, 68.8, 66.7, 66.3, 62.6, 62.1, 62.0, 59.8, 59.3, 50.6, 39.9, 37.0, 35.4, 35.4, 32.6, 32.2, 31.4, 30.0, 30.0, 30.0, 29.9, 29.9, 29.8, 29.7, 29.6, 29.6, 29.2, 25.9, 23.0, 22.8, 21.1, 21.0, 20.8, 14.4, 1.5, 1.4, 1.3; HRMS (ESI) *m*/*z*: found $[M+Na]^+$ 2347.1565, $C_{123}H_{172}F_3N_3O_{36}$ calcd for $[M+Na]^+$ 2347.1565.

(3-*O*-{2-[2-(2-Aminoethoxy)ethoxy]ethyl}-β-D-galactopyranosyl)-(1→3)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-octadecanamido-4-octadecene-1,3-diol (S1). To a solution of **S21** (17.5 mg, 7.36 μmol) in THF/MeOH (1.2 mL/1.2 mL) was added 1 M NaOH aq. (150 μL, 150 μmol) at room temperature. After stirring for 19 h at room temperature as the reaction was monitored by TLC ($CHCl_3$/MeOH/H_2O/AcOH = 5:5:1:0.05), the reaction mixture was co-evaporated with EtOH. The resulting residue was purified by column chromatography on silica gel using $CHCl_3$/MeOH/H_2O/28% NH_3 aq. (5:4:1:0.1) as the eluent to give **S1** (8.8 mg, 86%): $[\alpha]_D$ +10.3 (c 0.45, $CHCl_3$/MeOH = 1/1); ^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD = 1/1) δ 5.73–5.67 (m, 1 H, H-5^{Cer}), 5.45 (dd, 1 H, $J_{3,4}$ = 7.7 Hz, $J_{4,5}$ = 15.3 Hz, H-4^{Cer}), 4.73–2.83 (m, 44 H, H-1^{a}, H-2^{a}, H-3^{a}, H-4^{a}, H-5^{a}, H-6a^{a}, H-6b^{a}, H-1^{b}, H-2^{b}, H-3^{b}, H-4^{b}, H-5^{b}, H-6a^{b}, H-6b^{b}, H-1^{c}, H-2^{c}, H-3^{c}, H-4^{c}, H-5^{c}, H-6a^{c}, H-6b^{c}, H-1^{d}, H-2^{d}, H-3^{d}, H-4^{d}, H-5^{d}, H-6a^{d}, H-6b^{d}, H-1a^{Cer}, H-1b^{Cer}, H-2^{Cer}, H-3^{Cer}, 6 CH_2), 2.17 (t, 2 H, J_{vic} = J_{gem} = 7.6 Hz, $COCH_2^{Cer}$), 2.03 (dd, 2 H, J_{vic} = 7.2 Hz, J_{gem} = 14.7 Hz, H-6a^{Cer}, H-6b^{Cer}), 1.99 (s, 3 H, Ac), 1.59–1.57 (m, 2 H, $COCH_2CH_2^{Cer}$), 1.30–1.27 (m, 50 H, 25 CH_2^{Cer}), 0.90–0.88 (m, 6 H, 2 CH_3^{Cer}); ^{13}C NMR (200 MHz, CD_3OD) δ 176.1, 175.1, 135.7, 130.8, 105.1, 105.1, 104.4, 103.7, 84.7, 83.8, 83.2, 81.0, 79.1, 78.9, 78.7, 77.4, 76.8, 76.7, 76.4, 76.2,

74.7, 73.3, 71.7, 71.6, 71.5, 71.4, 71.1, 70.2, 70.0, 69.8, 69.7, 66.8, 62.8, 62.8, 62.7, 62.1, 56.5, 54.7, 50.7, 50.4, 50.3, 50.2, 50.1, 50.1, 50.0, 50.0, 49.9, 49.8, 49.6, 49.5, 37.8, 33.7, 33.3, 31.0, 31.0, 30.9, 30.9, 30.8, 30.7, 30.7, 30.6, 27.4, 24.0, 23.9, 15.1; HRMS (ESI) *m/z*: found $[M+Na]^+$ 1408.8647, $C_{68}H_{127}N_3O_{25}$ calcd for $[M+Na]^+$ 1408.8651.

ATTO594-lactotetraosylceramide (2). Compound **S1** (5.5 mg, 3.97 µmol) and ATTO594 *N*-succinimidyl ester (8.9 mg, 9.9 µmol) were dissolved in DMF/H_2O (360 µL/3 µL). To the solution, triethylamine (11 µL, 79 µmol) was added at room temperature. After stirring for 3 h at room temperature as the reaction was monitored by TLC ($CHCl_3$/MeOH/H_2O/AcOH = 8:5:1:0.05), the reaction mixture was concentrated. The resulting residue was purified by gel filtration column chromatography on Sephadex LH-20 using $CHCl_3$/MeOH (1:1) and preparative thin layer chromatography (PTLC) using $CHCl_3$/MeOH/H_2O (8:5:1) as the eluent to give **2** (5.0 mg, 58%): ^{1}H NMR (500 MHz, CD_3OD) δ 7.74–7.35 (m, 6 H, $Ar^{ATTO594}$), 6.80 (s, 2 H, $Ar^{ATTO594}$), 5.89 (s, 1 H, $CH^{ATTO594}$), 5.89 (s, 1 H, $CH^{ATTO594}$), 5.69–5.65 (m, 1 H, H-5^{Cer}), 5.44 (dd, 1 H, $J_{3,4}$ = 7.8 Hz, $J_{4,5}$ = 15.3 Hz, H-4^{Cer}), 4.75–3.21 (m, 54 H, H-1^c, H-2^c, H-3^c, H-4^c, H-5^c, H-6a^c, H-6b^c, H-1^b, H-2^b, H-3^b, H-4^b, H-5^b, H-6a^b, H-6b^b, H-1^d, H-2^d, H-3^d, H-4^d, H-5^d, H-6a^d, H-6b^d, H-1^a, H-2^a, H-3^a, H-4^a, H-5^a, H-6a^a, H-6b^a, H-1a^{Cer}, H-1b^{Cer}, H-2^{Cer}, H-3^{Cer}, 6 CH_2, 2 $CH_2SO_3^{ATTO594}$, 3 $NCH_2^{ATTO594}$), 2.72/2.64 (s, 3 H, $NCH_3^{ATTO594}$), 2.17 (t, 2 H, J_{vic} = J_{gem} = 7.5 Hz, $COCH_2^{Cer}$), 2.02 (m, 2 H, H-6a^{Cer}, H-6b^{Cer}), 1.96 (s, 3 H, Ac), 1.79–1.28 (m, 74 H, 26 CH_2^{Cer}, 4 $CH_3^{ATTO594}$, $NCH_2CH_2^{ATTO594}$, 2 $NCH_2CH_3^{ATTO594}$, $NCH_2CH_2CH_2^{ATTO594}$), 0.91–0.88 (m, 6 H, 2 CH_3^{Cer}); ^{13}C NMR (200 MHz, CD_3OD) δ 176.8, 175.9, 175.6, 172.1, 160.1, 155.9, 155.0, 139.2, 138.4, 135.9, 133.0, 132.6, 132.2, 132.0, 131.6, 129.8, 126.9, 125.0, 125.0, 123.8, 115.9, 106.0, 105.9, 105.3, 104.7, 97.9, 85.5, 84.3, 84.1, 81.4, 78.4, 77.7, 77.5, 77.1, 75.7, 73.9, 72.5, 72.5, 72.3, 72.3, 71.9, 71.4, 71.1, 70.8, 70.5, 67.6, 65.2, 63.4, 63.3, 63.3, 62.6, 62.6, 62.6, 57.3, 55.6, 54.9, 54.7, 54.7, 50.2, 50.1, 50.1, 50.0, 50.0, 49.9, 49.8, 49.6, 49.5, 48.4, 42.2, 41.3, 41.1, 39.6, 38.2, 34.6, 34.3, 34.0, 33.9, 33.5, 31.7, 31.7, 31.7, 31.7, 31.6, 31.6, 31.5, 31.4, 31.3, 31.3, 31.3, 31.3, 30.3, 30.3, 30.1, 28.0, 25.0, 24.6, 24.2, 15.3, 14.6, 14.6; HRMS (ESI) *m/z*: found $[M-H]^-$ 2172.1283, $C_{109}H_{173}N_6O_{35}S_2$ calcd for $[M-H]^-$ 2172.1283.

3. Supplemental References

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4. Copies of ^{1}H and ^{13}C NMR spectra

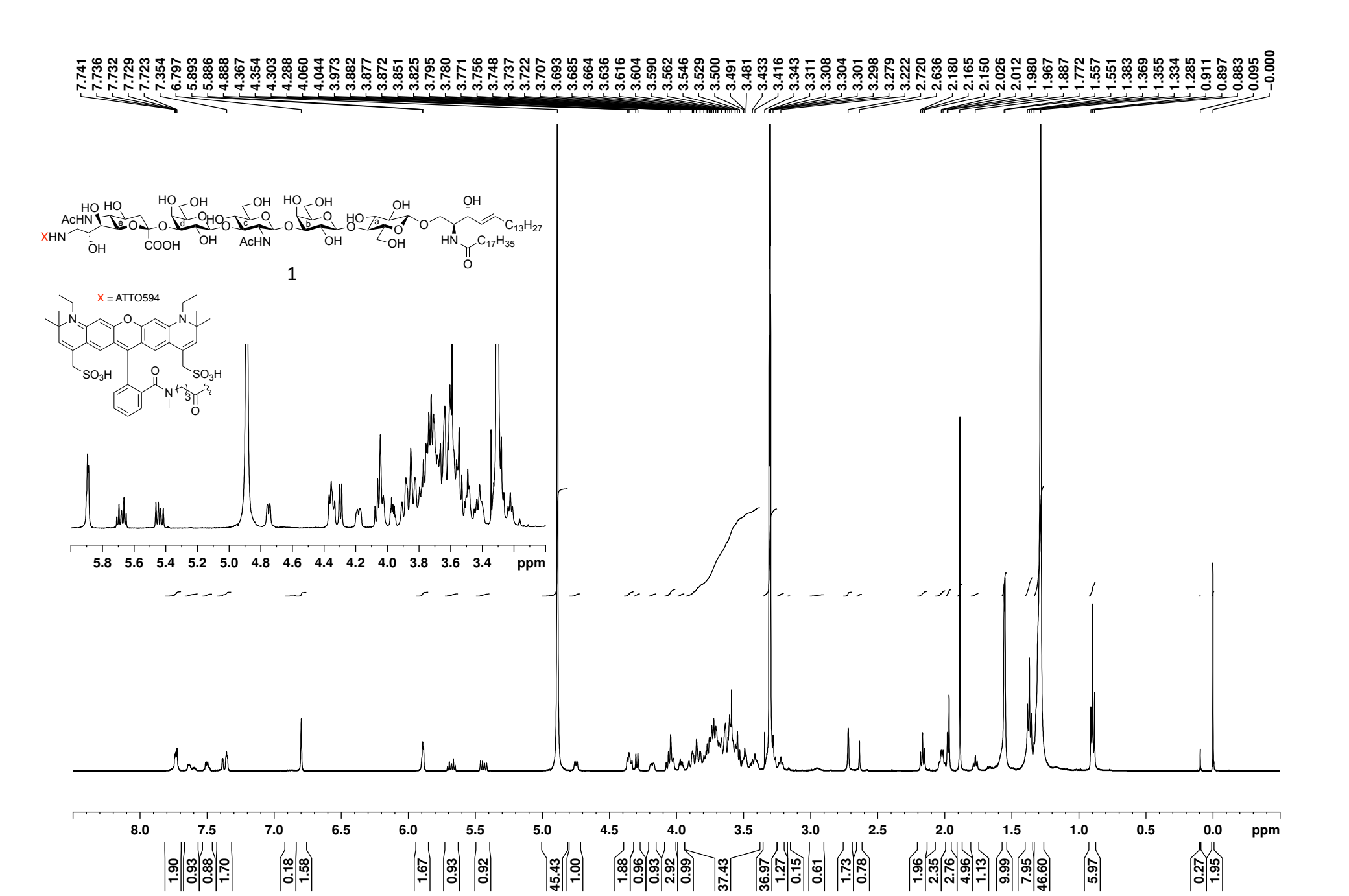

Figure S1. ^{1}H NMR spectrum (800 MHz, CD_3OD)

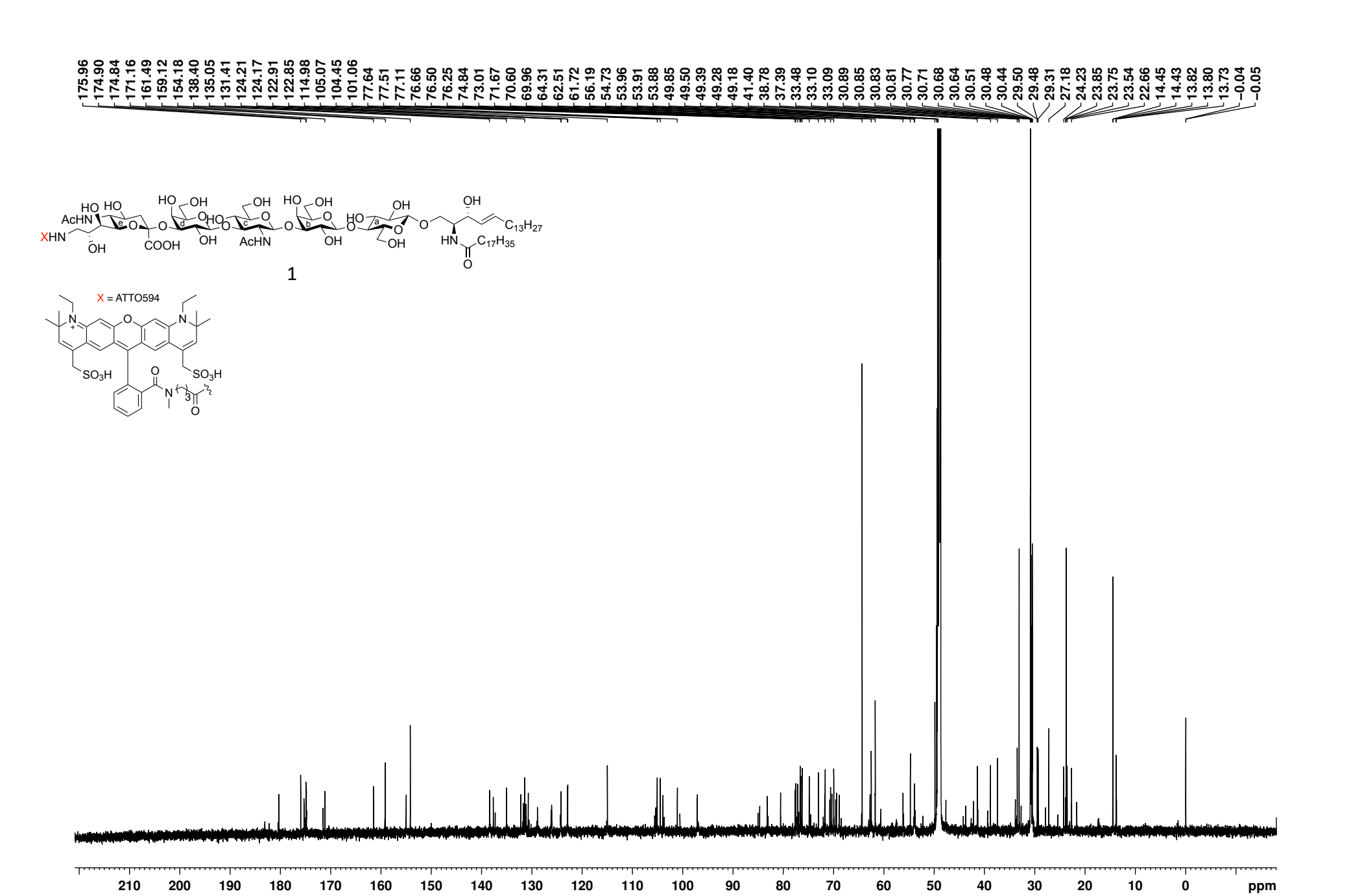

Figure S2. ^{13}C NMR spectrum (200 MHz, CD_3OD)

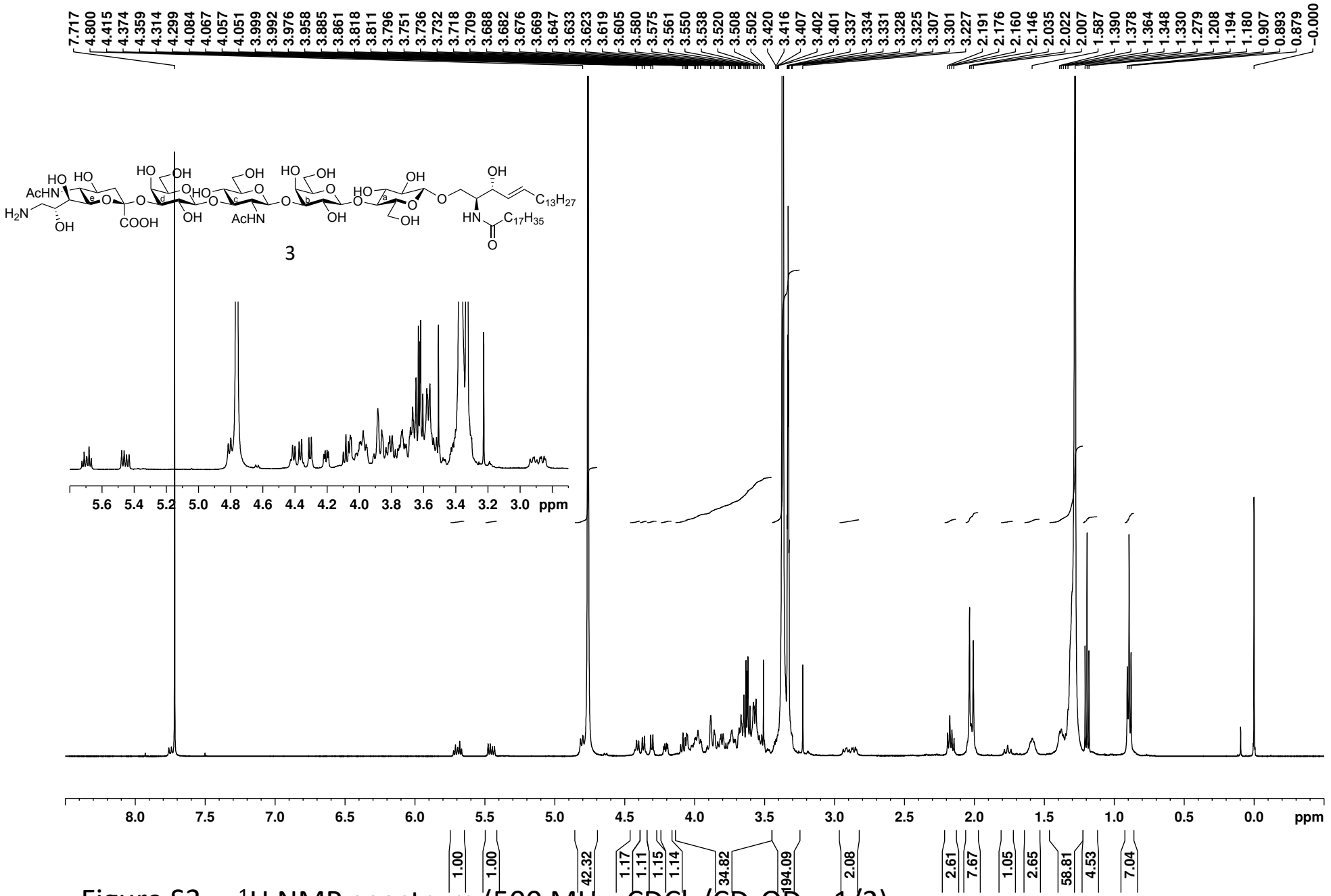

Figure S3. ^{1}H NMR spectrum (500 MHz, $CDCl_3/CD_3OD$ = 1/2)

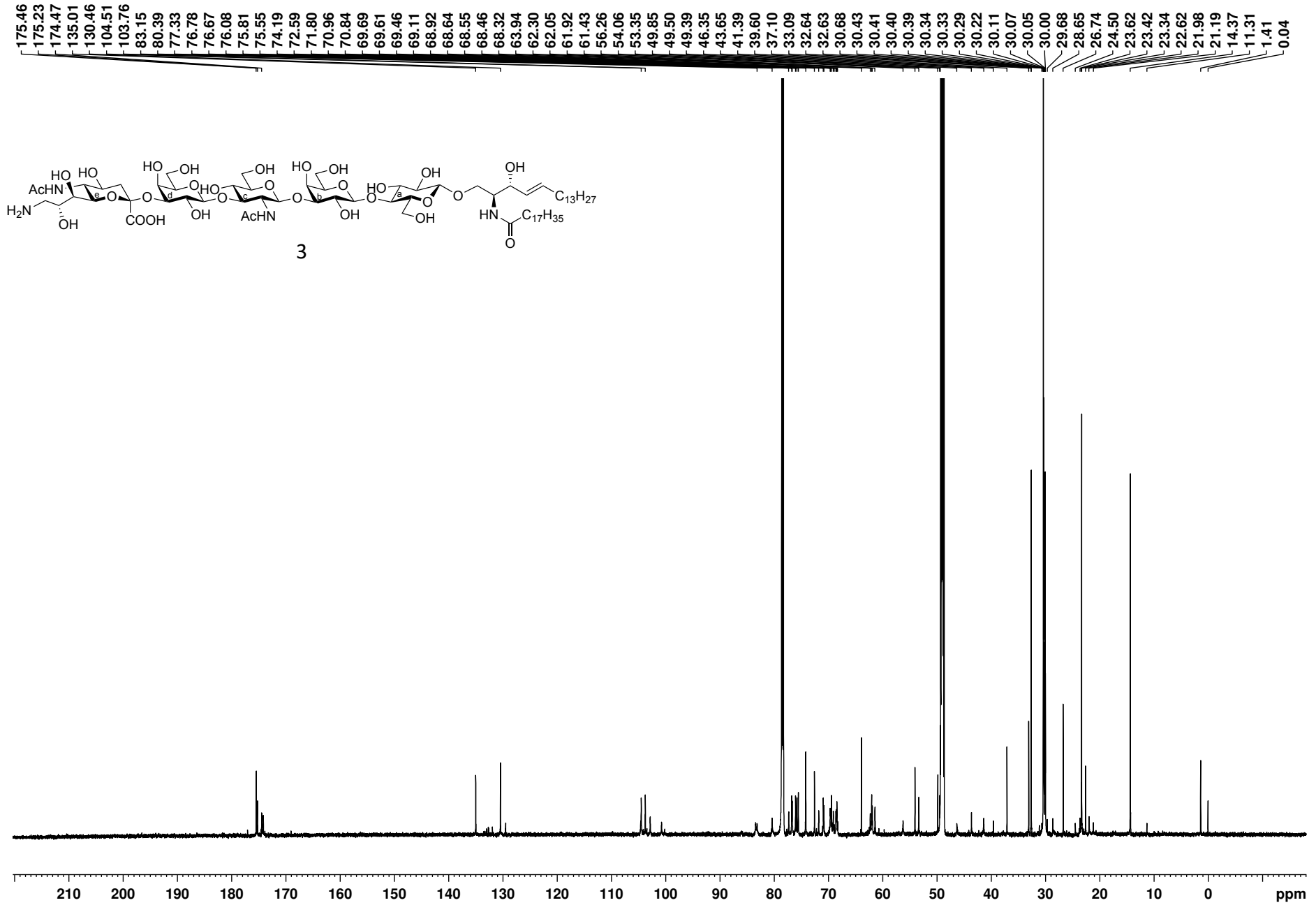

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Figure S4. ^{13}C NMR spectrum (200 MHz, $CDCl_3/CD_3OD$ = 1/2)

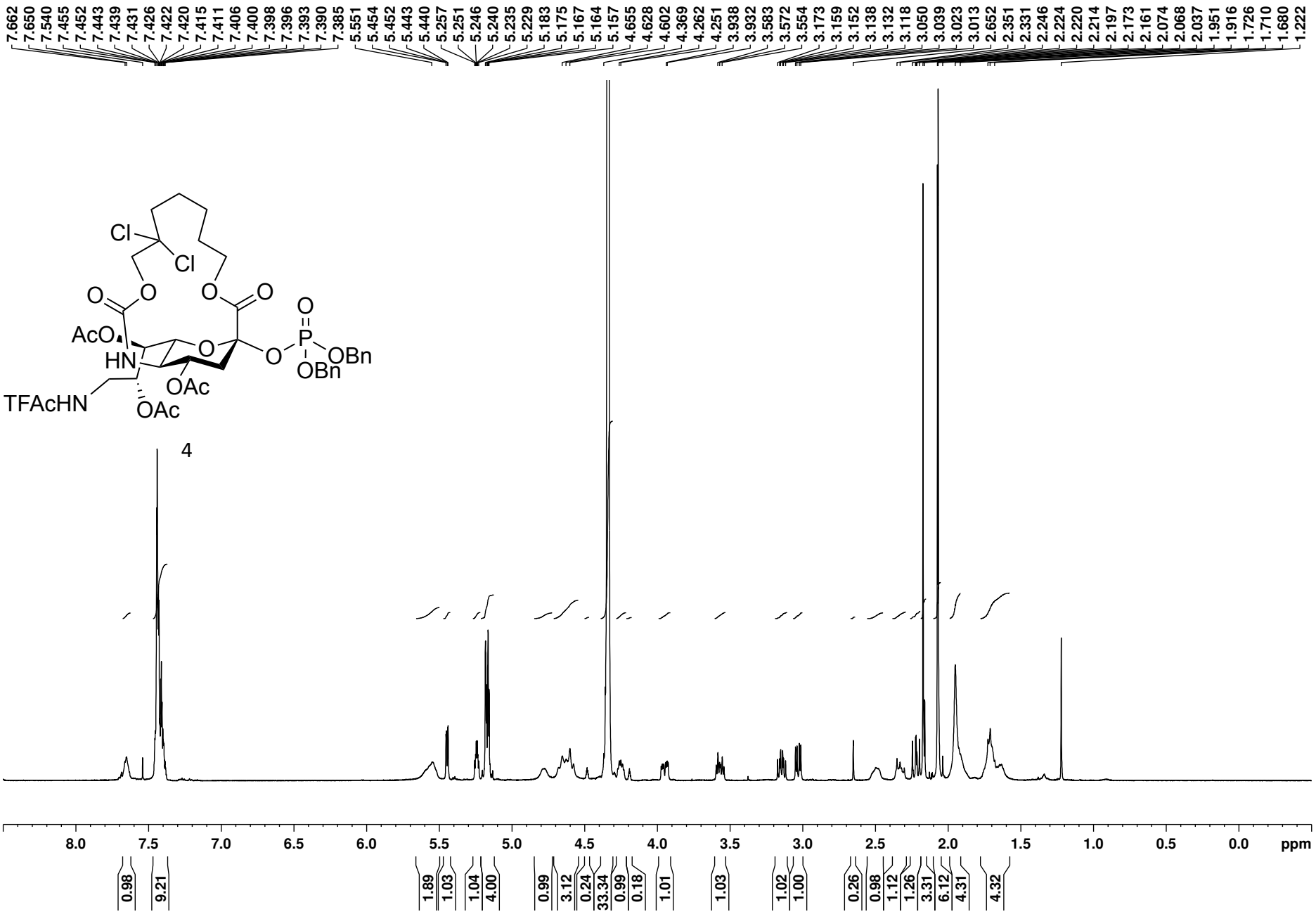


Figure S5. ^{1}H NMR spectrum (500 MHz, CD_3NO_2, 60 °C)

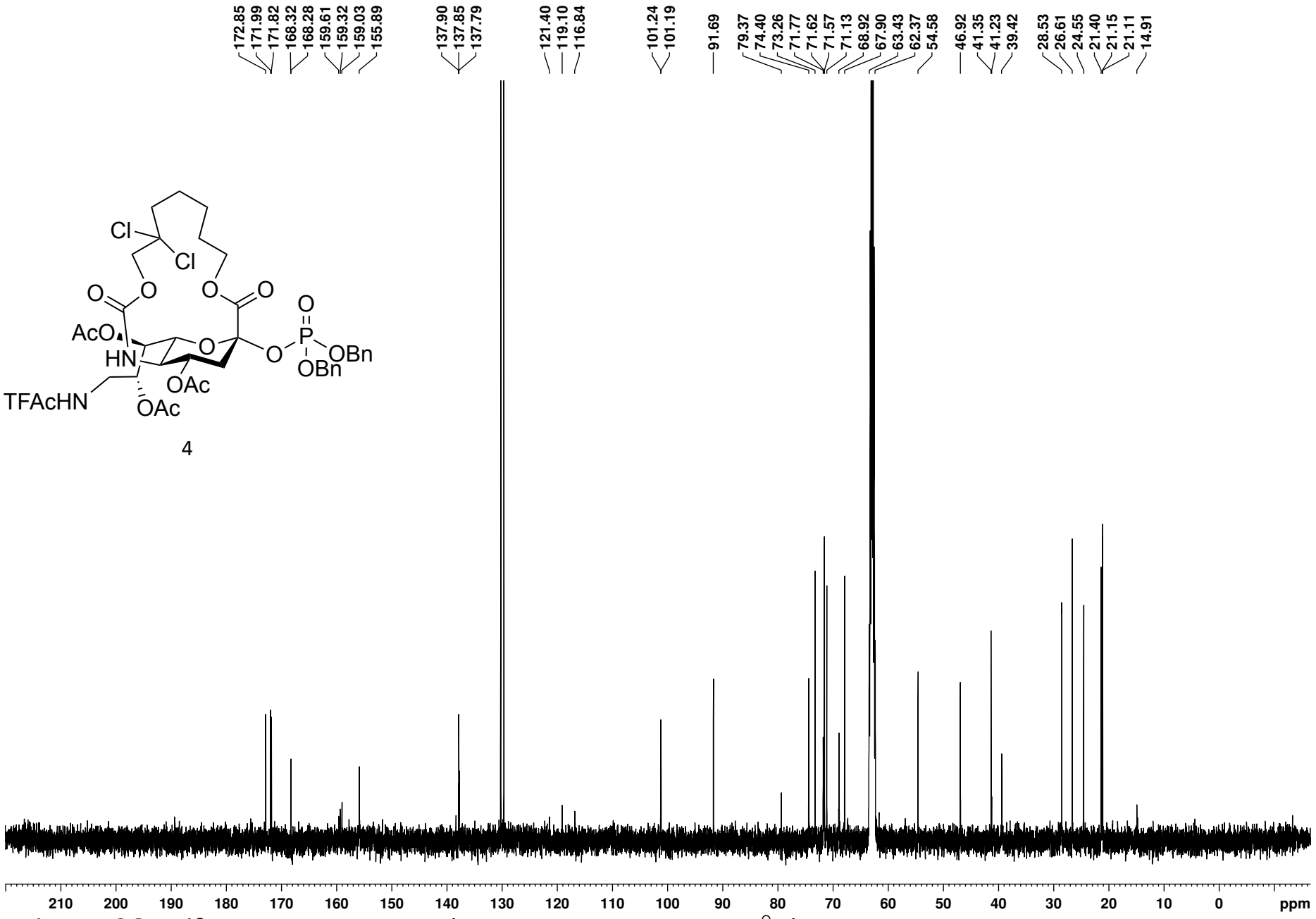


Figure S6. ^{13}C NMR spectrum (125 MHz, CD_3NO_2, 85 °C)

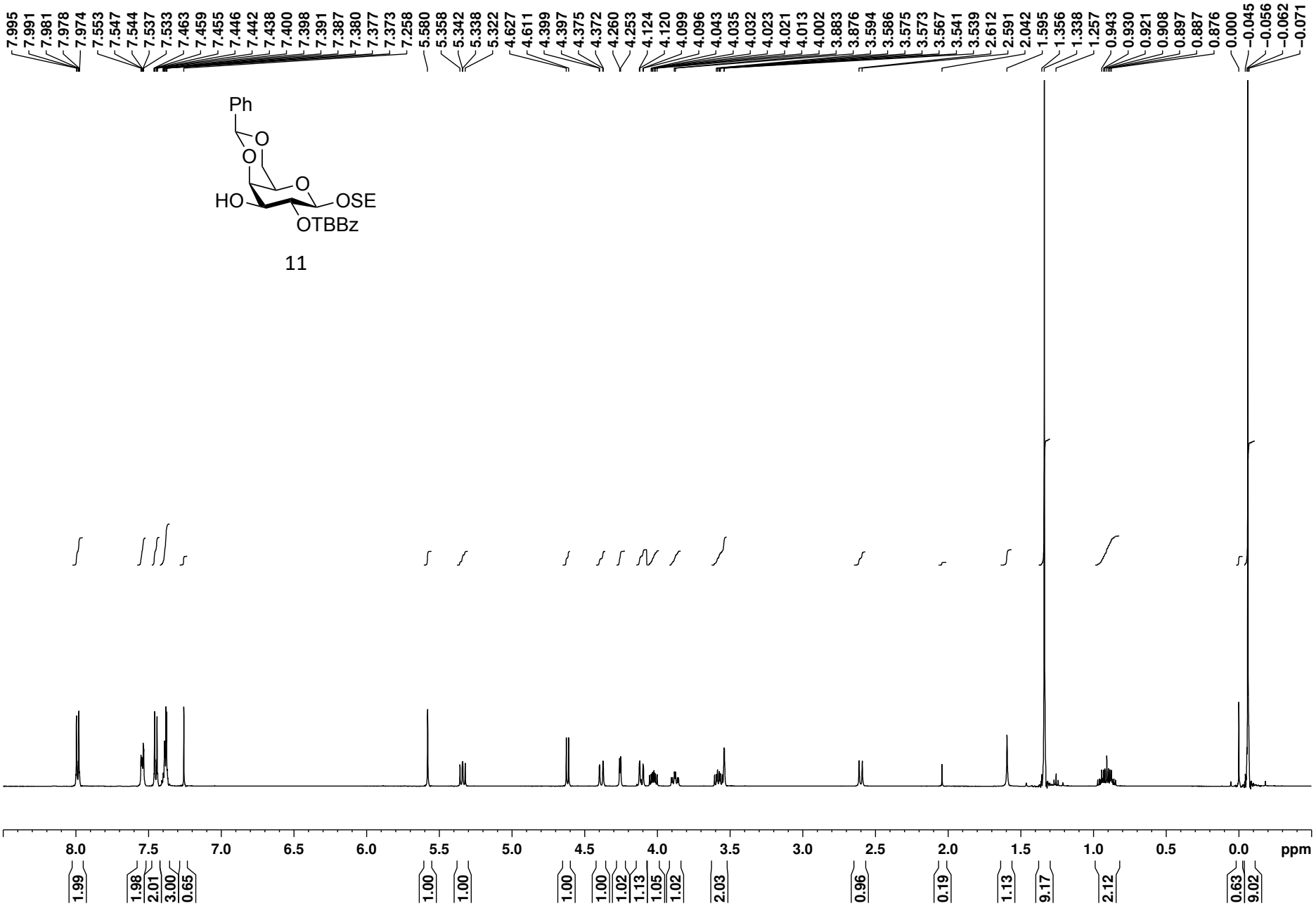


Figure S7. ^{1}H NMR spectrum (500 MHz, $CDCl_3$)

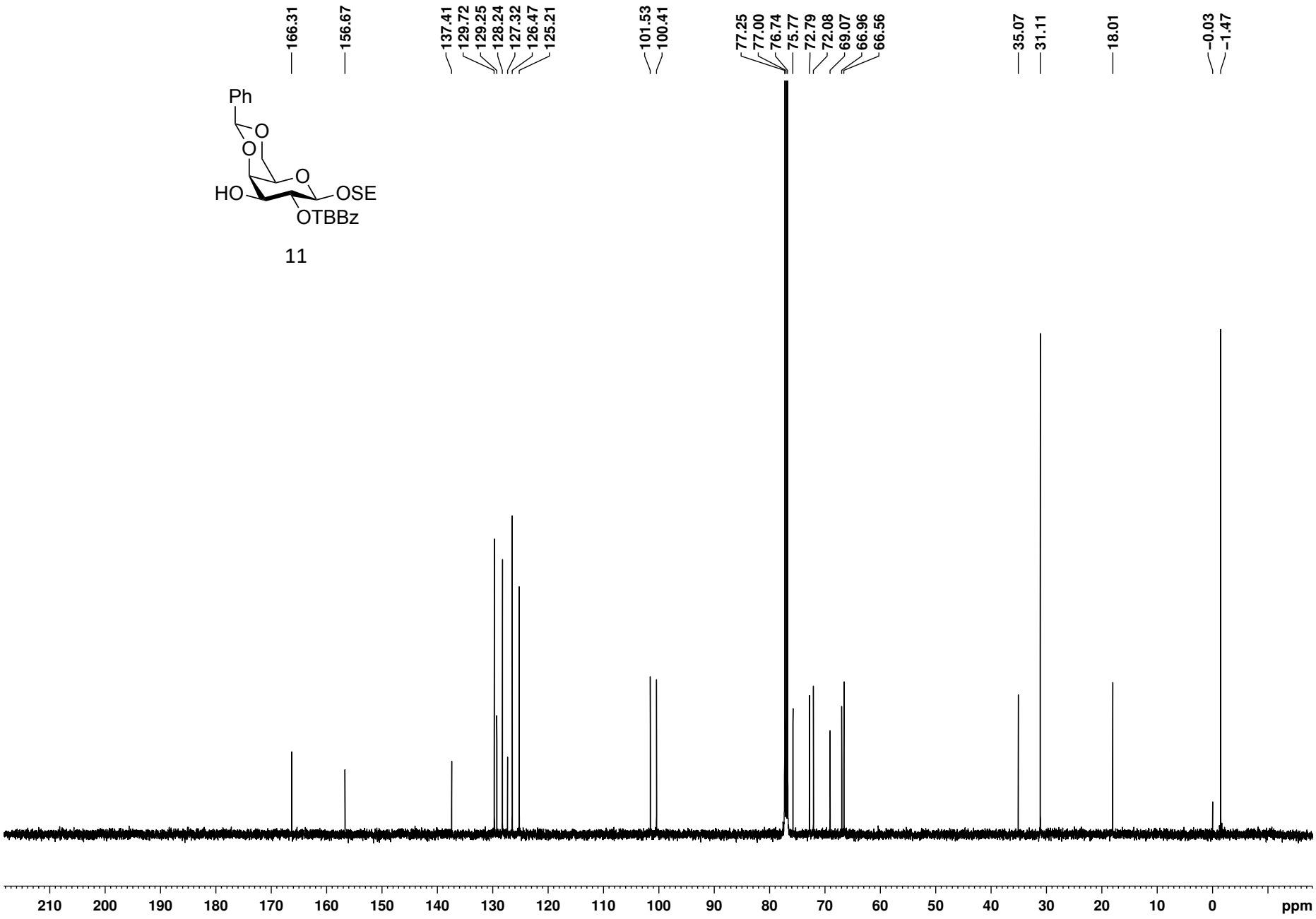
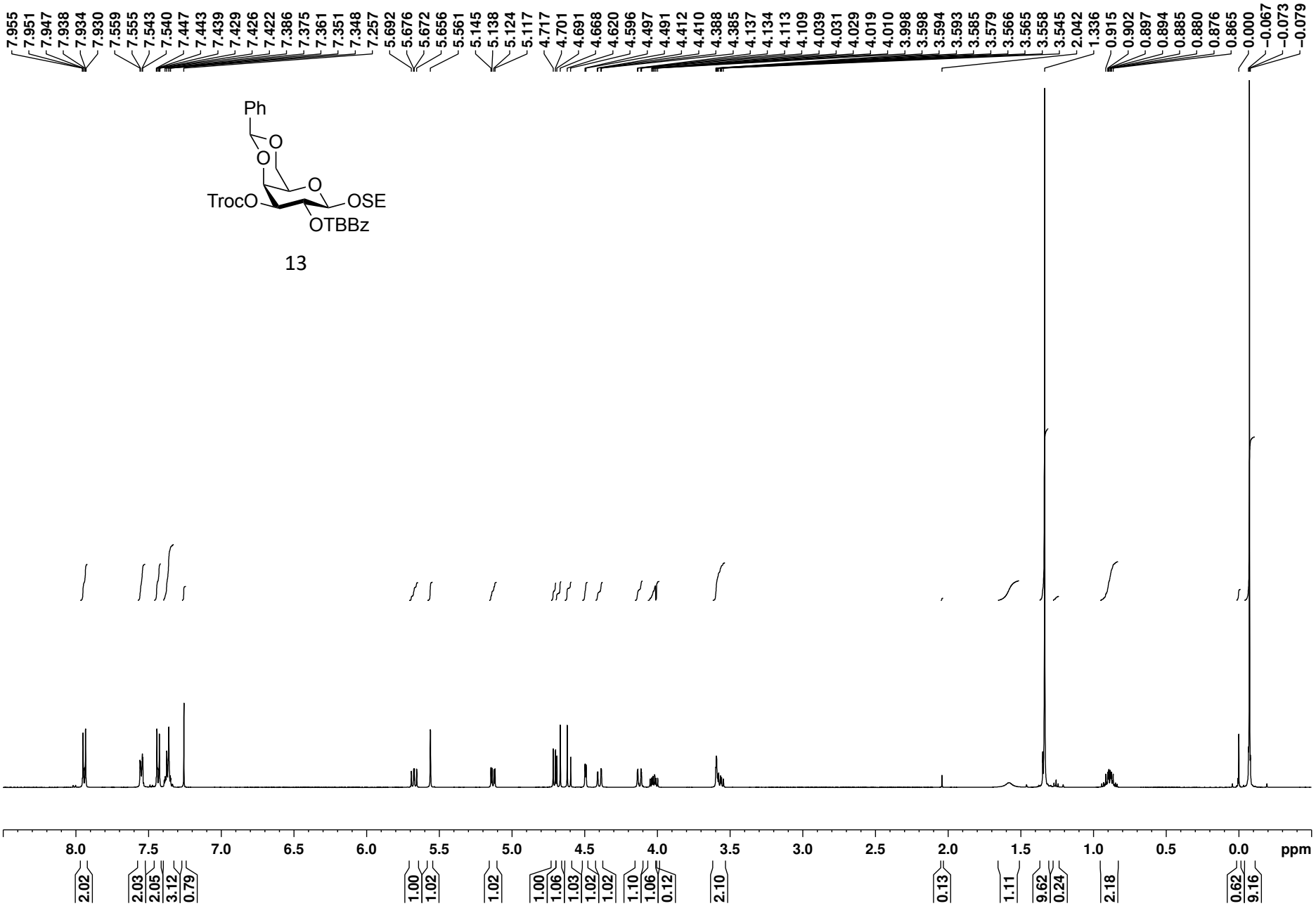


Figure S8. ^{13}C NMR spectrum (125 MHz, $CDCl_3$)



13

Figure S9. ^{1}H NMR spectrum (500 MHz, $CDCl_3$)

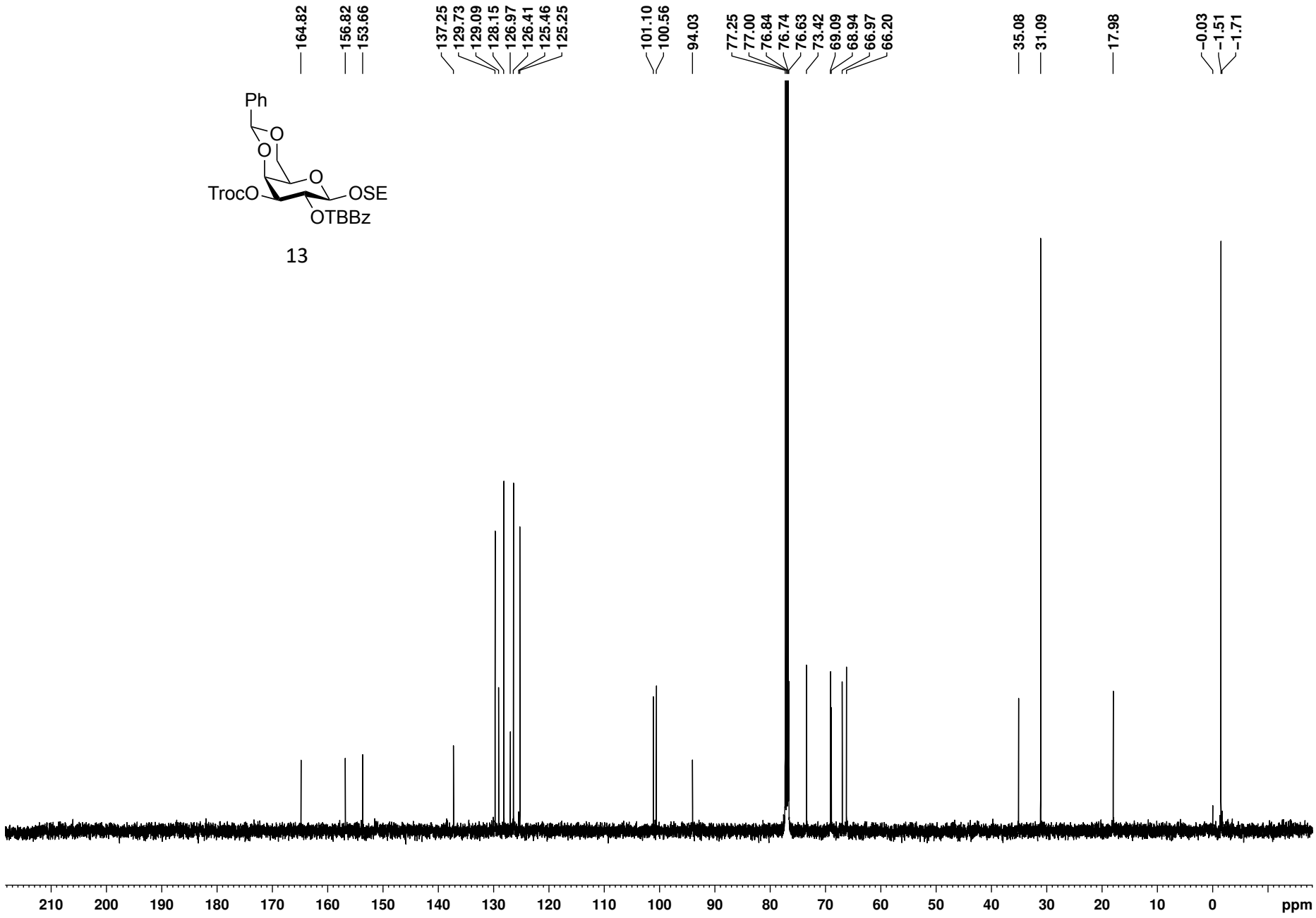


Figure S10. ^{13}C NMR spectrum (125 MHz, $CDCl_3$)

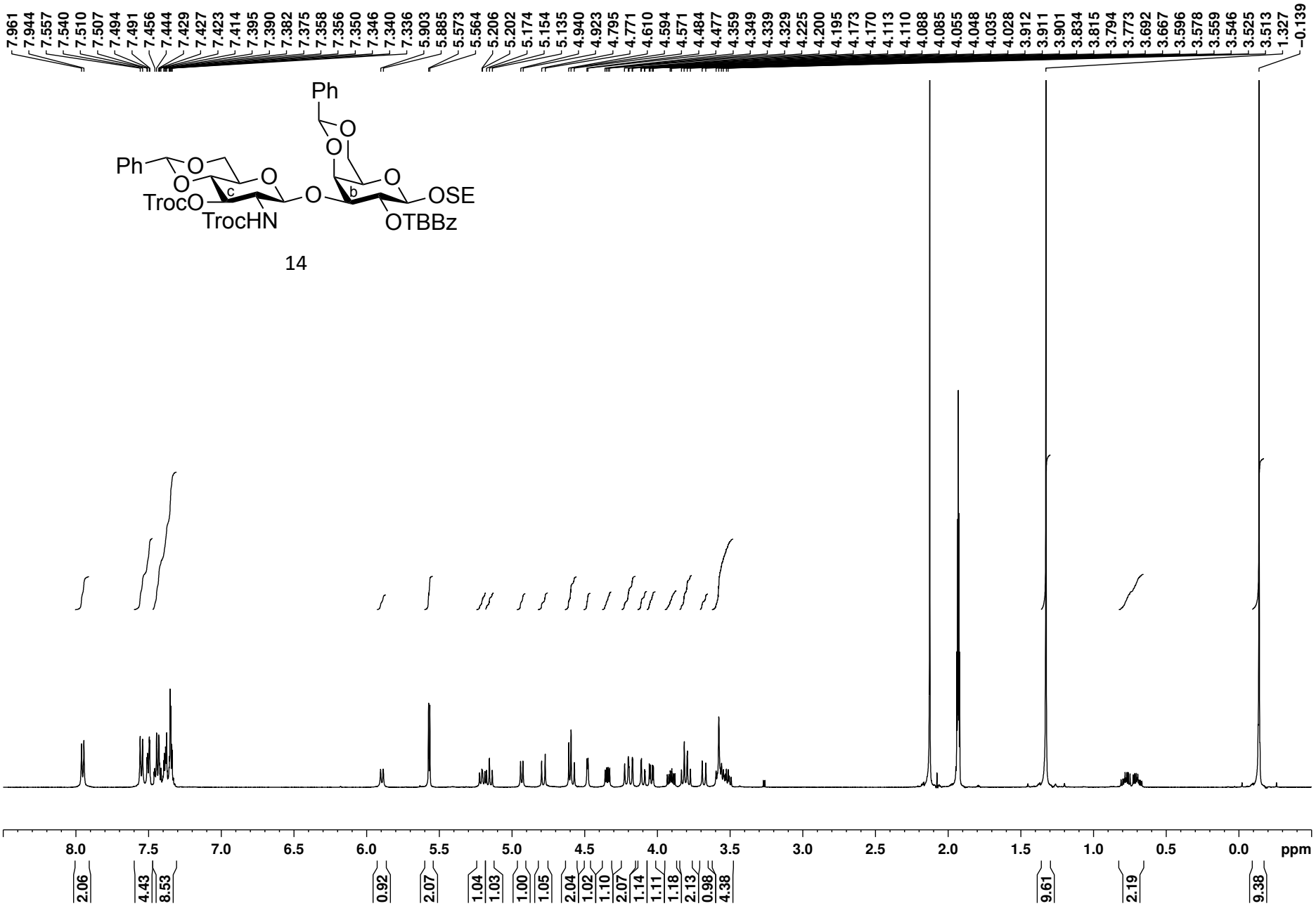


Figure S11. ^{1}H NMR spectrum (500 MHz, CD_3CN)

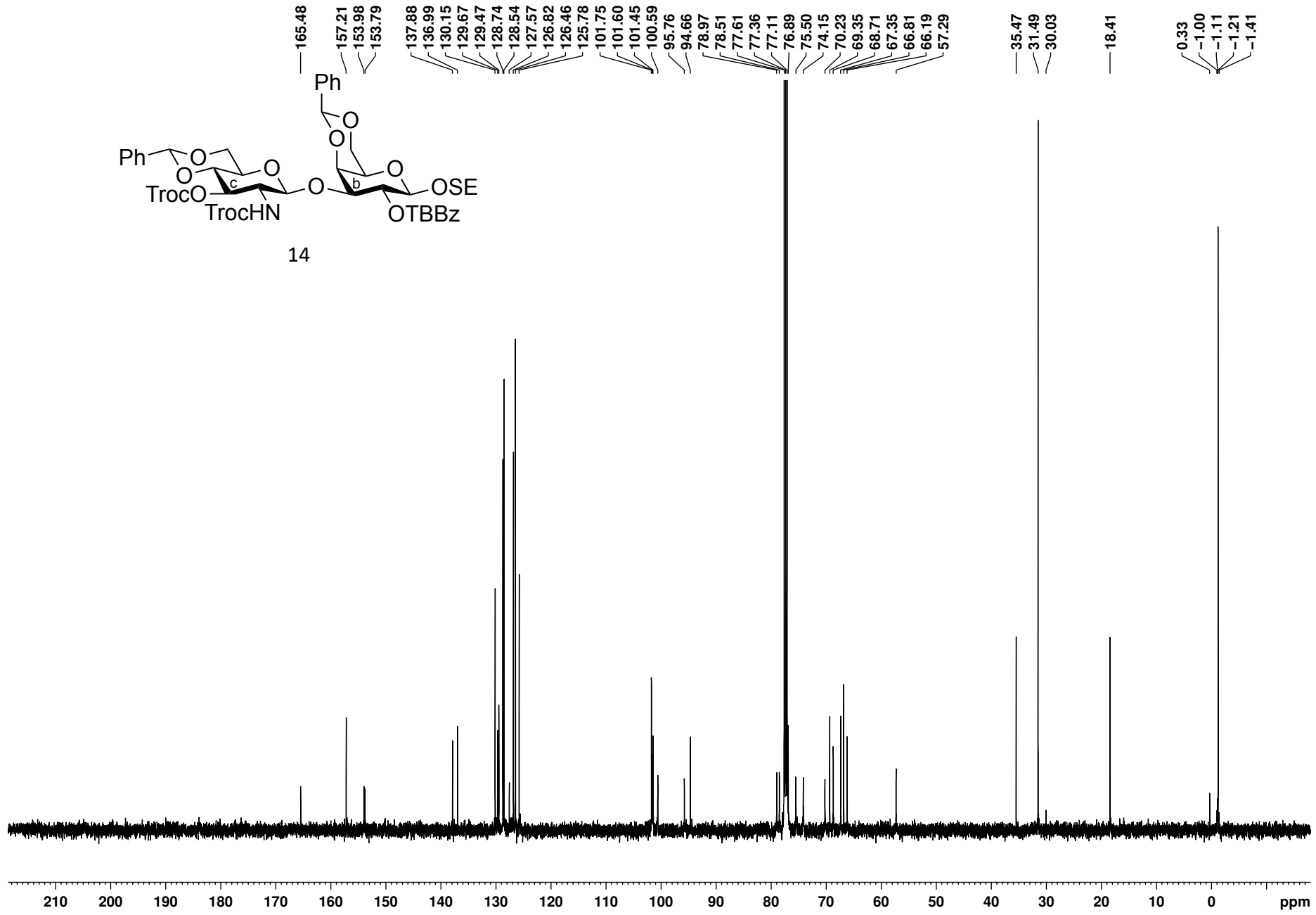


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Figure S12. ^{13}C NMR spectrum (125 MHz, $CDCl_3$)

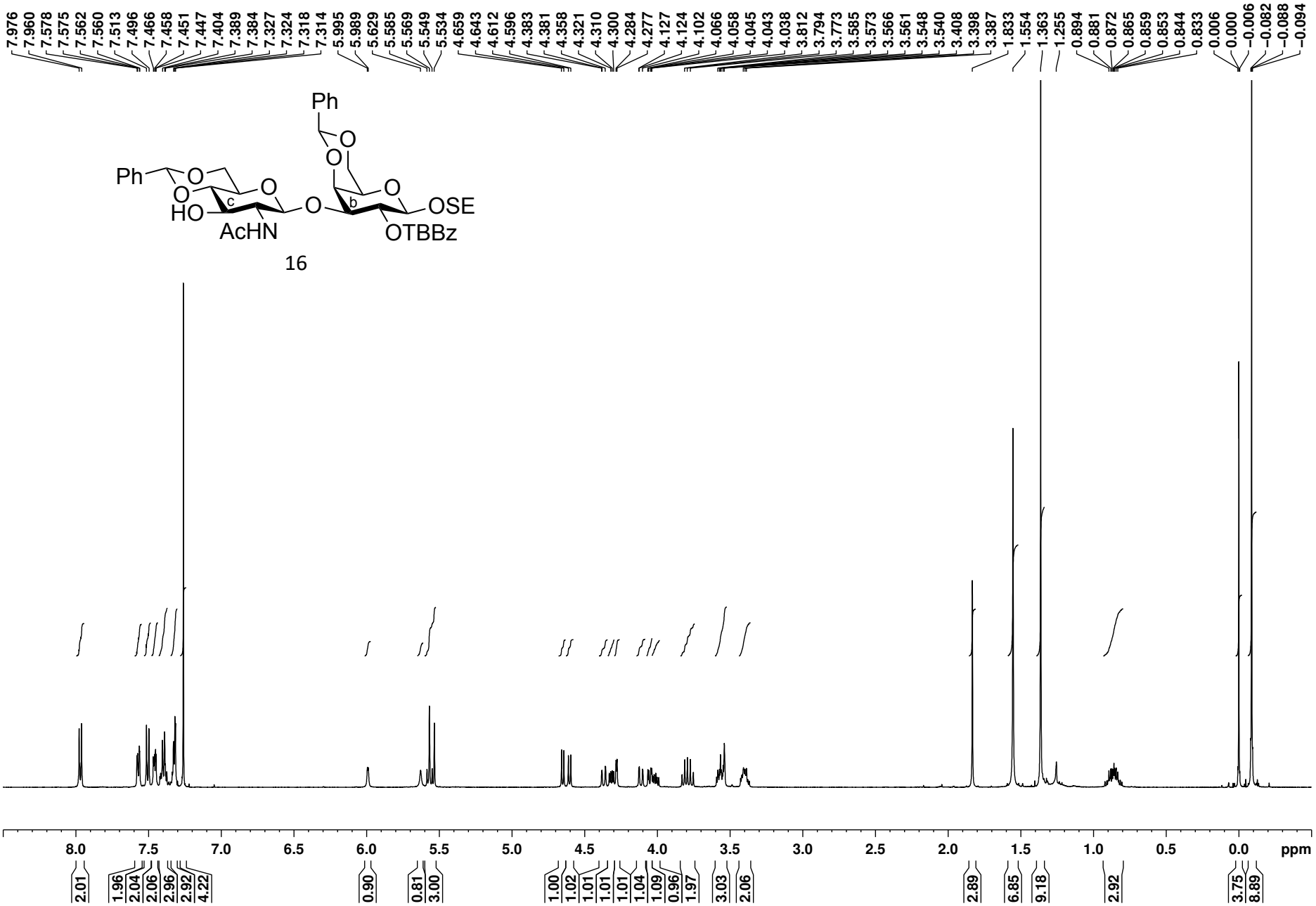

Figure S13. ^{1}H NMR spectrum (500 MHz, $CDCl_3$)

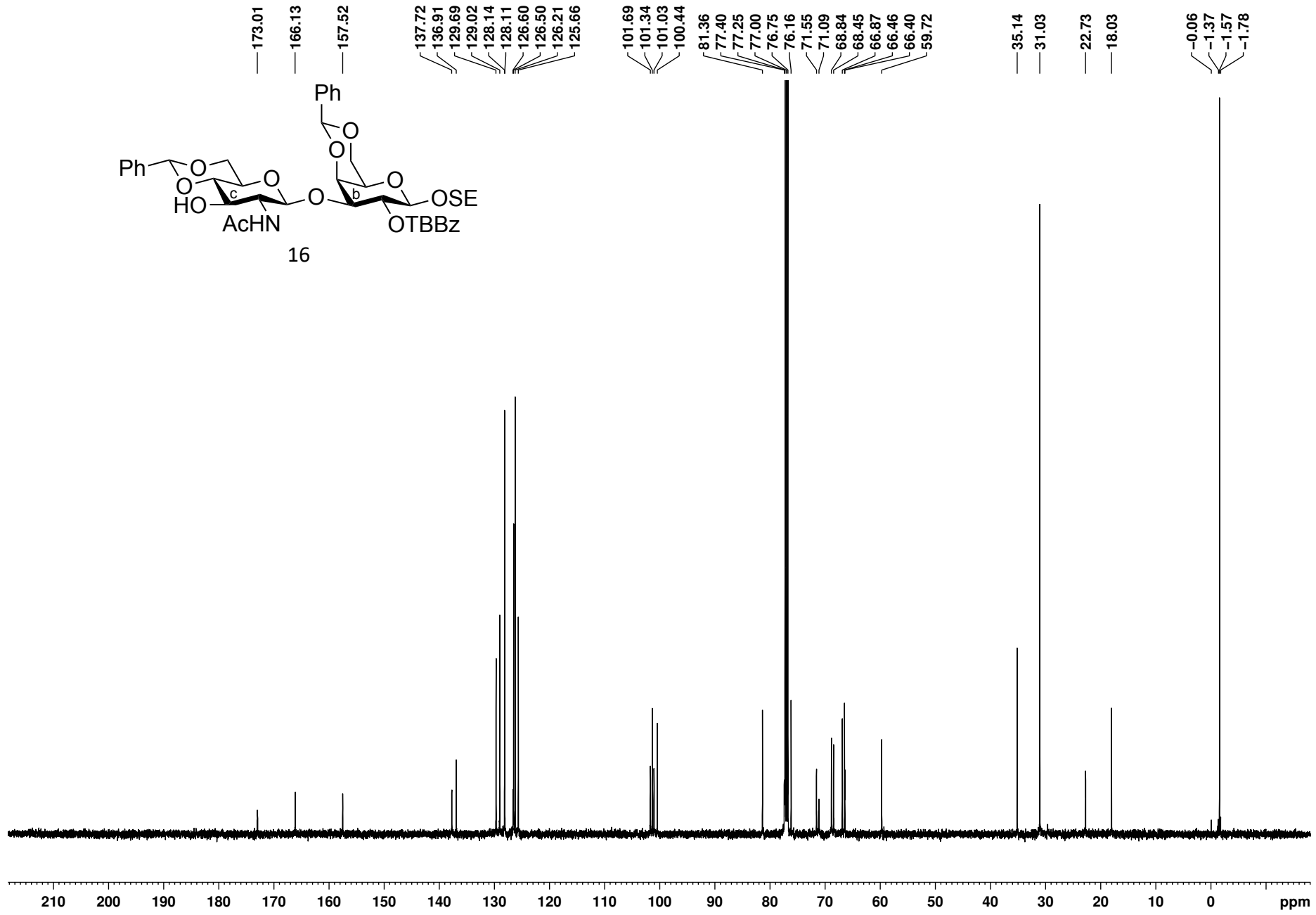

Figure S14. ^{13}C NMR spectrum (125 MHz, $CDCl_3$)

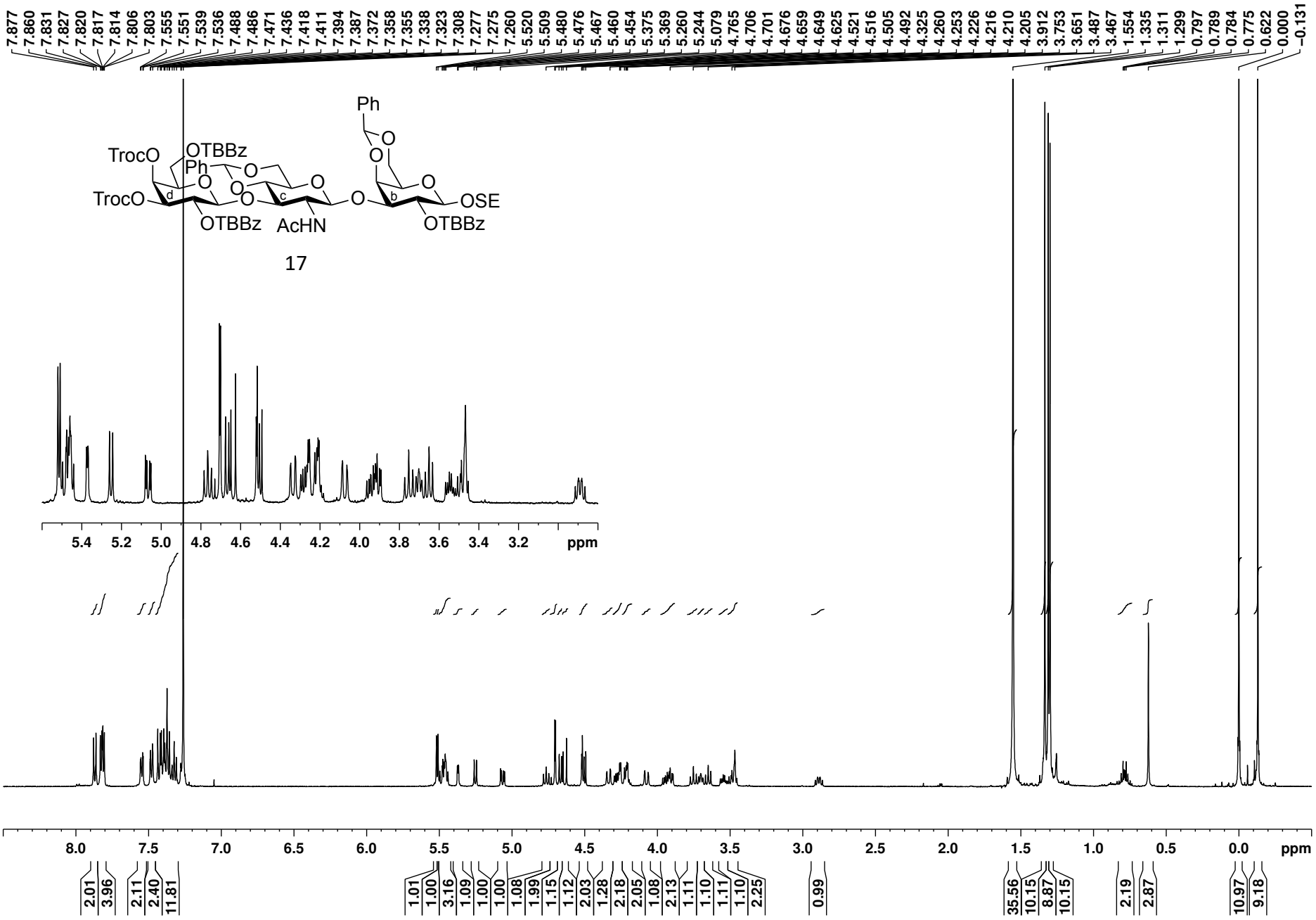

Figure S15. ^{1}H NMR spectrum (500 MHz, $CDCl_3$)

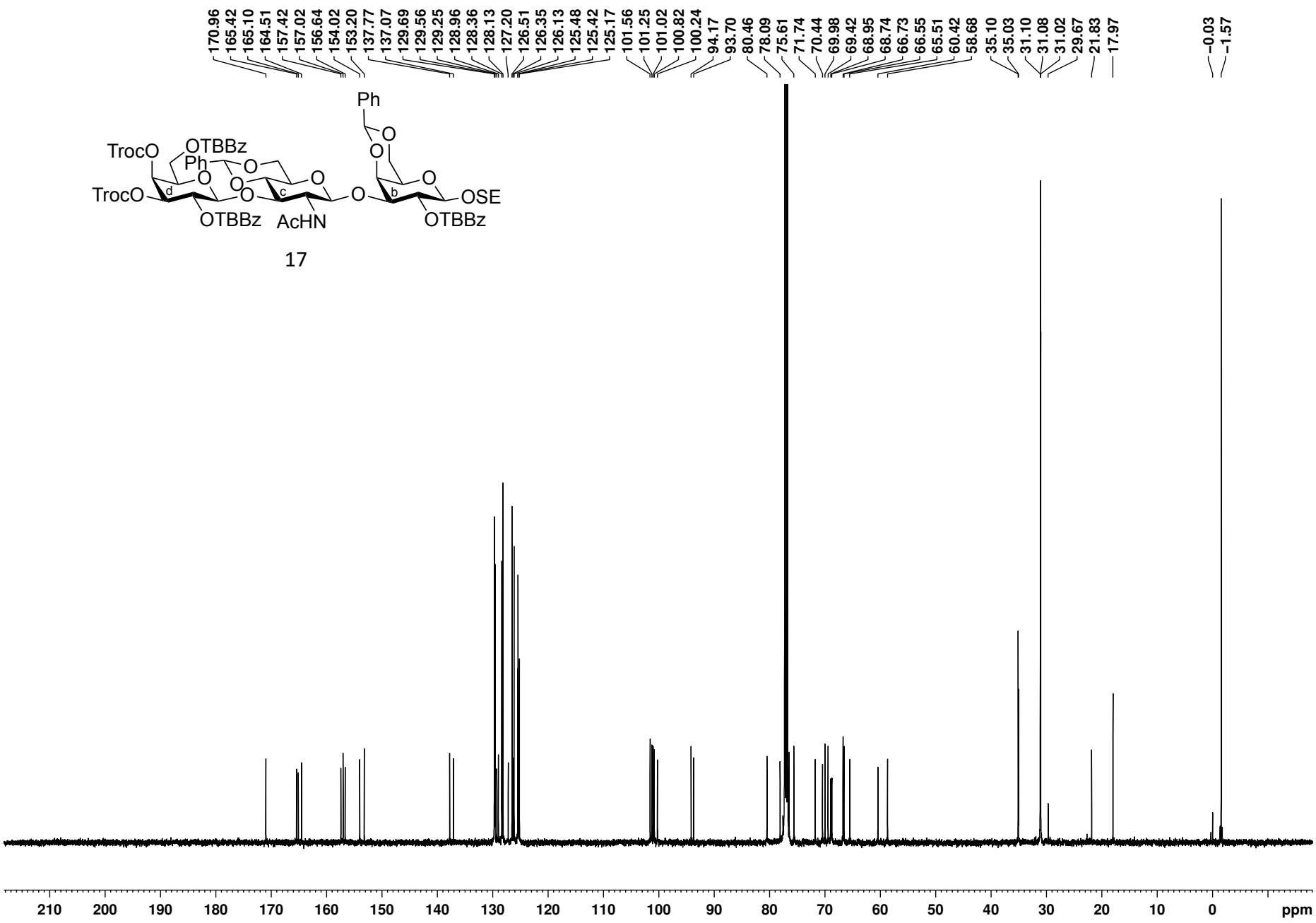

Figure S16. ^{13}C NMR spectrum (125 MHz, $CDCl_3$)

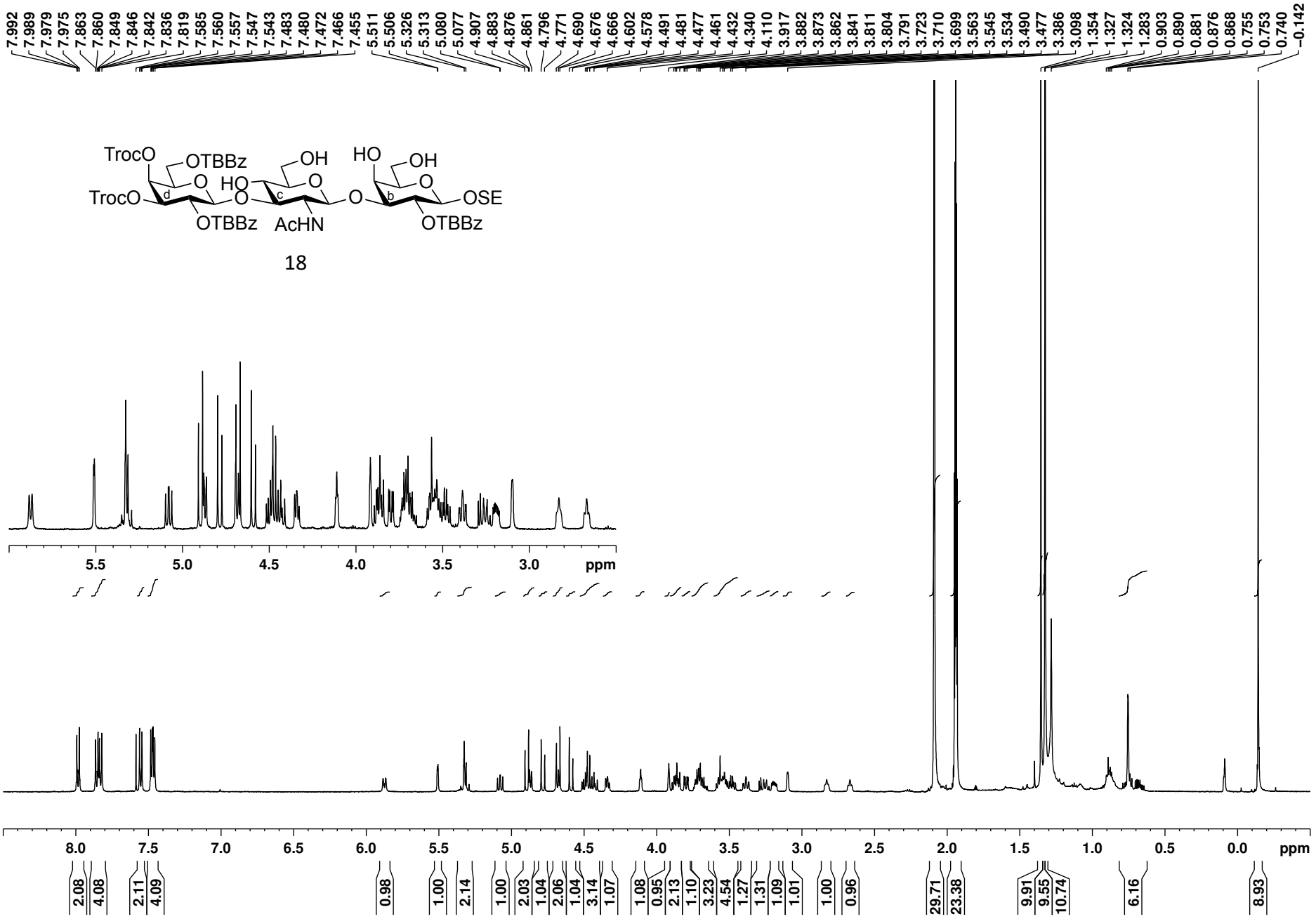

Figure S17. ^{1}H NMR spectrum (500 MHz, CD_3CN)

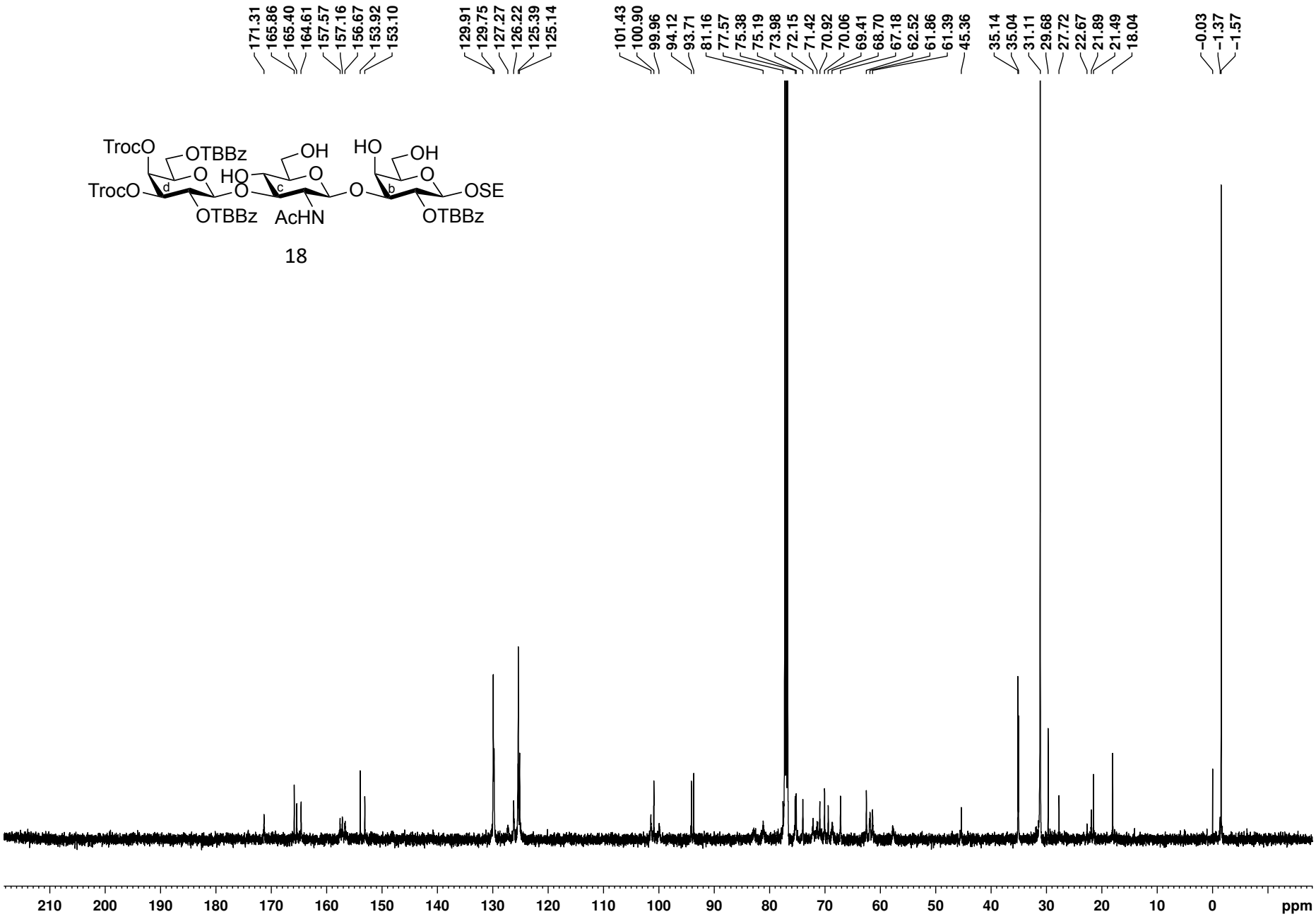

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Figure S18. ^{13}C NMR spectrum (125 MHz, $CDCl_3$)

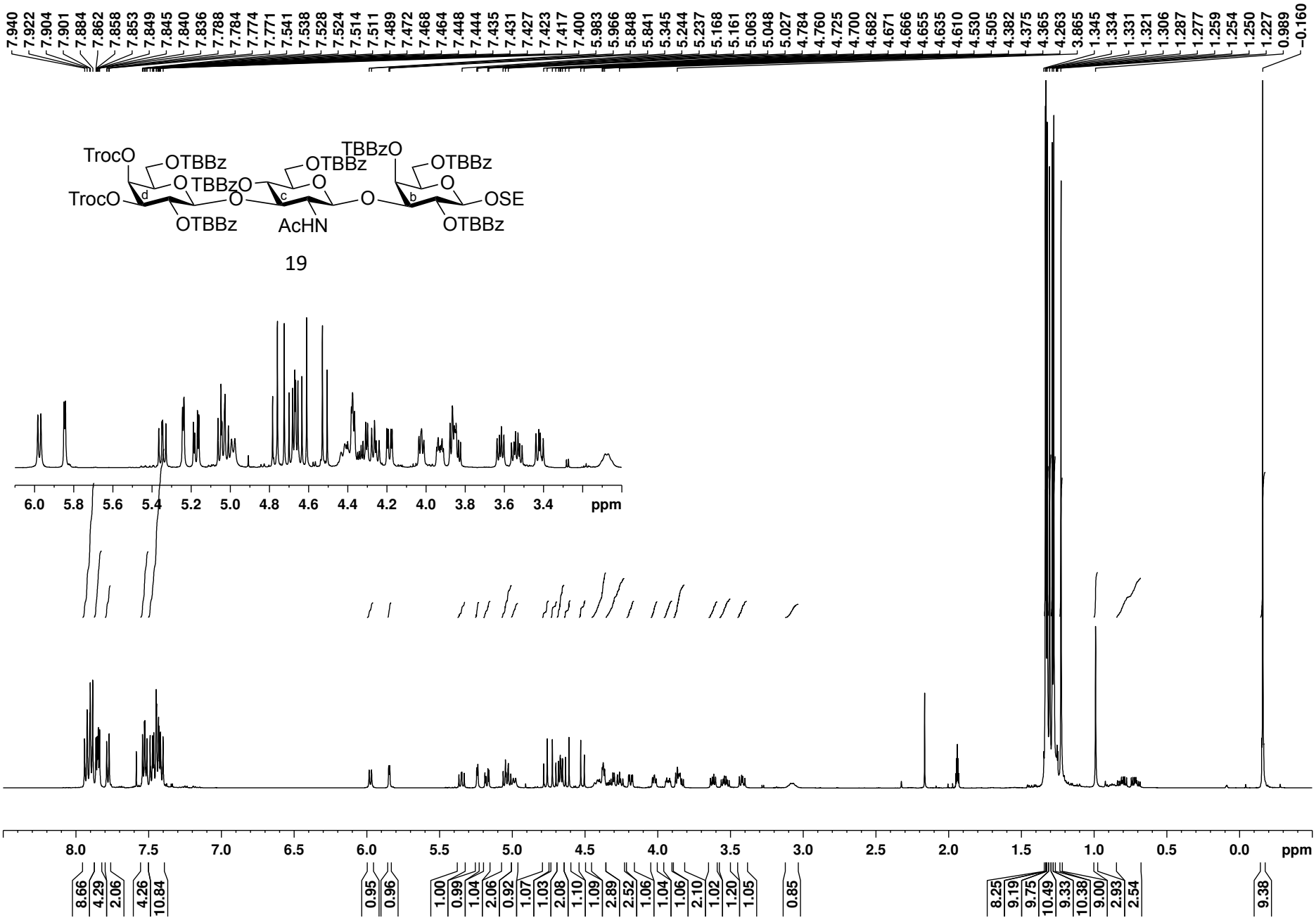

Figure S19. ^{1}H NMR spectrum (500 MHz, CD_3CN)

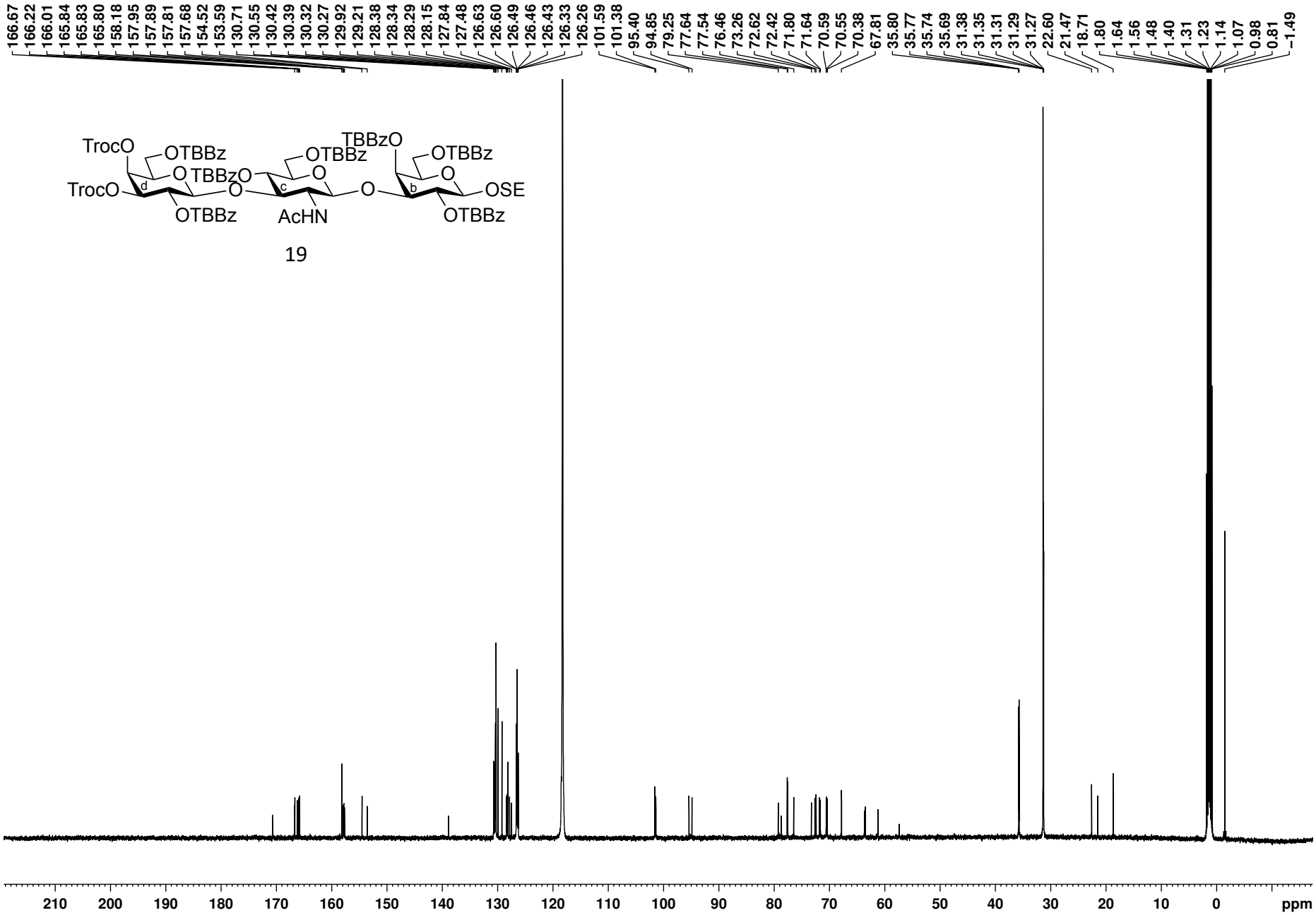

Figure S20. ^{13}C NMR spectrum (125 MHz, CD_3CN)

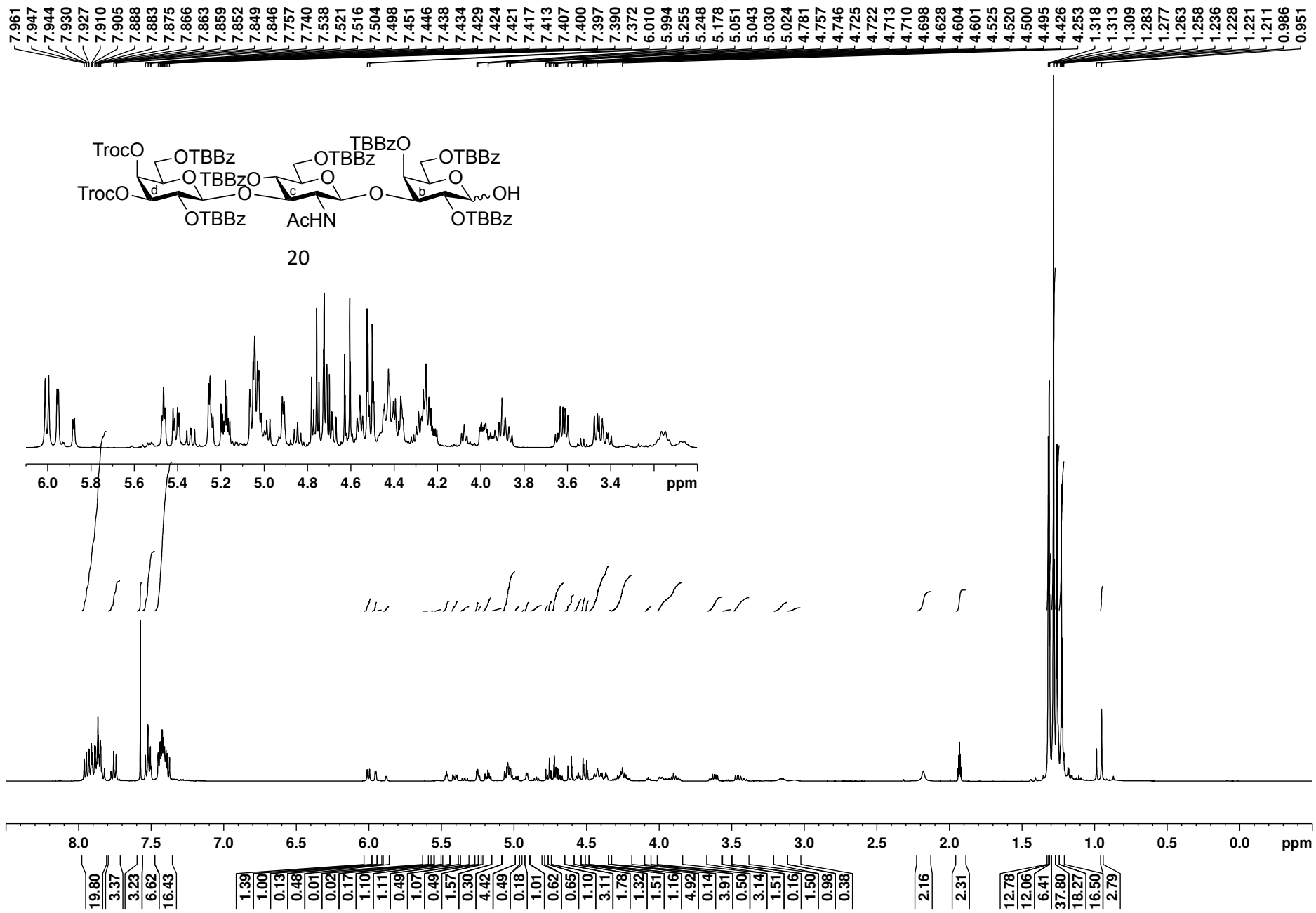

Figure S21. ^{1}H NMR spectrum (500 MHz, CD_3CN)

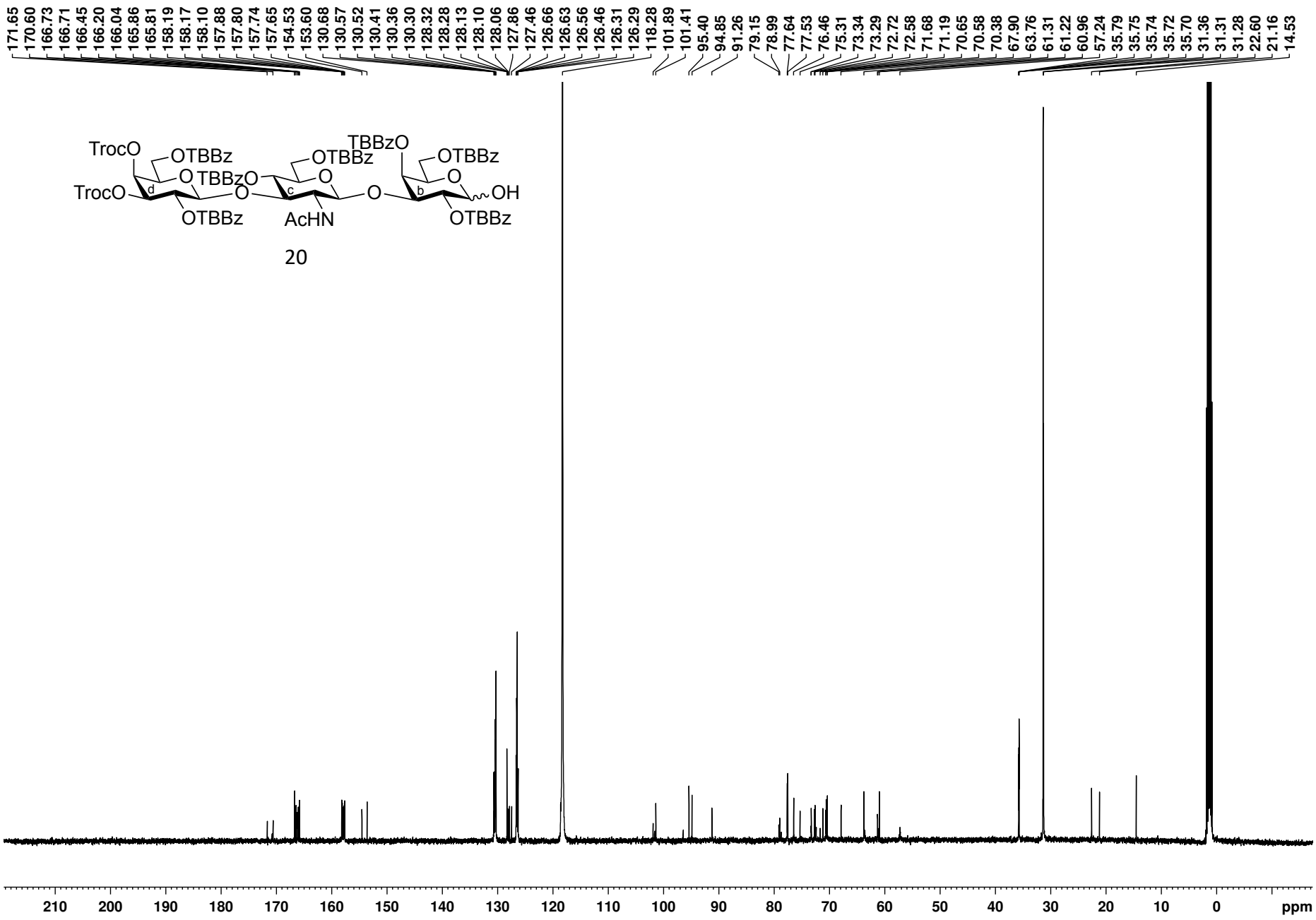

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Figure S22. ^{13}C NMR spectrum (125 MHz, CD_3CN)

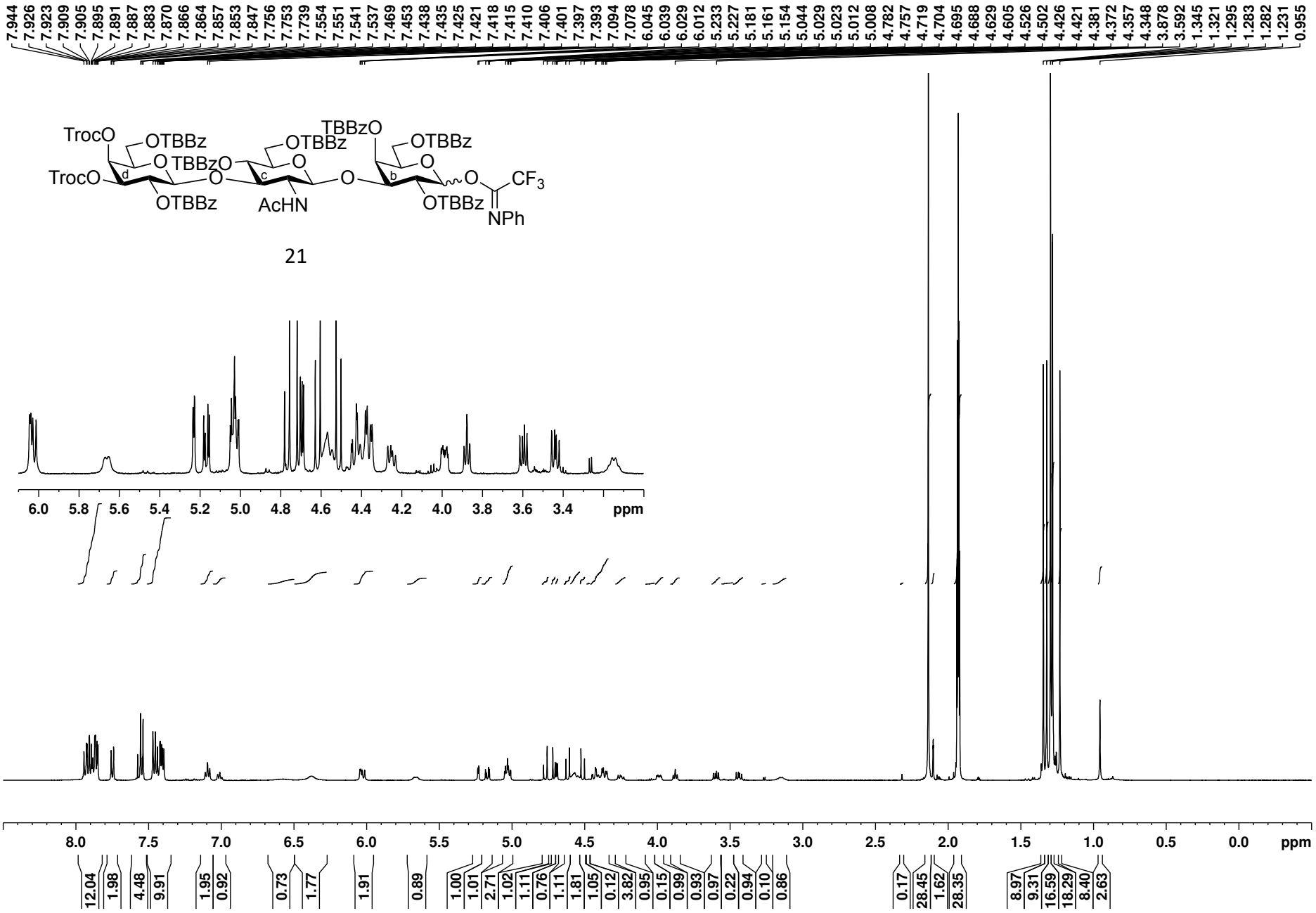

Figure S23. ^{1}H NMR spectrum (500 MHz, CD_3CN)

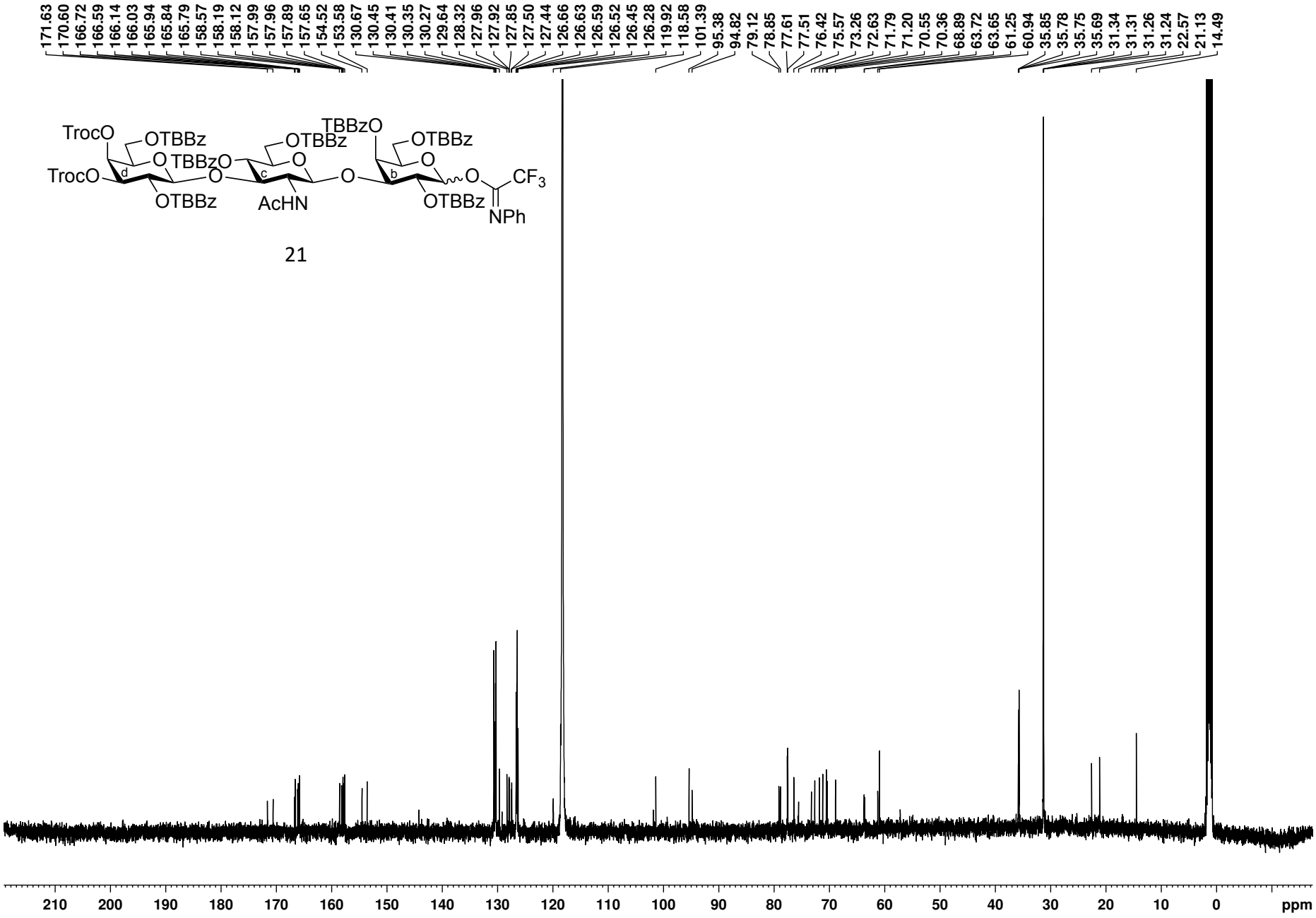

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Figure S24. ^{13}C NMR spectrum (125 MHz, CD_3CN)

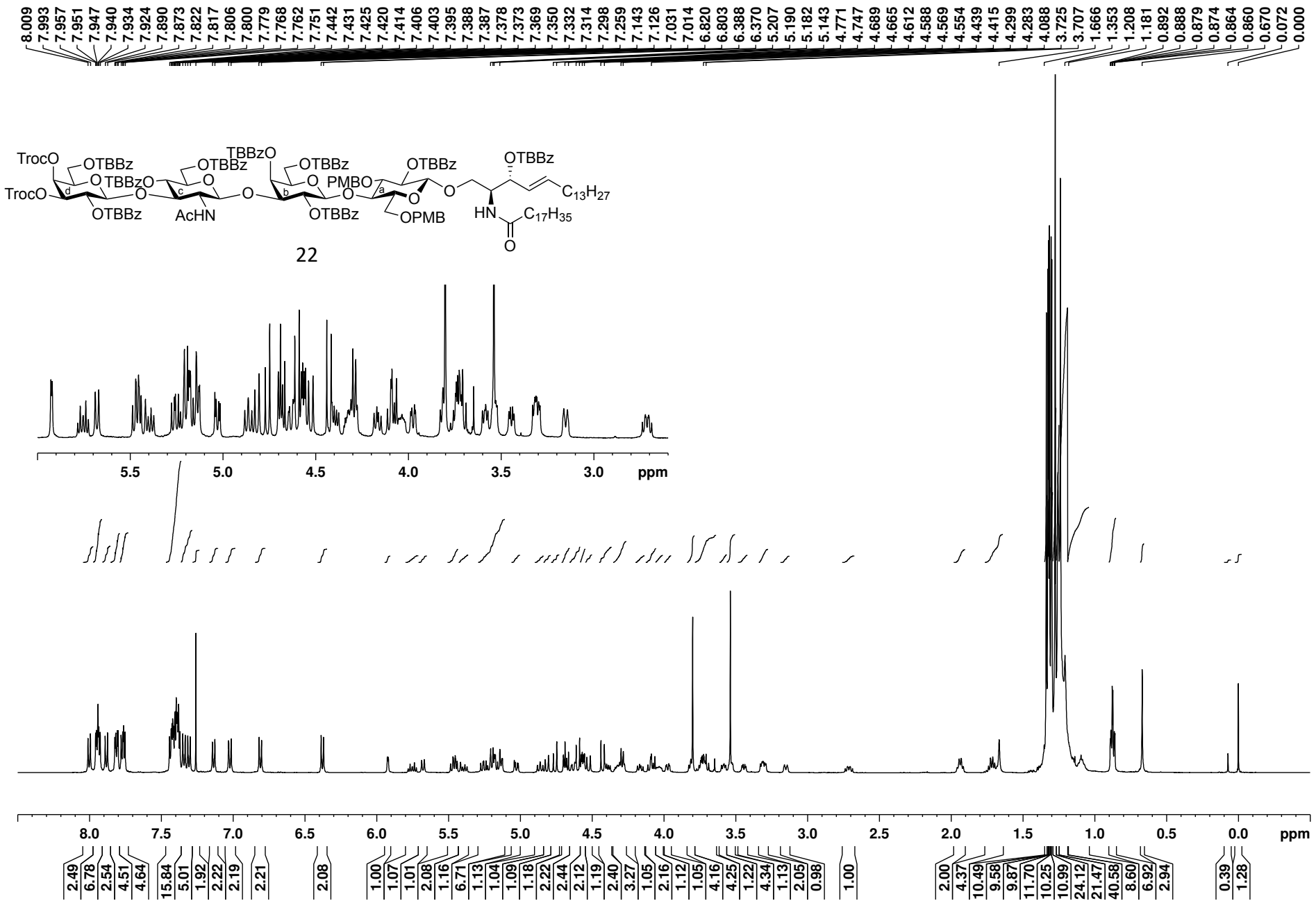

Figure S25. ^{1}H NMR spectrum (500 MHz, $CDCl_3$)

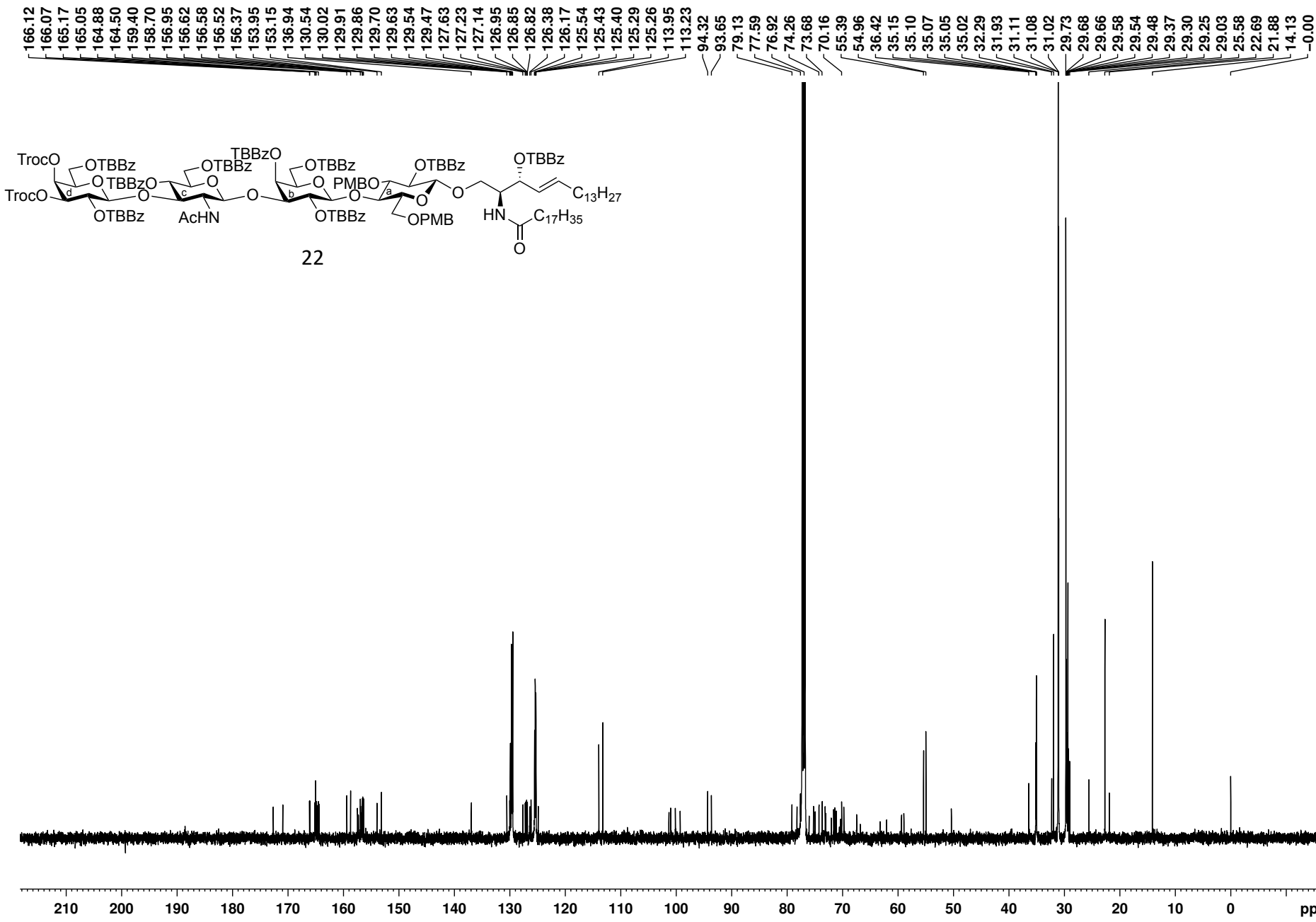

Figure S26. ^{13}C NMR spectrum (125 MHz, $CDCl_3$)

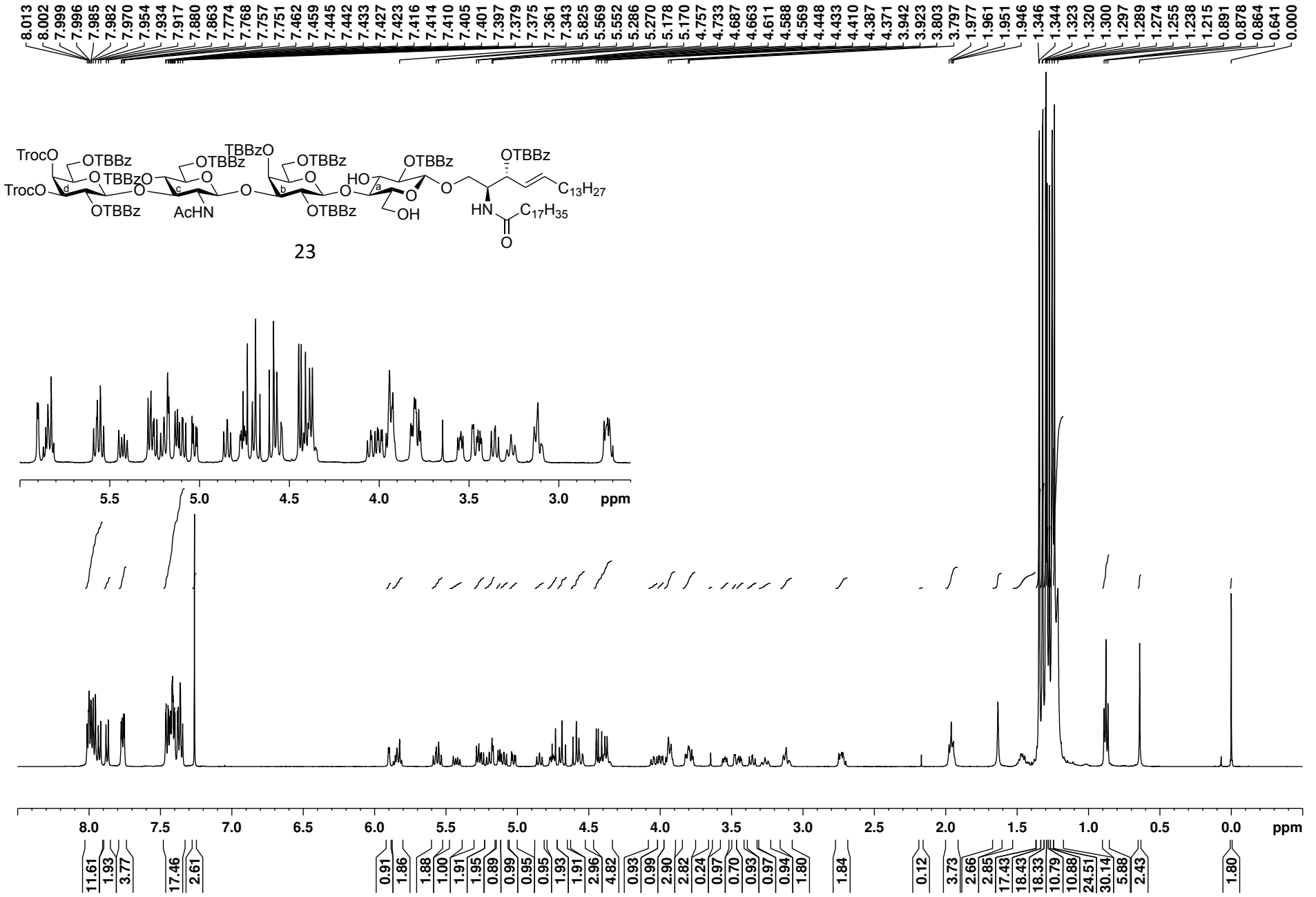

Figure S27. ^{1}H NMR spectrum (500 MHz, $CDCl_3$)

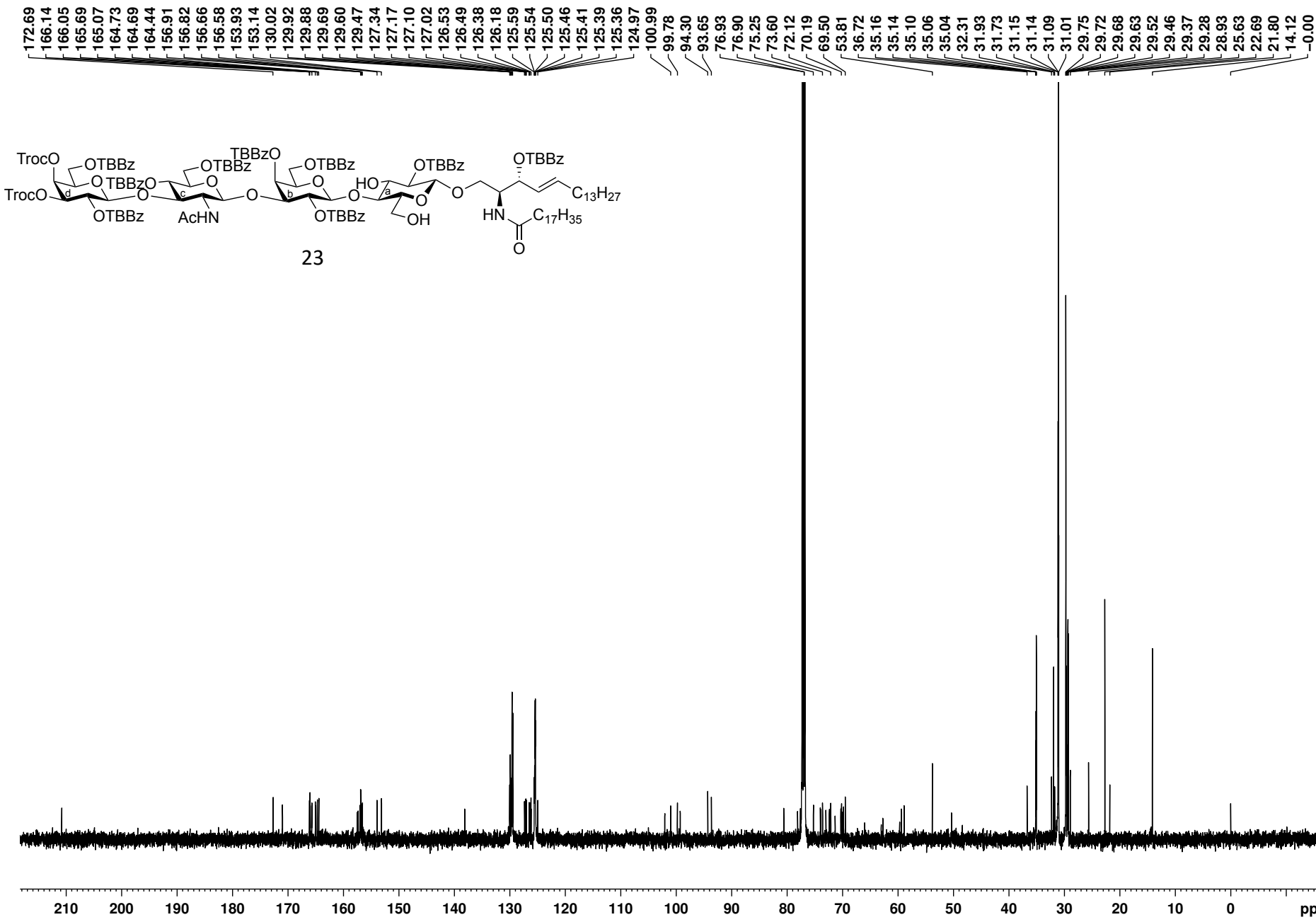

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Figure S28. ^{13}C NMR spectrum (125 MHz, $CDCl_3$)

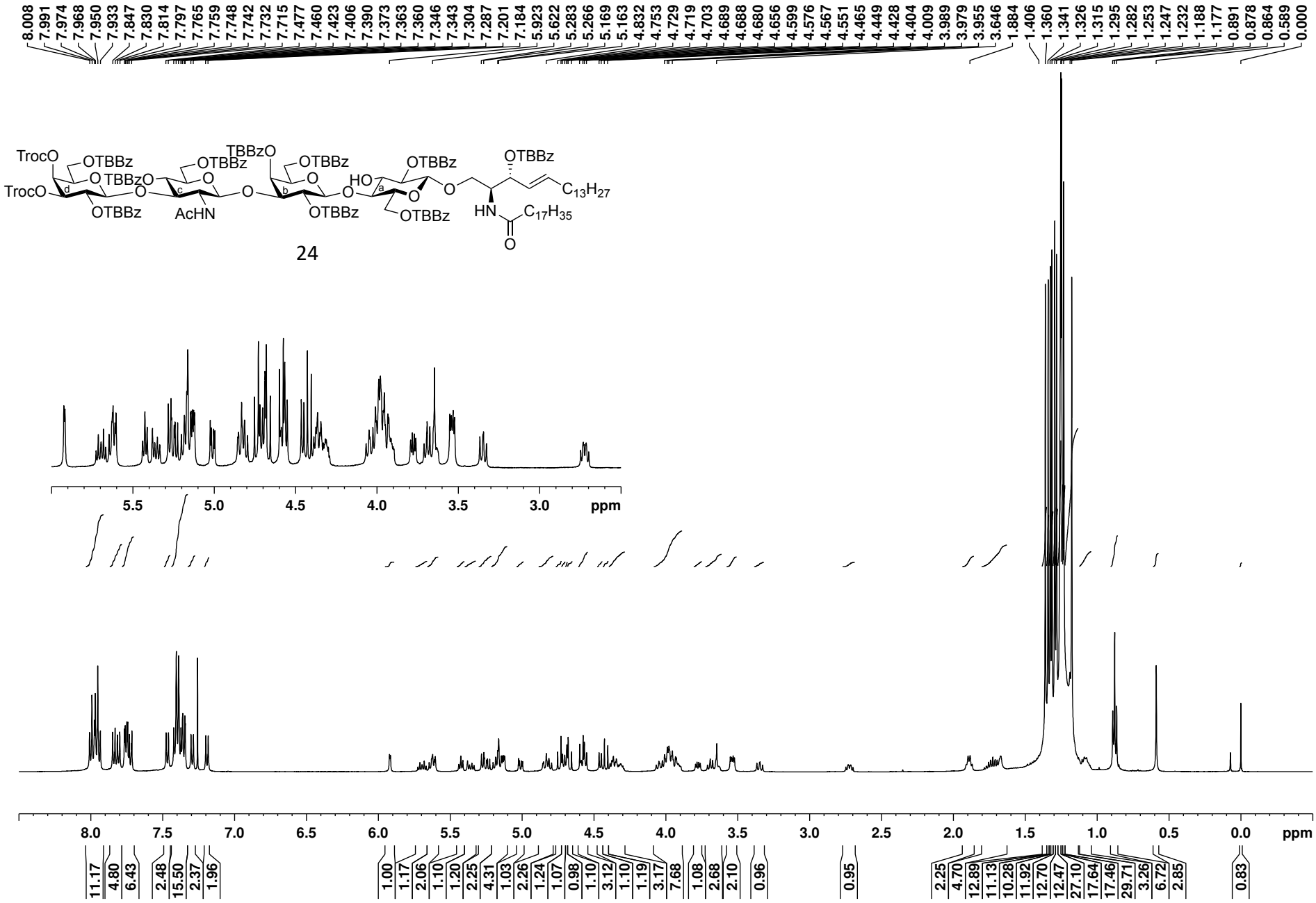

Figure S29. ^{1}H NMR spectrum (500 MHz, $CDCl_3$)

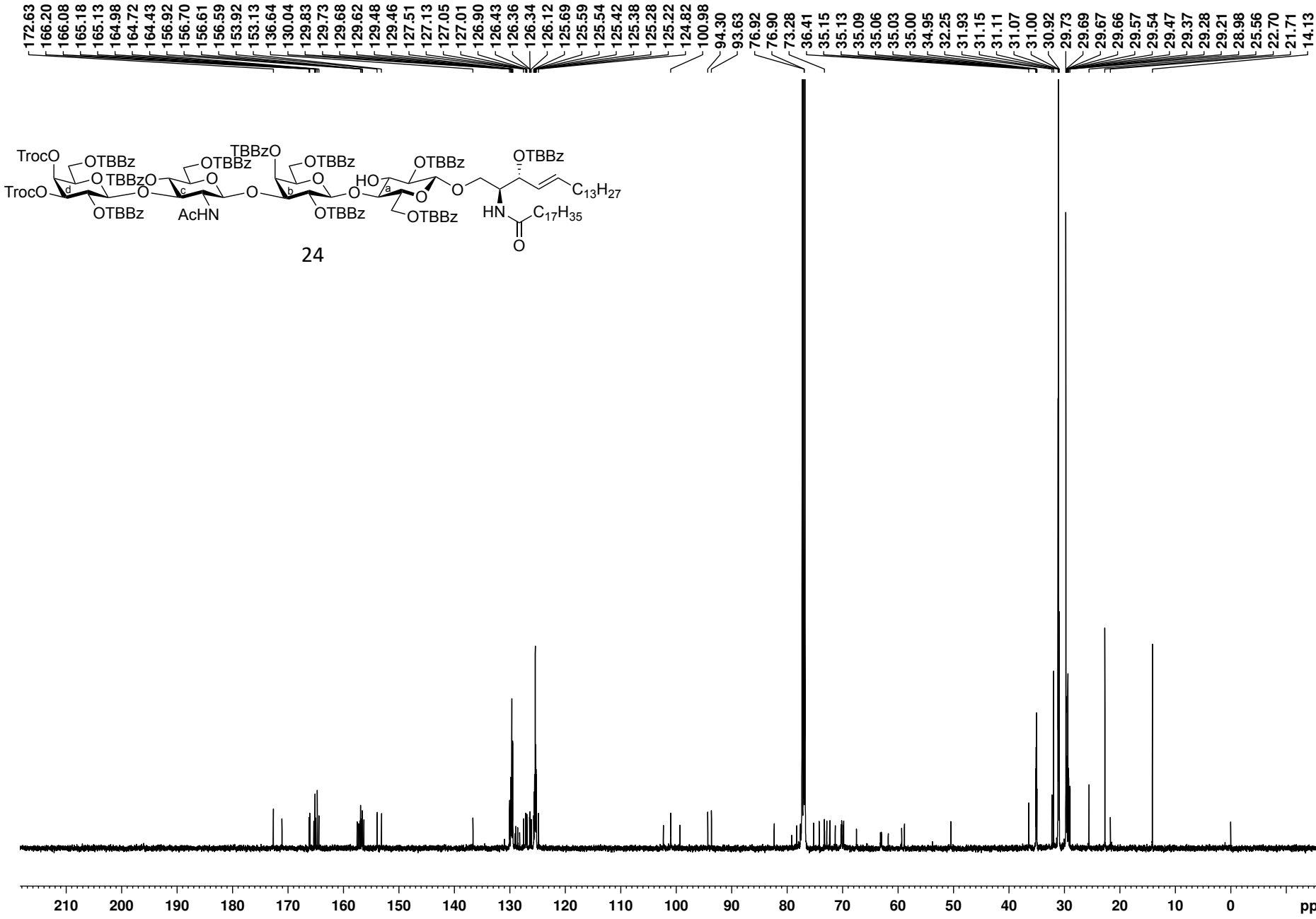

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Figure S30. ^{13}C NMR spectrum (125 MHz, $CDCl_3$)

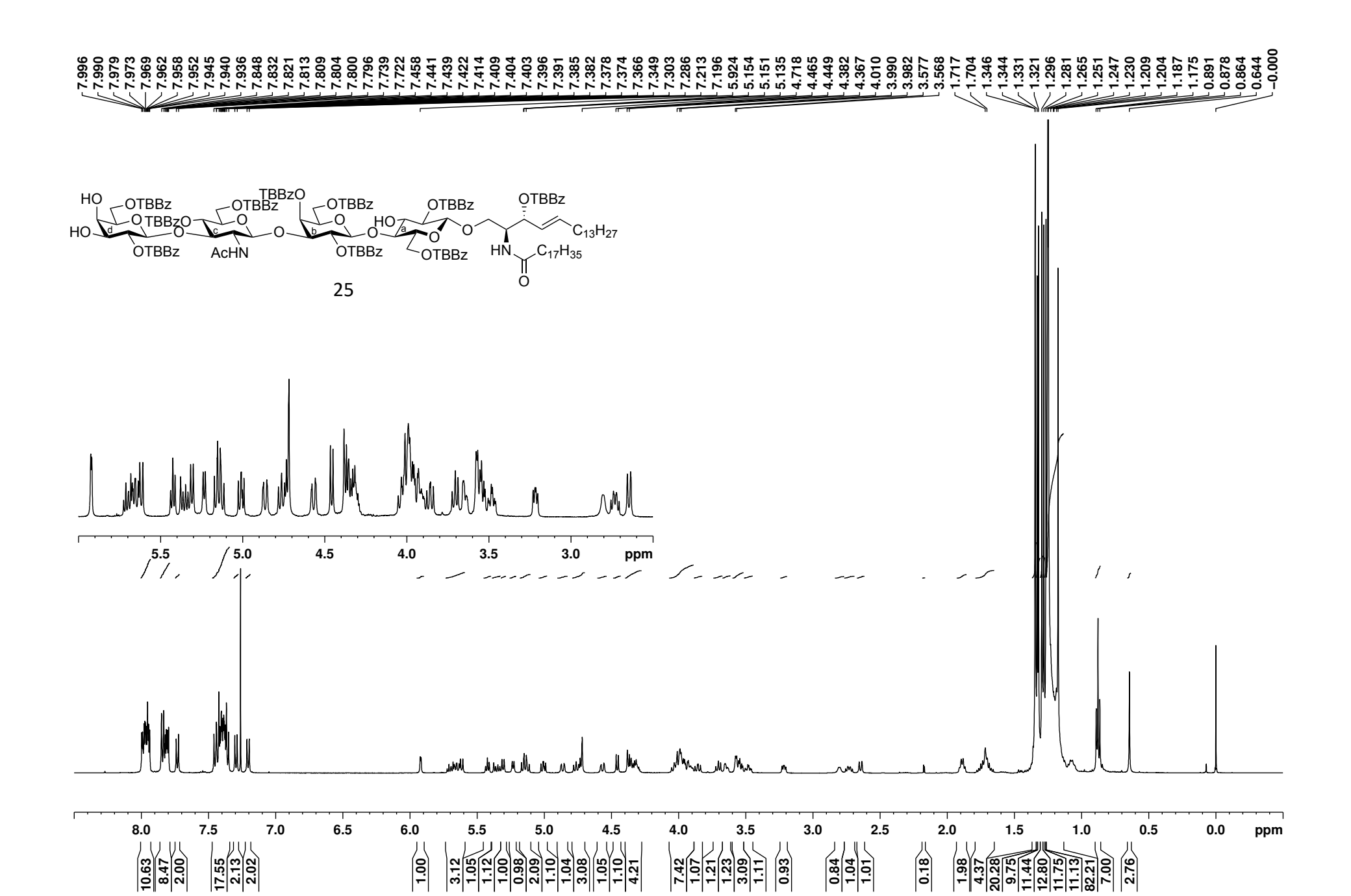

Figure S31. ^{1}H NMR spectrum (500 MHz, $CDCl_3$)

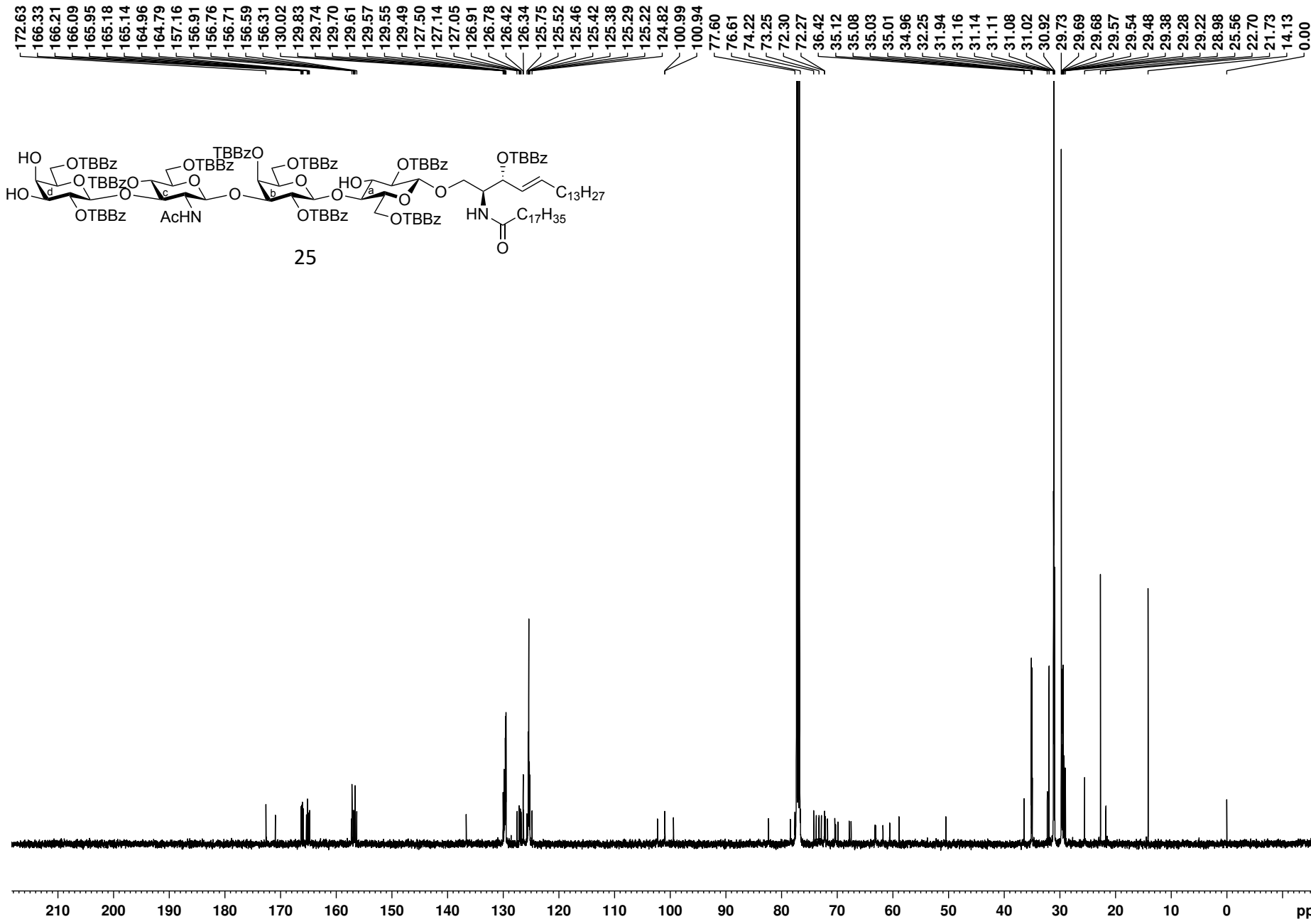

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Figure S32. ^{13}C NMR spectrum (125 MHz, $CDCl_3$)

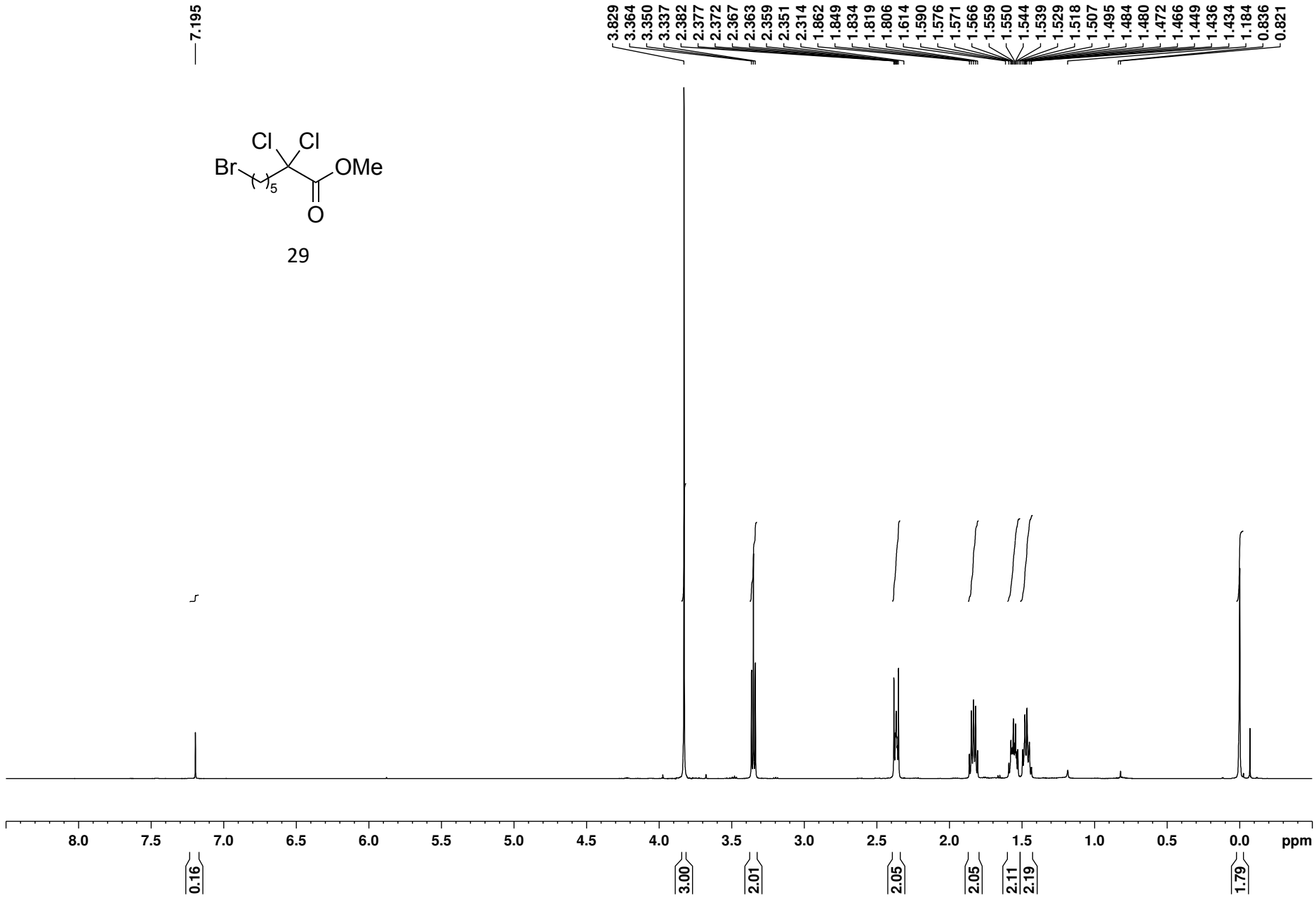

Figure S33. ^{1}H NMR spectrum (500 MHz, $CDCl_3$)

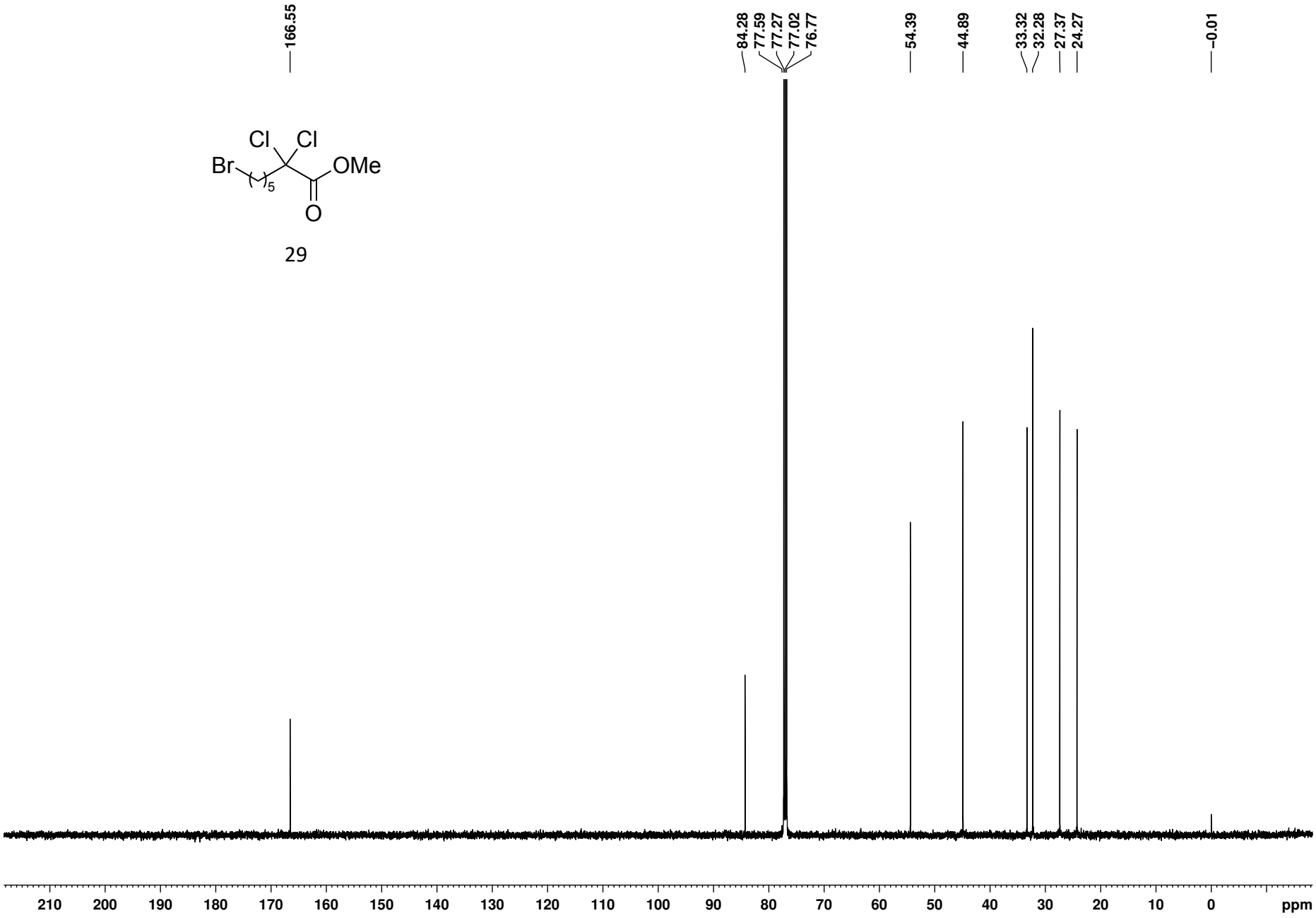

Figure S34. ^{13}C NMR spectrum (125 MHz, $CDCl_3$)

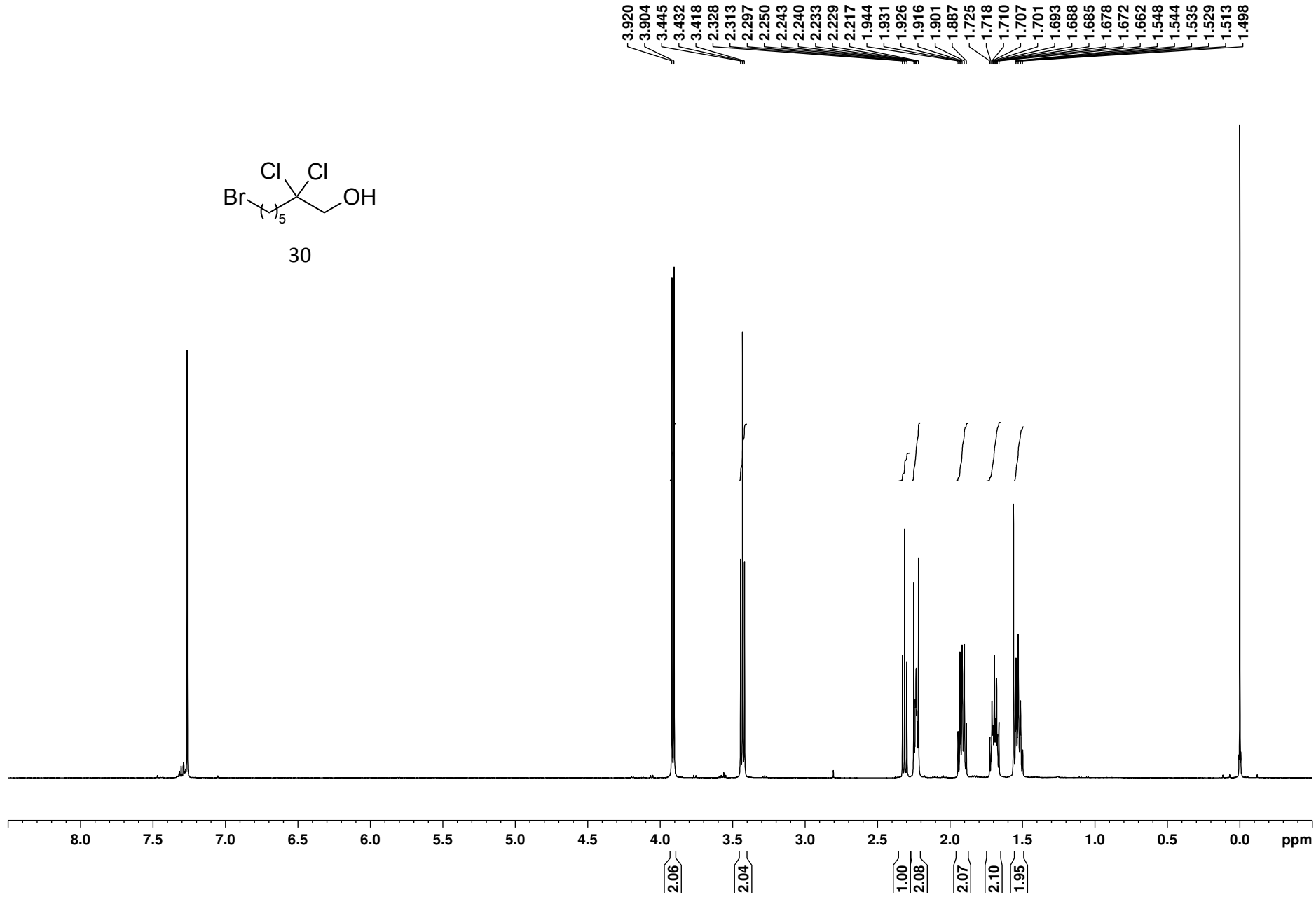

Figure S35. ^{1}H NMR spectrum (500 MHz, $CDCl_3$)

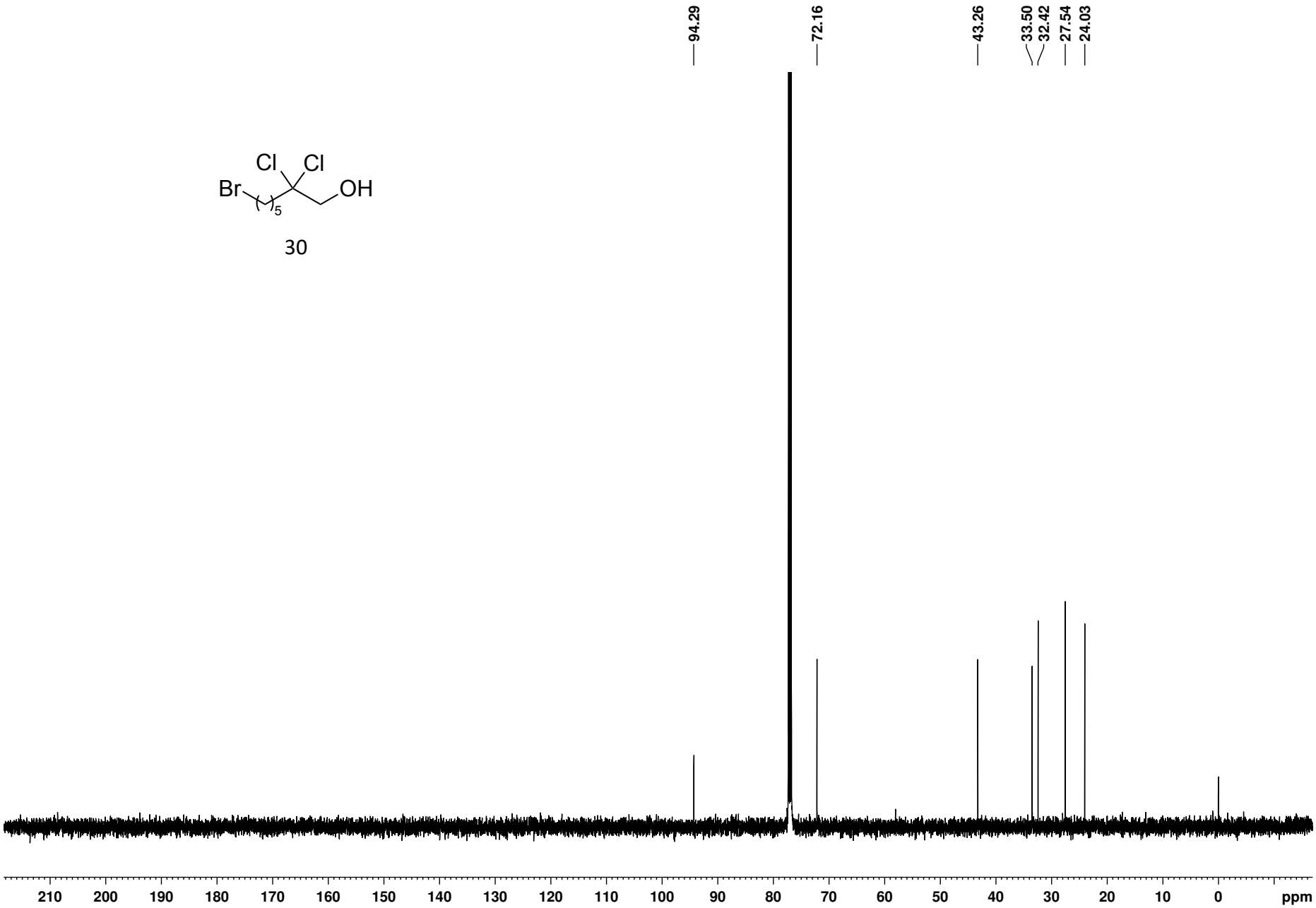

Figure S36. ^{13}C NMR spectrum (125 MHz, $CDCl_3$)

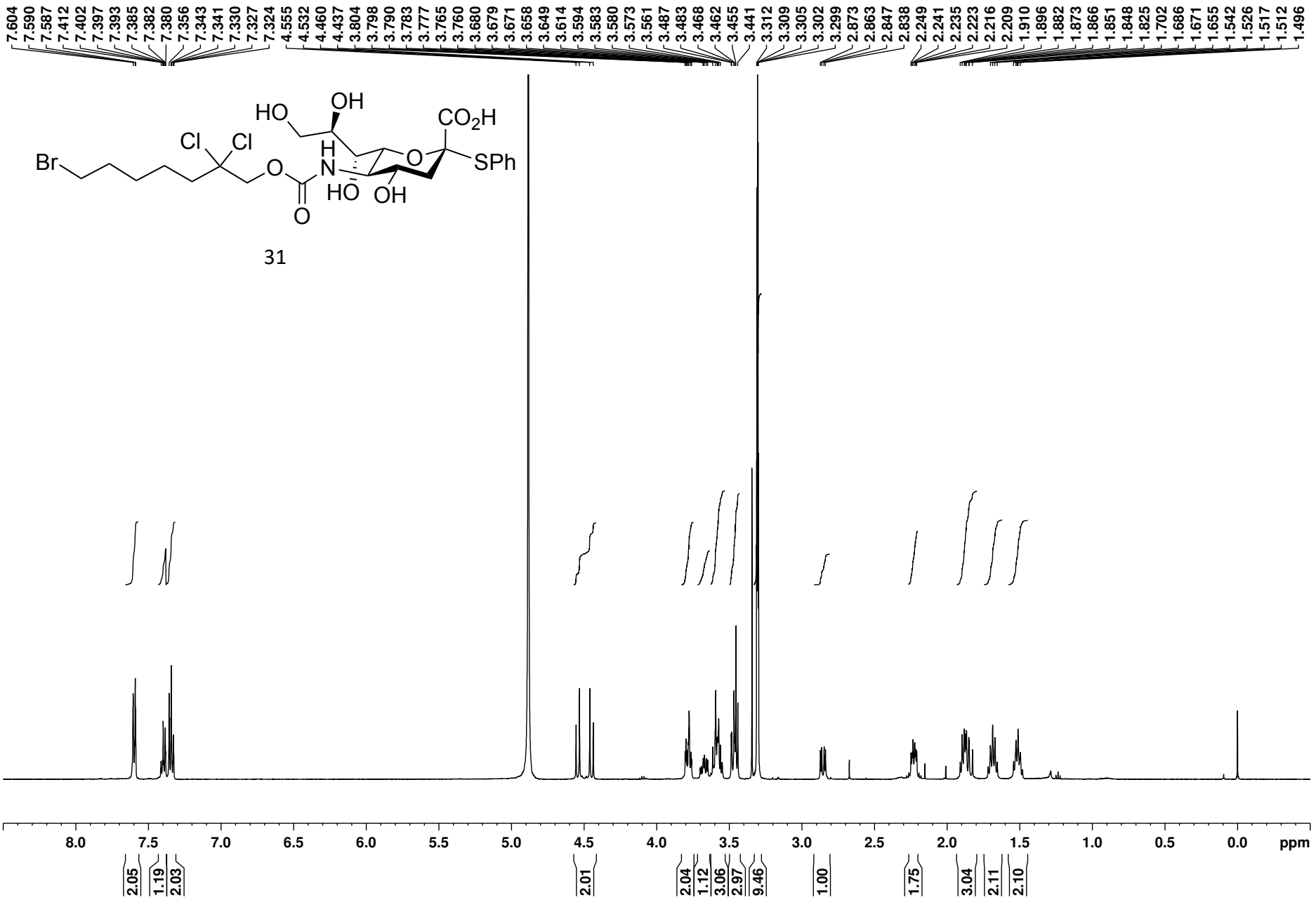

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Figure S37. ^{1}H NMR spectrum (500 MHz, CD_3OD)

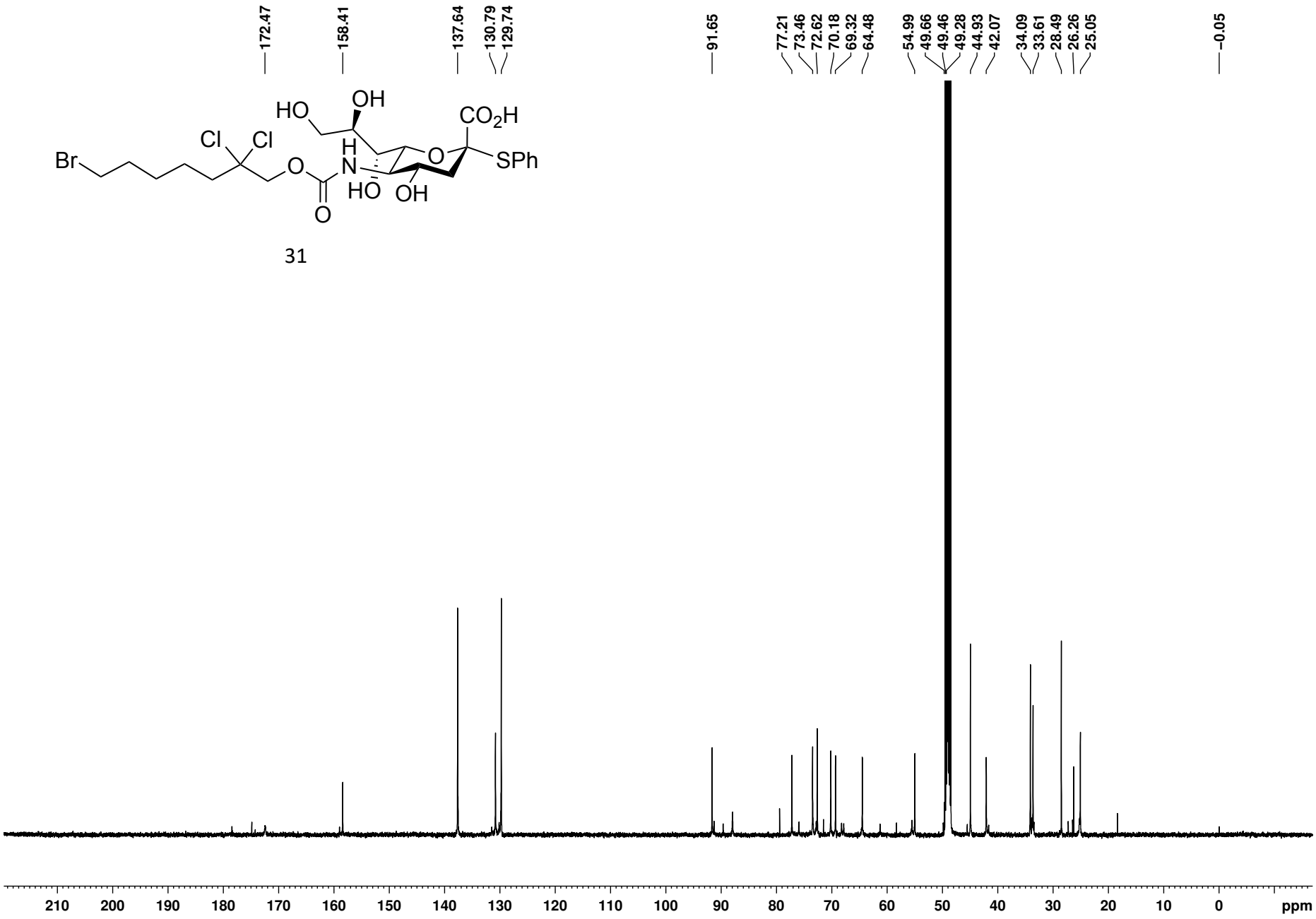

Figure S38. ^{13}C NMR spectrum (125 MHz, CD_3OD)

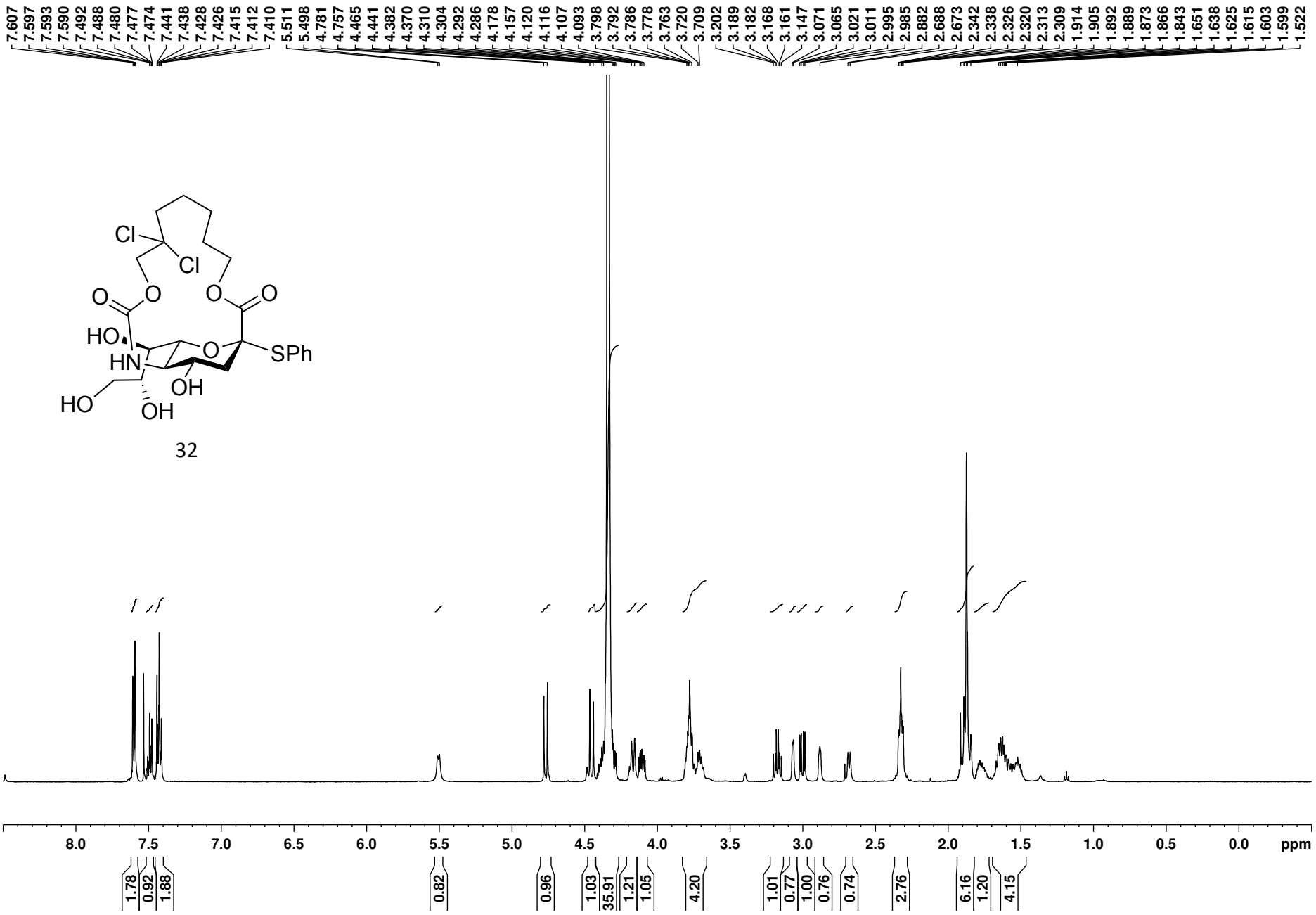

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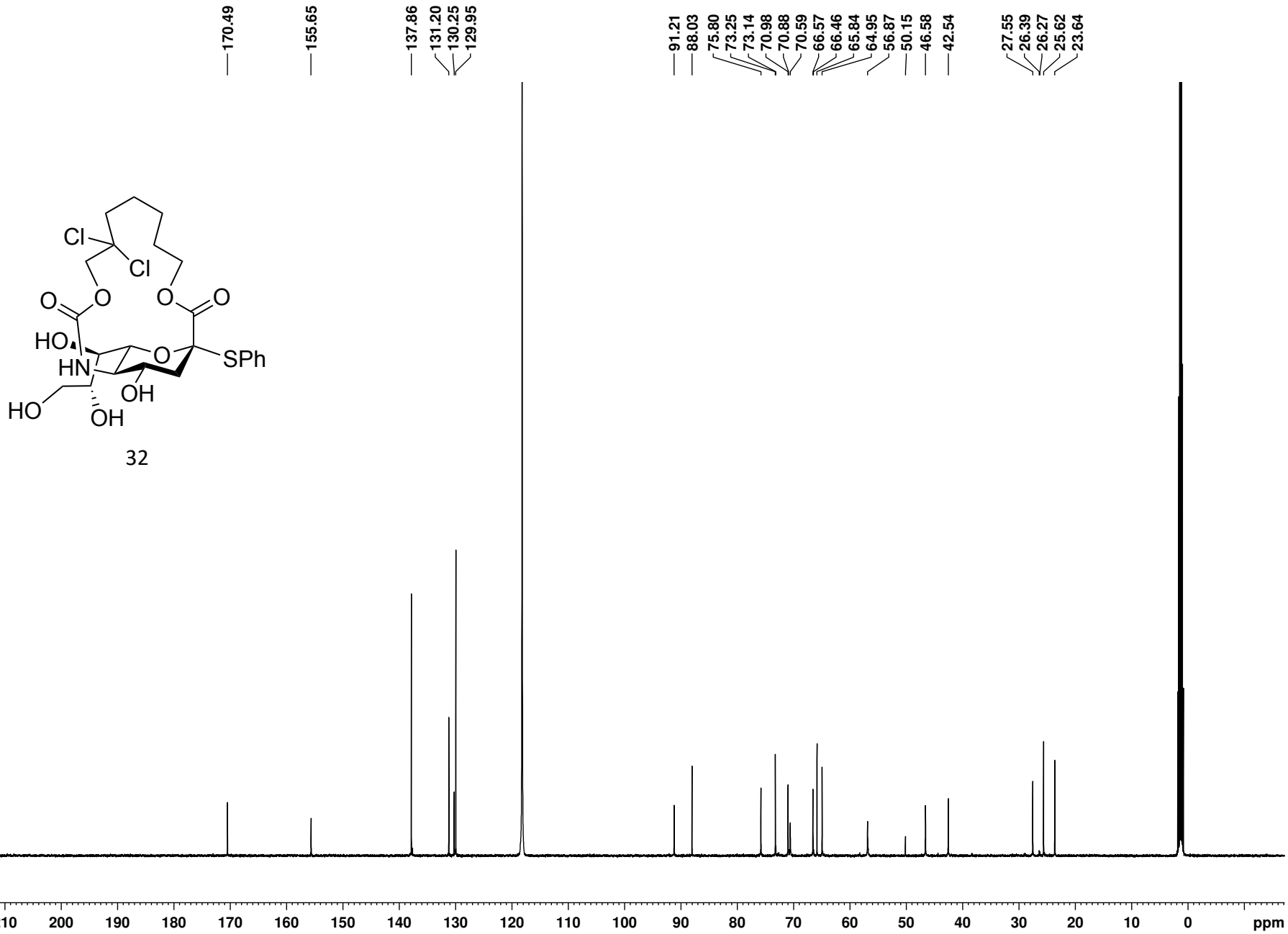
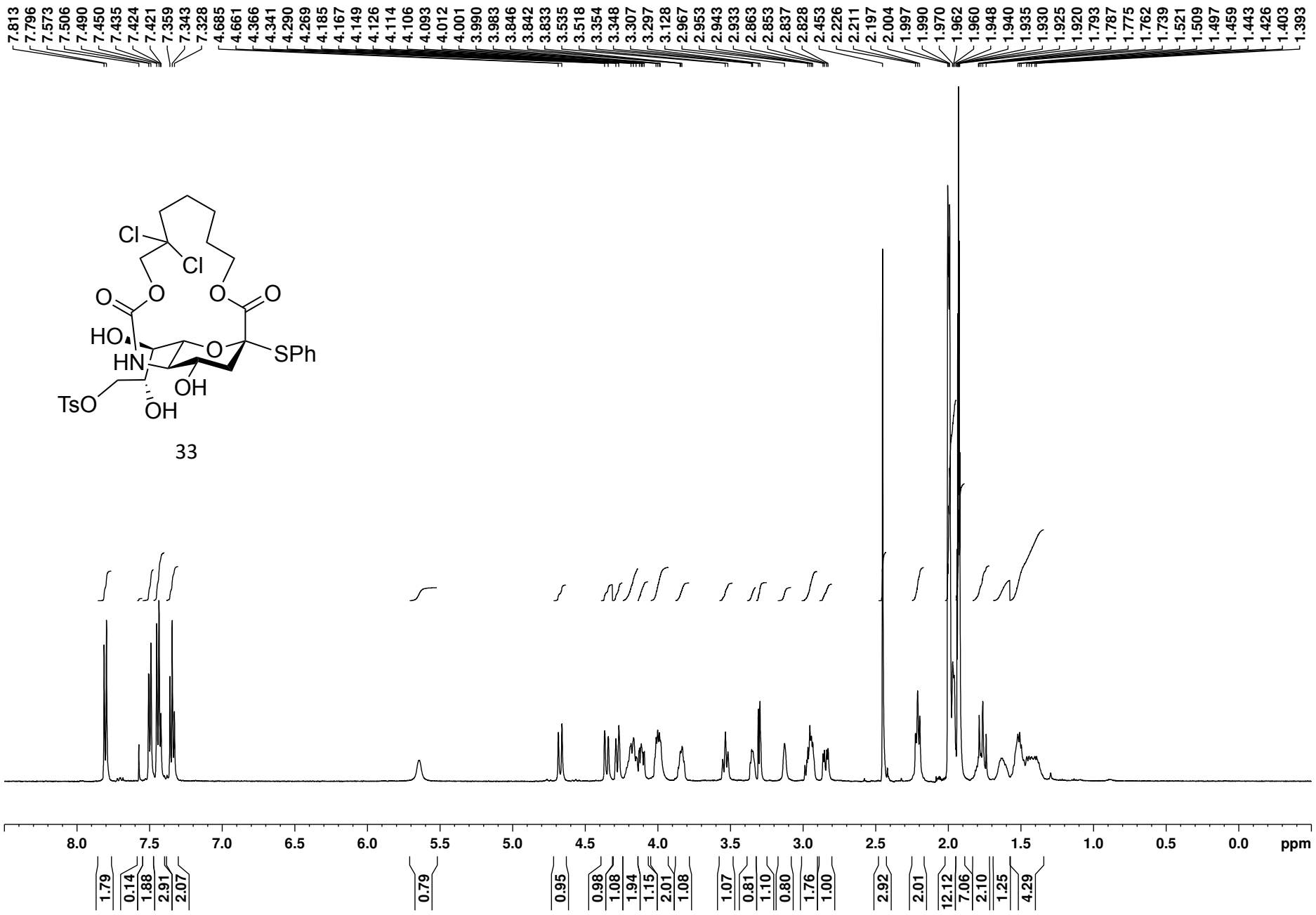
Figure S39. ^{1}H NMR spectrum (500 MHz, CD_3NO_2, 90 °C)



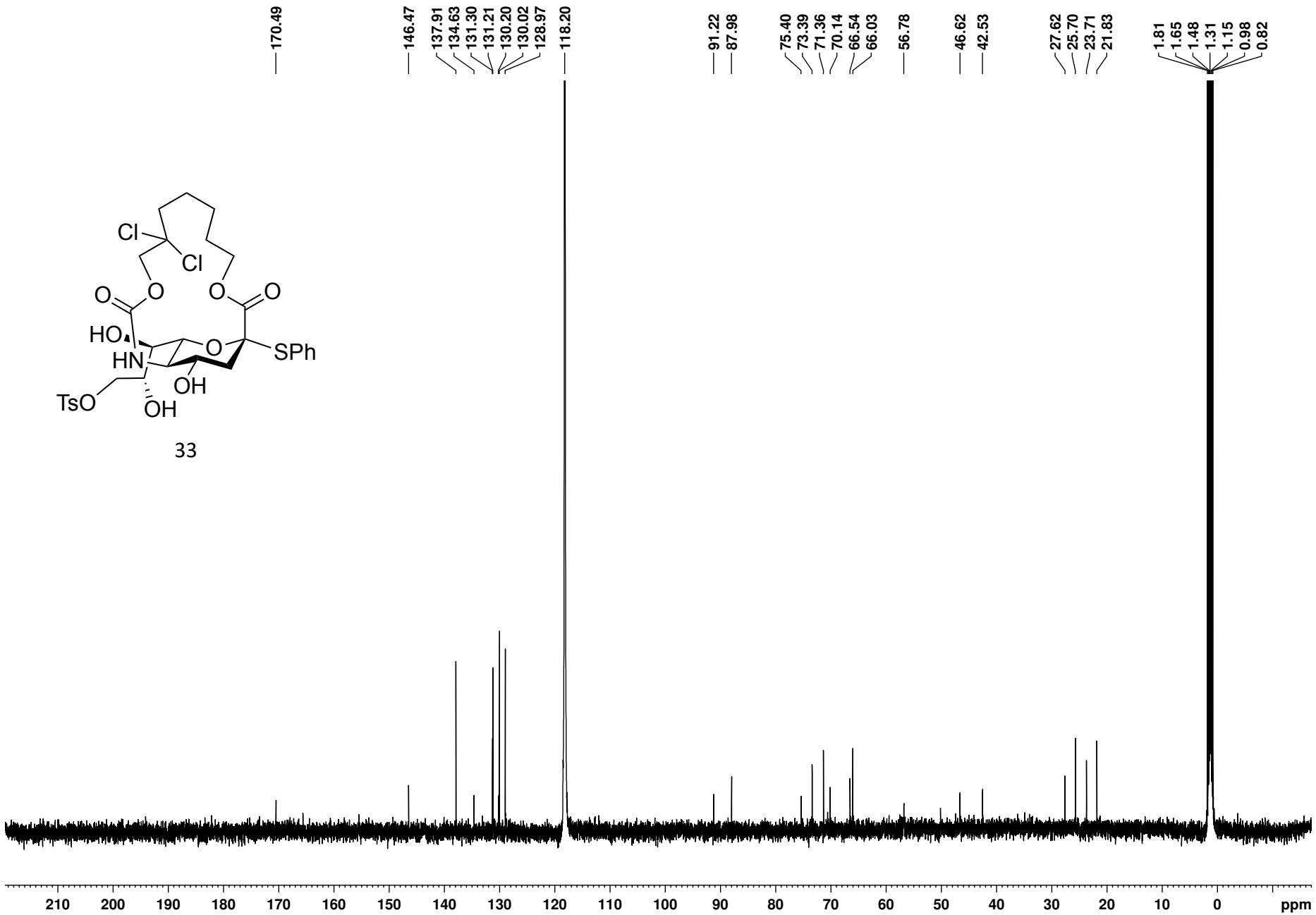
32

Figure S40. ^{13}C NMR spectrum (125 MHz, CD_3CN, 60 °C)



33

Figure S41. ^{1}H NMR spectrum (500 MHz, CD_3CN, 60 °C)



33

Figure S42. ^{13}C NMR spectrum (125 MHz, CD_3CN, 60 °C)

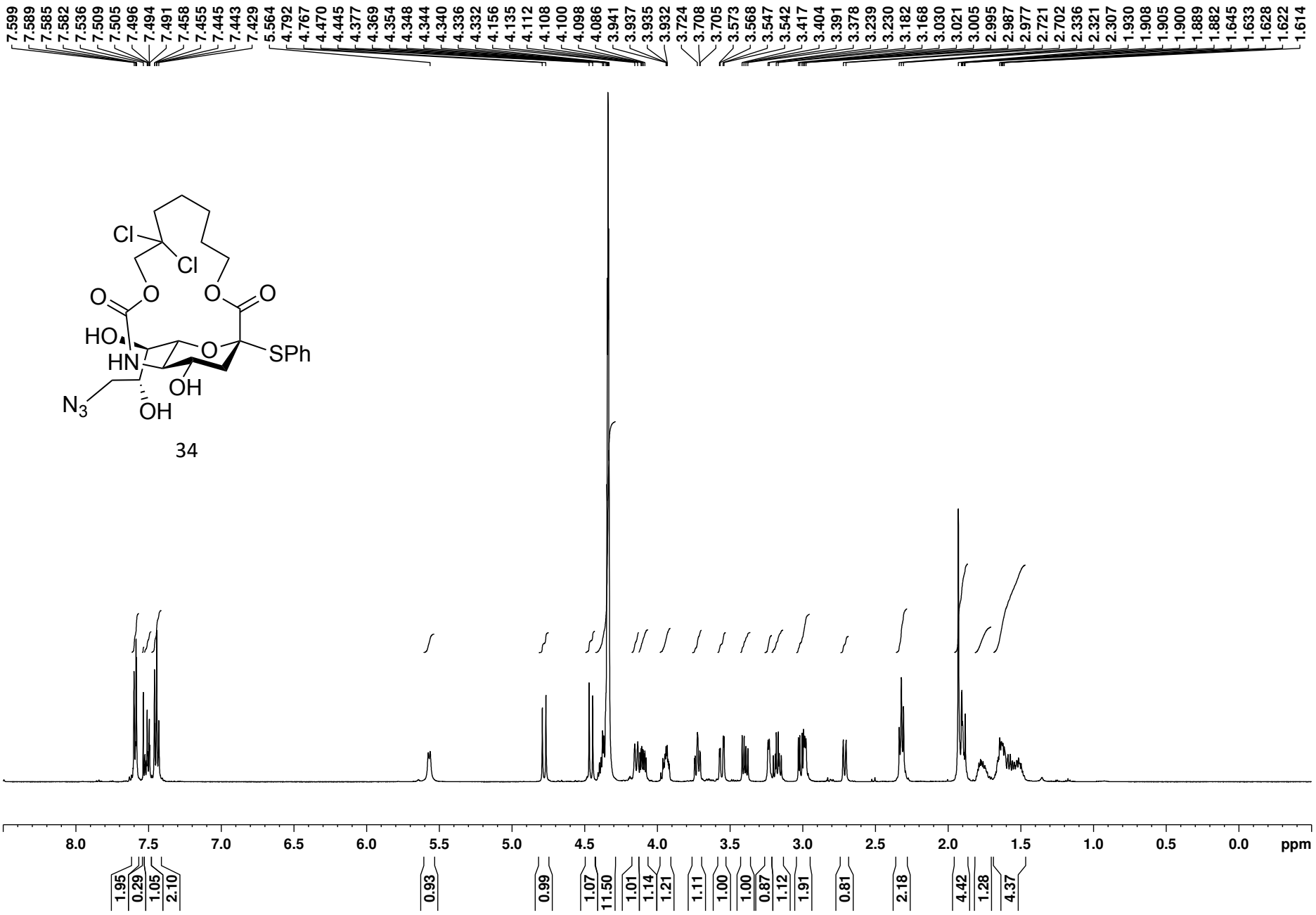


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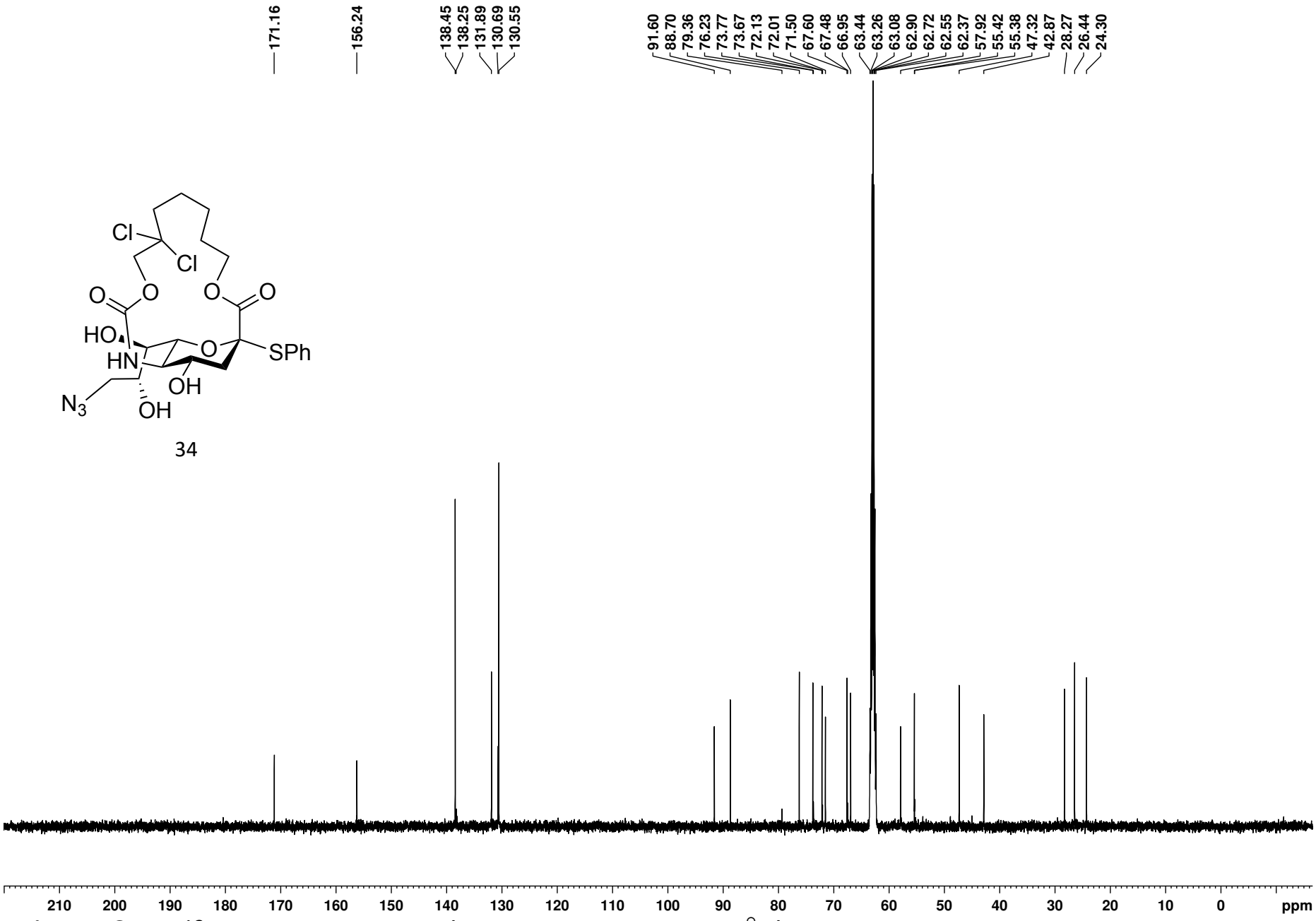
Figure S43. ^{1}H NMR spectrum (500 MHz, CD_3NO_2, 90 °C)



34

Figure S44. ^{13}C NMR spectrum (125 MHz, CD_3CN, 60 °C)

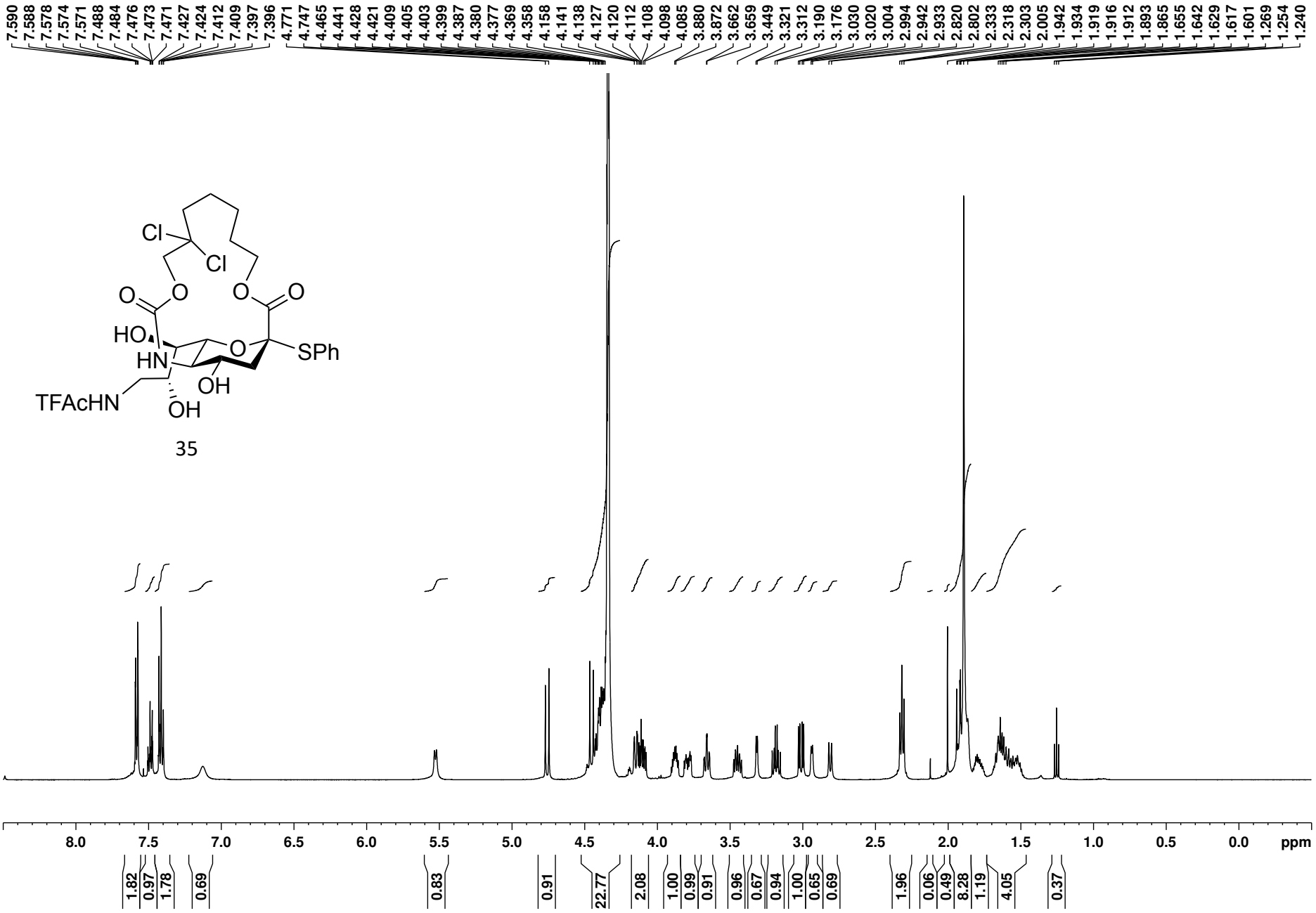


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Figure S45. ^{1}H NMR spectrum (500 MHz, CD_3NO_2, 90 °C)

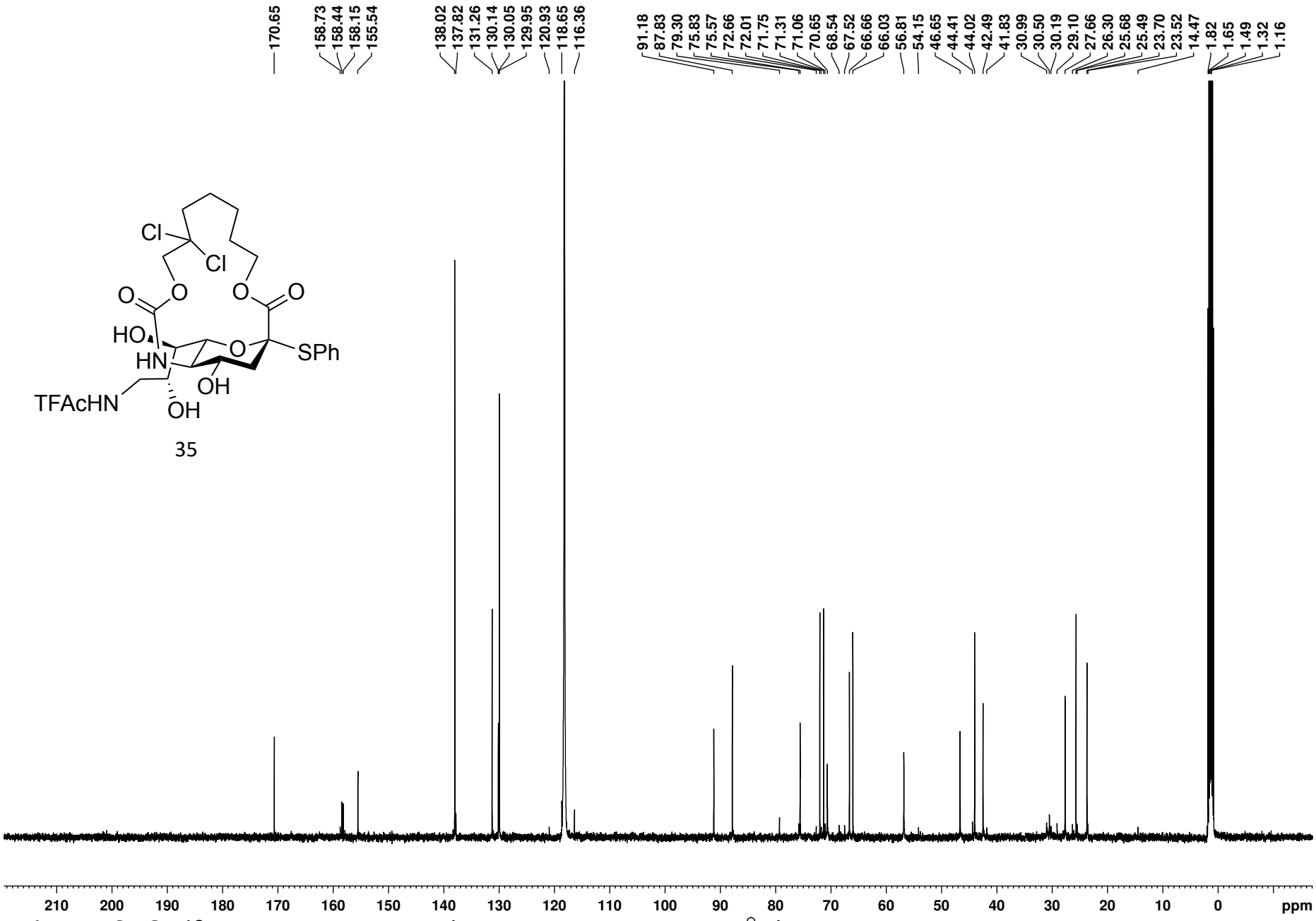

Figure S46. ^{13}C NMR spectrum (125 MHz, CD_3CN, 60 °C)

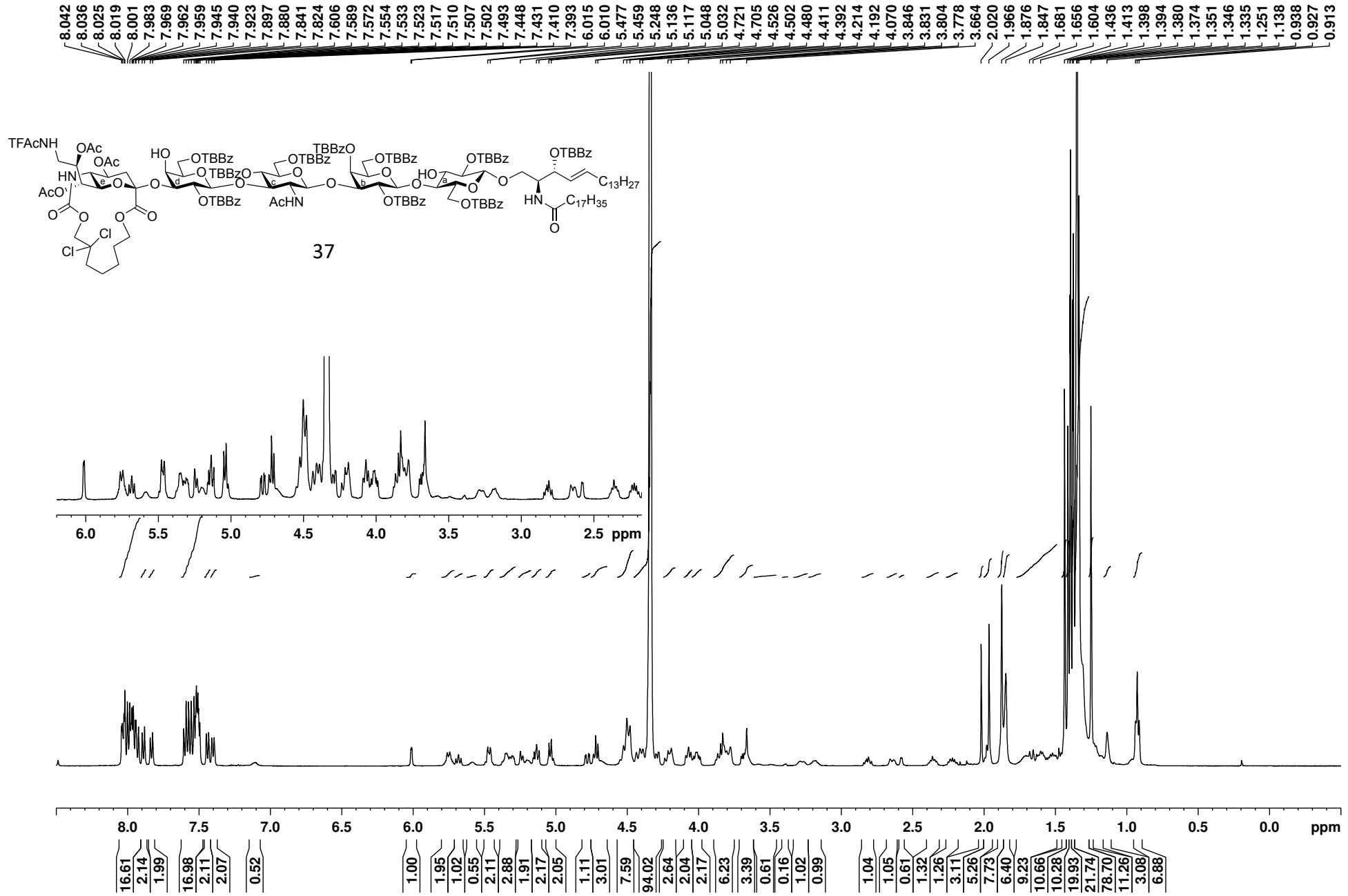

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Figure S47. ^{1}H NMR spectrum (500 MHz, CD_3NO_2, 90 °C)

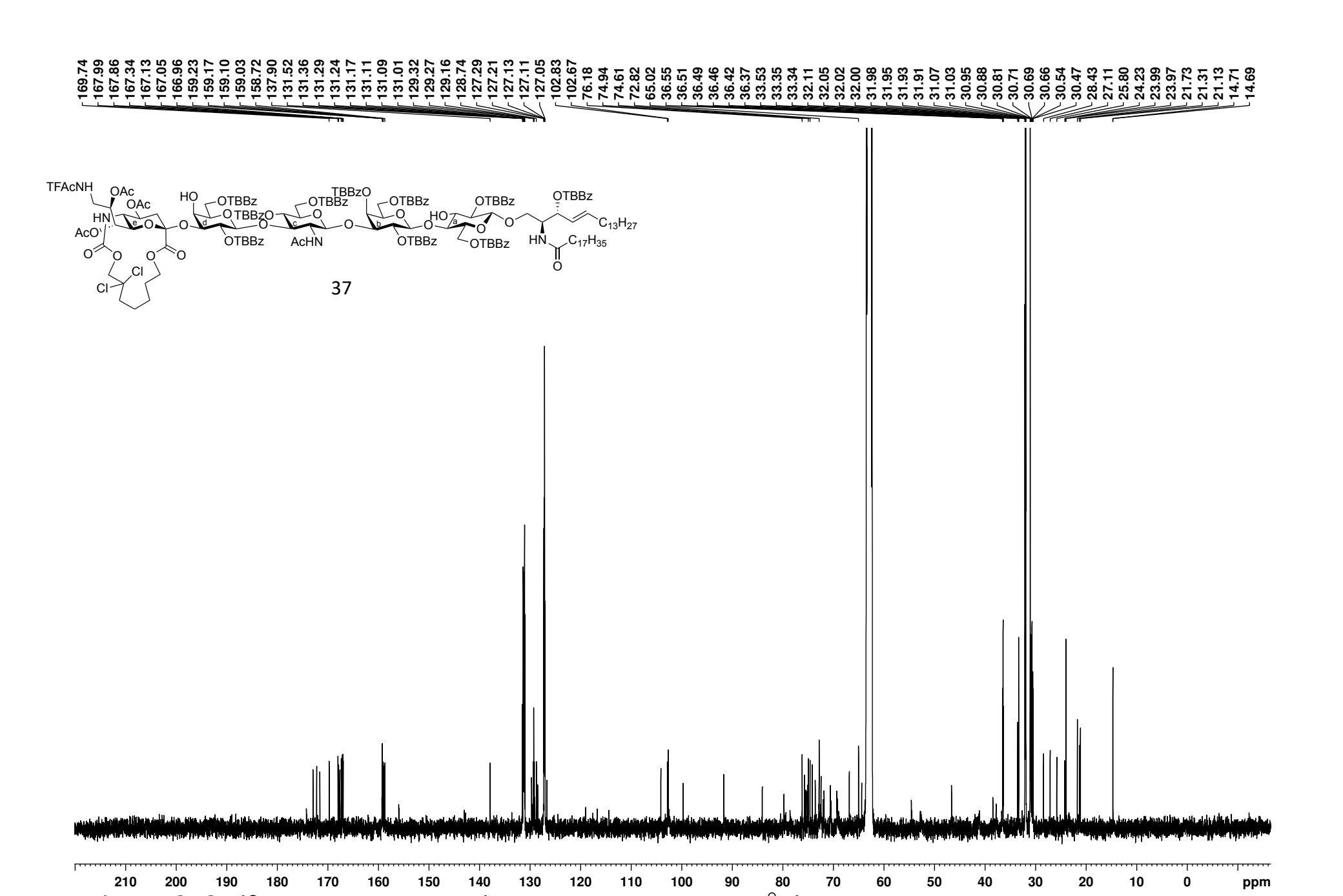

Figure S48. ^{13}C NMR spectrum (125 MHz, CD_3NO_2, 90 °C)

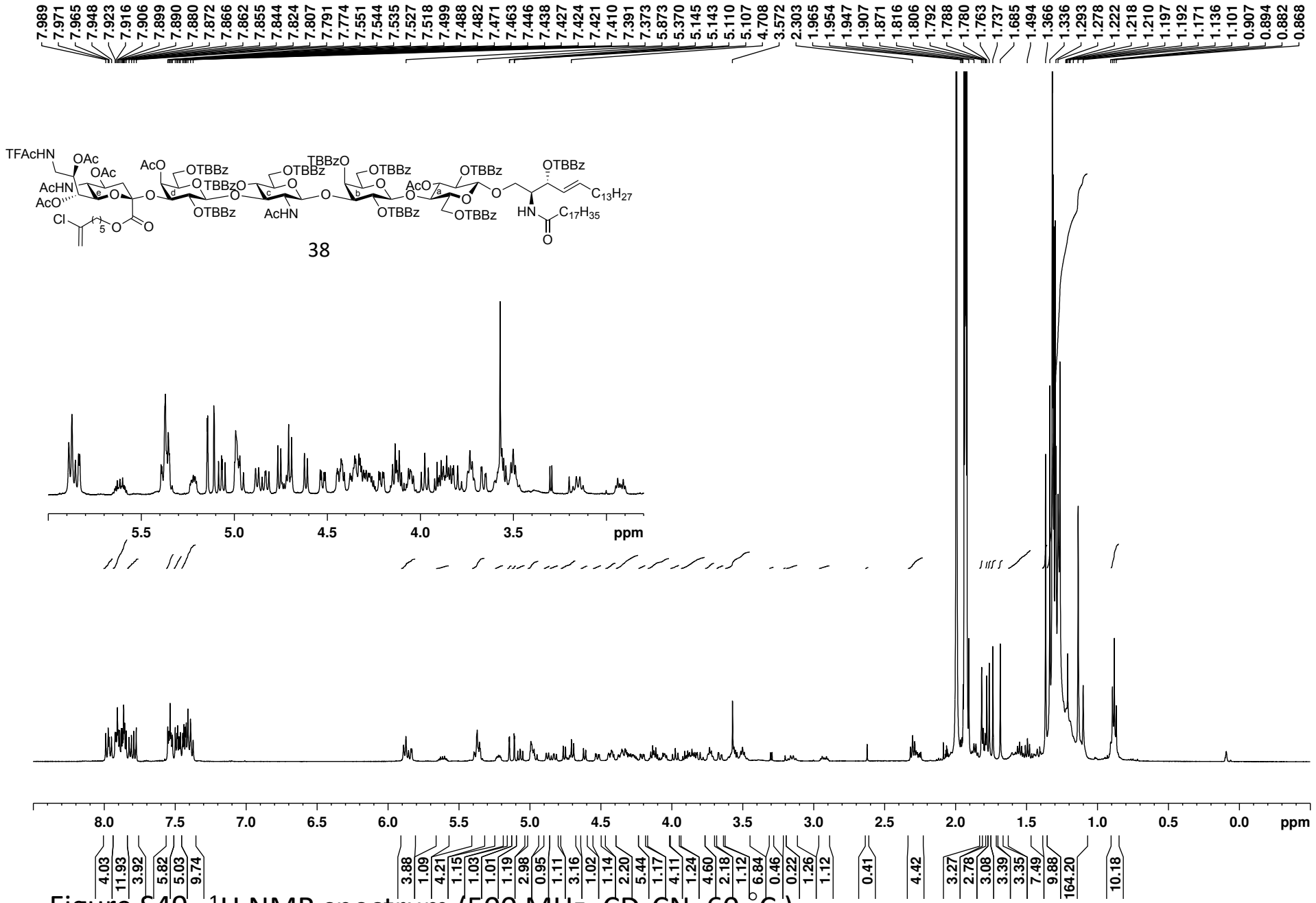

Figure S49. ^{1}H NMR spectrum (500 MHz, CD_3CN, 60 °C)

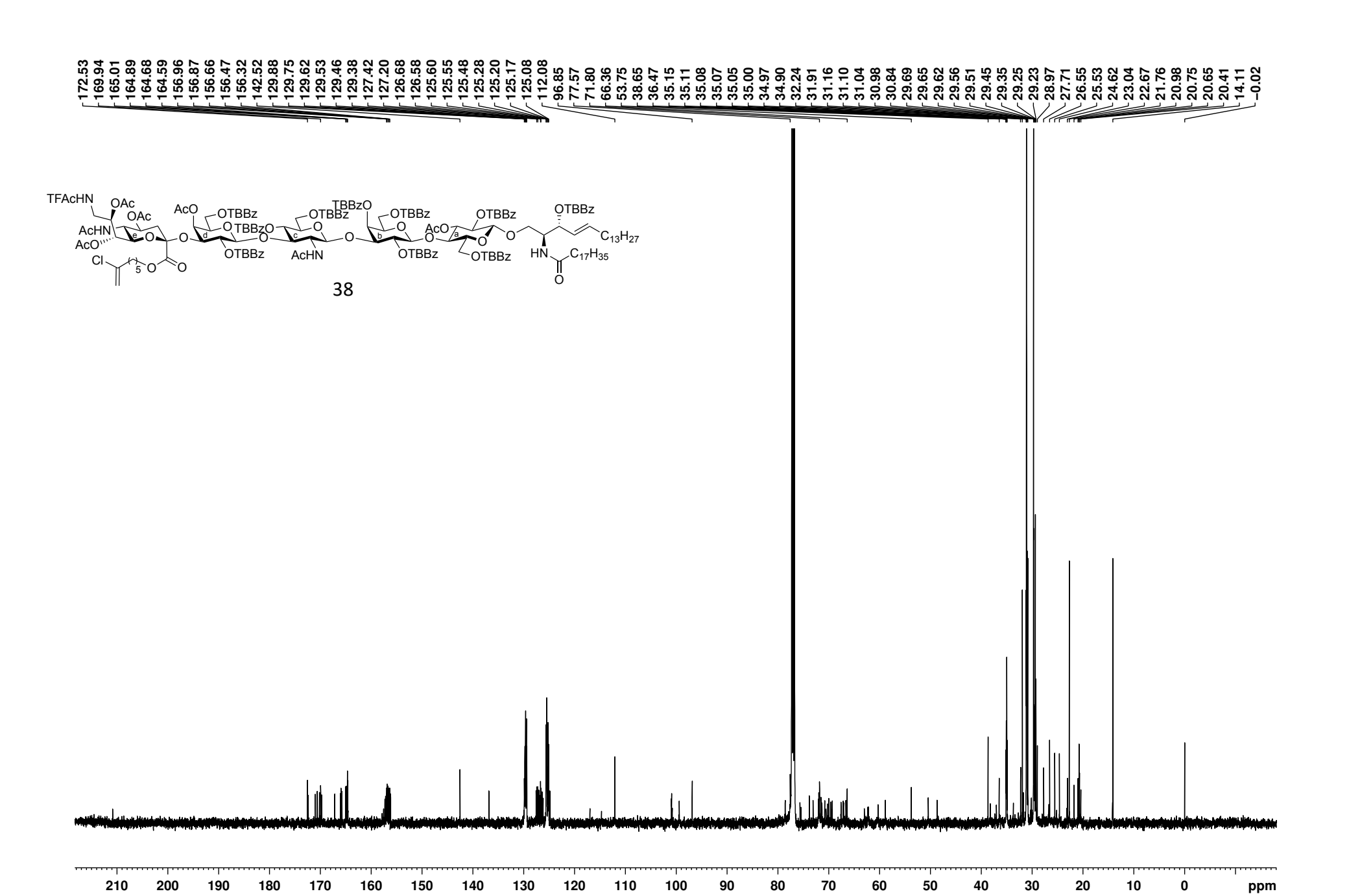

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Figure S50. ^{13}C NMR spectrum (125 MHz, $CDCl_3$)

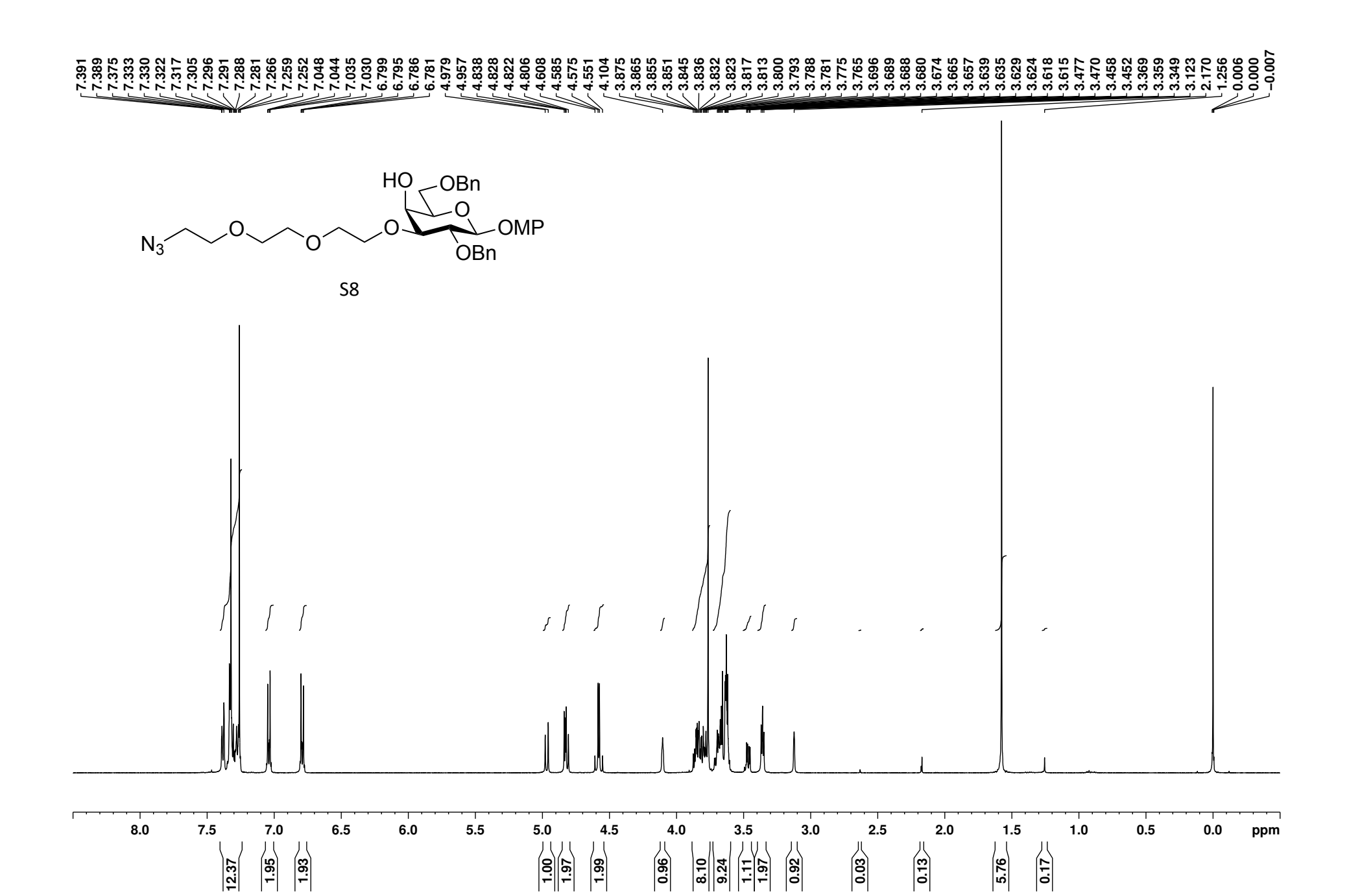


Figure S51. ^{1}H NMR spectrum (500 MHz, $CDCl_3$)

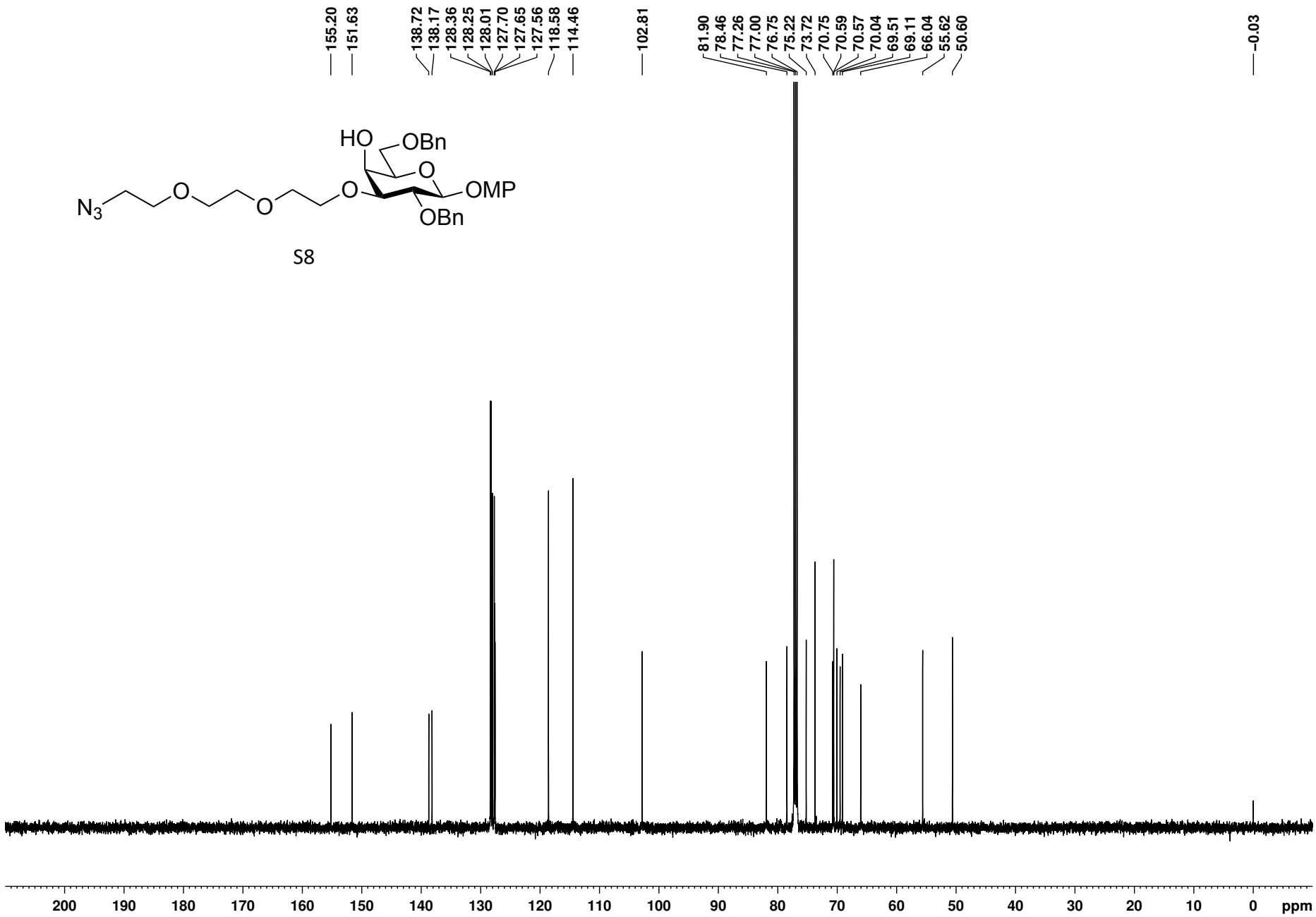


Figure S52. ^{13}C NMR spectrum (125 MHz, $CDCl_3$)

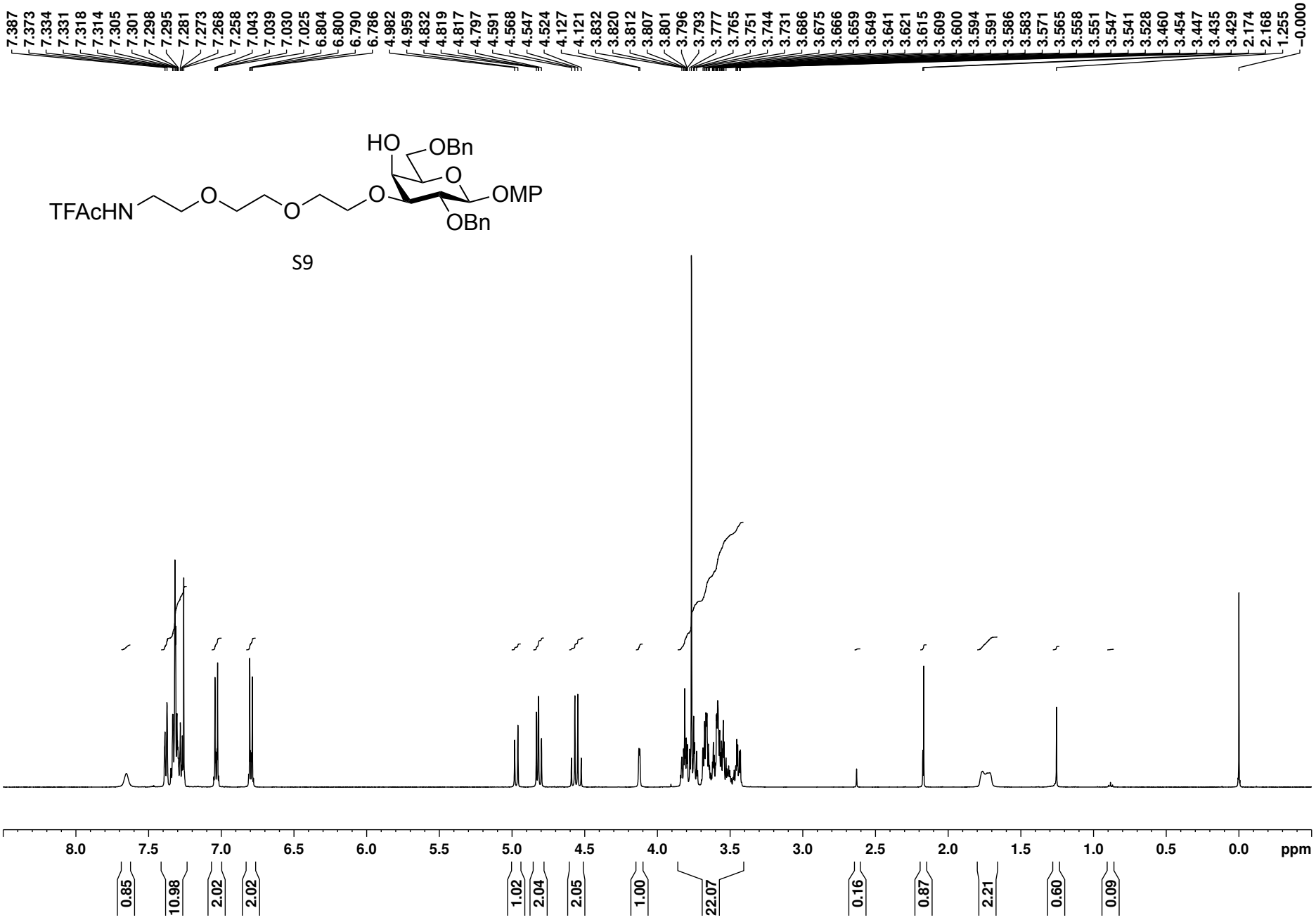


Figure S53. ^{1}H NMR spectrum (500 MHz, $CDCl_3$)

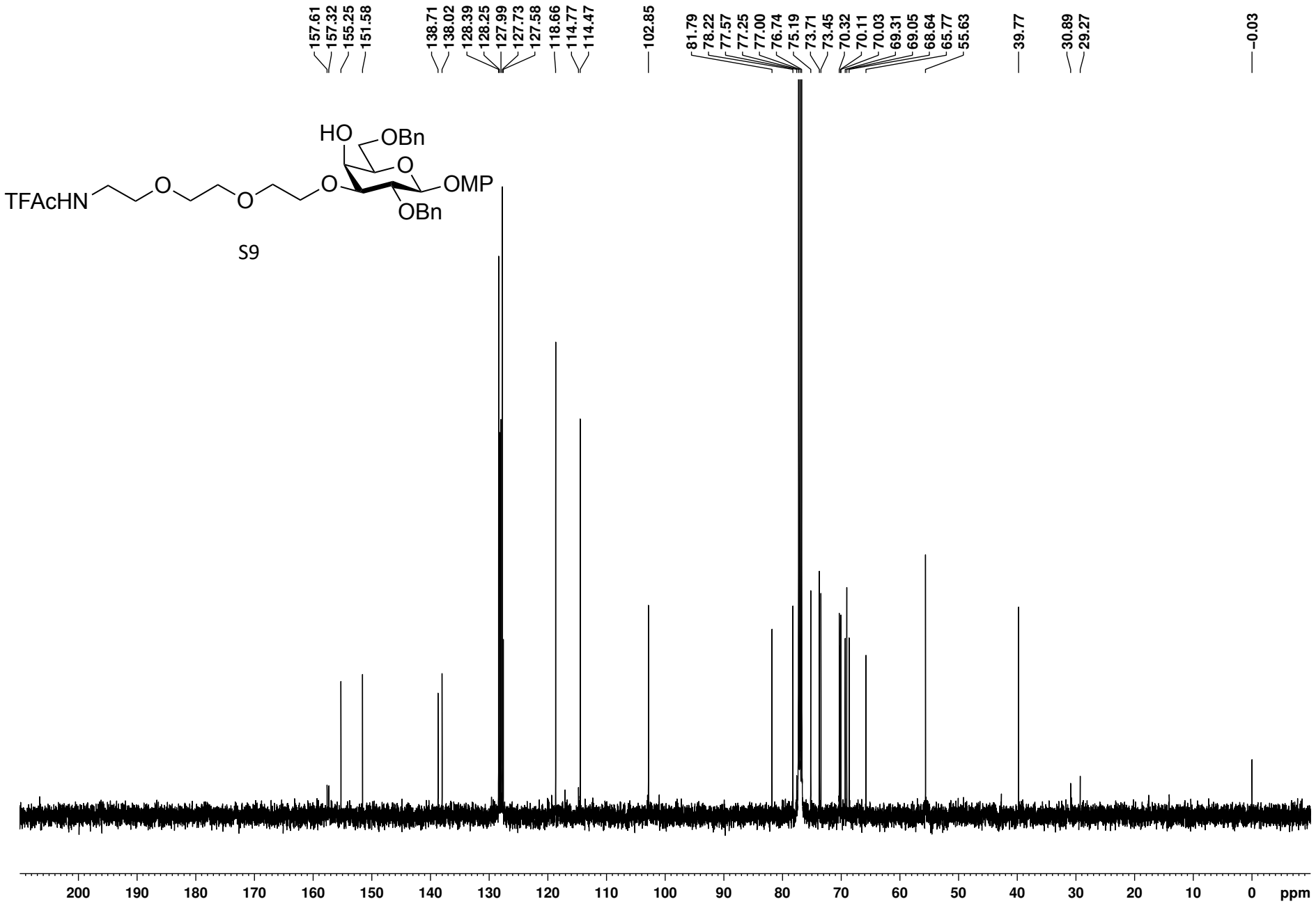


Figure S54. ^{13}C NMR spectrum (125 MHz, $CDCl_3$)

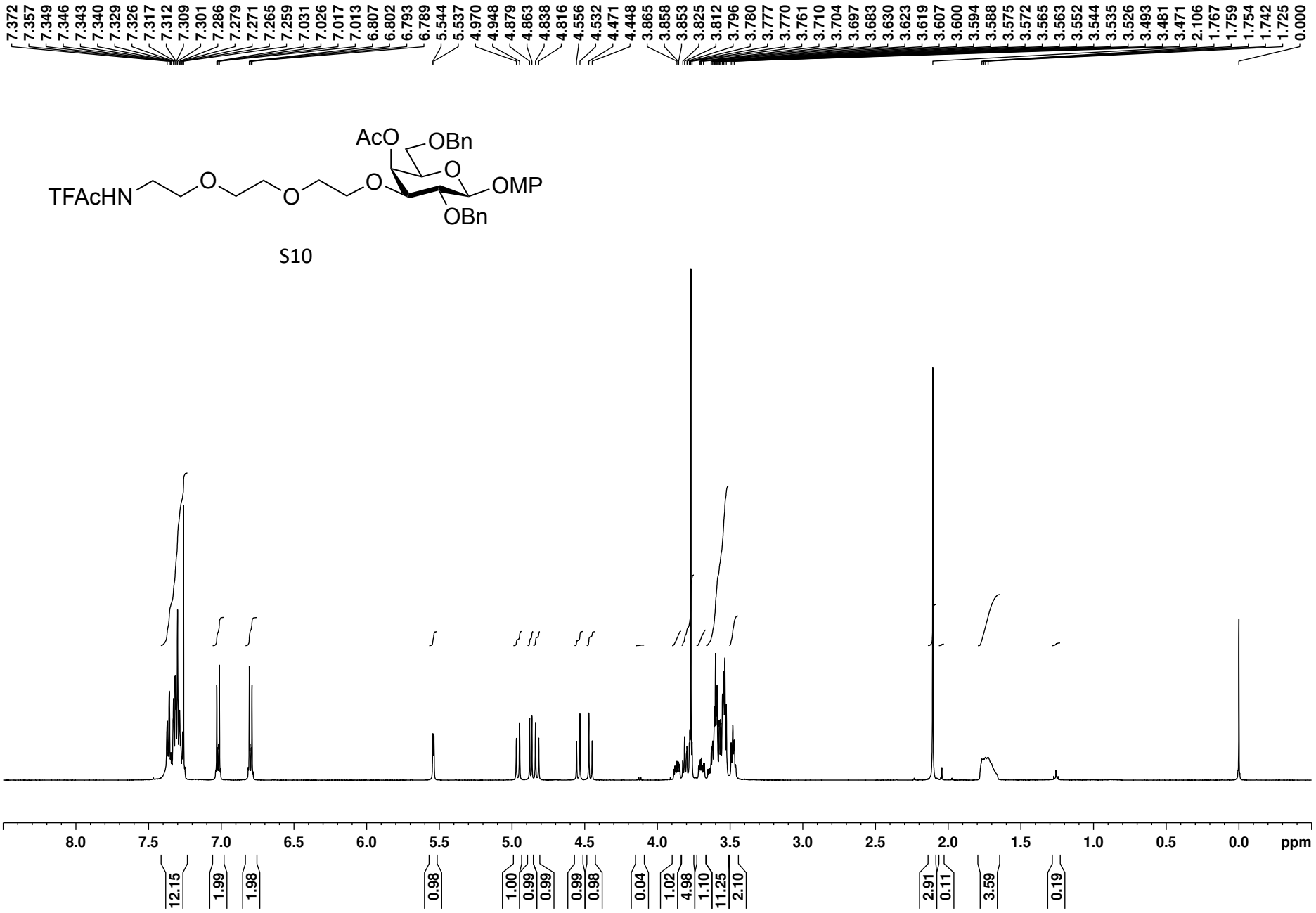


Figure S55. ^{1}H NMR spectrum (500 MHz, $CDCl_3$)

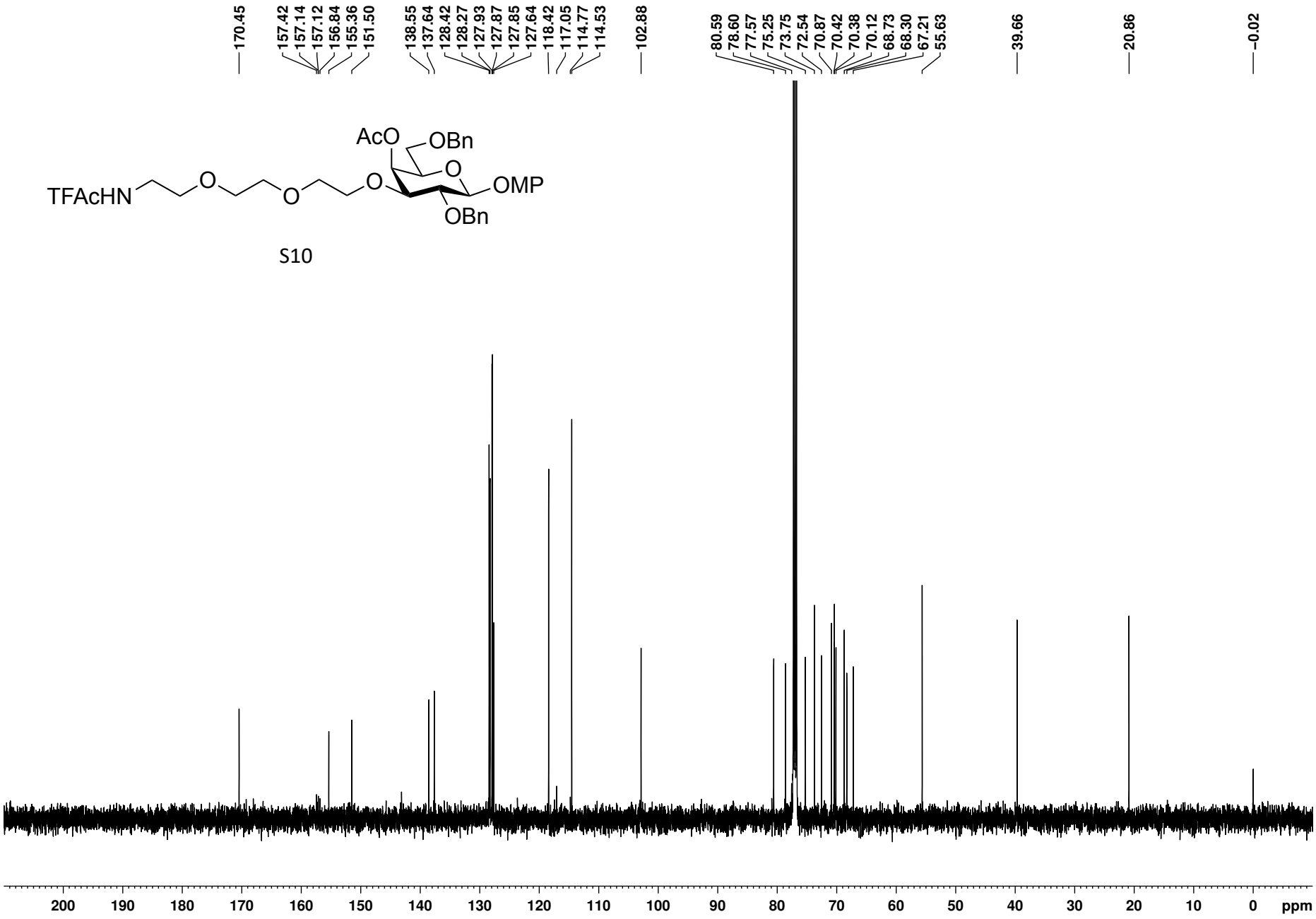


Figure S56. ^{13}C NMR spectrum (125 MHz, $CDCl_3$)

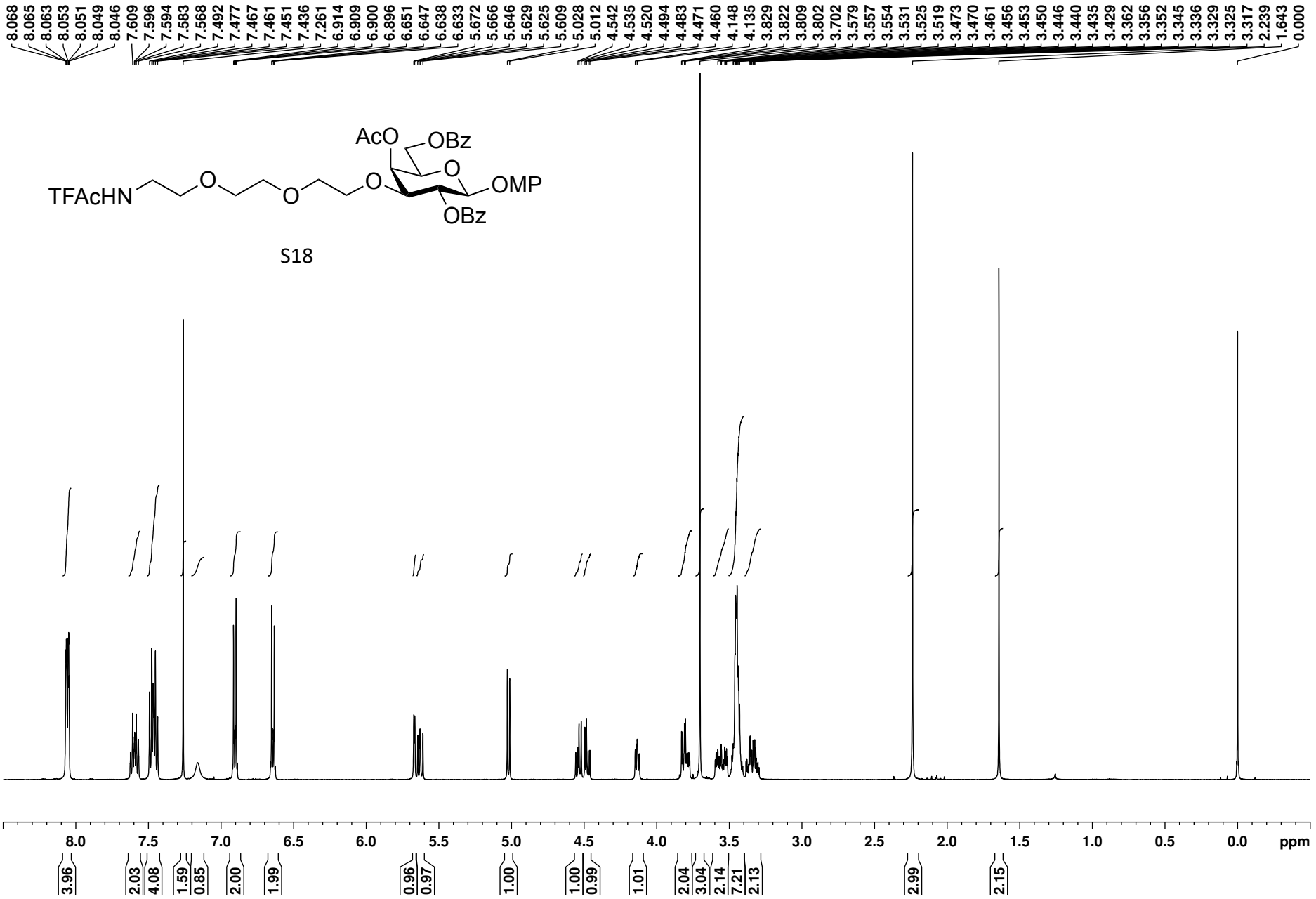


Figure S57. ^{1}H NMR spectrum (500 MHz, $CDCl_3$)

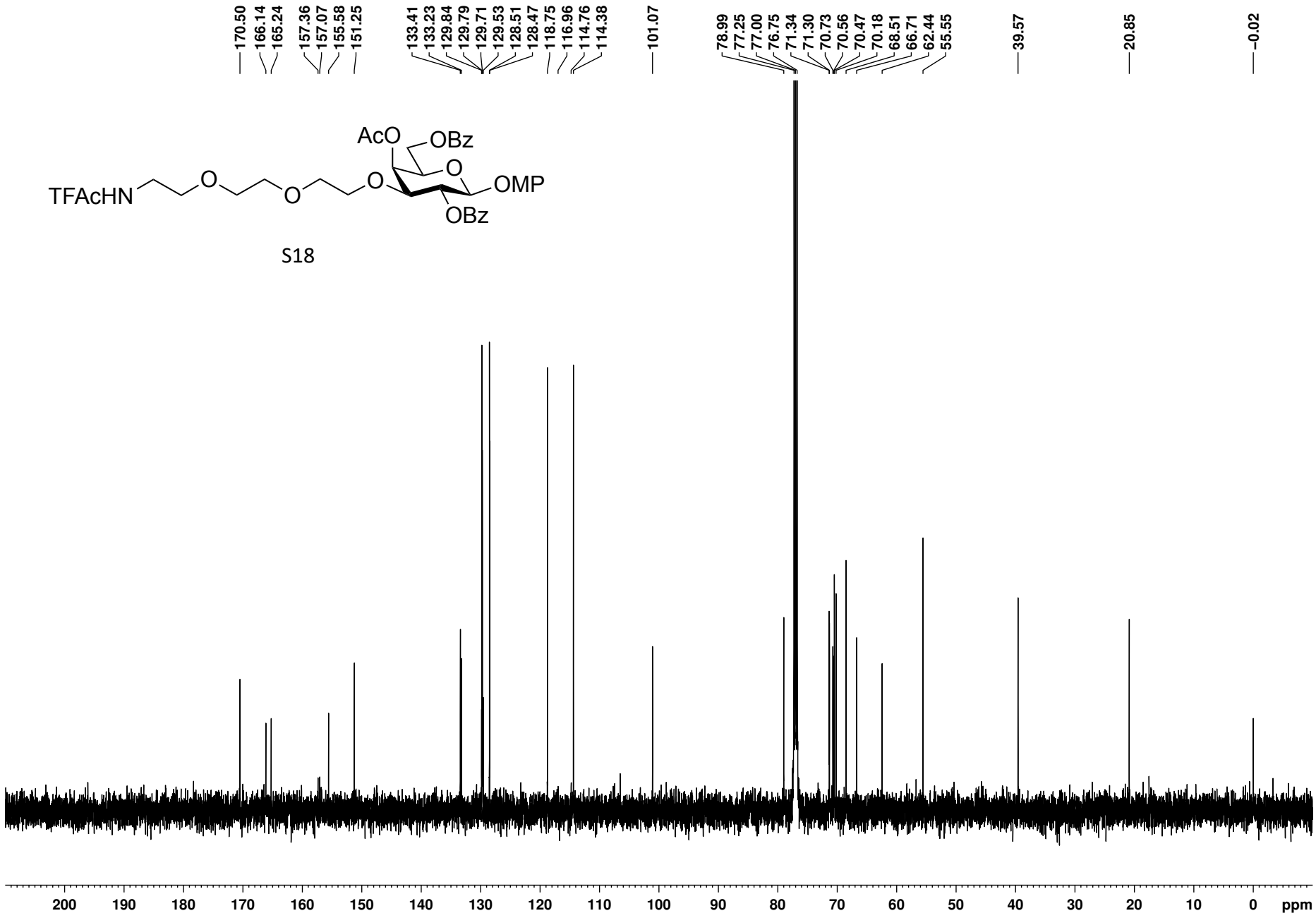


Figure S58. ^{13}C NMR spectrum (125 MHz, $CDCl_3$)

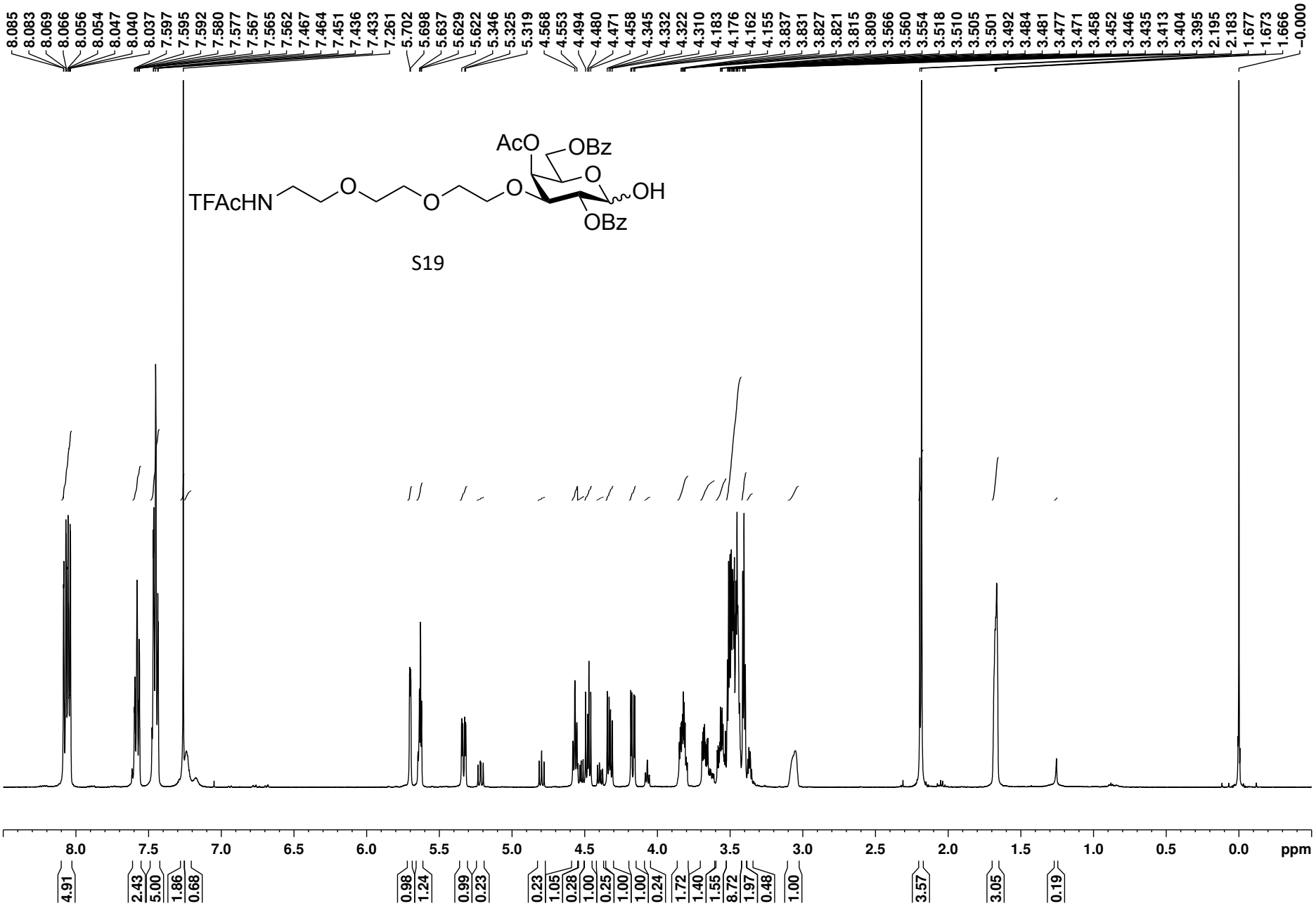


Figure S59. ^{1}H NMR spectrum (500 MHz, $CDCl_3$)

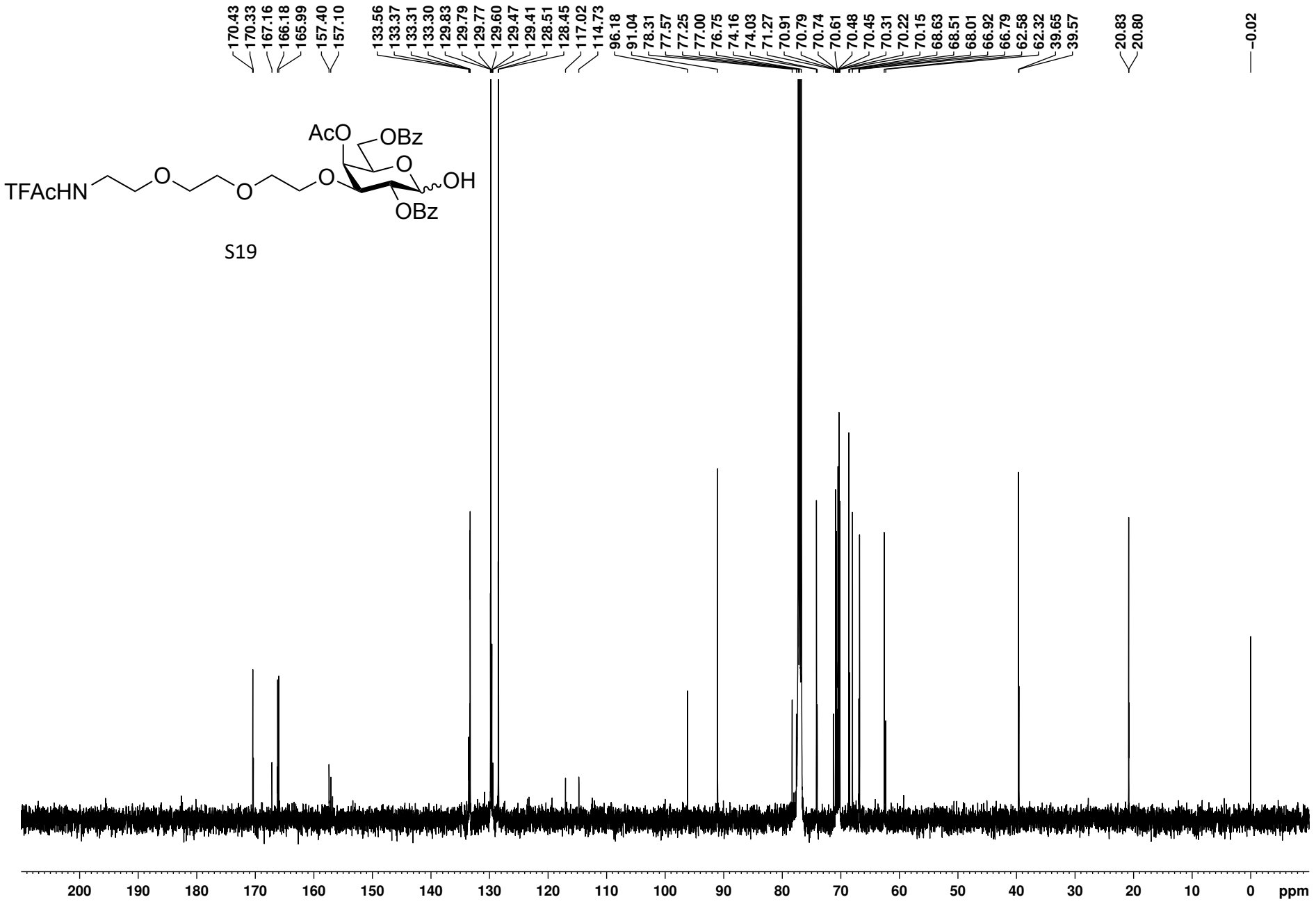


Figure S60. ^{13}C NMR spectrum (125 MHz, $CDCl_3$)

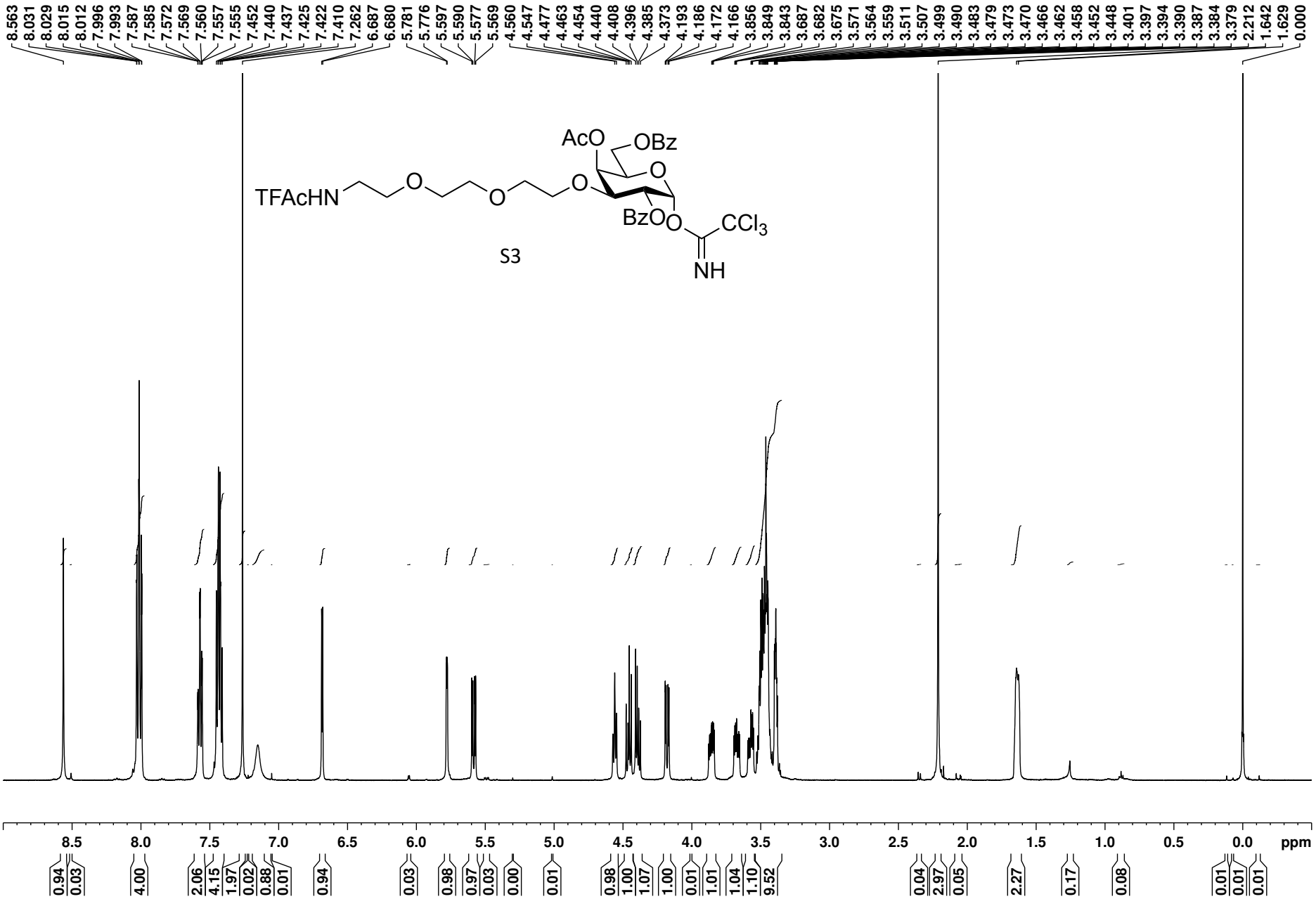


Figure S61. ^{1}H NMR spectrum (500 MHz, $CDCl_3$)

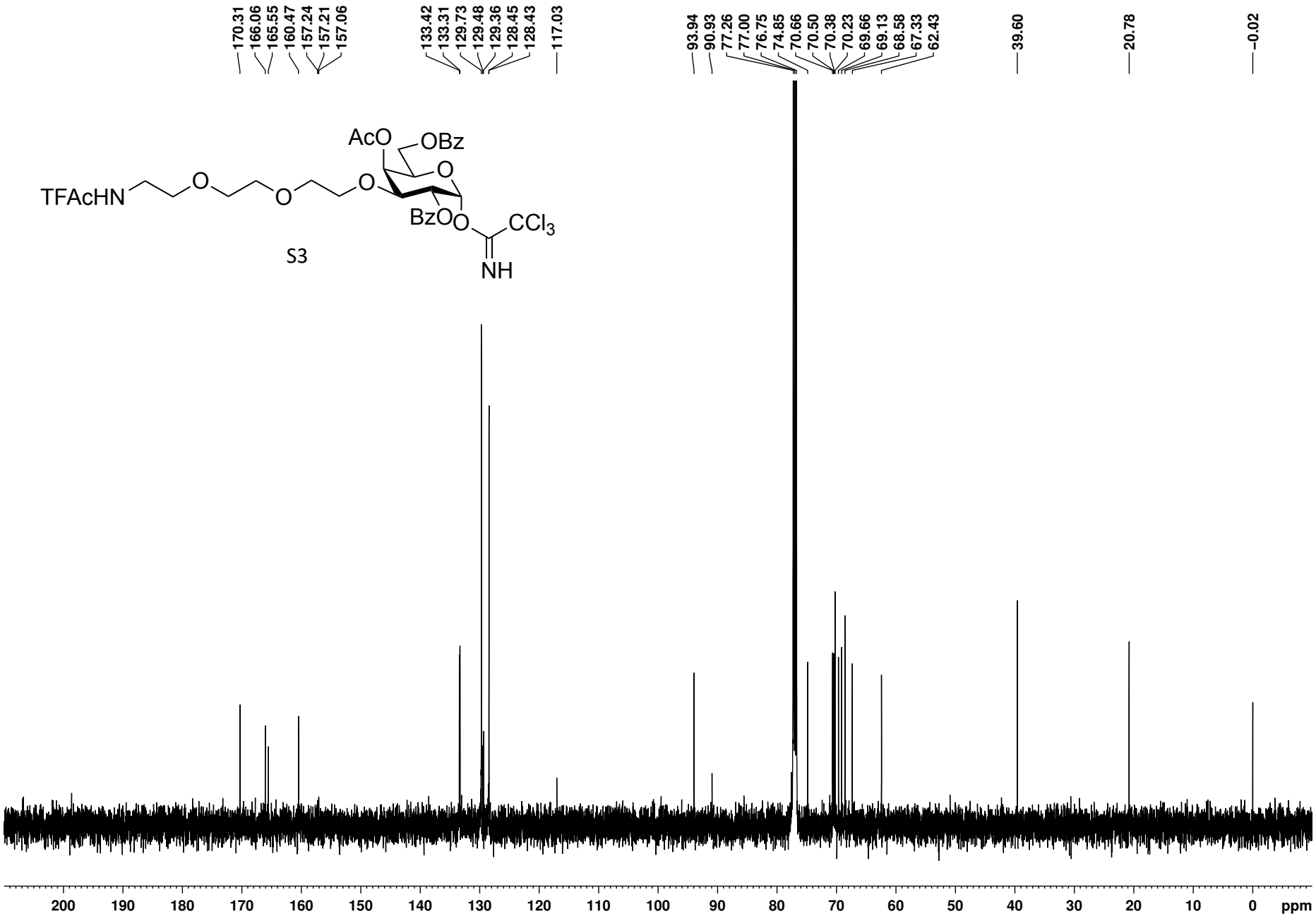


Figure S62. ^{13}C NMR spectrum (125 MHz, $CDCl_3$)

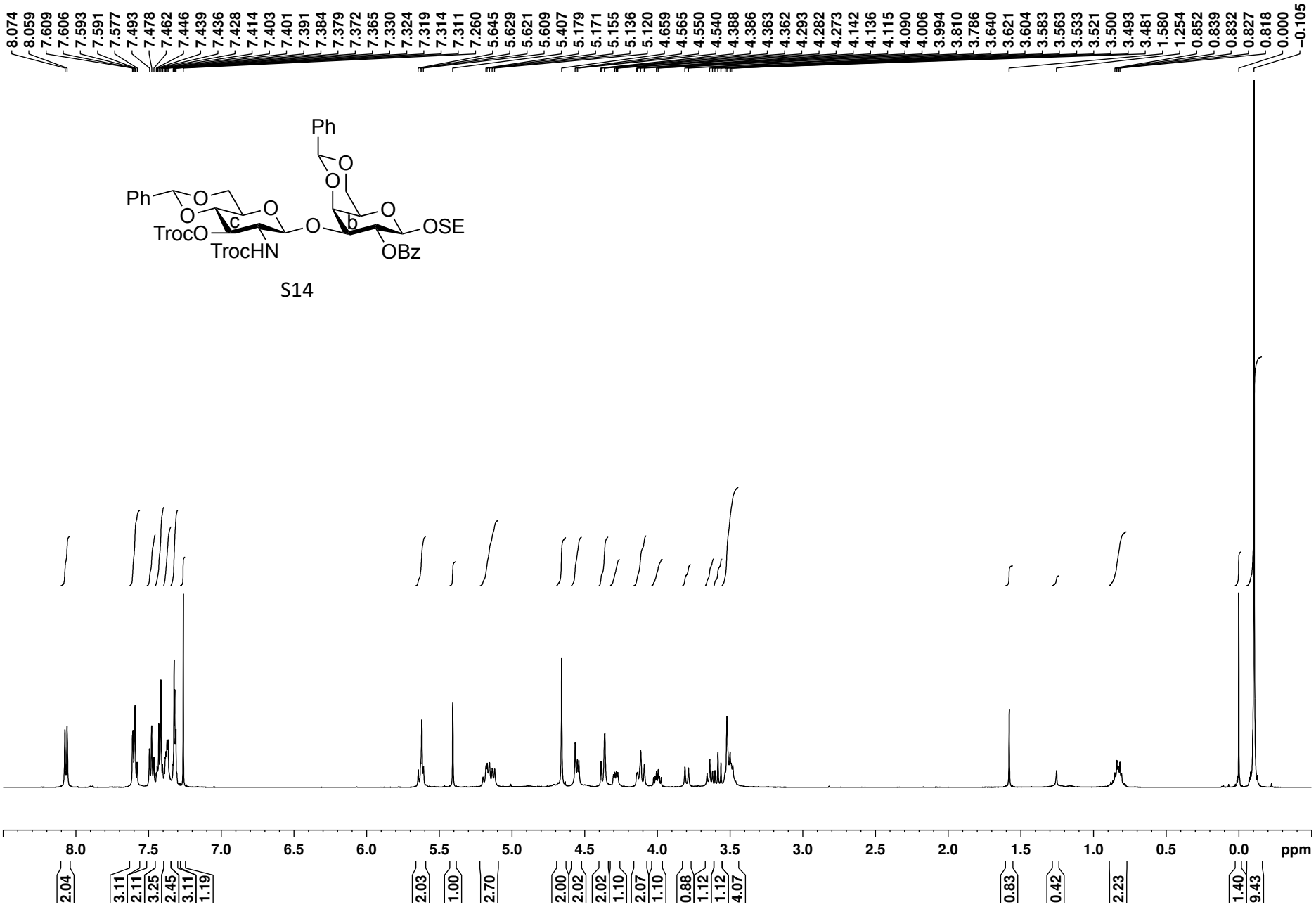


Figure S63. ^{1}H NMR spectrum (500 MHz, $CDCl_3$)

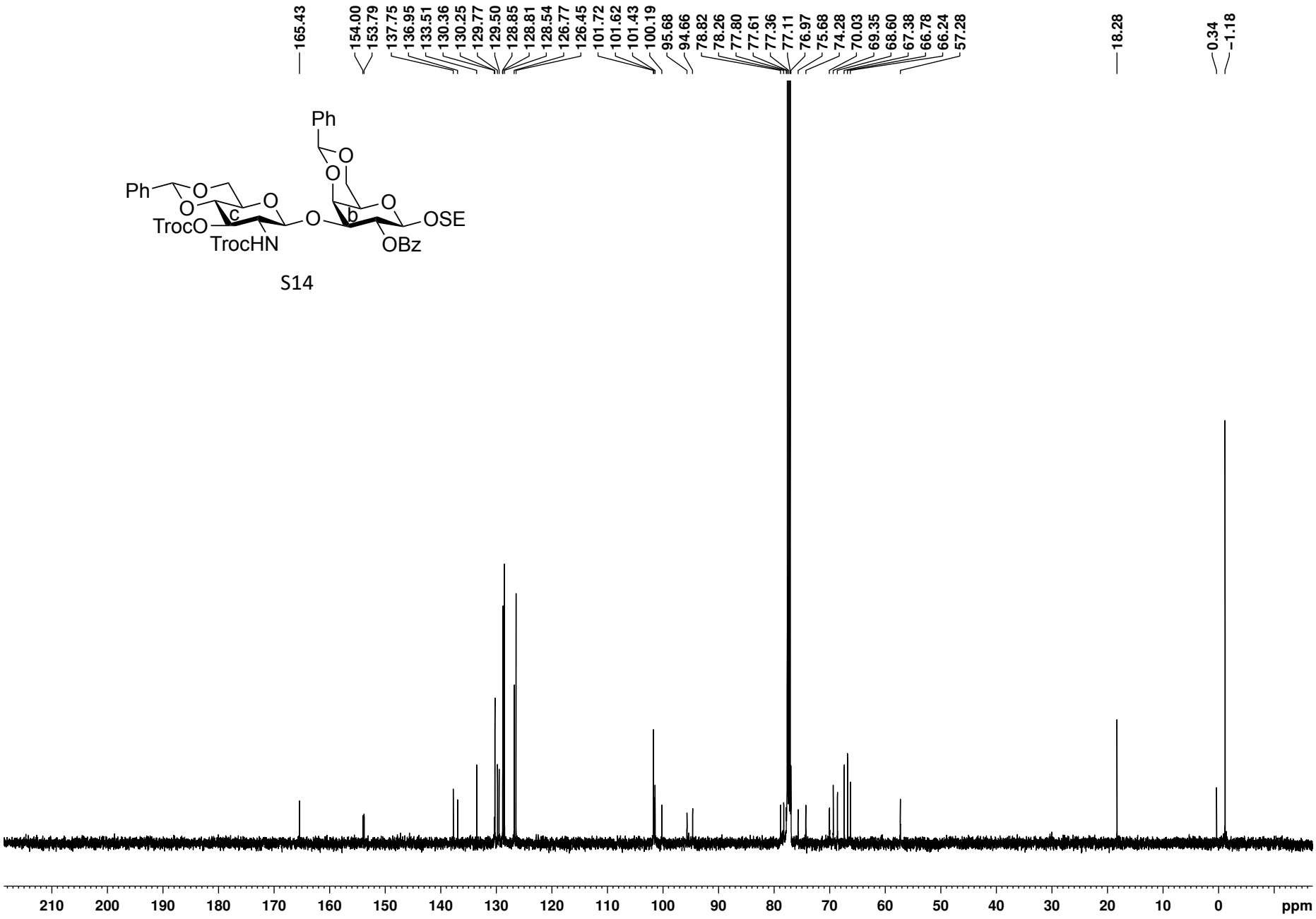


Figure S64. ^{13}C NMR spectrum (125 MHz, $CDCl_3$)

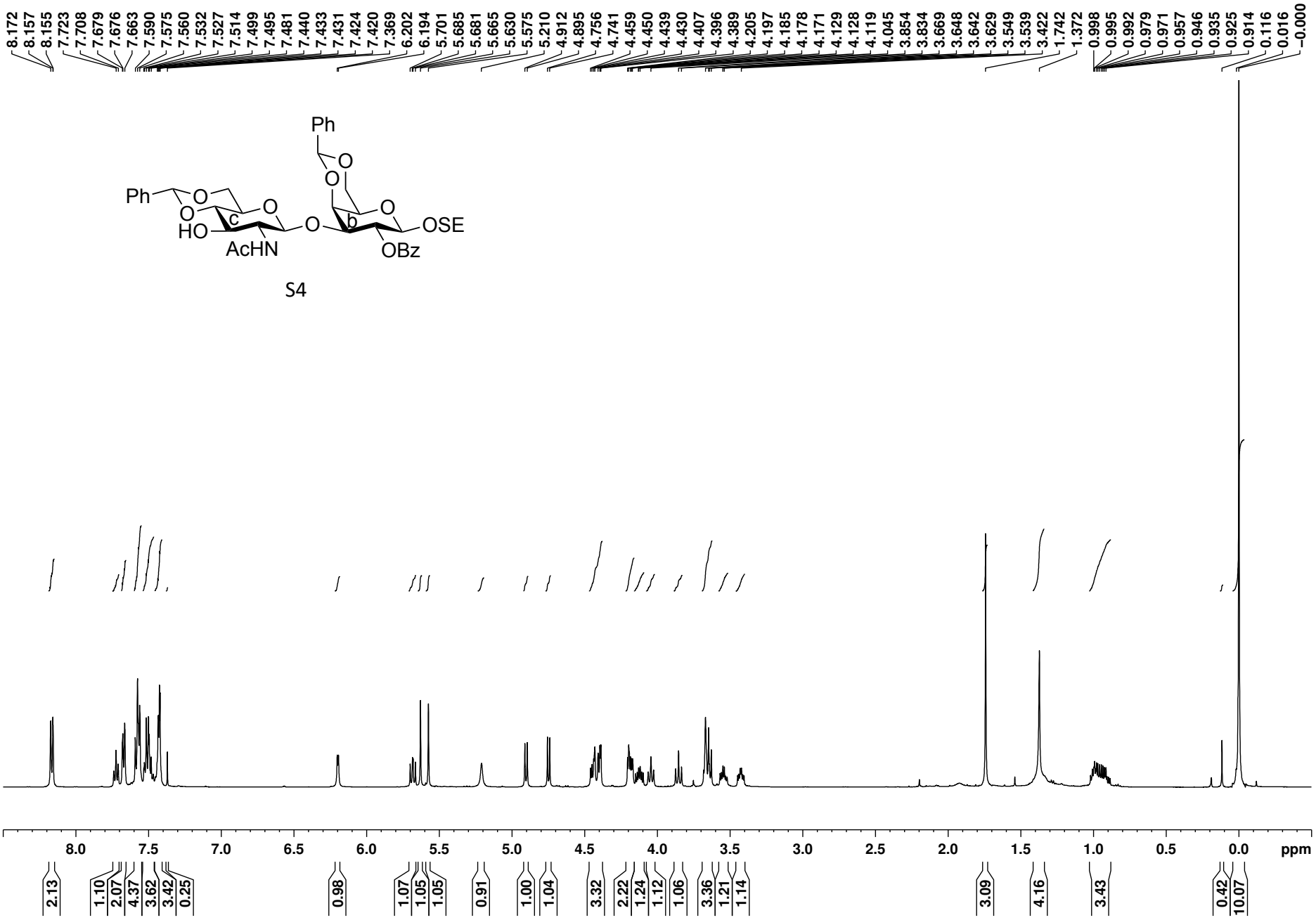


Figure S65. ^{1}H NMR spectrum (500 MHz, $CDCl_3$)

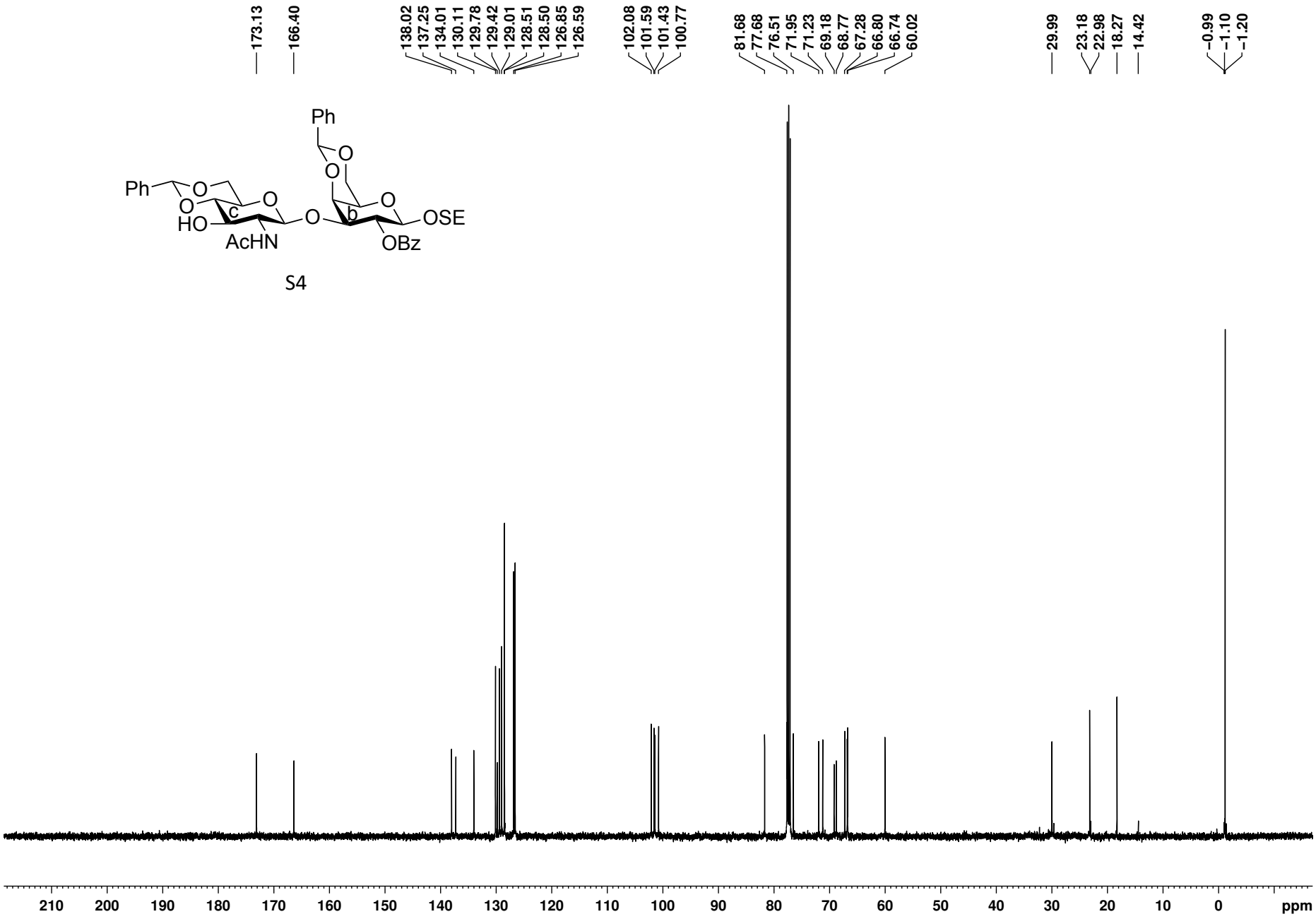


Figure S66. ^{13}C NMR spectrum (125 MHz, $CDCl_3$)

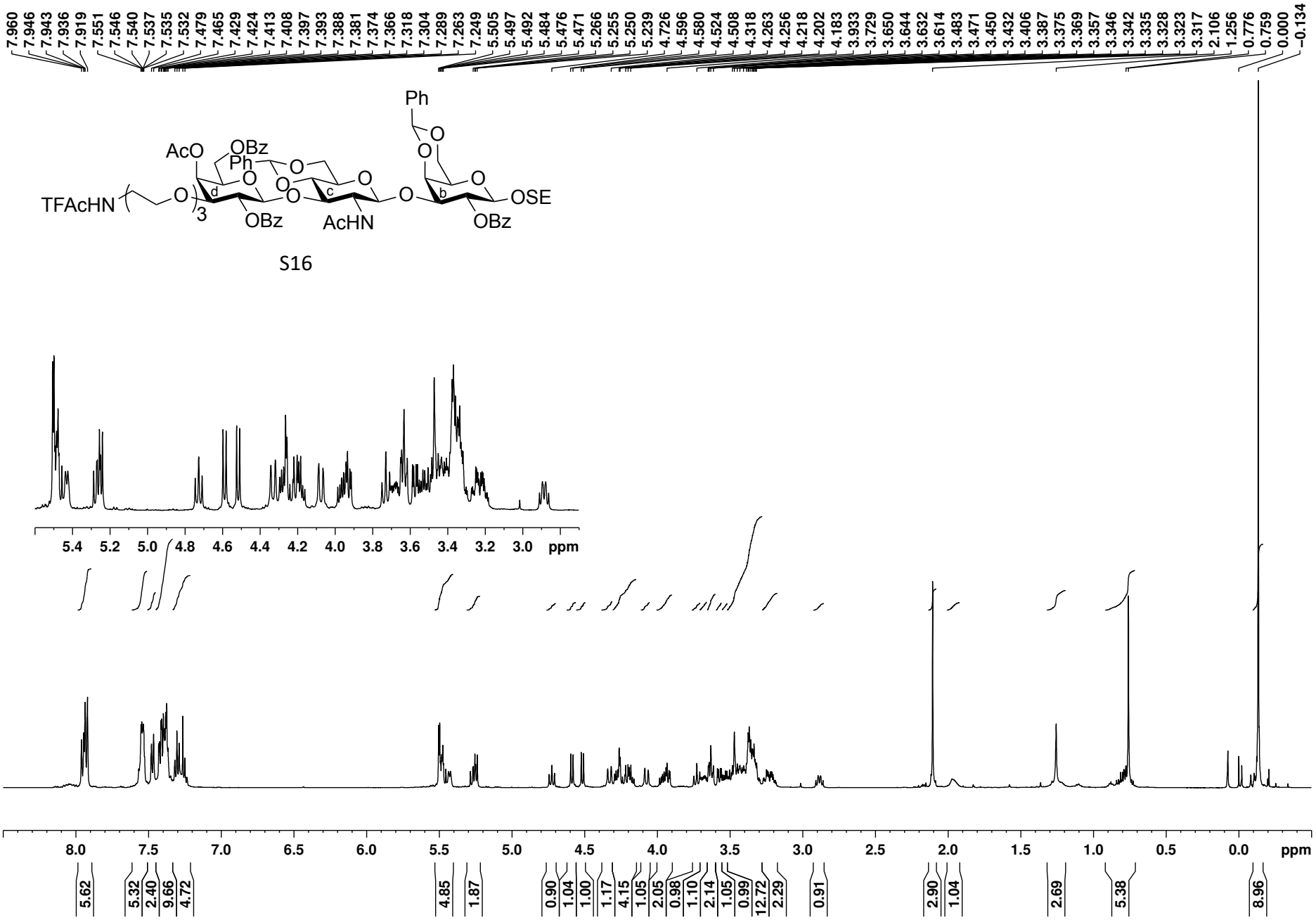


Figure S67. ^{1}H NMR spectrum (500 MHz, $CDCl_3$)

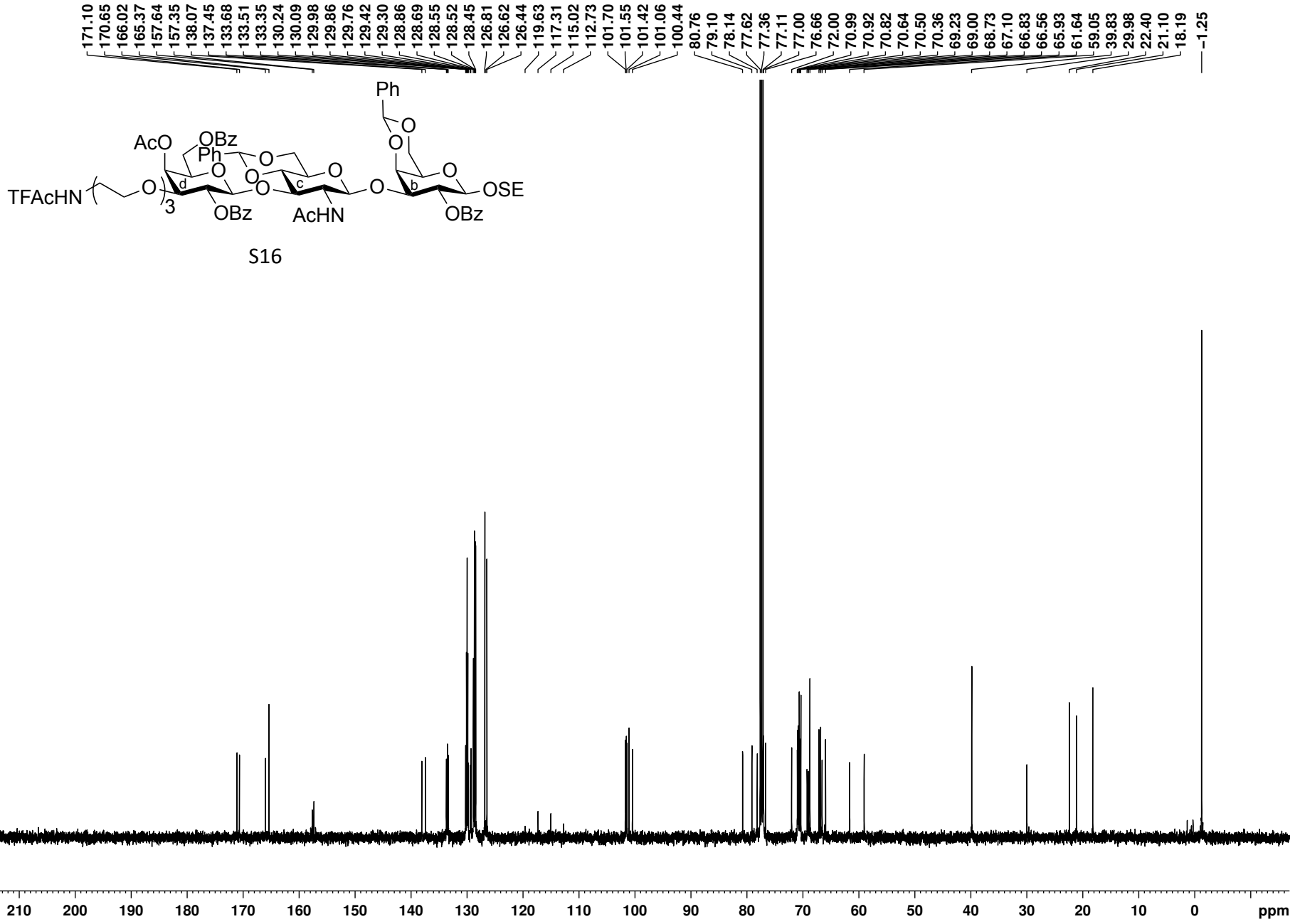
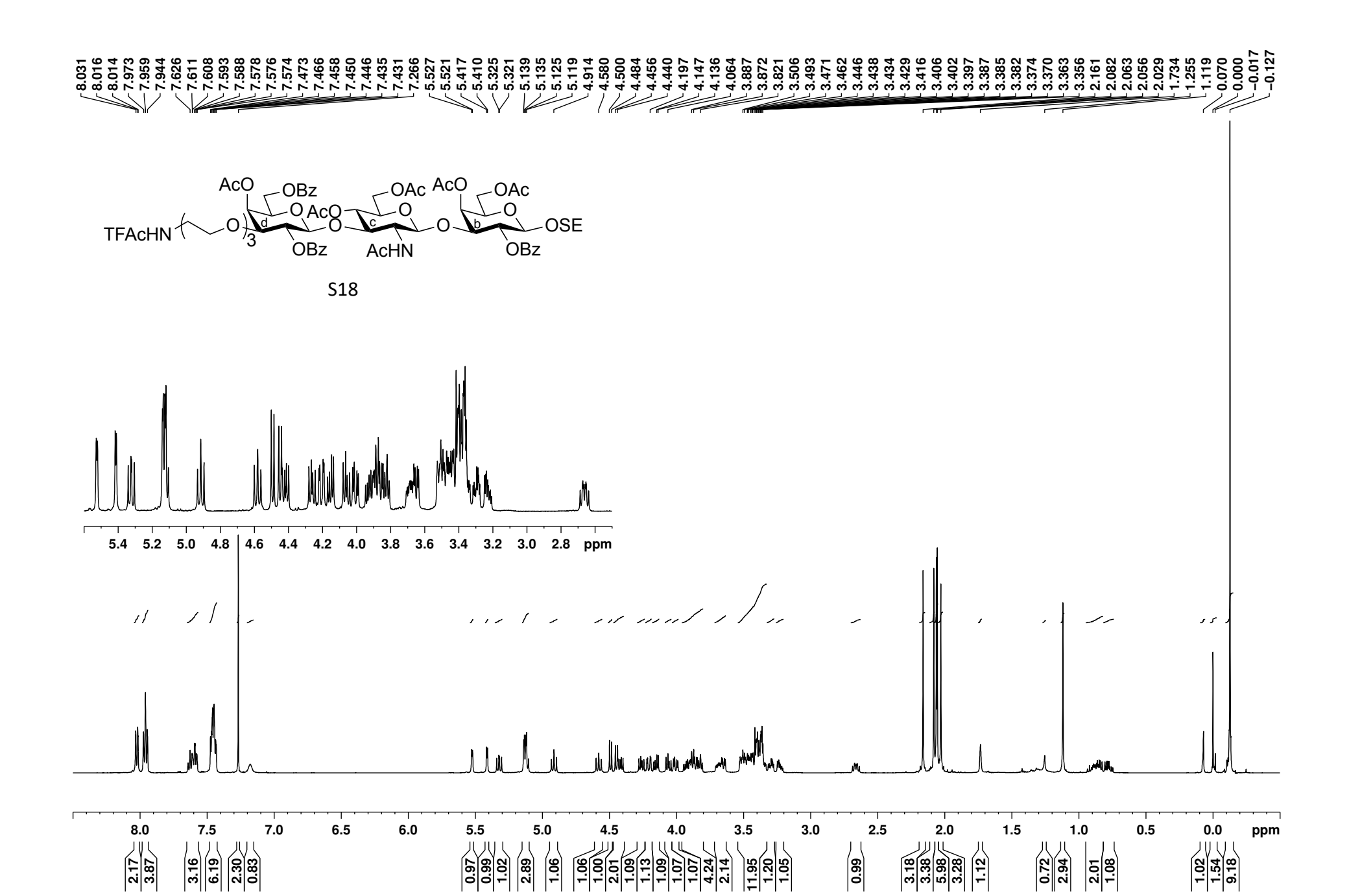


Figure S68. ^{13}C NMR spectrum (125 MHz, $CDCl_3$)



S18

Figure S69. ^{1}H NMR spectrum (500 MHz, $CDCl_3$)

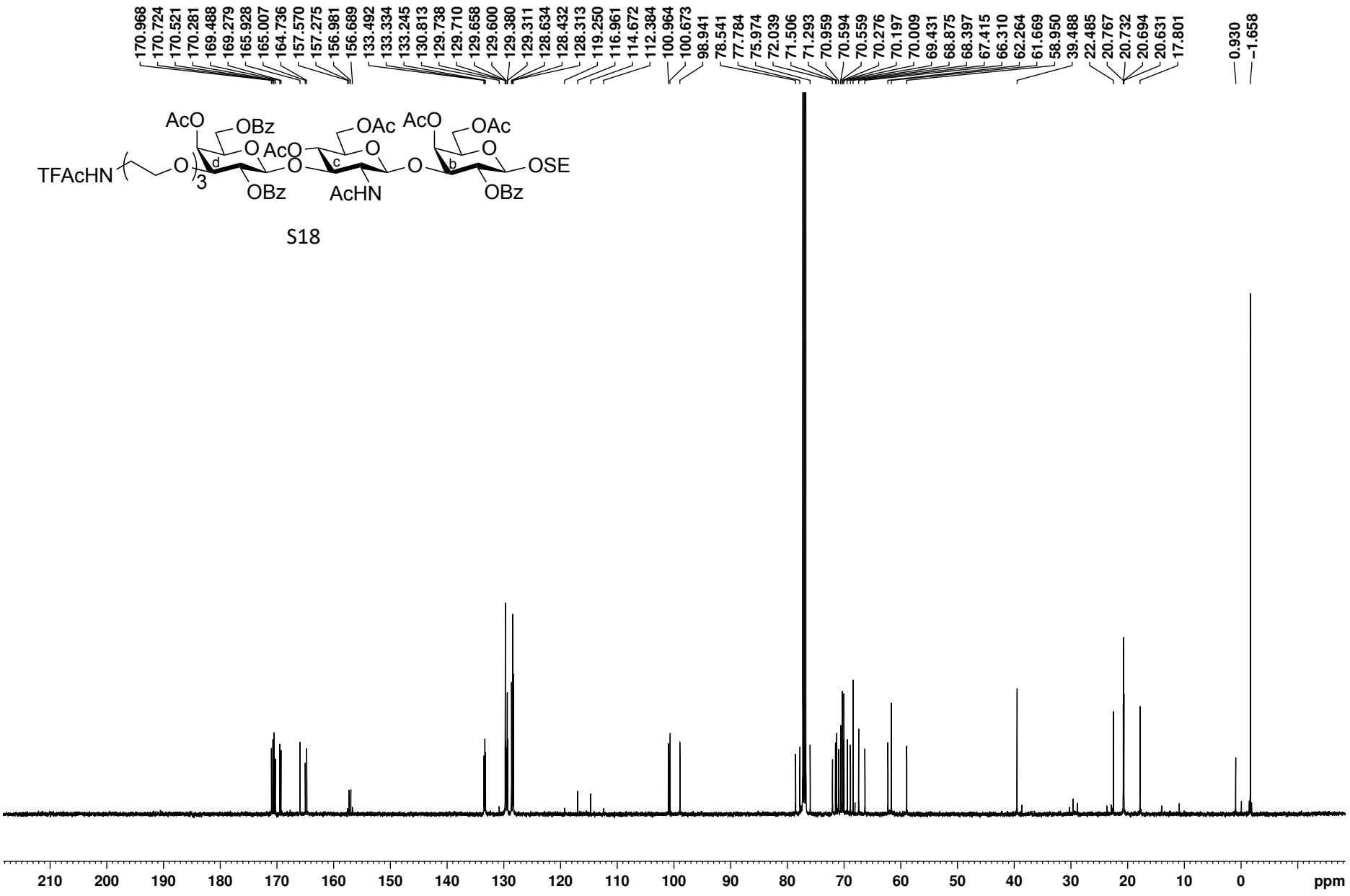
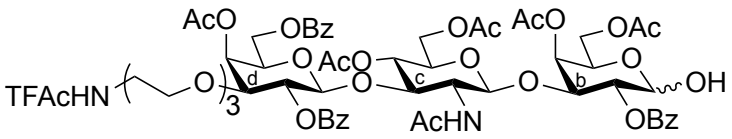


Figure S70. ^{13}C NMR spectrum (125 MHz, $CDCl_3$)



S19

Figure S71. ^{1}H NMR spectrum (500 MHz, $CDCl_3$)

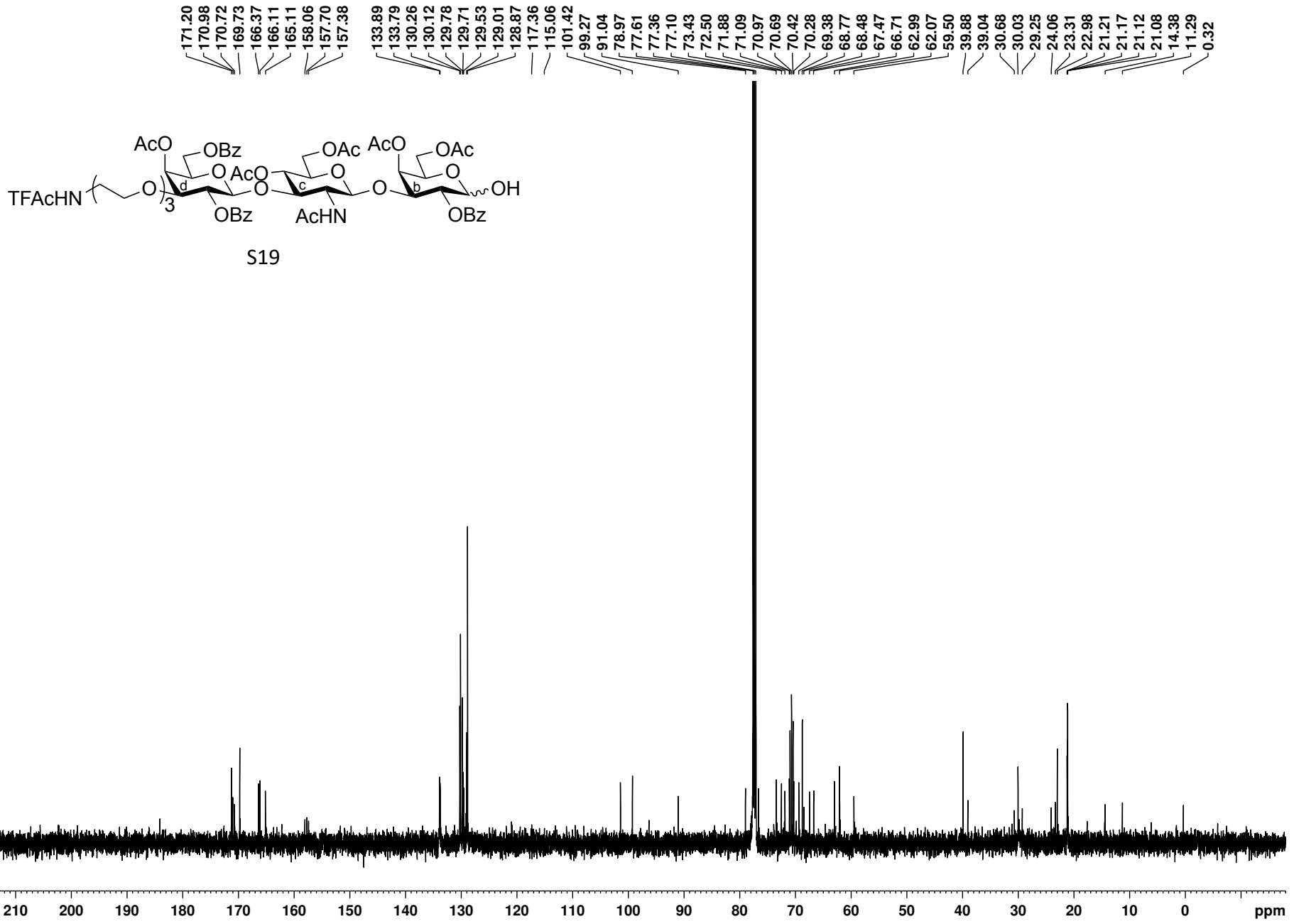


Figure S72. ^{13}C NMR spectrum (125 MHz, $CDCl_3$)

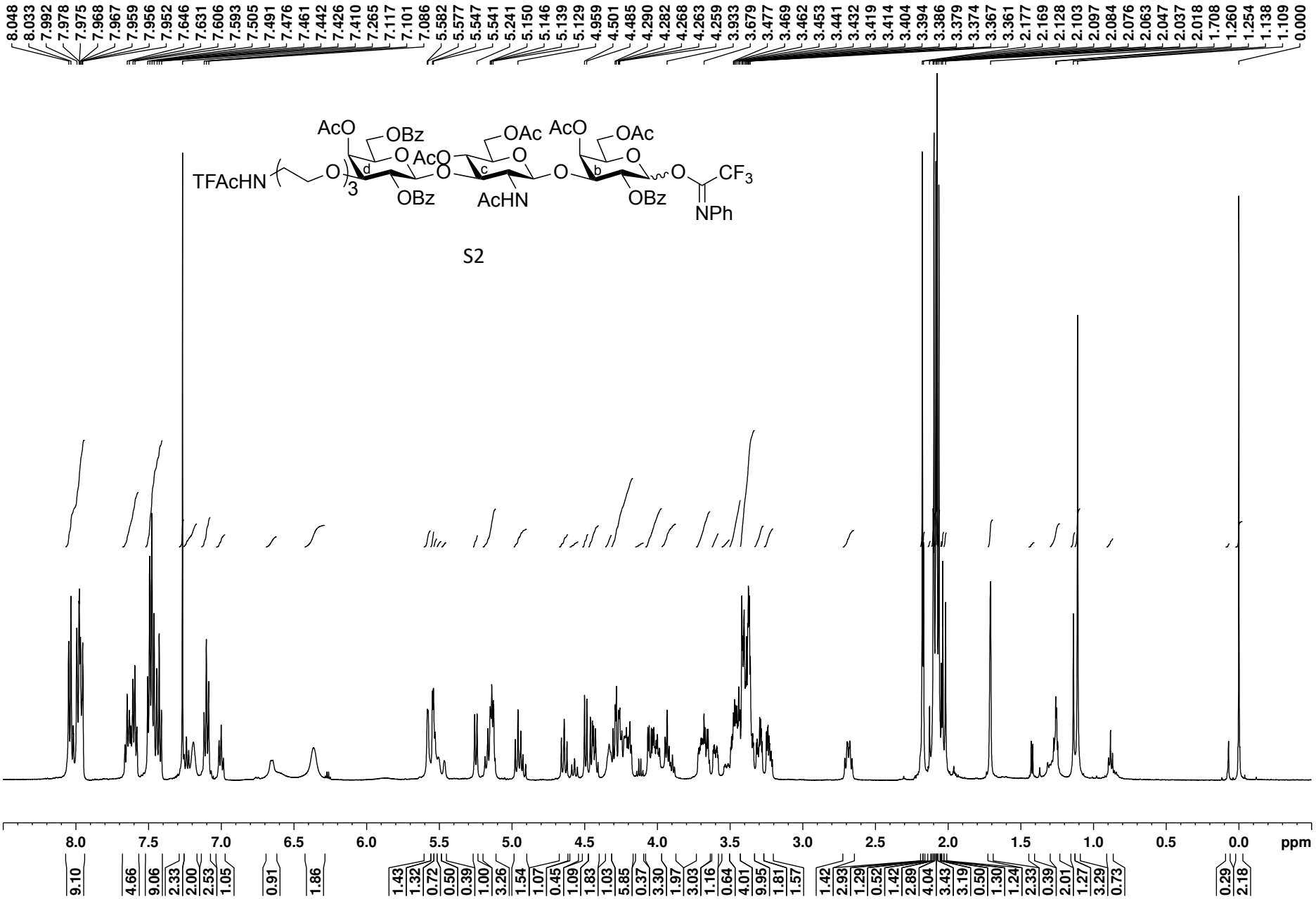


Figure S73. ^{1}H NMR spectrum (500 MHz, $CDCl_3$)

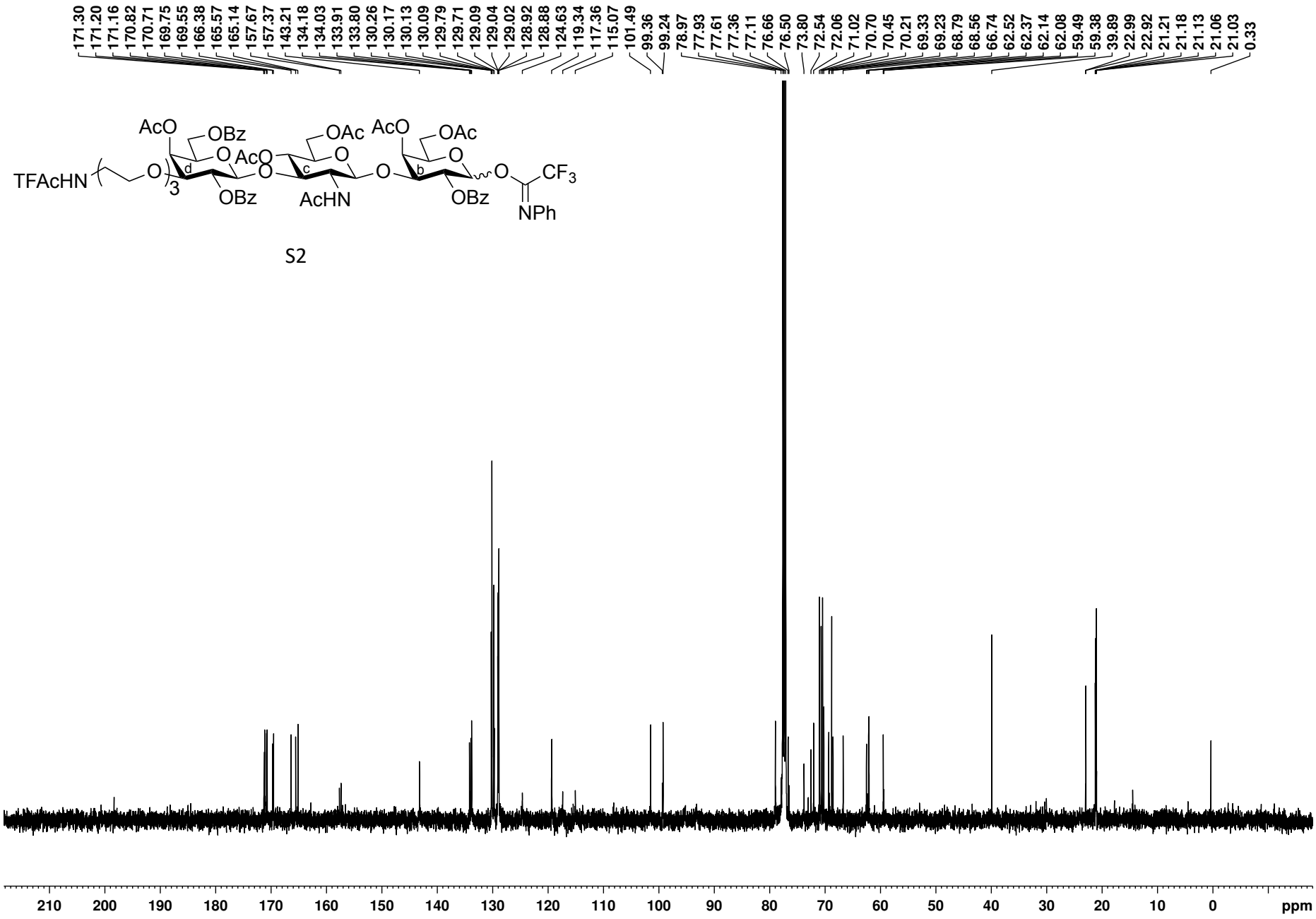


Figure S74. ^{13}C NMR spectrum (125 MHz, $CDCl_3$)

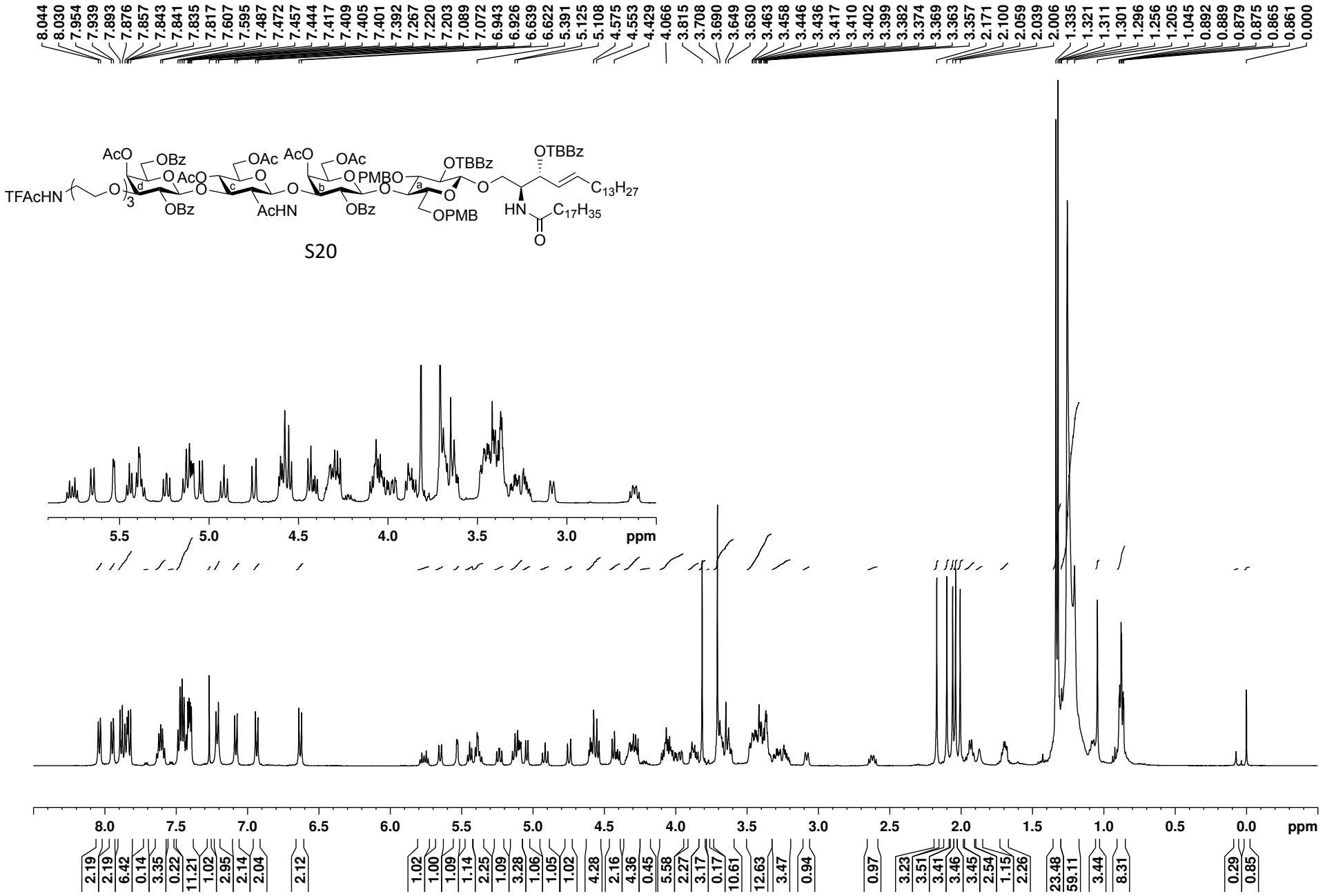


Figure S75. ^{1}H NMR spectrum (500 MHz, $CDCl_3$)

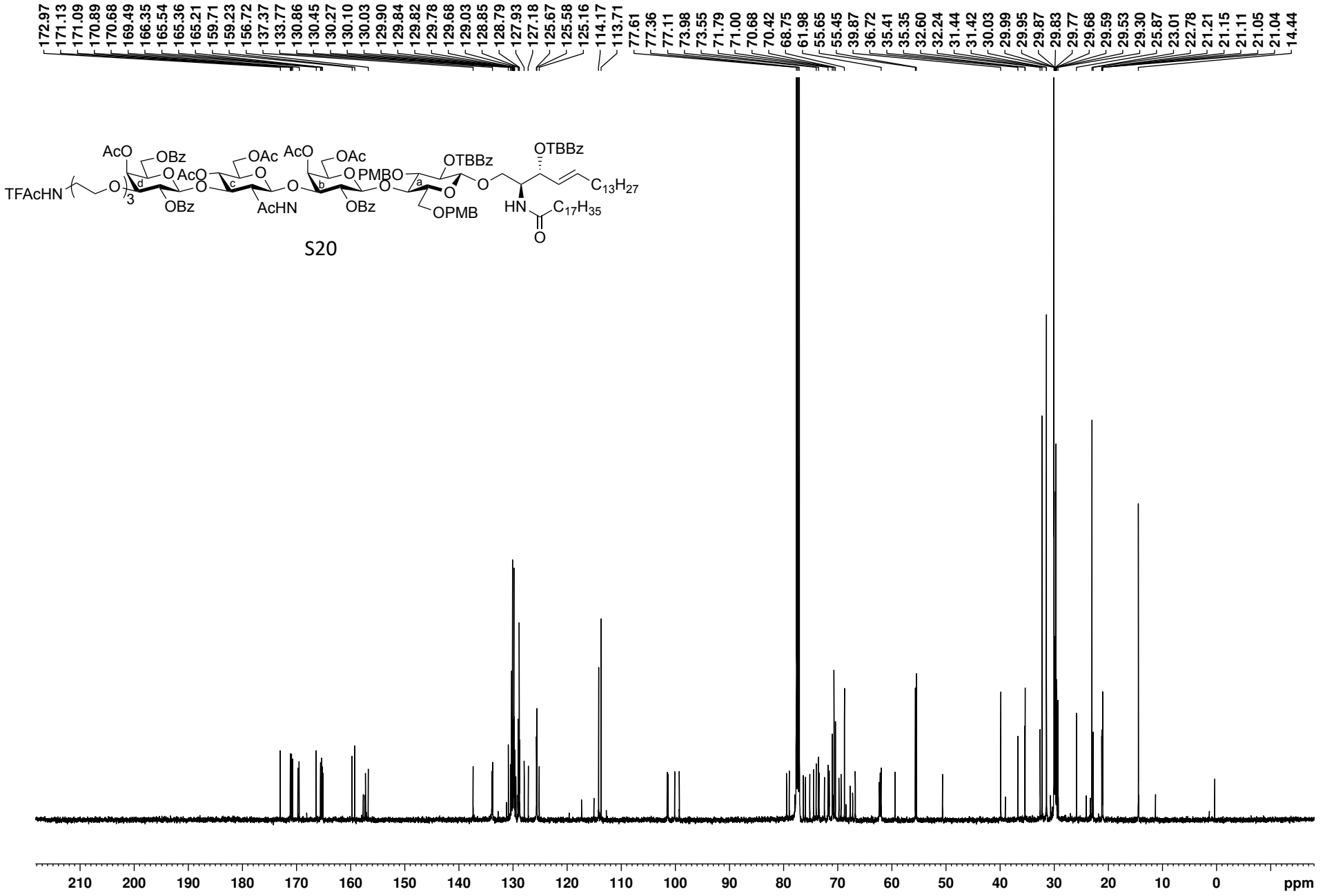


Figure S76. ^{13}C NMR spectrum (125 MHz, $CDCl_3$)

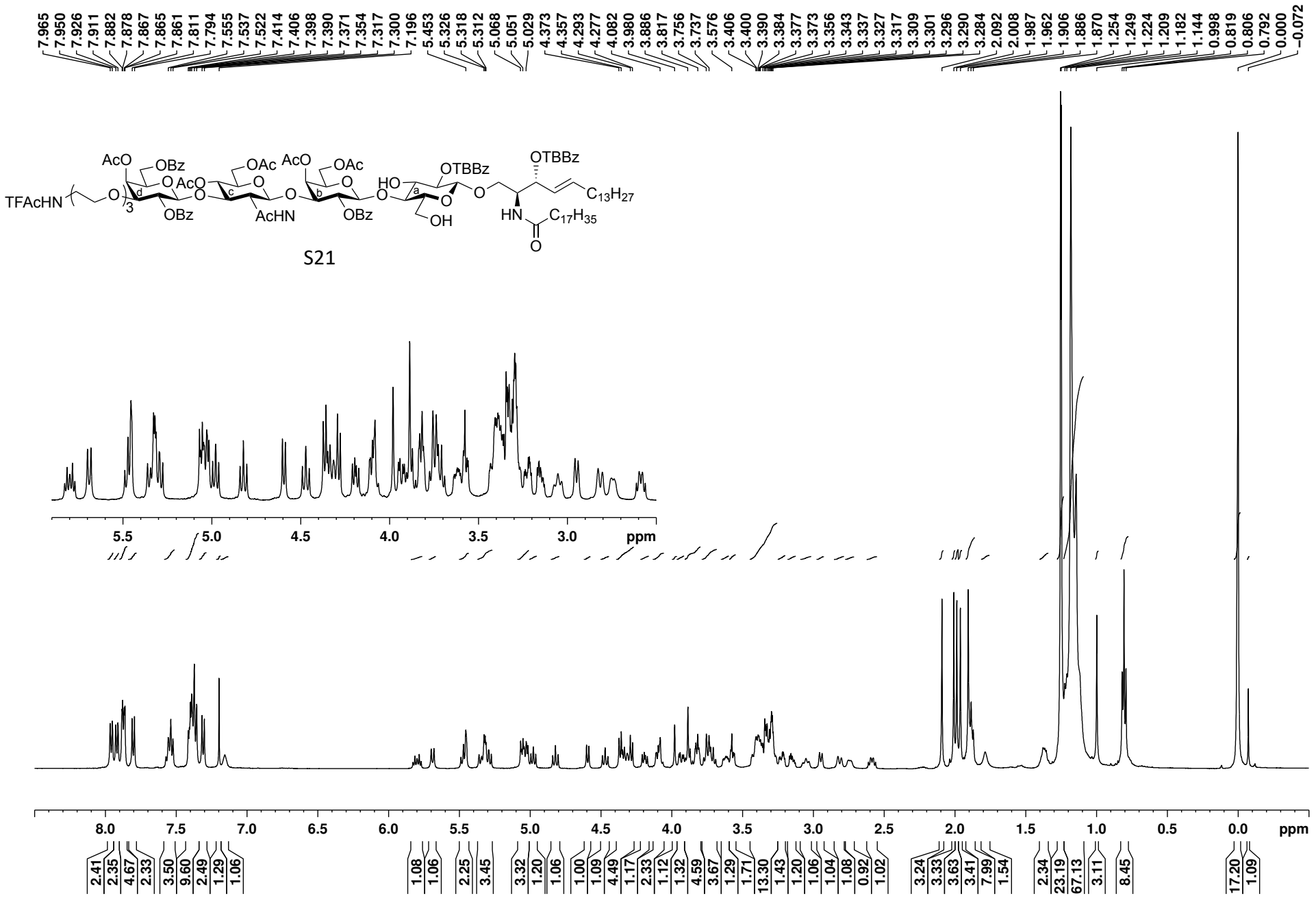


Figure S77. ^{1}H NMR spectrum (500 MHz, $CDCl_3$)

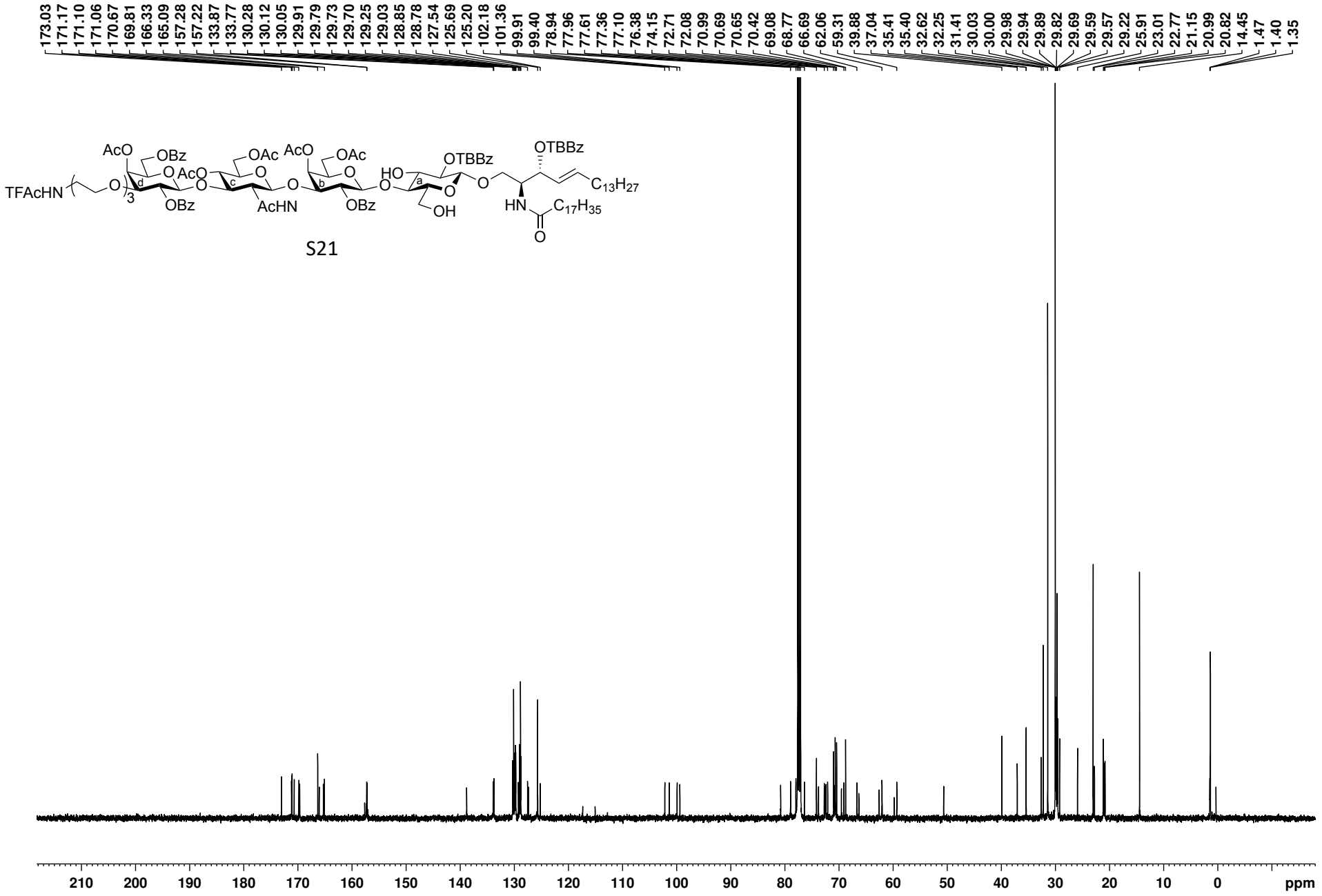


Figure S78. ^{13}C NMR spectrum (125 MHz, $CDCl_3$)

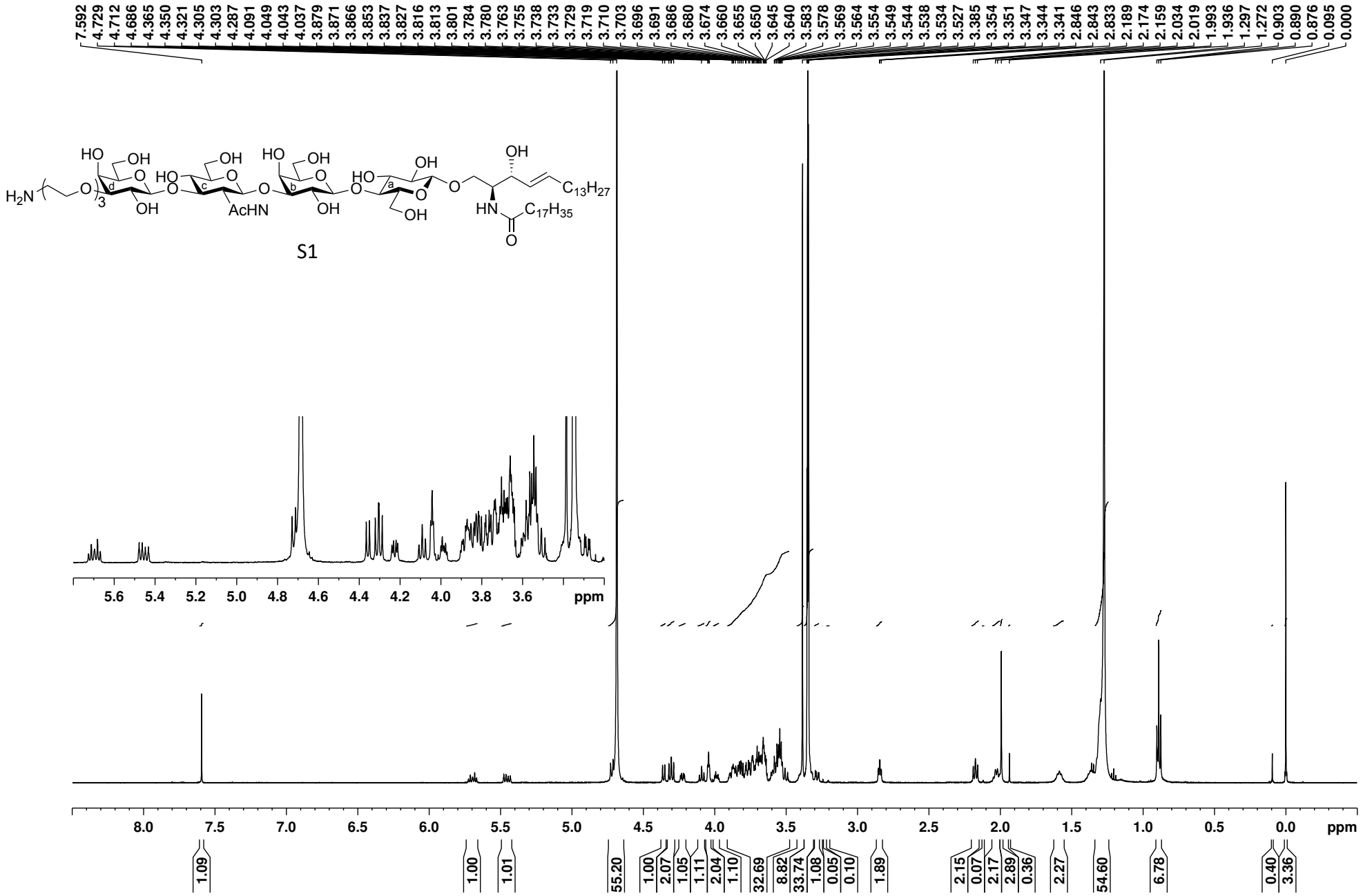


Figure S79. ^{1}H NMR spectrum (500 MHz, $CDCl_3/CD_3OD$ = 1/1)

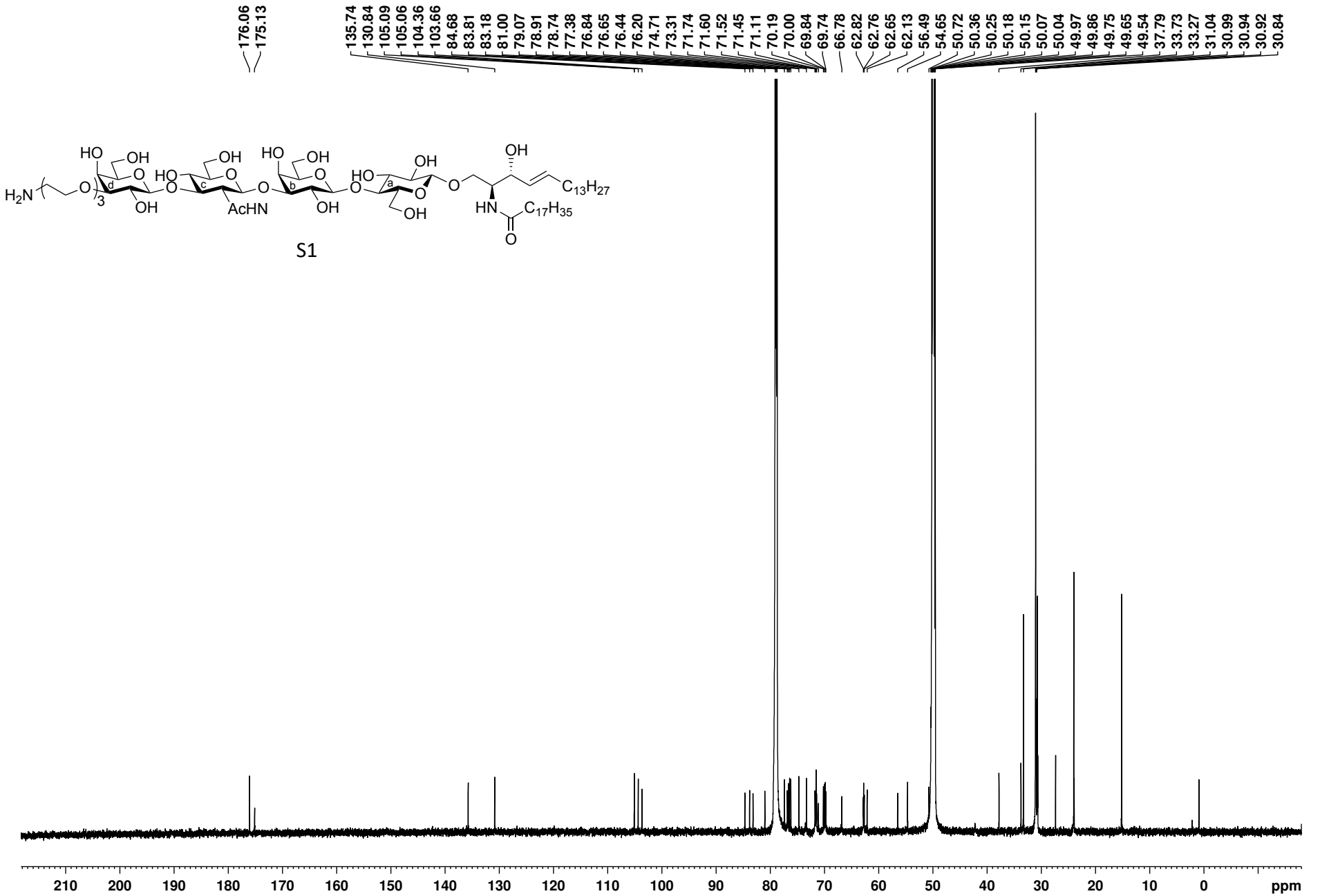


Figure S80. ^{13}C NMR spectrum (200 MHz, CD_3OD)

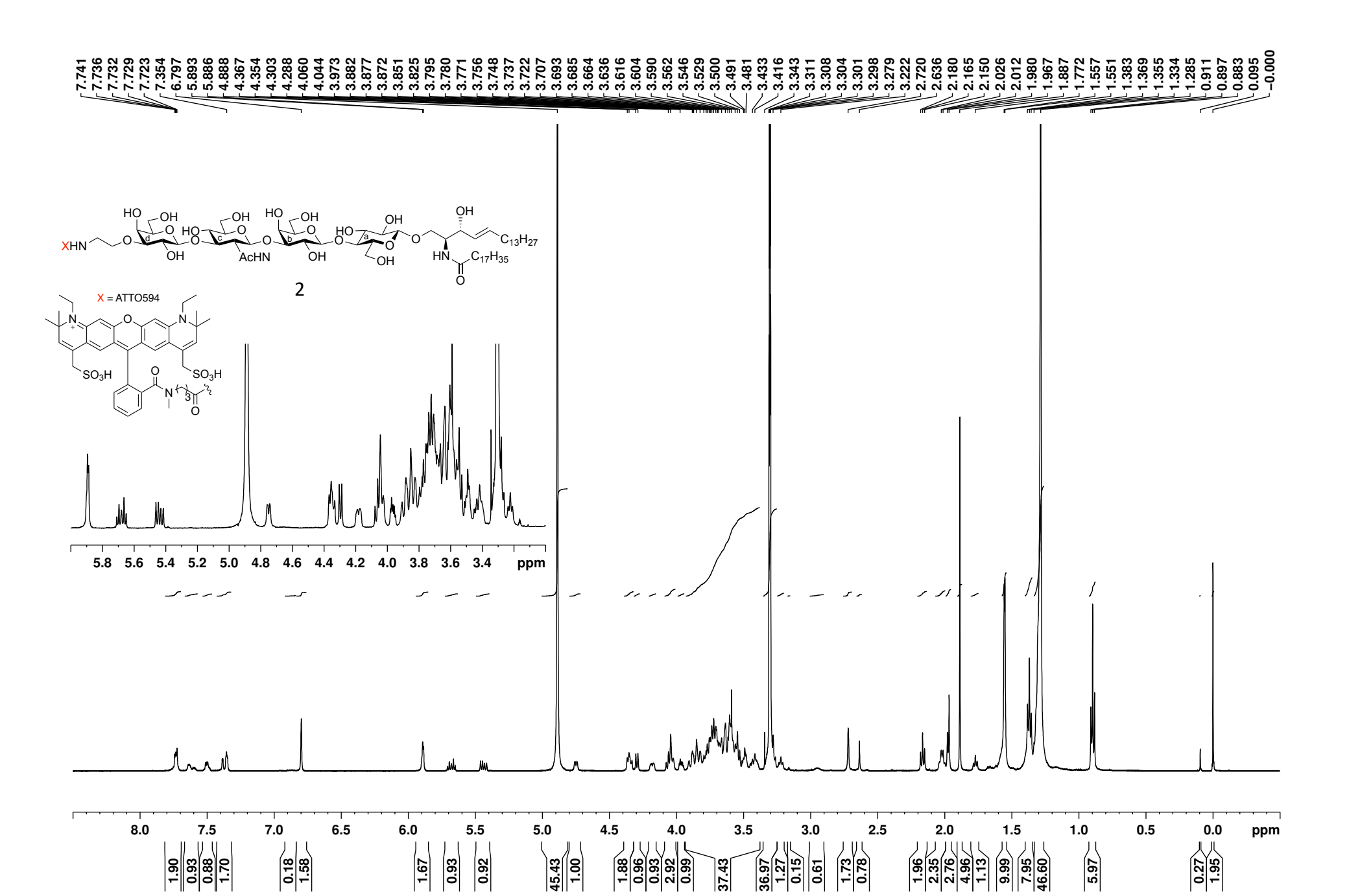


Figure S81. ^{1}H NMR spectrum (500 MHz, CD_3OD)

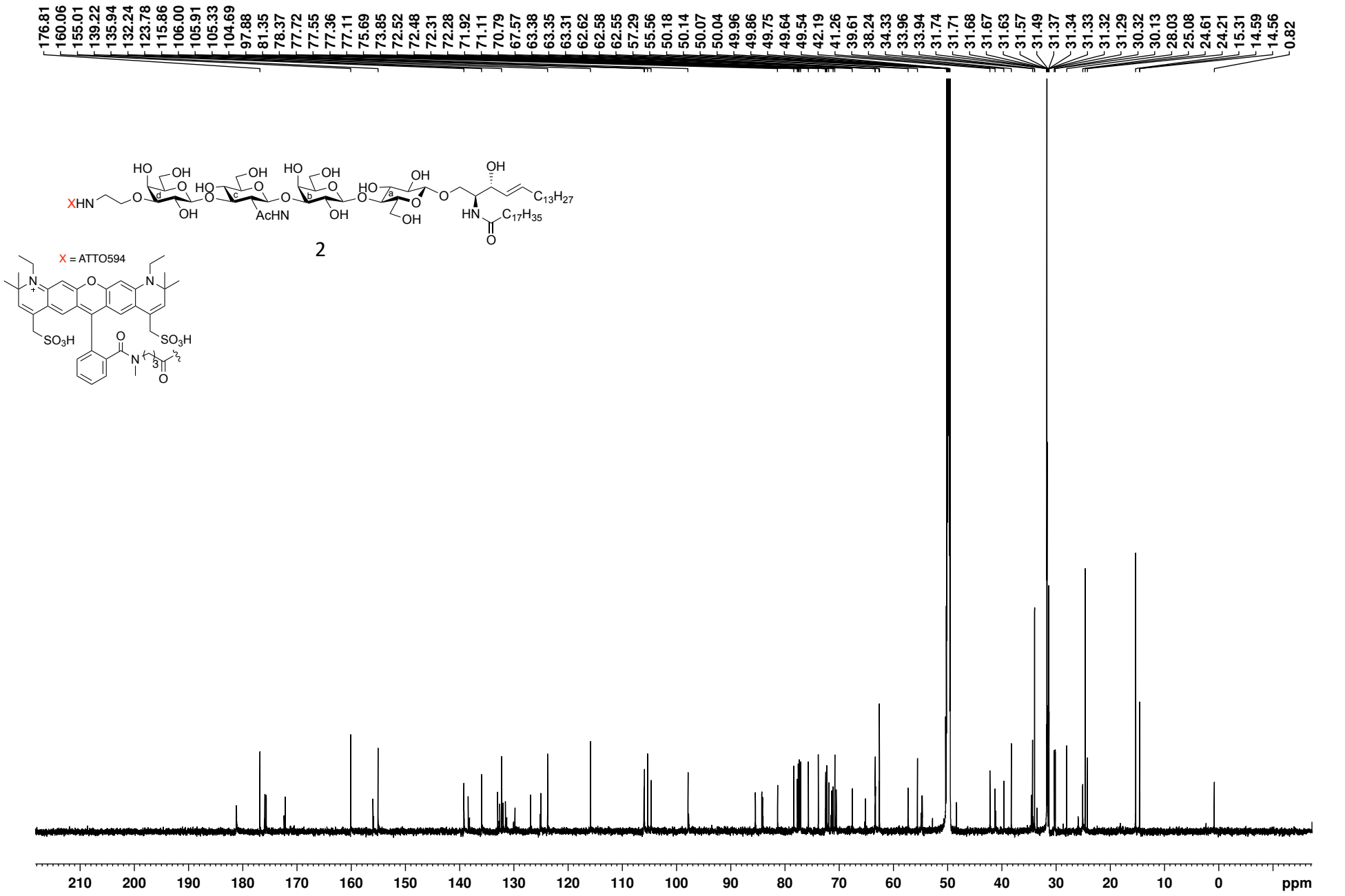


Figure S82. ^{13}C NMR spectrum (200 MHz, CD_3OD)