

Supplementary information

Acceptor-Mediated Regioselective Enzyme Catalyzed Sialylation:

Chemoenzymatic Synthesis of GAA-7 Ganglioside Glycan

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Materials and methods

All solvents were dried and distilled by standard techniques. *N,N*-Dimethylformamide (DMF) was vacuum-distilled over sodium hydride. Pyridine was distilled over sodium hydride. Tetrahydrofuran (THF) was distilled from sodium under nitrogen (N₂). Dichloromethane (DCM), toluene, and acetonitrile (ACN) were distilled from calcium hydride under N₂. The chemicals for the synthesis were all obtained from Acros, Merck, Fluka, or Sigma-Aldrich and used without further purification unless otherwise noted.

All reactions were carried out in oven-dried glassware (104 °C) and performed under anhydrous conditions with N₂ unless indicated otherwise. The reactions were monitored by analytical thin-layer chromatography (TLC) on Merck silica gel 60 F₂₅₄ plates (0.25 mm). Detection was accomplished by examination under UV light (254 nm) and by staining with *p*-anisaldehyde, ninhydrin, cerium molybdate, or potassium permanganate staining solution. Silica gel column chromatography was performed using a forced flow of the indicated solvent on silica gel 60 (Merck). Size exclusion column chromatography was performed by gravity on polymethacrylic polymer beads (Toyopearl HW-40F) with MeOH and polyacrylamide gel (Bio-Gel P-2) with deionized H₂O. C18 reverse-phase silica column (Sep-Pak Vac tC18 cartridge 20 cc/5 g 37-55 μm, Waters) and C18 reverse-phase silica gel (LiChroprep® RP-18 (40-63 μm), Merck) were used to perform reverse-phase column chromatography with methanol, acetonitrile, and deionized H₂O. DEAE Sepharose® Fast Flow (GE Healthcare Life Sciences) was used for anion exchange chromatography. ¹H and ¹³C NMR spectra were recorded by Bruker AV-400, AV-600, Varian MR400, Varian-Unity INOVA-500, or VNMRS-700. Chemical shifts are expressed in ppm using residual CDCl₃ (7.24 ppm), CD₃OD (3.31 or 4.87 ppm), or D₂O (4.79 ppm at 298 K) as internal standard in ¹H-NMR spectra. ¹³C-NMR spectra were recorded in either CDCl₃, CD₃OD or D₂O at 100, 125, 150, or 175 MHz, using the central resonances of CDCl₃ (77.0 ppm) and CD₃OD (49.0 ppm) as the internal references. 2D NMR (COSY, HSQC, HMBC, or HSQC-TOCSY) experiments were used to assist assignment of the products. Multiplicities are reported by using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad; *J* = coupling constant values are expressed in Hertz. Mass spectra were obtained in ESI mode.

Alkaline phosphatase from calf intestinal was purchased from Sigma-Aldrich. Chemical competent *E. coli* BL21(DE3) were purchased from Yeastern Biotechnology (Taipei, Taiwan). Vector plasmid pET-22b(+) was purchased from Merck (Darmstadt, Germany). *Nde*I restriction enzyme and *Xho*I restriction enzyme were purchased from New England Biolabs (Ipswich, MA, USA). QIAprep spin miniprep kit and QIAEX II gel extraction kit were purchased from Qiagen (Valencia, CA, USA). T4 DNA ligase and 1 kb DNA ladder were obtained from Thermo (Waltham, MA, USA). DNA primers for the polymerase chain reaction (PCR) were purchased from Mission Biotech (Taipei, Taiwan). Matrixes of bacterial cultural media were purchased from BD Bioscience (San Jose, CA, USA). IMPACT™ system (Intein Mediated Purification with Affinity Chitin binding Tag) was purchased from New England Biolabs. His-tag purification resin was purchased from Roche (Basel, Switzerland). Amylose resin was purchased from Cyrusbioscience (New Taipei City, Taiwan). Protein molecular weight standards and the *Pfu* DNA polymerase were purchased from Thermo Fisher Scientific (Waltham, MA, USA). The protein

concentrations were determined with the Bradford Protein Assay (Bio-Rad) and Pierce™ BCA protein assay kit (Thermo Fisher Scientific) using bovine serum albumin as the standard. Protein purification devices were using centrifugal filter devices (Vivaspin® Turbo 15 10 kDa MWCO, Satorius).

Cloning and Overexpression of Enzymes

Enzyme Resources. BNahK (*N*-acetylhexosamine 1-kinase from *Bifidobacterium longum* subsp. *longum*),¹ EcGlmU (*N*-acetylglucosamine 1-phosphate uridyltransferase from *Escherichia coli*),² AGX1 (recombinant human UDP-GalNAc pyrophosphorylase),³ NmLgtA (*N*-acetylglucosaminyltransferase from *Neisseria meningitidis*),⁴ NmCSS (CMP-sialic acid synthetase from *Neisseria meningitidis*),⁵ PmST1 (α (2,3)-sialyltransferase 1 from *Pasteurella multocida*),⁶ CjCstI (α (2,3)-sialyltransferase from *Campylobacter jejuni*),⁷ Pd2,6ST (α (2,6)-sialyltransferase from *Photobacterium damsela*),¹ and Psp2,6ST (α (2,6)-sialyltransferase from *Photobacterium sp. JH-ISH-224*)⁸ were cloned and overexpressed as previously reported procedures. The plasmid pMal-c2X containing the *lgtA* gene and pET-15b containing the *agx1* gene were generously provided by Professor Ching-Ching Yu.

Gene cloning. The genes for the PmST3 Δ 35 was optimized as previous described⁹ and synthesized by Genomics (New Taipei City, Taiwan), then isolated by PCR amplification using the following primers: PmST3 Δ 35-F: 5'- GAG ACAT ATG GAT AAA TTT GCC GAA CAT G-3' (*NdeI* restriction site is underlined) and PmST3 Δ 35-R: 5'- ATA TCT CGA GGC CCA GTT TTT CAT ACA CAA A-3' (*XhoI* restriction site is underlined). Briefly, PCR amplification of target gene was performed in a solution (50 μ L) that contained 50 ng of template DNA, 0.4 μ M of each primer, 0.2 mM dNTP, 1.5 mM MgCl₂, and 2.5 U *Pfu* DNA polymerase. PCR was performed with denaturation at 95 °C for 30 sec, annealing at 61.5 °C for 30 sec and extension at 72 °C for 30 sec. The target gene was digested from the amplified fragment with *NdeI* and *XhoI* and then was inserted into the *NdeI/XhoI*-treated vector pET-22b(+). The ligation mixture was then transformed into the *E. coli* DH5 α and BL21 (DE3) strains of *E. coli* DNA sequencing was used to confirm the in-frame cloning of the truncation of 105 base pair residues from the 3' end of the target gene.

Protein Overexpression and purification for PmST3. The protein overexpression procedure is similarly as previous report.¹ Positive plasmid was selected and subsequently transformed into *E. coli* BL21(DE3) chemical competent cells. The *E. coli* BL21 (DE3) harboring the recombinant plasmid was grown in LB rich medium containing ampicillin (100 μ g/mL) at 37 °C until the OD₆₀₀ reached 0.5-0.8. Protein expression was then induced by adding 0.5 mM of IPTG (isopropyl-1-thio- β -D-galactopyranoside) followed by incubation at 16 °C for 16-20 h with vigorous shaking at 200 rpm in a shaking incubator (Firstek S300R). The bacterial cells were harvested by centrifugation at 4 °C and 5000 x g for 15 min. The cell pellet was resuspended at 25 mL per liter of cell culture in column buffer (pH 8.0, 20 mM Tris-HCl containing 0.1% Triton X-100, 250 mM NaCl, and 0.1 mM EDTA). The cells were disrupted by sonication on ice for 30 min in 3-s intervals, and then the debris was removed by centrifugation for 60

min at 20000 x g and 4 °C. The supernatant was applied to 10 mL cOmplete™ HisTag Purification Resin bead column and then incubated for 30 mins. The resin was quickly washed with 20 column volumes of the same column buffer containing 10 mM imidazole. The bound protein was eluted out by 250 mM imidazole in column buffer. The fractions were analyzed by SDS-PAGE. Fractions with significant amounts of protein were pooled and concentrated using a centrifugal filter device, divided into aliquots, and stored at -20 °C.

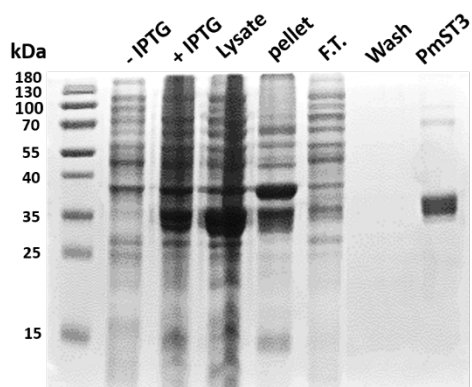


Fig. S1 SDS-PAGE analysis of PmST3Δ35. The gel of the purified protein shows an apparent band that is close to the calculated molecular weight (33 kDa) of the desired protein. Lanes: IPTG (-/+), whole cell extract before and after induction; Lysate, supernatant of the induced cell extract after centrifugation; pellet, cell debris after centrifugation; F.T., flow through of the cell lysate after His-tag purification by resin bead column chromatography; Wash, His-tag purification resin bead column washed with column buffer containing 10 mM imidazole after flow through of the cell lysate; and PmST3, column-purified protein PmST3Δ35.

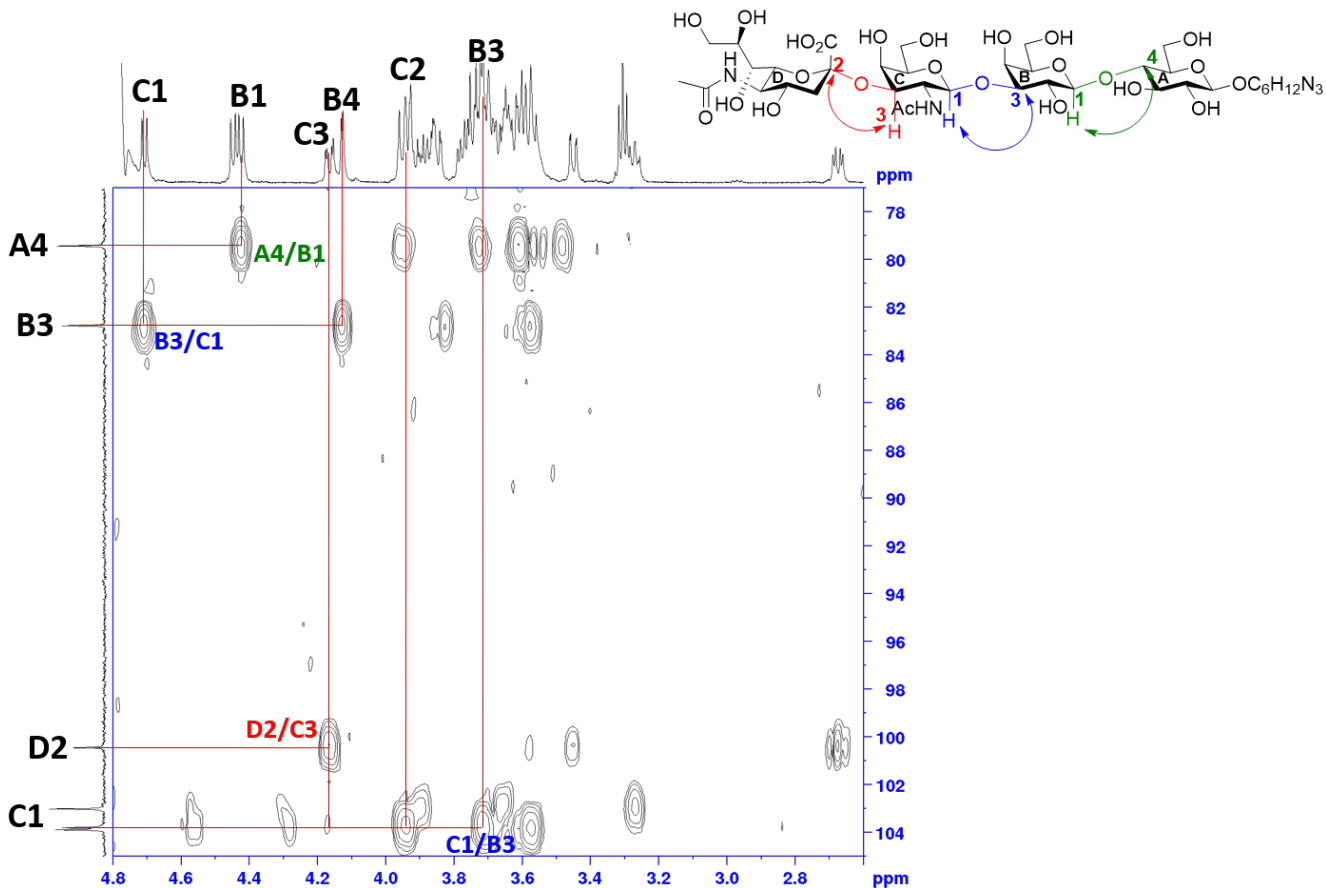


Fig. S2 ¹H-¹³C HMBC analysis of compound 4. Glycans A, B, C, and D represent Glc, Gal, GalNAc, and Neu5Ac, respectively. The number indicates the position of the carbon or proton atom. **GalNAc-β(1,3)-Gal linkage.** A cross signal for the anomeric proton of GalNAc (C1) and the 3-position carbon atom of Gal (B3) was observed (B3/C1). Moreover, a cross signal for the 3-position proton of Gal (B3) and the anomeric carbon atom of GalNAc (C1) was observed (C1/B3). The cross signal correlations reveal that the glycosidic linkage is a 1→3 linkage. **Neu5Ac-α(2,3)-GalNAc linkage.** A cross signal for the anomeric carbon of sialic acid (D2) and the 3-position proton atoms of GalNAc (C3) was observed (D2/C3). The cross signal correlation suggests that the glycosidic linkage is a 2→3 linkage.

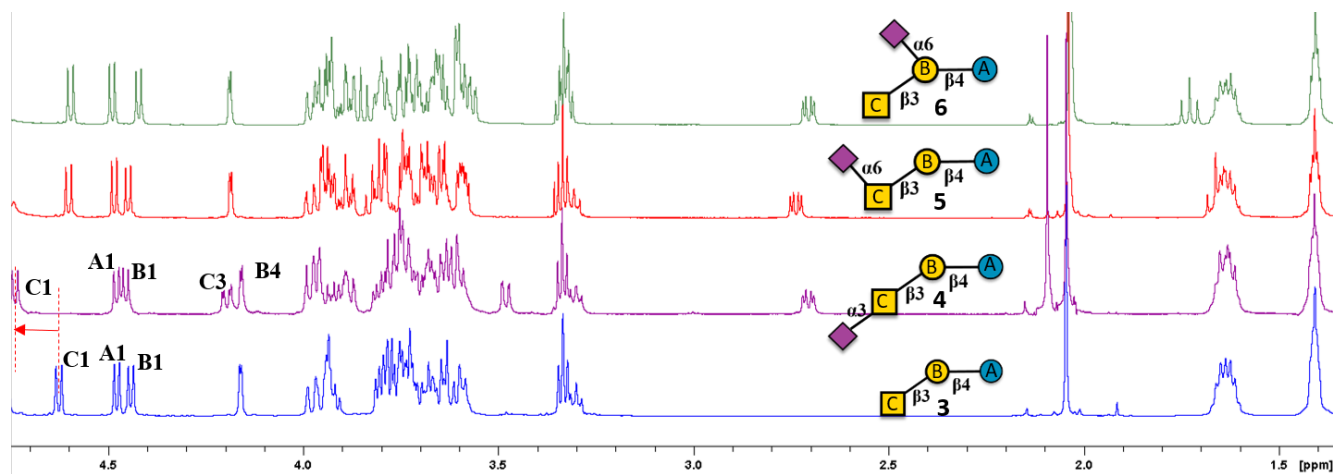


Fig. S3 ^1H NMR comparison of monosialylated compounds **4**, **5**, and **6**. Glycans A, B, C, and D represent Glc, Gal, GalNAc, and Neu5Ac, respectively. The number indicates the position of the proton atom. The H-1 signal of GalNAc (C1) was shifted downfield from 4.63 to 4.71 ppm after $\alpha(2,3)$ sialylation (**4**), while no significant signal shift was observed for the $\alpha(2,6)$ sialylated glycans **5** and **6**. Moreover, the H-3 signal of GalNAc (C3) was shifted downfield to 4.17 ppm in compound **4**. For $\alpha(2,6)$ sialylation on Gal (**6**), the H-1 signal of Gal (B1) was shifted upfield but no shift was observed for the $\alpha(2,6)$ sialylation on GalNAc (**5**).

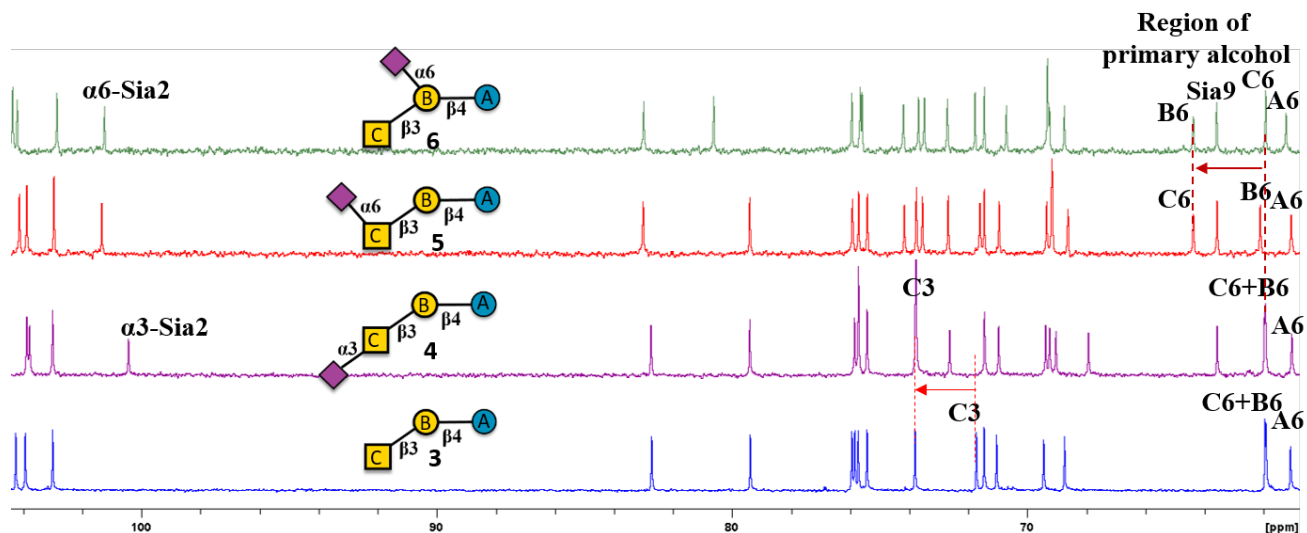


Fig. S4 ^{13}C NMR comparison of monosialylated compounds **4**, **5**, and **6**. Glycans A, B, and C represent Glc, Gal, and GalNAc, respectively. The number indicates the position of the carbon atom. Sia2 and Sia9 represent the anomeric and 9-position carbon atoms of sialic acid, respectively. The C-3 signal of GalNAc (C3) shifted downfield from 71.73 to 73.78 ppm after $\alpha(2,3)$ sialylation (**4**), but no shift for the $\alpha(2,6)$ -sialylated glycans **5** and **6** was observed. In contrast, in the primary alcohol region, there is no significant signal shift in **4** compared with **3** but there was an obvious chemical shift of the C-6 signal of GalNAc or Gal (C6 or B6) after $\alpha(2,6)$ sialylation.

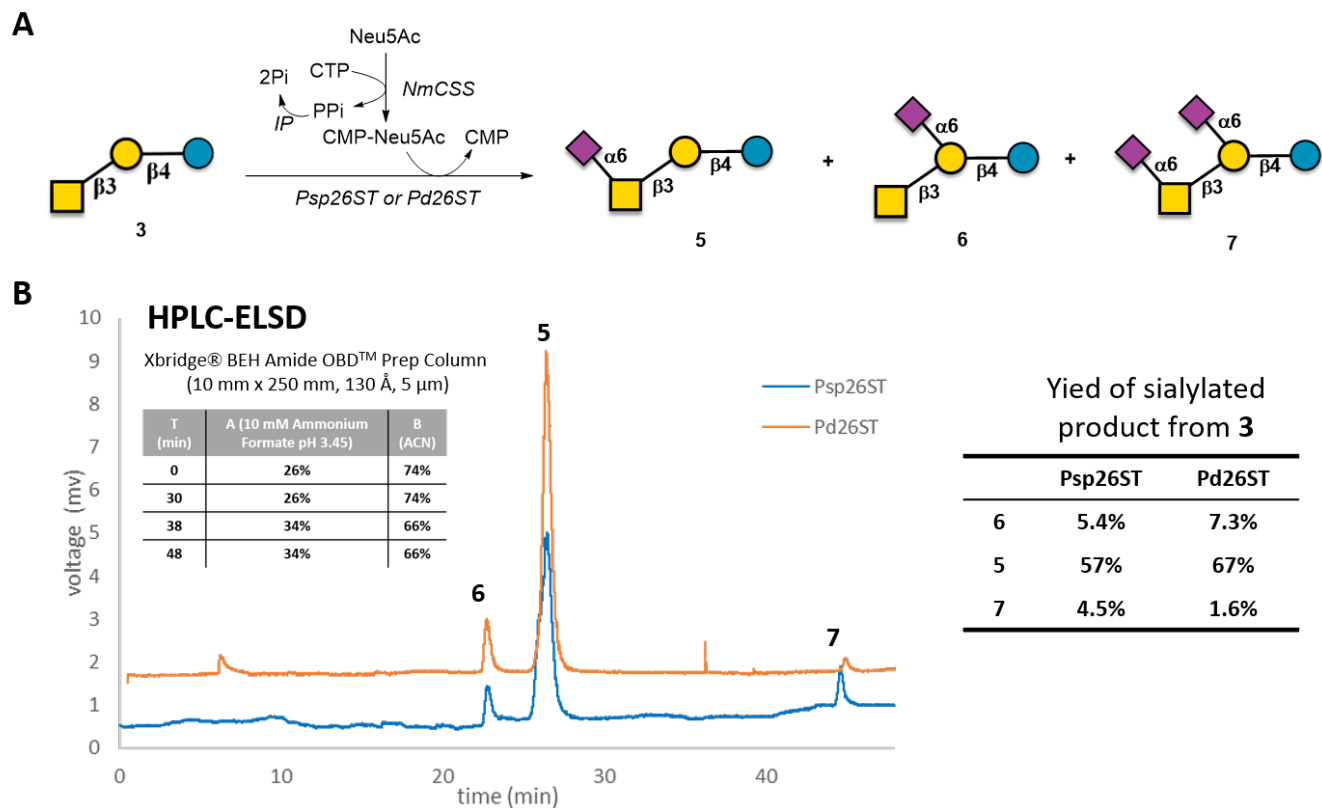


Fig. S5 Study of $\alpha(2,6)$ sialylation of compound **3**. HPLC-evaporative light scattering detector (ELSD) analysis of the sialylation of compound **3** by two kinds of $\alpha(2,6)$ STs with 1.2 equivalents of sialic acid. A. Schematic of the enzymatic sialylation of compound **3** by Psp2,6ST and Pd2,6ST. B. HPLC-ELSD analysis of the crude sialylated products and their corresponding yields.

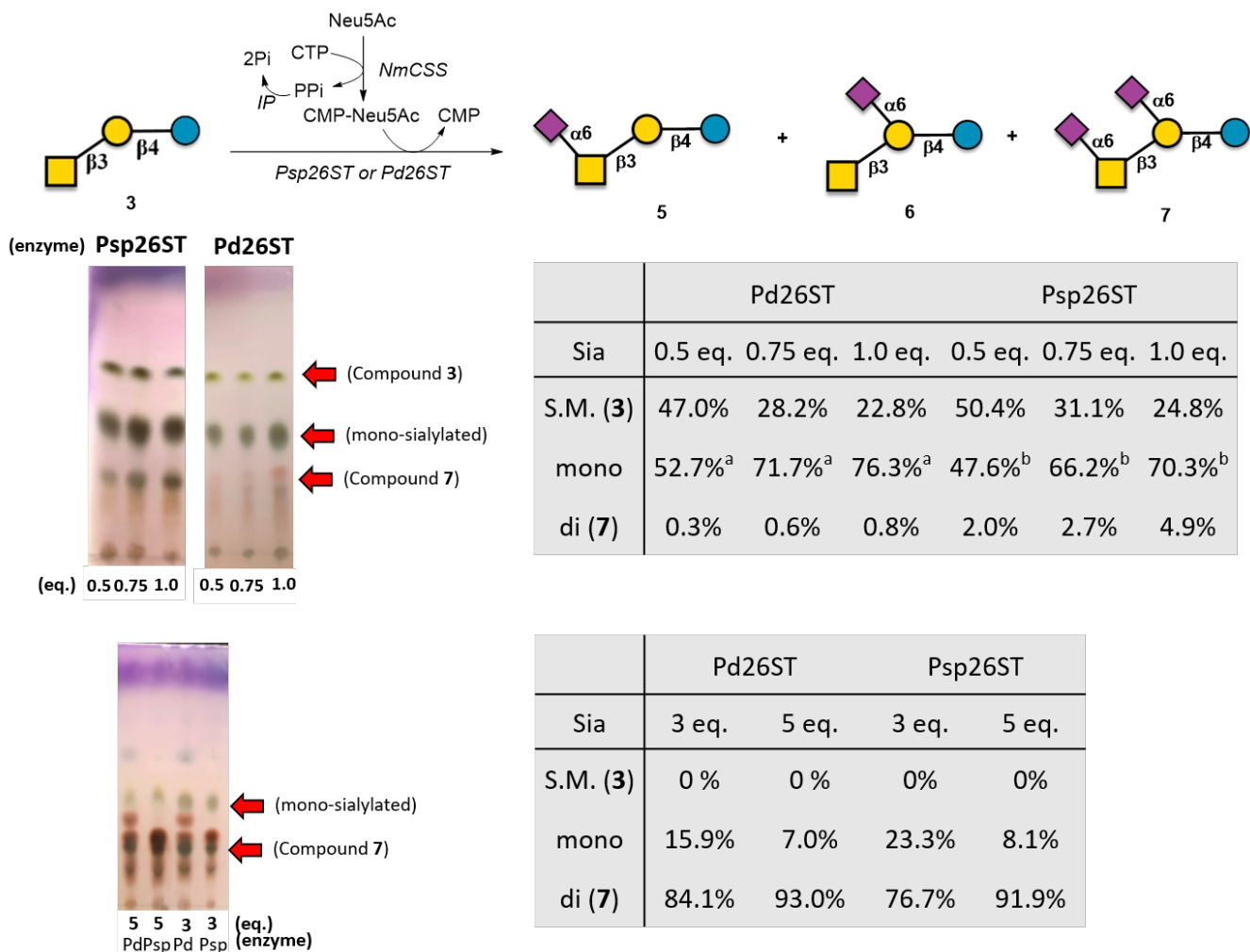


Fig. S6 Analysis of the sialylation of compound **3** by two kinds of $\alpha(2,6)$ STs with various equivalents of sialic acid (0.5, 0.75, 1.0, 3.0, and 5.0). The synthetic procedure was similar to the sialylation of compound **3** with 1.2 equivalents of sialic acid. The reaction was monitored by TLC (*n*-PrOH/H₂O/AcOH = 6/2/1 (v/v/v)). After all of the CMP-sialic acid was consumed, the reaction was quenched by the addition of the same reaction volume of acetonitrile. The reaction solution was centrifuged (10,000 × g, 5 min) to remove enzymes and insoluble precipitates. The supernatant was filtered through a 0.45- μ m PVDF filter (Millipore) followed by injection of the residues into the HPLC-ELSD instrument. The glycan peaks were integrated, and the content of each glycan was calculated as shown in the tables. ^aOnly compound **5** was observed. ^bCompounds **5** and **6** were observed (identified by HPLC-ELSD).

When using fewer equivalents of sialic acid, the $\alpha(2,6)$ sialylation of compound **3** by Pd2,6ST preferred the nonreducing sugar (GalNAc). However, Psp2,6ST did not show regioselectivity between GalNAc and Gal. When 5 equivalents of sialic acid were used, the reactions catalyzed by both Pd2,6ST and Psp2,6ST generated a small amount of monosialylated product.

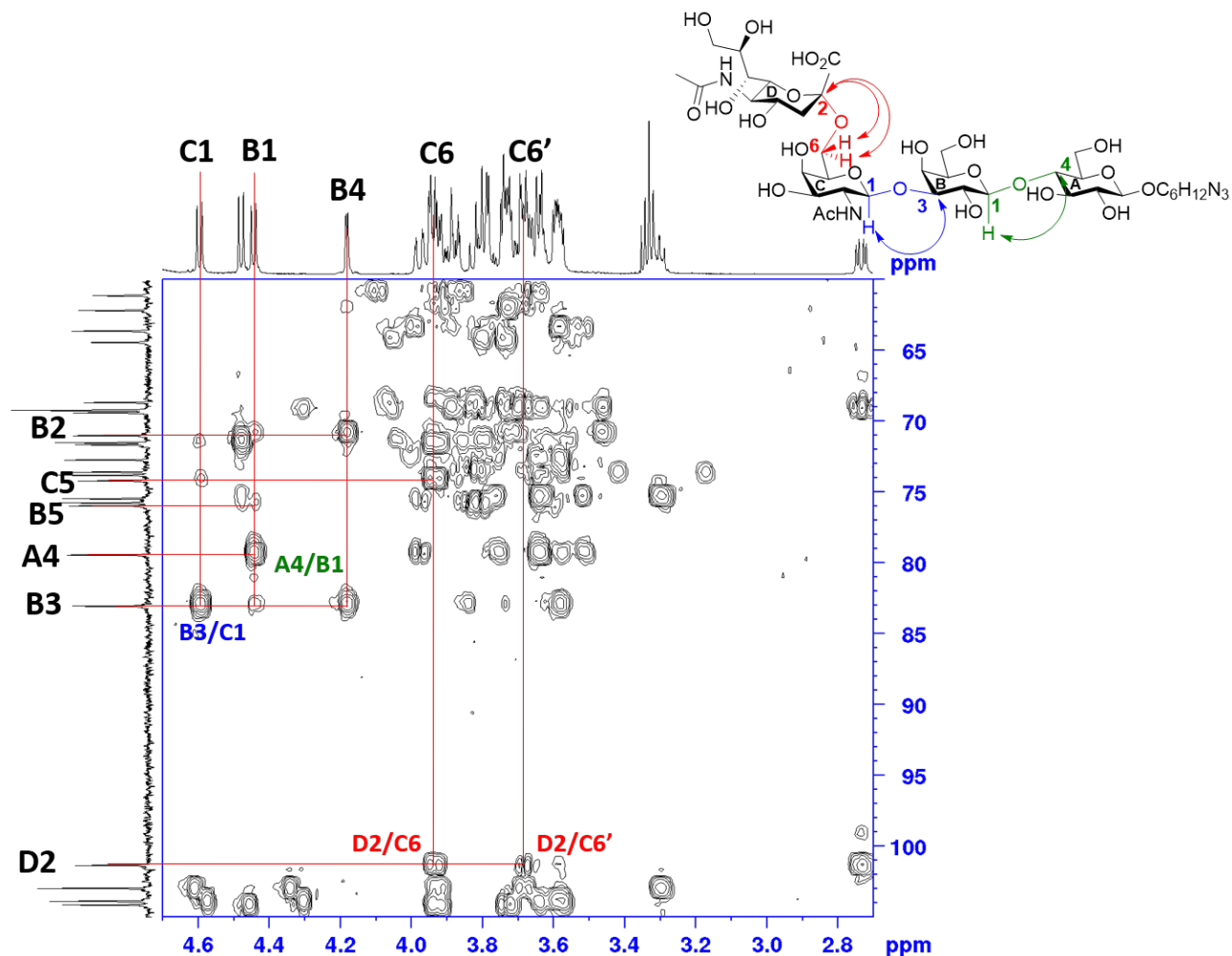


Fig. S7 ^1H - ^{13}C HMBC spectrum of **5**. Glycans A, B, C, and D represent Glc, Gal, GalNAc, and Neu5Ac, respectively. The number indicates the position of the carbon or proton atom. **Neu5Ac- α (2,6)-GalNAc linkage**. Cross signals for the anomeric carbon of sialic acid (D2) and both 6-position proton atoms of GalNAc (C6 and C6') were observed (D2/C6 and D2/C6'). Moreover, there were also cross signals for the C5 carbon and one of C6 protons of GalNAc (C5/C6) and the C5 carbon and the anomeric proton of GalNAc (C5/C1), supporting the assignment of the D2-C6-Hs cross signals. These signals reveal that the glycosidic linkage is a 2 \rightarrow 6 linkage between Neu5Ac and GalNAc.

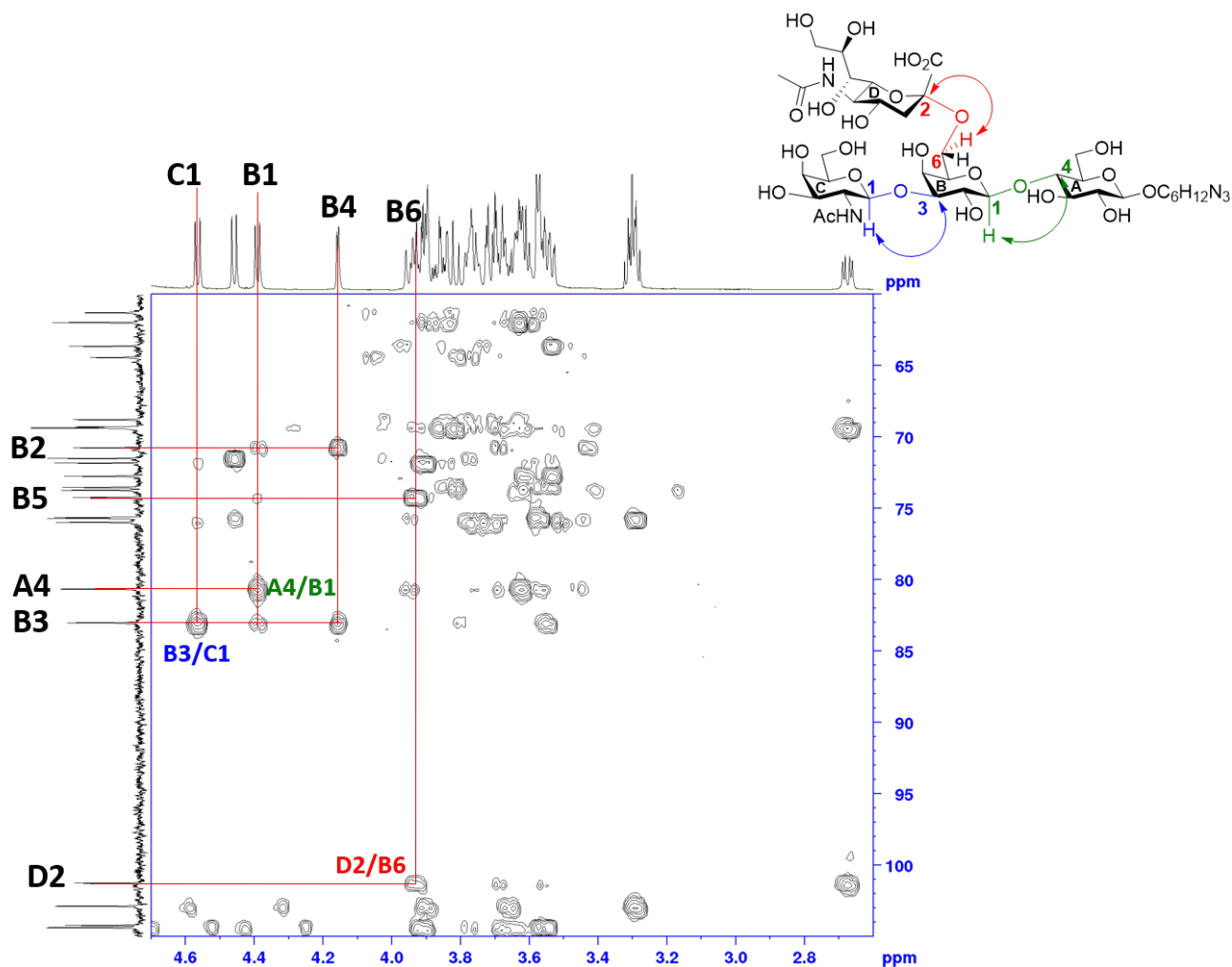


Fig. S8 ^1H - ^{13}C HMBC and ^1H - ^{13}C HSQC-TOCSY analysis of compound **6**. Glycans A, B, C, and D represent Glc, Gal, GalNAc, and Neu5Ac, respectively. The number indicates the position of the carbon or proton atom. **Neu5Ac- α (2,6)-Gal linkage**. A cross signal for the anomeric carbon of sialic acid (D2) and one of the B6 protons of Gal (B6) was observed (D2/B6). This suggests that the glycosidic linkage is a 2 \rightarrow 6 (Gal) linkage. There are also cross signals for the B5 carbon and one of the B6 protons of Gal (B5/B6) and the B5 carbon and the anomeric proton atom of Gal (B5/B1). These signals reveal that glycosidic linkage is a 2 \rightarrow 6 linkage between Neu5Ac and Gal.

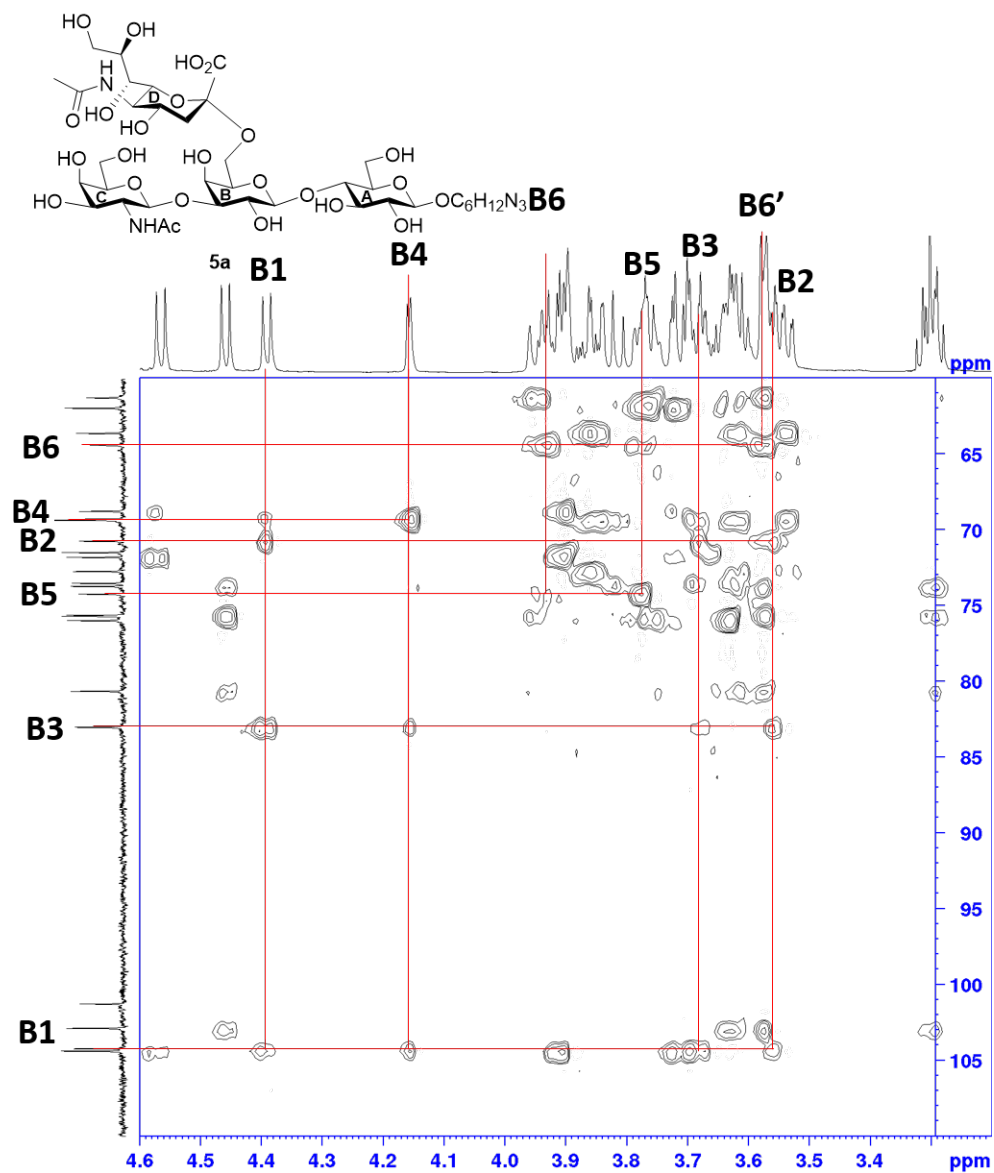


Fig. S9 Full carbon atom assignment of inner Gal from the ^1H - ^{13}C HSQC-TOCSY spectrum of **6**. The spectrum shows that the downfield shift signal of the 6-position carbon is on the Gal carbon rather than GalNAc carbon.

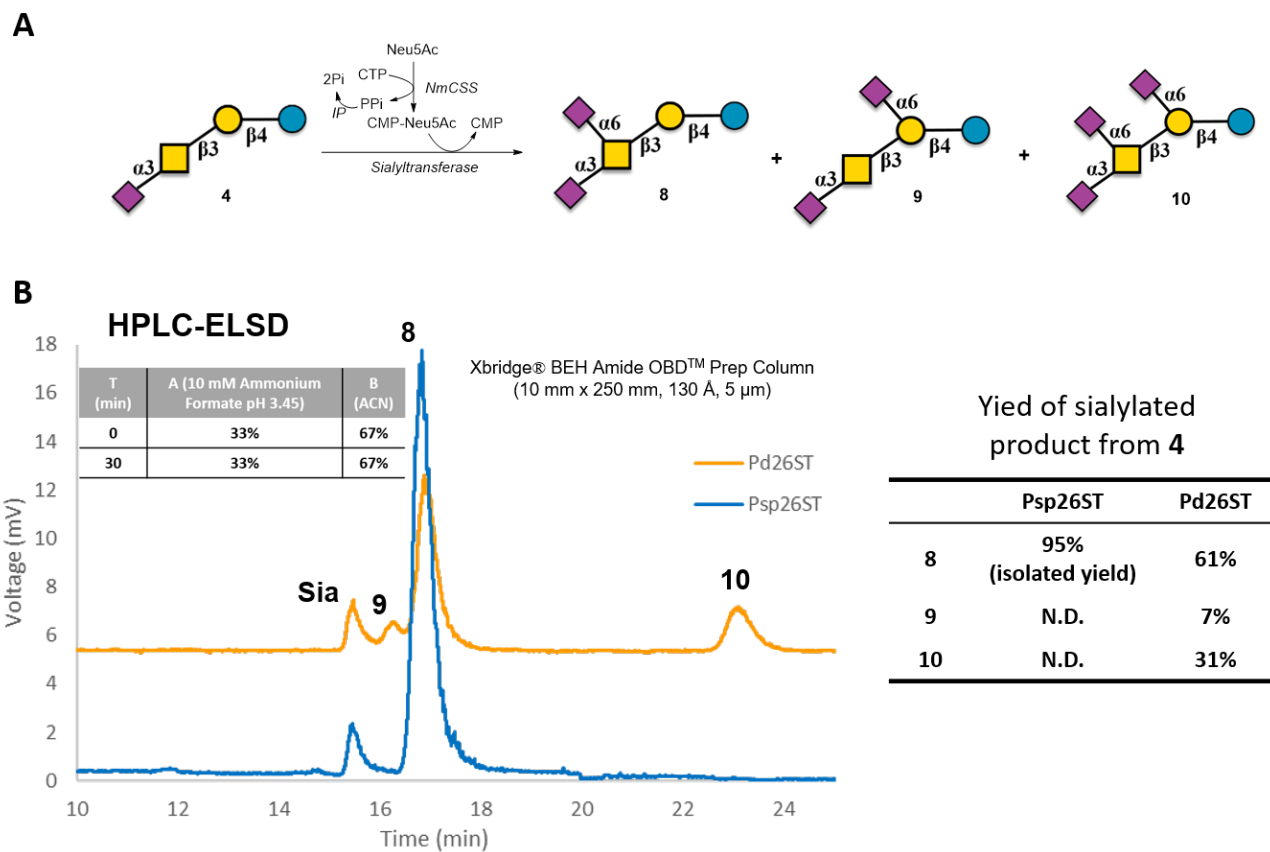
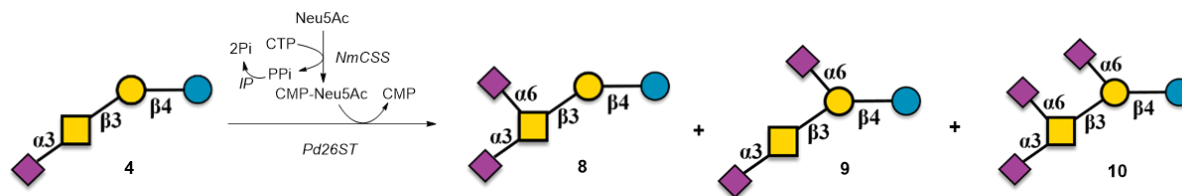


Fig. S10 Study of $\alpha(2,6)$ sialylation of compound **4**. HPLC-ELSD analysis of the sialylation of compound **4** by two different $\alpha(2,6)$ STs with 1.5 equivalents of sialic acid. A. Schematic of the enzymatic sialylation of compound **4** by Psp2,6ST and Pd2,6ST. B. HPLC-ELSD analysis of the crude sialylated products and corresponding yields.



Sia	Pd26ST		
	0.5 eq.	0.8 eq.	1.0 eq.
S.M. (4)	41.6%	21.1%	7.0%
8	47.5%	61.2%	68.9%
9	10.1%	10.5%	15.5%
10	0.7%	7.2%	8.6%

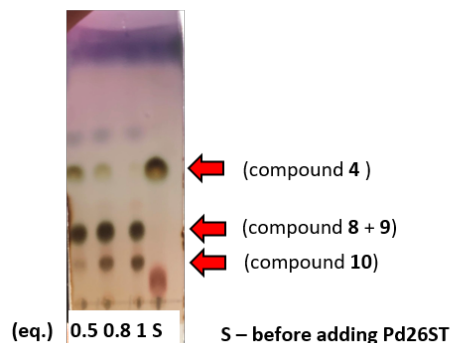


Fig. S11 Analysis of the sialylation of compound **4** by Pd_{2,6}ST with reduced equivalents of sialic acid (0.5, 0.8, 1.0). The reaction was monitored by TLC (*n*-PrOH/H₂O/AcOH = 6/2/1 (v/v/v)). After the CMP-sialic acid was consumed, the reaction was quenched by the addition of the same reaction volume of acetonitrile. The reaction solution was centrifuged (10,000 × *g*, 5 min) to remove enzymes and insoluble precipitates. The supernatant was filtered through a 0.45- μ m PVDF filter (Millipore) followed by injection the residues into an HPLC-ELSD instrument. The glycan peaks were integrated, and the content of each glycan was calculated as shown in the table.

When using less than one equivalent of sialic acid, the $\alpha(2,6)$ sialylation of compound **4** by Pd_{2,6}ST produced regioisomers **8** and **9** (identified by HPLC-ELSD). This result suggests that Pd_{2,6}ST does not have selectivity between GalNAc and Gal when compound **4** was used as the acceptor for sialylation.

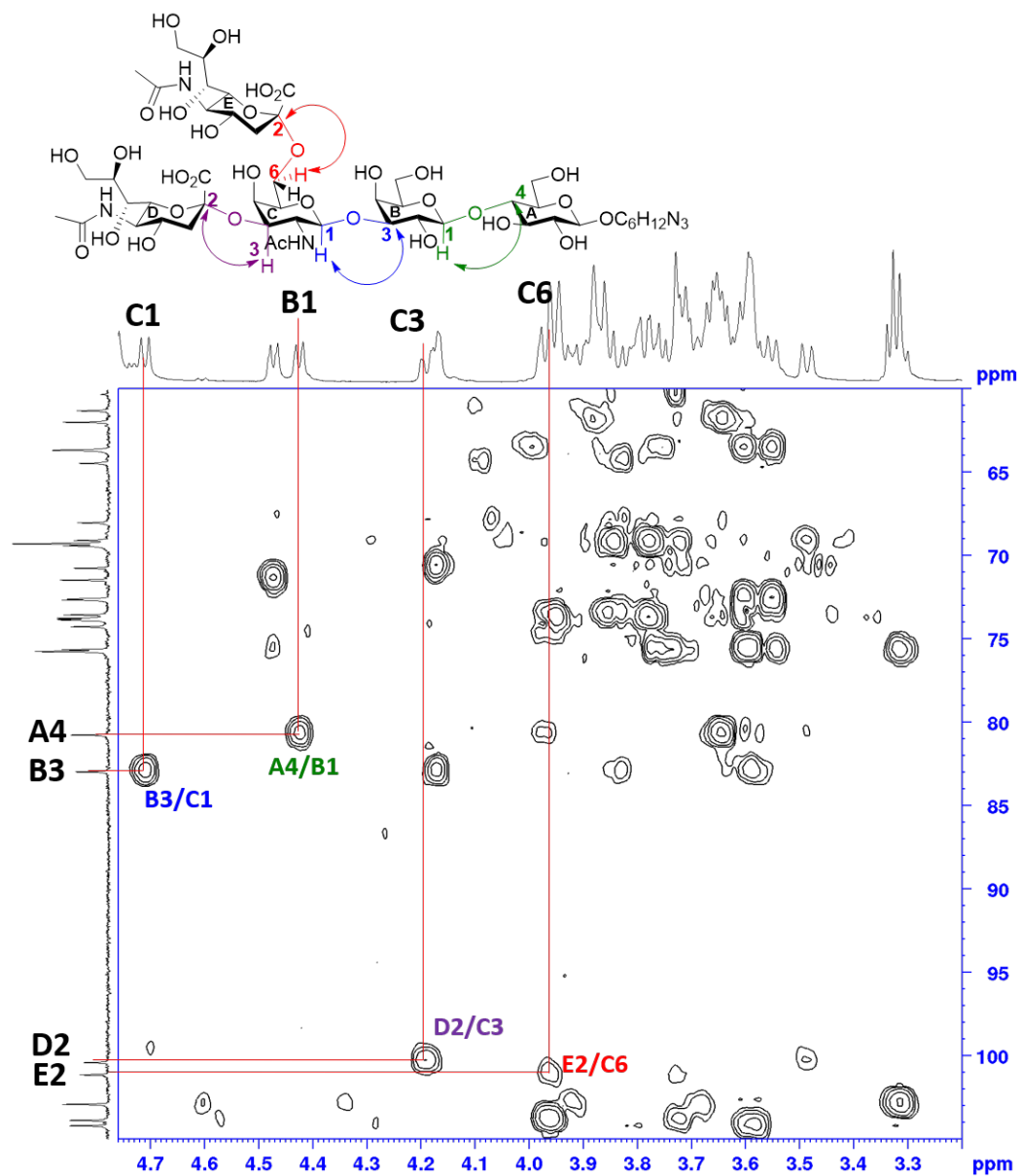


Fig. S12 ^1H - ^{13}C HMBC analysis of compound **8**. Glycans A, B, C, D, and E represent Glc, Gal, GalNAc, α 3Neu5Ac, and α 6Neu5Ac, respectively. The number indicates the position of the carbon or proton atom. **Neu5Ac- α (2,6)-GalNAc linkage.** A cross signal for the anomeric carbon of α 6Neu5Ac (D2) and the one of the C6 protons of GalNAc (C6) was observed (D2/C6). This suggests that the glycosidic linkage is a 2 \rightarrow 6 (GalNAc) linkage.

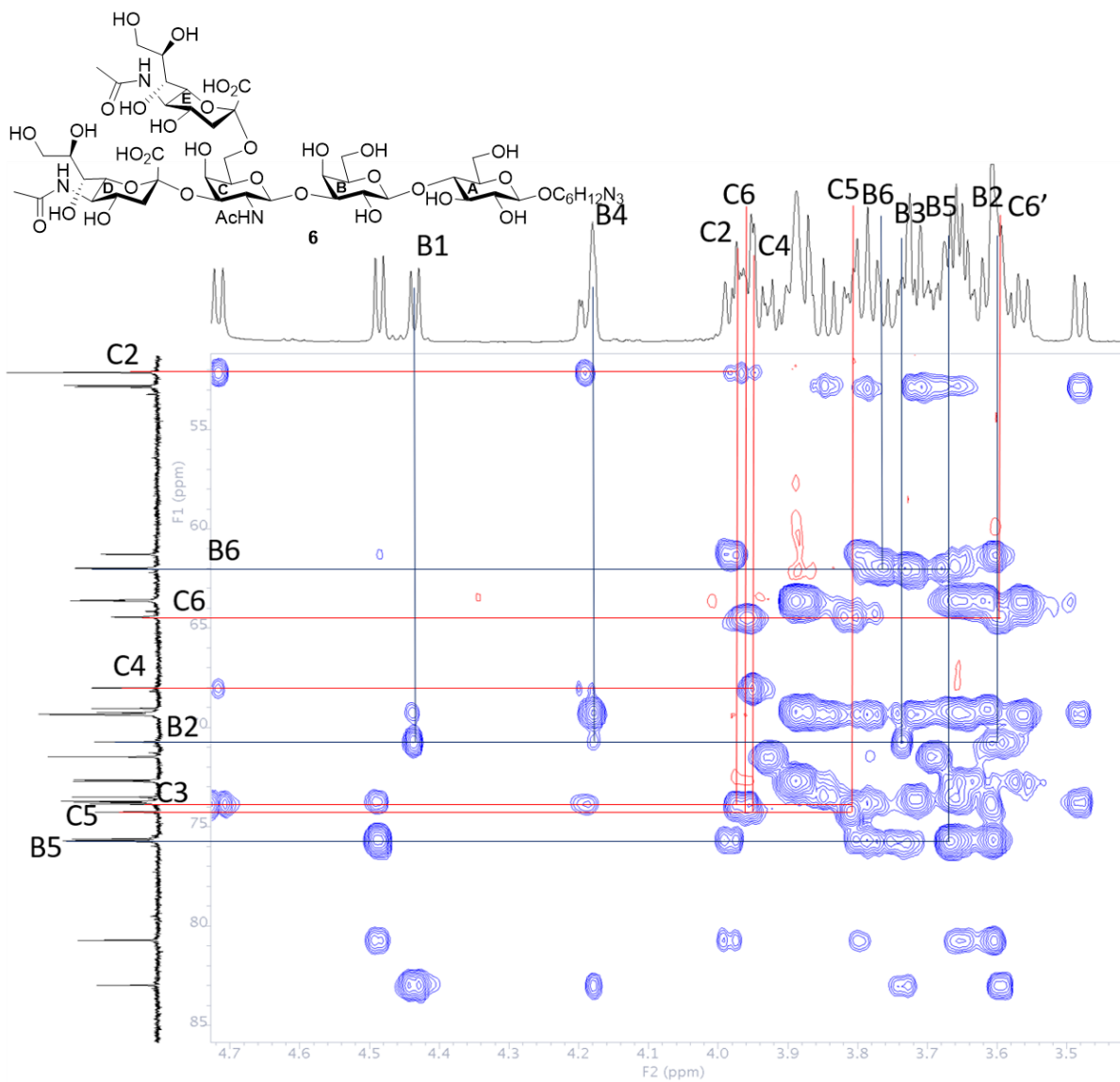


Fig. S13 Crucial carbon atom assignments of Gal and GalNAc on **8** by ^1H - ^{13}C HSQC-TOCSY. The results reveal that the downfield shifted signal of the 6-position carbon is on GalNAc rather than on Gal.

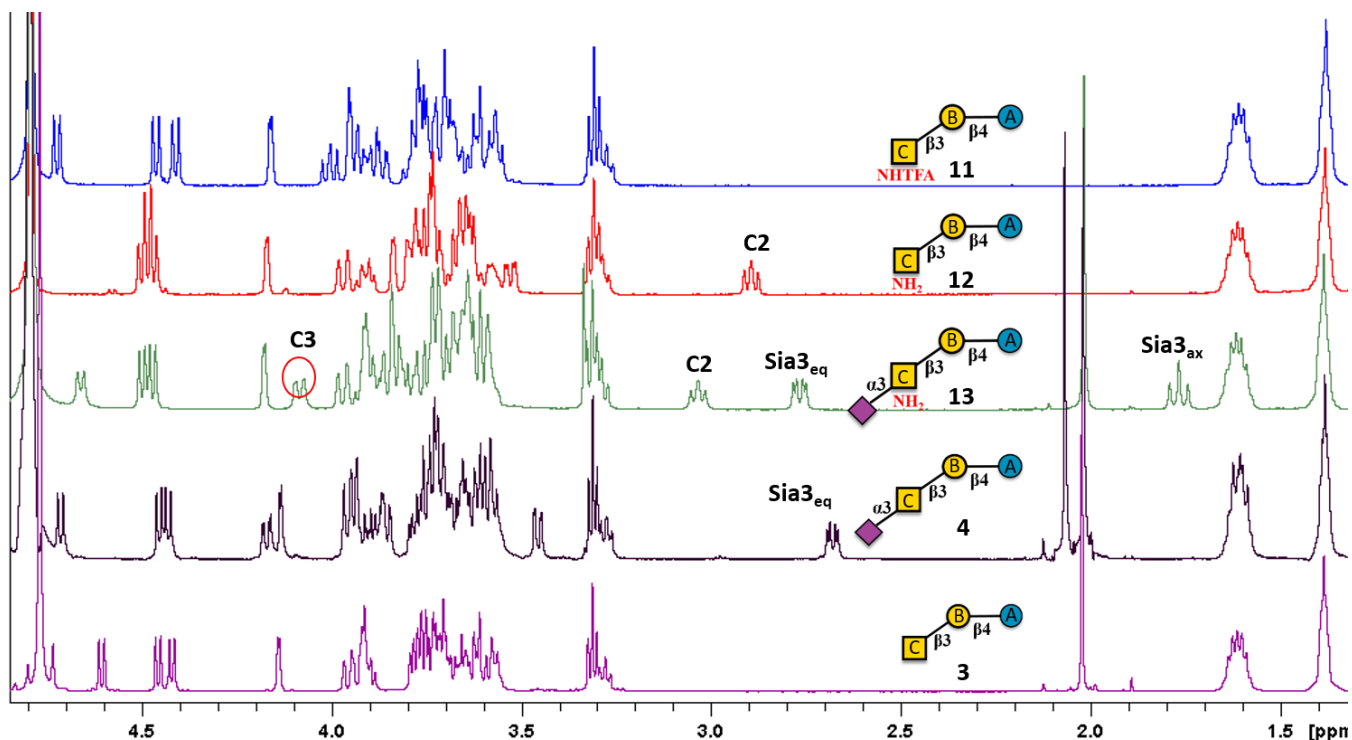


Fig. S14 $\alpha(2,3)$ sialylation on GalNH₂ as evidenced by ¹H NMR spectroscopy. Glycans A, B, and C represent Glc, Gal, and GalNAc, respectively. The number indicates the position of the carbon or proton atom. The C3-H on Neu5Ac is represented as Sia3_{eq} (equatorial) and Sia3_{ax} (axial). After deprotection of NHTFA, the C2-H signal of GalNH₂ (C2) shifted upfield to 2.89 ppm (**12**). In the green color spectrum (**13**), the C2 and Sia3_{eq} signals confirmed that the sialylation of GalNH₂ was successful. In addition, the C3-H signal of GalNH₂ (C3) was shifted downfield, as shown by the red circle. After *N*-acetylation of **13**, the ¹H NMR spectrum of the purified product was identical to that of **4**.

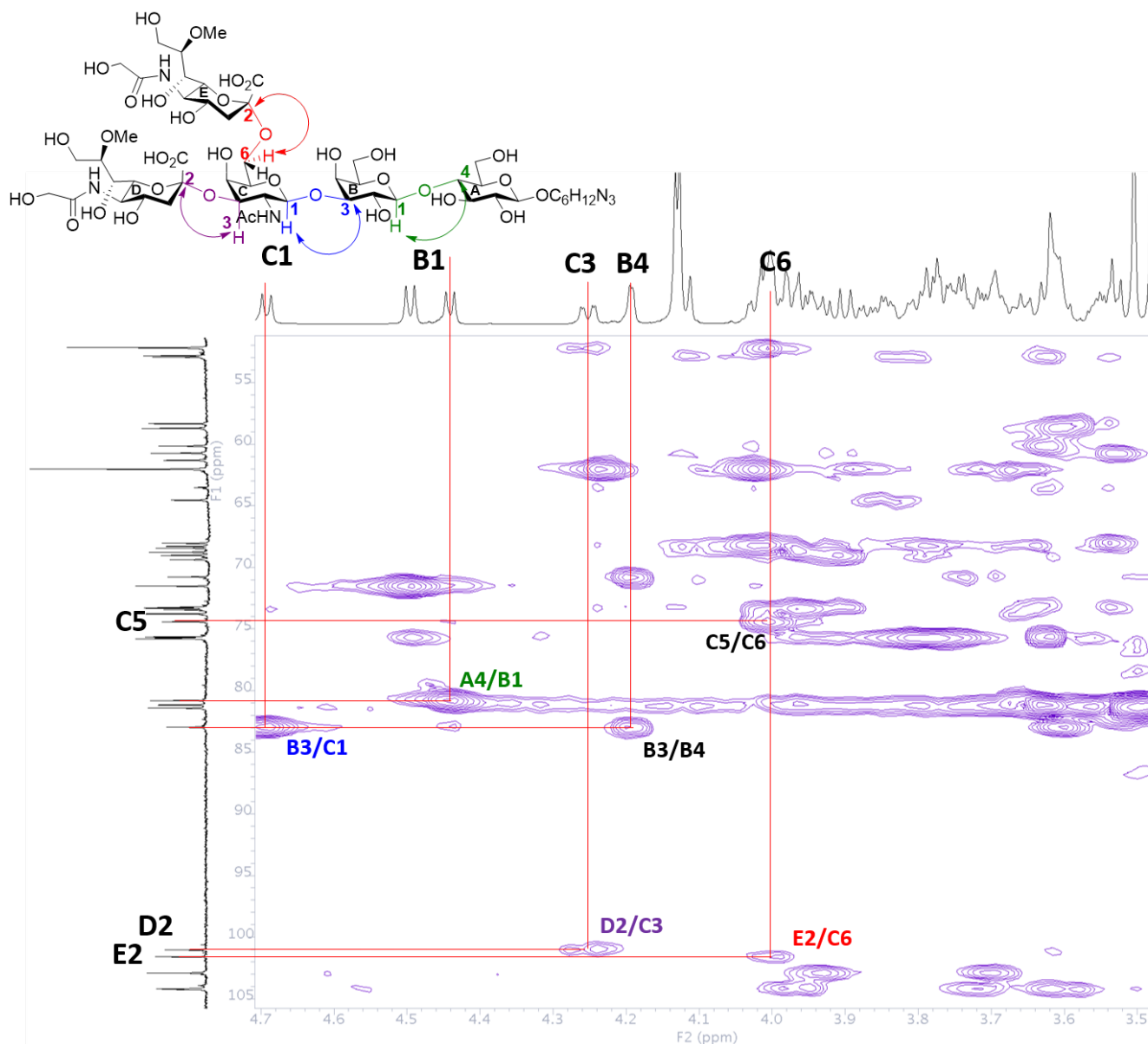


Fig. S15 ^1H - ^{13}C HMBC analysis of compound **1**. Glycans A, B, C, D, and E represent Glc, Gal, GalNAc, $\alpha 3\text{Neu}5\text{Gc}8\text{Me}$, and $\alpha 6\text{Neu}5\text{Gc}8\text{Me}$, respectively. The number indicates the position of the carbon or proton atom. **Neu5Gc8Me- α (2,6)-GalNAc linkage**. A cross signal for the anomeric carbon of $\alpha 6\text{Neu}5\text{Gc}8\text{Me}$ (E2) and one of the C6 protons of GalNAc (C6) was observed (E2/C6). This suggests that the glycosidic linkage is a 2 \rightarrow 6 (GalNAc) linkage.

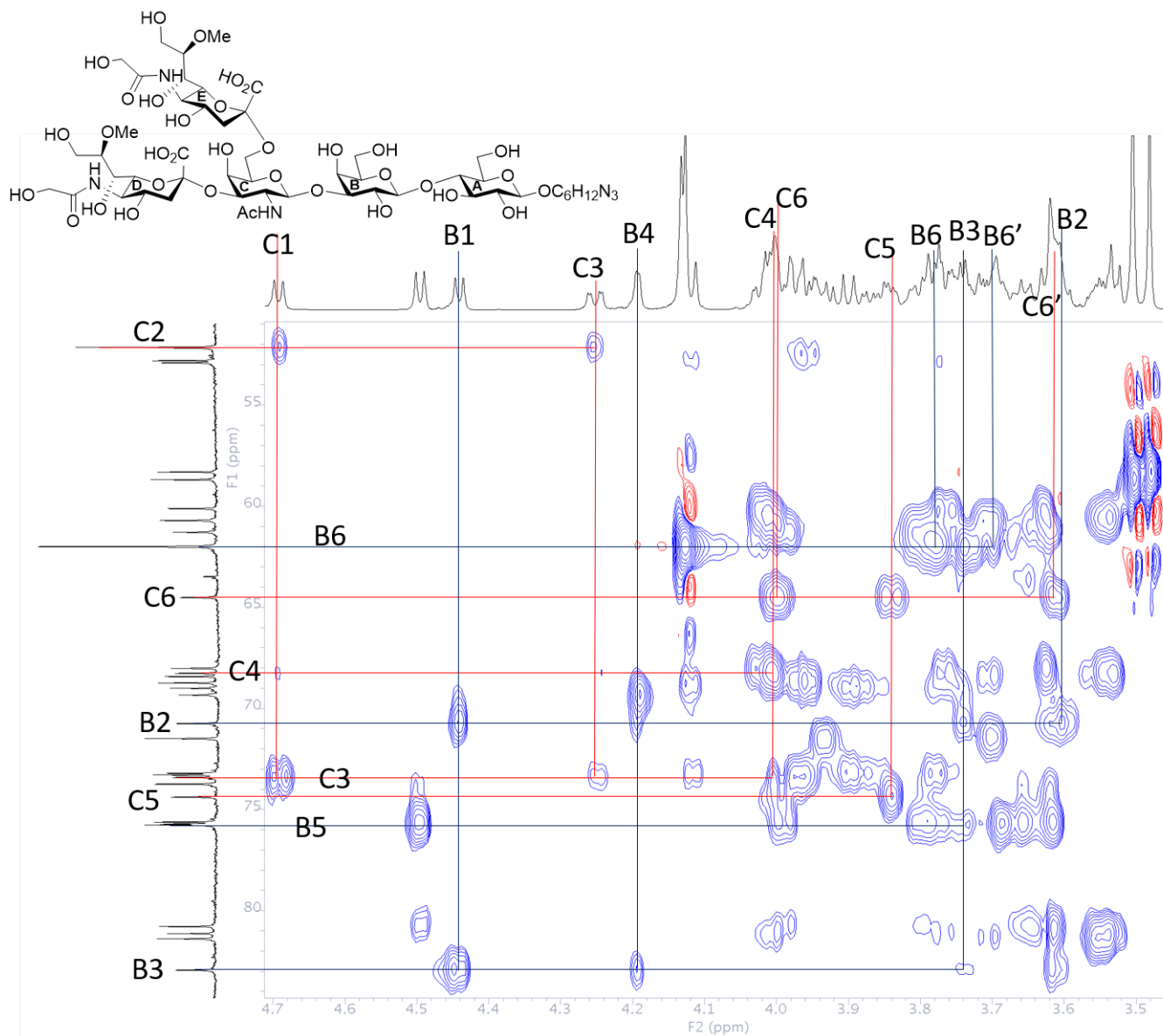


Fig. S16 Crucial carbon atom assignments of Gal and GalNAc in **1** by ^1H - ^{13}C HSQC-TOCSY. The results reveal that the downfield shifted signal of the 6-position carbon is on GalNAc rather than on Gal.

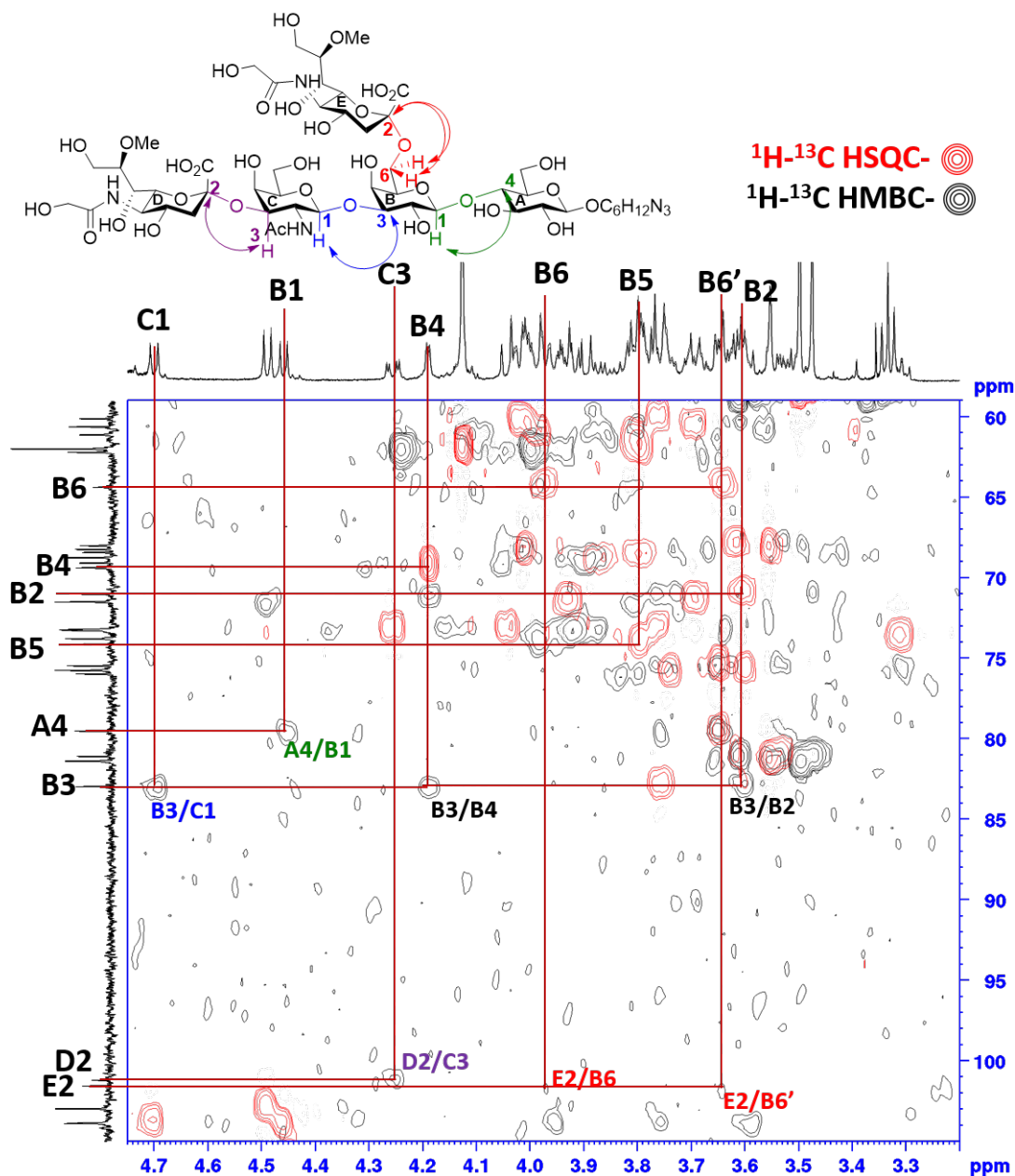


Fig. S17 ^1H - ^{13}C HSQC and ^1H - ^{13}C HMBC analysis of compound **1a**. The red and black contours represent the cross signals on the HSQC and HMBC spectra, respectively. Glycans A, B, C, D, and E represent Glc, Gal, GalNAc, $\alpha 3\text{Neu5Gc8Me}$, and $\alpha 6'\text{Neu5Gc8Me}$, respectively. The number indicates the position of the carbon or proton atom. **Neu5Gc8Me- α (2,6)-Gal linkage.** Cross signals for the anomeric carbon of $\alpha 6'\text{Neu5Gc8Me}$ (E2) and both of the 6-position proton atoms of Gal (B6 and B6') were observed (E2/B6 and E2/B6'). This result suggests that the glycosidic linkage is a 2 \rightarrow 6 linkage between Neu5Gc8Me and Gal.

General Procedure

General Enzymatic Synthetic Procedure 1: Sequential One Pot Enzymatic $\alpha(2,3)$ - or $\alpha(2,6)$ -Sialylation

The pH of a buffer solution (100 mM Tris-HCl, pH 8.5) containing 15 mM of Neu5Ac or Neu5Gc8Me, 30 mM of CTP, and 20 mM of MgCl₂ was adjusted to 8.5 by adding 2N NaOH_(aq.). Then, 0.2-0.8 mg/mL of CSS and 0.1-0.3 mg/mL of IP were added to the above solution. The resulting mixture was incubated at 37 °C with agitation at 600 rpm for 0.5-2 h and the formation of CMP-Sia was monitored by TLC analysis (*n*-PrOH/ H₂O/ AcOH = 6/2/1 (v/v/v), R_f = 0.06). After the completion of the reaction as indicated by the disappearance of all of Neu5Ac or a half of Neu5Gc8Me on TLC, 5-10 mM of acceptor was added and the pH of the reaction solution was adjusted (pH 8.0 for Psp2,6ST and pH 8.5 for PmST1, PmST3, Cst-I, or Pd2,6ST). Finally, 0.4-0.9 mg/mL of Psp2,6ST, 2.2 mg/ml of PmST1, 1.1 mg/ml of PmST3, 0.9-1.1 mg/ml of Cst-I, or 0.2-0.6 mg/ml of Pd2,6ST was added and the solution was incubated at 25 °C for Psp2,6ST or 37 °C for other four sialyltransferases. Furthermore, 1.9 U/mL of alkaline phosphatase was added for Cst-I or PmST3 to decompose potentially inhibitory CMP. More enzymes were added if necessary. The reaction was monitored by TLC analysis (*n*-PrOH/ H₂O/ AcOH = 6/2/1 (v/v/v)) and stained with *p*-anisaldehyde stain.

General Enzymatic Synthetic Procedure 2: for Sequential One Pot Enzymatic Assembly of $\beta(1,3)$ -GalNAc or GalNTFA

The pH of a buffer solution (100 mM HEPES, pH 8.5) containing 20 mM of GalNAc or GalNTFA, 30 mM of ATP, 30 mM of UTP, and 20 mM of MgCl₂ was adjusted to 7.5 by adding 2N NaOH_(aq.). Then, 0.1-0.2 mg/mL of NahK, 1.4 mg/mL of GlmU (only for GalNAc) or 0.03-0.1 mg/mL of AGX1, and 0.1-0.3 mg/mL of IP were added to the above solution. The resulting mixture was incubated at 37 °C with agitation at 600 rpm for 7 h and the formation of UDP-GalNAc or UDP-GalNTFA was monitored by TLC analysis (*n*-PrOH/ H₂O/ AcOH = 6/2/1 (v/v/v), R_f = 0.15 or 0.43). After the completion of the reaction as indicated by the disappearance of GalNAc-1-P on TLC, 10 mM of acceptor was added and the pH of the reaction solution was adjusted to pH 7.8 by addition of 2N NaOH_(aq.). Then, 15 mM of MnCl₂ and 0.6-1.4 mg/mL of LgtA were added and the solution was incubated at 37 °C. More enzymes were added if necessary. The reaction was monitored by TLC analysis (*n*-PrOH/ H₂O/ AcOH = 6/2/1 (v/v/v)) and stained with *p*-anisaldehyde stain.

General Procedure for Deprotection of NHTFA (*N*-trifluoroacetyl) Group

The pH of a H₂O solution containing NHTFA protected compound was adjusted to 12.0 by adding 2N NaOH_(aq.). The resulting mixture was incubated at 37 °C with agitation at 600 rpm for 1 h and the reaction was monitored by TLC analysis (*n*-PrOH/ H₂O/ AcOH = 6/2/1 (v/v/v)). After the completion of the reaction as indicated by the disappearance of starting material on TLC. The reaction was then neutralized with 4 N HCl, concentrated and purified by a C18 reverse-phase silica column (Sep-Pak Vac C18 cartridge 5 g, 55-105 μ m, Waters) using a solution followed by a gradient of 0-80% MeOH in H₂O as eluent.

General Procedure for *N*-Acetylation

Thirty mM of amine (1.0 equiv.) containing compound was dissolved in H₂O followed by adding solid NaHCO₃ (10.0 equiv.) and Ac₂O (10.0 equiv.). After being sonicated for 2 min, the reaction mixture was incubated at 37 °C for 1 h. After the completion of the reaction as indicated by the disappearance of starting material on TLC. The resulting solution was purified by size-exclusion column using H₂O as eluent (BioGel P2 gel packed in a column 1.6 cm x 50 cm, Biorad).

General Purification Procedure 1

If there is no regioisomer after enzymatic sialylation, the reaction mixtures are suitable for this purification procedure. The enzymatic reaction was quenched by addition of the same reaction volume of EtOH. The reaction solution was centrifuged (10,000 x g, 10 min) to remove enzymes and insoluble precipitates. The supernatant was collected, filtered (0.45- μ m PVDF filter; Millipore), and then concentrated. The resulting residue was purified by a C18 reverse-phase silica column (Sep-Pak Vac C18 cartridge 5 g, 55-105 μ m, Waters) using a solution followed by a gradient of 0-100% MeOH in H₂O as eluent. The fractions containing products were collected and directly loaded on to anion exchange chromatography (DEAE Sepharose Fast Flow resin, GE Healthcare). The column was washed with water (10 mL) followed by the elution with a gradient of aqueous sodium chloride (10-100 mM, increase 10 mM for each gradient and 10 mL for each gradient). The fractions containing products were further purified by size-exclusion column using H₂O as eluent (BioGel P2 gel packed in a column 1.6 cm x 50 cm, Biorad).

General Purification Procedure 2

If there are regioisomers after enzymatic sialylation, the reaction mixtures are suitable for this purification procedure. Follow the same procedure as described in general purification procedure 1, but the residues after C-18 reverse-phase silica column were pooled and purified by Xbridge® BEH Amide OBD™ Prep Column (10 x 250 mm, 130Å, 5 μ m) with a flow rate of 2 mL/min. HPLC purification was monitored by ELSD (evaporative light scattering detector), and glycans-containing fractions were analyzed by TLC. After pooled and concentrated, further purification by size-exclusion column using H₂O as eluent (BioGel P2 gel packed in a column 1.6 cm x 50 cm, Biorad).

HPLC mobile phase condition for compounds **1** and **1a**:

Using an elution of 65.5% acetonitrile in water containing 100 mM ammonium formate (pH = 3.45) for 30 mins.

HPLC mobile phase condition for compounds **5** and **6**:

Using an elution of 74% acetonitrile in water containing 10 mM ammonium formate (pH = 3.45) for 30 mins, 74%-66% for 8 mins, and 66% for 12 mins.

HPLC mobile phase condition for compounds **8** and **9**:

Using an elution of 66% acetonitrile in water containing 10 mM ammonium formate (pH = 3.45) for 30

mins.

HPLC mobile phase condition for further purification of compound **11**:

Using an elution of 86.5-82% acetonitrile in water containing 10 mM ammonium formate (pH = 3.45) for 25 mins.

HPLC mobile phase condition for purification of compound **13a** and its $\alpha(2,6)$ -sialylated regioisomer:

Using an elution of 74% acetonitrile in water containing 10 mM ammonium formate (pH = 3.45) for 30 mins.

HPLC mobile phase condition for purification of compounds **15a** and its $\alpha(2,6)$ -sialylated regioisomers:

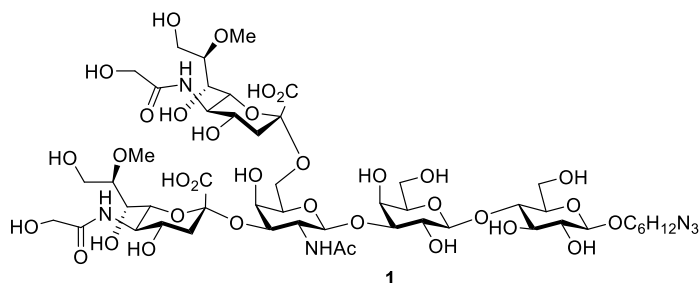
Using an elution of 76%-70% acetonitrile in water containing 100 mM ammonium formate (pH = 3.45) for 30 mins.

General Purification Procedure 3

Follow the same procedure as described in general purification procedure 1, but the residues after C-18 reverse-phase silica column were directly purified by size-exclusion column using H₂O as eluent (BioGel P2 gel packed in a column 1.6 cm x 50 cm, Biorad).

Synthetic procedures and characterization of new compounds

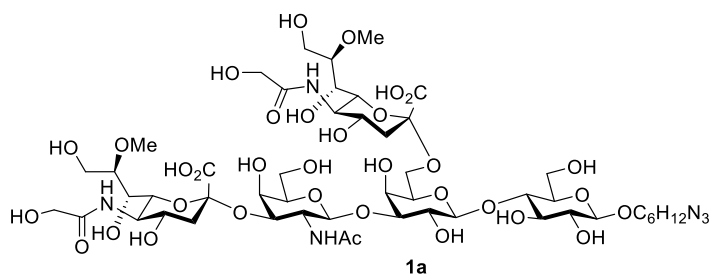
Compound **2**,¹⁰ and **2a**,¹¹ and Neu5Gc8Me¹² were reported previously.



GAA7-C₆H₁₂N₃ (Neu5Gc8Me α 2,3(Neu5Gc8Me α 2,6)-GalNAc β 1,3-Gal β 1,4-Glc β C₆H₁₂N₃ (**1**).

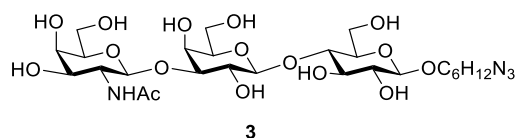
Sialylation catalyzed by Psp2,6ST. Compound **4a** (8.4 mg, 8.5 μ mol) and Neu5Gc8Me (8.6 mg, 25.3 μ mol) were used as acceptor and donor, respectively, by following the general enzymatic synthetic procedure 1. After being shaken for 13 h, the reaction was quenched and purified by following general purification procedure 1. After purification, the product was lyophilized to give compound **1** in 77.3% yield (8.6 mg).

Sialylation catalyzed by Pd2,6ST. Compound **4a** (8.6 mg, 8.7 μ mol) and Neu5Gc8Me (8.8 mg, 25.9 μ mol) were used as acceptor and donor, respectively, by following the general enzymatic synthetic procedure 1. After being shaken for 2 h, the reaction was quenched and purified by following general purification procedure 2. After purification, the product was lyophilized to give compound **1** in 29.8% yield (3.4 mg). $R_f = 0.21$ (*n*-PrOH/ H₂O/ AcOH = 6/2/1 (v/v/v)); ¹H NMR (600 MHz, D₂O) δ 4.68 (d, *J* = 8.5 Hz, 1H), 4.48 (d, *J* = 8.0 Hz, 1H), 4.43 (d, *J* = 7.9 Hz, 1H), 4.24 (dd, *J* = 2.9, 11.0 Hz, 1H), 4.18 (d, *J* = 3.2 Hz, 1H), 4.14-4.09 (m, 5H), 4.02-3.58 (m, 28H), 3.54-3.51 (m, 2H), 3.49 (s, 3H), 3.47 (s, 3H), 3.34-3.30 (m, 3H), 2.66-2.56 (m, 2H), 2.07 (s, 3H), 1.70 (t, *J* = 12.2 Hz, 1H), 1.67-1.59 (m, 5H), 1.43-1.36 (m, 4H); ¹³C NMR (150 MHz, D₂O) δ 176.77, 176.63, 175.92, 174.82, 174.62, 104.18, 104.13, 102.85, 101.54, 101.00, 82.91, 81.35, 81.09, 80.75, 75.72, 75.66, 75.58, 74.35, 73.70, 73.38, 73.24, 73.17, 71.45, 70.71, 69.30, 68.97, 68.70, 68.39, 68.24, 67.98, 64.48, 61.96(x3), 61.25, 60.66, 60.08, 58.65, 58.27, 52.87, 52.77, 52.10(x2), 41.22, 40.48, 29.54, 28.83, 26.62, 25.56, 23.30; HRMS (ESI) *m/z* calcd for C₅₀H₈₃N₆O₃₄ [M-H]⁻ 1311.4956, found 1311.4930.

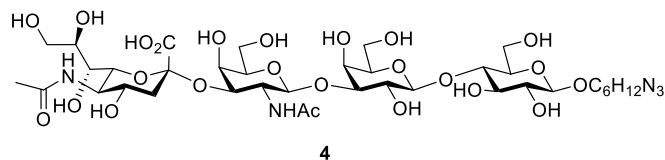


Neu5Gc8Me α 2,3-GalNAc β 1,3(Neu5Gc8Me α 2,6)-Gal β 1,4-Glc β C₆H₁₂N₃ (1a**).** The compound **1a** was synthesized from **4a** by following the same procedure as mentioned in the synthesis of compound **1**. After purification, the product was lyophilized to give compound **1a** in 20.2% yield (2.3 mg). $R_f = 0.21$ (*n*-

PrOH/ H₂O/ AcOH = 6/2/1 (v/v/v)); ¹H NMR (600 MHz, D₂O) δ 4.70 (d, *J* = 8.6 Hz, 1H), 4.49 (d, *J* = 8.1 Hz, 1H), 4.46 (d, *J* = 7.9 Hz, 1H), 4.26 (dd, *J* = 3.0, 11.0 Hz, 1H), 4.19 (d, *J* = 3.2 Hz, 1H), 4.13 (s, 4H), 4.05-3.83 (m, 12H), 3.82-3.73 (m, 8H), 3.73-3.58 (m, 9H), 3.58-3.47 (m, 9H), 3.35-3.30 (m, 3H), 2.65 (dd, *J* = 4.5, 12.3 Hz, 1H), 2.59 (dd, *J* = 4.2, 12.1 Hz, 1H), 2.08 (s, 3H), 1.71-1.59 (m, 6H), 1.45-1.37 (m, 4H); ¹³C NMR (150 MHz, D₂O) δ 176.74, 176.68, 175.90, 174.69, 174.53, 103.88, 103.84, 102.96, 101.54, 101.19, 82.94, 81.36, 81.07, 79.49, 75.94, 75.72, 75.44, 74.09, 73.76, 73.26, 73.21, 73.17, 71.47, 70.98, 69.35, 69.05, 68.72, 68.39, 68.20, 67.97, 64.35, 62.17, 61.96(x2), 61.08, 60.58, 60.09, 58.59, 58.27, 52.85(x2), 52.09(x2), 41.20, 40.28, 29.53, 28.82, 26.61, 25.55, 23.29; HRMS (ESI) *m/z* calcd for C₅₀H₈₃N₆O₃₄ [M-H]⁻ 1311.4956, found 1311.4912.



GalNAcβ1,3-Galβ1,4-GlcβC₆H₁₂N₃ (3). Compound **2** (70.0 mg, 149.7 μmol) and GalNAc (50.0 mg, 226.0 μmol) were used as acceptor and donor, respectively, by following the general enzymatic synthetic procedure 2. After being shaken for 30 h, the reaction was quenched and purified by following general purification procedure 3. After purification, the product was lyophilized to give compound **3** in 97.6% yield (98.0 mg). *R_f* = 0.63 (*n*-PrOH/ H₂O/ AcOH = 6/2/1 (v/v/v)); ¹H NMR (600 MHz, D₂O) δ 4.63 (d, *J* = 8.5 Hz, 1H), 4.48 (d, *J* = 8.0 Hz, 1H), 4.44 (d, *J* = 7.9 Hz, 1H), 4.16 (d, *J* = 3.0 Hz, 1H), 4.01-3.90 (m, 4H), 3.82-3.58 (m, 14H), 3.36-3.27 (m, 3H), 2.05 (s, 3H), 1.68-1.60 (m, 4H), 1.44-1.38 (m, 4H); ¹³C NMR (150 MHz, D₂O) δ 176.06, 104.26, 103.93, 103.01, 82.73, 79.39, 75.95, 75.85, 75.73, 75.43, 73.81, 71.73, 71.47, 71.05, 69.45, 68.75, 61.96, 61.92, 61.09, 53.56, 52.10, 29.56, 28.85, 26.63, 25.57, 23.19; HRMS (ESI) *m/z* calcd for C₂₆H₄₆N₄O₁₆Na [M+Na]⁺ 693.2801, found 693.2818.



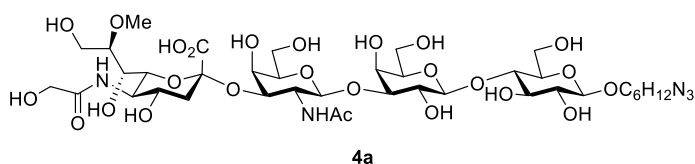
Neu5Acα2,3-GalNAcβ1,3-Galβ1,4-GlcβC₆H₁₂N₃ (4).

The sialylation catalyzed by Cst-I. Compound **3** (70.0 mg, 104.4 μmol) and Neu5Ac (161.4 mg, 521.9 μmol) were used as acceptor and donor, respectively, by following the general enzymatic synthetic procedure 1. After being shaken for 38 h, the reaction was quenched and purified by following general purification procedure 1. After purification, the product was lyophilized to give compound **4** in 70.2% yield (70.5 mg).

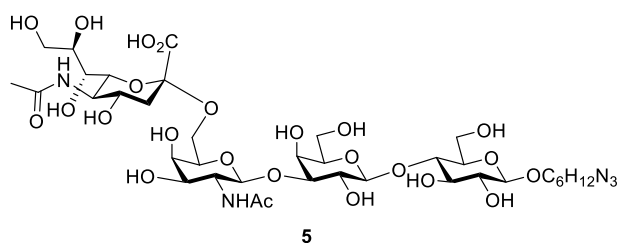
The sialylation catalyzed by PmST3. Compound **3** (7.2 mg, 10.7 μmol) was used as acceptor for sialylation by following the procedure described in the sialylation catalyzed by Cst-I, but the Cst-I enzyme was replaced with PmST3 in 1.1 mg/mL. After purification, the product was lyophilized to give compound **4** in 76.4% yield (7.9 mg).

The chemical *N*-acetylation of compound 13. Compound **13** (11.8 mg, 12.8 μmol), NaHCO₃ (6.7 mg,

80.0 μmol), and Ac_2O (7.6 μL , 80.0 μmol) were used by following the general *N*-acetylation procedure. Fractions containing the desired product were pooled and lyophilized to give compound **4** in 92.5% (11.4 mg). $R_f = 0.52$ (*n*-PrOH/ H_2O / AcOH = 6/2/1 (v/v/v)); ^1H NMR (600 MHz, D_2O) δ 4.71 (d, $J = 8.5$ Hz, 1H), 4.45 (d, $J = 8.0$ Hz, 1H), 4.42 (d, $J = 7.9$ Hz, 1H), 4.17 (dd, $J = 2.9, 10.9$ Hz, 1H), 4.13 (d, $J = 3.2$ Hz, 1H), 3.99-3.83 (m, 6H), 3.79-3.56 (m, 17H), 3.45 (dd, $J = 1.7, 10.4$ Hz, 1H), 3.34-3.25 (m, 3H), 2.67 (dd, $J = 4.6, 12.5$ Hz, 1H), 2.06 (s, 3H), 2.01 (s, 3H), 1.66-1.56 (m, 5H), 1.42-1.32 (m, 4H); ^{13}C NMR (150 MHz, D_2O) δ 176.16, 175.86, 175.08, 103.88, 103.79, 103.01, 100.44, 82.75, 79.40, 75.86, 75.72(x2), 75.43, 73.78(x3), 72.64, 71.46, 70.98, 69.39, 69.25, 69.04, 67.94, 63.58, 61.98, 61.94, 61.06, 52.88, 52.09(x2), 49.43, 49.29, 49.14, 49.00, 48.85, 48.71, 48.56, 40.69, 29.57, 28.86, 26.64, 25.58, 23.37, 22.93; HRMS (ESI) m/z calcd for $\text{C}_{37}\text{H}_{62}\text{N}_5\text{O}_{24}$ $[\text{M}-\text{H}]^-$ 960.3790, found 960.3767.



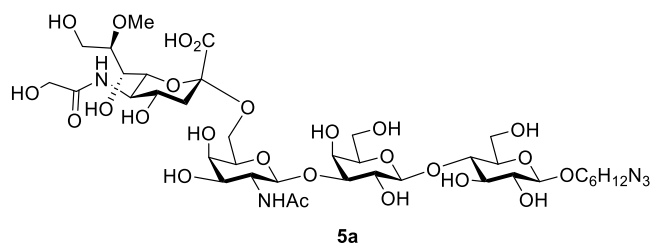
Neu5Gc8Mea2,3-GalNAc β 1,3-Gal β 1,4-Glc β C $_6$ H $_{12}$ N $_3$ (4a). Compound **13a** (10.0 mg, 10.5 μmol), NaHCO_3 (8.8 mg, 105 μmol), and Ac_2O (9.9 μL , 105 μmol) were used by following the general *N*-acetylation procedure. Fractions containing the desired product were pooled and lyophilized to give compound **4a** in 91.0% (9.5mg). $R_f = 0.36$ (*n*-PrOH/ H_2O / AcOH = 6/2/1 (v/v/v)); ^1H NMR (600 MHz, D_2O) δ 4.69 (d, $J = 8.5$ Hz, 1H), 4.46 (d, $J = 8.1$ Hz, 1H), 4.43 (d, $J = 7.9$ Hz, 1H), 4.24 (d, $J = 2.9, 11.0$ Hz, 1H), 4.14 (d, $J = 3.2$ Hz, 1H), 4.11 (s, 2H), 4.01-3.85 (m, 7H), 3.80-3.56 (m, 16H), 3.53-3.50 (m, 1H), 3.46 (s, 3H), 3.33-3.27 (m, 3H), 2.59 (dd, $J = 4.0, 12.2$ Hz, 1H), 2.07 (s, 3H), 1.66-1.60 (m, 5H), 1.41-1.38 (m, 4H); ^{13}C NMR (150 MHz, D_2O) δ 176.71, 175.84, 174.52, 104.01, 103.91, 103.01, 100.78, 82.66, 81.30, 79.56, 75.85, 75.71, 75.68, 75.45, 73.80, 73.32(x2), 71.42, 71.02, 69.42, 68.84, 68.19, 68.09, 61.98(x2), 61.90, 61.14, 60.30, 58.33, 52.91, 52.13, 52.10, 40.57, 29.54, 28.81, 26.60, 25.55, 23.29; HRMS (ESI) m/z calcd for $\text{C}_{38}\text{H}_{65}\text{N}_5\text{O}_{25}\text{Na}$ $[\text{M}+\text{Na}]^+$ 1014.3866, found 1014.3860.



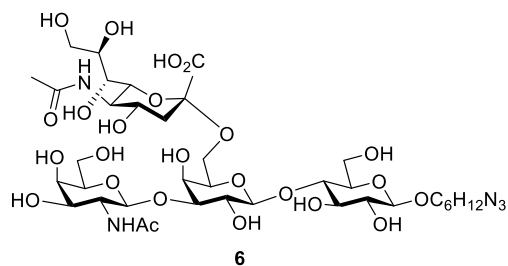
Neu5Ac α 2,6-GalNAc β 1,3-Gal β 1,4-Glc β C $_6$ H $_{12}$ N $_3$ (5).

The sialylation catalyzed by PmST1. Compound **3** (11.0 mg, 16.4 μmol) and Neu5Ac (25.4 mg, 82.0 μmol) were used as acceptor and donor, respectively, by following the general enzymatic synthetic procedure 1. After being shaken for 20 h, the reaction was quenched and purified by following general purification procedure 1. After purification, the product was lyophilized to give compound **5** in 82.2% yield (13.0 mg).

The sialylation catalyzed by Pd2,6ST or Psp2,6ST using 1.2 equiv. of sialic acid. Compound **3** (5.0 mg, 7.5 μ mol) and Neu5Ac (2.8 mg, 8.9 μ mol) were used as acceptor and donor, respectively, by following the general enzymatic synthetic procedure 1. After being shaken for 8 h, the reaction was quenched and purified by following general purification procedure 2. After purification, the product was lyophilized to give compound **5** in 66.9% yield (4.8 mg) by Pd2,6ST or 57.2% yield (4.1 mg) by Psp2,6ST. $R_f = 0.43$ (*n*-PrOH/ H₂O/ AcOH = 6/2/1 (v/v/v)); ¹H NMR (600 MHz, D₂O) δ 4.60 (d, *J* = 8.4 Hz, 1H), 4.48 (d, *J* = 8.0 Hz, 1H), 4.44 (d, *J* = 7.9 Hz, 1H), 4.18 (d, *J* = 3.3 Hz, 1H), 3.98 (dd, *J* = 1.9, 12.2 Hz, 1H), 3.96-3.86 (m, 6H), 3.83-3.77 (m, 4H), 3.75-3.61 (m, 11H), 3.60-3.57 (m, 3H), 3.35-3.28 (m, 3H), 2.73 (dd, *J* = 4.7, 12.4 Hz, 1H), 2.04 (s, 3H), 2.03 (s, 3H), 1.68-1.59 (m, 5H), 1.44-1.36 (m, 4H); ¹³C NMR (150 MHz, D₂O) δ 176.04, 175.99, 174.37, 104.13, 103.88, 102.97, 101.34, 83.02, 79.40, 75.93, 75.72, 75.42, 74.17, 73.76, 73.56, 72.69, 71.61, 71.47, 70.96, 69.35, 69.17(x2), 68.64, 64.38, 63.58, 62.12, 61.07, 53.44, 52.83, 52.08, 41.15, 29.54, 28.83, 26.61, 25.55, 23.18, 22.97; HRMS (ESI) *m/z* calcd for C₃₇H₆₂N₅O₂₄ [M-H]⁻ 960.3790, found 960.3756.



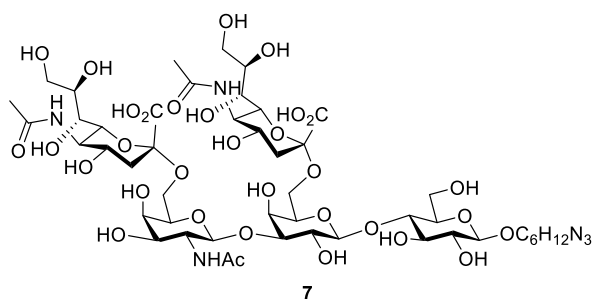
Neu5Gc8Mea2,6-GalNAc β 1,3-Gal β 1,4-Glc β C₆H₁₂N₃ (5a). Compound **3** (3.0 mg, 4.5 μ mol) and Neu5Gc8Me (4.6 mg, 13.4 μ mol) were used as acceptor and donor, respectively, by following the similar procedure as described in the synthesis of compound **5** by PmST1. After purification, the product was lyophilized to give compound **5a** in 69.9% yield (3.1 mg). $R_f = 0.33$ (*n*-PrOH/ H₂O/ AcOH = 6/2/1 (v/v/v)); ¹H NMR (500 MHz, D₂O) δ 4.58 (d, *J* = 8.4 Hz, 1H), 4.45 (d, *J* = 8.0 Hz, 1H), 4.41 (d, *J* = 7.9 Hz, 1H), 4.15 (d, *J* = 2.8 Hz, 1H), 4.09 (s, 2H), 4.03 (d, *J* = 10.5 Hz, 1H), 4.00-3.87 (m, 7H), 3.80-3.50 (m, 17H), 3.46 (s, 3H), 3.32-3.23 (m, 3H), 2.61 (dd, *J* = 4.3, 12.2 Hz, 1H), 2.01 (s, 3H), 1.67-1.53 (m, 5H), 1.43-1.30 (m, 4H); ¹³C NMR (125 MHz, D₂O) δ 176.74, 176.08, 174.62, 104.13, 103.90, 102.97, 101.63, 82.91, 81.43, 79.38, 75.93, 75.74, 75.43, 74.27, 73.77, 73.28, 71.59, 71.50, 71.04, 69.39, 68.65, 68.61, 68.11, 64.21, 62.14, 61.95, 61.07, 60.60, 58.59, 53.47, 52.86, 52.09, 41.15, 29.55, 28.85, 26.63, 25.57, 23.18; HRMS (ESI) *m/z* calcd for C₃₈H₆₄N₅O₂₅ [M-H]⁻ 990.3896, found 990.3884.



GalNAc β 1,3(Neu5Ac α 2,6-)-Gal β 1,4-Glc β C₆H₁₂N₃ (6).

The sialylation catalyzed by Pd2,6ST or Psp2,6ST. The compound **6** was synthesized from **3** by

following the procedure as described in the synthesis of compound **5**. After purification, the product was lyophilized to give compound **6** in 7.3% yield (0.5 mg) by Pd2,6ST or 5.4% yield (0.4 mg) by Psp2,6ST. $R_f = 0.42$ (*n*-PrOH/ H₂O/ AcOH) = 6/2/1 (v/v/v)); ¹H NMR (600 MHz, D₂O) δ 4.57 (d, *J* = 8.5 Hz, 1H), 4.46 (d, *J* = 8.1 Hz, 1H), 4.39 (d, *J* = 8.0 Hz, 1H), 4.16 (d, *J* = 3.2 Hz, 1H), 3.96-3.89 (m, 5H), 3.88-3.80 (m, 3H), 3.80-3.74 (m, 3H), 3.74-3.58 (m, 10H), 3.58-3.52 (m, 5H), 3.32-3.27 (m, 3H), 2.67 (dd, *J* = 4.7, 12.3 Hz, 1H), 2.01 (s, 3H), 2.00 (s, 3H), 1.70 (t, *J* = 12.3 Hz, 1H), 1.65-1.56 (m, 4H), 1.41-1.35 (m, 4H); ¹³C NMR (150 MHz, D₂O) δ 176.08, 175.87, 174.43, 104.36, 104.21, 102.86, 101.25, 82.99, 80.62, 75.95, 75.67, 75.60, 74.20, 73.70, 73.49, 72.71, 71.78, 71.46, 70.72, 69.32(x2), 69.26, 68.75, 64.38, 63.59, 61.94, 61.25, 53.52, 52.76, 52.10, 49.44, 49.29, 49.14, 49.00, 48.85, 48.71, 48.57, 41.11, 29.55, 28.84, 26.63, 25.58, 23.19, 23.00; HRMS (ESI) *m/z* calcd for C₃₇H₆₂N₅O₂₄ [M-H]⁻ 960.3790, found 960.3782.



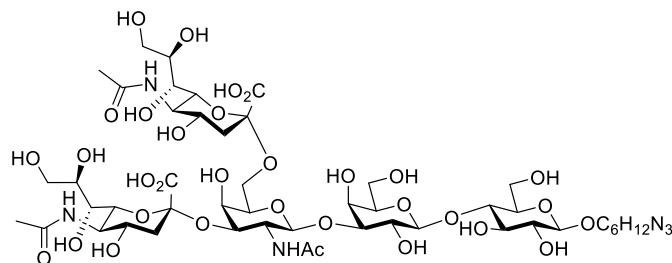
Neu5Ac α 2,6-GalNAc β 1,3(Neu5Ac α 2,6)-Gal β 1,4-Glc β C₆H₁₂N₃ (7).

The sialylation catalyzed by Pd2,6ST or Psp2,6ST using 1.2 equiv. of sialic acid. The compound **7** was synthesized from **3** by following the same procedure as described in the synthesis compound **5**. After purification, the product was lyophilized to give compound **7** in 1.6% yield (0.2 mg) by Pd2,6ST or 4.5% yield (0.4 mg) by Psp2,6ST.

The sialylation of compound 5 by Pd2,6ST. Compound **5** (2.3 mg, 2.4 μ mol) and Neu5Ac (3.7 mg, 12.0 μ mol) were used as acceptor and donor, respectively, by following the general enzymatic synthetic procedure 1. After being shaken for 8 h, the reaction was quenched and purified by following general purification procedure 1. After purification, the product was lyophilized to give compound **7** in 96.8% yield (2.9 mg).

The sialylation of compound 3 by Pd2,6ST. Compound **3** (13.0 mg, 19.4 μ mol) and Neu5Ac (29.9 mg, 96.9 μ mol) were used as acceptor and donor, respectively, by following the general enzymatic synthetic procedure 1. After being shaken for 8 h, the reaction was quenched and purified by following general purification procedure 1. After purification, the product was lyophilized to give compound **7** in 86.0% yield (20.9 mg). $R_f = 0.14$ (*n*-PrOH/ H₂O/ AcOH = 6/2/1 (v/v/v)); ¹H NMR (500 MHz, D₂O) δ 4.58 (d, *J* = 8.4 Hz, 1H), 4.46 (d, *J* = 8.1 Hz, 1H), 4.39 (d, *J* = 7.9 Hz, 1H), 4.12 (d, *J* = 2.6 Hz, 1H), 3.98-3.83 (m, 10H), 3.82-3.73 (m, 4H), 3.71-3.51 (m, 18H), 3.32-3.27 (m, 3H), 2.70 (dd, *J* = 4.5, 12.3 Hz, 1H), 2.68 (dd, *J* = 4.5, 12.3 Hz, 1H), 2.01 (s, 3H), 2.00 (s, 3H), 2.00 (s, 3H), 1.72 (t, *J* = 12.3 Hz, 1H), 1.65 (t, *J* = 12.3 Hz, 1H), 1.64-1.55 (m, 4H), 1.43-1.32 (m, 4H); ¹³C NMR (125 MHz, D₂O) δ 176.03, 176.01, 175.79, 174.42(x2), 104.17, 104.00, 102.82, 101.47, 101.23, 82.52, 80.84, 75.72, 75.56, 74.45, 74.10, 73.66, 73.62, 73.44, 72.68, 72.59, 71.65, 71.44, 70.91, 69.41, 69.37, 69.33, 69.16, 69.08, 68.56, 64.86, 64.16, 63.63,

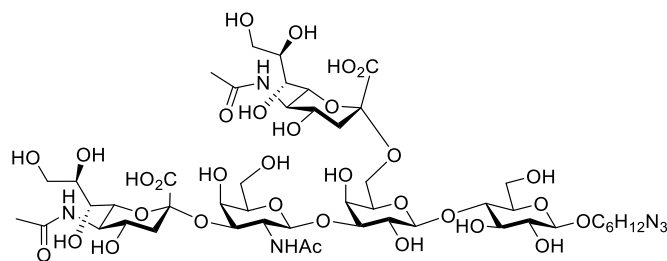
63.59, 61.29, 53.44, 52.95, 52.73, 52.09, 49.51, 49.34, 49.17, 49.00, 48.82, 48.65, 48.48, 41.23, 41.03, 29.54, 28.84, 26.62, 25.57, 23.19, 23.02, 22.99; HRMS (ESI) m/z calcd for $C_{48}H_{79}N_6O_{32}$ $[M-H]^-$ 1251.4744, found 1251.4728.



8

Neu5Aca2,3(Neu5Aca2,6)-GalNAcβ1,3-Galβ1,4-GlcβC₆H₁₂N₃ (8).

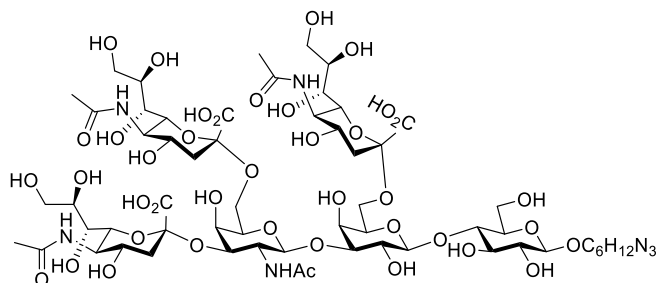
The sialylation catalyzed by Pd2,6ST or Psp2,6ST using 1.5 equiv. sialic acid. Compound 4 (4.2 mg, 4.4 μmol) and Neu5Ac (2.0 mg, 6.5 μmol) were used as acceptor and donor, respectively, by following the general enzymatic synthetic procedure 1. After being shaken for 10 h, the reaction was quenched and purified by following general purification procedure 2. The fractions with product were lyophilized to give compound 8 in 58.5% yield (3.2 mg) by Pd2,6ST or 95.0% yield (5.2 mg) by Psp2,6ST. $R_f = 0.25$ (n -PrOH/ H₂O/ AcOH = 6/2/1 (v/v/v)); ¹H NMR (700 MHz, D₂O) δ 4.72 (d, $J = 8.5$ Hz, 1H), 4.48 (d, $J = 8.0$ Hz, 1H), 4.43 (d, $J = 7.9$ Hz, 1H), 4.22-4.15 (m, 2H), 4.00-3.86 (m, 9H), 3.85-3.56 (m, 21H), 3.48 (d, $J = 10.4$ Hz, 1H), 3.37-3.29 (m, 3H), 2.75-2.67 (m, 2H), 2.08 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 1.73 (t, $J = 12.2$ Hz, 1H), 1.68-1.59 (m, 5H), 1.45-1.37 (m, 4H); ¹³C NMR (175 MHz, D₂O) δ 176.14, 175.86, 175.84, 175.06, 174.40, 104.14, 103.84, 102.85, 101.23, 100.51, 82.94, 80.68, 75.70, 75.66, 75.58, 74.21, 73.82, 73.73, 73.68, 73.47, 72.68, 72.61, 71.44, 70.68, 69.33, 69.30, 69.29, 69.21, 69.00, 67.97, 64.40, 63.58, 63.54, 61.94, 61.24, 52.82, 52.74, 52.08(x2), 41.08, 40.60, 29.53, 28.82, 26.61, 25.56, 23.36, 23.00, 22.94; HRMS (ESI) m/z calcd for $C_{48}H_{79}N_6O_{32}$ $[M-H]^-$ 1251.4744, found 1251.4745.



9

Neu5Aca2,3-GalNAcβ1,3(Neu5Aca2,6)-Galβ1,4-GlcβC₆H₁₂N₃ (9). Compound 4 (29.5 mg, 30.7 μmol) and Neu5Ac (12.3 mg, 39.9 μmol) were used as acceptor and donor, respectively. The experimental procedure is similar as described in the synthesis of compound 8 by Pd2,6ST. After purification, the product was lyophilized to give compound 9 in 16.1% yield (6.2 mg). $R_f = 0.25$ (n -PrOH/ H₂O/ AcOH = 6/2/1 (v/v/v)); ¹H NMR (600 MHz, D₂O) δ 4.71 (d, $J = 8.6$ Hz, 1H), 4.48 (d, $J = 8.0$ Hz, 1H), 4.46 (d, $J = 8.0$ Hz, 1H), 4.22-4.16 (m, 2H), 3.99-3.87 (m, 9H), 3.82-3.73 (m, 8H), 3.72-3.56 (m, 13H), 3.49 (dd, $J = 1.7, 10.4$, 1H), 3.35-3.28 (m, 3H), 2.72 (dd, $J = 4.7, 12.4$, 1H), 2.68 (dd, $J = 4.6, 12.4$, 1H), 2.08 (s, 3H),

2.034 (s, 3H), 2.032 (s, 3H), 1.68-1.60 (m, 6H), 1.43-1.38 (m, 4H); ^{13}C NMR (150 MHz, D_2O) δ 176.11, 175.95, 175.88, 174.75, 174.20, 103.86, 103.65, 102.98, 101.18, 100.59, 83.11, 79.45, 75.96, 75.74, 75.44, 73.97, 73.77(x2), 73.73, 73.56, 72.54(x2), 71.50, 70.90, 69.32(x2), 69.23, 69.22, 69.00, 68.07, 64.38, 63.62, 63.60, 62.18, 61.07, 52.80, 52.78, 52.10, 52.04, 41.01, 40.37, 29.55, 28.85, 26.63, 25.57, 23.35, 22.99, 22.96; HRMS (ESI) m/z calcd for $\text{C}_{48}\text{H}_{78}\text{N}_6\text{O}_{32}$ $[\text{M}-2\text{H}]^{2-}$ 625.2330, found 625.2277.

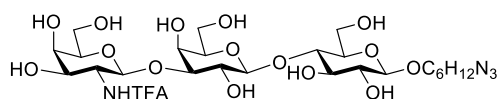


10

Neu5Ac α 2,3(Neu5Ac α 2,6)-GalNAc β 1,3(Neu5Ac α 2,6)-Gal β 1,4-Glc β C $_6\text{H}_{12}\text{N}_3$ (10).

The sialylation of compound **8** was catalyzed by Pd2,6ST. Compound **8** (8.2 mg, 6.5 μmol) and Neu5Ac (6.1 mg, 19.6 μmol) were used as acceptor and donor, respectively. The experimental procedure is similar as described in the synthesis of compound **8** by Pd2,6ST. After purification, the product was lyophilized to give compound **10** in 89.1% yield (9.0 mg).

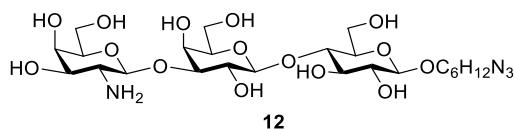
The sialylation of compound **4** by Pd2,6ST with 1.5 equiv. sialic acid. The compound **10** was synthesized from **4** by following the procedure as described in the synthesis of compound **8**. After purification, the product was lyophilized to give compound **10** in 5.9% yield (0.4 mg). $R_f = 0.12$ ($n\text{-PrOH}/\text{H}_2\text{O}/\text{AcOH} = 6/2/1$ (v/v/v)); ^1H NMR (500 MHz, D_2O) δ 4.71 (d, $J = 8.5$ Hz, 1H), 4.47 (d, $J = 8.1$ Hz, 1H), 4.41 (d, $J = 7.9$ Hz, 1H), 4.16 (dd, $J = 1.3, 11.0$ Hz, 1H), 4.13 (d, $J = 1.3$ Hz, 1H), 4.00-3.85 (m, 12H), 3.83-3.52 (m, 25H), 3.46 (d, $J = 10.3$, 1H), 3.35-3.27 (m, 3H), 2.73-2.62 (m, 3H), 2.05 (s, 3H), 2.01 (s, 9H), 1.74 (t, $J = 12.2$ Hz, 1H), 1.69-1.55 (m, 6H), 1.44-1.32 (m, 4H); ^{13}C NMR (125 MHz, D_2O) δ 176.09, 175.94, 175.84, 175.79, 174.97, 174.45, 174.40, 104.15, 103.49, 102.84, 101.44, 101.24, 100.73, 82.50, 80.95, 75.76, 75.58, 74.48, 73.94, 73.75(x2), 73.67, 73.61, 73.44, 72.63(x2), 72.58, 71.45, 70.91, 69.45, 69.41(x2), 69.30, 69.26, 69.15, 69.04, 68.03, 64.91, 64.20, 63.64(x2), 63.57, 61.31, 52.97, 52.79, 52.74, 52.10, 52.07, 49.52, 49.35, 49.17, 49.00, 48.83, 48.65, 48.48, 41.24, 41.04, 40.44, 29.56, 28.86, 26.64, 25.59, 23.38, 23.03, 23.01, 22.96; HRMS (ESI) m/z calcd for $\text{C}_{59}\text{H}_{94}\text{N}_7\text{Na}_2\text{O}_{40}$ $[\text{M}-3\text{H}+2\text{Na}]^-$ 1586.5337, found 1586.5340.



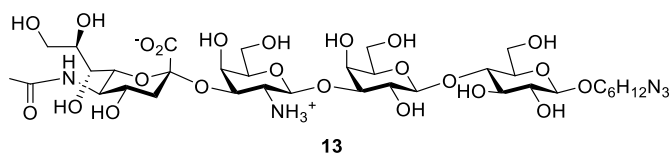
11

GalNTFA β 1,3-Gal β 1,4-Glc β C $_6\text{H}_{12}\text{N}_3$ (11). Compound **2** (10.0 mg, 21.4 μmol) and *N*-trifluoroacetyl galactosamine (GalNTFA) 13 (11.8 mg, 42.8 μmol) were used as acceptor and donor, respectively, by following the general enzymatic synthetic procedure 2. After being shaken for 30 h, the reaction was quenched and purified by following general purification procedure 2. The fractions with

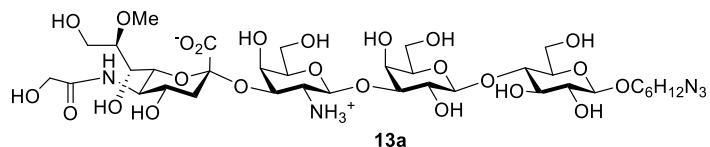
product were lyophilized to give compound **11** in 92.2% yield (14.3 mg). $R_f = 0.72$ (n -PrOH/ H₂O/ AcOH = 6/2/1 (v/v/v)); ¹H NMR (500 MHz, D₂O) δ 4.72 (d, $J = 8.4$ Hz, 1H), 4.46 (d, $J = 8.0$ Hz, 1H), 4.41 (d, $J = 7.8$ Hz, 1H), 4.16 (d, $J = 2.9$ Hz, 1H), 4.00 (dd, $J = 8.4, 10.9$ Hz, 1H), 3.95-3.89 (m, 3H), 3.86 (dd, $J = 3.2, 11.0$ Hz, 1H), 3.81-3.66 (m, 9H), 3.65-3.60 (m, 2H), 3.60-3.54 (m, 2H), 3.34-3.25 (m, 3H), 1.66-1.57 (m, 4H), 1.42-1.34 (m, 4H); ¹³C NMR (125 MHz, D₂O) δ 160.67 (q, $^2J_{CF} = 37$ Hz, NHCOCF₃), 116.88 (q, $^1J_{CF} = 286$ Hz, NHCOCF₃), 103.99, 103.54, 103.11, 83.14, 79.35, 76.12, 75.89, 75.76, 75.49, 73.88, 71.47, 71.05, 70.98, 69.40, 68.76, 61.96, 61.94, 61.11, 54.46, 52.12, 29.65, 28.94, 26.72, 25.66; HRMS (ESI) m/z calcd for C₂₆H₄₃F₃N₄O₁₆Na [M+Na]⁺ 747.2524, found 747.2500.



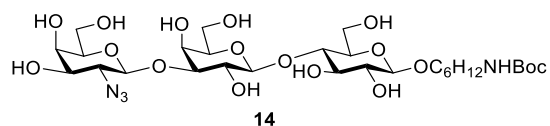
GalNH₂β1,3-Galβ1,4-GlcβC₆H₁₂N₃ (12). Compound **11** (14.3 mg, 19.7 μmol) was deprotected by following the general procedure for deprotection of NHTFA group. The fractions with product were lyophilized to give compound **12** in quantitative yield (12.2 mg). $R_f = 0.64$ (n -PrOH/ H₂O/ AcOH = 6/2/1 (v/v/v)); ¹H NMR (500 MHz, D₂O) δ 4.50 (d, $J = 8.2$ Hz, 1H), 4.48 (d, $J = 7.8$ Hz, 1H), 4.47 (d, $J = 7.9$ Hz, 1H), 4.17 (d, $J = 2.7$ Hz, 1H), 3.97 (d, $J = 11.8$ Hz, 1H), 3.94-3.88 (m, 1H), 3.84 (d, $J = 2.6$ Hz, 1H), 3.81-3.72 (m, 7H), 3.71-3.60 (m, 6H), 3.60-3.56 (m, 1H), 3.53 (dd, $J = 2.9, 10.4$ Hz, 1H), 3.34-3.26 (m, 3H), 2.89 (t, $J = 9.2$ Hz, 1H), 1.65-1.58 (m, 4H), 1.41-1.36 (m, 4H); ¹³C NMR (125 MHz, D₂O) δ 106.26, 103.66, 103.00, 83.07, 79.37, 76.15, 75.96, 75.70, 75.43, 73.87, 73.79, 71.50, 71.13, 69.34, 68.81, 62.08, 61.93, 61.07, 54.16, 52.09, 29.54, 28.84, 26.62, 25.56; HRMS (ESI) m/z calcd for C₂₄H₄₅N₄O₁₅ [M+H]⁺ 629.2876, found 629.2875.



Neu5Acα2,3-GalNH₂β1,3-Galβ1,4-GlcβC₆H₁₂N₃ (13). Compound **12** (10.0 mg, 15.9 μmol) and Neu5Ac (14.8 mg, 47.7 μmol) were used as acceptor and donor, respectively. The experimental procedure is similar as described in the synthesis of compound **5** by PmST1. After purification, the product was lyophilized to give compound **13** in 86.1% (12.6 mg). $R_f = 0.43$ (n -PrOH/ H₂O/ AcOH = 6/2/1 (v/v/v)); ¹H NMR (500 MHz, D₂O) δ 4.67 (d, $J = 8.1$ Hz, 1H), 4.50 (d, $J = 7.9$ Hz, 1H), 4.47 (d, $J = 8.0$ Hz, 1H), 4.17 (d, $J = 2.8$ Hz, 1H), 4.08 (dd, $J = 2.8, 10.4$ Hz, 1H), 3.96 (dd, $J = 11.4$ Hz, 1H), 3.93-3.56 (m, 22H), 3.33-3.26 (m, 3H), 3.03 (t, $J = 9.3$ Hz, 1H), 2.77 (dd, $J = 4.4, 12.1$ Hz, 1H), 2.02 (s, 3H), 1.77 (t, $J = 12.1$ Hz, 1H), 1.67-1.55 (m, 4H), 1.45-1.34 (m, 4H); ¹³C NMR (125 MHz, D₂O) δ 175.97, 174.59, 105.10, 103.53, 103.00, 100.52, 83.07, 79.27, 76.00, 75.94, 75.70, 75.42, 73.81(x2), 73.79, 72.66, 71.50, 71.11, 69.37, 69.30, 68.97, 67.15, 63.51, 61.96, 61.94, 61.06, 52.74, 52.68, 52.09, 40.95, 29.55, 28.84, 26.62, 25.57, 22.99; HRMS (ESI) m/z calcd for C₃₅H₆₁N₅O₂₃Na [M+Na]⁺ 942.3650, found 942.3677.

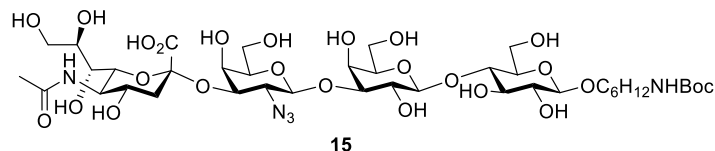


Neu5Gc8Mea2,3-GalNH₂β1,3-Galβ1,4-GlcβC₆H₁₂N₃ (13a). Compound **12** (60.0 mg, 95.4 μmol) and Neu5Gc8Me (81.0 mg, 238.6 μmol) were used as acceptor and donor, respectively. The experimental procedure is similar as described in the synthesis of compound **5** by PmST1, and the product was purified by following the general purification procedure 2. The fractions with product were lyophilized to give compound **13a** in 11.0% yield (10.0 mg). $R_f = 0.44$ (*n*-PrOH/ H₂O/ AcOH = 6/2/1 (v/v/v)); ¹H NMR (500 MHz, D₂O) δ 4.52 (d, *J* = 8.2 Hz, 1H), 4.48 (d, *J* = 7.9 Hz, 1H), 4.46 (d, *J* = 8.0 Hz, 1H), 4.16 (d, *J* = 2.9 Hz, 1H), 4.09 (s, 2H), 4.01 (dd, *J* = 2.8, 10.6 Hz, 1H), 3.98-3.94 (m, 2H), 3.93-3.86 (m, 4H), 3.80-3.59 (m, 14H), 3.58-3.51 (m, 3H), 3.46 (s, 3H), 3.32-3.25 (m, 3H), 2.95 (dd, *J* = 8.4, 10.2 Hz, 1H), 2.69 (dd, *J* = 4.3, 12.0 Hz, 1H), 1.72 (t, *J* = 12.0 Hz, 1H), 1.65-1.55 (m, 4H), 1.42-1.32 (m, 4H); ¹³C NMR (125 MHz, D₂O) δ 176.73, 174.55, 106.33, 103.58, 103.00, 100.94, 83.20, 80.92, 79.36, 76.17, 75.96, 75.92, 75.70, 75.43, 73.80, 73.26, 71.50, 71.14, 69.36, 68.81, 68.07, 67.43, 62.07, 61.96(x2), 61.06, 60.15, 58.42, 52.75, 52.64, 52.09, 41.08, 29.54, 28.84, 26.62, 25.56; HRMS (ESI) *m/z* calcd for C₃₆H₆₃N₅O₂₄Na [M+Na]⁺ 972.3755, found 972.3723.

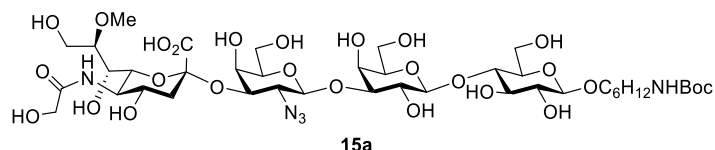


GalAzβ1,3-Galβ1,4-GlcβC₆H₁₂NHBoc (14). Compound **2a** (121.0 mg, 223.4 μmol) and GalNTFA (123.0 mg, 446.8 μmol) were used as acceptor and donor, respectively, by following the general enzymatic synthetic procedure 2. After being shaken for 36 h, the reaction was quenched and purified by following general purification procedure 3. The fractions with crude product were lyophilized by following the general procedure for deprotection of NHTFA group. The resulting residue was performed by diazo transfer reaction. 1*H*-Imidazole-1-sulfonyl azide hydrochloride¹⁴ (274.9 mg, 1.1 mmol) was added to a solution containing the crude product (223.4 μmol, 10 mM), NaHCO₃ (112.6 mg, 1.3 mmol), and CuSO₄·5H₂O (5.6 mg, 22.3 μmol) in H₂O and MeOH (1/1, v/v, 22.3 mL). The mixture was stirred at rt for 5 h and the progress of the reaction was monitored by TLC analysis (*n*-PrOH/ H₂O/ AcOH = 6/2/1 (v/v/v)). The mixtures were concentrated and purified by a C18 reverse-phase silica column (Sep-Pak Vac C18 cartridge 10 g, 55-105 μm, Waters) using a gradient solution of 0-100% MeOH in H₂O as eluent. After concentration of the fractions containing product, the resulting residue was further purified by size-exclusion column using H₂O as eluent (BioGel P2 gel packed in a column 1.6 cm x 50 cm, Biorad). The fractions with product were lyophilized to give compound **14** in 90.3% yield (147.0 mg). $R_f = 0.74$ (*n*-PrOH/ H₂O/ AcOH = 6/2/1 (v/v/v)); ¹H NMR (500 MHz, D₂O) δ 4.69 (d, *J* = 7.8 Hz, 1H), 4.47 (d, *J* = 8.0 Hz, 1H), 4.45 (d, *J* = 7.8 Hz, 1H), 4.16 (d, *J* = 2.8 Hz, 1H), 3.97 (d, *J* = 11.5 Hz, 1H), 3.93-3.87 (m, 2H), 3.85 (dd, *J* = 3.0, 10.0 Hz, 1H), 3.81-3.69 (m, 7H), 3.68-3.51 (m, 7H), 3.28 (t, *J* = 8.4 Hz, 1H), 3.04 (t, *J* = 6.6 Hz, 2H), 1.66-1.58 (m, 2H), 1.49-1.43 (m, 2H), 1.43-1.29 (m, 13H); ¹³C NMR (125 MHz, D₂O) δ

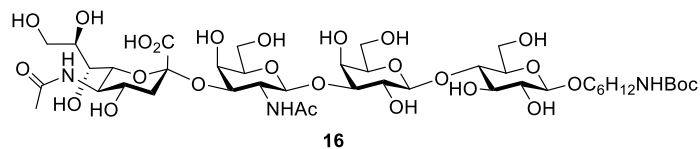
159.36, 104.49, 103.99, 103.07, 82.63, 81.63, 79.38, 76.10, 75.95, 75.79, 75.48, 73.84, 72.18, 71.55, 71.19, 69.40, 68.84, 64.44, 61.96, 61.90, 61.05, 40.88, 29.79, 29.65, 28.68, 26.61, 25.69; HRMS (ESI) m/z calcd for $C_{29}H_{52}N_4O_{17}Na [M+Na]^+$ 751.3220, found 751.3234.



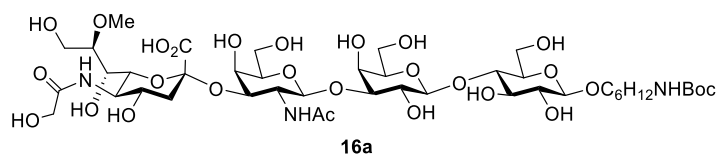
Neu5Ac α 2,3-GalAz β 1,3-Gal β 1,4-Glc β C₆H₁₂NHBoc (15). Compound **14** (7.4 mg, 10.2 μ mol) and Neu5Ac (9.4 mg, 30.5 μ mol) were used as acceptor and donor, respectively. The experimental procedure is similar as described in the synthesis of compound **5** by PmST1. After purification, the product was lyophilized to give compound **15** in 98.5% yield (10.2 mg). $R_f = 0.54$ (n -PrOH/ H₂O/ AcOH = 6/2/1 (v/v/v)); ¹H NMR (500 MHz, D₂O) δ 4.65 (d, $J = 8.2$ Hz, 1H), 4.46 (d, $J = 7.9$ Hz, 2H), 4.19-4.11 (m, 2H), 4.00-3.76 (m, 9H), 3.75-3.55 (m, 14H), 3.52 (dd, $J = 1.6, 10.5$ Hz, 1H), 3.28 (t, $J = 8.4$ Hz, 1H), 3.05 (t, $J = 6.6$ Hz, 2H), 2.77 (dd, $J = 4.5, 12.1$ Hz, 1H), 2.02 (s, 3H), 1.80 (t, $J = 12.1$ Hz, 1H), 1.66-1.58 (m, 2H), 1.50-1.28 (m, 6H), 1.41 (s, 9H); ¹³C NMR (125 MHz, D₂O) δ 176.08, 174.70, 159.26, 103.81, 103.68, 102.97, 100.63, 82.59, 81.69, 79.49, 75.87, 75.75, 75.69, 75.41, 74.88, 73.78, 73.74, 72.75, 71.48, 71.07, 69.28(x2), 69.15, 67.66, 63.52, 62.67, 61.87(x2), 61.07, 52.61, 40.89, 40.36, 29.69, 29.55, 28.64, 26.49, 25.58, 22.94; HRMS (ESI) m/z calcd for $C_{40}H_{68}N_5O_{25} [M-H]^-$ 1018.4203, found 1018.4229.



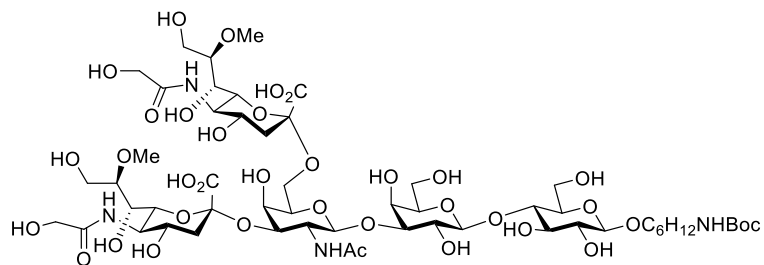
Neu5Gc8Me α 2,3-GalAz β 1,3-Gal β 1,4-Glc β C₆H₁₂NHBoc (15a). Compound **14** (47.5 mg, 65.2 μ mol) and Neu5Gc8Me (66.37 mg, 196 μ mol) were used as acceptor and donor, respectively. The experimental procedure is similar as described in the synthesis of compound **15**. The fractions with product were lyophilized to give compound **15a** in 35.5% yield (24.3 mg). $R_f = 0.49$ (n -PrOH/ H₂O/ AcOH = 6/2/1 (v/v/v)); ¹H NMR (500 MHz, D₂O) δ 4.70 (d, $J = 8.2$ Hz, 1H), 4.50 (d, $J = 7.9$ Hz, 2H), 4.19-4.16 (m, 2H), 4.15 (s, 2H), 4.06 (dd, $J = 2.4, 12.5$ Hz, 1H), 4.03-3.98 (m, 2H), 3.98-3.81 (m, 7H), 3.81-3.71 (m, 7H), 3.70-3.65 (m, 4H), 3.65-3.60 (m, 2H), 3.59-3.54 (m, 1H), 3.49 (s, 3H), 3.32 (t, $J = 7.7$ Hz, 1H), 3.08 (t, $J = 6.7$ Hz, 2H), 2.73 (dd, $J = 4.2, 12.2$ Hz, 1H), 1.84 (t, $J = 12.2$ Hz, 1H), 1.70-1.61 (m, 2H), 1.54-1.31 (m, 6H), 1.45 (s, 9H); ¹³C NMR (125 MHz, D₂O) δ 176.82, 174.63, 159.37, 104.14, 103.89, 103.00, 101.30, 82.53, 81.72, 81.52, 79.40, 75.93, 75.75(x2), 75.44, 74.50, 73.80, 73.39, 71.56, 71.16, 69.36, 68.85, 68.26, 68.06, 62.84, 61.94, 61.89, 61.04, 60.38, 58.29, 52.73, 40.88, 40.19, 29.72, 29.59, 28.65, 26.54, 25.63; HRMS (ESI) m/z calcd for $C_{41}H_{70}N_5O_{26} [M-H]^-$ 1048.4309, found 1048.4278.



Neu5Ac α 2,3-GalNAc β 1,3-Gal β 1,4-Glc β C₆H₁₂NHBoc (16). To a solution of compound **15** (10.2 mg, 10.0 μ mol) in THF (0.8 mL) was added 2 N NaOH_(aq) (50.0 μ l, 100.0 μ mol) and 1.0 M PMe₃ in THF (80.0 μ l, 80.0 μ mol). The reaction mixture was stirred at rt until TLC analysis (*n*-PrOH/ H₂O/ AcOH = 6: 2: 1 (v/ v/ v)) indicated the completion of the reaction after 4 h, the resultant was neutralized with AcOH. After removal of solvent under vacuum, the crude product was purified by size-exclusion column using H₂O as eluent (BioGel P2 gel packed in a column 1.6 cm x 50 cm, Biorad). The glycan fractions were acetylated by following the general *N*-acetylation procedure. Fractions containing the desired product were pooled and lyophilized to give compound **16** in 98.5% (10.2 mg). R_f = 0.50 (*n*-PrOH/ H₂O/ AcOH = 6/2/1 (v/v/v)); ¹H NMR (500 MHz, D₂O) δ 4.71 (d, J = 8.5 Hz, 1H), 4.45 (d, J = 8.1 Hz, 1H), 4.43 (d, J = 8.0 Hz, 1H), 4.17 (dd, J = 2.1, 11.0 Hz, 1H), 4.13 (d, J = 2.5 Hz, 1H), 3.98-3.83 (m, 6H), 3.80-3.68 (m, 9H), 3.67-3.55 (m, 8H), 3.45 (d, J = 10.3 Hz, 1H), 3.27 (t, J = 8.0 Hz, 1H), 3.04 (t, J = 6.6 Hz, 2H), 2.67 (dd, J = 4.5, 12.4 Hz, 1H), 2.06 (s, 3H), 2.01 (s, 3H), 1.66-1.56 (m, 3H), 1.50-1.27 (m, 6H), 1.40 (s, 9H); ¹³C NMR (125 MHz, D₂O) δ 176.17, 175.93, 175.11, 159.37, 103.87, 103.78, 102.99, 100.48, 82.76, 81.73, 79.37, 75.86, 75.72(x2), 75.42, 73.78(x2), 73.76, 72.65, 71.56, 70.96, 69.38, 69.30, 69.00, 67.94, 63.55, 61.97, 61.94, 61.04, 52.83, 52.11, 40.88, 40.64, 29.73, 29.60, 28.66, 26.55, 25.64, 23.37, 22.95; HRMS (ESI) m/z calcd for C₄₂H₇₂N₃O₂₆ [M-H]⁻ 1034.4410, found 1034.4382.



Neu5Gc8Me α 2,3-GalNAc β 1,3-Gal β 1,4-Glc β C₆H₁₂NHBoc (16a). Compound **15a** (13.5 mg, 12.9 μ mol) was used by following the procedures as described in the synthesis of compound **16**. After purification, the product was lyophilized to give compound **16a** in 94.8% (13.0 mg). R_f = 0.39 (*n*-PrOH/ H₂O/ AcOH = 6/2/1 (v/v/v)); ¹H NMR (500 MHz, D₂O) δ 4.68 (d, J = 8.5 Hz, 1H), 4.46 (d, J = 8.0 Hz, 1H), 4.43 (d, J = 7.9 Hz, 1H), 4.23 (dd, J = 2.5, 11.0 Hz, 1H), 4.14 (d, J = 2.7 Hz, 1H), 4.11 (s, 2H), 4.02-3.83 (m, 7H), 3.82-3.65 (m, 11H), 3.64-3.55 (m, 5H), 3.53-3.49 (m, 1H), 3.45 (s, 3H), 3.28 (t, J = 8.4 Hz, 1H), 3.04 (t, J = 6.7 Hz, 2H), 2.58 (dd, J = 4.4, 12.0 Hz, 1H), 2.06 (s, 3H), 1.67-1.57 (m, 3H), 1.50-1.28 (m, 6H), 1.41 (s, 9H); ¹³C NMR (125 MHz, D₂O) δ 176.81, 175.97, 174.84, 159.38, 104.07, 103.91, 102.99, 101.00, 82.69, 81.71, 81.13, 79.38, 75.86, 75.72, 75.67, 75.43, 73.78, 73.33, 73.18, 71.56, 71.01, 69.44, 68.96, 68.22, 67.96, 61.98, 61.95(x2), 61.05, 60.08, 58.28, 52.88, 52.14, 40.87, 40.48, 29.73, 29.60, 28.66, 26.55, 25.64, 23.30; HRMS (ESI) m/z calcd for C₄₃H₇₄N₃O₂₇ [M-H]⁻ 1064.4515, found 1064.4482.



17

Neu5Gc8Mea2,3(Neu5Gc8Mea2,6)-GalNAc β 1,3-Gal β 1,4-Glc β C₆H₁₂NHBoc (17, GAA7-C₆H₁₂NHBoc). Compound **16a** (3.6 mg, 3.4 μ mol) and Neu5Gc8Me (3.4 mg, 10.1 μ mol) were used as acceptor and donor, respectively. The experimental procedure is similar as described in the synthesis of compound **1** by Psp2,6ST. After purification, the product was lyophilized to give compound **17** in 74.7% yield (3.5 mg). $R_f = 0.22$ (*n*-PrOH/ H₂O/ AcOH = 6/2/1 (v/v/v)); ¹H NMR (500 MHz, D₂O) δ 4.67 (d, $J = 8.5$ Hz, 1H), 4.47 (d, $J = 8.0$ Hz, 1H), 4.42 (d, $J = 7.9$ Hz, 1H), 4.23 (dd, $J = 2.3, 10.8$ Hz, 1H), 4.17 (d, $J = 2.7$ Hz, 1H), 4.113 (s, 2H), 4.108 (s, 2H), 4.11-4.09 (m, 1H), 4.03-3.56 (m, 27H), 3.56-3.51 (m, 3H), 3.49 (s, 3H), 3.46 (s, 3H), 3.31 (t, $J = 8.4$ Hz, 1H), 3.05 (t, $J = 6.7$ Hz, 2H), 2.63-2.56 (m, 2H), 2.06 (s, 3H), 1.69 (t, $J = 12.3$ Hz, 1H), 1.67-1.61 (m, 3H), 1.50-1.32 (m, 6H), 1.42 (s, 9H); ¹³C NMR (125 MHz, D₂O) δ 176.79, 176.64, 175.94, 174.84, 174.65, 159.41, 104.21, 104.15, 102.86, 101.54, 101.01, 82.93, 81.75, 81.34, 81.07, 80.75, 75.74, 75.67, 75.59, 74.36, 73.70, 73.39, 73.24, 73.16, 71.52, 70.71, 69.31, 68.98, 68.71, 68.38, 68.23, 67.97, 64.50, 61.96(x2), 61.24, 60.65, 60.04, 58.66, 58.27, 52.87, 52.76, 52.13, 41.23, 40.87, 40.47, 29.71, 29.59, 28.65, 26.55, 25.64, 23.30; HRMS (ESI) m/z calcd for C₅₅H₉₃N₄O₃₆ [M-H]⁻ 1385.5575, found 1385.5581.

Reference

1. W.-T. Chien, C.-F. Liang, C.-C. Yu, C.-H. Lin, S.-P. Li, I. Primadona, Y.-J. Chen, K.-K. Mong and C.-C. Lin, *Chem. Commun.*, 2014, **50**, 5786-5789.
2. W. Guan, L. Cai, J. Fang, B. Wu and P. G. Wang, *Chem. Commun.*, 2009, **45**, 6976-6978.
3. W. Guan, L. Cai and P. G. Wang, *Chem. Eur. J.*, 2010, **16**, 13343-13345.
4. O. Blixt, I. van Die, T. Norberg and D. H. van den Eijnden, *Glycobiology*, 1999, **9**, 1061-1071.
5. C.-C. Yu, P.-C. Lin and C.-C. Lin, *Chem. Commun.*, 2008, **11**, 1308-1310.
6. C.-C. Yu, Y.-Y. Kuo, C.-F. Liang, W.-T. Chien, H.-T. Wu, T.-C. Chang, F.-D. Jan and C.-C. Lin, *Bioconjugate Chem.*, 2012, **23**, 714-724.
7. C. P. Chiu, L. L. Lairson, M. Gilbert, W. W. Wakarchuk, S. G. Withers and N. C. J. Strynadka, *Biochemistry*, 2007, **46**, 7196-7204.
8. P.-J. Li, S.-Y. Huang, P.-Y. Chiang, C.-Y. Fan, L.-J. Guo, D.-Y. Wu, T. Angata and C.-C. Lin, *Angew. Chem., Int. Ed.*, 2019, **58**, 11273-11278.
9. V. Thon, Y. Li, H. Yu, K. Lau and X. Chen, *Appl. Microbiol. Biotechnol.*, 2012, **94**, 977-985.
10. S.-P. Li, W.-C. Hsiao, C.-C. Yu, W.-T. Chien, H.-J. Lin, L.-D. Huang, C.-H. Lin, W.-L. Wu, S.-H. Wu and C.-C. Lin, *Adv. Synth. Catal.*, 2014, **356**, 3199-3213.
11. K. Suzuki, S. Daikoku, T. Ako, Y. Shioiri, A. Kurimoto, A. Ohtake, S. K. Sarkar and O. Kanie, *Anal. Chem.*, 2007, **79**, 9022-9029.
12. H. Yu, H. Z. Cao, V. K. Tiwari, Y. H. Li and X. Chen, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 5037-5040.
13. L. Wen, M. R. Gadi, Y. Zheng, C. Gibbons, S. M. Kondengaden, J. Zhang and P. G. Wang, *ACS Catal.*, 2018, **8**, 7659-7666.
14. E. D. Goddard-Borger and R. V. Stick, *Org. Lett.*, 2007, **9**, 3797-3800.

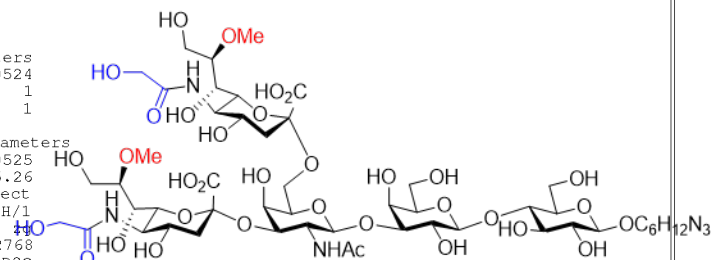
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THK-GAA7-N3

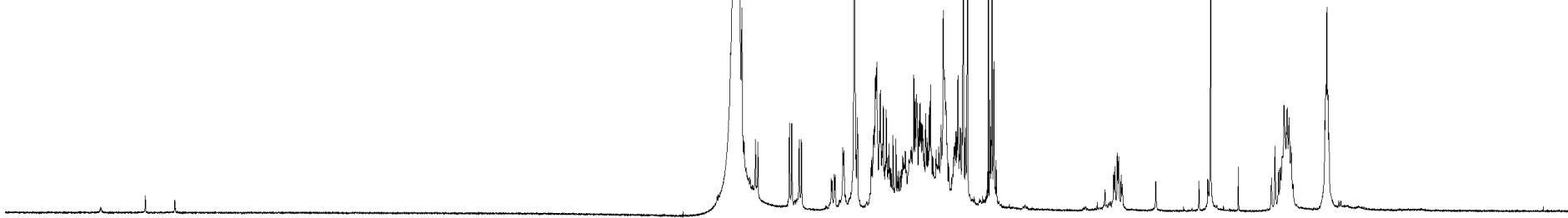
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¹H NMR spectrum of Compound 1 (600 MHz D₂O)



THK-GAA-7-N3

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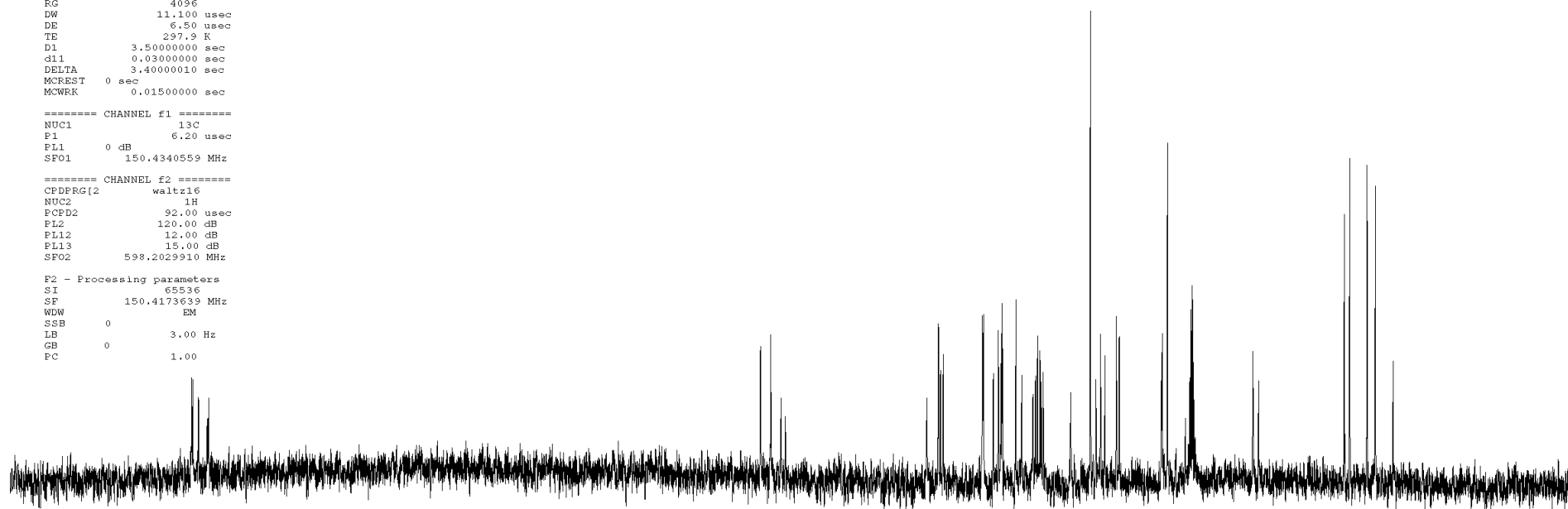
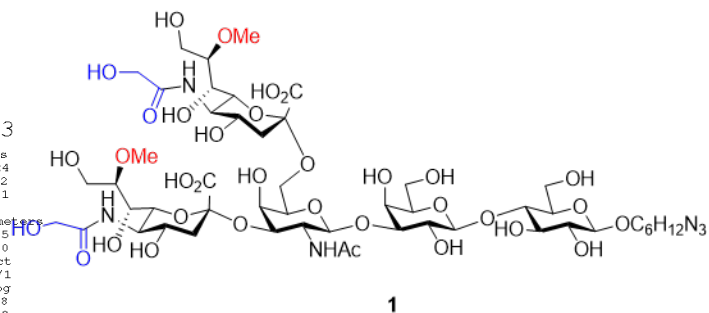
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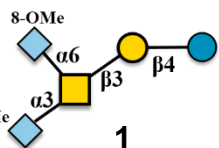
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¹³C NMR spectrum of Compound 1 (150 MHz D₂O)



THK-GAA-7-N3

Current Data Parameters
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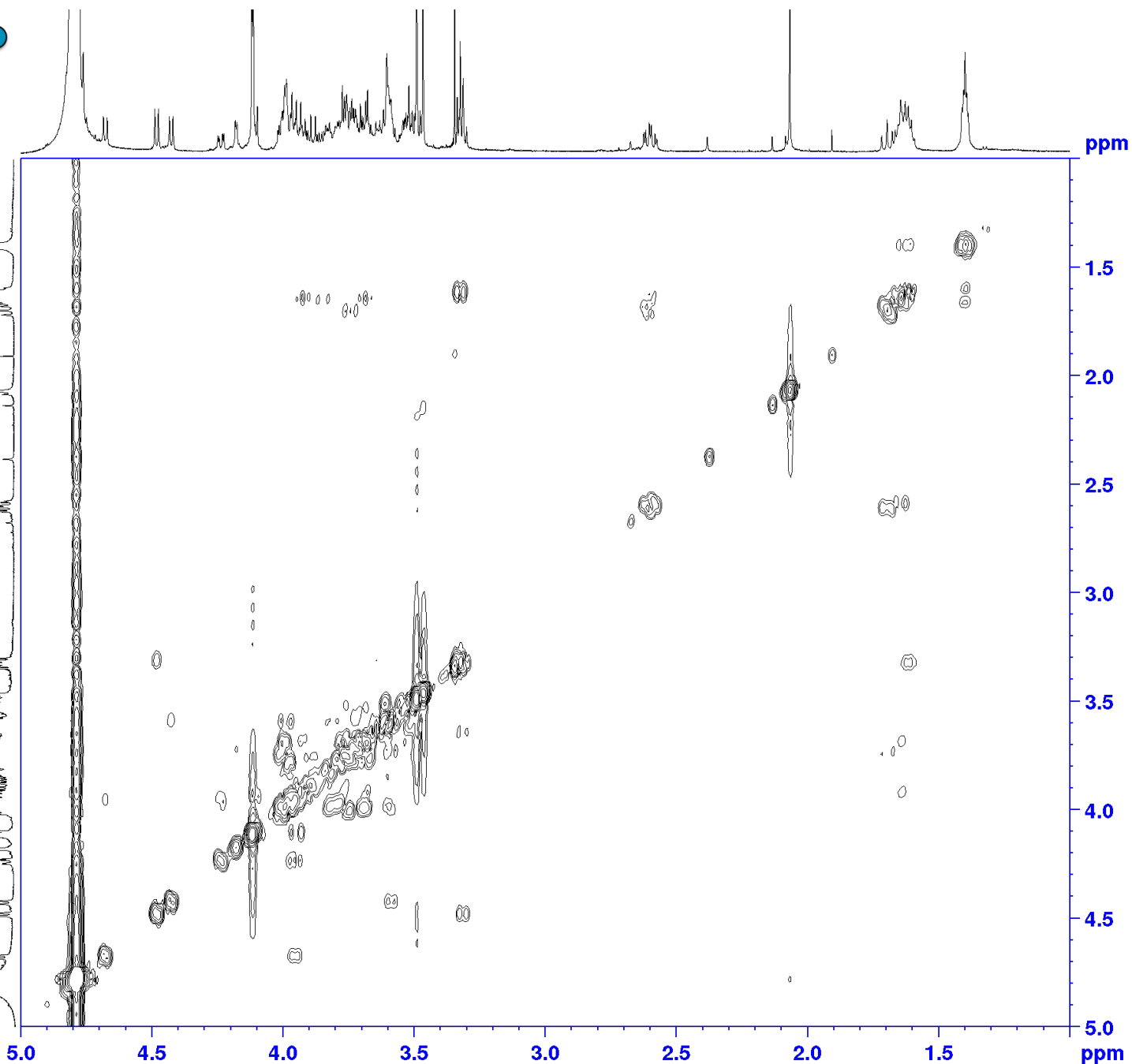
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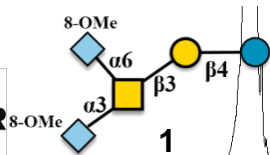
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^1H - ^1H COSY NMR spectrum of Compound 1 (600 MHz D_2O)



THK-GAA-7-N3

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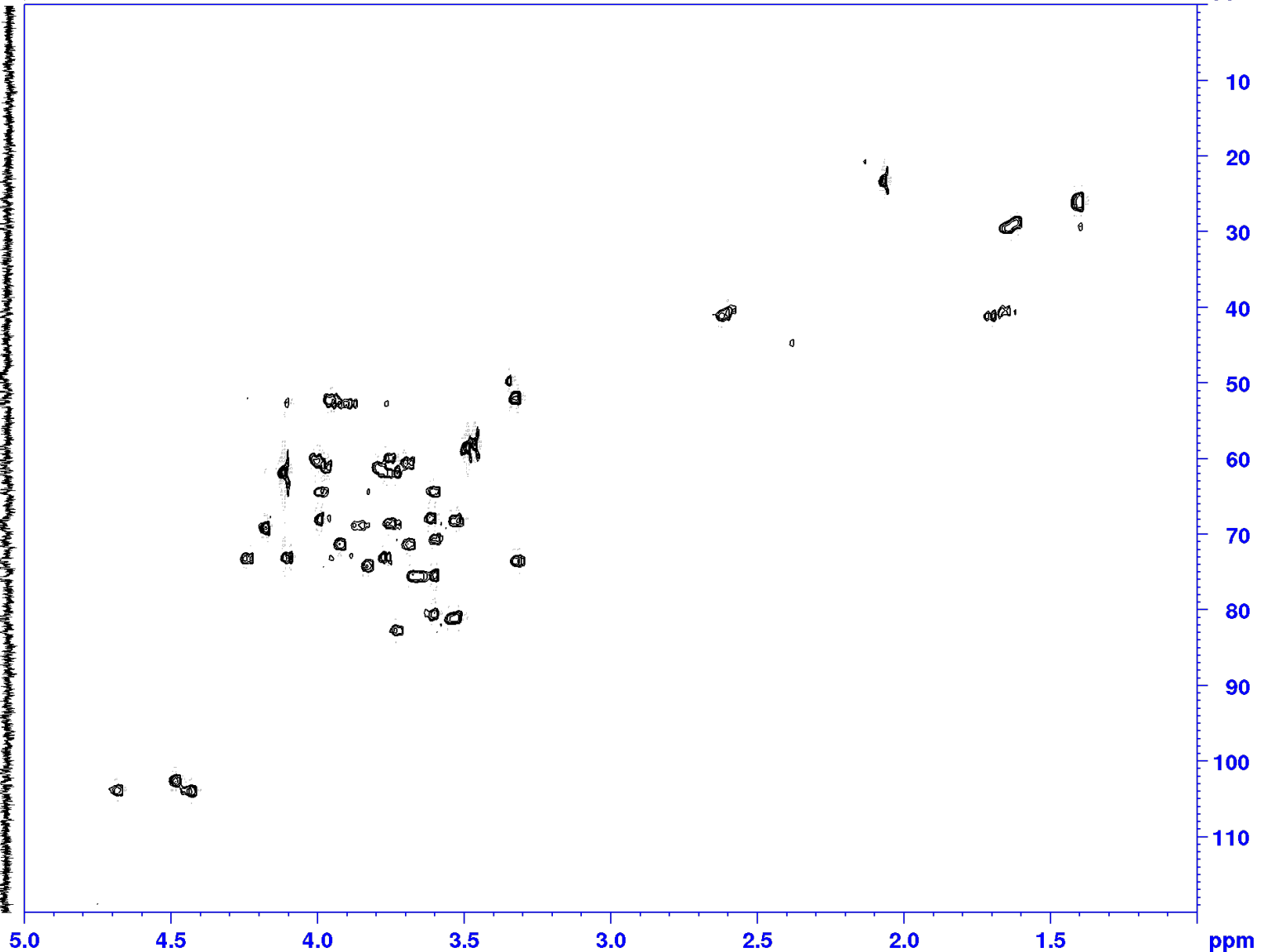
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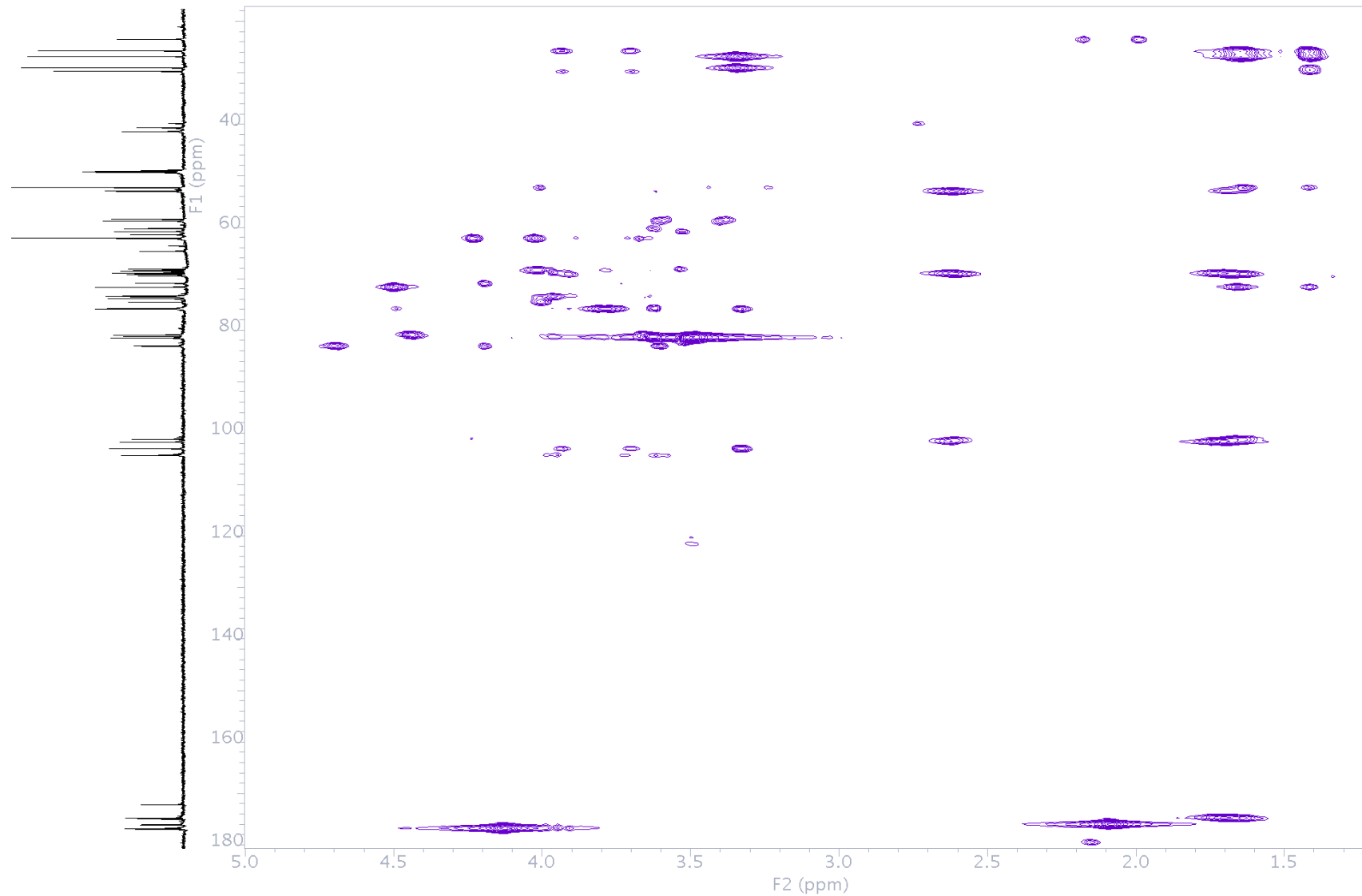
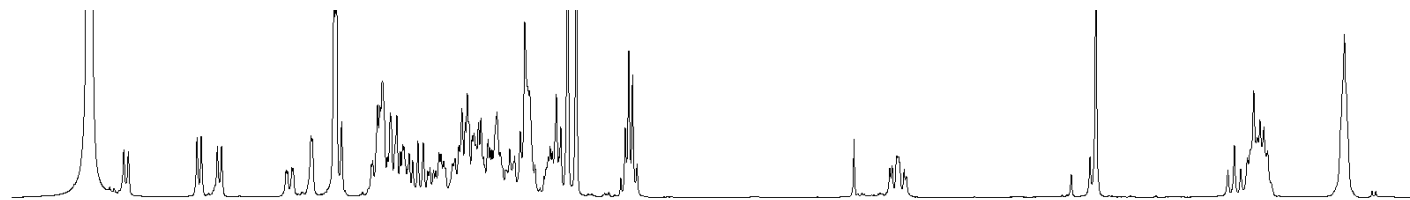
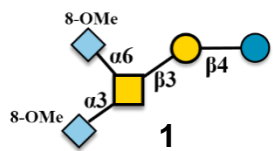
F1 - Acquisition parameters
ID        400
SFO1      150.4288 MHz
FIDRES    195.503418 Hz
SW        259.928 ppm
FMODE     Echo-Antiecho

E2 - Processing parameters
SI        2048
SF        598.1999600 MHz
WDW       QSINE
SSB       4
LB        0 Hz
GB        0
PC        1.00

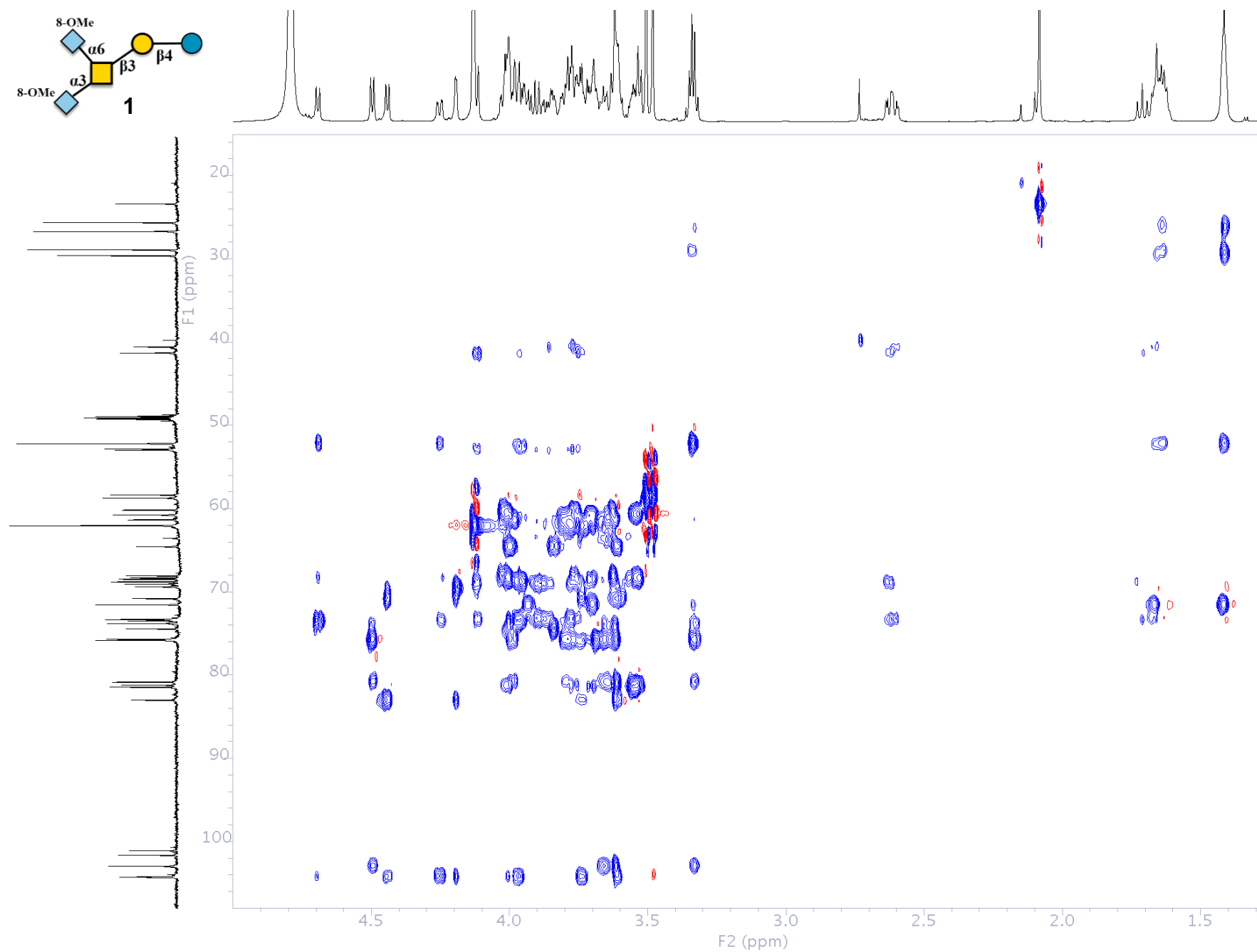
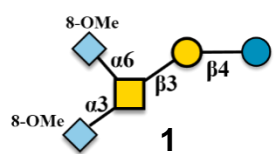
F1 - Processing parameters
SI        512
MC2       echo-antiecho
SF        150.4173639 MHz
WDW       QSINE
SSB       4
LB        0 Hz
GB        0
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^1H - ^{13}C HSQC NMR spectrum of Compound 1 (600 MHz/150 MHz D_2O)



^1H - ^{13}C HMBC NMR spectrum of Compound **1** (700 MHz/175 MHz D_2O)

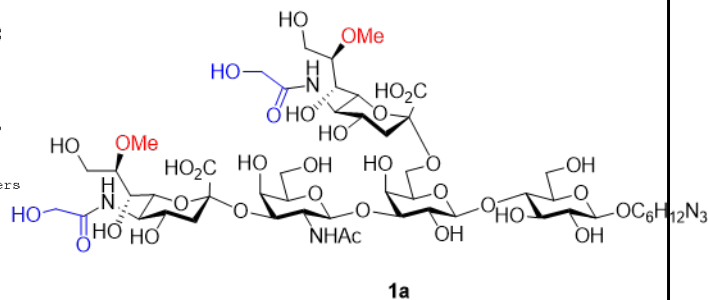


^1H - ^{13}C HSQC-TOCSY NMR spectrum of Compound 1 (700 MHz/175 MHz D_2O)

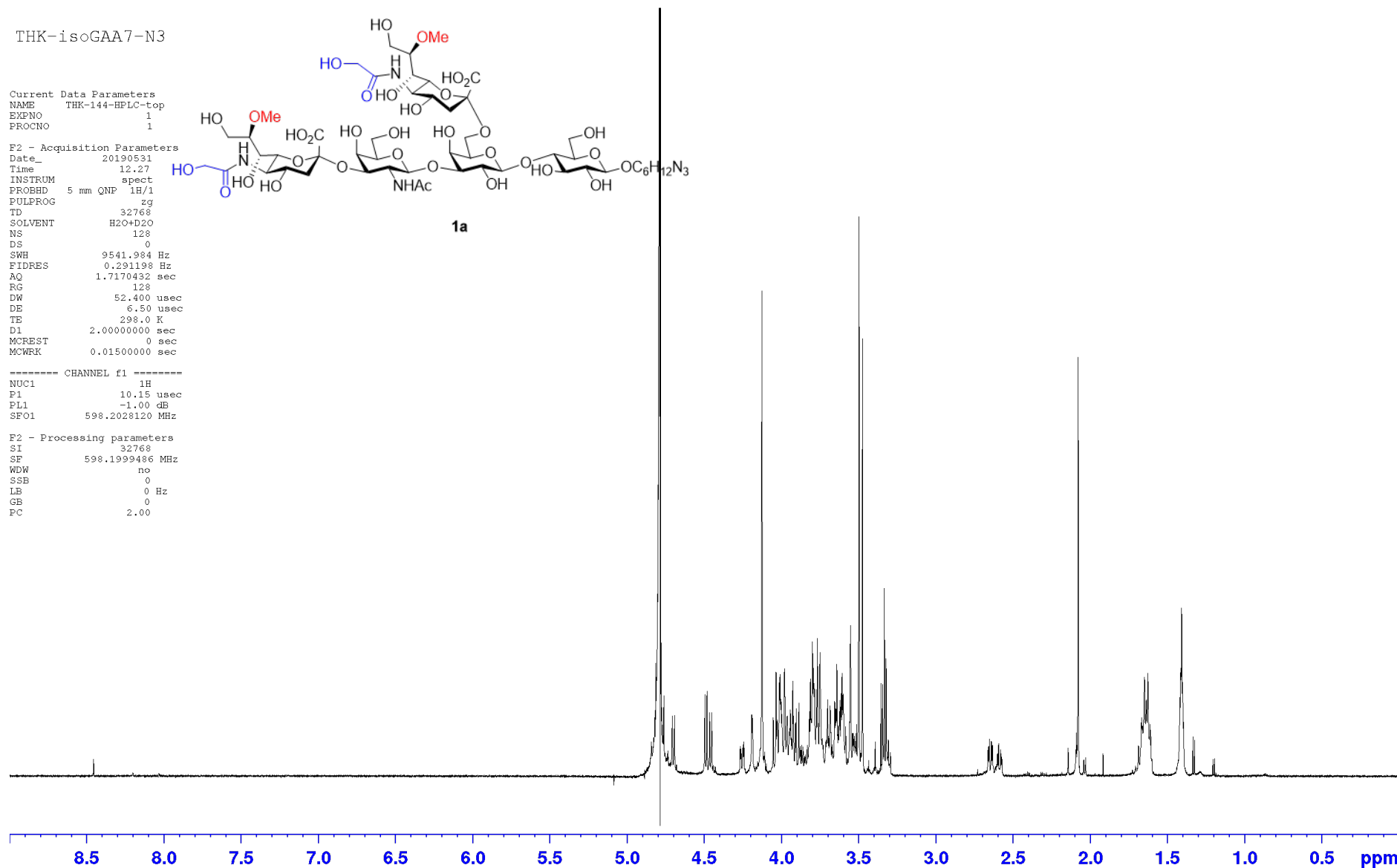
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4.6932
4.4961
4.4826
4.4655
4.4523
4.4373
4.4140
4.3440
4.3216
4.1873
4.1263
4.0529
4.0350
4.0258
4.0141
4.0094
4.0033
3.9978
3.9806
3.9629
3.9480
3.9435
3.9398
3.9345
3.9267
3.9230
3.9093
3.9041
3.8871
3.8790
3.8683
3.8607
3.8514
3.8438
3.8323
3.8184
3.8121
3.8072
3.7988
3.7840
3.7679
3.7473
3.7678
3.7625
3.7507
3.7354
3.7291
3.7119
3.7005
3.6950
3.6841
3.6722
3.6586
3.6498
3.6467
3.6409
3.6321
3.6206
3.6141
3.6068
3.6006
3.5845
3.5732
3.5531
3.5399
3.5340
3.5285
3.5207
3.5138
3.4979
3.4747
3.4560
3.4548
3.4450
3.4334
3.4218
3.4192
3.4161
3.4089
3.4072
3.3934
3.3609
3.3533
3.3404
3.3330
3.3007
2.9934
2.5801
2.5736
2.5736
2.0777
1.6872
1.6674
1.6614
1.6479
1.6360
1.6269
1.6123
1.6070
1.6014
1.4194
1.4131
1.4073
1.4012
1.3953

THK-isogAA7-N3

Current Data Parameters
 NAME THK-144-HPLC-top
 EXPNO 1
 PROCNO 1
 F2 - Acquisition Parameters
 Date_ 20190531
 Time 12.27
 INSTRUM spect
 PROBHD 5 mm QNP 1H/1
 PULPROG zg
 TD 32768
 SOLVENT H2O-D2O
 NS 128
 DS 0
 SWH 9541.984 Hz
 FIDRES 0.291198 Hz
 AQ 1.7170432 sec
 RG 128
 DW 52.400 usec
 DE 6.50 usec
 TE 299.0 K
 D1 2.00000000 sec
 MCREST 0 sec
 MCWRK 0.01500000 sec



----- CHANNEL f1 -----
 NUC1 1H
 P1 10.15 usec
 PL1 -1.00 dB
 SFO1 598.2028120 MHz
 F2 - Processing parameters
 SI 32768
 SF 598.1999486 MHz
 WDW no
 SSB 0
 LB 0 Hz
 GB 0
 PC 2.00



1.10
1.00
0.98
1.00
1.08
4.15
3.98
4.17
4.38
8.21
9.26
8.99
2.93
1.97
2.83
6.28
4.20

¹H NMR spectrum of Compound 1a (600 MHz D₂O)



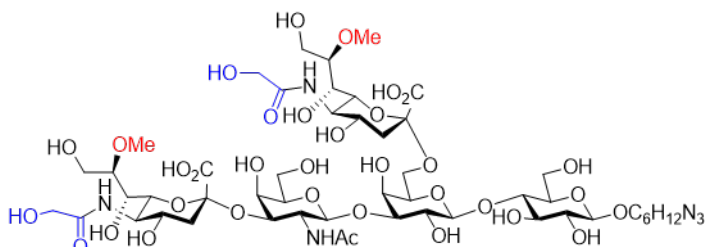
THK-isoGAA-7-N3

Current Data Parameters
NAME THK-144-HPLC-top
EXPMO 2
PROCNO 1

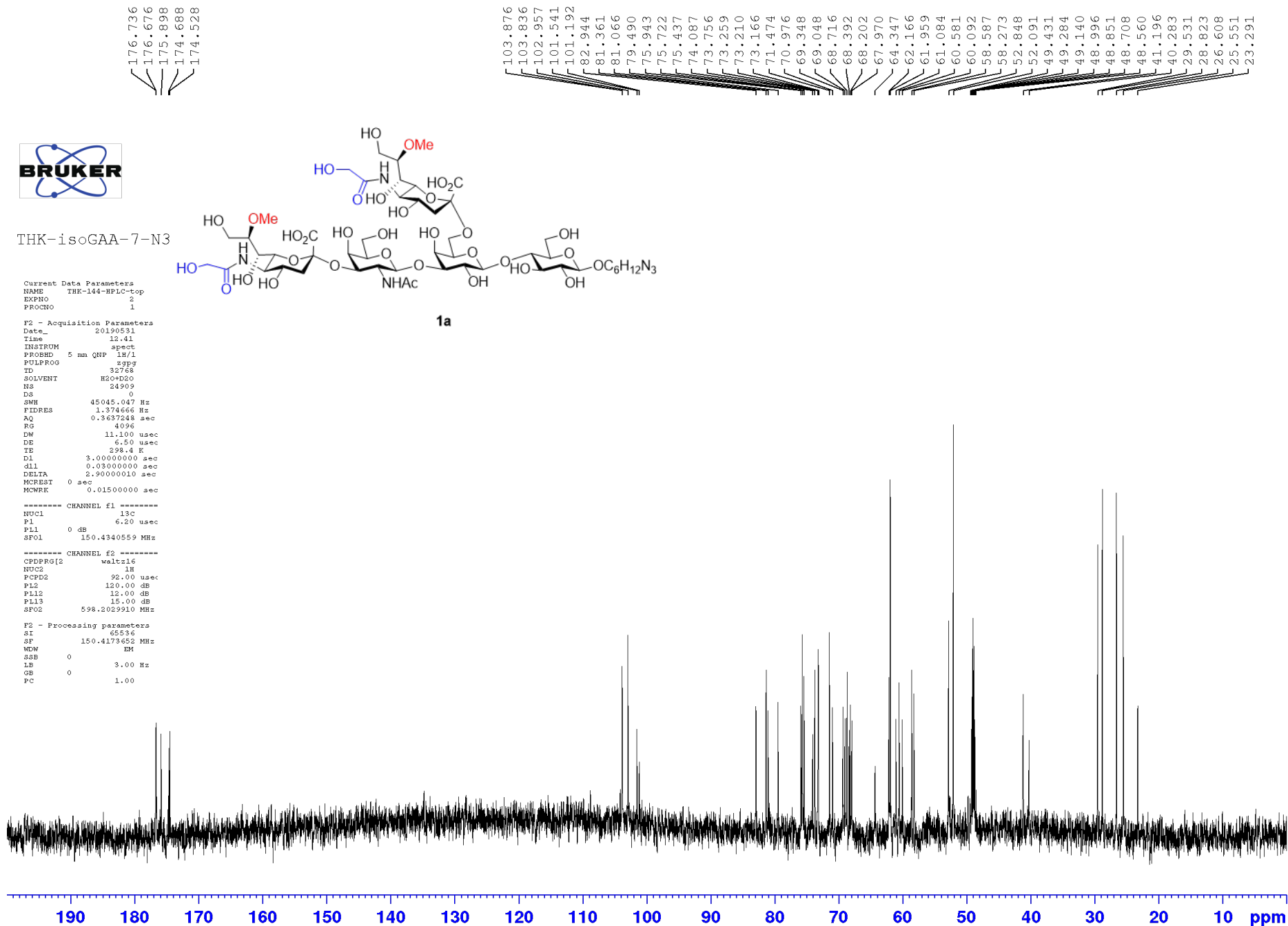
F2 - Acquisition Parameters
Date_ 20190531
Time 12.41
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG zgpg30
TD 32768
SOLVENT H2O-D2O
NS 24909
DS 0
SWH 45045.047 Hz
FIDRES 1.374666 Hz
AQ 0.3637248 sec
RG 4096
DW 11.100 usec
DE 6.50 usec
TE 298.4 K
D1 3.00000000 sec
d11 0.03000000 sec
DELTA 2.90000010 sec
MCREST 0 sec
MCWRK 0.01500000 sec

----- CHANNEL f1 -----
NUC1 13C
P1 6.20 usec
PL1 0 dB
SF01 150.4340559 MHz
----- CHANNEL f2 -----
CPDPRG2 waltz16
NUC2 1H
PCPD2 92.00 usec
PL2 120.00 dB
PL12 12.00 dB
PL13 15.00 dB
SF02 598.2029910 MHz

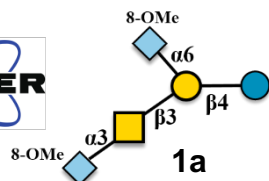
F2 - Processing parameters
SI 65536
SF 150.4173652 MHz
WDW EM
SFB 0
LB 3.00 Hz
GB 0
PC 1.00



1a



¹³C NMR spectrum of Compound 1a (150 MHz D₂O)



THK-isoGAA-7-N3

Current Data Parameters
NAME THK-144-HPLC-top
EXPNO 11
PROCNO 1

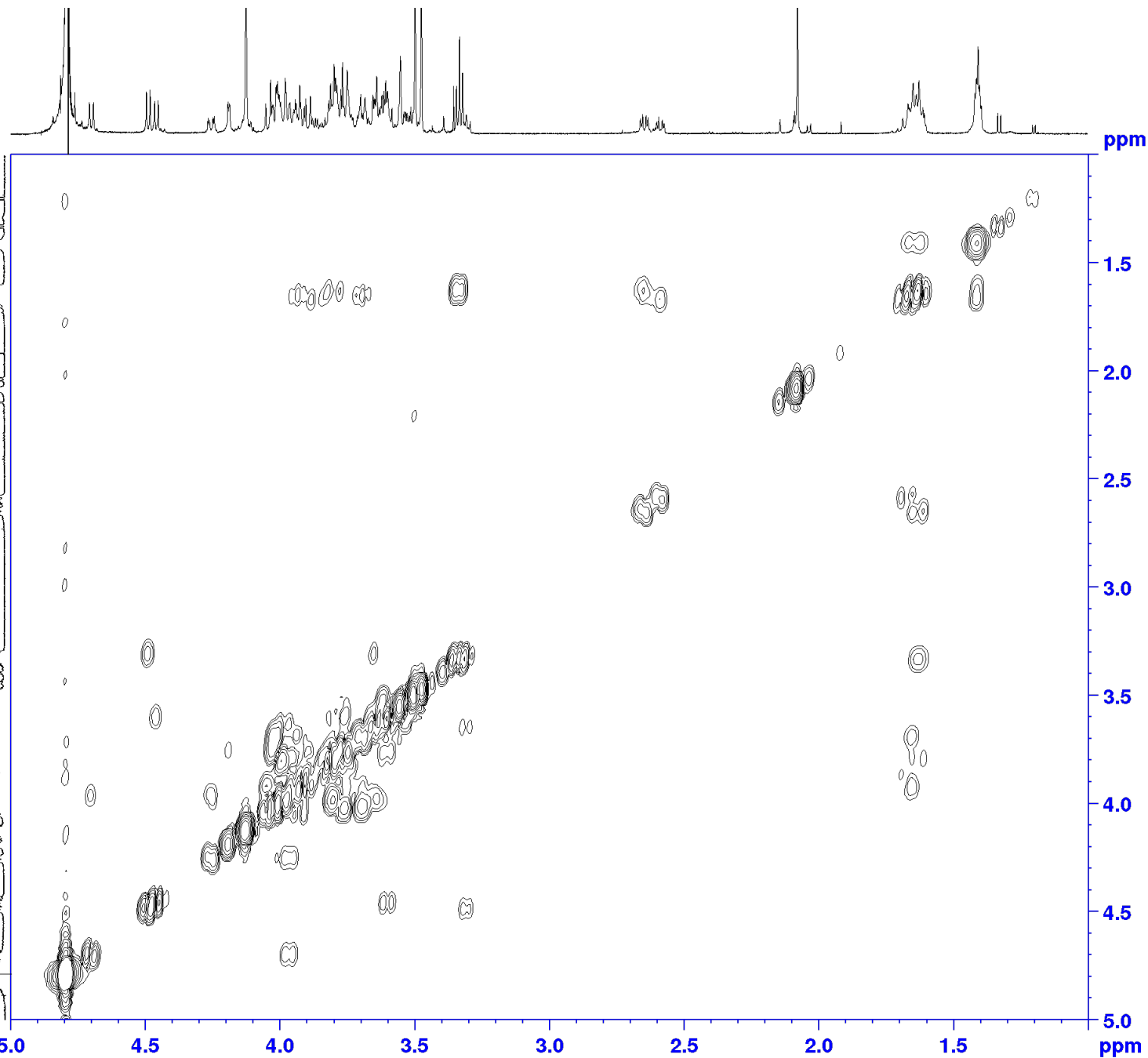
F2 - Acquisition Parameters
Date_ 20190601
Time 12.40
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG cosyqf
TD 2048
SOLVENT Acetone
NS 16
DS 0
SWH 6009.615 Hz
FIDRES 2.934392 Hz
AQ 0.1703936 sec
RG 128
DW 83.200 usec
DE 6.50 usec
TE 298.2 K
d0 0.00000300 sec
D1 1.50000000 sec
IN0 0.00016640 sec
MCREST 0 sec
MCWRK 1.50000000 sec

===== CHANNEL f1 =====
NUC1 1H
P0 13.00 usec
P1 10.50 usec
PL1 -1.00 dB
SFO1 598.2028140 MHz

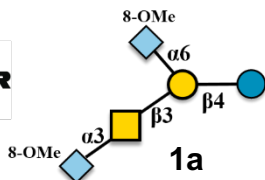
F1 - Acquisition parameters
TD 256
SFO1 598.2028 MHz
FIDRES 46.950119 Hz
SW 10.046 ppm
FhMODE QF

F2 - Processing parameters
SI 2048
SF 598.1999486 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0
PC 1.00

F1 - Processing parameters
SI 512
MC2 QF
SF 598.1999486 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0



^1H - ^1H COSY NMR spectrum of Compound 1a (600 MHz D_2O)



THK-isoGAA-7-N3

Current Data Parameters
NAME THK-144-RPLC-top
EXPNO 12
PROCNO 1

F2 - Acquisition Parameters
Date_ 20190601
Time 14:27
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG hsqcetgps1
TD 2048
SOLVENT CDCl3
NS 64
DS 8
SWH 6009.615 Hz
FIDRES 2.934382 Hz
AQ 0.1703936 sec
RG 32768
DW 83.200 usec
DE 6.50 usec
TE 297.8 K
CNST2 145.000000
d0 0.00000300 sec
d1 1.20000005 sec
d4 0.00172414 sec
d11 0.03000000 sec
d13 0.00000400 sec
d16 0.00050000 sec
D24 0.00089000 sec
DELTA 0.00157700 sec
DELTA1 0.00150800 sec
IN0 0.00001511 sec
MCREST 0 sec
MCMRK 0.20000041 sec
STCNT 0

----- CHANNEL f1 -----
NUC1 1H
P1 10.50 usec
P2 21.00 usec
P28 1000.00 usec
P11 -1.00 dB
SF01 598.2028140 MHz

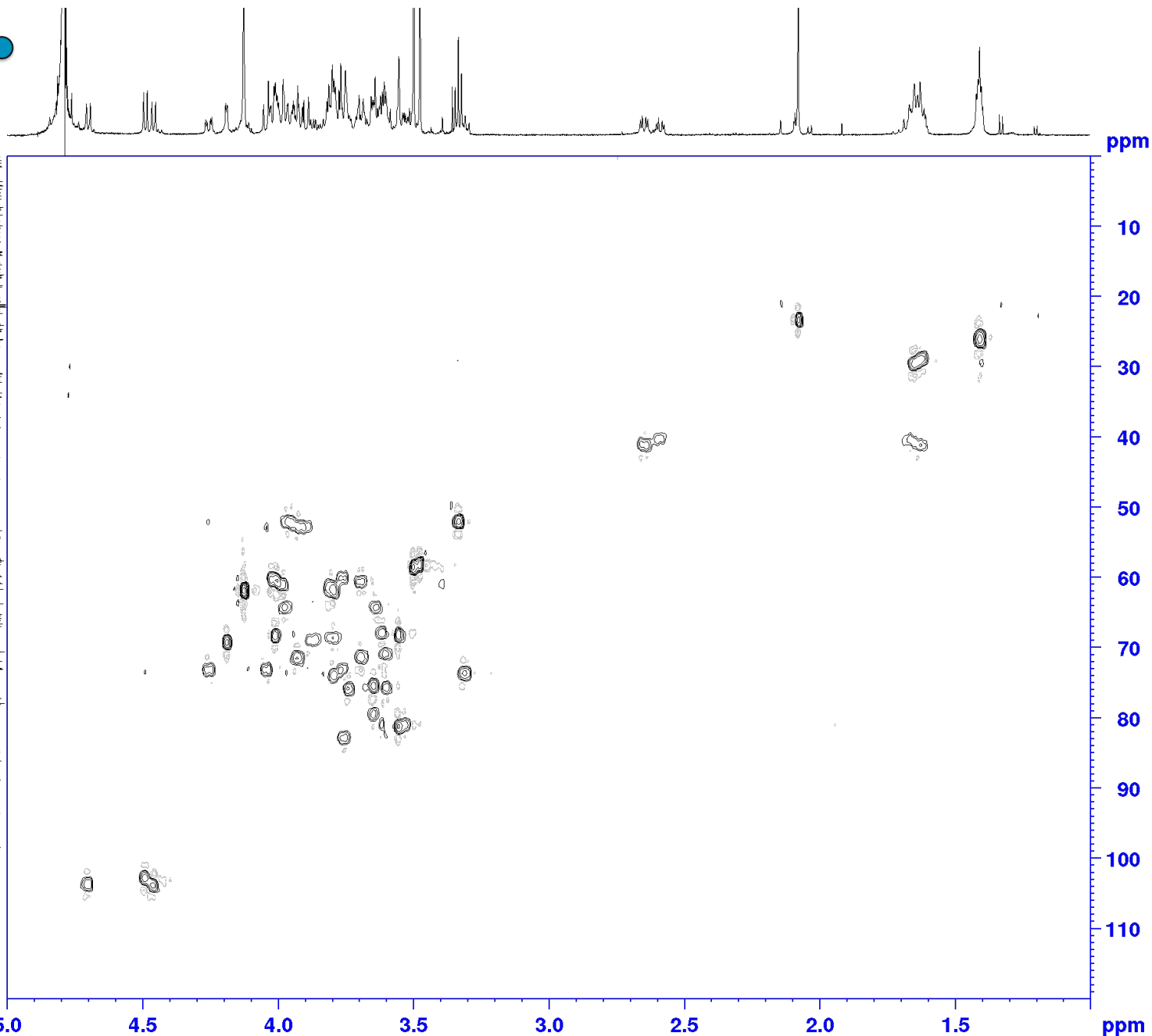
----- CHANNEL f2 -----
CPDPRG2 gamp
NUC2 13C
P3 10.00 usec
P4 20.00 usec
PCPD2 70.00 usec
P12 0 dB
P112 20.00 dB
SF02 150.4287913 MHz

----- GRADIENT CHANNEL -----
GPNAM[1] SINE.100
GPNAM[2] SINE.100
GPX1 0 %
GPX2 0 %
GPY1 0 %
GPY2 0 %
GPZ1 80.00 %
GPZ2 20.10 %
P16 1000.00 usec

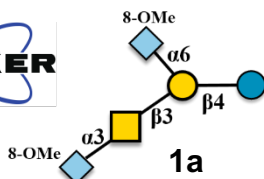
F1 - Acquisition parameters
TD 256
SF01 150.4288 MHz
FIDRES 258.478088 Hz
SW 219.939 ppm
FMODE Echo-Antiecho

F2 - Processing parameters
SI 2048
SF 598.1999730 MHz
WDW QSINE
SSB 4
LB 0 Hz
GB 0
PC 1.00

F1 - Processing parameters
SI 512
MC2 echo-antiecho
SF 150.4173652 MHz
WDW QSINE
SSB 4
LB 0 Hz
GB 0



^1H - ^{13}C HSQC NMR spectrum of Compound 1a (600 MHz/150 MHz D_2O)



THK-isoGAA-7-N3

Current Data Parameters
NAME THK-144-HPIC-top
EXPNO 13
PROCNO 1

F2 - Acquisition Parameters
Date_ 20190601
Time 20.59
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG hmcgpp1pdgdf
TD 2048
SOLVENT cdcl3
NS 128
DS 0
SWH 6009.615 Hz
FIDRES 2.934382 Hz
AQ 0.1703396 sec
RG 32768
DW 83.200 usec
DE 6.00 usec
TE 298.3 K
CNS12 145.000000
CNS13 12.000000
d0 0.00000300 sec
d1 1.00000000 sec
d2 0.00344828 sec
d5 0.04166667 sec
d16 0.00050000 sec
IN0 0.00001661 sec
MCREST 0 sec
MCWRK 1.00000000 sec

===== CHANNEL f1 =====
NUC1 1H
P1 10.50 usec
P2 21.00 usec
PL1 -1.00 dB
SFO1 598.2028140 MHz

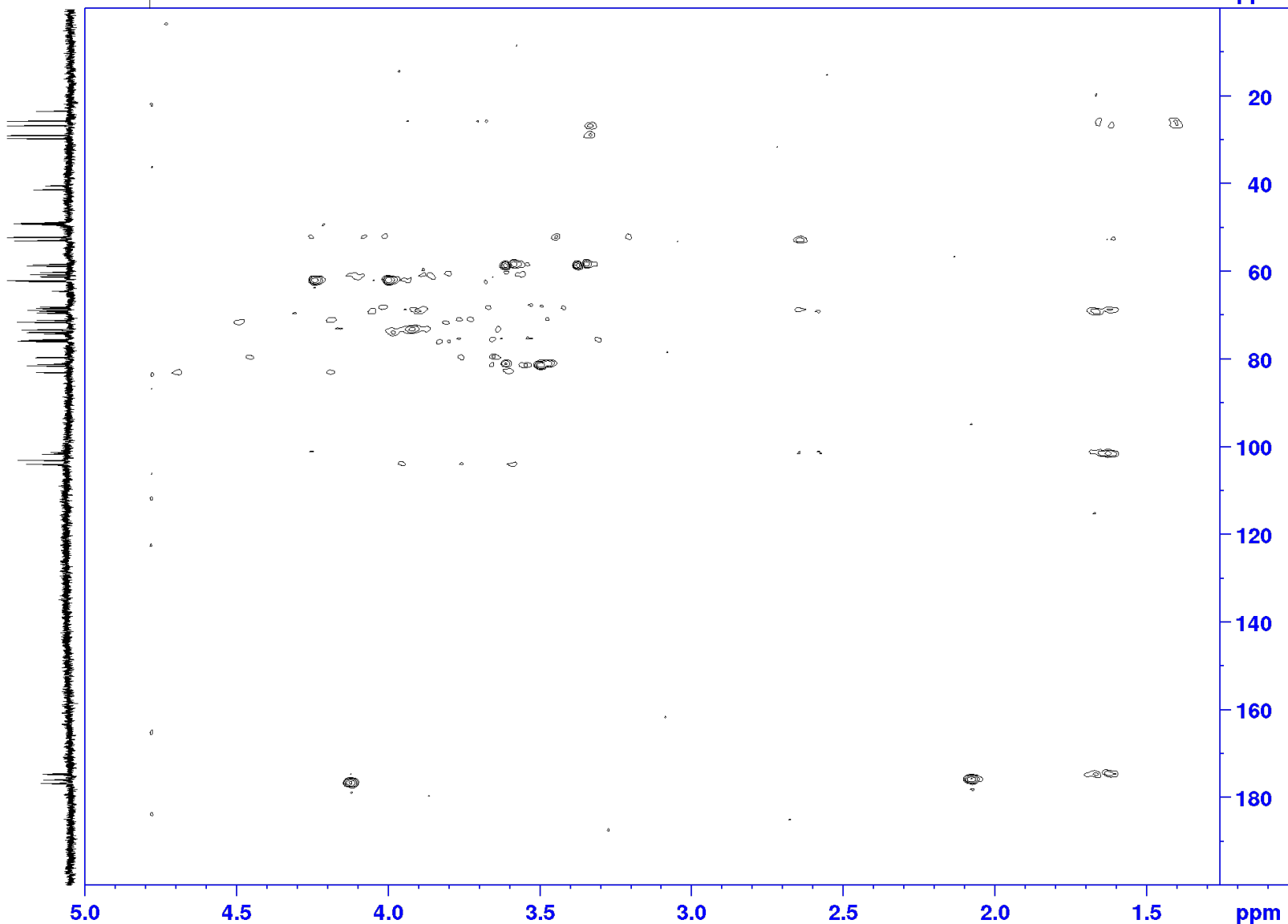
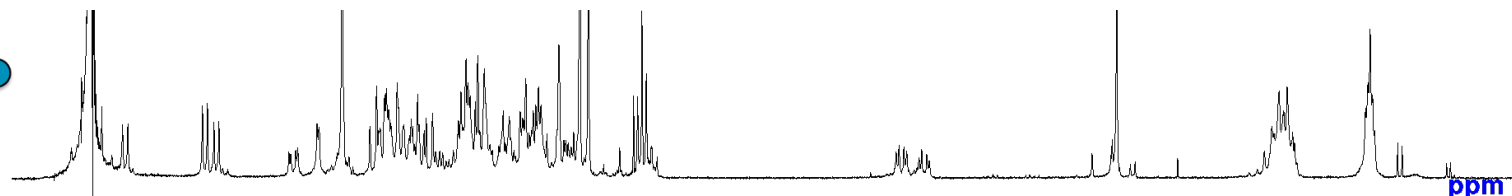
===== CHANNEL f2 =====
NUC2 13C
P3 12.00 usec
PL2 0 dB
SFO2 150.4310476 MHz

===== GRADIENT CHANNEL =====
GPNAM[1] SINE.100
GPNAM[2] SINE.100
GPNAM[3] SINE.100
GPX1 0 %
GPX2 0 %
GPX3 0 %
GPI1 0 %
GPV2 0 %
GPV3 0 %
GPZ1 50.00 %
GPZ2 30.00 %
GPZ3 40.10 %
PI6 1000.00 usec

F1 - Acquisition parameters
TD 256
SFO1 150.431 MHz
FIDRES 235.139206 Hz
SW 200.077 ppm
FhMODE QF

F2 - Processing parameters
SI 4096
SF 598.1999550 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.00

F1 - Processing parameters
SI 512
MC2 QF
SF 150.4173652 MHz
WDW SINE
SSB 0
LB 0 Hz
GB 0



^1H - ^{13}C HMBC NMR spectrum of Compound **1a** (600 MHz/150 MHz D₂O)

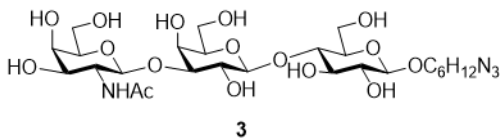
THK-GAA7-0

Current Data Parameters
NAME THK-015-170817
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20170820
Time 22.53
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG zg
TD 32768
SOLVENT H2O+D2O
NS 32
DS 0
SWH 9541.984 Hz
FIDRES 0.291198 Hz
AQ 1.7170432 sec
RG 512
DW 52.400 usec
DE 6.50 usec
TE 300.3 K
D1 2.00000000 sec
MCREST 0 sec
MCWRK 0.01500000 sec

==== CHANNEL f1 =====
NUC1 1H
P1 20.00 usec
PL1 0 dB
SFO1 598.4028152 MHz

F2 - Processing parameters
SI 32768
SF 598.3999460 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



4.7898
4.6332
4.6191
4.4843
4.4710
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4.4351
4.1634
4.1584
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3.9688
3.9409
3.9351
3.9237
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1.4141
1.4079
1.4020
1.3960



1.00
1.03
1.10
1.01
4.14
4.12
3.27
1.20
2.07
2.04
2.09
3.08

2.89
4.44
4.46

¹H NMR spectrum of Compound 3 (600 MHz D₂O)

176.056



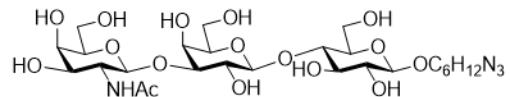
THK-0-GAA-7

Current Data Parameters
NAME THK-015-170817
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20170820
Time 23.01
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG zgpg
TD 32768
SOLVENT CDCl3
NS 5028
DS 0
SWH 45045.047 Hz
FIDRES 1.374666 Hz
AQ 0.3637248 sec
RG 4096
DW 11.100 usec
DE 6.50 usec
TE 300.4 K
D1 3.5000000 sec
d11 0.0300000 sec
DELTA 3.4000010 sec
MCREST 0 sec
MCWRK 0.0150000 sec

===== CHANNEL f1 =====
NUC1 13C
P1 4.80 usec
PL1 0 dB
SFO1 150.4835991 MHz
===== CHANNEL f2 =====
CPDPRG2 waltz16
NUC2 1H
PCPD2 92.00 usec
PL2 120.00 dB
PL12 6.00 dB
PL13 9.00 dB
SFO2 598.4029920 MHz

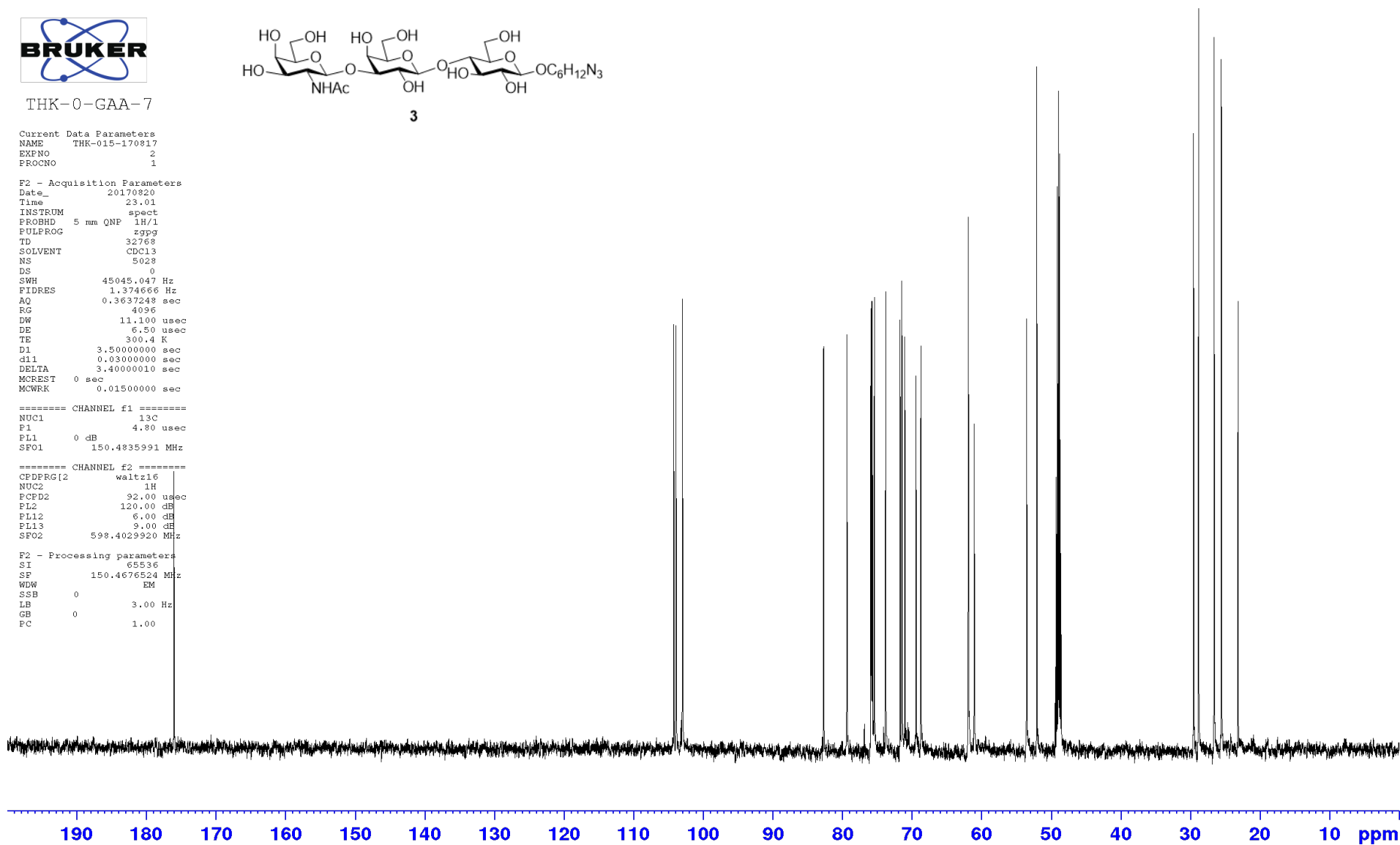
F2 - Processing parameters
SI 65536
SF 150.4676524 MHz
WDW EM
SSB 0
LB 3.00 Hz
GB 0
FC 1.00



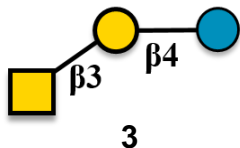
3

104.258
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103.009

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71.471
71.050
69.451
68.748
61.958
61.918
61.092
53.555
52.097
49.432
49.287
49.144
48.999
48.855
48.711
48.568
29.562
28.847
26.631
25.574
23.189



¹³C NMR spectrum of Compound 3 (150 MHz D₂O)



THK-0-GAA-7

Current Data Parameters
NAME THK-015-170817
EXPNO 11
PROCNO 1

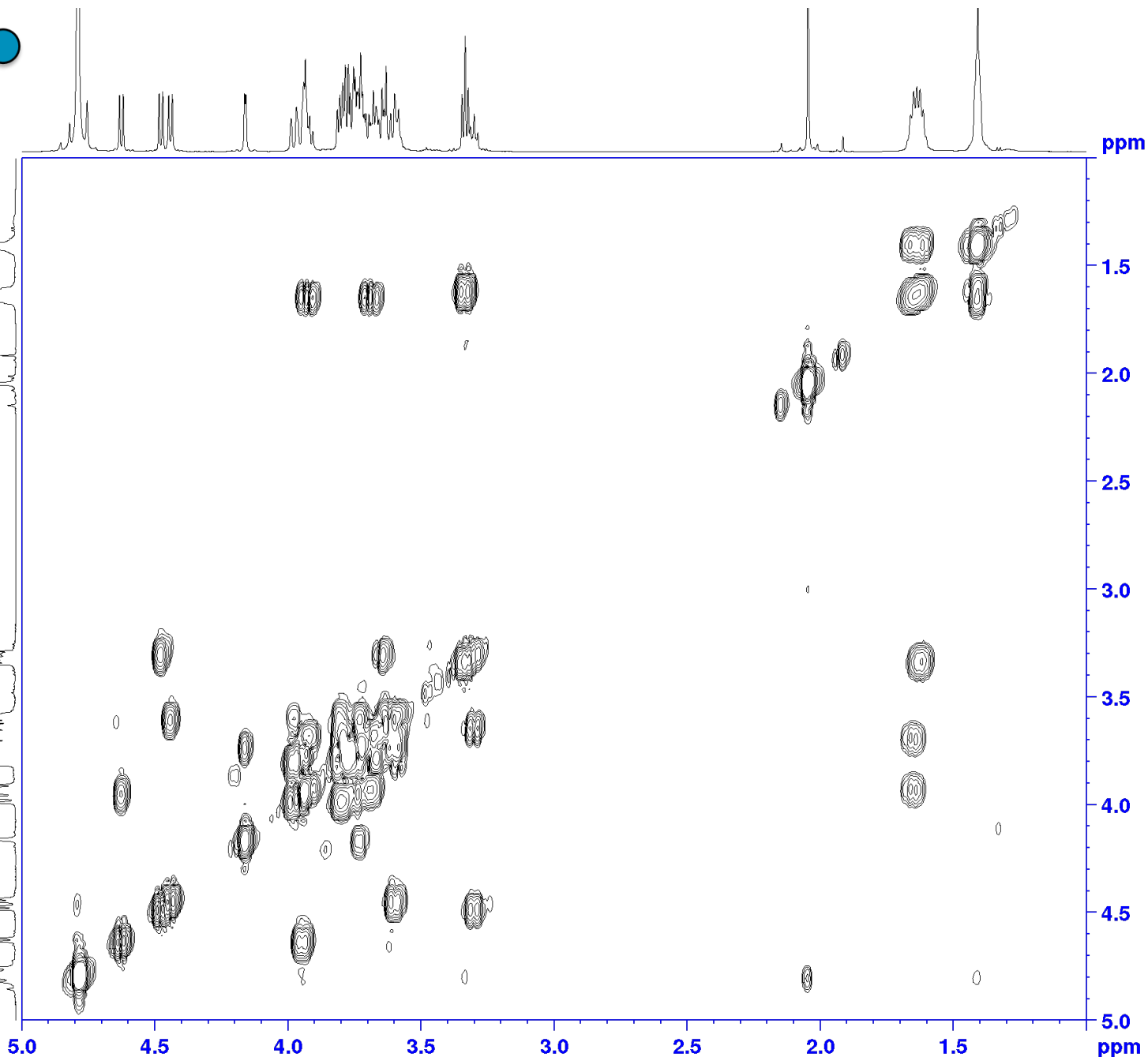
F2 - Acquisition Parameters
Date_ 20170821
Time 6.28
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG cosyprgf
TD 2048
SOLVENT CDCl3
NS 16
DS 0
SWH 6009.615 Hz
FIDRES 2.934382 Hz
AQ 0.1703936 sec
RG 128
DW 83.200 usec
DE 6.50 usec
TE 300.3 K
d0 0.0000300 sec
D1 1.5000000 sec
d11 0.0300000 sec
d12 0.0000200 sec
d13 0.0000400 sec
IN0 0.00016640 sec
MCREST 0 sec
MCWRK 0.03000000 sec

----- CHANNEL f1 -----
NUC1 1H
P0 10.00 usec
P1 11.70 usec
PL1 -2.00 dB
PL9 55.00 dB
SFO1 598.4028150 MHz

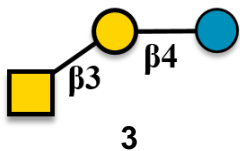
F1 - Acquisition parameters
TD 256
SFO1 598.4028 MHz
FIDRES 46.950119 Hz
SW 10.043 ppm
EnMODE QF

F2 - Processing parameters
SI 4096
SF 598.3999460 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0
PC 1.00

F1 - Processing parameters
SI 512
MC2 QF
SF 598.3999460 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0



^1H - ^1H COSY NMR spectrum of Compound 3 (600 MHz D_2O)



THK-0-GAA-7

```
Current Data Parameters
NAME THK-015-170817
EXPNO 12
PROCNO 1

F2 - Acquisition Parameters
Date_ 20170821
Time 8.26
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG hsqcetps1
TD 2048
SOLVENT CDCl3
NS 32
DS 8
SWH 6009.615 Hz
FIDRES 2.934382 Hz
AQ 0.1703926 sec
RG 16384
DM 83.200 usec
DE 6.50 usec
TE 300.7 K
CNS12 145.0000000
d0 0.0000300 sec
D1 1.20000005 sec
d4 0.00172414 sec
d11 0.03000000 sec
d13 0.0000400 sec
D16 0.00050000 sec
D24 0.00089000 sec
DELTA 0.00157940 sec
DELTA1 0.00150800 sec
INO 0.00001583 sec
MCKEET 0 sec
MCWRR 0.20000041 sec
ST1CNT 0

===== CHANNEL f1 =====
NUC1 1H
P1 11.70 usec
p2 23.40 usec
P2 1000.00 usec
PL1 -2.00 dB
SFO1 598.4028153 MHz

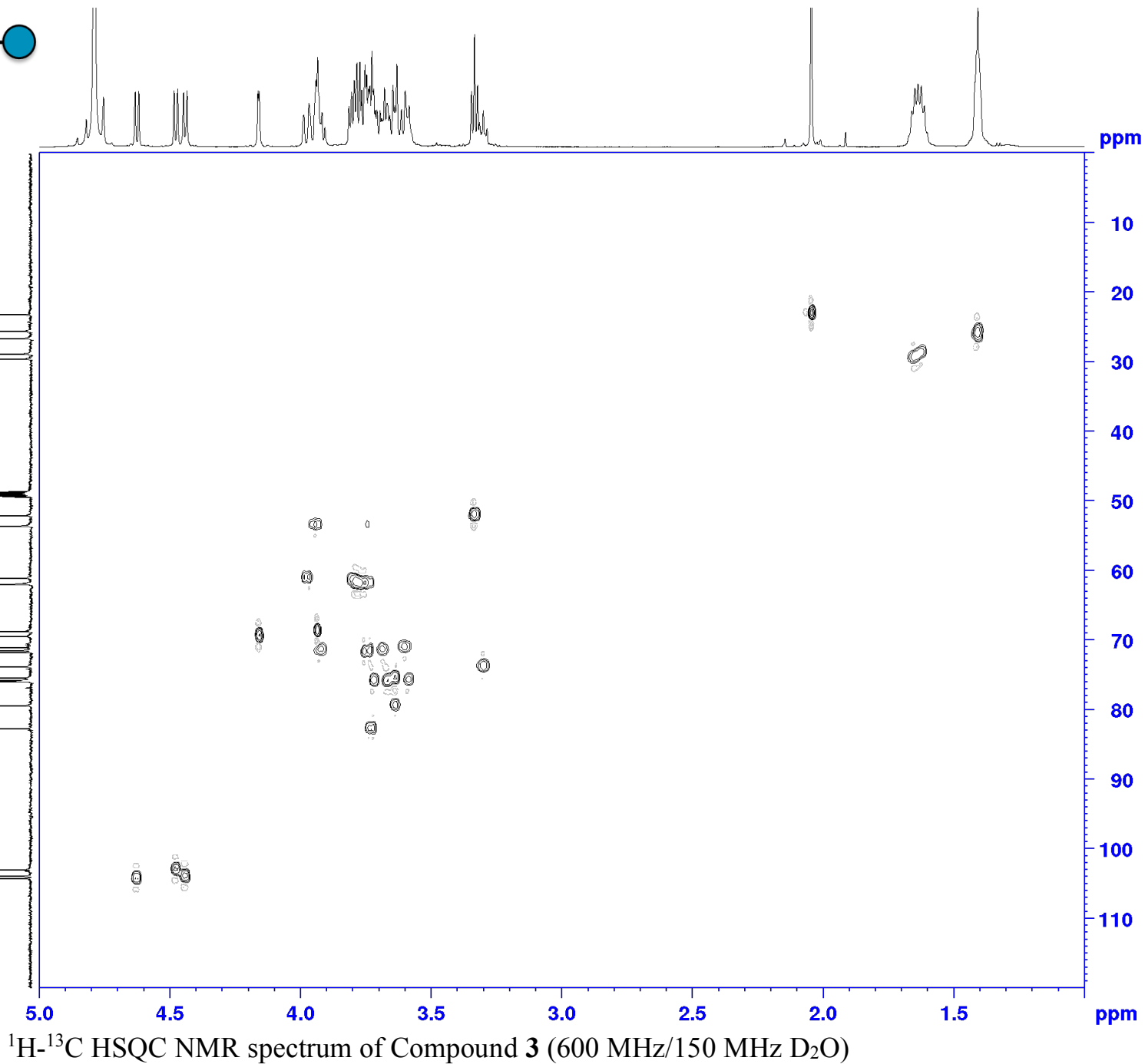
===== CHANNEL f2 =====
CPDPRG2 garp
NUC2 13C
P3 10.00 usec
p4 20.00 usec
PCPD2 70.00 usec
PL2 0 dB
PL12 20.00 dB
SFO2 150.4798374 MHz

===== GRADIENT CHANNEL =====
GPNM[1] SINE.100
GPNM[2] SINE.100
GPK1 0 %
GPK2 0 %
GPV1 0 %
GPV2 0 %
GPE1 80.00 %
GPE2 20.10 %
P16 1000.00 usec

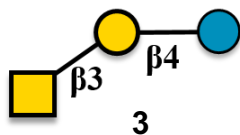
F1 - Acquisition parameters
TD 256
SFO1 150.4798 MHz
FIDRES 246.840459 Hz
SM 209.966 ppm
FaMODE Echo-Antiecho

F2 - Processing parameters
SI 2048
SF 598.3999540 MHz
WDW QSINE
SSB 4
LB 0 Hz
GB 0
PC 1.00

F1 - Processing parameters
SI 512
MC2 echo-antiecho
SF 150.4676524 MHz
WDW QSINE
SSB 4
LB 0 Hz
GB 0
```



^1H - ^{13}C HSQC NMR spectrum of Compound 3 (600 MHz/150 MHz D_2O)



THK-0-GAA-7

```
Current Data Parameters
NAME      THK-015-170817
EXPNO     13
PROCNO    1

F2 - Acquisition Parameters
Date_     20170821
Time      11.37
INSTRUM   spect
PROBHD    5 mm QNP 1H/1
PULPROG   hmbcpgpndqf
TD         2048
SOLVENT   CDCl3
NS         32
DS         0
SWH        6009.615 Hz
FIDRES     2.934382 Hz
AQ         0.1703936 sec
RG         32768
DM         83.200 usec
DE         6.00 usec
TE         301.4 K
CNST2     145.000000
CNST13    12.000000
d0         0.00000300 sec
d1         1.20000005 sec
d2         0.00344828 sec
d4         0.04166667 sec
d16        0.00050000 sec
INVO      0.00001661 sec
MCREST    0 sec
MCWRR     1.20000005 sec

----- CHANNEL f1 -----
NUC1       1H
P1         11.70 usec
P2         23.40 usec
PL1        -2.00 dB
SFO1       598.4028150 MHz

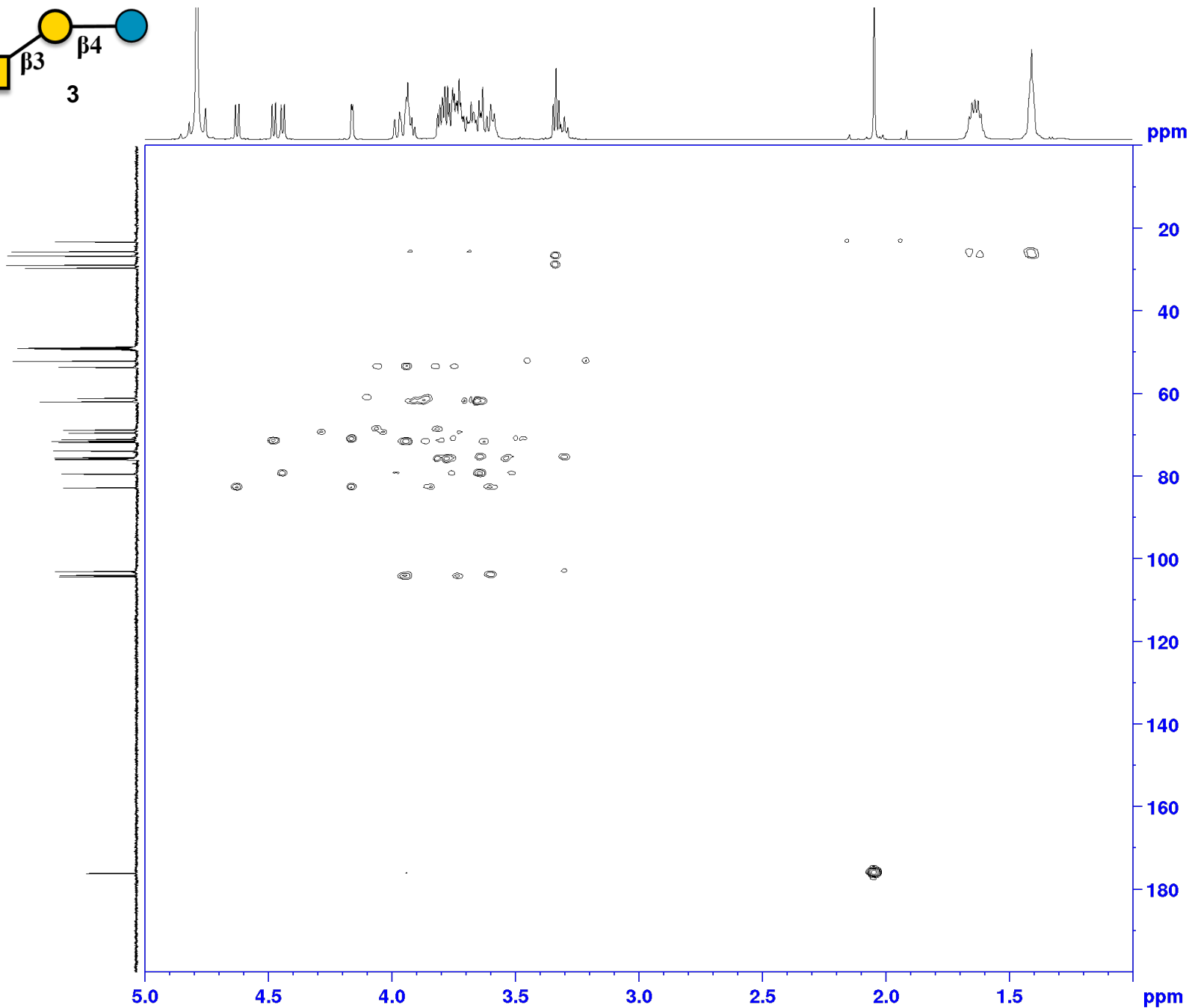
----- CHANNEL f2 -----
NUC2       13C
P3         12.00 usec
PL2         0 dB
SFO2       150.4813421 MHz

----- GRADIENT CHANNEL -----
GPNAM[1]   SINE.100
GPNAM[2]   SINE.100
GPNAM[3]   SINE.100
GPX1       0 %
GPX2       0 %
GPX3       0 %
GPY1       0 %
GPY2       0 %
GPY3       0 %
GPZ1       50.00 %
GPZ2       30.00 %
GPZ3       40.10 %
Pl6        1000.00 usec

F1 - Acquisition parameters
TD         256
SFO1       150.4813 MHz
FIDRES     235.139204 Hz
SW         200.010 ppm
FhMODE     QF

F2 - Processing parameters
SI         4096
SF         598.3999410 MHz
WDW        QSINE
SSB        2
LB         0 Hz
GB         0
PC         1.00

F1 - Processing parameters
SI         512
MC2        QF
SF         150.4676534 MHz
WDW        SINE
SSB        0
LB         0 Hz
GB         0
```



^1H - ^{13}C HMBC NMR spectrum of Compound 3 (600 MHz/150 MHz D_2O)

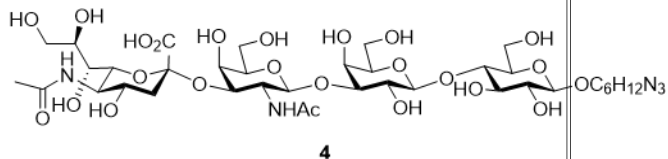
THK-3A-GAA7

Current Data Parameters
NAME THK-041-tetra-180116
EXPNO 1
PROCNO 1

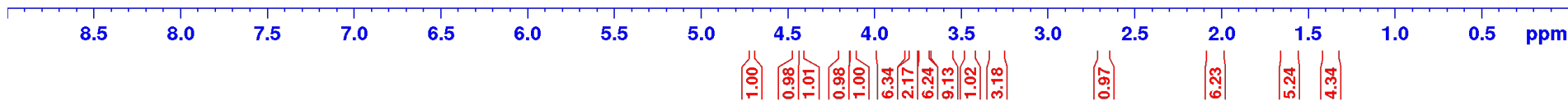
F2 - Acquisition Parameters
Date_ 20180119
Time 0.37
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG zg
TD 32768
SOLVENT D2O
NS 1
DS 0
SWH 8389.262 Hz
FIDRES 0.256020 Hz
AQ 1.9529728 sec
RG 512
DW 59.600 usec
DE 6.50 usec
TE 296.8 K
D1 2.00000000 sec
MCREST 0 sec
MCWRK 0.01500000 sec

==== CHANNEL f1 =====
NUC1 1H
P1 10.00 usec
PL1 -1.00 dB
SFO1 598.3028139 MHz

F2 - Processing parameters
SI 32768
SF 598.2999482 MHz
WDW no
SSB 0
LB 0 Hz
GB 0
PC 2.00



4.7898
4.7150
4.7008
4.4546
4.4412
4.4308
4.4176
4.1770
4.1721
4.1588
4.1539
4.1309
4.1256
3.9605
3.9424
3.9270
3.9069
3.9019
3.8955
3.8904
3.8783
3.8666
3.8619
3.8584
3.8417
3.8381
3.7897
3.7811
3.7694
3.7597
3.7529
3.7399
3.7357
3.7300
3.7224
3.7140
3.7082
3.6921
3.6861
3.6766
3.6653
3.6595
3.6542
3.6486
3.6404
3.6305
3.6177
3.6144
3.6016
3.5899
3.5755
3.5594
3.4610
3.4583
3.4438
3.4410
3.4172
3.3056
3.2941
3.2846
3.2693
3.2594
3.2556
2.8890
2.6813
2.6682
2.6606
2.0634
2.0142
1.6329
1.6205
1.6064
1.6008
1.5948
1.5821
1.3802
1.3842
1.3785
1.3730
1.3679



¹H NMR spectrum of Compound 4 (600 MHz D₂O)



THK-3A-GAA-7

Current Data Parameters
NAME THK-041-tetra-180116
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20180119
Time 0.42
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG zgpg3
TD 32768
SOLVENT D2O
NS 565
DS 0
SWH 45045.047 Hz
FIDRES 1.374666 Hz
AQ 0.3637248 sec
RG 4096
DW 11.100 usec
DE 6.50 usec
TE 296.9 K
DL 3.5000000 sec
dL1 0.03000000 sec
DELTA 3.40000010 sec
MCREST 0 sec
MCWRE 0.01500000 sec

----- CHANNEL f1 -----
NUC1 13C
P1 4.80 usec
PL1 0 dB
SFO1 150.4592037 MHz

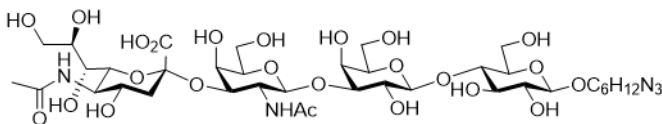
----- CHANNEL f2 -----
CPDPRG2 waltz16
NUC2 1H
PCPD2 92.00 usec
PL2 120.00 dB
PL12 5.50 dB
PL13 9.00 dB
SFO2 598.3029915 MHz

F2 - Processing parameters
SI 65536
SF 150.4425029 MHz
WDW EM
SSB 0
LB 3.00 Hz
GB 0
PC 1.00

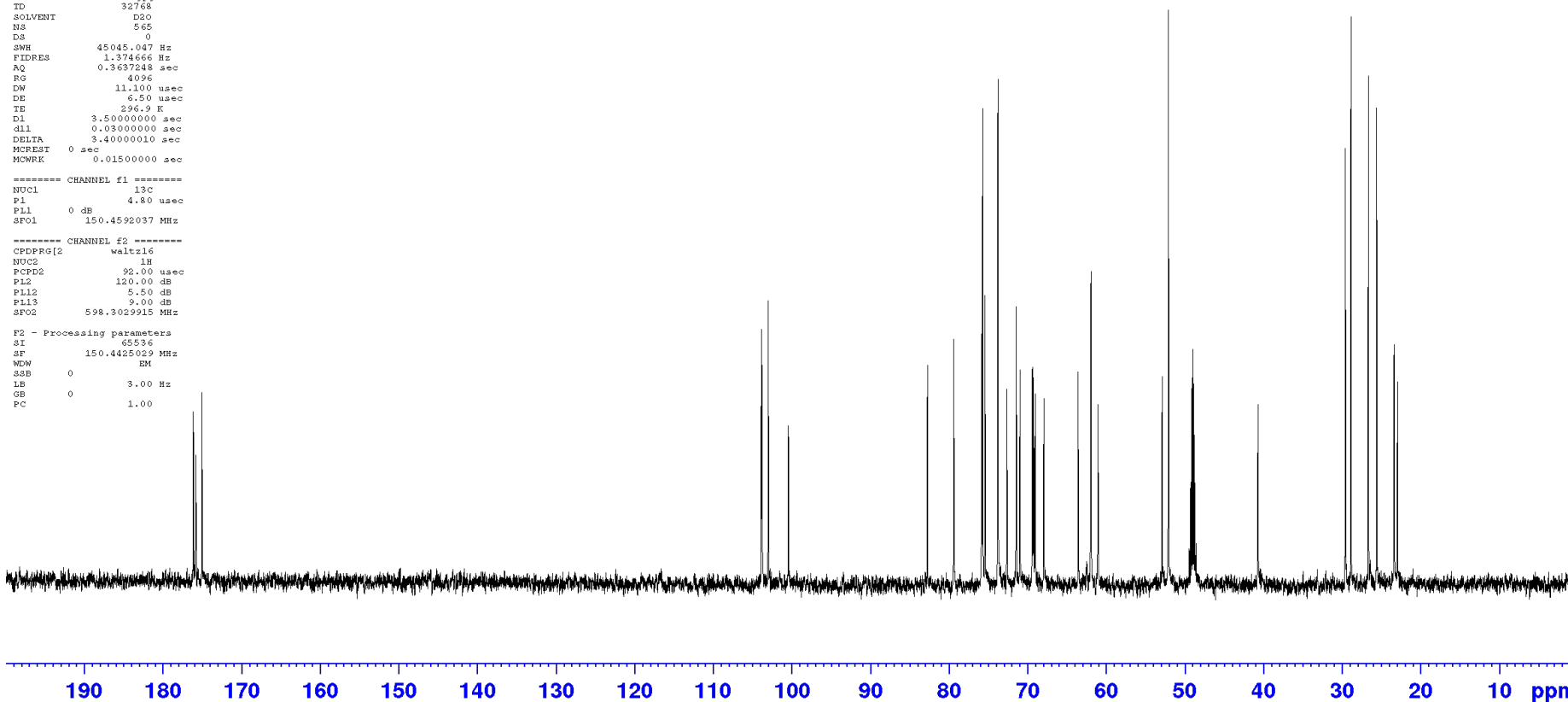
176.156
175.857
175.080

103.882
103.791
103.010
100.443

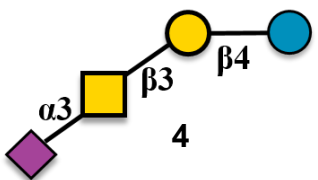
82.745
79.404
75.860
75.725
75.432
73.776
72.639
71.457
70.978
69.390
69.251
69.038
67.936
63.581
61.978
61.939
61.056
52.882
52.087
49.430
49.285
49.143
48.998
48.854
48.710
48.565
40.691
29.569
28.863
26.643
25.583
23.367
22.932



4



¹³C NMR spectrum of Compound 4 (150 MHz D₂O)



THK-3A-GAA-7

Current Data Parameters
NAME THK-041-tetra-180116
EXPNO 11
PROCNO 1

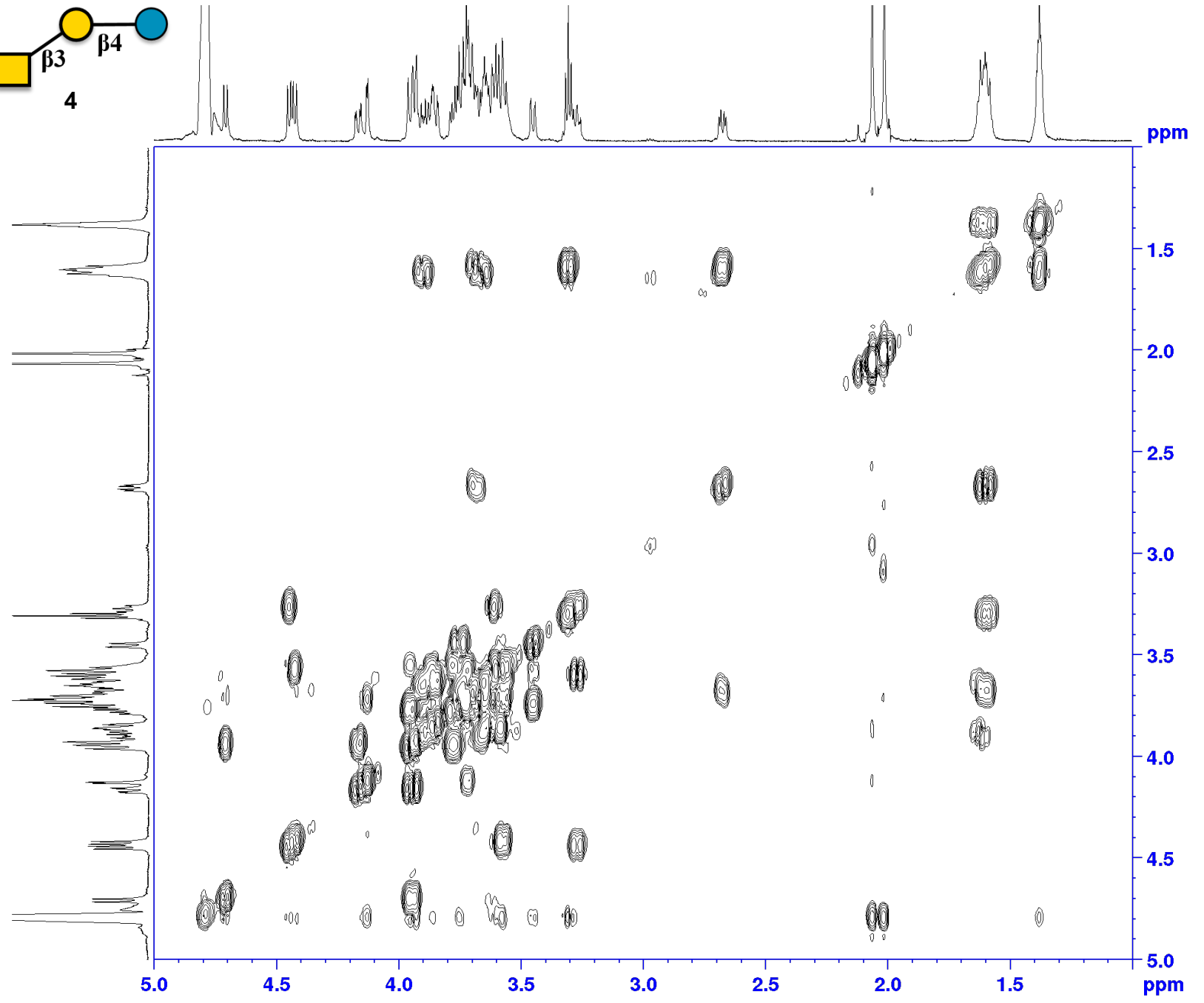
F2 - Acquisition Parameters
Date_ 20180119
Time 3.13
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG cosyprgf
TD 2048
SOLVENT CDCl3
NS 8
DS 0
SWH 6009.615 Hz
FIDRES 2.934382 Hz
AQ 0.1703936 sec
RG 64
DW 83.200 usec
DE 6.50 usec
TE 296.9 K
d0 0.00000300 sec
d1 1.50000000 sec
d11 0.03000000 sec
d12 0.00002000 sec
d13 0.00000400 sec
IN0 0.00016640 sec
MCREST 0 sec
MCWRK 0.03000000 sec

==== CHANNEL f1 =====
NUC1 1H
P0 10.00 usec
P1 11.70 usec
PL1 -2.00 dB
PL9 55.00 dB
SFO1 598.3028142 MHz

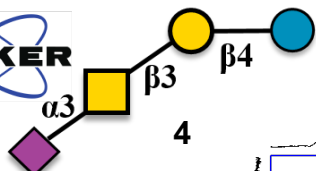
F1 - Acquisition parameters
TD 256
SFO1 598.3028 MHz
FIDRES 46.950119 Hz
SW 10.044 ppm
FhMODE QF

F2 - Processing parameters
SI 4096
SF 598.2999450 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0
PC 1.00

F1 - Processing parameters
SI 512
MC2 QF
SF 598.2999482 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0



^1H - ^1H COSY NMR spectrum of Compound 4 (600 MHz D_2O)



THK-3A-GAA-7

Current Data Parameters
NAME THK-041-tetra-180116
EXPNO 12
PROCNO 1

F2 - Acquisition Parameters
Date_ 20180119
Time 6.07
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG zgpg30psi
TD 2048
SOLVENT cdcl3
NS 8
DS 8
SWH 6009.615 Hz
FIDRES 2.934582 Hz
AQ 0.1703936 sec
RG 16384
DW 83.200 usec
DE 6.50 usec
TE 297.4 K
CST2 145.0000000
d0 0.00000300 sec
d1 1.20000005 sec
d4 0.0012414 sec
d11 0.03000000 sec
d13 0.00000400 sec
d16 0.00000000 sec
D24 0.00089000 sec
DELTA 0.00158400 sec
DELTA1 0.00150800 sec
INO 0.00001583 sec
MORFST 0 sec
MORPR 0.20000041 sec
SIUNT 0

***** CHANNEL f1 *****
NUC1 1H
P1 14.00 usec
p2 28.00 usec
P28 1000.00 usec
PL1 -2.00 dB
SFO1 598.3028142 MHz

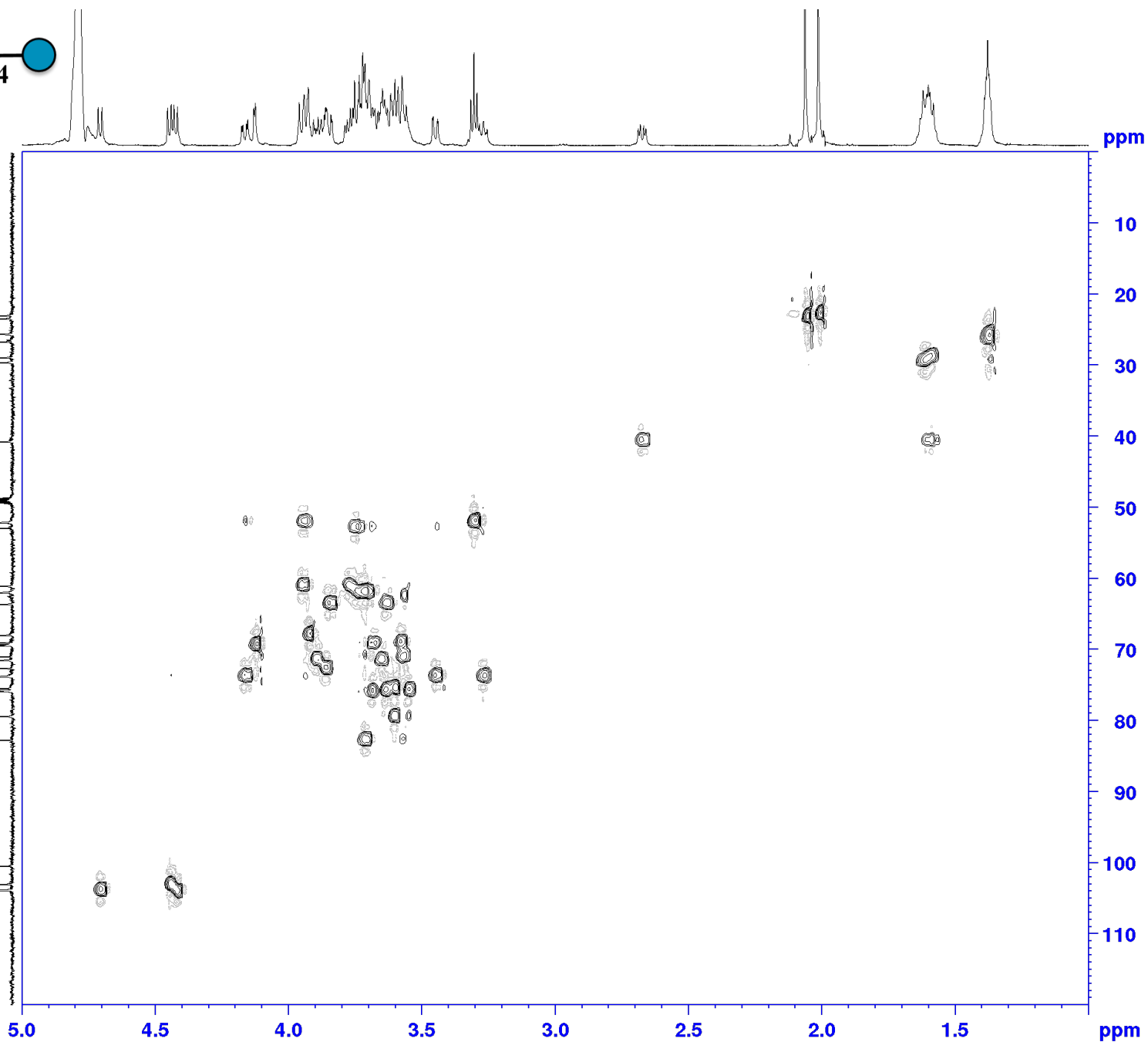
***** CHANNEL f2 *****
CPDPRG2 gexp
NUC2 13C
P3 10.00 usec
p4 20.00 usec
PCPD2 70.00 usec
PL2 0 dB
PL12 20.00 dB
SFO2 150.4546904 MHz

***** GRADIENT CHANNEL *****
GENAM(1) SINE.100
GENAM(2) SINE.100
GPX1 0 %
GPX2 0 %
GPY1 0 %
GPY2 0 %
GPE1 80.00 %
GPE2 20.10 %
PL6 1000.00 usec

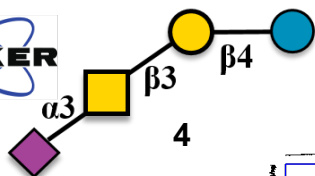
F1 - Acquisition parameters
TD 256
SFO1 150.4547 MHz
FIDRES 246.840439 Hz
SF 210.001 ppm
EnMODE Echo-Antiecho

F2 - Processing parameters
SI 2048
SF 598.2999550 MHz
WDM QSINE
SSB 4
LB 0 Hz
GB 0
PC 1.00

F1 - Processing parameters
SI 512
MC2 echo-antiecho
SF 150.4425029 MHz
WDM QSINE
SSB 4
LB 0 Hz
GB 0



^1H - ^{13}C HSQC NMR spectrum of Compound 4 (600 MHz/150 MHz D_2O)



THK-3A-GAA-7

Current Data Parameters
NAME THK-041-tetra-180116
EXPNO 13
PROCNO 1

F2 - Acquisition Parameters
Date_ 20180119
Time 6.55
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG hmcgplpndgf
TD 2048
SOLVENT CDCl3
NS 8
DS 0
SWH 6009.615 Hz
FIDRES 2.934382 Hz
AQ 0.1703936 sec
RG 32768
DM 83.200 usec
DE 6.00 usec
TE 298.0 K
CHST2 145.0000000
CHST13 12.0000000
d0 0.00000300 sec
D1 1.20000005 sec
d2 0.00344828 sec
d5 0.04166667 sec
D16 0.00050000 sec
IN0 0.00001661 sec
MCREST 0 sec
MCWEX 1.20000005 sec

==== CHANNEL f1 =====
NUC1 1H
F1 14.00 usec
P2 28.00 usec
PL1 -2.00 dB
SFO1 598.3028142 MHz

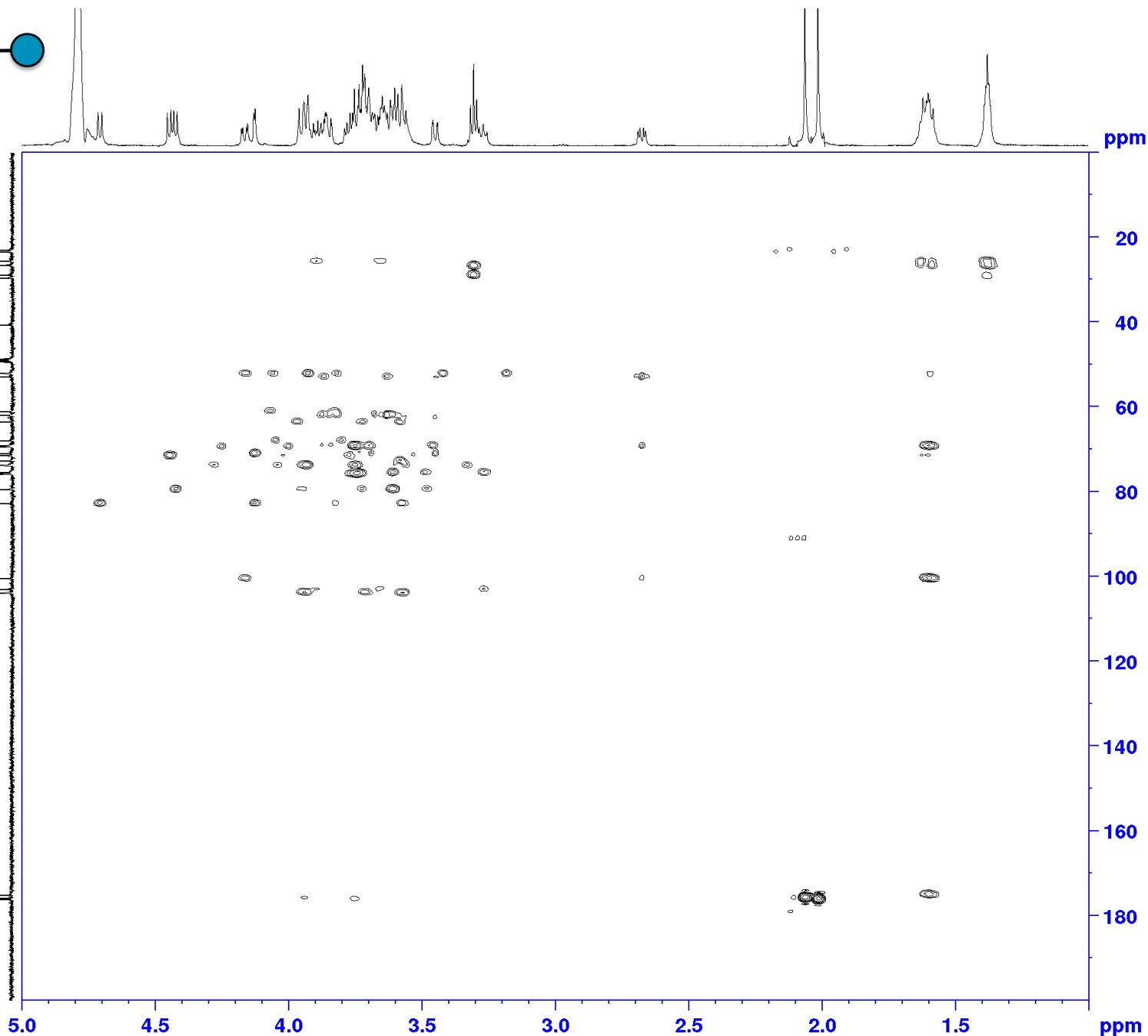
==== CHANNEL f2 =====
NUC2 13C
F2 12.00 usec
PL2 0 dB
SFO2 150.4561948 MHz

==== GRADIENT CHANNEL =====
GENAM(1) SINE.100
GENAM(2) SINE.100
GENAM(3) SINE.100
GPX1 0 %
GPX2 0 %
GPX3 0 %
GPY1 0 %
GPY2 0 %
GPY3 0 %
GPE1 50.00 %
GPE2 30.00 %
GPE3 40.10 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 256
SFO1 150.4562 MHz
FIDRES 235.139206 Hz
SW 200.044 ppm
FAMODE QF

F2 - Processing parameters
SI 4096
SF 598.299400 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.00

F1 - Processing parameters
SI 512
M2 QF
SF 150.4425029 MHz
WDW SINE
SSB 0
LB 0 Hz
GB 0



^1H - ^{13}C HMBC NMR spectrum of Compound 4 (600 MHz/150 MHz D_2O)

4.7898
4.6997
4.6884
4.4690
4.4586
4.4391
4.4299
4.2656
4.2688
4.2353
4.2305
4.1433
4.1380
4.1106
4.0124
4.0077
3.9974
3.9921
3.8971
3.9778
3.9688
3.9544
3.9361
3.9321
3.9206
3.9156
3.9092
3.8044
3.8890
3.8722
3.8647
3.8538
3.8463
3.8373
3.8296
3.8227
3.8019
3.7935
3.7869
3.7812
3.7738
3.7694
3.7646
3.7568
3.7450
3.7393
3.7326
3.7181
3.7133
3.7009
3.6919
3.6804
3.6744
3.6688
3.6635
3.6522
3.6437
3.6406
3.6302
3.6212
3.6160
3.6065
3.6011
3.5874
3.5844
3.5765
3.5713
3.5596
3.5463
3.5227
3.5172
3.5109
3.5038
3.4977
3.4932
3.4817
3.4731
3.4614
3.3307
3.3191
3.3075
3.3050
3.3020
3.3012
3.2983
3.2904
3.2841
3.2772
3.2694
2.6092
2.6019
2.5884
2.5819
2.0674
1.6599
1.6404
1.6346
1.6211
1.6089
1.5971
1.5857
1.4050
1.3989
1.3930
1.3873
1.3813

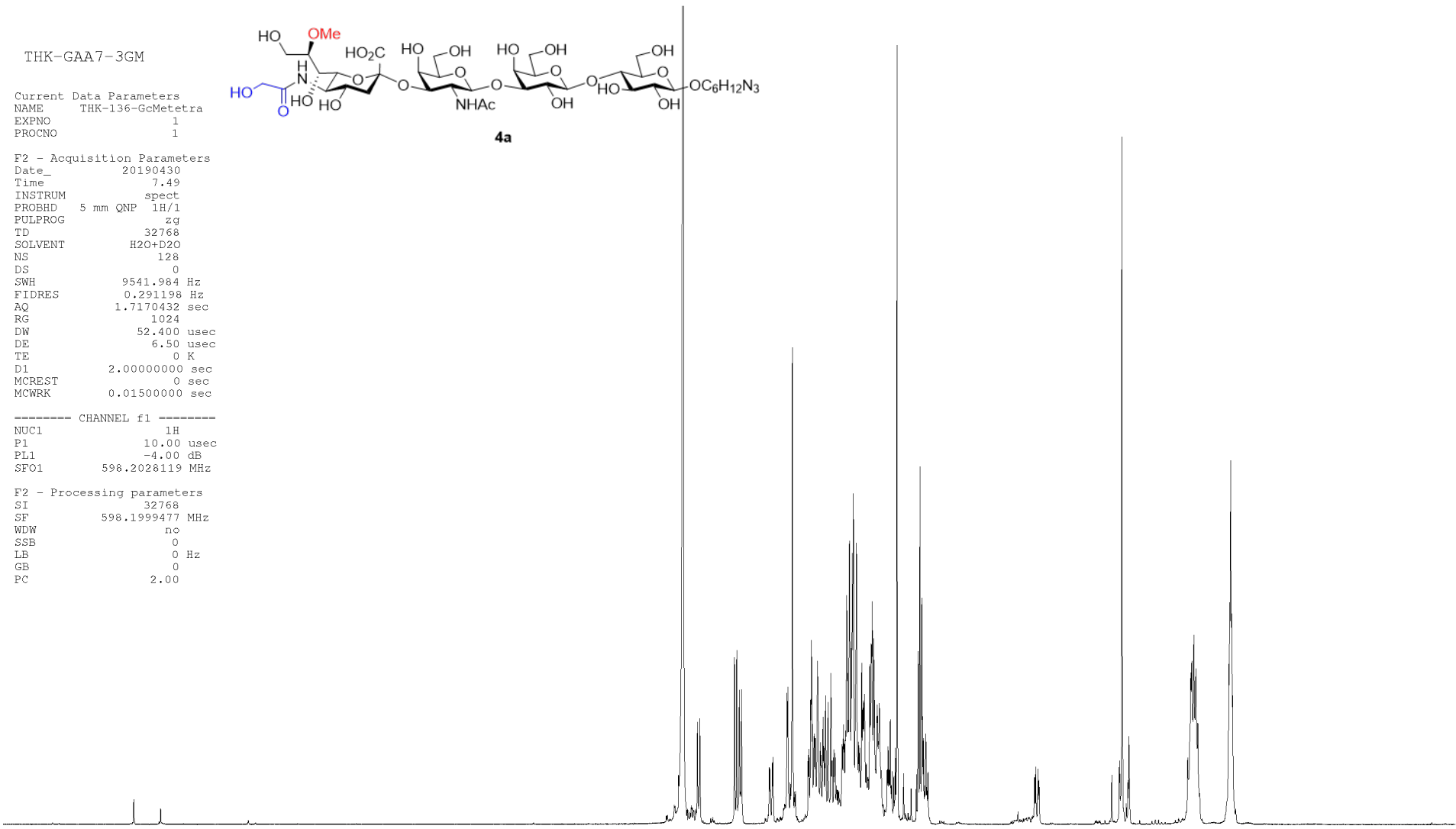
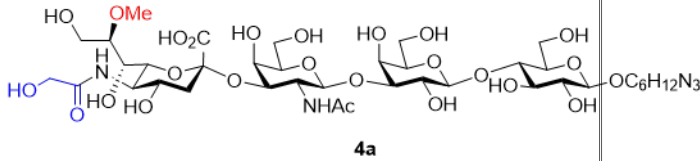
THK-GAA7-3GM

Current Data Parameters
NAME THK-136-GcMetetra
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20190430
Time 7.49
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG zg
TD 32768
SOLVENT H2O+D2O
NS 128
DS 0
SWH 9541.984 Hz
FIDRES 0.291198 Hz
AQ 1.7170432 sec
RG 1024
DW 52.400 usec
DE 6.50 usec
TE 0 K
D1 2.00000000 sec
MCREST 0 sec
MCWRK 0.01500000 sec

==== CHANNEL f1 =====
NUC1 1H
P1 10.00 usec
PL1 -4.00 dB
SFO1 598.2028119 MHz

F2 - Processing parameters
SI 32768
SF 598.1999477 MHz
WDW nc
SSB 0
LB 0 Hz
GB 0
PC 2.00



1.04
1.20
1.14
0.91
1.05
2.16
2.05
2.22
1.31
1.28
1.36
1.29
2.47
2.38
3.21
1.24
2.39
2.48
1.33
2.01
1.24
2.40
1.00
0.95
2.99
5.42
4.08

¹H NMR spectrum of Compound 4a (600 MHz D₂O)



THK-3G-GAA-7

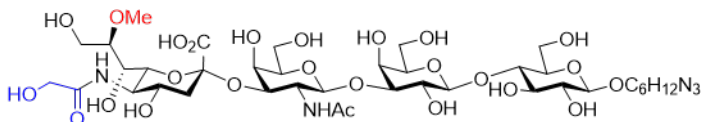
Current Data Parameters
NAME THK-136-GcMetetra
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20190428
Time 7.53
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG zgpg
TD 32768
SOLVENT D2O
NS 2048
DS 0
SWH 45045.047 Hz
FIDRES 1.374666 Hz
AQ 0.3637248 sec
RG 4096
DW 11.100 usec
DE 6.50 usec
TE 0 K
D1 3.5000000 sec
d11 0.0300000 sec
DELTA 3.4000010 sec
MCREST 0 sec
MCWRK 0.0150000 sec

===== CHANNEL f1 =====
NUC1 13C
P1 4.80 usec
PL1 0 dB
SFO1 150.4340559 MHz

===== CHANNEL f2 =====
CPDPRG2 waltz16
NUC2 1H
PCPD2 92.00 usec
PL2 120.00 dB
PL12 3.00 dB
PL13 5.00 dB
SFO2 598.2029910 MHz

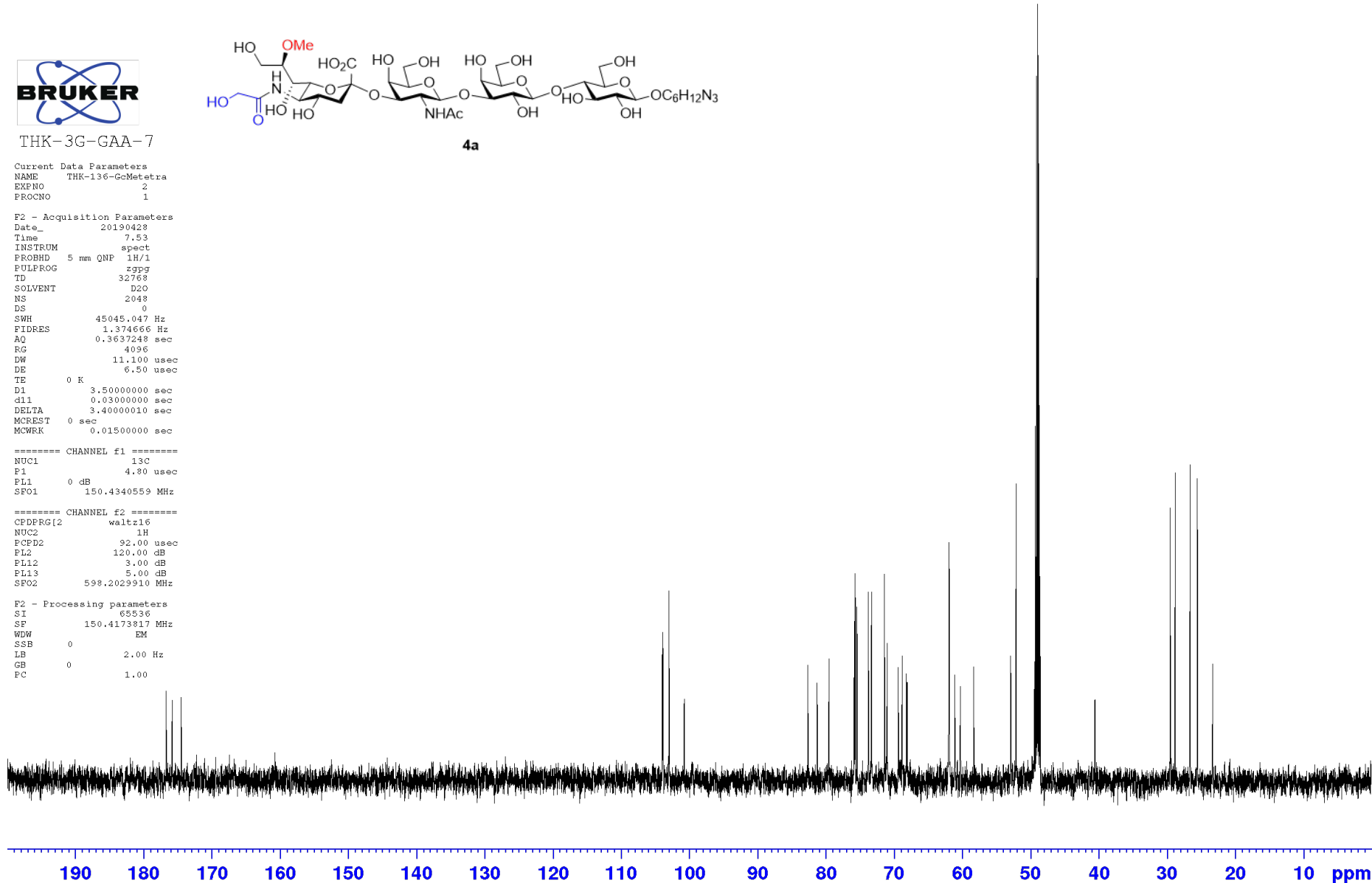
F2 - Processing parameters
SI 65536
SF 150.4173817 MHz
WDW EM
SSB 0
LB 2.00 Hz
GB 0
FC 1.00



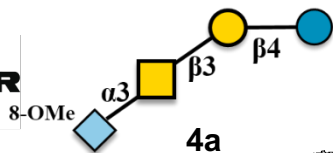
4a

176.713
175.845
174.518

104.006
103.913
103.006
100.780
82.658
81.298
79.557
75.848
75.709
75.679
75.455
73.805
73.325
71.419
71.021
69.417
68.838
68.187
68.093
61.979
61.904
61.137
60.303
58.327
52.909
52.128
52.095
49.431
49.287
49.143
48.999
48.855
48.710
48.566
40.569
29.544
28.805
26.604
25.546
23.295



¹³C NMR spectrum of Compound 4a (150 MHz D₂O)



THK-3GM-GAA-7

Current Data Parameters
NAME THK-136-GcMetetra
EXPNO 11
PROCNO 1

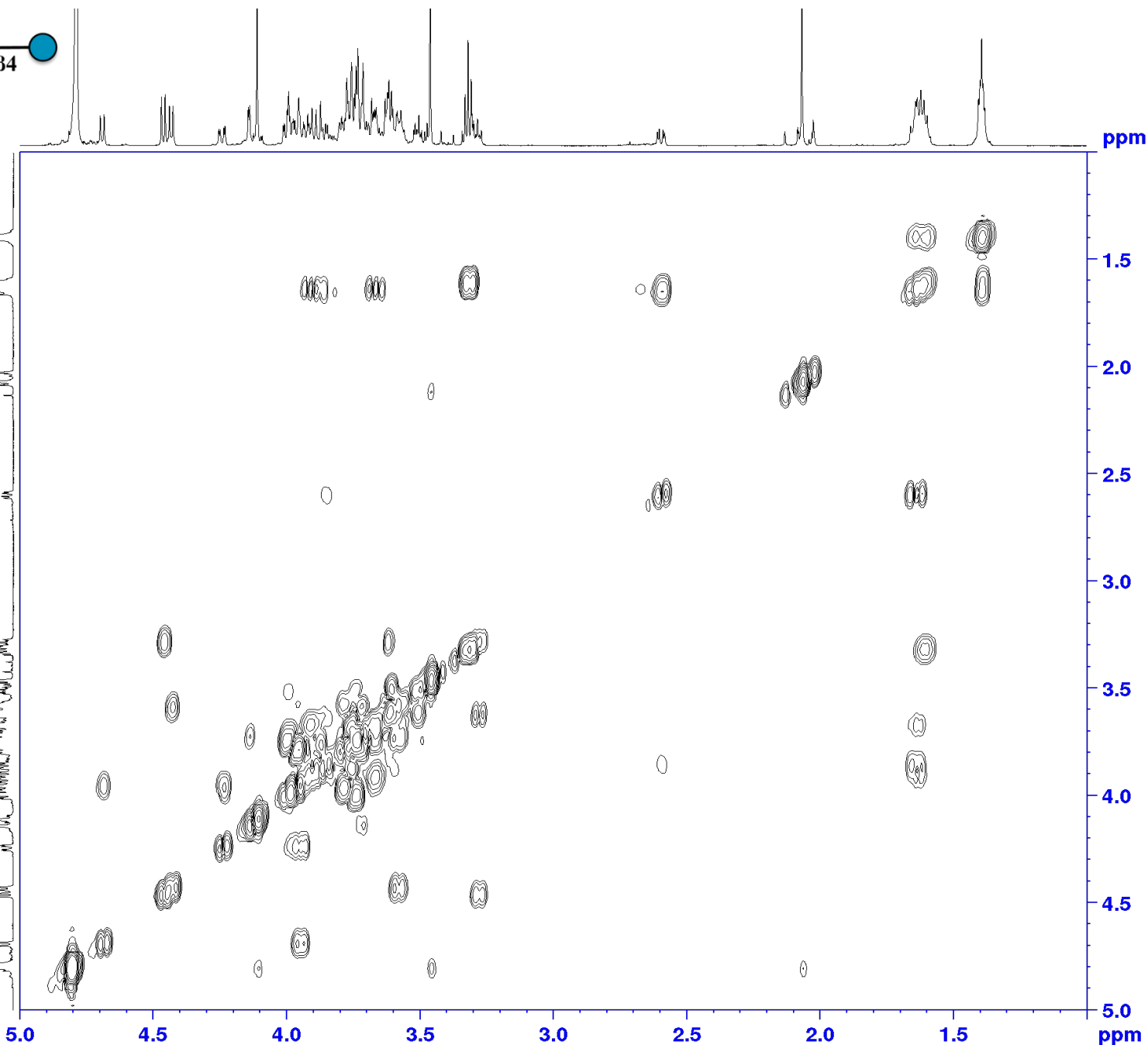
F2 - Acquisition Parameters
Date_ 20190428
Time 13.14
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG cosyqf
TD 2048
SOLVENT Acetone
NS 8
DS 0
SWH 6009.615 Hz
FIDRES 2.934382 Hz
AQ 0.1703936 sec
RG 128
DW 83.200 usec
DE 6.50 usec
TE 0 K
d0 0.00000300 sec
D1 1.50000000 sec
IN0 0.00016640 sec
MCREST 0 sec
MCWRK 1.50000000 sec

----- CHANNEL f1 -----
NUC1 1H
P0 13.00 usec
P1 15.90 usec
PL1 -5.00 dB
SFO1 598.2028100 MHz

F1 - Acquisition parameters
TD 256
SFO1 598.2028 MHz
FIDRES 46.950119 Hz
SW 10.046 ppm
FnMODE QF

F2 - Processing parameters
SI 2048
SF 598.1999350 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0
PC 1.00

F1 - Processing parameters
SI 512
MC2 QF
SF 598.1999350 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0



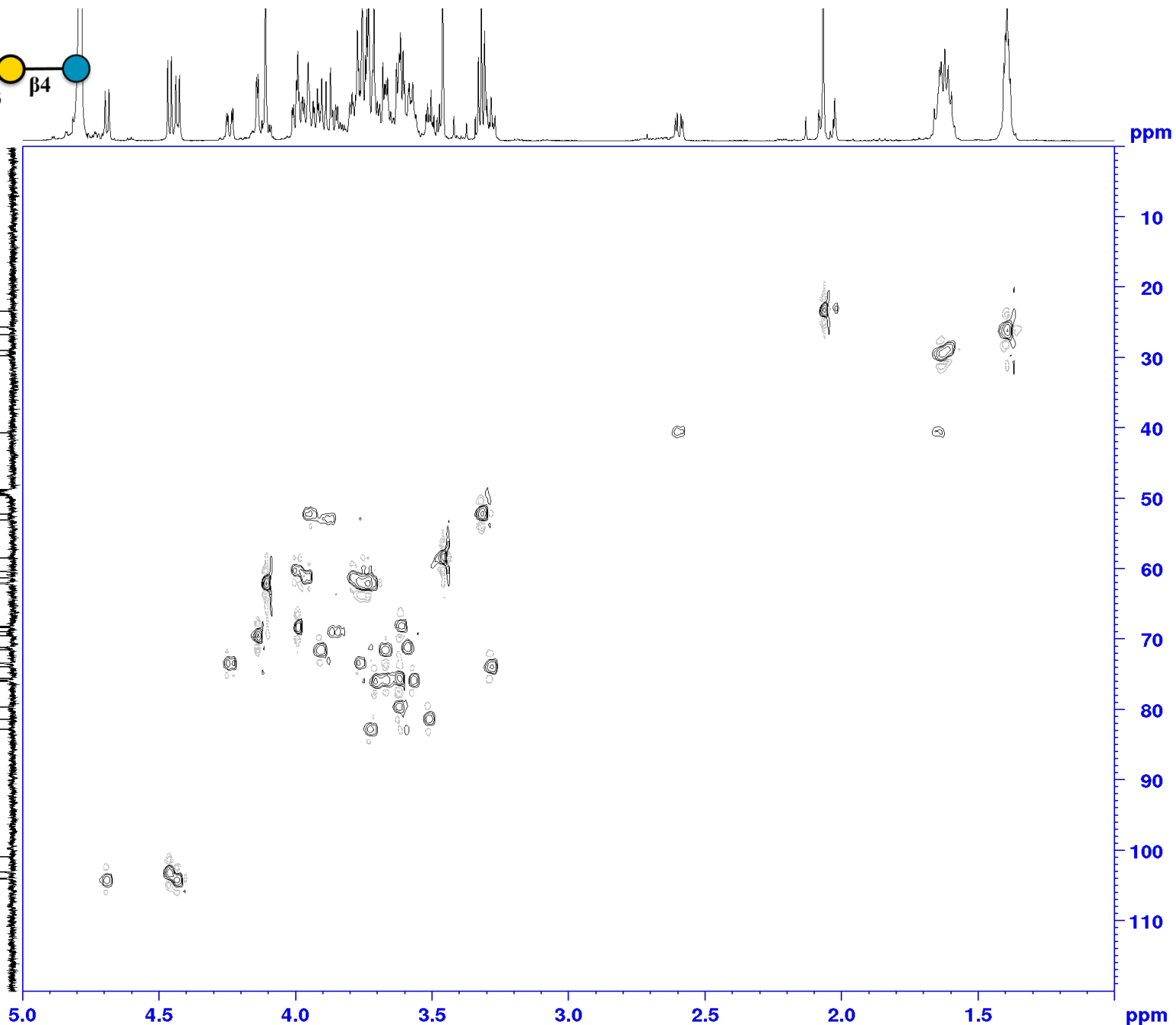
^1H - ^1H COSY NMR spectrum of Compound **4a** (600 MHz D_2O)



8-OMe

α 3

4a



THK-3GM-GAA-7

Current Data Parameters
NAME THK-136-GoMetaza
EXPNO 12
PROCNO 1

F2 - Acquisition Parameters
Date_ 20190428
Time 14.12
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG hsqcetppa
TD 2048
SOLVENT cdcl3
NS 8
DS 8
SWH 6009.615 Hz
FIDRES 2.934362 Hz
AQ 0.1703936 sec
RG 32768
DW 83.200 usec
DE 4.50 usec
TE 0 K

CHST2 145.0000000 sec
d0 0.00000300 sec
d1 1.20000005 sec
d4 0.0012444 sec
d11 0.03000000 sec
d13 0.00000400 sec
d16 0.00050000 sec
d24 0.00089000 sec
DELTA 0.00158780 sec
DELTA1 0.00150800 sec
IN0 0.00001511 sec
MORPH 0 sec
MORPH 0.20000041 sec
ST1CNT 0

***** CHANNEL f1 *****
NUC1 1H
P1 15.90 usec
p2 31.80 usec
P2S 1000.00 usec
PL1 -5.00 dB
SFO1 598.2028100 MHz

***** CHANNEL E2 *****
CPDPRG2 9acp
NUC2 13C
P3 10.00 usec
p4 20.00 usec
P4S 70.00 usec
PL2 0 dB
PL12 20.00 dB
SFO2 150.4287913 MHz

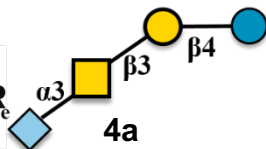
***** GRADIENT CHANNEL *****
GFNAM[1] SINE.100
GFNAM[2] SINE.100
GPX1 0 %
GPX2 0 %
GPY1 0 %
GPY2 0 %
GPH1 80.00 %
GPH2 20.10 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 256
SFO1 150.4288 MHz
FIDRES 258.478088 Hz
SW 219.939 ppm
FAMODE Echo-Antiecho

F2 - Processing parameters
SI 2048
SF 598.199520 MHz
WDW QSINE
SSB 4
LB 0 Hz
GB 0
PC 1.00

F1 - Processing parameters
SI 512
MC2 echo-antiecho
SF 150.4173600 MHz
WDW QSINE
SSB 4
LB 0 Hz
GB 0

^1H - ^{13}C HSQC NMR spectrum of Compound **4a** (600 MHz/150 MHz D_2O)



THK-3GM-GAA-7

Current Data Parameters
NAME THK-136-GMetetra
EXPNO 13
PROCNO 1

F2 - Acquisition Parameters
Date_ 20190428
Time 15.01
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG hmbcgp1pdqf
TD 2048
SOLVENT CDCl3
NS 64
DS 0
SWH 6009.615 Hz
FIDRES 2.934382 Hz
AQ 0.1703936 sec
RG 32768
DW 83.200 usec
DE 6.00 usec
TE 0 K

CHST2 145.0000000
CHST13 12.0000000
d0 0.00000300 sec
D1 1.00000000 sec
d2 0.00348828 sec
d6 0.04166667 sec
D16 0.00050000 sec
IND 0.00001661 sec
MCREST 0 sec
MCWRK 1.00000000 sec

----- CHANNEL f1 -----
NUC1 1H
P1 15.90 usec
P2 31.80 usec
PL1 -5.00 dB
SFO1 598.2028100 MHz

----- CHANNEL f2 -----
NUC2 13C
P3 12.00 usec
PL2 0 dB
SFO2 150.4310476 MHz

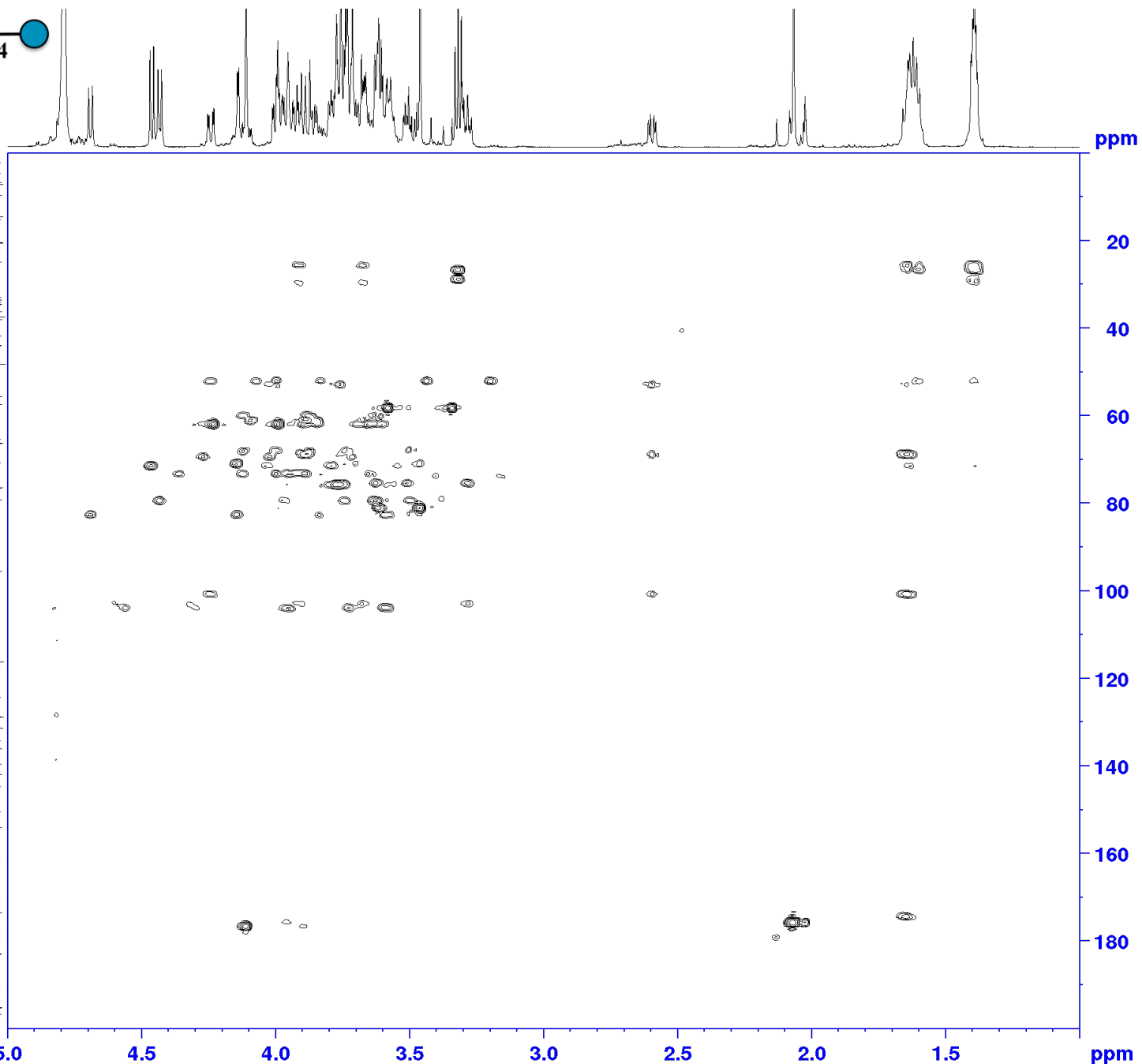
----- GRADIENT CHANNEL -----
GPNAM[1] SINE.100
GPNAM[2] SINE.100
GPNAM[3] SINE.100

GPX1 0 %
GPX2 0 %
GPX3 0 %
GPY1 0 %
GPY2 0 %
GPY3 0 %
GPZ1 50.00 %
GPZ2 30.00 %
GPZ3 40.10 %
P16 1000.00 usec

F1 - Acquisition parameters
ID 256
SFO1 150.431 MHz
FIDRES 235.139206 Hz
SW 200.077 ppm
FbMODE QF

F2 - Processing parameters
SI 4096
SF 598.1999270 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
FC 1.00

F1 - Processing parameters
SI 512
MC2 QF
SF 150.4173500 MHz
WDW SINE
SSB 0
LB 0 Hz
GB 0



^1H - ^{13}C HMBC NMR spectrum of Compound **4a** (600 MHz/150 MHz D_2O)

THK-6A-GAA7

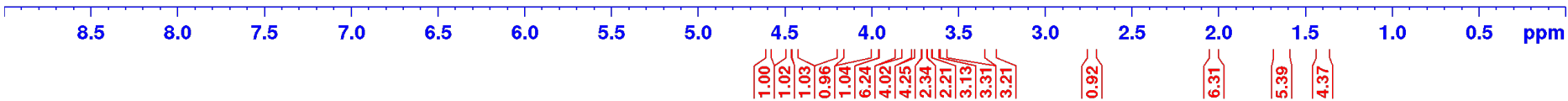
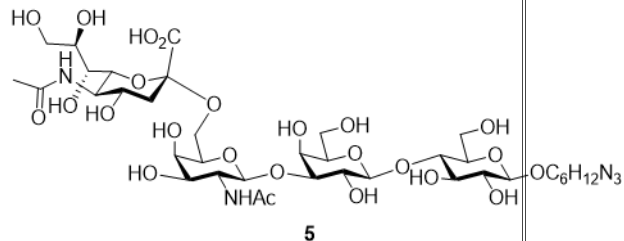
Current Data Parameters
NAME THK-297
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20200504
Time 13.06
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG zg
TD 32768
SOLVENT D2O
NS 32
DS 0
SWH 8389.262 Hz
FIDRES 0.256020 Hz
AQ 1.9529728 sec
RG 1024
DW 59.600 usec
DE 6.50 usec
TE 298.9 K
D1 2.00000000 sec
MCREST 0 sec
MCWRK 0.01500000 sec

===== CHANNEL f1 =====
NUC1 1H
P1 10.00 usec
PL1 -3.00 dB
SFO1 598.1031956 MHz

F2 - Processing parameters
SI 32768
SF 598.0999528 MHz
WDW no
SSB 0
LB 0 Hz
GB 0
PC 2.00

4.7899
4.6030
4.5889
4.4856
4.4722
4.4501
4.4369
4.41835
4.1780
3.9889
3.9855
3.9683
3.9653
3.9498
3.9444
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3.9277
3.9208
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3.9005
3.8964
3.8866
3.8816
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3.8637
3.8541
3.8472
3.8410
3.8062
3.8002
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3.7814
3.7680
3.7613
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3.7335
3.7281
3.7227
3.7173
3.7075
3.7052
3.6998
3.6931
3.6902
3.6839
3.6764
3.6727
3.6669
3.6591
3.6572
3.6465
3.6425
3.6373
3.6319
3.6268
3.6198
3.6003
3.5940
3.5916
3.5872
3.5839
3.5792
3.5764
3.5705
3.5412
3.5297
3.5181
3.5155
3.5019
3.2997
3.2862
3.2463
3.2385
3.2256
2.7178
2.0355
2.0341
1.6772
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1.6448
1.6364
1.6315



¹H NMR spectrum of Compound 5 (600 MHz D₂O)

THK-6A-GAA7

```

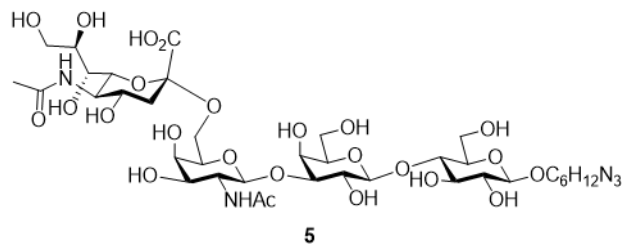
Current Data Parameters
NAME      THK-297
EXPNO    2
PROCNO   1

F2 - Acquisition Parameters
Date_    20200504
Time     13:43
INSTRUM spect
PROBHD   5 mm QNP 1H/1
PULPROG zgpg
TD       32768
SOLVENT  D2O
NS       500
DS       0
SWH      45045.047 Hz
FIDRES   1.374666 Hz
AQ       0.3637248 sec
RG       4096
DW       11.100 usec
DE       6.50 usec
TE       298.6 K
D1       3.5000000 sec
d11      0.03000000 sec
DELTA    3.40000010 sec
MCREST   0 sec
MCWRK    0.01500000 sec

===== CHANNEL f1 =====
NUC1     13C
P1       4.80 usec
PL1      0 dB
SFO1     150.4102296 MHz

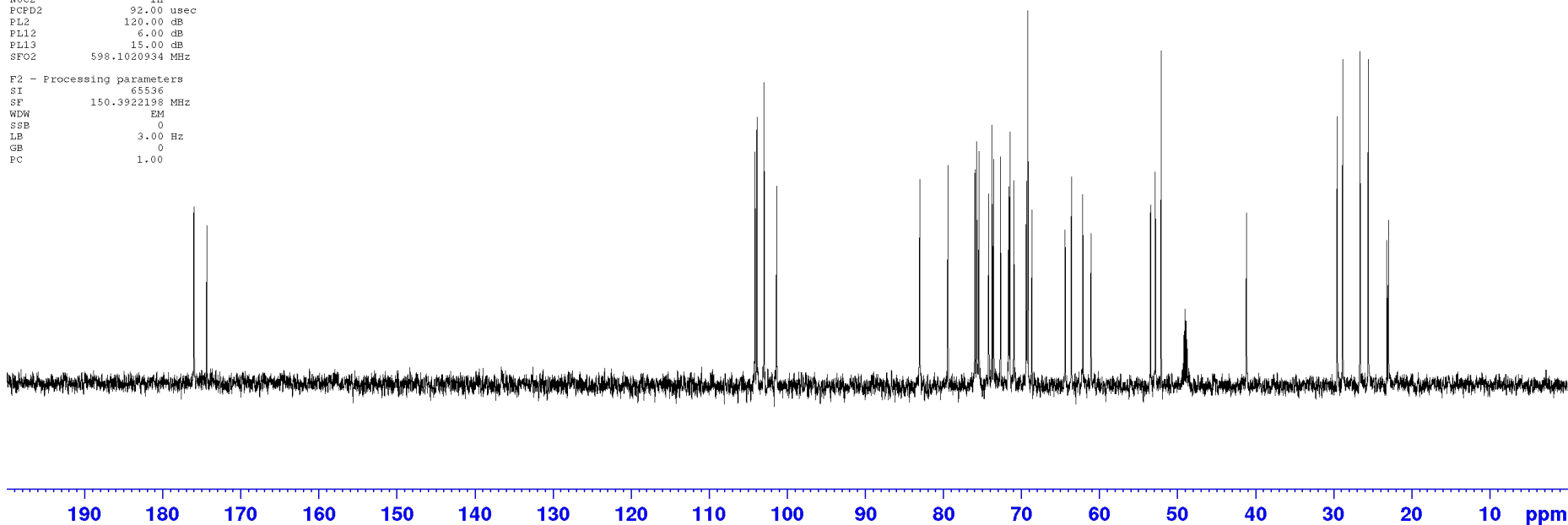
===== CHANNEL f2 =====
CPDPRG2  waltz16
NUC2     1H
PCPD2    92.00 usec
PL2      120.00 dB
PL12     6.00 dB
PL13     15.00 dB
SFO2     598.1020934 MHz

F2 - Processing parameters
SI       65536
SF       150.3922198 MHz
WDW      EM
SSB      0
LB       3.00 Hz
GB       0
PC       1.00
    
```



176.037
175.991
174.371

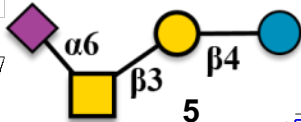
104.132
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79.404
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75.716
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74.172
73.763
73.558
72.687
71.613
71.465
70.965
69.175
68.635
64.384
63.580
62.122
61.075
53.439
52.830
49.432
49.286
49.142
48.998
48.854
48.709
48.569
41.154
29.536
28.830
26.612
25.554
23.177
22.967



¹³C NMR spectrum of Compound 5 (150 MHz D₂O)



THK-6A-GAA7



Current Data Parameters
NAME THK-297
EXPNO 11
PROCNO 1

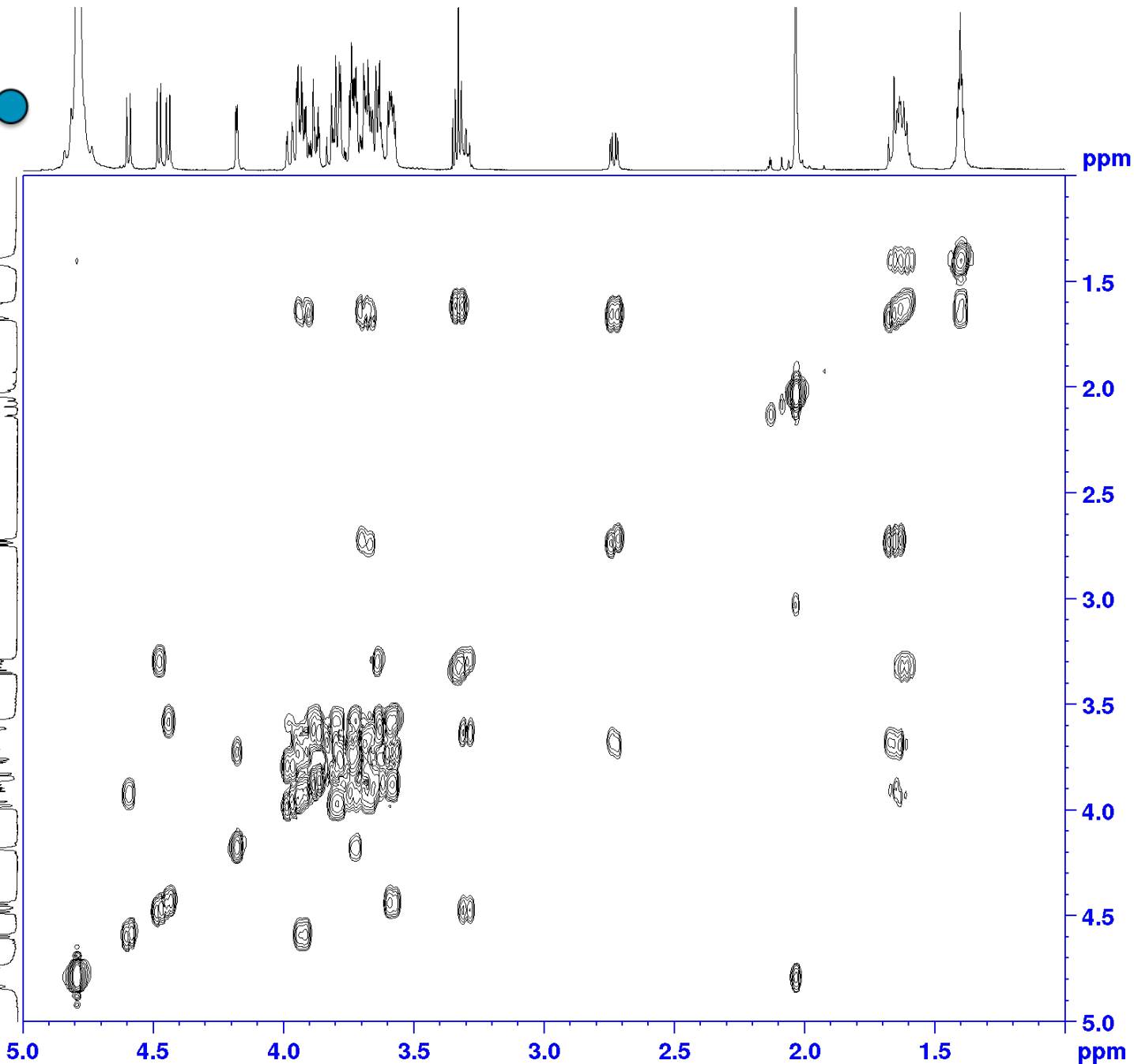
F2 - Acquisition Parameters
Date_ 20200504
Time 13.43
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG cosyqf
TD 2048
SOLVENT D2O
NS 16
DS 0
SWH 6009.615 Hz
FIDRES 2.934382 Hz
AQ 0.1703936 sec
RG 32
DW 83.200 usec
DE 6.50 usec
TE 298.6 K
d0 0.00000300 sec
D1 1.50000000 sec
IN0 0.00016640 sec
MCREST 0 sec
MCWRK 1.50000000 sec

==== CHANNEL f1 =====
NUC1 1H
P0 14.00 usec
P1 40.00 usec
PL1 -3.00 dB
SFO1 598.1028177 MHz

F1 - Acquisition parameters
TD 256
SFO1 598.1028 MHz
FIDRES 46.950119 Hz
SW 10.048 ppm
FnMODE QF

F2 - Processing parameters
SI 2048
SF 598.0999500 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0
PC 1.00

F1 - Processing parameters
SI 512
MC2 QF
SF 598.0999535 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0



^1H - ^1H COSY NMR spectrum of Compound 5 (600 MHz D_2O)



THK-6A-GAA7

Current Data Parameters
NAME THK-297
EXPNO 12
PROCNO 1

F2 - Acquisition Parameters
Date_ 20200505
Time_ 13.53
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG hsqcetgpa1
TD 2048
SOLVENT CH3NO2
NS 8
DS 8
SWH 6009.615 Hz
FIDRES 2.934382 Hz
AQ 0.1703936 sec
RG 16384
TW 83.200 usec
DE 6.50 usec
TE 297.9 K
CNST2 145.0000000
d0 0.00000300 sec
d1 1.20000005 sec
d4 0.00172414 sec
d11 0.03000000 sec
d13 0.00000400 sec
d16 0.00050000 sec
d24 0.00089000 sec
DELTA 0.00162000 sec
DELTA1 0.00150800 sec
INO 0.00001511 sec
MCRET 0 sec
MCMRK 0.20000041 sec
STCNT 0

===== CHANNEL f1 =====
NUC1 1H
F1 32.00 usec
p2 64.00 usec
F28 1000.00 usec
PL1 -3.00 dB
SFO1 598.1028177 MHz

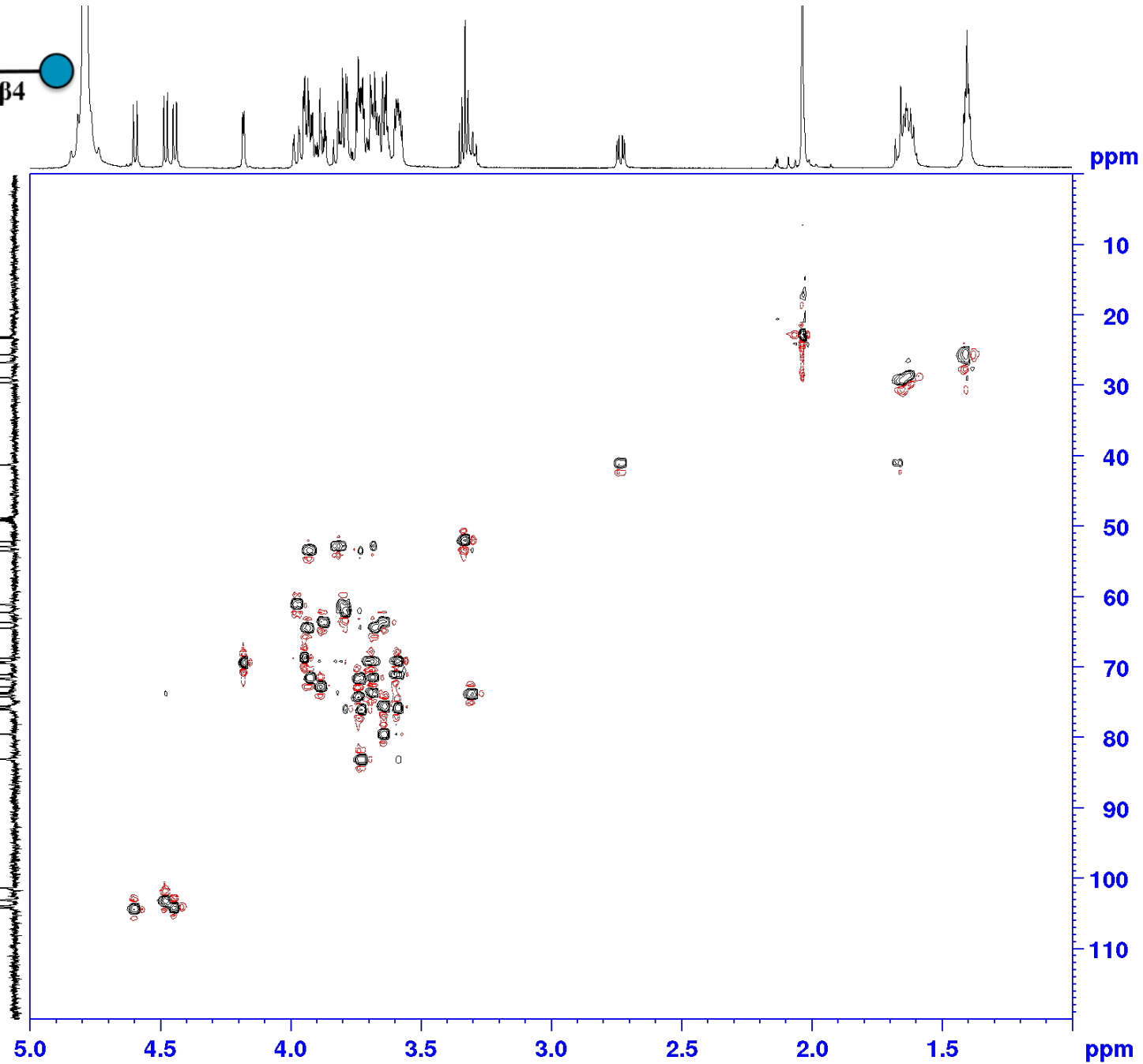
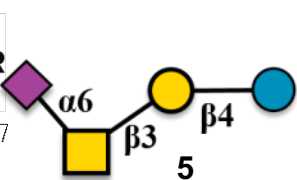
===== CHANNEL E2 =====
CPDPRG2 gcp
NUC2 13C
F3 10.00 usec
p4 20.00 usec
PCPD2 70.00 usec
PL2 0 dB
PL12 20.00 dB
SFO2 150.4036444 MHz

===== GRADIENT CHANNEL =====
GPNAM[1] SINE.100
GPNAM[2] SINE.100
GEX1 0 %
GEX2 0 %
GEY1 0 %
GEY2 0 %
GFX1 80.00 %
GFX2 20.10 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 360
SFO1 150.4036 MHz
FIDRES 183.806641 Hz
SW 219.976 ppm
EPMODE Echo-Antiecho

F2 - Processing parameters
SI 2048
SF 598.0999557 MHz
WDW QSINE
SSB 4
LE 0 Hz
GE 0
FC 1.00

F1 - Processing parameters
SI 512
MC2 echo-antiecho
SF 150.3922053 MHz
WDW QSINE
SSB 4
LE 0 Hz
GE 0



^1H - ^{13}C HSQC NMR spectrum of Compound 5 (600 MHz/150 MHz D_2O)



THK-6A-GAA7

Current Data Parameters
NAME THK-297
EXPNO 13
PROCNO 1

F2 - Acquisition Parameters
Date_ 20200505
Time 15:00
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG hmbcgplpdqf
TD 2048
SOLVENT CHAN02
NS 16
DS 0
SWH 6009.615 Hz
FIDRES 2.934382 Hz
AQ 0.1703936 sec
RG 327.68
DW 83.200 usec
DE 6.00 usec
TE 298.4 K
CNST2 145.0000000
CNST13 2.0000000
d0 0.00000300 sec
D1 1.20000005 sec
d2 0.00344828 sec
d6 0.06250000 sec
D16 0.00050000 sec
INO 0.00001583 sec
MCREST 0 sec
MCREK 1.20000005 sec

==== CHANNEL f1 =====
NUC1 1H
P1 32.00 usec
p2 64.00 usec
PL1 -3.00 dB
SFO1 598.1028177 MHz

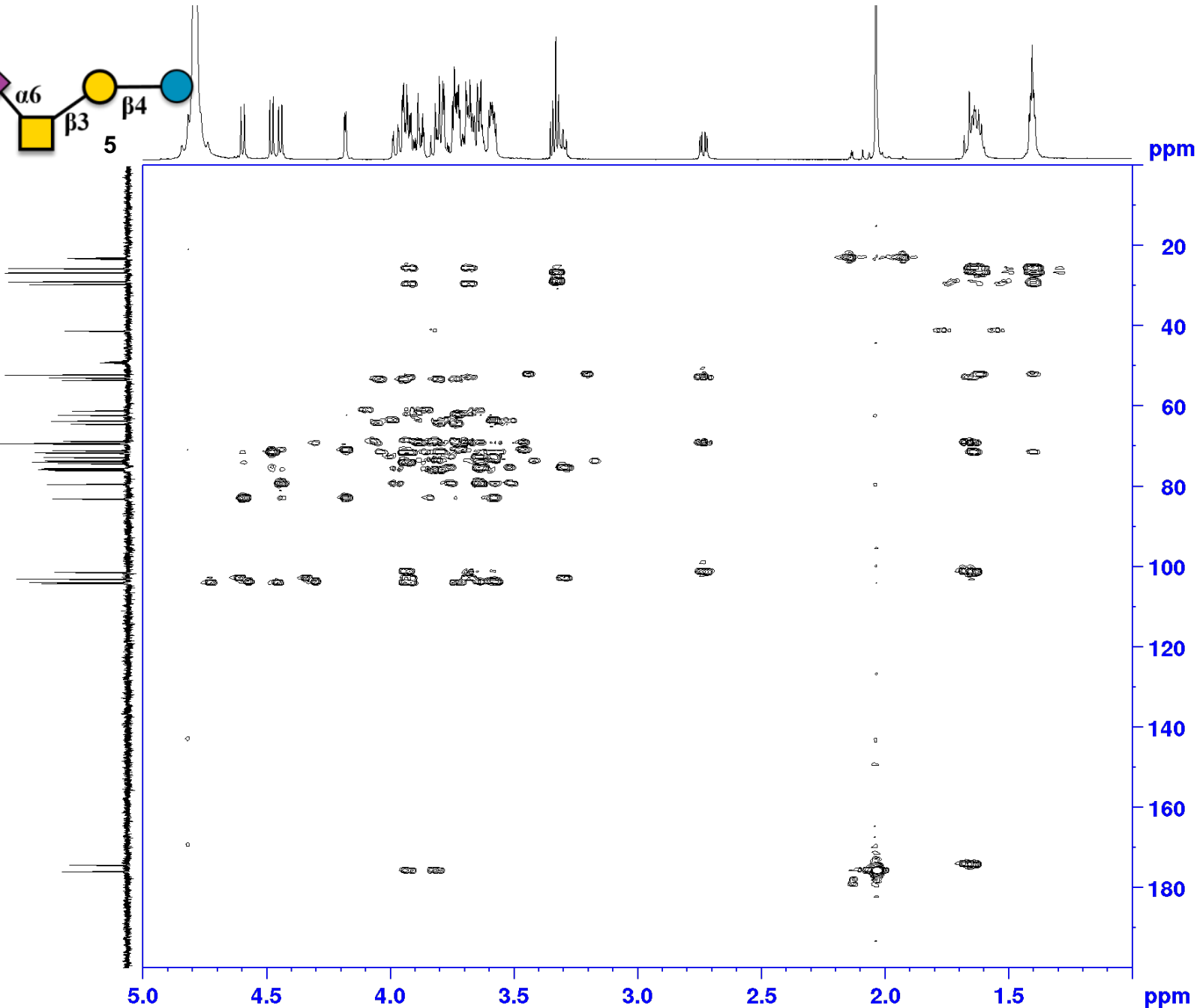
==== CHANNEL f2 =====
NUC2 13C
P3 10.50 usec
PL2 0 dB
SFO2 150.4059003 MHz

==== GRADIENT CHANNEL =====
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GENAM[2] SINE.100
GENAM[3] SINE.100
GPX1 0 %
GPX2 0 %
GPX3 0 %
GPY1 0 %
GPY2 0 %
GPY3 0 %
GPZ1 50.00 %
GPZ2 30.00 %
GPZ3 40.10 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 360
SFO1 150.4059 MHz
FIDRES 175.530975 Hz
SW 210.069 ppm
FMODE QF

F2 - Processing parameters
SI 4096
SF 598.0999360 MHz
MDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.00

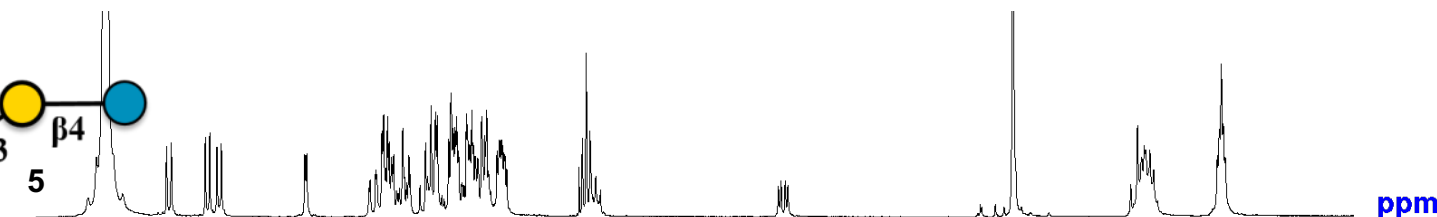
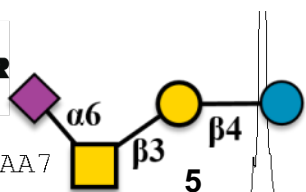
F1 - Processing parameters
SI 512
MC2 QF
SF 150.3922180 MHz
MDW SINE
SSB 0
LB 0 Hz
GB 0



^1H - ^{13}C HMBC NMR spectrum of Compound **5** (600 MHz/150 MHz D_2O)



THK-6A-GAA7



Current Data Parameters
NAME THK-297
EXPNO 15
PROCNO 1

F2 - Acquisition Parameters
Date_ 20200506
Time 3.44
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG hzgpgm1ph
TD 2048
SOLVENT cdcl3
NS 16
DS 8
SWH 6009.615 Hz
FIDRES 2.934392 Hz
AQ 0.1703906 sec
RG 32768
DW 83.200 usec
DE 6.50 usec
TE 569.7 K
CST2 145.0000000
d0 0.0000300 sec
d1 1.200000000 sec
d4 0.00172414 sec
d9 0.07500000 sec
d11 0.00000000 sec
d13 0.00000400 sec
d16 0.00100000 sec
DELTA 0.00200000 sec
DELTA1 0.00007000 sec
FACTOR1 3
IN0 0.00001385 sec
l1 18
MORST 0 sec
MORWK 0.60000002 sec
SCALEP 6

==== CHANNEL f1 =====
NUC1 1H
P1 32.00 usec
p2 64.00 usec
p3 46.69 usec
P6 70.00 usec
p7 140.00 usec
P17 1000.00 usec
P28 1000.00 usec
PL1 -3.00 dB
PL10 4.00 dB
SFO1 598.1028172 MHz

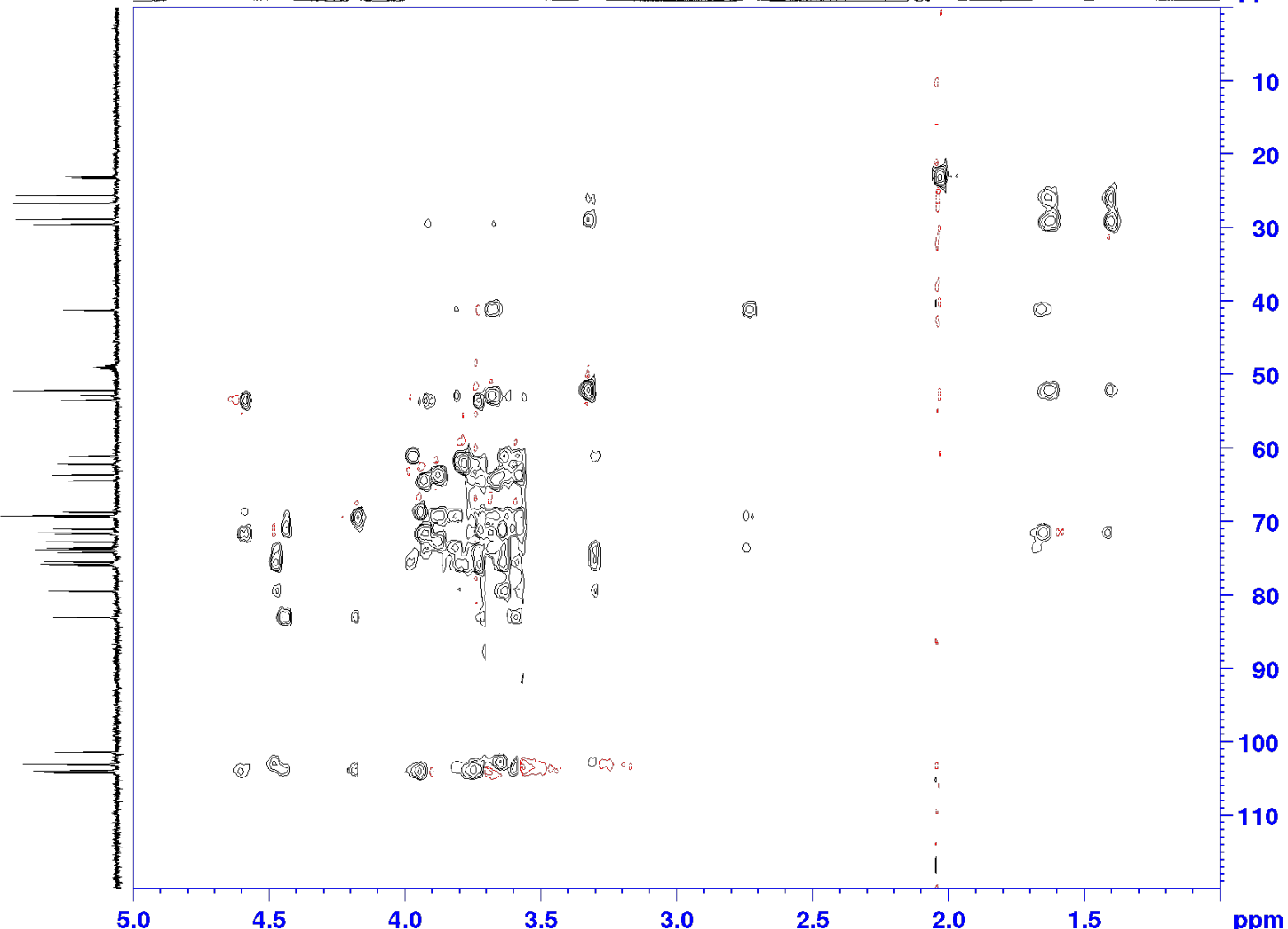
==== CHANNEL f2 =====
CPDPRG2 garrp
NUC2 13C
P3 10.00 usec
p4 20.00 usec
PFD2 70.00 usec
PL2 0 dB
PL12 20.00 dB
SFO2 150.4044237 MHz

==== GRADIENT CHANNEL =====
GPNAM[1] SINE.100
GPNAM[2] SINE.100
GPNAM[3] SINE.100
GFX1 0 %
GFX2 0 %
GFX3 0 %
GPY1 0 %
GPY2 0 %
GPY3 0 %
GPE1 80.00 %
GPE2 30.00 %
GPE3 20.10 %
PL6 1000.00 usec

F1 - Acquisition parameters
TD 300
SFO1 150.4044 MHz
FIDRES 240.673889 Hz
SW 240.027 ppm
F0MODE TFF1

F2 - Processing parameters
SI 2048
SF 598.0999514 MHz
WDW Q8SINE
SSB 2
LB 0 Hz
GB 0
FC 1.00

F1 - Processing parameters
SI 512
M02 TFF1
SF 150.3922204 MHz
WDW Q8SINE
SSB 2
LB 0 Hz
GB 0

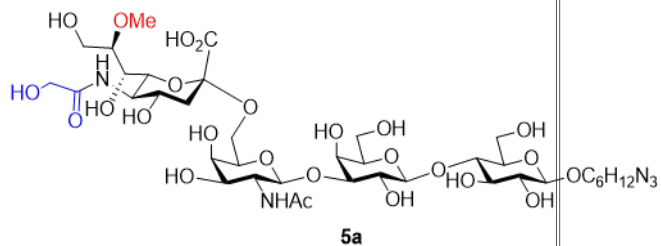


^1H - ^{13}C HSQC-TOCSY NMR spectrum of Compound 5 (600 MHz/150 MHz D_2O)

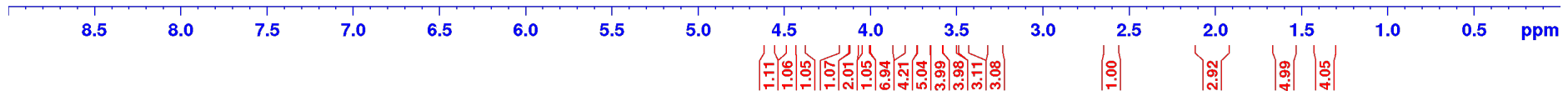
THK-6GM-GAA7

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EXPNO 1
PROCNO 1

F2 - Processing parameters
SI 16384
SF 499.7875562 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



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4.4439
4.4193
4.4036
4.1540
4.1485
4.0925
4.0354
4.0184
3.9965
3.9660
3.8592
3.8462
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3.8858
3.8767
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3.7446
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2.5936
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1.6270
1.6127
1.6013
1.5880
1.5742
1.5601
1.3862
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1.3642
1.3570



¹H NMR spectrum of Compound 5a (500 MHz D₂O)

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176.082
174.623

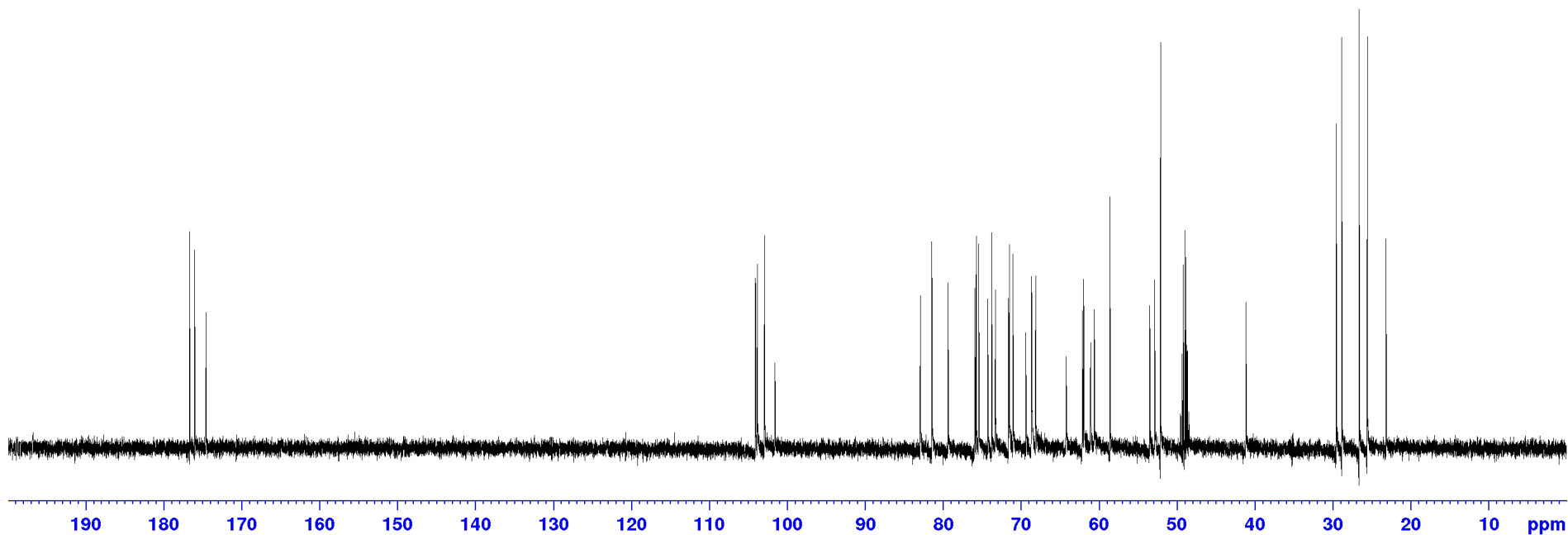
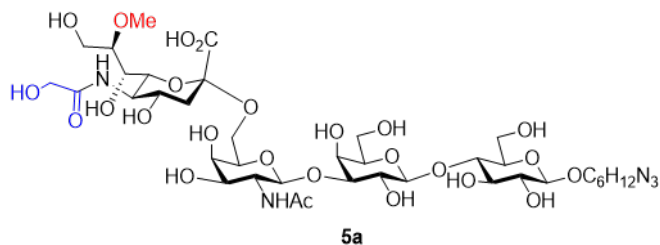
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49.342
48.169
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48.651
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28.849
26.626
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23.183

THK-6GM-GAA7

Current Data Parameters
NAME THK-283-13C-20200328.fid
EXPNO 1
PROCNO 1

F2 - Processing parameters
SI 65536
SF 125.6715636 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



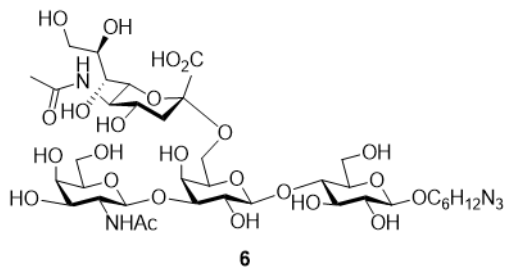
¹H NMR spectrum of Compound 5a (125 MHz D₂O)

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4.1349
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3.9455
3.9393
3.9317
3.9281
3.9215
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3.6200
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3.5447
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3.5266
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2.0019
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1.6965
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1.6314
1.6200
1.6083
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1.3639

THK-6A'-GAA7

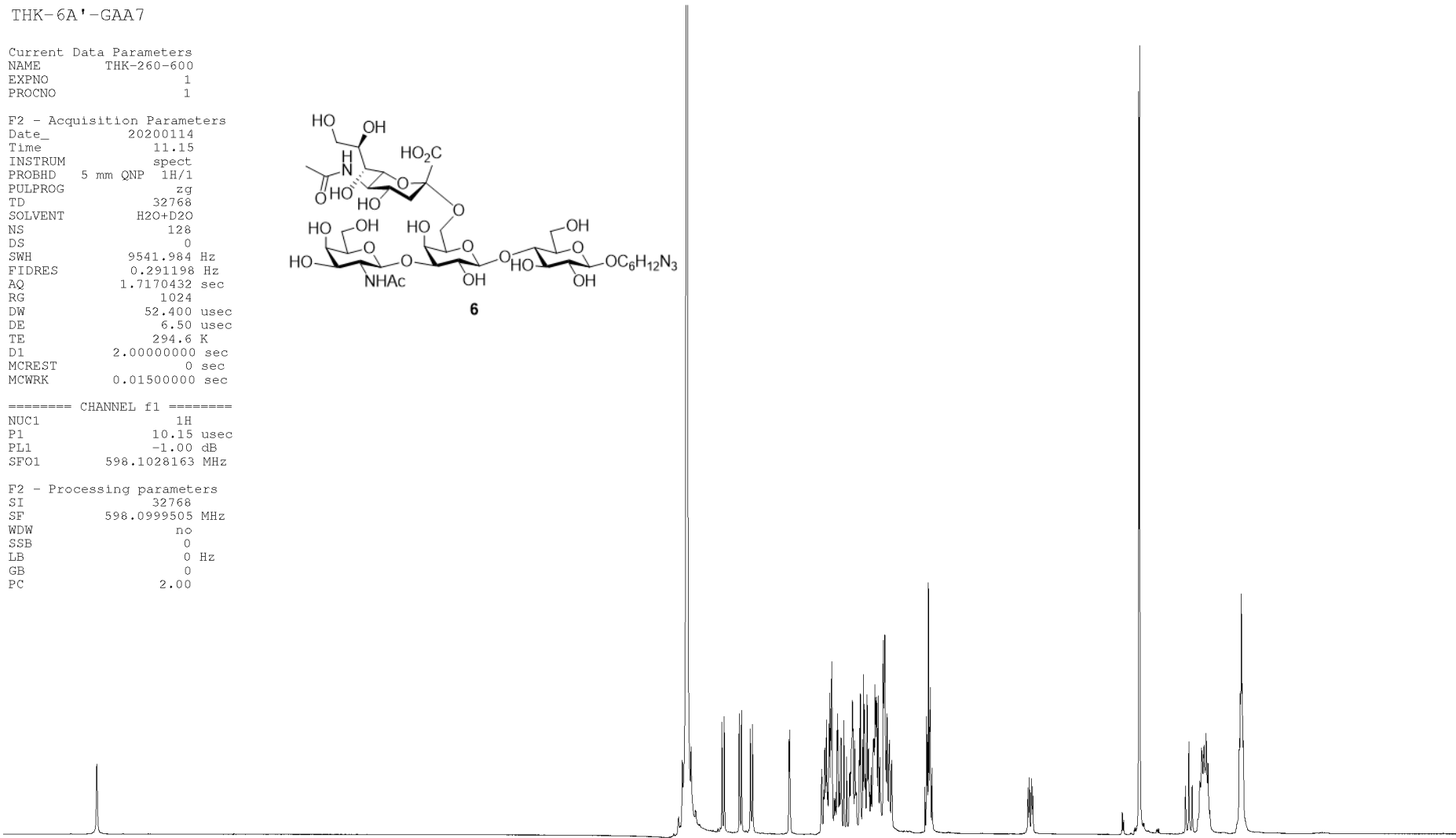
Current Data Parameters
NAME THK-260-600
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20200114
Time 11.15
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG zg
TD 32768
SOLVENT H2O+D2O
NS 128
DS 0
SWH 9541.984 Hz
FIDRES 0.291198 Hz
AQ 1.7170432 sec
RG 1024
DW 52.400 usec
DE 6.50 usec
TE 294.6 K
D1 2.00000000 sec
MCREST 0 sec
MCWRK 0.01500000 sec



===== CHANNEL f1 =====
NUC1 1H
P1 10.15 usec
PL1 -1.00 dB
SFO1 598.1028163 MHz

F2 - Processing parameters
SI 32768
SF 598.0999505 MHz
WDW no
SSB 0
LB 0 Hz
GB 0
PC 2.00



¹H NMR spectrum of Compound 6 (600 MHz D₂O)

THK-6A'-GAA7

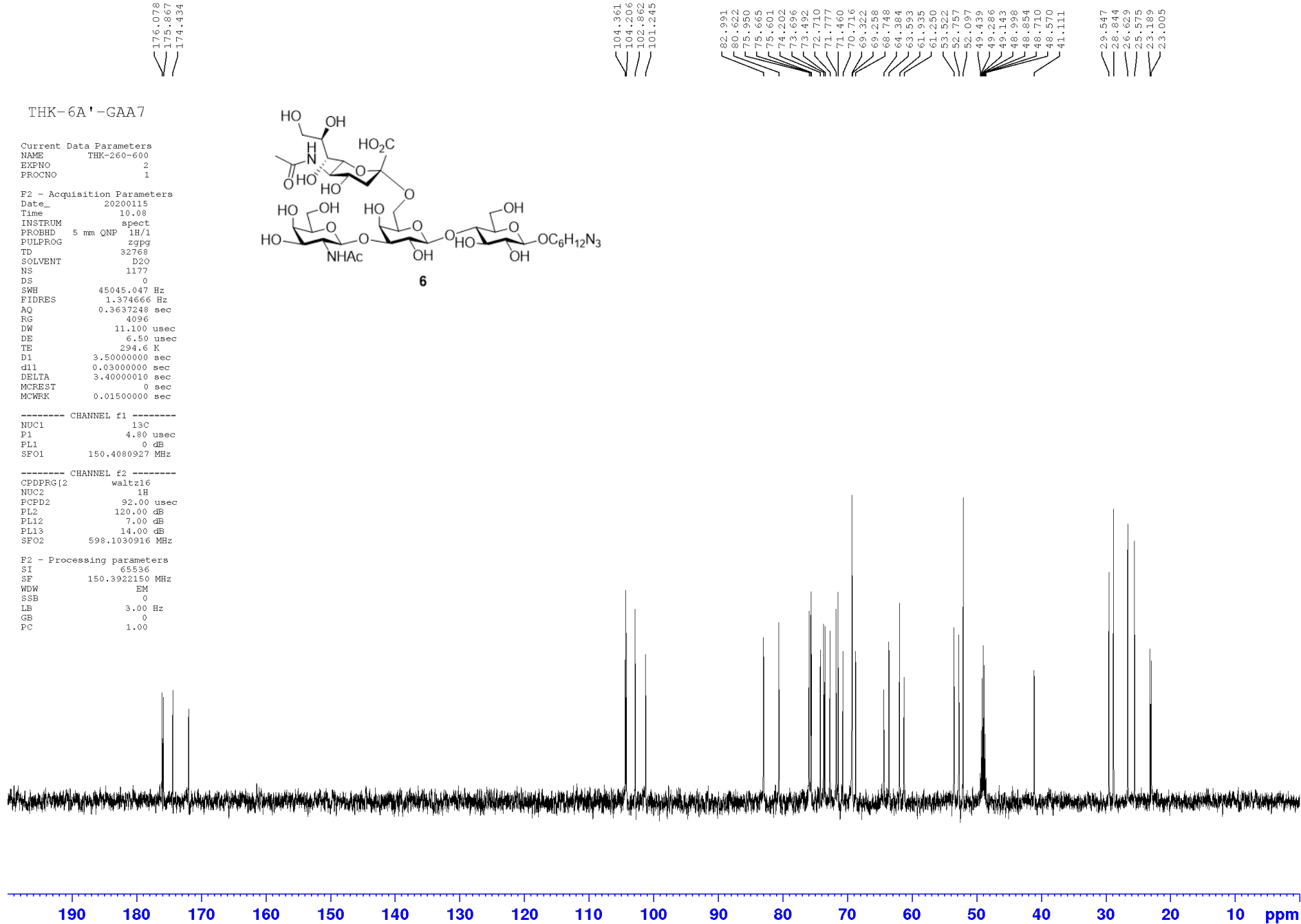
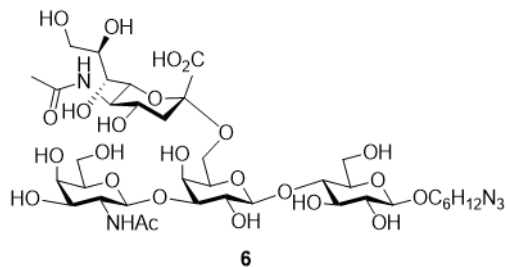
Current Data Parameters
NAME THK-260-600
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20200115
Time 10.08
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG zgpg
TD 32768
SOLVENT D2O
NS 1177
DS 0
SWH 45045.047 Hz
FIDRES 1.374666 Hz
AQ 0.3637248 sec
RG 4096
DW 11.100 usec
DE 6.50 usec
TE 294.6 K
D1 3.5000000 sec
d11 0.0300000 sec
DELTA 3.4000010 sec
MCREST 0 sec
MCWFK 0.0150000 sec

----- CHANNEL f1 -----
NUC1 13C
P1 4.80 usec
PL1 0 dB
SFO1 150.4080927 MHz

----- CHANNEL f2 -----
CPDPRG2 waltz16
NUC2 1H
PCPD2 92.00 usec
PL2 120.00 dB
PL12 7.00 dB
PL13 14.00 dB
SFO2 598.1030916 MHz

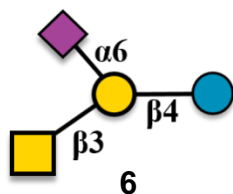
F2 - Processing parameters
SI 65536
SF 150.3922150 MHz
WDW EM
SSB 0
LB 3.00 Hz
GB 0
PC 1.00



¹³C NMR spectrum of Compound 6 (150 MHz D₂O)



THK-6 'A-GAA7



Current Data Parameters
NAME THK-260-600
EXPNO 11
PROCNO 1

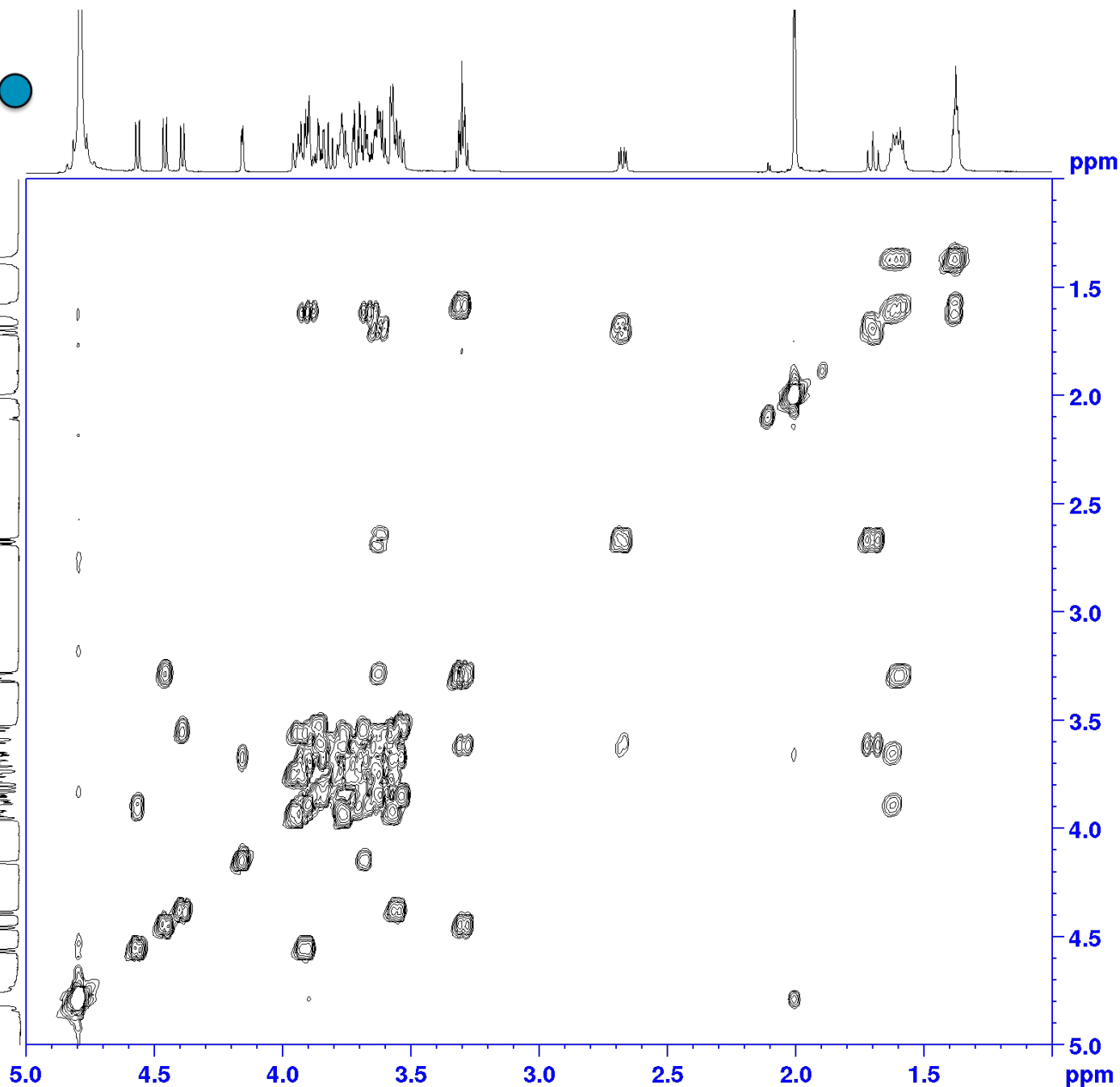
F2 - Acquisition Parameters
Date_ 20200115
Time 12.09
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG cosyqf
TD 2048
SOLVENT MeOD
NS 8
DS 0
SWH 6009.615 Hz
FIDRES 2.934382 Hz
AQ 0.1703936 sec
RG 128
DW 83.200 usec
DE 6.50 usec
TE 295.0 K
d0 0.00000300 sec
D1 1.50000000 sec
IN0 0.00016640 sec
MCREST 0 sec
MCWRK 1.50000000 sec

===== CHANNEL f1 =====
NUC1 1H
P0 14.00 usec
P1 12.80 usec
PL1 -3.00 dB
SFO1 598.1028152 MHz

F1 - Acquisition parameters
TD 400
SFO1 598.1028 MHz
FIDRES 30.048077 Hz
SW 10.048 ppm
FnMODE QF

F2 - Processing parameters
SI 2048
SF 598.0999443 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0
PC 1.00

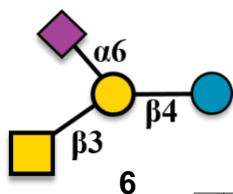
F1 - Processing parameters
SI 512
MC2 QF
SF 598.0999504 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0



^1H - ^1H COSY NMR spectrum of Compound 6 (600 MHz D_2O)



THK-6'A-GAA7



Current Data Parameters
NAME THK-260-600
EXPNO 12
PROCNO 1

F2 - Acquisition Parameters
Date_ 20200115
Time 13.40
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG hsqcetpsi
TD 2048
SOLVENT Acetone
NS 14
DS 8
SWH 6009.615 Hz
FIDRES 2.934382 Hz
AQ 0.1703936 sec
RG 14384
DW 83.200 usec
DE 6.50 usec
TE 294.7 K
CHST2 145.000000
d0 0.00000300 sec
D1 1.20000005 sec
d4 0.00172414 sec
d11 0.00000000 sec
d13 0.00000400 sec
D16 0.00050000 sec
D24 0.00089000 sec
DELTA 0.00158160 sec
DELTA1 0.00150800 sec
INO 0.00001511 sec
MCREST 0 sec
MCWREK 0.20000041 sec
STICNT 0

----- CHANNEL f1 -----
NUC1 1H
P1 12.80 usec
p2 25.60 usec
P28 1000.00 usec
FL1 -3.00 dB
SFO1 598.1028152 MHz

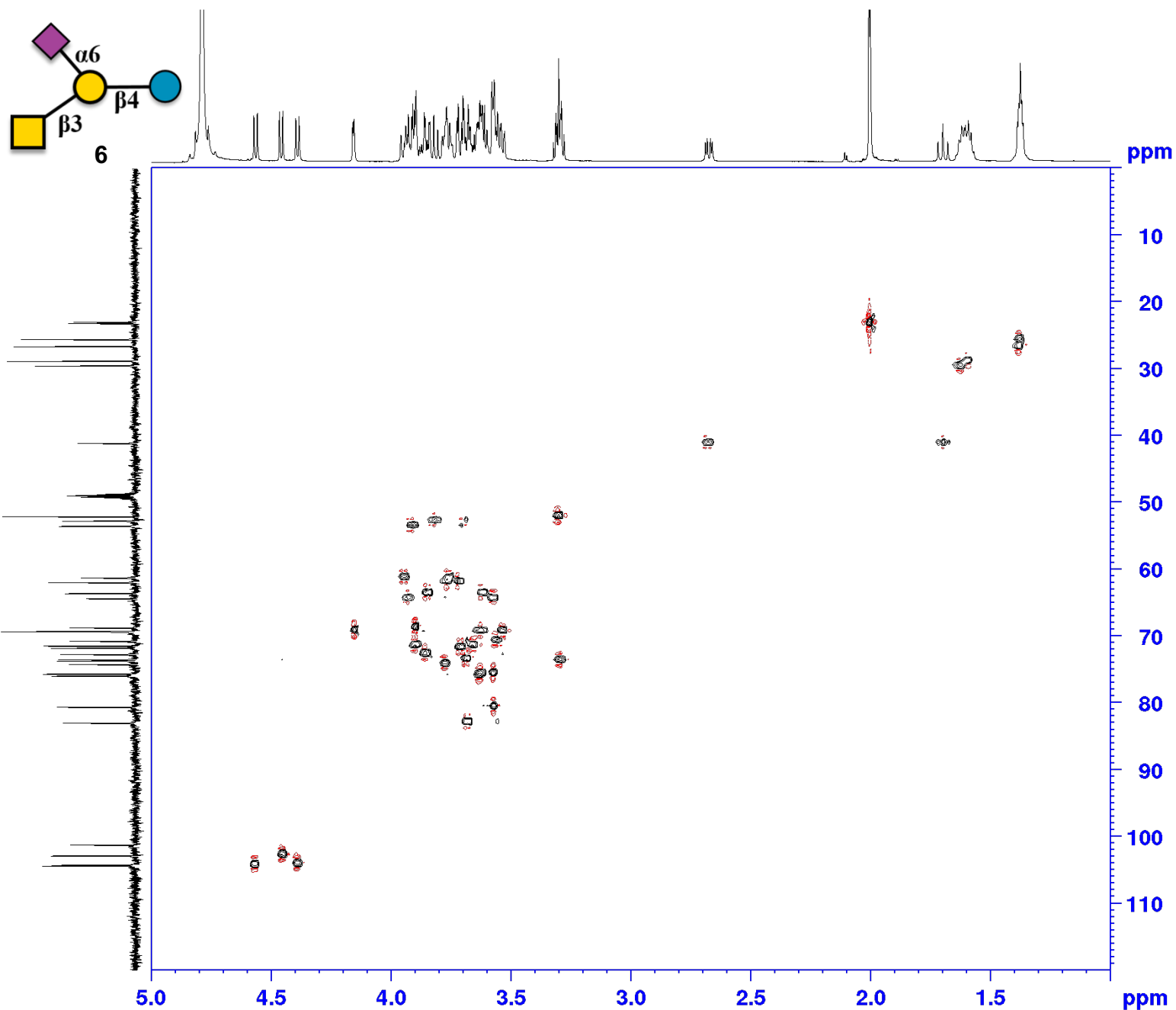
----- CHANNEL f2 -----
CPDPRG2 garp
NUC2 13C
P3 10.00 usec
p4 20.00 usec
PCPD2 70.00 usec
FL2 0 dB
FL12 20.00 dB
SFO2 150.4042733 MHz

----- GRADIENT CHANNEL -----
GPNAM[1] SINE.100
GPNAM[2] SINE.100
GPX1 0 %
GPK2 0 %
GPY1 0 %
GPY2 0 %
GPE1 80.00 %
GPE2 20.10 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 512
SFO1 150.4043 MHz
FIDRES 129.239044 Hz
SW 219.975 ppm
FaMODE Echo-Antiecho

F2 - Processing parameters
SI 2048
SF 598.0999701 MHz
WDW QSINE
SFB 4
LB 0 Hz
GB 0
FC 1.00

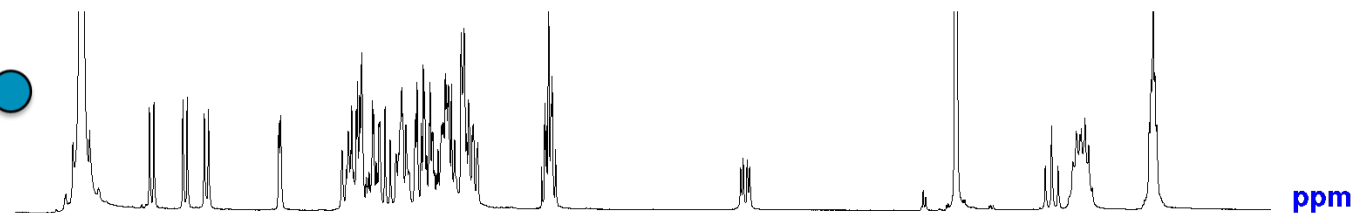
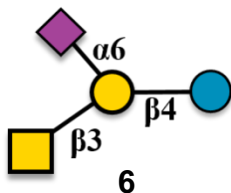
F1 - Processing parameters
SI 512
MC2 echo-antiecho
SF 150.3922119 MHz
WDW QSINE
SFB 4
LB 0 Hz
GB 0



^1H - ^{13}C HSQC NMR spectrum of Compound 6 (600 MHz/150 MHz D_2O)



THK-6'A-GAA7



```
Current Data Parameters
NAME      THK-260-600
EXPNO     13
PROCNO    1

F2 - Acquisition Parameters
Date_     20200115
Time      16.51
INSTRUM   spect
PROBHD    5 mm QNP 1H/1
PULPROG   hmbcpg1pdqdf
TD         2048
SOLVENT   Acetone
NS         32
DS         0
SWH        6009.615 Hz
FIDRES     2.934382 Hz
AQ         0.1703936 sec
RG         32768
DW         83.200 usec
DE         6.00 usec
TE         295.8 K
CNST12    145.0000000
CNST13    8.0000000
d0         0.00000300 sec
D1         1.20000005 sec
d2         0.00344828 sec
d6         0.06250000 sec
D16        0.00050000 sec
INO        0.00001662 sec
MCREST    0 sec
MCWREK    1.20000005 sec

===== CHANNEL f1 =====
NUC1       1H
P1         12.80 usec
p2         25.60 usec
PL1        -3.00 dB
SFO1       598.1028152 MHz

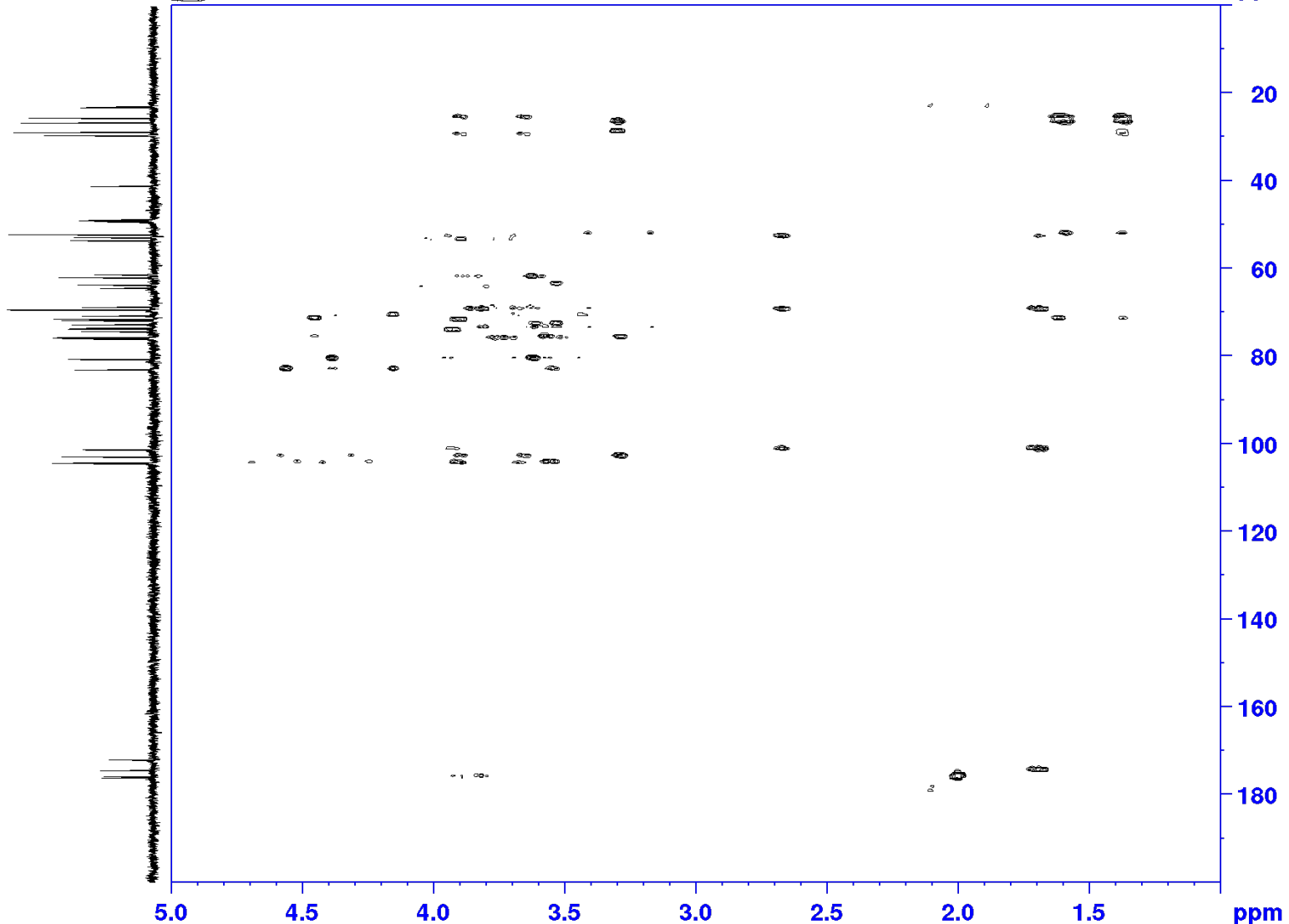
===== CHANNEL f2 =====
NUC2       13C
P3         10.50 usec
PL2         0 dB
SFO2       150.4042733 MHz

===== GRADIENT CHANNEL =====
GENAM[1]   SINE.100
GENAM[2]   SINE.100
GENAM[3]   SINE.100
GPX1        0 %
GPX2        0 %
GPX3        0 %
GPY1        0 %
GPY2        0 %
GPY3        0 %
GZ11        50.00 %
GZ22        30.00 %
GZ23        40.10 %
P16         1000.00 usec

F1 - Acquisition parameters
TD         512
SFO1       150.4043 MHz
FIDRES     117.481201 Hz
SW         199.962 ppm
FqMODE     QF

F2 - Processing parameters
SI         4096
SF         598.0999445 MHz
WDW        QSINE
SSB        2
LB         0 Hz
GB         0
PC         1.00

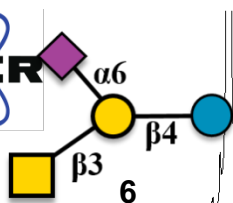
F1 - Processing parameters
SI         512
MC2        QF
SF         150.3922056 MHz
WDW        SINE
SSB        0
LB         0 Hz
GB         0
```



^1H - ^{13}C HMBC NMR spectrum of Compound 6 (600 MHz/150 MHz D_2O)



THK-6 'A-GAA7



Current Data Parameters
NAME THK-260-600
EXPNO 14
PROCNO 1

F2 - Acquisition Parameters
Date_ 20200115
Time 23:28
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG hzgpgp1ph
TD 2048
SOLVENT cdcl3
NS 32
DS 8
SWH 6009.615 Hz
FIDRES 2.794362 Hz
AQ 0.1703296 sec
RG 32768
DW 83.200 usec
DE 6.50 usec
TE 294.6 K
CNST2 145.0000000
d0 0.0000000 sec
d1 1.2000000 sec
d4 0.00172414 sec
d9 0.07500000 sec
d11 0.03000000 sec
d13 0.00000400 sec
d16 0.00100000 sec
DELTA 0.00200000 sec
DELTA1 0.00003160 sec
FACTOR1 8
LNO 0.00001511 sec
l1 48
MOREST 0 sec
MGATE 0.60000002 sec
SCALE1

===== CHANNEL f1 =====
NUC1 1H
P1 12.80 usec
p2 25.60 usec
p3 16.67 usec
p6 25.00 usec
p7 30.00 usec
P17 1000.00 usec
P28 1000.00 usec
PL1 -3.00 dB
PL10 4.00 dB
SFO1 598.1026152 MHz

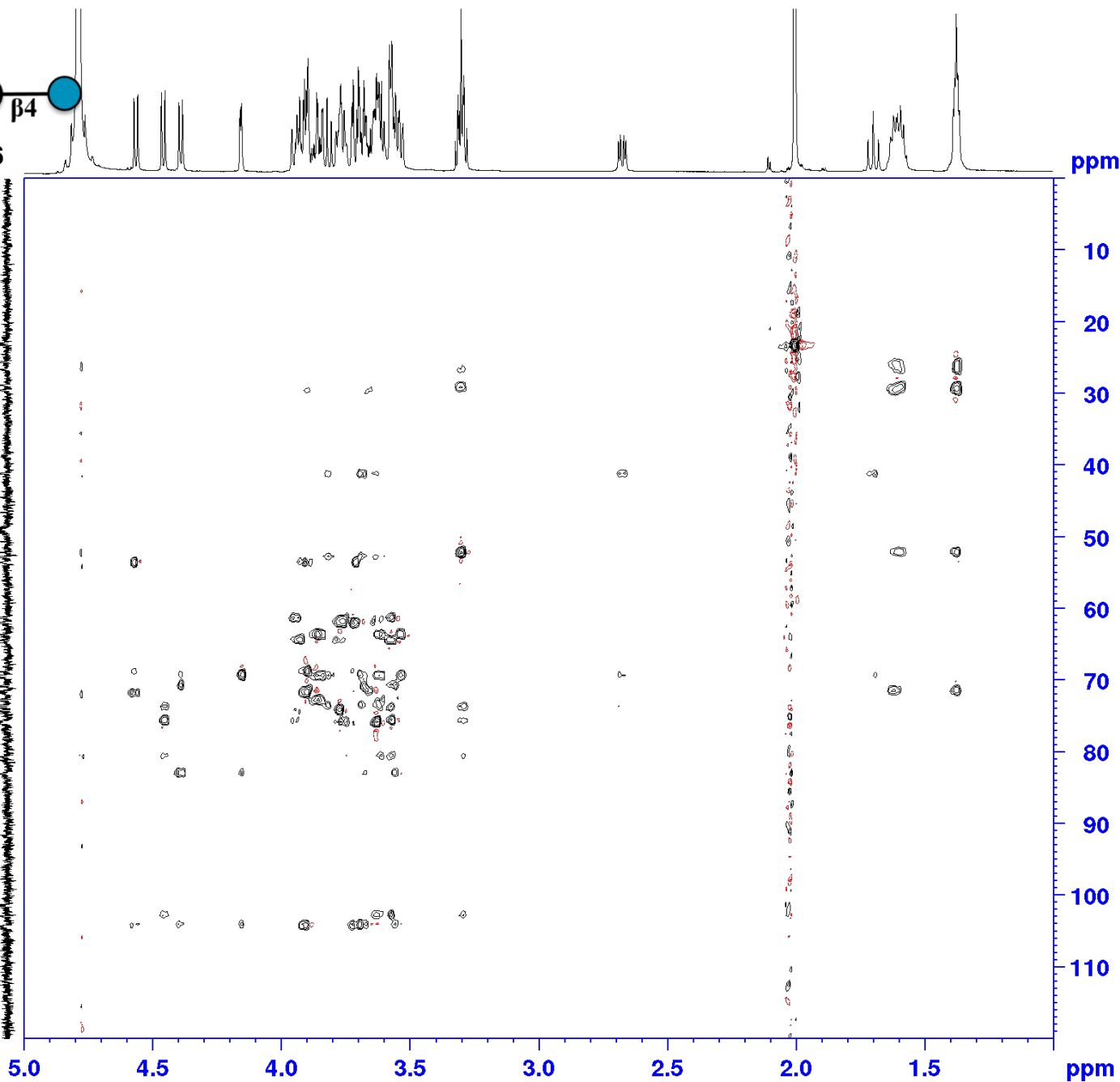
===== CHANNEL f2 =====
CPDPRG2 gars
NUC2 13C
P3 10.00 usec
p4 20.00 usec
PCPD2 70.00 usec
PL2 0 dB
PL12 20.00 dB
SFO2 150.4042753 MHz

===== GRADIENT CHANNEL =====
GENAM[1] SINE.100
GENAM[2] SINE.100
GENAM[3] SINE.100
GPR1 0 %
GPR2 0 %
GPR3 0 %
GPR4 0 %
GPR5 0 %
GPR6 0 %
GPR7 80.00 %
GPR8 30.00 %
GPR9 20.10 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 400
SFO1 150.4043 MHz
FIDRES 163.423964 Hz
SW 219.975 ppm
F1MODE TPFI

F2 - Processing parameters
SI 2048
SF 598.0999770 MHz
WDW QSINE
SSB 3
LB 0 Hz
GB 0
PC 1.00

F1 - Processing parameters
SI 512
MC2 TPFI
SF 150.3922131 MHz
WDW QSINE
SSB 3
LB 0 Hz
GB 0

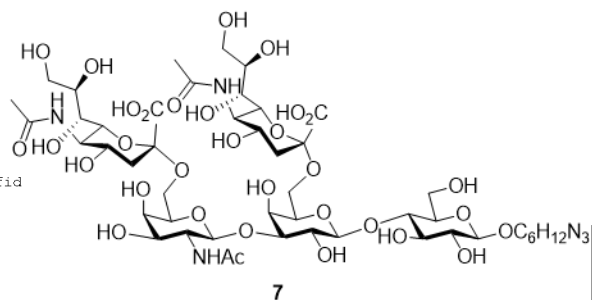


^1H - ^{13}C HSQC-TOCSY NMR spectrum of Compound 6 (600 MHz/150 MHz D_2O)

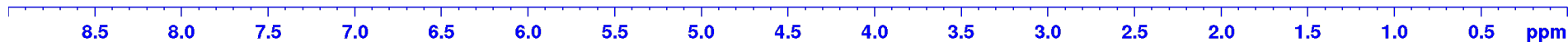
THK-6A6'A-GAA7

Current Data Parameters
NAME THK-261-20200201-1H.fid
EXPNO 1
PROCNO 1

F2 - Processing parameters
SI 16384
SF 499.7888646 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



4.7899
4.5881
4.5712
4.4638
4.4476
4.3945
4.3786
4.1181
4.1129
3.9841
3.9629
3.9505
3.9408
3.9339
3.9227
3.9171
3.9095
3.8886
3.8711
3.8631
3.8584
3.8427
3.8344
3.8137
3.8033
3.7994
3.7881
3.7793
3.7684
3.7483
3.7286
3.7234
3.7087
3.7003
3.6893
3.6735
3.6652
3.6476
3.6306
3.6164
3.6049
3.5917
3.5807
3.5688
3.5594
3.5413
3.5317
3.5262
3.5058
3.3132
3.2986
3.2848
2.7184
2.7094
2.6938
2.6850
2.6846
2.6830
2.6673
2.6585
2.0060
1.2.0004
1.1.9978
1.1.7468
1.1.7222
1.1.6977
1.1.6742
1.1.6496
1.1.6250
1.1.6176
1.1.6038
1.1.5901
1.1.5745
1.1.5622
1.1.3834
1.1.3809
1.1.3730
1.1.3659
1.1.3588



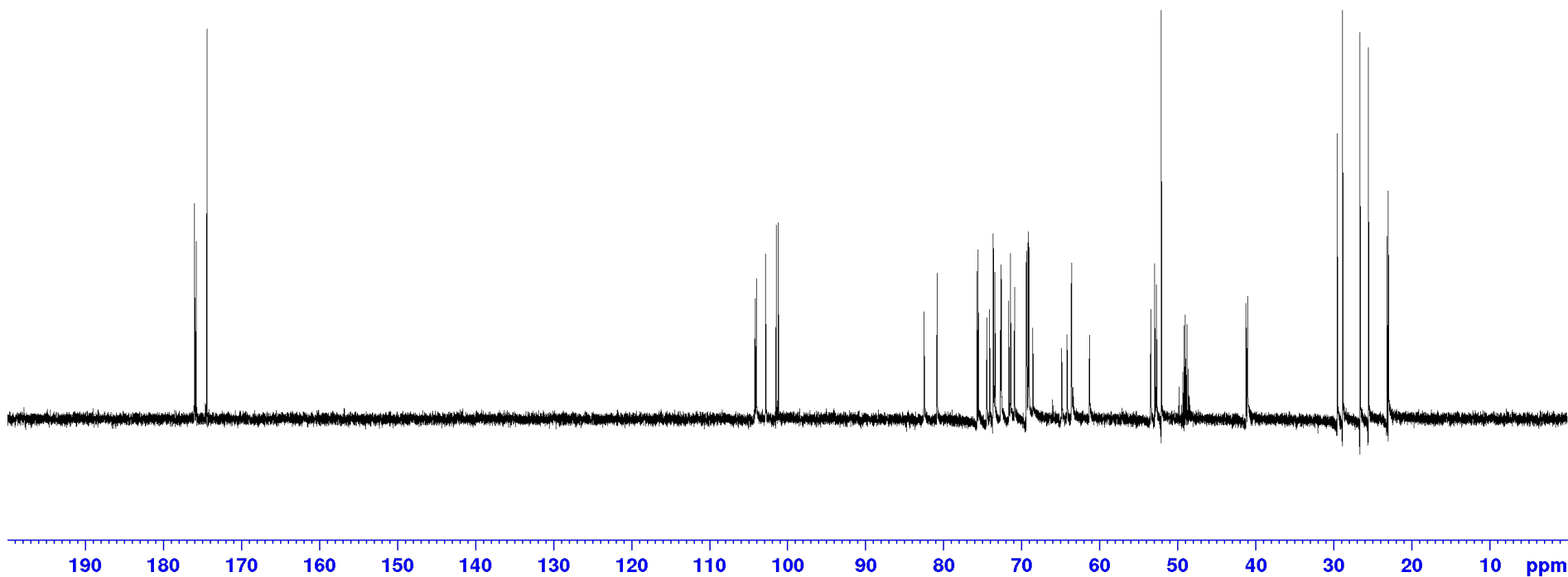
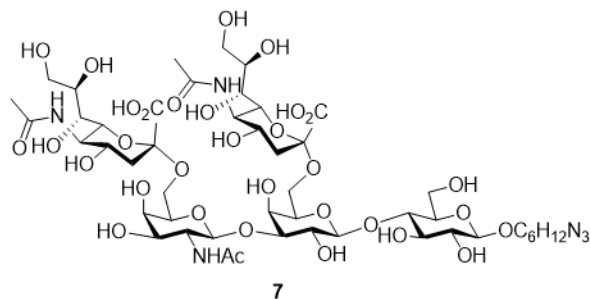
¹H NMR spectrum of Compound 7 (500 MHz D₂O)

176.026
176.007
175.792
174.425

104.166
103.997
102.820
101.466
101.231
82.520
80.842
75.721
75.563
74.450
74.104
73.663
73.622
73.437
72.678
72.595
71.648
71.437
70.914
69.414
69.375
69.333
69.161
69.079
68.555
64.861
64.163
63.633
63.595
61.252
53.443
52.948
52.730
52.087
49.514
49.342
49.169
48.996
48.824
48.651
48.479
41.230
41.034
39.539
28.839
26.624
25.570
23.186
23.017
22.990

THK-6A6A'-GAA7

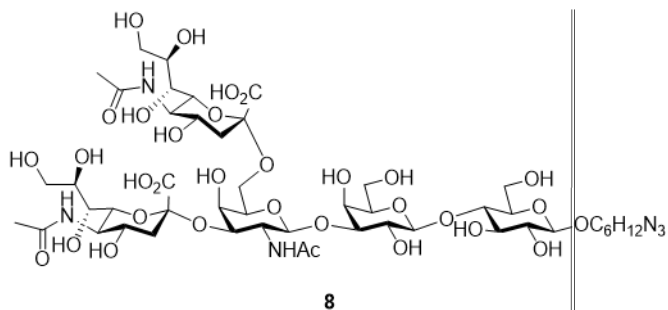
Current Data Parameters
 NAME THK-261-20200201-13c.fid
 EXPNO 1
 PROCNO 1
 F2 - Processing parameters
 SI 65536
 SF 125.6718952 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00



¹H NMR spectrum of Compound 7 (500 MHz D₂O)

THK-6A3A-GAA7

Current Data Parameters
NAME CCLin-THK-GAA7-AA-H.fic
EXPNO 1
PROCNO 1
F2 - Processing parameters
SI 65536
SF 699.7447850 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



4.7899
4.7219
4.7097
4.4909
4.4794
4.4400
4.4286
4.1981
4.1944
4.1796
3.9882
3.9788
3.9727
3.9667
3.9629
3.9517
3.9477
3.9353
3.9314
3.9213
3.9114
3.9013
3.8871
3.8701
3.8480
3.8334
3.8181
3.8128
3.8052
3.7997
3.7848
3.7707
3.7557
3.7419
3.7343
3.7242
3.7172
3.7091
3.6976
3.6937
3.6864
3.6834
3.6744
3.6660
3.6577
3.6489
3.6413
3.6348
3.6316
3.6202
3.6067
3.5931
3.5792
3.5686
3.5553
3.4879
3.4731
3.3415
3.3317
3.3224
3.3110
2.7158
2.7089
2.7055
2.6982
2.6912
2.6876
2.6809
2.0837
2.0386
2.0306
1.7439
1.7264
1.7090
1.6587
1.6488
1.6396
1.6315
1.6225
1.6135
1.6033
1.4154
1.4106
1.4055
1.4004
1.3949

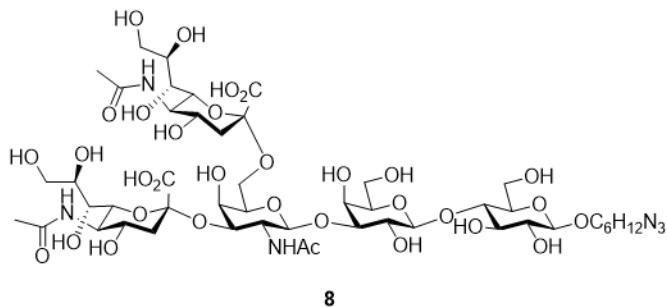
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm

1.13
1.01
1.00
1.96
9.35
5.37
10.32
6.24
0.99
3.10
2.02
8.90
1.01
5.13
4.11

^1H NMR spectrum of Compound 8 (700 MHz D_2O)

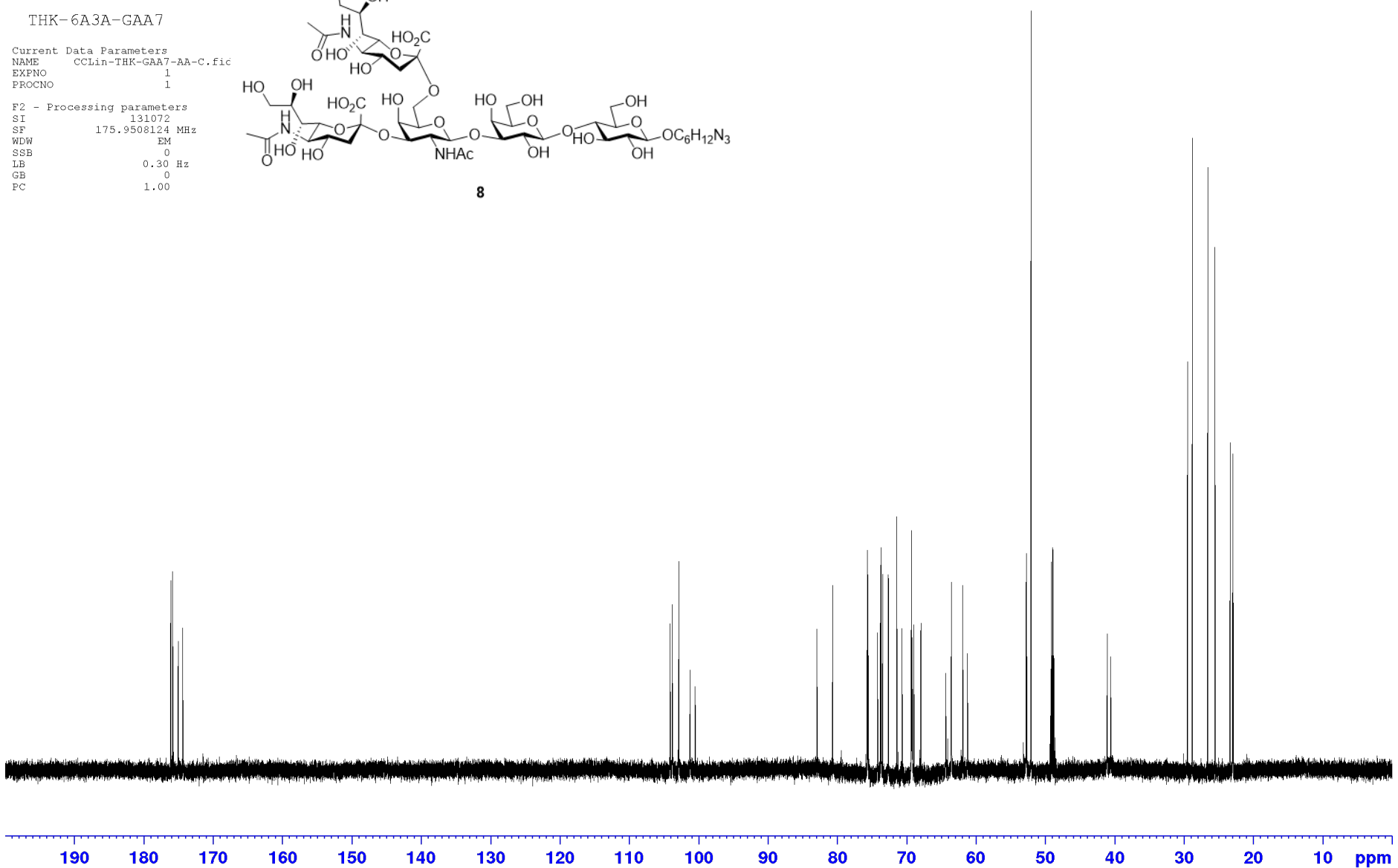
THK-6A3A-GAA7

Current Data Parameters
 NAME CCLin-THK-GAA7-AA-C.fic
 EXPNO 1
 PROCNO 1
 F2 - Processing parameters
 SI 131072
 SF 175.9508124 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00

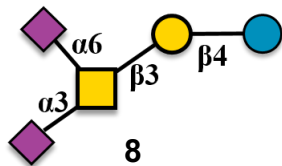


176.140
 175.864
 175.840
 175.065
 174.395

104.141
 103.837
 102.847
 101.235
 100.506
 82.944
 80.679
 75.696
 75.659
 75.576
 74.214
 73.818
 73.735
 73.678
 73.465
 72.679
 72.608
 71.436
 70.682
 69.326
 69.302
 69.292
 69.210
 68.999
 67.969
 64.399
 63.581
 63.541
 61.938
 61.237
 52.820
 52.742
 52.085
 49.368
 49.245
 49.121
 48.998
 48.875
 48.752
 48.628
 41.081
 40.597
 39.530
 28.824
 26.612
 25.557
 23.358
 23.000
 22.938



¹³C NMR spectrum of Compound 8 (175 MHz D₂O)



THK-6A3A-GAA-7

Current Data Parameters
NAME THK-GAA7-AA-180604
EXPNO 11
PROCNO 1

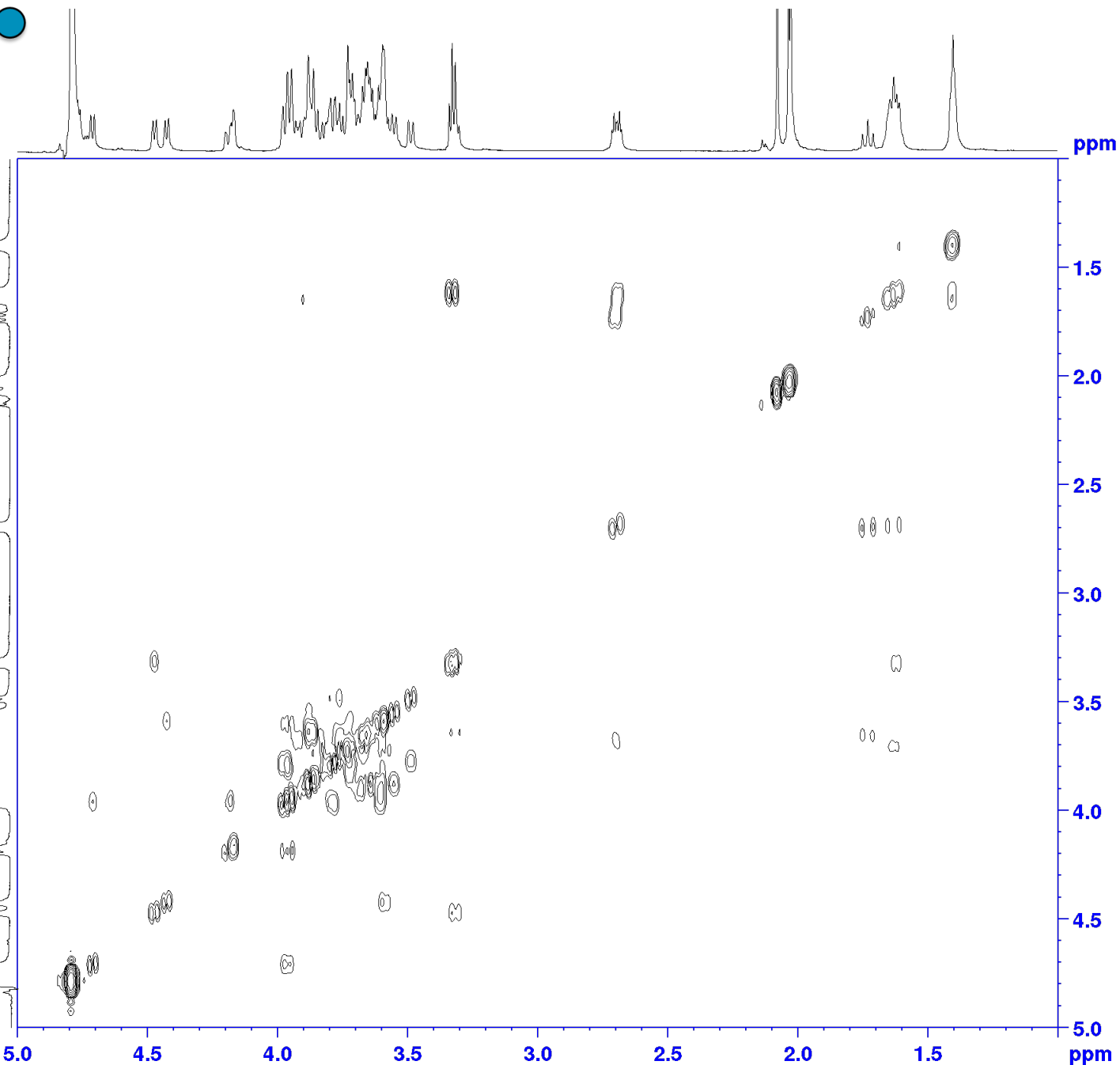
F2 - Acquisition Parameters
Date_ 20180604
Time 9.48
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG cosyqf
TD 2048
SOLVENT H2O+D2O
NS 16
DS 0
SWH 6009.615 Hz
FIDRES 2.934382 Hz
AQ 0.1703936 sec
RG 128
DW 83.200 usec
DE 6.50 usec
TE 299.8 K
d0 0.00000300 sec
D1 1.50000000 sec
IN0 0.00016640 sec
MCREST 0 sec
MCWRK 1.50000000 sec

===== CHANNEL f1 =====
NUC1 1H
P0 14.00 usec
P1 14.00 usec
PL1 -2.00 dB
SFO1 598.3028186 MHz

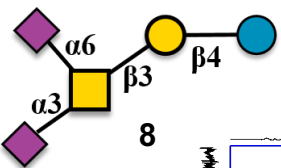
F1 - Acquisition parameters
TD 256
SFO1 598.3028 MHz
FIDRES 46.950119 Hz
SW 10.044 ppm
FnMODE QF

F2 - Processing parameters
SI 2048
SF 598.2999500 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0
PC 1.00

F1 - Processing parameters
SI 512
MC2 QF
SF 598.2999530 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0



^1H - ^1H COSY NMR spectrum of Compound **8** (600 MHz D_2O)



THK-6A3A-GAA-7

Current Data Parameters
NAME THK-GAA7-AA-180604
EXPNO 12
PROCNO 1

F2 - Acquisition Parameters
Date_ 20180605
Time 6.42
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG hsqcztgpa1
TD 2048
SOLVENT D2O
NS 8
DS 8
SWH 6009.615 Hz
FIDRES 2.984382 Hz
RQ 0.1703926 sec
RG 32768
DW 83.200 usec
DE 4.50 usec
TE 297.8 K
CHET2 145.0000000
d0 0.0000000 sec
D1 1.5000000 sec
d4 0.00172414 sec
d11 0.0300000 sec
d13 0.00000400 sec
d16 0.00080000 sec
E24 0.00089000 sec
DELTA 0.00157700 sec
DELTA1 0.00150800 sec
TMO 0.00001385 sec
MORSET 0 sec
MORWT 0.25000051 sec
STICNT 0

----- CHANNEL f1 -----
NUC1 1H
P1 10.50 usec
P2 21.00 usec
P28 1000.00 usec
PL1 -3.00 dB
SFO1 598.3028186 MHz

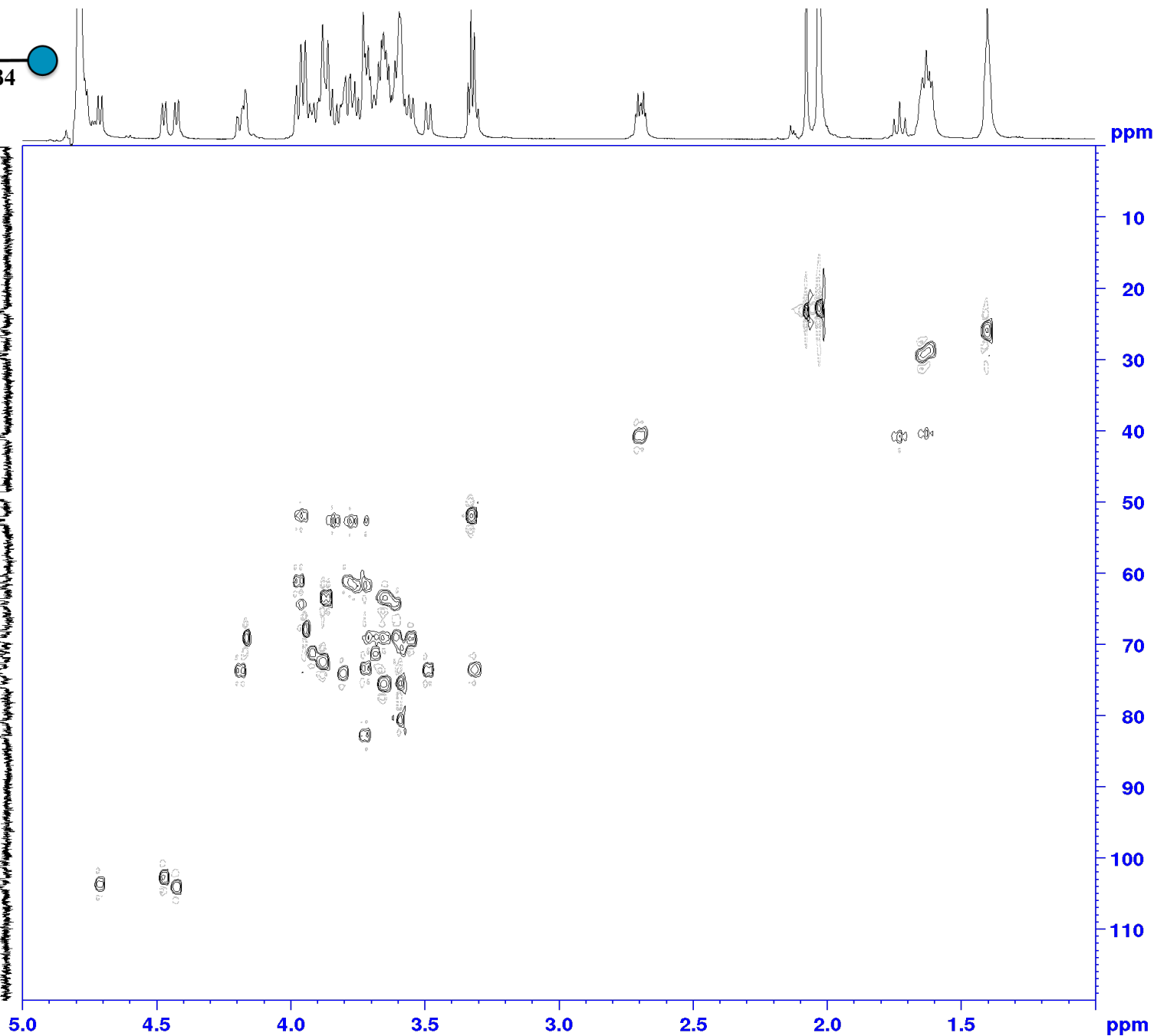
----- CHANNEL f2 -----
CPDPRG2 sarp
NUC2 13C
P3 10.00 usec
P4 20.00 usec
PCPD2 70.00 usec
PL2 0 dB
PL12 20.00 dB
SFO2 150.4539382 MHz

----- GRADIENT CHANNEL -----
GPNAM[1] SINE.100
GPNAM[2] SINE.100
GPA1 0 %
GPA2 0 %
GPA3 0 %
GPA4 0 %
GPE1 80.00 %
GPE2 20.10 %
PL6 1000.00 usec

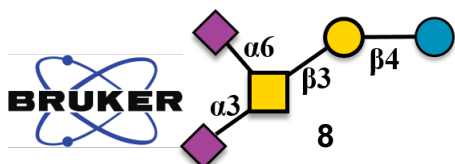
F1 - Acquisition parameters
TD 300
SFO1 150.4539 MHz
FIDRES 240.673889 Hz
SW 239.948 ppm
FAMODE Echo-Antiecho

F2 - Processing parameters
SI 2048
SF 598.2999530 MHz
WDW QSIINE
SSB 4
LB 0 Hz
GB 0
PC 1.00

F1 - Processing parameters
SI 512
MC2 echo-antiecho
SF 150.4425044 MHz
WDW QSIINE
SSB 4
LB 0 Hz
GB 0



^1H - ^{13}C HSQC NMR spectrum of Compound **8** (600 MHz/150 MHz D₂O)



THK-6A3A-GAA-7

```

Current Data Parameters
NAME THK-GA7-1A-150604
EXPNO 13
PROCNO 1

F2 - Acquisition Parameters
Date_ 20180605
Time 7.50
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG hmbcgp1pndgf
TD 2048
SOLVENT D2O
NS 16
DS 0
SWH 6009.615 Hz
FIDRES 2.934382 Hz
AQ 0.1703936 sec
RG 32768
DM 83.200 usec
DE 6.00 usec
TE 298.9 K
CNS12 145.0000000
CNS13 12.0000000
d0 0.00000300 sec
D1 1.50000000 sec
d2 0.00344828 sec
d6 0.04166667 sec
D16 0.00050000 sec
IN0 0.00001661 sec
MCREST 0 sec
MCMRK 1.50000000 sec

===== CHANNEL f1 =====
NUC1 1H
P1 10.50 usec
P2 21.00 usec
PL1 -3.00 dB
SFO1 598.3028186 MHz

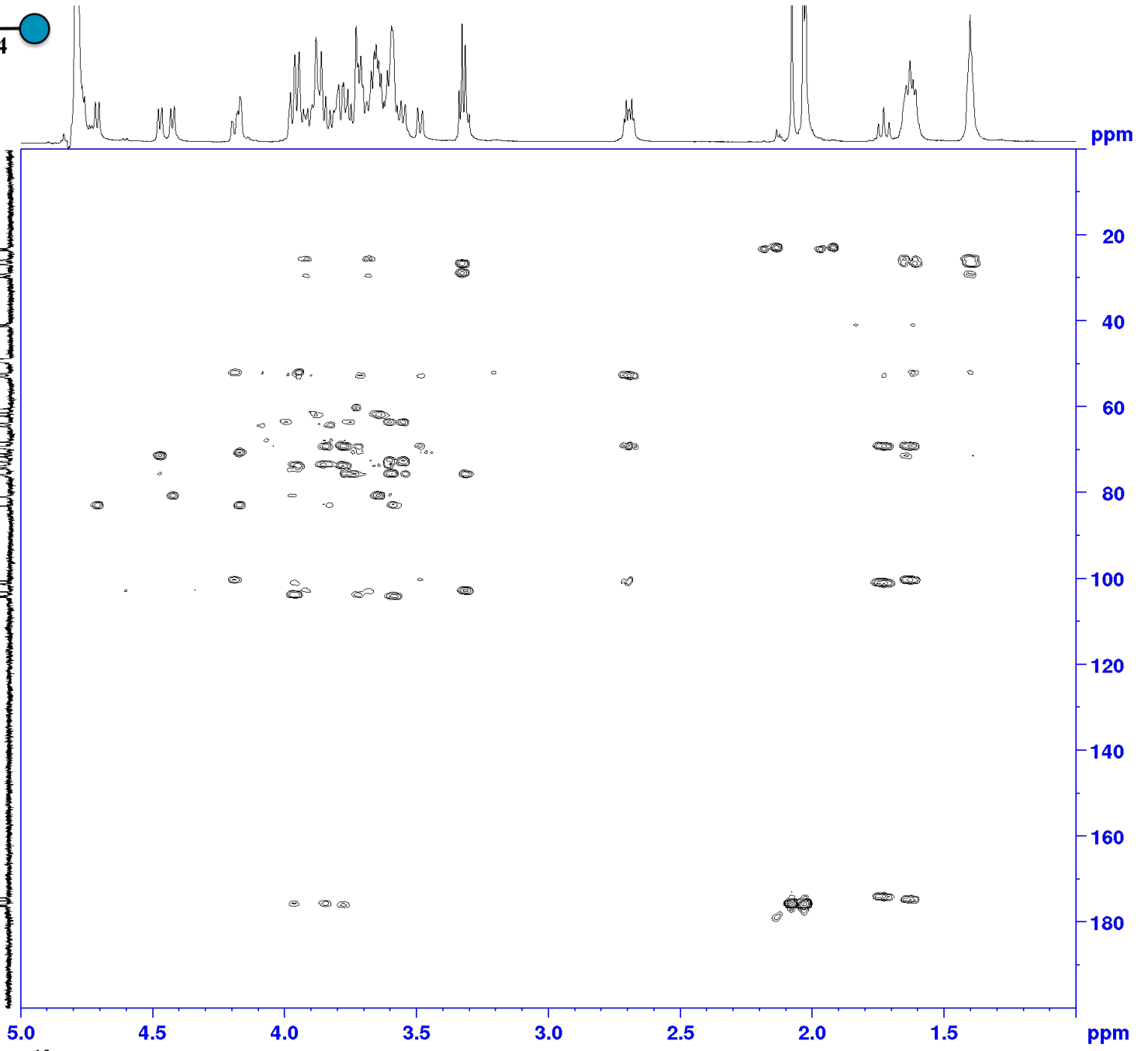
===== CHANNEL f2 =====
NUC2 13C
P3 10.00 usec
PL2 0 dB
SFO2 150.4561948 MHz

===== GRADIENT CHANNEL =====
GPNM[1] SINE.100
GPNM[2] SINE.100
GPNM[3] SINE.100
GPX1 0 %
GPX2 0 %
GPX3 0 %
GPY1 0 %
GPY2 0 %
GPY3 0 %
GPZ1 50.00 %
GPZ2 30.00 %
GPZ3 40.10 %
P16 1000.00 usec

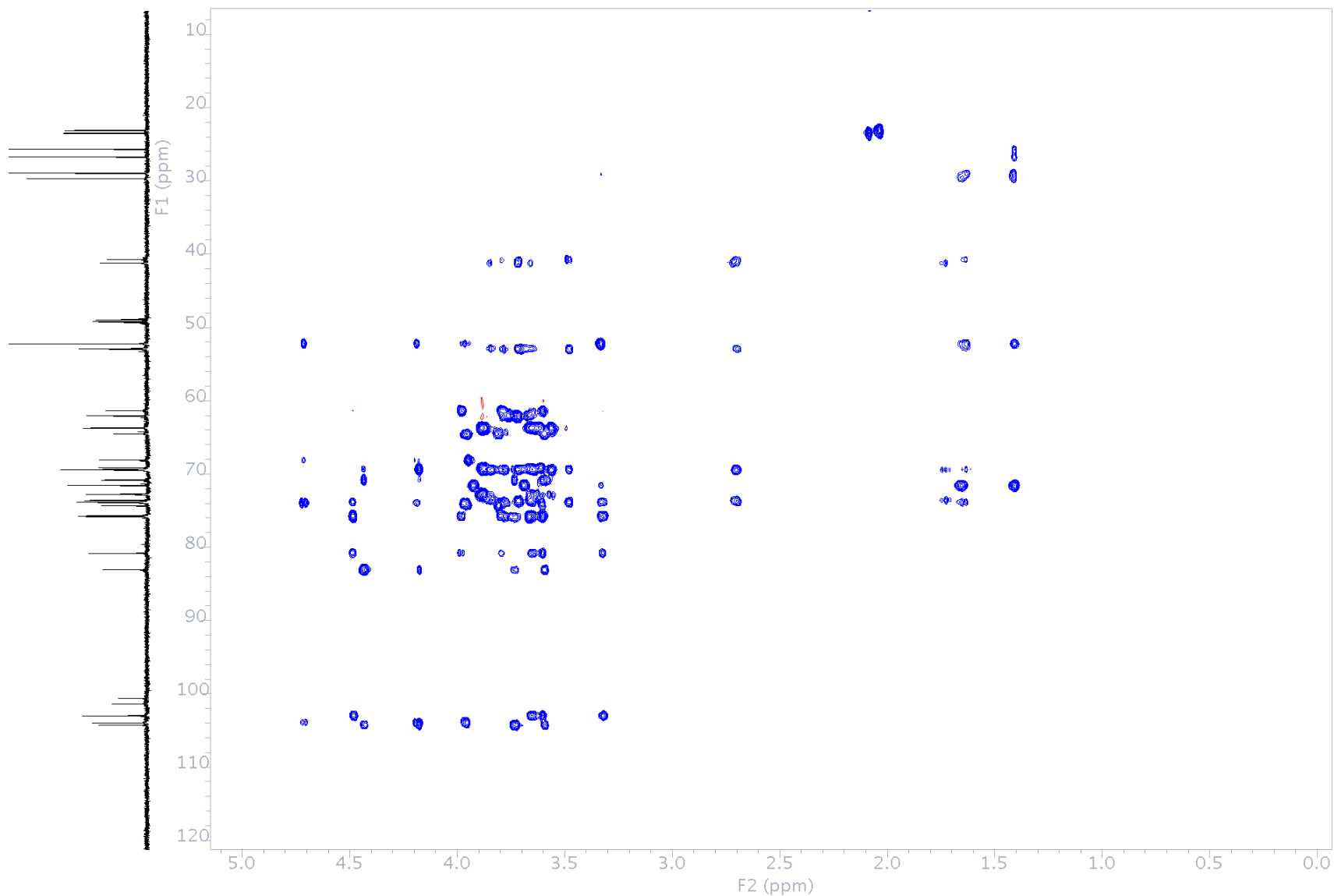
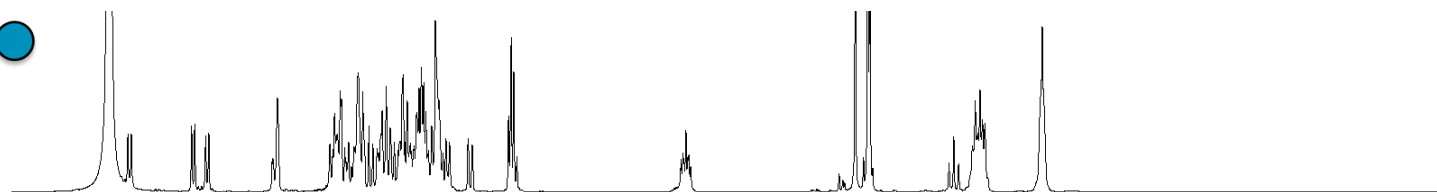
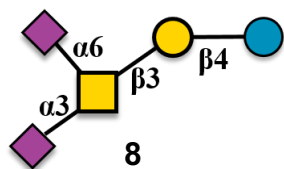
F1 - Acquisition parameters
TD 282
SFO1 150.4562 MHz
FIDRES 213.459702 Hz
SW 200.044 ppm
FMODE QF

F2 - Processing parameters
SI 4096
SF 598.2999340 MHz
WDW QSLINE
SSB 2
LB 0 Hz
GB 0
PC 1.00

F1 - Processing parameters
SI 512
MC2 QF
SF 150.4425046 MHz
WDW SINE
SSB 0
LB 0 Hz
GB 0
  
```



^1H - ^{13}C HMBC NMR spectrum of Compound **8** (600 MHz/150 MHz D_2O)



^1H - ^{13}C HSQC-TOCSY NMR spectrum of Compound **8** (700 MHz/175 MHz D_2O)



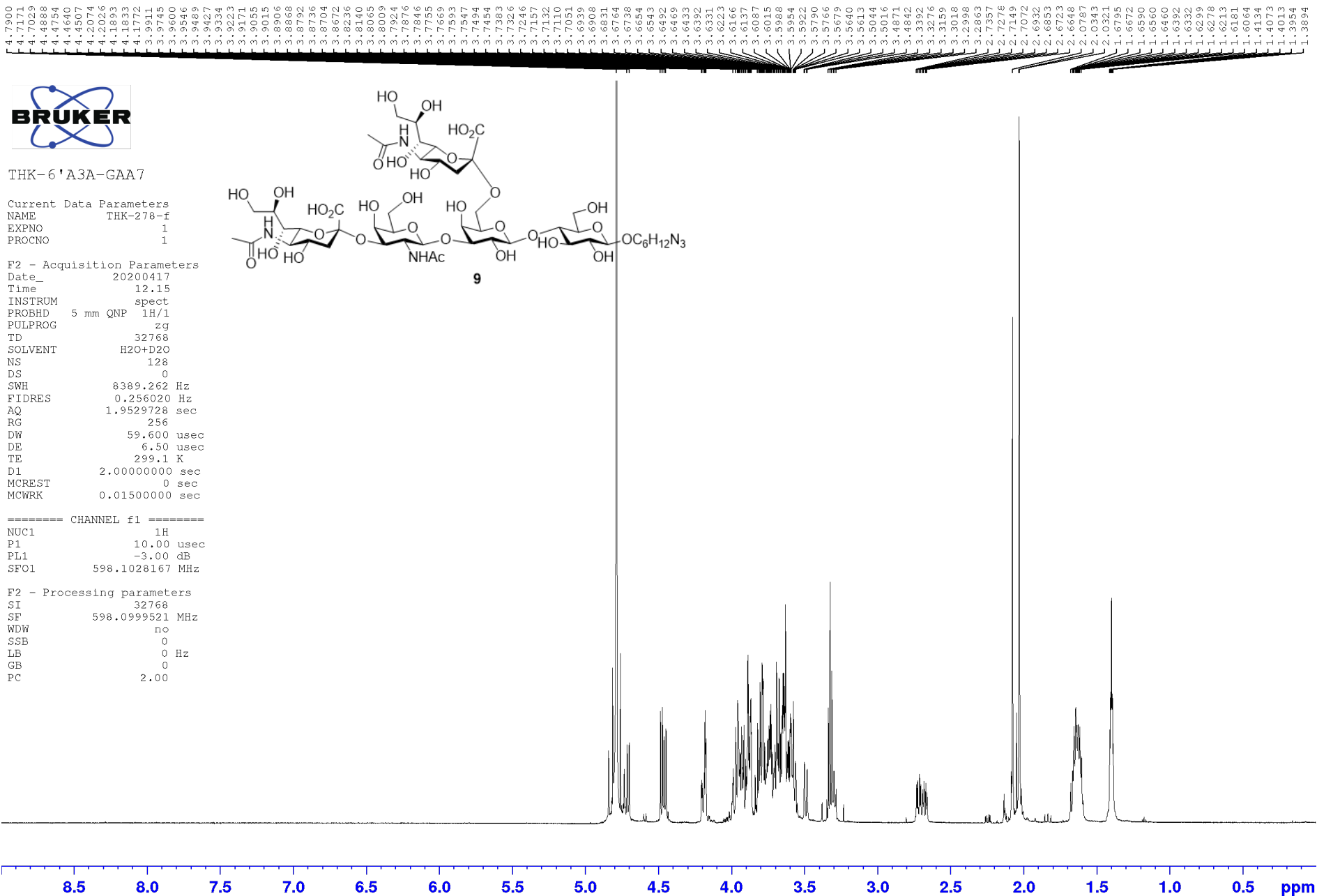
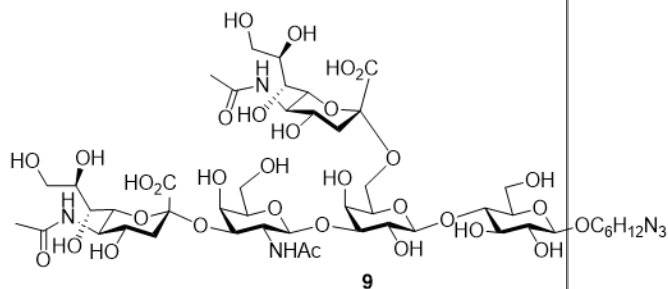
THK-6'A3A-GAA7

Current Data Parameters
NAME THK-278-f
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20200417
Time 12.15
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG zg
TD 32768
SOLVENT H2O+D2O
NS 128
DS 0
SWH 8389.262 Hz
FIDRES 0.256020 Hz
AQ 1.9529728 sec
RG 256
DW 59.600 usec
DE 6.50 usec
TE 299.1 K
D1 2.00000000 sec
MCREST 0 sec
MCWRK 0.01500000 sec

===== CHANNEL f1 =====
NUC1 1H
P1 10.00 usec
PL1 -3.00 dB
SFO1 598.1028167 MHz

F2 - Processing parameters
SI 32768
SF 598.0999521 MHz
WDW no
SSB 0
LB 0 Hz
GB 0
PC 2.00



¹H NMR spectrum of Compound 9 (600 MHz D₂O)



THK-6'A3A-GAA7

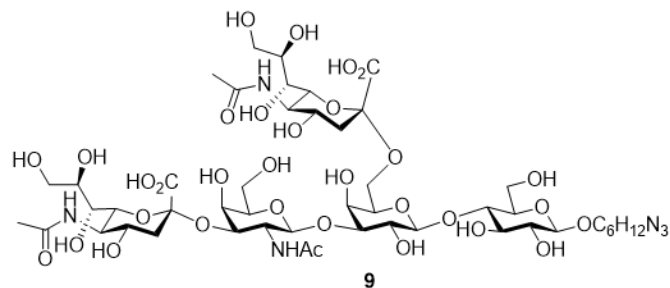
Current Data Parameters
NAME THK-278-f
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20200413
Time 2.38
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG zgpgg
TD 32768
SOLVENT D2O
NS 9765
DS 0
SWH 45045.047 Hz
FIDRES 1.374666 Hz
AQ 0.3637248 sec
RG 4096
DW 11.100 usec
DE 6.50 usec
TE 294.6 K
DL 3.50000000 sec
dL1 0.03000000 sec
DELTA 3.40000010 sec
MCREST 0 sec
MCHRK 0.01500000 sec

----- CHANNEL f1 -----
NUC1 13c
P1 4.80 usec
PL1 0 dB
SFO1 150.4092089 MHz

----- CHANNEL f2 -----
CPDPRG[2] waltz16
NUC2 1H
PCPD2 92.00 usec
PL2 120.00 dB
PL12 6.00 dB
PL13 15.00 dB
SFO2 598.1029905 MHz

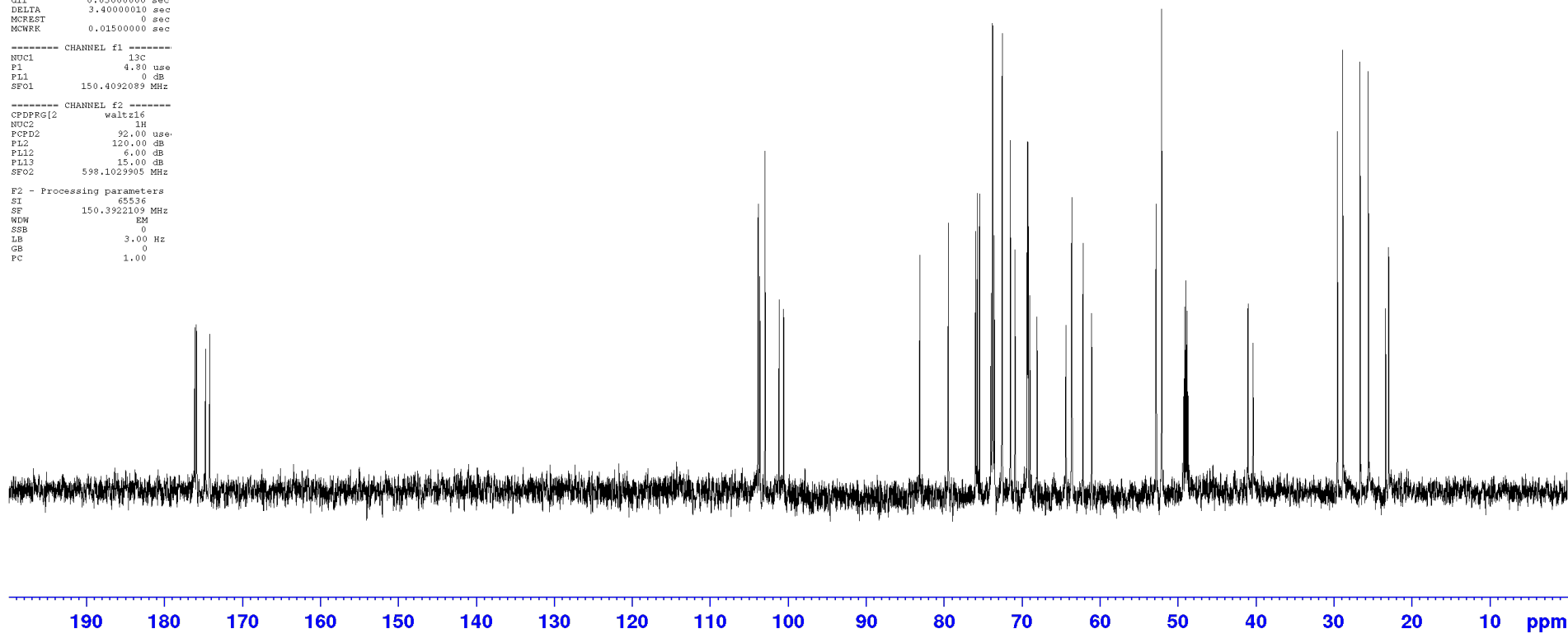
F2 - Processing parameters
SI 45536
SF 150.3922109 MHz
WDW EM
SSB 0
LB 3.00 Hz
GB 0
PC 1.00

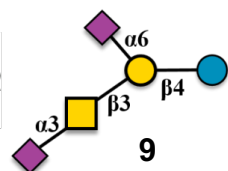


176.115
175.947
175.883
174.753
174.201

103.857
103.646
102.977
101.176
100.592

83.113
79.449
75.962
75.737
75.441
73.970
73.772
73.734
73.561
72.544
71.500
70.900
69.319
69.233
68.218
68.199
68.070
64.376
63.625
63.600
62.179
61.065
52.805
52.782
52.098
52.042
49.431
49.285
49.142
48.998
48.854
48.710
48.564
41.013
40.367
39.550
28.852
26.629
25.573
23.355
22.988
22.962





THK-6 'A3A-GAA7

Current Data Parameters
NAME THK-278-f
EXPNO 11
PROCNO 1

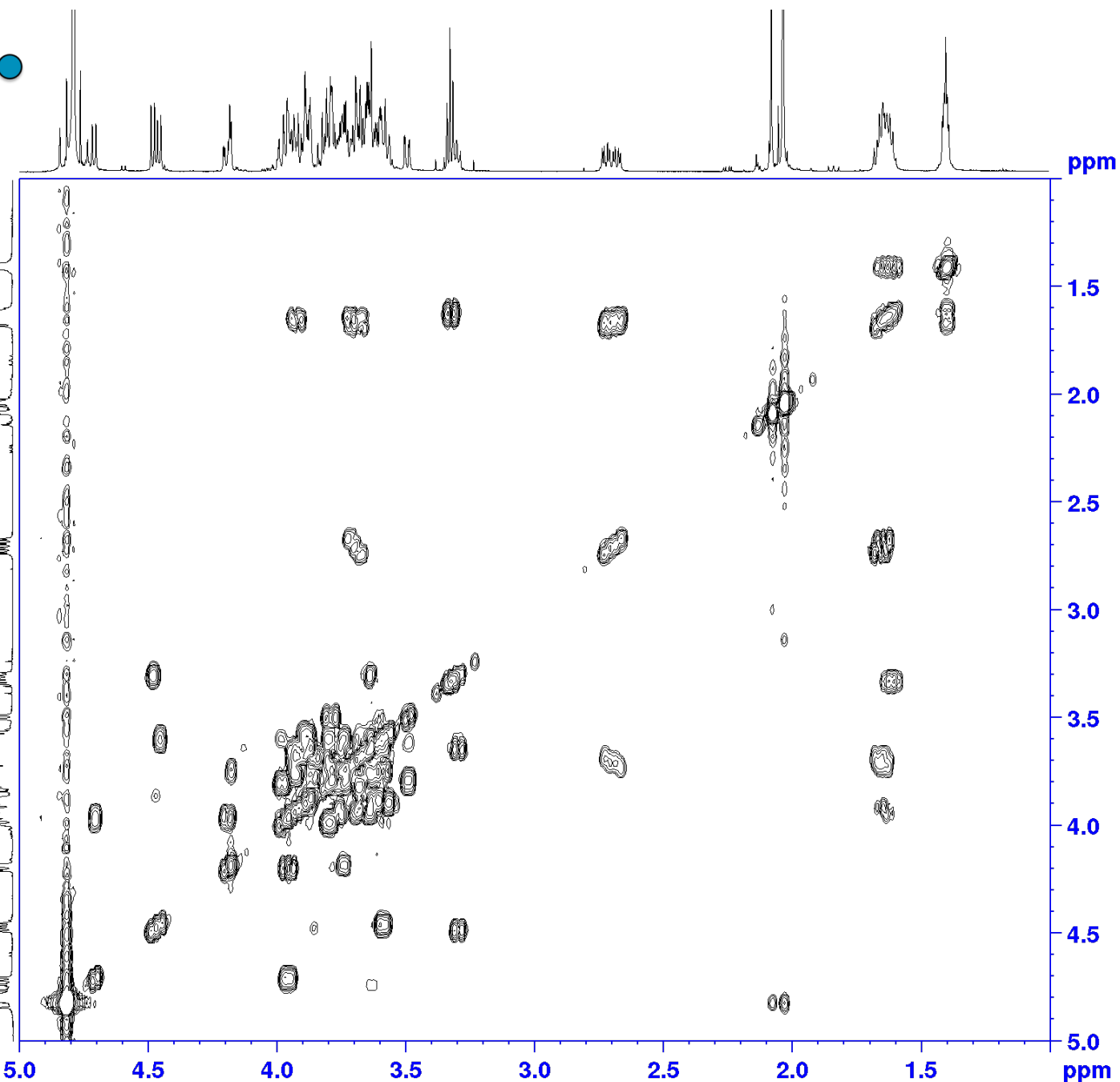
F2 - Acquisition Parameters
Date_ 20200412
Time 12.57
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG cosygqf
TD 2048
SOLVENT D2O
NS 18
DS 0
SWH 6009.615 Hz
FIDRES 2.934382 Hz
AQ 0.1703936 sec
RG 128
DW 83.200 usec
DE 6.50 usec
TE 294.8 K
d0 0.00000300 sec
D1 1.50000000 sec
IN0 0.00016640 sec
MCREST 0 sec
MCWRR 1.50000000 sec

----- CHANNEL f1 -----
NUC1 1H
P0 14.00 usec
P1 12.80 usec
PL1 -3.00 dB
SFO1 598.1028162 MHz

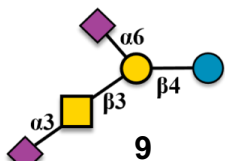
F1 - Acquisition parameters
TD 400
SFO1 598.1028 MHz
FIDRES 30.048077 Hz
SW 10.048 ppm
FnMODE QF

F2 - Processing parameters
SI 2048
SF 598.0999344 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0
PC 1.00

F1 - Processing parameters
SI 512
MC2 QF
SF 598.0999348 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0



^1H - ^1H COSY NMR spectrum of Compound 9 (600 MHz D_2O)



9

THK-6 'A3A-GAA

Current Data Parameters
NAME THK-278-f
EXPNO 102
PROCNO 1

F2 - Acquisition Parameters
Date_ 20200415
Time 3.50
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG haqcetgpa1
TD 2048
SOLVENT acetone
NS 16
DS 8
SWH 6009.615 Hz
FIDRES 2.934382 Hz
AQ 0.1703936 sec
RG 32768
DW 83.200 usec
DE 6.50 usec
TE 295.9 K
CNS12 145.000000
dc 0.0000000 sec
D1 1.20000005 sec
d4 0.00172414 sec
d11 0.03000000 sec
d13 0.00000400 sec
D16 0.00050000 sec
D24 0.00089000 sec
DELTA 0.00158200 sec
DELTA1 0.00150800 sec
INO 0.00001511 sec
MCPRST 0 sec
MCPK 0.20000041 sec
SICNT 0

==== CHANNEL f1 =====
NUC1 1H
P1 13.00 usec
P2 26.00 usec
P28 1000.00 usec
PL1 -3.00 dB
SFO1 598.1028152 MHz

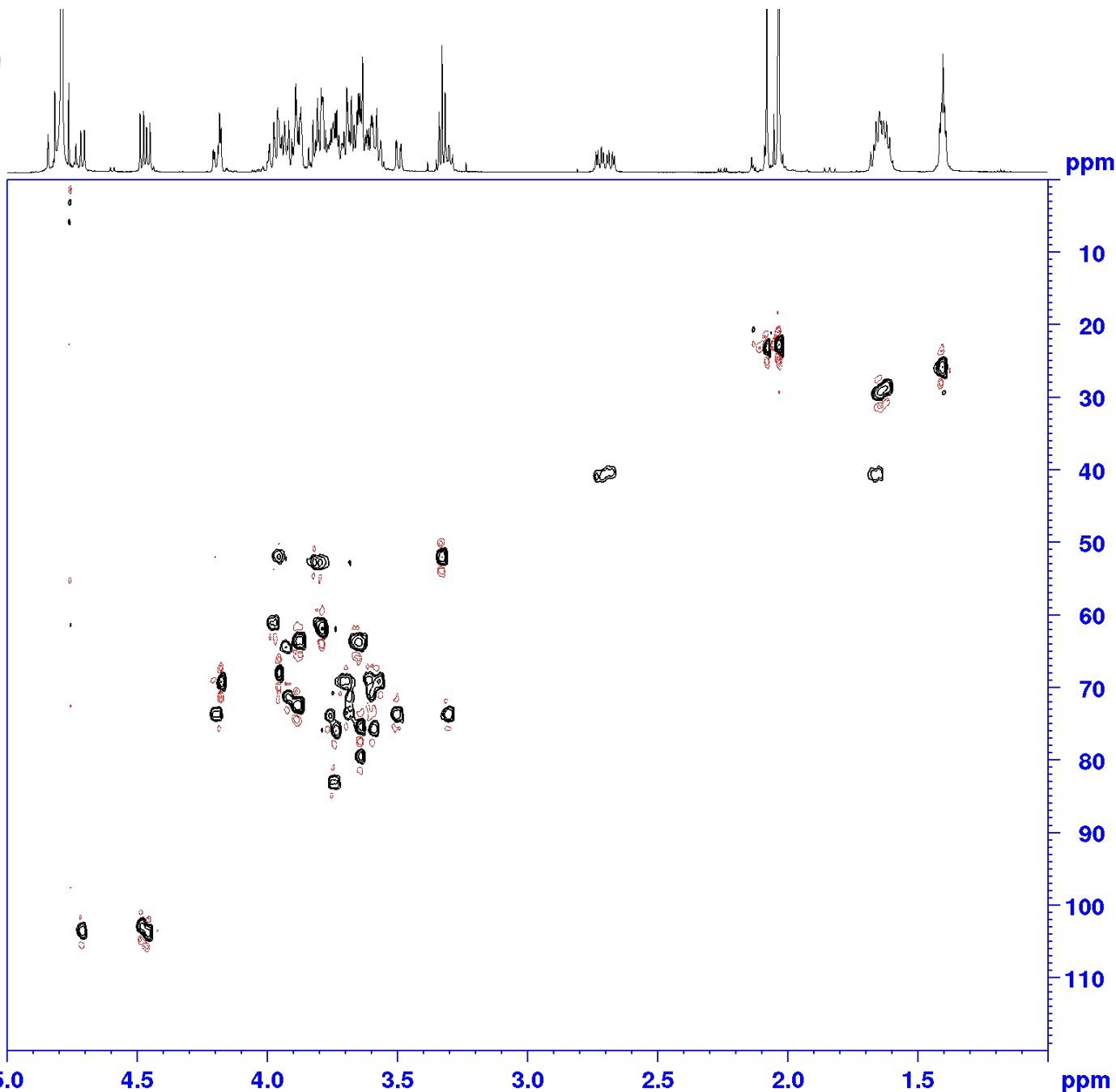
==== CHANNEL f2 =====
CPDPRG2 garp
NUC2 13C
P3 10.00 usec
p4 20.00 usec
PCPD2 70.00 usec
PL2 0 dB
PL12 20.00 dB
SFO2 150.4037948 MHz

==== GRADIENT CHANNEL =====
GPNM[1] SINE.100
GPNM[2] SINE.100
GPK1 0 %
GPK2 0 %
GPX1 0 %
GPX2 0 %
GPE1 80.00 %
GPE2 20.10 %
P16 1000.00 usec

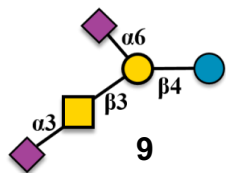
F1 - Acquisition parameters
TD 239
SFO1 150.4038 MHz
FIDRES 276.863556 Hz
SW 219.976 ppm
EnMODE Echo-Antiecho

F2 - Processing parameters
SI 2048
SF 598.0999706 MHz
WDW QSINE
SSB 4
LB 0 Hz
GB 0
FC 1.00

F1 - Processing parameters
SI 512
MC2 echo-antiecho
SF 150.3922135 MHz
WDW QSINE
SSB 4
LB 0 Hz
GB 0



^1H - ^{13}C HSQC NMR spectrum of Compound 9 (600 MHz/150 MHz D_2O)



THK-6'A3A-GAA7

Current Data Parameters
NAME THK-278-f
EXNO 13
PROCNO 1

F2 - Acquisition Parameters
Date_ 20200415
Time 13.03
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG hmbcgp1pdqf
TD 2048
SOLVENT Acetone
NS 96
DS 0
SWH 6009.615 Hz
FIDRES 2.934382 Hz
AQ 0.1703936 sec
RG 32768
DW 83.200 usec
DE 6.00 usec
TE 295.7 K
CNST2 145.000000
CNST13 2.0000000
d0 0.00000300 sec
d1 1.20000005 sec
d2 0.00344828 sec
d4 0.06250000 sec
d16 0.00050000 sec
TNO 0.00001662 sec
MCREST 0 sec
MCWRK 1.20000005 sec

==== CHANNEL f1 =====
NUC1 1H
P1 12.90 usec
p2 25.80 usec
FL1 -3.00 dB
SFO1 598.1028152 MHz

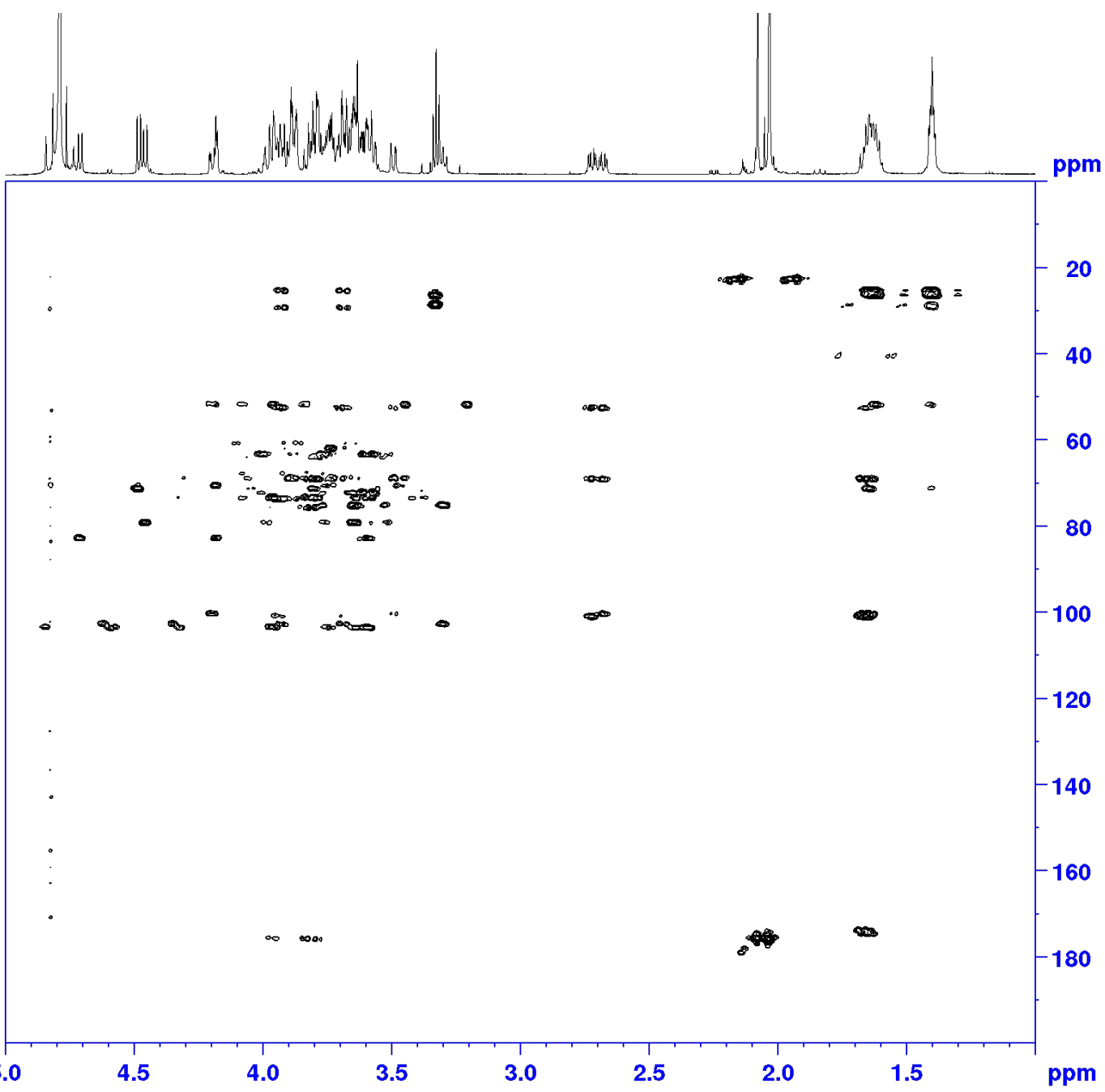
==== CHANNEL f2 =====
NUC2 13C
P3 10.50 usec
FL2 0 dB
SFO2 150.4042733 MHz

===== GRADIENT CHANNEL =====
GPNAM[1] SINE.100
GPNAM[2] SINE.100
GPNAM[3] SINE.100
GFX1 0 %
GFX2 0 %
GFX3 0 %
GPY1 0 %
GPY2 0 %
GPY3 0 %
GZF1 50.00 %
GZF2 30.00 %
GZF3 40.10 %
F16 1000.00 usec

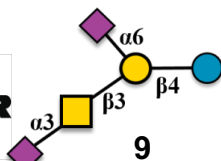
F1 - Acquisition parameters
TD 367
SFO1 150.4043 MHz
FIDRES 163.897476 Hz
SW 199.962 ppm
FnMODE QF

F2 - Processing parameters
SI 4096
SF 598.0999308 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.00

F1 - Processing parameters
SI 512
MC2 QF
SF 150.3922043 MHz
WDW SINE
SSB 0
LB 0 Hz
GB 0



^1H - ^{13}C HMBC NMR spectrum of Compound 9 (600 MHz/150 MHz D₂O)



THK-6 'A3A-GAA7

```
Current Data Parameters
NAME      THK-278-f
EXPNO    14
PROCNO   1

F2 - Acquisition Parameters
Date_    20200416
Time     12.46
INSTRUM  spect
PROBHD   5 mm QNP 1H/1
PULPROG  zgpg30
TD       2048
SOLVENT  CDCl3
NS       96
DS       8
SWH      6009.615 Hz
FIDRES   2.934382 Hz
AQ       0.1703936 sec
RG       32768
DW       83.200 usec
DE       6.50 usec
TE       297.1 K
CONST2   145.000000
d0       0.00000300 sec
D1       1.20000005 sec
d4       0.00172414 sec
D9       0.07500000 sec
d11      0.03000000 sec
d13      0.00000400 sec
D16      0.00100000 sec
DELTA    0.00200000 sec
DELTA1   0.00003160 sec
PRGTOP1  8
INO      0.00001511 sec
J1       48
MQUEST   0 sec
MCHRFK   0.60000002 sec
SCALEF   6

===== CHANNEL f1 =====
NUC1     1H
P1       12.60 usec
p2       25.60 usec
p3       16.67 usec
P6       25.00 usec
p7       50.00 usec
E17      1000.00 usec
P28      1000.00 usec
FL1      -3.00 dB
FL10     4.00 dB
SFO1     598.1026152 MHz

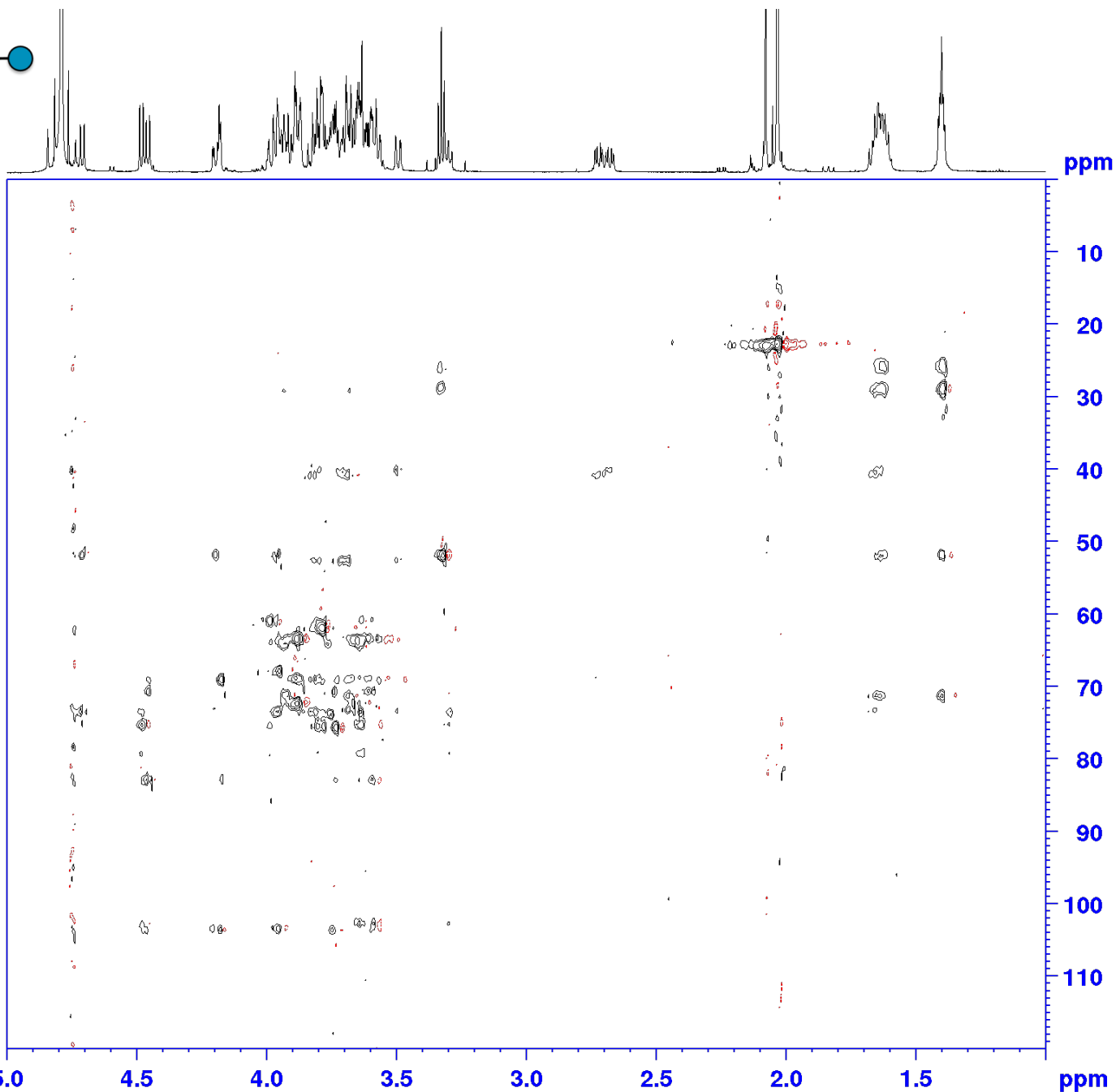
===== CHANNEL f2 =====
CPDPRG2  gspg
NUC2     13C
P3       10.00 usec
p4       20.00 usec
PCPD2    70.00 usec
FL2      0 dB
FL12     20.00 dB
SFO2     150.4059003 MHz

===== GRADIENT CHANNEL =====
GPMAM[1] SINE.100
GPMAM[2] SINE.100
GPMAM[3] SINE.100
GPX1     0 %
GPX2     0 %
GPX3     0 %
GPY1     0 %
GPY2     0 %
GPY3     0 %
GZ1      80.00 %
GZ2      30.00 %
GZ3      20.10 %
F16      1000.00 usec

F1 - Acquisition parameters
ID       394
SFO1     150.4059 MHz
FIDRES   167.945145 Hz
SW       167.973 ppm
P2MODE   TPFI

F2 - Processing parameters
SI       2048
SF       598.0929936 MHz
WDW      QSI
SSB      3
LB       0 Hz
GB       0
PC       1.00

F1 - Processing parameters
SI       512
MC2      TPFI
SF       150.3924224 MHz
WDW      QSI
SSB      2
LB       0 Hz
GB       0
```

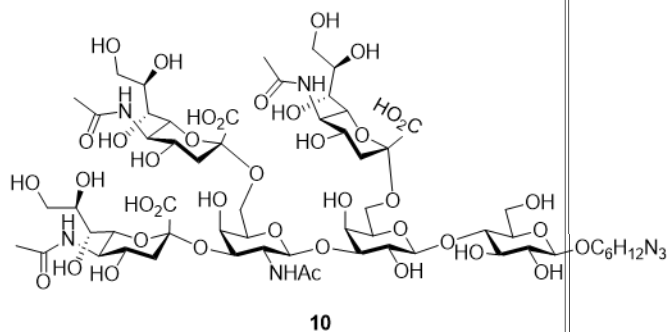


^1H - ^{13}C HSQC-TOCSY NMR spectrum of Compound 9 (600 MHz/150 MHz D_2O)

THK-6A6A'3A-GAA7

Current Data Parameters
NAME THK-263-20200202.fid
EXPNO 1
PROCNO 1

F2 - Processing parameters
SI 16384
SF 499.7888646 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



4.7897
4.7169
4.6999
4.4786
4.4625
4.4208
4.4051
4.1742
4.1718
4.1523
4.1496
4.1244
4.1219
3.9983
3.9773
3.9464
3.9357
3.8234
3.8115
3.8099
3.8799
3.8702
3.8596
3.8489
3.8256
3.8194
3.7981
3.7901
3.7790
3.7689
3.7590
3.7393
3.7236
3.7004
3.6836
3.6640
3.6550
3.6424
3.6300
3.6147
3.5933
3.5845
3.5698
3.5575
3.5365
3.5161
3.4680
3.4474
3.3248
3.3097
3.2950
3.2718
2.7139
2.6993
2.6914
2.6727
2.6650
2.6407
2.6382
2.0586
2.0146
2.0135
1.7600
1.7356
1.7114
1.6748
1.6452
1.6199
1.6003
1.5890
1.5751
1.4011
1.3940
1.3842
1.3752
1.3676

8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm

1.04
1.09
1.07
1.08
1.18
4.15
8.09
7.40
15.95
2.12
1.09
3.18
3.00
11.50
1.17
6.06
4.08

¹H NMR spectrum of Compound 10 (500 MHz D₂O)

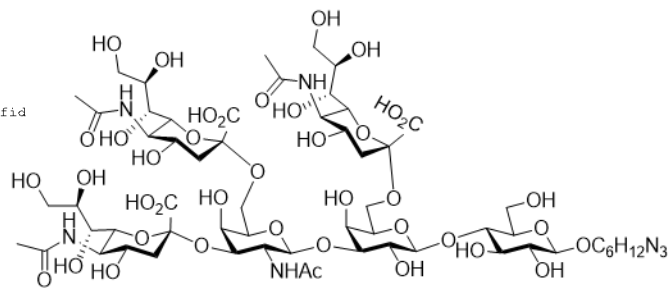
176.086
175.944
175.840
175.786
174.971
174.447
174.399

104.147
103.491
102.835
101.438
101.235
100.726
82.502
80.949
75.761
75.578
74.484
73.945
73.747
73.670
73.606
73.439
72.626
72.583
71.447
70.906
69.447
69.412
69.295
69.264
69.152
69.037
68.031
64.914
64.198
63.645
63.571
61.310
52.974
52.789
52.741
52.104
52.066
49.518
49.345
49.173
49.000
48.827
48.654
48.482
41.237
41.044
40.440
29.557
28.856
26.640
25.586
23.376
23.026
23.011
22.958

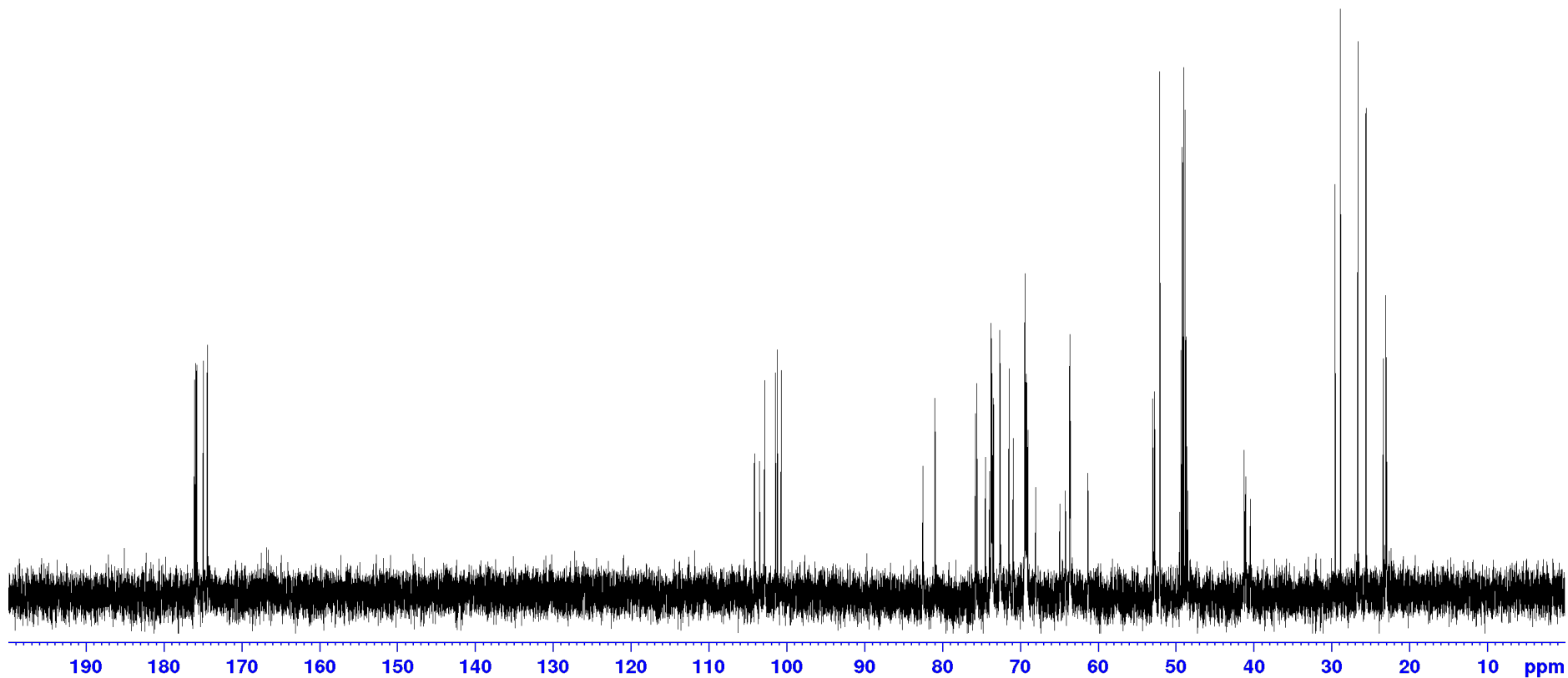
THK-6A6A'3A-GAA7

Current Data Parameters
NAME THK-263-20200202-13c.fid
EXPNO 1
PROCNO 1

F2 - Processing parameters
SI 65536
SF 125.6718930 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



10



¹³C NMR spectrum of Compound 10 (125 MHz D₂O)

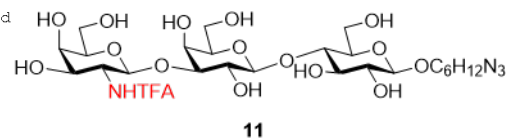
THK-0-TFA-GAA7

Current Data Parameters

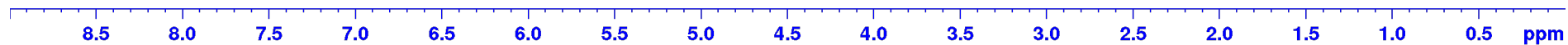
NAME THK-264-TFA-20200204-1H.fid
EXPNO 1
PROCNO 1

F2 - Processing parameters

SI 16384
SF 499.7888646 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



4.7897
4.7289
4.7122
4.4675
4.4515
4.4159
4.4002
4.1606
4.1547
4.0220
4.0056
4.0006
3.9835
3.9523
3.9461
3.9287
3.9137
3.9032
3.9003
3.8940
3.8790
3.8715
3.8560
3.8497
3.8091
3.7932
3.7855
3.7806
3.7698
3.7632
3.7546
3.7464
3.7298
3.7222
3.7001
3.6856
3.6782
3.6735
3.6592
3.6534
3.6392
3.6238
3.6143
3.6062
3.5830
3.5668
3.5477
3.5199
3.5062
3.2920
3.2733
3.2585
1.6508
1.6395
1.6221
1.6096
1.5965
1.5830
1.5698
1.5940
1.5871
1.5796
1.5727
1.5665



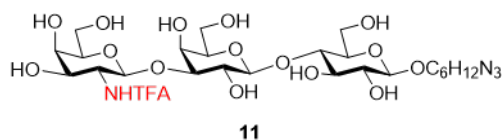
1.11
1.07
1.00
0.99
0.98
2.07
0.96
1.11
3.99
5.12
2.26
2.23
3.15
4.08
4.18

¹H NMR spectrum of Compound 11 (500 MHz D₂O)

THK-0-TFA-GAA7

Current Data Parameters
NAME THK-264-TFA-20200203-13C.fid
EXPNO 1
PROCNO 1

F2 - Processing parameters
SI 65536
SF 125.6718829 MHz
WDW EM
SSE 0
LB 0.30 Hz
GB 0
PC 1.00



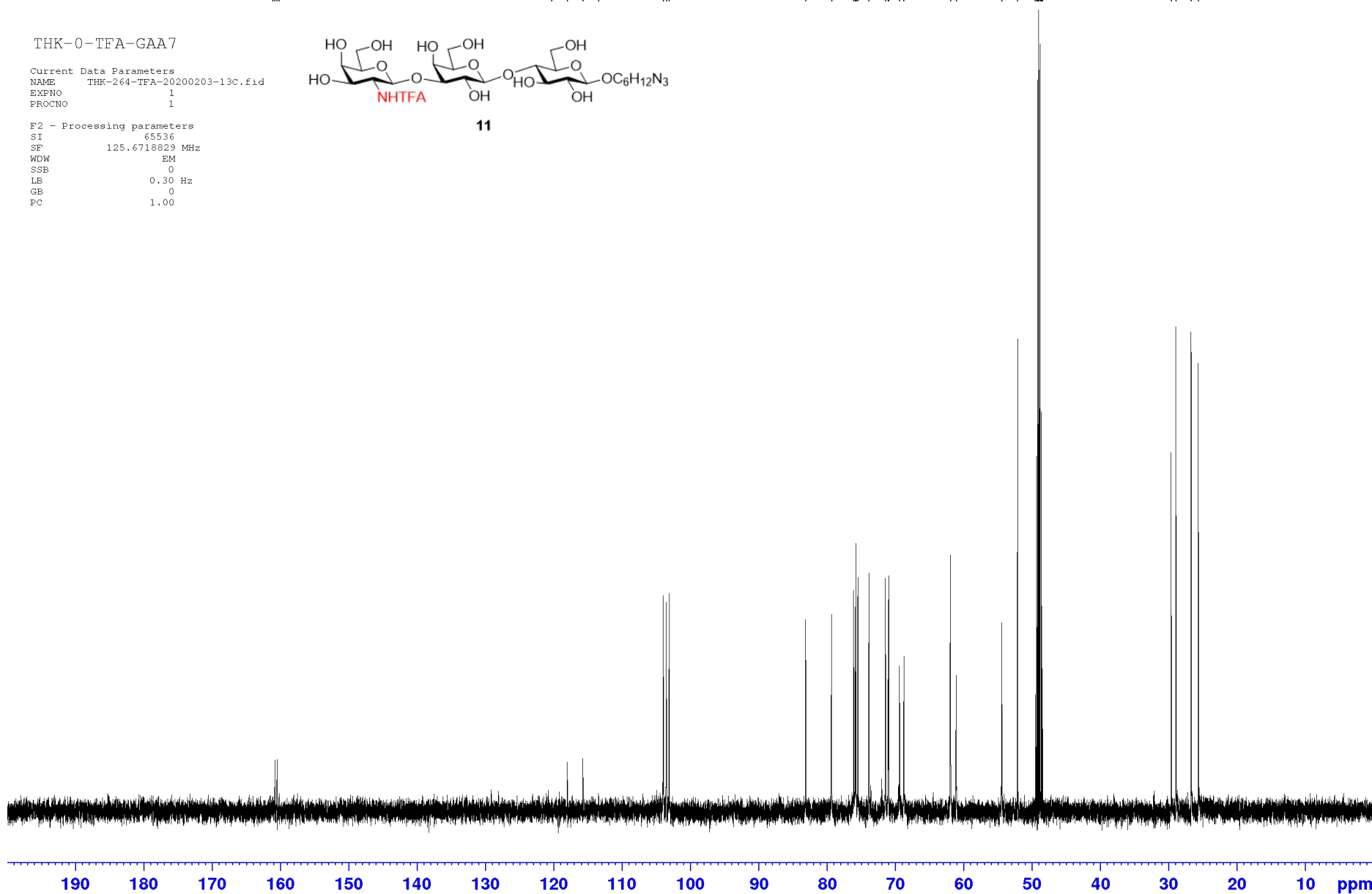
161.113
160.811
160.516
160.225

120.307
118.023
115.744
113.455

103.988
103.545
103.110

83.138
79.353
76.119
75.892
75.759
75.493
73.880
71.466
71.049
70.979
69.401
68.761
61.964
61.941
61.113
54.463
52.122
49.525
49.352
49.160
49.007
48.835
48.662
48.490

29.649
28.941
26.717
25.656

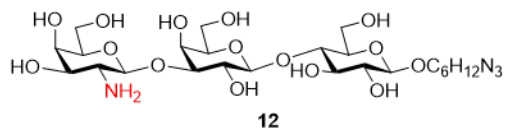


^{13}C NMR spectrum of Compound 11 (125 MHz D_2O)

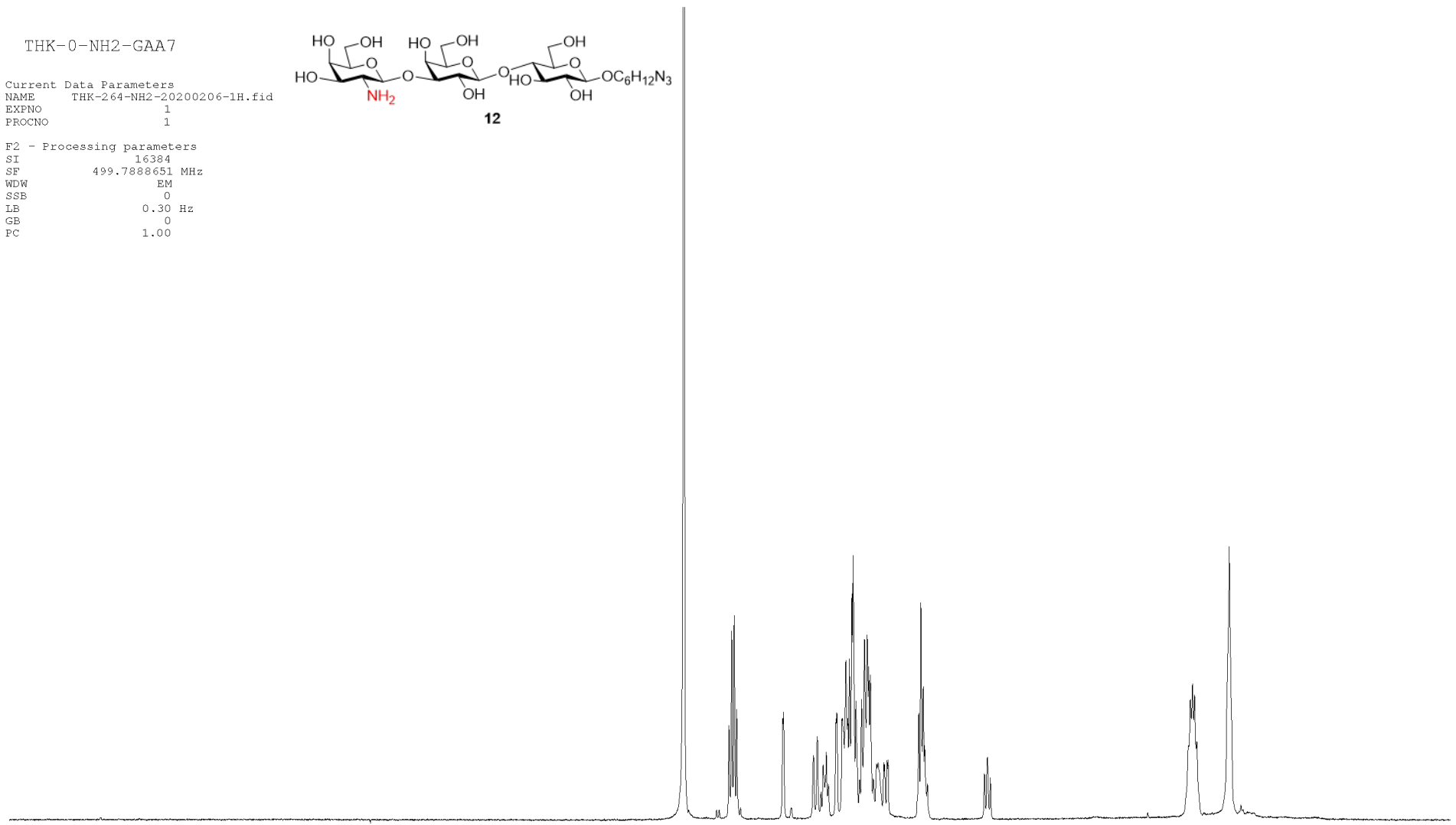
THK-0-NH2-GAA7

Current Data Parameters
NAME THK-264-NH2-20200206-1H.fid
EXPNO 1
PROCNO 1

F2 - Processing parameters
SI 16384
SF 499.7888651 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



4.7894
4.5059
4.4895
4.4739
4.4581
4.1709
4.1655
3.9787
3.9551
3.9317
3.8181
3.8083
3.8055
3.8382
3.8329
3.8002
3.7965
3.7762
3.7649
3.7543
3.7387
3.7305
3.7137
3.6908
3.6778
3.6601
3.6433
3.6332
3.6229
3.6046
3.5854
3.5750
3.5398
3.5341
3.5190
3.5133
3.3213
3.3075
3.2937
3.2843
3.2674
2.9108
2.8920
2.8739
1.6530
1.6372
1.6250
1.6118
1.5963
1.5850
1.5700
1.3968
1.3897
1.3819
1.3745
1.3665



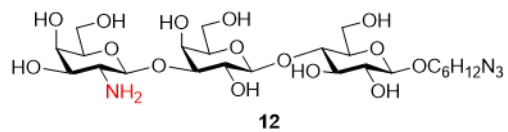
3.12
1.02
1.04
1.20
1.02
7.14
1.05
4.99
1.15
1.07
3.19
1.00
4.04
4.20

¹H NMR spectrum of Compound 12 (500 MHz D₂O)

THK-0-NH2-GAA7

Current Data Parameters
NAME THK-264-NH2-20200206-13c.fid
EXPNO 1
PROCNO 1

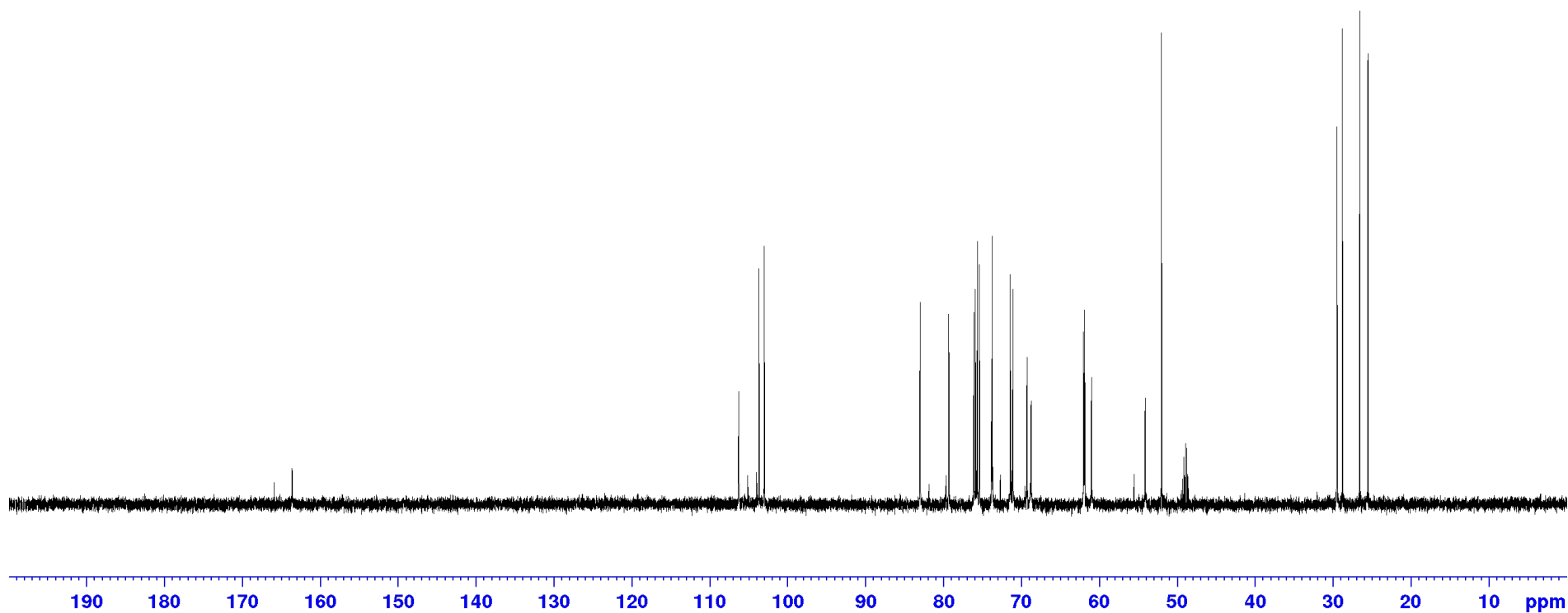
F2 - Processing parameters
SI 6536
SF 125.6718957 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



106.263
103.659
102.996

83.071
79.371
76.148
75.958
75.699
75.432
73.874
73.794
71.497
71.131
69.339
68.813
62.076
61.933
61.068
54.163
52.087
49.528
49.344
49.172
48.998
48.826
48.654
48.491

29.544
28.841
26.620
25.564

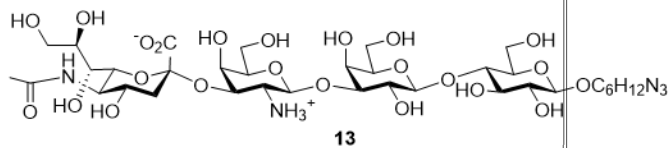


¹³C NMR spectrum of Compound 12 (125 MHz D₂O)

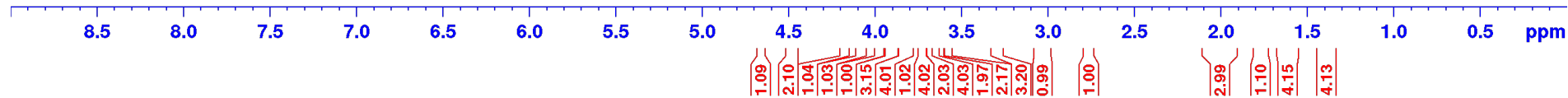
THK-3A-NH2-GAA7

Current Data Parameters
NAME THK-267-20200210.fid
EXPNO 1
PROCNO 1

F2 - Processing parameters
SI 16384
SF 499.7888646 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



4.7896
4.6658
4.6496
4.6333
4.5041
4.4883
4.4765
4.4605
4.1776
4.1720
4.0954
4.0900
4.0747
4.0687
3.9798
3.9570
3.9346
3.9207
3.9126
3.9071
3.9029
3.8881
3.8707
3.8596
3.8394
3.8186
3.8084
3.7989
3.7825
3.7720
3.7532
3.7325
3.7159
3.6948
3.6788
3.6698
3.6538
3.6389
3.6249
3.6060
3.5858
3.5626
3.3251
3.3112
3.2977
3.2865
3.2697
3.0514
3.0322
3.0140
2.7730
2.7576
2.7489
2.0164
1.7914
1.7671
1.7430
1.6556
1.6403
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1.5745
1.3998
1.3932
1.3853
1.3792
1.3714

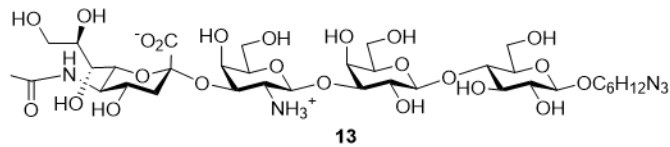


¹H NMR spectrum of Compound 13 (500 MHz D₂O)

THK-3A-NH2-GAA7

Current Data Parameters
NAME THK-267-a23-20200210-13C.fid
EXPNO 1
PROCNO 1

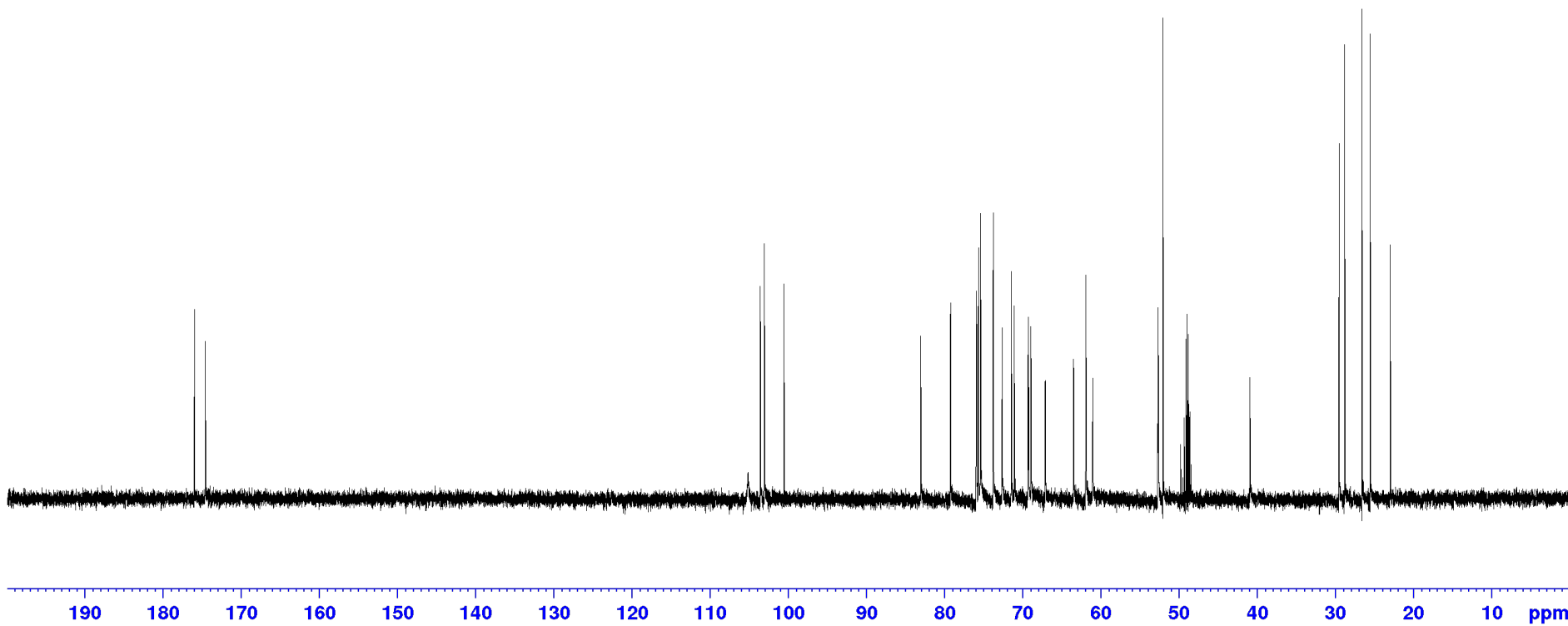
F2 - Processing parameters
SI 65536
SF 125.6718946 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



175.971
174.589

105.105
103.529
103.004
100.525

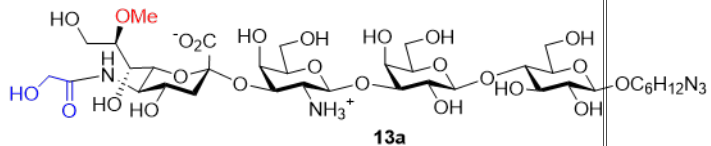
83.074
79.269
75.998
75.943
75.702
75.423
73.808
73.794
72.663
71.505
71.111
69.367
69.297
68.972
67.146
63.512
61.964
61.940
61.060
52.736
52.679
52.093
49.513
49.342
49.169
48.936
48.823
48.650
48.479
40.945
29.546
28.844
26.622
25.566
22.993



¹³C NMR spectrum of Compound **13** (125 MHz D₂O)

THK-3GM-NH2-GAA7

Current Data Parameters
NAME THK-268-2-20200227-1H.fid
EXPNO 1
PROCNO 1
F2 - Processing parameters
SI 16384
SF 499.7875556 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



4.7893
4.5286
4.5122
4.4875
4.4717
4.4633
4.4472
4.1612
4.1553
4.0894
4.0138
3.9983
3.9927
3.9774
3.9723
3.9484
3.9370
3.9199
3.9154
3.8934
3.8735
3.7968
3.7820
3.7746
3.7628
3.7576
3.7395
3.7192
3.7036
3.6974
3.6806
3.6794
3.6671
3.6578
3.6538
3.6472
3.6359
3.6287
3.6224
3.6171
3.6112
3.5949
3.5715
3.5589
3.5412
3.5318
3.5266
3.5210
3.5092
3.5042
3.4949
3.4648
3.4188
3.3110
3.2972
3.2855
3.2718
3.2547
2.9659
2.9491
2.9455
2.9286
2.7025
2.6937
2.6783
2.6699
1.7476
1.7235
1.6419
1.6275
1.6148
1.6016
1.5879
1.5744
1.5600
1.3855
1.3786
1.3717
1.3637
1.3562



1.06
1.07
1.05
1.07
2.02
1.15
2.19
4.17
4.36
4.34
3.00
3.14
3.15
3.16
3.23
1.00
1.00
1.10
4.17
4.21

¹H NMR spectrum of Compound 13a (500 MHz D₂O)

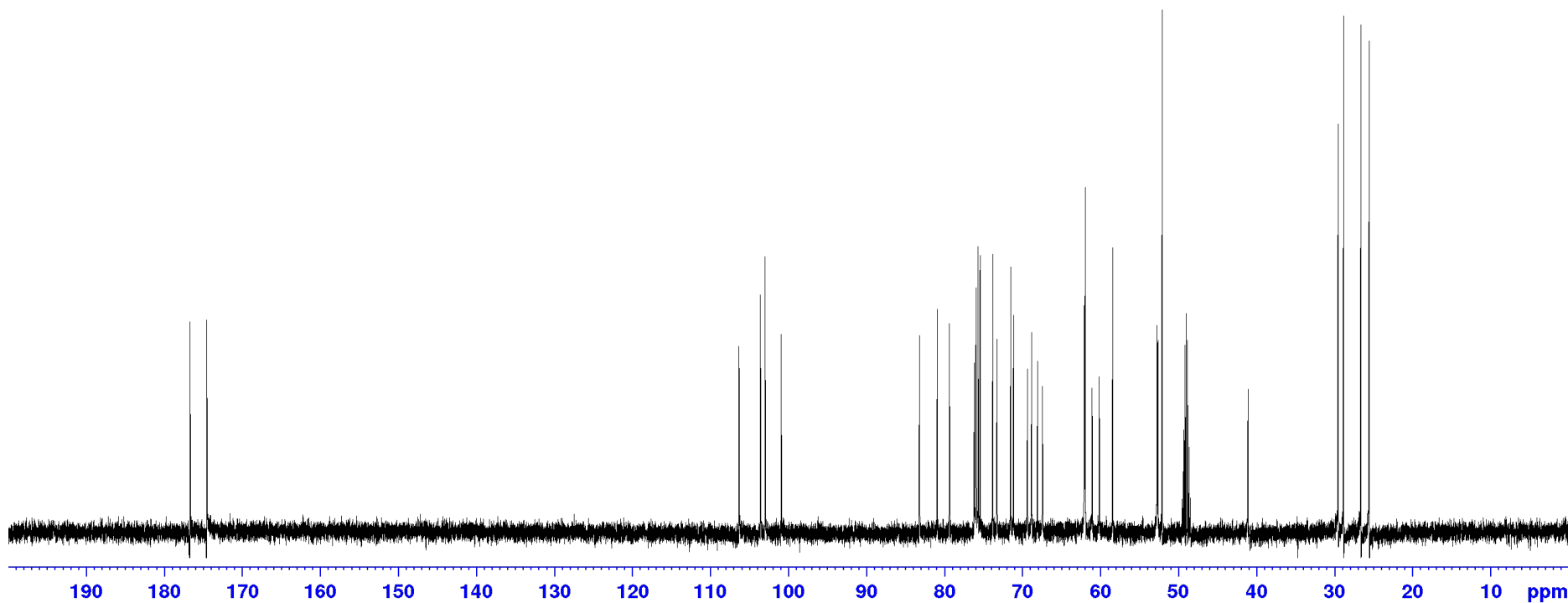
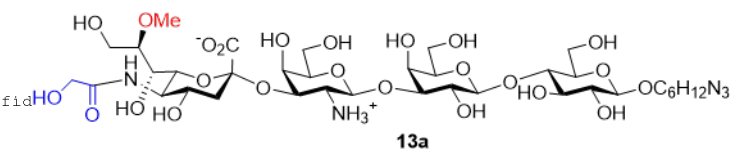
176.727
174.549

106.332
103.583
102.999
100.939
83.200
80.924
79.363
76.169
75.961
75.921
75.700
75.432
73.801
73.262
71.500
71.143
69.363
68.813
68.071
67.432
62.071
61.956
61.063
60.155
58.421
52.748
52.642
52.093
49.515
49.342
49.169
48.997
48.825
48.651
48.480
41.082
39.545
38.840
36.619
35.563

THK-3GM-NH2-GAA7

Current Data Parameters
NAME THK-268-2-20200227-13c.fid
EXPNO 1
PROCNO 1

F2 - Processing parameters
SI 65536
SF 125.6715669 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

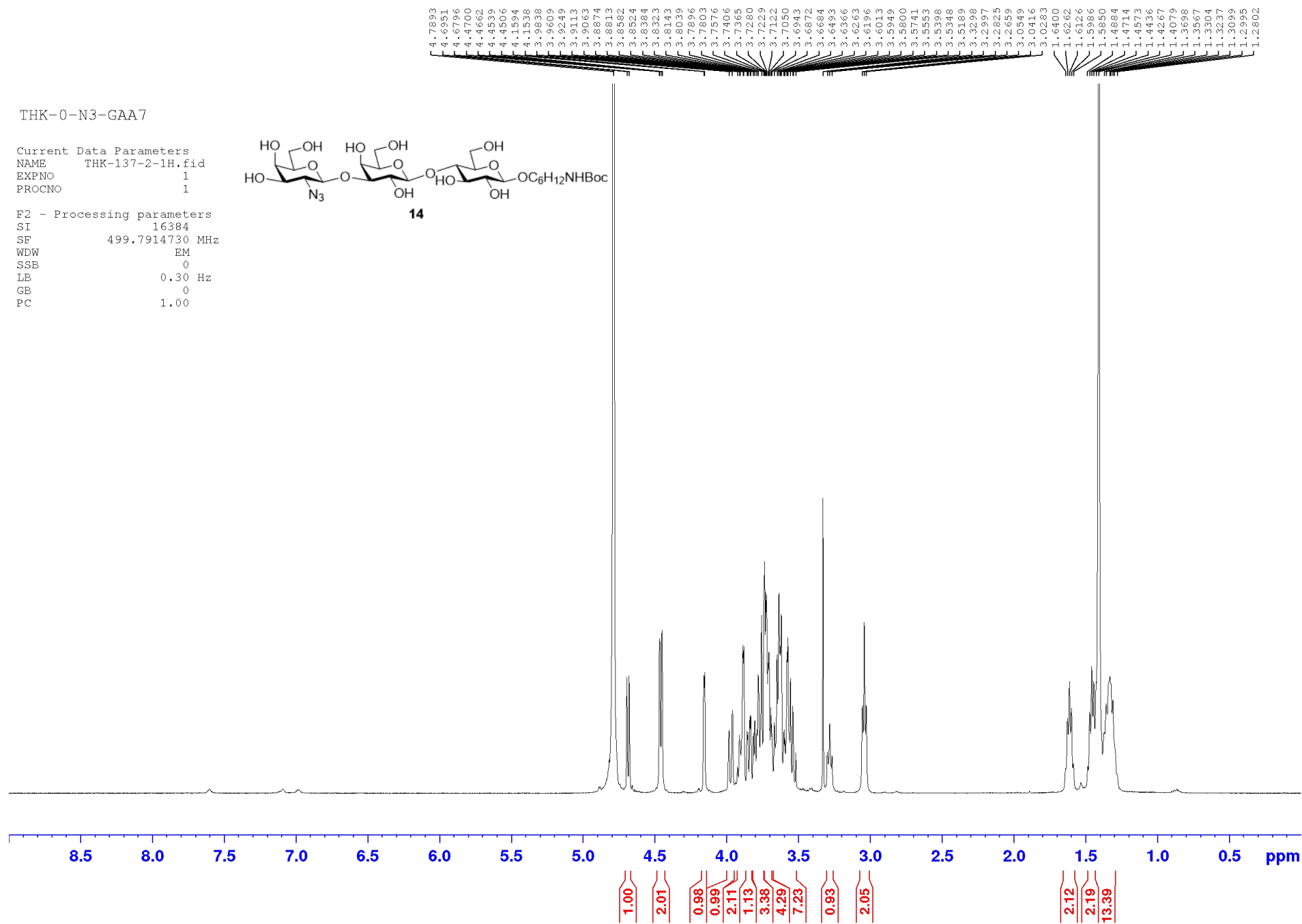
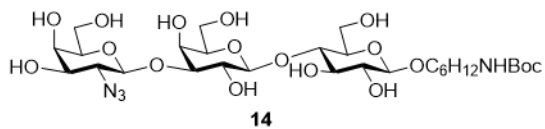


¹³C NMR spectrum of Compound 13a (125 MHz D₂O)

THK-0-N3-GAA7

Current Data Parameters
NAME THK-137-2-1H.fid
EXPNO 1
PROCNO 1

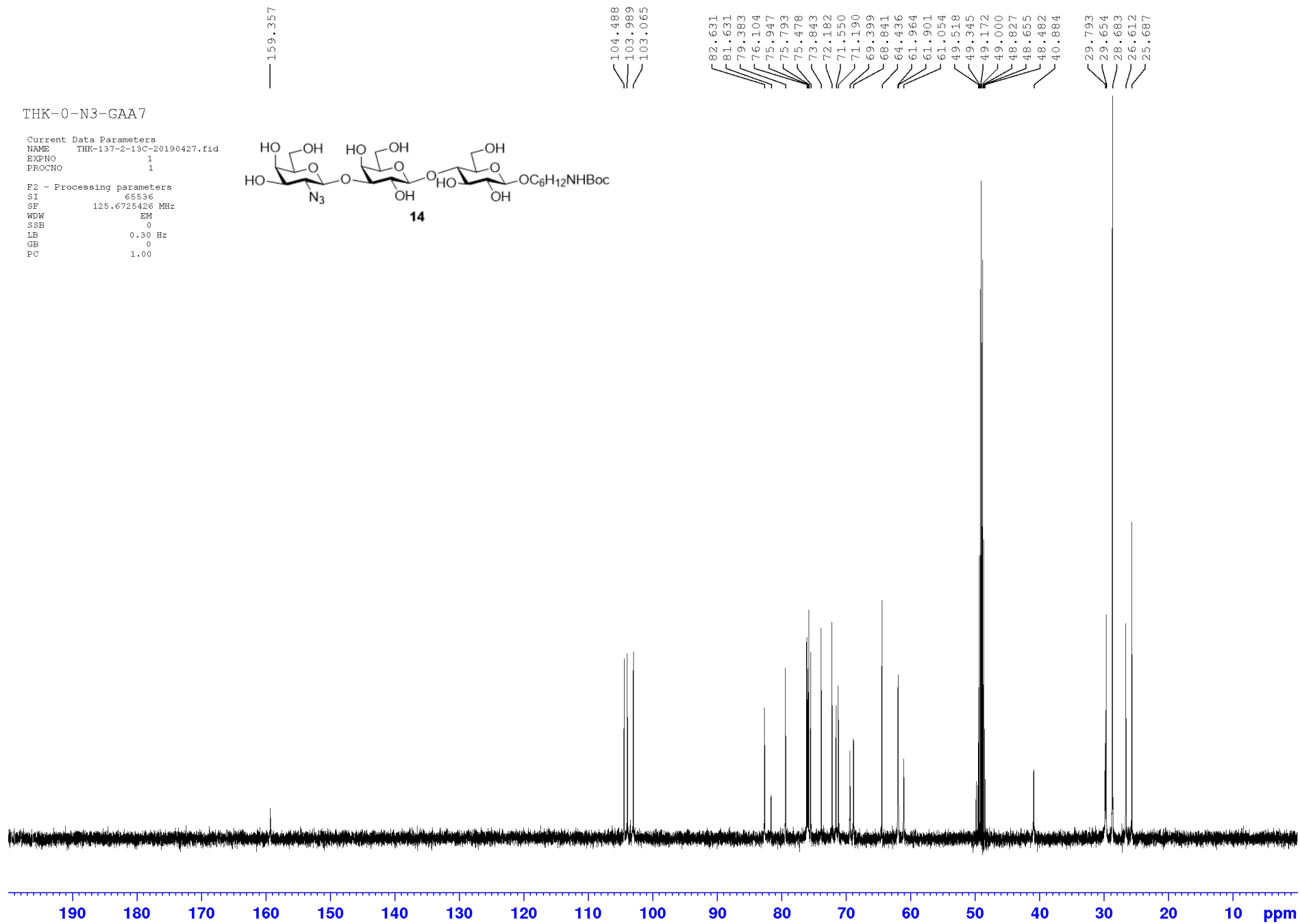
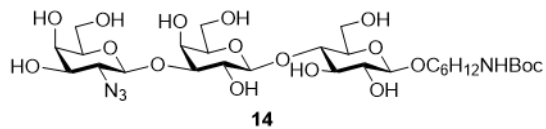
F2 - Processing parameters
SI 16384
SF 499.7914730 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



¹H NMR spectrum of Compound 14 (500 MHz D₂O)

THK-0-N3-GAA7

Current Data Parameters
NAME THK-137-2-13C-20190427.fid
EXPNO 1
PROCNO 1
F2 - Processing parameters
SI 65536
SF 125.6725426 MHz
WDW EM
SSB 0
LE 0.30 Hz
GB 0
PC 1.00



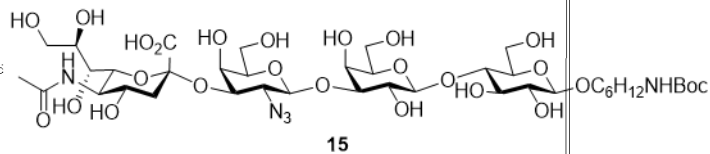
^{13}C NMR spectrum of Compound **14** (125 MHz D_2O)

8.4330

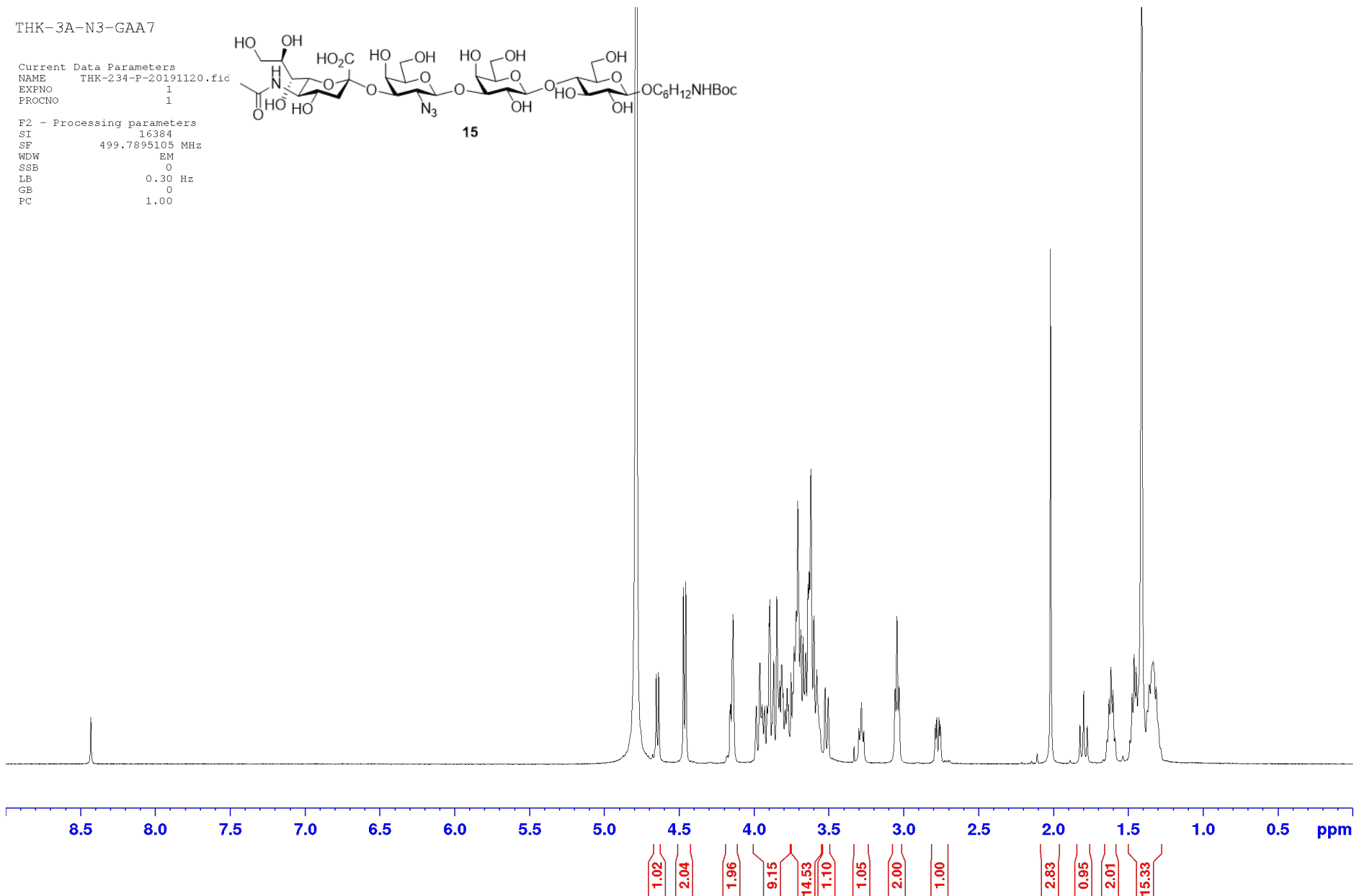
THK-3A-N3-GAA7

Current Data Parameters
NAME THK-234-P-20191120.fid
EXPNO 1
PROCNO 1

F2 - Processing parameters
SI 16384
SF 499.7895105 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



4.7893
4.6543
4.6378
4.4728
4.4569
4.1622
4.1568
4.1413
3.9851
3.9613
3.9522
3.9470
3.9291
3.9183
3.9166
3.8994
3.8805
3.8652
3.8483
3.8401
3.8285
3.8166
3.8065
3.7920
3.7798
3.7719
3.7540
3.7406
3.7334
3.7208
3.7178
3.7080
3.6920
3.6878
3.6724
3.6647
3.6550
3.6406
3.6317
3.6209
3.5994
3.5819
3.5755
3.5618
3.5289
3.5256
3.5077
3.5046
3.3003
3.2835
3.2669
3.0583
3.0451
3.0318
2.7895
2.7806
2.7653
2.7564
2.0195
1.8232
1.7991
1.7793
1.6426
1.6291
1.6155
1.6014
1.5879
1.4885
1.4745
1.4604
1.4468
1.4110
1.3727
1.3593
1.3335
1.3129
1.3001
1.2823

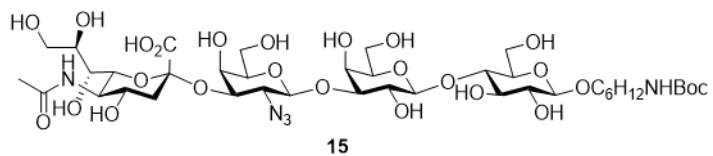


¹H NMR spectrum of Compound 15 (500 MHz D₂O)

THK-3A-N3-GAA7

Current Data Parameters
NAME THK-234-13c-20191121.fid
EXPNO 1
PROCNO 1

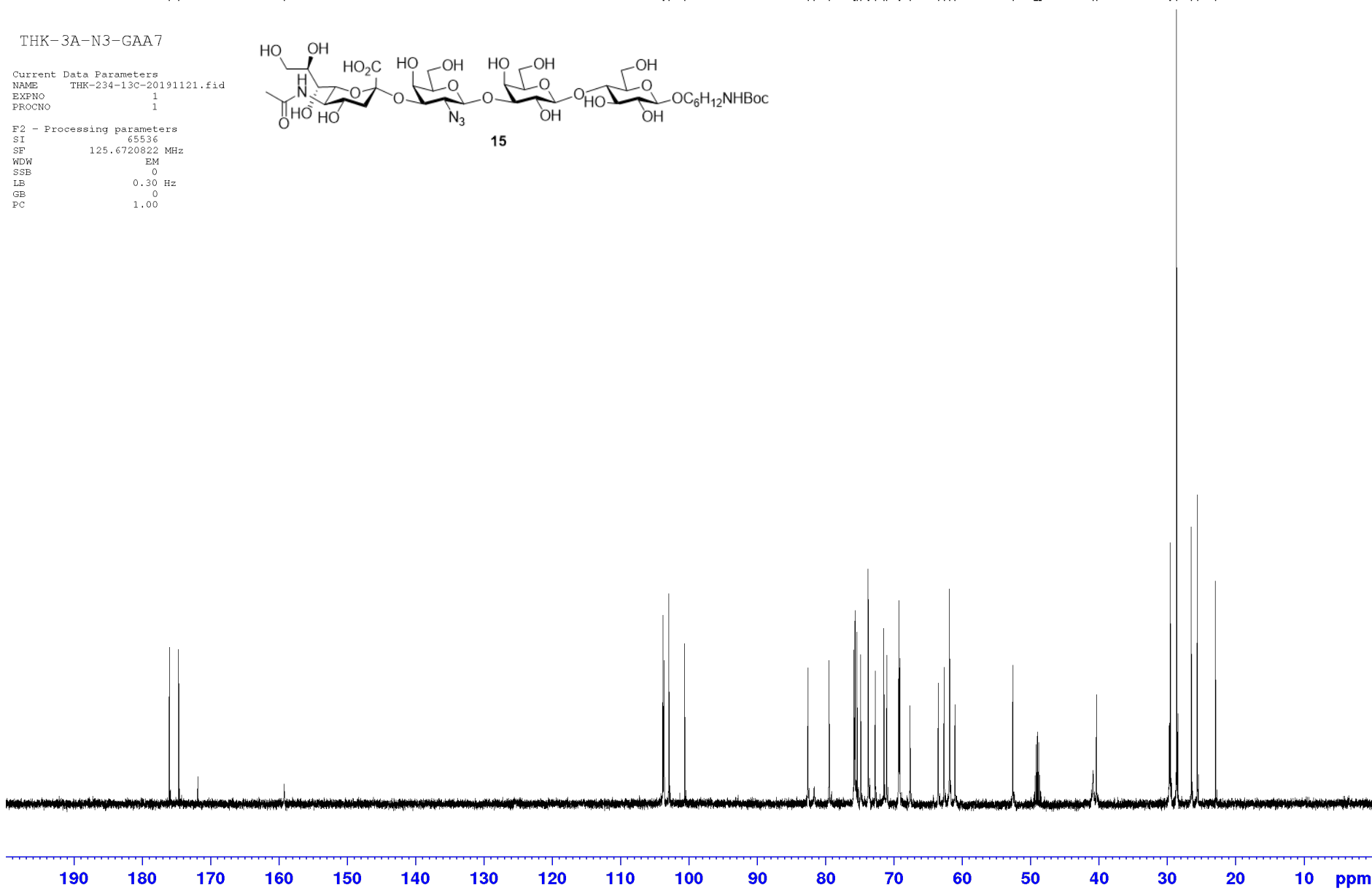
F2 - Processing parameters
SI 65536
SF 125.6720822 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



176.076
174.700

159.259

103.812
103.683
102.967
100.633
82.587
81.685
79.491
75.872
75.747
75.693
75.411
74.884
73.775
73.740
72.751
71.482
71.066
69.282
69.153
67.657
63.519
62.667
61.871
61.069
52.607
49.516
49.345
49.172
48.999
48.827
48.654
48.482
40.887
40.359
29.688
29.547
28.636
28.489
25.578
22.939



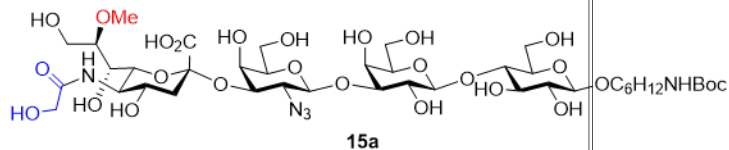
^{13}C NMR spectrum of Compound 15 (125 MHz D_2O)



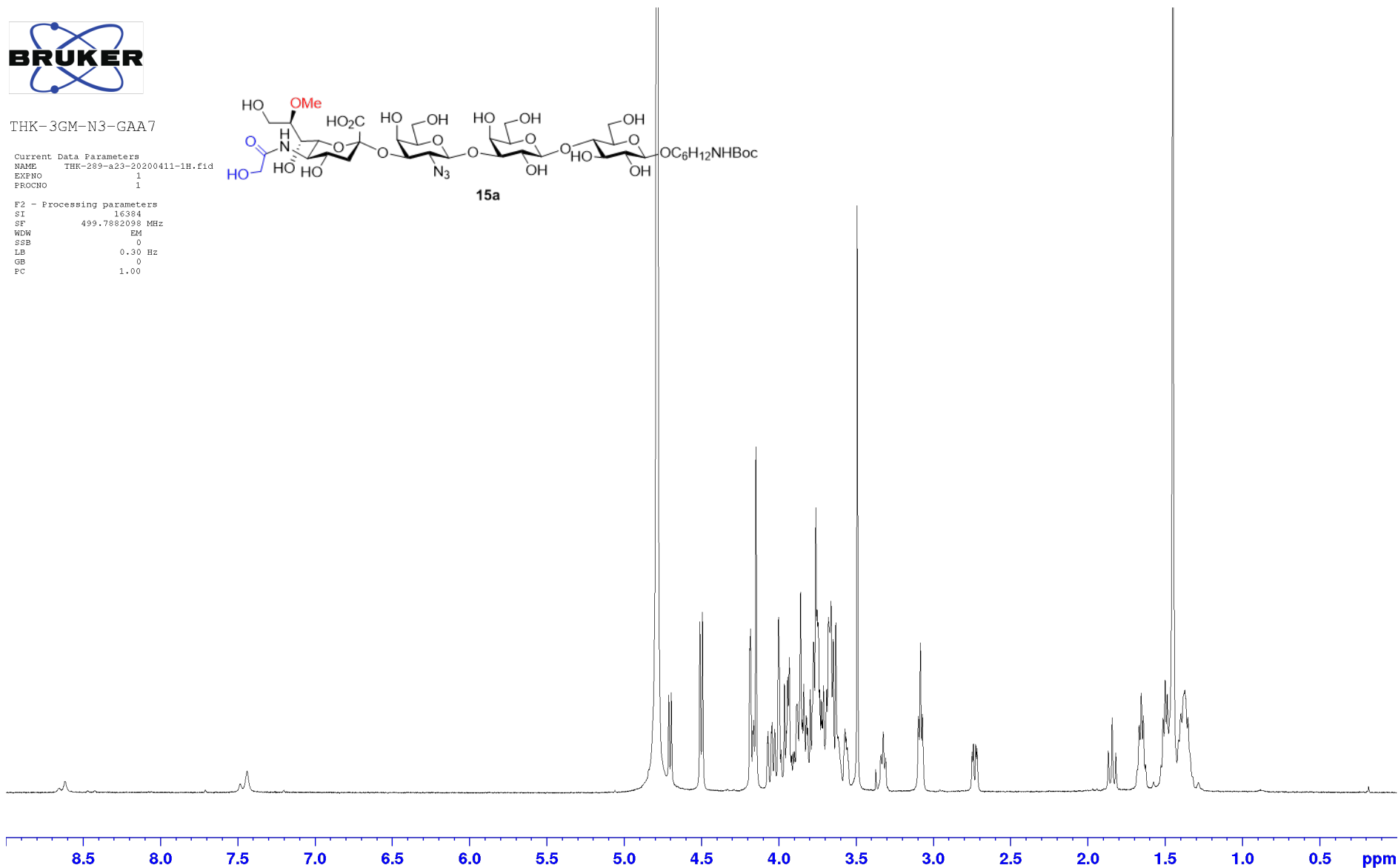
THK-3GM-N3-GAA7

Current Data Parameters
NAME THK-289-a23-20200411-1H.fid
EXPNO 1
PROCNO 1

F2 - Processing parameters
SI 16384
SF 499.7882098 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

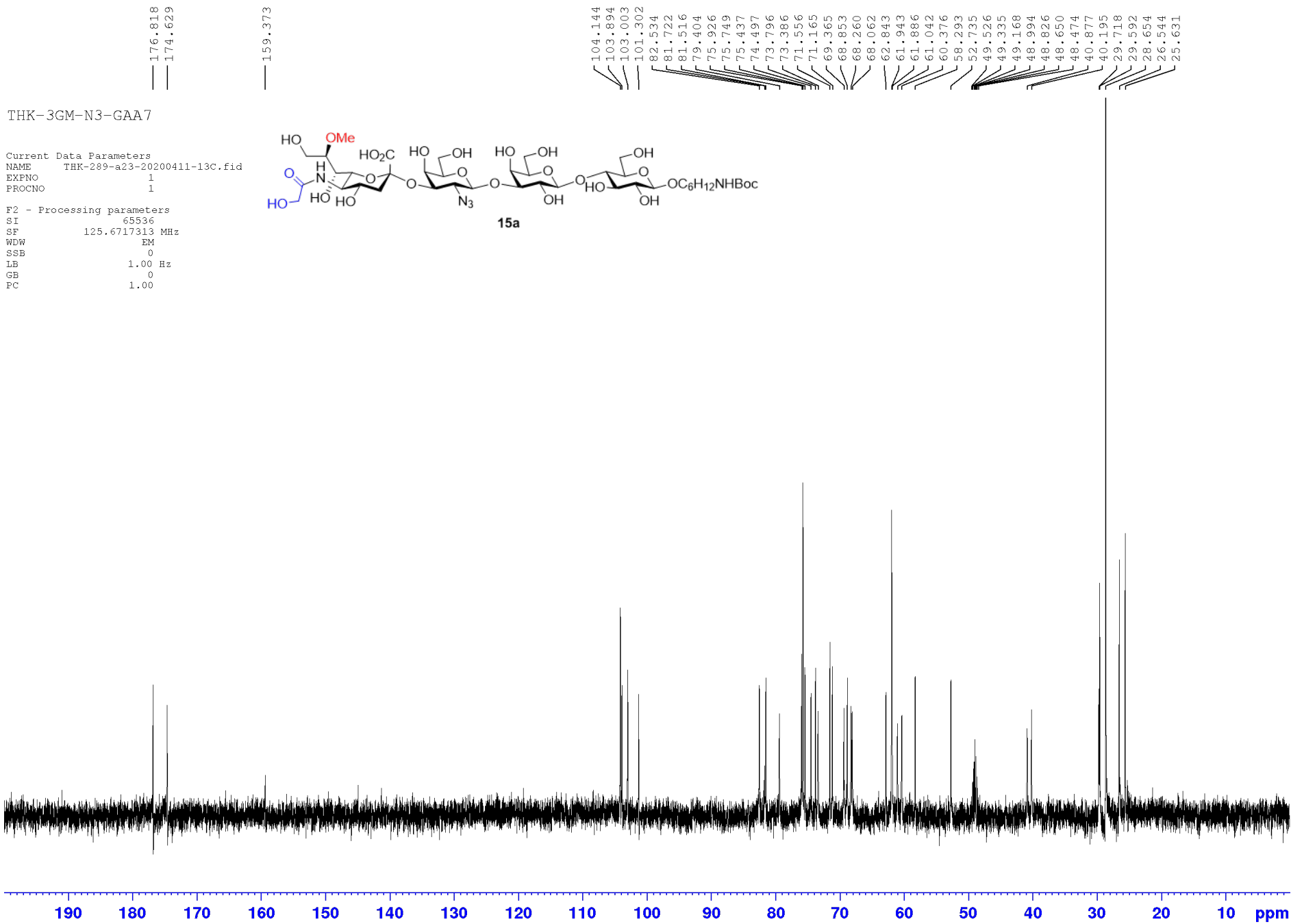


4.7891
4.7121
4.6957
4.5099
4.4941
4.1878
4.1830
4.1680
4.1627
4.1476
4.0738
4.0687
4.0486
4.0439
4.0260
4.0015
3.9840
3.9635
3.9507
3.9431
3.9319
3.9179
3.9067
3.8979
3.8842
3.8815
3.8588
3.8471
3.8378
3.8210
3.8141
3.7962
3.7746
3.7598
3.7513
3.7451
3.7358
3.7263
3.7190
3.7108
3.6895
3.6784
3.6616
3.6538
3.6483
3.6311
3.6149
3.5779
3.5713
3.5644
3.5561
3.5495
3.4926
3.3388
3.3236
3.3082
3.0963
3.0831
3.0637
2.7495
2.7410
2.7250
2.7166
1.8679
1.8433
1.8190
1.6823
1.6677
1.6542
1.6403
1.6267
1.6171
1.5131
1.4982
1.4885
1.4503
1.4112
1.3978
1.3788
1.3724
1.3660
1.3516
1.3389
1.3221



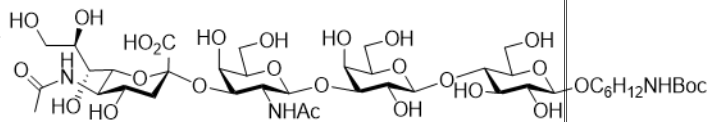
1.07
2.00
4.02
1.08
2.09
7.31
7.16
4.09
2.02
1.09
3.08
1.03
2.00
1.02
1.03
2.14
15.30

¹H NMR spectrum of Compound 15a (500 MHz D₂O)

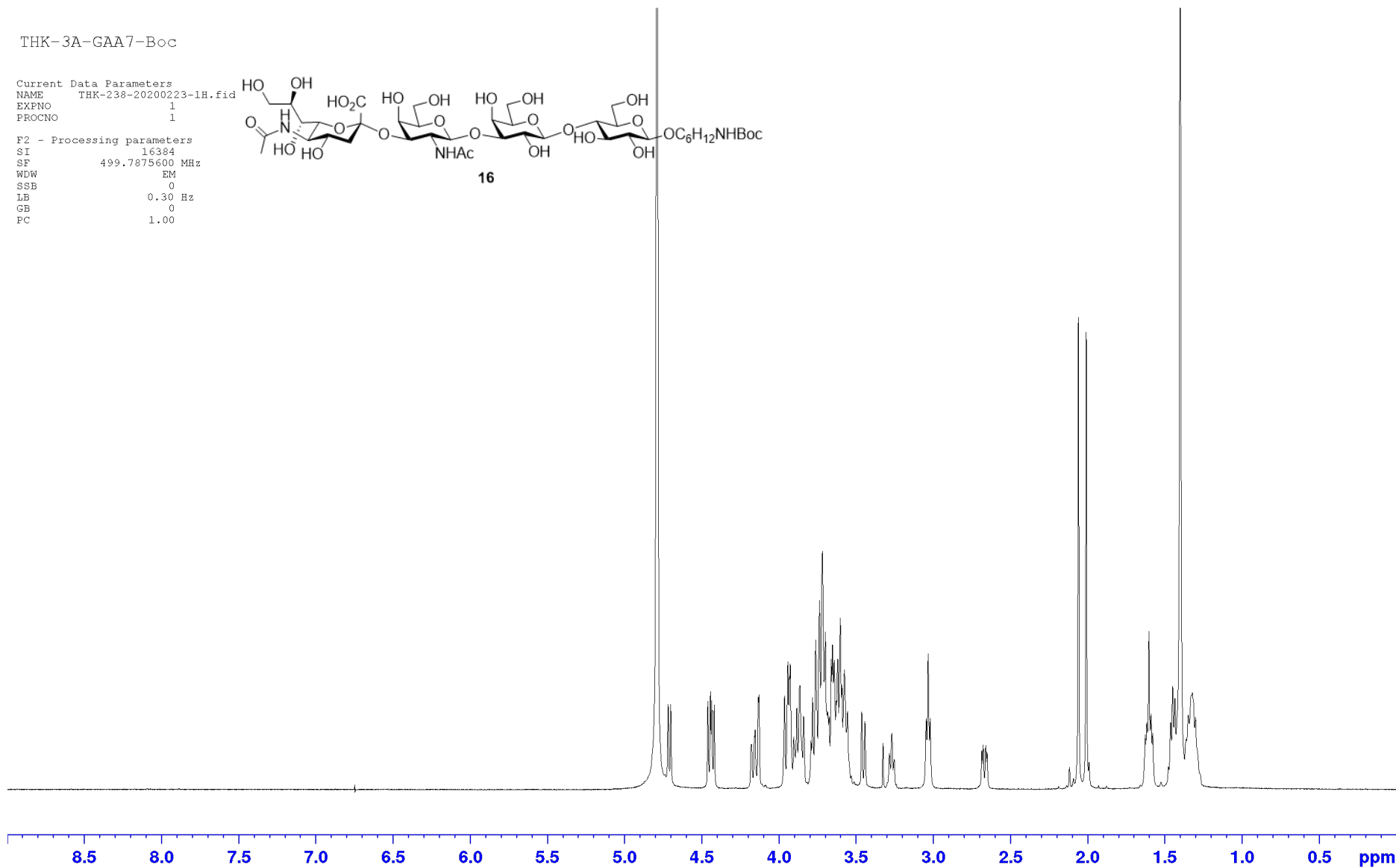


THK-3A-GAA7-Boc

Current Data Parameters
NAME THK-238-20200223-1H.fid
EXPNO 1
PROCNO 1
F2 - Processing parameters
SI 16384
SF 499.7875600 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



4.7893
4.7170
4.7000
4.4592
4.4430
4.4344
4.4185
4.1799
4.1757
4.1580
4.1556
4.1338
4.1293
3.9629
3.9414
3.9308
3.9253
3.9030
3.8831
3.8651
3.8395
3.7911
3.7811
3.7607
3.7364
3.7167
3.6989
3.6824
3.6739
3.6604
3.6524
3.6406
3.6290
3.6172
3.6017
3.5907
3.5751
3.5550
3.4626
3.4419
3.2840
3.2662
3.2521
3.0488
3.0356
3.0223
2.6879
2.6630
2.6541
2.0626
2.0115
1.6292
1.6191
1.6052
1.5916
1.5812
1.4789
1.4651
1.4511
1.4374
1.4023
1.3645
1.3500
1.3240
1.3037
1.2936
1.2753

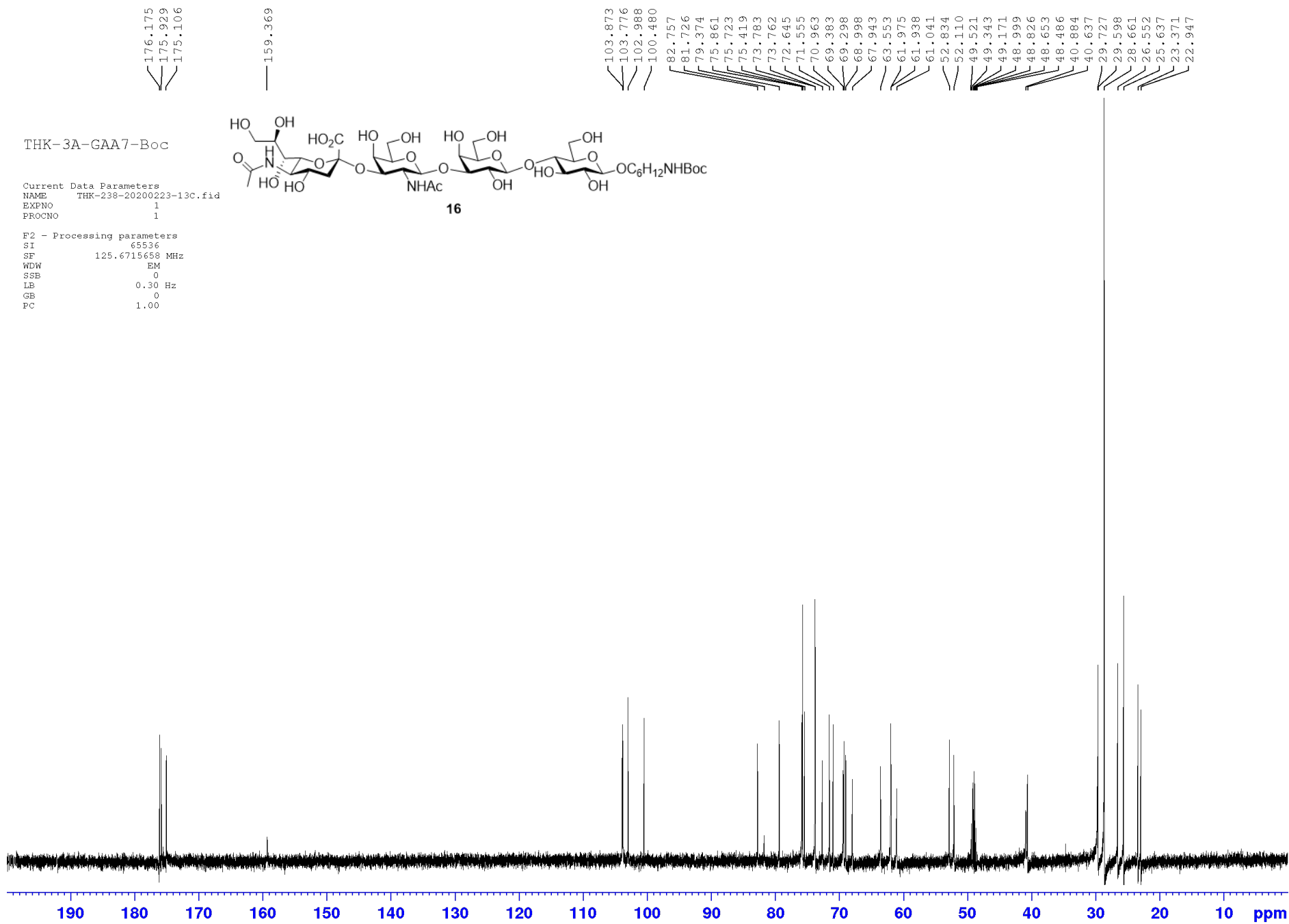
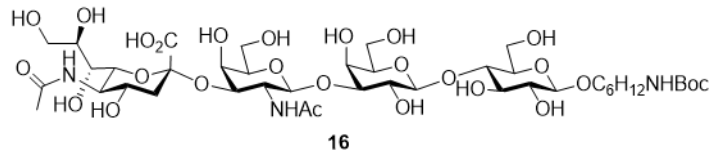


¹H NMR spectrum of Compound 16 (500 MHz D₂O)

THK-3A-GAA7-Boc

Current Data Parameters
NAME THK-238-20200223-13c.fid
EXPNO 1
PROCNO 1

F2 - Processing parameters
SI 65836
SF 125.6715658 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

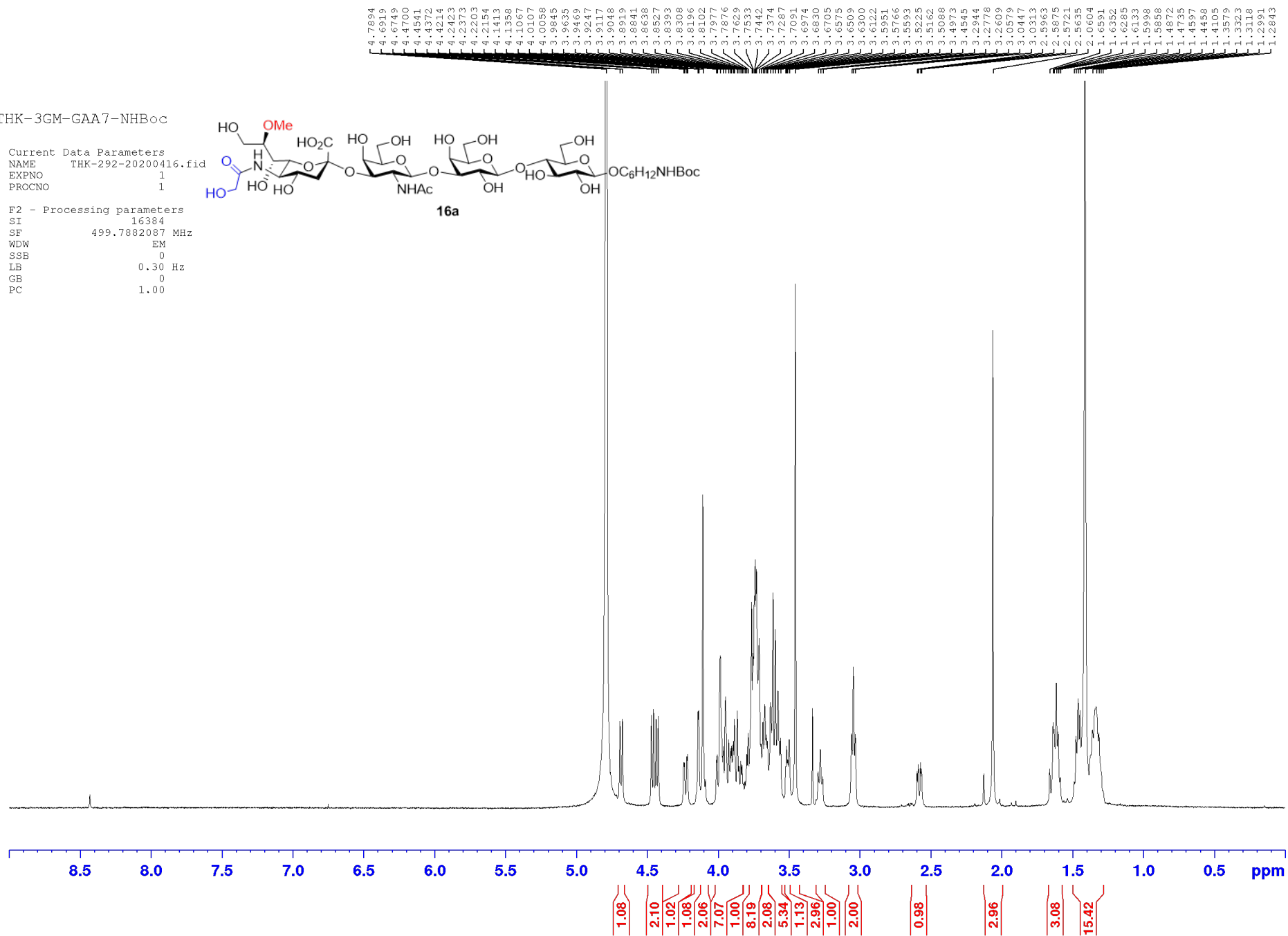
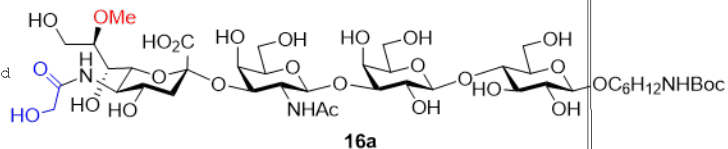


^{13}C NMR spectrum of Compound 16 (125 MHz D_2O)

THK-3GM-GAA7-NHBoc

Current Data Parameters
NAME THK-292-20200416.fid
EXPNO 1
PROCNO 1

F2 - Processing parameters
SI 16384
SF 499.7882087 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

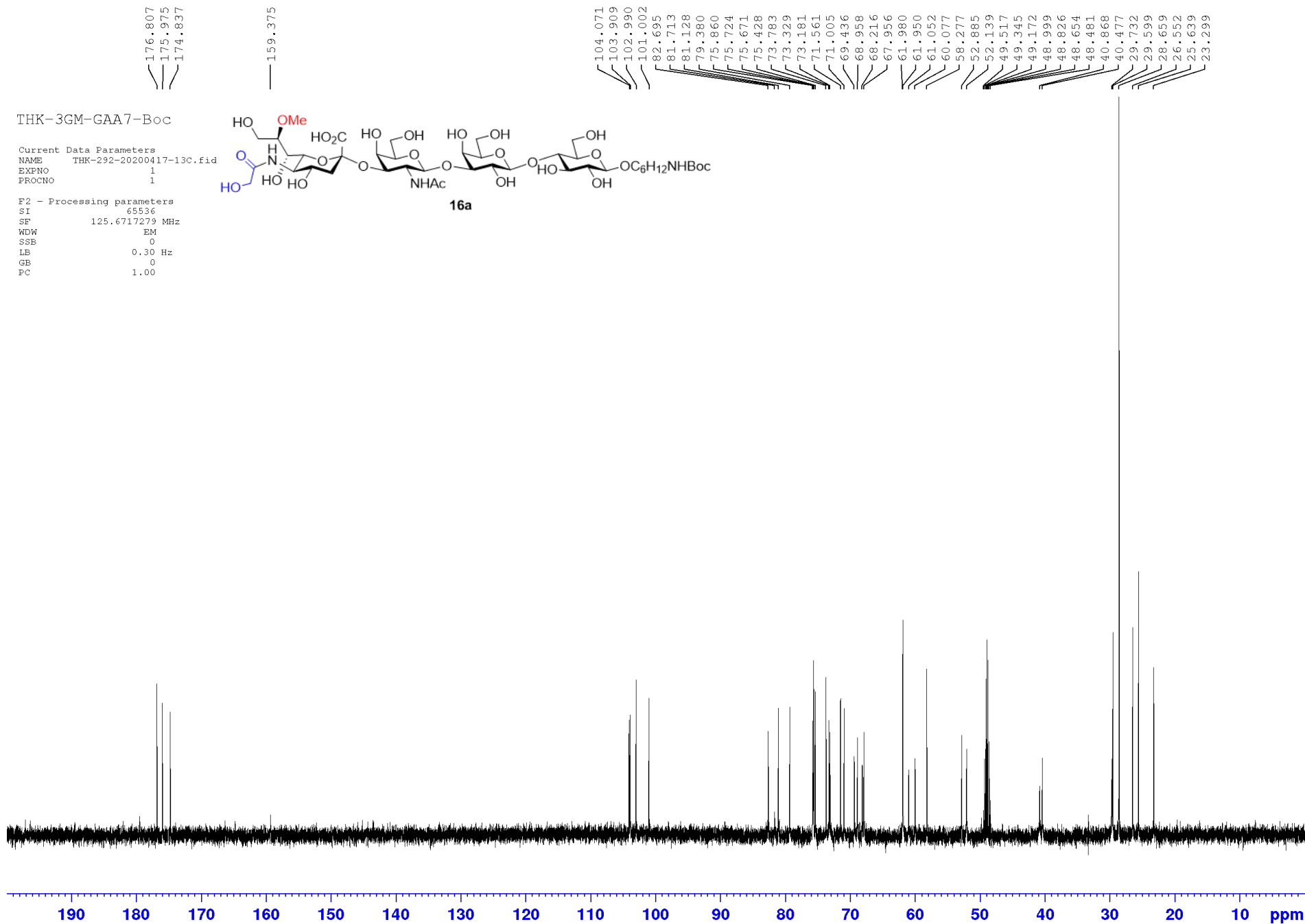
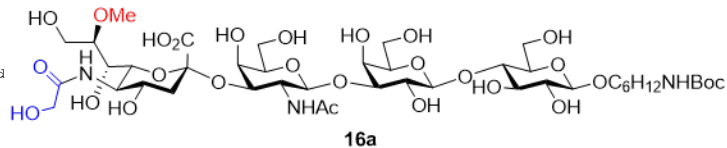


¹H NMR spectrum of Compound **16a** (500 MHz D₂O)

THK-3GM-GAA7-Boc

Current Data Parameters
NAME THK-292-20200417-13c.fid
EXPNO 1
PROCNO 1

F2 - Processing parameters
SI 65536
SF 125.6717279 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

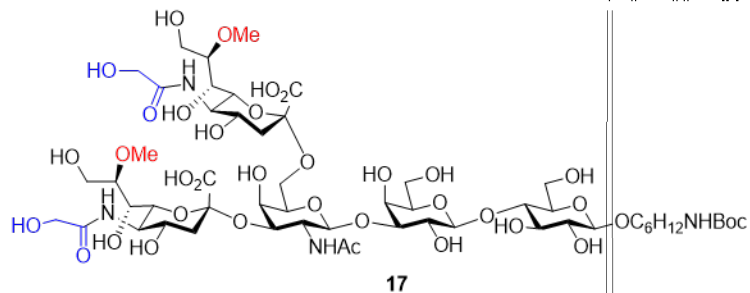


¹³C NMR spectrum of Compound **16a** (125 MHz D₂O)

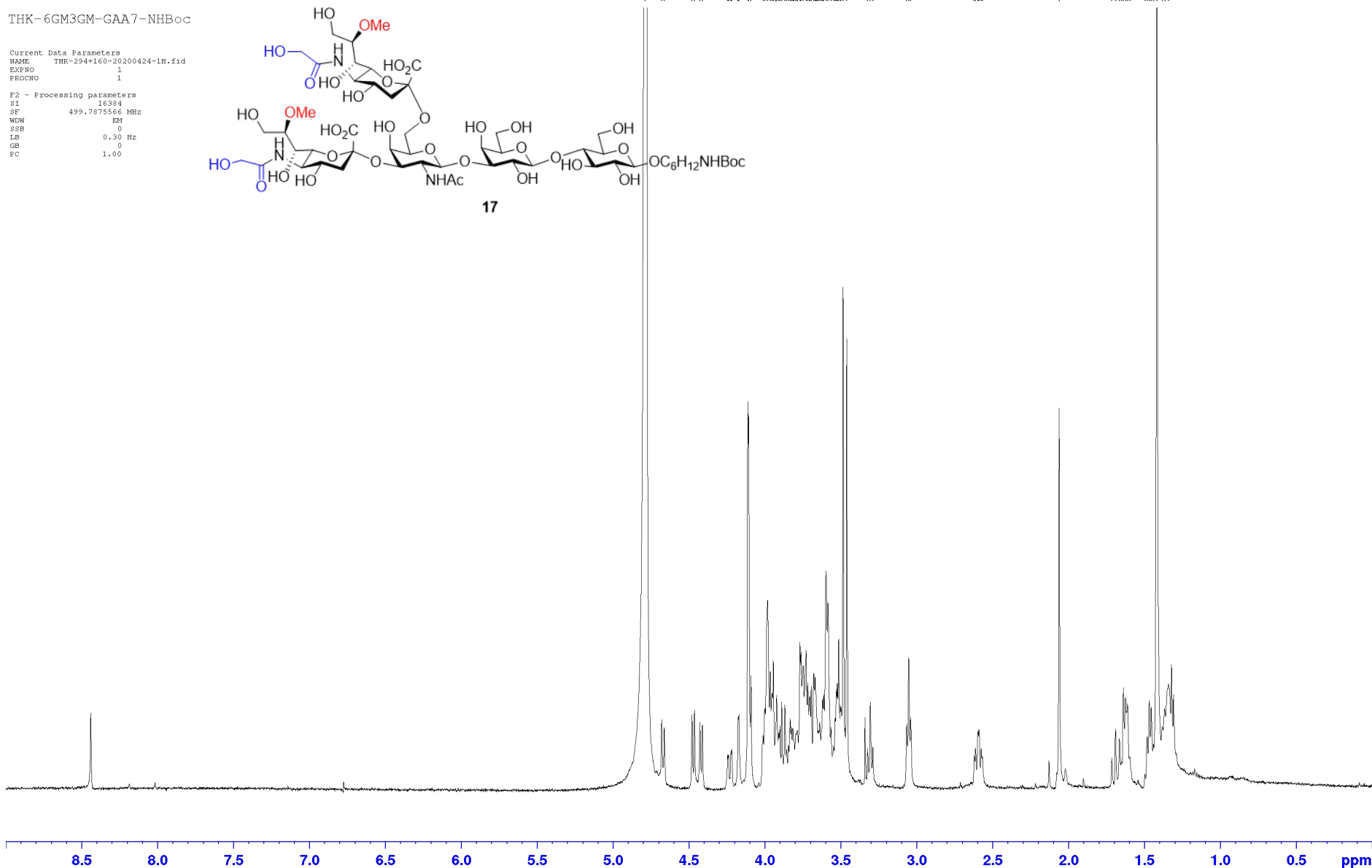
THK-6GM3GM-GAA7-NHBoc

Current Data Parameters
NAME THK-294+160-20200424-18.fid
EXPNO 1
PROCNO 1

F2 - Processing parameters
SI 16384
SF 499.7875566 MHz
WDW EM
SSB 0
DS 0.30 Hz
GB 0
PC 1.00



4.7895
4.6804
4.6634
4.4813
4.4602
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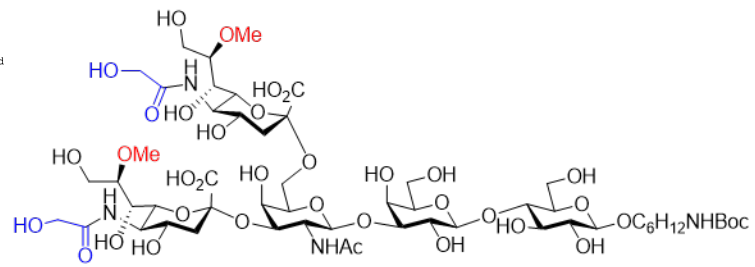
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1.05
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1.07
5.03
6.24
2.27
2.20
6.06
2.96
6.25
3.04
6.20
1.21
2.05
2.02
3.14
3.98
15.32

¹H NMR spectrum of Compound 17 (500 MHz D₂O)

THK-6GM3GM-GAA7NHBoc

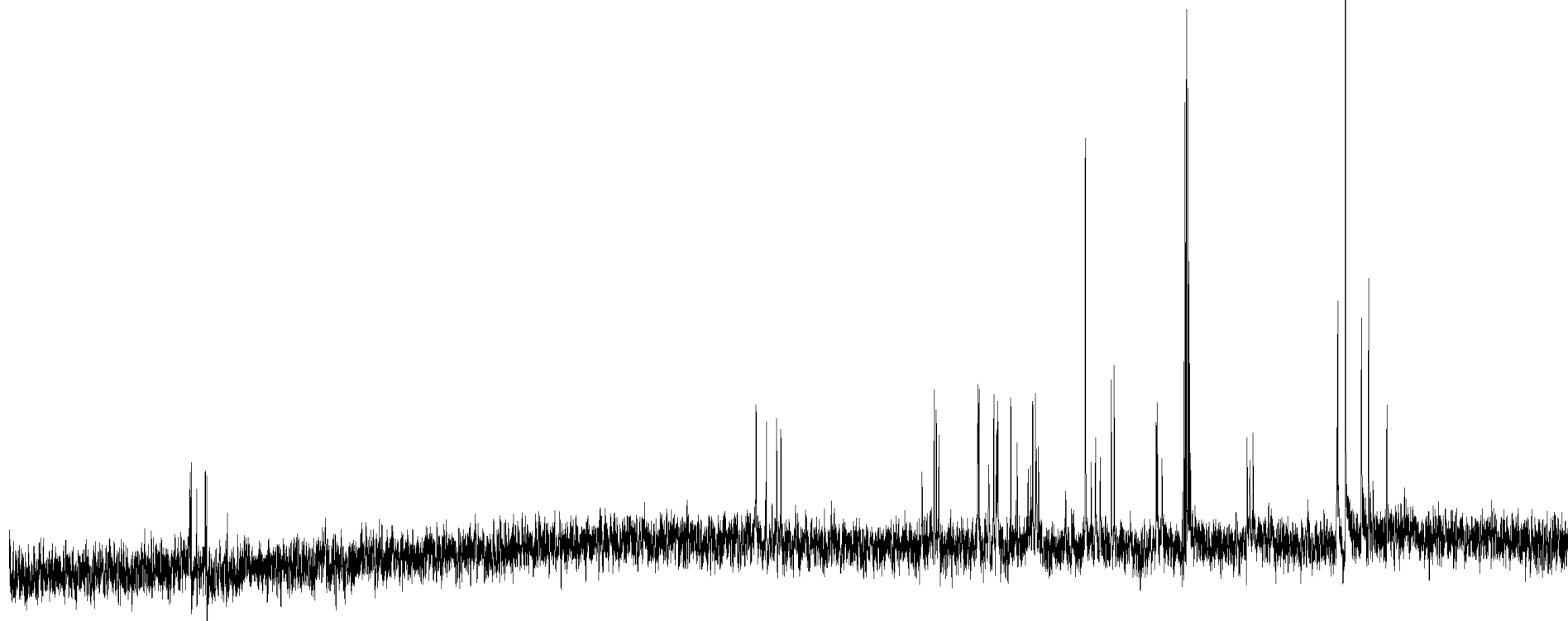
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 EXPNO 1
 PROCNO 1

F2 - Processing parameters
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 WDW EM
 SSB 0
 LB 2.00 Hz
 GB 0
 PC 1.00



17

176.794
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 174.652
 159.407
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 104.149
 102.865
 101.542
 101.006
 82.928
 81.748
 81.338
 81.073
 80.745
 75.737
 75.674
 75.585
 74.357
 73.704
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 40.871
 40.472
 29.712
 29.587
 28.652
 26.551
 25.642
 23.297



190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

¹³C NMR spectrum of Compound 17 (125 MHz D₂O)