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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 (N_2). Dichloromethane (DCM), toluene, and acetonitrile (ACN) were distilled from calcium hydride under N_2. 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 N_2 unless indicated otherwise. The reactions were monitored by analytical thin-layer chromatography (TLC) on Merck silica gel 60 F_{254} 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 H_2O. 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 H_2O. DEAE Sepharose® Fast Flow (GE Healthcare Life Sciences) was used for anion exchange chromatography. ^1H and ^{13}C 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 $CDCl_3$ (7.24 ppm), CD_3OD (3.31 or 4.87 ppm), or D_2O (4.79 ppm at 298 K) as internal standard in ^1H-NMR spectra. 13C-NMR spectra were recorded in either $CDCl_3$, CD_3OD or D_2O at 100, 125, 150, or 175 MHz, using the central resonances of $CDCl_3$ (77.0 ppm) and CD_3OD (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 polymeras 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. BlNahK (*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 PmST3Δ35 was optimized as previous described[9] and synthesized by Genomics (New Taipei City, Taiwan), then isolated by PCR amplification using the following primers: PmST3Δ35-F: 5'- GAG ACAT ATG GAT AAA TTT GCC GAA CAT G-3' (*Nde*I restriction site is underlined) and PmST3Δ35-R: 5'- ATA TCT CGA GGC CCA GTT TTT CAT ACA CAA A-3' (*Xho*I restriction site is underlined). Briefly, PCR amplification of target gene was performed in a solution (50 μL) that contained 50 ng of template DNA, 0.4 μM of each primer, 0.2 mM dNTP, 1.5 mM $MgCl_2$, 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 *Nde*I and *Xho*I and then was inserted into the *Nde*I/*Xho*I-treated vector pET-22b(+). The ligation mixture was then transformed into the *E. coli* DH5α 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 μg/mL) at 37 °C until the OD_{600} 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.

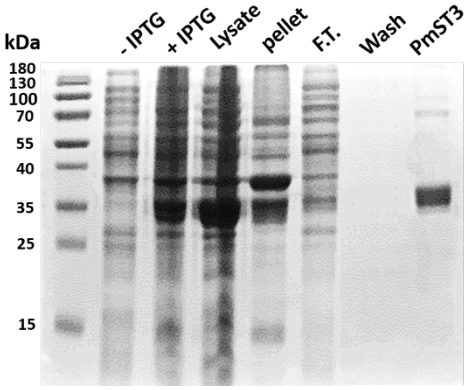


Fig. S1 SDS-PAGE analysis of PmST3Δ35. The gel of the purified protein shows an apparent band that is close to the calculated molecular weight (33 kDa) of the desired protein. Lanes: IPTG (-/+), whole cell extract before and after induction; Lysate, supernatant of the inducted cell extract after centrifugation; 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.

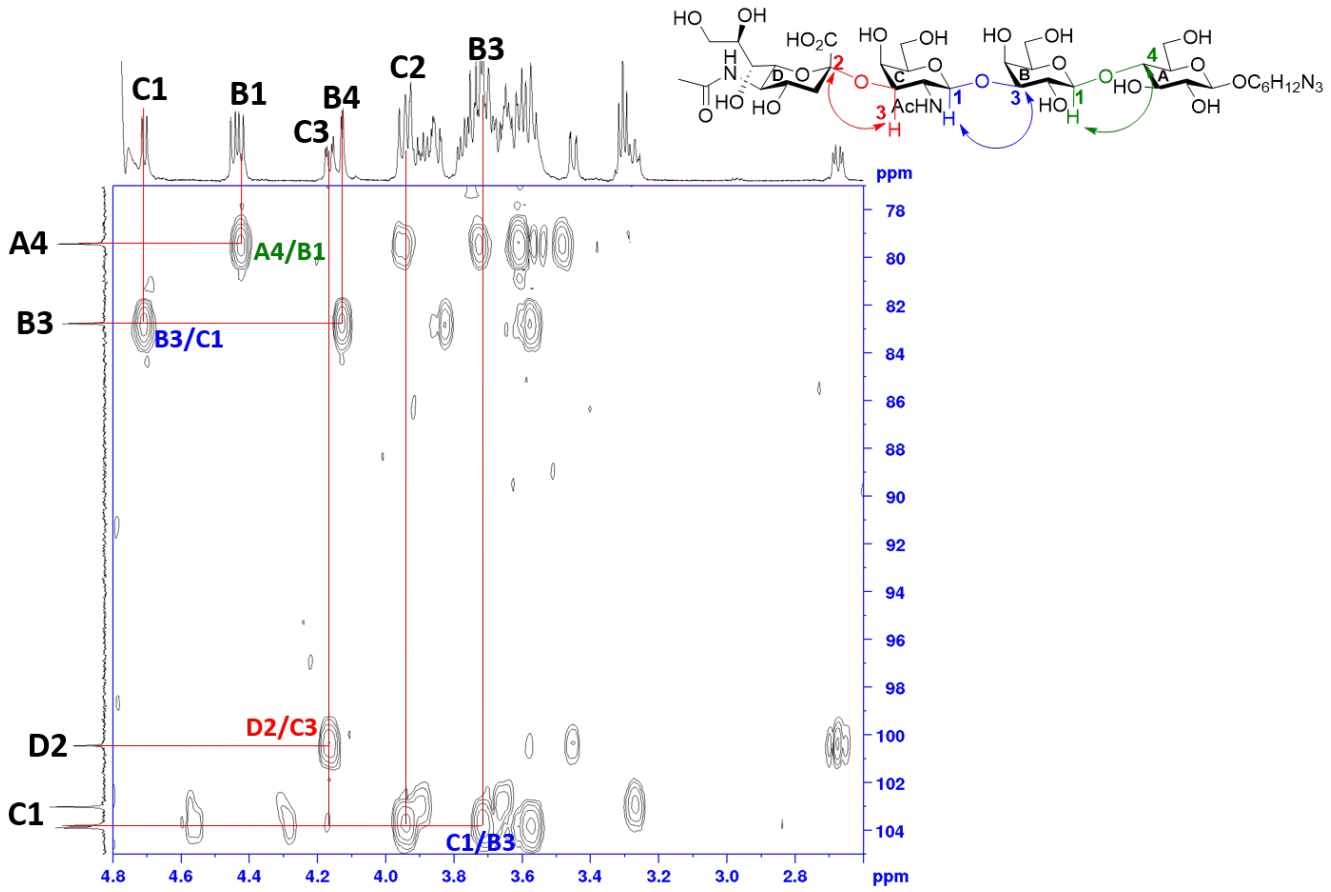


Fig. S2 ^{1}H-^{13}C HMBC analysis of compound **4**. Glycans A, B, C, and D represent Glc, Gal, GalNAc, and Neu5Ac, respectively. The number indicates the position of the carbon or proton atom. **GalNAc-β(1,3)-Gal linkage.** A cross signal for the anomeric proton of GalNAc (C1) and the 3-position carbon 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.

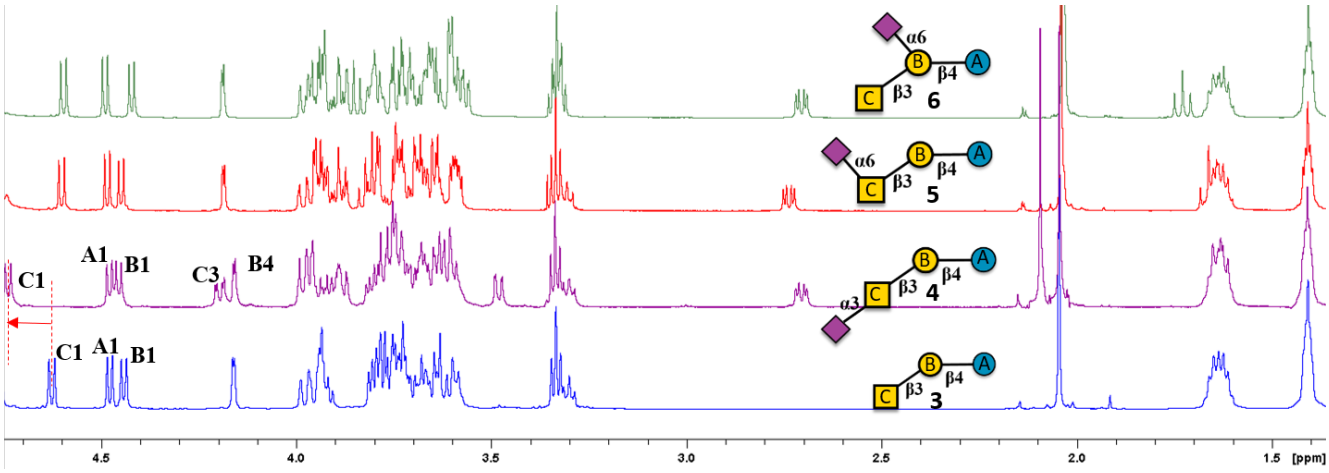


Fig. S3 ^{1}H NMR comparison of monosialylated compounds **4**, **5**, and **6**. Glycans A, B, C, and D represent Glc, Gal, GalNAc, and Neu5Ac, respectively. The number indicates the position of the proton atom. The H-1 signal of GalNAc (C1) was shifted downfield from 4.63 to 4.71 ppm after α(2,3) sialylation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

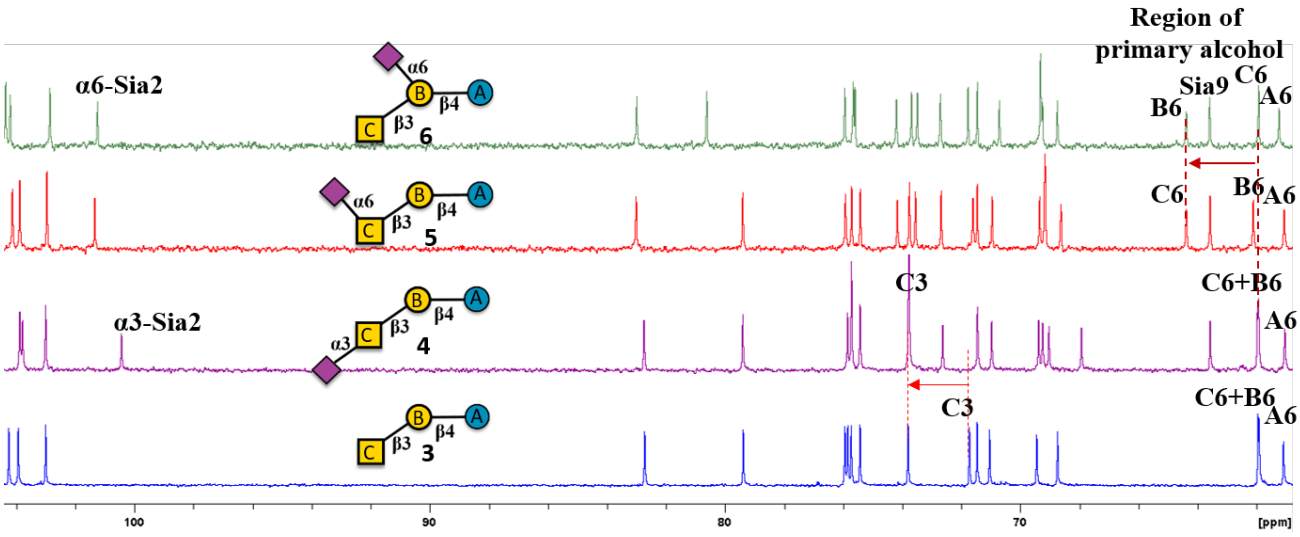
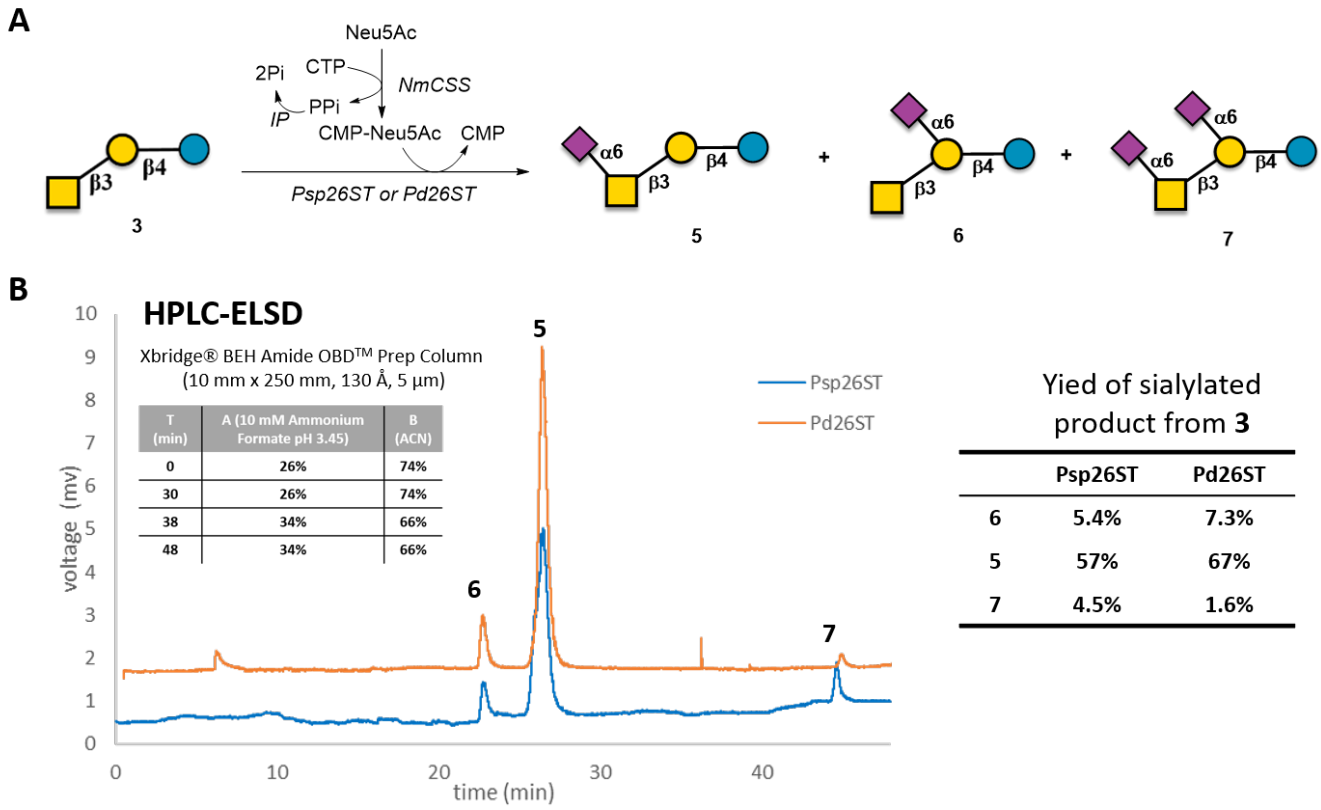


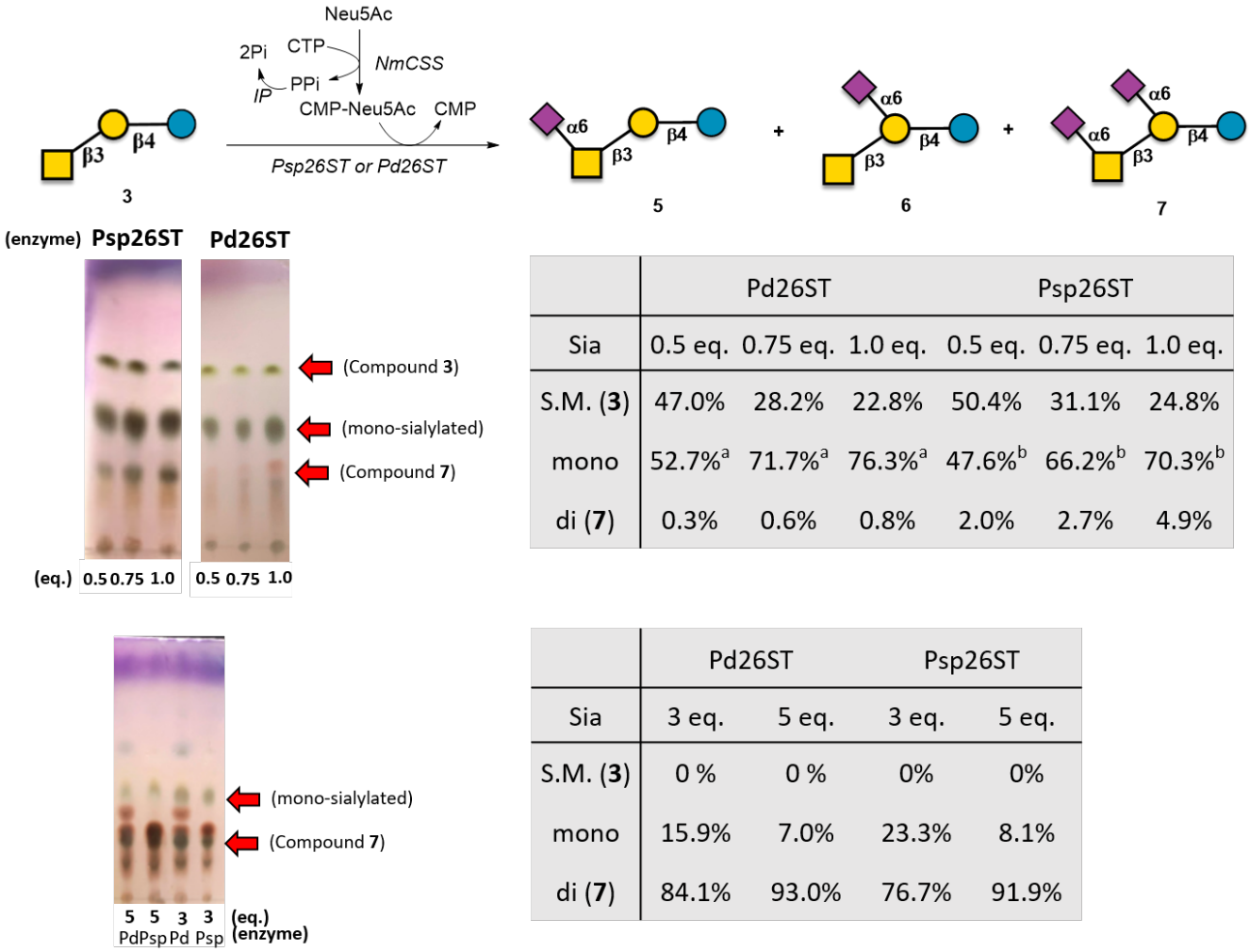
Fig. S4 ^{13}C NMR comparison of monosialylated compounds **4**, **5**, and **6**. Glycans A, B, and C represent Glc, Gal, and GalNAc, respectively. The number indicates the position of the carbon atom. Sia2 and Sia9 represent the anomeric and 9-position carbon atoms of sialic acid, respectively. The C-3 signal of GalNAc (C3) shifted downfield from 71.73 to 73.78 ppm after α(2,3) sialylation (**4**), but no shift for the α(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 α(2,6) sialylation.



Yied of sialylated product from **3**

	Psp26ST	Pd26ST
6	5.4%	7.3%
5	57%	67%
7	4.5%	1.6%

Fig. S5 Study of α(2,6) sialylation of compound **3**. HPLC-evaporative light scattering detector (ELSD) analysis of the sialylation of compound **3** by two kinds of α(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.



	Pd26ST			Psp26ST		
Sia	0.5 eq.	0.75 eq.	1.0 eq.	0.5 eq.	0.75 eq.	1.0 eq.
S.M. (**3**)	47.0%	28.2%	22.8%	50.4%	31.1%	24.8%
mono	52.7%[a]	71.7%[a]	76.3%[a]	47.6%[b]	66.2%[b]	70.3%[b]
di (**7**)	0.3%	0.6%	0.8%	2.0%	2.7%	4.9%

	Pd26ST		Psp26ST	
Sia	3 eq.	5 eq.	3 eq.	5 eq.
S.M. (**3**)	0 %	0 %	0%	0%
mono	15.9%	7.0%	23.3%	8.1%
di (**7**)	84.1%	93.0%	76.7%	91.9%

Fig. S6 Analysis of the sialylation of compound **3** by two kinds of α(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/H_2O/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-μm 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. [a]Only compound **5** was observed. [b]Compounds **5** and **6** were observed (identified by HPLC-ELSD).

When using fewer equivalents of sialic acid, the α(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.

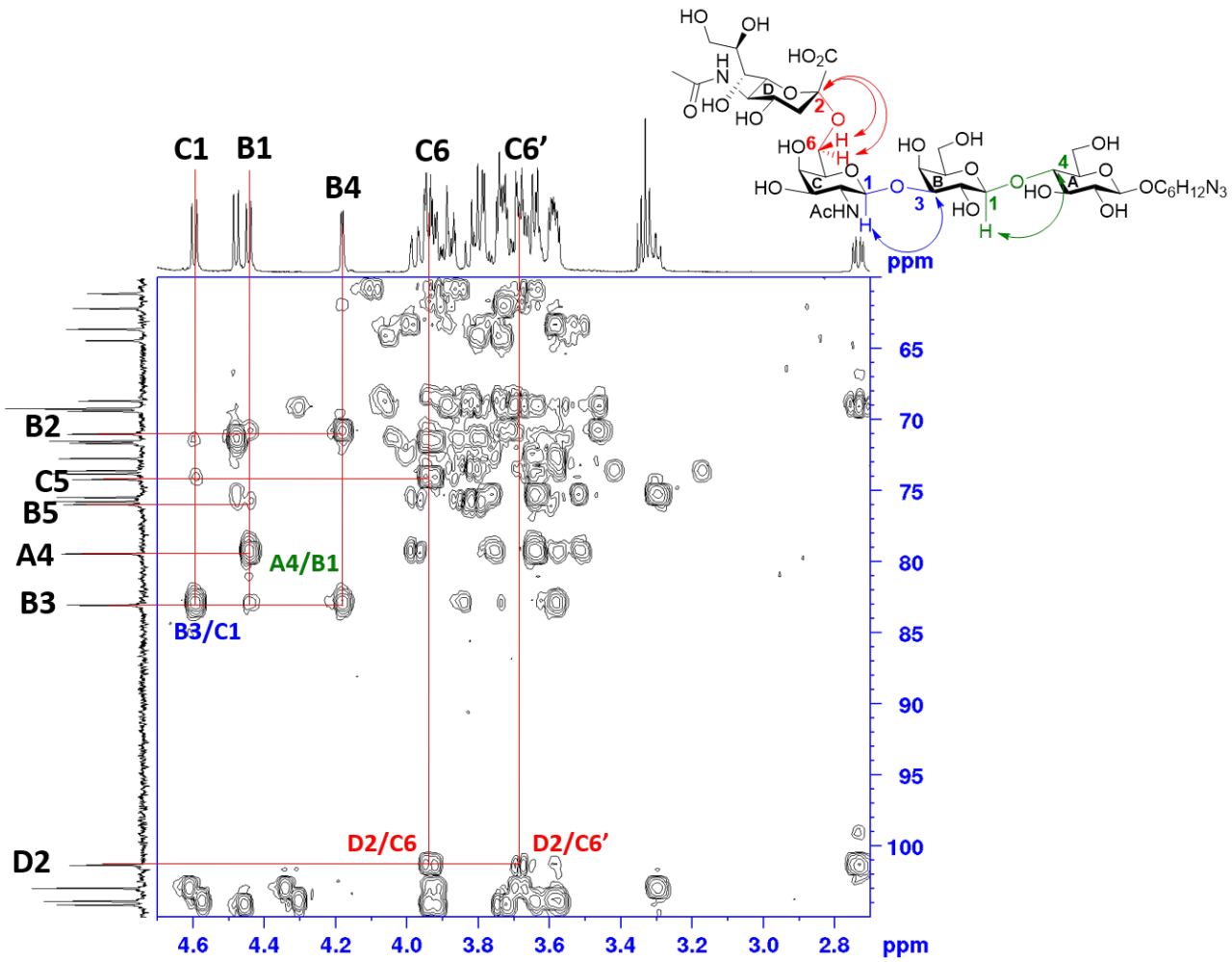


Fig. S7 ^{1}H-^{13}C HMBC spectrum of **5**. Glycans A, B, C, and D represent Glc, Gal, GalNAc, and Neu5Ac, respectively. The number indicates the position of the carbon or proton atom. **Neu5Ac-α(2,6)-GalNAc linkage.** Cross signals for the anomeric carbon of sialic acid (D2) and both 6-position proton 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→6 linkage between Neu5Ac and GalNAc.

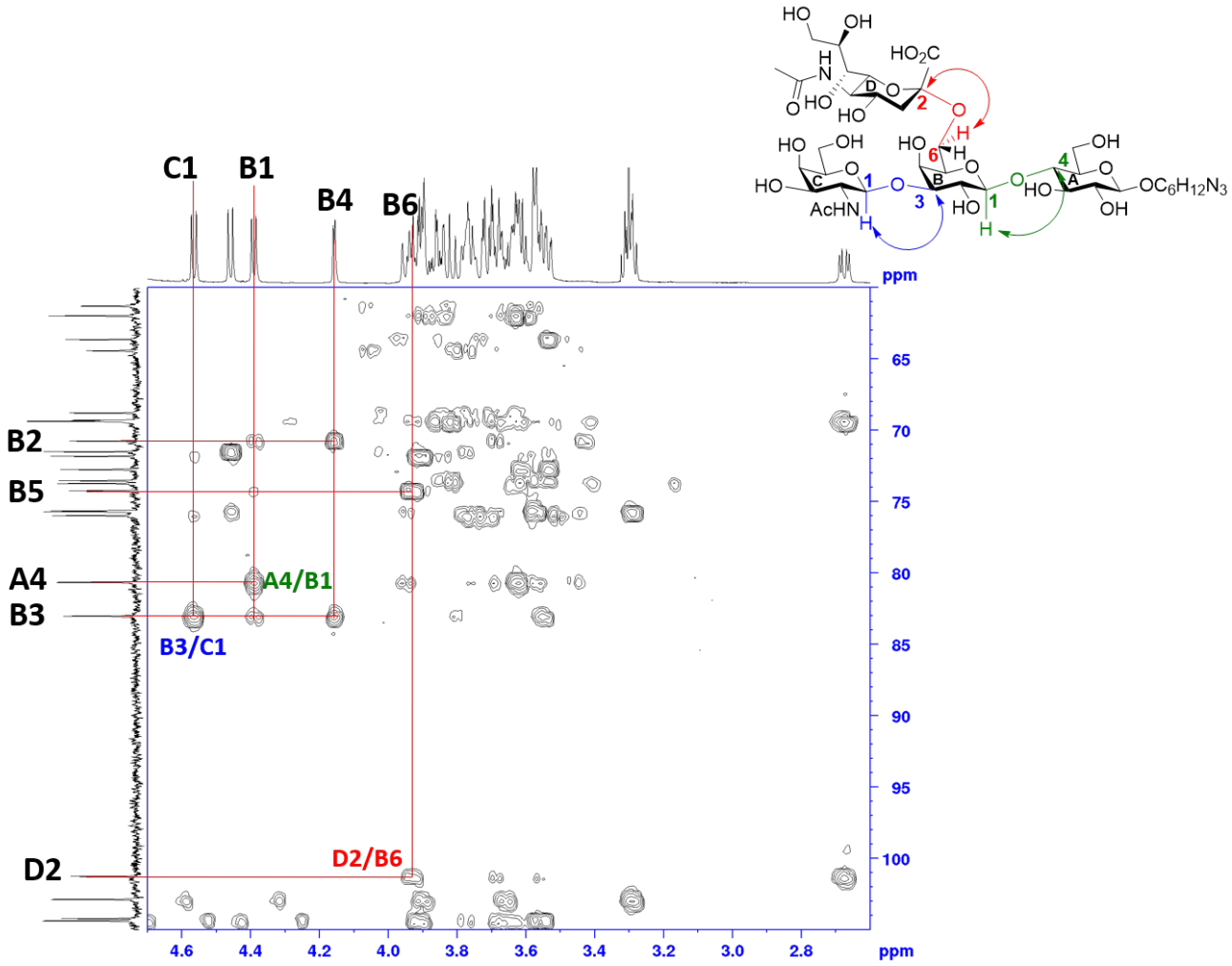


Fig. S8 ^{1}H-^{13}C HMBC and ^{1}H-^{13}C HSQC-TOCSY analysis of compound **6**. Glycans A, B, C, and D represent Glc, Gal, GalNAc, and Neu5Ac, respectively. The number indicates the position of the carbon or proton atom. **Neu5Ac-α(2,6)-Gal linkage.** A cross signal for the anomeric carbon of sialic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

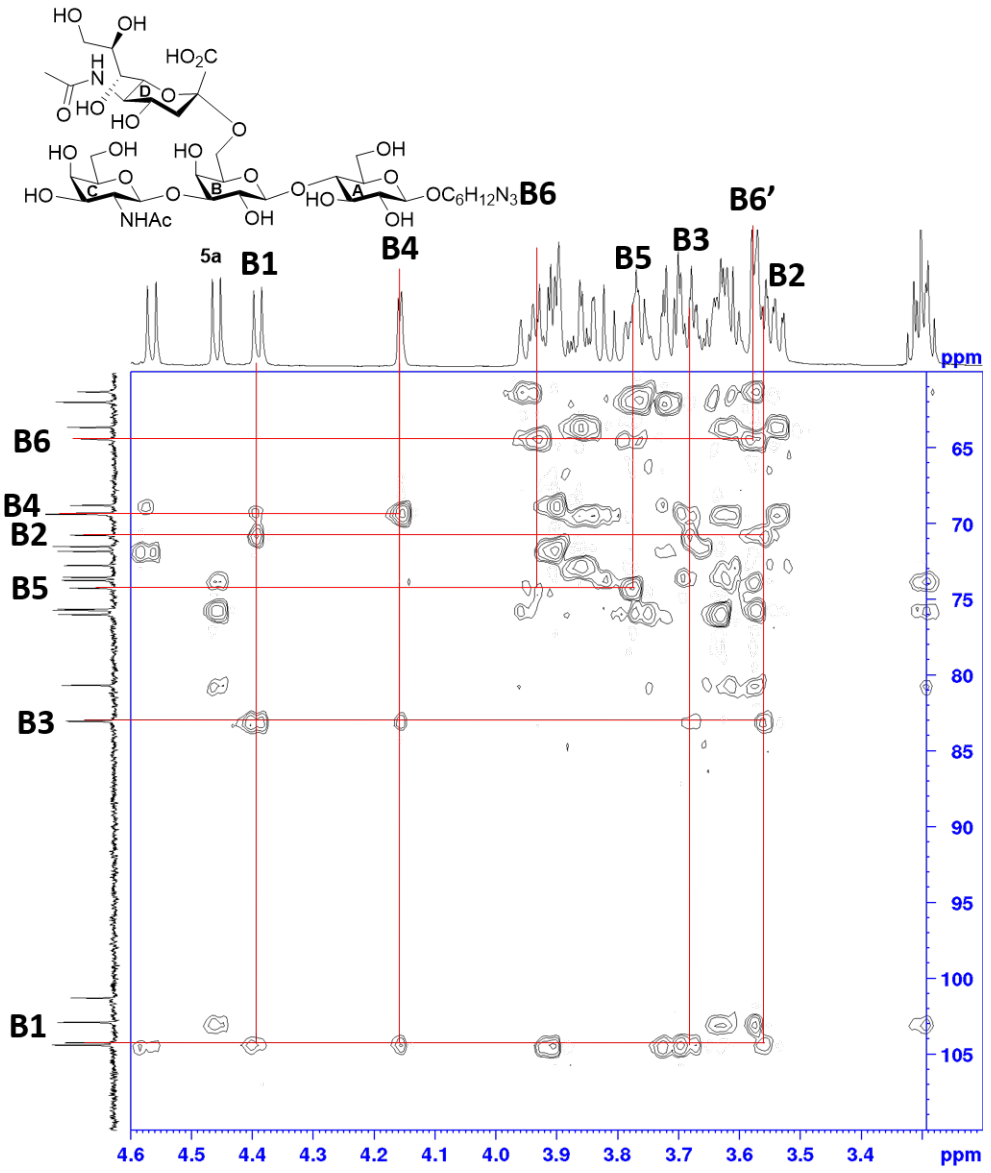
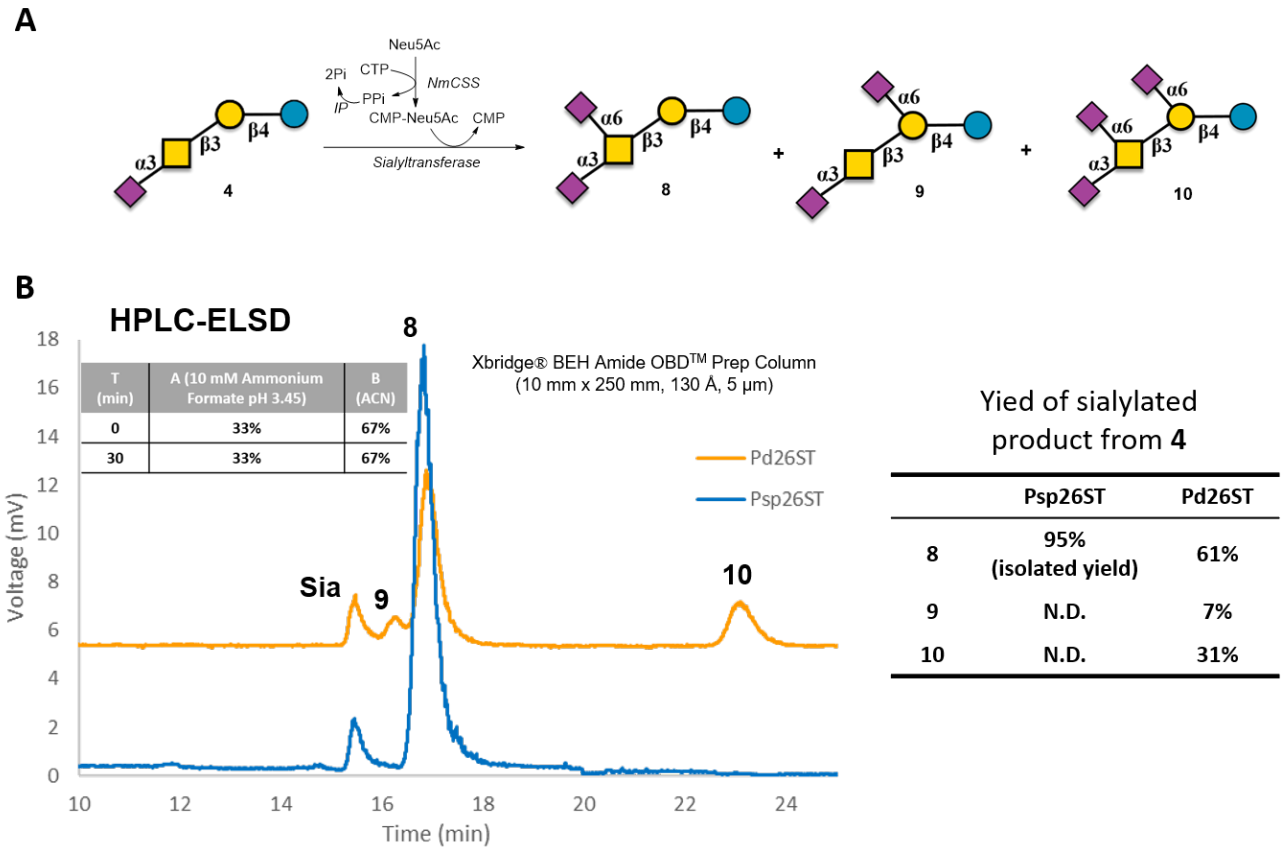


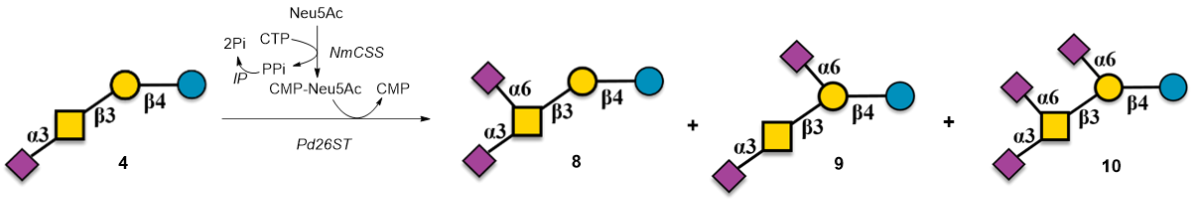
Fig. S9 Full carbon atom assignment of inner Gal from the ^{1}H-^{13}C HSQC-TOCSY spectrum of **6**. The spectrum shows that the downfield shift signal of the 6-position carbon is on the Gal carbon rather than GalNAc carbon.



Yied of sialylated product from **4**

	Psp26ST	Pd26ST
8	95% (isolated yield)	61%
9	N.D.	7%
10	N.D.	31%

Fig. S10 Study of α(2,6) sialylation of compound **4**. HPLC-ELSD analysis of the sialylation of compound **4** by two different α(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.



	Pd26ST		
Sia	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%

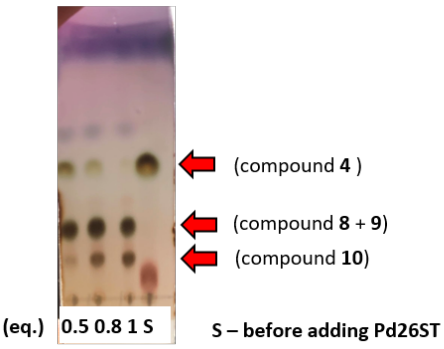


Fig. S11 Analysis of the sialylation of compound **4** by Pd2,6ST with reduced equivalents of sialic acid (0.5, 0.8, 1.0). The reaction was monitored by TLC (*n*-PrOH/H_2O/AcOH = 6/2/1 (v/v/v)). After the CMP-sialic acid was consumed, the reaction was quenched by the addition of the same reaction volume 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-μm 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 α(2,6) sialylation of compound **4** by Pd2,6ST produced regioisomers **8** and **9** (identified by HPLC-ELSD). This result suggests that Pd2,6ST does not have selectivity between GalNAc and Gal when compound **4** was used as the acceptor for sialylation.

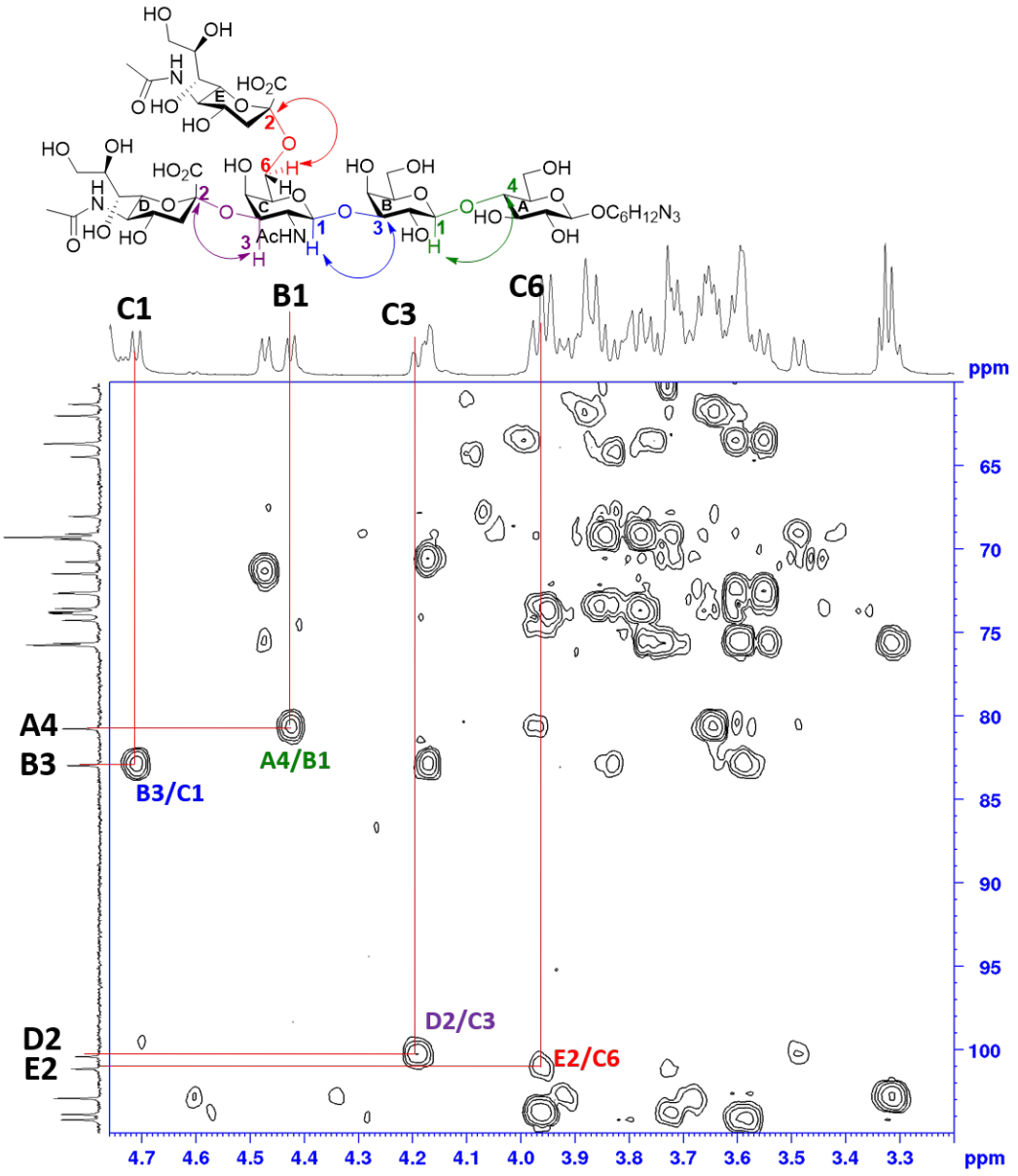


Fig. S12 ^{1}H-^{13}C HMBC analysis of compound **8**. Glycans A, B, C, D, and E represent Glc, Gal, GalNAc, α3Neu5Ac, and α6Neu5Ac, respectively. The number indicates the position of the carbon or proton atom. **Neu5Ac-α(2,6)-GalNAc linkage.** A cross signal for the anomeric carbon of α6Neu5Ac (D2) and the one of the C6 protons of GalNAc (C6) was observed (D2/C6). This suggests that the glycosidic linkage is a 2 →6 (GalNAc) linkage.

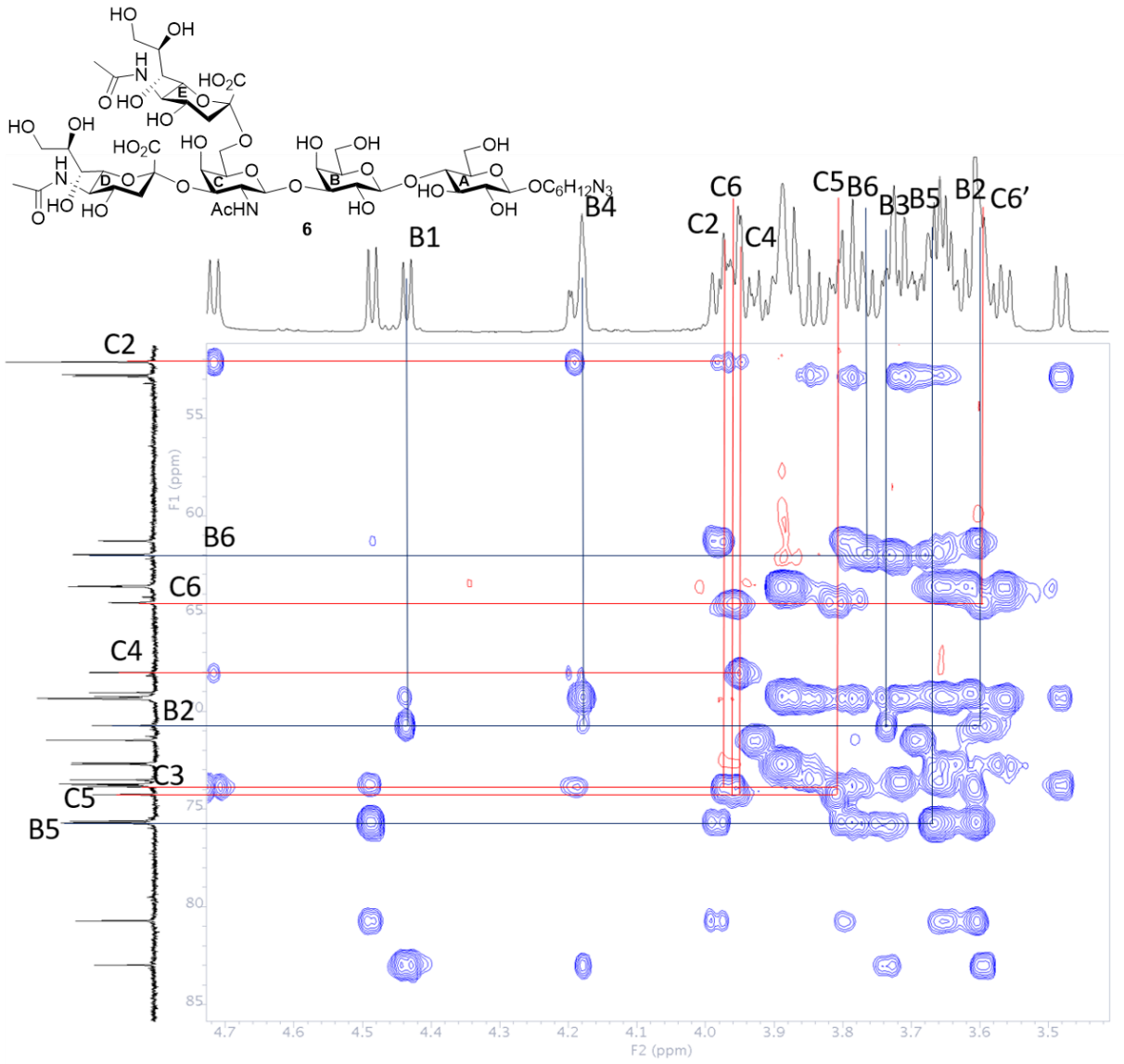


Fig. S13 Crucial carbon atom assignments of Gal and GalNAc on **8** by ^{1}H-^{13}C HSQC-TOCSY. The results reveal that the downfield shifted signal of the 6-position carbon is on GalNAc rather than on Gal.

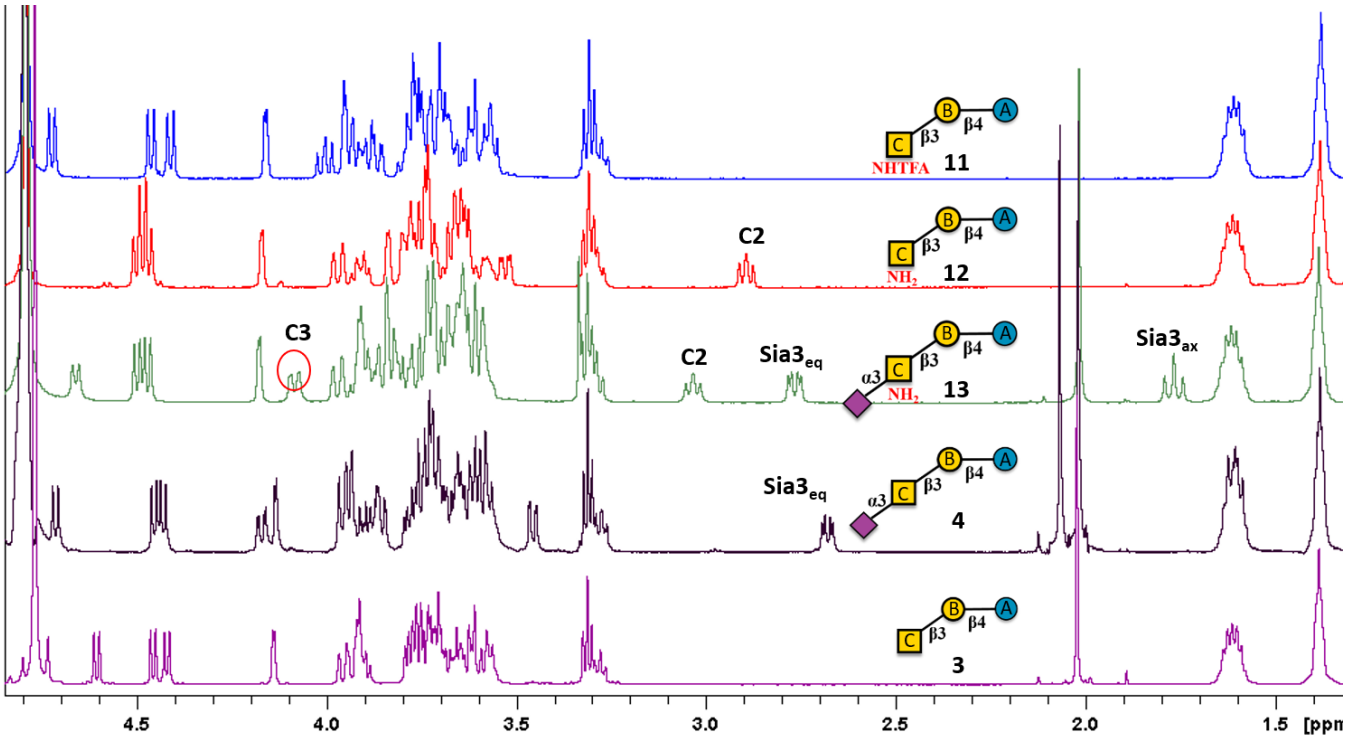


Fig. S14 α(2,3) sialylation on $GalNH_2$ as evidenced by ^1H NMR spectroscopy. Glycans A, B, and C represent Glc, Gal, and GalNAc, respectively. The number indicates the position of the carbon or proton atom. The C3-H on Neu5Ac is represented as $Sia3_{eq}$ (equatorial) and $Sia3_{ax}$ (axial). After deprotection of NHTFA, the C2-H signal of $GalNH_2$ (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 $GalNH_2$ was successful. In addition, the C3-H signal of $GalNH_2$ (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**.

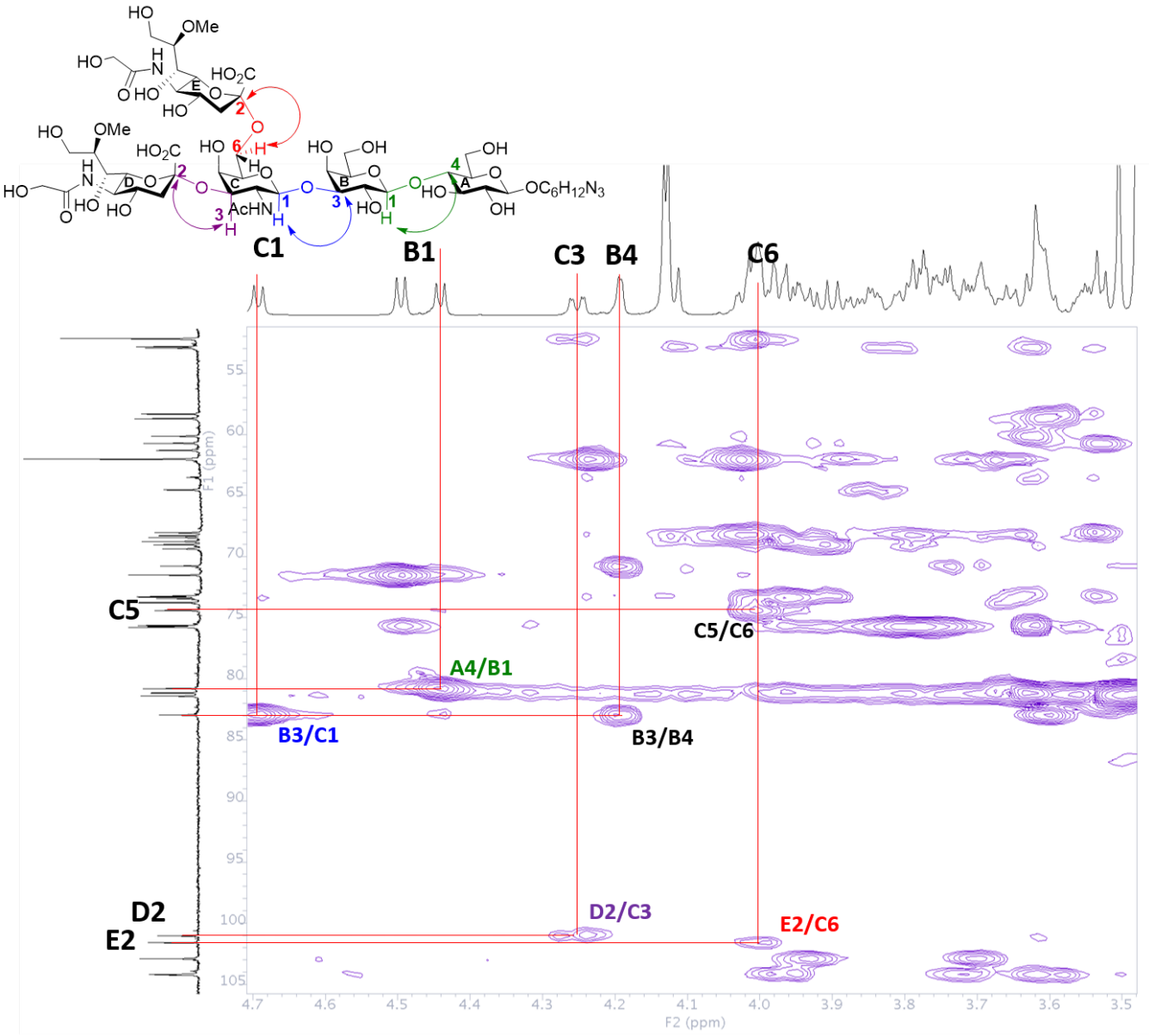


Fig. S15 ^{1}H-^{13}C HMBC analysis of compound **1**. Glycans A, B, C, D, and E represent Glc, Gal, GalNAc, α3Neu5Gc8Me, and α6Neu5Gc8Me, respectively. The number indicates the position of the carbon or proton atom. **Neu5Gc8Me-α(2,6)-GalNAc linkage**. A cross signal for the anomeric carbon of α6Neu5Gc8Me (E2) and one of the C6 protons of GalNAc (C6) was observed (E2/C6). This suggests that the glycosidic linkage is a 2→6 (GalNAc) linkage.

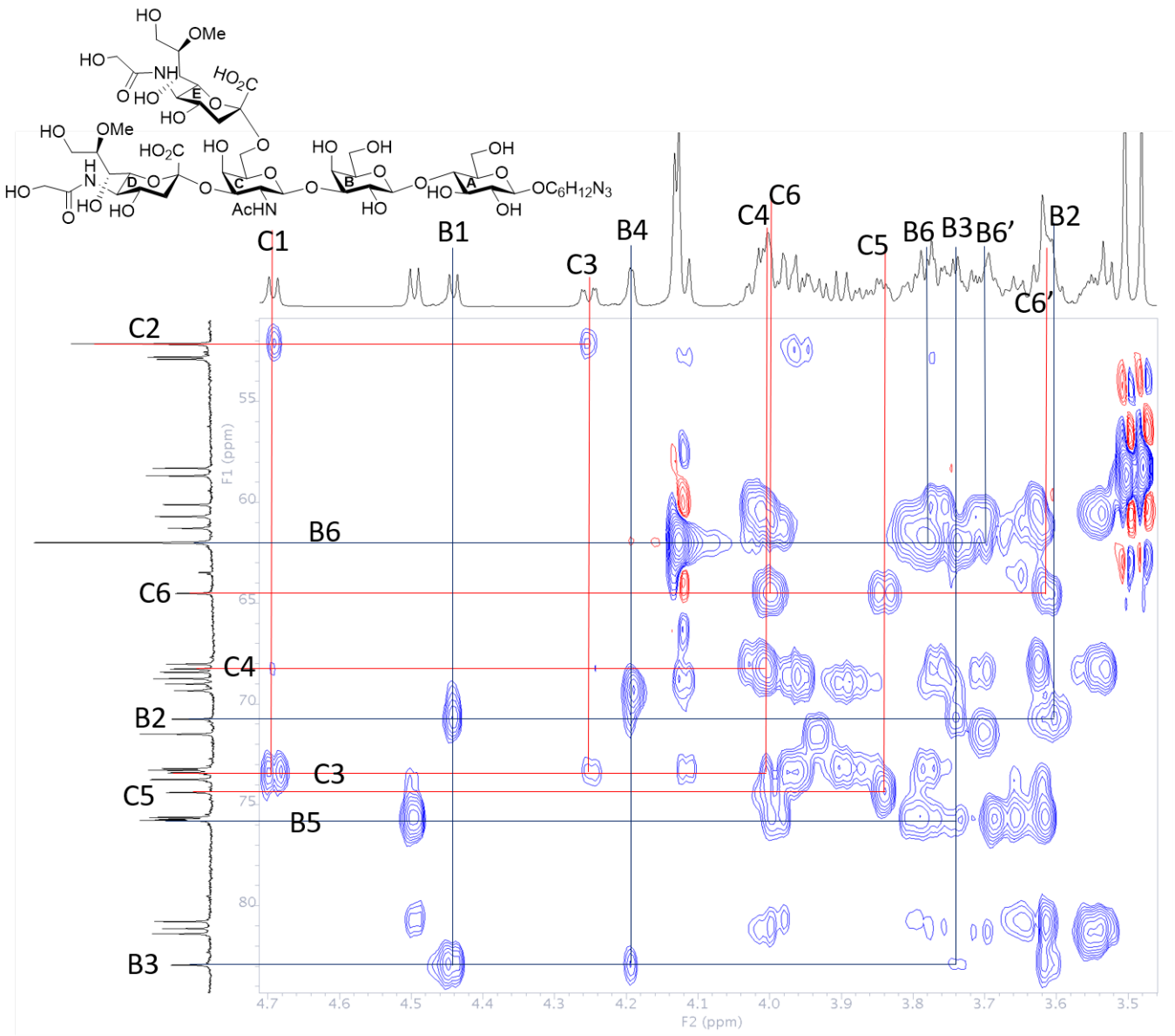


Fig. S16 Crucial carbon atom assignments of Gal and GalNAc in **1** by ^{1}H-^{13}C HSQC-TOCSY. The results reveal that the downfield shifted signal of the 6-position carbon is on GalNAc rather than on Gal.

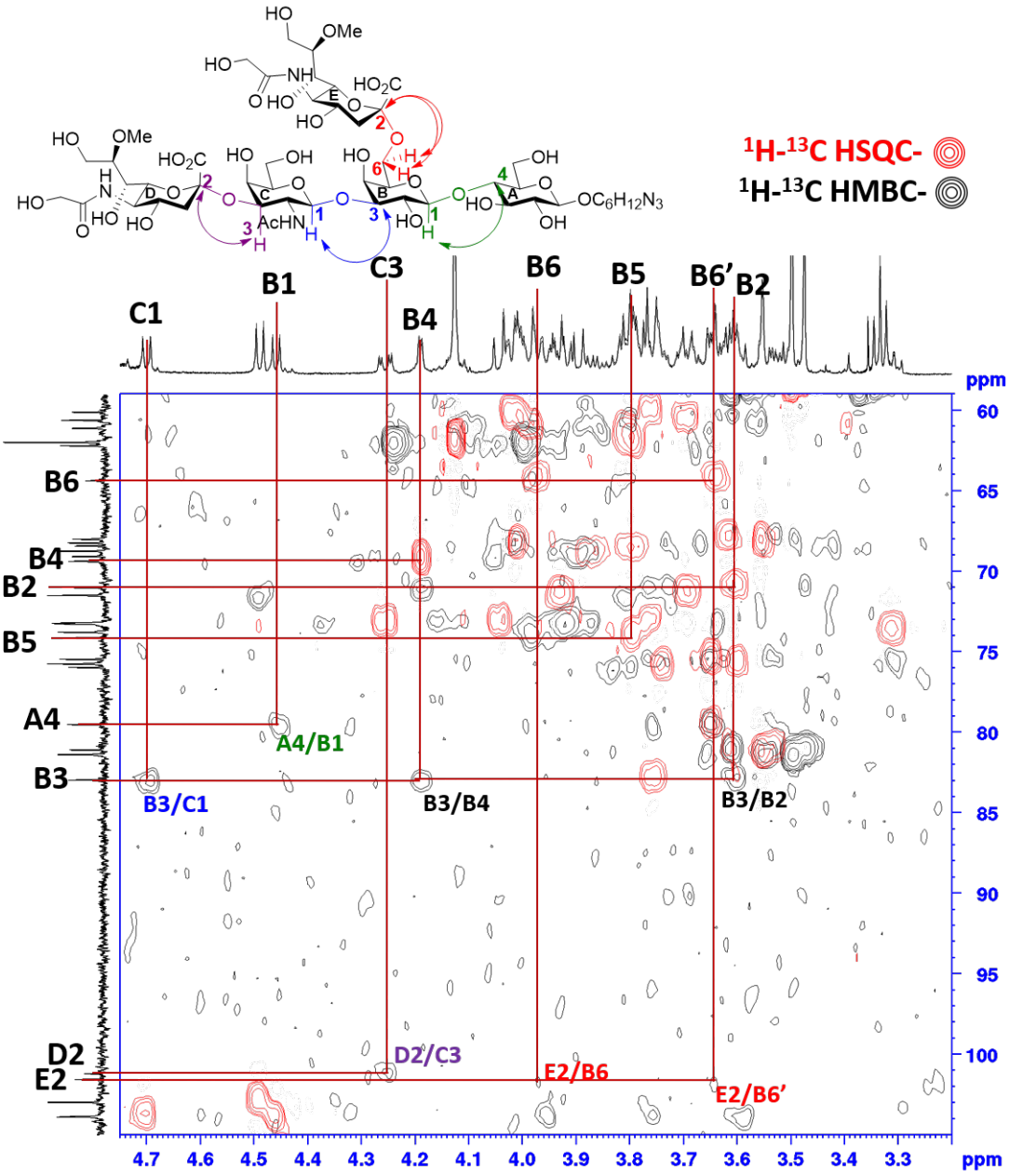


Fig. S17 ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC analysis of compound **1a**. The red and black contours represent the cross signals on the HSQC and HMBC spectra, respectively. Glycans A, B, C, D, and E represent Glc, Gal, GalNAc, α3Neu5Gc8Me, and α6'Neu5Gc8Me, respectively. The number indicates the position of the carbon or proton atom. **Neu5Gc8Me-α(2,6)-Gal linkage.** Cross signals for the anomeric carbon of α6'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→6 linkage between Neu5Gc8Me and Gal.

General Procedure

General Enzymatic Synthetic Procedure 1: Sequential One Pot Enzymatic α(2,3)- or α(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 $MgCl_2$ 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/ H_2O/ 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/ H_2O/ 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 β(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 $MgCl_2$ 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/ H_2O/ 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 $MnCl_2$ 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/ H_2O/ 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 H_2O 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/ H_2O/ 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 μm, Waters) using a solution followed by a gradient of 0-80% MeOH in H_2O as eluent.

General Procedure for *N*-Acetylation

Thirty mM of amine (1.0 equiv.) containing compound was dissolved in H_2O followed by adding solid $NaHCO_3$ (10.0 equiv.) and Ac_2O (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 H_2O 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-μm 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 μm, Waters) using a solution followed by a gradient of 0-100% MeOH in H_2O 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 H_2O 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, 5μm) 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 H_2O 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.

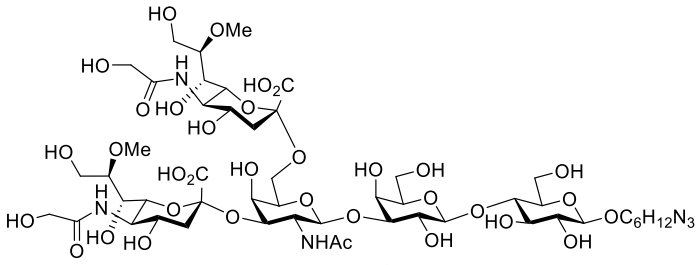
HPLC mobile phase condition for purification of compound **13a** and its α(2,6)-sialylated regioisomer:
Using an elution of 74% acetonitrile in water containing 10 mM ammonium formate (pH = 3.45) for 30 mins.

HPLC mobile phase condition for purification of compounds **15a** and its α(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 H_2O as eluent (BioGel P2 gel packed in a column 1.6 cm x 50 cm, Biorad).

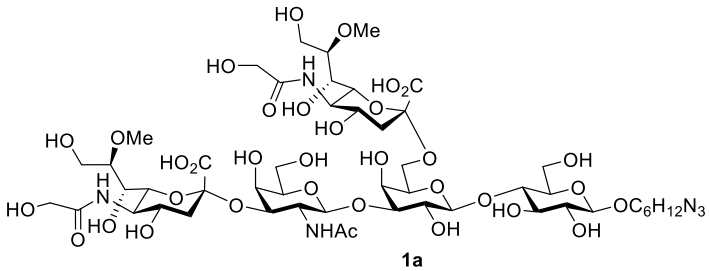
Synthetic procedures and characterization of new compounds

Compound **2**,[10] and **2a**,[11] and Neu5Gc8Me[12] were reported previously.



1

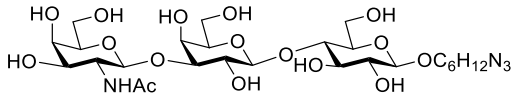
GAA7-$C_6H_{12}N_3$ (Neu5Gc8Meα2,3(Neu5Gc8Meα2,6-)-GalNAcβ1,3-Galβ1,4-GlcβC$_6$H$_{12}$N$_3$ (1).
Sialylation catalyzed by Psp2,6ST. Compound **4a** (8.4 mg, 8.5 μmol) and Neu5Gc8Me (8.6 mg, 25.3 μmol) were used as acceptor and donor, respectively, by following the general enzymatic synthetic procedure 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 μmol) and Neu5Gc8Me (8.8 mg, 25.9 μmol) 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/ H_2O/ AcOH = 6/2/1 (v/v/v)); ^{1}H NMR (600 MHz, D_2O) 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); ^{13}C NMR (150 MHz, D_2O) 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 $C_{50}H_{83}N_6O_{34}$ [M-H]$^-$ 1311.4956, found 1311.4930.



1a

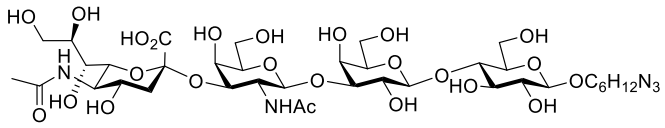
Neu5Gc8Meα2,3-GalNAcβ1,3(Neu5Gc8Meα2,6-)-Galβ1,4-GlcβC$_6$H$_{12}$N$_3$ (1a). The compound **1a** was synthesized from **4a** by following the same procedure as mentioned in the synthesis of compound **1**. After purification, the product was lyophilized to give compound **1a** in 20.2% yield (2.3 mg). R_f = 0.21 (*n*-

PrOH/ H_2O/ AcOH = 6/2/1 (v/v/v)); ^{1}H NMR (600 MHz, D_2O) 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); ^{13}C NMR (150 MHz, D_2O) 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 $C_{50}H_{83}N_6O_{34}$ $[M-H]^-$ 1311.4956, found 1311.4912.



3

GalNAcβ1,3-Galβ1,4-GlcβC$_6$H$_{12}$N$_3$ (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/ H_2O/ AcOH = 6/2/1 (v/v/v)); ^{1}H NMR (600 MHz, D_2O) 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); ^{13}C NMR (150 MHz, D_2O) 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 $C_{26}H_{46}N_4O_{16}Na$ $[M+Na]^+$ 693.2801, found 693.2818.



4

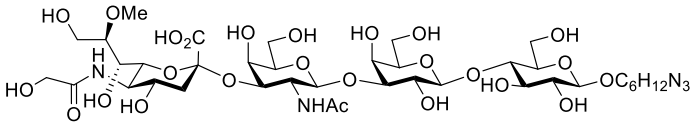
Neu5Acα2,3-GalNAcβ1,3-Galβ1,4-GlcβC$_6$H$_{12}$N$_3$ (4).

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).

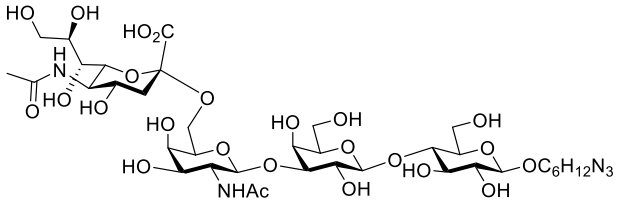
The chemical *N*-acetylation of compound 13. Compound **13** (11.8 mg, 12.8 μmol), $NaHCO_3$ (6.7 mg,

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)); ^{1}H 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 $C_{37}H_{62}N_5O_{24}$ $[M-H]^-$ 960.3790, found 960.3767.



4a

Neu5Gc8Meα2,3-GalNAcβ1,3-Galβ1,4-GlcβC$_6$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)); ^{1}H 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 $C_{38}H_{65}N_5O_{25}Na$ $[M+Na]^+$ 1014.3866, found 1014.3860.

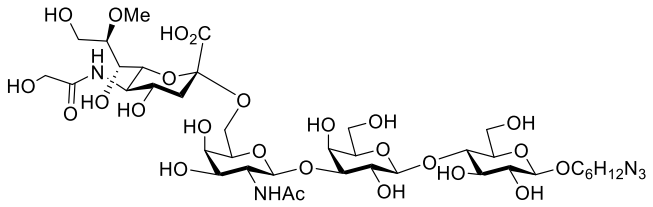


5

Neu5Acα2,6-GalNAcβ1,3-Galβ1,4-GlcβC$_6$H$_{12}$N$_3$ (5).

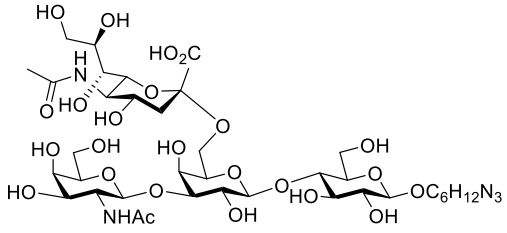
The sialylation catalyzed by PmST1. Compound **3** (11.0 mg, 16.4 μmol) and Neu5Ac (25.4 mg, 82.0 μmol) were used as acceptor and donor, respectively, by following the general enzymatic synthetic procedure 1. After being shaken for 20 h, the reaction was quenched and purified by following general purification 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 μmol) and Neu5Ac (2.8 mg, 8.9 μmol) 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/ H_2O/ AcOH = 6/2/1 (v/v/v)); ^{1}H NMR (600 MHz, D_2O) 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); ^{13}C NMR (150 MHz, D_2O) 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 $C_{37}H_{62}N_5O_{24}$ $[M-H]^-$ 960.3790, found 960.3756.



5a

Neu5Gc8Meα2,6-GalNAcβ1,3-Galβ1,4-GlcβC$_6$H$_{12}$N$_3$ (5a). Compound **3** (3.0 mg, 4.5 μmol) and Neu5Gc8Me (4.6 mg, 13.4 μmol) 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/ H_2O/ AcOH = 6/2/1 (v/v/v)); ^{1}H NMR (500 MHz, D_2O) 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); ^{13}C NMR (125 MHz, D_2O) 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 $C_{38}H_{64}N_5O_{25}$ $[M-H]^-$ 990.3896, found 990.3884.

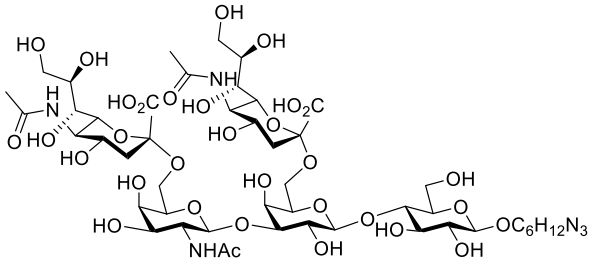


6

GalNAcβ1,3(Neu5Acα2,6-)-Galβ1,4-GlcβC$_6$H$_{12}$N$_3$ (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/ H_2O/ AcOH) = 6/2/1 (v/v/v)); ^{1}H NMR (600 MHz, D_2O) 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); ^{13}C NMR (150 MHz, D_2O) 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 $C_{37}H_{62}N_5O_{24}$ $[M-H]^-$ 960.3790, found 960.3782.



7

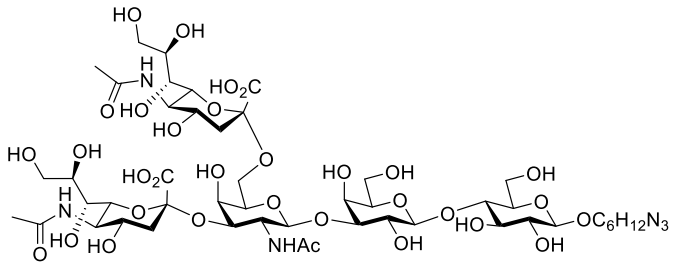
Neu5Acα2,6-GalNAcβ1,3(Neu5Acα2,6-)-Galβ1,4-GlcβC$_6$H$_{12}$N$_3$ (7).

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 μmol) and Neu5Ac (3.7 mg, 12.0 μmol) 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 μmol) and Neu5Ac (29.9 mg, 96.9 μmol) 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/ H_2O/ AcOH = 6/2/1 (v/v/v)); ^{1}H NMR (500 MHz, D_2O) 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); ^{13}C NMR (125 MHz, D_2O) 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,

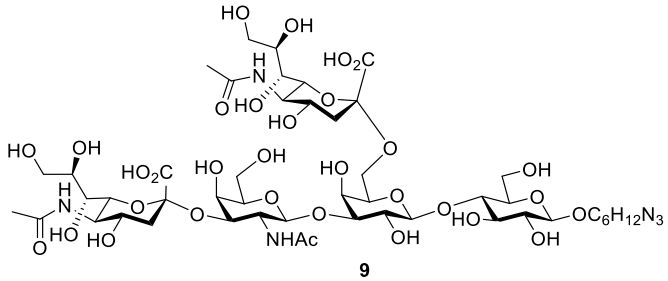
63.59, 61.29, 53.44, 52.95, 52.73, 52.09, 49.51, 49.34, 49.17, 49.00, 48.82, 48.65, 48.48, 41.23, 41.03, 29.54, 28.84, 26.62, 25.57, 23.19, 23.02, 22.99; HRMS (ESI) m/z calcd for $C_{48}H_{79}N_6O_{32}$ $[M-H]^-$ 1251.4744, found 1251.4728.



8

Neu5Acα2,3(Neu5Acα2,6-)-GalNAcβ1,3-Galβ1,4-GlcβC$_6$H$_{12}$N$_3$ (8).

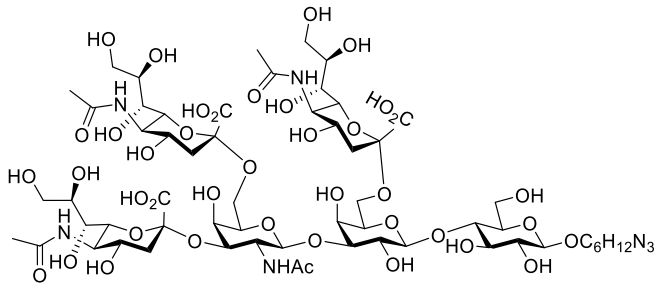
The sialylation catalyzed by Pd2,6ST or Psp2,6ST using 1.5 equiv. sialic acid. Compound **4** (4.2 mg, 4.4 μmol) and Neu5Ac (2.0 mg, 6.5 μmol) were used as acceptor and donor, respectively, by following the general enzymatic synthetic procedure 1. After being shaken for 10 h, the reaction was quenched 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/ H_2O/ AcOH = 6/2/1 (v/v/v)); ^{1}H NMR (700 MHz, D_2O) 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); ^{13}C NMR (175 MHz, D_2O) 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

Neu5Acα2,3-GalNAcβ1,3(Neu5Acα2,6-)-Galβ1,4-GlcβC$_6$H$_{12}$N$_3$ (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/ H_2O/ AcOH = 6/2/1 (v/v/v)); ^{1}H NMR (600 MHz, D_2O) 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 $C_{48}H_{78}N_6O_{32}$ $[M-2H]^{2-}$ 625.2330, found 625.2277.

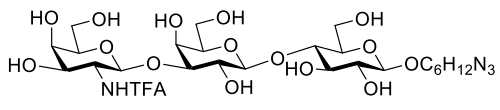


10

Neu5Acα2,3(Neu5Acα2,6-)-GalNAcβ1,3(Neu5Acα2,6-)-Galβ1,4-GlcβC$_6$H$_{12}$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).

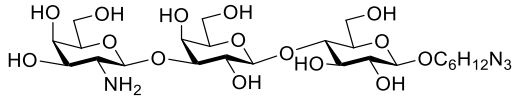
The sialylation of compound 4 by Pd2,6ST with 1.5 equiv. sialic acid. The compound **10** was synthesized from **4** by following the procedure as described in the synthesis of compound **8**. After purification, the product was lyophilized to give compound **10** in 5.9% yield (0.4 mg). R_f = 0.12 (*n*-PrOH/ H_2O/ AcOH = 6/2/1 (v/v/v)); ^{1}H 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 $C_{59}H_{94}N_7Na_2O_{40}$ $[M-3H+2Na]^-$ 1586.5337, found 1586.5340.



11

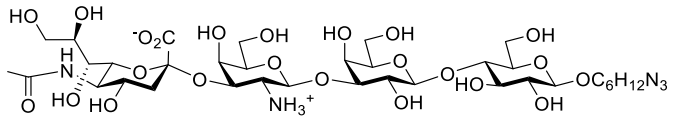
GalNTFAβ1,3-Galβ1,4-GlcβC$_6$H$_{12}$N$_3$ (11). Compound **2** (10.0 mg, 21.4 μmol) and *N*-trifluoroacetylgalactosamine (GalNTFA)[13] (11.8 mg, 42.8 μmol) were used as acceptor and donor, respectively, by following the general enzymatic synthetic procedure 2. After being shaken for 30 h, the reaction 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/ H_2O/ AcOH = 6/2/1 (v/v/v)); ^{1}H NMR (500 MHz, D_2O) 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); ^{13}C NMR (125 MHz, D_2O) δ 160.67 (q, $^2J_{CF}$ = 37 Hz, NH<u>C</u>OCF$_3$), 116.88 (q, $^1J_{CF}$ = 286 Hz, NHCO<u>C</u>F$_3$), 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 $C_{26}H_{43}F_3N_4O_{16}Na$ $[M+Na]^+$ 747.2524, found 747.2500.



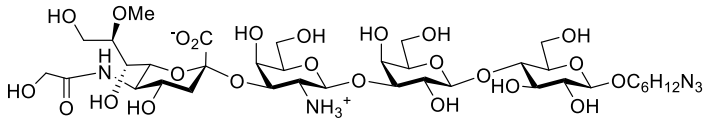
12

GalNH$_2$β1,3-Galβ1,4-GlcβC$_6$H$_{12}$N$_3$ (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/ H_2O/ AcOH = 6/2/1 (v/v/v)); ^{1}H NMR (500 MHz, D_2O) 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); ^{13}C NMR (125 MHz, D_2O) 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 $C_{24}H_{45}N_4O_{15}$ $[M+H]^+$ 629.2876, found 629.2875.



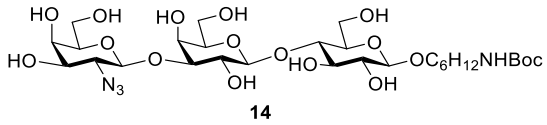
13

Neu5Acα2,3-GalNH$_2$β1,3-Galβ1,4-GlcβC$_6$H$_{12}$N$_3$ (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/ H_2O/ AcOH = 6/2/1 (v/v/v)); ^{1}H NMR (500 MHz, D_2O) 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); ^{13}C NMR (125 MHz, D_2O) 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 $C_{35}H_{61}N_5O_{23}Na$ $[M+Na]^+$ 942.3650, found 942.3677.



13a

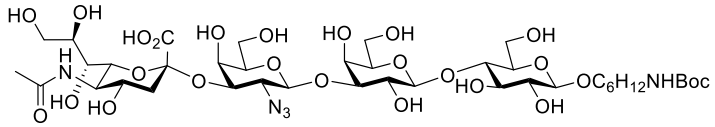
Neu5Gc8Meα2,3-GalNH$_2$β1,3-Galβ1,4-GlcβC$_6$H$_{12}$N$_3$ (13a). Compound **12** (60.0 mg, 95.4 μmol) and Neu5Gc8Me (81.0 mg, 238.6 μmol) were used as acceptor and donor, respectively. The experimental procedure is similar as described in the synthesis of compound **5** by PmST1, and the product was 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/ H_2O/ AcOH = 6/2/1 (v/v/v)); ^{1}H NMR (500 MHz, D_2O) 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); ^{13}C NMR (125 MHz, D_2O) 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 $C_{36}H_{63}N_5O_{24}Na$ $[M+Na]^+$ 972.3755, found 972.3723.



14

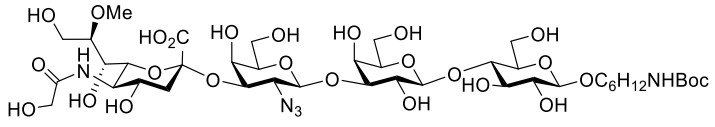
GalAzβ1,3-Galβ1,4-GlcβC$_6$H$_{12}$NHBoc (14). Compound **2a** (121.0 mg, 223.4 μmol) and GalNTFA (123.0 mg, 446.8 μmol) were used as acceptor and donor, respectively, by following the general enzymatic synthetic procedure 2. After being shaken for 36 h, the reaction was quenched and purified by following 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 hydrochloride[14] (274.9 mg, 1.1 mmol) was added to a solution containing the crude product (223.4 μmol, 10 mM), $NaHCO_3$ (112.6 mg, 1.3 mmol), and $CuSO_4 \cdot 5H_2O$ (5.6 mg, 22.3 μmol) in H_2O 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/ H_2O/ 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 H_2O as eluent. After concentration of the fractions containing product, the resulting residue was further purified by size-exclusion column using H_2O 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/ H_2O/ AcOH = 6/2/1 (v/v/v)); ^{1}H NMR (500 MHz, D_2O) 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); ^{13}C NMR (125 MHz, D_2O) 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.



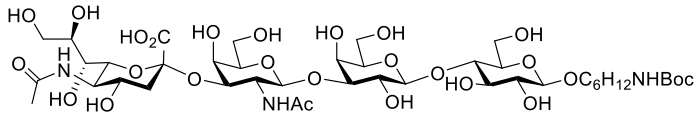
15

Neu5Acα2,3-GalAzβ1,3-Galβ1,4-GlcβC$_6$H$_{12}$NHBoc (15). Compound **14** (7.4 mg, 10.2 μmol) and Neu5Ac (9.4 mg, 30.5 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 **15** in 98.5% yield (10.2 mg). R_f = 0.54 (*n*-PrOH/ H_2O/ AcOH = 6/2/1 (v/v/v)); ^{1}H NMR (500 MHz, D_2O) 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); ^{13}C NMR (125 MHz, D_2O) 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.



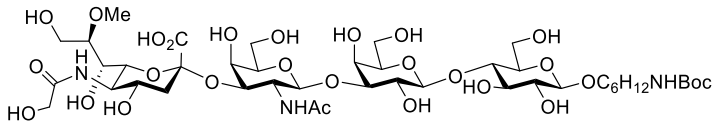
15a

Neu5Gc8Meα2,3-GalAzβ1,3-Galβ1,4-GlcβC$_6$H$_{12}$NHBoc (15a). Compound **14** (47.5 mg, 65.2 μmol) and Neu5Gc8Me (66.37 mg, 196 μmol) 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/ H_2O/ AcOH = 6/2/1 (v/v/v)); ^{1}H NMR (500 MHz, D_2O) 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); ^{13}C NMR (125 MHz, D_2O) 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.



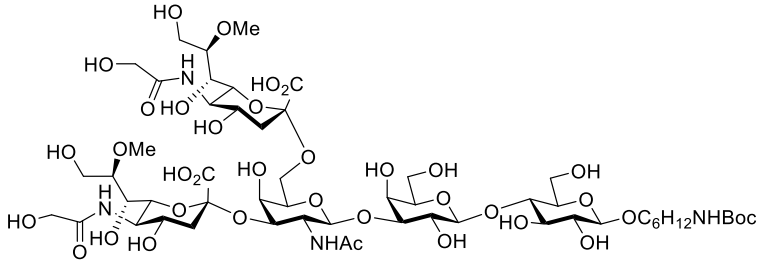
16

Neu5Acα2,3-GalNAcβ1,3-Galβ1,4-GlcβC$_6$H$_{12}$NHBoc (16). To a solution of compound **15** (10.2 mg, 10.0 μmol) in THF (0.8 mL) was added 2 N NaOH$_{(aq)}$ (50.0 μl, 100.0 μmol) and 1.0 M PMe$_3$ in THF (80.0 μl, 80.0 μmol). The reaction mixture was stirred at rt until TLC analysis (*n*-PrOH/ H$_2$O/ 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 H$_2$O 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/ H$_2$O/ AcOH = 6/2/1 (v/v/v)); ^{1}H NMR (500 MHz, D$_2$O) 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); ^{13}C NMR (125 MHz, D$_2$O) 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 C$_{42}$H$_{72}$N$_3$O$_{26}$ [M-H]$^-$ 1034.4410, found 1034.4382.



16a

Neu5Gc8Meα2,3-GalNAcβ1,3-Galβ1,4-GlcβC$_6$H$_{12}$NHBoc (16a). Compound **15a** (13.5 mg, 12.9 μmol) 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/ H$_2$O/ AcOH = 6/2/1 (v/v/v)); ^{1}H NMR (500 MHz, D$_2$O) 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); ^{13}C NMR (125 MHz, D$_2$O) 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 C$_{43}$H$_{74}$N$_3$O$_{27}$ [M-H]$^-$ 1064.4515, found 1064.4482.



17

Neu5Gc8Meα2,3(Neu5Gc8Meα2,6-)-GalNAcβ1,3-Galβ1,4-GlcβC$_6$H$_{12}$NHBoc (17, GAA7-C$_6$H$_{12}$NHBoc). Compound **16a** (3.6 mg, 3.4 μmol) and Neu5Gc8Me (3.4 mg, 10.1 μmol) 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/ H_2O/ AcOH = 6/2/1 (v/v/v)); ^{1}H NMR (500 MHz, D_2O) 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); ^{13}C NMR (125 MHz, D_2O) 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 $C_{55}H_{93}N_4O_{36}$ $[M-H]^-$ 1385.5575, found 1385.5581.

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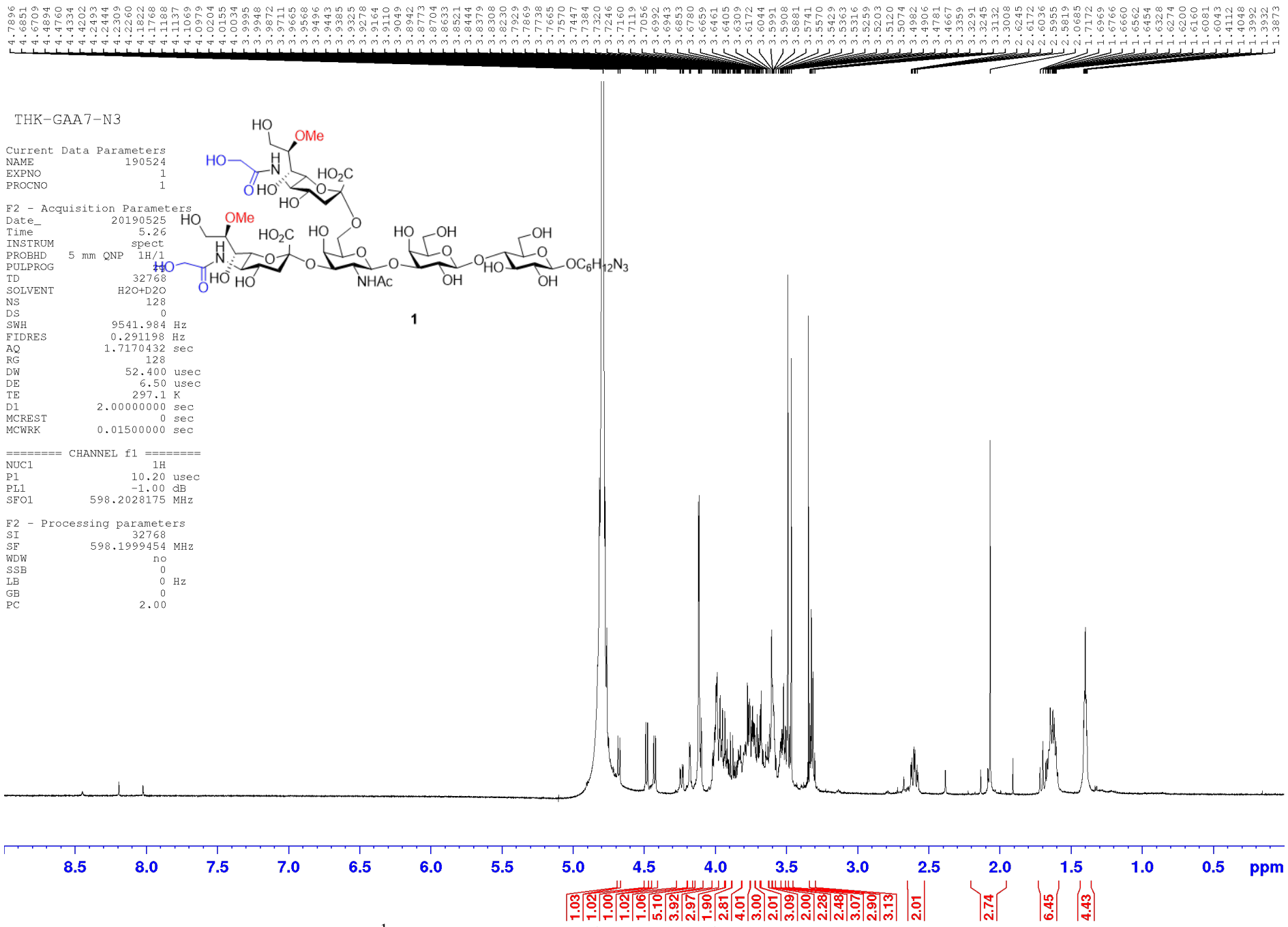
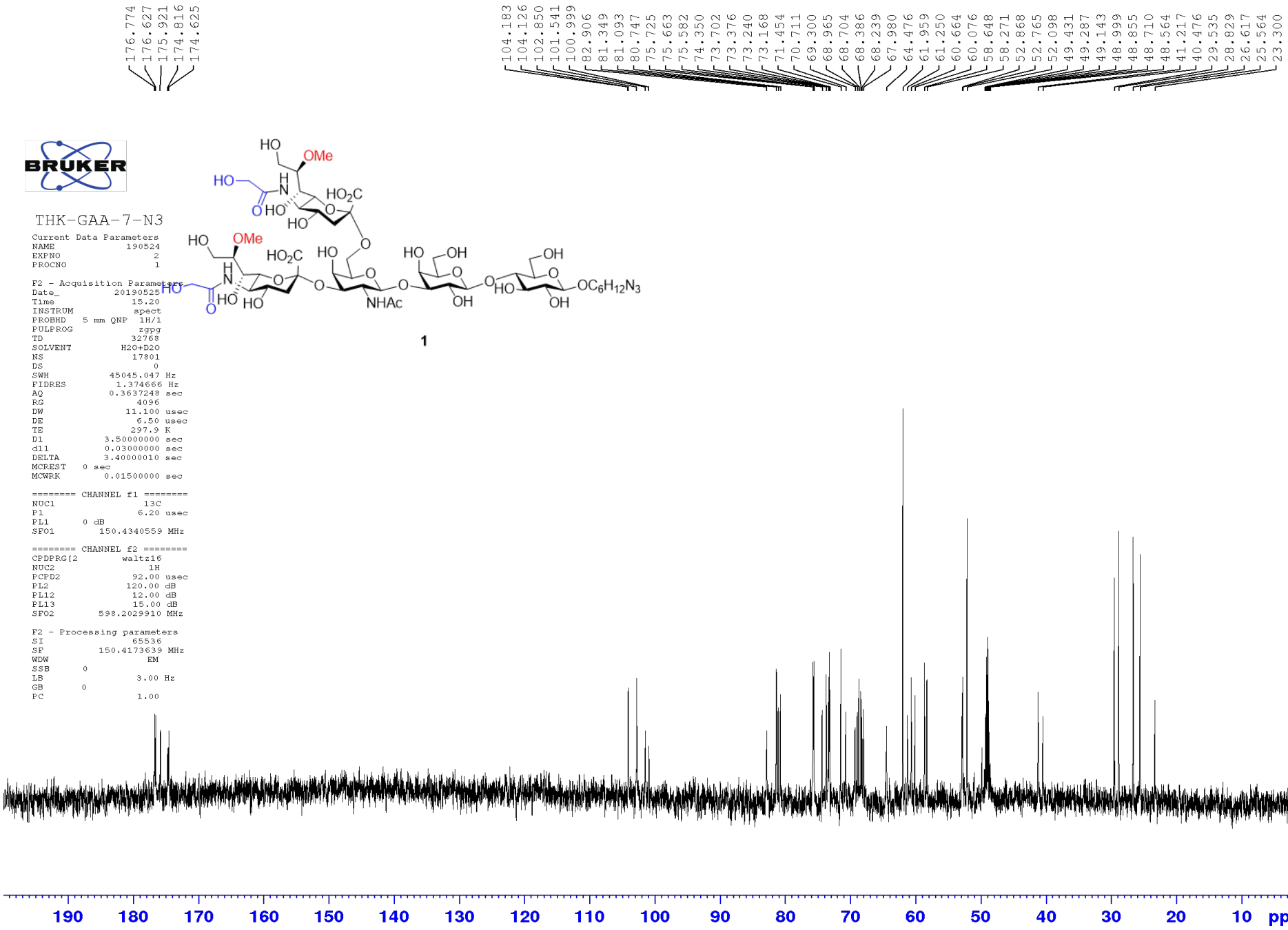
NMR Spectra

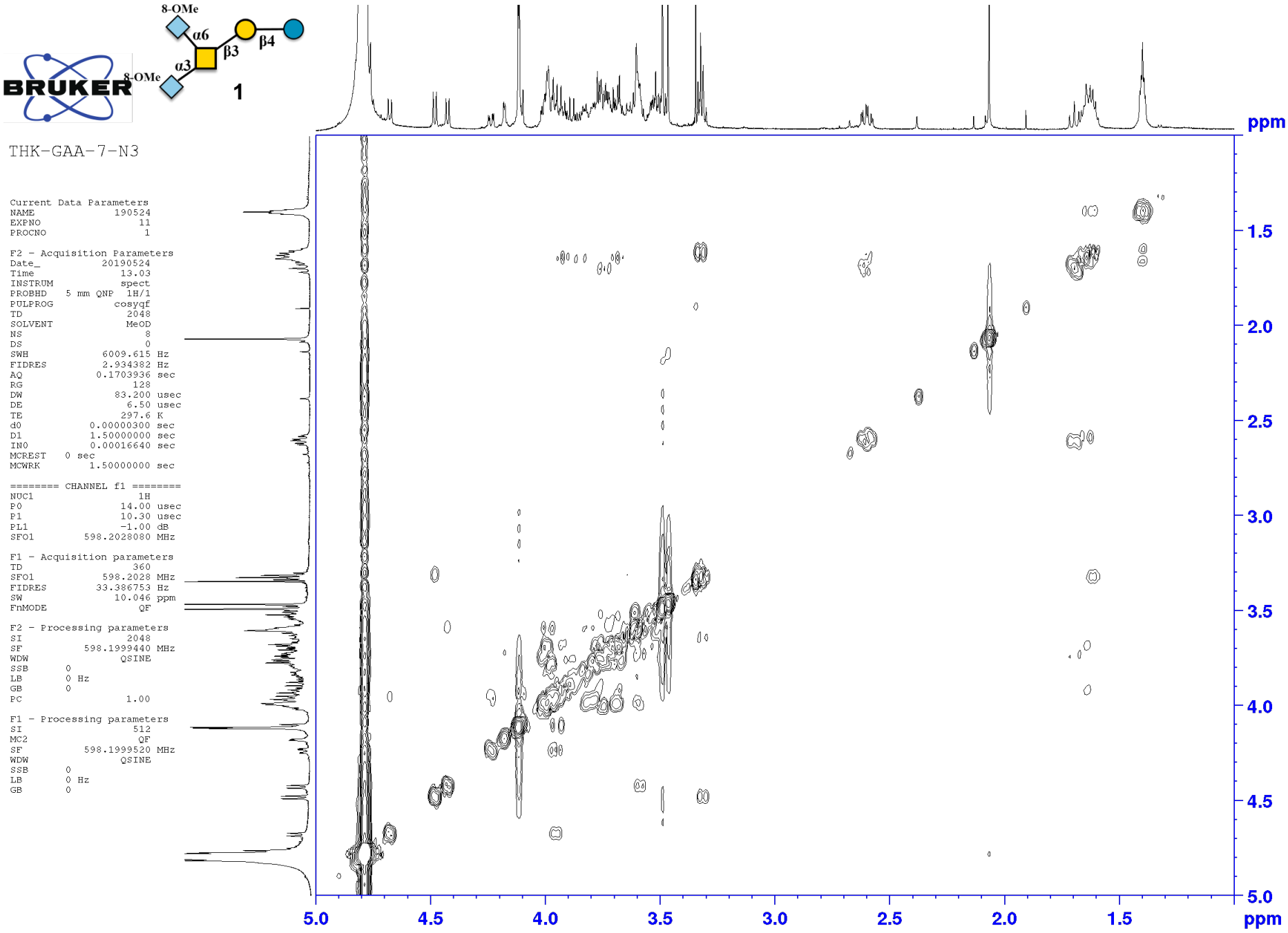


^{1}H NMR spectrum of Compound **1** (600 MHz D_2O)

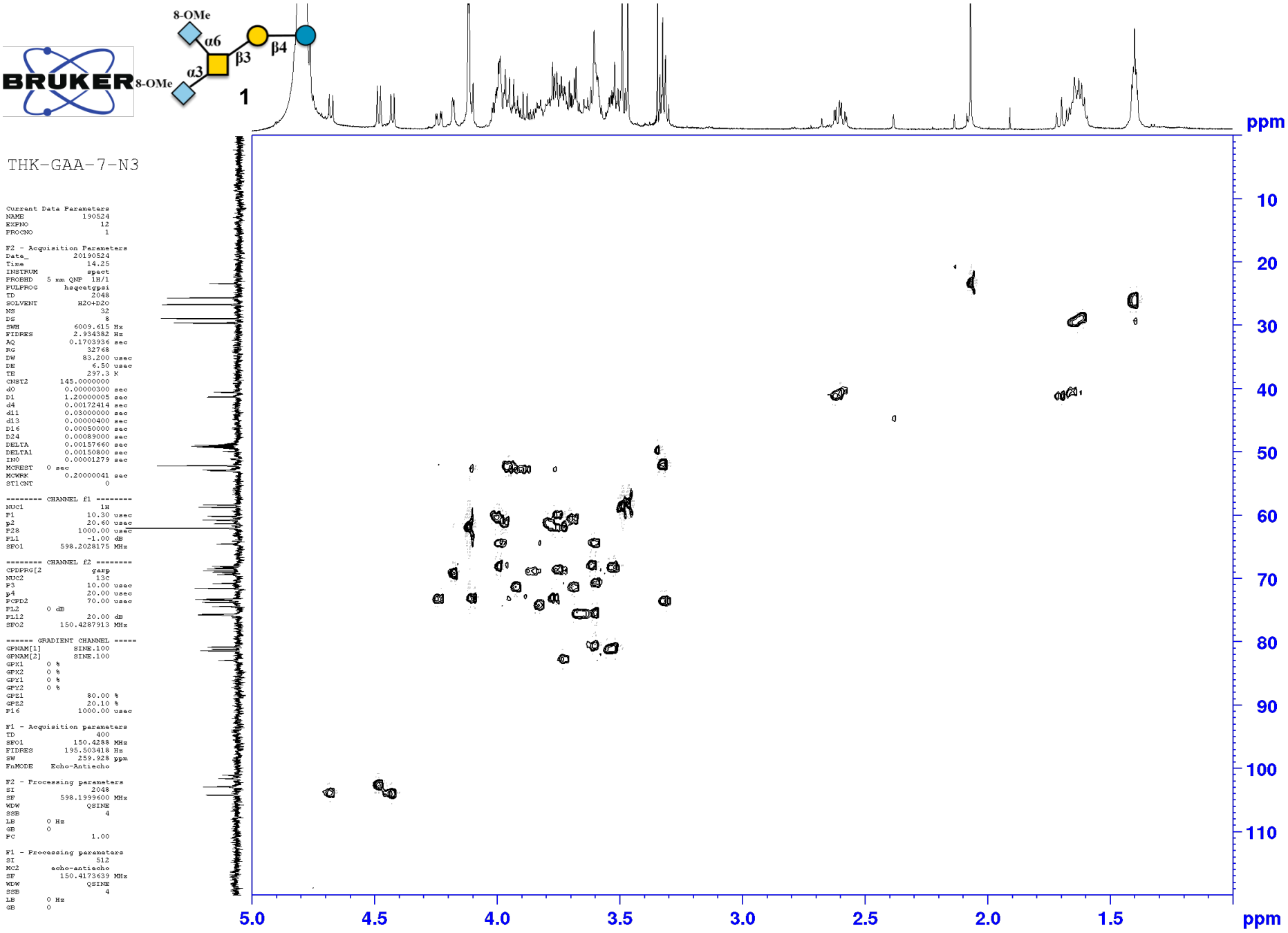


1

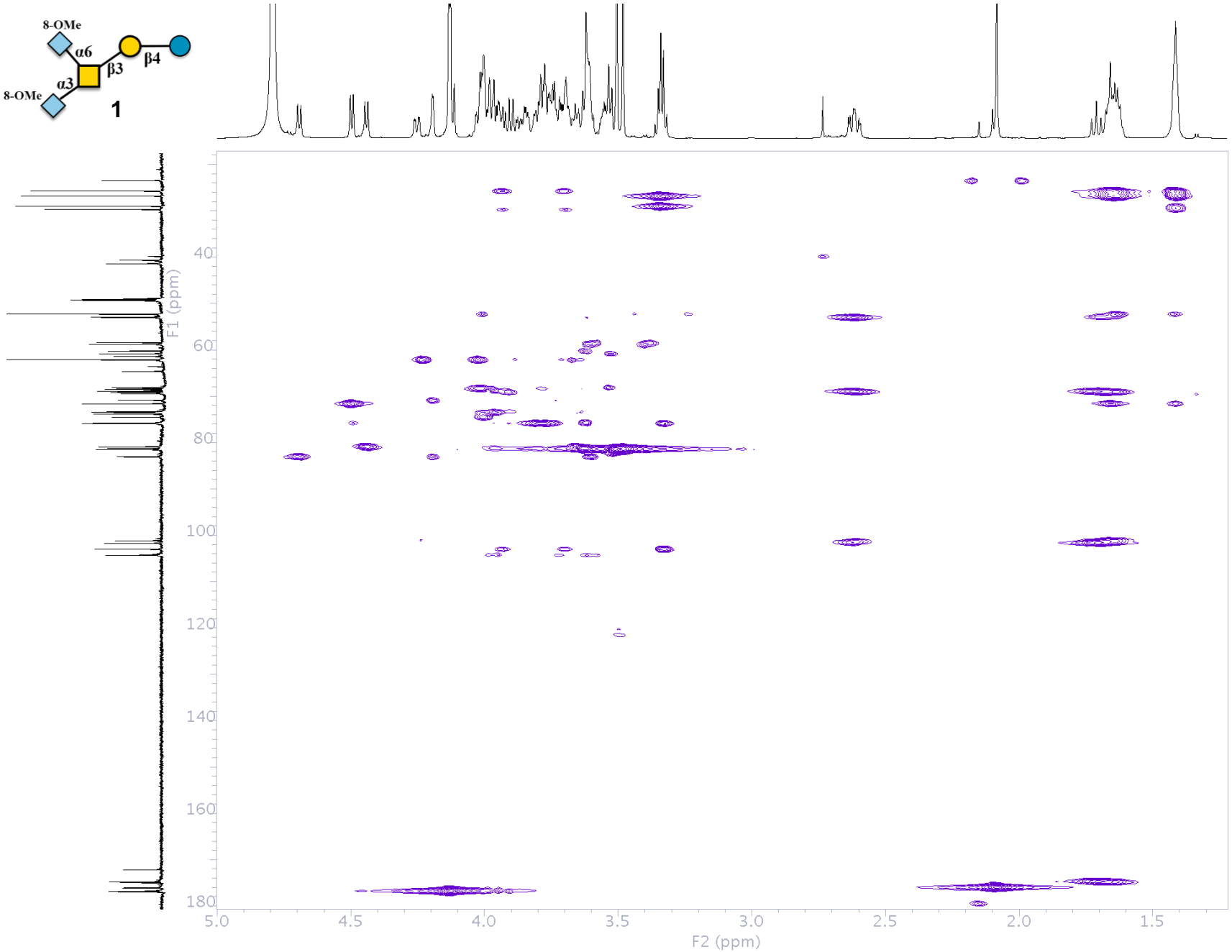
^{13}C NMR spectrum of Compound **1** (150 MHz D_2O)



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



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



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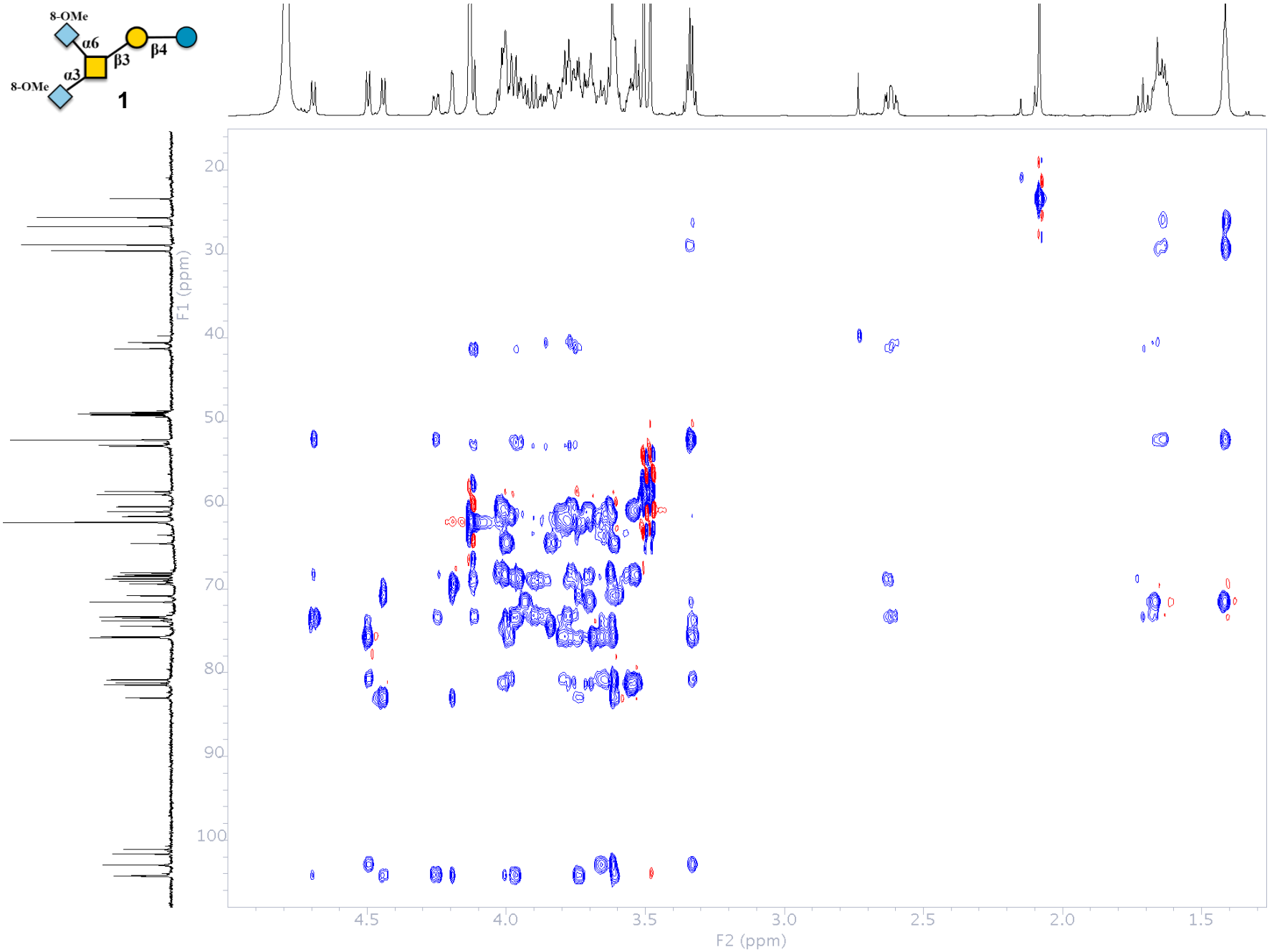

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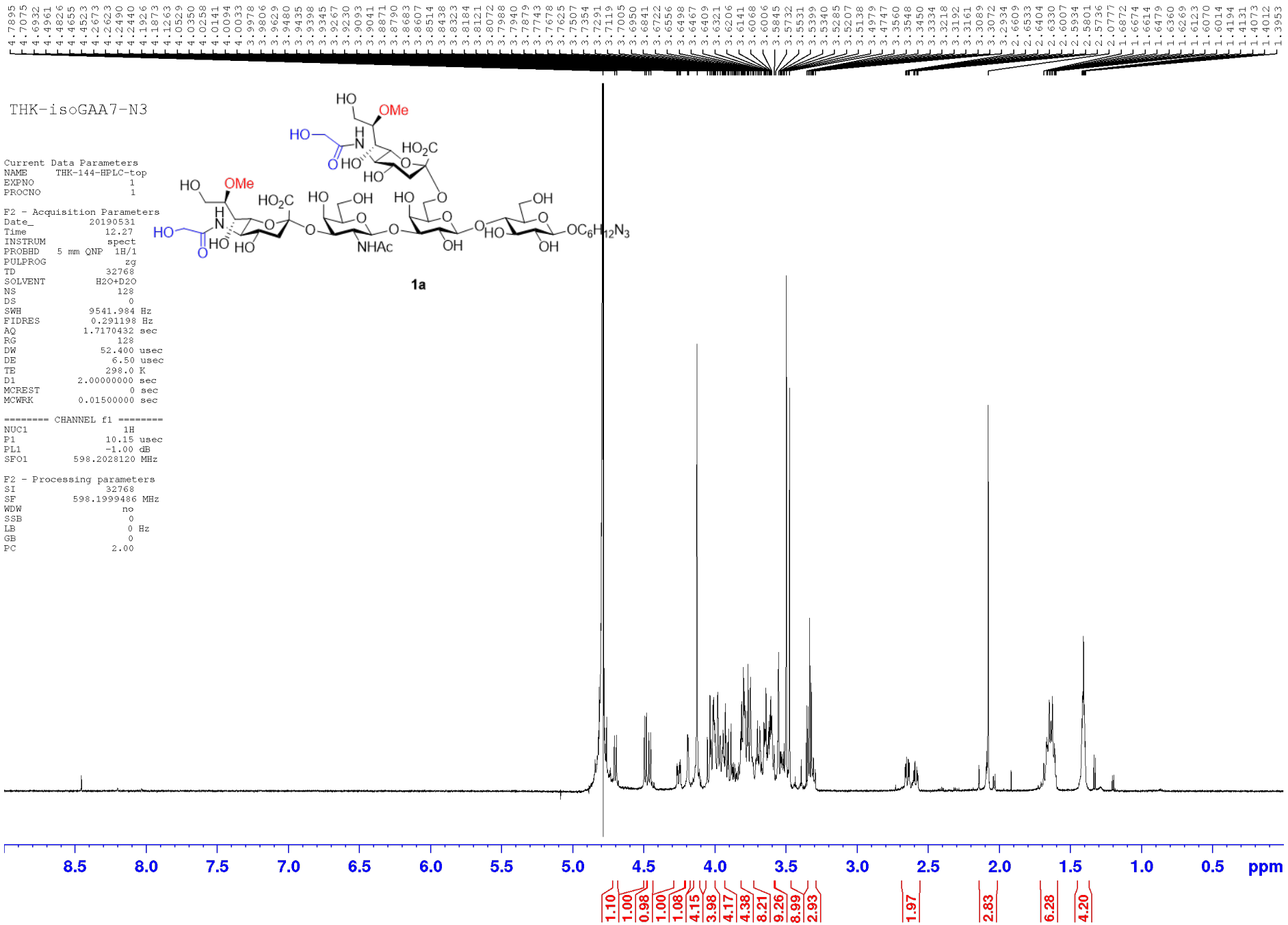
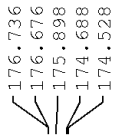
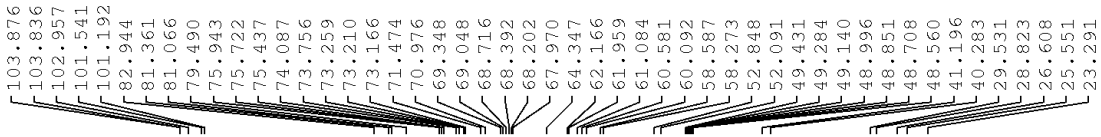
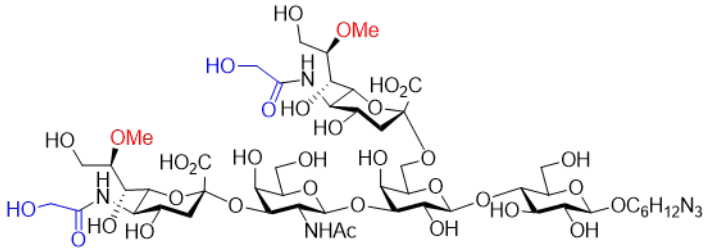


^{1}H NMR spectrum of Compound **1a** (600 MHz D_2O)





THK-isoGAA-7-N3



1a

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PROCNO                   1

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TD                 32768
SOLVENT          H2O+D2O
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DE                 6.50 usec
TE                298.4 K
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MCREST    0 sec
MCWRK         0.01500000 sec

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PL1        0 dB
SFO1         150.4340559 MHz

======== CHANNEL f2 ========
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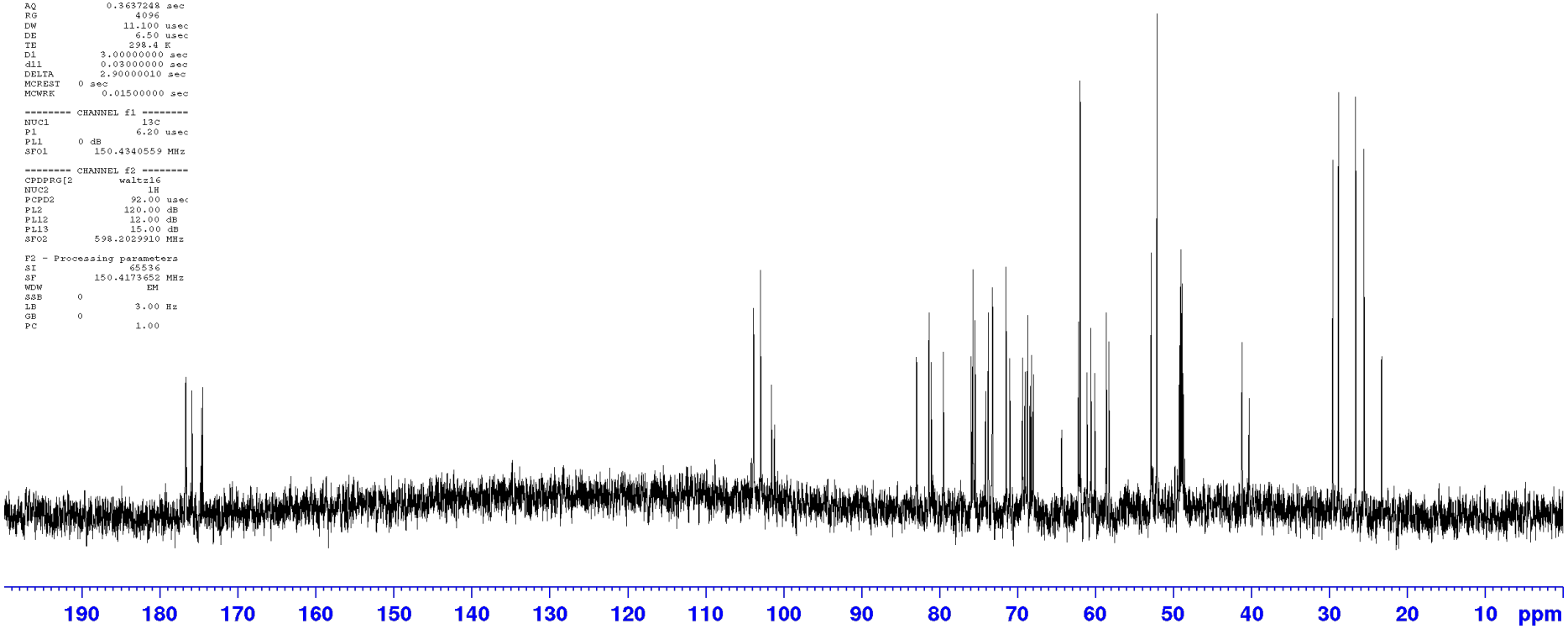
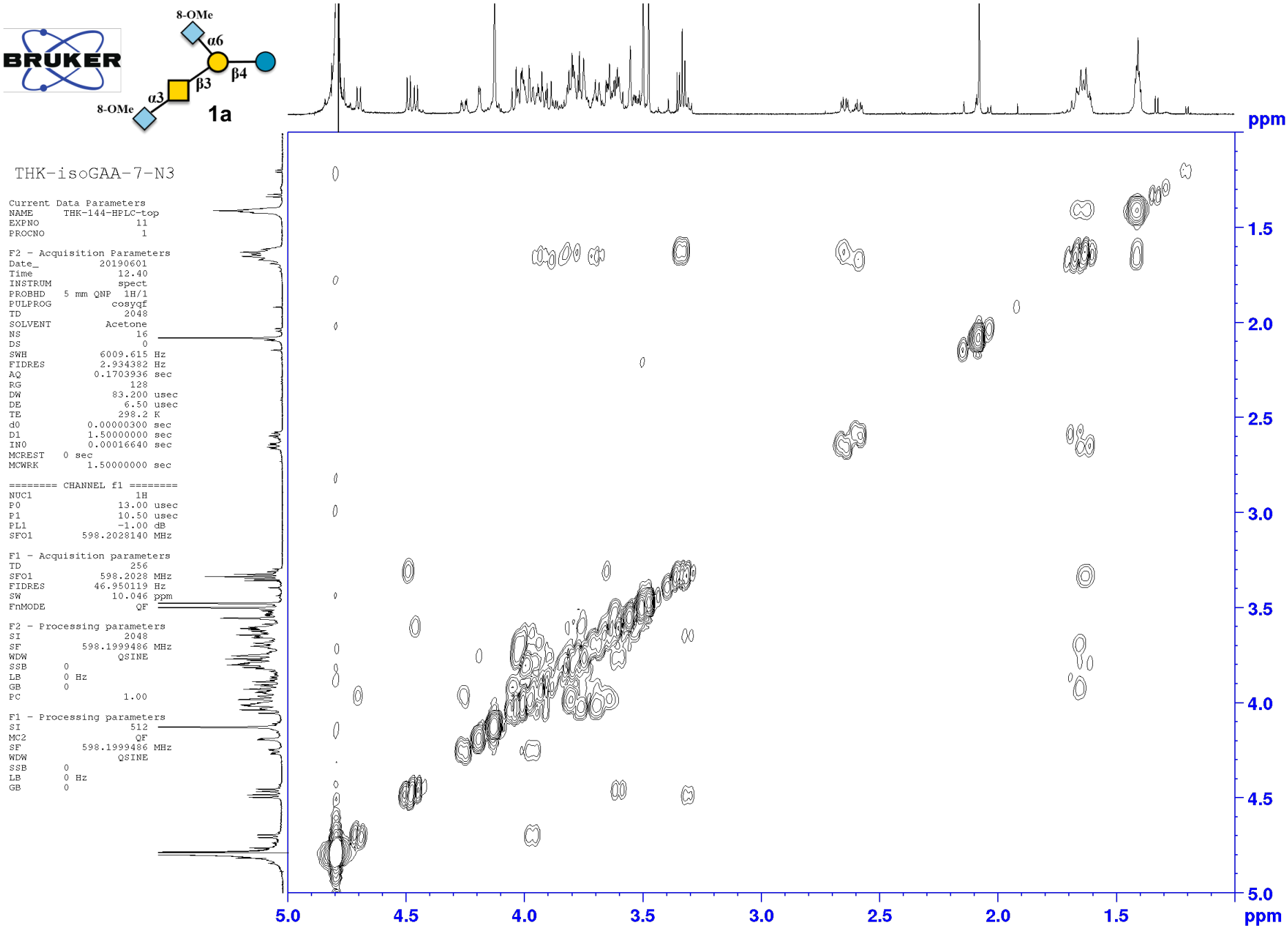
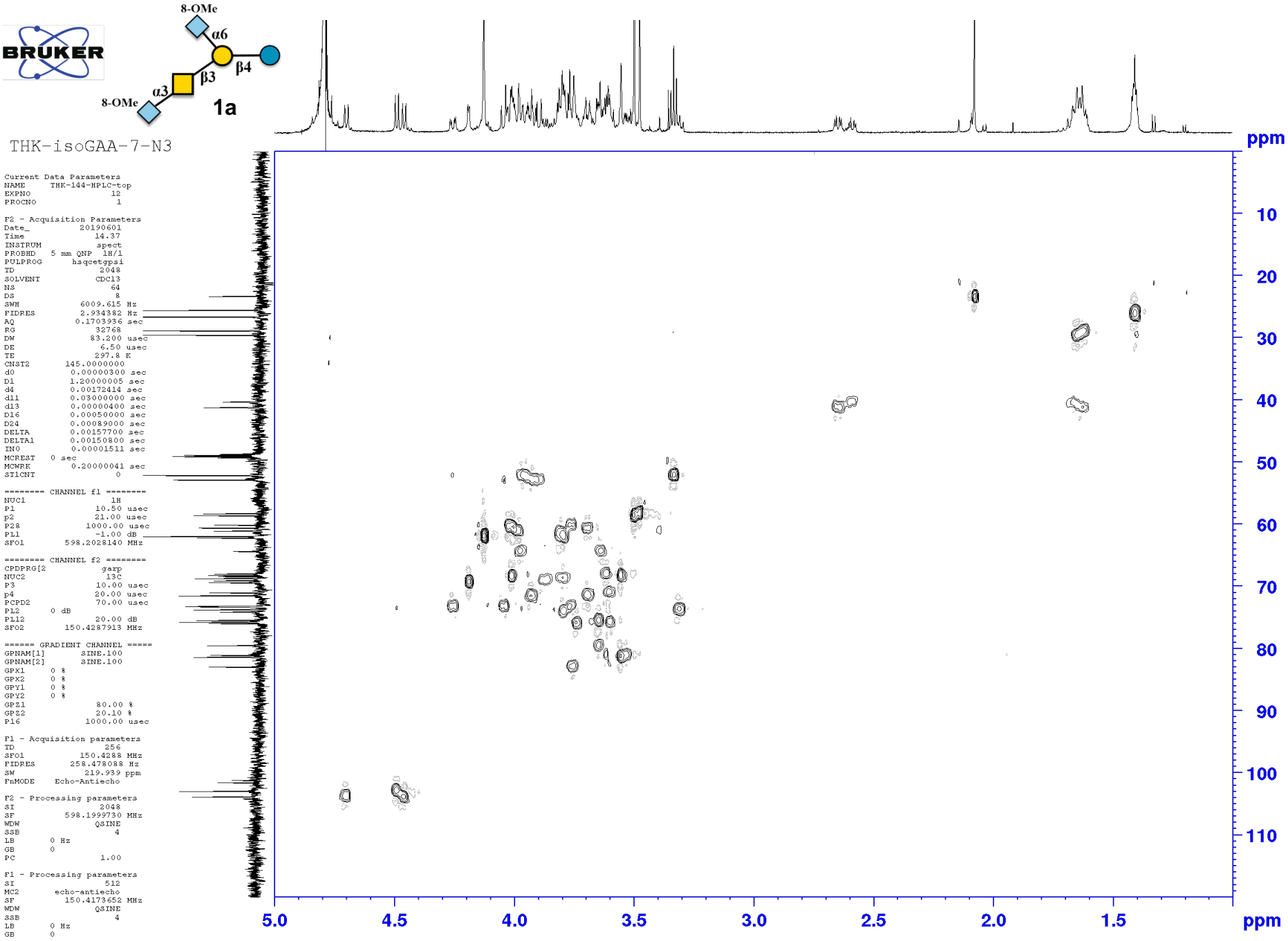


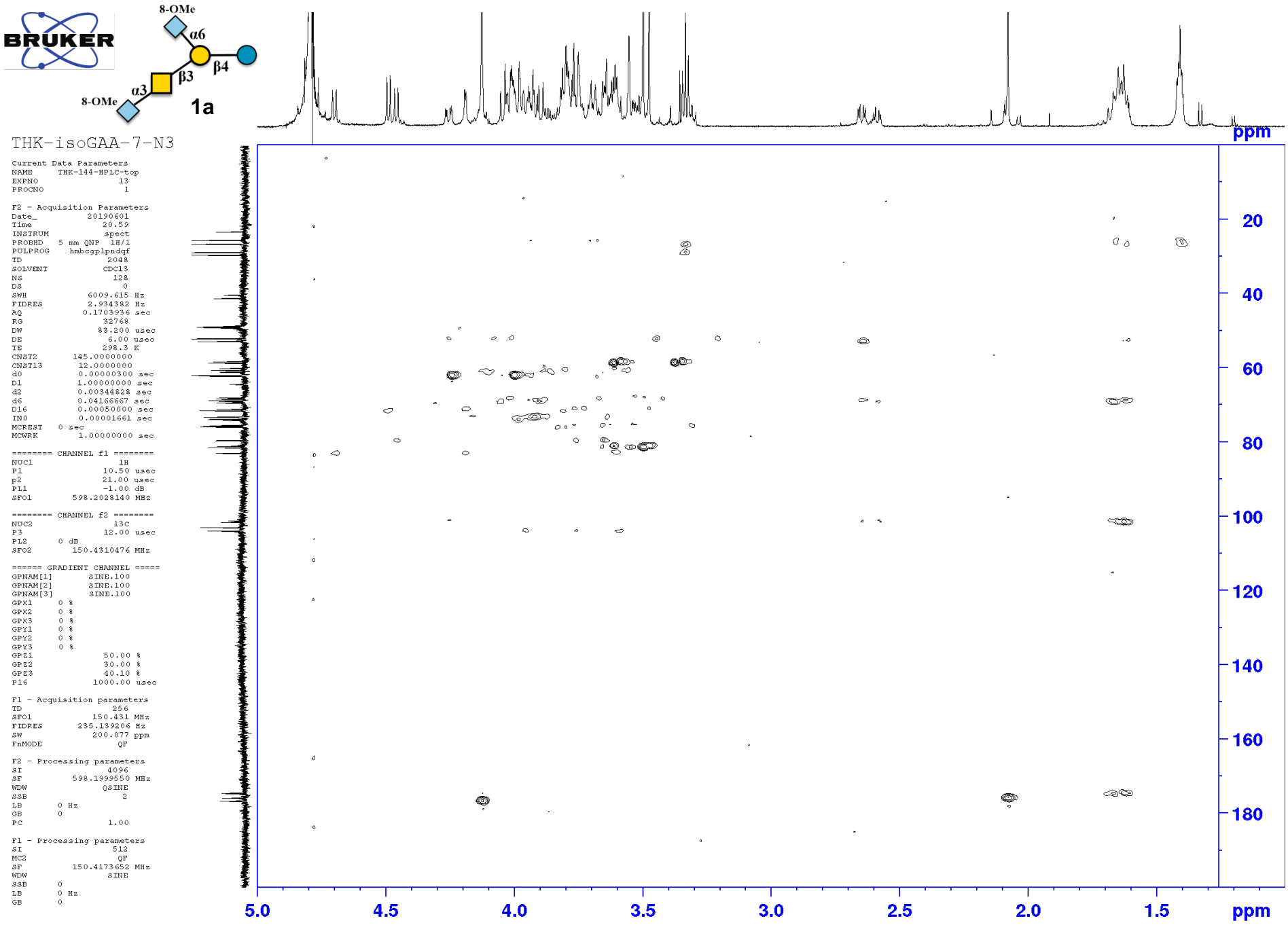
^{13}C NMR spectrum of Compound **1a** (150 MHz D_2O)



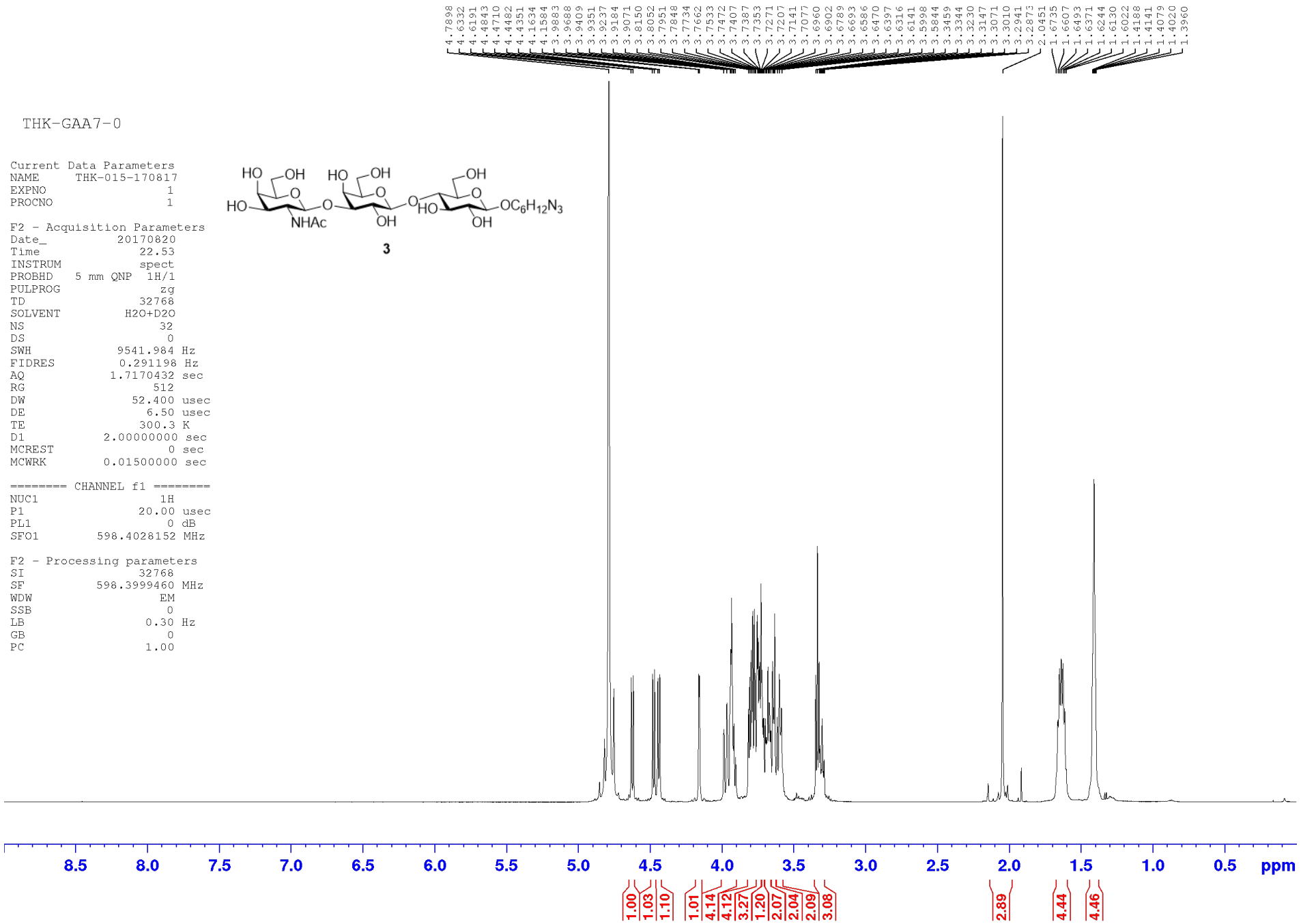
^{1}H-^{1}H COSY NMR spectrum of Compound **1a** (600 MHz D_2O)



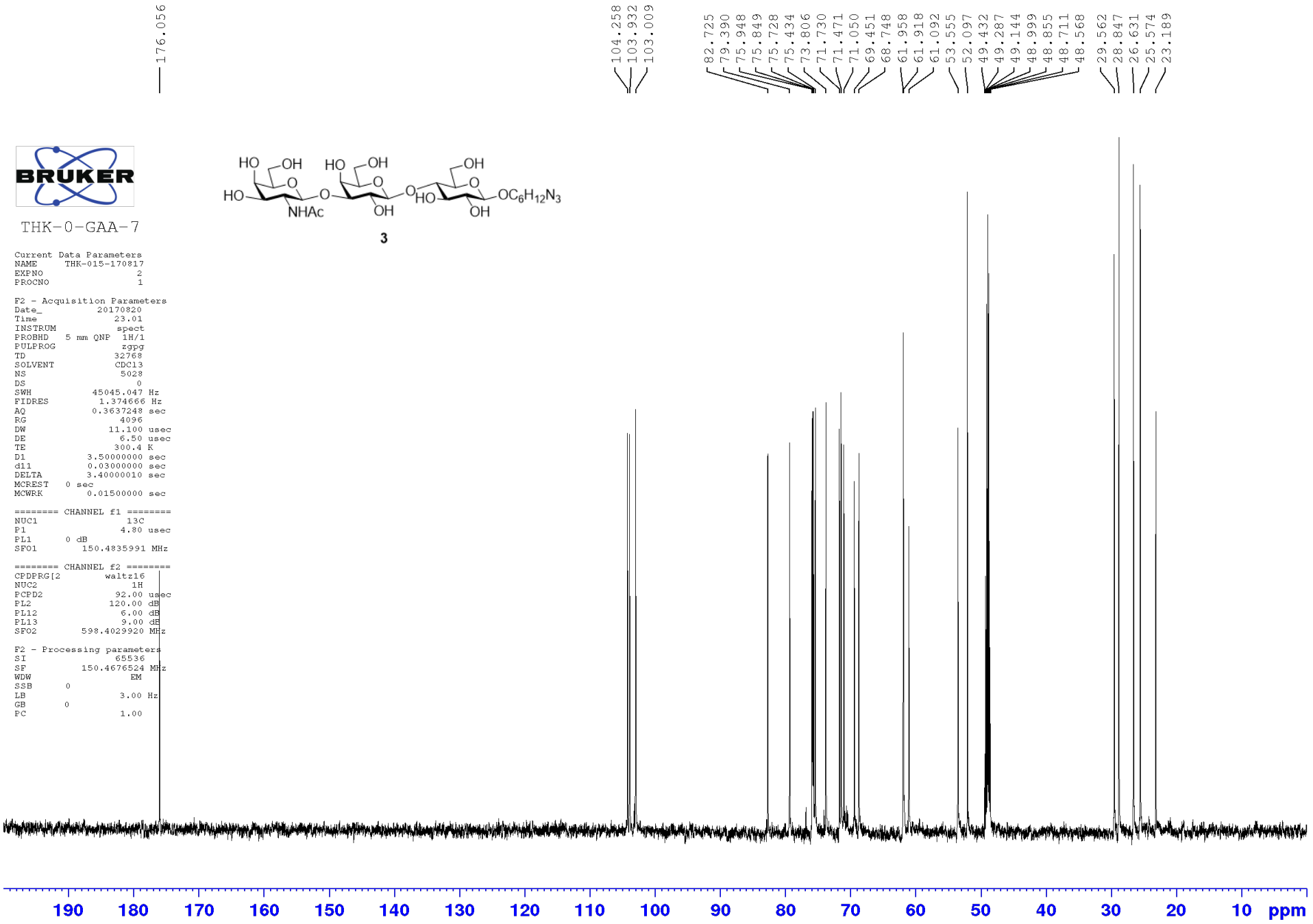
^{1}H-^{13}C HSQC NMR spectrum of Compound **1a** (600 MHz/150 MHz D_2O)



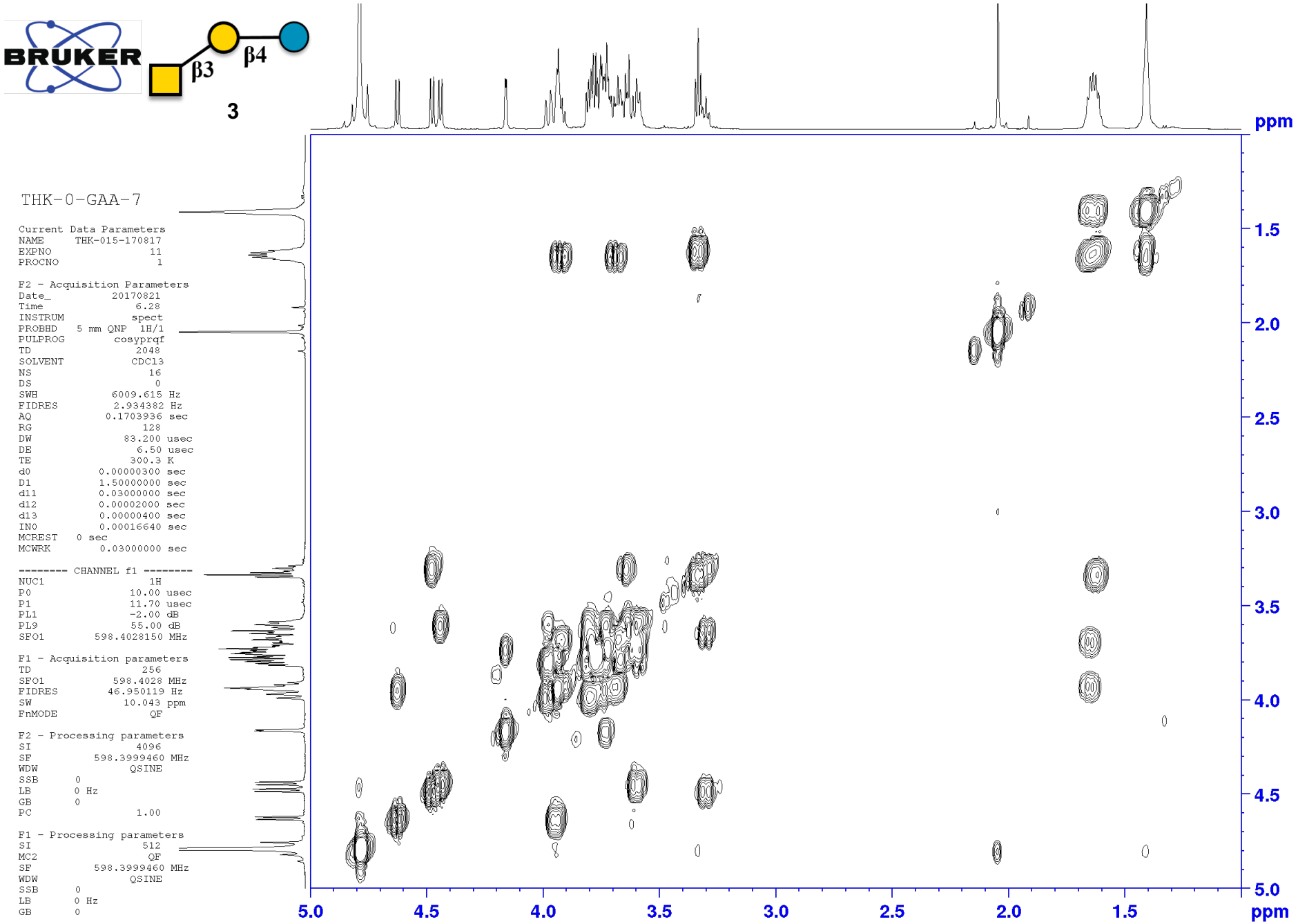
^{1}H-^{13}C HMBC NMR spectrum of Compound **1a** (600 MHz/150 MHz D_2O)



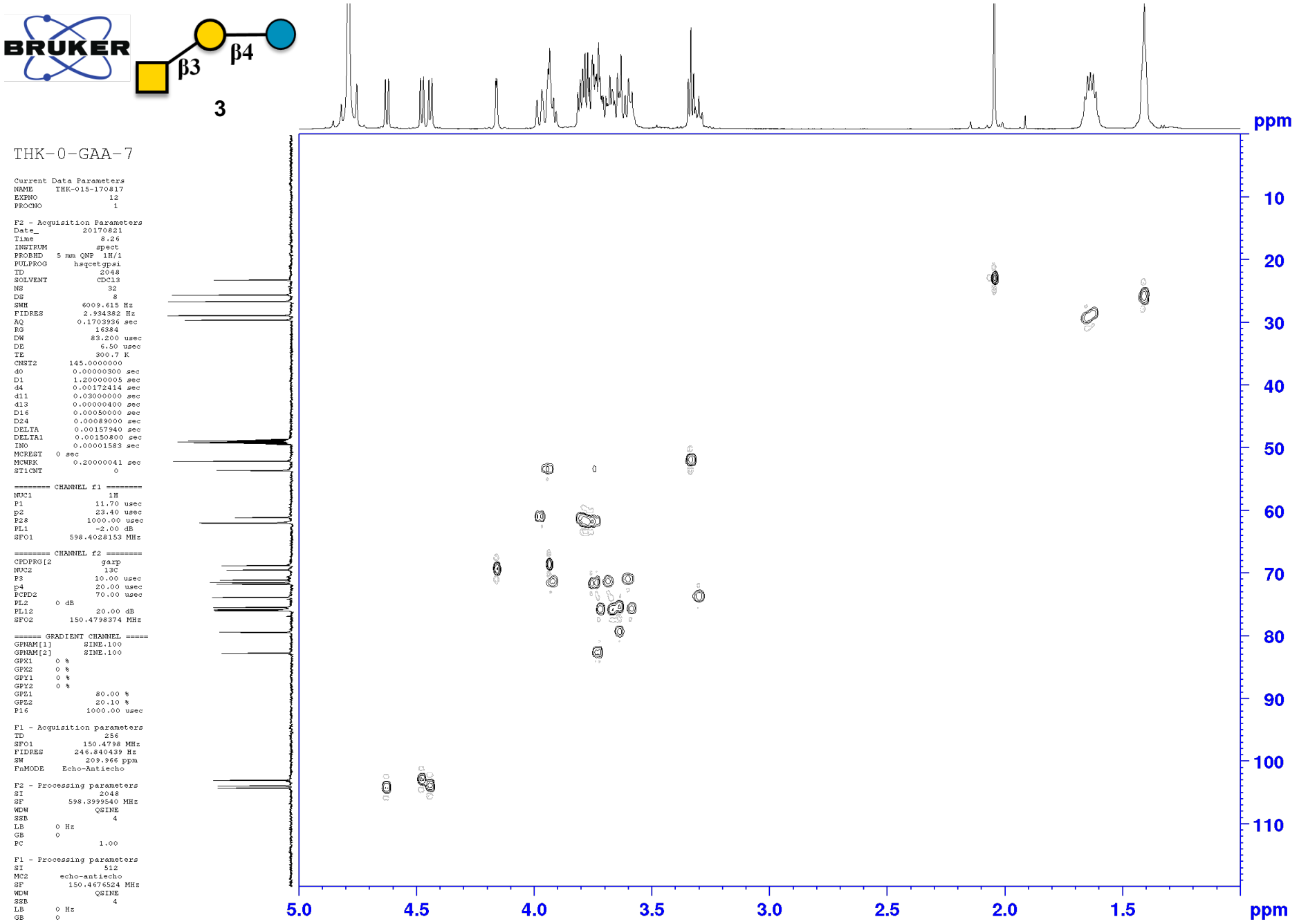
^{1}H NMR spectrum of Compound **3** (600 MHz D_2O)



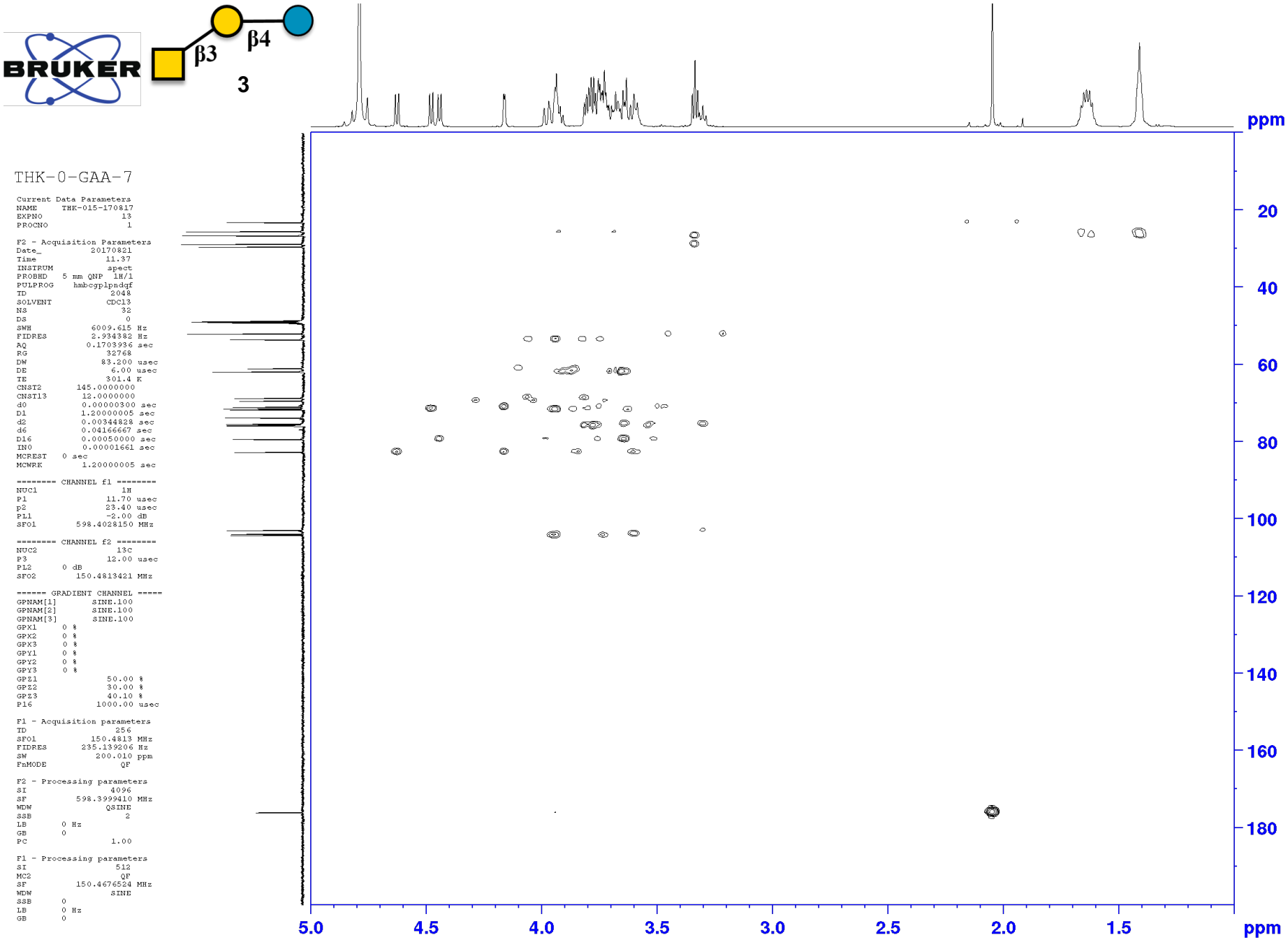
^{13}C NMR spectrum of Compound **3** (150 MHz D_2O)



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



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



THK-0-GAA-7

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PROCNO                 1

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Time              11.37
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PULPROG     hmbcgplpndqf
TD                 2048
SOLVENT           CDCl3
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               301.4 K
CNST2       145.0000000
CNST13       12.0000000
d0           0.00000300 sec
D1           1.20000005 sec
d2           0.00344828 sec
d6           0.04166667 sec
D16          0.00050000 sec
IN0          0.00001661 sec
MCREST    0 sec
MCWRK        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 %
P16            1000.00 usec

F1 - Acquisition parameters
TD                  256
SFO1           150.4813 MHz
FIDRES       235.139206 Hz
SW             200.010 ppm
FnMODE               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           150.4676524 MHz
WDW                SINE
SSB       0
LB        0 Hz
GB        0
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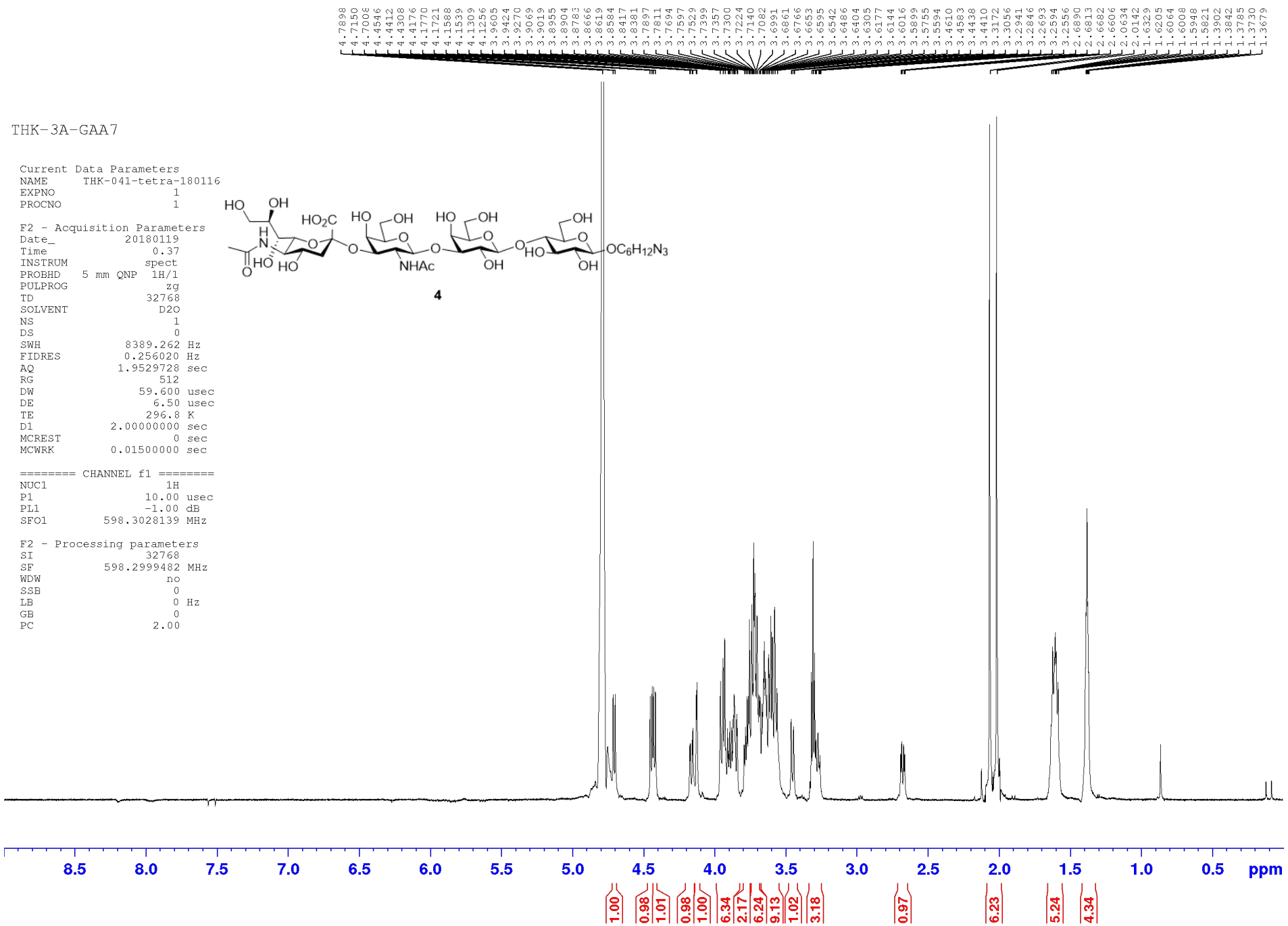
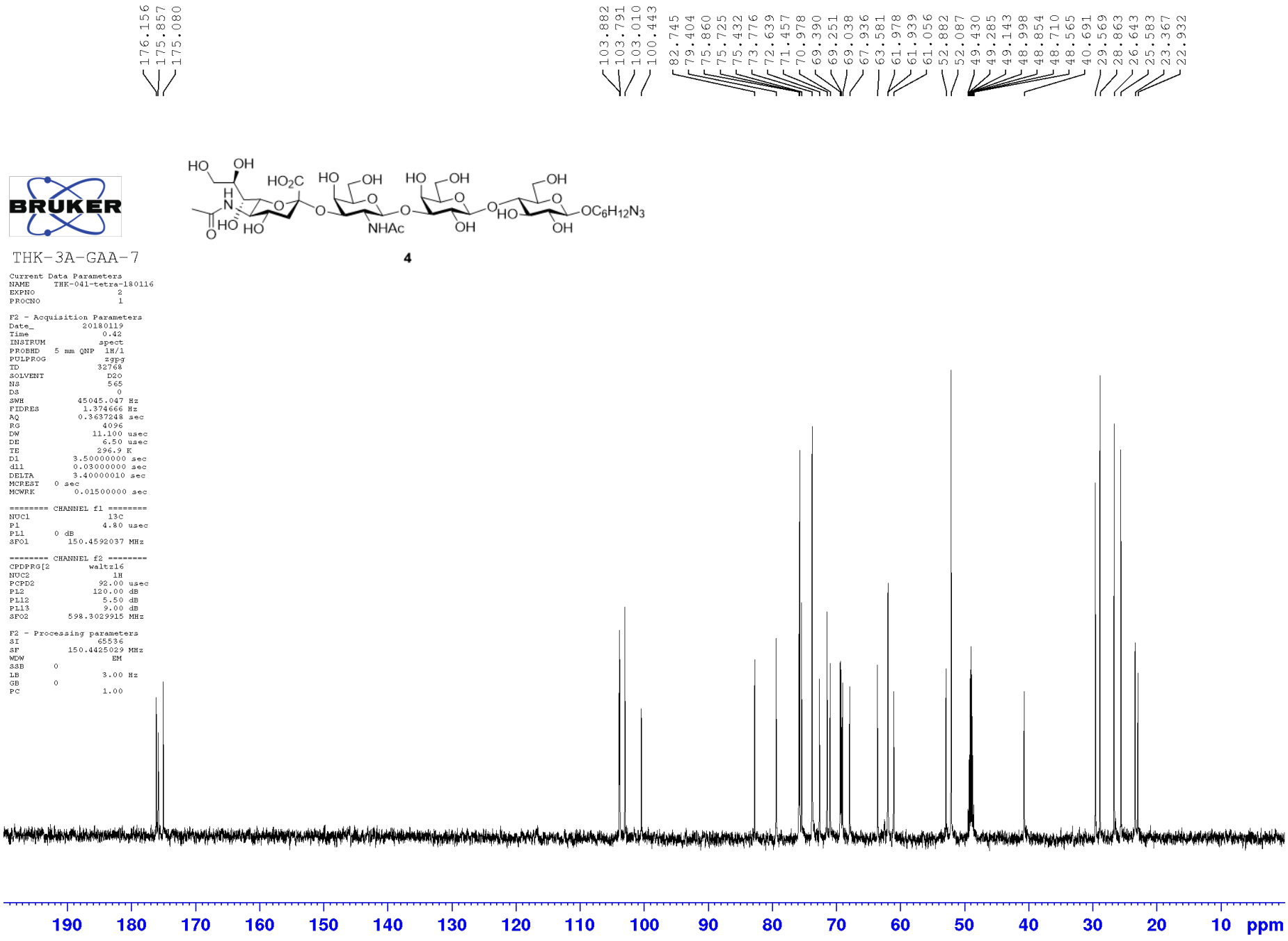
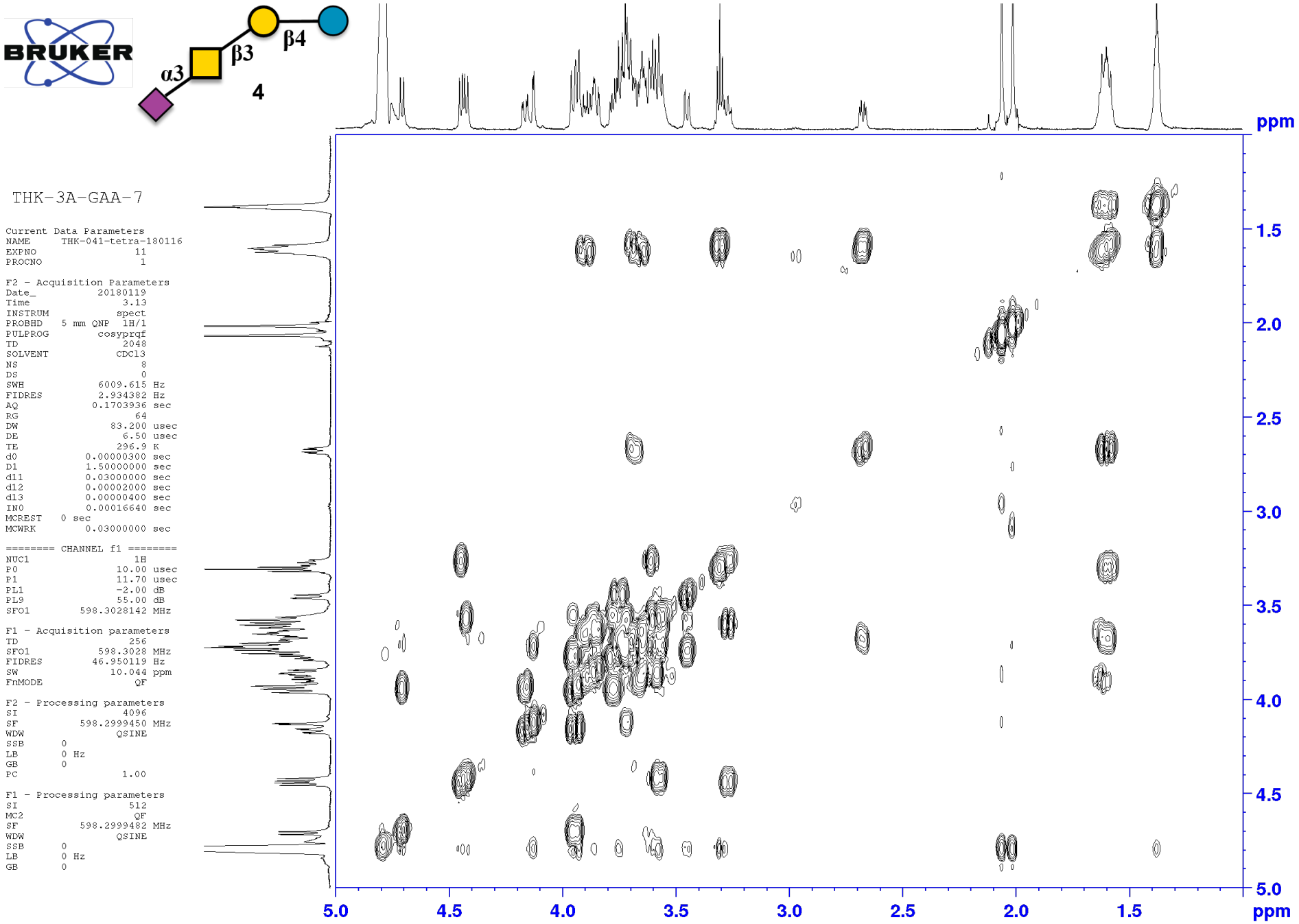
^{1}H-^{13}C HMBC NMR spectrum of Compound **3** (600 MHz/150 MHz D_2O)



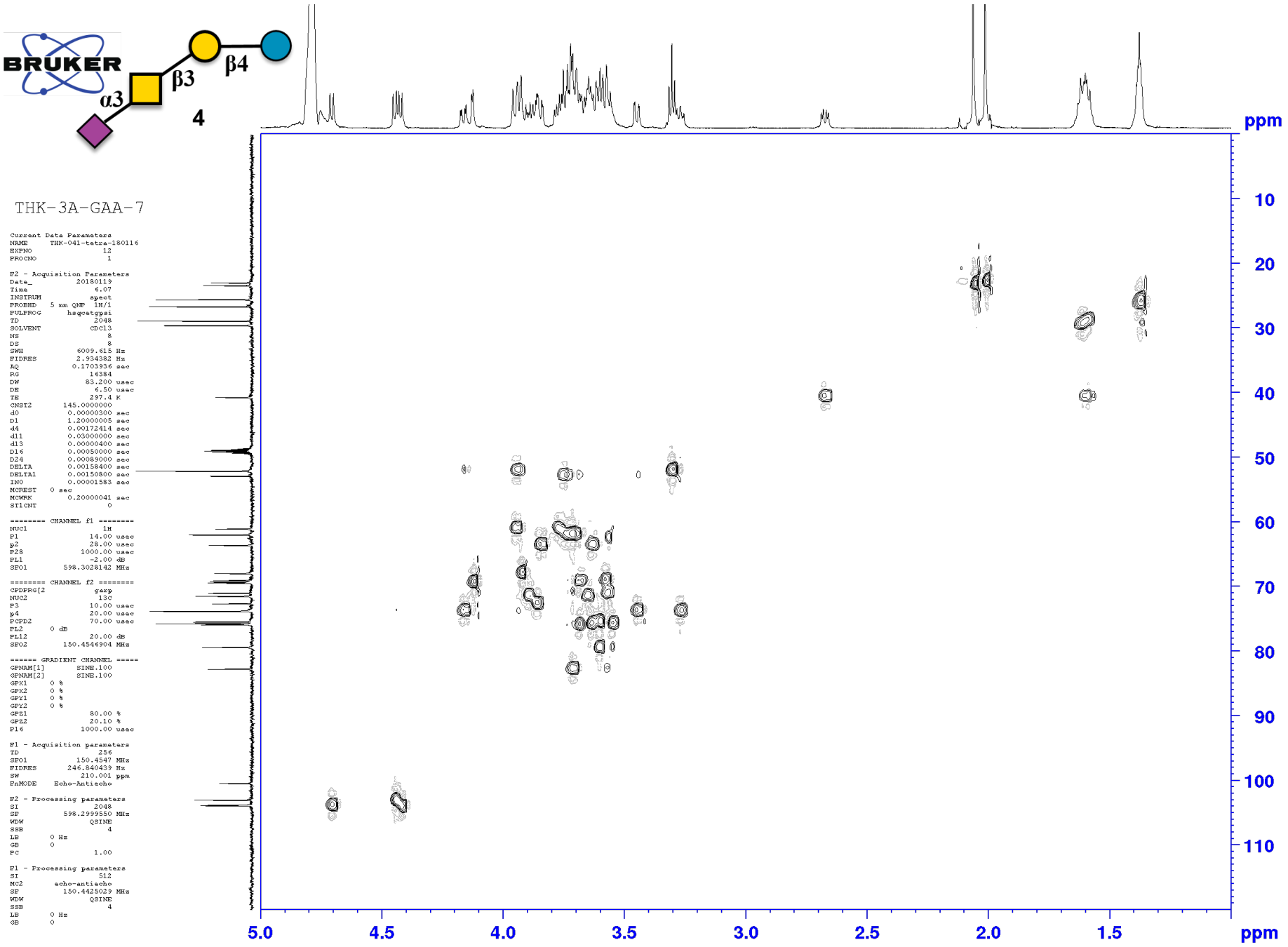
^{1}H NMR spectrum of Compound **4** (600 MHz D_2O)



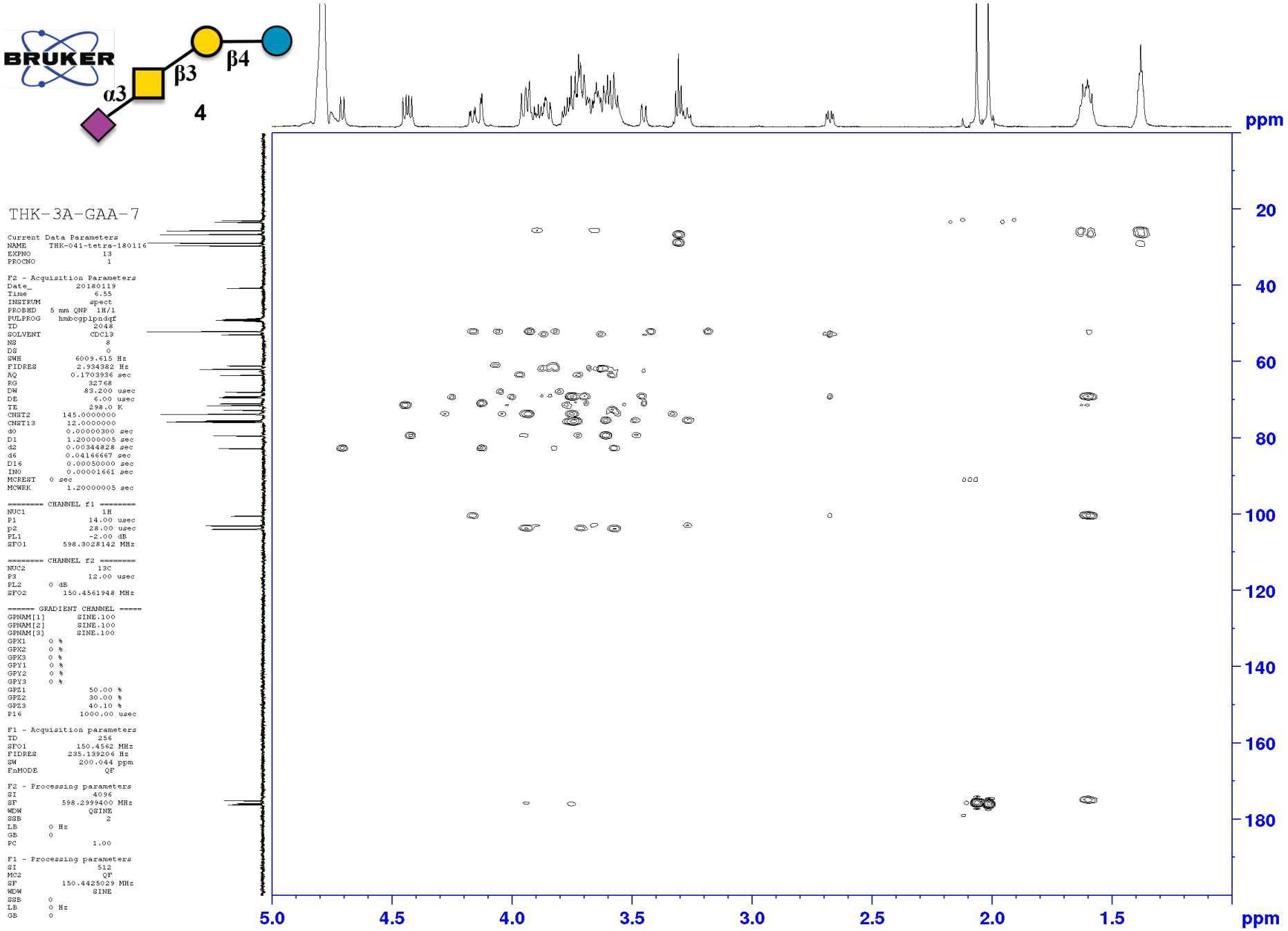
^{13}C NMR spectrum of Compound **4** (150 MHz D_2O)



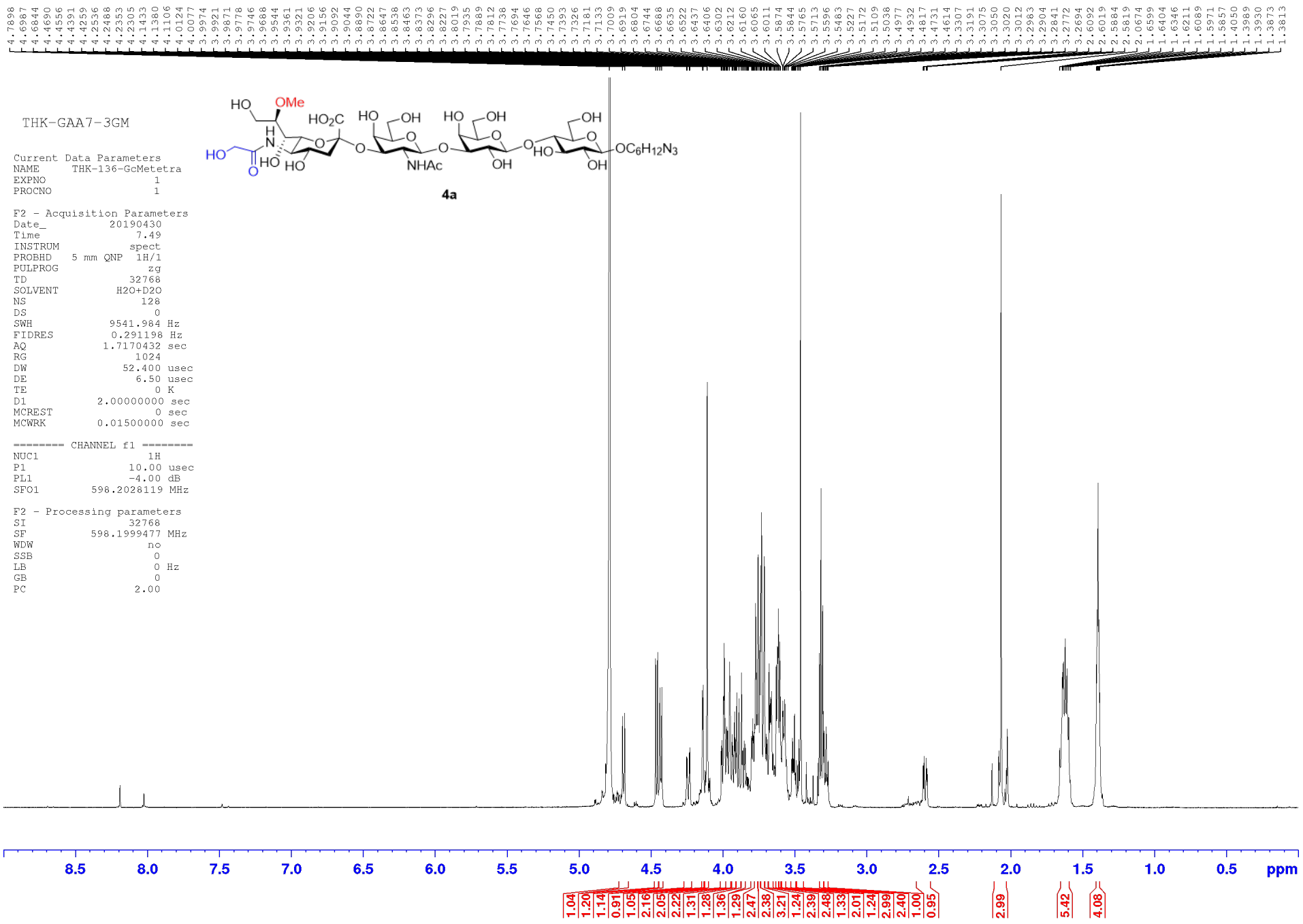
^{1}H-^{1}H COSY NMR spectrum of Compound **4** (600 MHz D_2O)



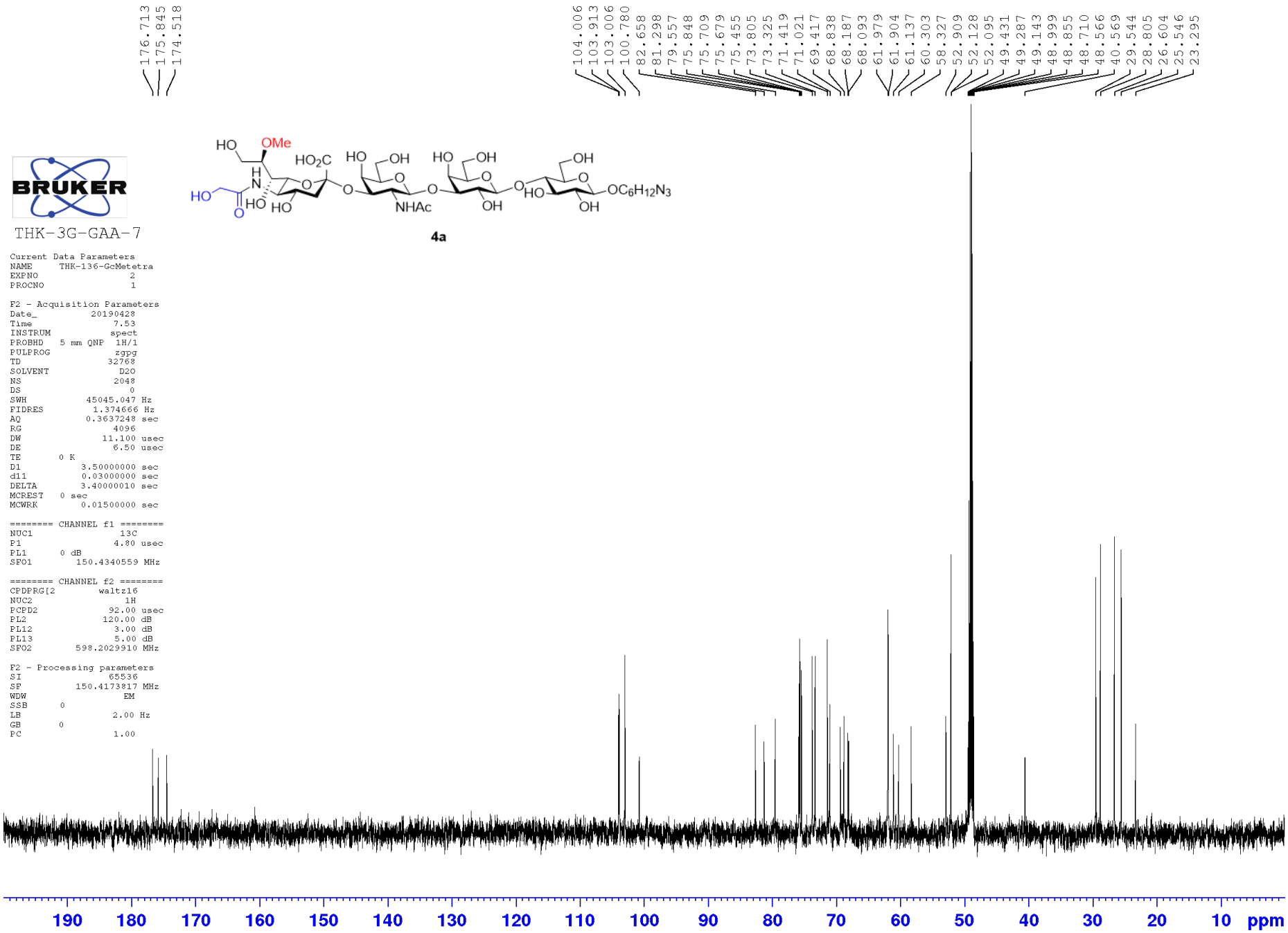
^{1}H-^{13}C HSQC NMR spectrum of Compound **4** (600 MHz/150 MHz D_2O)



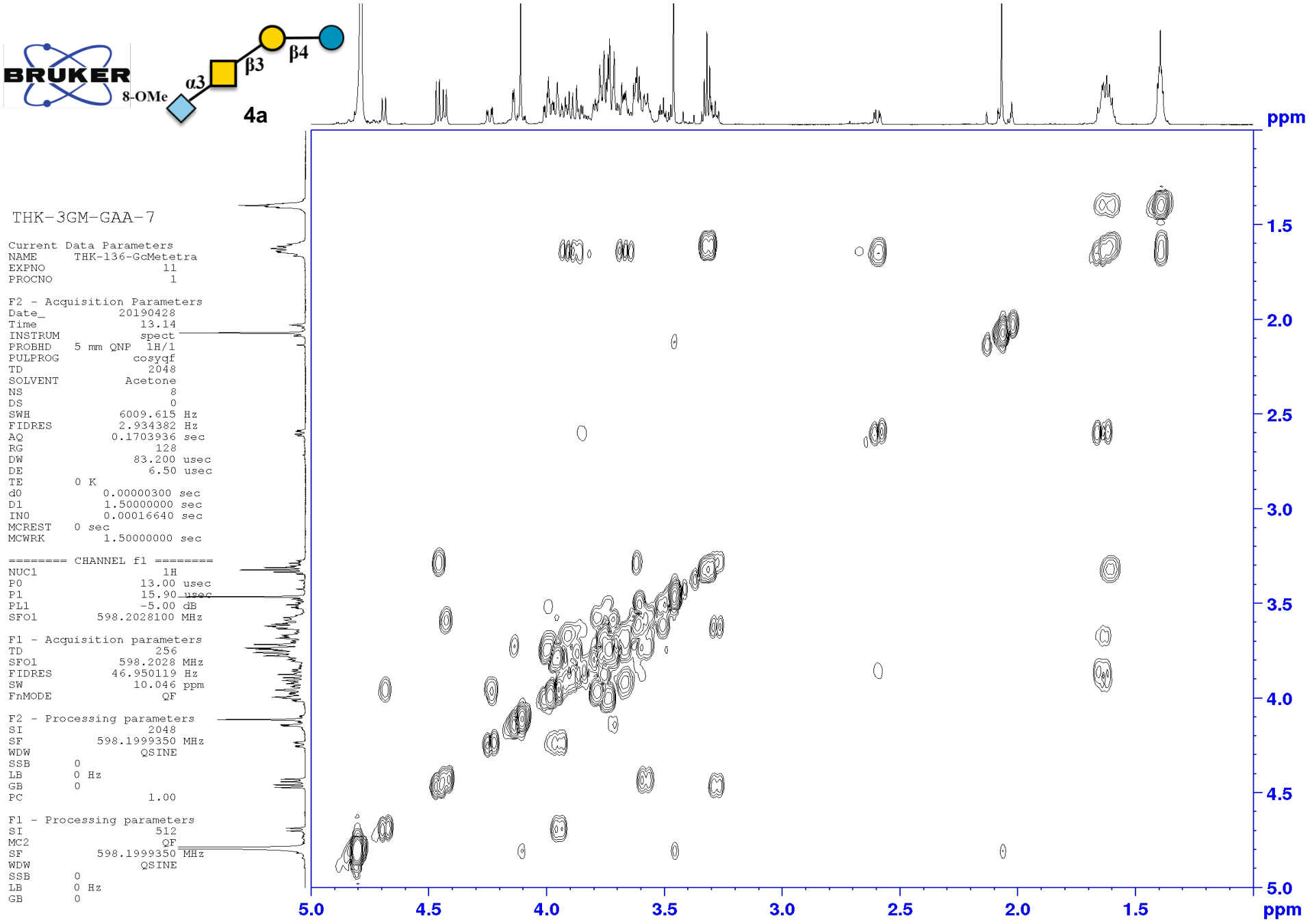
^{1}H-^{13}C HMBC NMR spectrum of Compound **4** (600 MHz/150 MHz D_2O)



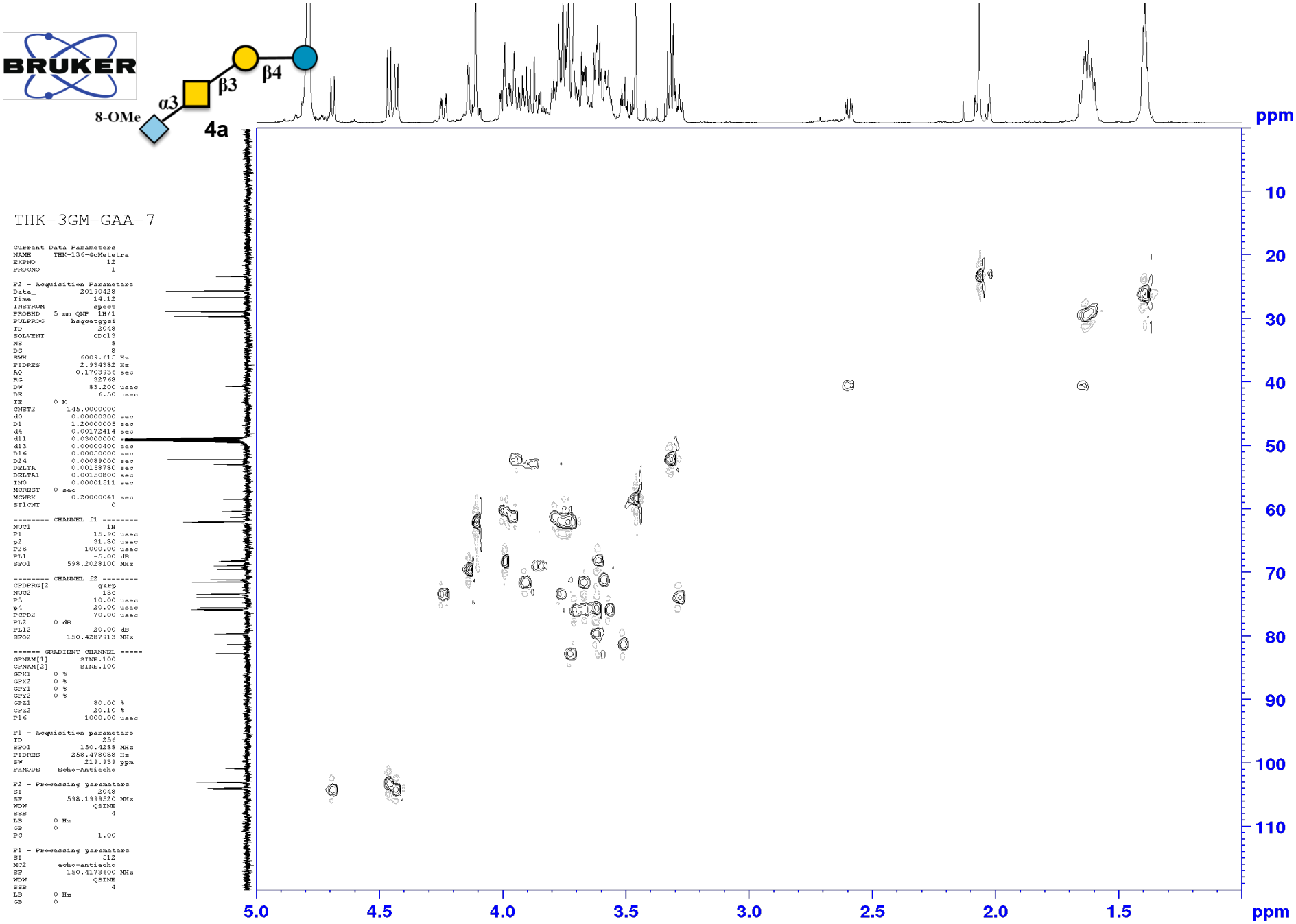
^{1}H NMR spectrum of Compound **4a** (600 MHz D_2O)



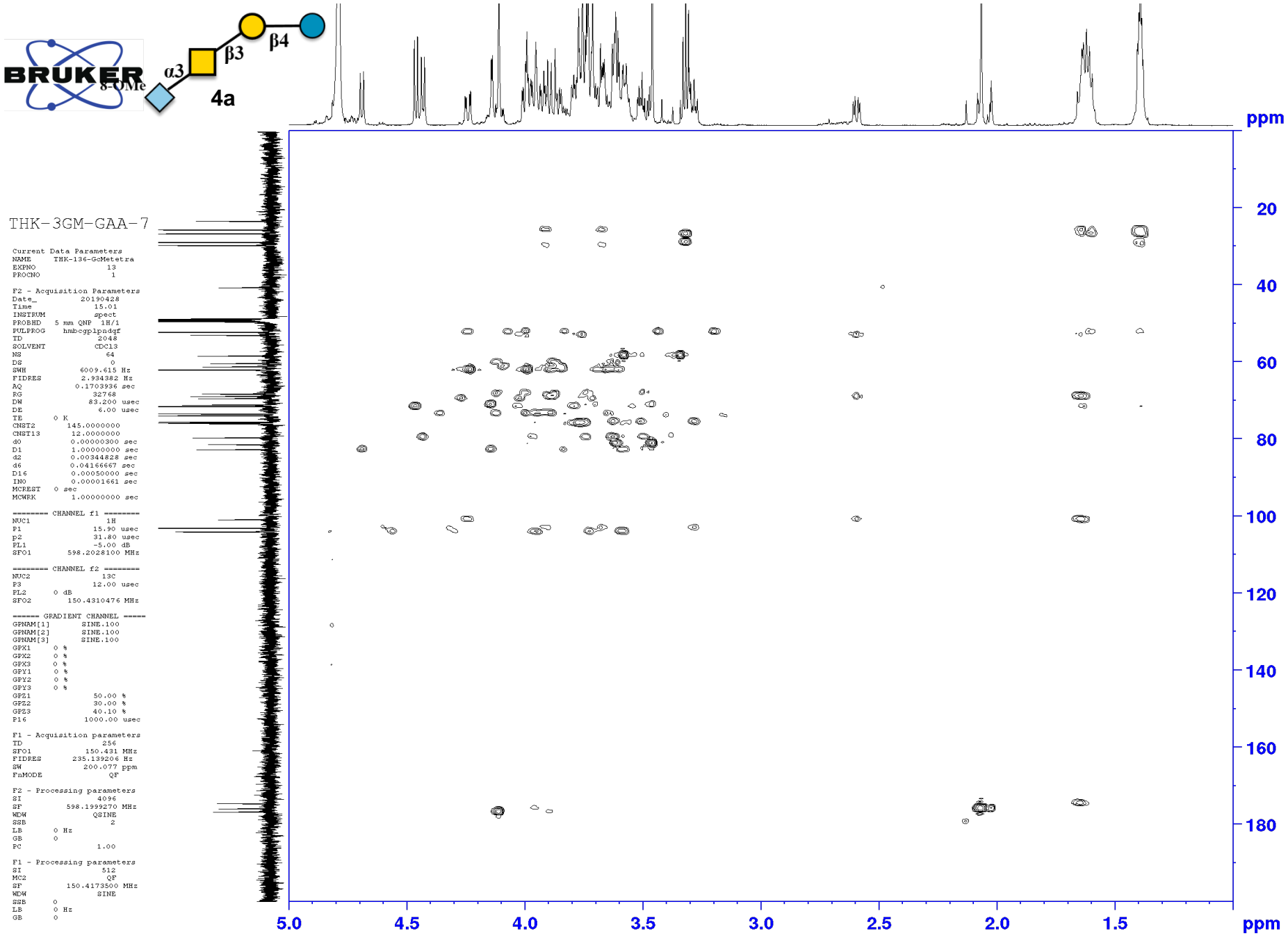
^{13}C NMR spectrum of Compound **4a** (150 MHz D_2O)



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



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



THK-3GM-GAA-7

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EXPNO                    13
PROCNO                    1

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Time                  15.01
INSTRUM               spect
PROBHD    5 mm QNP  1H/1
PULPROG     hmbcgplpndqf
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
CNST2           145.0000000
CNST13           12.0000000
d0              0.00000300 sec
D1              1.00000000 sec
d2              0.00344828 sec
d6              0.04166667 sec
D16             0.00050000 sec
IN0             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
TD                      256
SFO1                150.431 MHz
FIDRES          235.139206 Hz
SW                  200.077 ppm
FnMODE                   QF

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

F1 - Processing parameters
SI                       512
MC2                       QF
SF            150.4173500 MHz
WDW                    SINE
SSB         0
LB          0 Hz
GB          0
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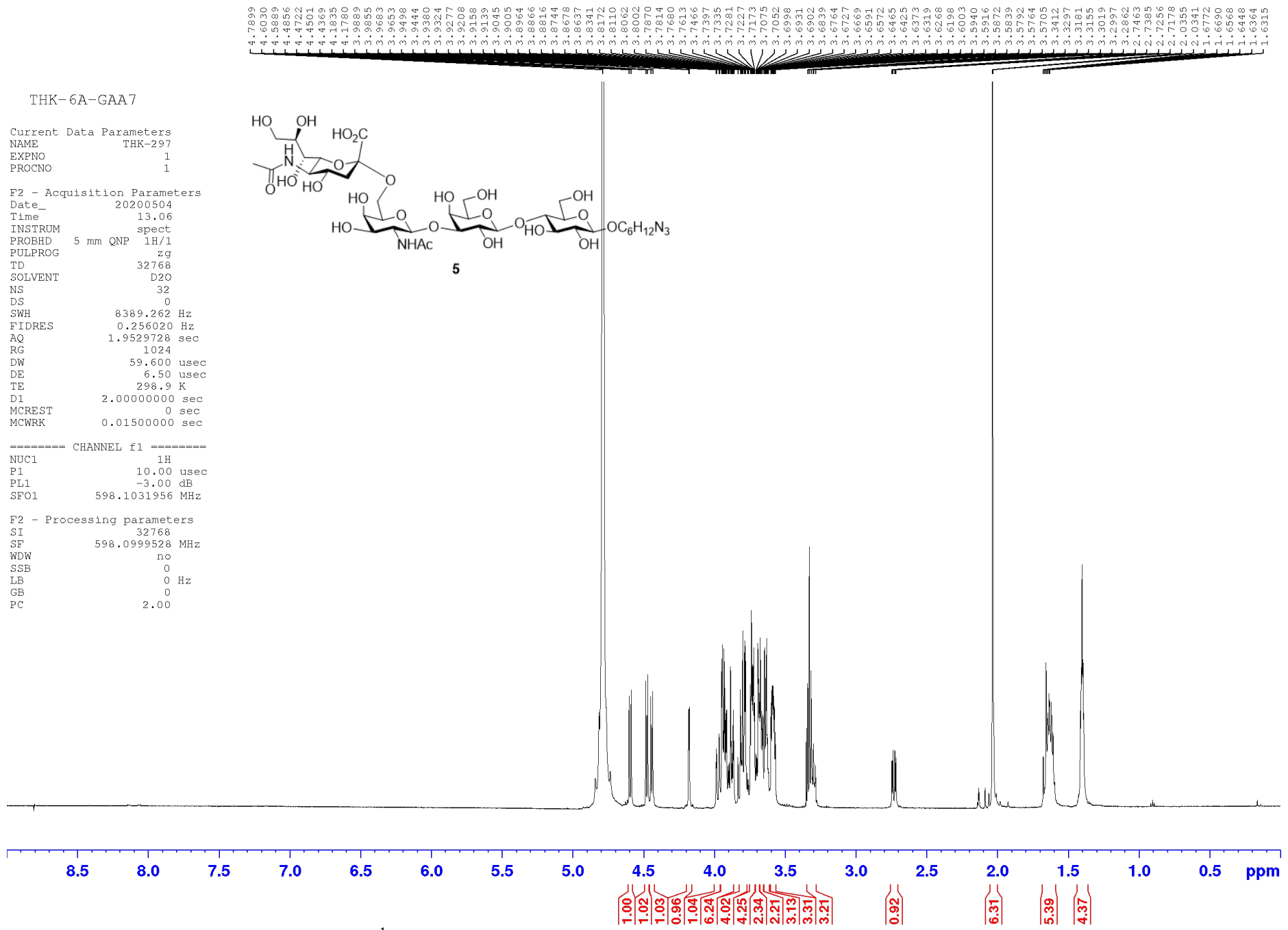
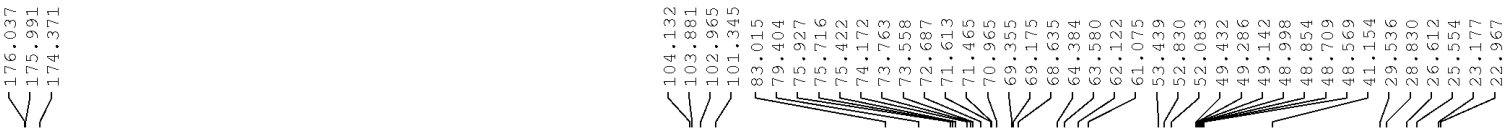
^{1}H-^{13}C HMBC NMR spectrum of Compound **4a** (600 MHz/150 MHz D_2O)



^{1}H NMR spectrum of Compound **5** (600 MHz D_2O)



THK-6A-GAA7

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Current Data Parameters
NAME              THK-297
EXPNO                   2
PROCNO                  1

F2 - Acquisition Parameters
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Time                13.43
INSTRUM             spect
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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           3.50000000 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 ========
CPDPRG[2         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
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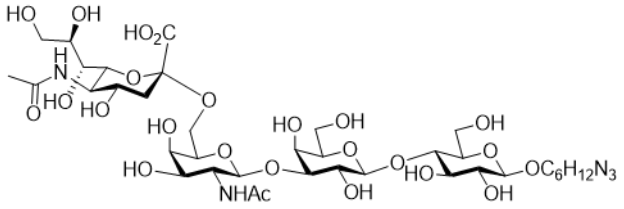
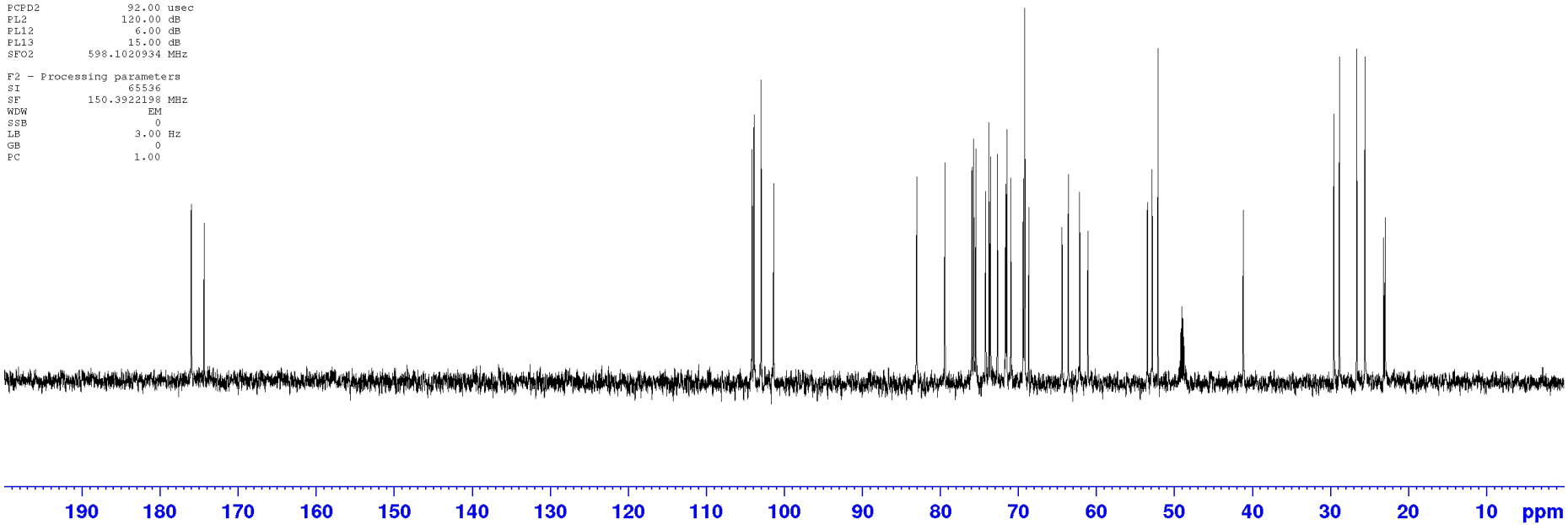
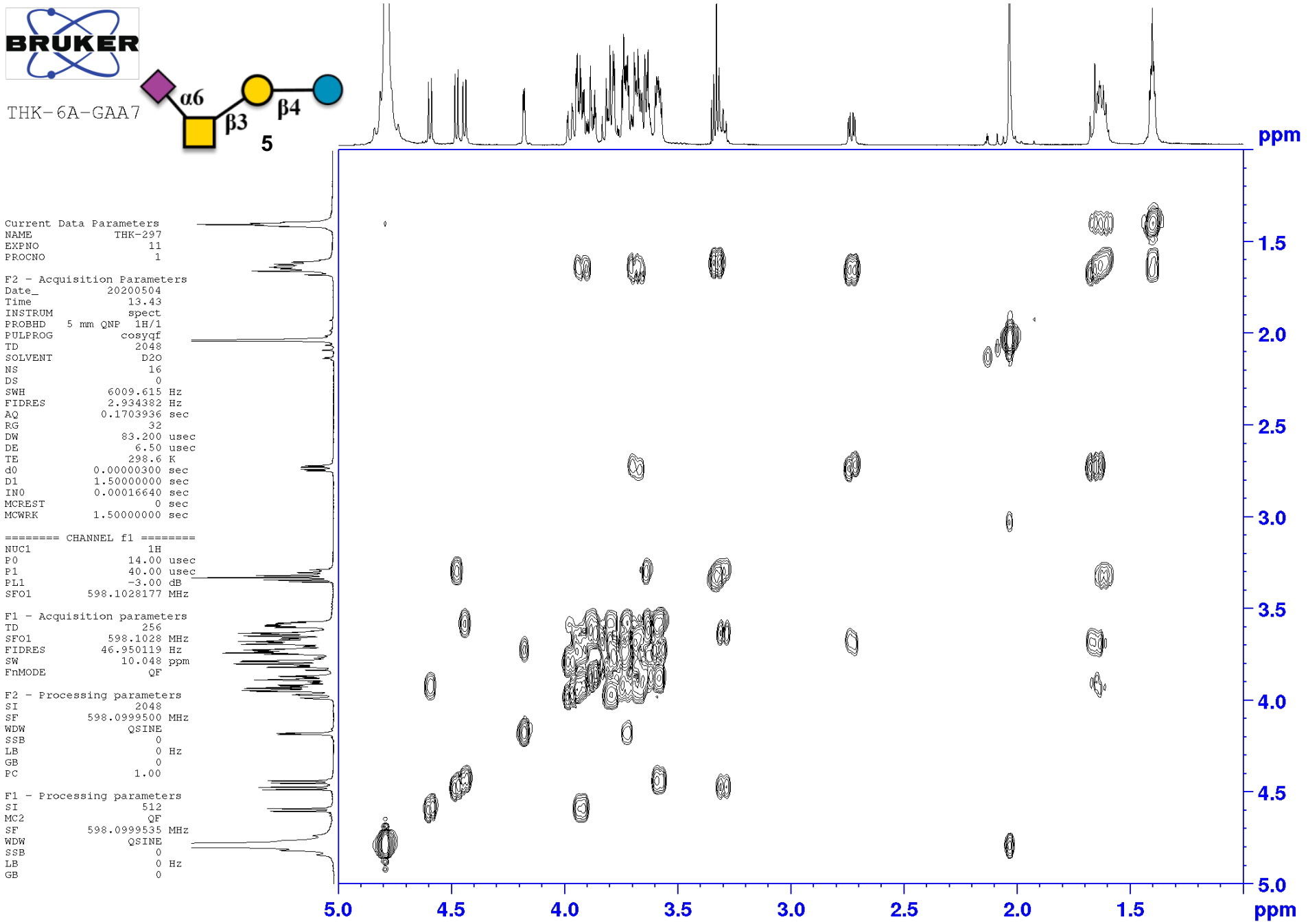


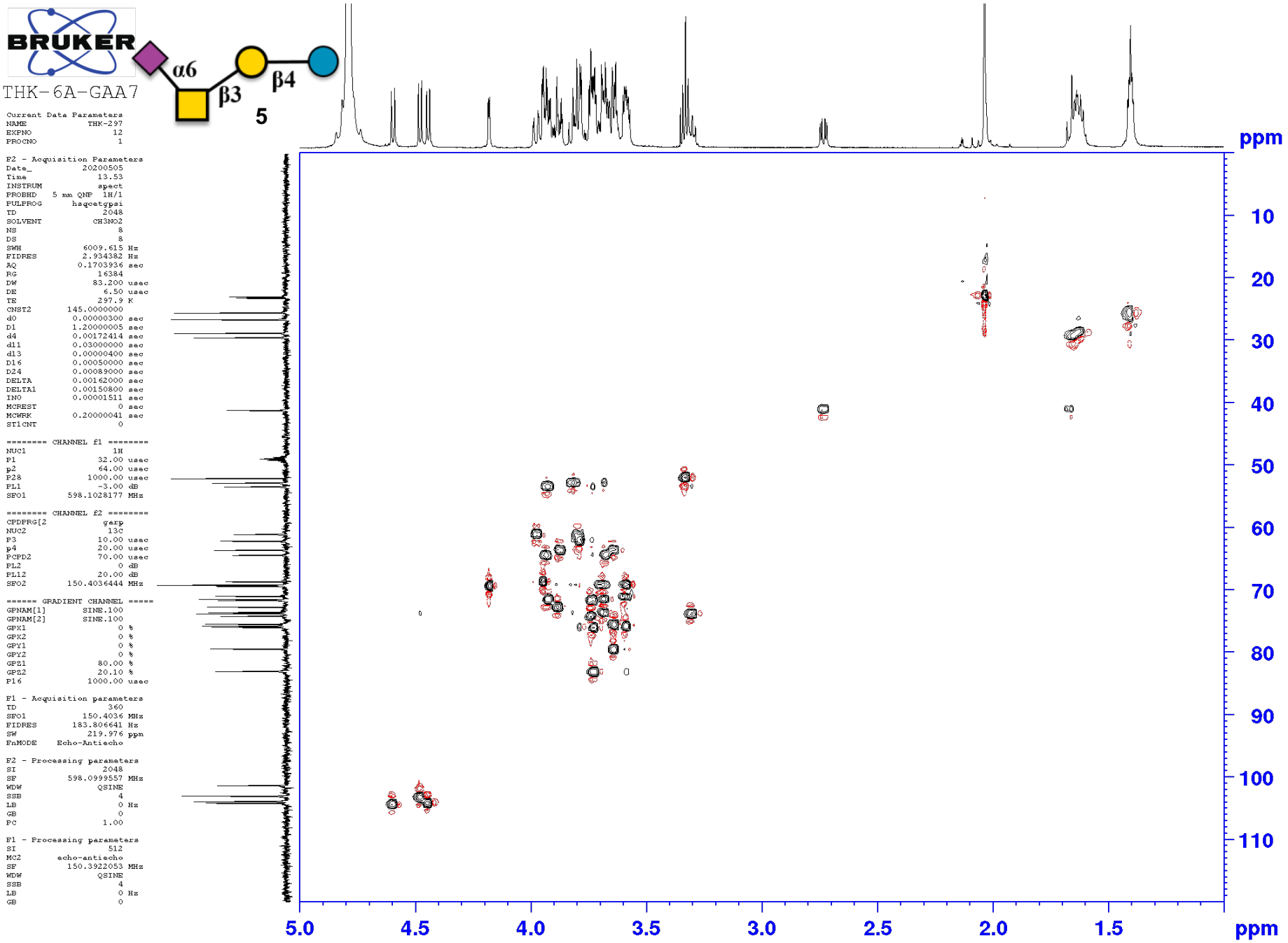
5



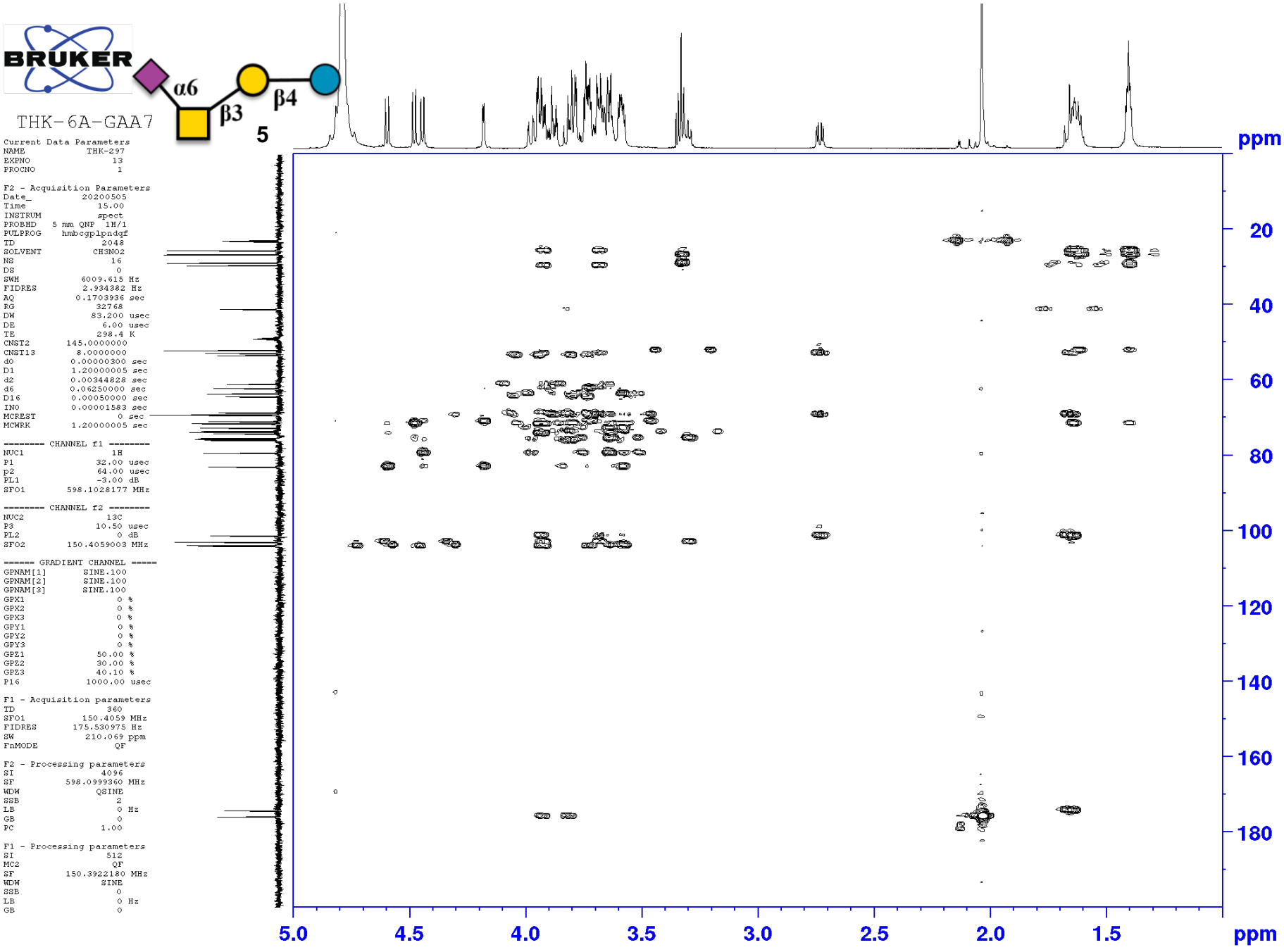
^{13}C NMR spectrum of Compound **5** (150 MHz D_2O)



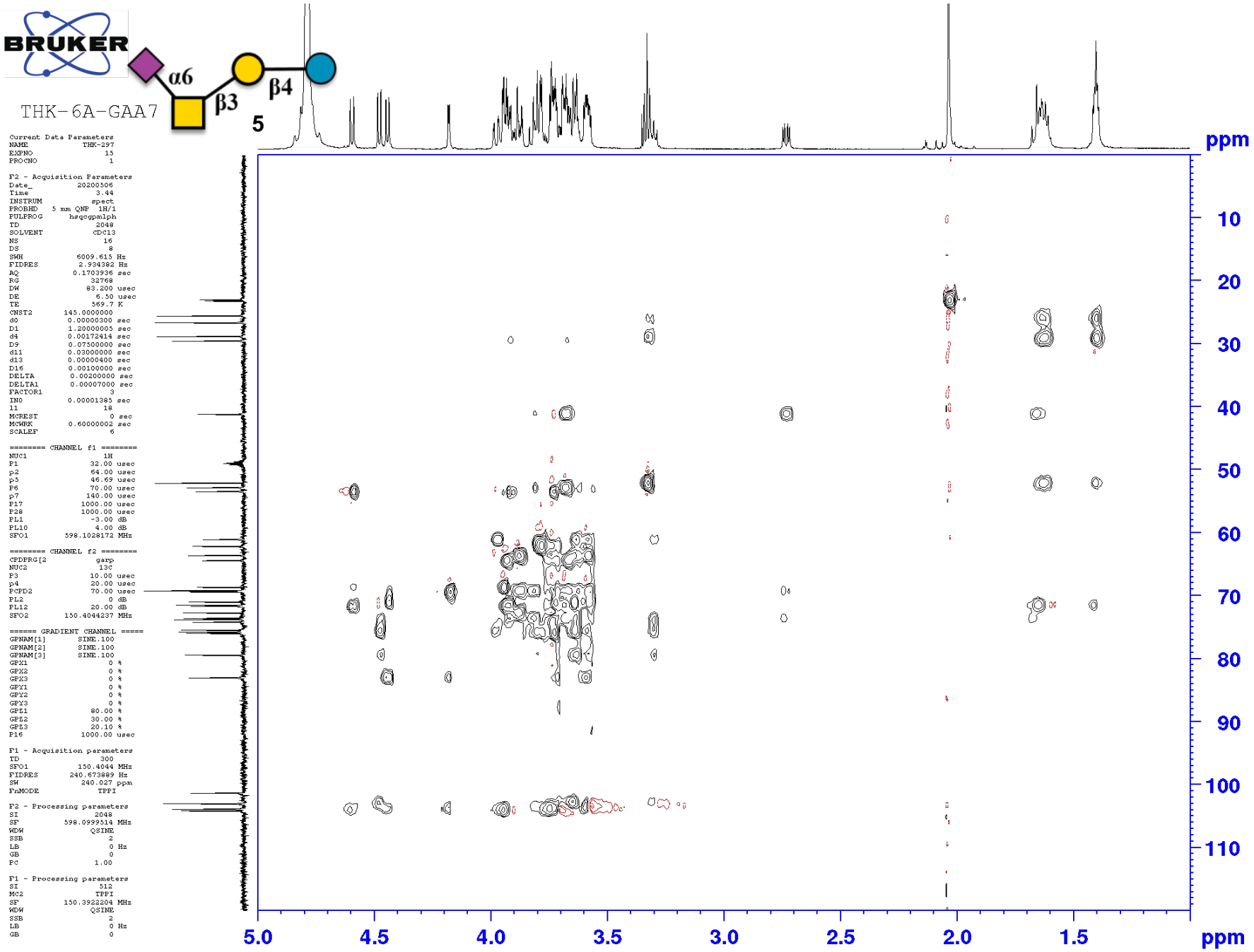
^{1}H-^{1}H COSY NMR spectrum of Compound **5** (600 MHz D_2O)



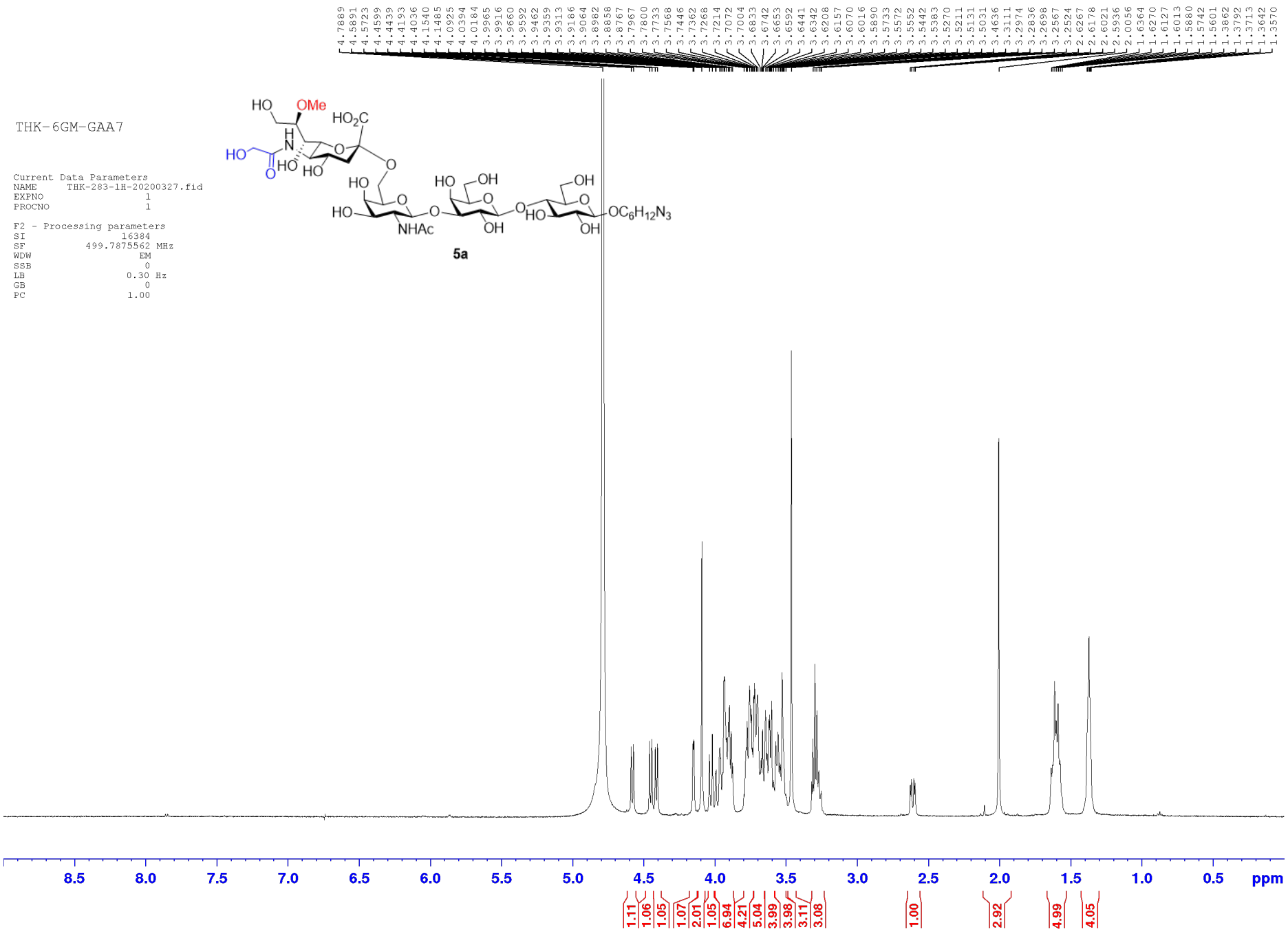
^{1}H-^{13}C HSQC NMR spectrum of Compound **5** (600 MHz/150 MHz D_2O)



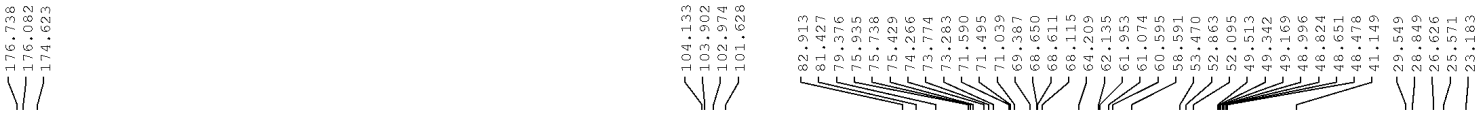
^{1}H-^{13}C HMBC NMR spectrum of Compound **5** (600 MHz/150 MHz D_2O)

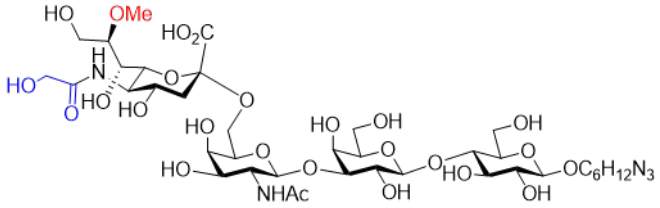


^{1}H-^{13}C HSQC-TOCSY NMR spectrum of Compound **5** (600 MHz/150 MHz D_2O)



^{1}H NMR spectrum of Compound **5a** (500 MHz D_2O)





5a

THK-6GM-GAA7

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

F2 - Processing parameters
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SSB                   0
LB                 0.30 Hz
GB                    0
PC                 1.00
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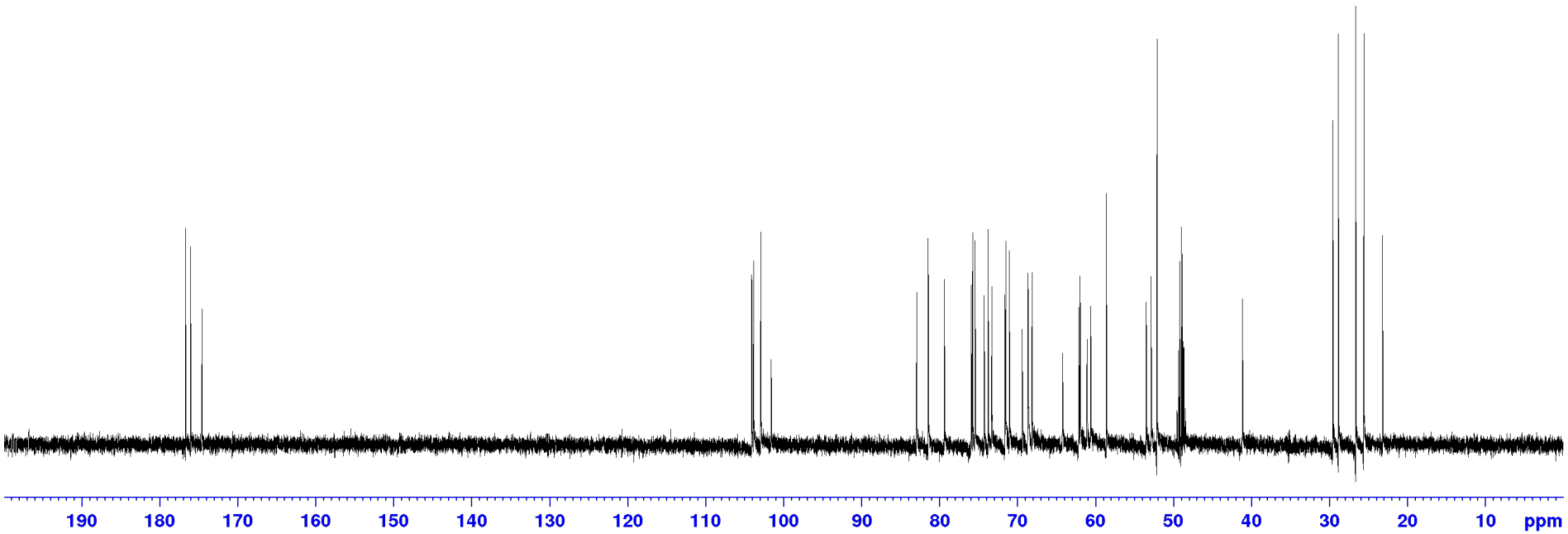
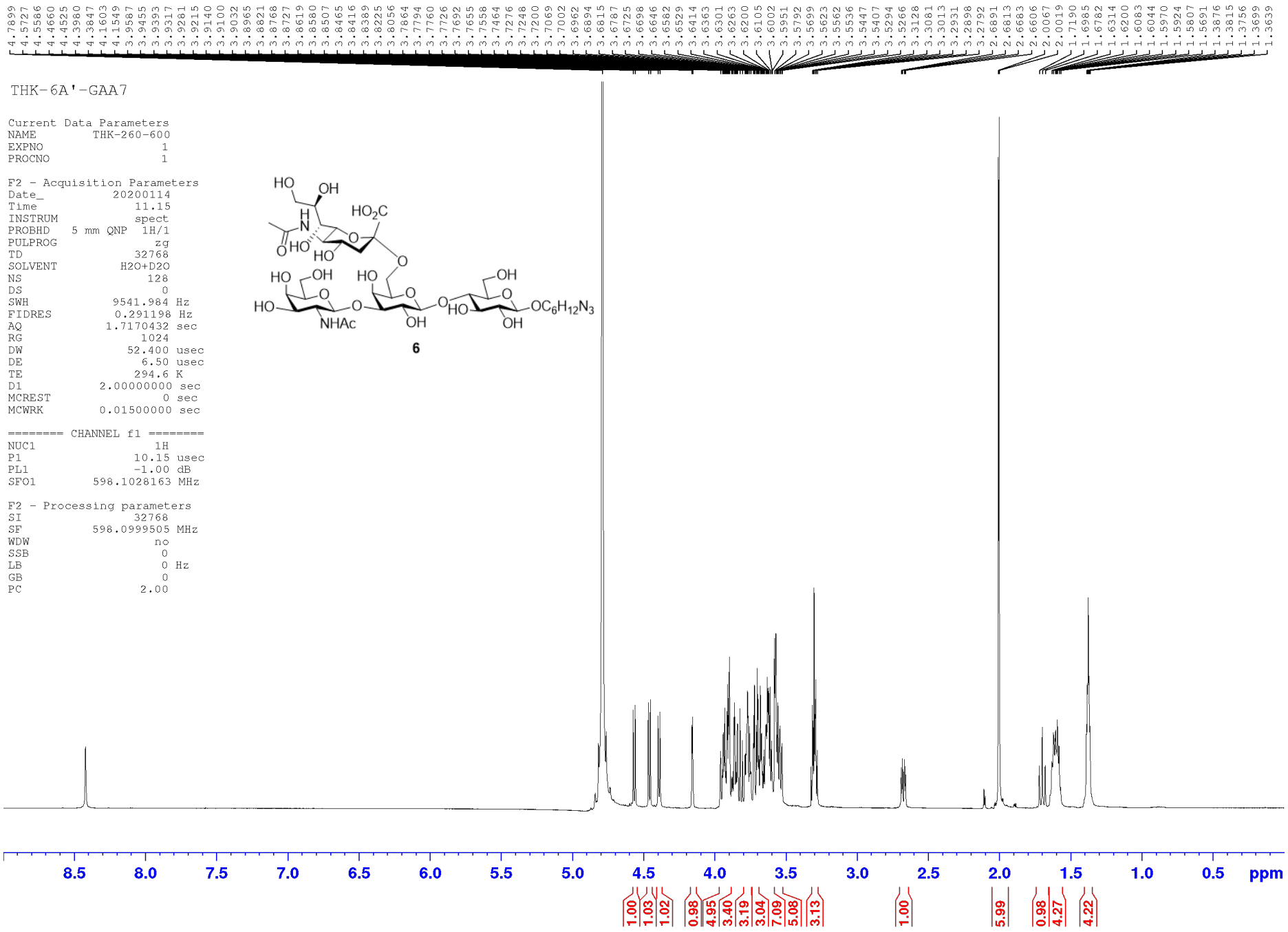
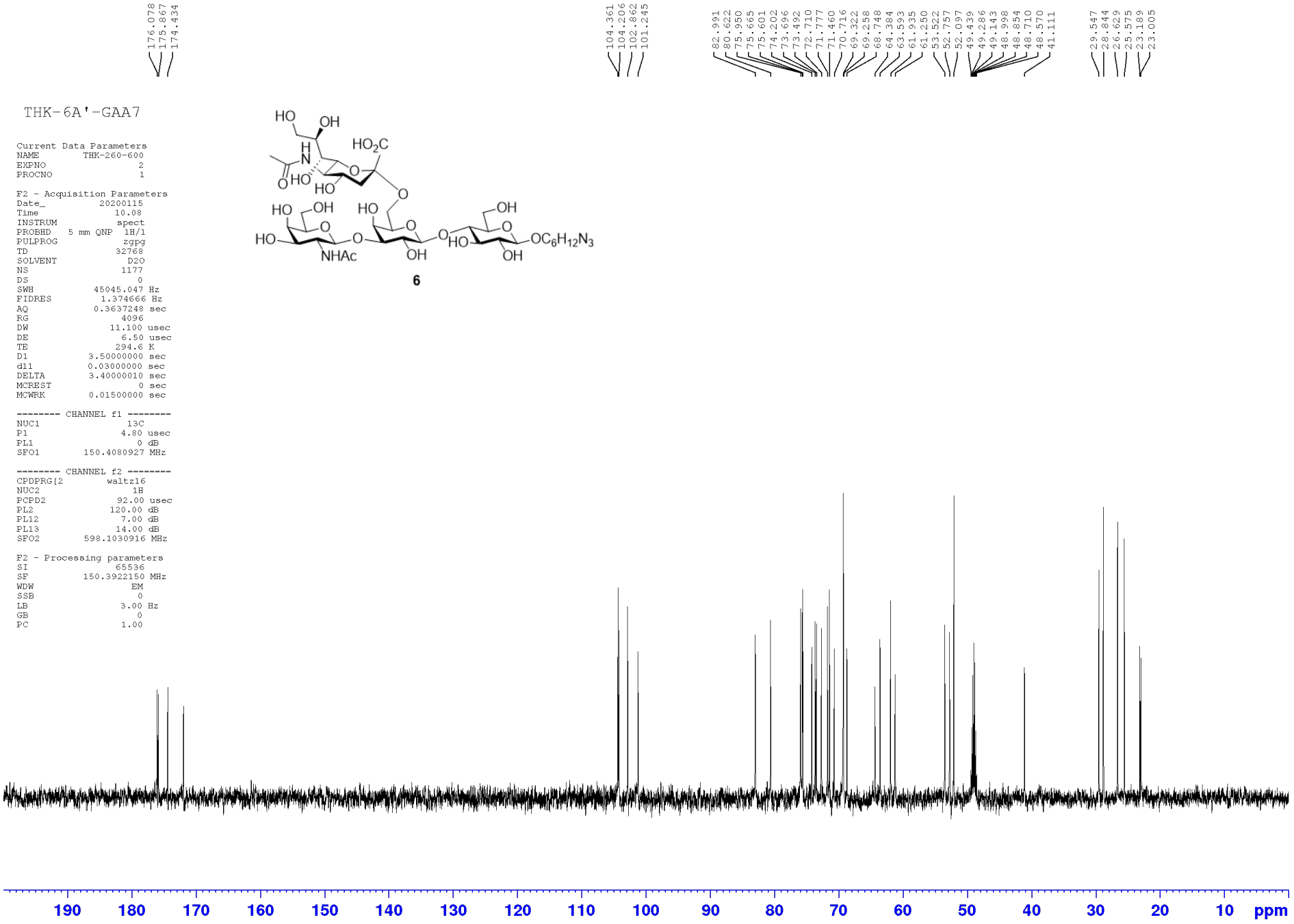


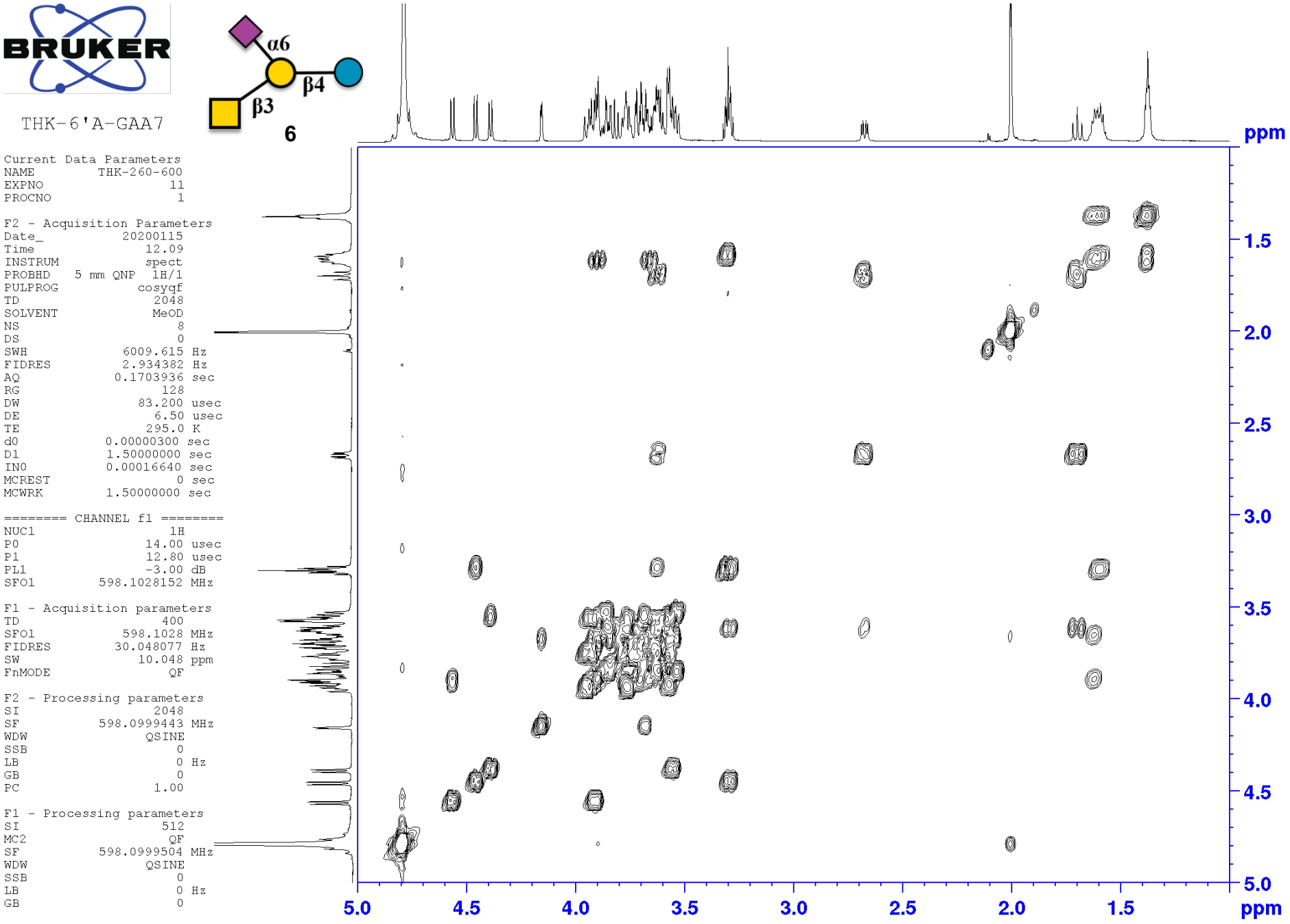
^{1}H NMR spectrum of Compound **5a** (125 MHz D_2O)



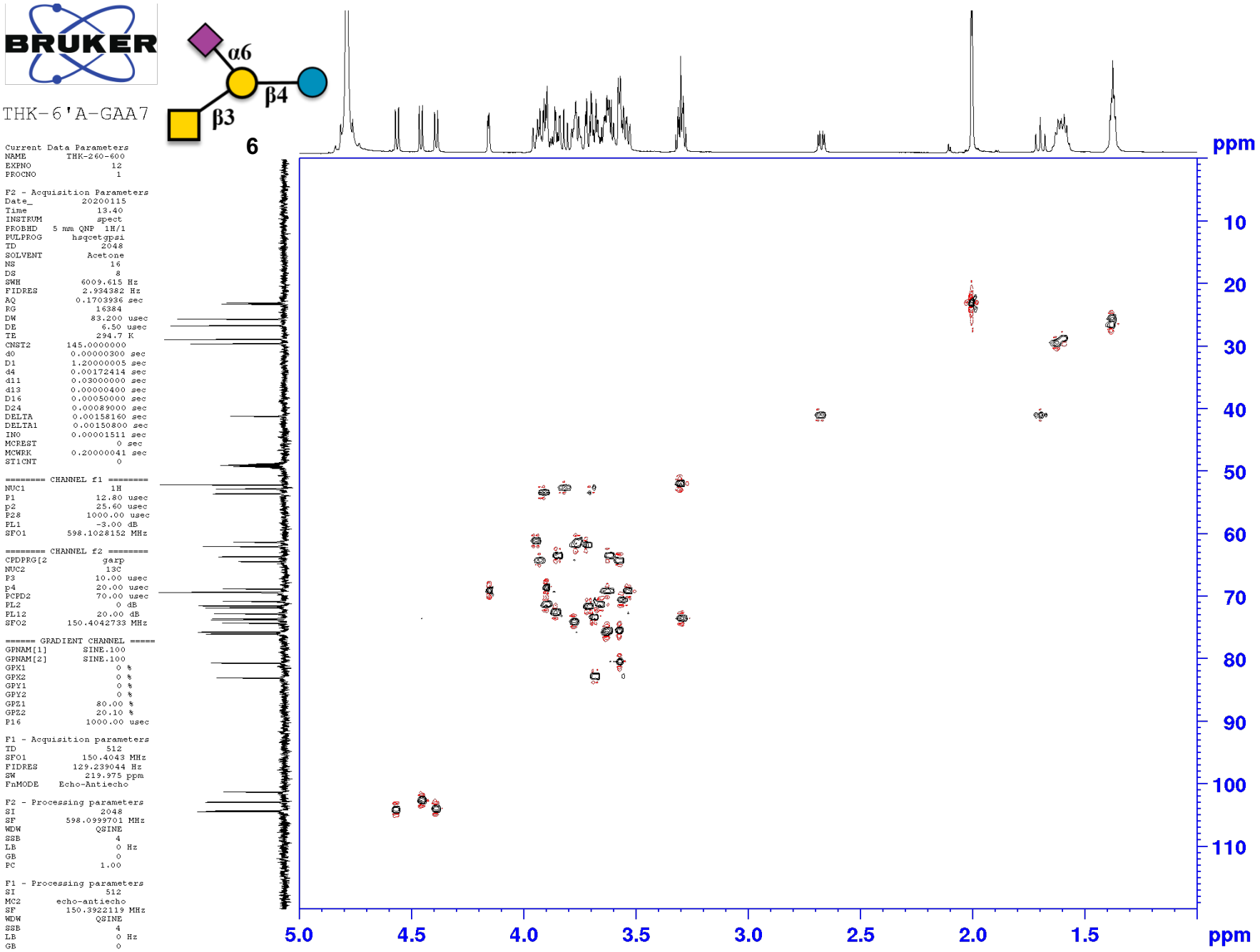
^{1}H NMR spectrum of Compound **6** (600 MHz D_2O)



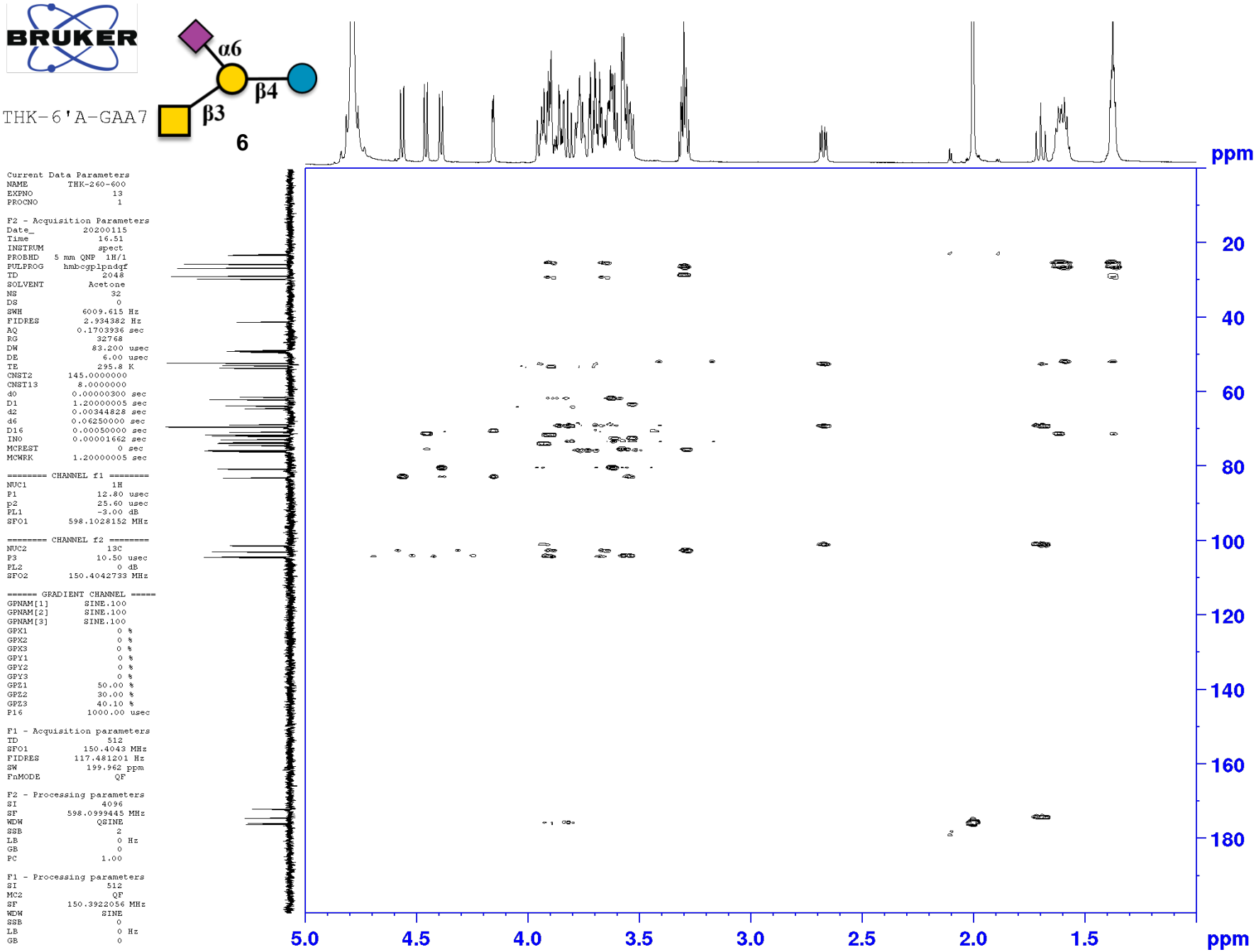
^{13}C NMR spectrum of Compound **6** (150 MHz D_2O)



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



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

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Current Data Parameters
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EXPNO                 13
PROCNO                 1

F2 - Acquisition Parameters
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INSTRUM             spect
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PULPROG     hmbcgplpndqf
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
CNST2        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
IN0            0.00001662 sec
MCREST                 0 sec
MCWRK          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 =====
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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
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SFO1           150.4043 MHz
FIDRES       117.481201 Hz
SW             199.962 ppm
FnMODE               QF

F2 - Processing parameters
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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
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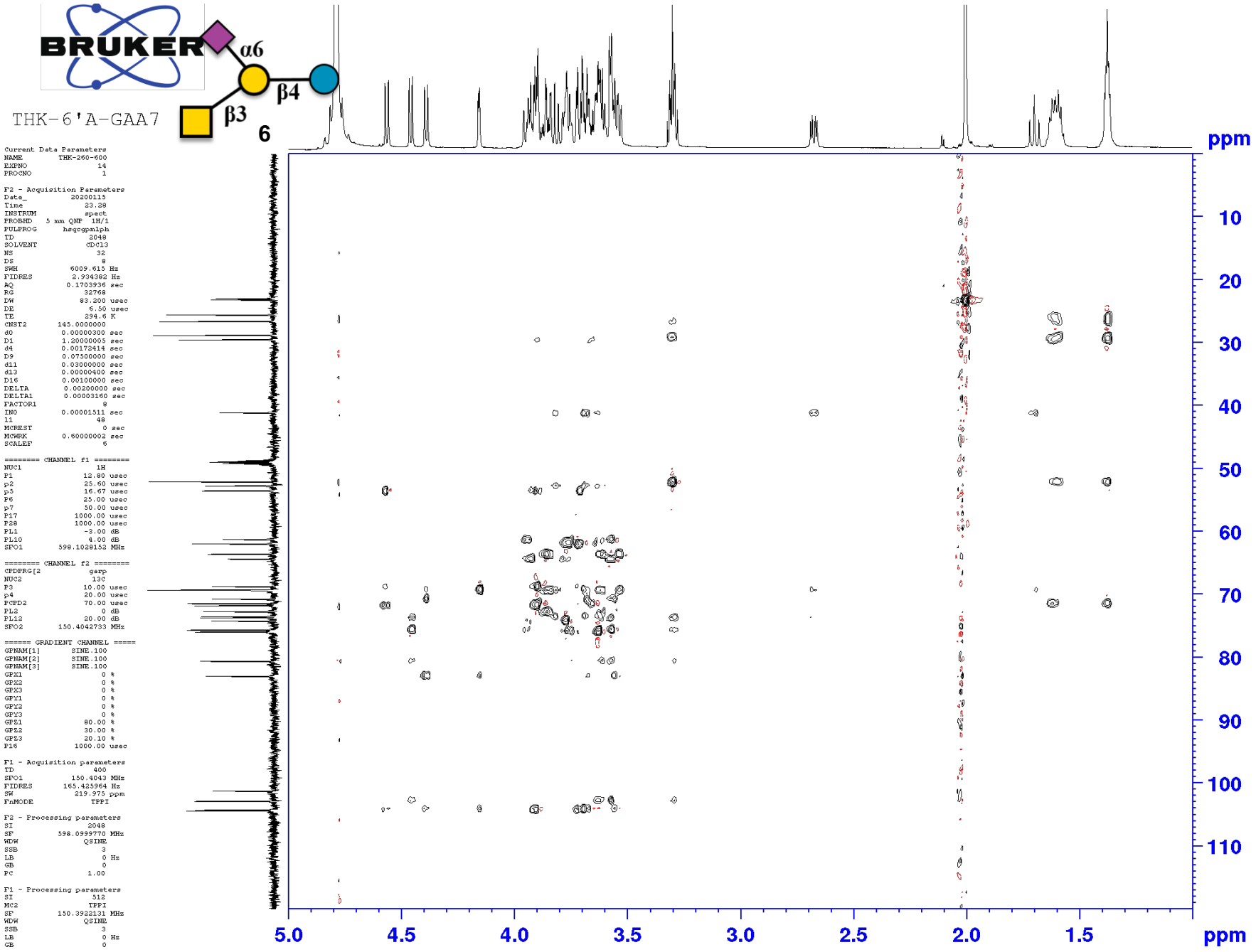
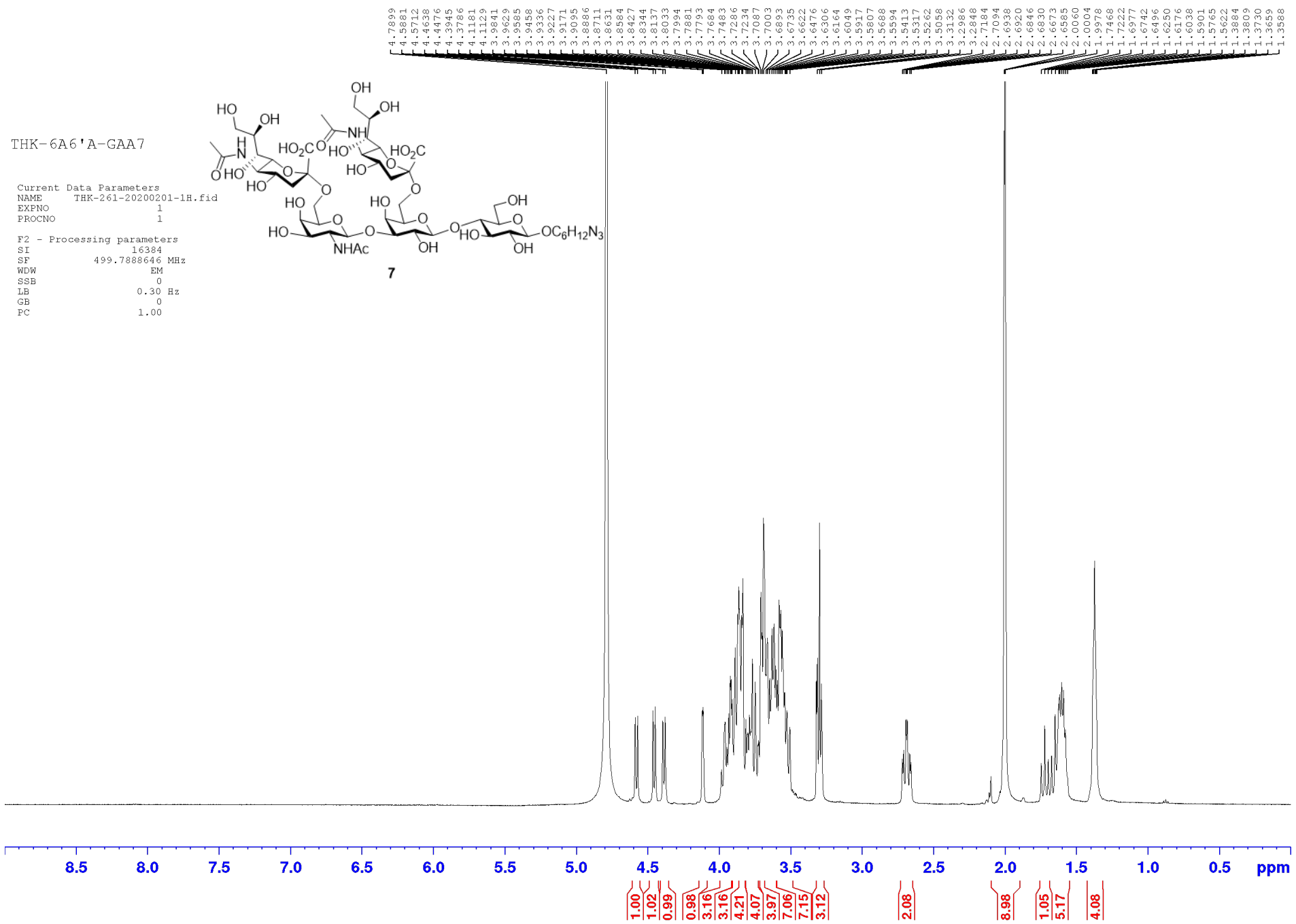
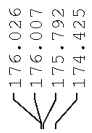
^{1}H-^{13}C HMBC NMR spectrum of Compound **6** (600 MHz/150 MHz D_2O)

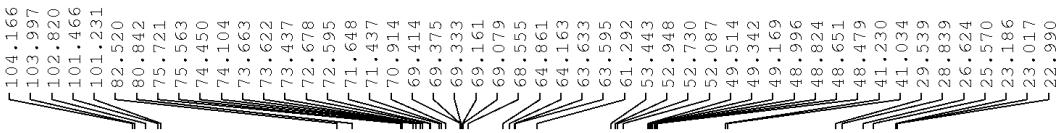


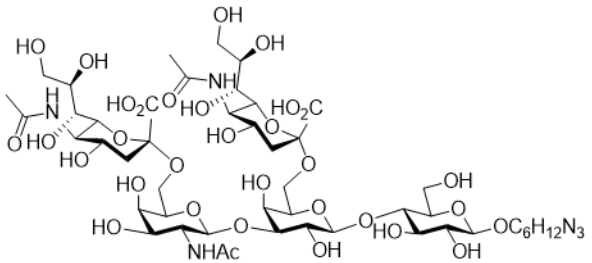
^{1}H-^{13}C HSQC-TOCSY NMR spectrum of Compound **6** (600 MHz/150 MHz D_2O)



^{1}H NMR spectrum of Compound **7** (500 MHz D_2O)







7

THK-6A6A'-GAA7

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PROCNO               1

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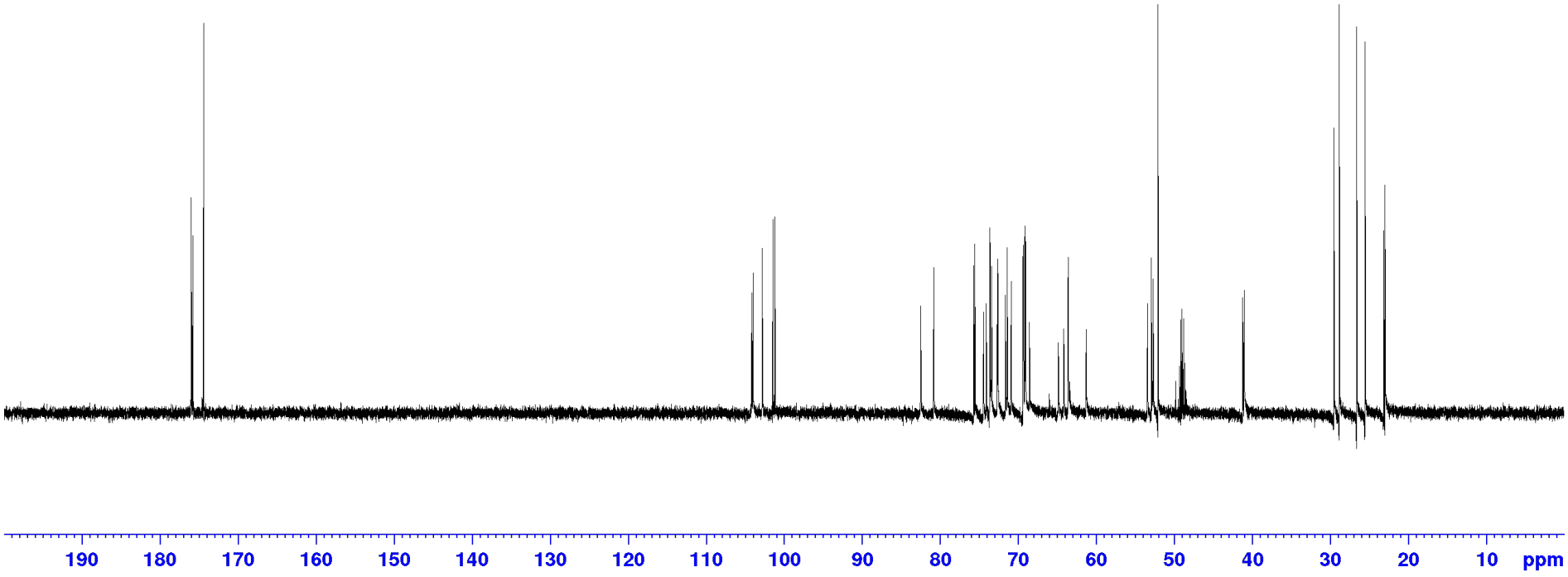
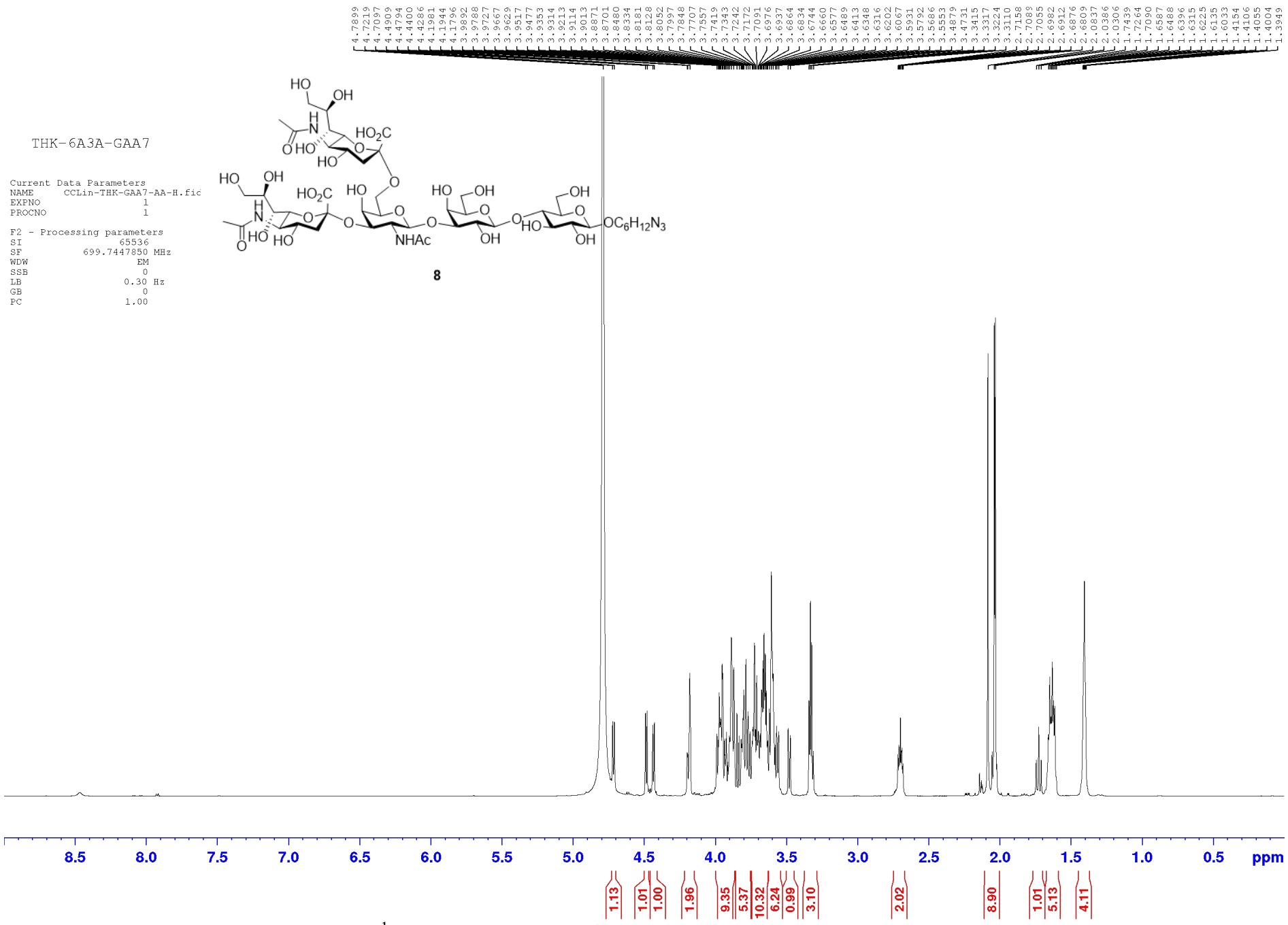
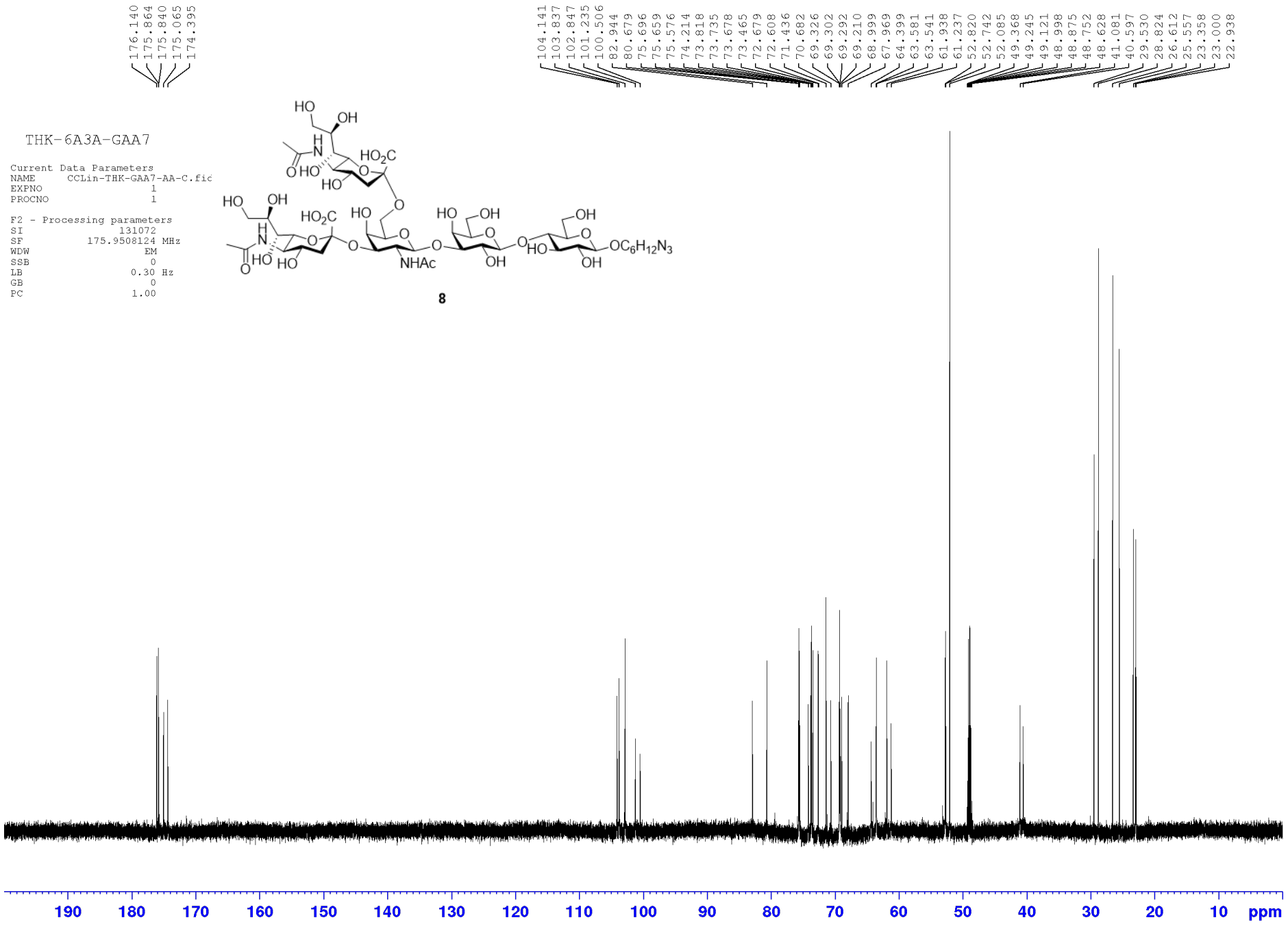


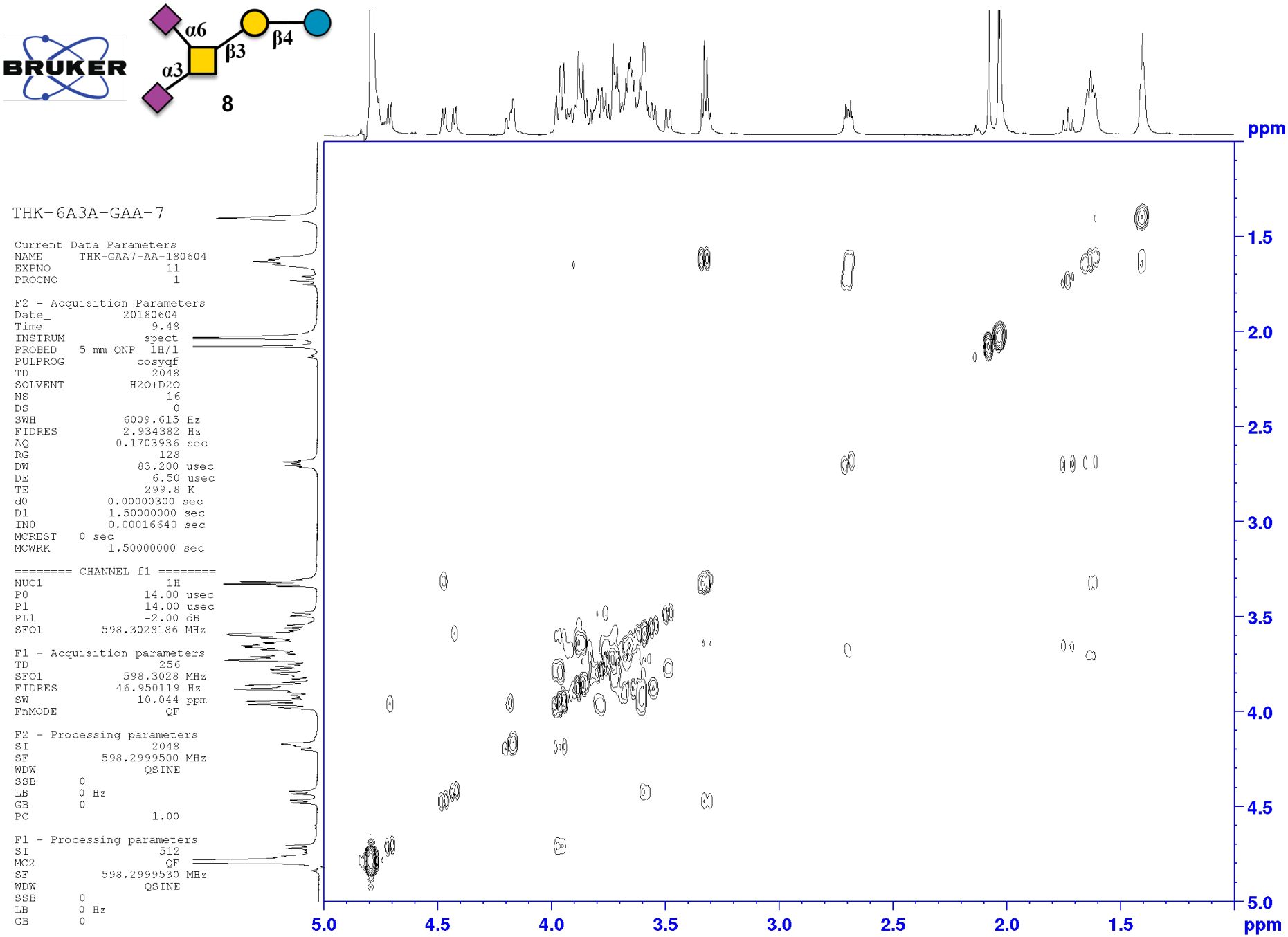
^{1}H NMR spectrum of Compound **7** (500 MHz D_2O)



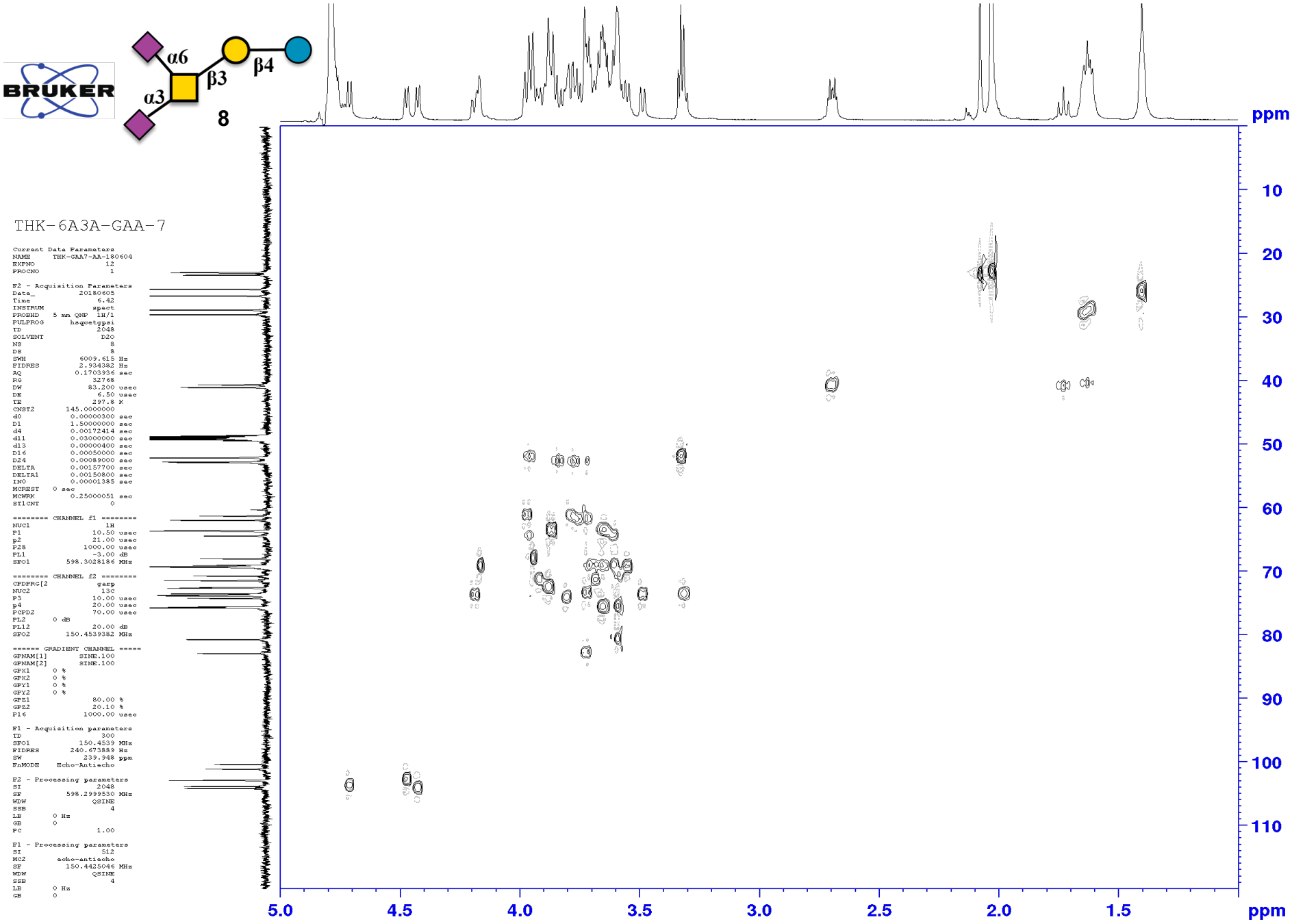
^{1}H NMR spectrum of Compound **8** (700 MHz D_2O)



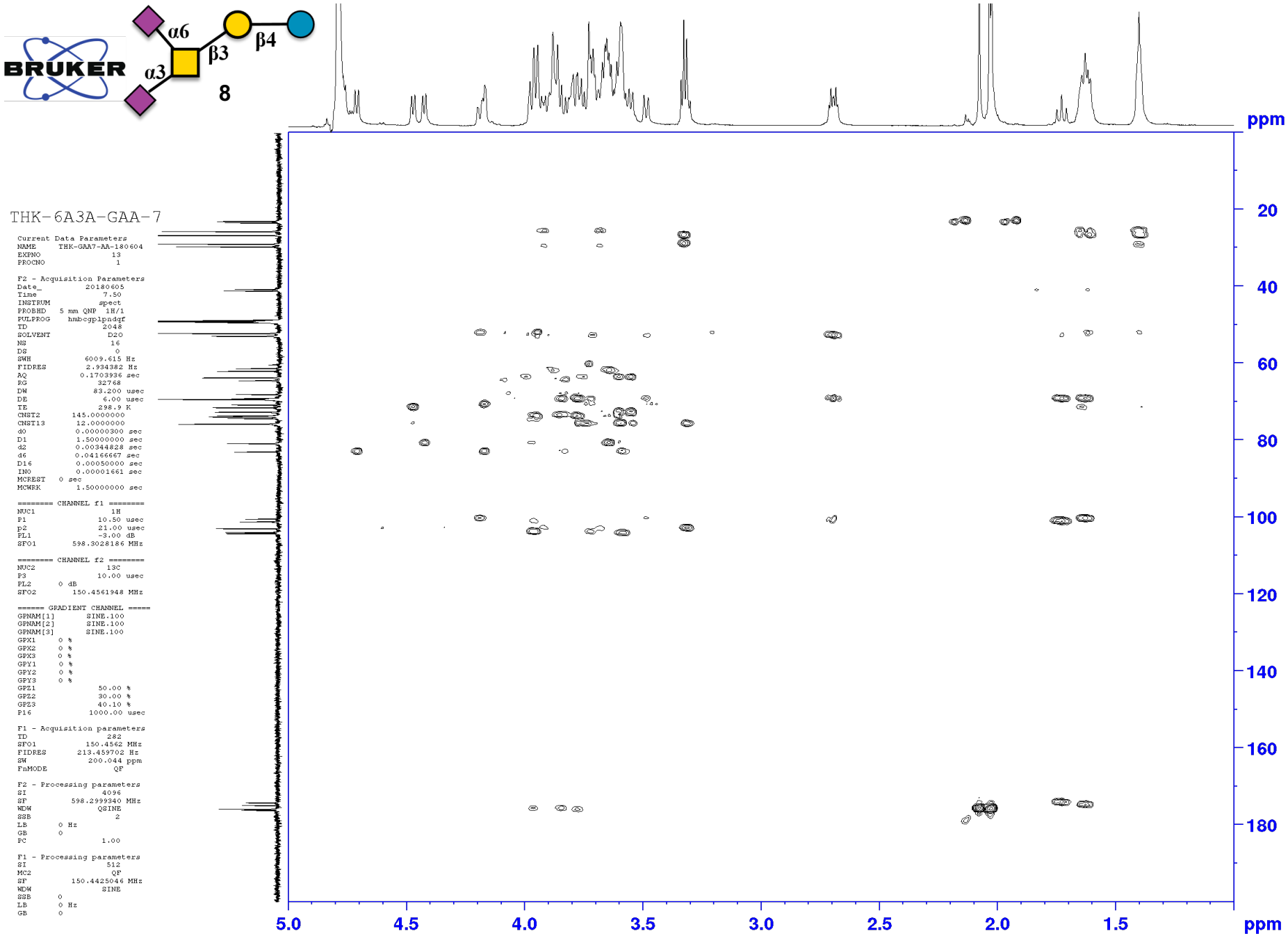
^{13}C NMR spectrum of Compound **8** (175 MHz D_2O)



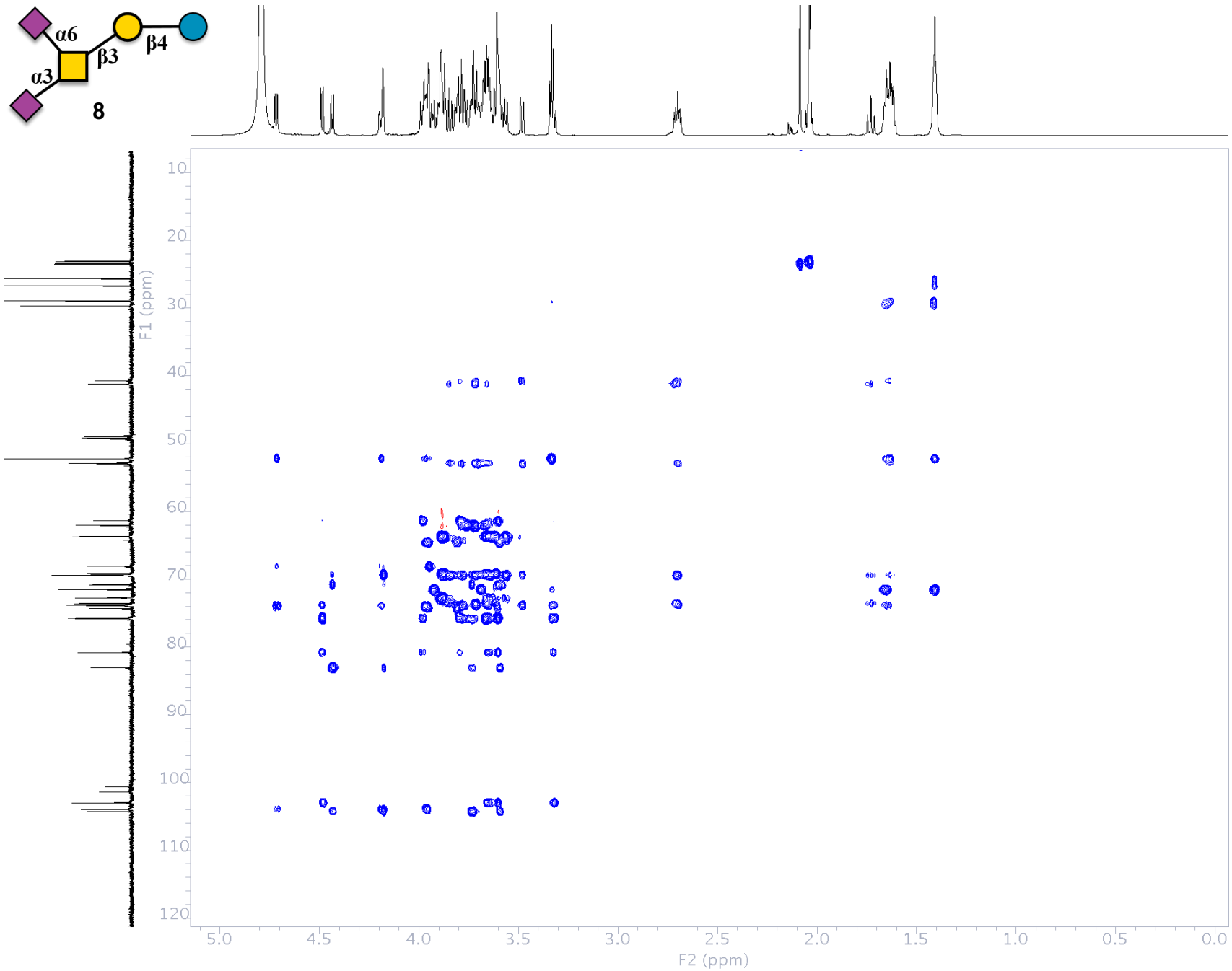
^{1}H-^{1}H COSY NMR spectrum of Compound **8** (600 MHz D_2O)



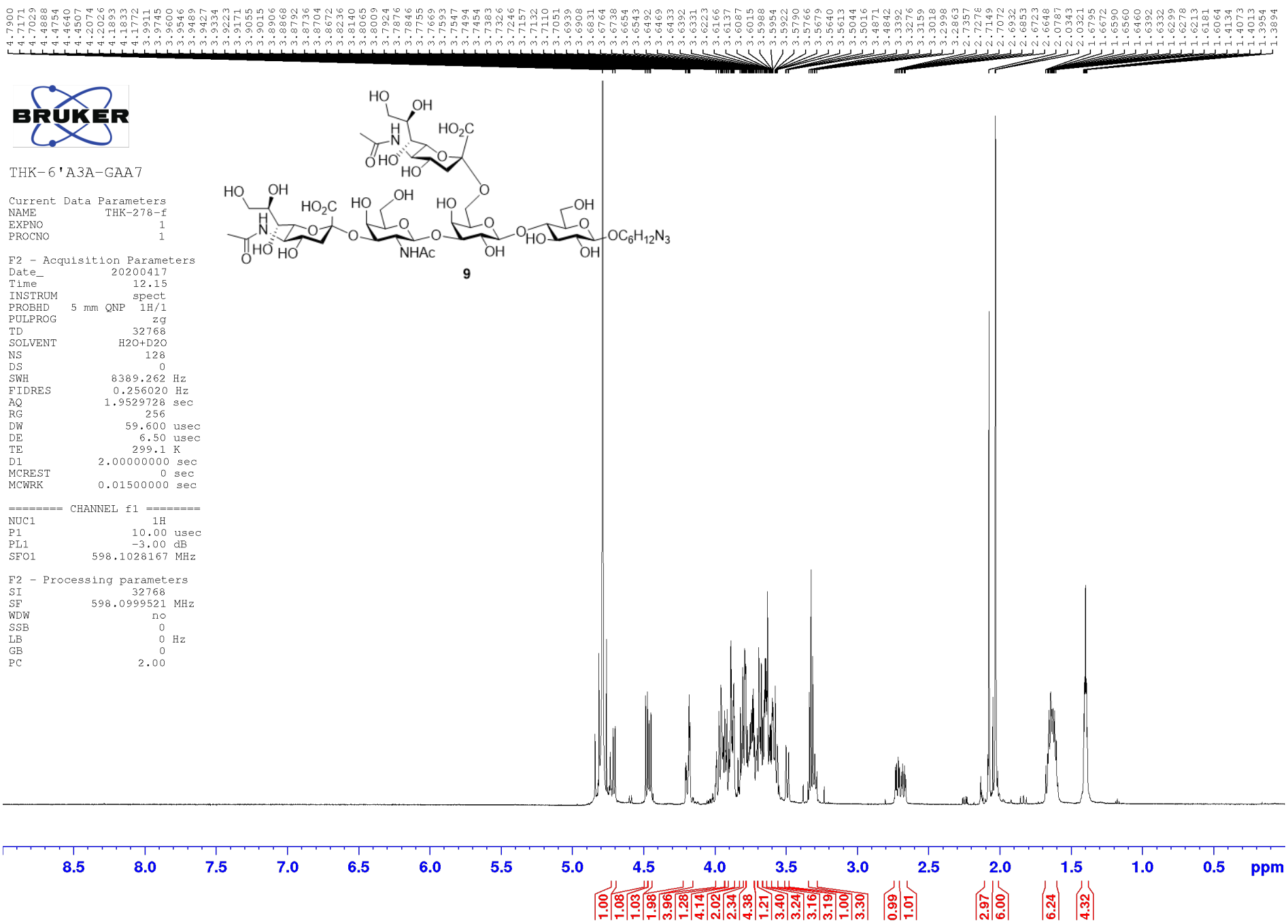
^{1}H-^{13}C HSQC NMR spectrum of Compound **8** (600 MHz/150 MHz D_2O)



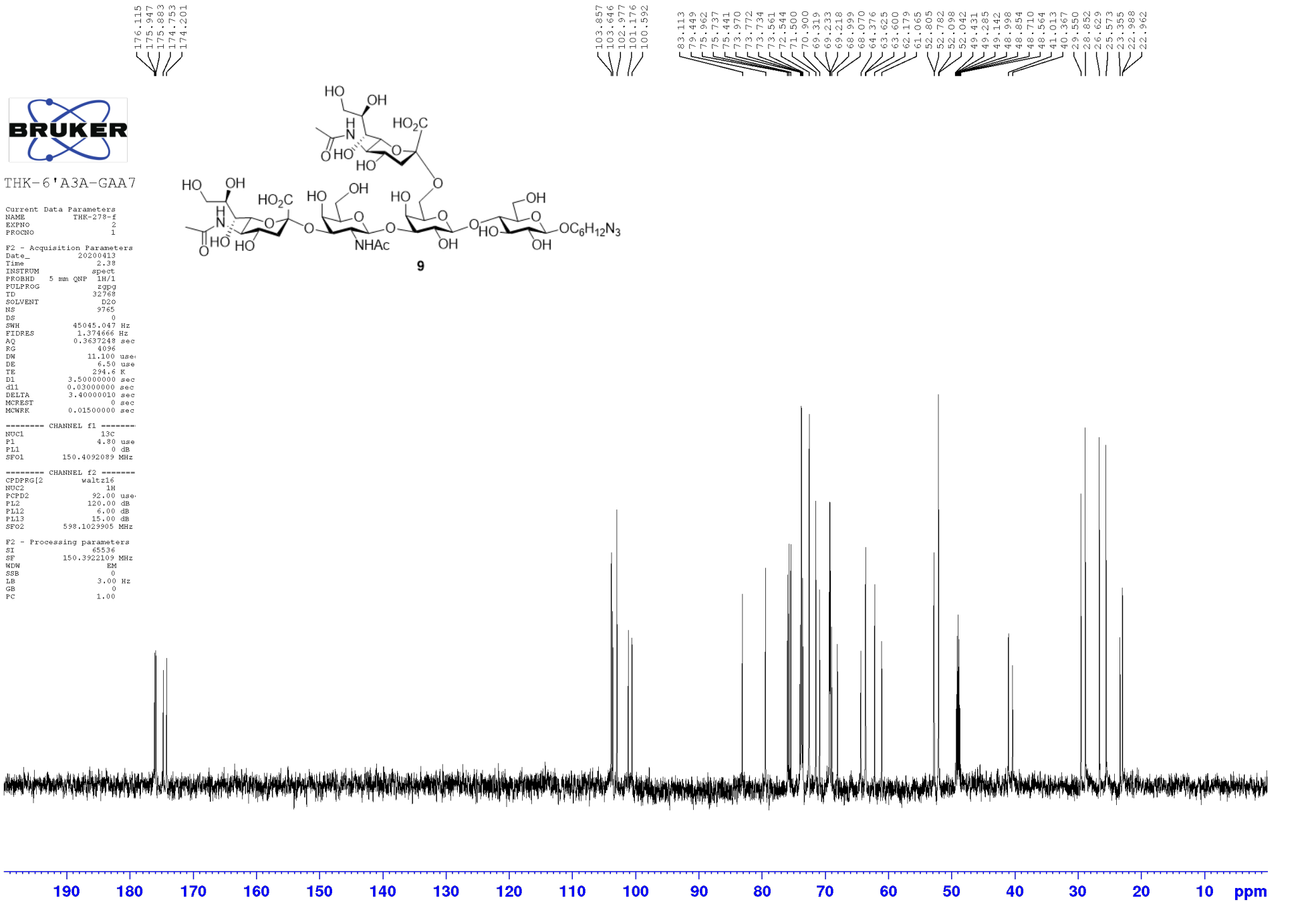
^{1}H-^{13}C HMBC NMR spectrum of Compound **8** (600 MHz/150 MHz D_2O)



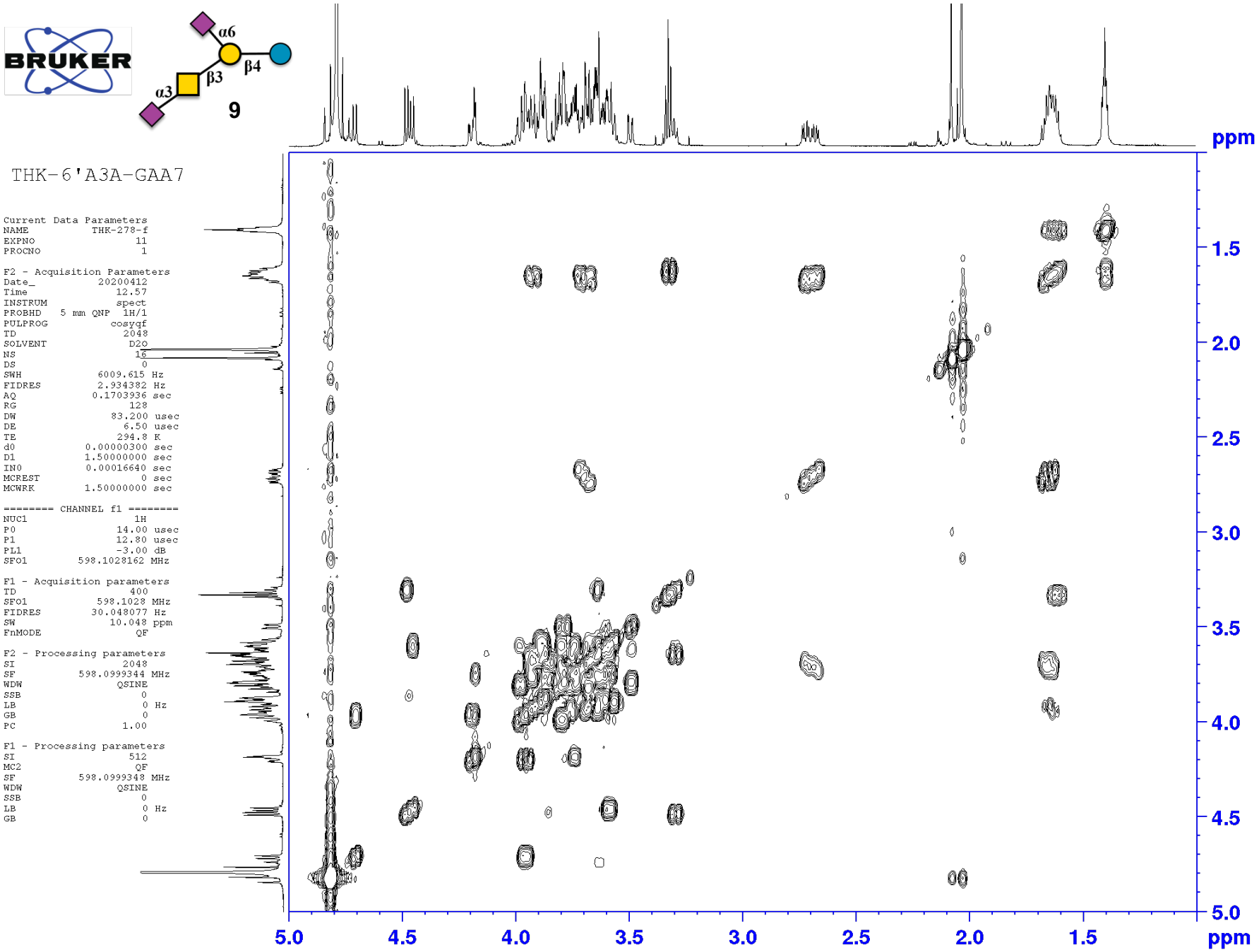
^{1}H-^{13}C HSQC-TOCSY NMR spectrum of Compound **8** (700 MHz/175 MHz D_2O)



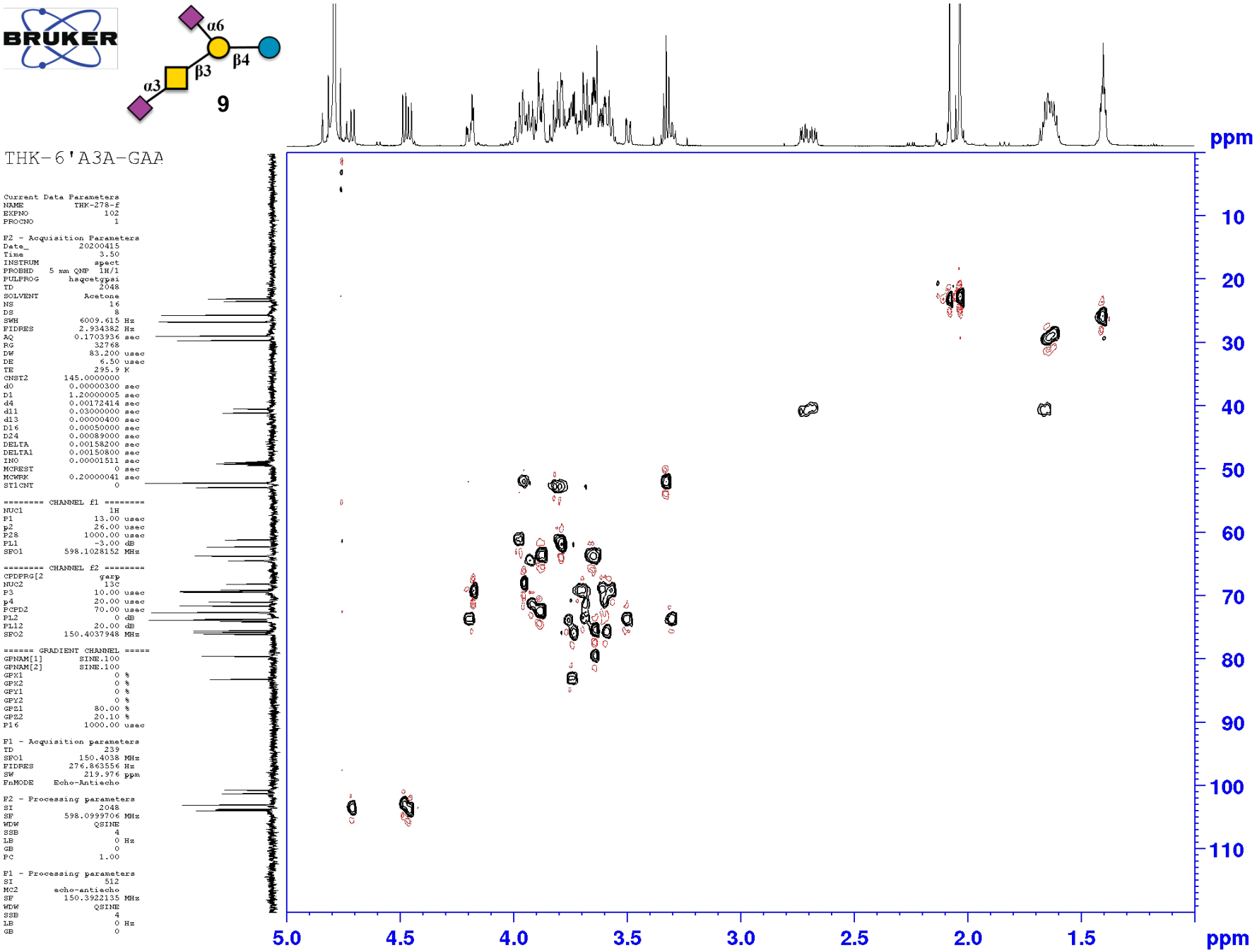
^{1}H NMR spectrum of Compound **9** (600 MHz D_2O)



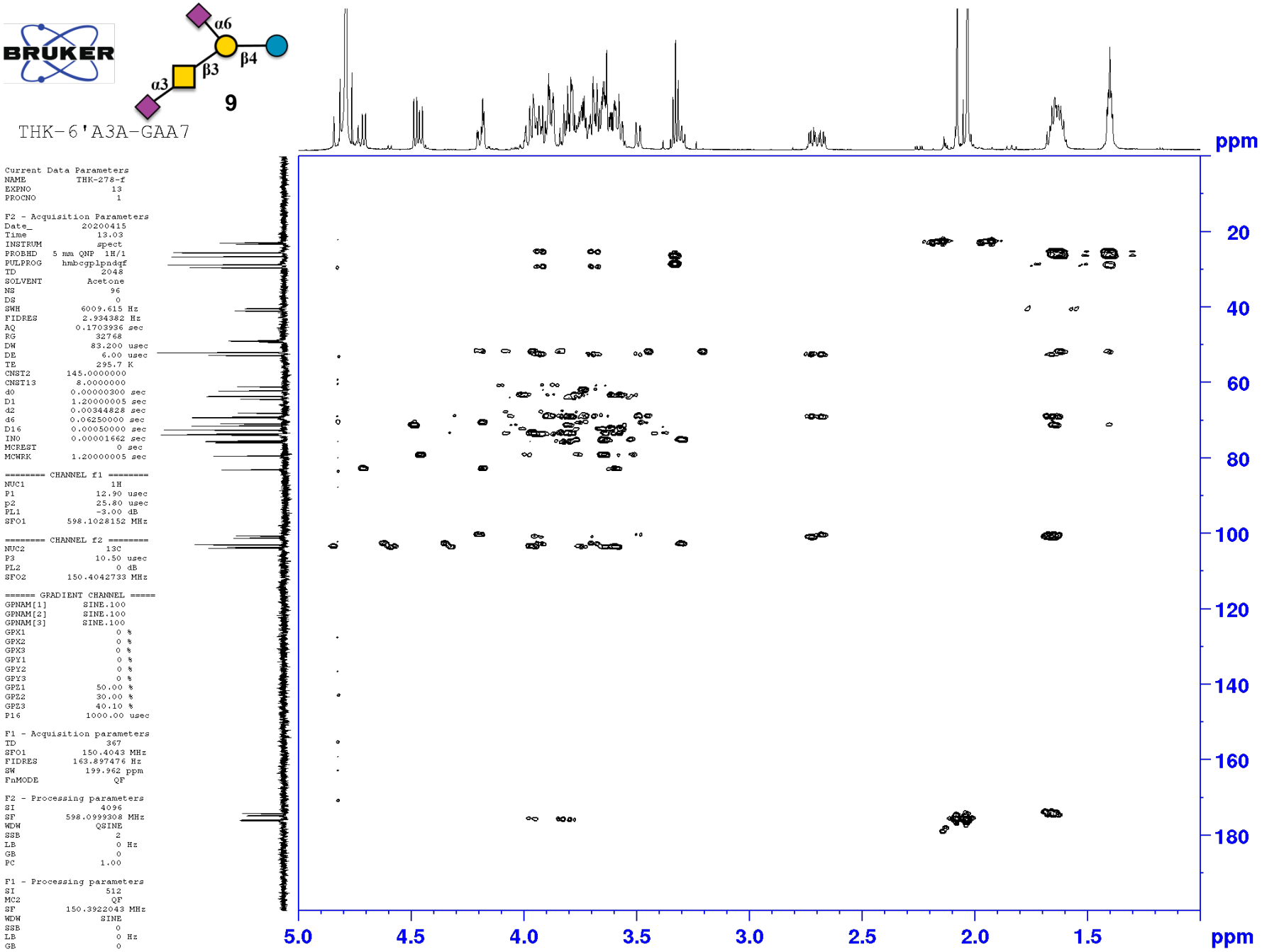
^{13}C NMR spectrum of Compound **9** (150 MHz D_2O)



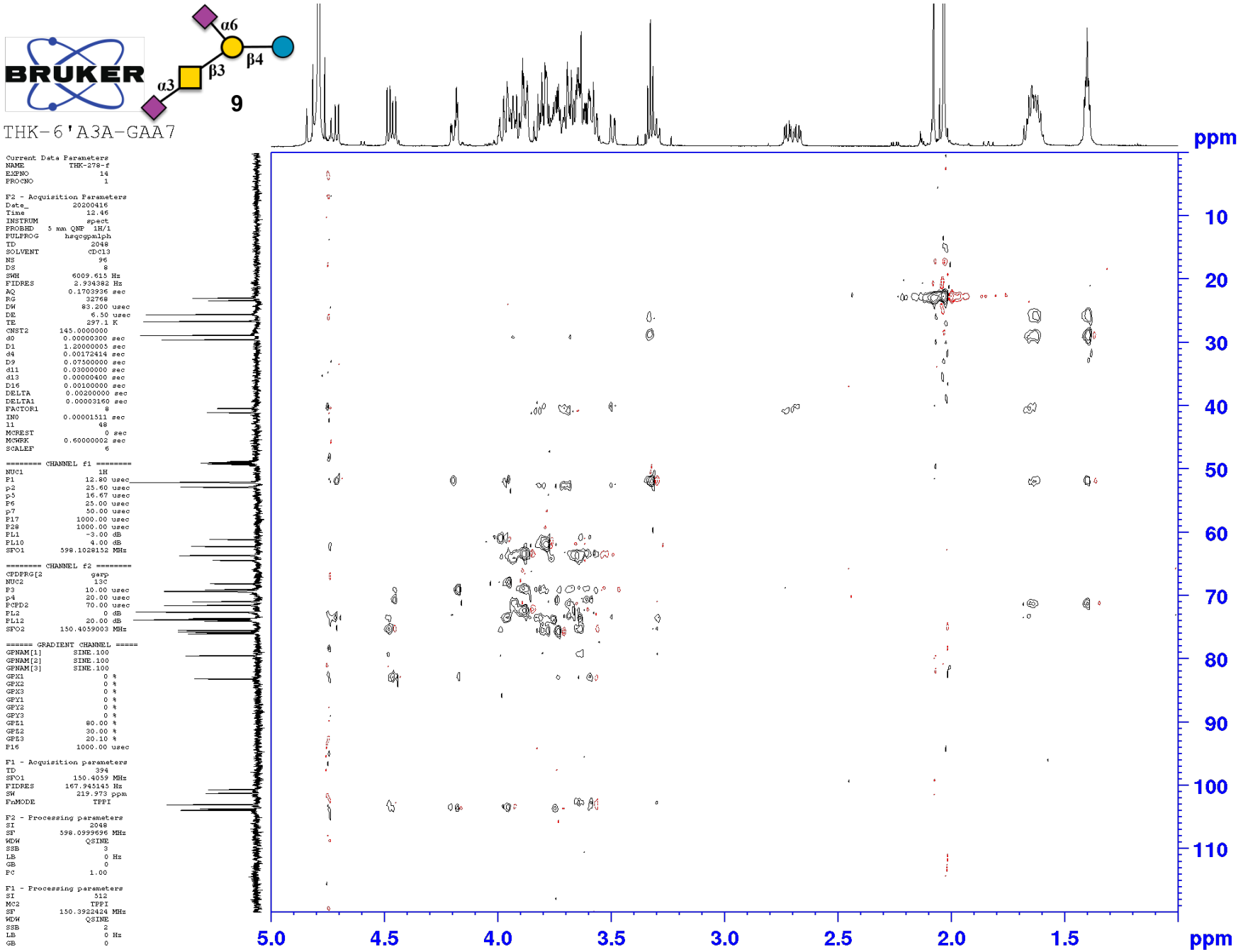
^{1}H-^{1}H COSY NMR spectrum of Compound **9** (600 MHz D_2O)



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



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



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PROCNO 1

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SWH 6009.615 Hz
FIDRES 2.934382 Hz
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RG 32768
DW 83.200 usec
DE 6.50 usec
TE 297.1 K
CNST2 145.0000000
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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
FACTOR1 8
IN0 0.00001511 sec
l1 48
MCREST 0 sec
MCWRK 0.60000002 sec
SCALEF 6

======== CHANNEL f1 ========
NUC1 1H
P1 12.80 usec
p2 25.60 usec
p5 16.67 usec
P6 25.00 usec
p7 50.00 usec
P17 1000.00 usec
P28 1000.00 usec
PL1 -3.00 dB
PL10 4.00 dB
SFO1 598.1028152 MHz

======== CHANNEL f2 ========
CPDPRG[2 garp
NUC2 13C
P3 10.00 usec
p4 20.00 usec
PCPD2 70.00 usec
PL2 0 dB
PL12 20.00 dB
SFO2 150.4059003 MHz

====== GRADIENT CHANNEL =====
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GPNAM[2] SINE.100
GPNAM[3] SINE.100
GPX1 0 %
GPX2 0 %
GPX3 0 %
GPY1 0 %
GPY2 0 %
GPY3 0 %
GPZ1 80.00 %
GPZ2 30.00 %
GPZ3 20.10 %
P16 1000.00 usec

F1 - Acquisition parameters
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FIDRES 167.945145 Hz
SW 219.973 ppm
FnMODE TPPI

F2 - Processing parameters
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WDW QSINE
SSB 3
LB 0 Hz
GB 0
PC 1.00

F1 - Processing parameters
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SF 150.3922424 MHz
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SSB 2
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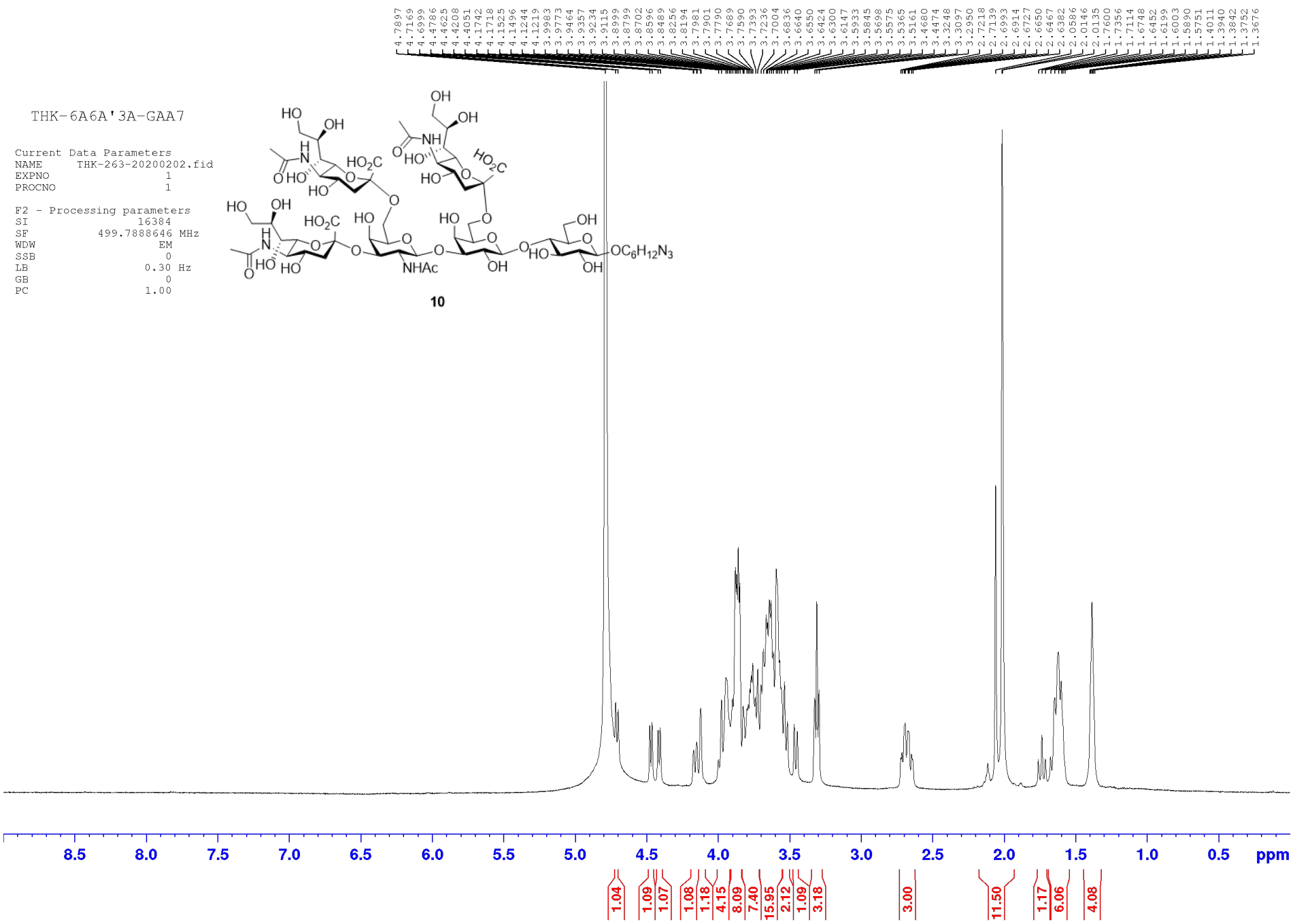
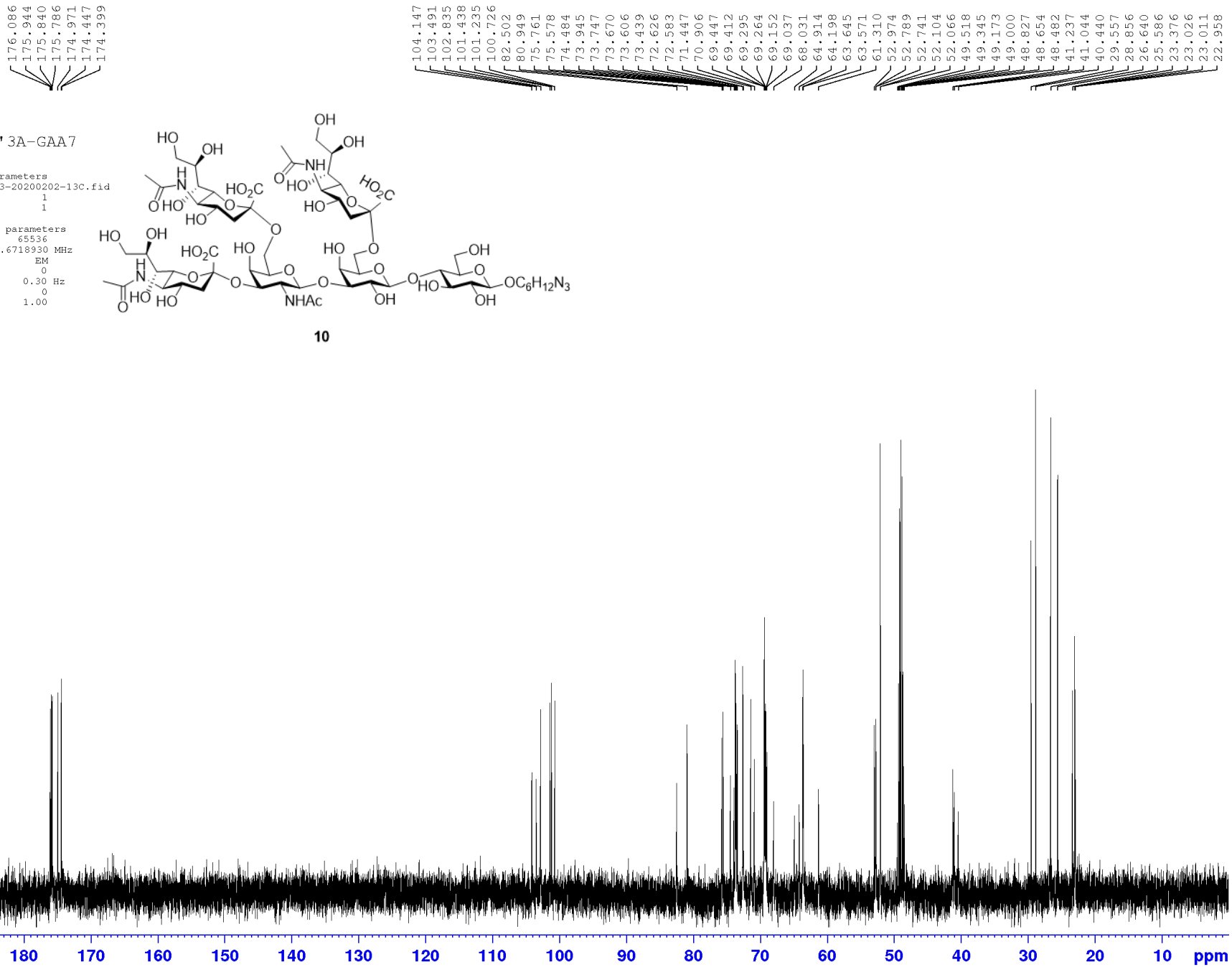
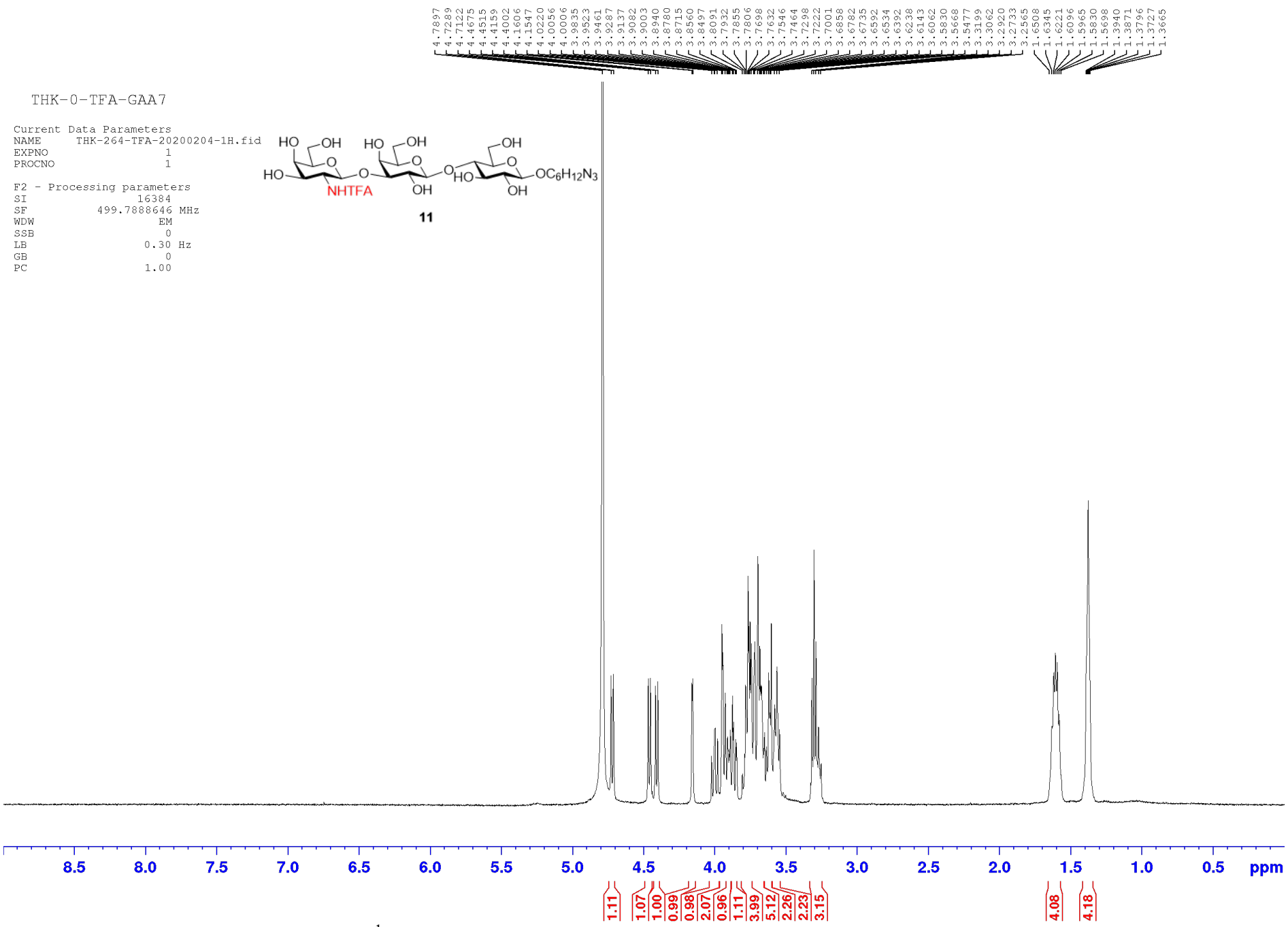
^{1}H-^{13}C HSQC-TOCSY NMR spectrum of Compound **9** (600 MHz/150 MHz D_2O)



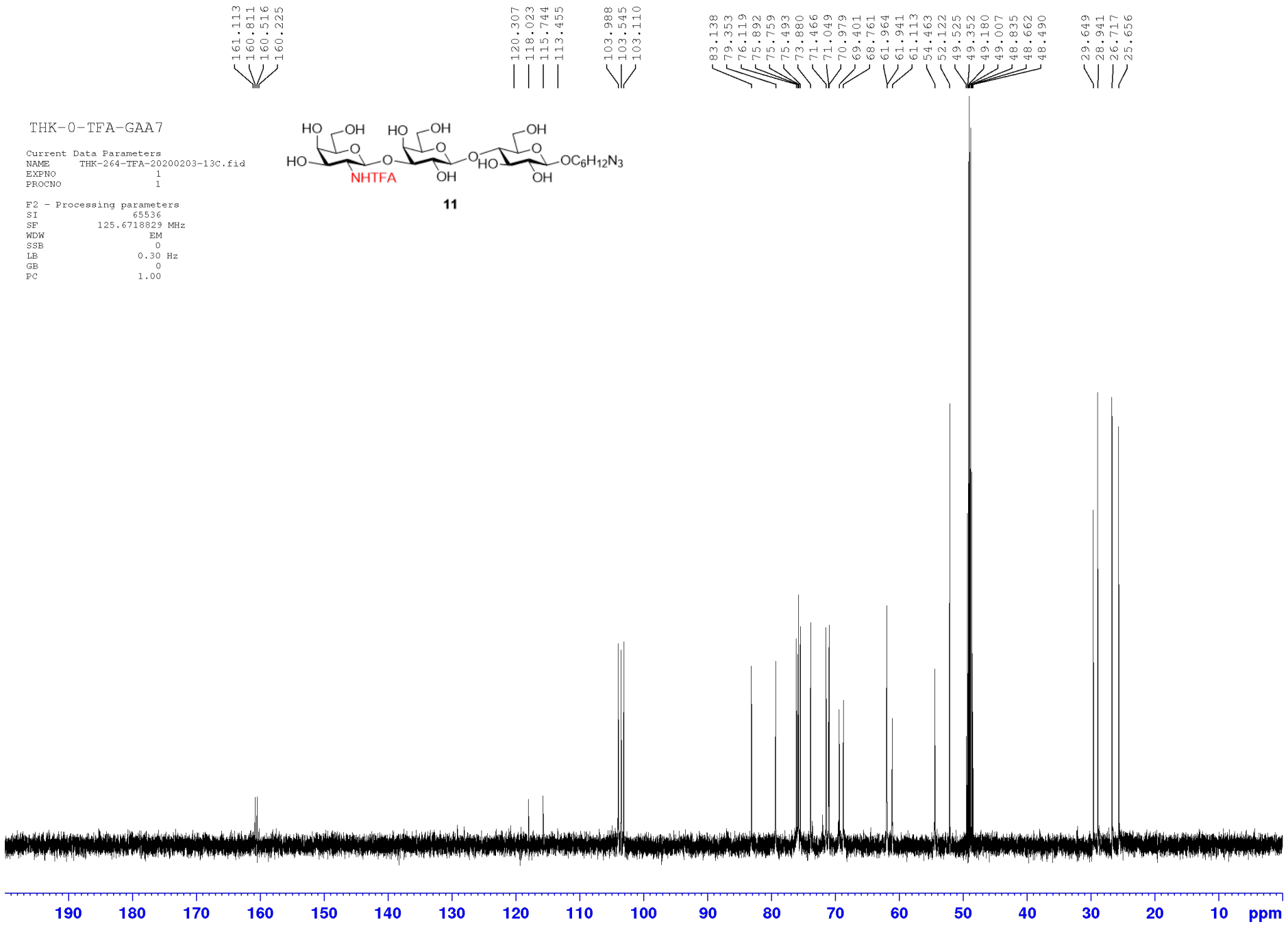
^{1}H NMR spectrum of Compound **10** (500 MHz D_2O)



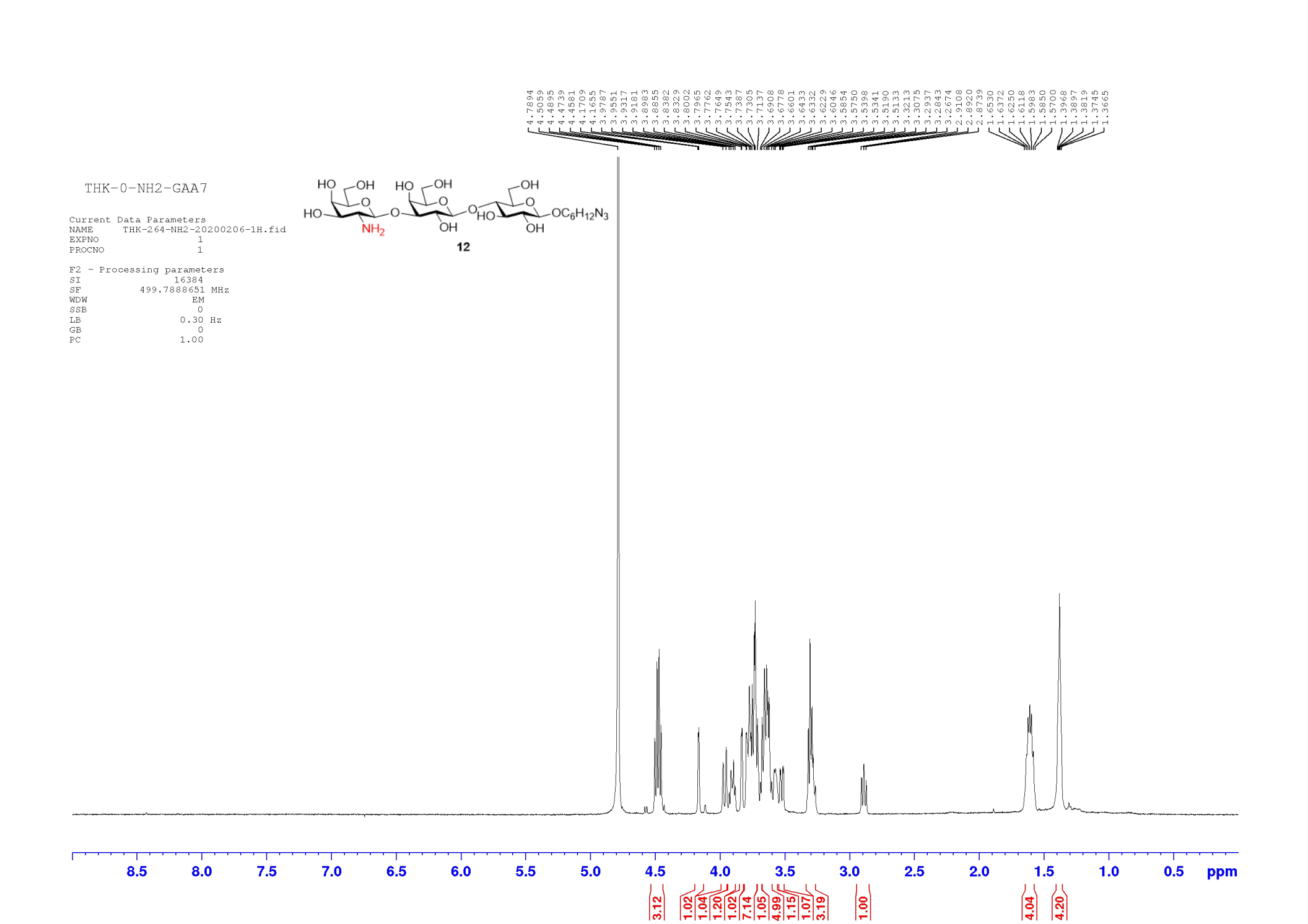
^{13}C NMR spectrum of Compound **10** (125 MHz D_2O)



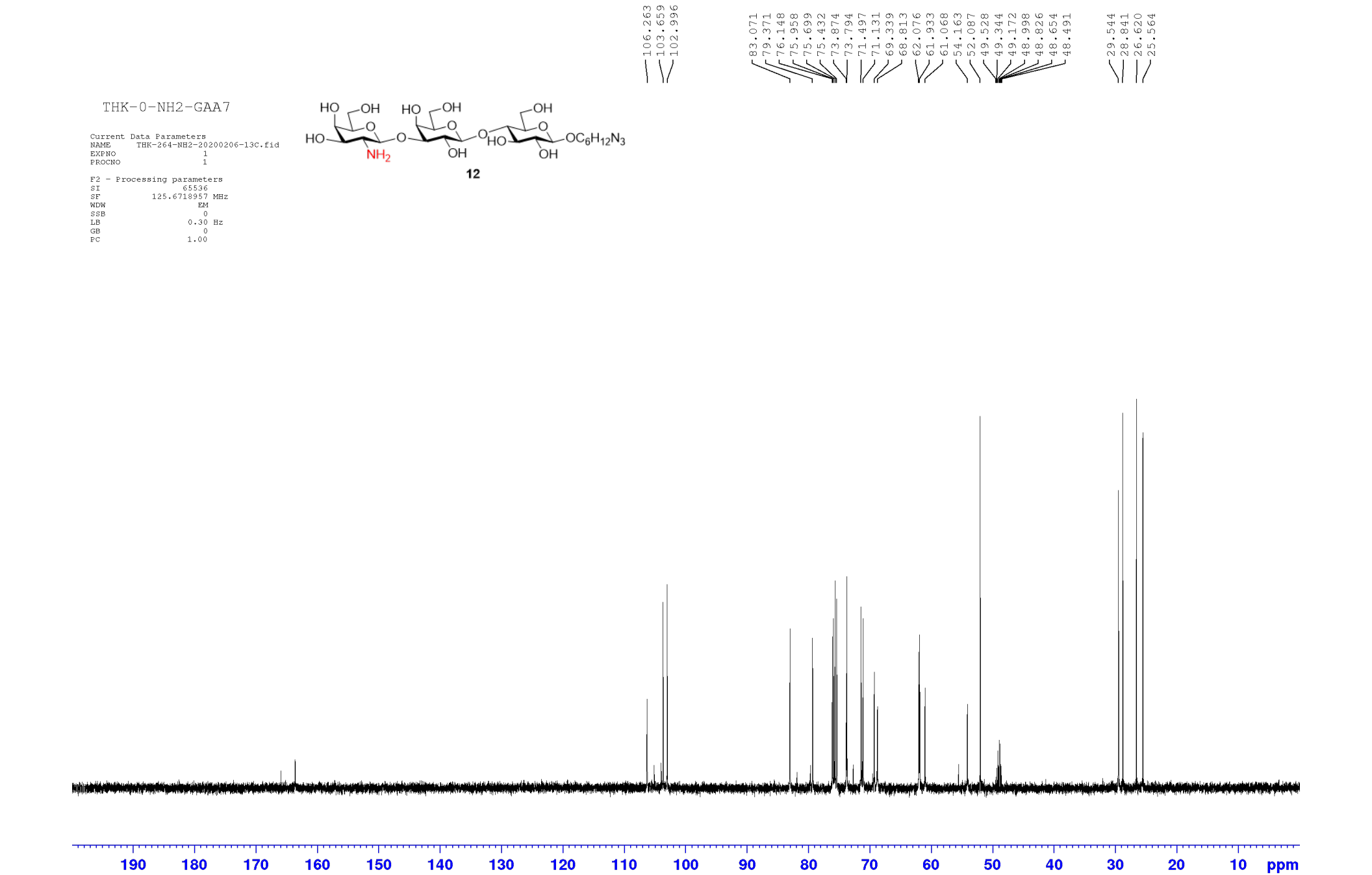
^{1}H NMR spectrum of Compound **11** (500 MHz D_2O)



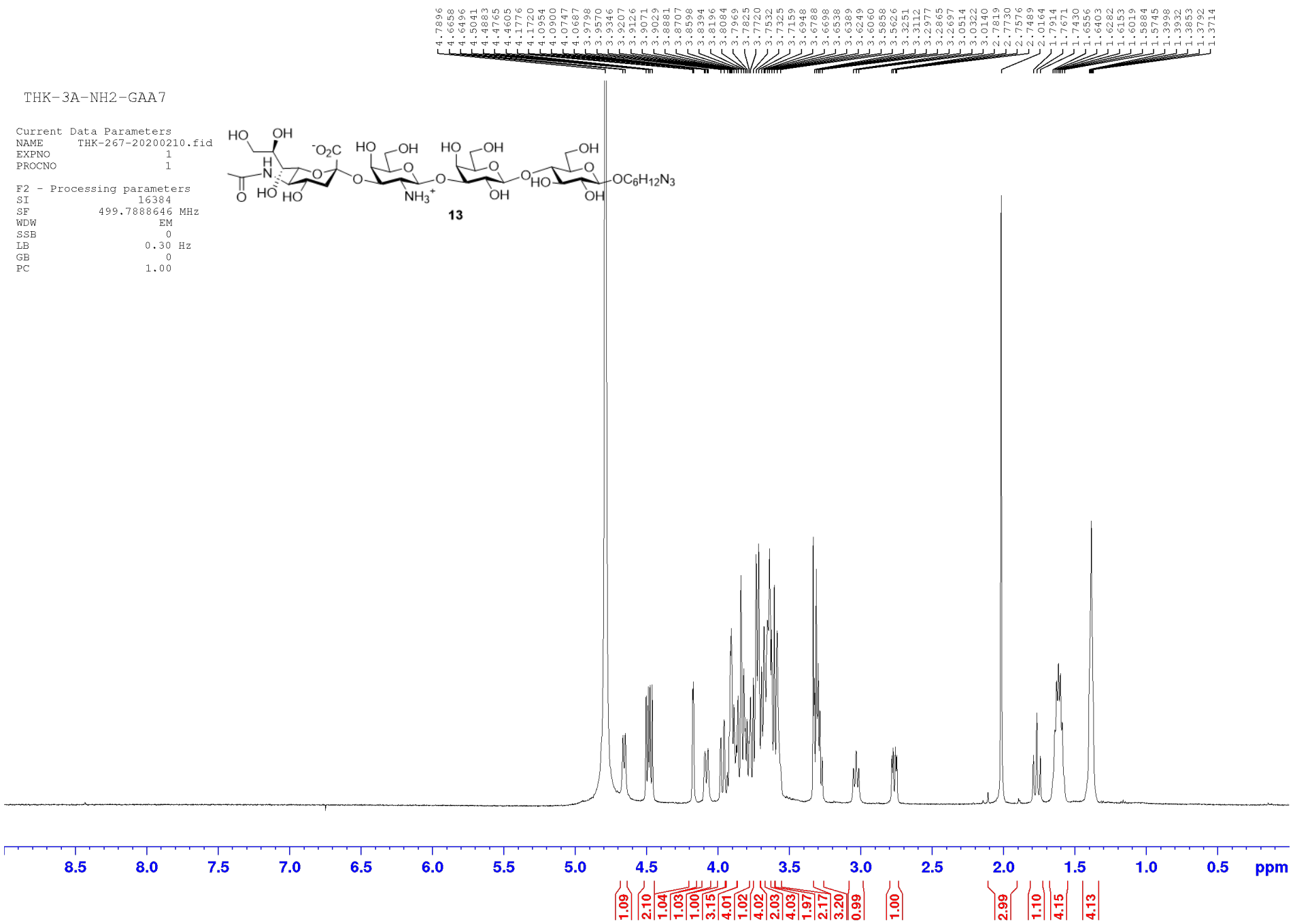
^{13}C NMR spectrum of Compound **11** (125 MHz D_2O)



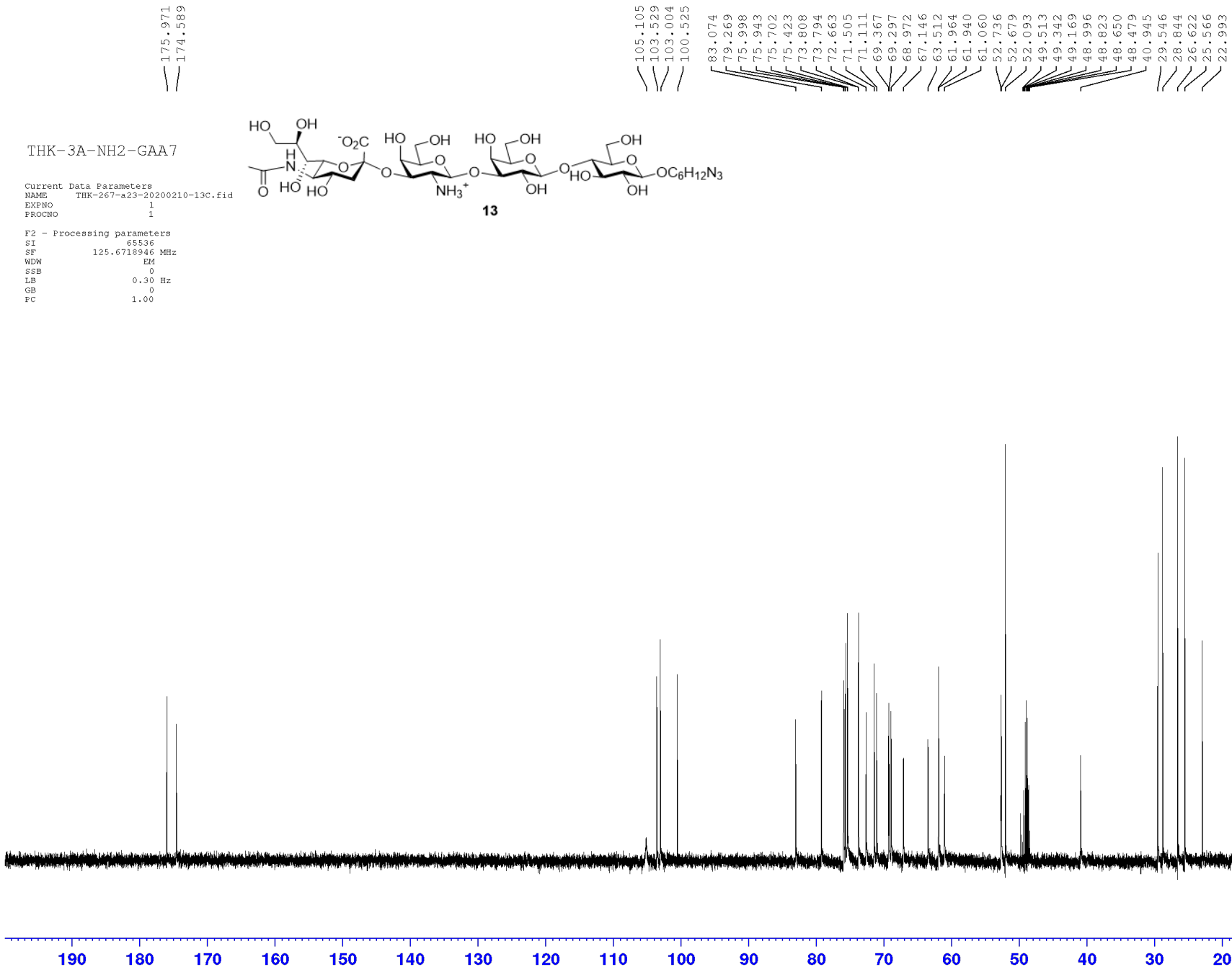
^{1}H NMR spectrum of Compound **12** (500 MHz D_2O)



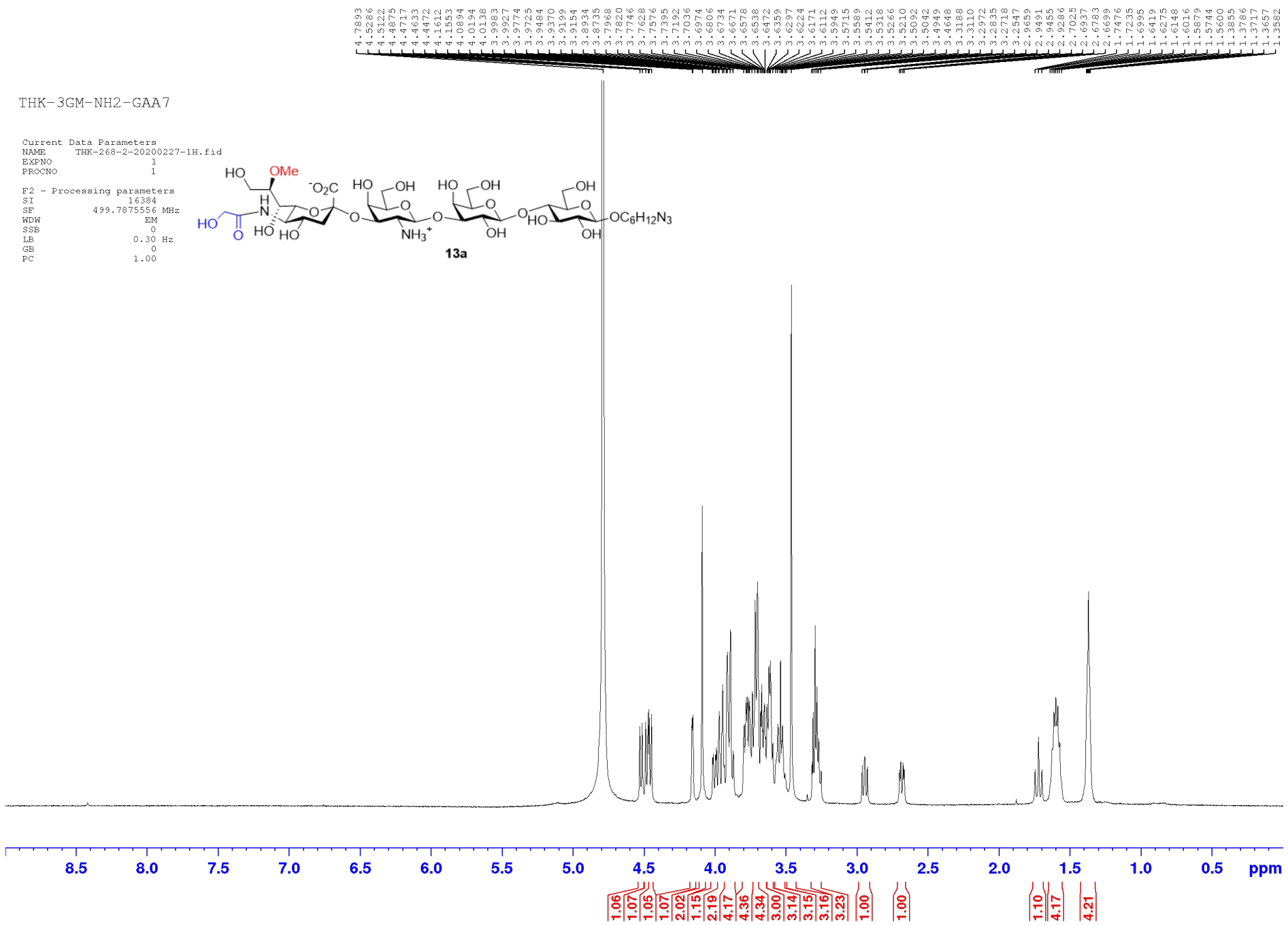
^{13}C NMR spectrum of Compound **12** (125 MHz D_2O)



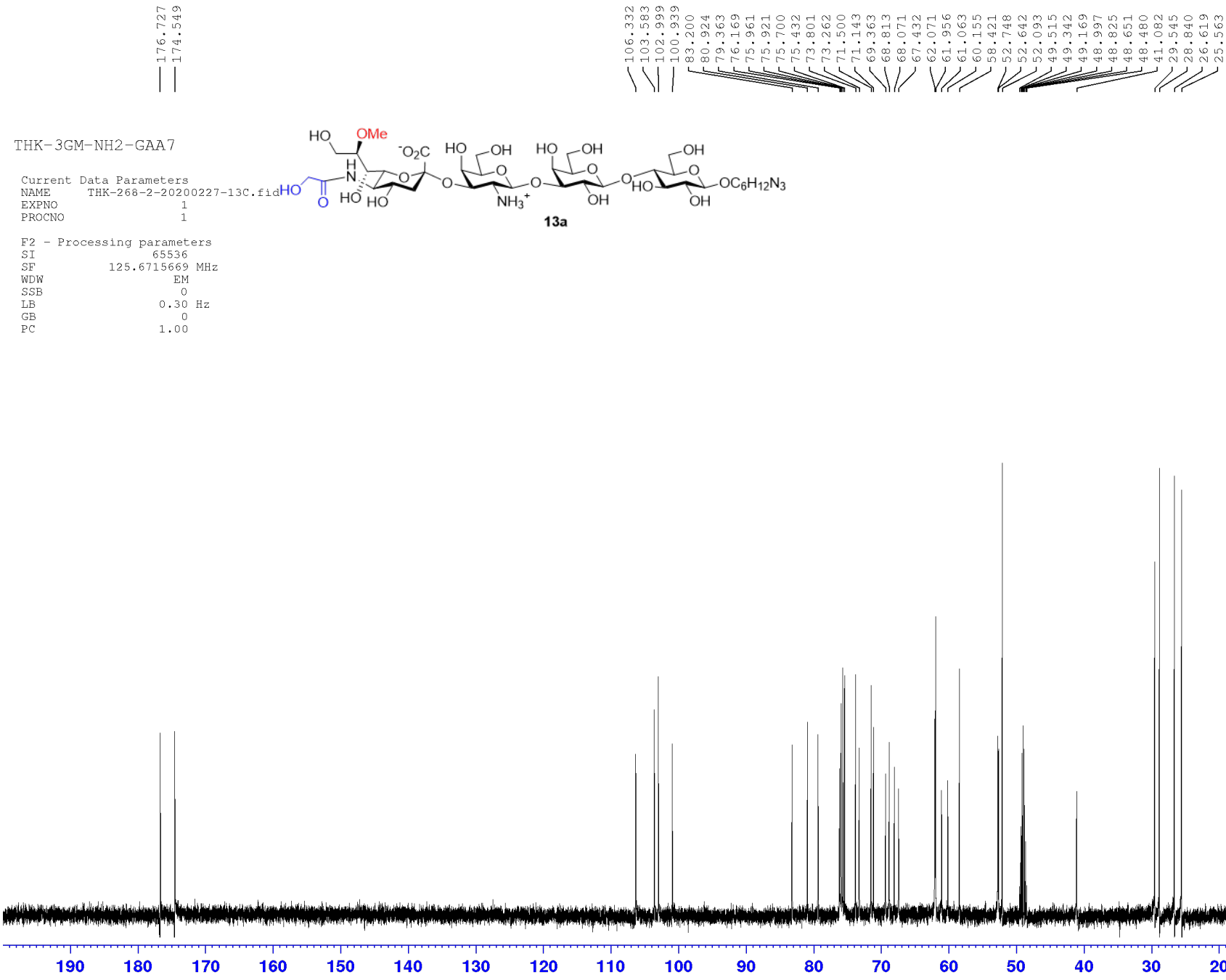
^{1}H NMR spectrum of Compound **13** (500 MHz D_2O)



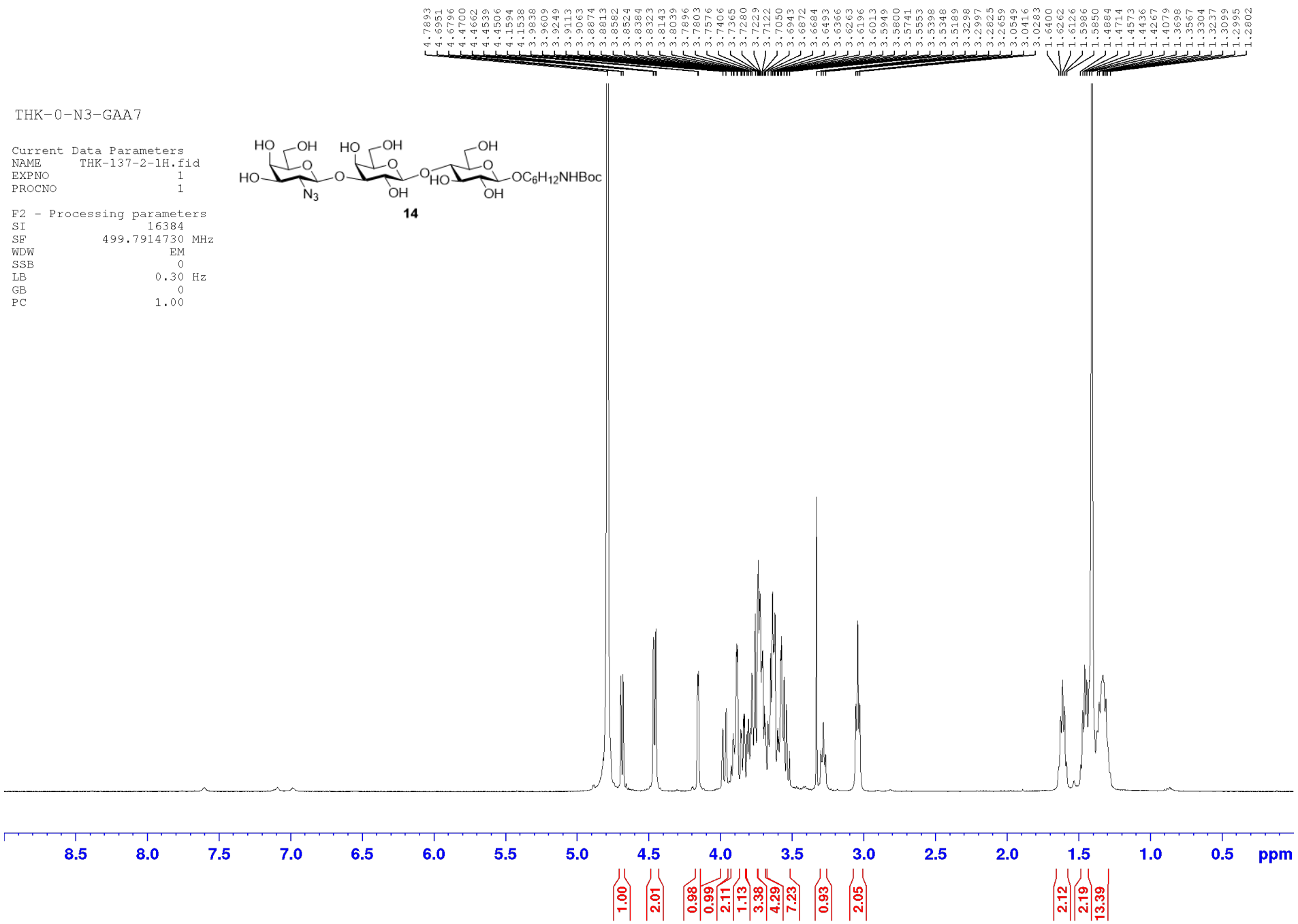
^{13}C NMR spectrum of Compound **13** (125 MHz D_2O)



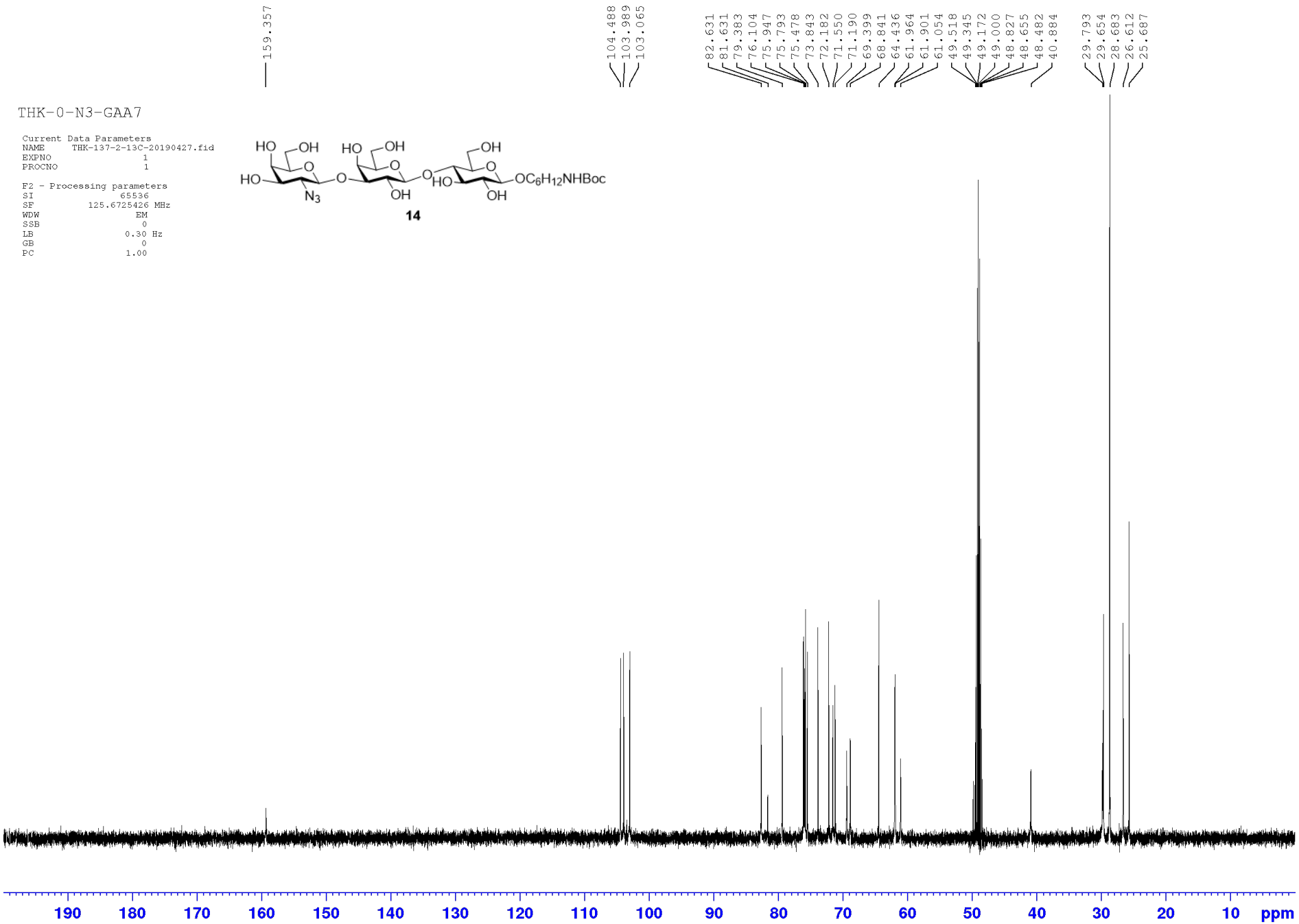
^{1}H NMR spectrum of Compound **13a** (500 MHz D_2O)



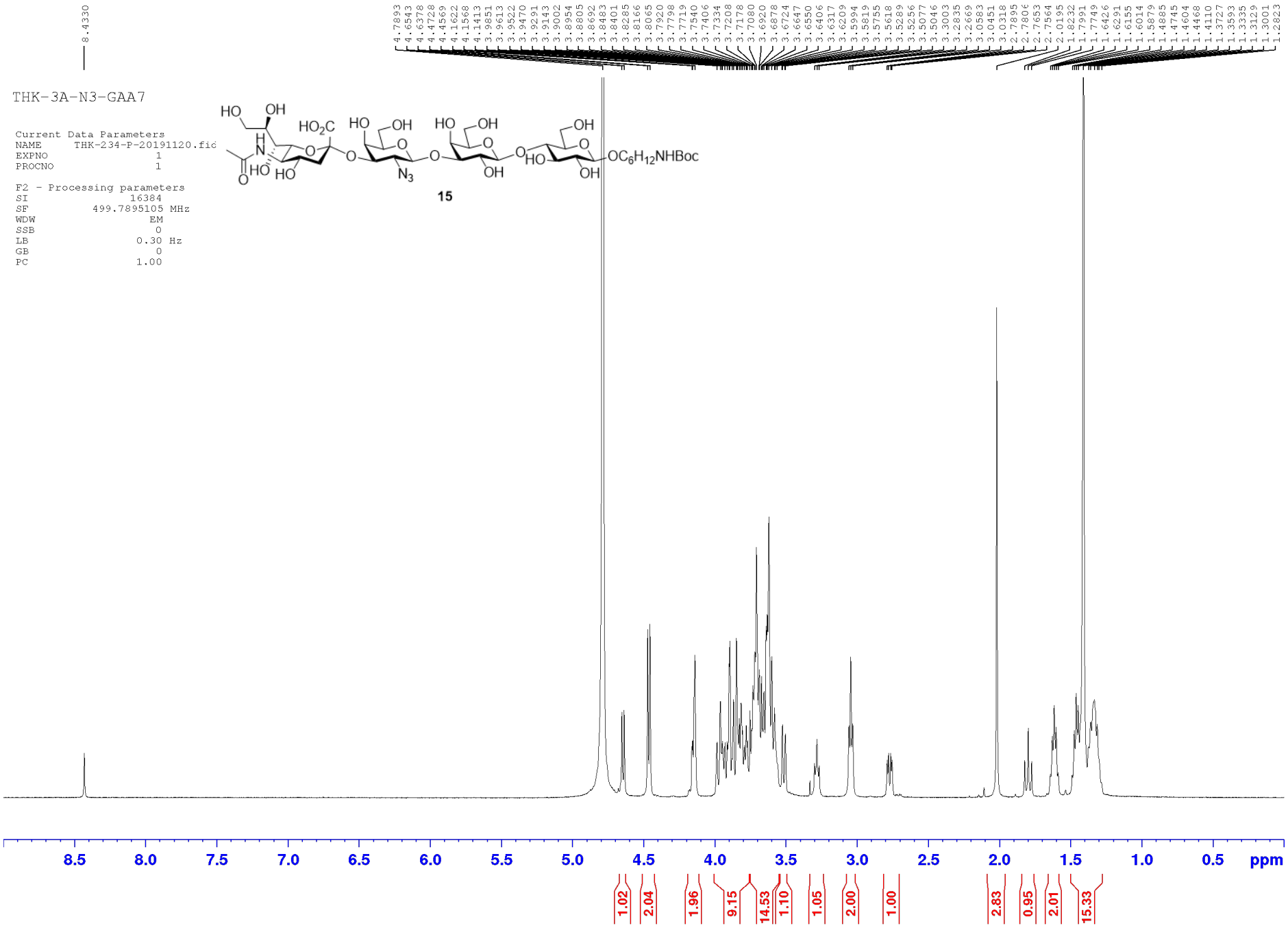
^{13}C NMR spectrum of Compound **13a** (125 MHz D_2O)



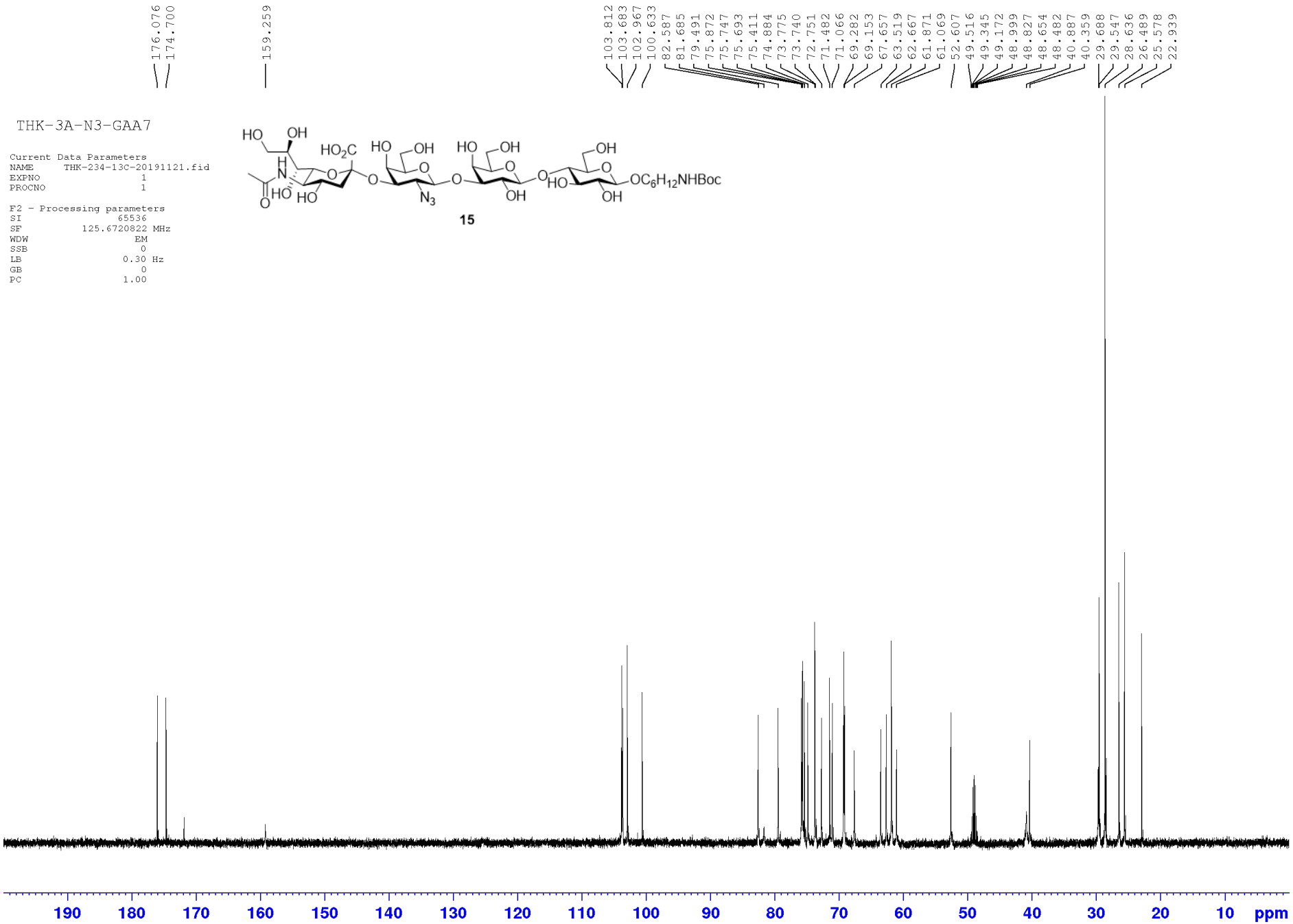
^{1}H NMR spectrum of Compound **14** (500 MHz D_2O)



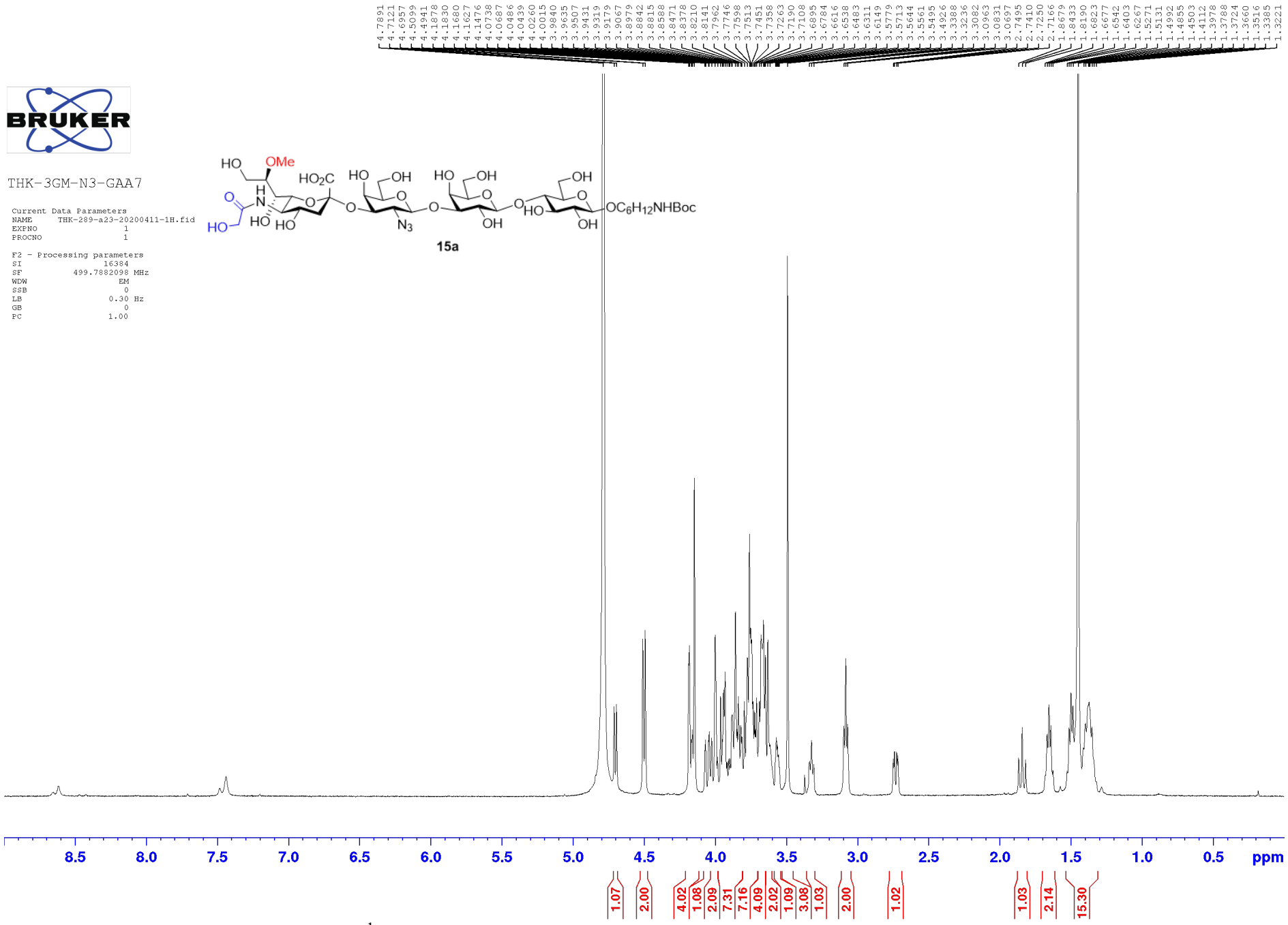
^{13}C NMR spectrum of Compound **14** (125 MHz D_2O)



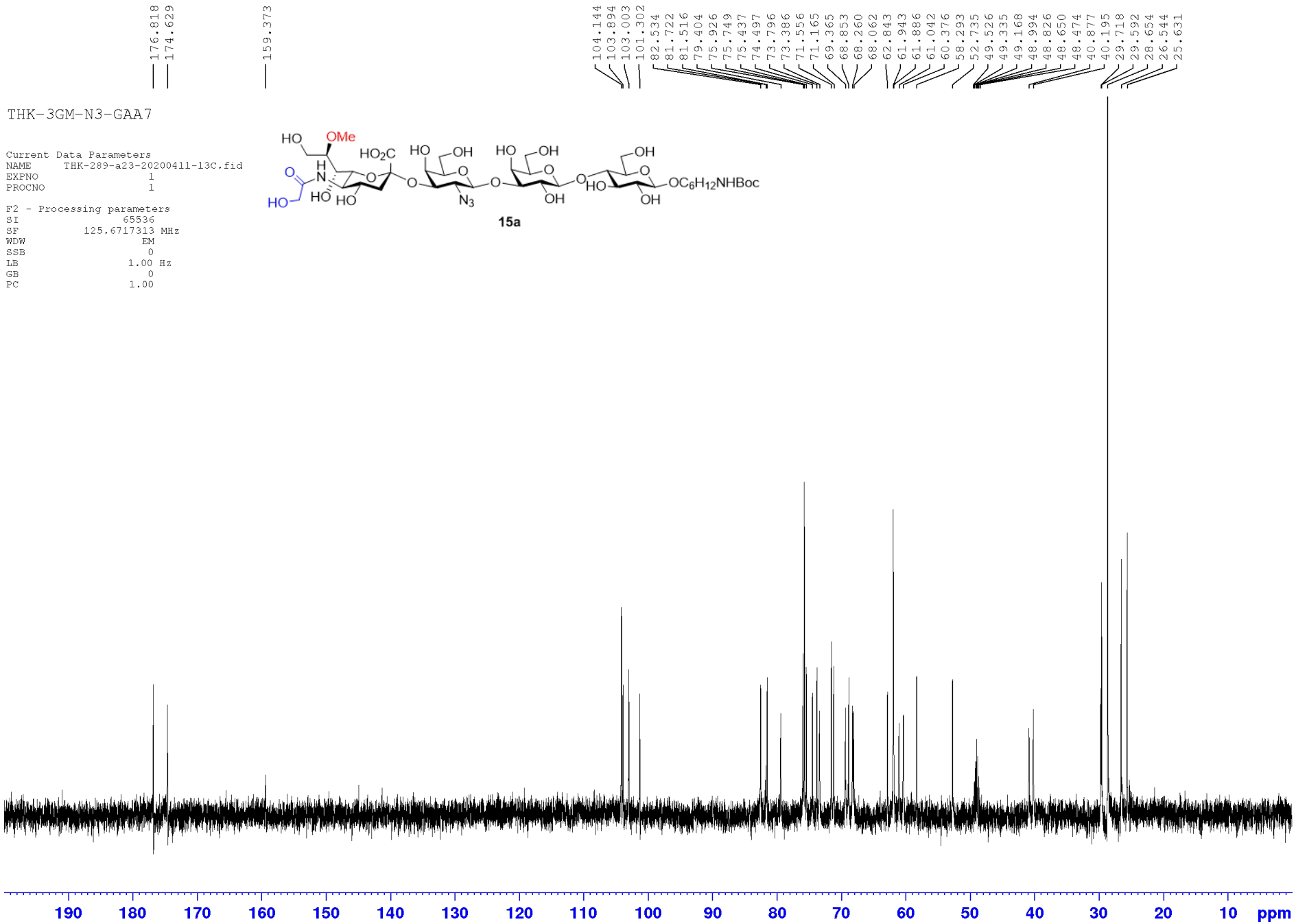
^{1}H NMR spectrum of Compound **15** (500 MHz D_2O)



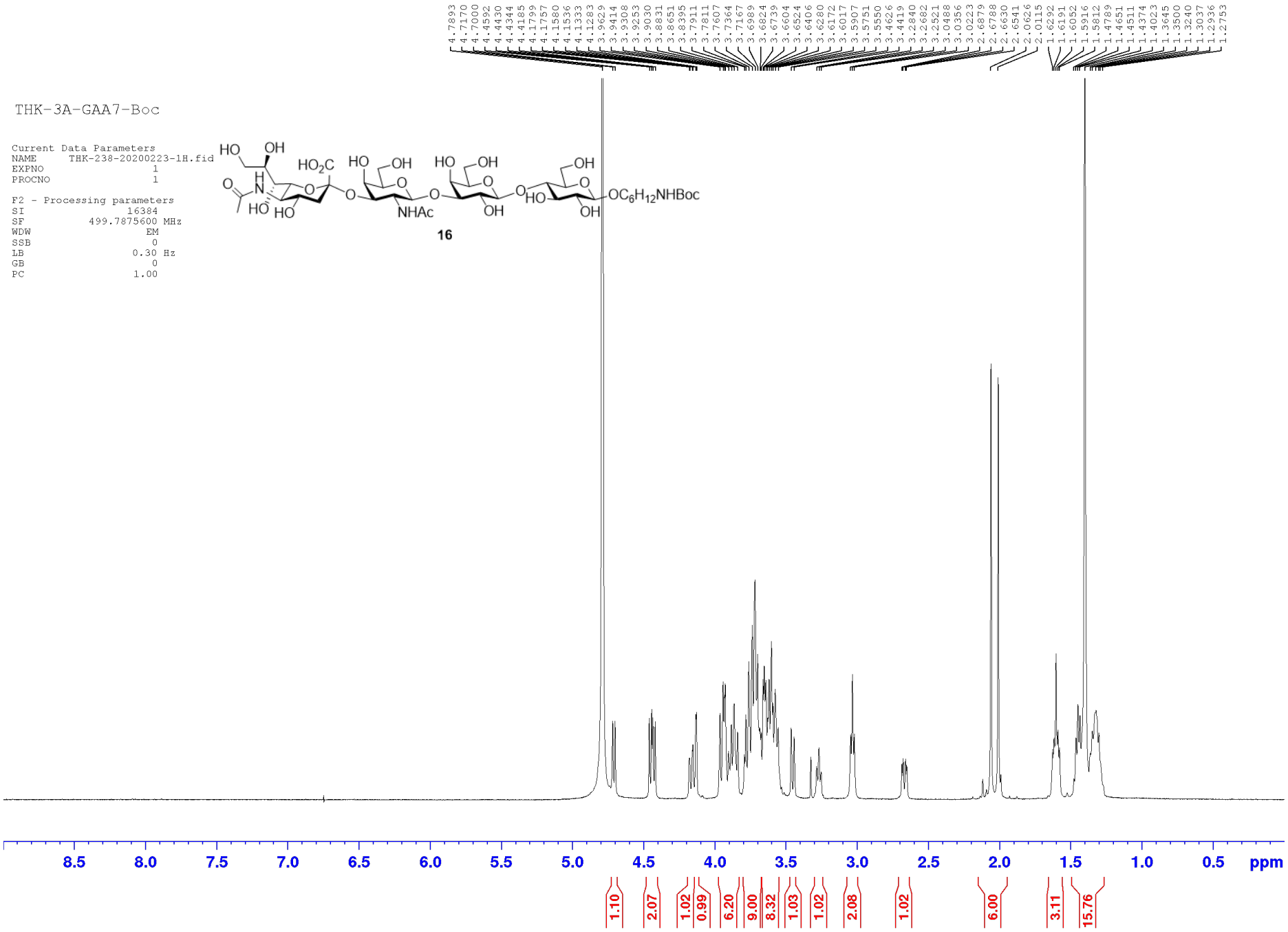
^{13}C NMR spectrum of Compound **15** (125 MHz D_2O)



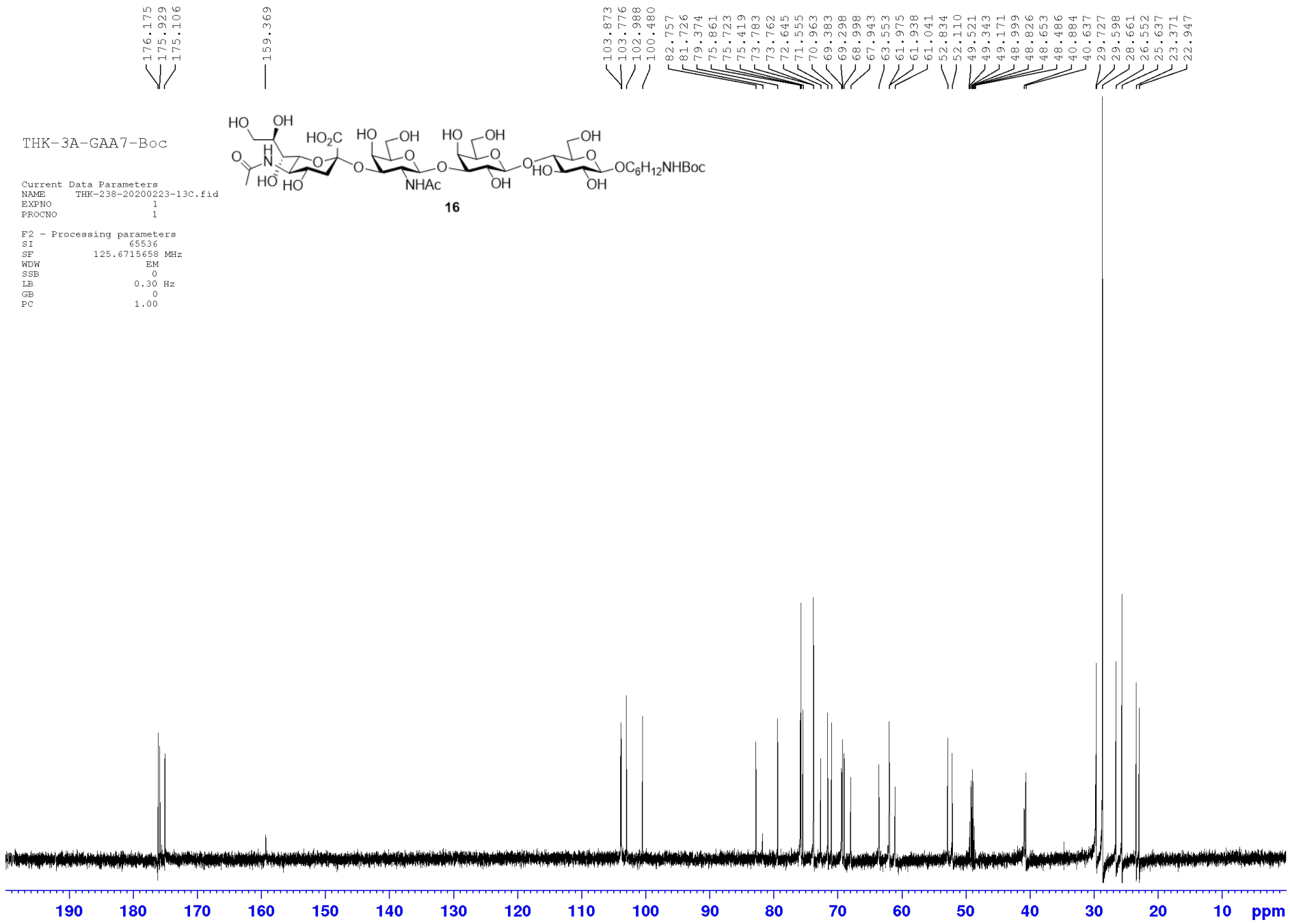
^{1}H NMR spectrum of Compound **15a** (500 MHz D_2O)



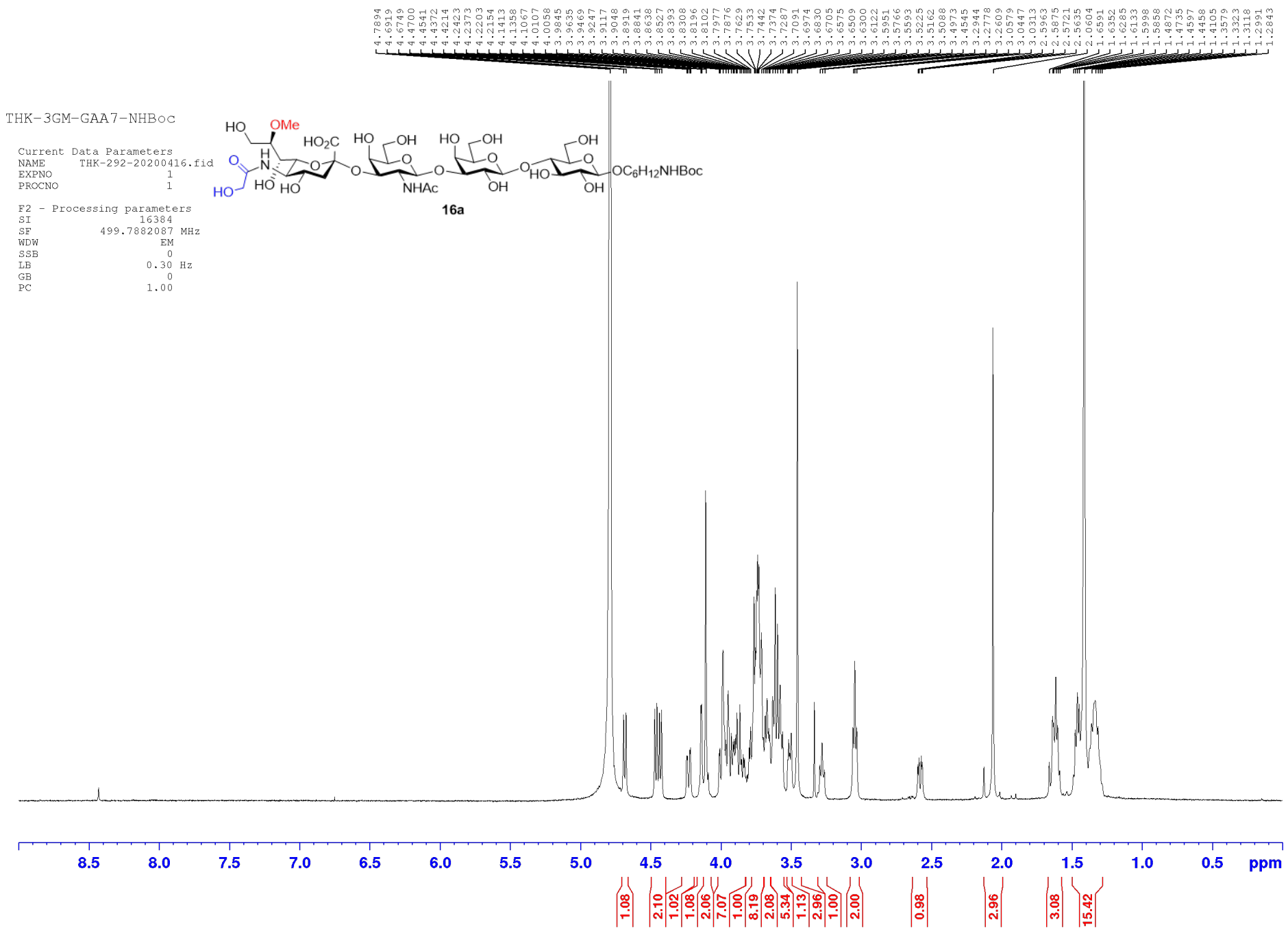
^{13}C NMR spectrum of Compound **15a** (125 MHz D_2O)



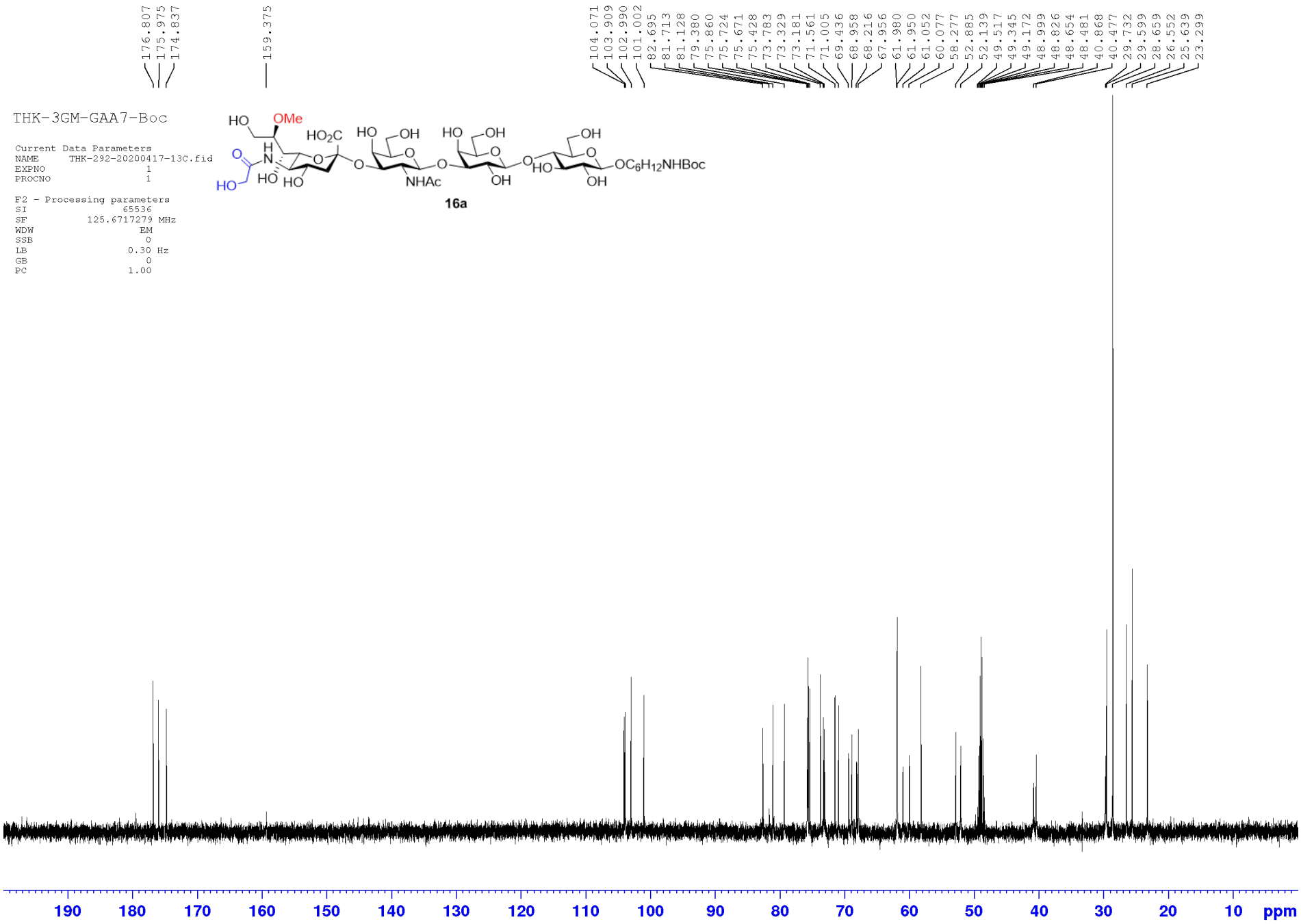
^{1}H NMR spectrum of Compound **16** (500 MHz D_2O)



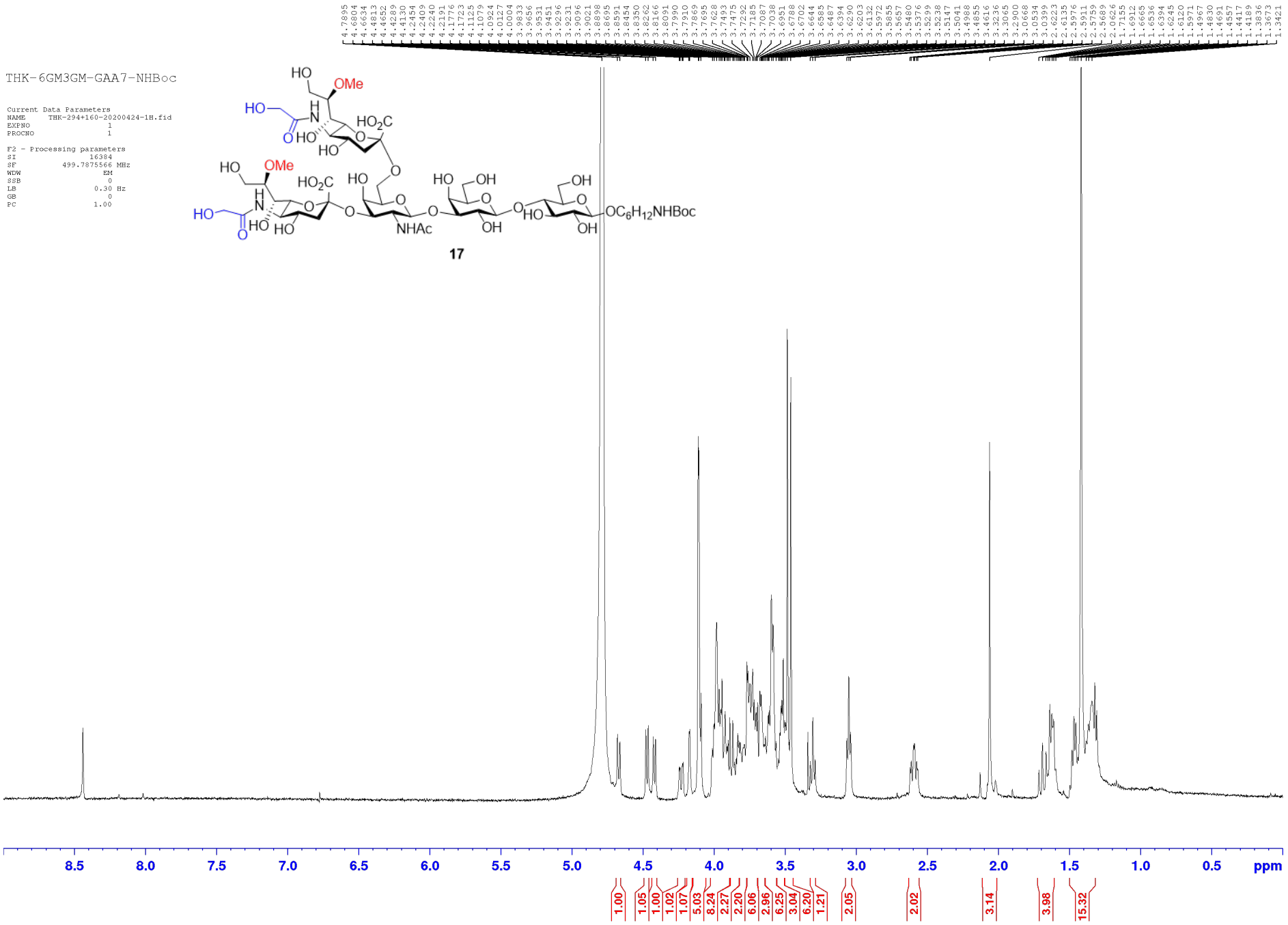
^{13}C NMR spectrum of Compound **16** (125 MHz D_2O)



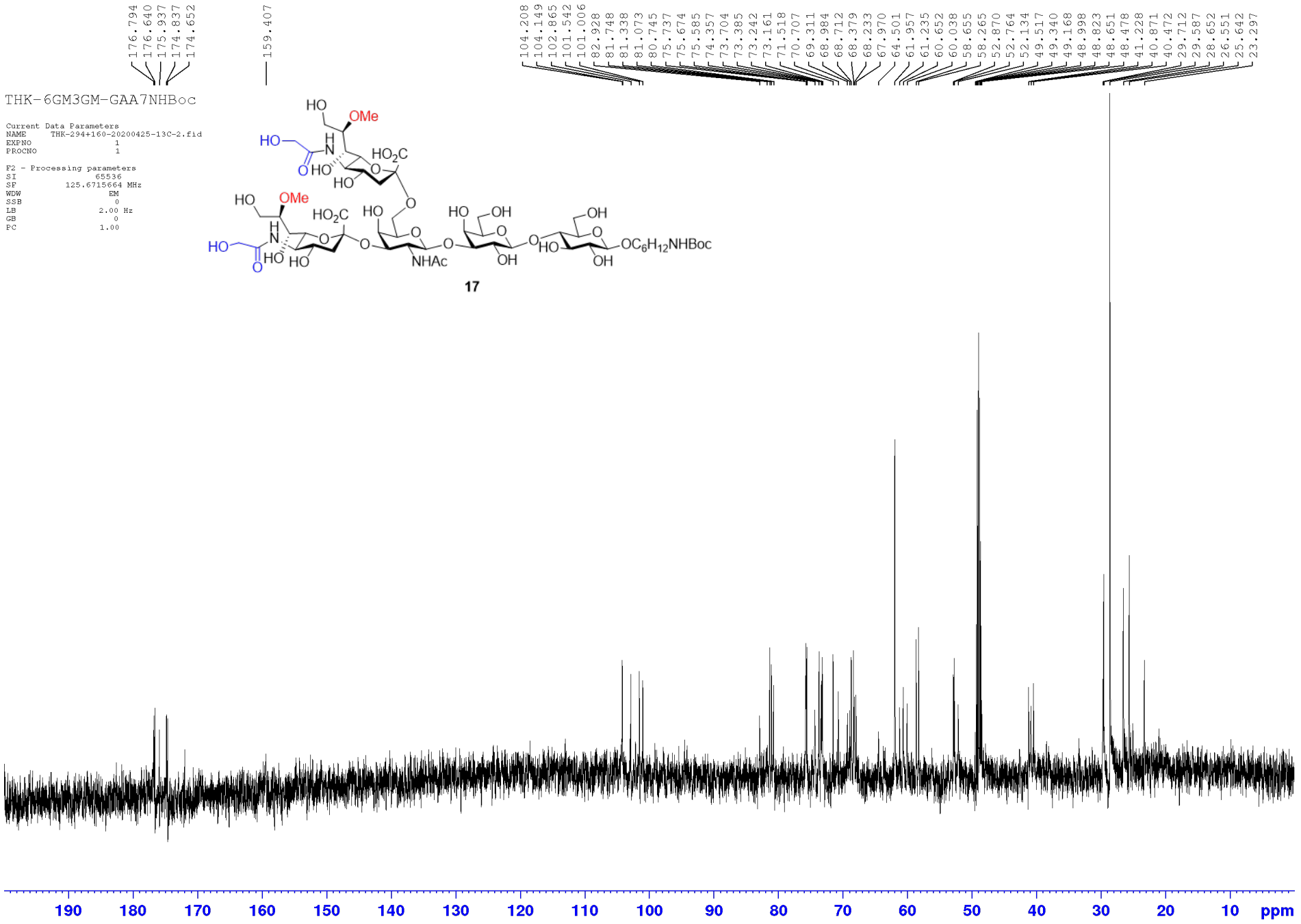
^{1}H NMR spectrum of Compound **16a** (500 MHz D_2O)



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



^{1}H NMR spectrum of Compound **17** (500 MHz D_2O)



^{13}C NMR spectrum of Compound **17** (125 MHz D_2O)