Supplementary information

For

Expeditious Synthesis of Multiglycopeptides with Heterogeneous Glycan Cores Derived from α-Dystroglycan Mucin-Like Domain

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Contents

Materials and methods

All chemicals purchased as reagent grade were used without further purification. AR and HPLC grade solvents were purchased from Bio-Lab ltd. 2-Azidogalactose tertaacetate phenylselenide **3** and 2,2,2-Trifluoro-*N*-phenylacetimidoyl Chloride were purchased from DSK InnoSciences. tert-Butyl 2,2,2-trichloroacetimidate was purchased from Merck KGaA and from Aaron Chemicals LLC. Flash chromatography (FC) was performed using Interchim PuriFlash XS420, using SiO_2 columns. NMR spectra were recorded on Bruker AV 400 and Bruker AV 500 spectrometers. Residual solvent peaks were used as internal references. Chemical shifts of the reference solvent were calibrated.

Analytical HPLC

HPLC analyses were performed on a Merck-Hitachi system equipped with an L-2130 pump, L-2400 UV detector, and an XTerra RP8 column (125Å, 3.5 µm, 4.6 mm X 150 mm), except for **αDG-n** library analyses (not including **αDG-9-Ac**), which were performed with an XTerra Shield RP18 column (125Å, 5 µm, 4.6 mm X 250 mm). All samples of synthetic products, as well as **D1-A** and **D1-N**, were dissolved in TDW/ACN 50:50, and all samples of other glycopeptides were dissolved in TDW. All samples were filtered through 0.22 µm filters (PTFE for samples dissolved in TDW/ACN 50:50 and nylon for samples dissolved in TDW) and injected into the reversed-phase analytical HPLC column. Chromatograms were recorded at room temperature with a flow rate of 1 mL/min. The mobile phase consisted of solution A: TDW (0.1% v/v TFA) and solution B: ACN (0.1% v/v TFA). GAAs were analyzed ramping from 5:95 to 30:70 ACN:TDW in 5 minutes and then from 30:70 to 95:5 ACN:TDW in 25 minutes, recorded at 254 nm. GPs were analyzed ramping from 3:97 to 60:40 ACN:TDW in 25 minutes, recorded at 220 nm. The HPLC conditions are presented below. The collected fractions were analyzed by MALDI-MS. The crude purity of each peptide was calculated by integration of the desired peak that was detected by MALDI-MS.

Preparative HPLC purification

GPs and GAAs were purified on a Waters HPLC system with a 2545 Binary Gradient Module and a 2849 UV detector (220 nm). Crude GPs after lyophilization were dissolved in 4 mL TDW and injected into the preparative reversed-phase HPLC (Phenomenex Luna C18(2) HPLC C18 column (5 µm, 250 x 21.2 mm)). Elution was performed with a gradient of 5:95 to 35:65 ACN:TDW in 30 min at room temperature with a flow rate of 10 mL/min. The mobile phase consisted of solution A: TDW (0.1% v/v TFA) and solution B: ACN (0.1% v/v TFA). GAAs were purified with a gradient of 40:60 to 95:5 ACN:TDW in 35 min at room temperature with a flow rate of 10 mL/min prior to NMR analysis.

Mass spectrometry

High-resolution mass spectra were recorded on a Sciex X500R QTOF mass spectrometer using ESI in positive mode. MALDI-MS spectra were acquired with a Bruker autoflex maX TOF/TOF, setting in positive reflective mode, using an α-Cyano-4-hydroxycinnamic acid matrix.

Synthetic procedures

Synthesis of 2-Azido-2-deoxy-1-*p*-tolylthio-3,4,6-tri-O-acetyl-D-galactopyranose **7**

2-Azidogalactose tertaacetate phenylselenide **3** (4.705 g, 10 mmol) was dissolved in 25 mL of a 1:1 water/THF mixture. N-iodosuccinimide (NIS, 11.249 g, 50 mmol) was added, and the reaction was allowed to stir overnight. The reaction was quenched with 10% $Na_2S_2O_3$ solution (350 mL) and washed with hexane (4x50 mL) to remove unwanted organic products. The aqueous phase was washed with EtOAc (5x50 mL). The combined EtOAc phase was dried over $MgSO_4$ and evaporated to give crude **4**, which was used without further purification. Crude **4** (4.13 g) was dissolved in pyridine (30 mL), added acetic anhydride (1.9 mL, 20 mmol) and was left to stir overnight. The reaction was diluted in dichloromethane (DCM, 50 mL), and the organic phase was washed with HCl 1N (3x150 mL), saturated $NaHCO_3$ solution (100 mL), and brine (100 mL). The

obtained mixture was adsorbed on silica gel (30 mL), then washed with hexane until the solution was no longer yellow. The silica was washed with EtOAc (140 mL) and the eluted solvent was evaporated to give crude **5**, which was used without further purification. Crude **5** (3.09 g) was mixed with p-thiocresol (1.54 g, 12.4 mmol) and co-evaporated with toluene twice. The mixture was dissolved in dry DCM (50 mL) under Ar and stirred in an ice bath. BF_3Et_2O (10.5 mL, 80 mmol) was added slowly. After 10 min the reaction was allowed to warm up to rt and left to stir overnight. The reaction was quenched with saturated $NaHCO_3$ solution (100 mL), separated, and the organic phase was washed again with saturated $NaHCO_3$ solution (50 mL) and brine (50 mL). The combined aqueous phase was extracted with DCM (2x40 mL). The DCM extracts were combined, dried over $MgSO_4$ and evaporated to give crude **7** as a viscous yellow liquid. Crude product was separated by column chromatography (Hexane/EtOAc 4:1) to give a sticky white solid of **7** as a 1:1 α\β mixture (1.632g, 45% yield over 3 steps). Pure **7** was separated by preparative HPLC in small quantity to its α and β anomers for analytical purposes. ^{1}H NMR (400 MHz, $CDCl_3$): α anomer – δ 7.41 (m, 2H; STol-Ar), 7.14 (m, 2H; STol-Ar), 5.61 (d, 1H J = 5.5); H-1), 5.48 (dd, 1H, J = 3.3, 1.3; H-4), 5.18 (dd, 1H, J = 11.1, 3.2; H-3), 4.77 (td, 1H, J = 6.4, 1.1; H-5), 4.23 (dd, 1H, J = 11.1, 5.5; H-2), 4.08 (d, 2H, J = 6.5; H-6), 2.34 (s, 3H; STol-CH_3), 2.16 (s, 3H; Ac), 2.07 (s, 3H; Ac), 2.00(s, 3H; Ac). β anomer - 7.51 (m, 2H; STol-Ar), 7.16 (m, 2H; STol-Ar), 5.34 (dd, 1H, J = 3.2, 0.8; H-4), 4.85 (dd, 1H, J = 10.3, 3.2; H-3), 4.46 (d, 1H, J = 10.1; H-1), 4.10 (m, 2H; H-6), 3.86 (td, 1H, J = 6.6, 1.1; H-5), 3.63 (t, 1H, J = 10.2; H-2), 2.36 (s, 3H; STol-CH_3), 2.09 (s, 3H; Ac), 2.05 (s, 3H; Ac), 2.03 (s, 3H; Ac). ^{13}C NMR (125 MHz, $CDCl_3$): α anomer – δ 170.5, 170.1, 169.8, 138.6, 133.3, 130.1, 128.7, 87.5, 70.3, 67.7, 67.5, 61.9, 21.3, 20.8, 20.8, 20.8. β anomer - 169.5, 169.0, 168.8, 137.9, 133.0, 128.7, 126.2, 85.7, 73.3, 72.0, 65.5, 60.5, 58.3, 20.2, 19.7, 19.6, 19.5. HRMS (ESI) *m/z* Calcd for $C_{19}H_{24}N_3O_7S$, 438.13295 $[M+H^+]^+$; found, 438.13262.

Synthesis of tert-Butyl N-Fmoc-O-(2-azido-2-deoxy-3,4,6-tri-O-acetyl-D-Galactopyranosyl)-L-Serinate **9**

Fmoc-Ser-COOtBu **8** (776 mg, 2 mmol) [1] was mixed with **7** (590 mg, 1.35 mmol) and co-evaporated with toluene twice and left under HV overnight. The mixture was dissolved in dry DCM (20 mL) and stirred under Ar with 4Å molecular sieve. After 1h of stirring, reaction was cooled down to -10 °C and recrystallized NIS (607 mg, 2.7 mmol) and trifluoromethanesulfonic acid (TfOH, 24μL, 0.27 mmol) were added. After 1h the reaction was quenched with saturated $NaHCO_3$ solution (10 mL) and filtered on celite. Organic phase was washed with 10% $Na_2S_2O_3$ solution (50 mL) and brine (2x50 mL). The combined aqueous phase was extracted with DCM (25 mL), and the combined organic phase was dried over $MgSO_4$ and evaporated to give crude **9** as a viscous yellow liquid. The crude product was separated by column chromatography (65:30:5 Hexane/EtOAc/DCM). The product was collected in a fraction of **9** as of pure α isomer (12%), a fraction containing a mixture of **9** and its isomer **9β** products (11%), and a fraction of pure **9β** isomer (2%). After lyophilization, pure **9** was obtained as a white powder (112 mg, 12%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.76 (m, 2H; Fmoc-Ar), 7.63 (m, 2H; Fmoc-Ar), 7.40 (m, 2H; Fmoc-Ar), 7.32 (m, 2H; Fmoc-Ar), 5.87 (N-H), 5.46 (m, 1H; H-4), 5.30 (dd, 1H, J = 11.1, 3.2; H-3), 4.95 (d, 1H; H-1), 4.42 (m, 3H; H-6, Fmoc-CH), 4.25 (t, 1H; Ser-CH), 4.20 (m, 1H; H-5), 4.10 (dd, 1H, J = 10.7, 3.2; Fmoc-CH_2), 4.05 (m, 2H; Ser-CH_2), 3.98 (dd, 1H, J = 10.7, 3.0; Fmoc-CH_2), 3.66 (dd, 1H; H-2), 2.15 (s, 3H; Ac), 2.06 (s, 3H; Ac), 1.98 (s, 3H; Ac), 1.51 (s, 9H; tBu). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.5, 170.1, 169.8, 168.6, 156.0, 144.0, 143.9, 141.4, 127.9, 127.2, 125.3, 120.1, 99.4, 83.3, 70.1, 68.1, 67.6, 67.4, 67.3, 61.8, 57.6, 55.1, 47.2, 28.1, 20.8, 20.7, 20.7. HRMS (ESI) *m/z* Calcd for $C_{34}H_{41}N_4O_{12}$, 697.27155 $[M+H^+]^+$; found, 697.26811.

Synthesis of tert-Butyl N-Fmoc-O-(2-azido-2-deoxy-3,4,6-tri-O-acetyl-D-Galactopyranosyl)-L-Threoninate **S1**

S1 was synthesized according to the same procedure used for the preparation of **9**. In short, **7** (1 g, 2.29 mmol) was treated with recrystallized NIS (1.029 g, 4.57 mmol), TfOH (40μL, 0.46 mmol), and Fmoc-Thr-COOtBu[1] (1.362 g, 3.43 mmol) to provide a mixture of **S1** and the **S1β** isomers. Separation of the crude **S1** mixture was done by column chromatography (15% EtOAc in toluene). The product was collected in a fraction of **S1** in a pure α form (15%), a fraction containing a mixture of **S1** and **S1β** (43%), and a fraction of pure **S1β** (15%).

After lyophilization, pure **S1** was obtained as a white powder (250 mg, 15%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (m, 2H; Fmoc-Ar), 7.64 (m, 2H; Fmoc-Ar), 7.40 (m, 2H; Fmoc-Ar), 7.32 (m, 2H; Fmoc-Ar), 5.66 (N-H), 5.47 (m, 1H; H-4), 5.35 (dd, 1H, J = 11.2, 3.2; H-3), 5.11 (d, 1H, J = 3.7; H-1), 4.46 (m, 1H; Thr-β-CH), 4.39 (m, 2H; Fmoc-CH_2), 4.29 (m, 3H; H-5, Thr-α-CH, Fmoc-CH), 4.10 (d, 2H, J = 6.5; H-6), 3.64 (dd, 1H, J = 11.2, 3.7; H-2), 2.15 (s, 3H; Ac), 2.08 (s, 3H; Ac), 2.05 (s, 3H; Ac), 1.51 (s, 9H; tBu), 1.35 (d, 3H, J = 6.5; Thr-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.5, 170.1, 170.0, 169.3, 157.0, 144.0, 144.0, 141.4, 127.8, 127.2, 125.4, 120.1, 120.1, 99.3, 76.5, 68.2, 67.6, 67.6, 67.2, 61.9, 59.3, 57.8, 47.2, 28.1, 20.8, 20.8, 20.7, 19.1. HRMS (ESI) *m/z* Calcd for $C_{35}H_{43}N_4O_{12}$, 711.28720 $[M+H^+]^+$; found, 711.28751.

Synthesis of N-Fmoc-O-(2-azido-2-deoxy-3,4,6-tri-O-acetyl-D-Galactopyranosyl)-L-Serine **2s**

9 (150 mg, 0.22 mmol) was dissolved in 10 mL of DCM. Added 2 mL of TFA and stirred at rt for 2.5h. Washed with 1 N HCl solution (10 mL), then extracted aqueous phase with DCM (10 mL). Combined organic phase was dried over $MgSO_4$ and evaporated. After lyophilization, pure **2s** was collected as a white powder (121 mg, 88%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.75 (m, 2H; Fmoc-Ar), 7.58 (N-H), 7.57 (m, 2H; Fmoc-Ar), 7.39 (m, 2H; Fmoc-Ar), 7.30 (m, 2H; Fmoc-Ar), 5.62 (dd, 1H, J = 11.2, 3.2; H-3), 5.54 (m, 1H; H-4), 5.05 (d, 1H, J = 3.4; H-1), 4.85 (d, 1H, J = 8.7; Ser-CH), 4.38 (dd, 1H, J = 10.0, 7.0; Fmoc-CH_2), 4.32 (m, 1H; H-5), 4.26 (m, 1H; Fmoc-CH_2), 4.21 (d, 1H, J = 7.3; Fmoc-CH), 4.16 (m, 1H; Ser-CH_2), 4.07 (m, 2H; H-6, Ser-CH_2), 3.63 (dd, 1H, J = 11.4, 4.7; H-6), 3.54 (dd, 1H, J = 11.2, 3.4; H-2), 2.12 (s, 3H; Ac), 2.09 (s, 3H; Ac), 1.75 (s, 3H; Ac). ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.8, 171.8, 170.6, 170.3, 156.1, 144.1, 143.6, 141.3, 127.8, 127.1, 125.1, 120.2, 98.0, 69.3, 68.8, 67.6, 67.4, 67.2, 61.8, 57.0, 54.0, 47.0, 21.0, 20.6, 20.3. HRMS (ESI) *m/z* Calcd for $C_{30}H_{33}N_4O_{12}$, 641.20895 $[M+H^+]^+$; found, 641.20593.

Synthesis of N-Fmoc-O-(2-azido-2-deoxy-3,4,6-tri-O-acetyl-D-Galactopyranosyl)-L-Threonine **2t**

2t was prepared from **S1** (250 mg, 0.35 mmol) following the same procedure used for **2s**. After lyophilization, pure **2t** was obtained as a white powder (222 mg, 97%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (m, 2H; Fmoc-Ar), 7.62 (m, 2H; Fmoc-Ar), 7.40 (m, 2H; Fmoc-Ar), 7.32 (m, 2H; Fmoc-Ar), 5.80 (N-H), 5.45 (m, 1H; H-4), 5.28 (dd, 1H, J = 11.1, 3.2; H-3), 5.15 (d, 1H, J = 3.7; H-1), 4.49 (dd, 1H, J = 8.9, 2.5; Thr-β-CH), 4.46 (m, 3H; Thr-α-CH, Fmoc-CH_2), 4.27 (m, 2H; H-5, Fmoc-CH), 4.09 (d, 2H, J = 6.6; H-6), 3.79 (dd, 1H, J = 11.1, 3.7; H-2), 2.15 (s, 3H; Ac), 2.06 (s, 3H; Ac), 2.03 (s, 3H; Ac), 1.34 (d, 3H, J = 6.4; Thr-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.0, 170.7, 170.3, 170.2, 156.9, 143.8, 143.8, 141.4, 127.9, 127.3, 125.3, 120.2, 98.9, 76.4, 68.9, 67.8, 67.5, 67.3, 61.9, 58.2, 58.1, 47.2, 20.8, 20.8, 20.8, 18.0. HRMS (ESI) *m/z* Calcd for $C_{31}H_{35}N_4O_{12}$, 655.22460 $[M+H^+]^+$; found, 655.22730.

Synthesis of tert-Butyl N-Fmoc-O-(2-azido-2-deoxy-3,4,6-tri-O-acetyl-D-Galactopyranosyl)-L-Serinate **9** through route I

Crude **4** (480 mg) was co-evaporated with toluene twice, then left overnight under HV. Added Cs_2CO_3 (562 mg, 1.6 mmol) and dissolved in dry DCM (10 mL) under an inert atmosphere. The reaction was cooled down to 0 °C and 2,2,2-Trifluoro-*N*-phenylacetimidoyl Chloride (670 μL, 4.2 mmol) was added. The reaction was allowed to warm up to rt and stirred under N_2 for 6 h. The crude product was filtered on celite and washed with DCM (4x15 mL). The collected solution was evaporated under reduced pressure at 25 °C, and the obtained product was triturated with hexane to remove excess reactant. Crude **6** (372 mg) was collected and used without further purification. Fmoc-Thr-COOtBu (312 mg, 0.81 mmol) was added to the crude **6** and the mixture was co-evaporated with toluene twice and left overnight under HV. The mixture was dissolved in Dry DCM/dioxane (1:1 v/v, 20 mL) and cooled to -20°C while stirring under N_2. Added Trimethylsilyl trifluoromethanesulfonate (TMSOTf, 15 μL, 0.08 mmol) and left to stir for 1 h at -20 °C. The reaction was quenched with saturated $NaHCO_3$ solution and diluted with EtOAc (30 mL). The organic phase was washed with brine (2x30 mL), separated, dried over $MgSO_4$ and evaporated to afford crude **9** as a viscous yellow liquid. NMR peaks were in agreement with **9** obtained by route II, and indicated a formation of **9** and **9β** in a 2:1 ratio.

Peptide Synthesis procedures

All syntheses were carried out in the same reactor, the design for which was described in previous papers.[2] All syntheses were done on 100 mg of TentaGel® resin (loading 0.18 mmol/g) produced by Rapp Polymere GmbH. Stirring was always done by an overhead stirrer at 1200 rpm. All stages were done at 90 °C unless stated otherwise.

Coupling solutions were prepared as described in table S1:

Table S1: Coupling solutions used for solid phase synthesis in this study

Solution Number*	Solute**	Concentration (mM)	Equivalents per addition	Addition volume (mL)***
(1)	Amino Acid	54	3	1
(2)	GAA	21.6	1.2	1
(3)	HATU	52.2	2.9	1
(4)	DIPEA	108	6	1

* The solution combinations are described in the coupling modules below.

** HATU - Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium; DIPEA - *N,N*-Diisopropylethylamine; Amino acids – standard Fmoc protected amino acids ; GAA – compounds **1s, 1t, 2s** or **2t**.

*** All solutions were prepared in *N,N*-Dimethylformamide (DMF).

Module description:

The specific washings before and after modules are described in the protocols section.

Module I – Fmoc deprotection

Resin was stirred for 30 sec with 3 mL of 20% v/v piperidine in DMF.

Module IIa – Coupling of Non-glycosylated AA

Solutions (1), (3), and (4) were added sequentially to the resin and the mixture stirred for 30 sec.

Module IIb – Coupling of Glycosylated AAs

Solutions (2), (3), and (4) were added sequentially to the resin and the mixture stirred for 60 sec.

Module III – Deacetylation

A solution of 160 mM NaOMe in methanol (3 mL) was injected into a reactor containing a resin with acetylated glycans. The mixture was stirred at rt for 5 minutes, evacuated, and washed once with 3 mL of methanol for 30 sec before adding the next NaOMe solution. This process was repeated two more times.

Module IV – Azide reduction

After drying, the resin was placed in a 15 mL single fritted reservoir (20 μm), added thioacetic acid (2 mL) and pyridine (1 mL) and left to shake overnight. After completion, the solution was evacuated from the vessel and the resin was washed 3 times with DCM.

Module V - Cleavage

After the resin was dried, cleavage was done by shaking the resin for 2 h with a solution of TFA (5.5 mL), TDW (0.3 mL), and triisopropylsilane (TIPS, 0.2 mL). Crude peptides were precipitated in a cold solution of diethyl ether and hexane (1:1) and centrifuged. The solvents were decanted out and the resulting crude peptide slurry was dissolved in TDW:ACN 1:1 solution and lyophilized.

Synthesis protocols

Protocols were executed according to the order of modules described below (Scheme S1). Before peptide assembly, the resin was placed in the reactor, added DMF and stirred for 20 min for swelling. The DMF in the reactor was evacuated and replaced with fresh solvent to wash the reactor. Between each coupling and deprotection module, a single 30 second wash with 3 mL of DMF was done while stirring.

EMGPS protocol:

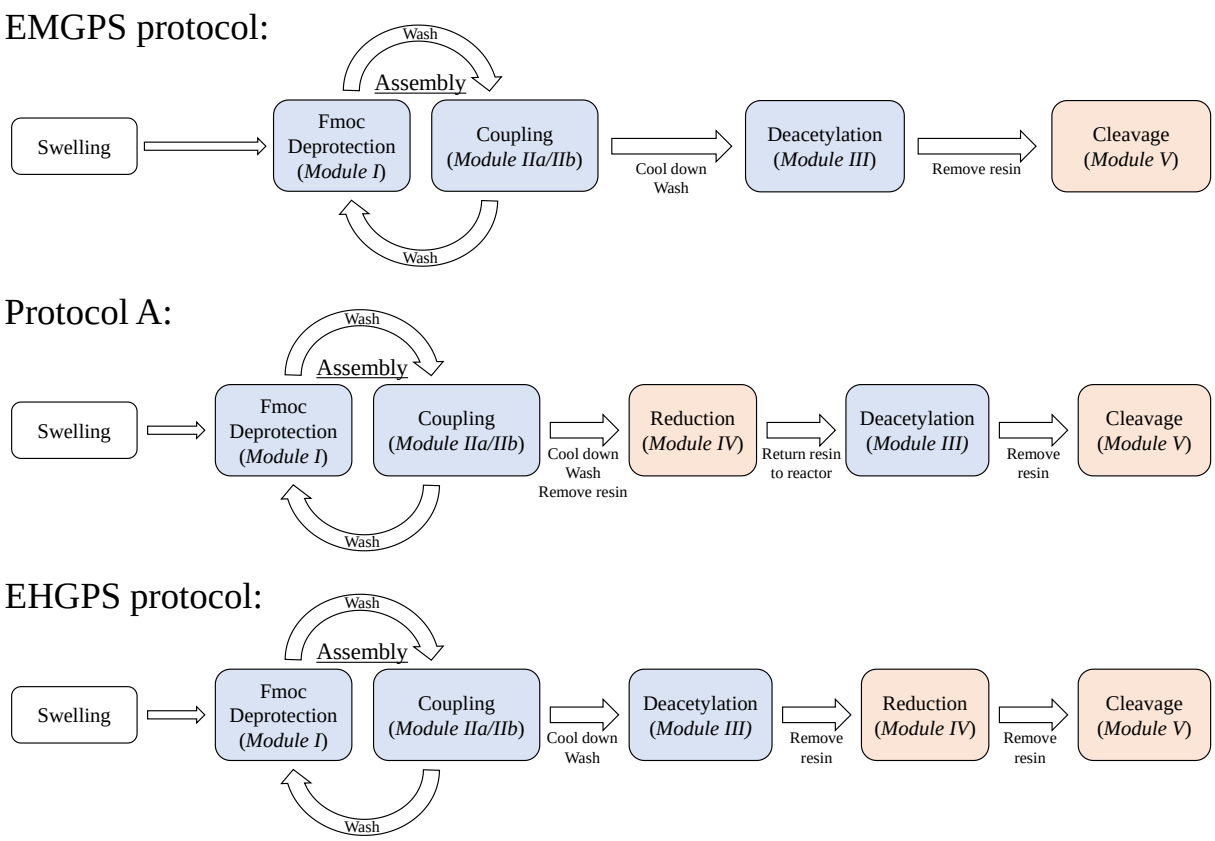
After swelling, peptides were assembled by repeating cycles of Fmoc deprotection (*Module I*) and coupling (*Module IIa* for aas or *Module IIb* for GAAs).[3] After each stage, the solvent was filtered out and then the new reactants were injected into the reactor. The cycles were repeated to complete the peptide assembly and the last aa was deprotected with *Module I*. The reactor was allowed to cool down while washing the resin with DMF. Resin was washed with DCM and methanol before deacetylation (*Module III*). Resin was washed with DCM and MeOH, removed from the reactor and cleaved (*Module V*).

Protocol A:

After swelling, peptides were assembled by repeating cycles of Fmoc deprotection (*Module I*) and coupling (*Module IIa* for aas or *Module IIb* for GAAs). After each stage, the solvent was filtered out and then the new reactants were injected into the reactor. The cycles were repeated to complete the peptide assembly without removing the Fmoc from the terminal amine group. The resin was washed with DMF, DCM, methanol, and Et_2O, dried, and removed from the reactor. Azide reduction was done (*Module IV*). Following reduction, the resin was placed back in the reactor for deacetylation and Fmoc removal (*Module III*). Resin was washed with DCM and MeOH, removed from the reactor and cleaved (*Module V*).

EHGPS Protocol (Protocol B):

After swelling, peptides were assembled by repeating cycles of Fmoc deprotection (*Module I*) and coupling (*Module IIa* for aas or *Module IIb* for GAAs). After each stage, the solvent was filtered out and then the new reactants were injected into the reactor. The cycles were repeated to complete the peptide assembly. The reactor was allowed to cool down while washing the resin with DMF. Resin was washed with DCM and methanol before deacetylation (*Module III*). The resin was washed with DCM, methanol, and Et_2O, dried, and removed from the reactor before azide reduction (*Module IV*), and cleavage (*Module V*) were consecutively executed.



Scheme S1 - Module organization in the different EGPS protocols.

Deviations from the general protocol

The synthesis of **αDG-1** followed EMGPS protocol using only *Module II* for coupling and skipping the deacetylation steps.

Analyses

Analyses of synthetic products

Analysis of 2-Azido-2-deoxy-1-*p*-tolylthio-3,4,6-tri-O-acetyl-D-galactopyranose **7**

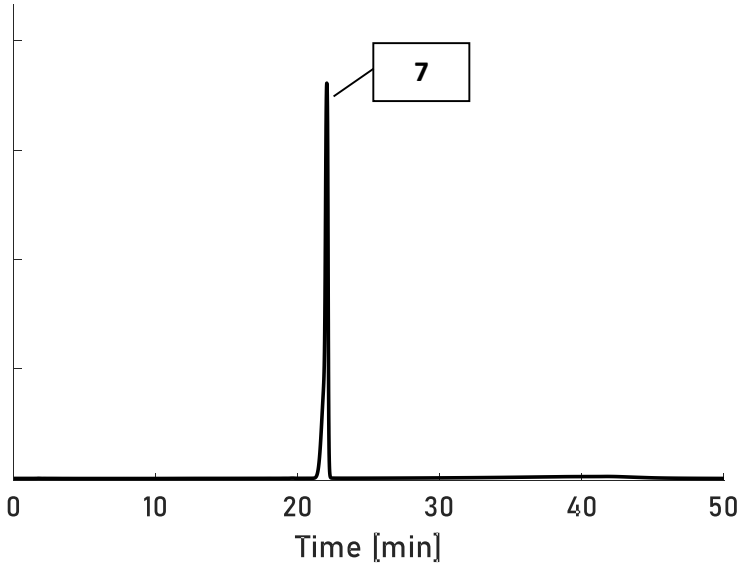


Figure S1 - Analytical HPLC chromatogram of purified **7** (recorded at 254nm).

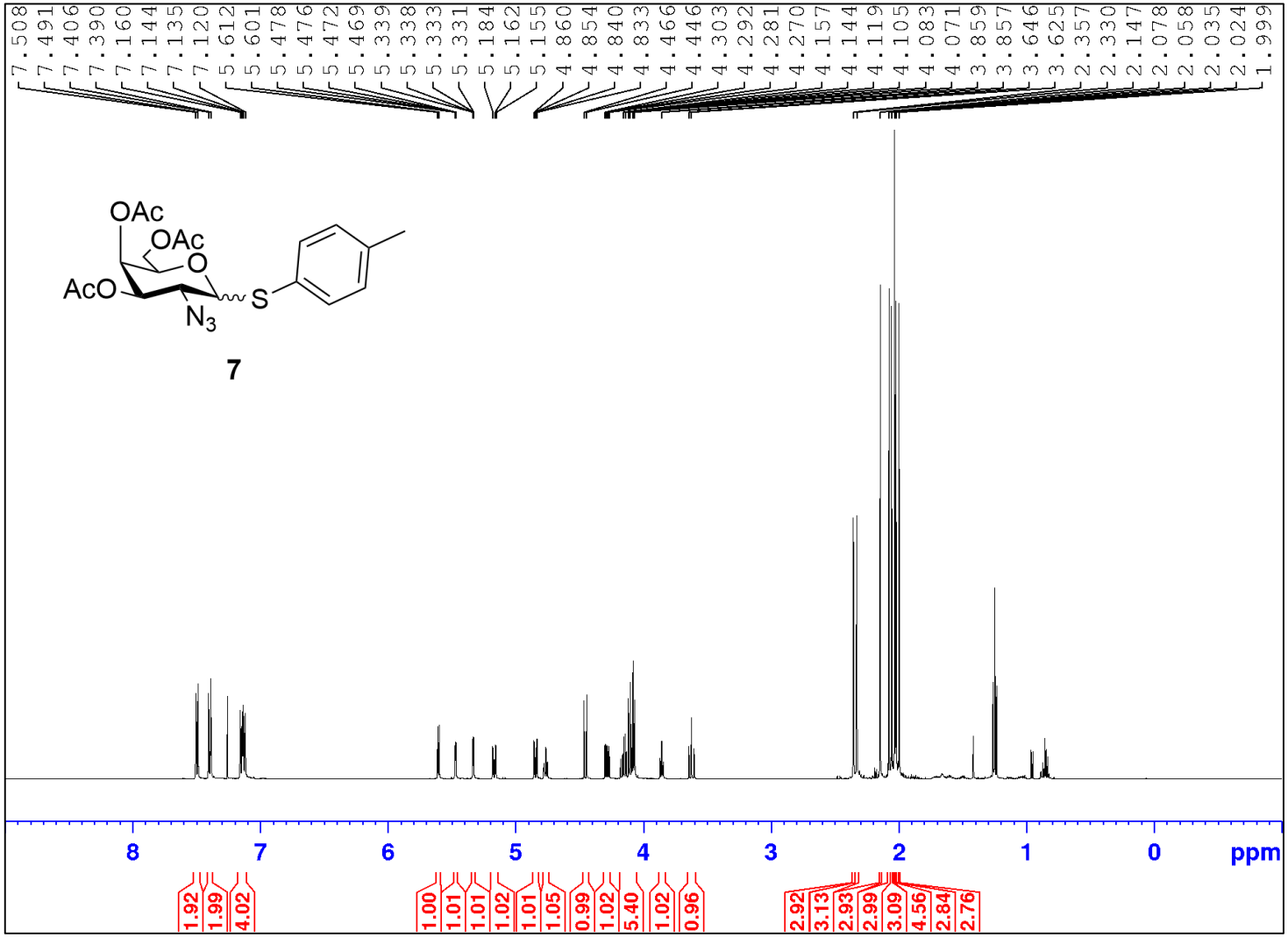


Figure S2 - ^{1}H NMR analysis of the anomeric mixture of **7** ($CDCl_3$, 500MHz). Residual peaks: δ 4.12, (q, EtOAc), 2.04 (s, EtOAc), 1.25 (t, EtOAc). The anomeric ratio was determined based on the integration of the doublet at 4.46 ppm (β anomer) and the doublet at 5.61 ppm (α anomer).

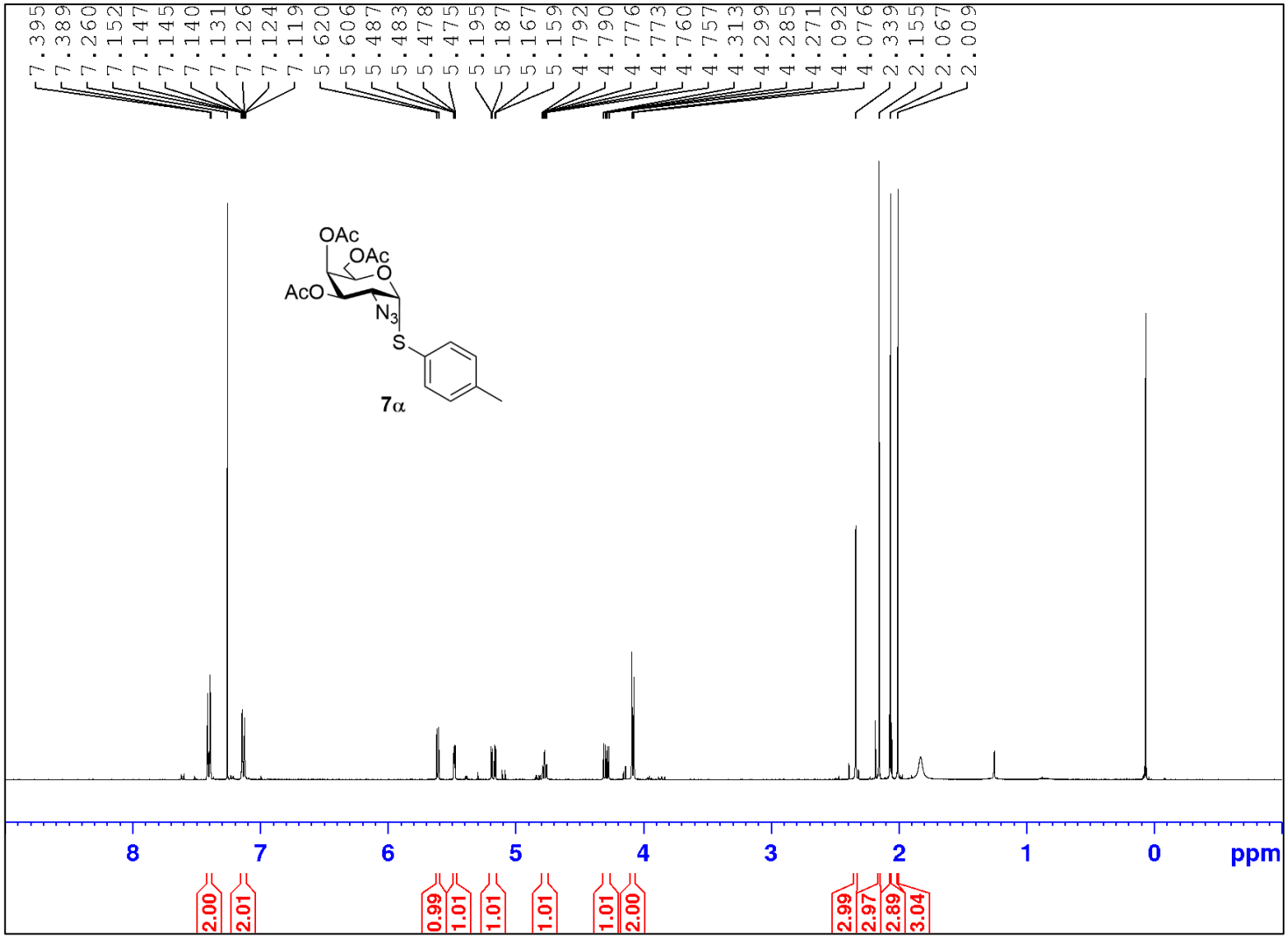


Figure S3 - ^{1}H NMR analysis of α anomer of **7** ($CDCl_3$, 400MHz). Residual peaks: δ 1.25 (s, grease), 0.07 (s, grease).

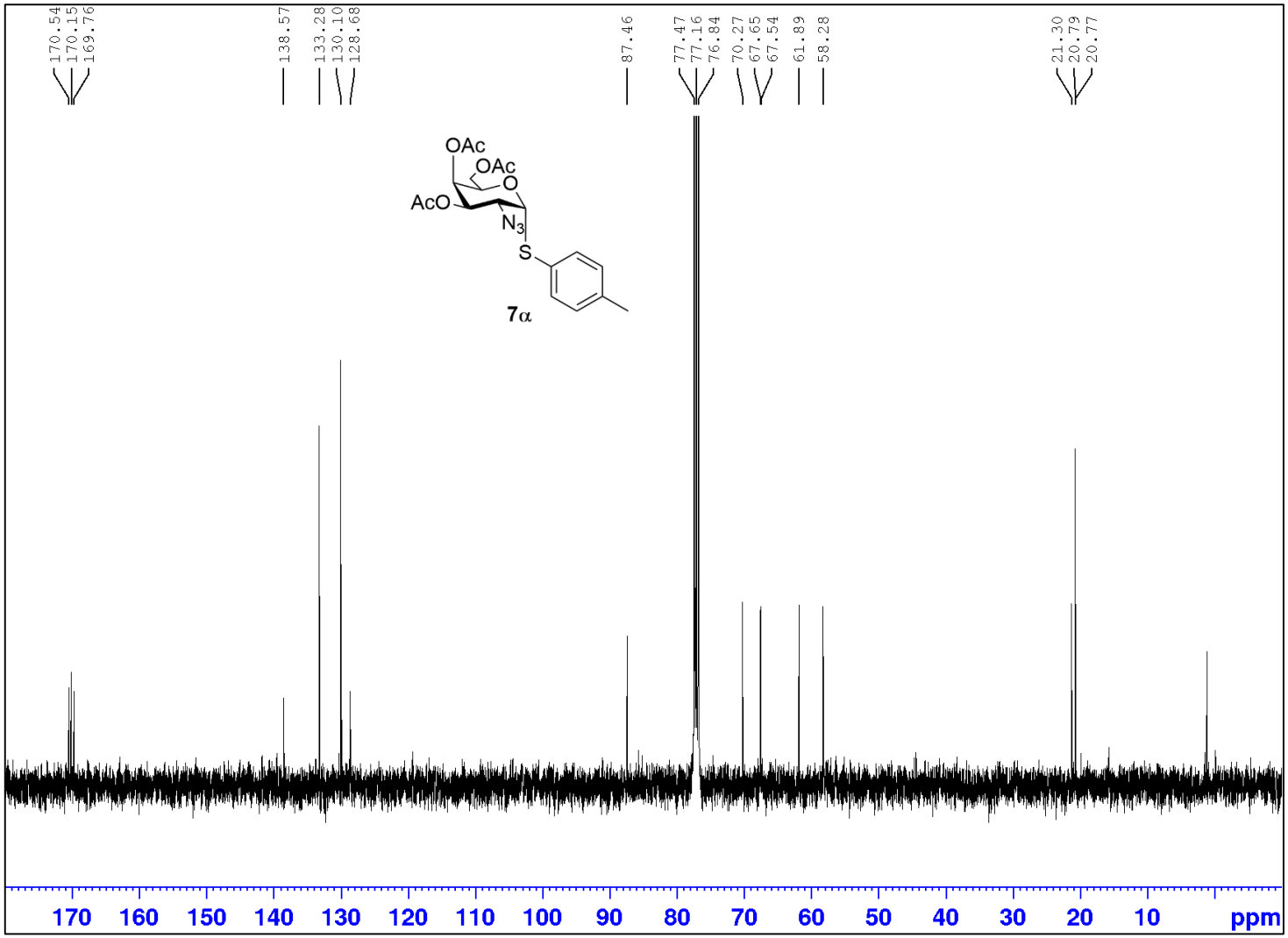


Figure S4 - ^{13}C NMR analysis of α anomer of **7** ($CDCl_3$, 100 MHz).

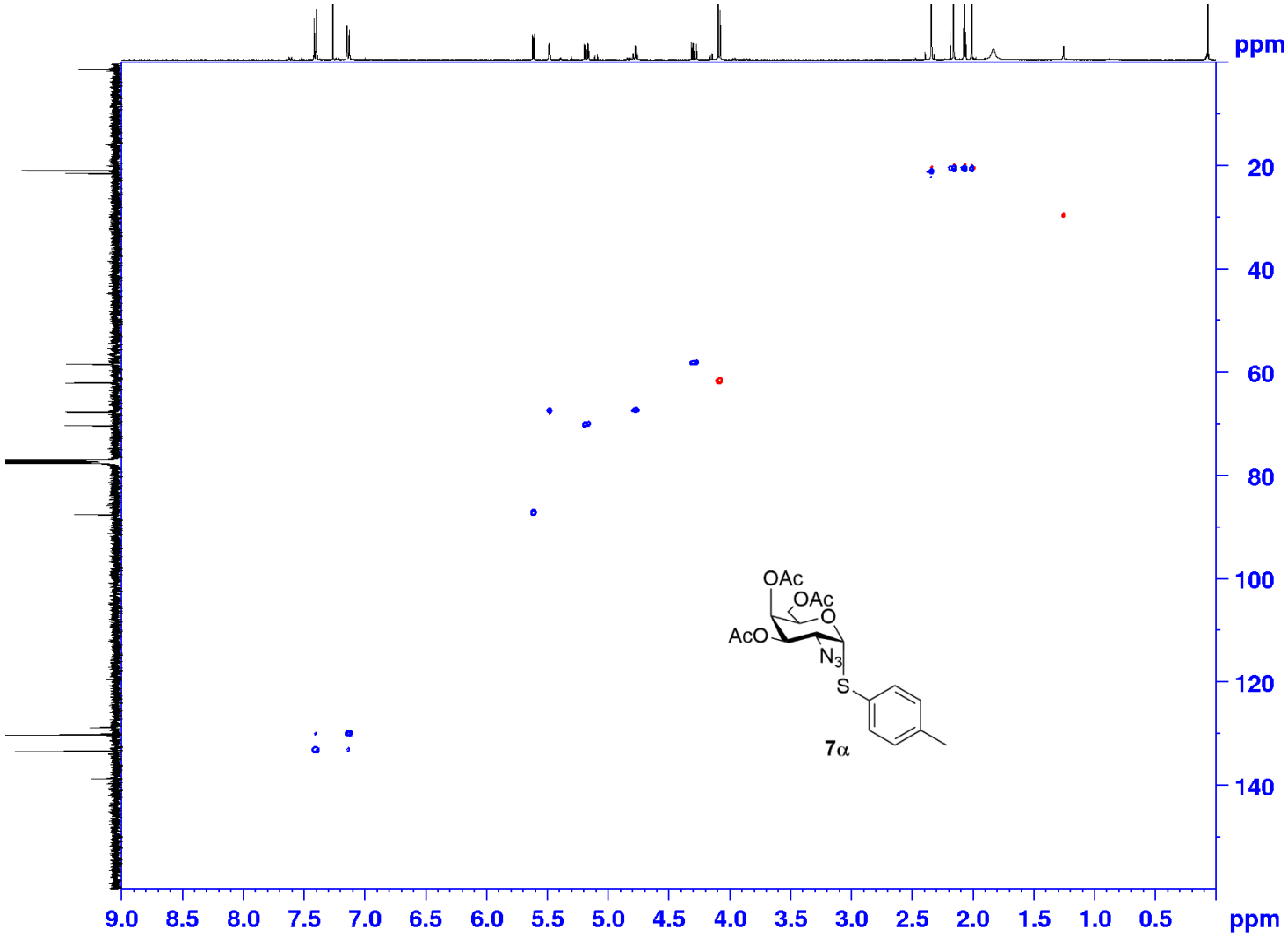


Figure S5 - HSQC NMR analysis of α anomer of **7** ($CDCl_3$, 400MHz).

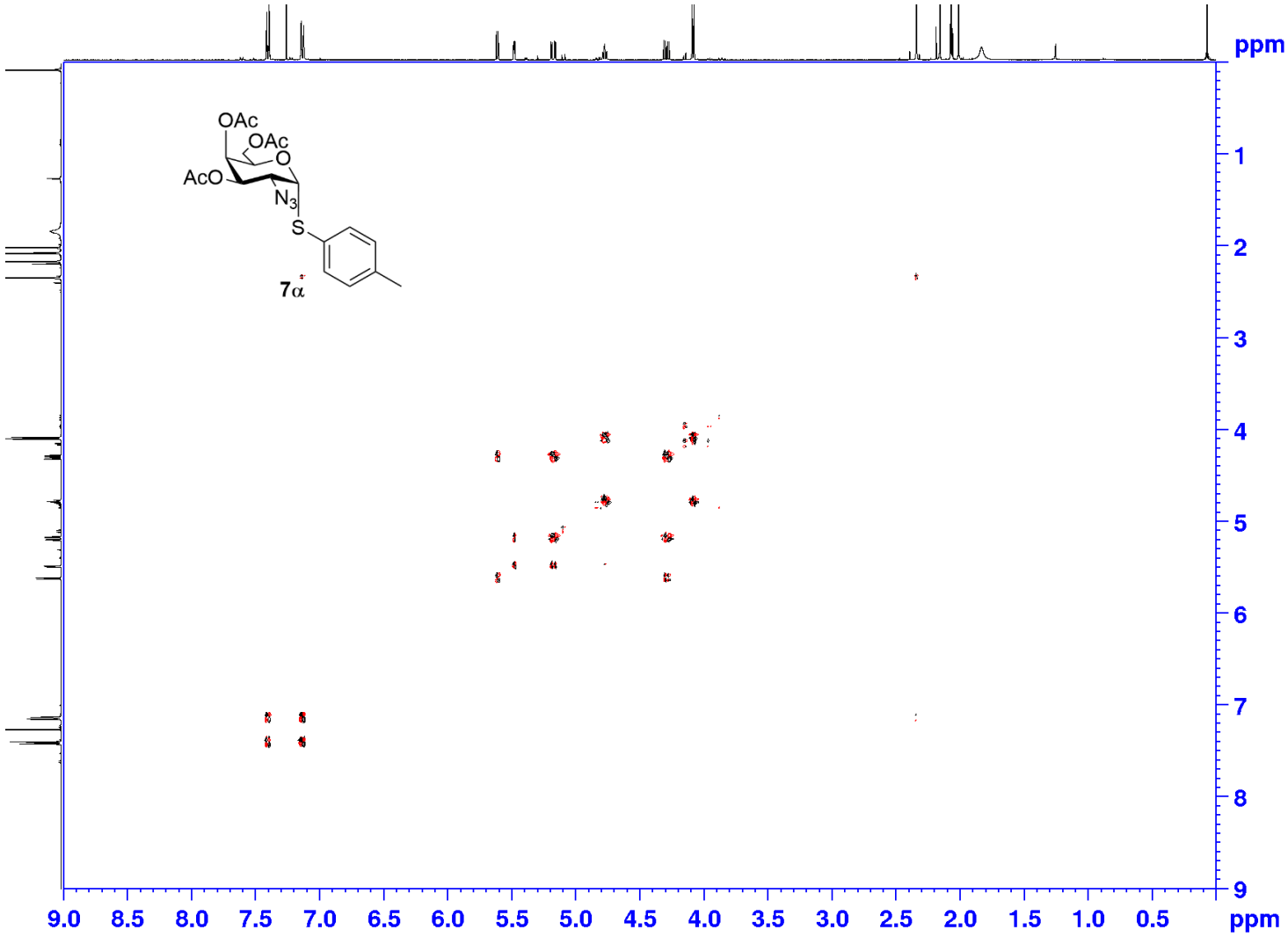


Figure S6 - COSY NMR analysis of α anomer of **7** ($CDCl_3$, 400MHz).

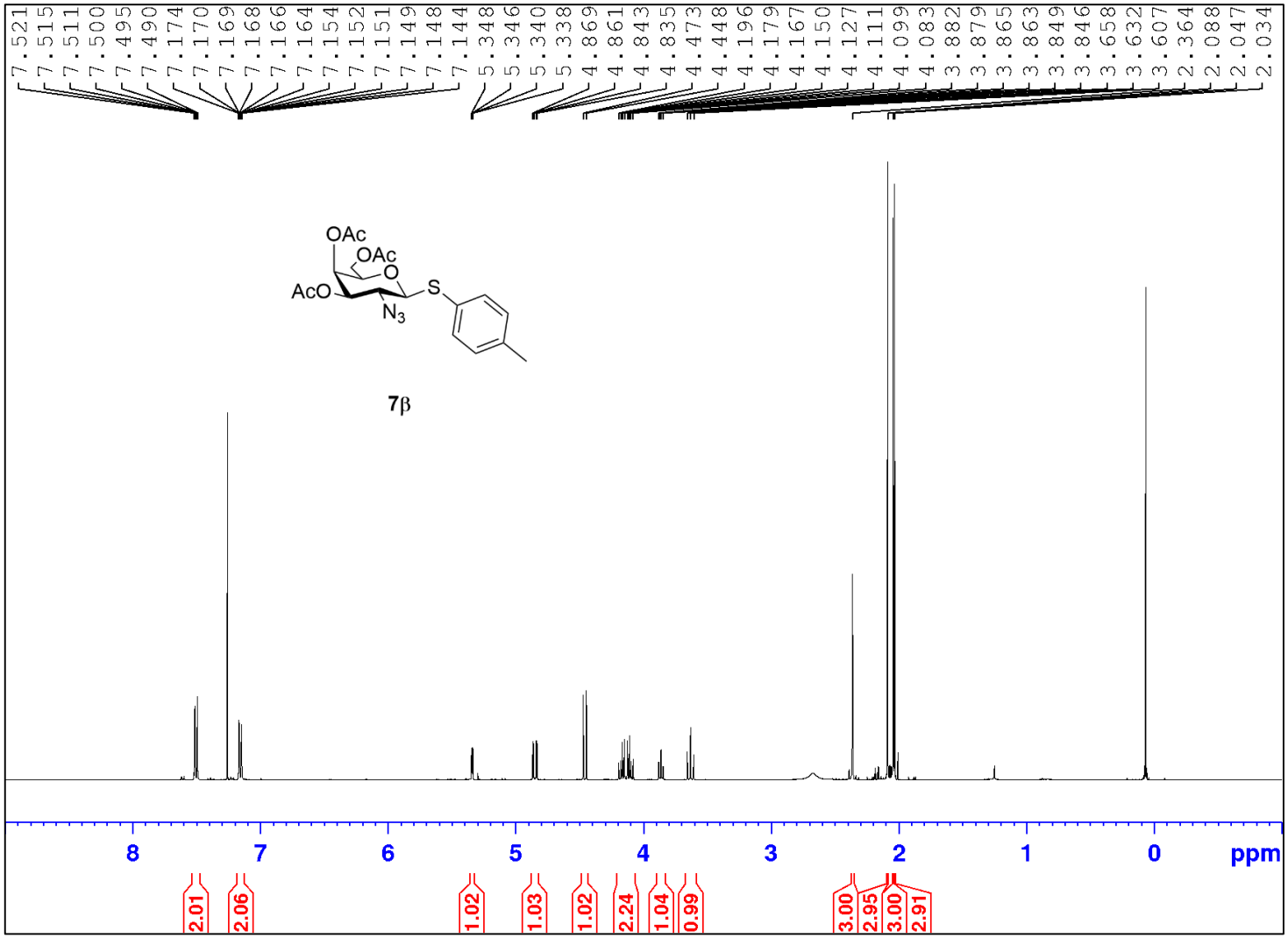


Figure S7 - ^{1}H NMR analysis of β anomer of **7** ($CDCl_3$, 400MHz). Residual peaks: δ 1.25 (s, grease), 0.07 (s, grease).

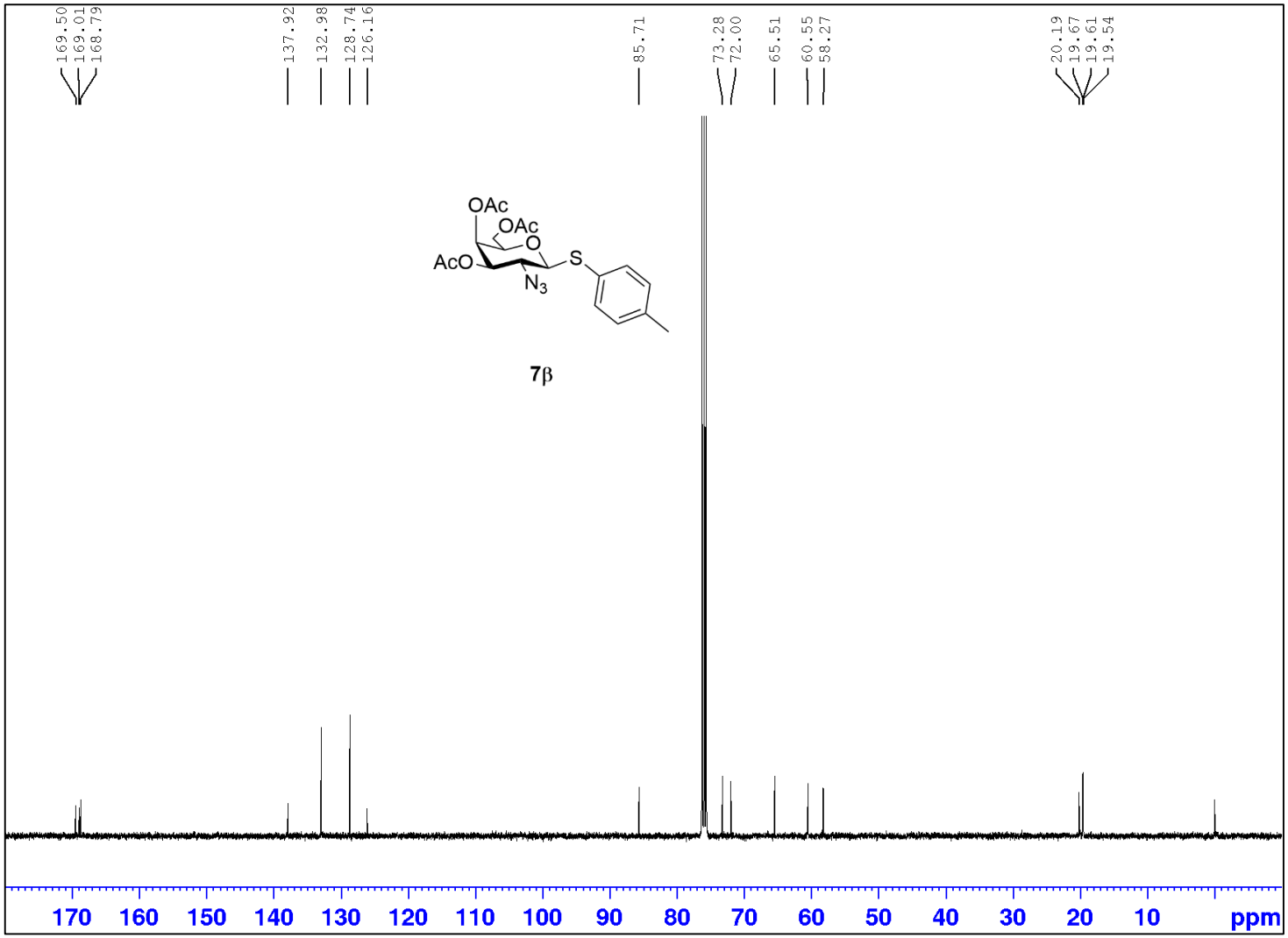


Figure S8 - ^{13}C NMR analysis of β anomer of **7** ($CDCl_3$, 100 MHz).

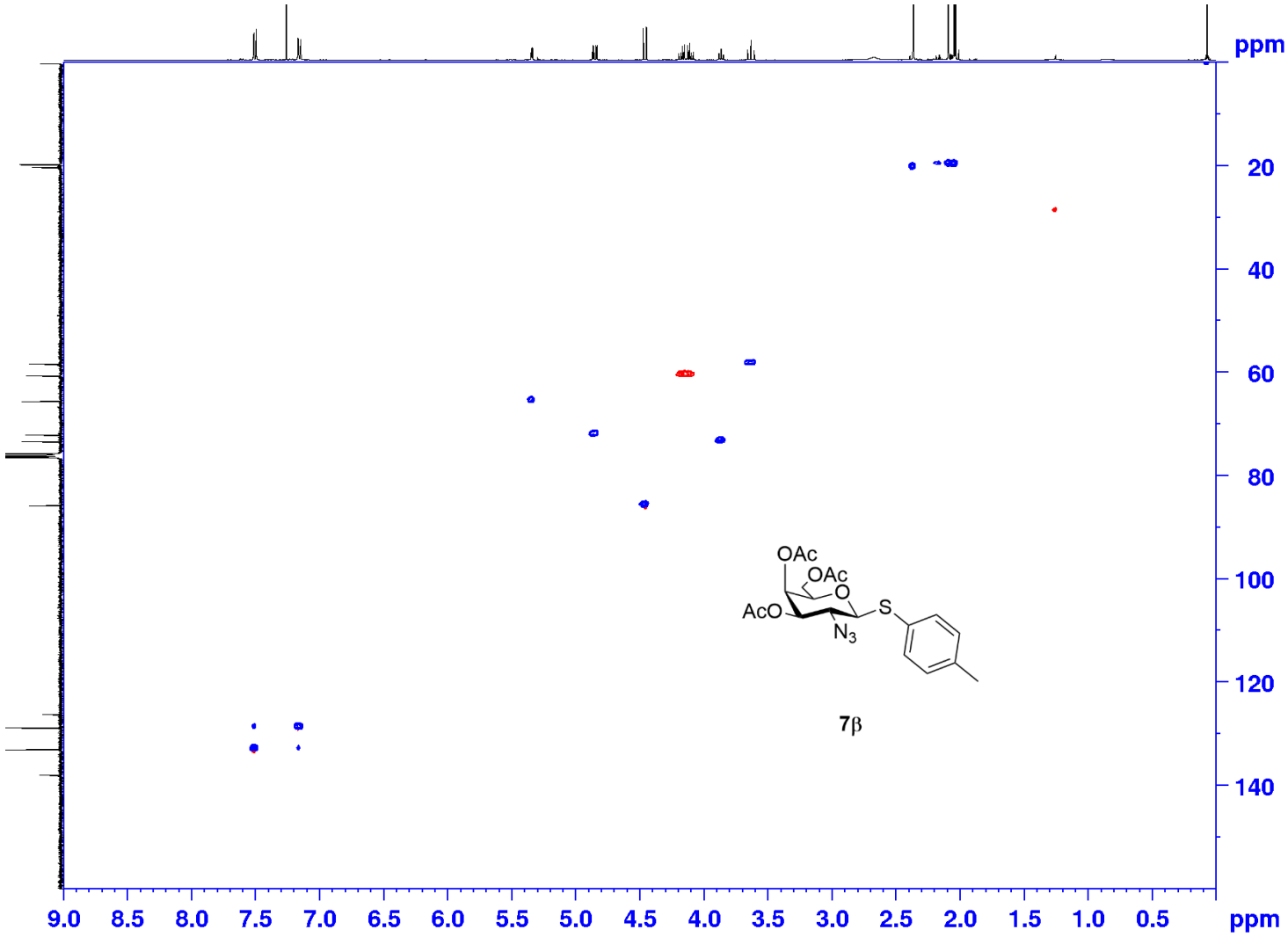


Figure S9 - HSQC NMR analysis of β anomer of **7** ($CDCl_3$, 400MHz).

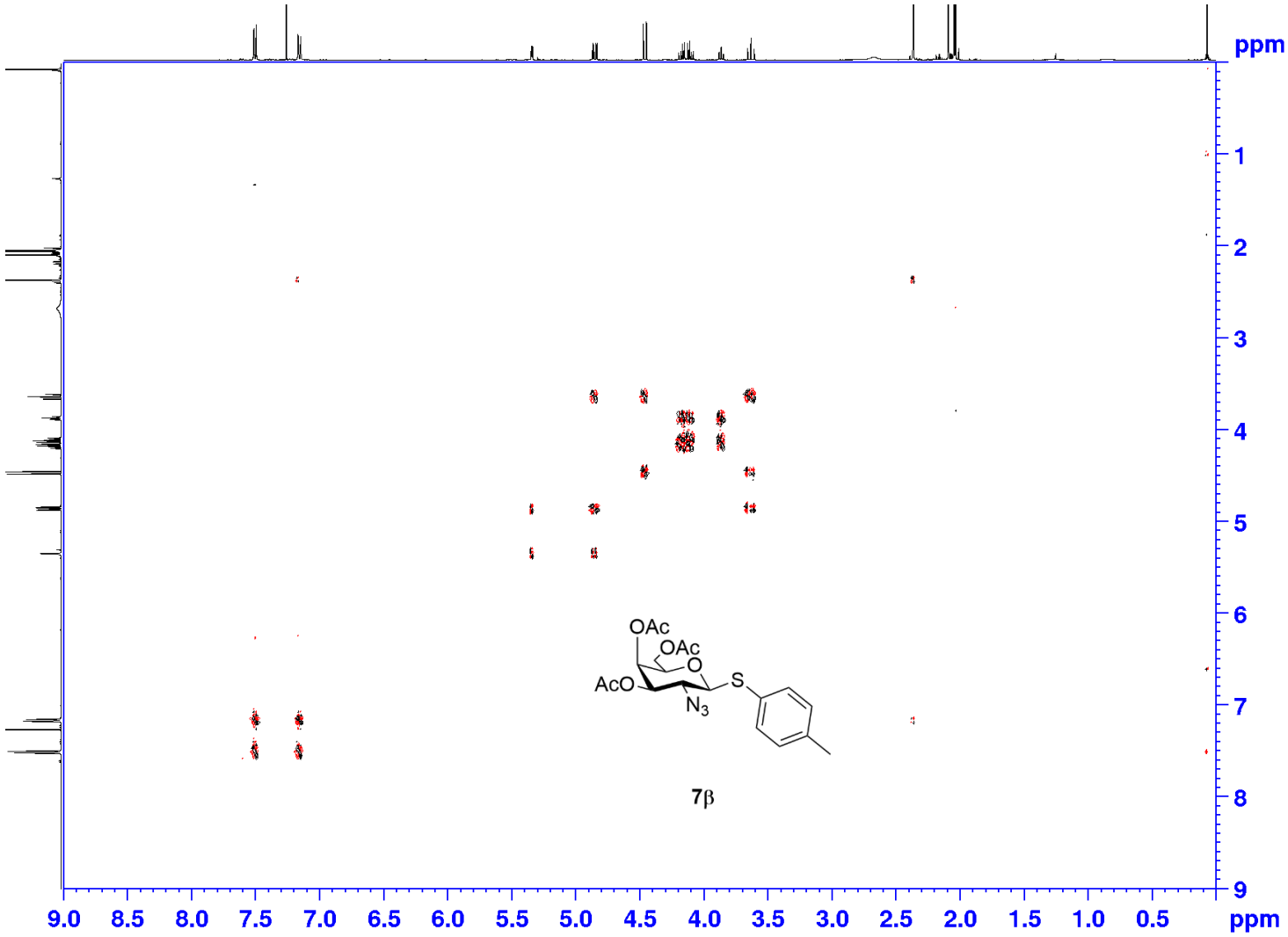


Figure S10 - COSY NMR analysis of β anomer of **7** ($CDCl_3$, 400MHz)

Analysis of tert-Butyl N-Fmoc-O-(2-azido-2-deoxy-3,4,6-tri-O-acetyl-D-Galactopyranosyl)-L-Serinate **9**

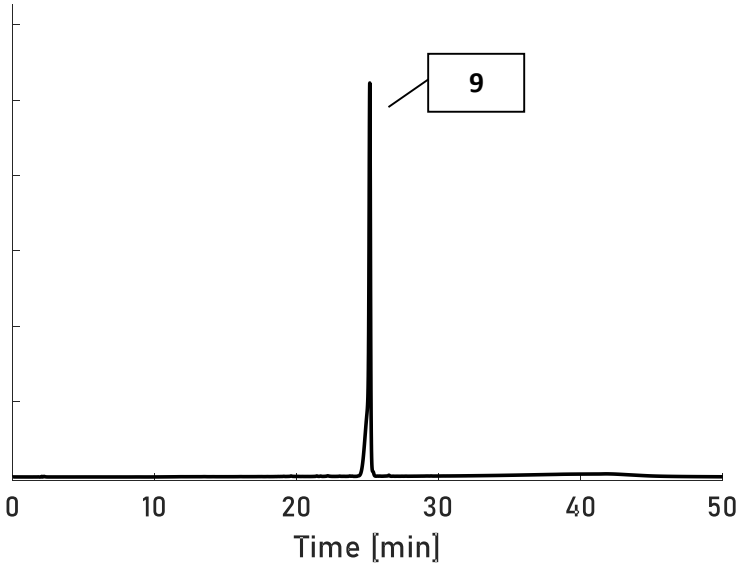


Figure S11 - Analytical HPLC chromatogram of purified **9** (recorded at 254nm).

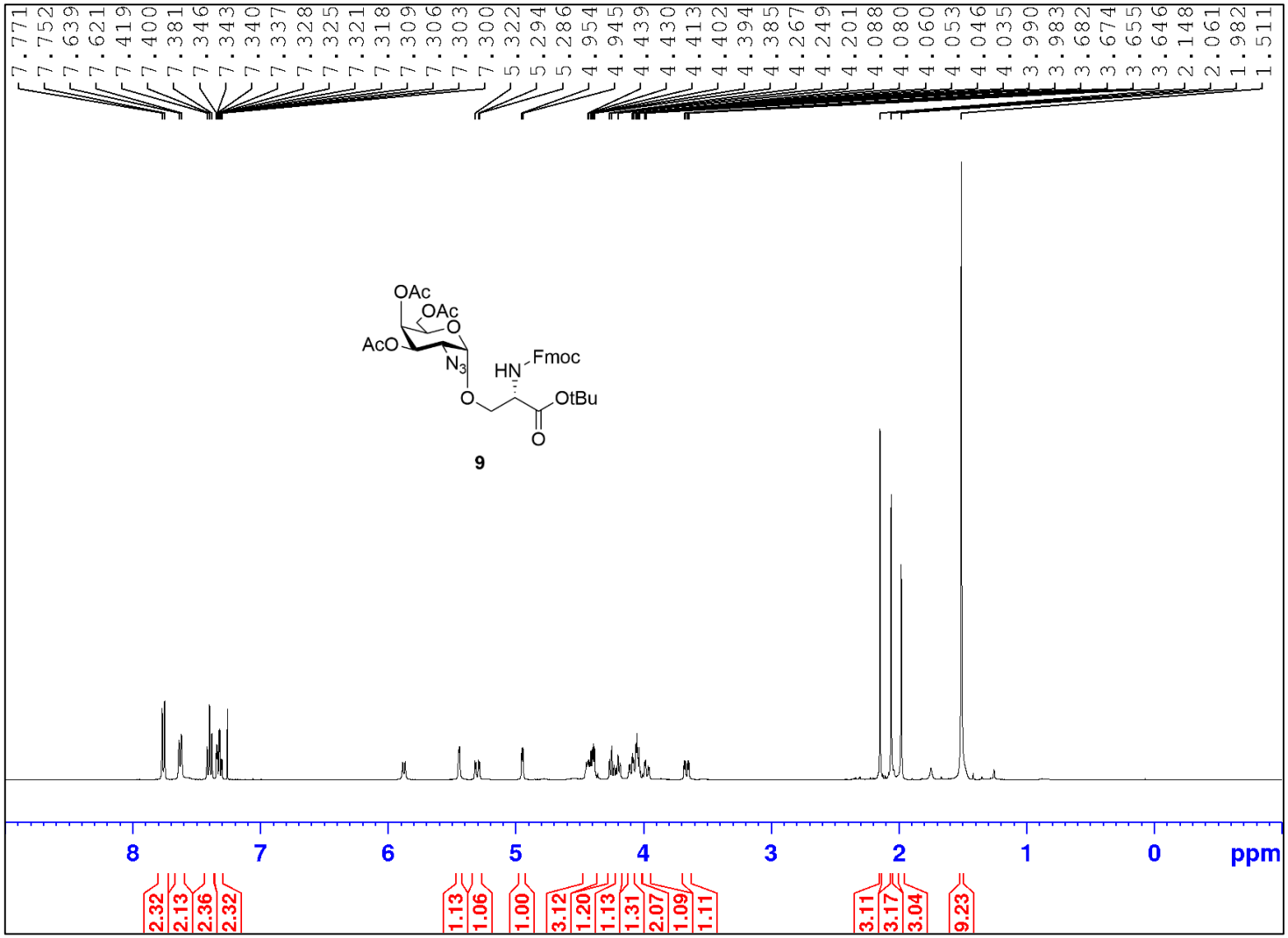


Figure S12 - ^{1}H NMR analysis of **9** ($CDCl_3$, 400 MHz).

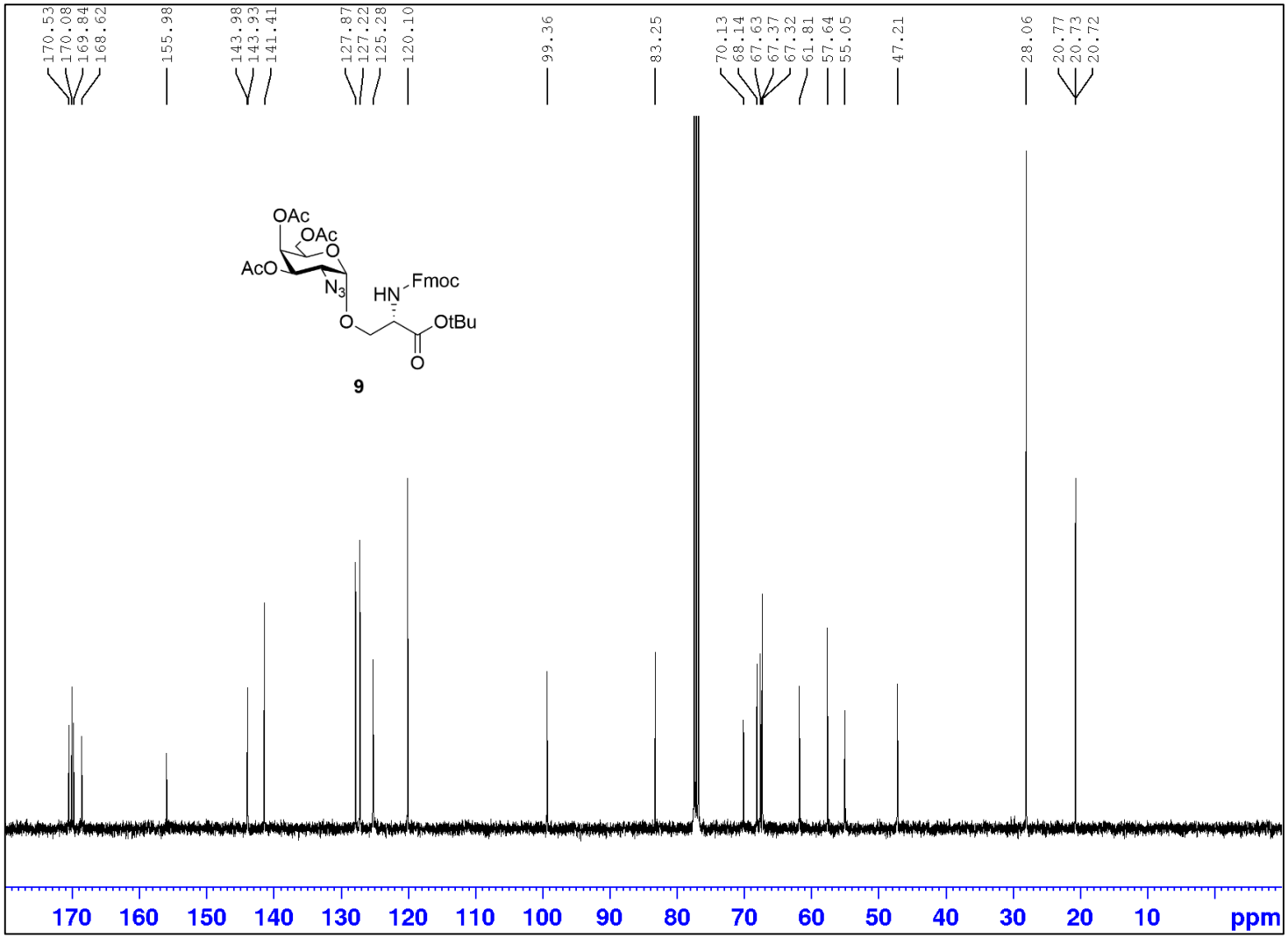


Figure S13 - ^{13}C NMR analysis of **9** ($CDCl_3$, 100 MHz).

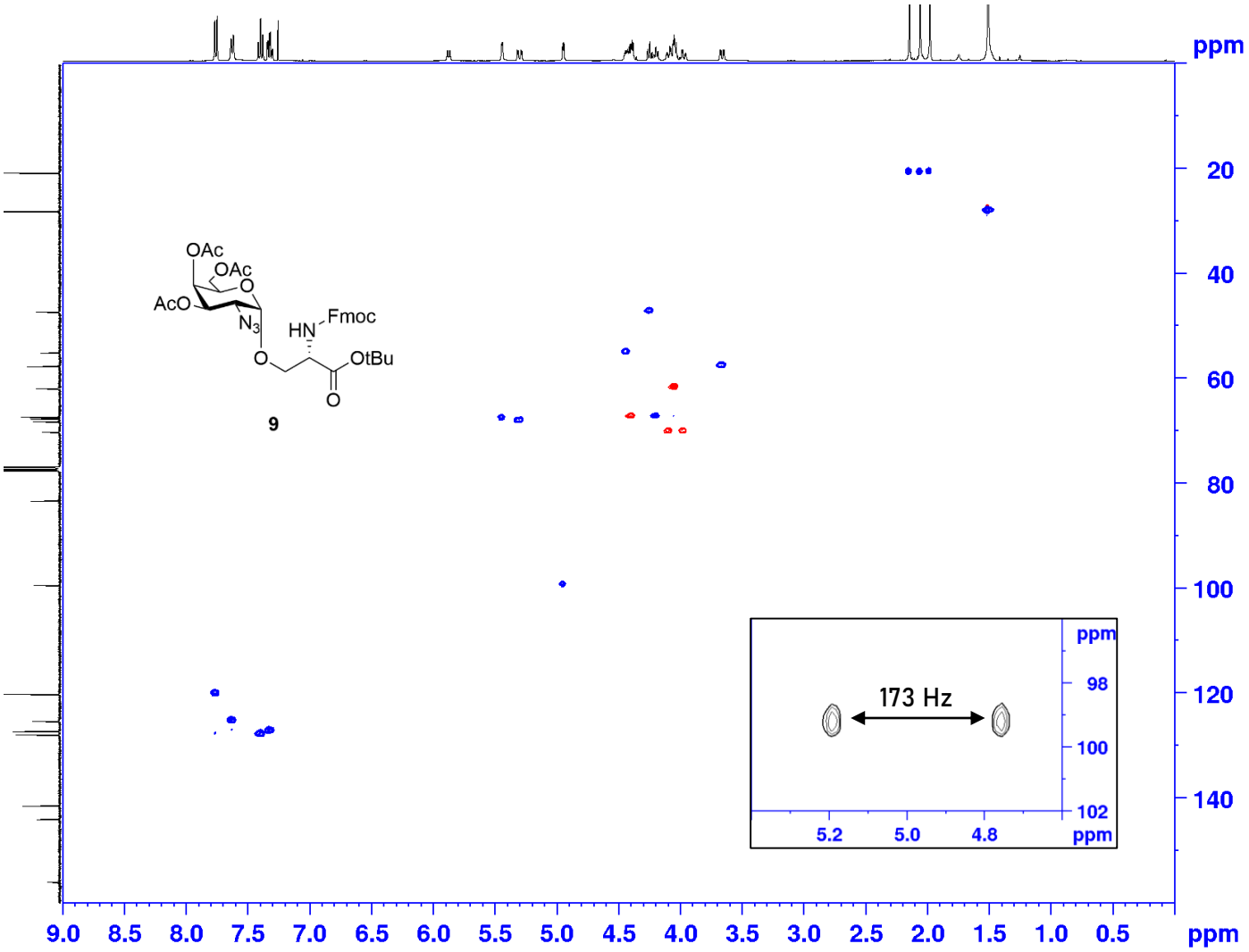


Figure S14 - HSQC NMR analysis of **9** ($CDCl_3$, 400MHz). Inset: Coupled HSQC NMR analysis of anomeric region ($CDCl_3$, 400 MHz).

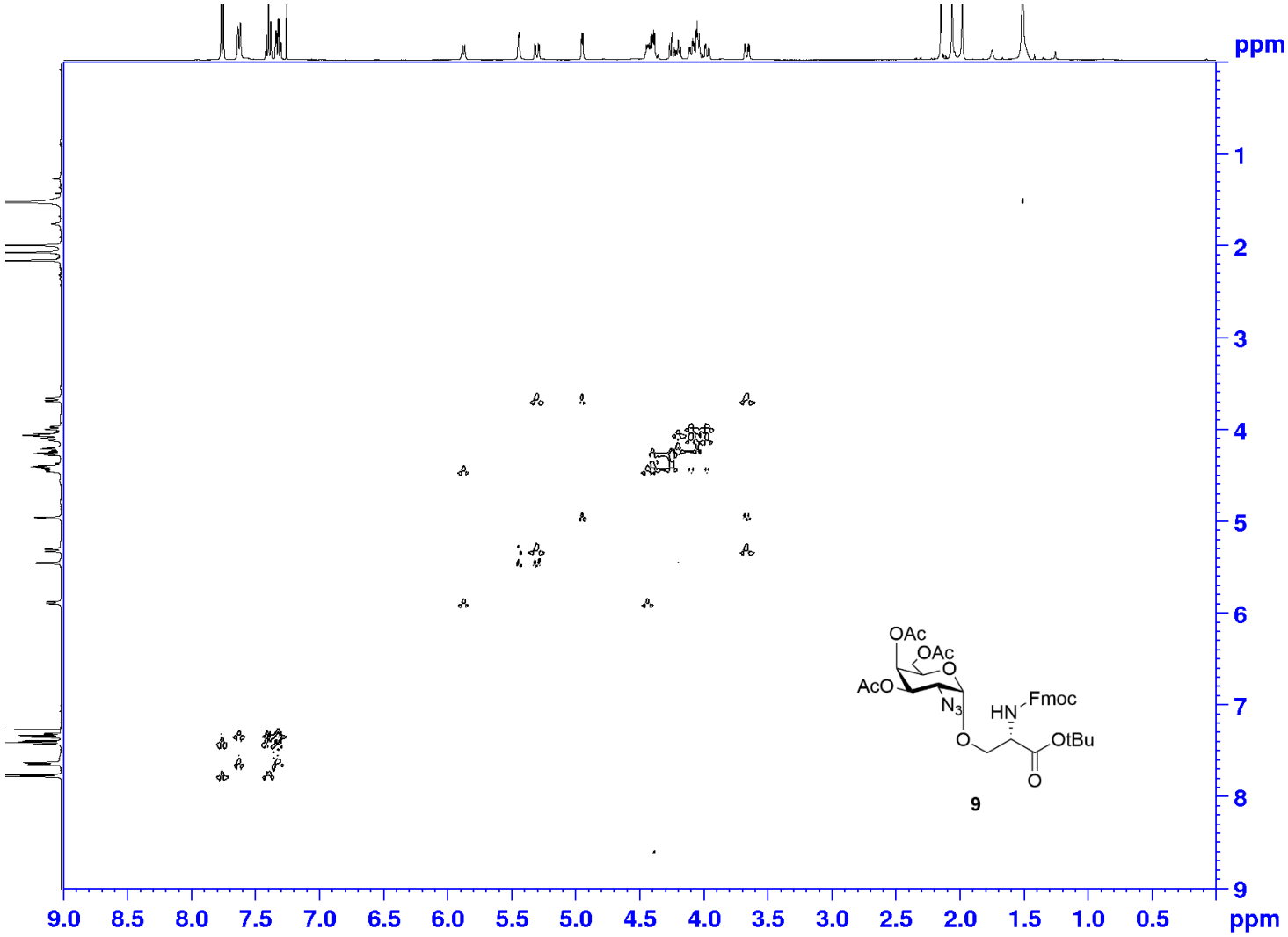


Figure S15 - COSY NMR analysis of **9** ($CDCl_3$, 400 MHz).

Analysis of tert-Butyl N-Fmoc-O-(2-azido-2-deoxy-3,4,6-tri-O-acetyl-D-Galactopyranosyl)-L-Threoninate **S1**

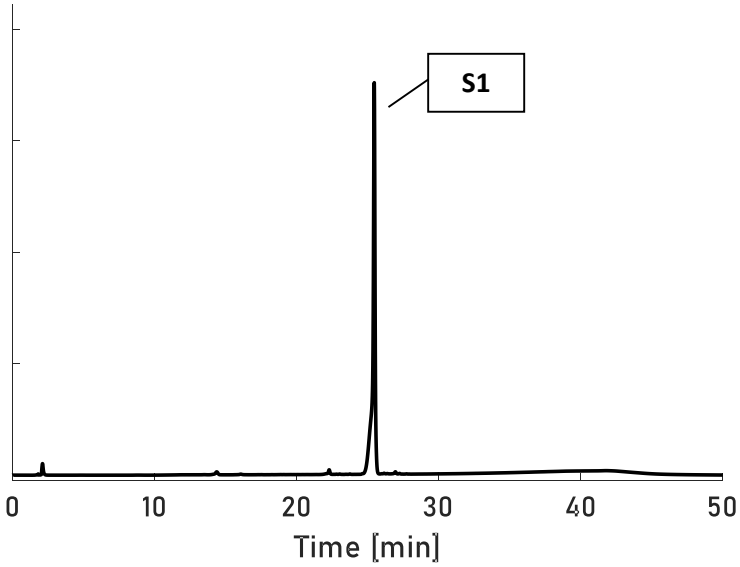


Figure S16 - Analytical HPLC chromatogram of purified **S1** (recorded at 254nm).

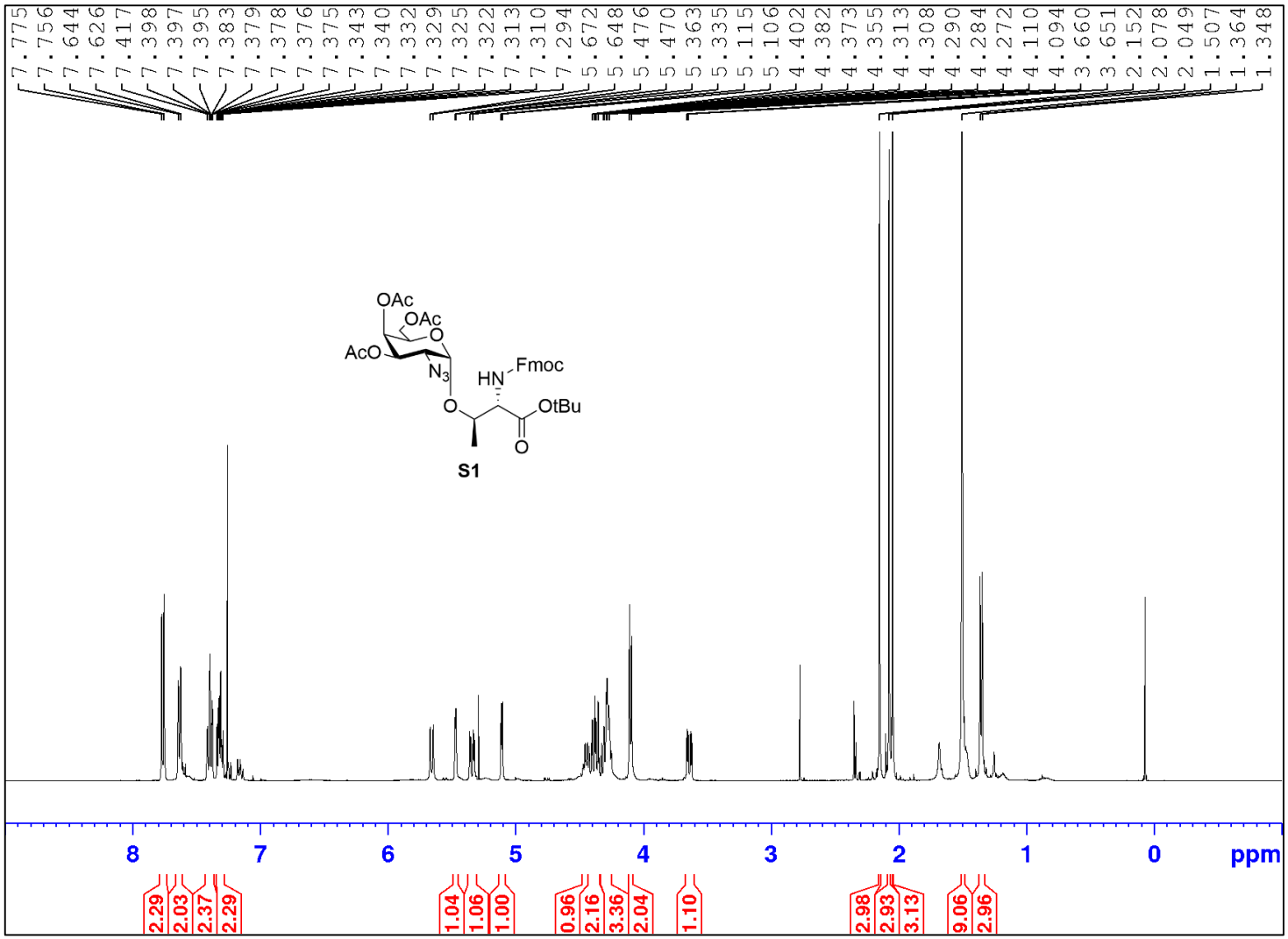


Figure S17 - ^{1}H NMR analysis of **S1** ($CDCl_3$, 400 MHz). Residual peaks: δ 7.25 (m, toluene), 7.17 (m, toluene), 2.77 (s, unknown artefact), 2.36 (s, toluene), 1.68 (s, unknown artefact), 1.25 (s, grease), 0.07 (s, grease).

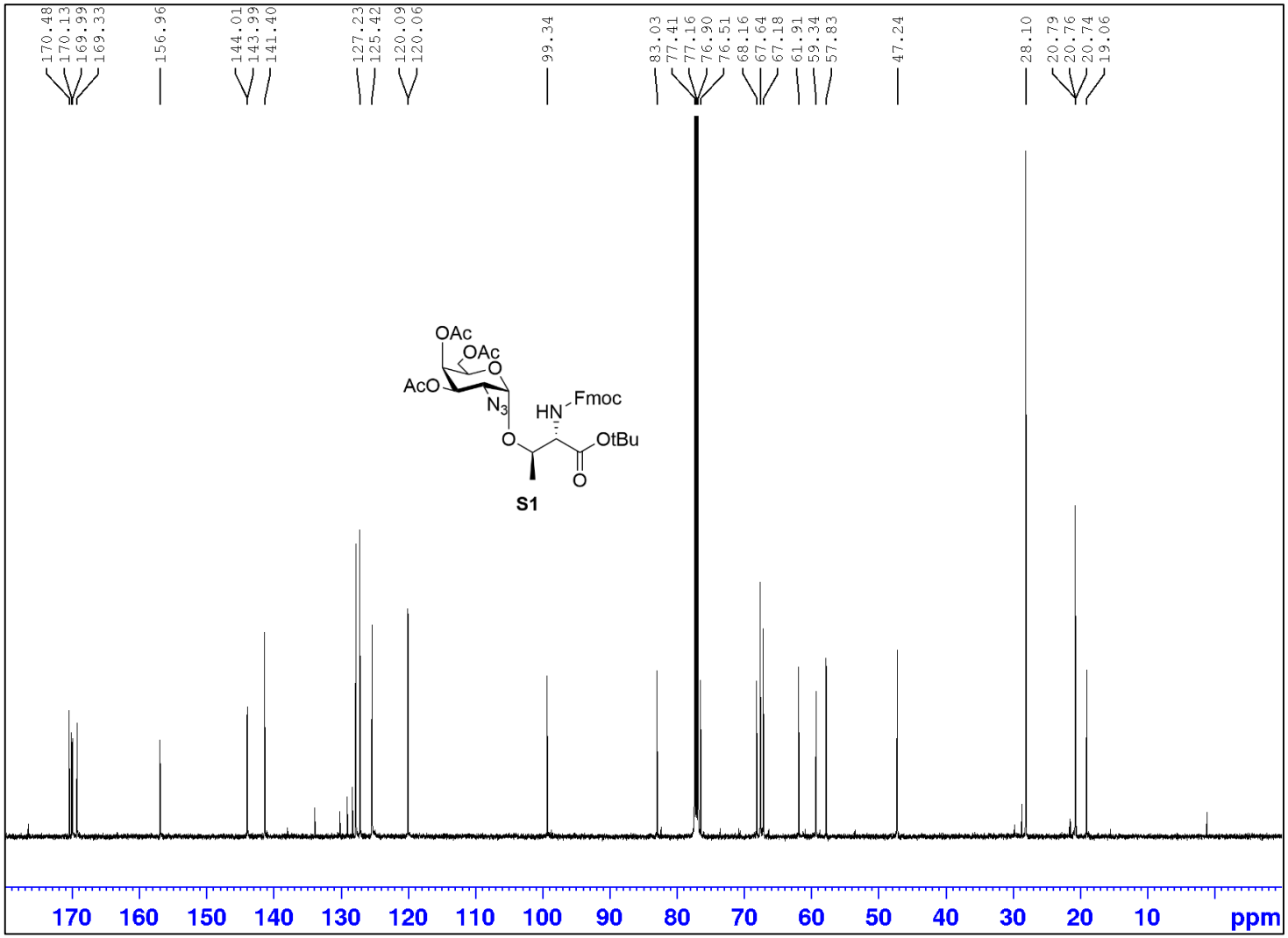


Figure S18 - ^{13}C NMR analysis of **S1** ($CDCl_3$, 125 MHz).

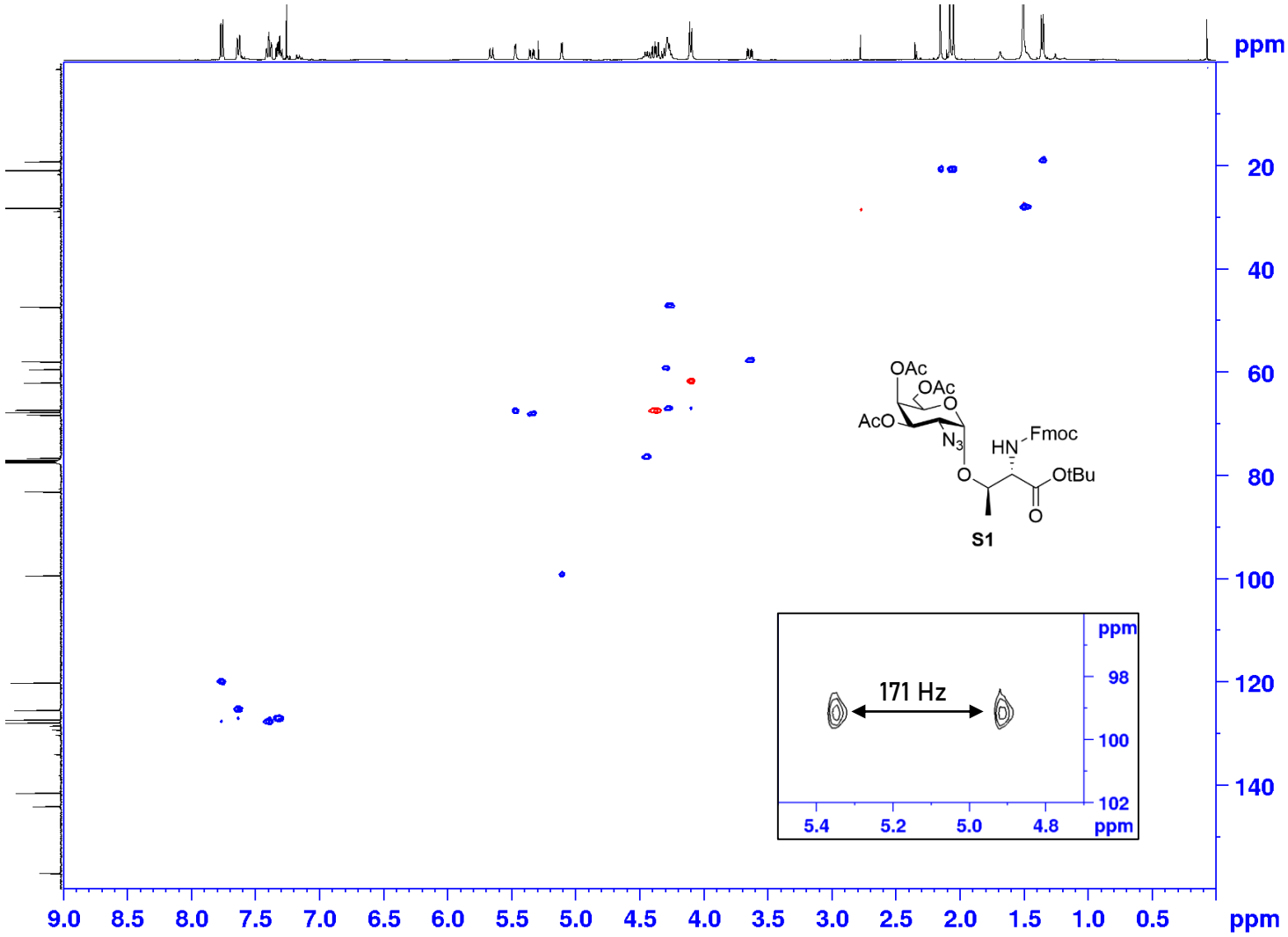


Figure S19 - HSQC NMR analysis of **S1** ($CDCl_3$, 400MHz). Inset: Coupled HSQC NMR analysis of anomeric region ($CDCl_3$, 400 MHz).

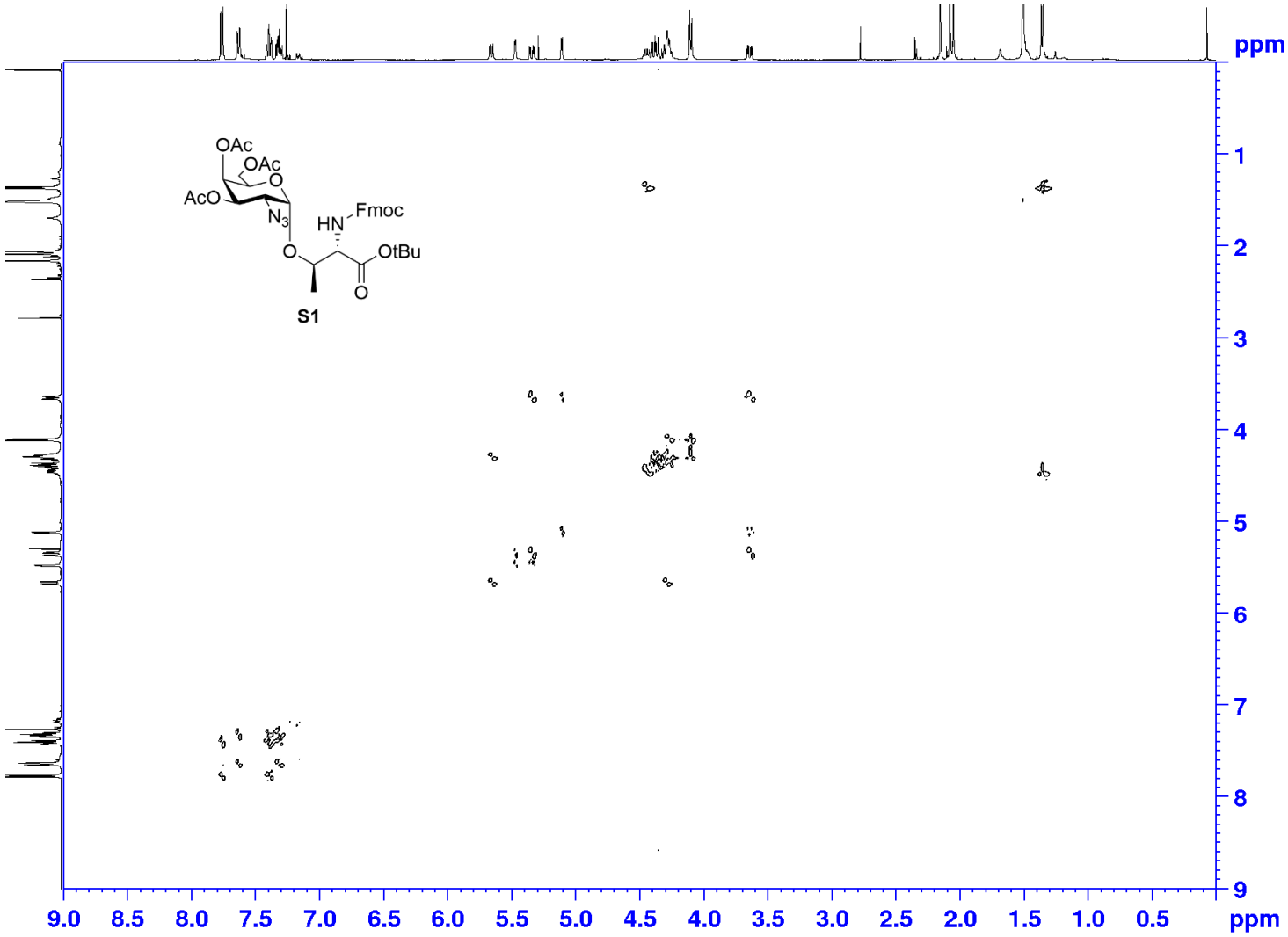


Figure S20 - COSY NMR analysis of **S1** ($CDCl_3$, 400 MHz).

Analysis of N-Fmoc-O-(2-azido-2-deoxy-3,4,6-tri-O-acetyl-D-Galactopyranosyl)-L-Serine **2s**

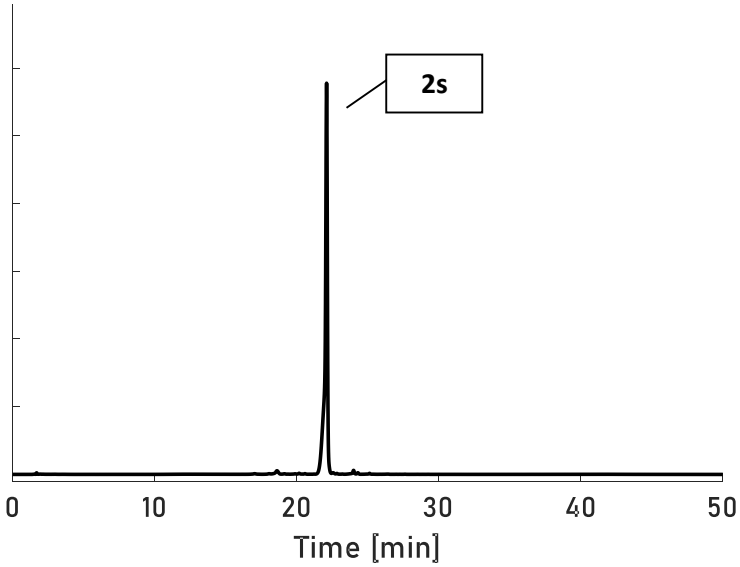


Figure S21 - Analytical HPLC chromatogram of purified **2s** (recorded at 254nm).

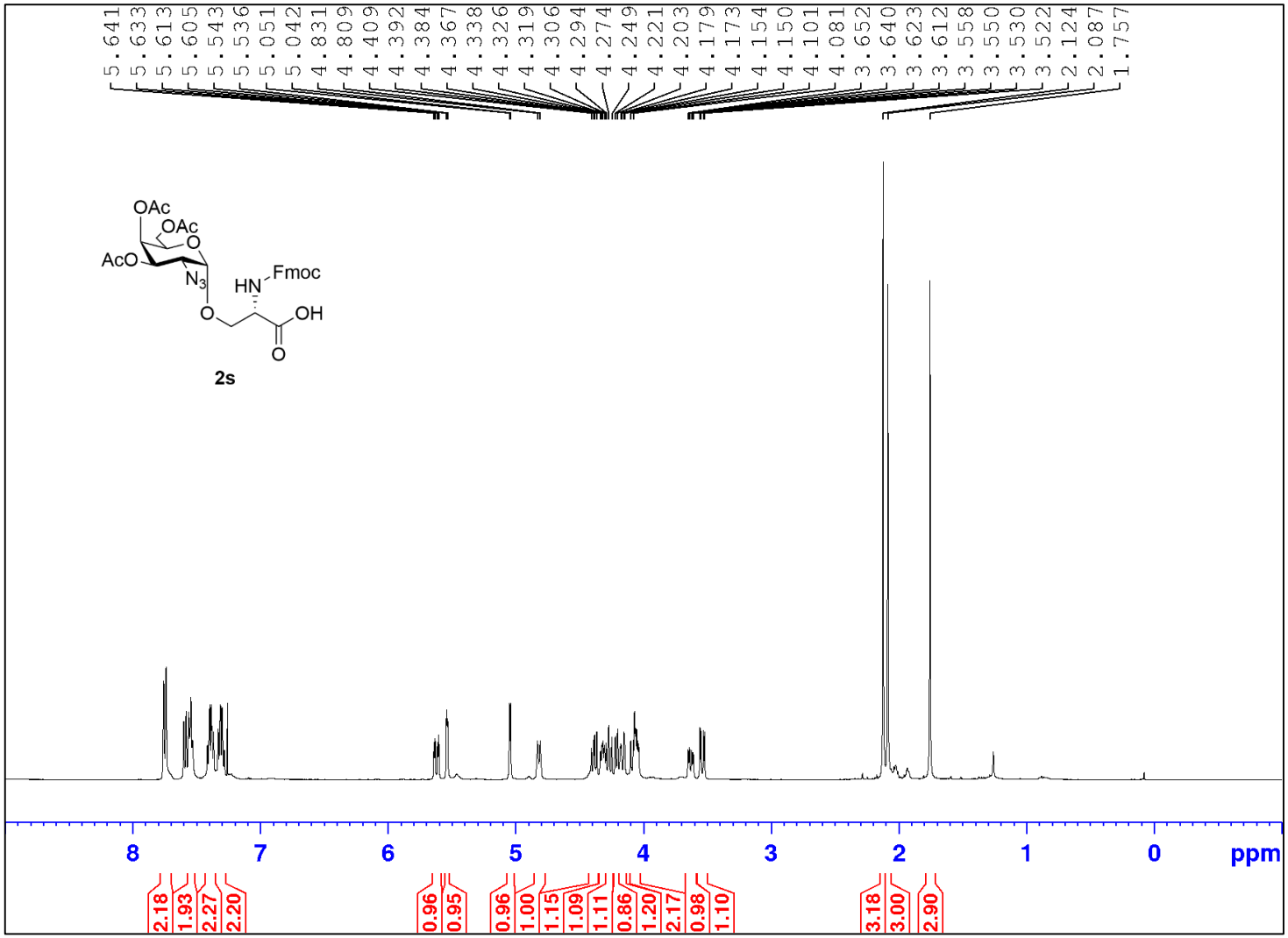


Figure S22 - ^{1}H NMR analysis of **2s** ($CDCl_3$, 400 MHz). Residual peaks: δ 1.25 (s, grease), 0.07 (s, grease).

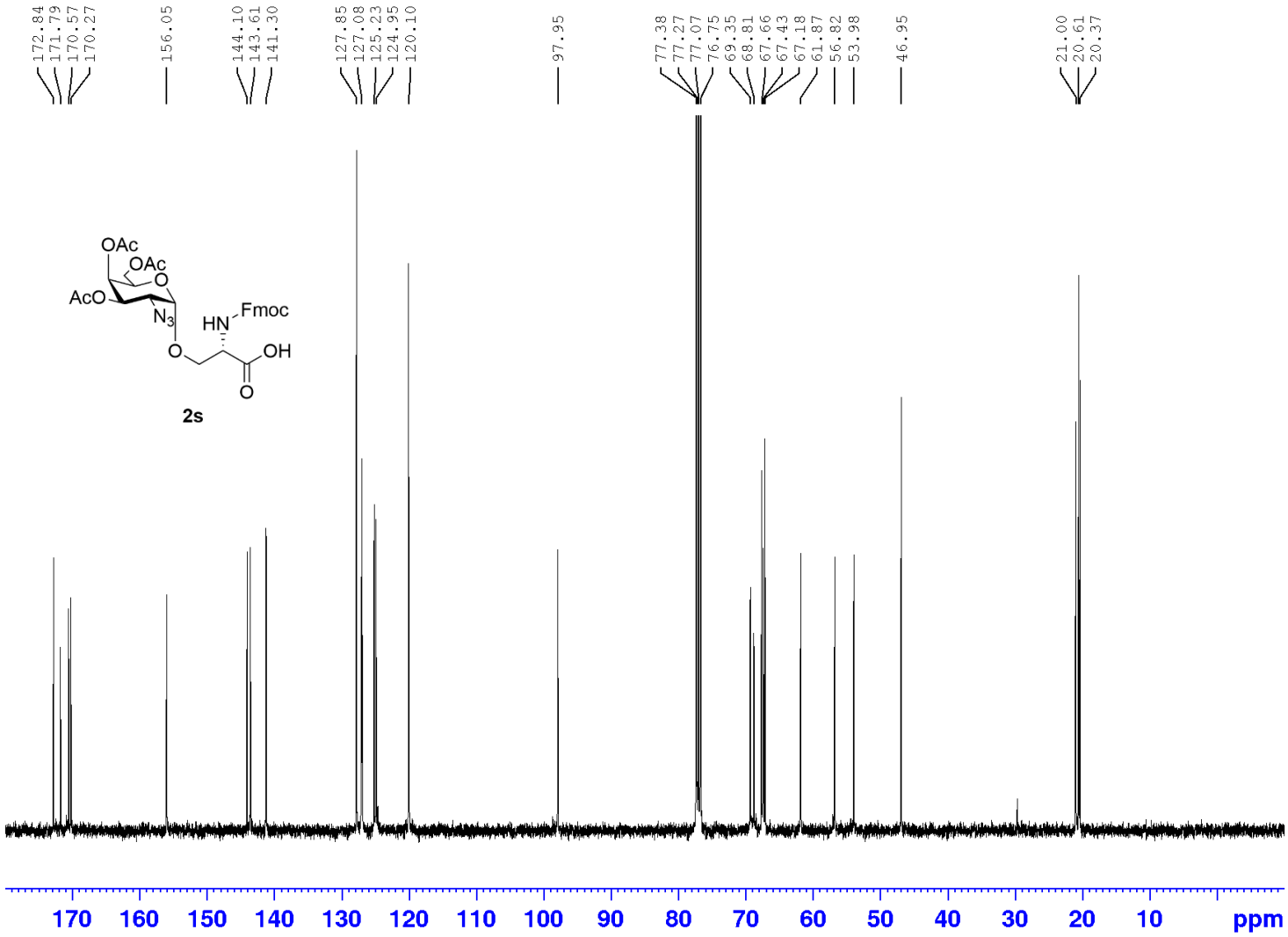


Figure S23 - ^{13}C NMR analysis of **2s** ($CDCl_3$, 100 MHz).

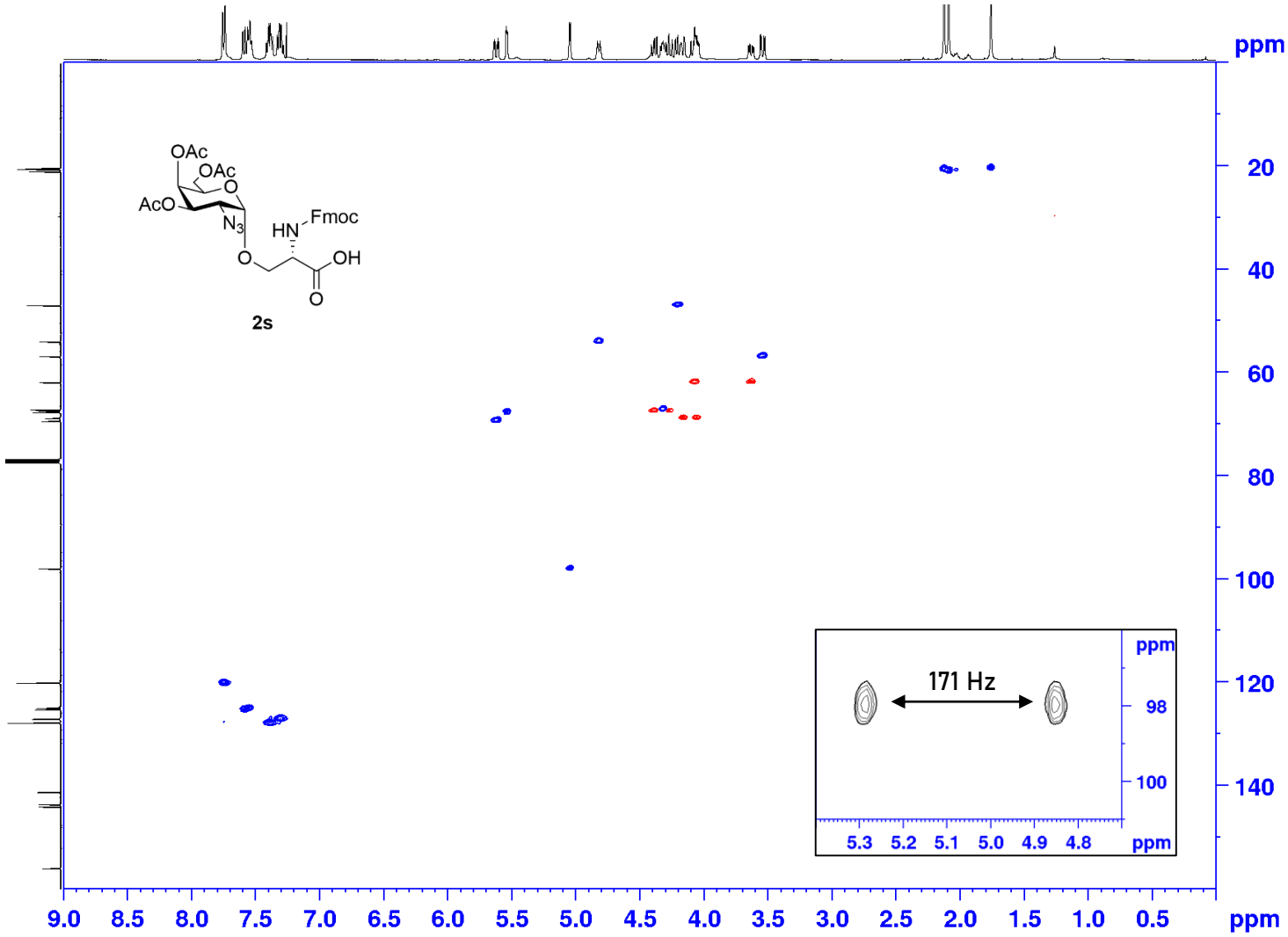


Figure S24 - HSQC NMR analysis of **2s** ($CDCl_3$, 400MHz). Inset: Coupled HSQC NMR analysis of anomeric region ($CDCl_3$, 400 MHz).

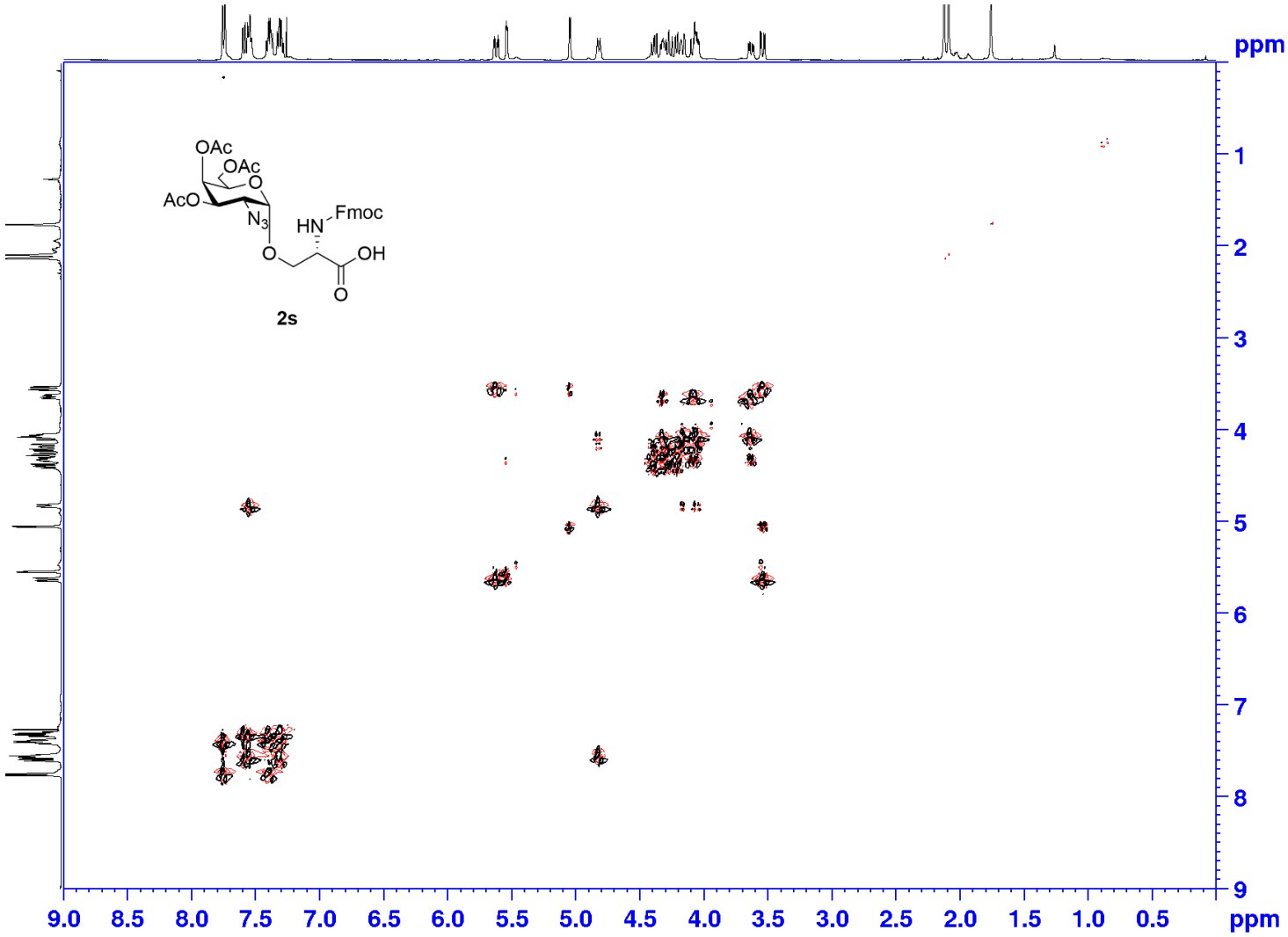


Figure S25 - COSY NMR analysis of **2s** ($CDCl_3$, 400 MHz).

Analysis of N-Fmoc-O-(2-azido-2-deoxy-3,4,6-tri-O-acetyl-D-Galactopyranosyl)-L-Threonine **2t**

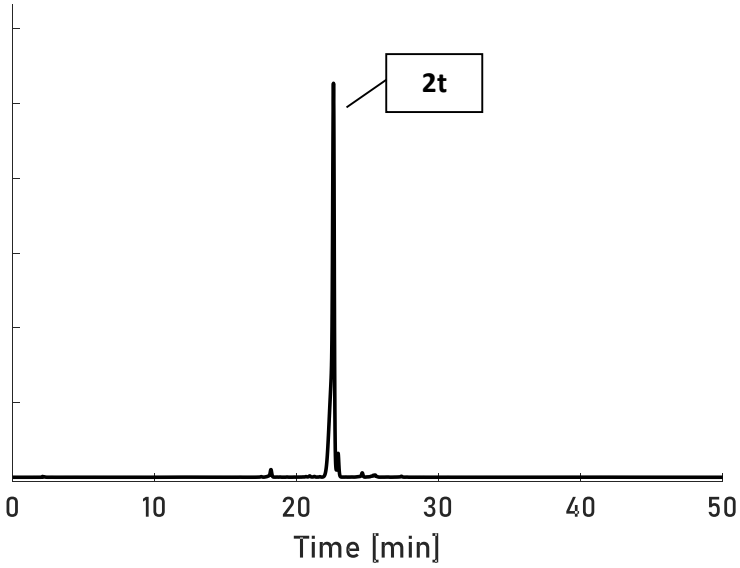


Figure S26 - Analytical HPLC chromatogram of purified **2t** (recorded at 254nm).

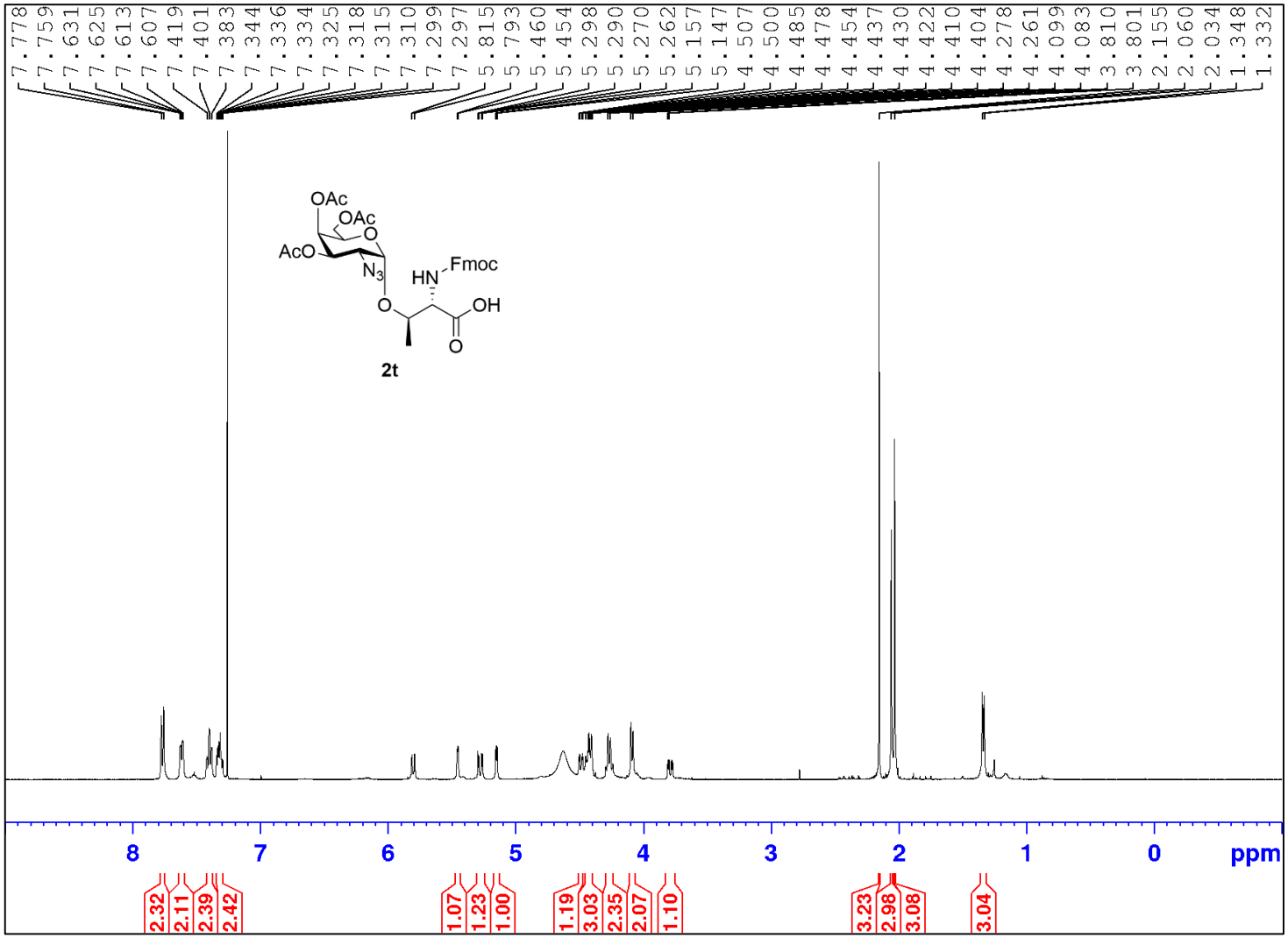


Figure S27 - ^{1}H NMR analysis of **2t** ($CDCl_3$, 400 MHz).

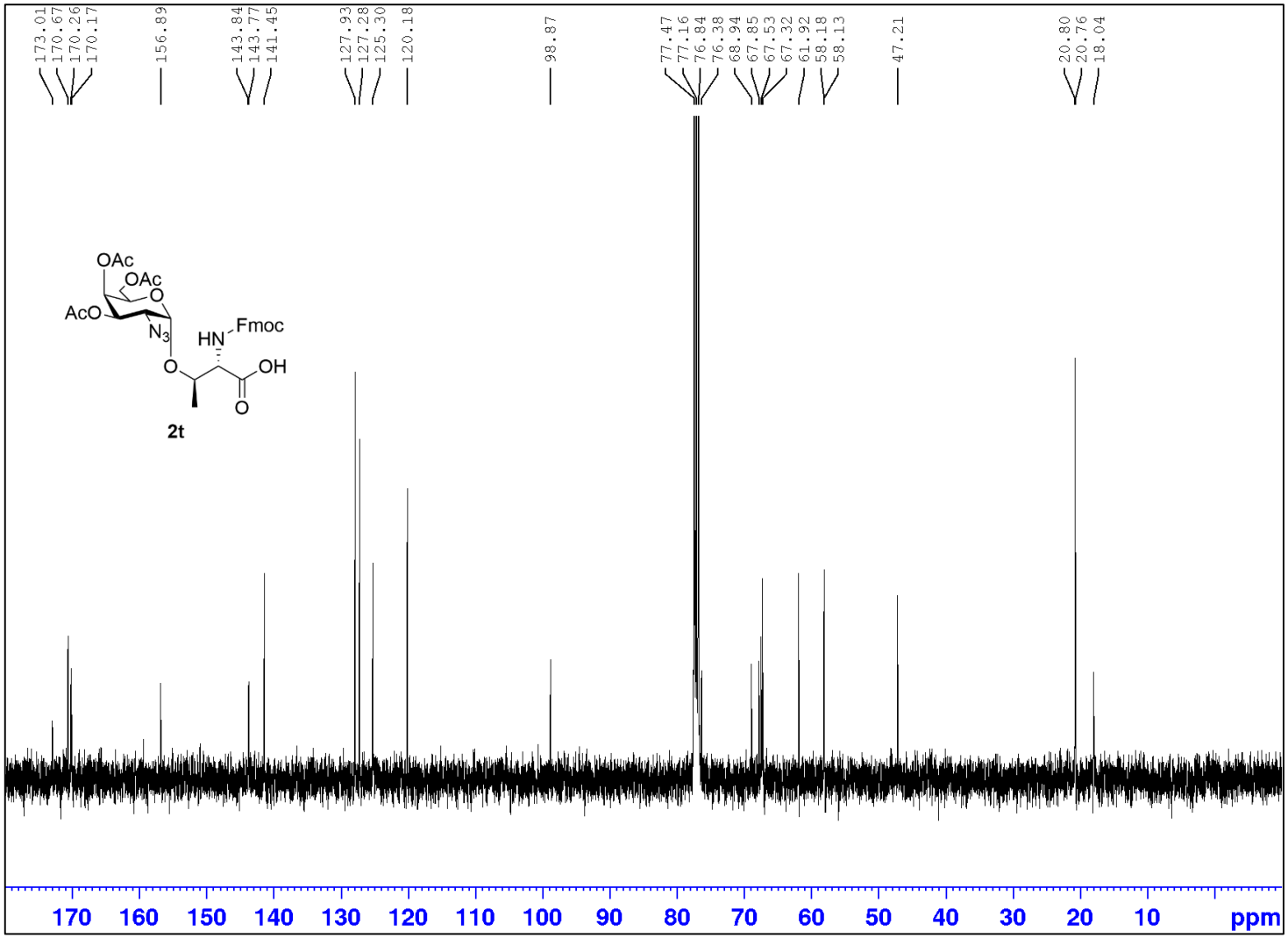


Figure S28 - ^{13}C NMR analysis of **2t** ($CDCl_3$, 100 MHz).

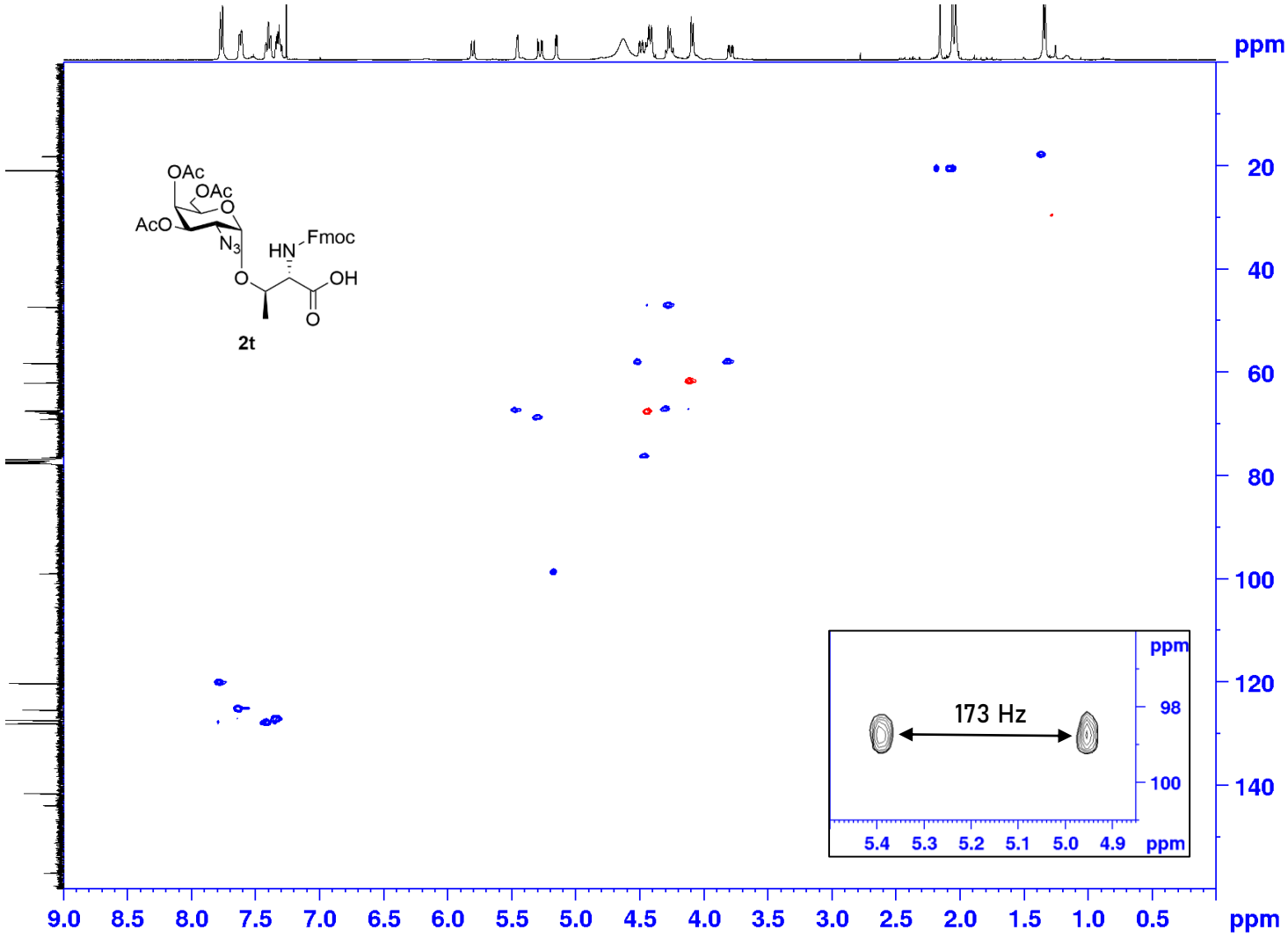


Figure S29 - HSQC NMR analysis of **2t** ($CDCl_3$, 400MHz). Inset: Coupled HSQC NMR analysis of anomeric region ($CDCl_3$, 400 MHz).

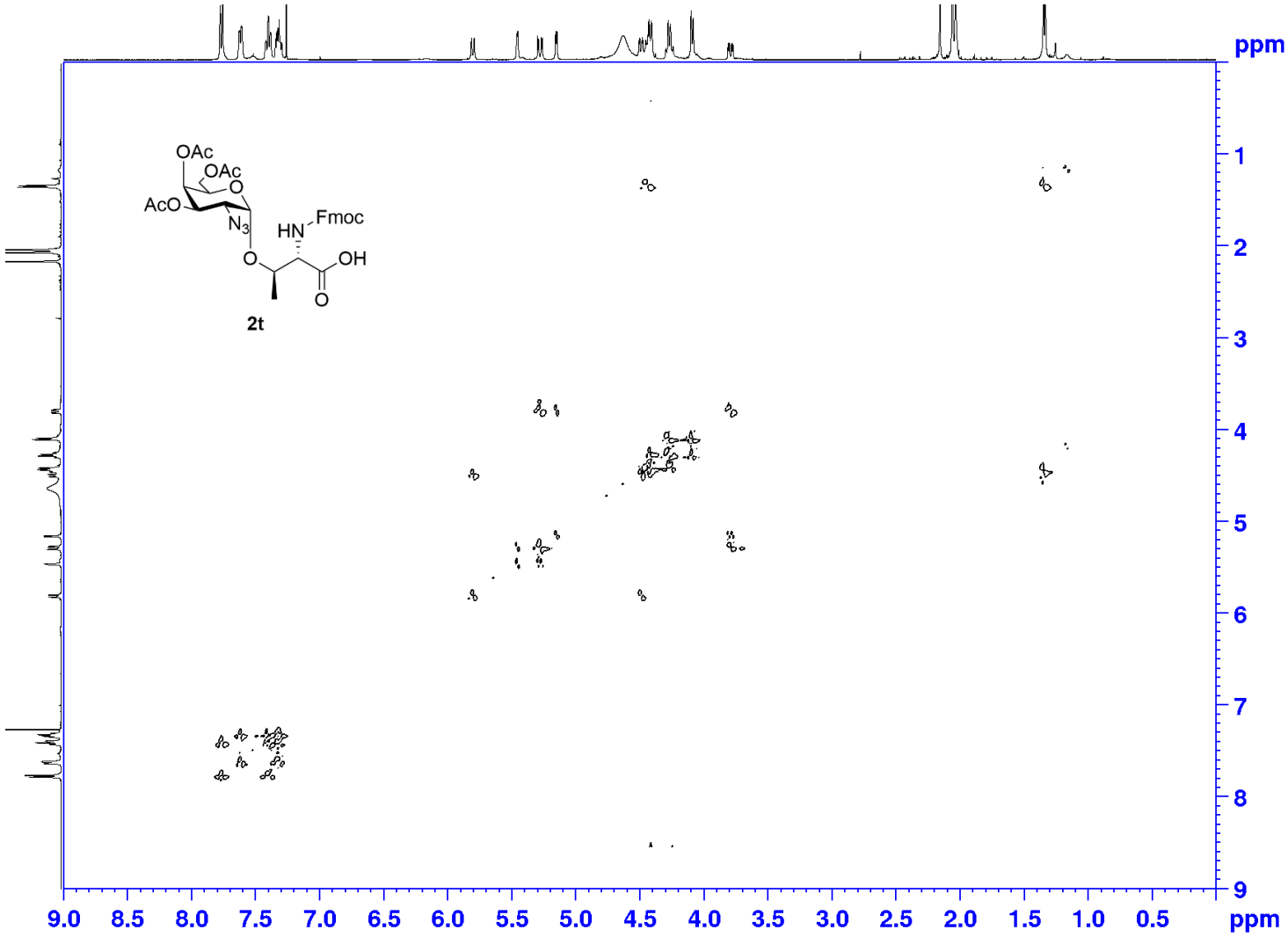


Figure S30 - COSY NMR analysis of **2t** ($CDCl_3$, 400 MHz).

Analyses of stages in the synthesis of **D1**

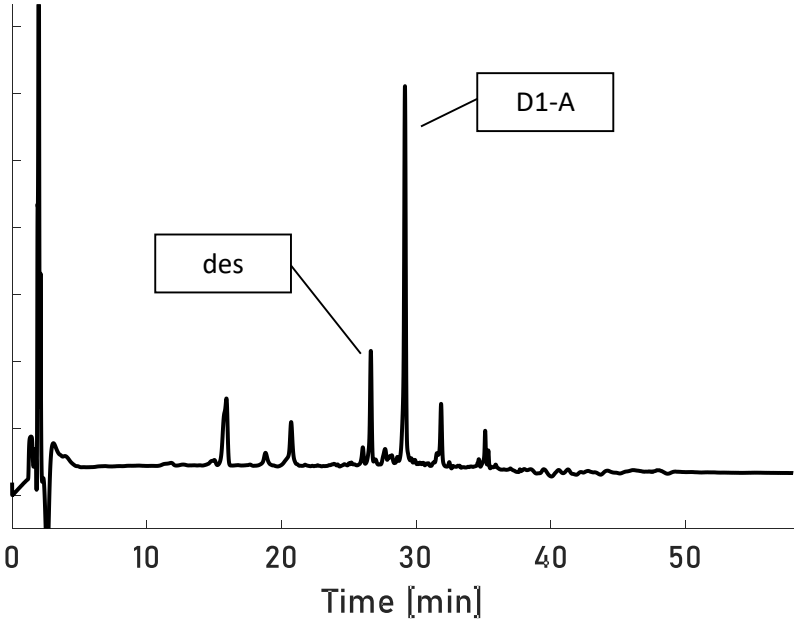


Figure S31 – Analytical HPLC chromatogram of the assembly of **D1-A** (recorded at 220nm)

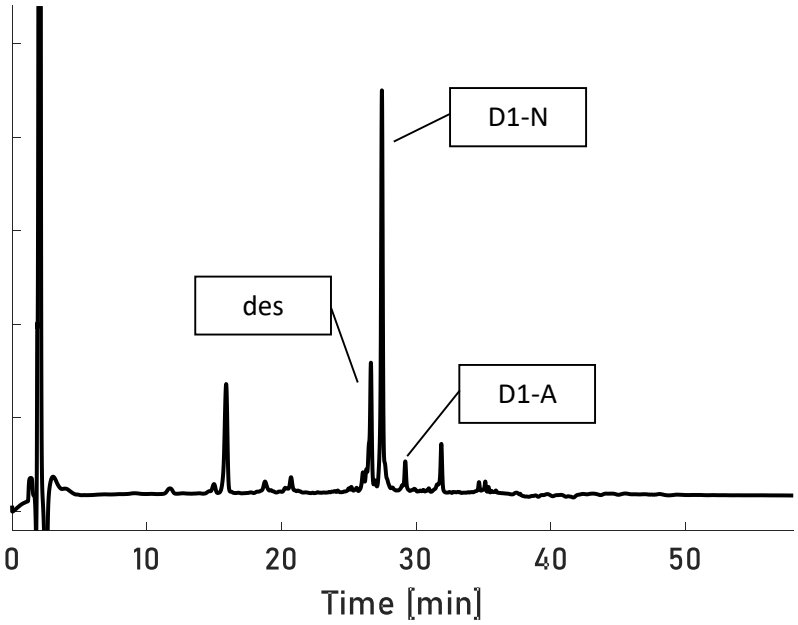


Figure S32 – Analytical HPLC chromatogram of overnight reduction of **D1-A** to **D1-N** (recorded at 220nm)

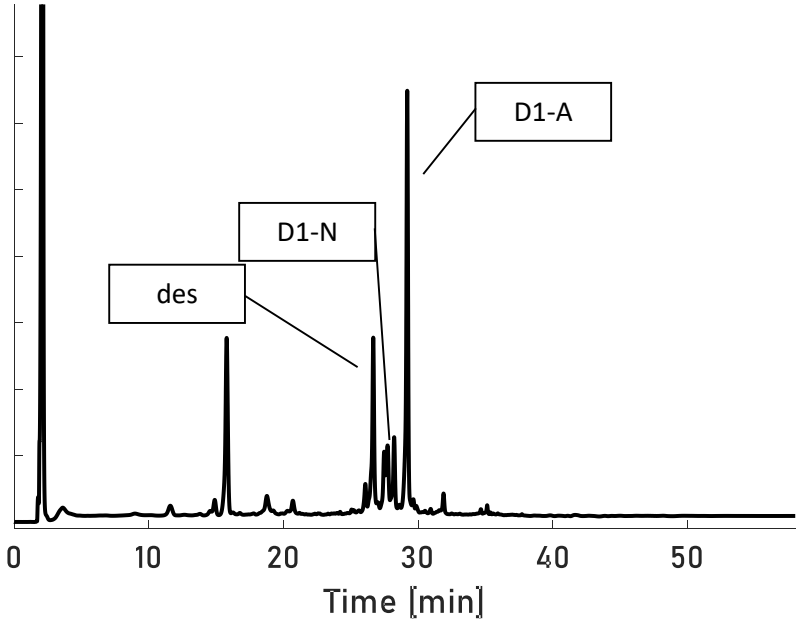


Figure S33 – Analytical HPLC chromatogram of 30 min reduction of **D1-A** to **D1-N** (recorded at 220nm)

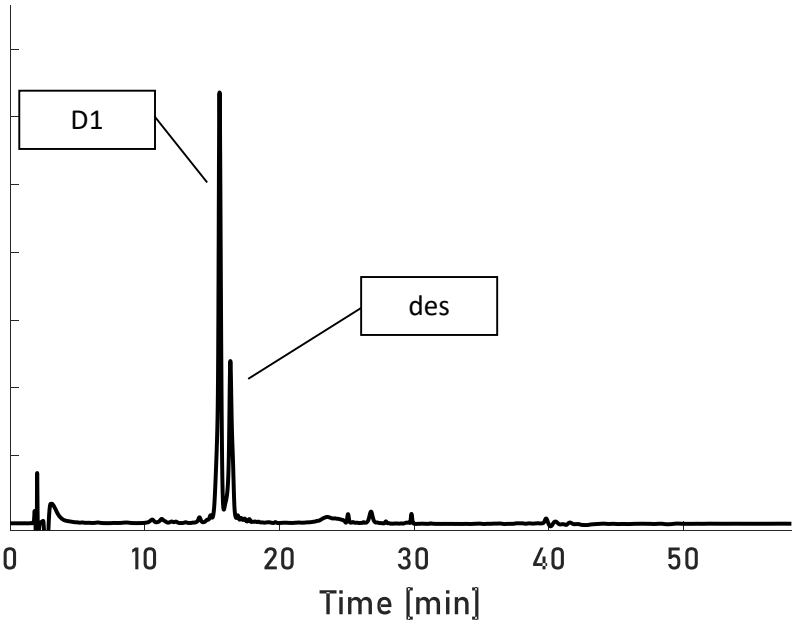


Figure S34 – Analytical HPLC chromatogram deacetylation of **D1-N** to **D1** (recorded at 220nm)

Analyses of αDG-n library

Analysis of **αDG-1**

αDG-1 was synthesized according to the protocol described above. It was obtained at a crude purity of 86%. After HPLC purification, **αDG-1** was isolated at 7 mg (48% yield). HRMS (ESI) *m/z* Calcd for $C_{35}H_{60}N_9O_{13}$, 814.43051 $[M+H^+]^+$; found, 814.4272.

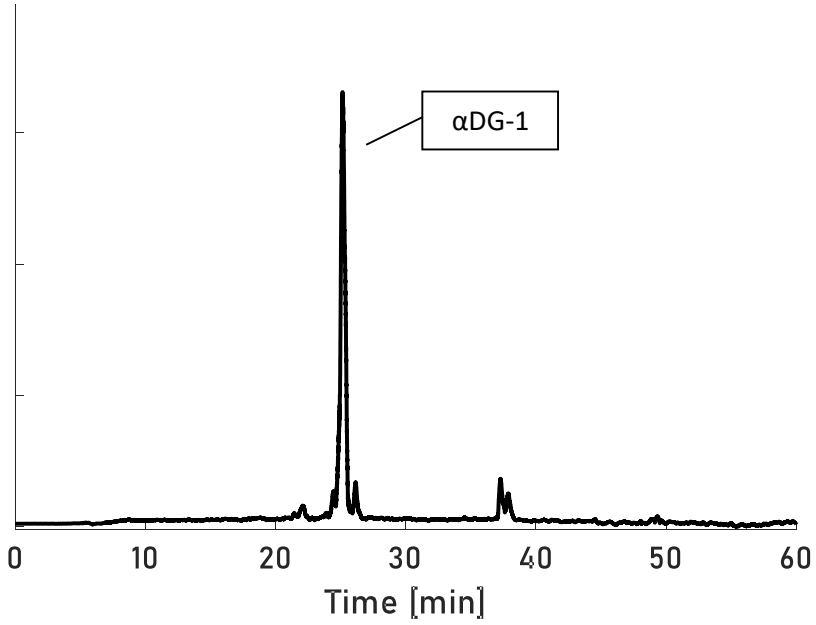


Figure S35 – Preparative HPLC chromatogram of crude **αDG-1** (recorded at 220nm)

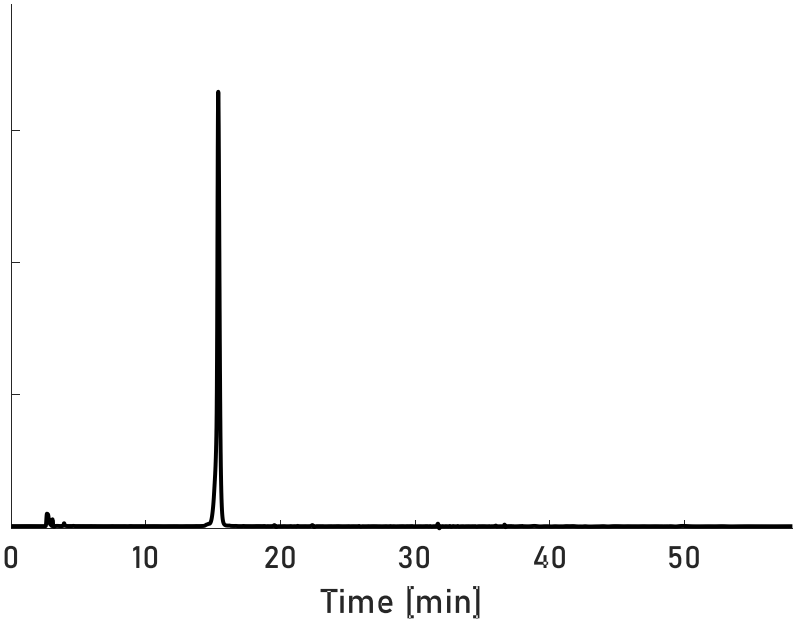


Figure S36 - Analytical HPLC chromatogram of pure **αDG-1** (recorded at 220nm).

Analysis of **αDG-2**

αDG-2 was synthesized according to the HMGPS protocol described above. It was obtained at a crude purity of 51%. After HPLC purification, **αDG-2** was isolated at 7 mg (40% yield). HRMS (ESI) *m/z* Calcd for $C_{41}H_{70}N_9O_{18}$, 976.48333 $[M+H^+]^+$; found, 976.48141.

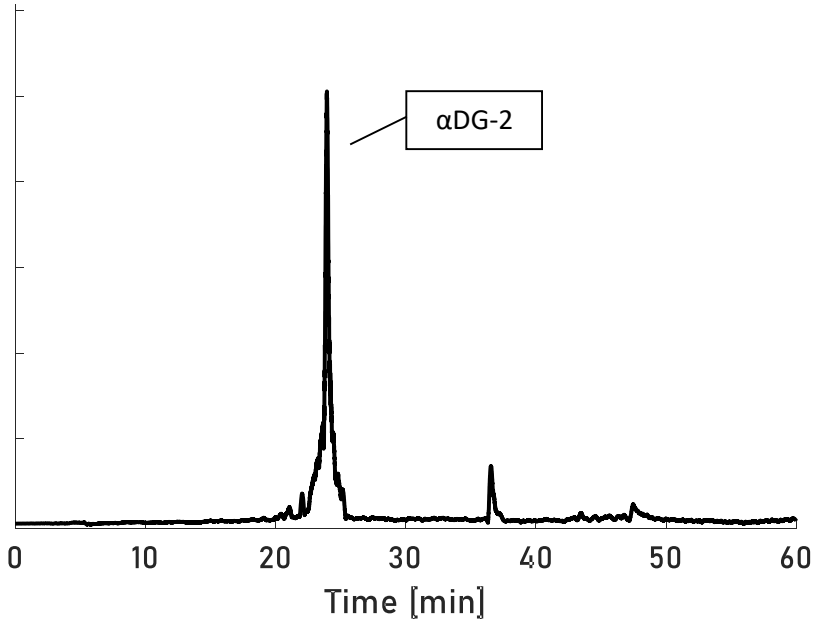


Figure S37 – Preparative HPLC chromatogram of crude **αDG-2** (recorded at 220nm)

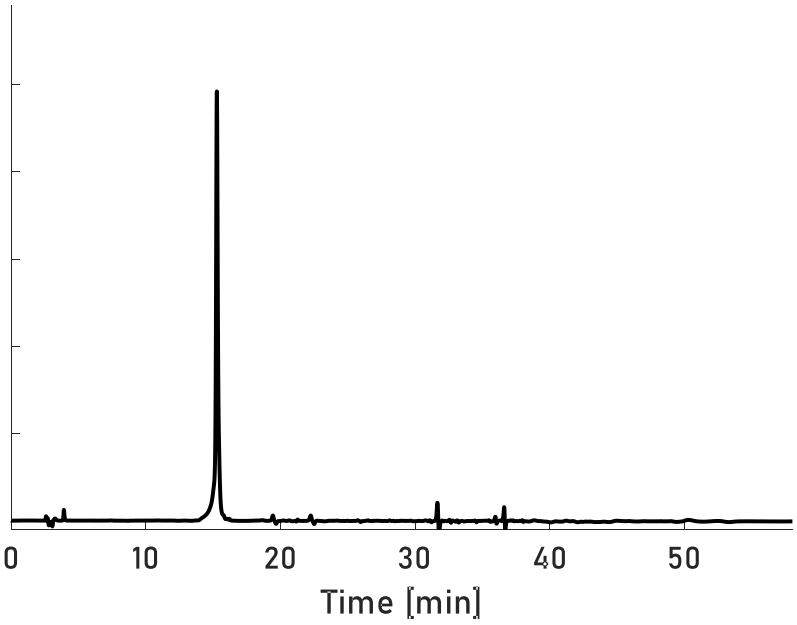


Figure S38 - Analytical HPLC chromatogram of pure **αDG-2** (recorded at 220nm).

Analysis of **αDG-3**

αDG-3 was synthesized according to the HMGPS protocol described above. It was obtained at a crude purity of 57%. After HPLC purification, **αDG-3** was isolated at 11 mg (62% yield). HRMS (ESI) *m/z* Calcd for $C_{41}H_{70}N_9O_{18}$, 976.48333 $[M+H^+]^+$; found, 976.48191.

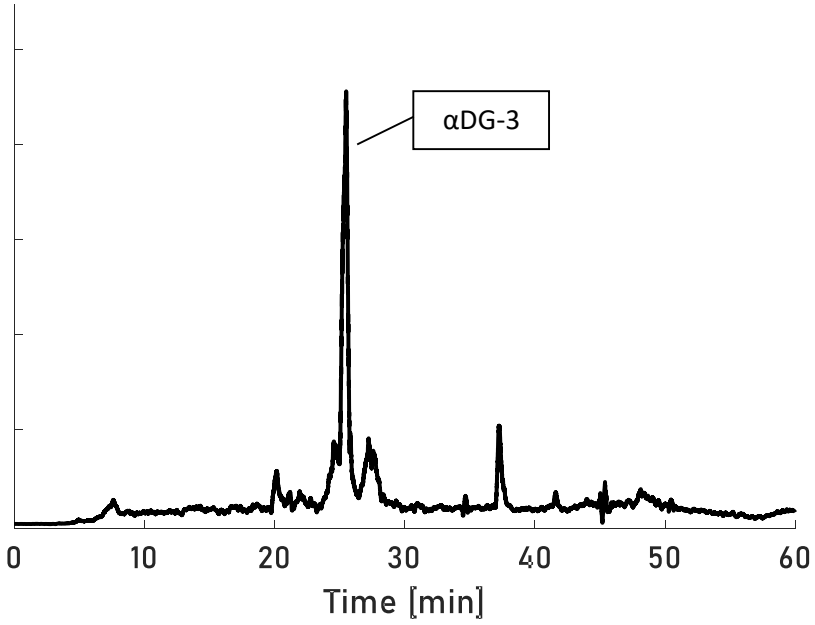


Figure S39 – Preparative HPLC chromatogram of crude **αDG-3** (recorded at 220nm)

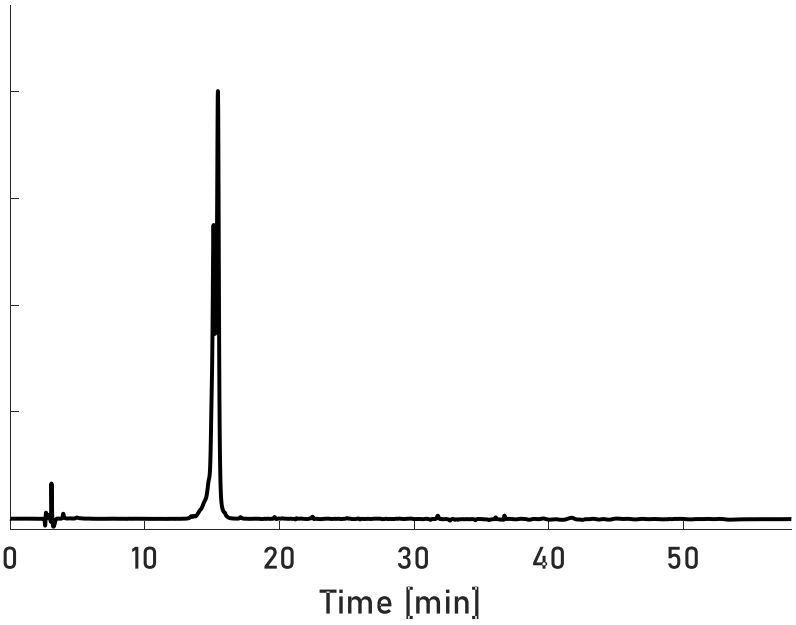


Figure S40 - Analytical HPLC chromatogram of pure **αDG-3** (recorded at 220nm).

Analysis of **αDG-4**

αDG-4 was synthesized according to Protocol A described above. It was obtained at a crude purity of 82%. After HPLC purification, **αDG-4** was isolated at 10 mg (54% yield). HRMS (ESI) *m/z* Calcd for $C_{43}H_{73}N_{10}O_{18}$, 1017.50988 $[M+H^+]^+$; found, 1017.50526.

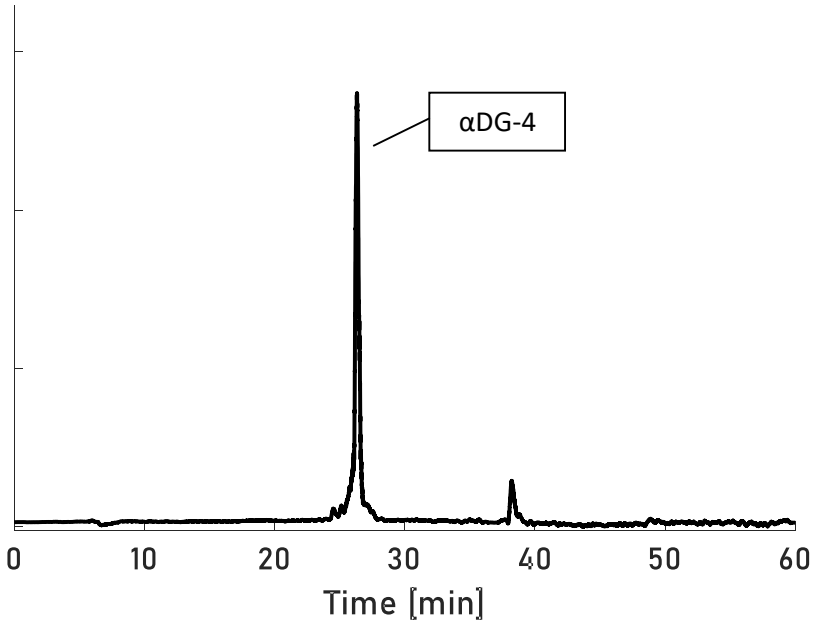


Figure S41 – Preparative HPLC chromatogram of crude **αDG-4** (recorded at 220nm)

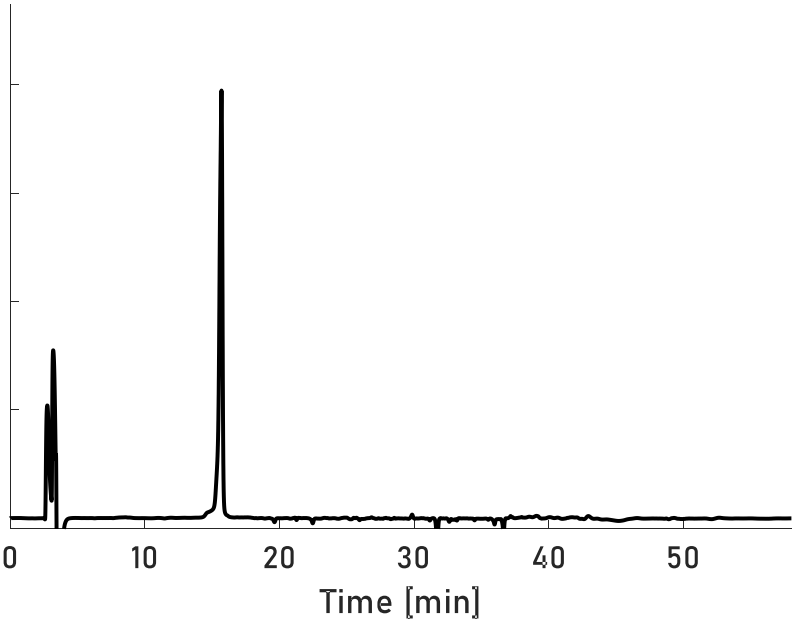


Figure S42 - Analytical HPLC chromatogram of pure **αDG-4** (recorded at 220nm).

Analysis of **αDG-5**

αDG-4 was synthesized according to Protocol A described above. It was obtained at a crude purity of 84%. After HPLC purification, **αDG-5** was isolated at 9 mg (49% yield). HRMS (ESI) *m/z* Calcd for $C_{43}H_{73}N_{10}O_{18}$, 1017.50988 $[M+H^+]^+$; found, 1017.50736.

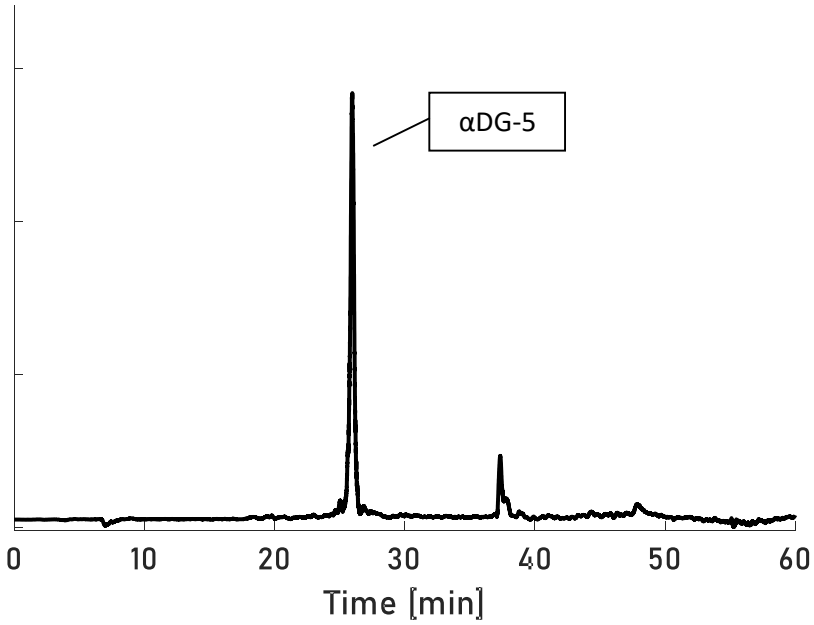


Figure S43 – Preparative HPLC chromatogram of crude **αDG-5** (recorded at 220nm)

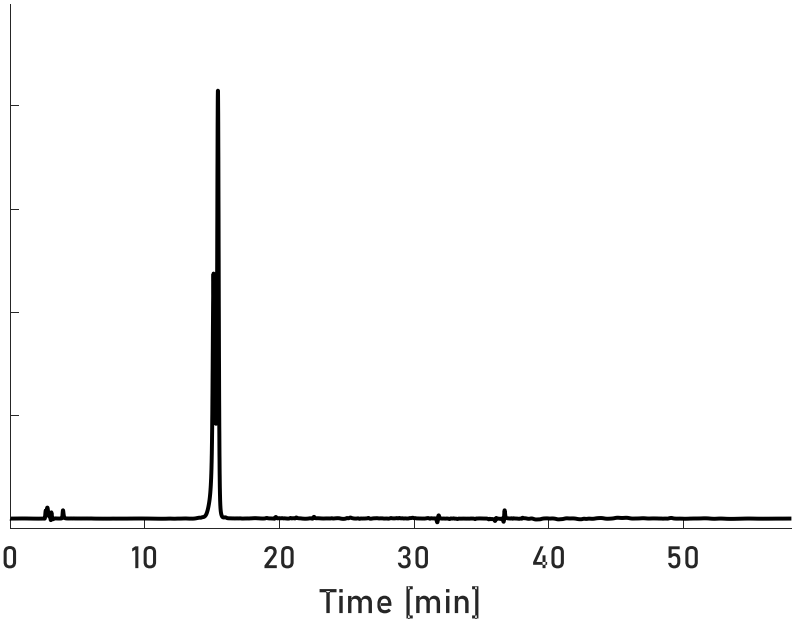


Figure S44 - Analytical HPLC chromatogram of pure **αDG-5** (recorded at 220nm).

Analysis of **αDG-6**

αDG-6 was synthesized according to the EMGPS protocol described above. It was obtained at a crude purity of 51%. After HPLC purification, **αDG-6** was isolated at 8 mg (39% yield). HRMS (ESI) *m/z* Calcd for $C_{47}H_{80}N_9O_{23}$, 1138.53616 $[M+H^+]^+$; found, 1138.53571.

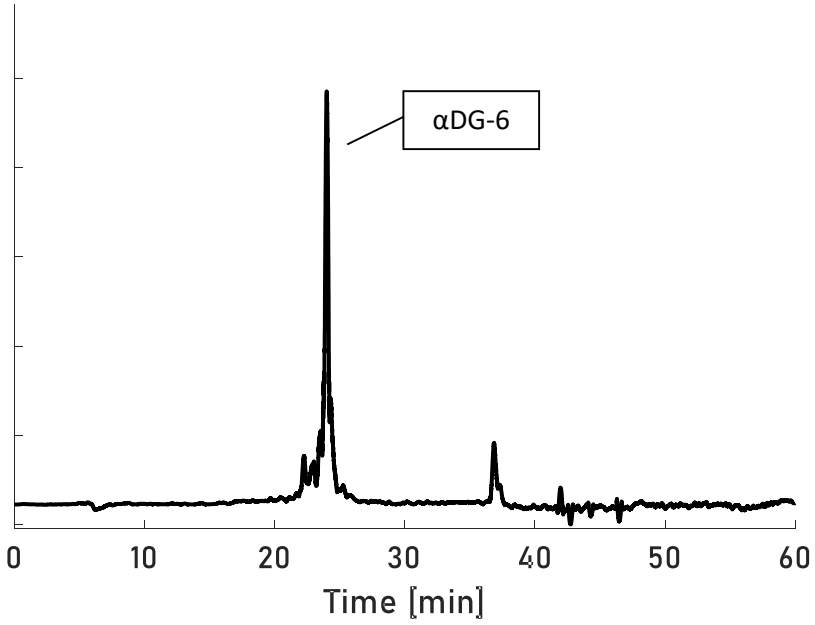


Figure S45 – Preparative HPLC chromatogram of crude **αDG-6** (recorded at 220nm)

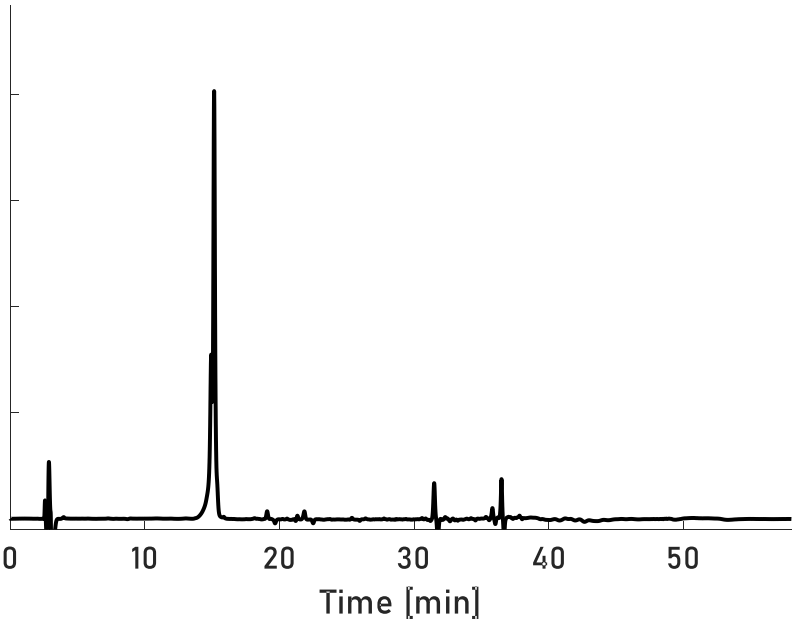


Figure S46 - Analytical HPLC chromatogram of pure **αDG-6** (recorded at 220nm).

Analysis of **αDG-7**

αDG-7 was synthesized according to Protocol A described above. It was obtained at a crude purity of 80%. After HPLC purification, **αDG-7** was isolated at 4.3 mg (20% yield). HRMS (ESI) *m/z* Calcd for $C_{51}H_{86}N_{11}O_{23}$, 1220.58925 $[M+H^+]^+$; found, 1220.58953.

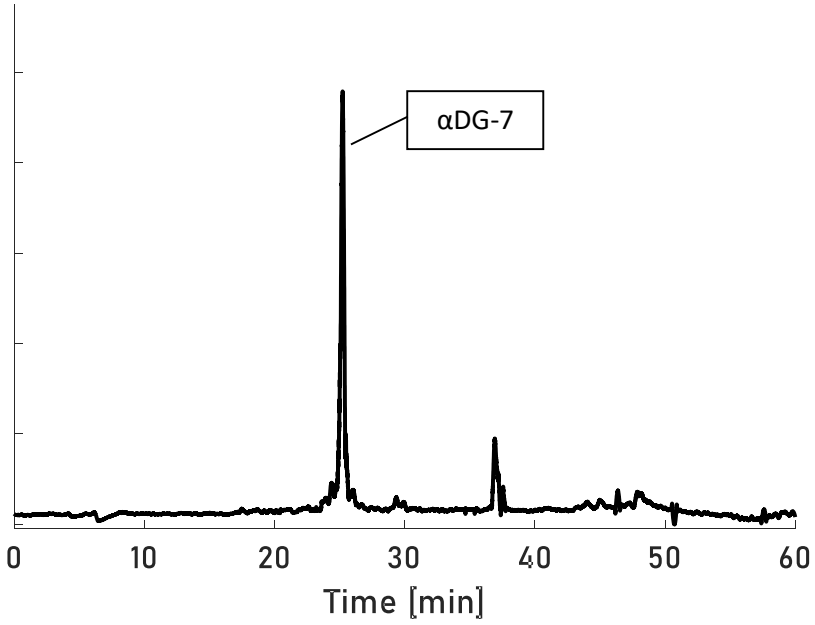


Figure S47 – Preparative HPLC chromatogram of crude **αDG-7** (recorded at 220nm)

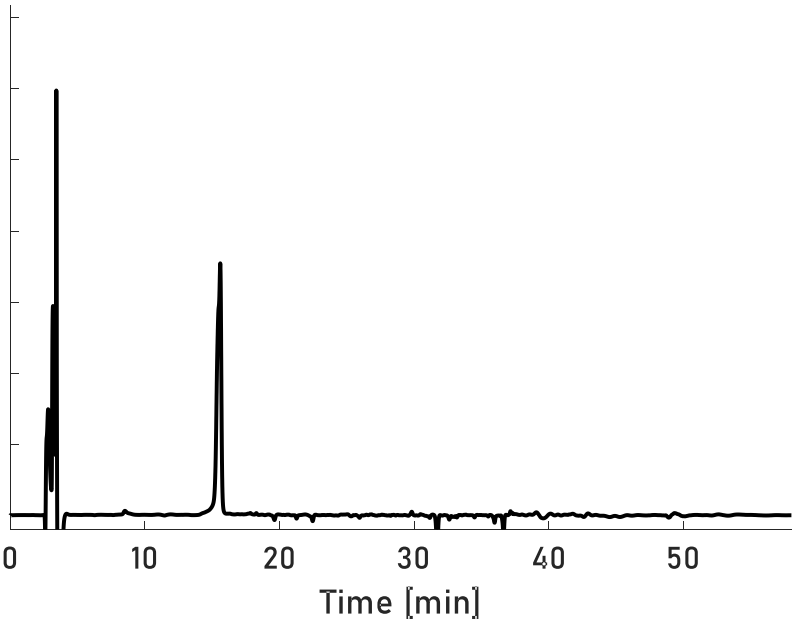


Figure S48 - Analytical HPLC chromatogram of pure **αDG-7** (recorded at 220nm).

Analysis of **αDG-8**

αDG-8 was synthesized according to Protocol A described above. It was obtained at a crude purity of 60%. After HPLC purification, **αDG-8** was isolated at 4.7 mg (22% yield).HRMS (ESI) *m/z* Calcd for $C_{49}H_{83}N_{10}O_{23}$, 1179.56271 $[M+H^+]^+$; found, 1179.55837.

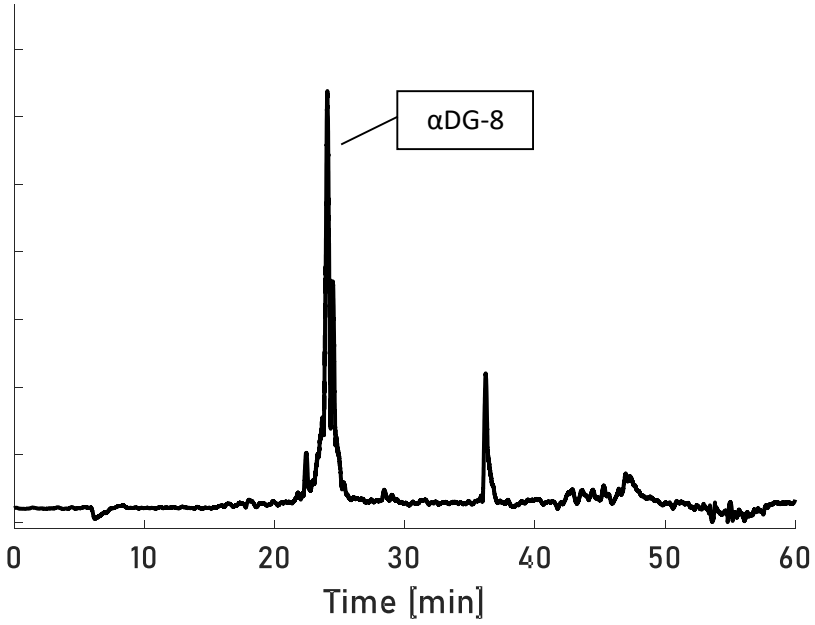


Figure S49 – Preparative HPLC chromatogram of crude **αDG-8** (recorded at 220nm)

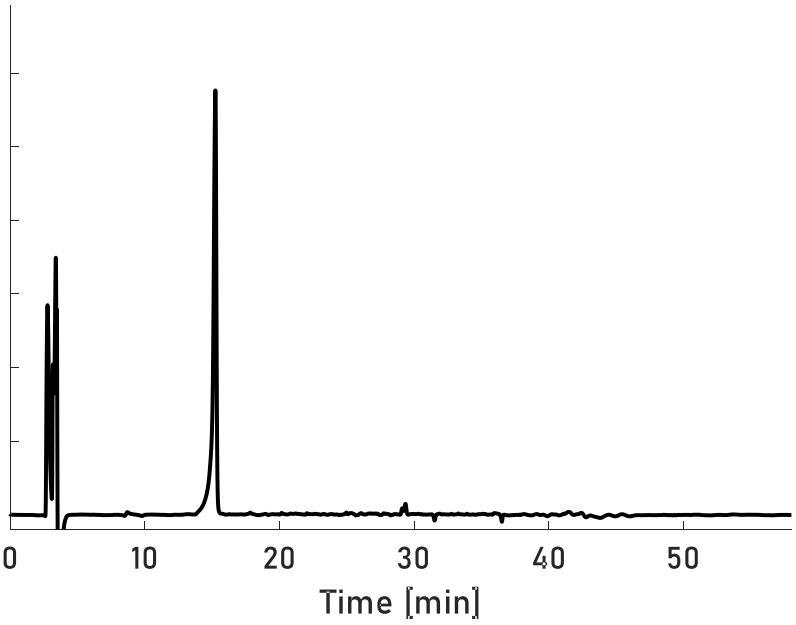


Figure S50 - Analytical HPLC chromatogram of pure **αDG-8** (recorded at 220nm).

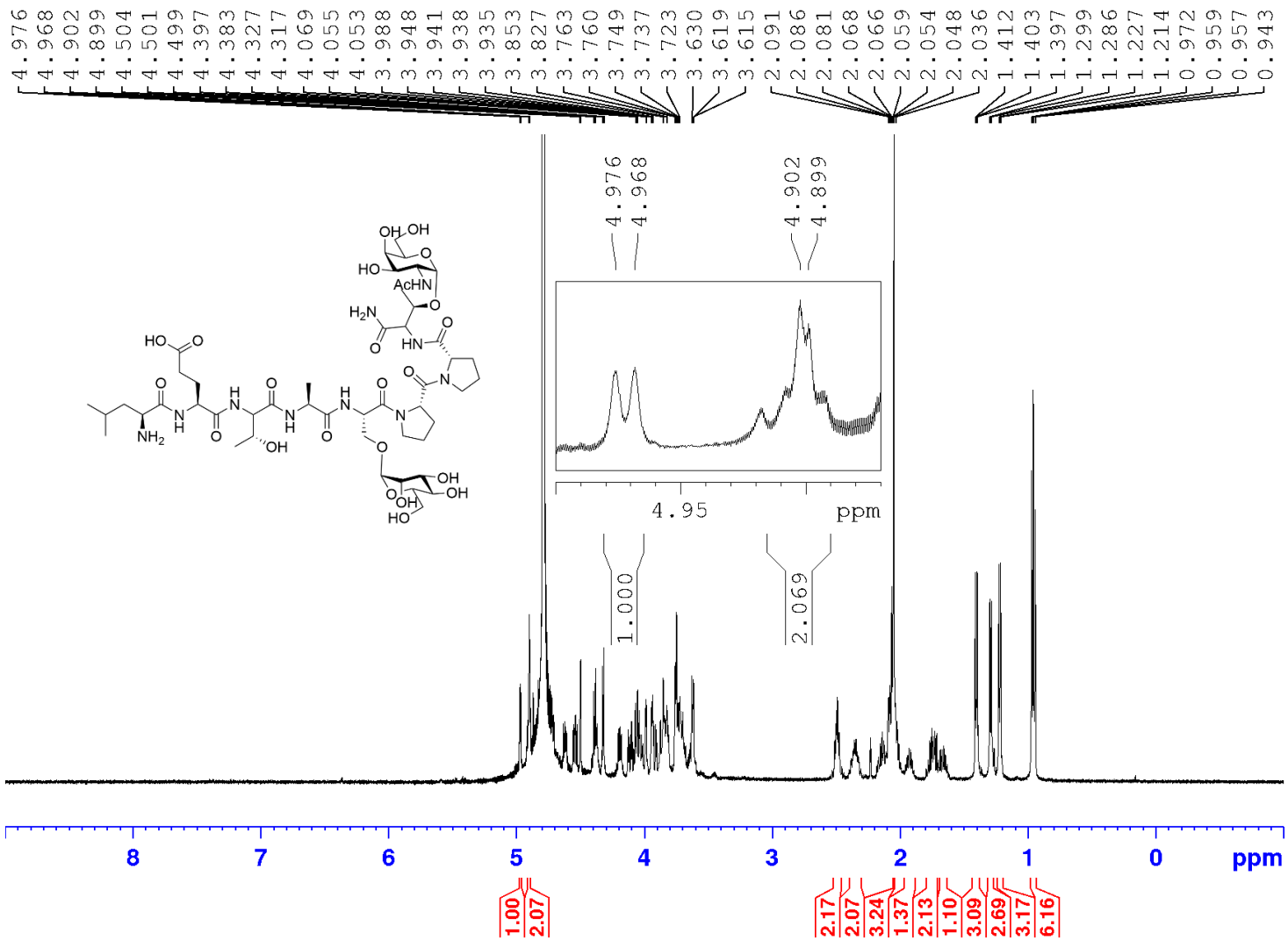


Figure S51 - ^{1}H NMR analysis of **αDG-8** (D_2O, 500MHz). Residual solvent peaks: δ 4.79 (water).

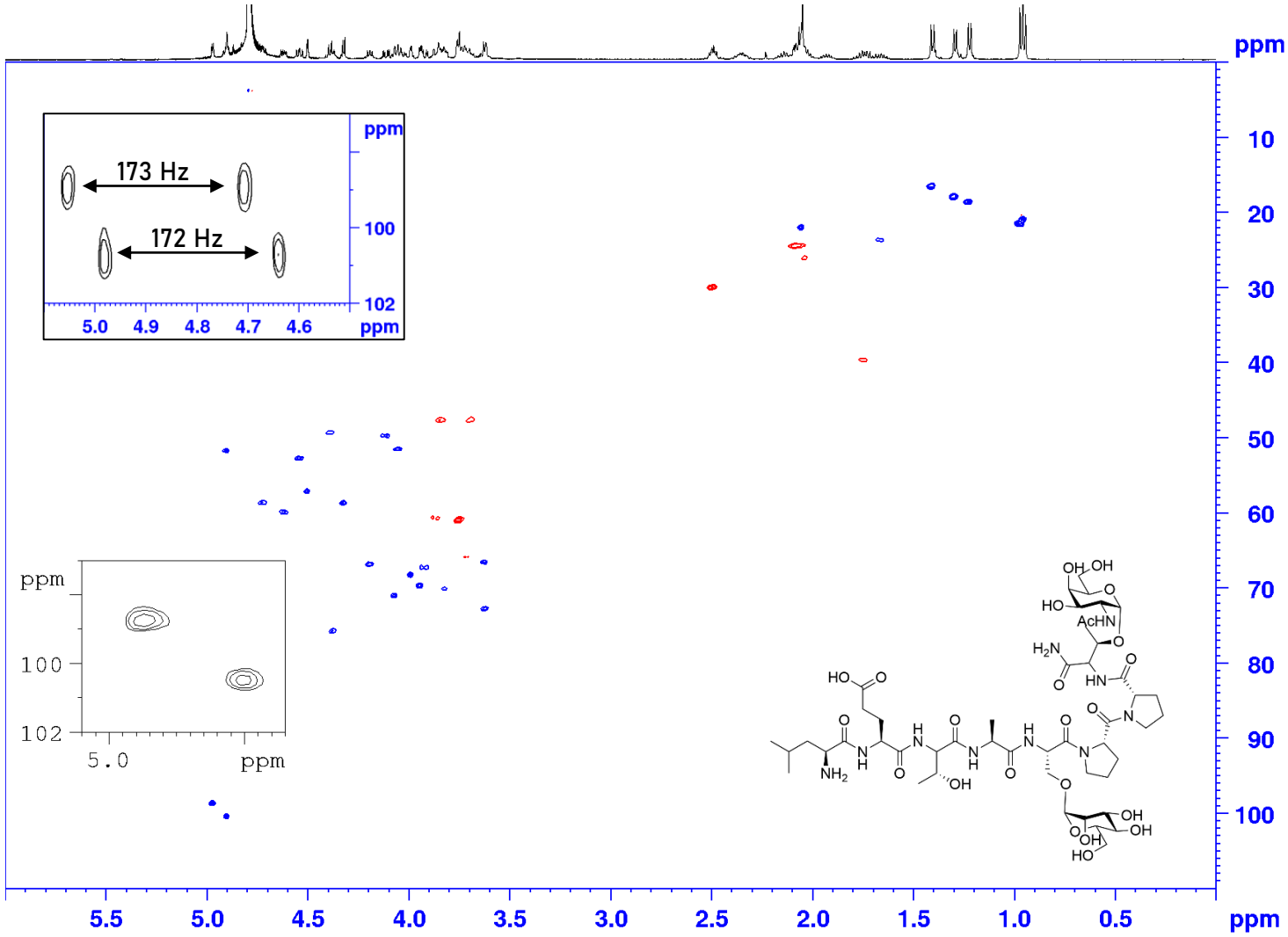


Figure S52 - HSQC NMR analysis of **αDG-8** (D_2O, 500MHz). Inset: Coupled HSQC NMR analysis of anomeric region (D_2O, 500 MHz).

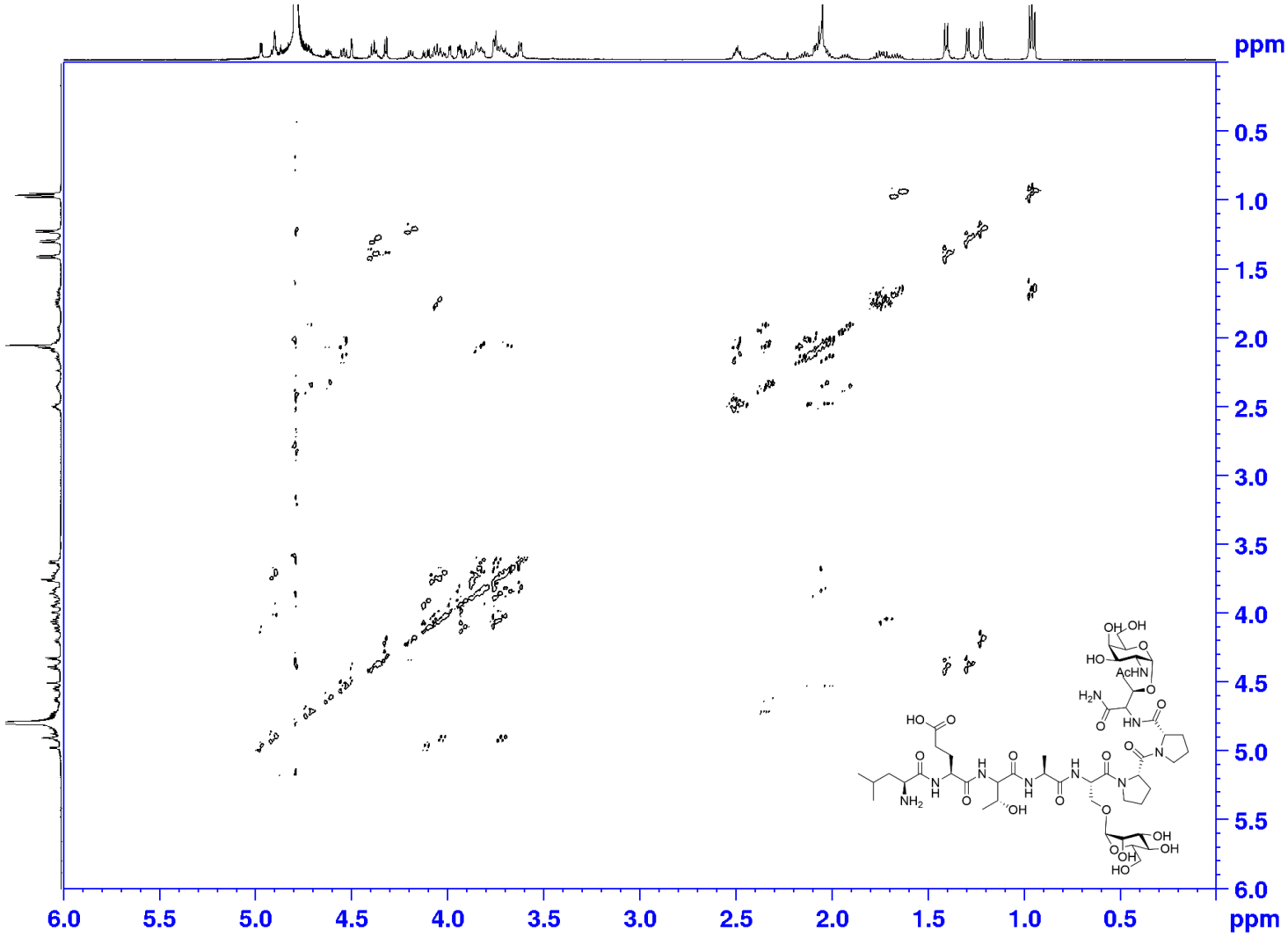


Figure S53 - COSY NMR analysis of **αDG-8** (D_2O, 500MHz).

Analysis of **αDG-9**

αDG-9 was synthesized according to Protocol A described above. It was obtained at a crude purity of 79%. After HPLC purification, **αDG-9** was isolated at 7 mg (33% yield). HRMS (ESI) *m/z* Calcd for $C_{49}H_{83}N_{10}O_{23}$, 1179.56271 $[M+H^+]^+$; found, 1179.56010.

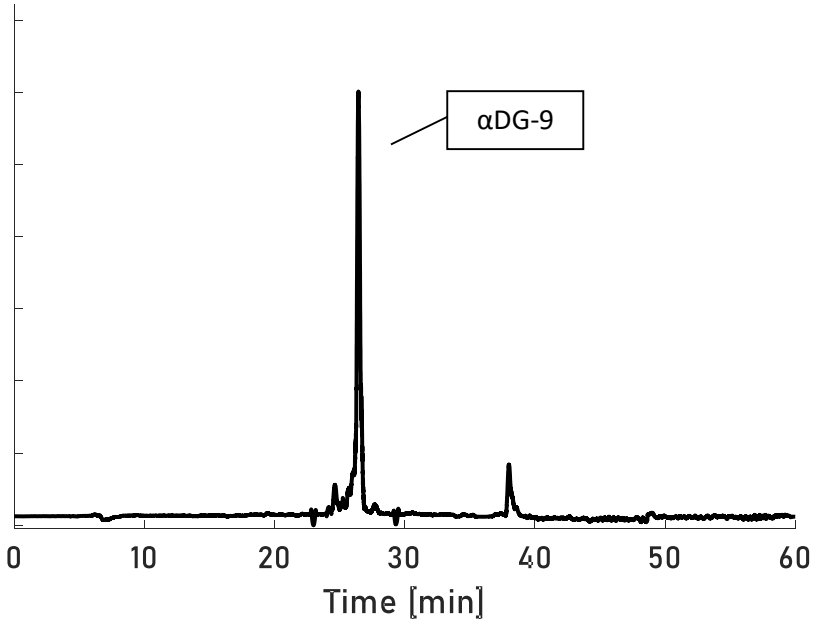


Figure S54 – Preparative HPLC chromatogram of crude **αDG-9** (recorded at 220nm)

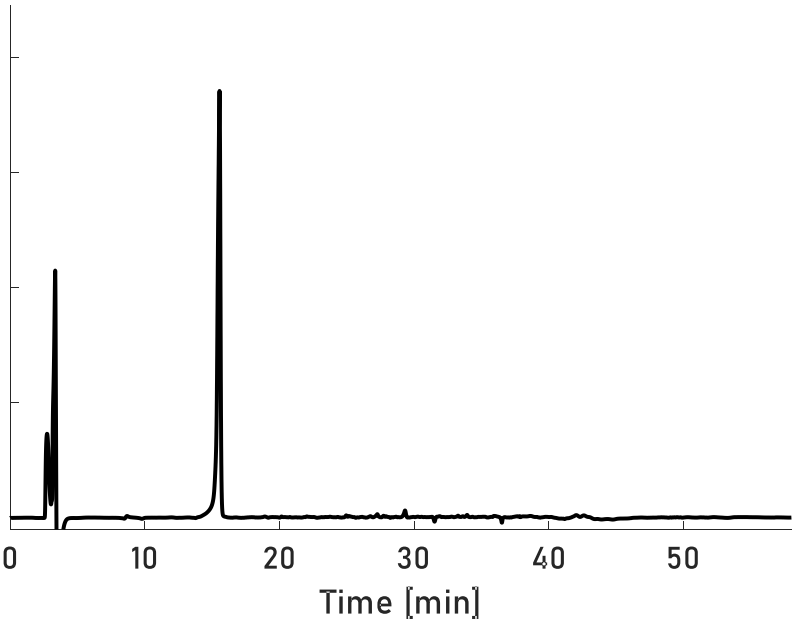


Figure S55 - Analytical HPLC chromatogram of pure **αDG-9** (recorded at 220nm).

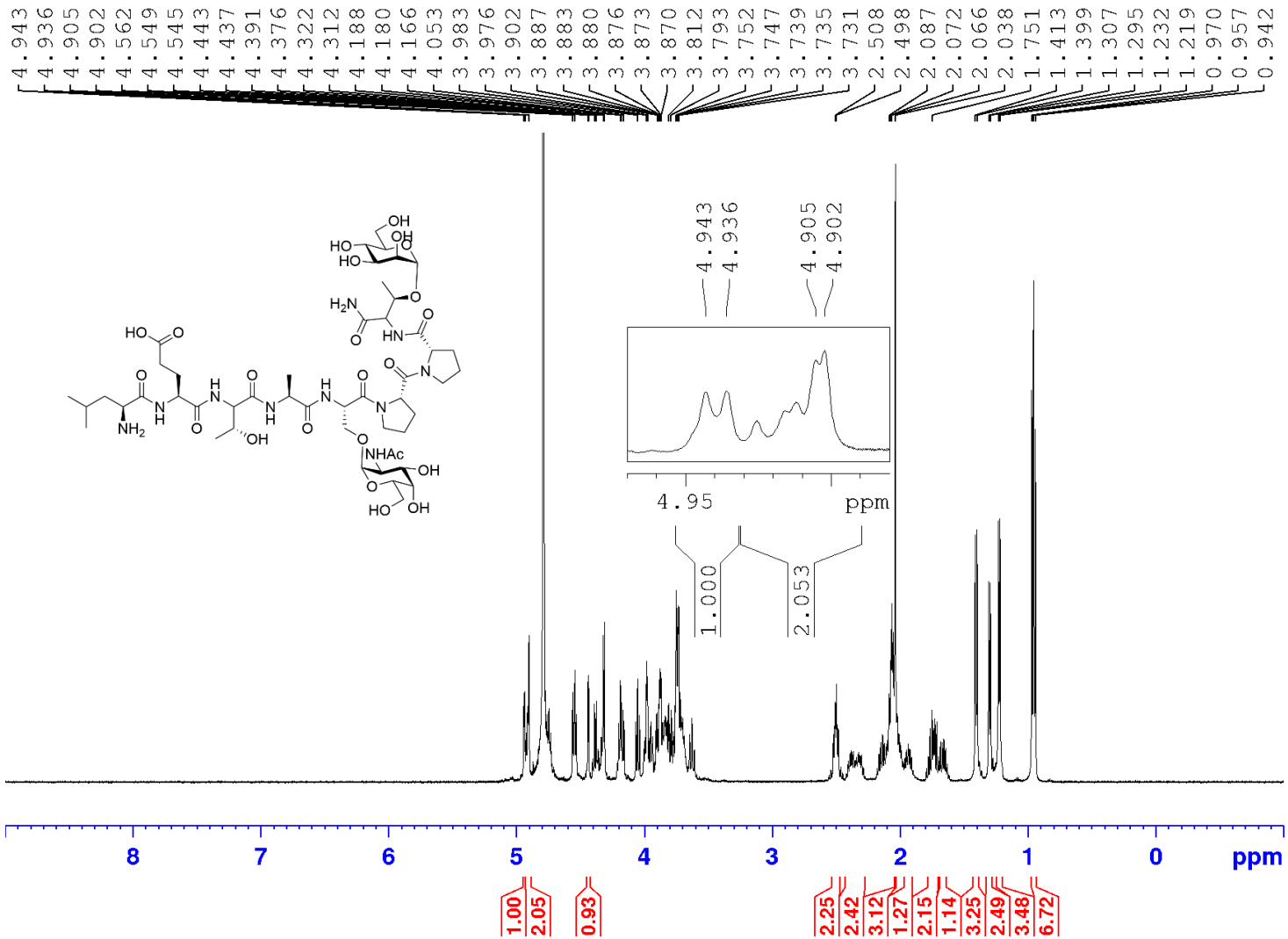


Figure S56 - ^{1}H NMR analysis of **αDG-9** (D_2O, 500MHz). Residual solvent peaks: δ 4.79 (water).

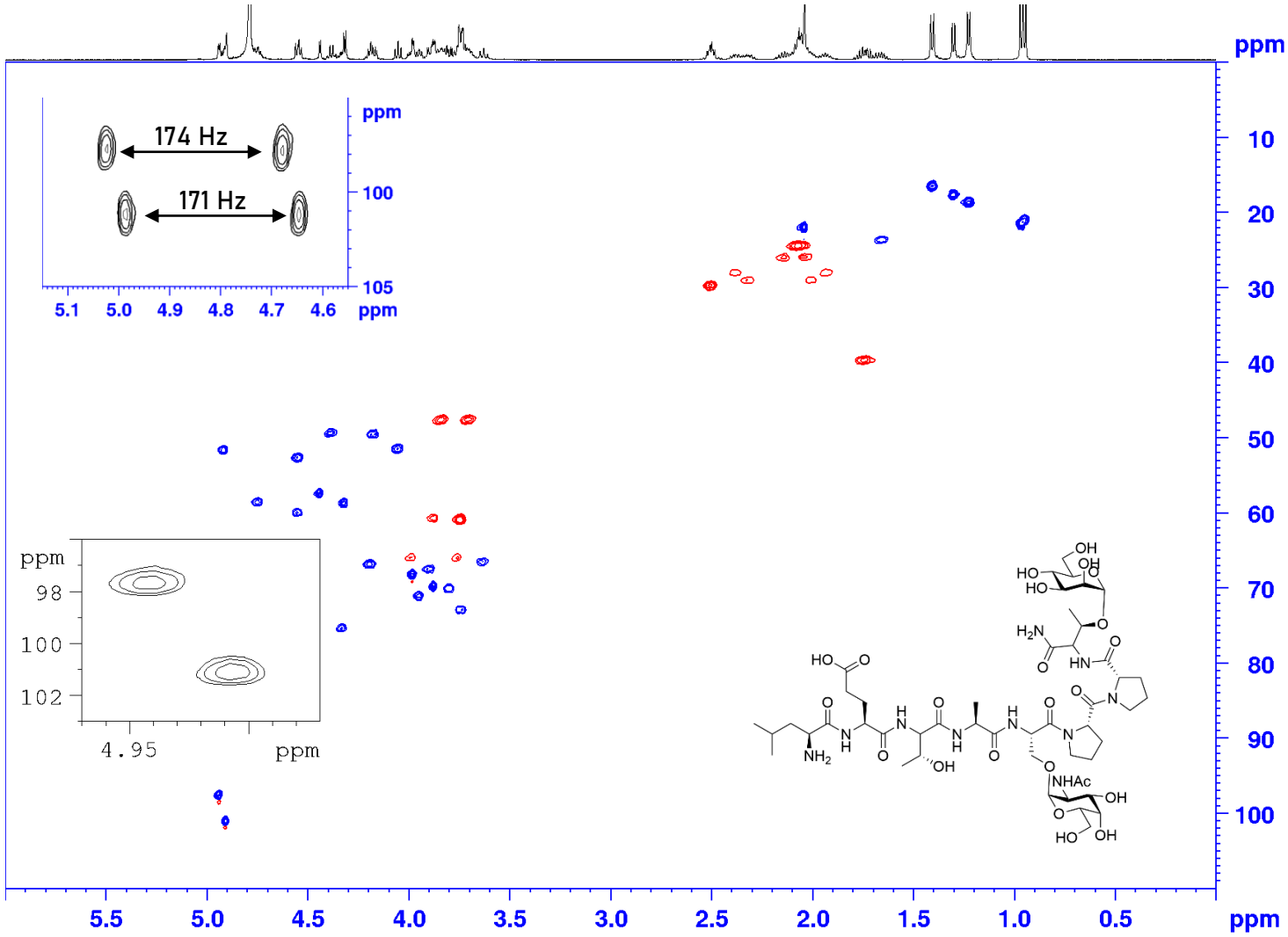


Figure S57 - HSQC NMR analysis of **αDG-9** (D_2O, 500MHz). Inset: Coupled HSQC NMR analysis of anomeric region (D_2O, 500 MHz).

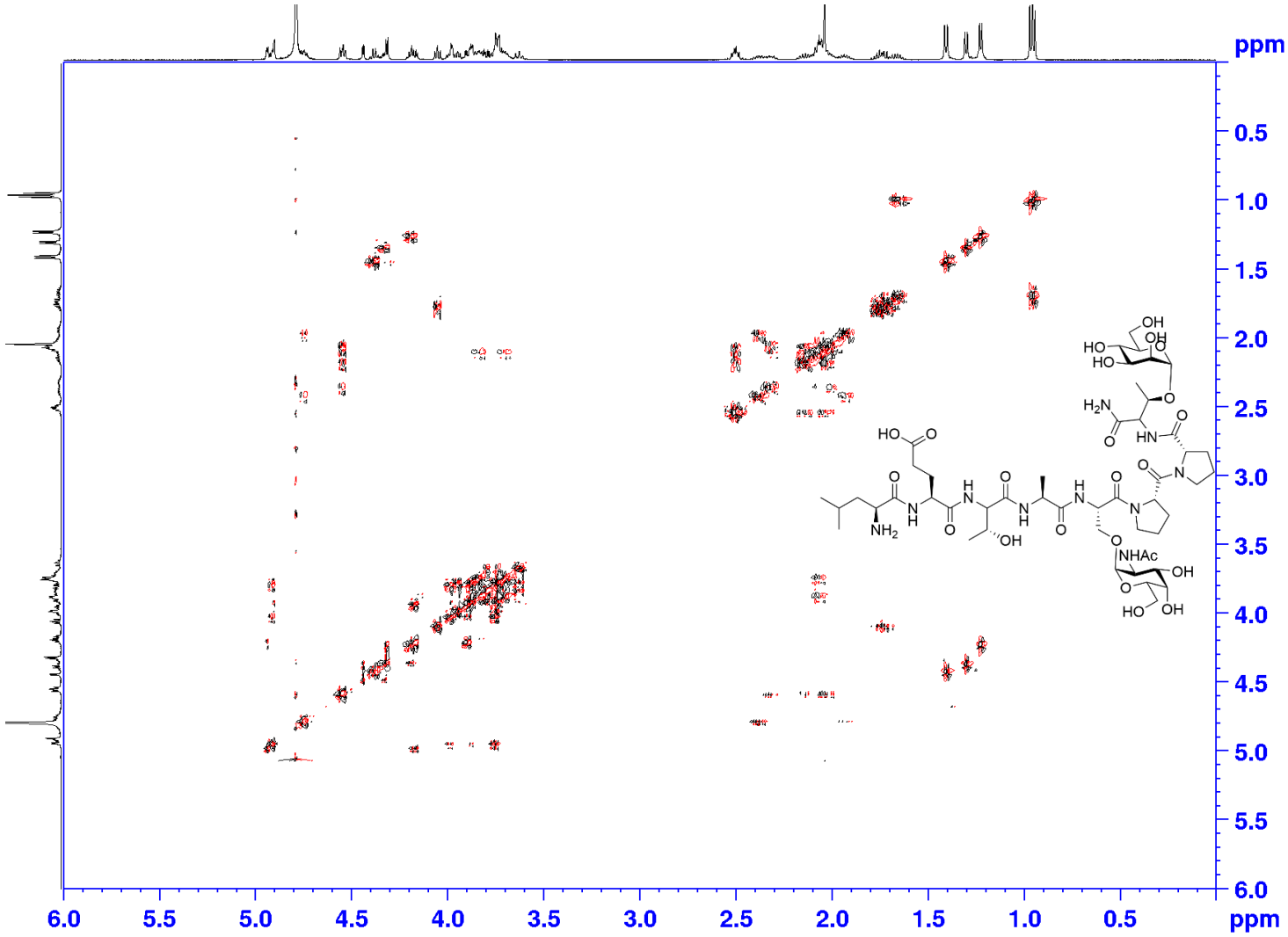


Figure S58 - COSY NMR analysis of **αDG-9** (D_2O, 500MHz).

Analysis of **αDG-9-Ac**

αDG-9-Ac was synthesized according to Protocol B described above. It was obtained at a crude purity of 51%. After HPLC purification, **αDG-9-Ac** was isolated at 6 mg (27% yield). HRMS (ESI) *m/z* Calcd for $C_{51}H_{85}N_{10}O_{24}$, 1221.57372 $[M+H^+]^+$; found, 1221.57018.

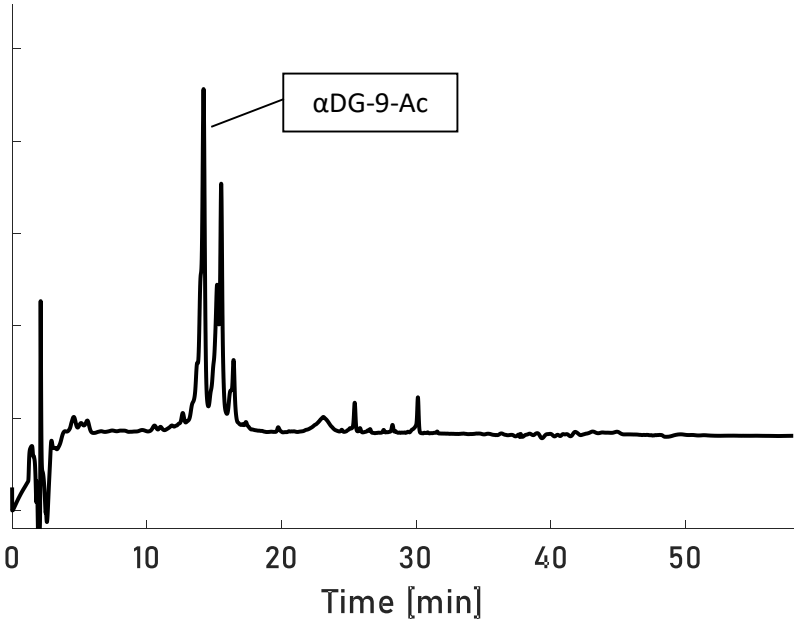


Figure S59 – Analytical HPLC chromatogram of crude **αDG-9-Ac** (recorded at 220nm)

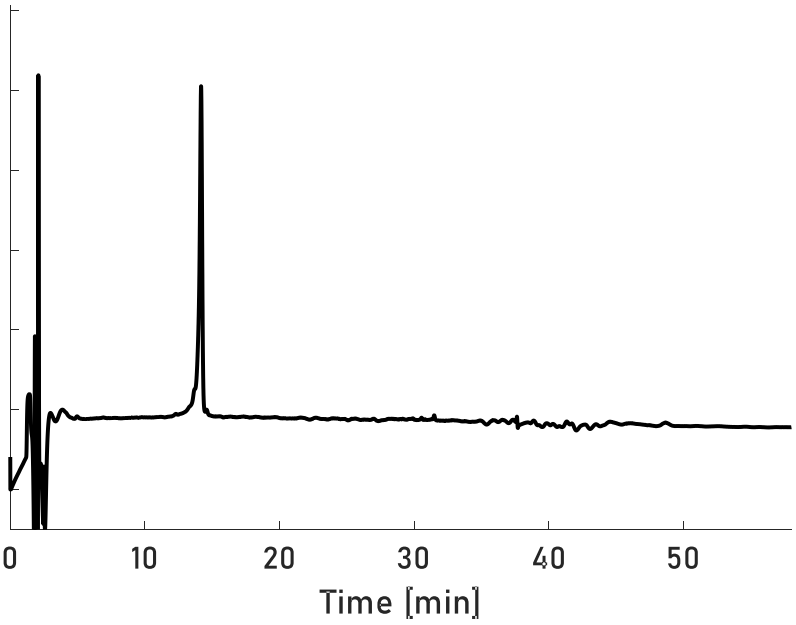


Figure S60 - Analytical HPLC chromatogram of pure **αDG-9-Ac** (recorded at 220nm).

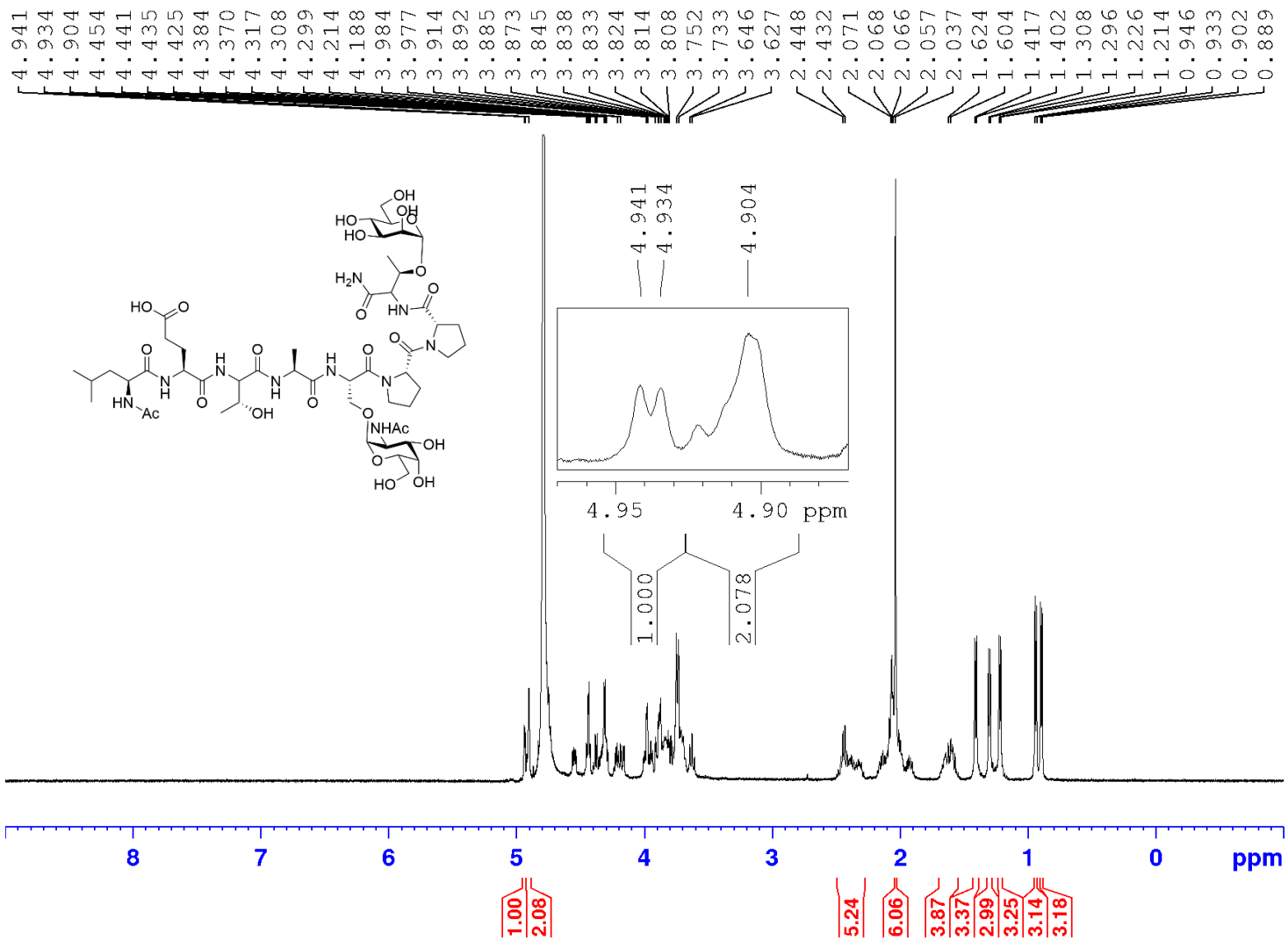


Figure S61 - ^{1}H NMR analysis of **αDG-9-Ac** (D_2O, 500MHz). Residual solvent peaks: δ 4.79 (water).

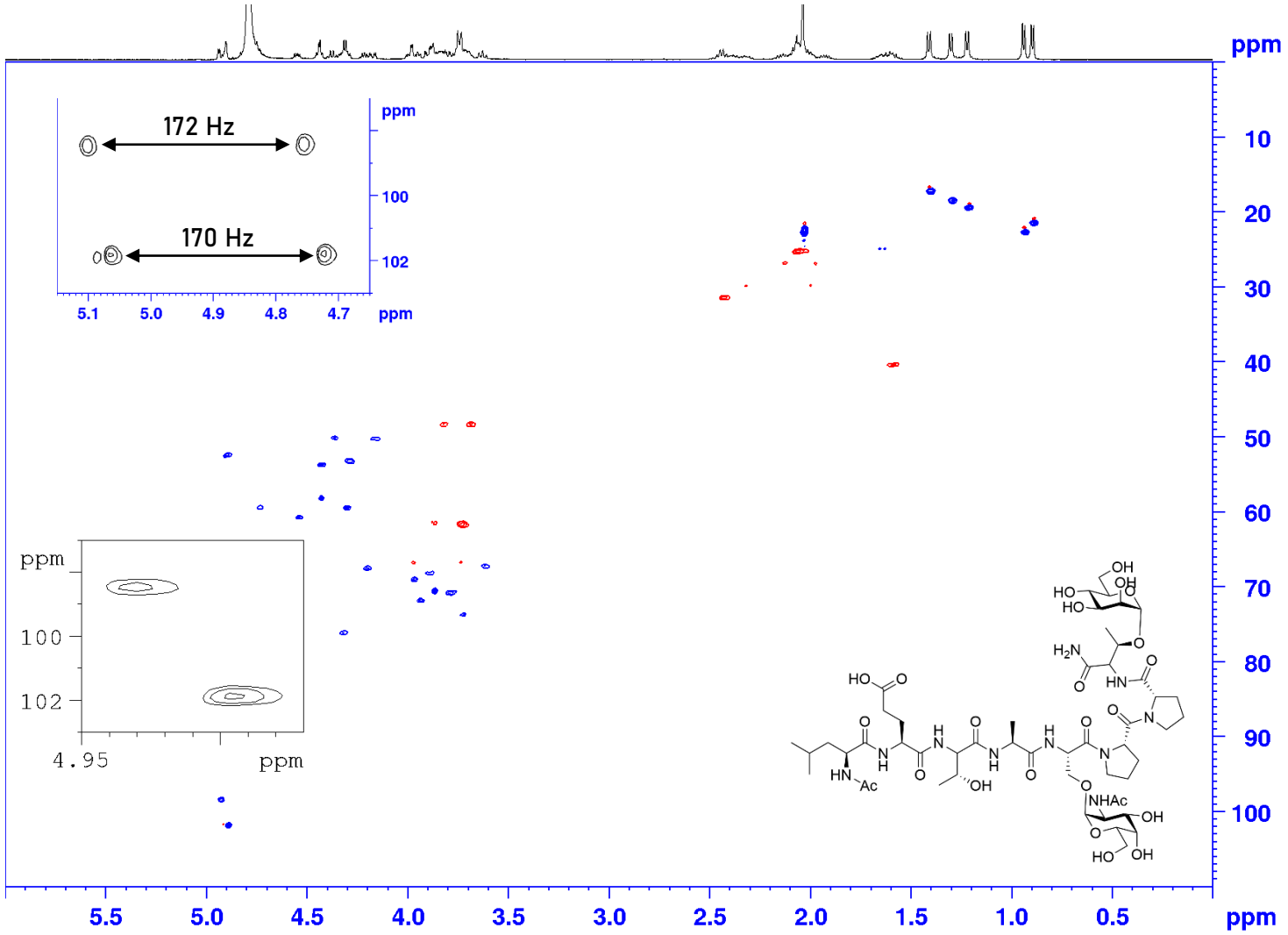


Figure S62 - HSQC NMR analysis of **αDG-9-Ac** (D_2O, 500MHz). Inset: Coupled HSQC NMR analysis of anomeric region (D_2O, 500 MHz).

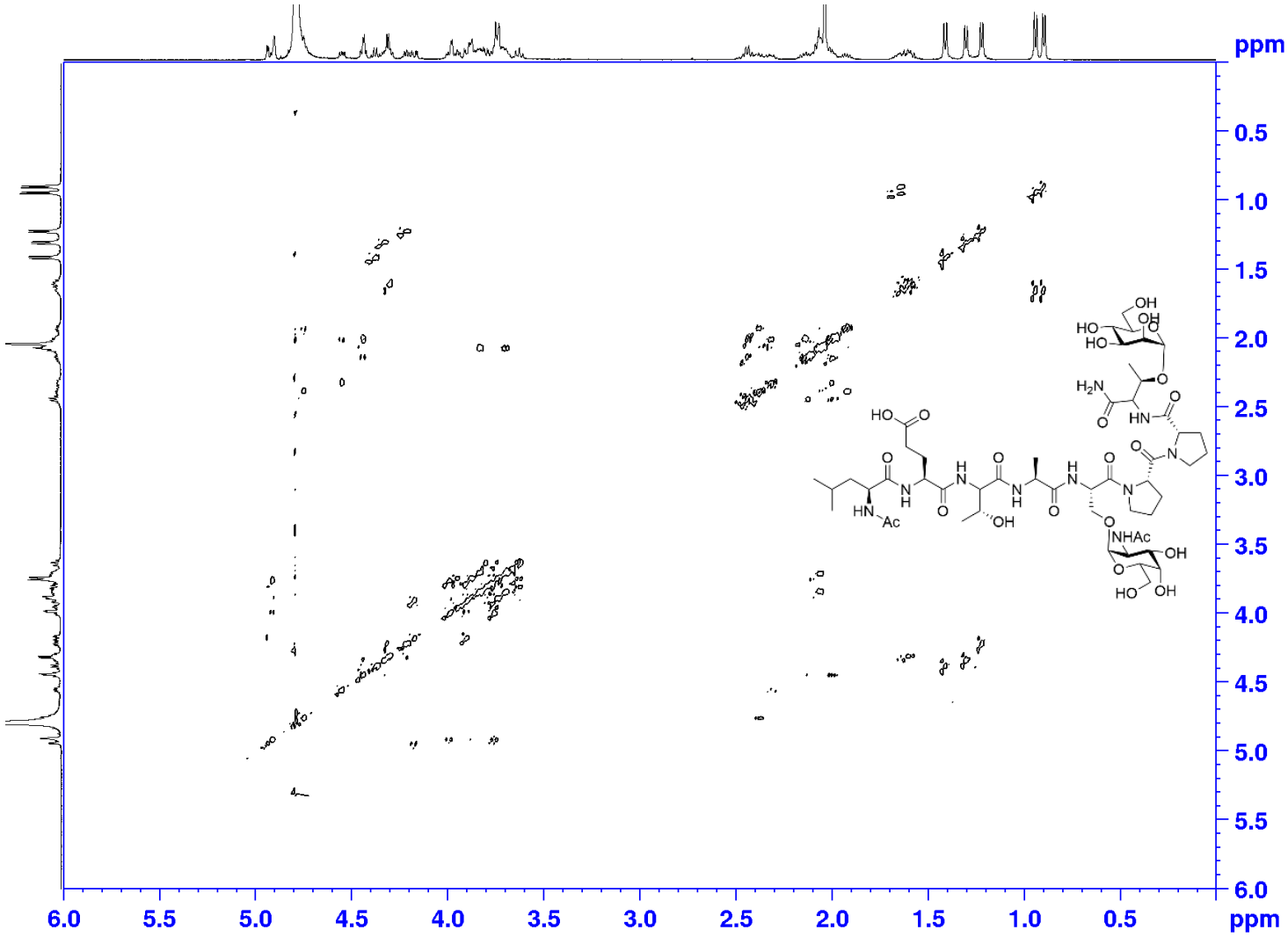


Figure S63 - COSY NMR analysis of **αDG-9-Ac** (D_2O, 500MHz).

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