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Glycotentacles: Synthesis of cyclic glycopeptides toward a tailored blocker of influenza virus hemagglutinin

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Materials and general methods

Unless otherwise stated, all commercially available solvents and reagents were used without purification. Fmoc amino acid derivatives and 2-chlorotrityl chloride resin were purchased from NOVA biochem Co. Ltd. α-2,3-(*N*)-Sialyltransferase was purchased from Calbiochem Co. Ltd. and transglutaminase from guinea pig liver was purchased from ORIENTAL YEAST Co. Ltd. Automatic peptide synthesis was performed with Applied Biosystem MODEL 433A peptide synthesizer. Optical rotation was measured with Perkin Elmer Polarimeter 343. NMR spectra were recorded with Bruker DRX 600 spectrometer at 600 MHz (^{1}H NMR) and 150 MHz (^{13}C NMR). Ring-proton assignments in NMR were made by first-order analysis of the spectra and supported by H-H COSY spectra. Preparative HPLC was performed on a Hitachi HPLC system equipped with an L-7400 intelligent pump, an L-7400 UV detector and reversed-phase C18 column, Inertsil® ODS-3 (10 x 250mm) at a flow rate of 3.0 ml min^{-1}. The eluant was monitored by UV absorption at 220nm. Chemical reactions were monitored by thin layer chromatography (TLC) on a precoated plates of

silica gel 60 F_{254} (layer thickness, 0.25 mm; E. Merck, Darmstadt), and Matrix-associated laser-desorption ionization time of fright mass spectrometry (MALDI-TOF-MASS ; Bruker REFLEXIII mass spectrometry). Column chromatography was performed on silica gel (Silica gel 60; 0.015-0.040 mm, E. Merck). Solvent extracts were dried with magnesium sulfate.

Molecular modeling

To confirm that the orientation of the sugar moieties can be fixed by cyclic peptide scaffold in solutions, conformation of cyclic glycopeptide having three sugar chains (**1-t**) was investigated using SYBYL molecular modeling software. First, energy minimized 3-D structure was calculated by Maximin 2 energy minimizer of SYBYL using Tripos force field parameters. Calculations were started from two model conformations with the longest distance (A-1) between each sialic acid and the shortest distance (B-1) in water at room temperature (T=300 K). The sugar chains of A-1 were roughly in a plane of the cyclic peptide and are nearly radiate in all direction. All sugar chains of B-1 were approximately perpendicular to the plane of the cyclic peptide.

For these two conformers, molecular dynamics (MD) calculations with *T*=300 K were carried out. The time interval of the MD was 100 ps and the time step was 1 fs. All calculations were performed using the software SYBYL 6.7 (Tripos Association, St. Louis, MO) on a Silicon Graphics Origin 200 workstation.[1,2] The MD calculations were performed with the force field developed by Tripos and with the atomic charges calculated by the Gasteiger-Hückel method.[3-5] The cyclic glycopeptide **1-t** was surrounded by 14644 water molecules for A-1 and 4507 water molecules for B-1, and the cyclic glycopeptide **1-t** and water molecules were in a box with the size of 33.8×63.0×67.1 Å for A-1 and 45.7×41.1×52.4 Å for B-1. The periodic boundary condition was used in the MD calculations.

Figure S-1 (A-2) and (B-2) show the geometry of A-1 and B-1 after 100 ps of MD simulations. The changes of the geometry were not drastic throughout the trajectories. The sides of the triangle made by the C_1 carbon of sialic acids in **1-t** are 60, 51 and 48 Å for A-2 and 37, 38 and 38 Å for B-2. These lengths were not so far from those between the sugar-binding sites of HA. Therefore, this suggests that the cyclic glycopeptide **1-t** may be expected to occupy the binding pockets of HA.

Second, the conformations entered on SYBYL cyclic glycopeptide (**1-t**)was docked to hemagglutinin extracted from the protein bank and the energy minimized 3-D structure of hemagglutinin-cyclic glycopeptide (**1-t**) complex was determined as shown in Figure 2 of the main text.

(Figure S-1)

Synthesis.

Cyclo(Ser-Gly-Gly-Gln-Ser-His-Asp)$_3$ (2)

The linear peptide was prepared on 2-chlorotrityl chloride resin (0.7 mmol Cl^-/g) using a common solid-phase peptide synthetic method. Fmoc-Asp-ODmab (0.75 g, 1.13 mmol) was coupled to the resin in the presence of diisopropylethylamine (5 ml) in dichloromethane (DCM) (20 ml) for 1 h at room temperature. To a solution was added with a mixture of methanol (0.8 ml) and diisopropylethylamine (0.2 ml) for endcapping and the mixture was stirred for 20 min at room temperature. The mixture was filtered off and the resin was washed with *N*,*N*′-dimethylformamide (40 ml),

2-propanol (30 ml), DCM (40 ml), and dried in vacuo. Fmoc-Asp-ODmab loaded to the resin was estimated to be 0.16 mmol/g by the ninhydrin method. The chain elongation was performed automatically using peptide synthesizer with 0.05 mmole scale. Fmoc amino acid derivatives, Fmoc-Asp(tBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Gly-OH, Fmoc-His(Trt)-OH, and Fmoc-Ser(tBu)-OH were employed. After completing the synthesis, the resin was washed with *N*-metyl pyrrolidone (NMP) (2 x 10 min) and DCM (3 x 10 min), and dried in vacuo.

Deprotection of the C-terminal Dmab group was carried out in a batch-wise manner. The peptide-resin was treated with 2% (v/v) hydrazine-H_2O in NMP (10 ml) for 15 min at room temperature. The reaction was monitored by spectrophotometer at 290 nm as the amount of the indazole by-product. The mixture was filtered off and the resin was washed with NMP (40ml) and DCM (40ml), and dried in vacuo.

To a solution of the partially protected peptide-resin in NMP (10 ml) was added diphenylphosphorylazide (300 mg, 1.1 mmol) in ice cold and dropwise triethylamine (140 mg, 1.4 mmol). The mixture was stirred for 15 min, and continuously for 24 h at room temperature. The mixture was filtered off and the resin was washed with

NMP (40 ml) and DCM (40 ml), dried in vacuo. The procedure of cyclization on the resin was repeated three times.

The protected cyclic peptide-resin was treated with the splitting mixture TFA/H_2O/ethanedithiol (10 ml, 9.50/0.25/0.25 (v/v)) for 2 h at room temperature to remove the peptide from resin and for deprotection of tBu and Trt groups. The mixture was filtered off and washed with TFA (2 ml) and DCM (2 ml). The solvent was evaporated and precipitated from cold dry *tert*-butylmethyl ether. To give pure cyclo(Ser-Gly-Gly-Gln-Ser-His-Asp)$_3$, the obtained white solid was purified by Sephadex G-25 gel filtration (1.5 cm x 60 cm, H_2O) and preparative HPLC [solvent A (0.1 % TFA in H_2O) and solvent B (0.1 % TFA in acetonitrile), gradient; increase from 0% to 70% B over 60 min with flow rate of 3 ml/min, monitoring at 220 nm]. The fractions containing cyclo(Ser-Gly-Gly-Gln-Ser-His-Asp)$_3$ were collected and lyophilized. The cyclicpeptide **1** was obtained as white powder. (14mg, overall yield 14%)

^{1}H-NMR (600 MHz; D_2O); 1.90, 2.05 (m, 6 H, Gln-β), 2.29 (m, 6 H, J = 7.49 Hz, Gln-γ), 2.71-2.80 (m, 6 H, Asp-β), 3.11-3.25 (m, 6 H, His-β), 3.67-3.85 (m, 12 H,

Ser-β), 3.89~3.91 (m, 12 H, Gly-α), 4.28-4.30 (m, 3 H, Gln-α), 4.35 (m, 6 H, Ser-α), 7.24 (s, 3 H His-im C_2), and 8.55 (s, 3 H His-im C_4)

Amino acid ratios of the acid hydrolysate; Asp 3.3 (3), Ser 5.2 (6), Glu 3.1 (Gln;3), Gly 3.1 (3), His 3.2 (3)

MALDI-TOF-MASS; Calculated for 2004.75, found $(M+H)^+$ 2005.738, $(M+Na)^+$ 2028.110, $(M+K)^+$ 2044.307, ESI-MASS (m/z); 2005.2 $(M+H)^+$

(Scheme S-1)

(Figure S-2)

6-(Benzyloxycarbonyl) aminohexan-1-ol (5)

To a solution of 6-aminohexan-1-ol (5.0 g, 0.043 mol) and sodium hydrogen carbonate (7.2 g, 0.086 mol) in water were added dropwisely a mixture of bezyloxycarbonyl chloride (8 ml, d=1.195, 0.056 mol) and ether (10 ml). The mixture was stirred for 3 h at room temperature. The mixture was filtered with celite, aorganic layer was extracted with ether, washed with water, dried, and evaporated. Recrystallization from ethanol gave the compound **5** (9.2 g, 85%).

^{1}H-NMR (600 MHz; $CDCl_3$); 1.35~1.57 (m, 8 H, CH_2), 3.20 (t, *J*=6.5 Hz, 2 H, NCH_2), 3.63 (t, *J*=6.5 Hz, 2 H, OCH_2), 5.09(s, 2 H, PhC*H* $_2$), and 7.30-7.37 (m, 5 H, aromatic).

6-Aminohexyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2, 3, 4, 6-tetra-*O*-benzoyl-β -D-galactopyranosyl)-β-D–glucopyranoside (6)

To a solution of 1,2,3,6-tetra-*O*-benzoyl-4-*O*-(2, 3, 4, 6-tetra-*O*-benzoyl-D-galactopyranosyl)-α/β-D-glucopyranose (3.0 g, 2.6 mmol) in dry 1,2-dichloroethane (15 ml) was added dropwisely 30% HBr-acetic acid (3.5 ml) at -20°C and stirred for 6 h. The mixture was poured into ice and water, extracted with chloroform, and washed with H_2O, $NaHCO_3$ and brine. After evaporation, the residue was dissolved and recrystalized from methanol to give bromo 2,3,6-tri-*O*-benzoyl-4-*O*-(2, 3, 4, 6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-β-D-glucopyranose **7** (2.6 g). To a solution of compound **7** (2.1 g, 1.85 mmol) and **5** (500 mg, 2.00 mmol) in dry CH_2Cl_2 (20 ml) was added silvertrifluoromethanesufonate (532 mg, 2.03 mmol) under a nitrogen atmosphere at -20 °C and the reaction mixture was stirred for 16 h at room temperature. The mixture was filtered with celite, and extracted with ether. The extract was washed

with water, dried, and evaporated. Since this compound was unstable, deprotection of benzoyl and benzyloxycarbonyl groups was performed without further purification.

6-Aminohexyl-(4-*O*-β-D-galactopyranosyl)-*O* –β-D-glucopyranoside (4)

Perprotected **6** (2.2 g) was treated with sodium methoxide (50 mg) in methanol-tetrahydrofuran (3:1, v/v) for 16 h at room temperature. The mixture was neutralized with DOWEX 50W X-8 (H^+) and filtered with celite. Then, the solution was evaporated. To the residual solution dissolved in methanol-water (20:1) was added palladium-activated carbon (Pd=10%) (50mg). The mixture was stirred at room temperature under hydrogen gas for overnight. After filtration and evaporation, the residue was dissolved and recrystallized from methanol to give compound **4**. (410 mg, overall yield from perbenzoylated lactose=36%)

$[\alpha]_D$ (c=0.5, H_2O) -18.7°, ^{1}H-NMR (600 MHz, D_2O); 1.42 (m, 4 H, CH_2), 1.60~1.68 (m, 4 H, CH_2), 2.56 (t, *J* = 7.21 Hz, 2 H, C*H*$_2$NH$_2$), 3.33 (t, *J* = 7.81 Hz, 2 H, H-2'), 3.57~3.81 (m, 10 H, H-2, H-4', H-3, H-3', H-6'a, OCH_2, H-5, H-5', H-6a, H-6b), 3.95~4.01 (m, 3 H, OCH_2, H-4, H-6'b), 4.37(d, *J* = 7.21 Hz, 1H, H-1), 4.40 (d, *J* = 7.81

Hz, 1 H, H-1').

(^{13}C-NMR, D_2O) 25.2, 25.9, 28.8, 29.0 ($\underline{C}H_2$), 40.3 ($N\underline{C}H_2$), 60.7 (C-6'), 61.5 (C-6), 69.1 (C-4), 71.0 (C-5), 71.5 (C-2), 73.1 ($O\underline{C}H_2$), 73.4 (C-2'), 75.0 (C-3'), 75.3 (C-4'), 75.9 (C-5'), 79.1 (C-3), 102.6 (C-1'), 103.5 (C-1).

FAB-MASS (m/z); Calculated for 441.22, Found 442 $[M+H]^+$

(Figure S-3)

Synthesis of cyclic glycopeptides

To a solution of 6-aminohexyl-(4-*O*-D-galactopyranosyl)-*O* -D-glucopyranoside **4** (30 mg, 67.9 μmol) and compound **1** (3 mg, 1.49 μmol) in 0.2 M Tris-HCl buffer containing 10 mM calcium chloride (pH=7.5, 500 μl) was added transglutaminase from guinea pig liver (2.0 U) and incubated for 24 h at 37 °C. The reaction was monitored with MALDI-TOF mass spectrometry. Although a variety of reaction conditions were examined for this step, a mixture of mono-, di-, and trivalent cyclic glycopeptide intermediates was obtained. The mixture was applied to Sephadex G-25 gel filtration (1.5 cm x 60 cm, H_2O) directly to remove enzyme, excess of compound **4**,

and buffer solution. The fractions containing glycosylated products with starting material **1** were collected and lyophilized. Since the mixture of these intermediates could not be separated by using common chromatographic purifications, we decided to use them for the next sugar elongation reaction without further purification.

Bovine serum albumin (BSA) (2 mg/ml) and manganese (II) chloride tetrahydrate (1.58 mM) were dissolved in sodium cacodylate buffer (50 mmol) and pH of this solution was adjusted with 0.25 M HCl (aq) to pH 7.40. To a solution of cyclic glycopeptide intermediate (staring from 3 mg of **1**) in buffer solution were added Triton CF-54, Cytidine-5'-mono-phospho-*N*-acetylneuraminic acid (CMP-NANA) (7 mg, 11μmol), carfintestinal alkaline phosphate (CIAP) (20 U) and α-(*N*)-2,3-sialyltransfarase (100 mU) and the mixture was incubated for 3 days at 37 °C. The reaction was monitored with MALDI-TOF mass spectrometry. The mixture was treated with Sephadex G-25 (1.5 x 60 cm, H_2O) to remove enzyme and salt. The mixture was subjected to the lectin affinity chromatography (Agarose immobilizing *R. communis agglutinin*) (H_2O, gradient; 1 mM lactose aq.) to remove intermediates carrying terminal galactose residues and ion exchanged chromatography

(DEAE-Sepharose; elution with 0.5 M NH_4HCO_3 aq.) to remove triton CF-54 and Sephadex G-25 (1.5 cm x 60 cm, H_2O) to remove NH_4HCO_3. To isolate the target compound **1-m**, **1-b**, and **1-t**, a preparative HPLC [solvent A (H_2O) and solvent B (acetonitrile), gradient; increase from 0% to 60% B over 60 min with flow rate of 3 ml/min, monitoring at 220 nm] was finally performed. (**1-m** = 489μg, 12%, **1-b** = 514μg, 10%, **1-t** = 497μg, 8%)

1-m: ^{1}H-NMR (600 MHz; D_2O) 1.26 (m, 4 H, CH_2), 1.41 (m, 2 H, CH_2), 1.53 (m, 2 H, CH_2), 1.72 (t, *J* = 12.4 Hz, 1 H, NeuNAc-3ax), 1.90, 2.05 (m, 6 H, Gln-β), 1.96 (s, 3 H, NHCOC*H*$_3$), 2.24 (m 2 H, Gln-β linked sugar chain), 2.29 (m, 4 H, Gln-γ), 2.70-2.80 (m, 7 H, Asp-β, NeuNAc-3eq), 3.10 (m, 5 H, His-β, C*H*$_2$NHCO), 3.25 (m, 4 H, His-β, H-2'), 3.51-3.56 (m, 8 H, H-2, H-3, H-3', H-5, H-5', H-6, NeuNAc-4, NeuNAc-5), 3.67-3.85 (m, 11 H, H-4, H-4', H-6, Ser-β), 3.89~3.91 (m, 12 H, Gly-α), 4.28-4.39 (m, 11 H, Gln-α, Ser-α, H-1', H-6'), 4.45 (d, 1 H, *J* = 7.8, H-1), 7.24 (s, 3 H, His-im C_2), 8.55 (s, 3 H, His-im C_4)

MALDI-TOF MASS; Calculated for 2720.04, Found 2721.47 $[M+H]^+$

(Figure S-4)

1-b: ^{1}H-NMR (600 MHz; D_2O) 1.26 (m, 8 H, CH_2), 1.41 (m, 4 H, CH_2), 1.53 (m, 4 H, CH_2), 1.72 (m, 2 H, NeuNAc-3ax), 1.90, 2.05 (m, 6 H, Gln-β), 1.96 (s, 6 H, $NCOCH_3$), 2.24 (m 4 H, Gln-γ linked sugar chain), 2.29 (m, 2 H, Gln-γ), 2.70-2.80(m, 8 H, Asp-β NeuNAc-3eq), 3.10 (m, 7 H, His-β, CH_2NHCO), 3.25 (m, 5 H, His-β H-2'), 3.51-3.56 (m, 16H, H-2, H-3, H-3', H-5, H-5', H-6, NeuNAc-4, NeuNAc-5), 3.67-3.85 (m, 16 H, H-4, H-4', H-6, H-6', Ser-β), 3.89~3.91 (m, 12 H, Gly-α), 4.28-4.39 (m, 11 H, Gln-α, Ser-α, H-1'), 4.45 (d, 2 H, J = 7.9, H-1), 7.24 (s, 3 H, His-im C_2), 8.55(s, 3 H, His-im C_4)

MALDI-TOFMASS; Calculated for 3435.33, Found: 3436.41 $[M+H]^+$

(Figure S-5)

1-t: ^{1}H-NMR (600 MHz; D_2O) 1.26 (m, 12 H, CH_2), 1.41 (m, 6 H, CH_2), 1.53 (m, 6 H, CH_2), 1.72 (t, J =12.2 Hz, 3 H, NeuNAc-3ax), 1.90, 2.05 (m, 6 H, Gln-β), 1.96 (s, 9 H, $NCOCH_3$), 2.24 (m 6 H, Gln-γ linked sugar chain), 2.70-2.80 (m, 9 H, Asp-β NeuNAc-3eq), 3.10 (m, 9 H, His-β, CH_2NHCO), 3.25 (m, 6 H, His-β, H-2'), 3.51-3.56 (m, 16 H H-2, H-3, H-3', H-5, H-5', H-6, NeuNAc-4, NeuNAc-5), 3.67-3.85 (m, 24 H, H-4, H-4', H-6, H-6', Ser-β), 3.89~3.91 (m, 12 H, Gly-α), 4.26 (m, 3 H, Gln-α,)

4.28-4.38 (m, 6 H, Ser-α,), 4.40 (d, 3 H, J = 6.1, H-1'), 4.45 (d, 3 H J 6.1, H-1), 7.24 (s, 3 H, His-im C_2), 8.55 (s, 3 H, His-im C_4)

MALDI-TOFMASS: Calculated for 4150.62, Found 4150.78 $[M+H]^+$

(Figure S-6)

Cyclo(Gly- Ser -Ser -Gln -Ser-Ser -Gly)$_3$ (2)

First, the linear peptide was prepared automatically on peptide synthesizer (0.1 mmol scale by stepwise coupling of Fmoc amino acid derivatives). The HMP (4-hydroxymethyl-phenoxy-methyl-copolystyrene-1% divinyl benzene) resin preloaded with Fmoc-Gly-OH was used. *Tert*-butyl (tBu) group was used as side chain protection for Ser, trityl (Trt) group was used as side chain protection for Gln. Fmoc amino acid derivatives (1 mmol) were coupled for 1 h in the presence of 1 M *N*,*N'*-dicyclocarbodiimide (DCC)/ NMP (2 ml) and 1 M 1-hydroxybenzotriazol (HOBt)/NMP (2 ml). The Fmoc protecting groups were removed by treatment with 20% piperidine in NMP. The reaction was monitored spectrophotometrically at 570 nm by following release of the piperidine-carbomate salt as by-product. After

completing the synthesis, protected peptide-resin was cleaved with the splitting mixture of TFA /H_2O/ethanedithiol (10 ml, 9.50/0.25/0.25) for 2 h at room temperature to remove the peptide from resin and for deprotection of tBu and Trt groups. The mixture was filtered off and washed with TFA (2 ml) and DCM (2 ml). The solvent was evaporated and precipitated from cold dry *tert*-butylmethyl ether. To give pure H_2N-(Gly-Ser-Ser-Gln-Ser-Ser-Gly)$_3$-OH, the obtained white solid was dissolved in water and chromatographed on Sephdex-25 gel filtration column (1.5 cm x 60 cm, 1% acetic acid in H_2O) and on preparative HPLC (Inertsil® C-18 column, 22 mm x 25 cm). The HPLC was performed with linear gradient obtained by mixing solvent A (0.1 % TFA in H_2O) and solvent B (0.08 % TFA in acetonitrile), and monitored at 220 nm. The gradient was programmed to increase from 0% to 60% B over 60 min with flow rate of 3 ml/min. The fractions of H_2N-(Gly-Ser-Ser-Gln-Ser-Ser-Gly)$_3$-OH were collected and lyophilizedto give pure white powder [66mg, overall yield 37% ; Amino acid ratios of the acid hydrolysate; Gln 3.1 (3), Ser 10.1 (12), and Gly 6.3 (6)]. MALDI-TOF-MASS; Calculated for 1788.70, Found: $(M+Na)^+$ 1811.7, $(M+K)^+$ 1827.3).

Next, to a solution of H_2N-(Gly-Ser-Ser-Gln-Ser-Ser-Gly)$_3$-OH (41 mg, 23 μmol) in distilled H_2O (50ml) and ethanol (30 ml) was added dropwisely *N* -ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) (300 mg, 1.2 mmol) in ethanol (20 ml) and the mixture was stirred for 30 min at 0 °C and for 4 h at room temperature until ninhydrin reaction became negative. After evaporation, the residue was purified by Sephadex G-25 gel filtration (1.5 cm x 60 cm, 1% acetic acid in H_2O) and by preparative HPLC [solvent A (0.1 % TFA in H_2O) and solvent B (0.1 % TFA in methanol), gradient; increase from 0% to 70% B over 60 min with flow rate of 3 ml/min, monitoring at 220 nm] to give cyclo(Gly-Ser-Ser-Gln-Ser-Ser-Gly)$_3$ **2** (7.2 mg, 18%).

Amino acid ratios of the acid hydrolysate; Gln 2.9 (3), Ser 10.8 (12), Gly 6.1 (6)

^{1}H-NMR (600 MHz; D_2O): 1.86, 2.00 (m, 6 H, Gln-β), 2.23 (dd, 6 H, *J*=7.49 Hz, Gln-γ), 3.70~3.86 (m, 18 H, Ser-β), 3.70~3.76 (m, 12 H, Gly-α), 4.31~4.37 (m, 9 H, Ser-α).

MALDI-TOF-MASS: Calculated for 1771.65 $(M+H)^+$, Found: $(M+Na)^+$ 1794.9

Synthesis of cyclic glycopeptide from cyclo(GSSQSSG)$_3$

Cyclic glycopeptide having trivalent GM3 oligosaccharides based on cyclo(GSSQSSG)$_3$ was synthesized by similar procedure of the synthesis of compound **1**. To a solution of cyclo(GSSQSSG)$_3$ (3 mg, 1.69 μmol) in 0.2 M Tris-HCl buffer (containing 10 mM calcium chloride, pH=7.5, 500 μl) was added compound **4** (30mg, 68 μmol) and guinea pig liver transglutaminase (2.1 U). Next, sugar elongation was performed in 50mM sodium cacodylate buffer (2 mg/ml BSA, 1.58 mmol $MgCl_2$, 0.1% Triton CF-54) by α-2,3-(*N*)-sialyltransferase (100mU, CMP-NANA; 13 mg, 19.7 μmol, CIAP ; 20 U). The products were isolated according to the procedures described in the synthesis **1-m**, **1-b**, and **1-t**. (**2-m**=829 μg, 20%; **2-b**=400 μg, 7%; **2-t**=600 μg, 9%).

(Figure S-7)

Synthesis of cyclo(GSQSSG)$_3$ 3 and cyclic glycopeptide (3-m, 3-b and 3-t).

Synthesis of cyclo(GSQSSG)$_3$ **3** and cyclic glycopeptide (**3-m**, **3-b** and **3-t)** was synthesized by similar procedure of the synthesis of cyclic peptide **3** and cyclic glycopeptide **2-m**, **2-b** and **2-t**.

(**3**, 24%; **3-m**, 8%; **3-b**, 7%; **3-t**, 33%)

Evaluation of the inhibitory effect by cyclic glycopeptides on hemagglutination

Influenza virus, A/PR/8/34(H1N1) was used to evaluate the inhibition of hemagglutination. This assay was carried out using 96-well microtiter plates. Phosphate buffer saline (pH 6.5) containing 0.01% gelatin was used as a dilution buffer. Chicken erythrocytes were used as indicator cells. Virus suspension (4 HA units in 0.025 ml of PBS) was added to each well containing synthetic sialyllactose peptide derivatives in two-fold serial dilutions with the dilution buffer. The plates were incubated at 4ºC for 1 h after 0.05 ml of 0.5% (v/v) chicken erythrocytes in PBS were added to the plates, the plates were then kept at 4°C for 1 h. The maximum dilution concentration of the samples showing complete inhibition of the hemagglutination was defined as the concentration of hemagglutination inhibition assay. Fetuin was used for contorol.

Binding assay using SPR

All binding experiments were carried out on a BIAcore biosensor system (BIAcore X) using a carboxy-methylated Dextran-coated gold sensor surface (CM-5).

Hemagglutinins (recombinant influenza virus hemagglutinin purchased from KATAKURA INDUSTRIES Co., LTD; A/Equine/La Plata/93) were covalently immobilized on the dextran polymer. Carboxyl groups of the dextran polymer were activated with 80μl of a 50:50 (v/v) solution of *N*-hydroxysuccinimide (NHS) and *N*-ethyl-*N'*-(3-diethylaminopropyl)carbodiimide (EDC), at a flow rate of 20 μl/min. After the injection loop was washed with PBS buffer (pH 7.2), hemagglutinin (1.25 mg/ml) in PBS buffer (pH 7.2) was injected over the activated sensor surface for 6 min at flow rate of 5μl/min. The remaining activated residues were blocked with ethanolamine (1M, pH 8.0). Synthetic cyclic peptides and cyclic glycopeptides were diluted in PBS buffer to a final concentration of 50-500 μM and exposed to the immobilized hemagglutinin on the sensor surface for 5 min at a flow rate of 2 μl/min (association phase). During the dissociation phase, the sensor surface was exposed to PBS buffer at a flow rate of 2 μl/min. The values of K_d was determined from **Figure S-9**.

NMR spectroscopy for conformational analysis.

The NMR measurements were performed on a Bruker DRX 600 spectrometer operating at 600 MHz for the proton frequency equipped with triple resonance probehead. The sample pH was adjusted to 5.0 and spectra were recorded with a sample temperature of 293 K. In order to achieve the assignment completely and collect the conformational

restraints, two-dimensional DQF-COSY,[6] TOCSY with MLEV-17 spin-lock sequence,[7,8] and NOESY [9] spectra were recorded in a phase sensitive mode. All two-dimensional spectra were recorded with 2048 x 256 time data points and zero-filled to yield 2048 x 2048 data matrices except for high resolution DQF-COSY recorded for determination of the coupling constant of NH and $^{\alpha}$H ($^{3}J_{HN\alpha}$). High resolution measurement was recorded with 4096 x 256 data points. Time domain data in both dimensions were multiplied by a sine-bell window function with 90° phase shift prior to Fourier transformation. The spectral width of all two-dimensional measurements was 6001.13 Hz, and the water signal was suppressed by WATERGATE method with 3-9-19 pulse sequence. [10,11]

All NMR spectra were processed by NMRPipe, [12] and the signals were assigned with the program XEASY [13] on a Silicon Graphics workstation. DQF-COSY and TOCSY spectra recorded with the samples dissolved in H_2O were used to identify the spin systems of all residues. And one-dimensional spectra were also used to identify the amide protons protected from solvent exchange using sample that had been lyophilized from H_2O solution and resuspended in D_2O. In order to complete

assignments, the sequential assignments that connect *i* th $^{\alpha}H$ ($^{\alpha}H_i$) to *i*+1 th amide proton (NH_{i+1}) *via* NOEs observed between these protons using the fingerprint region of NOESY spectra were conducted.

(Figure S-8)

Structure calculations.

Three-dimensional structures of cyclic peptide **1** and **3** were calculated with the program X-PLOR 3.851 [14] on R10000 processors of a Silicon Graphics workstation. A total of 67 distance restraints, 12 dihedral angle ϕ restraints were used to calculate the family of structures at cyclic peptide **1**. A total of 49 distance restraints, 9 dihedral angle ϕ restraints were used to calculate the family of structures at cyclic peptide **3**. Distance restraints for calculations were estimated from the cross-peak intensities in NOESY spectra with mixing time of 300 ms, and these were classified in tri-level as strong, medium and weak and were assigned upper limits of 3.0, 3.6 and 5.0 Å, respectively. For the lower limits, 1.8 Å was assigned to all restrains. Restrains of dihedral angle ϕ were based on $^{3}J_{HN\alpha}$ coupling constants measured in high resolution DQF-COSY. When $^{3}J_{HN\alpha}$ was more than 8.0 Hz, the dihedral angle ϕ was constricted

to -120±20°. Hydrogen bonds restraints were used as distance constraints of 1.5-2.5Å between amide protons and carbonyl oxygen, and 2.5-3.5 Å between amide nitrogen and amide protons, respectively. All analysis of RMSD values, secondary and tertiary structures of cyclic peptide **1** and **3** were performed with MOLMOL. [15]

(Table S-1, Table S-2, Table S-3, Table S-4)

References

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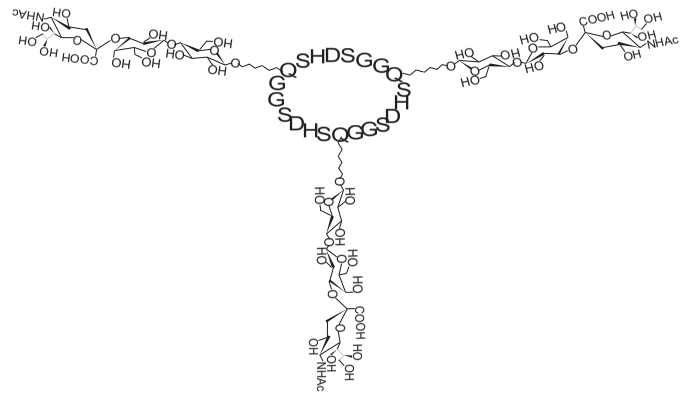
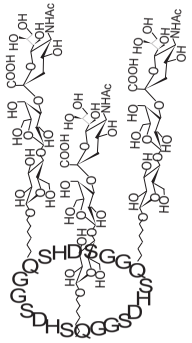
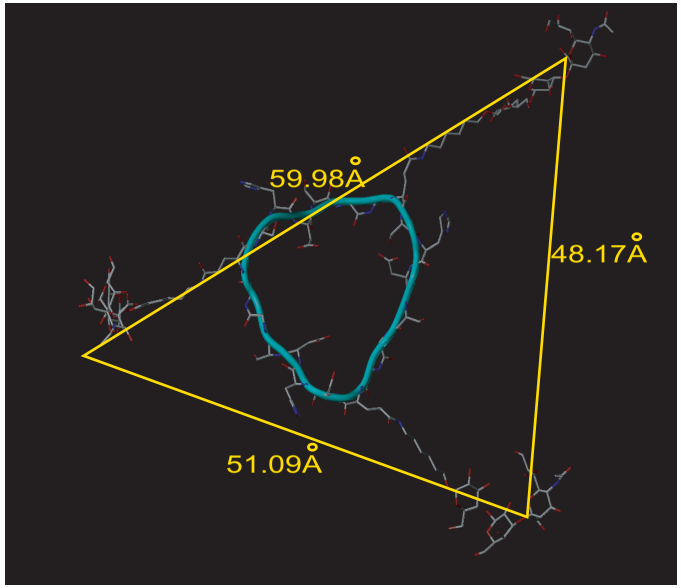


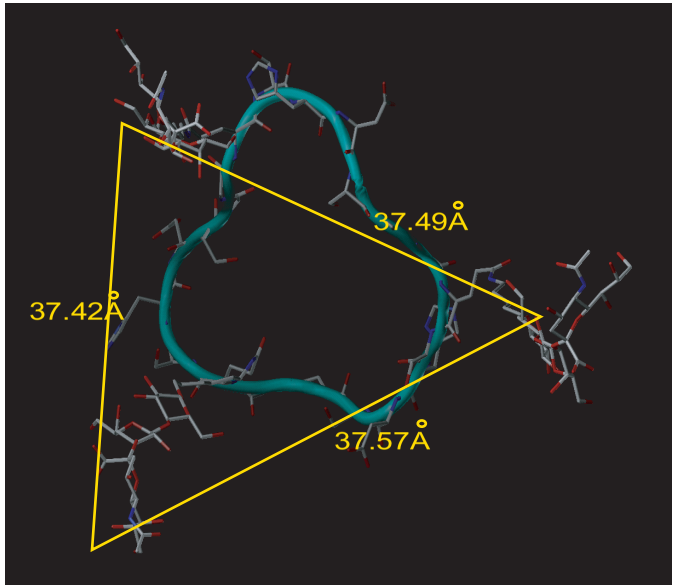
A-1



B-1

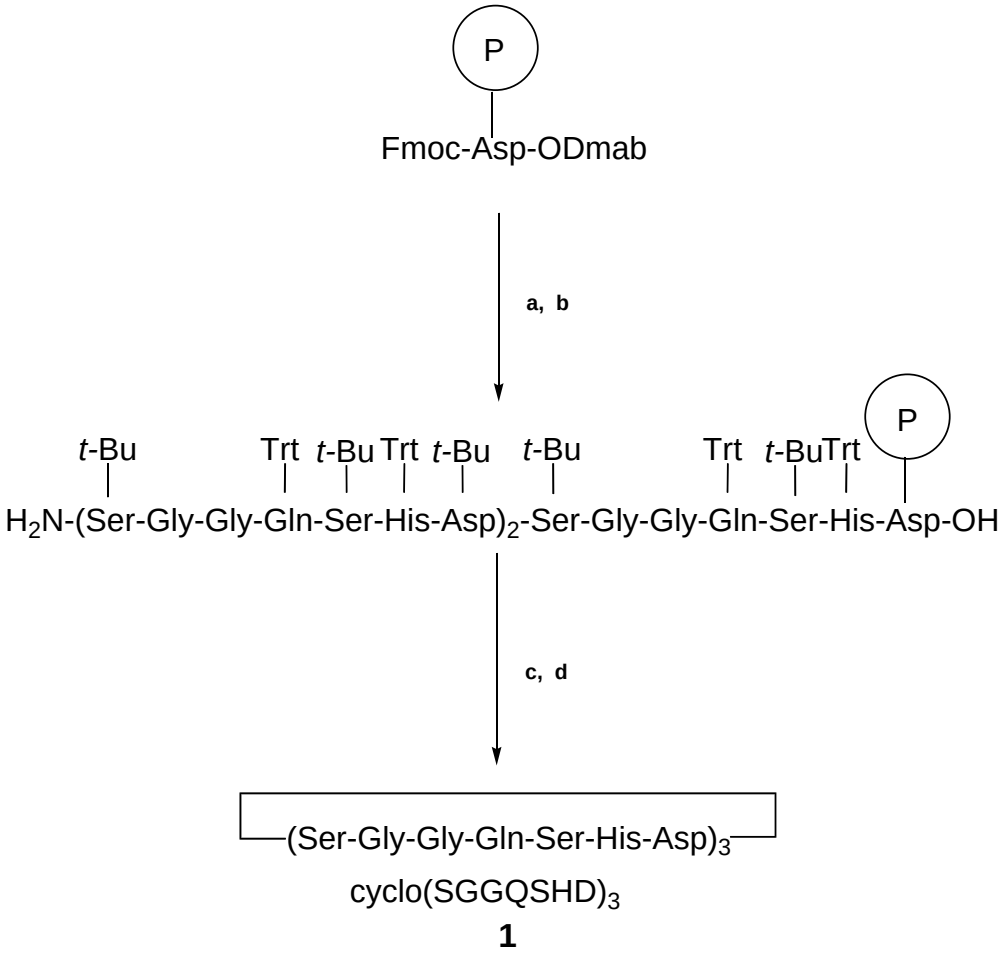


A-2



B-2

Figure S-1. Sugar-sugar distance calculated by MD method .



Scheme S-1. Synthesis of cyclo(SGGQSHD)$_3$: (a) Fmoc-AA-OH, *N*,*N'*-dicyclocarbodiimide, *N*-hydroxybenzotriazole, *N*-methylpyrrolidone; (b) 2% N_2H_4/*N*,*N*-dimethylformamide, 15 min; (c) diphenylphospholylazide/Et_3N, DMF, 48 h; (d) trifluoroaceticacid/H_2O/ethandithiol (95/2.5/2.5), 3 h (14% yield from preloaded resin). P=2-chlorotritylresin, ODmab=4-{N-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]amino}benzyl ester.

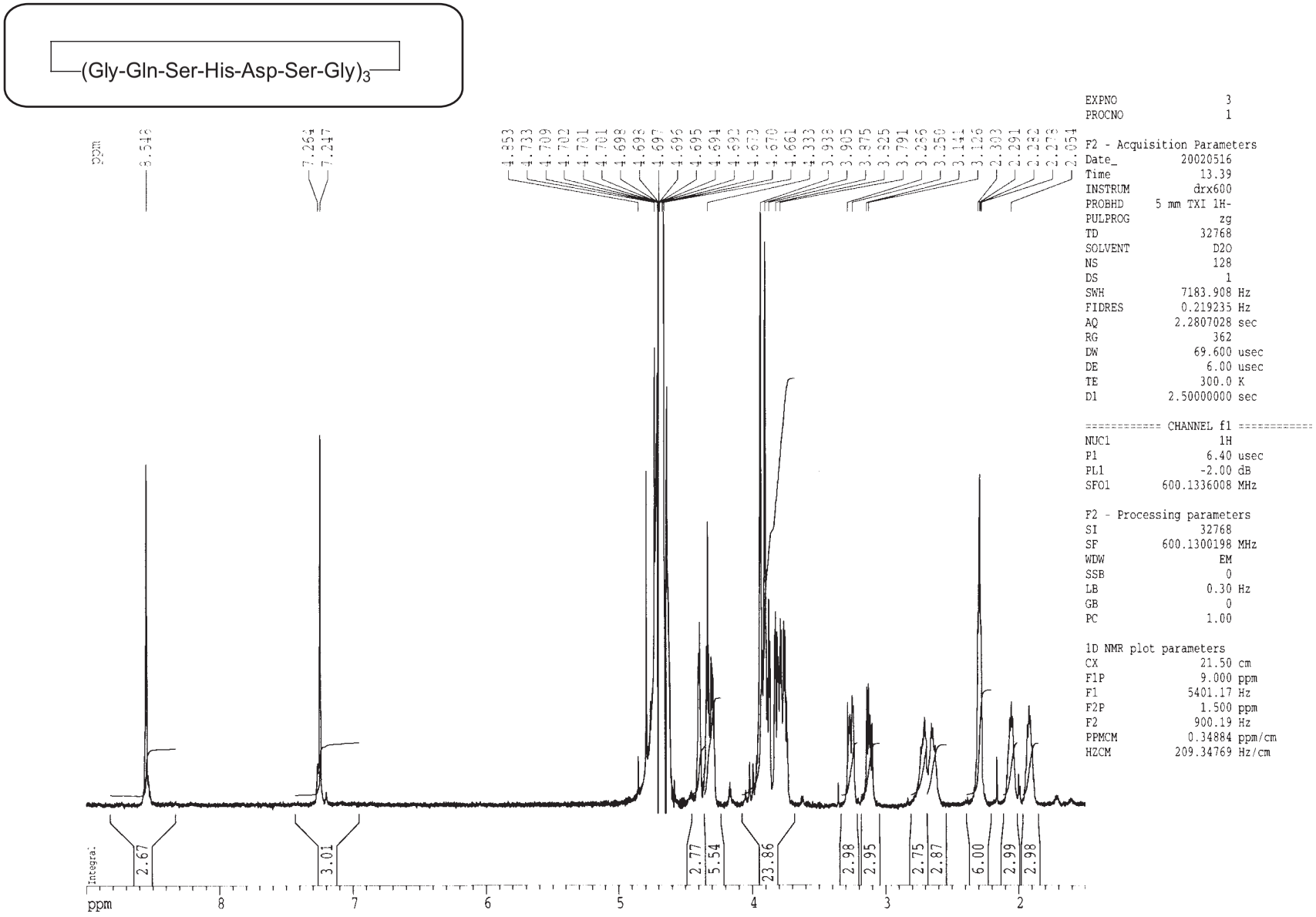


Figure S-2a. ^{1}H NMR spectrum (D_2O, 600 MHz, 300 K) of cyclic peptide **1**

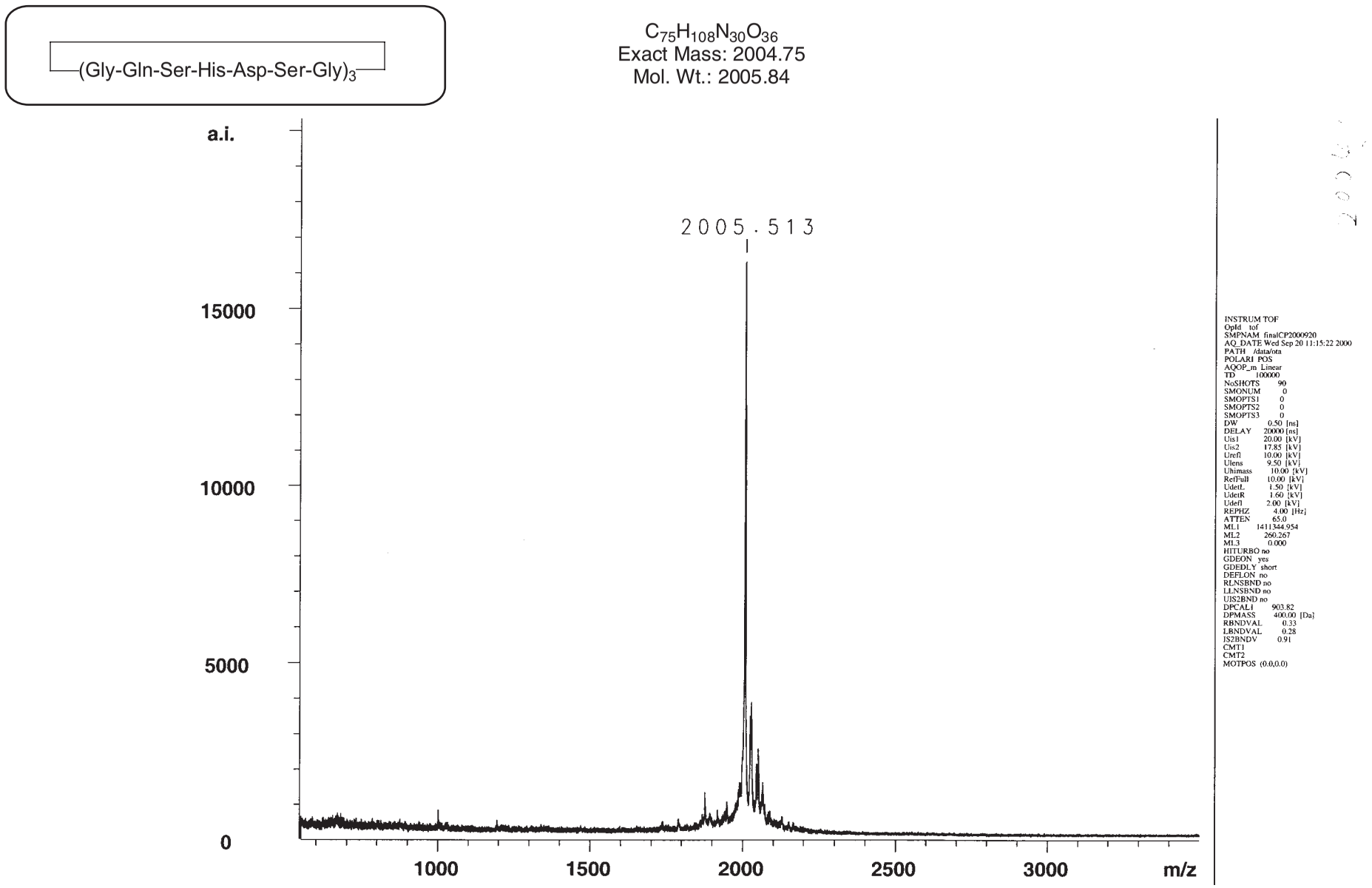


Figure S-2b. MALDI-TOF-MASS spectrum of cyclic peptide **1** (1 μM, 0.1% trifluoroacetic acid H_2O/CH_3CN = 2/1 (v/v) , matrix ; α-cyano-4-hydroxy cinnamic acid)

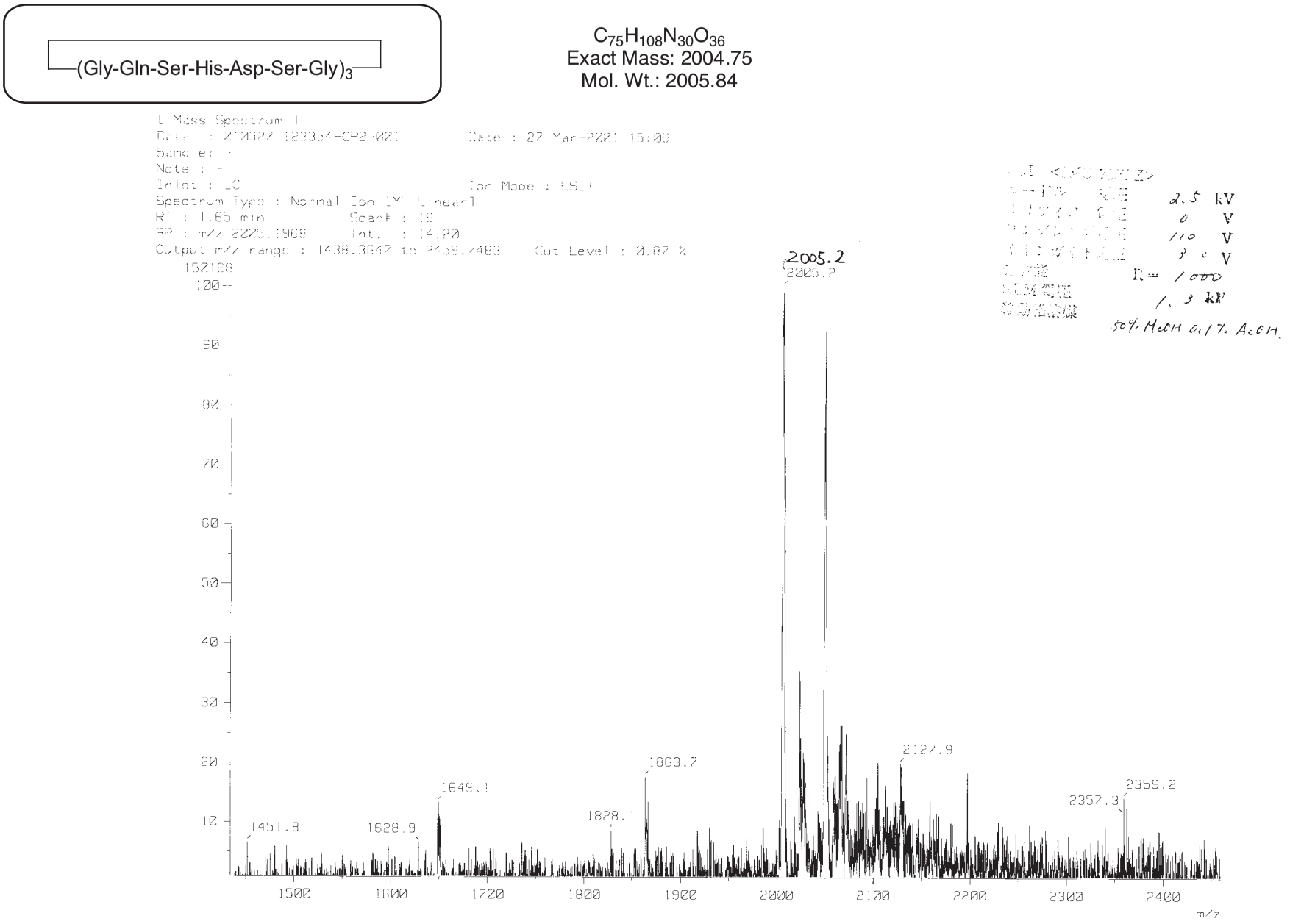
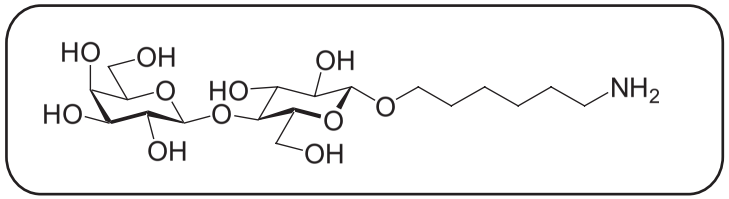
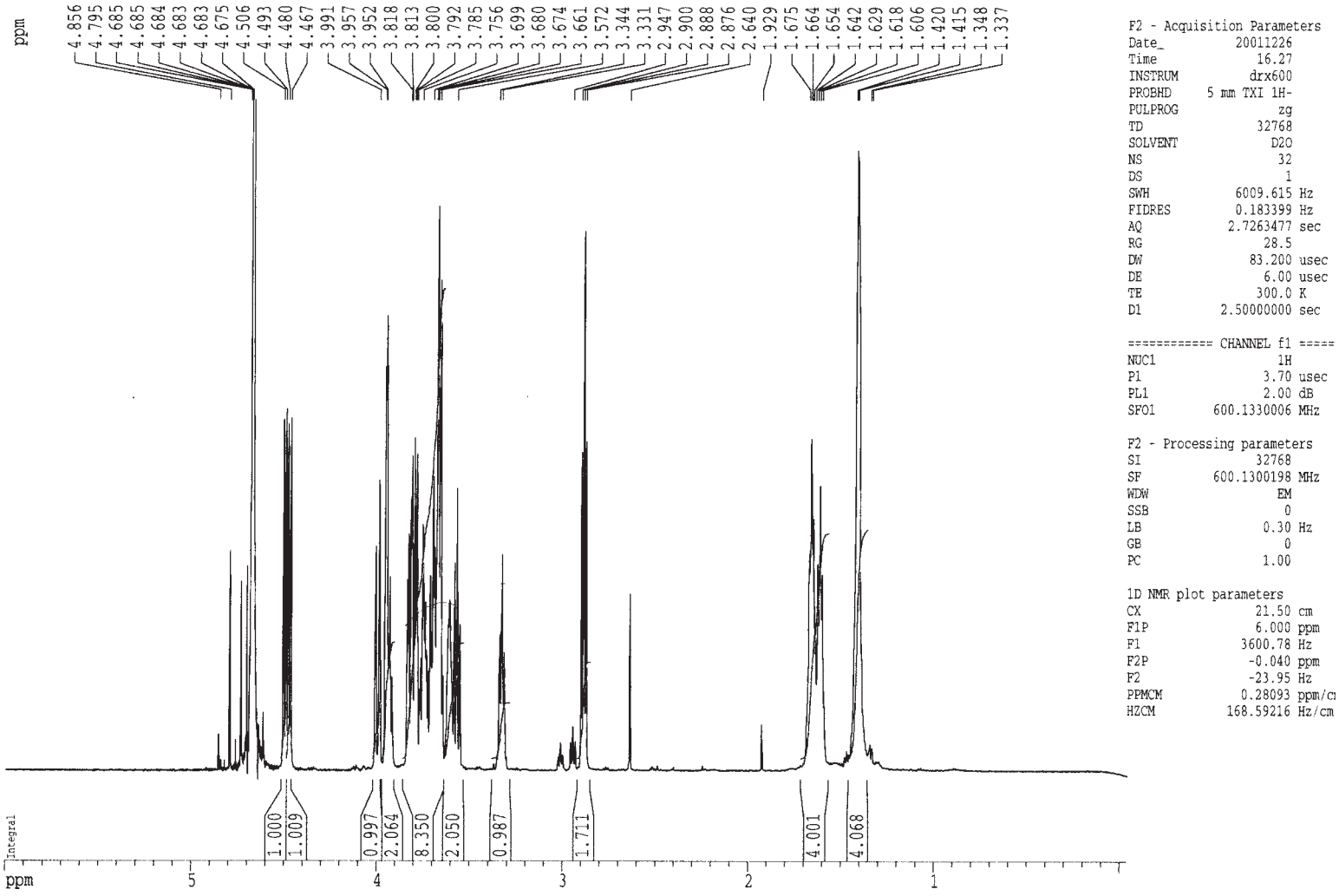


Figure S-2c. ESI-MASS-spectrum of cyclic peptide **1** (positive).



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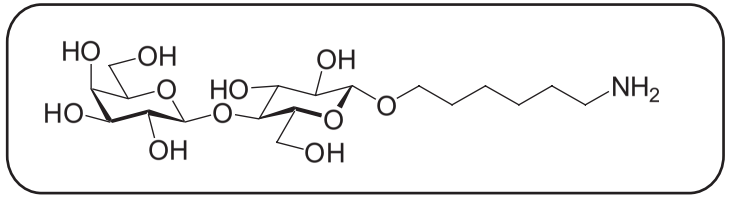
PULPROG                zg
TD                  32768
SOLVENT               D2O
NS                     32
DS                      1
SWH             6009.615 Hz
FIDRES          0.183399 Hz
AQ              2.7263477 sec
RG                   28.5
DW                 83.200 usec
DE                  6.00 usec
TE                 300.0 K
D1             2.50000000 sec

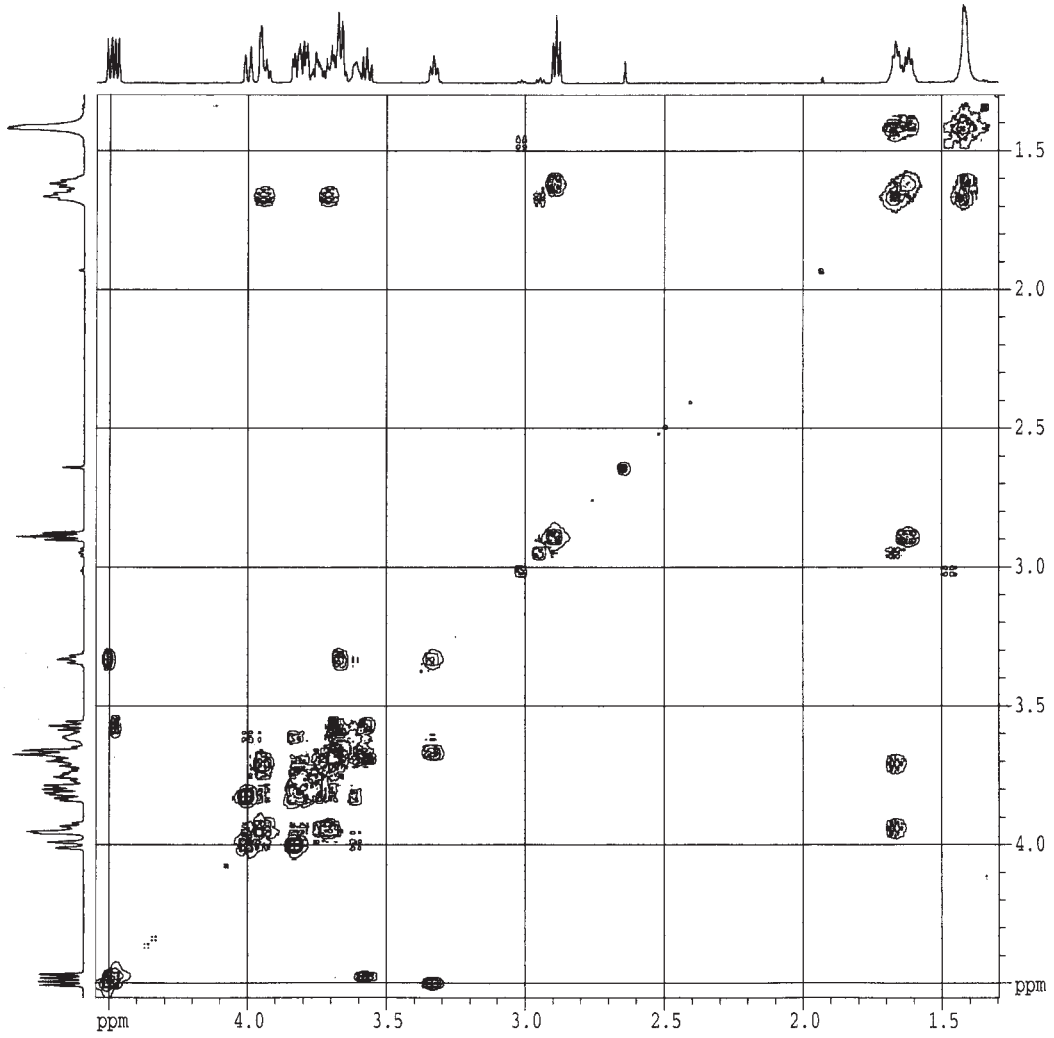
============ CHANNEL f1 =====
NUC1                   1H
P1                   3.70 usec
PL1                  2.00 dB
SFO1          600.1330006 MHz

F2 - Processing parameters
SI                  32768
SF            600.1300198 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00

1D NMR plot parameters
CX                  21.50 cm
F1P                 6.000 ppm
F1                3600.78 Hz
F2P                -0.040 ppm
F2                 -23.95 Hz
PPMCM           0.28093 ppm/cm
HZCM          168.59216 Hz/cm
```

Figure S-3a. ^{1}H NMR spectrum (D_2O, 600 MHz, 300 K) of lactose derivative having amino hexanol to reduing terminal **4.**



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EXPNO                        2
PROCNO                       1

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Time                    18.01
INSTRUM                drx600
PROBHD     5 mm TXI 1H-
PULPROG                cosygs
TD                       2048
SOLVENT                  DMSO
NS                          4
DS                          8
SWH                  2765.487 Hz
FIDRES               1.350335 Hz
AQ                   0.3703284 sec
RG                       322.5
DW                     180.800 usec
DE                       6.00 usec
TE                      300.0 K
D0                   0.00090320 sec
D1                   1.00000000 sec
D13                  0.00000300 sec
D16                  0.00010000 sec
IN0                  0.00036160 sec

========== CHANNEL f1 ==========
NUC1                        1H
P0                       11.20 usec
P1                       11.20 usec
PL1                       2.00 dB
SFO1                600.1313803 MHz

========== GRADIENT CHANNEL ======
P16                    1000.00 usec

      F1 - Acquisition parameters
ND0                         1
TD                        128
SFO1                600.1314 MHz
FIDRES               21.605366 Hz
SW                        4.608 ppm

      F2 - Processing parameters
SI                       1024
SF                  600.1300175 MHz
WDW                        SINE
SSB                           0
LB                         0.00 Hz
GB                            0
PC                         1.00

      F1 - Processing parameters
SI                       1024
MC2                        QF
SF                  600.1300175 MHz
WDW                        SINE
SSB                           0
LB                         0.00 Hz
GB                            0

      2D NMR plot parameters
CX2                      15.00 cm
CX1                      15.00 cm
F2PLO                     4.548 ppm
F2LO                   2729.33 Hz
F2PHI                     1.294 ppm
F2HI                    776.74 Hz
F1PLO                     4.552 ppm
F1LO                   2732.03 Hz
F1PHI                     1.299 ppm
F1HI                    779.44 Hz
F2PPMCM                0.21691 ppm/cm
F2HZCM               130.17233 Hz/cm
F1PPMCM                0.21691 ppm/cm
F1HZCM               130.17233 Hz/cm
```
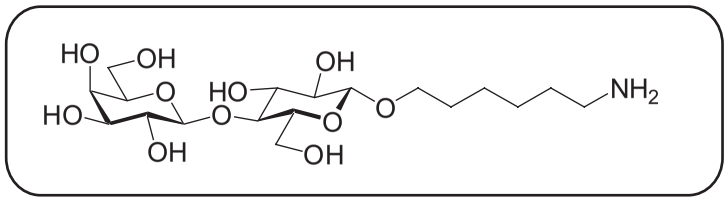
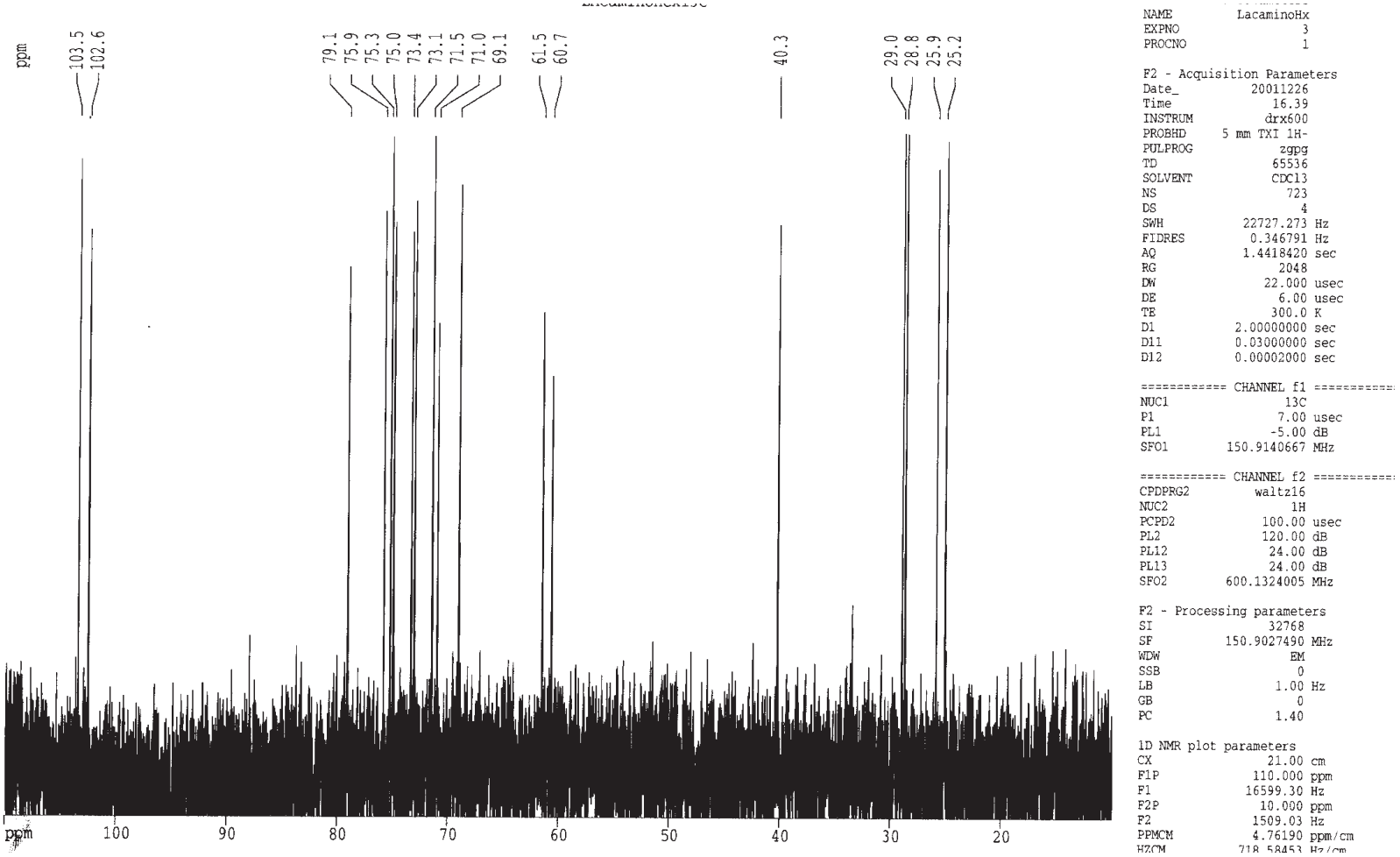

Figure S-3b. [^{1}H, ^{1}H] COSY NMR spectrum (D_2O, 300 K) of lactose derivative having amino hexanol to reduing terminal **4.**



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PROCNO                 1

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PULPROG            zgpg
TD                65536
SOLVENT           CDCl3
NS                  723
DS                    4
SWH           22727.273 Hz
FIDRES         0.346791 Hz
AQ             1.4418420 sec
RG                 2048
DW               22.000 usec
DE                6.00 usec
TE               300.0 K
D1             2.00000000 sec
D11            0.03000000 sec
D12            0.00002000 sec

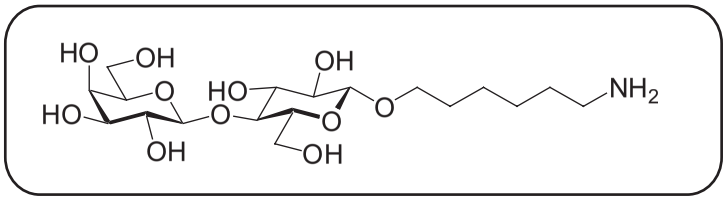
============ CHANNEL f1 ============
NUC1                13C
P1                 7.00 usec
PL1               -5.00 dB
SFO1         150.9140667 MHz

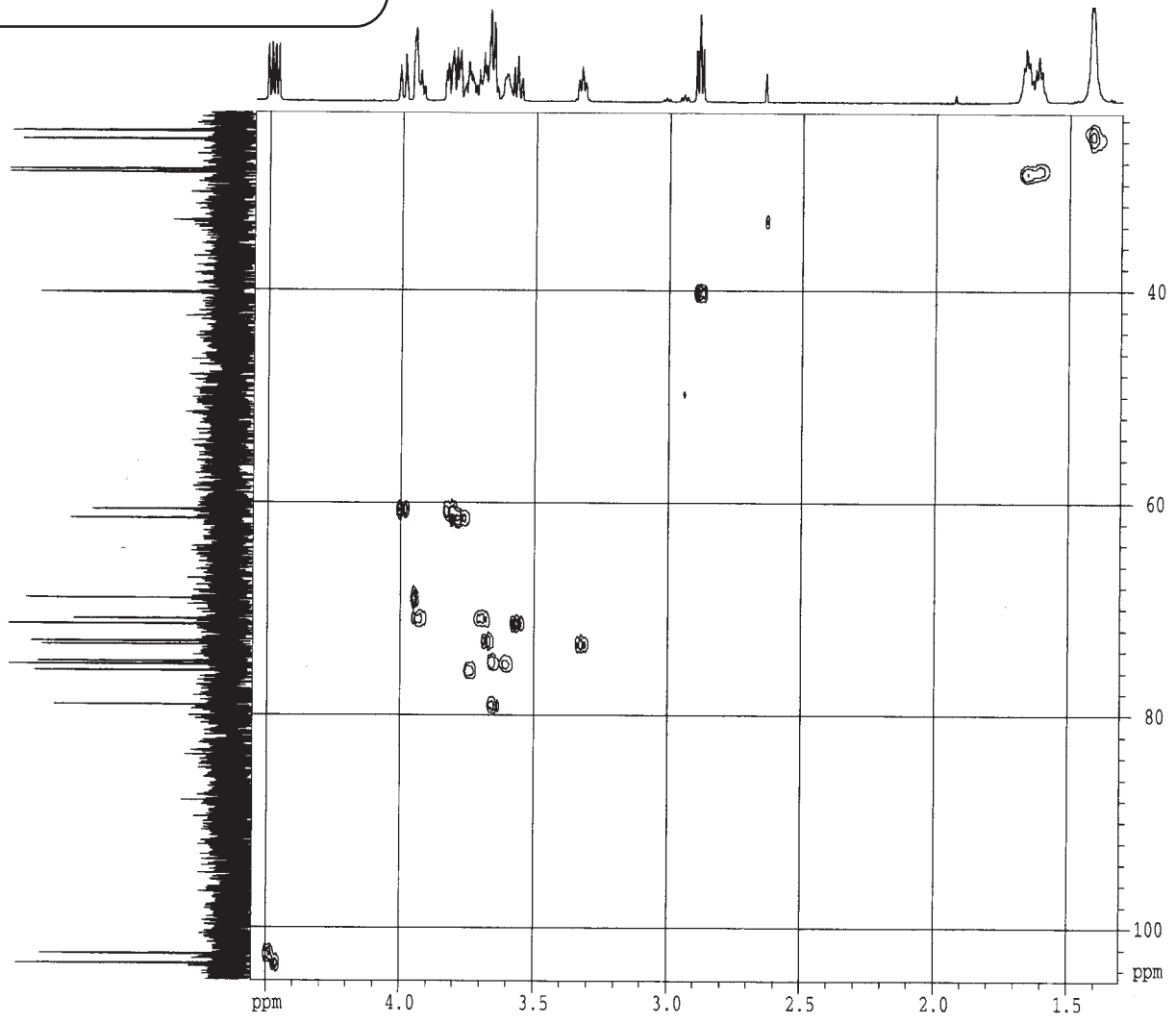
============ CHANNEL f2 ============
CPDPRG2          waltz16
NUC2                1H
PCPD2            100.00 usec
PL2              120.00 dB
PL12              24.00 dB
PL13              24.00 dB
SFO2         600.1324005 MHz

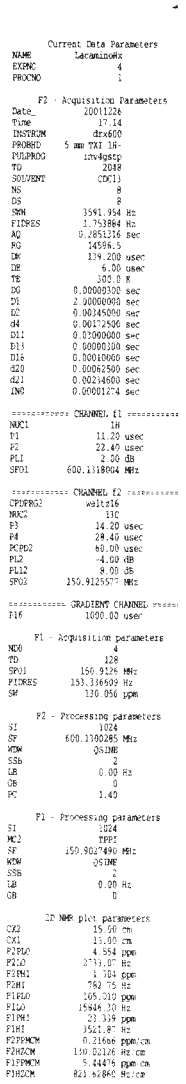
F2 - Processing parameters
SI                 32768
SF           150.9027490 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40

1D NMR plot parameters
CX                 21.00 cm
F1P              110.000 ppm
F1             16599.30 Hz
F2P               10.000 ppm
F2              1509.03 Hz
PPMCM            4.76190 ppm/cm
HZCM           718.58453 Hz/cm
```

Figure S-3c. ^{13}C NMR spectrum (D_2O, 125 MHz, 300 K) of lactose derivative having amino hexanol to reduing terminal **4**







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PROCNO 1

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PULPROG inv4gstp
TD 2048
SOLVENT CDCl3
NS 8
DS 8
SWH 3591.954 Hz
FIDRES 1.753884 Hz
AQ 0.2851316 sec
RG 14596.5
DW 139.200 usec
DE 6.00 usec
TE 303.0 K
D0 0.00000300 sec
D1 2.00000000 sec
D2 0.00345000 sec
d4 0.00172500 sec
D11 0.03000600 sec
D13 0.00000300 sec
D16 0.00010000 sec
d20 0.00062500 sec
d21 0.00214600 sec
DN2 0.00001274 sec

========== CHANNEL f1 ==========
NUC1 1H
P1 11.20 usec
P2 22.40 usec
PL1 2.00 dB
SFO1 600.1318004 MHz

========== CHANNEL f2 ==========
CPDPRG2 waltz16
NUC2 13C
P3 14.20 usec
P4 28.40 usec
PCPD2 80.00 usec
PL2 -4.00 dB
PL12 8.00 dB
SFO3 150.9125775 MHz

========== GRADIENT CHANNEL ==========
P16 1000.00 usec

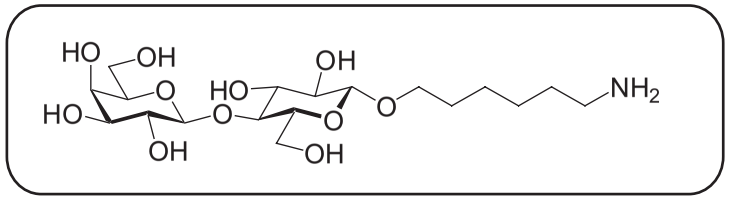
F1 - Acquisition parameters
ND0 4
TD 128
SFO1 150.9126 MHz
FIDRES 153.336609 Hz
SW 130.056 ppm

F2 - Processing parameters
SI 1024
SF 600.1300285 MHz
WDW QSINE
SSB 2
LB 0.00 Hz
GB 0
PC 1.40

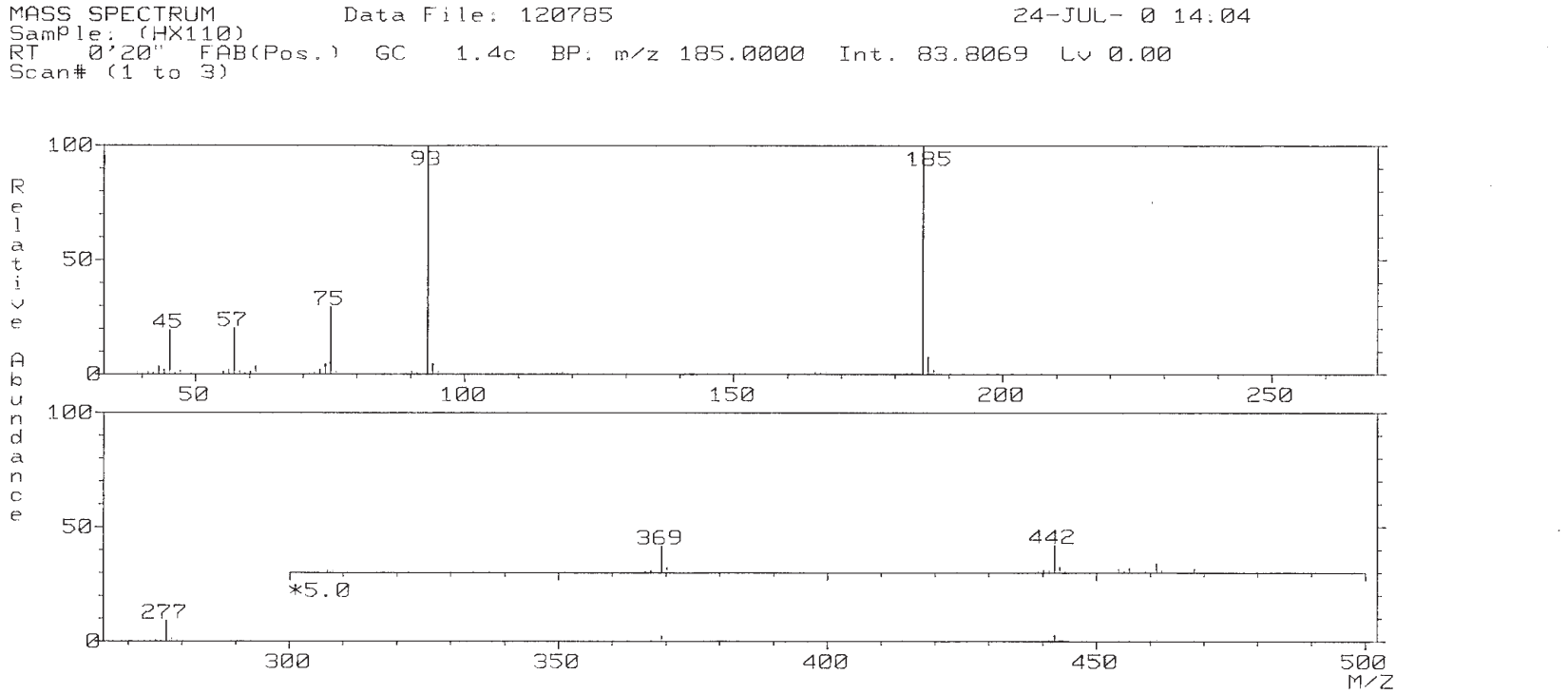
F1 - Processing parameters
SI 1024
MC2 TPPI
SF 150.9027490 MHz
WDW QSINE
SSB 2
LB 0.00 Hz
GB 0

2D NMR plot parameters
CX2 15.50 cm
CX1 15.00 cm
F2PLO 4.554 ppm
F2LO 2733.07 Hz
F2PH1 1.704 ppm
F2HI 762.75 Hz
F1PLO 105.210 ppm
F1LO 15948.30 Hz
F1PH1 23.339 ppm
F1HI 1521.87 Hz
F2PPMCM 0.21666 ppm/cm
F2HZCM 130.02126 Hz/cm
F1PPMCM 5.44476 ppm/cm
F1HZCM 821.62860 Hz/cm

Figure S-3d. [^{1}H, ^{13}C] HMQC NMR spectrum (D_2O, 300 K) of lactose derivative having amino hexanol to reduing terminal **4.**



$C_{18}H_{35}NO_{11}$
Exact Mass: 441.22
Mol. Wt.: 441.47

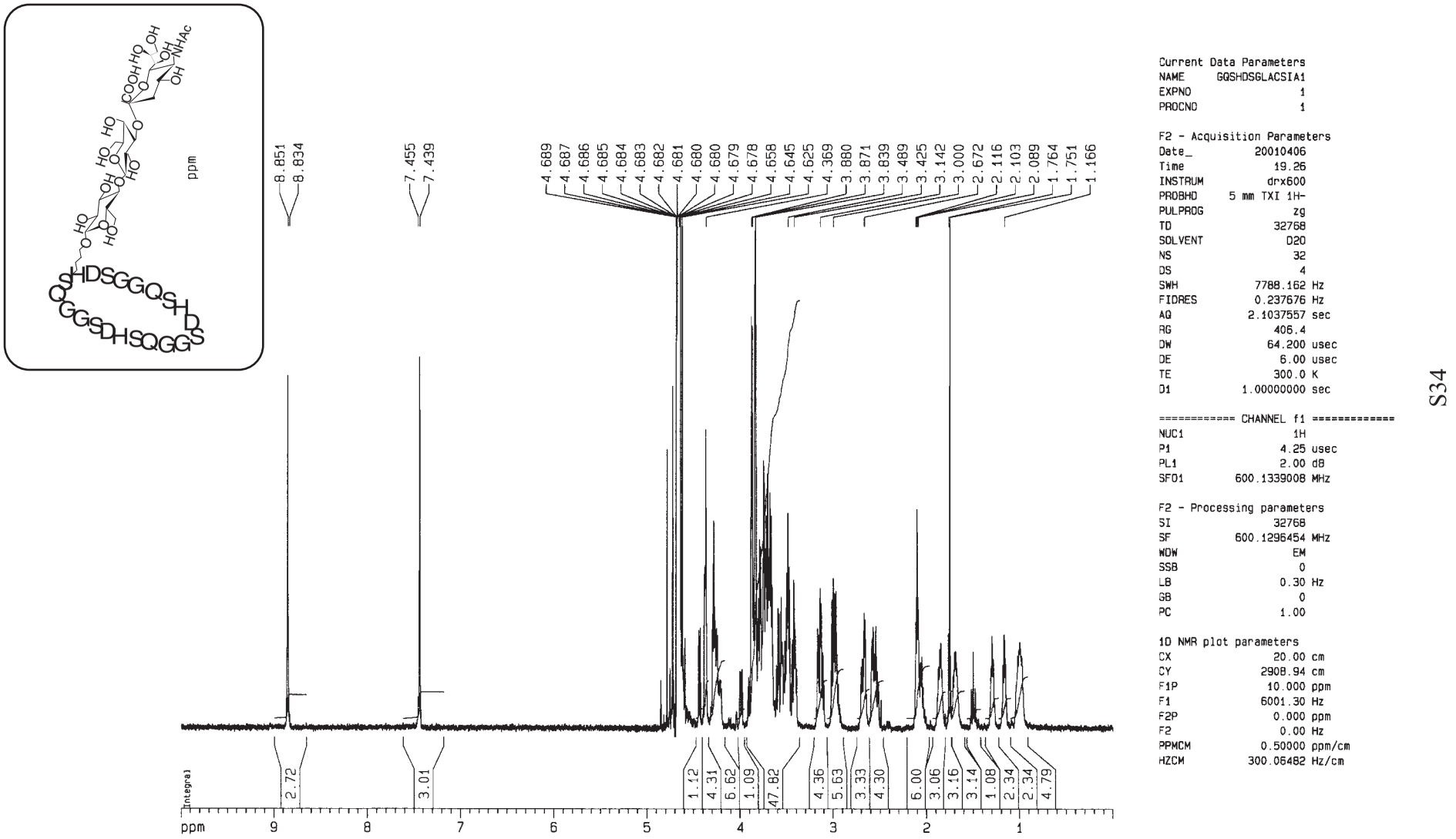


Solvent MeOH
Matrix Gly
Emiss **Cur.** 5mA
Ion **Multi.** 1.2kV

EGT 5 5 0

S33

Figure S-3e. FAB MASS spectrum (positive, CH_3OH, matrix ; glycerol) of lactose derivative having amino hexanol to reduing terminal **4.**

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PROCNO                 1

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PULPROG               zg
TD                 32768
SOLVENT              D20
NS                    32
DS                     4
SWH             7788.162 Hz
FIDRES          0.237676 Hz
AQ             2.1037557 sec
RG                 406.4
DW                64.200 usec
DE                 6.00 usec
TE                300.0 K
D1             1.00000000 sec

============ CHANNEL f1 =============
NUC1                  1H
P1                 4.25 usec
PL1                2.00 dB
SFO1         600.1339008 MHz

F2 - Processing parameters
SI                 32768
SF          600.1296454 MHz
WDW                   EM
SSB                    0
LB                 0.30 Hz
GB                     0
PC                 1.00

1D NMR plot parameters
CX                20.00 cm
CY              2908.94 cm
F1P              10.000 ppm
F1               6001.30 Hz
F2P               0.000 ppm
F2                0.00 Hz
PPMCM          0.50000 ppm/cm
HZCM          300.06482 Hz/cm
```
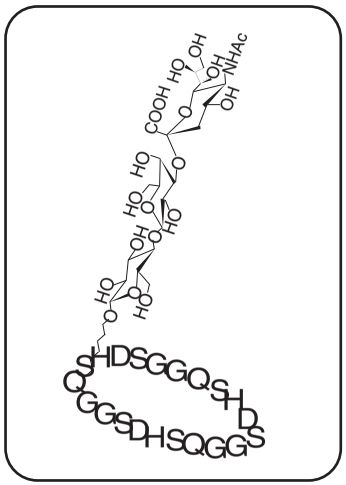
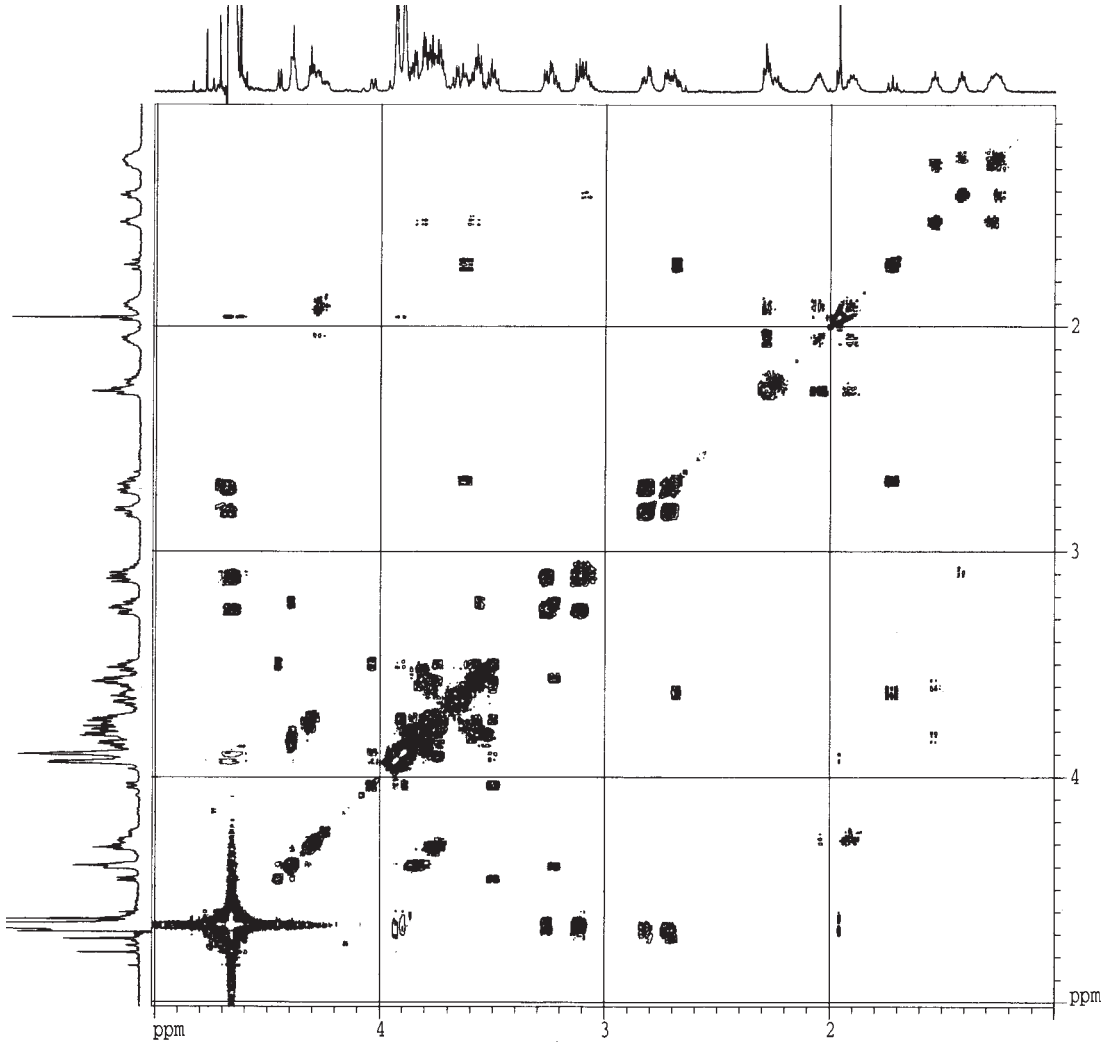

Figure S-4a. ^{1}H NMR spectrum (D_2O, 600 MHz, 300 K) of cyclic glycopeptide **1-m.**



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PROCNO                1

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PULPROG          cosygs
TD                2048
SOLVENT           DMSO
NS                   4
DS                  16
SWH            3591.954 Hz
FIDRES         1.753884 Hz
AQ             0.2851316 sec
RG                1626.5
DW              139.200 usec
DE                6.50 usec
TE              300.0 K
D0             0.00000309 sec
D1             2.00000000 sec
D13            0.00000300 sec
D16            0.00010000 sec
IN0            0.00017840 sec

 ============ CHANNEL f1 =============
NUC1                  1H
P0                  8.50 usec
P1                  8.50 usec
PL1                 2.00 dB
SFO1         600.1318004 MHz

============ GRADIENT CHANNEL ========
P16              1000.00 usec

      F1 - Acquisition parameters
NDO                   1
TD                  256
SFO1            600.1318 MHz
FIDRES         14.031071 Hz
SW              5.985 ppm

      F2 - Processing parameters
SI                  2048
SF           600.1300295 MHz
WDW                 SINE
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00

      F1 - Processing parameters
SI                  2048
MC2                   QF
SF           600.1300295 MHz
WDW                 SINE
SSB                    0
LB                  0.00 Hz
GB                     0

      2D NMR plot parameters
CX1                15.00 cm
CY1                15.00 cm
F2PLC               5.013 ppm
F2LO             3008.43 Hz
F2PHI               0.997 ppm
F2HI              598.59 Hz
F1PLC               5.022 ppm
F1LO             3013.69 Hz
F1PHI               1.015 ppm
F1HI              609.11 Hz
F2PPMCM          0.26770 ppm/cm
F2HZCM         160.65576 Hz/cm
F1PPMCM          0.26712 ppm/cm
F1HZCM         160.30498 Hz/cm
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Figure S-4b. [^{1}H, ^{1}H] NMR spectrum (D_2O, 600 MHz, 300 K) of cyclic glycopeptide **1-m.**

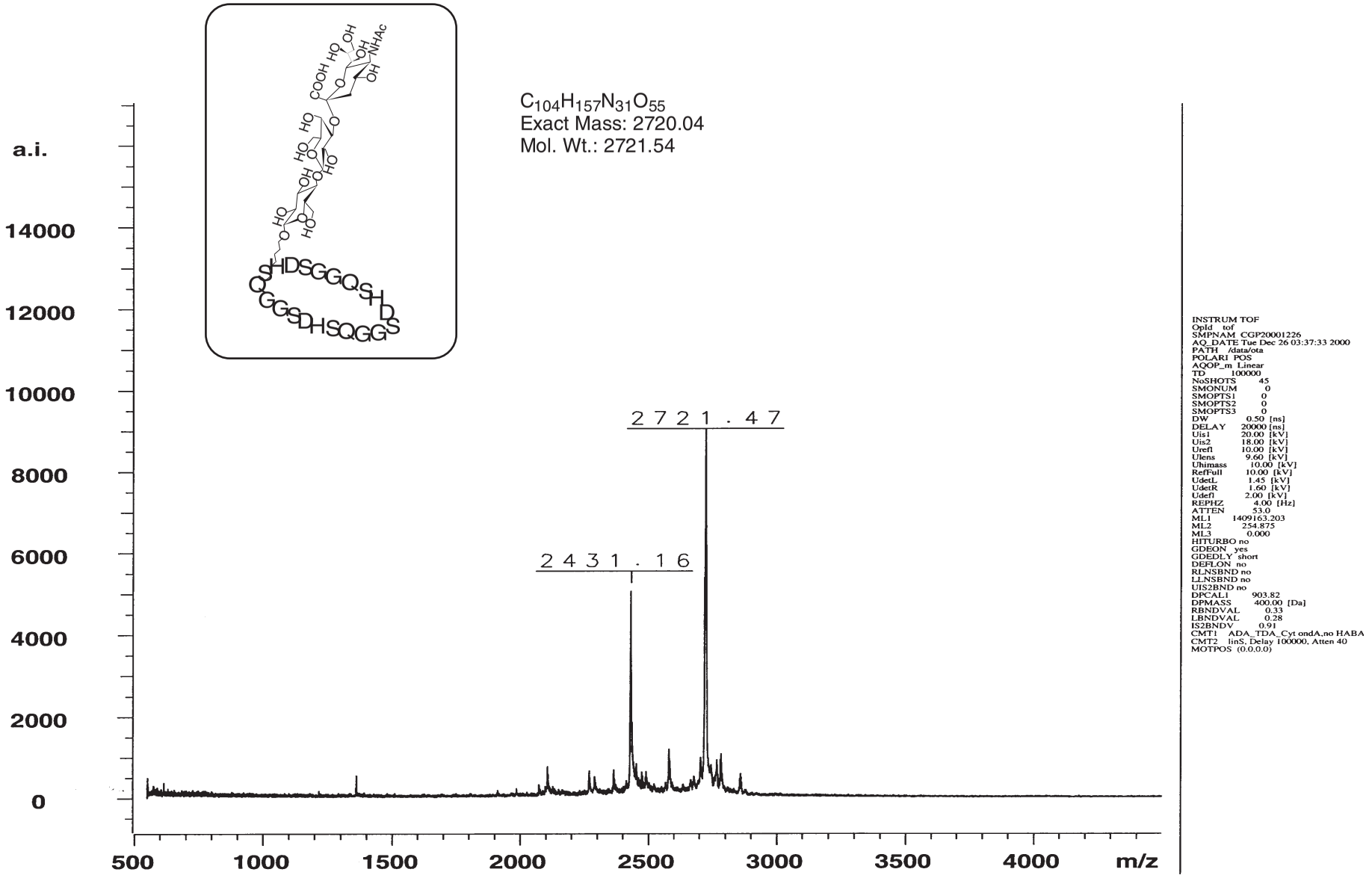


Figure S-4c. MALDI-TOF-MASS spectrum of cyclic glycopeptide **1-m** (1 µM, 0.1% trifluoroacetic acid H_2O/CH_3CN = 2/1 (v/v) , matrix ; 2,5-dihydroxybenzoic acid).

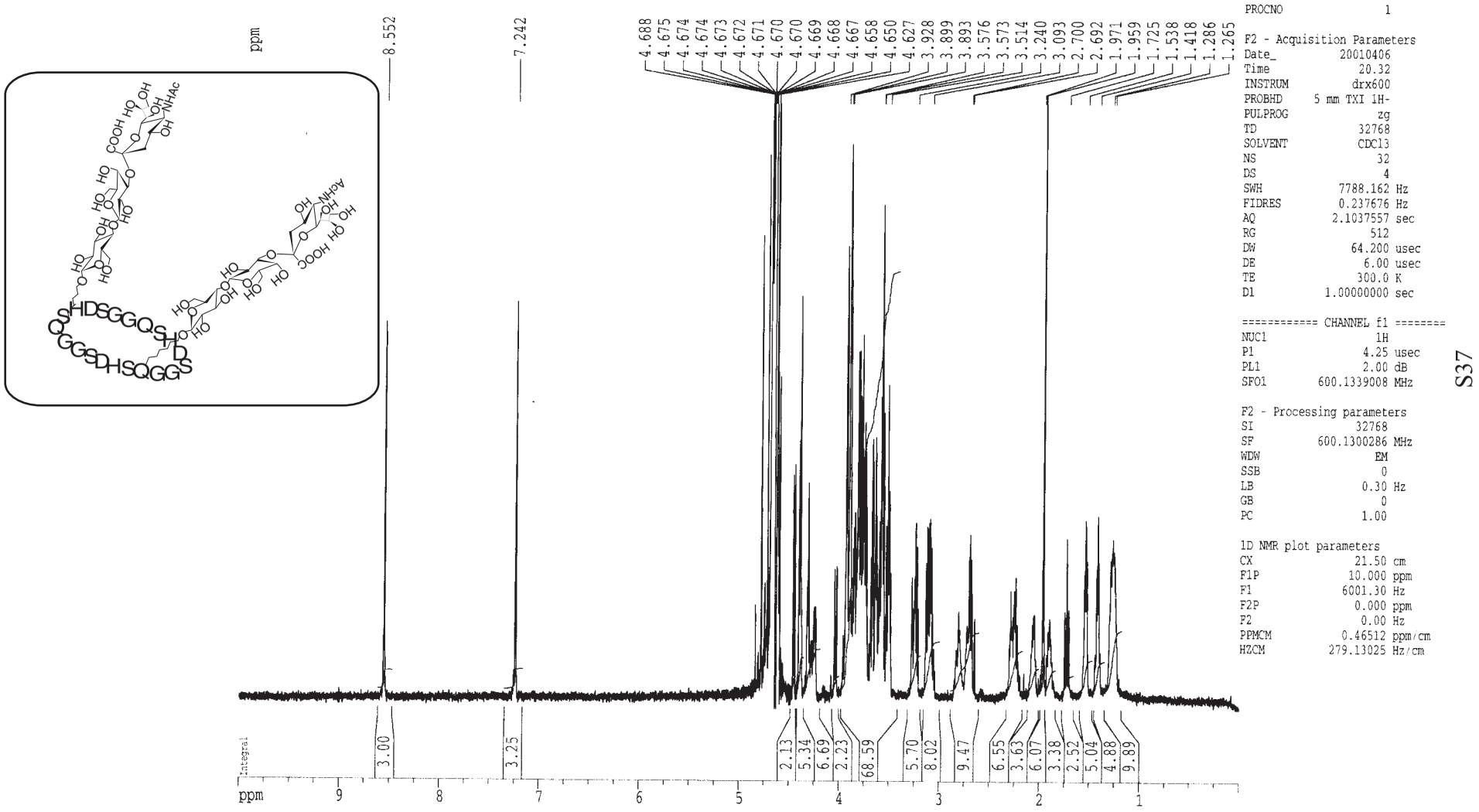


Figure S-5a. ^{1}H NMR spectrum (D_2O, 600 MHz, 300 K) of cyclic glycopeptide **1-b.**

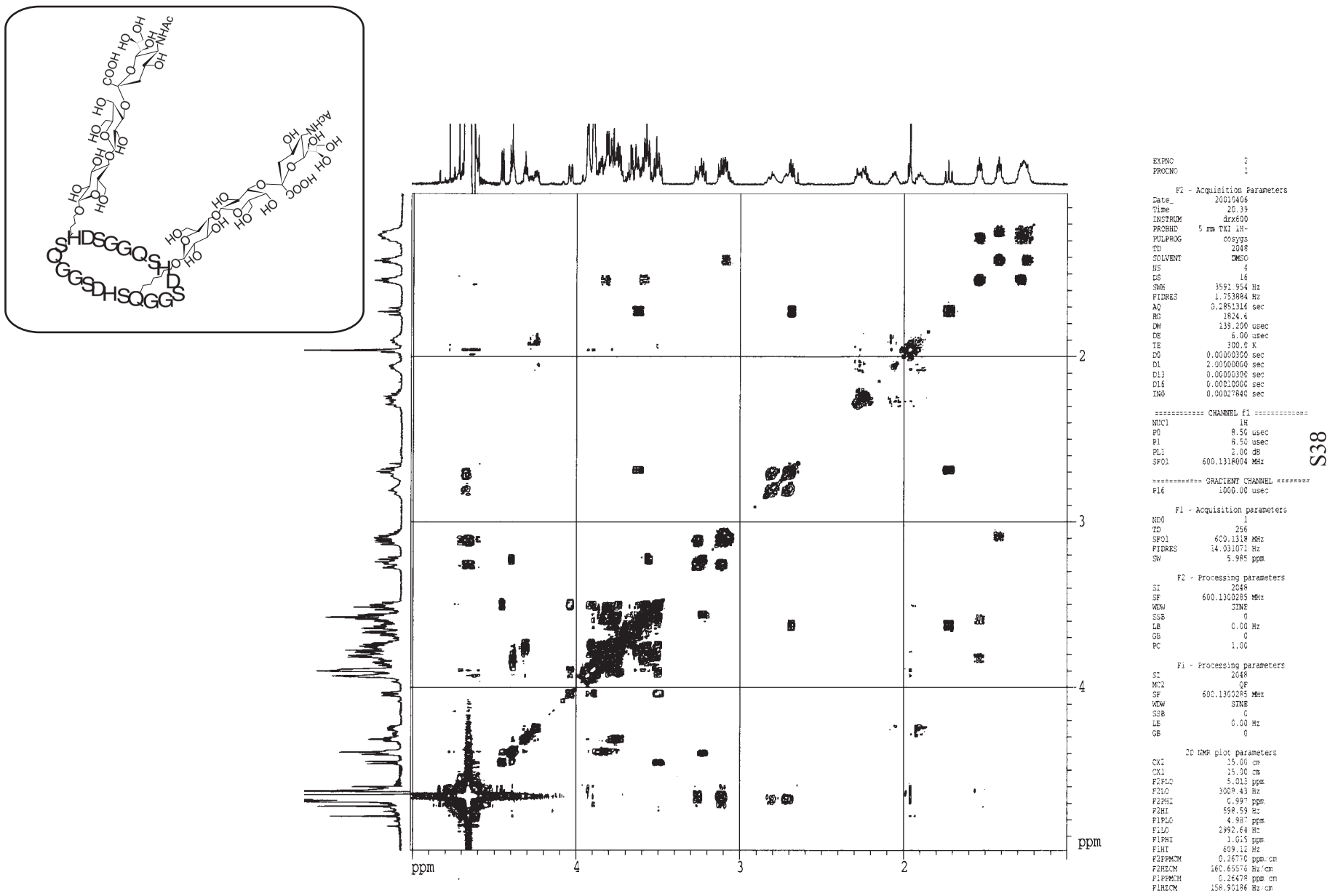


Figure S-5b. [^{1}H, ^{1}H] COSY NMR spectrum (D_2O, 600 MHz, 300 K) of cyclic glycopeptide **1-b.**

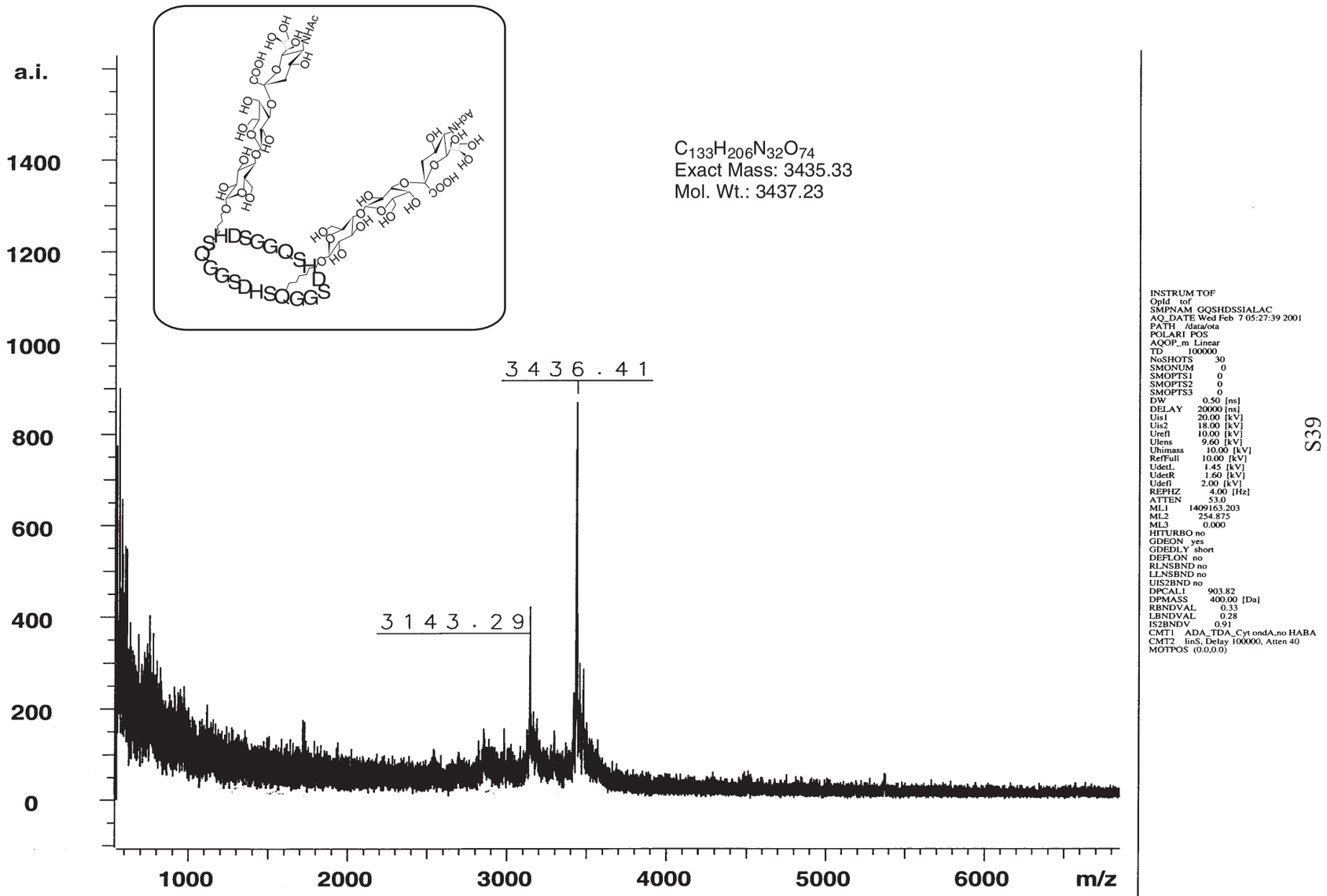


Figure S-5c. MALDI-TOF-MASS spectrum of cyclic glycopeptide **1-b** (1 µM, 0.1% trifluoroacetic acid H_2O/CH_3CN = 2/1 (v/v) , matrix ; 2,5-dihydroxybenzoic acid).

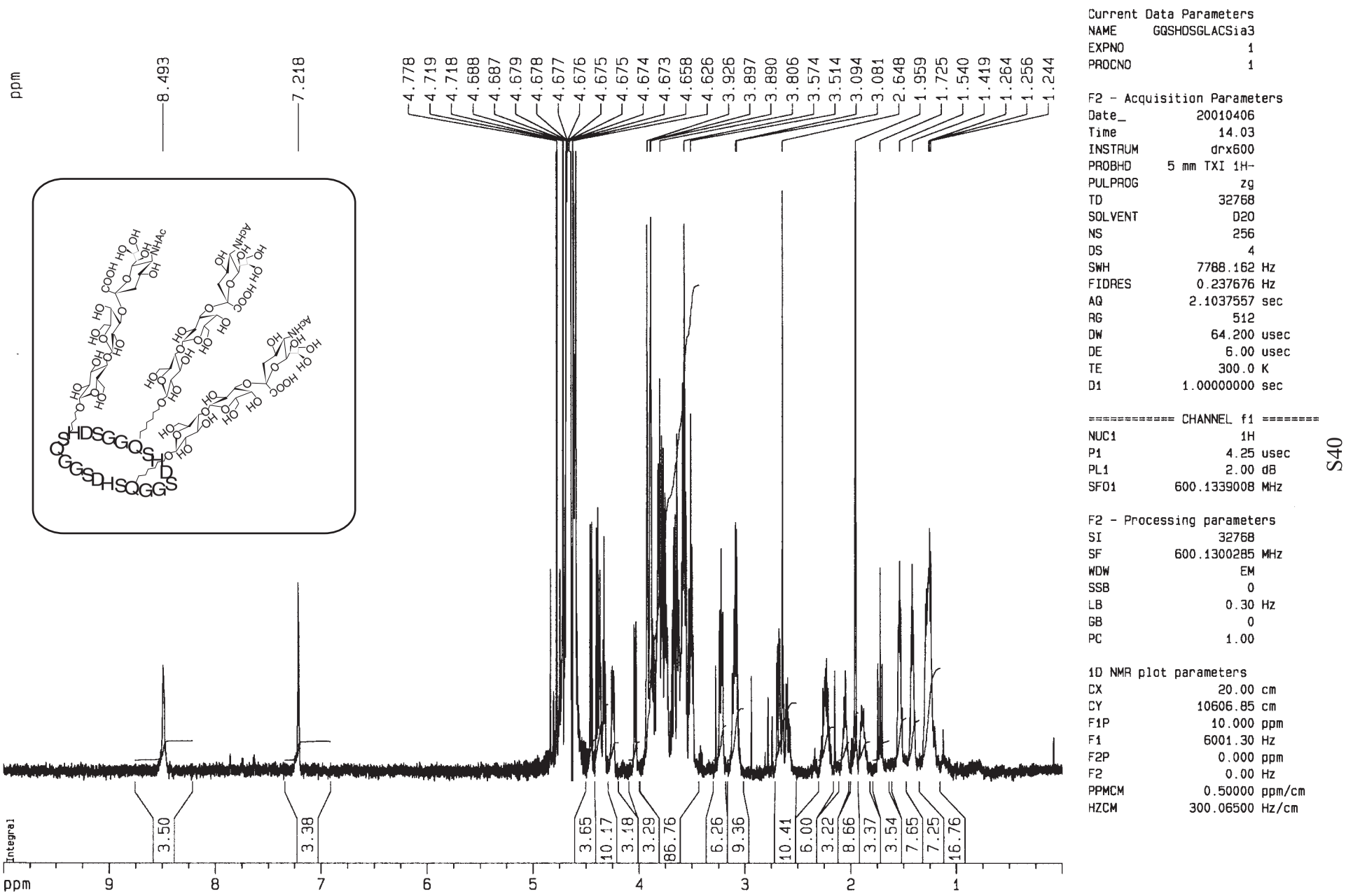


Figure S-6a. ^{1}H NMR spectrum (D_2O, 600 MHz, 300 K) of cyclic glycopeptide **1-t.**

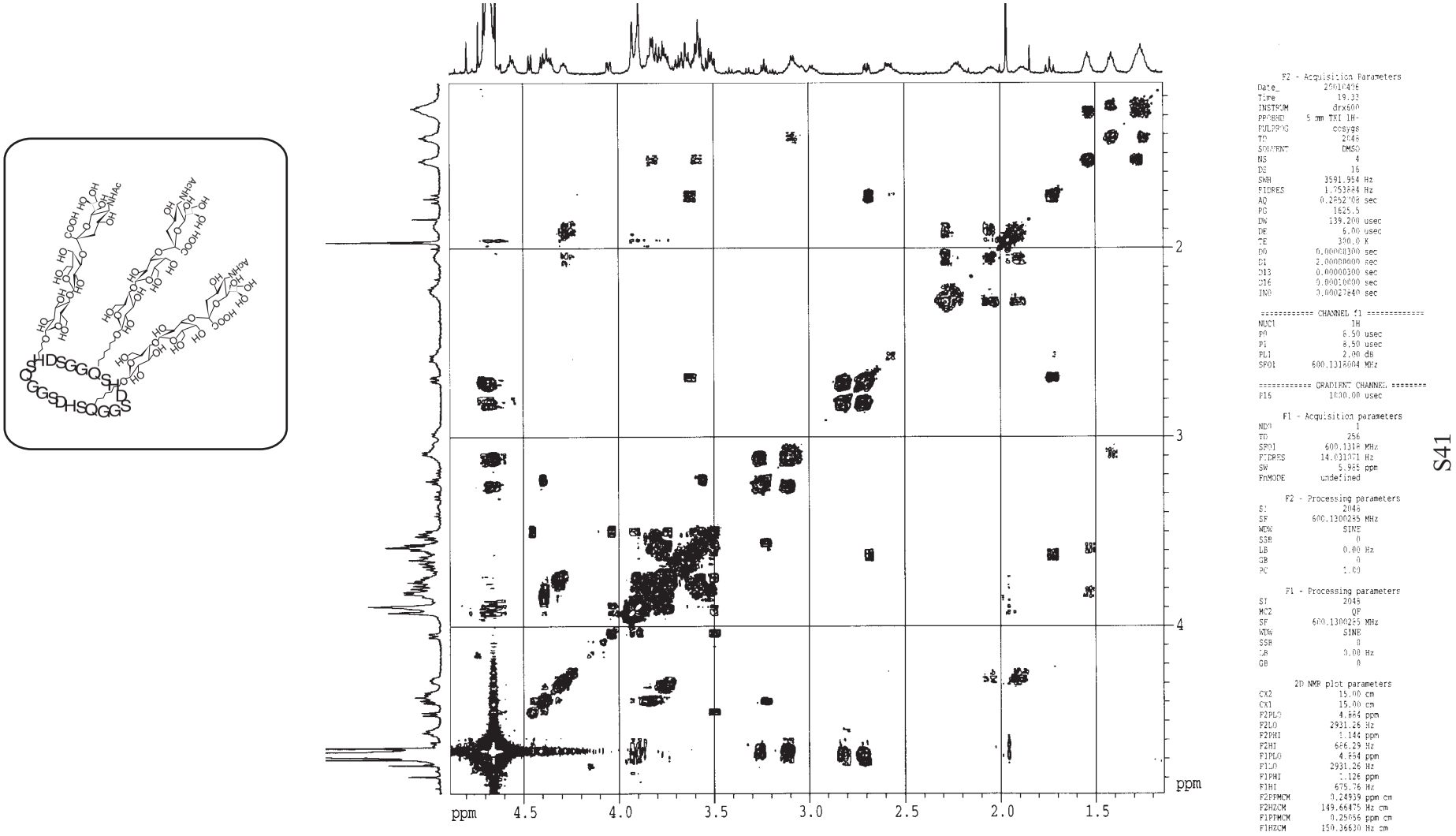


Figure S-6b. [^{1}H, ^{1}H] COSY NMR spectrum (D_2O, 600 MHz, 300 K) of cyclic glycopeptide **1-t.**

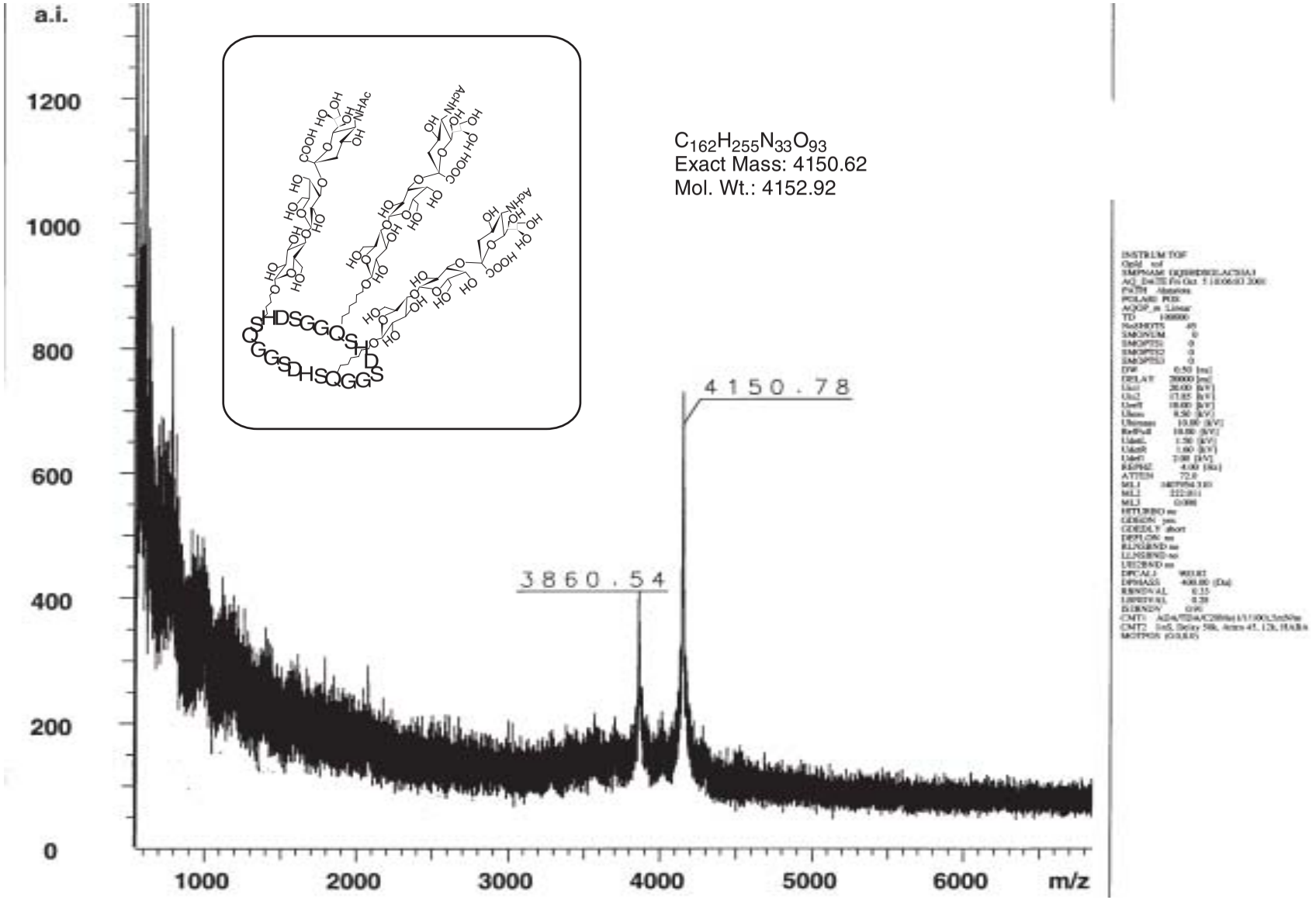


Figure S-6c. MALDI-TOF-MASS spectrum of cyclic glycopeptide **1-t** (1 μM, 0.1% trifluoroacetic acid H_2O/CH_3CN = 2/1 (v/v) , matrix ; 2,5-dihydroxybenzoic acid).

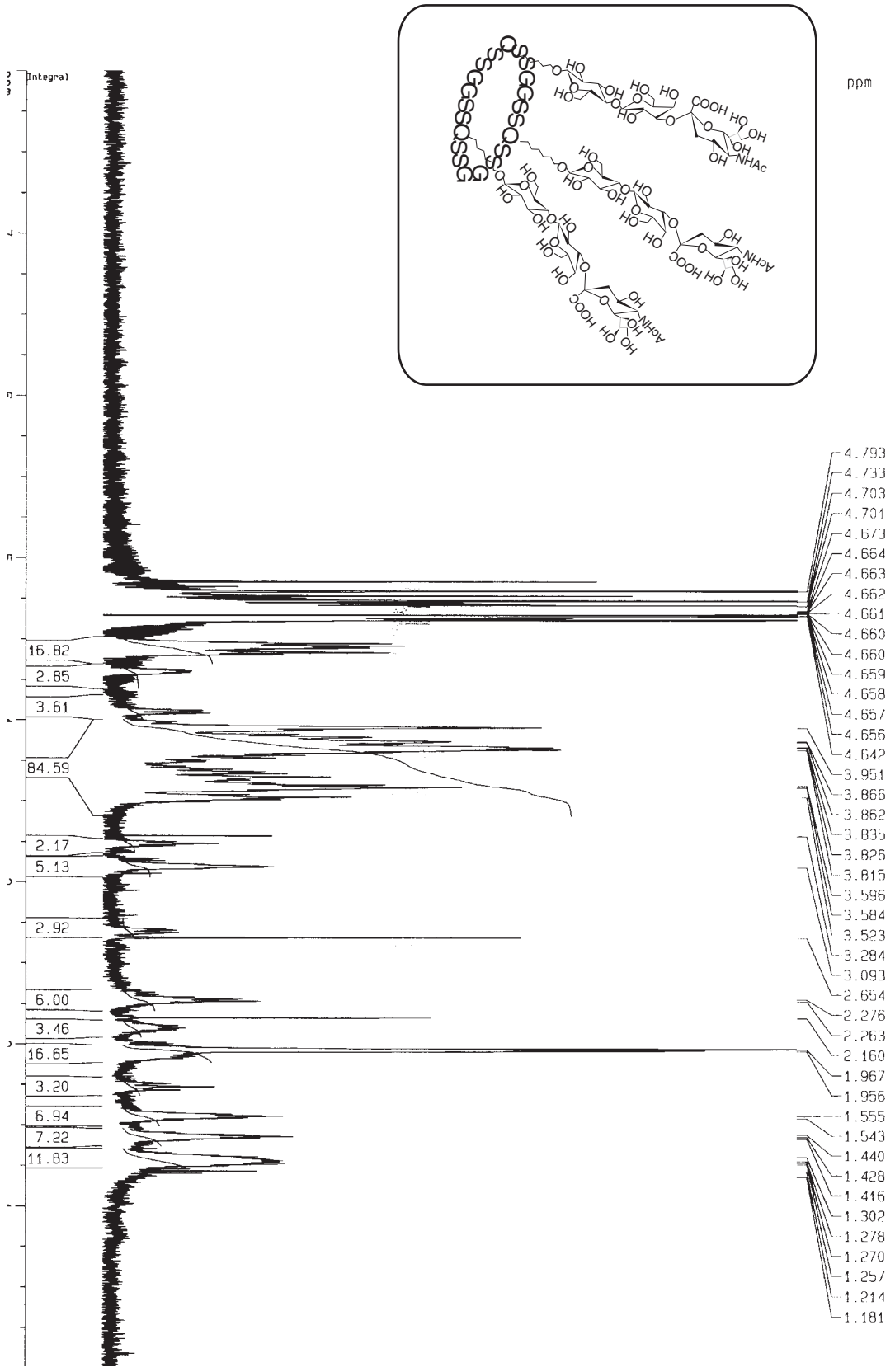
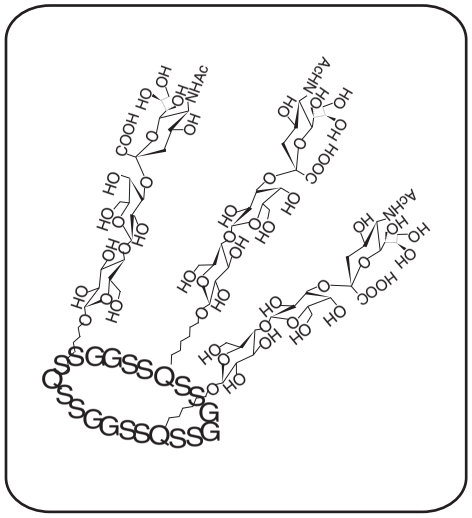
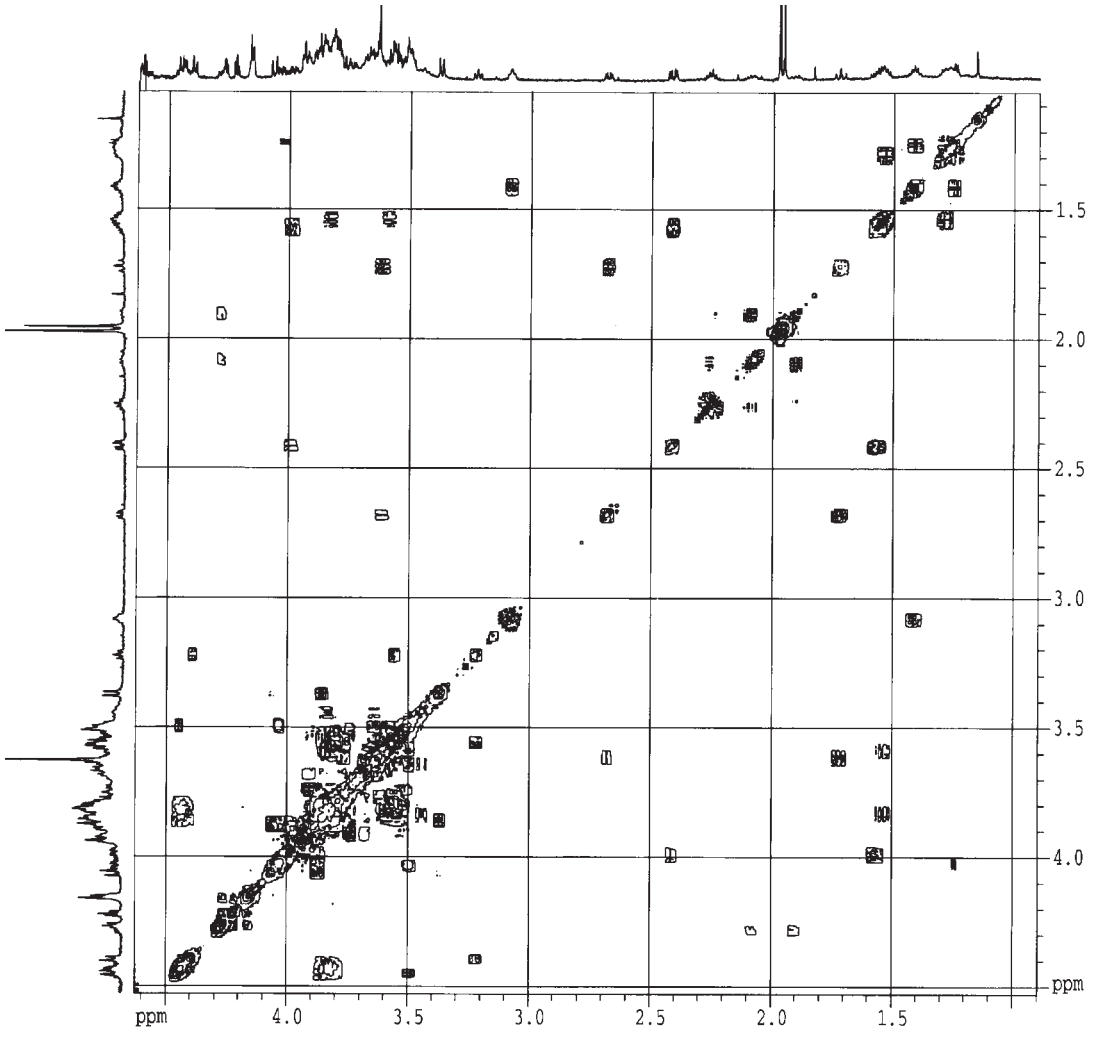


Figure S-7a. ^{1}H NMR spectrum (D_2O, 600 MHz, 300 K) of cyclic glycopeptide having cyclo(GSSQSSG)$_3$.



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                Current Data Parameters
NAME      GSSQSSSGLACSIA
EXPNO                  2
PROCNO                 1

        F2 - Acquisition Parameters
Date_          20010714
Time               18.54
INSTRUM         drx600
PROBHD     5 mm TXI 1H-
PULPROG          cosygs
TD                 2048
SOLVENT            DMSO
NS                   32
DS                    8
SWH           3591.954 Hz
FIDRES        1.753884 Hz
AQ            0.2851316 sec
RG               5792.6
DW             139.200 usec
DE               6.00 usec
TE              300.0 K
D0          0.00000300 sec
D1          2.00000000 sec
D13         0.00000300 sec
D16         0.00010000 sec
IN0         0.00027840 sec

============ CHANNEL f1 ========
NUC1                1H
P0               8.50 usec
P1               8.50 usec
PL1              2.00 dB
SFO1        600.1318004 MHz

============ GRADIENT CHANNEL ===
P16           1000.00 usec

        F1 - Acquisition parameters
ND0                  1
TD                 128
SFO1        600.1318 MHz
FIDRES      28.062141 Hz
SW           5.985 ppm

        F2 - Processing parameters
SI                2048
SF          600.1300285 MHz
WDW               SINE
SSB                  0
LB            0.00 Hz
GB                   0
PC                1.00

        F1 - Processing parameters
SI                2048
MC2                 QF
SF          600.1300285 MHz
WDW               SINE
SSB                  0
LB            0.00 Hz
GB                   0

        2D NMR plot parameters
CX2              15.00 cm
CX1              15.90 cm
F2PLC            4.627 ppm
F2LC          2776.92 Hz
F2PHI            0.895 ppm
F2HI           537.21 Hz
F1PLC            4.531 ppm
F1LO          2719.04 Hz
F1PHI            1.047 ppm
F1HI           628.41 Hz
F2PPMCM       0.24880 ppm/cm
F2HZCM      149.31396 Hz/cm
F1PPMCM       0.23224 ppm/cm
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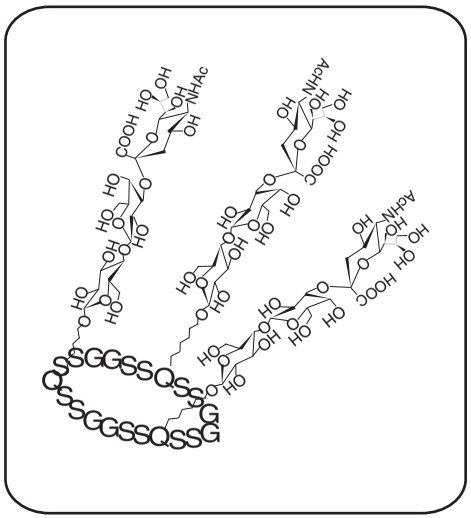
Figure S-7b. [^{1}H, ^{1}H] COSY NMR spectrum (D_2O, 600 MHz, 300 K) of cyclic glycopeptide having cyclo(GSSQSSG)3.



C150H249N27O93
Exact Mass: 3916.56
Mol. Wt.: 3918.71

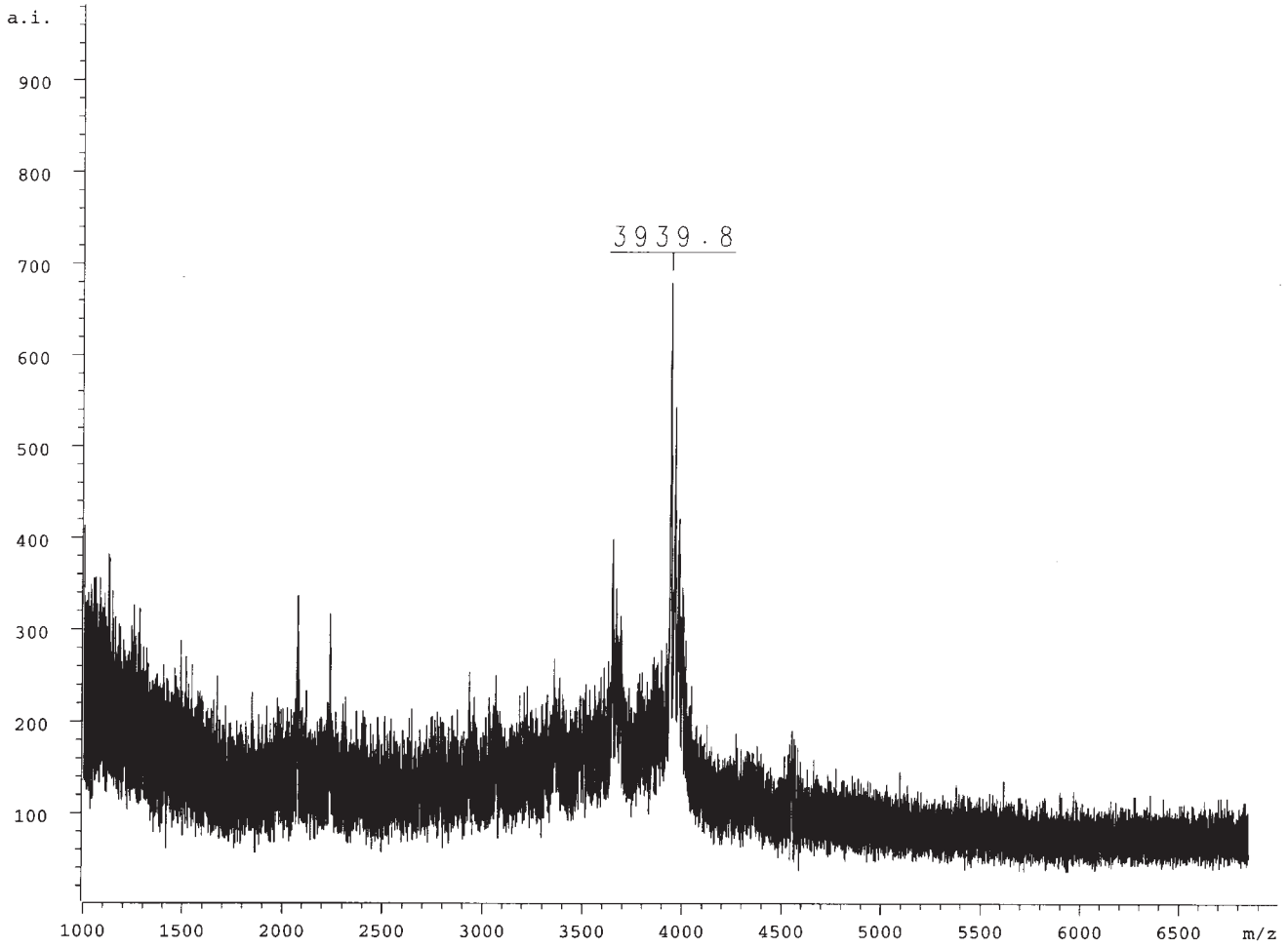


Figure S-7c. MALDI-TOF-MASS spectrum of cyclic glycopeptide having cyclo(GSSQSSG)3. (1 μM, 0.1% trifluoroacetic acid H_2O/CH_3CN = 2/1 (v/v) , matrix ; 2,5-dihydroxybenzoic acid) .

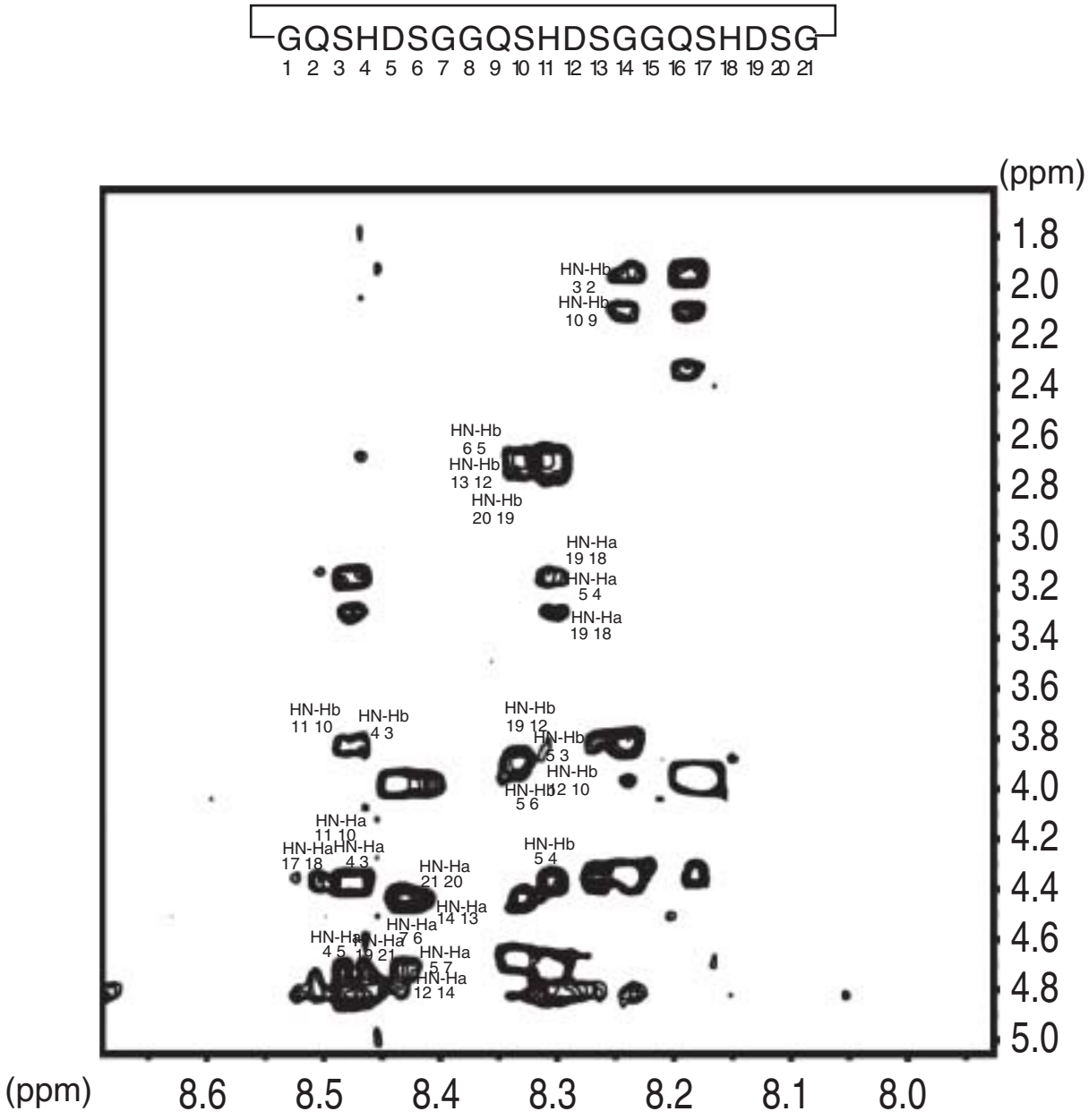


Figure S-8. NOE analysis in the fingerprint region of 600 MHz NOESY spectrum. All NMR signals were assigned by the combinations of DQF-COSY,TOCSY and NOESY. This spectrum was recorded at pH 6.0, 293K.

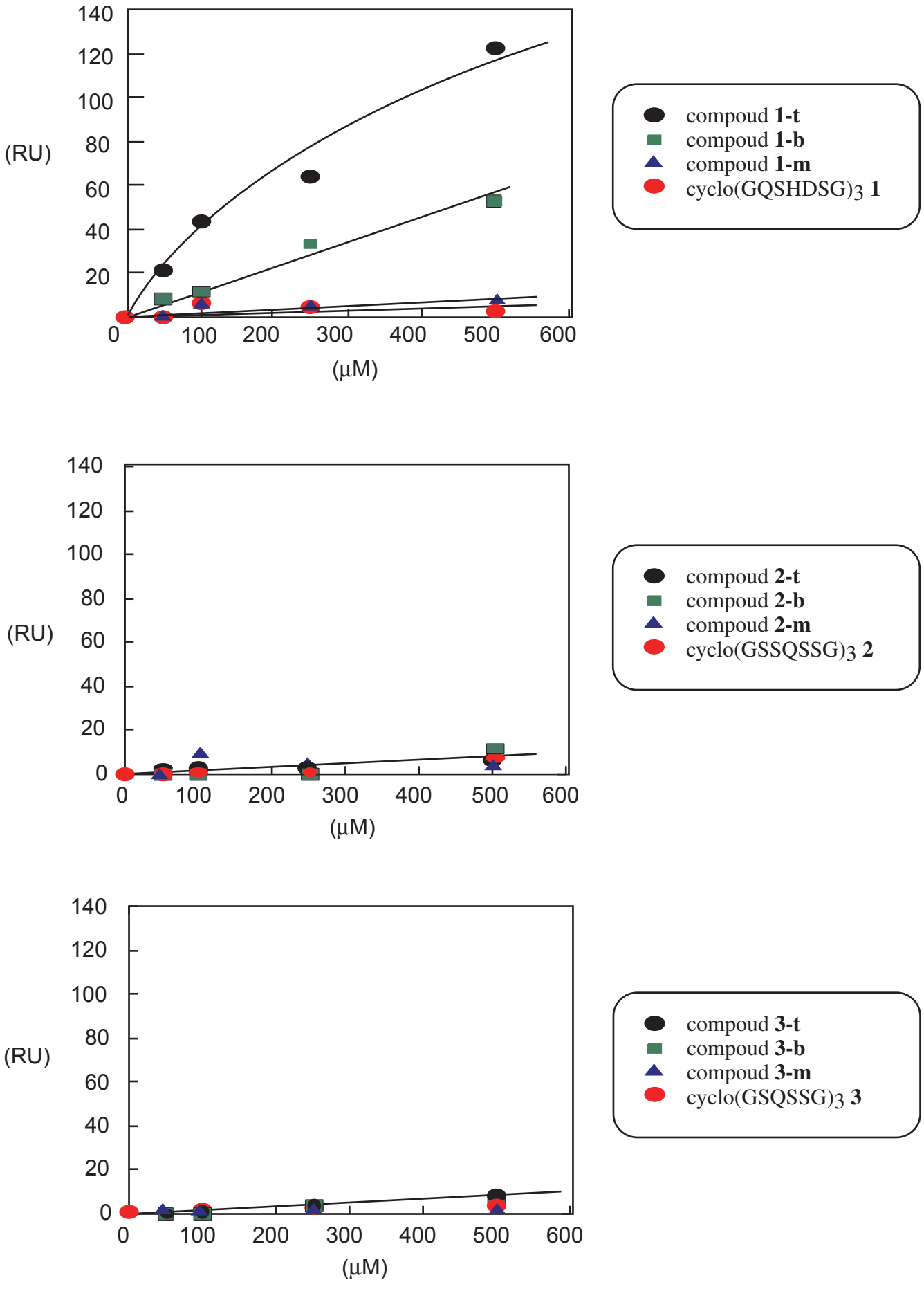


Figure S-9. SPR binding assay for determination of the binding constants between HA and cyclic glycopeptide.

Table S-1. Structure statistics for 30 structures of cyclic peptide **2** ; All energies and r. m. s. of difference values were calculated using the programs X-PLOR 3.1 and MOLMOL, respectively

Parameters	Values
Average potential energies (kcal/mol)[a]	
E_{total}	15.28 ± 0.045
E_{bonds}	0.283 ± 0.005
E_{angle}	12.114 ± 0.025
E_{impr}	2.870 ± 0.017
E_{VDW}[b]	0.010 ± 0.025
E_{NOE}[b]	0.0019 ± 0.0036
E_{cdih}[b]	0.000089 ± 0.00033
r. m. s. of differences from idealized geometry	
Bonds (Å)	0.00100 ± 0.00001
Angle (degrees)	0.4291 ± 0.0004
Impropers (degrees)	0.3656 ± 0.0011
Pairwise r. m. s. of difference of 30 structures (Å)	
Backbone atoms	3.56 ± 0.70 Å
All heavy atoms	4.99 ± 0.78 Å

[a] E_{impr}, E_{VDW}, E_{NOE} and E_{cdih} are the energy of improper torsion angles, the van der Waals repulsion energy, the square well NOE potential energy, and the dihedral potential energy, respectively.
[b] The force constants for the calculations of E_{VDW}, E_{NOE} and E_{cdih} were 4.0 kcal mol^{-1} $Å^{-4}$, 50 kcal mol^{-1} and 200kcal mol^{-1} rad^{-2}, respectively

Table S-2. Inter/intra residual constraints of cyclic peptide **1**.

intra

residue	atom	residue	atom	upper distance (Å)*	residue	atom	residue	atom	upper distance (Å)*
1	αH	1	NH	3.0	11	βH	11	NH	5.0
2	αH	2	NH	3.0	12	αH	12	NH	3.0
2	βH	2	NH	5.0	12	βH	12	NH	3.6
2	γH	2	NH	3.6	13	αH	13	NH	3.0
3	αH	3	NH	3.0	13	βH	13	NH	3.6
3	βH	3	NH	3.6	14	αH	14	NH	3.0
4	αH	4	NH	3.0	15	αH	15	NH	3.0
4	βH	4	NH	5.0	16	αH	16	NH	3.0
5	αH	5	NH	3.0	16	βH	16	NH	5.0
5	βH	5	NH	3.0	16	γH	16	NH	3.6
6	αH	6	NH	3.0	17	αH	17	NH	3.0
6	βH	6	NH	3.0	17	βH	17	NH	3.6
7	αH	7	NH	3.0	18	αH	18	NH	3.0
8	αH	8	NH	3.0	18	βH	18	NH	5.0
9	αH	9	NH	3.0	19	αH	19	NH	3.0
9	βH	9	NH	5.0	19	βH	19	NH	3.6
9	γH	9	NH	5.0	20	αH	20	NH	3.0
10	αH	10	NH	3.0	20	βH	20	NH	3.6
10	βH	10	NH	3.6	21	αH	21	NH	3.0
11	αH	11	NH	3.0					

inter

residue	atom	residue	atom	upper distance (Å)*	residue	atom	residue	atom	upper distance (Å)*
2	βH	3	NH	5.0	10	βH	11	NH	5.0
3	αH	4	NH	3.0	10	βH	12	NH	3.6
3	βH	4	NH	3.6	11	αH	12	NH	3.6
3	βH	5	NH	3.6	12	βH	13	NH	3.6
4	αH	5	NH	3.6	12	αH	14	NH	5.0
4	αH	7	NH	5.0	12	αH	15	NH	5.0
5	αH	4	NH	5.0	13	αH	14	NH	3.6
5	βH	6	NH	3.0	17	αH	18	NH	3.6
5	αH	8	NH	5.0	17	βH	19	NH	5.0
6	βH	5	NH	5.0	18	αH	19	NH	3.0
6	αH	7	NH	3.6	19	βH	20	NH	5.0
9	αH	8	NH	3.0	19	αH	21	NH	5.0
9	βH	10	NH	5.0	19	αH	1	NH	5.0
10	αH	11	NH	3.6	20	αH	21	NH	3.6

*The lower distance of all residual constraints is 1.8Å.

1

Table S-3. Inter/intra residual constraints of cyclic peptide **3**.

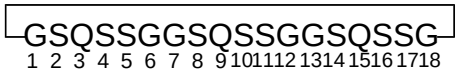
intra

residue	atom	residue	atom	upper distance (Å)*	residue	atom	residue	atom	upper distance (Å)
1	αH	1	NH	3.0	10	αH	10	NH	3.0
2	αH	2	NH	3.0	10	βH	10	NH	3.6
2	βH	2	NH	3.0	11	αH	11	NH	3.0
3	αH	3	NH	3.0	11	βH	11	NH	5.0
3	βH	3	NH	3.0	12	αH	12	NH	3.0
3	γH	3	NH	3.6	13	βH	13	NH	3.6
4	αH	4	NH	3.0	14	αH	14	NH	3.0
4	βH	4	NH	3.0	14	βH	14	NH	3.6
5	αH	5	NH	3.0	15	αH	15	NH	3.0
5	βH	5	NH	3.0	15	βH	15	NH	3.0
6	αH	6	NH	3.0	15	γH	15	NH	3.0
7	αH	7	NH	3.0	16	αH	16	NH	5.0
8	αH	8	NH	3.0	16	βH	16	NH	3.6
8	βH	8	NH	3.0	17	αH	17	NH	3.0
9	αH	9	NH	3.0	17	βH	17	NH	3.6
9	βH	9	NH	5.0	18	αH	18	NH	3.0
9	γH	9	NH	3.0					

inter

residue	atom	residue	atom	upper distance (Å)*	residue	atom	residue	atom	upper distance (Å)
2	βH	1	NH	3.6	8	βH	7	NH	5.0
3	αH	1	NH	5.0	9	αH	12	NH	3.6
3	βH	1	NH	3.6	10	βH	9	NH	5.0
3	βH	4	NH	5.0	12	αH	11	NH	5.0
3	αH	6	NH	3.6	14	βH	13	NH	5.0
4	βH	3	NH	5.0	15	αH	18	NH	3.6
4	βH	3	NH	5.0	16	βH	15	NH	5.0
6	αH	5	NH	5.0	18	αH	17	NH	5.0

*The lower distance of all residual constraints is 1.8Å.



3

Table S-4. the coupling constant and dihedral restraints of cyclic peptide **1** and **3**.

cyclo(GQSHDSG)$_3$ **1**			cyclo(GSQSSG)$_3$ **3**		
residue	coupling constant	dihedral restraints	residue	coupling constant	dihedral restraints
1	11.74	-120±20	1	11.73	-120±20
2	7.33	-	2	7.33	-
3	5.87	-60±30	3	7.43	-
4	7.33	-	4	5.83	-
5	7.32	-	5	5.32	-60±30
6	5.87	-60±30	6	11.73	-120±20
7	11.74	-120±20	7	11.73	-120±20
8	11.74	-120±20	8	7.33	-
9	7.33	-	9	7.43	-
10	5.87	-60±30	10	5.83	-
11	7.33	-	11	5.32	-60±30
12	7.32	-	12	11.73	-120±20
13	5.87	-60±30	13	11.73	-120±20
14	11.74	-120±20	14	7.33	-
15	11.74	-120±20	15	7.43	-
16	7.33	-	16	5.83	-
17	5.87	-60±30	17	5.32	-60±30
18	7.33	-	18	11.73	-120±20
19	7.32	-			
20	5.87	-60±30			
21	11.74	-120±20			

1

3