Synthetic Studies on Nephritogenic Glycosides. Synthesis of *N*-(β-L-Aspartyl)-α-D-glucopyranosylamine†

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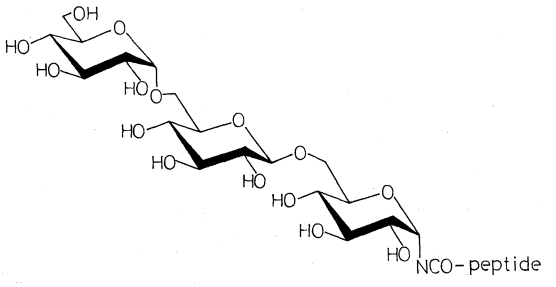
A route for the stereoselective synthesis of *N*-(β-L-aspartyl)-α-D-glucopyranosylamine, a part structure of the nephritogenic glycopeptide, was developed by using 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl azide as a key intermediate.

In 1969, Shibata *et al.*[2] isolated, from the glomerular basement-membrane of rats, a new glycopeptide having nephritogenic activity.[3] In 1980, Shibata and Nakanishi[4] proposed **1** as the structure of the glycopeptide from the analysis of ^{13}C-NMR data and the results of a concanavalin A test.



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The proposed structure **1** is quite unusual compared with the conventional glycan structure of glycopeptides,[5] in the following ways: (i) α-D-glucopyranose, instead of 2-acetamido-2-deoxy-β-D-glucopyranose, is directly linked to the amide group of L-asparagine or L-glutamine of the peptide, and (ii) the glycan chain is composed of only three glucopyranosyl residues.

In order to clarify the relationship between the chemical structure and the nephritogenic activity, we embarked upon synthetic studies of the model structure related to **1**. In 1980, the total synthesis of the part structure of the glycopeptide **1**, containing three glucopyranoses and asparagine, was first reported in a preliminary communication.[6] In this and following papers we report in detail the synthesis of model glycosyl peptides related to **1**.

Several synthetic studies on D-glucopyranosyl peptides have been reported. In 1961, Coutsogeorgopoulos and Zervas[7] reported the first synthesis of *N*-(L-β-aspartyl)-β-D-glucopyranosylamine **3** by the condensation of **4** with **5** in the presence of ethyl chloroformate and triethylamine with subsequent deprotection in a 57% overall yield. An alternative synthesis of **3** was also reported by Marks and Neuberger[8] by the condensation of peracetylated glucopyranosylamine **6** with **5** in the presence of dicyclohexylcarbodiimide with subsequent deprotection in a 30% overall yield. In 1975, Garg and Jeanloz[9] employed glycosylamine **7** as the substrate for the synthesis of β-asparagine derivatives. Similarly, the condensation of per-

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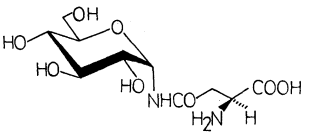

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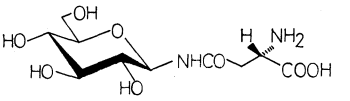

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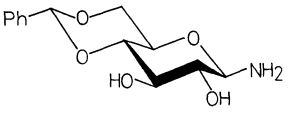

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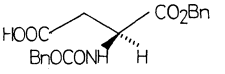

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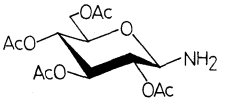

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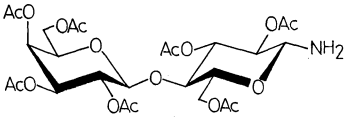

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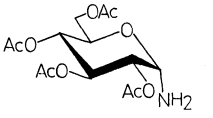

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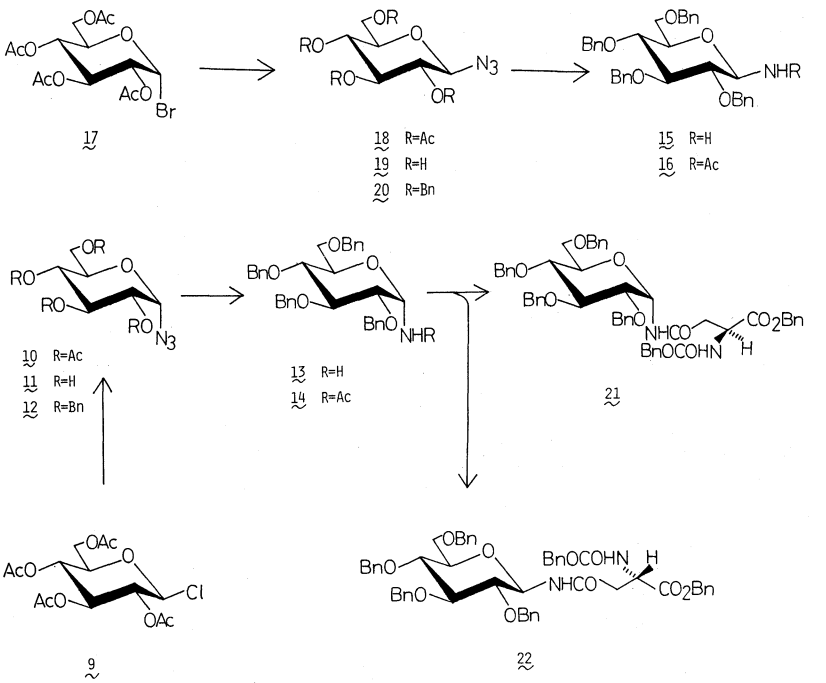
acetylated α-D-glucopyranosylamine **8** with α-ethyl benzyloxycarbonyl-L-aspartate was reported to give **2** in 1980 by Takeda *et al.*[10]

This paper describes the stereocontrolled synthesis of **2** *via* the intermediate perbenzylated glucopyranosylamine **13**. Treatment of the β-chloride **9**[11] with sodium azide in hexamethylphosphoric triamide (HMPA) at 20° afforded a 79% yield of α-azide **10**[12] containing a trace of β-axide **18**, which could not be separated from **10** by crystallization from diisopropyl ether. The configuration at C-1 of **10** was assigned by ^{1}H-NMR spectrum which showed a signal for H-1 at δ 5.58 as a doublet with $J_{12}=4$ Hz. The formation of 1,2-*cis* azide as the major product was in agreement with the observation made by Gyorgydeak and Paulsen.[13] Deacetylation of **10** containing a trace of **18** and crystallization of the product from ethanol afforded pure **11** in an 85% yield. Benzylation of **11** with sodium hydride and benzylbromide afforded α-azide **12** in a 65% yield. The α-D-Configuration at C-1 of **12** was supported by NMR data which revealed a signal for H-1 as a doublet with $J_{12}=4$ Hz at δ 5.20 and a signal for C-1 as a doublet with $^1J_{CH}=166.2$ Hz at δ_C 87.91. The β-anomer **20** was prepared from acetobromoglucose **17** in a similar way; (i) treatment with sodium azide in DMF at 50° to give **18**[14] (77%), (ii) deacetylation, and (iii) benzylation in a 61% overall yield from **17**.

Catalytic hydrogenation of the α-azide **12** in the presence of Lindlar catalyst in THF according to the method of Corey *et al.*[15] and TLC examination of the products showed a single spot at *Rf* 0.37 in 2:1 toluene–EtOAc. Hydrogenation of the β-anomer **20** under similar conditions also showed the same single spot on TLC at *Rf* 0.37. In order to see the anomeric purity, both glucopyranosylamines were *N*-acetylated with pyridine–Ac_2O. The product from the β-azide **20** showed a single spot at *Rf* 0.64 in 2:1 toluene–EtOAc. The products from the α-azide **12**, however, showed two spots on TLC at *Rf* 0.78 and *Rf* 0.64, which correspond to the α- and β-anomer, respectively. The ratio of α- to β-anomer was determined to be 5:4 by integrating the $NCOCH_3$ signals at δ 2.00 (α) and δ 1.78 (β) in the ^{1}H-NMR spectrum. Therefore, it is evident that in the course of the catalytic hydrogenation of the α-azide into α-amine, the stereochemical purity at C-1 was lost. This epimerization at C-1 was found to be mostly avoided by performing the hydrogenation in the presence of triethylamine. Thus, hydrogenation of the α-azide **12** in the presence



of Et_3N, and subsequent condensation of **13** with α-benzyl *N*-benzyloxycarbonyl-L-aspartate **5**[16] in the presence of diethylphosphocyanidate[17] afforded a mixture of the α- and β-amide (**21** and **22** in a ratio of 6:1) in a 78.7% yield. Catalytic hydrogenolysis of **21** in the presence of 10% Pd-C afforded the desired compound **2**. The α-D-configuration at C-1 of **2** was assignable according to the NMR data, which contained the signal for H-1 at δ 5.51 as a doublet with $J_{12}=5$ Hz, and the signal for C-1 at δ_C 76.7 with $^1J_{CH}=166.0$ Hz. In a similar way, hydrogenation of **22** afforded the β-anomer **3**. The β-D-configuration at C-1 of **3** was supported by NMR data which contained the signal for H-1 at δ 4.93 as a doublet with $J_{12}=9$ Hz and the signal for C-1 at δ_C 79.4 with $^1J_{CH}=155.0$ Hz.

In conclusion, synthetic sequences for the stereo-controlled formation of the α-linkage between the D-glucopyranosyl residue and the amide group of L-asparagine were developed.

EXPERIMENTAL

Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were determined with a Perkin-Elmer 241MC Polarimeter for solutions in $CHCl_3$ at 25°C unless otherwise noted. IR spectra were recorded with an EPI-G2 Hitachi Spectrophotometer, as KBr disks for the crystalline samples and as neat films for the liquid samples. ^{1}H-NMR spectra were recorded with a Varian HA-100 NMR spectrometer, and ^{13}C NMR spectra were recorded with a JNM-FX 100FT NMR spectrometer operated at 25.05 MHz. The values of δ_C and δ_H are expressed in ppm downwards from the internal standard, tetramethylsilane, for the solutions in $CDCl_3$ unless otherwise noted. Columm chromatography was performed on columns of Silica Gel Merck (70~230 mesh; E. Merck, Darmstadt, Germany). This layer chromatography was performed on precoated plates (layer thickness, 0.25 mm, E. Merck, Darmstadt, Germany) of Silica Gel 60 F_{254}.

2,3,4,6-Tetra-O-acetyl-α-O-glucopyranosyl azide **10**. A mixture of **9** (81 g, 0.22 M) and NaN_3 (28.7 g, 0.44 M) in HMPA (400 ml) was stirred for 18 hr at 20°C, poured into ice–water, and extracted with EtOAc. The organic layer was washed with water, dried ($MgSO_4$), and evaporated *in vacuo*. The residual oil was crystallized from *i*-Pr_2O to give **10** (65 g, 79%), which was pure enough for the next step, but found to contain a trace of the β-anomer **18** at *Rf* 0.44. An analytical sample was obtained after purification with pTLC. *Rf* 0.49 in 2:1 toluene–EtOAc, mp 98~99.5° (*i*-Pr_2O), $[\alpha]_D$ +174.2° ($c=0.6$), δ_H: 5.58 (H-1, d, $J=4$ Hz), 5.36 (H-3, t, $J=10$ Hz), 5.02 (H-4, t, $J=10$ Hz), 4.92 (H-2, dd, $J=4$, 10 Hz), 2.10, 2.04 and 2.02 (3 singlets for 4 Ac).

Anal. Calcd. for $C_{14}H_{19}N_3O_9$: C, 45.04; H, 5.13; N, 11.26. Found: C, 44.99; H, 5.11; N, 10.90.

α-D-Glucopyranosyl azide **11**. A solution of **10** (65 g, 0.174 M) in MeOH (400 ml) containing a trace of NaOMe was stirred for 18 hr at 20°C, neutralized with Amberlist 15, and filtered. The filtrate was evaporated *in vacuo* and the residue was crystallized from EtOH to give **11** (30.3 g 85%), *Rf* 0.44 in 25:6 CH_2Cl_2–MeOH, (a trace of the *β*-anomer **19** at *Rf* 0.50 completely disappeared upon one crystallization from EtOH). mp 181.5~182.5° (EtOH), $[\alpha]_D$ +266.7° (c=0.69, H_2O), $\delta_H(D_2O)$: 5.51 (H-1, d, J=3.5 Hz). $\delta_C(D_2O)$: 89.51 (C-1, $^1J_{CH}$=168.8 Hz).

Anal. Calcd. for $C_6H_{11}N_3O_5$: C, 35.12; H, 5.40; N, 20.48. Found: C, 34.96; H, 5.39; N, 20.44.

2,3,4,6-Tetra-O-benzyl-α-D-glucopyranosylazide **12**. To the solution of **11** (2.04 g, 10 mM) in DMF (60 ml) was added portionwise 50% NaH (2.16 g, 45 mM) at 5°C and the mixture was stirred for 30 min. To this mixture was added dropwise benzyl bromide (4.86 ml, 41 mM) at 0~5°C and the reaction mixture was stirred for 3 hr at 20°C. Excess NaH was decomposed by adding MeOH and the reaction mixture was partitioned between H_2O and EtOAc. The organic layer was washed with H_2O, dried ($MgSO_4$) and evaporated *in vacuo*. The residual oil was chromatographed over SiO_2 (300 g, in 49:1 toluene–EtOAc to give **12** (3.69 g, 65%) as a syrup, *Rf* 0.90 in 4:1 toluene–EtOAc, $[\alpha]_D$ +84.6° (c=0.41), δ_H: 5.20 (H-1, d, J=4 Hz). δ_C: 87.91 (C-1, $^1J_{CH}$=166.2 Hz).

Anal. Calcd. for $C_{34}H_{35}N_3O_5$: C, 72.19; H, 6.24; N, 7.43. Found: C, 72.39; H, 6.23, N, 7.41.

2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl azide **18**. To the solution of acetobromoglucose **17** (85 g, 207 mM) in DMF (350 ml) was added NaN_3 (27 g, 415 mM). The mixture was stirred for 3.5 hr at 50°C and poured into ice-water to give crystals, which were collected, washed with cold water and air dried. **18**: 59.6 g (77.3%) *Rf* 0.25 in 3:1 toluene–EtOAc, mp 124~125° (*i*-Pr_2O), $[\alpha]_D$ −28.1° (c=0.41), δ_H: 4.68 (H-1, d, J=8 Hz), 2.08, 2.06, 2.02 and 2.00 (4 singlets for 4 Ac).

Anal. Calcd. for $C_{14}H_{19}N_3O_9$: C, 45.04; H, 5.13; N, 11.26. Found: C, 45.15; H, 5.14; N, 11.32.

β-D-Glucopyranosyl azide **19**. A solution of **18** (6.0 g) in 0.2 N NaOMe in MeOH (100 ml)–DME (50 ml) was stirred for 1.5 hr at 15°C, neutralized with silica gel (10 g), and then filtered (celite). Evaporation of the filtrate afforded an oily **19** (3.29 g, 99%), *Rf* 0.50 in 25:6 CH_2Cl_2–MeOH, $\delta_C(D_2O)$: 90.37 (C-1, $^1J_{CH}$=160.1 Hz).

2,3,4,6-Tetra-O-benzyl-β-D-glucopyranosyl azide **20**. Benzylation of **19** as described for **11** afforded **20** as a syrup in an 80% yield. *Rf* 0.48 in 19:1 toluene–EtOAc, $[\alpha]_D$ +4.52° (c=1.55), δ_H: 7.5~7.0 (20 H, m, aromatic).

Anal. Calcd. for $C_{34}H_{35}N_3O_5$: C, 72.19; H, 6.24; N, 7.43. Found: C, 72.28; H, 6.24; N, 7.45.

2,3,4,6-Tetra-O-benzyl-β-D-glucopyranosyl amine **15**. A mixture of **20** (1.03 g) and Lindlar catalyst (275 mg) in EtOH (20 ml) was stirred under H_2 for 18 hr at 15°C. Usual processing afforded **15** (0.7 g, 17.4%) as a syrup, *Rf* 0.37 in 2:1 toluene–EtOAc, $[\alpha]_D$ +7.92° (c=0.51), δ_H: 4.10 (H-1, d, J=9 Hz).

Anal. Calcd. for $C_{34}H_{37}NO_5$: C, 75.66; H, 6.91; N, 2.60. Found: C, 75.66; H, 6.80; N, 2.53.

2,3,4,6-Tetra-O-benzyl-N-(O-benzyl-N-benzyloxycarbonyl-L-β-aspartyl)α-D-glucopyranosylamine **21** *and 2,3,4,6-tetra-O-benzyl-N-(O-benzyl-N-benzyloxycarbonyl-L-β-aspartyl)-β-D-glucopyranosylamine* **22**. A mixture of **12** (452 mg, 0.8 mM), Et_3N (0.24 ml, 1 mM), and Lindlar catalyst (240 mg) in THF (7 ml) was stirred under H_2 for 1 hr at 20°C. After further addition of Et_3N (0.5 ml), the mixture was filtered and the filtrate was evaporated *in vacuo* below 20°C (bath). To the solution of the oily residue in DMF (10 ml)–Et_3N (0.4 ml) was added α-benzyl benzyloxycarbonyl-L-aspartate (314 mg, 0.88 mM) and diethyl phosphorocyanidate (75% purity, Wako Chemicals, 0.2 ml) at 5°C. The reaction mixture was stirred for 30 min at 5°, for 1.5 hr at 20°C, and was then diluted with EtOAc. The organic layer was washed successively with H_2O, 0.1 N aq. HCl, H_2O, aq. $NaHCO_3$, dried ($MgSO_4$), and evaporated *in vacuo*. The residual oil was treated with MeOH to give **22** as solids (80 mg, 11.4%), *Rf* 0.28 in 4:1 toluene–EtOAc, mp 188~202° (acetone–EtOH), $[\alpha]_D$ +18.9° (c=0.28, THF), δ_H (DMSO-d_6); 8.88 (NHCO, d, J=8 Hz), 7.70 (NHCO, d, J=8 Hz).

Anal.[19] Calcd. for $C_{53}H_{54}N_2O_{10}$: C, 72.42; H, 6.19; N, 3.19. Found: C, 71.86; H, 6.09; N, 3.11.

The mother liquid of **22** was evaporated and the residue was chromatographed over SiO_2 (50 g) in 4:1 toluene–EtOAc to give **21** (473 mg, 67.3%) as a syrup, *Rf* 0.37 in 4:1 toluene–EtOAc, $[\alpha]_D$ +40.8° (c=0.60), δ_H: 6.50 (NHCO, d, J=7 Hz), 6.50 (NHCO, d, J=8 Hz), 3.0~2.75 ($COCH_2CH$, m).

Anal. Calcd. for $C_{53}H_{54}N_2O_{10}$: C, 72.42; H, 6.19; N, 3.19. Found: C, 72.14; H, 6.25; N, 3.49.

N-(β-L-Aspartyl)-α-D-glucopyranosylamine **2**. A mixture of **21** (217 mg, 0.25 mM) and 10% Pd-C (200 mg) in THF (4 ml)-EtOAc (3 ml) was stirred under H_2 at 20°C. After 16 hr, water (1.5 ml) was added, and the mixture was stirred for 18 hr. After further addition of water (4 ml), the mixture was stirred under H_2 for 14 hr at 40°C. Usual processing afforded **2** (77 mg, 99%) as a syrup, *Rf* 0.35 in 2:4:1 *n*-BuOH–AcOH–H_2O, $[\alpha]_D$ +106.9° (c=0.28, H_2O), $\delta_H(D_2O)$: 5.50 (H-1, d, J=5 Hz). $\delta_C(D_2O)$: 173.53 (CO), 76.65 (C-1, $^1J_{CH}$=166.0 Hz), 73.14 (C-3), 72.85 (C-5), 69.49 (C-2 and C-4), 60.67 (C-6), 51.22 (–CHNH–), 35.28 (–CH_2CO–).

Anal.[19] Calcd. for $C_{10}H_{18}N_2O_8$: C, 40.81; H, 6.17; N, 9.52. Found: C, 40.75; H, 6.48; N, 8.68.

N-(β-L-Aspartyl)-β-D-glucopyranosylamine **3**. A mixture of **22** (90 mg, 0.10 mM) and 10% Pd-C (100 mg) in THF (2 ml) was stirred under H_2 for 9 hr at 20°C. After adding water (0.4 ml), the mixture was further stirred for 16 hr at 20°C. Usual processing afforded **3** (30 mg, quantitative) as a syrup, *Rf* 0.35 in 2 : 4 : 1 *n*-BuOH–AcOH–H_2O, $[\alpha]_D$ −11.9° (c=1.55, H_2O), $\delta_H(D_2O)$: 4.93 (H-1, d, J= 9 Hz), 3.1 ~ 2.8 ($COC\underline{H}_2CH$, m). $\delta_C(D_2O)$: 173.53 (CO), 173.23 (CO), 79.44 (C-1, $^1J_{CH}$=155.0 Hz), 77.87 (C-5), 76.75 (C-3), 72.07 (C-2), 69.49 (C-4), 60.82 (C-6), 51.36 ($\underline{C}HNH$), 35.57 ($-CO\underline{C}H_2$).

Anal.[19] Calcd. for $C_{10}H_{18}N_2O_8 \cdot 2H_2O$: C, 36.36; H, 6.71; N, 8.48. Found: C, 36.64; H, 6.66; N, 7.93.

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19) Repeated analyses for these samples did not improve the relative errors observed.