Carbohydrate Research, 86 (1980) C7–C10
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

Preliminary communication

Synthetic studies on nephritogenic glycosides*. Synthesis of α-Glc-(1→6)-β-Glc-(1→6)-α-Glc-(1→Asn

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(Received August 30th, 1980; accepted for publication, September 15th, 1980)

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

α-Glc-(1→6)-β-Glc-(1→6)-α-Glc-(1→NHCO—peptide

1

α-Glc-(1→6)-β-Glc-(1→6)-α-Glc-(1→Asn

2

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-glucose, 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 D-glucopyranosyl residues.

As part of the experiments directed toward the synthesis of **1**, we report here a total synthesis of the smallest glycopeptide (**2**) in a regio- and stereo-controlled manner.

Treatment of β-chloride[6] (**3**) with NaN_3 in hexamethylphosphoric triamide (HMPA) for 16 h at 20° gave a 79% yield of α-azide[7] (**4**), m.p. 98–99.5°, $[\alpha]_D$ +174.2° (*c* 0.6, $CHCl_3$); R_F 0.49 (2:1 toluene–EtOAc); δ_H ($CDCl_3$): 5.58 (d, H-1, *J* 4 Hz). Subsequent Zemplén deacetylation of **4** afforded an 85% yield of **5**: m.p. 181.5–182.5°, $[\alpha]_D$ +266.7° (*c* 0.69, H_2O); R_F 0.44 (25:6 CH_2Cl_2–MeOH); δ_H (D_2O): 5.51 (d, H-1,

*Part 2 in the series "Synthetic Studies on Cell-surface Glycans". For Part 1, see ref. 1.
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0008-6215/80/0000–0000/$ 02.25,

J 3.5 Hz); δ_C (D_2O): 89.5 (C-1, $^1J_{CH}$ 168.9 Hz) and 60.7 (C-6). Compound **5** was converted, in 3 steps, into **6** in 74.8% yield: (*i*) TrCl in pyridine during 16 h at 55°; (*ii*) Ac_2O–pyridine during 2 h at 20°; and (*iii*) 4:1 AcOH–H_2O during 6 h at 55°. Compound **6**: $[\alpha]_D$ +155.2° (*c* 0.62, $CHCl_3$); R_F 0.25 in 2:1 toluene–EtOAc; δ_H ($CDCl_3$): 5.62 (d, H-1, *J* 4 Hz). Glycosidation of **6** with bromide **8** in $ClCH_2CH_2Cl$, in the presence[8] of $HgBr_2$ and powdered molecular sieve 4A, at 70–80°, afforded, in 72% yield, **10**: m.p. 116–118°, $[\alpha]_D$ + 76.6° (*c* 0.53, $CHCl_3$); δ_H ($CDCl_3$): 5.59 (d, H-1, *J* 4 Hz) and 4.55 (d, H-1′, *J* 8 Hz). The formation of a (1→6)-β-glycosidic linkage in **10** without acetyl migration was confirmed by its ^{13}C-n.m.r. spectrum, which showed the deshielded signal[9] for C-6 at δ 67.2 and the signal for C-1′ at δ 100.6, with $^1J_{CH}$ 164.8 Hz, as well as the signal for C-1 at[10] δ 86.1 with $^1J_{CH}$ 170.9 Hz.

Zemplén deacetylation of **10** afforded **11** {$[\alpha]_D$ +88.2° (*c* 0.425, H_2O), R_F 0.42 in 2:1 $CHCl_3$–MeOH}. Structure **11** was confirmed by the following ^{1}H- and ^{13}C-n.m.r. data: δ_H (D_2O): 5.52 (d, H-1, *J* 3 Hz) and 4.49 (d, H-1′, *J* 8 Hz); δ_C (D_2O): 103.0 (C-1′, $^1J_{CH}$ 162.1 Hz), 89.5 (C-1, $^1J_{CH}$ 168.9 Hz), 68.7 (C-6), and 61.0 (C-6′).

Tritylation of **11** and acetylation to give **12**, and detritylation* of **12** with 40:8:1 AcOH–H_2O–NaI during 2.5 h at 65° afforded **13** in an 83% overall yield from **10**. Compound **13** had $[\alpha]_D$ +94.9° (*c* 0.725, $CHCl_3$); R_F 0.40 in 1:2 toluene–EtOAc; δ_H ($CDCl_3$): 5.59 (d, H-1, *J* 4 Hz) and 4.59 (d, H-1′, *J* 8 Hz); δ_C ($CDCl_3$): 100.0 (C-1′, $^1J_{CH}$ 163.6 Hz) and 85.7 (C-1, $^1J_{CH}$ 170.9 Hz). Glycosidation of **13** with **9** under Hanessian–Banoub conditions[11] afforded a 74% yield of a mixture of **14** and **15** in the ratio of 3:2. The anomers were readily separated by flash column-chromatography[12]. Compound **14** had $[\alpha]_D$ +82.5° (*c* 0.93, $CHCl_3$); R_F 0.55 in 40:7 $CHCl_3$–EtOAc; δ_C ($CDCl_3$): 100.4 (C-1′, $^1J_{CH}$ 161.1 Hz), 97.1 (C-1″, $^1J_{CH}$ 169.7 Hz), and 86.1 (C-1, $^1J_{CH}$ 169.5 Hz). Compound **15** had $[\alpha]_D$ +61.3° (*c* 0.6, $CHCl_3$); R_F 0.49 in 40:7 $CHCl_3$–EtOAc; δ_C ($CDCl_3$): 103.9 (C-1″, $^1J_{CH}$ 159.9 Hz), 100.4 (C-1′, $^1J_{CH}$ 162.4 Hz), and 86.1 (C-1, $^1J_{CH}$ 171.2 Hz).

The α anomer **14** was deacetylated to **16**: $[\alpha]_D$ +60.3° (*c* 0.76, $CHCl_3$); R_F 0.63 in 6:1 $CHCl_3$–MeOH. Benzylation of **16** afforded **17** (in 62.3% yield from **14**). Compound **17** had $[\alpha]_D$ +58.0° (*c* 0.35, $CHCl_3$); R_F 0.45 in 10:1 toluene–EtOAc. Catalytic hydrogenation of the azide group in **17** in oxolane (THF)–Et_3N** in the presence of Lindlar catalyst[13] gave a mixture of **18** and **19** (a single spot in t.l.c., R_F 0.41 in 10:1 $CHCl_3$–EtOAc) which was immediately treated with **20** and diethylphosphorocyanidate[14] in $HCONMe_2$–Et_3N, to give a 75% yield of a mixture of **21** and its β anomer (at C-1) **22** in the ratio of 5:1. Compound **21** was purified by flash column-chromatography[12]: $[\alpha]_D$ +40° (*c* 0.98, $CHCl_3$); R_F 0.50 in 10:1 $CHCl_3$–EtOAc.

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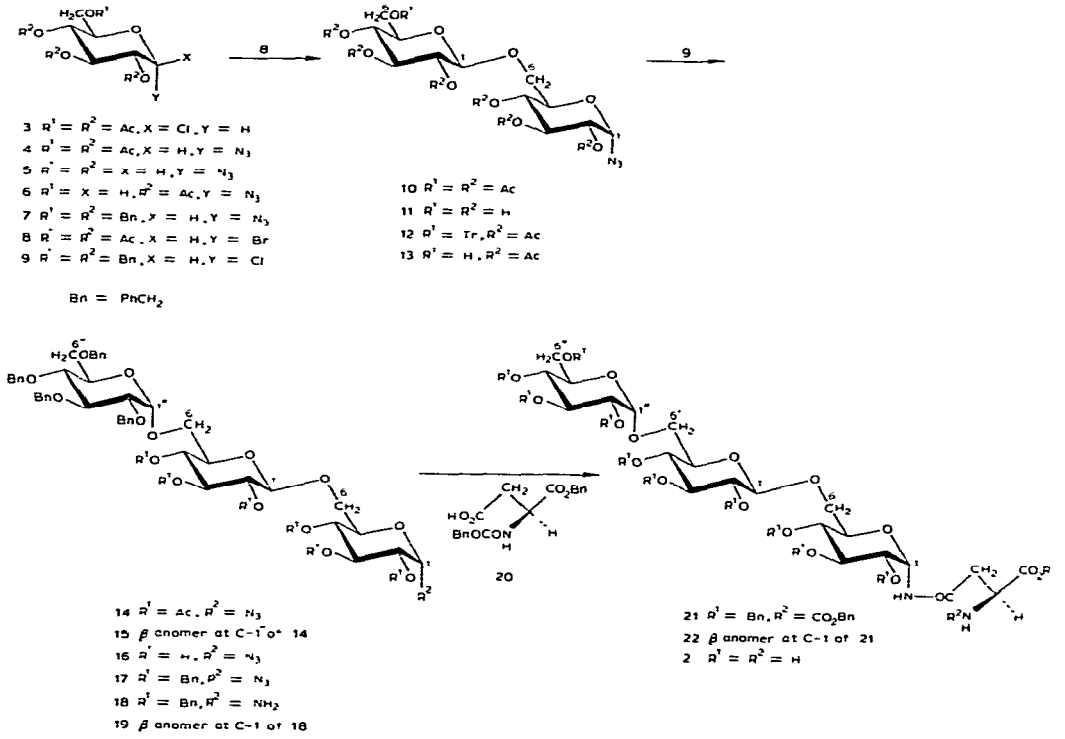
*Hydrolysis of the trityl group of **12** was improved by the presence of NaI in aq. AcOH. In the absence of added NaI, the hydrolysis required 48 h, and gave rise to a mixture of **13** and its regioisomer, due to acetyl migration.

In the absence of added Et_3N, **7 was hydrogenated to a mixture of α- and β-amine in the ratio of 1:1. Addition of Et_3N is crucial in obtaining α-amine as the major product.



Catalytic hydrogenolysis of **21** over 10% Pd–C in THF–EtOH–H_2O gave the target compound **2**, $[\alpha]_D$ +72.8° (*c* 2.0, H_2O); R_F 0.35 in 2:4:1 BuOH–AcOH–H_2O. The structure of **2** was deduced from the synthetic sequence, and was confirmed by the following ^{1}H- and ^{13}C-n.m.r. data: δ_H (D_2O): 5.58 (d, H-1, *J* 4 Hz), 4.93 (d, H-1″, *J* 3.5 Hz), 4.50 (d, H-1′, *J* 7.5 Hz), and 2.9–3.1 (m, $COCH_2CH$); δ_C (D_2O): 102.7 (C-1′, $^1J_{CH}$ 162.4 Hz), 97.9 (C-1″, $^1J_{CH}$ 169.7 Hz), 76.7 (C-1, $^1J_{CH}$ 166 Hz), 68.5 (C-6), 65.6 (C-6′), 60.5 (C-6″), 51.2 ($COCH_2CH$), and 35.1 ($COCH_2CH$).

The small discrepancy found between the ^{13}C-n.m.r. data for natural **1** and for the synthetic product (**2**) may be attributable to the presence of a longer peptide moiety in **1**.

ACKNOWLEDGMENTS

We thank Dr. H. Homma and his staff for the elemental analyses, and Dr. J. Uzawa and Mrs. T. Chijimatsu for recording and measuring the n.m.r. spectra. We also thank Miss A. Sone for her technical assistance.

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