THE NEPHRITOGENIC GLYCOPEPTIDE FROM RAT GLOMERULAR BASEMENT MEMBRANE. XI.[1] TOTAL SYNTHESIS OF NEPHRITOGENOSIDE

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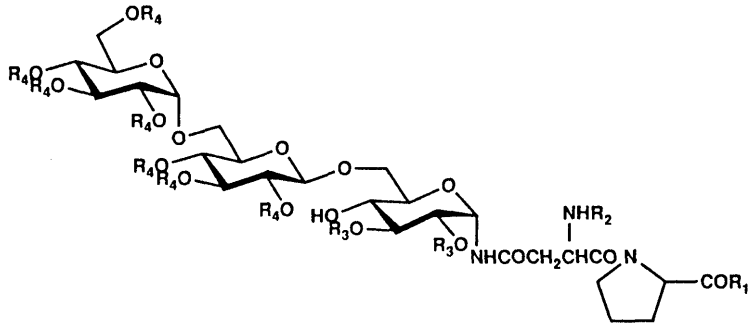
Nephritogenoside has been prepared by coupling of the acylazide derivative of N-triglycosyl dipeptide with nonadecapeptide, followed by deprotection of a desirable protected nephritogenoside

KEYWORDS nephritogenoside; glomerulonephritis; glycopyranosylamine; triglycosyl dipeptide; hydrazide; acylazide; nonadecapeptide; coupling; solid phase peptide synthesis

Shibata et al.[2] isolated and purified from the glomerular basement membrane of rats a compound named nephritogenoside that was active for the induction of glomerulonephritis in homologous animals.[3] Nephritogenoside is composed of three D-glucose units, α-D-Glc$_p$-(1→6)-β-D-Glc$_p$-(1→6)-D-Glc$_p$, and 21 amino acids [^{1}Asn-Pro-Leu-Phe-Gly-Ile-Ala-Gly-Glu-Asp-Gly-Pro-Thr-Gly-Pro-Ser-Gly-Ile-Val-Gly-^{21}Gln], and the reducing α-D-glucose unit is linked N-glycosidically to the N-terminal asparagine unit.[4] The synthesis of model glycoproteins and glycopeptides is important because these compounds may have significant biological properties. In our previous paper,[1] we reported the synthesis of an N-triglycosyl dipeptide by condensation of the corresponding dipeptide derivative with triglycosylamine. N-Triglycosyl dipeptide derivative, O-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-(1→6)-O-(2,3,4-tri-O-acetyl-β-D-glucopyranosyl)-(1→6)-2,3-di-O-benzyl-N-[N-(tert-butoxycarbonyl)-L-aspart-1-oyl-(methyl L-prolinate)-4-oyl]-α,β-D-glucopyranosylamine 1,[5] was converted into the hydrazide by the procedure of Yajima et al.[6] The isomers 2α[7] (68.2%), and 2β (13.8%) were separated by silica gel column chromatography in the ratio of 5:1. The acylazide (3) derived from the corresponding hydrazide by the Honzl and Rudinger[8] procedure was allowed to react with the triethylammonium salt of nonadecapeptide 7, obtained by the procedure described as follows, to give a desirable protected nephritogenoside 4 in 81.2% yield. Fmoc-Gln(Mbh)-O-polymer (purchased from Kokusan Chem. Works Ltd.) was subjected to the usual procedures of solid phase peptide synthesis[9] to give the protected resin corresponding to nonadecapeptide 7. Reaction of the peptide resin with TFA and purification of the product by HPLC[10] gave 7[11] in excellent yield. Removal of the tert-butoxycarbonyl group with 90% TFA gave

5,[12] removal of the benzyl group of this compound with 10% Pd-C afforded the target compound, nephritogenoside 6[13] ($[\alpha]_D^{22}$ -31.3°) in 88.8% yield. The configuration of nephritogenoside was confirmed by ^{1}H-NMR spectroscopy; signals for H-1, H-1', and H-1" were observed at δ 5.63(J=5.49), 4.30(J=7.15), and 4.94(J=3.67 Hz), respectively.



1 R_1=OMe, R_2=Boc, R_3=Bn, R_4=Ac
2 R_1=$NHNH_2$, R_2=Boc, R_3=Bn, R_4=H
3 R_1=N_3, R_2=Boc, R_3=Bn, R_4=H
4 R_1=Leu-Phe-Gly-Ile-Ala-Gly-Glu-Asp-Gly-Pro-Thr-Gly-Pro-Ser-Gly-Ile-Val-Gly-Gln-OH, R_2=Boc, R_3=Bn, R_4=H
5 R_1=nonadecapeptide, R_2=H, R_3=Bn, R_4=H
6 R_1=nonadecapeptide, R_2=R_3=R_4=H
7 H-Leu-Phe-Gly-Ile-Ala-Gly-Glu-Asp-Gly-Pro-Thr-Gly-Pro-Ser-Gly-Ile-Val-Gly-Gln-OH

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4) S. Shibata, T. Takeda, and Y. Natori, *J. Biol. Chem.*, **263**, 12483 (1988); T. Takeda, M. Sawaki, Y. Ogihara, and S. Shibata, *Chem. Pharm. Bull.*, **37**, 54 (1989).

5) Mixture of α and β anomers of O-(2,3,4,6-tetra-O-acetyl- α -D-glucopyranosyl)-(1→6)-O-(2,3,4-tri-O-acetyl-β-D-glucopyranosyl)-(1→6)- 2,3-di-O-benzyl-D-glucopyranosylamine was coupled with the dipeptide, N-(tert-butoxycarbonyl)-L-asparty1-L-proline methyl ester, in the presence of O,O-diethylcyanophosphonate to give 1 in 64.1% yield.

6) N. Fujii and H. Yajima, *Chem. Pharm. Bull.*, **23**, 1596 (1975). To a solution

of 1 (100 mg) in MeOH (6 ml), aqueous 80% hydrazine hydrate (0.3 ml) was added and stirred for 12h at room temperature (yield; 82.0%).

7) 2α :TLC : R_F 0.51 ($CHCl_3$-MeOH-H_2O 65:35:10 lower layer); ^{1}H-NMR (d_4-MeOH) :δ 5.77 (1H,d, J=5.31 Hz, H-1), 4.37 (1H,d, J=7.69 Hz, H-1'), 4.89 (1H,d, J=3.42 Hz, H-1").

8) J. Honzl and J. Rudinger, *Collect. Czech. Chem. Commun.*, **26**, 2333 (1961). To a solution of the hydrazide (25.0 mg) in DMF (0.3 ml), 6.9 N HCl-DMF (35 μl) and isoamylnitrite (70 μl) were added and the mixture was stirred at -80°C for 20 min. When the hydrazine test became negative, the solution, after neutralization with Et_3N (30 μl), was combined with a solution of 7 (21.6 mg) in DMF (0.4 ml) containing Et_3N (30 μl), and the mixture was stirred at 4°C for 48 h. The solvent was evaporated, and the resulting solid residue was chromatographed on silica gel to give 27.2 mg (81.2%) of the protected nephritogenoside.

9) Using an Applied Biosystems (ABI) Model 431A synthesizer employing Fmoc amino acids purchased from ABI.

10) HPLC was performed on an Applied Biosystems HPLC 150A separation system equipped with a Aquapore Prep-10, C-8 (20 μm) column (10 mm i.d.x 250 mm) [solvent: CH_3CN-H_2O containing 0.1% TFA: 0-100% gradient]: flow rate:2 ml/min, detection: 220 nm.

11) $[\alpha]_D^{22}$-79.8° (c=0.62,H_2O): The N-terminal sequence of the synthetic peptide 7 was determined by an automated Edman degradation using an Applied Biosystems model 477A automated gas phase sequencer.

12) 5: $[\alpha]_D^{19}$ -55.8°(c=0.19, H_2O), ^{1}H-NMR (D_2O):δ5.75 (1H,d, J=5.60, H-1), 4.26 (1H,d, J=7.33, H-1'), 4.94 (1H,d, J=3.48, H-1").

13) 6: $[\alpha]_D^{22}$ -31.3°(c=0.44, H_2O), HPLC was performed on an ABI HPLC 130A separation system equipped with an Aquapore RP-300, C-8(7 μm);[solvent: CH_3CN-H_2O containing 0.1% TFA(0-30%, 5min, 30-40%, 25 min)]: flow rate 200 μl/min, detection:220 nm.: Retention time was 9.52 min for nephritogenoside, 10.02 min for nonadecapeptide.

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