Tetrahedron Letters, Vol. 33, No. 3, pp. 363-366, 1992
Printed in Great Britain

0040-4039/92 $3.00 + .00
Pergamon Press plc

TOTAL SYNTHESIS OF NEPHRITOGENIC GLYCOPEPTIDE, NEPHRITOGENOSIDE

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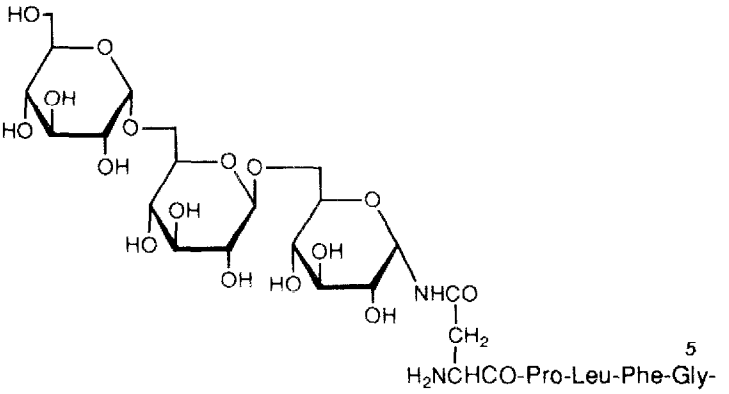
Key Words: nephritogenoside; glycopeptide; allyloxycarbonyl group; palladium complex; nephritogenic activity

Abstract: Nephritogenoside (**1**), proposed as a nephritogenic triglycosylhenicosapeptide with asparagine N-α-glycoside linkage, was first synthesized by the coupling of triglycosyldipeptide active ester with the nonadecapeptide (3-21) using allyloxycarbonyl group as the final protecting group. A β-anomeric glycopeptide was also prepared.

Nephritogenoside was isolated as an active principle of nephritogenicity from the basement membrane of rats by S. Shibata in 1981[1]. The structure was determined by S. Shibata and his collaborators in 1988 to be a glycopeptide in which the trisaccharide composed of three glucose moieties is linked to the peptide of 21 amino acids *via N*-glycoside bond on the asparagine residue.[2] Although syntheses of the trisaccharide part connected with an amino acid or a short peptide have been reported,[3] a total synthesis of whole structure of nephritogenoside has not yet been accomplished. In order to confirm the proposed structure and to elucidate the biological activity in molecular level, we performed the first total synthesis of nephritogenoside.



Structure of Nephritogenoside (**1**)

In our synthetic strategy, allyloxycarbonyl (Aloc) group[4] which is removable by palladium complex under neutral conditions was chosen as the final protecting group of amino and hydroxyl groups, taking account of general instability of glycopeptide under acidic and basic conditions. No existence of basic amino acid residues in the peptide chain makes possible the free peptide to be coupled with glycosyl moiety. The

peptide used in this study was synthesized by ABI 430A peptide synthesizer. Boc group was used as protection for α-amino group, and cyclohexyl ester for ω-carboxyl group of Asp and Glu. The final deprotection and cleavage from the resin were carried out by the treatment with HF - *p*-cresol (8:2). After purification by HPLC, the peptide obtained was directly used for the coupling with the glycosyl moiety.

Heptaacetylisomaltosyl fluoride (**2**) was prepared from isomaltose by peracetylation followed by treatment with 60% HF in pyridine, and then coupled with 2,3,4-triacetylglucopyranosyl-α-azide (**3**)[3b] to give trisaccharide azide **4α** as shown in Fig. 1. First, a formation of N-glycoside linkage of this trisaccharide with asparagine moiety was attempted. Thus the compound **4α** was reduced by catalytic hydrogenation using Pd-C, and then coupled with Aloc-Asp(OH)-O^{t}Bu[5] to give an anomeric mixture of *N*-glycosides **5α** and **5β**. Under reduction conditions, an anomerization at C-1 carbon occurred to give a mixture of diastereomers which were separated and used for the following synthetic steps respectively.

Replacement of acetyl group in compound **5α** or **5β** with Aloc group gave **6α** or **6β**, which was converted to N-hydroxysuccinimide (OSu) ester **7α** or **7β** by TFA treatment followed by active esterification as shown in Fig. 2. However, the active ester **7α** or **7β** did not react with the eicosapeptide (2-21) at all but cyclized itself to give the succinimide derivative.

In order to avoid the succinimide formation of the active ester **7α** or **7β** during the coupling reaction, we next tried a coupling reaction of glycosyldipeptide active ester with free nonadecapeptide (3-21) as shown in

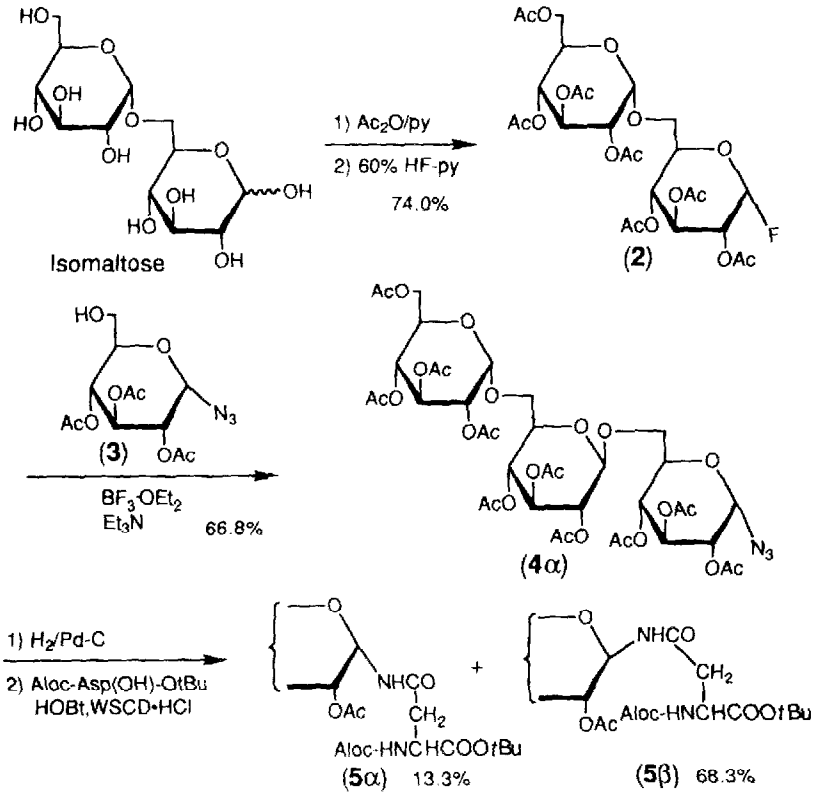


Fig.1

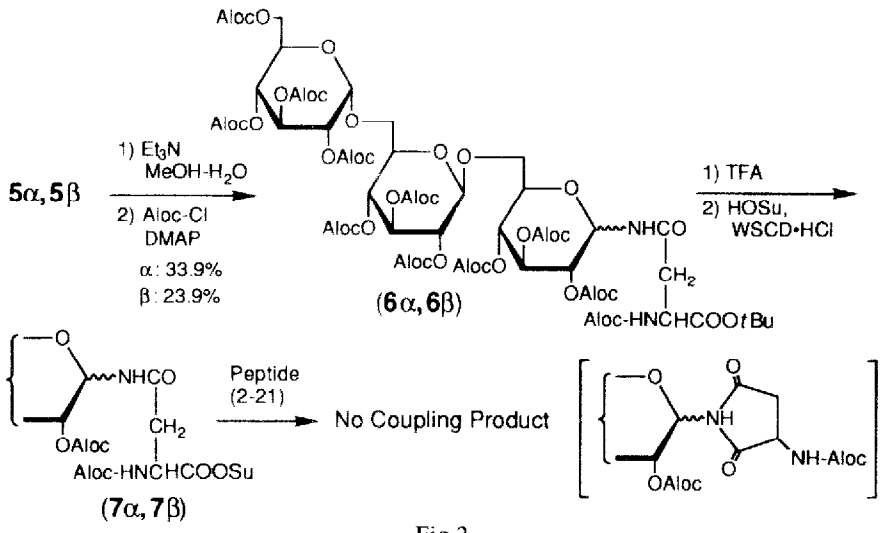


Fig.2

Fig. 3. The glycosyl dipeptide can be prepared from glycosylasparagine and the protected proline residue by another coupling method rather than the active ester method. Thus, the compound **5α** was treated with TFA to remove *t*-butyl ester and then coupled with H-Pro-O*t*Bu by 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSCD•HCl) and 1-hydroxybenzotriazole (HOBt) method. Acetyl group of triglycosyldipeptide (**8α**) thus obtained was replaced with Aloc group to give **9α**. *t*-Butyl ester group in **9α** was cleaved

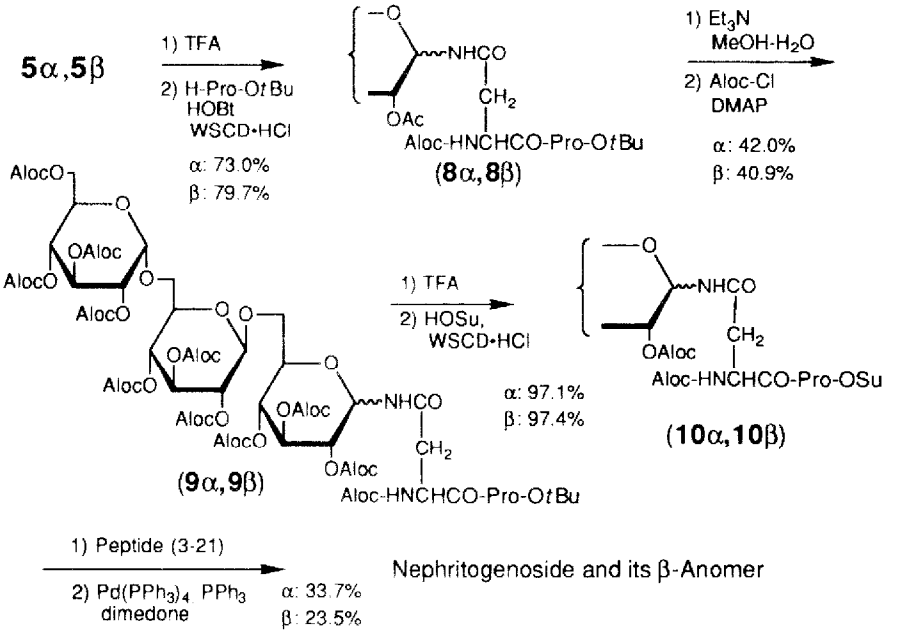


Fig. 3

with TFA and converted to the active ester (**10**α). Finally, **10**α was reacted with the nonadecapeptide (3-21) to give us a desirable protected nephritogenoside without cyclization at asparagine residue as in compound **7**. After deprotection of all Aloc groups by palladium complex, the crude product was purified by HPLC to give free nephritogenoside.[6)]

Moreover, the β-anomer of nephritogenoside[7)] was also synthesized from **5**β by the same manner as that of nephritogenoside. Biological tests of nephritogenoside and its β-anomer are now being undertaken.

In this study, we established the general strategy for the synthesis of glycopeptide with N-glycosyl bond of asparagine which is linked to a relatively long peptide chain: (i) The glycosyl moiety is first coupled with asparagine residue. (ii) The glycosylasparagine in a protected form is coupled with amino acid or peptide[9)]. (iii) The glycosylpeptide thus obtained is coupled with the longer peptide by the active ester method. (iv) The Aloc group is used as the final protecting group.

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5) Aloc-Asp(OH)-O*t*Bu was prepared from H-Asp(OBzl)-OH through three steps. [1) Aloc-Cl, $NaHCO_3$, 2) $(CH_3)_2C=CH_2$, H_2SO_4, 3) NaOH, total yield: 75.4%]

6) Amino acid analysis[8a)], Asp(2): 2.00, Thr(1): 0.92, Ser(1): 0.91, Glu(2): 1.96, Pro(3): 2.90, Gly(6): 5.91, Ala(1): 0.96, Val(1): 0.58, Ile(2): 1.56, Leu(1): 1.00, Phe(1): 0.98, NH_3(2): 2.35; PD-MS[8b)], M+H : 2471.3, M+Na : 2493.7; ^{1}H-NMR[8c)], 0.78-0.95 (24H), 1.05-1.23 (3H), 1.19 (3H, d, 6.3 Hz), 1.37 (3H, d, 7.3 Hz), 1.35-1.60 (5H), 1.70-2.48 (24H), 2.75-4.55 (59H), 4.92 (1H, d, 3.7 Hz), 5.60 (1H, d, 5.4 Hz), 7.20-7.39 (5H); HPLC[8d)]: 15.7 min.

7) Amino acid analysis[8a)], Asp(2): 2.00, Thr(1): 0.95, Ser(1): 0.97, Glu(2): 2.04, Pro(3): 3.03, Gly(6): 5.87, Ala(1): 1.04, Val(1): 0.57, Ile(2): 1.50, Leu(1): 1.01, Phe(1): 1.00, NH_3(2): 2.67; PD-MS[8b)], M+H : 2471.6, M+Na : 2493.9; ^{1}H-NMR[8c)], 0.78-0.95 (24H), 1.05-1.20 (3H), 1.19 (3H, d, 6.4 Hz), 1.33 (3H, d, 7.1 Hz), 1.35-1.60 (5H), 1.65-2.50 (24H), 2.75-4.55 (59H), 4.89 (1H, d, 3.9 Hz), 4.98 (1H, d, 9.0 Hz), 7.20-7.39 (5H); HPLC[8d)]: 15.7 min.

8) a) Hydrolysis conditions: 6M HCl, 110°C, 22 h. Under this condition, only about 60 % of Ile-Val in the peptide sequence was hydrolyzed. Theoretical values of amino acid ratios were shown in parentheses. b) Plasma desorption mass spectrometry. The calculated molecular weight, M+H: 2470.5, M+Na: 2492.5. c) δ(ppm)(270 MHz ^{1}H-NMR in D_2O). The peak of HDO was used as the reference (δ=4.70 ppm). d) Retention time of HPLC (Cosmosil 5C_{18}, 10 x 250 mm, CH_3CN-H_2O containing 0.1% TFA, gradient 10 - 60 % CH_3CN (25 min), flow rate: 3 ml/min, detection: 210 nm).

9) The C-terminal amino acid of the glycosylpeptide is recommended to be either Gly or Pro to avoid the racemization in further fragment condensation.

(Received in Japan 17 October 1991)