Tetrahedron Letters, Vol.32, No.47, pp 6873-6876, 1991
Printed in Great Britain

0040-4039/91 $3.00 + .00
Pergamon Press plc

An Efficient and Stereocontrolled Synthesis of the Nephritogenoside Core Structure

Makoto Sasaki* **and Kazuo Tachibana**

Department of Chemistry, Faculty of Science, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan

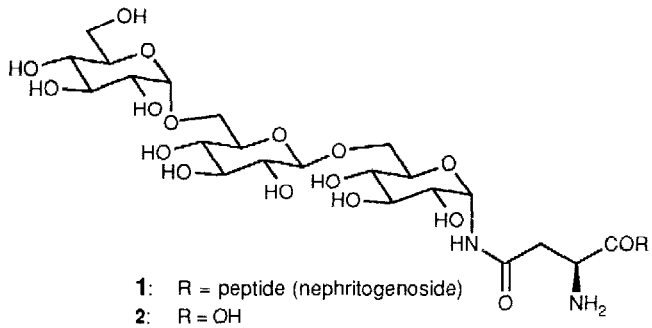
Hiroshi Nakanishi

National Chemical Laboratory for Industry, Tsukuba, Ibaraki 305, Japan

Key Words: nephritogenoside; glycopeptide; glomerulonephritis; β-selective glycosylation; α-nitrilium ion

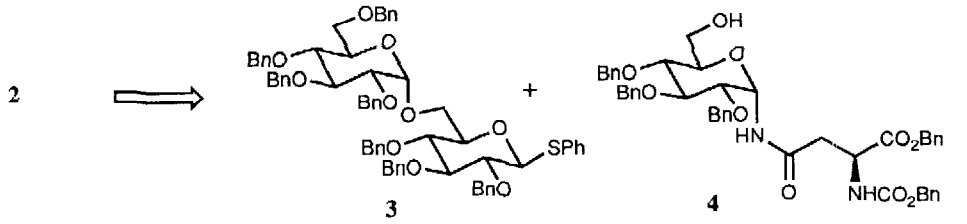
Abstract: *An efficient and stereocontrolled synthesis of the core trisaccharide* ***2*** *of nephritogenic glycopeptide, nephritogenoside* ***1*** *is described. Key to our synthetic strategy was β-selective glycosylation without neighboring group participation.*

Nephritogenoside[1] **1** was isolated as a minor component from the glomerular basement membrane of normal rats after exhaustive proteolytic digestions. This compound exhibits an activity for the induction of progressive chronic glomerulonephritis in homologous animals by a single footpad injection with incomplete Freund's adjuvant. Structurally, the glycopeptide is uniquely distinct from common glycoproteins; namely, the trisaccharide core is *N*-glycosidically linked to the peptide chain through α-D-glucose.[2] The full sequence of the 21 amino acid residues in the peptide chain was proposed recently.[3] In view of the unambiguous structural confirmation and biological investigations, we initiated a synthetic program directed toward nephritogenoside **1**.[4] In this communication we wish to report an efficient and stereocontrolled synthesis of the asparagine-linked core trisaccharide **2** of this novel nephritogenic glycopeptide, based on β-selective glycosylation without neighboring group participation.



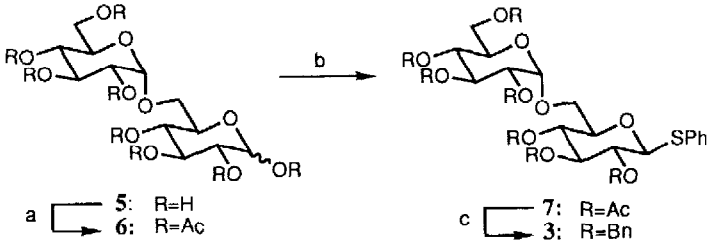
1: R = peptide (nephritogenoside)
2: R = OH

Our convergent synthetic plan is outlined in Scheme 1. For the overall simplicity and efficiency, we planned stereoselective construction of the β-glycosidic bond in **2** from benzyl-protected phenyl thioglycoside **3** and asparagine-linked monosaccharide alcohol **4**. In connection with the crucial coupling reaction, we applied the putative α-nitrilium ion as an intermediate in order to control the β-stereoselectivity[5] in the glycosylation of **4** without anchimeric assistance.



Scheme 1

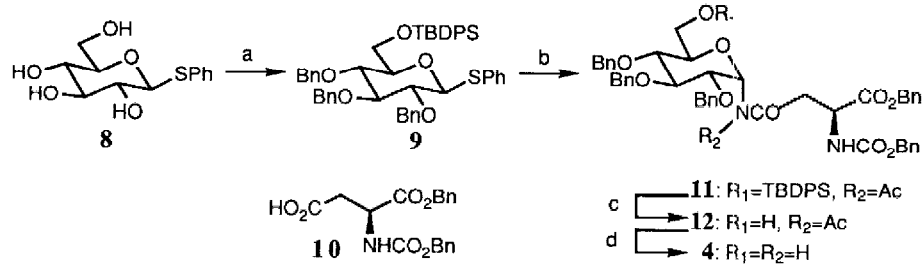
For the synthesis of the left half **3**, we chose isomaltose **5**[6] as the starting material (Scheme 2). Acetylation of **5** afforded octaacetate **6**, which upon treatment with (trimethylsilyl)thiophenol according to the method of Hanessian[7] gave phenyl thioglycoside **7**, $[\alpha]_D^{27}$ + 76.5 (*c* 1.40, $CHCl_3$). Under these conditions, no cleavage of the internal α-glycosidic bond was observed. This compound was converted into the desired isomaltosyl derivative **3**, $[\alpha]_D^{25}$ +25.1 (*c* 1.03, $CHCl_3$), in the usual manner.



(a) Ac_2O, 4-dimethylaminopyridine (DMAP), r.t. (98%); (b) $PhSSiMe_3$, ZnI_2, *n*-Bu_4NI, $ClCH_2CH_2Cl$, 60 °C (98%); (c) (i) NaOMe, MeOH, r.t.; (ii) NaH, BnBr, *n*-Bu_4NI, DMF, r.t. (90% overall).

Scheme 2

The right half **4**, also used as an intermediate in the synthesis of **2** by Ratcliffe *et al.*,[4d] was prepared according to essentially the same procedure with the exception that we employed the readily available phenyl thioglycoside (Scheme 3). Selective protection of the primary alcohol of the known thioglycoside **8**[8] as its *t*-butyldiphenylsilyl ether and subsequent benzylation afforded compound **9**, $[\alpha]_D^{22}$ -13.2° (*c* 1.83, $CHCl_3$). Conversion of **9** into α-imide **11**, $[\alpha]_D^{26}$ -7.7 (*c* 1.29, $CHCl_3$), proceeded *via* the α-acetonitrilium ion under the influence of *N*-iodosuccinimide (NIS) in acetonitrile at room temperature in higher yield (85%) than that described for the corresponding pent-4-enyl glycoside.[4d] When *N*-bromosuccinimide (NBS) was used as a source of halonium ion in place of NIS, none of the imide formation proceeded and an approximately 1 : 1 anomeric mixture of the corresponding glycosyl esters was formed instead. The imide **11** was converted into the right half **4**, mp 143-143.5 °C; $[\alpha]_D^{26}$ +33.1 (c 0.68, $CHCl_3$), by sequential deprotection in two steps.

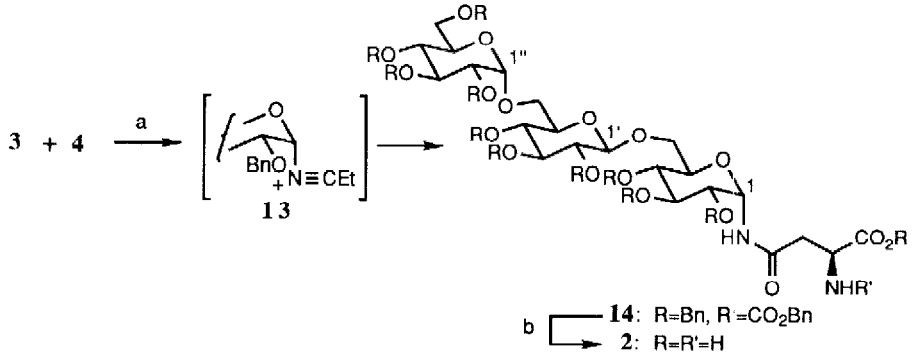


(a) (i) *t*-BuPh$_2$SiCl, Et$_3$N, DMAP, CH$_2$Cl$_2$, r.t. (98%); (ii) NaH, BnBr, *n*-Bu$_4$NI, DMF, r.t. (71%); (b) **10**, NIS, CH$_3$CN, r.t. (85%); (c) HF•pyridine, pyridine, THF, r.t. (87%); (d) piperidine, DMF, r.t. (92%).

Scheme 3

With the desired left and right halves **3** and **4** available, attention was directed toward the crucial coupling of these two fragments to assemble the glycosidic linkage in **2**. Among the reagents and conditions tested using a model compound phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside, the most satisfactory result was observed when a combination of NBS and trifluoromethanesulfonic acid (TfOH) was employed.[9] Thus, coupling of alcohol **4** with 1.5 equivalent of phenyl thioglycoside **3** under the influence of NBS-TfOH in propionitrile at -78 °C proceeded smoothly *via* the putative α-propionitrilium ion **13** to give trisaccharide **14**, $[\alpha]_D^{28}$ +28.7 (*c* 1.50, $CHCl_3$), and its α-anomer with a ratio of 96:4 in 74% combined yield.[12] Stereochemistry of the newly generated glycosidic bond in **14** was confirmed to be β by C-H COSY experiment.[13]

Finally, hydrogenolysis of **14** over 20% palladium hydroxide on carbon deprotected all the protecting groups to furnish the target compound **2**, $[\alpha]_D^{25}$ +73.2 (*c* 0.097 H_2O), in quantitative yield. Three anomeric protons resonated at δ 5.60 (d, J=5.5 Hz, 1-H), 4.95 (d, J=4.1 Hz, 1"-H), and 4.50 (d, J=8.0 Hz, 1'-H), whereas three anomeric carbons resonated at δ 79.22 (C-1), 105.32 (C-1'), and 100.48 (C-1"). Other ^{1}H and ^{13}C NMR signals were traced in the H-H, and C-H COSY spectra to confirm the above assignments and thus the structure of **2** was unambiguous.[14]



(a) NBS, TfOH, CH_3CH_2CN, -78 °C, 1 h (74%, α:β = 96:4); (b) H_2, 20% Pd(OH)$_2$-C, THF, EtOH, H_2O, r.t. (quant.).

Scheme 4

The described synthetic route to the core trisaccharide **2** of nephritogenoside **1** based on the stereocontrolled construction of β-glycosidic bond is considerably shorter and more efficient than those previously reported and rendered a large quantity of this compound available in a pure form for further biological investigations.[15] Further extensions of this strategy for the synthesis of nephritogenoside **1** and its analogues are currently in progress in these laboratories.

References and Notes

1. (a) Shibata, S.; Miyagawa, Y.; Naruse, T.; Takuma, T. *J. Immunol.* **1969**, *102*, 593. (b) Shibata, S.; Nagasawa, T. *J. Immunol.* **1971**, *106*, 1284.
2. Shibata, S.; Nakanishi, H. *Carbohydr. Res.* **1980**, *81*, 345; **1980**, *86*, 316.
3. (a) Shibata, S.; Takeda, T.; Natori, Y. *J. Biol. Chem.* **1988**, *263*, 12483. (b) Takeda, T.; Sawaki, M.; Ogihara, Y.; Shibata, S. *Chem. Pharm. Bull.* **1989**, *37*, 54.
4. For the earlier synthetic works, see: (a) Ogawa, T.; Nakabayashi, S.; Shibata, S. *Carbohydr. Res.* **1980**, *86*, C7. (b) Ogawa, T.; Nakabayashi, S.; Shibata, S. *Agric. Biol. Chem.* **1983**, *47*, 1213. (c) Takeda, T.; Sugiura, Y.; Hamada, C.; Fujii, R.; Suzuki, K,; Ogihara, Y.; Shibata, S. *Chem. Pharm. Bull.* **1981**, *29*, 3196. (d) Ratcliffe, A. J.; Konradsson, P.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1990**, *112*, 5665. (e) Takeda, T.; Utsuno, A.; Okamoto, N.; Ogihara, Y.; Shibata, S. *Carbohydr. Res.* **1990**, *207*, 71.
5. (a) Ratcliffe, A. J.; Fraser-Reid, B. *J. Chem. Soc., Perkin Trans. 1* **1990**, 747. (b) Schmidt, R. R.; Behrendt, M.; Toepfer, A. *Synlett* **1990**, 694, and references cited therein.
6. Purchased from Sigma Chemical Company, St. Louis, MO, U.S.A..
7. Hanessian, S.; Guindon, Y. *Carbohydr. Res.* **1980**, *86*, C3.
8. Phenyl thioglycoside **8** was prepared according to the reported method in Nicolaou, K. C.; Randall, J. L.; Furst, G. T. *J. Am. Chem. Soc.* **1985**, *107*, 5556.
9. When *N*-iodosuccinimide (NIS)-TfOH, which allowed acyl-protected ("disarmed") pent-4-enyl glycoside[10] and 1-thioglycosides[11], was used in place of NBS-TfOH, the coupling reaction was found to take longer reaction time at -78 °C. Sasaki, M; Gama, Y.; Ishigami, Y. unpublished results.
10. (a) Konradsson, P.; Mootoo, D. R.; McDevitt, R. E.; Fraser-reid, B. *J. Chem. Soc., Chem. Commun.* **1990**, 270. (b) Konradsson, P.; Udodong, U. E.; Fraser-Reid, B. *Tetrahedron Lett.* **1990**, *31*, 4313.
11. Veeneman, G. H.; van Leeuwen, S. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331.
12. The ratio was determined by HPLC analysis (Inertsil ODS column, 4.6 x 250 mm; eluent 5% tetrahydrofuran in methanol; UV 254 nm; flow rate 1.2 mL/min).
13. ^{1}H NMR ($CDCl_3$, 500MHz) δ 5.74 (dd, J=7.2, 4.7 Hz, 1-H), 5.15 (d, J=3.1 Hz, 1"-H), and 4.36 (d, J=7.7 Hz, 1'-H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 74.33 (C-1), 103.23 (C-1'), and 97.17 (C-1").
14. NMR assignments of **2**: ^{1}H (D_2O, 500 MHz) δ 3.02 (dd, 1H, J=17.3, 7.0 Hz, Asn β-Ha), 3.08 (dd, 1H, J=17.3, 4.1 Hz, Asn β-Hb), 3.30 (t, 1H, J=8.5 Hz, 2'-H), 3.43 (t, 1H, J=3.4 Hz, 4"-H), 3.46-3.53 (3H, H-3', 4-H, and 4'-H), 3.55 (dd, 1H, J=9.8, 3.7 Hz, 2"-H), 3.60-3.87 (10H, 2-H, 3-H, 3"-H, 5-H, 5'-H, 5"-H, 6-Ha, 6'-Ha, and 6"-H_2), 3.95 (dd, 1H, J=11.1, 4.6 Hz, 6'-Hb), 4.10 (dd, 1H, J=11.4, 1.7 Hz, 6-Hb), 4.15 (dd, 1H, J=7.0, 4.1 Hz, Asn α-H), 4.50 (d, 1H, J=8.0Hz, 1'-H), 4.95 (d, 1H, J=4.1 Hz, 1"-H), and 5.60 (d, 1H, J=5.5 Hz, 1-H); ^{13}C (D_2O, 125 MHz) δ 37.45 (Asn β-C), 53.23 (Asn α-C), 63.10 (C-6"), 68.13 (C-6'), 71.03 (C-6), 71.81 (C-4'*), 71.96 (C-2), 72.06 (C-4*), 72.11 (C-4"), 74.17 (C-2"), 74.51 (C-5"), 74.54 (C-5), 75.62 (C-3), 75.73 (C-2'), 75.77 (C-3"), 76.97 (C-5'), 78.49 (C-3'), 79.22 (C-1), 100.48 (C-1"), 105.32 (C-1'), 175.23 (CO_2H), and 175.76 (CONH). *Assignments may be reversed.
15. Biological activity of synthetic **2** is currently under evaluation.

(Received in Japan 9 August 1991)