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Syntheses of nephritogenoside and related compounds †

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ABSTRACT

Nephritogenoside has been prepared by coupling of the acyl azide derivative of a *N*-triglycosyl dipeptide, derived from the corresponding hydrazide derivative 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-1-*N*-[*N*-(*tert*-butoxycarbonyl)-L-aspart-1-oyl-(L-proline methyl ester)-4-oyl]-α-D-glucopyranosylamine, with a nonadecapeptide, followed by deprotection of the desired protected nephritogenoside. The *N*-triglycosyl pentapeptide also has been prepared as a model compound.

INTRODUCTION

Shibata et al.[2] isolated and purified, from the glomerular basement membrane of rats, a compound named nephritogenoside that caused 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]. The reducing α-D-glucose unit is *N*-glycosidically linked to the *N*-terminal asparagine unit[4]. Syntheses of model glycopeptides and nephritogenoside itself are important because these compounds may have significant biological properties.

A part of the study has been reported in a preliminary communication on a total synthesis of nephritogenoside[1], in which detailed procedures for the synthesis were not reported. Shiba and co-workers[5] have reported a total synthesis of nephritogenoside using the Aloc group as the final protecting group.

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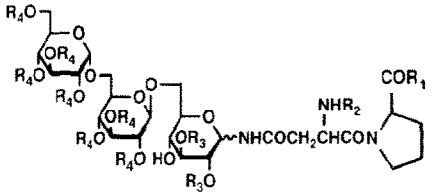
† Part XII. The Nephritogenic Glycopeptide from Rat Glomerular Basement Membrane. For Part XI, see ref 1.

Herein, we describe details of the total synthesis of nephritogenoside and its β anomer and also the synthesis of the triglycosyl pentapeptide as a model compound.

RESULTS AND DISCUSSION

In our previous paper[6], we described the synthesis of an *N*-triglycosyl dipeptide, *O*-α-D-glucopyranosyl-(1 → 6)-O-β-D-glycopyranosyl-(1 → 6)-1-*N*-[L-aspart-1-oyl-(L-proline)-4-oyl]-α-D-glucopyranosylamine. The *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-1-*N*-[*N*-(*tert*-butoxycarbonyl)-L-aspart-1-oyl-(L-proline methyl ester)-4-oyl]-α,β-D-glucopyranosylamine (**1**α and **1**β)[6] was converted into the hydrazide by the procedure of Fujii and Yajima[7]. Isomers **2**α (68.2%) and **2**β (13.8%) were separated by silica gel column chromatography in the ratio 5:1. The presence of a doublet at δ 5.76 (J 5.5 Hz) in the ^{1}H NMR spectrum of **2**α established the α-D-configuration of the reducing-end residue. The β-D-linked anomer (**2**β) showed a signal at δ 5.14 (J 9.0 Hz). The acyl azide (**3**α) derived from the corresponding hydrazide (**2**α) by the Honzl and Rudinger[8]



1α,β R_1 = OMe, R_2 = Boc, R_3 = Bn, R_4 = Ac

2α,β R_1 = $NHNH_2$, R_2 = Boc, R_3 = Bn, R_4 = H

3α,β R_1 = N_3, R_2 = Boc, R_3 = Bn, R_4 = H

4α,β 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

5α,β R_1 = nonadecapeptide, R_2 = H, R_3 = Bn, R_4 = H

6α,β 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

8α,β R_1 = Leu-Phe-Gly-OMe, R_2 = Boc, R_3 = Bn, R_4 = H

9α,β R_1 = Leu-Phe-Gly-OMe, R_2 = H, R_3 = Bn, R_4 = H

10α,β R_1 = Leu-Phe-Gly-OH, R_2 = H, R_3 = Bn, R_4 = H

11α,β R_1 = Leu-Phe-Gly-OH, R_2 = R_3 = R_4 = H

12 Z-Phe-Gly-OMe

13 Z-Leu-Phe-Gly-OMe

14 H-Leu-Phe-Gly-OMe

procedure was allowed to react with the triethylammonium salt of nonadecapeptide **7**, which was obtained by the procedure described below, to give the desired protected nephritogenoside **4α** in 81.2% yield.

The signal for the *tert*-butoxy methyl groups appeared at δ 1.42, and that for the alanine β methyl and the threonine γ methyl groups appeared at δ 1.40 (*J* 7.3 Hz) and 1.72 (*J* 6.2 Hz), respectively. The other ^{1}H NMR and ^{13}C NMR data were in accordance with the proposed structure. Fmoc-Gln(Mbh)-*O*-polymer (purchased from Kokusan Chemical Works, Ltd.) was subjected to the usual procedures of solid-phase peptide synthesis using an Applied Biosystems (ABI) Model 431A Synthesizer, employing Fmoc amino acids purchased from ABI, to give the protected resin corresponding to nonadecapeptide **7**. Reaction of the peptide resin with trifluoroacetic acid, and purification of the product by high-performance liquid chromatography (HPLC), gave **7** in an excellent yield.

Removal of the *tert*-butoxycarbonyl group of **4α** with 90% TFA gave **5α**, followed by removal of the benzyl group with 10% Pd–C, afforded the target compound, nephritogenoside **6α** ($[\alpha]_D$ −31.3°) in 88.8% yield. The configuration of nephritogenoside was confirmed by ^{1}H NMR spectroscopy, with signals for H-1, H-1′, and H-1″ being observed at δ 5.63 (*J* 5.5 Hz), 4.30 (*J* 7.2 Hz), and 4.94 (*J* 3.7 Hz), respectively. HPLC was performed on an ABI HPLC 130A Separation System equipped with an Aquapore RP-300, C_8 (7 μm) column. Retention times were 9.52 min for nephritogenoside and 10.02 min for the nonadecapeptide. The β anomer of the nephritogenoside was also prepared according to the method described for the α anomer. The configuration of the β anomer of nephritogenoside (**6β**) was confirmed by ^{1}H NMR spectroscopy, signals for H-1, H-1′, and H-1″ being observed at δ 5.01 (*J* 9.2 Hz), 4.32 (*J* 7.5 Hz), and 4.93 (*J* 3.7 Hz), respectively.

Next, we synthesized *N*-triglycosyl pentapeptide (**11α**) and its β anomer (**11β**). *O*-α-D-Glucopyranosyl-(1 → 6)-*O*-β-D-glucopyranosyl-(1 → 6)-2,3-di-*O*-benzyl-1-*N*-[*N*-(*tert*-butoxycarbonyl)-L-aspart-1-oyl-(L-proline hydrazide)-4-oyl]-α-D-glucopyranosylamine (**2α**) was transformed into the acyl azide (**3α**), and attachment of acyl azide to tripeptide derivative, L-leucyl-L-phenylalanyl-glycine methyl ester gave the *N*-triglycosyl pentapeptide derivative (**8α**). Removal of the *tert*-butoxycarbonyl group of **8α** with 90% TFA gave **9α**, and removal of the benzyl group of this compound with 10% Pd–C afforded the *N*-triglycosyl pentapeptide, *O*-α-D-glucopyranosyl-(1 → 6)-*O*-β-D-glucopyranosyl-(1 → 6)-*N*-[L-aspart-1-oyl-(L-prolyl-L-leucyl-L-phenylalanyl-glycine-4-oyl]-α-D-glucopyranosylamine (**11α**). The tripeptide, L-leucyl-L-phenylalanyl-glycine methyl ester (**14**), was synthesized by stepwise elongation from the carboxy terminus by way of the diethyl cyanophosphonate (Et_2PC) method[9]. Coupling of benzyloxycarbonyl-L-phenylalanine with glycine methyl ester gave the dipeptide **12**, which was treated with 10% Pd–C to give the dipeptide. This compound was then coupled with benzyloxycarbonyl-L-leucine to form a tripeptide **13**. Treatment of **13** with 10% Pd–C gave **14**.

The β anomer of this *N*-triglycosyl pentapeptide (**11β**) was also prepared according to the method described for the α anomer. The configuration of the α

TABLE I

^{13}C NMR data (δ) for selected compounds

Carbon atom	Compound									
	2α	**8α**	**9α**	**10α**	**11α**	**2β**	**8β**	**9β**	**10β**	**11β**
C-1	78.0	76.0	77.9	78.6	79.5	80.0	80.3	80.4	81.4	82.2
2	79.0	78.6	79.0	79.8	72.2	82.4	82.2	81.9	83.0	74.4 [a]
3	82.8	82.8	82.8	83.5	75.9 [a]	86.9	87.6	86.9	87.3	79.2
4	71.2	71.1	71.3	71.9	72.1 [b]	71.9 [a]	71.5 [a]	71.6 [a]	72.2 [a]	72.2 [b]
5	75.3 [a]	75.3 [a]	75.3 [a]	76.0	76.0	76.4	76.3	76.0	77.2	79.5
6	69.9	70.2	70.2	71.4	71.4	69.9	69.8	70.0	71.0	71.5
1′	104.6	105.1	104.6	105.7	105.6	104.2	104.8	104.7	105.6	105.6
2′	75.1 [a]	75.2 [a]	75.1 [a]	77.1 [a]	75.8 [a]	75.2	75.1 [b]	75.2	74.7	74.6 [a]
3′	76.3 [b]	78.1	76.3 [b]	77.3 [a]	78.7	78.2	78.1	78.2	78.8	78.7
4′	71.5 [c]	71.5	71.6 [c]	72.2 [b]	72.2 [b]	71.7 [a]	71.7 [a]	71.7 [a]	72.3 [a]	72.2 [b]
5′	76.1 [b]	76.4	76.2 [b]	76.0	77.2	78.8	78.7	78.7	78.8	78.7
6′	67.2	67.4	66.9	68.2	68.3	67.4	67.4	67.4	69.7	68.3
1″	99.7	99.8	99.8	100.7	100.7	99.7	99.8	99.8	100.7	100.7
2″	73.6 [d]	73.7 [b]	73.8	74.4	74.4	73.7 [b]	73.7 [c]	73.7 [b]	75.9	74.7
3″	73.8	74.0	74.4	74.7	74.9	75.2	75.2 [b]	75.2	76.0	76.0 [c]
4″	71.7 [c]	71.8	71.7 [c]	72.3 [b]	72.3 [b]	71.7 [a]	71.6 [a]	71.6 [a]	72.2 [a]	72.3 [b]
5″	73.7 [d]	73.8 [b]	75.1 [a]	74.4	74.7	73.5 [b]	73.6 [c]	73.5 [b]	74.4	75.9 [c]
6″	62.6	62.7	62.6	63.3	63.3	62.6	62.5	62.5	63.3	63.3
Asp-α	50.4	51.0	– [e]	51.6	51.6	50.1	50.5	50.0	51.8	51.7
β	38.4	40.0	36.1	37.1	39.6	39.5	40.0	36.6	36.2	38.2
Pro-α	60.8	63.0	61.9	63.7	63.4	61.2	62.9	62.3	64.0	63.5
β	30.8	30.8	30.8	32.3	32.3	30.6	30.7	30.7	32.2	32.3
γ	25.6	26.0	25.9	27.3	27.4	25.4	26.2	25.9	27.4	27.4
δ	– [e]	– [e]	– [e]	49.3	50.7	– [e]	– [e]	– [e]	50.8	50.8
Leu-α		54.5	53.9	55.8	55.7		54.3	53.8	55.8	55.7
β		40.7	41.5	42.3	42.4		40.4	41.3	42.1	42.4
γ		26.0	26.0	27.2	27.4		26.2	26.0	27.2	27.2
δ		21.6	22.1	23.6	23.6		21.4	21.8	23.4	23.6
δ		23.7	23.5	25.1	24.8		24.0	23.7	25.3	25.0
Phe-α		55.9	55.5	57.0	57.4		55.9	55.6	57.2	57.4
β		38.6	39.0	39.2	39.9		38.7	39.0	40.0	39.9
Gly		42.0	41.9	46.3	46.2		42.0	42.0	46.3	46.2
–OMe		52.7	52.7				52.8	52.7		
Boc-Me	28.7	28.8				28.7	28.8			

[a,b,c,d] These values in each column may be interchanged. [e] These values were concealed by solvent peaks.

anomer **11α** was confirmed by ^1H NMR spectroscopy, the signals for H-1, H-1′, and H-1″ being observed at δ 5.63 (J 5.5 Hz), 4.52 (J 8.1 Hz), and 4.96 (J 3.5 Hz), respectively, whereas, in the case of β anomer **11β**, the H-1, H-1′, and H-1″ signals were observed at δ 5.01 (J 9.0 Hz), 4.51 (J 7.9 Hz), and 4.92 (J 3.7 Hz), respectively. The ^{13}C NMR data were in accordance with the proposed structure (see Table I).

Nephritogenic activity tests of nephritogenoside and related compounds are now being carried out and will be reported elsewhere.

EXPERIMENTAL

General methods.—Melting points were measured with a Yanagimoto micro melting-point apparatus and are uncorrected. Optical rotations were determined with a Jasco DIP-140 digital polarimeter. ^{1}H NMR and ^{13}C NMR spectra were recorded with Jeol EX-270 and GSX-400 spectrometers, Me_4Si was the internal standard for solutions in $CDCl_3$ and CD_3OD, and sodium 4,4-dimethyl-4-silapentane-1-sulfonate for solutions in D_2O. Plasma-desorption mass spectroscopy (PDMS) was carried out with a Bio-Ion 20 mass spectrometer (ABI). TLC was performed on Silica Gel-60 (E. Merck), and the compounds were detected by the quenching of UV fluorescence and by spraying with either 10% H_2SO_4 or 5% methanolic ninhydrin solution. Column chromatography was carried out on Silica Gel-60 (E. Merck).

Preparation 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-1*-N-*[*N-*(*tert*-*butoxycarbonyl)*-L-*aspart-1-oyl-(*L-*proline methyl ester)-4-oyl]-α,β*-D-*glucopyranosylamine* (**1*α*** *and* **1*β***).—This compound was obtained by the procedure described in a previous paper[6].

O-*α*-D-*Glucopyranosyl-(1 → 6)*-O-*β*-D-*glucopyranosyl-(1 → 6)-2,3-di*-O-*benzyl-1*-N-*[*N-*(*tert*-*butoxycarbonyl)*-L-*aspart-1-oyl-(*L-*proline hydrazide)-4-oyl]-α,β*-D-*glucopyranosylamine* (**2*α*** *and* **2*β***).—A solution consisting of **1*α*** and **1*β*** (100 mg, 0.08 mmol) in MeOH (6 mL) and aq 80% hydrazine hydrate (0.3 mL) was stirred at room temperature for 24 h and concentrated to a syrup that was chromatographed on a column of silica gel. The first eluate was evaporated to dryness to give **2*α*** (52.8 mg, 68.2%), and the latter eluate gave **2*β*** (10.7 mg, 13.8%). Physicochemical data for **2*α***: mp 150–152°C; $[\alpha]_D^{16}$ + 37° (*c* 0.7, MeOH); TLC, (65 : 35 : 10 $CHCl_3$–MeOH–H_2O, lower layer) R_f 0.39; ^{1}H NMR (CD_3OD, 50°C): δ 7.38–7.23 (m, 10 H, Ar), 5.76 (d, 1 H, *J* 5.5 Hz, H-1), 4.85 (d, 1 H, *J* 3.7 Hz, H-1″), 4.73–4.64 (m, 1 H, Asp*α*), 4.47 (dd, 1 H, *J* 3.1, 11.0 Hz, Pro*α*), 4.38 (d, 1 H, *J* 7.5 Hz, H-1′), 2.80–2.76 (m, 2 H, Asp*β*), 2.21–2.02 (m, 4 H, Pro*β*,*γ*), and 1.44 (s, 9 H, Boc-Me). Anal. Calcd for $C_{46}H_{67}N_5O_{20} \cdot 3H_2O$: C, 51.92; H, 6.35; N, 6.58. Found: C, 51.89; H, 6.61; N, 6.32.

Physicochemical data for **2*β***: mp 145–148°C; $[\alpha]_D^{22}$ + 15° (*c* 0.8, MeOH); TLC, 65 : 35 : 10 $CHCl_3$–MeOH–H_2O, lower layer) R_f 0.31; ^{1}H NMR (CD_3OD, 50°C): δ 7.38–7.22 (m, 10 H, Ar), 5.14 (d, 1 H, *J* 9.0 Hz, H-1), 4.86 (d, 1 H, *J* 3.5 Hz, H-1″), 4.66–4.61 (m, 1 H, Asp*α*), 4.45 (dd, 1 H, *J* 3.6, 8.3 Hz, Pro*α*), 4.36 (d, 1 H, *J* 7.9 Hz, H-1′), 2.81 (dd, 1 H, *J* 9.7, 15.4 Hz, Asp*β*-Ha), 2.51 (dd, 1 H, *J* 5.0, 15.4 Hz, Asp*β*-Hb), 2.11–1.92 (m, 4 H, Pro*β*,*γ*), and 1.42 (s, 9 H, Boc-Me). Anal. Calcd for $C_{46}H_{67}N_5O_{20} \cdot 3H_2O$: C, 51.92; H, 6.35; N, 6.58. Found: C, 51.55; H, 6.34; N, 6.23.

O-*α*-D-*Glucopyranosyl-(1 → 6)*-O-*β*-D-*glucopyranosyl-(1 → 6)-2,3-di*-O-*benzyl-1*-N-*[*N-*(*tert*-*butoxycarbonyl)*-L-*aspart-1-oyl-(*L-*prolyl*-L-*leucyl*-L-*phenylalanyl-glycyl*-L-*isoleucyl*-L-*alanyl-glycyl*-L-*glutamyl*-L-*aspartyl-glycyl*-L-*prolyl*-L-*threonyl-glycyl*-L-*prolyl*-L-*seryl-glycyl*-L-*isoleucyl*-L-*valyl-glycyl*-L-*glutamine)-4-oyl]-α*-D-*glucopyranosyl-*

amine (**4α**).—To a solution of the hydrazide **2α** (25.0 mg) in DMF (0.3 mL), 6.9 N HCl in DMF (35 μL) and isoamyl nitrite (70 μL) were added. The solution was cooled for 20 min to −78°C. When the hydrazine test became negative, the solution, after neutralization with Et_3N (30 μL), was combined with a solution of the nonadecapeptide **7** (21.6 mg) in DMF (0.4 mL) containing Et_3N (30 μL), and the mixture was stirred for 48 h at 4°C and concentrated. The residue was chromatographed on silica gel with 65:35:10 $CHCl_3$–MeOH–H_2O (lower layer). The eluate was evaporated to dryness to give **4α** (27.2 mg, 81.2%): mp 208–210°C, $[\alpha]_D^{27}$ −44° (*c* 0.8, H_2O); TLC (5:4:1 $CHCl_3$–MeOH–H_2O R_f 0.41; ^1H NMR (D_2O): δ 7.39–7.19 (m, 15 H, Ar), 5.69 (d, 1 H, *J* 4.8 Hz, H-1), 4.94 (d, 1H, *J* 3.5 Hz, H-1″), 1.42 (s, 9 H, Boc-Me), 1.40 (d, 3 H, *J* 7.3 Hz, Alaβ), 1.72 (d, 3 H, *J* 6.2 Hz, Thrγ), 0.98 (d, 3 H, *J* 6.4 Hz, Valγ), 0.96 (d, 3 H, *J* 6.6 Hz, Valγ), and 0.94–0.83 (18 H, 2 × Leuδ, 2 × Ileβ-Me, 2 × Ileδ).

β Anomer (**4β**).—This compound was prepared as described for **4α**; yield 27.0 mg (72.6%): mp 216–219°C; $[\alpha]_D^{27}$ −38° (*c* 0.9, H_2O); TLC (5:4:1 $CHCl_3$–MeOH–H_2O) R_f 0.41; ^1H NMR (D_2O): δ 7.37–7.20 (m, 15 H, Ar), 5.03 (d, 1 H, *J* 7.9 Hz, H-1), 4.89 (d, 1 H, *J* 3.1 Hz, H-1″), 1.40 (d, 3 H, *J* 7.2 Hz, Alaβ), 1.35 (s, 9 H, Boc-Me), 1.23 (d, 3 H, *J* 6.4 Hz, Thrγ), 0.96 (d, 3 H, *J* 6.6 Hz, Valγ), 0.95 (d, 3 H, *J* 6.8 Hz, Valγ), and 0.91–0.83 (18 H, 2 × Leuδ, 2 × Ileβ-Me, 2 × Ileδ).

O-α-D-Glucopyranosyl-(1 → 6)-O-β-D-glucpyranosyl-(1 → 6)-2,3-di-O-benzyl-N-[L-aspart-1-oyl-(L-prolyl-L-leucyl-L-phenylalanyl-glycyl-L-isoleucyl-L-analyl-glycyl-L-glutamyl-L-aspartyl-glycyl-L-prolyl-L-threonyl-glycyl-L-prolyl-L-seryl-glycyl-L-isoleucyl-L-valyl-glycyl-L-glutamine)-4-oyl]-α-D-glucopyranosylamine (**5α**).—The *tert*-butoxycarbonyl group in **4α** (12 mg) was cleaved with 90% CF_3CO_2H (1 mL) at room temperature for 1 h to give **5α** (10.8 mg, 93.4%): mp 175–178°C; $[\alpha]_D^{9}$ −56° (*c* 0.2, H_2O); TLC (5:4:1, $CHCl_3$–MeOH–H_2O) R_f 0.36; ^1H NMR (D_2O): δ 7.38–7.18 (m, 15 H, Ar), 5.75 (d, 1 H, *J* 5.6 Hz, H-1), 4.94 (d, 1 H, *J* 3.5 Hz, H-1″), 4.26 (d, 1 H, *J* 7.3 Hz, H-1′), 1.40 (d, 3 H, *J* 7.3 Hz, Alaβ), 1.22 (d, 3 H, *J* 6.2 Hz, Thrγ), 0.96 (d, 3 H, *J* 8.6 Hz, Valγ), 0.94 (d, 3 H, *J* 7.0 Hz, Valγ), and 0.92–0.82 (18 H, 2 × Leuδ, 2 × Ileβ-Me, 2 × Ileδ).

β Anomer (**5β**).—This compound was prepared as described for **5α**; yield 13.6 mg (91.4%): mp 167–170°C; $[\alpha]_D^{24}$ −44° (*c* 0.7, H_2O); TLC (5:4:1 $CHCl_3$–MeOH–H_2O) R_f 0.36; ^1H NMR (D_2O): δ 7.42–7.20 (m, 15 H, Ar), 5.07 (d, 1 H, *J* 9.2 Hz, H-1), 4.91 (d, 1 H, *J* 3.7 Hz, H-1″), 1.40 (d, 3 H, *J* 6.6 Hz, Alaβ), 1.22 (d, 3 H, *J* 6.2 Hz, Thrγ), 0.96 (d, 3 H, *J* 8.4 Hz, Valγ), 0.94 (d, 3 H, *J* 7.2 Hz, Valγ), and 0.92–0.82 (18 H, 2 × Leuδ, 2 × Ileβ-Me, 2 × Ileδ).

O-α-D-Glucopyranosyl-(1 → 6)-O-β-D-glucopyranosyl-(1 → 6)-N-[L-aspart-1-oyl-(L-prolyl-L-leucyl-L-phenylalanyl-glycyl-L-isoleucyl-L-alanyl-glycyl-L-glutamyl-L-aspartyl-glycyl-L-prolyl-L-threonyl-glycyl-L-prolyl-L-seryl-glycyl-L-isoleucyl-L-valyl-glycyl-L-glutamine)-4-oyl]-α-D-glucopyranosylamine (nephritogenoside (**6α**).—To a solution of **5α** (7.9 mg) in 1:1 EtOH–H_2O (2 mL) was added 10% Pd–C (20 mg). The suspension was stirred for 24 h under H_2 and then filtered and concentrated to dryness. The residue was chromatographed on Sephadex G-10, and the water

eluate was lyophilized to give **6α** (7.0 mg, 95.1%): mp 158–160°C; $[\alpha]_D^{22}$ −31° (*c* 0.4, H_2O); TLC (1:1:1:1 BuOH–AcOH–EtOAc–H_2O) R_f 0.15; ^1H NMR (D_2O): δ 7.37–7.24 (m, 5 H, Ar), 5.63 (d, 1 H, *J* 5.5 Hz, H-1), 4.94 (d, 1 H, *J* 3.7 Hz, H-1″), 4.30 (d, 1 H, *J* 7.2 Hz, H-1′), 1.40 (d, 3 H, *J* 7.3 Hz, Alaβ), 1.22 (d, 3 H, *J* 6.2 Hz, Thrγ), 0.96 (d, 3 H, *J* 7.0 Hz, Valγ), 0.95 (d, 3 H, *J* 8.2 Hz, Valγ), and 0.92–0.84 (18 H, 2 × Leuδ, 2 × Ileβ-Me, 2 × Ileδ); ^{13}C NMR (D_2O): δ 79.6 (C-1), 105.7 (C-1′), and 100.8 (C-1″). Amino acid ratios in a 6 N HCl hydrolysate: Asp 1.83, Glu 2.02, Ser 0.90, Gly 6.00, Thr 0.92, Ala 1.01, Pro 2.93, Val 0.63, Ile 1.67, Leu 0.97, Phe 1.02. Plasma-desorption mass spectrometry (PDMS): Calcd mol wt, M + H: 2470.5 found 2472.3.

β Anomer (**6β**).—This compound was prepared as described for **6α**, yield 15.8 mg (97.5%): mp 167–170°C; $[\alpha]_D^{19}$ −43° (*c* 0.8, H_2O); TLC (1:1:1:1 BuOH–AcOH–EtOAc–H_2O) R_f 0.15; ^1H NMR (D_2O): δ 7.37–7.24 (m, 5 H, Ar), 5.01 (d, 1 H, *J* 9.2 Hz, H-1), 4.93 (d, 1 H, *J* 3.7 Hz, H-1″), 4.32 (d, 1 H, *J* 7.5 Hz, H-1′), 1.41 (d, 3 H, *J* 7.0 Hz, Alaβ), 1.22 (d, 3 H, *J* 6.2 Hz, Thrγ), 0.97 (d, 3 H, *J* 7.1 Hz, Valγ), 0.95 (d, 3 H, *J* 7.5 Hz, Valγ), and 0.93–0.85 (18 H, 2 × Leuδ, 2 × Ileβ-Me, 2 × Ileδ); ^{13}C NMR (D_2O): δ 82.3 (C-1), 105.7 (C-1′), and 100.8 (C-1″). Amino acid ratios in a 6 N HCl hydrolysate: Asp 1.90, Glu 2.01, Ser 0.92, Gly 6.00, Thr 0.90, Ala 1.00, Pro. 2.98, Val 0.62, Ile 0.97, Phe 1.01.

L-Leucyl-L-phenylalanyl-glycyl-L-isoleucyl-L-alanyl-glycyl-L-glutamyl-L-aspartyl-glycyl-L-prolyl-L-threonyl-glycyl-L-prolyl-L-seryl-glycyl-L-isoleucyl-L-valyl-glycyl-L-glutamine (**7**).—Using an Applied Biosystems (ABI) Model 431A peptide synthesizer employing Fmoc amino acids, Fmoc-Gln(Mbh)-*O*-polymer was subjected to the usual procedures of solid phase peptide synthesis to give the protected resin corresponding to the nonadecapeptide. Reaction of the peptide resin with CF_3CO_2H, and purification of the product by HPLC [ABI 150A separation system; Aquapore Prep-10, C_8 (20 μm) column (10 mm i.d. × 250 mm); solvent, MeCN–H_2O containing 0.1% CF_3CO_2H (0–100% gradient); flow rate, 2 mL/min; detection, 220 nm] gave **7**. The sequence was comfirmed by an Applied Biosystems (ABI) Model 477A protein sequencer. Physicochemical data: $[\alpha]_D^{22}$ −80° (*c* 0.62, H_2O); ^1H NMR (D_2O) δ: 7.39–7.26 (m, 5 H, Ar), 1.39 (d, 3 H, *J* 7.3 Hz, Alaβ), 1.21 (d, 3 H, *J* 6.4 Hz, Thrγ), 0.96 (d, 3 H, *J* 6.8 Hz, Valγ), 0.94 (d, 3 H, *J* 6.8 Hz, Valγ), and 0.92–0.82 (18 H, 2 × Leuδ, 2 × Ileβ-Me, 2 × Ileδ); HPLC [ABI 130A separation system; column, Aquapore RP-300, C_8 (7 μm); solvent, MeCN–H_2O containing 0.1% CF_3CO_2H (0–30%, 5 min; 30–40%, 25 min.); flow rate, 200 μL/min; detection, 220 nm] retention time for **6α**, 9.52 min; for natural nephritogenoside, 9.52 min; for **7**, 10.02 min. Amino acid ratios in a 6 N HCl hydrolysate: Asp 0.98, Glu 2.01, Ser 0.94, Gly 6.00, Thr 0.95, Ala 1.00, Pro 1.96, Val 0.70, Ile 1.78, Leu 0.99, Phe 1.00.

O-α-D-Glucopyranosyl-(1 → 6)-O-β-D-glucopyranosyl-(1 → 6)-2,3-di-O-benzyl-1-N-[N-(tert-butoxycarbonyl)-L-aspart-1-oyl-[L-prolyl-L-leucyl-L-phenylalanyl-(glycine methyl ester)-4-oyl]-α-D-glucopyranosylamine (**8α**).—To a solution of the hydrazide **2α** (23.0 mg) in DMF (0.3 mL) were added 6.9 N HCl in DMF (40 μL) and

isoamyl nitrite (70 μL). The solution was cooled to −20°C for 20 min. When the hydrazine test became negative, the solution was neutralized with Et_3N (40 μL) and combined with a solution of **14** (20 mL) in DMF (0.3 mL) containing Et_3N (30 μL). The mixture was stirred for 48 h at 4°C, diluted with $CHCl_3$, and washed with water. Drying, followed by evaporation, gave a syrup that was chromatographed on silica gel with 4:1 $CHCl_3$–MeOH. The eluate was evaporated to dryness to give **8α** (21.2 mg, 70.2%): mp 112–115°C; $[\alpha]_D^{22}$ +5° (*c* 0.7, MeOH); TLC (65:35:10 $CHCl_3$–MeOH–H_2O lower layer) R_f 0.60; ^1H NMR (CD_3OD): δ 7.39–7.15 (m, 15 H, Ar), 5.75 (d, 1 H, *J* 5.4 Hz, H-1), 4.84 (d, 1 H, *J* 3.8 Hz, H-1″), 4.70–4.63 (m, 2 H, Pheα, Aspα), 4.53–4.42 (m, 1 H, Proα), 4.35 (d, 1 H, *J* 7.8 Hz, H-1′), 4.22 (dd, 1 H, *J* 4.7, 10.5 Hz, Leuα), 3.70 (s, 3 H, OMe), 3.27–3.19 (m, 1 H, Pheβ-Ha), 2.95 (dd, 1 H, *J* 9.3, 14.1 Hz, Pheβ-Hb), 2.78–2.74 (m, 2 H, Aspβ), 1.45 (s, 9 H, Boc-Me), 0.89 (d, 3 H, *J* 6.6 Hz, Leuδ), 0.83 (d, 3 H, *J* 6.6 Hz, Leuδ). Anal. Calcd for $C_{64}H_{90}N_6O_{24} \cdot 4H_2O$: C, 54.93; H, 7.06; N, 6.01. Found: C, 55.34; H, 7.26; N, 5.55.

β Anomer (**8β**).—This compound was prepared as described for **8α**; yield 42.5 mg (60.4%): mp 148–151°C; $[\alpha]_D^{25}$ +10.5° (*c* 0.6, MeOH); TLC (65:35:10 $CHCl_3$–MeOH–H_2O, lower layer) R_f 0.60; ^1H NMR (CD_3OD): δ 7.37–7.18 (m, 15 H, Ar), 5.04 (d, 1 H, *J* 9.1 Hz, H-1), 4.87 (d, 1 H, *J* 3.6 Hz, H-1″), 4.65 (dd, 1 H, *J* 6.1, 9.0, Pheα), 4.42–4.38 (m, 1 H, Proα), 4.35 (d, 1 H, *J* 7.9 Hz, H-1′), 4.21 (dd, 1 H, *J* 4.1, 11.1 Hz, Leuα), 3.70 (s, 3 H, OMe), 3.27–3.22 (m, 1 H, Pheβ-Ha), 2.97 (dd, 1 H, *J* 9.0, 14.0 Hz, Pheβ-Hb), 2.81–2.75 (m, 1 H, Aspβ-Ha), 2.56–2.52 (m, 1 H, Aspβ-Hb), 1.44 (s, 9 H, Boc-Me), 0.96 (d, 3 H, *J* 6.4 Hz, Leuδ), and 0.85 (d, 3 H, *J* 6.5 Hz, Leuδ). Anal. Calcd for $C_{64}H_{90}N_6O_{24} \cdot 4H_2O$: C, 54.93; H, 7.06; N, 6.01. Found: C, 55.38; H, 7.20; N, 5.48.

O-α-D-Glucypyranosyl-(1 → 6)-O-β-D-glucopyranosyl-(1 → 6)-2,3-di-O-benzyl-N-[L-aspart-1-oyl-{L-prolyl-L-leucyl-L-phenylalanyl-(glycine methyl ester)}-4-oyl]-α-D-glucopyranosylamine (**9α**).—The *tert*-butoxycarbonyl group of **8α** (18.6 mg) was cleaved with 90% CF_3CO_2H (2 mL) for 1 h at room temperature to give **9α** (16.2 mg, 94.2%): mp 150–153°C; $[\alpha]_D^{22}$ +12° (*c* 0.8, MeOH); TLC (65:35:10 $CHCl_3$–MeOH–H_2O, lower layer) R_f 0.32; ^1H NMR (CD_3OD, 50°C): δ 7.39–7.15 (m, 15 H, Ar), 5.75 (d, 1 H, *J* 5.1 Hz, H-1), 4.86 (d, 1 H, *J* 3.5 Hz, H-1″), 4.49 (dd, 1 H, *J* 4.9, 8.3 Hz, Proα), 4.44 (d, 1 H, *J* 7.5 Hz, H-1′), 4.28 (dd, 1 H, *J* 5.7, 9.5 Hz, Leuα), 3.19–3.15 (m, 2 H, Pheβ), 3.69 (s, 3 H, OMe), 3.03–2.87 (m, 2 H, Aspβ), 0.92 (d, 3 H, *J* 6.6 Hz, Leuδ), and 0.87 (d, 3 H, *J* 6.4 Hz, Leuδ).

β Anomer (**9β**).—This compound was prepared as described for **9α**; yield 24.5 mg (93.8%): mp 154–157°C; $[\alpha]_D^{27}$ +11° (*c* 1.2, MeOH); TLC (65:35:10 $CHCl_3$–MeOH–H_2O, lower layer) R_f 0.32; ^1H NMR (CD_3OD): δ 7.37–7.19 (m, 15 H, Ar), 5.07 (d, 1 H, *J* 9.2 Hz, H-1), 4.46–4.40 (m, 1 H, Aspα, Proα), 4.36 (d, 1 H, *J* 7.9 Hz, H-1′), 4.27 (dd, 1 H, *J* 5.0, 10.3 Hz, Leuα), 3.70 (s, 3 H, OMe), 0.94 (s, 3 H, *J* 6.6 Hz, Leuδ), and 0.86 (d, 3 H, *J* 6.4 Hz, Leuδ).

O-α-D-Glucopyranosyl-(1 → 6)-O-β-D-glucopyranosyl-(1 → 6)-2,3-di-O-benzyl-N-[L-aspart-1-oyl-(L-prolyl-L-leucyl-L-phenylalanyl-glycine)-4-oyl]-α-D-glucopyranosyl-

amine (**10α**).—To a solution of **9α** (15.0 mg) in MeOH (4 mL) and water (0.2 mL) was added NaOMe (10 mg), and the mixture was stirred for 5 h at room temperature. After neutralization with Amberlite IR-120B (H^+), the resin was filtered off, and the filtrate was concentrated to give a syrup which was chromatographed on a column of silica gel to give **10α** (11.1 mg, 80.1%): mp 170–172°C; $[\alpha]_D^{17}$ +9.5° (*c* 0.5, H_2O); TLC (5:4:1 $CHCl_3$–MeOH–H_2O) R_f 0.43; ^1H NMR (D_2O, 60°C): δ 7.40–7.12 (m, 15 H, Ar), 5.73 (d, 1 H, *J* 5.1 Hz, H-1), 4.95 (d, 1 H, *J* 3.7 Hz, H-1″), 3.22 (dd, 1 H, *J* 5.3, 14.2 Hz, Pheβ-Ha), 2.97 (dd, 1 H, *J* 9.3, 14.2 Hz, Pheβ-Hb), 2.79–2.59 (m, 2 H, Aspβ), 2.30–2.23 (m, 1 H, Proβ-Ha), 2.03–1.80 (m, 3 H, Proβ-Hb, Proγ), 1.55–1.32 (m, 2 H, Leuβ-Ha, Leuγ), 0.91–0.86 (m, 1 H, Leuβ-Hb), 0.85 (d, 3 H, *J* 6.2 Hz, Leuδ), and 0.79 (d, 3 H, *J* 6.4 Hz, Leuδ).

β Anomer (**10β**).—This compound was prepared as described for **10α**; yield 14.4 mg (82.3%): mp 173–175°C; $[\alpha]_D^{16}$ +8.5° (*c* 0.7, H_2O); TLC (5:4:1 $CHCl_3$–MeOH–H_2O) R_f 0.43; ^1H NMR (D_2O, 60°C): δ 7.40–7.16 (m, 15 H, Ar), 5.09 (d, 1 H, *J* 8.8 Hz, H-1), 4.97 (d, 1 H, *J* 3.7 Hz, H-1″), 3.29–3.19 (m, 2 H, Pheβ), 3.04–2.91 (m, 2 H, Aspβ), 2.29–2.25 (m, 1 H, Proβ-Ha), 1.92–1.74 (m, 3 H, Proβ-Hb, Proγ), 1.54–1.38 (m, 3 H, Leuβ-Ha, Leuγ), 0.93–0.85 (m, 1 H, Leuβ-Hb), 0.85 (d, 3 H, *J* 5.9 Hz, Leuδ), and 0.78 (d, 3 H, *J* 6.1 Hz, Leuδ).

O-α-D-*Glucopyranosyl-(1 → 6)*-O-β-D-*glucopyranosyl-(1 → 6)*-N-[L-*aspart-1-oyl-(*L-*prolyl-*L-*leucyl-*L-*phenylalanyl-glycine)-4-oyl]*-α-D-*glucopyranosylamine* (**11α**).—To a solution of **10α** (9.3 mg) in 1:1 EtOH–H_2O (2 mL) was added 10% Pd–C (20 mg). The suspension was stirred for 24 h under H_2, and then filtered and concentrated to dryness. The residure was chromatographed on Sephadex G-10. The water eluate was lyophilized to give a white powder (7.5 mg, 95.2%): mp 175–180°C; $[\alpha]_D^{20}$ + 4° (*c* 0.3, H_2O); TLC (5:4:1 $CHCl_3$–MeOH–H_2O) R_f 0.17; ^1H NMR (D_2O): δ 7.39–7.26 (m, 5 H, Ar), 5.63 (d, 1 H, *J* 5.5 Hz, H-1), 4.96 (d, 1 H, *J* 3.5 Hz, H-1″), 4.52 (d, 1 H, *J* 8.1 Hz, H-1′), 4.47 (m, 2 H, Aspα, Proα), 3.25 (dd, 1 H, *J* 5.5, 14.0 Hz, Pheβ-Ha), 3.03 (dd, 1 H, *J* 9.3, 14.0 Hz, Pheβ-Hb), 2.99 (dd, 1 H, *J* 5.7, 16.7 Hz, Aspβ-Ha), 2.81 (dd, 1 H, *J* 8.4, 16.7 Hz, Aspβ-Hb), 2.33–2.25 (m, 1 H, Proβ-Ha), 2.03–1.99 (m, 2 H, Proγ), 1.90–1.84 (m, 1 H, Proβ-Hb), 1.57–1.38 (m, 3 H, Leuβ, Leuγ), 0.92 (d, 3 H, *J* 6.1 Hz, Leuδ), and 0.84 (d, 3 H, *J* 6.0 Hz, Leuδ). Amino acid ratios in a 6 N HCl hydrolysate: Asp 0.96, Gly 1.00, Pro 0.96, Leu 0.96, Phe 0.96.

β Anomer (**11β**).—This compound was prepared as described for **11α**; yield 8.3 mg (94.3%): mp 183–185°C; $[\alpha]_D^{20}$ −12.5° (*c* 0.4, H_2O); TLC (5:4:1 $CHCl_3$–MeOH–H_2O) R_f 0.17; ^1H NMR (D_2O): δ 7.40–7.26 (m, 5 H, Ar), 5.01 (d, 1 H, *J* 9.0 Hz, H-1) 4.92 (d, 1 H, *J* 3.7 Hz, H-1″), 4.51 (d, 1 H, *J* 7.9 Hz, H-1′), 4.45 (dd, 1 H, *J* 5.8, 8.3 Hz, Proα), 4.38 (dd, 1 H, *J* 5.5, 7.8 Hz, Aspα), 3.29–3.23 (m, 1 H, Pheβ-Ha), 3.02 (dd, 1 H, *J* 5.5, 16.0 Hz, Pheβ-Hb), 2.87 (dd. 1 H, *J* 5.5, 16.0 Hz, Aspβ-Ha), 2.71 (dd, 1 H, *J* 8.2, 16.0 Hz, Aspβ-Hb), 2.32–2.23 (m, 1 H, Proβ-Ha), 2.08–1.99 (m, 2 H, Proγ), 1.90–1.82 (m, 1 H, Proβ-Hb), 1.61–1.50 (m, 2 H, Leuβ-Ha, Leuγ), 1.43–1.40 (m, 1 H, Leuβ-Ha), 0.91 (d, 3 H, *J* 6.0 Hz, Leuδ) and 0.84 (d, 3 H, *J* 6.2 Hz, Leuδ).

Benzyloxycarbonyl-L-phenylalanyl-glycine methyl ester (**12**).—To a solution of glycine methyl ester hydrochloride (1.7 g), benzyloxycarbonyl-L-phenylalanine (4.0 g), and Et_3N (2.6 mL) in 9:1 CH_2Cl_2–oxolane (30 mL) was added diethyl cyanophosphonate (2.6 mL) at −20°C, and after 24 h at room temperature, the mixture was extracted with $CHCl_3$. This solution was washed successively with water, 10% citric acid, satd aq $NaHCO_3$, and water, and dried (Na_2SO_4). After evaporation of the soluvent in vacuo, the residue was crystallized from MeOH to give **12** (4.4 g, 89.5%): mp 122–125°C; $[\alpha]_D^{24}$ +28° (*c* 0.2, $CHCl_3$); TLC (10:1 $CHCl_3$; TLC (10:1 $CHCl_3$–MeOH) R_f 0.60; ^1H NMR ($CDCl_3$): δ 7.37–7.12 (m, 10 H, Ar), 5.05 (d, 2 H, *J* 2.6 Hz, Z-CH_2), 4.55–4.46 (m, 1 H, Pheα), 4.01 (dd, 1 H, *J* 5.6, 18.1 Hz, Gly-Ha), 3.89 (dd, 1 H, *J* 5.3, 18.1 Hz, Gly-Hb), 3.71 (s, 3 H, OMe), and 3.16–3.05 (m, 2 H, Pheβ). Anal. Calcd for $C_{20}H_{22}N_2O_5$: C, 64.85; H, 5.99; N, 7.56. Found: C, 64.85; H, 5.68; N, 7.29.

Benzyloxycarbonyl-L-leucyl-L-phenylalanyl-glycine methyl ester (**13**).—To a solution of **12** (100 mg) in oxolane (1 mL) was added 10% Pd–C (10 mg). The suspension was stirred for 24 h under H_2 at room temperature. After filtration of the catalyst oxolane (5 mL) and Et_3N (0.11 mL) were added to the filtrate. To this solution were added benzyloxycarbonyl-L-leucine · 0.5 piperazine (100 mg) in DMF (0.6 mL), and diethyl cyanophosphonate (0.09 mL) in oxolane (0.5 mL). The mixture was stirred for 24 h at room temperature, diluted with CH_2Cl_2, and washed with water, 10% critic acid solution, satd $NaHCO_3$, and water, and dried (Na_2SO_4). After evaporation of the solvent in vacuo, the residue was chromatographed on silica gel with 10:1 $CHCl_3$–MeOH as the eluent to give **13** (70 mg, 53.6%): mp 94–97°C; $[\alpha]_D^{25}$ −43° (*c* 0.3, $CHCl_3$); TLC (10:1 $CHCl_3$–MeOH) R_f 0.57; ^1H NMR ($CDCl_3$): δ 7.38–7.17 (m, 10 H, Ar), 5.08 (d, 2 H, *J* 12.0 Hz, Z-CH_2), 4.78–4.72 (m, 1 H, Pheα), 4.16–4.08 (m, 1 H, Leuα), 4.03 (dd, 1 H, *J* 5.4, 17.7 Hz, Gly-Ha), 3.87 (dd, 1 H, *J* 4.6, 17.7 Hz, Gly-Hb), 3.71 (s, 3 H, OMe), 3.16 (dd, 1 H, *J* 6.6, 13.7 Hz, Pheβ-Ha), 3.04 (dd, 1 H, *J* 7.1, 13.7 Hz, Pheβ-Hb), 1.62–1.49 (m, 2 H, Leuβ), 1.41–1.25 (m, 1 H, Leuγ), 0.90 (d, 3 H, *J* 6.6 Hz, Leuδ), and 0.86 (d, 3 H, *J* 6.3 Hz, Leuδ). Anal. Calcd for $C_{26}H_{33}N_3O_6$: C, 64.58; H, 6.88; N, 8.69. Found: C, 64.60; H, 6.81; N, 8.53.

L-Leucyl-L-phenylalanyl-glycine methyl ester (**14**).—A solution of **13** (255 mg) in MeOH (3 mL) was hydrogenolyzed in the presence of 10% Pd–C (12 mg) for 24 h at room temperature, and then filtered and concentrated to dryness. The residue was chromatographed on silica gel with 1:1 benzene–acetone as the eluent to give **14** (130 mg, 70.6%): mp 91–96°C; $[\alpha]_D^{25}$ −35° (*c* 0.3, $CHCl_3$); TLC (1:1 benzene–acetone) R_f 0.46; ^1H NMR ($CDCl_3$): δ 7.29–7.19 (m, 5 H, Ar), 4.78–4.72 (m, 1 H, Pheα), 3.99 (dd, 1 H, *J* 5.7, 18.1 Hz, Gly-Ha), 3.91 (dd, 1 H, *J* 5.5, 18.1 Hz, Gly-Hb), 3.71 (s, 3 H, OMe), 3.35 (dd, 1 H, *J* 4.4, 9.7 Hz, Leuα), 3.20 (dd, 1 H, *J* 6.4, 13.9 Hz, Pheβ-Ha), 3.01 (dd, 1 H, *J* 8.2, 13.9 Hz, Pheβ-Hb), 1.68–1.58 (m, 1 H, Leuβ-Ha), 1.50–1.43 (m, 1 H, Leuβ-Hb), 1.16–1.09 (m, 1 H, Leuγ), 0.88 (d, 1 H, *J* 6.6 Hz, Leuδ), and 0.85 (d, 1 H, *J* 6.6 Hz, Leuδ). Anal. Calcd for $C_{18}H_{27}N_3O_4$: C, 61.87; H, 7.79; N, 12.03. Found: C, 61.51; H, 7.87; N, 11.83.

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