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Synthesis of *O*-[2-acetamido-2-deoxy-6-*O*-stearoyl- and -6-*O*-(2-tetradecylhexadecanoyl)-β-D-glucopyranosyl]-(1 → 4)-*N*-acetylnormuramoyl-L-α-aminobutanoyl-D-isoglutamine, lipophilic disaccharide analogues of MDP

Miroslav Ledvina [a], Jan Ježek [a], David Šaman [a], Tomáš Vaisar [a] and Věra Hříbalová [b]

[a] *Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6 (Czechoslovakia)*

[b] *National Institute of Public Health, 100 42 Prague 10 (Czechoslovakia)*

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ABSTRACT

Silver triflate-promoted condensation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (**1**) with benzyl 2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-(methoxycarbonyl)methyl-α-D-glucopyranoside (**4**) afforded the key compound, benzyl 2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-(methoxycarbonyl)methyl-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-glucopyranoside (**5**), which after deprotection was transformed into acid **10**. Condensation of **10** with the benzyl ester of L-α-aminobutanoyl-D-isoglutamine and deisopropylidenation of the product **11** afforded the benzyl ester of *N*-{2-*O*-[benzyl 2-acetamido-4-*O*-(2-acetamido-3-*O*-benzyloxymethyl-2-deoxy-β-D-glucopyranosyl)-6-*O*-benzyl-2,3-dideoxy-α-D-glucopyranosid-3-yl]glycoloyl}-L-α-aminobutanoyl-D-isoglutamine (**12**). Partial *O*-acylation of **12** and hydrogenolysis of protecting groups gave the 6-*O*-stearoyl- and 6-*O*-(2-tetradecylhexadecanoyl)-disaccharide-dipeptides **17** and **18**, respectively. Pyrogenicity and adjuvant activity in cell-mediated immunity are reported.

INTRODUCTION

Some time ago, we described[1,2] the synthesis of *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-*N*-acetylnormuramoyl-L-α-aminobutanoyl-D-isoglutamine [β-D-GlcNAc-(1 → 4)-norMurNAc-L-Abu-D-isoGln] *, an analogue of the basic repeating disaccharide–dipeptide subunit of peptidoglycan [GMDP, β-D-GlcNAc-(1 → 4)-MurNAc-L-Ala-D-isoGln], modified both in the sugar and the peptide part

* Normuramic acid (norMur) is the trivial name for 2-amino-3-*O*-carboxymethyl-2-deoxy-D-glucopyranose; Abu, α-aminobutanoic acid.

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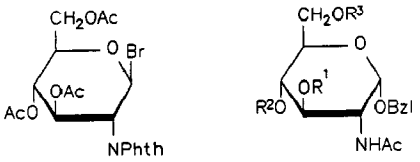
of the molecule. This compound displays higher immunoadjuvant activity than MDP (MurNAc-L-Ala-D-isoGln) and GMDP, and is without their unwanted side effects such as pyrogenicity and thrombocytolysis. In order to potentiate the biological activity in vivo and to improve the incorporation into liposomes, we prepared[3] its lipophilic derivative [β-D-GlcNstearoyl-(1 → 4)-norMurNAc-L-Abu-D-isoGln] bearing a stearoyl residue on the NH_2 group of the GlcNAc subunit.

This work describes the synthesis of lipophilic analogues of β-D-GlcNAc-(1 → 4)-norMurNAc-L-Abu-D-isoGln with bulky groups of the type of fatty and mycolic acids on the primary hydroxyl group in the GlcNAc subunit. Besides the potentiation of biological effects in vivo connected with the introduction of a lipophilic residue into the molecule, such a derivative of the GlcNAc subunit contains 2-acetamido-2-deoxy-6-*O*-mycoloyl-D-glucopyranose, which has immunoadjuvant activity similar to trehalose dimycolate (TDM), but lower toxicity than TDM[4]. This can result in a strong synergistic effect, because it is known that muramoyl dipeptides in combination with TDM display strong antitumour activity[5].

RESULTS AND DISCUSSION

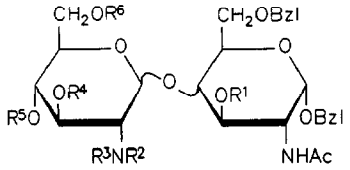
Reductive opening of the acetal ring in the methyl ester of *N*-acetyl-1-α-*O*-benzyl-4,6-*O*-benzylidenenormuramic acid (**3**) with sodium cyanoborohydride[6] afforded the glycosyl acceptor **4**. Compound **3** was obtained by *O*-alkylation of the sodium hydride-generated sodium salt of benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (**2**) by sodium chloroacetate in dioxane[7] followed by esterification with diazomethane. Benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (**2**) was obtained by a modified literature procedure[8].

The key disaccharide **5** was prepared by the triflate approach without base[1], because the base acts as an inhibitor of the glycosidation of hydroxyl groups of low reactivity[9,10]. Reaction of glycosyl donor **1** and glycosyl acceptor **4** in the presence of silver triflate (molar ratios 2:1:2) in dichloromethane at −45°C afforded benzyl 2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-(methoxycarbonyl)methyl-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-glucopyranoside (**5**) (yield 93%). Disaccharide **5** was *O*-deacetylated with sodium methoxide in methanol, the ester group was saponified with aqueous sodium hydroxide, and the phthaloyl group was detached by heating with butylamine in methanol. The deblocked product was selectively *N*-acetylated with acetic anhydride in methanol and esterified with diazomethane, yielding benzyl 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-6-*O*-benzyl-2-deoxy-3-*O*-(methoxycarbonyl)methyl-α-D-glucopyranoside (**6**), which was characterized as a crystalline triacetate **7**. Compound **6** reacted under trifluoromethanesulfonic acid catalysis with 2,2-dimethoxypropane in acetone to give the 4′,6′-*O*-isopropylidene derivative **8**. Reaction of this compound with benzyl chloromethyl ether in the presence of *N*,*N*-diisopropylethylamine in dichloromethane afforded benzyl 2-acetamido-4-*O*-(2-acetamido-3-*O*-benzyloxymethyl-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-6-*O*-benzyl-2-de-



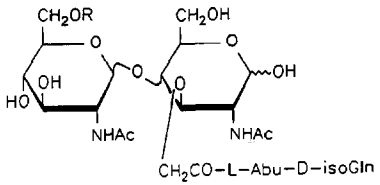
1

2 R^1=H, R^2+R^3=CHPh
3 R^1=CH_2COOCH_3, R^2+R^3=CHPh
4 R^1=CH_2COOCH_3, R^2=H, R^3=Bzl



5 R^1=CH_2COOCH_3, R^2+R^3=Phth,R^4=R^5=R^6=Ac
6 R^1=CH_2COOCH_3, R^2=R^4=R^5=R^6=H, R^3=Ac
7 R^1=CH_2COOCH_3, R^2=H, R^3=R^4=R^5=R^6=Ac
8 R^1=CH_2COOCH_3, R^2=R^4=H, R^3=Ac, R^5+R^6=$(CH_3)_2C$
9 R^1=CH_2COOCH_3, R^2=H, R^3=Ac, R^4=BOM, R^5+R^6=$(CH_3)_2C$
10 R^1=CH_2COOH, R^2=H, R^3=Ac, R^4=BOM, R^5+R^6=$(CH_3)_2C$
11 R^1=CH_2CO-L-Abu-D-isoGln(OBzl), R^2=H, R^3=Ac, R^4=BOM, R^5+R^6=$(CH_3)_2C$
12 R^1=CH_2CO-L-Abu-D-isoGln(OBzl), R^2=H, R^3=Ac, R^4=BOM, R^5=R^6=H
13 R^1=CH_2CO-L-Abu-D-isoGln(OBzl), R^2=H, R^3=Ac, R^4=BOM, R^5=H, R^6=stearoyl
14 R^1=CH_2CO-L-Abu-D-isoGln(OBzl), R^2=H, R^3=Ac, R^4=BOM, R^5=R^6=stearoyl
15 R^1=CH_2CO-L-Abu-D-isoGln(OBzl), R^2=H, R^3=Ac, R^4=BOM, R^5=H, R^6=2-tetradecylhexadecanoyl
16 R^1=CH_2CO-L-Abu-D-isoGln(OBzl), R^2=H, R^3=Ac, R^4=BOM, R^5=R^6=2-tetradecylhexadecanoyl

oxy-3-*O*-(methoxycarbonyl)methyl-α-D-glucopyranoside (**9**). The methyl ester **9** was saponified by sodium hydroxide and the resulting acid **10** was condensed with the trifluoroacetate of L-α-aminobutanoyl-D-isoglutamine benzyl ester[1] by means of 1,3-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole, yielding the protected glycopeptide **11**. This was transformed into the benzyl ester of *N*-{2-*O*-[benzyl 2-acetamido-4-*O*-(2-acetamido-3-*O*-benzyloxymethyl-2-deoxy-β-D-glucopyranosyl)-6-*O*-benzyl-2,3-dideoxy-α-D-glucopyranosid-3-yl]-glycoloyl}-L-α-aminobutanoyl-D-isoglutamine (**12**) by splitting off the isopropylidine group in 50% acetic acid. Partial *O*-acylation[11] of compound **12** with stearic acid in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC) and 4-dimethyl-



17 R = stearoyl
18 R = 2-tetradecylhexadecanoyl

BOM = benzyloxymethyl, isoGln(OBzl) = isoglutamine benzyl ester
Phth = phthaloyl

aminopyridine (DMAP) in *N*,*N*-dimethylformamide at 45°C afforded the 6′-*O*-acyl- and 4′,6′-di-*O*-acyl derivatives **13** and **14**, respectively, in the molar ratio 3:1; DCC as a condensation reagent was not efficient under these conditions. By using the benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) reagent[12], the condensation proceeded with low conversion, and after addition of DMAP with low selectivity. Acylation of **12** with stearoyl chloride in the presence of triethylamine did not proceed and in the presence of pyridine or DMAP displayed low selectivity. Attempts to introduce fatty acids branched on C-2 into the molecule of **12** by reaction with 2-tetradecylhexadecanoic acid[13] in the presence of WSC and DMAP or via the acyl chloride with DMAP catalysis were not successful. The desired 6′-*O*-(2-tetradecylhexadecanoyl) derivative **15** was obtained in a yield of 51% by silver triflate-promoted acylation of **12** with 2-tetradecylhexadecanoyl chloride in dichloromethane at −45°C. Raising the reaction temperature to 0°C leads, besides the monoacyl derivative **15**, to the 4′,6′-di-*O*-acyl derivative **16**. Hydrogenolysis of the benzyl groups in **13** and **15** led to the target *O*-[2-acetamido-2-deoxy-6-*O*-stearoyl-β-D-glucopyranosyl]-(1 → 4)-*N*-acetylnormuramoyl-L-α-aminobutanoyl-D-isoglutamine (**17**) and *O*-[2-acetamido-2-deoxy-6-*O*-(2-tetradecylhexadecanoyl)-β-D-glucopyranosyl]-(1 → 4)-*N*-acetylnormuramoyl-L-α-aminobutanoyl-D-isoglutamine (**18**), respectively.

Pyrogenicity was tested on Chinchilla rabbits in doses of 40, 200, and 1000 nM per rabbit. 6-*O*-Stearoyl derivative **17** was weakly pyrogenic ($\Delta T > 0.5$°C) only at the highest dose tested, 6-*O*-(2-tetradecylhexadecanoyl) derivative **18** was completely apyrogenic. In the tested sequence of MDP derivatives, the apyrogenic character was detected already with β-D-GlcNAc-(1 → 4)-norMurNAc-L-Abu-D-isoGln[1,2] and its stearoyl derivative β-D-GlcNstearoyl-(1 → 4)-norMurNAc-L-Abu-D-isoGln[3], while MDP, norMurNAc-L-Abu-D-isoGln, and GMDP were highly and comparably pyrogenic even at 200 nM per rabbit. Certain differences in pyrogenicity of the last three substances were visible only at 40 nM per rabbit, with MDP being the most and norMurNAc-L-Abu-D-isoGln the least pyrogenic.

Adjuvant activity was tested by induction of experimental allergic encephalomyelitis, which is, besides the delayed type skin reaction, the most frequently used test of adjuvant activity of muramoyl peptides in cell-mediated immunity. In this test, β-D-GlcNstearoyl-(1 → 4)-norMurNAc-L-Abu-D-isoGln and norMurNAc-L-Abu-D-isoGln displayed the highest activity. A weaker effect of MDP, GMDP, and β-D-GlcNAc-(1 → 4)-norMurNAc-L-Abu-D-isoGln was mutually comparable. Compound **18** with the bulkiest lipophilic part of the molecule displayed the lowest activity. Details of the above-mentioned data and other biological activities will be published elsewhere.

The structure of the compounds synthesized was shown by NMR spectrometry. Characteristic ^{1}H and/or ^{13}C NMR data for compounds **5–9** and **11–18** are presented in Tables I–V. In the case of compounds **7** and **11**, for a better understanding of spectra and uninterchangeable assignment of signals, 2D homonuclear ^{1}H–^{1}H and heteronuclear ^{1}H–^{13}C COSY spectra were also measured.

TABLE I

^{1}H NMR parameters of *N*-acetylnormuramoyl and glucosaminide moieties in compounds **5–9**, **11**, and **12** (in $CDCl_3$, 125.8 MHz, $CDCl_3$ = 77.00 ppm)

Parameter	5	6 [a]	7	8	9	11	12 [a]
δ (H-1)	5.32 d	4.80 d	5.39 d	5.32 d	5.36 d	4.88 d	4.83 d
δ (H-2)	3.80 m	3.75 m	3.80 m	3.82 m	3.83 m	4.22 m	3.83 m
δ (H-3)	3.67 dd	3.58 dd	3.69 dd	3.70 dd	3.67 dd	3.62 dd	3.59–3.68 m
δ (H-4)	4.09 dd	3.70 dd	3.85 dd	3.81 dd	3.86 dd	3.91 dd	3.93 t
δ (H-5)	3.43 m	3.57–3.68 m	3.58 m	3.64 m	3.54 m	3.63 m	3.59–3.68 m
δ (H-6a)	3.28 dd		3.34 dd	3.48 dd	3.31 dd	3.46 dd	
δ (H-6b)	3.35 dd	3.67 dd	3.51 dd	3.61 dd	3.51 dd	3.63 dd	
δ (H-1′)	5.39 d	4.55 d	4.25 d	4.38 d	4.19 d	4.47 d	4.28 d
δ (H-2′)	4.25 dd	3.70 q	3.97 m	3.63 m	3.88 bq	3.74 bq	3.59–3.68 m
δ (H-3′)	5.81 dd		3.41 dd	3.41 dd	3.82 dd	3.66 dd	
δ (H-4′)	4.85 dd	3.57–3.68 m	3.57 dd	3.53 dd	3.60 dd	3.53 dd	3.61 dd
δ (H-5′)	3.57 m		3.56 m	3.12 m	3.13 m	3.06 m	3.09 m
δ (H-6′a)	3.96 dd		4.00 dd	3.68 t	3.64 t	3.66 t	3.59–3.68 m
δ (H-6′b)	4.40 dd	4.43 dd	4.41 dd	3.94 dd	3.96 dd	3.85 dd	
δ (NH*Ac*)	1.85 s	1.82 s	1.99 s	1.79 s	1.99 s	1.90 s	1.83 s
δ (N*H*Ac)	5.39 d	7.86 d	7.81 d	7.70 d	7.70 d	6.30 d	8.16 d
J(1, 2)	3.4	3.4	3.3	3.5	3.5	3.7	3.5
J(2, 3)	11.0	11.0	11.0	11.0	11.0	10.6	11.0
J(3, 4)	8.8	8.6	8.8	8.8	8.8	9.0	[b]
J(4, 5)	10.0	9.3	9.9	9.7	9.8	9.7	9.3
J(5, 6a)	3.1	[b]	2.0	4.3	2.2	2.0	[b]
J(5, 6b)	1.3	1.7	2.4	2.6	2.3	2.3	[b]
J(1′, 2′)	8.4	9.5	9.7	8.4	8.6	8.5	8.6
J(2′, 3′)	10.8	9.8	10.6	9.8	10.3	9.6	[b]
J(3′, 4′)	9.0	[b]	9.3	9.0	9.0	9.0	9.0
J(4′, 5′)	10.2	[b]	10.0	10.0	9.9	9.9	9.8
J(5′, 6′a)	2.4	[b]	2.5	10.5	10.4	10.6	[b]
J(5′, 6′b)	4.2	5.0	4.5	5.3	5.4	5.3	[b]

[a] Spectra measured in $(CD_3)_2SO$ [$(CD_3)_2SO$ = 2.50 ppm]. [b] Value not determined.

TABLE II

^{13}C NMR chemical shifts of *N*-acetylnormuramoyl and glucosaminide moieties in compounds **5–9** and **11–18** ($CDCl_3$, 125.8 MHz, $CDCl_3$ = 77.00 ppm)

Compound	C-1	C-2	C-3	C-4	C-5	C-6	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′
5	96.40	51.99	77.14	76.59	70.09	67.68	97.01	55.14	68.68	70.46	71.34	61.38
6 [a]	95.97	51.59	78.25	76.79	70.49	68.67	101.18	56.38	70.57	74.01	76.65	61.20
7	96.66	51.89	78.66	76.90	69.86	66.85	100.84	54.19	68.26	71.23	72.85	61.55
8	96.33	51.96	78.35	77.18	70.32	68.64	100.20	56.42	71.33	75.42	75.93	62.07
9	96.58	51.98	78.09	77.15	70.15	67.30	100.63	54.24	70.99	85.24	75.00	63.12
11	96.47	52.31	79.53	76.30	70.18	67.66	100.47	54.81	70.51	82.12	75.57	61.50
12 [a]	95.97	52.69	78.28	76.60	70.45	68.62	100.25	53.55	70.64	80.06	75.59	60.90
13	96.64	52.64	80.33	73.72	70.23	67.73	99.71	55.08	70.36	82.73	75.99	62.85
14	96.78	52.75	78.28	73.41	70.38	67.90	99.30	55.19	70.57	79.72	75.64	62.15
15 [a]	95.82	51.71	77.55	73.65	70.46	68.20	99.81	55.18	70.92	79.67	75.71	63.47
16 [a]	95.73	51.64	76.79	76.20	70.35	68.02	98.77	56.23	70.63	77.45	75.74	62.38
17 [a]	90.28	51.96	76.73	73.91	70.61	63.37	101.32	56.04	70.97	78.04	73.53	59.75
18 [a]	90.25	52.17	77.36	74.16	71.21	63.87	101.47	56.33	71.11	77.82	74.01	60.00

[a] Spectra measured in $(CD_3)_2SO$ [$(CD_3)_2SO$ = 39.70 ppm].

TABLE III

^{13}C NMR chemical shifts of the nonsugar moiety in compounds **5–9** and **11–18** ($CDCl_3$, 125.8 MHz, $CDCl_3$ = 77.00 ppm)

Carbon	**5** [a]	**6** [b,c]	**7** [d]	**8** [e]	**9** [f]	**11**	**12** [b]	**13**	**14**	**15** [b]	**16** [b]	**17** [b]	**18** [b]
$NHCOCH_3$	170.91	169.56	169.58	172.23	170.98	171.04	169.73	170.33	170.34	169.47	169.77	169.63	169.69
		169.42	169.33	171.26	169.83	170.81	169.30	170.42	170.43	169.43	169.33	169.44	169.64
	22.99	23.30	23.00	22.76	23.28	22.98	23.13	23.34	23.29	23.10	22.89	23.19	23.41
		22.77	23.00	22.76	23.00	22.75	22.72	23.34	23.29	22.69	22.48	22.83	23.06
CH_2COOR	173.80	171.57	173.94	173.09	173.40	172.54	171.33	171.17	171.32	171.24	171.33	170.55	171.52
	72.71	72.25	73.93	74.00	73.77	73.61	72.14	73.48	73.35	72.16	72.06	70.02	70.18
OCH_2OBzl					95.91	95.66	95.22	95.90	95.72	95.28	95.34		
L-Abu-D-isoGln(OBzl)						54.97	53.55	55.93	55.15	53.58	53.55	53.54	54.28
						24.80	25.78	24.94	24.88	25.89	25.81	24.58	25.92
						9.96	10.03	10.32	10.34	9.98	9.76	9.87	10.24
						171.54	170.32	170.42	170.71	170.93	171.17	171.32	171.62
						52.50	52.69	52.81	52.73	53.31	53.55	53.48	53.96
						173.10	172.28	172.14	172.09	172.26	172.04	173.34	173.51
						26.21	27.05	26.25	26.35	27.14	27.07	27.11	27.36
						30.52	30.27	30.72	30.70	30.24	30.11	30.38	30.60
						173.98	173.12	173.28	173.35	173.06	173.81	174.03	174.22
						69.73	68.35	[a]	66.65	68.69	69.18		

[a] $COOCH_3$ 54.07. [b] Spectra measured in $(CD_3)_2SO$ [$(CD_3)_2SO$ = 39.70 ppm]. [c] $COOCH_3$ 52.81. [d] $COOCH_3$ 53.69. [e] $COOCH_3$ 53.45. [f] $COOCH_3$ 53.86.

TABLE IV

^{13}C NMR chemical shifts of the nonsugar moiety in compounds **13–18** [in $(CD_3)_2SO$, 125.8 MHz, $(CD_3)_2SO$ = 39.70 ppm]

Carbon	**13** [a]	**14** [a,b]	**15**	**16** [b]	**17**	**18**
OStear	174.47	173.75			174.03	
	34.22	33.83			33.57	
	31.90	31.90			31.50	
	29.70	29.70			29.27	
	29.54	29.56			29.25	
	29.34	29.35			29.17	
	29.22	29.21			28.90	
	14.09	14.09			14.16	
OTDHC [c]			175.50	174.58		175.87
			44.70	44.34		44.89
			31.48	31.40		31.71
			29.23	29.18		29.46
			29.16	29.18		29.12
			26.70	26.60		26.98
			22.27	22.13		22.50
			14.08	13.63		14.34

[a] Measured in $CDCl_3$ ($CDCl_3$ = 77.00 ppm). [b] Double intensity of all signals of acyl residue. [c] OTDHC = 2-tetradecylhexadecanoyl.

Fast atom bombardment mass spectra showed a sodium-cationized molecular ion for all compounds (even when the spectrum was recorded in pure commercial 3-nitrobenzyl alcohol) and only little fragmentation. Formation of this type of ion is well-known for saccharides[15], where the sodium cation is probably chelated on one of the C-1 carbon-bonded oxygens and ether oxygens. Further addition of sodium cation in the form of sodium iodide causes an increase in the intensity of cationized molecular ion and the appearance of several fragment ions of medium intensity due to cleavages of glycosidic bonds between sugar units[16].

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Optical rotations were measured with a Perkin–Elmer 141 polarimeter at 25°C. NMR spectra were recorded with a Varian UNITY-500 spectrometer in the FT mode at 499.8 MHz (^1H spectra) and at 125.6 MHz (^{13}C spectra) in $CDCl_3$, using Me_4Si as internal standard for the ^1H NMR spectrum and $CDCl_3$ (δ 77.0) or $(CD_3)_2SO$ (δ 39.7) signals (for compounds **15** and **16**) as standards for the ^{13}C NMR spectrum. Chemical shifts are given in ppm (δ scale) and coupling constants (J) in Hz. FAB mass spectra were measured on a BEqQ geometry mass spectrometer ZAB-EQ (VG Analytical, Manchester, UK), using an M-Scan FAB gun (Xe, energy 8 keV) (Ascott, UK) at an accelerating voltage of 8 kV. Data acquisition of the spectra was controlled by a VG data system 11-250J [computer PDP 11/73(DEC, USA)] and VG-MS software. The spectra were acquired using the MultiChannel Analysis

TABLE V

^{13}C NMR chemical shifts of the nonsugar moiety in compounds **5–9** and **11–18** ($CDCl_3$, 125.8 MHz, $CDCl_3$ = 77.00 ppm)

Carbon	**5** [a,b]	**6** [a]	**7** [c]	**8**	**9**	**11**	**12** [a]	**13**	**14**	**15** [a]	**16** [a]
OCH_2Ph	69.98	69.02	70.31	70.14	70.32	70.70	70.39	70.18	69.88	69.88	69.49
	68.77	68.67	68.94	68.78	68.98	69.73	68.88	69.84	69.88	68.94	68.74
OCH_2OCH_2Ph					[d]	66.52	66.57	66.55	65.32	65.65	65.45
C_6H_5	138.32	138.85	137.63	137.29	137.99	137.81	138.39	138.09	137.45	137.77	137.79
	137.56	137.73	137.63	137.19	137.56	137.06	137.76	136.78	136.87	136.33	136.21
	128.18	128.46	129.13	128.77	128.96	128.45	128.60	128.64	128.60	128.58	128.30
	127.56	127.69	128.28	127.81	127.93	127.79	127.70	128.10	128.46	128.02	128.18
	127.46	127.52	127.65	127.80	127.72	127.65	127.34	127.89	127.44	127.16	127.18

[a] Spectra measured in $(CD_3)_2SO$ [$(CD_3)_2SO$ = 39.70 ppm]. [b] $OCOCH_3$: 170.54, 169.98, 169.53, 20.63, 20.58, 20.37. [c] 170.87, 170.66, 170.66, 170.60, 20.71, 20.69, 20.69, 20.55. [d] Signal not observed.

utility of VG software, cumulating ca. 5 spectra. Samples were dissolved in $CHCl_3$ prior to addition of 1 μL of this saturated solution to the matrix (3-nitrobenzyl alcohol) on the target. Extensive sodium cationization was achieved by addition of sodium iodide to the sample on the target. CD spectra were recorded with a dichrographe Jobin–Yvon Mark V in MeOH, using software Dichrosoft written by Dr. P. Maloň. The measurements were done in cells of 0.1- and 0.02-cm path length in the range 200–260 nm. Thin-layer chromatography was performed on Silufol UV_{254} sheets, and column chromatography on silica gel Silpearl (both Kavalier, Votice, Czechoslovakia). High-performance liquid chromatography was carried out on columns (250 × 4 mm or 250 × 17 mm) packed with Separon SGX C18 (5 and 10 μm, respectively; Laboratorní přístroje, Prague, Czechoslovakia). Solutions were evaporated on a rotary vacuum evaporator. Amino acid analyses were obtained with a Durrum amino acid analyser (samples were hydrolyzed with 4 M HCl for 8 h at 110°C. Analytical samples were dried at 6.5 Pa and 25°C for 8 h.

Benzyl 2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (**2**).—To a stirred suspension of benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside[8] (49.6 g, 160 mmol) in a mixture of DMF (160 mL) and dioxane (160 mL) were added ethyl orthoformate (80 mL, 480 mmol), benzaldehyde (64 mL, 630 mmol), and trifluoromethanesulfonic acid (2 mL, 22 mmol), and the mixture was stirred for 8 h at room temperature. After standing overnight, the mixture first dissolved and then the product gradually precipitated. After neutralization with Et_3N (4 mL, 28.6 mmol) the mixture was stirred with ether (400 mL), and the product was filtered off and washed with ether, to yield **2** (50 g, 78%); mp 261°C (pyridine); $[\alpha]_D$ +109° (*c* 0.9, pyridine); lit.[14]: mp 262°C; $[\alpha]_D$ +114° (*c* 1.1, pyridine).

Benzyl 2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-(methoxycarbonyl)methyl-α-D-glucopyranoside (**3**).—Compound **2** (52 g, 130 mmol) and NaH (18.0 g, 750 mmol) were heated in dioxane (900 mL) for 2 h at 95°C. After cooling to room temperature, chloroacetic acid (17.3 g, 184 mmol) was added and the mixture was stirred for 6 h at 65°C. To the mixture (cooled to room temperature) was added solid CO_2, excess of NaH was decomposed by water, and solvents were evaporated. The residue was dissolved in water (1 L), and the stirred and ice-cooled solution was neutralized with KH_2PO_4, which had been adjusted to pH 3 by H_2SO_4 (120 g of KH_2PO_4) and 80 g of H_2SO_4 in 400 mL of water). The precipitated product was extracted with EtOAc (2 × 1 L), and the extracts were dried (Na_2SO_4) and concentrated to 400 mL. To this stirred ice-cooled solution was added diazomethane in ether until a yellow coloration persisted. After 30 min at room temperature, the excess of diazomethane was decomposed with AcOH and the mixture was concentrated. Crystallization from MeOH afforded **3** (41 g, 67%); mp 205°C; $[\alpha]_D$ +141° (*c* 0.2, $CHCl_3$). Anal. Calcd for $C_{25}H_{29}NO_8$ (471.5): C, 63.68; H, 6.20; N, 2.97. Found: C, 63.78; H, 6.15; N, 3.07.

Benzyl 2-acetamido-6-O-benzyl-2-deoxy-3-O-(methoxycarbonyl)methyl-α-D-glucopyranoside (**4**).—To a stirred suspension of **3** (28.5 g, 60 mmol) and $NaBH_3CN$ (15.08 g, 240 mmol) in dry THF (1 L) at room temperature was slowly added a

saturated solution of HCl in diethyl ether to give an acidic pH (pH paper). After stirring for 1 h, another portion of $NaBH_3CN$ (11.31 g, 180 mmol) was added, followed by HCl in diethyl ether to keep the pH acidic, and the mixture was stirred for an additional 8 h. Then aq 20% NaOAc was added to give a neutral pH (pH paper) and the mixture was concentrated in vacuo. Chloroform was added (750 mL), and the mixture was extracted with water (2 × 200 mL), dried (Na_2SO_4), and evaporated. Chromatography of the residue on a silica gel column (750 g) in 1:3 toluene–EtOAc afforded 24.8 g (87%) of **4**. Crystallization from toluene afforded **4** (19.5 g, 69%); mp 124°C $[\alpha]_D$ +87° (*c* 0.2, $CHCl_3$). Anal. Calcd for $C_{25}H_{31}NO_8$ (473.2): C, 63.39; H, 6.60; N, 2.95. Found: C, 63.41; H, 6.52; N, 2.94.

Benzyl 2-acetamido-6-O-benzyl-2-deoxy-3-O-(methoxycarbonyl)methyl-4-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-glucopyranoside (**5**).—A mixture of **4** (18.94 g, 40 mmol) and silver trifluoromethanesulfonate (20.56 g, 80 mmol) was dried, with intensive stirring in an apparatus equipped with a septum, for 4 h at room temperature and 1.32 Pa. The apparatus was flushed with Ar (2 ×), and dry CH_2Cl_2 (140 mL) was added through the septum. After dissolution, the stirred mixture was cooled to −45°C. A solution of bromide **1**[1] (39.88 g, 80 mmol) in dry CH_2Cl_2 (140 mL) was gradually added through the septum during 1 h and the mixture was stirred for another 1 h at −45°C and 20 min at −20°C. At −20°C, pyridine (20 mL) was added and after raising to room temperature the precipitated AgBr was filtered off and washed with $CHCl_3$ (500 mL). The filtrate was diluted by $CHCl_3$ (2 L), and the solution was extracted with satd aq $NaHCO_3$ (2 × 300 mL) and water (300 mL), dried (Na_2SO_4), and evaporated. Chromatography on a silica gel column (2000 g) in 2:3 toluene–EtOAc afforded 33.0 g (93%) of **5** as a solid foam; $[\alpha]_D$ +47° (*c* 0.4, $CHCl_3$). Anal. Calcd for $C_{45}H_{50}N_2O_{17}$ (890.4): C, 60.64; H, 5.66; N, 3.14. Found: C, 60.32; H, 5.61; N, 3.33.

Benzyl 2-acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-6-O-benzyl-2-deoxy-3-O-(methoxycarbonyl)methyl-α-D-glucopyranoside (**6**).—A solution of **5** (22.5 g, 25.3 mmol) in 0.01 M NaOMe in MeOH (500 mL) was kept for 12 h at 5°C, then neutralized with Dowex 50 (H^+), and the resin was filtered off and washed with MeOH. The filtrate was evaporated and the residue heated with stirring in 2:1 MeOH–1 M NaOH (600 mL) for 3 h at 60°C. After cooling, the mixture was neutralized with Dowex 50 (H^+), and the suspension was placed on a column of the same ion-exchange resin (500 mL) which was then eluted with aq 60% MeOH (2.5 L). The eluate was evaporated, and the residue was dried for 3 h at room temperature and 1.32 Pa, dissolved in a mixture of 4:1 MeOH–butylamine (200 mL), and heated in a pressure bottle for 15 h at 85°C. After cooling, the mixture was evaporated and the residue was extracted with ether (3 × 200 mL). The insoluble residue was dissolved in 90% MeOH (200 mL), and the solution was adjusted to pH 4 with formic acid and then poured onto a column of Dowex 50 (NH_4^+) resin (800 mL). The column was washed with 90% MeOH (3 L), the product was desorbed with a mixture of 1:9 aq 25% ammonia–MeOH, and the eluate was evaporated. The residue was dissolved in MeOH (120 mL), and Ac_2O

(12 mL) was added with stirring. After 30 min at room temperature, another portion of Ac_2O (12 mL) was added and after 2 h the mixture was evaporated. The residue was codistilled with toluene (3 × 100 mL) and dried for 3 h at room temperature and 1.32 Pa. The residue was dissolved in MeOH (120 mL) and diazomethane was added with stirring at 0°C until a yellow coloration persisted. After 30 min at 0°C, the excess of diazomethane was decomposed with AcOH and the mixture was evaporated. The residue was chromatographed on a column of silica gel (500 g) in 20:2:2:1 EtOAc–acetone–EtOH–water, to yield solid **6** (10.5 g, 61%); $[\alpha]_D$ +72° (*c* 0.4, MeOH). Anal. Calcd for $C_{33}H_{44}N_2O_{13}$ (676.4): C, 58.54; H, 6.55; N, 4.13. Found: C, 58.26; H, 6.41; N, 4.36.

Benzyl 2-acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-6-O-benzyl-2-deoxy-3-O-(methoxycarbonyl)methyl-α-D-glucopyranoside (**7**).—A solution of **6** (677 mg, 1.0 mmol) in 2:1 pyridine–Ac_2O (6 mL) was kept for 12 h at room temperature. Excess of Ac_2O was decomposed with MeOH and the mixture was evaporated. The residue was dissolved in $CHCl_3$ (50 mL), and the solution was extracted with water (2 × 10 mL), dried (Na_2SO_4), and evaporated. Crystallization form $CHCl_3$–ether afforded **7** (520 mg, 65%); mp 245–248°C; $[\alpha]_D$ +68° (*c* 0.4, $CHCl_3$). Anal. Calcd for $C_{39}H_{50}N_2O_{16}$ (802.4): C, 58.32; H, 6.27; N, 3.48. Found: C, 58.08; H, 6.29; N, 3.69.

Benzyl 2-acetamido-4-O-(2-acetamido-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranosyl)-6-O-benzyl-2-deoxy-3-O-(methoxycarbonyl)methyl-α-D-glucopyranoside (**8**).—To a stirred suspension of **6** (5.3 g, 7.8 mmol) in a mixture of 2,2-dimethoxypropane (40 mL) and acetone (160 mL) was added trifluoromethanesulfonic acid (250 μL, 2.8 mmol). After stirring at room temperature for 6 h, the solution was neutralized with Et_3N (1 mL) and evaporated. The residue was dissolved in $CHCl_3$ (300 mL), and the solution was extracted with water (2 × 100 mL), dried (Na_2SO_4), and evaporated. Chromatography on a silica gel column (250 g) in 40:2:2:1 EtOAc–acetone–EtOH–water afforded 4.1 g (73%) of solid **8**; $[\alpha]_D$ +65° (*c* 0.4, $CHCl_3$). Anal. Calcd for $C_{36}H_{48}N_2O_{13}$ (716.4): C, 60.30; H, 6.75; N, 3.90. Found: C, 59.94; H, 6.62; N, 4.02.

Benzyl 2-acetamido-4-O-(2-acetamido-3-O-benzyloxymethyl-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranosyl)-6-O-benzyl-2-deoxy-3-O-(methoxycarbonyl)methyl-α-D-glucopyranoside (**9**).—Compound **8** (3.4 g, 4.74 mmol) was dissolved in CH_2Cl_2 (20 mL), and *N*,*N*-diisopropylethylamine (3.8 mL, 21.8 mmol), benzyl chloromethyl ether (2.0 mL, 14.4 mmol), and 4A molecular sieves (Fluka, 4.0 g) were added. The mixture was stirred at room temperature for 48 h. Methanol (2.0 mL) was added, the mixture was stirred for 30 min, the molecular sieves were then filtered off, the filtrate was evaporated, and the residue was codistilled with toluene (2 × 20 mL). The residue was dissolved in $CHCl_3$ (150 mL), and the solution was extracted with water (2 × 30 mL), dried (Na_2SO_4), and evaporated. Chromatography on silica gel (200 g) in 3:1 EtOAc–toluene afforded 3.4 g (86%) of solid **9**; $[\alpha]_D$ +87° (*c* 0.4, $CHCl_3$). Anal. Calcd for $C_{44}H_{56}N_2O_{14}$ (836.4): C, 63.12; H, 6.74; N, 3.34. Found: C, 63.49; H, 6.86; N, 3.19.

Benzyl 2-acetamido-4-O-(2-acetamido-3-O-benzyloxymethyl-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranosyl-6-O-benzyl-2-deoxy-3-O-carboxymethyl-α-D-glucopyranoside (**10**).—Compound **9** (3.0 g, 3.58 mmol) was dissolved in dioxane (60 mL), 0.5 M NaOH (20 mL) was added, and the mixture was stirred for 12 h at room temperature and then neutralized with Dowex 50 (pyridine form) resin. The resin was filtered off and washed with dioxane. The filtrate was evaporated and the residue was codistilled with toluene (3 × 50 mL), to yield 2.93 g (99%) of solid **10**, which, without further purification, was condensed with the trifluoroacetate of the benzyl ester of L-α-aminobutanoyl-D-isoglutamine.

N-{2-O-[*Benzyl 2-acetamido-4-O-(2-acetamido-3-O-benzyloxymethyl-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranosyl)-6-O-benzyl-2,3-dideoxy-α-D-glucopyranosid-3-yl]-glycoloyl*}-L-α-*aminobutanoyl-D-isoglutamine benzyl ester* (**11**).—A solution of (*tert*-butoxycarbonyl)-L-α-aminobutanoyl-D-isoglutamine benzyl ester[1] (1.68 g, 4.0 mmol) in a mixture of 17:3 CH_2Cl_2–CF_3CO_2H (50 mL) was kept at room temperature for 50 min. After evaporation, the syrupy residue was extracted with ether (2 × 100 mL), and the insoluble portion was dried for 2 h at room temperature and 1.32 Pa. The syrup obtained was dissolved in dry dioxane (65 mL) and the resulting solution of the trifluoroacetate of L-α-aminobutanoyl-D-isoglutamine benzyl ester was used immediately for coupling with the acid **10**.

To a stirred solution of **10** (2.96 g, 3.6 mmol) and 1-hydroxybenzotriazole monohydrate (500 mg, 3.7 mmol) in CH_2Cl_2 (25 mL) at 0° was added 1 M DCC in CH_2Cl_2 (3.6 mL). After 1 h, a solution of the trifluoroacetate of L-α-aminobutanoyl-D-isoglutamine benzyl ester and Et_3N (0.7 mL) were added. The mixture was stirred for 2 h at 0°C, kept for 12 h at room temperature, and filtered, and the filtrate was evaporated. A solution of the residue in $CHCl_3$ (400 mL) was washed with satd aq $NaHCO_3$ (2 × 100 mL) and water (100 mL), dried (Na_2SO_4), and evaporated. The residue was stirred with $CHCl_3$ (50 mL), *N,N'*-dicyclohexylurea was filtered off and washed with $CHCl_3$, and the filtrate was evaporated. Chromatography on a silica gel column (350 g) in 300:10:1 $CHCl_3$–MeOH–Et_3N afforded 3.8 g (94%) of **11** as a solid foam; $[\alpha]_D$ +48° (*c* 0.4, $CHCl_3$). Anal. Calcd for $C_{59}H_{75}N_5O_{17}$ (1125.6): C, 62.89; H, 6.71; N, 6.21. Found: C, 62.82; H, 6.75; N, 6.26.

N-{2-O-[*Benzyl 2-acetamido-4-O-(2-acetamido-3-O-benzyloxymethyl-2-deoxy-β-D-glucopyranosyl)-6-O-benzyl-2,3-dideoxy-α-D-glucopyranosid-3-yl]-glycoloyl*}-L-α-*aminobutanoyl-D-isoglutamine benzyl ester* (**12**).—Compound **11** (3.3 g, 2.93 mmol) was heated for 1.5 h at 40°C with stirring in aq 50% AcOH (140 mL). During this time, **11** dissolved and then the product precipitated. The mixture was evaporated and codistilled with toluene (3 × 50 mL); yield: 3.06 g (96%) of solid **12**, chromatographically homogeneous in TLC (silica gel) in 10:1 $CHCl_3$–MeOH; $[\alpha]_D$ +63° (*c* 0.4, pyridine). FABMS: *m/z* 1108.7 (M + Na). Anal. Calcd for $C_{56}H_{71}N_5O_{17}$ (1086.2): C, 61.92; H, 6.59; N, 6.45. Found: C, 61.57; H, 6.43; N, 6.40.

N-{2-O-[*Benzyl 2-acetamido-4-O-(2-acetamido-3-O-benzyloxymethyl-2-deoxy-6-O-stearoyl-β-D-glucopyranosyl)-6-O-benzyl-2,3-dideoxy-α-D-glucopyranosid-3-yl]-gly-*

coloyl}-L-*α-aminobutanoyl*-D-*isoglutamine benzyl ester* (**13**) *and* N-*{2*-O-*[benzyl 2-acetamido-4*-O-*(2-acetamido-3*-O-*benzyloxymethyl-2-deoxy-4,6-di*-O-*stearoyl-β*-D-*glucopyranosyl)-6*-O-*benzyl-2,3-dideoxy-α*-D-*glucopyranosid-3-yl]-glycoloyl}*-L-*α-aminobutanoyl*-D-*isoglutamine benzyl ester* (**14**).—A mixture of **12** (260 mg, 0.24 mmol), stearic acid (85 mg, 0.30 mmol), WSC (58 mg, 0.3 mmol), and 4-dimethylaminopyridine (29 mg, 0.24 mmol) in DMF (10 mL) was heated with stirring for 3 h at 45°C. Another portion of stearic acid (28 mg, 0.1 mmol), WSC (19 mg, 0.1 mmol), and 4-dimethylaminopyridine (12 mg, 0.1 mmol) were added and the mixture was stirred at 45°C for another 2 h. After cooling to room temperature, MeOH (2 mL) was added and 1 h later the mixture was evaporated. The residue was chromatographed on a silica gel column (40 g) in 20:1 $CHCl_3$–MeOH.

Lyophilization from AcOH of homogeneous fractions with higher R_f value afforded 90 mg (23%) of **14**; $[\alpha]_D$ +29° (*c* 0.5, $CHCl_3$). FABMS: *m/z* 1641.7 (M + Na). Anal. Calcd for $C_{92}H_{139}N_5O_{19}$ (1618.1): C, 68.22; H, 8.65; N, 4.32. Found: C, 68.30; H, 8.59; N, 4.16.

Lyophilization from AcOH of homogeneous fractions with lower R_f value afforded **13** (218 mg, 67%); $[\alpha]_D$ +25° (*c* 0.4, $CHCl_3$). FABMS: *m/z* 1374.9 (M + Na). Anal. Calcd for $C_{74}H_{105}N_5O_{18}$ (1351.8): C, 65.68; H, 7.82; N, 5.17. Found: C, 65.40; H, 7.64; N, 4.94.

N-*[2*-O-*{Benzyl 2-acetamido-4*-O-*[2-acetamido-3*-O-*benzyloxymethyl-2-deoxy-6*-O-*(2-tetradecylhexadecanoyl)-β*-D-*glucopyranosyl]-6*-O-*benzyl-2,3-dideoxy-α*-D-*glucopyranosid-3-yl}-glycoloyl]*-L-*α-aminobutanoyl*-D-*isoglutamine benzyl ester* (**15**).—Compound **12** (435 mg, 0.4 mmol) and silver trifluoromethanesulfonate (154 mg, 0.6 mmol) were dried for 8 h at room temperature and 1.32 Pa in an apparatus provided with a septum. The apparatus was purged twice with Ar, and CH_2Cl_2 (8 mL) was added through the septum. The mixture was cooled to −45°C and, with stirring, a solution of 2-tetradecylhexadecanoyl chloride (283 mg, 0.6 mmol) in dry CH_2Cl_2 (8 mL) was added through the septum during 1 h. The mixture was stirred for another 30 min at −45°C and 30 min at −20°C, pyridine (1.5 mL) was added, and the mixture was withdrawn from the cooling bath; when the mixture reached ambient temperature, it was diluted with $CHCl_3$ (60 mL), and the suspension was filtered. The filtrate was washed with satd aq $NaHCO_3$ (2 × 20 mL) and water (20 mL), dried (Na_2SO_4), and evaporated. The residue was chromatographed on silica gel (25 g) with 20:1 $CHCl_3$–MeOH and homogeneous fractions were lyophilized from benzene, to yield **15** (309 mg, 51%); $[\alpha]_D$ +25° (*c* 0.4, $CHCl_3$). FABMS: *m/z* 1543.2 (M + Na). Anal. Calcd for $C_{86}H_{129}N_5O_{18}$ (1520.3): C, 67.89; H, 8.55; N, 4.60. Found: C, 67.92; H, 8.63; N, 4.41.

Washing the column with 10:1 $CHCl_3$–MeOH and evaporation of homogeneous fractions yielded 130 mg (30%) of starting compound **12**.

It is important to maintain strictly the reaction temperature given above, because when the temperature was allowed to rise to 0°C, the chromatography afforded, besides **15**, *N*-[2-*O*-{benzyl 2-acetamido-4-*O*-[2-acetamido-3-*O*-benzyloxymethyl-2-deoxy-4,6-di-*O*-(2-tetradecylhexadecanoyl)-β-D-glucopyranosyl]-6-*O*-

benzyl-2,3-dideoxy-α-D-glucopyranosid-3-yl}-glycoloyl]-L-α-aminobutanoyl-D-isoglutamine benzyl ester (**16**), which was obtained after lyophilization from benzene; $[\alpha]_D$ +37° (*c* 0.5, $CHCl_3$). FABMS: *m/z* 1978.5 (M + Na). Anal. Calcd for $C_{116}H_{187}N_5O_{19}$ (1954.5): C, 71.22; H, 9.64; N, 3.58. Found: C, 71.45; H, 9.52; N, 3.41.

O-(2-Acetamido-2-deoxy-6-O-stearoyl-β-D-glucopyranosyl)-(1 → 4)-N-acetylnormuramoyl-L-α-aminobutanoyl-D-isoglutamine (**17**).—Compound **13** (200 mg, 0.148 mmol) was hydrogenolyzed in AcOH (14 mL) in the presence of 10% Pd–C catalyst (230 mg) for 15 h at room temperature. At the end of the hydrogenolysis, the apparatus was evacuated, and purged with N_2. The catalyst was filtered off, then washed with AcOH (25 mL), and the filtrate was evaporated. The residue was chromatographed on a silica gel C_{18} column in 4:1 MeOH–water. Homogeneous fractions were lyophilized from AcOH to yield **17** (85 mg, 62%). CD spectrum (MeOH; deg.cm^2.dmol^{-1}): Θ_{212} −4600. Amino acid analysis: glutamic acid, 1.00; α-aminobutyric acid, 0.96; normuramic acid, 0.97; glucosamine, 0.92. FABMS: *m/z* 984.9 (M + Na). Anal. Calcd for $C_{45}H_{79}N_5O_{17}$ (961.6): C, 56.21; H, 8.87; N, 7.28. Found: C, 56.22; H, 8.81; N, 7.30.

O-[2-Acetamido-2-deoxy-6-O-(2-tetradecylhexadecanoyl)-β-D-glucopyranosyl]-(1 → 4)-N-acetylnormuramoyl-L-α-aminobutanoyl-D-isoglutamine (**18**).—Compound **15** (150 mg, 0.098 mmol) was hydrogenolyzed in AcOH (10 mL) in the presence of 10% Pd–C catalyst (150 mg) for 15 h at room temperature. At the end of the hydrogenolysis, the apparatus was evacuated, and purged with N_2. The catalyst was filtered off, then washed with AcOH (25 mL), and the filtrate was evaporated. The residue was chromatographed on a silica gel C18 column in 9:1 MeOH–water. Homogeneous fractions were lyophilized from AcOH, to yield **18** (74 mg, 67%). CD spectrum (MeOH; deg.cm^2.dmol^{-1}): Θ_{212} −5000. Amino acid analysis: glutamic acid, 1.00; α-aminobutyric acid, 0.98; normuramic acid, 0.94; glucosamine, 0.92. FABMS: *m/z* 1153.1 (M + Na). Anal. Calcd for $C_{57}H_{103}N_5O_{17}$ (1129.8): C, 60.54; H, 9.18; N, 6.19. Found: C, 60.31; H, 9.34; N, 6.09.

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