SYNTHESIS AND IMMUNOMODULATING ACTIVITY OF LIPOPHILIC ANALOGS OF *N*-ACETYLNORMURAMOYL-L-2-AMINOBUTANOYL-D-ISOGLUTAMINE*

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Received January 15, 1998
Accepted March 14, 1998

N-Acetylnormuramoyl-L-2-aminobutanoyl-D-isoglutamine (**7**) and its lipophilic 6-*O*-octadecanoyl (**8**) and 6-*O*-(2-tetradecylhexadecanoyl) (**9**) derivatives were prepared and their immunoadjuvant activity and pyrogenicity were tested. Compounds **8** and **9** are less pyrogenic than muramoyl-dipeptide (MDP) and norMDP analog **7**. Both lipophilic derivatives **8** and **9** are better adjuvants than MDP in cell mediated immunity.

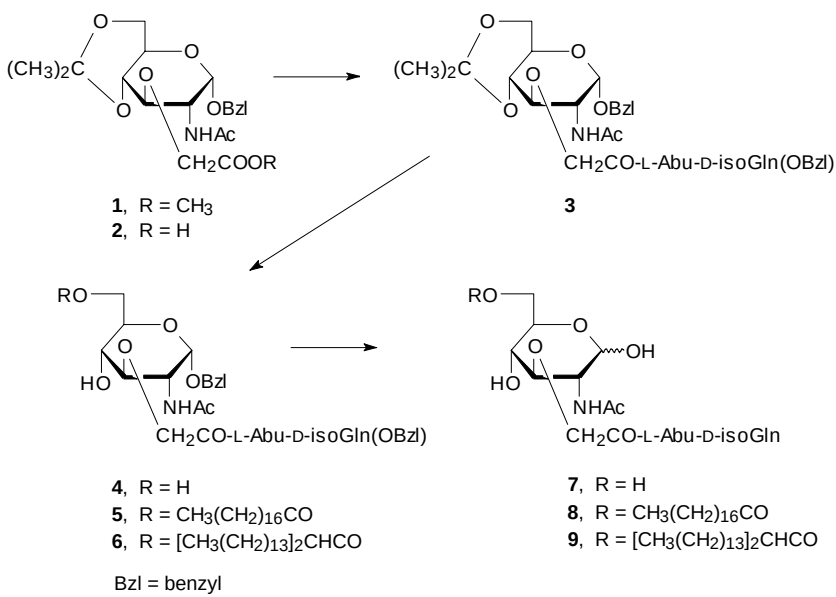
Key words: Carbohydrates; Glycosides; Aminosugars; Muramyl glycopeptides; Immunoadjuvants.

A recess from natural vaccines and changeover to semisynthetic, recombinant and synthetic vaccines craved a development of nontoxic, structurally defined adjuvants. In this connection, attention has been focused to the molecule of *N*-acetylmuramoyl-L-alanyl-D-isoglutamine (muramoyl-dipeptide, MDP), a minimal unit of peptidoglycan of bacterial cell wall, which still possess immunoadjuvant activity. A large number of MDP analogs was synthesized with the aim to suppress undesirable side effects, namely pyrogenicity, and to potentiate immunoadjuvant activity (for review, see refs[1,2]) Introduction of bulky lipophilic groups onto 6-OH group of the sugar unit led to a decrease in pyrogenicity and an increase in immunoadjuvant activity, especially for cellular immunity[2–5]. These results and the fact that *N*-acetylnormuramoyl-L-2-aminobutanoyl-D-isoglutamine, an analog of MDP modified both in the sugar and peptide parts, is less pyrogenic and has a higher immunoadjuvant activity[6,7] motivated us to prepare its lipophilic analogs bearing bulky acyl groups on the primary OH group. The aim was to

* Normuramic acid is the trivial name for 2-amino-3-*O*-carboxymethyl-2-deoxy-D-glucopyranose. The symbols and abbreviations obey the published recommendations (*Biochemical Nomenclature and Related Documents.* International Union of Biochemistry, London 1978).

obtain new adjuvants with better immunopharmacological parameters compared with the mentioned lipophilic derivatives of MDP, and to improve their incorporation into liposomes.

As a starting compound for the preparation of the sugar unit **2**, we utilized the previously described benzyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-3-*O*-methoxycarbonylmethyl-α-D-glucopyranoside (**1**; ref.[8]) (Scheme 1). Methyl ester **1** was hydrolyzed with 0.2 M solution of sodium hydroxide in a mixture methanol–water (3 : 2) to give benzyl 2-acetamido-3-*O*-carboxymethyl-2-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranoside (**2**). Coupling of acid **2** with trifluoroacetate of L-2-aminobutanoyl-D-isoglutamine benzyl ester[9] by means of *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBT) afforded *N*-[2-*O*-(benzyl 2-acetamido-2,3-dideoxy-4,6-*O*-isopropylidene-α-D-glucopyranosid-3-yl)-glycoloyl]-L-2-aminobutanoyl-D-isoglutamine benzyl ester (**3**) in 66% yield. The isopropylidene protecting group was removed from the glycopeptide **3** by heating with 50% acetic acid at 60 °C, yielding the key intermediate *N*-[2-*O*-(benzyl 2-acetamido-2,3-dideoxy-α-D-glucopyranosid-3-yl)-glycoloyl]-L-2-aminobutanoyl-D-isoglutamine benzyl ester (**4**). Partial *O*-acylation[10] of compound **4** with stearic acid in the presence of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (WSC) and 4-dimethylaminopyridine (DMAP) in *N*,*N*-dimethylformamide (molar ratios 1 : 1.3 : 1.9 : 2; WSC and DMAP were added in two portions) at room temperature gave *N*-[2-*O*-(benzyl 2-acetamido-2,3-dideoxy-6-*O*-octadecanoyl-α-D-glucopyranosid-3-yl)glycoloyl]-L-2-aminobutanoyl-D-isoglutamine



1, R = CH_3
2, R = H
3
4, R = H
5, R = $CH_3(CH_2)_{16}CO$
6, R = $[CH_3(CH_2)_{13}]_2CHCO$
7, R = H
8, R = $CH_3(CH_2)_{16}CO$
9, R = $[CH_3(CH_2)_{13}]_2CHCO$

Bzl = benzyl

SCHEME 1

benzyl ester (**5**) in 48% yield. Introduction of fatty acid branched on C-2 required a higher reaction temperature. In the same way promoted reaction of compound **4** with 2-tetradecylhexadecanoic acid[11] at 50 °C afforded the required *N*-{2-*O*-[benzyl 2-acetamido-2,3-dideoxy-6-*O*-(2-tetradecylhexa-decanoyl)-α-D-glucopyranosid-3-yl]glycoloyl}-L-2-aminobutanoyl-D-isoglutamine benzyl ester (**6**) in 54% yield. Hydrogenolysis of compound **4** on Pd/C catalyst in acetic acid afforded *N*-acetylnormuramoyl-L-2-aminobutanoyl-D-isoglutamine (**7**). This approach is an alternative to described procedure[6]. Hydrogenolysis of benzyl protecting groups in compounds **5** and **6** as described above afforded the target 6-*O*-octadecanoyl-*N*-acetylnormuramoyl-L-2-aminobutanoyl-D-isoglutamine (**8**) and 6-*O*-(2-tetradecylhexadecanoyl)-*N*-acetylnormuramoyl-L-2-aminobutanoyl-D-isoglutamine (**9**).

Pyrogenicity was tested on Chinchilla rabbits in doses of 40, 200 and 1 000 nmol per rabbit. In the tested series of MDP and its derivatives, pyrogenicity decreases in the order MDP > norMDP analog **7** > stearoyl derivative **8** > 2-tetradecylhexadecanoyl derivative **9**. Compound **9** is apyrogenic even at the highest dose tested.

The adjuvant activity was tested by induction of experimental allergic encephalomyelitis, which is, besides the delayed type skin reaction, the most frequently used test for the adjuvant activity of muramoyl peptides in cell-mediated immunity. In this test, the adjuvant activity of stearoyl derivative **8** was lower than that of norMDP analog **7** and higher than that of MDP, whereas the 2-tetradecylhexadecanoyl derivative **9** was the most active compound from all compounds tested. Details of the above mentioned data and other immunopharmacological parameters will be published elsewhere.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Optical rotations were measured on a Perkin–Elmer 141 polarimeter at 22 °C. NMR spectra were recorded with a Varian UNITY-500 spectrometer in the FT mode at 499.8 MHz (^{1}H) and at 125.6 MHz (^{13}C) in CD_3SOCD_3, using the central line of solvent for standardization (δ 2.50 for ^{1}H and 39.5 for ^{13}C). Chemical shifts are given in ppm (δ-scale) and coupling constants (*J*) in Hz. Positive-ion FAB mass spectra were measured on a BEqG geometry mass spectrometer ZAB-EQ (VG Analytical, Manchester, U.K.), using an M-Scan FAB gun (Xe, energy 8 keV) at an accelerating voltage of 8 kV. Samples were dissolved in chloroform or methanol, and the mixture glycerol–thioglycerol was used as matrix. Thin-layer chromatography (TLC) was performed on Silufol UV_{254} sheets, and column chromatography on silica gel Silpearl (both Kavalier, Votice, Czech Republic). Analytical RP HPLC was performed with a Spectra-Physics 8700 apparatus (Darmstadt, Germany) equipped with a column (250 × 4 mm) of Separon SGX-RPS (C18), particle size 10 nm (Tessek, Prague, Czech Republic). Preparative RP HPLC was performed with a Knauer apparatus (Bad Homburg, Germany) equipped with a column (250 × 10 mm) filled with Separon SGX-RPS (C18), particle size 10 nm (Tessek, Prague, Czech Republic). Solutions were evaporated on rotatory vacuum evaporator. Samples for amino acid analysis were hydrolyzed in 4 M HCl at 110 °C for 8 h and analyzed on D-500 Durrum Amino Acid Analyzer (Durrum Corporation, Palo Alto, CA). Analytical samples were dried at 6.5 Pa and 25 °C for 8 h.

Dichloromethane was distilled from phosphorus pentoxide and stored over molecular sieves 4A.

Benzyl 2-Acetamido-3-*O*-carboxymethyl-2-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranoside (**2**)

To a stirred solution of methyl ester[8] **1** (4.235 g, 10 mmol) in MeOH (60 ml) 0.5 M NaOH (40 ml, 20 mmol) was added. After 4 h the mixture was neutralized by addition of Dowex 50 (pyridinium cycle). The ion-exchanger was filtered off, washed with 50% MeOH (3 × 50 ml) and the filtrate was evaporated *in vacuo*. The residue was coevaporated with benzene (3 × 100 ml) and lyophilized from benzene. Yield 4.05 g (99%) of compound **2**, TLC-homogeneous in solvent systems ethyl acetate–toluene (2 : 1) and in butanol–acetic acid–water (4 : 1 : 1), $[\alpha]_D$ +90° (*c* 0.6, chloroform). For $C_{20}H_{27}NO_8$ calculated: relative molecular mass 409.4, monoisotopic mass 409.1. FAB MS, *m/z*: 410.1 $[M + H]^+$, 432.1 $[M + Na]^+$. For $C_{20}H_{27}NO_8$ (409.4) calculated: 58.67% C, 6.64% H, 3.42% N; found: 58.75% C, 6.50% H, 3.57% N.

N-[2-*O*-(Benzyl 2-Acetamido-2,3-dideoxy-4,6-*O*-isopropylidene-α-D-glucopyranosid-3-yl)-glycoloyl]-L-2-aminobutanoyl-D-isoglutamine Benzyl Ester (**3**)

A solution of *tert*-butoxycarbonyl-L-2-aminobutanoyl-D-isoglutamine benzyl ester[9] (4.22 g, 10.0 mmol) in a mixture of dichloromethane–trifluoroacetic acid (1 : 1, 40 ml) was kept at room temperature for 30 min. The mixture was evaporated, the syrupy residue was triturated with diethyl-ether (3 × 50 ml) and the insoluble portion was coevaporated with CH_2Cl_2 (2 × 50 ml) and dried at room temperature and 1.32 Pa for 2 h. The syrup obtained was dissolved in DMF (20 ml) and the resulting solution of the trifluoroacetate of L-2-aminobutanoyl-D-isoglutamine benzyl ester was used immediately for coupling with the acid **2**.

To a stirred solution of **2** (4.094 g, 10 mmol) and 1-hydroxybenzotriazole monohydrate (1.531 g, 10 mmol) in a mixture of DMF (10 ml) and dichloromethane (40 ml) at 0 °C and 1 M solution of *N*,*N*′-dicyclohexylcarbodiimide in dichloromethane (10 ml, 10 mmol), was added. After 1 h at 0 °C, the precipitated *N*,*N*′-dicyclohexylurea was filtered off (2 g, 89%), and the filtrate was concentrated to approximately 20 ml. The above mentioned solution of the trifluoroacetate of L-2-aminobutanoyl-D-isoglutamine benzyl ester and *N*,*N*-diisopropylethylamine (1.88 ml, 11 mmol) were added at 0 °C and the mixture was stirred for 1 h at 0 °C and kept overnight at room temperature (pH must be 7–8). Ethyl acetate (500 ml) was added and the overnight precipitated *N*,*N*′-dicyclohexylurea was filtered off. The filtrate was cooled in an ice bath and extracted with a saturated solution of sodium hydrogen carbonate (3 × 100 ml) and 5% solution of sodium chloride (3 × 100 ml), dried over anhydrous magnesium sulfate and concentrated to approximately 30 ml. Petroleum ether (80 ml) was added, the mixture was left overnight at +3 °C and the separated crystalline product (6.46 g) was recrystallized from a mixture of ethyl acetate and petroleum ether. Yield 4.71 g (66%) of compound **3**, TLC homogeneous in solvent systems chloroform–methanol (10 : 1) and butanol–acetic acid–water (4 : 1 : 1); m.p. 136–137 °C, $[\alpha]_D$ +63° (*c* 0.6, chloroform). For ^1H and ^{13}C NMR data, see Tables I and II. For $C_{36}H_{48}N_4O_{11}$ calculated: relative molecular mass 712.8, monoisotopic mass 712.3. FAB MS, *m/z*: 713.6 $[M + H]^+$. Amino acid analysis: glutamic acid 1.03, 2-aminobutanoic acid 1.04, normuramic acid 0.96. For $C_{36}H_{48}N_4O_{11}$ (712.8) calculated: 60.66% C, 6.78% H, 7.86% N; found: 60.49% C, 6.88% H, 7.65% N.

N-[2-*O*-(Benzyl 2-Acetamido-2,3-dideoxy-α-D-glucopyranosid-3-yl)glycoloyl]-L-2-aminobutanoyl-D-isoglutamine Benzyl Ester (**4**)

The fully protected compound **3** (6.36 g, 8.92 mmol) was heated under stirring in 50% aqueous acetic acid (140 ml) at 60 °C for 2 h. The solvents were evaporated and the residue was coevaporated with toluene (3 × 100 ml). Diethyl ether and petroleum ether were added and the mixture was left in a refrigerator overnight. The solid product was chromatographed on a silica gel C18 column in a

TABLE I
^{1}H NMR data of compounds **3–9** (CD_3SOCD_3, chemical shifts in ppm, coupling constants *J* in Hz)

Parameter	**3**[a]	**4**	**5**[b]	**6**[c]	**7**[d]	**8**[e]	**9**[f]
H-1	4.84 d	4.72 d	4.74 d	4.73 d	4.91 d	4.91 d	4.89 d
H-2	4.01 ddd	3.86 ddd	3.87 ddd	3.88 ddd	3.74 ddd	3.76 ddd	3.75 dd
H-3	3.59 dd	3.53 dd	3.53 dd	3.53 dd	3.53 dd	3.49 dd	3.49 dd
H-4	3.78 t	3.40 dt	3.41 bt	3.39 ddd	3.34 dd	3.34 bt	3.34 dd
H-5	3.56 dt	3.51–3.56 m	3.74 ddd	3.75 ddd	3.48–3.53 m	3.85 ddd	3.83 ddd
H-6a	3.72 dd	3.51–3.56 m	4.09 dd	4.08 dd	3.48–3.53 m	4.05 dd	4.03 dd
H-6b	3.76 dd	3.67 dt	4.34 dd	4.40 dd	3.48–3.53 m	4.30 dd	4.37 dd
N**H**Ac	8.13 d	8.11 d	8.13 d	8.11 d	8.05 d	8.02 d	8.01 d
NH**Ac**	1.85 s	1.84 s	1.84 s	1.85 s	1.84 s	1.84 s	1.83 s
H-1a′	4.01 d	4.11 d	4.12 d	4.11 d	4.09 d	4.10 d	4.09 d
H-1b′	4.12 d	4.23 d	4.23 d	4.23 d	4.20 d	4.21 d	4.21 d
H-3′	4.28 bq	4.20 bq	4.20 bq	4.20 bq	4.18 bq	4.21 bq	4.20 m
H-4a′	1.56 m	1.57 m	1.59 m	1.58 m	1.59 m	1.59 m	1.59 m
H-4b′	1.67 m	1.67 m	1.67 m	1.66 m	1.70 m	1.67 m	1.67 m
H-5′	0.81 t	0.81 t	0.82 t	0.82 t	0.84 t	0.85 t	0.85 t
H-7′	4.23 dt	4.20 ddd	4.19 dt	4.20 dt	4.15 ddd	4.17 ddd	4.17 ddd
H-8a′	1.78 ddt	1.76 ddt	1.77 dq	1.77 dq	1.70 m	1.72 m	1.72 m
H-8b′	2.03 ddt	2.05 ddt	2.04 ddt	2.04 ddt	1.97 m	1.99 ddt	1.99 m
H-9′	2.37 t	2.37 t	2.36 t	2.36 t	2.20 t	2.29 t	2.21 t
H-3′,N**H**	[g]	[g]	7.97 d	7.91 d	8.01 d	7.93 d	7.94 d
H-7′,N**H**	8.29 d	8.26 d	8.34 d	8.26 d	8.29 d	8.24 d	8.25 d
$CONH_2$	7.05 s	7.07 bs	7.04 bs	7.06 bs	[g]	[g]	[g]
OC**H**$_2$-Ph	4.48 d	4.45 d	4.44 d	4.41 d	–	–	–

TABLE I
(*Continued*)

Parameter	**3**[a]	**4**	**5**[b]	**6**[c]	**7**[d]	**8**[e]	**9**[f]
OC**H**$_2$-Ph	4.68 d	4.68 d	4.64 d	4.65 d	–	–	–
OC**H**$_2$-Ph	5.08 s (2 H)	5.08 s (2 H)	5.07 s (2 H)	5.07 s (2 H)	–	–	–
arom.H	7.28–7.40 m	7.27–7.40 m	7.27–7.38 m	7.27–7.38 m	–	–	–
J(1,2)	3.7	3.4	3.5	3.5	3.4	3.2	3.4
J(2,3)	10.3	10.8	10.9	10.5	10.5	10.5	10.5
J(3,4)	9.3	8.7	8.6	8.9	8.9	8.8	8.8
J(4,5)	9.6	8.9	10.0	9.9	9.8	9.7	9.8
J(5,6a)	10.2	[g]	6.3	6.8	[g]	5.8	5.1
J(5,6b)	5.5	5.6	1.8	1.8	[g]	2.1	2.2
J(6a,6b)	10.5	10.8	11.8	11.8	[g]	11.8	11.9
J(1a′,1b′)	15.5	16.0	16.0	16.0	16.0	16.0	15.9
J(3′,4a′)	6.9	6.8	7.0	7.2	6.9	7.1	6.9
J(3′,4b′)	6.9	6.8	7.0	7.2	6.9	7.1	6.9
J(4a′,5′)	7.5	7.5	7.5	7.5	7.4	7.6	7.6
J(7′,8a′)	9.1	9.3	8.0	8.5	9.5	9.2	9.2
J(7′,8b′)	4.8	4.9	5.0	4.9	4.9	4.9	4.7
*J*8a′,9a′)	7.8	7.8	7.9	7.9	7.6	7.8	7.9
J(8a′,9b′)′	7.8	7.8	7.9	7.9	7.6	7.8	7.9
J(8a′,8b′)	13.5	13.5	14.2	14.4	13.5	14.2	14.2
J(NH,2)	8.3	8.5	8.6	8.6	8.5	8.8	8.5
*J*NH,3′)	[g]	[g]	7.2	7.2	7.3	7.1	7.2
J(NH,7′)	8.3	8.3	8.3	8.3	8.0	8.3	8.3
J(OCH$_2$)	12.4	12.3	12.1	12.0	–	–	–

[a] Other signals: O(CH$_3$)$_2$: 1.32 s, 1.47 s; [b] OCOC$_{17}$H$_{35}$: H-2″ 2.31 t (7.4) (2 H), H-3″ to H-17″ 1.18–1.28 m (30 H), H-18″ 0.85 t (6.7); [c] OCOC$_{29}$H$_{59}$: H-2″ 2.30 m, H-3″ to H-17″ 1.20–1.29 m (46 H), 0.85 t (6.9) (6 H); [d] values for α-anomer, ratio α/β *ca* 2 :1; [e] OCOC$_{17}$H$_{35}$: H-2″ 2.21 t (7.3) (2 H), H-3″ to H-17″ 1.22–1.29 m (30 H), H-18″ 0.85 t (6.9); [f] OCOC$_{29}$H$_{53}$: H-2″ 2.30 m, H-3″ to H-17″ 1.20–1.29 m (46 H), 0.85 t (6.9) (6 H).

solvent system water–methanol (linear gradient: 50→80%/60 min), yield 4.8 g (80%) of solid product **4**, which was crystallized from a mixture methanol–ether–petroleum ether. Yield 4.25 g (71 %) of compound **4**; m.p. 208 °C, $[\alpha]_D$ +63° (*c* 0.2, methanol). For ^1H and ^{13}C NMR data, see Tables I and II. For $C_{33}H_{44}N_4O_{11}$ calculated: relative molecular mass 672.7, monoisotopic mass 672.3. FAB MS, *m/z*: 673.4 $[M + H]^+$, 695.3 $[M + Na]^+$. For $C_{33}H_{44}N_4O_{11}$ (672.7) calculated: 58.91% C, 6.59% H, 8.32% N; found: 58.66% C, 6.48% H, 8.40% N.

TABLE II
^{13}C NMR data of compounds **3–9** (CD_3SOCD_3, chemical shifts in ppm)

Carbon	Compound						
	3[a]	**4**[b]	**5**[c]	**6**[d]	**7**	**8**[e]	**9**[f]
C-1	97.0	96.1	96.2	96.2	90.8	90.8	90.8
C-2	52.8	52.8	52.7	52.7	53.4	53.2	53.2
C-3	77.4	80.8	80.5	80.5	80.7	80.5	80.5
C-4	73.9	69.7	69.9	70.1	69.9	69.3	69.4
C-5	63.6	73.1	70.1	70.2	72.2	70.0	69.9
C-6	61.5	60.5	63.0	63.0	60.7	63.3	62.7
C-1′	70.5	70.6	70.7	70.6	70.5	70.6	70.5
C-2′	173.0	173.0	172.8	173.0	174.1	173.8	173.8
C-3′	53.3	53.9	53.9	53.8	54.0	53.8	53.9
C-4′	25.6	25.0	25.1	25.1	25.0	25.1	25.0
C-5′	9.7	9.9	9.9	9.9	10.0	9.9	9.9
C-6′	172.1	172.2	172.1	172.1	173.3	173.2	173.1
C-7′	51.5	51.5	51.6	51.5	52.9	51.6	51.6
C-8′	27.0	26.7	26.7	26.7	26.8	26.8	26.8
C-9′	30.1	30.1	30.1	30.1	30.5	30.2	30.2
C-10′	171.0	171.3	171.3	171.3	171.4	171.3	171.3
C-11′	169.3	169.5	169.5	169.4	169.4	169.4	169.3
CH_2Ph	65.5	65.5	65.4	65.4	–	–	–
	68.7	68.0	68.3	68.4	–	–	–
$NHCOCH_3$	169.6	170.5	170.4	170.4	170.7	170.5	170.6
	22.5	22.5	22.5	22.4	22.6	22.6	22.6

[a] C-1″ 99.3, C-2″ 19.1, C-3″ 29.0, aromatic carbons: 137.6, 136.2, 128.4 (2 C), 128.3 (2 C), 128.0, 127.9 (2 C), 127.8 (2 C), 127.7; [b] aromatic carbons: 137.7, 136.2, 128.4 (2 C), 128.3 (2 C), 128.0, 127.9 (2 C), 127.7 (2 C), 127.6; [c] $OCOC_{17}H_{35}$: 173.1, 33.5, 31.5, 28.9 (9 C), 28.8 (3 C), 28.6, 28.4, 13.9 aromatic carbons: 137.4, 136.2, 128.3 (2 C), 128.2 (2 C), 127.9, 127.8 (4 C), 127.6; [d] $OCOC_{29}H_{59}$: 175.2, 44.7, 31.6, 31.2, 29.0 (10 C), 28.8 (5 C), 28.7 (5 C), 26.6 (3 C), 22.0, 13.8 (2 C), aromatic carbons: 137.3, 136.1, 128.4 (2 C), 128.2 (2 C), 127.9, 127.8 (2 C), 127.7 (2 C), 127.6; [e] $OCOC_{17}H_{35}$: 172.9, 33.4, 31.2, 29.0 (9 C), 28.7 (3 C), 28.4, 24.4, 13.9; [f] $OCOC_{29}H_{59}$: 175.4, 44.7, 31.6, 31.2, 29.0 (10 C), 28.9 (5 C), 28.8 (5 C), 26.6 (3 C), 22.0, 13.7 (2 C).

N-[2-*O*-(Benzyl 2-Acetamido-2,3-dideoxy-6-*O*-octadecanoyl-α-D-glucopyranosid-3-yl)-glycoloyl]-L-2-aminobutanoyl-D-isoglutamine Benzyl Ester (**5**)

To a stirred solution of compound **4** (1.345 g, 2 mmol) in dry *N*,*N*-dimethylformamide (80 ml) stearic acid (740 mg, 2.6 mmol), WSC (728 mg, 3.8 mmol) and DMAP (489 mg, 4 mmol) were added. After 6 h stirring at room temperature, another portion of WSC (728 mg, 3.8 mmol) and DMAP (489 mg, 4 mmol) were added and the stirring continued for 14 h. The reaction course was checked by TLC in chloroform–methanol (10 : 1). Methanol (5 ml) was added to the reaction mixture, the mixture was stirred for 30 min at room temperature and the solvents were distilled off. The residue was dissolved in $CHCl_3$ (200 ml) and the solution was washed twice with a solution of potassium dihydrogen phosphate, the pH of which was adjusted to 3 with sulfuric acid (KH_2PO_4 (6 g) and concentrated H_2SO_4 (2 ml) in water (40 ml)) and twice with 5% solution of sodium chloride (60 ml), dried over anhydrous magnesium sulfate and evaporated *in vacuo*. Chromatography of the residue on silica gel C18 column in a solvent system water–methanol (linear gradient: 90→100%/60 min) afforded 900 mg (48%) of solid compound **5**, $[\alpha]_D$ +33° (*c* 0.4, chloroform). For ^1H and ^{13}C NMR data, see Tables I and II. For $C_{51}H_{78}N_4O_{12}$ calculated: relative molecular mass 939.2, monoisotopic mass 938.6. FAB MS, *m/z*: 939.3 $[M + H]^+$, 961.3 $[M + Na]^+$. For $C_{51}H_{78}N_4O_{12}$ (939.2) calculated: 65.22% C, 8.37% H, 5.96% N; found: 65.41% C, 8.23% H, 6.13% N.

N-{2-*O*-[Benzyl 2-Acetamido-2,3-dideoxy-6-*O*-(2-tetradecylhexadecanoyl)-α-D-glucopyranosid-3-yl]-glycoloyl}-L-2-aminobutanoyl-D-isoglutamine Benzyl Ester (**6**)

To a stirred solution of compound **4** (1.345 g, 2 mmol) in dry *N*,*N*-dimethylformamide (80 ml) 2-tetradecylhexadecanoic acid[11] (1.177 g, 2.6 mmol), WSC (728 mg, 3.8 mmol) and DMAP (489 mg, 4 mmol) were added and the mixture was stirred at 50 °C for 6 h. Another portion of WSC (728 mg, 3.8 mmol) and DMAP (489 mg, 4 mmol) were added and the stirring continued at the same temperature for another 6 h. The reaction course was checked by TLC in chloroform–methanol (10 : 1). After cooling to room temperature, methanol (5 ml) was added, the mixture was stirred at rooom temperature for 30 min and the solvents were distilled off. The residue was dissolved in $CHCl_3$ (200 ml) and the solution was washed as described above, dried over anhydrous magnesium sulfate and evaporated *in vacuo*. Chromatography of the residue on silica gel C18 column in a solvent system water–methanol (linear gradient: 95→100%/60 min) afforded 1.2 g (54%) of solid compound **6**, $[\alpha]_D$ +30° (*c* 0.6, chloroform). For ^1H and ^{13}C NMR data, see Tables I and II. For $C_{63}H_{102}N_4O_{12}$ calculated: relative molecular mass 1 107.5, monoisotopic mass 1 106.7. FAB MS, *m/z*: 1 107.7 $[M + H]^+$, 1 130.3 $[M + Na]^+$. For $C_{63}H_{102}N_4O_{12}$ (1 107.5) calculated: 68.32% C, 9.28% H, 5.05% N; found: 68.52% C, 9.13% H, 5.15% N.

N-Acetylnormuramoyl-L-2-aminobutanoyl-D-isoglutamine (**7**)

Compound **4** (673 mg, 1 mmol) in acetic acid (150 ml) was hydrogenolyzed in the presence of 10% Pd/C catalyst (200 mg) at room temperature for 24 h. The catalyst was filtered off, washed with acetic acid (3 × 30 ml), and the filtrate was lyophilized. The product was chromatographed on a silica gel C18 column in methanol–water (1 : 19). The homogeneous fractions corresponding to α- and β-anomer in the ratio 2 : 1 were concentrated *in vacuo* and the residue was lyophilized from water, to give 480 mg (91%) of dihydrate **7**, $[\alpha]_D$ +34° (*c* 0.5, methanol) and $[\alpha]_D$ +11° (*c* 0.5, water, after 24 h). Literature[6]: m.p. 133–144 °C (crystallization with 2.5 molecules of water), $[\alpha]_D$ +33° (*c* 0.3, methanol). For ^1H and ^{13}C NMR data, see Tables I and II. For $C_{19}H_{32}N_4O_{11}$ calculated: relative molecular mass 492.5, monoisotopic mass 492.2. FAB MS, *m/z*: 493.1 $[M + H]^+$, 515.1 $[M + Na]^+$. Amino acid ana-

lysis: glutamic acid 1.05, 2-aminobutanoic acid 1.03, normuramic acid 0.94. For $C_{19}H_{32}N_4O_{11}\cdot 2\ H_2O$ (528.5) calculated: 43.17% C, 6.86% H, 10.60% N; found: 43.21% C, 6.75%H, 10.74% N.

6-*O*-Octadecanoyl-*N*-acetylnormuramoyl-L-2-aminobutanoyl-D-isoglutamine (**8**)

Compound **5** (380 mg, 0.4 mmol) in acetic acid (50 ml) was hydrogenolyzed in the presence of 10% Pd/C catalyst (400 mg) at room temperature for 16 h and worked up analogously to compound **7**. The lyophilized product was chromatographed on a silica gel C18 column in methanol–water (9 : 1). The homogeneous fractions were concentrated *in vacuo* and the residue was lyophilized from acetic acid to give 200 mg (66%) of compound **8**, $[\alpha]_D$ +17° (*c* 0.1, acetic acid). For ^1H and ^{13}C NMR data see Tables I and II. For $C_{37}H_{66}N_4O_{12}$ calculated: relative molecular mass 759.0, monoisotopic mass 758.5. FAB MS, *m/z*: 759.4 $[M + H]^+$, 781.4 $[M + Na]^+$. For $C_{37}H_{66}N_4O_{12}$ (759.0) calculated: 58.55% C, 8.76% H, 7.38% N; found: 58.67% C, 8.61% H, 7.20% N.

[6-*O*-(2-Tetradecylhexadecanoyl)-*N*-acetylnormuramoyl]-L-2-aminobutanoyl-D-isoglutamine (**9**)

Compound **6** (1.2 g, 1.084 mmol) in acetic acid (50 ml) was hydrogenolyzed in the presence of 10% Pd/C catalyst (400 mg) at room temperature for 16 h and worked up analogously to compound **7**. The lyophilized product was chromatographed on a silica gel C18 column in methanol–water (9 : 1). Homogeneous fractions were concentrated *in vacuo* and the residue was lyophilized from acetic acid to yield 698 mg (69%) of compound **9**, $[\alpha]_D$ +13° (*c* 1.0, acetic acid). For ^1H and ^{13}C NMR data, see Tables I and II. For $C_{49}H_{90}N_4O_{12}$ calculated: relative molecular mass 927.3, monoisotopic mass 926.7. FABMS, *m/z*: 927.9 $[M + H]^+$, 949.9 $[M + Na]^+$. For $C_{49}H_{90}N_4O_{12}$ (927.3) calculated: 63.46% C, 9.78% H, 6.04% N; found: 63.68% C, 9.96% H, 5.81% N.

The authors would like to express their thanks to Dr V. Pechanec for elemental analyses, Dr K. Ubik for FAB MS spectra and to Mr J. Zbrozek for amino acid analyses. Part of this work was supported by grant No. 203/96/0309 of the Grant Agency of the Czech Republic.

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