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Synthesis and Protective Anti-Infective Action of Anomeric Lipophilic Glycosides of *N*-Acetylmuramyl-*L*-Alanyl-*D*-Isoglutamine

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Abstract—Anomeric pairs of α- and β-dodecyl, α- and β-(1-pentylhexyl), and α- and β-cyclododecyl glycosides of *N*-acetylmuramyl-*L*-alanyl-*D*-isoglutamine (MDP) were synthesized. The starting β-*D*-glucosaminides were obtained by the oxazoline method, and the corresponding α-isomers, by the mercuric iodide–catalyzed glycosylation of alcohols with α-glucosaminyl chloride peracetate in nitromethane at ~90°C. No reliable differences between the stimulation of mouse resistance to the infection with *Staphylococcus aureus* (doses of 2, 20, and 200 μg/mouse) and *Escherichia coli* (doses of 0.05, 1, and 20 μg/mouse) with the MDP α- and β-glycosides were found.

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Key words: anti-infective resistance, glycopeptides, muramyldipeptide, muramyldipeptide glycosides

INTRODUCTION

Comparison of the immunostimulating action of anomeric pairs of amphiphilic *N*-acetylmuramyl-*L*-alanyl-*D*-isoglutamine (muramyldipeptide) glycosides,[2] namely, α- and β-butyl- [1], α- and β-heptyl-, and α-and β-cyclohexyl muramyldipeptides [2, 3], showed that α-glycosides both in vitro and in vivo are less active than the corresponding muramyldipeptide β-glycosides and even MDP itself. The β-anomers of MDP glycosides exhibit a more intensive adjuvant action than the corresponding α-glycosides also in the case of methyl [4] and benzyl [5] glycosides of MDP. The effect of glycoside center configuration on the biological activity was not studied for the predominantly lipophilic MDP glycosides, although it is known that changes in lipophilicity can cardinally alter the mechanism of glycopeptide penetration through the macrophage membranes. In particular, the processes of lipoglycopeptide incorporation into lipid membrane and exit from it change.

RESULTS AND DISCUSSION

We synthesized α- and β-dodecyl-, α- and β-(1-pentylhexyl)-, and α- and β-cyclododecyl glycosides of MDP (**IXa**)–(**IXf**) from aliphatic linear primary and symmetric secondary alcohols and cycloaliphatic alcohols as aglycones, in order to determine how the configuration of anomeric center and the structure and nature of aglycones of MDP glycosides affect their immunostimulating activity. On the one hand, these aglycons have similar lipophilicities ($C\log P$*);[3] they are 4.95, 4.20, and 4.52 for the corresponding alcohols. On the other hand, the conformational mobilities of hydrocarbon chains and the effective surface in this series of aglycons decrease, which undoubtedly affects the interaction with biomembranes. In particular, a relatively rigid double-stranded structure could form due to hydrophobic interactions for the aglycones of glycopeptides (**IXc**)–(**IXd**) in aqueous solutions.

The starting peracetylated β-*N*-acetylglucosaminides (**IIIa**), (**IIIc**), and (**IIIe**) were synthesized by the glycosylation of alcohols with an excess oxazoline (**I**) [6] in dichloroethane at ~90°C in the presence of catalytic amounts of TosOH. The corresponding α-anomers (**IIIb**), (**IIId**), and (**IIIf**) were synthesized by the reaction of the alcohols with α-glucosaminyl chloride (**II**) in nitromethane at ~90°C in the presence of mercuric iodide [7].

The structures of glycosides (**IIIa**)–(**IIIf**) were confirmed by ^{1}H NMR spectra, in which all protons were completely assigned (see Table 1). Methylene protons in the aglycones of (**IIIa**)–(**IIIf**) resonate in the region

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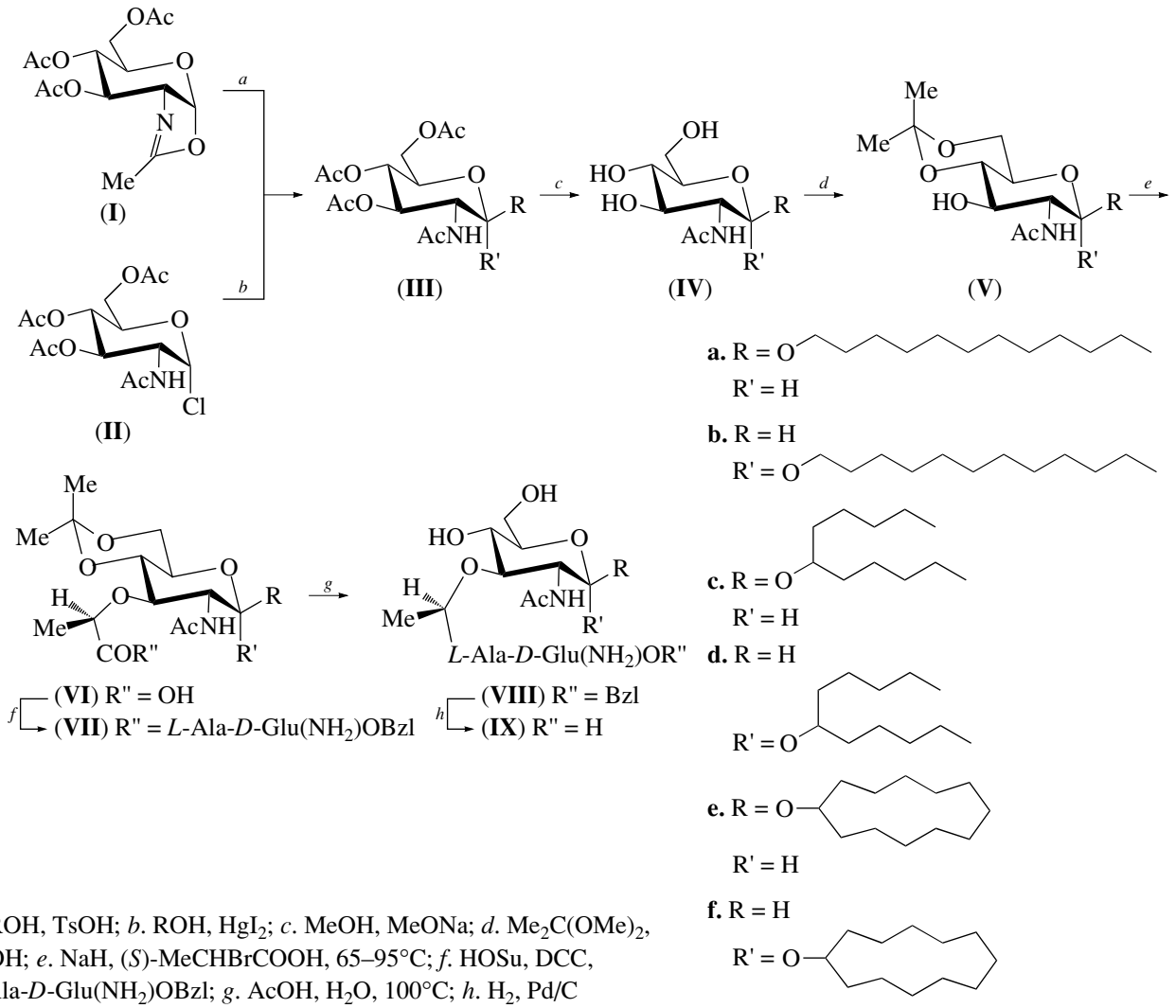
[2] Abbreviations: HONSu, *N*-hydroxysuccinimide; MDP, muramyldipeptide, *N*-acetylmuramyl-*L*-alanyl-*D*-isoglutamine.

[3] The calculated value of the logarithm of the *n*-octanol/water distribution coefficient.



of 1.19–1.63 ppm. High-field signals of the protons of terminal methyl groups (δ from 0.80 to 0.88 ppm) are represented by a triplet for each of (**IIIa**) and (**IIIb**) and by two triplets for glycosides (**IIIc**) and (**IIId**). The signals of the methyne proton coupled with oxygen atom at C1 are shifted to a low field: δ 3.48 and 3.52 ppm for (**IIIc**) and (**IIId**) and 3.71 ppm for (**IIIe**) and (**IIIf**). Nonequivalent protons of the α-methylene group of the aglycone of glycosides (**IIIa**) and (**IIIb**) are represented by two doublets of triplets with δ 3.44–3.46 and 3.62–3.86 ppm.

The β-configuration of *D*-glucosaminides (**IIIa**), (**IIIc**), and (**IIIe**) follows from the doublet of anomeric proton at 4.68–4.77 ppm with a spin coupling constant of 8.5 Hz characteristic of 1,2-*trans*-glycoside bond. The signals of anomeric protons in the case of α-*D*-glucosaminides (**IIIb**), (**IIId**), and (**IIIf**) are shifted to low field (δ 4.75–4.87 ppm) and have spin coupling constants of 3.5 Hz characteristic of 1,2-*cis*-glycosides. In addition, the spectra of the latter compounds exhibit a typical downfield shift of the proton signal at C2 (δ 4.25–4.27 ppm as compared with 3.64–3.80-ppm shift for the corresponding β-anomers) and the amide proton at δ 5.56–5.59 vs. 5.45–5.50 ppm, respectively.

The scheme of further synthesis (see the scheme) involved the successive deacetylation of peracetates (**IIIa**)–(**IIIf**) by Zemplen and isopropylidenation of triols (**IVa**)–(**IVf**) with 2,2-dimethoxypropane. Free hydroxyl groups at C3 in (**Va**)–(**Vf**) were treated with sodium hydride in dioxane and alkylated with *L*-2-bromopropionic acid as described in [8] to give *N*-acetyl-*D*-muramic acid derivatives (**VIa**)–(**VIf**). The muramic acids were activated with HONSu and DCC and then coupled with *L*-alanyl-*D*-isoglutamine benzyl ester. The isopropylidene protection was removed from glycopeptides (**VIIa**)–(**VIIf**) by acidic hydrolysis.

The benzyl groups were removed from the isoglutamine residues of glycopeptides (**VIIIa**)–(**VIIIf**) by catalytic hydrogenolysis to give the target MDP glycosides (**IXa**)–(**IXf**). The structures of (**VIIIa**)–(**VIIIf**) and (**IXa**)–(**IXf**) were confirmed by their ^{1}H NMR spectra, in which the signals of protons belonging to the

Table 1. ^{1}H NMR spectra of glycosides (**IIIa**)–(**IIIf**) in C_2HCl_3

Group or atom	Chemical shift, ppm (spin coupling constant, Hz)					
	(**IIIa**)	(**IIIb**)	(**IIIc**)	(**IIId**)	(**IIIe**)	(**IIIf**)
H1 ($J_{1,2}$)	4.69 d (8.5)	4.75 d (3.5)	4.68 d (8.5)	4.87 d (3.5)	4.77 d (8.5)	4.87 d (3.5)
H2 ($J_{2,3}$)	3.80 ddd (10.5)	4.27 ddd (10)	3.64 ddd (10.5)	4.25 ddd (10)	3.71 m (10.5)	4.25 ddd (10.5)
H3 ($J_{3,4}$)	5.32 dd (9.5)	5.15 dd (9.5)	5.29 dd (9.5)	5.13 dd (9.5)	5.37 dd (9.5)	5.13 dd (9.5)
H4 ($J_{4,5}$)	5.07 dd (9.5)	5.05 dd (9.5)	4.96 dd (9.5)	5.03 dd (9.5)	5.02 dd (9.5)	5.03 dd (9.5)
H5 ($J_{5,6a}$; $J_{5,6b}$)	3.70 ddd (2; 5)	3.87 ddd (2; 4.5)	3.61 ddd (2.5; 5)	3.95 ddd (2; 4.5)	3.71 ddd (2; 5)	3.98 ddd (2; 5)
H6a, b ($J_{6a,6b}$)	4.13 dd, 4.27 dd (12.5)	4.02 dd, 4.17 dd (12.5)	4.05 dd, 4.14 dd (12)	3.99 dd, 4.16 dd (12.5)	4.12 dd, 4.22 dd (12.5)	4.00 dd, 4.17 dd (12.5)
NAc, OAc	1.95 s, 2.02 s, 2.03 s, 2.09 s	1.88 s, 1.95 s, 1.96 s, 2.03 s	1.86 s, 1.96 s (6H), 2.00 s	1.86 s, 1.95 s, 1.96 s, 2.02 s	1.94 s, 2.03 s (6H), 2.08 s	1.87 s, 1.95 s, 1.96 s, 2.02 s
NH ($J_{2,NH}$)	5.50 d (8.5)	5.59 d (9)	5.45 d (8.5)	5.59 d (9)	5.50 d (8)	5.56 d (9.5)
C1-OCH	3.46 dt, 3.86 dt	3.44 dt, 3.62 dt	3.48 quintet	3.52 quinte	3.71 m	3.71 m
$\underline{CH_3}CH_2$	0.88 t	0.81 t	0.80 t, 0.82 t	0.82 t, 0.83 t		
$(CH_2)_n$	1.25 m, 1.56 m	1.20 m, 1.54 m	1.19 m, 1.36 m, 1.46 m	1.20 m, 1.38 m, 1.46 m	1.32 m, 1.44 m, 1.63 m	1.27 m, 1.48 m, 1.61 m

main moieties of the molecules: aglycone, glycoside residue, and lactyldipeptide component were assigned (see Table 2). The signals of protons of benzyl protecting groups were absent from the spectra of deblocked glycopeptides (**IXa**)–(**IXf**).

It is correct to assess the action of lipophilic muramyldipeptides on the immune system in vivo rather than using in vitro experiments. We used the test involving the stimulation of antibacterial resistance of mice after the intraperitoneal injection of our preparations [9]. This permits one to ignore the problem of solubility of lipoglycopeptides in aqueous solutions and possible aggregation. The study of stimulation of the mouse resistance to the infection with *Staphylococcus aureus* (Fig. 1*a*) showed that there is no reliable difference in the immunostimulating action between α- and β-dodecyl-MDPs (**IXa**) and (**IXb**) and between α- and β-cyclododecyl-MDPs (**IXe**) and (**IXf**). Moreover, unlike the MDP glycosides with alkylalicyclic, alkylaryl, cycloalkyl, and arylethyl aglycones described in the earlier communication [10], all of these glycopeptides demonstrated 100% protective effect. Presumably, the influence of the aglycone lipophilicity on the protective action dominates over the effect of configuration of its glycoside center.

The protective effect of glycosides (**IXa**)–(**IXf**) against the infection of mice with *E. coli* was studied using lower doses and a greater dilution step (Fig. 1*b*). However, the testing did not reveal any distinct influence of either the configuration of the glycoside center or the administration of lipophilic aglycones on the protective anti-infectious action of lipophilic MDP glycosides. Presumably, this is related to the fact that, under the conditions of the in vivo model when the MDP derivatives and then a lethal dose of Gram-negative bacteria are administered to the animal body, the tested compounds begin to act, depending on dose, in synergism/antagonism with various bacterial products and components, e.g., lipopolysaccharide. We plan further studies to unambiguously find a relationship between the configuration of the anomeric center of lipoglycopeptides and their biological action.

EXPERIMENTAL

Melting points were measured on a PTP device, and optical rotation was registered at 20–25°C on a Polamat-A polarimeter at λ 546 nm. ^{1}H NMR spectra were recorded on a Varian VXR-300 spectrometer (300 MHz) with Me_4Si as internal standard. Chemical shifts in δ-scale (ppm) and spin coupling constants (*J*, Hz) are presented. Signals were assigned using the methods of double homonuclear resonance, the spectra of compounds obtained previously (including those reported in [3, 7, 10]), and the published data [11].

TLC was carried out on Sorbfil-AFV-UV plates (Sorbpolimer, Russia). Substances were detected using a 5% sulfuric acid solution in ethanol followed by heating at 200–300°C. The following solvent systems were used: (*A*) 10 : 1 benzene–isopropanol, (*B*) 19 : 1 diethyl ether–isopropanol, (*C*) 10 : 1 chloroform–isopropanol, (*D*) 5 : 1 chloroform–isopropanol, and (*E*) 3 : 1 chloroform–isopropanol. Column chromatography was carried out on silica gel 60 (63–200 μm; Merck, Germany) columns [(A) 1.8 × 12 cm and (B) 1.2 × 12 cm]. The data of elemental analysis of key compounds correspond to the calculated values.

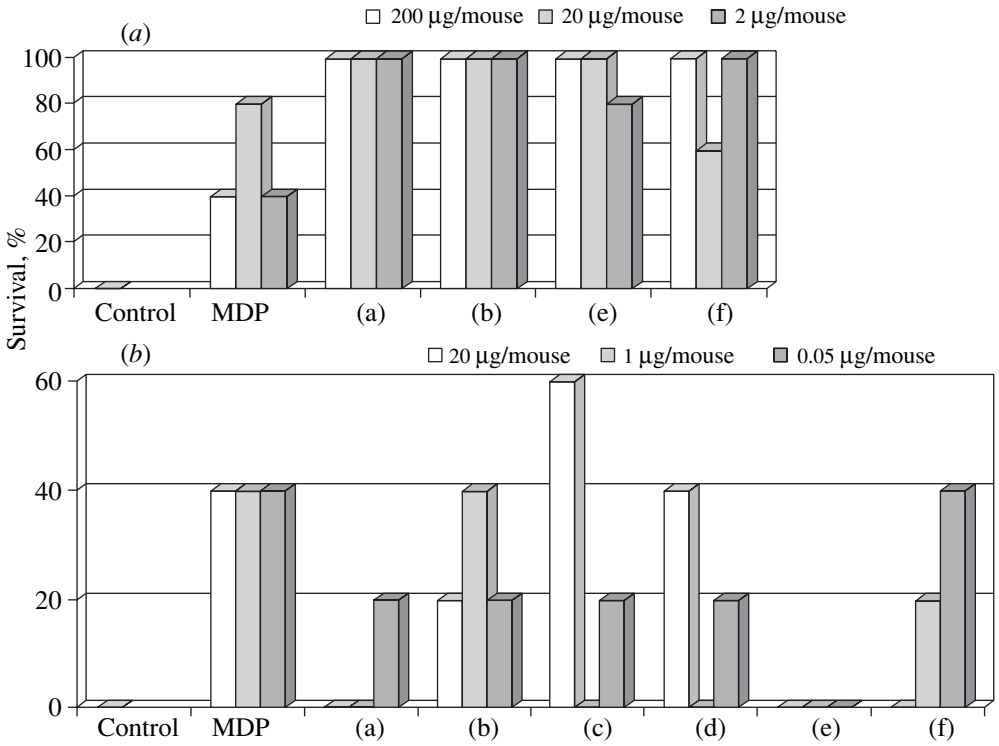


Fig. 1. Effect of MDP glycosides on the protection against intraperitoneal infection of mice with (*a*) *St. aureus* (10^9 cells/mouse) and (*b*) *E. coli* (2×10^7 cells/mouse). Doses are given in the figures.

Dodecanol-1 (Novocherkassk, Russia), undecanol-6 (Lancaster), and cyclododecanol (Acros) were used.

The biological activity was examined as described in [9]. White nonbred mice of 12–14-g body mass (Central Nursery of Experimental Animals, branch Kryukovo, Russia) of 20–25-day-old (five animals in each group) were used.

A test preparation dissolved in 0.9% NaCl was injected intraperitoneally (final volume of 0.5 ml) at doses of 200, 20, and 2 μg per mouse. Mice of the control group were injected intraperitoneally with 0.5 ml of 0.9% NaCl. After 24 h, the animals were infected either with a culture of *S. aureus* (Wood 46 strain) or an 18-h culture of *E. coli* (strain 264). The culture was administered in 0.2% agar to enhance the virulent properties of the microorganisms. The animals were under observation for 6 days. The efficiency of the preparation was estimated from the number of survived animals (in percent of animals taken into experiment).

The amounts of microbial bodies necessary for infection (10^9 for *St. aureus* and 2×10^7 for *E. coli*) were determined in preliminary experiments; they were the minimal doses inducing, upon intraperitoneal injection, 100% death of animals for the first three days.

Dodecyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranoside (IIIa). Dodecanol-1 (0.70 g, 3.79 mmol) and anhydrous TosOH (25 mg) were added to a solution of 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-*D*-glucopyrano)-[2,1-*d*]-2-oxazoline (**I**) (1.87 g, 5.87 mmol) [6] in dry dichloroethane (15 ml). The reaction was carried out at 85–90°C (bath temperature) until the complete decomposition of oxazoline under the monitoring with TLC in systems *A* and *B*. The reaction mixture was neutralized by pyridine (50 μl), and dichloroethane (15 ml) was added. The organic layer was washed with water (10 ml), and the extract was dried with anhydrous Na_2SO_4 and evaporated. The separation of the residue by column chromatography (column A, stepwise gradient of the eluent benzene–isopropanol: 100 : 1, 75 : 1, and 50 : 1; volume of a step 100 ml) and subsequent crystallization of the product from diethyl ether yielded 1.60 g (82%) of glycoside (**IIIa**); mp 103–105°C, $[\alpha]_{546}$ –15° (*c* 1.0; chloroform); for ^{1}H NMR, see Table 1. Similarly, the following compounds were synthesized: *(1-pentylhexyl) 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside* (**IIIc**), yield 0.61 g (48%); mp 178–182°C, $[\alpha]_{546}$ –10° (*c* 1.0; chloroform); for ^{1}H NMR, see Table 1; and *cyclododecyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside (***IIIe***)*: yield 0.70 g (45%); mp 160–162°C, $[\alpha]_{546}$ –21° (*c* 1.0; chloroform); for ^{1}H NMR, see Table 1.

Dodecyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-*D*-glucopyranoside (IIIb). Mercuric iodide (2.88 g, 6.34 mmol) and dodecanol-1 (1.0 g, 5.37 mmol) were added to a solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-*D*-glucopyranosyl chloride (**II**) [12] (2.0 g, 5.47 mmol) in dry nitromethane (30 ml). The reaction

Table 2. Characteristic signals of ^{1}H NMR spectra of (**VIIIa**)–(**VIIIf**) and (**IXa**)–(**IXf**) in DMSO-d_6

Group or atom		Chemical shift, ppm (spin coupling constant, Hz)											
		(**VIIIa**)	(**VIIIb**)	(**VIIIc**)	(**VIIId**)	(**VIIIe**)	(**VIIIf**)	(**IXa**)	(**IXb**)	(**IXc**)	(**IXd**)	(**IXe**)	(**IXf**)
Alk:	$\underline{CH_3}CH_2$	0.85 t	0.85 t	0.85 t	0.85 t	–	–	0.85 t	0.85 t	0.85 t	0.85 t	–	–
	$(CH_2)_n$	1.23 m, 1.43 m	1.24 m, 1.49 m	1.24 m, 1.33 m, 1.46 m	1.24 m, 1.33 m, 1.46 m	1.28 m, 1.48 m	1.28 m, 1.47 m	1.24 m, 1.43 m	1.24 m, 1.51 m	1.23 m, 1.35 m, 1.46 m	1.24 m, 1.37 m, 1.47 m	1.29 m, 1.48 m	1.29 m, 1.48 m
GlcNAc:	H1	4.25 d	4.68 d	4.29 d	4.78 d	4.33 d	4.81 d	4.26 d	4.68 d	4.30 d	4.78 d	4.33 d	4.82 d
($J_{1,2}$ Hz)		(8.5)	(3.5)	(8)	(3.5)	(8)	(3.5)	(8.5)	(3.5)	(8)	(3.5)	(8)	(3.5)
	N$\underline{Ac}$	1.75 s	1.78 s	1.73 s	1.77 s	1.74 s	1.78 s	1.75 s	1.79 s	1.73 s	1.77 s	1.74 s	1.78 s
	$\underline{NH}$Ac	7.80 d	7.99 d	7.83 d	8.01 d	7.77 d	7.98 d	7.79 d	7.95 d	7.78 d	7.94 d	7.78 d	7.96d
	C4-OH	5.26 d	5.27 brd	5.22 d	5.29 d	5.18 d	5.26 brd	5.25 brd	5.24 d	5.15 d	5.24 brd	5.18 brd	nd
	C6-OH	4.59 t	4.30 t	4.44 t	4.54 t	4.44 brt	4.31 t	4.54 brt	4.49 t	4.37 t	4.46 brt	4.27 ÛÚ	nd
$\underline{CH_3}CHCO$		1.23 m	1.24 m	1.23 d	1.22 d	1.17 d	1.22 d	1.24 m	1.24 m	1.22 d	1.22 d	1.23 d	1.22 d
Ala:	CH_3	1.23 m	1.24 m	1.23 d	1.22 d	1.17 d	1.22 d	1.24 m	1.24 m	1.25 d	1.25 d	1.25 d	1.25 d
	NH	7.39 d	7.59 d	7.39 d	7.61d	7.39 d	7.64 d	7.39 d	7.56 d	7.36 d	7.56 d	7.37 d	7.63 d
Glu:	CO_2R''	5.08 s, 7.36 m	5.08 s, 7.36 m	5.08 s, 7.36 m	5.08 s, 7.36 m	5.08 s, 7.36 m	5.08 s, 7.36 m	nd	12.06 brs	12.04 brs	12.05 brs	nd	nd
	γ-CH_2	2.36 t	2.36 t	2.36 t	2.36 t	2.36 t	2.36 t	2.19 t	2.21 t	2.19 t	2.20 t	2.19 t	2.19 t
	β-CH_2	1.77 m, 2.02 m	1.78 m, 2.01 m	1.76 m, 2.02 m	1.79 m, 2.02 m	1.79 m, 2.01 m	1.78 m, 2.01 m	1.71 m, 1.97 m	1.73 m, 1.96 m	1.70 m, 1.95 m	1.72 m, 1.95 m	1.70 m, 1.96 m	1.73 m, 1.94 m
	$CONH_2$	7.13 s, 7.32 s	7.07 s, 7.33 s	7.13 s, 7.32 s	7.08 s, 7.32 s	7.09 s, 7.32 s	7.06 s, 7.31 s	7.05 s 7.30 s	7.01 s, 7.30 s	7.04 s, 7.28 s	7.00 s, 7.29 s	7.05 s, 7.30 s	6.98 s, 7.27 s
	NH	8.12 d	8.15 d	8.13 d	8.17 d	8.08 d	8.16 d	8.10 d	8.09 d	8.03 d	8.09 d	8.05 d	8.27 d

* Abbreviations: brd, broadened doublet; brt, broadened triplet; brs, broadened singlet; nd, not detected.

mixture was stirred in the presence of molecular sieves 3 Å at 80–90°C until the glycosyl donor disappeared (monitoring by TLC in systems *A* and *B*) and then for additional 30 min (total time 1.5 h). Molecular sieves and the catalyst were filtered off, and the filtrate was evaporated. The residue was dissolved in chloroform (50 ml) and washed with 10% potassium iodide solution (20 ml) and water. The organic layer was dried with anhydrous Na_2SO_4 and evaporated. The residue was purified by chromatography on column A as described for (**IIIa**) to give 1.2 g (43%) of glycoside (**IIIb**); an amorphous powder; $[\alpha]_{546}$ +138° (*c* 1.0; chloroform); for ^{1}H NMR, see Table 1. Similarly, the following compounds were synthesized: *(1-pentylhexyl) 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranoside* (**IIId**); yield 0.79 g (38%); mp 63°C, $[\alpha]_{546}$ +94° (*c* 1.0, chloroform); for ^{1}H NMR, see Table 1, and *cyclododecyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranoside* (**IIIf**); yield 0.95 g (34%); mp 108–110°C, $[\alpha]_{546}$ +118° (*c* 0.67, chloroform); for ^{1}H NMR, see Table 1.

Dodecyl 2-acetamido-2-deoxy-β-*D*-glucopyranoside (IVa). A 0.1 N sodium methylate solution (1 ml) in methanol was added to a solution of (**IIIa**) (1.55 g, 3.01 mmol) in dry methanol (50 ml), and the mixture was kept for 16 h. The precipitate was filtered off and washed with cold methanol. The mother liquor was neutralized by cation exchanger KU-2 (H^+); the resin was washed with methanol; and the filtrate was evaporated. Total yield of (**IVa**) was 1.1 g (94%); mp 165–167°C, $[\alpha]_{546}$ –29° (*c* 1.0; ethanol). Similarly, the following compounds were synthesized: *dodecyl 2-acetamido-2-deoxy-α-D-glucopyranoside* (**IVb**); yield 0.75 g (95%); mp 144–145°C, $[\alpha]_{546}$ +117° (*c* 1.0; ethanol); *(1-pentylhexyl) 2-acetamido-2-deoxy-β-D-glucopyranoside* (**IVc**); yield 0.6 g (95%); mp 178–182°C, $[\alpha]_{546}$ –21° (*c* 1.0; ethanol); *(1-pentylhexyl) 2-acetamido-α-D-glucopyranoside* (**IVd**); yield 0.58 g (98%); mp 163–168°C, $[\alpha]_{546}$ +160° (*c* 1.0; ethanol); *cyclododecyl 2-acetamido-2-deoxy-β-D-glucopyranoside* (**IVe**); yield 0.5 g (96%); mp 204–207°C, $[\alpha]_{546}$ –15° (*c* 1.0, ethanol); and *cyclododecyl 2-acetamido-2-deoxy-α-D-glucopyranoside* (**IVf**); yield 0.5 g (89%); mp 207–212°C; $[\alpha]_{546}$ +158° (*c* 1.0, ethanol).

Dodecyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-*D*-glucopyranoside (Va). 2,2-Dimethoxypropane (1.0 ml) and TosOH (10 mg) were added to a suspension of (**IVa**) (0.5 g, 1.29 mmol) in dry THF (20 ml) under stirring and heating at 50–55°C. After 1 h (monitoring by TLC in system *A*), the reaction mixture was cooled, neutralized with pyridine, and evaporated. The residue was purified by column chromatography (column B, stepwise gradient of isopropanol in benzene 1 : 50, 1 : 25, and 1 : 10 (a step volume of 75 ml). Crystallization of the product from ether yielded 0.32 g (58%) of acetal (**Va**) as a glassy solid; $[\alpha]_{546}$ –71° (*c* 1.0, chloroform). Similarly, the following compounds were obtained: *dodecyl 2-acetamido-2-deoxy-4,6-O-isopropylidene-α-D-glucopyranoside* (**Vb**); yield 0.5 g (88%); $[\alpha]_{546}$ +96° (*c* 1.0, chloroform); *(1-pentylhexyl) 2-acetamido-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranoside* (**Vc**); yield 0.55 g (86%); $[\alpha]_{546}$ –62° (*c* 0.67, chloroform); *(1-pentylhexyl) 2-acetamido-2-deoxy-4,6-O-isopropylidene-α-D-glucopyranoside* (**Vd**); 0.5 g (81%); $[\alpha]_{546}$ +93° (*c* 0.67, chloroform); *cyclododecyl 2-acetamido-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranoside* (**Ve**); yield 0.36 g (67%); $[\alpha]_{546}$ –85° (*c* 1.0, chloroform); and *cyclododecyl 2-acetamido-2-deoxy-4,6-O-isopropylidene-α-D-glucopyranoside* (**Vf**); yield 0.45 g (85%); $[\alpha]_{546}$ +110° (*c* 1.0, chloroform).

Benzyl ester of *O*-(dodecyl 2-acetamido-2,3-dideoxy-β-*D*-glucopyranoside-3-yl)-*D*-lactoyl-*L*-alanyl-*D*-isoglutamine (VIIIa). Sodium hydride (4 equiv) was portionwise added to a stirred suspension of (**Va**) (0.26 g, 0.61 mmol) in dry dioxane (10 ml). The reaction mixture was heated to 95°C and kept at this temperature for 1 h. After cooling to 65°C, (*S*)-2-bromopropionic acid (0.075 ml, 0.9 mmol) was added, and the mixture was kept at 65°C for additional 3 h. After cooling, the sodium hydride excess was decomposed by ethanol, and the mixture was concentrated and poured into cold water (30 ml). The solution was acidified with 1 N HCl to pH 3–4, and muramic acid (**VIa**) was extracted with chloroform (3 × 20 ml). The extract was dried with anhydrous Na_2SO_4 and evaporated. The product (**VIa**) (230 mg, 75%) was dissolved without additional purification in dry THF (10 ml), and HONSu (58 mg, 0.50 mmol) and DCC (105 mg, 0.50 mmol) were added under stirring. After 5 h, the dicyclohexylurea residue was filtered off and washed with the solvent. *L*-Alanyl-*D*-isoglutamine benzyl ester trifluoroacetate [13] [obtained by the treatment of the corresponding Boc-derivative (205 mg, 0.50 mmol) with TFA followed by evaporation to dryness] and triethylamine were added to the filtrate to pH 8. After the reaction was over (monitored by TLC in system *C*), the reaction mixture was evaporated. The residue was dissolved in chloroform (50 ml), and the solution was washed with 1 N HCl, a saturated $NaHCO_3$ solution, and water (15 ml each). The organic layer was dried with anhydrous Na_2SO_4 and evaporated. The resulting glycopeptide (**VIIa**) was dissolved in 70% acetic acid (10 ml) upon heating on a boiling water bath and kept at this temperature for 15 min (monitoring by TLC in systems *C* and *D*). The solution was evaporated to dryness, and the residue was coevaporated with toluene. The residue was purified by chromatography on column B (stepwise gradient of chloroform–isopropanol 50 : 1, 25 : 1, 10 : 1, and 5 : 1; volume of a step 75 ml) and crystallized by adding ether. Yield of glycopeptide (**VIIIa**) 160 mg [35% from (**Va**)]; mp 174–180°C (dec.); $[\alpha]_{546}$ –22° (*c* 0.67, THF); for ^{1}H NMR, see Table 2.

Similarly, the following compounds were obtained: *benzyl ester of O-(dodecyl 2-acetamido-2,3-dideoxy-α-D-glucopyranosid-3-yl)-D-lactoyl-L-alanyl-D-isoglutamine* (**VIIIb**); yield 400 mg (44%); mp 178–

183°C, $[\alpha]_{546}$ +77° (*c* 1.0, ethanol); for ^{1}H NMR, see Table 2; *benzyl ester of O-[(1-pentylhexyl) 2-acetamido-2,3-dideoxy-β-D-glucopyranosid-3-yl]-D-lactoyl-L-alanyl-D-isoglutamine* (**VIIIc**); yield 340 mg (35%); mp 210–213°C, $[\alpha]_{546}$ –12° (*c* 0.67, ethanol); ^{1}H NMR: Table 2; *benzyl ester of O-[(1-pentylhexyl) 2-acetamido-2,3-dideoxy-α-D-glucopyranosid-3-yl)-D-lactoyl-L-alanyl-D-isoglutamine* (**VIIId**); yield 360 mg (32%); mp 186°C, $[\alpha]_{546}$ +19° (*c* 0.33, ethanol–chloroform 2 : 1); ^{1}H NMR: Table 2; *benzyl ester of O-(cyclododecyl 2-acetamido-2,3-dideoxy-β-D-glucopyranosid-3-yl)-D-lactoyl-L-alanyl-D-isoglutamine* (**VIIIe**); 180 mg (37%); mp 149–155°C (dec.); $[\alpha]_{546}$ +6° (*c* 067, ethanol); ^{1}H NMR: Table 2; *benzyl ester of O-(cyclododecyl 2-acetamido-2,3-dideoxy-α-D-glucopyranosid-3-yl)-D-lactoyl-L-alanyl-D-isoglutamine* (**VIIIf**); yield 260 mg (34%); mp 209–215°C (dec.); $[\alpha]_{546}$ +83° (*c* 1.0, ethanol); ^{1}H NMR: Table 2.

***O*-(Dodecyl 2-acetamido-2,3-dideoxy-β-*D*-glucopyranosid-3-yl)-*D*-lactoyl-*L*-alanyl-*D*-isoglutamine (IXa).** Benzyl ester (**VIIIa**) (165 mg, 0.22 mmol) was dissolved in 9 : 1 THF–water mixture (15 ml) and subjected to hydrogenolysis over 10% Pd/C (50 mg) at room temperature for 4 h (monitoring by TLC in system *E*). The catalyst was filtered off and washed with 5 ml of a mixture of solvents, and the filtrate was evaporated to dryness. The residue was triturated under a layer of ether to get 120 mg (83%) of amorphous glycopeptide (**IXa**); $[\alpha]_{546}$ +4° (*c* 0.67, ethanol).

Similarly, the following compounds were obtained: *O-(dodecyl 2-acetamido-2,3-dideoxy-α-D-glucopyranosid-3-yl)-D-lactoyl-L-alanyl-D-isoglutamine* (**IXb**); yield 180 mg (86%); amorphous powder, $[\alpha]_{546}$ +85° (*c* 1.0, ethanol); *O-[(1-pentylhexyl) 2-acetamido-2,3-dideoxy-β-D-glucopyranosid-3-yl]-D-lactoyl-L-alanyl-D-isoglutamine* (**IXc**); yield 250 mg (96%); mp 174–175°C, $[\alpha]_{546}$ +2° (*c* 1.0, ethanol); *O-[(1-pentylhexyl) 2-acetamido-2,3-dideoxy-α-D-glucopyranosid-3-yl)-D-lactoyl-L-alanyl-D-isoglutamine* (**IXd**); yield 150 mg (78%); mp 185–187°C, $[\alpha]_{546}$ +90° (*c* 0.67, ethanol); *O-(cyclododecyl 2-acetamido-2,3-dideoxy-β-D-glucopyranosid-3-yl)-D-lactoyl-L-alanyl-D-isoglutamine* (**IXe**); yield 70 mg (98%); amorphous powder, $[\alpha]_{546}$ +3° (*c* 1.0, ethanol–chloroform 2 : 1); and *O-(cyclododecyl 2-acetamido-2,3-dideoxy-α-D-glucopyranosid-3-yl)-D-lactoyl-L-alanyl-D-isoglutamine* (**IXf**); yield 200 mg (91%); amorphous powder, $[\alpha]_{546}$ +93° (*c* 1.0, ethanol).

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