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N-Acetylmuramyl-*L*-Alanyl-*D*-Isoglutamine Glycosides: Effect of Glycoside Bond Configuration and Aglycone on Biological Activity

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Abstract—Hexyl, octyl, and cyclohexyl β-glycosides and heptyl and cyclohexyl α-glycosides of muramyl dipeptide (MDP) were synthesized. Tests *in vitro* and *in vivo* revealed lower immunostimulating activities of MDP α-glycosides in comparison with the corresponding β-glycosides and MDP itself. In the case of alkyl β-glycosides, differences in hydrocarbon chain lengths (C_4–C_8) and in aglycone (aliphatic chain and aliphatic or aromatic ring) exerted no substantial effect on the immunostimulating activity.

Key words: *glycopeptides, muramyl dipeptide, muramyl dipeptide glycosides, immunostimulating activity, nonspecific infectious resistance*

Methyl α- and β-glycosides of *N*-acetylmuramyl-*L*-alanyl-*D*-isoglutamine had been synthesized before the beginning of our studies in order to use glycosylation for fixing the configuration of the anomeric center [1] or the furanose form of MDP [2].[2] We used *O*-glycosylation of MDP as a method of chemical modification, which can affect the biological activity of this compound. Various (alkyl [3, 4], 2,3-didodecyloxypropyl [5], cholesteryl [6], and aryl [7]) β-glycosides of MDP possess high immunostimulating activities both *in vitro* [8, 9] and *in vivo* [9–11]. With the goal to reveal structural factors that affect the biological activities of these MDP derivatives, we synthesized in this work hexyl, octyl, and cyclohexyl β-glycosides and heptyl and cyclohexyl α-glycosides of MDP (**VIIa**)–(**VIIe**). Butyl [3], heptyl [4], and phenyl [7] β-glycosides of MDP synthesized earlier were also used in biological tests.

The MDP glycosides were synthesized according to the known scheme (see scheme). The starting α- and β-glycosides of *N*-acetylglucosamine (**Ia**)–(**Ie**) were obtained by glycosylation of the corresponding alcohols with 2-acetamido-3,4,6-tri-*O*-acetyl-α-*D*-glucopyranosyl chloride by a modified Koenigs–Knorr method using mercury(II) iodide as catalyst [12]. Then they were deacetylated to (**IIa**)–(**IIe**), in which 4,6-diol moiety was protected by the treatment with 2,2-dimethoxypropane. Free hydroxy group at C3 was alkylated with (*S*)-2-bromo- or (*S*)-2-chloropropionic acid in dioxane in the presence of sodium hydride to give the derivatives of *N*-acetyl-*D*-muramic acid (**IVa**)–(**IVe**), which were then coupled with *L*-alanyl-*D*-glutamine benzyl ester by the *N*-hydroxysuccinimide method. The two-step deprotection (acidic hydrolysis of the acetal moiety and catalytic hydrogenolysis of the benzyl ester in the glutamine residue) resulted in the target glycosides (**VIIa**)–(**VIIe**).

A comparison of ^{1}H NMR spectra of deacetylated glycosides (**IIa**)–(**IIe**) with those of glycosyl glycopeptides (**VIa**)–(**VIe**) confirmed the introduction of the lactylpeptide fragment. In particular, this was proven by the presence of resonances of benzyl ester protons (singlets of methylene groups at δ 5.02–5.08 ppm and multiplets of phenyl protons at δ 7.35–7.36 ppm), triplets of the γ-methylene group at δ 2.20–2.36 ppm, and two singlets of amide protons of isoglutamine residue in a range of 7.06–7.13 and 7.29–7.34 ppm (see table for details).

Activation of the production of such important for antitumor and antibacterial immunity mono- and lymphokines as TNF and ILs is the most characteristic indicator of the immunostimulating activity of MDP and its derivatives *in vitro*. The effect of MDP glycosides on the production of IL-1, IL-2, and TNF by murine peritoneal macrophages are given in Fig. 1. The total effect of muramyl dipeptides on immune system can be easily estimated in the test of stimulation of the nonspecific

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[2] Abbreviations: ConA, concanavalin A; IL, interleukin; LPS, lipopolysaccharide; MDP, muramyl dipeptide, *N*-acetylmuramyl-*L*-alanyl-*D*-isoglutamine; TNF, tumor necrosis factor. Aglycone designations and other abbreviations correspond to IUPAC–IUB recommendations.

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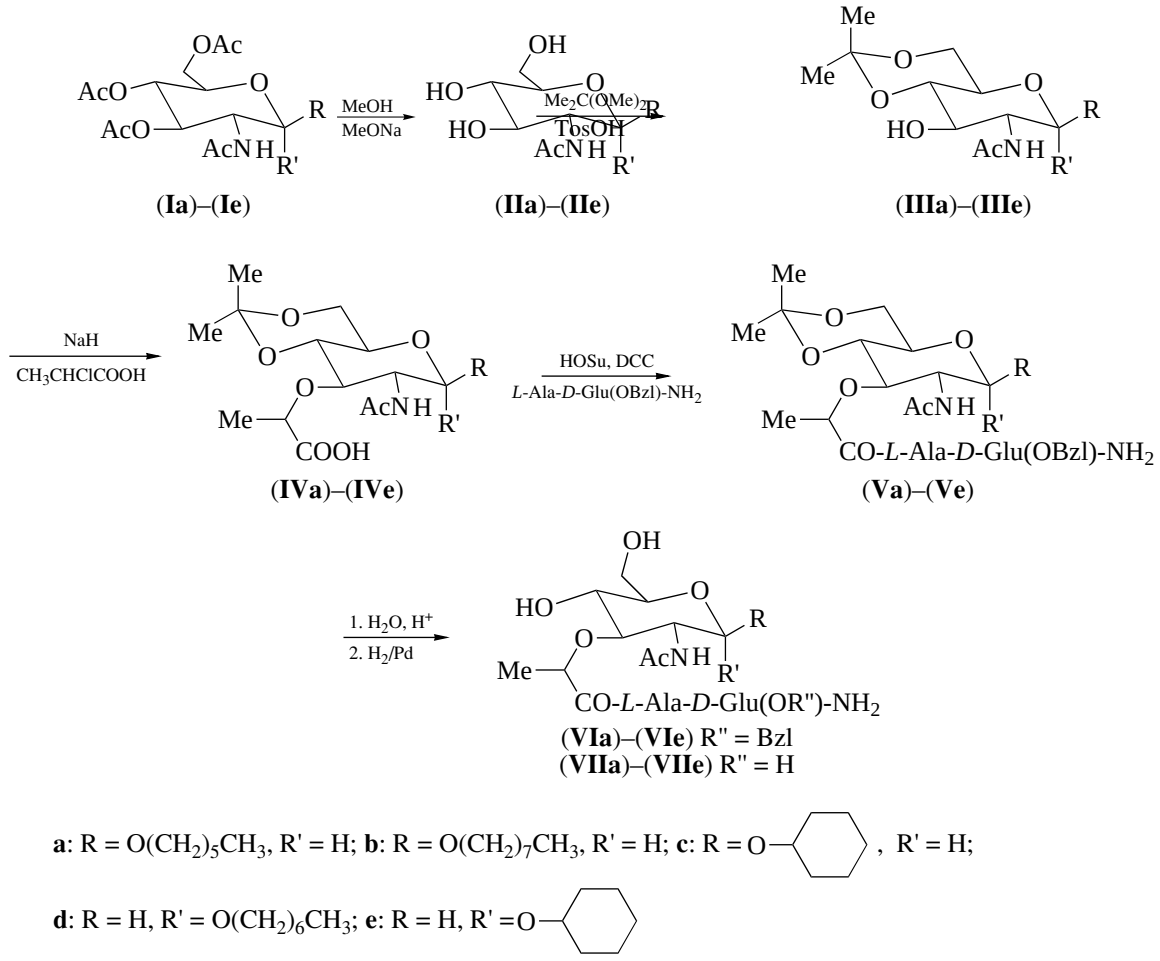

Scheme 1.

antiinfectious resistance in mice using the model of peritonitis caused by the intraperitoneal administration of *Salmonella typhi* at a dose of 100 LD_{50} (Fig. 2).[3]

A comparison of the immunostimulating activity of MDP heptyl and cyclohexyl α- and β-glycosides demonstrated using both *in vitro* and *in vivo* tests that α-glycosides are less active than both the corresponding β-glycosides and MDP itself. A similar effect was observed in the case of MDP butyl α-glycoside in the study of activation of T- and B-cell immune response to HIV-1 proteins [9]. A higher adjuvant activity of MDP methyl β-glycoside over that of the corresponding α-glycoside in the test for the induction of experimental allergic encephalomyelitis was reported in [13]; MDP benzyl β-glycoside showed a stronger adjuvant action than benzyl α-glycoside in the test for delayed hypersensitivity [14]. It seems likely that the β-anomer of MDP is the active form. This assumption helps explain a lower stimulating effect of free MDP, which exists in solution as a mixture of α- and β-anomers, than that of MDP β-glycosides. A low adjuvant capacity of α-1-*O*-acyl MDP [15] compared to that of the mixture of α- and β-1-*O*-acyl-MDP [16] also becomes clear.

A comparison of stimulating effects of MDP alkyl β-glycosides with various lengths of hydrocarbon chain and also MDP glycosides with aglycones with the same number of carbon atoms but different chemical structures (aliphatic chain, aliphatic ring, or aromatic ring) showed that all of them possess comparable immunostimulating activity.

EXPERIMENTAL

Melting points were taken on a PTP apparatus. The values of optical rotation were measured on a Polamat-A) polarimeter at λ 546 nm and 20–25°C. ^{1}H NMR spectra were registered on a Varian VXR-300 (300 MHz) instrument in DMSO-d_6 using Me_4Si as an internal standard. Chemical shifts (δ, ppm) and coupling constants (*J*, Hz) are given.

[3] The studies were performed in cooperation with the researches from the Mechnikov Research Institute of Vaccines and Sera, Russian Academy of Medical Sciences, Moscow.

^{1}H NMR spectra of compounds (**IIa**)–(**IIe**) and (**VIa**)–(**VIe**)*

Group or atom	**(IIa)**	**(VIa)**	**(IIb)**	**(VIb)**	**(IIc)**	**(VIc)**	**(IId)**	**(VId)**	**(IIe)**	**(VIe)**
R	0.86t, 1.27m, 1.47m	0.85t, 1.24m, 1.43m	0.86t, 1.24m, 1.43m	0.86t, 1.24m, 1.44m	1.26m, 1.46m, 1.67m	1.23m, 1.41m, 1.60m	0.85t, 1.24m, 1.45m	0.86t, 1.26m, 1.51m	1.22m, 1.45m, 1.66m	1.22m, 1.43m, 1.66m
H1 ($J_{1,2}$)	4.28d (8.0)	4.35d (8.0)	4.25d (8.0)	4.25d (8.0)	4.43d (8.5)	4.37d (8.0)	4.72d (3.0)	4.69d (3.0)	4.79d (3.0)	4.84d (3.5)
NAc	1.79s	1.75s	1.78s	1.75s	1.82s	1.74s	1.80s	1.79s	1.82s	1.78s
C4-OH, C6-OH	4.72d, 4.32bt	5.28d, 4.61t	4.85d, 4.47bt	4.25d, 4.57bt	4.89bt, 4.53bt	5.24d, 4.56t	4.72d, 4.52t	5.27d, 4.53t	4.66d, 4.46t	5.28d, 4.53t
C$\underline{H}_3$CH	–	1.24m	–	1.24m	–	1.23m	–	1.22d, 1.23d	–	1.22d, 1.25d
C$\underline{H}$CH$_3$	–	4.22q	–	4.22q	–	4.23q	–	4.29q	–	4.29m
CH(Ala)	–	4.18dq	–	4.12dq	–	4.17dq	–	4.28dq	–	4.29m
CH(*i*Gln)	–	4.10ddd	–	3.69ddd	–	4.09ddd	–	4.18ddd	–	4.18ddd
β-CH$_2$(*i*Gln)	–	1.77m, 2.01m	–	1.71m, 1.94m	–	1.77m, 2.01m	–	1.76m, 2.02m	–	1.73m, 2.01m
γ-CH$_2$(*i*Gln)	–	2.35t	–	2.20t	–	2.35t	–	2.36t	–	2.36t
CONH$_2$(*i*Gln)	–	7.13s 7.29s	–	7.09s 7.33s	–	7.12s 7.32s	–	7.06s 7.32s	–	7.08s 7.34s
CH$_2$Ph	–	5.08s, 7.36m	–	5.02s, 7.36m	–	5.07s, 7.35m	–	5.08s, 7.36m	–	5.08s, 7.36m
NH	7.50d	7.39d, 7.80d, 8.13d	7.63d	7.39d, 7.80d, 8.09d	7.54d	7.41d, 7.77d, 8.12d	7.56d	7.59d, 7.98d, 8.14d	7.62d	7.65d, 7.98d, 8.17d

* Abbreviations: bd, broadened doublet; bt, broadened triplet; dd, doublet of doublets; dq; doublet of quartets; and ddd, doublet of doublets of doublets.

TLC was performed on Silufol UV-254 (Kavalier) and Kieselgel 60 F_{254} (Merck) precoated plates. Spots were visualized by charring at 300°C (Silufol) or treating with 2% H_2SO_4 in *n*-butanol and subsequent heating at 150°C (Kieselgel). The following developing systems were used: (*A*) 15 : 1 and (*B*) 5 : 1 chloroform–ethanol, (*C*) 10 : 1 : 1 chloroform–benzene–ethanol, (*D*) 100 : 10 : 10 : 10 : 3 ethanol–*n*-butanol–pyridine–water–acetic acid; and (*E*) 3 : 1 : 1 *n*-butanol–acetic acid–water. Column chromatography was carried out on Silica gel 70–300 mesh (Aldrich) and 230–400 mesh (Merck).

Elemental analysis data for key compounds correspond to calculated values. Biological activity was studied by the techniques reported in [8, 10].

A number of general synthetic procedures were used in this study.

Zemplen deacetylation. Sodium methylate (0.1 N solution in methanol, 0.01–0.05 equiv) was added to a solution of acetate (**Ia**)–(**Ie**) in anhydrous methanol (10 ml/g). The reaction mixture was kept for 12–24 h and neutralized with KU-2 (H^+) cation exchanger. The resin was filtered off and washed with methanol. The combined filtrate was concentrated in a vacuum.

Isopropylidenation. 2,2-Dimethoxypropane (3 equiv) and dry TosOH were added to a solution of triol (**IIa**)–(**IIe**) in dioxane or THF (50 ml/g). After 1 h (TLC monitoring in systems *A* and *C*), the reaction mixture was neutralized with pyridine and evaporated in a vacuum. The residue was dissolved in chloroform (50 ml) and washed with water (3 × 25 ml). The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness.

Synthesis of muramic acids. Sodium hydride (4 equiv) was added in portions to a stirred solution of acetal (**IIIa**)–(**IIIe**) in anhydrous dioxane (20 ml/g). The reaction mixture was heated to 95°C, kept for 1 h at this temperature, and cooled to 65°C. (*S*)-2-Bromopropionic acid (1.5 equiv) or (*S*)-2-chloropropionic acid (2 equiv) was added, and the mixture was kept at 65°C for another 1 h. The reaction mixture was cooled, the excess of sodium hydride was decomposed with ethanol, and the mixture was concentrated and poured in cold water. The solution was acidified with 1 N hydrochloric acid to pH 2–3, and muramic acid was extracted with chloroform. The extract was dried over anhydrous Na_2SO_4 and concentrated in a vacuum.

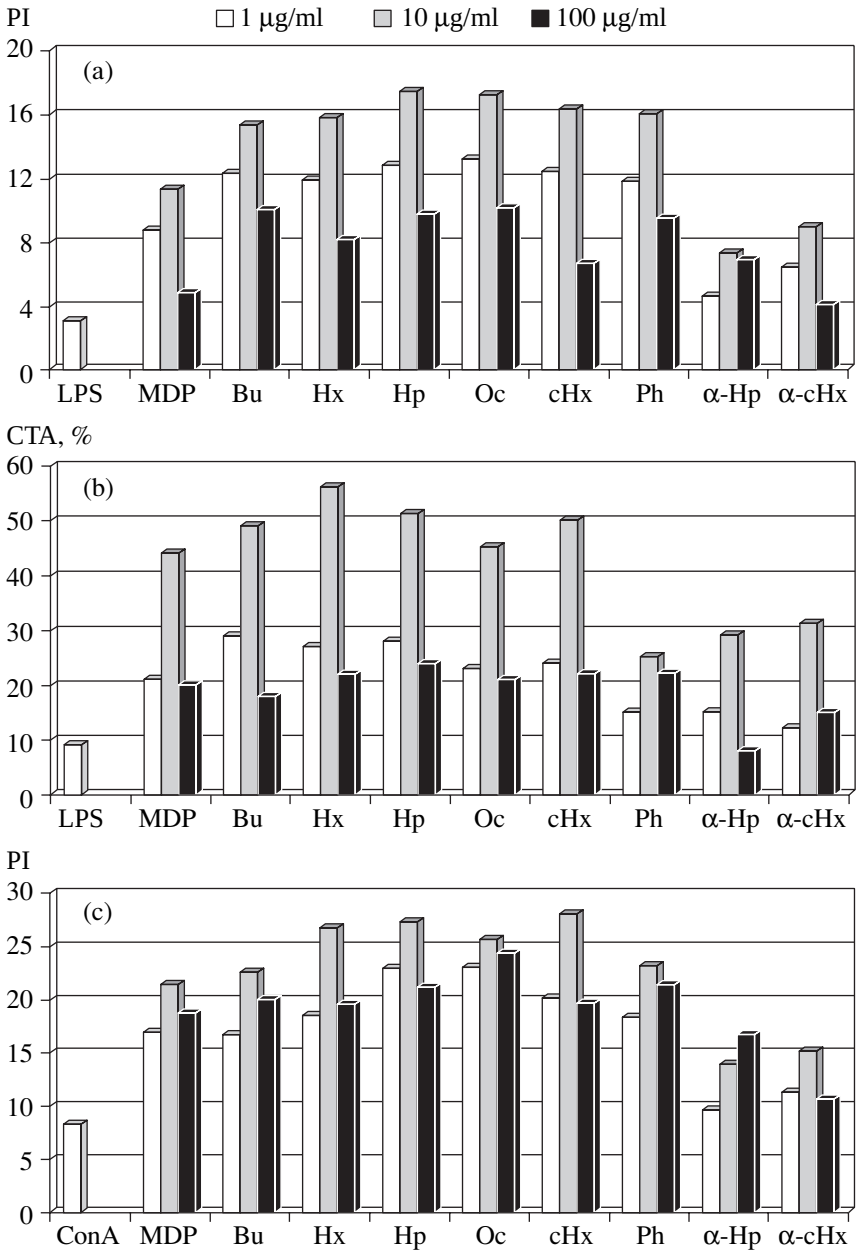

Fig. 1. The effect of MDP glycosides (in doses of 1, 10, and 100 μg/ml; the corresponding aglycone is given under the abscissa axis) in combination with suboptimal dose of (a, b) LPS (20 ng/ml) and (c) concanavalin A (1 μg/ml) on the production of (a) IL-1, (b) TNF by peritoneal macrophages, and (c) IL-2 by murine splenocytes C57BL/6. The experiment was carried out as described earlier [8]. PI, proliferation index; CTA, cytotoxic activity. All glycosides belong to β-series except for heptyl (α-Hp) and cyclohexyl (α-cHx) glycosides.

Synthesis of glycopeptides by the N-hydroxysuccinimide method. *N*-Hydroxysuccinimide (1.1 equiv) and DCC (1.1 equiv) were added to a solution of muramic acid (**IVa**)–(**IVe**) in anhydrous dioxane or THF (10 ml/g) under stirring. After 3–5 h (TLC monitoring in systems *A* and *C*), the precipitate of dicyclohexylurea was filtered off and washed with the solvent. *L*-Alanyl-*D*-isoglutamine benzyl ester trifluoroacetate [17] (1 equiv) and triethylamine (to pH 8) were added to the filtrate. After the reaction was finished (TLC monitoring in systems *A* and *C*), the solvent was evaporated.

Hydrolysis of isopropylidene groups. Alkylidene derivatives (**Va**)–(**Ve**) were dissolved in 80% acetic acid (10 ml/g) under heating in boiling water bath, kept for 5–15 min at this temperature (TLC monitoring in systems *A* and *C*), and evaporated to dryness.

Hydrogenolysis of benzyl protecting groups. Benzyl esters (**VIa**)–(**VIe**) were dissolved in ethanol (50–100 ml/g) and hydrogenated over 10% Pd/C (cata-

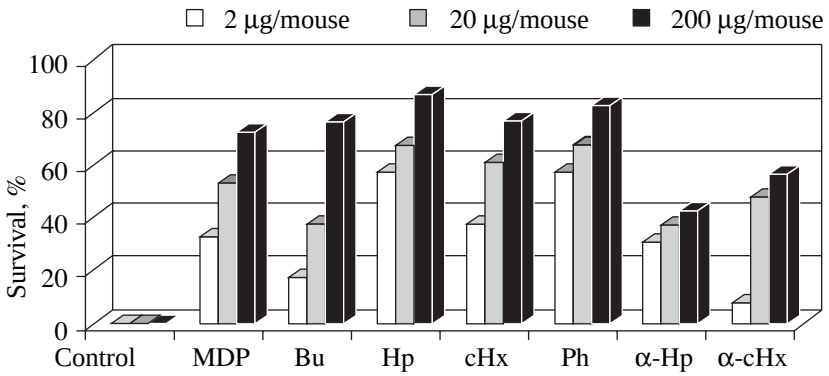

Fig. 2. The effect of MDP glycosides (in doses of 2, 20, and 200 μg/mouse) on the protective effect upon the i.p. administration of *S. typhi* (10^3 cell/mouse) to mice. The experiment was carried out as described earlier [10]. The same abbreviations as in Fig. 1 are used.

lyst–substance ratio of 1 : 5) at room temperature for 3–6 h. After the reaction was finished (TLC monitoring in systems *D* and *E*), the catalyst was filtered off and washed with the solvent. The filtrate was evaporated in a vacuum.

***O*-(Hexyl 2-acetamido-2,3-dideoxy-β-*D*-glucopyranosid-3-yl)-*D*-lactoyl-*L*-alanyl-*D*-isoglutamine (VIIa).** *Hexyl-2-acetamido-2-deoxy*-β-*D-glucopyranoside (**IIa**)* was obtained by deacetylation of acetate (**Ia**) (1.54 g, 3.57 mmol); yield 1.0 g (92%); mp 160–162°C; $[\alpha]_{546}$ –29° (*c* 1.0, ethanol). For ^{1}H NMR data, see table.

Hexyl 2-acetamido-2-deoxy-4,6-O-isopropylidene-β-*D-glucopyranoside (**IIIa**)* was synthesized by isopropylidenation of triol (**IIa**) (1.0 g, 3.28 mmol); yield after column chromatography (elution with chloroform ⟶ 20 : 1 chloroform–ethanol) 0.61 g (54%); glassy substance, $[\alpha]_{546}$ –88° (*c* 1.0, chloroform).

Hexyl 2-acetamido-2-deoxy-4,6-O-isopropylidene-3-O-(D-1-carboxyethyl)-β-*D-glucopyranoside (**IVa**)* was obtained by alkylation of acetal (**IIIa**) (485 mg, 1.41 mmol) with (*S*)-bromopropionic acid; yield 0.57 g (97%); amorphous substance; $[\alpha]_{546}$ –15° (*c* 1.0, chloroform).

O-Hexyl 2-acetamido-2,3-dideoxy-β-*D-glucopyranosid-3-yl)-D-lactoyl-L-alanyl-D-isoglutamine benzyl ester (**VIa**).* Muramic acid (**IVa**) (0.55 g, 1.32 mmol) was condensed with dipeptide. The isopropylidene protecting group of the resulting (**Va**) was removed by hydrolysis. The target compound (**VIa**) was isolated by column chromatography (elution with chloroform ⟶ 10 : 1 chloroform–ethanol); yield 0.48 g (55%); amorphous powder; $[\alpha]_{546}$ +2° (*c* 1.0, ethanol). For ^{1}H NMR data, see table.

O-(Hexyl 2-acetamido-2,3-dideoxy-β-*D-glucopyranosid-3-yl)-D-lactoyl-L-alanyl-D-isoglutamine (**VIIa**)* was obtained by catalytic hydrogenolysis of benzyl ester (**VIa**) (250 mg, 0.38 mmol); yield 170 mg (78%); amorphous powder; $[\alpha]_{546}$ +5° (*c* 0.83, ethanol).

***O*-Octyl 2-acetamido-2,3-dideoxy-β-*D*-glucopyranosid-3-yl)-*D*-lactoyl-*L*-alanyl-*D*-isoglutamine (VIIb).** *O-Octyl 2-acetamido-2-deoxy*-β-*D-glucopyranoside (**IIb**)* was obtained by deacetylation of acetate (**Ib**) (2.5 g, 5.45 mmol); yield 1.80 g (99%); mp 185–188°C, $[\alpha]_{546}$ –42° (*c* 1.0, DMF). For ^{1}H NMR data, see table.

Octyl 2-acetamido-2-deoxy-4,6-O-isopropylidene-β-*D-glucopyranoside (**IIIb**)* was synthesized by isopropylidenation of triol (**IIb**) (1.65 g, 4.95 mmol); yield after column chromatography (elution with chloroform ⟶ 25 : 1 chloroform–ethanol) 1.55 g (84%); amorphous substance; $[\alpha]_{546}$ –71° (*c* 1.0, chloroform).

Octyl 2-acetamido-2-deoxy-4,6-O-isopropylidene-3-O-(D-1-carboxyethyl)-β-*D-glucopyranoside (**IVb**)* was obtained by alkylation of acetal (**IIIb**) (2.2 g, 5.9 mmol) with (*S*)-bromopropionic acid; yield after column chromatography (elution with chloroform ⟶ 25 : 1 chloroform–ethanol) 2.4 g (92%); glassy substance, $[\alpha]_{546}$ –12° (*c* 1.0, chloroform).

***O*-(Octyl 2-acetamido-2,3-dideoxy-β-*D*-glucopyranosid-3-yl)-*D*-lactoyl-*L*-alanyl-*D*-isoglutamine benzyl ester (VIb).** Condensation of muramic acid (**IVb**) (2.0 g, 4.49 mmol) with dipeptide resulted in glycopeptide (**Vb**) (2.0 g, 61%). The resulting compound (**Vb**) (1.0 g, 1.36 mmol) was deprotected by acidic hydrolysis, and the target (**VIb**) was isolated by column chromatography (elution with chloroform ⟶ 5 : 1 chloroform–ethanol); yield 0.72 g (76%); amorphous powder; $[\alpha]_{546}$ +9° (*c* 1.0, ethanol). For ^{1}H NMR data, see table.

O-(Octyl 2-acetamido-2,3-dideoxy-β-*D-glucopyranosid-3-yl)-D-lactoyl-L-alanyl-D-isoglutamine (**VIIb**)* was obtained by catalytic hydrogenolysis of benzyl ester (**VIb**) (350 mg, 0.51 mmol); yield 290 mg (95%); amorphous powder; $[\alpha]_{546}$ +12° (*c* 0.93, ethanol).

***O*-(Cyclohexyl 2-acetamido-2,3-dideoxy-β-*D*-glucopyranosid-3-yl)-*D*-lactoyl-*L*-alanyl-*D*-isoglutamine (VIIc).** *Cyclohexyl 2-acetamido-2-deoxy*-β-*D-glucopyranoside (**IIc**)* was obtained by deacetylation of

acetate (**Ic**) (2.8 g, 6.5 mmol); yield 1.9 g (96%). An analytical sample was crystallized from methanol; mp 167–169°C, $[\alpha]_{546}$ –37° (*c* 0.67, methanol). For ^{1}H NMR data, see table.

*Cyclohexyl 2-acetamido-2-deoxy-4,6-O-isopropylidene-*β*-D-glucopyranoside (**IIIc**)* was synthesized by isopropylidenation of triol (**IIc**) (830 mg, 2.74 mmol); yield after column chromatography (elution with 75 : 1 benzene–ethanol ⟶ 25 : 1 benzene–ethanol) 765 mg (81%); glassy substance; $[\alpha]_{546}$ –69° (*c* 1.0, chloroform).

*Cyclohexyl-2-acetamido-2-deoxy-4,6-O-isopropylidene-3-O-(D-1-carboxyethyl)-*β*-D-glucopyranoside (**IVc**)* was obtained by alkylation of acetal (**IIIc**) (750 mg, 2.19 mmol) with (*S*)-bromopropionic acid; yield 850 mg (94%); amorphous powder, $[\alpha]_{546}$ –6° (*c* 1.0, chloroform).

*O-Cyclohexyl 2-acetamido-2,3-dideoxy-*β*-D-glucopyranosid-3-yl)-D-lactoyl-L-alanyl-D-isoglutamine benzyl ester (**VIc**).* Muramic acid (**IVc**) (835 g, 2.01 mmol) was condensed with the dipeptide. The isopropylidene protecting group was removed from the resulting compound (**Vc**) by hydrolysis. The target compound (**VIc**) was isolated by column chromatography (elution with chloroform ⟶ 10 : 1 chloroform–ethanol); yield 635 mg (48%); amorphous powder; $[\alpha]_{546}$ +6° (*c* 1.0, ethanol). For ^{1}H NMR data, see table.

*O-(Cyclohexyl 2-acetamido-2,3-dideoxy-*β*-D-glucopyranosid-3-yl)-D-lactoyl-L-alanyl-D-isoglutamine (**VIIc**)* was obtained by catalytic hydrogenolysis of benzyl ester (**VIc**) (325 mg, 0.49 mmol); yield 265 mg (92%); amorphous powder; $[\alpha]_{546}$ +6° (*c* 0.67, ethanol).

***O*-(Heptyl 2-acetamido-2,3-dideoxy-α-*D*-glucopyranosid-3-yl)-*D*-lactoyl-*L*-alanyl-*D*-isoglutamine (VIId).** *Heptyl 2-acetamido-2-deoxy-*α*-D-glucopyranoside (**IId**)* was obtained by deacetylation of acetate (**Id**) (800 mg, 1.80 mmol); yield 500 mg (87%). An analytical sample was crystallized from methanol; mp 163–165°C, $[\alpha]_{546}$ +131° (*c* 1.0, methanol). For ^{1}H NMR data, see table.

*Heptyl 2-acetamido-2-deoxy-4,6-O-isopropylidene-*α*-D-glucopyranoside (**IIId**)* was synthesized by isopropylidenation of triol (**IId**) (1.0 g, 3.30 mmol); after column chromatography (elution with 75 : 1 benzene–ethanol ⟶ 25 : 1 benzene–ethanol), yield 925 mg (82%); glassy substance; $[\alpha]_{546}$ +100° (*c* 1.0, chloroform).

*Heptyl 2-acetamido-2-deoxy-4,6-O-isopropylidene-3-O-(D-1-carboxyethyl)-*α*-D-glucopyranoside (**IVd**)* was obtained by alkylation of acetal (**IIId**) (250 mg, 0.70 mmol) with (*S*)-2-chloropropionic acid; yield 228 mg (76%); amorphous powder, $[\alpha]_{546}$ +85° (*c* 1.0, chloroform).

*O-Heptyl 2-acetamido-2,3-dideoxy-*α*-D-glucopyranosid-3-yl)-D-lactoyl-L-alanyl-D-isoglutamine benzyl ester (**VId**).* Muramic acid (**IVd**) (228 g, 0.53 mmol) was condensed with the dipeptide. The isopropylidene protecting group of the resulting glycopeptide (**Vd**) was removed by hydrolysis. The target compound (**VId**) was isolated by precipitation with diethyl ether; yield 290 mg (78%); amorphous substance; $[\alpha]_{546}$ +95° (*c* 0.83, ethanol). For ^{1}H NMR data, see table.

*O-(Heptyl 2-acetamido-2,3-dideoxy-*α*-D-glucopyranosid-3-yl)-D-lactoyl-L-alanyl-D-isoglutamine (**VIId**)* was obtained by catalytic hydrogenolysis of benzyl ester (**VId**) (190 mg, 0.29 mmol); yield 150 mg (90%); amorphous powder; $[\alpha]_{546}$ +96° (*c* 0.67, ethanol).

***O*-(Cyclohexyl 2-acetamido-2,3-dideoxy-α-*D*-glucopyranosid-3-yl)-*D*-lactoyl-*L*-alanyl-*D*-isoglutamine (VIIe).** *Cyclohexyl 2-acetamido-2-deoxy-*α*-D-glucopyranoside (**IIe**)* was obtained by deacetylation of (**Ic**) (3.43 g, 3.22 mmol); yield 2.42 g (100%). An analytical sample was crystallized from methanol; mp 206–207°C, $[\alpha]_{546}$ +192° (*c* 1.0, ethanol). For ^{1}H NMR data, see table.

*Cyclohexyl 2-acetamido-2-deoxy-4,6-O-isopropylidene-*α*-D-glucopyranoside (**IIIe**)* was synthesized by isopropylidenation of triol (**IIe**) (1.0 g, 3.30 mmol); after column chromatography (elution with benzene ⟶ 20 : 1 benzene–ethanol), yield 925 mg (82%); amorphous powder; $[\alpha]_{546}$ +100° (*c* 1.0, chloroform).

*O-(Cyclohexyl 2-acetamido-2,3-dideoxy-*α*-D-glucopyranosid-3-yl)-D-lactoyl-L-alanyl-D-isoglutamine benzyl ester (**VIe**).* Acetal (**IIIe**) (910 mg, 2.65 mmol) was alkylated with (*S*)-2-chloropropionic acid, and the resulting muramic acid (**IVe**) was condensed with the dipeptide. The isopropylidene protecting group of the resulting compound (**Ve**) was removed by hydrolysis. The target compound (**VIe**) was isolated by column chromatography (elution with chloroform ⟶ 20 : 1 chloroform–ethanol); yield 0.78 g (44%); amorphous powder; $[\alpha]_{546}$ +98° (*c* 1.0, ethanol). For ^{1}H NMR data, see table.

*O-(Cyclohexyl 2-acetamido-2,3-dideoxy-*α*-D-glucopyranosid-3-yl)-D-lactoyl-L-alanyl-D-isoglutamine (**VIIe**)* was obtained by catalytic hydrogenolysis of benzyl ester (**VIe**) (380 mg, 0.57 mmol); yield 330 mg (98%); amorphous powder; $[\alpha]_{546}$ +93° (*c* 1.0, ethanol).

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