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Synthesis of *N*-Acetylmuramyl-*L*-Alanyl-*D*-Isoglutamine Aryl β-Glycosides

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Abstract—Synthesis of *N*-acetylmuramyl-*L*-alanyl-*D*-isoglutamine phenyl and (2-naphthyl) β-glycosides, novel muramyl dipeptide derivatives with phenolic aglycons, was reported. The starting *N*-acetylglucosamine aryl glycosides were obtained by glycosylation of phenols with peracetylated α-glucosaminyl chloride under the conditions of phase-transfer catalysis and used for the synthesis of 4,6-*O*-isopropylidene-*N*-acetylmyramic acid aryl β-glycosides. Condensation of these derivatives with a dipeptide and subsequent deprotection resulted in the intended glycopeptides.

Key words: glycopeptides, muramyl dipeptide, N-acetylglucosamine aryl glycosides, glycosylation

INTRODUCTION

Glycosides of *N*-acetylmuramyl-*L*-alanyl-*D*-isoglutamine exhibited the activity exceeding the effect of MDP itself in a number of tests.[2] For example, MDP heptyl β-glycoside [1] is a potent stimulator of the interleukin-1 and tumor necrosis factor production [2]; β-butyl [3] and β-cholesteryl MDP[4] displayed a strong adjuvant effect upon immunization with HIV-1 rgp160 and rgp120 glycoproteins [5]. In previous studies we synthesized MDP phenyl and naphthyl-2 β-glycosides, new MDP glycoside derivatives with phenolic aglycons to investigate the correlation between the structures of MDP glycosides and their biological activity and to look for new promising immunomodulators. Earlier, only synthesis of MDP β-*p*-aminophenylglycoside was reported [6], whose condensation with glutaric aldehyde resulted in the "macromolecular" MDP with a high activity [7]. Synthesis of phenyl α- and β-glycosides of *N*-acetylmuramic acid, which can be referred to as the carbohydrate moiety of the corresponding glycopeptides, has also been reported [8].

RESULTS AND DISCUSSION

In recent years, the traditional methods of synthesis of *N*-acetylglucosamine aryl glycosides (oxazoline synthesis [9] and interaction of phenolates with peracetylated α-glucosaminyl chloride (**I**) in polar solvents [10]) have been enriched with facile and mild PTC syntheses [11, 12]. We glycosylated phenol and β-naphthol with α-chloride (**I**) at room temperature in chloroform–1.25 N NaOH in the presence of triethylbenzylammonium chloride, a PTC catalyst, to prepare peracetylated *N*-acetylglucosamine phenyl and naphthyl-2 β-glycosides (**IIa, IIb**) in yields, after crystallization, 66 and 69%, respectively (see the scheme). The structures of aryl glycosides (**IIa, IIb**) were confirmed by the ^{1}H NMR spectral data: in particular, signals of five and seven aromatic protons, respectively, were observed in a range of 6.96–7.80 ppm (see the Experimental section). The doublets of anomeric protons in these compounds (δ 5.28 and 5.40 ppm) are shifted downfield relative to those of *N*-acetylglucosamine aliphatic glycosides (δ 4.6–4.8 ppm) [13]), and the coupling constant of 8 Hz indicates the β-configuration of the glycoside bond.

Compounds (**IIa, IIb**) were deacetylated according to Zemplen, and the β-diol moiety of the resulting triols (**IIIa, IIIb**) was protected by treatment with 2,2-dimethoxypropane. Alkylation of the free 3-hydroxyl group in (**IVa, IVb**) with (2*S*)-bromopropionic acid in anhydrous dioxane in the presence of NaH gave 4,6-*O*-isopropylidene-*N*-acetyl-*D*-muramic acid aryl β-glycosides (**Va, Vb**). Condensation of the muramic acid activated esters (**Va, Vb**) with *L*-alanyl-*D*-isoglutamine benzyl ester yielded protected glycopeptides (**VIa, VIb**). The acetal protecting group was removed by acid hydrolysis.

Comparison of the ^{1}H NMR spectra of glycopeptides (**VIa, VIb**) with the spectra of glycosides of acids (**Va, Vb**) and glycosides (**IIIa, IIIb**) proves the presence of lactyl dipeptide fragments in (**VII**), which can be inferred from the proton signals of the lactic acid,

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[2] Abbreviations: MDP, muramyl dipeptide, *N*-acetylmuramyl-*L*-alanyl-*D*-isoglutamine; PTC, phase-transfer catalysis.

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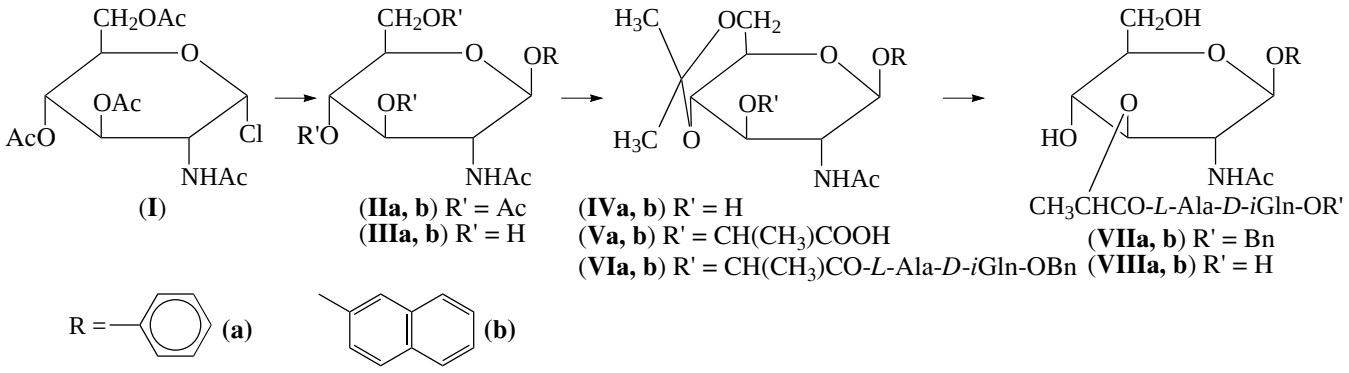

alanine, isoglutamine, and benzyl ester residues (see Table 1). The final catalytic hydrogenolysis of benzyl esters (**VIIa, VIIb**) led to the target MDP aryl glycosides (**VIIIa, VIIIb**).

The study of MDP aryl β-glycosides in the nonspecific anti-infection resistance stimulation test in mice with model peritonitis induced by intraperitoneal administration of *Salmonella typhi* in a dose of

Table 1. Characteristic signals in the ^{1}H NMR spectra of (**IIIa, IIIb**), (**Va, Vb**), and (**VIIa, VIIb**) (DMSO-d_6)

Resonances from fragments	Compound					
	(**IIIa**)	(**Va**)	(**VIIa**)	(**IIIb**)	(**Vb**)	(**VIIb**)
C1-OAr	6.97 m	6.97 m	6.98 m	7.17 m (1H)	7.18 m (1H)	7.17 m (1H)
	7.29 m	7.29 m	7.30 m	7.42 m (3H)	7.45 m (3H)	7.45 m (3H)
				7.83 m (3H)	7.86 m (3H)	7.86 m (3H)
H1 ($J_{1,2}$, Hz)	4.96 d (8)	5.25 d (6)	4.95 d (8)	5.14 d (8)	5.42 d (7)	5.12 d (8)
NAc, NH	1.81 s	1.80 s	1.78 s	1.82 s	1.81 s	1.79 s
	7.80 d	7.84 d	7.47 d	7.85 d	7.90 d	7.81 d
			7.91 d			7.96 d
			8.14 d			8.15 d
>CMe_2		1.33 s			1.35 s	
		1.48 s			1.50 s	
C4-OH, C6-OH	5.09 d		5.40 br. s	5.11 d		5.43 d
	4.59 t		4.69 br. s	4.62 br. s		4.72 t
CH_3CH		1.24 d	1.25 d		1.27 d	1.26 d
		4.19 q	4.26 q		4.22 q	4.25 q
CH_3 (Ala)			1.27 d			1.28 d
CH (Ala)			4.18 dq			4.21 dq
γ-CH_2 (Glu)			2.36 t			2.36 t
β-CH_2 (Glu)			1.80 m			1.82 m
			2.02 m			2.01 m
CH (Glu)			3.90 ddd			3.96 ddd
$CONH_2$ (Glu)			7.13 s			7.14 s
			7.32 s			7.32 s
CO_2CH_2Ph			5.08 s			5.08 s
			7.36 m			7.36 m

Table 2. The effect of MDP glycosides on the nonspecific resistance of mice to intraperitoneal infection with *S. typhi* (10^3 cells/mouse)*

Dose of glycopeptide, μg/mouse	Survival, %				
	Control	MDP	β-heptyl MDP	(**VIIIa**)	(**VIIIb**)
2	0	40	60	80	40
20	0	80	80	40	60
200	0	80	80	80	80

* The experiment was performed as described in [14].

100 LD_{50} [14] showed that the β-aryl aglycons in the MPD molecule did not have marked effect on the immunostimulating activity (Table 2).[3] At the same time, a high protective effect of small doses (2 μg/mouse) of phenyl glycoside (**VIIIa**) should be noted.

EXPERIMENTAL

Melting points were determined on a PTP instrument. Optical rotation (λ_{546}) was measured on a Polamat-A polarimeter at 20–22°C. ^{1}H NMR spectra were registered on a Varian VXR-300 (300 MHz) spectrometer using Me_4Si as the internal standard. Chemical shifts (δ, ppm) and coupling constants (*J*, Hz) are given. TLC was performed on precoated Silufol UV-254 (Kavalier) and Kieselgel 60 F_{254} (Merck) plates. The spots were visualized by carbonization at 300°C (Silufol) or by spraying with 5% H_2SO_4 and subsequent heating to 200–300°C (Kieselgel). The following developing systems were used: (A) 15 : 1 chloroform–ethanol, (B) 5 : 1 chloroform–ethanol, (C) 3 : 1 chloroform–ethanol, and (D) 10 : 1 benzene–ethanol. Chromatography was carried out on a silica gel (70–230 mesh, Aldrich or 240–400 mesh, Merck) column (1.8 × 12 cm) The elemental analysis data for compounds synthesized matched the calculated values.

Phenyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-*D*-glucopyranoside (IIa). A mixture of 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-*D*-glucopyranosyl chloride (**I**) [15] (1.0 g, 2.74 mmol), phenol (0.52 g, 5.48 mmol), triethylbenzylammonium chloride (0.52 g, 2.29 mmol), 1.25 N NaOH (3.5 ml, 4.38 mmol), and chloroform (10 ml) was stirred vigorously at room temperature until glycosyl donor disappeared (**I**) (TLC monitoring in systems A and D). The mixture was diluted with water (10 ml) and chloroform (10 ml); the organic layer was separated and washed with 1 N NaOH (2 × 5 ml) and water (10 ml). The chloroform extract was dried over anhydrous Na_2SO_4 and evaporated. The residue was crystallized from isopropanol to give 0.76 g (66%) of glycoside (**IIa**): mp 205–206°C, $[\alpha]_{546}$ –13° (*c* 0.95, chloroform). Lit. [9]: [mp 203–204°C, $[\alpha]_D$ –14.5° (chloroform); ^{1}H NMR (C^2HCl_3): 1.95, 2.05, 2.06, and 2.08 (12 H, all s, NAc and 3 OAc); 3.88 (1 H, ddd, $J_{5,6a}$ = 5, $J_{5,6b}$ = 2.5, H5); 4.14 (1 H, ddd, $J_{2,3}$ = 10, H2); 4.16 and 4.29 (2 H, both dd, $J_{6a,6b}$ = 12, H6a and H6b); 5.15 (1 H, dd, $J_{4,5}$ = 9.5, H4); 5.28 (1 H, d, $J_{1,2}$ = 8, H1); 5.42 (1 H, dd, $J_{3,4}$ = 9.5, H3); 5.75 (1 H, d, $J_{2,NH}$ = 9, NH); and 7.01 and 7.28 (5 H, both m, CH_{arom}).

2-Naphthyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-*D*-glucopyranoside (IIb) was synthesized as described for (**IIa**) from α-chloride (**I**) (1.0 g, 2.74 mmol) and naphthol-2 (0.79 g, 5.48 mmol). Yield 0.98 g (69%); mp 217–218°C, $[\alpha]_{546}$ –8° (*c* 1.05, chloroform). Lit. [11]: mp 220.1–220.5°C, $[\alpha]_D$ –65.8° (chloroform). ^{1}H NMR (C^2HCl_3): 1.95, 2.05, 2.06, and 2.08 (12 H, all s, NAc and 3 OAc); 3.94 (1 H, ddd, $J_{5,6a}$ = 5.5, $J_{5,6b}$ = 2.5, H5); 4.19 (1 H, ddd, $J_{2,3}$ = 10, H2); 4.20 and 4.30 (2 H, both dd, $J_{6a,6b}$ = 12, H6a and H6b); 5.16 (1 H, dd, $J_{4,5}$ = 9, H4); 5.40 (1 H, d, $J_{1,2}$ = 8, H1); 5.45 (1 H, dd, $J_{3,4}$ = 9, H3); 5.82 (1 H, d, $J_{2,NH}$ = 8.5, NH); and 7.18 (1 H, m, CH_{arom}); 7.41 (3 H, m, CH_{arom}); and 7.77 (3 H, m, CH_{arom}).

Phenyl 2-acetamido-2-deoxy-β-*D*-glucopyranoside (IIIa). Acetate (**IIa**) (0.76 g, 1.8 mmol) was dissolved in boiling anhydrous methanol, the solution was cooled to ~40°C, and 0.1 N sodium methylate in methanol (0.5 ml) was added. The precipitate formed was filtered off and washed with cold methanol to give 0.47 g (87%) of (**IIIa**); mp 232–232.5°C, $[\alpha]_{546}$ –6° (*c* 1.0, methanol). For ^{1}H NMR data, see Table 1. Lit. [16]: mp 249°C (decomp.).

2-Naphthyl 2-acetamido-2-deoxy-β-*D*-glucopyranoside (IIIb) was synthesized as described for (**IIIa**) by deacetylation of acetate (**IIb**) (0.95 g, 2.0 mmol) to yield 0.46 g of the target compound. The mother liquor was neutralized with KU-2 (H^+) cation exchanger, the resin was washed with methanol, and the filtrate was evaporated. The total yield of (**IIIb**) was 0.62 g (89%); mp 236–237°C, $[\alpha]_{546}$ +8° (*c* 1.0, methanol). For the ^{1}H NMR data, see Table 1. Lit. [11]: mp 239.8–240.3°C, $[\alpha]_D$ +15.8° (DMSO).

[3] The results of biological tests have been presented by O.V. Kalyuzhin (Institute of Human Morphology, Russian Academy of Sciences, Moscow, Russia).

Phenyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-*D*-glucopyranoside (IVa). A suspension of compound (**IIIa**) (0.47 g, 1.57 mmol) in 10 ml of anhydrous dioxane was heated to 50–55°C at stirring, and 2,2-dimethoxypropane (1.5 ml) and *p*-toluenesulfonic acid (10 mg) were added. After 1 h (TLC monitoring in system B), the reaction mixture was cooled, neutralized with pyridine, and precipitated by the addition of diethyl ether to yield 0.47 g (89%) of acetal (**IVa**); mp 153–154°C, $[\alpha]_{546}$ +55° (*c* 0.8, 3 : 1 chloroform–methanol).

2-Naphthyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-*D*-glucopyranoside (IVb) was obtained as described above from 0.44 g (1.27 mmol) of triol (**IIIb**). Yield 0.47 g (96%); amorphous powder, $[\alpha]_{546}$ –48° (*c* 1.0, chloroform).

Phenyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-3-*O*-(*D*-1-carboxyethyl)-β-*D*-glucopyranoside (Va). Sodium hydride (4 eq) was added portionwise at stirring to a suspension of (**IVa**) (0.45 g, 1.36 mmol) in anhydrous dioxane (10 ml). The mixture was heated to 95°C, kept for 1 h, and cooled to 65°C. (2*S*)-Bromopropionic acid (180 μl, 2 mmol) was added, and the mixture was kept at 65°C for further 3 h. The mixture was cooled, the excess sodium hydride was quenched with ethanol, and the mixture was concentrated and poured into 50 ml of cold water. The solution was acidified with 2 N HCl to pH 3–4, and the muramic acid glycoside was extracted with chloroform (3 × 20 ml). The extract was dried over anhydrous Na_2SO_4 and evaporated. The residue was crystallized by the addition of diethyl ether to give 0.42 g (76%) of acid (**Va**); mp 194–196°C (decomp.), $[\alpha]_{546}$ –4° (*c* 1.0, chloroform). For the ^{1}H NMR data, see Table 1.

2-Naphthyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-3-*O*-(*D*-1-carboxyethyl)-β-*D*-glucopyranoside (Vb) was obtained as described for (**Va**) by treating acetal (**IVb**) (0.47 g, 1.12 mmol) with NaH (4 eq.) and (2*S*)-bromopropionic acid (160 μl, 1.82 mmol). Yield 0.49 g (88%); mp 115–117°C, $[\alpha]_{546}$ +8° (*c* 1.0, chloroform). For the ^{1}H NMR data, see Table 1.

***O*-(Phenyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-*D*-glucopyranosid-3-yl)-*D*-lactoyl-*L*-alanyl-*D*-isoglutamine benzyl ester (VIa).** *N*-Hydroxysuccinimide (84 mg, 0.73 mmol) and DCC (150 mg, 0.73 mmol) were added to a solution of acid (**Va**) (270 mg, 0.66 mmol) in anhydrous dioxane (10 ml) at stirring. After 3–5 h, the precipitate of dicyclohexylurea was filtered off and washed with dioxane. *L*-Alanyl-*D*-isoglutamine benzyl ester trifluoroacetate (obtained by treatment of the corresponding Boc-derivative (270 mg, 0.66 mmol) with trifluoroacetic acid and subsequent evaporation to dryness) and triethylamine to pH 8 were added to the filtrate. After the reaction was over (TLC monitoring in system B), the reaction mixture was concentrated, and diethyl ether (30 ml) was added. The product was filtered off and chromatographed on silica gel (elution with 50 : 1 ⟶ 10 : 1 chloroform–ethanol) to yield 270 mg (59%) of glycopeptide (**VIa**) as amorphous powder, $[\alpha]_{546}$ +8° (*c* 1.0, chloroform).

***O*-(2-Naphthyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-*D*-glucopyranosid-3-yl)-*D*-lactoyl-*L*-alanyl-*D*-isoglutamine benzyl ester (VIb)** was synthesized as described above from acid (**Vb**) (210 mg, 0.46 mmol) and Boc-*L*-alanyl-*D*-isoglutamine benzyl ester (182 mg, 0.46 mmol). Yield 295 mg (86%); amorphous powder, $[\alpha]_{546}$ +21° (*c* 1.0, chloroform).

***O*-(Phenyl 2-acetamido-2-deoxy-β-*D*-glucopyranosid-3-yl)-*D*-lactoyl-*L*-alanyl-*D*-isoglutamine benzyl ester (VIIa).** Alkylidene derivative (**VIa**) (190 mg, 0.27 mmol) was dissolved in 80% acetic acid (3 ml) at heating on boiling water bath and kept at this temperature for 5 min (TLC monitoring in system C). The solution was evaporated, the residue was co-evaporated with toluene and triturated with diethyl ether to yield 179 mg (100%) of diol (**VIIa**); mp 212–213°C (decomp.), $[\alpha]_{546}$ +25° (*c* 0.67, ethanol). For the ^{1}H NMR data, see Table 1.

***O*-(2-Naphthyl 2-acetamido-2-deoxy-β-*D*-glucopyranosid-3-yl)-*D*-lactoyl-*L*-alanyl-*D*-isoglutamine benzyl ester (VIIb)** was obtained as described for compound (**VIIa**) by treatment (**VIb**) (275 mg, 0.37 mmol) with acetic acid. Yield 260 mg (100%); mp 218–219°C (decomp.), $[\alpha]_{546}$ +56° (*c* 0.67, ethanol). For the ^{1}H NMR data, see Table 1.

***O*-(Phenyl 2-acetamido-2-deoxy-β-*D*-glucopyranosid-3-yl)-*D*-lactoyl-*L*-alanyl-*D*-isoglutamine (VIIIa).** Benzyl ester (**VIIa**) (180 mg, 0.27 mmol) was dissolved in ethanol (10 ml) and hydrogenated over 10% Pd/C (100 mg) at room temperature for 4 h. The catalyst was filtered off and washed with ethanol (5 ml), he filtrate was concentrated, and the residue was triturated with diethyl ether to give 130 mg (84%) of glycopeptide (**VIIIa**) as amorphous powder.

***O*-(2-Naphthyl 2-acetamido-2-deoxy-β-*D*-glucopyranosid-3-yl)-*D*-lactoyl-*L*-alanyl-*D*-isoglutamine (VIIIb)** was obtained as described for compound (**VIIIa**) by hydrogenolysis of benzyl ester (**VIIb**) (136 mg, 0.19 mmol) in 92% yield (110 mg) as amorphous powder.

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