Chemistry of Natural Compounds, Vol. 30, No. 3, 1994

SYNTHESIS OF ALKYL β-GLYCOSIDES OF 6-(N-ACETYLMURAMOYL-L-ALANYL—D-ISOGLUTAMINYLAMINO)HEXANOIC ACID AND ITS 4-AMINOBUTYL ESTER

V. O. Kur'yanov, V. V. Tsikalov, A. E. Zemlyakov, and V. Ya. Chirva

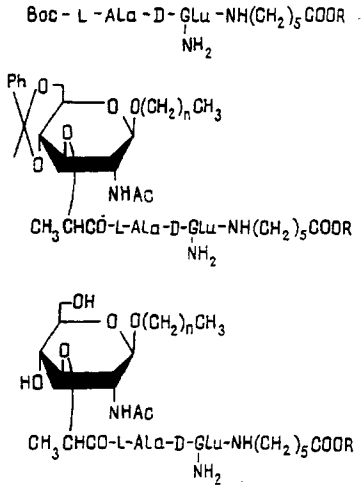
UDC 547.963.1

The synthesis has been effected of alkyl β-glycosides containing spacers with amino and carboxy groups at the end. As "prespacers" we used benzyl and 4-azidobutyl esters of 6-(L-alanyl-D-isoglutaminylamino)hexanoic acid. The use of the butyl and hexadecyl β-glycosides of N-acetylmuramic acid has enabled us to obtain glycopeptides with different hydrophilic–lipophilic balances.

Derivatives of N-acetylmuramoyl-L-alanyl-D-isoglutamine (muramoyldipeptide, MDP) having spacers with active functional groups (usually, NH_2, more rarely COOH) at the end are widely used for immobilization on polymeric matrices [1, 2], and for conjugation with proteins and peptides [3, 4], with mono- and polysaccharides [5, 6], and with vitamins [7]. In this way, highly active macromolecular glycopeptides [1, 2, 8], new immunomodulators [5, 7], and synthetic vaccines [3, 4] have been obtained, and the immunogenicity of antigens has been raised [6].

We have previously synthesized glycopeptides with ω-aminoalkyl spacers at the glycosidic center [9] and ω-amino- and ω-carboxyalkyl spacers at the γ-carboxy group of isoglutamine [10]. From them, highly active conjugates of polyacrylamide with MDP and also with MDP and phosphatidylethanolamine have been obtained [2]. Continuing work on the synthesis and study of biologically active MDP derivatives, we have now synthesized muramoyl peptides containing spacers with terminal carboxy or amino groups in the isoglutamine residues (**1**, **2**, A, B).

As spacers we proposed to use 6-aminohexanoic acid and its 4-aminobutyl ester. The butyl and hexadecyl β-glycosides of muramic acid were used as the carbohydrate moieties. This, on the one hand, ensured a high biological action (alkyl β-glycosides of MDP have activities comparable with that of MDP itself [2]) and, on the other hand, eliminated the necessity for introducing and removing temporary protection of the glycosidic hydroxyl, while side reactions of the carbonyl groups of the carbohydrates with the amino spacers became impossible and, in addition, the possibility was presented of changing the lipophilicity of the conjugates through the aglycon.



3. R=(CH2)4N3
4. R=Bzl
5. R=(CH2)4N3
6. R=Bzl
7. R=(CH2)4N3
8. R=Bzl
1. R=H
2. R=(CH2)4NH2

A. n=3 B. n=15

Simferopol' State University. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 424-429, May-June, 1994. Original article submitted October 22, 1993.

 0009-3130/94/3003-0390$12.50

TABLE 1. PMR Spectra of Compounds (**3**, **4**, **7** A,B, **8** A,B, and **9** A,B)*

Compound	O(CH2)nCH3		N Ac Mur			Ala		Glu			NH(CH2)5COOR				Solvent
	CH3CH2	(CH2)n	NAc	H-1 (J1,2, Hz)	NH	CH3CH	NH	γ—CH2	CONH2	NH	NHCH2	CH2COO	NHCH2	R	
3						1.32 d	5.28 d	2.30 m	5.72 s 7.09 s	7.78 d	3.20 q	2.28 t	6.31 t	3.29 t 4.07 t	CDCl3
4						1.34 d	5.29 d	2.31 t	5.73 s 7.13 s	7.80 d	3.22 q	2.37 t	6.31 t	5.11 s 7.35 m	CDCl3
7 A	0.86 t	1.36 m 1.61 m	1.95 s	4.65 d (8)	7.45 d	1.34 d	6.99	2.34 t	5.80 s 6.86 s	8.03 d	3.20 q	2.30 t	6.34 t	3.30 t 4.07 t	CDCl3
7 B	0.86 t	1.23 m 1.50 m	1.75 s	4.26 d (8)	7.77 d	1.23 m	7.38 d	2.07 t	7.04 s 7.29 s	8.07 d	2.98 q	2.28 t	7.74 t	3.30 t 4.03 t	DMSO-d_6
8 A	0.86 t	1.29 m 1.53 m	1.76 s	4.26 d (8)	7.78 d	1.24 d	7.38 d	2.06 t	7.05 s 7.29 s	8.07 d	2.99 q	2.34 t	7.74 t	5.08 s 7.36 m	DMSO-d_6
8 B	0.86 t	1.24 m 1.54 m	1.75 s	4.26 d (8)	7.78 d	1.24 m	7.38 d	2.06 t	7.04 s 7.29 s	8.07 d	2.99 q	2.33 t	7.73 t	5.08 s 7.35 m	DMSO-d_6
9 A	0.90 t	1.34 m 1.55 m	1.95 s	4.68 d (8.5)	5.59 d										CDCl3
9 B	0.91 t	1.29 m 1.64 m	1.95 s	4.68 d (8.5)	5.45 d										CDCl3

*Working frequency 500 MHz or, for compound **9** B, 100 MHz.

To obtain compounds (**2** A, B) we used 4-azidobutyl 6-aminohexanoate as a "prespacer." The replacement of the chloride atom in 4-chlorobutyl acetate by an azido group was achieved by the action of sodium azide in boiling aqueous dioxane or acetonitrile in the presence of Et_4NBr. In the latter case, the yield of 4-azidobutyl acetate was close to quantitative. Its IR spectrum contained an intense absorption band at 2080 cm^{-1}, which is characteristic for the azido group. Subsequent deacylation with sodium methanolate in methanol and azeotropic esterification of the alcohol with 6-aminohexanoic acid in the presence of TsOH led to the desired "prespacer." Its condensation with Boc-L-Ala-D-Glu-NH_2 was conducted with the use of N-hydroxysuccinimide (HOSu) and dicyclohexylcarbodiimide (DCC) as activating reagents. The IR and PMR spectra of peptide (**3**) were close to those of an ester of 6-(tert-butoxycarbonyl-L-alanyl-D-isoglutaminylamino)hexanoic acid synthesized previously [10] (Table 1) and corresponded to its structure. In the PMR spectrum of compound (**3**) in addition to the signals of the protons of the L-alanine and D-isoglutamine residues, we observed the signals of the protons of the 6-aminohexanoic acid residue; a triplet of the amide proton with the CS 6.31 ppm, a quartet of the α-methylene group with CS 3.20 ppm, and a triplet of the ω-methylene group with CS 2.28 ppm, and also the signals of the 4-azidobutyl residue: triplets of the α-methylene protons with a CS of 4.07 ppm and of the ω-methylene protons with a CS of 3.29 ppm.

Peptides (**3**), and (**4**) were condensed with the butyl and hexadecyl alkylidene-β-glycosides of N-acetylmuramic acid, using the N-hydroxysuccinimide method. The yields of the glycopeptides (**5**, **6** A, B) amounted to 57-82%. The alkylidene protective groups were eliminated by acid hydrolysis. In the PMR spectra of compounds (**7**, **8** A, B) we identified characteristic signals of the protons of the peptide and carbohydrate components, which were comparable, respectively, with the signals of the protons of peptides (**3**) and (**4**) and those of the peracetates of the butyl and hexadecyl β-glycosides of N-acetylglucosamine (**9** A, B) (see Table 1). The concluding catalytic hydrogenolysis of the benzyl esters in compounds (**8** A, B) and the hydrogenation of the azido groups in compounds (**7** A, B) over PdO enabled the desired glycopeptides (**1**, **2** A, B) to be obtained. The IR spectra of these compounds lacked the absorption bands of the protective groups.

The amines (**2** A, B) were used for conjugation with poly(4-nitrophenyl acrylate) [11]. The amino components were introduced in a ratio to the matrix of 1:5. The activated esters that had not reacted were converted into amides by the action of ammonia. After purification by gel filtration on Sephadex G-15, macromolecular muramoylpeptides with differing hydrophilic-lipophilic balances were obtained.

EXPERIMENTAL

For general observations, see [10].

TLC was conducted in the following solvent systems: 1) chloroform–ethanol (5:1); 2) chloroform–ethanol (15:1); 3) butanol–acetic acid–water (3:1:1).

4-Azidobutyl 6-Aminohexanoate. A solution of 5.5 g (37.0 mmoles) of 4-chlorobutyl acetate [12] in 50 ml of acetonitrile was treated with 4.8 g (74.0 mmoles) of sodium azide and 0.4 g (1.1 mmoles) of Et_4NBr. The reaction mixture was boiled for 9 h, and the solvent was evaporated off. The residue was treated with 100 ml of benzene, and salts were filtered off. The filtrate was washed with water (30 × 30 ml) [sic], and the organic layer was dried with $NaSO_4$ and evaporated.

The 4-azidobutyl acetate obtained (5.7 g, 99%) was treated with a solution of 2.1 g (37.0 mmoles) of KOH in 40 ml of ethanol. After 12 h, the mixture was neutralized with acetic acid and evaporated. The residue was dissolved in 100 ml of chloroform, and the solution was washed with water (3 × 30 ml). The chloroform extract was dried with Na_2SO_4 and evaporated. The yield of 4-azidobutanol was 4.15 g (99%).

A solution of 1.56 g (14 mmoles) of 4-azidobutanol in 30 ml of dichloroethane was treated with 1.3 g (10 mmoles) of 6-aminohexanoic acid and 1.83 g (11 mmoles) of TsOH. The reaction mixture was boiled for 36 h with a Dean–Stark trap filled with $CaCl_2$ (control by TLC in system 1). The mixture was evaporated, the residue was dissolved in 100 ml of chloroform, and the solution was washed with saturated $NaHCO_3$ solution. The organic layer was dried with Na_2SO_4 and evaporated. By column chromatography with chloroform as eluent, the residue yielded 1.28 g (56%) of 4-azidobutyl 6-aminohexanoate.

4-Azidobutyl 6-(tert-Butoxycarbonyl-L-alanyl-D-isoglutaminylamino)hexanoate (3). A solution of 0.25 g (0.79 mmole) of tert-butoxycarbonyl-L-alanyl-D-isoglutamine [13] in a mixture of 5 ml of dry dioxane and 0.4 ml of DMFA was treated with 0.11 g (0.95 mmole) of HOSu and 0.195 g (0.95 mmole) of DCC. After the end of activation (monitoring by TLC in system 2), the precipitate of N,N′-dicyclohexylurea that had deposited was filtered off and was washed with dioxane

(2 × 2 ml). The filtrate was treated with a solution of 0.27 g (1.18 mmoles) of 4-azidobutyl 6-aminohexanoate and with triethylamine to pH 8. After 24 h, the reaction mixture was evaporated and the residue was subjected to column chromatography with the eluent chloroform → chloroform–ethanol (25:1)), to give 0.32 g (77%) of compound (**3**), mp 93-94°C, $[\alpha]_{546}$ +12.9° (*c* 1.23; chloroform) ν_{max}^{KBr} (cm^{-1}): 3300, 3380 (NH), 2960, 2920, 2850 (CH_2, CH_3), 2080 (azide), 1720 (ester), 1680, 1540 (amide).

4-Azidobutyl 6-{[O-(Hexadecyl 2-Acetamido-2-deoxy-β-D-glucopyranosid-3-yl)-D-lactoyl]-L-alanyl-D-isoglutaminylamino}hexanoate (7 B). A solution of 0.19 g (0.31 mmole) of the hexadecyl β-glycoside of 4,6-O-benzylidene-N-acetylmuramic acid [14] in 10 ml of dry dioxane and 0.8 ml of DMFA was treated with 40 mg (0.35 mmole) of HOSu and 71 mg (0.35 mmole) of DCC. After the end of activation (monitoring by TLC in system 2), the precipitate of dicyclohexylurea was filtered off and was washed with 4 ml of dioxane–DMFA (10:1), and the filtrate was treated with a solution of 0.20 g (0.38 mmole) of the trifluoroacetate of 4-azidobutyl 6-(L-alanyl-D-isoglutaminylamino)hexanoate (obtained by treating the Boc derivative (**3**) with trifluoroacetic acid followed by evaporation to dryness) in 5 ml of dioxane and with triethylamine to pH 8.

After 16 h, the reaction mixture was evaporated to 2/3 volume, and the residue was crystallized from ether. The precipitate was filtered off and dried in the air. This gave 263 mg (82%) of 4-azidobutyl 6-{[O-(hexadecyl 2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosid-3-yl)-D-lactoyl]-L-alanyl-D-isoglutaminylamino}hexanoate (**5** B), a sparingly soluble substance with mp 238-239°C (decomp.), ν_{max}^{KBr} cm^{-1}: 3300 (NH), 2930, 2850 (CH_2, CH_3), 2100 (azide), 1720 (ester), 1660, 1520 (amide) 740, 690 (Ph).

In a similar way, 100 mg (0.23 mmole) of the butyl β-glycoside of 4,6-O-benzylidene-N-acetylmuramic acid yielded 150 mg (78%) of glycopeptide (**5** A) with mp 218°C, $[\alpha]_{546}$ +33.3° (*c* 0.5; DMSO); ν_{max}^{KBr} (cm^{-1}): 3320, 3240 (NH), 2920, 2840 (CH_2, CH_3) 2100 (azide, 1720 (ester), 1650, 1580 (amide), 740, 690 (Ph).

By the same procedure, using benzyl 6-(tert-butoxycarbonyl-L-alanyl-D-isoglutaminylamino)hexanoate [10], we obtained 0.5 g (57%) of benzyl 6-{[O-(butyl 2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosid-3-yl)-D-lactoyl]-L-alanyl-D-isoglutaminylamino}hexanoate (**6** A), a sparingly soluble amorphous substance, ν_{max}^{KBr} (cm^{-1}): 3380, 3280 (NH), 2950, 2840 (CH_2, CH_3), 1720 (ester), 1640, 1530 (amide), 730, 680 (Ph), and 0.25 g (59%) of glycopeptide (**6** B), a sparingly soluble amorphous substance, ν_{max}^{KBr} (cm^{-1}) 3380, 3260 (NH), 2900, 2840 (CH_2, CH_3), 1720 (ester), 1630, 1530 (amide), 730, 680 (Ph).

A solution of glycopeptide (**5** B) (0.2 g, 0.2 mmole) in 5 ml of 80% acetic acid was heated in the boiling water bath for 30 min and was then evaporated to dryness. The residue was crystallized from ether. The yield of the diol (**7** B) was 0.17 g (72%), mp 182-183°C (decomp.) $[\alpha]_{546}$ 0° (*c* 0.67; DMSO), ν_{max}^{KBr} (cm^{-1}): 3400-3290 (OH, NH), 2920, 2840 (CH_2, CH_3), 2100 (azide, 1720 (ester), 1660, 1540 (amide). In a similar way we obtained: the diol (**7** A), 45 mg (78%), mp 210°C (decomp.), $[\alpha]_{546}$ −3.8° (*c* 1.1; chloroform—ethanol (1:5)), ν_{max}^{KBr} (cm^{-1}): 3450-3280 (OH, NH), 2920, 2830 (CH_2, CH_3), 2100 (azide), 1720 (ester), 1640, 1520 (amide); the diol (**8** A), 0.42 g (92%), $[\alpha]_{546}$ +3.1° (*c* 0.67; acetic acid), ν_{max}^{KBr} (cm^{-1}): 3400-3280 (OH, NH), 2920, 2850 (CH_2, CH_3) 1720 (ester), 1630, 1540 (amide, 730, 690 (Ph); and the diol (**8** B), 0.185 g (92%), ν_{max}^{KBr} (cm^{-1}): 3420-3280 (OH, NH), 2930, 2840 (CH_2, CH_3), 1740 (ester), 1650, 1540 (amide), 730, 690 (Ph).

6-{[O-(Hexadecyl 2-Acetamido-2-deoxy-β-D-glycopyranoside-3-yl)-D-lactoyl]-L-alanyl-D-isoglutaminylamino}hexanoic Acid (1 B). In solution in 20 ml of dioxane–ethanol–water (100:100:5), 0.175 g (0.19 mmole) of the diol (**8** B) was subjected to hydrogenolysis over 0.1 g of Pd/C. After the end of the reaction (monitoring by TLC in system 3), the catalyst was filtered off, the solvent was distilled off in vacuum, and the residue was crystallized from ether. Yield 0.136 g (86%), amorphous, $[\alpha]_{546}$ +1.05° (*c* 0.95; acetic acid; ν_{max}^{KBr} (cm^{-1}); 3400-3282 (OH, NH), 2900, 2830 (CH_2, CH_3), 1700 (C=O), 1670, 1540 (amide). By the same procedure, from diol (**8** A) we obtained the acid (**1** A), yield 0.18 g (93%), amorphous $[\alpha]_{546}$ +1.8° (*c* 1.06; acetic acid); ν_{max}^{KBr} (cm^{-1}; 3420-3280 (OH, NH) 2940, 2850 (CH_2, CH_3), 1700 (C=O), 1650, 1540 (amide).

Conjugate of the 4-Aminobutyl Ester of the Hexadecyl β-Glycoside of 6-(N-Acetylmuramoyl-L-alanyl-D-isoglutaminylamino)hexanoic Acid with Polyacrylamide. To a solution of 36 mg (186 meq.) of poly(4-nitrophenyl acrylate) (fraction with a molecular mass of 20,000-100,000 Da) in 1 ml of DMFA were added 34 mg (38 μmoles) of compound (**2** B) (obtained by the hydrogenation of 35 mg of the diol (**7** B) over 70 mg of PdO in ethanol) in 0.5 ml of DMFA, and two drops of triethylamine. The reaction mixture was stirred at room temperature for 12 h (TLC in system showed the absence of derivative (**2** B)). The solution was treated with 0.05 ml of 25% aqueous ammonia, and after 2 h the reaction mixture was diluted with 10 ml of water, and the conjugate was isolated by gel filtration on Sephadex G-15 with a yield of 40 mg (85%).

A conjugate of the 4-aminobutyl ester of the butyl β-glycoside of 6-(N-acetylmuramoyl-L-alanyl-D-isoglutaminylamino)hexanoic acid with polyacrylamide was obtained similarly with a yield of 80%.

REFERENCES

1. L. Chedid, M. Parant, F. Parant, F. Audibert, P. Lefrancier, J. Choay, and M. Sela, Proc. Natl. Acad. Sci. USA, **76**, 6557 (1979).
2. A. É. Medvedev, B. B. Fuks, N. V. Bovin, and A. E. Zemlyakov, Byull. Éksp. Biol., No. 12, 626 (1992).
3. C. Carelli, F. Audibert, J. Gaillard, and L. Chedid, Proc. Natl. Acad. Sci. USA, **79**, 5392 (1982).
4. F. Audibert, M. Jolivet, L. Chedid, R. Arnon, and M. Sela, Proc. Natl. Acad. Sci. USA, **79**, 5042 (1982).
5. Y. Fujishima, K. Kigawa, Y. Ogawa, M. Kiso, A. Hasegawa, H. Ishida, and I. Azuma, Carbohydr. Res., **167**, 317 (1987).
6. M. M. Ponpipom and K. M. Rupprecht, Carbohydr. Res., **113**, 57 (1983).
7. S. Kobayashi, T. Fukuda, H. Yukimasa, M. Fujino, I. Azuma, and Y. Yamamura, Bull. Chem. Soc. Jpn., **57**, 3182 (1984).
8. M. Parant, C. Damais, F. Audibert, F. Parant, L. Chedid, E. Sache, P. Lefrancier, J. Choay, and E. Lederer, J. Infect. Dis., **138**, 378 (1978).
9. A. E. Zemlyakov, E. S. Kakayan, and V. Ya. Chirva, Bioorg. Khim., **15**, 1527 (1989).
10. V. O. Kur'yanov, T. F. Zhelobetskaya, A. E. Zemlyakov, and V. Ya. Chirva, Khim. Prir. Soedin., 122 (1993).
11. T. V. Zemlyanukhina and N. V. Bovin, Bioorg. Khim., **16**, 1096 (1990).
12. C. Weygand and G. Hilgetag, Organisch-chemische Experimentierkunst, J. A. Barth, Leipzig (1964). [English translation as Weygand/Hilgetag's Preparative Organic Chemistry, Wiley, New York (1972)].
13. S. Kusumoto, Y. Tarumi, K. Ikenaka, and T. Shiba, Bull. Chem. Soc. Jpn., **49**, 533 (1976).
14. A. E. Zemlyakov and V. Ya. Chirva, Khim. Prir. Soedin., 714 (1986).