SYNTHESIS OF LIPOPHILIC DERIVATIVES OF N-ACETYLMURAMOYL-L-ALANYL-D-GLUTAMIC ACID

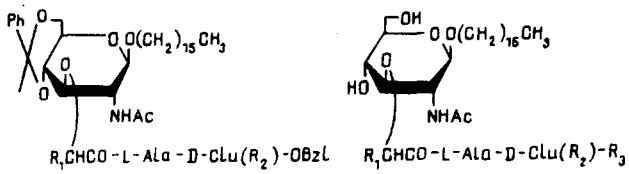
V. O. Kur'yanov, A. E. Zemlyakov, and V. Ya. Chirva

UDC 547.963.1

The synthesis has been achieved of the hexadecyl β-glycosides of N-acetylmuramoyl-L-alanyl-D-glutamic acid and N-acetyl-nor-muramoyl-L-alanyl-D-glutamic acid and of the α-octadecylamide of N-acetylmuramoyl-L-alanyl-D-glutamic acid – lipophilic glycopeptides of differing hydrophilic–lipophilic balance.

Lipophilic derivatives of N-acetylmuramoyl-L-alanyl-D-isoglutamine (muramoyldipeptide, MDP) possess high immunoadjuvant, antitumoral, and antiinfection activities [1]. It is known that N-acetylmuramoyl-L-alanyl-D-glutamic acid (MDP-A) [2-4], N-acetyl-nor-muramoyl-L-alanyl-D-isoglutamine (nor-MDP), a glycopeptide in which the lactoyl fragment has been replaced by a glycoloyl residue [5], and nor-MDP-A [2] have biological activities comparable with those of MDP [6]. The hexadecyl β-glycoside of MDP, which we have synthesized previously [7], is an active stimulator of turmor necrosis factor (TNF) [8] and possesses an absolute adjuvancy comparable with that of O-(N-acetylglucosaminyl)-(β1 → 4)-MDP (GMDP).* According to the preliminary results, the α-octadecylamide of MDP-A [9] also exhibits a high activity in the stimulation of the production to TNF by mouse macrophages.†

Continuing a study of the influence of the hydrophilic–lipophilic balance of MDP derivatives on their biological activity, and also with the aim of finding MDP derivatives having a potential immunostimulating activity, we have synthesized the hexadecyl β-glycosides of MDP-A (IX) and of nor-MDP-A (VIII) and the α-octadecylamide of MDP-A (X).



II. R_1=H; R_2=OBzl
III. R_1=CH_3; R_2=OBzl
IV. R_1=CH_3; R_2=$NHC_{16}H_{37}$
V. R_1=H; R_2=R_3=OBzl
VI. R_1=CH_3; R_2=R_3=OBzl
VII. R_1=CH_3; R_2=$NHC_{16}H_{37}$; R_3=OBzl
VIII. R_1=H; R_2=R_3=OH
IX. R_1=CH_3; R_2=R_3=OH
X. R_1=CH_3; R_2=$NHC_{16}H_{37}$; R_3=OH

Hexadecyl 2-acetamido-4,6-O-benzylidene-2-deoxy-β-glucopyranoside was alkylated with monochloroacetic acid by the procedure of [2]. The nor-muramic acid derivative (I) obtained was condensed with the dibenzyl ester of L-alanyl-D-glutamic acid (L-Ala-D-Glu(OBzl)$_2$), using N-hydroxysuccinimide and dicyclohexylcarbodiimide as activating reagents. The reactions of

*The trials were conducted by A. E. Meshcheryakova and T. M. Andronova (Institute of Bioorganic Chemistry, Academy of Sciences of the USSR, Moscow).
†Results of Prof. B. B. Fuks (Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow).

M. V. Frunze Simferopol' State University. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 553-557, July-August, 1991. Original article submitted November 22, 1990.

 0009-3130/91/2704-0484$12.50

TABLE 1. PMR Spectra of Boc-L-Ala-D-Glu(OBzl)$_2$ and of Compounds (II)-(IV)

	Boc-L-Ala-D-Glu (OBzl)$_2$	II	III	IV
$\underline{CH_3}CH_2$		0,88 t	0,88 t	0,89 t
$(\underline{CH_2})_n$		1,24 m	1,25 m	1,28 m
$\underline{CH_2}CH$	1,34 d	1,37 d	1,36 d; 1,37 d	1,41 d (6H)
NAc		1,88 s	1,90 s	1,96 s
$O\underline{CH_2}CH_2$		3,76 t	3,77 t	3,78 t
H-1 ($J_{1,2}$, Hz)		4,84 d (8)	4,92 d; (8)	4,88 d (8)
$COOCH_3$	5,10 s; 5,14 d; 5,17 d	5,14 d; 5,06 d; 5,10 d	5,09 s; 5,13 d; 5,15 d	5,15 s
CHPh		5,51 s	5,52 s	5,55 s
Ph	7,35 m	7,32 m	7,31 m	7,36 m
NH	4,95 d; 6,89 d	6,13 d; 6,94 d; 7,42 d	6,40 d; 7,10 d; 7,45 d	6,49 d; 7,06 d; 7,09 d; 7,47 d,

the hexadecyl β-glycoside of 4,6-O-benzylidene-N-acetylmuramic acid with L-Ala-D-Glu(Bzl)$_2$ and with the γ-benzyl ester of L-Ala-D-Glu were performed similarly. The protected glycopeptides (II)-(IV) were isolated by column chromatography with yields of 62-67%. The structures of these compounds were confirmed by their IR and PMR spectra (Table 1). The coincidence of the signals in the PMR spectrum of the protected dipeptide Boc-L-Ala-D-Glu(OBzl)$_2$ with the signals in the spectrum of glycopeptide (III) confirmed the introduction of the peptide fragment. In the PMR spectrum of compound (II) it was possible to identify, in addition to the signals in the spectrum of compound (III), two singlets of the methylene group of a glycoloyl residue (CSs 4.35 and 4.38 ppm), and in the spectrum of compound (IV) the triplet of the amide proton of the octadecylamide with CS 6.49 ppm. The signals of the glycosidic protons, with CSs of 4.88-4.92 ppm and SSCCs of 8 Hz, corresponded to a β-glycosidic bond. The benzylidene protective groups in substances (II)-(IV) were eliminated by acid hydrolysis. The concluding catalytic hydrogenolysis of the benzyl ester groups in the peptide moieties of the diols (V)-(VII) led to the desired MDP analogues (VIII)-(X).

EXPERIMENTAL

Melting points were determined on a PTP instrument, and optical rotations at 20-22°C on a Polamat polarimeter. PMR spectra were obtained on a Bruker WM-500 (500 MHz) spectrometer with TMS as internal standard and $CDCl_3$ as solvent. IR spectra were recorded on a Specord 75-IR spectrophotometer (KBr tablets). Column chromatography was conducted on washed silica gel L 100-250 μm (Czechslovakia). For all the compounds the analytical results corresponded to the calculated figures.

Hexadecyl 2-Acetamido-4,6-O-benzylidene-3-O-carboxymethyl-2-deoxy-β-D-glucopyranoside (I). A solution of 230 mg (0.43 mmole) of hexadecyl 2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside [7] in 10 ml of dry dioxane was treated with 52 mg (1.72 mmoles) of sodium hydride (80% suspension in oil) and the mixture was heated with stirring to 95°C. It was kept at this temperature for 2 h and was then cooled to 65°C, and 82 mg (0.86 mmole) of monochloroacetic acid was used. After being stirred at this temperature for 4 h, the mixture was cooled to room temperature, and 1 ml of ethanol was added. The solution was evaporated, the residue was dissolved in 100 ml of cold water and the solution was acidified to pH 2 with 1 N HCl. The liberated acid was extracted with chloroform. The organic layer was dried with Na_2SO_4 and evaporated. Crystallization from ether–chloroform gave 230 mg (91%) of the acid (I) with mp 196°C, $[\alpha]_{546}$ −21° (c 0.70; $CHCl_3$); ν_{max}^{KBr}, cm^{-1}: 3280 (OH, NH); 2920, 2840 (CH_2); 1720 (C=O); 1650, 1560 (amide), 740, 690 (Ph).

Dibenzyl O-(Hexadecyl 2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosid-3-yl)-glycoloyl-L-alanyl-D-glutamate (III). The acid (I) (100 mg, 0.17 mmole) was dissolved in 10 ml of dioxane–DMFA (9:1) and was activated with 21 mg (0.19 mmole) of N-hydroxysuccinimide and 38 mg (0.19 mmole) of dicyclohexylcarbodiimide. After 3 h the precipitate of dicyclohexylurea was filtered off and was washed with 3 ml of dioxane. The combined filtrate was treated with L-Ala-D-Glu(OBzl)$_2$ trifluoroacetate (obtained by treating 93 mg (0.19 mmole)

of Boc-L-Ala-D-Glu(OBzl)$_2$ [2, 4] with 2 ml of trifluoroacetic acid, followed by evaporation to dryness) and 2 drops of triethylamine. After 24 h, the reaction mixture was evaporated, and column chromatography [chloroform → chloroform–ethanol (50:1)] yielded 115 mg (63%) of the amorphous glycopeptide (II), $[\alpha]_{546}$ −23° (c 0.75; $CHCl_3$); ν_{max}^{KBr}, cm^{-1}: 3290 (NH); 2900, 2830 (CH_2); 1720 (C=O); 1610, 1560 (amide), 730, 690 (Ph).

Similarly, 450 mg (0.74 mmole) of hexadecyl 2-acetamido-4,6-O-benzylidene-3-O-(D-1-carboxyethyl)-2-deoxy-β-D-glucopyranoside [7] and 407 mg (0.82 mmole) of Boc-L-Ala-D-Glu(OBzl)$_2$ gave 500 mg (67%) of the glycopeptide (III), $[\alpha]_{546}$ −13° (c 1.34; $CHCl_3$); ν_{max}^{KBr}, cm^{-1}: 3260 (NH); 2920, 2840 (CH_2); 1730 (C=O); 1630, 1540 (amide), 740, 690 (Ph).

By the same procedure, from 150 mg (0.25 mmole) of the β-hexadecyl derivative of muramic acid and 180 mg (0.27 mmole) of the α-octadecylamide of Boc-L-Ala-D-Blu(OBzl) [9] was synthesized 177 mg (62%) of the glucopeptide (IV), $[\alpha]_{546}$ +8° (c 1.03; $CHCl_3$); ν_{max}^{KBr}, cm^{-1}: 3280 (NH); 2920, 2840 (CH_2); 1730 (C=O); 1630, 1550 (amide), 730, 690 (Ph).

Dibenzyl O-(Hexadecyl 2-Acetamido-2-deoxy-β-D-glucopyranosid-3-yl)-glycoloyl-L-alanyl-D-glutamate (V). With heating on the boiling water bath, 81 mg (0.082 mmole) of the acetal (II) was dissolved in 2 ml of 80% acetic acid, and the solution was kept for 30 min. Then it was evaporated to dryness, and the addition of hexane precipitated 70 mg (95%) of the diol (V), $[\alpha]_{546}$ −15° (c 1.02; EtOH); ν_{max}^{KBr}, cm^{-1}: 3290 (OH, NH); 2910, 2830 (CH_2); 1720 (C=O); 1610, 1560 (amide), 730, 690 (Ph).

By a similar method, 270 mg (0.27 mmole) of compound (III) yielded 210 mg (85%) of diol (VI), $[\alpha]_{546}$ −2° (c 1.14; EtOH–$CHCl_3$, 3:1), ν_{max}^{KBr}, cm^{-1}: 3250 (OH, NH); 2900, 2830 (CH_2); 1720 (C=O); 1630, 1530 (amide), 730, 690 (Ph), while 150 mg (0.13 mmole) of compound (IV) gave 121 mg (87%) of the diol (VII), $[\alpha]_{546}$ +9° (c 1.25; EtOH–$CHCl_3$, 3:1), ν_{max}^{KBr}, cm^{-1}: 3250 (OH, NH); 2910, 2840 (CH_2); 1730 (C=O); 1630, 1550 (amide), 710, 690 (Ph).

O-(Hexadecyl 2-Acetamido-2-deoxy-β-D-glucopyranosid-3-yl)-glycoloyl-L-alanyl-d-glutamic Acid (VIII). A solution of 60 mg (0.067 mmole) of the dibenzyl ester (V) in 6 ml of dioxane–ethanol (1:2) was subjected to catalytic hydrogenolysis over 50 mg of 10% Pd/C at room temperature. After 10 h the catalyst was filtered off and was washed with 3 ml of ethanol, the filtrate was evaporated and the residue was treated with hexane, to give 39 mg (71%) of the amorphous compound (VIII), $[\alpha]_{546}$ −20° (c 0.75; EtOH); ν_{max}^{KBr}, cm^{-1}: 3250-3360 (OH, NH); 2910, 2840 (CH_2); 1710 (C=O); 1640, 1550 (amide).

Analogously, from 110 mg (0.12 mmole) of the diester was synthesized 72 mg (82%) of compound (IX), $[\alpha]_{546}$ −11° (c 1.19; EtOH–$CHCl_3$, 3:1); ν_{max}^{KBr}, cm^{-1}: 3230-3330 (OH, NH); 2910, 2840 (CH_2); 1710 (C=O), 1620, 1550 (amide), and from 50 mg (0.047 mmole) of the ester (VII) 31 mg (75%) of compound (X), $[\alpha]_{546}$ +15° (c 0.45; EtOH–$CHCl_3$, 3:1); ν_{max}^{KBr}, cm^{-1}: 3250-3320 (OH, NH); 2910, 2830 (CH_2); 1710 (C=O); 1630, 1550 (amide).

LITERATURE CITED

1. E. Lederer, Prospects of Bioorganic Chemistry and Molecular Biology [in Russian], Nauka, Moscow (1986), p. 294.
2. N. C. Chaturvedi, M. S. Khosla, and N. J. Anand, J. Med. Chem., 9, No. 11, 971 (1966).
3. S. Kusumoto, Y. Tarumi, K. Ikenaka, and T. Shiba, Bull. Chem. Soc. Jpn., 49, No. 2, 533 (1976).
4. P. Lefrancier, J. Choay, M. Derrien, and I. Lederman, Int. J. Peptide Protein Res., 9, 249 (1977).
5. P. Lefrancier, M. Derrien, I. Lederman, F. Nief, J. Choay, and E. Lederer, Int. J. Peptide Protein Res., 11, 289 (1978).
6. E. Lederer, J. Med. Chem., 23, No. 8, 819 (1980); A. Adam, M. Devys, M. Souvannavong, P. Lefrancier, J. Choay, and E. Lederer, Biochem. Biophys. Res. Commun., 72, No. 1, 339 (1976).
7. A. E. Zemlyakov and V. Ya. Chirva, Khim. Prir. Soedin., No. 5, 714 (1987).
8. A. L. Rakhmilevich, T. L. Migdal, M. S. Rakhimova, M. A. Shneiderova, V. Ya. Chirva, and A. E. Zemlyakov, Antibiot. Khim., 34, No. 11, 836 (1989).
9. A. E. Zemlyakov, V. V. Terekohov, and V. Ya. Chirva, Khim. Prir. Soedin., No. 2, 249 (1990).