precipitate that had deposited was filtered off and was washed on the filter with 66 ml of cooled ethanol. In the case of trityl protection of the mercapto function of cysteine, the crystallization was carried out from 65 ml of hot ethyl acetate.

3. Preparation of the Hydrochlorides of the Tetra-, Penta-, and Hexapeptides. A. A solution of 10 mmole of a tetra- or pentapeptide in 20 ml of acetic acid was treated with 6 ml (40 mmole of HCl) of a solution of HCl in dioxane (c = 0.25 g/ml). The mixture was kept at 17 ±5°C for 30 min, and then the peptide was precipitated by the addition of 50 ml of dry ether. The solution was decanted off and the precipitate was treated with 20 ml of dry ether, transferred to a filter, and washed on the filter with 20 ml of dry ether. The dry powder was dried further in a vacuum drying cabinet over KOH to constant weight. This gave 10 mmole of the tetra- or pentapeptide hydrochloride. Yield 100%.

B. The hexapeptide hydrochloride was obtained similarly, but 14 ml of methanol was used in place of acetic acid and 8.5 ml of HCl in dioxane instead of 6 ml. The mixture was allowed to stand for 15 min and the peptide was precipitated by the addition of 18 ml of ethyl acetate. The subsequent operations were as in paragraph A. this gave 10 mmole of the hexapeptide hydrochloride. Yield 100%.

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SYNTHESIS OF ω-AMINO- AND ω-CARBOXYALKYLAMIDES OF N-ACETYLMURAMOYL-L-ALANYL-D-ISOGLUTAMINE

V. O. Kur'yanov, T. F. Zhelobetskaya, A. E. Zemlyakov, and V. Ya. Chirva

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The synthesis has been effected of derivatives of MDP having a spacer with an amino or carboxy group. The final stage of the synthesis was the condensation of Boc-L-Ala-D-iGln with 6-aminohexanol (followed by the two-stage replacement of the hydroxy by an azido group) or with benzyl 6-aminohexanoate.

Derivatives of N-acetylmuramoyl-L-alanyl-D-isoglutamine (muramoyldipeptide, MDP) having a spacer with active functional groups are widely used to obtain highly active "macromolecular" glycopeptides [1], conjugates with proteins, peptides, and polysaccharides [2, 4], and new immunomodulators [5, 6]. Spacers have been described which are attached to the MDP molecule at the glycosidic center [1, 4], at the primary hydroxyl [3, 5, 6], and in the dipeptide fragment [2, 7].

We have previously reported the synthesis of β-(ω-aminoalkyl)glycosides of MDP [8]. In continuation of work on the synthesis and study of the biological activity of muramoyldipeptide derivatives, we have obtained derivatives of MDP having an alkyl spacer with an amino or carboxy group at the end (I, a, b). In investigations published previously, amino acids (L-lysine, L-alanine, etc.) have usually been used as spacers [2, 7]. Thr 6-N-acryloylhexamethylenediamide of MDP synthesized by Khorlin et al. [9] can be used only for obtaining copolymers [9, 10]. The ω-amino- and ω-carboxyalkamides of MDP that we have ob-

Simferopol' State University.

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tained can be used both for immobilization on natural or synthetic polymers and in combinations with a wide range of biologically active compounds.

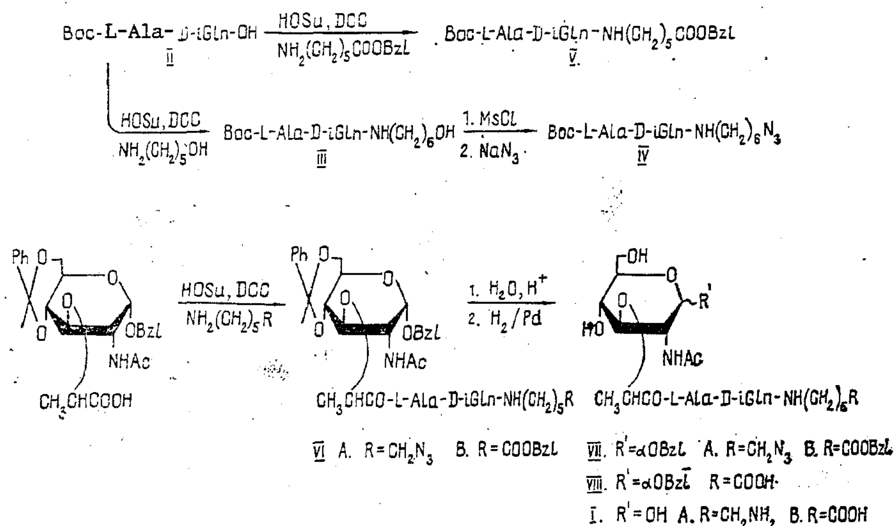

Fig. 1

The scheme for the synthesis of compounds (IA, B) is shown in Fig. 1. The N-protected dipeptide (II) [11], obtained by the hydrogenolysis of the corresponding benzyl ester, was condensed with 6-aminohexan-1-ol. The hydroxy group in compound (III) was mesylated, and then, by heating with sodium azide, the mesylate group was replaced by an azido group. The IR spectra of the azide (IV) showed an intense absorption band of the azido group (2100 cm^{-1}). Compound (V) was synthesized similarly by the condensation of the dipeptide (II) with benzyl ε-aminocaproate. Its IR spectrum contained the absorption band of the phenyl group (690 cm^{-1}).

In the PMR spectra of derivatives (IV) and (V), in addition to the signals of the dipeptide fragment (see the Experimental part) it was possible to identify signals of the protons of the spacer grouping: the triplet of the amide proton (6.31 and 6.37 ppm), the quartet of the α-methylene group (3.22 and 3.23), multiplets of methylene protons (1.51, 1.66, and 1.52, 1.60 ppm), and the triplet of the ω-methylene group (2.37 and 3.27 ppm). In addition, for compound (IV) we observed the signals of the protons of the benzyl ester group: the singlet of a methylene group (5.11 ppm) and the multiplet of phenyl protons (7.35 ppm).

1-O-Benzyl-4,6-O-benzylidene-N-acetyl-α-muramic acid [12] was activated with N-hydroxysuccinimide (HOSU) and N,N'-dicyclohexylcarbodiimide (DCC), and then the N-deblocked peptides were added. The protected glycopeptides (VIA, B) were isolated by column chromatography. The benzylidene protections were eliminated by acid hydrolysis. Subsequent hydrogenolysis of the benzyl glycoside and simultaneous reeuction of the azido function to an amino function in compound (VIIA) were carried out over PdO. In the case of compound (VIIB) the catalytic hydrogenolysis of the benzyl ester and the benzyl glycoside over PdO/C was carried out stepwise. First (reaction time about 1 h), the acid (VIII) was formed, and after 24 h the desired compound (IB) was isolated. The IR spectra of derivatives (IA, B) lacked the absorption bands of the protective groups.

EXPERIMENTAL

Melting points were determined on a PTP instrument, and optical rotations on a Polamat-A polarimeter at 20-22°C. PMR spectra were obtained on a Bruker WM-500 (500 MHz) spectrometer with TMS as internal standard. IR spectra were recorded on a Specord 75-IR spectrophotometer (KBr tablets). TLC was conducted on Silufol UV-254 plates (Czechoslovakia) with the zones being detected by carbonization at 300°C or, for the peptides and compound (I), by spraying with alcoholic solutions of ninhydrin followed by heating. The following solvent systems were used: 1) chloroform—ethanol (15:1) (A), (10:1), (B), and (5:1) (C); and 2) butanol—acetic acid—water (3:1:1) (D). Column chromatography was performed on washed silica gels L 40-100 μm and L 100-250 μm (Czechoslovakia). The elementary analyses of all the compounds corresponded to the calculated figures.

6-(tert-Butoxycarbonyl-L-alanyl-D-isoglutaminyl)hexan-1-ol (III). With stirring, 200 mg (1.74 mmole) of N-hydroxysuccinimide and 358 mg (1.74 mmole) of dicyclohexylcarbodiimide were added to a solution of 500 mg (1.58 mmole) of the N-protected dipeptide (II) in 10 ml of tetrahydrofuran. After the end of the reaction (monitoring by TLC, system B), the precipitate of dicyclohexylurea that had deposited was filtered off and was washed with two portions of THF. With stirring, 203 mg (1.7 mmole) of 6-aminohexan-1-ol in TMF was added to the filtrate. After 3 h, the reaction mixture was evaporated, and the residue was purified by column chromatography (eluent: chloroform–ethanol (50:1)), giving 350 mg (53%) of compound (III) $[\alpha]_{546}$ −12.8° (c 0.39; chloroform) mp 90-91°, R_f 0.17 (syst. B); ν_{max}^{KBr} (cm^{-1}): 3400-3290 (OH, NH_2, NH); 2930, 2840 (CH_2); 1670, 1640, 1620, 1530 (amide).

Benzyl 6-(tert-butoxycarbonyl-L-alanyl-D-isoglutaminylamino)haxanoate (V) was obtained similarly with a yield of 85%, mp 102-103°, $[\alpha]_{546}$ − 6.5° (c 0.6; chloroform); R_f 0.49 (syst. B); ν_{max}^{KBr} (cm^{-1}1): 3400, 3310 (NH_2, NH); 2920, 2860 (CH_2); 1725 (ester); 1680, 1640, 1615, 1540 (amide); 690 (Ph). PMR (500 MHz, $CDCl_3$): 1.34 (3H, d, $J_{CH_3,CH}$ = 7 Hz, CH_3CH − Ala), 1.42 (9H, s, Me_3C), 1.51,1.66 (m, $(CH_2)_n$), 2.37 (2H, t, CH_2COOBzl), 3.22 (2H, q, $NHCH_2CH_2$), 4.07 (1H, m, CH−iGln), 4.41 (1H, q, CH−CH_3−Ala), 5.11 (2H, s, $COOCH_2Ph$), 5.29 (1H, d, NH−iGln), 5.73 and 7.13 (2H, 2s, $CONH_2$), 6.31 (1H, t, $NHCH_2$), 7.35 (5H, m, Ph), 7.80 (1H, d, NH−Ala).

6-(tert-Butoxycarbonyl-L-alanyl-D-isoglutaminylamino)hexyl Azide (IV). With stirring and cooling to −10°C, a solution of 0.9 ml (1.16 mmole) of mesyl chloride in 2 ml of methylene chloride was added in small portions to a solution of 350 mg (0.84 mmole) of compound (III) in 2 ml of pyridine. The reaction mixture was left at room temperature for 3 h and was then diluted with chloroform and was washed with saturated sodium bicarbonate solution and with two portions of water. The organic layer was dried with sodium sulfate and evaporated. The mesylate obtained (290 mg, 0.56 mmole), without purification, was dissolved in 2 ml of dimethylformamide and, with stirring, this solution was heated with 114 g (1.75 mmole) of sodium azide. The reaction mixture was kept at 80°C for 2 h and was then evaporated, and, by column chromatography (eluent: chloroform−methanol (50:1)), 218 mg (85%) of the azide (IV) was isolated in the form of an amorphous powder, $[\alpha]_{546}$ − 3.7° (c 0.8; chloroform): R_f 0.43 (syst. B); ν_{max}^{KBr} (cm^{-1}): 3360, 3300 (NH_2, NH); 2920, 2860 (CH_2); 2100 (azide); 1680, 1630, 1620, 1540 (amide), PMR (500 MHz, $CDCl_3$): 1.35 (3H, d, $J_{CH_3,CH}$ = 7 Hz, CH_3CH−Ala), 1.43 (9H, s, Me_3C), 1.52 and 1.60 (m, $CH_2)_n$), 3.23 (2H, q, $NHCH_2CH_2$), 3.27 (2H, t, $CH_2CH_2N_3$), 4.08 (1H, m, CH−iGln), 4.41 (1H, q, $CHCH_3$−Ala), 5.36 (1H, d, NH−iGln), 5.84 and 7.15 (2H, 2s, $CONH_2$), 6.37 (1H, t, $NHCH_2$), 7.82 (1H, d, NH−Ala).

Benzyl 6-[O-(Benzyl 2-Acetamido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranosid-3-yl)-lactoyl-L-alanyl-D-isoglutaminylamino]hexanoate (VIB). With stirring 63 mg (0.55 mmole) of N-hydroxysuccinimide and 114 mg (0.55 mmole) of dicyclohexylcarbodiimide were added to a solution of 200 mg (0.42 mmole) of 1-O-benzyl-4,6-benzylidene-N-acetyl-α-muramic acid in 10 ml of dioxane. After 4 h, the precipitate of dicyclohexylurea that had deposited was filtered off and was washed on the filter with 5 ml of dioxane, and then the filtrate was treated with solution of 295 mg (0.55 mmole) of benzyl 6-(L-alanyl-D-isoglutaminylamino)hexanoate (obtained by treating compound (V) with 3 ml of trifluoroacetic acid followed by evaporation to dryness) in 5 ml of dioxane and 0.35 ml of triethylamine. After 24 h, the precipitate of glycopeptide that had deposited was filtered off and was purified by column chromatography (eluent: chloroform−ethanol (10:1)).

This gave 230 mg (62%) of the glycopeptide (VIB) in the form of an amorphous powder, $[\alpha]_{546}$ + 72° (c 1.3; DMFA), R_f 0.26 (syst. A), ν_{max}^{KBr} (cm^{-1}): 3400, 3270 (NH_2, NH), 2920, 2860 (CH_2); 1725 (ester); 1620, 1530 (amide); 720, 695 (Ph). Glycopeptide (VIA) was obtained similarly with a yield of 55%, R_f 0.25 (syst. A); ν_{max}^{KBr} (cm^{-1}): 3390, 3260 (NH_2, NH); 2915, 2840 (CH_2); 2090 (azide), 1630, 1520 (amide); 715, 690 (Ph).

Benzyl [O-(Benzyl 2-Acetamido-2-deoxy-α-D-glucopyranosid-3-yl)-D-lactoyl-L-alanyl-D-isoglutaminylamino)hexanoate (VIIB). With heating in the boiling water bath, 350 mg (0.40 mmole) of glycopeptide (VIB) was dissolved in 5 ml of 80% acetic acid, and the solution was heated for another 30 min and was then evaporated to dryness. The residue was triturated with ether, giving 300 mg (96%) of the diol (VIIB), mp 210°C (decomp.), $[\alpha]_{546}$ + 81.9° (c 0.55; acetic acid), R_f 0.43 (syst. B), ν_{maxr}^{KBr} (cm^{-1}); 3380-3260 (OH, NH_2, NH); 2915, 2850

(CH_2); 1725 (ester); 1630, 1530 (amide): 715, 690 (Ph). Diol (VIIA) was obtained similarly yield 90%, mp 205°C (decomp), $[\alpha]_{546}$ + 100° (c 0.7; acetic acid), R_f 0.58 (syst. D); ν_{max}^{KBr} (cm^{-1}): 3400-3290 (OH, NH_2, NH); 2915, 2850 (CH_2); 2090 (azide); 1630, 1540 (amide); 720, 690 (Ph).

6-[O-(2-Acetamido-2-deoxy-D-glucopyranos-3-yl)-D-lactoyl-L-alanyl-D-isoglutaminamino)-hexanoic Acid (IB). The diol (VIIB) (80 mg 0.10 mmole) was dissolved in 5 ml of 80% acetic acid and was subjected to hydrogenolysis at room temperature over 30 mg of 10% Pd/C. After 30 min, the catalyst was filtered off and was washed with 3 ml of 80% acetic acid. The filtrate was evaporated, and the residue was triturated in acetone, giving 59 mg (83%) of compound (VIII), $[\alpha]_{546}$ + 91° (c 1.13; acetic acid), R_f 0.52 (syst. D); ν_{max}^{KBr} (cm^{-1}): 3390-3290 (OH, NH_2, NH); 2910, 2840 (CH_2); 1640, 1540 (amide); 720, 690 (Ph).

The benzyl glycoside (VIII) (50 mg, 0.07 mmole) was dissolved in 3 ml of 80% acetic acid and subjected to hydrogenolysis over 40 mg of 10% Pd/C for 8 h. then the catalyst was filtered off and was washed on the filter with 3 ml of 80% acetic acid, and the filtrate was evaporated. The residue was triturated in acetone, giving 36 mg (83%) of the desired glycopeptide (IB), $[\alpha]_{546}$ + 4.4° (c 1.1; ethanol), R_f 0.27 (syst. D); ν_{max}^{KBr} (cm^{-1}): 3400-3250 (OH, NH_2, NH); 2910, 2850 (CH_2); 1640, 1530 (amide).

The glycopeptide (Ia) was obtained similarly by the exhaustive hydrogenolysis of the diol (VIIA) over PdO with the simultaneous reduction of the azido group to an amino group. Yield: 86%, amorphous powder, $[\alpha]_{546}$ + 81.2° (c 0.8; DMSO), R_f 0.1 (syst. D); ν_{max}^{KBr} (cm^{-1}): 3500-3200 (OH, NH_2, NH); 2950, 2880 (CH_2); 1650, 1550 (amide).

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