SYNTHESIS OF MURAMOYLTRIPEPTIDES AND THEIR ISOTOPE-LABELED ANALOGUES

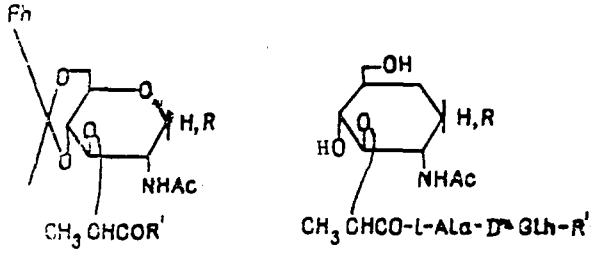
A. E. Zemlyakov, I. M. Andryakova, and V. Ya. Chirva

UDC 547.455.623'233.1'472.3'466.23'64'22.057

The synthesis has been effected of the methyl ester of N-acetylmuramoyl-L-alanyl-D-isoglutamylglycine and its hexadecyl β-glycoside and the corresponding [1-^{14}C]glycine analogues.

N-Acetylmuramoyl-L-alanyl-D-isoglutamine (muramoyldipeptide, MDP) and many of its derivatives exhibit immunoadjuvant activity and stimulate nonspecific anti-infection resistance. They have found use in "classical" and synthetic vaccines and also in the immunotherapy of tumors [1]. For the study of the mechanism of the action of MDP and its analogues on biological systems and the metabolism use has been made of [6-^{3}H]-N-acetylmuramoyl-L-alanyl-D-isoglutamine [2], N-acetylmuramoyl-[U-^{14}C]-L-alanyl-D-isoglutamine [3], and muramoyl peptides labeled with ^{14}C and C_1 of the lactoyl residue [4].

The aim of the present work was to develop a method for synthesizing ^{14}C-labeled MDP derivatives. Since it is known that the presence of a third amino acid (glycine, D- or L-alanine, L-lysine) in the peptide component of glycopeptides does not affect their biological activity [5], it appeared possible to introduce an isotopic label into the isoglutamine residue of various derivatives of MDP. We have performed the synthesis of the methyl ester of N-acetylmuramoyl-L-alanyl-D-isoglutamylglycine (X) and its hexadecyl β-glycoside (VIII) and the corresponding [1-^{14}C]glycine analogues [(IX) and (XI)].



III. R = αOBzl; R′ = OH
IV. R = βC$_{16}$H$_{33}$; R′ = OH
V. R = αOBzl; R′ = *L*-Ala-*D*-iGln-Gly-OMe
VI. R = βC$_{16}$H$_{33}$; R′ = *L*-Ala-*D*-iGln-Gly-OMe

VII. R = αOBzl; R′ = Gly-OMe
VIII. R = βC$_{16}$H$_{33}$; R′ = Gly-OMe
IX. R = βC$_{16}$H$_{33}$; R′ = [1-^{14}C]-Gly-OMe
X. R = OH; R′ = Gly-OMe
XI. R = OH; R′ = [1-^{14}C]-Gly-OMe

Using the traditional scheme for the synthesis of glycopeptides, we first obtained the tripeptide (I) by condensing tert-butoxycarbonyl-L-alanyl-D-glutamine [6] with the hydrochloride of glycine methyl ester, employing N-hydroxysuccinimide (HOSu) and dicyclohexylcarbodiimide (DCC) as activating reagents. The protected muramic acids (III) and (IV) were condensed with the tripeptide (I) using the N-hydroxysuccinimide method. The corresponding glycopeptides (V) and (VI) were isolated by column chromatography with yields of 84-88%. Their structures were shown by their PMR spectra, which contained the signals of the aglycons, of an acetamide group, and of the benzylidene protection in the carbohydrate moiety of the molecule. The structure of the peptide fragment was confirmed by the signals of the protons of the methyl ester group (three-proton singlet with a CS of 3.58-3.60 ppm), and of glycine (doublet of a methylene group with a CS of 3.79-3.81 ppm and triplet of an amide proton with a CS of 8.27-8.28 ppm), and of the γ-methylene group of isoglutamine (triplet with the CS of 2.12-2.15 ppm). The presence of one-proton doublets with the CSs 4.85 ppm (SSCC 3.5 Hz) and

M. V. Frunze Simferapol' State University. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 245-248, March-April, 1990. Original article submitted May 4, 1989.

4.47 ppm (9 Hz) confirmed the retention of the α- and β-configurations of the anomeric protons in compounds (V) and (VI), respectively. The subsequent elimination of the benzylidene protection in these substances and the catalytic hydrogenolysis of the benzyl glycoside in the diol (VII) led to the muramoyl tripeptides (VIII) and (X) [sic]. The removal of the temporary protective groups was confirmed by their IR spectra. By an alternative variant of the synthesis of muramoyl tripeptides, the glycine fragment was attached to partially protected muramoyl dipeptides. Thus, the condensation of glycine methyl ester with α-benzyl-4,6-O-isopropylidene-N-acetylmuramoyl-L-alanyl-D-isoglutamine [7] and the subsequent acid hydrolysis of the isopropylidene group gave the diol (VII). At the same time, the addition of glycine methyl ester to the hexadecyl β-glycoside of MDP [8] took place with low yield.

Starting from [1-^{14}C]glycine the isotopically labeled muramoyl tripeptides (IX) and (XI) were synthesized by the above-described procedures with specific radioactivities of 0.25 and 0.36 mBq/mg, respectively.

EXPERIMENTAL

For general observations see [8]. PMR spectra were taken on Bruker WM-250 (250 MHz) and Bruker WM-500 (500 MHz) spectrometers in DMSO-d_6. Solvent systems for TLC: 1) butanol—acetic acid—water (3:1:1); 2) benzene—ethanol (4:1); 3) and 4) chloroform—ethanol (10:1) and (5:1). The analyses of all the compounds corresponded to the calculated figures.

[1-^{14}C]Glycine from the USSR and unlabeled glycine from Reanal (Hungary) were used.

Methyl Ester of tert-Butoxycarbonyl-L-alanyl-D-isoglutamylglycine (I). A solution of 300 mg (0.95 mmole) of tert-butoxycarbonyl-L-alanyl-D-isoglutamine [6] in 15 ml of THF was treated with 120 mg (1.04 mmole) of HOSu and 225 mg (1.09 mmole) of DCC, and the mixture was stirred for 4 h. The precipitate of dicyclohexylurea was filtered off. The filtrate was treated with a solution of 130 mg (1.04 mmole) of glycine methyl ester hydrochloride (obtained by boiling a methanolic solution in the presence of thionyl chloride; crystallized from methanol; mp 170-173°C, R_f 0.28 (system 1); according to the literature [9]: mp 175°C) in 2 ml of DMFA and 0.01 ml of triethylamine. After 2 days, the reaction mixture was evaporated, and crystallization from ether—hexane yielded 310 mg (65%) of the tripeptide (I); mp 108°C, $[\alpha]_{546}$ −27° (c 1.25; $CHCl_3$), R_f 0.44 (system 2). IR (cm^{-1}): 3400, 3290 (NH_2, NH); 2970 (CH_3); 1760 (ester); 1720 (urethane); 1670, 1650, 1550 (amide).

The methyl ester of tert-butoxycarbonyl-L-alanyl-D-isoglutamyl[1-^{14}C]glycine (II) was obtained similarly.

Methyl Ester of 2-Acetamido-1-O-benzyl-4,6-O-benzylidene-2-deoxy-α-D-glucopyranosyl-(3→O)-D-lactoyl-L-alanyl-D-isoglutamylglycine (V). Benzyl 2-acetamido-4,6-O-benzylidene-3-O-(D-1-carboxyethyl)-2-deoxy-α-D-glucopyranoside (III) [10] (120 mg; 0.25 mmole) was dissolved in 6 ml of THF and was activated with 32 mg (0.28 mmole) of HOSu and 62 mg (0.30 mmole) DCC. After 4 h, the activated ester was filtered into a flask containing a solution of the methyl ester of L-alanyl-D-isoglutamylglycine (obtained by treating 100 mg (0.26 mmole) of peptide (I) with trifluoroacetic acid (1 ml) followed by evaporation to dryness and neutralization with triethylamine) in 5 ml of THF. After 30 h, the gel that had deposited was filtered off and was washed with ether. Column chromatography [chloroform→chloroform—ethanol (20:1)] led to the isolation of 160 mg (84%) of (V); mp 268-270°C, $[\alpha]_{546}$ +90° (c 0.25; DMFA), R_f 0.45 (system 3). IR (cm^{-1}): 3400, 3280 (NH_2, NH); 1740 (ester); 1660, 1540 (amide); 700, 670 (Ph). PMR (250 MHz): 1.19 and 1.22 (6H, 2d, $J_{Me,CH}$ 7 Hz, 2 MeCH), 1.81 (3H, s, NAc), 2.12 (2H, t, γ-CH_2—iGln), 3.60 (3H, s, COOMe), 3.79 (2H, d, CH_2-Gly), 4.50 and 4.70 (2H, 2d, J_{gem} = 12 Hz, O$\underline{CH_2}$Ph), 4.85 (1H, d, $J_{1,2}$ = 3.5 Hz, H-1), 5.70 (1H, s, $\underline{CH}$Ph), 7.32-7.41 (10H, m, 2Ph), 7.06, 7.55, 8.13, 8.16, (5H, 1s, 3d, NH_2 and 3NH), 8.28 (1H, t, NH-Gly).

Methyl Ester of 2-Acetamido-1-O-hexadecyl-4,6-O-benzylidene-2-deoxy-α-D-glucopyranosyl-(3→O)-D-lactoyl-L-alanyl-D-isoglutamylglycine (VI). Similarly, from 95 mg (0.16 mmole) of hexadecyl-2-acetamido-4,6-O-benzylidene-3-O-(D-1-carboxyethyl)-2-deoxy-β-D-glucopyranoside (IV) [8] and 45 mg (0.16 mmole) of the tripeptide (I) was obtained 120 mg (88%) of the glycopeptide (VI), mp 240°C, decomp., $[\alpha]_{546}$ −17° (c 0.70; $CHCl_3$—EtOH, 4:1), R_f 0.67 (system 4). IR (cm^{-1}): 3410, 3280 (NH_2, NH); 2930, 2850 (CH_2); 1750 (ester); 1660, 1550 (amide); 750, 700 (Ph). PMR (500 MHz): 0.85 (3H, t, $\underline{Me}CH_2$), 1.24 (m, CH_2, MeCH), 1.80 (3H, s, NAc), 2.15 (2H, t, ν-CH_2—iGln), 3.58 (3H, s, COOMe), 3.81 (2H, d, CH_2—Gly), 4.47 (1H, d, $J_{1,2}$ = 9 Hz, H-1), 5.69 (1H, s, $\underline{CH}$Ph), 7.31 (5H, m, Ph), 7.06, 7.38, 7.91, 8.09 (5H, s and 3d, NH_2 and 3NH), 8.27 (1H, t, $\underline{NH}$—Gly).

Methyl Ester of 2-Acetamido-1-O-benzyl-2-deoxy-α-D-glucopyranosyl-(3→O)-D-lactoyl-L-alanyl-D-isoglutamylglycine (VII). With heating in the boiling water bath, 100 mg (0.12 mmole) of compound (V) was dissolved in 15 ml of 80% acetic acid, and the solution was kept at the same temperature for 30 min. Then it was evaporated, and the addition of 50 ml of hexane led to the precipitation of 70 mg (80%) of the diol (VII); mp 195°C, $[\alpha]_{546}$ +130° (c 0.80; 90% ethanol–EtOH), R_f 0.35 (system 1). IR (cm^{-1}): 3360-3270 (OH, NH_2, NH): 1710 (ester); 1640, 1530 (amide); 700, 670 (phenyl).

1-O-benzyl-2-acetamido-4,6-O-isopropylidene-2-deoxy-α-D-glucopyranosyl-(3→O)-D-lactoyl-L-alanyl-D-isoglutamine [7] (100 mg; 0.16 mmole) was activated as described in the synthesis of compound (I) and was condensed with 25 mg (0.19 mmole) of glycine methyl ester hydrochloride with the addition of 2 drops of triethylamine. After 30 h, the reaction mixture was diluted with ether and the precipitate was filtered off. The dried substance was dissolved with heating in the boiling water bath in 10 ml of 80% acetic acid. The solution was evaporated, giving 85 mg (76%) of the diol (VII).

Methyl Ester of 1-O-Hexadecyl-2-acetamido-2-deoxy-β-D-glucopyranosyl-(3→O)-D-lactoyl-L-alanyl-D-isoglutamylglycine (VIII). Compound (VI) (95 mg; 0.11 mmole) was debenzylidenated in a similar way to glycopeptide (V), giving 70 mg (77%) of the diol (VIII); mp 195°C, $[\alpha]_{546}$ −6° (c 0.70; $CHCl_3$–EtOH, 4:1), R_f 0.22 (system 1). IR (cm^{-1}): 3320 (OH, NH_2, NH); 2930, 2850 (CH_2); 1740 (ester); 1660, 1630, 1580 (amide).

By the procedure described above, 45 mg (74%) of the isotope-labeled muramoyl tripeptide (IX) was synthesized from 50 mg (83 μmole) of the acid (IV) and 30 mg (77 μmole) of the tripeptide (II).

The hexadecyl β-glycoside of N-acetylmuramoyl-L-alanyl-D-isoglutamine [8] (40 mg; 56 μmole) was activated as in the procedure for the synthesis of compound (I) and was condensed with 7 mg (62 μmole) of glycine methyl ester hydrochloride. After 3 days, 18 mg (41%) of the tripeptide derivative (VIII) was filtered off.

Methyl Ester of 2-Acetamido-2-deoxy-D-glucopyranosyl-(3→O)-D-lactoyl-L-alanyl-D-isoglutamylglycine (X). A solution of 50 mg (76 μmole) of the diol (VII) in 5 ml of ethanol was subjected to catalytic hydrogenolysis over 100 mg of 10% Pd/C. After 3 days, the catalyst was filtered off and was washed with 5 ml of ethanol. The filtrate was evaporated and ether precipitated 39 mg (75%) of the glycopeptide (X); mp 125-127°C, $[\alpha]_{546}$ +24° (c 0.90; 90%-EtOH); R_f 0.16 (system 1). IR (cm^{-1}): 3400-3300 (OH; NH_2, NH); 1730 (ester); 1660, 1550 (amide).

By the same method, 60 mg (0.13 mmole) of the acid (III) and 50 mg (0.13 mmole) of the isotope-labeled tripeptide (II) gave 56 mg (78%) of the muramoyl tripeptide (XI).

LITERATURE CITED

1. A. Adam, J.-F. Petit, P. Lefrancier, and E. Lederer, Mol. Cell. Biochem., 40, 27 (1981); E. Lederer, in: Perspectives in Bioorganic Chemistry and Molecular Biology [in Russian], Nauka, Moscow (1964), p. 294.
2. P. L. Durette, A. Rosegay, M. A. R. Walsh, and T. Y. Shen, Tetrahedron Lett., No. 4, 291 (1979).
3. S. Kusumoto, K. Ikenaka, and T. Shiba, Tetrahedron Lett., No. 46, 4055 (1977); S. Kusumoto, K. Ikenaka, and T. Shiba, Bull. Chem. Soc. Jpn., 52, No. 4, 1177 (1979).
4. M. Parant, F. Parant, L. Chedid, A. Yapo, J.-F. Petit, and E. Lederer, Int. J. Immunopharmacol., 1, 35 (1979).
5. Y. Nagai, K. Akiyama, S. Kotani, Y. Watanabe, T. Shimono, T. Shiba, and S. Kusumoto, Cell. Immunol., 35, 168 (1978); S. Kotani, F. Kinishita, Y. Watanabe, I. Morisaki, T. Shimono, K. Kato, T. Shiba, S. Kusumoto, K. Ikenaka, and Y. Tarumi, Biken. J., 20, No. 3-4, 125 (1977).
6. S. Kusumoto, Y. Tarumi, K. Ikenaka, and T. Shiba, Bull. Chem. Soc. Jpn., 49, No. 2, 533 (1976).
7. A. E. Zemlyakov and V. Ya. Chirva, Khim. Prir. Soedin., No. 6, 892 (1987).
8. A. E. Zemlyakov and V. Ya. Chirva, Khim. Prir. Soedin., No. 5, 714 (1987).
9. I. Heilbron et al., Dictionary of Organic Compounds, 2nd edn., Eyre and Spottiswood, London [Russian translation, Izd. Inostr. Lit., Moscow, Vol. 2 (1949), p. 125].
10. T. Osawa and R. W. Jeanloz, J. Org. Chem., 30, No. 2, 448 (1965).