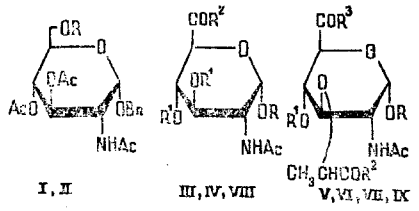
SYNTHESIS OF URONOYLDIPEPTIDE DERIVATIVES OF N-ACETYLGLUCOSAMINE AND OF N-ACETYLMURAMOYLDIPEPTIDE

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

UDC 547.963.1

Starting from benzyl 3,4-di-O-acetyl-6-O-trityl-N-acetyl-β-glucosamine and benzyl α-benzyl-4-O-acetyl-N-acetylmuramate, 2-acetamido-2-deoxy-D-glucopyranuronyl-L-alanyl-D-isoglutamine and O-(2-acetamido-2-deoxy-D-glucopyranuronyl-L-alanyl-D-isoglutamin-3-yl)-D-lactoyl-L-alanyl-D-isoglutamine — uronoyldipeptide analogs of N-acetylglucosamine and N-acetylmuramoyl dipeptides — have been synthesized.

Investigations of the synthesis on analogs of N-acetylmuramoyl-L-alanyl-D-isoglutamine (muramoyldipeptide, MDP), the minimum immunoactive structural unit of the peptidoglycan of the cell walls of bacteria have been carried out widely in the last two decades [1, 2]. In a number of MDP derivatives, the method and position of attachment of the dipeptides to the carbohydrate moiety of a molecule have a substantial influence on its biological activity. Thus, isomers of muramoyldipeptide having the lactylpeptide fragment at C-4 and C-6 [3, 4] are inactive in the test for hypersensitivity of the delayed type [3]. At the same time, the use in place of the lactyl fragment of a hydroxyacetic acid residue [5] or a 2-hydroxy-3-methylpentanoic acid residue [6] had little effect on biological activity [1, 6]. The use of a thiocarbamoyl group at C-1 of the glucosamine residue as a binding unit [7] preserves the adjuvant activity.



I. R=Tr
II. R=H

III. R=Bn; R^1=Ac; R^2=OH
IV. R=Bn; R^1=Ac; R^2=*a*
VIII. R=R^1=H; R^2=*b*

V. R=Bn; R^1=Ac; R^2=OBn; R^3=OH
VI. R=Bn; R^1=Ac; R^2=R^3=*a*
VII. R=Bn; R^1=H; R^2=R^3=*b*
IX. R=R^1=H; R^2=R^3=*b*

a=*L*-Ala-*D*-iGln-OBn; *b*=*L*-Ala-*D*-iGln

We have previously [8] described the synthesis of O-(2-acetamido-2-deoxy-D-glucopyranuronos-3-yl)-D-lactoyl-L-alanyl-D-isoglutamine — a convenient initial compound for obtaining new analogs of muramoyldipeptide by modifying the carboxy group of the monosaccharide moiety. In the present paper we propose a synthesis of uronoyldipeptide analogs of N-acetylglucosamine and of MPD differing by the position and method of attachment of the dipeptide to the monosaccharide.

Compound (I) was obtained by the successive tritylation and acetylation of the benzyl α-glycoside of N-acetylglucosamine in dry pyridine. The detritylation of (I) with pyridinium perchlorate in a mixture of methanol and nitromethane [9] led to the alcohol (II). Derivative (II) and benzyl 2-acetamido-4-acetyl-3-O-[D-1-(benzyloxycarbonyl)ethyl]-2-deoxy-α-D-glucopyranoside [8] were converted by oxidation with chromium trioxide in H_2SO_4 in acetone into the corresponding uronic acids (III) and (IV). Acid (III) was activated with N-hydroxysuccinimide (HONSu) and with dicyclohexylcarbodiimide (DCC) and was condensed with the γ-benzylester of L-alanyl-D-isoglutamine. The glycopeptide (V) was isolated by column chroma-

M. V. Frunze Simferopol' State University. Translated from Khimiya Prirodynkh Soedinenii, No. 6, pp. 850-855, November-December, 1988. Original article submitted December 14, 1987.

0009-3130/88/2406-0725$12.50

tography with a yield of 70%. The introduction of the dipeptide was confirmed by the PMR spectrum, which showed the signals of the protons of the methyl group of L-alanine and of the protons of the γ-CH_2 group of D-isoglutamine, and a singlet of the methylene protons of the γ-benzyl protection of the isoglutamine.

The selective hydrogenolysis of the benzyl ester protection in the acid (IV) gave a quantitative yield of the biacid which, without purification, was condensed with the γ-benzyl ester of L-alanyl-D-isoglutamine. The structure of glycopeptide (VI) was confirmed by its PMR spectrum (see the Experimental part).

The ester protections in compounds (V) and (VI) were saponified with 1 M aqueous KOH. The benzyl 2-acetamido-2-deoxy-α-D-glucopyranosiduronyl-L-alanyl-D-isoglutamine, without purification was subjected to hydrogenolysis over 10% Pd/C, and the benzyl glycoside (VII) was isolated by column chromatography. In the PMR spectrum of (VII), the signals of the nine protons of three methyl groups, the multiplets of four protons of γ-CH_2 groups of isoglutamine, and the signals of the five phenyl protons of the glycosidic benzyl group were identified. The glycosidic protection in compound (VII) was removed by catalytic hydrogenolysis. The hydrogenolysis gave the desired products (VIII) and (IX). The structure of (VIII) was confirmed by its PMR and IR spectra. The IR spectrum of (IX) lacked the absorption bands of an aromatic group and showed the absorption bands of amide groups and also of NH, NH_2, and OH groups, which corresponds to the structure of the glycopeptide (IX).

EXPERIMENTAL

Melting points were determined on a PTP instrument. Optical rotations were measured at 20-22°C on a Polamat-A automatic polarimeter. PMR spectra were taken on Bruker WM-500 (500 MHz) and Varian XL-100 (100 MHz) spectrometers relative to TMS. IR spectra were recorded on a Specord IR-75 spectrophotometer in KBr tablets. Thin-layer chromatography was conducted on Silufol UV-254 (Kavalier) and Kieselgel 60F-254 (Merck) plates in the systems $CHCl_3$–EtOH (25:1) (1), $CHCl_3$–EtOH (15:1) (2), $CHCl_3$–EtOH (10:1) (3), BuOH–AcOH–H_2O (3:1:1) (4), and BuOH–AcOH–H_2O (4:2:3) (5). The zones on the chromatogram were detected by carbonization at 400°C (for Silufol UV-254) and by spraying with 5-10% ethanolic H_2SO_4 (for Kieselgel 60F-254) followed by carbonization. Washed silica gel L 100/250 and 40/100 μm was used for column chromatography. All the solvents were previously redistilled. Solutions were evaporated in vacuum at a temperature of 40°C. The results of elementary analysis corresponded to the calculated figures.

Benzyl 2-Acetamido-3,4-di-O-acetyl-6-O-trityl-2-deoxy-α-D-glucopyranoside (I). A solution of 4 g (12.86 mmole) of benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside and 5.37 g (19.28 mmole) of chlorotriphenylmethane in 40 ml of dry pyridine was heated at 40°C under reflux until benzyl 2-acetamido-6-O-trityl-2-deoxy-α-D-glucopyranoside had been formed (monitoring by TLC in system 1). Then the reaction mixture was cooled to room temperature, treated with 40 ml of acetic anhydride, and left overnight. The resulting solution was evaporated, and the residue was diluted with benzene and purified by column chromatography, elution [benzene → benzene–$CHCl_3$ (1:25)] giving 6.9 g of (III). Yield 84%. Amorphous powder $[\alpha]_{546}$ +204° (c 1.0; chloroform); R_f 0.86 (system 1). ν_{max}^{KBr} (cm^{-1}): 3420, 3300 (NH), 1780 and 1260 (ester); 1690 and 1550 (amide), 740 (phenyl).

Benzyl 2-Acetamido-3,4-di-O-acetyl-2-deoxy-α-D-glucopyranoside (II). In a flask fitted with a reflux condenser, 6.9 g (10.83 mmole) of (I) was dissolved in a mixture of 94 ml of methanol and 16 ml of nitromethane, and 5.6 g (31.2 mmole) of pyridinium perchlorate was added. The mixture was heated at 60-70°C for 5 h. After the end of the reaction (monitoring by TLC in system 1), the solution was evaporated, and the residue was purified on a column with elution by benzene–$CHCl_3$ (1:1) → benzene–$CHCl_3$ (1:25). Yield 3.6 g (84%); mp 143-145°C; $[\alpha]_{546}$ +126° (c 0.5; chloroform), R_f 0.42 (system 1). ν_{max}^{KBr} (cm^{-1}): 3470, 3390 (OH, NH), 1750 and 1250 (ester), 1620 and 1540 (amide), 700 and 680 (phenyl).

Benzyl (2-Acetamido-3,4-di-O-acetyl-2-deoxy-α-D-glucopyranosid)uranoic acid (III). With cooling to 0-5°C, 4.15 ml of a solution of 0.96 g of CrO_3 (9.63 mmole) in 3.5 M H_2SO_4 was added in portions to a stirred solution of 1.8 g (4.56 mmole) of compound (II) in 30 ml of acetone. After the addition of the whole amount of oxidant, the reaction mixture was stirred at the given temperature for 15 min, and at room temperature for 2-3 h. Then it was diluted with water and extracted with chloroform (3 × 50 ml). The combined extract was washed with water, dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue was

dissolved in chloroform and chromatographed on a column of silica gel with elution by $CHCl_3 \rightarrow CHCl_3$–EtOH (100:1). Yield 1.15 g (62%), mp 135-137°C; $[\alpha]_{546}$ +148° (c 1.0; chloroform); R_f 0.69 (system 3); ν_{max}^{KBr} (cm^{-1}): 3390 (OH; NH); 1780 and 1260 (ester); 1650 and 1550 (amide); 750, 700 (phenyl).

γ-Benzyl Ester of (Benzyl 2-Acetamido-3,4-di-O-acetyl-2-deoxy-α-D-glucopyranosid)uronyl-L-alanyl-D-isoglutamine (IV). A stirred solution of 1.0 g (2.44 mmole) of (benzyl 2-acetamido-3,4-di-O-acetyl-2-deoxy-α-D-glucopyranosid)uranic acid (III) in 20 ml of dry THF was treated with 0.337 g (2.92 mole) of HONSu and 0.628 g (3.05 mole) of DCC. After 3 h, the precipitate of dicyclohexylurea that had deposited was filtered off and was washed with THF, and to the filtrate were added 0.46 ml of Et_3N and 1.29 g (3.17 mmole) of the γ-benzyl ester of trichloroacetyl-L-alanyl-D-isoglutamine. After 24 h, the precipitate of the glycopeptide (VI) that had deposited was filtered off and was purified by column chromatography with elution by $CHCl_3 \rightarrow CHCl_3$–EtOH (20:1). Yield 1.2 g (70%); mp 235-236°; $[\alpha]_{546}$ +94° (c 0.73; dimethylformamide); R_f 0.59 (system 2); PMR [100 MHz; (C^2H_3SO): 1.27 d (3H; $J_{CH_3,CH}$ = 7 Hz; CH_3CH); 1.87 s (3H, NAc); 1.94 s, 1.96 s (6H, 2OAc); 2.40 t (2H, γ-CH_2); 4.52 d, 4.74 d (2H; J_{gem} = 12 Hz, OCH_2Ph); 4.91 d (1H, $J_{1,2}$ = 3 Hz; H-1); 5.10 s (2H; $COOCH_2Ph$); 7.08 s (2H; $CONH_2$); 7.36 m (10H, 2Ph); 7.95 d, 8.11 d, 8.33 d (3H, 3NH). ν_{max}^{KBr} (cm^{-1}): 3390, 3300 (NH, NH_2); 1780, 1260 (ester); 1680, 1570 (amide); 730, 700 (phenyl).

2-Acetamido-2-deoxy-D-glucopyranuronoyl-L-alanyl-D-isoglutamine γ-Benzyl Ester (VIII). A suspension of 1.2 g (1.72 mmole) of (IV) in 25 ml of ethanol was treated with 5.16 ml of 1 M aqueous caustic potash. After the solid matter had dissolved, the solution was stirred for another 1 h and was then treated with JU-2 cation-exchange resin (H^+). The resin was filtered off and washed with ethanol. The saponification product was subjected to hydrogenolysis over 10% Pd–C (1 g). After 48 h, the catalyst was filtered off and was washed with 15 ml of 90% aqueous ethanol, and the filtrate was evaporated to dryness. The residue was triturated with acetone. Yield 0.6 g (80%); amorphous powder; $[\alpha]_{546}$ +12° (c 0.67; water); R_f 0.22 (svstem 5: Kieselgel 60 F-254); PMR : (500 MHz, ^2H_2O): 1.22 d (3H; CH_3CH), 1.83 s (3H, NAc), 2.00 m (2H, β-CH_2), 2.13 t (2H, γ-CH_2). ν_{max}^{KBr} (cm^{-1}): 3300 (OH, NH, NH_2), 1660 and 1560 (amide).

{Benzyl 2-Acetamido-4-O-acetyl-3-O-[D-1-(benzyloxycarbonyl)ethyl]-2-deoxy-α-D-glucopyranosid}uronic Acid (V). In portions, 4.6 ml of a solution of 1.06 g (1.06 mmole) of CrO_3 in 3.5 M H_2SO_4 was added to a stirred solution, cooled to 0°C, of 2.0 g (3.97 mmole) of benzyl 2-acetamido-4-O-acetyl-3-O-[D-1-(benzyloxycarbonyl)ethyl]-2-deoxy-α-D-glucopyranoside [8] in 33 ml of acetone. Then the synthesis and working up were carried out in the same way as for (III). The reaction product was purified by column chromatography with elution by $CHCl_3 \rightarrow CHCl_3$–EtOH (50:1). Yield 1.7 g (82%); mp 198-199°C, $[\alpha]_{546}$ +138° (c 1.0; chloroform); R_f 0.38 (system 2); ν_{max}^{KBr} (cm^{-1}): 3300 (OH, NH), 1730, 1250 (ester), 1660, 1540 amide), 730, 690 (phenyl).

γ-Benzyl Ester of the O-[γ-Benzyl Ester of (Benzyl 2-Acetamido-4-O-acetyl-2-deoxy-α-D-glucopyranosid)uronyl-L-alanyl]-D-isoglutamin-3-yl)-D-lactoyl-L-alanyl-D-isoglutamine (VI). A soluton of 0.50 g (0.96 mmole) of the uraonic acid (V) in 20 ml of THF was subjected to selective hydrogenolysis over 0.1 g of 10% Pd/C. After 1 h, the catalyst was filtered off, and washed with THF, the solution was evaporated to dryness, and the residue was triturated with ether. The chromatographically pure benzyl 2-acetamido-4-O-acetyl-3-O-(D-1-carboxyethyl)-2-deoxy-α-D-glucopyranosiduronic acid obtained (0.4 g, 0.93 mmole) was dissolved in a mixture of 10 ml of dry dioxane and 5 ml of DMF and, with stirring, 0.257 g (2.23 mmole) of HONSu and 0.462 g (2.24 mmole) of DCC were added. After the end of activation, the precipitate of dicyclohexylurea that had deposited was filtered off and was washed with 10 ml of dioxane–DMF (2:1). Then, with stirring, 0.31 ml of Et_3N and a solution of 0.913 g (2.24 mmole) of the γ-benzyl ester of trifluoroacetyl-L-alanyl-D-isoglutamine in dioxane were added to the filtrate. After 48 h, the precipitate of the glycopeptide (VI) that had deposited was filtered off and was washed with dioxane–DMF (2:1) and with ether and was dried and recrystallized from $CHCl_3$–ethanol (5:1). Yield 0.64 g (68%); mp 245°C (decomp.); $[\alpha]_{546}$ + 85° (c 1.0; dimethylformamide); R_f 0.73 (system 4). PMR [500 MHz; $(C^2H_3)_2SO$]: 1.07 d, 1.18 d (9H; J_{CH_3CH} = 7 Hz; $3CH_3CH$); 1.72 s (3H; NAc); 1.96 s (3H; OAc); 2.31 m (4H; 2γ-CH_2); 4.44 d, 4.61 d (2H; J_{gem} = 12 Hz; OCH_2Ph); 4.81 d (1H; $J_{1,2}$ = 3 Hz; H-1α); 4.96 t (1H; H-4); 5.02 s (4H; $2COOCH_2Ph$); 7.31 m (15H; 3Ph); 7.03 s, 7.24 s, 7.34 d, 7.45 d, 8.02 d, 8.09 d, 8.11

d (9H; 5NH, $2NH_2$). ν_{max}^{KBr} (cm^{-1}): 3400, 3290 (NH, NH_2); 1740, 1250 (ester); 1650, 1550 amide); 730, 700 (phenyl).

O-[(Benzyl 2-Acetamido-2-deoxy-α-D-glucopyranosid)uronoyl-L-alanyl-D-isoglutamin-3-yl]-D-lactoyl-L-alanyl-D-isoglutamine (VII). With stirring, 2.2 ml of a 1 M solution of KOH in water was added to a solution of 0.64 g (0.64 mmole) of (VI) in 20 ml of ethanol. After the end of the reaction (TLC, Kieselgel, system 4), the reaction mixture was treated with KU-anion-exchange resin (H^+), the resin was filtered off, the filtrate was evaporated to dryness, and the residue was triturated with acetone. The precipitate obtained was rechromatographed on a column of silica gel with elution by acetone → ethanol. Yield 0.35 g (70%); amorphous powder: $[\alpha]_{546}$ +63° (c 0.46; water). R_f 0.15 (system 4). PMR (500 MHz $(C^2H_3)_2SO$): 1.19 d, 1.23 d (9H, $J_{CH_3,CH}$ = 7 Hz, $3CH_3CH$); 1.77 s (3H; NAc); 2.03 m (4H; 2γ-CH_2); 4.60 d, 4.82 d (2H, J_{gem} = 12 Hz, OCH_2Ph); 7.31 (5H, Ph). ν_{max}^{KBr} (cm^{-1}): 3400-3200 (NH, NH_2, OH); 1650, 1540 (amide); 720, 690 (phenyl).

O-(2-Acetamido-2-deoxy-D-glucopyranuronosyl-L-alanyl-D-isoglutamin-3-yl)-D-lactoyl-L-alanyl-D-isoglutamine (IX). The catalytic hydrogenolysis of 0.30 g (0.38 mmole) of compound (VII) in solution in 25 ml of 90% aqueous ethanol was performed over 0.300 g of 10% Pd—C. After 48 h, the catalyst was filtered off and was washed with 90% aqueous ethanol. The filtrate was evaporated, and the residue was triturated with acetone. Yield 0.220 g (45%); amorphous powder; $[\alpha]_{546}$ −36° (c 0.55; water); R_f 0.17 (system 5). ν_{max}^{KBr} (cm^{-1}): 3400-3200 (OH, NH, NH_2); 1650, 1530 (amide).

CONCLUSIONS

Uronoyldipeptide derivatives of N-acetylglucosamine and of N-acetylmuramoyldipeptide differing in the position and method of the grafting of the peptide to the carbohydrate moiety have been synthesized.

LITERATURE CITED

1. P. Lefrancier and E. Lederer, Prog. Chem. Org. Nat. Prod., 40, 1 (1981).
2. A. Adam, J.-F. Petit, P. Lefrancier, and E. Lederer, Mol. Cell. Biochem., 41, 27 (1981).
3. M. Kiso, Y. Kaneda, Y. Goh, A. Hasegawa, and I. Azuma, Agric. Biol. Chem., 44, No. 8, 1971 (1980).
4. Yu. P. Abashev, T. M. Andronova, S. É. Zurabyan, and A. Ya. Khorlin, Bioorg. Khim., 7, No. 7, 980 (1981).
5. N. C. Chaturvedi, M. C. Khosla, and N. Anand, J. Med. Chem., 9, 971 (1966).
6. S. Kobayashi, T. Fukuda, H. Yukimura, M. Fujino, J. Azuma, and Y. Yamamura, Bull. Chem. Soc. Jpn., 53, 2570 (1980).
7. Yu. P. Abashev, T. M. Andronova, S. É. Zurabyan, V. P. Mal'kova, I. B. Sorokina, and A. Y. Khorlin, Bioorg. Khim., 10, No.1, 18 (1984).
8. A. E. Zemlyakov, V. O. Kur'yanov, V. Ya. Chirva, and A. Ya. Khorlin, Bioorg. Khim., 12, No. 7, 929 (1986).
9. N. K. Kochetkov, B. A. Dmitriev, N. É. Bairamova, and A. V. Nikolaev, Izv. Akad. Nauk SSSR, Ser. Khim., No. 3, 652 (1978).