Chemistry of Natural Compounds, Vol. 33, No. 5, 1997

SYNTHESIS OF CONJUGATES OF GLYCO AMINO ACIDS AND GLYCOPEPTIDES WITH CARBOXYLIC ACID HYDRAZIDES

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

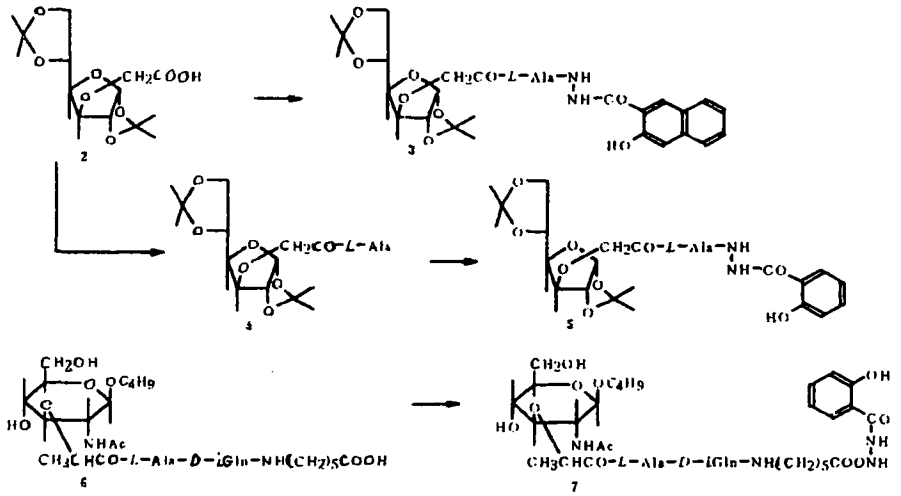
UDC 547.963.057

It is proposed to use a hydrazine "bridge" for obtaining conjugates of glycopeptides with biologically active carboxylic acids. Diacylhydrazines have been synthesized from glucofuranosylglycoloyl-L-alanine and β-butylmuramoyltripeptide.

Analogs of N-acetylmuramoyl-*L*-alanyl-*D*-isoglutamine (MDP) acylated by biologically active carboxylic acids exhibit high adjuvant and antitumoral activity [1]. At the same time, the ester type of bond in such compounds breaks down in biological media considerably faster than the amide bond in conjugates with such a bond.

We have proposed to use the bifunctional hydrazine molecule as a "bridge" permitting the linkage of carboxylic acids with the terminal carboxy group of a glyco amino acid or a glycopeptide of the MDP type. Several schemes for the synthesis of such conjugates have been tested.

The diacylhydrazine (**1**) has been obtained by the action of the N-hydroxysuccinimide ester of Boc-*L*-alanine on 3-hydroxy-2-naphthoic acid hydrazide. After N-deblocking, compound (**1**) was condensed with the glucofuranosylglycolic acid (**2**) [2], dipeptide derivatives of which are active immunomodulators [3], using dicyclohexylcarbodiimide (DCC) and N-hydroxysuccinimide (HOSu) as activating agents. The structure of the conjugate obtained (**3**) was confirmed by its PMR spectrum (see the Experimental part) in which, in particular, we identified the four signals of the methyl groups of the isopropylidene protective groupings (1.34-1.51 ppm), two doublets of the *AB*-system of nonequivalent protons of the glycoloyl fragment in the 4.22-4.28 interval, and a one-proton doublet with a CS of 5.92 ppm and a SSCC of 3.5 Hz corresponding to an anomeric proton with the α-configuration of a 1,2-O-alkylidene protective grouping. The signals of the protons of the hydrazine "bridge" appeared in the form of two broadened singlets with CSs of 9.35 and 9.54 ppm.



Simferopol' State University, 333036, Ukraine, Crimea, Simferopol', ul. Yaltinskaya, 4. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 731-734, September-October, 1997. Original article submitted March 17, 1997.

0009-3130/97/3305-0568$18.00

Alternatively the activated glucofuranosylglycolic ester (2) was condensed with the sodium salt of *L*-alanine. The interaction of the glyco amino acid (4) so obtained with salicyloylhydrazine was conducted by the carbodiimide method, giving the corresponding diacylhydrazine (5). The IR spectra of compounds (3) and (5) were similar.

A third variant of the synthesis has been proposed for unprotected glycopeptides. The β-butylmuramoylpeptide (6) that we had obtained previously [4] was condensed with salicyloylhydrazine in water in the presence of a water-soluble carbodiimide, and conjugate (7) was isolated.

In the PMR spectrum of (7) we determined proton signals characterizing the carbohydrate fragment: a triplet of three terminal methyl groups of the butyl aglycon with the CS 0.85 ppm, the singlet of an acetamide group with the CS 1.72 ppm, and the doublet of a glycosidic proton with a CS of 4.35 ppm and an SSCC of 8 Hz, corresponding to the β-configuration of the glycosidic bond. The structure of the tripeptide fragment was confirmed by the presence of triplets with CSs of 2.07 and 2.20 ppm from the protons of the γ- and α-methylene groups of the glutamic and aminohexanoic acid residues respectively. The salicylic acid hydrazide residue was characterized by two broadened singlets from the protons of the hydrazine "bridge," with CSs of 10.00 and 10.41 ppm, a singlet of a proton of a phenolic hydroxyl with a CS of 11.80 ppm, and, in addition, the signals of aromatic protons in the 6.93-7.68 ppm interval.

EXPERIMENTAL

Melting points were determined on a PTP instrument, and optical rotations at 20-22°C on a Polamat-A polarimeter. PMR spectra were obtained on Bruker WP-200 (200 MHz) and Bruker WM-500 (500 MHz) instruments, with tetramethylsilane as internal standard; chemical shifts are given in ppm, δ-scale. IR spectra were recorded on a Specord 75-IR spectrophotometer (KBr tablets). TLC was conducted on Silufol UV-254 plates (Kavalier). The substances were revealed by carbonization at 300°C or, in the case of amino acids, by means of solution of ninhydrin in butanol. The following systems were used: 1) chloroform–isopropyl alcohol (15:1); 2) toluene–isopropyl alcohol (10:1) and 3) (5:1); and 4) butan-1-ol–pyridine–water (3:1:1). Column chromatography was conducted on Aldrich 70-230 mesh silica gel. The elementary analyses of the compounds synthesized corresponded to the calculated values.

In the syntheses we used cyclohexylcarbodiimide from Ferak, N-hydroxysuccinimide from Merck, and 3-hydroxy-2-naphthoic acid from Reanal.

The hydrazides of salicylic and 3-hydroxy-2-naphthoic acids were obtained by the hydrazinolysis of the corresponding methyl esters.

N-(3-Hydroxy-2-naphthoyl)-N′-(*tert*-butoxycarbonyl-*L*-alanyl)hydrazine (1). With stirring, 187 mg (1.63 mmole) of HOSu and 336 mg (1.63 mmole) of DCC were added to a solution of 230 mg (1.48 mmole) of Boc-*L*-alanine in 5 ml of dry acetonitrile. After the end of the reaction (monitoring by TLC in system 1), the dicyclohexylurea precipitate was filtered off and was washed with 3 ml of acetonitrile, and a solution of 300 mg (1.48 mmole) of 3-hydroxy-2-naphthoic acid hydrazide in 3 ml of acetonitrile was added. The white crystalline precipitate resulting from the reaction was filtered off. The yield of the diacylhydrazine (1) was 235 mg (50%); mp 208-209°C, $[\alpha]_{546}$ −22° (*c* 1.0; dimethyl sulfoxide). IR spectrum (ν, cm^{-1}): 3300 (NH), 3250 (OH), 1665, 1520 (amide), 750 (CH-arom.).

N-(3-Hydroxy-2-naphthoyl)-N′-[O-(1,2:5,6-di-O-isopropylidene-α-*D*-glucofuranos-3-yl)glycoloyl-*L*-alanyl]hydrazine (3). 1,2:5,6-Di-O-isopropylidene-3-carboxymethyl-α-*D*-glucofuranose (2) (177 mg, 0.53 mmole) was dissolved in 5 ml of dichloroethane and activated with 67 mg (0.58 mmole) of HOSu and 121 mg (0.58 mmole) of DCC. After the end of activation the dicyclohexylurea was filtered off and was washed with 3 ml of solvent, and the filtrate was treated with N-(3-hydroxy-2-naphthoyl)-N′-(*L*-alanyl)hydrazine (obtained by treating 200 mg (0.54 mmole) of compound (1) with 2 ml of trifluoroacetic acid, followed by evaporation to dryness and neutralization with triethylamine). After the end of the reaction (monitoring by TLC in system 2), the solvent was evaporated off, and by column chromatography of the residue (eluent: hexane-chloroform (2:1) → chloroform–ethyl acetate (50:1) we isolated 226 mg (70%) of conjugate (3); mp 108°C, $[\alpha]_{546}$ −132° (*c* 0.1; chloroform). IR spectrum (ν, cm^{-1}): 3400-3500 (NH, OH), 1650, 1520 (amide), 820 (CH-arom). PMR spectrum (500 MHz, C^2HCl_3): 1.34, 1.39, 1.43, 1.54 (12 H, 2 Me_2C, s), 1.54 (3H, CH_3-Ala, d), 4.10 and 4.12 (2H, H-6a, H-6b; $J_{6a,6b}$ 9 Hz, dd), 4.15 (1H, H-4; $J_{4,5}$ 9.0 Hz, dd), 4.21 (1H, H-3; $J_{3,4}$ 3.0 Hz, dd), 4.22 and 4.28 (2H, OCH_2CO; J_{gem} 16 Hz, d). 4.40 (1H, H-5; $J_{5,6a}$ 3.5 Hz, $J_{5,6b}$ 5.5 Hz, ddd), 4.64 (1H, H-2; $J_{2,3}$ 0 Hz, d), 4.80 (1H, CH-Ala; $J_{CH,Me}$ 7 Hz, m), 5.22 (1H, H-1; $J_{1,2}$ 3.5 Hz, d), 7.14 (1H, H_{arom}, s), 7.26 (1H, H_{arom}, dd), 7.44 (1H, H_{arom}, dd). 7.57 (1H, H_{arom}, d), 7.61 (1H, H_{arom}, d), 7.73 (1H, NH-Ala; $J_{CH,NH}$ 8 Hz, d), 8.13 (1H, H_{arom}, s), 9.35 and 9.54 (2H, NH-NH, br.s), 10.69 (1H, OH-arom, s).

O-(1,2:5,6-Di-O-isopropylidene-α-*D*-glucofuranos-3-yl)glycoloyl-*L*-alanine(4). 1,2:5,6-Di-O-isopropylidene-3-carboxymethyl-α-*D*-glucofuranose (**2**) (300 mg; 0.90 mmole), dissolved in 20 ml of acetonitrile, was activated as described above with 114 mg (0.99 mmole) of HOSu and 205 mg (0.99 mmole) of DCC. The solution of the activated ester was treated with 10 ml of an aqueous solution of 80 mg (0.90 mmole) of *L*-alanine and 150 mg (1.71 mmole) of $NaHCO_3$. After the end of the reaction (monitoring by TLC in system 3), the solvent was evaporated off, and a solution of the residue in 20 ml of ethyl acetate was washed with dilute HCl and then with water. The organic layer was dried with anhydrous Na_2SO_4 and was evaporated, and, after column chromatography (eluent: chloroform → chloroform–ethanol (50:1)), we obtained 320 mg (70%) of the glyco amino acid (**4**); mp 52-55°C, $[\alpha]_{546}$ −79° (*c* 0.46; dimethyl sulfoxide). IR spectrum (ν, cm^{-1}) 3250-3500 (NH, OH), 1700 (C=O), 1665, 1500 (amide).

N-Salicyloyl-N′-[O-(1,2:5,6-di-O-isopropylidene-α-*D*-glucofuranos-3-yl)glycoloyl-*L*-alanyl]hydrazine (5). To a solution of 290 mg (0.72 mmole) of the acid (**4**) in 20 ml of acetonitrile were added 109 mg (0.72 mmole) of salicylic acid hydrazide and 148 mg (0.72 mmole) of DCC (monitoring by TLC in system 2). The precipitate of dicyclohexylurea was filtered off, the filtrate was evaporated, and from the residue by column chromatography (eluent: methylene chloride → methylene chloride–isopropyl alcohol (25:1)) we isolated 235 mg (64%) of the conjugate (**5**): amorphous white powder, $[\alpha]_{546}$ −117° (*c* 0.66; chloroform). IR spectrum (ν, cm^{-1}): 3250-3400 (NH, OH), 1630, 1510 (amide), 820 (CH-arom.).

N-Salicyloyl-N′-{6-[(butyl 2-acetamido-2-deoxy-β-*D*-glucopyranosid-3-yl)-*D*-lactoyl-*L*-alanyl-*D*-isoglutaminyl-amino]hexanoyl}hydrazine (7). A solution of 40 mg (0.06 mmole) of 6-{[O-(butyl 2-acetamido-2-deoxy-β-*D*-glucopyranos-3-yl)-*D*-lactoyl-*L*-alanyl-*D*-isoglutaminyl]amino}hexanoic acid and 12 mg (0.06 mmole) of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide in 1 ml of water was treated with 0.5 ml of an aqueous solution of salicylic acid hydrazide (9 mg, 0.06 mmole). The reaction mixture was kept at 40°C until the reaction was complete (monitoring by TLC in system 4), and then 4 ml of *n*-butyl alcohol was added and stirring was carried out for 1 h. The organic layer was separated off, dried with anhydrous Na_2SO_4, and evaporated. The product was twice reprecipitated with ether from solution in isopropyl alcohol. The yield of conjugate (**7**), an amorphous light gray powder, amounted to 15 mg (31%). PMR spectrum (200 MHz, DMSO-d_6); 0.85 (3H, $\underline{CH_3}CH_2$, t), 1.26 (6H, 2 $\underline{CH_3}CH$, d), 1.72 (3H, NAc, s), 2.07 (2H, γ-CH_2-iGln, t), 2.20 (2H, α-CH_2CO, t), 4.33 (1H, H-1; $J_{1,2}$ 8 Hz, d), 6.93 (3H, H_{arom}, $CONH_a$, m), 7.20 (1H, H_{arom}, br.s), 7.37 (2H, H_{arom}, $CONH_b$, m), 7.68 (1H, $\underline{NH}CH_2$, d), 7.87 and 7.94 (2H, NH, d), 10.00 and 10.41 (2H, NH-NH, br.s), 11.80 (1H, OH-arom, s).

REFERENCES

1. S. Kobayashi, T. Fukuda, I. Imada, M. Fujino, I. Azuma, and Y. Yamamura, Chem. Pharm. Bull., **27**, No. 12, 3193 (1979); S. Kobayashi, T. Fukuda, H. Yukimasa, I. Imada, M. Fujino, I. Azuma, and Y. Yamamura, Bull. Chem. Soc. Jpn., **53**, No. 10, 2917 (1980); S. Kobayashi, T. Fukuda, H. Yukimasa, M. Fujino, I. Azuma, and Y. Yamamura, Bull. Chem. Soc. Jpn., **57**, No. 11, 3182 (1984).
2. H. Chikashita and T. Ouchi, J. Heterocycl. Chem., **19**, No. 5, 981 (1982).
3. W. Gruszecki, K. N. Masihi, H. Labischinski, and H. Bradaczek, Adv. Biosciences, **68**, 415 (1988).
4. V. O. Kur'yanov, V. V. Tsikalov, A. E. Zemlyakov, and V. Ya. Chirva, Khim. Prir. Soedin., 424 (1994).