Chemistry of Natural Compounds, Vol. 34, No. 1, 1998

SYNTHESIS OF γ-BENZYL *tert*-BUTOXYCARBONYL-*L*-ALANYL-*D*-GLUTAMATE AND ITS DERIVATIVES

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UDC 547.466.23 64.057

A convenient method of obtaining Boc-L-Ala-D-iGln-OBzl and its amide analogs by condensing the N-hydroxysuccinimidyl ester of Boc-L-Ala with the γ-benzyl ester of D-Glu in the presence of $NaHCO_3$, followed by amidation of the resulting Boc-L-Ala-D-Glu-γ-OBzl, is proposed. The use of l-adamantylamine and octadecylamine as amino components has enabled the corresponding α-adamantylamide and octadecylamide of the dipeptide to be obtained.

The dipeptide *L*-alanyl-*D*-isoglutamine is one of the components responsible for the adjuvant activity of glucopeptide analogs of N-acetylmuramoyl-*L*-alanyl-*D*-isoglutamine (MDP, muramoyldipeptide) and of lipophilic peptides of the type of pimelautide ($C_{11}H_{23}CO$-*L*-Ala-*D*-Glu-γ-*L*,*L*-Dap*-(Gly)-NH_2) and triglimik (*L*-Ala-*D*-*i*Gln-*L*-Ala-$OCH_2CH(OH)CH_2OMyc$) [1]. The traditional synthesis of the dipeptide in protected form (Boc-*L*-Ala-*D*-*i*Gln-OBzl, **1**) comprises 6-7 stages [2, 3]. The greatest consumption of time and reagents takes place at the stage of converting the initial *D*-glutamic acid into *D*-isoglutamine, which usually includes the preparation of the γ-benzyl ester of *D*-Glu (**2**), blocking the amino function (most frequently with Boc protection), activating the α-carboxy group, and converting it into the amide.

An alternative approach was realized by P. Lefrancier et al. [4] in the synthesis of the dipeptide Boc-*L*-Ala-*D*-Glu-(γ-OBzl)-OMe, in which the key stage was the condensation of ester (**2**) with the N-hydroxysuccinimide ester of Boc-*L*-alanine (**3**), using salt protection for the α-carboxy group of the *D*-Glu. The peptide obtained, Boc-*L*-Ala-*D*-Glu-γ-OBzl (**4**), was then methylated with diazomethane.

We have proposed to use this approach for the synthesis of dipeptide (**1**) and its amide analogs. The free carboxy group in dipeptide (**4**) was converted into a carboxamide group by the simultaneous action of N-hydroxysuccinimide (HOSu), dicyclohexylcarbodiimide (DCC), and aqueous ammonia. The complete synthesis of dipeptide (**1**) included three stages, and its overall yield calculated on the *D*-Glu amounted to 52%. Treatment of the activated dipeptide ester (**4**) with octadecylamine led to the α-octadecylamide (**5**), previously synthesized by a standard procedure and used in the synthesis of the α-octadecylamide of N-acetylmuramoyl-*L*-alanyl-*D*-glutamic acid [5]. The action of *l*-adamantylamine and DCC on peptide (**4**) gave the α-adamantylamide (**6**). In the PMR spectrum of compound (**6**), in addition to the signals of the protons of the dipeptide fragment, we also identified the signals of the protons of the adamantyl residue: multiplets of methylene and methine protons with CSs of 1.33, 1.60, and 1.91 ppm and also the singlet of the amide proton with the CS 6.87 ppm, which confirmed its structure. It must be mentioned that when the PMR spectrum was taken in deuterochloroform the nonequivalence of the skeletal protons of *D*-Glu residue clearly appeared, although this was not observed in deuterated dimethyl sulfoxide (see the Experimental part).

EXPERIMENTAL

For general observations, see [6].

*Abbreviations: Glu) glutamic acid; *i*Gln) isoglutamine; Dap) diaminopimelic acid; Myc) mycolic acid.

Simferopol' State University, 4 Yaltinskaya ul., Simferopol', Crimea, Ukraine 333036. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 101-103, January-February, 1998. Original article submitted September 10, 1997.

 0009-3130/98/3401-0078$20.00

The following solvent systems were used: 1) chloroform–propan-2-ol (20:1); 2) toluene–acetone (20:1). The substances were revealed with a 2% solution of ninhydrin in ethanol. The reactants were Sigma Boc-*L*-Ala, Aldrich DCC and *l*-adamantylamine, Merck HOSu, and Kavalier *l*-octadecylamine.

γ-Benzyl *tert*-Butoxycarbonyl-*L*-alanyl-*D*-glutamate (4). The activated ester Boc-*L*-Ala obtained by the interaction of 0.5 g (2.65 mmole) of Boc-*L*-Ala (**3**) with 335 mg (2.92 mmole) of HOSu and 600 mg (2.92 mmole) of DCC in 5 ml of acetonitrile was added to a solution of 630 mg (2.65 mmole) of the γ-benzyl ester of *D*-Glu (**2**) [7] in 10 ml of water containing 445 mg (5.3 mmole) of $NaHCO_3$. After the end of the reaction (TLC monitoring in system 1), the acetonitrile was evaporated off, and the product was extracted with 75 ml of ethyl acetate. The organic layer was washed with 1 N HCl to pH 3-4 and then with a saturated solution of NaCl and was dried with anhydrous Na_2SO_4 and evaporated. Crystallization of the residue from ether yielded 900 mg (83%) of dipeptide (**4**), mp 79-81°C $[\alpha]_{546}$ −18° (*c* 5.0; chloroform). Lit. [4]: mp 70-72°C, $[\alpha]_D$ −12° (methanol).

γ-Benzyl Ester of *tert*-Butoxycarbonyl-*L*-alanyl-*D*-glutamine (1). To 200 mg (0.5 mmole) of dipeptide (**4**) in 5 ml of acetonitrile were added 65 mg (0.55 mmole) of HOSu, 115 mg (0.55 mmole) of DCC, and 100 μl of 25% ammonia solution. The reaction mixture was kept for 1 h (TLC monitoring in system 1), the precipitate was filtered off, the filtrate was evaporated, and the residue was dissolved in 50 m of ethyl acetate. The organic layer was washed with water, dried with anhydrous Na_2SO_4, and evaporated. The product was crystallized from ether to give 175 mg (83%) of dipeptide (**1**), mp 138°C, $[\alpha]_{546}$ −10° (*c* 1.13; methanol). Lit. [3]: mp 139-140°C, $[\alpha]_D$ −9° (methanol).

Octadecylamide of γ-Benzyl *tert*-Butoxycarbonyl-*L*-alanyl-*D*-glutamate (5). A solution of 100 mg (0.25 mmole) of dipeptide (**4**) in 5 ml of acetonitrile was treated with 31 mg (0.27 mmole) of HOSu and 56 mg (0.27 mmole) of DCC. After the completion of activation (monitoring by TLC in system 1), the precipitate of dicyclohexylurea was filtered off, and a solution of 67 mg (0.25 mmole) of octadecylamine in 2 ml of dichloroethane and also triethylamine to pH 8-9 were added to the filtrate. After 24 h (monitoring by TLC in system 2) the solvent was evaporated off and the residue was dissolved in 50 ml of ethyl acetate. The extract was washed with 1 N HCl and then with a solution of $NaHCO_3$ to pH 7 and with water. The organic layer was dried with anhydrous Na_2SO_4 and evaporated. After purification by column chromatography (eluent: benzene) 148 mg (88%) was obtained of the α-octadecylamide (**5**), mp 101-102°C, $[\alpha]_{546}$ +10° (*c* 1.0; chloroform). Lit. [5]: mp 100-102°C, $[\alpha]_{546}$ +9° (chloroform).

α-Adamantylamide of γ-Benzyl *tert*-Butoxycarbonyl-*L*-alanyl-*D*-glutamate (6). To 300 ml (0.74 mmole) of dipeptide (**4**) in 5 ml of acetonitrile were added 115 mg (0.74 mmole) of *l*-adamantylamine and 155 mg (0.74 mmole) of DCC. After the completion of the reaction (monitoring by TLC in system 2), the precipitate of dicyclohexylurea was filtered off, the filtrate was evaporated, and the residue was dissolved in 70 ml of ethyl acetate. The solution was washed with 1 N HCl, and then with $NaHCO_3$ solution to pH 7 and with water. The organic layer was dried with anhydrous Na_2SO_4 and evaporated. The reaction product was purified by column chromatography (eluent: benzene → benzene–acetone (25:1)). Crystallization from ether gave 255 mg (64%) of the dipeptide (**6**), mp 60-62°C, $[\alpha]_{546}$ −6° (*c* 5.0; chloroform). PMR spectrum (200 MHz, C^2HCl_3): 1.28 (3H, MeCH-Ala, d, J_{MeCH} 7.5 Hz), 1.33 (9H, Me_3C, s), 1.33, 1.60 and 1.91 (CH_2, CH-Ada, m), 2.00 and 2.08 (2H, β-CH_2-Glu, m), 2.42 (2H, γ-CH_2-Glu, m), 4.04 (1H, MeCH-Ala, dq), 4.27 (1H, CH-Glu, ddd), 5.03 and 5.10 (2H, CH_2Ph, d, J_{gem} 12.5 Hz), 5.94 (1H, NH-Ala, br.d), 6.87 (1H, NH-Ada, br.s), 7.22 (1H, NH-Glu, br.d), 7.30 (5H, Ph, m).

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