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β-Alanine, γ-Aminobutyric Acid Analog of Bradykinin

V. Škarić, M. Topić-Bulić, and Đ. Škarić

Laboratory of Stereochemistry and Natural Products, »Ruđer Bošković« Institute, 41001 Zagreb, Croatia, Yugoslavia

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The synthesis of β-alanyl-L-prolyl-L-prolyl-glycyl-L-phenyl-alanyl-γ-aminobutyryl-L-prolyl-L-phenylalanyl-β-alanine (I), as an analog of Bradykinin, and β-alanyl-glycyl-glycyl-β-alanyl-glycyl-glycine (II) by the solid phase method were described.

INTRODUCTION

The structural alterations of naturally occurring peptides by the insertion of β- or γ-amino acids could clarify the relationship between their primary structures and the accessibilities of their active centres. Namely, the homologation of a peptide chain to a more flexible oligomer by lengthening the chain of some amino acid unit could bring the active centre of the adjoining α-amino acid outwards from the body of the peptide chain and disclose it for biological interactions[1].

The structure of naturally occurring di- and tri-peptides already containing β-alanine and γ-aminobutyric acid[2–5] stimulated our search toward homologous tri- and hexa-peptides[6]. This paper deals with the preparation of nonapeptide, Bradykinin analog (I), bearing β-alanine in position *1* and *9* in place of arginine and γ-aminobutyric acid in position *6* in place of serine. Thus, the biological potency of Bradykinin, first isolated from a plasma globulin[7] as hypotensive factor

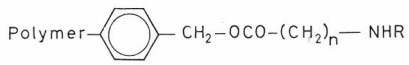
H-β-Ala-L-Pro-L-Pro-Gly-L-Phe-γ-ABut-L-Pro-L-Phe-β-Ala-OH
1 2 3 4 5 6 7 8 9

(I)

and excitant of smooth muscles, could also emerge from this synthesis, especially in respect to its biologically active centres.

Many Bradykinin analogs containing α-amino acids were described by several authors[8–11]. Bradykinin itself has been synthesized by conventional method[12–15] and more efficiently by the solid-phase method developed by Merrifield[16]. In the present work, the adoption of the improved solid-phase procedure[17,18] was shown to be the most convenient one for the synthesis of nonapeptide I. Namely, *N*-*t*-butoxycarbonyl derivatives of β-alanine[19] and γ-aminobutyric acid[20] in condensation processes behaved similarly as α-amino acids. It is worth noting that the solid-phase method made feasible the synthesis of hitherto unknown β-alanyl-diglycyl-β-alanyl-glycyl-glycine (II).

In order to synthesize the Bradykinin analog I, *N*-*t*-butoxycarbonyl-β--alanine[19] was first coupled with chloromethylcopolystyrene-divinylbenzene (98 : 2) in ethyl acetate and in the presence of triethylamine. The thus obtained *N*-*t*-butoxycarbonyl-amino acid ester (III) was *N*-deprotected in 1 molar hydrochloric acid — acetic acid solution generating the corresponding hydro-



(III) R=BOC, n=2
(IV) R=H · HCl, n=2
(V) R=H, n=2
(VI) R=BOC, n=1
BOC = *N*-*t*-butoxycarbonyl

chloride IV and then the free base V by treatment with triethylamine. The coupling of polymer-amino acid ester V with an excess of *N*-*t*-butoxycarbonyl-L-phenylalanine[21] was allowed to proceed for 2 h in dimethylformamide in the presence of dicyclohexylcarbodi-imide.

The lengthening of the peptide chain by one amino acid residue was completed in about 4 h using *N*-*t*-butoxycarbonyl-amino acid as the units. Namely, the milder conditions for each deacylation of the thus protected polymerpeptide intermediates are decisive in preserving the initial ester[22] bond of polymer-amino acid V. Thus, in exactly the same way and in the same vessel the peptide chain was smoothly lengthened by the stepwise addition of the *N*-*t*-butoxycarbonyl derivatives of L-proline[20], γ-aminobutyric acid[20], L-phenylalanine[21], glycine[21], L-proline, L-proline, and β-alanine.[19] Finally, *N*-*t*-butoxycarbonyl-β-alanyl-L-prolyl-L-prolyl-glycyl-L-phenylalanyl-γ-aminobutyryl-L--prolyl-L-phenylalanyl-β-alanine was cleaved from solid support by treatment with HBr trifluoroacetic acid.

The crude product was chromatographed and successfully purified on a Dowex 50 X5 column, by a gradient elution at various ionic concentrations (pH of buffer solutions) of pyridine in acetic acid (pH = 5.6 — 3.1). The structure and the purity of the thus obtained product, R_f ca. 0.58, (TLC on cellulose and development by amyl alcohol-pyridine-water), was evaluated by mass spectroscopy and amino acid and elemental analyses.

The mass spectrum fragmentation pattern of nonapeptide I indicated »aminoacyl type« cleavage[23,24] evidenced by the corresponding peaks for fragments of Pro-Phe, Pro-Pro, Pro-β-Ala, Pro-Gly, γ-ABut, β-Ala, and Gly. In addition the fragmentation of L-phenylalanine exhibited the tropilium kation at *m/e* 91. An acid hydrolysate of nonapeptide I was analyzed on a Beckman-Unichrom Amino Acid Analyzer. The following molar ratios for amino acids were obtained: 3.03 (Pro), 1.03 (Gly), 2.10 ((β-Ala), 2.16 (Phe), and 1.00 (γ-ABut).

The polymeric ester used in the synthesis of β-alanine-hexapeptide (II) was preformed in ethyl acetate by coupling the *N*-*t*-butoxycarbonyl-glycine with chloromethylcopolystyrene-divinylbenzene (2%) in the presence of triethylamine. The thus obtained ester VI was lengthened by condensations with *N*-*t*-butoxycarbonyl derivatives of glycine[21] and β-alanine[19] in the properly chosen order using the DCCI method in activation, and 1 molar HCl—HOAc in *N*-deprotections processes. The cleavage of the product II from the supporting

polymer was affected by a stream of HBr in trifluoroacetic acid. The liberation of the thus obtained hydrobromide through an Amberlite IR-4B (OH) column afforded hitherto unknown β-alanyl-glycyl-glycyl-β-alanyl-glycyl-glycine (II) in 59% yield, identified by mass spectrum and elemental analysis.

EXPERIMENTAL

The same techniques and apparatus were used as described previously[25]. In addition, the »DC-fertigplatten cellulose F« was used for TLC. The content of amino acids was determined by Beckman-Unichrom Amino Acid Analyzer.

Chloromethylcopolymer

The cross-linked copolymer of styrene and divinylbenzene at — molar ratio of 98 : 2 (10 g) (400 mesh beads, Dow Chemical Co.) was suspended in 1 molar NaOH (68 ml), stirred for 2 h at room temperature and washed with water. The resin was then suspended in 1 molar HCl (68 ml), stirred at room temperature for 3 h, washed with water and successively with dimethylformamide (3 × 15 ml) and methanol (3 × 15 ml). The beads were dried at 80 °C and 10^{-3} mm Hg* for 48 h and suspended in freshly distilled chloromethylmethyl ether[26] (50 ml). To this suspension stirred for (*a*) 60 min or (*b*) 90 min at room temperature, cooled at — 8 °C, a solution of $SnCl_4$ (1 ml) in chloromethyl ether (20 ml) was added and stirred at 0 °C for an additional 60 min. The solvent was removed under reduced pressure and the polymeric chloromethylated products under *a*) and *b*) successively washed with dioxane — water (3 : 1, 200 ml), dioxane — 3 molar HCl (3 : 1, 200 ml), dioxane — water (1 : 1, 200 ml), dioxane — ethanol (1 : 1, 200 ml), and finally with methanol (200 ml), then dried for 48 h at 80 °C and 10^{-3} mm Hg.

Anal. found: Cl *a*) 4.62%
b) 7.26%

N-t-*Butoxycarbonyl-glycyl-copolymer* (VI)

a) To a suspension of chloromethylated copolymer (3 g; Cl 4.62%) in ethyl acetate (50 ml) *N-t*-butoxycarbonyl-glycine (685 mg, 3.9 mmol) and triethylamine (0.45 ml, 3.2 mmol) were added, heated and stirred under reflux for 48 h. The product was separated by suction and successively washed with ethyl acetate (200 ml), ethanol (200 ml), water (200 ml), and methanol (200 ml), and then dried at room temperature and $5 \cdot 10^{-2}$ mm Hg for 24 h.

Anal. found: N 0.27% (0.193 mmol/g copolymer)

b) From chloromethylated copolymer (3 g; Cl 7.26%) the product was obtained under the above described conditions.

Anal. found: N 0.43% (0.31 mmol/g copolymer)

N-t-*Butoxycarbonyl-β-alanyl-copolymer* (IV)

To a suspension of chloromethylated copolymer (10 g; Cl 6.95%) in ethyl acetate (70 ml) *N-t*-butoxycarbonyl-β-alanine (3.7 g, 19.6 mmol) and triethylamine (2.5 ml, 17.8 mmol) were added and worked up as described for ester VI.

Anal. found: N 1.49% (1.06 mmol/g copolymer)

β-*Alanyl-glycyl-glycyl-β-alanyl-glycyl-glycine* (II)

N-t-Butoxycarbonyl-glycyl-copolymer (VI) (3 g, 0.93 mmol) was washed with acetic acid (20 ml) in a specially constructed vessel[18], treated with 1 molar HCl in acetic acid (20 ml) by shaking at room temperature for 15 min, filtered through a fritted glass disk, successively washed with acetic acid, anhydrous ethanol and dimethylformamide (each 3 × 20 ml) by 5 min shaking period and filtration after each wash.

* 1 mm Hg ≈ 133.322 Pa

The resin — ester was then suspended in dimethylformamide (20 ml) and triethylamine (2.0 ml) by shaking to be separated by suction and washings with dimethylformamide (3 × 20 ml) and methylene chloride (3 × 20 ml). To the thus purified resin-ester suspended in dimethylformamide (20 ml), *N*-*t*-butoxycarbonyl-glycine (472 mg, 2.7 mmol) and dicyclohexylcarbodi-imide (558 mg, 2.7 mmol) dissolved in dimethylformamide (2 ml) were added. The suspension was shaken at room temperature for 2 h, the solvent removed by suction and the product washed with 10 ml portions of dimethylformamide and acetic acid (each 3 times). The subsequent deprotections and couplings with *N*-*t*-butoxycarbonyl derivatives of β-Ala, Gly, Gly, and finally β-Ala followed the above described procedure. The final deprotection by 1 molar HCl in acetic acid afforded a product which, after having been washed and dried, was suspended in trifluoroacetic acid (20 ml). A slow stream of anhydrous hydrogen bromide was bubbled through this suspension for 90 min, and then set aside at room temperature for 60 min. The resin was filtered off and washed with trifluoroacetic acid (3 × 20 ml). The filtrate and washings were evaporated to dryness under reduced pressure (at 25 °C), triturated with water, and the filtrate lyophilized. The water solution of the thus obtained product (330 mg) was passed through an Amberlite IR-4B (OH) (45 ml) column. It afforded a crystalline product (223 mg, 59%), m. p. 220—230 °C (from water—ethanol), R_f ca. 0.38 (butanol—acetic acid — water = 1 : 1 : 1).

Anal. $C_{14}H_{24}N_6O_7 \cdot H_2O$ (406.40) calc'd.: C 41.38; H 6.46; N 20.68%
found: C 41.19; H 6.23; N 20.36%

IR spectrum: ν_{max} 3333, 1613 br, and 1563 br cm^{-1}. MS (*m/e*): 128 ($HNCH_2CH_2CONHCH_2CO$), 114 ($HNCH_2CONHCH_2CO$), 71 ($HNCH_2CH_2CO$), 57 ($HNCH_2CO$), 42 (CH_2CO), and 30 (H_2NCH_2).

β-*Alanyl*-L-*prolyl*-L-*prolyl*-*glycyl*-L-*phenylalanyl*-γ-*aminobutyryl*-L-*prolyl*-L-*phenylalanyl*-β-*alanine* (I)

N-*t*-Butoxycarbonyl-β-alanyl-copolymer (III) (5 g, 5.3 mmol) containing 1.49% N (1.06 mmol/g copolymer), was washed with acetic acid (30 ml) and then treated with 1 molar HCl in acetic acid (20 ml) as already described. β-Alanyl-resin was washed with 30 ml portions of acetic acid, ethanol, and methylene chloride (3 times by each) by shaking for 5 min followed by filtration. After treatment with triethylamine (2 ml) in methylene chloride (20 ml), filtration and washing with methylene chloride (4 × 30 ml) *N*-*t*-butoxycarbonyl-L-phenylalanine (4.22 g, 15.9 mmol) in methylene chloride (20 ml) was added and shaken for 10 min. To this suspension *N*,*N*-dicyclohexylcarbodi-imide (DCCI) (3.28 g, 15.9 mmol) was added and shaken for an additional 2 h to be filtered and washed with methylene chloride, ethanol, and acetic acid (each 3 × 30 ml). The subsequent seven amino acids [-Pro(7), -γ-ABut(6), -Phe(5), -Gly(4), -Pro(3), -Pro(2), and -β-Ala(1)] as *N*-*t*-butoxycarbonyl derivatives were coupled in the presence of DCCI (3.28 g, 15.9 mmol) and triethylamine (2 ml) in methylene chloride (20 ml) and *N*-deprotected in 1 molar HCl — acetic acid (20 ml) except after β-alanine (*1*) had been coupled.

The *N*-protected nonapeptide-polymer was dried and suspended in anhydrous trifluoroacetic acid (30 ml). The suspension was treated by bubbling anhydrous hydrogen bromide at room temperature for 90 min, and then setting it aside for additional 60 min. The freeze drying of filtrate and trifluoroacetic and acetic acids washings afforded the crude product (3.2 g, 59.7%).

The solution of crude nonapeptide (I) (200 mg) in 0.2 mmol/dm^3 pyridine-acetic acid (pH 3.1) was purified on a 2.5 × 100 cm Dowex 50 × 5 (pyridinium form) column, thermostated at 30 °C. The column was performed by a suspension of resin in 0.2 molar pyridine-acetic acid buffer (pH 3.1) (1 : 3 v/v), packed under moderate pressure. The gradient elution (220 fractions) at a rate of 16/h combining — molar pyridine — acetic acid (pH 5.6, 2000 ml) and 0.2 molar pyridine-acetic acid (pH 3.1, 2000 ml) separated the pure product, R_f ca. 0.58 (cellulose in ethanol—pyridine—water = = 1.2 : 1.2 : 1). (The first 824 ml of eluates did not contain the desired product). The sample was dissolved in water, filtered, and freeze dried, m. p. 155—157 °C.

Anal. $C_{45}H_{64}N_9O_{10} \cdot 2\,CH_3COOH \cdot H_2O$ (1011.038)
calc'd.: C 55.89; H 7.08; N 11.98%
found: C 55.74; H 7.06; N 12.17%

IR spectrum: ν_{max} 3333, 3077 br, 2941 br, 1653, and 1534 cm^{-1}. MS (*m/e*): 245 (HNPro-PheCO), 195 (HNPro-ProCO), 169 (HNPro-β-AlaCO), 154 (HNPro-GlyCO), 91 (tropilium cation), 85 [$HN(CH_2)_3CO$], 72 ($HNCH_2CH_2CO$), 57 ($HNCH_2CO$).

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SAŽETAK

Analogon bradikinina sa β-alaninom i γ-aminomaslačnom kiselinom

V. Škarić, M. Topić-Bulić i Đ. Škarić

Opisane su sinteze β-alanil-L-prolil-L-prolil-glicil-L-fenilalanil-γ-aminobutiril-L-prolil-L-fenilalanil-β-alanina i β-alanil-glicil-glicil-β-alanil-glicil-glicina metodom krute faze.

LABORATORIJ ZA STEREOKEMIJU
I PRIRODNE SPOJEVE
INSTITUT »RUĐER BOŠKOVIĆ«
41001 ZAGREB

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