# The Synthesis of l-Lysine, l-Ornithine, l-Citrulline, l-Glutamic Acid, and l-Desarginine Bradykinin

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*Received June 10, 1963*

The synthesis of four analogs of bradykinin is described in which the N-terminal arginine is replaced by lysine, ornithine, citrulline, and glutamic acid. The preparation of l-desarginine bradykinin is also reported. The biological activity of these five peptides when compared to bradykinin is summarized.

The determination of structural requirements necessary for retaining biological activity in a peptide has usually included studies on the C and N terminal portions of the molecule. Although this approach does not provide information regarding the mode of action of the peptide if one is to consider receptor sites, it does provide an effective way to establish minimum amino acid or functional group characteristics of the drug.

Since it was evident that removal of the C-terminal arginine in bradykinin led to complete loss of biological activity,[1] it was considered important to evaluate the requirements of the N-terminal part of the molecule.

The present report is concerned with the synthesis of the octapeptide l-desarginine bradykinin and four nonapeptides related to bradykinin in which the l-arginine has been replaced by lysine, ornithine, citrulline, and glutamic acid. The synthetic approach used for the preparation of these five compounds is shown in Scheme I. The starting material for all of the analogs was the octapeptide carbobenzoxy-L-prolyl-L-prolylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanylnitro-L-arginine methyl ester.[2]

Hydrolysis of carbobenzoxyoctapeptide methyl ester I with sodium hydroxide, followed by catalytic hydrogenation, gave the l-desarginine bradykinin II. The four nonapeptides were obtained by treating the decarbobenzoxylated octapeptide III with the appropriate protected amino acid *p*-nitrophenyl ester. The resulting carbobenzoxynonapeptide methyl esters were hydrolyzed with alkali and then hydrogenated to give the desired products.

The biological activity of the five analogs is presented in Table I.[3] These results appear to indicate that the C-terminal arginine is not essential for kinin-like activity and also the size of the molecule need not absolutely be that of a nonapeptide. Although decreasing the basicity of the first amino acid results in a decrease in bronchoconstriction and hypotensive activity, a small amount of hypotensive activity is still retained when the arginine is replaced by an acidic amino acid such as glutamic acid. The large decrease in the bronchoconstrictor activity compared with the less dramatic hypotensive activity decline would suggest that the N-terminal arginine is required for the strong bronchoconstrictor action exhibited by bradykinin. An important feature of the 1-citrulline analog is that its bronchoconstrictor activity in the guinea pig is not antagonized by aspirin.[4] This would appear to mean that its activity is not entirely kinin-like as was also found with the 6-threonine analog,[5] but because of the very low order of activity of these analogs such results may be misleading.

Table I

Biological Activity of Bradykinin Analogs

| Peptide | Bronchoconstrictor activity,[a] Guinea pig | Hypotensive activity Guinea pig | Dog[b] |
|---|---|---|---|
| 1-Desarginine bradykinin | <1/2000 | 1/80 | 1/50 |
| 1-Lysine bradykinin | 1/62 | 1/10 | 1/30 |
| 1-Ornithine bradykinin | 1/1000 | 1/50 | 1/100 |
| 1-Citrulline bradykinin | <1/2000 | 1/250 | |
| 1-Glutamic acid bradykinin | <1/2000 | 1/300 | |
| Bradykinin | 1 | 1 | 1 |

[a] H. O. J. Collier, J. A. Holgate, M. Schachter, and P. G. Shorley, *Brit. J. Pharmacol.*, **15,** 290 (1960). [b] For a description of this test method see L. Beck, *Circulation,* **17,** 798 (1958).

## Experimental[6]

**L-Prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-L-arginine Diacetate: 1-Desarginine Bradykinin (II).**—To a solution of 250 mg. ($2.2 \times 10^{-4}$ mole) of carbobenzoxy-L-prolyl-L-prolylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanylnitro-L-arginine methyl ester (I)[2] in 20 ml. of methanol was added 1 ml. of 2 *N* NaOH. The solution was stirred 1 hr. at room temperature, diluted with 75 ml. of water, and 1.5 ml. of *N* HCl was added. The precipitate was removed, washed with water, and dried; wt. 200 mg. The solid was dissolved in 30 ml. of glacial acetic acid–methanol (2:1) and was hydrogenated over 250 mg. of palladium black catalyst for 24 hr. at slight pressure. The catalyst was removed and the filtrate was evaporated to an oil. The oil was dissolved in 50 ml. of water, shell frozen, and lyophilized, leaving 155 mg. of white powder, $[\alpha]^{23}D$ −83.7° (*c* 0.43, water).

*Anal.* Calcd. for $C_{48}H_{69}N_{11}O_{14}\cdot 4H_2O$: C, 52.59; H, 7.08; N, 14.06. Found: C, 52.49; H, 7.00; N, 14.27.

**Dicarbobenzoxy-L-lysyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanylnitro-L-arginine Methyl Ester (IVa).**—A solution of 2.1 g. (0.0019 mole) of the carbobenzoxyoctapeptide methyl ester I was treated with 3 g. of anhydrous HBr in 50 ml. of glacial acetic acid for 2 hr. The solution was poured into ether and the precipitate was removed and dried *in vacuo*. The crude product was dissolved in 30 ml. of dimethylformamide, cooled to 0°, and 1 ml. of triethylamine was added. The solution was filtered and to the filtrate was added 1.5 g. (0.0027 mole) of dicarbobenzoxy-L-lysine *p*-nitrophenyl ester. The solution was stirred for 2 days at 25°, evaporated to 10 ml., and ethyl acetate was added giving a yellow solid. The product was twice recrystallized from methanol–

(1) D. F. Elliott, G. P. Lewis, and E. W. Horton, *Biochem. Biophys. Res. Commun.*, **3,** 87 (1960).

(2) E. D. Nicolaides and H. A. DeWald, *J. Org. Chem.*, **26,** 3872 (1961).

(3) We are indebted to Dr. D. A. McCarthy for the results of the dog experiments and to Dr. H. O. J. Collier, Miss P. G. Shorley, and Miss R. A. Hamilton for the guinea pig tests.

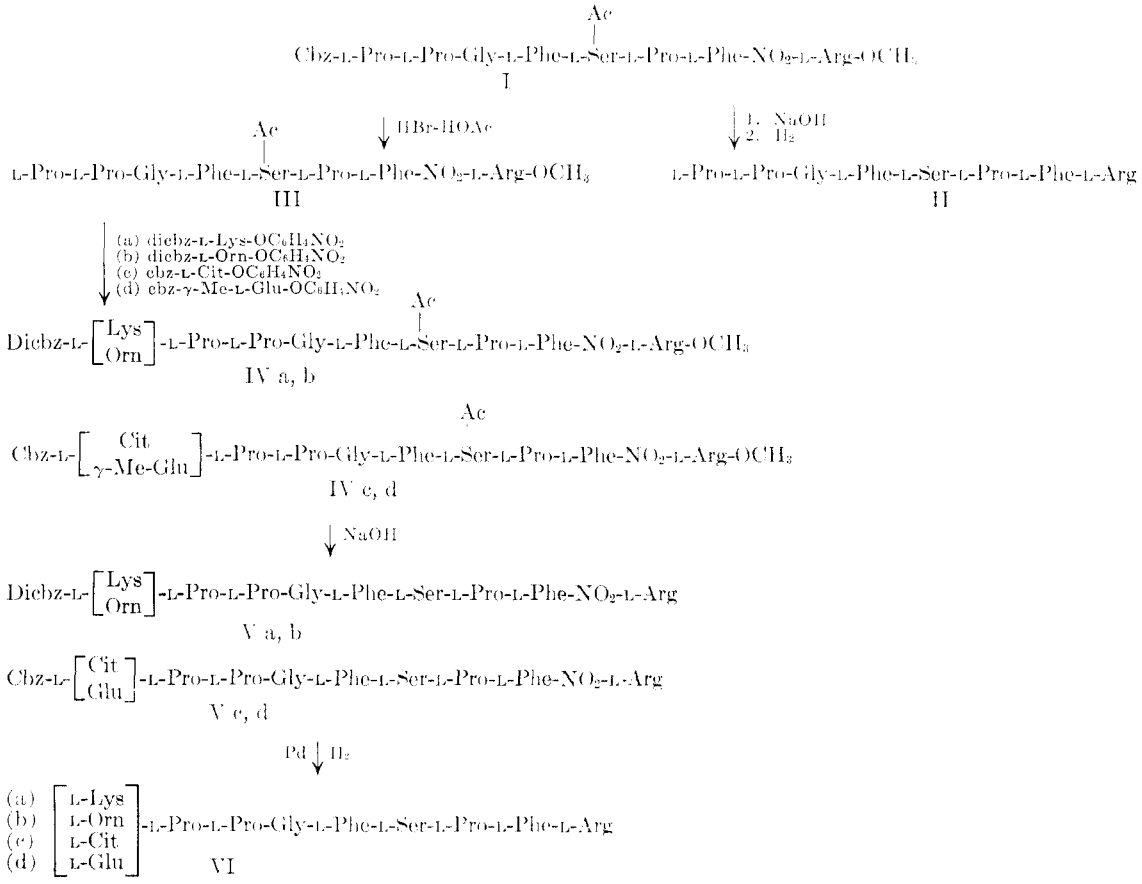
(4) H. O. J. Collier, *Ann. N. Y. Acad. Sci.*, **104,** 290 (1963).

(5) H. A. DeWald, M. K. Craft, and E. D. Nicolaides, *J. Med. Chem.*, **6,** 741 (1963).

(6) Melting points were taken using a Thomas–Hoover capillary melting point apparatus and are corrected.

SCHEME 1

SYNTHETIC SCHEME FOR I-LYS, I-ORN, I-CIT, AND I-GLU BRADYKININ




ether yielding 2 g. (77%) of cream colored solid, m.p. 140–145°, $[\alpha]^{23}D$ −76° (*c* 1, methanol).

*Anal.* Calcd. for $C_{68}H_{88}H_{14}O_{18}\cdot 3H_2O$: C, 56.58; H, 6.56; N, 13.59. Found: C, 56.13; N, 6.56; N, 14.12.

**Dicarbobenzoxy-L-lysyl-L-prolyl-L-prolyglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanylnitro-L-arginine (Va).**—To a solution of 1.8 g. (0.00132 mole) of the carbobenzoxynonapeptide methyl ester (IVa) in 30 ml. of methanol was added 1.5 ml. of 2 *N* NaOH. After 1 hr. the solution was diluted with 75 ml. of water, 1.6 ml. of 2 *N* HCl was added, and the precipitate was removed and dried. The product was recrystallized from ethanol–ether; yield, 1.5 g. (84%), m.p. 155–160°, $[\alpha]^{23}D$ −70° (*c* 1, methanol).

*Anal.* Calcd. for $C_{66}H_{84}N_{14}O_{17}\cdot H_2O$: C, 58.14; H, 6.36; N, 14.38. Found: C, 58.12; H, 6.37; N, 14.54.

**L-Lysyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-L-arginine Triacetate: 1-Lysine Bradykinin (VIa).**—A solution of 600 mg. ($4.45 \times 10^{-4}$ mole) of the carbobenzoxynonapeptide Va was dissolved in 50 ml. of glacial acetic acid–methanol (3:2) and the solution was hydrogenated over 200 mg. of palladium black catalyst at slight pressure for 24 hr. The catalyst was removed and the filtrate was evaporated to dryness. The residue was taken up in 50 ml. of water, shell frozen, and lyophilized, leaving 514 mg. of white powder.

*Anal.* Calcd. for $C_{56}H_{86}N_{13}O_{17}\cdot H_2O$: C, 54.66; H, 7.13; N, 14.80. Found: C, 54.24; H, 7.38; N, 15.30.

**Dicarbobenzoxy-L-ornithyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanylnitro-L-arginine-Methyl Ester (IVb).**—Two grams (0.0019 mole) of L-prolyl-L-prolylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanylnitro-L-arginine methyl ester hydrobromide was dissolved in 30 ml. of dimethylformamide, the solution cooled to 0° and 1 ml. of triethylamine was added. After 5 min., the mixture was filtered and 1.5 g. of dicarbobenzoxy-L-ornithine *p*-nitrophenyl ester was added to the filtrate. The solution was kept 2 days at 25°, evaporated to 10 ml., and ethyl acetate was added giving a yellow solid which was washed with water, dilute HCl, dilute $NH_4OH$, and water, and was dried and recrystallized from methanol–ether as a cream colored solid; m.p. 140–145°, $[\alpha]^{23}D$ −81.5° (*c* 1, methanol), yield, 2.4 g. (82%).

*Anal.* Calcd. for $C_{67}H_{86}N_{14}O_{18}$: C, 58.50; H, 6.30; N, 14.26. Found: C, 58.19; H, 6.69; N, 14.17.

**Dicarbobenzoxy-L-ornithyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanylnitro-L-arginine (Vb).**—The methyl ester was hydrolyzed from 2 g. (0.00149 mole) of the dicarbobenzoxynonapeptide IVb with 2 *N* NaOH in methanol. After acidification the product was removed, washed with water, and was recrystallized from ethanol–ether as a white solid, m.p. 145–150°, $[\alpha]^{23}D$ −74° (*c* 1, methanol), yield, 1.7 g. (86%).

*Anal.* Calcd. for $C_{65}H_{82}N_{14}O_{17}\cdot H_2O$: C, 57.85; H, 6.27; N, 14.54. Found: C, 57.89; H, 6.32; N, 14.48.

**L-Ornithyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-L-arginine Triacetate: 1-Ornithine Bradykinin (VIb).**—The dicarbobenzoxynonapeptide Vb (500 mg., $3.76 \times 10^{-4}$ mole) was hydrogenated for 24 hr. over palladium black catalyst. The catalyst was removed, the filtrate was evaporated, and the residual oil was taken up in 50 ml. of water, frozen, and lyophilized. A cream colored solid, 467 mg., was obtained, $[\alpha]^{23}D$ −87° (*c* 1, water).

*Anal.* Calcd. for $C_{55}H_{84}N_{13}O_{17}\cdot 3H_2O$: C, 52.70; H, 7.24; N, 14.53. Found: C, 51.79; H, 7.31; N, 15.05.

**Carbobenzoxy-L-citrullyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanylnitro-L-arginine (Vc).**—The protected octapeptide I (1.2 g., 0.0012 mole) was treated with glacial acetic acid–hydrogen bromide in the usual manner to yield 1.4 g. of the hydrobromide salt. The salt was dissolved in 5 ml. of dimethylformamide and was treated with 0.7 ml. of triethylamine at 4°. The triethylamine hydrobromide was removed by filtering and 0.6 g. (0.0014 mole) of carbobenzoxy-L-citrulline *p*-nitrophenyl ester was added. The mixture was kept at 40° for 3 days, then was diluted with ethyl acetate, and was washed with aqueous potassium carbonate and aqueous saturated sodium chloride as a slowly solidifying oil separated. The solid (1.2 g.) was redissolved in methanol and was precipitated with ether to yield 0.8 g. of amorphous solid.

*The nonapeptide (0.7 g.) was dissolved in 5 ml. of methanol* and 1.4 ml. of *N* sodium hydroxide was added dropwise. The solution was kept at room temperature for 2.5 hr., then acidified, and the methanol was evaporated *in vacuo* to yield 0.6 g. of amorphous solid, $[\alpha]^{23}D$ −48° (*c* 0.5, dimethylformamide).

*Anal.* Calcd. for $C_{58}H_{77}N_{15}O_{16}\cdot 2H_2O$: C, 54.59; H, 6.40; N, 16.46. Found: C, 54.54; H, 6.68; N, 16.40.

**L-Citrullyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-L-arginine. (1-Cit Bradykinin) (VIc).**—The nonapeptide Vc (500 mg.) was dissolved in methanol–acetic acid and hydrogenated in the presence of palladium black in the usual manner. The mixture was filtered and the filtrate was evaporated *in vacuo*. The residue was redissolved in water and filtered; the filtrate was shell frozen and lyophilized to yield 300 mg. of a colorless solid, $[\alpha]^{23}D$ −93° (*c* 1, *N* acetic acid), lit.[7] $[\alpha]^{20}D$ −91.2° (*c* 1, *N* acetic acid).

**Carbobenzoxy-γ-methyl-L-glutamic Acid *p*-Nitrophenyl Ester.**—To a cold (5°) solution of 5 g. (0.017 mole) of carbobenzoxy-γ-methyl-L-glutamic acid in 100 ml. of ethyl acetate was added 2.5 g. of *p*-nitrophenol and 3.6 g. of dicyclohexylcarbodiimide. The mixture was kept 2 hr. at 5°, filtered, evaporated to an oil, the oil was taken up in ether, and cyclohexane was added. The white precipitate was removed, washed with cold ethanol, and was dried; yield, 6 g. (85%), m.p. 103–104°.

*Anal.* Calcd. for $C_{20}H_{20}N_2O_8$: C, 57.68; H, 4.84; N, 6.73. Found: C, 57.83; H, 4.90; N, 6.88.

**Carbobenzoxy-L-glutamyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanylnitro-L-arginine (Vd).**—To a cold (5°) solution of 2.5 g. (0.0024 mole) of the octapeptide methyl ester hydrobromide III in 50 ml. of dimethylformamide was added 1.3 g. of triethylamine. The mixture was filtered and 1 g. (0.0024 mole) of carbobenzoxy-γ-methyl-L-glutamic acid *p*-nitrophenyl ester was added to the filtrate. The solution was stirred 2 days at 30°, evaporated to 10 ml., and ethyl acetate was added. An oil formed which solidified on trituration with ether. The solid was dissolved in 50 ml. of methanol and 5 ml. of 2 *N* NaOH was added. The solution was kept 1 hr. at 25°, diluted with water, and 6 ml. of 2 *N* HCl was added. The precipitate was removed and was reprecipitated twice from methanol with ether as a white solid, m.p. 175–180°, $[\alpha]^{23}D$ −61.6° (*c* 1, methanol), yield, 1.1 g.

*Anal.* Calcd. for $C_{57}H_{73}N_{13}O_{17}\cdot 2H_2O$: C, 54.84; H, 6.22; N, 14.59. Found: C, 54.73; H, 6.34; N, 14.81.

**L-Glutamyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-L-arginine Triacetate Salt: 1-Glutamic Acid Bradykinin (VId).**—Five hundred milligrams ($4.12 \times 10^{-4}$ mole) of the carbobenzoxynonapeptide Vd in 50 ml. of glacial acetic acid–methanol (3:2) was hydrogenated over palladium black catalyst for 24 hr. as previously described. The mixture was filtered, evaporated to an oil, and the oil was dissolved in 50 ml. of water and freeze-dried, leaving 450 mg. of a cream colored solid, $[\alpha]^{23}D$ −72.8° (*c* 1.03, water).

*Anal.* Calcd. for $C_{49}H_{68}N_{12}O_{13}\cdot 4H_2O$: C, 53.24; H, 6.93; N, 15.21. Found: C, 52.91; H, 6.62; N, 15.23.

For the paper chromatography of the analogs two different solvent systems were employed: (A) *t*-butyl alcohol–acetic acid–water (2:1:1); (B) isopropyl alcohol–ammonium hydroxide–water (70:5:25). The peptides appeared homogenous after development of the spots with brom phenol blue and Sakaguchi reagents with the following $R_f$ values: 1-Lys (A) 0.71, (B) 0.53; 1-Orn (A) 0.79, (B) 0.51; 1-Desarg (A) 0.74, (B) 0.60; 1-Glu (A) 0.74, (B) 0.66; 1-Cit (A) 0.72, (B) 0.61. Paper eletrophoresis in acetate buffer, pH 5.6, 3 hr. at 30 ma., produced single spots with all of the analogs except the 1-Glu derivative which showed the presence of a minor, faster moving component.

**Acknowledgment.**—We are indebted to Mr. C. E. Childs and his staff for the microanalyses reported herein and Dr. J. M. Vandenbelt, Mrs. Carola Spurlock, and Mrs. Vivien Lee for the optical rotations.

(7) M. A. Ondetti, *J. Med. Chem.*, **6**, 10 (1963).

# The Synthesis of 6-O-Carbamyl-L-Serine, 6-D-Serine, and 6-L-Threonine Bradykinin

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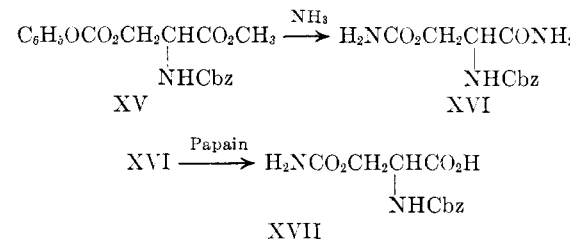
*Received May 27, 1963*

The synthesis of three analogs of bradykinin is described in which the serine of position 6 has been changed to L-threonine, D-serine, and O-carbamyl-L-serine. The biological activity of the analogs compared to bradykinin is reported.

In a previous paper[1] the preparation of three analogs of bradykinin, in which the phenylalanine amino acid in position 8 of the molecule was replaced by D-phenylalanine, *p*-fluoro-L-, and *p*-fluoro-D-phenylalanine, was described. As part of a continuing effort to investigate what effect subtle changes in the bradykinin structure have in relation to its biological activity, this paper describes three analogs which have variations in the serine portion of the molecule; these three new nonapeptides are the 6-O-carbamyl-L-serine, 6-D-serine, and 6-L-threonine bradykinins.

The synthetic method used for the preparation of the 6-O-carbamylserine analog is shown in Scheme I. The required intermediate O-carbamyl-N-carbobenzoxy-L-serine (XVII)[2] was obtained by ammonolysis of the O-phenylcarbonate ester of carbobenzoxy-L-serine methyl ester (XV). The resulting O-carbamyl-N-carbobenzoxy-L-serine amide (XVI) was hydrolyzed enzymatically with papain to yield XVII.




The *p*-nitrophenyl ester of the carbobenzoxy-O-carbamylserine was prepared and subsequent reaction with L-prolyl-L-phenylalanylnitro-L-arginine *p*-nitrobenzyl ester gave the carbobenzoxytetrapeptide X. The *p*-nitrobenzyl ester was utilized, since it was easily removed by hydrogenation and alkaline hydrolysis was to be avoided. The next two steps leading to the carbobenzoxyheptapeptide XII were *p*-nitrophenyl ester reactions and the heptapeptide was obtained in a crystalline state. The fully protected nonapeptide

(1) E. D. Nicolaides, M. K. Craft, and H. A. DeWald, *J. Med. Chem.*, **6**, 524 (1963).

(2) We are indebted to Dr. M. S. Morgan, Mellon Institute, for the use of his unpublished procedure for preparing O-carbamyl-N-carbobenzoxy-L-serine.