SYNTHESIS OF SOME PEPTIDES OF ε-N-ACETYL-L-LYSINE

LEO BENOITON
Department of Biochemistry, Faculty of Medicine, University of Ottawa, Ottawa, Ontario
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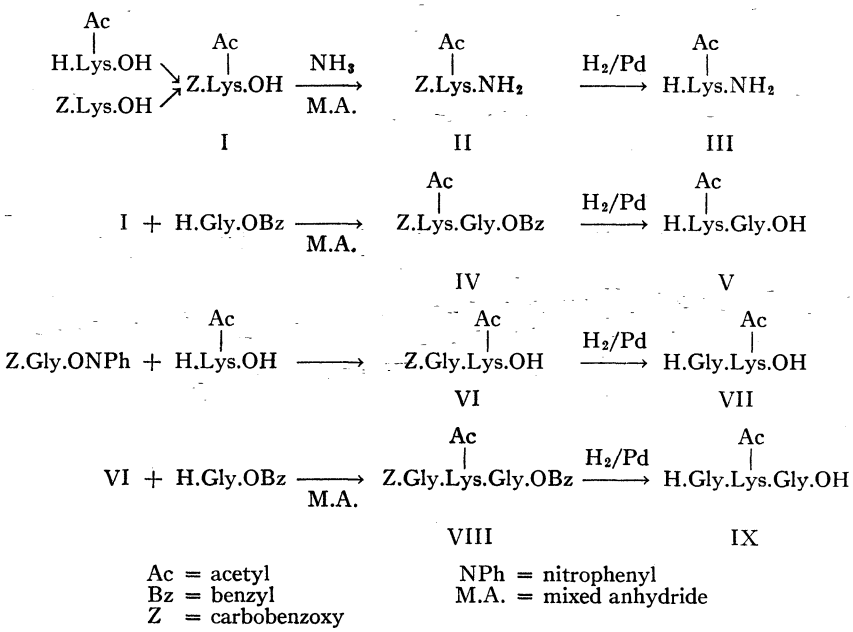
ABSTRACT

ε-*N*-Acetyl-L-lysinamide, α-*N*-glycyl-ε-*N*-acetyl-L-lysine, ε-*N*-acetyl-L-lysylglycine, α-*N*-glycyl-ε-*N*-acetyl-L-lysylglycine, and ε-*N*-acetylglycyl-L-lysine have been synthesized for testing as substrates for the enzyme ε-lysine acylase.

Several years ago, the enzyme ε-lysine acylase was identified and partially purified from rat kidney (1). We have now purified ε-lysine acylase from a much more convenient source, hog kidney, and have tested its hydrolytic action on several derivatives of lysine including some peptides of ε-*N*-acetyl-L-lysine (2). This paper describes the synthesis of the peptides.

The compounds III, V, VII, and IX, and the synthetic routes employed, are illustrated in the accompanying scheme. In addition, the isomeric peptide ε-*N*-acetylglycyl-L-lysine was also prepared.



ε-*N*-Acetyl-L-lysine, prepared by acetylation of the copper salt of lysine (3), was carbobenzoxylated to the disubstituted derivative I, which was crystallized as the dicyclohexylammonium salt. I was also prepared by acetylation of α-*N*-carbobenzoxy-L-lysine, obtained through the ε-*N*-benzylidene derivative (4); however, our inability to obtain the reported yields for α-*N*-carbobenzoxy-L-lysine rendered this route less attractive. The mixed anhydride (5) of I, by treatment with anhydrous ammonia or glycine benzyl ester, gave the protected amide II and dipeptide IV respectively. Catalytic hydrogenolysis of these provided the ε-*N*-acetyl-L-lysine amide III and the ε-*N*-acetyl-L-lysine dipeptide

V. The isomeric glycyl dipeptide VII was made by hydrogenation of the protected intermediate VI, obtained in one step by the coupling of carbobenzoxyglycine *p*-nitrophenyl ester with ε-*N*-acetyl-L-lysine in hot acetic acid (6). The mixed anhydride (5) of VI plus glycine benzyl ester yielded the protected tripeptide VIII, which gave the free ε-*N*-acetyl tripeptide IX on hydrogenation. ε-*N*-Acetylglycyl-L-lysine was prepared directly by using a general procedure for the synthesis of ε-*N*-lysine peptides (7), namely, the coupling of the mixed anhydride of acetylglycine with the copper salt of lysine in aqueous solution.

Several of the optical rotations observed were extremely low, with one (for VII) actually being equal to zero. However, this was not due to racemization. If racemization had occurred, it would have been during the synthesis of VI by the nitrophenyl ester method. The optical purity of VI was established by measuring the optical rotation of a sample after hydrolysis in 5 *N* hydrochloric acid. The observed rotation was identical with that of a solution of lysine of corresponding concentration.

Enzymatic studies (2) have shown that the peptides are cleaved by ε-lysine acylase at rates of 85, 24, 109, and 70 for III, V, VII, and IX respectively, relative to a rate of 100 for ε-*N*-acetyl-L-lysine.

EXPERIMENTAL

General

Melting points were determined by the capillary method, using a thermometer calibrated against standard substances. Optical rotations were determined with a Rudolph Model 62 polarimeter using a 2-dm tube. Compounds were dried at 60° before analysis. Acetonitrile and chloroform were purified and distilled before use. The R_f values recorded are for the following solvents: R_f^B, *n*-butanol – acetic acid – water (4:1:5); R_f^F, *tert*-butanol – formic acid – water (70:15:15).

Starting Materials

ε-*N*-acetyl-L-lysine, $[\alpha]_D^{25}$ +22.7° (1, 5 *N* HCl), was prepared by acetylation of the copper salt of lysine with acetic anhydride (3) using two equivalents of lithium hydroxide as base (8). Yields of recrystallized, chromatographically pure material averaged 45%. Yields of 60%, obtained when using only one equivalent of base, have been reported (8). In our hands this procedure gave a product which was too highly contaminated with lysine to be purified by recrystallization. The original procedure (3) calls for two equivalents of base.

Carbobenzoxyglycine *p*-nitrophenyl ester (9) was prepared by the action of dicyclohexylcarbodiimide (10) on carbobenzoxyglycine (11) and *p*-nitrophenol in pyridine. After 12 hours, the mixture was filtered into ice water, and the precipitate which formed was washed by trituration with 1 *N* hydrochloric acid, water, and aq. sodium bicarbonate, and finally recrystallized from ethanol. Yield: 81%, m.p. 128°.

Glycine benzyl ester hydrochloride (12) was prepared from the corresponding *p*-toluenesulphonate (13) as described (14) except that the product was recrystallized from benzyl alcohol–ether. Yield: 80%.

Acetylglycine (15) was prepared by adding acetic anhydride to a hot solution of glycine in acetic acid.

α-N-Carbobenzoxy-ε-N-acetyl-L-lysine (Dicyclohexylammonium Salt (I))

(*a*) To a vigorously stirred cold solution of ε-*N*-acetyl-L-lysine (7.52 g; 0.04 mole) containing sodium bicarbonate (10 g) was added carbobenzoxy chloride (7 g) in four portions. After 3 hours, the solution was acidified (HCl) and extracted with ethyl acetate, the extract dried (Na_2SO_4) and evaporated to dryness. The residue was dissolved in ethyl acetate, and excess dicyclohexylamine was added. Crystals appeared immediately. After cooling, the product was filtered, recrystallized from acetonitrile, and washed with ether. Yield: 5.54 g (55%), m.p. 132–133°, $[\alpha]_D^{25}$ +8.6° (2, ethanol). Found: C, 66.7; H, 9.0; N, 8.3. $C_{28}H_{47}N_3O_5$ (503.7) requires: C, 66.8; H, 9.0; N, 8.3%.

(*b*) To a vigorously stirred solution of α-*N*-carbobenzoxy-L-lysine (4) (5.6 g; 0.02 mole) and sodium bicarbonate (3.78 g) in water (5°) was added, dropwise, acetic anhydride (2.3 g; 0.022 mole) during 30 minutes. Stirring was continued an additional hour at 25°, then the solution was acidified and the compound crystallized as described above. Yield: 5.54 g (55%), m.p. 132–133°.

α-N-Carbobenzoxy-ε-N-acetyl-L-lysinamide (II)

To I (3.02 g; 0.006 mole) suspended in chloroform was added 1 *N* sulphuric acid (6 ml). After shaking, the organic layer was taken and washed twice with water, dried overnight (Na_2SO_4), and concentrated down. The solution was cooled (0°), and triethylamine (0.83 ml; 0.006 mole) followed by ethyl chloroformate (0.54 ml; 0.006 mole) was added. After 30 minutes, anhydrous ammonia was bubbled through for 15 minutes,

the mixture being stirred by a magnetic stirrer. The mixture was kept an additional 15 minutes at 25°, then cooled and filtered. The product was washed with cold chloroform, and recrystallized from acetonitrile. Yield: 1.1 g (60%), m.p. 179°, $[\alpha]_D^{25}$ +3.4° (1, dimethylformamide). Found: C, 59.8; H, 7.1; N, 13.0. $C_{16}H_{23}N_3O_4$ (321.4) requires: C, 59.8; H, 7.2; N, 13.1%.

ε-N-Acetyl-L-lysinamide Hydrochloride (III)

A solution of II (0.96 g) in ethanol (25 ml) containing 1 *N* hydrochloric acid (3 ml) was hydrogenated over palladium black. The catalyst was filtered off, the solution evaporated to dryness, and the residue crystallized from ethanol containing a few drops of water. Ether was then added. Yield: 0.53 g (80%), m.p. 243°, $[\alpha]_D^{25}$ +17.9° (1, water), R_f^B 0.32, R_f^F 0.41. Found for a sample recrystallized from water–ethanol: C, 43.2; H, 8.1; N, 18.2. $C_8H_{18}ClN_3O_2$ (223.7) requires: C, 43.0; H, 8.1; N, 18.8%.

α-N-Carbobenzoxy-ε-N-acetyl-L-lysylglycine Benzyl Ester (IV)

The mixed anhydride of *α-N*-carbobenzoxy-*ε-N*-acetyl-L-lysine was prepared from I (4.02 g; 0.008 mole) as described for the synthesis of the amide (II). After 30 minutes, a cold chloroform solution of glycine benzyl ester hydrochloride (1.61 g; 0.008 mole) and triethylamine (1.11 ml; 0.008 mole) was added. The solution was kept at 0° for 1 hour, and at 25° overnight. The solution was then washed successively with 1 *N* hydrochloric acid, water, aq. sodium bicarbonate, and finally water, dried (Na_2SO_4), and evaporated to dryness. The residue, which solidified on trituration with ether, was collected and recrystallized from chloroform–ether. Yield: 2.85 g (80%), m.p. 113–114°. A sample recrystallized for analysis had m.p. 115–116°, $[\alpha]_D^{25}$ −6.3° (1, dimethylformamide). Found: C, 63.8; H, 6.8; N, 8.9. $C_{25}H_{31}N_3O_6$ (469.5) requires: C, 63.95; H, 6.65; N, 8.95%.

ε-N-Acetyl-L-lysylglycine (V)

A solution of IV (1.41 g) in ethanol (35 ml) containing water (5 ml) and acetic acid (2 ml) was hydrogenated over palladium black. After removal of the catalyst, the solution was evaporated down and the residue was crystallized from water–ethanol–ether. Yield: 0.59 g (80%). Recrystallization from water–ethanol gave 0.41 g (56%), m.p. 225–226°, $[\alpha]_D^{25}$ +74.3° (1, water), R_f^B 0.32, R_f^F 0.49. Found: C, 48.8; H, 8.0; N, 16.9. $C_{10}H_{19}N_3O_4$ (245.3) requires: C, 49.0; H, 7.8; N, 17.1%.

α-N-Carbobenzoxyglycyl-ε-N-acetyl-L-lysine (VI)

A mixture of *ε-N*-acetyl-L-lysine (3.76 g; 0.02 mole) and carbobenzoxyglycine *p*-nitrophenyl ester (7.2 g; 0.021 mole) in dry acetic acid was refluxed for 3 hours. The solvent was distilled off, and the residue was distributed between ethyl acetate and aq. sodium bicarbonate. The aqueous layer was acidified and extracted with ethyl acetate, the extract was dried (Na_2SO_4) and then evaporated to a white solid. The product was filtered with the aid of ether and recrystallized from acetonitrile. Yield: 4.4 g (58.7%), m.p. 126°, $[\alpha]_D^{25}$ −3.6° (1, dimethylformamide). Found: C, 56.9; H, 6.8; N, 11.2. $C_{18}H_{25}N_3O_6$ (379.4) requires: C, 57.0; H, 6.6; N, 11.1%. After hydrolysis of a sample (129.6 mg) in 5 ml of 5 N hydrochloric acid, α was +0.52° corresponding to $[\alpha]_D^{25}$ +26.0° when calculated for a lysine content of 49.9 mg. A solution of lysine had $[\alpha]_D^{25}$ +26.4° (1, 5 *N* HCl).

α-N-Glycyl-ε-N-acetyl-L-lysine (VII)

A solution of VI (1.54 g) in ethanol (50 ml) containing water (10 ml) and acetic acid (2 ml) was hydrogenated over palladium black. After removal of the catalyst by filtration, and solvent by evaporation, the residue was crystallized from water–ethanol. Yield: 0.7 g (72%), m.p. 220–221°, $[\alpha]_D^{25}$ 0 (water, or 5 *N* HCl), R_f^B 0.31, R_f^F 0.46. Found: C, 48.8; H, 7.9; N, 17.2. $C_{10}H_{19}N_3O_4$ (245.3) requires: C, 49.0; H, 7.8; N, 17.1%.

α-N-Carbobenzoxyglycyl-ε-N-acetyl-L-lysylglycine Benzyl Ester (VIII)

To VI (1.89 g; 0.005 mole) and triethylamine (0.70 ml; 0.005 mole) in dry chloroform at 0° was added ethyl chloroformate (0.45 ml; 0.005 mole). After 30 minutes, a cold chloroform solution of glycine benzyl ester hydrochloride (1.01 g; 0.005 mole) and triethylamine (0.70 ml; 0.005 mole) was added. After 1 hour at 0°, and 12 hours at 25°, the solution was successively washed with 1 *N* hydrochloric acid, water, aq. sodium bicarbonate, water, and then dried. The chloroform was evaporated off and the white residue was crystallized twice from acetonitrile. Yield: 1.2 g (46%), m.p. 170–171°, $[\alpha]_D^{25}$ −7.2° (1, dimethylformamide) Found: C, 61.4; H, 6.6; N, 10.7. $C_{27}H_{34}N_4O_7$ (526.6) requires: C, 61.6; H, 6.5; N, 10.6%.

α-N-Glycyl-ε-N-acetyl-L-lysylglycine (IX)

A solution of VIII (1.25 g) was hydrogenated as described for the preparation of VII. The product was crystallized twice from water–ethanol. Yield: 0.53 g (74%), m.p. 245–246°, $[\alpha]_D^{25}$ +1.5° (1, water), R_f^B 0.28, R_f^B 0.36. Found: C, 47.45; H, 7.4; N, 18.3. $C_{12}H_{22}N_4O_5$ (302.3) requires: C, 47.7; H, 7.3; N, 18.5%.

ε-N-Acetylglycyl-L-lysine

The mixed anhydride prepared from acetylglycine (1.17 g; 0.01 mole) in tetrahydrofuran was added quickly to a cold aqueous solution of the copper salt of lysine (1.83 g; 0.01 mole) containing 5 ml of 2 *N* lithium hydroxide. The mixture was kept cold and stirred for 2 hours. Next morning, the blue precipitate was collected, washed with water, then dissolved in hot water, and hydrogen sulphide was bubbled through

the solution. After subsequent charcoal treatment, the remaining solution was evaporated down and the residue crystallized twice from water–ethanol. Yield: 1.23 g (50%), m.p. 245–247°, $[\alpha]_D^{25}$ +17.1° (1, 5 *N* HCl), R_f^B 0.28, R_f^F 0.52. Found: C, 48.8; H, 7.9; N, 16.9. $C_{10}H_{19}N_3O_4$ (245.3) requires: C, 49.0; H, 7.8; N, 17.1%.

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REFERENCES

1. W. K. Paik, L. Bloch-Frankenthal, S. M. Birnbaum, M. Winitz, and J. P. Greenstein. Arch. Biochem. Biophys. **69**, 56 (1957).
2. W. K. Paik and L. Benoiton. Can. J. Biochem. Physiol. **41**, 1643 (1963).
3. A. Neuberger and F. Sanger. Biochem. J. **37**, 515 (1943).
4. B. Bezas and L. Zervas. J. Am. Chem. Soc. **83**, 719 (1961).
5. R. A. Boissonas. Helv. Chim. Acta, **34**, 874 (1951). J. R. Vaughan and R. L. Osata. J. Am. Chem. Soc. **73**, 553 (1951).
6. F. Weygand and W. Steglich. Ber. **93**, 2983 (1960).
7. D. M. Theodoropoulus. J. Org. Chem. **23**, 140 (1958).
8. J. P. Greenstein and M. Winitz. Chemistry of the amino acids. Vol. 3. J. Wiley & Sons, Inc., New York, N.Y. 1961. p. 2117.
9. B. Iselin, W. Rittel, P. Sieber, and R. Schwyzer. Helv. Chim. Acta, **46**, 373 (1957).
10. M. Rothe and F. W. Kunitz. Ann. **609**, 88 (1957). D. F. Elliot and D. W. Russel. Biochem. J. **66**, 49-P (1957).
11. H. E. Carter, R. L. Frank, and H. W. Johnston. Org. Syn. **23**, 13 (1943).
12. C. R. Harrington and T. H. Mead. Biochem. J. **30**, 1598 (1936).
13. L. Zervas, M. Winitz, and J. P. Greenstein. J. Org. Chem. **22**, 1515 (1957).
14. P. Tailleur and L. Berlinguet. Can. J. Chem. **39**, 1309 (1961).
15. H. D. Dakin. J. Biol. Chem. **82**, 443 (1929).