

Synthesis of Acyl Derivatives of β -Lysine for Peptide Synthesis

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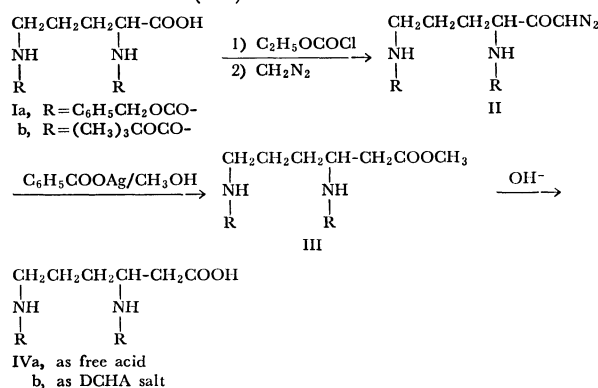
Synopsis. Dibenzoyloxycarbonyl- and di-*t*-butyloxycarbonyl- β -lysine were synthesized from corresponding diacyl-L-ornithine by modified Arndt-Eistert reaction involving diazoketone preparation *via* the mixed carbonic carboxylic anhydride. These diacyl- β -lysine derivatives are required for total- or semi-syntheses of peptide antibiotics, tuberactinomycins or streptothricins.

β -Lysine had been first found as a naturally occurring amino acid in the hydrolyzate of viomycin,¹⁾ and thereafter isolated from streptolin,²⁾ streptothricin,³⁾ and similar antibiotics. In our recent studies on tuberculostatic peptide, tuberactinomycins, the presence of this amino acid was confirmed in tuberactinomycin B, *i.e.* viomycin, and also in tuberactinomycin O.⁴⁾

Synthesis of the amino acid had been achieved by van Tamelen *et al.* for the purpose of a determination of its chemical structure.^{2,3)} In their method, N^{α},N^{δ} -diphthalyl-L-ornithine was elongated by Arndt-Eistert reaction and then hydrolyzed to 3,6-diaminohexanoic acid, *i.e.* β -lysine. In the course of this reaction, two amino groups had to be blocked with phthalyl group for the complete protection against a possible change with the acid chloride reagent which was used in the preparation of the diazoketone. On the other hand, benzyloxycarbonyl or *t*-butyloxycarbonyl group is preferable from a standpoint of the peptide synthesis where easily removable protecting groups are required. If the use of the acid chloride can be avoided by application of the mixed carbonic carboxylic anhydride method for the preparation of acylaminodiazo ketone as in the case of simple diazoketones,⁵⁾ those alkyloxycarbonyl type protections may be employed instead of phthalyl group. Actually, Penke *et al.* synthesized several diazoketones from benzyloxycarbonyl or *t*-butyloxycarbonyl amino acid using the mixed anhydride method or dicyclohexylcarbodiimide method.⁶⁾ More recently, Ondetti *et al.* prepared *t*-butyloxycarbonyl derivative of 3-amino-4-phenylbutyric acid by Arndt-Eistert reaction employing the mixed anhydride method from *t*-butyloxycarbonyl-phenylalanine. This derivative had been further used to a synthesis of the β -amino acid peptide.⁷⁾ In the present paper, N^{α},N^{δ} -dibenzoyloxycarbonyl- and N^{α},N^{δ} -di-*t*-butyloxycarbonyl- β -lysine were synthesized based on the same principle of combination of the protecting group and the mixed anhydride method (Scheme 1). These compounds are important starting materials for synthetic studies of tuberactinomycins and streptothricins containing β -lysine residues.

N^{α},N^{δ} -Dibenzoyloxycarbonyl-L-ornithine (Ia) and N^{α},N^{δ} -di-*t*-butyloxycarbonyl-L-ornithine (Ib) were first converted to their ethoxycarbonic anhydrides in the presence of *N*-methylmorpholine or triethylamine. The mixed anhydrides were immediately allowed to react with diazomethane for the preparation of

diazoketones(II). Following Wolff rearrangement in methanol gave acyl- β -lysine methyl esters (III) as crystals, which were successfully saponified to their acid derivatives (IV).



Scheme 1.

Preparation of such diacyl- β -lysine opened a way to the semi-synthesis of tuberactinomycin analogues. Thus, as reported in our recent study, dialkyloxycarbonyl- β -lysine 1-succinimidyl esters were coupled with tuberactinamine N, a cyclic peptide moiety isolated from tuberactinomycin N by selective liberation of the branched γ -hydroxy- β -lysine with acid treatment, and then diacyl groups were removed under mild conditions.⁸⁾ Synthetic tuberactinomycin O thus obtained was identical with natural compound in all respects. Consequently, dibenzoyloxycarbonyl- or di-*t*-butyloxycarbonyl- β -lysine newly prepared *via* modified Arndt-Eistert reaction from the corresponding diacyl-L-ornithine proved to be very useful derivatives for syntheses of its peptides.

Experimental

All melting points are uncorrected. The specific rotations were measured with a Perkin-Elmer 141 polarimeter.

N^{α},N^{δ} -Dibenzoyloxycarbonyl-L- β -lysine Methyl Ester (IIIa). N^{α},N^{δ} -Dibenzoyloxycarbonyl-L-ornithine⁹⁾ (4.00 g, 0.01 mol) was dissolved in 200 ml of ethyl acetate and the solution was cooled on ice-salt bath. To a cold solution, *N*-methylmorpholine (1.11 g, 0.011 mol) and ethyl chloroformate (1.20 g, 0.011 mol) were added under stirring. After 3 hr, precipitated amine hydrochloride was rapidly filtered off in the cold. Then diazomethane in ether was added to the filtrate and stirred at 0 °C overnight. Excess diazomethane was removed by warming at 50 °C and thereafter the reaction mixture was evaporated *in vacuo*. Oily diazoketone* thus

* When oily product was stored in refrigerator, diazoketone was crystallized. Recrystallization from ethyl acetate and *n*-hexane gave pale yellow needles, mp 79–80 °C or 93–94 °C (polymorphism). Found: C, 62.20; H, 5.70; N, 13.12%. Calcd for $\text{C}_{22}\text{H}_{24}\text{O}_5\text{N}_4$: C, 62.25; H, 5.70; N, 13.20%. Since the diazoketone was generally unstable, oily product was immediately used for next Wolff rearrangement.

obtained was dissolved in 80 ml of methanol, and silver benzoate (0.50 g, 0.002 mol) in 5 ml of triethylamine was added. The mixture was stirred overnight in the dark and then concentrated *in vacuo*. The residue was dissolved in ethyl acetate and insoluble material was filtered off. The filtrate was washed with saturated sodium hydrogencarbonate solution, saturated sodium chloride solution, 1 M hydrochloric acid, and then finally saturated sodium chloride solution to neutral. Organic layer was dried over anhydrous sodium sulfate and evaporated *in vacuo*. Crystalline residue was recrystallized from ethyl acetate and *n*-hexane, yield 3.64 g (85%), mp 105–107 °C, $[\alpha]_D^{25} - 5.0^\circ$ (*c* 0.3, dimethylformamide).

Found: C, 64.38; H, 6.59; N, 6.58%. Calcd for $C_{23}H_{28}O_6N_2$: C, 64.47; H, 6.59; N, 6.54%.

N^β, N^ϵ -Di-*t*-butyloxycarbonyl-L- β -lysine Methyl Ester (IIIb).

N^α, N^δ -Di-*t*-butyloxycarbonyl-L-ornithine(Ib)** was derived to IIIb in a similar way *via* its diazoketone (IIb)*** except the use of citric acid solution in place of hydrochloric acid for washing. Methyl ester (IIIb) was obtained in a 86% yield, mp 60–61 °C, $[\alpha]_D^{25} - 5.8^\circ$ (*c* 1, dimethylformamide).

Found: C, 56.47; H, 8.89; N, 7.80%. Calcd for $C_{17}H_{32}O_6N_2$: C, 56.64; H, 8.95; N, 7.77%.

N^β, N^ϵ -Dibenzoyloxycarbonyl-L- β -lysine (IVa). Methyl ester IIIa (0.86 g, 2 mmol) was dissolved in a small amount of dioxane and 1.5 ml of 1.5 M aqueous sodium hydroxide was added. After stirring at 0 °C for 30 min and then at room temperature for 1 hr, the reaction mixture was diluted with water, and extracted with ethyl acetate. Aqueous layer was acidified with 1 M hydrochloric acid and extracted with ethyl acetate. Organic layer was washed with saturated sodium chloride solution to neutral and dried over anhydrous sodium sulfate. The extract was concentrated *in vacuo* to give IVa, which was recrystallized from ethyl acetate, in a

yield of 0.68 g (82%), mp 155 °C, $[\alpha]_D^{25} + 1.0^\circ$ (*c* 1, dimethyl formamide).

Found: C, 63.79; H, 6.36; N, 6.78%. Calcd for $C_{22}H_{28}O_6N_2$: C, 63.75; H, 6.32; N, 6.76%.

N^β, N^ϵ -Di-*t*-butyloxycarbonyl-L- β -lysine (IVb). Corresponding methyl ester IIIb (0.90 g, 2.5 mmol) was dissolved in 3 ml of methanol and 2 ml of 1.5 M aqueous sodium hydroxide was added. The reaction mixture was treated as in the preparation of IIIa except use of citric acid for acidification. Oily product was crystallized as dicyclohexylammonium salt which was recrystallized from ether and *n*-hexane, 1.18 g (89%), mp 106–107 °C, $[\alpha]_D^{25} - 3.5^\circ$ (*c* 1, 95% ethanol).

Found: C, 62.93; H, 10.19; N, 7.73%. Calcd for $C_{28}H_{53}O_6N_3 \cdot 1/2 H_2O$: C, 62.65; H, 10.14; N, 7.83%.

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** N^α, N^δ -Di-*t*-butyloxycarbonyl-L-ornithine was prepared by acylation of L-ornithine hydrochloride with *t*-butyl azidoformate¹⁰ or *t*-butyl 4,6-dimethylpyrimidyl-2-thiol carbonate¹¹ in the usual manner, and the yield was 77% and 87%, respectively. Mp 120–121 °C, $[\alpha]_D^{25} - 6.0^\circ$ (*c* 1, dimethylformamide). Found: C, 54.23; H, 8.49; N, 8.34%. Calcd for $C_{15}H_{28}O_6N_2$: C, 54.20; H, 8.49; N, 8.43%.

*** Diazoketone (IIb) crystallized as well as in the case of IIa. It was recrystallized from ethyl acetate and *n*-hexane. Mp 114–115 °C, $[\alpha]_D^{25} - 43.1^\circ$ (*c* 1, dimethylformamide). Found: C, 53.71; H, 7.92; N, 15.66%. Calcd for $C_{16}H_{28}O_5N_4$: C, 53.91; H, 7.92; N, 15.72%.