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AN IMPROVED SYNTHESIS OF N^α-BENZYLOXYCARBONYL-L-LYSINE

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AN IMPROVED SYNTHESIS OF *N*^α-BENZYLOXYCARBONYL-L-LYSINE

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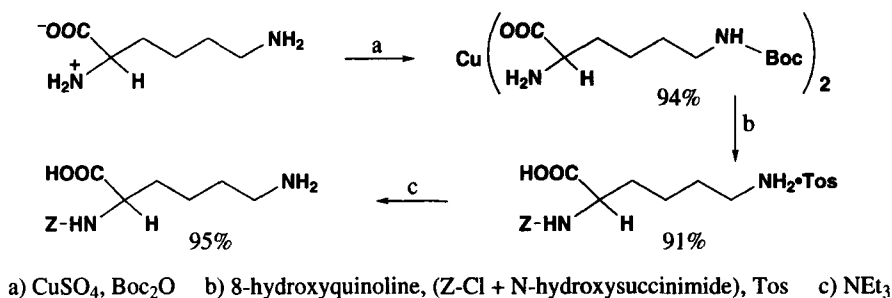
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Lysine contains two amino groups and thus may form peptides (α -peptides) and isopeptides (ϵ -peptides). The former (α -peptides) occur universally and their synthesis¹ and the production of the suitable derivatives for them² has been elaborated. Those latter occur first of all in fungi and bacteria.^{3,4} Since ϵ -peptides containing lysine are less common, it is anticipated that future studies will be focused on these isopeptides.

Z-Lys⁵ belongs to the type of derivatives useful in ϵ -peptide synthesis.⁶ The compound is produced on a large scale in a two-step process *via* blocking with the *N*^ε-benzylidene group; *N*^ε-benzylidene-L-lysine is isolated and subjected to the action of benzyl chlorocarbonate followed by treatment with hydrochloric acid to remove the *N*^ε-benzylidene protection.⁷ This sequence gives only 66% yield of Z-Lys, based on lysine. Moreover, the process has severe inconveniences resulting from the great instability of the benzylidene derivative. Introduction of the benzylidene group must be performed under nitrogen and argon. All reactions and operations during the preparation of *N*^ε-benzylidene-L-lysine and *N*^α-benzyloxycarbonyl-*N*^ε-benzylidene-L-lysine require low temperatures. Its maintenance is troublesome, particularly during benzyloxycarbonylation, an exothermic process accompanied by local overheating. In our hands, the synthesis of *N*^ε-benzylidene-L-lysine failed.

We report herein the synthesis of Z-Lys *via* *N*^ε-*t*-butoxycarbonyl blocking, whose introduction and manipulation of the resulting compound is without problem compared to the introduction and manipulation of *N*^ε-benzylidene protection.

The basic process encompasses two steps. (i) In the first, Lys(Boc) is obtained as the copper complex in 94% yield, based on lysine. Compared to the existing procedures,⁷ this constitutes a simple preparation of the complex of high quality (homogenous by TLC, correct elemental analysis).² (ii) The second stage is a one-pot procedure, in which the complex is decomposed with 8-hydroxyquinoline,² and the resulting Lys(Boc) is *N*^α-benzyloxycarbonylated with benzyl *N*-succinimidyl carbonate,⁸ prepared in a separate vessel from benzyl chlorocarbonate and *N*-hydroxysuccinimide and used *in situ*.² Although the commercial carbonate was used in initial experiments, this proved unnecessary as



the reagent prepared *in situ* works as well. The Z-Lys(Boc) is *N*^ε-deprotected with *p*-toluenesulfonic acid to provide Z-Lys(•Tos) in 91% yield (overall 86% yield, based on lysine) of 100% purity by HPLC and of correct elemental analysis. We tried a variety of inorganic and organic acids for the acidic removal of the Boc-group. However, none was as good as *p*-toluenesulfonic acid which deprotects quantitatively, without any side-reaction and moreover, gives a well-crystallized salt. This is directly useful in ϵ -peptide synthesis. If Z-Lys is needed in the free form as its inner salt, the action of triethylamine is sufficient to liberate Z-Lys(•Tos) from *p*-toluenesulfonic acid and give Z-Lys in 95% yield (99.8% purity by HPLC). The overall yield for the sequence amounts to 81% in comparison to 66% *via* the temporary *N*^ε-benzylidene protection.

The present method of the synthesis of Z-Lys displays a high degree of convenience and practicality.

EXPERIMENTAL SECTION

Mps were determined on a Boëtius heating block and are uncorrected. Reactions were monitored and the products checked on silica gel plates (DC Alufolien Kieselgel, 0.25 Merck # 5553) in the following solvent systems (v/v): A = *n*-butanol-acetic acid-water (4:1:1), B = *n*-butanol-acetic acid-ethyl acetate-water (1:1:1:1) and C = ethyl acetate-pyridine-acetic acid-water (30:20:6:11). All products are homogenous. HPLC analyses were carried out using a Beckman System Gold chromatograph, a 5 μ L loop, an Alltech Alltima, C₁₈, 5 μ m, 150 x 4.6 mm column, mobile phase: 0.1% trifluoroacetic acid-acetonitrile (80:20), a flow rate of 1 mL/min and detection at 210 nm. Specific rotations were measured at a Jasco DIP-1000 polarimeter.

Copper(II) Complex of *N*^ε-*t*-Butoxycarbonyl-L-lysine ([Lys(Boc)]₂Cu)².- To a stirred solution of HCl•Lys (365 g, 2 mol) in 2 M aqueous NaHCO₃ (2.0 L), a solution of CuSO₄•5H₂O (250 g, 1 mol) in water (2.0 L) was added. Thereafter NaHCO₃ (168 g, 2 mol) was added, followed by a solution of 96% Boc₂O (590 g, 2.6 mol) in acetone of technical quality (2.4 L). After 24 h, methanol (0.5 L) was introduced and stirring was continued for 12 h. Water (2.0 L) and ethyl acetate (2.0 L) were added and the precipitate was collected. The precipitate was suspended in water (5.0 L) and collected. These operations were repeated twice more. The resulting precipitate was air-dried to yield 522 g (94%) of the title compound as fine, light blue solid. TLC: R_f: A - 0.38, R_f: B - 0.73.

Anal. Calcd for C₂₂H₄₂CuN₄O₈: C, 47.68; H, 7.64; N, 10.11. Found: C, 47.57; H, 7.82; N, 9.99

***N*^α-Benzyloxycarbonyl-L-lysine Tosylate (Z-Lys(•Tos)).**- To a vigorously stirred suspension of [Lys(Boc)]₂Cu (55.4 g, 0.100 mol), wetted with acetone (0.2 L), in a 10% aqueous Na₂CO₃ solution (0.4 L), was added 8-hydroxyquinoline (29.9 g, 0.206 mol), and the whole was stirred for 1 h (mixture 1). To a solution of *N*-hydroxysuccinimide (25.3 g, 0.220 mol) in water (0.125 L) in a separate vessel, Na₂CO₃ (11.7 g, 0.110 mol) was introduced and followed by acetone (0.1 L). Benzyl chlorocarbonate of 96% purity (30 mL, 0.200 mol) was added dropwise and the mixture was left standing for 0.5 h with occasional stirring (mixture 2). Mixture 2 was poured into stirred mixture 1. After 1 h, the precipitate was filtered off and washed with water (4 x 0.1 L). The filtrate was acidified with 1 M HCl to pH 2 (with stirring) and extracted with ethyl acetate (0.5 L). The dried organic layer was evaporated *in vacuo* on a rotary evaporator at a bath temperature not exceeding 45°. The oily residue was dissolved in acetone (0.4 L), evaporated, dissolved once more in acetone (0.8 L) and *p*-toluenesulfonic acid hydrate (76.08 g, 0.4 mol) was added. The next day, the precipitate was collected and washed with acetone (0.5 L) to give 82.68 g (91%) of the title compound as a colorless solid, mp. 179-181°. TLC: R_f: B - 0.70, R_f: C - 0.50, HPLC: tR 7.69 min, 100% purity, [α]_D^{28.6} = -7.06±0.03° (c = 2, methanol), [α]₅₄₆^{28.6} = -7.72±0.00° (c = 2, methanol).

Anal. Calcd. for C₂₁H₂₈N₂O₇S: C, 55.74; H, 6.24; N, 6.19. Found: C, 56.01; H, 6.48; N, 5.99

¹H NMR [(CD₃)₂SO] (Varian 200 MHz): δ 1.371, 1.552 (βCH₂CH₂CH₂^δ, m,m, 6H), 2.290 (CH₃, s, 3H), 2.748 (CH₂^ε, m, 2H), 3.942 (CH^α, m, 1H), 5.042 (PhCH₂, s, 2H), 7.140 (CH₃C₆H₄, d <7.5>, 2H), 7.356 (C₆H₅, m, 5H), 7.530 (C₆H₄SO₃⁻, d <7.5>, 2H), 7.604 (CONH, bs, 1H), 7.704 (NH₃⁺, COOH, m, 4H).

¹³C NMR [(CD₃)₂SO] (Varian 200 MHz): δ 20.686 (CH₃), 22.420 (βCH₂CH₂^γ), 26.368 (βCH₂CH₂CH₂^δ), 30.090 (βCH₂CH₂CH₂CH₂^ε), 53.588 (CH^α), 65.344 (PhCH₂), 125.393, 127.644, 127.731, 128.117, 128.256 (CH_{arom}), 136.887, 137.980, 144.987 (C_{arom}), 156.117 (CO_{ureth}), 173.737 (COOH).

***N*^α-Benzyloxycarbonyl-L-lysine (Z-Lys).**- To a suspension of Z-Lys(•Tos) (22.63 g, 50 mmol) in a mixture of acetone-water (32 mL + 18 mL), was added triethylamine (8.4 mL, 60 mmol). After 1 h, acetone in portions was added (total 194 mL) and the whole left standing overnight. The next day, the precipitate was collected and washed with acetone (4 x 25 mL) to furnish 13.32 g (95%) of Z-Lys, mp. 228-230° (dec.), lit. mp. 228-229° (dec.),⁷ ca 235° (dec.),⁹ 226° (dec.),¹⁰ 226-230° (dec.).¹¹ TLC: R_f: B - 0.70, R_f: C - 0.50, HPLC: tR 7.69 min, 99.8% purity, [α]_D²⁰ = -13.4±0.05° (c = 2, 0.2 M HCl),

lit. $[\alpha]_D^{20} -12 \pm 1^\circ$ ($c = 2, 0.2 \text{ M HCl}$),^{9,11} -13° ($c = 2, 0.2 \text{ M HCl}$),¹⁰ $-13.7 \pm 0.5^\circ$ ($c = 2, 0.2 \text{ M HCl}$).¹²

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5. Abbreviations used: Boc = *t*-butoxycarbonyl, Z = benzyloxycarbonyl, Tos = *p*-toluenesulfonic acid, Lys = L-lysine.
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