A Large Scale Synthesis of Mono- and Di-urethane Derivatives of Lysine[1)]

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In the search for a practical route to lysine diurethane derivatives useful for peptide synthesis, we elaborated the synthesis of N^{ε}-*tert*-butoxycarbonyl-L-lysine copper(II) complex (1). This served as substrate for obtaining N^{ε}-*tert*-butoxycarbonyl-L-lysine (2), N^{α}-benzyloxycarbonyl-N^{ε}-*tert*-butoxycarbonyl-L-lysine dicyclohexylamine salt (3) and N^{α}-(9-fluorenyl)methoxycarbonyl-N^{ε}-*tert*-butoxycarbonyl-L-lysine (4).

Key words N^{ε}-*tert*-butoxycarbonyl-L-lysine; N^{α}-benzyloxycarbonyl-N^{ε}-*tert*-butoxycarbonyl-L-lysine; N^{α}-(9-fluorenyl)methoxycarbonyl-N^{ε}-*tert*-butoxycarbonyl-L-lysine; N^{ε}-*tert*-butoxycarbonyl-L-lysine copper(II) complex; large scale peptide synthesis; 8-quinolinol

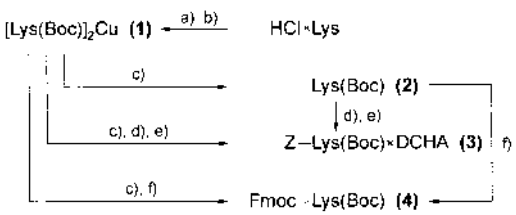
Orthogonally protected diurethane derivatives of lysine are valuable materials for peptide syntheses. An example is Z-Lys(Boc), which is exploited in the industrial production of certain well-established peptide drugs.[3,4)] Another derivative is Fmoc-Lys(Boc), which is in common use in the laboratory synthesis of peptides.[5)] The simplest route to these lysine derivatives seems to be using the copper complex for simultaneous protection of the α-amino and α-carboxyl function, N^{ε}-*tert*-butoxycarbonylation and then copper detachment. The obtained Lys(Boc) might be then subjected to N^{α}-benzyloxycarbonylation.

Earlier attempts in the above direction were plagued by low and often irreproducible yields.[4)] More recent approach[4)] does not give full satisfaction either. The formation of the copper complex of Lys requires several operations and, moreover, is associated with some limitations. The copper complex of Lys(Boc) is an amorphous precipitate that has to be centrifuged. This precipitate contains di(*tert*-butyl) pyrocarbonate, used in excess for N^{ε}-*tert*-butoxycarbonylation, and methanol must be applied for its decomposition, but the thorough washing out is rather difficult. This prevents isolation of the complex as a well-characterized entity. The copper was detached with a chelating ion exchange resin, Na^{+} Chelex 100, which is rather expensive. Furthermore, its conditioning and regeneration consume labor, time and money. The overall yield of Lys(Boc) amounted to 76%. Simple N^{α}-benzyloxycarbonylation of Lys(Boc) proceeds in only modest yield. Taken together, these difficulties forced authors[4)] to abandon this route *via* the copper complex.[4)]

Z-Lys(Boc) was eventually obtained *via* a temporary N^{ε}-benzylidene blockage.[4)] N^{ε}-benzylidene-lysine was synthesized, isolated and subjected to reaction with benzyl chlorocarbonate followed by hydrochloric acid, which removed the N^{ε}-benzylidene protection. The resulting N^{α}-Z-Lys was isolated and N^{ε}-*tert*-butoxycarbonylated with *tert*-butyl *p*-nitrophenyl carbonate. The product was then converted into its DCHA salt. The three step yield is only 61% and the process has severe problems resulting from the great instability of the benzylidene derivatives. Introduction of the benzylidene group must be done under nitrogen and argon. Reactions and all operations during the preparation of N^{ε}-benzylidene-lysine and N^{α}-Z-N^{ε}-benzylidene-Lys require a low temperature. Maintenance of temperature in the whole body of the reaction mass is troublesome, particularly during benzyloxycarbonylation, which is an exothermic process accompanied by local overheating.[4)] In our hands, the synthesis of N^{ε}-benzylidene-lysine failed.

At this juncture, to produce Z-Lys(Boc) we returned to the copper complex and have now elaborated an improved procedure for obtaining $[Lys(Boc)]_2Cu$ (**1**) compound. **1** then served as a very valuable substrate for the synthesis of Lys(Boc) (**2**), Z-Lys(Boc) (**3**) and Fmoc-Lys(Boc) (**4**) (Chart 1). Besides delineating of the preparation of complex **1**, we report herein the critical modifications of the described procedure for the production of compound **2** and present our own new procedures for the production of **3** and **4**.

We synthesized Lys_2Cu using the well-defined $CuSO_4 \cdot 5H_2O$ instead of using basic copper carbonate of approximate formula $CuCO_3 \cdot Cu(OH)_2 \cdot H_2O$, as originally applied.[4)] This route to the complex does not require heating and filtration as described in the literature.[4)] Boc_2O in slight excess was added as a solution in acetone of technical quality, which is much less expensive than the previously applied dioxane.[4)] The unreacted pyrocarbonate was decomposed by methanol introduced directly into the post-reaction medium before $[Lys(Boc)]_2Cu$ was filtered. After this, a mixture of ethyl acetate–water (1 : 1) was added to improve of the condition of the $[Lys(Boc)]_2Cu$ precipitate. This allows the complex to be easy filtrated and gives the product **1** in 94% yield, homogenous by TLC and of correct elemental analysis. We removed copper from **1** using 8-quinolinol easily, quantitatively and inexpensively. This furnishes Lys(Boc) (**2**) in 96% yield, homogenous by TLC and of correct elemental analysis. The overall yield of the two steps is somewhat over 90%, as opposed to the literature yield of only 76%.[4)]



a) $CuSO_4$, b) Boc_2O, c) 8-quinolinol, d) Z–Cl + *N*-hydroxysuccinimide
e) DCHA, f) Fmoc–Cl + *N*-hydroxysuccinimide

Chart 1. Synthesis of Mono- and Di-urethane Derivatives of Lysine

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We confirmed that N^{α}-benzyloxycarbonylation of **2** with benzyl chlorocarbonate proceeded as described in the literature[4] in moderate yield. The product of this process contained impurities. However, employment of benzyl *N*-succinimidyl carbonate[6] led to an excellent outcome: 96% yield and 99.9% purity of crude **3** by HPLC. Further, we recognized that the reaction can be carried out directly with $[Lys(Boc)]_2Cu$ in the presence of 8-quinolinol in alkaline aqueous-acetone solution. Benzyl *N*-succinimidyl carbonate may be used *in situ* as well, prepared in a separate vessel from benzyl chlorocarbonate and *N*-hydroxysuccinimide also in an alkaline aqueous-acetone solution. Eventually, we synthesized Z-Lys(Boc) in a one pot procedure starting with $[Lys(Boc)]_2Cu$, reacting it with 8-quinolinol first and next, adding benzyl *N*-succinimidyl carbonate, prepared *in situ*. Yield amounted to 91% of product as the DCHA salt of 99.5% purity by HPLC. Yield, based on Lys, was 82% as opposed to the literature value 61%.[4]

The elaborated one pot method was applied to the useful lysine diurethane derivative Fmoc-Lys(Boc) (**4**). Also with 9-fluorenylmethyl *N*-succinimidyl carbonate used *in situ*, yield amounted to 90% of product of 100% purity by HPLC. The overall yield, based on lysine, was 81%.

All described methods display a high degree of convenience and practicality.

Experimental

General Experimental Procedures Reactions were monitored and the homogeneity of products was checked, on silica gel plates (DC Alufolien Kieselgel, 0.25 Merck # 5553) using the following solvent systems (v/v): A, C_6H_6 : MeOH (4 : 1); B, $CHCl_3$: dioxane : MeOH : NH_3 concd. (12 : 5 : 7 : 1); C, $CHCl_3$: MeOH : AcOH (95 : 5 : 3); D, *n*-BuOH : AcOH : H_2O (4 : 1 : 1); E, *n*-BuOH : AcOH : AcOEt : H_2O (1 : 1 : 1 : 1); F, C_6H_6 : MeOH : acetone : pyridine : AcOH (24 : 4 : 2 : 2 : 1). Melting points were determined on a Boëtius heating block and are uncorrected. HPLC analyses were carried out using a Beckman System Gold chromatograph, a 5 μl loop, an Alltech Alltima, C_{18}, 5 μm, 150×4.6 mm column, detection at 210 nm and a flow rate of 1 ml/min.

Copper(II) Complex of N^{ε}-*tert*-Butoxycarbonyl-L-lysine ($[Lys(Boc)]_2$-Cu) (1) To a stirred solution of HCl·Lys (365 g, 2 mol) in 2 M aqueous $NaHCO_3$ (2.0 l), a solution $CuSO_4 \cdot 5H_2O$ (250 g, 1 mol) in water (2.0 l) was added. Thereafter $NaHCO_3$ (168 g, 2 mol) was added followed by a solution of 96% Boc_2O (590 g, 2.6 mol) in acetone of technical quality (2.4 l). After 24 h, methanol (0.5 l) was introduced and stirring continued for 12 h. Water (2.0 l) and ethyl acetate (2.0 l) were added and the precipitate was filtered off. The precipitate was suspended in water (5.0 l) and filtered. These operations were repeated twice more. The resulting fine, light blue solid was air-dried to yield **1** (522 g, 94.2%). *Rf* (D) 0.38, *Rf* (E) 0.73. *Anal*. Calcd for $C_{22}H_{42}CuN_4O_8$: C, 47.68; H, 7.64; N, 10.11. Found: C, 47.57; H, 7.82; N, 9,99.

N^{α}-*tert*-Butoxycarbonyl-L-lysine (Lys(Boc)) (2) To an intensively stirred suspension of $[Lys(Boc)_2]Cu$ (277 g, 0.5 mol) in water (10 l), 8-quinolinol (189 g, 1.3 mol) was added. After 5 h, copper(II) 8-quinolinolate was filtered off and washed with water (0.5 l). The precipitate was suspended in water (5.0 l) and left standing for several hours. It was filtered off and washed with water (2×0.5 l). All combined filtrates and washings were extracted with ethyl acetate (2×3.0 l) (discarded). The aqueous phase was evaporated *in vacuo* on a rotary evaporator at a bath temperature not exceeding 45 °C to give **2** (237 g, 96%). *Rf* (D) 0.38, *Rf* (E) 0.73. *Anal*. Calcd for $C_{11}H_{22}N_2O_4$: C, 53.64; H, 9.00; N, 11.37. Found: C, 53.38; H, 9.24; N, 11.19.

N^{α}-Benzyloxycarbonyl-N^{ε}-*tert*-butoxycarbonyl-L-lysine Dicyclohexylamine Salt (Z-Lys(Boc)·DCHA) (3) To an intensively stirred suspension of $[Lys(Boc)]_2Cu$ (55.4 g, 0.10 mol) in acetone (0.2 l) and 10% aqueous Na_2CO_3 solution (0.4 l), 8-quinolinol (29.9 g, 0.206 mol) was added. The mixture, resulting after 1 h, is called reaction mixture 1 and was used subsequently. To a solution of *N*-hydroxysuccinimide (25.3 g, 0.220 mol) in water (0.125 l) placed in a separate vessel, Na_2CO_3 (11.7 g, 0.110 mol) was introduced, followed by acetone (0.1 l). Benzyl chlorocarbonate of 96% purity (30 ml, 0.200 mol) was added dropwise and the whole was left standing for 0.5 h with occasional stirring to give reaction mixture 2. This was poured into stirred reaction mixture 1. After 1 h, the precipitate was filtered off and washed with water (4×0.1 l). The filtrate was acidified with 1 M HCl under stirring to pH 2 and extracted with ethyl acetate (0.8 l). The organic phase was washed with water, dried and treated with DCHA (40 ml, 0.200 mol). After several hours, the crystalline solid was filtered off and washed with a mixture of ethyl acetate : hexane (1 : 1) to furnish **3** (102.6 g, 91%), mp 152—154 °C (lit.[4] mp 154—155 °C); *Rf* (A) 0.26; *Rf* (B) 0.47; *Rf* (C) 0.46; *Rf* (F) 0.61; t_R (0.1% trifluoroacetic acid : acetonitrile (50 : 50), v/v) 5.60 min, 99.5% purity.

N^{α}-(9-Fluorenyl)methoxycarbonyl-N^{ε}-*tert*-butoxycarbonyl-L-lysine (Fmoc-Lys(Boc)) (4) Reaction mixture 1 was obtained exactly as in the case of **3**. To a solution of *N*-hydroxysuccinimide (24.3 g, 0.211 mol) in water (0.120 l) placed in a separate vessel, Na_2CO_3 (11.2 g, 0.106 mol) was introduced, followed by acetone (0.1 l). 9-Fluorenylmethyl chlorocarbonate (49.6 g, 0.192 mol) was added and the whole was left standing for 0.5 h with occasional stirring to give reaction mixture 2. This, diluted with acetone (0.34 l), was poured into stirred reaction mixture 1. After 1 h, the precipitate was filtered off and washed with water (4×0.1 l). The filtrate was acidified with 1 M HCl under stirring to pH 2. The resulting precipitate was filtered off, washed with water, dried and crystallized from ethyl acetate/*n*-hexane to give **4** (80.5 g, 89.5%), mp 129—130 °C (lit.[7] mp 125—129 °C); *Rf* (F) 0.70, t_R (0.1% trifluoroacetic acid : acetonitrile (70 : 30), v/v) 3.37 min, 100% purity.

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References and Notes

1) The methods for preparing the described compounds are the object of Polish Patent Applications P 330 243, P 330 246 and P 330 245 (1998).
2) Abbreviations: Lys=lysine, Boc=*tert*-butoxycarbonyl, Z=benzyloxycarbonyl, Fmoc=9-fluorenylmethoxycarbonyl, DCHA=dicyclohexylamine.
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