Improved Scalable Syntheses of Mono- and Bis-Urethane Derivatives of Ornithine[1)]

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Received March 5, 2001; accepted April 22, 2001

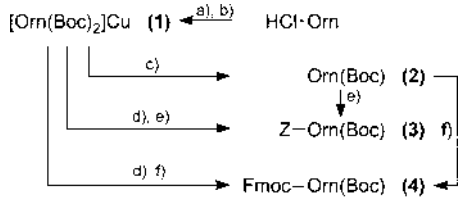
In the search for a practical route to ornithine bisurethane derivatives useful for peptide synthesis, we elaborated the simple and efficient (86% yield) synthesis of N^{ε}-*tert*-butoxycarbonyl-L-ornithine copper(II) complex(1). This served as substrate for obtaining N^{ε}-*tert*-butoxycarbonyl-L-ornithine (2), N^{α}-benzyloxycarbonyl-N^{ε}-*tert*-butoxycarbonyl-L-ornithine (3) and N^{α}-(9-fluorenyl)methoxycarbonyl-N^{ε}-*tert*-butoxycarbonyl-L-ornithine (4). These were synthesized in 94—95% yields and with a purity above 99%.

Key words N^{δ}-Boc-L-ornithine; N^{α}-Z-N^{δ}-Boc-L-ornithine; N^{α}Fmoc-N^{δ}-Boc-L-ornithine; N^{δ}-Boc-L-ornithine copper(II) complex; peptide synthesis; 8-quinolinol

Ornithine[2)] is a non-coded amino acid which is frequently applied as a modifier in bioactive peptides, including drugs obtained industrially, to improve their biological properties (*e.g.*[3—8)]), and as the precursor of a coded amino acid, arginine to circumvent problems posed with its guanidino function through peptide syntheses.[9)] Ornithine is also commonly used in place of the natural folate glutamic acid residue in research programs aimed at new effective antifolate drugs (*e.g.* ref. 10—14). Recent advances in the area of drug delivery have caused a resurgence of interest in the large scale production of peptides as pharmaceuticals.[8)] Therefore, economically viable methods are desirable for the manufacturing of amino acid derivatives, among others of ornithine derivatives, useful to this end. The simplest route to orthogonally masked derivatives of ornithine is the simultaneous blockage of the α-amino and α-carboxyl function by copper complex, then δ-amino group acylation, copper detachment and the introduction of further protection.

We report the simple, scalable syntheses of four ornithine compounds **1**—**4** (Chart 1, Fig. 1), of which N^{δ}-*tert*-butoxycarbonyl-L-ornithine copper(II) complex (**1**) serves for obtaining N^{δ}-*tert*-butoxycarbonyl-L-ornithine (**2**) and for direct one-pot, almost quantitative preparation of N^{α}-benzyloxycarbonyl-N^{δ}-*tert*-butoxycarbonyl-L-ornithine (**3**) and N^{α}-(9-fluorenyl)methoxycarbonyl-N^{δ}-*tert*-butoxycarbonyl-L-ornithine (**4**). The described methods are based on those recently elaborated in our laboratories for the parallel derivatives of the congeneric amino acid, lysine,[15)] but, owing to quite significant differences in the solubility properties between the corresponding lysine and ornithine compounds, some critical modifications were either possible or needed, which are delineated herein.

The majority of procedures for preparing Orn_2Cu[3,4,16,17)] apply a basic copper carbonate of approximate formula $CuCO_3 \cdot Cu(OH)_2 \cdot H_2O$ as a copper source and $NaHCO_3$ as an alkaline factor. This route to the complex requires heating and filtration and leads after N^{δ}-*tert*-butoxycarbonylation to $[Orn(Boc)]_2Cu$ (**1**) in 66% yield to the maximum.[17)] Another copper reagent is $CuSO_4 \cdot 5H_2O$.[13,15,18)] For the preparation of **1**, isolated in 79% yield,[18)] as well as of $[Lys(Boc)]_2Cu$, isolated in 94% yield,[15)] we previously just employed $CuSO_4$ and either NaOH[18)] or $NaHCO_3$,[15)] respectively, for the complex formation followed by Boc_2O and $NaHCO_3$ for the N^{δ}-*tert*-butoxycarbonylation. An attempt to produce $[Orn(Boc)]_2Cu$ according to ref. 15 also gave 80% yield only. The cause of this is that the water solubility of this compound is better than that of the lysine one. Therefore, in the present work on the synthesis of the ornithine copper complex $(Orn)_2Cu$, we used $Cu(CH_3COO)_2$ and NaOH,[11)] which first allowed us to conduct the reaction in less water. Secondly, the resulting CH_3COONa confers the appropriate pH on the reaction medium. This permitted us to avoid the extra alkali always applied[3,4,15—18)] for the catalysis of the subsequent N^{δ}-*tert*-butoxycarbonylation process. Thirdly and most importantly, the *tert*-butoxycarbonylation could be carried out in an organic-aqueous medium, acetone–water (4 : 3) instead of an aqueous-organic one, water–acetone (2 : 1) characteristic of the manufacturing of $[Lys(Boc)]_2Cu$.[15)] This offered a significant facilitation in operability, in the form of a filtration-friendly $[Orn(Boc)]_2Cu$ precipitate. This way, the decomposition of unreacted Boc_2O with methanol, needed for the high purity of $[Lys(Boc)]_2Cu$,[15)] could be eliminated here, because only stirring the $[Orn(Boc)]_2Cu$ complex with acetone and washing the resulting filtered-off precipitate with a small amount of this solvent was sufficient. As the net result of all these modifications, the protocol for the $[Orn(Boc)]_2Cu$ synthesis was simplified as much as possible and furnished **1** in 86% yield (lit.[4,17,18)] 53%, 66% and 79% yield, respectively). The product was homogenous by TLC and had correct elemental analysis.



a) NaOH, $Cu(CH_3COO)_2$. b) Boc_2O. c) 8-quinolinol.
d) Na_2CO_3, 8-quinolinol. e) Z–Cl + N-hydroxysuccinimide.
f) Fmoc–Cl + N-hydroxysuccinimide

Chart 1. Synthesis of Mono- and Bis-Urethane Derivatives of Ornithine

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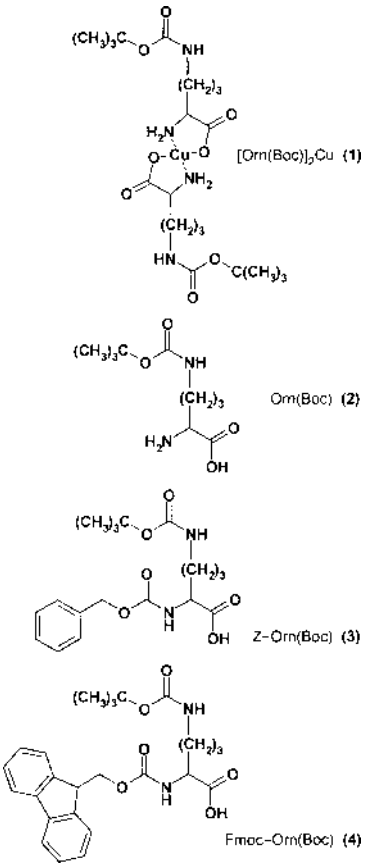

Fig. 1. Structures of the Compounds Synthesized in this Work

We removed copper from **1** in an aqueous suspension with 8-quinolinol, originally proposed and previously well verified by us as a new complexone in peptide chemistry.[15] Advantageously, the present product, Orn(Boc) **2** is much more water-soluble than Lys(Boc) and does not precipitate at copper quinolinate as the lysine derivative did.[15] Therefore, we were able (i) to use less water as the suspension medium than needed in Lys(Boc) production, and (ii) to avoid the several-hour maceration of copper quinolinate in water required for a high Lys(Boc) yield. The first (i) is feasible provided that prior to due copper exchange, complex **1** is pre-wetted with small amounts of acetone and then of water. The second (ii) is dispensable, because ordinary washing of the quinolinate with water was sufficient to secure Orn(Boc) in 94% yield (81% based on Orn), homogenous by TLC and of correct elemental analysis. With a classical copper sequestrator, the yields amount to only 65% (34% based on Orn[4]) and 69% (46% based on Orn[17]). The product is useful for preparing Z-Orn(Boc) (**3**) and Fmoc-Orn(Boc) (**4**) applied, respectively, in the α-Z-ω-*t*-Bu[19] and α-Fmoc-ω-*t*-Bu[20] peptide synthesis strategy, for preparing other bisurethane derivatives including those of a new generation, *e.g.* Bsmoc-Orn(Boc),[21] as well as for preparing antifolate N^{δ}-masked ornithine analogs.[13,14]

The bisurethane ornithine derivatives, Z-Orn(Boc) **3** and Fmoc-Orn(Boc) **4** can be directly obtained from copper complex $[Orn(Boc)]_2Cu$ **1** in the presence of 8-quinolinol, omitting the above separate copper removal step. To gain products of high quality, as was the case for the related Lys derivative,[15] benzyl or 9-fluorenylmethyl *N*-succinimidyl carbonate is needed to introduce the δ-urethane group. As previously, these reagents can be applied *in situ* prepared in a separate vessel. Details of the work-up procedures for the production of **3** and **4**, however, had to be modified. The sodium salts of **3** and **4** being well water-soluble, at the same time, strongly opposed to the congeneric Lys compounds, are also well (**3**) or even very well (**4**) soluble in ethyl acetate, commonly used for extractive procedures in amino acid derivative and peptide chemistry. The sodium salts of **3** and **4** proved, however, to be insoluble in dichloromethane and toluene, respectively, which allowed for extracting from their aqueous solutions minute quantities of organic post-reaction impurities, collected through all steps of these one-pot procedures. Eventually, this leaves very pure final products, **3** and **4**, above 99% as determined by HPLC, and in high yields, 94% and 95%, respectively.

All described methods display a high degree of convenience and practicality and lend themselves to be scaled up.

Experimental

General Experimental Procedures HCl·Orn came from Fluka (#75 470). 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, *n*-BuOH : AcOH : AcOEt : H_2O (1 : 1 : 1 : 1); B, $CHCl_3$: MeOH : AcOH (95 : 5 : 3). Spots were visualised with ninhydrin and chlorine-KI-tolidine reagent. Organic solutions were dried over anhydrous Na_2SO_4. The solvents from reaction mixtures were removed *in vacuo* on a rotatory evaporator at bath temperatures not exceeding 45 °C. Melting points were determined by means of differential scanning calorimetry (DSC) in a calorimeter DSC-2010 (Thermal Analysis Instruments) under nitrogen in a closed copper vessel with a heating rate of 10 °C/min. 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, 0.1% trifluoroacetic acid : acetonitrile (50 : 50; v/v) as a mobile phase with a flow rate of 1 ml/min and detection at 210 nm. Specific rotations were measured on a Jasco DIP-1000 polarimeter.

Copper(II) Complex of N^{δ}-*tert*-Butoxycarbonyl-L-ornithine ($[Orn(Boc)]_2Cu$) (1) To a stirred solution of HCl·Orn (16.862 g, 100 mmol) in 2 M NaOH (100 ml), a solution $Cu(CH_3COO)_2 \cdot H_2O$ (9.982 g, 50 mmol) in water (50 ml) was introduced followed by a solution of 96% Boc_2O (28.73 g, 130 mmol) in acetone of technical quality (200 ml). After 24 h, the additional portion of acetone (100 ml) was introduced and stirring continued for 20 h. The precipitate was filtered off and washed with a mixture of acetone : water (2 : 1) (200 ml) and water (2×500 ml). The resulting fine, light blue solid was air-dried to yield **1** (22.57 g, 86%), mp 245.89 °C (lit.[17] mp 244 °C); *Rf* (A) 0.68. *Anal.* Calcd for $C_{20}H_{38}CuN_4O_8$: C, 45.66; H, 7.28; N, 10.65. Found: C, 45.47; H, 7.49; N, 10.43.

N^{δ}-*tert*-Butoxycarbonyl-L-ornithine (Orn(Boc)) (2) A suspension of $[Orn(Boc)_2]Cu$ (13.152 g, 25 mmol) in acetone (50 ml) was intensively stirred for 15 min, water (50 ml) added and stirring continued for 10 min. Then, water (300 ml) and 8-quinolinol (9.45 g, 65 mmol) were introduced and stirring was continued for a further 4 h. The precipitate of copper(II) quinolinolate was filtered off and washed with water (2×25 ml). The filtrate and washings were combined and acetone was evaporated. The residual aqueous solution was extracted with ethyl acetate (3×100 ml) (discarded) and evaporated to give **2** (10.90 g, 94%), mp 209.75 °C and then dec. 235.30 °C (lit.[3] mp 180 °C, lit.[10] mp 220—222 °C); $[\alpha]_D^{20} = 15.2°$ (*c* 1.0, AcOH) (lit.[4] $[\alpha]_D^{20} = 13.4 \pm 0.2°$ (*c* 1.0, AcOH)); *Rf* (A) 0.68. *Anal.* Calcd for $C_{10}H_{20}N_2O_4$: C, 51.71; H, 8.68; N, 12.06. Found: C, 51.64; H, 8.81; N, 12.13.

N^{α}-Benzyloxycarbonyl-N^{δ}-*tert*-butoxycarbonyl-L-ornithine (Z-Orn(Boc)) (3) A suspension of $[Orn(Boc)]_2Cu$ (10.52 g, 20 mmol) in acetone (40 ml) was intensively stirred for 15 min, water (40 ml) added and stirring continued for 10 min. Then, 10% aqueous Na_2CO_3 solution (80 ml) and 8-quinolinol (5.99 g, 41.2 mmol) were introduced. The mixture resulting after 1 h is called reaction mixture 1 and was subsequently used. To a solution of *N*-hydroxysuccinimide (5.06 g, 44 mmol) in water (24 ml) placed in a separate vessel, Na_2CO_3 (2.32 g, 22 mmol) was added, followed by acetone (40 ml) and the mixture was cooled to −5 °C. Benzyl chlorocarbonate of 96% purity (6.2 ml, 40 mmol) was introduced in portions to maintain this

temperature. The whole was left standing at −5 °C for a half hour with occasional stirring to give reaction mixture 2. This was poured into stirred reaction mixture 1. After 1 h, the precipitate of copper quinolinate was filtered off and washed with water. The filtrate and washings were combined and acetone was evaporated. The residual aqueous solution was extracted with dichloromethane (3×50 ml) (discarded), acidified under stirring with 1 M HCl to pH 2 and extracted with ethyl acetate (3×70 ml). The acetate phase was washed with 0.25 M HCl (50 ml) and brine, dried, evaporated and crystallized from ethyl acetate/hexane to furnish **3** (13.86 g, 95%), *Rf* (B) 0.60; t_R 5.12 min, 99.1% purity.

For analytical purposes, the product (1.00 g) was crystallized from ethyl acetate (3 ml)/hexane (5 ml) to give **3** (0.92 g), mp 106.27 °C (lit.[3] mp 101 °C, lit.[18] mp 99.5—101 °C, lit.[22] mp 97—99 °C); $[\alpha]_D^{20} = -2.27 \pm 0.06°$ (*c* 1.0, methanol) (lit.[3] $[\alpha]_D^{20} = -3.2 \pm 0.6°$ (*c* 2.45, methanol)); 100% purity by HPLC. *Anal.* Calcd for $C_{18}H_{26}N_2O_4$: C, 59.00; H, 7.15; N, 7.65. Found: C, 58.95; H, 7.21; N, 7.53.

***N*$^{\alpha}$-(9-Fluorenyl)methoxycarbonyl-*N*$^{\delta}$-*tert*-butoxycarbonyl-L-ornithine (Fmoc-Orn(Boc)) (4)** Reaction mixture 1 was obtained in exactly the same way as for **3**. To a solution of *N*-hydroxysuccinimide (4.86 g, 42.24 mmol) in water (24 ml) placed in a separate vessel, Na_2CO_3 (2.24 g, 21.2 mmol) was introduced, followed by 9-fluorenylmethyl chlorocarbonate (9.92 g, 38.4 mmol) in acetone (40 ml). The whole was left standing for a half hour with occasional stirring to give reaction mixture 2. This was poured into stirred reaction mixture 1. After 1 h, the precipitate of copper quinolinate was filtered off and washed with water (100 ml). The filtrate and washings were combined and acetone was evaporated. The residual aqueous solution was extracted with toluene (2×40 ml and 1×15 ml) (discarded) and then with ethyl acetate (3×70 ml). The acetate layer was extracted with 0.5 M HCl (100 ml), 0.25 M HCl (2×60 ml) and brine, dried and thoroughly evaporated. Ethyl acetate (80 ml) was added to give a product like lyogel. The solvent was evaporated to leave needles. These were re-dissolved in ethyl acetate (80 ml) under reflux. The solution was cooled to 20 °C and hexane (60 ml) added. The whole was left standing in a refrigerator overnight. The resulting crystals were filtered off, washed with a mixture of ethyl acetate : hexane (1 : 3) to furnish **4** (16.72 g, 94%), mp 112.51 °C (lit.[23] mp 113—116 °C); $[\alpha]_D^{20} = -7.95 \pm 0.07°$ (*c* 1.0, DMF) (lit.[23] $[\alpha]_D^{20} = -6.8°$ (*c* 1.0, DMF)); *Rf* (B) 0.57; t_R 14.37 min, 100% purity. *Anal.* Calcd for $C_{25}H_{30}N_2O_6 \cdot 0.5H_2O$: C, 64.78; H, 6.74; N, 6.04. Found: C, 64.49; H, 6.96; N, 5.94.

Acknowledgement The authors acknowledge financial support for this work by a grant-in-aid from the Polish State Committee for Scientific Research (KBN).

References and Notes

1) The methods for preparing the described compounds are the object of Polish Patent Application P 344 809, P 344 810 (2000) and P 345 912 (2001).
2) Abbreviations: Orn=L-ornithine, Lys=L-lysine, Boc=*tert*-butoxycarbonyl, Z=benzyloxycarbonyl, Fmoc=9-fluorenylmethoxycarbonyl, Bsmoc=1,1-dioxobenzo[*b*]thiophene-2-ylmethyloxycarbonyl.
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