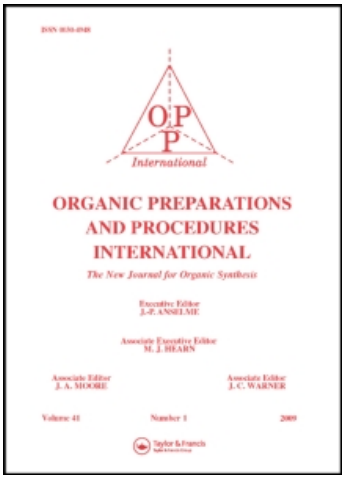

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SCALABLE SYNTHESES OF N^{α}-BENZYLOXYCARBONYL-*l*-ORNITHINE AND OF N^{α}-(9-FLUORENYLMETHOXY)CARBONYL-*l*-ORNITHINEE

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SCALABLE SYNTHESES OF N^{α}-BENZYLOXYCARBONYL-*L*-ORNITHINE AND OF N^{α}-(9-FLUORENYLMETHOXY)CARBONYL-*L*-ORNITHINE

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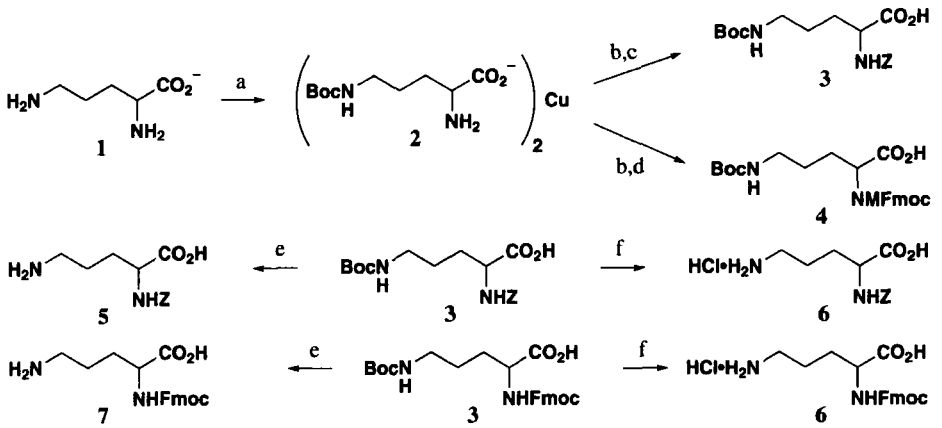
The last few years have seen a large increase in the importance of ornithine (Orn,[1] **1**), a non-coded amino acid. It is used as a modifier of biologically active peptides, including drugs produced industrially,[2] and is frequently utilized for the preparation of some amino acids and their derivatives. In the latter context, it should be mentioned first and foremost, that N^{α}-Z- or N^{α}-Fmoc-ornithine (Z-Orn, **5** and Fmoc-Orn, **7**) serve as precursors of N^{ω},$N^{\omega'}$-diprotected and N^{ω}-modified-$N^{\omega'}$-protected arginines that are building blocks for Boc/Fmoc/Z peptide chemistry.[3-7] Z-Orn is also a siderophore substrate, into which the N^{δ}-hydroxy function is introduced.[8-10] Moreover, it is commonly employed in place of the natural folate glutamic acid residue in research programs aimed at new effective anti-cancer antifolate drugs.[11-15] Fmoc-Orn has found application for anchoring a monomethoxypoly(ethyleneglycol) chain which, in turn, allows the synthesis of modified peptides with markedly improved biological properties.[16,17] Therefore simple methods for the production of Z-Orn and Fmoc-Orn seem desirable and we now report scalable syntheses of these two compounds in the form of their inner salts **5**, **7** and their hydrochlorides **6**, **8**.

The most common path to the N^{α}-protected derivatives of diamino acids proceeds through a ready available N^{ω}-protected derivative thereof.[18] Z-Orn hitherto is synthesized *via* N^{δ}-benzylidene blocking. N^{δ}-benzylidene-ornithine is isolated and subjected to the action of benzyl chlorocarbonate for the Z-group introduction. Treatment with hydrochloric acid removes N^{δ}-protection.[9,19,20] This synthesis is based on the procedure for the preparation of Z-lysine,[21] but no experimental detail has been reported. The process has severe inconveniences, among which are the definite alkalinity of reaction media and the low temperatures required during the preparation of both N^{δ}-benzylidene-ornithine itself and N^{α}-benzyloxycarbonyl-N^{δ}-benzylidene-ornithine. It has also an inherent defect, *viz.* the introduction of the Z-group is not regioselective and a mixture of Z-Orn and Orn(Z) with a large content of the latter is formed.[22] Fmoc-Orn is known only in commercial catalogues as its hydrochloride[23] and is not described in the literature.

We decided to prepare Z-Orn (**5**) and Fmoc-Orn (**7**) *via* N^{δ}-*t*-butoxycarbonyl blocking; the introduction of the *t*-butoxycarbonyl group and manipulation of the resulting derivative present no problem in contrast to the introduction of N^{δ}-benzylidene protection and the manipulation of the N^{δ}-benzylidene derivative. The syntheses are based on our earlier work[24] on the preparation of Z-Orn(Boc) and Fmoc-Orn(Boc) and on the patent application on the removal of the Boc-group (*Scheme 1*).[25] This four-step chemistry has been developed into a convenient, cascade-like, high yielding process without purification of any intermediate.[26]



a) $Cu(CH_3COO)_2 \cdot H_2O$, Boc_2O b) 8-quinolinol c) (benzyl chlorocarbonate + *N*-hydroxysuccinimide); d) (9-fluorenylmethyl chlorocarbonate + *N*-hydroxysuccinimide); e) *p*-toluenesulfonic acid, NEt_3 f) 5M HCl

Scheme 1

Orn(Boc) was isolated as the copper complex (**2**), and the copper was removed with 8-quinolinol, a new copper sequestrator;[24,27] Orn(Boc) was then converted to both bisurethane derivatives (**3** and **4**) by reaction with benzyl or 9-fluorenylmethyl *N*-succinimidyl carbonate (prepared *in situ* in a separate vessel[28]). For N^{δ}-deprotection, we attempted a variety of inorganic and organic acids, solvents and conditions. In general, an excess of an acid and elevated temperatures had been found to be necessary. For the production of **5** and **7**, none of the acids was as efficient as 1.5–2.0 equivalents of *p*-toluenesulfonic acid. This is mainly due to the fact that after the Boc cleavage, the subsequent removal of this acid from a post-reaction medium poses no problem. With triethylamine, the acid gives triethylamine tosylate, which is soluble in the medium and from which Z-Orn (**5**) or Fmoc-Orn (**7**) being inner salts precipitated, thus making isolation of **5** and **7** simple. Unfortunately however, both **5** and **7** are amorphous solids. For the synthesis of final crystalline compounds, we tested hydrochloric acid and obtained crystalline hydrochlorides **6** and **8**. Essential details for the Boc cleavage to furnish pure compound **5-8** in high yields are given below.

Z-Orn(Boc) (**3**) is quite soluble in acetone and its Boc group can be removed in this solvent with either two equivalents of *p*-toluenesulfonic acid or five equivalents of 5M HCl at reflux for 1 hr. The crude Z-Orn (**5**) of 98.8% purity by HPLC was then converted into its tosylate salt and neutralized with triethylamine to give a readily filtered precipitate. The overall yield of Z-Orn (**5**) and Z-Orn(•HCl) (**6**), based on Orn•HCl, amounts to 73% and 75%, respectively, and the compounds have 99.8% purity. The N^{δ}-deprotection of Fmoc-Orn(Boc) (**4**) has to be performed in acetic acid as solvent. To this end, either 1.5 equivalent of *p*-toluenesulfonic acid or five equivalents of 5M HCl and heating at 70° for 0.5 hr were used. A larger excess of *p*-toluenesulfonic acid is difficult to be removed. The crude Fmoc-Orn(•HCl) is 99.5% pure, but it still binds solvents and requires thorough re-precipitation to become crystalline. The overall yield of Fmoc-Orn (**7**) and Fmoc-Orn(•HCl) (**8**) of better than 99.5% purity, based on Orn•HCl, amounts to 72% and 78% respectively.

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

EXPERIMENTAL SECTION

Orn•HCl 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 solvent systems (v/v): *n*-butanol-acetic acid-ethyl acetate-water (1:1:1:1). Spots were visualized 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 rotary evaporator at bath temperatures not exceeding 30°. Melting points were determined by 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°/min. HPLC analyses were carried out using a Beckman System Gold chromatograph, a 5 µL loop, an Alltech Alltima, C_{18}, 5 µm, 150 x 4.6 mm column, 0.1% trifluoroacetic acid-acetonitrile (70:30; v/v) as a mobile phase with a flow rate of 1 mL/min and detection at 210 nm. Specific rotations were measured at a Jasco DIP-1000 polarimeter.

N^{α}-Benzyloxycarbonyl-*L*-ornithine (5).- To a stirred solution of Orn•HCl (16.862 g, 100 mmol) in 2M NaOH (100 mL), a solution of $Cu(CH_3COO)_2 \cdot H_2O$ (9.982 g, 50 mmol) in water (50 mL) was added followed by a solution of 96% Boc_2O (28.73 g, 130 mmol) in acetone of technical quality (200 mL). After 24 h, an additional portion of acetone (100 mL) was added and stirring continued for 20 h. The precipitate was collected and washed with a mixture of acetone-water (2:1) (200 mL) and water (2 x 500 mL). The wet complex was suspended in acetone (90 mL) and stirred vigorously. After 15 min, 10% Na_2CO_3 (180 mL) and 8-quinolinol (13.06 g, 90 mmol) were added and stirring was continued for 1.5 h. The resulting mixture (*reaction mixture 1*) was used subsequently.

To a solution of *N*-hydroxysuccinimide (10.43 g, 90 mmol) in water (45 mL) in a separate flask, cooled at –10°, was added Na_2CO_3 (4.77 g, 45 mmol), followed by a solution of benzyl chlorocarbonate of 96% purity (14 mL, 90 mmol) in acetone (45 mL). The suspension (*reaction mixture 2*) was left standing at –10° for 0.5 h with occasional stirring; it then was poured into *reaction mixture 1* with stirring at room temperature. After 1.5 h, the precipitate of copper quinolinate was filtered off

and washed with water (3 x 45 mL). The filtrate and washings were combined and the acetone was evaporated. The residual aqueous solution was extracted with dichloromethane (3 x 70 mL) (discarded), acidified under stirring with 2M HCl to pH 3 and extracted with ethyl acetate (3 x 100 mL). The acetate phase was washed with 0.5M HCl (2 x 50 mL) and water, dried and evaporated. The oily residue was dissolved in acetone (50 mL) and the solvent evaporated. The dissolution and evaporation processes were repeated twice to give crude Z-Orn(Boc) (**3**) as a white solid. This solid was dissolved in acetone (270 mL), *p*-toluenesulfonic acid hydrate (34.22 g, 180 mmol) was added and the solution was refluxed for 1 h. After cooling to room temperature, triethylamine (25 mL, 180 mmol) was introduced and the whole was left standing overnight. The gelatinous precipitate was collected and soon turned into a powder (20.36 g, 76% yield); the powder was dissolved in a mixture of acetone (48 mL) and a solution of *p*-toluenesulfonic acid hydrate (29.0 g, 150 mmol) in water (24 mL). Then with stirring, triethylamine (21.25 mL, 150 mmol) was added portionwise. After 1 h, acetone (400 mL) was added and the whole left standing overnight. A resulting precipitate was collected, washed with acetone to afford the title compound (**5**) as a white powder (19.55 g, 73% yield based on Orn•HCl), mp. 204.83°, *lit.* mp. 209-210°,[19] 196-198°,[20] 206-208°.[9] TLC: R_f: 0.63; $[\alpha]_D^{20} = -15.94°$ ±0.03 (c = 1, HCl 0.5M), [*lit.*[19] $[\alpha]_D^{25} = -8.4°$ (c = 1, HCl 5M); *lit.*[9] $[\alpha]_D^{15} = -7°$ (c = 2,HCL 5M)]; $[\alpha]_D^{20} = -3.0°$ ±0.02 (c = 1, H_2O); HPLC: t_R 2.60 min, 99.8% purity.

Anal. Calcd for $C_{13}H_{18}N_2O_4$: C, 58.63, H, 6.81, N, 10.52. Found: C, 58.40, H, 6.76, N, 10.50

N^{α}-Benzyloxycarbonyl-*L*-ornithine Hydrochloride (6).- To a solution of the crude Z-Orn(Boc) (**3**) obtained exactly as described for **5** in acetone (270 mL) was added 5M hydrochloric acid (84 mL) and the whole refluxed for 1 h. The solvents were evaporated to dryness and acetone (100 mL) was added to the solid residue and evaporated. The addition and evaporation of acetone were repeated twice. Then, acetone (200 mL) was added and the colorless crystalline solid was collected (22.76 g, 75% yield based on Orn•HCl); mp. 195.31°, *lit.* mp. 187-189°.[29] TLC: R_f: 0.63; $[\alpha]_D^{20} = -15.18°$ ±0.02 (c = 1, H_2O) [*lit.*[29] $[\alpha]_D^{25} = -11.5°$ (c = 1, H_2O)]; HPLC: t_R 2.60 min, 99.8% purity.

Anal. Calcd for $C_{13}H_{18}N_2O_4$•HCl: C,51.57, H, 6.33, N, 9.25. Found: C, 51.40, H, 6.48, N, 9.27

N^{α}-(9-Fluorenylmethoxy)carbonyl-*L*-ornithine (7).- To a solution of *N*-hydroxysuccinimide (12.43 g, 108 mmol) in water (50 mL) in a separate vessel, was added Na_2CO_3 (5.724 g, 54 mmol) followed by a solution of (9-fluorenylmethyl) chlorocarbonate (23.284 g, 90 mmol) in acetone (45 mL). The whole was left standing for 1.5 h with occasional stirring to furnish *reaction mixture 3*, which was poured into stirred *reaction mixture 1* prepared earlier (see compound **5**). After 1.5 h, the precipitate of copper quinolinate was collected and washed with water (100 mL). The filtrate and washings were combined and acetone was evaporated. The residue was dissolved in water (200 mL) and extracted with toluene (4 x 140 mL) (discarded). The aqueous solution was then extracted with ethyl acetate (2 x 200 mL and 100 mL) and the acetate extract was washed with 0.5M HCl (200 mL), 0.25M HCl (100 mL) and finally with water, dried and evaporated to dryness. The solid residue was dissolved in acetone (100 mL) and evaporated to leave crude Fmoc-Orn(Boc) (**4**). Crude **4** was dissolved in acetic acid (340 mL) and *p*-toluenesulfonic acid hydrate (24.80 g, 130 mmol) added. The solution was

heated in a water bath of 70° for 0.5 h. Acetic acid was evaporated and the oily residue was dissolved with heating in a mixture of water (216 mL) and acetone (72 mL), followed by addition of triethylamine (20 mL, 144 mmol) in portions; then after 15 min, acetone was added (114 mL). After 2 h in the refrigerator, the precipitated solid was collected and washed with a mixture of acetone (100 mL) and water (100 mL), water (200 mL) and acetone (200 mL) to afford the title compound (**7**) as a white solid (25.64 g, 72% yield based on Orn•HCl); mp. 165.95°. TLC: R_f: 0.66; $[\alpha]_D^{20} = -3.34° \pm 0.05$ (c = 1, AcOH); HPLC: t_R 12.14 min, 99.6% purity.

Anal. Calcd for $C_{20}H_{22}N_2O_4$: C, 67.76, H, 6.26, N, 7.91: Found: C, 67.50, H, 6.11, N, 7.78

N^{α}-(9-Fluorenylmethoxy)carbonyl-*L*-ornithine Hydrochloride (8).- To crude Fmoc-Orn(Boc) (**4**), obtained as previously described for compound **7**, in acetic acid (340 mL) was added 5M hydrochloric acid (84 mL) and the solution heated at a bath of 70° for 0.5 h. Evaporation of acetic acid left a solid residue which was dissolved in acetone (100 mL) with heating and evaporated. The dissolution and evaporation were repeated twice. The residue was dissolved in methanol (30 mL), ethyl acetate was added (200 mL) and after 10 min another portion of ethyl acetate was added (200 mL). After storage in a refrigerator overnight, the precipitate was collected, dissolved in methanol (32 mL) and precipitated by adding ethyl acetate (200 mL) to give the title compound (**8**) as a white, crystalline compound (31.0 g, 78% yield based on Orn•HCl); mp. 97.86°. TLC: R_f: 0.66; $[\alpha]_D^{20} = -2.43° \pm 0.07$ (c = 1, MeOH); HPLC: t_R 12.14 min, 99.8% purity.

Anal. Calcd for $C_{20}H_{22}N_2O_4$•HCl•0.5H_2O: C, 60.06, H, 6.05, N, 7.01. Found: C, 59.96, H, 6.29, N, 6.86

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1. Abbreviations used: Boc = *t*-butoxycarbonyl, Fmoc = (9-fluorenylmethoxy)carbonyl, Orn = ornithine, Z = benzyloxycarbonyl.

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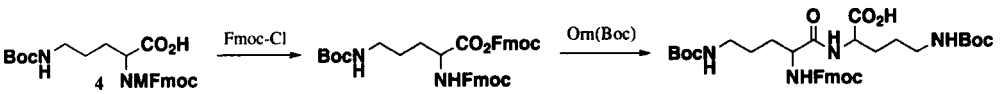
28. While the reaction of benzyl chlorocarbonate with *N*-hydroxysuccinimide (HOSu) in equimolar quantities was complete after half an hour, that of 9-fluorenylmethyl chlorocarbonate (Fmoc-Cl) with HOSu requires 20% excess of HOSu and one hour and half to be completed. If there

remains unreacted Fmoc–Cl, it reacts with the main product Fmoc-Orn(Boc) (**4**) to give a reactive mixed anhydride, which in turn reacts with still unreacted Orn(Boc) to furnish dipeptide Fmoc-Orn(Boc)-Orn(Boc). This, after the Boc removal process, yields dipeptide Fmoc-Orn-Orn that cannot be removed from the final derivative, Fmoc-Orn (**7**) which remains as 2% contaminant.



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A NOVEL AND EFFICIENT BIOMIMETIC HYDROLYSIS OF OXIRANES TO 1,2-DIOLS CATALYSED BY β-CYCLODEXTRIN IN WATER UNDER NEUTRAL CONDITIONS†

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The 1,2-diol functionality has great significance in pharmaceutical and industrial chemistry[1] due to its utility in the synthesis of bacteriostatic[2] and antifogging agents,[3] β-blockers,[4] perfumes[5] and in the polymer industry.[6] However, the facile synthesis of these commercially important 1,2-diols consists of the ring opening of the easily accessible epoxides under acidic conditions at room temperature or at high temperatures under basic conditions.[7] Since these harsh experimental conditions involving either acid or base are not suitable for the epoxides bearing labile substituents, there is need to develop milder methods. Cyclodextrins (CDs) which are cyclic oligosaccharides, exert microenvironmental effect leading to selective reactions. They catalyze reactions by supramolecular catalysis through non-covalent bonding as in enzymes. These biomimetic reactions can be carried out efficiently in water.[8] This biomimetic approach of chemical reactions involving supramolecular catalysis has several advantages over chemical methodologies since these reactions can be carried out under mild and neutral conditions in water. Hence, in our effort to develop biomimetic approaches of chemical reactions involving cyclodextrins,[9] the synthesis of 1,2-diols from epoxides (derived from cyclic and terminal alkenes) catalyzed by cyclodextrins (α and β) in water under neutral conditions was developed.