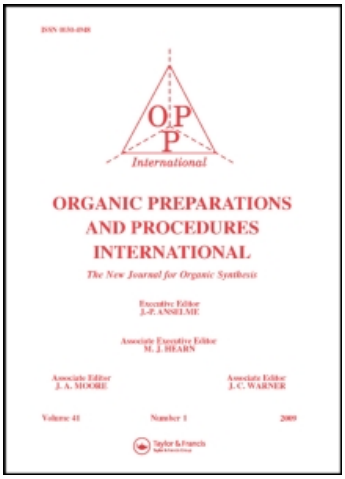

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DIRECT SYNTHESIS OF N^{α}-BENZYLOXYCARBONYL-N^{ε}-t-BUTYLOXY-CARBONYL-L-LYSINE FROM L-LYSINE

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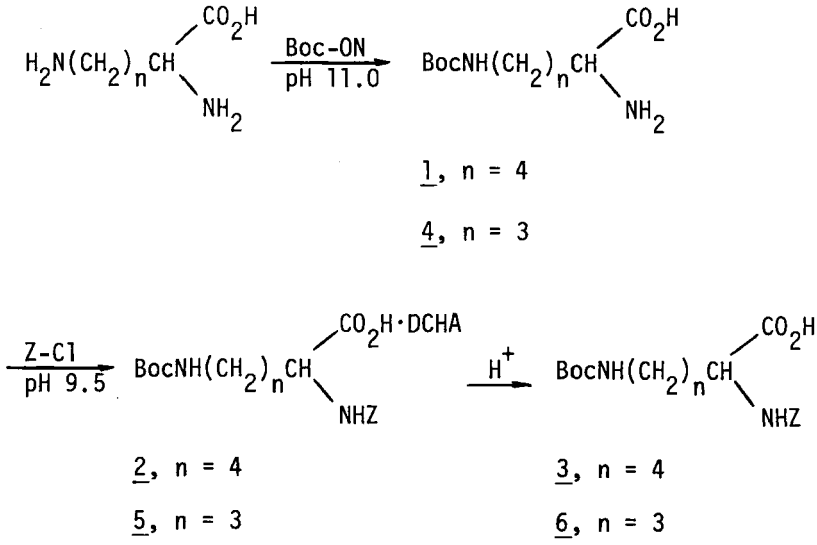
N^{ε}-t-Butyloxycarbonyl-L-Lysine [H-Lys(Boc)-OH] and N^{α}-benzyloxycarbonyl-N^{ε}-t-butyloxycarbonyl-L-lysine [Z-Lys(Boc)-OH] are important intermediates which are useful for the solution synthesis of peptides. Several syntheses of Z-Lys(Boc)-OH have been reported previously usually requiring the use of a partially protected intermediate, N^{ε}-benzylidene-L-lysine.[1,2] The synthesis of H-Lys(Boc)-OH has been reported through the use of the copper complex of L-lysine[3] with subsequent conversion to Z-Lys(Boc)-OH with benzyloxycarbonyl chloride.[4] More recently the copper complex of L-lysine was converted to H-Lys(Boc)-OH using di-t-butyldicarbonate[5] in better yield.[6] Although the copper chelate method has been effective for the preparation of Z-Lys(Boc)-OH or other N^{ε}-acylated L-lysine intermediates[7] it suffers a major drawback in that the intermediate copper complexes must be decomposed with H_2S or through the use of ion-exchange resins.[3,5]

Selective t-butyloxycarbonylation of the ε-amino group of L-lysine has not been previously reported [e.g. reaction of L-lysine with t-

butylphenyl carbonate[8] was reported to give $N^{\alpha,\varepsilon}$-di-tert-butyloxycarbonyl-L-lysine, Boc-Lys(Boc)-OH[9]]. We have observed that direct reaction of L-lysine at 25° for 24 hrs with 2-(t-butyloxycarbonyloxyimino)-2-phenylacetonitrile(Boc-ON)[10] at pH 11.0 (autotitrator) resulted in the selective synthesis of N^{ε}-t-butyloxycarbonyl-L-lysine (Scheme 1). Subsequent N^{α}-carbobenzoxylation (pH 9.5) of the H-Lys(Boc)-OH gave Z-Lys(Boc)-OH in 68% overall yield (as the DCHA salt).



Scheme 1

The new two-step procedure for the preparation of Z-Lys(Boc)-OH is more convenient than those previously reported and compares favorably to published yields. We have also converted L-ornithine to δ-t-butyloxycarbonyl-L-ornithine [H-Orn(Boc)-OH] and subsequently prepared Z-Orn(Boc)-OH by analogous procedures in 77% overall yield (Scheme 1). These observations led us to the conclusion that this procedure is generally applicable for the ω-t-butyloxycarbonylation of diamino acids.

EXPERIMENTAL SECTION

Materials and Methods

L-lysine·HCl was purchased from Ajinomoto, Inc. and L-ornithine·HCl was obtained from Chemical Dynamics Corp. 2-(t-Butyloxycarbonyloxyimino)-2-phenylacetonitrile [Boc-ON] was purchased from Aldrich Chemical Co. and was recrystallized from MeOH (mp. 84-85.5°). Benzyloxycarbonyl chloride [ZCl] and dicyclohexylamine (sequalog grade) were obtained from Chemical Dynamics Corp. All other solvents were of reagent grade purity and used without further purification.

TLC determinations (100 μg load) were carried out on silica gel G plates (Analtech, Inc.) and developed with chlorine-TDM (4,4'-tetramethyldiaminodiphenylmethane). Melting points were determined on a Hoover Uni-Melt apparatus and are corrected. Infrared and NMR spectra were measured and found to be compatible for all products and intermediates synthesized. Optical rotations were measured in a jacketed 1-dm cell on a Perkin Elmer Model 141 Polarimeter. An autotitrator Model TTT2 equipped with an autoburette Model ABU12 (Radiometer, Copenhagen) was used to maintain the pH for the reactions with Boc-ON and ZCl.

N^{α}-Benzyloxycarbonyl-N^{ϵ}-t-Butyloxycarbonyl-L-lysine [Z-Lys(Boc)-OH] (3).

A. N^{ϵ}-t-Butyloxycarbonyl-L-lysine [H-Lys(Boc)-OH] (1).- A solution of L-lysine·HCl (9.14 g, 0.05 mol) in H_2O (75 mL) and dioxane (75 mL) was adjusted to pH 11.0 [autotitrator using 2N NaOH] and stirred (mechanically) at 25°. A solution of Boc-ON (13.6 g, 0.055 mol, 1.1 equiv.) in dioxane (75 mL) was added portionwise while the pH was maintained at 11.0. Stirring continued at 25° for 24h and the reaction mixture was extracted with ether (5 x 250 mL). The aqueous layer was acidified to pH 2.0 (2M HCl), extracted with EtOAc (3 x 250 mL) and readjusted to pH 5.15 (2N NaOH). A portion of this material was evaporated to dryness and desalted on a G-10 column using 30% MeOH-H_2O. Evaporation of appropriate fractions gave N^{ϵ}-t-butyloxycarbonyl-L-lysine, mp. 248-251° (dec.), lit.[11] mp. 237-255°; $[\alpha]_D^{25}$= +6.67° (c 1, 2N NH_4OH), lit.[3,11] $[\alpha]_D^{25}$= +4.7° (c 1, 2N NH_4OH); $[\alpha]_D^{25}$= +14.85° (c 1, 0.1M HCl), lit.[11] $[\alpha]_D^{25}$= +14.4° (c 1, 0.1M HCl);

R_f 0.65 (BuOH-AcOH-EtOAc-H_2O; 1:1:1:1); R_f 0.64 (PrOH-H_2O; 7:3); R_f 0.74 (EtOH-H_2O; 7:3).

Anal. Calcd. for $C_{11}H_{22}N_2O_4 \cdot 1/2\ H_2O$ (255.3): C, 51.82; H, 9.08; N, 10.99. Found: C, 52.10, H, 8.97; N, 11.67.

B. N^{α}-Benzyloxycarbonyl-N^{ε}-t-butyloxycarbonyl-L-lysine dicyclohexylammonium salt [Z-Lys(Boc)-OH·DCHA] (2).- The aqueous pH 5.15 solution of 1 was adjusted to pH 9.5 (2N NaOH), cooled to 0°, stirred (mechanically) and reacted with benzyloxycarbonyl chloride (7.2 mL, 0.0522 mol, 1.16 equiv.). The pH was maintained at 9.5 (autotitrator using 2N NaOH). Stirring continued at 25° for 40h and the reaction mixture was extracted with ether (3 x 250 mL). The aqueous layer was acidified to pH 2.0 (2M HCl) and extracted with EtOAc (6 x 250 mL). The organic layers were combined, dried ($MgSO_4$), filtered and evaporated to an oil (17.2 g) and taken up in EtOAC (88 mL). Dicyclohexylamine (10.8 mL, 0.055 mol, 1.1 equiv.) was added and crystallization proceeded at 0° (24h). The product was filtered, washed with EtOAc and dried in vacuo to give 19.18 g overall yield (68.3%) of white crystalline product in 2 crops. The analytical sample was obtained by recrystallization from MeOH-H_2O, mp. 147-150°, lit.[2] mp. 150-153°; $[\alpha]_D^{25}$= +7.47° (c 1, EtOH), lit.[12] $[\alpha]_D^{25}$= +7.82° (c 1, EtOH).

Anal. Calcd. for $C_{31}H_{51}N_3O_6$ (561.8): C, 66.28; H, 9.15; N, 7.48. Found: C, 66.05; H, 9.11; N, 7.47.

C. N^{α}-Benzyloxycarbonyl-N^{ε}-t-butyloxycarbonyl-L-lysine [Z-Lys(Boc)-OH] (3). A 1.0 g (0.00178 mol) aliquot of Z-Lys(Boc)-OH·DCHA (2) was stirred with a mixture of 0.05M H_2SO_4 (50 mL) and EtOAc (50 mL) at 0° for 30 min. The layers were separated and the aqueous layer was extracted with EtOAc

(4 x 30 mL). The organic layers were combined, dried ($MgSO_4$), filtered, evaporated and dried in vacuo to give an oil, 677 mg (100%). $[\alpha]_D^{25}$= -4.80° (c, 1, MeOH), lit.[13] $[\alpha]_D^{25}$= -5.87° (c 1, MeOH); $[\alpha]_D^{25}$= -3.48° (c 1, AcOH), lit.[4] $[\alpha]_D^{25}$= -3.0° (c 1, AcOH); R_f 0.82 (BuOH-AcOH-EtOAc-H_2O; 1:1:1:1); R_f 0.73 (PrOH-H_2O; 7.3); R_f 0.83 (EtOH-H_2O; 7:3).

Anal. Calcd. for $C_{19}H_{28}N_2O_6$ (380.45): C, 59.99; H, 7.42; N, 7.36. Found: C, 59.52; H, 7.35; N, 7.20.

N^{α}-Benzyloxycarbonyl-N^{δ}-t-Butyloxycarbonyl-L-Ornithine [Z-Orn(Boc)-OH] (6)

A. N^{δ}-t-Butyloxycarbonyl-L-ornithine [H-Orn(Boc)-OH] (4).- A solution of L-ornithine·HCl (8.44 g, 0.05 mol) was reacted with Boc-ON as described above for the synthesis of H-Lys(Boc)-OH. The reaction was followed by tlc which confirmed that after 23h ornithine was consumed and one major product was formed corresponding to N^{δ}-t-butyloxycarbonyl-L-ornithine, 4; R_f 0.64 (PrOH-H_2O; 7:3); R_f 0.76 (EtOH-H_2O; 7:3); R_f 0.53 (CH_3CN-0.1M NH_4Ac; 7:3). These tlc systems were capable of separating the isomeric impurity, N^{α}-t-butyloxycarbonyl-L-ornithine, [R_f 0.57 (PrOH-H_2O; 7:3); R_f 0.72 (EtOH-H_2O; 7:3); R_f 0.40 (CH_3CN-0.1M NH_4Ac; 7:3)] which was found to be present in only trace amounts.

B. N^{α}-Benzyloxycarbonyl-N^{δ}-t-butyloxycarbonyl-L-ornithine dicyclohexylammonium salt [Z-Orn(Boc)-OH·DCHA] (5).- Carbobenzoxylation of 4 was carried out for 69h as described above for the synthesis of Z-Lys(Boc)-OH and converted to the DCHA salt by the same procedure. The product was filtered, washed with petroleum ether and dried in vacuo to give 21.1 g (77.0% overall yield) of white crystalline product in 3 crops. The analytical sample was obtained by recrystallization from MeOH-H_2O, mp. 121-126°,

lit.[14,15] mp. 128-129°; $[\alpha]_D^{25}$= +4.36° (c 1, MeOH), lit.[14] $[\alpha]_D^{25}$= +5.1° (c 1, MeOH); $[\alpha]_D^{25}$= +7.24° (c 1, EtOH), lit.[14] $[\alpha]_D^{25}$= +7.9° (c 1, EtOH). Anal. Calcd. for $C_{30}H_{49}N_3O_6$ (547.7): C, 65.79; H, 9.02; N, 7.67. Found: C, 66.07; H, 8.91; N, 7.49.

C. N^{α}-Benzyloxycarbonyl-N^{δ}-t-butyloxycarbonyl-L-ornithine [Z-Orn(Boc)-OH] (6).- A 1.0 g (0.00183 mol) aliquot of the DCHA salt (5) was stirred with a mixture of 0.1M HCl (50 mL) and EtOAc (50 mL) at 0° for 30 min. The layers were separated and the aqueous layer extracted with EtOAc (4 x 30 mL). The combined organic layers were dried ($MgSO_4$), filtered, evaporated and dried in vacuo to give an oil, 669 mg (100%). $[\alpha]_D^{25}$= -8.51° (c 1, pyridine), lit.[15] $[\alpha]_D^{25}$= -8.8° (c 1, pyridine); R_f 0.85 (BuOH-AcOH-EtOAc-H_2O; 1:1:1:1); R_f 0.72 (PrOH-H_2O; 7:3); R_f 0.90 (EtOH-H_2O; 7:3); R_f 0.69 (CH_3CN-0.1M NH_4Ac; 7:3).

Anal. Calcd. for $C_{18}H_{26}N_2O_6$ (366.4): C, 59.00; H, 7.15; N, 7.64. Found: C, 58.87; H, 7.05; N, 7.42.

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† Abbreviations follow the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature [Biochem. J., 126, 773-780 (1972)]. Additional abbreviations: AcOH, acetic acid; BuOH, n-butanol; PrOH, n-propanol; DMF, dimethylformamide; EtOAc, ethyl acetate; DCHA, dicyclohexylamine.

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