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A PROCEDURE FOR THE LARGE SCALE PREPARATION OF N^{ε}-ALLOC-LYSINE AND N^{ε}-ALLOC-N^{α}-FMOC-LYSINE

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Abstract: This report describes a convenient procedure for a 100 g scale preparation of Fmoc-Lys(Alloc) in 85% yield. This method can be easily adapted to a near one-pot procedure with slightly reduced yields, without requiring the isolation of the intermediate Lys(Alloc).

The allyloxycarbonyl (Alloc) group was first introduced for the protection of the ε-amino group of lysine by Stevens and Watanabe[1]. Its application to solid phase peptide synthesis has been of interest recently because of its compatibility with both the Fmoc and tBoc methodologies. It is especially useful for the orthogonal protection of peptides that require selective deprotection of the lysine side chain, such as in the synthesis of lactam cyclized peptides that are to be cyclized between the ε-amino group of lysine and the ω-carboxyl groups of either glutamic or aspartic acid, while the peptide is still anchored to the solid support[2].

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In this scheme the ω-carboxylic acid of glutamic or aspartic acid can be protected as the allyl ester[3], and the allyl and Alloc groups can be selectively deprotected using a palladium catalyst[4], leaving all other protecting groups intact.

Selective side chain protection of lysine can be performed after initial complexation of the α-amino and carboxylic acid groups with copper[5]. After ε-amino protection, the copper can be removed using H_2S gas[6] or EDTA[7]. The use of excess H_2S gas is inconvenient, and procedures using EDTA rely on the differential solubilities of the derivatized lysine and the EDTA-copper complex. However, we have found that Lys(Alloc) is not easily separated from the EDTA-copper complex, and Fmoc protection in the presence of EDTA gives poor yields. We have optimized the procedure of Taylor *et al.*[8], using thioacetamide, for the removal the copper by precipitating it as CuS, and we have integrated this step into a convenient, near "one-pot" procedure for the large scale (100 g) preparation of Fmoc-Lys(Alloc) in 72% yield. The yield is improved to 85% by the isolation of the hydrochloride salt of the intermediate Lys(Alloc). Both methods are described below.

Table 1. Physical characteristics of N^{ε}-allyloxycarbonyl derivatives of L-lysine

		% yield	$[\alpha]_D^{20}$ (lit.)[ref.]	Mp, °C (lit.)[ref.]	R_f
1	L-Lys(Alloc)	98	+ 9.2 (+ 9.0)[10]	228-231, dec. (230)[10]	0.60[c]
2	Fmoc-L-Lys(Alloc)	85[a], 72[b]	- 11.8	84-86 (80-85)[11]	0.30[d]

[a] Method (a)
[b] Method (b)
[c] 1: 1: 1: 1 H_2O: EtOAc: n-BuOH: MeOH
[d] 60: 40: 1 EtOAc: hexane: acetic acid

Experimental Section

Lysine hydrochloride was purchased from Schweizerhall (New Jersey), allylchloroformate was purchased from Aldrich Chemical; all other chemicals were obtained from either Aldrich Chemical or BDH (Canada).

Typical Procedure:

N^{ε}-Allyloxycarbonyl-L-lysine hydrochloride (1): A solution of lysine hydrochloride (50.0 g, 0.28 mol) and basic copper (II) carbonate (63.5 g, 0.29 mol) in H_2O (500 ml) was refluxed for 30 min[1]. Solids formed during reflux were removed by hot filtration. The filtrate was cooled to 0°C and adjusted to pH 9 by the addition of solid $Na_2CO_3.H_2O$ (about 5 g was required). Allylchloroformate (42 ml, 0.41 mol) was added dropwise over one hour, while the solution stirred at 0°C. During this addition, the reaction mixture was maintained at pH 9 by the addition of solid $Na_2CO_3.H_2O$ (a total of 70 g was added). The reaction mixture was allowed to warm to room temperature, and after 12 h, there was no starting material left by TLC (1:1:1:1 H_2O: EtOAc: n-BuOH: MeOH). The blue solid product formed during the reaction (99.1 g, 0.38 mol) was collected by filtration in quantitative yield. The solid copper salt of Lys(Alloc) collected above (0.28 mol) was suspended in H_2O (1 L) and two equivalents of thioacetamide (42.1 g, 0.56 mol) were added[8]. The alkaline suspension was stirred at 50°C for 3 h, during which time the solid slowly dissolved. The solution was then acidified to pH 2 with 2 M HCl, and was further boiled for 5 min. The precipitated CuS was removed by filtration. The filtrate was concentrated under vacuum to about 300 ml, at which point the product hydrochloride salt of Lys(Alloc) precipitated as a white solid, and was recovered by filtration in quantitative (74.7 g, 0.28 mol) yield.

Mp, °C (lit.[10]) 228-231, dec. (230, dec.); $[\alpha]_D^{20}$ (lit.[10]) +9.2 (+9.0) (c=1.0, 8% aq. K_2CO_3); R_f 0.60 (1:1:1:1 H_2O: EtOAc: n-BuOH: MeOH); ^{1}H-NMR (D_2O, 250 MHz): δ 1.21-1.48 (m, 4H, γ,δ-C$\underline{H}_2$), 1.78 (m, 2H, β-C$\underline{H}_2$), 3.02 (t, 2H, *J*=6.4, ε-C$\underline{H}_2$), 3.74 (t, 1H, *J*=6.2, α-C$\underline{H}$), 4.42 (d, 2H, *J*=4.6, C$\underline{H}_2$-CH=), 5.11 (d, 1H, J_{cis}=17.8, CH=C$\underline{H}_2$), 5.17 (d, 1H, J_{trans}=11.2, CH=C$\underline{H}_2$), 5.82 (m, 1H, C$\underline{H}$=CH$_2$).

N^{ε}-allyloxycarbonyl-N^{α}-9-fluorenylmethoxycarbonyl-L-lysine (2) Method (a): The product Lys(Alloc) ' ' ined above (0.28 mol) was dissolved in 10% Na_2CO_3 (500 ml) and dioxane (1ᵕᵕ ml), and 1.1 equivalents of Fmoc-succinimide (104 g, 0.31 mol), dissolved in dioxane (200 ml), were added dropwise to the amino acid solution over one hour, while stirring at 0°C [9]. The reaction mixture was allowed to warm to roo nperature, while stirring overnight (18 h). The solids formed were removed l ltration and the filtrate was poured into cold H_2O (1 L), and extracted with l)Ac (3 x 500 ml). The aqueous phase was cooled to 0°C and acidified to pH 2 with conc HCl. The cold, acidified aqueous phase was extracted with EtOAc (3 x 500 ml). The EtOAc extracts were pooled, washed with brine, dried over $MgSO_4$, and evaporated to an oil. The final product Fmoc-Lys(Alloc) (108 g, 0.24 mol) was crystallized from CH_2Cl_2/pet. ether (30°-60°) for an overall yield of 85% from lysine hydrochloride.

Mp, °C (lit.[11]) 84-86 (80-85); $[\alpha]_D^{20}$ -11.8 (c=1.0, DMF); R_f 0.30 (60:40:1 EtOAc: hexane: acetic acid) ; ^{1}H-NMR ($CDCl_3$, 250 MHz): δ 1.25-1.51 (m, 4H, γ,δ-C$\underline{H}_2$), 1.83 (m, 2H, β-C$\underline{H}_2$), 3.19 (m, 2H, ε-C$\underline{H}_2$), 4.21 (t, 1H, *J*=6.3, Ph_2-C$\underline{H}$), 4.40 (d, 2H, *J*=6.6, C$\underline{H}_2$CH=), 4.56 (dd, 2H, *J*=4.6, 14.0, Ph_2-CH-C$\underline{H}_2$), 4.86 (m, 1H, α-C$\underline{H}_2$), 5.18 (d, 1H, J_{cis}=10.5, CH=C$\underline{H}_2$), 5.27 (d, 1H, J_{trans}=16.8, CH=C$\underline{H}_2$), 5.61 (d, 1H, *J*=7.5, α-NH), 5.88 (m, 1H, C$\underline{H}$=CH_2).

N^{α}-Allyloxycarbonyl-N^{ε}-9-fluorenylmethoxycarbonyl-L-lysine (2) Method (b): *Alternate Method Not Requiring Isolation of Intermediate Products*

The copper salt of Lys(Alloc) was prepared as above but this intermediate product, which precipitated during and after the addition of allylchloroformate, was not isolated. The resulting alkaline suspension containing the solid Lys(Alloc) was stirred at 50°C for 1h, while the solid gradually dissolved. Two equivalents of thioacetamide (42.1 g, 0.56 mol) were added, and the suspension was stirred for an additional 3 h at 50°C. The pH was lowered to pH 2 with 2 M HCl, and the mixture was boiled for 5 min. The precipitated CuS was removed by filtration. The filtrate was cooled to 0°C, adjusted to pH 9 by the addition of

solid $Na_2CO_3.H_2O$ (about 50 g was added), and dioxane (100 ml) was added. Fmoc-succinimide (104 g, 0.31 mol), dissolved in dioxane (200 ml), was added dropwise to the amino acid solution over one hour, while stirring at 0°C. The reaction mixture was stirred overnight (18 h), while warming to room temperature. The product was isolated as in the work-up described in Method (a), for an overall 72% yield from lysine hydrochloride.

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