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Modified Benzyloxycarbonyl Groups for Protection of ε-Amino Group of Lysine

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The benzyloxycarbonyl[1] group has been found suitable for protection of the ε-amino group of lysine in peptide synthesis by either the solution or solid phase method. Since the use of ε-Z group is compatible with that of BOC group in the solid phase method, there have been many reports regarding the use of α-BOC-ε-Z-L-lysine.[2] However, there have been suggestions that ε-Z group was partially cleaved by either N hydrogen chloride-acetic acid or 4N hydrogen chloride-dioxane used for the removal of the α-BOC group during the synthesis of oligopeptide by the solid phase method.[3] Deprotection of ε-Z group was avoided by the use of the α-N protecting group which is much more readily removed than BOC group; such sensitive α-groups so far reported are 2-(*p*-biphenyl)isopropoxycarbonyl[4] and 2-benzoyl-1-methylvinyl.[5]

Another approach to avoid partial deprotection from ε-amino group is the use of a protecting group more stable than Z. The present paper will describe the syntheses of some ε-Z-L-lysine derivatives in which phenyl is substituted with negative group and their stabilities toward treatment with hydrogen chloride as compared with ε-Z-lysine. Behavior of these protecting groups toward liquid hydrogen fluoride will also be described.

It has been observed that several ε-Z groups sub-

1) The following abbreviations are used; Z, benzyloxycarbonyl; Z(*p*-Cl), Z(*m*-Cl), Z(*p*-CN), Z(*p*-NO_2), *e. g. p*-chlorobenzyloxycarbonyl for Z(*p*-Cl); BOC, *t*-butyloxycarbonyl.

2) For example, B. Gutte and R. B. Merrifield, *J. Amer. Chem. Soc.*, **91**, 501 (1969).

3) A. Yaron and F. Schlossman, *Biochemistry*, **7**, 2673 (1968); D. A. Ontjes and C. B. Anfinsen, The 1st American Peptide Symposium, Yale Univ., New Haven, Conn., Aug., 1968.

4) P. Sieber and B. Iselin, *Helv. Chim. Acta*, **51**, 622 (1968).

5) G. L. Southard, G. S. Brooke and J. M. Pettee, *Tetrahedron Lett.*, **1969**, 3505.

Table 1. ε-*N*-Modified benzyloxycarbonyl-L-lysine

ε-*N*-Protected-L-lysine	Yield %	Mp, °C (decomp.)	$[\alpha]_D^{25}$ (*c* 2, 5N HCl)	Formula	Calcd, % C	H	N	Found, % C	H	N
Z(*p*-Cl)-Lys	81	243—245	+8.0°[a]	$C_{14}H_{19}O_4N_2Cl$	53.41	6.08	8.90	53.55	5.91	9.03
Z(*m*-Cl)-Lys	83	245—248	+10.0°	$C_{14}H_{19}O_4N_2Cl$	53.41	6.08	8.90	53.15	6.15	8.84
Z(*p*-CN)-Lys	89	240—242	+12.7°	$C_{15}H_{19}O_4N_3$	59.00	6.27	13.76	58.64	6.39	13.68
Z(*p*-NO_2)-Lys	86	233—235	+12.9°	$C_{14}H_{19}O_6N_3$	51.68	5.89	12.92	51.43	6.02	12.66

a) The solvent used was AcOH with *c* 1. Z(*p*-Cl)-Lys was hardly soluble even in hydrochloric acid.

stituted with *p*-chloro,[6] *p*-cyano,[6] and *p*-nitro,[7] are more stable toward hydrogen bromide than ε-Z group. We observed a similar tendecny regarding the stabilities toward 4N hydrochloric acid-dioxane (Table 3). The results indicate that the modified ε-Z groups can be satisfactorily used in the solid phase method if the groups are readily removed by hydrogen fluoride from a finished peptide polymer. The behavior of these groups toward hydrogen fluoride was therefore investigated. Sakakibara and Shimonishi have reported that ε-Z group is easily removed with hydrogen fluoride.[8] Sakakibara observed recently that ε-Z(*p*-Cl) group was also removed by the treatment of hydrogen fluoride.[9] We found that ε-Z groups having *p*-cyano and *p*-nitro group were highly resistant against deprotection, whereas ε-Z with *p*-chloro and *m*-chloro were removed completely within 2 to 3 hr. The ε-Z(*m*-Cl) group seemed to be slightly more resistant than ε-Z(*p*-Cl) (Table 4). Syntheses of several oligopeptides containing lysine residue by the solid phase method using α-BOC-ε-Z(*p*- or *m*-Cl)-L-lysine as a starting material are in progress in this laboratory.

Experimental

Modified Benzyloxycarbonyl Chlorides. Z(*p*-Cl)-chloride[10] and Z(*p*-NO_2)-chloride[7] were prepared as described in literature. Other chlorides were prepared as follows.

To a chilled solution of *p*-cyanobenzyl alcohol (2.66 g, 0.02 mol) or *m*-chlorobenzyl alcohol (2.85 g, 0.02 mol) in dry ether (10 ml) was added a solution of phosgene (10 g, 0.1 mol) in dry dioxane (10 ml) at −10°C. The reaction mixture was left to stand for 2 hr at −10°C and overnight at 0°C, and then evaporated *in vacuo*. The oily product, 3.92 g for Z(*p*-CN)-chloride or 4.01 g for Z(*m*-Cl)-chloride, thus obtained was used without further purification in the following reaction.

ε-*N*-Modified Benzyloxycarbonyl-L-lysines. As an example, preparation of Z(*p*-CN)-Lys is described. Others were prepared by a procedure essentially the same. To a hot solution of L-lysine hydrochloride (0.91 g, 0.005 mol) in water (15 ml) was added cupric carbonate (1.25 g) portionwise. The mixture was refluxed for 20 min, and the insoluble material was filtered off. To the filtrate was added sodium bicarbonate (1.2 g) at 0°C. To the solution was added Z(*p*-CN)-chloride (1.6 g, about 0.008 mol) in 3 portions over a period of 30 min under vigorous stirring. Stirring was continued for 5 hr. The alkoxycarbonylated copper complex which deposited was collected, washed successively with water, acetone and ether. After the complex was dissolved in dilute hydrochloric acid, the solution was treated with hydrogen sulfide and cupric sulfide was filtered off. The pH of the filtrate was adjusted to 6 with 6N ammonium hy-

Table 2. R_f of ε-*N*-protected-lysine on TLC[a]

ε-*N*-Protected-L-lysine	R_f[b] Solvent I	Solvent II
Z-Lys	0.79	0.58
Z(*p*-Cl)-Lys	0.81	0.63
Z(*m*-Cl)-Lys	0.81	0.63
Z(*p*-CN)-Lys	0.79	0.51
Z(*p*-NO_2)-Lys	0.79	0.53
Lysine·HCl	0.25	0.03

a) Thin layer plates (20×20 cm; Merck Silica Gel G, No. 7731; thickness of Gel, approximately 0.2 mm) prepared in the usual way were used throughout these experiments. Each spot was detected by spraying 0.2% ninhydrin in acetone.
b) Solvent I, *n*-butanol-acetic acid-pyridine-water (4 : 1 : 1 : 2, v/v); solvent II, *n*-butanol-acetic acid-water (4 : 1 : 2, v/v).

Table 3. Stability of ε-*N*-modified benzyloxycarbonyl-L-lysine in 4N HCl-dioxane at 25°C[a]

ε-*N*-Protected-L-lysine	Appearance of Lys after different time intervals (hr) 0.5	2	12	24
Z-Lys	±	+	++	++
Z(*p*-Cl)-Lys	−	±	+	+
Z(*m*-Cl)-Lys	−	−	±	+
Z(*p*-CN)-Lys	−	−	±	±
Z(*p*-NO_2)-Lys	−	−	−	±

a) Explanation of signs: −, not cleaved; ±, cleaved about 1%; +, about 2—4%; ++, about 5—10%.

6) J. Meienhofer, "Peptides-Proceeding of the 6th European Symposium," Pergamon Press, London (1965), p. 55.
7) F. H. Carpenter and D. T. Gish, *J. Amer. Chem. Soc.*, **74**, 3818 (1952).
8) S. Sakakibara and Y. Shimonishi, This Bulletin, **38**, 1412 (1965).
9) S. Sakakibara, private communication.
10) O. L. Kisfaludy and S. Pualszky, *Acta Chim. Acad. Sci. Hung.*, **24**, 301 (1960).

Table 4. Removal of modified benzyloxycarbonyl group from ε-*N*-protected-lysine with HF and 36% HBr-acetic acid[a)]

ε-*N*-Protected-L-lysine	HF at 0°C	36% HBr-acetic acid at 25°C
	Reaction time	
Z-Lys	○ (less than 10 min)	○ (less than 0.5 hr)
Z(*p*-Cl)-Lys	○ (about 2 hr)	○ (about 1 hr)
Z(*m*-Cl)-Lys	○ (about 3 hr)	○ (about 1.5 hr)
Z(*p*-CN)-Lys	± (after 12 hr)	○ (about 4 hr)
Z(*p*-NO_2)-Lys	± (after 12 hr)	○ (about 8 hr)

a) ○, Complete deprotection; ±, deprotected about 1%.

droxide. The precipitate was collected after being left for several hours at 0°C, and washed with water and ethanol. It was recrystallized from dilute hydrochloric acid - dilute ammonium hydroxide; yield, 1.35 g (89%). Analytical data on this compound are listed in Table 1, together with those on the other derivatives.

R_f values on TLC of each ε-protected-lysine thus prepared and lysine hydrochloride are shown in Table 2.

Procedure for Treatment of ε-*N*-Protected-lysine with 4N HCl-dioxane. Fifty μmol (about 15 mg) of each of the ε-protected-lysines were dissolved in 4N HCl-dioxane (1 m*l*) at 25°C. At various time intervals, an aliquot (about 1 μ*l*) of the reaction solution was chromatographed on a plate of Silica gel G using two different solvent systems. On the same plate, aliquots (each 1 μ*l*) of lysine hydrochloride solution at various concentrations were applied as controls; thus, the amount of lysine produced from ε-protected lysine after a definite time could be estimated semiquantitatively. The results are summarized in Table 3.

Reaction of ε-*N*-Protected-lysine with Hydrogen Fluoride and 36% Hydrogen Bromide-Acetic Acid. To fifty μmol each of the ε-protected-lysines was added anhydrous HF (about 3 m*l*) at 0°C or 36% HBr - acetic acid (about 4 m*l*) at 25°C. After a certain time, the solution was evaporated *in vacuo* at 0°C (in the case of HF) or at room temperature (36% HBr), and the residue was dissolved in 10% acetic acid (1 m*l*). An aliquot (1 μ*l*) was subjected to TLC as described before, and the amount of free lysine produced by the deprotection or ε-protected-lysine remaining was estimated semiquantitatively. The results are summarized in Table 4, giving optimum reaction time for complete deprotection on each protecting group.