Notes

Application of the 2-(phenylsulfonyl)ethoxycarbonyl Group for the ε-Amino Function of Lysine in Liquid Phase Peptide Synthesis

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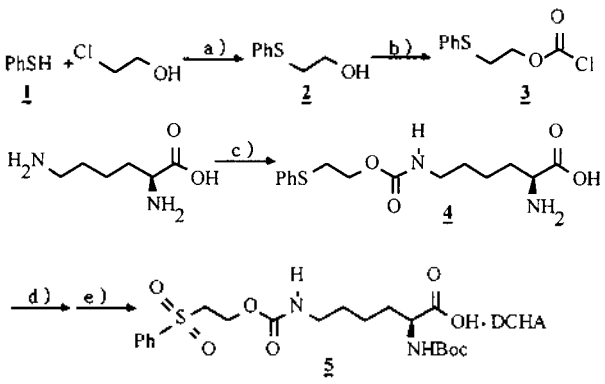
Since the reactivities of the α-/ε-amino groups of lysine are not sufficiently different for regio-specific acylation, the ε-amino function should be protected in peptide synthesis. The ε-amino protecting group has to be used orthogonally both to the α-amino group and to the α-carboxyl group: selectively removable α-position protecting groups, and a semipermanently stable ε-amino pretecting group during a peptide chain elongation.

Various groups, for example benzyloxycarbonyl(Z), *t*-butoxycarbonyl(Boc), and trityl(Trt), have been proposed for the ε-amino function of lysine. The most popular combination is the protection of α-amino/ε-amino functions with the Boc/Z groups which can be removed by acidolysis/catalytic hydrogenolysis, respectively. But the combination is imperfect because of low stability of the Z group with acidic reagents which are needed for the removal of the Boc group. To increase the resistance to acid, the Z group has been modified by the introduction of electron-withdrawing substituents.[1] The modified Z groups, however, require stronger acids for their removal which may give rise to side reactions.

As an alternative to the Z group, we planed to introduce new base-labile protecting group with acid stability and with resistance to catalytic hydrogenation to the ε-amino function. Even though several base-labile amino protecting groups such as 2-(*p*-tolylsulfonyl)ethoxycarbonyl,[2a] 2-(methylsulfonyl)ethoxycarbonyl,[2b] and 2-(*p*-nitrophenylsulfonyl)ethoxycarbonyl[2c-d] on the basis of substituted 2-sulfonylethanols have been developed, these groups have been used not for semipermanent protection on the ε-position but only temporary protection on the α-position. We therefore tried to apply substituted 2-sulfonylethanol group, 2-(phenylsufonyl)ethoxycarbonyl(Psc) group, for the orthogonal protection of the ε-amino function of lysine in conjuction with the Boc group. Since it was considered that the Psc group could afford higher stability than the other groups, which had no negative inductive effect from ortho- or para-substituent on phenyl ring.[3]

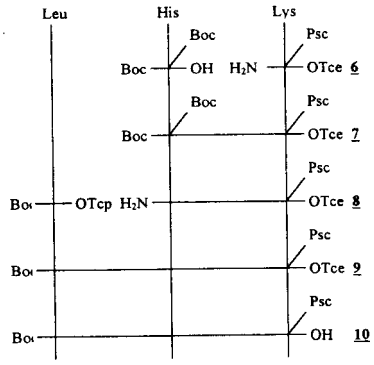
The reagent, 2-(phenylthio)ethyl chloroformate(Ptc-Cl, **3**), used for the ε-amino protection of lysine was obtained quantitatively by treatment of phenylsulfide with 2-chloroethanol in the presence of base, and subsequent reaction with phosgene.[4] The Ptc group was introduced in the ε-amino position of lysine by the urethane-type reaction with **3** whilst the α-groups were chelated to Cu^{2+} to give H-Lys(Ptc)-OH (**4**) in moderate yield.[5] The α-amino function of **4** was then protected with the Boc group, oxidized by hydrogen peroxide in the presence of sodium molibdate,[6] and solidified with dicyclohexylamine(DCHA) to afford the corresponding Boc-Lys(Psc)-OH·DCHA (**5**) which showed a nearly pure form on analytical HPLC in 95% yield (Scheme 1).



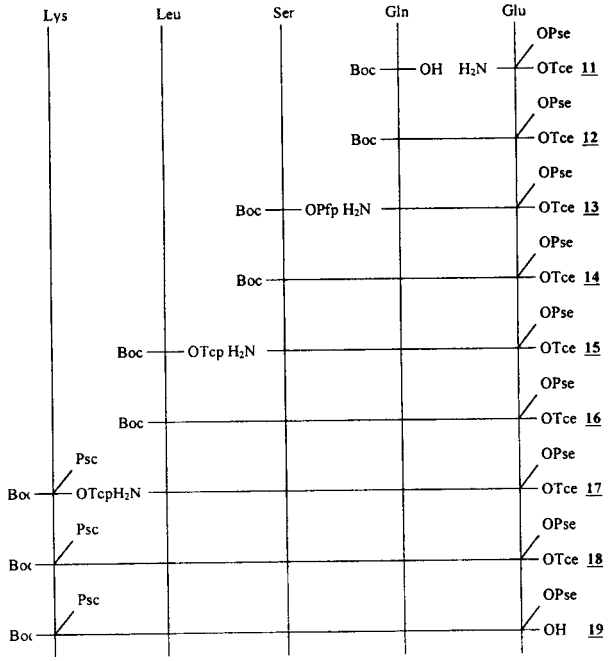
Scheme 1. [a] 2 N NaOH, rt → 50 °C, quantitative. [b] $COCl_2$, THF, -78 °C → rt, quantitative. [c] i) $CuCO_3$ $Cu(OH)_2$, water ii) 2 N NaOH, **3**, dioxane, rt iii) 2 N HCl, EDTA → pH 4, 77%. [d] 2 M K_2CO_3, Boc_2O, *i*-PrOH, 50 °C. [e] 0.3 M Na_2MoO_4, H_2O_2, acetone, DCHA, 50 °C, 95%.

In order to check the applicability of Boc-Lys(Psc)-OH in liquid phase peptide synthesis, two peptide fragments, Boc-Leu-His-Lys(Psc)-OH (**10**) and Boc-Lys(Psc)-Leu-Ser-Gln-Glu(OPse)-OH (**19**), which were key intermediates for a convergent synthesis of salmon calcitonin[7] by liquid phase peptide synthesis were synthesized (Scheme 2, 3). Syntheses of **10** and **19** started with the protection of the C-terminal with trichloroethyl(Tce) group which could be removed by catalytic hydrogenolysis. Elongations of the peptide chains were performed either by preactivation with DCC/HOBt or activated esters of appropriate N^{α}-Boc-amino acids. Removal of the Boc group was carried out by treatment with HCl/AcOH or TFA. After completion of the peptide chain elongation, the Tce group was deprotected by the catalytic hydrogenolysis using 10% Pd-C to give **10** and **19** in overall yields of 81% and 58%, respectively.

No difficulties were encountered in the synthesis of the two fragments using this new lysine derivative: the Psc



Scheme 2. Synthesis of Boc-Leu-His-Lys(Psc)-OH



Scheme 3. Synthesis of Boc-Lys(Psc)-Leu-Ser-Gln-Glu(OPse)-OH.

group was sufficiently stable during both the coupling and the Boc/Tce group deprotection. These two synthesized fragments showed high degree of purities(98%, 99%) on analytical HPLC and had good solubility in common organic solvents used for the fragment assembly. The Psc group was successfully removed by piperidine for a short period of time after completing the peptide chain(1-32) to give high quality salmon calcitonin.[8]

In conclusion, we have demonstrated that the Psc group is a very powerful protecting group for the ε-amino function of lysine in conjunction with the Boc group. Easy and selective removal of the Psc group from ε-amino function of lysine has made the Psc group a promising candidate for the protection of histidine and arginine residues during peptide synthesis.

Experimental Section

General. All protected and/or activated amino acids were prepared by known procedures except Lys/Glu derivatives.[9] Melting points were taken on a Buchi apparatus. Optical rotations were measured on a Jasco DIP 1000. TLC was done on silicagel 60 F-254 precoated plates (Merck). Analytical HPLC was carried out on a Hewlett Packard 1100, preparative HPLC on a Merck GP-900, FT-IR on a Jasco 300E, and ^{1}H NMR on a Bruker AMX500 (500 MHz).

2-(Phenylthio)ethyl chloroformate(3). To a mixture of thiophenol (103 mL, 1.0 mol) and 2 N NaOH (550 mL, 1.1 mol), 2-chloroethanol (74 mL, 1.1 mol) was added at room temperature. After 1 h, the mixture was stirred for 4 h at 50 °C, cooled to room temperature, and diluted with 300 mL of chloroform. Organic layer was separated, washed with 200 mL of water and 200 mL of brine, and dried over anhydrous $MgSO_4$. The solvent was evaporated *in vacuo* to give oil (2, 155.6 g, quantitative). Condensed solution of 40 mL of phosgene in 100 mL of THF to -78 °C was added to 2 (38.5 g, 0.25 mol), and stood at room temperature overnight. To remove excess phosgene, the mixture was bubbled with N_2, and evaporated *in vacuo* to afford 58.0 g (quantitative) of 3 as colorless oil.

N$^{\varepsilon}$-2-(Phenylthio)ethoxycarbonyl-*L*-lysine(4) A mixture of L-lysine (20.1 g, 0.11 mol), copper basic carbonate (12.0 g, 0.10 mol), and water (250 mL) was boiled for 20 min with stirring, filtered, and washed with 2×200 mL of water. To the filtrate 125 mL of dioxane and 55 mL of 2 N NaOH were added. After cooling the mixture, a solution of 2 N NaOH (50 mL) and 3 (21.7 g, 0.10 mol) in 50 mL of dioxane were added during 1.5 h, and stirred for additional 2 h. Copper complex formed was filtered, and washed with 200 mL of water, 300 mL of acetone, and finally with 300 mL of ether. The complex was dissolved in 500 mL of 2 N HCl, and then a suspension of 30.0 g of EDTA in 1 L of water was added to the solution with stirring. The mixture was neutralized to pH 4 with 2 N NaOH to form white precipitate. The precipitate was filtered, washed with 200 mL of water, and dried on air to afford 26.0 g (77%) of 4 as white powder; mp 257-259 °C; $[\alpha]_D^{20}$+12.9 (c 1.0, 0.5 N HCl); HPLC (C-18, ODS hypersil, 5 μm, 4.6×200 mm; gradient 0% to 100% of acetonitrile (0.1% TFA) in 45 min; 1 mL/min; 216 nm) R_T 19.5 min; IR (KBr, cm^{-1}) 3343, 2947, 1683, 1578, 1533, 1418, 1325, 1236, 734; ^{1}H NMR (D_2O, DCl) δ 6.96(d, *J*=7.5 Hz, 2H), 6.87(t, *J*=7.6 Hz, 2H), 6.79(t, *J*=7.3 Hz, 1H), 3.76(t, *J*=6.2 Hz, 2H), 3.66(t, *J*=7.6 Hz, 1H), 2.73(t, *J*=6.2 Hz, 2H), 2.58(t, *J*=6.1 Hz, 2H), 1.46-1.58(m, 2H), 0.92-1.07(m, 2H)

N$^{\alpha}$-*t*-butoxycarbonyl-N$^{\varepsilon}$-2-(Phenylthio)ethoxycarbonyl-*L*-lysine DCHA(5). To a suspension of 4 (19.6 g, 0.06 mol), K_2CO_3 (12.5 g, 0.09 mol), water (200 mL), and *i*-PrOH (150 mL), 16 mL (0.07 mol) of Boc_2O was added for 2 h at 50 °C with stirring, and stirred for additional 3 h. The mixture was evaporated, diluted with 500 mL of water, and washed with 2×150 mL of ether. 200 mL of EtOAc was added to the aqueous layer, followed by acidification to pH 2. More 150 mL of EtOAc was added, and the organic layer was separated, washed with 150 mL of water and 100 mL of brine, and dried over anhydrous Na_2SO_4. The filtrate was evaporated to give 26.0 g of oil. The oil was diluted with 300 mL of acetone, and 15 mL of 0.3 M Na_2MoO_4 and 14 mL of H_2O_2 were added to the mixture. After 1 h, the mixture was stirred for 5 h at 50 °C, and evaporated. The residue was diluted with 300 mL of water

and 300 mL of EtOAc, and the organic layer was separated, and washed with 150 mL of water, 150 mL of 0.5 N HCl, and finally 100 mL of brine. Combined organic layer was dried over anhydrous Na_2SO_4, solvent evaporated, and the residue triturated with DCHA. Precipitate formed was filtered, and dried *in vacuo* to give 36.5 g (95%) of **5** as white powder: mp 109-112 °C; $[\alpha]_D^{20}$ +7.2 (c 1.0, 10% AcOH); HPLC (C-18, ODS hypersil, 5 μm, 4.6×200 mm; gradient 0% to 100% of acetonitrile(0.1% TFA) in 45 min ; 1 mL/min; 216 nm) R_T 21.4 min; IR(KBr, cm^{-1}) 3393, 2933, 2858, 1698, 1636, 1560, 1398, 1318, 1254, 1149, 1051, 728, 692; ^1H NMR (D_2O, DCl) δ 7.89 (d, *J*=7.6 Hz, 2H), 7.75 (d, *J*=7.4 Hz, 1H), 7.65 (t, *J*=7.7 Hz, 2H), 6.92 (m, 1H), 6.13 (m, 1H), 4.17 (t, *J*=6.0 Hz, 2H), 3.60-3.64 (m, 2H), 2.82-2.86 (m, 4H), 1.92 (m, 4H), 1.69 (m, 4H), 1.58 (d, *J*=12.1 Hz, 2H), 1.40-1.48 (m, 2H), 1.36 (m, 9H), 1.25-1.31 (m, 12H), 1.07 (t, *J*=6.9 Hz, 2H)

Synthesis of the peptide fragments: Boc-Leu-His-Lys(Psc)-OH (10) and Boc-Lys(Psc)-Leu-Ser-Gln-Glu(Pse)-OH (19). Syntheses were carried out by the procedure described in Scheme 2 and 3. At first, the carboxyl groups of C-terminal were protected by treatment with trichloroethanol. Peptide couplings were carried out by the activation with HOBt/DCC or with active esters, and monitored by TLC and Sarin test.[10] Deprotections of the Boc groups were performed by usual method (HCl/AcOH or TFA). After final deprotections of Tce groups of **9** and **18**, the resulting peptides were purified by recrystallization to afford **10** of >98% purity in overall 81% yield and **19** of >99% purity in overall 58% yield, respectively. The results are summarized in Table 1 and 2. **10**: mp 155-158 °C; $[\alpha]_D^{25}$ +7.2 (c 0.5, MeOH); HPLC(C-18, ODS hypersil, 5 μm, 4.6×200 mm; gradient 0% to 100% of acetonitrile (0.1% TFA) in 45 min; 1 mL/min; 216 nm) R_T 23.6 min **19**: mp 148-152 °C; $[\alpha]_D^{25}$ -28.4 (c 0.5, MeOH); HPLC(C-18, ODS hypersil, 5 μm, 4.6×200 mm; linear gradient, 0-100% AcCN, 0.1% TFA, 45 min; 1 mL/min; 216 nm) R_T 16.4 min

Table 1.

1	2	deprotection /coupling	product	yield	R_f**
Boc-His(Boc)-OTcp	**6**	/active ester	**7**(solid)	94%	0.63[3]
Boc-Leu-OTcp	**8**	HCl, AcOH* /active ester	**9**(oil)	93%	0.32[2]
	9	Zn, AcOH/	**10**(solid)	93%	0.66[1]

*For the deprotection of the Boc group of **7**
**1. *n*-BuOH/H_2O/AcOH=4/1/1
2. $CHCl_3$/MeOH/AcOH=90/10/3
3. $CHCl_3$/MeOH/AcOH=95/5/3

Table 2.

1	2	deprotection /coupling	product	yield	R_f**
Boc-Gln-OH	**11**	TFA /DCC,HOBt	**12**(solid)	93%	0.42[2]
Boc-Ser-OPfp	**13**	TFA* /DCC,HOBt	**14**(solid)	90%	0.33[2]
Boc-Leu-OTcp	**15**	TFA* /active ester	**16**(solid)	88%	0.43[2]
Boc-Lys(Psc)-OTcp	**17**	TFA* /active ester	**18**(solid)	93%	0.342
	18	Zn, AcOH/	**19**(solid)	85%	0.731

*For the deprotections of the Boc groups of **12**, **14**, and **16**, respectively.
**1. *n*-BuOH/H_2O/AcOH=4/1/1
2. $CHCl_3$/MeOH/AcOH=90/10/3

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7. Salmon calcitonin is a cyclic peptide hormone of 32 amino acids(*vide infra*) exhibiting hypocalcemic potency.

   H-Cys-Ser-Asn-Leu-Ser-Thr-Cys-Val-Leu-Gly-Lys-Leu-Ser-Gln-Glu-Leu-His-Lys-Leu-Gln-Thr-Tyr-Pro-Arg-Thr-Asn-Thr-Gly-Ser-Gly-Thr-Pro-NH_2 (1,7-disulfide bond)
8. After completion of the peptide chain (1-32) by fragment assembly, and deprotection of the Boc group, the resulting side chain-protected peptide chain was treated with piperidine in DMSO for 5 min to afford crude salmon calcitonin, which was purified by preparative HPLC (C-18, LiChrospher 300, 15 μm, 50×250 mm, column for packing stand NW 50; gradient 20% to 50% of acetonitrile (0.2% TFA) in 30 min; 30 mL/min; 214 nm) in a purity of >98% according to analytical HPLC. The purified one showed high hypocalcemic potency of about 4000 IU/mg measured in rats by MRC method.
9. Boc-Glu(OPse)-OH was prepared by esterification of the γ-carboxyl function of L-glutamic acid with 2-(phenylthio)ethanol in the presence of sulfuric acid, followed by protection of the α-amino group with Boc_2O and oxidation of 2-(phenylthio)ethyl group by hydrogen peroxide with the catalyst of 0.3 M Na_2MoO_4. The procedure will be discussed in detail elsewhere.
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