Communications to the Editor

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A FLUORIDE ION DEPROTECTION STRATEGY IN PEPTIDE SYNTHESIS.[1] COMBINATION WITH SELECTIVE DEPROTECTION USING THE DILUTE METHANESULFONIC ACID OF α-AMINO PROTECTING GROUPS

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We have developed a novel orthogonal protection methodology in peptide synthesis which involves two classes of acid-labile 'temporary' N^{α}-protecting groups and acid-stable but fluoride ion-labile 'permanent' protecting groups. This fluoride ion final deprotection strategy was successfully applied to the synthesis of bradykinin potentiating peptide 5a in combination with selective deprotection using dilute methanesulfonic acid of the N^{α}-t-butoxycarbonyl group on the acid-stable phenacyl ester linkage-resin cleavable with fluoride ion. The more acid-labile N^{α}-p-methoxybenzyloxycarbonyl group on the p-(carbamoylmethyl)-benzyl ester linkage-resin was also used.

KEYWORDS — fluoride ion deprotection; dilute methanesulfonic acid selective deprotection; orthogonal protection scheme; N^{in}-diphenylphosphinothioyl; N^{ε}-9-fluorenylmethoxycarbonyl; phenacyl ester linkage-resin; p-(carbamoylmethyl)-benzyl ester linkage-resin; peptide synthesis

Acidic conditions have been widely used for the final deprotection in peptide synthesis. Most frequently, strong acids such as HF[2] and trifluoromethanesulfonic acid (TFMSA)[3] have been used for the final deprotection in combination with selective deprotection using the trifluoroacetic acid (TFA) of temporary α-amino protecting groups, based on graduated acidolysis. Recently, alkaline conditions have been used for selective deprotection of temporary α-amino protecting 9-fluorenylmethyloxycarbonyl (Fmoc) group[4] and TFA has been used for the final deprotection.

Here, we describe a final deprotection strategy under mild conditions using tetra-n-butylammonium fluoride trihydrate (TBAF) in dimethylformamide (DMF) in combination with selective deprotection using the dilute methanesulfonic acid (MSA) of temporary α-amino protecting groups by a different deprotection mode. This strategy is

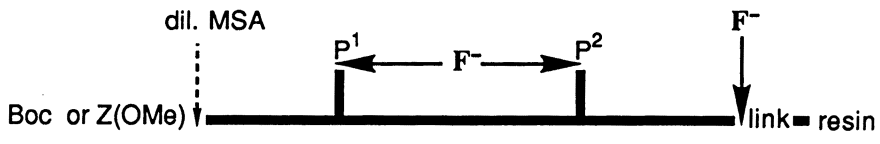

Fig.1. A Two-Dimensional Orthogonal Protection Scheme for Peptide Synthesis by a Fluoride Ion Final Deprotection Strategy

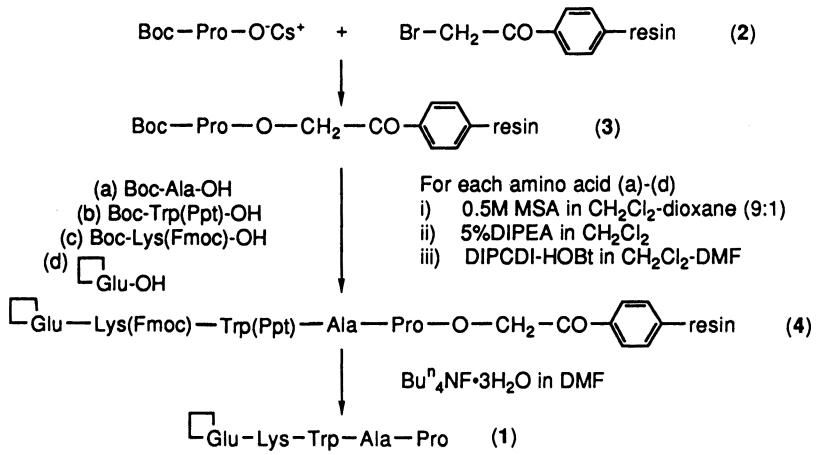

Chart 1. Solid Phase Synthesis of BPP5a Using a Pac-Resin by a Fluoride Ion Final Deprotection Strategy

based on a mild two-dimensional orthogonal protection scheme; that is, a combination of acid-labile 'temporary' N^{α}-protecting group [Boc=t-butoxycarbonyl or Z(OMe)=p-methoxybenzyloxycarbonyl in Fig.1] and acid-stable but fluoride ion-labile 'permanent' side-chain protecting groups (P^1, P^2, and anchoring link in Fig.1).

We synthesized bradykinin potentiating peptide 5a (BPP5a)[5] (1) by this strategy using an acid-stable phenacyl ester linkage (Pac)-resin[6] (Chart 1). In combination with the acid-labile Boc group for N^{α}-protection, we used amino acid derivatives bearing protecting groups removable with fluoride ion, *i.e.*, Boc-Trp(Ppt)-OH[7,8] (Ppt=diphenylphosphinothioyl) and Boc-Lys(Fmoc)-OH[8-10]. It is known that the N^{in}-Ppt group[7,8], N-Fmoc group[8,11,12] and phenacyl ester linkage[13] can be removed by TBAF in DMF.

The N^{in}-Ppt deprotection was complete within 2 min with 0.1 M TBAF (10 eq) in DMF at 25°C. In the presence of a small amout (0.5-1.0 eq) of ethane-1,2-dithiol (EDT), this deprotection reaction was complete within 5 min, but the presence of 5-10 eq EDT served to slow down the rate of deprotection reaction considerably and the deprotection was incomplete even after 2 h. The phenacyl ester of Z(OMe)-Gly was completely cleaved within 2 min by 0.1 M TBAF (10 eq) in DMF at 25°C, and the addition of 0.5-5.0 eq EDT had little effect on the cleavage reaction. Also, the ester cleavage reaction of Boc-Gly-Pac-resin was nearly quantitative (98.5% yield; calculated by amino acid analysis) with 0.1 M TBAF (10 eq) in DMF at 25°C for 30 min. The N-Fmoc deprotection was completed within 2 min with 0.1 M TBAF (10 eq) in DMF at 25° in the absence or presence of EDT[12](0.5-30 eq), while a cleavage product from the Fmoc group, dibenzofulvene had to be scavenged by adding water, alcohols or thiols when the N-Fmoc was deprotected by the use of higher concentration (1 M) TBAF in a longer period.[11]

Boc-Pro-Pac-resin (Pro, 0.43 meq/g) (3) was prepared from bromoacetylated polystyrene-1% divinylbenzene (2), and the solid phase peptide synthesis was accomplished using a Biosearch 9500 automated synthesizer and diisopropylcarbodiimide (DIPCDI)-1-hydroxybenzotriazole (HOBt) activation at 25°C. To deprotect the N^{α}-Boc group selectively, we examined several acidic conditions (Table I) and used 0.5 M MSA in CH_2Cl_2-dioxane(9:1) containing 2% anisole.[8,14] For neutralization, 5% N,N-di-isopropylethylamine (DIPEA) in CH_2Cl_2 was used. The N^{ε}-Fmoc group, the N^{in}-Ppt group and the Pac-resin ester linkage were stable under those conditions employed in the solid phase synthesis.

The protected peptide resin (4) thus obtained was deprotected and cleaved from resin with 0.1 M TBAF (30 eq)-DMF in the presence of EDT (1 eq) at 25°C for 30 min. After the resin was separated from the solution by filtration and washed with 50% aqueous acetic acid, the combined filtrate and washing were concentrated *in vacuo*. After the residue

Table I. Deprotection Reaction of Boc-Phe, Z(OMe)-Phe, Lys(Z)and Ser(Bzl) under Several Acidic Conditions

Acid	Solvent[a]	% Parent amino acid regenerated			
		Boc-Phe 15min[b]	Z(OMe)-Phe 15min[b]	Lys(Z) 20h[b]	Ser(Bzl) 50h[b]
1.0M MSA	DOX-DCM(3:7)	100	100	2.8	N.T.[c]
0.8M MSA	DOX-DCM(3:7)	>98	100	2.5	N.T.
0.5M MSA	DOX-DCM(1:9)	100	100	5.9	0.7
0.3M MSA	DOX-DCM(1:9)	>98	100	1.7	N.T.
0.05M MSA and 0.5M TFA	DCM	100	100	29.7	N.T.
45%TFA	DCM	100	100	70.2	4.4
25%TFA	DCM	>98	100	22.1	1.9

DOX=dioxane, DCM=dichloromethane. a)Containing 2% anisole. b)At 25°C. c)Not tested.

was converted to TFA salt and washed with ether, the crude product was purified by fast protein liquid chromatography (FPLC) on a YMC-ODS AQ120A(S-50) column. The purified BPP5a (1) was obtained in an overall yield of 35% (based on starting Boc-Pro-Pac-resin) and had physicochemical properties identical with an authentic sample.[7,8]

Alternatively, we prepared Boc-Ala-Pro-Pac-resin from Boc-Ala-Pro-O$^-$Cs$^+$ and (2), because the termination in the amino acid-Pac-resin has been known to occur to some extent.[15] Starting from this dipeptide-resin, the solid phase synthesis, deprotection and purification were performed in essentially the same manner as above. The homogeneous peptide (1) thus obtained (overall yield 63% based on starting dipeptide-resin) was identical with an authentic sample.

In addition, since the p-(carbamoylmethyl)-benzyl ester linkage (Pam) has been found to be cleaved not only by HF, but also by TBAF in DMF,[8,13] we synthesized BPP5a using the more acid-labile N$^\alpha$-Z(OMe) group and Pam-resin[16] by a similar deprotection strategy (Chart 2). In combination with the acid-labile Z(OMe) group for N$^\alpha$-protection, new amino acid derivatives bearing protecting groups removable with fluoride ion were used, *i.e.*, Z(OMe)-Trp(Ppt)-OH and Z(OMe)-Lys(Fmoc)-OH.

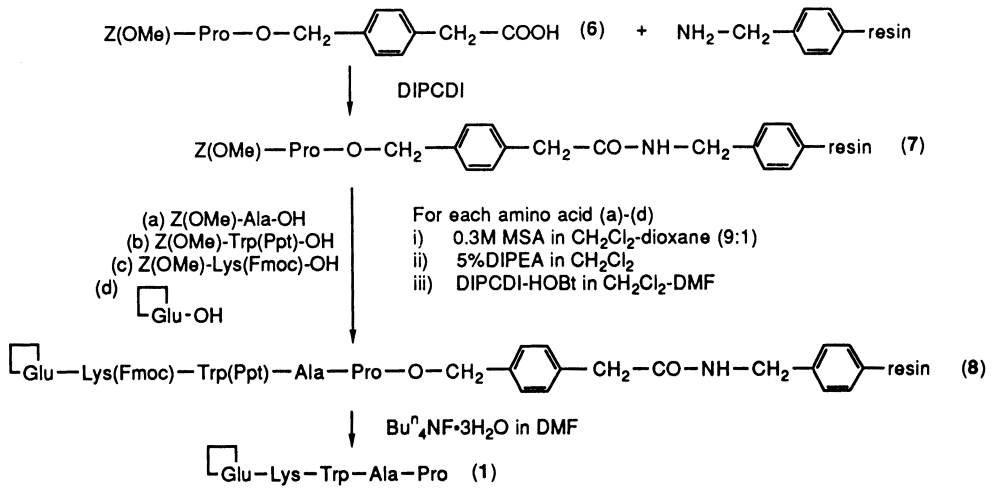

Chart 2. Solid Phase Synthesis of BPP5a Using a Pam-Resin by a Fluoride Ion Final Deprotection Strategy

Z(OMe)-Trp(Ppt)-OH [m.p. 88-89°C, $[\alpha]_D^{18}$ -17.6(*c*0.46, DMF)] was prepared from Z(OMe)-Trp-OMe and Ppt-Cl (1.5 eq) in CH_2Cl_2 in the presence of tetra-n-butylammonium hydrogen sulfate (0.01 eq) and pulverized NaOH, followed by saponification. Z(OMe)-Lys(Fmoc)-OH [m.p. 147-148°C, $[\alpha]_D^{19}$ -6.8(*c*1.92, DMF)] was prepared from Lys•1/2Cu and Fmoc-OSu (1.1 eq) followed by the reaction of Z(OMe)-N_3.

To deprotect the N^{α}-Z(OMe) group selectively, we used a new reagent [0.3M MSA in CH_2Cl_2-dioxane, 9:1 (containing 2% anisole)].[14] As shown in Table I, 0.3 M MSA reagent had selective deprotecting ability. The cleavage of the Pam-type p-(benzylcarbamoylmethyl)-benzyl (Bcmb) ester, Boc-Val-O-CH_2-Ph-CH_2-CO-NH-CH_2-Ph (**5**) (prepared from Boc-Val-O-CH_2-Ph-CH_2-COOH by condensation with benzylamine) was at less than detectable levels (0.02%) in 0.3 M MSA (in CH_2Cl_2-dioxane, 9:1) for 5 h at 25°C, while in 0.5 M MSA (in CH_2Cl_2-dioxane, 9:1) for 5 h at 25°C, 0.09% of Val was detected. This Pam-type Bcmb ester (**5**) was completely cleaved by 0.1 M TBAF (10 eq) in DMF at 25°C within 1 h, but the presence of water, alcohols and thiols served to slow down the rate of deprotection reaction considerably. Thus, in the presence of EDT (0.5 eq), this cleavage reaction was complete within 2 h and in the presence of 1 eq EDT, it was incomplete even after 2 h.

Z(OMe)-Pro-Pam-resin (Pro, 0.27 meq/g) (**7**) was prepared from aminomethylated polystyrene-1% divinylbenzene and (**6**) by DIPCDI followed by acetylation using acetic anhydride for capping. The protected peptide-resin (**8**) was prepared from Z(OMe)-Pro-Pam-resin (**7**) using a Biosearch 9500 automated synthesizer and DIPCDI-HOBt activation. After MSA deprotection of Z(OMe) group, 5% DIPEA in CH_2Cl_2 was used for neutralization. The protected peptide-resin (**8**) thus obtained was deprotected and cleaved from resin with 0.1M TBAF (10 eq) in DMF in the presence of EDT (0.5eq) at 25°C for 3 h. The product was purified as described above, and the homogeneous BPP5a (**1**) was obtained in a good overall yield of 64% (based on starting Z(OMe)-Pro-Pam-resin) and had properties identical with an authentic sample.

These good results show that the fluoride ion final deprotection strategy based on a new two-dimensional orthogonal protection scheme is a useful and mild method for synthesizing free peptide without exposure to strong acid.

REFERENCES AND NOTES

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