 Chem. Pharm. Bull. 38(1) 270—272 (1990) Vol. 38, No. 1

EFFICIENT SOLID PHASE PEPTIDE SYNTHESIS ON A PHENACYL-RESIN BY A METHANE-SULFONIC ACID α-AMINO DEPROTECTING PROCEDURE[1)]

Yoshiaki KISO,* Tooru KIMURA, Yoichi FUJIWARA, Hiroshi SAKIKAWA and Kenichi AKAJI

Department of Medicinal Chemistry, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607, Japan

We have developed an efficient method for solid phase peptide synthesis which consists of N^{α}-selective deprotection by dilute methanesulfonic acid, *in situ* neutralization, and rapid coupling reaction using benzotriazol-1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate or new 2-(benzotriazol-1-yl)oxy-1,3-dimethylimidazolidinium hexafluorophosphate. This method was successfully used to synthesize several peptides using a new derivative, Boc-Tyr(Dpp) (Dpp: diphenylphosphinyl) and Boc-Arg(HCl) on a phenacyl-resin. For this method, we employed a fluoride ion final deprotection strategy based on a two-dimensional orthogonal protection scheme.

KEYWORDS solid phase peptide synthesis; fluoride ion final deprotection; *in situ* neutralization; methanesulfonic acid; coupling reagent; O-diphenylphosphinyl-tyrosine

We reported previously that the methanesulfonic acid (MSA) system [0.5 M MSA in dichloromethane-dioxane(9:1)][2-4)] is suitable for the removal of N^{α}-*tert*-butyloxycarbonyl (Boc) groups in solid phase peptide synthesis (SPPS), and is superior to trifluoroacetic acid (TFA) systems in terms of stability of semipermanent side chain-protecting groups and undesired pyroglutamyl formation from N-terminal glutamine in peptide-resin.[3)] Herein we report an efficient method for solid phase peptide synthesis using this MSA deprotection system on an acid-stable phenacyl ester linkage (Pac)-resin[5)] cleavable with fluoride ion.[2,6)] This method consists of *in situ* neutralization and the rapid coupling reaction using benzotriazol-1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP)[7)] or new 2-(benzotriazol-1-yl)oxy-1,3-dimethylimidazolidinium hexafluorophosphate (BOI)[1)] reagent activation. For this method, we used a fluoride ion final deprotection strategy based on a two-dimensional orthogonal protection scheme.[2,4)]

First, we synthesized bradykinin potentiating peptide 5a (BPP5a;<Glu-Lys-Trp-Ala-Pro)[8)] by this method. The termination in the amino acid Pac-resin occurs during N^{α}-deprotection and neutralization cycle of SPPS[9,10)] (Fig. 1), and diketopiperadine formation and loss of loaded peptides from the Pac-resin are also observed.[9)] These side reactions result from the high susceptibility of phenacyl linkage to aminolysis. To reduce the side reactions, we omitted the base-wash neutralization cycle using diisopropylethylamine (DIEA). After N^{α}-Boc deprotection by 0.5 M MSA, Boc-amino acid (2.5eq), BOP (2.5eq) and DIEA (4.5eq) were added to the N^{α}-deprotected resin, in which the α-amino group was masked as its MSA salt (Fig. 2). Then appropriate amounts of DIEA were added manually to adjust the reaction mixture to pH 7-8. After the coupling reaction, 0.3 M decanoic anhydride was used for capping. The protected BPP5a-resin [<Glu-Lys(Fmoc)-Trp(Ppt)-Ala-Pro-O-CH_2CO-C_6H_4-resin] (Fmoc: 9-fluorenylmethyloxycarbonyl; Ppt: diphenylphosphinothioyl) was obtained from Boc-Ala-Pro-Pac-resin[2)] using a

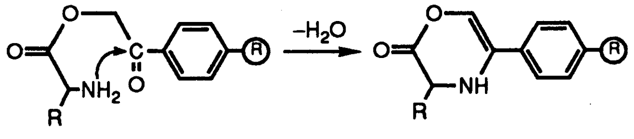

Fig. 1

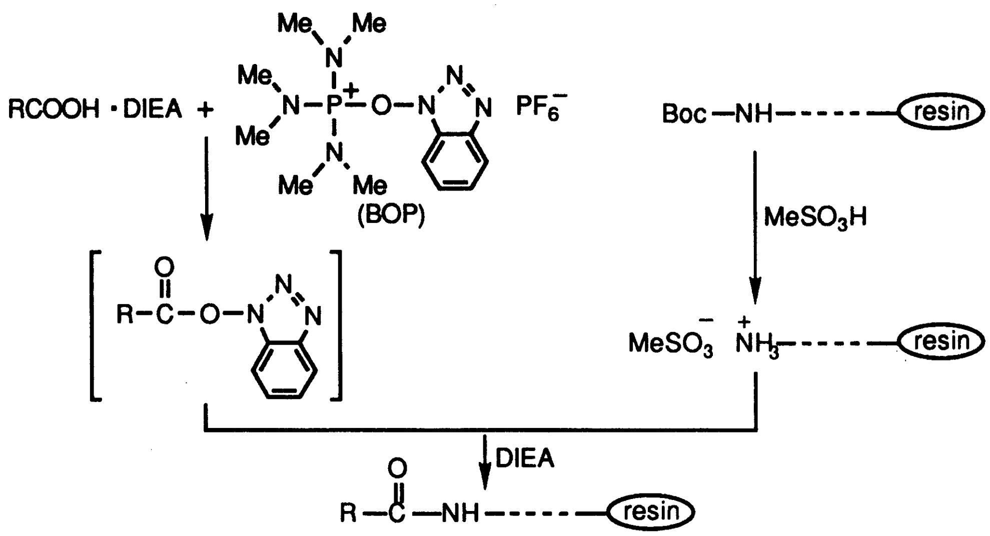

Fig. 2. Coupling Pathway of Efficient SPPS Employing *in situ* Neutralization

Beckman 990E synthesizer at 25°C employing amino acid derivatives bearing side-chain protecting groups removable with fluoride ion in combination with the acid-labile N^{α}-Boc group, *i.e.*, Boc-Lys(Fmoc)[2] and Boc-Trp(Ppt).[4,11] The protected BPP5a-resin was deprotected by fluoride ion and purified as previously described.[2] The homogeneous peptide[12] was obtained in excellent yield (72% based on starting Boc-Ala-Pro-Pac-resin) compared with the previous synthesis using carbodiimide.[2]

To apply this *in situ* neutralization method to the fully automated synthesis (Table), we employed an additional pyridine-wash (step 6) to remove the trace amount of free MSA remaining on the resin. After this treatment, 1 eq of MSA remains in the resin as the salt to mask the α-amino function. The protected BPP5a-resin was synthesized according to the Table using a Biosearch 9500 synthesizer, and pure BPP5a was obtained by the same manner (overall yield 72%). This *in situ* neutralization method reduced side reactions such as cyclization, because the terminal amino group was less exposed as a nucleophile by MSA masking and the rapid coupling reaction using BOP or BOI reagent.

By this method, we successfully synthesized Leu-enkephalin (Tyr-Gly-Gly-Phe-Leu) and neuromedin N (Lys-Ile-Pro-Tyr-Ile-Leu)[13] using a new Tyr derivative with the fluoride ion-labile O-diphenylphosphinyl (Dpp) group, Boc-Tyr(Dpp)-OH, [mp. 69-70°C; $[\alpha]_D^{17}$+16.2°(c=0.56, MeOH); satisfactory elemental analyses were obtained for $C_{26}H_{28}NO_6P \cdot 0.5H_2O$] prepared by the reaction of Boc-Tyr-OPac

Table. Schedule for Efficient Solid Phase Peptide Synthesis

1	DCM (x3)	0.3 min
2	0.5M MSA in DCM-dioxane(9:1)	1min and 20 min
3	dioxane-DCM(1:2) (x3)	0.7 min
4	DCM-DMF(1:1) (x2)	0.3 min
5	DCM (x3)	0.3 min
6	2% pyridine in DCM (x2)	0.7 min
7	DCM (x4)	0.3 min
8	DCM-DMF(1:1)	1.5 min
9	Boc-amino acid(2.5eq), BOP or BOI(2.5eq) and DIEA(4.5eq) in DCM-NMP(1:2)	60min or 90 min
10	DCM-DMF(1:1) (x2)	0.3 min
11	DCM (x2)	0.3 min
12	if recoupling was nesessary then go to step 8	
13	DCM-DMF(1:1) (x2)	0.3 min
14	0.3M decanoic anhydride in DCM-NMP(1:1)	30 min
15	DCM-DMF(1:1) (x2)	0.3 min

DCM: dichloromethane, DMF: *N,N*-dimethylformamide, NMP: *N*-methylpyrrolidone.

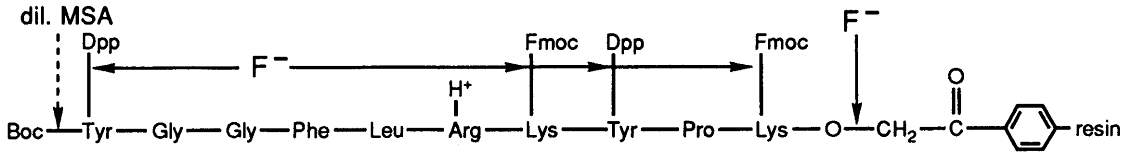

Fig. 3. A Two-Dimensional Orthogonal Protection Scheme for α-Neo-endorphin Synthesis by a Fluoride Ion Final Deprotection Strategy

and diphenylphosphinyl chloride (1.2 eq) in the presence of triethylamine (1.2 eq), followed by zinc-acetic acid treatment in DMF. The O-Dpp group was stable to such conditions as TFA (r.t., 24 h), 0.5 M MSA system (r.t., 24 h) and 5% DIEA in DMF (r.t., 24 h), while it could be readily removed with 0.1 M tetra-*n*-butylammonium fluoride trihydrate in DMF within 5 min at r.t. The protected peptide-resins[14] prepared from Boc-Leu-Pac-resin according to Table were treated with 0.5 M MSA system to remove the terminal Boc group, prior to fluoride ion final deprotection. The deprotected peptides were purified in the same way as BPP5a. The purified Leu-enkephalin and neuromedin N were obtained in 79% and 77% overall yield (based on the starting Boc-Leu-Pac-resin), respectively. The synthetic peptides had properties identical with authentic samples.

We applied this new method to the synthesis of an arginine-containing peptide, α-neo-endorphin (Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro-Lys),[15] using Boc-Arg(HCl)-OH, the guanidine group of which was protected by protonation. The fully protected α-neo-endorphin-resin[16] prepared from Boc-Lys(Fmoc)-Pac-resin according to the Table using a two-dimensional orthogonal protection scheme as shown in Fig. 3 was deprotected and purified as described above. The homogeneous α-neo-endorphin identical with an authentic sample was obtained in 46% yield [based on the starting Boc-Lys(Fmoc)-Pac-resin].

This method reduces side reactions such as cyclizations resulting from acid- or base-catalyzed intramolecular aminolysis. These excellent results show the potential of the method for SPPS on phenacyl ester linkage-resin in combination with fluoride ion final deprotection.

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(Received October 23, 1989)