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Synthetic Study of Phosphopeptides Related to Heat Shock Protein HSP27

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Abstract—Two kinds of phosphoserine-containing peptides related to HSP27 were synthesized by the Boc- or Fmoc-mode solid-phase method based on prephosphorylation strategy. In the case of the Boc strategy, the *O*-phosphono group of the phosphoserine residue was protected with the cyclopentyl or cyclohexyl group. On the other hand, N^{α}-Fmoc-*O*-[(benzyloxy)-hydroxyphosphinyl]serine was employed in case of the Fmoc strategy. Consequently, it has become feasible to utilize conventional solid-phase methods for synthesizing any phosphopeptides which are required to elucidate biochemical significance of protein phosphorylation. Copyright © 1997 Elsevier Science Ltd

Introduction

Phosphorylation and dephosphorylation of proteins are presently known to be quite important phenomena in our life process. Understanding the biological role of protein phosphorylation is now a very attractive theme in the research of protein chemistry. In terms of further progress in biochemical study on protein phosphorylation, biological chemists investigating this field are required to prepare many kinds of phosphopeptides related to natural phosphoproteins. It is therefore a quite urgent subject to establish a versatile method for synthesizing phosphopeptides. In the initial study on this subject, many efforts were generally devoted to find the acid stable *O*-phosphono-protecting groups such as phenyl,[1–3] trichloroethyl,[4] and allyl[5,6] for employing the Boc strategy which was believed to be an appropriate methodology for synthesizing phosphopeptides. Unfortunately, these protecting groups were not so helpful in developing the study of phosphopeptide synthesis.[7]

We recently reported a versatile procedure for synthesizing phosphopeptides based on the Boc strategy using not only the acid stable cyclohexyl (*c*Hex) group, but also the acid labile benzyl (Bzl) group for protecting the *O*-phosphono group.[8–13] The most striking advantage of our procedure is that the *c*Hex and Bzl groups are removable by treatment with TFMSA[14] and additives in TFA;[15] thus cleavage of the synthetic peptide from the resin and removal of all of the protecting groups can be concurrently carried out.[8,10,11] However, we realized that the removal of the *c*Hex group requires a longer period of hard acid treatment than usual,[11,15] and the acid labile Bzl group tends to be gradually cleaved by repeated treatment with TFA for de-*tert*-butoxycarbonylation.[16,17] In order to overcome the disadvantages observed in the use of these protecting groups, we newly proposed to use the cyclopentyl (*c*Pen) and 3-chlorobenzyl [Bzl(3Cl)] groups for protecting the *O*-phosphono group; both are sufficiently stable to TFA, and more easily removable than the *c*Hex group by treatment with TFMSA and additives in TFA.[18] So far as we examined, *O*-(dicyclopentyloxyphosphinyl) derivatives obtained as crystalline compounds are more favorable than oily *O*-[di(3-chlorobenzyloxy)phosphinyl] ones for use as building blocks for an automated solid-phase peptide synthesis.[19]

Although the Boc strategy is well established as a conventional method for peptide synthesis, the Fmoc strategy is also presently a very common methodology. In particular, the latter method seems to be rather advantageous for investigators who are not so familiar with peptide synthesis. However, the Fmoc-mode prephosphorylation strategy has so far been believed to be inadequate for synthesizing phosphoserine- or phosphothreonine-containing peptides, since β-elimination of phosphate in these phosphoamino acid residues readily occurs during a standard cycle for removing the Fmoc group with piperidine.[20–22] As a result of our recent examination concerning base stability of the bis-protected *O*-phosphono group in the phosphoserine residue, we succeeded to propose an efficient methodology for synthesizing phosphopeptides using N^{α}-Fmoc-*O*-[(benzyloxy)hydroxyphosphinyl] β-hydroxy α-amino acid derivatives.[23]

In the present paper we precisely describe the preparations of novel phosphoamino acid derivatives with the *c*Pen and Bzl(3Cl) groups for protecting the *O*-phosphono group. Furthermore, the syntheses of two kinds of phosphoserine-containing peptides related to a 27 kDa mammalian heat shock protein HSP27[24,25] were carried out to demonstrate the usefulness of our methodologies for synthesizing phosphopeptides.

Results and Discussion

Synthesis of phosphopeptides based on the Boc strategy

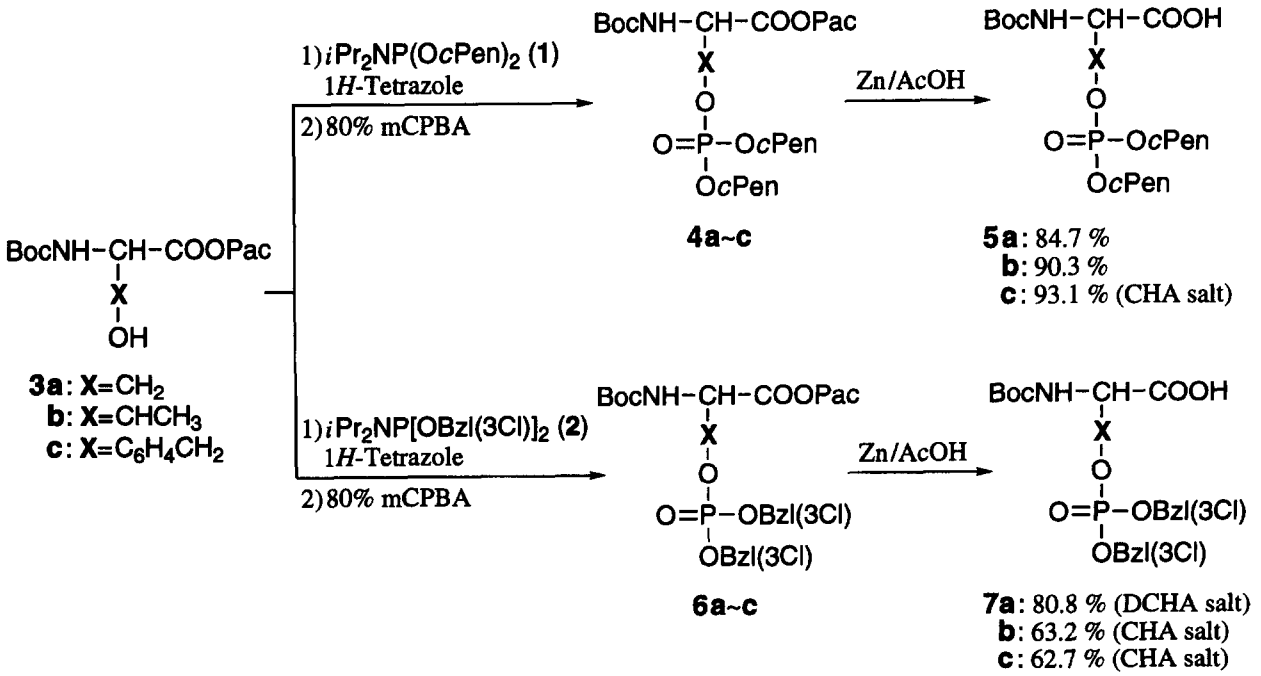
N^{α}-Boc-*O*-(dicyclopentyloxyphosphinyl) and N^{α}-Boc-*O*-[di(3-chlorobenzyloxy)phosphinyl] hydroxy α-amino acid derivatives were first prepared by amidite method as shown in Scheme 1: two amidites as phosphite-forming reagents, iPr$_2$NP(O*c*Pen)$_2$ (**1**) and iPr$_2$NP[OBzl(3Cl)]$_2$ (**2**), were obtained by a general method.[26,27] Of these phosphoamino acid derivatives, Boc-Ser[PO(O*c*Pen)$_2$]-OH (**5a**) and Boc-Thr-[PO(O*c*Pen)$_2$]-OH (**5b**) were obtained as crystalline compounds; other derivatives, **5c** and **7a–7c**, were prepared as crystalline DCHA or CHA salts.

HSP27(79–89)-Cys-NH$_2$ (H-Arg-Ala-Leu-Asn-Arg-Gln-Leu-PSer-Ser-Gly-Val-Cys-NH$_2$ **9**) was synthesized by the use of Boc-Ser[PO(O*c*Pen)$_2$]-OH (**5a**) as shown in Scheme 2. DCC-HOBt method was applied to the elongation of peptide chain; the use of BOP as coupling reagent resulted in the occurrence of undesirable reactions as shown in Figure 1.[23,28] The synthesis of **9** was also carried out by the use of Boc-Ser[PO(O-*c*Hex)$_2$]-OH (**8**) for comparison. The analysis of each crude product by reversed-phase HPLC (RPHPLC; Fig. 2) shows that the use of **5a** gave a little better result than the use of **8**; the yield of **9** after purification by means of preparative RPHPLC was of 29 and 24%, respectively.

Judging from the above-mentioned results concerning the synthesis of **9**, both **5a** and **8** are useful as building blocks for synthesizing phosphopeptides.[29] However, we now suggest that the *O*-(dicyclopentyloxyphosphinyl) derivatives are rather favorable for use as building blocks, since the *c*Pen group is removable under milder conditions compared with the *c*Hex group.

Synthesis of phosphopeptides based on the Fmoc strategy

Although the syntheses of Fmoc-Ser[PO(OBzl)(OH)]-OH (**10**) and Fmoc-Ser[PO(OBzl)(OH)]-OH (**11**) for use in the Fmoc strategy were reported in a previous paper,[30] we herein present an improved result for preparing iPr$_2$NP(OBzl)(OTce) (**13**) to be utilized as a phosphite-forming reagent. The amidite **13** is conveniently prepared from trichlorophosphine (PCl_3) via benzyl tetraisopropylphosphorodiamidite [(iPr$_2$N)$_2$P(OBzl)] (**12**) (Scheme 3). As shown in Table 1, the yield of **12** increased with extending a period of reflux for reaction of PCl_3 with diisopropylamine (DIPA). Furthermore, the subsequent reaction of TceOH with **12** purified by column chromatography gave **13** in an almost quantitative yield; the overall yield of **13** was thus improved from 48% in the previous study up to 83%. This result is quite helpful in

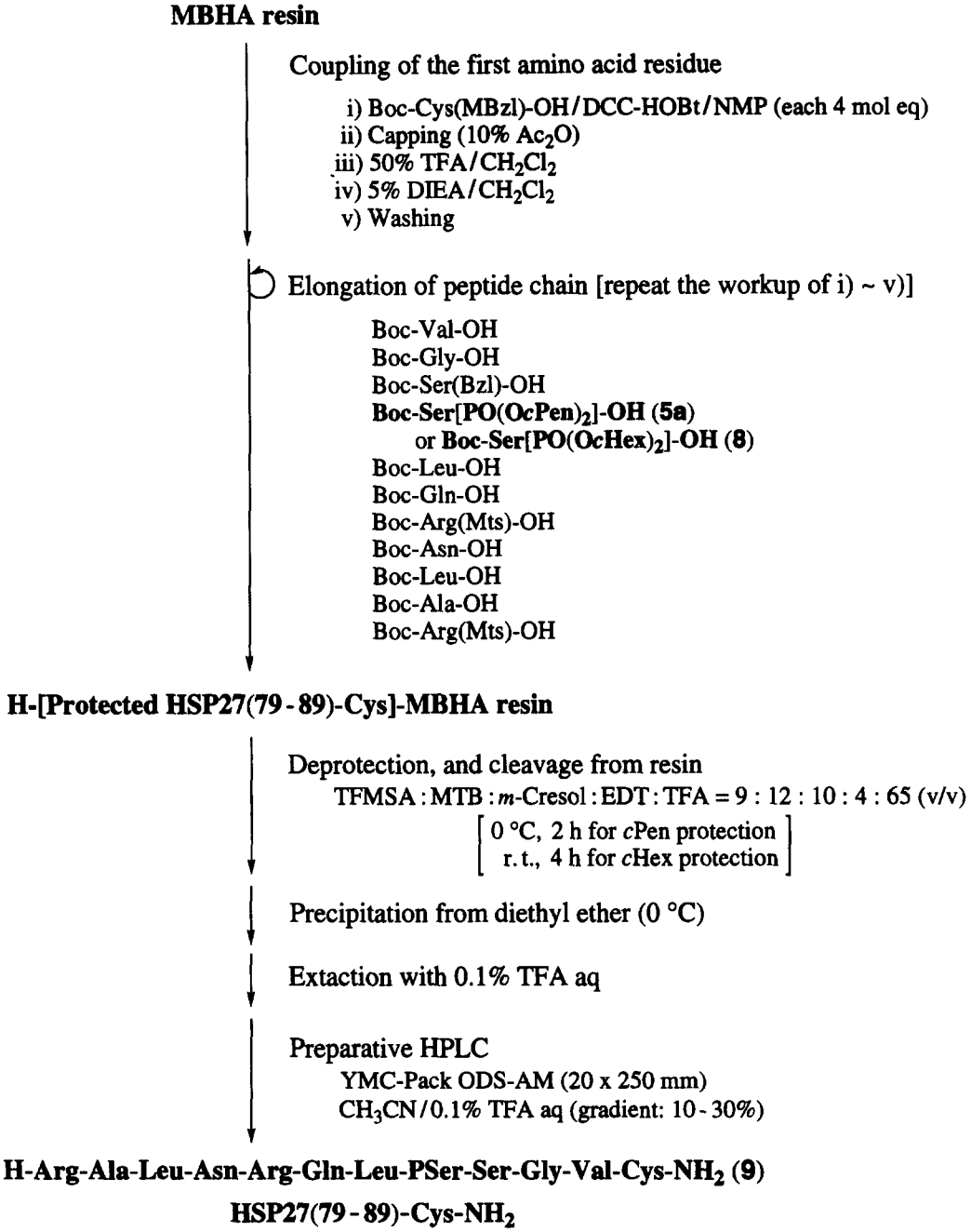


Scheme 1.

proposing our protocol[30] to use as a general and practical one for preparing not only **10** and **11**,[31] but the corresponding phosphotyrosine derivative.

The synthesis of HSP27(10–20)-Cys-NH_2 (H-Leu-Leu-Arg-Ser-Pro-PSer-Trp-Glu-Pro-Phe-Arg-Cys-NH_2, **14**) by the use of **10** was carried out as shown in Scheme 4. Protected peptide resin was treated with reagent K, i.e. TFA:PhOH:H_2O:MTB:EDT = 82.5:5:5:5:2.5 (v/v).[32] RPHPLC analysis of crude product (Fig. 3) clearly shows that the Fmoc strategy is also applicable to the synthesis of phosphopeptides. We finally obtained the purified peptide **14** in a 49% yield after preparative RPHPLC. So far as we compare the yield of **14** with that of **9** prepared by the Boc strategy as mentioned above, the use of the Fmoc strategy is favorable for investigators who are not so familiar with peptide synthesis. Biochemical studies using both **9** and **14** are currently being undertaken, and the results will be reported in the near future.

Consequently, we suggest that both the Boc- and Fmoc-mode prephosphorylation strategies are applicable to the synthesis of phosphopeptides by the use of phosphoamino acid derivatives with suitable *O*-phosphono-protection; (1) the *c*Pen group is an excellent *O*-phosphono-protecting group for the Boc strategy; (2) N^{α}-Fmoc-*O*-[(benzyloxy)hydroxyphosphinyl]β-hydroxy



Scheme 2.

α-amino acid derivatives are confirmed to be quite useful building blocks for the Fmoc strategy.[33]

Experimental

All of the mps are uncorrected and were measured by a Yanaco MP-J3 (Yanaco Co Ltd, Kyoto, Japan). Silica-gel column chromatography was carried out with Merck silica gel 60 (Art. 7734, 70–230 mesh). Boc-Arg(Mts)-OH and Rink amide resin (100–200 mesh, Fmoc-NH/0.48 mmol eq/g) were purchased from Calbiochem-Novabiochem (Japan) Ltd (Tokyo, Japan). All other general protected amino acid derivatives and *p*-methylbenzhydrylamine (MBHA) resin (100–200 mesh, NH_2/0.41 mmol eq/g) were purchased from the Peptide Institute Inc. (Osaka, Japan); all of the amino acids used are of L-forms, except for glycine. Solid-phase peptide synthesis was carried out by use of Peptide Synthesizer 430A and 433A (Applied Biosystems Inc., Foster City, CA, U.S.A.) for the Boc strategy and the Fmoc strategy, respectively. Analytical RPHPLC was performed on Cosmosil $5C_{18}$-AR (4.6 × 250 mm, Nacalai Tesque, Kyoto, Japan), and preparative one was performed on YMC-Pack ODS-AM (20 × 250 mm, YMC Co Ltd, Kyoto, Japan).

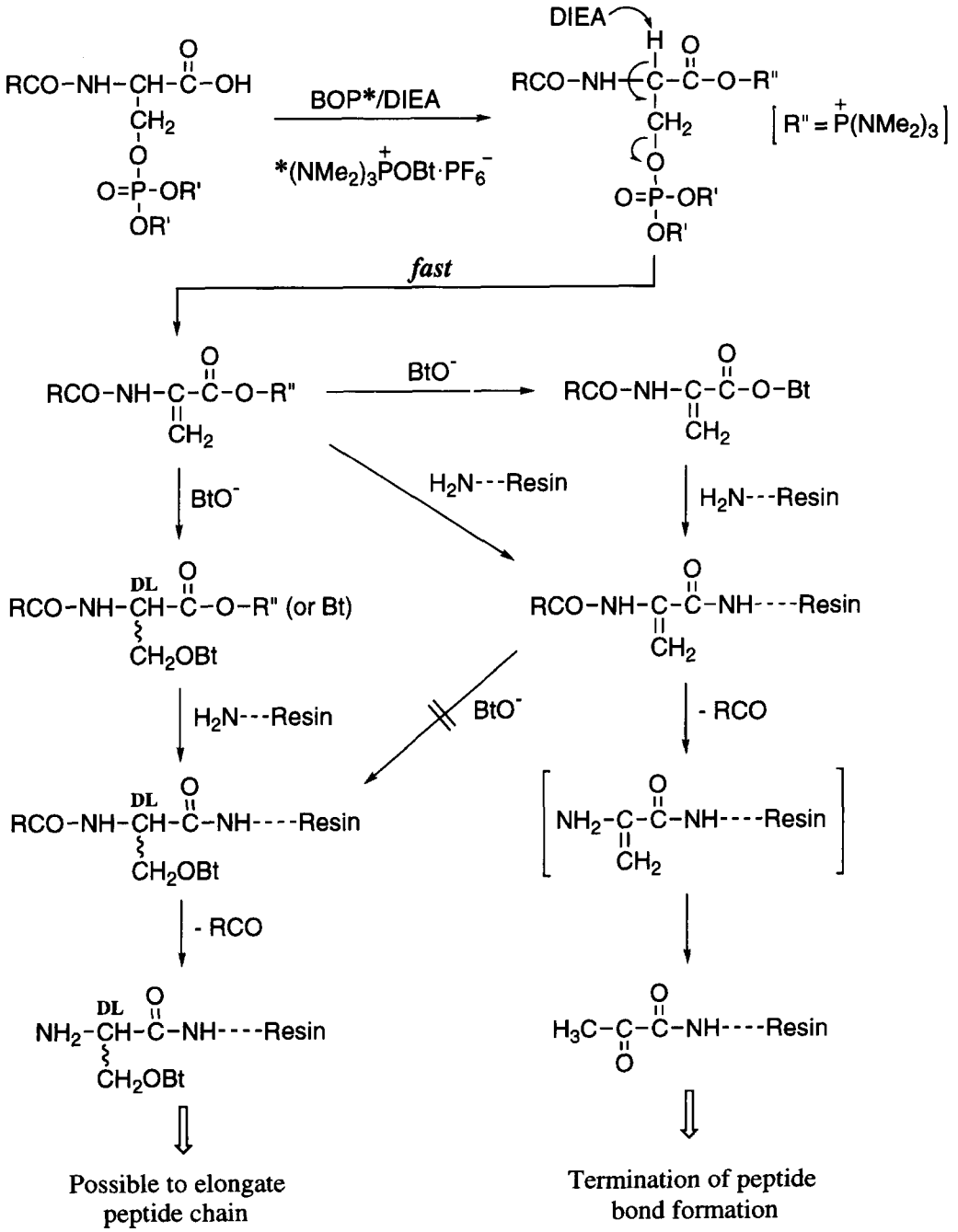
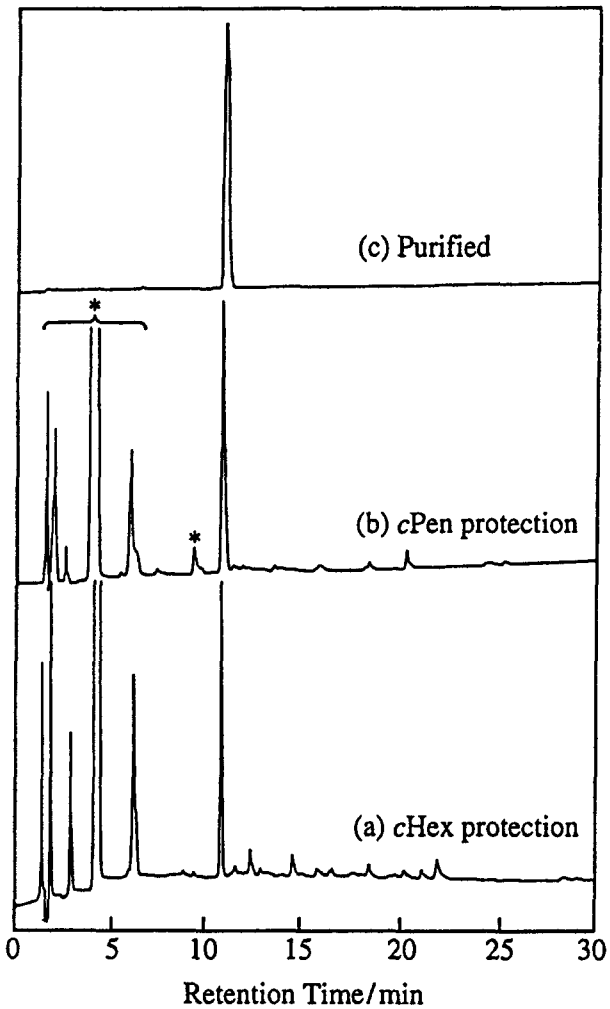


Figure 1. Plausible pathways of undesirable reactions occurring through the activation of *O*-(dialkyloxyphosphinyl)serine derivatives by the use of BOP for peptide bond formation.

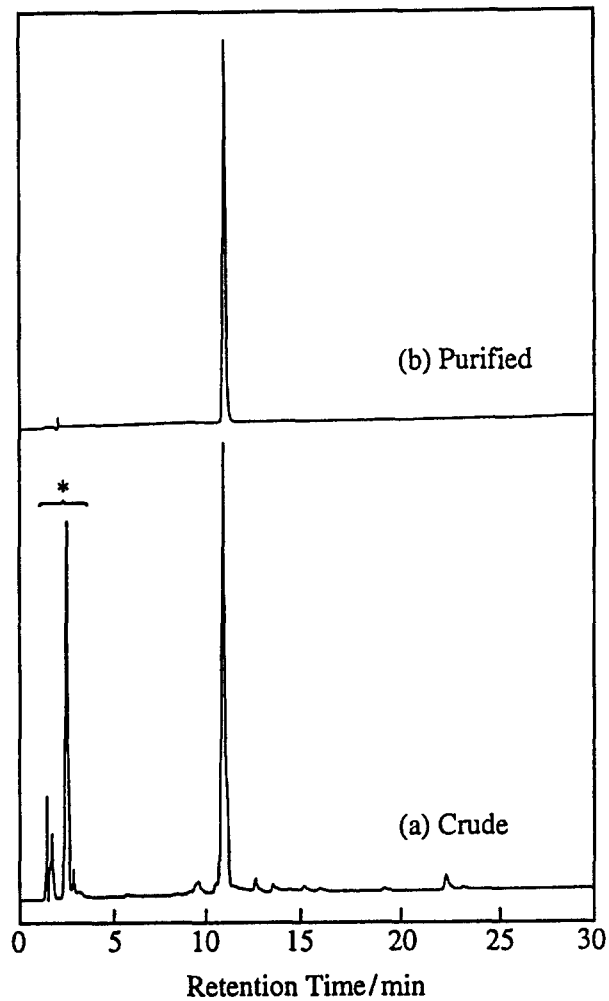
^{1}H NMR (270 MHz) and ^{31}P NMR (202.35 MHz) spectra were recorded on JNM-EX 270 and JNM-LA 500 spectrometers (JEOL Co Ltd, Tokyo, Japan), respectively. The chemical shifts in ^{1}H NMR are given in δ values from TMS used as an internal standard, and those in ^{31}P NMR from 75% aq phosphoric acid used as an external standard. The peptide mass number was determined by FABMS using a JMS SX-270 or JMS-HX 100 mass spectrometer (JEOL Co Ltd, Tokyo, Japan). Amino acid analysis was carried out by JLC-300 amino acid analyzer (JEOL Co Ltd, Tokyo, Japan). Samples (0.5 mg) for the analysis were hydrolyzed with constant boiling 6 M HCl (0.2 mL) in evacuated sealed tubes at 110 °C for 22 h; Trp-containing peptide was hydrolyzed in the presence of thioglycolic acid (8 μL). The compounds containing phosphorus were characterized by a color reaction with the Dittmer–Lester reagent.[34]

Dicyclopentyl diisopropylphosphoramidite (1). To a solution of diisopropylphosphoramidous dichloride (iPr$_2$NPCl$_2$)[35,36] (11.4 g, 56.3 mmol) in anhydrous tetrahydrofuran (THF; 120 mL) was added dropwise a solution of cyclopentyl alcohol (9.69 g, 113 mmol) and triethylamine (TEA; 13.6 g, 135 mmol) in THF (80 mL) with stirring at 0 °C in an atmosphere of nitrogen. After stirring for 2 h at 0 °C and 30 min at rt, insoluble TEA·HCl was filtered off, and the filtrate was concentrated in vacuo. To the residue were added ethyl acetate (AcOEt) and satd aq $NaHCO_3$. The organic layer was separated, washed with aq $NaHCO_3$ and NaCl, dried over anhyd $MgSO_4$, and evaporated in vacuo. The thus obtained oily residue was purified by silica-gel column chromatography [75 g; silica-gel was



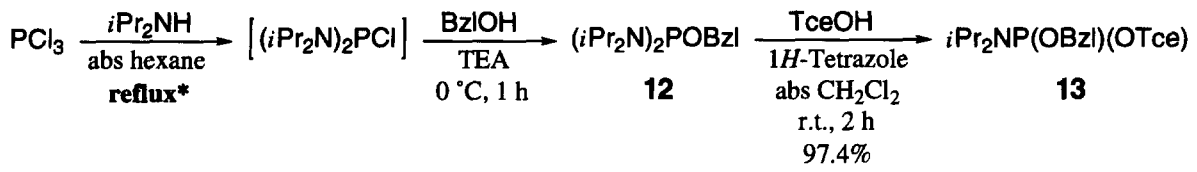
Column : Cosmosil 5C$_{18}$-AR (4.6 × 150 mm)
Elution : CH_3CN (containing 0.1% TFA)/0.1% TFA aq (10-40% gradient; 1%/min)
Flow Rate : 1.0 ml/min
Detection : UV at 220 nm

Figure 2. HPLC profiles of HSP27(79–89)-Cys-NH_2: (a) crude product prepared by cHex protection, (b) crude product prepared by cPen protection, and (c) purified peptide. Peaks asterisked appeared after treatment with DTT.



Column : Cosmosil 5C$_{18}$-AR (4.6 × 150 mm)
Elution : CH_3CN (containing 0.1% TFA)/0.1% TFA aq (20-50% gradient; 1%/min)
Flow Rate : 1.0 ml/min
Detection : UV at 220 nm

Figure 3. HPLC profiles of HSP27(10–20)-Cys-NH_2: (a) crude product and (b) purified peptide. Peaks asterisked appeared after treatment with DTT.



Scheme 3.

Table 1. Relationship between the yield of **12** and a period of reflux

Entry	Period of reflux	Yield of **12** (%)[a]
1	19 h	65.5
2	2 days	74.8
3	3 days	83.8
4	4 days	85.4

[a] After purification by silica-gel column chromatography.

sufficiently pre-washed with hexane:TEA (10:1 v/v)]; pure amidite was obtained by elution with hexane:TEA (100:1 v/v) as a colorless oil [yield 13.0 g (76.9%)]. ^{1}H NMR (DMSO-d_6): δ 1.12 [d, 12H, J=6.8 Hz, $CH(C\underline{H}_3)_2 \times 2$], 1.48–1.77 (m, 16H, CH_2/*c*Pen × 8), 3.43–3.62 [m, 2H, $C\underline{H}(CH_3)_2 \times 2$], and 4.20–4.29 (m, 2H, CH/*c*Pen × 2). ^{31}P NMR (DMSO-d_6): δ 144.77 (s).

Di-(3-chlorobenzyl) diisopropylphosphoramidite (2). Amidite **2** was prepared by the reaction of iPr$_2$NPCl$_2$ (8.20 g, 40.6 mmol) in THF (80 mL) with 3-chlorobenzyl alcohol (11.6 g, 81.2 mmol) and TEA (9.84 g, 97.4 mmol) in THF (30 mL) under the same conditions as mentioned in the preparation of **1**. Purification by silica-gel column chromatography (70 g) was also carried out in a similar manner as described above to obtain the amidite **2** as a colorless oil. Yield 14.2 g (81.1%). ^{1}H NMR (DMSO-d_6): δ 1.17 [m, 12H, $CH(C\underline{H}_3)_2 \times 2$), 3.51–3.73 [m, 2H, $C\underline{H}(CH_3)_2 \times 2$), 4.71 and 4.72 [d, each 1H, J=9 Hz, $C\underline{H}_2$Ph(3Cl)], 5.15 and 5.16 [d, each 1H, J=9.5 Hz, $C\underline{H}_2$Ph(3Cl)], and 7.37–7.93 [m, 8H, $CH_2\underline{Ph}$(3Cl) × 2]. ^{31}P NMR (DMSO-d_6): δ 149.67 (s).

***N*$^{\alpha}$-*tert*-Butoxycarbonyl-*O*-(dicyclopentyloxyphosphinyl) hydroxy α-amino acid phenacyl ester (4).** Boc-Ser[PO(*Oc*Pen)$_2$]-OPac (**4a**), as a general procedure: To a solution of Boc-Ser-OPac (**3a**)[13] (4.52 g, 14.0 mmol) in anhyd THF (30 mL) were added the amidite **1** (5.90 g, 19.6 mmol) and 1*H*-tetrazole (2.94 g, 42.0 mmol). After stirring for 2 h at rt, to the reaction mixture was added 80% mCPBA (7.51 g, 35.0 mmol) at 0 °C, and the mixture was stirred for 10 min at 0 °C. The oxidation was quenched by addition of $NaHSO_3$ (3.64 g, 35.0 mmol) in H_2O (3 mL), and THF was then evaporated in vacuo. The residue dissolved in AcOEt (60 mL) was washed successively with 10% aq citric acid (10 mL × 3), brine (10 mL × 1), satd aq $NaHCO_3$ (10 mL × 3), and brine (10 mL × 3). The organic layer was dried over anhyd $MgSO_4$, and concentrated in vacuo. The phenacyl ester obtained as an oily substance was subjected to the subsequent cleavage reaction of the Pac group without further purification.

Boc-Thr[PO(*Oc*Pen)$_2$]-OPac (**4b**) and Boc-Tyr[PO-(*Oc*Pen)$_2$]-OPac (**4c**) were prepared by the same method as described above. The compound **4b** was also obtained as an oily substance, and subjected to the subsequent reaction without further purification.

The compound **4c** was crystallized by trituration with hexane; the thus obtained crystalline product (yield 90.4%) was pure enough to be subjected to the subsequent reaction. A part of the product was recrystallized from AcOEt:hexane to obtain an analytical sample: mp 108–110 °C, $[\alpha]_D^{25}$ −6.8° (*c* 1.1, EtOH). ^{1}H NMR (DMSO-d_6): δ 1.33 (s, 9H, *t*Bu), 1.51–1.83 (m, 16H, CH_2/*c*Pen × 8), 2.94 (dd, 1H, J=9.9 and 14.2 Hz, CH_2/Tyr × 1/2), 3.18 (dd, 1H, J=4.5 and 14.2 Hz, CH_2/Tyr × 1/2), 4.35 (broad m, 1H, CH/Tyr), 4.84–4.92 (m, 2H, CH/*c*Pen × 2), 5.45 and 5.55 (d, each 1H, J=17Hz, CH_2/Pac), 7.08–8.00 (m, 10H, Ph/Tyr, NH, Ph/Pac).

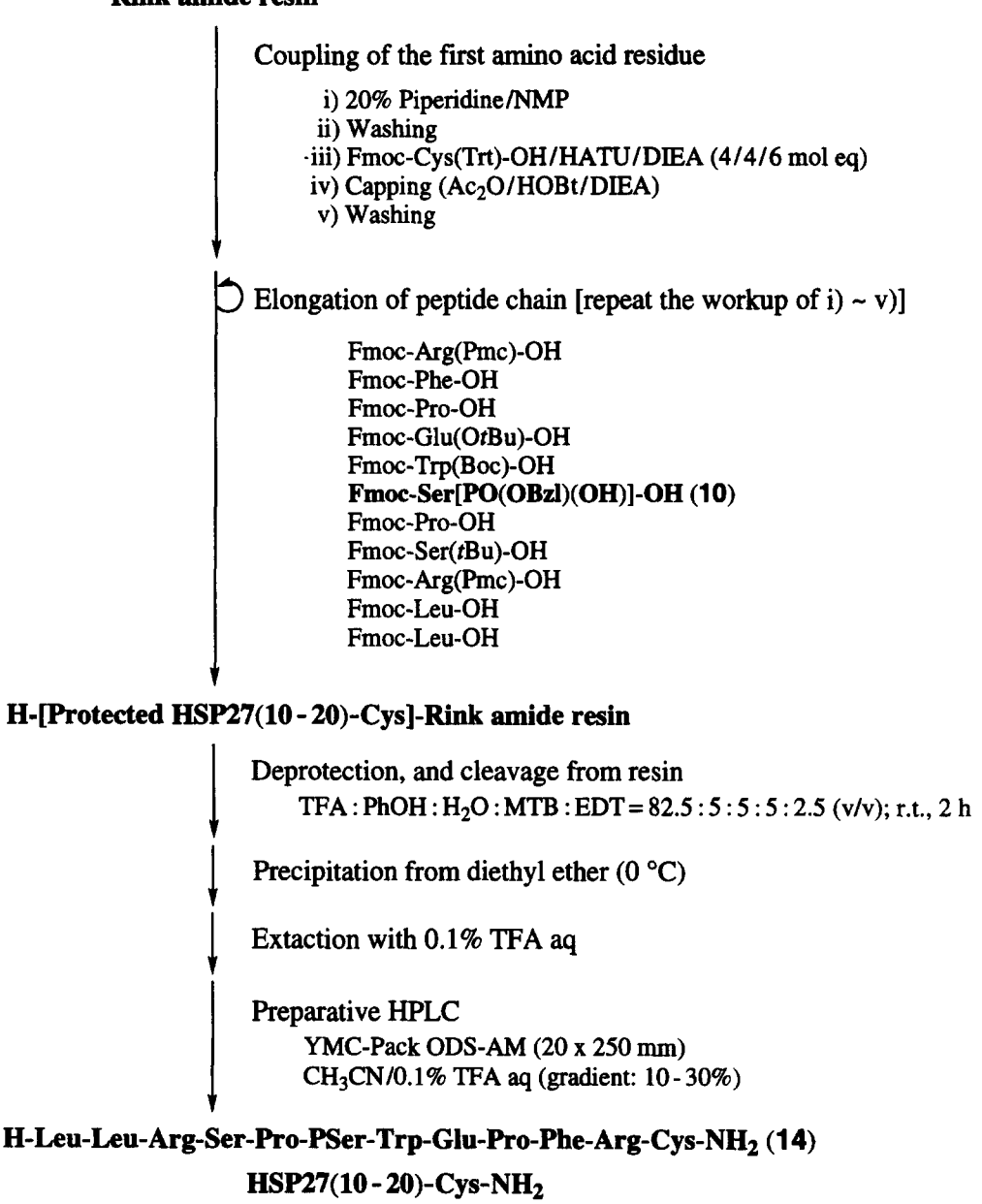
***N*$^{\alpha}$-*tert*-Butoxycarbonyl-*O*-(dicyclopentyloxyphosphinyl) hydroxy α-amino acid (5).** Boc-Ser[PO(*Oc*Pen)$_2$]-OH (**5a**), as a general procedure: To a solution of Boc-Ser[PO(*Oc*Pen)$_2$]-OPac (**4a**) in 90% acetic acid (80 mL) was added zinc dust (13.7 g, 210 mmol) in several portions. After stirring for 1 h, insoluble inorganic materials were filtered off, and the filtrate was concentrated in vacuo. The residue was dissolved in diethyl ether (130 mL), and insoluble materials were filtered off again; the inorganic materials combined were treated with 0.5 M HCl (40 mL) and diethyl ether (10 mL × 3). The separated diethyl ether layer and the first ethereal filtrate were combined; the ether solution was washed with 10% aq citric acid (20 mL × 3), and brine (20 mL × 3). The organic layer was dried over anhyd $MgSO_4$, and concentrated in vacuo. An oily residue was crystallized by trituration with hexane; the thus obtained crystalline product was collected by filtration, and recrystallized from AcOEt:hexane. Yield 4.99 g (84.7% from **3a**); mp 110–113 °C; $[\alpha]_D^{28}$ +15.0° (*c* 1.01, EtOH). Anal. calcd for $C_{18}H_{32}NO_8P$: C, 51.30, H, 7.65; N, 3.32; found: C, 51.18; H, 7.73; N, 3.41%. ^{1}H NMR (DMSO-d_6): δ 1.39 (s, 9H, ^{t}Bu), 1.51–1.76 (m, 16H, CH_2/*c*Pen × 8), 4.05–4.19 (m, 2H, CH_2/Ser), 4.23 (broad m, 1H, CH/Ser), 4.75 (m, 2H, CH/*c*Pen × 2), and 6.77 (broad s, 1H, NH). ^{31}P NMR (DMSO-d_6): δ−2.67 (s).

Boc-Thr[PO(*Oc*Pen)$_2$]-OH (**5b**) was obtained as a crystalline compound by the same method as described above. Yield 90.3% (from **3b**); mp 120–123 °C; $[\alpha]_D^{25}$ +18.2° (*c* 1.00, EtOH). Anal. calcd for $C_{19}H_{34}NO_8P$: C, 52.40, H, 7.87; N, 3.22. Found: C, 52.11; H, 7.90; N, 3.36%. ^{1}H NMR (DMSO-d_6): δ 1.30 (d, 3H, J=6.5 Hz,

CH_3/Thr), 1.41 (s, 9H, ^{t}Bu), 1.50–1.79 (m, 16H, CH_2/*c*Pen × 8), 4.16 (m, 1H, α-CH/Thr), 4.71–4.77 (m, 3H, β-CH/Thr and CH/*c*Pen × 2), and 6.46 (broad *s*, 1H, NH). ^{31}P NMR (DMSO-d_6): δ–3.68 (s).

Boc-Tyr[PO(O*c*Pen)$_2$]-OH (**5c**) was prepared from **4c** (3.00 g, 4.87 mmol) by the same method as described above. The thus obtained oily product was dissolved in a small amount of diethyl ether. After CHA (589 μL, 4.87 mmol) was added to the solution, the mixture was allowed to stand in a refrigerator overnight. The precipitated crystalline CHA salt was collected by filtration and recrystallized from diethyl ether. Yield 2.51 g (93.1%); mp 126–130 °C (decomp); $[\alpha]_D^{28}$ +33.3 ° (*c* 1.09, EtOH). Anal calcd for $C_{30}H_{49}N_2O_8P \cdot 0.4H_2O$: C, 59.67; H, 8.31; N, 4.64. Found: C, 59.62; H, 8.37; N, 4.77%. ^{1}H NMR (DMSO-d_6): δ 1.06–1.88 (m, 35H, CH_2/*c*Pen × 8, CH_2/CHA × 5, and *t*Bu), 2.87–3.07 (m, 3H, CH/CHA and CH_2/Tyr), 3.81 (dd, 1H, J = 6 and 12 Hz, CH/Tyr), 4.87 (m, 2H, CH/*c*Pen × 2), 5.88 (broad s, 1H, NH), and 6.99 and 7.14 (d, each 2H, J = 8 Hz, Ph/Tyr). ^{31}P NMR (DMSO-d_6): δ −7.72 (s).

***N*$^{\alpha}$-*tert*-Butoxycarbonyl-*O*-[di(3-chlorobenzyloxy)phosphinyl] hydroxy α-amino acid phenacyl ester (6).** Boc-Ser(PO[OBzl(3Cl)]$_2$)-OPac (**6a**), Boc-Thr(PO-[OBzl(3Cl)]$_2$)-OPac (**6b**), and Boc-Tyr(PO[OBzl(3-Cl)]$_2$)-OPac (**6c**) were prepared by the same method as described in the preparation of *O*-(dicyclopentyloxyphosphinyl) derivatives. The compound **6a** was obtained as a crystalline substance in a yield of 88.6%. An analytical sample was obtained by recrystallization



Scheme 4.

from AcOEt; mp 79–80 °C; $[\alpha]_D^{25}$ −9.7° (*c* 1.0, EtOH). ^{1}H NMR (DMSO-d_6): δ 1.38 (s, 9H, *t*Bu), 4.26–4.48 (m, 2H, CH_2/Ser), 4.55 (broad s, 1H, CH/Ser), 5.07 and 5.10 (s, each 2H, $C\underline{H}_2$Ph(3Cl) × 2), 5.47 and 5.56 (d, each 1H, *J* = 17 Hz, CH_2/Pac), 7.32–7.42 (m, 8H, $CH_2\underline{Ph}$(3Cl) × 2), and 7.51–7.95 (m, 5H, Ph/Pac).

Both **6b** and **6c** were prepared from **3b** (2.01 g, 5.96 mmol) and **3c** (1.60 g, 4.00 mmol), respectively. The thus obtained oily products were subjected to the subsequent cleavage reaction of the Pac group without further purification.

***N*$^{\alpha}$-*tert*-Butoxycarbonyl-*O*-[di(3-chlorobenzyloxy)phosphinyl] hydroxy α-amino acid DCHA or CHA salt (7). Boc-Ser(PO[OBzl(3Cl)]$_2$)-OH·DCHA (7a).** To a solution of Boc-Ser(PO[OBzl(3Cl)]$_2$)-OPac (**6a**; 3.00 g, 4.60 mmol) in 90% acetic acid (40 mL) was added zinc dust (4.51 g, 69.0 mmol) in several portions. After the same work up as described in the preparation of *O*-(dicyclopentyloxyphosphinyl) derivatives, the thus obtained oily substance was dissolved in a small amount of diethyl ether and hexane (1:1 v/v). DCHA (914 μL, 4.60 mmol) was added to the solution, and the mixture was allowed to stand in a refrigerator overnight. The thus precipitated crystalline DCHA salt was collected by filtration and recrystallized from diethyl ether. Yield: 3.00 g (91.2%); mp 105–108 °C; $[\alpha]_D^{25}$ +20.6° (*c* 1.06, EtOH). Anal. calcd for $C_{34}H_{49}N_2O_8Cl_2P$: C, 57.06; H, 6.90; N, 3.91. Found: C, 57.25; H, 7.04; N, 4.00%. ^{1}H NMR (CD_3OD): δ 1.15–2.06 (m, 29H, *t*Bu and CH_2/DCHA × 2), 3.11–3.21 (m, 2H, CH/DCHA × 2), 4.17 (t × 2,[37] 1H, *J* = 3.6 Hz, CH/Ser), 4.40 and 4.42 (d, each 1H,[37] *J* = 3.6 Hz, CH_2/Ser), 5.02–5.06 [d × 3,[37] 4H, *J* = 8 Hz, $C\underline{H}_2$Ph(3-Cl) × 2], and 7.28–7.38 [m, 8H, $CH_2\underline{Ph}$(3Cl) × 2]. ^{31}P NMR (CD_3OD): δ −1.00 (s).

Boc-Thr(PO[OBzl(3Cl)]$_2$)-OH·CHA (7b). Boc-Thr(PO[OBzl(3Cl)]$_2$)-OPac (**6b**) prepared as mentioned above was worked up in a similar manner as the preparation of **7a**. In this case, however, Boc-Thr(PO[OBzl(3Cl)]$_2$)—OH was first purified chromatographically as follows. An oily crude product was dissolved in satd aq $NaHCO_3$ (100 mL), and diluted with water (4 L); the solution was then applied to a column of Diaion HP 20 (Mitsubishi Chemical Co., Tokyo, 4.0 × 14 cm). The column was thoroughly washed with water, and Boc-Thr(PO[OBzl(3Cl)]$_2$)-ONa adsorbed on the resin was then eluted with 60% MeOH; the eluate was concentrated in vacuo. The residue was suspended in AcOEt (50 mL), and acidified with 10% aq citric acid. The AcOEt layer was washed with brine several times, dried over anhyd $MgSO_4$, and concentrated in vacuo. The thus obtained oily product (2.74 g, 5.00 mmol, 83.8%) was dissolved in a small amount of diethyl ether. CHA (605 μL, 5.00 mmol) was added to the solution, and the mixture was allowed to stand in a refrigerator overnight. The thus precipitated crystalline CHA salt **7b** was collected by filtration, and recrystallized from diethyl ether and hexane. Yield: 2.44 g (63.2% from **3b**); mp 106–109 °C; $[\alpha]_D^{19}$ +10.6° (*c* 1.01, EtOH). Anal. calcd for $C_{29}H_{41}N_2O_8Cl_2P$: C, 53.79, H, 6.38; N, 4.33. Found: C, 54.05; H, 6.61; N, 4.38%. ^{1}H NMR (CD_3OD): δ 1.15–2.01 (m, 22H, *t*Bu, CH_3/Thr, and CH_2/CHA × 5), 2.96–3.07 (m, 1H, CH/CHA), 4.07 and 4.09 (each d,[37] 1H in total, *J* = 3 Hz, α-CH/Thr), 4.98–5.11 [m, 5H, $C\underline{H}_2$Ph(3Cl) × 2 and β-CH/Thr], and 7.25–7.39 [m, 8H, $CH_2\underline{Ph}$(3Cl) × 2]. ^{31}P NMR (CD_3OD): δ −2.09 (s).

Boc-Tyr(PO[OBzl(3Cl)]$_2$)-OH·CHA (7c). Boc-Tyr(PO[OBzl(3Cl)]$_2$)-OPac (**6c**) prepared as mentioned above was worked up in a similar manner as the preparation of **7a**. In this case, the combined ether solution containing Boc-Tyr(PO[OBzl(3Cl)]$_2$)-OH after Zn reduction were washed successively with 10% aq citric acid, brine, 1% aq $NaHCO_3$, brine, 10% aq citric acid, and brine. The organic layer was dried over anhyd $MgSO_4$, and concentrated in vacuo. The thus obtained oily product was dissolved in a small amount of diethyl ether, and CHA (484 μL, 4.00 mmol) was added to the solution; the mixture was allowed to stand in a refrigerator overnight. The precipitated crystalline CHA salt was collected by filtration, and recrystallized from diethyl ether. Yield: 1.78 g (62.7% from **3c**); mp 120–124 °C; $[\alpha]_D^{26}$ +25.8° (*c* 1.05, EtOH). Anal. calcd for $C_{34}H_{43}N_2O_8Cl_2P$: C, 57.54; H, 6.11; N, 3.95. Found: C, 57.52; H, 6.22; N, 4.07%. ^{1}H NMR (CD_3OD): δ 1.30–2.00 (m, 19H, *t*Bu and CH_2/CHA × 5), 2.90 (dd, 1H, *J* = 13.5 and 7 Hz, 1/2 × CH_2/Tyr), 2.97–3.07 (m, 1H, CH/CHA), 3.15 (dd, 1H, *J* = 13.5 and 5 Hz, 1/2 × CH_2/Tyr), 4.17 (dd, 1H, *J* = 5 and 7 Hz, CH/Tyr), 5.10 and 5.14 [s, each 2H, $C\underline{H}_2$Ph(3Cl) × 2], and 7.02–7.34 [m, 12H, Ph/Tyr and $CH_2\underline{Ph}$(3Cl) × 2]. ^{31}P NMR (CD_3OD): δ −5.79 (s).

Benzyl tetraisopropylphosphorodiamidite [(*i*Pr$_2$N)$_2$P(OBzl)] (12)[30]. Under optimum conditions: To a solution of diisopropylamine (DIPA; 50.0 g, 544 mmol) in anhyd hexane (230 mL) was added dropwise a solution of trichlorophosphine (18.7 g, 136 mmol) in anhyd hexane (30 mL) over 40 min with stirring at 0 °C in an atmosphere of N_2. The mixture was stirred for 3 h at rt, and then heated under reflux for 4 days. To the reaction mixture neutralized with TEA was added a solution of benzyl alcohol (BzlOH; 14.7 g, 136 mmol) and TEA (13.8 g, 136 mmol) in anhyd diethyl ether (150 mL) over 1 h with stirring at 0 °C in an atmosphere of N_2. After stirring for 30 min at rt, the precipitated salt was filtered off, and the filtrate was concentrated in vacuo. The residue was dissolved in hexane (310 mL), and the solution was washed with acetonitrile (30 mL × 3), followed by evaporation in vacuo. An oily residue was purified by silica-gel column chromatography (400 g; silica gel was sufficiently pre-washed with hexane:TEA 10:1) and pure (*i*Pr$_2$N)$_2$P(OBzl) was obtained by elution with hexane:TEA (100:1) as a colorless oil. Yield 39.3 g (85.4%).

Benzyl 2,2,2-trichloroethyl diisopropylphosphoramidite [*i*Pr$_2$NP(OBzl)(OTce)] (13). To a solution of **12** (16.9 g, 50.0 mmol) and 1*H*-tetrazole (3.5 g, 50.0 mmol) in anhyd CH_2Cl_2 (200 mL) was added dropwise TceOH

(7.47 g, 50.0 mmol); the solution was stirred for 2 h at rt in an atmosphere of N_2. The reaction mixture was washed with satd aq $NaHCO_3$ (40 mL × 2), dried over anhyd $MgSO_4$ in an atmosphere of N_2, and concentrated in vacuo. An oily residue was purified by silica-gel column chromatography (500 g; silica gel was sufficiently pre-washed with hexane:TEA 10:1) and pure *i*Pr_2NP(OBzl)(OTce) was obtained by elution with hexane:TEA 100:1 as a colorless oil. Yield 18.9 g (97.4%).

HSP27(79–89)-Cys-NH_2 (9). Use of Boc-Ser-[PO(O*c*Pen)$_2$]-OH (**5a**), as a general procedure: Solid-phase synthesis of the target peptide was carried out by the 0.5 mmol scale standard protocol of the benzotriazole active ester method in the system software version 1.40 NMP/HOBt *t*-Boc.[38] The synthesis was started from *p*-methylbenzhydrylamine (MBHA) resin (1.22 g, 0.500 mmol), and 2.19 g (theoretical: 2.30 g) of protected peptide resin was obtained.

To a part of the thus obtained protected peptide resin (101 mg) were added TFMSA (408 μL), MTB (544 μL), *m*-cresol (453 μL), EDT (181 μL), and TFA (2.95 mL) (9:12:10:4:65 v/v). After stirring for 2 h at 0 °C, the mixture was filtered through a glass filter in an atmosphere of nitrogen, and the resin on the filter was washed with TFA (4 mL). The filtrate was poured into diethyl ether (200 mL) at 0 °C, and allowed to stand for 1 h in an ice bath. The thus obtained precipitates were collected by filtration using Fluoropore® filter (Sumitomo Electric Inc., Type: FP-022, Pore size: 0.22 μm), and dried over NaOH in vacuo. The crude peptide was dissolved in 0.1% TFA aq, and filtered through Millipore® filter (Millipore Corp., Type: HA, Pore size: 0.45 μm); the filtrate was then lyophilized. To a solution of the thus obtained powder (35.1 mg) in 0.1% TFA aq was added DTT (179 mg, 1.16 mmol), and pH of the solution was adjusted to 7–8 with ammonium acetate. After stirring for 1 h at room temperature, the crude product was purified by preparative RPHPLC. Each fraction containing the desired peptide was combined, and lyophilized to obtain **9** as a powder. Yield 11.4 mg (29% as 3TFA salt). FABMS (negative): $m/z = 1380$ $(M-H)^-$ (calcd for $C_{52}H_{95}N_{21}O_{19}SP-H$: 1379.7), 1494 $(M+TFA-H)^-$, 1608 $(M+2TFA-H)^-$. Amino acid analysis: $Asp_{1.02}Ser_{1.70}$*$Glu_{1.01}Gly_{1.03}Ala_{1.05}Val_{1.00}Cys_{0.53}Leu_{2.12}Arg_{2.07}$ (*PSer is analysed as Ser, although a part of this residue changes to the dehydroalanine residue under acidic conditions for hydrolysis, followed by rapid decomposition).

The synthesis of **9** using Boc-Ser[PO(O*c*Hex)$_2$]-OH (**8**)[13] was carried out by the same method as described above, except the protected peptide resin was treated with a mixture of TFMSA and additives in TFA for 4 h at rt (25 °C), and resulted in a 24% yield after preparative RPHPLC.

HSP27(10–20)-Cys-NH_2 (14). Fmoc-Ser[PO(OBzl)-(OH)]-OH used for the experiment was prepared as reported in a previous paper.[30] Solid-phase synthesis of the target peptide was carried out by the 0.25 mmol scale standard protocol of FastMoc 0.25 MonPvPk (Monitor Previous Peak). The synthesis was started from Rink amide resin (0.521 g, 0.250 mmol), and 1.046 g (theoretical: 1.128 g) of protected peptide resin was obtained.

To a part of the thus obtained protected peptide resin (100 mg) were added TFA (1.65 mL), PhOH (100 μL), H_2O (100 μL), MTB (100 μL), and EDT (50 μL) (82.5:5:5:5:2.5 v/v). After stirring for 2 h at rt (20 °C), the mixture was filtered through a glass filter in an atmosphere of nitrogen, and the resin on the filter was washed with TFA (4 mL). The filtrate was worked up in a similar manner as described in the synthesis of **9**. Yield 22.5 mg (49% as 3TFA salt). FABMS (negative): $m/z = 1568$ $(M-H)^-$ (calcd for $C_{68}H_{105}N_{20}O_{19}SP-H$: 1567.7), 1682 $(M+TFA-H)^-$. Amino acid analysis: $Ser_{1.64}$*$Glu_{1.01}Pro_{2.02}Cys_{0.92}Leu_{2.03}Phe_{1.00}Trp_{0.61}Arg_{2.00}$ (*including Ser from PSer).

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References

1. Perich, J. W.; Johns, R. B. *J. Org. Chem.* **1983**, *53*, 4103; *Aust. J. Chem.* **1990**, *43*, 1609; **1991**, *44*, 397, 405, 1683.

2. Perich, J. W.; Alewood, P. F.; Johns, R. B. *Aust. J. Chem.* **1991**, *44*, 233, 253; Perich, J. W.; Johns, R. B.; Reynolds, E. C. *ibid* **1992**, *45*, 385; Perich, J. W.; Kelly, D. P.; Reynolds, E. C. *Int. J. Pept. Protein Res.* **1992**, *40*, 81.

3. Tsukamoto, M.; Kato, R.; Ishiguro, K.; Uchida, T.; Sato, K. *Tetrahedron Lett.* **1991**, *32*, 7083.

4. Paquet, A.; Johns, M. *Int. J. Pept. Protein Res.* **1990**, *36*, 97; Paquet, A. *ibid.* **1992**, *39*, 82.

5. Bannwarth, W.; Küng, E. *Tetrahedron Lett.* **1989**, *30*, 4219.

6. Ueno, Y.; Suda, F.; Taya, Y.; Noyori, R.; Hayakawa, H.; Hata, T. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 823.

7. Two step reactions are required for final deprotection procedure, and the use of the phenyl group which must be removed by catalytic hydrogenation over PtO_2 is restricted to peptides devoid of aromatic or sulfur-containing amino acids.

8. Wakamiya, T.; Kawahara, M.; Kusumoto, S.; Imajoh-Ohmi, S.; Kanegasaki, S. In *Peptide Chemistry 1991*; Suzuki, A. Ed.; Protein Research Foundation: Osaka, 1992; pp 383–388.

9. Wakamiya, T. *Chem. Express* **1992**, *7*, 577.

10. Wakamiya, T.; Saruta, K.; Kusumoto, S.; Nakajima, K.; Yoshizawa-Kumagaye, K.; Imajoh-Ohmi, S.; Kanegasaki, S. *Chem. Lett.* **1993**, 1401.

11. Wakamiya, T.; Saruta, K.; Kusumoto, S.; Aimoto, S.; Yoshizawa-Kumagaye, K.; Nakajima, K. In *Peptide Chemistry 1993*, Okada, Y. Ed. Protein Research Foundation, Osaka, 1994; pp 17–20.

12. Sato, K.; Ishiguro, K.; Omori, A.; Kim, J. I.; Uchida, T.; Saruta, K.; Wakamiya, T. In *Peptide Chemistry 1993*, Okada, Y. Ed.; Protein Research Foundation: Osaka, 1994; pp 109–112.

13. Wakamiya, T.; Saruta, K.; Yasuoka, J.; Kusumoto, S. *Bull. Chem. Soc. Jpn* **1995**, *68*, 2699.

14. Abbreviations used: Boc, *tert*-butoxycarbonyl; BOP, *O*-(benzotriazol-1-yl)tris(dimethylamino)phosphonium hexafluorophosphate; *t*Bu: *tert*-butyl; CHA, cyclohexylamine; mCPBA, *m*-chloroperbenzoic acid; DCC, dicyclohexylcarbodiimide; DCHA, dicyclohexylamine; DIEA, diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; DTT, dithiothreitol; EDT, ethanedithiol; Fmoc, 9-fluorenylmethoxycarbonyl; HATU, *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; MTB, methylthiobenzene (thioanisole); NMP, *N*-methylpyrrolidone; Pac, phenacyl; Pmc, 2,2,5,7,8-pentamethylchroman-6-sulfonyl; *i*Pr, isopropyl; PSer, phosphoserine; Tce, 2,2,2-trichloroethyl; TFA, trifluoroacetic acid; TFMSA, trifluoromethanesulfonic acid; Trt, triphenylmethyl (trityl).

15. Fujii, N.; Funakoshi, S.; Sasaki, T.; Yajima, H. *Chem. Pharm. Bull.* **1977**, *25*, 3096; Fujii, N.; Otaka, A.; Ikemura, O.; Hatano, M.; Okamachi, A.; Funakoshi, S.; Sakurai, M.; Shioiri, T.; Yajima, H. *Chem. Pharm. Bull.* **1987**, *35*, 3447. A hard acid treatment for removing all of the protecting groups and cleaving of peptides from the resin is generally carried out at 0 °C for 2 h.

16. A partial cleavage of one of the Bzl groups results in a formation of *O*-[(monobenzyloxy)hydroxyphosphinyl] derivative which is still usable for synthesizing phosphopeptides. Thus, we had succeeded to synthesize phosphopeptides related to EGF receptor protein, Cys-[EGFRP-(649–659)][8,10,11] and HSP27-(85–94)-Cys-NH_2[11] by the use of Boc-Thr[PO(OBzl)$_2$]-OH and Boc-Ser[PO(OBzl)$_2$]-OH, respectively. On the other hand, the phosphoamino acid residue with the free *O*-phosphono group formed by a complete cleavage of the *O*-phosphono-protecting groups tends to cause an undesirable side reaction.[23]

17. There are several reports concerning the synthesis of phosphopeptides using phosphoamino acid derivatives with the free *O*-phosphono group: Zardeneta, G.; Chen, D.; Weintraub, S. T.; Klebe, R. *Anal. Biochem.* **1990**, *190*, 340; Chatterjee, S.; Goldstein, B. J.; Csermely, P.; Shoelson, S. E. In *Peptides*; Smith, J.; Rivier, J. Eds.; ESCOM: Leiden, 1992; pp 553–555; Ottinger, E. A.; Shekels, L. L.; Bernlohr, D. A.; Barany, G. *Biochemistry* **1993**, *32*, 4354. Ottinger et al. reported a successful synthesis of phosphopeptides by the use of Fmoc-phosphotyrosine with the free *O*-phosphono group, while Zardeneta et al. recommended to protect this group. Furthermore, we observed a formation of dimer peptide with pyrophosphate structure in a synthesis of phosphoserine-containing peptide by the use of Fmoc-phosphoserine with the free *O*-phosphono group.[23]

18. A part of this work was presented at the 33rd Japanese Peptide Symposium, Sapporo, October, 1995, and published in *Peptide Chemistry 1995*; Nishi, N. Ed.; Protein Research Foundation: Osaka, 1996; pp 17–20.

19. Otaka et al. have recently reported the use of *O*-(dimethyloxyphosphinyl) β-hydroxy α-amino acid derivatives as building blocks for the Boc strategy: Otaka, A.; Miyoshi, K.; Roller, P. R.; Burke, T.; Tamamura, H.; Fujii, N. *J. Chem. Soc. Chem. Commun.* **1995**, 387. Although the methyl group is removable by trimethylsilyl trifluoromethanesulfonate (TMSOTf) and additives in TFA, two-step deprotection protocol is required for the complete removal.

20. Lacombe, J.-M.; Andriamanampisoa, F.; Pavia, A. A. *Int. J. Pept. Protein Res.* **1990**, *36*, 275.

21. Fmoc-strategy is basically applicable to the synthesis of phosphotyrosine-containing peptides. Kitas, E. A.; Knorr, R.; Trzeciak, A.; Bannwarth, W. *Helv. Chim. Acta* **1991**, *74*, 1314; Bannwarth, W.; Kitas, E. A. *Helv. Chim. Acta* **1992**, *75*, 707.

22. β-Elimination of phosphate does not occur during repeated treatment with an organic tertiary amine for washing the resin after de-*tert*-butoxycarbonylation with TFA; an excess amount of bulky DIEA is generally used for this purpose. Other organic bases such as *N*-methylmorpholine or triethylamine are also usable so far as we examined.[8,10] Furthermore, we suggest that the phosphoamino acid residue at the *N*-terminus is generally stable during base treatment after de-*tert*-butoxycarbonylation; in fact, we have never observed a side reaction due to the β-elimination of phosphate at this stage.

23. Wakamiya, T.; Saruta, K.; Yasuoka, J.; Kusumoto, S. *Chem. Lett.* **1994**, 1099; In *Peptide Chemistry 1994*; Ohno, M. Ed.; Protein Research Foundation: Osaka, 1995; pp 5–8.

24. Gaestel, M.; Schröder, W.; Benndorf, R.; Lipmann, C.; Buchner, K.; Hucho, F.; Erdmann, V. A.; Bielka, H. *J. Biol. Chem.* **1991**, *266*, 14721.

25. Landry, J.; Lambert, H.; Zhou, M.; Lavoie, J. N.; Hickey, E.; Weber, L. A.; Anderson, C. W. *J. Biol. Chem.* **1992**, *267*, 794.

26. Dreef, C. E.; Elie, C. J. J.; Hoogerhout, P.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1988**, *29*, 6513.

27. Perich, J. W.; Johns, R. B. *Aust. J. Chem.* **1990**, *43*, 1623; **1991**, *44*, 389.

28. Plausible pathways of the undesirable reactions are briefly explained as follows: (1) the first intermediate RCO-Ser[PO(OR′)$_2$]-$OP^+(NMe_2)_3$ formed by reaction of a protected phosphoamino acid with BOP is instantly converted into dehydroalanine (Dha) derivative, i.e. RCO-Dha-$OP^+(NMe_2)_3$, through β-elimination of phosphate in the presence of DIEA; (2) the thus formed Dha derivative partly couples with amine component as it is or after changing to Bt ester; (3) the remaining RCO-Dha-$OP^+(NMe_2)_3$ is converted into RCO-Ser(Bt)-$OP^+(NMe_2)_3$ by addition of HOBt to the α,β-unsaturated bond, followed by coupling with amine component as it is or as Bt ester; (4) the Dha residue is not subject to the addition of HOBt after changing to Bt ester or coupling with amine component. Consequently, we now suggest that the highly activated species with $P^+(NMe_2)_3$ ester is susceptible to the β-elimination of phosphate or the addition of HOBt to the Dha residue.

29. The synthesis of HSP27(85–94)-Cys-NH_2 had been carried out by the use of Boc-Ser[PO(OBzl)$_2$]-OH.[11] Comparison of this result with that obtained by the use of **5a** or **8** is currently undertaken.

30. Wakamiya, T.; Nishida, T.; Togashi, R.; Saruta, K.; Yasuoka, J.; Kusumoto, S. *Bull. Chem. Soc. Jpn* **1996**, *69*, 465. Compound **10** was obtained in an 81% yield through four reaction steps from Fmoc-Ser-OH as follows: (1) esterification with PacBr; (2) phosphite-forming reaction with amidite **13** (3) oxidation with mCPBA to give Fmoc-Ser[PO(OBzl)(OTce)]-OPac; and (4) simultaneous removal of the Pac and Tce groups with Zn/AcOH. Fmoc-Thr[PO(OBzl)(OH)]-OH (**11**) was similarly prepared in an 86% yield.

31. An alternative method for preparing **10** and **11** from the corresponding N^{α}-Fmoc-β-hydroxy α-amino acids has recently

been reported: Vorherr, T.; Bannwarth, W. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2661. The synthesis was carried out as follows: (1) esterification with allyl bromide (AllBr); (2) phosphite-forming reaction with $i Pr_2NP(OBzl)_2$; (3) oxidation with *t*BuOOH to give Fmoc-Ser/Thr[$PO(OBzl)_2$]-OAll; (4) removal of the All group with $Pd(PPh_3)_4/Bu_3SnH$, and (5) selective cleavage of one of the *O*-phosphono-protecting groups with NaI. The overall yield of each derivative isolated as Na salt was of 37% (**10**) and 38% (**11**), respectively.

32. King, D. S.; Fields, C. G.; Fields, G. B. *Int. J. Pept. Protein Res.* **1990**, *36*, 255.

33. The use of N^{α}-allyloxycarbonyl (Alloc) derivative of phosphoamino acids was recently reported: Lacombe, J. M.; Andriamanampisoa, F.; Pavia, A. A. *Int. J. Pept. Protein Res.* **1990**, *36*, 275; Shapiro, G.; Büchler, D.; Dalvit, C.; Fernandez, C.; Gomez-Lor, B.: Pombo, E.; Stauss, U.; Swoboda, R. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 409. More examinations may be required to establish a methodology for synthesizing phosphopeptides based on the Alloc or combined Alloc-Fmoc strategy.

34. Dittmer, J. D.; Lester, R. L. *J. Lipid Res.* **1964**, *5*, 126.

35. Shimidzu, T.; Yamana, K.; Maikuma, S. *Tetrahedron Lett.* **1984**, *25*, 4237.

36. Tanaka, T.; Tamatsukuri, S.; Ikehara, M. *Tetrahedron Lett.* **1986**, *27*, 199.

37. The presence of conformers can be suggested.

38. The synthesis was carried out according to the standard protocol which is originally specified in the system software. Therefore, the synthetic result of each peptide mentioned in the present study is reproducible so far as we use the same software.

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