Preparation of Phosphoamino Acid Derivatives with Acid Stable *O*-Phosphono-Protection for the Boc-Mode Solid-Phase Synthesis of Phosphopeptides[1)]

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Boc-phosphoamino acid derivatives with *O*-[di(4-nitrobenzyl)- or dicyclohexylphosphono]-protection were prepared for application to the Boc-mode solid-phase synthesis of phosphopeptides. These protecting groups are both stable to TFA, but removable with a combination of trifluoromethanesulfonic acid and methylthiobenzene in TFA. Of these derivatives, N^{α}-Boc-*O*-(dicyclohexylphosphono)serine and N^{α}-Boc-*O*-(dicyclohexylphosphono)threonine were obtained as crystalline compounds to be favorably utilized as starting materials for solid-phase synthesis using an automated peptide synthesizer. On the other hand, N^{α}-Boc-*O*-(dicyclohexylphosphono)tyrosine and all of the *O*-[di(4-nitrobenzyl)]phosphono derivatives were prepared as crystalline cyclohexylammonium or dicyclohexylammonium salts.

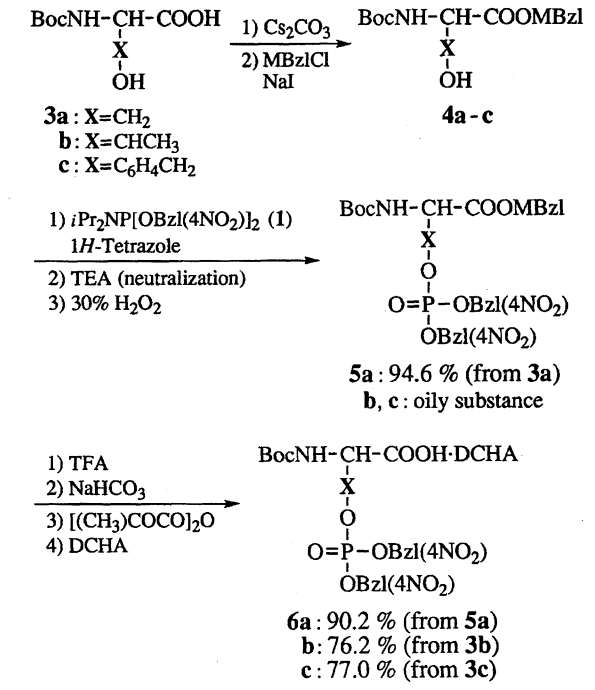
The synthesis of phosphopeptides based on the Boc strategy requires proper Boc-phosphoamino acid derivatives with acid-stable *O*-phosphono-protection; the phenyl group has been most widely used for this purpose. However, the application of the phenyl group was limited to the synthesis of peptides devoid of aromatic or sulfur-containing amino acids, since it must be removed by catalytic hydrogenation over PtO_2 under pressure.[2—9)]

We recently proposed the 4-nitrobenzyl (4-nitrophenylmethyl, Bzl($4NO_2$)) or cyclohexyl (*c*Hex) group as an alternative to the phenyl group.[10)] In general, introducing an electron-withdrawing substituent to the phenyl ring increases the acid stability of the benzyl-type protecting groups.[11)] Indeed, the Bzl($4NO_2$) group shows remarkable stability to any acidic conditions tested for removing the Boc group. Furthermore, we confirmed that the Bzl($4NO_2$) group is readily removable by the hard acid deprotection procedure using a combination of trifluoromethanesulfonic acid (TFMSA), methylthiobenzene (MTB) and trifluoroacetic acid (TFA) without cleavage of the phosphoric part.[10)] Similar acid stability and removability were also observed in the *c*Hex group, which is generally utilized for protecting the side-chain carboxyl group in the aspartic acid or glutamic acid residue to prevent imide formation under acidic conditions during peptide synthesis.[12)] In the present paper we describe convenient procedures for preparing Boc-phosphoamino acid derivatives whose phosphoric residues are protected with the Bzl($4NO_2$) or *c*Hex group.

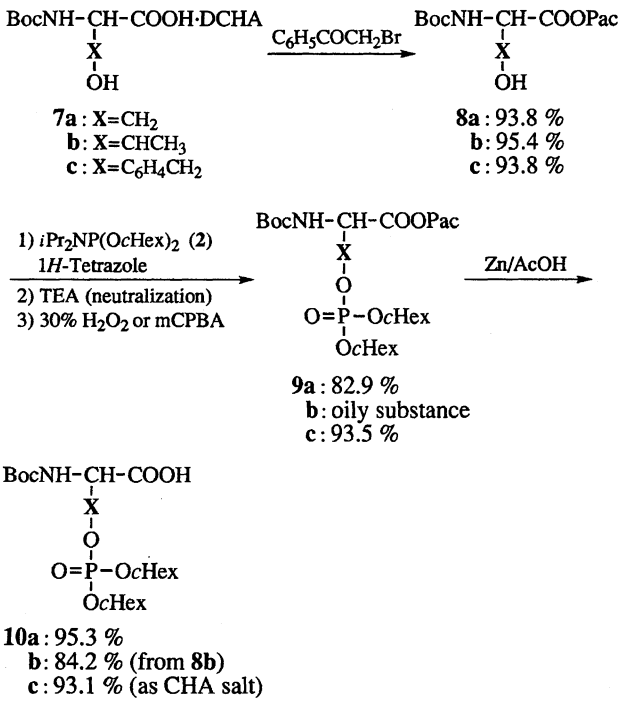
Results and Discussion

Two amidites as phosphitylation reagents, i.e., $i\mathrm{Pr_2NP[OBzl(4NO_2)]_2}$ (**1**) and $i\mathrm{Pr_2NP(O}c\mathrm{Hex)_2}$ (**2**), were first prepared by general methods.[13,14)] Of these reagents, the former was obtained as a crystalline compound, which can be easily purified by recrystallization. The phosphoamino acid derivatives protected by the Bzl($4NO_2$) group were prepared as shown in Scheme 1. In preparing Boc-hydroxyamino acid 4-methoxybenzyl (4-methoxyphenylmethyl, MBzl) esters (**4a—c**), the reaction of Boc-amino acids (**3a—c**) Cs salts with 4-methoxybenzyl chloride (MBzlCl) was effectively accelerated by the addition of NaI. The phosphitylation of **4a—c** with amidite **1** was carried out in the presence of 1*H*-tetrazole. The phosphite formed was then oxidized with 30% H_2O_2 or *m*-chloroperbenzoic acid (mCPBA).[15)] The phosphorylation products **5a—c** were treated once with TFA to remove both the Boc and MBzl groups; the amino group was then reprotected with the Boc group. The thus-obtained N^{α}-Boc-*O*-[di(4-nitrobenzyl)phosphono]hydroxyamino acids were finally isolated as crystalline dicyclohexylammonium (DCHA) salts **6a—c** (Scheme 1).

N^{α}- Boc- *O*- (dicyclohexylphosphono)hydroxyamino acids (**10a—c**) were next prepared by phosphorylation of the corresponding Boc-hydroxyamino acid phenacyl (Pac) esters (**8a—c**) with amidite **2**. The Pac group in the phosphorylation products **9a—c** was selectively removed by Zn/AcOH reduction to give **10a—c**, respectively (Scheme 2). Of these derivatives, **10a** and **10b** were obtained as crystalline compounds, while **10c**



Scheme 1.



Scheme 2.

was crystallized only as cyclohexylammonium (CHA) salt.[16,17]

In previous papers[1,10] we had noted that the Bzl($4NO_2$) protection might be practical for the synthesis of phosphopeptides by the Boc strategy, since the phosphitylation reagent **1** can be obtained as a crystalline compound. However, we now recommend the utilization of *O*-dicyclohexylphosphono derivatives **10a**—**c**, which are obtainable as crystalline or powderly substances,[17] for the Boc-mode solid-phase method using an automated peptide synthesizer. Indeed, a part of these derivatives had already been applied to the solid-phase synthesis of phosphopeptides related to heat-shock protein and tau protein kinase I.[18,19] Although details concerning a synthetic study of phosphopeptides will be reported in the near future, the following important facts are briefly given:

1) both the *c*Hex and Bzl($4NO_2$) groups are sufficiently stable under general acidic conditions for removing the Boc group;

2) no β-elimination of the phosphates was observed under basic conditions for neutralization with *N*,*N*-diisopropylamine after de-*t*-butoxycarbonylation with TFA during the course of the peptide-chain elongation procedure;

and 3) the *c*Hex and Bzl($4NO_2$) groups are removable with TFMSA : MTB : *m*-cresol : 1,2-ethanedithiol : TFA (8 : 10 : 9 : 4 : 69 v/v) within 4 h at 0 °C—r.t. and 2 h at 0 °C, respectively.[18]

Experimental

All of the melting points are uncorrected. The specific rotations were measured on a Perkin–Elmer 241 polarimeter. ^{1}H NMR and ^{31}P NMR spectra were recorded on JEOL JNM-EX 270 and JNM-GSX 400 NMR spectrometers, respectively. The chemical shifts in ^{1}H NMR are given in δ values from TMS used as the internal standard, and those in ^{31}P NMR from 75% aqueous phosphoric acid used as the external standard. Fast-atom bombardment mass spectra (FAB-MS) were obtained on a JEOL JMS SX-270 mass spectrometer. Silica-gel column chromatography was carried out with Merck silica gel 60 (Art. 9385, 230—400 mesh) at medium pressure (1—5 kg cm^{-2}). The compounds containing phosphorus were characterized by a color reaction with the Dittmer–Lester reagent.[20]

***N*,*N*-Diisopropyl-di(4-nitrobenzyl)phosphoramidite (1).** To a solution of dichloro-*N*,*N*-diisopropylphosphoramidite[21,22] (4.10 g, 20.3 mmol) in anhydrous tetrahydrofuran (THF) (50 ml) was added dropwise a solution of 4-nitrobenzyl alcohol (6.21 g, 40.6 mmol) and triethylamine (TEA) (4.52 g, 44.7 mmol) in THF (30 ml) with stirring at 0 °C in an atmosphere of nitrogen. After stirring for 2 h at 0 °C, the reaction mixture was concentrated in vacuo. To the residue were added ethyl acetate (AcOEt) and 5% aqueous $NaHCO_3$. The organic layer separated was washed with 5% aqueous $NaHCO_3$ (×2), dried over anhydrous $MgSO_4$, and evaporated in vacuo. The thus-obtained solid residue was triturated with hexane and collected by filtration. The crude product (8.41 g, 95.1%) was dissolved in AcOEt (80 ml) and hexane (600 ml) containing TEA (3 ml). After active charcoal (1 g) was added to the solution, the resulting mixture was allowed to stand for a few minutes. Charcoal was filtered off, and the filtrate was concentrated in vacuo. The residue was dissolved in a small amount of AcOEt and reprecipitated by the addition of hexane to produce pale-yellow prisms. Yield 7.06 g (79.9%); mp 78—79 °C. Found: C, 55.19; H, 6.00; N, 9.65%. Calcd for $C_{20}H_{26}N_3O_6P$: C, 55.17; H, 6.01; N, 9.65%. FAB-MS *m*/*z* 436.0 [(M+H)$^+$] (calcd 436.2). ^{1}H NMR ($CDCl_3$) δ=1.23 (12H, d, *J*=6.5 Hz, CH-

($C\underline{H}_3)_2\times2$), 3.68—3.77 (2H, m, $C\underline{H}(CH_3)_2\times2$), 4.80—4.86 (4H, m, $C\underline{H}_2Ph(4NO_2)\times2$), and 7.51 and 8.19 (each 4H, d, $J=8.4$ Hz, $Ph(4NO_2)\times2$). ^{31}P NMR (DMSO-d_6) $\delta=9.30$ (s).

Dicyclohexyl-*N,N*-diisopropylphosphoramidite (2). The amidite **2** was prepared by the reaction of dichloro-*N,N*-diisopropylphosphoramidite (20.2 g, 100 mmol) in THF (300 ml) with cyclohexyl alcohol (20.0 g, 200 mmol) and TEA (24.5 g, 240 mmol) in THF (30 ml) under the same conditions as mentioned in the preparation of **1**. The thus-obtained oily residue was dissolved once in hexane; the formed insoluble material was then filtered off. The filtrate was concentrated in vacuo, and the concentration was finally carried out at 40—50 °C in order to remove any remaining cyclohexyl alcohol. The resulting oily amidite **2** (30.3 g, 92.1%) was used for a subsequent reaction without further purification. FAB-MS m/z 330.0 [$(M+H)^+$] (calcd 330.2). ^{1}H NMR (DMSO-d_6) $\delta=1.11$—1.43 (24H, m, $CH_2\times6$ and $CH(C\underline{H}_3)_2\times2$), 1.65—1.74 (8H, m, $CH_2\times4$), and 3.49—3.74 (4H, m, CH/*c*Hex×2 and $C\underline{H}(CH_3)_2\times2$). ^{31}P NMR (DMSO-$d_6$) $\delta=5.71$ (s).

N^α-*t*-Butoxycarbonyl-*O*-[di-(4-nitrobenzyl)phosphono]hydroxyamino Acid 4-Methoxybenzyl Ester (5). Boc–Ser(PO[OBzl(4NO$_2$)]$_2$)–OMBzl (5a) (as a General Procedure): A solution of Boc–Ser–OH (1.19 g, 5.80 mmol) and Cs_2CO_3 (945 mg, 2.90 mmol) in H_2O (5 ml) and MeOH (15 ml) was evaporated in vacuo. The residue was dissolved in *N,N*-dimethylformamide (DMF) (30 ml), followed by evaporation to dryness in vacuo; these operations were repeated two more times, respectively. The thus-prepared Boc–Ser–OCs was dissolved in DMF (12 ml); to the solution were added MBzlCl (999 mg, 6.38 mmol), NaI (956 mg, 6.38 mmol), and Cs_2CO_3 (94.5 mg, 290 μmol). The mixture was first stirred at room temperature overnight, and then an insoluble inorganic salt was filtered off. After the filtrate was concentrated in vacuo, the residue was purifed by silica-gel column chromatography (silica gel: 100 g, column: 23×480 mm, eluent: $CHCl_3$) to afford Boc–Ser–OMBzl (**4a**).

To a solution of the oily product **4a** and the amidite **1** (3.00 g, 6.90 mmol) in THF (11 ml) was added 1*H*-tetrazole (1.21 g, 17.3 mmol). The solution was first stirred for 1 h, and then concentrated in vacuo. The residue was dissolved in AcOEt (20 ml), and the solution was neutrallized with TEA. To the solution was added 30% H_2O_2 (0.60 ml, 7.6 mmol) at 0 °C. After stirring for 1.5 h, water (5 ml) was added to the reaction mixture; the precipitated N^α-Boc-*O*-[di-(4-nitrobenzyl)phosphono]serine MBzl ester (Boc–Ser(PO[OBzl(4NO$_2$)]$_2$)–OMBzl) (**5a**) was collected by filtration. The organic layer separated from the filtrate was washed with water several times, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was triturated with diethyl ether containing a small amount of methanol to obtain a second crop of **5a**. The combined crops of **5a** (3.65 g, 94.6%) were subjected to the preparation of **6a** without further purification. A part of the product was recrystallized from $CHCl_3$-diethyl ether to obtain an analytical sample. Mp 103.5—104 °C; $[\alpha]_D^{24}-1.8°$ (*c* 1.1, $CHCl_3$). Found: C, 53.08; H, 5.03; N, 6.30%. Calcd for $C_{30}H_{34}N_3O_{13}P$: C, 53.34; H, 5.07; N, 6.22%. ^{1}H NMR (DMSO-d_6) $\delta=1.35$ (9H, s, *t*-Bu), 3.71 (3H, s, Ph(4O$\underline{Me}$)), 4.25—4.29 (2H, m, CH_2/Ser), 4.40—4.42 (1H, m, CH/Ser), 5.05 (2H, s, $C\underline{H}_2$Ph(4OMe)), 5.19 and 5.20 (each 2H, d, $J=8.2$ Hz, $C\underline{H}_2Ph(4NO_2)\times2$), 6.87 and 7.25 (each 2H, d, $J=8.6$ Hz, $\underline{Ph}$(4OMe)), 7.43 (1H, d, $J=7.9$ Hz, NH), and 7.59 and 8.19 (each 4H, d, $J=8.8$ Hz, $Ph(4NO_2)\times2$). ^{31}P NMR (DMSO-d_6) $\delta=-0.84$ (s).

Boc–Thr(PO[OBzl(4NO$_2$)]$_2$)–OMBzl (**5b**) and Boc–Tyr(PO[OBzl(4NO$_2$)]$_2$)–OMBzl (**5c**) were prepared by the same method as described above. In these cases, however, each reaction mixture in AcOEt after the oxidation procedure was directly washed with water several times, since no precipitate was observed. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The oily **5b** and **5c** were subjected to the preparation of **6b** and **6c**, respectively, after purification by silica-gel column chromatography ($CHCl_3$: acetone=20 : 1 v/v).

N^α-*t*-Butoxycarbonyl-*O*-[di-(4-nitrobenzyl)phosphono]hydroxyamino Acid Dicyclohexylammonium Salt (6). Boc–Ser(PO[OBzl(4NO$_2$)]$_2$)–OH·DCHA (6a) (as a General Procedure): To a solution of **5a** (1.50 g, 2.22 mmol) in CH_2Cl_2 (5 ml) was added TFA (5 ml) at 0 °C. The solution was stirred for 1 h at room temperature, and then concentrated in vacuo. The residue was first washed with diethyl ether by trituration and decantation (×3), and then dissolved in 50% aqueous dioxane (24 ml). To the solution adjusted to pH 8 with TEA was added di-*t*-butyl dicarbonate (581 mg, 2.66 mmol). After stirring for 2 h, the reaction mixture was acidified with citric acid and concentrated in vacuo to remove dioxane. The aqueous solution was extracted with AcOEt (×3), and the combined extracts were washed with brine (×3). The organic layer was first dried over Na_2SO_4 and then concentrated in vacuo. To a solution of the residue in diethyl ether (10 ml) was added dicyclohexylamine (DCHA) (403 mg, 2.22 mmol); the mixture was then allowed to stand overnight in a refrigerator. The crystalline product was collected by filtration and recrystallized from ethanol–diethyl ether–hexane to obtain pure Boc–Ser(PO[OBzl(4NO$_2$)]$_2$)–OH·DCHA (**6a**). Yield 1.48 g (90.2%); mp 116.5—120 °C (decomp); $[\alpha]_D^{24}+20.7°$ (*c* 1.02, EtOH). Found: C, 55.34; H, 6.78; N, 7.50%. Calcd for $C_{34}H_{49}N_4O_{12}P$: C, 55.43; H, 6.70; N, 7.60%. ^{1}H NMR (DMSO-d_6) $\delta=1.06$—1.94 (29H, m, CH_2/DCHA×10 and *t*-Bu), 2.99 (2H, m, CH/DCHA×2), 3.82 (1H, m, CH/Ser), 4.27—4.35 (2H, m, CH_2/Ser), 5.20 (4H, m, $C\underline{H}_2Ph(4NO_2)\times2$), 6.15 (1H, d, NH), and 7.65 and 8.20 (each 4H, d, $J=8.6$ Hz, $Ph(4NO_2)\times2$). ^{31}P NMR (DMSO-d_6) $\delta=-0.61$ (s).

Boc–Thr(PO[OBzl(4NO$_2$)]$_2$)–OH·DCHA (**6b**) and Boc–Tyr(PO[OBzl(4NO$_2$)]$_2$)–OH·DCHA (**6c**) were prepared by the same method as described above.

6b: Yield 76.2% from **3b**; mp 147—148 °C; $[\alpha]_D^{24}+9.2°$ (*c* 1.0, EtOH). Found: C, 56.21; H, 6.86; N, 7.45%. Calcd for $C_{35}H_{51}N_4O_{12}P$: C, 55.99; H, 6.85; N, 7.46%. ^{1}H NMR (DMSO-d_6) $\delta=1.06$—1.97 (32H, m, CH_2/DCHA×10, *t*-Bu, and CH_3/Thr), 2.96 (2H, m, CH/DCHA×2), 3.80—3.83 (1H, m, α-CH/Thr), 4.96—5.02 (1H, m, β-CH/Thr), 5.13—5.20 (4H, m, $C\underline{H}_2Ph(4NO_2)\times2$), 5.89 (1H, d, $J=7.8$ Hz, NH), and 7.66 and 8.19 (each 4H, d, $J=8.6$ Hz, $Ph(4NO_2)\times2$). ^{31}P NMR (DMSO-d_6) $\delta=-1.86$ (s).

6c: Yield 77.0% from **3c**; mp 118—121 °C; $[\alpha]_D^{24}+25.2°$ (*c* 1.01, EtOH). Found: C, 58.86; H, 6.56; N, 6.86%. Calcd for $C_{35}H_{51}N_4O_{12}P$: C, 59.11; H, 6.57; N, 6.89%. ^{1}H NMR (DMSO-d_6) $\delta=1.10$—1.93 (29H, m, CH_2/DCHA×10 and *t*-Bu), 2.93—3.08 (4H, m, CH_2/Tyr and CH/DCHA×2),

3.86—3.88 (1H, m, CH/Tyr), 5.32 (4H, d, J=8.6 Hz, CH_2Ph(4NO_2)×2), 6.03 (1H, d, J=5.8 Hz, NH), 7.05 and 7.17 (each 2H, d, J=8.4 Hz, Ph/Tyr), and 7.61 and 8.20 (each 4H, d, J=8.8 Hz, Ph(4NO_2)×2). ^{31}P NMR (DMSO-d_6) δ=−5.69 (s).

N^{α}-(*t*-Butoxycarbonyl)hydroxyamino Acid Phenacyl Ester (8). Boc–Ser–OPac (8a) (as a General Procedure): To a solution of Boc–Ser–OH·DCHA (**7a**) (5.25 g, 13.6 mmol) in DMF (70 ml) were added phenacyl bromide (PacBr) (2.98 g, 15.0 mmol) and TEA (137 mg, 1.36 mmol). After stirring for 3 h, the precipitated DCHA·HBr was filtered off. The filtrate was concentrated in vacuo, and the residue was dissolved in AcOEt, followed by filtration to remove insoluble DCHA·HBr. The filtrate was washed with saturated aqueous $NaHCO_3$ (×2) and water (×2), dried over Na_2SO_4, and concentrated in vacuo. To a solution of the residue in diethyl ether (10 ml) was added hexane (10—20 ml). The solution was allowed to stand overnight in a refrigerator. The crystalline product was collected by filtration and recrystallized from AcOEt–hexane to obtain pure Boc–Ser–OPac (**8a**). Yield 4.13 g, (93.8%), mp 90—91 °C, $[\alpha]_D^{24}$ −30.3° (c 1.00, EtOH). Found: C, 59.29; H, 6.59; N, 4.39%. Calcd for $C_{16}H_{21}NO_6$: C, 59.43; H, 6.55; N, 4.33%. ^{1}H NMR (DMSO-d_6) δ=1.40 (9H, s, t-Bu), 3.67—3.82 (2H, m, CH_2/Ser), 4.22—4.29 (1H, m, CH), 4.89 (1H, t, OH), 5.48 and 5.56 (each 1H, d, J=17.0 Hz, CH_2/Pac), 6.98 (1H, d, NH), and 7.52—7.98 (5H, m, Ph).

Boc–Thr–OPac (**8c**) and Boc–Tyr–OPac (**8c**) were prepared by the same method as described above.

8b: Yield 95.4%; mp 110—112 °C; $[\alpha]_D^{24}$ −30.4° (c 1.02, MeOH). Found: C, 60.53; H, 6.65; N, 4.06%. Calcd for $C_{17}H_{23}NO_6$: C, 60.52; H, 6.87; N, 4.15%. ^{1}H NMR (DMSO-d_6) δ=1.17 (3H, d, CH_3/Thr), 1.41 (9H, s, t-Bu), 4.02—4.16 (2H, m, α- and β-CH/Thr), 4.75 (1H, bs, OH), 5.52 (2H, s, CH_2/Pac), 6.61 (1H, d, NH), and 7.53—7.99 (5H, m, Ph).

8c: Yield 93.8%; mp 106—107 °C, $[\alpha]_D^{24}$ −15.8° (c 1.00, MeOH). Found: C, 66.00; H, 6.29; N, 3.43%. Calcd for $C_{22}H_{25}NO_6$: C, 66.15; H, 6.31; N, 3.51%. ^{1}H NMR (DMSO-d_6) δ=1.33 (9H, s, t-Bu), 2.83 and 3.12 (each 1H, dd, CH_2/Ser), 4.25 (1H, m, CH), 5.47 and 5.60 (each 1H, d, CH_2/Pac), 6.68 and 7.08 (each 2H, d, Ph/Tyr), 7.22 (1H, d, NH), 7.53—8.00 (5H, m, Ph/Pac), and 9.16 (1H, s, OH).

N^{α}- *t*- Butoxycarbonyl- *O*- (dicyclohexylphosphono)hydroxyamino Acid Phenacyl Ester (9). Boc–Ser[PO(O*c*Hex)$_2$]–OPac (9a) (as a General Procedure): To a solution of Boc–Ser–OPac (**8a**) (4.44 g, 13.7 mmol) in anhydrous THF (30 ml) were added the amidite **2** (6.79 g, 20.6 mmol) and 1H-tetrazole (2.88 g, 41.1 mmol). After stirring for 2 h at room temperature, the reaction mixture was neutralized with TEA; to the solution was added 30% H_2O_2 (1.62 ml, 20.6 mmol) at 0 °C. After stirring for 30 min at 0 °C and for 40 min at room temperature, the oxidation was quenched by addition of $NaHSO_3$ (2.49 g, 22.7 mmol) in H_2O (5 ml); THF was then evaporated in vacuo. The residue dissolved in AcOEt (20 ml) was washed successively with aqueous 10% citric acid, brine, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. To the residue dissolved in a small amount of diethyl ether was added hexane. After allowing to stand overnight in a refrigerator, the precipitated crystalline Boc–Ser[PO(O*c*Hex)$_2$]–OPac (**9a**) was collected by filtration, and then recrystallized from diethyl ether–hexane. Yield 6.45 g (82.9%), mp 88—89 °C, $[\alpha]_D^{24}$ −11.6° (c 1.01, EtOH). Found: C, 59.10; H, 7.47; N, 2.57%. Calcd for $C_{28}H_{42}NO_9P$: C, 59.25; H, 7.46; N, 2.47%. ^{1}H NMR (DMSO-d_6) δ=1.18—2.04 (29H, m, CH_2/*c*Hex×10, t-Bu), 4.29—4.53 (4H, m, CH/*c*Hex×2 and CH_2/Ser), 4.68 (1H, m, CH/Ser), 5.41 (2H, s, CH_2/Pac), 5.65 (1H, d, NH), and 7.47—7.91 (5H, m, Ph). ^{31}P NMR (DMSO-d_6) δ=−2.29 (s).

Boc–Thr[PO(O*c*Hex)$_2$]–OPac (**9b**) and Boc–Tyr[PO(O*c*Hex)$_2$]–OPac (**9c**) were prepared by the same method as described above, though 30% H_2O_2 was replaced with 55% mCPBA in the oxidation.[23]

9b: Since this compound was obtained as an oily substance, it was subjected to the following cleavage reaction of the Pac group without further purification.

9c: Yield 93.5%; mp 111—112 °C; $[\alpha]_D^{21}$ −6.4° (c 1.0, EtOH). Found: C, 63.58; H, 7.00; N, 2.15%. Calcd for $C_{34}H_{46}NO_9P$: C, 63.43; H, 7.20; N, 2.18%. ^{1}H NMR (DMSO-d_6) δ=1.09—1.84 (29H, m, CH_2/*c*Hex and t-Bu), 2.93—3.21 (2H, dd×2, CH_2/Tyr), 4.29—4.45 (3H, m, CH/*c*Hex×2 and CH/Tyr), 5.49 and 5.63 (each 1H, d, CH_2/Pac), 7.11 and 7.32 (each 2H, d, Ph/Tyr), 7.33 (1H, d, NH), and 7.54—8.00 (5H, m, Ph/Pac). ^{31}P NMR (DMSO-d_6) δ=−7.33 (s).

N^{α}- *t*- Butoxycarbonyl- *O*- (dicyclohexylphosphono)hydroxyamino Acid (10). Boc–Ser[PO(O*c*Hex)$_2$]–OH (10a) (as a General Procedure): To a solution of Boc–Ser[PO(O*c*Hex)$_2$]–OPac (**9a**) (5.43 g, 9.57 mmol) in 90% acetic acid (150 ml) was added zinc dust (9.39 g, 144 mmol) in several portions. After stirring for 1 h, an insoluble inorganic material was filtered off, and the filtrate was concentrated in vacuo. The residue was dissolved in diethyl ether (50 ml), and an insoluble material was filtered off. The filtrate was first washed with 10% aqueous citric acid (×3) and brine (×3), and then extracted with saturated aqueous $NaHCO_3$ several times. The combined alkaline extracts were acidified with citric acid. The acidified solution was thoroughly extracted with AcOEt. The combined extracts were washed with brine (×3), dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was dissolved in a small amount of diethyl ether. After the addition of hexane, until the appearance of turbidity, the solution was allowed to stand overnight in a refrigerator. Boc–Ser[PO(O*c*Hex)$_2$]–OH (**10a**) precipitated as fine prisms was collected by filtration. Yield 4.10 g (95.3%); mp 124—125 °C (decomp); $[\alpha]_D^{23}$ +17.4° (c 1.04, EtOH). Found: C, 53.43; H, 8.14; N, 3.16%. Calcd for $C_{20}H_{36}NO_8P$: H, 53.44; H, 8.07; N, 3.12%. ^{1}H NMR (DMSO-d_6) δ=1.16—1.91 (29H, m, CH_2/*c*Hex×10 and t-Bu), 4.10—4.28 (5H, m, CH/*c*Hex×2, CH/Ser, and CH_2/Ser), and 6.96 (1H, d, NH). ^{31}P NMR (DMSO-d_6) δ=−2.30 (s).

Boc–Thr[PO(O*c*Hex)$_2$]–OH (**10b**) was obtained as a crystalline compound by the same method as described above. Yield 84.2% (from **8b**); mp 129—130 °C; $[\alpha]_D^{25}$ +21.5° (c 1.02, EtOH). Found: C, 54.30; H, 8.24; N, 3.10%. Calcd for $C_{21}H_{38}NO_8P$: H, 54.42; H, 8.26; N, 3.02%. ^{1}H NMR (DMSO-d_6) δ=1.17—1.81 (32H, m, CH_2/*c*Hex×10, t-Bu, and CH_3/Thr), 4.17—4.25 (3H, m, CH/*c*Hex×2 and α-CH/Thr), 4.77—4.79 (1H, m, β-CH/Thr), and 6.63 (1H, d, NH). ^{31}P NMR (DMSO-d_6) δ=−3.11 (s).

Boc–Tyr[PO(O*c*Hex)$_2$]–OH (**10c**), obtained as an oily substance, was dissolved in a small amount of diethyl

ether–hexane (1 : 1 v/v). After CHA (1.05 equiv) was added to the solution, the mixture was allowed to stand in a refrigerator overnight. The precipitated crystalline CHA salt was collected by filtration. Yield 93.1%. An analytical sample was obtained by recrystallization from diethyl ether–hexane. Mp 111—113 °C (decomp); $[\alpha]_D^{21}$ +32.6° (*c* 1.01, EtOH). Found: C, 60.89; H, 8.65; N, 4.51%. Calcd for $C_{32}H_{53}N_2O_8P \cdot 0.5H_2O$: C, 60.65; H, 8.59; N, 4.42%. ^{1}H NMR (DMSO-d_6) δ=1.07—1.89 (39H, m, CH_2/*c*Hex×10, CH_2/CHA×5, and *t*-Bu), 2.87—3.10 (3H, m, CH/CHA and CH_2/Tyr), 3.83 (1H, dd, CH/Tyr), 4.36—4.39 (2H, m, CH/*c*Hex×2), 5.80 (1H, dd, NH/Thr), and 7.10 and 7.13 (each 2H, d, Ph/Tyr). ^{31}P NMR (DMSO-d_6) δ=−5.61 (s).

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15) Although 30% H_2O_2 is easy and safe to handle, the dephosphitylation during oxidation procedure occurs more rapidly than that with mCPBA.

16) In a previous paper[1] we reported that both **10a** and **10b** were crystallized as DCHA salts, while **10c** was not crystallized as either DCHA or CHA salt.

17) Compound **10c** with the free carboxyl group can be obtained as a powdery substance by lyophilization from benzene.

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