

***N,N'*-Dialkyldiamide-Type Phosphate Protecting Groups for Fmoc Synthesis of Phosphotyrosine-Containing Peptides: Optimization of the Alkyl Group¹⁾**

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Synthesis and evaluation of three Fmoc-phosphotyrosine derivatives with a phosphate group protected as *N,N'*-dialkyldiamide (alkyl = Pr^{*i*}, Pr^{*i*}, and Bu^{*i*}) were studied. All the derivatives were obtained as crystals, among which the Bu^{*i*} derivative (**4c**) was the best in ease of preparation and excellence in cleavage property. Solid phase synthesis of a methionine-containing peptide, H-Tyr(PO₃H₂)-Val-Pro-Met-Leu-OH, could be done without any problems.

Phosphorylation of many key cellular proteins is implicated in a variety of biochemical pathways. Synthetic phosphopeptides and phosphopeptide mimetics can be useful for the study of the biological and chemical properties of such proteins.²⁾

Various phosphate-protecting groups, most of which are based on ester formation, have been developed for phosphopeptide synthesis.²⁾ However, the number of protecting groups applicable to the Fmoc³⁾ strategy synthesis is limited. This is due to the monodealkylation of phosphate diester groups,^{4,5)} dephosphorylation (phosphotyrosine),⁶⁾ and β -elimination of protected phosphoamino acid residues (phosphoserine and phosphothreonine)⁷⁾ under the basic conditions used in the removal of the Fmoc group. Instability of the side chain protecting groups under acidic conditions is another problem. In Fmoc synthesis Bu^{*t*}-based acid-labile side-chain protecting groups are generally used. The acid lability of carboxylate esters is governed mainly by the cation stability of the ester alkyl groups; however, deciding the acid lability of phosphate esters is not so simple. Since two acid-labile bonds, P–O and O–C, exist in phosphate esters, Fmoc-Tyr[P(O)(OBu^{*t*})₂]-OH should be stored at –70 °C under anhydrous conditions to avoid decomposition by the P–O bond cleavage.⁸⁾ Those problems described above are substantial in protection of the phosphate group by esterification and could not be overcome only by changing the structure of the ester residues.

The acid-labile and base-stable properties of the P–N bonds are common in phosphoric amides and related organophosphorus amide compounds and were previously used by us for protection of the α -amino function of amino acids.⁹⁾ The acid lability of P–N bonds is more pronounced in the trivalent state than in the pentavalent state. This phenomenon has been used in global phosphorylation methodology, consisting of phosphorylation and oxidation, in phosphopeptide

synthesis.¹⁰⁾ Since the acid lability of P–N bonds generally increases as the bulk of substituents on the amide nitrogen decreases, the most popular reagents for phosphorylation are *N,N*-diethyl- and *N,N*-diisopropylphosphoramidite esters. The fact that these compounds allow easy replacement of the amino moiety by a weakly acidic hydroxy function at the phosphorus suggested the possibility of the existence of acid-labile phosphoric amide groups suitable for protection of phosphoamino acids. However, it was not known whether these properties would be suitable for phosphopeptide synthesis by the Fmoc strategy using excess base during peptide chain elongation and acids for final deprotection.¹¹⁾

Chao et al. compared the acid lability of five phosphorodiamidate derivatives of phosphotyrosine in HCl in dioxane and observed rapid cleavage in *N,N*-dimethyl- and *N*-propylamides.¹²⁾ Based on these results, they demonstrated the utility of bis-*N,N*-dimethylamide as a phosphate-protecting group through syntheses of several peptides containing phosphotyrosine.¹²⁾ However, our observation was somewhat different. We also compared rates of acid degradation of model compounds with the structure of PhOP(O)(NRR')₂ in 95% TFA solution, which is often used at the final deprotection step of solid phase synthesis by the Fmoc strategy. While monoalkylamides (R = H, R' = Et and Pr^{*i*}) could be cleaved completely within 1 h, 57% of the unreacted *N,N*-dimethylamide derivative (R = R' = Me) was recovered.^{1a)} We then pursued the application of *N,N'*-dipropyl- and *N,N'*-diisopropyl-diamide-type phosphate protecting groups for synthesis of phosphotyrosine-containing peptides and the results have already been reported in a preliminary communication.^{1c)} At that stage, we could not show any difference between these two groups; however, in the continuing study we observed some difference between the two. In this study we added *N*-isobutylamide (R = H, R' = Bu^{*i*}) and tried optimization of the *N*-substituting alkyl groups.

Results and Discussion

Introduction of *N,N'*-dialkyldiaminophosphinoyl moieties to the tyrosine side-chain was done as sketched in Scheme 1. *N,N'*-Dipropylphosphorodiamidic chloride (**1a**) was obtained by the reaction of propylamine with phosphoryl chloride as reported.¹³ This compound could be purified by sublimation to give colorless crystals. Preparation of *N,N'*-diisopropyl derivative **1b** required a two-step reaction and the product was obtained as an oil. In the case of *N,N'*-diisobutyl derivative **1c**, a one-step reaction easily gave crude products with sufficient purity for the following reaction.

Reactions of **1a–c** with *Z*-Tyr-OBzl (**2**) required strong bases for activation of **2**. When **2** was activated with 1.1 mol amt. of LDA and subsequently treated with 1.8 mol amt. of **1a**, *Z*-Tyr[P(O)(NHPrⁿ)₂]-OBzl (**3a**) was obtained in 79% yield. This compound was homogeneous on TLC and used without purification for the following reaction. Catalytic hydrogenolysis of **3a** followed by reaction with Fmoc-OSu under the usual conditions gave the corresponding Fmoc derivative **4a**. Analysis of the thus obtained materials by HPLC found a trace of a closely-eluting by-product, which could be removed by repeated gel chromatography on Sephadex LH-20. The structure of the by-product, gathered from many runs, was determined as an over-reaction product **4'a** (Fig. 1) by ¹H, ¹³C, and ³¹P NMR spectra and mass spectrometry. Since purification of preparative amounts of **4a** by gel chromatography was not easy, suppression of this side reaction at the step of formation of **3a** was tried.

While **3a** was homogeneous as far as TLC was concerned, an impurity could be separated from the early eluting fractions in gel chromatography on LH-20. The structure of the impurity was assigned to **3'a** (Fig. 1) by spectrometric analyses. Since the chloride **1a** used was pure, showing a single peak on the ³¹P NMR spectrum, it is clear that the side reaction occurred as an over-reaction of **3a** in the presence of the excess strong base and **1a**. In this reaction, use of a strong base is indispensable for activation of the side-chain

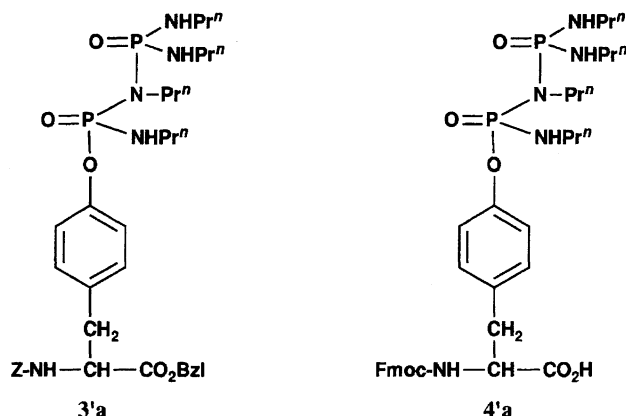
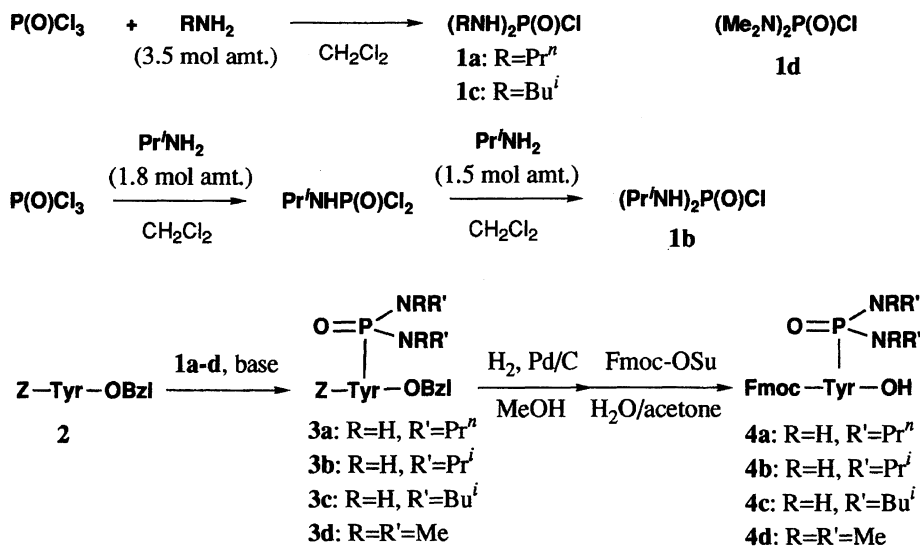


Fig. 1. Structures of side-products.

hydroxy group of **2**; therefore, optimization study of the reaction conditions was focused on the kind and the extent of excess of bases and the results are summarized in Table 1.

When LDA was used as the base, formation of the by-product **3'a** was minimal, but detectable (Entry 1). Reduction of the amount of **1a** resulted in lowering of the yield without affecting the purity (Entries 2 and 3). While scale-up to the 2.0 mmol scale with increase of the yield was possible (Entry 4), the product was obtained as an oil. On the other hand, when **1a** (2.0 mol amt.) was treated with **2** in the presence of DBU (1.5 mol amt.) and DMAP (2.0 mol amt.), a higher yield was obtained with some loss of the purity (Entry 7). Sufficient purity was obtained, with considerable loss of the yield, when the amounts of both chloride **1a** and the bases were decreased (Entry 10). It should be noted that, when the chlorides **1b** and **1c** with more bulky *N*-alkyl groups were used, side-products like **3'a** could not be detected in either LDA (Entries 5 and 6) or DBU-DMAP (Entries 12 and 13) method. Especially, when **1c** was used, a preparative-scale run gave crude **3c** as crystals, which were purified easily by recrystallization (Entries 6 and 13).

Catalytic hydrogenolysis of **3a–c** followed by introduc-



Scheme 1.

Table 1. Effects of Reaction Conditions on Yields and Purity of **3a—c**

Entry	Reagent		Scale mmol	Product	Yield % ^{a)}	Purity % ^{b)}
	1a—c /mol amt.	Base(s)/mol amt.				
1	1a /1.8	LDA/1.1	0.25	3a	79	99.7
2	1a /1.5	LDA/1.1	0.25	3a	75	99.6
3	1a /1.3	LDA/1.1	0.25	3a	50	99.7
4	1a /1.8	LDA/1.1	2.0	3a	94	—
5	1b /1.8	LDA/1.1	5.6	3b	86	ND ^{c)}
6	1c /1.8	LDA/1.1	2.0	3c	94	ND
7	1a /2.0	DBU/1.5+DMAP/2.0	0.8	3a	87	96.8
8	1a /2.0	DBU/1.5	0.25	3a	37	99.6
9	1a /1.5	DBU/1.5+DMAP/1.5	0.25	3a	68	96.7
10	1a /1.5	DBU/1.3+DMAP/1.5	0.25	3a	43	ND
11	1a /2.0	DBU/1.5+DMAP/2.0	3.0	3a	94	—
12	1b /2.0	DBU/1.5+DMAP/2.0	1.5	3b	76	ND
13	1c /2.0	DBU/1.5+DMAP/2.0	1.5	3c	94	ND

a) Yield indicates that of a crude product. b) Purity indicates that of the crude product determined by HPLC (Column: 5 μ m μ Bondasphere C18 (3.9 mm $\times$ 150 mm); gradient: 0.1% TFA–acetonitrile 50/50 to 20/80 over 20 min; flow rate: 1 ml min⁻¹; detection: 254 nm; retention times: 7.8 min (**3a**), 6.0 min (**3b**), 9.7 min (**3c**), 9.1 min (**3'a**). c) ND = not detected.

tion of the Fmoc group in the usual manner gave Fmoc derivatives **4a—c**. In this step, as in the former step, **4c** was obtained most easily as pure colorless crystals. Yields in preparative scale syntheses, physical properties, and ³¹P NMR data of **3a—c** and **4a—c** are summarized in Table 2. All the derivatives were quite stable and could be stored for more than a year at room temperature without change.

Racemization of the tyrosine residue during introduction of the diaminophosphinoyl moiety was checked by Marfey's method.¹⁴⁾ Compound **3c** was deprotected by hydrogenolysis and converted to the diastereomeric derivative with *N* α -(2,4-dinitro-5-fluorophenyl)-L-alaninamide (Marfey's reagent). In an HPLC trace of this derivative shown in Fig. 2, the D-tyrosine form could not be detected.

Requisite for side-chain protecting groups for Fmoc synthesis would be complete stability toward a base and cleavability under acidic conditions without any accompanying side-reactions.

The stability of the amide-type phosphate protecting groups under the conditions for repeated Fmoc deprotection was checked using **3a**. When **3a** was treated with 20% piperidine in DMF at R.T. for 72 h, no change could be observed on HPLC. We reported before that tetrabutylam-

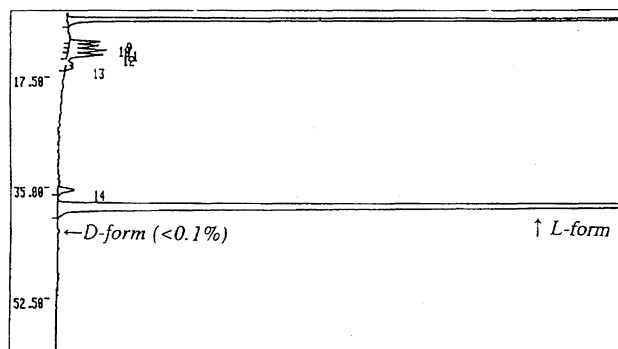


Fig. 2. Racemization test by Marfey's method.

monium fluoride hydrate (TBAF·xH₂O) could be used for Fmoc deprotection.¹⁵⁾ When **3a** was treated with 2 mol amt. of TBAF·xH₂O in DMF at R.T. for 30 min, dephosphorylation of **3a** proceeded to the extent of 79%. Therefore, piperidine should be used for Fmoc deprotection.

The cleavage potential of the three new phosphate protecting groups was then compared using the model dipeptide compounds. For this purpose *N,N,N',N'*-tetramethyldiaminophosphinoyl derivative, Fmoc-Tyr[P(O)(NMe₂)₂]-OH (**4d**),¹²⁾ was also prepared. Compounds **4a—d** were cou-

Table 2. Yields, Physical Constants, and ³¹P NMR Data of **3a—c** and **4a—c**

Compound	Yield (%)	Mp (°C)	[α] _D	³¹ P NMR (ppm) ^{b)}
3a	94 (Method A) ^{a)}	Oil	+4.3° (c 1.0, CHCl ₃ , 31 °C)	9.71
3a	94 (Method B)			
3b	86 (Method A)	Oil	+3.8° (c 1.0, CHCl ₃ , 31 °C)	9.41
3b	76 (Method B)			
3c	94 (Method A)	98—99	+4.6° (c 1.0, CHCl ₃ , 31 °C)	10.05
3c	96 (Method B)			
4a	89	146—147	−8.3° (c 0.75, DMF, 25 °C)	13.50
4b	79	150—151	−15.7° (c 1.0, DMF, 27 °C)	10.58
4c	89	171	−10.0° (c 1.0, DMF, 33 °C)	11.07

a) For methods see Experimental. b) Chemical shifts were measured relative to external H₃PO₄ assigned at zero.

pled, respectively, with H-Val-OMe using various coupling reagents to give the corresponding dipeptide derivatives **5a–d** (Scheme 2). For **4a–c**, the corresponding pentafluorophenyl esters **6a–c** were also prepared and used for comparison. As summarized in Table 3, all the coupling methods tested gave the desired products in high yields. Therefore, in the efficacy of coupling there was no difference among the four protecting groups.

Rates of selective phosphate deprotection of **5a–d** in 95% TFA solution were traced at room temperature using HPLC and the results are shown in Fig. 3. Both *N*-propyl- and *N*-isobutylamides could be deprotected completely within 4 h. However, *N*-isopropylamide was cleaved rather slowly and took 12 h for complete deprotection. As expected, deprotection of *N,N*-dimethylamide was incomplete after 12 h. These experiments are the first that showed a difference in deprotection behavior of *N*-monoalkylamides.

In the case of **5c**, the deprotection product was isolated to identify its structure. Retention time in HPLC and the ³¹P NMR spectrum (Fig. 4) of the product were consistent with those of the authentic sample obtained by coupling of Fmoc-Tyr(PO₃H₂)-OH¹⁶ with H-Val-OMe.

It was tentatively concluded from all the results using the model reactions described above that *N*-isobutylamide would be most useful as a phosphate protecting group of phosphotyrosine. This conclusion was further substantiated by applications of the new protecting groups to solid phase

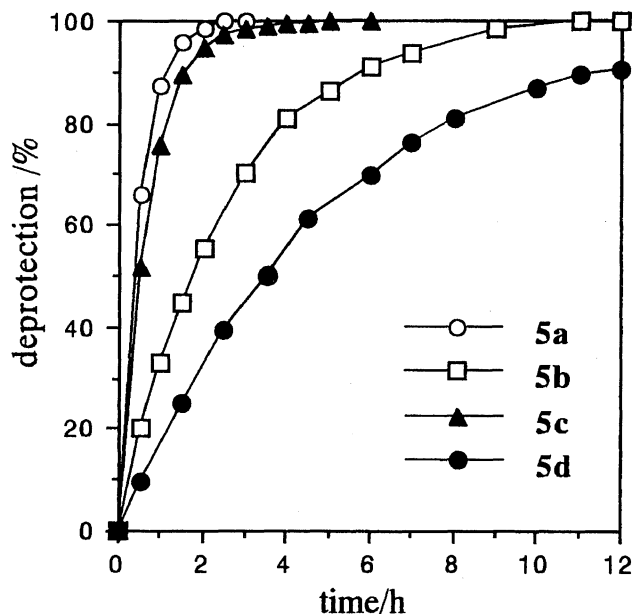


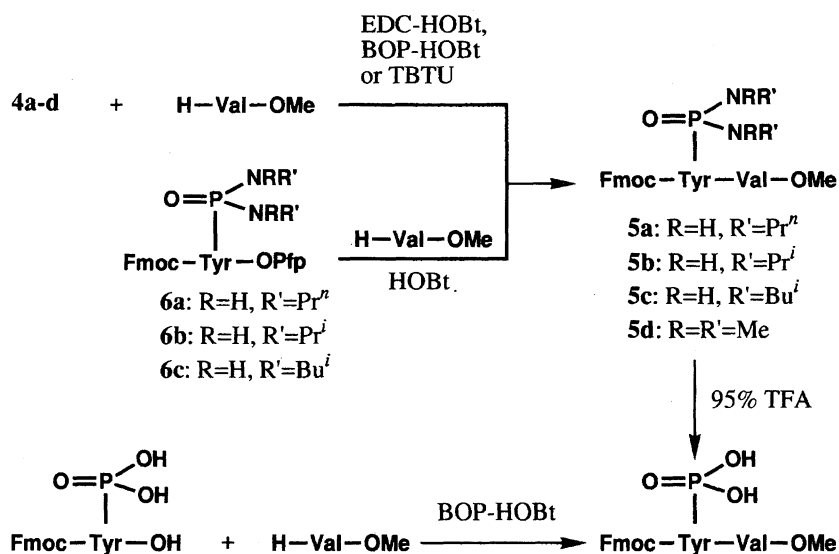
Fig. 3. Deprotection of amide-type phosphate protecting groups of Fmoc-Tyr[P(O)(NRR')₂]-Val-OMe (**5a–d**) with 95% TFA.

synthesis.

Syntheses of a model peptide, H-Gly-Val-Tyr(P)-Ala-Ala-Ser-Gly-OH (**7**),⁸ using **4a–d**, respectively, were used to evaluate the four amide-type phosphate protecting groups. An authentic sample of **7** necessary for this purpose was obtained by solid phase synthesis using **4c** on the Applied Biosystems 430A peptide synthesizer. Total deprotection and release with 95% TFA solution followed by the usual workup gave 88% of the crude product with a peptide content of 71%. Purification by preparative HPLC easily gave the pure material, the identity of which was established by ³¹P NMR, FAB-MS, and amino acid analysis. Then, four separate solid phase syntheses were done using **4a–d** as a building block on an Advanced ChemTech ACT

Table 3. Preparation of Fmoc-Tyr[P(O)(NRR')₂]-Val-OMe (**5a–d**) Using Various Coupling Methods

Compound	Coupling yield (%) / Coupling method			
	EDC/HOBt	BOP/HOBt	TBTU	Pfp ester/HOBt
5a	Quant.	Quant.	92	97
5b	94	95	Quant.	Quant.
5c	Quant.	93	Quant.	95
5d	Quant.	—	—	—



Scheme 2.

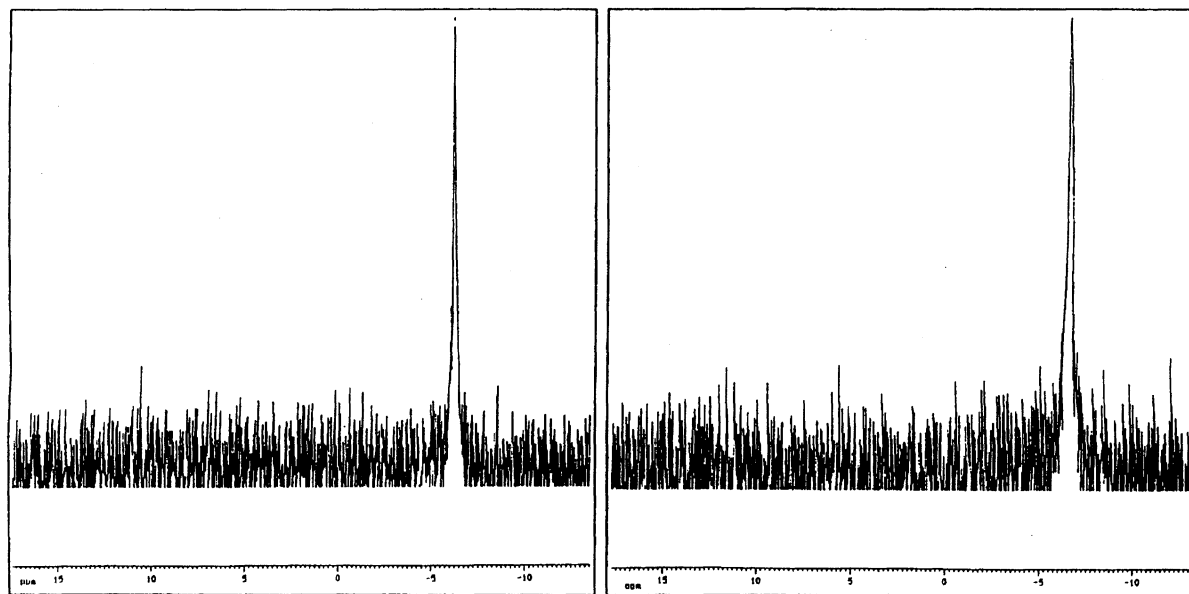


Fig. 4. ^{31}P NMR spectra of the deprotection product of **5c** (left) and the authentic sample obtained by coupling of Fmoc-Tyr(PO_3H_2)-OH with Val-OMe (right).

350 multiple peptide synthesizer. The peptides were totally deprotected and simultaneously released from the resins by treatment with 95% TFA solution for 4 h and analyzed by HPLC. As shown in HPLC traces in Fig. 5, except in the case using **4b** ($\text{R} = \text{H}$, $\text{R}' = \text{Pr}^i$), complete deprotection occurred to give the desired product with a retention time of 4.1 min, accompanied by an undefined methylated side-product, as ascertained by mass spectrometry, which showed a minor peak (retention time: 5.5 min). In the case of **4b**, about 41% of the phosphate protecting group remained unaffected, however no peak corresponding to the intermediate monoamide could be detected. This is probably because the hydrolysis of the second amide would be accelerated by intramolecular proton transfer as sketched in Scheme 3. The lowered acid-lability of the N,N' -diisopropyldiaminophosphinoyl moiety on this heptapeptide must be related to a structural change initiated by this moiety. However, further studies are needed

to explain it.

The most important feature of the new amide-type phosphate protecting groups is the cleavage under the acidic conditions without generating a cationic species. To demonstrate this feature, a methionine-containing peptide was selected as the target for the next synthesis. In a synthesis of a native platelet-derived growth factor- β -receptor (PDGF- β) related phosphopeptide, H-Tyr(*P*)-Val-Pro-Met-Leu-OH (**8**), using Boc-Tyr[P(O)(OBzl) $_2$]-OH at the *N*-terminal, formation of high content of an *S*-benzylated by-product at the final deprotection and release from the resin with Reagent R (90% TFA, 5% thioanisole, 3% 1,2-ethanedithiol, and 2% anisole) was reported.¹⁷⁾ Cleavage of the peptide with Reagent K (82.5% TFA, 5% phenol, 5% H_2O , 5% thioanisole and 2.5% 1,2-ethanedithiol) did not reduce the amount of the by-product. This peptide was then synthesized using **4c**. Starting from the Fmoc-Leu-Wang resin, peptide chain

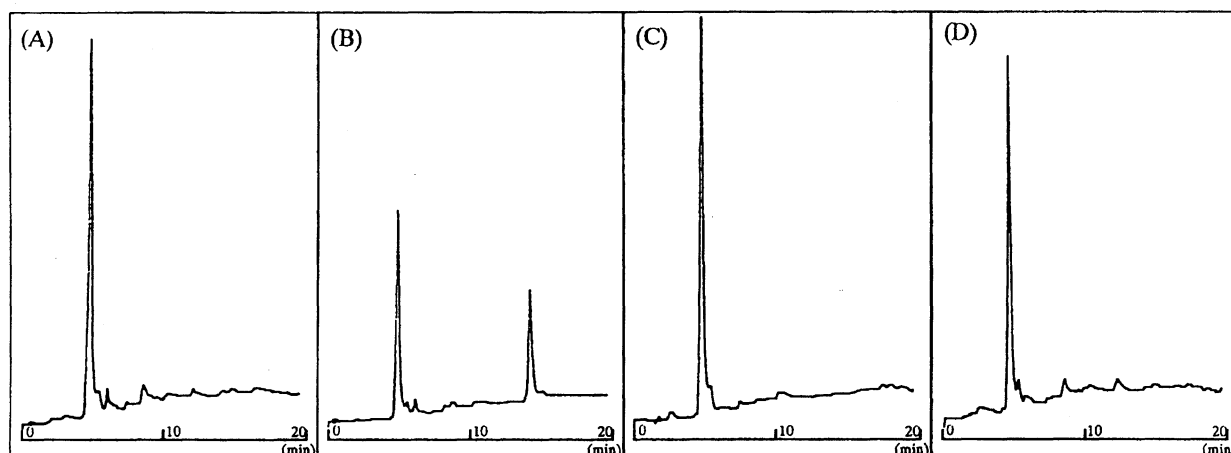
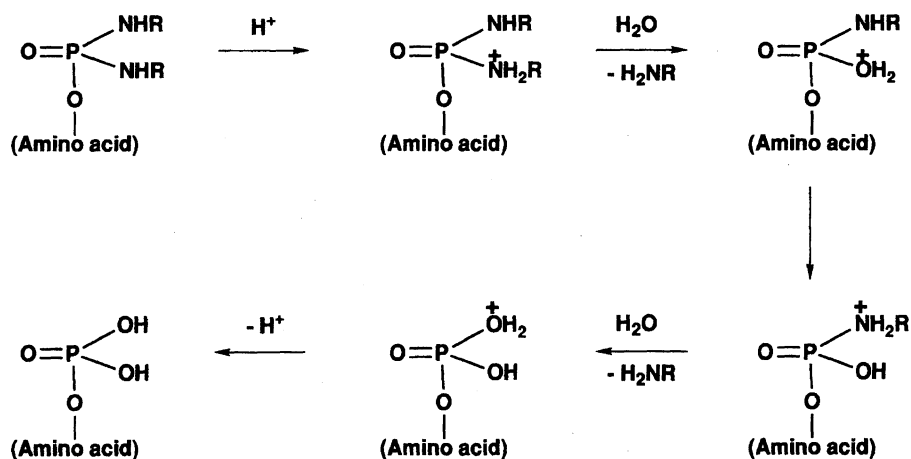


Fig. 5. HPLC profiles of the crude materials of phosphopeptide **7** obtained by deprotection and cleavage of the protected peptide resins synthesized using **4a** (A), **4b** (B), **4c** (C), and **4d** (D), respectively.



Scheme 3.

elongation was done on the Applied Biosystems 430A peptide synthesizer by the FastMoc program using the Fmoc/*O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU) chemistry.¹⁸⁾ The peptide was deprotected and released simultaneously by treating the resin with Reagent K for 4 h at room temperature. The crude peptide was precipitated with ether and lyophilized. Pure materials were easily obtained by preparative HPLC. HPLC profiles of the crude and purified products are shown in Fig. 6.

In conclusion, we were able to establish *N,N'*-diisobutyldiamide as the optimized form of *N,N'*-dialkyldiamide-type phosphate protecting groups of phosphotyrosine. The building block **4c** for synthesis of phosphotyrosine-containing peptides by the Fmoc strategy could be obtained easily as stable crystals and used for coupling in both solution-phase and solid-phase syntheses without any problems. Most importantly, the new protecting group accomplished clear deprotection with trifluoroacetic acid without generating any cationic species. Further applications to synthesis of phosphoserine and phosphothreonine-containing peptides are in progress in our laboratory.

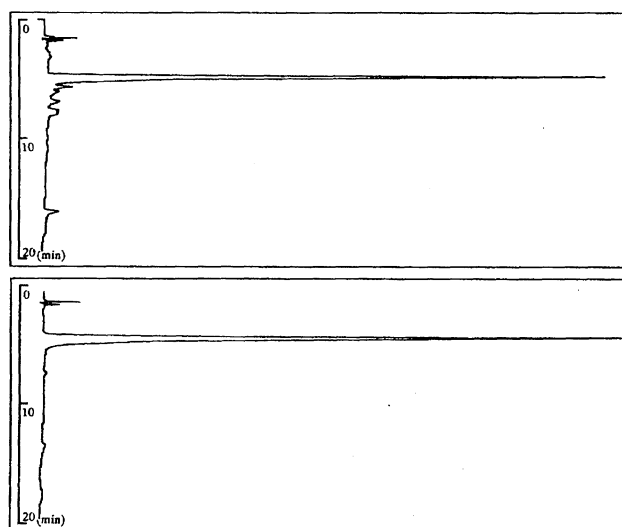


Fig. 6. HPLC profiles of the crude (upper) and purified (lower) phosphopeptide **8**.

Experimental

TLC was done using Merck silica gel plates 60F₂₅₄ in the following systems: (a) chloroform–methanol (10 : 1), (b) chloroform–methanol (8 : 1), (c) chloroform–methanol (2 : 1), (d) chloroform–methanol–acetic acid (85 : 10 : 5), (e) hexane–ethyl acetate (1 : 1), (f) ethyl acetate–methanol (9 : 1), and (g) chloroform–methanol (15 : 1). Column chromatography was done on a Wakogel C-300 (Wako Pure Chemical Industries, Ltd., Osaka). Analytical HPLC was done on Waters 625 LC system containing 5 μ m μ Bondasphere C18 (3.9 mm $\times$ 150 mm) with a Waters 484 tunable absorbance detector. Preparative HPLC was done using Waters 600E LC system containing 5 μ m μ Bondasphere C18 (19 mm $\times$ 150 mm). ¹H NMR spectra were recorded on a JEOL JNM-EX270L FT-NMR system (270 MHz), a Bruker AVANCE DPX300 (300 MHz), or a JEOL JNM-EX400L FT-NMR system (400 MHz) spectrometer using tetramethylsilane (TMS) as the internal standard. ¹³C NMR spectra were recorded on a JEOL JNM-EX270L FT-NMR system operating at 67.8 MHz, a Bruker AVANCE DPX300 spectrometer operating at 75.5 MHz, or a JEOL JNM-EX400L FT-NMR system operating at 100 MHz. Samples were dissolved in the solvent indicated and chemical shifts were measured relative to CDCl₃ assigned at 77.00 ppm. ³¹P NMR spectra were recorded on either a Bruker AVANCE DPX300 spectrometer operating at 121.5 MHz or a JEOL JNM-EX400L FT-NMR system operating at 161.7 MHz. Chemical shifts were measured relative to external phosphoric acid assigned at zero. Mass spectra were obtained using a JEOL JMS-AX505HA spectrometer. Optical rotations were measured in a JASCO DIP-360 apparatus. Melting points were measured on a Ishii-shoten melting point apparatus without correction. Elemental analyses were done on a YANACO MT-5 apparatus. Amino acid analyses were done on a Hitachi L-8500 Amino Acid Analyzer. Solid phase peptide syntheses were done using either an Applied Biosystems 430A peptide synthesizer or an Advanced ChemTech 350 multiple peptide synthesizer.

***N,N'*-Dipropylphosphorodiamidic Chloride (PrⁿNH)₂P(O)-Cl (1a) and *N,N'*-Diisobutylphosphorodiamidic Chloride (BuⁱNH)₂P(O)Cl (1c):** To a stirred solution of phosphorus oxychloride (5.0 ml, 53.6 mmol) in 100 ml of dichloromethane at -80°C under an argon atmosphere was added a solution of the corresponding alkyl amine (a: R = Prⁿ, 15.4 ml, c: R = Buⁱ, 18.7 ml, 188 mmol) in 120 ml of dichloromethane over 6 h, and the mixture stirred at -80°C for 2 h. After evaporation, the residue was taken up in ethyl acetate and the solution washed with cooled wa-

ter (twice), and saturated NaCl solution, and dried over anhydrous sodium sulfate. The solvent was removed to give a solid mass, which was washed thoroughly with petroleum ether. Only compound **1a** was sublimated at 130 °C/2 mmHg (1 mmHg = 133.322 Pa).

1a: Yield 7.60 g (71%), mp 71 °C, R_f^a 0.55, R_f^c 0.77. ^{31}P NMR (121.5 MHz, CDCl_3) δ = 21.90 (s).

1c: Yield 10.06 g (83%), mp 94–95 °C, R_f^a 0.67, R_f^c 0.89. ^{31}P NMR (121.5 MHz, CDCl_3) δ = 19.53 (s).

N,N' -Diisopropylphosphorodiamidic Chloride ($\text{Pr}^i\text{NH})_2\text{P}(\text{O})\text{Cl}$ (1b**):** To a stirred solution of phosphorus oxychloride (5.0 ml, 53.6 mmol) in 100 ml of dichloromethane at –80 °C under an argon atmosphere was added a solution of isopropylamine (8.22 ml, 96.5 mmol) in 120 ml of dichloromethane over 6 h, and the mixture stirred at –80 °C for 2 h. After evaporation, the residue was taken in ethyl acetate, and the solution washed with cooled water (twice), and saturated NaCl solution, and dried over anhydrous sodium sulfate. The solvent was removed to give isopropylphosphoramidic dichloride, $\text{Pr}^i\text{NHP}(\text{O})\text{Cl}_2$, as solid mass, which was washed thoroughly with petroleum ether.

Yield 8.51 g, (93%), mp 62–71 °C.

To a solution of the above dichloride (8.50 g, 48.3 mmol) in 100 ml of dichloromethane at –80 °C under an argon atmosphere was added a solution of isopropylamine (6.17 ml, 72.5 mmol) in 120 ml of dichloromethane over 6 h, and the mixture was stirred at –80 °C for 2 h. After washing as above, evaporation of the solvent in vacuo gave **1b** as an oil.

Yield 8.54 g, (89%), R_f^a 0.65. ^{31}P NMR (121.5 MHz, CDCl_3) δ = 16.25 (s).

N^α -Benzoyloxycarbonylphosphotyrosine Benzyl Ester O^p -(N,N' -Dialkylphosphorodiamide) (3**). **Z-Tyr[P(O)(NHBuⁱ)₂]-OBzl (**3c**)-Method A (as a General Procedure Using LDA as Base):** LDA solution was prepared by adding *n*-BuLi (1.38 ml, 2.20 mmol) in hexane to a solution of diisopropylamine (0.317 ml, 2.42 mmol) in THF (5 ml) at 0 °C under an argon atmosphere. After 30 min of stirring at 0 °C, the solution was cooled to –60 °C and transferred to a cooled solution of Z-Tyr-OBzl (0.811 g, 2.00 mmol) in THF (5 ml). Cooling bath was removed and the mixture was stirred at R.T. for 30 min. The solution was cooled again to –60 °C and to this **1c** (0.816 g, 3.60 mmol) was added. The mixture was warmed up to R.T. and stirred for 5 h. Then the reaction was quenched with 5% NaHCO_3 (1 ml), and the solvent was evaporated. The residue was taken up in ethyl acetate and the solution washed with water, 5% citric acid, 5% NaHCO_3 , and saturated NaCl solutions and dried over anhydrous sodium sulfate. After evaporation, the residue was purified by silica-gel column chromatography using chloroform–methanol for elution and then recrystallization from ethyl acetate–petroleum ether to give the desired product as a white crystalline solid.**

Yield 1.117 g, (94%), mp 98–99 °C, $[\alpha]_D^{31} + 4.6^\circ$ (c 1.0, CHCl_3), R_f^a 0.87, R_f^c 0.22.

^1H NMR (300 MHz, CDCl_3) δ = 0.88 (6H, d, J = 6.6 Hz, CH_3/Bu^i), 0.89 (6H, d, J = 6.6 Hz, CH_3/Bu^i), 1.68 (2H, septet, J = 6.6 Hz, CH/Bu^i), 2.75–2.80 (6H, m, CH_2/Bu^i , NHBu^i), 2.99–3.12 (2H, m, CH_2/Tyr), 4.62–4.69 (1H, m, CH/Tyr), 5.08 (2H, s, CH_2Ph), 5.13 (2H, d, J = 7.2 Hz, CH_2Ph), 5.36 (1H, d, J = 7.3 Hz, NH/Tyr), 6.93 (2H, d, J = 7.9 Hz, aromatic 2, 6H/Tyr), 7.07 (2H, d, J = 8.1 Hz, aromatic 3, 5H/Tyr), 7.32–7.35 (10H, m, CH_2Ph).

^{13}C NMR (75.5 MHz, CDCl_3) δ = 19.84 (CH_3/Bu^i), 29.74, 29.82 (CH/Bu^i), 37.13 (CH_2/Tyr), 48.78 (CH_2/Bu^i), 54.70 (CH/Tyr), 66.84, 67.14 (CH_2Ph), 120.31 (aromatic C3, C5/Tyr), 127.96, 128.06, 128.40, 128.46, 128.53 (CH_2Ph), 130.36 (aromatic C2,

C6/Tyr), 131.34 (aromatic C1/Tyr), 134.94, 136.10 (CH_2Ph), 150.20 (aromatic C4/Tyr), 155.55 (CO/urethane), 171.16 (CO/Tyr).

^{31}P NMR (121.5 MHz, CDCl_3) δ = 10.05 (s). FAB-MS: Found: m/z 596.7. Calcd for $\text{C}_{32}\text{H}_{43}\text{N}_3\text{O}_6\text{P}$: ($\text{M}+\text{H}$)⁺, 596.7. Found: C, 64.51; H, 7.35; N, 7.07%. Calcd for $\text{C}_{32}\text{H}_{42}\text{N}_3\text{O}_6\text{P}$: C, 64.52; H, 7.11; N, 7.05%.

3a and 3b were obtained as colorless oil.

3a ($\text{R}=\text{H}$, $\text{R}'=\text{Pr}^n$): Yield 1.08 g, (94%), $[\alpha]_D^{31} + 4.3^\circ$ (c 1.0, CHCl_3), R_f^a 0.59, R_f^c 0.15.

^1H NMR (270 MHz, CDCl_3) δ = 0.89 (6H, t, J = 7.3 Hz, CH_3/Pr^n), 1.42–1.56 (4H, m, $\beta\text{-CH}_2/\text{Pr}^n$), 2.73–2.86 (2H, m, NHPr^n), 2.89–2.94 (4H, m, $\alpha\text{-CH}_2/\text{Pr}^n$), 3.05 (2H, dd, J = 4.5 Hz, 5.6 Hz, CH_2/Tyr), 4.64–4.67 (1H, m, CH/Tyr), 5.08 (2H, s, CH_2Ph), 5.13 (2H, d, J = 5.6 Hz, CH_2Ph), 5.38–5.41 (1H, m, NH/Tyr), 6.93 (2H, d, J = 8.2 Hz, aromatic 2, 6H/Tyr), 7.06 (2H, d, J = 8.2 Hz, aromatic 3, 5H/Tyr), 7.32–7.36 (10H, m, CH_2Ph).

^{13}C NMR (67.8 MHz, CDCl_3) δ = 11.21 (CH_3/Pr^n), 24.98 (d, J = 7.3 Hz, $\beta\text{-CH}_2/\text{Pr}^n$), 37.25 (CH_2/Tyr), 43.07 ($\alpha\text{-CH}_2/\text{Pr}^n$), 54.82 (CH/Tyr), 66.94, 67.24 (CH_2Ph), 120.43 (d, J = 4.9 Hz, aromatic C3, C5/Tyr), 128.07, 128.16, 128.50, 128.55, 128.62 (CH_2Ph), 130.44 (aromatic C2, C6/Tyr), 131.46 (aromatic C1/Tyr), 135.04, 136.21 (CH_2Ph), 150.31 (d, J = 7.3 Hz, aromatic C4/Tyr), 155.67 (CO/urethane), 171.30 (CO/Tyr).

^{31}P NMR (121.5 MHz, CDCl_3) δ = 9.71 (q, J = 9.3 Hz). FAB-MS: Found: m/z 568.8. Calcd for $\text{C}_{30}\text{H}_{39}\text{N}_3\text{O}_6\text{P}$: ($\text{M}+\text{H}$)⁺, 568.8. Found: N, 7.46%. Calcd for $\text{C}_{30}\text{H}_{38}\text{N}_3\text{O}_6\text{P}$: N, 7.40%.

3b ($\text{R}=\text{H}$, $\text{R}'=\text{Pr}^i$): Yield 2.734 g, (86%) (5.6 mmol scale), $[\alpha]_D^{29} + 3.8^\circ$ (c 1.0, CHCl_3), R_f^b 0.61, R_f^c 0.18.

^1H NMR (300 MHz, CDCl_3) δ = 1.13–1.16 (12H, m, CH_3/Pr^i), 2.46 (2H, brs, NHPr^i), 3.06 (2H, dd, J = 5.8 Hz, 6.0 Hz, CH_2/Tyr), 3.44–3.48 (2H, m, CH/Pr^i), 4.64–4.67 (1H, m, CH/Tyr), 5.08 (2H, s, CH_2Ph), 5.13 (2H, d, J = 6.3 Hz, CH_2Ph), 5.30 (1H, d, J = 8.1 Hz, NH/Tyr), 6.93 (2H, d, J = 8.3 Hz, aromatic 2, 6H/Tyr), 7.08 (2H, d, J = 8.3 Hz, aromatic 3, 5H/Tyr), 7.26–7.35 (10H, m, CH_2Ph).

^{13}C NMR (75.5 MHz, CDCl_3) δ = 25.35, 25.44, 25.52 (CH_3/Pr^i), 37.22 (CH_2/Tyr), 43.69 (CH/Pr^i), 54.75 (CH/Tyr), 66.89, 67.19 (CH_2Ph), 120.25 (d, J = 4.5 Hz, aromatic C3, C5/Tyr), 128.00, 128.10, 128.45, 128.50, 128.58 (CH_2Ph), 130.35 (aromatic C2, C6/Tyr), 131.18 (aromatic C1/Tyr), 134.98, 136.15 (CH_2Ph), 150.51 (d, J = 6.4 Hz, aromatic C4/Tyr), 155.58 (CO/urethane), 171.18 (CO/Tyr).

^{31}P NMR (121.5 MHz, CDCl_3) δ = 9.41 (s). Found: N, 7.41%. Calcd for $\text{C}_{30}\text{H}_{38}\text{N}_3\text{O}_6\text{P}$: N, 7.40%.

Method B (as a General Procedure Using Organic Base):

To an ice-cooled solution of Z-Tyr-OBzl (1.216 g, 3.00 mmol) in dichloromethane (5 ml) under an argon atmosphere were added DBU (0.6727 ml, 4.50 mmol), DMAP (0.733 g, 6.00 mmol), and **1c** (1.360 g, 6.00 mmol) and the mixture was stirred for 1 h at 0 °C, and then for 5 h at R.T. Workup and purification were similar to the method A described above. Yield 1.714 g, (96%).

3a and 3b were prepared as **3c**.

3a ($\text{R}=\text{H}$, $\text{R}'=\text{Pr}^n$): Yield 1.601 g, (94%).

By-product of **3a** (**3'a**) was separated by gel chromatography on Sephadex LH-20 using methanol as solvent.

3'a: R_f^c 0.60. ^1H NMR (300 MHz, CDCl_3) δ = 0.79–0.95 (12H, m, CH_3/Pr^n), 1.26–1.72 (8H, m, $\beta\text{-CH}_2/\text{Pr}^n$), 2.62 (3H, brs, NH/Pr^n), 2.81–3.02 (6H, m, $\alpha\text{-CH}_2/\text{Pr}^n$), 3.05–3.32 (4H, m, CH_2/Tyr , $\alpha\text{-CH}_2/\text{Pr}^n$), 4.63–4.70 (1H, m, CH/Tyr), 5.09 (2H, s, CH_2Ph), 5.27 (1H, d, J = 8.0 Hz, NH/Tyr), 6.94 (2H, d, J = 8.0 Hz, aromatic 2, 6H/Tyr), 7.12 (2H, d, J = 8.1 Hz, aromatic 3, 5H/Tyr), 7.28–7.38 (10H, m, CH_2Ph).

^{13}C NMR (75.5 MHz, CDCl_3) δ = 11.05, 11.22 (CH_3/Pr^n), 24.52, 24.61 ($\beta\text{-CH}_2/\text{Pr}^n$), 25.04, 25.11 ($\beta\text{-CH}_2/\text{Pr}^n$), 37.24 (CH_2/Tyr), 42.78 ($\alpha\text{-CH}_2/\text{Pr}^n$), 43.09 ($\alpha\text{-CH}_2/\text{Pr}^n$), 48.48 ($\alpha\text{-CH}_2/\text{Pr}^n$), 52.46 (CH/Tyr), 67.25 (CH_2Ph), 120.71 (aromatic C3, C5/Tyr), 128.02, 128.16, 128.48, 128.56, 128.61 (CH_2Ph), 130.37 (aromatic C1, C2, C6/Tyr), 134.94 (CH_2Ph), 150.54 (aromatic C4/Tyr), 156.87 (CO/urethane), 171.12 (CO/Tyr).

^{31}P NMR (121.5 MHz, CDCl_3) δ = 8.87 (s), 12.32 (s). HRMS: Found: m/z 729.3439. Calcd for $\text{C}_{36}\text{H}_{53}\text{N}_5\text{O}_7\text{P}_2$: M^+ , 729.3420. Found: N, 9.67%. Calcd for $\text{C}_{36}\text{H}_{53}\text{N}_5\text{O}_7\text{P}_2$: N, 9.68%.

3b (R=H, R'=Prⁿ): Yield 0.647 g, (76%) (1.5 mmol scale).

N^α-9-Fluorenylmethoxycarbonylphosphotyrosine O^p-(N,N'-Dialkylphosphorodiamide) (4). **Fmoc-Tyr[P(O)(NH-Buⁱ)₂]-OH (4c) (as a General Procedure):** Compound **3c** (1.644 g, 2.76 mmol) in methanol (10 ml) was hydrogenolyzed under a slight hydrogen stream over 10% Pd-C for 2 h. The catalyst was removed by filtration, the filtrate evaporated, and the residue dissolved in water, then lyophilized. The crystalline residue was dissolved in water (10 ml) and to this NaHCO_3 (0.255 g, 3.04 mmol) and acetone (10 ml) were added. Then Fmoc-Osu (1.025 g, 3.04 mmol) was added to the solution as it was stirred for over 1 h at 0 °C and the mixture stirred for 5 h at R.T. After evaporation of acetone, the solution was washed with either (twice), acidified to pH 2–3 with citric acid, and extracted with ethyl acetate for several times. The extracts were washed with water and saturated NaCl solution and dried over anhydrous sodium sulfate. After evaporation of the solvent, the residue was crystallized by trituration with petroleum ether and collected by filtration as colorless crystals.

Yield 1.444 g, (89%), mp 171 °C, $[\alpha]_D^{33}$ –10.0° (c 1.0, DMF), R_f^0 0.34, R_f^d 0.62.

^1H NMR (300 MHz, CDCl_3) δ = 0.84 (3H, d, J = 6.6 Hz, CH_3/Bu^i), 0.85 (3H, d, J = 6.3 Hz, CH_3/Bu^i), 0.91 (3H, d, J = 6.6 Hz, CH_3/Bu^i), 0.92 (3H, d, J = 6.6 Hz, CH_3/Bu^i), 1.57–1.75 (2H, m, CH/Bu^i), 2.69–2.81 (4H, m, CH_2/Bu^i), 3.11–3.29 (2H, m, CH_2/Tyr), 3.77 (2H, brs, NH/Bu^i), 4.24 (1H, t, J = 6.9 Hz, CH/Fmoc), 4.32–4.53 (2H, m, CH_2/Fmoc), 4.65–4.71 (1H, m, CH/Tyr), 7.10 (4H, s, aromatic/Tyr), 7.30–7.43 (4H, m, aromatic/Fmoc), 7.59 (2H, d, J = 7.4 Hz, aromatic/Fmoc), 7.77 (2H, d, J = 7.4 Hz, aromatic/Fmoc).

^{13}C NMR (75.5 MHz, CDCl_3) δ = 19.94 (CH_3/Bu^i), 29.82, 29.91 (CH/Bu^i), 36.76 (CH_2/Tyr), 47.25 (CH/Fmoc), 48.59, 48.79 (CH_2/Bu^i), 54.40 (CH/Tyr), 66.76 (CH_2/Fmoc), 119.61 (aromatic/Fmoc), 119.98 (aromatic C3, C5/Tyr), 125.10, 127.04, 127.69, 128.10 (aromatic/Fmoc), 130.88 (aromatic C2, C6/Tyr), 132.12 (aromatic C1/Tyr), 141.32, 143.79, 143.94 (aromatic/Fmoc), 149.99 (aromatic C4/Tyr), 155.47 (CO/urethane), 173.70 (CO/Tyr).

^{31}P NMR (121.5 MHz, CDCl_3) δ = 11.07 (s). HRMS: Found: m/z 593.2667. Calcd for $\text{C}_{32}\text{H}_{40}\text{N}_3\text{O}_6\text{P}$: M^+ , 593.2665. Found: N, 7.33%. Calcd for $\text{C}_{32}\text{H}_{40}\text{N}_3\text{O}_6\text{P}$: N, 7.08%.

4a (R=H, R'=Prⁿ): Yield 1.631 g, (89%) (3.24 mmol scale), mp 146–147 °C, $[\alpha]_D^{25}$ –8.3° (c 0.75, DMF), R_f^d 0.32.

^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ = 0.82 (6H, t, J = 7.3 Hz, CH_3/Pr^n), 1.37–1.41 (4H, m, $\beta\text{-CH}_2/\text{Pr}^n$), 2.71–2.75 (4H, m, $\alpha\text{-CH}_2/\text{Pr}^n$), 2.95 (2H, dd, J = 5.3 Hz, 5.7 Hz, CH_2/Tyr), 3.22–3.42 (2H, m, NH/Pr^n), 4.10–4.28 (3H, m, CH/Fmoc , CH_2/Fmoc), 4.58–4.64 (1H, m, CH/Tyr), 7.08 (2H, d, J = 8.3 Hz, aromatic 3, 5H/Tyr), 7.22 (2H, d, J = 8.3 Hz, aromatic 2, 6H/Tyr), 7.31 (2H, dd, J = 7.3 Hz, 7.3 Hz, aromatic/Fmoc), 7.38 (2H, dd, J = 7.3 Hz, 7.3 Hz, aromatic/Fmoc), 7.42–7.53 (1H, m, NH/Tyr), 7.65–7.73 (2H, m, aromatic/Fmoc), 7.89 (2H, d, J = 7.3 Hz, aromatic/Fmoc), 12.74 (1H, brs, CO_2H).

^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ = 11.68 (CH_3/Pr^n), 24.88

(d, J = 5.5 Hz, $\beta\text{-CH}_2/\text{Pr}^n$), 36.08 (CH_2/Tyr), 42.92 ($\alpha\text{-CH}_2/\text{Pr}^n$), 46.94 (CH/Fmoc), 56.01 (CH/Tyr), 66.00 (CH_2/Fmoc), 120.35 (aromatic/Fmoc), 120.48 (d, J = 3.7 Hz, aromatic C3, C5/Tyr), 125.64, 127.45, 128.00 (aromatic/Fmoc), 130.26 (aromatic C2, C6/Tyr), 133.39 (aromatic C1/Tyr), 141.05, 144.14 (aromatic/Fmoc), 150.71 (d, J = 7.4 Hz, aromatic C4/Tyr), 156.32 (CO/urethane), 173.73 (CO/Tyr).

^{31}P NMR (161.7 MHz, $\text{DMSO}-d_6$) δ = 13.50 (s). FAB-MS: Found: m/z 566.1. Calcd for $\text{C}_{30}\text{H}_{37}\text{N}_3\text{O}_6\text{P}$: ($\text{M}+\text{H}$)⁺, 566.6. Found: C, 62.76; H, 6.67; N, 7.14%. Calcd for $\text{C}_{30}\text{H}_{36}\text{N}_3\text{O}_6\text{P}$ (+1/2H₂O): C, 62.71; H, 6.49; N, 7.31%.

By-product of **4a** (**4'a**) was separated by gel chromatography on Sephadex LH-20 using methanol for elution.

4'a: ^1H NMR (300 MHz, CDCl_3) δ = 0.75–0.92 (12H, m, CH_3/Pr^n), 1.29–1.78 (8H, m, $\beta\text{-CH}_2/\text{Pr}^n$), 2.75–2.94 (6H, m, $\alpha\text{-CH}_2/\text{Pr}^n$), 3.12–3.30 (4H, m, CH_2/Tyr , $\alpha\text{-CH}_2/\text{Pr}^n$), 3.85 (3H, brs, NH/Pr^n), 4.22 (1H, t, J = 6.7 Hz, CH/Fmoc), 4.29–4.49 (2H, m, CH_2/Fmoc), 4.57–4.66 (1H, m, CH/Tyr), 5.42–5.48 (1H, m, NH/Tyr), 7.11 (4H, s, aromatic/Tyr), 7.30 (2H, dd, J = 7.3 Hz, 7.3 Hz, aromatic/Fmoc), 7.39 (2H, dd, J = 7.3 Hz, 7.3 Hz, aromatic/Fmoc), 7.58 (2H, d, J = 7.3 Hz, aromatic/Fmoc), 7.76 (2H, d, J = 7.3 Hz, aromatic/Fmoc).

^{13}C NMR (75.5 MHz, CDCl_3) δ = 11.48, 11.62, 11.72 (CH_3/Pr^n), 24.93, ($\beta\text{-CH}_2/\text{Pr}^n$), 25.45, 25.49, 25.58 ($\beta\text{-CH}_2/\text{Pr}^n$), 37.57 (CH_2/Tyr), 43.28 ($\alpha\text{-CH}_2/\text{Pr}^n$), 43.61 ($\alpha\text{-CH}_2/\text{Pr}^n$), 47.64 (CH/Fmoc), 49.03 ($\alpha\text{-CH}_2/\text{Pr}^n$), 55.20 (CH/Tyr), 67.17 (CH_2/Fmoc), 120.37 (aromatic/Fmoc), 121.00 (d, J = 5.4 Hz, aromatic C3, C5/Tyr), 125.44, 127.46, 128.11 (aromatic/Fmoc), 131.20, (aromatic C2, C6/Tyr), 133.30 (aromatic C1/Tyr), 141.72, 144.19 (aromatic/Fmoc), 150.39 (d, J = 7.4 Hz, aromatic C4/Tyr), 155.97 (CO/urethane), 173.61 (CO/Tyr).

^{31}P NMR (121.5 MHz, CDCl_3) δ = 11.05 (s), 15.17 (s). HRMS: Found: m/z 728.3317. Calcd for $\text{C}_{36}\text{H}_{52}\text{N}_5\text{O}_7\text{P}_2$: ($\text{M}+\text{H}$)⁺, 728.3341.

4b (R=H, R'=Prⁱ): Yield 0.742 g, (79%) (1.66 mmol scale), mp 150–151 °C, $[\alpha]_D^{27}$ –15.7° (c 1.0, DMF), R_f^0 0.61, R_f^d 0.82.

^1H NMR (300 MHz, CD_3OD) δ = 1.12–1.16 (12H, m, CH_3/Pr^i), 2.80–2.84 (2H, brs, NH/Pr^i), 3.01–3.18 (2H, m, CH_2/Tyr), 3.36–3.45 (2H, m, CH/Pr^i), 4.16 (1H, t, J = 6.9 Hz, CH/Fmoc), 4.29–4.43 (2H, m, CH_2/Fmoc), 4.54–4.59 (1H, m, CH/Tyr), 7.11 (4H, s, aromatic/Tyr), 7.31 (2H, dd, J = 7.3 Hz, 7.3 Hz, aromatic/Fmoc), 7.39 (2H, dd, J = 7.3 Hz, 7.3 Hz, aromatic/Fmoc), 7.56–7.63 (2H, m, aromatic/Fmoc), 7.76 (2H, d, J = 7.4 Hz, aromatic/Fmoc).

^{13}C NMR (75.5 MHz, CD_3OD) δ = 25.41, 25.48, 25.56 (CH_3/Pr^i), 37.43 (CH_2/Tyr), 44.02 (CH/Pr^i), 47.47 (CH/Fmoc), 55.16 (CH/Tyr), 67.23 (CH_2/Fmoc), 120.29 (aromatic/Fmoc), 120.51 (d, J = 4.8 Hz, aromatic C3, C5/Tyr), 125.44, 127.59, 128.31 (aromatic/Fmoc), 130.88 (aromatic C2, C6/Tyr), 132.69 (aromatic C1/Tyr), 141.63, 144.15 (aromatic/Fmoc), 150.62 (d, J = 6.7 Hz, aromatic C4/Tyr), 155.48 (CO/urethane), 173.73 (CO/Tyr).

^{31}P NMR (121.5 MHz, CD_3OD) δ = 10.58 (s). FAB-MS: Found: m/z 566.1. Calcd for $\text{C}_{30}\text{H}_{37}\text{N}_3\text{O}_6\text{P}$: ($\text{M}+\text{H}$)⁺, 566.6. Found: C, 63.83; H, 6.72; N, 7.34%. Calcd for $\text{C}_{30}\text{H}_{36}\text{N}_3\text{O}_6\text{P}$: C, 63.71; H, 6.42; N, 7.43%.

N^α-Benzyloxycarbonyl-D-phosphotyrosine Benzyl Ester O^p-(N,N'-Diisobutylphosphorodiamide) Z-D-Tyr[P(O)(NH-Buⁱ)₂]-OBzl (D-3c): This compound was prepared quite as same as **3c**. Yield 80%, mp 93–94 °C, $[\alpha]_D^{25}$ –3.9° (c, 1.0, CHCl_3).

^1H NMR (300 MHz, CDCl_3) δ = 0.89 (6H, d, J = 6.6 Hz, CH_3/Bu^i), 0.90 (6H, d, J = 6.5 Hz, CH_3/Bu^i), 1.69 (2H, septet, J = 6.6 Hz, CH/Bu^i), 2.65 (2H, brs, NH/Bu^i), 2.73–2.82 (4H, m, CH_2/Bu^i), 3.00–3.08 (2H, m, CH_2/Tyr), 4.63–4.69 (1H, m, CH/Tyr), 5.08

(2H, s, CH₂Ph), 5.13 (2H, d, $J=7.1$ Hz, CH₂Ph), 5.29 (1H, d, $J=8.1$ Hz, NH/Tyr), 6.93 (2H, d, $J=8.3$ Hz, aromatic 2, 6H/Tyr), 7.07 (2H, d, $J=8.1$ Hz, aromatic 3, 5H/Tyr), 7.33–7.35 (10H, m, CH₂Ph).

¹³C NMR (75.5 MHz, CDCl₃) $\delta=19.87$, (CH₃/Bu^t), 29.80, 29.88 (CH/Buⁱ), 37.20 (CH₂/Tyr), 48.83 (CH₂/Buⁱ), 54.72 (CH/Tyr), 66.90, 67.20 (CH₂Ph), 120.32 (d, $J=4.8$ Hz, aromatic C3, C5/Tyr), 128.01, 128.11, 128.45, 128.51, 128.58 (CH₂Ph), 130.40 (aromatic C2, C6/Tyr), 131.33 (aromatic C1/Tyr), 134.98, 136.14 (CH₂Ph), 150.32 (d, $J=6.3$ Hz, aromatic C4/Tyr), 155.56 (CO/urethane), 171.16 (CO/Tyr).

³¹P NMR (121.5 MHz, CDCl₃) $\delta=9.80$ (s). FAB-MS: Found: m/z 596.4. Calcd for C₃₂H₄₃N₃O₆P: (M+H)⁺, 596.7.

Evaluation of Optical Purity of 3c Using Marfey's Reagent: H-L-D-Tyr[P(O)(NH-Bu^t)₂]-OH (1 mg, 2.69 μ mol) obtained by catalytic hydrogenolysis of **3c** or **D-3c**, respectively, was dissolved in 0.1 M (1 M = 1 mol dm⁻³) NaHCO₃ (1 ml) and added to freshly prepared *N*^α-(2,4-dinitro-5-fluorophenyl)-L-alaninamide (Marfey's Reagent¹⁴) (0.73 mg, 2.69 μ mol) in acetone (1 ml). The solution was kept at 40 °C for 1 h with frequent mixing. One milliliter of 0.2 M HCl was then added to the cooled solution. After degassing and filtration, the solution was analyzed by HPLC (Column: 5 μ m μ Bondasphere C18 (3.9 mm $\times$ 150 mm); Gradient: 0.1% TFA-acetonitrile 70/30 to 50/50 over 70 min; Flow rate: 1 ml min⁻¹; Detection at 340 nm). Retention times are 38.3 min (L-form) and 41.5 min (D-form), respectively.

Dipeptide Formation Using 4a—d. Fmoc-Tyr[P(O)(NH-Bu^t)₂]-Val-OMe (5c) (as a General Procedure): a) **EDC-HOBt Method:** HCl-H-Val-OMe (15.36 mg, 0.1 mmol) was dissolved in dichloromethane (2 ml) and cooled at 0 °C. To this were added DIEA (17.42 μ l, 0.1 mmol), **4c** (59.37 mg, 0.1 mmol), HOBt-H₂O (16.84 mg, 0.11 mmol) and EDC-HCl (21.09 mg, 0.11 mmol) and the mixture was stirred for 1 h at 0 °C, then 6 h at R.T. After evaporation, the desired product was obtained by preparative thin-layer chromatography on silica gel. Yield 70.4 mg, (99%), mp 133 °C, $[\alpha]_D^{25} -8.2^\circ$ (c 1.0, CH₃OH), R_f^a 0.52.

¹H NMR (300 MHz, CDCl₃) $\delta=0.82$ —0.90 (18H, m, CH₃/Buⁱ, CH₃/Val), 1.67—1.71 (2H, m, CH/Buⁱ), 2.06—2.12 (1H, m, β -CH/Val), 2.53 (2H, brs, NH-Bu^t), 2.75—2.84 (4H, m, CH₂/Buⁱ), 3.01—3.09 (2H, m, CH₂/Tyr), 3.70 (3H, s, -OCH₃), 4.19 (1H, t, $J=6.9$ Hz, CH/Fmoc), 4.34—4.48 (4H, m, CH₂/Fmoc, CH/Tyr, α -CH/Val), 5.54 (1H, d, $J=7.5$ Hz, NH/Tyr), 6.45 (1H, d, $J=7.7$ Hz, NH/Val), 7.14 (4H, s, aromatic/Tyr), 7.31 (2H, dd, $J=7.3$ Hz, 7.3 Hz, aromatic/Fmoc), 7.40 (2H, dd, $J=7.3$ Hz, 7.3 Hz, aromatic/Fmoc), 7.55 (2H, d, $J=7.0$ Hz, aromatic/Fmoc), 7.76 (2H, d, $J=7.4$ Hz, aromatic/Fmoc).

¹³C NMR (75.5 MHz, CDCl₃) $\delta=18.26$, 19.26 (CH₃/Val), 20.36 (CH₃/Buⁱ), 30.26 (CH/Buⁱ), 31.62 (β -CH/Val), 36.76 (CH₂/Tyr), 47.48 (CH/Fmoc), 49.31 (CH₂/Buⁱ), 52.60 (-OCH₃), 55.00 (CH/Tyr), 57.74 (α -CH/Val), 67.57 (CH₂/Fmoc), 120.39 (aromatic/Fmoc), 120.92 (aromatic C3, C5/Tyr), 125.47, 127.51, 128.15 (aromatic/Fmoc), 130.96 (aromatic C2, C6/Tyr), 132.72 (aromatic C1/Tyr), 141.68, 144.13 (aromatic/Fmoc), 150.62 (aromatic C4/Tyr), 155.57 (CO/urethane), 170.18 (CO/Tyr, CO/Val).

³¹P NMR (121.5 MHz, CDCl₃) $\delta=10.10$ (s). FAB-MS: Found: m/z 707.4. Calcd for C₃₈H₅₂N₄O₇P: (M+H)⁺, 707.8. Found: C, 64.16; H, 7.39; N, 7.66%. Calcd for C₃₈H₅₁N₄O₇P(+1/2CH₃OH): C, 63.97; H, 7.39; N, 7.75%.

5a, 5b, and 5d were prepared as **5c**.

5a (R=H, R'=Prⁱ): Yield 66.7 mg, (98%), mp 127—128 °C, $[\alpha]_D^{26} -6.3^\circ$ (c 1.0, CH₃OH), R_f^a 0.55.

¹H NMR (300 MHz, CDCl₃) $\delta=0.84$ —0.88 (12H, m, CH₃/Prⁱ,

CH₃/Val), 1.45—1.49 (4H, m, β -CH₂/Prⁱ), 2.04—2.15 (1H, m, β -CH/Val), 2.85—2.96 (6H, m, α -CH₂/Prⁱ, NHPrⁱ), 3.01—3.03 (2H, m, CH₂/Tyr), 3.68 (3H, s, -OCH₃), 4.17 (1H, t, $J=7.0$ Hz, CH/Fmoc), 4.23—4.38 (2H, m, CH₂/Fmoc), 4.44—4.53 (2H, m, CH/Tyr, α -CH/Val), 6.03 (1H, d, $J=8.0$ Hz, NH/Tyr), 7.00 (1H, d, $J=8.4$ Hz, NH/Val), 7.12 (4H, s, aromatic/Tyr), 7.28 (2H, dd, $J=6.4$ Hz, 6.4 Hz, aromatic/Fmoc), 7.38 (2H, dd, $J=7.4$ Hz, 7.4 Hz, aromatic/Fmoc), 7.45—7.66 (2H, m, aromatic/Fmoc), 7.74 (2H, d, $J=7.4$ Hz, aromatic/Fmoc).

¹³C NMR (75.5 MHz, CDCl₃) $\delta=11.08$ (CH₃/Prⁱ), 17.80, 18.74 (CH₃/Val), 24.76, 24.85 (β -CH₂/Prⁱ), 30.97 (β -CH/Val), 37.44 (CH₂/Tyr), 42.94 (α -CH₂/Prⁱ), 46.90 (CH/Fmoc), 51.94 (-OCH₃), 55.94 (CH/Tyr), 57.26 (α -CH/Val), 66.94 (CH₂/Fmoc), 119.78 (aromatic/Fmoc), 120.30 (d, $J=4.8$ Hz, aromatic C3, C5/Tyr), 124.99, 126.93, 127.54 (aromatic/Fmoc), 130.39 (aromatic C2, C6/Tyr), 132.44 (aromatic C1/Tyr), 141.07, 143.62, 143.68 (aromatic/Fmoc), 150.05 (d, $J=6.7$ Hz, aromatic C4/Tyr), 155.89 (CO/urethane), 171.10 (CO/Tyr), 171.87 (CO/Val).

³¹P NMR (121.5 MHz, CDCl₃) $\delta=9.82$ (s). FAB-MS: Found: m/z 679.0. Calcd for C₃₆H₄₇N₄O₇P: M⁺, 678.8. Found: C, 63.13; H, 7.04; N, 8.15%. Calcd for C₃₆H₄₇N₄O₇P(+1/2CH₃OH): C, 63.10; H, 7.11; N, 8.06%.

5b (R=H, R'=Prⁱ): Yield 63.7 mg, (94%), mp 138—139 °C, $[\alpha]_D^{30} -7.0^\circ$ (c 1.0, CH₃OH), R_f^a 0.62.

¹H NMR (300 MHz, CDCl₃) $\delta=0.84$ (3H, d, $J=7.9$ Hz, CH₃/Val), 0.86 (3H, d, $J=7.6$ Hz, CH₃/Val), 1.12 (6H, d, $J=5.9$ Hz, CH₃/Prⁱ), 1.14 (6H, d, $J=6.1$ Hz, CH₃/Prⁱ), 2.05—2.10 (1H, m, β -CH/Val), 2.70—2.76 (2H, m, NHPrⁱ), 3.00—3.02 (2H, m, CH₂/Tyr), 3.39—3.51 (2H, m, CH/Prⁱ), 3.68 (3H, s, -OCH₃), 4.16 (1H, t, $J=6.8$ Hz, CH/Fmoc), 4.23—4.38 (2H, m, CH₂/Fmoc), 4.44—4.53 (2H, m, CH/Tyr, α -CH/Val), 5.89 (1H, d, $J=7.3$ Hz, NH/Tyr), 6.89 (1H, d, $J=7.6$ Hz, NH/Val), 7.12 (4H, s, aromatic/Tyr), 7.28 (2H, dd, $J=7.3$ Hz, 7.3 Hz, aromatic/Fmoc), 7.38 (2H, dd, $J=7.3$ Hz, 7.3 Hz, aromatic/Fmoc), 7.48—7.57 (2H, m, aromatic/Fmoc), 7.74 (2H, d, $J=7.3$ Hz, aromatic/Fmoc).

¹³C NMR (75.5 MHz, CDCl₃) $\delta=17.83$, 18.78 (CH₃/Val), 25.23, 25.31, 25.41 (CH₃/Prⁱ), 31.05 (β -CH/Val), 37.51 (CH₂/Tyr), 43.65 (CH/Prⁱ), 46.92 (CH/Fmoc), 52.02 (-OCH₃), 55.97 (CH/Tyr), 57.29 (α -CH/Val), 67.03 (CH₂/Fmoc), 119.84 (aromatic/Fmoc), 120.21 (d, $J=5.0$ Hz, aromatic C3, C5/Tyr), 125.02, 126.99, 127.60 (aromatic/Fmoc), 130.40 (aromatic C2, C6/Tyr), 132.22 (aromatic C1/Tyr), 141.13, 143.67 (aromatic/Fmoc), 150.28 (d, $J=6.7$ Hz, aromatic C4/Tyr), 155.93 (CO/urethane), 171.10 (CO/Tyr), 171.89 (CO/Val).

³¹P NMR (121.5 MHz, CDCl₃) $\delta=9.82$ (s). FAB-MS: Found: m/z 679.0. Calcd for C₃₆H₄₇N₄O₇P: M⁺, 678.8. Found: C, 63.07; H, 7.02; N, 8.18%. Calcd for C₃₆H₄₇N₄O₇P(+1/2CH₃OH): C, 63.10; H, 7.11; N, 8.06%.

5d (R=R'=Me): Yield 64.1 mg, (99%), mp 58 °C, $[\alpha]_D^{29} -11.0^\circ$ (c 1.0, CH₃OH), R_f^a 0.67.

¹H NMR (300 MHz, CDCl₃) $\delta=0.83$ (3H, d, $J=7.0$ Hz, CH₃/Val), 0.86 (3H, d, $J=6.9$ Hz, CH₃/Val), 2.07—2.13 (1H, m, β -CH/Val), 2.69 (12H, d, $J=10.1$ Hz, CH₃/NCH₃), 3.00—3.08 (2H, m, CH₂/Tyr), 3.70 (3H, s, -OCH₃), 4.19 (1H, t, $J=6.9$ Hz, CH/Fmoc), 4.29—4.49 (4H, m, CH₂/Fmoc, CH/Tyr, α -CH/Val), 5.64 (1H, d, $J=7.5$ Hz, NH/Tyr), 6.60 (1H, d, $J=7.7$ Hz, NH/Val), 7.07—7.13 (4H, m, aromatic/Tyr), 7.30 (2H, dd, $J=7.3$ Hz, 7.3 Hz, aromatic/Fmoc), 7.39 (2H, dd, $J=7.3$ Hz, 7.3 Hz, aromatic/Fmoc), 7.55 (2H, d, $J=7.3$ Hz, aromatic/Fmoc), 7.75 (2H, d, $J=7.4$ Hz, aromatic/Fmoc).

¹³C NMR (75.5 MHz, CDCl₃) $\delta=17.76$, 18.78 (CH₃/Val), 31.05 (β -CH/Val), 36.56 (CH₃/NCH₃), 37.42 (CH₂/Tyr), 46.96

(CH/Fmoc), 52.07 (–OCH₃), 55.94 (CH/Tyr), 57.28 (α -CH/Val), 67.05 (CH₂/Fmoc), 119.87 (aromatic/Fmoc), 120.23 (d, J = 4.8 Hz, aromatic C3, C5/Tyr), 124.98, 127.00, 127.64 (aromatic/Fmoc), 130.51 (aromatic C2, C6/Tyr), 132.10 (aromatic C1/Tyr), 141.16, 143.62 (aromatic/Fmoc), 150.28 (d, J = 6.0 Hz, aromatic C4/Tyr), 155.90 (CO/urethane), 170.74 (CO/Tyr), 171.72 (CO/Val).

³¹P NMR (121.5 MHz, CDCl₃) δ = 15.78 (septet, J = 10.1 Hz). FAB-MS: Found: m/z 651.0. Calcd for C₃₄H₄₃N₄O₇P: M⁺ 650.7. Found: C, 61.92; H, 6.84; N, 8.56%. Calcd for C₃₄H₄₃N₄O₇P-(+1/2CH₃OH): C, 62.15; H, 6.80; N, 8.40%.

b) BOP-HOBt Method: HCl-H-Val-OMe (15.36 mg, 0.1 mmol) was dissolved in dichloromethane (2 ml) and cooled at 0 °C. To this were added DIEA (52.26 μ l, 0.3 mmol), **4c** (59.37 mg, 0.1 mmol), HOBt·H₂O (16.84 mg, 0.11 mmol), and benzotriazol-1-yl-oxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) (48.67 mg, 0.11 mmol) and the mixture was stirred for 1 h at 0 °C, then 6 h at R.T. After evaporation, the desired product was obtained by preparative thin-layer chromatography on silica gel. Reactions of **4a**, **4b**, and **4d** were done in the same manner and the results were summarized in Table 3.

c) TBTU Method: HCl-H-Val-OMe (15.36 mg, 0.1 mmol) was dissolved in dichloromethane (2 ml) and cooled at 0 °C. To this were added DIEA (34.84 μ l, 0.2 mmol), **4c** (59.37 mg, 0.1 mmol), and TBTU (35.32 mg, 0.11 mmol) and the mixture was stirred for 1 h at 0 °C, then 6 h at R.T. After evaporation, the desired product was obtained by preparative thin-layer chromatography on silica gel. Reactions of **4a**, **4b**, and **4d** were done in the same manner and the results are summarized in Table 3.

d) Pfp-Ester Method: Fmoc-Tyr[P(O)(NHR)₂]-OPfp. Fmoc-Tyr[P(O)(NHBu^{*i*})₂]-OPfp (**6c**) (as a General Procedure): Compound **4c** (118.74 mg, 0.2 mmol) was dissolved in ethyl acetate (5 ml) and cooled at 0 °C. To this were added pentafluorophenol (40.48 mg, 0.22 mmol) and DCC (44.88 mg, 0.22 mmol), and the mixture was stirred for 3 h at 0 °C. Precipitate was removed by filtration and the filtrate evaporated. The residue was triturated with petroleum ether and collected by filtration as a white crystalline solid. Yield 174.4 mg, (quant), mp 156–158 °C, $[\alpha]_D^{24}$ –10.6° (c 1.0, CHCl₃), R_f^0 0.87.

¹H NMR (300 MHz, DMSO-*d*₆) δ = 0.85 (12H, d, J = 6.2 Hz, CH₃/Bu^{*i*}), 1.56–1.67 (2H, m, CH/Bu^{*i*}), 2.64 (4H, dt, J = 6.6 Hz, 9.8 Hz, CH₂/Bu^{*i*}), 3.06–3.26 (2H, m, CH₂/Tyr), 4.19 (1H, t, J = 6.5 Hz, CH/Fmoc), 4.26–4.42 (4H, m, CH₂/Fmoc, NHBu^{*i*}, CH/Tyr), 5.54 (1H, d, J = 7.6 Hz, NH/Tyr), 7.14 (2H, d, J = 7.9 Hz, aromatic 3, 5H/Tyr), 7.26–7.40 (6H, m, aromatic/Fmoc, aromatic 2, 6H/Tyr), 7.64 (2H, d, J = 7.0 Hz, aromatic/Fmoc), 7.82 (2H, d, J = 7.3 Hz, aromatic/Fmoc), 8.20 (1H, d, J = 8.3 Hz, NH/Tyr).

¹³C NMR (75.5 MHz, DMSO-*d*₆) δ = 19.96 (CH₃/Bu^{*i*}), 29.42, 29.50 (CH/Bu^{*i*}), 35.31 (CH₂/Tyr), 47.55 (CH/Fmoc), 48.45 (CH₂/Bu^{*i*}), 55.41 (CH/Tyr), 65.83 (CH₂/Fmoc), 119.82 (aromatic/Fmoc), 120.22 (aromatic C3, C5/Tyr), 124.99, 125.44, 126.86, 127.45 (aromatic/Fmoc), 129.87 (aromatic C2, C6/Tyr), 131.44 (aromatic C1/Tyr), 139.10, 140.69, 143.53 (aromatic/Fmoc), 150.69 (aromatic C4/Tyr), 155.89 (CO/urethane), 168.30 (CO/Tyr).

³¹P NMR (121.5 MHz, DMSO-*d*₆) δ = 11.11 (quintet, J = 10.0 Hz). FAB-MS: Found: m/z 782.1. Calcd for C₃₈H₃₉F₅N₃NaO₆P: (M+Na)⁺, 782.7.

6a and **6b** were prepared as **6c**.

6a (R=H, R'=Pr^{*n*}): Yield 155.5 mg, (quant.), mp 147–148 °C, $[\alpha]_D^{26}$ –11.6° (c 1.0, CHCl₃), R_f^0 0.77.

¹H NMR (300 MHz, DMSO-*d*₆) δ = 0.82 (6H, d, J = 7.3 Hz, CH₃/Pr^{*n*}), 1.41 (4H, sextet, J = 7.1 Hz, β -CH₂/Pr^{*n*}), 2.70–2.80 (4H, m, α -CH₂/Pr^{*n*}), 3.04–3.24 (2H, m, CH₂/Tyr), 4.21 (1H, t, J = 6.8

Hz, CH/Fmoc), 4.26–4.39 (2H, m, CH₂/Fmoc), 4.59–4.70 (3H, m, CH/Tyr, NHP^{*n*}), 7.11 (2H, d, J = 8.3 Hz, aromatic 3, 5H/Tyr), 7.27–7.33 (2H, m, aromatic 2, 6H/Tyr, aromatic/Fmoc), 7.37–7.42 (2H, m, aromatic/Fmoc), 7.64–7.67 (2H, m, aromatic/Fmoc), 7.87 (2H, d, J = 7.4 Hz, aromatic/Fmoc), 8.26 (1H, d, J = 7.7 Hz, NH/Tyr).

¹³C NMR (75.5 MHz, DMSO-*d*₆) δ = 11.34 (CH₃/Pr^{*n*}), 24.55, 24.63 (β -CH₂/Pr^{*n*}), 33.45 (CH₂/Tyr), 42.62 (α -CH₂/Pr^{*n*}), 46.62 (CH/Fmoc), 55.40 (CH/Tyr), 65.88 (CH₂/Fmoc), 120.11 (aromatic/Fmoc), 120.34 (aromatic C3, C5/Tyr), 125.17, 127.06, 127.65 (aromatic/Fmoc), 130.09 (aromatic C2, C6/Tyr), 131.58 (aromatic C1/Tyr), 140.79, 143.72 (aromatic/Fmoc), 150.66 (aromatic C4/Tyr), 156.01 (CO/urethane), 168.46 (CO/Tyr).

³¹P NMR (121.5 MHz, DMSO-*d*₆) δ = 10.83 (quintet, J = 10.5 Hz). FAB-MS: Found: m/z 732.3. Calcd for C₃₆H₃₆F₅N₃O₆P: (M+H)⁺, 732.7.

6b (R=H, R'=Pr^{*i*}): Yield 161.1 mg, (quant.), mp 154–155 °C, $[\alpha]_D^{24}$ –8.1° (c 1.0, CHCl₃), R_f^0 0.83.

¹H NMR (300 MHz, DMSO-*d*₆) δ = 1.05 (12H, d, J = 6.3 Hz, CH₃/Pr^{*i*}), 3.03–3.28 (4H, m, CH₂/Tyr, CH/Pr^{*i*}), 4.20 (1H, t, J = 6.7 Hz, CH/Fmoc), 4.25–4.38 (2H, m, CH₂/Fmoc), 4.49 (2H, dd, J = 10.1 Hz, 10.1 Hz, NHP^{*i*}), 4.62–4.70 (1H, m, CH/Tyr), 7.12 (2H, d, J = 8.4 Hz, aromatic 3, 5H/Tyr), 7.27–7.32 (4H, m, aromatic 2, 6H/Tyr, aromatic/Fmoc), 7.37–7.42 (2H, m, aromatic/Fmoc), 7.63–7.67 (2H, m, aromatic/Fmoc), 7.88 (2H, d, J = 7.4 Hz, aromatic/Fmoc), 8.26 (1H, d, J = 7.7 Hz, NH/Tyr).

¹³C NMR (75.5 MHz, DMSO-*d*₆) δ = 25.01, 25.13, 25.20 (CH₃/Pr^{*i*}), 33.45 (CH₂/Tyr), 42.90 (CH/Pr^{*i*}), 46.62 (CH/Fmoc), 55.52 (CH/Tyr), 65.90 (CH₂/Fmoc), 120.20 (aromatic C3, C5/Tyr, aromatic/Fmoc), 125.21, 127.13, 127.71 (aromatic/Fmoc), 130.07 (aromatic C2, C6/Tyr), 131.38 (aromatic C1/Tyr), 140.83, 143.76 (aromatic/Fmoc), 150.90 (aromatic C4/Tyr), 156.00 (CO/urethane), 173.73 (CO/Tyr).

³¹P NMR (121.5 MHz, DMSO-*d*₆) δ = 8.02 (quintet, J = 9.7 Hz). FAB-MS: Found: m/z 732.2. Calcd for C₃₆H₃₆F₅N₃O₆P: (M+H)⁺, 732.7.

To an ice-cooled solution of HCl-H-Val-OMe (15.36 mg, 0.1 mmol) in dichloromethane (2 ml) were added DIEA (17.42 μ l, 0.1 mmol), HOBt·H₂O (16.84 mg, 0.11 mmol), and **6c** (98.77 mg, 0.13 mmol), and the mixture was stirred for 1 h at 0 °C, then 6 h at R.T. After evaporation, the desired product was obtained by preparative thin-layer chromatography on silica gel. Reactions of **6a** and **6b** were done in the same manner and the results were summarized in Table 3.

Selective Phosphate Deprotection of 5a–d: Compound **5** (20 mg) and Fmoc-Phe-OMe or Fmoc-Leu-OMe (10 mg) as internal standard were dissolved in 95% TFA aq (1 ml). Samples of the reaction mixture were taken at various times up to 12 h and analyzed by HPLC. (Column: 5 μ m μ Bondasphere C18 (3.9 mm $\times$ 150 mm); Gradient: 0.1% TFA-acetonitrile 50/50 to 20/80 over 20 min; Flow rate: 1 ml min^{–1}; Detection at 254 nm; Retention times: 8.1 min (**5a**), 7.8 min (**5b**), 10.4 min (**5c**), 7.3 min (**5d**), 3.3 min (Fmoc-Tyr-[PO₃H₂]-Val-OMe), 6.1 min (Fmoc-Tyr-Val-OMe)).

From the reaction mixture with **5c**, deprotection product, Fmoc-Tyr[PO₃H₂]-Val-OMe, was obtained in 97% yield. ³¹P NMR (121.5 MHz, CD₃OD) δ = –6.29 (s).

Fmoc-Tyr[PO₃H₂]-Val-OMe (Authentic Sample): To an ice-cooled solution of HCl-H-Val-OMe (30.72 mg, 0.2 mmol) in dichloromethane (2 ml) were added DIEA (104.52 μ l, 0.6 mmol), Fmoc-Tyr(PO₃H₂)-OH⁽¹⁶⁾ (96.68 mg, 0.2 mmol), HOBt·H₂O (33.68 mg, 0.22 mmol), and BOP (97.34 mg, 0.22 mmol), and the mixture was stirred for 1 h at 0 °C, then 6 h at R.T. After evapo-

ration, the residue was purified by gel chromatography on Sephadex LH-20 using methanol for elution to give the desired product as a white solid.

Yield 84.71 mg, (71%), mp 142–145 °C, $[\alpha]_D^{27} -2.2^\circ$ (c 1.0, CH₃OH), R_f^2 0.11.

¹H NMR (300 MHz, CD₃OD) δ = 0.92 (6H, d, J = 6.8 Hz, CH₃/Val), 2.09–2.16 (1H, m, β -CH/Val), 2.84–3.13 (2H, m, CH₂/Tyr), 3.70 (3H, s, -OCH₃), 4.17 (1H, t, J = 6.7 Hz, CH/Fmoc), 4.29–4.38 (3H, m, CH₂/Fmoc, α -CH/Val), 4.43–4.47 (1H, m, CH/Tyr), 7.13 (2H, d, J = 7.8 Hz, aromatic 3, 5H/Tyr), 7.21 (2H, d, J = 8.1 Hz, aromatic 2, 6H/Tyr), 7.29 (2H, dd, J = 7.3 Hz, 7.3 Hz, aromatic/Fmoc), 7.38 (2H, dd, J = 7.3 Hz, 7.3 Hz, aromatic/Fmoc), 7.58 (2H, d, J = 7.3 Hz, aromatic/Fmoc), 7.76 (2H, d, J = 7.5 Hz, aromatic/Fmoc).

¹³C NMR (75.5 MHz, CD₃OD) δ = 18.67, 19.64 (CH₃/Val), 31.94 (β -CH/Val), 38.36 (CH₂/Tyr), 48.23 (CH/Fmoc), 52.83 (-OCH₃), 55.04 (CH/Tyr), 57.37 (α -CH/Val), 68.04 (CH₂/Fmoc), 120.93 (aromatic/Fmoc), 121.23 (aromatic C3, C5/Tyr), 126.14, 128.19, 128.80 (aromatic/Fmoc), 131.51 (aromatic C2, C6/Tyr), 134.43 (aromatic C1/Tyr), 142.47, 144.97 (aromatic/Fmoc), 151.59 (d, J = 6.0 Hz, aromatic C4/Tyr), 158.03 (CO/urethane), 173.31 (CO/Tyr), 173.91 (CO/Val).

³¹P NMR (121.5 MHz, CD₃OD) δ = -6.48 (s). FAB-MS: Found: m/z 619.4. Calcd for C₃₀H₃₃N₂NaO₉P: (M+Na)⁺, 619.6.

Solid Phase Synthesis. a) **H-Gly-Val-Tyr(PO₃H₂)-Ala-Ala-Ser-Gly-OH (7):** Synthesis using **4c** as building block was done on an Applied Biosystems 430A peptide synthesizer. The peptide resin obtained by FastMoc cycles starting with an Fmoc-Gly-Wang resin (0.5108 g, 0.49 mmol g⁻¹) was simultaneously deprotected and cleaved with a mixture of TFA/H₂O (95 : 5 v/v) for 4 h at R.T. The crude peptide was precipitated with ether and lyophilized. Yield 0.1541 g, (88%) (content of peptide 7: 71%). To get the pure product, we used preparative HPLC with a 5 μ m μ Bondasphere C18 column (19 mm $\times$ 150 mm).

³¹P NMR (121.5 MHz, D₂O) δ = -6.43 (s). FAB-MS: Found: m/z 703.6 (M)⁺, 726.2 (M+Na)⁺. Calcd for C₂₇H₄₂N₇O₁₃P: M⁺, 703.3.

Amino acid ratios (4% HSCH₂CO₂H in 6 M HCl, 120 °C, 48 h): Gly 2.07 (2), Val 0.98 (1), Tyr 1.02 (1), Ala 2.13 (2), Ser 0.81 (1).

Alternative syntheses of peptide **7** using **4a**, **b**, **c**, and **d**, respectively, as building blocks were done on an Advanced ChemTech 350 multiple peptide synthesizer, starting with Fmoc-Gly-Wang resin (50 mg, 0.49 mmol g⁻¹). Couplings were done with 6 mol amt. of Fmoc-amino acid with *N,N'*-diisopropylcarbodiimide (DIC) and HOBt in 1-methyl-2-pyrrolidinone (NMP), and the Fmoc group was cleaved after each coupling cycle using 40% piperidine in DMF. The peptide was deprotected and released from the resin with a mixture of TFA/H₂O (95 : 5 v/v) at R.T. for 4 h. The resin was filtered and washed with dichloromethane (5 $\times$ 2 ml) and the filtrate extracted with 10% AcOH aq (2 $\times$ 3 ml). The aqueous AcOH extracts containing crude peptide were combined and lyophilized.

7 using 4a: Yield 19.4 mg, (content of peptide **7**: 83%). Amino acid ratios (4% HSCH₂CO₂H in 6 M HCl, 120 °C, 24 h): Gly 2.16 (2), Val 0.93 (1), Tyr 0.88 (1), Ala 2.36 (2), Ser 0.67 (1).

7 using 4b: Yield 22.7 mg, (content of peptide **7**: 43%).

Amino acid ratios (4% HSCH₂CO₂H in 6 M HCl, 120 °C, 24 h): Gly 2.18 (2), Val 0.88 (1), Tyr 0.79 (1), Ala 2.25 (2), Ser 0.90 (1).

7 using 4c: Yield 25.6 mg, (content of peptide **7**: 79%).

7 using 4d: Yield 13.8 mg, (content of peptide **7**: 72%).

Amino acid ratios (4% HSCH₂CO₂H in 6 M HCl, 120 °C, 24 h): Gly 2.11 (2), Val 0.82 (1), Tyr 0.79 (1), Ala 2.50 (2), Ser 0.78 (1).

b) A Phosphopeptide with PDGF- β Receptor Sequence,

H-Tyr(PO₃H₂)-Val-Pro-Met-Leu-OH (8): Peptide **8** was synthesized on an Applied Biosystems 430A peptide synthesizer. Starting with an Fmoc-Leu-Wang resin (0.446 g, 0.56 mmol g⁻¹), a FastMoc cycle was used on Fmoc-amino acids containing **4c**. The peptide was simultaneously deprotected and released from the resin with a mixture of TFA/H₂O/EDT/PhSMe/PhOH (8.25 : 0.5 : 0.25 : 0.5 : 0.75, v/v/v/v/w) for 4 h at R.T. The crude peptide was precipitated with ether and lyophilized. Yield 0.1168 g, (67%) (content of peptide **8**: 85%).

Purification was done by preparative HPLC on a 5 μ m μ Bondasphere C18 (19 mm $\times$ 150 mm) to yield the pure product.

³¹P NMR (121.5 MHz, CD₃OD) δ = -7.24 (s). FAB-MS: Found: m/z 724.9. Calcd for C₃₀H₄₈N₅NaO₁₀PS: (M+Na)⁺, 724.8.

Amino acid ratios (4% HSCH₂CO₂H in 6 M HCl, 120 °C, 48 h): Tyr 0.97 (1), Val 0.99 (1), Pro 1.21 (1), Met 1.01 (1), Leu 1.03 (1).

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- 3) Abbreviations: Symbols for amino acids and peptides are in accordance with the recommendations of the IUPAC-IUB Joint Commission on Biochemical Nomenclature: *Biochem. J.*, **219**, 345 (1984). The following other abbreviations were used: Boc, *t*-butoxycarbonyl; Buⁱ, isobutyl; BuLi, butyl lithium; Bzl, benzyl; DBU, 1, 8-diazabicyclo[5.4.0]undec-7-ene; DIEA, *N,N*-diisopropylethylamine; DMAP, 4-dimethylaminopyridine; EDC-HCl, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; EDT, 1, 2-ethanedithiol; FAB-MS, fast atom bombardment mass spectrum; Fmoc, 9-fluorenylmethyloxycarbonyl; HOBt, 1-hydroxybenzotriazole; HPLC, high-performance liquid chromatography; HRMS, high resolution mass spectrum; LDA, lithium diisopropylamide; NMR, nuclear magnetic resonance; OPfp, pentafluorophenyl ester; OSu, *N*-hydroxysuccinimide ester; PhSMe, thioanisole; Prⁱ, isopropyl; Prⁿ, propyl; TBTU, *O*-(benzotriazol-1-yl)-*N,N,N'*, *N'*-tetramethyluronium tetrafluoroborate; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TLC, thin-layer chromatography; Tyr(*P*), phosphotyrosine; Z, benzyloxycarbonyl.
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