Synthesis and Application of Bis-Silylethyl-Derived Phosphate-Protected Fmoc-Phosphotyrosine Derivatives for Peptide Synthesis

Hann-Guang Chao,* Michael S. Bernatowicz, Paul D. Reiss, and Gary R. Matsueda

Department of Macromolecular Biochemistry, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, New Jersey 08543

Received May 12, 1994[⊗]

Three Fmoc-phosphotyrosine derivatives with silylethyl-based phosphate protection were synthesized and evaluated for use in peptide synthesis. The stability of these derivatives toward piperidine/DMF (1:4) treatment and their cleavability with TFA solutions were examined. On the basis of these data, the bis[(methyldiphenylsilyl)ethyl]-protected Fmoc-phosphotyrosine derivative or Fmoc-Tyr(PO_3MDPSE_2)-OH, was shown to be the most suitable candidate for the production of phosphotyrosine peptides. The syntheses of phosphotyrosine peptides including one containing Met and Cys are described.

Introduction

With the discovery of *Src* homology 2 (SH2) domain proteins and the importance of their interactions with phosphotyrosine motifs in biochemical signal transduction, there has been an increasing interest in using synthetic phosphotyrosine peptides to study protein–protein interactions at the molecular level.[1,2] Currently two approaches are commonly used to synthesize phosphotyrosine peptides, (a) the post-assembly approach[3] where the phosphate group is added after peptide chain assembly, and (b) the building-block approach[4] where a protected phosphotyrosine is used during stepwise peptide assembly. Limitations to the post-assembly approach include degradation of Met, Cys, and Trp by the oxidation step used when phosphitylating agents are used.[3b,c] The use of protected phosphotyrosine derivatives (building-blocking approach) is more desirable for phosphotyrosine peptides containing these amino acid residues and perhaps more convenient for general use. In the past, several protected Fmoc-phosphotyrosine derivatives and more recently Fmoc-Tyr(PO_3H_2)-OH[5] itself have been used for the preparation of phosphotyrosine peptides. However, premature cleavage with piperidine,[4d,4i] instability on storage,[4e] or incomplete cleavage with TFA[4f,4i] are some of the drawbacks associated with these derivatives.

The goal of this work was to prepare more suitably protected Fmoc-phosphotyrosine derivatives that would be stable under conditions used for Fmoc removal yet readily removable with TFA and ideally stable to long term storage. Such derivatives would be particularly beneficial for the synthesis of longer, complex phosphotyrosine peptides.

Here we report the synthesis and application of the (methyldiphenylsilyl)ethyl (MDPSE) protected Fmoc-phosphotyrosine derivative and several analogs for the production of phosphotyrosine peptides. The MDPSE-phosphate group was shown to be stable under the conditions used for repetitive deprotection of the Fmoc group and readily removable with TFA. In addition, it was stable at room temperature for several months.

Results and Discussion

There are three important requirements in the design of fully protected phosphotyrosine building blocks useful for stepwise Fmoc/*tert*-butyl-based solid phase synthesis. They are: (1) stability during piperidine treatment, (2) cleavability with TFA, and (3) stability during long term storage. Phosphate trialkyl esters are known to undergo mono-dealkylation by reaction with amines with rates dependent on the bulkiness of alkyl groups employed for protection.[6] The rate dependence of mono-dealkylation was clearly observed when the *tert*-butyl, benzyl, and methyl protected phosphotyrosine derivatives were used

[⊗] Abstract published in *Advance ACS Abstracts,* September 15, 1994.

(1) (a) Ullrich, A.; Schlessinger, J. *Cell* **1990**, *61*, 203. (b) Zhou, S.; Shoelson, S. E.; Chaudhuri, M.; Gish, G.; Pawson, T.; Haser, W. G.; King, F.; Roberts, T.; Ratnofsky, S.; Lechleider, R. J.; Neel, B. G.; Birge, R. B.; Fajardo, J. E.; Chou, M. M.; Hanafusa, H.; Schaffhausen, B.; Cantley, L. C. *Cell* **1993**, *72*, 767.

(2) Abbreviations for amino acids and nomenclature of peptide structures follow the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (*J. Biol. Chem.* **1971**, *247*, 997). Other abbreviations are as follow: AAA = amino acid analysis; BOP = (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate; Cbz = benzyloxycarbonyl; DIEA = diisopropylethylamine; DMF = *N,N*-dimethylformamide; DMPSE = (dimethylphenylsilyl)ethyl; HATU = *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-bis(tetramethylene)uronium hexafluorophosphate; LDA = lithium diisopropylamide; MS/FAB = mass spectrometry/fast atom bombardment ; Fmoc = 9-fluorenylmethyloxycarbonyl; HOBt = 1-hydroxybenzotriazole; HPLC = high-performance liquid chromatography; Linker AM = 2-(4-Fmoc-aminomethyl(2,4–dimethoxyphenyl)phenoxy)acetic acid; MDPSE = (methyldiphenylsilyl)ethyl; OSu = N-succinimidyloxy; TBDMS = *tert*-butyldimethylsilyl; TFA = trifluoroacetic acid; THF = tetrahydrofuran; TLC = thin layer chromatography; TMSE = (trimethylsilyl)ethyl.

(3) (a) Chao, H.-G.; Bernatowicz, M. S.; Klimas, C. E.; Matsueda, G. R. *Tetrahedron Lett.* **1993**, *34*, 3377. (b) Perich, J. W. *Int J. Pept. Protein Res.* **1992**, *40*, 134. (c) Andrews, D. M.; Kitchin, J.; Seale, P. W. *Int. J. Pept. Protein Res.* **1991**, *38*, 469. (d) de Bont, H. B. A.; van Boom, J. H.; Liskamp, R. M. J. *Tetrahedron Lett.* **1990**, 2497. (e) Perich, J. W.; Johns, R. B. *Aust. J. Chem.* **1990**, *43*, 1623.

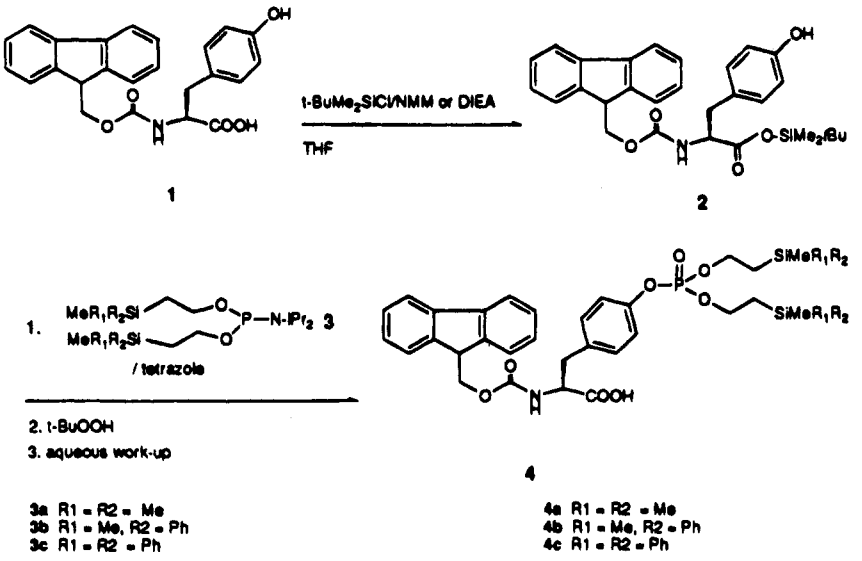
(4) (a) Perich, J. W.; Ruzzene, M.; Pinna, L. A.; Reynolds, E. C. *Int. J. Pept. Protein Res.* **1994**, *43*, 39. (b) Bannwarth, W.; Kitas, E. A. *Helv. Chim. Acta* **1992**, *75*, 707. (c) Kitas, E. A.; Knorr, R.; Trzeciak, A.; Bannwarth, W. *Helv. Chim. Acta* **1991**, *74*, 1314. (d) Kitas, E. A.; Wade, J. D.; Johns, R. B.; Perich, J. W.; Tregear, G. W. *J. Chem. Soc. Chem. Commun.* **1991**, 338. (e) Perich, J. W.; Reynolds, E. C. *Int. J. Pept. Protein Res.* **1991**, *37*, 572. (f) Kitas, E. A.; Perich, J. W.; Tregear, G. W.; Johns, R. B. *J. Org. Chem.* **1990**, *55*, 4181. (g) Zardeneta, G.; Chen, D.; Weintraub, S. T.; Klebe, R. J. *Anal. Biochem.* **1990**, *190*, 340. (h) Valerio, R. M.; Alewood, P. F.; Johns, R. B.; Kemp, B. E. *Int. J. Pept. Protein Res.* **1989**, *33*, 428. (i) Kitas, E. A.; Perich, J. W.; Wade, J. D.; Johns, R. B.; Tregear, G. W. *Tetrahedron Lett.* **1989**, *30*, 6229. (j) Perich, J. W.; Johns, R. B. *J. Org. Chem.* **1988**, *53*, 4103. (k) Bannwarth, W.; Trzeciak, A. *Helv. Chim. Acta* **1987**, *70*, 175.

(5) Ottinger, E. A.; Shekels, L. L.; Bernlohr, D. A.; Barany, G. *Biochemistry* **1993**, *32*, 4354.

(6) Gray, M. D. M.; Smith, D. J. H. *Tetrahedron Lett.* **1980**, *21*, 859. (b) Christodoulou, C.; Reese, C. B. *Tetrahedron Lett.* **1983**, *24*, 951.

0022-3263/94/1959-6687$04.50/0

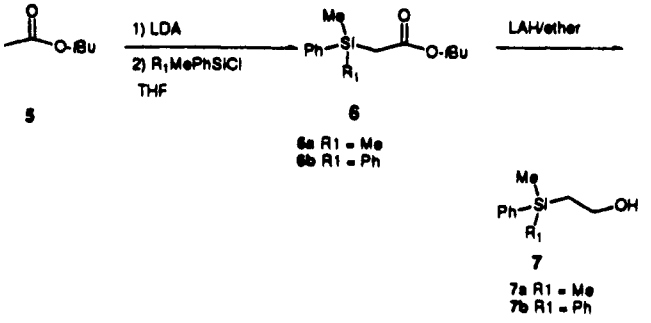
Scheme 1



in Fmoc-based solid phase peptide synthesis. For the *tert*-butyl- protected phosphate, protection remained intact after piperidine treatment whereas a rapid dealkylation was observed for the methyl- or benzyl-protected phosphates.[4b,4i] Recently we reported the synthesis of a novel phosphitylating agent, bis[2-(trimethylsilyl)ethyl] *N,N*-diisopropylphosphoramidite.[3a] This reagent is fully compatible with the Fmoc/*tert*-butyl strategy and has been successfully used in the preparation of several phosphotyrosine-containing peptides by the postassembly approach. These results led to the design and synthesis of protected Fmoc-phosphotyrosine derivatives using bis-(2-trisubstituted silyl)ethyl moieties with the hope that sufficient bulkiness of the trisubstituted silylethyl groups would provide enough protection against base-promoted dealkylation.

Due to the commercial availability of the starting silyl alcohol needed for its preparation, we first chose the (trimethylsilyl)ethyl (TMSE) group for stability studies. The key phosphitylating agent **3a** used for the synthesis of the desired fully protected phosphotyrosine derivative was prepared according to a published procedure.[3a,7] Fmoc-Tyr(PO_3TMSE_2)-OH (**4a**) was synthesized from **3a** according to the procedure reported by Perich and Reynolds (Scheme 1).[8] In this procedure, the *tert*-butyldimethylsilyl (TBDMS) group was used for transient carboxyl protection and the protected intermediate, without isolation, was phosphitylated and oxidized to give the fully protected Fmoc-phosphotyrosine derivative. Although this method is straightforward, in our hands introduction of the TBDMS group at the carboxyl function was not as specific as expected and attempts to improve this step using various silylating agents or bases were unsuccessful. Nevertheless Fmoc-Tyr(PO_3TMSE_2)-OH (**4a**) could be obtained using this one-pot procedure in yields of 40−50% as a crystalline solid after purification by column chromatography.[9] The crystalline Fmoc-Tyr(PO_3TMSE_2)-OH derivative, however, was found to completely decompose on standing at room temperature for 2−3 days. The decomposition product was identified as mono-trimethylsilylethyl protected phosphate diester

Scheme 2

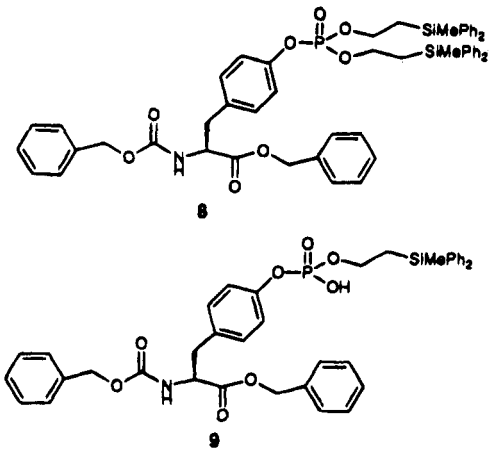


by MS/FAB. These results suggest that although Fmoc-Tyr(PO_3TMSE_2)-OH (**4a**) may be useful for the preparation of phosphotyrosine peptides, it lacks sufficient long term stability. The instability of **4a** is presumably due to the intrinsic acidity of its free carboxylic acid. Conversion of **4a** to the corresponding 2,4,5-trichloro or pentachlorophenyl esters was found to preserve the integrity of the protected phosphate but with the disadvantage of an additional step required for their preparation.

It has been shown that the (dimethylphenylsilyl)ethyl (DMPSE) group is more acid resistant as an oxygen protectant than the (trimethylsilyl)ethyl group.[10] The increased acid stability is presumably due to the electron-withdrawing ability and steric hindrance of the phenyl group. The use of DMPSE for phosphate protection was therefore examined. To synthesize the DMPSE protected phosphotyrosine derivative, 2-(dimethylphenylsilyl)-ethanol (**7a**) was first prepared by reaction of dimethylphenylsilyl chloride with *tert*-butyllithioacetate followed by $LiAlH_4$ reduction (Scheme 2).[10,11] The phosphoramidite **3b** and Fmoc-Tyr(PO_3DMPSE_2)-OH (**4b**) were then synthesized as described and were obtained in 85 and 60% yield, respectively. As expected, **4b** was found to be more stable than **4a**. Compound **4b** generated only 5−10% of the phosphate diester on standing at room temperature for 2 days as determined by NMR.

(7) Perich, J. W.; Johns, R. B. *Synthesis* **1988**, 142.
(8) Perich J. W.; Reynolds, E. C. *Synlett* **1991**, 577.
(9) Crystalline **4a** was only obtained once. We have not been able to reproduce crystalline **4a** in several attempts.
(10) Fotouhi, N.; Kemp, D. S. *Int. J. Pept. Protein Res.* **1993**, *41*, 153.
(11) Crude *tert*-butyl -α-(dimethylphenylsilyl) or α-(methyldiphenylsilyl) acetate were used directly without purification and the resulting alcohols were obtained, after $LiAlH_4$ reduction, in good yields and purity.

Scheme 3



Under similar conditions **4a** was completely decomposed to the phosphate diester. Unfortunately **4b** could only be obtained as an oil and therefore could not be handled with ease. As an alternative, (methyldiphenylsilyl)ethyl (MDPSE) protection, used in nucleotide synthesis,[12] was examined. In order to synthesize **4c**, 2-(methyldiphenylsilyl)ethanol **7b** was first prepared following the procedure as described for **7a**. The corresponding phosphoramidite **3c** and Fmoc-Tyr(PO_3MDPSE_2)-OH (**4c**) were then synthesized in 90 and 70% yield, respectively. Fortunately **4c** was obtained as an amorphous powder. A solid sample of **4c** was left standing at room temperature for 2 weeks with little or no decomposition as determined by NMR and TLC.

In order to determine TFA cleavability and the stability under piperidine treatment of the MDPSE protection, Z-Tyr(PO_3MDPSE_2)-OBzl (**8**) was synthesized from Z-Tyr-OBzl using phosphoramidite **3c** followed by oxidation with *tert*-BuOOH. When **8** was treated with 50% TFA in CH_2Cl_2 or TFA/phenol (95:5) solution, complete removal of MDPSE groups were observed within 30 min. Complete removal of the MDPSE groups were also observed within 1 h with 25% TFA in CH_2Cl_2.

In order to examine the stability under piperidine treatment, a solution of **8** (40 mg in 0.5 mL (4:1) of DMF-d_7–piperidine solution) was monitored by ^{31}P NMR (−4.28 ppm, Figure 1A). Compound **8** was shown to be stable for 24 h (Figure 1B); however, an additional peak (−3.07 ppm) was observed afterwards and approached 45% in 7 days (Figure 1C). Similar results were obtained on the TMSE or DMPSE protected analogs. The solution used for the NMR study was evaporated to give a residue which was examined by MS/FAB. The MS/FAB analysis of the mixture showed in addition to **8**, two additional components with peaks at $(M - H)^- = 708$ and $(M + H)^+ = 310$. The $(M + H)^+ = 310$ peak was assigned as N-MDPSE piperidine. The component with $(M - H)^- = 708$ was isolated and assigned as Z-Tyr($PO_3HMDPSE$)-OBzl (**9**) the mono-MDPSE-protected analog of **8**, on the basis of ^{31}P (−3.07 ppm) and ^1H NMR. Given the fact that mono-dealkylation of the MDPSE protected phosphate derivative could only be observed with 20% piperidine/DMF after 24 h, the stability of the MDPSE protection is sufficient to, theoretically, allow the synthesis of a 100-amino acid residue phosphotyrosine peptide (based on a 10-min deprotection cycle).

To demonstrate the utility of Fmoc-Tyr(PO_3MDPSE_2)-OH, three octapeptide amides containing phosphotyrosine, PDDY(PO_3H_2)EDEN-NH_2 (**10**), ENLY(PO_3H_2)-EGLN-NH_2 (**11**), and CSMY(PO_3H_2)EDIS-NH_2 (**12**), derived from MB-1 protein,[13] were synthesized. Peptide chains were assembled using the Fmoc/*tert*-butyl strategy. Coupling reactions were carried out using BOP[14] or HATU[15] reagents. Generally, peptide chain elongation proceeded smoothly as determined by the ninhydrin test except for **12** where difficult couplings were observed starting with the glutamic acid residue. Double couplings were required to reach ninhydrin-negative endpoints for the remainder of this synthesis.[16] Peptides **10**–**12** were purified to homogeneity by HPLC. The yields of the isolated peptides were 44, 38, and 25% as determined by quantitative amino acid analysis. The purity and identity of these three phosphotyrosine peptides were established by MS/FAB.

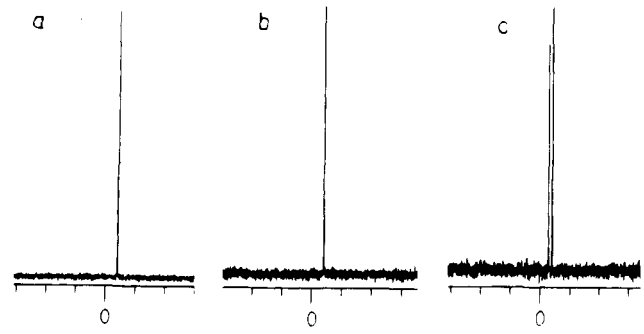


Figure 1. Stability study of the bis-MDPSE protected phosphotyrosine derivative. A solution of Z-Tyr(PO_3MDPSE_2)-OBzl (**8**), 40 mg in 0.5 mL of 4:1 DMF-d_7/piperidine solution, was monitored by ^{31}P NMR. (a) Time zero, (b) 1 day, and (c) 7 days.

Conclusions

Here is the first report of using substituted silylethyl moieties for phosphate protection in peptide synthesis. Three silylethyl-based fully protected Fmoc-phosphotyrosine derivatives were prepared and examined. Among them, Fmoc-Tyr(PO_3MDPSE_2)-OH was found to be the most suitable for the production of phosphotyrosine peptides as determined by its physical and chemical properties. Several phosphotyrosine peptides were synthesized using this derivative and were obtained in high purity and good yields. We also anticipate that the β-elimination propensity for the silylethyl-based protecting groups during TFA treatment should make it particularly attractive for sequences containing tryptophan residue which suffers from alkylation side reactions.[17]

Experimental Section

General. Normal workup from an organic solvent involved drying over $MgSO_4$ and rotary evaporation. Melting points were obtained using a micro hot plate apparatus and are

(12) Honda, S.; Hata, T. *Tetrahedron Lett.* **1981**, *22*, 2093.

(13) Campbell, K. S.; Hager, E. J.; Friedrich, R. J. Cambier, J. C. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 3982.

(14) Castro, B.; Dormoy, J.-R.; Evin, G.; Selve, C. *Tetrahedron Lett.* **1975**, 1219.

(15) (a) Carpino, L. A.; El-Faham, A.; Minor, C. A.; Albericio, F. *J. Chem. Soc. Chem. Commun.* **1994**, 201. (b) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397.

(16) Unphosphorylated peptide **11** was also prepared similarly using Fmoc-Tyr-OH and difficult couplings were likewise observed starting at same residue and throughout the synthesis.

(17) (a) Chao, H.-G.; Bernatowicz, M. S.; Reiss, P. D.; Klimas, C. E.; Matsueda, G. R. *J. Am. Chem. Soc.* **1994**, *116*, 1746. (b) Chao, H.-G.; Bernatowicz, M. S.; Matsueda, G. R. *J. Org. Chem.* **1993**, *38*, 2640.

uncorrected. All NMR spectra were recorded on a Varian XL-300 NMR spectrophotometer at 300 MHz (^{1}H), 75 HMz (^{13}C), or 121 MHz (^{31}P). The chemical shifts were reported relative to tetramethylsilane for proton and carbon and to 85% phosphoric acid for phosphorus NMR. Mass spectra were obtained by CI, FAB, or ion spray instrumentation. Hydrolysis of peptides for amino acid composition analysis was performed according to the procedure of Liu and Boykins.[18] Amino acid analysis was carried out using the Pico Tag method.[19] TLC was performed on precoated silica gel 60 F254 plates. Compounds on TLC plates were visualized with UV light, iodine, or 2% ninhydrin in EtOH. Analytical HPLC was performed using a YMC ODS-AQ column, 4 mm × 100 mm, 3 μm particle, (Morris Plains, NJ) and a commercial pumping system with high pressure mixing. The column effluent was monitored with a photodiode array detector. Solvents used for HPLC elution were the following: A, 0.1% (w/v) TFA in H_2O; B, 0.07% (w/v) TFA in acetonitrile/H_2O (95:5). The column was eluted with a linear gradient of 6–64% solvent B over the course of 9 min at a flow rate of 2.0 mL/min. For preparative HPLC, a Waters (Milford, MA) PrepPak (C18, 25 × 300 mm, 15 μm, 100 Å pore) column was eluted with the same gradient over the course of 36 min at 20 mL/min. The column effluent was monitored at 215 nm with a variable wavelength detector.

All organic reagents or solvents were used as supplied unless otherwise specified. DIEA and piperidine were purchased from Fluka (Buchs, Switzerland). 2-(Trimethylsilyl)-ethanol, *tert*-butyldimethylsilyl chloride, $LiAlH_4$, 1*H*-tetrazole, 90% tert-BuOOH, and dichloro(diisopropylamino)phosphine were purchased from Aldrich (Milwaukee, WI). Phenyldimethylchlorosilane and diphenylmethylchlorosilane were purchased from Hüls America (Bristol, PA). Fmoc-OSu was purchased from Bachem (Torrance, CA). Fmoc-Linker AM and BOP reagent were purchased from Milligen/Biosearch (Burlington, MA). THF was distilled from sodium/benzophenone prior to use. Sequencing grade DMF was purchased from Fisher Scientific (Fair Lawn, NJ). All other solvents were analytical reagent grade or better and were used as supplied.

N^{α}-(9-Fluorenylmethyloxycarbonyl)tyrosine (1). To a suspension of 18.1 g (0.1 mol) of tyrosine in 150 mL of 10% $NaHCO_3$ was added 34 g (0.1 mol) of Fmoc-OSu in 300 mL of acetone. The resulting mixture was stirred at room temperature for 20 h and acetone was removed by rotary evaporation. Upon extraction with ether, the sodium salt of Fmoc-tyrosine precipitated and was collected by filtration; the solid material was washed thoroughly with H_2O then EtOAc and dried *in vacuo*. This sodium salt was suspended in 300 mL of H_2O and acidified with 6 N HCl. The free acid was extracted twice with 100 mL of EtOAc and the organic solution was washed with brine. Normal workup furnished a residue which was recrystallized from EtOAc/hexane (3:2) to give 29.6 g (73.4%) of the title compound as white crystals: mp 184–185 °C. (lit.[20a] mp 98–107 °C; lit.[20b] mp 184–185 °C).

General Procedure for the Preparation of Phosphoramidites. To a solution of 10 g (47 mmol) of dichloro-(diisopropylamino)phosphine in 40 mL of dry ether at 0 °C was added dropwise a solution of 80 mL of dry ether containing 98 mmol of silyl-alcohol and 100 mmol of triethylamine. After addition was completed, the resulting solution was allowed to gradually warm to room temperature and stirred overnight. The precipitated hydrochloride salt was removed by filtration and washed thoroughly with ether. The combined organic phase was washed with 10% $NaHCO_3$ and brine. After removal of solvent the residue was purified by silica gel column chromatography with elution by hexane/EtOAc/triethylamine (90:10:4).

Bis[2-(trimethylsilyl)ethyl] *N,N*-diisopropylphosphoramidite (3a) was obtained in 81% yield as a clear liquid: ^{1}H NMR ($CDCl_3$) δ 0.0 (s, 18H), 1.02 (m, 4H), 1.15 (d, J = 7 Hz, 12 H), 3.56–3.73 (m, 6H); ^{13}C NMR ($CDCl_3$) −1.49, 19.97, 20.05, 24.41, 24.51, 42.47, 42.63, 60.43, 60.67; ^{31}P NMR ($CDCl_3$) 143.55 ppm. Anal. Calcd for $C_{16}H_{40}NO_2Si_2P$: C,52.56; H, 11.02; N, 3.83. Found: C, 52.31; H, 10.68; N, 3.81.

Bis[2-(dimethylphenylsilyl)ethyl] *N,N*-diisopropylphosphoramidite (3b) was obtained in 85% yield as a clear liquid: ^{1}H NMR ($CDCl_3$) δ 0.4 (s, 12H), 1.22 (d, J = 7 Hz, 12H), 1.33–1.40 (m, 4H), 3.62–3.89 (m, 6H), 7.41–7.63 (m, 10H); ^{13}C NMR ($CDCl_3$) −2.66, 19.26, 19.34, 24.51, 24.61, 42.62, 42.79, 60.38, 60.63, 127.76, 128.92, 133.48, 138.71; ^{31}P NMR ($CDCl_3$) 144.02 ppm. Anal. Calcd for $C_{26}H_{44}NO_2Si_2P$: C, 63.76; H, 9.06; N, 2.86. Found: C, 63.66; H, 8.91; N, 2.80.

Bis[2-(methyldiphenylsilyl)ethyl] *N,N*-diisopropylphosphoramidite (3c) was obtained in 90% yield as an oil: ^{1}H NMR ($CDCl_3$) δ 0.71 (s, 6H), 1.21 (d, J = 7 Hz, 12H), 1.69–1.77 (m, 4H), 3.62–3.79 (m, 2H) 3.83–3.95 (m, 4H), 7.42–7.51 (m, 12H), 7.63–7.67 (m, 8H); ^{13} C NMR ($CDCl_3$) −3.85, 17.98, 18.07, 24.53, 24.63, 42.63, 42.79, 60.29, 60.53, 127.91, 129.31, 134.45, 136.56; ^{31}P NMR ($CDCl_3$) 144.05 ppm. Anal. Calcd for $C_{36}H_{48}NO_2Si_2P$: C, 70.43; H, 7.88; N, 2.28. Found C, 70.14; H, 7.91; N, 2.39.

General Procedure for the Synthesis of Bis(2-trisubstituted silyl)ethyl Protected Fmoc-tyrosine Derivatives. To a solution of 2.02 g (5 mmol) of Fmoc-Tyr-OH in 15 mL of dry THF was added NMM or DIEA (5 mmol) followed by TBDMS-Cl (5 mmol). The resulting mixture was stirred at room temperature for 15 min. To this mixture there was added tetrazole (15 mmol) followed by phosphoramidite **3** (10 mmol) in 5 mL of THF. The resulting solution was stirred at room temperature for 2 h and cooled to 0 °C ,and *tert*-BuOOH (90%, 15 mmol) was added in one portion. After being stirred at 0 °C for 3 h, solvent was removed and the residue dissolved in 50 mL of EtOAc. The organic solution was washed with 5% $KHSO_4$ and brine and dried. The residue, after evaporation, was purified by silica gel column chromatography with elution by $CHCl_3$/MeOH/HOAc (9:1:0.1).

N^{α}-(9-Fluorenylmethyloxycarbonyl)-*O*-[Bis[2-(trimethylsilyl)ethyl]phosphono]tyrosine (Fmoc-Tyr(PO_3TMSE_2)-OH, 4a). The title compound was obtained as a solid in 44% yield: ^{1}H NMR ($CDCl_3$) δ 0.0 (s, 18H, 2 × $SiMe_3$), 1.10 (t, 4H, J = 8.3 Hz, 2 × CH_2Si) 3.14 (d, 2H, CH_2), 4.12–4.48 (m, 7H, $CHCH_2$ + 2 × OCH_2), 4.65 (m, 1H, α-CH) 5.57 (d, 1H, NH), 7.05 (s, 4H, aromatic, tyrosine), 7.13–7.80 (m, 8H, aromatic); ^{31}P NMR ($CDCl_3$) −5.91 ppm; MS/FAB (M + H$^+$) 684, calcd 683 (M). Anal. Calcd for $C_{34}H_{46}NO_8Si_2P$: C, 59.71; H, 6.78; N, 2.05. Found: C, 59.31; H, 6.43; N, 1.82.

N^{α}-(9-Fluorenylmethyloxycarbonyl)-*O*-[Bis[2-(dimethylphenylsilyl)ethyl]phosphono]tyrosine (Fmoc-Tyr(PO_3DMPSE_2)-OH, (4b). The title compound was obtained as an oil in 60% yield: ^{1}H NMR (DMSO-d_6) δ 0.21 (s, 12H, 2 × $SiMe_2$), 1.19 (t, 4H, J = 8.3 Hz, 2 × CH_2Si) 2.8–3.1 (m, 2H, CH_2), 4.0–4.3 (m, 8H, $CHCH_2$, + α-CH + 2 ×OCH_2), 6.91–7.85 (m, 22H, aromatic); ^{31}P NMR (DMSO-d_6) −5.79 ppm; MS/FAB (M + H$^+$) 808, calcd 807 (M). Anal. Calcd for $C_{44}H_{50}NO_8Si_2P$: C, 65.40; H, 6.23; N, 1.73. Found: C, 65.79; H, 6.03; N, 1.71.

N^{α}-(9-Fluorenylmethyloxycarbonyl)-*O*-[Bis[2-(methyldiphenylsilyl)ethyl]phosphono]tyrosine (Fmoc-Tyr(PO_3MDPSE_2)-OH, (4c). The title compound was Lyophilized from dioxane to give a white powder in 70% yield: ^{1}H NMR (DMSO-d_6) δ 0.54 (s, 6H, 2 × CH_3Si), 1.54 (t, 4H, J = 8.5 Hz, 2 × CH_2Si), 2.75–3.1 (m, 2H, CH_2Ph), 4.04–4.18 (m, 8H, $CHCH_2$ + α-CH + 2 × OCH_2), 6.94 (d, 2H, aromatic), 7.21–7.84 (m, 30H, aromatic); ^{13}C NMR (DMSO-d_6) −4.7, 16.5, 16.6, 36.5, 46.6, 56.3, 65.5, 65.9, 66.1, 119.3, 120.0, 125.2, 125.3, 127.0, 127.6, 128.0, 129.5, 130.5, 134.1, 135.6, 140.7, 143.8, 155.8, 173.91; ^{31}P NMR (DMSO-d_6) −5.84 ppm. Anal. Calcd for $C_{54}H_{54}NO_8Si_2P$: C, 69.57; H, 5.84; N, 1.50. Found: C, 69.77; H, 5.57; N, 1.61.

Synthesis of 2-(Dimethylphenylsilyl)ethanol (7a). To the freshly prepared LDA solution (0.15 mmol) at −78 °C was added dropwise 17.4 g (0.15 mol) of *tert*-butyl acetate in 30 mL of THF over 30 min. The resulting solution was stirred at −78 °C for 1 h and 24.75 g (0.145 mol) of dimethylphenylchlorosilane in 30 mL of THF was added. After addition was completed, the reaction mixture was allowed to gradually

(18) Liu, T.-Y.; Boykins, R. A. *Anal. Biochem.* **1989**, *182*, 383.
(19) Heinrickson, R. L.; Meridith, S. C. *Anal. Biochem.* **1984**, *136*, 65.
(20) (a) Schön, I.; Kisfaludy, L. *Synthesis* **1986**, 303. (b) Sadat-Aalaee, D. Ph.D. Thesis, University of Massashusetts, Amherst, MA, 1990.

warm to room temperature, stirred overnight, and quenched with 50 mL of saturated NH_4Cl solution. The aqueous phase was extracted twice with 50 mL of ether and the combined organic solution was washed with 10% $NaHCO_3$ and brine. After removal of solvent, the crude product was dissolved in 250 mL of dry ether and cooled to 0 °C. To the solution, 6 g of $LiAlH_4$ was added portionwise over 20 min. The reaction mixture was gradually warmed to room temperature, stirred for 1 h, and refluxed for 2 h. The reaction mixture was allowed to cool to 0 °C and quenched by slow addition of 18 mL of THF/H_2O (2:1) solution, followed by 6 mL of 15% NaOH solution. The aluminum complex which had precipitated was removed by filtration and the filtrate was washed with saturated NH_4Cl, 10% $NaHCO_3$, and brine. After removal of solvent, the silyl alcohol was purified by vacuum distillation to give a clear liquid in 66% yield (17.3 g): (87–95 °C, 1.5 mmHg; lit.[10] 95 °C, 6 mmHg); ^1H NMR ($CDCl_3$) δ 0.3 (s, 6 H), 1.23 (m, 2H), 1.81–2.05 (bs, 1H, OH), 3.74 (m, 2H), 7.42(m, 3H), 7.57 (m, 2H); ^{13}C NMR ($CDCl_3$) −2.75, 21.02, 59.63, 127.8, 129.0, 133.4, 138.6.

2-(Methyldiphenylsilyl)ethanol (7b) was prepared following the procedure described for **7a**. After $LiAlH_4$ reduction, the reaction mixture was purified by column chromatography (400 g silica gel) with elution by 20% acetone in hexane to give 24.4 g (69%) of the pure alcohol as a colorless oil: ^1H NMR ($CDCl_3$) δ 0.63 (s, 3H), 1.55–1.60 (m, 2H), 1.8 (bs, 1H, OH), 3.85 (m, 2H), 7.45 (m, 6H), 7.57 (m, 4H); ^{13}C NMR ($CDCl_3$) −4.0, 19.6, 59.7, 127.9, 129.3, 134.3, 136.4. Anal. Calcd for $C_{15}H_{18}OSi$: C, 74.33; H, 7.48. Found: C, 74.04; H, 7.40.

***N*$^{\alpha}$-(Benzyloxycarbonyl)-*O*-[Bis[2-(methyldiphenylsilyl)-ethyl]phosphono]tyrosine Benzyl Ester (8).** To a solution of 405 mg (1 mmol) of Z-Tyr-OBzl and 245 mg (3.5 mmol) of tetrazole in 5 mL of THF was added 1.2 g (2 mmol) of phosphitylating agent **3c**. The resulting mixture was stirred at room temperature for 2 h and cooled to 0 °C. To this mixture was added 310 mg (90%; 3 mmol) of *tert*-BuOOH and it was stirred at 0 °C for 2 h. After aqueous workup, the residue was purified by column chromatography (50 g of silica gel) eluting with 30% acetone in hexane to give 714 mg (77%) of the title compound as a colorless oil: ^1H NMR ($CDCl_3$) δ 0.58 (s, 6H), 1.61–1.66 (t, 4H, J = 7.4 Hz), 3.08 (m, 2H), 4.18–4.28 (m, 4H), 4.65 (m, 1H), 5.02–5.17 (m, 4H), 5.22 (t, 1H), 6.93–6.95 (m, 4H), 7.27–7.51 (m, 30H); ^{13}C NMR ($CDCl_3$) −4.1, 17.4, 17.5, 37.5, 54.8, 66.5, 66.6, 67.1, 67.3, 119.9, 120, 127.9, 128.0, 128.1, 128.4, 128.5, 128.6, 129.5, 130.4, 134.2, 135.4, 156.3, 171.2; ^{31}P NMR ($CDCl_3$) −5.78 ppm; (DMF-d_7) −4.27 ppm. Anal. Calcd for $C_{54}H_{56}NO_8Si_2P$: C, 69.43; H, 6.04; N, 1.50. Found: C, 69.33; H, 5.98; N, 1.54.

Synthesis of PDDY(PO_3H_2)EDEN-NH_2 (10). The peptide synthesis was carried out manually using 0.1 mmol (312 mg, 0.32 mmol Fmoc/g substitution level) of the Fmoc-LinkerAM-(aminomethyl)polystyrene support. The peptide chain was assembled using 4 equiv of Fmoc-amino acids/BOP/DIEA. Tyrosine was coupled as Fmoc-Tyr(PO_3MDPSE_2)-OH. Generally, the coupling reactions required 20–30 min to reach completion judging by a ninhydrin test. For deprotection 20% piperidine in DMF was used (10 min). Upon completion of the synthesis, the peptide resin was washed with DMF and MeOH and dried *in vacuo* overnight. The peptide resin was treated with 10 mL of TFA/phenol/Et_3SiH (95:3:2) solution at room temperature for 2 h. After removal of TFA, 100 mL of ether was added and the resulting mixture was kept at 4 °C for 3 h. The crude peptide was isolated and purified by reversed-phase HPLC to give 58.6 mg (44%) of the homogenous peptide as a white solid. MS/FAB ($M + H^+$) 1075, calcd 1074 (M); amino acid analysis Asp 4.0 (4), Glu 2.1 (2), Pro 1.0 (1), Tyr 0.8 (1).

Synthesis of ENLY(PO_3H_2)EGLN-NH_2 (11). The peptide synthesis was carried out manually according to the protocol as previously described for **10** using 0.1 mmol (312 mg, 0.32 mmol Fmoc/g substitution level) of the Fmoc-LinkerAM-(aminomethyl)polystyrene support except HATU[15] was substituted for BOP reagent. The peptide resin was treated with 10 mL of TFA/phenol/Et_3SiH (95:3:2) for 2 h. The crude peptide was isolated and purified to furnish 73.7 mg (38%) of the homogenous peptide as a white solid: MS/FAB ($M + H^+$) 1030, calcd 1029 (M); amino acid analysis Asp 2.0 (2), Glu 2.2 (2), Gly 1 (1) Leu 2 (2), Tyr 0.9 (1).

Synthesis of CSMY(PO_3H_2)EDIS-NH_2 (12). The peptide synthesis was carried out manually according to the protocol as previously described for **10** using 0.1 mmol (223 mg, 0.45 mmol Fmoc/g substitution level) of the Fmoc-LinkerAM-(aminomethyl)polystyrene support. After coupling Glu, double couplings were required to reach a negative ninhydrin test endpoint. The peptide resin was treated with 10 mL of TFA/phenol/Et_3SiH (95:3:2) for 2 h. The crude peptide was isolated and purified to furnish 35 mg (25%) of the homogenous peptide as a white solid: MS/FAB ($M + H^+$) 1026, calcd 1025 (M); amino acid analysis Asp 1.1 (1), Glu 1.0 (1), Ser 1.9 (2), Ile 1.1 (1), Tyr 1.0 (1), Met 1.0 (1).

Acknowledgment. We thank Clifford E. Klimas for skillful analytical assistance, Dr. Mark Witmer for helpful discussions, and Dr. Salvatore A. Triolo (Millipore) for the generous gift of HATU reagent.