Efficient Synthesis of a Phosphinate Bis-Amino Acid and Its Use in the Construction of Amphiphilic Peptides

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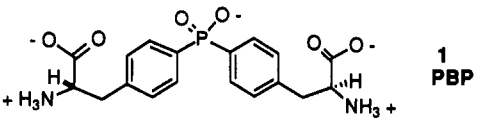
A new amphiphilic bis-amino acid has been designed and its convergent, asymmetric synthesis achieved in differentially protected form. A convenient preparation of iodophenylalanine, a generally useful starting material, is disclosed. Sequential palladium-catalyzed couplings of aryl iodides to phosphinate lead directly to the target protected bis-amino acid. Controlled peptide coupling of the new bis-amino acid is also demonstrated.

Introduction

Unusual amino acids are of considerable interest.[1] In particular, bis-amino acids, components of several peptide-derived antibiotics,[2–4] provide the conformational limitation that confers structure and activity to these compounds.[5] In the field of protein design, multiple amino acids,[6] cross-linking functionality,[6–17] and amphiphilic structures[18,19] are powerful tools for the design of peptide sequences that will fold into predictable conformations. Considerable recent activity has centered around the preparation of amino acids,[20] including natural[21–25] and unnatural[26,27] bis-amino acids.

We chose the phosphinylidene bis-phenylalanine (PBP, **1**) structure as an attractive target.[28] As an amino acid,



1
PBP

it has the properties needed for incorporation into peptides and other molecules using well-precedented chemistry. The structure is interesting as it has a single atom connection between the aromatic rings, a structural motif common to the peptide-derived antibiotics[2–4] and to a large range of synthetic macrocyclic molecules capable of binding and orienting aromatic guests.[29–35] In addition, in contrast to the known bis-amino acids, the diarylphosphinic acid provides a hydrophilic convex surface to complement the hydrophobic concavity, a particularly useful feature for construction of water-soluble binding sites.[30–32,36] We have shown that these phosphinates are appropriate for the construction of simple macrocyclic binding sites,[37] and have communicated the use of metal complexes of PBP as self-assembling receptors.[28] We are interested in the possibility of building binding sites from small peptides and therefore require the amino acid in protected form so that we can incorporate it into such peptides.

We report herein the novel synthesis of this new amphiphilic bis-amino acid, optically pure, in differentially protected form **6** suitable for peptide synthesis, in five steps from phenylalanine. Our sequence is shown in Scheme 1.

Results and Discussion

4-Iodophenylalanine (I-Phe, **2**), a known compound,[38,39] was prepared much more conveniently and cheaply by

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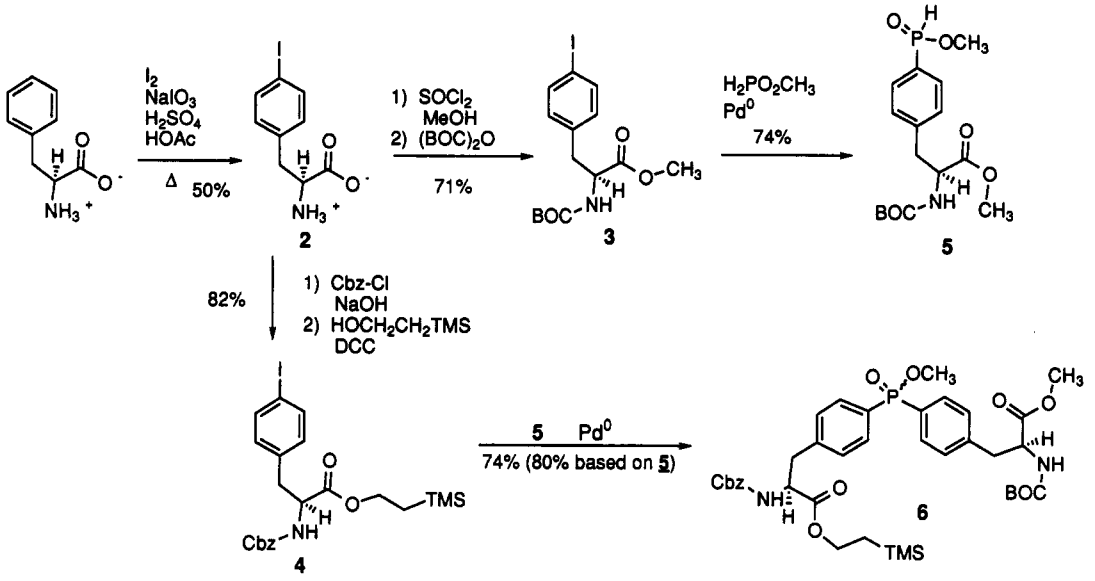
Scheme 1



direct iodination of phenylalanine in acetic acid in the presence of I_2, $NaIO_3$, and sulfuric acid. Precipitation from water by neutralization of an acidic solution and crystallization from acetic acid gave pure (*S*)-I-Phe in 50% yield. TLC on a chiral support[40] showed none of the (*R*) enantiomer. We have easily prepared hundreds of grams of iodophenylalanine by our route. A similar yield of I-Phe was obtained when the iodine oxidation was carried out with peracetic acid,[41] but the iodate oxidation was considered safer for large-scale work. Oxidation of the amino acid functionality is prevented by protonation of the amine. If the reaction mixture is heated slowly to reflux, the reaction is sometimes complete after a few minutes reflux but sometimes is incomplete after overnight reflux. Apparently this is dependent on the rate of heating; at reflux temperature in acetic acid the iodate is consumed by processes that do not lead to I-Phe.

Consequently, we carry out the reaction at 70 °C using close to a stoichiometric amount of iodate. Oxidation of the starting amino acid produces only a trace of organic extractable side product; the major loss of material is from the purification. The bulk of the remaining phenylalanine appears to have been converted to a mixture of the ortho and para isomers of I-Phe but is not easily isolated from the crystallization mother liquors.

Iodophenylalanine is itself a useful and versatile compound. It is a more hydrophobic amino acid than phenylalanine and has been shown to enhance biological activity in certain peptides.[42] Aryl iodides are particularly reactive substrates for transition metal-catalyzed transformations,[22,43,44] so many other derivatives of phenylalanine should be preparable from the iodide.

As we planned to assemble the diarylphosphinic acid by our palladium-catalyzed process,[44] ordinary peptide synthetic protecting groups were expected to survive the assembly. This led to a particularly direct method for differential protection of the two amino acid moieties of the phosphinylidene bis-phenylalanine: they can be protected separately before linkage. Classical routes involving Grignard or Friedel–Crafts chemistry[45,46] would have required a much less direct route to the amino acids, and the differential protection would have been more difficult.

Conversion of I-Phe to protected derivatives was carried out in high yield by standard procedures.[47] I-Phe was converted to the methyl ester hydrochloride with thionyl chloride in methanol and then acylated with di-*tert*-butyl dicarbonate to yield BOC-I-Phe-OMe (**3**). I-Phe was also acylated under Schotten–Baumann conditions with benzyl chlorocarbonate, and the resulting Cbz-I-Phe was esterified with 2-(trimethylsilyl)ethanol using DCC as a coupling agent, providing Cbz-I-Phe-OCH2CH$_2$SiMe$_3$ (**4**).

Application of our new procedure[44] for the preparation of monoaryl and diaryl phosphinic acid derivatives gave the desired bis-phenylalanyl phosphinates: A solution of t-BOC-I-Phe-OMe (**3**) in CH_3CN was treated under N_2 with excess propylene oxide (as a HI scavenger), 0.05 equiv of bis(triphenylphosphine)palladium chloride, and then with a solution containing 3 equiv of methyl phosphinate obtained by reaction of anhydrous phosphinic acid (hypophosphorous acid) with excess trimethyl orthoformate.[48] One hour reflux at ~75 °C provided monoaryl phosphinic acid methyl ester **5** which was not isolated but was freed of excess methyl phosphinate and decomposition products thereof by washing an ethyl acetate solution with $NaHCO_3$.[49] Subjection of this material to Cbz-I-Phe-OCH$_2$CH$_2$Si(CH$_3$)$_3$ (**4**), again under palladium catalysis in the presence of propylene oxide, led to formation of the desired differentially protected phosphinylidene bis-phenylalanine **6**. Both P–C bonds of the diarylphosphinate have been formed by the mild

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(49) Dimethyl phosphonate (dimethyl phosphite), formed by decomposition of methyl phosphinate, is not completely removed by filtration through silica as originally recommended, so we now routinely use aqueous bicarbonate to minimize contamination of product with the dimethyl arylphosphonate that results from palladium-catalyzed reaction of dimethyl phosphonate with the second aryl iodide.

palladium-catalyzed process, and all protecting groups needed for peptide synthesis are intact. The intermediate in this process, not usually isolated, but obtainable in pure form by chromatography, can potentially be converted to a variety of amino acid structures, for example by oxidation,[46] acylation, vinylation, or alkylation.[46]

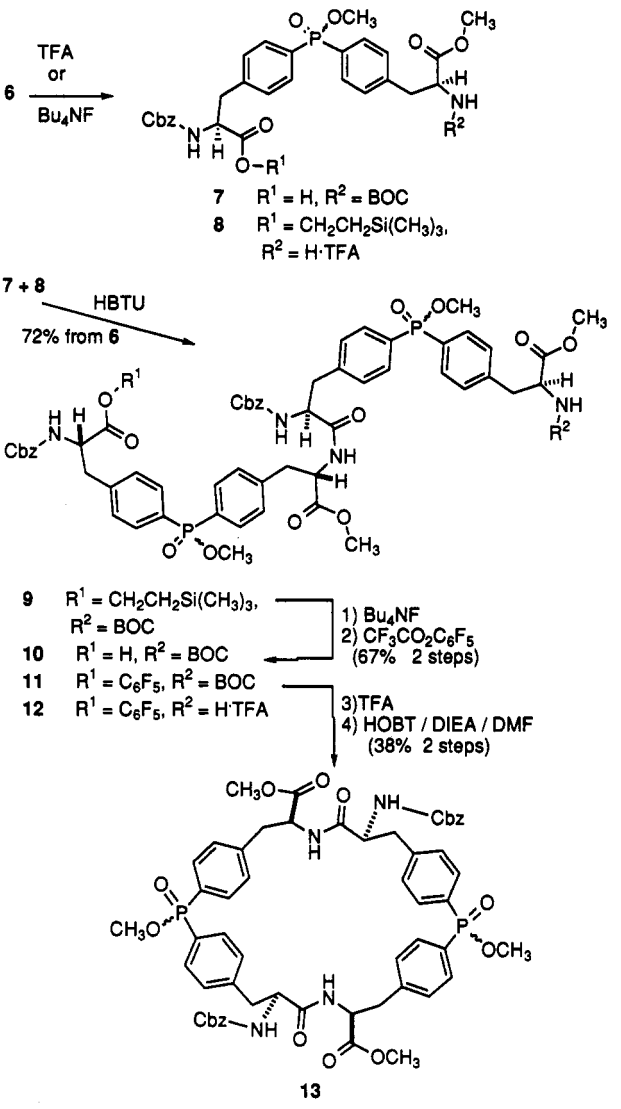
The protecting groups chosen for this preparation are differentiable. The Cbz group can be removed by hydrogenolysis over palladium, the trimethylsilylethyl ester by fluoride, and the BOC group by trifluoroacetic acid, each without disturbing any of the other groups. The phosphinic methyl ester may be cleaved by bromotrimethylsilane in the presence of the other groups, though the BOC group is also somewhat sensitive to the cleavage conditions, (as is the Cbz group, to a much lesser extent, which does not cause interference.) The carboxylic methyl ester can be saponified with hydroxide without cleavage of the phosphinic ester, but the trimethylsilylethyl ester is competitively cleaved. Nonetheless, considerable synthetic flexibility is commanded by this array of protecting groups, and other groups may be installed by similar routes. Installation of such diverse functionality in so small a molecule is noteworthy. Differential protection alone of an unprotected bis-amino acid would require more steps than our synthetic route to protected amino acid. Evans's elegant preparation of protected amino acids by enantioselective α azidation of enolates[21] leads to a very versatile preparation of bis-amino acids.[24] The sequence, however, is quite lengthy compared to a convergent route, as our and Boger's[22] work demonstrates.

We have demonstrated the peptide coupling of this new material by construction of a macrocyclic bis-dipeptide. Phosphinate **6** was converted into carboxylic acid **7** with tetrabutylammonium fluoride, and in a separate reaction the BOC was removed from **6** with trifluoroacetic acid to produce **8** (Scheme 2). Coupling of these with hydroxybenzotriazolyl tetramethyluronium hexafluorophosphate (HBTU) gave dipeptide **9** in 77% yield. It is interesting to note that, even though each of the diarylphosphinate derivatives is a mixture of diastereoisomers at phosphorus, no doubling of peaks in the ^1H or ^{13}C NMR spectra is observed for compound **6**. Only the free acid **7** shows doubling of peaks, presumably because of hydrogen bonding of the acid to the phosphinate, which provides a magnetically distinct environment for the two diastereomers.

Deprotection of the dipeptide with fluoride is again uneventful, and acid **10** is converted to pentafluorophenyl ester **11** in 65% yield using pentafluorophenyl trifluoroacetate. Deprotection of the amine of activated ester **11** with trifluoroacetic acid, and syringe pump addition of the resulting salt to a DMF solution of hydroxybenzotriazole and diisopropylethylamine, gave macrocycle **13** in up to 38% yield. The ^{31}P NMR spectrum of this material shows three signals, which we interpret to be diastereomers because of the cleanliness of the ^1H NMR, mass spectra, and HPLC. In consonance with this interpretation, deprotection of the phosphinic acids using trimethylsilyl chloride and sodium iodide, and isolation by HPLC, gives a species which shows a single resonance in the ^{31}P NMR.

In summary, we have developed a succinct route to an unusual new bis-amino acid, in an appropriately functionalized form for peptide synthesis. We have demonstrated the necessary transformations that allow incorporation of the new amphiphilic amino acid into peptides. Study of the interactions of such peptides with other molecules is underway.

Scheme 2



Experimental Section

^1H, ^{13}C, and ^{31}P NMR spectra were obtained at 300, 75.43, and 121.42 MHz, respectively, in $CDCl_3$ unless otherwise indicated. ^{13}C NMR spectra are ^1H decoupled; the coupling constants reported are for doublets due to ^{31}P. Flash chromatography was carried out as described.[50] CH_3CN used for reactions (not chromatography) was freshly distilled from P_4O_{10}. CH_2Cl_2, pyridine, THF, *N*-methylmorpholine, and diisopropylethylamine used for reactions were freshly distilled from CaH_2. HBTU was obtained from Applied Biosystems. Bis(triphenylphosphine)palladium dichloride was prepared as described.[43] Other materials were used as received, except for drying *in vacuo*.

4-Iodo-L-Phenylalanine (2). L-Phenylalanine (40.15 g, 243 mmol) in 220 mL of HOAc and 29.0 ml (542 mmol) of concentrated H_2SO_4 was stirred while powdered I_2 (24.65 g, 97.0 mmol) and $NaIO_3$ (10.18 g, 51.4 mmol) were added. The mixture was heated to 70 °C until TLC (16:3:2.5 MEK:HOAc:H_2O/ninhydrin, product $R_f = 0.54$, Phe $R_f = 0.46$) showed the reaction to be complete, which took 21 h and the addition of two 1.0-g aliquots of additional $NaIO_4$ at this 40-g scale. Completion was indicated by the I_2 color fading to orange.

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HOAc was removed by rotary evaporation at 35 °C, and the residual viscous oil was diluted with 400 mL of water and washed twice each with 100-mL aliquots of Et_2O and CH_2Cl_2. After decolorization with 5 g of Norit, the aqueous solution was neutralized with NaOH to precipitate the crude product, which, after chilling, was filtered and rinsed with 800 mL of water and then 300 mL of ethanol. The damp precipitate was crystallized from 200 mL of HOAc to yield 35.5 g (50%) of *p*-iodophenylalanine, mP 261–262 °C. A second crop was only 2.0 g (53% overall). This material was identical by ^1H NMR, TLC, and chiralplate[40] TLC to commercial I-Phe from Serva: ^1H NMR (D_2O/DCl) δ 7.82 (d, J = 8.1 Hz, 2H), 7.15 (d, J = 8.1 Hz, 2H), 4.43 (apparent t, J = 6.2 Hz, 1H), 3.36 (dd, J = 5.8, 14.7 Hz, 1H), 3.25 (dd, J = 7.5, 14.4 Hz, 1H); ^{13}C NMR (D_2O + DCl) δ 175.34, 159.02, 136.84, 132.19, 130.15, 52.47, 19.34; IR (KBr, cm^{-1}) 2918, 1718, 1701, 1585, 1522, 1487, 1396.

4-Iodo-L-phenylalanine Methyl Ester Hydrochloride. Thionyl chloride (10.2 g, 0.086 mol) was added dropwise to 10 mL of methanol that was stirring on an ice bath. (*S*)-4-Iodophenylalanine (5.0 g, 0.017 mol) was added, and the yellow solution was refluxed for 2 h and then rotary evaporated to a white solid. Crystallization from 10 mL of MeOH by addition of 50 mL of ether gave (*S*)-4-iodophenylalanine methyl ester hydrochloride (5.40 g, 92%): mp 199.5–200.5 °C; ^1H NMR (CD_3OD) δ 7.73 (d, J = 6.0 Hz, 2H), 7.05 (d, J = 6.0 Hz, 2H), 4.31 (t, J = 6.9 Hz, 1H), 3.81 (s, 3H), 3.22 (dd, J = 15.0, 6.0 Hz, 1H), 3.12 (dd, J = 15.0, 7.5 Hz, 1H). Anal. Calcd for $C_{10}H_{13}ClINO_2$: C, 35.16, H, 3.84, N 4.10 Found: C, 35.17, H, 3.88, N, 4.15.

***N*-t-BOC-4-Iodo-L-phenylalanine Methyl Ester (3).** (*S*)-4-Iodophenylalanine methyl ester hydrochloride (5.40 g, 0.0158 mol) in 30 mL of CH_2Cl_2 was treated with *N*-methylmorpholine (4.8 g, 47.8 mmol) and di-*tert*-butyl dicarbonate (4.49 g, 0.0206 mol) at rt under N_2 for 5 h. H_2O was added and CH_2Cl_2 removed under reduced pressure. The resulting yellow oil was dissolved in EtOAc, washed with saturated $NaHCO_3$, 50 mM citric acid, H_2O, and saturated NaCl, dried over Na_2SO_4, filtered, and rotary evaporated. Crystallization from 10 mL of CH_2Cl_2 and 50 mL of hexanes gave 5.1 g (77%) of **3**: mp 74–76 °C; ^1H NMR ($CDCl_3$) δ 7.61 (d, J = 8.4 Hz, 2H), 6.88 (d, J = 8.4 Hz, 2H), 5.04–4.95 (m, 1H), 4.61–4.52 (m, 1H), 3.72 (s, 3H), 3.08 (q, J = 5.7, 13.8 Hz, 1H), 2.98 (q, J = 5.7, 14.1 Hz, 1H), 1.42 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 172.06, 155.01, 137.61 (2C), 136.09, 135.74, 131.34(2C), 92.55, 54.21, 52.36, 37.93, 28.31; IR (NaCl, cm^{-1}) 3358, 2978, 1744, 1715, 1485, 1366, 1215, 1165, 1059, 1009. Anal. Calcd for $C_{15}H_{20}INO_4$: C, 44.44, H, 5.01, N 3.46. Found: C, 44.40, H, 5.02, N, 3.40.

***N*-[(Benzyloxy)carbonyl]-4-iodo-L-phenylalanine.** To (*S*)-4-Iodophenylalanine (20.0 g, 68.7 mmol) and NaOH (4.25 g, 106 mmol) in 50 mL of H_2O were added benzyl chloroformate (13.48 g, 79.0 mmol) and 50 mL of NaOH solution (4.75 g, 118.8 mmol) in approximately 10 portions alternately over a 30-min period. The cloudy reaction mixture was stirred at rt for 1 h until completion and washed with ether, and concd. HCl was added to pH 2. Precipitated product was filtered, rinsed with water and hexanes, dried in vacuo, and crystallized from 5:1 CH_2Cl_2/CH_3CN to yield 26.43 g (91%) of(*S*)-*N*-[(benzyloxy)carbonyl]-4-Iodophenylalanine: mp 150–152 °C; ^1H NMR (CD_3CN) δ 7.63 (d, J = 8.1 Hz, 2H), 7.31 (m, 5H), 7.01 (d, J = 7.5 Hz, 2H), 5.89 (m, 1H), 5.02 (s, 1H), 5.00 (s, 1H), 4.39 (m, 1H), 3.13 (dd, J = 13.8, 4.5 Hz, 1H), 2.87 (dd, J = 13.8, 9.0 Hz, 1H). An analytical sample was obtained by crystallization from methanol, mp 161–162 °C. Anal. Calcd for $C_{17}H_{16}INO_4$: C, 48.02; H, 3.79; N, 3.29. Found: C, 48.29; H, 3.92; N, 3.46.

***N*-[(Benzyloxy)carbonyl]-4-iodo-L-phenylalanine (Trimethylsilyl)ethyl Ester (4).** To *N*-[(benzyloxy)carbonyl]-4-iodo-L-phenylalanine (2.45 g, 5.78 mmol) in 20 mL of CH_3CN and 1.0 mL of pyridine on an ice bath was added 2-(trimethylsilyl)ethanol (0.82 g, 6.94 mmol) and DCC (1.33 g, 6.36 mmol). The mixture was stored in a refrigerator overnight. Oxalic acid (0.15 mL of a 5 M solution in DMF) was added and stirred for 1 h to consume any remaining DCC. The precipitate was removed by filtration and washed with EtOAc. The organic filtrate was washed with 0.5 N HCl and $NaHCO_3$ (saturated), dried with $MgSO_4$, and filtered, and the solvent was removed by rotary evaporation. Flash chromatography (1:9 EtOAc:hexanes) gave product as a white solid in 88% yield: mp 45–46.5 °C (2.68 g); ^1H NMR ($CDCl_3$) δ 7.57 (d, J = 8.4 Hz, 2H), 7.40–7.28 (m, 5H), 6.85 (d, J = 8.4 Hz, 2H), 5.28 (d, J = 8.4 Hz, 1H), 5.11 (d, J = 12.3 Hz, 1H), 5.06 (d, J = 12.3 Hz, 1H), 4.59 (q, J = 5.7, 13.8 Hz, 2H), 3.09 (q, J = 6.0, 14.1 Hz, 1H), 2.99 (q, J = 6.3, 13.8 Hz, 1H), 0.95 (d, J = 6.6 Hz, 1H), 0.92 (d, J = 6.6 Hz, 1H), 0.036 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 171.30, 155.51, 139.68, 137.56(2C), 136.19, 135.55, 131.34(2C), 128.52(2C), 128.21, 128.08(2C), 92.55, 66.95, 64.06, 54.69, 37.81, 17.34; IR (NaCl, cm^{-1}) 3730, 3584, 3554, 2953, 1726, 1514, 1250, 1059, 839, 696. Anal. Calcd for $C_{22}H_{28}INO_4Si$: C, 50.29, H, 5.37, N, 2.67. Found: C, 50.14, H, 5.54, N, 2.67.

Protected Bis-Amino Acid 6. Anhydrous phosphinic acid (28.4 mg, 0.43 mmol) (obtained by rotary evaporation of the commercial 50% aqueous solution at room temperature, followed by evacuation (<1 torr) for 2 days) was treated with trimethyl orthoformate (228 mg, 2.15 mmol) at rt under N_2 for 1 h to provide methyl phosphinate.[48] The methyl phosphinate solution was added by syringe to a solution of *N*-BOC-iodophenylalanine methyl ester (**3**) (64.9 mg, 0.14 mmol), bis(triphenylphosphine)palladium chloride (5.0 mg, 0.0072 mmol), and propylene oxide (87 mg, 1.5 mmol) in 0.3 mL of CH_3CN under N_2. The reaction mixture was heated at reflux for 4 h until TLC (EtOAc, product R_f = 0.35, sm R_f = 0.91) showed completion. A volume of 10 mL of EtOAc was added, the mixture was washed with saturated $NaHCO_3$ (5×), H_2O, and saturated NaCl, and dried over Na_2SO_4, and solvent was removed under reduced pressure. This crude **5** was carried on without purification. Spectra were acquired on a sample purified by flash chromatography (EtOAc): ^1H NMR ($CDCl_3$) δ 7.73 (dd, J = 18.0, 9.0 Hz), 7.53 (d, J = 555 Hz, 1H), 7.29 (m, 2H), 5.04 (m, 1H), 4.63 (m, 1H), 3.80 (d, J = 12.0 Hz, 3H), 3.73 (s, 3H), 3.18 (m, 2H), 1.40 (s, 9H).

To the crude **5** was added **4** (60.1 mg, 0.114 mmol) and tetrakis(triphenylphosphine)palladium (12.8 mg, 0.0114 mmol), propylene oxide (87 mg, 1.5 mmol), and 1.0 mL of CH_3CN under N_2. The brown solution was refluxed 4 h until **5** was consumed (TLC EtOAc, product R_f = 0.56, sm R_f = 0.35). Rotary evaporation, followed by flash chromatography twice (3:7 CH_3CN:CH_2Cl_2 then 5:1 EtOAc:hexanes) gave **6** in 54% yield (46 mg): ^1H NMR ($CDCl_3$) δ 7.71 (m, 4H), 7.33 (m, 5H), 7.23 (m, 4H), 5.27 (m, 1H), 5.08 (s, 2H), 5.00 (m, 1H), 4.61 (m, 2H), 4.19 (dd, J = 8.4, 17.4 Hz, 2H), 3.74 (d, J = 11.7 Hz, 3H), 3.70 (s, 3H), 3.12 (m, 4H), 1.40 (s, 9H), 0.95 (t, 2H), 0.031 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 171.93, 171.31, 155.58, 154.96, 140.95, 140.76, 136.15, 131.98, 131.84, 129.57 (d, J = 137.3 Hz), 129.78 (d, J = 1.5 Hz), 129.59 (d, J = 1.5 Hz), 128.57, 128.28, 128.13, 80.15, 77.27, 67.06, 64.21, 54.65, 54.17, 52.40, 51.61, 38.42, 38.20, 28.28, 17.41, −1.50; IR (NaCl, cm^{-1}) 3273, 2951, 1718, 1526, 1366, 1215, 1032; MS (CI, NH_3) for $C_{38}H_{51}N_2O_{10}SiP$, observed $[M + H]^+$ 755.6, $[M + NH_4)]^+$ 772.6; FAB MS (thioglycerol/TFA/H_2O) $[M + Na]^+$ 777.2948 calcd for $C_{38}H_{51}N_2O_{10}SiPNa$, found 777.2947.

Peptide Phosphinate 9. Compound **6** (35.3 mg, 0.0468 mmol) in 2 mL of 5% TFA/CH_2Cl_2 was stirred at rt for 1 h. The solution was rotary evaporated to dryness. The residue, dissolved in 5 mL of EtOAc, was washed with H_2O and dried over Na_2SO_4. Solvent was removed under reduced pressure to yield amino acid TFA salt **8** as colorless oil in 100% yield (35.9 mg): ^1H NMR ($CDCl_3$) δ 8.03 (m, 2H), 7.70 (m, 4H), 7.30 (m, 9H), 5.46 (m, 1H), 5.08 (m, 2H), 4.63 (m, 1H), 4.33 (m, 1H), 4.18 (m, 2H), 3.78 (m, 6H), 3.24 (m, 4H), 0.93 (m, 2H), 0.028 (s, 9H).

Compound **6** (71.8 mg, 0.095 mmol) in 0.4 mL of DMF was treated with 1 N tetrabutylammonium fluoride in THF (0.24 mL, 0.24 mmol) at rt for 30 min. H_2O and EtOAc were added, the mixture was acidified with 2 N HCl to pH 3, and the EtOAc layer was washed with H_2O 4× and saturated NaCl, dried over Na_2SO_4, filtered, and evaporated to leave **7** as a colorless oil (59.3 mg, 95%): ^1H NMR ($CDCl_3$) δ 7.65 (m, 4H), 7.28 (m, 9H), 5.46 (m, 1H), 5.11 (m, 1H), 4.99 (m, 2H), 4.72 (m, 1H), 4.60 (m, 1H), two diasteriomers, 3H total: 3.73 (d, J = 11.1 Hz) and 3.71 (d, J = 11.4 Hz), 3.70 (s, 3H), 3.10 (m, 4H), 1.37 (s, 9H); ^{31}P NMR ($CDCl_3$) δ 35.76, 35.58.

Trifluoroacetate **8** (921.3 mg, 1.2 mmol) and acid **7** (803.9 mg, 1.23 mmol) were dissolved in 4.0 mL of DMF, followed by HBTU[51] (780 mg, 2.02 mmol) and *N*-methylmorpholine (263 mg, 2.6 mmol). The reaction mixture was stirred at room temperature under N_2 until it became ninhydrin negative (1.5 h). The mixture was diluted with 20 mL of EtOAc, washed with saturated $NaHCO_3$, citric acid (50 mM), H_2O, and saturated NaCl, dried with Na_2SO_4, filtered, and rotary evaporated. Flash chromatography (1:1 acetone:CH_2Cl_2) gave **9** in 77% yield (1.12 g) as a colorless oil: ^1H NMR ($CDCl_3$) δ 7.69 (m, 8H), 7.32 (m, 10H), 7.22 (m, 8H), 6.65 (m, 1H), 5.47 (m, 1H), 5.37 (m, 1H), 5.06 (s, 1H), 5.04 (s, 1H), 4.78 (m, 1H), 4.64 (m, 1H), 4.42 (m, 1H), 4.19 (m, 2H), 3.68 (m, 6H), 3.69 (s, 6H), 3.10 (m, 8H), 1.44 (s, 9H), 0.95 (m, 2H), 0.028 (s, 9H); ^{31}P NMR ($CDCl_3$) δ 33.56; IR (NaCl, cm^{-1}) 3254, 3038, 2953, 1717, 1529, 1445, 1252, 1217, 1036, 733. FAB MS (thioglycerol/TFA/H_2O) $[M + H]^+$ 1291.4791 calcd for $C_{66}H_{81}N_4O_{17}P_2Si$, found 1291.4841.

Macrocyclic Bis-Dipeptide 13. Dipeptide **9** (774 mg, 0.60 mmol) in 1 mL of DMF was treated with tetra-*n*-butylammonium fluroide (1.48 mmol as 1.48 mL of 1 M THF solution) at 23 °C for 1 h. H_2O and EtOAc were added, and the pH of the mixture was adjusted to 3 by adding 0.2 N HCl. The EtOAc layer was washed 3× with citric acid (50 mM), H_2O, and saturated NaCl, dried over Na_2SO_4, filtered, and solvent rotary evaporated. Crude **10**, obtained as a colorless oil (694 mg, 97%), was carried on without further purification: ^1H NMR ($CDCl_3$) δ 7.61 (m, 8H), 7.25 (m, 18H), 5.91 (m, 1H), 5.53 (m, 1H), 5.03 (m, 6H), 4.83 (m, 2H), 4.60 (m, 2H), 3.65 (m, 6H), 3.69 (s, 6H), 3.01 (m, 8H), 1.43 (s, 9H).

Dipeptide free acid **10** (415 mg, 0.349 mmol), dried by dissolution in 0.5 mL of pyridine and evacuation to dryness, in 0.8 mL of THF and 120 mg (1.5 mmol) of pyridine at 23 °C under N_2 was treated with pentafluorophenyl trifluoroacetate[52] (293 mg, 1.05 mmol). Upon completion, (<30 min) the reaction mixture was rotary evaporated and **11** obtained as a colorless oil in 65% yield (0.308 g) by flash chromatography (6:4 acetone:CH_2Cl_2): ^1H NMR ($CDCl_3$) δ 7.76 (m, 8H), 7.31 (m, 10H), 7.15 (m, 8H), 6.79 (m, 1H), 5.75 (m, 1H), 5.66 (m, 2H), 5.06 (s, 4H), 4.94 (m, 1H), 4.75 (m, 1H), 4.56 (m, 1H), 4.40 (m, 1H), 3.65 (m, 12H), 3.11 (m, 8H), 1.37 (s, 9H); IR (NaCl, cm^{-1}) 3279, 2934, 1717, 1533, 1518, 1215, 1016.

Dipeptide pentafluorophenyl ester **11** (340 mg, 0.251 mmol) was treated with 3 mL of 30% TFA/CH_2Cl_2 solution at rt until completion (1.5 h). The solution was rotary evaporated and vacuum-dried to leave **12** (343 mg, 100%): ^1H NMR ($CDCl_3$) δ 9.81 (m, 2H), 7.68 (m, 8H), 7.28 (m, 18H), 6.63 (m, 1H), 5.91 (m, 1H), 5.43 (s, 1H), 5.11 (s, 2H), 5.03 (s, 2H), 4.78 (m, 2H), 4.48 (m, 1H), 4.34 (m, 1H), 3.79 (s, 6H), 3.70 (m, 6H), 3.14 (m 8H).

DMF (peptide synthesis grade, 300 mL) and hydroxybenzotriazole hydrate (34 mg, 0.251 mmol) were stirred with activated 3-Å molecular sieves at room temperature for 3 h and filtered, and DIEA (130 mg, 1 mmol) was added. Dipeptide **11** (343 mg, 0.251 mmol) in 13 mL of DMF was added at 23 °C via syringe pump over 22 h. (TLC: silica, acetone, product $R_f = 0.66$, sm $R_f = 0$). DMF was removed by rotary evaporation at 40 °C, the yellow residue in 15 mL of CH_2Cl_2 was washed twice each with 20 mL of 20 mM citric acid, saturated $NaHCO_3$, H_2O, and saturated NaCl, dried over Na_2SO_4, filtered, and rotary evaporated. Crude product was purified by flash chromatography (85:15 acetone: 2-propanol) to yield **13** (103 mg, 38%) as a colorless oil: ^1H NMR ($CDCl_3$) δ 7.70–7.59 (m, 8H), 7.38–7.08 (m, 18H), 6.95 (m, 8H), 6.85 (m, 1H), 6.50 (m, 1H), 5.55 (m, 1H), 5.40 (m, 1H), 5.00 (s, 4H), 4.81 (m, 2H), 4.29 (m, 2H), 3.66 (m, 12H), 2.88 (m, 8H); ^{31}P NMR ($CDCl_3$) δ 32.62, 32.42, 32.28; IR (NaCl, cm^{-1}) 3267, 2953, 1744, 1726, 1680, 1564, 1217, 1130, 1036; FAB MS [M + H]$^+$ (thiogycerol/TFA/H_2O) calcd for $C_{56}H_{59}N_7O_{14}P_2$ 1073.3503, observed 1073.3550.

To confirm that the multiple ^{31}P signals were due to diastereomers, a small sample (ca. 10 mg) of **13** was treated with Me_3SiCl/NaI in refluxing CH_3CN. Evaporation of solvent, addition and evaporation of methanol, and neutralization with pH 7 potassium phosphate gave an aqueous solution. The major component of the resulting mixture eluted at 14.47 min on a 250 × 4 mm C-18 column eluted at 0.5 mL/min with a 30-min gradient of 20–40% CH_3CN in 1 mM pH 7 potassium phosphate: ^{31}P NMR (D_2O) δ 21.980 (also shows phosphate at 1.590); FAB MS $[M - H]^+$ calcd for $C_{54}H_{53}N_4O_{14}P_2$ 1043.3, observed 1043.6, $[M - 2H + K]^+$ calcd for $C_{54}H_{52}N_4O_{14}P_2K$ 1081.2, observed 1081.5.

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Supplementary Material Available: Copies of ^1H and ^{13}C NMR spectra of **6** and **9** and copies of ^1H and ^{31}P NMR spectra of **13** (6 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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