Stereoselective Synthesis and Peptide Incorporation of a Pyridoxal Coenzyme–Amino Acid Chimera†

Barbara Imperiali* and Ranabir Sinha Roy

Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125

Received November 15, 1994

The *de novo* design of polypeptide motifs of defined secondary structure has met with success in recent years.[1-4] However, the development of new *functional* proteins has been limited by an inability to duplicate the *precise* array of functional groups required to effect a particular transformation. Strategically, this "protein folding problem" may be addressed by utilizing coenzyme-amino acid chimeras as modular functional components within polypeptides.[5] Herein we describe the synthesis of a pyridoxol amino acid derivative (*S*)-2-amino-N^{α}-(9-fluorenylmethyloxycarbonyl)-3-(α^4,3-*O*-isopropylidenepyridox-5-yl)propanoic acid (**6**), its incorporation into four model hexapeptides, and subsequent oxidation to the pyridoxal analog. The synthesis of gram quantities of optically pure amino acid, suitably protected for peptide incorporation, underscores the potential for utilizing coenzyme amino acid chimeras in the construction of functional polypeptide motifs.

The synthesis of (*S*)-2-amino-N^{α}-(9-fluorenylmethyloxycarbonyl)-3-(α^4,3-*O*-isopropylidenepyridox-5-yl)propanoic acid (**6**) from α^4,3-*O*-isopropylidenepyridoxol[6] (**1**) is outlined in Scheme 1. The key step involves a stereoselective alkylation of *N*-(diphenylmethylene)-glycine *tert*-butyl ester (**3**) with the bromide **2** using the phase transfer catalyst (PTC) (8*S*,9*R*)-(−)-*N*-benzylcinchonidinium chloride according to the method of O'Donnell et al.[7] The transformation proceeded with modest asymmetric induction and afforded a 52% ee[8] of the alkylated product (*S*)-**4**.[9] Enantiomerically pure (>99% ee) material was obtained by crystallizing the racemate from a solution of the optically enriched product. Treatment of (*S*)-**4** with anhydrous HCl in acetone afforded the isopropylidenepyridoxol–amino acid dihydrochloride **5**. The N^{α}-(9-fluorenylmethyloxycarbonyl) derivative **6** was prepared for solid phase peptide synthesis by treatment with 9-(fluorenylmethyloxycarbonyl)succinimidyl carbonate.[10]

Scheme 1

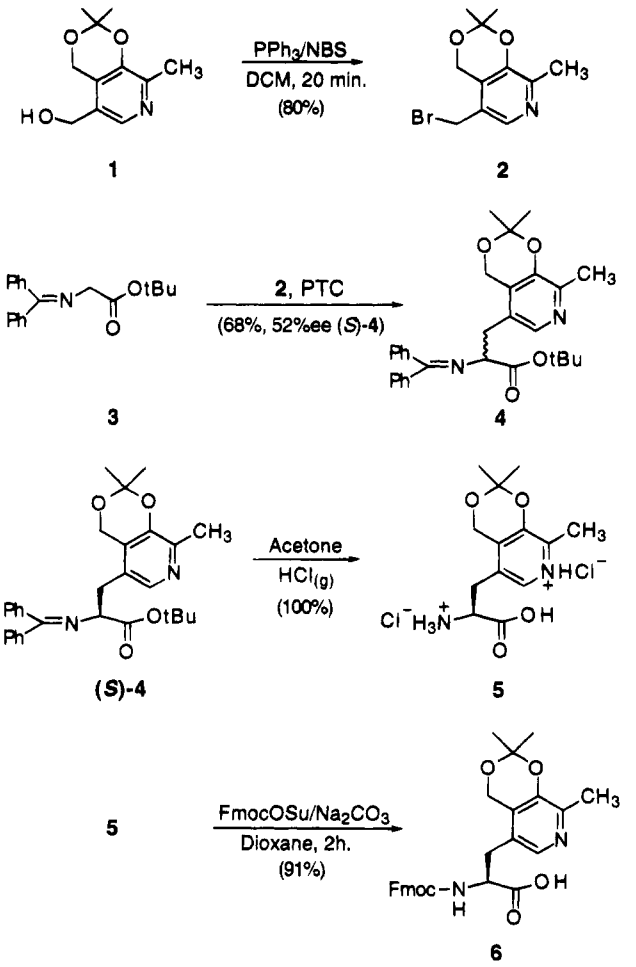

Chart 1

Position:	1	2	3	4	5	6	
P1:	Ac	Thr	**Val**	Pro	DAla	**Pol**	Gly NH2
P2:	Ac	Thr	**His**	Pro	DAla	**Pol**	Gly NH2
P3:	Ac	Thr	**Pyr**	Pro	DAla	**Pol**	Gly NH2
P4:	Ac	Thr	His	Gly	Gly	Pol	Gly NH2

Pyr = 3-(3-pyridyl)alanine

The efficient syntheses[11] of hexapeptides **P1**–**P4** (Chart 1), incorporating the pyridoxol amino acid (Pol) derivative **6**, demonstrate the compatibility of the residue with solid phase methods. After peptide assembly, acidolytic cleavage of the peptides from the solid support proceeded with simultaneous deprotection of the Pol moiety. Subsequently, the Pol residue was oxidized to the electrophilic pyridoxal moiety (Pal) in modest yields, using γ-MnO_2[12] in dimethyl sulfoxide (Scheme 2). The oxidation is selective in the presence of many functionalized residues with the potential exceptions of cysteine and methionine. This scheme therefore allows access to a wide variety of polypeptide motifs incorporating the pyridoxal coenzyme as an integral constituent of the peptide backbone.

Pyridoxal-dependent enzymes mediate a variety of transformations that are facilitated by general acid/base

† Contribution No. 8992.

(1) Yan, Y.; Erickson, B. E. *Protein Sci.* **1994**, *3*, 1069.

(2) Fezoui, Y.; Weaver, D. L.; Osterhout, J. J. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 3675.

(3) Pessi, A.; Bianchi, E.; Crameri, A.; Venturini, S.; Tramontano, A.; Sollazzo, M. *Nature* **1993**, *362*, 367.

(4) Ho, S. P.; DeGrado, W. F. *J. Am. Chem. Soc.* **1987**, *109*, 6751.

(5) Imperiali, B.; Sinha Roy, R. *J. Am. Chem. Soc.* **1994**, *116*, 12083.

(6) Korytnyk, W.; Wiedeman, W. *J. Chem. Soc., Chem. Comm.* **1962**, 2531.

(7) O'Donnell, M. J.; Bennett, W. D.; Wu, S. *J. Am. Chem. Soc.* **1989**, *111*, 2353.

(8) The optical purity of the underivatized amino acid was determined directly on a Crownpak CR (+) column (Daicel).

(9) The absolute stereochemical outcome of the asymmetric alkylation has been demonstrated to be quite predictable with a wide variety of electrophiles.[7] In addition, the change in the direction of the optical rotation of the amino acid in going to an acidic aqueous solution [+4.7° (*c* 0.3, H_2O); +19.8° (*c* 0.3, 0.2 N HCl)] is in keeping with the Clough–Lutz–Jirgenson rule (see: Greenstein, J. P.; Winitz, M. *Chemistry of the Amino Acids*; John Wiley & Sons, New York, 1961; p 85). Finally, the order of elution from the Crownpak CR(+) column is also consistent with this assignment.

(10) Paquet, A. *Can. J. Chem.* **1982**, *60*, 976.

(11) For a review of Fmoc-based solid phase peptide synthesis see: Fields, G. B.; Noble, R. L. *Int. J. Pep. Protein Res.* **1990**, *35*, 161.

(12) Fatiadi, A. J. *Synthesis* **1976**, 65.

0022-3263/95/1960-1891$09.00/0

Scheme 2

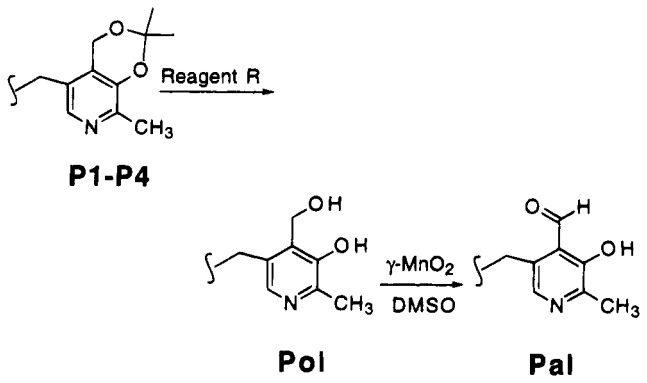

Table 1

coenzyme system	$k_{obs} \times 10^5$ (min^{-1})	$t_{1/2}$ (h)
P1	165	7.0
P2	225	5.1
P3	278	4.1
P4	62	18.7
DPal	59	19.6

catalysis.[13] The availability of a pyridoxal coenzyme amino acid chimera allows an investigation of these processes in functionalized peptidyl constructs. A β-turn motif forms the structural basis of the hexapeptide sequences (**P1**–**P4**). This motif is important in protein design since it can provide a template for organizing functionality within a relatively short peptide. The L-Pro-D-Ala sequence in peptides **P1**–**P3** promotes a type-II reverse-turn conformation in model peptides,[14] which brings the flanking residues proximal to each other (highlighted in Chart 1). Reactions effected by the coenzyme chimera (Pal) might therefore be amenable to catalysis by a general base[15] situated across the reverse-turn (His, **P2**; 3-(3-pyridyl)alanine (Pyr), **P3**). The Val analog (peptide **P1**) and a nonpeptidyl coenzyme (5′-deoxypyridoxal,[16] DPal) were assayed as controls. The participation of an internal base (His) in the absence of a reverse-turn motif was investigated in peptide **P4**, which incorporates a flexible Gly-Gly spacer. The Pal hexapeptides were assayed in the presence of copper[17] for the transamination of L-alanine to pyruvic acid, under single turnover conditions.

The rates of transamination for hexapeptides **P1**–**P4** and DPal are listed for comparison in Table 1. The enhanced reactivities of peptides **P2** and **P3** may reflect their capacity to deliver an internal base to the Pal chimera via a type-II β-turn conformation. The increased acidity of the 3-(3-pyridyl)alanine residue (pK_a 5.2, **P3**) relative to histidine (pK_a 6.5, **P2**) and valine (**P1**) paralleled the reactivities of these peptides under the reaction conditions (pH 4.0). The low transamination rate for **P4** was consistent with the absence of a reverse-turn motif in this peptide and ruled out an *intermolecular* participation of the general base. The importance of a defined peptidyl architecture for enhancing coenzyme reactivity was further highlighted by the poor transamination rate of DPal.

In conclusion, this paper describes the synthesis, peptide incorporation, and analysis of a pyridoxal coenzyme–amino acid chimera. The ability to elicit and modulate activity within synthetic peptides demonstrates the potential of this new class of amino acids in the *de novo* design of functional proteins.

Experimental Section

Optical rotations were recorded at room temperature in a 1-dm path length cell. Nuclear magnetic resonance (NMR) spectra were obtained at 300/500 MHz for proton frequency and 75/125 MHz for carbon frequency. Chemical shifts are reported in δ units (ppm) relative to dimethyl sulfoxide-d_6 or chloroform-d as indicated. Ultraviolet–visible (UV–vis) spectra were acquired at 25 °C. Mass spectra were obtained using fast atom bombardment (FAB) or plasma desorption (PD) ionization. Thin layer chromatography was carried out on hard TLC plates with fluorescence indicator from EM Reagents (SiO_2 60, F-254). R_f values are reported in one of the following solvent systems: (A) 5:1 chloroform/methanol, (B) 2:1 ether/hexane. Flash column chromatography was performed according to the procedure of Still[18] using J. T. Baker flash silica gel (~40 μm).

α^4,3-*O*-Isopropylidene-α^5-pyridoxyl Bromide (2). Isopropylidenepyridoxine[6] (1.92 g, 9.2 mmol) was dissolved in 20 mL of dichloromethane and cooled to 0 °C. Alternately, small amounts of triphenylphosphine (2.40 g, 9.2 mmol) and *N*-bromosuccinimide (NBS, 1.63 g, 9.2 mmol) were added over 5 min. The reaction mixture was stirred for 20 min and concentrated *in vacuo*. The crude product was purified by flash chromatography (eluent ether/hexanes 2:1) to yield **2** as an oil (2.0 g, 80%). The unstable product was used immediately. Mass spectral analysis was not possible due to rapid decomposition of the product: R_{fB} = 0.36; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50 (s, 6H), 2.30 (s, 3H), 4.25 (s, 2H), 4.80 (s, 2H), 7.90 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.6, 18.7, 24.9, 27.0, 58.2, 100.2, 125.7, 126.6, 140.3, 146.3, 149.0.

(*S*)-2-Amino-*N*$^\alpha$-diphenylmethylene-3-(α^4,3-*O*-isopropylidenepyridox-5-yl)propanoic Acid *tert*-Butyl Ester (4). *N*-(Diphenylmethylene)glycine *tert*-butyl ester (**3**, 1.66 g, 5.61 mmol) and (8*S*,9*R*)-(−)-*N*-benzylcinchonidinium chloride (472 mg, 1.12 mmol) were dissolved in a suspension of 20 mL of toluene/dichloromethane (7:3) and 8.2 mL of 50% aqueous sodium hydroxide at 5 °C. The bromide **2** (2.288 g, 8.41 mmol) was added dropwise and stirred for 2 h using a Teflon impeller operating at 500 rpm. The organic phase was separated and the aqueous layer extracted with dichloromethane (5 × 40 mL). The combined organic phase was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by flash chromatography (eluant ether/hexanes 2:1). The purified amino acid derivative was obtained as an oil (1.86 g, 68%) and crystallized from hexanes. The supernatant was decanted and concentrated to yield 687 mg (25%) of the *S* enantiomer (>99% ee). The asymmetric induction in this reaction was determined by HPLC analysis of the corresponding amino acid (obtained by acidolytic hydrolysis of the imine **4**), on a chiral Crownpack CR(+) column: R_{fB} = 0.36; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.15 (s, 3H), 1.45 (overlapping s, 12H), 2.35 (s, 3H), 3.0 (m, 2H), 4.05 (m, 1H), 4.65 (d, 1H), 4.85 (d, 1H), 7.20–7.60 (overlapping m, 10H), 7.70 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.5, 23.7, 25.7, 28.3, 32.4, 59.3, 67.3, 81.8, 99.6, 126.4, 127.4, 127.7, 128.3, 128.6, 128.7, 129.0, 130.5, 130.7, 136.1, 139.2, 141.5, 145.8, 145.9, 170.4, 171.0; FAB-HRMS [MH$^+$] calcd for $C_{30}H_{35}O_4N_2$ 487.2597, obsd 487.2599; $[\alpha]^{25}_D$ −143.9° (*c* 0.5, $CHCl_3$).

(*S*)-2-Amino-3-(α^4,3-*O*-isopropylidenepyridox-5-yl)propanoic Acid Dihydrochloride (5). The (*S*)-imine **4** (1.22 g, 2.51 mmol) was dissolved in 20 mL of anhydrous acetone and cooled in an ice bath (0 °C). The solution was saturated with anhydrous hydrogen chloride and stirred at room temperature

(13) Bender, M. L.; Bergeron, R. J.; Komiyama, M. *The Bioorganic Chemistry of Enzymatic Catalysis*; Wiley-Interscience: New York, 1984; pp 159–190.

(14) Imperiali, B.; Fisher, S. L.; Moats, R. A.; Prins, T. J. *J. Am. Chem. Soc.* **1992**, *114*, 3128.

(15) Chmielewski, J.; Breslow, R. *Heterocycles* **1987**, *25*, 533.

(16) Iwata, C. *Biochem. Prep.* **1968**, *12*, 117.

(17) Although pyridoxal-dependent transaminases do not require metal ions, the formation of a metal–Schiff base chelate in simple model systems positions the C_α–H bond of the substrate orthogonal to the plane of the pyridoxal ring and extends the conjugation of the subsequent carbanion intermediate (see: Martell, A. E. *Acc. Chem. Res.* **1989**, *22*, 115). For the Pal-hexapeptides, transamination proceeds 14–53% slower in the absence of Cu^{II}.

(18) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

for 30 min. After the solvent was removed *in vacuo*, the amino acid dihydrochloride **5** was neutralized with 10% sodium carbonate (20 mL) and washed with hexane (5 × 15 mL). The aqueous phase was utilized directly without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 (s, 6H), 2.54 (s, 3H), 3.27 (d, 2H), 4.32 (m, 1H), 5.14 (m, 2H), 8.23 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2, 28.1, 30.3, 53.5, 60.6, 86.0, 103.8, 131.1, 132.7, 138.1, 144.0, 150.1, 168.0; FAB-HRMS [M^+] for neutralized amino acid calcd for $C_{13}H_{19}O_4N_2$ 267.1345, obsd 267.1346.

(*S*)-2-Amino-N^{α}-(9-fluorenylmethyloxycarbonyl)-3-(α^4,3-*O*-isopropylidenepyridox-5-yl)propanoic acid (6). A solution of 9-(fluorenylmethyloxycarbonyl)succinimidyl carbonate (FmocOSu, 931 mg, 2.76 mmol) in 26 mL of *p*-dioxane was added dropwise to a solution of amino acid **5** (849 mg, 2.51 mmol) in 20 mL of 10% aqueous sodium carbonate at 5 °C over 5 min. The reaction mixture was then allowed to warm to room temperature and stirred for 12 h. The mixture was diluted with 200 mL of water and extracted with ether (5 × 50 mL). The aqueous phase was cooled in an ice bath and acidified to pH 3 with concentrated hydrochloric acid. The suspension was extracted with ethyl acetate (6 × 50 mL). The organic phase was dried (Na_2SO_4) and the solvent removed *in vacuo* to yield the Fmoc-amino acid (1.11 g, 91% overall yield from **4**: R_{fA} = 0.14. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.36 (s, 6H), 2.11 (s, 3H), 2.65 (dd, 1H), 2.89 (dd, 1H), 4.05 (m, 1H), 4.1 (d, 2H), 4.15 (t, 1H), 4.75 (d, 1H), 4.85 (d, 1H), 7.2–7.8 (overlapping m, 18 H, includes contributions from different rotamers of the Fmoc ring system); ^{13}C NMR (75 MHz, DMSO-d_6) δ 18.7, 24.6, 25.2, 30.8, 40.8, 47.1, 54.6, 58.6, 66.3, 99.8, 120.6, 125.7, 125.8, 126.3, 127.5, 127.6, 128.1, 141.2, 144.2, 144.8, 145.5, 156.4; FAB-HRMS [MH^+] calcd for $C_{28}H_{29}O_6N_2$ 489.2026, obsd 489.2014; $[\alpha]^{25}_D$ +18.8° (*c* 0.5, $CHCl_3$).

Peptide Synthesis. Peptides were synthesized on a 0.124–0.146 mmol scale, using N^{α}-(9-fluorenylmethyloxycarbonyl) (Fmoc) amino protection on an automated peptide synthesizer. PAL-PEG-PS resin (Millipore) was used to obtain carboxyl terminal primary amides. *In situ* activated esters were generated from the free acid derivatives using 1-hydroxybenzotriazole/*N,N'*-diisopropylcarbodiimide (HOBT/DIPCDI) chemistry. For the commercially available residues, 3 equiv of amino acid derivative was used with a minimum coupling time of 30 min; however, for Pol, only 2 equiv were employed. Fmoc deprotection was performed using a 7 min 20% piperidine/DMF wash. Coupling efficiencies were monitored using the counterion distribution monitoring system[19] (Millipore). Residual terminal amines at the end of each coupling cycle were capped using a 10 min wash with 0.3 M acetic anhydride/HOBT solution in *N,N*-dimethylformamide (DMF)/dichloromethane (9:1). All peptides were acetyl capped on the resin using 21 equiv of acetic anhydride and 6 equiv of triethylamine in 3 mL of DMF. The peptides were cleaved using 5 mL of reagent R (trifluoroacetic acid/thioanisole/ethanedithiol/anisole, 90:5:3:2)[20] and lyophilized from water. The crude peptides were purified by reversed phase (C_{18}) high-pressure liquid chromatography (HPLC) on a dual pump system (solvent A, 0.1% TFA/H_2O; Solvent B, 0.1% TFA/MeCN).

Oxidation of the Pol-Peptides. Freshly prepared γ-MnO_2 (50 mg) was added to a solution of Pol-peptide in dimethyl sulfoxide (2 mg/120 μL) and stirred at room temperature for 12 h in the dark. The slurry was filtered through Celite and the column rinsed with double-distilled water to ensure complete collection of the oxidized peptide. Trace particulates were removed with a 1.2 μm filter, and the filtrate was lyophilized. The oxidized Pal-peptide was obtained as a yellow oil in 15–40% yield. The product was dissolved in an appropriate buffer, quantified by the unique absorption of the pyridoxal moiety in 0.1 N NaOH (ϵ_{390} = 6400 cm^{-1} M^{-1})[21] and used immediately without further purification.

Characterization of the Schiff Base Intermediate. The chemical identity of the Pal moiety was further verified for representative Pal-peptides by borohydride reduction of the Schiff base intermediate and characterization of the trapped derivative. L-Isoleucine (0.7 mg, 5.3 μmol) and phenylethylamine (PEA, 24.2 mg, 0.2 mmol in 69 μL methanol) were added to solutions of **P1** (176 nmol Pal-peptide in 200 μL of 0.1 M phosphate buffer, pH 7.8) and **P3** (200 nmol Pal-peptide in 31μL of H_2O), respectively. The solutions were stirred at room temperature for 1 h before treatment with sodium borohydride (2.6 mg, 68.7 μmol). After 10 min, the solutions were desalted by HPLC (isocratic elution, 11% solvent B), and the reduced Schiff bases were characterized by mass spectroscopy.

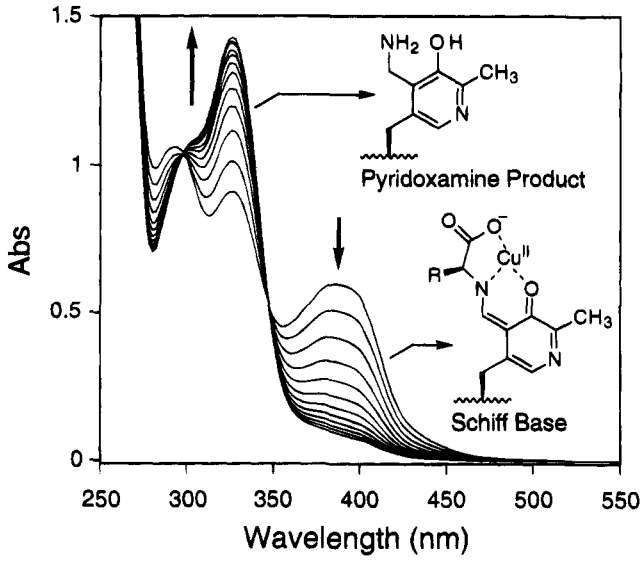

Figure 1.

Transamination Assays. Transamination was monitored by UV spectroscopy at 25 °C according to the procedure of Vazquez et al.[22] Stock solutions of peptide, D-Pal and L-alanine were made up in acetate buffer (0.1–1.0 M, pH 4.0, ionic strength adjusted to 0.29 with KCl). The initial concentrations of all species in the assay were as follows: Pal-peptide, 0.1 mM; Cu^{II} ($CuCl_2$), 0.1 mM; L-Ala, 1.0 M. UV–vis spectra were acquired every 20 min for at least 2.5 half-lives. Spectra from a typical assay (peptide **P3**) are shown in Figure 1 at 60 min intervals. The pseudo-first-order rate constants (k_{obs}) were calculated from the rates of disappearance of aldimine at 390 nm, which were directly proportional to the rates of appearance of product (pyridoxamine) at 324 nm. First-order fits were obtained in all cases to greater than 90% completion of reaction. The pH change during the entire assay was less than 0.02 units. Repeat runs were accurate to within 10%.

Ac-Thr-Val-Pro-D-Ala-Pol-Gly-NH_2 (P1): t_R (linear gradient 0–70% Solvent B over 25 min) = 20.2 min; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.85 (d, 6H), 1.0 (d, 3H), 1.05 (d, 3H), 1.8 (m, 2H), 1.9 (s, 3H), 2.05 (overlapping d, 2H), 2.35 (m, 1H), 2.5 (s, 3H), 2.95 (t, 1H), 3.0–3.8 (absorbed water), 3.8 (t, 1H), 3.9 (q, 1H), 4.2 (overlapping m, 6H), 4.55 (m, 1H), 4.85 (s, 1H), 7.05 (s, 1H), 7.30 (s, 1H), 7.65 (d, 1H), 7.30 (d, 1H), 7.90 (d, 1H), 8.15 (overlapping d, 2H); ^{13}C (125 MHz, DMSO-d_6) δ 20.8, 21.2, 21.4, 22.2, 22.8, 24.6, 25.6, 27.7, 28.8, 32.2, 33.2, 45.1, 50.6, 58.8, 61.2, 63.0, 69.7, 82.1, 136.0, 137.3, 144.0, 145.2, 155.2, 161.2, 172.9, 173.3, 173.8, 174.4, 174.5, 174.6, 175.2; FAB-HRMS [MH^+] calcd for $C_{31}H_{49}O_{10}N_8$ 693.3572, obsd 693.3604; UV (0.1 N NaOH), λ_{max} (nm) = 308. **Pal-P1:** UV (0.1 N NaOH), λ_{max} (nm) = 392. FAB-HRMS [MH^+] for **Pal-P1**: calcd for $C_{31}H_{47}O_{10}N_8$ 691.3415, obsd 691.3427. FAB-HRMS [MH^+] for **Pal-P1-L-Ile** trapped Schiff base: calcd for $C_{37}H_{60}O_{11}N_9$ 806.4412, obsd 806.4396.

Ac-Thr-His-Pro-D-Ala-Pol-Gly-NH_2 (P2): t_R (linear gradient 0–70% solvent B over 25 min) = 15.9 min; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.00 (d, 3H), 1.03 (d, 3H), 1.60–1.85 (overlapping m, 3H), 2.05 (m, 1H), 2.45 (s, 3H), 2.95 (m, 2H), 3.08 (dd, 1H), 3.15 (dd,1H), 3.65 (overlapping m, 3H), 3.90 (m, 1H), 4.15 (m, 1H), 4.25 (t, 1H), 4.35 (m, 1H), 4.62 (m, 1H), 4.85 (s, 2H), 7.05 (s, 1H), 7.25 (s, 1H), 7.37 (s, 1H), 7.70 (d, 1H), 7.95 (s, 1H), 8.10 (d, 1H), 8.17 (d, 1H), 8.25 (overlapping s, 2H), 8.90 (s, 1H); ^{13}C (125 MHz, 90:10 H_2O:D_2O) δ 17.3, 19.7, 22.5, 25.3, 26.7, 30.2,

(19) Young, S. C.; White, P. D.; Davies, J. W.; Owen, D. E. I. A.; Salisbury, S. A.; Tremeer, E. J. *Biochem. Soc. Trans.* **1990**, *18*, 1311.
(20) Biosearch Technical Bulletin No. 9000-02.
(21) Vazquez, M. A.; Donoso, J.; Munoz, F. *Helv. Chim. Acta* **1990**, *73*, 1991.
(22) Vazquez, M. A.; Munoz, F.; Donoso, J. *J. Mol. Catal.* **1991**, *68*, 105.

31.9, 39.5, 43.0, 48.8, 50.4, 51.2, 54.6, 57.0, 60.0, 61.5, 67.8, 100.4, 118.4, 129.0, 131.3, 133.5, 134.4, 141.9, 143.7, 156.2, 170.2, 172.6, 173.2, 174.5, 174.8, 175.3, 175.7; FAB-HRMS [MH^+] calcd for $C_{32}H_{47}O_{10}N_{10}$ 731.3476, obsd 731.3469; UV (0.1 N NaOH), λ_{max} (nm) = 309. **Pal-P2:** UV (0.1 N NaOH), λ_{max} (nm) = 390. FAB-MS [MH^+] for **Pal-P2:** *m/z* 729.

Ac-Thr-Pyr-Pro-D-Ala-Pol-Gly-NH_2 (P3): t_R (linear gradient 0–60% solvent B over 25 min) = 21.1 min; ^1H NMR (500 MHz, DMSO-d_6) δ 0.93 (d, 3H), 1.00 (d, 3H), 1.78–1.87 (overlapping m, 6H), 1.99 (m, 1H), 2.28 (s, 3H), 2.81 (m, 2H), 3.10 (m, 2H), 3.12 (m, 1H), 3.53 (m, 2H), 3.67 (m, 2H), 3.81 (m, 1H), 4.12 (t, 1H), 4.21 (t, 1H), 4.26 (m, 1H), 4.45 (m, 1H), 4.73 (s, 2H), 7.08 (s, 1H), 7.14 (s, 1H), 7.20 (t, 1H), 7.63–7.66 (overlapping t, 2H), 7.74 (s, 1H), 8.01–8.03 (overlapping m, 2H), 8.12 (t, 1H), 8.17 (d, 1H), 8.36 (d, 1H), 8.43 (s, 1H); ^{13}C (125 MHz, DMSO-d_6) δ 18.2, 19.4, 20.0, 22.8, 24.8, 29.3, 31.6, 31.8, 33.0, 34.1, 42.3, 47.3, 48.5, 51.7, 53.9, 56.5, 58.5, 60.2, 66.9, 123.4, 129.8, 133.2, 137.3, 145.0, 147.8, 149.8, 150.6, 169.7, 170.3, 171.0, 171.2, 171.5, 171.7, 172.5; FAB-HRMS [MH^+] calcd for $C_{34}H_{48}O_{10}N_9$ 742.3524, obsd 742.3516; UV (0.1 N NaOH), λ_{max} (nm) = 310. **Pal-P3:** UV (0.1 N NaOH), λ_{max} (nm) = 394. PDMS [MH^+] for **Pal-P3-PEA** trapped Schiff base *m/z* 845.

Ac-Thr-His-Gly-Gly-Pol-Gly-NH_2 (P4): t_R (linear gradient 0–60% solvent B over 25 min) = 19.6 min; ^1H NMR (500 MHz, DMSO-d_6) δ 1.00 (d, 3H), 1.88 (s, 3H), 2.47 (s, 3H), 3.00 (overlapping m, 2H), 3.22 (overlapping m, 2H), 3.69 (overlapping m, 6H), 3.90 (t, 1H), 4.15 (m, 1H), 4.59 (overlapping m, 2H), 4.81 (s, 2H), 7.06 (s, 1H), 7.25 (s, 1H), 7.38 (s, 1H), 7.84 (d, 1H), 8.06 (s, 1H), 8.19 (m, 2H), 8.25 (m, 3H), 8.94 (s, 1H); ^{13}C (125 MHz, 90:10 H_2O:D_2O) δ 15.6, 19.5, 22.5, 26.9, 31.7, 39.5, 43.1, 53.2, 54.5, 57.0, 60.1, 67.7, 100.8, 118.1, 129.3, 131.4, 133.5, 134.4, 142.0, 143.5, 155.9, 172.1, 172.5, 172.9, 173.2, 174.5, 175.5; FAB-HRMS [MH^+] calcd for $C_{28}H_{41}O_{10}N_{10}$ 677.3007, obsd 677.2999; UV (0.1 N NaOH), λ_{max} (nm) = 310. **Pal-P4:** UV (0.1 N NaOH), λ_{max} (nm) = 370. FAB-MS [MH^+] for **Pal-P4:** *m/z* 675.

Acknowledgment. This research was supported by the Office of Naval Research and the Beckman Foundation. The award of a Glaxo Summer Fellowship (1993–94) to R.S.R. and support from the Alfred P. Sloan Foundation, the Camille and Henry Dreyfus Teacher Scholar Program, and Zeneca, Inc., to B.I. is gratefully acknowledged. The authors also acknowledge Tarun Kapoor for assistance in the early stage of the project.

Supplementary Material Available: Copies of ^1H NMR spectra of **2**, **4**, and **6**. Crownpak CR (+) HPLC analysis of **4** and (*S*)–**4** (4 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.

JO941928A