Materials. Fmoc-β-alanine-p-benzyloxybenzyl alcohol resin (Fmoc-β-Wang-resin), 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), and Boc-γ-Aminobutyric Acid were purchased from Peptides International. N,N-Diisopropylethylamine (DIEA), piperidine, and N-methylpyrrolidone (NMP), were purchased from Applied Biosystems. Dichloromethane (DCM) and triethylamine (TEA) were reagent grade from EM, 9-Fluorenylmethyl chloroformate (Fmoc-Cl), thiophenol (PhSH), (dimethylamino)propylamine, trichloroacetylchloride, *N*-methylpyrrole, and *N*-methylimidazole were from Aldrich, and trifluoroacetic acid (TFA) was from Halocarbon. All reagents were used without further purification.

^{1}H NMR spectra were recorded on a General Electric-QE NMR spectrometer at 300 MHz in DMSO-d_6, with chemical shifts reported in parts per million relative to residual solvent. UV spectra were measured in water on a Hewlett-Packard Model 8452A diode array spectrophotometer. Matrix-assisted, laser desorption/ionization time of flight mass spectrometry (MALDI-TOF) electrospray mass spectrometry were performed at the Protein and Peptide Microanalytical Facility at the California Institute of Technology. HPLC analysis was performed on either on a HP 1090M analytical HPLC or a Beckman Gold system using a RAINEN C_{18}, Microsorb MV, 5μm, 300 x 4.6 mm reversed phase column in 0.1% (wt/v) TFA with acetonitrile as eluent and a flow rate of 1.0 mL/min, gradient elution 1.25% acetonitrile/min. Preparatory reversed phase HPLC was performed on a Beckman HPLC with a Waters DeltaPak 25 x 100 mm, 100 μm C18 column equipped with a guard, 0.1% (wt/v) TFA, 8.0 ml/min, 0.25% acetonitrile/min. 18MW water was obtained from a Millipore MilliQ water purification system, and all buffers were 0.2 μm filtered.

Monomer Synthesis.

***tert*-Butyl 4-Nitro-1-methylpyrrole-2-carboxylate (5a).** To a suspension of 4-nitro-2-(trichloroacetyl)-1-methylpyrrole (**5a**) (50 g, 19.5 mmol) in 500 ml of *tert*-butyl alcohol was added sodium *tert*-butoxide (25 g) over 1 hour. The suspension was refluxed under argon for 5 hours. The mixture was quenched with 500 ml of water and extracted with chloroform (3 × 500 ml). The chloroform was concentrated *in vacuo* to provide **6a** (39.4 g, 94.6% yield). TLC (3:1 hexanes: ethyl acetate) R_f 0.52 ^{1}H NMR (DMSO-d_6) δ 8.18 (d, 1 H, J = 2.4), 7.18 (d, 1 H, J = 2.4), 3.97 (s, 3 H), 1.47 (s, 9 H). FABMS m/e 226.096 (226.095 calcd for $C_{10}H_{14}N_2O_4$).

***tert*-Butyl 4-[(9-Fluorenylmethoxycarbonyl)amino]-1-methylpyrrole-2-carboxylate (6a).** To a solution of *tert*-Butyl 4-Nitro-1-methylpyrrole-2-carboxylate (**5a**) (20.0 g, 88.4 mmol) in 100 ml of DMF was added 10% Pd/C (2 g) in 20 ml of DMF. The mixture was vigorously stirred for 2 h under 500 psi of hydrogen, filtered though Celite, and the Celite was washed with DMF (1 × 200 ml). The two filtrates were combined, Fmoc-Cl (25.3 g, 92.8 mmol) and DIEA (35 ml, 200 mmol) were added and the solution was stirred for 10 hours. Water (250 ml) was added, and the mixture extracted with ethyl ether (2 × 500 ml). The ether was dried (sodium sulfate) and concentrated *in vacuo* to ~200 ml. Hexanes was added to preciptitate a brown solid which was washed with cold methanol/$H_2$0 to yield **6a** as a white solid (24.3 g, 66% yield). TLC (3:1 hexanes/ethyl acetate) R_f 0.39 ^{1}H NMR (DMSO-d_6) δ 9.45 (s, 1 H), 7.92 (m, 2 H), 7.71 (m, 2 H), 7.44 (m, 2 H), 7.36 (m, 2 H), 7.03 (d, 1 H, J = 2.4), 6.63 (d, 1 H, J = 2.4), 4.45 (d, 2 H, J = 6.3), 4.31 (t, 1 H, J = 6.3), 3.78 (s, 3 H), 1.50 (s, 9 H). FABMS m/e 418.189 (418.189 calcd for $C_{25}H_{26}N_2O_4$).

4-[(9-Fluorenylmethoxycarbonyl)amino]-1-methylpyrrole-2-carboxylic acid (8a). *tert*-Butyl 4-[(9-Fluorenylmethoxycarbonyl)amino]-1-methylpyrrole-2-carboxylate (7a) (5.0 g, 12.0 mmol) was dissolved in 100 ml of dichloromethane and cooled to 0° C.

$TiCl_4$ (25 ml, 1.0 M in dichloromethane) was added dropwise to the solution. The mixture was stirred for 30 min. 1 M HCl (250 ml) was cooled to 4°C and added dropwise. The resulting white precipitate (**8a**) was collected by vacuum filtration and washed with a small amount of cold water and dried *in vacuo* (3.8 g, 88% yield). TLC (1:1 hexanes/ethyl acetate) R_f 0.15 ^{1}H NMR (DMSO-d_6) δ 12.16 (bs, 1 H), 9.45 (s, 1 H), 7.92 (m, 2 H), 7.74 (m, 2 H), 7.44 (m, 2 H), 7.36 (m, 2 H), 7.07 (d, 1 H, J = 2.4), 6.64 (d, 1 H, J = 2.4), 4.46 (d, 2 H, J = 6.3), 4.30 (t, 1 H, J = 6.3), 3.80 (s, 3 H). FABMS m/e 362.127 (362.126 calcd for $C_{21}H_{18}N_2O_4$).

4-Nitro-2-(trichloroacetyl)-1-methylimidazole (3b). A solution of 2-(tricholoroacteyl)-1-methylimidazole (700 g, 3.1 mol) in 4 L of acetic anhydride was cooled to -40° C. Fuming nitric acid (500 ml), followed by conc. sulfuric acid (25 ml) was added over 2 h, and the reaction mixture was stirred for 12 hours. The mixture was poured into a tray and allowed to crystallize over two days. The product was collected by vacuum filtration, washed (cold water, 1 × 100 ml), and dried *in vacuo* to yield **4b** as a white crystalline solid (472 g, 56 % yield). TLC (1:1 hexanes/ethyl acetate) R_f 0.57 ^{1}H NMR (DMSO-d_6) δ 8.84 (s, 1 H), 4.04 (s, 3 H). FABMS m/e 270.932 (270.932 calcd for $C_6H_4N_3O_3$).

***tert*-Butyl 4-Nitro-1-methylimidazole-2-carboxylate (4b).** To a mixture of 4-nitro-2-(trichloroacetyl)-1-methylimidazole (**5b**) (50 g, 0.18 mol) in 500 ml of *tert*-butyl alcohol was added sodium *tert*-butoxide (20 g) over 1 hour. The reaction was refluxed under argon for 5 hours, then quenched with 500 ml of water and extracted with chloroform (3 × 500 ml). The chloroform was removed *in vacuo* to provide **6b** (36.4 g, 84% yield). TLC (1:1 hexanes/ethyl acetate) R_f 0.59 ^{1}H NMR (DMSO-d_6) δ 8.62 (s, 1 H), 3.98 (s, 3 H), 1.58 (s, 9 H). FABMS m/e 227.091 (227.091 calcd for $C_9H_{13}N_3O_4$).

***tert*-Butyl 4-[(9-Fluorenylmethoxycarbonyl)amino]-1-methylimidazole-2-carboxylate (6b).** *tert*-Butyl 4-Nitro-1-methylimidazole-2-carboxylate (**6b**) (9.2 g, 40 mmol) was dissolved in 100 ml of DMF. 10% Pd/C (1 g) in 20 ml of DMF was added.

The mixture was vigorously stirred for 2 h under 500 psi of Hydrogen and then filtered though Celite. The Celite was rinsed with DMF (200 ml). Fmoc-Cl (10.7 g, 41 mmol) was added and the solution was stirred for 3 hour. Water was added to the DMF and the mixture was extracted with diethyl ether. The ether was extracted 2 × water and 1 × brine, dried over sodium sulfate and concentrated by rotoevaporation to yield a crude yellow solid which was dissolved in a minimum amount of diethyl ether. Purified by column chromatography (3:1 hexanes/ethyl acetate, 5.9 g, 34.4% yield). TLC (1:1 hexanes/ethyl acetate) R_f 0.64 ^{1}H NMR (DMSO-d_6) δ 10.4 (s, 1 H), 7.9 (d, 2 H), 7.7 (d, 2 H), 7.4 (m, 2 H), 7.3 (m, 2 H), 7.2 (s, 1H),), 4.4 (d, 2 H, J = 6), 4.3 (t, 1 H, J = 6), 3.9 (s, 3 H), 1.6 (s, 9 H). FABMS m/e 419.185 (419.184 calcd for $C_{24}H_{25}N_3O_4$).

4-[(9-Fluorenylmethoxycarbonyl)amino]-1-methylimidazole-2-carboxylic Acid (7b). *tert*-Butyl 4-[(9-Fluorenylmethoxycarbonyl)amino]-1-methylimidazole-2-carboxylate (**7b**) (5.0 g, 11.9 mmol) was dissolved in 100 ml of dichloromethane and cooled to 0° C. $TiCl_4$ (25 ml, 1.0 M in dichloromethane) was added dropwise to the solution. The mixture was stirred for 1 hour. Cold HCl (1 M, 500 ml) was added. The white precipitate was collected by vacuum filtration (3.7 g, 86% yield). ^{1}H NMR (DMSO-d_6) δ 12.4 (bs, 1 H), 10.4 (s, 1 H), 7.9 (d, 2 H), 7.7 (d, 2 H), 7.4 (m, 2 H), 7.3 (m, 2 H), 7.2 (s, 1H),), 4.4 (d, 2 H, J = 6), 4.3 (t, 1 H, J = 6), 3.9 (s, 3 H). FABMS m/e 364.131 (M+H) (364.122 calcd for $C_{20}H_{17}N_3O_4$).

Solid Phase Synthesis. Activation of Fmoc-Imidazole Acid. Activation of Imidazole-2-carboxylic acid, Fmoc-Pyrrole acid, Fmoc-Imidazole acid and γ-aminobutyric acid. The appropriate amino acid or acid (2 mmol) was dissolved in 2 mL of DMF. HBTU (720 mg, 1.9 mmol) was added followed by DIEA (1 mL) and the solution was lightly shaken for at least 5 min.

Machine-Assisted Protocols. Machine-assisted synthesis was performed on an ABI 430A synthesizer on a 0.69 mmol scale (800 mg resin; 0.86 mmol/g). Each cycle of amino acid addition involved deprotection with approximately 80% Piperidine/NMP for 3 min, draining the reaction vessel, and then deprotection for 17 min; two NMP washes; a DCM wash; an NMP wash; draining the reaction vessel; coupling for 1 h (8 h when coupling to imidazole), addition of dimethyl sulfoxide (DMSO)/NMP, coupling for 30 min; draining the reaction vessel; washing with DCM, taking a resin sample for evaluation of the progress of the synthesis for HPLC analysis; capping with acetic anhydride/DIEA in DCM for 6 min; and washing with DCM.

The ABI 430A synthesizer was left in the standard hardware configuration for NMP-HOBt protocols. Reagent position 1 was piperidine, reagent position 2 was not used, reagent position 3 was 70% ethanolamine/methanol, reagent position 4 was acetic anhydride, reagent position 5 was DMSO/NMP, reagent position 6 was methanol, reagent position 7 was DIEA, and reagent position 8 was NMP.

Fmoc-Im acid was added manually. Fmoc-imidazole acid (363 mg, 1 mmol) and HBTU (378 mg, 1 mmol) were combined in 2 mL of NMP. DIEA (1 ml) was then added, and the reaction mixture was allowed to stand for 5 min. At the initiation of the coupling cycle the synthesis was interrupted, the reaction vessel vented, and the activated monomer added directly to the reaction vessel. When manual addition was necessary, an empty synthesis cartridge was used.

Fmoc-Py acid (362 mg, 1 mmol) and aliphatic amino acids (2 mmol) were placed in a synthesis cartridge with 0.9 equiv. HBTU. NMP (3 ml) was added using a calibrated delivery loop from reagent bottle 8, followed by a calibrated delivery of 1 mL of DIEA from reagent bottle 7, and a 3 min mixing of the cartridge.

The activator cycle was written to transfer activated monomer directly from the cartridge to the concentrator vessel, bypassing the activator vessel. After transfer, 1 mL of DIEA was measured into the cartridge using a calibrated delivery loop, and the DIEA

solution was combined with the activated monomer solution in the concentrator vessel. The activated ester in 2:1 DMF/DIEA was then transferred to the reaction vessel. All lines were emptied with argon before and after solution transfers.

ImPyPyPy-γ-PyPyPyPy-β-Dp (1). ImPyPyPy-γ-PyPyPyPy-β-Wang resin was prepared by machine-assisted synthesis protocols. A sample of resin (1 g, 0.47 mmol/g) was placed in a 20 mL glass scintillation vial. 4 mL of *N,N*-(dimethylamino) propylamine added, and the solution heated at 55 °C for 18 h. Resin was removed by filtration through a disposable propylene filter, and 16 mL of water was added. The polyamide/amine mixture was purified directly by preparatory HPLC, and the appropriate fractions were lyophilized to provide the trifluoroacetate salt of ImPyPyPy-γ-PyPyPyPy-β-Dp (240 mg, 38% recovery) as a white powder. MALDI-TOF-MS (monoisotopic), 1221.5 (1221.6 calc. for M+H).

ImPyPyPy-γ-ImPyPyPy-β-Dp (2). ImPyPyPy-γ-PyPyPyPy-β-Wang resin was prepared by machine-assisted synthesis protocols. A sample of resin (1 g, 0.47 mmol/g) was placed in a 20 mL glass scintillation vial. 4 mL of (dimethylamino)propylamine added, and the solution heated at 55 °C for 18 h. Resin was removed by filtration through a disposable propylene filter, and 16 mL of water was added. The polyamide/amine mixture was purified directly by preparatory HPLC, and the appropriate fractions were lyophilized to provide the trifluoroacetate salt of ImPyPyPy-γ-ImPyPyPy-β-Dp (57 mg, 9% recovery) as a white powder. MALDI-TOF-MS (monoisotopic), 1222.5 (1222.6 calc. for M+H).

Stepwise HPLC Analysis. A resin sample (ca. 4 mg) was placed in a 4 mL glass test tube. 200 mL of (*N*,*N*-dimethylamino)propylamine was added, and the mixture was heated at 100 °C for 5 min. The cleavage mixture was filtered and a 25 mL sample analyzed by analytical HPLC at 254 nm.