Solid Phase Synthesis of Polyamides Containing Imidazole and Pyrrole Amino Acids

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Abstract: The solid phase synthesis of sequence specific DNA binding polyamides containing *N*-methylimidazole (Im) and *N*-methylpyrrole (Py) amino acids is described. Two monomer building blocks, Boc-Py-OBt ester and Boc-Im acid, are prepared on a 50 g scale without column chromatography. Using commercially available Boc-*β*-alanine-Pam resin, cycling protocols were optimized to afford high stepwise coupling yields (>99%). Deprotection by aminolysis affords up to 100 mg quantities of polyamide. Solid phase methodology increases both the number and complexity of minor groove binding polyamides which can be synthesized and analyzed with regard to DNA binding affinity and sequence specificity. The solid phase synthesis of a representative eight-residue polyamide is reported.

Introduction

Efforts to discover a universal set of simple chemical rules for the digital readout of double-helical DNA by artificial molecules have met with encouraging success.[1,2] Polyamides containing *N*-methylimidazole (Im) and *N*-methylpyrrole (Py) amino acids can be combined in antiparallel side-by-side dimeric complexes with the minor groove of DNA.[2−4] The DNA sequence specificity of these small molecules can be controlled by the linear sequence of pyrrole and imidazole amino acids. An imidazole ring on one ligand complemented by a pyrrole ring on the second ligand recognizes a G·C base pair, while a pyrrole/ imidazole combination targets a C·G base pair.[2,4] A pyrrole/pyrrole pair is degenerate for A·T or T·A base pairs.[2−4] The utility of the 2:1 model is demonstrated by the four-ring polyamide ImPyImPy-Dp (Dp = (*N*,*N*-dimethylamino)propylamide), which binds the four base pair core sequence 5′-GCGC-3′, a complete reversal of the natural specificity of netropsin and distamycin.[5]

Covalently linking polyamide heterodimers and homodimers within the 2:1 motif has led to designed ligands with both increased affinity and specificity.[6,7] A simple polyamide "hairpin" motif with *γ*-aminobutyric acid (*γ*) serving as a "turn monomer" provides a synthetically accessible method of linking polyamide units within the 2:1 motif.[7] The polyamide ImPyPy-*γ*-PyPyPy-Dp (**1**) was found to bind the designated target site 5'-TGTTA-3' with high specificity and an approximate 300-fold binding enhancement over the individual unlinked polyamide pair, ImPyPy and PyPyPy.[7]

While the limits of the 2:1 model for the design of polyamides for the recognition of any sequence of any site size were being explored, the synthetic effort emerged as a limiting step. The process of expanding the 2:1 motif to include longer sequences recognized by increasingly complex polyamides is demanding. For example, using previously described multistep solution phase chemistry, the total synthesis of hairpin polyamides such as ImPyPy-*γ*-PyPyPy-Dp (**1**) and ImPyPy-*γ*-PyPyPy-*β*-Dp (**2**) (*β* = *β*-alanine) would require more than a month's effort for each polyamide (Figure 1). We report here general protocols for manual and machine-assisted Boc-chemistry solid phase synthesis of the pyrrole−imidazole polyamides which reduce the synthetic investment from months to days.

Synthesis of Pyrrole−Imidazole Polyamides. Distamycin and its analogs have previously been considered targets of traditional multistep synthetic chemistry.[8] The repeating amide of distamycin is formed from an aromatic carboxylic acid and an aromatic amine. The aromatic acid is often unstable to decarboxylation, and the aromatic amines have been found to be air and light sensitive.[9] The variable coupling yields, long reaction times (often >24 h), numerous side products, and reactive intermediates (acid chlorides and trichloro ketones) characteristic of the traditional solution phase coupling reactions make the synthesis of the aromatic carboxamides problematic.[10]

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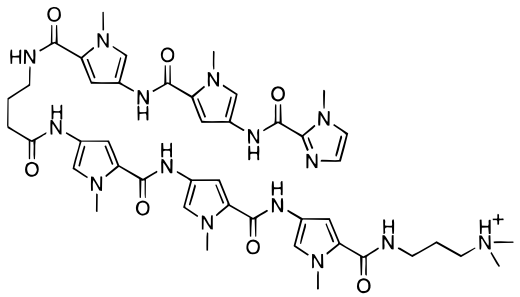
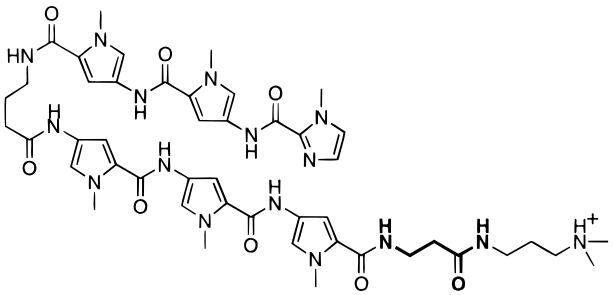


ImPyPy-γ-PyPyPy-Dp (1)



ImPyPy-γ-PyPyPy-β-Dp (2)

Figure 1. ImPyPy-γ-PyPyPy-Dp prepared by multistep solution phase synthesis (top) and the solid phase analog ImPyPy-γ-PyPyPy-β-Dp (bottom) containing a C-terminal β-alanine residue to facilitate synthesis.

Solid Phase Synthesis. In order to implement an efficient solid phase methodology[11−16] for the synthesis of the pyrrole−imidazole polyamides, the following components were developed: (1) a synthesis which provides large quantities of appropriately protected monomer or dimer building blocks in high purity, (2) optimized protocols for forming an amide in high yield from a support-bound aromatic amine and an aromatic carboxylic acid, (3) methods for monitoring reactions on the solid support, and (4) a stable resin linkage agent that can be cleaved in high yield upon completion of the synthesis.

Results and Discussion

Monomer Syntheses. The synthesis of Boc-Py-OBt ester **7**[17] and Boc-Im acid **11**[18] has been previously described. Available procedures[17−22] provide only milligram to gram quantities of monomer while requiring difficult column chromatography and the use of toxic chlorofluorophosgene for introduction of the Boc group. An optimized synthesis, using inexpensive starting materials, has been developed allowing Boc-Py-OBt ester **7** and Boc-Im acid **11** monomers to be prepared on 50 g scale without the use of column chromatography (Figure 2 and 3). Two dimeric building blocks have also been prepared, Boc-Py-Im acid **12** and Boc-γ-Im acid **13** (Figure 4).

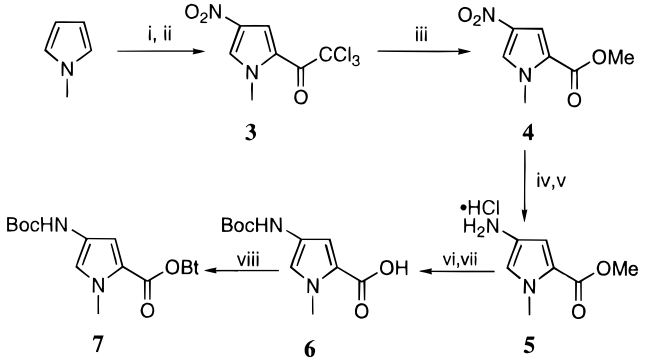


Figure 2. (i) Trichloroacetyl chloride, ethyl ether; (ii) 90% nitric acid, Ac_2O; (iii) NaOMe/MeOH; (iv) 1 atm of H_2, 10% Pd/C, EtOAc; (v) HCl, ethyl ether; (vi) 10% Na_2CO_3 (aqueous), Boc-anhydride; (vii) NaOH, MeOH, water, 60 °C; (viii) DCC, HOBt, DMF.

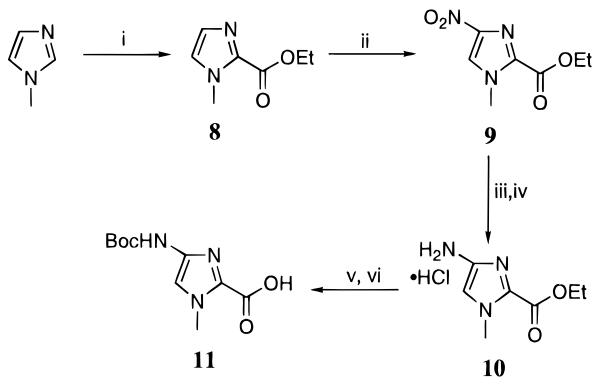


Figure 3. (i) Ethyl chloroformate, TEA, CH_3CN, −20 °C; (ii) H_2SO_4 (concentrated), 90% nitric acid; (iii) 1 atm of H_2, 10% Pd/C, EtOAc/EtOH; (iv) HCl, ethyl ether; (v) Boc-anhydride, DIEA, DMF, 60 °C; (vi) 1 M NaOH (aqueous).

Resin Linkages. For solid phase synthesis, the polyamide is attached to an insoluble matrix by a linkage which is cleaved in a single-step which introduces a positive charge into the polyamide. The addition of an aliphatic amino acid at the C-terminus of the pyrrole−imidazole polyamides allows the use of Boc-β-alanine-Pam-resin which is commercially available in appropriate substitution levels (0.2 mmol/g) (Figure 1).[23,24] Aminolysis of the resin ester linkage provides a simple and efficient method for cleaving the polyamide from the support.[25] The DNA binding affinity and sequence specificity of polyamides containing a C-terminal β-alanine spacer such as ImPyPy-γ-PyPyPy-β-Dp (**2**) are not greatly altered from those of solution phase polyamides such as ImPyPy-γ-PyPyPy-Dp (**1**). Detailed thermodynamic characterization is described elsewhere.[26]

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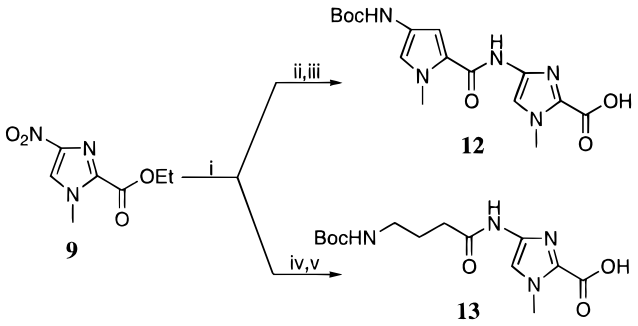

Figure 4. (i) 500 psi of H_2, 10% Pd/C, DMF; (ii) Boc−pyrrole acid (activated *in situ* with DCC/HOBt), DIEA, DMF, 60 °C; (iii) NaOH, MeOH, water, 60 °C; (iv) Boc-γ-aminobutyric acid (activated *in situ* with DCC/HOBt), DIEA, DMF, 60 °C; (v) NaOH, MeOH, water, 60 °C.

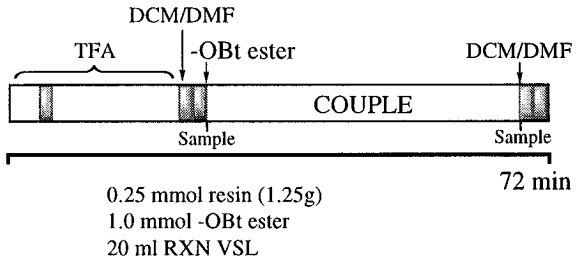

Figure 5. Protocol for the solid phase synthesis of a pyrrole−imidazole polyamide.

Table 1. Standard Protocol for Manual Solid Phase Synthesis of Pyrrole−Imidazole Polyamides

synthesis cycle		time/mode
1. deprotect	80% TFA/DCM, 0.5 M PhSH	1 min shake 30 s flow 20 min shake
2. wash	DCM DMF (take resin sample for monitoring)	1 min flow 30 s flow
3. couple	OBt ester, DIEA (take resin sample for monitoring)	45 min shake
4. wash	DMF DCM	2 × 30 s flow 30 s flow

Solid Phase Polyamide Synthesis Protocols. Solid phase polyamide synthesis protocols were modified from the *in situ* neutralization Boc-chemistry protocols recently reported by Kent and co-workers.[27] Coupling cycles are rapid, 72 min per residue for manual synthesis or 180 min per residue for machine-assisted synthesis, and require no special precautions beyond those used for ordinary solid phase peptide synthesis. Manual solid phase synthesis of a pyrrole−imidazole polyamide consists of a dichloromethane (DCM) wash, removal of the Boc group with trifluoroacetic acid (TFA)/DCM/thiophenol (PhSH), a DCM wash, a DMF wash, taking a resin sample for analysis, addition of activated monomer, addition of DIEA if necessary, coupling for 45 min, taking a resin sample for analysis, and a final DMF wash (Figure 5, Table 1). In addition, the manual solid phase protocol for synthesis of pyrrole−imidazole polyamides has been adapted for use on an ABI 430A peptide synthesizer.

Monitoring the Synthesis. The aromatic amine of the pyrrole and imidazole do not react in the quantitative ninhydrin test.[28] Stepwise cleavage of a sample of resin and analysis by HPLC indicates that high stepwise yields (>99%) are routinely achieved. We note that acylation of imidazole amine with Boc-Py-OBt ester was not satisfactory. However, acylation with Boc-Py symmetrical anhydride/DMAP ester (DCC, DMAP, DCM) proceeds to completion within 3 h. Alternatively, the preparation of a Boc-PyIm dimer unit avoids the difficult coupling of pyrrole to imidazole.

Synthesis of Eight-Residue Polyamide 2. ImPyPy-γ-PyPyPy-β-Dp was prepared in 14 steps using the protocols described in the Experimental Section (Figure 6). The yield of each individual coupling step was established as >98% by HPLC analysis. The resin was cleaved in high yield (>90%) by aminolysis with (*N*,*N*-dimethylamino)propylamine. A single HPLC separation of the eight-residue polyamide was sufficient to obtain a final purity greater than 98% as determined by a combination of analytical HPLC, ^{1}H NMR, and mass spectroscopy.

Conclusion. Pyrrole−imidazole polyamide−DNA complexes provide a potentially general model for the design of non-natural ligands for the sequence specific recognition of the minor groove of DNA. The large number of polyamides made available by solid phase synthetic methodology should accelerate the elucidation of the scope and limitations of this approach.

Experimental Section

Materials. Boc-β-alanine-(4-carbonylaminomethyl)-benzyl-ester-copoly(styrene−divinylbenzene) resin (Boc-β-Pam-resin), dicyclohexylcarbodiimide (DCC), hydroxybenzotriazole (HOBt), 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), Boc-glycine, and Boc-β-alanine were purchased from Peptides International. *N*,*N*-Diisopropylethylamine (DIEA), *N*,*N*-dimethylformamide (DMF), *N*-methylpyrrolidone (NMP), and DMSO/NMP were purchased from Applied Biosystems. Boc-γ-aminobutyric acid was from NOVA Biochem, dichloromethane (DCM) and triethylamine (TEA) were reagent grade from EM, thiophenol (PhSH), (dimethylamino)propylamine, trichloroacetyl chloride, *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 GE 300 instrument operating at 300 MHz. Chemical shifts are reported in parts per million relative to the solvent residual signal. UV spectra were measured on a Hewlett-Packard Model 8452A diode array spectrophotometer. IR spectra were recorded on a Perkin-Elmer FTIR spectrometer. High-resolution FAB mass spectra were recorded at the Mass Spectroscopy Laboratory at the University of California, Riverside. Matrix-assisted, laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) was carried out at the Protein and Peptide Microanalytical Facility at the California Institute of Technology. HPLC analysis was performed either on a HP 1090M analytical HPLC or on a Beckman Gold system using a RAINEN C_{18}, Microsorb MV, 5 μm, 300 × 4.6 mm reversed-phase column in 0.1% (w/v) TFA with acetonitrile as eluent and a flow rate of 1.0 mL/min, gradient elution 1.25% acetonitrile/min. Preparatory HPLC was carried out on a Beckman HPLC using a Waters DeltaPak 25 × 100 mm, 100 μm C_{18} column equipped with a guard, 0.1% (w/v) TFA, 0.25% acetonitrile/min. 18MΩ water was obtained from a Millipore MilliQ water purification system, and all buffers were 0.2 μm filtered. Thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} precoated plates. Reagent-grade chemicals were used unless otherwise stated.

Monomer Syntheses. 4-Nitro-2-(trichloroacetyl)-1-methylpyrrole (3). To a well-stirred solution of trichloroacetyl chloride (1 kg, 5.5 mol) in 1.5 L of ethyl ether in a 12 liter flask was added dropwise over a period of 3 h a solution of *N*-methylpyrrole (0.45 kg, 5.5 mol) in 1.5 L of anhydrous ethyl ether. The reaction mixture was stirred for an additional 3 h, and the reaction was quenched by the dropwise addition of a solution of 400 g of potassium carbonate in 1.5 L of water. The layers were separated, and the ether layer was concentrated

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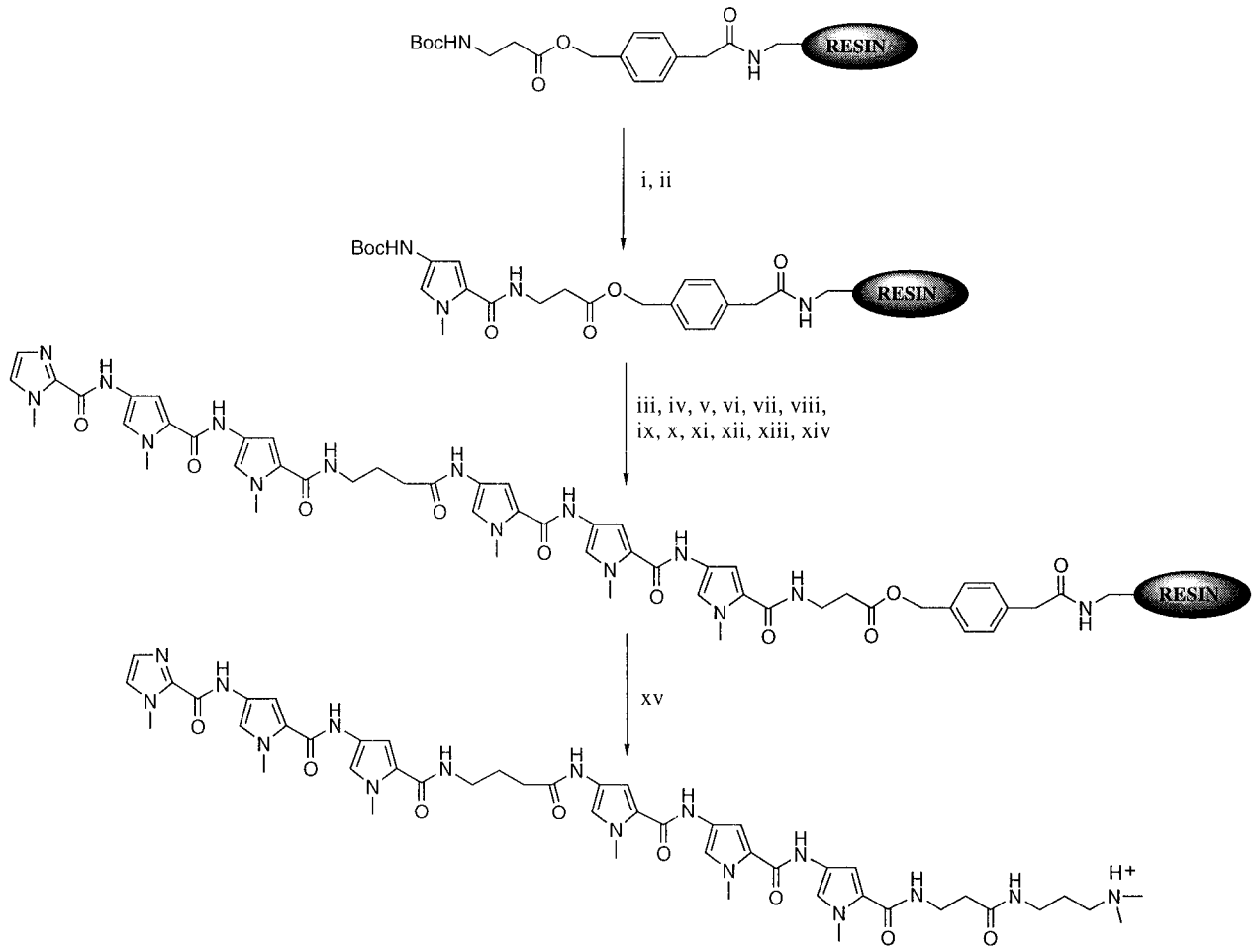

Figure 6. Solid phase synthetic scheme for ImPyPy-γ-PyPyPy-β-Dp starting from commercially available Boc-β-Pam-resin: (i) 80% TFA/DCM, 0.4 M PhSH; (ii) Boc-Py-OBt, DIEA, DMF; (iii) 80% TFA/DCM, 0.4 M PhSH; (iv) Boc-Py-OBt, DIEA, DMF; (v) 80% TFA/DCM, 0.4 M PhSH; (vi) Boc-Py-OBt, DIEA, DMF; (vii) 80% TFA/DCM, 0.4 M PhSH; (viii) Boc-γ-aminobutyric acid (HBTU, DIEA); (ix) 80% TFA/DCM, 0.4 M PhSH; (x) Boc-Py-OBt, DIEA, DMF; (xi) 80% TFA/DCM, 0.4 M PhSH; (xii) Boc-Py-OBt, DIEA, DMF; (xiii) 80% TFA/DCM, 0.4 M PhSH; (xiv) imidazole-2-carboxylic acid (HBTU/DIEA); (xv) (*N*,*N*-dimethylamino)propylamine, 55 °C.

in vacuo to provide 2-(trichloroacetyl)pyrrole (1.2 kg, 5.1 mol) as a yellow crystalline solid sufficiently pure to be used without further purification. To a cooled (−40 °C) solution of 2-(trichloroacetyl)pyrrole (1.2 kg, 5.1 mol) in acetic anhydride (6 L) in a 12 L flask equipped with a mechanical stirrer was added 440 mL of fuming nitric acid over a period of 1 h while a temperature of (−40 °C was maintained). The reaction mixture was carefully allowed to warm to room temperature and stirred for an additional 4 h. The mixture was cooled to −30 °C and isopropyl alcohol (6 L) added. The solution was stirred at −20 °C for 30 min, during which time a white precipitate formed. The solution was allowed to stand for 15 min and the resulting precipitate collected by vacuum filtration to provide **3** (0.8 kg, 54% yield): TLC (7:2 benzene/ethyl acetate) R_f 0.7; ^{1}H NMR (DMSO-d_6) δ 8.55 (d, 1 H, J = 1.7 Hz), 7.77 (d, 1 H, J = 1.7 Hz), 3.98 (s, 3 H); ^{13}C NMR (DMSO-d_6) δ 173.3, 134.7, 133.2, 121.1, 116.9, 95.0, 51.5; IR (KBr) 1694, 1516, 1423, 1314, 1183, 1113, 998, 750; FABMS *m/e* 269.936 (M + H 269.937 calcd for $C_7H_5N_2O_3Cl_3$).

Methyl 4-Nitropyrrole-2-carboxylate (4). To a solution of **3** (800 g, 2.9 mol) in 2.5 L of methanol in a 4 L Erlenmeyer flask equipped with a mechanical stirrer was added dropwise a solution of NaH (60% dispersion in oil) (10 g, 0.25 mol) in 500 mL of methanol. The reaction mixture was stirred for 2 h at room temperature, and the reaction was quenched by the addition of concentrated sulfuric acid (25 mL). The reaction mixture was then heated to reflux and allowed to slowly cool to room temperature as **4** crystallized as white needles, which were collected by vacuum filtration and dried *in vacuo* (450 g, 47% yield). TLC (ethyl acetate) R_f 0.8; ^{1}H NMR (DMSO-d_6) δ 8.22 (d, 1 H, J = 1.7 Hz), 7.22 (d, 1 H, J = 1.6 Hz), 3.88 (s, 3 H), 3.75 (s, 3 H); ^{13}C NMR (DMSO-d_6) δ 37.8, 52.2, 112.0, 123.0, 129.9, 134.6, 160.3; IR-(KBr) 3148, 1718, 1541, 1425, 1317, 1226, 1195, 1116, 753; FABMS *m/e* 184.048 (M + H 184.048 calcd for $C_7H_8N_2O_4$).

Methyl 4-Amino-1-methylpyrrole-2-carboxylate Hydrochloride (5). Methyl 4-nitropyrrole-2-carboxylate (**4**) (450 g, 2.8 mol) was dissolved in ethyl acetate (8 L). A slurry of 40 g of 10% Pd/C in 800 mL of ethyl acetate was then added and the mixture stirred under a slight positive pressure of hydrogen (ca. 1.1 atm) for 48 h. Pd/C was removed by filtration through Celite and washed with 1 × 50 mL ethyl acetate, and the volume of the mixture was reduced to ca. 500 mL; 7 L of cold ethyl ether was added and HCl gas gently bubbled through the mixture. The precipitated amine hydrochloride was then collected by vacuum filtration to yield 380 g (81.6%) of **5** as a white powder: TLC (ethyl acetate) R_f (amine) 0.6, R_f salt (0.0); ^{1}H NMR (DMSO-d_6) δ 10.23 (br s, 3 H), 7.24 (d, 1 H, J = 1.9 Hz), 6.79 (d, 1 H, J = 2.0 Hz), 3.83 (s, 3 H), 3.72 (s, 3 H); ^{13}C NMR (DMSO-d_6) δ 160.8, 124.3, 121.2, 113.4, 112.0, 51.8, 37.1; IR (KBr) 3095, 2693, 1709, 1548, 1448, 1266, 1102, 802, 751; FABMS *m/e* 154.075 (154.074 calcd for $C_7H_{10}N_2O_2$).

4-[(*tert*-Butoxycarbonyl)amino]-1-methylpyrrole-2-carboxylic Acid (6). The hydrochloride salt of the pyrrole amine **5** (340 g, 1.8 mol) was dissolved in 1 L of 10% aqueous sodium carbonate in a 3 L flask equipped with a mechanical stirrer; di-*tert*-butyl dicarbonate (400 g, 2.0 mmol) slurried in 500 mL of dioxane was added over a period of 30 min while a temperature of 20 °C was maintained. The reaction was allowed to proceed for 3 h and was determined complete by TLC; the mixture was cooled to 5 °C for 2 h and the resulting white precipitate collected by vacuum filtration. The Boc-pyrrole ester contaminated with Boc-anhydride was dissolved in 700 mL of MeOH; 700 mL of 2 M NaOH was added and the solution heated at 60 °C for 6 h. The

reaction was cooled to room temperature and washed with ethyl ether (4 × 1000 mL), the pH of the aqueous layer reduced to ca. 3 with 10% (v/v) H_2SO_4, and the mixture was extracted with ethyl acetate (4 × 2000 mL). The combined ethyl acetate extracts were dried (sodium sulfate) and concentrated *in vacuo* to provide a tan foam. The foam was dissolved in 500 mL of DCM and 2 L petroleum ether added, and the resulting slurry was concentrated *in vacuo*. The reaction mixture was redissolved and concentrated three additional times to provide 320 g (78% yield) of **6** as a fine white powder: TLC (7:2 benzene/ethyl acetate v/v) R_f (ester) 0.8, R_f (acid) 0.1. (ethyl acetate), R_f (acid) 0.6; ^{1}H NMR (DMSO-d_6) δ 12.10 (s, 1 H), 9.05 (s, 1 H), 7.02 (s, 1 H), 6.55 (s, 1 H), 3.75 (s, 3H), 1.41 (s, 9 H); ^{13}C NMR (DMSO-d_6) δ 162.4, 153.2, 123.3, 120.1, 119.2, 107.9, 78.9, 36.6, 28.7; IR(KBr) 3350, 2978, 1700, 1670, 1586, 1458, 1368, 1247, 1112, 887, 779; FABMS *m/e* 241.119 (M + H 241.119 calcd for $C_{11}H_{17}N_2O_4$).

1,2,3-Benzotriazol-1-yl 4-[(*tert*-Butoxycarbonyl)amino]-1-methylpyrrole-2-carboxylate (7). Boc-Py-acid **6** (31 g, 129 mmol) was dissolved in 500 mL of DMF, and HOBt (17.4 g, 129 mmol) was added followed by DCC (34 g, 129 mmol). The reaction mixture was stirred for 24 h and then filtered dropwise into a well-stirred solution of 5 L of ice water. The precipitate was allowed to sit for 15 min at 0 °C and then collected by filtration. The wet cake was dissolved in 500 mL of DCM, and the organic layer was added slowly to a stirred solution of cold petroleum ether (4 °C). The mixture was allowed to stand at −20 °C for 4 h and then collected by vacuum filtration and dried *in vacuo* to provide 39 g (85% yield) of **7** as a finely divided white powder: TLC (7:2 benzene/ethyl acetate v/v) R_f 0.6; ^{1}H NMR (DMSO-d_6) δ 9.43 (s, 1 H), 8.12 (d, 1 H, J = 8.4 Hz), 7.80 (d, 1 H, J = 8.2 Hz), 7.64 (t, 1 H, J = 7.0 Hz), 7.51 (m, 2 H), 7.18 (s, 1 H), 3.83 (s, 3 H), 1.45 (s, 9 H); ^{13}C NMR (DMSO-d_6) δ 156.5, 153.3, 143.2, 129.6, 129.2, 125.7, 125.2, 124.6, 120.3, 112.8, 110.3, 109.8, 79.5, 36.8, 28.6; IR (KBr) 3246, 3095, 2979, 1764, 1711, 1588, 1389, 1365, 1274, 1227, 1160, 1101, 999, 824, 748; FABMS *m/e* 358.152 (M + H 358.151 calcd for $C_{17}H_{20}N_5O_4$).

Ethyl 1-Methylimidazole-2-carboxylate (8). *N*-Methylimidazole (320 g, 3.9 mol) was combined with 2 L of acetonitrile and 1 L of triethylamine in a 12 L flask equipped with a mechanical stirrer, and the solution was cooled to −20 °C. Ethyl chloroformate (1000 g, 9.2 mol) was added with stirring, the temperature being kept between −20 and −25 °C. The reaction mixture was allowed to *slowly* warm to room temperature and stirred for 36 h. Precipitated triethylamine hydrochloride was removed by filtration and the solution concentrated *in vacuo* at 65 °C. The resulting oil was purified by distillation under reduced pressure (2 Torr, 102 °C) to provide **8** as a white solid (360 g, 82% yield): TLC (7:2 benzene/ethyl acetate v/v) R_f 0.2; ^{1}H NMR (DMSO-d_6) δ 7.44 (d, 1 H, J = 2.8 Hz), 7.04 (d, 1 H, J = 2.8 Hz), 4.26 (q, 2 H, J = 3.5 Hz), 3.91 (s, 3 H), 1.26 (t, 3 H, J = 3.5 Hz); ^{13}C NMR (DMSO-d_6) δ 159.3, 129.1, 127.7, 61.0, 36.0, 14.5; IR(KBr) 3403, 3111, 2983, 1713, 1480, 1422, 1262, 1134, 1052, 922, 782, 666; FABMS *m/e* 155.083 (M + H 155.083 calcd for $C_7H_{11}N_2O_2$).

Ethyl 1-Methyl-4-nitroimidazole-2-carboxylate (9). Compound **8** was carefully dissolved in 1000 mL of concentrated sulfuric acid cooled to 0 °C; 90% nitric acid (1 L) was slowly added, a temperature of 0 °C being maintained. The reaction mixture was then refluxed with an efficient condenser (−20 °C) in a well-ventilated hood for 50 min. The reaction mixture was cooled with an ice bath and quenched by pouring onto 10 L of ice. The resulting blue solution was then extracted with 20 L of DCM, and the combined extracts were dried (sodium sulfate) and concentrated *in vacuo* to yield a tan solid, which was recrystallized from 22 L of 21:1 carbon tetrachloride/ethanol. The resulting white crystals were collected by vacuum filtration to provide pure **9** (103 g, 22% yield): TLC (7:2 benzene/ethyl acetate v/v) R_f 0.5; ^{1}H NMR (DMSO-d_6) δ 8.61 (s, 1 H), 4.33 (q, 2 H, J = 6.4 Hz), 3.97 (s, 3 H), 1.29 (t, 3 H, J = 6.0 Hz); ^{13}C NMR (DMSO-d_6) δ 158.2, 145.4, 135.3, 127.4, 62.2, 37.3, 14.5; IR (KBr) 3139, 1719, 1541, 1498, 1381, 1310, 1260, 1122, 995, 860, 656; FABMS *m/e* 200.066 (M + H 200.067 calcd for $C_7H_{10}N_3O_4$).

Ethyl 4-Amino-1-methylimidazole-2-carboxylate Hydrochloride (10). The nitroimidazole ethyl ester **9** (103 g, 520 mmol) was dissolved in 5 L of 1:1 ethanol/ethyl acetate, 20 g of 10% Pd/C slurried in 500 mL of ethyl acetate was added, and the mixture was stirred under a slight positive pressure of hydrogen (ca. 1.1 atm) for 48 h. The reaction mixture was filtered concentrated *in vacuo* to a volume of 500 mL, and 5 L of cold anhydrous ethyl ether was added. Addition of HCl gas provided a white precipitate. The solution was cooled at −20 °C for 4 h and the precipitate collected by vacuum filtration and dried *in vacuo* to provide 75 g (78% yield) of **10** as a fine white powder: TLC (7:2 benzene/ethyl acetate) R_f (amine) 0.3, R_f (salt) 0.0; ^{1}H NMR (DMSO-d_6) δ 10.11 (br s, 3 H), 7.43 (s, 1 H), 4.28 (q, 2 H, J = 7.1 Hz), 3.92 (s, 1 H), 1.28 (t, 3 H, J = 7.1 Hz) ^{13}C NMR (DMSO-d_6) δ 157.6, 132.6, 117.4, 117.3, 61.8, 36.6, 14.5; IR (KBr) 3138, 2883, 1707, 1655, 1492, 1420, 1314, 1255, 1152, 1057, 837, 776; FABMS *m/e* 169.085 (169.084 calcd for $C_7H_{11}N_3O_2$).

4-[(*tert*-Butoxycarbonyl)amino]-1-methylimidazole-2-carboxylic Acid (11). The imidazole amine **10** (75 g, 395 mmol) was dissolved in 200 mL of DMF. DIEA (45 mL, 491 mmol) was added followed by di-*tert*-butyl dicarbonate (99 g, 491 mmol). The mixture was shaken at 60 °C for 18 h, allowed to assume room temperature, and partitioned between 500 mL of brine and 500 mL of ethyl ether. The ether layer was extracted (2 × 200 mL each) with 10% citric acid, brine, saturated sodium bicarbonate, and brine, dried over sodium sulfate, and concentrated *in vacuo* to yield the Boc-ester contaminated with 20% Boc-anhydride as indicated by ^{1}H NMR. The Boc-ester, used without further purification, was dissolved in 200 mL of 1 M NaOH. The reaction mixture was allowed to stand for 3 h at 60 °C with occasional agitation. The reaction mixture was cooled to 0 °C and carefully neutralized with 1 M HCl to pH 2, at which time a white gel formed. The gel was collected by vacuum filtration and frozen before drying, and remaining water was lyophilized to yield **10** as a white powder (51 g, 54% yield): ^{1}H NMR (DMSO-d_6) δ 9.47 (s, 1 H), 7.13 (s, 1 H), 3.85 (s, 3 H), 1.41 (s, 9 H); ^{13}C NMR (DMSO-d_6) δ 160.9, 152.9, 137.5, 134.5, 112.4, 79.5, 35.7, 28.6; IR (KBr) 3448, 2982, 1734, 1654, 1638, 1578, 1357, 1321, 1249, 1163, 799; FABMS *m/e* 241.105 (241.106 calcd for $C_{10}H_{15}N_3O_4$).

4-[[[4-[(*tert*-Butoxycarbonyl)amino]-1-methylpyrrol-2-yl]carbonyl]amino]-1-methylimidazole-2-carboxylic acid (12) was prepared as described below for **13** substituting Boc-Pyrrole acid for Boc-γ-aminobutyric acid (4.1 g, 91% yield): ^{1}H NMR (DMSO-d_6) δ 10.58 (s, 1 H), 9.08 (s, 1 H), 7.57 (s, 1 H), 6.97 (s, 1 H), 6.89 (s, 1 H), 3.89 (s, 3 H), 3.75 (s, 3 H), 1.35 (s, 9 H); ^{13}C NMR (DMSO-d_6) δ 160.36, 159.1, 153.4, 137.9, 132.3, 122.8, 122.3, 118.5, 115.5, 105.5, 105.4, 78.8, 28.7, 24.9; IR 3346, 2929, 1685, 1618, 1529, 1342, 1274, 1179, 997, 761; FABMS *m/e* 364.161 (364.162 calcd for $C_{16}H_{22}N_5O_5$).

4-[[[3-[(*tert*-Butoxycarbonyl)amino]propyl]carbonyl]amino]-1-methylimidazole-2-carboxylic Acid (13). To a solution of Boc-γ-aminobutyric acid (10 g, 49 mmol) in 40 mL of DMF was added 1.2 equiv of HOBt (7.9 g, 59 mmol) followed by 1.2 equiv of DCC (11.9 g, 59 mmol). The solution was stirred for 24 h and the DCU removed by filtration. Separately, to a solution of ethyl 4-nitro-1-methylimidazole-2-carboxylate (9.8 g, 49 mmol) in 20 mL of DMF was added Pd/C catalyst (10%, 1 g), and the mixture was hydrogenated in a Parr bomb apparatus (500 psi of H_2) for 2 h. The catalyst was removed by filtration through Celite and the filtrate immediately added to the OBt ester solution. An excess of DIEA (15 mL) was then added and the reaction mixture stirred at 60 °C for 8 h. The reaction mixture was then added dropwise to a stirred solution of ice water and the resulting precipitate collected by vacuum filtration to provide crude ethyl 4-[[[3-[(*tert*-butoxycarbonyl)amino]propyl]carbonylamino]-1-methylimidazole-2-carboxylate (5 g, 14.1 mmol). To the crude ester dissolved in 50 mL of methanol was added 50 mL of 1 M NaOH, and the resulting mixture was stirred for 6 h at 60 °C. Excess methanol was removed *in vacuo* and the resulting solution acidified by the addition of 1 M HCl. The resulting precipitate was collected by vacuum filtration and dried *in vacuo* to yield a brown powder (4.4 g, 89% yield): ^{1}H NMR (DMSO-d_6) δ 10.50 (s, 1 H), 7.45 (s, 1 H), 6.82 (t, 1 H, J = 3.6 Hz), 3.86 (s, 3 H), 2.86 (q, 2 H, J = 4.6 Hz), 2.22 (t, 2 H, J = 7.4 Hz), 1.57 (quintet, 2 H, J = 5.9 Hz), 1.29 (s, 9 H); IR 3416, 2950, 2841, 1650, 1538 1449, 1392, 1250, 1165, 1108; FABMS *m/e* 326.160 (326.159 calcd for $C_{14}H_{22}N_4O_5$).

Solid Phase Syntheses. Activation of Imidazole-2-carboxylic acid,[2a] γ-aminobutyric acid, Boc-glycine, and Boc-β-alanine. 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 lightly shaken for at least 5 min.

Activation of Boc−Imidazole Acid. Boc−imidazole acid (257 mg, 1 mmol) and HOBt (135 mg, 1 mmol) were dissolved in 2 mL of DMF, DCC (202 mg, 1 mmol) was then added, and the solution was allowed to stand for at least 5 min.

Activation of Boc−γ−Imidazole Acid and Boc−Pyrrole−Imidazole Acid. The appropriate dimer (1 mmol) and HBTU (378 mg, 1 mmol) were combined in 2 mL of DMF. DIEA (1 mL) was then added, and the reaction mixture was allowed to stand for 5 min.

Activation of Boc−Pyrrole Acid (for Coupling to Imidazole Amine). Boc−pyrrole acid (514 mg, 2 mmol) was dissolved in 2 mL of dichloromethane, DCC (420 mg, 2 mmol) was added, the solution was allowed to stand for 10 min, DMAP (101 mg, 1 mmol) was added, and the solution was allowed to stand for 1 min.

Acetylation Mix. DMF (2 mL), DIEA (710 μL, 4.0 mmol), and acetic anhydride (380 μL, 4.0 mmol) were combined immediately before use.

Manual Synthesis Protocol. Boc-β-alanine-Pam-resin (1.25 g, 0.25 mmol) was placed in a 20 mL glass reaction vessel and shaken in DMF for 5 min, and the reaction vessel was drained. The resin was washed with DCM (2 × 30 s) and the Boc group removed with 80% TFA/DCM/0.5 M PhSH, 1 × 30 s, 1 × 20 min. The resin was washed with DCM (2 × 30 s) followed by DMF (1 × 30 s). A resin sample (5−10 mg) was taken for analysis. The vessel was drained completely and activated monomer added, followed by DIEA if necessary. The reaction vessel was shaken vigorously to make a slurry. The coupling was allowed to proceed for 45 min, and a resin sample was taken. The reaction vessel was then washed with DCM, followed by DMF.

Machine-Assisted Protocols. Machine-assisted synthesis was performed on a ABI 430A synthesizer on a 0.18 mmol scale (900 mg of resin; 0.2 mmol/g). Each cycle of amino acid addition involved deprotection with approximately 80% TFA/DCM/0.4 M PhSH for 3 min, draining the reaction vessel, and then deprotection for 17 min; two dichloromethane flow washes; an NMP flow wash; draining the reaction vessel; coupling for 1 h with *in situ* neutralization, addition of dimethyl sulfoxide (DMSO)/NMP, coupling for 30 min, addition of DIEA, coupling for 30 min; draining the reaction vessel; washing with DCM, taking a resin sample for evaluation of the progress of the synthesis by HPLC analysis; capping with acetic anhydride/DIEA in DCM for 6 min; and washing with DCM. A double couple cycle is employed when coupling aliphatic amino acids to imidazole; all other couplings are performed with single couple cycles.

The ABI 430A synthesizer was left in the standard hardware configuration for NMP−HOBt protocols. Reagent positions 1 and 7 were DIEA, reagent position 2 was TFA/0.5 M thiophenol, reagent position 3 was 70% ethanolamine/methanol, reagent position 4 was acetic anhydride, reagent position 5 was DMSO/NMP, reagent position 6 was methanol, and reagent position 8 was DMF. New activator functions were written, one for direct transfer of the cartridge contents to the concentrator (switch list 21, 25, 26, 35, 37, 44), and a second for transfer of reagent position 8 directly to the cartridge (switch list 37, 39, 45, 46).

Boc-Py-OBt ester (357 mg, 1 mmol) was dissolved in 2 mL of DMF and filtered into a synthesis cartridge. Boc-Im acid monomer was activated (DCC/HOBt), filtered, and placed in a synthesis cartridge. Imidazole-2-carboxylic acid was added manually. 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 through the resin sampling loop via syringe. When manual addition was necessary, an empty synthesis cartridge was used. Aliphatic amino acids (2 mmol) and HBTU (1.9 mmol) were placed in a synthesis cartridge. DMF (3 mL) was added using a calibrated delivery loop from reagent bottle 8, followed by 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.

ImPyPy-γ-ImPyPy-β-Dp (2). ImPyPy-γ-PyPyPy-β-Pam-resin was prepared by machine-assisted synthesis protocols. A sample of resin (1 g, 0.17 mmol[29]) 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 yield a white powder (103 mg, 61% recovery): 24.1; UV $\lambda_{max}(H_2O)$ (ϵ), 234 (39 300), 304 nm (52 000); ^{1}H NMR (DMSO-d_6) δ 10.47 (s, 1 H), 9.91 (s, 1 H), 9.89 (s, 1 H), 9.87 (s, 1 H), 9.84 (s, 1 H), 9.2 (br s, 1 H), 8.08 (m, 3 H), 7.38 (s, 1 H), 7.26 (d, 1 H, J = 1.0 Hz), 7.20 (d, 1 H, J = 1.0 Hz), 7.14 (m, 4 H), 7.04 (d, 1 H, J = 1.1 Hz), 7.02 (d, 1 H, J = 1.1 Hz), 6.89 (d, 1 H, J = 1.0 Hz), 6.85 (m, 2 H), 3.97 (s, 3 H), 3.82 (m, 6 H), 3.81 (s, 3 H), 3.77 (m, 6 H), 3.34 (m, 2 H, J = 3.9 Hz), 3.18 (m, 2 H, J = 5.5 Hz), 3.06 (m, 2 H, J = 5.7 Hz), 2.95 (m, 2 H, J = 4.9 Hz), 2.71 (d, 6 H, J = 4.6 Hz), 2.30 (m, 6 H), 1.75 (m, 4 H); MALDI-TOF-MS 978.0 (978.1 calcd for M + H).

Stepwise HPLC Analysis. A resin sample (ca. 4 mg) was placed in a 4 mL glass test tube, 200 μL 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 μL sample analyzed by analytical HPLC at 254 nm.

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Supporting Information Available: HPLC and MALDI-TOF mass spectral characterization of the synthesis of ImPyPy-γ-PyPyPy-β-Dp (2 pages). See any current masthead page for ordering and Internet access instructions.

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(29) Resin substitution is calculated as L_{new}(mmol/g) = $L_{old}/(1 + L_{old}(W_{new - Wold}) \times 10^{-3})$; L is the loading, and W is the molecular weight of the polyamide attached to the resin. See: Barlos, K.; Chatzi, O.; Gatos, D.; Stravropoulos, G. *Int. J. Pept. Protein Res.* **1991**, *37*, 513.