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Fmoc Solid Phase Synthesis of Polyamides Containing Pyrrole and Imidazole Amino Acids

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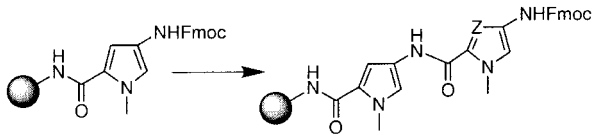
ABSTRACT



Polyamides containing *N*-methylimidazole (Im) and *N*-methylpyrrole (Py) amino acids are synthetic ligands that have an affinity and specificity for DNA comparable to those of many naturally occurring DNA binding proteins. A machine-assisted Fmoc solid phase synthesis of polyamides has been optimized to afford high stepwise coupling yields (>99%). Two monomer building blocks, Fmoc-Py acid and Fmoc-Im acid, were prepared in multigram scale. Cleavage by aminolysis followed by HPLC purification affords up to 200 mg quantities of polyamide with purities and yields greater than or equal to those reported using Boc chemistry. A broader set of reaction conditions will increase the number and complexity of minor groove binding polyamides which may be prepared and help ensure compatibility with many commercially available peptide synthesizers.

Small molecules specifically targeted to any predetermined DNA sequence would be useful tools in molecular biology and potentially in human medicine. Polyamides containing *N*-methylpyrrole (Py), 3-hydroxylpyrrole (Hp), and *N*-methylimidazole (Im) are synthetic ligands that have an affinity and specificity for DNA comparable to those of naturally occurring DNA binding proteins. DNA recognition depends on side-by-side aromatic amino acid pairings oriented N → C with respect to the 5′ → 3′ direction of the DNA helix in the minor groove. An antiparallel pairing of Im opposite Py (Im/Py pair) recognizes a G·C base pair, while a Py/Im combination recognizes C·G. A Hp/Py pair prefers T·A over A·T. A Py/Py pair is degenerate and recognizes either an A·T or T·A base pair.[1]

The large repertoire of pyrrole−imidazole polyamides synthesized hallmarks the development of Boc-chemistry solid phase polyamide synthesis.[2] However, to ensure compatibility with most commercially available peptide synthesizers, we set out to develop an alternative solid phase synthesis incorporating a different protecting group that would provide a route to polyamides with comparable purity and recovery to Boc chemistry. The synthetic schemes made available by this new chemistry could make available a greater variety of bifunctional polyamide conjugates and derivatives. As a minimum first step, we report here monomer synthesis and general protocols for machine-assisted Fmoc-chemistry solid phase synthesis of pyrrole−imidazole polyamides, ImPyPyPy-γ-PyPyPyPy-β-Dp **1** and ImPyPyPy-γ-ImPyPyPy-β-Dp **2** (Figure 1), which are identical or superior in recovery, purity, and coupling times to Boc-chemistry protocols.

The synthesis of Fmoc-Py acid has previously been reported.[3] We report a convenient multigram scalable synthesis of Fmoc-Py acid with a higher overall yield without the use of column chromatography, as well as the first-reported synthesis of Fmoc-Im acid.

(1) Bürli, R. W.; Dervan, P. B. *Curr. Opin. Chem. Biol.* **1999**, and references therein.

(2) Baird, E. E.; Dervan, P. B. *J. Am. Chem. Soc.* **1996**, *118*, 6141−6146.

(3) (a) Vázquez, E.; Caamaño, A. M.; Castedo, L.; Mascareñas, J. L. *Tetrahedron Lett.* **1999**, *40*, 3621. (b) König, B.; Rödel, M. *Synth. Commun.* **1999**, *29*, 943.

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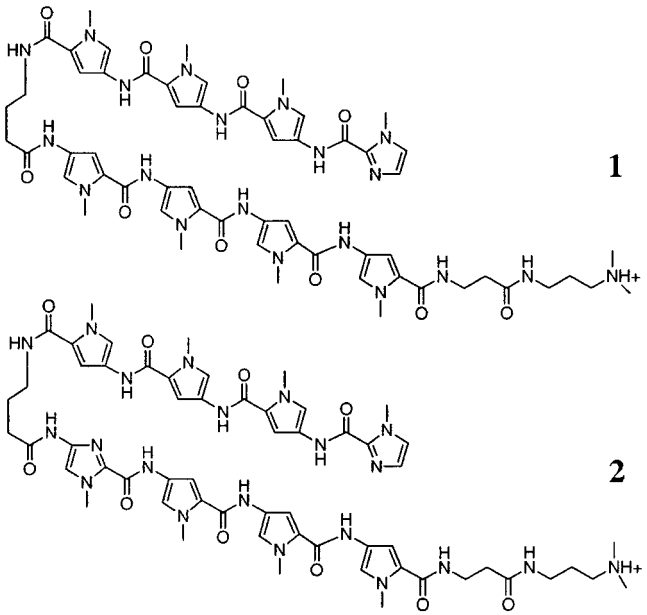

Figure 1. ImPyPyPy-γ-PyPyPyPy-β-Dp (top) and ImPyPyPy-γ-PyPyPyPy-β-Dp (bottom) prepared by multistep solid phase synthesis.

Pyrrole monomer precursors **3a** and **4a** were synthesized using literature protocols.[2] Previously reported 2-(trichloroacetyl)-1-methylimidazole **3b** was converted to *tert*-butyl 1-methylimidazole-2-carboxylate **4b** using modifications of previously published procedures.[4] Compound **3b** in acetic anhydride was cooled to 0 °C followed by the addition of fuming nitric acid and sulfuric acid over 2 h (Figure 2).

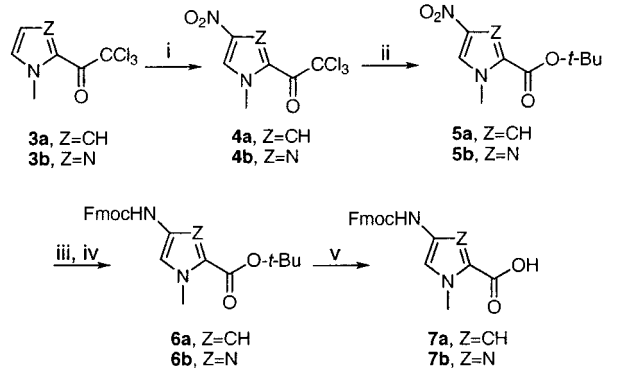

Figure 2. (i) Fuming nitric acid, sulfuric acid, Ac_2O; (ii) NaO*t*-Bu/*t*-BuOH, (iii) 500 psi of H_2, 10% Pd/C, DMF; (iv) Fmoc-Cl, DIEA; (v) $TiCl_4$, CH_2Cl_2.

Crystallization provided 528 g of **4b** in 56% yield. *tert*-Butyl esters **5a** and **5b** were synthesized from **4a** and **4b** with *tert*-butoxide in refluxing *tert*-butyl alcohol yielding **5a** and **5b** in 95% and 84% yields, respectively. Reduction of **5a** and **5b** in DMF in the presence of 10% Pd/C under 500 psi of hydrogen proceeds to completion in 3 h. After filtering the reaction mixture through a pad of Celite, Fmoc-Cl and diisopropylethylamine were added to provide **6a** and **6b** in 66% and 35% yields, respectively. The *tert*-butyl esters were deprotected with $TiCl_4$ in dichloromethane at 0 °C to prevent the decarboxylation, which was observed with standard trifluoroacetic acid deprotection. Precipitation with 1.0 M HCl afforded the final products **7a** and **7b** in 88% and 86% yields, respectively. The overall yields from commercially available starting materials for the Fmoc-Py acid and Fmoc-Im acid are 33% and 11%, respectively.

Additionally, the first two steps of the Fmoc-Im acid synthesis provide a safer, higher yielding route to the Boc-Im acid monomer. The original published synthesis[2] of the Boc-Im acid required acylation of *N*-methyl imidazole with ethyl chloroformate, followed by a highly exothermic nitration at the 4- and 5- positions in sulfuric acid and fuming nitric acid. The isomers were separated by a difficult recrystallizaton to provide the desired ethyl 1-methyl-4-nitroimidazole-2-carboxylate **8**. In the new synthetic route, nitration of trichloroketone **3b** in acetic anhydride provides the desired isomer, 4-nitro-2-(trichloroacetyl)-1-methylimidazole **4b**, by milder nitration conditions. The product was esterified with sodium ethoxide in ethanol to yield ethyl 1-methyl-4-nitroimidazole-2-carboxylate **8** in 95% yield (Figure 3).

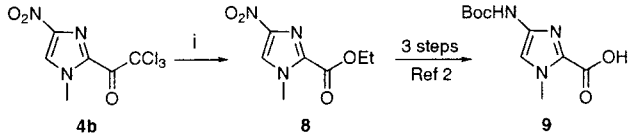

Figure 3. (i) NaOEt/EtOH.

This intermediate was converted in three steps into the desired Boc-Im acid **9** using previously reported reactions[2] in an improved overall yield of 18%, compared to the 8% overall yield in the original synthesis.

For Fmoc solid phase synthesis, the polyamide was attached to an insoluble matrix by a linkage, which is cleaved in a single step, introducing a positive charge into the polyamide. Fmoc-β-alanine−Wang resin is commercially available in appropriate substitution levels (0.86 mmol/g). Manual solid phase synthesis uses HBTU/DIEA activation protocols as previously reported.[2]

Fmoc-chemistry machine-assisted solid phase polyamide synthesis protocols were modified from the Boc-chemistry protocols reported for use on an ABI 430A peptide synthesizer. Coupling cycles for machine-assisted synthesis are rapid, 180 min per residue, and require no special precautions beyond those used for ordinary solid phase peptide synthesis. Machine-assisted solid phase synthesis of a pyrrole−imida-

(4) Nishiwaki, E.; Tanaka, S.; Lee, H.; Shibuya, M. *Heterocycles* **1988**, *27*, 1945.

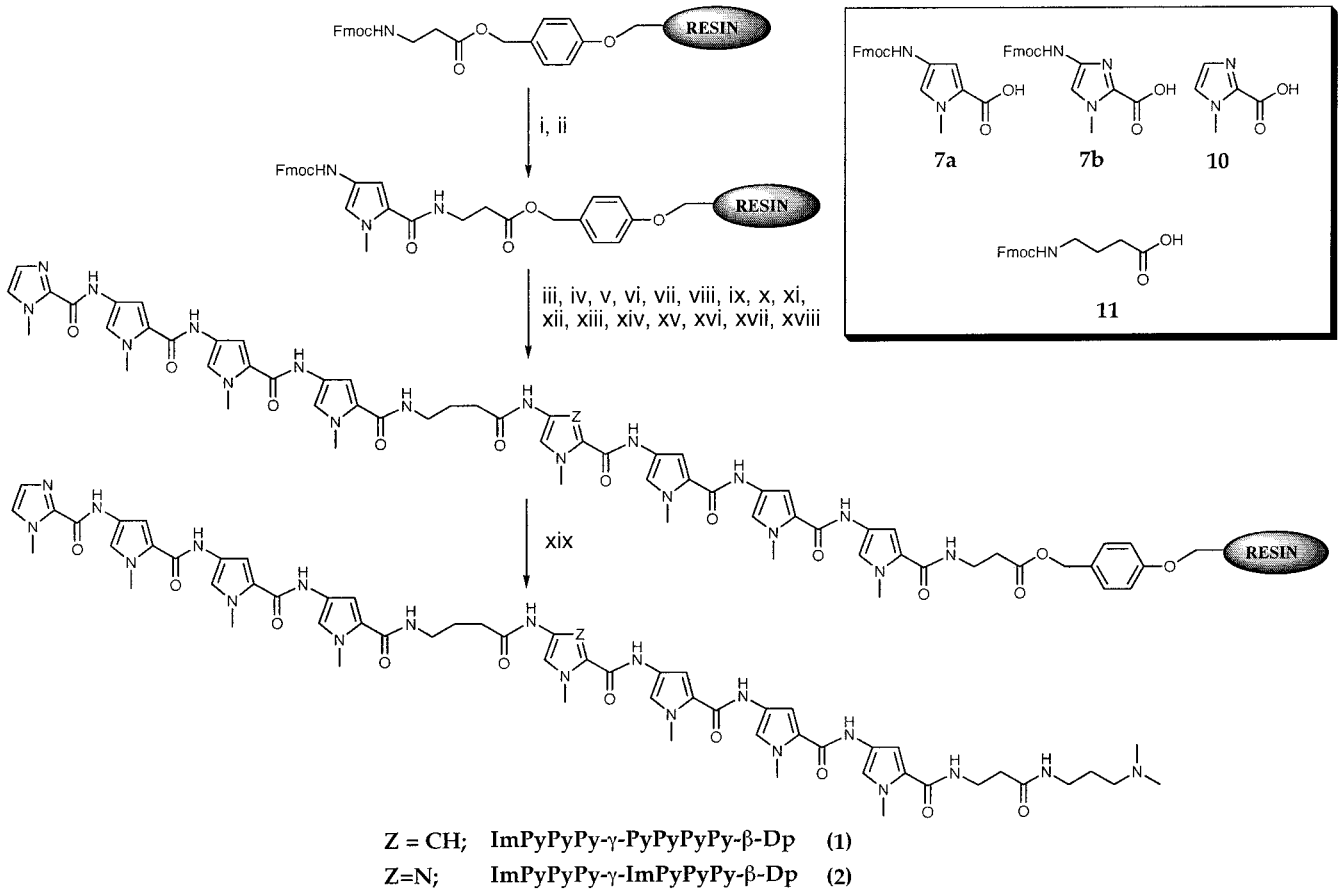

Figure 4. Fmoc solid phase synthetic scheme for polyamides **1** and **2** starting from commercially available Fmoc-β-alanine−Wang resin: (i) 20% piperidine/NMP; (ii) Fmoc-Py acid, HBTU, DIEA; (iii) 20% piperidine/NMP; (iv) Fmoc-Py acid, HBTU, DIEA; (v) 20% piperidine/NMP; (vi) Fmoc-Py acid, HBTU, DIEA; (vii) 20% piperidine/NMP; (viii) Fmoc-Py acid (for **1**) or Fmoc-Im acid (for **2)**, HBTU, DIEA; (ix) 20% piperidine/NMP; (x) Fmoc-γ-aminobutyric acid, HBTU, DIEA; (xi) 20% piperidine/NMP; (xii) Fmoc-Py acid, HBTU, DIEA; (xiii) 20% piperidine/NMP; (xiv) Fmoc-Py acid, HBTU, DIEA; (xv) 20% piperidine/NMP; (xvi) Fmoc-Py acid, HBTU, DIEA; (xvii) 20% piperidine/NMP; (xviii) Im-acid, HBTU, DIEA; (xix) *N*,*N*-dimethylaminopropylamine, 55 °C.

zole polyamide consists of a DCM wash, an NMP wash, removal of the Fmoc group with piperidine/NMP, an NMP wash, a DCM wash, an NMP wash, addition of activated monomer, coupling for 180 min, capping with acetic anhydride, taking a resin sample for analysis, and a final NMP wash.

Because the aromatic amine of the pyrrole and imidazole do not react in the quantitative ninhydrin test, stepwise cleavage of a sample of resin and analysis by HPLC are used to indicate that high stepwise yields (>99%) are routinely achieved. We note that coupling of the imidazole amine with Fmoc-Py-OBt ester was not satisfactory. However, acylation with Boc-Py anhydride ester (synthesized in situ using DCC, DMAP in DCM) proceeds to completion within 3 h.[2]

ImPyPyPy-γ-PyPyPyPy-β-Dp **1** and ImPyPyPy-γ-ImPyPyPy-β-Dp **2** were prepared in 19 steps using the protocols described in the Experimental Section (Figure 4). The yield of each individual coupling step was established as >99% by HPLC analysis. The resin was cleaved in high yield (>90%) by aminolysis with (*N*,*N*-dimethylamino)propylamine. A single HPLC purification of the eight-ring polyamide was sufficient to obtain a recovery of 38% and 9% for **1** and **2**, respectively, and a final purity for both compounds greater than 98% as determined by a combination of analytical HPLC, ^{1}H NMR, and mass spectroscopy.

Pyrrole−imidazole polyamides are a promising class of compounds used to target a predetermined DNA sequences. The synthetic schemes made available by Fmoc-chemistry solid phase synthesis will allow a greater variety of bifunctional conjugates and derivatives to be synthesized.

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Supporting Information Available: Complete experimental details and product characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

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