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Facile Dimer Synthesis for DNA-Binding Polyamide Ligands

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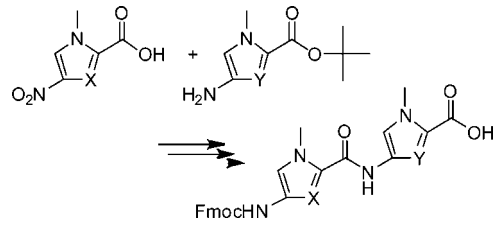
ABSTRACT



Pyrrole-imidazole polyamide ligands are highly sequence specific synthetic DNA-binding ligands that bind with high affinity. To counter the synthetic difficulties associated with coupling the electron-rich heterocyclic acids to the electron-deficient nucleophilic imidazole amine, a novel approach is described for synthesis of Fmoc-protected dimers for solid-phase peptide synthesis (SPPS). This method produces the dimers in high yields, is broadly applicable to other heterocyclic-containing polyamides, and results in improved ligand yields and synthesis times.

Synthetic polyamide ligands containing *N*-methylpyrrole (Py) and *N*-methylimidazole (Im) have affinity and specificity for DNA similar to DNA binding proteins.[1] Over the past decade preparation of these ligands through standard Boc-[2] and Fmoc-based[3] solid-phase peptide synthesis (SPPS) techniques has been described.

Due to the increasing complexity and length of recently reported DNA-binding ligands,[4] faster methods to access these increasingly complicated molecules in fewer steps would save both time and purification effort. Furthermore, the continued use of solution-based synthesis for these ligands in recent years[5−9] demonstrates that significant room remains for improving their synthetic accessibility with use of SPPS.

Both SPPS ligand synthesis[2,3] and solution dimer synthesis for SPPS[2] couple an amino-protected monomer (**1** or **2**) to a resin-bound amine or an amine-bearing monomer (**3** or **4**, Figure 1). However, the imidazole amine **4** is a poor nucleophile, with reported yields as low as 5% for on-resin coupling.[10] Common synthetic approaches include varying the activation chemistry,[3,11,12] heating the resin,[13] or prolonging individual coupling steps to as long as 60 h.[11,13] However, none of these strategies directly address the inherent difficulty of coupling an electron-rich electrophile to an electron-poor nucleophile.

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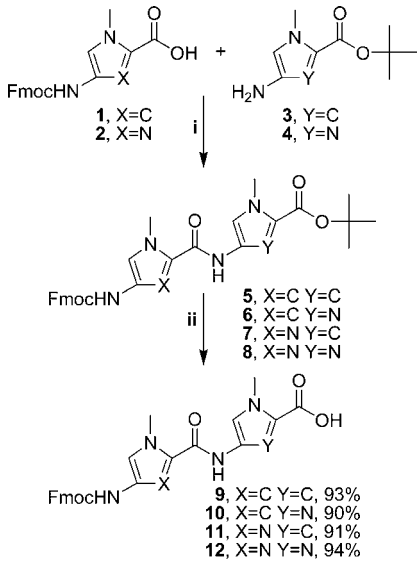

Figure 1. Traditional route for dimer synthesis:[2] (i) HBTU, DIEA, DMF; (ii) $TiCl_4$, CH_2Cl_2.

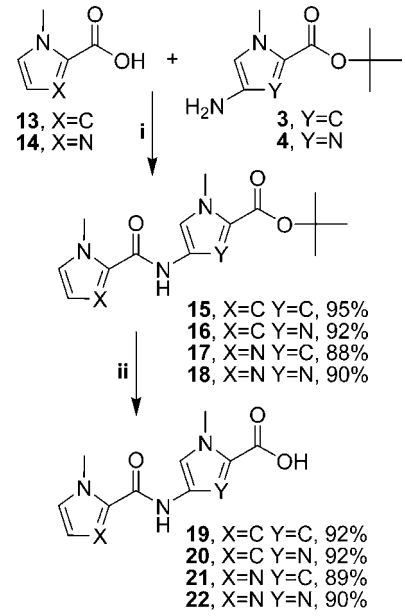

Figure 2. Terminal dimer synthesis: (i) HBTU, DIEA, DMF; (ii) $TiCl_4$, CH_2Cl_2.

To facilitate faster synthesis of polyamide ligands with reduced need for purification, as well as to obviate the difficulties traditionally encountered with on-resin coupling to Im monomers,[2,3,11,12] we report a simple and uniform synthesis of the eight terminal and Fmoc-protected Py and Im dimer combinations, as well as representative syntheses for two previously unpublished polyamide ligands, using these dimers. This work represents the first report of Fmoc-protected Py and Im dimers. Our synthetic approach circumvents the difficulty traditionally associated with SPPS and solution coupling to the imidazole amine, and should be applicable to many of the variant heterocycles reported as possible components of these ligands.[14,15]

Ligand synthesis with dimers requires two dimer sets: amino-protected dimers used in most of the couplings (**9**−**12**), and *N*-terminal dimers lacking an amine (**19**−**22**, Figure 2). To synthesize the terminal dimers, the terminal Py and Im acids **13** and **14**[2] were coupled to the amines **3** or **4** by using standard HBTU coupling protocols in DMF to yield compounds **15**−**18**. The esters **15**−**18** were then deprotected by using $TiCl_4$ in CH_2Cl_2, similar to previously reported deprotections,[3] yielding compounds **19**−**22**.

In synthesizing the nonterminal dimers **9**−**12**, we utilized the highly facile solution coupling to a nitro-derivatized intermediate that dates to the earliest synthetic efforts with this class of molecules.[16] Compounds **23**[3] and **24**[17] (Figure 3) were saponified to **25** and **26**, respectively, which were then coupled to amines **3** and **4** as described for **13** and **14** above, yielding the nitro-dimers **27**−**30**. These dimers were reduced to their corresponding amines with 1000 psi of H_2 and 10% Pd/C in DMF, then protected with Fmoc-chloroformate in situ yielding esters **5**−**8**. Subsequent $TiCl_4$ deprotection of the esters provided the final Fmoc-protected dimers **9**−**12**.

The use of nitro-bearing rings as electron-deficient electrophiles greatly facilitates dimer formation, resulting in couplings that are complete in seconds to minutes by NMR spectroscopy (results not shown), in contrast to incomplete couplings even after 18 h for the coupling of **1** to **4** (Supporting Information, Figure S1). Compared to the previously reported dimer synthetic strategy for Boc-based SPPS,[2] this dimer synthesis saves one synthetic step, improves the atom economy compared to using monomers (by 25% for the Fmoc dimers and 260% for the terminal dimers), and results in significantly higher overall yields for the final dimers (12% overall for **10** versus 2.9% for the comparable Boc-dimer[2]). In addition to the specific compounds reported here, the ease of coupling with this synthetic method should apply to other currently used[14,15] and future heterocycle building blocks for polyamide ligands.

Two new polyamide ligands (Figure 4) were synthesized by using these dimers, with modifications to previously reported Fmoc SPPS procedures.[3] Briefly, dimers and Fmoc-aminobutyric acid were activated with HOBt/HBTU in the presence of DIEA. Coupling times of 60 min were used instead of 3−8 h[3] without capping. The long coupling times (8−60 h) and alternative activation protocols previously employed to facilitate the coupling to the imidazole amine[3,11,12] were unnecessary, and stepwise resin analysis to confirm efficiency of coupling was not performed. Deprotections used 20% 4-methylpiperdine in DMF. Final yields for the polyamide ligands **31** and **32** were 64% and

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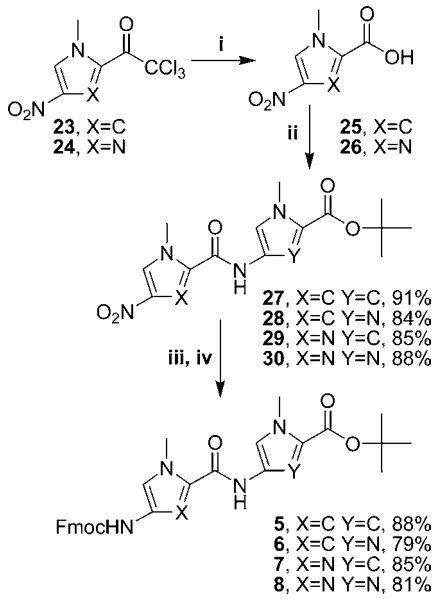

Figure 3. New dimer synthesis route: (i) KOH, 1:1 CH_3OH:H_2O; (ii) **3** or **4**, HBTU, DIEA, DMF; (iii) 1000 psi of H_2, 10% Pd/C, DMF; (iv) Fmoc-Cl, DIEA, DMF.

54%, respectively, after a single HPLC purification step, in contrast with reported yields as low as 9% for similar ligands.[3] The final purity of the ligands **31** and **32** was greater than 97% as confirmed by HPLC, NMR spectroscopy, and HRMS. Notably, ligand **32** represents the most difficult possible ligand to synthesize in terms of the traditional poor coupling unto imidazole amines.

Pyrrole-imidazole-containing polyamides are a versatile class of DNA-binding ligands. The syntheses of dimeric building blocks reported herein solve the difficulties associated with direct imidazole coupling instead of moving them to a different step,[3,11,12] reduce ligand synthesis times by half, may be broadly applicable for synthesis of other heterocyclic polyamides,[14,15] and result in overall ligand yields higher than previously reported,[3] thereby allowing facile synthesis of this useful class of compounds.

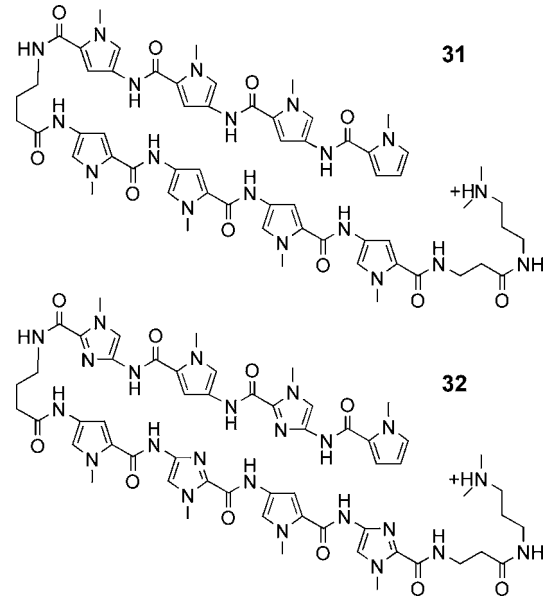

Figure 4. PyPyPyPy-γ-PyPyPyPy-β-Dp (**31**, top) and PyImPyIm-γ-PyImPyIm-β-Dp (**32**, bottom) prepared from dimers with Fmoc SPPS.

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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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