Oligomerization Route to Py-Im Polyamide Macroycles

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Abstract

Cyclic eight-ring pyrrole-imidazole polyamides are sequence-specific DNA-binding small molecules that are cell permeable and can regulate endogenous gene expression. Syntheses of cyclic polyamides have been achieved by solid-phase and solution-phase methods. A rapid solution-phase oligomerization approach to 8-ring symmetrical cyclic polyamides yields 12 and 16 membered macrocycles as well. A preference for DNA binding by the 8 and 16 membered oligomers was observed over the 12-ring macrocycle, which we attributed to a conformational constraint not present in the smaller and larger systems.

Pyrrole-imidazole polyamides are a class of cell-permeable oligomers that target the minor groove of DNA in a sequence specific manner.\textsuperscript{1,2} Antiparallel arrangements of $N$-methylpyrrole (Py) and $N$-methylimidazole (Im) carboxamides (Im/Py) recognize G•C from C•G base pairs, whereas Py/Py specifies for both T•A and A•T.\textsuperscript{3} Hairpin Py-Im polyamides have been programmed for a broad repertoire of DNA sequences with high affinities.\textsuperscript{4} These cell permeable\textsuperscript{5} ligands can influence gene transcription by disrupting protein-DNA interfaces,\textsuperscript{2} and have been shown to control transcription of genes important in human disease.\textsuperscript{6} Py-Im polyamides have also been used for a variety of applications ranging from fluorescence-based DNA detection,\textsuperscript{7} transcriptional activation with artifical transcription factor mimics,\textsuperscript{5e,8} and self-assembly of DNA nano-architectures.\textsuperscript{9}

We recently reported solution-phase methods for the synthesis of hairpin\textsuperscript{10a} and cyclic polyamides.\textsuperscript{11a} Key to the cyclic polyamide synthesis was a macrocyclization from an acyclic precursor that yielded cyclic polyamide 1z (Figure 1). Activation of the C-terminal Py amino acid of 1z as a pentafluorophenyl ester allowed efficient macrocyclization by the γ-NHz on the turn moiety under dilute reaction conditions. Our studies of polyamide 1 revealed it possessed very high DNA binding affinities and could modulate gene expression in cell culture from which we infer 8-ring cycles are cell permeable.\textsuperscript{11a}

Supporting Information Available Compound characterization, Figure S1, Figure S2, and experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.
An orthogonal polymerization/oligomerization strategy for the synthesis of 1 and related polyamides is reported here. This method affords symmetrical Py-Im polyamide macrocycles from simple Py-Im building blocks in a convergent manner (Scheme 1). As serendipitous minor products higher order oligomers such as the 12-membered (2) and 16-membered (3) cyclic polyamides are also produced by this method. In addition to describing the synthetic chemistry to prepare 1–3, we examined the DNA binding properties of such expanded polyamide macrocycles.

Our strategy for this oligomerization route relied on the palindromic nature of polyamide 1. Disconnection of 1 at both γ-amino turns affords two identical halves of the molecule. Bimolecular coupling between two molecules, followed by intramolecular ring closure delivers cyclic Py-Im polyamides. Bifunctional oligomer 4 contains every atom needed to construct cyclic polyamides 1–3 by this process (Scheme 1).

The pentafluorophenyl ester 4 was prepared in one step from the previously reported carboxylic acid of 4. Acidic deprotection of the γ-amino functionality of 4 followed by drying in vacuo yields intermediate 5 which is the substrate for the homodimerization/oligomerization reaction. To initiate this sequence, the protected trifluoroacetate salt 5 was diluted with DMSO, then treated with an organic base (DIEA) to unmask the nucleophilic primary γ-amine. The ensuing oligomerization/macrocyclization process provides benzylcarbamate protected cyclic polyamides 1z, 2z, 3z, and trace amounts of unisolated higher-order oligomers. A distribution of uncyclized intermediates corresponding to the dimer (8-ring cycle, 1z), trimer (12-ring cycle, 2z), tetramer (16-ring cycle, 3z), and higher order adducts can be observed at early time points, as evidenced by HPLC analysis at 2 hr (Figure S1). Extended reaction times (20 hours) reveals cyclized polyamides 1z, 2z, and 3z in a ratio of 6.6:2.6:1 almost exclusively (Figure 2). Isolation of 1z (13.9%), 2z (5.5%), and 3z (2.1%) by preparative HPLC, followed by Cbz-deprotection under acidic conditions (solution of CF3CO2H and CF3SO3H) provides polyamide macrocycles 1–3.

Quantitative DNase I footprint titrations have historically been utilized to measure polyamide-DNA binding affinities and specificities. However, this method is limited to measuring Ka values ≤ 2 × 10^10 M⁻¹, which invalidates this technique for quantifying the exceptionally high DNA-binding affinities of cycles 1 and 3. The magnitude of DNA thermal stabilization (ΔTm) of DNA-polyamide complexes has been utilized to rank order polyamides with high DNA binding affinities and we have employed melting temperature analysis (ΔTm) for evaluating DNA-binding ability in this study. Polyamide 1, which targets the DNA sequence 5′-WGWWCW-3′ (W = A or T) through pairing of two antiparallel ImPyPyPy strands, increases the melting temperature of 14-mer dsDNA containing one binding site by 23.6 °C. With polyamide macrocycles 2 and 3 in hand we evaluated their ability to bind duplex DNA relative to cycle 1. Trimeric macrocycle 2 failed to bind its target double stranded DNA sequence as evidenced by the complete lack of ligand-promoted thermal stabilization of duplex DNA melting (Table 1). This result is presumably due to inherent geometrical constraints of 2, preventing the side-by-side antiparallel alignment of the ImPyPyPy strands, a motif well accomodated by the DNA minor groove. Remarkably, in the case of tetrameric macrocycle 3, dsDNA binding and thermal stabilization was restored to a comparable value to dimer 1. We hypothesize that an even number of ImPyPyPy strands allows 3 to possess a collapsed or folded tetramer geometry, with two adjacent, antiparallel ImPyPyPy strands followed by an idential repeat of this motif linked through two intervening turn units (Fig. S2).

In summary, we have demonstrated that macrocyclic polyamides can be synthesized by oligomerization of a bifunctional polyamide to yield a distribution of cyclic polyamide oligomers. Additionally, we show that certain large cyclic polyamide geometries are able to bind dsDNA, a result which could be utilized in the design of highly specific molecules for...
targeting larger binding site sizes. Due to the size of the 16-ring cycle, the larger series are likely not cell permeable and therefore have limited utility in gene regulation studies. Rather, 16-ring cycles may add to the repertoire of DNA binding motifs for use in DNA nanotechnology.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

### Acknowledgments

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### References


Figure 1.
Structures of macrocyclic polyamides 1z–3z and 1–3, and their ball-and-stick models. Polyamide shorthand code: closed circles, Im monomer; open circles, Py monomer.
Figure 2.
Reverse phase HPLC analysis (20 hr) of the oligomerization reaction revealing products 1z, 2z, and 3z. Peaks were identified by high-resolution mass spectrometry following isolation and Cbz-deprotection.
Scheme 1.
Synthesis of macrocyclic polyamides 1–3 and higher order cycles by oligomerization of bifunctional intermediate 5.
**Table 1**

$T_m$ values for cycles 1–3 in the presence of DNA.

<table>
<thead>
<tr>
<th>dsDNA sequence = $5'$TTGC TGGTC GCG $3'$ $3'$AACG ACAAGA CGT $5'$</th>
<th>polyamide cycle</th>
<th>$T_m/°C$</th>
<th>$\Delta T_m/°C$</th>
</tr>
</thead>
<tbody>
<tr>
<td>--</td>
<td>--</td>
<td>60.0 (±0.3)</td>
<td>--</td>
</tr>
<tr>
<td>$5'$TTGC TGGTC GCG $3'$AACG ACAAGA CGT $5'$</td>
<td>(1)</td>
<td>83.5 (±0.5)</td>
<td>23.6</td>
</tr>
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</table>
dsDNA sequence =

5′-TTGC TGTTCT GCAA
3′-AACG ACAAGA CGTT

polyamide cycle

<table>
<thead>
<tr>
<th>$T_m/°C$</th>
<th>$\Delta T_m/°C$</th>
</tr>
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<tbody>
<tr>
<td>60.1 (±0.6)</td>
<td>0.1 (±0.6)</td>
</tr>
<tr>
<td>83.0 (±0.3)</td>
<td>23.1 (±0.4)</td>
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All values reported are derived from at least three melting temperature experiments with standard deviations indicated in parentheses. $\Delta T_m$ values are given as $T_m$(DNA/polyamide) - $T_m$(DNA). The propagated error in $\Delta T_m$ measurements is the square root of the sum of the square of the standard deviations for the $T_m$ values.