## Oligomerization Route to Py–Im Polyamide Macrocycles

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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 solutionphase oligomerization approach to eight-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.<sup>1,2</sup> 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.<sup>3</sup> Hairpin Py–Im polyamides have been programmed for a broad repertoire of DNA sequences with high affinities.<sup>4</sup> These cell-permeable<sup>5</sup> ligands can influence gene transcription by disrupting protein—DNA interfaces<sup>2</sup> and have been shown to control transcription of genes important in human disease.<sup>6</sup> Py–Im polyamides have also been used for a variety of applications

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such as fluorescence-based DNA detection,<sup>7</sup> transcriptional activation with artifical transcription factor mimics,<sup>5e,8</sup> and self-assembly of DNA nanoarchitectures.<sup>9</sup>

We recently reported solution-phase methods for the synthesis of hairpin<sup>10a</sup> and cyclic polyamides.<sup>11a</sup> 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  $\gamma$ -NH<sub>2</sub> on the turn moiety under dilute reaction conditions. Our studies of polyamide **1** revealed it possessed

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very high DNA binding affinities and could modulate gene expression in cell culture from which we infer eight-ring cycles are cell permeable.<sup>11a</sup>

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

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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.

manner (Scheme 1). As serendipitous minor products, higherorder oligomers such as the 12-membered (2) and 16membered (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  $\gamma$ -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**.<sup>11a</sup> Acidic deprotection of the  $\gamma$ -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  $\gamma$ -amine. The ensuing oligomerization/macrocyclization process provides benzylcarbamate protected cyclic polyamides 1z, 2z, and 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 h (Figure S1, Supporting Information). Extended reaction times (20 h) 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  $CF_3CO_2H$  and  $CF_3SO_3H$ ), provides polyamide macrocycles **1**–**3**.

Scheme 1. Synthesis of Macrocyclic Polyamides 1–3 and Higher-Order Cycles by Oligomerization of Bifunctional Intermediate 5



Quantitative DNase I footprint titrations have historically been utilized to measure polyamide-DNA binding affinities and specificities.<sup>12</sup> However, this method is limited to measuring Ka values  $\leq 2 \times 10^{10} \text{ M}^{-1}$ , which invalidates this technique for quantifying the exceptionally high DNAbinding affinities of cycles 1 and 3. The magnitude of DNA thermal stabilization ( $\Delta T_{\rm m}$ ) of DNA-polyamide complexes has been utilized to rank order polyamides with high DNA binding affinities, <sup>5g,11a</sup> and we have employed melting temperature analysis ( $\Delta T_{\rm m}$ ) 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.<sup>11a</sup> 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 accommodated 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 identical repeat of this motif linked through two intervening turn units (Figure S2, Supporting Information).



Figure 2. Reverse-phase HPLC analysis (20 h) of the oligomerization reaction revealing products 1z, 2z, and 3z. Peaks were identified by high-resolution mass spectrometry following isolation and Cbz-deprotection.

**Table 1.**  $T_{\rm m}$  Values for Cycles 1–3 in the Presence of DNA<sup>*a*</sup>

dsDNA sequence =		5'-TTGC 3'-AACG	TGTTCT ACAAGA	GCAA-3' CGTT-5'
polyamide cycle		$T_{\rm m}/$	°C	$\Delta T_{\rm m}$ / °C
_		60.0 (:	±0.3)	_
*H <sub>3</sub> N <sup>++</sup>	(1)	83.5 (:	±0.5)	23.6 (±0.6)
	(2)	60.1 (:	±0.6)	0.1 (±0.6)
	(3)	83.0 (:	±0.3)	23.1 (±0.4)

<sup>*a*</sup> All values reported are derived from at least three melting temperature experiments with standard deviations indicated in parentheses.  $\Delta T_{\rm m}$  values are given as  $T_{\rm m}$ (DNA/polyamide) –  $T_{\rm m}$ (DNA). The propagated error in  $\Delta T_{\rm m}$  measurements is the square root of the sum of the square of the standard deviations for the  $T_{\rm m}$  values.

In summary, we have demonstrated that macrocyclic polyamides can by 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

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not cell permeable and therefore have limited utility in gene regulation studies. Rather, 16-ring cycles may add to the repetoire of DNA binding motifs for use in DNA nanotechnology.<sup>9</sup>

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**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.

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