Oligomerization Route to Py-Im Polyamide Macrocycles

David M. Chenoweth, **Daniel A. Harki**, and **Peter B. Dervan**[*]
Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125

Abstract

(n = 1, 2, 3, ...)
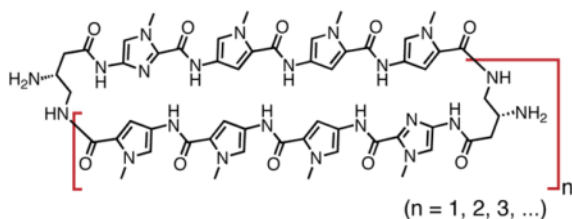
Cyclic eight-ring pyrrole-imidazole polyamides are sequence-specific DNA-binding small molecules that are cell permeable and can regulate endogenous gene expression. Syntheses of cyclic polyamides have been achieved by solid-phase and solution-phase methods. A rapid solution-phase oligomerization approach 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.[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.[3] Hairpin Py-Im polyamides have been programmed for a broad repertoire of DNA sequences with high affinities.[4] These cell permeable[5] ligands can influence gene transcription by disrupting protein-DNA interfaces,[2] and have been shown to control transcription of genes important in human disease.[6] Py-Im polyamides have also been used for a variety of applications ranging from fluorescence-based DNA detection,[7] transcriptional activation with artifical transcription factor mimics,[5e,8] and self-assembly of DNA nano-architectures.[9]

We recently reported solution-phase methods for the synthesis of hairpin[10a] and cyclic polyamides.[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-NH$_2$ 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.[11a]

*dervan@caltech.edu.

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**.[11a] 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 preprative HPLC, followed by Cbz-deprotection under acidic conditions (solution of CF_3CO_2H and CF_3SO_3H) provides polyamide macrocycles **1–3**.

Quantitative DNase I footprint titrations have historically been utilized to measure polyamide-DNA binding affinities and specificities.[12] However, this method is limited to measuring Ka values $\leq 2 \times 10^{10}\,M^{-1}$, 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[5g,11a] 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.[11a] 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 identical repeat of this motif linked through two intervening turn units (Fig. S2).

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 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. 9

Supplementary Material

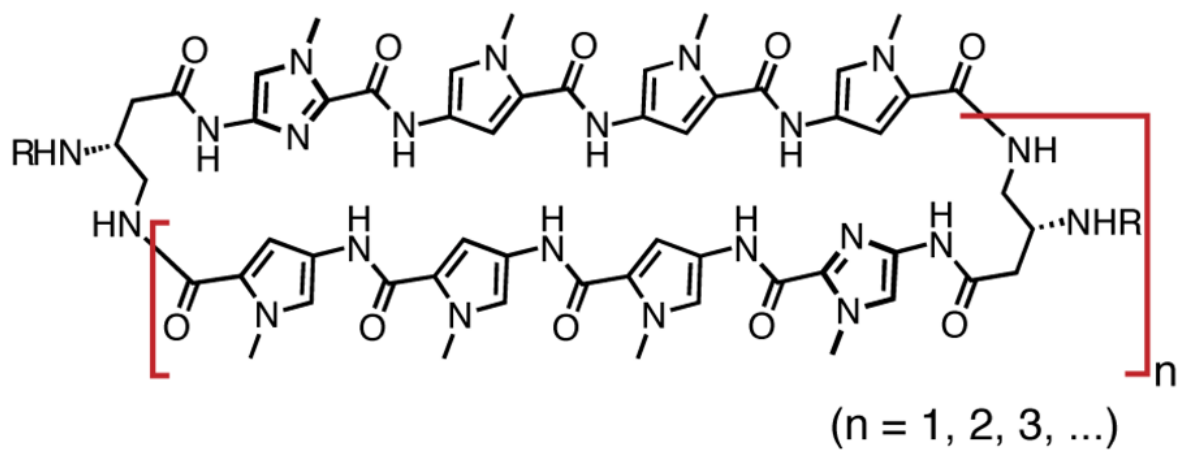Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References

1. Dervan PB. Bioorg Med Chem 2001;9:2215–2235. [PubMed: 11553460]

2. Dervan PB, Edelson BS. Curr Opin Struct Biol 2003;13:284–299. [PubMed: 12831879]

3. (a) Trauger JW, Baird EE, Dervan PB. Nature 1996;382:559–561. [PubMed: 8700233] (b) White S, Szewczyk JW, Turner JM, Baird EE, Dervan PB. Nature 1998;391:468–470. [PubMed: 9461213] (c) Kielkopf CL, Baird EE, Dervan PB, Rees DC. Nat Struct Biol 1998;5:104–109. [PubMed: 9461074] (d) Kielkopf CL, White S, Szewczyk JW, Turner JM, Baird EE, Dervan PB, Rees DC. Science 1998;282:111–115. [PubMed: 9756473]

4. Hsu CF, Phillips JW, Trauger JW, Farkas ME, Belitsky JM, Heckel A, Olenyuk BZ, Puckett JW, Wang CC, Dervan PB. Tetrahedron 2007;63:6146–6151. [PubMed: 18596841]

5. (a) Belitsky JM, Leslie SJ, Arora PS, Beerman TA, Dervan PB. Bioorg Med Chem 2002;10:3313–3318. [PubMed: 12150878] (b) Crowley KS, Phillion DP, Woodard SS, Scheitzer BA, Singh M, Shabany H, Burnette B, Hippenmeyer P, Heitmeier M, Bashkin JK. Bioorg Med Chem Lett 2003;13:1565–1570. [PubMed: 12699756] (c) Best TP, Edelson BS, Nickols NG, Dervan PB. Proc Natl Acad Sci U S A 2003;100:12063–12068. [PubMed: 14519850] (d) Edelson BS, Best TP, Olenyuk B, Nickols NG, Doss RM, Foister S, Heckel A, Dervan PB. Nucleic Acids Res 2004;32:2802–2818. [PubMed: 15155849] (e) Xiao X, Yu P, Lim HS, Sikder D, Kodadek T. Angew Chem Int Ed Engl 2007;46:2865–2868. [PubMed: 17351994] (f) Nickols NG, Jacobs CS, Farkas ME, Dervan PB. Nucleic Acids Res 2007;35:363–370. [PubMed: 17175539] (g) Dose C, Farkas ME, Chenoweth DM, Dervan PB. J Am Chem Soc 2008;130:6859–6866. [PubMed: 18459783] (h) Hsu CF, Dervan PB. Bioorg Med Chem Lett 2008;18:5851–5855. [PubMed: 18524590]

6. (a) Olenyuk BZ, Zhang GJ, Klco JM, Nickols NG, Kaelin WG, Dervan PB. Proc Natl Acad Sci U S A 2004;101:16768–16773. [PubMed: 15556999] (b) Kageyama Y, Sugiyama H, Ayame H, Iwai A, Fujii Y, Huang LE, Kizaka-Kondoh S, Hiraoka M, Kihara K. Acta Oncol 2006;45:317–324. [PubMed: 16644575] (c) Nickols NG, Jacobs CS, Farkas ME, Dervan PB. ACS Chem Biol 2007;2:561–571. [PubMed: 17708671] (d) Nickols NG, Dervan PB. Proc Natl Acad Sci U S A 2007;104:10418–10423. [PubMed: 17566103] (e) Matsuda H, Fukuda N, Ueno T, Tahira Y, Ayame H, Zhang W, Bando T, Sugiyama H, Saito S, Matsumoto K, et al. J Am Soc Neph 2006;17:422–432. (f) Yao EH, Fukuda N, Ueno T, Matsuda H, Matsumoto K, Nagase H, Matsumoto Y, Takasaka A, Serie K, Sugiyama H, Sawamura T. Hypertension 2008;52:86–92. [PubMed: 18519843] (g) Takahashi T, Asami Y, Kitamura E, Suzuki T, Wang X, Igarashi J, Morohashi A, Shinojima Y, Kanou H, Saito K, Takasu T, Nagase H, Harada Y, Kuroda K, Watanabe T, Kumamoto S, Aoyama T, Matsumoto Y, Bando T, Sugiyama H, Yoshida-Noro C, Fukuda N, Hayashi N. Chem Biol 2008;15:829–841. [PubMed: 18721754] (h) Hochhauser D, Kotecha M, O'hare C, Morris PJ, Hartley JM, Taherbhai Z, Harris D, Forni C, Mantovani R, Lee M, Hartley JA. Mol Cancer Ther 2007;6:346–354. [PubMed: 17237293]

7. (a) Rucker VC, Foister S, Melander C, Dervan PB. J Am Chem Soc 2003;125:1195–1202. [PubMed: 12553822] (b) Chenoweth DM, Viger A, Dervan PB. J Am Chem Soc 2007;129:2216–2217. [PubMed: 17279754] (c) Fujimoto J, Bando T, Minoshima M, Kashiwazaki G, Nishijima S, Shinohara K, Sugiyama H. Bioorg Med Chem 2008;16:9741–9744. [PubMed: 18930403]

8. (a) Arndt HD, Hauschild KE, Sullivan DP, Lake K, Dervan PB, Ansari AZ. J Am Chem Soc 2003;125:13322–13323. [PubMed: 14583004] (b) Kwonj Y, Arndt HD, Qian M, Choi Y, Kawazoe Y, Dervan PB, Uesugi M. J Am Chem Soc 2004;126:15940–15941. [PubMed: 15584709] (c) Hauschild KE, Metzler RE, Arndt HD, Moretti R, Raffaelle M, Dervan PB, Ansari AZ. Proc Natl Acad Sci U S A 2005;102:5008–5013. [PubMed: 15781856] (d) Stafford RL, Arndt HD, Brezinski ML, Ansari AZ, Dervan PB. J Am Chem Soc 2007;129:2660–2668. [PubMed: 17290996] (e) Stafford RL, Dervan PB. J Am Chem Soc 2007;129:14026–14033. [PubMed: 17949089]

9. (a) Cohen JD, Sadowski JP, Dervan PB. Angew Chem Int Ed 2007;46:7956–7959. (b) Schmidt TL, Nandi CK, Rasched G, Parui PP, Brutschy B, Famulok M, Heckel A. Angew Chem Int Ed 2007;46:4382–4384. (c) Cohen JD, Sadowski JP, Dervan PB. J Am Chem Soc 2008;130:402–403. [PubMed: 18081289]

10. (a)Chenoweth DM, Harki DA, Dervan PB. J Am Chem Soc 2009;131:7175–7181.7181 [PubMed: 19413320]For additional solution-phase polyamide synthesis studies see: (b)Mrksich M, Parks ME, Dervan PB. J Am Chem Soc 1994;116:7983–7988.7988(c)Xiao J, Yuan G, Huang W, Chan AS, Lee KL. J Org Chem 2000;65:5506–5513.5513 [PubMed: 10970288](d)Harris D, Stewart M, Sielaff A, Mulder K, Brown T, Mackay H, Lee M. Heterocycl Commun 2007;13:17–23.23(e)Xiao JH, Huang WQ, Tang FL, Yuan G, Chan ASC, Lee KLD. Chin J Chem 2000;18:603–607.607(f)Boger DL, Fink BE, Hedrick MP. J Am Chem Soc 2000;122:6382–6394.6394(g)Mamidyala SK, Firestine SM. Tetrahedron Lett 2006;47:7431–7434.7434(h)Sinyakov AN, Feshchenko MV, Ryabinin VA. Russian J Bioorg Chem 2004;30:98–99.99(i)Yuan G, Tang F. ARKIVOC 2003;ii:32–37.37(j)Xiao JH, Yuan G, Huang WQ, Tang FL, Chan ASC, Lee KLD. Chinese Chem Lett 2000;11:207–208.208For additional solid-phase hairpin polyamide synthesis studies see: (k)Baird EE, Dervan PB. J Am Chem Soc 1996;118:6141–6146.6146(l)Belitsky JM, Nguyen DH, Wurtz NR, Dervan PB. Bioorg Med Chem 2002;10:2767–2774.2774 [PubMed: 12057666](m)Wurtz NR, Turner JM, Baird EE, Dervan PB. Org Lett 2001;3:1201–1203.1203 [PubMed: 11348194](n)Krutzik PO, Chamberlin AR. Bioorg Med Chem Lett 2002;12:2129–2132.2132 [PubMed: 12127520](o)Krutzik PO, Chamberlin AR. Methods Mol Biol 2002;201:77–92.92 [PubMed: 12357940](p)Ayame H, Saito T, Bando T, Fukuda N, Sugiyama H. Nucleic Acids Res Suppl 2003:67–68.68 [PubMed: 14510383](q)Moore MJB, Cuenca F, Searcey M, Neidle S. Org Biomol Chem 2006;4:3479–3488.3488 [PubMed: 17036143]

11. (a)Chenoweth DM, Harki DA, Phillips JW, Dose C, Dervan PB. J Am Chem Soc 2009;131:7182–7188.7188 [PubMed: 19413319]For additional solid-phase cyclic polyamide synthesis studies see: (b)Cho J, Parks ME, Dervan PB. Proc Natl Acad Sci USA 1995;92:10389–10392.10392 [PubMed: 7479790](c)Zhang Q, Dwyer TJ, Tsui V, Case DA, Cho J, Dervan PB, Wemmer DE. J Am Chem Soc 2004;126:7958–7966.7966 [PubMed: 15212545](d)Herman DM, Turner JM, Baird EE, Dervan PB. J Am Chem Soc 1999;121:1121–1129.1129(e)Melander C, Herman DM, Dervan PB. Chem Eur J 2000;6:4487–4497.4497

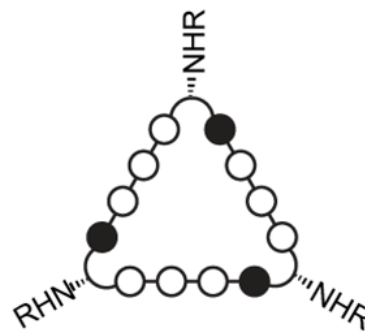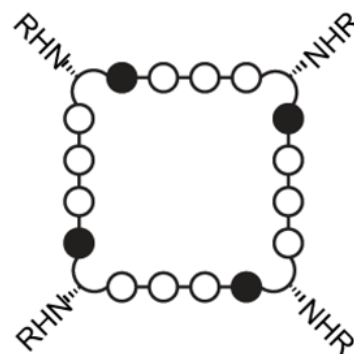12. Trauger JW, Dervan PB. Methods Enzymol 2001;340:450–466. [PubMed: 11494863]

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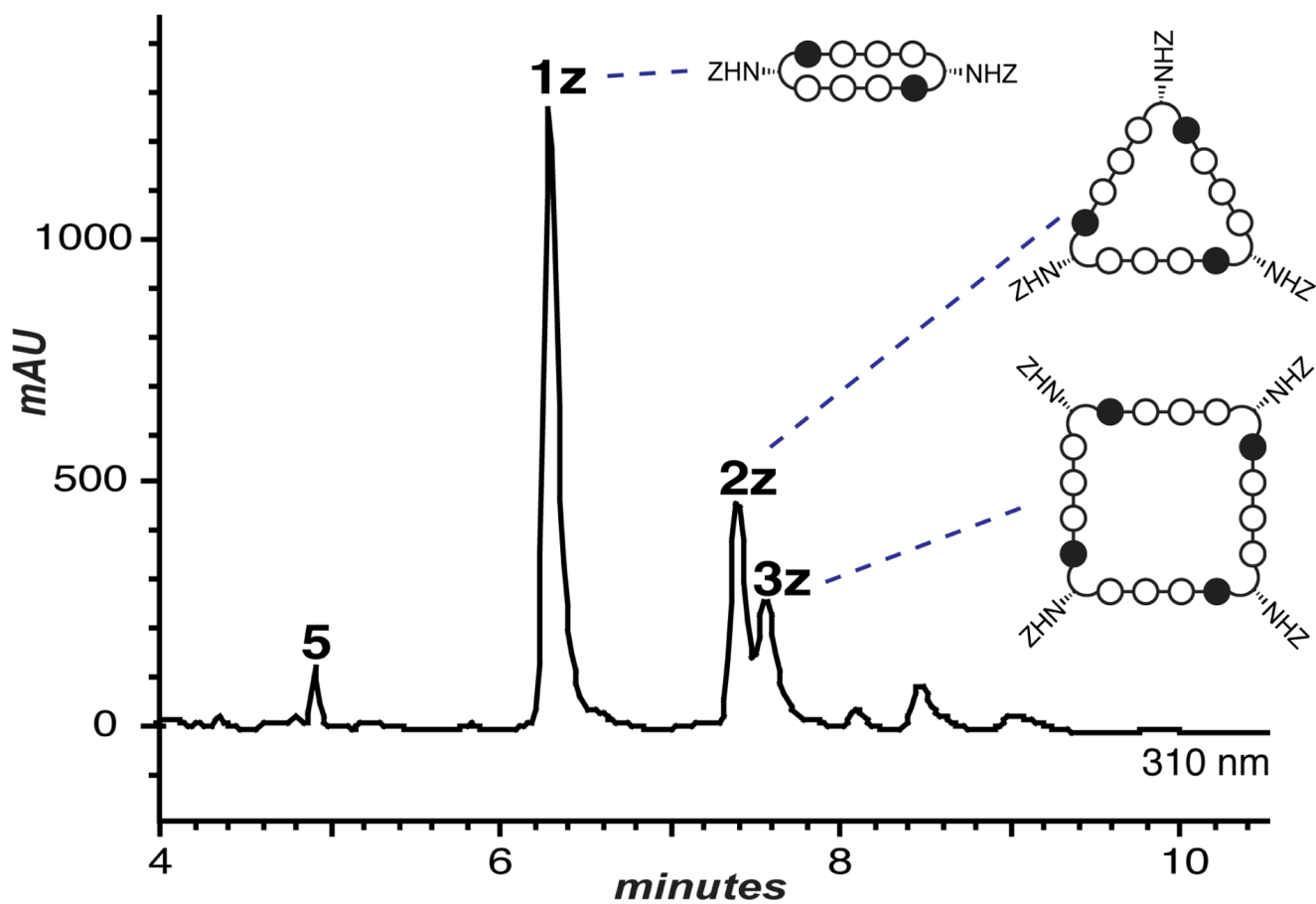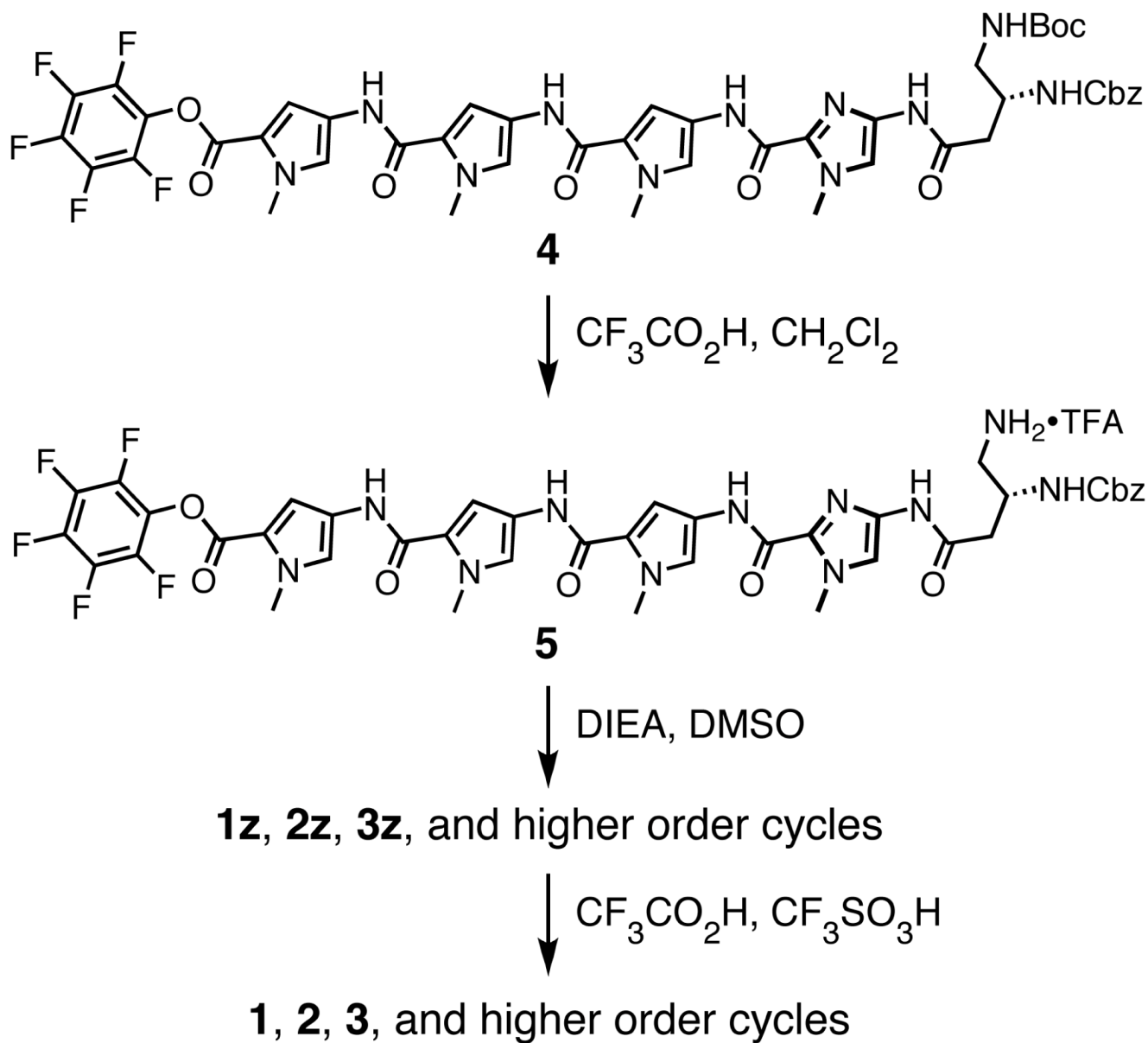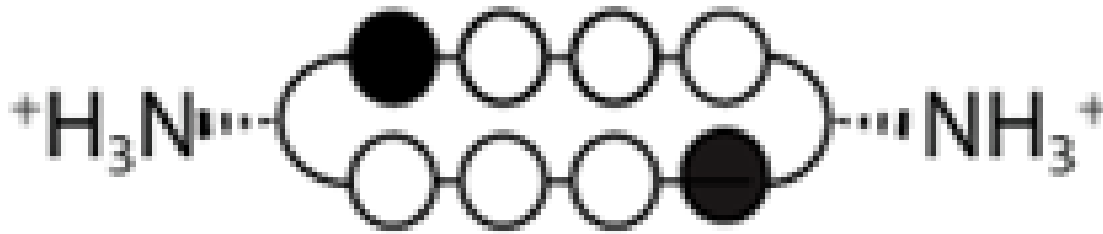
Figure 2.
Reverse phase HPLC analysis (20 hr) of the oligomerization reaction revealing products **1z**, **2z**, and **3z**. Peaks were identified by high-resolution mass spectrometry following isolation and Cbz-deprotection.

Scheme 1.
Synthesis of macrocyclic polyamides **1–3** and higher order cycles by oligomerization of bifunctional intermediate **5**.

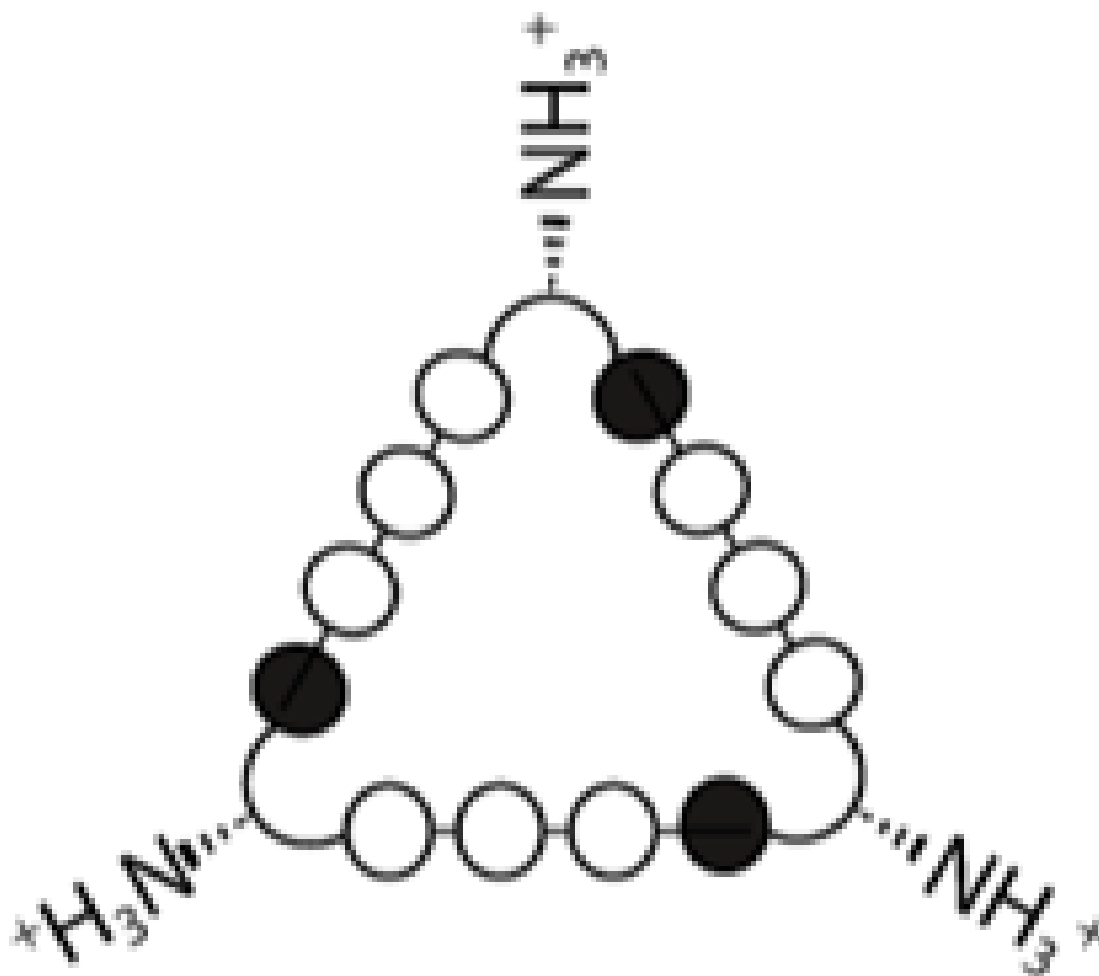**Table 1**

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

dsDNA sequence =	5′–TTGC TGTTCT GC... 3′–AACG ACAAGA CG... 5′	
polyamide cycle	$T_m/°C$	ΔT
—	60.0 (±0.3)	

(**1**) — 83.5 (±0.5) — 23.6

dsDNA sequence =	5′–TTGC TGTTCT GC... 3′–AACG ACAAGA CC... 5′	
polyamide cycle	$T_m/°C$	ΔT
 (**2**)	60.1 (±0.6)	0.1
 (**3**)	83.0 (±0.3)	23.1
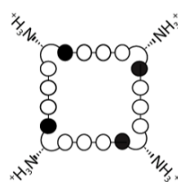
All values reported are derived from at least three melting temperature experiments with standard deviations indicated in parentheses. ΔT_m values are given as T_m(DNA/polyamide) - T_m(DNA). The propagated error in ΔT_m measurements is the square root of the sum of the square of the standard deviations for the T_m values.