SUPPORTING INFORMATION

Oligomerization Route to Py-Im Polyamide Macrocycles

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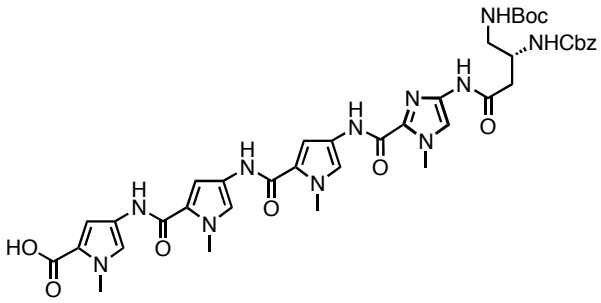
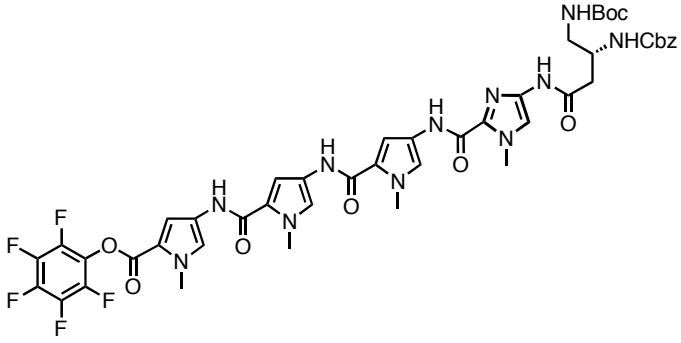
General. Chemicals were purchased from Sigma-Aldrich and were used without further purification. (*R*)-3,4-Cbz-Dbu(Boc)-OH was purchased from Senn Chemicals AG (code number 44159). Bulk grade solvents were from Fisher Scientific. Analytical HPLC analysis was conducted on a Beckman Gold instrument equipped with a Phenomenex Gemini analytical column (250 × 4.6 mm, 5 μm), a diode array detector, and the mobile phase consisted of a gradient of acetonitrile (MeCN) in 0.1% (v/v) aqueous CF_3CO_2H. Preparative HPLC was performed on an Agilent 1200 system equipped with a solvent degasser, diode array detector, and a Phenomenex Gemini column (250 × 21.2 mm, 5 μm). A gradient of MeCN in 0.1% (v/v) aqueous CF_3CO_2H was utilized as the mobile phase. NMR spectroscopy was performed on a Varian instrument operating at 499.8 MHz (for ^1H) at ambient temperature. All NMR analyses were performed in DMSO-d_6, and chemical shifts are reported in parts per million relative to the internal solvent peak referenced to 2.49 (for ^1H). High-resolution mass spectrometry (HRMS) was recorded in positive-ion mode by fast-atom bombardment (FAB^+) on a JOEL JMS-600H instrument or by matrix-assisted, LASER desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) on an Applied Biosystems Voyager DE-Pro spectrometer using α-cyano-4-hydroxycinnamic acid as matrix.



BocHN-(*R*)$^{\beta\text{-CbzHN}}$γ-ImPyPyPy-CO_2H. Synthesized as previously described.[1]



BocHN-(*R*)$^{\beta\text{-CbzHN}}$γ-ImPyPyPy-CO_2Pfp (4). A solution of BocHN-(*R*)$^{\beta\text{-CbzHN}}$γ-ImPyPyPy-CO_2H (100 mg, 0.119 mmol) and DCC (49 mg, 0.238 mmol) in CH_2Cl_2 (5.2 mL) was stirred at 23 ºC for 45 min. The solution was then treated with DMAP (1.4 mg, 0.012 mmol) followed by pentafluorophenol (131.2 mg, 0.713 mmol) and stirred at 23 ºC for 12 h. The reaction mixture was then loaded onto a silica gel column with CH_2Cl_2 and eluted with step gradients of 100%

CH_2Cl_2 to 100% acetone with incremental steps of 5% acetone. The product was concentrated *in vacuo* to yield BocHN-$(R)^{\beta\text{-CbzHN}}\gamma$-ImPyPyPy-$CO_2$Pfp **4** as an off-white solid (84 mg, 71%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.16 (s, 1H), 10.08 (s, 1H), 9.99 (s, 1H), 9.97 (s, 1H), 7.74 (d, J = 1.8 Hz, 1H), 7.44 (s, 1H), 7.33 – 7.30 (m, 5H), 7.29 (d, J = 1.9 Hz, 1H), 7.27 (d, J = 1.8 Hz, 1H), 7.25 (d, J = 1.8 Hz, 1H), 7.14 (d, J = 1.8 Hz, 1H), 7.13 (d, J = 1.8 Hz, 1H), 7.03 (d, J = 8.4 Hz, 1H), 6.80 (t, J = 5.8 Hz, 1H), 4.98 (s, 2H), 3.95 (m, 4H), 3.89 (s, 3H), 3.860 (s, 3H), 3.856 (s, 3H), 3.03 (m, 2H), 2.46 (m, 2H), 1.36 (s, 9H). HRMS (FAB$^+$) calc′d for $C_{46}H_{47}N_{11}O_{10}F_5$ $[M+H]^+$ 1008.343, found 1008.342.

Oligomerization procedure. A glass vial (1 dram) was charged with **4** (5.0 mg, 4.96 μmole) and treated with a solution of CF_3CO_2H in CH_2Cl_2 (1:1 CF_3CO_2H:CH_2Cl_2, 1 mL) and stirred at 23 °C for 10 min. The solvent was removed *in vacuo* and the residual solid was dried under high vacuum for 20 min. The solid was diluted with DMSO (500 μL) followed by DIEA (80 μL) and the solution was stirred at 23 °C for 20 hr. After 20 hr the reaction was complete by analytical HPLC analysis. The reaction was diluted to a final volume of 10 mL by addition of a solution of DMF in aqueous CF_3CO_2H (2:3 DMF:0.1% aqueous CF_3CO_2H). NOTE: A small amount of yellow insoluble material was observed and discarded. Purification by RP-HPLC (a gradient of 20% to 100% MeCN in 0.1% (v/v) aqueous CF_3CO_2H was utilized as the mobile phase) yielded **1z** (13.9 % yield), **2z** (5.5 % yield), and **3z** (2.1 % yield). The yield of **1z** is calculated from the mass of the purified and isolated material (0.5 mg). Yields for **2z** and **3z** were calculated based on **1z** using the relative product distribution as measured by integration of the preparative HPLC chromatogram at 310 nm (product distribution: 6.6:2.6:1.0 ratio of **1z**:**2z**:**3z**; UV integral values were normalized to the number of ImPyPyPy strands contained in each cyclic oligomer). The benzyl carbamate (Cbz) protecting groups of **1z**-**3z** were removed as previously described[2] and compounds **1**, **2**, and **3** were purified as described for compound **1**.[2] Characterization data for dimer **1** has been reported previously.[1] Dimer **1** ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.56 (s, 2H), 9.91 (s, 4H), 9.88 (s, 2H), 8.17 (t, J = 5.6 Hz, 2H), 7.96 (m, 6H), 7.40 (s, 2H), 7.31 (d, J = 1.6 Hz, 2H), 7.27 (d, J = 1.6 Hz, 2H), 7.19 (d, J = 1.6 Hz, 2H), 7.00 (d, J = 1.7 Hz, 2H), 6.96 (d, J = 1.6 Hz, 2H), 6.94 (d, J = 1.7 Hz, 2H), 3.94 (s, 6H), 3.83 (s, 12H), 3.80 (s, 6H), 3.71-3.66 (m, 2H), 3.49-3.27 (m, 4H, partially obstructed by H_2O peak), 2.79 (dd, J = 16.1 Hz, 6.0 Hz, 2H), 2.60 (dd, J = 15.2 Hz, 5.2 Hz, 2H); UV-vis (H_2O) λmax (ε): 312 nm (55540). Trimer **2** HRMS (MALDI-TOF) calc′d for $C_{81}H_{94}N_{33}O_{15}$ $[M+H]^+$ 1768.7607, found 1768.7566; UV-vis (H_2O) λmax (ε): 312 nm (83310). Tetramer **3** HRMS (MALDI-TOF) calc′d for $C_{108}H_{125}N_{44}O_{20}$ $[M+H]^+$ 2358.0112, found 2358.0143; UV-vis (H_2O) λmax (ε): 312 nm (111080).

UV Absorption Spectrophotometry. Melting temperature analysis was performed on a Varian Cary 100 spectrophotometer equipped with a thermo-controlled cell holder possessing a cell path length of 1 cm. A degassed aqueous solution of 10 mM sodium cacodylate, 10 mM KCl, 10 mM $MgCl_2$, and 5 mM $CaCl_2$ at pH 7.0 was used as analysis buffer. DNA duplexes and hairpin polyamides were mixed in 1:1 stoichiometry to a final concentration of 2 μM for each experiment. Prior to analysis, samples were heated to 90 °C and cooled to a starting temperature of 25 °C with a heating rate of 5 °C/min for each ramp. Denaturation profiles were recorded at λ = 260 nm from 25 °C to 90 °C with a heating rate of 0.5 °C/min. The reported melting temperatures were defined as the maximum of the first derivative of the denaturation profile.

References

(1) Chenoweth, D. M.; Harki, D. A.; Phillips, J. W.; Dose, C.; Dervan, P. B. *J. Am. Chem. Soc.* **2009**, *131*, 7182-7188.
(2) Dose, C.; Farkas, M. E.; Chenoweth, D. M.; Dervan, P. B. *J. Am. Chem. Soc.* **2008,** *130*, 6859-6866.

Solvent	dmso
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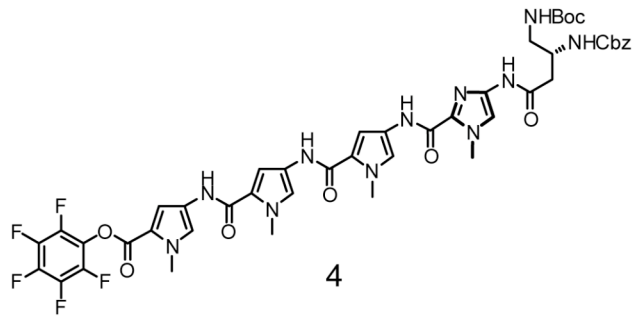
Temperature	25.0
Number of Scans	64
Spectrometer Frequency	499.76
Nucleus	1H



4

exp3 Proton

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rsys/data/auto_200~		hst	0.008
9.01.19/3201.fid		pw90	9.900
ACQUISITION		alfa	6.600
sw	8000.0	**FLAGS**	
at	2.048	il	n
np	32762	in	n
fb	not used	dp	y
bs	32	hs	nn
ss	2	**PROCESSING**	
d1	1.000	lb	0.20
nt	800	fn	65536
ct	800	**DISPLAY**	
TRANSMITTER		sp	-8.0
tn	H1	wp	6008.8
sfrq	499.772	rfl	2248.0
tof	499.6	rfp	1244.4
tpwr	61	rp	142.2
pw	4.950	lp	-0.2
DECOUPLER		**PLOT**	
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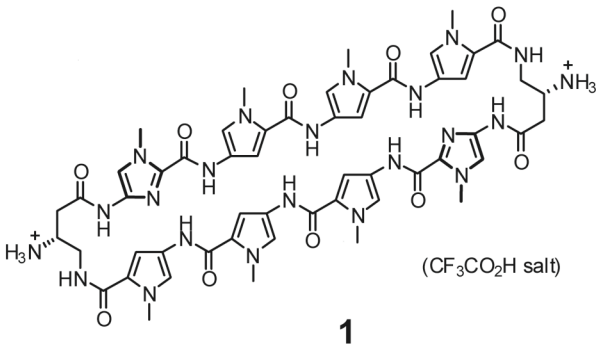
dmm	c	th	1
dpwr	35	ai cdc ph	
dmf	32258		



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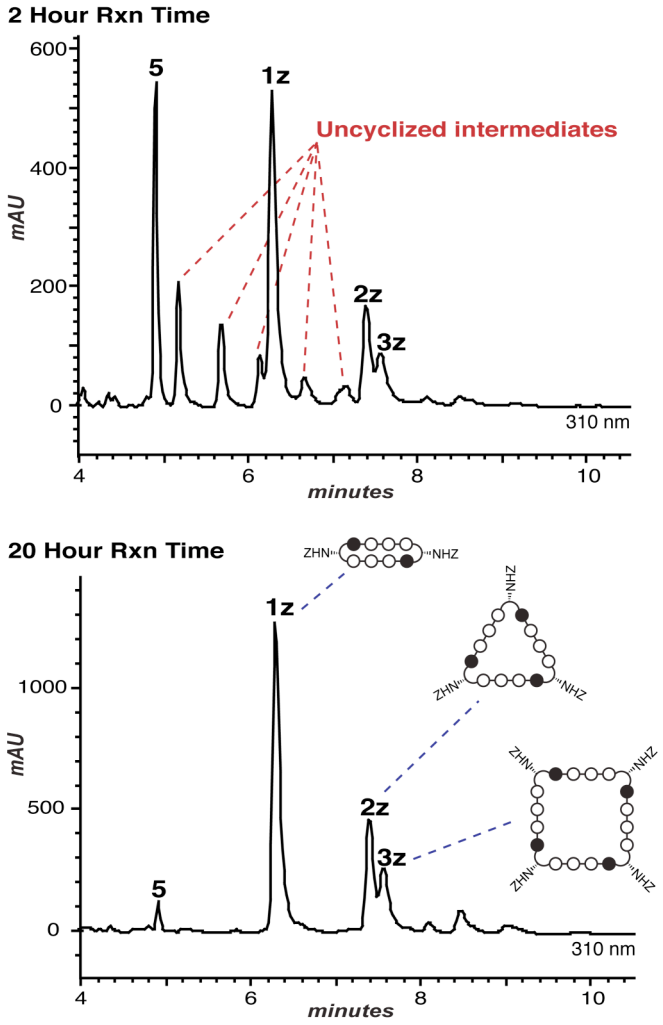
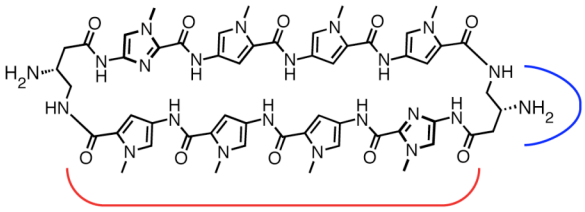


Figure S1. (Top) Reverse phase HPLC analysis (2 hr) of the oligomerization reaction showing starting material **5**, macrocycle products **1z**, **2z**, **3z**, and reaction intermediates. (Bottom) Analysis (20 hr) of the oligomerization reaction revealing products **1z**, **2z**, **3z**, and the consumption of starting material **5** and reaction intermediates. Peaks were identified by high-resolution mass spectrometry following isolation and Cbz-deprotection.



Each turn unit allows for conformational mobility and folding of the two amide linked heterocycle strands as in the case of the macrocycle dimer **1** and macrocyclic tetramer **3**.

Each strand of amide linked heterocycles imposes a conformational constraint with a distance of ~20 Å as observed in Xray structures of 2:1 binding polyamides. (See reference 3d in paper, PDB ID 365D)

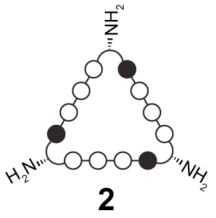
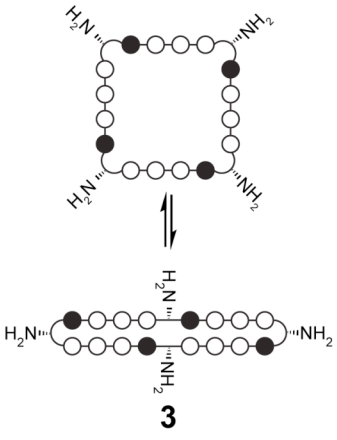
Compound	DNA Binding Model	Polyamide Conformation
 **1**	5′ – W G W W C W – 3′ 3′ – W C W W G W – 5′	The collapse or side-by-side alignment of two adjacent antiparallel ImPyPyPy stands allows binding of **1** in the minor groove of DNA.
 **2**	No DNA binding observed by thermal melting temperature analysis.	Introduction of a third ImPyPyPy stand acts as a conformational constraint preventing the collapse or side-by-side alignment of two adjacent antiparallel ImPyPyPy stands as in compound **1**.
 **3**	5′ – W G W W C N^X G W W C W – 3′ 3′ – W C W W G N^X C W W G W – 5′ N = any base pair (A, T, G, or C) and X = the number of base pairs between the two binding sites. (W = A or T)	Collapsed or folded tetramer geometry with two adjacent antiparallel ImPyPyPy stands followed by an identical repeat of this motif linked through two intervening turn units.

Figure S2. (Top) Anatomy of a polyamide macrocycle showing constrained heterocyclic strands and flexible linker regions. (Bottom) Proposed DNA binding models and macrocycle conformation for polyamides **1**, **2**, and **3**.