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Solution-Phase Synthesis of Pyrrole−Imidazole Polyamides

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Abstract: Pyrrole−imidazole polyamides are DNA-binding molecules that are programmable for a large repertoire of DNA sequences. Typical syntheses of this class of heterocyclic oligomers rely on solid-phase methods. Solid-phase methodologies offer rapid assembly on a micromole scale sufficient for biophysical characterizations and cell culture studies. In order to produce gram-scale quantities necessary for efficacy studies in animals, polyamides must be readily synthesized in solution. An 8-ring hairpin polyamide **1**, which targets the DNA sequence 5′-WGWWCW-3′, was chosen for our synthesis studies as this oligomer exhibits androgen receptor antagonism in cell culture models of prostate cancer. A convergent solution-phase synthesis of **1** from a small set of commercially available building blocks is presented which highlights principles for preparing gram quantities of pyrrole−imidazole oligomers with minimal chromatography.

Introduction

Pyrrole−imidazole polyamides are a class of small molecules that bind the minor groove of DNA sequence-specifically.[1,2] Encoded by side-by-side arrangements of *N*-methylpyrrole (Py) and *N*-methylimidazole (Im) carboxamide monomers, Im/Py pairs distinguish G·C from C·G base pairs, whereas Py/Py pairs are degenerate for T·A and A·T.[3] Hairpin Py-Im polyamides have been shown to bind a broad repertoire of DNA sequences,[4] permeate cell membranes and traffic to the nucleus,[5] access chromatin,[6] and disrupt protein−DNA interfaces.[2] Hairpin polyamide inhibition of transcription factor−DNA binding of HIF-1α,[7] androgen receptor (AR),[8] and AP-1[9] has been exploited for controlling expression of medically relevant genes such as VEGF, PSA, TGF-β1, and LOX-1 in cell culture experiments.

An underpinning to transition polyamide studies from cell culture to small animal disease models is the ability to synthesize Py-Im polyamides on gram-scale. Over the years advances in polyamide solid-phase synthesis have been reported, including Boc- and Fmoc-based approaches from our laboratories and others.[10] Solid-phase methodologies offer many advantages for milligram-scale polyamide syntheses, including rapid and reliable amino acid couplings and facile purifications owing to immobilization of the polyamide oligomer on a solid support. However, these techniques intrinsically limit the scale of synthesis. Conversely, efficient gram-scale solution-phase meth-

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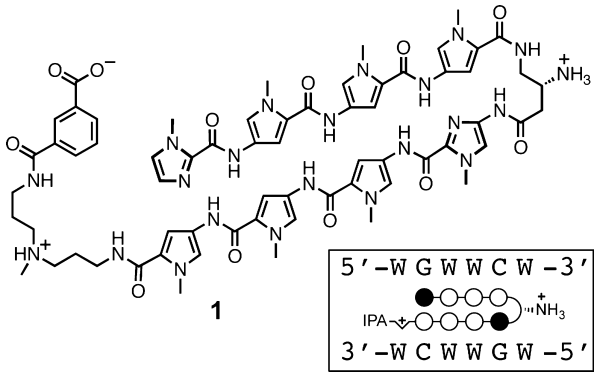

Figure 1. Structure of Py-Im hairpin polyamide **1** targeting the DNA sequence 5′-WGWWCW-3′ and its ball-and-stick representation.

ods for polyamide synthesis that avoid arduous chromatographic purifications and employ commercially available Py-Im amino acid building blocks as reagents are less well developed. Remarkably, solution-phase synthesis of hairpin polyamides was the standard in our laboratory prior to the development of solid-phase methodologies,[11a] and many variations on this theme have been published.[11] However, laborious chromatographic purifications and modest reaction yields are commonplace. Therefore, we sought to develop a general solution-phase polyamide synthesis method that would allow access to gram quantities of material in high yield with minimal chromatography.

We report a proof-of-principle study demonstrating that hairpin Py-Im polyamides can be synthesized in solution from a small set of building blocks on large scale with minimal use of chromatography. This method involves Boc-protected dimers, trimers, and tetramers of heterocycles suitable for convergent syntheses. By exploiting differences in the physical solubility properties of starting materials versus products, a solution-phase synthesis of an 8-ring hairpin Py-Im polyamide **1** (Figure 1) has been achieved. Notably, our synthesis permits core polyamide **2** (Figure 2) to be prepared without a single chromatographic purification, thereby providing large quantities of **2** for subsequent modification at the C-terminus such as **1**. Py-Im polyamide **1**, which targets the DNA sequence 5′-WGWWCW-3′, was selected for our studies because it antagonizes AR binding to androgen response elements (ARE) in gene promoters and regulates a subset of AR-driven genes, such as PSA.[5g] The regulation of aberrant AR-activated gene expression in prostate cancer is a promising strategy for developing novel therapeutics.[8] This biological activity, coupled with our desire to conduct small animal efficacy experiments with **1**, renders this polyamide an ideal candidate for scale-up and optimization studies. In addition, we discuss unifying principles for planning solution-phase polyamide syntheses of different Py-Im arrangements.

Results and Discussion

Our retrosynthetic approach for the preparation of an 8-ring hairpin polyamide, ImPyPyPy-(*R*)$^{\beta\text{-}H_2N}\gamma$-ImPyPyPy-(+)-IPA (**1**), is shown in Figure 2. Sequential couplings of ImPyPyPy tetramer **3** to turn moiety **5**, followed by ester saponification and coupling to PyPyPy trimer **4**, afford polyamide **2** in a convergent manner. Advanced intermediates **3**−**5** were prepared from building blocks **6**−**10**,[12] which have been previously synthesized by our laboratory and others.[10a,c,13] The cornerstone of our synthesis strategy capitalizes on the disparate physical properties of starting materials versus products, which permit purification of each intermediate to be achieved by combinations of precipitation, trituration, and crystallization. Such details are described below.

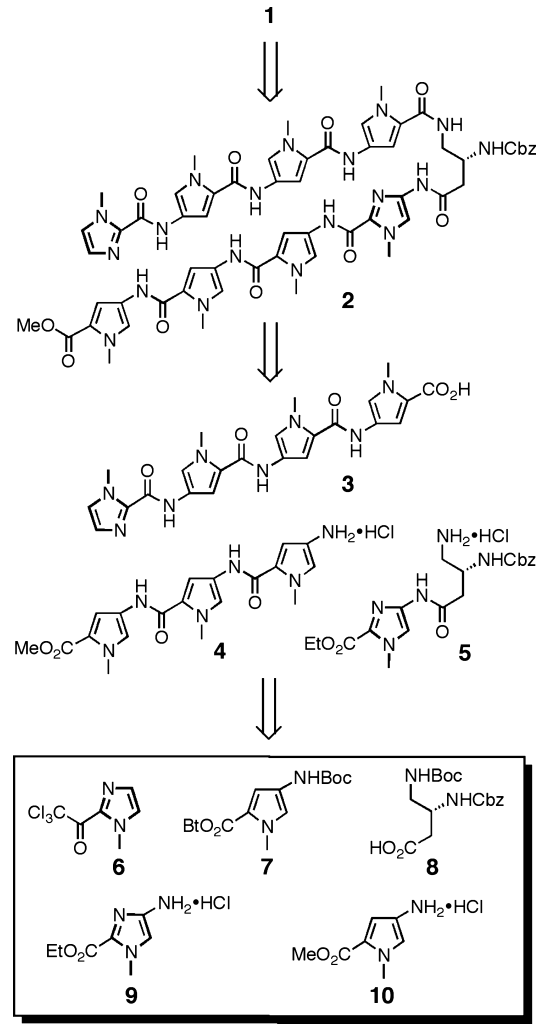

Figure 2. Retrosynthetic strategy for the convergent solution-phase synthesis of polyamide **1**. **7**−**10** are commercially available building blocks. Boc = *tert*-butyl carbamate, Bt = benzotriazole, Cbz = benzyl carbamate.

The synthesis of pyrrole trimer **4** begins with pyrrole amine salt **10** (Scheme 1). Amide coupling of **10** with activated pyrrole monomer **7** affords dimer **11** in 93% yield. The utilization of a small excess of **10** relative to **7** drives the reaction to completion, and residual **10** is readily separated from **11** following precipitation in water and aqueous washing of the residual solid **11**. Reaction of dimer **11** with anhydrous HCl in diethyl ether removes the carbamate protecting group and facilitates precipi-

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(12) Sources for commercially available building blocks **7**−**10** shown in Figure 2: **7**, Fluorochem, cat. no. 019570; **8**, Fluka, cat. no. 17974; **9**, Bachem, cat. no. F-3480; **10**, Bachem, cat. no. F-3485.

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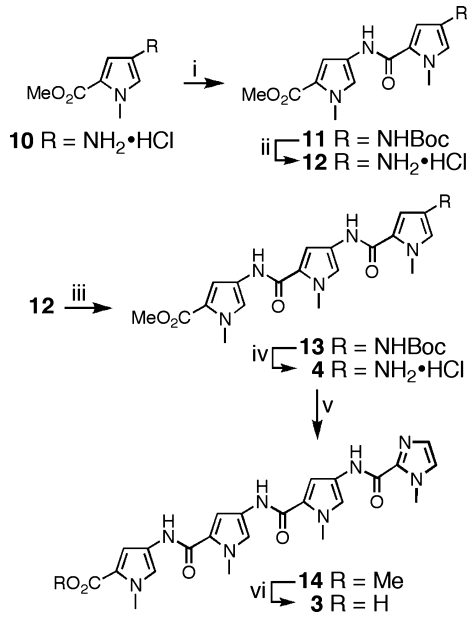
Scheme 1. Preparation of **3** and **4**[a]

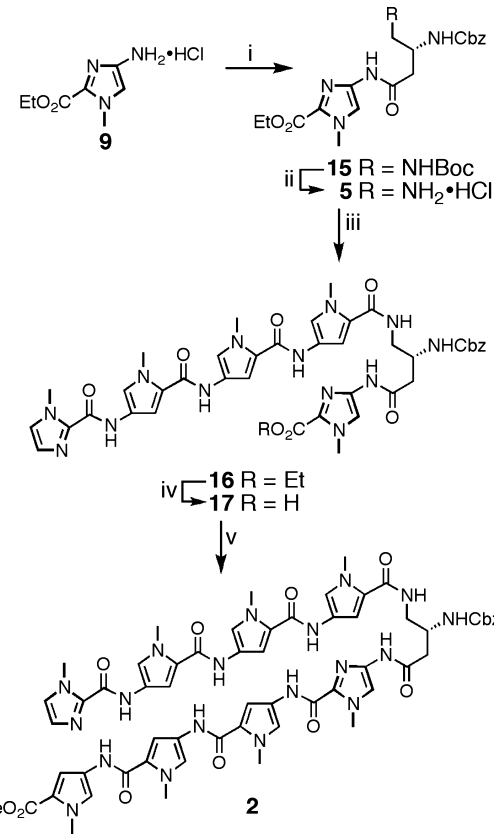


[a] Reagents and conditions: (i) DMF, DIEA, **7**, 23 °C, 8 h, 93%; (ii) 2.0 M HCl in Et_2O, 23 °C, 18 h, 99%; (iii) DMF, DIEA, **7**, 23 °C, 8 h, 96%; (iv) 4.0 M HCl in 1,4-dioxane, 23 °C, 18 h, 99%; (v) DMF, DIEA, **6**, 23 °C, 2 h, 83%; (vi) NaOH(aq), 1,4-dioxane, 42 °C, 2 h, 93%.

tation of **12** as the HCl salt during the course of the reaction. Isolation of solid **12** by filtration, followed by washing of the residual solid with excess Et_2O, provides the amine HCl salt in 99% yield. By exploiting the aqueous solubility of **10** versus insolubility of **11**, PyPy dimer **11** is easily purified from a small excess of **10** by precipitation, whereas deprotected **12** is separable from Boc-protected **11** by virtue of the Et_2O solubility of **11** versus insolubility of **12**. This reaction sequence highlights our synthesis strategy: exploiting the different solubility profiles of reactants versus products for chromatography-free purifications. Accordingly, pyrrole trimer **4** was obtained from dimer **12** by coupling with **7**, followed by acidic deprotection to yield **4** in 95% yield (two steps) from dimer **12**. The ImPyPyPy tetramer **3** was synthesized in two steps from trimer **4**. 1-Methyl-2-trichloroacetylimidazole (**6**), prepared in one step from *N*-methylimidazole,[10c,13] was allowed to react with a small excess of **4** to deliver tetramer **14** in 83% yield following precipitation in H_2O, trituration with Et_2O, and drying *in vacuo*. Saponification of **14** with aqueous NaOH in 1,4-dioxane, followed by neutralization with aqueous HCl, precipitation, and Et_2O trituration, afforded tetramer **3** in 77% overall yield from trimer **4**.

The Im-turn fragment **5** was synthesized in two steps from the Im•HCl salt monomer[10a] **9** by coupling to PyBOP-activated (*R*)-3,4-Cbz-Dbu(Boc)-OH (**8**), yielding protected dimer **15** in 95% yield (Scheme 2). The utilization of a small excess of **9** drives the coupling reaction to completion and is easily separated in the aqueous wash step. Removal of the carbamate protecting group with anhydrous HCl in 1,4-dioxane yielded the final synthon for our studies, imidazole-turn dimer **5**, in quantitative yield following filtration and washing of the residual salt. With compound **5** in hand, the assembly of core polyamide **2** was initiated. PyBOP-mediated coupling of tetramer **3** to a small excess of water-soluble Im-turn dimer **5** yielded the advanced intermediate **16** in 97% yield. Saponification of **16** to acid **17**, followed by amide coupling with an excess of water-soluble trimer **4**, delivered core Py-Im hairpin polyamide **2** in 88% yield

Scheme 2. Preparation of **5** and Assembly of Core Polyamide **2**[a]



[a] Reagents and conditions: (i) DMF, DIEA, PyBOP, (*R*)-3,4-Cbz-Dbu(Boc)-OH (**8**), 23 °C, 8 h, 95%; (ii) HCl in 1,4-dioxane, 23 °C, 16 h, 99%; (iii) DMF, DIEA, PyBOP, **3**, 23 °C, 2 h, 97%; (iv) KOH(aq), MeOH, 1,4-dioxane, 42 °C, 2 h, 92%; (i) DMF, DIEA, PyBOP, **4**, 23 °C, 10 h, 96%.

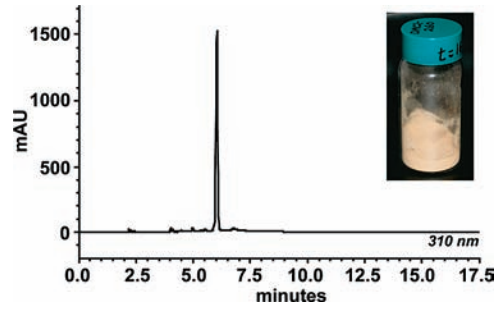
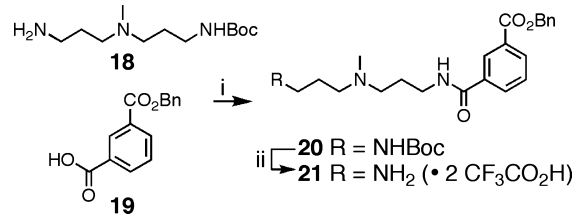


Figure 3. Analysis of polyamide **2** purity by analytical HPLC. Wavelength shown is 310 nm.

for the two steps. Consistent with the previously discussed strategy for synthesizing intermediates **3**−**5**, the differences in solubility of reactants versus products were exploited to isolate pure material by precipitation, washing, and trituration. In most cases a low-boiling-point solvent was employed in the final trituration step to facilitate efficient solvent removal *in vacuo*. Core polyamide **2** was synthesized without a single chromatographic purification in high overall purity, as depicted by the analytical HPLC analysis of **2** shown in Figure 3. Multigram quantities of **2** have been readily synthesized by this method, providing a stockpile of material for elaboration at the C-terminus into discrete polyamide conjugates, such as **1**.

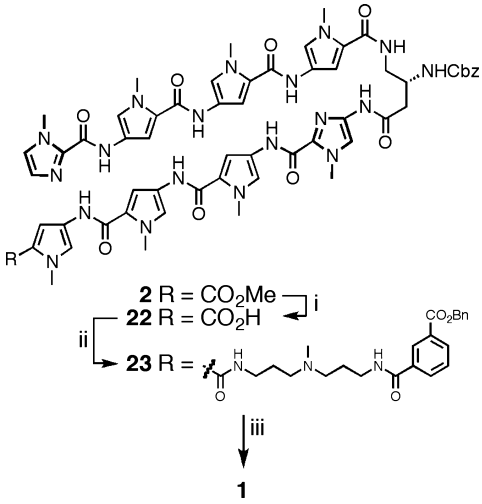
Py-Im polyamide **1** was synthesized in solution from advanced core **2** by coupling the preassembled C-terminal tail moiety **21** with saponified core **22**. This convergent approach

Scheme 3. Preparation of **21**[a]



[a] Reagents and conditions: (i) DMF, DIEA, PyBOP, 23 °C, 3 h, 98%; (ii) CF_3CO_2H, CH_2Cl_2, 23 °C, 20 min, concentration *in vacuo* and used crude.

Scheme 4. Final Steps for the Synthesis of Py-Im Polyamide **1**[a]



[a] Reagents and conditions: (i) NaOH(aq), 1,4-dioxane, 23 °C, 11 h, 89%; (ii) DIEA, DMF, PyBOP, **21**, 23 °C, 12 h, 87%; (iii) DMF, Pd/C, H_2 (1 atm), 23 °C, 48 h, 81%.

begins by preparing Boc-protected C-terminus moiety **20** (Scheme 3). PyBOP-mediated coupling of mono-Boc-protected triamine linker **18**[14] with monobenzyl-protected isophthalic acid **19**[15] afforded Boc-protected **20** in 98% yield. Deprotection of **20** with anhydrous CF_3CO_2H in dichloromethane (1:1) yielded amine **21**, which was used immediately following concentration under high vacuum. Saponification of core polyamide **2** with aqueous NaOH in 1,4-dioxane at 23 °C yielded 8-ring acid **22** in 89% yield (Scheme 4). The transformation of **2** to **22** proved somewhat difficult in early studies due to formation of an unidentified side product. Avoiding reaction temperatures above 23 °C suppresses most of the byproduct formation, whereas a screen of aqueous bases commonly used for ester saponification (KOH and LiOH) failed to identify a better reagent. Coupling of residual acid **22** with freshly prepared **21** delivered the penultimate oligomer **23** in 87% yield. Unfortunately, crude oligomer **23** could not be satisfactorily purified by our standard method and required chromatography on silica gel to achieve pure material. Hundreds of milligrams of **23** have been prepared by this method in a single reaction sequence. Global deprotection of **23** by hydrogenation (Pd/C, ~1 atm H_2) at 23 °C for 48 h yields Py-Im polyamide **1** in 81% yield. Final product **1** can be separated from residual catalyst by solid-phase extraction and then purified by preparative reverse-phase HPLC.

With multi-milligram quantities of **1** on hand, we investigated in detail the UV properties of Py-Im polyamide **1** in a variety of laboratory and biologically relevant solvents. As shown in Figure 4, a strong solvent influence on the molar extinction coefficient of **1** is observed as the amount of organic cosolvent is increased. For example, an extinction coefficient (ε, M^{-1} cm^{-1}) of 26 500 was measured for **1** in distilled and deionized H_2O, whereas this value doubled to 54 800 in 50% acetonitrile in aqueous CF_3CO_2H (0.1% v/v CF_3CO_2H), a widely utilized laboratory solvent for purifying and quantifying peptides. A stock solution frequently encountered for preparing biological samples, 10% DMSO in DEPC-treated H_2O yielded an intermediary value of 40 900 $M^{-1}cm^{-1}$. Hence, care must be taken to consider the solvent system utilized when performing UV spectroscopy to determine polyamide concentrations.

Conclusion

A solution-phase synthesis of Py-Im hairpin polyamide **1** is presented, highlighting unifying principles for the preparation of related polyamides. A convergent synthesis was developed, requiring no chromatographic purifications to arrive at core 8-ring polyamide **2** on multigram scale. Final elaboration of the C-terminus affords AR polyamide antagonist **1** in high yield. The synthetic methodology permits gram-scale synthesis of Py-Im polyamides, a minimum next step as we transition these small molecules to animal models for biological efficacy.

Experimental Section

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. Centrifugation was performed in a Beckman Coulter benchtop centrifuge (Allegra 21R) equipped with a Beckman swing-out rotor (model S4180). Analytical HPLC analysis was conducted on a Beckman Gold instrument equipped with a Phenomenex Gemini analytical column (250 × 4.6 mm, 5 μm) and 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, a 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. UV−vis measurements were made on a Hewlett-Packard diode array spectrophotometer (model 8452 A). NMR spectroscopy was performed on a Varian instrument operating at 499.8 MHz (for ^1H) or 125.7 MHz (for ^{13}C) 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) or 39.5 (for ^{13}C). High-resolution mass spectrometry (HRMS) was recorded in positive-ion mode by fast-atom bombardment (FAB^+) on a JEOL JMS-600H instrument or by electrospray ionization (ESI^+) on a Waters Acquity UPLC-LCT Premiere XE TOF-MS system.

$HCl \cdot H_2N$-Py-CO_2Me (10). Prepared as previously described.[10a] ^1H NMR: δ 10.09 (br s, 3H), 7.25 (d, J = 2.0 Hz, 1H), 6.80 (d, J = 2.2 Hz, 1H), 3.85 (s, 3H), 3.74 (s, 3H). ^{13}C NMR: δ 160.2, 123.7, 120.8, 113.9, 111.4, 51.3, 36.6. HRMS (FAB^+): calcd for $C_7H_{11}N_2O_2$ $[M + H]^+$, 155.0821; found, 155.0847.

BocHN-Py-CO_2Me (11). A solution of BocHN-Py-OBt **7** (6.16 g, 17.2 mmol) and $HCl \cdot H_2N$-Py-CO_2Me **10** (3.61 g, 19.0 mmol) in DMF (39 mL) and DIEA (6 mL, 34.4 mmol) was stirred at 23 °C for 8 h. The solution was then added to distilled H_2O

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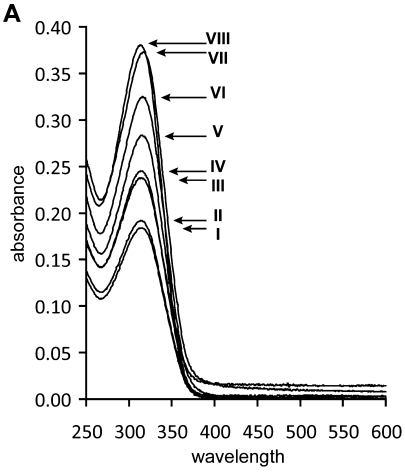
A



B

	Solvent System	Molar Extinction Coefficient ($M^{-1}cm^{-1}$)
I	H_2O (distilled & deionized)	26,500
II	H_2O (DEPC)	27,600
III	2% DMSO in H_2O (DEPC)	34,300
IV	5% EtOH in H_2O (DEPC)	35,400
V	10% DMSO in H_2O (DEPC)	40,900
VI	10% MeCN in H_2O (0.1 % CF_3CO_2H)	46,800
VII	10% DMF in H_2O (0.1% CF_3CO_2H)	53,800
VIII	50% MeCN in H_2O (0.1% CF_3CO_2H)	54,800

Figure 4. UV properties of polyamide **1** in solvent systems I−VIII. (A) UV traces of I−VIII from 250−600 nm and (B) tabular form of data. Molar extinction coefficients were calculated from the λ_{max} for each individual system, which ranged from 313 to 317 nm. DEPC = diethylpyrocarbonate-treated H_2O.

(500 mL) preacidified with aqueous HCl (1 N, 300 mL, 300 mmol), yielding a precipitate that was isolated by centrifugation (∼4500 rpm). The residual solid was again suspended in distilled H_2O (80 mL) and collected by centrifugation (repeated 2×). The resultant solid, which contained a small amount of residual H_2O, was frozen and lyophilized to dryness. Drying of the light-brown solid *in vacuo* yielded dimer **11** (6.03 g, 93%). ^{1}H NMR: δ 9.84 (s, 1H), 9.10 (s, 1H), 7.44 (d, J = 1.7 Hz, 1H), 6.88 (m, 2H), 6.82 (s, 1H), 3.82 (s, 3H), 3.79 (s, 3H), 3.72 (s, 3H), 1.44 (s, 9H). ^{13}C NMR: δ 160.8, 158.4, 152.8, 123.0, 122.6, 122.4, 120.7, 118.4, 117.1, 108.3, 103.8, 78.3, 50.9, 36.1, 36.0, 28.2. HRMS (FAB$^+$): calcd for $C_{18}H_{25}N_4O_5$ [M + H]$^+$, 377.1825; found, 377.1835.

HCl·H$_2$N-PyPy-CO$_2$Me (12). Dimer **11** (4.0 g, 10.6 mmol) in a solution of anhydrous HCl in Et_2O (2.0 M, 400 mL) was stirred at 23 °C for 18 h. The mixture was then diluted with 400 mL of anhydrous Et_2O and filtered over a sintered glass funnel. The resultant solid was washed with copious amounts of anhydrous Et_2O and dried *in vacuo* to yield dimer **12** as a tan solid (3.3 g, 99%). ^{1}H NMR: δ 10.12 (s, 1H), 10.07 (br s, 3H), 7.46 (d, J = 2.0 Hz, 1H), 7.10 (d, J = 2.0 Hz, 1H), 7.00 (d, J = 2.0 Hz, 1H), 6.91 (d, J = 2.0 Hz, 1H), 3.87 (s, 3H), 3.83 (s, 3H), 3.72 (s, 3H). ^{13}C NMR: δ 160.8, 157.7, 124.6, 122.6, 121.7, 120.8, 118.6, 113.1, 108.4, 107.2, 51.0, 36.6, 36.2. HRMS (FAB$^+$): calcd for $C_{13}H_{17}N_4O_3$ [M + H]$^+$, 277.1301; found, 277.1292.

BocHN-PyPyPy-CO$_2$Me (13). A solution of BocHN-Py-OBt **7** (3.1 g, 8.7 mmol) and dimer **12** (3.0 g, 9.59 mmol) in DMF (20 mL) and DIEA (3 mL, 17.4 mmol) was stirred at 23 °C for 8 h. The solution was then added to distilled H_2O (250 mL) preacidified with aqueous HCl (1 N, 150 mL, 150 mmol), yielding a precipitate that was isolated by centrifugation (∼4500 rpm). The residual solid was again suspended in distilled H_2O (40 mL) and collected by centrifugation (repeated 2×). The resultant solid, which contained a small amount of residual H_2O, was frozen and lyophilized to dryness. Drying of the light-brown solid *in vacuo* yielded trimer **13** (4.17 g, 96%). ^{1}H NMR: δ 9.91 (s, 1H), 9.86 (s, 1H), 9.09 (s, 1H), 7.46 (d, J = 2.0 Hz, 1H), 7.21 (d, J = 1.7 Hz, 1H), 7.05 (d, J = 1.5 Hz, 1H), 6.89 (m, 2H), 6.83 (s, 1H), 3.83 (s, 6H), 3.80 (s, 3H), 3.73 (s, 3H), 1.45 (s, 9H). ^{13}C NMR: δ 160.8, 158.5, 158.4, 152.8, 123.0, 122.8, 122.4, 122.30, 122.29, 120.7, 118.48, 118.47, 117.0, 108.3, 104.8, 103.8, 78.3, 50.9, 36.2, 36.05, 36.04, 28.2. HRMS (FAB$^+$): calcd for $C_{24}H_{30}N_6O_6$ [M$^•$]$^+$, 498.2227; found, 498.2233.

HCl·H$_2$N-PyPyPy-CO$_2$Me (4). Dimer **13** (4.0 g, 8.02 mmol) in a solution of anhydrous HCl in 1,4-dioxane (4.0 M, 300 mL) was stirred at 23 °C for 18 h. The mixture was then diluted with 600 mL of anhydrous Et_2O and filtered over a sintered glass funnel. The resultant solid was washed with copious amounts of anhydrous Et_2O and dried *in vacuo* to yield trimer **4** as a brown-orange solid (3.45 g, 99%). ^{1}H NMR: δ 10.16 (s, 3H), 10.13 (s, 1H), 9.97 (s, 1H), 7.46 (d, J = 2.0 Hz, 1H), 7.25 (d, J = 1.7 Hz, 1H), 7.11 (d, J = 2.0 Hz, 1H), 7.08 (d, J = 2.0 Hz, 1H), 7.02 (d, J = 2.0 Hz, 1H), 6.91 (d, J = 2.0 Hz, 1H), 3.88 (s, 3H), 3.84 (s, 3H), 3.82 (s, 3H), 3.72 (s, 3H). ^{13}C NMR: δ 160.8, 158.4, 157.7, 124.8, 123.0, 122.6, 121.9, 121.6, 120.8, 118.7, 118.5, 113.0, 108.4, 107.2, 104.8, 51.0, 36.6, 36.2, 36.1. HRMS (FAB$^+$): calcd for $C_{19}H_{22}N_6O_4$ [M$^•$]$^+$, 398.1702; found, 398.1685.

ImPyPyPy-CO$_2$Me (14). A solution of trimer **4** (1.019 g, 2.34 mmol) and 1-methyl-2-trichloroacetylimidazole (**6**)[10c,13] (478 mg, 2.10 mmol) in DMF (4.5 mL) and DIEA (910 μL, 5.22 mmol) was stirred at 23 °C for 2 h. The solution was then added to distilled H_2O (40 mL) preacidified with aqueous HCl (1N, 910 μL, 0.91 mmol), yielding a precipitate that was isolated by centrifugation (∼4500 rpm). The residual solid was again suspended in distilled H_2O (40 mL) and collected by centrifugation (repeated 1×). The resultant solid, which contained a small amount of residual H_2O, was frozen and lyophilized to dryness. The solid was triturated with anhydrous Et_2O and filtered over a sintered glass funnel. The resultant solid was washed with copious amounts of anhydrous Et_2O and dried *in vacuo* to yield tetramer **14** as a light brown solid (886 mg, 83%). ^{1}H NMR: δ 10.68 (s, 1H), 10.00 (s, 1H), 9.94 (s, 1H), 7.48 (s, 1H), 7.47 (d, J = 2.0 Hz, 1H), 7.31 (d, J = 1.7 Hz, 1H), 7.24 (d, J = 1.7 Hz, 1H), 7.18 (s, 1H), 7.17 (d, J = 2.0 Hz, 1H), 7.08 (d, J = 2.0 Hz, 1H), 6.91 (d, J = 1.7 Hz, 1H), 4.01 (s, 3H), 3.86 (s, 3H), 3.84 (s, 3H), 3.83 (s, 3H), 3.73 (s, 3H). ^{13}C NMR: δ 160.8, 158.5, 158.4, 155.0, 138.2, 126.4, 125.6, 123.1, 123.0, 122.5, 122.2, 121.2, 120.7, 118.8, 118.6, 118.5, 108.3, 104.9, 104.8, 50.9, 36.18, 36.17, 36.1, 35.4. HRMS (FAB$^+$): calcd for $C_{24}H_{27}N_8O_5$ [M + H]$^+$, 507.2104; found, 507.2116.

ImPyPyPy-CO$_2$H (3). A solution of tetramer **14** (804 mg, 1.59 mmol) in 1,4-dioxane (8 mL) and aqueous NaOH (1 N, 8.0 mL, 8.00 mmol) was stirred at 42 °C for 2 h. The solution was then added to distilled H_2O (40 mL) preacidified with aqueous HCl (1 N, 8 mL, 8.00 mmol), yielding a precipitate that was isolated by centrifugation (∼4500 rpm). The residual solid was again suspended in distilled H_2O (40 mL) and collected by centrifugation (repeated 1×). The resultant solid, which contained a small amount of residual H_2O, was frozen and lyophilized to dryness. The solid was triturated with anhydrous Et_2O and filtered over a sintered glass funnel. The resultant solid was washed with copious amounts of anhydrous Et_2O and dried *in vacuo* to yield tetramer **3** as a brown solid (728 mg, 93%). ^{1}H NMR: δ 10.45 (s, 1H), 9.97 (s, 1H), 9.91 (s, 1H), 7.43 (s, 1H), 7.38 (s, 1H), 7.29 (s, 1H), 7.24 (s, 1H), 7.17 (s, 1H), 7.07 (s, 1H), 7.03 (s, 1H), 6.85 (s, 1H), 3.99 (s, 3H), 3.85 (s, 3H), 3.84 (s, 3H), 3.82 (s, 3H). ^{13}C NMR: δ 162.0, 158.48, 158.46, 156.1, 138.8, 127.0, 126.4, 123.0, 122.7, 122.6, 122.2, 121.5, 120.3, 119.5,

118.7, 118.5, 108.4, 105.0, 104.8, 36.14, 36.07, 35.1. HRMS (FAB^+): calcd for $C_{23}H_{25}N_8O_5$ $[M + H]^+$, 493.1948; found, 493.1952.

BocHN-(R)$^{\beta\text{-CbzHN}}\gamma$-Im-$CO_2$Et (15). A solution of (*R*)-3,4-Cbz-Dbu(Boc)-OH **8** (1.03 g, 2.93 mmol) and PyBOP (1.83 g, 3.51 mmol) in DMF (12 mL) and DIEA (1.5 mL, 8.8 mmol) was stirred at 23 °C for 22 min. The solution was then added to solid (powdered) HCl·H_2N-Im-CO_2Et **9** (850 mg, 3.15 mmol) and stirred at 23 °C for 8 h. This solution was then added to distilled H_2O (30 mL) preacidified with aqueous HCl (1 N, 9 mL, 9 mmol), yielding a precipitate that was isolated by centrifugation (~ 4500 rpm). The residual solid was again suspended in distilled H_2O (40 mL) and collected by centrifugation (repeated 2×). The resultant solid, which contained a small amount of residual H_2O, was frozen and lyophilized to dryness. Drying of the brown solid *in vacuo* yielded dimer **15** (1.4 g, 95%). ^{1}H NMR: δ 10.57 (s, 1H), 7.51 (s, 1H), 7.31−7.27 (m, 5H), 7.02 (d, J = 8.5 Hz, 1H), 6.79 (m, 1H), 4.97 (s, 2H), 4.25 (q, J = 7.2 Hz, 2H), 3.93 (m, 1H), 3.89 (s, 3H), 3.01 (m, 2H), 2.44−2.35 (m, 2H), 1.35 (s, 9H), 1.27 (t, J = 7.2 Hz, 3H). ^{13}C NMR: δ 167.6, 158.4, 155.8, 155.4, 137.4, 137.1, 130.7, 128.2, 127.6, 127.5, 114.8, 77.7, 65.1, 60.5, 48.6, 43.5, 38.1, 35.4, 28.2, 14.0. HRMS (FAB^+): calcd for $C_{24}H_{34}N_5O_7$ $[M + H]^+$, 504.2458; found, 504.2462.

HCl·H_2N-(R)$^{\beta\text{-CbzHN}}\gamma$-Im-$CO_2$Et (5). Dimer **15** (500 mg, 0.993 mmol) in a solution of anhydrous HCl in 1,4-dioxane (4.0 M, 10 mL) and anhydrous Et_2O (4 mL) was stirred at 23 °C for 16 h. The mixture was then diluted with 20 mL of anhydrous Et_2O and filtered over a sintered glass funnel. The resultant solid was washed with copious amounts of anhydrous Et_2O and dried *in vacuo* to yield dimer **5** as a white solid (432 mg, 99%). ^{1}H NMR: δ 10.75 (s, 1H), 8.05 (m, 3H), 7.52 (s, 1H), 7.38 (d, J = 8.5 Hz, 1H), 7.34−7.28 (m, 5H), 5.01 (m, 2H), 4.25 (q, J = 7.1 Hz, 2H), 4.13 (m, 1H), 3.90 (s, 3H), 2.96−2.84 (m, 2H), 2.60−2.51 (m, 2H), 1.27 (t, J = 7.1 Hz, 3H). ^{13}C NMR: δ 166.8, 158.4, 155.7, 137.2, 136.8, 130.9, 128.3, 127.8, 127.7, 114.9, 65.5, 60.6, 46.6, 42.1, 38.2, 35.4, 14.0. HRMS (FAB^+): calcd for $C_{19}H_{26}N_5O_5$ $[M + H]^+$, 404.1934; found, 404.1928.

ImPyPyPy-(R)$^{\beta\text{-CbzHN}}\gamma$-Im-$CO_2$Et (16). A solution of tetramer **3** (1.28 g, 2.59 mmol) and PyBOP (1.49 g, 2.86 mmol) in DMF (8 mL) and DIEA (1.8 mL, 10.4 mmol) was stirred at 23 °C for 20 min. The solution was then treated with solid (powdered) HCl·H_2N-(R)$^{\beta\text{-CbzHN}}\gamma$-Im-$CO_2$Et **5** (1.2 g, 2.73 mmol) and stirred at 23 °C for 2 h. This solution was then added to distilled H_2O (30 mL) preacidified with aqueous HCl (1 N, 20 mL, 20 mmol), yielding a precipitate that was isolated by centrifugation (~4500 rpm). The residual solid was again suspended in distilled H_2O (40 mL) and collected by centrifugation (repeated 2×). The resultant solid, which contained a small amount of residual H_2O, was frozen and lyophilized to dryness. The solid was triturated with anhydrous Et_2O and filtered over a sintered glass funnel. The resultant solid was washed with copious amounts of anhydrous Et_2O and dried *in vacuo* to yield ImPyPyPy-(R)$^{\beta\text{-CbzHN}}\gamma$-Im-$CO_2$Et **16** as a tan solid (2.2 g, 97%). ^{1}H NMR: δ 10.60 (s, 1H), 10.46 (s, 1H), 9.96 (s, 1H), 9.91 (s, 1H), 7.97 (t, J = 5.6 Hz, 1H), 7.52 (s, 1H), 7.39 (s, 1H), 7.29−7.26 (m, 6H), 7.24 (m, 1H), 7.18−7.14 (m, 3H), 7.04 (m, 2H), 6.90 (m, 1H), 4.98 (m, 2H), 4.24 (q, J = 7.1 Hz, 2H), 4.10 (m, 1H), 3.99 (s, 3H), 3.89 (s, 3H), 3.845 (s, 3H), 3.839 (s, 3H), 3.77 (s, 3H), 3.29 (m, 2H), 2.49 (m, 2H, obstructed by NMR solvent), 1.27 (t, J = 7.2 Hz, 3H). ^{13}C NMR: δ 167.7, 161.5, 158.49, 158.48, 158.45, 156.0, 155.6, 138.7, 137.4, 137.1, 130.8, 128.2, 127.7, 127.6, 126.8, 126.4, 123.0, 122.8, 122.7, 122.24, 122.17, 121.4, 118.7, 118.5, 118.0, 114.9, 105.0, 104.7, 104.4, 65.2, 60.5, 48.6, 42.1, 38.2, 36.13, 36.11, 36.0, 35.4, 35.2, 14.0. HRMS (FAB^+): calcd for $C_{42}H_{48}N_{13}O_9$ $[M + H]^+$, 878.3698; found, 878.3668.

ImPyPyPy-(R)$^{\beta\text{-CbzHN}}\gamma$-Im-$CO_2$H (17). A solution of ImPyPyPy-(R)$^{\beta\text{-CbzHN}}\gamma$-Im-$CO_2$Et **16** (2.0 g, 2.28 mmol) dissolved in 1,4-dioxane (2 mL), MeOH (6 mL), and aqueous KOH (1N, 9.1 mL, 9.1 mmol) was stirred at 42 °C for 2 h. The solution was then acidified with aqueous HCl (1 N, ~9.1 mL, ~9.1 mmol) to a pH = 4.5, yielding a precipitate that was isolated by centrifugation (~4500 rpm). The residual solid was again suspended in distilled H_2O (10 mL) and collected by centrifugation (repeated 1×). The resultant solid, which contained a small amount of residual H_2O, was frozen and lyophilized to dryness. The solid was triturated with anhydrous Et_2O and filtered over a sintered glass funnel. The resultant solid was washed with copious amounts of anhydrous Et_2O and dried *in vacuo* to yield ImPyPyPy-(R)$^{\beta\text{-CbzHN}}\gamma$-Im-$CO_2$H **17** as a tan solid (1.78 g, 92%). ^{1}H NMR: δ 10.50 (s, 1H), 10.47 (s, 1H), 9.96 (s, 1H), 9.92 (s, 1H), 7.98 (m, 1H), 7.48 (s, 1H), 7.40 (s, 1H), 7.30−7.27 (m, 6H), 7.24 (m, 1H), 7.19−7.14 (m, 3H), 7.05 (m, 2H), 6.90 (m, 1H), 4.99 (m, 2H), 4.10 (m, 1H), 3.99 (s, 3H), 3.88 (s, 3H), 3.845 (s, 3H), 3.838 (s, 3H), 3.77 (s, 3H), 3.30 (m, 2H), 2.49 (m, 2H, obstructed by NMR solvent). ^{13}C NMR: δ 167.7, 161.5, 160.0, 158.5, 155.8, 155.6, 138.6, 137.1, 131.6, 128.3, 127.7, 127.6, 126.7, 126.4, 123.0, 122.8, 122.7, 122.23, 122.16, 121.4, 118.7, 118.5, 118.0, 114.6, 105.0, 104.7, 104.4, 65.2, 48.6, 42.2, 38.2, 36.13, 36.10, 36.0, 35.4, 35.2. HRMS (FAB^+): calcd for $C_{40}H_{44}N_{13}O_9$ $[M + H]^+$, 850.3385; found, 850.3383.

ImPyPyPy-(R)$^{\beta\text{-CbzHN}}\gamma$-ImPyPyPy-$CO_2$Me (2). A solution of ImPyPyPy-(R)$^{\beta\text{-CbzHN}}\gamma$-Im-$CO_2$H **17** (1.5 g, 1.77 mmol) and PyBOP (546 mg, 1.85 mmol) in DMF (8.8 mL) and DIEA (922 μL, 5.3 mmol) was stirred at 23 °C for 10 min. The solution was then treated with solid (powdered) HCl·H_2N-PyPyPy-CO_2Me **4** (806 mg, 1.85 mmol) and stirred at 23 °C for 10 h. This solution was then added to distilled H_2O (35 mL) preacidified with aqueous HCl (1 N, 5 mL, 5 mmol), yielding a precipitate that was isolated by centrifugation (~4500 rpm). The residual solid was again suspended in distilled H_2O (40 mL) and collected by centrifugation (repeated 3×). The resultant solid, which contained a small amount of residual H_2O, was frozen and lyophilized to dryness. The solid was triturated with anhydrous Et_2O and filtered over a sintered glass funnel. The resultant solid was washed with copious amounts of anhydrous Et_2O and dried *in vacuo* to yield ImPyPyPy-(R)$^{\beta\text{-CbzHN}}\gamma$-ImPyPyPy-$CO_2$Me **2** as a tan solid (2.09 g, 96%). ^{1}H NMR: δ 10.66 (s, 1H), 10.21 (s, 1H), 10.00 (s, 1H), 9.98 (s, 1H), 9.96 (s, 1H), 9.93 (s, 1H), 9.92 (s, 1H), 8.00 (m, 1H), 7.48 (s, 1H), 7.46 (d, J = 2.0 Hz, 1H), 7.45 (s, 1H), 7.31−7.27 (m, 7H), 7.23 (m, 2H), 7.20−7.14 (m, 5H), 7.06 (m, 2H), 6.92 (m, 1H), 6.90 (d, J = 2.0 Hz, 1H), 5.00 (m, 2H), 4.11 (m, 1H), 4.00 (s, 3H), 3.95 (s, 3H), 3.854 (s, 3H), 3.850 (s, 3H), 3.842 (s, 3H), 3.837 (s, 3H), 3.828 (s, 3H), 3.78 (s, 3H), 3.73 (s, 3H), 3.32 (m, 2H), 2.53 (m, 2H). ^{13}C NMR: δ 167.9, 161.6, 160.8, 158.5, 158.42, 158.38, 155.8, 155.6, 137.6, 137.1, 136.0, 134.0, 128.3, 127.7, 127.6, 126.4, 123.3, 123.1, 123.0, 122.80, 122.77, 122.5, 122.26, 122.24, 122.1, 121.2, 120.9, 120.8, 118.9, 118.70, 118.64, 118.5, 118.0, 114.1, 108.4, 104.9, 104.79, 104.76, 104.5, 65.2, 51.0, 48.8, 42.2, 38.4, 36.25, 36.21, 36.19, 36.13, 36.10, 36.0, 35.8, 35.0. HRMS (FAB^+): calcd for $C_{59}H_{64}N_{19}O_{12}$ $[M + H]^+$, 1230.498; found, 1230.504.

ImPyPyPy-(R)$^{\beta\text{-CbzHN}}\gamma$-ImPyPyPy-$CO_2$H (22). A solution of polyamide **2** (500 mg, 0.406 mmol) dissolved in 1,4-dioxane (8 mL) and aqueous NaOH (1 N, 8.0 mL, 8.0 mmol) was stirred at 23 °C for 11 h. The solution was then cooled to 0 °C in an ice bath and the pH adjusted to pH = 4.0 with aqueous HCl (1 N, ~8 mL, 8 mmol), yielding a precipitate that was isolated by centrifugation (~4500 rpm). The residual solid was again suspended in distilled H_2O (40 mL) and collected by centrifugation (repeated 2×). The resultant solid, which contained a small amount of residual H_2O, was frozen and lyophilized to dryness. The solid was triturated with anhydrous Et_2O and filtered over a sintered glass funnel. The resultant solid was washed with copious amounts of anhydrous Et_2O and dried *in vacuo* to yield ImPyPyPy-(R)$^{\beta\text{-CbzHN}}\gamma$-ImPyPyPy-$CO_2$H **22** as a tan solid (442 mg, 89%). ^{1}H NMR: δ 10.52 (s, 1H), 10.22 (s, 1H), 10.00 (s, 1H), 9.97 (s, 1H), 9.95 (s, 1H), 9.93 (s, 1H), 9.90 (s, 1H), 8.00 (m, 1H), 7.45 (s, 1H), 7.42 (m, 2H), 7.30−7.27 (m, 7H), 7.24 (m, 2H), 7.19−7.14 (m, 4H), 7.09 (s, 1H), 7.06 (m, 2H), 6.92 (m, 1H), 6.84 (d, J = 2.0 Hz, 1H), 5.01 (m, 2H), 4.11 (m, 1H), 3.99 (s, 3H), 3.95 (s, 3H), 3.851 (s, 3H), 3.848 (s, 3H), 3.843

(s, 3H), 3.838 (s, 3H), 3.81 (s, 3H), 3.79 (s, 3H), 3.32 (m, 2H), 2.53 (m, 2H). ^{13}C NMR: δ 167.9, 162.0, 161.6, 158.48, 158.47, 158.44, 158.40, 155.8, 155.6, 138.6, 137.1, 136.0, 134.0, 128.3, 127.7, 127.6, 126.4, 123.06, 123.05, 122.8, 122.74, 122.70, 122.6, 122.24, 122.19, 122.15, 121.4, 121.2, 120.3, 119.5, 118.7, 118.55, 118.47, 118.0, 114.1, 108.4, 104.9, 104.86, 104.80, 104.75, 104.5, 65.2, 48.7, 42.2, 38.3, 36.2, 36.13, 36.10, 36.07, 35.97, 35.2, 34.9. HRMS (FAB$^+$): calcd for $C_{58}H_{62}N_{19}O_{12}$ [M+H]$^+$, 1216.483; found, 1216.487.

BocHN-(+)-^{BnO}IPA (20). A solution of acid **19**[15] (211 mg, 0.824 mmol) and PyBOP (624 mg, 1.2 mmol) in DMF (3 mL) and DIEA (211 μL, 1.2 mmol) was stirred at 23 °C for 10 min. Protected triamine **18**[14] (373 mg, 1.52 mmol) was then added to the solution, and stirring was continued at 23 °C for 3 h. The solution was then added to distilled H_2O (15 mL), and a viscous oil was isolated following centrifugation (~4500 rpm) and decanting of the aqueous layer. The residual oil was again washed with distilled H_2O (10 mL) and collected by centrifugation (repeated 3×). Drying of the residual oil *in vacuo* yielded BocHN-(+)-^{BnO}IPA **20** as a viscous amber oil (391 mg, 98%). ^{1}H NMR: δ 8.77 (t, J = 5.5 Hz, 1H), 8.43 (app t, J = 1.5 Hz, 1H), 8.14−8.09 (m, 2H), 7.63 (app t, J = 7.8 Hz, 1H), 7.48−7.34 (m, 5H), 6.85 (t, J = 4.9 Hz, 1H), 5.38 (s, 2H), 3.30 (m, 2H), 3.00 (m, 1H), 2.94 (m, 2H), 2.72−2.51 (m, 4H), 2.38 (br s, 3H), 1.75 (m, 2H), 1.59 (m, 2H), 1.34 (s, 9H). ^{13}C NMR: δ 165.3, 165.2, 155.6, 136.0, 135.0, 131.9, 131.7, 129.8, 129.0, 128.6, 128.2, 128.1, 127.9, 77.5, 66.4, 54.3, 54.0, 40.6, 37.7, 37.3, 28.2, 25.9, 25.5. HRMS (FAB$^+$): calcd for $C_{27}H_{38}N_3O_5$ [M+H]$^+$, 484.2811; found, 484.2793.

ImPyPyPy-(R)$^{\beta\text{-CbzHN}}\gamma$-ImPyPyPy-(+)-^{BnO}IPA (23). A solution of ImPyPyPy-(R)$^{\beta\text{-CbzHN}}\gamma$-ImPyPyPy-$CO_2H$ **22** (192 mg, 0.158 mmol) and PyBOP (99 mg, 0.189 mmol) in DMF (2 mL) and DIEA (220 μL, 1.3 mmol) was stirred at 23 °C for 30 min. In a separate vial, BocHN-(+)-^{BnO}IPA **20** (122 mg, 0.252 mmol) was deprotected by treating with a solution of CH_2Cl_2:CF_3CO_2H (1:1, 2 mL) for 20 min at 23 °C, followed by concentration to dryness *in vacuo*. This residual material was then treated with the preactivated solution of ImPyPyPy-(R)$^{\beta\text{-CbzHN}}\gamma$-ImPyPyPy-$CO_2H$ **22** and allowed to stir at 23 °C for 12 h. The solution was then added to distilled H_2O (30 mL) preacidified with aqueous HCl (1 N, 2 mL, 2 mmol), yielding a precipitate that was isolated by centrifugation (~4500 rpm). The residual solid was again suspended in distilled H_2O (40 mL) and collected by centrifugation (repeated 3×). The resultant solid, which contained a small amount of residual H_2O, was frozen and lyophilized to dryness. The residual brown solid was purified by SiO_2 chromatography with the mobile phase consisting of a step gradient of 49:1 CH_2Cl_2:MeOH to 44:5:1 CH_2Cl_2:MeOH:NH_3 to provide ImPyPyPy-(R)$^{\beta\text{-CbzHN}}\gamma$-ImPyPyPy-(+)-^{BnO}IPA **23** as a tan solid (217 mg, 87%) after drying under high vacuum. ^{1}H NMR: δ 10.43 (s, 1H), 10.20 (s, 1H), 9.98 (s, 1H), 9.95 (s, 1H), 9.93 (s, 1H), 9.92 (s, 1H), 9.87 (s, 1H), 8.73 (t, J = 5.5 Hz, 1H), 8.41 (app t, J = 1.6 Hz, 1H), 8.11−8.07 (m, 2H), 8.03−7.98 (m, 2H), 7.60 (app t, J = 7.7 Hz, 1H), 7.48−7.32 (m, 7H), 7.30−7.23 (m, 9H), 7.19−7.14 (m, 5H), 7.06 (d, J = 1.7 Hz, 1H), 7.05 (d, J = 1.7 Hz, 1H), 7.03 (d, J = 1.0 Hz, 1H), 6.92 (d, J = 1.2 Hz, 1H), 6.84 (d, J = 1.7 Hz, 1H), 5.37 (s, 2H), 5.00 (m, 2H), 4.11 (m, 1H), 3.99 (s, 3H), 3.95 (s, 3H), 3.85 (s, 3H), 3.84 (m, 6H), 3.83 (s, 3H), 3.79 (s, 3H), 3.77 (s, 3H), 3.30 (m, 4H), 3.18 (m, 2H), 2.53 (m, 2H), 2.35 (m, 4H), 2.16 (br s, 3H), 1.71−1.60 (m, 4H). ^{13}C NMR: δ 167.9, 165.20, 165.16, 161.6, 161.2, 158.50, 158.46, 158.42, 156.1, 155.8, 155.6, 138.8, 137.1, 136.0, 135.2, 134.0, 131.9, 131.6, 129.7, 129.0, 128.5, 128.3, 128.2, 128.1, 128.0, 127.8, 127.7, 127.6, 127.00, 126.99, 126.3, 123.1, 123.0, 122.80, 122.77, 122.75, 122.26, 122.18, 122.14, 122.11, 121.4, 121.2, 118.7, 118.5, 118.0, 117.7, 114.1, 105.0, 104.9, 104.8, 104.5, 104.1, 66.4, 65.2, 55.1, 55.0, 48.8, 42.2, 41.6, 38.3, 37.9, 37.0, 36.2, 36.10, 36.09, 36.07, 35.96, 35.89, 35.1, 34.9, 26.9, 26.6. HRMS (ESI$^+$): calcd for $C_{80}H_{89}N_{22}O_{14}$ [M + H]$^+$, 1581.6929; found, 1581.6992.

ImPyPyPy-(R)$^{\beta\text{-H}_2\text{N}}\gamma$-ImPyPyPy-(+)-IPA (1). A solution of ImPyPyPy-(R)$^{\beta\text{-CbzHN}}\gamma$-ImPyPyPy-(+)-^{BnO}IPA **23** (25 mg, 0.016 mmol) and Pd/C (10 wt % dry, 10 mg) in DMF (2 mL) was stirred at 23 °C for 48 h under H_2 (~1 atm, balloon). [Note: The protecting groups are cleaved at different rates, and early reaction aliquots will reveal a monoprotected compound.] The reaction was then filtered through a Sep-Pak cartridge (5 g of C-18 sorbent), and the Sep-Pak was washed with DMF (4 mL), aqueous MeCN (50%, 20 mL), MeCN (250 mL), and MeOH (250 mL). The filtrate was then concentrated *in vacuo*, purified by reverse-phase HPLC, and lyophilized to dryness. The solid was triturated with anhydrous Et_2O and filtered over a sintered glass funnel. The resultant solid was washed with copious amounts of anhydrous Et_2O and dried *in vacuo* to yield ImPyPyPy-(R)$^{\beta\text{-H}_2\text{N}}\gamma$-ImPyPyPy-(+)-IPA **1** as a light tan solid (18.9 mg, 81%). ^{1}H NMR: δ 10.61 (s, 1H), 10.46 (s, 1H), 9.95 (s, 2H), 9.94 (s, 1H), 9.92 (s, 1H), 9.89 (s, 1H), 9.46 (br s, 1H), 8.82 (t, J = 5.8 Hz, 1H), 8.42 (app t, J = 1.4 Hz, 1H), 8.20 (m, 1H), 8.15 (m, 1H), 8.08 (d, J = 1.7 Hz, 1H), 8.07 (d, J = 1.7 Hz, 1H), 7.97 (m, 3H), 7.60 (app t, J = 7.6 Hz, 1H), 7.45 (s, 1H), 7.40 (d, J = 0.7 Hz, 1H), 7.28 (d, J = 2.0 Hz, 1H), 7.26 (d, J = 1.7 Hz, 1H), 7.22 (d, J = 2.0 Hz, 1H), 7.21 (d, J = 1.7 Hz, 1H), 7.18 (d, J = 1.7 Hz, 1H), 7.16 (m, 2H), 7.15 (d, J = 1.7 Hz, 1H), 7.08 (m, 2H), 7.05 (d, J = 1.0 Hz, 1H), 7.03 (d, J = 1.7 Hz, 1H), 6.94 (d, J = 1.8 Hz, 1H), 3.99 (s, 3H), 3.96 (s, 3H), 3.85 (s, 3H), 3.844 (s, 3H), 3.842 (s, 3H), 3.83 (s, 3H), 3.82 (s, 3H), 3.79 (s, 3H), 3.69 (m, 1H), 3.44 (m, 2H), 3.35 (m, 2H), 3.24 (m, 2H), 3.18−3.12 (m, 2H), 3.11−3.04 (m, 2H), 2.76 (m, 3H), 2.50 (m, 2H, obstructed by NMR solvent), 1.95−1.91 (m, 2H), 1.90−1.83 (m, 2H). ^{13}C NMR: δ 166.84, 166.82, 165.7, 162.1, 161.6, 158.52, 158.49, 158.40, 158.1, 157.8, 156.0, 155.7, 138.7, 135.6, 134.6, 134.2, 131.9, 131.5, 131.0, 128.7, 128.0, 126.9, 126.3, 123.1, 123.0, 122.8, 122.7, 122.5, 122.4, 122.24, 122.16, 122.10, 121.4, 121.1, 118.7, 118.5, 118.3, 118.0, 115.7, 105.0, 104.9, 104.8, 104.54, 104.52, 59.2, 53.3, 48.2, 36.5, 36.2, 36.08, 36.07, 35.99, 35.6, 35.38, 35.35, 35.1, 35.0, 24.3, 24.0. HRMS (ESI$^+$): calcd for $C_{65}H_{77}N_{22}O_{12}$ [M + H]$^+$, 1357.6086; found, 1357.6091.

Calculation of Molar Extinction Coefficients. Solid **1** (0.51 mg, 0.35 μmol) was weighed into a tared vial on a microbalance (Sartorius Micro M4). A molecular weight of 1471.46 was utilized for **1**, which assumes **1** exists as the CF_3CO_2H salt. The material was dissolved in distilled and deionized H_2O (1000 μL), yielding a 0.35 μM stock solution. Fifty-fold dilutions of this stock solution were made into the appropriate solvent systems (Figure 4). Data were collected at 23 °C in a 1 cm quartz cuvette, and molar extinction coefficients are based on the λ_{max} for each solvent system (range: 313−317 nm). The instrument was blanked on each discrete solvent system prior to data collection. Duplicate analysis of each sample yielded similar results (data not shown). A second polyamide stock solution was generated by dissolving solid **1** in 50% MeCN in aqueous CF_3CO_2H (0.1% v/v), followed by 50-fold dilution into the individual solvent systems, and data collection yielded results similar to those shown in Figure 4 (data not shown). DEPC-treated H_2O (RNase- and DNase-free) was purchased from USB Corp. EtOH was absolute grade from Pharmaco-AAPER, and DMSO was molecular biology grade from Sigma-Aldrich. DMF was anhydrous synthesis grade from Sigma-Aldrich, and acetonitrile was HPLC grade from Fisher.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra for synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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