Chapter 11

Solid-Phase Synthesis of DNA-Binding Polyamides Using Safety Catch Hydrazine Resin

The text of this chapter was taken in part from a manuscript coauthored with Alexander Heckel and Professor Peter B. Dervan (Caltech)

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Abstract.

DNA binding energetics and nuclear trafficking profiles of polyamides are influenced in part by the functionality at the C-terminal tail position of the molecule. 4-Hydrazinobenzoyl safety catch resin was chosen as a novel synthetic platform for the development of next generation polyamides. Using the hydrazine resin, a series of polyamides with an array of functionality at the tail position were readily synthesized in moderate to good yields.

Introduction.

Selectively regulating individual genes using therapeutic agents is a formidable problem that remains to be solved. Polyamides that bind predetermined DNA sequences with high affinity and fidelity using modular cofacial pairings of heterocyclic amino acids may offer one solution to this difficult problem.[1-3] Recently, research in our group has been focussed in two key areas: elucidating the mechanisms of cellular entry and localization of polyamides, [4-6] and developing novel heterocyclic amino acids for improved molecular recognition of DNA.[7-11] Common to both goals is the need for a versatile resin platform for the solid-phase synthesis of next generation polyamides. To fully explore the structure-activity relationship between polyamides, the cellular environment, and ultimately DNA, a resin system that possesses two specific traits is required. First, the resin must be tolerant to a wide range of chemical conditions. Second, the installation of a broad range of functionality at the tail position must be facile.

Previously, solid-phase methods using Boc-protected monomers for polyamide synthesis have depended on Boc-β-Ala-PAM resin, which affords a β-alanine-Dp tail at the C-terminus upon cleavage with *N*,*N*-dimethylaminopropylamine (Dp).[12] While the PAM resin is thermally stable and provides polyamides in good yields, functionalization at the tail position is not easily accomplished. Further, the β-Dp tail requires DNA binding sites to be composed of the sequence 5'-WW(N)$_x$W-3' (W = A,T; N = A,T,G,C; x = # of ring pairings) due to a steric clash when the tail is positioned opposite the exocyclic amine of guanine (Figure 11.1).[13] Gene promoter regions are known to have elevated G,C content and the necessity for A,T base pairs flanking the polyamide core is

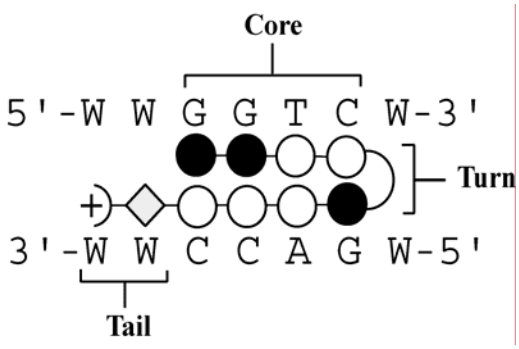


Figure 11.1. Ball and stick representation of polyamide with DNA. Core, tail and turn binding regions are labeled. Facile manipulation of the tail region to target DNA sequences other than 5'-WW(N)$_x$W-3' (W = A, T; N = A, T, G, C x = # or ring pairs) is desirable. Filled circles denote imidazole while open circles represent pyrrole. The diamond represents β-alanine and dimethylaminopropylamine (Dp) is indicated by a half circle with a plus sign.

clearly a limitation.[14, 15] In addition, unpublished results demonstrate that in several cases, the β-Dp tail may negatively effect nuclear localization of polyamides.

To address the limitation of the PAM resin, Belitsky et al. adapted polyamide synthesis to work with the Kaiser oxime resin.[16-18] This resin allows for the incorporation of a variety of truncated tails, removing the DNA sequence requirements mandated by the PAM resin. However, the oxime resin is intolerant of extended acid treatment, elevated coupling temperatures and many nucleophilic reagents. Thus, many of the novel heterocycles and polyamide conjugates that are under development require solid phase conditions that are incompatible with the oxime resin.

The synthesis of polyamides on a safety catch sulfonamidobutyryl (SAB) resin has also been recently reported in the literature by Fattori et al.[19] The SAB resin was used to prepare polyamides with amino acyl tails similar to Dp, and thioester tails as a prerequisite functionality for Native Chemical Ligation. While satisfactory yields were reported, the ability to synthesize a series of polyamides with a variety of truncated tails was not demonstrated. Thus, the polyamides prepared on the SAB resin exhibit the same DNA sequence restrictions as those prepared on the PAM resin, requiring polyamide

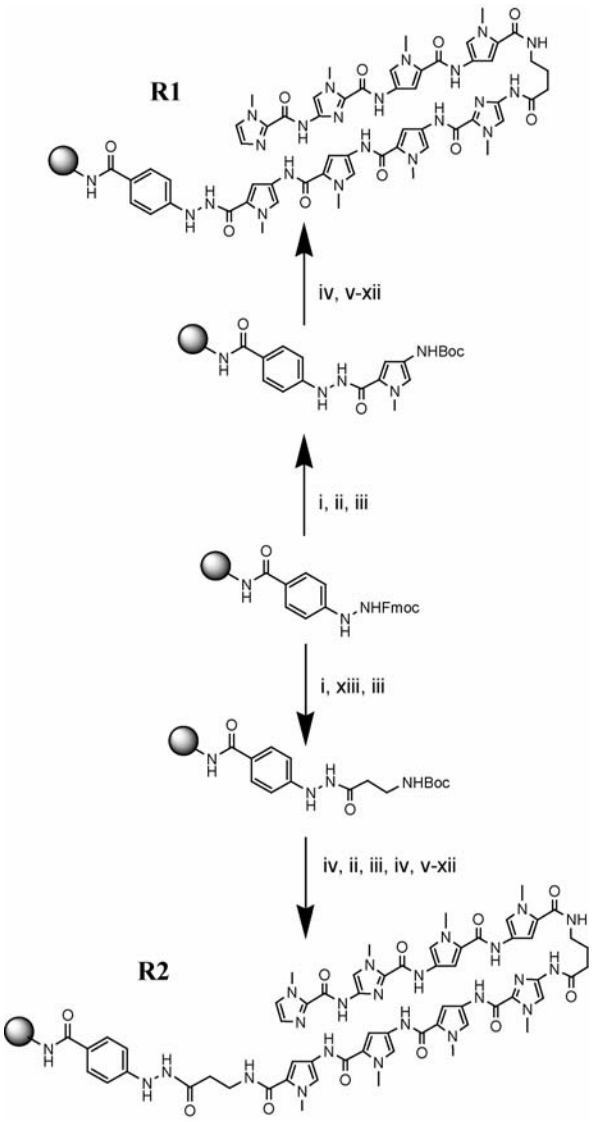


Figure 11.2. Solid phase synthesis of Im-Im-Py-Py-γ-Im-Py-Py-Py-HN-NH-Res (**R1**) and Im-Im-Py-Py-γ-Im-Py-Py-Py-β-HN-NH-Res (**R2**). **(**i) 20% piperedine/DMF; (ii) Boc-Py-OBt, DIEA, DMF; (iii) 10% pivalic anhydride, DIEA, DMF; (iv) 80% TFA/DCM; (v) repeat steps ii-iv (x2); (vi) Boc-γ-Im-OH, HBTU, DIEA, DMF; (vii) 80% TFA/DCM; (viii) repeat steps ii-iv (x2); (ix) Boc-Im-OH, HBTU, DIEA, DMF; (x) 10% pivalic anhydride, DIEA, DMF; (xi) 80% TFA/DCM; (xii) Im-$COCCl_3$, DIEA, DMF; (xiii) Boc-β-OH, HBTU, DIEA, DMF.

binding sites to conform to 5'-$WW(N)_xW$-3' as described above. Furthermore, activation of the SAB safety catch linker requires the use of strong alkylating reagents such as diazomethane, TMS-diazomethane, or iodoacetonitrile, a substantial limitation.

Results/Discussion.

As a possible alternative, we have found that the commercially available hydrazine resin combines the best qualities of both PAM and Oxime resins, and is a substantial improvement over the SAB resin. The hydrazine resin is stable to both strong acids and bases, making it ideal for both Boc and Fmoc chemistry. Also, the resin is stable to coupling at elevated temperatures (40 ^oC) for prolonged periods of time (48 h), a prerequisite for the

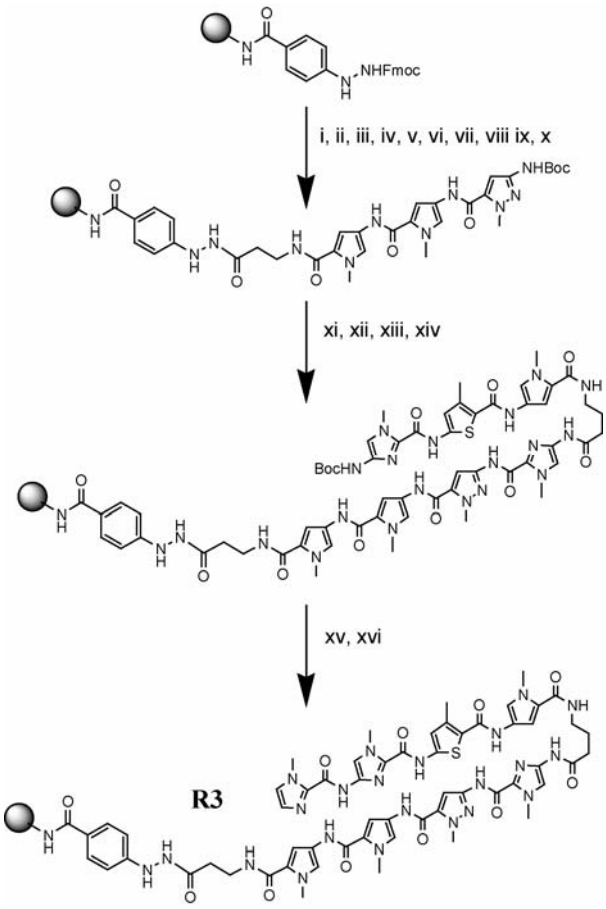


Figure 11.3. Solid Phase Synthesis of Im-Im-**Tn**-Py-γ-Im-**Pz**-Py-Py-HN-NH-Res (**R3**). (i) 20% piperedine/DMF; (ii) Boc-β-OH, HBTU, DIEA, DMF; (iii) 10% pivalic anhydride, DIEA, DMF; (iv) 80% TFA/DCM; (v) Boc-Py-OBt, DIEA, DMF; (vi) 10% pivalic anhydride, DIEA, DMF; (vii) 80% TFA/DCM; (viii) repeat steps v-vii. (x1); (ix) Boc-Pz-OH, HBTU, DIEA, DMF, couple 24 h. at 40 ^{o}C; (x) 10% pivalic anhydride, DIEA, DMF; (xi) 80% TFA/DCM; (xii) Boc-γ-Im-OH, HBTU, DIEA, DMF; (xiii) repeat steps iii-vii (x1); (xiii) Boc-Im-Tn-OH, HBTU, DIEA, DMF, couple 48 h. at 40 ^{o}C; (xiv) 10% pivalic anhydride, DIEA, DMF; (xv) 80% TFA/DCM; (xvi) Im-$COCCl_3$, DIEA, DMF.

incorporation of many novel heterocycles.[9, 10] Finally, the hydrazine safety-catch linker is activated using a mild chemoselective oxidation, negating unwanted side reactions with the polyamide. Using the hydrazine resin as a platform for polyamide synthesis, polyamide resins **R1** and **R2** were synthesized using standard Boc/Fmoc protocols and previously described monomers (Figure 11.2).[12] The Boc-protected heterocyclic amino acids were pre-activated with DCC/HOBt or were activated in situ using HBTU. Following activation, coupling proceeded at room temperature for 1.5 h. Following coupling, capping was accomplished with pivalic anhydride to avoid acylation of the hydrazine linker.

To further demonstrate the utility of the hydrazine resin, as well as its stability to rigorous coupling conditions, polyamide resin **R3** was synthesized using novel pyrazole

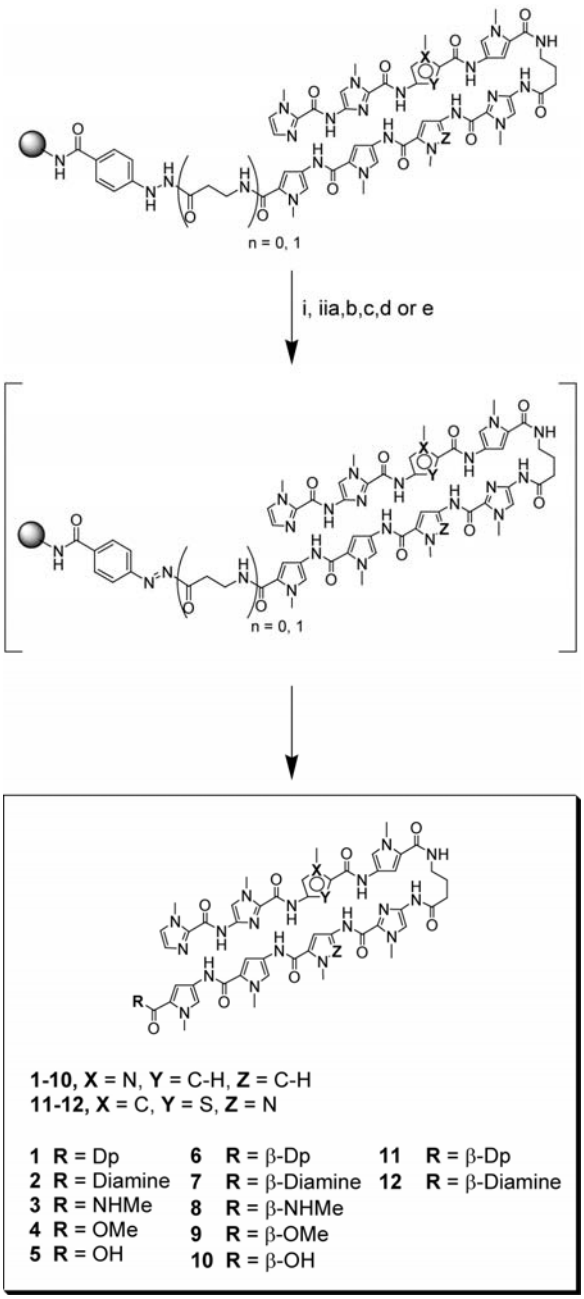


Figure 11.4. Cleavage of polyamides from hydrazine resin. (i) $Cu(OAc)_2$ (40 mol%); (iia) Dp, pyridine; (iib) $MeN((CH_2)_3NH_2)_2$, pyridine; (iic) $MeNH_2$/THF, pyridine; (iid) MeOH/DMF, pyridine; (iie) THF/TEMED/H_2O, DBU, LiBr. β = beta-Alanine, Dp = $NH(CH_2)_3N(CH_3)_2$, Diamine = $NH(CH_2)_3NCH_3(CH_2)_3NH_2$.

(**Pz**) and thiophene (**Tn**) heterocyclic amino acids (Figure 11.3).[9, 10] Incorporation of the **Pz** and **Tn** heterocycles onto the growing polyamide chain was accomplished using HBTU activation, however, coupling temperatures of 40 ^oC for periods of 24-48 h were required. Due to the inability of the oxime resin to accommodate these conditions, the synthesis of next generation polyamides containing novel heterocycles like those mentioned above must proceed using either the PAM, SAB, or hydrazine resins. Of these resins, only the hydrazine resin allows for the facile incorporation of a broad range of functionality at the polyamide C-terminus.

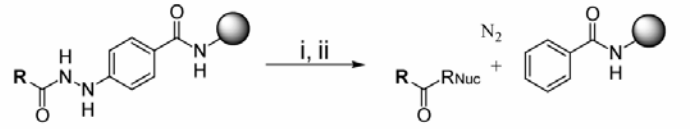
After installation of the terminal monomer, resins **R1**, **R2,**

and **R3** were subject to a mild oxidation with copper acetate. While several other oxidative conditions have been reported in the literature, the copper acetate conditions worked cleanly and were chemoselective for the hydrazine linker. [20, 21] Upon oxidation, the linker is activated and the polyamide may be cleaved from the resin using a variety of nucleophiles to provide esters, amides, or free carboxylic acid polyamides **1-12** (Figure 11.4).

Thus, the hydrazine resin offers the stability of the PAM resin while maintaining the functionality of the oxime resin. Further, while the PAM and Oxime resins require heating and the use of nucleophiles in large excess (often neat), the hydrazine resin can be cleaved at mild temperatures with near-stochiometric quantities of nucleophile. Lower cleavage temperatures may allow for the synthesis of compounds that have previously been found to be unstable at elevated temperatures due to nucleophilic fragmentation. More specifically, electron-rich heterocycles, such as 5-membered oxazole carboxamides, when treated with strong nucleophiles at elevated temperatures are subjected to decomposition by nucleophilic addition to the carboxamide bond. Use of near stoichiometric reagents for resin cleavage also provides two significant benefits. First, treating the resin with nucleophile/DMF mixtures may allow solid nucleophiles of increasing molecular complexity, such as nuclear localization signals or other biologically relevant small molecules, to be directly incorporated at the tail position of the polyamide. This benefit has significant implications for nuclear trafficking studies.[4-6] Second, reagents that would otherwise have to be used in large excess are readily conserved, making cleavage with expensive and highly functionalized reagents economically feasible.

Following cleavage from the resin, polyamides were diluted in 0.1% TFA and purified by reverse-phase preparatory HPLC. Compounds were then characterized using matrix-assisted, laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). In all cases, final recoveries of polyamides from the hydrazine resin were competitive with yields reported for PAM and Oxime resins (Figure 11.5).

Conclusion.



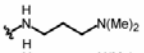
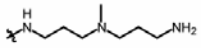
Cleavage Mixture[a]	RNuc	%Yield[b] R = Aryl/Alkyl
Dp, py		11.2 / 12.1
DMF/Dp (1.5 eq.), py		10.6 / 11.0
$MeN((CH_2)_3NH_2)_2$, py		9.3 / 10.3
THF/$MeNH_2$, py		9.4 / 11.4
1:1 DMF/MeOH, py		10.1 / 8.6
1:1:1 THF/TEMED/H_2O, DBU, LiBr		10.1 / 9.7

Figure 11.5. Yield of polyamides following linker oxidation and nucleophilic cleavage from hydrazine resin. (i) $Cu(OAc)_2$ (40 mol%), (ii) Cleavage mixture. [a]Dp = dimethylamino propylamine, py = pyridine, DBU = diazabicycloundecene. TEMED = *N*,*N*,*N*',*N*' - tetramethylethylenediamine. [b]Yield of pure compound after recovery off HPLC.

In conclusion, we have found that the hydrazine resin is a novel and highly useful platform for polyamide synthesis. The hydrazine resin effectively addressed the weaknesses of previously described PAM, Oxime and SAB resins. Namely, the hydrazine resin is stable to elevated coupling temperatures, is amenable to both Boc and Fmoc chemistry, and is cleaved rapidly at moderate temperatures by a wide range of nucleophiles following a mild and selective oxidation protocol. Resin cleavage provides polyamides with a wide range of C-terminus functionality in competitive yields. Going forward, this resin may prove useful in the preparation of next-generation polyamides that contain novel heterocycles and tail

functionalities that enhance both molecular recognition of the DNA and the nuclear trafficking and permeability properties within the cellular environment.

Acknowledgments.

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Experimental.

General. *N*,*N*-dimethylformamide (DMF), *N*,*N*-diisopropylethylamine (DIEA), *N*,*N*-dimethylaminopropylamine (Dp), methylamine (2M in THF), pyridine, and copper(II) acetate were purchased from Aldrich. *N*,*N*,*N*',*N*'-tetramethylethylenediamine (TEMED) was purchased from Invitrogen. 4-Fmoc-Hydrazinobenzoyl AM NovaGel Resin, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), and Boc-β-alanine were purchased from NOVA Biochem. Trifluoroacetic acid (TFA) was purchased from Halocarbon. All other solvents were reagent-grade from EM. All reagents were used without further purification. All heterocyclic Boc-protected amino acids were prepared as previously described in the literature.[9, 10, 12]

UV spectra were measured on a Hewlett-Packard Model 8452A diode array spectrophotometer. Matrix-assisted, laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) was conducted at the Mass Spectroscopy Facility at the California Institute of Technology.

Representative Solid-Phase Synthesis.

Im-Im-Py-Py-γ-Im-Py-Py-Py-HN-NH-Resin **R1** was generated by manual solid-phase synthesis from Fmoc-Hydrazinobenzoly AM NovaGel resin (0.5 g at 0.45 meq/g) using previously described Boc-protected monomers.[12] The resin was placed into a siliconized shaker and a solution of piperidine in DMF (20%) was drained over the resin for 15 sec., followed by shaking for 25 min to provide the free hydrazine amine **H_2N**-NH-Resin. The **Py** monomer was loaded onto the resin using pre-activated Boc-Py-OBt (291 mg, 0.785 mmol), in DIEA (204 mg, 274 μl, 1.58 mmol) and DMF (1.3 mL). The mixture was added to the reaction vessel containing **H_2N**-NH-Resin, and coupling was allowed to proceed for 12 h at room temperature. The resin was then drained and washed with DMF. Acetylation of the resin was accomplished by shaking in a mixture of 10% pivalic anhydride and DIEA (2 equiv.) in DMF for 15 min. Note: pivalic anhydride is substituted for acetic anhydride to reduce the risk of acylating the hydrazine linker. However, treatment of the resin with acetic anhydride does not seem to affect the linker to any appreciable extent. The resin was acylated following each round of coupling. The resin was drained and washed with DCM, and the Boc-group removed upon treatment with 80% TFA in DCM for 25 min. The resin was filtered and washed with DCM, followed by additional washing with 10% DIEA in DMF to neutralize the free amine, providing H_2N-**Py**-HN-NH-Resin for further coupling. The additional **Py** monomers were incorporated as described above, but complete coupling was achieved in 1.5 h, followed by the acetylation and deprotection steps also outlined. The **γ-Im** dimer was incorporated by activating Boc-γ-Im-OH (257 mg, 785 μmol) with HBTU (284 mg, 745

μmol), DIEA (204 mg, 274 μL, 1.58 mmol) and DMF (1.3 mL) for 10 min. at room temperature. The mixture was then added to the reaction vessel containing the H_2N-Py-Py-Py-HN-NH-Resin, and coupled for 1.5 h at room temperature, followed by acetylation and deprotection to provide H_2N-γ-Im-Py-Py-Py-HN-NH-Resin. Additional **Py** monomers were incorporated as described above. The penultimate **Im** monomer was incorporated as described for the γ-Im dimer. The terminal **Im** cap was installed using Im-$COCCl_3$ (510 mg, 2.25 mmol), in DIEA (116 mg, 157 μL, 900 μmol), and DMF (2 mL). The mixture was added to the reaction vessel containing the H_2N-Im-Py-Py- γ-Im-Py-Py-Py-HN-NH-Resin, and coupled for 2 h at 40 ^oC to provide Im-Im-Py-Py- γ-Im-Py-Py-Py-HN-NH-Resin **R1**.

Im-Im-Py-Py-γ-Im-Py-Py-Py-β-HN-NH-Resin **R2** was synthesized as described above. However, β is loaded onto the resin by activating Boc-β-OH (300 mg, 1.57 mmol) with HBTU (567 mg, 1.49 mmol), DIEA (407 mg, 548 μL, 3.15 mmol) and DMF (2.5 mL) for 10 min. at room temperature. The mixture was then added to the deprotected **H_2N**-NH-Resin, and coupling was allowed to proceed for 12 h at room temperature.

Im-Im-**Tn**-Py-γ-Im-**Pz**-Py-Py-β-HN-NH-Resin **R3** was synthesized as described above for **R2** and **R3**. However, the Boc-**Pz**-OH monomer is loaded onto the resin by activating Boc-**Pz**-OH (190 mg, 788 μmol) with HBTU (283 mg, 748 μmol), DIEA (203 mg, 274 μL, 1.58 mmol) and DMF (2.5 mL) for 10 min. at room temperature.[3a] The mixture was then added to the deprotected **H_3N**-Py-Py-β-HN-NH-Resin, and coupling was allowed to proceed for 24 h at 40 ^oC. The Boc-Im-**Tn**-OH dimer (257 mg, 675 μmol) was activated with HBTU (243 mg, 641 μmol), DIEA (174 mg, 235 μL, 1.35

mmol) and DMF (2.0 mL) for 10 min. at room temperature.[3b] The mixture was then added to the deprotected **H_3N**-Py-γ-Im-**Pz**-Py-Py-β-HN-NH-Resin, and coupling was allowed to proceed for 48 h at 40 ^oC.

Cleavage Procedures.

General Notes: Using 40-100 mol% catalyst works well and provides the greatest yields. Cleavages attempted using less than 40% $Cu(OAc)_2$ provided lower yields. Cleavage times may be extended longer than 4 h and if weaker nucleophiles are being used, longer cleavage times may be necessary to maintain good yields. Over oxidation of the polyamide was never observed by mass spec. All samples were purified by reversed phase HPLC using a Beckman system and Waters C18 column (Eluent A = 0.1% TFA and B = ACN). At a flow rate of 8 mL/min, the method consisted of sample injection at 0% A and ramping to 60% B over 180 min. Lyophilization of the appropriate fractions provided the following compounds. In all cases, yields were competitive with the previously published protocols for Pam and Oxime resins.[12, 16]

Dp Tail: $NH(CH_2)_3N(CH_3)_2$, (polyamides **1**, **6** and **11**): To the resin (0.1 g at 0.45 meq/g) was added Dp (1 mL) followed by $Cu(OAc)_2$ (3.2 mg, 18 μmol), and pyridine (5.3 mg, 5.4 μL, 67 μmol). The resin was shaken well at room temperature for 4 h and then filtered into a 15 mL falcon tube. The resin filtrate was then diluted to a total volume of 8 mL using 0.1% TFA and purified by reverse-phase HPLC to provide Dp tail polyamides **1**, **6** and **11**. Im-Im-Py-Py-γ-Im-Py-Py-Py-**Dp** (**1**) (5.8 mg, 11.2% recovery) as a fine white powder. MALDI-TOF-MS, 1152.52 (M+H calcd. for 1152.54 $C_{54}H_{66}N_{21}O_9$). Im-Im-Py-Py-γ-Im-Py-Py-Py-**β-Dp** (**6**) (6.7 mg, 12.1% recovery) as a fine

white powder. MALDI-TOF-MS, 1223.57 (M+H calcd for 1223.57 $C_{57}H_{71}N_{22}O_{10}$). Im-Im-**Tn**-Py-γ-Im-**Pz**-Py-Py-**β-Dp** (**11**) (3.1 mg, 5.6% recovery) as a fine white powder. MALDI-TOF-MS, 1241.54 (M+H calcd. for 1241.53 $C_{56}H_{69}N_{22}O_{10}S$). Alternatively, the resin may be cleaved using only a slight excess of nucleophile (1.5-2 equiv of Dp 0.25 M in DMF), followed by shaking at room temperature over night.

Diamine Tail: $NH(CH_2)_3NCH_3(CH_2)_3NH_2$, (polyamides **2, 7** and **12**): To the resin (0.1 g at 0.45 meq/g) was added $MeN(CH_2CH_2NH_2)_2$ (1 mL) followed by $Cu(OAc)_2$ (12 mg, 65 μmol), and pyridine (19.5 mg, 20 μL, 248 μmol). The resin was shaken well at room temperature for 12 h and then filtered into a 15 mL falcon tube. The resin filtrate was then diluted to a total volume of 8 mL using 0.1% TFA and purified by reverse phase HPLC to provide diamine tail polyamides **2, 7,** and **12**. Im-Im-Py-Py-γ-Im-Py-Py-Py-**Diamine** (**2**) (4.9 mg, 9.3% recovery) as a fine white powder. MALDI-TOF-MS, 1195.58 (M+H calcd for 1195.58 $C_{56}H_{71}N_{22}O_9$). Im-Im-Py-Py-γ-Im-Py-Py-Py-**β-Diamine** (**7**) (5.7 mg, 10.3% recovery) as a fine white powder. MALDI-TOF-MS, 1266.63 (M+H calcd for 1266.61 $C_{59}H_{76}N_{23}O_{10}$). Im-Im-**Tn**-Py-γ-Im-**Pz**-Py-Py-**β-Diamine** (**12**) (2.0 mg, 4.4% recovery) as a fine white powder. MALDI-TOF-MS, 1256.55 (M+H calcd. for 1284.57 $C_{58}H_{74}N_{23}O_{10}S$).

Methylamide Tail: NHMe, (polyamides **3** and **8**): To the resin (0.1 g at 0.45 meq/g) was added 2M methylamine in THF (1 mL) followed by $Cu(OAc)_2$ (3.2 mg, 18 μmol), and pyridine (5.3 mg, 5.4 μL, 67 μmol). The resin was shaken well at room temperature for 4 h and then filtered into a 15 mL falcon tube. The resin filtrate was then diluted to a total volume of 8 mL using 0.1% TFA and purified by reverse-phase HPLC to provide **NHMe** tail polyamides **3** and **8**. Im-Im-Py-Py-γ-Im-Py-Py-Py-**NHMe** (**3**) (4.6

mg, 9.4% recovery) as a fine white powder. MALDI-TOF-MS, 1081.48 (M+H calcd for 1081.46 $C_{50}H_{57}N_{20}O_9$). Im-Im-Py-Py-γ-Im-Py-Py-Py-**β-NHMe** (**8**) (5.8 mg, 11.4% recovery) as a fine white powder. MALDI-TOF-MS, 1152.48 (M+H calcd. for 1152.50 $C_{53}H_{62}N_{21}O_{10}$).

Methoxy Tail: OMe, (polyamides **4** and **9**): To the resin (0.1 g at 0.45 meq/g) was added a 1:1 mixture of anhydrous methanol and DMF (1 mL) followed by $Cu(OAc)_2$ (6.2 mg, 32 μmol), and pyridine (10.6 mg, 10.8 μL, 134 μmol). The resin was shaken well at room temperature for 12 h and then filtered into a 15 mL falcon tube. The addition of DMF promotes improved swelling of the resin. Of note, using a protic solvent mixture seems to decrease the solubility of the copper species and longer cleavage times (8-12 h) are necessary for maintaining good yields. The resin filtrate was then diluted to a total volume of 8 mL using 0.1% TFA and purified by reverse phase HPLC to provide **OMe** tail polyamides **4** and **9**. Im-Im-Py-Py-γ-Im-Py-Py-Py-**OMe** (**4**) (5.0 mg, 10.1% recovery) as a fine white powder. MALDI-TOF-MS, 1082.37 (M+H calcd for 1082.45 $C_{50}H_{56}N_{19}O_{10}$). Im-Im-Py-Py-γ-Im-Py-Py-Py-**β-OMe** (**9**) (4.5 mg, 8.6% recovery) as a fine white powder. MALDI-TOF-MS, 1153.51 (M+H calcd. for 1153.48 $C_{53}H_{61}N_{20}O_{11}$).

Carboxy Tail: OH, (polyamides **5** and **10**): **Procedure 1.** To the resin (0.1 g at 0.45 meq/g) was added a 1:1 mixture of water and DMF (1 mL) followed by $Cu(OAc)_2$ (6.2 mg, 32 μmol), and pyridine (10.6 mg, 10.8 μL, 134 μmol). The resin was shaken well at room temperature for 12 h and then filtered into a 15 mL falcon tube. The addition of DMF promotes improved swelling of the resin. Again, using a protic solvent mixture seems to decrease the solubility of the copper species and longer cleavage times

(8-12 h) are necessary for maintaining good yields. The resin filtrate was then diluted to a total volume of 8 mL using 0.1% TFA and purified by reverse phase HPLC to provide **OH** tail polyamides **5** and **10**. Im-Im-Py-Py-γ-Im-Py-Py-Py-**OH** (**5**) (3.4 mg, 7.0% recovery) as a fine white powder. MALDI-TOF-MS, 1068.51 (M+H calcd for 1068.43 $C_{49}H_{54}N_{19}O_{10}$). Im-Im-Py-Py-γ-Im-Py-Py-Py-β-**OH** (**10**) (3.8 mg, 7.4% recovery) as a fine white powder. MALDI-TOF-MS, 1139.53 (M+H calcd for 1139.47 $C_{52}H_{59}N_{20}O_{11}$). Alternatively, a second procedure was optimized to increase yields. **Procedure 2.** To the resin (0.1 g at 0.45 meq/g) was added a 1:1:1 mixture of THF:H_2O:TEMED (2 mL), followed by $Cu(OAc)_2$ (12 mg, 68 μmol), LiBr (23 mg, 270 μmol) and DBU (12.3 mg, 12 μL, 81 μmol). The resin was then shaken at 37 ^oC for 72 h. Purification as described above provided **5** and **10** in 10.1% and 9.7% yield respectively. In procedure 2 TEMED is substituted for pyridine and appears to improve the solubility of the copper species in solution. The LiBr/DBU mixture has been previously used for saponifying or transesterifying cleavages from PAM and Wang resins.[22]

Monitoring Polyamide Synthesis. To monitor the progress of the resin synthesis, a small amount of resin (3-5 mg) may be taken from the vial and placed in a 1.7 mL centrifuge tube. Cleavage conditions are scaled within reason. Often, a significant excess of catalyst, pyridine and nucleophile are used due to the difficulty of weighing out μg quantities of material. Analytical samples are then vortexed and allowed to stand at room temperature for 2 h. Samples are then diluted in 0.1% TFA (100-120 μL), filtered and loaded onto the analytical HPLC.

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