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Parallel Synthesis of H-pin Polyamides by Alkene Metathesis on Solid Phase

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Abstract: A small library of H-pin polyamides with variable aliphatic bridge lengths $(CH_2)_n$, where $n = 4-8$, connecting a central Py/Py pair was prepared via parallel synthesis with Ru-catalyzed alkene metathesis on solid phase as a complexity-generating cross-linking reaction. DNA binding affinities and sequence specificities were analyzed for each member of the library to determine the optimum linker length. An H-pin polyamide with a six-methylene bridge was found to have the highest affinity to its match site with high selectivity over a 1-bp mismatch site. The relationship between the number of methylenes in the linker $(CH_2)_n$ and affinity is $n = 6 > 4 > 7 > 5 > 8$. These results indicate that 6 followed by 4 methylene-bridged polyamides represent the optimum spacer length for the H-pin motif in the DNA minor groove. Importantly, the H-pin is competitive with hairpin polyamides with respect to affinity and specificity. The metathesis-based convergent synthetic route to H-pin polyamides expands the scope of readily available DNA recognition motifs for small molecule-based gene regulation studies.

Introduction

The development of DNA-binding ligands capable of targeting a broad range of sequences with high affinity and sequence specificity is essential for the regulation of gene expression by chemical methods.[1] Synthetic polyamides comprising *N*-methylpyrrole (Py), *N*-methylimidazole (Im), and 3-hydroxypyrrole (Hp) amino acids represent a set of molecular recognition tools with potential for transcriptional modulation,[2a−e] chromatin modification,[2f] promoter scanning,[2g] and chromosome mapping.[2h,i] Recognition of a specific DNA sequence by two antiparallel polyamide strands depends on a code of side-by-side aromatic amino acid pairs in the minor groove, usually oriented N to C with respect to the 5′ to 3′ direction of the DNA helix.[3] Enhanced affinity and specificity of polyamide DNA binding is accomplished by covalently linking the antiparallel strands.[4−6] The "hairpin motif" connects the N and C termini of the two strands with a γ-aminobutyric acid unit (γ-turn) to form a folded *linear* chain.[4] The "H-pin motif" connects the antiparallel strands across a central ring/ring pair[5] by a short, flexible polymethylene bridge (Figure 1). The *branched* nature of H-pin polyamides made solid-phase synthesis of this motif less convenient, hindering a broad exploration of its potential regarding DNA-binding properties and biological applications. In this paper, we present a convergent synthesis of H-pin polyamides based on alkene metathesis as the key step for cross-linking the two polyamide strands. To investigate the optimum distance for the flexible linker, we used this metathesis strategy to create a small library of H-pin polyamides with incrementally increasing aliphatic linker lengths, from $(CH_2)_4$ to $(CH_2)_8$ (Figure 2).[6] The parallel synthesis allows the preparation of five 10-ring H-pin polyamides **1**−**5**. A control hairpin polyamide ImPyPyPyPy-γ-ImPyPyPyPy-β-Dp **6** was also synthesized by standard solid-phase protocol.[7] Subsequent DNase I footprint titration experi-

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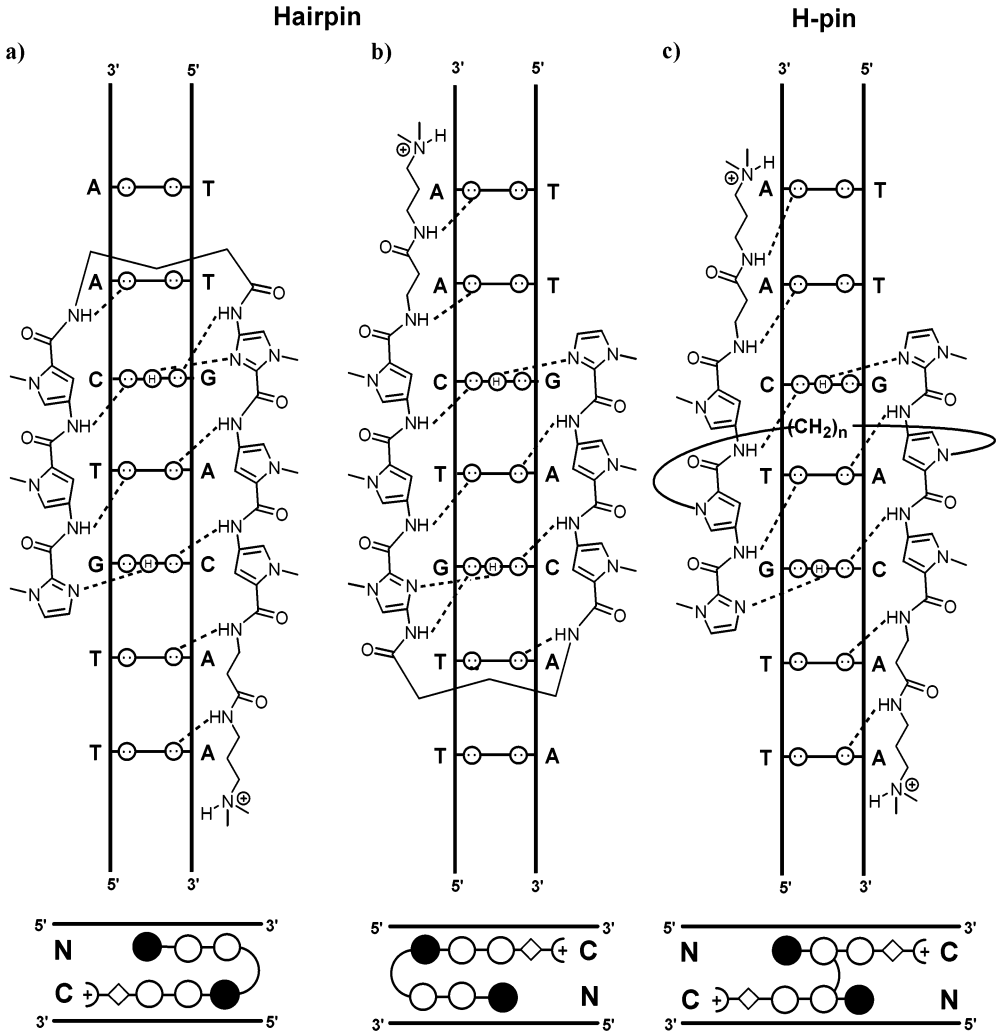

Figure 1. DNA binding model for two polyamide motifs utilizing antiparallel pyrrole−imidazole strands: asymmetric hairpin (a, b) and symmetric H-pin (c). (Top) Circles with dots represent lone pairs of N3 of purines and O2 of pyrimidines. Circles containing H represent the N2 hydrogens of guanine. Putative hydrogen bonds are illustrated by dotted lines. (Bottom) Solid circles represent imidazoles, open circles represent pyrroles, and diamonds denote β-alanines. The aliphatic linkers of the H-pin and the γ-aminobutyric acid of the hairpin are depicted as curved lines. Curved lines with plus signs represent *N*,*N*-[(dimethylamino)propyl]amine tails.

ments allowed the relationship of bridge distance to be correlated with binding affinity and specificity.

Results and Discussion

Parallel Synthesis via Metathesis on Resin. Solid-phase syntheses of pyrrole−imidazole polyamides involve stepwise addition of an activated ester of the corresponding protected amino acid to the resin-bound amine at the end of the growing polyamide chain.[7] This approach, useful in the preparation of linear hairpin polyamides, has limitations when applied to the synthesis of certain branched structures, such as H-pin polyamides. In the synthetic strategy described here, we start with simultaneous synthesis of the two C-terminal sections of the H-pin on solid support, followed by the cross-linking reaction of a middle monomer on resin. After the cross-linking step, the synthesis can be continued with the appropriate terminal residues being added to the growing chain in the usual manner. We chose olefin metathesis using the Grubbs catalyst as the cross-linking reaction,[8] due to the ease of preparation of the alkenyl−pyrrole precursors and the high functional group tolerance of the Ru catalyst.[9] This method has been used previously in the solution-phase synthesis of dimeric molecules[10] and libraries of homo- and heterodimeric compounds[11] and in the solid-phase synthesis of natural products.[12]

Cross-linking via intrasite interactions on solid support have been demonstrated,[13] albeit infrequently, since the introduction of solid-phase chemistry. Despite the initial belief about the paucity of these interactions, cross-reactivity between the individual peptide chains, in some cases with excellent yields,

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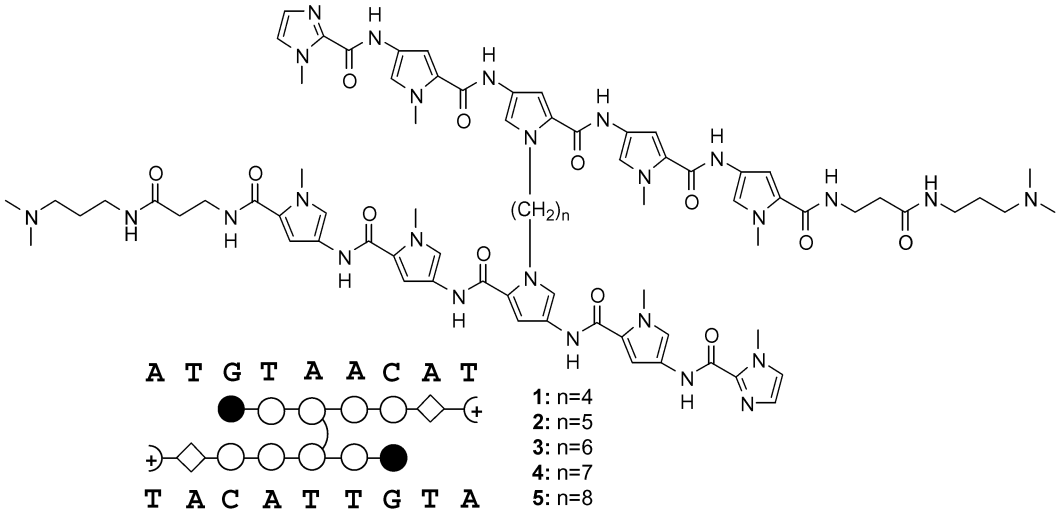

Figure 2. (Top) Structures of 10-ring H-pin polyamides with aliphatic linkers of 4−8 methylenes. (Bottom) Schematic illustration of H-pin polyamide bound to predetermined match site (symbols are as described for Figure 1).

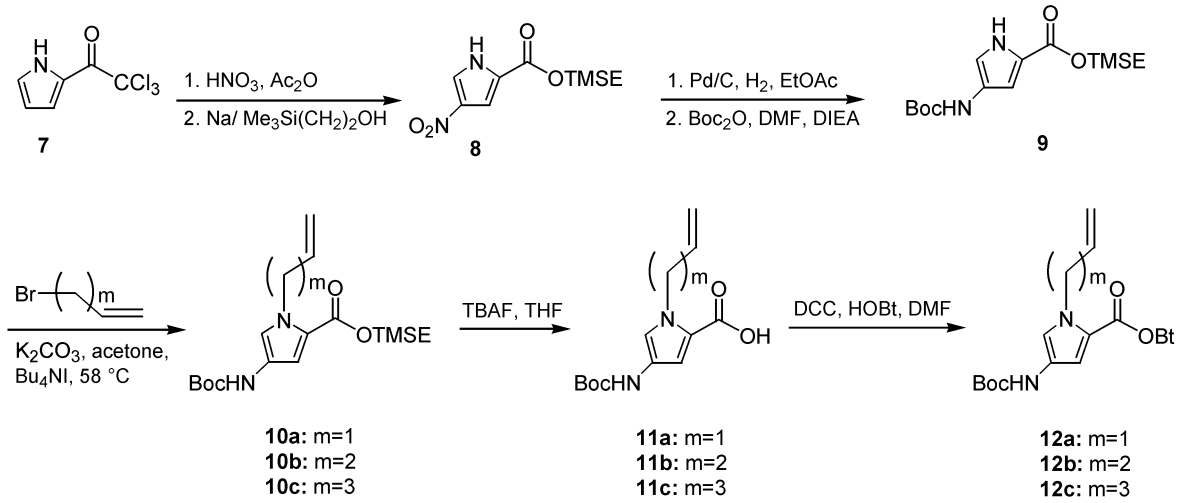

Figure 3. Synthesis of the pyrrole precursors containing variable alkenyl chains.

was quickly established and became an obstacle in synthesis of large peptide fragments. To circumvent these problems, resins with lower loading (0.05−0.2 mmol/g) and a high degree of cross-linking with divinylbenzene (10−20%) were employed, sometimes in solvents and conditions that prevent maximal swelling of the resin. Therefore, we chose commercially available PAM resins with a low degree of cross-linking (∼1% divinylbenzene) and high loads (0.88−1.2 mmol/g) with dichloromethane as a solvent to obtain high cross-linking yields. Similar conditions have been reported in solid-phase synthesis of libraries of dimeric molecules with short peptide backbones.[11e]

When two different terminal alkenes with similar reactivities toward the catalyst are employed in the olefin cross-metathesis, the result is a mixture of three products: two homodimers and one heterodimer. This often unwanted side effect of the typical cross-metathesis reaction could be an advantageous step when mixed products are desirable. Indeed, if several terminal alkenes with variable chain lengths are used simultaneously, then upon completion of the metathesis reaction, a mixture of homo- and heterodimeric products resulting from all possible combinations of chain lengths in precursors will be formed. Therefore, for our purposes, a library of five different cross-linked products can be generated from three common precursors.

Three N-alkenyl substituted pyrrole amino acids **11a**−**c** were synthesized as described in Figure 3. Nitration of the commercially available 2-trichloroacetyl-1*H*-pyrrole **7**, followed by haloform reaction, provided 1,1,1-trimethylsilylethyl (TMSE) ester **8**. It was hydrogenated and converted into Boc-protected amine **9**, which was then separately alkylated with three alkenyl bromides resulting in TMSE esters of alkenyl pyrroles **10a**−**c**. They were deprotected with TBAF to yield the acids **11a**−**c**. A mixture of corresponding activated esters **12a**−**c** was added to the growing chain containing two pyrrole monomers **13** attached

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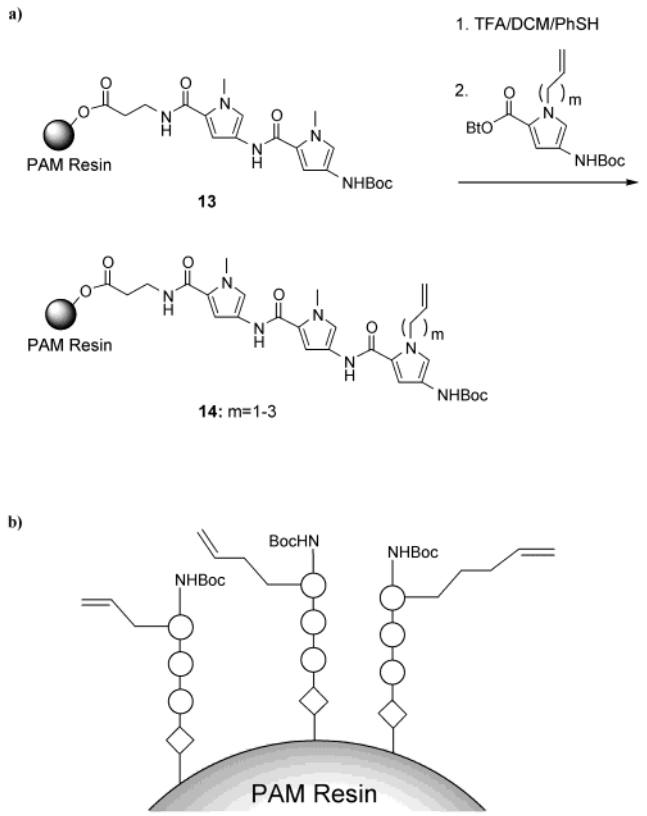

Figure 4. (a) Preparation of a resin-bound mixture of three-ring polyamides via standard solid-phase synthetic protocols utilizing Boc chemistry. (b) A mixture of three pyrrole precursors with variable alkenyl chains lengths was added in the final step.

to the β-alanine-PAM resin (Figure 4) in accordance with previous solid-phase procedures for polyamides.[7] Treatment of alkenyl-derivatized resin **14** with 12−18 mol % of Grubbs's ruthenium benzylidene catalyst[8,9] in dichloromethane under Ar, followed by an additional 12 mol % catalyst and 12 h under Ar and rigorous washing, produced a small library of cross-metathesis products (Figures 5 and 6). The time course of the reaction and the purity of the resulting library products were monitored by cleaving a resin sample with *N*,*N*′-[(dimethylamino)propyl]amine (Dp) followed by HPLC analysis and identification of each individual peak by MALDI-TOF mass spectrometry. After satisfactory conversion of the individual chains to cross-linked dimers, the resin was washed with dichloromethane and treated with 1,3-bis(diphenylphosphino)propane (dppp) in dichloromethane to remove catalyst decomposition products, and the solid-phase synthesis was continued. After deblocking, activated pyrrole monomer was added and the coupling was carried out as before, followed by another deblocking and coupling of an activated imidazole acid. The solid-phase synthesis was concluded by cleaving the contents of the resin with Dp (Figure 6). As expected, after cross-linking the resin exhibited a lesser degree of swelling in organic solvents, but it is worth noting that this did not preclude successful coupling in the subsequent steps. After cleavage from the solid support, the excess of cleaving diamine was removed first in high vacuum and subsequently by passing the crude mixture dissolved in 50% acetonitrile−0.1% aqueous TFA through a Sep-Pak (Waters). The products were reduced by use of a mixture of palladium acetate−ammonium formate in ethanol. H-pin polyamides of sequence composition (ImPyPyPyPy-β-Dp)$_2$(CH$_2$)$_{4-8}$ **1**−**5** were separated by semipreparative HPLC and their masses were confirmed by MALDI-TOF mass spectrometry. Remarkably, despite the structural similarity, each compound was separated on reverse-phase C_{18} HPLC columns with a relatively slow gradient (Figure 6). The prepared amounts of **1**−**5** (100−200 nM of each H-pin polyamide compound per 100 mg of resin) were sufficient for further characterization as well as DNA-binding studies.

Alternative Synthesis on Resin with Cross-Linked Monomers. Having established the utility of metathesis-based cross-linking for the synthesis of a library of H-pin polyamides, we examined an alternative cross-linking strategy, which could be scalable and compatible with standard conditions used in automated solid-phase peptide synthesis where transition metal chemistry might be more difficult to implement. Our alternative approach involved preparation of the dimeric building block where two pyrroles are linked through ring nitrogens (Figure 7). As in the case of the library synthesis, olefin metathesis was used to prepare the dimer **17** from *N*-alkenyl-substituted pyrrole monomer **16** (Figure 7), which provided the Boc-protected amine **18**, diacid **19**, and finally the activated ester **20** after several straightforward synthetic transformations. Solid-phase synthesis was performed as depicted in Figure 8. For convenience, we used separately prepared BocPyPyCOOBt and ImPyCOOBt building blocks[7a] that allowed us to reduce the number of steps on solid phase, although stepwise addition of one monomer at a time may be utilized. On high-loading resin with a low cross-link rate (0.88 mmol/g, 1% divinylbenzene), reaction of the dimeric residue **20** with resin **21** proceeds with remarkable efficiency to produce cross-linked product **22**. Further deprotection and coupling steps were performed without significant modifications to the standard polyamide protocol.[7] Removal from the solid support followed by HPLC purification provided the desired product **3**. Notably, the described method requires roughly half the synthetic steps compared to the synthesis of a 10-ring hairpin. In addition, using high-load resin provides a larger amount of product for a given amount of the reagents and therefore is more economical and amenable to large-scale synthesis.

Binding Affinities and Sequence Specificities. To compare the binding affinity of the 10-ring H-pin polyamides **1**−**5** with the variable methylene bridge as well as a control 10-ring hairpin **6** to their respective match and mismatch sites, a restriction fragment of plasmid pDHN2 was used (Figure 9). It contains three sites: a match 5′-ATGTTACAT-3′, a single bp mismatch 5′-ATG**G**TACAT-3′, and a double bp mismatch 5′-ATG**G**TC-**C**AT-3′. Quantitative DNase I footprint titrations were performed to determine the equilibrium association constants for each polyamide-DNA complex (K_a) according to previously published protocols.[14,15]

Comparison of the equilibrium association constants of **1**−**5** reveals that H-pin polyamide **3** (ImPyPyPyPy-β-Dp)$_2$(CH$_2$)$_6$ has the highest affinity in the library to its match site ($K_a = 4.3 \times 10^{10}$ M^{-1}) and displays a 73-fold lower affinity ($K_a = 5.9 \times$

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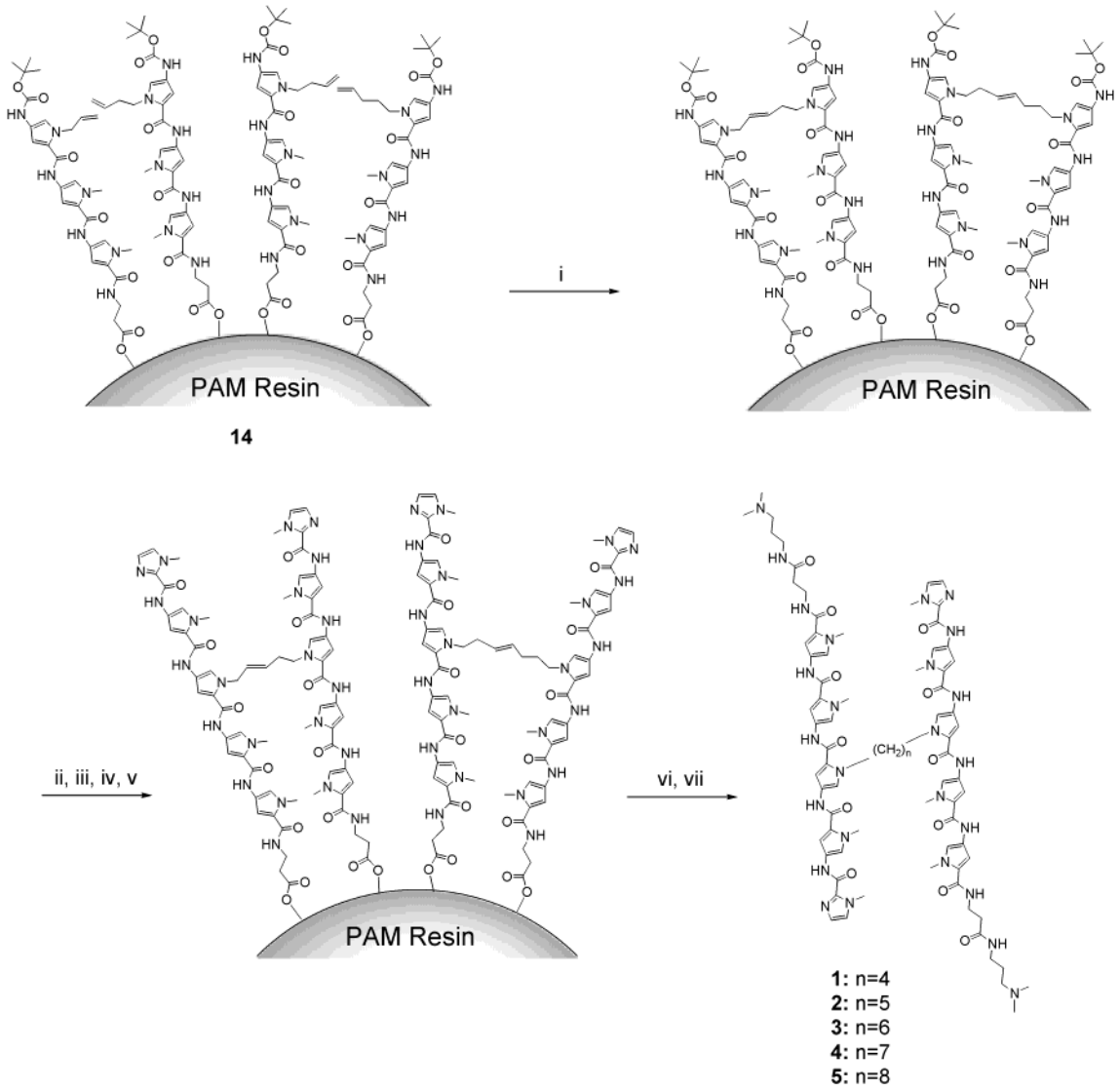

Figure 5. Synthesis of H-pin polyamides via on-resin cross-linking. (i) Ru catalyst $Cl_2(PCy_3)_2Ru{=}CHPh$, DCM, 40 °C, 24−40 h, then second batch of Ru catalyst, 40 °C, 24 h; (ii) TFA/DCM, 20 min, (iii) BocPyCOOBt, DMF, DIEA, 2 h; (iv) TFA/DCM, 20 min, (v) ImCOOBt, DMF, DIEA, 1 h; (vi) Dp, 60 °C, 6 h; (vii) ammonium formate, $Pd(OAc)_2$, EtOH, 12 h.

10^8 M^{-1}) for the 1-bp mismatch site (Figure 10). The $(CH_2)_4$-linked H-pin polyamide **1** (ImPyPyPyPy-β-Dp)$_2(CH_2)_4$ binds the match site with 2-fold lower affinity, $K_a = 1.5 \times 10^{10}$ M^{-1}. The relationship between the number of methylenes in the linker $(CH_2)_n$ and affinity is $n = 6 > 4 > 7 > 5 > 8$ (Table 1). The analogous hairpin polyamide **6** (ImPyPyPyPy-γ-ImPyPyPyPy-β-Dp) binds the match site with slightly lower affinity (K_a = 2.5×10^{10} M^{-1}) than H-pin polyamide **3**. In the sequence context, specificity of the H-pin polyamide **3** was found to be higher than that of control hairpin **6**. In addition, nonspecific DNA binding for H-pin **3** appears at 50 nM, whereas hairpin **6** reveals nonspecific binding at lower concentrations (~10 nM).

The first DNA-binding studies of H-pin polyamides were completed before the sequence specificities of the polyamide tails (β and Dp) were rigorously established. At the C-terminus, the tail units β and Dp specify for A/T with an energetic penalty for G/C, presumably due to steric reasons.[4d] In an early study, 10-ring H-pin polyamides with $(CH_2)_4$ linkers were tested on a DNA restriction fragment derived from a plasmid (pJK7) containing the match sequence for symmetrical 10-ring aromatic core plus two A/T sites downstream but only *one* A/T site upstream. As a result, the control hairpin polyamide bound the designed site with high affinity, as expected for the complete match sequence interaction. H-pin polyamides, however, have two positively charged tails. On the tested sequence, the first tail was favorably positioned over the first two A/T sites, whereas the second tail was situated over one A/T site and one G/C site. The resulting unfavorable interaction of the positively charged Dp moiety with the exocyclic guanine of the second base pair diminished the binding affinity of the H-pin polyamide. As a result, the observed equilibrium association constant (K_a = 4.4×10^8 M^{-1}) for the $(CH_2)_4$ bridged H-pin was lower than observed in the current study, where a complete sequence match of both tails, in addition to the aromatic core, was achieved.

Among all members of the small library, the C_6-linked H-pin was found to have the highest equilibrium association constant, followed closely by the C_4-linked H-pin. We did not observe a gradual increase or decrease of the binding affinity as we changed the bridge length. This may be attributed to the

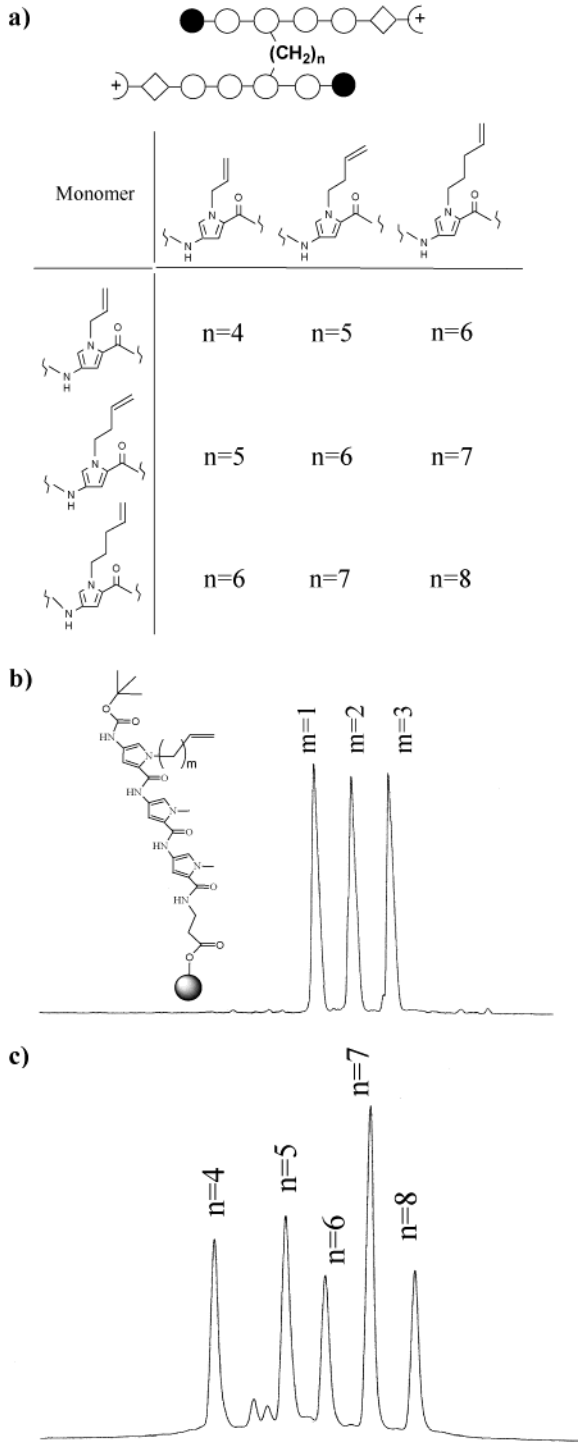

Figure 6. (a) Illustration of the cross-metathesis products of five different lengths generated via a combination of three alkenyl chains in precursors. (b) Analytical HPLC trace after cleavage of resin-bound precursor mixture **14** with Dp. (c) Analytical HPLC trace of the crude mixture of products obtained after the reduction step.

constrained conformation that each aliphatic chain may have in the dimer upon binding to DNA.

Conclusions

We developed a convergent solid-phase parallel synthesis of a library of H-pin polyamides with variable methylene linker lengths by olefin cross-metathesis. The reported synthetic strategy may be used for simultaneous preparation of DNA-

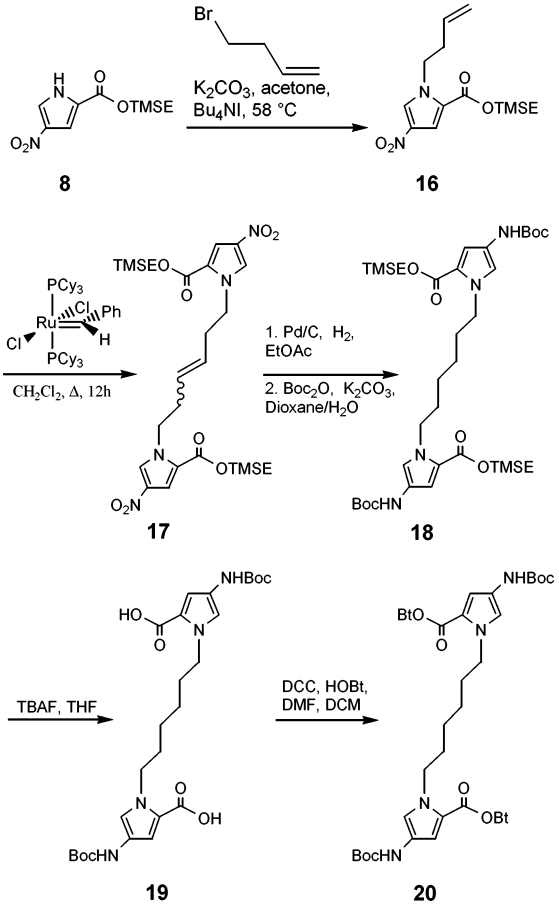

Figure 7. Preparation of Boc-protected bispyrrole dimer and its activated ester.

binding polyamides and other libraries of dimeric small molecules with gradually increased linker lengths. Each H-pin polyamide shows subnanomolar DNA binding affinity for its match site with the optimum bridges being $(CH_2)_6$, closely followed by $(CH_2)_4$. The current study is restricted to H-pin polyamides with C_2 symmetry, but we are optimistic that the synthetic strategy can be modified to allow convergent synthesis of unsymmetrical H-pins. This report sets the stage for the synthesis of H-pins lacking β-Dp tails capable of targeting pure GC binding sites without the inherent A/T-specific interaction of the hairpin γ-turn, as well as issues surrounding application of H-pin polyamides in gene regulation studies.

Experimental Section

General. *N*,*N*′-dicyclohexylcarbodiimide (DCC), *N*-hydroxybenzotriazole (HOBt), Boc-β-alanine-(4-carboxamidomethyl)-benzyl-ester-copoly(styrene−divinylbenzene) resin (Boc-β-PAM-resin), and 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) were purchased from Peptides International.

N,*N*-Diisopropylethylamine (DIEA) and *N*,*N*-dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) were purchased from Applied Biosystems. Reagent-grade dichloromethane (DCM) was from EM, and trifluoroacetic acid (TFA) was from Halocarbon. All other chemicals were obtained reagent-grade from Aldrich (unless otherwise stated) and used without further purification. ^{1}H NMR spectra were recorded on a Varian Mercury 300 instrument. Chemical shifts are reported in parts per million downfield from the signal for Me_4Si, with reference to the solvent residual signal. UV spectra were measured on a Hewlett-Packard model 8452A diode array spectrophotometer. Matrix-assisted, laser

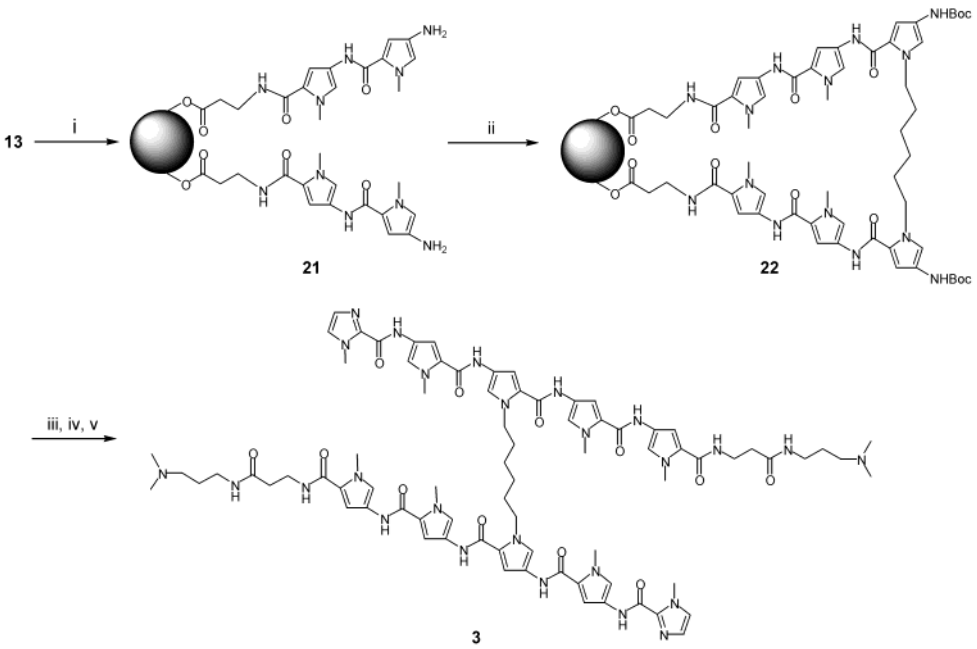

Figure 8. Synthesis of H-pin polyamide **3** via on-resin cross-linking through peptide bond formation. (i) 80% TFA/DCM, 20 min; BocPyPyCOOBt, DMF, DIEA, 1 h, 37 °C; 80% TFA/DCM, 20 min; (ii) **15**, DMF, DIEA, 12 h; (iii) 80% TFA/DCM, 20 min; (iv) ImPyCOOBt, DMF, DIEA, 4 h, 37 °C; (v) Dp, 6 h, 37 °C.

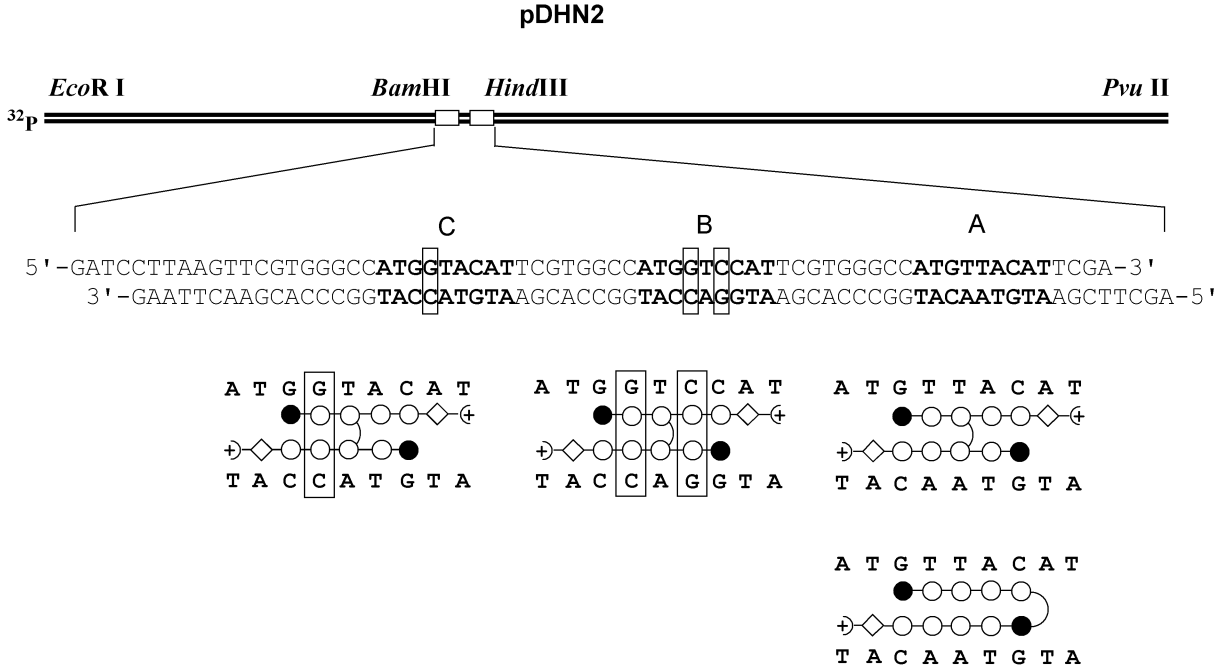

Figure 9. Illustration of the *Eco*RI/*Pvu*II restriction fragment with the *Bam*HI/*Hin*dIII insertion sites indicated. The sequence of the synthesized insert from the plasmid pDHN2 is shown with the polyamide binding sites boxed and in boldface type: match site A, double mismatch site B, and single mismatch site C. Ball and stick models are shown as in Figure 1.

desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry was carried out on Voyager DE-Pro mass spectrometer from PerSeptive Biosystems. Electrospray ionization mass spectrometry was carried out on an LC-Q mass spectrometer from Finnigan Mat. Fast atom bombardment mass spectrometry was carried out on a Jeol mass spectrometer. HPLC analysis was performed on a Beckman Gold system using a Rainin C_{18}, Microsorb-MV, 5 μm, 300 × 4.6 mm reversed-phase column in 0.1% (v/v) TFA with acetonitrile as eluent and a flow rate of 1.0 mL/min, gradient elution 1.70% acetonitrile/min unless otherwise stated. Preparatory HPLC was carried out on a Beckman HPLC apparatus with a Waters DeltaPak 100 × 25 mm, 100 μm C_{18} column, 0.1% (w/v) TFA, 0.25% acetonitrile/min unless otherwise stated. Water (18 MΩ) was obtained from a Millipore MilliQ water purification system, and all buffers were 0.2 μm filtered.

Glycogen (20 mg/mL), dNTPs (PCR nucleotide mix), and all enzymes (unless otherwise stated) were purchased from Roche Diagnostics and used with their supplied buffers. Plasmid pUC19 was from New England Biolabs. Deoxyadenosine [α-^{32}P]triphosphate and thymidine [α-^{32}P]triphosphate were from NEN. Calf thymus DNA (sonicated, deproteinized) and DNase I (7500 units/mL, FPLC pure) were from Amersham Pharmacia Biotech. HEPES was from Sigma.

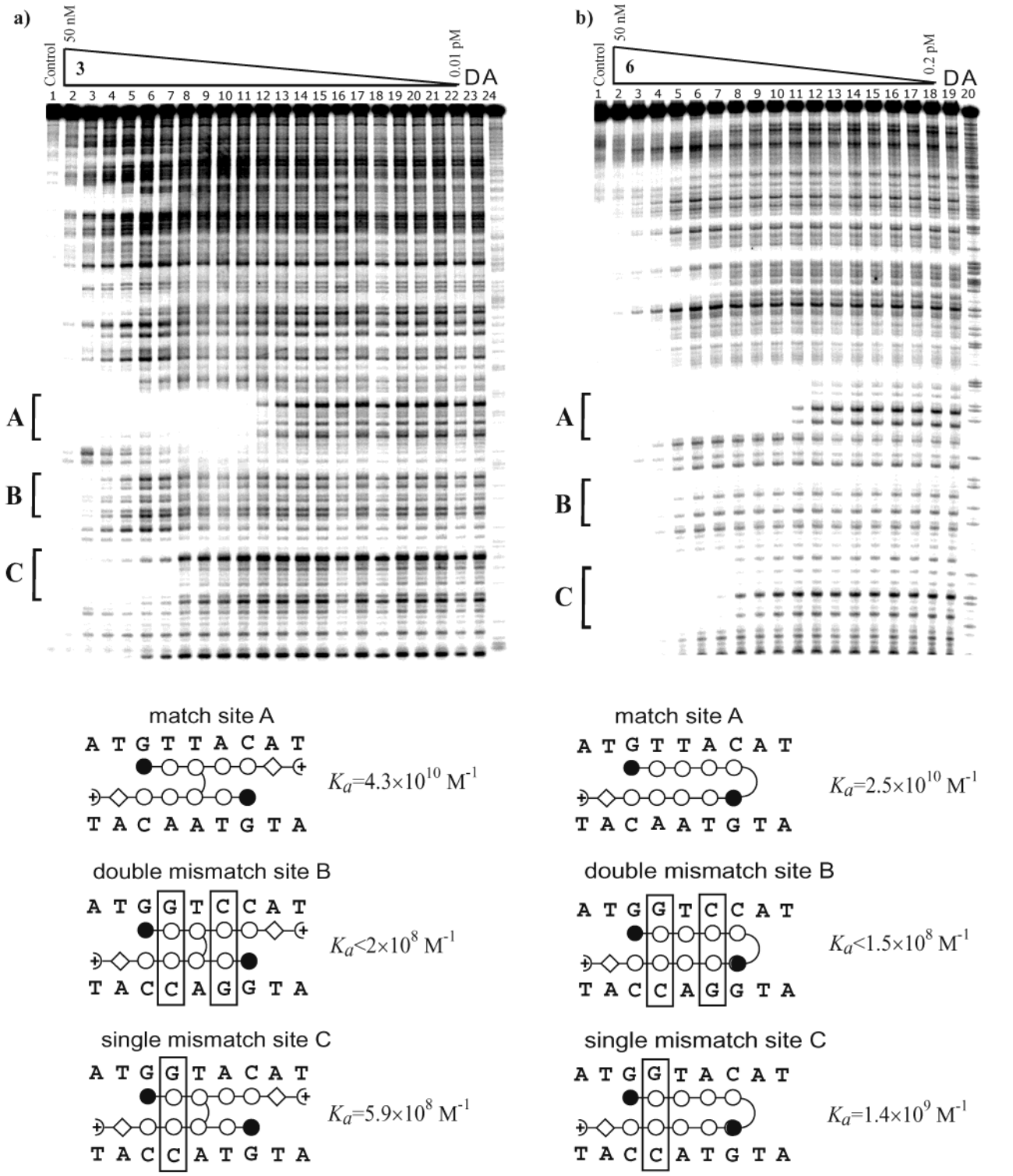

Figure 10. (a) Storage phosphor autoradiogram from quantitative DNase I footprint titration experiment with H-pin **3** on the radiolabeled restriction fragment derived from plasmid pDHN2. Lane 1, intact DNA; lanes 2−22, DNase I digestion products in the presence of 50 nM, 20 nM, 10 nM, 5 nM, 2 nM, 1 nM, 500 pM, 200 pM, 100 pM, 50 pM, 20 pM, 10 pM, 5 pM, 2 pM, 1 pM, 0.5 pM, 0.2 pM, 0.1 pM, 0.05 pM, 0.02 pM, and 0.01 pM polyamide, respectively; lane 23, DNase I standard; lane 24, A reaction. (b) Storage phosphor autoradiogram from quantitative DNase I footprint titration experiment with control hairpin **6** on the same restriction fragment. Lane 1, intact DNA; lanes 2−18, DNase I digestion products in the presence of 50 nM, 20 nM, 10 nM, 5 nM, 2 nM, 1 nM, 500 pM, 200 pM, 100 pM, 50 pM, 20 pM, 10 pM, 5 pM, 2 pM, 1 pM, 0.5 pM, and 0.2 pM polyamides, respectively; lane 19, DNase I standard; lane 20, A reaction. Footprints near the 5′-end of the gel are tail mismatch sequences 5′-**C**TGTTTC**C**T-3′ and 5′-TTGTTA**TCC**-3′ from the pDHN2 plasmid.

Tris-HCl, dithiothreitol (DTT), RNase-free water (used for all DNA manipulations), and 0.5 M EDTA were from U.S. Biochemical Corp. Ethanol (200 proof) was from Equistar, and 2-propanol was from Mallinckrodt. Calcium chloride, potassium chloride, and magnesium chloride were from Fluka and purchased in Microselect quality. Formamide was from Gibco, and premixed Tris−borate−EDTA for gel running buffer was from Sigma. Bromophenol blue and xylene cyanol FF were from Acros Organics. 1,1,1-Trimethylsilylethyl 4-nitropyrrole-2-carboxylate **8** was prepared according to the published procedure.[16]

Table 1. Equilibrium Association Constants[a] for H-pin Polyamides **1**−**5** and Control Hairpin **6** on a Restriction Fragment Derived from Plasmid pDHN2

polyamide	match 5′-ccATGTTACATtc-3′	1-bp mismatch 5′-ccATGGTACATtc-3′	specificity over 1-bp mismatch
1	1.5 (± 0.18) × 10^{10}	5.0 (± 0.15) × 10^{9}	30
2	4.9 (± 0.25) × 10^{9}	2.5 (± 0.28) × 10^{8}	20
3	4.3 (± 0.20) × 10^{10}	5.9 (± 0.22) × 10^{8}	73
4	1.1 (± 0.35) × 10^{10}	4.6 (± 0.38) × 10^{9}	24
5	2.1 (± 0.50) × 10^{9}	1.8 (± 0.48) × 10^{8}	12
6	2.5 (± 0.23) × 10^{10}	1.4 (± 0.20) × 10^{9}	18

[a] The reported equilibrium association constants (in reciprocal molar units) are the mean values obtained from three DNase I footprint titration experiments. Assays were carried out in the presence of 10 mM Tris-HCl, 10 mM KCl, 10 mM $MgCl_2$, and 5 mM $CaCl_2$ at pH 7.0 and 22 °C (See Figure 10 and Supporting Information).

General Procedure for Deprotection of 1,1,1-Trimethylsilylethyl Esters (10). The solution of 2.0 mmol an appropriate precursor in 2.0 mL of dry THF was placed under argon and cooled to 0 °C on an ice bath. To this solution, TBAF (4.0 mL of 1 M in THF) was added via a syringe with stirring. The solution was allowed to slowly warm to ambient temperature, and the mixture was stirred overnight under Ar. The solvent was removed under reduced pressure, and the residue was dissolved in Et_2O, washed with 0.5 M citric acid (3 × 100 mL) and saturated NaCl solution (4 × 100 mL), dried (anhydrous $MgSO_4$), filtered, and evaporated in vacuo. The products were dissolved in a minimum amount of dichloromethane and precipitated with cold petroleum ether. The solvent was removed by filtration and the obtained white fine powder solid was dried in vacuo.

1,1,1-Trimethylsilylethyl 4-[(*tert*-Butoxycarbonyl)amino]pyrrole-2-carboxylate (9). The 1,1,1-trimethylsilylethyl 4-nitropyrrole-2-carboxylate **8**[16] (7.50 g, 29.3 mmol) was dissolved in 35 mL of 6:1 EtOAc/MeOH and treated with Pd/C (2.0 g, 10%) under Ar. The mixture was pressurized with H_2 (100 psi) and stirred at room temperature for 40 h. The reaction mixture was filtered through a short pad of Celite and the solvent was removed in vacuo. The mixture was dissolved in cold diethyl ether (150 mL) and precipitated with HCl (1 M in diethyl ether). The precipitate was collected, washed with diethyl ether, and dried in vacuo. The crude amine salt was then suspended in 15 mL of EtOAc and 7.5 mL of DIEA followed by 11.0 g (50.4 mmol) of di-*tert*-butyl dicarbonate. The mixture was stirred at 37 °C for 12 h and then diluted to 30 mL with EtOAc. The mixture was washed with 0.5 M citric acid (2 × 200 mL) followed by sodium bicarbonate (3 × 100 mL) and saturated sodium chloride (2 × 100 mL), dried over anhydrous $MgSO_4$, and filtered. The solvent was removed in vacuo and the residue was dissolved in 8 mL of dichloromethane. Cold petroleum ether was added to the product and the precipitate was stored at −20 °C for 4 h. The off-white powder was collected by filtration, washed with the minimum amount of cold petroleum ether, and dried in vacuo. Yield was 7.28 g (76%) for two steps. ^{1}H NMR ($CDCl_3$, TMS): δ 7.84 (br s, 1H), 7.12 (br s, 1H), 6.62 (d, 1H, J = 1.8 Hz), 6.42 (br s, 1H), 4.26 (t, 2H, J = 8.4 Hz), 1.47 (s, 9H), 1.03 (t, 2H, J = 8.4 Hz), 0.03 (s, 9H). FAB-MS calcd for $C_{15}H_{26}N_2O_4Si$ ($M^{+\bullet}$): 326.1662. Found: 326.1676.

4-[(*tert*-Butoxycarbonyl)amino]-1-allylpyrrole-2-carboxylic acid (11a). A solution of 1,1,1-trimethylsilylethyl 4-[(*tert*-butoxycarbonyl)-amino]pyrrole-2-carboxylate **9** (3.2 g, 9.8 mmol) in dry acetone (30 mL), was treated sequentially with allyl bromide (5.00 g, 41.3 mmol), anhydrous K_2CO_3 (5.20 g, 37.6 mmol), and tetra-*n*-butylammonium iodide (300 mg, 0.812 mmol). The heterogeneous mixture was stirred at reflux for 12 h. The solvent was removed in vacuo and the residue was partitioned between Et_2O (150 mL) and water (150 mL). The layers were separated and the organic phase was washed with water (2 × 100 mL), saturated $NaHCO_3$ (3 × 100 mL), and saturated NaCl solution (2 × 50 mL) and then dried over anhydrous $MgSO_4$ and filtered. Removal of solvent in vacuo provided the crude product **10a**, which was purified on silica gel with a gradient of 0−5% EtOAc−hexane as an eluent, affording 2.80 g (78%) of product. ^{1}H NMR ($CDCl_3$, TMS): δ 7.12 (br s, 1H), 6.62 (d, 1H, J = 1.8 Hz), 6.42 (br s, 1H), 5.83−6.02 (m, 1H), 5.05 (dd, 1H, J = 17.7, 1.6 Hz), 5.03 (dd, 1H, J = 10.2, 1.5 Hz), 4.87 (d, 2H, J = 5.7 Hz), 4.26 (t, 2H, J = 8.4 Hz), 1.47 (s, 9H), 1.03 (t, 2H, J = 8.4 Hz), 0.03 (s, 9H). ^{13}C NMR ($CDCl_3$, TMS): δ 160.8, 152.9, 134.3, 122.4, 119.5, 118.2, 116.6, 107.8, 80.1, 62.0, 50.9, 28.4, 17.4, −1.3. The silyl ester **10a** was deprotected as above, affording 1.83 g (90%) of **11a**. ^{1}H NMR ($CDCl_3$, TMS): δ 7.25 (br s, 1H), 6.75 (s, 1H), 6.36 (br s, 1H), 5.88−6.03 (m, 1H), 5.14 (d, 1H, J = 10.2 Hz), 5.03 (d, 1H, J = 17.1, 0.9 Hz), 4.88 (d, 2H, J = 5.7 Hz), 1.49 (s, 9H). ^{13}C NMR ($CDCl_3$, TMS): δ 165.1, 152.8, 134.0, 122.8, 119.8, 118.3, 116.9, 109.5, 80.2, 51.2, 28.4. FAB-MS calcd for $C_{13}H_{18}N_2O_4$ ($M^{+\bullet}$): 266.1266. Found: 266.1267.

4-[(*tert*-Butoxycarbonyl)amino]-1-but-3-enylpyrrole-2-carboxylic acid (11b). A solution of 1,1,1-trimethylsilylethyl 4-[(*tert*-butoxycarbonyl)amino]pyrrole-2-carboxylate (3.2 g, 9.8 mmol) in dry acetone (30 mL), was treated sequentially with 1-bromo-2-butene (5.6 g, 42 mmol), anhydrous K_2CO_3 (5.2 g, 38 mmol), and tetrabutylammonium iodide (300 mg, 0.812 mmol) and stirred at reflux for 24 h. The solvent was removed in vacuo, and **10b** was isolated and purified as above. Yield: 2.5 g (58%). ^{1}H NMR ($CDCl_3$, TMS): δ 7.12 (br s, 1H), 6.60 (d, 1H, J = 2.1 Hz), 6.23 (br s, 1H), 5.65−5.88 (m, 1H), 5.05 (dd, 1H, J = 16.8, 1.8 Hz), 5.02 (dd, 1H, J = 10.2, 1.5 Hz), 4.29, (t, 2H, J = 8.4 Hz), 4.28 (d, 2H, J = 8.1 Hz), 2.49 (d, 2H, J = 14.4, 7.2 Hz), 1.49 (s, 9H), 1.06 (t, 2H, J = 8.4 Hz), 0.06 (s, 9H). ^{13}C NMR ($CDCl_3$, TMS): δ 160.7, 152.9, 134.2, 122.1, 119.0, 118.3, 116.8, 107.8, 79.6, 61.8, 48.4, 35.8, 28.3, 17.3, −1.4. The silyl ester was deprotected as above. Yield: 1.7 g (92%). ^{1}H NMR ($CDCl_3$, TMS): δ 7.23 (br s, 1H), 6.74 (s, 1H), 6.33 (br s, 1H), 5.72−5.85 (m, 1H), 5.06 (d, 1H, J = 8.1 Hz), 5.04 (d, 1H, J = 17.4, 1.5 Hz), 4.88 (t, 2H, J = 6.3 Hz), 2.50 (d, 2H, J = 14.4, 6.9 Hz), 1.50 (s, 9H). ^{13}C NMR ($CDCl_3$, TMS): δ 165.1, 152.8, 134.3, 122.6, 120.1, 117.3, 116.2, 109.5, 80.1, 48.9, 35.9, 28.5. FAB-MS calcd for $C_{14}H_{20}N_2O_4$ ($M^{+\bullet}$): 280.1423. Found: 280.1417.

4-[(*tert*-Butoxycarbonyl)amino]-1-pent-4-enylpyrrole-2-carboxylic acid (11c). A solution of 1,1,1-trimethylsilylethyl 4-[(*tert*-butoxycarbonyl)amino]-pyrrole-2-carboxylate (3.45 g, 1.06 mmol) in 30 mL of dry acetone was treated sequentially with 1-bromo-3-pentene (5.30 g, 35.6 mmol), tetrabutylammonium iodide (300 mg, 0.812 mmol), and anhydrous K_2CO_3 (5.20 g, 37.6 mmol) and stirred at reflux for 40 h. The solvent was removed in vacuo, and **10c** was isolated and purified as above. Yield: 2.97 g (71%). ^{1}H NMR ($CDCl_3$, TMS): δ 7.06 (br s, 1H), 6.92 (br s, 1H), 6.60 (d, 1H, J = 2.1 Hz), 5.61−5.73 (m, 1H), 4.91 (dd, 1H, J = 21.0, 1.5 Hz), 4.87 (dd, 1H, J = 10.5, 0.9 Hz), 4.20 (t, 2H, J = 9.0 Hz), 4.14 (t, 2H, J = 8.8 Hz), 1.94 (d, 2H, J = 14.0, 7.2 Hz), 1.70−1.76 (m, 2H), 1.38 (s, 9H), 0.96 (t, 2H, J = 8.4 Hz), −0.04 (s, 9H). ^{13}C NMR ($CDCl_3$, TMS): δ 160.8, 153.0, 137.3, 122.3, 119.1, 118.4, 115.0, 107.9, 79.5, 61.8, 48.4, 30.6, 30.5, 28.3, 17.3, −1.4. Deprotection of the silyl ester yielded 1.77 g (80%) of **11c**. ^{1}H NMR ($CDCl_3$, TMS): δ 7.24 (br s, 1H), 6.73 (s, 1H), 6.31 (br s, 1H), 5.72−5.86 (m, 1H), 5.04 (d, 1H, J = 16.8, 2.1 Hz), 4.99 (d, 1H, J = 11.1 Hz), 4.25 (t, 2H, J = 7.0 Hz), 2.04 (d, 2H, J = 14.0, 7.5 Hz), 1.80−1.89 (m, 2H), 1.50 (s, 9H). ^{13}C NMR ($CDCl_3$, TMS): δ 165.1, 152.9, 137.4, 122.6, 121.1, 116.2, 115.2, 109.4, 82.3, 48.8, 30.7, 30.6, 28.5. FAB-MS calcd for $C_{15}H_{22}N_2O_4$ ($M^{+\bullet}$): 294.1580. Found: 294.1575.

1,1,1-Trimethylsilylethyl (4-nitro-1-but-3-enyl)pyrrole-2-carboxylate (16). To a solution of 1,1,1-trimethylsilylethyl 4-nitropyrrole-2-carboxylate **8** (15.3 g, 59.8 mmol) in 50 mL of acetone was added 10.1 g (74.8 mmol) of 1-bromo-2-butene, followed by 20.6 g (0.15 mmol) of anhydrous potassium carbonate and 1.07 g (2.90 mmol) of tetrabutylammonium iodide. The mixture was stirred at reflux for 15 h and cooled to room temperature, and the precipitate was filtered out. The solvent was removed in vacuo and the residue was partitioned

(16) Rucker, V.; Foister, S.; Melander, C.; Dervan, P. B. *J. Am. Chem. Soc.* **2003**, *125*, ASAP article.

between diethyl ether (300 mL) and water (200 mL). The organic phase was separated, washed with water (2 × 200 mL) and saturated NaCl (200 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated. The product was purified by column chromatography on silica with hexane/EtOAc (10:1) as the eluent. Yield: 12.7 g (68%). ^{1}H NMR ($CDCl_3$, TMS): δ 7.58 (d, J = 2.1 Hz, 1H), 7.37 (d, J = 2.1 Hz, 1H), 5.73 (m, 1H), 5.03 (m, 1H), 5.03 (d, J = 17.9 Hz, 1H), 4.40 (t, J = 6.6 Hz, 2H), 4.34 (t, J = 6.9 Hz, 2H), 2.52 (m, 2H), 1.09 (t, J = 6.9 Hz, 2H), 0.04 (s, 9H). ^{13}C NMR ($CDCl_3$, TMS): δ 160.2, 135.3, 133.2, 126.9, 122.6, 118.6, 113.0, 63.5, 50.0, 35.5, 17.7, −1.2. FAB-MS calcd for $C_{14}H_{23}N_2O_4Si$ (MH^+): 311.1427. Found: 311.1422.

1,6-Di-[4-nitro-2-[[(1,1,1-trimethylsilylethyl)oxy]carbonyl]-*N*-pyrrolyl]-3-hexene (17). [1,1,1-Trimethylsilylethyl (4-nitro-1-but-3-enyl)]-pyrrole-2-carboxylate **16** (12.3 g, 39.6 mmol) was dissolved in dry dichloromethane (90 mL) and the solution was purged with Ar for 15 min. Ru catalyst (0.760 g, 1.98 mmol, 2.5 mol %) was added to the solution and stirred at reflux under Ar for 24 h. Another 0.76 g of catalyst was added, and the stirring continued for another 12 h until reaction was complete as determined by TLC. The solvent was removed in vacuo and the residue was chromatographed on silica with hexane/EtOAc = 5:1 as an eluent. Two isomers (*E* and *Z*) were collected and used in the next step without further separation. Yield for *E* + *Z* isomers: 8.71 g (74%). ^{1}H NMR ($CDCl_3$, TMS): δ 7.65 (d, J = 2.1 Hz, 1H), 7.54 (d, J = 2.1 Hz, 1H), 7.40 (s, 2H), 5.52 (m, 1H), 5.39 (m, 1H), 4.34 (m, 8H), 2.48 (m, 4H), 1.09 (t, J = 6.9 Hz, 9H), 0.07 (s, 18H). ^{13}C NMR ($CDCl_3$, TMS): δ 160.2, 129.0, 128.0, 126.9, 126.7, 122.6, 113.0, 63.7, 50.0, 34.5, 29.3, 17.7, −1.2. FAB-MS calcd for $C_{26}H_{40}N_4O_8Si_2$ ($M^{+\bullet}$): 592.2384. Found: 592.2372.

1,6-Di-[4-[(*tert*-butoxycarbonyl)amino]-2-[[(1,1,1-trimethylsilylethyl)oxy]carbonyl]-*N*-pyrrolyl]hexane (18). A solution of **17** (8.60 g, 14.5 mmol) in 2:1 EtOAc/EtOH (300 mL) was treated with 3.00 g of 10% Pd/C and stirred under H_2 (400 psi) at room temperature for 15 h. After reaction was complete, the solution was filtered through a pad of Celite and concentrated in vacuo. The product was precipitated with HCl (40 mL, 1 M in diethyl ether), collected by filtration under N_2, and dried in vacuo to afford crude amine hydrochloride in 89% yield. The amine salt was suspended in dioxane (150 mL) and 10% aqueous potassium carbonate (150 mL) and treated with Boc_2O (6.0 g, 28 mmol) in dioxane (30 mL) over a period of 20 min. The reaction was further stirred for 6 h, then cooled to 0 °C, and the precipitated product was collected and dried in vacuo. Yield: 65%. ^{1}H NMR ($CDCl_3$, TMS): δ 7.08 (br s, 1H), 6.61 (d, J = 2.1 Hz, 1H), 6.29 (br s, 1H), 4.27 (t, J = 8.1 Hz, 2H), 4.20 (t, J = 6.6 Hz, 2H), 1.68 (m, 2H), 1.52 (s, 9H), 1.27 (m, 2H), 1.05 (t, J = 8.1 Hz, 2H), 0.05 (s, 9H). ^{13}C NMR ($CDCl_3$, TMS): δ 161.1, 153.2, 122.2, 119.5, 118.7, 108.0, 62.2, 49.2, 31.7, 28.7, 27.7, 26.6, 17.7, −1.1. FAB-MS calcd for $C_{36}H_{62}N_4O_8Si_2$ ($M^{+\bullet}$): 734.4106. Found: 734.4112.

1,6-Di-[4-[(*tert*-butoxycarbonyl)amino]-2-carboxy-*N*-pyrrolyl]-hexane (19). Trimethylsilyl ester **18** (5.0 g, 6.8 mmol) was dissolved in 20 mL of dry THF, placed under Ar, cooled to 0 °C, and treated with 20 mL of 1 M TBAF in THF. The solution was allowed to warm to room temperature and stirred for 12 h. The solvent was removed under reduced pressure, and the compound was dissolved in 100 mL of DCM. The organic layer was washed with 1 M citric acid (3 × 100 mL), saturated sodium bicarbonate (3 × 100 mL), and saturated NaCl (2 × 100 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo to provide the product as a tan solid. Yield: 90%. ^{1}H NMR (DMSO-d_6, TMS): δ 12.02 (br s, 1H), 9.03 (s, 1H), 7.05 (s, 1H), 6.6 (s, 1H), 4.15 (t, J = 7.2 Hz, 2H), 1.57 (m, 2H), 1.41 (s, 9H), 1.18 (m, 2H). ^{13}C NMR (DMSO-d_6, TMS): δ 161.4, 152.5, 122.7, 118.6, 117.6, 107.7, 78.3, 47.8, 31.2, 28.2, 25.6. FAB-MS calcd for $C_{26}H_{39}N_4O_8$ ($M^{+\bullet}$): 534.2676. Found: 534.2679.

1,6-Di-[4-[(*tert*-butoxycarbonyl)amino]-2-[[(1-hydroxybenzotriazolyl)oxy]carbonyl]-*N*-pyrrolyl]hexane (20). A solution of **19 (**2.94 g, 5.50 mmol) in 15 mL of DMF was sequentially treated with 1-hydroxybenzotriazole (3.00 g, 22.2 mmol) and DCC (2.27 g, 11.0 mmol). The solution was stirred overnight to ensure the complete formation of the activated ester. The solution was then filtered into a stirring mixture of 100 g of water and 50 g of ice. The precipitate was collected, washed with a minimum amount of cold water, and dried in vacuo. Yield: 79%. ^{1}H NMR (DMSO-d_6, TMS): δ 9.42 (s, 1H), 8.12 (d, J = 8.1 Hz, 1H), 7.75 (d, J = 8.1 Hz, 1H), 7.59 (t, J = 8.1 Hz, 1H), 7.54 (s, 2H), 7.51 (t, J = 8.1 Hz, 1H), 7.50 (s, 1H), 7.19 (s, 1H), 4.15 (t, J = 7.2 Hz, 2H), 1.57 (m, 2H), 1.44 (s, 9H), 1.18 (m, 2H). ^{13}C NMR (DMSO-d_6, TMS): δ 155.5, 152.6, 142.6, 129.0, 127.0, 125.0, 124.3, 123.2, 119.7, 111.3, 110.1, 109.1, 78.9, 48.4, 34.5, 30.8, 28.1. FAB-MS calcd for $C_{38}H_{45}N_4O_8$ (MH^+): 769.3421. Found: 769.3419.

Solid-Phase Synthesis. Boc-Py-Py-β-Ala-PAM resin **13** was prepared from Boc-β-Ala-PAM resin (0.88 mmol/g) according to the standard solid-phase synthetic protocol as described by Baird and Dervan.[7a] A mixture of acids **11a**−**c** (150 mg each) was simultaneously activated with DCC (220 mg, 1.07 mmol) and HOBt (300 mg, 2.22 mmol) in 2 mL of DMF. The mixture was stirred at room temperature for 1 h. The precipitate was filtered off and the solution containing a mixture of activated esters **12a**−**c** was mixed with 1 mL of dry DIEA. Dried Boc-Py-Py-β-Ala-PAM resin (100 mg) was deblocked (80% TFA in DCM, 1 × 30 s + 1 × 20 min) and washed (DCM), and activated esters **12a**−**c** were added. After 4 h at room temperature, the reaction vessel was drained and the resin was rigorously washed with DMF, DCM, methanol, and diethyl ether and then dried overnight in vacuo.

Metathesis. The entire batch of dried resin from above step was placed in a 100 mL round-bottom flask, and 3 mL of dry DCM was added under Ar. Ruthenium catalyst (20 mg) was added to the heterogeneous mixture, which was stirred at reflux for 24 h at 40 °C under an Ar stream to remove the formed ethylene. A fresh portion of catalyst (10 mg) was then added and the stirring continued for another 24 h. The resin was collected and washed with 10% dppp in DCM, then with DCM, and finally with methanol. The extent of cross-linking was monitored by reverse-phase HPLC with a 0.1% TFA gradient in acetonitrile (0−60% in 35 min). The Boc group was deprotected with 25% TFA in DCM (2 × 10 min) and the synthesis was continued as described previously.[7a] Both activated esters of BocPyOBt and ImOBt were coupled as above. The mixture was cleaved from the solid support with Dp at 37 °C for 12 h. The excess of Dp was removed in vacuo and the residue was dissolved in absolute ethanol and passed through a Waters Sep-Pak. The EtOH solution was then treated with NH_4HCO_2 (150 mg) and $Pd(OAc)_2$ (25 mg) and stirred for 24 h at room temperature. After reduction was completed, the deposited Pd was filtered off and the solvent was removed in vacuo. The residue was dissolved in a mixture of 20% MeOH + 0.1% TFA/H_2O (v/v) and purified by reverse-phase HPLC on a Varian semipreparative C_{18} column. Each separated product was analyzed twice on analytical HPLC on a 100 Å C_{18} analytical column with gradients of 0.1% TFA-acetonitrile of 0−60% in 35 min and 0−55% in 60 min.

Cross-Linking. Boc-PyPy-β-Ala-PAM resin **13** was prepared from Boc-β-Ala-PAM resin (100 mg, 0.88 mmol/g) as described above. After deblocking (80% TFA in DCM, 1 × 30 s + 1 × 20 min) and washing (DCM), activated ester **20** (68 mg, 0.088 mmol) was added in 0.5 mL of DMF and 0.25 mL of DIEA. The extent of cross-linking was monitored by reverse-phase HPLC. The resin was shaken at 37 °C for 12 h, drained, and washed with DMF. Further coupling steps, cleavage from the solid support, and purification by HPLC were carried out as previously desribed.[7a] Yield of **3**: 48 mg, 30% recovery.

(ImPyPyPyPy-β-Dp)$_2$(CH$_2$)$_4$ (1). ^{1}H NMR ($CDCl_3$, TMS): δ 10.45 (s, 2H, arom NH), 9.98 (s, 2H, arom NH), 9.97 (s, 2H, arom NH), 9.90 (s, 2H, arom NH), 9.4 (br s, 2H, CF_3COOH), 8.05 (m, 4H, aliph NH), 7.40 (s, 2H, CH), 7.29 (m, 2H, CH), 7.27 (m, 2H, CH), 7.15 (m, 4H, CH), 7.04 (m, 4H, CH), 7.00 (m, 4H, CH), 6.86 (m, 2H, CH), 4.31 (br m, 4H, linker CH_2), 3.97 (s, 6H, NCH_3), 3.83 (s, 6H, NCH_3), 3.82 (s, 6H, NCH_3), 3.78 (s, 6H, NCH_3), 3.36 (m, 4H, CH_2), 3.08 (m, 4H, CH_2), 2.98 (m, 4H, CH_2), 2.72 (d, J = 4.8 Hz, 4H, N(CH_3)$_2$), 2.33 (t, J = 7.2 Hz, 4H, aliph CH_2), 1.70 (m, 8H, aliph CH_2 + linker CH_2).

MALDI-TOF-MS (monoisotopic) calcd for $C_{76}H_{97}N_{26}O_{12}$ (MH^+): 1565.8. Found: 1565.9.

(ImPyPyPyPy-*β*-Dp)$_2$(CH$_2$)$_6$ (3). ^{1}H NMR ($CDCl_3$, TMS): δ 10.49 (s, 2H, arom NH), 9.97 (s, 2H, arom NH), 9.94 (s, 2H, arom NH), 9.89 (s, 2H, arom NH), 9.45 (br s, 2H, CF_3COOH), 8.05 (m, 4H, aliph NH), 7.41 (s, 2H, CH), 7.27 (m, 2H, CH), 7.27 (m, 2H, CH), 7.16 (m, 4H, CH), 7.06 (m, 4H, CH), 7.03 (m, 4H, CH), 6.87 (m, 2H, CH), 4.27 (br m, 4H, linker CH_2), 3.98 (s, 6H, NCH_3), 3.92 (s, 6H, NCH_3), 3.83 (s, 6H, NCH_3), 3.78 (s, 6H, NCH_3), 3.36 (m, 4H, CH_2), 3.09 (m, 4H, CH_2), 2.99 (m, 4H, CH_2), 2.72 (d, J = 4.8 Hz, 4H, N(CH_3)$_2$), 2.33 (t, J = 7.3 Hz, 4H, aliph CH_2), 1.70 (m, 8H, aliph CH_2 + linker CH_2), 1.24 (m, 4H, linker CH_2). MALDI-TOF-MS (monoisotopic) calcd for $C_{76}H_{97}N_{26}O_{12}$ (MH^+): 1593.8. Found: 1593.7.

(ImPyPyPyPy-*β*-Dp)$_2$(CH$_2$)$_5$ (2). MALDI-TOF-MS (monoisotopic) calcd for $C_{76}H_{97}N_{26}O_{12}$ (MH^+): 1579.8. Found: 1579.9.

(ImPyPyPyPy-*β*-Dp)$_2$(CH$_2$)$_7$ (4). MALDI-TOF-MS (monoisotopic) calcd for $C_{76}H_{97}N_{26}O_{12}$ (MH^+): 1607.8. Found: 1607.9.

(ImPyPyPyPy-*β*-Dp)$_2$(CH$_2$)$_8$ (5). MALDI-TOF-MS (monoisotopic) calcd for $C_{76}H_{97}N_{26}O_{12}$ (MH^+): 1621.8. Found: 1621.3.

Preparation of 3′- and 5′-End-Labeled DNA Restriction Fragments. All cloning was carried out according to published procedures.[17] Plasmid pDHN2 was constructed by inserting the hybridized inserts 5′-GAT CCT TAA GTT CGT GGG CCA TGG TAC ATT CGT GGC CAT GGT CCA TTC GTG GGC CAT GTT ACA TTC GA-3′ and 5′-AGC TTC GAA TGT AAC ATG GCC CAC GAA TGG ACC ATG GCC ACG AAT GTA CCA TGG CCC ACG AAC TTA AG-3′ into the *Bam*HI/*Hin*dIII polycloning site in pUC19.[18] The insert was obtained by annealing complementary synthetic oligonucleotides and was then ligated to the large *Bam*HI/*Hin*dIII restriction fragment of pUC19 with T4 DNA ligase. The ligated plasmid was then used to transform *Escherichia coli* XL-1 Blue Supercompetent cells. Colonies were selected for α-complementation on 25-mL Luria−Bertani agar plates containing 50 mg/mL ampicillin and treated with X-Gal and IPTG solutions and grown overnight at 37 °C. Well-defined white colonies were transferred into 100 mL of Luria−Bertani medium containing 50 mg/mL ampicillin. Cells were harvested after overnight growth at 37 °C. Medium-scale plasmid purification was performed with Qiagen purification kits. The presence of the desired insert was determined by dideoxy sequencing. DNA concentration was determined at 260 nm by use of the relation 1 OD unit = 50 mg/mL duplex DNA. Plasmid pDHN2 was simultaneously cut with *Eco*RI and *Pvu*II and then radiolabeled by 3′ fill-in with 1 mCi of [α-^{32}P]dATP, 1 mCi of [α-^{32}P]-dTTP, and the Klenow fragment of DNA polymerase II at 37 °C for 25 min. The product mixture was purified on a 7% nondenaturing preparative polyacrylamide gel (5% cross-link), and the desired fragment was isolated after visualization by autoradiography. The DNA was precipitated with 2-propanol (1.5 volumes). The pellet was washed with 75% ethanol, lyophilized to dryness, and then resuspended in 10 mL of RNase-free H_2O. Chemical sequencing reactions were performed according to published protocols.[19]

Quantitative DNase I Footprint Titrations. All reactions were carried out in a volume of 400 μL according to the published procedure.[15] We note explicitly that no carrier DNA was used in these reactions until after DNase I cleavage. The concentration of radiolabeled DNA was calculated to remain below the concentration of polyamide in all but the most diluted reaction. Polyamide stock solution (or water for reference and intact lanes) was added to an assay buffer where the final concentrations were 10 mM Tris·HCl buffer (pH 7.0), 10 mM KCl, 10 mM $MgCl_2$, 5 mM $CaCl_2$, and 8 kcpm of 3′-radiolabeled DNA. The solutions were allowed to equilibrate for 18−24 h at 22 °C. Cleavage was initiated by the addition of 10 μL of a DNase I solution (diluted with 1 mM dithiothreitol to 1.5 units/mL) and allowed to proceed for 7 min at 22 °C. The reactions were stopped by adding 50 μL of a solution containing 2.25 M NaCl, 150 mM EDTA, 0.6 mg/mL glycogen, and 30 μM base-pair calf thymus DNA and then precipitated with EtOH (2.1 volumes) and centrifuged at 14 krpm for 25 min. The pellets were washed with 75% ethanol, resuspended in 15 μL of RNase-free H_2O, lyophilized to dryness, and then resuspended in 10 μL of 100 mM Tris−borate−EDTA/80% formamide loading buffer, denatured at 90 °C for 10 min, and cooled in ice. A 5 μL sample (ca. 4.5 kcpm) was loaded onto a prerun (30 min at 2000 V) 8% denaturing polyacrylamide gel (5% cross-link, 7 M urea) at 2000 V for 1.5 h. The gels were dried in vacuo at 80 °C and then exposed to a storage phosphor screen (Molecular Dynamics) at 22 °C in the dark for 12−24 h. Storage phosphor screens were then scanned with a Storm 820 phosphorimager or Typhoon 8900 variable-mode imager (Molecular Dynamics). Equilibrium association constants were determined as previously described.[14,15]

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Supporting Information Available: Two figures showing quantitative DNase I footprint titration experiments for compounds **1**, **2**, **4**, and **5** in Table 1 and equilibrium binding isotherms for **3** and **6** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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