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Solid-Phase Synthesis of DNA Binding Polyamides on Oxime Resin

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Abstract—Control of the energetics and specificity of DNA binding polyamides is necessary for inhibition of protein–DNA complex formation and gene regulation studies. Typically, solid-phase methods using Boc monomers for synthesis have depended on Boc-β-Ala-PAM resin which affords a β-alanine-Dp tail at the C-terminus, after cleavage with *N,N*-dimethylaminopropylamine (Dp). To address the energetic consequences of this tail for DNA minor groove binding, we describe an alternative solid phase method employing the Kaiser oxime resin which allows the synthesis of polyamides with incrementally shortened C-terminal tails. Polyamides without Dp and having methyl amide tails rather than β-alanine show similar affinity relative to the standard β-Dp tail. The truncated tail diminishes the A,T base pair energetic preference of the β-Dp tail which will allow a greater variety of DNA sequences to be targeted by hairpin polyamides.

Introduction

Hairpin polyamides containing *N*-methylpyrrole (Py), *N*-methylimidazole (Im), and *N*-methyl-3-hydroxypyrrole (Hp) residues bind specific predetermined sequences in the minor groove of DNA with affinities and specificities comparable to naturally occurring DNA-binding proteins.[1] DNA recognition depends on the side-by-side amino acid pairings in the minor groove that stack the aromatic rings against each other and the walls of the groove allowing backbone amide hydrogens and the substituents at the 3-position of the Py, Im, and Hp residues to make specific contacts with the edges of the DNA base pairs. An Im/Py and Py/Im specifies for G·C and C·G, respectively. A Py/Py pair binds A·T and T·A and a Hp/Py pair discriminates T·A over A·T base pairs. These modular ring pairs may be considered the 'core' of the polyamide recognition motif. For hairpins an alkyl amino acid, either γ-aminobutyric acid (GABA, γ), or the chiral, amine-functionalized derivative (*R*)-2,4,-diaminobutyric acid [DABA, $(R)^{H_2N}\gamma$], serves as the covalent linker region (referred to as the 'turn') between the N-terminal and C-terminal strands and is specific for A·T and T·A base pairs. The 'tail' or C-terminal portion of a typical hairpin polyamide is related to the method of synthesis and comprises β-alanine (β) and *N,N*-dimethylaminopropylamine (Dp), which specify for A,T base pairs at the N-1 and N-2 positions (Fig. 1).[1]

The β-Dp tail originated with the C→N synthesis of polyamides by solid phase methods using Boc monomers on Boc-β-alanine-PAM-resin.[2,3] An alkyl primary amine such as Dp affords efficient cleavage from the solid support by aminolysis of the β-alanine ester linkage to the resin. The sequence requirements of the β-Dp tail for A,T versus G,C at the N-1 and N-2 positions[4] presumably results from a steric clash with the exocyclic NH_2 of a G,C base pair on the floor of the minor groove of DNA (Fig. 1). Replacement of the β-Dp tail with a shorter propanol amide, generated by the reductive cleavage of the β-alanine-PAM ester from the solid support,[5] removes the preference for an A,T base pair at the N-2 position while maintaining the binding affinity for these sites, despite the loss of an energetically favorable positive charge.[4] This suggests that the Dp tail interaction with the minor groove may be sterically unfavorable. A deeper understanding of the effect of incremental changes to the tail on the energetics of DNA binding would provide insight on the molecular determinant for A,T versus G,C specificity at the N-1 position.

In order to incrementally reprogram the polyamide C-terminus in the minor groove of DNA, new solid-phase methodology would be required since the

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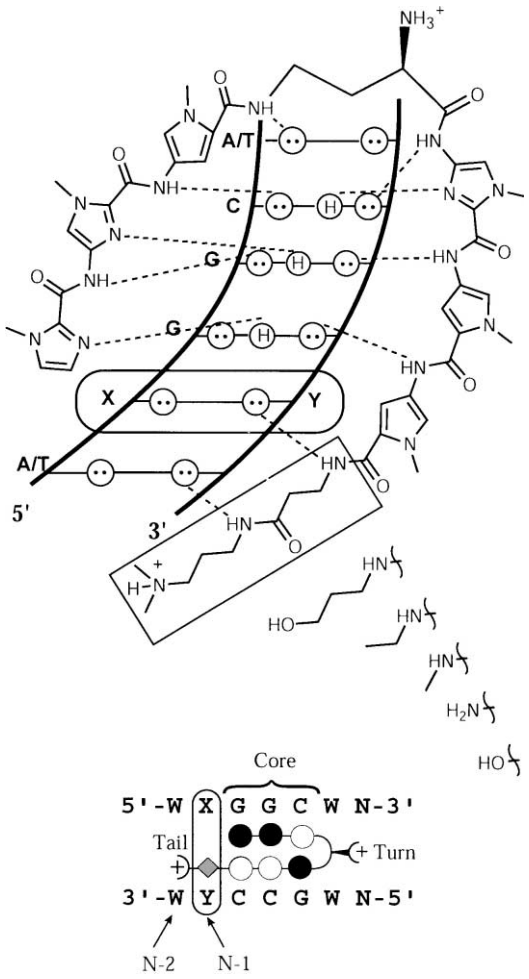


Figure 1. Binding model for polyamide **1** with DNA. (Top) Circles with dots represent lone pairs of N3 of purines and O2 of pyrimidines. Circles containing a H represent the N2 hydrogens of guanine. Putative hydrogen bonds are illustrated by dotted lines. The series of truncated tails to be examined are denoted. (Bottom) A ball and stick representation of polyamide **1** with DNA. Filled circles denote imidazole while open circles represent pyrrole. The diamond represents β-alanine. (*R*)-diaminobutyric acid [$(R)^{H_2N}\gamma$] and dimethylaminopropylamine (Dp) are depicted as a curved line and a plus sign, respectively. W signifies A or T, N represents any nucleotide. The N-2, N-1, core, turn positions for the polyamide/DNA complex are defined in the text.

propanol amide tail is the shortest C-terminus that can be generated from cleavage of Boc-β-Ala-PAM resin. Since the reductive cleavage to generate the propanol amide tail results in poor overall recovery, we generally do not use it. Therefore, for high affinity recognition, hairpin polyamides are synthesized with β-Dp tails which require DNA binding sites to be composed of the sequence 5′-WW(N)$_x$W-3′ (W = A,T; N = A,T,G,C; and x = the number of ring pairs in the core of the polyamide). While a large number of biologically relevant sequences have been targeted by the current generation of hairpin polyamides,[6] the necessity for A,T base pairs flanking the polyamide core is a limitation. Promoters are known to have high G,C content associated with CpG islands,[7] and many transcription factors bind G,C rich sequences.[8] In order to effectively inhibit these transcription factors, polyamides that can readily target all base pairs at the tail positions with high affinity are desirable.

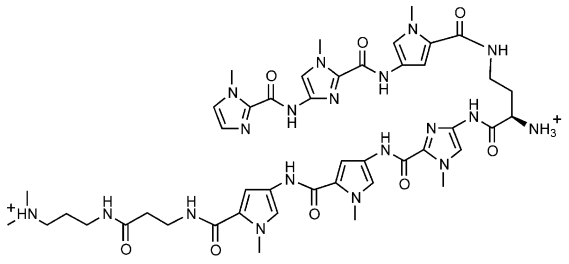
We describe here the solid-phase synthesis of polyamides with a pyrrole unit directly linked to the Kaiser oxime resin.[9–11] Six-ring polyamide hairpins with the common core sequence ImImPy-$(R)^{H_2N}\gamma$-ImPyPy-**X** (**3**–**6**) were synthesized where X represents four different C-terminal tails, ethyl amide, methyl amide, primary amide, and carboxylic acid. The DNA-binding affinity and specificity at the N-1 position was compared to the parent polyamides having either a β-Dp (**1**) or propanol amide tail (**2**) (Fig. 2).

Results

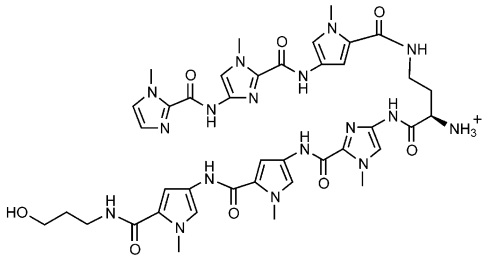
Polyamide synthesis

Polyamides ImImPy-$(R)^{H_2N}\gamma$-ImPyPy-β-Dp (**1**) and ImImPy-$(R)^{H_2N}\gamma$-ImPyPy-PrOH (**2**) were synthesized by solid phase methods on the usual Boc-β-alanine-PAM-resin. Four new polyamides **3**–**6** were synthesized by solid-phase methods on the Kaiser oxime resin with standard Boc-pyrrole and Boc-imidazole monomers (Fig. 3). Developed by Kaiser and DeGrado,[9,10] the oxime resin is a versatile polystyrene solid support that is amenable to Boc chemistry and allows for the synthesis of a variety of carboxylic acid derivatives by nucleophilic cleavage from the resin. Peptides can be cleaved from oxime resin to yield primary amide,[10] alkyl amide,[12] and carboxylic acid[13,14] C-termini. The oxime linker is reported to be somewhat acid labile, and solutions of less than 25% TFA are recommended for the deprotection of Boc groups. Consequently, a 20% TFA/CH_2Cl_2 solution was used for 30 min to remove the Boc groups from pyrrole and aliphatic amines. However, it was found that Boc-imidazole residues could not be fully deprotected under these conditions. Longer deprotection times resulted in increasing degradation of the resin bound polyamide. Boc groups could be efficiently removed from imidazoles in 30 min with a 50% TFA/CH_2Cl_2 solution. Remarkably, no premature cleavage of the oxime linker was observed at this higher concentration of acid. Attachment of the first pyrrole unit results in a bulky aromatic oxime ester that apparently stabilizes the linker relative to the aliphatic oxime ester present when conventional amino acids are used.

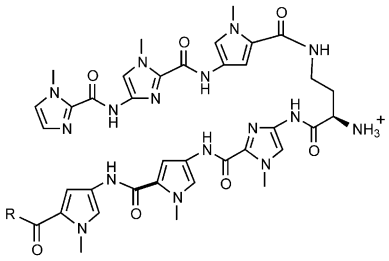
Polyamides **3**–**6** were generated from the common intermediate, ImImPy-$(R)^{FmocHN}\gamma$-ImPyPy-Oxime resin **11** (Fig. 3). Commercially available oxime resin was allowed to react directly with Boc-Py-OBt (**7**) in *N*-methylpyrrolidone (NMP) and diisopropylethylamine (DIEA) overnight at room temperature. The subsequent deprotections and couplings were carried out in a stepwise manner using the previously outlined deprotection conditions. All other monomers used; namely **8**, **9**, and **10**; were activated with HBTU and coupled in NMP/DIEA for 1.5–8 h at either room temperature or 37 °C. All cleavages of resin **11** required a co-solvent (CH_2Cl_2 or DMF) to swell the resin and/or solubilize the polyamide. Based on literature methods,[10] cleavage of resin **11** with a saturated solution of ammonia in THF/CH_2Cl_2 yielded polyamide **5**. However, even after more than 60 h at 37 °C only 60% cleavage was



1 ImImPy-$(R)^{H_2N}$γ-ImPyPy-β-Dp



2 ImImPy-$(R)^{H_2N}$γ-ImPyPy-PrOH



3 ImImPy-$(R)^{H_2N}$γ-ImPyPy-CO- R=NHEt

4 ImImPy-$(R)^{H_2N}$γ-ImPyPy-CO- R=NHMe

5 ImImPy-$(R)^{H_2N}$γ-ImPyPy-CO- $R=NH_2$

6 ImImPy-$(R)^{H_2N}$γ-ImPyPy-CO- R=OH

Figure 2. Structures of polyamides ImImPy-$(R)^{H_2N}$γ-ImPyPy-β-Dp (**1**), ImImPy-$(R)^{H_2N}$γ-ImPyPy-PrOH (**2**), ImImPy-$(R)^{H_2N}$γ-ImPyPy-CONHEt (**3**), ImImPy-$(R)^{H_2N}$γ-ImPyPy-CONHMe (**4**), ImImPy-$(R)H_2N$ImPyPy-$CONH_2$ (**5**) and ImImPy-$(R)^{H_2N}$γ-ImPyPy-CO_2H (**6**).

observed. Addition of a large excess of DBU, which had been used as a transacylation catalyst to generate peptide acids and esters from oxime resin previously,[13] resulted in quantitative cleavage of the resin to generate polyamide **5**. The C-terminal carboxylic acid polyamide **6** was generated in a similar manner with 1:1 H_2O/DMF, 30 equiv DBU for 60 h at 37 °C. This method was superior to acidic, basic, or reductive cleavage conditions. It is interesting to note that the standard transacylation catalyst, DMAP, was ineffective as a replacement for DBU in these cleavage reactions. Both polyamides **3** and **4** were generated from resin **11** with a 1.0 M solution in THF/CH_2Cl_2 of ethylamine or methylamine, respectively, overnight at 37 °C. The oxime resin is a versatile way to generate modified polyamides under mild conditions in good yield while remaining amenable to Boc chemistry.

Quantitative DNase I footprinting

It has been shown previously that elimination of Dp in the β-Dp tail in polyamide **1** diminishes the requirement for A,T preference at the N-2 position.[4] While empirical observations have suggested that the propanol amide tail, like the β-Dp tail (**2**) is A,T specific at the N-1 position, this had not been investigated in detail. Quantitative DNase I footprinting titrations[15] were performed to determine the equilibrium association constants (K_a) of polyamides **2**–**6** for four sites on a restriction fragment corresponding to the sequences 5′-AXGGCTA-3′ (where X = A,T,G,C) at the N-1 position (Fig. 4A–D, Table 1). The change from β-Dp (**1**) to propanol amide (**2**) results in similar affinity and specificity. This retention of affinity upon removal of the positively charged Dp moiety has previously been observed.[16,17] Presumably, the steric clash of propanol amide versus β-Dp with the exocyclic NH_2 of G at N-1 is similar.

Polyamides **3**–**5** afford similar DNA binding affinity versus the parent β-Dp while diminishing the sequence preference for A,T versus G,C at N-1. Polyamide **3** (ethyl amide) maintains a strong preference for A,T versus G,C, while polyamide **4** (methyl amide) less so. Polyamide **5** (primary amide) shows a loss in affinity relative to **1**–**4**, and little sequence discrimination between the four Watson–Crick base pairs. Polyamide **6** (carboxylic acid) reveals poor affinity and little sequence discrimination at the N-1 position (Table 1). The decreased affinity of polyamides **5** and **6** suggests that the increased Van der Waal contacts with the walls of the minor groove, made by polyamides **1**–**4**, may be important.

Discussion

In sequences with an A·T or T·A at the N-1 position, structural evidence[18–22] suggests that the C-terminal pyrrole secondary amide makes a hydrogen bond with the N3 of adenine and/or the O2 of thymine (Fig. 1). However, hydrogen bonds could also be made to the N3 of guanine and/or the O2 of cytosine. Assuming these hydrogen bonds are similar energetically, the discrimination of A,T versus G,C is likely due to steric reasons. Thus, the methylene groups of the propanol amide and β-Dp tails clash with the exocyclic amine of guanine and by default incur a lower energetic penalty at A,T. The influence of the exocyclic amine of guanine on specificity can be inferred from the observation that the sequence XY = C·G was the lowest affinity site for all of the polyamides examined in this study. At this site the guanine base is on the bottom strand of the DNA proximal to the C-terminus of the hairpin where the steric clash between the tail and the exocyclic NH_2 of guanine should be most severe.

Polyamides **3** and **4** with the ethyl and methyl amide tails provide a way to investigate whether specificity could be assigned to a particular methylene group of the propanol amide tail. The specific hydrogen bond made

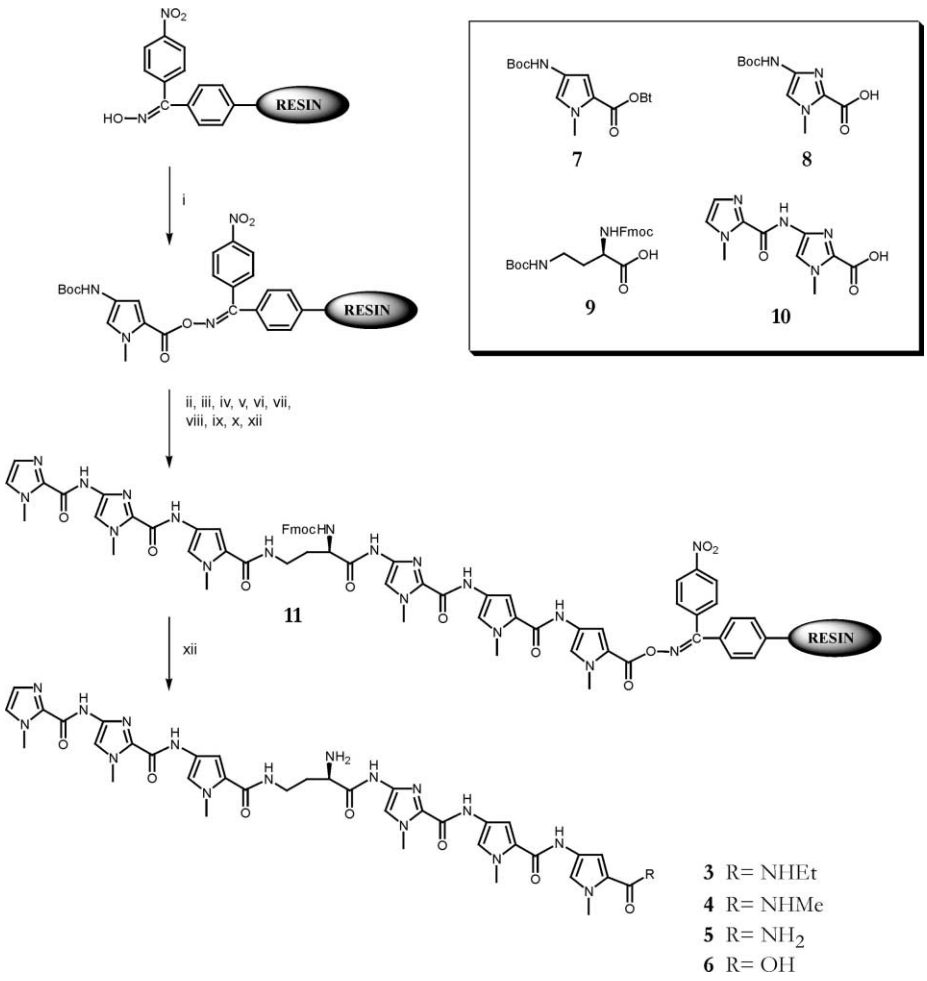
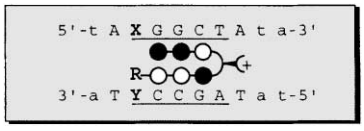


Figure 3. Synthesis of polyamide **3**–**6** from oxime resin using Boc-protected monomers: (i) **7**, DIEA, NMP; (ii) 20% TFA/DCM; (iii) **7**, DIEA, NMP; (v) 20% TFA/DCM; (v) **8**, HBTU, DIEA, NMP; (vi) 50% TFA/DCM; (vii) **9**, HBTU, DIEA, NMP, 37 °C; (viii) 20% TFA/DCM; (ix) **7**, DIEA, NMP; (x) 20% TFA/DCM; (xi) **10**, HBTU, DIEA, NMP; (xii) (a) R = NHEt, 2.0 M $CH_3CH_2NH_2$/THF, DCM, 37 °C; (b) R = NHMe, 2.0 M CH_3NH_2/THF, DCM, 37 °C; (c) R = NH_2, NH_3/THF, DCM, DBU, 37 °C; or (d) R = OH, H_2O, DMF, DBU, 37 °C.

Table 1. Polyamide **1**–**6** at the N-1 position[a]



	R = -β-Dp (**1**)	-$NH(CH_2)_3OH$ (**2**)	-$NHCH_2CH_3$ (**3**)	-$NHCH_3$ (**4**)	-NH_2 (**5**)	-OH (**6**)
X·Y						
A·T	1.7×10^9 (±0.5)	5.9×10^8 (±0.4)	1.1×10^9 (±0.1)	1.1×10^9 (±0.1)	1.9×10^8 (±0.2)	2.1×10^7 (±0.1)
T·A	1.6×10^9 (±0.2)	4.7×10^8 (±0.4)	9.2×10^8 (±0.3)	1.4×10^9 (±0.3)	2.1×10^8 (±0.2)	1.2×10^7 (±0.2)
G·C	5.8×10^7 (±1.4)	2.1×10^7 (±0.3)	8.2×10^7 (±1.3)	2.4×10^8 (±0.1)	6.8×10^7 (±0.5)	1.1×10^7 (±0.2)
C·G	3.2×10^7 (±0.3)	6.2×10^6 (±0.7)	3.0×10^7 (±0.5)	1.1×10^8 (±0.2)	3.5×10^7 (±0.2)	4.9×10^6 (±0.2)

[a]Equilibrium association constants (M^{-1}) for polyamide **1**–**6** with restriction fragment pSES-TL1.[4] Values reported are the mean values from at least three DNase I footprinting titration experiments with the standard deviation for each data set in parentheses. Equilibrium association constants for polyamide **1** at the N-1 position were obtained from Swalley et al.[4] Assays were performed at 22 °C at pH 7.0 in the presence of 10 mM Tris·HCl, 10 mM KCl, 10 mM $MgCl_2$, and 5 mM $CaCl_2$.

by the amide of the C-terminal pyrrole would point the first methylene group away from the floor of the minor groove, such that the energetic penalty and hence specificity might be attributable to the second methylene group. However, the methyl amide did exhibit about 8-fold preference for binding A,T or G,C. The ethyl amide tail disfavors both G·C and C·G sites by about 3-fold more than the methyl amide tail. Clearly, both alkyl groups contribute to A,T specificity, and even greater steric bulk beyond the second carbon is likely necessary to account for the greater than 50-fold specificity of the β-Dp and propanol amide tails. The high affinity of polyamides **3** and **4** suggest an energetic balance between increased Van der Waals contacts with the

walls of the minor groove relative to the primary amide tail (**5**) and decreased steric clashing relative to the β-Dp tail (**1**).

Polyamides with C-terminal carboxylic acid tails have been synthesized as intermediates to cyclic polyamides[23,24] but their DNA binding properties have not been investigated The carboxylic acid tail polyamide (**6**) binds all four sites with low affinity and poor sequence discrimination. To act as a hydrogen bond donor in analogy to the amides, the carboxylic acid would have to be protonated in the minor groove. The acid is likely deprotonated in the minor groove and the 10-fold lower affinity compared to the primary amide tail may be due to loss of this hydrogen bond. Additionally, in water polyamide **6** is expected to be a zwitterion with a net charge of zero which one would expect to have a lower affinity for DNA than the positively charged polyamides **1**–**5**.

In summary, hairpin polyamides with incrementally truncated C-terminal tails can be synthesized using Boc chemistry, in high yield, on an oxime resin by solid-phase methods. The polyamides are lower molecular weight than the corresponding β-Dp polyamide and bind all four base pairs at the N-1 position with high

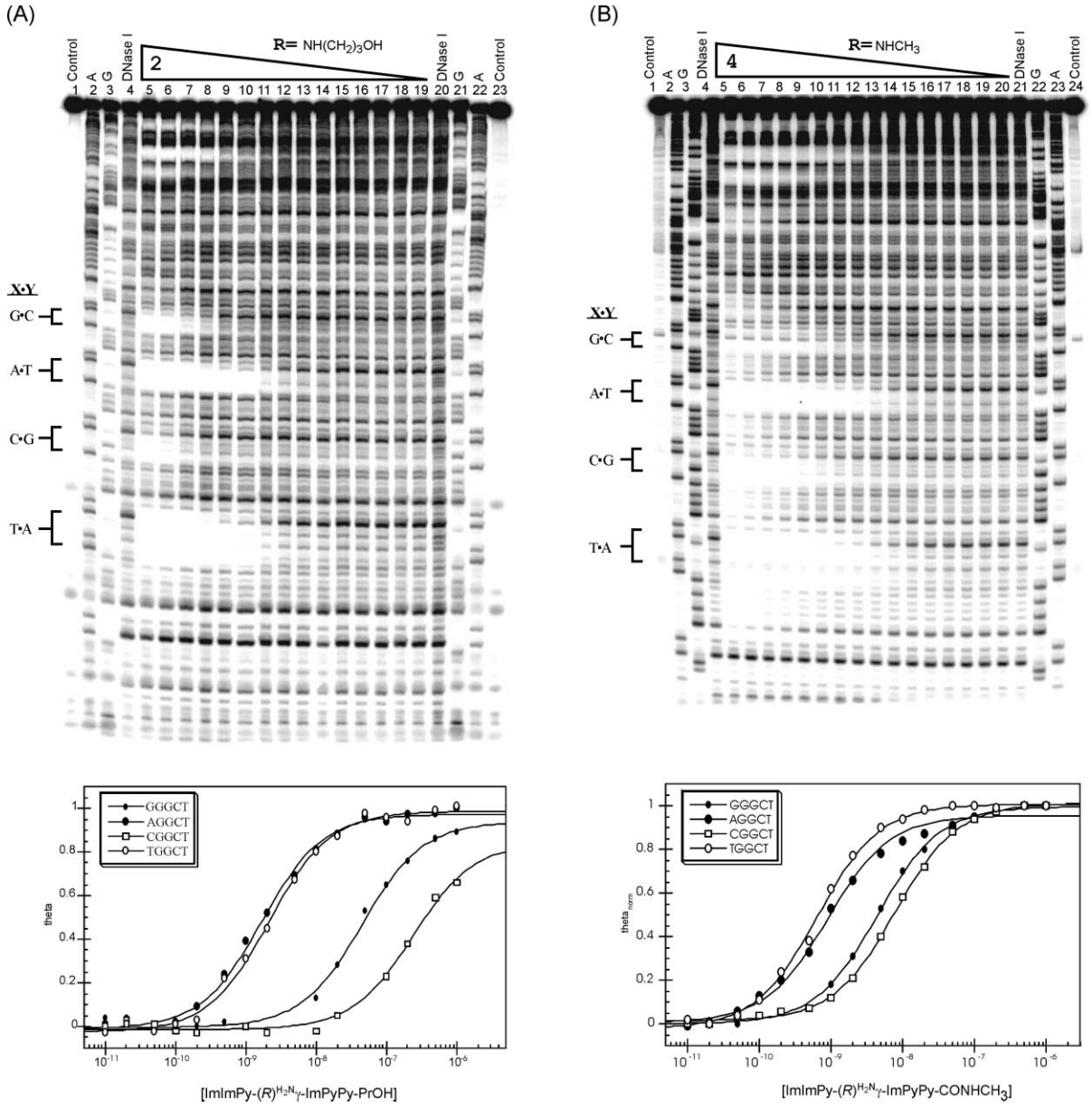


Figure 4. (A) Quantitative DNase I footprint titration experiment with ImImPy-$(R)^{H_2N}\gamma$-ImPyPyPrOH (**2**) on the 3'-^{32}P-labeled 286-bp restriction fragment pSES-TL1[4]: lane 1 & 23, intact DNA; lane 2 & 22, A reaction; lane 3 & 21, G reaction; lane 4 & 20 DNase I standard; lanes 5–19 DNase I digestion products in the presence of 1 μM, 500 nM, 200 nM, 100 nM, 20 nM, 10 nM, 5 nM, 2 nM, 1 nM, 500 pM, 200 pM, 100 pM, 50 pM, 20 pM, 10 pM polyamide respectively. (B) Quantitative DNase I footprint titration experiment with ImImPy-$(R)^{H_2N}\gamma$-ImPyPyCONHCH$_3$ (**4**) on the 3-^{32}P-labeled 286-bp restriction fragment pSES-TL1[4]: lane 1 & 24, intact DNA; lane 2 & 23, A reaction; lane 3 & 22, G reaction; lane 4 & 21 DNase I standard; lanes 5–20 DNase I digestion products in the presence of 1 μM, 500 nM, 200 nM, 100 nM, 50 nM, 20 nM, 10 nM, 5 nM, 2 nM, 1 nM, 500 pM, 200 pM, 100 pM, 50 pM, 20 pM, 10 pM polyamide, respectively.

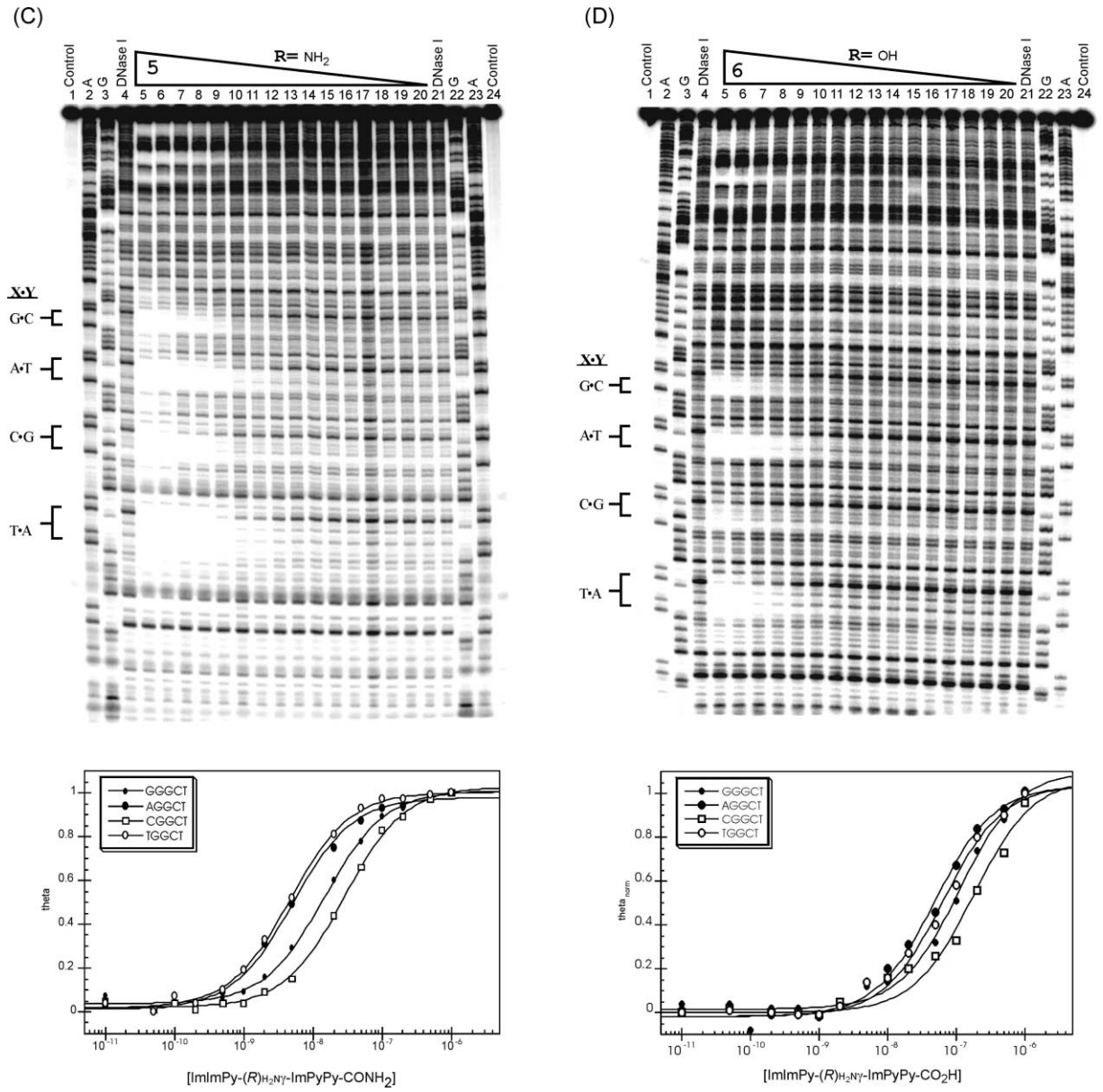


Figure 4. (C) Quantitative DNase I footprint titration experiment with ImImPy-$(R)^{H_2N}\gamma$-ImPyPyCONH$_2$ (**5**) on the 3′-^{32}P-labeled 286-bp restriction fragment pSES-TL1:[4] lanes 1 and 24, intact DNA; lanes 2 and 23, A reaction; lanes 3 and 22, G reaction; lanes 4 and 21 DNase I standard; lanes 5–20 DNase I digestion products in the presence of 1 μM, 500 nM, 200 nM, 100 nM, 50 nM, 20 nM, 10 nM, 5 nM, 2 nM, 1 nM, 500 pM, 200 pM, 100 pM, 50 pM, 20 pM, 10 pM polyamide respectively. All reactions contain 15 kcpm restriction fragment, 10 mM Tris·HCl (pH 7.0), 10 mM KCl, 10 mM $MgCl_2$, and 5 mM $CaCl_2$. Data was obtained for the four binding sites indicated at the left of the gel, 5′-GGGCT-3′, 5′-AGGCT-3′, 5′-CGGCT-3′, and 5′-TGGCT-3′, and is shown in the isotherm plot below. θ_{norm} points were obtained using storage phosphor autoradiography and processed by standard methods. Each data point shows the average value obtained from three footprinting experiments. The solid curves are best-fit Langmuir binding titration isotherms obtained from nonlinear least squares algorithm where $n = 1$ as previously described.[3] (D) Quantitative DNase I footprint titration experiment with ImImPy-$(R)^{H_2N}\gamma$-ImPyPyCO$_2$H (**6**) on the 3′-^{32}P-labeled 286-bp restriction fragment pSES-TL1:[4] lanes 1 and 24, intact DNA; lanes 2 and 23, A reaction; lanes 3 and 22, G reaction; lanes 4 and 21 DNase I standard; lanes 5–20 DNase I digestion products in the presence of 1 μM, 500 nM, 200 nM, 100 nM, 50 nM, 20 nM, 10 nM, 5 nM, 2 nM, 1 nM, 500 pM, 200 pM, 100 pM, 50 pM, 20 pM, 10 pM polyamide, respectively.

affinity which will enable targeting of new biologically relevant sequences by hairpin polyamides. If at the N-1 position, one desires specificity for A,T > G,C while maintaining high affinity, then the ethyl amide (**3**) is the end group of choice. If at the N-1 position, one prefers less sequence preference with high affinity, then the methyl amide is the best choice. It remains to be seen whether we can design an energetically favorable end group compatible with solid-phase methods with the criteria of efficient cleavage from solid phase resin which favors G,C > A,T.

Experimental

Materials

Polyamides **1** and **2**, and the restriction fragment pSES-TL1 have been previously described.[4] DNase I footprinting titrations on the ^{32}P-labeled restriction fragment pSES-TL1 were performed as previously described.[4] 2.0 M methylamine in THF, 2.0 M ethylamine in THF, DBU and anhydrous ammonia gas were purchased from Aldrich. Oxime resin was purchased

from Nova Biochem. All other synthetic and footprinting reagents were as previously described.[2,15,16] ^{1}H NMR were recorded on a Varian Mercury 300 instrument. UV spectra were measured on a Beckman Coulter DU 7400 diode array spectrophotometer. Autoradiography was performed with a Molecular Dynamics Typhoon Phosphorimager. Matrix-assisted, laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry was carried out at the Peptide and Protein Microanalytical Facility at the California Institute of Technology. HPLC analysis was performed on a Beckman Gold system using a RAINEN C_{18}, Microsorb MV, 5 μm, 300×4.6 mm reversed-phase column in 0.1% (w/v) TFA with CH_3CN as eluent and a flow rate of 1.0 mL/min, gradient elution 1.25% CH_3CN/min. Preparatory HPLC was carried out on a Beckman HPLC using a Waters DeltaPak 25×100 mm, 100 μm C_{18} column, 0.1% (w/v) TFA, 0.56% CH_3CN/min. 18 MΩ water was obtained from a Millipore MilliQ water purification system, and all buffers were 0.2 μm filtered. Reagent-grade chemicals were used unless otherwise stated.

ImImPy-(*R*)^{H_2N}γ-ImPyPyCONHEt (3). ImImPy-(*R*)^{FmocNH}γ-ImPyPyCO-Oxime resin (**11**) was generated by manual solid-phase synthesis from oxime resin (1 g, 0.48 mmol/g) using previously described Boc-protected monomers.[2] Boc-Py-OBt (**7**) (358 mg, 1 mmol) was dissolved in 2 mL of NMP and added to 1 g of oxime resin followed by 1 mL of DIEA. The coupling was allowed to proceed overnight at room temperature. The resin was then acetylated with 3 mL of acetic anhydride (Ac_2O), 4 mL of NMP and 1 mL of DIEA for 30 min at room temperature. The Boc group was removed upon treatment with 20% TFA/DCM for 30 min. The second pyrrole residue was coupled in the same fashion as the first but complete coupling could be achieved in 2 h at room temperature followed by the acetylation and deprotection steps outlined above. Boc-Im-OH (**8**) (241 mg, 1 mmol) was dissolved in 2 mL of NMP to which 360 mg (1 mmol) of HBTU and 1 mL of DIEA was added for activation of this monomer. Coupling was allowed to proceed for 1.5 h at room temperature followed by acetylation. The Boc-Im residue was deprotected using a 50% TFA/DCM solution for 30 min at room temperature. The next residue, α-Fmoc-γ-Boc-(*R*)-diaminobutyric acid (DABA) (**9**) (660 mg, 1.5 mmol) was activated with HBTU (540 mg, 1.5 mmol) in 2 mL of NMP and 1 mL of DIEA. Coupling of this residue onto the resin took 2 h at 37 °C. After acetylation and treatment with 20% TFA/DCM for 30 min, the next Boc-Py-OBt was attached in exactly the same manner as the second residue. The terminal two imidazoles were added as an ImIm-OH dimer.[2] Two hundred and forty nine milligrams (1 mmol) of the ImIm-OH dimer (**10**) was activated with HBTU (360 mg, 1 mmol) in 2 mL of NMP and 1 mL of DIEA and allowed to couple overnight at room temperature. It should be noted that the progress of the stepwise couplings were all monitored by analytical HPLC. The resin was washed thoroughly with DMF, DCM, MeOH and Et_2O then dried in vacuo. A 75-mg sample of dried resin was suspended in 2 mL of CH_2Cl_2 to which was added 2 mL of 2.0 M ethylamine in THF. This cleavage mixture was placed in a 37 °C oven and allowed to stand overnight in a sealed scintillation vial. The resin was filtered, the eluant concentrated in vacuo, then purified by reverse-phase HPLC. ImImPy-(*R*)H_2Nγ-ImPyPyCONHCH$_3$ (1.9 mg, 2.2 μmol, 10.6% recovery) was recovered upon lyophilization of the appropriate fractions as a white powder; UV (H_2O) λ_{max} 310 (52140). ^{1}H NMR (DMSO-d_6) δ 11.02 (s, 1H), 10.36 (s, 1H), 10.10 (s, 1H), 9.89 (s, 1H), 9.71 (s, 1H), 8.20 (m, 1H), 7.98 (m, 1H), 7.56 (s, 1H), 7.52 (s, 1H), 7.45 (d, 1H, J = 0.9 Hz), 7.25 (d, 1H, J = 1.5 Hz), 7.23 (d, 1H, J = 1.5 Hz), 7.15 (d, 1H, J = 1.8 Hz), 7.11 (d, 1H, J = 1.8 Hz), 7.06 (d, 1H, J = 0.9 Hz), 7.03 (d, 1H, J = 1.8 Hz), 6.83 (d, 1H, J = 1.8 Hz), 3.99 (s, 3H), 3.98 (s, 3H), 3.96 (s, 3H), 3.83 (s, 3H), 3.80 (s, 3H), 3.78 (s, 3H), 3.17 (dd, 2H, J = 6.6 Hz), 2.42 (m, 2H), 2.26 (m, 1H), 1.99 (m, 2H), 1.62 (m, 1H), 1.05 (t, 3H J = 6.6 Hz), MALDI-TOF-MS 866.5 (866.39 calcd for [M + H] $C_{39}H_{48}N_{17}O_7^+$).

ImImPy-(*R*)^{H_2N}γ-ImPyPyCONHMe (4). A 35-mg sample of dried resin **11** was suspended in 2 mL of CH_2Cl_2 to which was added 2 mL of 2.0 M methylamine in THF. This cleavage mixture was placed in a 37 °C oven and allowed to stand overnight in a sealed scintillation vial. The resin was filtered, the eluant concentrated in vacuo, then purified by reverse-phase HPLC. ImImPy-(*R*)^{H_2N}γ-ImPyPyCONHCH$_3$ (850 μg, 996 nmol, 8.9% recovery) was recovered upon lyophilization of the appropriate fractions as a white powder; UV (H_2O) λ_{max} 310 (52140). ^{1}H NMR (DMSO-d_6) δ 11.01 (s, 1H), 10.35 (s, 1H), 10.09 (s, 1H), 9.90 (s, 1H), 9.70 (s, 1H), 8.19 (m, 1H), 7.92(m, 1H), 7.56 (s, 1H), 7.51 (s, 1H), 7.45 (d, 1H, J = 0.9 Hz), 7.25 (d, 1H, J = 1.8 Hz), 7.23 (d, 1H, J = 1.8 Hz), 7.16 (d, 1H, J = 1.5 Hz), 7.10 (d, 1H, J = 1.5 Hz), 7.05 (d, 1H, J = 0.9 Hz), 7.03 (d, 1H, J = 1.8 Hz), 6.79 (d, 1H, J = 1.8 Hz), 3.99 (s, 3H), 3.98 (s, 3H), 3.96 (s, 3H), 3.82 (s, 3H), 3.79 (s, 3H), 3.77 (s, 3H), 2.66 (m, 1H), 2.41 (m, 2H), 2.25 (m, 1H), 1.98 (m, 2H), 1.62 (m, 2H), MALDI-TOF-MS 852.5 (852.38 calcd for [M + H] $C_{38}H_{46}N_{17}O_7^+$).

ImImPy-(*R*)^{H_2N}γ-ImPyPyCONH$_2$ (5). A 30-mg sample of dried resin **11** was placed into a pressure tolerant screw cap test tube, suspended in 4 mL of dry THF and cooled for 20 min in a −10 °C ice/brine bath. Anhydrous NH_3 gas was bubble into the suspension at a steady rate for 30 min to generate a saturated ammonia solution. 1.5 mL of a 10% v/v solution of DBU in CH_2Cl_2, cooled to −20 °C was quickly added to the cleavage mixture and the tube immediately sealed. The cleavage was allowed to proceed in a shaker at 37 °C for 72 h. The mixture is filtered and concentrated in vacuo, then purified by reverse-phase HPLC. ImImPy-(*R*)^{H_2N}γ-ImPyPyCONH$_2$ (330 μg, 390 nmol, 4.1% recovery) was recovered upon lyophilization of the appropriate fractions as a white powder; UV (H_2O) λ_{max} 310 (52140). ^{1}H NMR (DMSO-d_6) δ 10.65 (s, 1H), 10.32 (s, 1H), 10.20 (s, 1H), 9.90 (s, 1H), 9.75 (s, 1H), 8.09 (bs, 1H), 7.55 (s, 3H), 7.47 (s, 1H), 7.44 (s, 1H), 7.25 (d, 1H, J = 2.1 Hz), 7.21 (d, 1H, J = 2.1 Hz), 7.19 (bs 2H), 7.11 (d, 1H, J = 1.8 Hz), 7.05 (d, 1H, J = 1.2 Hz), 6.98 (d, 1H, J = 1.2 Hz), 6.83 (d, 1H, J = 1.5 Hz), 6.65 (d, 1H, J = 1.2 Hz), 6.53 (d, 1H, J = 2.4 Hz), 3.99 (s, 3H), 3.98 (s, 3H), 3.96 (s, 3H), 3.82 (s, 3H), 3.79 (s, 1H), 3.77 (s, 3H), 2.97

(m, 1H), 1.96 (m, 2H), 1.43 (m, 2H), MALDI-TOF-MS 838.4 (838.35 calcd for $[M+H]$ $C_{37}H_{44}N_{17}O_7^+$)

ImImPy-(*R*)^{H_2N}γ-ImPyPyCO$_2$H (6). A 35-mg sample of dried resin **11** was cleaved by treatment with 1 mL of H_2O, 1 mL DMF, and 75 μL DBU (0.48 mmol) at 37 °C for 60 h. After filtration the cleavage solution was concentrated in vacuo and purified by reverse phase HPLC. ImImPy-(*R*)^{H_2N}γ-ImPyPyCO$_2$H (294 μg, 350 nmol, 3.1% recovery) was recovered upon lyophilization of the appropriate fractions as a white powder; UV (H_2O) λ_{max} 310 (52140). ^{1}H NMR (DMSO-d_6) δ 12.67 (bs, 1H), 11.53 (s, 1H), 10.88 (s, 1H), 10.62 (s, 1H), 10.43 (s, 1H), 10.22 (s, 1H), 8.83 (m, 1H), 8.07 (s, 1H), 8.03 (s, 1H), 7.96 (d, 1H, $J=0.9$ Hz), 7.93 (d, 1H, $J=1.8$ Hz), 7.76 (d, 1H, $J=0.9$ Hz), 7.75 (d, 1H, $J=1.8$ Hz), 7.64 (d, 1H, $J=1.2$ Hz), 7.57 (d, 1H, $J=1.2$ Hz), 7.54 (d, 1H, $J=1.8$ Hz), 7.34 (d, 1H, $J=1.8$ Hz), 4.50 (s, 3H), 4.50 (s, 3H), 4.47 (s, 3H), 4.34 (s, 3H), 4.31 (s, 3H), 4.31 (s, 3H), 2.50 (m, 2H), 2.11 (m, 2H), 2.06 (m, 3H), MALDI-TOF-MS 839.4 (839.34 calcd for $[M+H]$ $C_{37}H_{43}N_{16}O_8^+$).

Quantitative DNase I footprinting. As previously reported.[15]

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References and Notes

1. Dervan, P. B.; Bürli, R. W. *Curr. Opin. Chem. Biol.* **1999**, *3*, 688.
2. Baird, E. E.; Dervan, P. B. *J. Am. Chem. Soc.* **1996**, *118*, 6141.
3. Parks, M. E.; Baird, E. E.; Dervan, P. B. *J. Am. Chem. Soc.* **1996**, *118*, 6147.
4. Swalley, S. E.; Baird, E. E.; Dervan, P. B. *J. Am. Chem. Soc.* **1999**, *121*, 1113.
5. Trauger, J. W.; Baird, E. E.; Dervan, P. B. *Angew. Chem., Int. Edit. Engl.* **1998**, *37*, 1421.
6. Gottesfeld, J. M.; Turner, J. M.; Dervan, P. B. *Gene Expr.* **2000**, *9*, 77.
7. Ioshikhes, I. P.; Zhang, M. Q. *Nat. Genet.* **2000**, *26*, 61.
8. Pabo, C. O.; Sauer, R. T. *Annu. Rev. Biochem.* **1992**, *61*, 1053.
9. DeGrado, W. F.; Kaiser, E. T. *J. Org. Chem.* **1980**, *45*, 1295.
10. DeGrado, W. F.; Kaiser, E. T. *J. Org. Chem.* **1982**, *47*, 3258.
11. Behrens, C.; Nielson, P. E. *Combin. Chem. High Throughput Screening* **1998**, *1*, 127.
12. Voyer, N.; Lavoie, A.; Pinette, M.; Bernier, J. *Tetrahedron Lett.* **1994**, *35*, 355.
13. Pichette, A.; Voyer, N.; Larouche, R.; Meillon, J.-C. *Tetrahedron Lett.* **1997**, *38*, 1279.
14. Nakagawa, S. H.; Kaiser, E. T. *J. Org. Chem.* **1983**, *48*, 678.
15. Trauger, J. W.; Dervan, P. B. *Methods Enzymol.* **2001**, *340*, 450.
16. Herman, D. M.; Baird, E. E.; Dervan, P. B. *J. Am. Chem. Soc.* **1998**, *120*, 1382.
17. Bremer, R. E.; Wurtz, N. R.; Szewczyk, J. W.; Dervan, P. B. *Bioorg. Med. Chem.* **2001**, *9*, 2093.
18. deClairac, R. P. L.; Geierstanger, B. H.; Mrksich, M.; Dervan, P. B.; Wemmer, D. E. *J. Am. Chem. Soc.* **1997**, *119*, 7909.
19. deClairac, R. P. L.; Seel, C. J.; Geierstanger, B. H.; Mrksich, M.; Baird, E. E.; Dervan, P. B.; Wemmer, D. E. *J. Am. Chem. Soc.* **1999**, *121*, 2956.
20. Kielkopf, C. L.; Baird, E. E.; Dervan, P. D.; Rees, D. C. *Nat. Struct. Biol.* **1998**, *5*, 104.
21. Kielkopf, C. L.; Bremer, R. E.; White, S.; Szewczyk, J. W.; Turner, J. M.; Baird, E. E.; Dervan, P. B.; Rees, D. C. *J. Mol. Biol.* **2000**, *295*, 557.
22. Kielkopf, C. L.; White, S.; Szewczyk, J. W.; Turner, J. M.; Baird, E. E.; Dervan, P. B.; Rees, D. C. *Science* **1998**, *282*, 111.
23. Cho, J.; Parks, M. E.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 10389.
24. Herman, D. M.; Turner, J. M.; Baird, E. E.; Dervan, P. B. *J. Am. Chem. Soc.* **1999**, *121*, 1121.