Experimental

Materials. Methylamine, piperidine, and dimethylaminopropylamine were purchased from Aldrich. Dimethylformamide (DMF) and diisopropylethylamine (DIEA), and dimethylsulfoxide/N-methylpyrrolidone (DMSO/NMP) (1:1, v/v) were purchased from Applied Biosystems. N,N-dimethylaminopropylamine, 3,3'-diamino-N-methyldipropylamine, ethylenediamine-tetraacetic dianhydride, triethylamine, and acetic acid were purchased from Aldrich. Acetic anhydride and acetonitrile were from EM. Trifluoroacetic acid (TFA) was from Halocarbon. (R)-2-Fmoc-4-Boc-diaminobutyric acid was purchased from Bachem. Boc-β-Pam resin was purchased from Peptides International. Calcuim chloride, magnesium chloride, and potassium chloride were from Fluka. Glycogen (20 mg/mL), dNTP's (PCR nucleotide mix), and all enzymes (unless otherwise stated) were purchased from Boehringer-Mannheim and used with their supplied buffers. pUC19 was from New England Biolabs. Deoxyadenosine [γ-^{32}P] triphosphate was from ICN. Calf thymus DNA (sonicated, deproteinized) and DNase I (7500 u/mL, FPLC pure) were from Amersham-Pharmacia. AmpliTaq DNA polymerase was from Perkin Elmer and used with the supplied buffers. HEPES was from Sigma. Tris-HCl, dithiothreitol (DTT), RNase-free water (used for all DNA manipulations), and 0.5 M EDTA were from US Biochemicals. Ethanol (200 proof) was from Equistar. Calcium chloride, potassium chloride, and magnesium chloride were from Fluka. Formamide and pre-mixed tris-borate-EDTA (Gel-Mate, used for gel running buffer) were from Gibco. Bromophenol blue was from Acros. All reagents were used without further purification. Centricon YM-3 dialysis filters were from

Amicon. D_2O ("100%") was from Cambridge Isotope Laboratories. NMR tubes were type 535 from Wilmad.

HPLC analysis was performed on a Beckman Gold system using a Rainin C_{18}, Microsorb MV, 5 μm, 300 x 4.6 mm reversed phase column in 0.1% (wt/v) TFA with acetonitrile as eluent and a flow rate of 1.0 mL/min, gradient elution 1.25% acetonitrile/min. Preparatory reversed phase HPLC was performed on a Beckman HPLC with a Waters DeltaPak 25 x 100 mm, 100 μm C18 column equipped with a guard, 0.1% (wt/v) TFA, 0.25% acetonitrile/min. Oligonucleotides were synthesized at the Biopolymer Synthesis Center at the California Institute of Technology (Caltech). Plasmid sequencing was carried out by Davis Sequencing (Davis, CA) or by the Sequence/Structure Analysis Facility at Caltech. Matrix-Assisted-Laser-Desorption-Ionization-Time-Of-Flight Mass Spectrometry (MALDI-TOF-MS) was performed at the Protein and Peptide Microanalytical Facility at Caltech. UV-Visible Spectra were obtained on a Beckman DU-7400 spectrophotometer. FPLC was performed on a Pharmacia LKB system. All buffers were 0.2 μm filtered. DNA manipulations were performed according to standard protocols (Sambrook et al., 1989).

Solid-Phase Synthesis of Polyamides. Published protocols were used to synthesize the Boc-protected amino acid monomers and dimers for all polyamides studied (Baird and Dervan, 1996; Urbach et al., 1999). Solid phase synthesis was performed by manual stepwise coupling on Boc-β-PAM resin using Boc-protected amino acid monomers and dimers as previously described (Baird and Dervan, 1996; Herman et al., 1998; Urbach et al., 1999). Couplings were monitored by analytical HPLC, and all polyamides except **18** were cleaved from solid support using dimethylaminopropylamine at 100 °C for 2h, and purified by preparatory HPLC. Special considerations for polyamides **4**, **18**, and **19** are described below. The fractions were analyzed by analytical HPLC, and the appropriate fractions were lyophilized to dryness.

ImPyImPy-β-ImPyImPy-β-Dp (1). 70% recovery; UV (H_2O) λ_{max} 244 nm (ε = 28 500, measured), 308 nm (ε = 44 300, measured); ^{1}HNMR (DMSO-*d6*) δ 10.41 (s, 1 H), 10.33 (s, 1 H), 10.21 (s, 2 H), 10.01 (s, 1 H), 9.99 (s, 1 H), 9.91 (s, 1 H), 9.18 (br s, 1 H), 8.06 (m, 3 H), 7.53 (s, 1 H), 7.52 (s, 1 H), 7.46 (s, 1 H), 7.38 (m, 2 H), 7.36 (d, 1 H, *J* = 1.8 Hz), 7.21 (d, 1 H, *J* = 1.8 Hz), 7.19 (d, 1 H, *J* = 1.8 Hz), 7.14 (d, 1 H, *J* = 1.8 Hz), 7.12 (d, 1 H, *J* = 1.8 Hz), 7.04 (s, 1 H), 6.94 (m, 2 H), 3.97 (s, 3 H), 3.95 (s, 6 H), 3.94 (s, 3 H), 3.84 (s, 3 H), 3.84 (s, 3 H), 3.80 (s, 3 H), 3.78 (s, 3 H), 3.42 (m, 2 H), 3.35 (m, 2 H), 3.09 (q, 2 H, *J* = 6.3 Hz), 2.98 (m, 2 H), 2.72 (d, 6 H, *J* = 4.5 Hz), 2.57 (t, 2 H, *J* = 6.6 Hz), 2.33 (t, 2 H, *J* = 6.3 Hz), 1.71 (m, 2 H); MALDI-TOF-MS (monoisotopic), 1210.6 (1210.5 calc. for $C_{55}H_{68}N_{23}O_{10}$);

Im-β-ImPy-β-Im-β-ImPy-β-Dp (2). 24% recovery; UV (H_2O) λ_{max} 248 (ε = 27 000, measured), 290 (ε = 28 200, measured); ^{1}HNMR (DMSO-*d6*) δ 10.36 (s, 1 H), 10.33 (s, 2 H), 9.32 (br s, 1 H), 9.26 (s, 2 H), 8.40 (t, 1 H, *J* = 6 Hz), 8.05 (m, 3 H), 7.89 (t, 1 H, *J* = 6 Hz), 7.43 (m, 2 H), 7.39 (s, 1 H), 7.34 (s, 1 H), 7.19 (d, 2 H), 6.98 (s, 1 H), 6.92 (s, 1 H), 6.90 (s, 1 H), 3.91 (m, 9 H), 3.89 (s, 3 H), 3.78 (s, 6 H), 3.47 (m, 4 H), 3.36 (m, 4 H), 3.08 (q, 2 H, *J* = 6 Hz), 2.98 (m, 2 H), 2.72 (d, 6 H, *J* = 4.5 Hz), 2.58 (t, 4 H, *J* = 6.6 Hz), 2.53 (t, 3 H, *J* = 7.2 Hz), 2.32 (t, 2 H, *J* = 7.2 Hz), 1.71 (m, 2 H); MALDI-TOF-MS (monoisotopic), 1108.6 (1108.5 calc. for $C_{49}H_{66}N_{21}O_{10}$);

Im-β-ImPyPyIm-β-ImPy-β-Dp (3). 11% recovery; UV (H_2O) λ_{max} 254 nm (ε = 32 200, measured), 308 nm (ε = 43 700, measured); ^{1}HNMR (DMSO-*d6*) δ 10.34 (s, 1H), 10.33 (s, 2H), 10.00 (s, 1 H), 9.94 (s, 2 H), 9.22 (br s, 1 H), 8.38 (t, 1 H, *J* = 5.7 Hz), 8.04 (m, 2 H), 7.91 (t, 1 H, *J* = 6.0 Hz), 7.50 (d, 1 H, *J* = 1.2 Hz), 7.45 (d, 1 H, *J* = 1.2 Hz), 7.41 (d, 1 H, *J* = 1.2 Hz), 7.33 (s, 1 H), 7.31 (s, 1 H), 7.25 (s, 1 H), 7.19 (s, 1 H), 7.13 (s, 1 H), 7.07 (s, 1 H), 6.96 (t, 1 H, *J* = 1.2 Hz), 6.91 (s, 1 H), 3.94 (s, 3 H), 3.93 (m, 6 H), 3.92 (s, 3 H), 3.82 (s, 6 H), 3.77 (s, 3 H), 3.49 (m, 4 H), 3.35 (q, 2 H, *J* = 6.0 Hz), 3.08 (q, 2 H, *J* = 6.0 Hz), 2.97 (m, 2 H), 2.71 (d, 6 H, *J* = 3.9 Hz), 2.59 (m, 4 H), 2.32 (t, 2 H, *J* = 6.9 Hz), 1.71 (m, 2 H); MALDI-TOF-MS (monoisotopic), 1159.6 (1159.5 calc. for $C_{52}H_{67}N_{22}O_{10}$).

Im-β-ImHp-β-Im-β-ImPy-β-Dp (4). The hydroxypyrrole-containing polyamide **4** was prepared using the methyl ether-protected hydroxypyrrole amino acid, Boc-Op-OH as described previously (Urbach et al., 1999). The protected polyamide, Im-β-ImOp-β-Im-β-ImPy-β-Dp, was synthesized as described above. 14% recovery, UV (H_2O) λmax 290 (ε~28,000); ^{1}H NMR (DMSO-d_6) δ 10.50 (s, 1H), 10.42 (s, 1 H), 10.33 (s, 1 H), 9.91 (s, 1 H), 9.27 (br s, 1 H), 8.91 (s, 1 H), 8.41 (t, 1 H, J = 6.0 Hz), 8.04 (t, 2 H, J = 6.0 Hz), 7.89 (t, 1 H, J = 6.0 Hz), 7.49 (s, 1 H), 7.42 (s, 1 H), 7.41 (s, 1 H), 7.34 (s, 1 H), 7.19 (s, 1 H), 7.14 (s, 1 H), 6.99 (s, 1 H), 6.92 (s, 1 H), 3.29 (s, 3 H), 3.92 (m, 6 H), 3.90 (s, 3 H), 3.78 (s, 3 H), 3.75 (s, 3 H), 3.69 (s, 3 H), 3.49 (m, 6 H), 3.36 (q, 2 H, J = 5.3 Hz), 3.09 (q, 2 H, J = 6.3 Hz), 2.97 (t, 2 H, J = 5.7 Hz), 2.72 (d, 6 H, J = 4.8 Hz), 2.58 (m, 6 H), 2.33 (t, 2 H, J = 6.9 Hz), 1.72 (quintet, 2 H, J = 8.4 Hz); MALDI-TOF-MS (monoisotopic), 1138.58 (1138.54 calcd. for $C_{50}H_{68}N_{21}O_{11}^+$).

In order to remove the methoxy protecting group, a sample of Im-β-ImOp-β-Im-β-ImPy-β-Dp, (9 mg, 7.9 mmol) was treated with sodium thiophenoxide at 100 °C for 2 h as described previously (Urbach et al., 1999). DMF (1.0 mL) and thiophenol (0.5 mL) were placed in a (13 x 100 mm) disposable Pyrex screw cap culture tube. A 60% dispersion of sodium hydride in mineral oil (100 mg) was slowly added. Upon completion of the sodium hydride addition, Im-β-ImOp-β-Im-β-ImPy-β-Dp (5 mg) dissolved in DMF (0.5 mL) was added. The solution was agitated, and heated to 100 °C for 2 h. Upon completion of the reaction, the reaction mixture was cooled to 0 °C, and 7 mL of a 20% (wt/v) aqueous solution of trifluoroacetic acid added. The aqueous layer is separated from the resulting biphasic solution. In order to remove the last trace of thiophenol, the aqueous layer was extracted three times with diethyl ether. The deprotected polyamide was purified by reversed phase preparatory HPLC. Im-β-ImHp-β-Im-β-ImPy-β-Dp **4** is recovered upon lyophilization of the appropriate fractions as a white powder (1.5 mg, 18% recovery). UV (H_2O) λmax 290 (ε ~ 28,000), ; ^{1}H NMR (DMSO-d_6) δ 10.38 (s, 1 H), 10.37 (s, 1 H), 10.33(s, 1 H), 9.93 (s, 1 H), 9.90 (s, 1 H), 9.61 (s, 1 H), 9.18 (br s, 1 H), 8.37 (t, 1 H, J = 6.0 Hz), 8.04 (m, 2 H), 7.90 (t, 1 H, J = 6.0 Hz), 7.56 (t, 1 H, J = 5.9 Hz), 7.48 (s, 1 H), 7.43 (s, 1 H), 7.40 (s, 1 H), 7.31 (s, 1 H), 7.19 (s, 1 H), 7.12 (s, 1 H), 6.94 (s, 1 H), 6.93 (s, 1 H), 3.92 (m, 9 H), 3.90 (s, 3 H), 3.78 (s, 3 H), 3.74 (s, 3 H), 3.48 (m, 6 H), 3.09 (q, 2 H, J = 5.7 Hz), 2.99 (m, 6 H), 2.56 (m, 4 H), 2.48 (d, 6 H, J = 5.4 Hz), 2.32 (t, 2 H, J = 5.9 Hz), 1.61 (quintet, 2 H, J = 5.4 Hz); MALDI-TOF-MS (monoisotopic), 1124.6 (1124.5 calcd. for $C_{49}H_{66}N_{21}O_{11}^+$).

ImPy-β-Im-β-ImPy-β-Dp (13). 29% recovery, UV (H_2O) λmax 290 (ε~30 000, measured); MALDI-TOF-MS (monoisotopic), 914.5 (914.5 calc. for $C_{41}H_{56}N_{17}O_8^+$); This compound is the one characterized in complex with DNA as described in the Results and Discussion section. The complete assignment of all protons by ^{1}HNMR is given in Table 5.

Im-β-ImPy-β-Im-β-Dp (14). 64% recovery, UV (H_2O) λmax 270 (ε~23 300, measured); MALDI-TOF-MS (monoisotopic), 792.4 (792.4 calc. for $C_{35}H_{50}N_{15}O_7^+$).

ImPy-β-Im-β-Dp (15). 55% recovery, UV (H_2O) λmax 280 (ε~19 500, measured); MALDI-TOF-MS (monoisotopic), 598.2 (598.3 calc. for $C_{27}H_{40}N_{11}O_5^+$).

Im-β-ImPy-β-Dp (16). 64% recovery, UV (H_2O) λmax 270 (ε~20 200, measured); MALDI-TOF-MS (monoisotopic), 598.3 (598.3 calc. for $C_{27}H_{40}N_{11}O_5^+$).

Im-β-ImPy-(R)^{H_2N}γ-Im-β-ImPy-β-Me (18). Im-β-ImPy-(R)^{H2N}γ-Im-β-ImPy-β-Pam resin was synthesized in a stepwise fashion from Boc-β-Pam resin using manual solid-phase protocols (Baird and Dervan, 1996). The chiral diaminobutyric acid "turn" residue (Herman et al., 1998) was incorporated by coupling (R)-2-Fmoc-4-Boc-diaminobutyric acid (10 equivalents) to 300 mg Boc-Im-Py-Py-Py-β-Pam resin in 2 mL DMF with 1.1 equivalents of DIEA at 37 °C for 2 h, followed by an acetylation wash (1 mL acetic anhydride, 1 mL DIEA, 2 mL DMF, 5 min, room temp). Subsequent coupling steps used 1.1 equivalents of DIEA and 45 min coupling times at room temperature to minimize Fmoc deprotection. Im-β-ImPy-(R)^{Fmoc}γ-Im-β-ImPy-β-Pam resin was treated with piperidine for 20 min at room temperature to remove the Fmoc group. 100 mg (38 μmol) of vacuum-dried Im-β-ImPy-(R)^{H2N}γ-Im-β-ImPy-β-Pam resin was cleaved in 30 mL condensed methylamine in a Parr bomb apparatus at 50 °C for

2 h, then overnight at room temperature. The methylamine was allowed to evaporate at ambient pressure and temperature, and the resin was suspended in 2 mL acetonitrile, followed by 7 mL 0.1% (wt/v) $TFA_{(aq)}$. The suspension was filtered, and the filtrate was purified by reversed phase preparatory HPLC to afford **18** as a white powder (7.1 μmol, 19% recovery) upon lyophilization of the appropriate fractions. MALDI-TOF-MS (monoisotopic), 1066.49 (1066.48 calcd. for $C_{46}H_{60}N_{21}O_{10}^+$).

Im-β-ImPy-(R)^{Ac}γ-Im-β-ImPy-β-Dp (19). Im-β-ImPy-(R)^{H_2N}γ-Im-β-ImPy-β-Pam resin was synthesized as described above for **18**. The resin was washed with acetic anhydride in DMF and DIEA for 10 min at room temperature and then dried *in vacuo*. 100 mg (37 μmol) Im-β-ImPy-(R)^{Ac}γ-Im-β-ImPy-β-Pam resin was treated with dimethylaminopropylamine at 100 °C for 2 h. The resin was removed by filtration, and the filtrate was diluted to 10 mL with 0.1 % (wt/v) $TFA_{(aq)}$ and purified by reversed phase HPLC. **19** was obtained as a white powder (11% recovery) upon lyophilization of the appropriate fractions. MALDI-TOF-MS (monoisotopic), 1179.57 (1179.57 calcd. for $C_{52}H_{71}N_{22}O_{11}^+$).

ImPyPyPy-β-ImPyPyPy-β-Dp (20). MALDI-TOF-MS (monoisotopic), 1208.6 (calcd. 1208.6 for $C_{57}H_{70}N_{21}O_{10}^+$).

Synthesis of EDTA Conjugates. A single-step aminolysis of the resin ester linkage was accomplished using 3,3'-diamino-N-methyldipropylamine to afford polyamides with a primary amine at the C-terminus (**X-NH$_2$**). After purification by HPLC these were allowed to react with excess EDTA dianhydride, and the EDTA conjugates (**XE**) were purified by reversed phase HPLC.

ImPyImPy-β-ImPyImPy-β-Dp-NH$_2$ (1-NH$_2$) A sample of ImPyImPy-β-ImPyImPy-β-Pam-resin resin (300 mg, 59 mmol) was placed in a 20 mL glass scintillation vial and treated with 3,3'-diamino-N-methyldipropylamine (2 mL) at 55 °C for 12 h. Resin was removed by filtration, and the filtrate diluted to a total volume of 10 mL with 0.1% (wt/v) aqueous TFA. The resulting solution was purified by reversed phase preparatory HPLC (analytical r.t. = 30.5 min.) to provide ImPyImPy-β-ImPyImPy-β-NH$_2$ (**1-NH$_2$**) as a white powder upon lyophilization of the appropriate fractions (32.6 mg, 26 mmol, 44% recovery). ^{1}HNMR (DMSO-*d6*) δ 10.42 (s, 1 H), 10.33 (s, 1 H), 10.21 (s, 1 H), 10.20 (s, 1 H), 10.01 (s, 1 H), 10.00 (s, 1 H), 9.91 (s, 1 H), 9.48 (br s, 1 H), 8.09 (m, 3 H), 7.80 (br s, 3 H), 7.53 (s, 1 H), 7.52 (s, 1 H), 7.46 (s, 1 H), 7.39 (s, 1 H), 7.38 (s, 1 H), 7.36 (s, 1 H), 7.21 (s, 1 H), 7.19 (s, 1 H), 7.14 (s, 1 H), 7.13 (s, 1 H), 7.05 (s, 1 H), 6.95 (d, 1 H, *J* = 1.2 Hz), 6.94 (d, 1 H, 1.5 Hz), 3.97 (s, 3 H), 3.95 (s, 6 H), 3.94 (s, 3 H), 3.84 (s, 6 H), 3.80 (s, 3 H), 3.78 (s, 3 H), 3.42 (m, 2 H), 3.36 (q, 2 H, *J* = 6.3 Hz), 3.10 (m, 4 H), 3.02 (m, 2 H), 3.83 (q, 2 H, *J* = 6.0 Hz), 2.71 (d, 3 H, *J* = 4.8 Hz), 2.58 (t, 2 H, *J* = 6.9 Hz), 2.34 (t, 2 H, *J* = 7.5 Hz), 1.87 (m, 2 H), 1.75 (m, 2 H); MALDI-TOF-MS (monoisotopic), 1253.6 (1253.6 calc. for $C_{57}H_{73}N_{24}O_{10}$).

ImPyImPy-β-ImPyImPy-β-Dp-EDTA (1E) EDTA-dianhydride (50 mg) was dissolved in a solution of DMSO/NMP (mL) and DIEA (1 mL) by heating at 55 °C for 5 min. The dianhydride solution was added to ImPyImPy-β-ImPyImPy-β-NH$_2$ (**1-NH$_2$**) (15.3 mg, 10.0 mmol) dissolved in 750 mL DMSO. The mixture was heated at 55 °C for 25 min and then treated with 0.1 N aqueous

NaOH (3 mL) for 10 min at 55 °C. Aqueous TFA (0.1% v/v) was then added to a total volume of 10 mL and the solution was purified directly by reversed phase preparatory HPLC to yield **1E** as a white powder (2.4 mg, 1.6 mmol, 16% recovery) upon lyophilization of the appropriate fractions. MALDI-TOF-MS (monoisotopic), 1527.7 (1527.7 calc. for $C_{67}H_{87}N_{26}O_{17}$).

Im-β-ImPy-β-Im-β-ImPy-β-Dp-NH$_2$ (2-NH$_2$) 27% recovery, ^{1}HNMR (DMSO-*d6*) δ 10.36 (s, 1 H), 10.32 (s, 2 H), 9.93 (s, 1 H), 9.92 (s, 1 H), 9.56 (br s, 1 H), 8.37 (t, 1 H, *J* = 6.3 Hz), 8.07 (m, 3 H), 7.89 (t, 1 H, *J* = 5.7 Hz), 7.83 (br s, 3 H), 7.44 (s, 1 H), 7.43 (s, 1 H), 7.39 (s, 1 H), 7.32 (s, 1 H), 7.19 (s, 2 H), 6.95 (s, 1 H), 6.92 (s, 1 H), 6.90 (d, 1 H, *J* = 2.1 Hz), 3.91 (s, 9 H), 3.89 (s, 3 H), 3.78 (s, 3 H), 3.48 (m, 4 H), 3.37 (m, 4 H), 3.10 (q, 2 H, *J* = 6.0 Hz), 3.01 (m, 2 H), 2.83 (q, 2 H, *J* = 6.3 Hz), 2.71 (d, 3 H, *J* = 4.8 Hz), 2.58 (m, 4 H), 2.52 (m, 4 H), 2.33 (t, 2 H, *J* = 7.2 Hz), 1.86 (m, 2 H), 1.74 (m, 2 H); MALDI-TOF-MS (monoisotopic), 1151.6 (1151.6 calc. for $C_{51}H_{71}N_{22}O_{10}$).

Im-β-ImPy-β-Im-β-ImPy-β-Dp-EDTA (2E) 12.5% recovery, MALDI-TOF-MS (monoisotopic), 1425.8 (1425.7 calc. for $C_{61}H_{85}N_{24}O_{17}$).

Im-β-ImPyPyIm-β-ImPy-β-Dp-NH$_2$ (3-NH$_2$) 8.6% recovery, ^{1}HNMR (DMSO-*d6*) δ 10.34 (s, 1 H), 10.32 (s, 2 H), 10.00 (s, 1 H), 9.95 (s, 1 H), 9.94 (s, 1 H), 9.31 (br s, 1 H), 8.37 (t, 1 H, *J* = 6.0 Hz), 8.07 (m, 2 H), 7.92 (t, 1 H, *J* = 5.1 Hz), 7.73 (br s, 3 H), 7.50 (s, 1 H), 7.46 (s, 1 H), 7.45 (s, 1 H), 7.32 (s, 1 H), 7.30 (d, 1 H, *J* = 1.8 Hz), 7.25 (d, 1 H, *J* = 1.5 Hz), 7.19 (d, 1 H, *J* = 1.5 Hz), 7.13 (d, 1 H, *J* = 1.5 Hz), 7.07 (d, 1 H, *J* = 1.5 Hz), 6.95 (s, 1 H), 6.92 (d, 1 H, 1.8 Hz), 3.93 (s, 3 H), 3.92 (s, 3 H), 3.92 (s, 6 H), 3.83 (s, 3 H), 3.82 (s, 3 H), 3.77 (s, 3 H), 3.49 (m, 4 H), 3.35 (q, 2 H, *J* = 6.0 Hz), 3.10 (q, 2 H, *J* = 6.9 Hz), 3.02 (m, 4 H), 2.82 (q, 2 H, *J* = 6.3 Hz), 2.71 (d, 3

H, *J* = 4.8 Hz), 2.59 (m, 4 H), 2.33 (t, 2 H, *J* = 6.9 Hz), 1.86 (t, 2 H, *J* = 8.7 Hz), 1.74 (t, 2 H, *J* = 8.1 Hz); MALDI-TOF-MS (monoisotopic), 1202.7 (1202.6 calc for $C_{54}H_{72}N_{23}O_{10}$).

Im-β-ImPyPyIm-β-ImPy-β-Dp-EDTA (3E) 7.5% recovery, MALDI-TOF-MS (monoisotopic), 1476.8 (1476.7 calc. for $C_{64}H_{86}N_{25}O_{17}$).

ImPy-β-Im-β-ImPy-β-Dp-NH$_2$ (13-NH$_2$) MALDI-TOF-MS (monoisotopic), 957.5 (957.5 calc. for $C_{43}H_{61}N_{18}O_8^+$).

ImPy-β-Im-β-ImPy-β-Dp-EDTA (13-E) MALDI-TOF-MS (monoisotopic), 1231.3 (1231.6 calc. for $C_{53}H_{75}N_{20}O_{15}^+$).

Construction of Plasmid DNA. Plasmids were constructed by inserting the following hybridized inserts into the *Bam*HI/*Hin*DIII polycloning site in pUC19 (match sites bolded for convenience):

pAU9 5'-GATCCGGCC**AAAGAGAAGAG**AAACCGGGGCC**TAGCGCAGCGCTA**GGCCA-3' • 5'-AGCTTGGCCTAGCGCTGCGCTAGGCCCCGGTTTCTCTTCTCTTTGGCCG-3'

pAU8 5'-GATCCGGCC**AAAGAGAAGAG**AAACCGGGGC**CAAAGACAAGAG**AAACCGGGGCC**AAAGAAAAGAG**AAACCGGGGCC**AAAGATAAGAG**AAACCGGA-3' • 5'-AGCTTCCGGTTTCTCTTATCTTTGGCCCCGGTTTCTCTTTTCTTTGGCCCCGGTTTCTCTTGTCTTTGGCCCCGGTTTCTCTTCTCTTTGGCCG-3'

pAU12 5'-GATCCGGCC**AAAGAGAAGAG**AAACCGGGGCC**AAAGAGATGAG**AAACCGGGGCC**AAAGAGAGGA**

GAAACCGGGGC**CAAAGAGACGAG**AAACCGGA-3' • 5'-AGCTTCCGGTTTCTCGTCTCTTTGGCCCCGGTTTCTCCTCTCTTTGGCCCCGGTTTCTCATCTCTTTGGCCCCGGTTTCTCTTCTCTTTGGCCG-3'

pAU13 5'-GATCCGGCC**AAAGAGAAGAG**AAACCGGGGCC**AAAGAGTAGAG**AAACCGGGGCC**AAAGAGGAGAG**AAACCGGGGC**CAAAGAGCAGAG**AAACCGGA-3' • 5'-AGCTTCCGGTTTCTCTGCTCTTTGGCCCCGGTTTCTCTCCTCTTTGGCCCCGGTTTCTCTACTCTTTGGCCCCGGTTTCTCTTCTCTTTGGCCG-3'

pAU15 5'-GATCCGGCC**AAAGAGAAGAG**AAACCGGGGC**CAAAGTGAAGAG**AAACCGGGGCC**AAAGGGAAGAG**AAACCGGGGCC**AAAGCGAAGAG**AAACCGGA-3' • 5'-AGCTTCCGGTTTCTCTTCGCTTTGGCCCCGGTTTCTCTTCCCTTTGGCCCCGGTTTCTCTTCACTTTGGCCCCGGTTTCTCTTCTCTTTGGCCG-3'

pAU16 5'-GATCCGGCC**AAAGAGAAGAG**AAACCGGGGC**CAAAGAGAACAG**AAACCGGGGCC**AAAGAGAAGAA**AAACCGGGGCC**AAAGAGAATAG**AAACCGGA-3' • 5'-AGCTTCCGGTTTCTATTCTCTTTGGCCCCGGTTTCTTTTCTCTTTGGCCCCGGTTTCTGTTCTCTTTGGCCCCGGTTTCTCTTCTCTTTGGCCG-3'

pAU18 5'-GATCCGGCC**AAAGAGAAGAG**AAACCGGGGCC**AAACACAACAC**AAACCGGGGCC**AAAAAAAAAAA**AAACCGGGGCC**AAATATAATAT**AAACCGGA-3' • 5'-A

GCTTCCGGTTTATATTATATTTGGCCCCGGTTTTTTTTTTTTTTGGCCCCGGTTTGTGTTGTGTTTGGCCCCGGTTTCTCTTCTCTTTGGCCG-3'

pAU20 5'-GATCCGGCC**AAAGAGAAG**CGGGTTGGCC**AAAGAGAAGA**CGGGTTGGCC**AAAGAGAAGAA**CGGGTTGGCCAAGAGAAGAACGGGGA-3' • 5'-AGCTTCCCCGTTCTTCTCTTGGCCAACCCGTTCTTCTCTTTGGCCAACCCGTCTTCTCTTTGGCCAACCCGCTTCTCTTTGGCCG-3'

pAU27 5'-GATCCGGGGCC**AAAAAGAAAAG**ACTGACTGAC**TAGTACT**GACGACTGACC**AAAGAGAAGAG**ACTGACTGAC**TAGCGCT**GACTGA-3' • 5'-AGCTTCAGTCAGCGCTAGTCAGTCAGTCTCTTCTCTTTGGTCAGTCGTCAGTACTAGTCAGTCAGTCTTTTCTTTTTGGCCCCG-3'

The ligated plasmids were subsequently transformed in to JM109 subcompetent cells (Promega) and amplified by cell growth, according to standard protocols (Sambrook et al., 1989). Plasmid DNA was isolated using WizardPlus Midi Preps from Promega.

Preparation of 5'- and 3′-End-Labeled Restriction Fragments. These procedures are described in detail in Trauger and Dervan, 2001. For 3'-labeling: pUC19 was *Pvu*II/*Eco*RI linearized and then 3'-filled with deoxyadenosine 5′-[a-^{32}P] and thymidine 5′-[a-^{32}P] triphosphates using Klenow polymerase. For 5'-labelling: two 20 base pair primer oligonucleotides, 5'-AATTCGAGCTCGGTACCCGG-3' (forward) and 5'-CTGGCACGACAGGTTTCCCG-3' (reverse) were constructed to complement the pUC19 *EcoRI* and *PvuII* sites, respectively, such that amplification by PCR would mimic the long, 3'-filled, pUC19 *EcoRI/PvuII* restriction fragment. The forward primer was radiolabelled using γ-^{32}P-dATP and polynucleotide kinase. Labeled fragments were purified on a 7% nondenaturing preparatory polyacrylamide gel (5% crosslink) and isolated. Chemical sequencing reactions were performed according to published protocols (Iverson and Dervan, 1987; Maxam and Gilbert, 1980).

MPE•Fe(II) Footprinting. This procedure is also described in Trauger and Dervan, 2001. All reactions were carried out in a volume of 400 μL. A polyamide stock solution or water (for reference lanes) was added to an assay buffer where the final concentrations were: 28.6 mM HEPES, 285.7 mM NaCl buffer (pH 7.0), and 20 kcpm 3´- or 5'-radiolabelled DNA. The solutions were allowed to equilibrate for 12 hours. A fresh 5 μM MPE•Fe(II) solution was prepared from equal volumes of 10 μM MPE and 10 μM ferrous ammonium sulfate ($Fe(NH_4)_2(SO_4)_2 \bullet 6H_2O$) solutions. MPE•Fe(II) solution (0.5 μM) was added to the equilibrated solutions, and the reactions were allowed to proceed for 15 min. Cleavage was initiated by the addition of dithiothreitol (5 mM) and allowed to proceed for 30 min. Reactions were stopped by ethanol precipitation, and the pellets were washed in 75% ethanol, dried *in vacuo*, resuspended in 15 mL H_2O, lyophilized to dryness, and resuspended in 100 mM tris-borate-EDTA/80% formamide loading buffer. The solutions were then denatured at 90 °C for 9 min, and a 5 μL sample (~ 8 kcpm) was loaded onto an 8% denaturing polyacrylamide gel (5% crosslink, 7 M urea) and run at 2000 V.

Affinity Cleaving. This procedure is also described in Trauger and Dervan, 2001. All reactions were carried out in a volume of 400 μL. A polyamide stock solution or water (for reference lanes) was added to an assay buffer where the final concentrations were: 28.6 mM HEPES/85.7 mM NaCl buffer (pH 7.0), 200 mM NaCl, and 20 kcpm 3´- or 5'-radiolabelled DNA. The solutions were allowed to equilibrate for 12 hours. A fresh solution of ferrous ammonium sulfate ($Fe(NH_4)_2(SO_4)_2\bullet 6H_2O$) (1 μM) was added to the equilibrated solutions, and the reactions proceeded for 15 min. Cleavage was initiated by the addition of dithiothreitol (5 mM) and allowed to proceed for 11 min. Reactions were stopped by ethanol precipitation, and the pellets were washed in 75% ethanol, dried *in vacuo*, resuspended in 15 mL H_2O, lyophilized to dryness, and resuspended in 100 mM tris-borate-EDTA/80% formamide loading buffer. The solutions were then denatured at 90 °C for 9 min, and a 5 μL sample (~ 8 kcpm) was loaded onto an 8% denaturing polyacrylamide gel (5% crosslink, 7 M urea) and run at 2000 V.

Quantitative DNase I Footprint Titrations. This procedure is described in detail in Trauger and Dervan, 2001. All reactions were carried out in a volume of 400 mL. We note explicitly that no carrier DNA was used in these reactions until after DNase I cleavage. A 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 20 kcpm 5′-radiolabelled DNA. The solutions were allowed to equilibrate for 18 hours at 22 °C. Cleavage was initiated by the addition of 10 mL of a DNase I solution (diluted with 1 mM DTT to 1.5 u/mL) and allowed to proceed for 7 min at 22 °C. The reactions were stopped by adding 50 mL of a solution containing 2.25M NaCl, 150 mM EDTA, 0.6 mg/mL glycogen, and 30 mM base-pair calf thymus DNA, and then ethanol precipetated (2.1 volumes) at 14 krpm for 23 min. The pellets were washed with 75% ethanol, resuspended in 15 mL RNase-free water, lyophilized to dryness, and then resuspended in 100 mM tris-borate-EDTA/80% formamide loading buffer (with bromophenol blue as dye), denatured at 90 °C for 10 min, and loaded directly onto a pre-run 8% denaturing polyacrylamide gel (5% crosslink, 7 M urea) at 2000 V for 1.2 h. The gels were dried *in vacuo* at 80°C and then exposed to a storage phosphor screen (Molecular Dynamics).

Determination of Equilibrium Constants. At least three sets of data were used in determining each association constant. Data from the footprint titration gels were obtained using a Molecular Dynamics Typhoon 8600 PhosphorImager followed by quantitation using ImageQuant software (Molecular Dynamics). Background-corrected volume integration of rectangles encompassing the footprint sites and a reference site at which DNase I reactivity was invariant across the titration generated values for the site intensities (I_{site}) and the reference intensity (I_{ref}). The apparent fractional occupancy (θ_{app}) of the sites was calculated using the equation:

$$\theta_{app} = 1 - \frac{I_{site}/I_{ref}}{I_{site}^{\circ}/I_{ref}^{\circ}} , \tag{1}$$

where I_{site}^{o} and I_{ref}^{o} are the site and reference intensities, respectively, from a control lane to which no polyamide was added.

The ($[L]_{tot}$, θ_{app}) data points were fit to a general Hill equation (eq 2) by minimizing the difference between θ_{app} and θ_{fit}:

$$\theta_{fit} = \theta_{min} + (\theta_{max} - \theta_{min}) \frac{K_a^n [L]^n_{tot}}{1 + K_a^n [L]^n_{tot}} , \tag{2}$$

where $[L]_{tot}$ is the total polyamide concentration, K_a is the apparent first-order association constant, and θ_{min} and θ_{max} are the experimentally determined site saturation values when the site is unoccupied or saturated, respectively. The data were fit using a nonlinear least-squares fitting procedure with K_a, θ_{max}, and θ_{min} as the adjustable parameters, and with either n=2 or n=1 depending on which value of n gave the better fit. We note explicitly that treatment of the data in this manner does not represent an attempt to model a binding mechanism. The binding isotherms were normalized using the following equation:

$$\theta_{norm} = \frac{\theta_{app} - \theta_{min}}{\theta_{max} - \theta_{min}} \tag{3}$$

High Resolution Multidimensional NMR Studies

DNA Purification. The dimethoxytrityl-protected complementary oligonucleotides 5'-CCAAAGAGAAGCG-3' (purine strand) and 5'-CGCTTCTCTTTGG-3' (pyrimidine strand) were synthesized at the Biopolymer Synthesis Center at Caltech. Each strand was purified separately by reversed phase preparatory HPLC in 100 mM triethylammonium acetate (TEAA), pH 6.5, with a gradient of 1% acetonitrile/min. The appropriate fractions were combined, lyophilized to dryness, and resuspended in RNAse-free water. The pH was lowered to 5.0 with acetic acid to remove the DMT group, and complete deprotection was observed by analytical HPLC after 12 hours. The deprotected single-stranded oligonucleotides were quantified by their UV absorbance at 260 nm using the calculated extinction coefficients, $\varepsilon = 137{,}300\ M^{-1}cm^{-1}$ (purine strand) and $\varepsilon = 107{,}200\ M^{-1}cm^{-1}$ (pyrimidine strand). 7.0 μmol of each strand was combined, and the single strands were hybridized by heating the 4.9 mL sample at 90 °C for 10 min., with slow cooling to room temperature over 6 hours. Duplex DNA was separated from slight excess of single stranded DNA by FPLC purification using a HiTrap Q strong anion exchange column (Pharmacia) in 200 mM KCl with a 1% per min gradient of 1.5 M KCl. The appropriate fractions were combined and concentrated using a Centricon YM-3, 3000 molecular-weight-cutoff filter from Amicon. The filter was used to dialyze the DNA sample, reducing the KCl concentration to approximately 1 μM. The sample was lyophilized to dryness and then dissolved in 10 mM sodium phosphate (pH 7.0), quantitated by UV absorbance, distributed to four 2.83 μmol aliquots, and then lyophilized to dryness. A 4.35 mM NMR sample was prepared by dissolving a 2.83 μmol aliquot of purified duplex DNA in 650 μL of 9:1 H_2O/D_2O.

1:1 Polyamide:DNA Complex Preparation. Polyamide concentration was estimated using the molar extinction coefficient, $\varepsilon = 31{,}000$. A polyamide stock solution was prepared by dissolving purified polyamide **13** in 10 mM sodium phosphate (pH 7.0) in 9:1 H_2O/D_2O. The polyamide stock was titrated into the 4.35 mM DNA sample in very small increments in order to determine empirically a 1:1 stoichiometry by observing complete disappearance of free DNA peaks.

Final complex concentration was 3.67 mM. For experiments carried out in D_2O, the complex was lyophilized twice from D_2O.

NMR Experiments and Data Processing. NMR experiments were performed at 25 °C on a Varian INOVA 600 MHz spectrometer. 1D spectra and 2D NOESY spectra in 9:1 H_2O/D_2O were acquired using WATERGATE (Piotto et al., 1992) water suppression containing a W5 pulse element (Liu et al., 1998). Presaturation of the residual solvent signal was used in the acquisition of 1D spectra and 2D double-quantum-filter (DQF)-COSY, TOCSY, and NOESY for the sample in D_2O. TOCSY spectra were acquired with mixing times of 40 and 100 ms, and NOESY spectra with mixing times of 50, 75, 100, and 150 ms. All 2D spectra were acquired on the 1:1 polyamide-DNA complex. Spectral widths were 12,500 Hz for the sample in protiated solvent and 6000 Hz for the sample in dueterated solvent. All spectra were recorded with $512t_1 * 2048t_2$ complex points. Data were processed on a SUN Ultra 10 workstation using VNMR (Varian, Inc.) or NMRPipe (Delaglio et al., 1995). Resonance assignment was performed using NMRVIEW 4.1.2 (Johnson and Blevins 1994). Chemical shifts were referenced relative to TSP via the residual solvent resonance at 4.7718.

Distance Constraints. In the initial round of structure calculations, resolved and unambiguously assigned crosspeak volumes from the 75 ms D_2O NOESY spectrum were converted to upper-bound distance constraints using the isolated spin-pair approximation, plus 20% as an error approximation (Barsukov and Lian, 1993). Once the first set of reasonable structures was obtained, a set of representative structures was used as the starting point for two rounds of hybrid relaxation matrix calculations using the MARDIGRAS program (Borgias and James, 1990). NOE crosspeak intensities from the 150 ms D_2O NOESY spectrum were input to the MARDIGRAS program. The uncertainty in these intensities was accounted for by adjusting the upper bound distances in proportion to their magnitude as follows: <2.0, +0.35 Å; 2 – 3, +0.7 Å; 3 – 4, +1.1 Å; 4 – 5, +1.5 Å; >5.0, +1.9 Å (Eis et al., 1997). The constraints were supplemented with additional distance constraints from labile protons identified in the 100 ms NOESY spectrum in protiated solvent. All distance constraints assigned from NOEs were given a lower bound of 1.8 Å as the sum of van der Waals radii. Overlapped crosspeaks from the D_2O and H_2O NOESY spectra were assigned upper bound distance constraints of 5.5 and 6.0 Å, respectively, based on calculated distances for the smallest observable crosspeak intensities. Methylene and methyl groups were restrained as Q and M pseudoatoms, respectively, as defined by MARDIGRAS and AMBER 6.0 software (Borgias and James, 1990; Kollman et al., 2000).

Watson–Crick hydrogen bonding constraints were included on the basis of characteristic crosspeaks observed at 12-14 ppm between labile protons in the 100 ms H_2O NOESY spectrum. Upper and lower bound constraints were assigned for each guanosine imino, thymidine imino, and cytosine amino group in all but the terminal base pairs. N • • • O or N • • • N distances (Schmitz et al., 1997), as well as H • • • N and H • • • O distances were enforced as follows: G – O6 to C – N4, 2.81 – 3.01 Å; G – N1 to C – N3, 2.85 – 3.05 Å; G – N1H to C – N3, 1.80 – 2.20 Å; G – O6 to C – N4H1, 1.76 – 2.16 Å; A – N1 to T – N3, 2.72 – 2.92 Å; A – N1 to T – N3H, 1.67 – 2.07 Å.

Structure Calculations. Calculation strategy was based on previously reported protocols (Smith et al., 1996; Eis et al., 1997). Restrained molecular dynamics (rMD) calculations were performed on a Dell Dimension 8100 workstation using the AMBER 6.0 software package (Kollman et al., 2000). All calculations were carried out *in vacuo* with a distance-dependent dielectric and a cutoff distance of 15.0 Å for nonbonded interactions. A force constant of 20 kcalmol^{-1}A^{-2} was applied to interproton distances exceeding the upper and lower constraints, in a smooth parabolic fashion for 0.5 Å and then linearly.

Forty starting DNA structures with variable x-displacement, incline, rise, and twist values were constructed using Nucleic Acids Builder (NAB) software (Macke, 1996). While holding base-pair geometry constant, the DNA backbone was relaxed using 1000 steps of steepest-descent minimization (average pairwise all-atom RMSD = 3.46 Å). The polyamide was constructed using the LEaP module of the AMBER 6.0 software package (Kollman et al., 2000), with partial charges assigned on the basis of an AM1 calculation using the AMPAC module of InsightII (Molecular Simulations, Inc.).

The polyamide was aligned with the DNA minor groove and positioned approximately 12 Å from the helix using NAB. Docking was performed using a 15 ps rMD simulated annealing (SA) calculation consisting of 4 ps of linear heating to 600 K, 5 ps of high temperature dynamics, and 6 ps of linear cooling to 0 K. Further rMD SA calculations did not improve the total energy or RMSD. The family of 40 docked structures had an all atom pairwise RMSD of 1.37 Å. The final structures were ranked by increasing residual constraint violation energy, and a calculation was performed using FINDFAM, which indicated that a minimum of 12 structures would be adequate to accurately represent the input data (Smith et al., 1996). Accordingly, the 12 structures of lowest violation energy were chosen as the final structural ensemble (Figure 27). The core binding site of the final ensemble, which is defined as polyamide residues 1-7 (ImPy-β-Im-β-ImPy) and DNA purine tract residues A4 – G11 and C16 – T23, has an all atom RMSD of 0.80 Å and RMSD from the mean of 0.54 Å. The ensemble coordinates are available from the Brookhaven Data Bank under accession code 1LEJ.

Distances and angles in the final structural ensemble were examined using Insight II software. DNA helical parameters and groove dimensions were calculated using CURVES 5.3 (Lavery and Sklenar, 1998; Stofer and Lavery, 1994). All helical parameters are listed and plotted in Appendix C. The molecular structures in Figures 27, 29, 30, 31, 32, and 33 were rendered in GRASP (Nicholls et al., 1991).