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Supporting Information

Recognition of DNA by Hairpin Polyamides with Hydroxypyrrole / Pyrrole Pairs at the Terminal Position

We describe here a series of eight-ring polyamide hairpins with the general sequence XPyPyPy-(R)$^{H_2N}\gamma$-ImPyPyPy-β-Dp with the N-terminal position X= 3-hydroxy-1-methylpyrrole substituted at the 4-position with groups of increasing size H, formamide, acetamide (Hp-**1**, Hp-**2**, Hp-**3**) (Figure 1). Since Py is in the eighth position, the terminal pairs in the hairpin conformation are Hp-**1**/Py, Hp-**2**/Py and Hp-**3**/Py. For controls the parent pyrrole series without the 3-hydroxy was also synthesized, 1-methylpyrrole substituted at the 4-position with H, formamide and acetamide (Py-**1**, Py-**2**, Py-**3**) afford end cap pairs Py-**1**/Py, Py-**2**/Py and Py-**3**/Py (Figure 1). In addition, X= Im at the N-terminal was included to establish a baseline with previously published work. Seven eight-ring hairpin polyamides, differing in the terminal position were synthesized for this study by solid-phase methods (Figure S2).[5] The plasmid pCW15 was designed to contain the four six-base pair recognition sites 5'-T**T**TACA-3', 5'-T**A**TACA-3', 5'-T**G**TACA-3' and 5'-T**C**TACA-3' which differ at a single common position allowing for comparison of the affinities between different terminal pairs and the four Watson Crick base pairs in the minor groove of DNA (Figure 5).

Results

Synthesis. The 3-methoxy-1-methylpyrrole unit was introduced at the N-terminal position as a dimeric building block **S11** which was synthesized in six steps from *t*-butyl 3-hydroxy-1-methylpyrrole-2-carboxylate (**S8**) (Figure S2).[10] The hydroxy ester **S8** was benzoylated using benzoyl chloride/pyridine to give compound **S9**. The *t*-butyl ester was

hydrolyzed using TMSOTf/DIEA[11] and pyrrole acid **S10** was then coupled with an excess of methyl 4-amino-1-methylpyrrole-2-carboxylate.[5] Subsequent debenzoylation, methylation and hydrolysis of the methyl-ester afforded the dimeric building block **S11**.

The polyamide resins Boc-PyPy-(R)^{Fmoc}γ-ImPyPyPy-β-PAM-resin and Boc-PyPyPy-(R)^{Fmoc}γ-ImPyPyPy-β-PAM-resin were synthesized in a stepwise manner from Boc-β-alanine-PAM resin (0.55 mmol/g) using Boc-chemistry solid-phase methodology (Figure S3).[5] The terminal monomers and dimer were then coupled to the deprotected resins. Hydroxypyrrole amino acid residues were introduced as orthogonally protected 3-methoxy-1-methylpyrrole (Op)[12] derivatives. A sample of the resin was then cleaved by a single-step aminolysis reaction with ((dimethyl)amino)propylamine (55°C, 16 h) and subsequently purified by reversed-phase HPLC to provide (Py-**1**)PyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (**S1**), (Py-**2**)PyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (**S2**), (Py-**3**)PyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (**S3**), and ImPyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (**S7**). Polyamides containing 3-methoxypyrrole at the N-terminus were deprotected by treatment with sodium ethanethiolate in DMF (100°C, 1 h) and purified by reversed-phase HPLC to provide (Hp-**1**)PyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (**S4**), (Hp-**2**)PyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (**S5**) and (Hp-**3**)PyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (**S6**).

Quantitative DNase I footprinting titrations. Quantitative DNase I footprint titrations (10 mM Tris•HCl, 10 mM KCl, 10 mM $MgCl_2$ and 5 mM $CaCl_2$, pH 7.0, 22°C) were performed on the 3'-^{32}P-end-labeled 284 base pair *Eco*RI/*Pvu*II restriction fragment of the pCW15 plasmid to determine the equilibrium association constant (K_a) of each eight-ring hairpin polyamide to the four different binding sites 5'-TNTACA-3' (where N=T, A, G, C)(Table S1). Polyamide **S1**-**S3**, with Py/Py pairs in the terminal position, bound to A•T and T•A with higher affinities than for G•C or C•G but did not distinguish

T•A from A•T as expected. Remarkably, within the Hp/Py series, only polyamide **S6** with the Hp-**3**/Py terminal pairs showed a preference for T•A over A•T.

Discussion.

Polyamide **S1** with a Py/Py pair in the terminal position, binds the sequences 5'-T**T**TACA-3' and 5'-T**A**TACA-3' with comparable affinity (3.6×10^{10} M^{-1} vs 3.0×10^{10} M^{-1}). Polyamide **S1** binds 5'-T**G**TACA-3' and 5'-T**C**TACA-3' sequences with 4.3 and 9.7 times lower affinity. The discrimination of T•A /A•T over G•C / C•G at the N-terminal position is comparable to results shown when the Py/Py pair is placed in internal positions of the hairpin polyamide.[8] The introduction of an acetamide group on the 1-methylpyrrole does not change the specificity for T•A /A•T over G•C / C•G but lowers the affinity for all four binding sites by a factor of 3, probably due to steric interactions of the methyl group with the floor of the minor groove or the adjacent β-alanine. Changing the acetamide group to formamide which reduces the steric bulk of the substituent restores the affinity.

For the 3-hydroxy series, polyamide **S4** (Hp-**1**)PyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp was expected to bind the sequence 5'-T**T**TACA-3' more tightly than 5'-T**A**TACA-3' due to the (Hp-**1**)/Py pair at the N-terminal position. However no sequence preference was observed. One possible explanation could be rotation of the terminal hydroxypyrrole to form a hydrogen bond between the 3-hydroxyl hydrogen and the carbonyl oxygen. This conformation would place the key hydroxyl group away from the minor groove (Figure 2). On the other hand polyamide **S6** binds the sequence 5'-T**T**TACA-3' with higher affinity than 5'-T**A**TACA-3', a 3.4-fold specificity. Perhaps the amide at the 4-position forms an hydrogen bond with the thymine-O2 and thereby locks the orientation of the terminal hydroxypyrrole in the proper configuration (Figure 2). As in the Py/Py series the

introduction of the acetamide group in polyamide **S6** decreases the overall binding affinity. Replacing the acetamide with formamide in polyamide **S5** restored the binding affinity but the specificity was lost. Both Hp-**2**/Py and Hp-**3**/Py pairs at the terminal position afforded poor sequence discrimination as demonstrated by the similar affinities at the sequences 5'-T**T**TACA-3' and 5'-T**G**TACA-3'. In conclusion Hp/Py pairs may be limited to internal positions for T•A discrimination and there is a need for the design of new pairs for the terminal position for distinguishing T•A from A•T (and both from G•C and C•G) in the minor groove of DNA.

Experimental Section

***t*-Butyl 3-benzoyloxy-1-methylpyrrole-2-carboxylate (S9).** *t*-Butyl 3-hydroxy-1-methylpyrrole-2-carboxylate[10] (**S8**) (627 mg, 3.2 mmol) was dissolved in DCM (3 mL) and pyridine (0.515 mL, 6.4 mmol) was added. The mixture was cooled to 0°C and benzoyl chloride (0.553 mL, 4.8 mmol) was added during 5 minutes. The temperature was raised to room temperature during 1 hour. The mixture was partitioned between water and Et_2O and the water layer extracted with Et_2O two times. The combined organic phases were dried ($MgSO_4$) and concentrated. The crude product was purified by chromatography (SiO_2, 2:1 hexanes-EtOAc) to afford **S9** (928 mg, 97%). ^{1}H NMR ($CDCl_3$) δ 8.10-8.25 (m, 2 H), 7.40-7.70 (m, 3 H), 6.68 (d, 1 H, J = 3.0 Hz), 6.02 (d, 1 H, J = 3.0 Hz), 3.88 (s, 3 H), 1.31 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 164.93, 160.07, 141.45, 134.72, 133.56, 130.75, 130.43, 129.89, 129.06, 128.60, 126.22, 102.16, 81.04, 38.35, 28.69. HRMS calcd. for $C_{17}H_{19}NO_4$: 301.1314; found: 301.1322.

3-Benzoyloxy-1-methylpyrrole-2-carboxylic acid (S10). *t*-Butylester **S9** (886 mg, 2.9 mmol) was dissolved in dioxane (19 mL) and DIEA (1.07 mL, 6.2 mmol) was added followed by TMSOTf (1.193 mL, 6.0 mmol) during 20 minutes under argon. The

mixture was allowed to stir for 4 h at room temperature and then partitioned between water and Et_2O and the water layer extracted with Et_2O two times. The combined organic phases were dried ($MgSO_4$) and concentrated. The crude product was purified by chromatography (SiO_2, 1:1 hexanes-EtOAc) to give **S10** (438 mg, 61%) together with unreacted starting material (201 mg, 23%). ^1H NMR ($CDCl_3$) δ 8.10-8.30 (m, 2 H), 7.40-7.60 (m, 3 H), 6.77 (d, 1 H, J = 2.8 Hz), 6.20 (d, 1 H, J = 3.0 Hz), 3.88 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 164.99, 163.22, 143.27, 133.48, 133.21, 130.44, 130.06, 129.81, 128.61, 128.48, 127.23, 112.58, 102.42, 38.38 HRMS calcd. for $C_{13}H_{11}NO_4$: 245.0688; found: 245.0697.

4-[[[3-methoxy-1-methylpyrrole-2-yl]carbonyl]amino]-1-methylpyrrole-2-carboxylic acid (S11). To a solution of **S10** (438 mg, 1.8 mmol) in DMF (3 mL) was added DCC (361 mg, 1.8 mmol) and HOBt (229 mg, 1.7 mmol). The mixture was allowed to stir at room temperature for 1.5 h and the DCU was removed by filtration. Separately, to a solution of methyl 4-nitro-1-methylpyrrole-2-carboxylate[5] (1.00 g, 5.8 mmol) in DMF (4 mL) was added Pd/C (10%, 100 mg) and the mixture was hydrogenated in a Parr bomb apparatus (500 psi of H_2) for 1 h. The catalyst was removed by filtration through Celite and the two solutions mixed and allowed to stir at 37°C for 6.5 h and then at 55°C for 3 h. The reaction mixture was poured on ice and extracted with Et_2O three times. The organic phase was dried ($MgSO_4$) and concentrated. The residue was dissolved in NaOMe-MeOH (0.05 M, 20mL) and allowed to stir at room temperature for 1.5 h and then neutralized by Amberlite IR-120 H^+. The mixture was concentrated and the residue dissolved in DMF (10 mL) and K_2CO_3 (1.24 g, 9.0 mmol) was added followed by a solution of MeI (0.277 mL, 3.0 mmol in 1 mL of DMF) during 10 min. The mixture was allowed to stir at room temperature for 14 h and then filtered and partitioned between water and DCM. The water phase was extracted with CH_2Cl_2 twice. The combined organic phases were dried ($MgSO_4$), concentrated and the

ester was isolated by chromatography (SiO_2, 2:1 hexanes-EtOAc). The ester was dissolved in MeOH (10 mL), NaOH (aq, 1 M, 10 mL) was added, and the mixture was heated to 50°C for 20 h. All volatiles were removed *in vacuo* and the resulting solution was acidified by addition of HCl (aq, 3 M). The solid was removed by filtration and was dissolved in DCM/MeOH and subsequently purified by chromatography (SiO_2, 15:1 DCM/MeOH) to afford **S11** (130 mg, 26%). ^1H NMR ($CDCl_3$) δ 8.74 (s, 1 H), 7.54 (d, 1 H, J = 1.8 Hz), 6.82 (d, 1 H, J = 2.1 Hz), 6.54 (d, 1 H, J = 2.7 Hz), 5.82 (d, 1 H, J = 3.0 Hz), 3.94, 3.92, 3.90 (s, 3 H each) ^{13}C NMR ($CDCl_3$); δ 165.60, 159.11, 150.63, 125.48, 122.65, 122.36, 119.04, 110.12, 109.78, 92.97, 58.54, 38.28, 37.28. HRMS calcd. for $C_{13}H_{15}N_3O_4$: 277.1063; found: 277.1052.

Boc-PyPy-(R)^{Fmoc}γ-ImPyPyPy-β-PAM-resin. Boc-PyPy-(R)^{Fmoc}γ-ImPyPyPy-β-PAM-resin (0.350 mmol/g) was synthesized in a stepwise fashion from 0.55 mmol/g Boc-β-PAM-resin by manual solid-phase methods.[5]

Deprotection of 3-methoxy-1-methylpyrrole polyamides. To a slurry of NaH (80 mg, 60% in mineral oil) in DMF (0.50 mL) was added a solution of ethanethiol (0.32 mL) in DMF (0.50 mL) and the mixture was heated to 100°C for 5 minutes in a sealed tube. The polyamide, dissolved in DMF (1.00 mL) was added to the ethanethiolate solution and the mixture was heated to 100°C for 1h in a sealed tube and then cooled to 0°C. The mixture was added to HOAc (3 mL) and all volatiles were removed (high vacuum, 40°C). The residue was dissolved in CH_3CN (1 mL) and TFA (7 mL, 0.1 % (w/v)) and purified by preparative HPLC

(Py-1)PyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (S1). 1-methylpyrrole-2-carboxylic acid (50 mg, 0.40 mmol) was dissolved in DMF (1 mL) and HBTU (75 mg, 0.20 mmol) and DIEA (0.5 mL) were added and the mixture stirred for 10 minutes and added to deprotected Boc-PyPyPy-(R)^{Fmoc}γ-ImPyPyPy-β-PAM-resin (100 mg) and the mixture shaken for 4 h at

22°C. The compound was then cleaved from the resin according to the general procedure. Preparative HPLC gave **S1** (20 mg, 49% isolated yield). UV λ_{max} (H_2O) 240, 308 (69500); ^{1}H NMR (DMSO-d_6) δ 10.97, 10.06, 9.90, 9.89, 9.84, 9.78 (s, 1 H each), 8.28 (bs, 3 H), 8.13 (t, 1 H, J = 5.5 Hz), 7.99 (q, 1 H, J = 6.0 Hz), 7.48 (s, 1 H), 7.20 (d, 1 H, J = 1.5 Hz), 7.17 (t, 1 H, J = 1.8 Hz), 7.14 (d, 1 H, J = 1.5 Hz), 7.12 (t, 1 H, J = 1.8 Hz), 7.09 (d, 1 H, J = 1.8 Hz), 7.02 (s, 2 H), 6.99 (d, 1 H, J = 1.8 Hz), 6.91 (d, 1 H, J = 1.8 Hz), 6.89 (t, 1 H, J = 2.1 Hz), 6.85 (dd, 1 H, J = 3.9, 1.8 Hz), 6.82 (d, 1 H, J = 1.8 Hz), 5.99 (dd, 1 H, J = 3.9, 2.7 Hz), 3.92, 3.81, 3.80, 3.79, 3.78, 3.77, 3.75, 3.74 (s, 3 H each), 3.20-3.40 (m, 4 H), 3.05 (q, 2 H, J = 6.3 Hz), 2.90-3.00 (m, 2 H), 2.67 (d, 6 H, J = 4.5 Hz), 2.29 (t, 2 H, J = 7.2 Hz), 1.90-2.00 (m, 2 H), 1.68 (p, 2 H, J = 7.8 Hz); MALDI-TOF-MS calcd. for $C_{59}H_{73}N_{21}O_{10}$ (M + H): 1236.6; found: 1236.7.

(Py-2)PyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (S2). Boc-Pyrrole-OBt ester[5] (50 mg, 0.14 mmol) was dissolved in DMF (0.5 mL) and DIEA (0.25 mL) and added to the deprotected Boc-PyPyPy-(R)^{Fmoc}γ-ImPyPyPy-β-PAM-resin (50 mg) and the mixture shaken for 4 h at 22°C. The resin was washed with DMF (2 × 30 s) and DCM (2 × 30 s) and the Boc group cleaved with 80% TFA/DCM/0.5 M PhSH (1 × 30 s, 1 × 20 min). The resin was washed with DCM (2 × 30 s) followed by DMF (2 × 30 s). Formic acid (1 mL) and Ac_2O (0.2 mL) were added and the mixture shaken for 1 h. The compound was then cleaved from the resin according to the general procedure. Preparative HPLC gave **S2** (4.5 mg, 21% isolated yield). UV λ_{max} (H_2O) 240, 312 (69500); ^{1}H NMR (DMSO-d_6) δ 11.01, 10.10, 10.05 (s, 1 H each), 9.90-10.00 (m, 4 H), 9.88 (s, 1 H), 8.32 (bs, 3 H), 8.18 (t, 1 H, J = 5.7 Hz), 8.10 (d, 1 H, J = 1.8 Hz), 8.03 (q, 2 H, J = 5.7 Hz), 7.52 (s, 1 H), 7.24 (d, 1 H, J = 1.5 Hz), 7.20 (d, 2 H, J = 1.5 Hz), 7.19 (d, 1 H, J = 1.5 Hz), 7.15-7.18 (m, 3 H), 7.13 (d, 1 H, J = 1.5 Hz), 7.04-7.08 (m, 3 H), 6.96 (d, 1 H, J = 1.5 Hz), 6.90 (d, 1 H, J = 2.1 Hz), 6.87 (d, 1 H, J = 1.5 Hz), 3.96, 3.84 (s, 3 H each), 3.83, 3.82 (s, 6 H each), 3.79, 3.78 (s, 3 H each), 3.09 (q, 2 H,

J = 5.4 Hz), 2.94-3.04 (m, 2 H), 2.75, 2.74 (s, 1 H each), 2.71 (d, 6 H, *J* = 4.8 Hz), 2.33 (t, 2 H, *J* = 7.5 Hz), 1.20-2.00 (m, 8 H). MALDI-TOF-MS calcd. for $C_{60}H_{74}N_{22}O_{11}$ (M + H): 1279.6; found: 1279.6.

(Py-3)PyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (S3). Boc-Pyrrole-OBt ester[5] (100 mg, 0.28 mmol) was dissolved in DMF (1 mL) and DIEA (0.5 mL) and added to the deprotected Boc-PyPyPy-(R)^{Fmoc}γ-ImPyPyPy-β-PAM-resin (100 mg) and the mixture shaken for 4 h at 22°C. The resin was washed with DMF (2 × 30 s) and DCM (2 × 30 s) and the Boc group cleaved with 80% TFA/DCM/0.5 M PhSH (1 × 30 s, 1 × 20 min). The resin was washed with DCM (2 × 30 s) followed by DMF (2 × 30 s). DMF (1 mL) and Ac_2O (0.5 mL) were added and the mixture shaken for 5 min. The compound was then cleaved from the resin according to the general procedure. Preparative HPLC gave **S3** (15 mg, 35% isolated yield). UV λ_{max} (H_2O) 240, 308 (69500); ^{1}H NMR (DMSO-d_6) δ 10.97, 10.07, 9.90 (s, 1 H each), 9.88 (s, 2 H), 9.85, 9.84, 9.78 (s, 1 H each), 8.27 (bs, 3 H), 8.13 (t, 1 H, *J* = 4.8 Hz), 8.00 (q, 2 H, *J* = 6.0 Hz), 7.48 (s, 1 H), 7.21 (d, 1 H, *J* = 1.5 Hz), 7.16 (t, 1 H, *J* = 1.2 Hz), 7.14 (d, 1 H, *J* = 1.5 Hz), 7.12 (bs, 1 H), 7.08 (dd, 1 H, *J* = 3.0, 1.2 Hz), 7.02 (d, 1 H, *J* = 1.8 Hz), 7.01 (d, 1 H, *J* = 1.8 Hz), 6.91 (d, 1 H, *J* = 1.8 Hz), 6.82 (d, 1 H, *J* = 1.5 Hz), 6.80 (d, 1 H, *J* = 2.1 Hz), 3.92, 3.79 (s, 3 H each), 3.78 (s, 6 H), 3.77, 3.76, 3.75, 3.74 (s, 3 H each), 3.20-3.35 (m, 4 H), 3.04 (q, 2 H, *J* = 6.6 Hz), 2.94 (p, 2 H, *J* = 6.6 Hz), 2.28 (t, 2 H, *J* = 7.5 Hz), 1.91 (s, 3 H), 1.67 (p, 2 H, *J* = 7.5 Hz). MALDI-TOF-MS calcd. for $C_{61}H_{76}N_{22}O_{11}$ (M + H): 1293.6; found: 1293.7.

(Hp-1)PyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (S4). Compound **S11** (22 mg, 0.08 mmol) was dissolved in DMF (0.3 mL) and HOBt (9 mg, 0.07 mmol) and DCC (15 mg, 0.07 mmol) were added and the mixture was allowed to stir for 20 minutes and then added to the deprotected Boc-PyPy-(R)^{Fmoc}γ-ImPyPyPy-β-PAM-resin (50 mg) followed by DIEA (0.1 mL) and the mixture shaken for 2.0 h at 22°C and then 2.0 h at 37°C. The compound

was then cleaved from the resin according to the general procedure. Preparative HPLC gave methyl-protected (Hp-1)PyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (9 mg, 39% isolated yield).UV λ_{max} (H_2O) 240, 312 (69500); ^{1}H NMR (DMSO-d_6) δ 10.97, 10.06, 9.90, 9.89, 9.84, 9.82, 8.73 (s, 1 H each), 8.27 (bs, 3 H), 8.13 (t, 1 H, J = 5.7 Hz), 8.00 (q, 2 H, J = 5.7 Hz), 7.48 (s, 1 H), 7.23 (d, 1 H, J = 1.8 Hz), 7.20 (d, 1 H, J = 1. 8 Hz), 7.15 (bs, 1 H), 7.12 (bs, 1 H), 7.09 (d, 1 H, J = 1. 8 Hz), 7.02 (bs, 2 H), 6.93 (d, 1 H, J = 1.8 Hz), 6.91 (d, 1 H, J = 1.8 Hz), 6.82 (d, 1 H, J = 1.8 Hz), 6.80 (d, 1 H, J = 2.7 Hz), 5.90 (d, 1 H, J = 2.7 Hz), 3.92, 3.80, 3.79 (s, 3 H each), 3.78 (bs, 9 H), 3.76, 3.75, 3.74 (s, 3 H each), 3.04 (q, 2 H, J = 6.0 Hz), 2.94 (p, 2 H, J = 5. 0 Hz). 2.67 (d, 6 H, J = 4.5 Hz), 2.28 (t, 2 H, J = 7.2 Hz), 1.92 (bs, 2 H), 1.67 (p, 2 H, J = 7.2 Hz). MALDI-TOF-MS calcd. for $C_{60}H_{75}N_{21}O_{11}$ (M + H): 1266.6; found: 1266.7. The methyl-protected (Hp-1)PyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (2.1 mg, 1.7 μmol) was deprotected according to the general procedure to give **S4** (1.3 mg, 63% isolated yield). UV λ_{max} (H_2O) 240, 310 (69500); ^{1}H NMR (DMSO-d_6) δ 11.12, 10.44, 10.19, 10.03, 10.02, 9.96, 9.89, 9.09 (s, 1 H each), 8.39 (bs, 3 H), 8.26 (t, 1 H, J = 2.2 Hz), 8.12 (q, 2 H, J = 2.1 Hz), 7.61 (s, 1 H), 7.36 (d, 1 H, J = 1.5 Hz), 7.33 (d, 1 H, J = 1.5 Hz), 7.28 (d, 2 H, J = 1.5 Hz), 7.25 (bd, 2 H, J = 1.8 Hz), 7.20 (d, 1 H, J = 1.5 Hz), 7.12-7.18 (m, 2 H), 7.05 (t, 2 H, J = 2.1 Hz), 6.96 (d, 1 H, J = 1.5 Hz), 6.80 (d, 1 H, J = 2.1 Hz), 5.73 (d, 1 H, J = 2.7 Hz), 4.05, 3.93, 3.92, 3.91, 3.90, 3.88 (s, 3 H each), 3.87 (s , 6 H), 3.17 (q, 2 H, J = 6.0 Hz), 3.06 (bt, 2 H, J = 8.1 Hz), 2.80 (s, 3 H), 2.42 (t, 2 H, J = 7.2 Hz), 2.06 (bs, 2 H), 1.80 (bt, 2 H, J = 7.8 Hz), 1.30 (s, 1 H); MALDI-TOF-MS calcd. for $C_{59}H_{73}N_{21}O_{11}$ (M + H): 1252.6; found: 1252.6.

(Hp-2)PyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (S5). Boc-Op acid[12] (50 mg, 0.18 mmol) was dissolved in DMF (1.0 mL) and HBTU (60 mg, 0.16 mmol) and DIEA (0.5 mL) were added and the mixture stirred for 10 minutes and added to the deprotected Boc-PyPyPy-(R)^{Fmoc}γ-ImPyPyPy-β-PAM-resin (50 mg) and the mixture shaken for 4.0 h at 22°C. The

resin was washed with DMF (2 × 30 s) and DCM (2 × 30 s) and the Boc group cleaved with 80% TFA/DCM/0.5 M PhSH (1 × 30 s, 1 × 20 min). The resin was washed with DCM (2 × 30 s) followed by DMF (2 × 30 s). Formic acid (1 mL) and Ac_2O (0.2 mL) were added and the mixture shaken for 1 h. The compound was then cleaved from the resin according to the general procedure. Preparative HPLC gave methyl-protected (Hp-2)PyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (6 mg, 27% isolated yield). UV λ_{max} (H_2O) 240, 312 (69500); ^{1}H NMR (DMSO-d_6) δ 11.10, 10.19, 10.03, 10.02, 9.99, 9.96, 9.88, 9.21 (s, 1 H each), 8.41 (bs, 3 H), 8.27 (t, 1 H, J = 6.0 Hz), 8.23 (d, 1 H, J = 1.5 Hz), 8.12 (q, 2 H, J = 6.0 Hz), 7.61 (s, 1 H), 7.36 (d, 1 H, J = 1.8 Hz), 7.33 (d, 1 H, J = 1.5 Hz), 7.29 (s, 1 H), 7.28 (s, 2 H), 7.25 (s, 2 H), 7.22 (d, 1 H, J = 1.5 Hz), 7.15 (d, 2 H, J = 1.8 Hz), 7.14 (d, 1 H, J = 1.5 Hz), 7.04 (d, 1 H, J = 1.5 Hz), 6.96 (d, 1 H, J = 1.5 Hz), 4.05 (s, 3 H), 3.93 (s, 6 H), 3.91, 3.90, 3.88,3.87, 3.86, 3.85 (s, 3 H each), 3.17 (q, 2 H, J = 6.3 Hz), 3.07 (p, 2 H, J = 5.4 Hz), 2.80 (d, 6 H, J = 4.5 Hz), 2.42 (t, 2 H, J = 7.5 Hz), 2.02-2.12 (m, 2 H), 1.81 p, 2 H, J = 7.8 Hz); MALDI-TOF-MS calcd. for $C_{61}H_{76}N_{22}O_{12}$ (M + H): 1309.6; found: 1309.7. The methyl-protected (Hp-2)PyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (1.8 mg, 1.4 μmol) was deprotected according to the general procedure to give **S5** (0.7 mg, 37% isolated yield). UV λ_{max} (H_2O) 240, 312 (69500); ^{1}H NMR (DMSO-d_6) δ 10.96, 10.60, 10.21, 10.06 (s, 1 H each), 9.89 (bs, 2 H), 9.83, 9.79 (s, 1 H each), 8.99 (s, 1 H), 8.25 (bs, 3 H), 8.13 (bs, 1 H), 7.99 (bs, 3 H), 7.48, 7.24, 7.20 (s, 1 H each), 7.14, 7.11 (s, 2 H each), 7.08, 7.02, 7.00, 6.94, 6.91 (s, 1 H each), 6.83 (s, 2 H), 3.91 (s, 3 H), 3.78, 3.77, 3.75, (s, 3 H each), 3.73 (s, 6 H), 3.04 (q, 2 H, J = 6.6 Hz), 2.95 (p, 2 H, J = 6.6 Hz), 2.67 (d, 6 H, J = 3.9 Hz), 2.28 (t, 2 H, J = 7.2 Hz), 1.90-2.00 (m, 2 H), 1.67 (p, 2 H, 6.8 Hz), 1.17 (s, 1 H); MALDI-TOF-MS calcd. for $C_{60}H_{74}N_{22}O_{12}$ (M + H): 1295.6; found: 1295.8

(Hp-3)PyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (S6). Boc-Op acid[12] (50 mg, 0.18 mmol)

was dissolved in DMF (1.0 mL) and HBTU (60 mg, 0.16 mmol) and DIEA (0.5 mL) were added and the mixture stirred for 10 minutes and added to the deprotected Boc-PyPyPy-(R)^{Fmoc}γ-ImPyPyPy-β-PAM-resin (100 mg) and the mixture shaken for 4.0 h at 22°C. The compound was then cleaved from the resin according to the general procedure. Preparative HPLC gave methyl-protected (Hp-3)PyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (15 mg, 46% isolated yield). UV λ_{max} (H_2O) 240, 312 (69500); ^{1}H NMR (DMSO-d_6) δ 10.99, 10.20, 9.92, 9.90 (s, 1 H each), 9.85 (s, 2 H), 9.34, 9.02 (s, 1 H each), 8.38 (bs, 3 H), 8.17 (t, 1 H, J = 5.9 Hz), 7.96-8.10 (m, 2 H), 7.47 (s, 1 H), 7.23 (d, 1 H, J = 1.2 Hz), 7.21 (bs, 1 H), 7.17 (d, 1 H, J = 1.2 Hz), 7.16 (bs, 2 H), 7.13 (d, 1 H, J = 1.2 Hz), 7.09-7.12 (m, 4 H), 7.05 (s, 1 H), 7.02 (bs, 2 H), 6.99 (d, 1 H, J = 1.8 Hz), 6.90 (d, 1 H, J = 1.2 Hz), 6.81 (bs, 2 H), 3.92 (s, 3 H), 3.89 (s, 1 H), 3.79, 3.78 (s, 6 H each), 3.75 (s, 3 H each), 3.73 (s, 6 H), 3.71 (s, 3 H), 3.20-3.35 (m, 4 H), 3.04 (q, 2 H, J = 6.3 Hz), 2.93 (p, 2 H, J = 4.5 Hz), 2.65 (d, 6 H, J = 4.5 Hz), 2.28 (t, 2 H, J = 6.6 Hz), 1.96 (s, 1 H), 1.95 (s, 3 H), 1.69 (p, 2 H, J = 7.2 Hz). MALDI-TOF-MS calcd. for $C_{62}H_{78}N_{22}O_{12}$ (M + H): 1323.6; found: 1323.8. The methyl-protected (Hp-3)PyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (3.1 mg, 2.4 μmol) was deprotected according to the general procedure to give **S6** (0.7 mg, 23% isolated yield). UV λ_{max} (H_2O) 240, 308 (69500); ^{1}H NMR (DMSO-d_6) δ 11.12, 10.97, 10.17, 10.06, 9.92, 9.89, 9.84, 9.78 (s, 1 H each), 9.24 (bs, 1 H), 8.26 (bs, 3 H), 8.13 (t, 1 H, J = 5.6 Hz), 8.00 (q, 2 H, J = 5.67 Hz), 7.48, 7.24, 7.20 (s, 1 H each), 7.15, 7.11 (s, 2 H each), 7.09, 7.02, 7.00, 6.93, 6.91, 6.83, 6.76 (s, 1 H each), 3.91, 3.79 (s, 3 H each), 3.78 (s, 6 H), 3.77, 3.74, 3.73, 3.72 (s, 3 H each), 3.04 (q, 2 H, J = 6.0 Hz), 2.94 (p, 2 H, J = 3.9 Hz), 2.68 (d, 6 H, J = 3.3 Hz), 2.28 (t, 2 H, J = 6.6 Hz), 1.99 (s, 3 H), 1.94 (bs, 2 H), 1.67 (p, 2 H, J = 5.8 Hz), 1.17 (s, 1 H); MALDI-TOF-MS calcd. for $C_{61}H_{76}N_{22}O_{12}$ (M + H): 1309.6; found: 1309.7.

ImPyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (S7). Imidazole acid (50 mg, 0.40 mmol) was dissolved in DMF (1.0 mL) and HBTU (140 mg, 0.37 mmol) and DIEA (0.5 mL) were

added and the mixture stirred for 10 minutes and added to the deprotected Boc-PyPyPy-(R)^{Fmoc}γ-ImPyPyPy-β-PAM-resin (100 mg) and the mixture shaken for 2.5 h at 22°C. Preparative HPLC gave **S7** (20 mg, 50% isolated yield). UV λ_{max} (H_2O) 240, 312 (69500); ^{1}H NMR (DMSO-d_6) δ 10.97, 10.42, 10.06 (s, 1 H each), 9.91 (bs, 2 H), 9.88, 9.84 (s, 1 H each), 8.28 (bs, 2 H), 8.13 (t, 1 H, J = 5.7 Hz), 7.99 (q, 2 H, J = 5.7 Hz), 7.48, 7.34 (s, 1 H each), 7.23 (d, 1 H, J = 1.6 Hz), 7.20 (d, 1 H, J = 1.4 Hz), 7.17 (d, 1 H, J = 1.4 Hz) 7.15 (bs, 1 H), 7.10-7.20 (m, 2 H), 7.09 (bs, 1 H), 7.02 (bs, 2 H), 6.99 (t, 1 H, J = 1.2 Hz), 6.91 (bs, 1 H), 6.83 (bs, 1 H), 3.93 (s, 6 H), 3.92, 3.79, 3.78, 3.77, 3.75, 3.73 (s, 3 H each), 3.31 (q, 2 H, J = 5.7 Hz), 3.23 (q, 2 H, J = 5.7 Hz), 3.04 (q, 2 H, J = 6.0 Hz), 2.90-2.97 (m, 2 H), 2.68 (d, 6 H, J = 4.8 Hz), 2.28 (t, 2 H, J = 7.2 Hz), 1.88-2.00 (m, 2 H), 1.67 (p, 2 H, J = 8.1 Hz). MALDI-TOF-MS calcd. for $C_{58}H_{72}N_{22}O_{10}$ (M + H): 1237.6; found: 1237.7.

Figure captions

Figure S1. Structures of polyamides. (Py-**1**)PyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (**S1**), (Py-**2**)PyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (**S2**), (Py-**3**)PyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (**S3**), (Hp-**1**)PyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (**S4**), (Hp-**2**)PyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (**S5**), (Hp-**3**)PyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (**S6**), ImPyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (**S7**).

Figure S2. (i) Pyridine, BzCl, DCM. (ii) TMSOTf, DIEA, dioxane. (iii) DCC, HOBt, DMF, DIEA, methyl 4-amino-1-methylpyrrole-2-carboxylate. (iv) 0.05 M NaOMe/MeOH. (v) K_2CO_3, MeI, DMF. (vi) 1M aq. NaOH, MeOH.

Figure S3. Solid-phase synthetic scheme for (Hp-**1**)PyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (**S4**) starting from commercially available Boc-β-PAM-resin: (i) 80% TFA/DCM, 0.4 M PhSH. (ii) Boc-Py-OBt, DIEA, DMF. (iii) 80% TFA/DCM, 0.4 M PhSH. (iv) Boc-Py-OBt, DIEA, DMF. (v) 80% TFA/DCM, 0.4 M PhSH. (vi) Boc-Py-OBt, DIEA, DMF. (vii) 80% TFA/DCM, 0.4 M PhSH. (viii) Boc-Im-acid, DCC, HOBt, DIEA, DMF. (ix) 80% TFA/DCM, 0.4 M PhSH. (x) Fmoc-α-Boc-γ-diaminobutyric acid, HBTU, DIEA, DMF.(xi) 80% TFA/DCM, 0.4 M PhSH. (xii) Boc-Py-OBt, DIEA, DMF. (xiii) 80% TFA/DCM, 0.4 M PhSH. (xiv) Boc-Py-OBt, DIEA, DMF. (xv) 80% TFA/DCM, 0.4 M PhSH. (xvi) **S11**, DCC, HOBt, DIEA, DMF, r.t. 2h and then 37°C 2h.(xvii) Piperidine:DMF 3:1. (xviii) (Dimethylamino)propylamine, 55°C, 16h. (xix) NaH, EtSH, DMF, 100°C, 1h.

Figure S4. (a) Quantitative DNase I footprint titration experiment with (Py-**3**)PyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (**S3**) on the 284 bp *Eco*RI/*Pvu*II restriction fragment from plasmid pCW15: lane 1, intact DNA; lane 2, A specific reaction; lane 3, DNase I standard; lanes 4-13, 10 pM, 20 pM, 50 pM, 100 pM, 200 pM, 500 pM, 1 nM, 2 nM, 5 nM, 10 nM (Py-**3**)PyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (**S3**); (b) Quantitative DNase I footprint titration experiment with (Hp-**3**)PyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (**S6**) on the 284 bp *Eco*RI/*Pvu*II restriction fragment from plasmid pCW15: lane 1, intact DNA; lane 2, A specific reaction; lane 3, DNase I standard; lanes 4-13, 500 pM, 1 nM, 2 nM, 5 nM, 10 nM, 20 nM, 50 nM, 100 nM, 200 nM, 500 nM (Hp-**3**)PyPyPy-(R)^{H_2N}γ-ImPyPyPy-β-Dp (**S6**); The four sites 5'-T**T**TACA-3', 5'-T**A**TACA-3', 5'-T**G**TACA-3' and 5'-T**C**TACA-3' sites that were analyzed are shown on the right side of the gel.

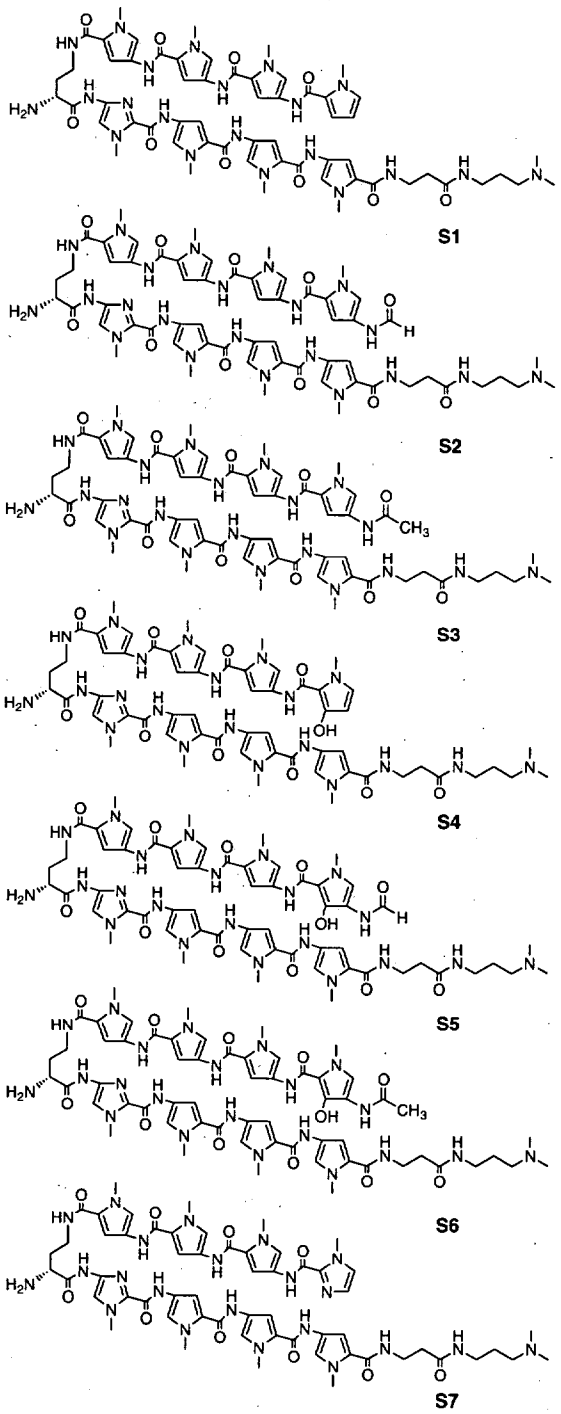

Figure S1

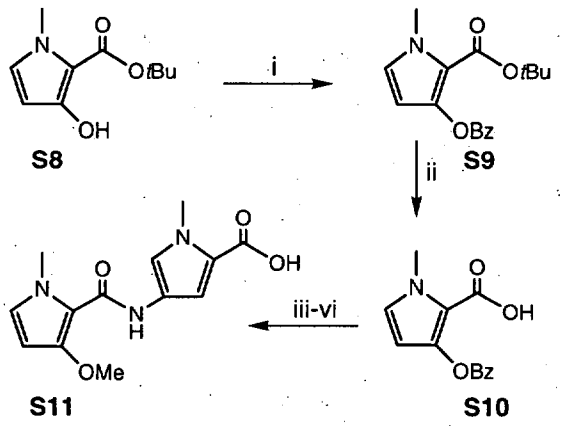

Figure S2

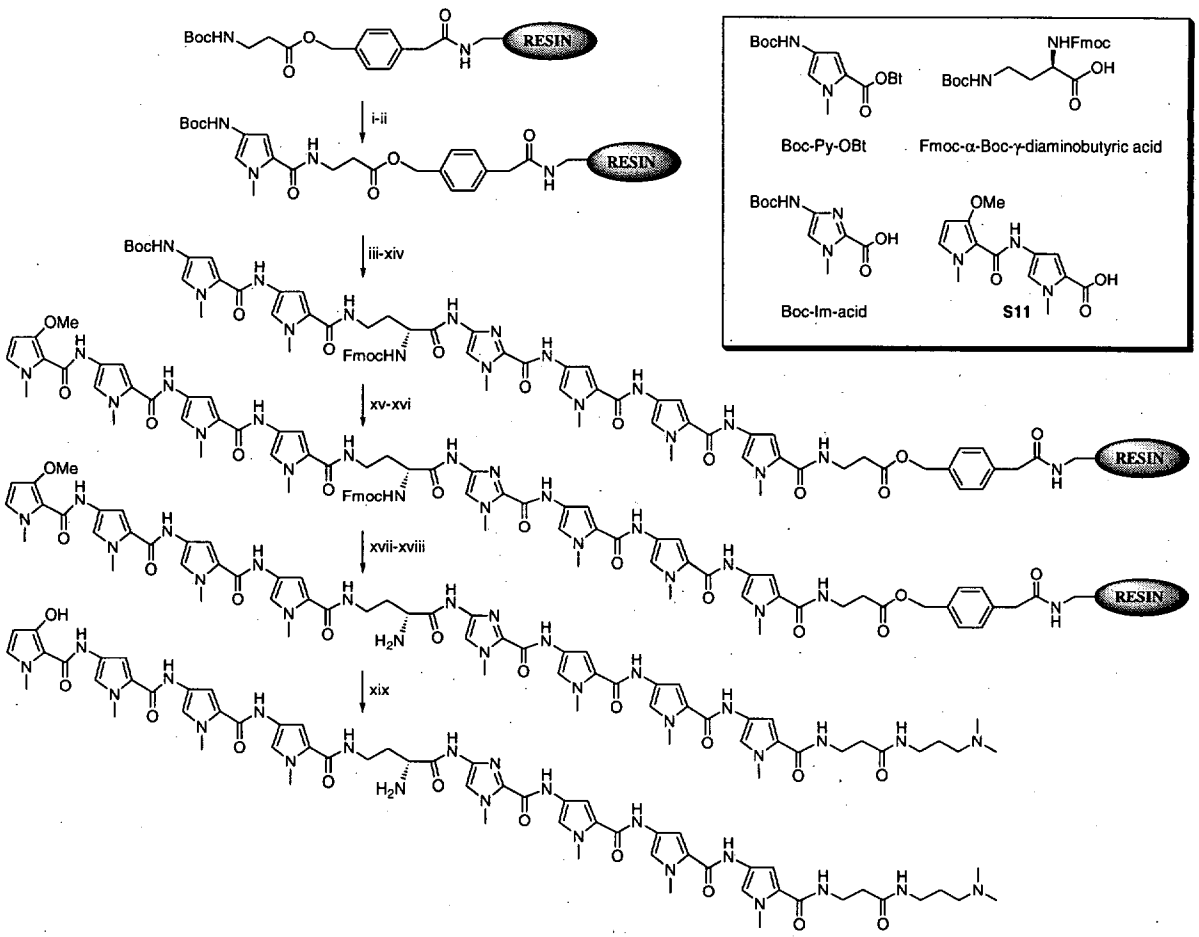
BocHN
RESIN
i-ii
iii-xiv
FmocHN
OMe
xv-xvi
xvii-xviii
OH
H_2N
xix
Boc-Py-OBt
Fmoc-α-Boc-γ-diaminobutyric acid
Boc-Im-acid
S11

Figure S3

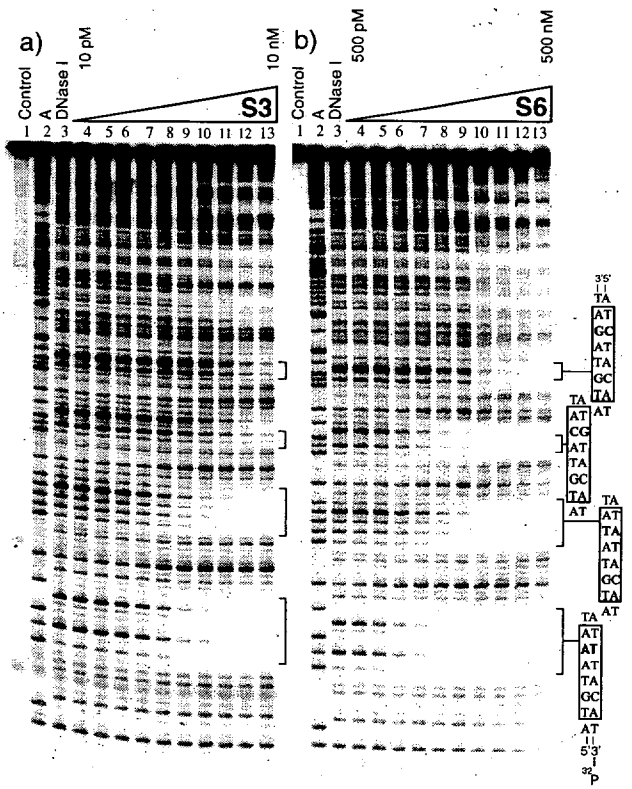

Figure S4

Table S1. Equilibration Association Constants (M^{-1})[a]

Polyamide	5'-TTTACA-3'	5'-TATACA-3'	5'-TGTACA-3'	5'-TCTACA-3'
S1	$3.6\ (\pm0.7) \times 10^{10}$	$3.0\ (\pm0.3) \times 10^{10}$	$8.4\ (\pm0.9) \times 10^{9}$	$3.7\ (\pm0.9) \times 10^{9}$
S2	$5.6\ (\pm0.8) \times 10^{10}$	$1.2\ (\pm0.2) \times 10^{11}$	$1.5\ (\pm0.2) \times 10^{10}$	$9.3\ (\pm1.2) \times 10^{9}$
S3	$1.1\ (\pm0.2) \times 10^{10}$	$9.4\ (\pm2.0) \times 10^{9}$	$1.9\ (\pm0.1) \times 10^{9}$	$1.3\ (\pm0.2) \times 10^{9}$
S4	$1.3\ (\pm0.2) \times 10^{10}$	$1.1\ (\pm0.3) \times 10^{10}$	$2.7\ (\pm0.9) \times 10^{9}$	$3.0\ (\pm3.0) \times 10^{8}$
S5	$1.3\ (\pm0.8) \times 10^{10}$	$1.1\ (\pm0.9) \times 10^{10}$	$1.2\ (\pm0.9) \times 10^{10}$	$1.8\ (\pm1.0) \times 10^{9}$
S6	$2.1\ (\pm0.2) \times 10^{9}$	$6.2\ (\pm1.6) \times 10^{8}$	$1.1\ (\pm0.3) \times 10^{9}$	$1.5\ (\pm0.4) \times 10^{8}$
S7	$2.0\ (\pm0.5) \times 10^{10}$	$1.1\ (\pm0.2) \times 10^{10}$	$2.9\ (\pm0.5) \times 10^{11}$	$2.9\ (\pm0.5) \times 10^{10}$

[a]Values reported are mean values from at least three DNase I footprint titration experiments, with the standard deviation for each data set indicated in parenthesis. The 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 $CaCl_2$.

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