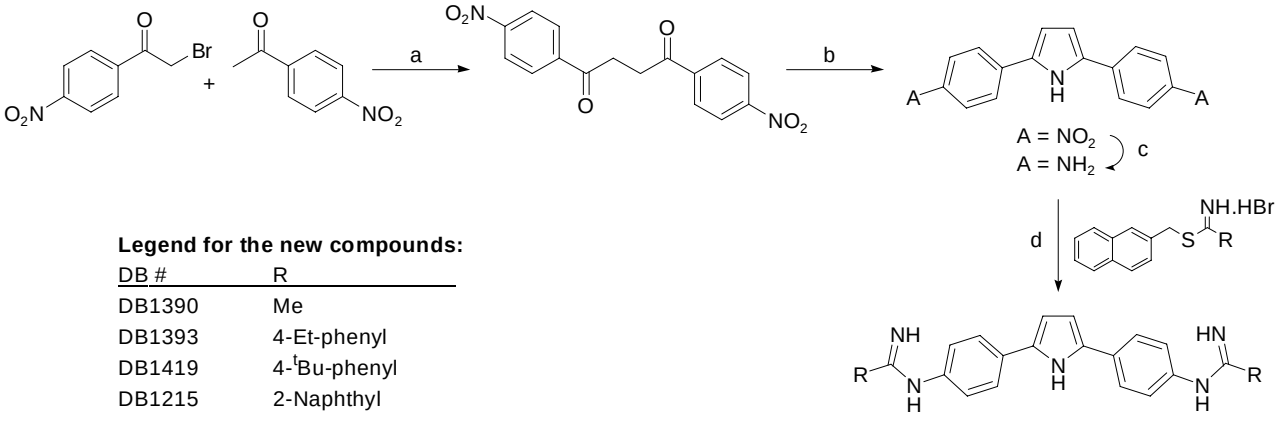
Supplemental Material Section For

Induced Fit Conformational Changes of a "Reversed Amidine" Heterocycle: Optimized Interactions in a DNA Minor Groove Complex

Manoj Munde,[†] Michael Lee,[‡] Stephen Neidle,[‡,*] Reem Arafa,[†] David W. Boykin,[†,*] Yang Liu,[†] Christian Bailly,[x,#] W. David Wilson[†,*]

Scheme 1



Legend for the new compounds:

DB #	R
DB1390	Me
DB1393	4-Et-phenyl
DB1419	4-^{t}Bu-phenyl
DB1215	2-Naphthyl
DB1480	4-Biphenyl

Reagents and reaction conditions: a. $ZnCl_2$, benzene, *t*-BuOH, TEA, r.t.; b. NH_4OAc, EtOH, HOAc, reflux, 5 h; c. Pd/C (10%), H_2, EtOH, EtOAc, 6 h; d. MeCN, EtOH, r.t.

Chemistry.

Melting points were recorded using a Thomas-Hoover (Uni-Melt) capillary melting point apparatus and are uncorrected. TLC analysis was carried out on silica gel 60 F_{254} precoated aluminum sheets and detected under UV light. ^{1}H and ^{13}C NMR spectra were recorded employing a Varian Unity Plus 300 spectrometer, and chemical shifts (δ) are in

ppm relative to TMS as internal standard. Mass spectra were recorded on a VG analytical 70-SE spectrometer. Elemental analyses were obtained from Atlantic Microlab Inc. (Norcross, GA) and are within ±0.4 of the theoretical values, except for DB1419**,** which was validated by HRMS. The compounds reported as salts frequently analyzed for fractional moles by water and/or ethanol of solvation. In each case proton NMR showed the presence of indicated solvent(s). All chemicals and solvents were purchased from Aldrich Chemical Co., Fisher Scientific or Lancaster.

DB1390 2,5-Bis[4-(acetimidoylamino)phenyl]pyrrole. Yield 83%, mp 175-7 ^{0}C; ^{1}H-NMR (DMSO-d_6) δ 2.21 (s, 6H), 6.55 (br s, 4H), 6.62 (s, 2H), 7.15 (d, J = 8.4 Hz, 4H), 7.82 (d, J = 8.4 Hz, 4H), 11.31 (br s, 1H).

Salt: mp 248-50 ^{0}C; ^{1}H-NMR (DMSO-d_6) δ 2.36 (s, 6H), 6.71 (s, 2H), 7.30 (d, J = 8.4 Hz, 4H), 9.97 (d, J = 8.4 Hz, 4H), 8.56 (br s, 2H), 9.58 (br s, 2H),11.58 (br s, 3H); ^{13}C-NMR δ 164.0, 132.6, 132.0, 131.7, 125.2, 108.7, 18.8. HRMS Calc. for $C_{20}H_{22}N_5$ ms 332.1875; observed 332.1860. Anal. Calcd. for $C_{20}H_{21}N_5$-2HCl-1.6H_2O-0.8C_2H_5OH (470.01): C % 55.19, H % 6.64, N % 14.90. Found: C % 55.35, H % 6.87, N % 14.52.

DB1393 2,5-Bis[4-(4-ethylbenzimido)aminophenyl]pyrrole. Yield 63%, mp 252-4 ^{0}C; ^{1}H-NMR (DMSO-d_6) δ 1.20 (t, J = 7.5 Hz, 6H), 2.65 (q, J = 7.5 Hz, 4H), 6.29 (br s, 4H), 6.46 (s, 2H), 6.88 (d, J = 8.1 Hz, 4H), 7.26 (d, J = 8.1 Hz, 4H), 7.71 (d, J = 8.1 Hz, 4H), 7.88 (d, J = 8.1 Hz, 4H), 11.02 (br s, 1H). ^{13}C-NMR δ 153.9, 148.0, 145.8, 133.3, 132.7, 127.3, 127.0, 126.6, 124.7, 121.8, 106.2, 27.9, 15.4.

Salt: mp >300 ^{0}C; ^{1}H-NMR (DMSO-d_6) δ 1.22 (t, J = 7.5 Hz, 6H), 2.73 (q, J = 7.5 Hz, 4H), 6.76 (s, 2H), 7.45-7.52 (m, 8H), 7.85-8.05 (m, 8H), 8.98 (br s, 2H), 9.80 (br s, 2H),

11.46 (br s, 2H), 11.62 (br s, 1H). HRMS Calc. for $C_{34}H_{34}N_5$ ms 512.2814; observed 512.2800. Anal. Calcd. for $C_{34}H_{33}N_5$-2HCl-2H_2O-0.25C_2H_5OH (632.13): C % 65.55, H % 6.45, N % 11.07. Found: C % 65.66, H % 6.08, N % 10.75.

DB1419 2,5-Bis[4-(4-*t*-butylbenzimido)aminophenyl]pyrrole. Yield 59%, mp 269-71 ^{0}C; ^{1}H-NMR (DMSO-d_6) δ 1.30 (s, 18H), 6.26 (br s, 4H), 6.47 (s, 2H), 6.87 (d, J = 8.4 Hz, 4H), 7.44 (d, J = 8.4 Hz, 4H), 7.71 (d, J = 8.4 Hz, 4H), 7.89 (d, J = 8.4 Hz, 4H), 11.03 (br s, 1H). MS (ESI) m/e (rel. int.): 568 (M^+ + 1, 14), 409 (15), 284 (100).

Salt: mp 265-7 ^{0}C; ^{1}H-NMR (DMSO-d_6) δ 1.34 (s, 18H), 6.76 (s, 2H), 7.46 (d, J = 8.1 Hz, 4H), 7.68 (d, J = 8.1 Hz, 4H), 7.88 (d, J = 8.1 Hz, 4H), 8.03 (d, J = 8.1 Hz, 4H), 9.01 (br s, 2H), 9.80 (br s, 2H), 11.45 (br s, 2H), 11.62 (br s, 1H). ^{13}C-NMR δ 162.8, 157.1, 132.7, 132.2, 128.7, 125.8, 125.6, 125.2, 108.9, 35.0, 30.8. HRMS Calc. for $C_{38}H_{42}N_5$ ms 568.3440; observed 568.3433. Anal. Calcd. for $C_{38}H_{41}N_5$-2HCl-2.75H_2O (690.22): C % 66.12, H % 7.08, N % 10.14. Found: C % 65.77, H % 6.54, N % 10.07.

DB1480 2,5-Bis[4-(4-biphenylimino)aminophenyl]pyrrole Yield 54%, mp >300 ^{0}C; ^{1}H-NMR (DMSO-d_6) δ 6.41 (br s, 4H), 6.49 (s, 2H), 6.90 (d, J = 8.4 Hz, 4H), 7.36-7.51 (m, 8H), 7.72-7.75 (m, 10H), 8.07 (d, J = 8.4 Hz, 4H), 11.05 (br s, 1H). HRMS Calc. for $C_{42}H_{34}N_5$ ms 608.2814; observed 608.2827.

Salt: mp 252-4 ^{0}C; ^{1}H-NMR (DMSO-d_6) δ 6.78 (s, 2H), 7.46-7.56 (m, 10H), 7.81 (d, J = 8.1Hz, 4H), 7.97-8.04 (m, 12H), 9.07 (br s, 2H), 9.93 (br s, 2H), 11.64 (br s, 3H). ^{13}C-NMR δ 162.6, 145.0, 138.2, 132.6, 132.0, 129.3, 128.9, 128.4, 127.1, 126.7, 126.6, 125.4, 125.0, 119.7, 108.5. Anal. Calcd. for $C_{42}H_{33}N_5$-2HCl-1.75H_2O (712.19): C % 70.83, H % 5.44, N % 9.83. Found: C % 70.84, H % 5.45, N % 9.84.

***S*-(2-Naphthylmethyl)-4-ethylbenzothioimidate.HBr.** Yield 75%, mp 190-2 ^{0}C; ^{1}H-NMR (DMSO-d_6) δ 1.18 (t, J = 7.5 Hz, 3H), 2.70 (q, J = 7.5 Hz, 4H), 4.93 (s, 2H), 7.47-7.65 (m, 5H), 7.83-7.99 (m, 5H), 8.07 (s, 1H). MS (ESI) m/e (rel. int.): 307 (M^+ + 2, 16), 306 (M^+ + 1, 100).

***S*-(2-Naphthylmethyl)-4-*t*-butylbenzothioimidate.HBr.** Yield 60%, mp 193-4 ^{0}C; ^{1}H-NMR (DMSO-d_6) δ 1.29 (s, 9H), 4.91 (s, 2H), 7.52-7.61 (m, 3H), 7.65 (d, J = 8.4 Hz, 2H), 7.85 (d, J = 8.4 Hz, 2H), 7.90-7.99 (m, 3H), 8.06 (s, 1H). MS (ESI) m/e (rel. int.): 335 (M^+ + 2, 22), 334 (M^+ + 1, 100).

***S*-(2-Naphthylmethyl)-2-naphthylthioimidate.HBr.** Yield 65%, mp 192-4 ^{0}C; ^{1}H-NMR (DMSO-d_6) δ 4.91 (s, 2H), 7.51-7.57 (m, 4H), 7.71-7.85 (m, 6H), 8.01-8.15 (m, 3H), 8.58 (s, 1H). MS (ESI) m/e (rel. int.): 329 (M^+ + 2, 23), 328 (M^+ + 1, 100).

***S*-(2-Naphthylmethyl)-4-biphenylthioimidate.HBr.** Yield 73%, mp 215-6 ^{0}C; ^{1}H-NMR (DMSO-d_6) δ 4.93 (s, 2H), 7.45-7.57 (m, 5H), 7.65 (d, J = 8.4 Hz, 1H), 7.78 (d, J = 8.4 Hz, 2H), 7.91-8.02 (m, 7H), 8.08 (s, 1H). MS (ESI) m/e (rel. int.): 335 (M^+ + 2, 10), 354 (M^+ + 1, 100).

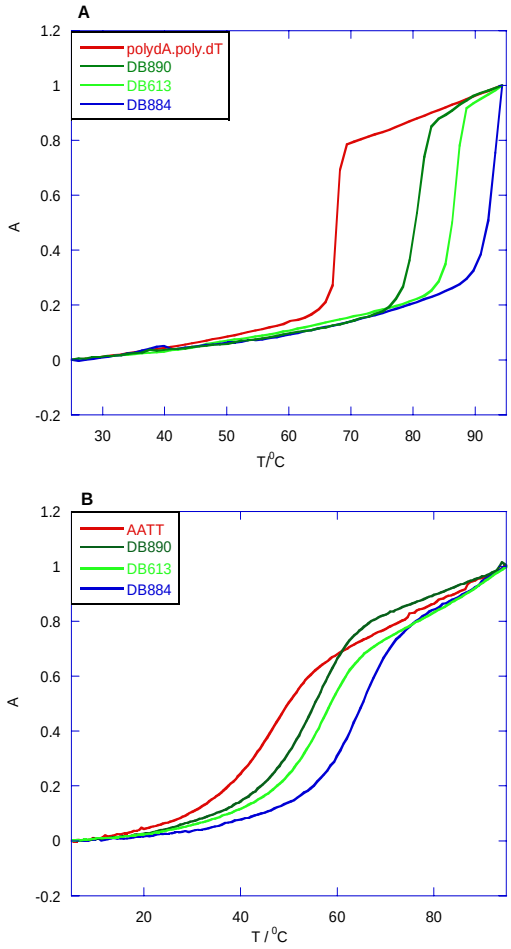


Figure S1. (A)Thermal melting curves of polydA.polydT in the absence and presence of DB884, DB613 and DB890 in cacodylic acid buffer. The ratio of compound to base pair of DNA is 0.3:1. (B)Thermal melting curves of DNA oligomer containing AATT sites in the absence and presence of DB884, DB613 DB890 and Db1390 at 1:1 ratio of compound to DNA oligomer.

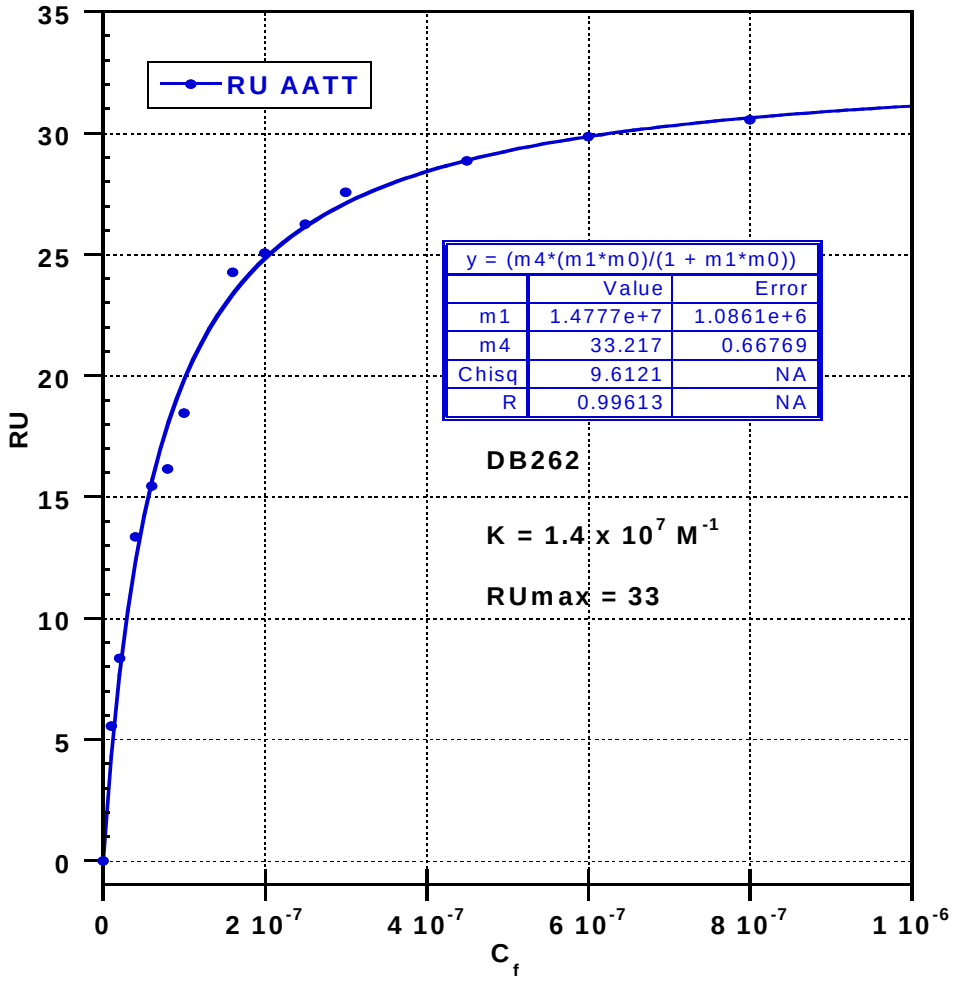


Figure S2. RU values are plotted against the unbound compound concentration, C_f (flow solution) for DB262 binding to AATT DNA hairpin. The data was fitted to one site model using equation 1.

Supporting Information Table 1. Crystallographic data.

Structure	DB 884 - d(CGCGAATTCGCG)$_2$
Space group	*P* $2_12_12_1$
Unit cell dimensions (Å)	*a* = 24.380, *b* = 40.102, *c* = 65.627
Date of collection	10-Feb-2006
Wavelength (Å)	1.54178
Resolution range (Å)	25.00 - 1.86
Data cut-off (σ)	0.0
Measured reflections	15784
Unique reflections	5536
I/σ (I) for the data (in outer shell)	35.93 (4.31)
Data redundancy	2.85
R_{merge} (in outer shell) (%)	4.3 (21.4)
Completeness (in outer shell) (%)	94.7 (91.3 for 1.93 - 1.86)
Free R (%)	31.0
R-factor (%)	21.9
R.m.s.d. bond lengths (Å)	0.006
R.m.s.d. bond angle distances (Å)	0.025
DNA atoms	486
DB 884 atoms	35
Hydrated magnesium atoms	7
Full occupancy water molecules	38
DNA B-factor (Å^2)	30.3
DB 884 B-factor (Å^2)	54.3
Hydrated magnesium B-factor (Å^2)	26.0
Water molecules B-factor (Å^2)	40.6