

SUPPLEMENTARY DATA

SELECTIVE RECOGNITION OF PYRIMIDINE-PYRIMIDINE DNA MISMATCHES BY DISTANCE-CONSTRAINED MACROCYCLIC BISINTERCALATORS

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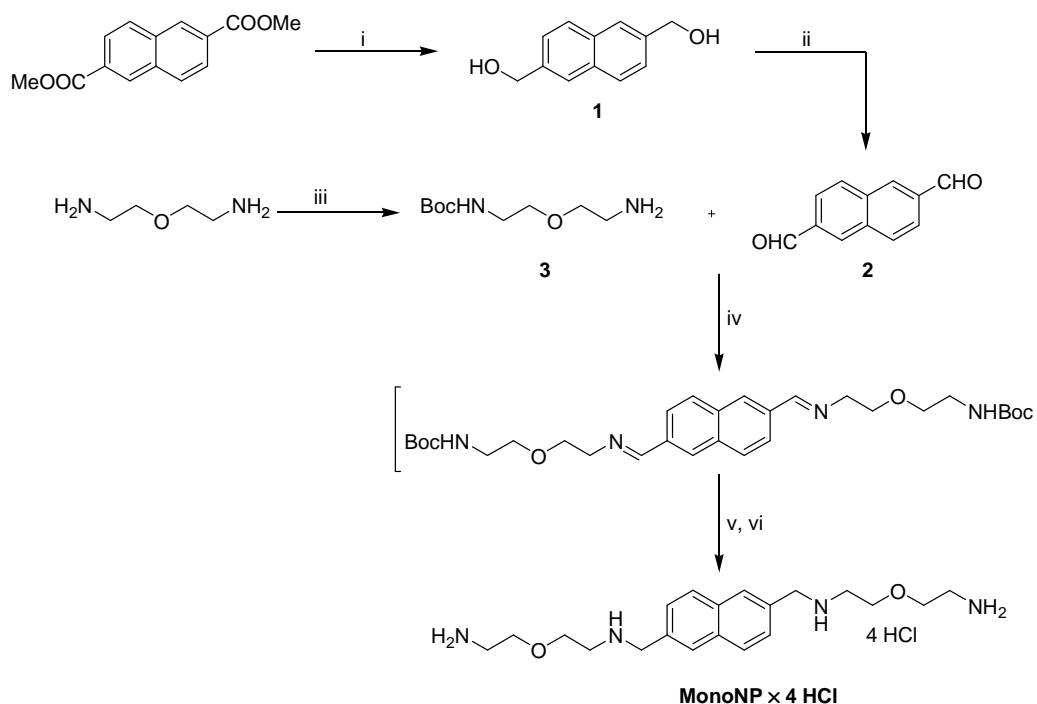
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Synthesis and characterization data of compounds MonoNP and BisA-NH₂

General Remarks

All commercially available chemicals were reagent grade and used without further purification. Solvents were purified and dried according to standard procedures. The melting points were measured with an Electrothermal IA 9100 digital melting point instrument (Barnstead International) and are uncorrected. ESI mass spectra (positive-ion mode) were recorded with a Waters ZQ instrument (source voltage 50–75 kV). NMR spectra were measured on a Bruker Avance 300 (¹H: 300 MHz, ¹³C: 75 MHz) spectrometer at 25 °C; chemical shifts are given in ppm (δ) values (internal standards methanol, $\delta_{\text{H}} = 3.34$, $\delta_{\text{C}} = 49.5$ ppm for D₂O,¹ and TMS for the other solvents). Elemental microanalyses of the new compounds were performed by the *Service de Microanalyse*, CNRS–ICSN, Gif-sur-Yvette, France.



Scheme S1. Synthesis of the model compound **MonoNP**. Reagents and conditions: (i) DIBAL-H, hexanes, room temp., 36 h, 97%; (ii) PCC, CH₂Cl₂, reflux, 1 h, 68%; (iii) BocOPh, EtOH, reflux, 24 h, 53%; (iv) benzene, reflux, 18 h; (v) NaBH₄, MeOH–CH₂Cl₂, room temp., 3 h; (vi) HCl, MeOH, reflux, 2 h, 87% over three steps.

¹ Gottlieb, H. E.; Kotlyar, V.; Nudelman, A. *J. Org. Chem.* **1997**, *62*, 7512–7515.

2,6-Bis(hydroxymethyl)naphthalene (1): To a solution of DIBAL-H (0.7–1.3 M in hexanes, 300 mL, ~0.3 mol), stirred under argon at room temperature, solid dimethyl 2,6-naphthalenedicarboxylate (12.2 g, 50.0 mmol) was added in several portions over 1 h. The reaction mixture was vigorously stirred at room temperature for 36 h. The reaction flask was cooled in an ice bath and a mixture of MeOH (50 mL) and H₂O (20 mL) was carefully added, followed by 6 M aqueous HCl (180 mL). The mixture was filtered and the solid was thoroughly washed with water and dried, to give **1** (9.10 g, 97%) as a white solid, m.p. 172–173 °C (lit.² 170–171 °C); ¹H-NMR (DMSO-*d*₆): δ = 4.66 (d, ³*J* = 5 Hz, 4 H, CH₂), 5.30 (t, ³*J* = 5 Hz, 2 H, OH), 7.45 (d, ³*J* = 8 Hz, 2 H, 3-H, 7-H), 7.79 (s, 2 H, 1-H, 5-H), 7.84 (d, ³*J* = 8 Hz, 2 H, 4-H, 8-H); ¹³C-NMR (DMSO-*d*₆): δ = 63.0 (CH₂), 124.2 (CH), 125.3 (CH), 127.4 (CH), 132.0 (C_q), 139.7 (C_q).

Naphthalene-2,6-dicarbaldehyde (2): To a suspension of pyridinium chlorochromate (19.4 g, 90.0 mmol) in anhydrous CH₂Cl₂ (120 mL), stirred at reflux temperature under argon, a suspension of **1** (5.65 g, 30.0 mmol) in anhydrous CH₂Cl₂ (80 mL) was added in one portion. The reaction mixture was rigorously stirred for 1 h at reflux temperature, cooled and poured into diethyl ether (500 mL). The mixture was triturated until the tar solidified and then filtered through a large pad of silica, eluting with an additional portion of ether (1 L). The solvents were removed in vacuo, to give **2** (3.74 g, 68%) as fine colorless needles, m.p. 167–170 °C (lit.³ 173.4–174.0 °C); ¹H-NMR (CDCl₃): δ = 8.06 (dd, ³*J* = 8.5 Hz, ⁴*J* = 1 Hz, 2 H, 3-H, 7-H), 8.13 (d, ³*J* = 8.5 Hz, 2 H, 4-H, 8-H), 8.41 (d, ⁴*J* = 1 Hz, 2 H, 1-H, 5-H), 10.22 (s, 2 H, CHO); ¹³C-NMR (CDCl₃): δ = 124.1 (CH), 130.6 (CH), 133.7 (CH), 135.7 (C_q), 136.2 (C_q), 191.8 (CH).

tert-Butyl-2-(2-aminoethoxy)ethylcarbamate (3): A solution of 2,2'-oxydiethylamine (2.05 g, 19.7 mmol) and *tert*-butyl phenyl carbonate (3.63 g, 18.7 mmol) in abs. EtOH (100 mL) was heated under reflux for 24 h, cooled, and the volatiles were removed in vacuo. Water (60 mL) was added, and the pH was adjusted to ~3 by addition of 0.5 M HCl. The mixture was extracted with CH₂Cl₂ (3 × 50 mL). The organic fractions contained phenol and the diprotected diamine and were discarded. The aqueous phase was made strongly alkaline with NaOH and extracted with CH₂Cl₂ (4 × 50 mL). The

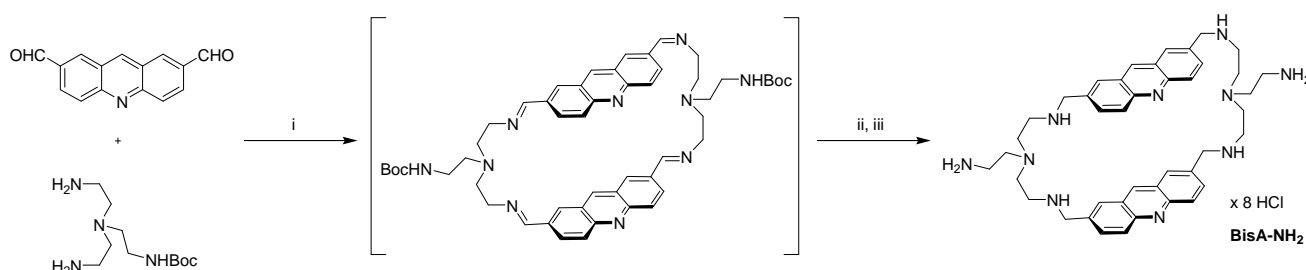
² (a) Storms, P. W. ; Taussig, P. R.; *J. Chem. Eng. Data* **1966**, *11*, 272–273. (b) Vanderwerff, W. D. (Sun Oil Co., Philadelphia, PA), U.S. Pat. 3,288,823, **1966**.

³ Hagiya, K.; Mitsui, S.; Taguchi, H. *Synthesis* **2003**, 823–828.

organic fractions were pooled, dried over anhydrous K_2CO_3 , evaporated in vacuo, and the residue was purified by flash chromatography (SiO_2 ; eluent: $CHCl_3$ –MeOH– NH_4OH , 80:17.5:2.5), to give the amine **3** (2.01 g, 53%) as colorless mobile oil; 1H -NMR ($CDCl_3$): δ = 1.45 (s, 9 H, CH_3), 1.49 (br s, 2 H, NH_2), 2.86 (t, 3J = 5 Hz, 2 H, CH_2NH_2), 3.30–3.35 (m, 2 H, $BocNHCH_2$), 3.46–3.54 (m, 4 H, CH_2OCH_2), 5.02 (br s, 1 H, $NHBoc$); ^{13}C -NMR ($CDCl_3$): δ = 28.4 (CH_3), 40.4 (CH_2), 41.8 (CH_2), 70.0 (CH_2), 73.2 (CH_2), 79.3 (C_q), 156.0 (C_q); MS (ESI^+): m/z (%) = 205 (100) [$M + H$] $^+$, 227 (13) [$M + Na$] $^+$.

2,6-Bis[[2-(2-aminoethoxy)ethylamino]methyl]naphthalene tetrahydrochloride (MonoNP \times 4 HCl): A solution of the dialdehyde **2** (0.90 g, 4.90 mmol) and amine **3** (2.00 g, 9.80 mmol) in benzene (20 mL) was heated under reflux for 18 h and then evaporated to dryness, to give the *diimine* in quantitative yield; 1H -NMR ($CDCl_3$): δ = 1.39 (s, 18 H, CH_3), 3.29–3.33 (m, 4 H, CH_2NHBoc), 3.57 (t, 3J = 5 Hz, 4 H, $CH=NCH_2$), 3.80–3.87 (m, 8 H, CH_2OCH_2), 4.97 (br s, 2 H, $NHBoc$), 7.91 (d, 3J = 8.5 Hz, 2 H, 3-H, 7-H), 8.02 (d, 3J = 8.5 Hz, 2 H, 4-H, 8-H), 8.06 (s, 2 H, 1-H, 5-H), 8.47 (s, 2 H, $CH=N$); ^{13}C -NMR ($CDCl_3$): δ = 28.4 (CH_3), 40.3 (CH_2), 61.2 (CH_2), 70.1 (CH_2), 70.4 (CH_2), 79.1 (C_q), 124.5 (CH), 129.1 (CH), 129.7 (CH), 134.4 (C_q), 134.8 (C_q), 155.9 (C_q), 162.6 (CH); MS (ESI^+): m/z (%) = 557 (100) [$M + H$] $^+$, 579 (46) [$M + Na$] $^+$. The diimine was dissolved in a mixture of MeOH (20 mL) and CH_2Cl_2 (10 mL), cooled in an ice bath, and $NaBH_4$ (0.57 g, 15 mmol) was added. The mixture was stirred at room temperature for 3 h and then evaporated to dryness. Aqueous NaOH (1 M, 20 mL) was added to the residue, and the mixture was extracted with $CHCl_3$ (5 \times 40 mL). The organic fractions were pooled, washed with 5% aqueous Na_2CO_3 solution, dried over anhydrous K_2CO_3 , and the solvent was removed in vacuo, to give the Boc-protected intermediate (2.75 g) as viscous pale-yellow oil; 1H -NMR ($CDCl_3$): δ = 1.43 (s, 18 H, CH_3), 2.83 (t, 3J = 5 Hz, 4 H, $ArCH_2NHCH_2$), 3.29–3.33 (m, 4 H, CH_2NHBoc), 3.50 (t, 3J = 5 Hz, 4 H, OCH_2), 3.58 (t, 3J = 5 Hz, 4 H, OCH_2), 3.97 (s, 4 H, $ArCH_2NH$), 5.02 (br s, 2 H, $NHBoc$), 7.46 (d, 3J = 8 Hz, 2 H, 3-H, 7-H), 7.74 (s, 2 H, 1-H, 5-H), 7.78 (d, 3J = 8 Hz, 2 H, 4-H, 8-H); ^{13}C -NMR ($CDCl_3$): δ = 28.4 (CH_3), 40.4 (CH_2), 48.6 (CH_2), 54.0 (CH_2), 70.0 (CH_2), 70.4 (CH_2), 79.2 (C_q), 126.3 (CH), 126.8 (CH), 127.9 (CH), 132.6 (C_q), 137.4 (C_q), 155.9 (C_q); MS (ESI^+): m/z (%) = 461 (12) [$M - tBuOCO$] $^+$, 561 (100) [$M + H$] $^+$, 583 (12) [$M + Na$] $^+$. This compound was dissolved in MeOH (30 mL), brought to reflux, and HCl (~7.5 M in EtOH, 30 mL) was added. The mixture was heated under reflux for 2 h, while white precipitate has formed, and then evaporated to dryness in vacuo. The residue was recrystallized from MeOH– H_2O , to give **MonoNP \times 4 HCl** (2.16 g, 87%) as white microcrystalline solid; m.p. (dec.) 307–307 °C; 1H -NMR

(D₂O): δ = 3.23 (t, 3J = 5 Hz, 4 H, CH₂CH₂NH₂), 3.38 (t, 3J = 5 Hz, 4 H, ArCH₂NHCH₂), 3.78 (t, 3J = 5 Hz, 4 H, CH₂CH₂NH₂), 3.85 (t, 3J = 5 Hz, 4 H, NHCH₂CH₂O), 4.49 (s, 4 H, ArCH₂), 7.66 (d, 3J = 8 Hz, 2 H, 3-H, 7-H), 8.07 (d, 3J = 8 Hz, 2 H, 4-H, 8-H), 8.09 (s, 2 H, 1-H, 5-H); ¹³C-NMR (D₂O): δ = 39.7 (CH₂), 47.1 (CH₂), 51.6 (CH₂), 66.0 (CH₂), 67.1 (CH₂), 128.2 (CH), 129.9 (CH), 130.0 (C_q), 130.4 (CH), 133.6 (C_q); MS (ESI⁺): m/z (%) = 383 (11) [$M + Na$]⁺, 361 (100) [$M + H$]⁺, 257 (39) [$M - H_2N(CH_2)_2O(CH_2)_2$]⁺; anal. calcd for C₂₀H₃₂N₄O₂ × 4 HCl (506.3): C, 47.44; H, 7.17; N, 11.07; Cl, 28.01; found: C, 47.28; H, 6.96; N, 10.97; Cl, 27.98.



Scheme S2. Synthesis of the macrocycle **BisA-NH₂**. Reagents and conditions: (i) CH₂Cl₂–MeOH, room temp., 24 h, 38%; (ii) NaBH₄, MeOH–CH₂Cl₂, 0 °C, 3 h; (iii) HCl, MeOH, room temp., 60% over two steps.

5,24-Bis(2-Aminoethyl)-2,5,8,21,24,27-hexaaza[9,9](2,7)acridinophane octahydrochloride (BisA-NH₂ × 8 HCl). A solution of acridine-2,7-dicarboxaldehyde⁴ (50.0 mg, 0.212 mmol) in a mixture of CH₂Cl₂ and MeOH (1:1, 28 mL) was added dropwise within 3 h at room temperature under argon to a well-stirred solution of bis(2-aminoethyl)-2-(*tert*-butoxycarbonylamino)ethylamine⁵ (52.0 mg, 0.212 mmol) in the same solvent mixture (4 mL). The resulting solution was stirred for 24 h after the end of the addition. The solvents were evaporated to give a brown powder. The residue was triturated with diethyl ether, and the solid was collected by filtration, dissolved in a mixture of CH₂Cl₂ and MeOH (1:2, 150 mL), filtered and concentrated in vacuo to give the *tetraimine* (36 mg, 38%) as yellow powder. NaBH₄ (26.0 mg, 0.673 mmol) was added to a solution of the tetraimine (100 mg, 0.112 mmol)

⁴ Teulade-Fichou, M.-P.; Vigneron, J. P.; Lehn, J. M. *Supramol. Chem.* **1995**, *5*, 139–147.

⁵ Benito, J. M.; Gómez-García, M.; Mellet, C. O.; Baussanne, I.; Defaye, J.; García Fernández, J. M. *J. Am. Chem. Soc.* **2004**, *126*, 10355–10363.

dissolved in CH₂Cl₂-MeOH (1:2, 40 mL), cooled at 0 °C. After stirring for 3 h the solvents were evaporated, the residue dissolved in water (2 mL) and extracted with CH₂Cl₂-MeOH (9:1). The organic phase was dried and concentrated by evaporation. The brown residue was dissolved in a mixture of MeOH and HCl (1 M), precipitated by adding diethyl ether and collected by filtration. The precipitate was recrystallized from a mixture of EtOH and HCl (1 M), to give **BisA-NH₂ × 8 HCl** (66 mg, 60%) as yellow powder; ¹H-NMR (D₂O): δ = 2.88 (m, 12 H), 3.14 (t, *J* = 6.6 Hz, 4 H), 3.28 (t, *J* = 6 Hz, 8 H), 4.28 (s, 8 H), 8.04 (d, *J* = 9 Hz, 4 H), 8.20 (d, *J* = 9 Hz, 4 H), 8.35 (s, 4 H), 9.56 (s, 2 H); ¹³C-NMR (D₂O): δ = 36.8 (CH₂), 45.6 (CH₂), 49.7 (CH₂), 50.7 (CH₂), 51.2 (CH₂), 121.5 (CH), 126.7 (C_q), 131.3 (C_q), 132.9 (CH), 139.3 (CH), 140.9 (C_q), 150.8 (CH); MS (ESI⁺): *m/z* (%) = 699 (100) [*M* + H]⁺.

Fitting of titration curves

Curve fitting of the competitive fluorescence titrations was performed by minimizing the sum of squared errors between the observed fluorescence intensities and the fluorescence intensities calculated from the model (see below) at all given enzyme concentrations (data points) using the Solver module of Microsoft Excel 2000.⁶ For FID titrations, the equilibrium between DNA and ethidium bromide has been neglected because the binding affinity of ethidium bromide to DNA is much lower than that of the investigated ligands. Thus, these titrations have been fitted according to the one binding equilibrium model described below.

Titration with one binding equilibrium

Since the total (indexed by “0”) concentrations (indicated by brackets) are known at each data point of the titration, a total of three equations can be formulated for titrations with one binding equilibrium:

$$K_{D1} = \frac{c(A) c(B)}{c(A \cdot B)} \quad (1)$$

$$c(A)_0 = c(A \cdot B) + c(A) \quad (2)$$

$$c(B)_0 = c(A \cdot B) + c(B) \quad (3)$$

In these three equations, the three variables $c(A)$, $c(B)$ and $c(A \cdot B)$ are unknown and, thus, can be expressed by the known parameters $c(A)_0$ and $c(B)_0$, as well as the parameter K_{D1} which is supposed to be determined later by curve fitting. Combination of the three equations (1) to (3) leads to a quadratic equation of the general form

$$ax^2 + bx + c = 0 \quad (4)$$

with the variable x as one of the unknown concentrations $c(A)$, $c(B)$ and $c(A \cdot B)$. Based on equation (4) an expression for the calculated fluorescence intensity F_{calc} can be derived. Minimizing the sum of squared errors between the observed fluorescence intensities and F_{calc} by adjusting (among others) the

⁶ Pingoud, A.; Urbanke, C.; Hogget, J.; Jeltsch, A. *Biochemical Methods*, Wiley-VCH, 2002.

fitting parameter K_{D1} then leads to the set of parameters that most accurately describes the experimental data points. This approach will be exemplified in more detail for the more complex case of a system with competitive binding and thus having two binding equilibria that need to be considered.

Titration with two binding equilibria

In analogy to equations (1) to (3), a total of five equations can be formed for a model with two ligands B and C competing for one binding site A:

$$K_{D1} = \frac{c(A) c(B)}{c(A \cdot B)} \quad (5)$$

$$K_{D2} = \frac{c(A) c(C)}{c(A \cdot C)} \quad (6)$$

$$c(A)_0 = c(A) + c(A \cdot B) + c(A \cdot C) \quad (7)$$

$$c(B)_0 = c(A \cdot B) + c(B) \quad (8)$$

$$c(C)_0 = c(A \cdot C) + c(C) \quad (9)$$

Combination of the five equations leads to a cubic equation of the general form

$$ax^3 + bx^2 + cx + d = 0 \quad (10)$$

The variable x , which this cubic equation will be solved for, was chosen to be $c(C)$ by cancellation of the other unknown variables $c(B)$, $c(A)$, $c(A \cdot B)$ and $c(A \cdot C)$. Then a , b , c , and d only consist of known $[c(B)_0, c(C)_0, \text{ and } c(A)_0]$ or fitting parameters (K_{D1} and K_{D2}):

$$a = K_{D2} - K_{D1} \quad (11)$$

$$b = [c(B)_0 + c(C)_0 - c(A)_0 - K_{D2}](K_{D1} - K_{D2}) - c(B)_0 K_{D1} - c(C)_0 K_{D2} \quad (12)$$

$$c = c(C)_0 K_{D2} (c(B)_0 + c(C)_0 - c(A)_0 + K_{D1} - 2K_{D2}) \quad (13)$$

$$d = [c(C)_0 K_{D2}]^2 \quad (14)$$

The solution of cubic equations of this general type is known and described in mathematical textbooks. The discriminant D is then defined as

$$D = (q / 2)^2 + (p / 3)^3, \quad (15)$$

where

$$p = (3ac - b^2) / 3a^2 \text{ and} \quad (16)$$

$$q = (2b^3 / 27a^3) - (bc / 3a^2) + (d / a). \quad (17)$$

The sign of the discriminant decides the following cases:

- If $D < 0$, then there exist three distinct real solutions.
- If $D = 0$, then there exist either one double real and one single real solution or alternatively one triple real solution.
- If $D > 0$, then there exist one real solution and a pair of complex conjugate solutions.

Only the case $D < 0$ (*casus irreducibilis*) was found in the present study with only one of the three distinct real solutions being physically meaningful:

$$x_{1/2/3} = 2 (-p / 3)^{1/2} \cos(\varphi / 3 + k^2/3 \pi) - (b / 3a), \quad (18)$$

where

$$\varphi = \arccos\{-0.5 q [-(p / 3)^3]^{-1/2}\} \text{ and } k = 0, 1, 2. \quad (19)$$

Explicitly, in the present study, only solution x_1 ($k = 0$) was physically meaningful for $K_{D1} > K_{D2}$ and only solution x_3 ($k = 2$) was physically meaningful for $K_{D1} < K_{D2}$. The other solutions gave either negative or too high concentrations [$x = c(C) > c(C)_0$]. The value $c(A \cdot C)$ was then calculated from equation (9) after insertion of $c(C) = x$, $c(A)$ from equation (6) after insertion of $c(C)$ and $c(A \cdot C)$, $c(A \cdot B)$ from equation (7) after insertion of $c(A)$ and $c(A \cdot C)$, and $c(B)$ from equation (8) after insertion of $c(A \cdot B)$.

The calculated value of the fluorescence intensity F_{calc} was comprised of fluorescence intensity factors (f_i) for each fluorescent species, i.e. **16-2T** (B), **16-2T**·M.TaqI (A·B), **17-TT**·M.TaqI (A·C) and M.TaqI (A) in the case of 2Ap-titrations (the Trp fluorescence of the enzyme is also partly detected at the wavelength pair chosen for 2Ap detection), and the concentration of the corresponding species plus a constant for the fluorescence background f_{bg} of the apparatus and buffer:

$$F_{\text{calc}} = f_1 c(\mathbf{B}) + f_2 c(\mathbf{A} \cdot \mathbf{B}) + f_3 c(\mathbf{A} \cdot \mathbf{C}) + f_4 c(\mathbf{A}) + f_{\text{bg}} \quad (20)$$

The sum of the squared errors SSE between the observed fluorescence intensities F_{obs} and the calculated fluorescence intensities F_{calc} was then calculated according to the equation

$$SSE = \sum (F_{\text{obs}} - F_{\text{calc}})^2, \quad (21)$$

where the sum was taken over all enzyme concentrations for which the fluorescence intensity was recorded. The SSE was minimized during the fit upon varying the parameters f_i , f_{bg} , and, importantly, the dissociation constants to be determined K_{D1} and K_{D2} .

Binding model for numerical simulation of titration (III) in Figure 6

Three binding equilibria have to be considered for titration (III) in Figure 6:

$$K_{\text{D1}} = \frac{c(\mathbf{16-2T}) c(\mathbf{M.TaqI})}{c(\mathbf{16-2T} \cdot \mathbf{M.TaqI})} \quad (22)$$

$$K_{\text{D2}} = \frac{c(\mathbf{17-TT}) c(\mathbf{M.TaqI})}{c(\mathbf{17-TT} \cdot \mathbf{M.TaqI})} \quad (23)$$

$$K_{\text{D3}} = \frac{c(\mathbf{17-TT}) c(\mathbf{BisNP})}{c(\mathbf{17-TT} \cdot \mathbf{BisNP})} \quad (24)$$

Four other equations can be formed to get a total of seven equations:

$$c(\mathbf{16-2T})_0 = c(\mathbf{16-2T}) + c(\mathbf{16-2T} \cdot \mathbf{M.TaqI}) \quad (25)$$

$$c(\mathbf{17-TT})_0 = c(\mathbf{17-TT}) + c(\mathbf{17-TT} \cdot \mathbf{M.TaqI}) + c(\mathbf{17-TT} \cdot \mathbf{BisNP}) \quad (26)$$

$$c(\mathbf{BisNP})_0 = c(\mathbf{BisNP}) + c(\mathbf{17-TT} \cdot \mathbf{BisNP}) \quad (27)$$

$$c(\mathbf{M.TaqI})_0 = c(\mathbf{M.TaqI}) + c(\mathbf{16-2T} \cdot \mathbf{M.TaqI}) + c(\mathbf{17-TT} \cdot \mathbf{M.TaqI}) \quad (28)$$

The concentrations $c(\mathbf{16-2T})$, $c(\mathbf{17-TT})$, $c(\mathbf{M.TaqI})$, $c(\mathbf{BisNP})$, $c(\mathbf{17-TT} \cdot \mathbf{M.TaqI})$, $c(\mathbf{16-2T} \cdot \mathbf{M.TaqI})$ and $c(\mathbf{17-TT} \cdot \mathbf{BisNP})$ have then been numerically adjusted with Maple in a way that equations (22) to (28) are fulfilled (with an accuracy of about 1×10^{-10}) for a given set of K_{D} parameters.

Table S1. Ligand-induced changes of melting temperature (ΔT_m) of mismatch-containing duplexes **12-TX** (5'-GTTCGTAGTAAC / 5'-GTTACTXCGAAC) and of the fully matched control (**12-TA**).^a

Ligand	Ligand-induced $\Delta T_m / ^\circ\text{C}$ of the duplex, at a ligand-to-duplex ratio q								
	X = T		X = C		X = G		X = A		
	$q = 1$	$q = 2$	$q = 1$	$q = 2$	$q = 1$	$q = 2$	$q = 1$	$q = 2$	$q = 3$
BisA	4.7	8.7	6.6	10.6	1.7	4.4	-0.1	0	0.2
BisA-NH₂	7.2	11.8	15.1	15.3	4.9	8.7	-0.2	-0.6	-0.5
BisNP	15.8	16.8	14.1	14.4	7.6	8.1	1.0	1.3	0.8
DMA1	8.6	11.1	13.2	14.9	5.5	7.8	1.1	3.5	2.9
MonoNP	12.9	15.0	7.2	9.3	7.6	9.8	3.2	4.9	6.0
Melting temperatures of duplexes in the absence of ligands, $T_m / ^\circ\text{C}$									
20.8		17.9		27.3		36.0			

^a Experimental conditions: Sodium cacodylate buffer (10 mM NaAsO₂Me₂, 50 mM NaCl, pH 6.0); [duplex] = 3 μM ; estimated error of ± 1.0 $^\circ\text{C}$.

Table S2. Ligand-induced changes of melting temperature (ΔT_m) of mismatch-containing duplexes **17-TX** (5'-CCAGTTCGTAGTAACCC / 5'-GGGTTACTXCGAACTGG) and of the fully matched control (**17-TA**).^a

Ligand	Ligand-induced $\Delta T_m / ^\circ\text{C}$ of the duplex, at a ligand-to-duplex ratio q							
	X = T		X = C		X = G		X = A	
	$q = 1$	$q = 2$	$q = 1$	$q = 2$	$q = 1$	$q = 2$	$q = 1$	$q = 2$
BisA ^b	5.8	6.8	5.8	7.1	2.6	3.1	0	0
BisNP	8.8	9.9	7.1	7.8	4.9	6.4	1.6	2.3
DMA1	5.3	8.1	5.8	8.4	4.4	6.8	3.5	5.1
MonoNP	8.3	14.1	5.2	10.2	6.2	12.4	4.9	10.6
Melting temperatures of duplexes in the absence of ligands, $T_m / ^\circ\text{C}$								
	38.3		36.7		41.9		46.5	

^a Experimental conditions: Sodium cacodylate buffer (10 mM NaAsO₂Me₂, 10 mM NaCl, pH 6.0); [duplex] = 6 μM ; estimated error of ± 0.5 $^\circ\text{C}$. ^b Taken from: David, A., Bleimling, N., Beuck, C., Lehn, J.M., Weinhold, E., and Teulade-Fichou, M. P. (2003) DNA mismatch-specific base flipping by a bisacridine macrocycle, *ChemBioChem* 4, 1326–1331.

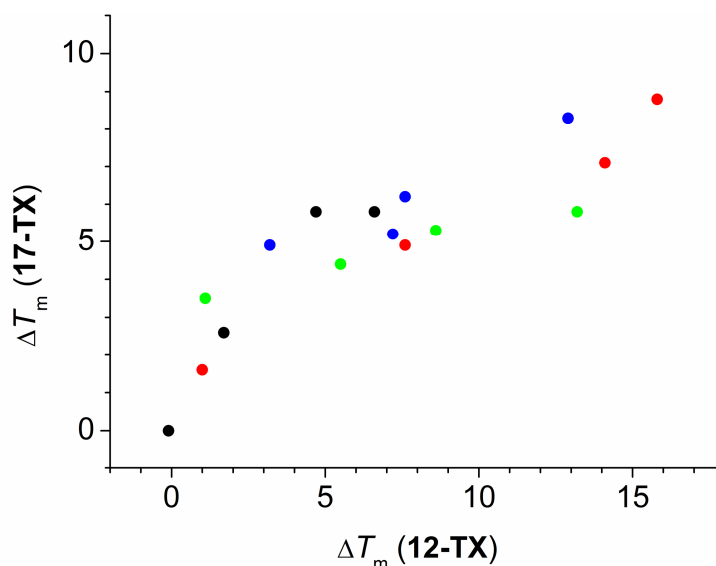


Figure S1. Correlation between the ligand-induced changes of melting temperature (ΔT_m) of duplexes **17-TX** and **12-TX** for the ligands **BisA** (black), **BisNP** (red), **DMA1** (green) and **MonoNP** (blue) at $q = 1$.

Table S3. Background-corrected relative decrease (%) of fluorescence intensity upon addition of **BisNP** (1.2 equivalents) to the **17-YX** duplexes in the presence of ethidium bromide.^a

17-YX	Fluorescence intensity decrease / %			
	Y = T	Y = C	Y = G	Y = A
X = T	15.1	17.1	6.7	5.3
X = C	12.1	12.5	2.5	4.6
X = G	4.6	3.5	4.5	2.9
X = A	3.1	7.2	4.5	4.1

^a Experimental conditions: M.TaqI binding buffer (20 mM Tris acetate, 10 mM Mg(OAc)₂, 50 mM KOAc, 1 mM DTT, 0.01% Triton X-100, pH 7.9); [**BisNP**] = 120 nM; [duplex] = 100 nM; [ethidium bromide] = 1 μM; excitation wavelength $\lambda_{\text{ex}} = 520$ nm; detection wavelength $\lambda_{\text{em}} = 615$ nm; estimated error of $\pm 0.5\%$.

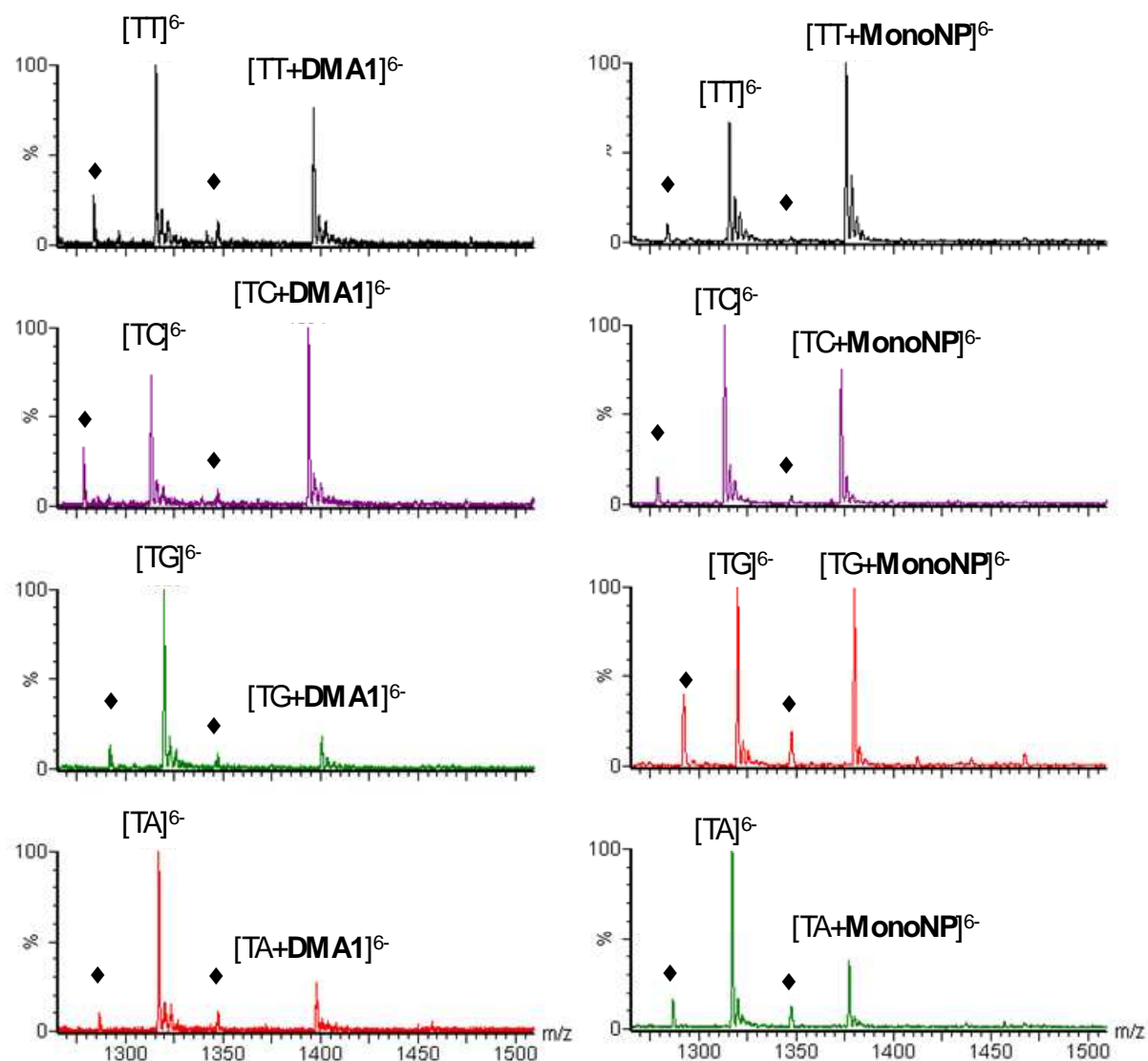


Figure S2. ESI-MS spectra of duplexes **14-TX** in the presence of control compounds **DMA1** and **MonoNP**. $[14\text{-TX}] = 5 \mu\text{M}$ and $[\text{ligand}] = 5 \mu\text{M}$. The signals of free duplexes are labeled as $[\text{TX}]^{6-}$ and the ones of 1:1 complexes as $[\text{TX} + \text{ligand}]^{6-}$. The diamonds indicate peaks corresponding to the triply charged single strands.

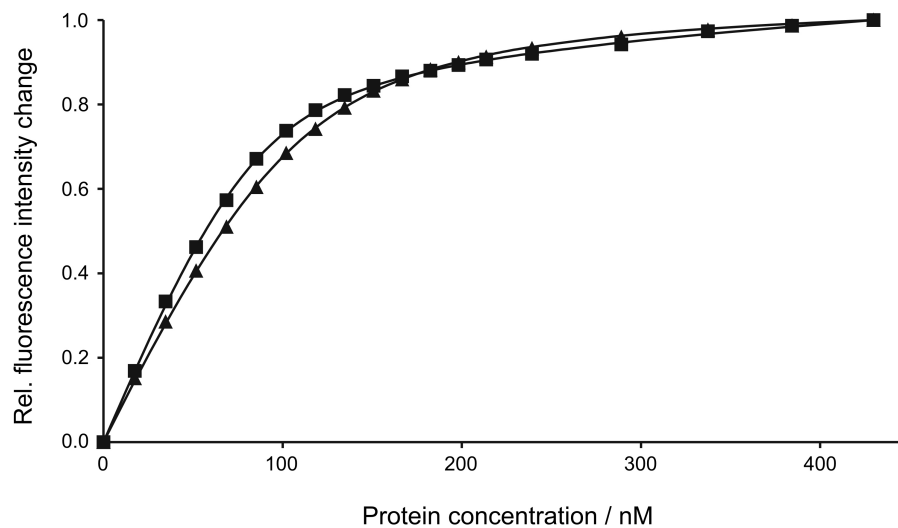


Figure S3. Binding of M.TaqI to **16-2T** (100 nM) (triangles) or **16-2T** (100 nM) in the presence of **BisNP** (2 μ M) (squares) (excitation at 320 nm and emission at 384 nm). Solid lines represent analytical curve fittings for a single-binding equilibrium model. Binding affinities of M.TaqI and **16-2T** were $60 \times 10^6 \text{ M}^{-1}$ in the absence and $80 \times 10^6 \text{ M}^{-1}$ in the presence of **BisNP**.

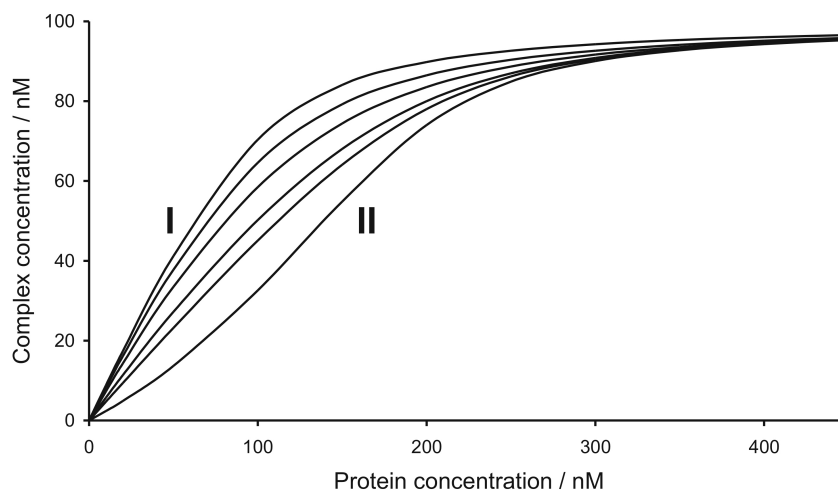


Figure S4. Numerical simulations of binding competition between **BisNP** and M.TaqI for a TT-mismatch in DNA. Curves **(I)** and **(II)** represent the analytical curve fittings for titrations of **16-2T** (100 nM) **(I)** and **16-2T** as well as **17-TT** (100 nM each) **(II)** with M.TaqI taken from Figure 6. In between are four numerical fits (performed with Maple according to the binding model with three binding equilibria described above) in the additional presence of **BisNP** (2 μM). Simulations were performed using K_a values for **BisNP-17-TT** of $20 \times 10^6 \text{ M}^{-1}$ (for the curve that is closest to curve **(I)**), $6.7 \times 10^6 \text{ M}^{-1}$, $2.2 \times 10^6 \text{ M}^{-1}$ and $1.1 \times 10^6 \text{ M}^{-1}$ (approaching curve **(II)** in this order). As further parameters, $K_a(\text{M.TaqI} \times \mathbf{17-TT}) = 280 \times 10^6 \text{ M}^{-1}$ and $K_a(\text{M.TaqI} \cdot \mathbf{16-2T}) = 100 \times 10^6 \text{ M}^{-1}$ were used for all simulations.

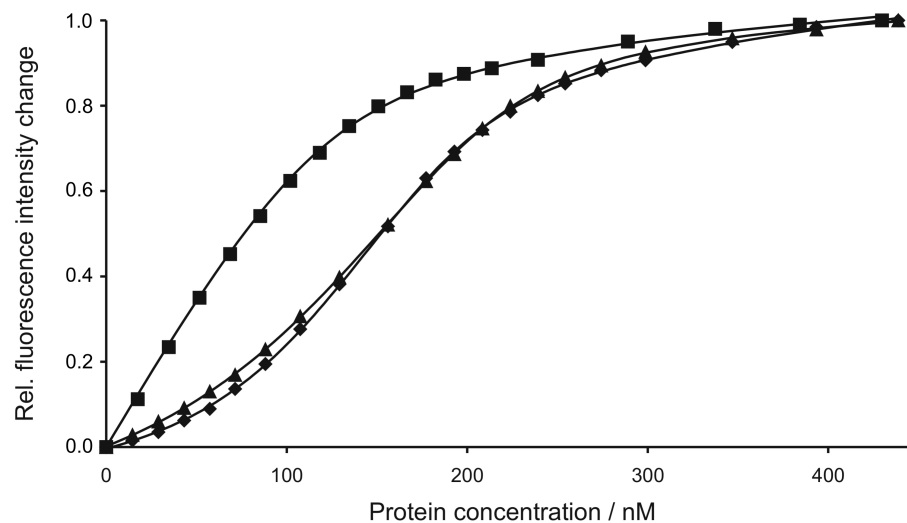


Figure S5. Fluorescence titrations (excitation at 320 nm and emission at 384 nm) of **16-2T** (100 nM) (squares), **16-2T** and **17-TA** (100 nM each) (triangles) or **16-2T** and **17-TA** (100 nM each) in the presence of **BisNP** (2 μ M) (diamonds) with M.TaqI. Solid lines represent analytical curve fittings. The binding affinity of M.TaqI to **17-TA** was determined to be $460 \times 10^6 \text{ M}^{-1}$ and the *apparent* binding affinity to **17-TA** in the presence of **BisNP** was found to be $750 \times 10^6 \text{ M}^{-1}$.

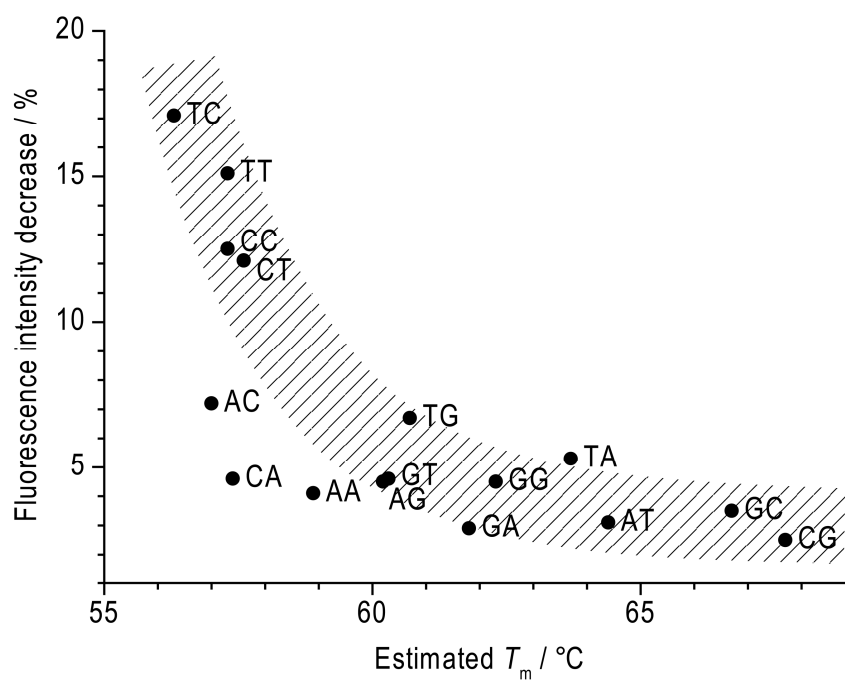


Figure S6. Correlation between background-corrected relative decrease (%) of fluorescence intensity upon addition of **BisNP** (1.2 equivalents) to the **17-YX** duplexes (100 nM) in the presence of ethidium bromide (1 μ M) and the thermodynamic stability of the duplexes, represented by their estimated melting temperature (T_m) under conditions of the FID experiments, which was calculated with HyTher software (<http://ozone2.chem.wayne.edu/>).