Pergamon

Bioorganic & Medicinal Chemistry 9 (2001) 2381–2385

BIOORGANIC & MEDICINAL CHEMISTRY

Improved Selectivity for the Binding of Naphthyridine Dimer to Guanine–Guanine Mismatch☆

Kazuhiko Nakatani,* Shinusuke Sando and Isao Saito*

Department of Synthetic Chemistry and Biological Chemistry, Faculty of Engineering, Kyoto University, CREST, Kyoto 606-8501, Japan

Received 10 February 2001; accepted 2 May 2001

Abstract—Naphthyridine dimer composed of two 2-amino-1,8-naphthyridines and a connecting linker strongly binds to guanine–guanine (G–G) mismatch in duplex DNA. In order to improve G–G selectivity for the binding, we have examined structure modification of the linker. A new naphthyridine dimer possessing 3,6-diazaoctanedioic acid linker binds to G–G mismatch with an association constant of $1.18\times10^7\,M^{-1}$, which is somewhat weaker than that of the original naphthyridine dimer having a shorter connecting linker. However, the binding of the modified naphthyridine dimer to G–A mismatch was almost negligible as compared to that of the original. This results in a net increase of the selectivity for the binding to G–G mismatch by 4-folds.

Introduction

Cross-hybridization of two duplex DNAs containing only one nucleotide difference produces duplex DNAs containing a mismatch base pair in addition to the original duplexes.[1–3] Detection of mismatch-containing DNAs and determination of mismatch bases are one of the important methods for detecting single nucleotide polymorphisms (SNPs) and identifying types of genetic mutation.[4–9] We have recently reported a novel approach for the detection of guanine–guanine (G–G) mismatches in duplex DNA by designing mismatch binding ligand naphthyridine dimmer (**N1**) composed of two 2-amino-1,8-naphthyridines and a connecting linker.[10] Mismatch recognition molecule **N1** strongly binds to G–G mismatch especially in the sequence of d(CGG)/d(CGG). Since 2-amino-1,8-naphthyridine has hydrogen bonding functional groups fully complementary to those of G,[11–14] **N1** was expected to selectively bind to G–G mismatch. In fact, **N1** bind to G–G mismatch more effectively than G–A (adenine) and G–T (thymine) mismatches and cannot bind to fully complementary duplex. We developed a sensor chip that can detect G–G mismatches in duplex DNA by means of surface plasmon resonance (SPR).[15,16] The sensor was prepared by immobilizing **N1** onto the carboxylated dextran matrix on the gold surface. By using this sensor, strong signal of SPR was obtained for DNAs containing G–G mismatch. However, weaker but distinct signal was also observed for DNAs containing G–A and G–T mismatches.[10] Affinity to other mismatches may lower the fidelity of G–G mismatch detection by naphthyridine dimer. In order to improve the selectivity for G–G mismatch, we have examined the structural modification of **N1** especially regarding the connecting linker of two naphthyridine rings. A new naphthyridine dimer **N2** possessing 3,6-diazaoctanedioic acid linker showed a weaker binding to G–G mismatch than **N1** but almost negligible binding to G–A mismatch. This resulted in a net increase of G–G mismatch selectivity against G-A mismatch. We report here that naphthyridine dimer **N2** is an improved drug as compared to **N1** in terms of the G–G selectivity. The use of **N2** instead of **N1** for the SPR sensor can eliminate the unnecessary binding to DNA containing G–A mismatch.

Results and Discussion

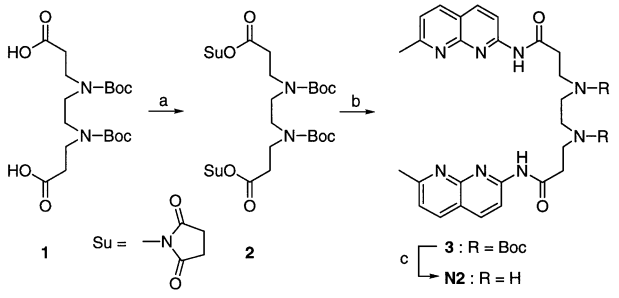
While we have little information on the structure of the complex produced from **N1** and G–G mismatch,[17] it is anticipated that extension of linker length has a

*Corresponding authors. Tel.: +81-75-753-4813; fax: +81-75-753-5676; e-mail: nakatani@sbchem.Kyoto-u.ac.jp; saito@sbchem.Kyoto-u.ac.jp

☆Dedicated to Professor Peter B. Dervan on receipt of the Tetrahedron Prize.

0968-0896/01/$ - see front matter
PII: S0968-0896(01)00160-2

considerable effect on the binding of **N1** to G–G mismatch with respect to the binding thermodynamics and kinetics. We have synthesized **N2** that has an additional secondary amino group and two methylenes in a linker connecting two naphthyridine rings as compared to that of **N1**. Synthetic procedure of **N2** is outlined in Scheme 1. Reaction of *N,N′*-di-*tert*-butoxycarbonylethylenediamine-*N,N′*-dipropionic acid[18] (**1**) with *N*-hydroxysuccinimide in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride gave activated diester **2**. Condensation of **2** with 2-amino-7-methyl-1,8-naphthyridine afforded Boc-protected dimeric naphthyridine **3**, which was treated with hydrogen chloride in ethyl acetate to give hydrochloride salt of dimeric naphthyridine **N2**.



Scheme 1. Reagents: (a) *N*-hydroxysuccinimide, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, DMF, 70%; (b) 2-amino-7-methyl-1,8-naphthyridine, $CHCl_3$, 82%; (c) HCl, AcOEt, $CHCl_3$, 62%.

Binding of **N2** to G–G mismatch was investigated by measuring melting temperatures (T_m) of 11-mer duplexes d(CTA AC**X** GAA TG)/d(CAT TC**Y** GTT AG) containing G–G (**X** = **Y** = G) and G-A (**X** = G, **Y** = A) mismatches in the presence and absence of **N2**. T_m increase (ΔT_m) of the duplex containing G–G mismatch was 12.2 °C in the presence of two equivalent moles of **N2** (Table 1). Under the identical conditions, ΔT_m of 16.4 °C was obtained in the presence of **N1**. The difference of ΔT_m ($\Delta\Delta T_m$) between these two drugs for G–G and G–A mismatches was −4.2 and −2.3 °C, respectively. $\Delta\Delta T_m$ of −4.2 °C for G–G mismatch showed that modification of the linker structure of **N1** to that of **N2** has negative effects for the thermal

Table 1. T_m of mismatch-containing duplexes in the presence and absence of naphthyridine dimer[a]

	ΔT_m (°C)[b]		
X–Y	**N1**[c]	**N2**	$\Delta\Delta T_{m\ (N2-N1)}$
G–G	16.4	12.2	−4.2
G–A	2.3	~0.0	−2.3
G–C	−0.3	0.2	0.5

[a]The UV–melting curve was measured for a duplex of d(CTA AC**X** GAA TG)/d(CAT TC**Y** GTT AG) at a total base concentration of 100 μM in 10 mM sodium cacodylate buffer (pH 7.0) containing 0.1 M NaCl in the absence and presence of drugs (9.1 μM). T_m (°C) was calculated as the maximum in a plot of $\Delta Abs_{260}/\Delta T$ versus temperature. Temperature was increased at a rate of 1 °C/min.
[b]ΔT_m is calculated as a difference of T_m in the presence and absence of drugs.
[c]Data reported in ref 10.

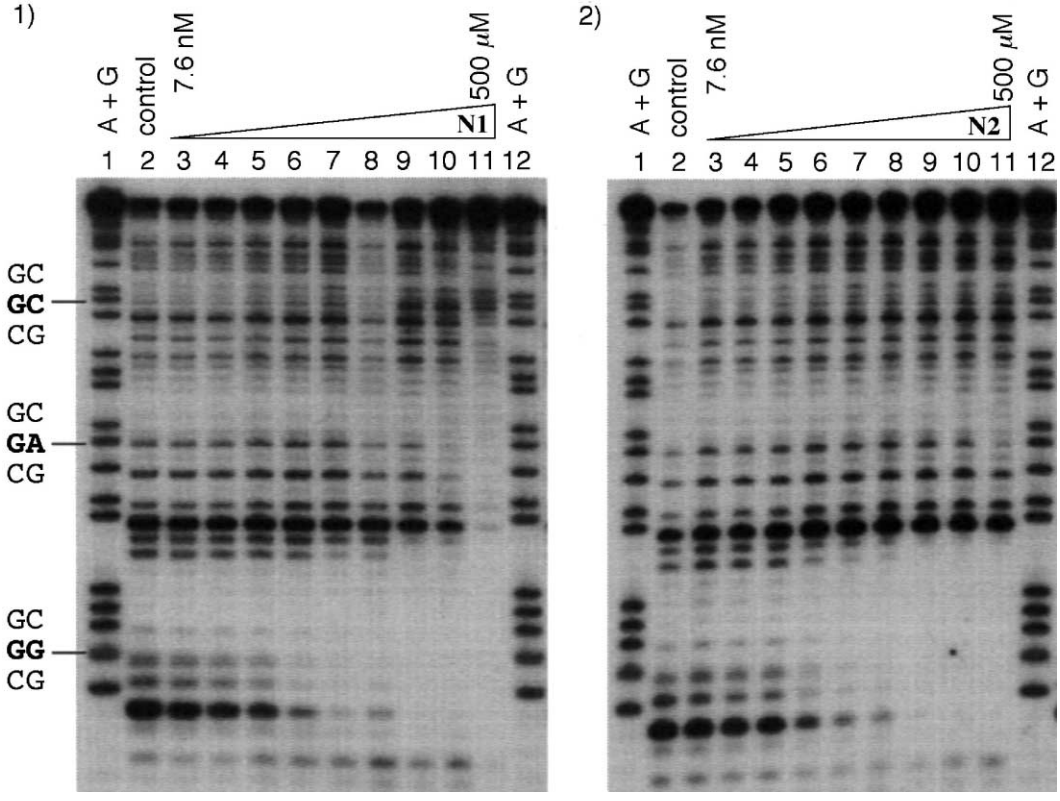


Figure 1. DNase I footprinting analysis of the complex of (1) ***N1*** and (2) ***N2*** with 5′-^{32}P-end-labeled 52-mer DNA containing G–G and G–A mismatches. Lanes 1 and 12, Maxam–Gilbert A + G sequencing reaction; lanes 2–11, 0, 7.6 nM, 31 nM, 122 nM, 488 nM, 2.0 μM, 7.8 μM, 31 μM, 125 μM, 500 μM of drug. Mismatches are shown on the left of the gel.

stabilization of G–G mismatch. However, it was also found that thermal stability of G–A mismatch was not so sensitive to the presence of **N2**. This observation is markedly distinct from ΔT_m of 2.3 °C obtained for G–A mismatch in the presence of **N1**.[11]

In order to quantitatively determine the affinity of **N2** to G–G and G–A mismatches, DNase I footprint titration for **N2** binding was then investigated.[19] We used 52-mer ODN d(GTT ACA GAA TCT *CGG* AAG CCT AAT A*CG G*TT GAC ATT CAC GGT TAC CAG TGT C) and its complementary strand d(GAC ACT GGT AAC CGT GAA TGT CAA *CAG* TAT TAG GCT T*CG G*AG ATT CTG TAA C). Upon annealing, both G–G (X = G) and G–A (X = A) mismatches were produced in the sequence of d(CGG)/d(CXG) as shown in italic. Our previous studies indicated that binding of **N1** to G–G mismatch was much more favorable with the flanking GC base pairs than with flanking AT base pairs.[10] Polyacrylamide gels obtained by DNase I footprint titration for **N1** and **N2** binding were shown in Figure 1. Distinct footprints by the binding of **N2** were observed at G–G mismatch, but it was not apparent at G–A mismatch even with the concentration of 500 μM. Quantitative analysis as determined by densitometry showed that association constants of **N2** to G–G mismatch was $1.18\times10^7\,M^{-1}$ (Fig. 2). Due to a poor water-solubility of **N2** at high concentration, association constant to G–A mismatch was estimated as $2.67\times10^3\,M^{-1}$ by curve fitting of the available data shown in Figure 1b. Association constants of **N1** to G–G and G–A mismatches were also determined as 2.41×10^7 and $2.13\times10^4\,M^{-1}$, respectively (Table 2).[20] Weaker binding of **N2** to G–G mismatch than of **N1** is well consistent with the results obtained for T_m measurements of G–G mismatch containing duplex in the presence of these two drugs. Affinity to G–A mismatch is about eight folds lower for **N2** than for **N1**. With regard to the selectivity for G–G mismatch, **N2** is a superior drug than **N1**. Selectivity of G–G mismatch versus G–A mismatch obtained by $K_{a(G-G)}/K_{a(G-A)}$ is about 4-fold higher for **N2** than for **N1**.

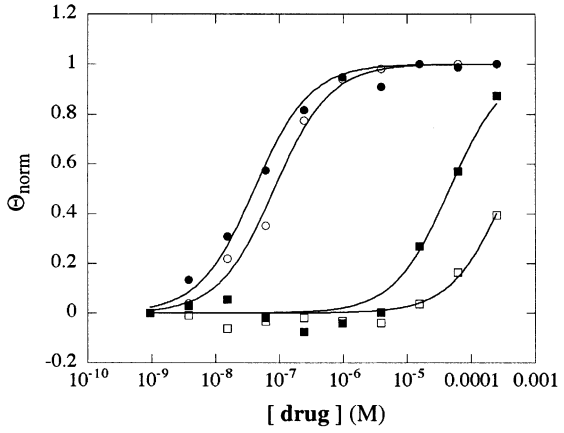


Figure 2. Data from quantitative DNase I footprinting titration experiments for the binding of dimeric naphthyridines to G–G and G–A mismatches. The Φ_{norm} points were obtained using autoradiography shown in Figure 2. The data for the binding of ***N1*** to G–G and G–A mismatch sites is indicated by closed cicles and closed squares, respectively. The data for the binding of ***N2*** to G–G and G–A mismatch sites is indicated by open circles and open squares, respectively. The solid line is the best-fit Langmuir binding titration isotherm obtained from a nonlinear least-squares algorithm as reported.[19]

Table 2. Association constants of dimeric naphthyridines to G–G and G–A mismatches[a]

Drug	K_a (M^{-1})		
	d(CGG)/d(GGC)	d(CGG)/d(GAC)	$K_{a(G-G)}/K_{a(G-A)}$
N1	2.41×10^7	2.13×10^4	1.1×10^3
N2	1.18×10^7	2.67×10^3	4.4×10^3

[a]Association constants for the binding of dimeric naphthyridine to mismatches were determined by Dnase I footprinting titration.

From the viewpoint of molecular design of drugs targeting mismatch base pairs, the results obtained by the modification of linker structure of naphthyridine dimers are especially suggestive. In general, stronger association of drugs to negatively charged DNA is expected with an increasing number of positively charged groups incorporated in the molecule. However, it is not the case for the structure modification of **N1** to **N2**. The present results may indicate that positively charged ammonium groups of **N2** do not directly contact to the negatively charged phosphates in the complex. Thus, electrostatic interaction between drugs and DNA does not contribute significantly to the thermal stability of the complex. It is also conceivable that the energy necessary for the conformational change to form the complex with mismatch DNA is larger for **N2** than for **N1** due to the extension of the linker length. In contrast to the binding to G–G mismatch, the structural modification significantly suppressed the binding of **N2** to G–A mismatch. Factors weakening the binding of **N2** to G–A mismatch are not obvious at this moment, but these observations are still useful for molecular design of drugs targeting G–A mismatch.

Conclusion

A new naphthyridine dimer **N2** showed a weaker binding to G–G mismatch than **N1**, but almost negligible binding to G–A mismatch. This resulted in the net increase of G–G mismatch selectivity against G–A mismatch. Studies described here clearly showed that the use of **N2** instead of **N1** for the SPR sensor can eliminate the unnecessary binding to DNA containing G–A mismatch. Furthermore, structure–activity relationships for G–A mismatch binding are very important for molecular design of G–A mismatch targeting drugs.

Experimental

***N,N′*-Di-*tert*-butoxycalbonylehtylenediamine-*N,N′*-di(N″-(7-methylpyridino[3,2-e]pyridine-2-yl)propanamide) (3).** To a solution of *N,N′*-di-*tert*-butoxycalbonylethylenediamine-*N,N′*-dipropionic acid **1** (1.50 g, 3.7 mmol) in dry DMF (30 mL) were added *N*-hydroxysuccinimide

(0.94 g, 8.2 mmol) and 1-(3-dimethyl-aminopropyl)-3-ethylcarbodiimide hydrochloride (1.57 g, 8.2 mmol) and the mixture was stirred for 12 h. Then ethyl acetate (30 mL) was added to the reaction mixture and the suspension thus formed was stirred for 1 h. The suspension was filtered to give **2** (1.55 g, 70%) as a white solid: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.40 (t, 4H, J = 6.8 Hz), 3.29 (s, 4H), 2.90 (t, 4H, J = 6.8 Hz), 2.80 (s, 8H), 1.37 (s, 18H); FAB-MS (NBA), m/e 599 [$(M+H)^+$]. To a solution of **2** (375 mg, 0.63 mmol) in dry DMF (5 mL) was added 2-amino-7-methyl-1,8-naphthyridine (200 mg, 1.26 mmol) and the mixture was stirred for 12 h at 45 °C. The solvent was evaporated in vacuo and the crude residue was purified by silica gel column chromatography to give **3** (355 mg, 82%) as a white solid: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.37 (d, 2H, J = 7.2 Hz), 8.24 (d, 2H, J = 7.2 Hz), 8.16 (d, 2H, J = 6.6 Hz), 7.37 (d, 2H, J = 6.6 Hz), 3.65 (t, 4H, J = 5.0 Hz), 3.47 (s, 4H), 2.76 (t, 4H, J = 5.0 Hz), 2.71 (s, 6H), 1.44 (s, 18H); ^{13}C NMR (CD_3OD, 100 MHz) δ 173.1, 164.3, 157.1, 155.6, 155.4, 140.2, 138.7, 122.8, 120.0, 115.8, 81.5, 58.3, 45.2, 28.7, 25.0, 18.3; FAB-MS (NBA), m/e 687 [$(M+H)^+$]; HR-MS calcd for $C_{36}H_{47}O_6N_8$ [$(M+H)^+$] 687.3616, found 687.3606.

Ehtylenediamine-*N*,*N*′-di(*N*″-(7-methylpyridino[3,2-e]pyridine-2-yl)propanamide) (N2). To a solution of **3** (120 mg, 0.17 mmol) in ethyl acetate (3 mL) and $CHCl_3$ (3 mL) was added ethyl acetate containing 4 M HCl (1.0 mL) at 0 °C and the mixture was stirred at 0 °C for 1.5 h, then stirred at room temperature for another 1 h. Solvent was evaporated to dryness to give hydrochloride of **N2** (quantitative yield) as a white solid. Hydrochloride of **N2** was dissolved in H_2O, basified with addition of 28% aqueous ammonia solution, and extracted with $CHCl_3$. Suspension in $CHCl_3$ turned to clear solution by addition of MeOH. The solution was dried with $MgSO_4$, and solvent was evaporated in vacuo to give **N2** (51 mg, 62%) as a pale yellow solid: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.13 (d, 2H, J = 8.8 Hz), 7.96 (d, 2H, J = 8.8 Hz), 7.91 (d, 2H, J = 8.0 Hz), 7.21 (d, 2H, J = 8.0 Hz), 3.26 (t, 4H, J = 5.7 Hz), 3.14 (s, 4H), 2.86 (t, 4H, J = 5.7 Hz), 2.67 (s, 6 H); ^{13}C NMR (CD_3OD, 100 MHz) δ 173.1, 164.1, 155.1, 155.0, 140.2, 138.5, 122.7, 119.6, 115.3, 46.6, 44.3, 35.6, 25.1; FAB-MS (NBA), m/e 487 [$(M+H)^+$]; HR-MS calcd for $C_{26}H_{31}O_2N_8$ [$(M+H)^+$] 487.2568, found 487.2592.

Measurements of melting temperatures of mismatch-containing duplexes

Dimeric naphthyridine (9.1 μM, 2 equiv to mismatch base pair) was dissolved in a sodium cacodylate buffer (10 mM, pH 7.0) containing 11-mer mismatch duplex d(CTA AC*G* GAA TG)/d(CAT TC*G* GTT AG) (100 μM, base concentration) and NaCl (100 mM). Thermal denaturation profile for absorption at 260 nm was recorded on a JASCO V-550 spectrometer equipped with a Peltier temperature controller. The absorbance of the sample was monitored at 260 nm from 2 to 70 °C with a heating rate of 1 °C/min. The T_m value was determined as the maximum in a plot of $\Delta A_{260}/\Delta T$ versus temperature.

Quantitative DNase I footprinting titration

^{32}P-5′-end-labeled 52-mer ODN 5′-d(GTT ACA GAA TCT CGG AAG CCT AAT ACG GTT GAC ATT CAC GGT TAC CAG TGT C)-3′ (20 pmol) was annealed to a 2-fold excess of 52-mer ODN 5′-d(GAC ACT GGT AAC CGT GAA TGT CAA CAG TAT TAG GCT TCG GAG ATT CTG TAA C)-3′ in a Tris–HCl buffer (10 mM, pH 7.6) containing NaCl (100 mM). The duplex was purified by nondenaturing polyacrylamide gel electrophoresis. All reactions were executed in a total volume of 50 μL with final concentrations of each species as indicated. The ^{32}P-5′-end-labeled DNA duplex (4 nM, 10×10^3 cpm) was incubated with various concentrations of dimeric naphthyridine (7.6 nM–500 μM) at 4 °C for 12 h in a Tris–HCl buffer (10 mM, pH 7.6) containing NaCl (100 mM) and $MgCl_2$ (5 mM). The mixture was incubated with DNase I (0.2 U) for 8 min at 25 °C. The reaction was quenched by addition of a 10 μL aqueous solution containing EDTA (70 mM), NaOAc (0.6 M), and calf thymus DNA (5 μM). After quenching, the reaction mixture was ethanol precipitated with 900 μL of ethanol. The precipitated DNA was washed with 100 μL of 80% cold ethanol, dried in vacuo, resuspended in 10 μL of 80% formamide loading buffer (a solution of 80% v/v formamide, 1 mM EDTA, 0.1% xylene cyanol, and 0.1% bromophenol blue). All DNA samples obtained above and the Maxam–Gilbert G + A sequencing marker were heat denatured at 90 °C for 1 min and quick-chilled on ice. The samples (1 μL, 1×10^3 cpm) were loaded onto 12% (19:1) polyacrylamide and 7 M urea sequencing gel and electrophoresed at 1500 V for approximately 2.5 h. The gel was dried and exposed to X-ray film with an intensifying sheet at −70 °C.

References and Notes

1. Cotton, R. D. *Mutat. Res.* **1993**, *285*, 125.
2. Nataraj, A. J.; Olivos-Glander, I.; Kusukawa, N.; Highsmith, W. E., Jr *Electrophoresis* **1999**, *20*, 1177.
3. Taylor, G. R. *Electrophoresis* **1999**, *20*, 1125.
4. Schafer, A. J.; Hawkins, J. R. *Nat. Biotechnol.* **1998**, *16*, 33.
5. Collins, F. S.; Guyer, M. S.; Chakravarti, A. *Science* **1997**, *278*, 1580.
6. Wang, D. G.; Fan, J.-B.; Siao, C.-J.; Berno, A.; Young, P.; Sapolsky, R.; Ghandour, G.; Perkins, N.; Winchester, E.; Spencer, J.; Kruglyak, L.; Stein, L.; Hsie, L.; Topaloglou, T.; Hubbell, E.; Robinson, E.; Mittmann, M.; Morris, M. S.; Shen, N.; Kilburn, D.; Rioux, J.; Nusbaum, C.; Rozen, S.; Judson, T. J.; Lipshutz, R.; Chee, M.; Lander, E. S. *Science* **1998**, *280*, 1077.
7. Marth, G. T.; Korf, I.; Yandell, M. D.; Yeh, R. T.; Gu, Z.; Zakeri, H.; Stitziel, N. O.; Hillier, L.; Kwok, P.-Y.; Gish, W. *Nat. Genet.* **1999**, *23*, 452.
8. Laken, S. J.; Jackson, P. E.; Kinzler, K. W.; Vogelstein, B.; Strickland, P. T.; Groopman, J. D.; Friesen, M. D. *Nat. Biotechnol.* **1999**, *16*, 1352.
9. Alderborn, A.; Kristofferson, A.; Hammerling, U. *Genome Res.* **2000**, *10*, 1249.
10. Nakatani, K.; Sando, S.; Saito, I. *Nat. Biotechnol.* **2001**, *19*, 51.
11. Nakatani, K.; Sando, S.; Saito, I. *J. Am. Chem. Soc.* **2000**, *122*, 2172.

12. Corbin, P. S.; Zimmerman, S. C. *J. Am. Chem. Soc.* **1998**, *120*, 9710.
13. Murray, T. J.; Zimmerman, S. C. *J. Am. Chem. Soc.* **1992**, *114*, 4010.
14. Kelly, T. R.; Zhao, C.; Bridger, G. J. *J. Am. Chem. Soc.* **1989**, *111*, 3744.
15. Nice, E. C.; Catimel, B. *Bioessays* **1999**, *21*, 339.
16. Fivash, M.; Towler, E. M.; Fisher, R. *J. Curr. Opin. Biotechnol.* **1998**, *9*, 97.
17. Preliminary NMR studies of **N1**-G–G mismatch showed that both naphthyridine rings intercalated into duplex DNA. Considerable upfield shifts of hydrogens on naphthyridine rings of **N1** are observed upon complex formation with a duplex containing G–G mismatch. These results will be published separately.
18. McMurry, T. J.; Brechbiel, M.; Kumar, K.; Gansow, O. A. *Bioconjugate Chem.* **1992**, *3*, 108.
19. Brenowitz, M.; Senear, D. F.; Shea, M. A.; Ackers, G. K. *Methods Enzymol.* **1986**, *130*, 132.
20. These data are consistent with those reported earlier within an experimental variability.[10]