CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.200802421

# A Fluorescent Bisanthracene Macrocycle Discriminates between Matched and Mismatch-Containing DNA

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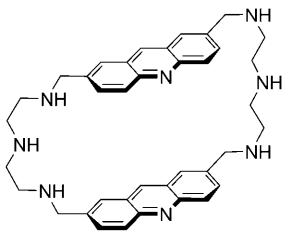
*Dedicated to Professor Jean-Marie Lehn*

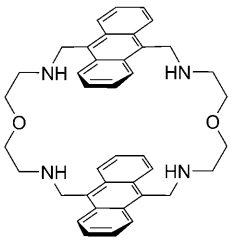
Detection of single nucleotide polymorphisms (SNPs) is important for the realization of personalized diagnostics and medication, as these mutations often represent genetic variants associated with susceptibility to various diseases and responses to various drugs.[1] Currently, a number of SNP detection methods are established,[2] based either on the fidelity of enzymatic reactions or on the use of fluorophore-modified oligonucleotides, which provide fluorescence response upon allele-specific hybridization.[3,4] Recently, the use of oligonucleotide probes incorporating base-discriminating fluorescent nucleoside analogues has been described, the fluorescence of which is modulated by the base that is paired opposite in the duplex.[5,6] However, in all these methods the covalently modified oligonucleotides have to be synthesized for each particular SNP site, which is not convenient for large-scale SNP screening.

An alternative approach, which does not require the covalent modification of the probe oligonucleotides, relies on the detection of the base-mismatch-containing DNA sequences, which are formed upon annealing of the mutant, SNP-containing DNA sample to a wild-type probe (*heteroduplex analysis*).[7] Especially attractive is the use of small-molecule ligands (*mismatch binders*) that selectively bind to the mismatched base pairs in heteroduplexes. This binding event may be monitored by a number of techniques, such as site-specific DNA photocleavage,[8] surface plasmon resonance,[9,10] and electrochemical methods,[11] provided the mismatch binder is endowed with relevant functional units. Since fluorescence-based methods are particularly rapid, highly sensitive, and may be easily automated, it is desirable to develop mismatch-selective ligands, the fluorescence response of which would be modulated upon binding to mismatched sites in the DNA. To date, only one ligand of this type has been described, represented by a bulky rhodium-based metalloinsertor tethered to a fluorophore; however, its versatility towards all possible base mismatches has not been investigated, so far.[12] A similar approach relies on the changes of fluorescent properties of a naphthyridine ligand, accommodated in a bulge region close to the matched or mismatched base pair, which is being detected.[13]

We have previously found that the bisacridine macrocycle **1** selectively binds to thymine-containing mismatched sites in DNA by a unique mode,[14] which involves ejection of a thymine residue into an extrahelical position (*base flipping*).[15] However, the fluorescence quantum yield of **1** is rather low ($4.2\times10^{-3}$) and changes to a relatively small extent upon interaction with nucleic acids.[16] We therefore proposed that replacement of the acridine units in **1** with highly emissive anthracene residues,[17] such as in macrocycle **2**, would give a ligand the fluorescence properties of which could be modulated upon binding to the mismatched base pairs in DNA.




**1**




**2**

The macrocycle **2** was readily synthesized by a [2+2]-type cyclocondensation of anthracene-9,10-dicarboxaldehyde with 2,2′-oxybis(ethylamine), followed by the reduction of the macrocyclic tetraimine and conversion of the amine to a water-soluble hydrochloride salt (Supporting Informa-

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.200802421.

tion).[18] Its binding affinity to the fully matched and mismatch-containing DNA was investigated by the thermal denaturation experiments with a set of 17-mer duplexes **I-YX** (5′-CCAG TTC G**Y**A GTA ACCC-3′/5′-GGGT TAC T**X**C GAA CTGG-3′),[19] containing either a matched (**I-TA**) or a mispaired (**I-TG**, **I-TC**, **I-TT**) thymine residue (**Y** = T) in the center of the middle triplet 5′-G**Y**A-3′/5′-T**X**C-3′ (Table 1).

Table 1. Binding affinities of **2** to the fully matched and mismatch-containing oligonucleotides **I-TX** from thermal denaturation studies.[a]

| | $T_m^0$ [°C][b] | Ligand-induced $\Delta T_m$ [°C] at ligand-to-duplex ratio $q$ | |
|---|---|---|---|
| | | $q=1$ | $q=2$ |
| X = A | 46.5 | 4.7 | 8.9 |
| X = G | 42.0 | 13.0 | 16.5 |
| X = C | 36.6 | 14.6 | 17.0 |
| X = T | 38.4 | 13.8 | 16.2 |

[a] Experimental conditions: $c$(DNA) = 6 μM in sodium cacodylate buffer, pH 6; experimental error ±0.6 °C. [b] Melting temperature in the absence of the ligand.

In contrast to the bisacridine macrocycle **1**, which had almost no effect on the fully matched duplex **I-TA**,[14] the macrocycle **2** induced a significant stabilization ($\Delta T_m \approx 5$ °C at a ligand-to-duplex ratio $q=1$), which became even more pronounced upon addition of the second equivalent of the ligand. However, an even higher degree of stabilization was observed in the case of mismatch-containing duplexes **I-TG**, **I-TC**, and **I-TT** ($\Delta T_m \approx 13$–15 °C at $q=1$), which was significantly larger than the one induced by the acridine analogue **1** ($\Delta T_m \approx 3$–7 °C under identical conditions) and was further increased upon addition of the second equivalent of the ligand (Table 1). These results demonstrate that, unlike the macrocycle **1**, the anthracene derivative **2** binds to the matched duplex **I-TA**; at the same time, it binds to the mismatch-containing duplexes with higher affinity than macrocycle **1**, as may be seen from significantly larger $\Delta T_m$ values.

Further information on binding stoichiometry of macrocycle **2** to the matched and mismatch-containing duplexes was obtained from UV/Vis and circular-dichroism (CD) spectrophotometric titrations. Addition of DNA resulted in significant changes in absorption spectrum of the anthracene derivative **2** (Figure 1), namely, hypochromism and red-shift of the absorption bands of the anthracene chromophore. In the case of fully matched duplex **I-TA**, the changes in the absorption spectrum were almost monotonous in the course of titration (Figure 1a), and a plot of absorbance at the long-wavelength absorption maximum (398 nm) versus concentration of DNA (Figure 1c) allowed us to determine that the binding was saturated at a ligand-to-duplex ratio of $q=4$.[20] This value, that is, four molecules of ligand per 17-mer duplex DNA, is in an excellent agreement with a binding-site size $n=4$ base pairs, determined from titration of highly polymerized calf thymus DNA to **2** (Supporting Information, Figure S1).

However, when the titration was performed with a mismatch-containing duplex **I-TC**, two binding modes could be identified (Figure 1b). Similarly to the previous case, the absorption of the ligand rapidly decreased upon addition of

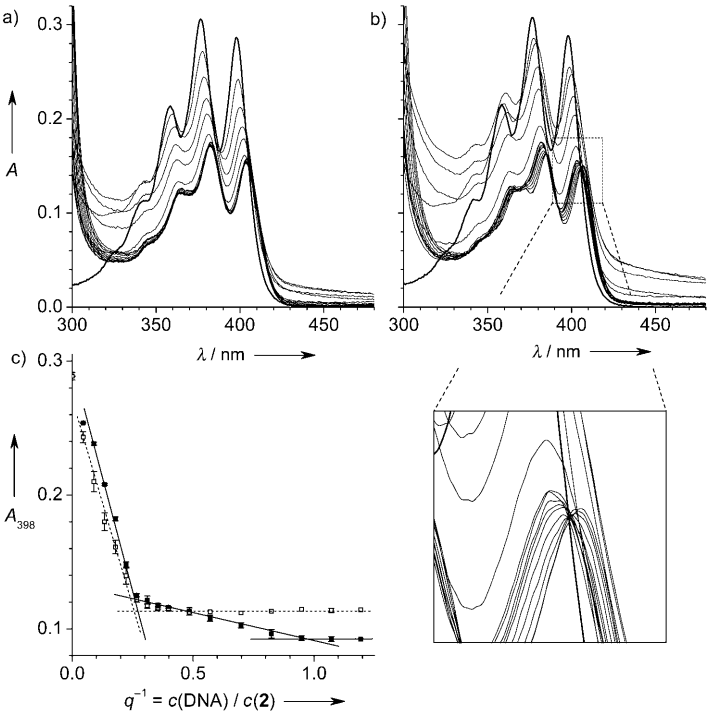


Figure 1. Spectrophotometric titrations of duplexes of a) **I-TA** and b) **I-TC** to solutions of macrocycle **2** (20 μM in sodium cacodylate buffer, pH 6). The solid dark curves represent the absorption spectrum of **2** in the absence of DNA. c) Binding isotherms of **I-TA** (dashed lines and empty points) and **I-TC** (solid lines and filled points) to **2**, as determined by absorbance at 398 nm.

**I-TC** up to a ligand-to-DNA ratio of $q=4$, which corresponds to binding of four ligand molecules to the duplex. However, as the concentration of DNA was increased from a ligand-to-DNA ratio $q=4$ to $q=1$, further changes in the absorption spectra were observed, which were not detected in the case of **I-TA**: the long-wavelength maximum of the absorption spectrum was red-shifted without a large hypochromic effect, and an isosbestic point was observed (inset in Figure 1b). Upon further addition of DNA ($q \geq 1$), no more changes in the absorption spectra were detected. We assume that this second binding mode, which takes place at $4 > q \geq 1$, may be attributed to the redistribution of the ligand from the sites of lower affinity (Watson–Crick base pairs) to the single binding site of higher affinity (mismatched base pair).[21]

This conclusion was confirmed by the results of spectropolarimetric titrations, which were performed in an inverse manner, that is, aliquots of the ligand **2** were added to the solutions of duplexes **I-TA** and **I-TC** (Figure 2). In the former case, a monotonous increase of the induced circular-dichroism (ICD) signal of **2** was observed, characterized by sharp positive bands. Although a precise qualitative analysis of the ICD signals is not possible due to excitonic interactions between two identical anthracene chromophores in the molecule of **2**, the large amplitude of the ICD signals may be an evidence of the binding mode, in which at least one anthracene residue is placed in the groove of DNA. Indeed,

  

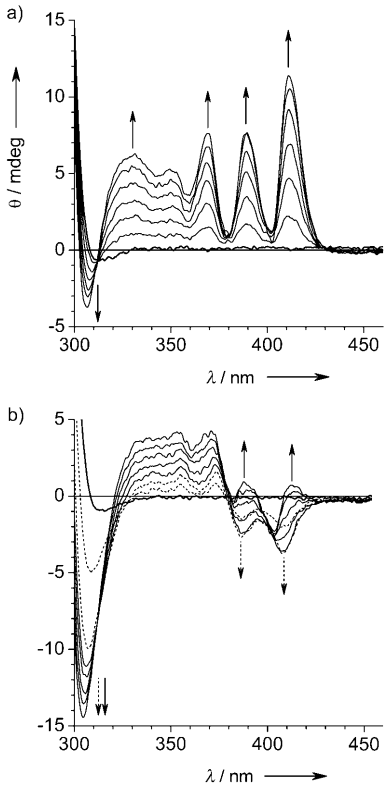


Figure 2. Induced CD spectra of **2** upon its titration to solutions of duplexes of a) **I-TA** and b) **I-TC** (50 μM in sodium cacodylate buffer). a) $c(\mathbf{2})=25$–$150$ μM; b) $c(\mathbf{2})=25$–$50$ μM (dashed curves); $c(\mathbf{2})=75$–$175$ μM (solid gray curves). The solid dark curves represent CD spectra of duplexes in the absence of the ligand.

the chromophores that are bound in the grooves of double-stranded DNA give rise to strong positive ICD signals, whereas the ones intercalated between base pairs may give weak negative or positive ICD signals, depending on the mutual orientation of the transition dipole moments of the chromophore and the neighboring base pairs.[22] Moreover, the monotonous increase of the ICD signals in the case of **I-TA** during whole course of titration ($0<q\leq3$) gives evidence that one type of complex is formed with matched DNA.

In the case of mismatch-containing duplex **I-TC**, however, the CD spectroscopy revealed a significantly different behavior. Thus, as the concentration of the macrocycle **2** was increased up to $q=1$, weak negative ICD signals were detected (dashed curves in Figure 2b). However, as further amounts of the ligand were added ($1<q\leq3.5$), the intensity of the negative ICD signals decreased and new positive bands were detected, with maxima at wavelength corresponding to the signals, which were observed in the presence of **I-TA**. This behavior may be explained by the binding of additional amounts of **2** to the matched base pairs in **I-TC** and superposition of the resulting ICD signals.

Even more drastic changes were observed in the fluorescence properties of the ligand **2** upon its binding to fully matched and mismatch-containing duplexes. In aqueous solutions in the absence of DNA the macrocycle **2** displays a yellow-green emission with a broad band centered at $\lambda\approx480$ nm, which corresponds to the intramolecular excimer emission of the anthracene chromophores held in a close proximity by the macrocyclic framework (Figure 3, red

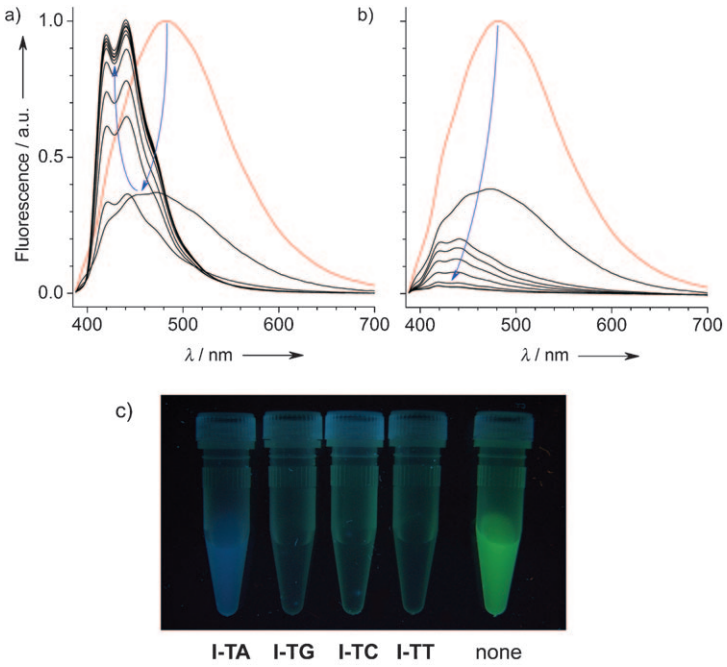


Figure 3. Spectrofluorimetric titrations of duplexes of a) **I-TA** and b) **I-TC** (0–6 μM) to solutions of macrocycle **2** (5 μM in sodium cacodylate buffer, excitation wavelength $\lambda_{ex}=385$ nm). The red curves correspond to the fluorescence spectrum of **2** in the absence of DNA; the arrows represent the changes in the spectra in the course of titrations. c) Image of solutions of **2** in the presence of indicated duplexes (5 μM each) and without DNA under UV illumination ($\lambda=312$ nm).

curves).[17] Upon addition of the matched duplex **I-TA**, the emission of the excimer form was reduced and a new structured emission band with two maxima at $\lambda=419$ and 440 nm was detected (Figure 3a). This blue-shifted emission band, with an integral emission intensity of about 50% of that of the initial excimer band, may be attributed to the fluorescence of monomeric anthracene chromophore.[17,23] This may suggest that, in the case of duplex **I-TA**, one anthracene moiety of the ligand intercalates into the base stack of DNA, which reduces its interaction with the second moiety, and is in agreement with ICD results, which show that one anthracene moiety is placed in the groove. However, when the mismatch-containing duplex **I-TC** was added to a solution of **2**, the emission of the excimer form was quenched without simultaneous increase of the monomer fluorescence (Figure 3b). Thus, upon addition of one equivalent of **I-TC**, the fluorescence of **2** was quenched about 80-fold, as determined by the integration of the fluorescence signals. Essentially the same behavior, that is, efficient quenching of fluorescence, was observed in the case of mismatch-containing duplexes **I-TT** and **I-TG** (Supporting Information, Figure S2). Most remarkably, this drastic difference in fluorescence properties of macrocycle **2** upon interaction with fully

  

matched (**I-TA**) and mismatch-containing (**I-TC**, **I-TT**, **I-TG**) duplexes allows a "naked-eye" discrimination between them (Figure 3c).

In a further experiment, the changes of fluorescence properties of macrocycle **2** upon interaction with all 16 base-pair combinations of the duplexes **I-YX** were determined. These measurements were performed with equimolar concentrations of the ligand and duplexes ($q=1$). In all cases, the emission of the excimer form of the macrocycle was efficiently quenched; however, the intensity of the monomer band was dependent on the base-pair combination of the duplex (Figure 4a and Supporting Information, Figure S3). Thus, the fluorescence emission was almost completely quenched (80–100-fold, that is, down to about 1% of the integral emission of the excimer form in the absence of DNA) upon interaction of **2** with thymine-containing mismatched duplexes **I-TT**, **I-TC** and **I-CT**, as well as **I-TG** and **I-GT**. At the same time, bright monomer emission (40–50% of the intensity of initial excimer emission) was detected in the case of matched duplexes **I-TA** and **I-AT**, as well as in the case of duplexes in which the central adenine was mispaired with adenine or cytosine (**I-AA** and **I-AC/CA**). In the case of duplexes **I-AG/GA**, as well as the matched duplex **I-CG**, moderate monomer emission was observed (~20%). Notably, a pronounced difference between fully matched duplexes **I-CG** and **I-GC** was observed, because the latter quenched the fluorescence of **2** down to 5% of its initial emission intensity; the effect of the other base-pair combinations was much less sensitive to the polarity of the strands (e.g. AC≈CA, etc).

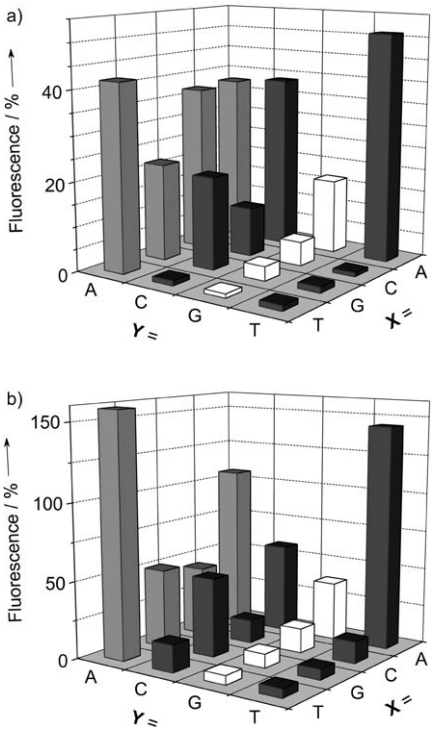


Figure 4. Integral fluorescence intensity of macrocycle **2** (5 μM) in the presence of one equivalent of duplexes of a) **I-YX** and b) **II-YX**, normalized to the integral fluorescence intensity of **2** in the absence of DNA.

Altogether, these results demonstrate that the fluorescence of the macrocycle **2** is efficiently quenched by the base pairs in which the thymine residue is mispaired, and switched to the monomer emission by the adenine-containing Watson–Crick and mismatched base pairs (AT/TA > AA≈AC/CA > AG/GA), allowing an easy fluorimetric discrimination between them. The effect of the other base-pair combinations was ambiguous.

We expected that the effect of the mismatched base pairs on the binding and fluorescence properties of the ligand might depend on the neighboring base pairs, since the stability of the matched and mismatched base pairs, which is usually reversibly proportional to their affinity to external ligands, depends on their sequence context.[24] Moreover, the extent of the fluorescence quenching may be influenced by the bases neighboring the binding site; for example, it may be expected that guanine residues in the proximity of the bound ligand would efficiently quench its fluorescence by an electron-transfer process, since quenching of fluorescence of anthracene derivatives by the guanine bases in the DNA is well-documented.[23] To investigate the influence of the sequence context on the DNA-recognition properties of the macrocycle **2**, we used a set of 16 oligonucleotides **II-YX**, in which the central base pair **YX** was placed in a triplet other than the one used in the previous experiments (5′-A**Y**A-3′/5′-T**X**T-3′ in **II-YX** versus 5′-G**Y**A-3′/5′-T**X**C-3′ in **I-YX**), while the rest of the nucleotide sequence was conserved. The changes of fluorescence properties of the probe **2** upon interaction with these duplexes (Figure 4b) were similar to the ones observed with the duplexes **I-YX**: in all cases the emission of the excimer form disappeared in the presence of DNA. A bright monomer emission was observed in the presence of the fully matched duplexes **II-AT** and **II-TA**, which, in this case, was more intense than in the case of **I-AT** and **I-TA** (~150% of the fluorescence intensity in the absence of DNA). The duplexes with mismatched adenine bases were better discriminated from **II-AT/TA** than within the first duplex set (110% of initial fluorescence intensity for **II-AA** and ~40–60% for **II-CA/AC** and **II-GA/AG**). Most importantly, the duplexes with mismatched thymine residues quenched the fluorescence of **2**, albeit less efficiently than in the case of **I-TX** (~5% residual fluorescence for **II-TT** and **II-TG/GT** and ~15% for **II-TC/CT**). Generally, brighter residual fluorescence was observed in the case of oligonucleotides **II-YX**, which gives evidence that the quenching of fluorescence of bound macrocycle **2** is in part due to the neighboring guanine residue (absent in the duplexes **II-YX**). However, the distal guanines also quench the fluorescence of the bound probe, though less efficiently, presumably by electron migration to a photoexcited anthracene residue through the base stack.

In conclusion, we have demonstrated that the macrocyclic anthracene derivative **2** binds to matched and mismatched base pairs in DNA by distinct binding modes that may be differentiated by UV/Vis and CD spectroscopy. Moreover,

  

when the mismatched base is thymine, binding of the probe **2** results in a quasi-complete quenching of its fluorescence, which allows an easy differentiation from the sequences in which the middle thymine is matched with adenine. Thus, the probe **2** is able to signal the presence of a single mismatched thymine residue in the duplexes that contain 16 other AT and GC base pairs. The selective recognition of thymine-containing mismatches is relevant in the context of SNP detection, since thymine is involved in 49% of point mutations in human genome, with the C–T transition representing about 33% of all substitutions.[25] Although the use of the anthracene fluorophore in the design of base-discriminating oligonucleotide probes has been described,[6a,e] this is one of the first examples of a non-covalently bound (external) probe which allows a simple "mix-and-measure" method for detection of mismatched base pairs in DNA.

## Acknowledgements

Generous financial support by the Centre National de la Recherche Scientifique is gratefully acknowledged.

**Keywords:** anthracenes · DNA mismatches · DNA recognition · fluorescence · macrocycles

[1] a) A.-C. Syvänen, *Nat. Rev. Genet.* **2001**, *2*, 930–942; b) R. M. Twyman, *Curr. Top. Med. Chem.* **2004**, *4*, 1423–1431; c) X. Chen, P. F. Sullivan, *Pharmacogenomics J.* **2003**, *3*, 77–96.

[2] a) P. Y. Kwok, *Annu. Rev. Genomics Hum. Genet.* **2001**, *2*, 235–258; b) B. W. Kirk, M. Feinsod, R. Favis, R. M. Kliman, F. Barany, *Nucleic Acids Res.* **2002**, *30*, 3295–3311; c) K. Nakatani, *ChemBioChem* **2004**, *5*, 1623–1633; d) B. Sobrino, M. Brion, A. Carracedo, *Forensic Sci. Int.* **2005**, *154*, 181–194; e) M. Strerath, A. Marx, *Angew. Chem.* **2005**, *117*, 8052–8060; *Angew. Chem. Int. Ed.* **2005**, *44*, 7842–7849.

[3] R. T. Ranasinghe, T. Brown, *Chem. Commun.* **2005**, 5487–5502.

[4] a) T. S. Kumar, J. Wengel, P. J. Hrdlicka, *ChemBioChem* **2007**, *8*, 1122–1125; b) E. V. Bichenkova, X. Yu, P. Bhadra, H. Heissigerova, S. J. A. Pope, B. J. Coe, S. Faulkner, K. T. Douglas, *Inorg. Chem.* **2005**, *44*, 4112–4114; c) E. V. Bichenkova, H. E. Savage, A. R. Sardarian, K. T. Douglas, *Biochem. Biophys. Res. Commun.* **2005**, *332*, 956–964; d) A. Yamane, *Nucleic Acids Res.* **2002**, *30*, e97; e) L. Valis, N. Amann, H. A. Wagenknecht, *Org. Biomol. Chem.* **2005**, *3*, 36–38; f) U. Asseline, M. Chassignol, Y. Aubert, V. Roig, *Org. Biomol. Chem.* **2006**, *4*, 1949–1957.

[5] a) A. Okamoto, Y. Saito, I. Saito, *J. Photochem. Photobiol. C* **2005**, *6*, 108–122; b) I. Saito, Y. Saito, K. Hanawa, K. Hayashi, K. Motegi, S. S. Bag, C. Dohno, T. Ichiba, K. Tainaka, A. Okamoto, *Pure Appl. Chem.* **2006**, *78*, 2305–2312.

[6] For recent examples of SNP detection with base-discriminating probes, see: a) N. Moran, D. M. Bassani, J. P. Desvergne, S. Keiper, P. A. S. Lowden, J. S. Vyle, J. H. R. Tucker, *Chem. Commun.* **2006**, 5003–5005; b) Y. Saito, S. S. Bag, Y. Kusakabe, C. Nagai, K. Matsumoto, E. Mizuno, S. Kodate, I. Suzuka, I. Saito, *Chem. Commun.* **2007**, 2133–2135; c) A. Okamoto, K. Tainaka, Y. Ochi, K. Kanatani, I. Saito, *Mol. BioSyst.* **2006**, *2*, 122–126; d) K. Tainaka, K. Tanaka, S. Ikeda, K. Nishiza, T. Unzai, Y. Fujiwara, I. Saito, A. Okamoto, *J. Am. Chem. Soc.* **2007**, *129*, 4776–4784; e) Y. Saito, K. Motegi, S. S. Bag, I. Saito, *Bioorg. Med. Chem.* **2008**, *16*, 107–113.

[7] a) V. N. Kristensen, D. Kelefiotis, T. Kristensen, A. L. Borresen-Dale, *BioTechniques* **2001**, *30*, 318–332; b) A. J. Nataraj, I. Olivos-Glander, N. Kusukawa, W. E. Highsmith, *Electrophoresis* **1999**, *20*, 1177–1185.

[8] a) B. A. Jackson, J. K. Barton, *Biochemistry* **2000**, *39*, 6176–6182; b) H. Junicke, J. R. Hart, J. Kisko, O. Glebov, I. R. Kirsch, J. K. Barton, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 3737–3742; c) J. R. Hart, M. D. Johnson, J. K. Barton, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 14040–14044.

[9] a) K. Nakatani, S. Sando, I. Saito, *Nat. Biotechnol.* **2001**, *19*, 51–55; b) A. Kobori, S. Horie, H. Suda, I. Saito, K. Nakatani, *J. Am. Chem. Soc.* **2004**, *126*, 557–562; c) S. Hagihara, H. Kumasawa, Y. Goto, G. Hayashi, A. Kobori, I. Saito, K. Nakatani, *Nucleic Acids Res.* **2004**, *32*, 278–286.

[10] E. R. Lacy, K. K. Cox, W. D. Wilson, M. Lee, *Nucleic Acids Res.* **2002**, *30*, 1834–1841.

[11] X. Li, H. Song, K. Nakatani, H. B. Kraatz, *Anal. Chem.* **2007**, *79*, 2552–2555.

[12] B. M. Zeglis, J. K. Barton, *J. Am. Chem. Soc.* **2006**, *128*, 5654–5655.

[13] F. Takei, H. Suda, M. Hagihara, J. Zhang, A. Kobori, K. Nakatani, *Chem. Eur. J.* **2007**, *13*, 4452–4457.

[14] a) A. David, N. Bleimling, C. Beuck, J. M. Lehn, E. Weinhold, M.-P. Teulade-Fichou, *ChemBioChem* **2003**, *4*, 1326–1331; b) M. Bahr, V. Gabelica, A. Granzhan, E. Weinhold, M.-P. Teulade-Fichou, *Nucleic Acids Res.* **2008**, *36*, 5000–5012.

[15] a) R. J. Roberts, X. D. Cheng, *Annu. Rev. Biochem.* **1998**, *67*, 181–198; b) U. D. Priyakumar, A. D. MacKerell, Jr, *Chem. Rev.* **2006**, *106*, 489–505.

[16] A. Slama-Schwok, M. P. Teulade-Fichou, J. P. Vigneron, E. Taillandier, J. M. Lehn, *J. Am. Chem. Soc.* **1995**, *117*, 6822–6830.

[17] See for example, : a) F. Fages, J.-P. Desvergne, K. Kampke, H. Bouas-Laurent, J.-M. Lehn, M. Meyer, A.-M. Albrecht-Gary, *J. Am. Chem. Soc.* **1993**, *115*, 3658–3664; b) G. Nishimura, H. Maehara, Y. Shiraishi, T. Hirai, *Chem. Eur. J.* **2008**, *14*, 259–271.

[18] Synthesis of an analogue of **2** with diethylenetriamine linking chains has been described: L. Fabbrizzi, M. Licchelli, N. Marcotte, F. Stomeo, A. Taglietti, *Supramol. Chem.* **2002**, *14*, 127–132.

[19] These duplexes were used in our previous studies (ref. [14]) and include a central four base-pair recognition sequence of the M.TaqI enzyme.

[20] The values of the binding constants could not be determined from this experiments due to relatively high concentrations, which were necessary to obtain reliable data on the stoichiometry; however, a binding constant of $K = 2.5 \times 10^6\,\text{M}^{-1}$ (bp) to calf thymus DNA was determined (Supporting Information, Figure S1).

[21] In both cases an increase of absorption in the long-wavelength region of the spectrum, ($\lambda > 440$ nm), was observed at high ligand-to-duplex loadings ($q > 5$), which disappeared as further amounts of DNA were added. We attribute this behavior to formation of aggregates of **2** on the exterior of the duplexes, which results in enhanced scattering, but does not lead to precipitation of the ligand–DNA complexes.

[22] a) M. Eriksson, B. Nordén, *Methods Enzymol.* **2001**, *340*, 68–98; b) B. Nordén, T. Kurucsev, *J. Mol. Recognit.* **1994**, *7*, 141–156; c) N. C. Garbett, P. A. Ragazzon, J. B. Chaires, *Nat. Protoc.* **2007**, *2*, 3166–3172.

[23] a) C. V. Kumar, E. H. Asuncion, *J. Am. Chem. Soc.* **1993**, *115*, 8547-8553; b) A. Rodger, S. Taylor, G. Adlam, I. S. Blagbrough, I. S. Haworth, *Bioorg. Med. Chem.* **1995**, *3*, 861–872; c) C. V. Kumar, E. H. A. Punzalan, W. B. Tan, *Tetrahedron* **2000**, *56*, 7027–7040.

[24] a) N. Peyret, P. A. Seneviratne, H. T. Allawi, J. SantaLucia, Jr., *Biochemistry* **1999**, *38*, 3468–3477; b) A. Tikhomirova, I. V. Beletskaya, T. V. Chalikian, *Biochemistry* **2006**, *45*, 10563–10571.

[25] a) F. Zhang, Z. Zhao, *Genomics* **2004**, *84*, 785–795; b) Y. Guo, D. C. Jamison, *BMC Genomics* **2005**, *6*, 140.

Received: November 20, 2008
Published online: December 29, 2008

  