CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.200901989

Macrocyclic DNA-Mismatch-Binding Ligands: Structural Determinants of Selectivity

Anton Granzhan, Eric Largy, Nicolas Saettel, and Marie-Paule Teulade-Fichou*[a]

Abstract: A collection of 15 homodimeric and 5 heterodimeric macrocyclic bisintercalators was prepared by one- or two-step condensation of aromatic dialdehydes with aliphatic diamines; notably, the heterodimeric scaffolds were synthesized for the first time. The binding of these macrocycles to DNA duplexes containing a mispaired thymine residue (TX), as well as to the fully paired control (TA), was investigated by thermal denaturation and fluorescent-intercalator-displacement experiments. The bisnaphthalene derivatives, in particular, the 2,7-disubstituted ones, have the highest selectivity for the TX mismatches, as these macrocycles show no apparent binding to the fully paired DNA. By contrast, other macrocyclic ligands, as well as seven conventional DNA binders, show lesser or no selectivity for the mismatch sites. The study demonstrates that the topology of the ligands plays a crucial role in determining the mismatch-binding affinity and selectivity of the macrocyclic bisintercalators.

Keywords: intercalations · bisintercalators · DNA mismatches · DNA recognition · macrocycles

Introduction

Mismatched base pairs in DNA represent defects in the double-helical DNA structure that are permanently formed in living cells due to statistical errors in the course of DNA replication (1 per 10^9–10^{10} base pairs per cell division). They may also be generated by incorporation of chemically damaged nucleotides or by incorporation of normal nucleotides opposite damaged bases in the DNA template.[1] The rate of generation of DNA mismatches can be greatly increased by exogenous factors, such as genotoxic chemicals or UV radiation.[2] If not repaired, mismatched base pairs lead to an enhanced frequency of mutations (50–1000-fold increase in organisms with a genetically inactivated mismatch-repair system), which leads to an accumulation of mutations and is harmful for the organism. Therefore, each organism possesses an ensemble of systems responsible for the maintenance of genomic integrity. In particular, the mismatch-repair (MMR) system, consisting of many proteins, recognizes and repairs the mismatched base pairs in the DNA. Notably, many cancers, including hereditary nonpolyposis colon cancers, are associated with deficient mismatch repair;[3] in turn, the inhibition of repair is currently investigated as a novel approach to potentiate the effect of anticancer drugs.[4] Thus, molecules that specifically bind to mismatched base pairs in DNA may interfere with mismatch-recognizing machineries and provide a basis for novel chemotherapeutic agents.[5] Indeed, it was shown that mismatch-selective rhodium complexes preferentially inhibit proliferation of mismatch-repair-deficient cell lines, compared to those lines which are MMR proficient.[6] Therefore, the search for other types of small molecules that selectively recognize mismatched base pairs in DNA is currently a challenging task.

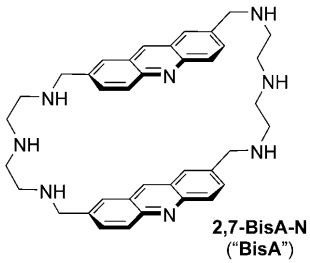
Currently, only a limited number of compounds that selectively bind to DNA mismatches and not to the fully paired DNA are known. The most well-studied examples are represented by the aforementioned metalloinsertors.[6,7] The bulkiness of these cationic metal complexes hinders their association with fully matched DNA and provides them with selectivity towards mismatched base pairs, particularly cytosine-containing mismatches,[8] and other pairing defects, such as abasic sites and single-base bulges,[9] by taking advantage of the local thermodynamic destabilization of such defects relative to the Watson–Crick base pairs. On the other hand, further increase of the steric bulk of metalloinsertors by introduction of π-extended aromatic ligands decreases the selectivity towards mismatches because the resulting complexes also bind to the well-matched sites, which poses a

[a] Dr. A. Granzhan, E. Largy, Dr. N. Saettel, Dr. M.-P. Teulade-Fichou
UMR176 CNRS, Institut Curie, Centre de Recherche
Centre Universitaire, 91405 Orsay (France)
Fax: (+33) 169-07-53-81
E-mail: marie-paule.teulade-fichou@curie.fr

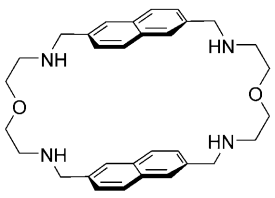
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.200901989.

possible limitation of this class.[10] Another important class of DNA-mismatch binders is represented by the homo- and heterodimeric derivatives of naphthyridine, which selectively bind to GG, AG, or CC mismatches by intercalation of the naphthyridine units into the base stack and formation of complementary hydrogen bonds with the guanine, adenine, or cytosine residues, respectively.[11–13] These compounds were also used to detect mismatched base pairs in heteroduplex DNA by analytical methods, such as surface plasmon resonance[12–14] or affinity chromatography.[15] This class of mismatch binders is very interesting due to the high fidelity of the hydrogen-bonding-mediated recognition of both mismatched bases; however, studies of the medicinal activity of these compounds remain preliminary.[16] A third class of well-studied mismatch binders is the imidazole-containing polyamides, which selectively bind to TG-mismatched base pairs by recognition of the hydrogen-bonding pattern of the bases in the minor groove. The structural and thermodynamic parameters of this binding were thoroughly determined.[17] This class of agents is important due to the high biological significance of the TG mismatch,[18] which also represents a particularly hard target for recognition by small molecules because it is only slightly less stable than the Watson–Crick base pairs.[19]

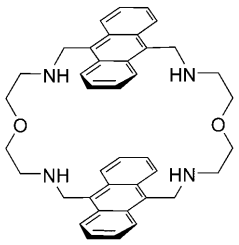
Over the past few years, we have shown that a macrocyclic bisacridine compound (**BisA**, Scheme 1) recognizes



2,7-BisA-N
("**BisA**")



2,6-BisNP



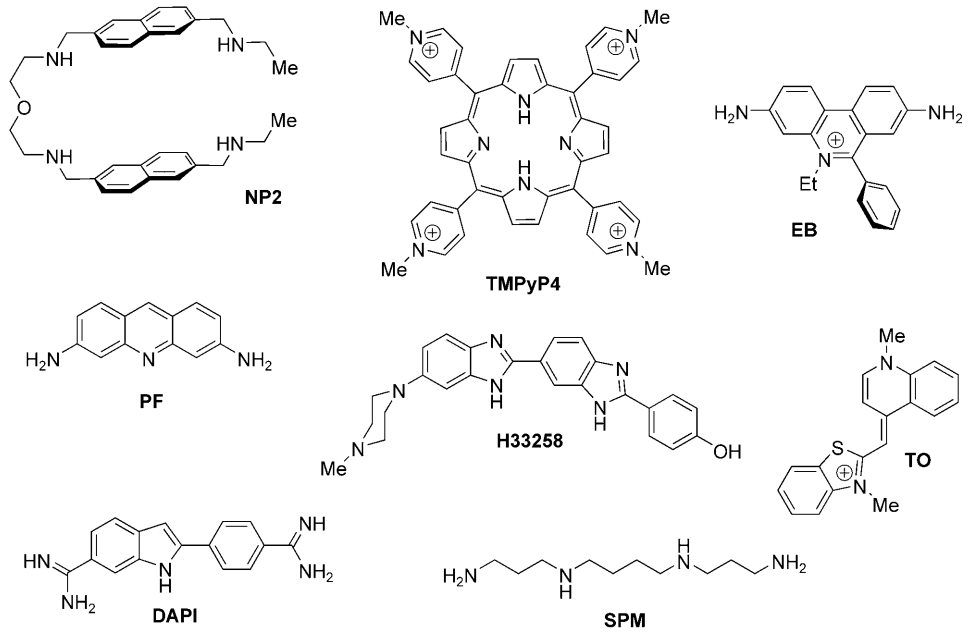
9,10-BisAN

Scheme 1. Mismatch-binding macrocycles identified in previous studies. Presumably, all three compounds are protonated at the four benzylamino groups at pH 6–7.[21,25,26]

base-pairing defects, like abasic sites[20] and thymine-containing mismatches,[21] through a putative threading bisintercalation mode. Moreover, insertion of **BisA** at TX-mismatch sites induces a displacement ("flipping") of the thymine into an extrahelical position. This represented the first example of a small molecule flipping the mismatched base out of the DNA base stack, in a manner similar to that of DNA methyltransferases and DNA glycosylases.[22] Subsequently, several studies have shown that other mismatch binders, such as naphthyridine dimers[23] and bulky rhodium-based metalloinsertors,[8b,24] are also able to displace bases into extrahelical positions. In a more recent study, we have shown that a bisnaphthalene macrocyclic compound, **2,6-BisNP**, which is structurally related to **BisA**, binds to TX mismatches with even higher affinity and selectivity than the latter.[25] Conversely, we observed that an analogous macrocycle containing anthracene residues, **9,10-BisAN**, is much less selective towards TX-mismatched duplexes and also binds to the fully paired duplexes. However, this occurs through different binding modes, as shown by the drastic differences in the fluorescence properties of the bound compound, which allows a "naked-eye" discrimination between the mismatched and fully paired duplexes.[26] Subsequently, it was found that a ruthenium-based DNA metallointercalator also binds to well-matched and mismatched base pairs by different binding modes, with an impact on its luminescence properties.[27]

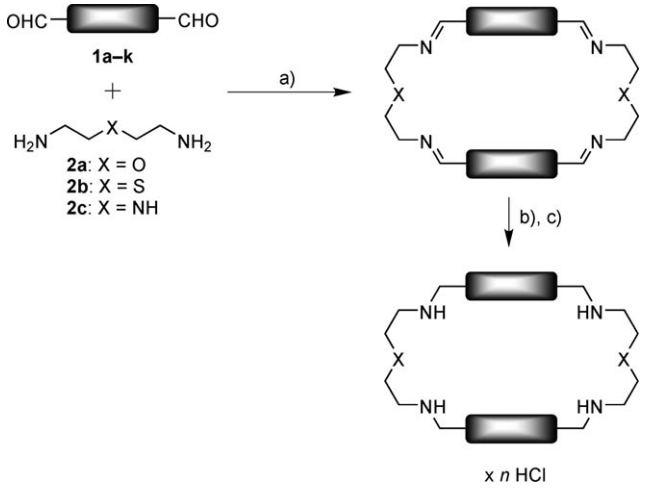
These studies led us to conclude that both the aromatic units and the connectivity between them are structural determinants for the selective recognition of mismatched sites. Consequently, with the aim of establishing comprehensive structure–binding relationships and further optimization of the mismatch-binding ligands in terms of affinity and selectivity towards TX mismatches, we prepared an extended series of macrocyclic compounds, containing two identical (homodimers) or different (heterodimers) aromatic units, connected by linking chains of the same length. Herein, we report the synthesis of these compounds, including heterodimers that represent rare examples in the chemistry of macrocycles, as well as a study of their interaction with double-stranded oligonucleotides containing one TX-mismatched site. The DNA-binding affinities and selectivities towards mismatches were determined by means of thermal-denaturation experiments and, for the most representative macrocycles, fluorescent-intercalator-displacement (FID) titration experiments were performed to confirm the binding characteristics.

To firmly establish that the macrocyclic scaffold is necessary for selective mismatch binding and to estimate the contribution of nonspecific binding, we included in our study a number of well-known "classical" DNA binders, such as compounds that bind by the prevailing intercalative mode: ethidium bromide (**EB**, Scheme 2),[28–30] proflavine hemisulfate (**PF**),[31] and thiazole orange tosylate (**TO**),[32,33] as well as representative DNA-minor-groove binders, namely the bisbenzimidazole derivative Hoechst 33258 trihydrochloride (**H33258**)[29a,34] and 4′,6-diamidino-2-phenylindole dihydro-



Scheme 2. Nonmacrocyclic DNA binders used in this study. Counterions and protonation sites are omitted for clarity.

chloride (**DAPI**).[35] Our interest in these compounds was, in part, stimulated by the recent reports on **H33258** binding to bulged and mismatched RNA,[36,37] as well as to DNA duplexes containing TG-mismatched base pairs.[38] Moreover, binding of **DAPI** to a DNA duplex containing a TT mismatch was demonstrated by NMR spectroscopy.[39] At the same time, it was claimed that **EB** does not bind to short duplexes containing TG mismatches.[38] We also included the tetracationic porphyrin *meso*-tetrakis(4-*N*-methylpyridinium)porphyrin tetratosylate (**TMPyP4**), which binds to duplex DNA either by intercalation between GC base pairs or the grooves of the AT-rich sequences[40] and whose Cu^{II} complex is able to flip the nucleic acid bases out of well-matched DNA.[41] Additionally, we included a naturally occurring tetraamine spermine (**SPM**), which binds to double-stranded DNA without sequence or base selectivity through electrostatic interactions, mainly in the major groove,[42] as well as the bisnaphthalene derivative **NP2**, which represents an open-chain analogue of **2,6-BisNP**.



Scheme 3. Synthesis of homodimeric macrocycles. For assignment of the aromatic units, see Table 1. Reagents and conditions: a) MeCN, RT, 5–7 days; b) $NaBH_4$, CH_2Cl_2/MeOH, RT, 3 h; c) HCl, MeOH or EtOH, 20–90% over 3 steps.

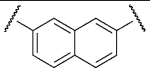
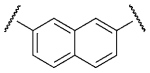
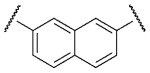
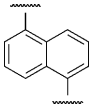
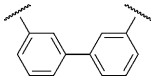
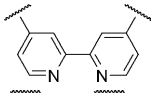
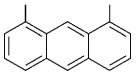
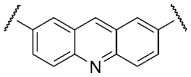
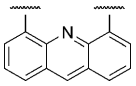
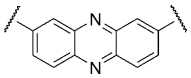
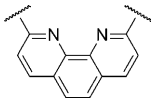
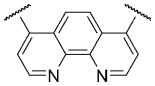
Results

Synthesis of macrocyclic ligands and reference compounds

Synthesis of homodimeric macrocycles: These macrocycles were obtained by a straightforward [2+2]-type cyclocondensation of aromatic dialdehydes **1a–k** with aliphatic diamines **2a–c** (Scheme 3, Table 1).[43] In a similar manner to the previously reported synthesis of macrocycles **2,7-BisA-N**, **2,6-BisNP**, and **9,10-BisAN** (Scheme 1),[26,44] the cyclocondensation was performed in acetonitrile under moderate to high dilution conditions. In this way, the tetraimine intermediates precipitated directly from the mixture (average purity >90%, as determined by ^{1}H NMR spectroscopy) and were thus used without further manipulation. In some cases, these intermediate tetraimines were barely soluble or underwent decomposition in common NMR solvents, which prevented their complete characterization. The reduction of the tetraimine intermediates with $NaBH_4$, followed by conversion of the macrocyclic amines into hydrochloride salts and purification by recrystallization or chromatography (see the Supporting Information), gave the corresponding polyammonium macrocycles (Table 1), the structure and purity of which were confirmed by ^{1}H and ^{13}C NMR spectroscopy, LC–MS, and elemental analysis data. It should be noted that in several cases the NMR spectra of the macrocyclic polyamines displayed broad signals, which did not allow proper signal assignment, due to conformational restrictions imposed by the macrocyclic scaffold; in such cases, single peaks in the HPLC chromatograms and correct MS and elemental analysis data were used as a proof of identity and purity for the samples.

Synthesis of heterodimeric macrocycles: Our general approach to the synthesis of heterodimers containing two dif-

Table 1. Homodimeric macrocycles prepared according to Scheme 1.

Macrocycle	Aldehyde		X
2,7-BisNP	**1a**		O
2,7-BisNP-S	**1a**		S
2,7-BisNP-N	**1a**		NH
1,5-BisNP	**1b**		O
3,3′-BisBP	**1c**		O
4,4′-BisBPy	**1d**		O
1,8-BisAN	**1e**		O
2,7-BisA[a]	**1f**		O
4,5-BisA	**1g**		O
2,8-BisPZ	**1h**		O
2,9-BisPN[b]	**1i**		O
4,7-BisPN	**1j**		O

[a] Synthesis was described previously.[45] [b] Although the synthesis of this macrocycle was recently described,[46] we obtained a material with a composition ($C_{36}H_{40}N_8O_2 \cdot 6\,HCl \cdot 8\,H_2O$) that was slightly different from that given in the literature ($C_{36}H_{40}N_8O_2 \cdot 5\,HCl \cdot 3\,H_2O$).

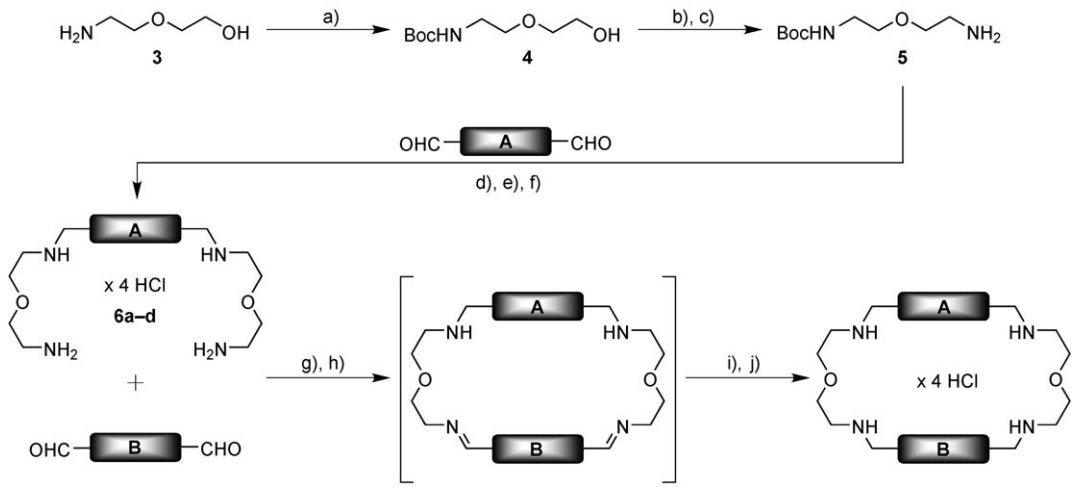
ferent aromatic residues **A** and **B** relies on fragment-to-fragment assembly from two aromatic aldehydes (Scheme 4, Table 2). Although a number of unsymmetrical macrocycles have been described,[43b] the heterodimeric-macrocycles of the type presented in Table 2 have yet to be reported. For the sake of clarity, macrocycles containing two identical units but linked at different positions of each aromatic ring were also included in the heterodimer category (Table 2).

For the synthesis of the heterodimeric macrocycles presented in Table 2, a common intermediate, monoprotected diamine **5**, was essential. Although the preparation of this compound by monoprotection of 2,2′-oxydiethylamine had been described,[47] the yield of the monoprotected diamine was only about 50% in our hands.[25] Taking into account the limited availability of 2,2′-oxydiethylamine, we developed an alternative preparation for amine **5** by starting from inexpensive 2-(2-aminoethoxy)ethanol (**3**; Scheme 4). After initial Boc protection of the amino group,[48] the hydroxy group of derivative **4** was converted into a primary amino group by a Mitsunobu reaction with phthalimide, followed by deprotection of the phthalimide group with hydrazine. This allowed us to obtain intermediate **5** in 81% yield from amino alcohol **3**. The reaction of **5** with selected aromatic dialdehydes (OHC–**A**–CHO; Scheme 4, Table 2) gave the corresponding tetraamine derivatives **6a–c**, which were isolated, characterized, and handled as hydrochloride salts. For the macrocyclization reaction, the salts were quantitatively converted into free bases by treatment with ion-exchange resin and were then treated with the second dialdehyde fragment (OHC–**B**–CHO), to give the amino-imino heterodimers contaminated by higher-order oligomers and products of polymerization, which rendered isolation and purification of these intermediates impossible. The crude intermediates were reduced with $NaBH_4$, and the desired macrocycles were isolated by flash chromatography, either directly or after conversion into lipophilic tetra-*N*-Boc-protected derivatives,[49] which after purification and deprotection with HCl gave the macrocycles as pure hydrochloride salts. The structure and purity of the products were confirmed by ^1H and ^{13}C NMR spectroscopy, LC–MS, and elemental analysis data.

Synthesis of NP2: The nonmacrocyclic analogue of macrocycle **2,6-BisNP**, the tetraamine derivative **NP2**, was synthesized by reductive amination with ethylamine of diformyl derivative **7**, which, in turn, was prepared in 8 steps from 6-bromo-2-naphthoic acid as described elsewhere (Scheme 5).[49]

DNA-mismatch-binding affinities and selectivities

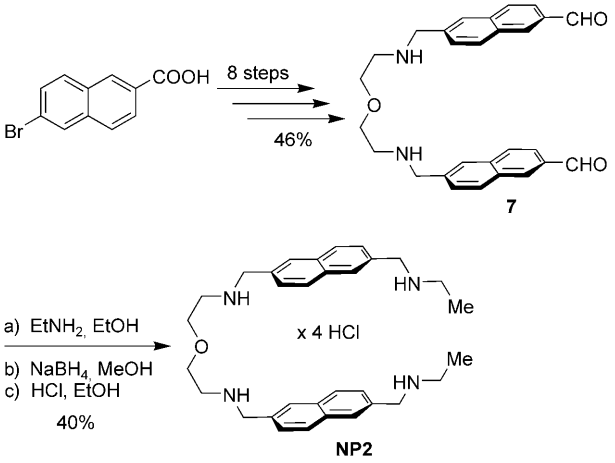
Thermal denaturation experiments: Thermal denaturation of DNA is a rapid and straightforward method for determination of the stabilization effect of ligands towards a given DNA structure. The extent of the ligand-induced stabilization provides a semiquantitative evaluation of the ligand affinity towards duplex DNA.[50] Base mismatches significantly reduce the thermodynamic stability of DNA duplexes, and the extent of this destabilization is dependent on the duplex length. As a consequence, the stabilization effect of mismatch-binding ligands becomes more pronounced under conditions of low duplex stability (low ionic strength, short duplexes). On the other hand, longer duplexes have sharper melting profiles, which allow more precise determination of melting temperatures, and offer more potential binding sites (intercalation between base pairs, groove and surface binding) to enable better determination of mismatch selectivity.[25] Hence, we chose to use 17-mer duplexes, which represent a good compromise, with a general sequence **TX** (5′-CCAG TTC G**T**A GTA ACCC-3′/5′-GGGT TAC T**X**C GAA CTGG-3′), containing either a matched (**TA**) or a mispaired (**TG**, **TC**, **TT**) thymine residue in the center of the middle triplet. The reference oligonucleotide (**TA**) was used in previous studies and corresponds to a biological se-



Scheme 4. Synthesis of heterodimeric macrocycles. Reagents and conditions: a) Boc_2O, CH_2Cl_2, RT, 18 h, 91%; b) phthalimide, PPh_3, DIAD, THF, RT, 18 h; c) N_2H_3OH, MeOH, reflux, 6 h, 89% over 2 steps; d) benzene, 18 h, reflux; e) $NaBH_4$, CH_2Cl_2/MeOH, RT, 3 h; f) HCl, EtOH or MeOH, 40–65 °C, 80–90% over 3 steps; g) Amberlite IRA-420 (OH^-), MeOH; h) MeCN, RT, 5–7 days; i) $NaBH_4$, CH_2Cl_2/MeOH, RT, 3 h; j) HCl, MeOH or EtOH, 20–60% over 4 steps. Boc: *tert*-butoxycarbonyl; DIAD: diisopropylazodicarboxylate; THF: tetrahydrofuran.

Table 2. Heterodimeric macrocycles prepared according to Scheme 2.

Macrocycle	Linear intermediate	A	B
1,5/2,6-BisNP	**6a**		
2,7-NP/9,10-AN	**6b**		
1,8/9,10-BisAN	**6b**		
2,7-NP/2,7-A	**6c**		
2,7-NP/1,1′-FC	**6d**		



Scheme 5. Synthesis of the reference compound **NP2**.

quence recognized by the DNA methyltransferase enzyme M.TaqI. Under the conditions employed (pH 6, $[Na^+]$ = 20 mM), the fully matched duplex **TA** has a melting temperature of 46.5 °C, whereas the mismatch-containing duplexes **TG**, **TT**, and **TC** denaturate at significantly lower temperatures (42.0, 38.4 and 36.6 °C, respectively). The ligand-induced changes in the melting temperatures (ΔT_m) at ligand-to-duplex ratios (q) of 1 and 2 are listed in Table S1 in the Supporting Information and are represented as bar graphs in Figures 1–4 to facilitate direct comparison between the various ligands. The value of the ligand-induced increase in the melting temperature of the duplex serves as a measure of the ligand binding affinity, whereas the difference in the values observed with one and two equivalents of ligand per duplex indicates whether saturation is reached with one equivalent of the ligand or not. Thus, ligands that occupy a single site with high affinity should show little or almost no increase in the ΔT_m value upon an increase in the ligand concentration, whereas ligands with lower affinity and/or several binding sites should show a larger increase in the ΔT_m value.

Nonmacrocyclic ("classical") DNA binders: The results of the thermal denaturation of oligonucleotides **TX** in the presence of "classical" DNA binders are presented in Figure 1. The intercalators **EB**, **TMPyP4**, and **PF** strongly bind to fully matched and mismatch-containing DNA duplexes, as indicated by the large ΔT_m values (5–7 °C at $q = 1$), without significant selectivity toward one of the four duplexes. Moreover, the large increase in the ΔT_m values upon an increase in the ligand concentration indicates that saturation of the potential site(s) is not reached at $q = 1$. The absence of specific stabilization of a particular duplex by ethidium

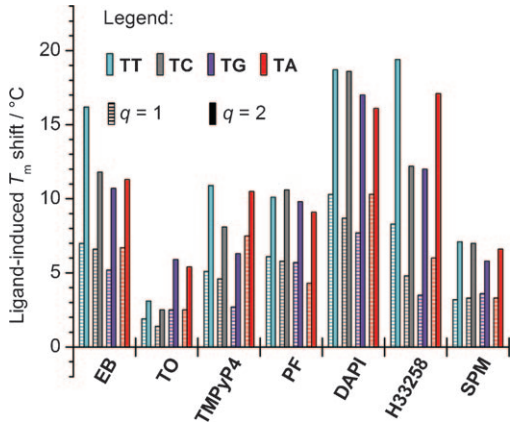


Figure 1. Results of thermal-denaturation experiments with duplexes **TX** ($c=6\,\mu M$ in cacodylate buffer, pH 6.0, $[Na^+]=20\,mM$) and nonmacrocyclic DNA binders at ligand-to-duplex ratios of $q=1$ and $q=2$. Estimated error in T_m determinations is $\pm 0.6\,°C$.

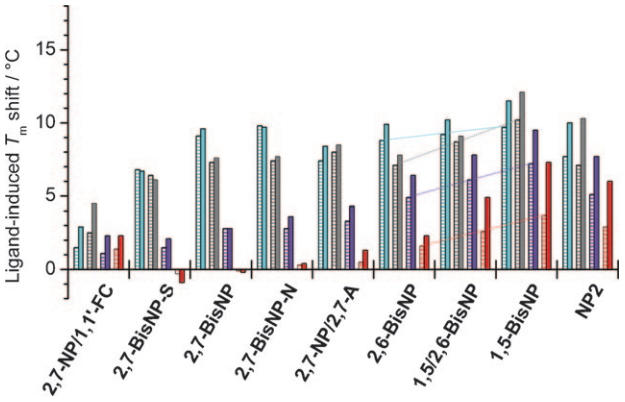
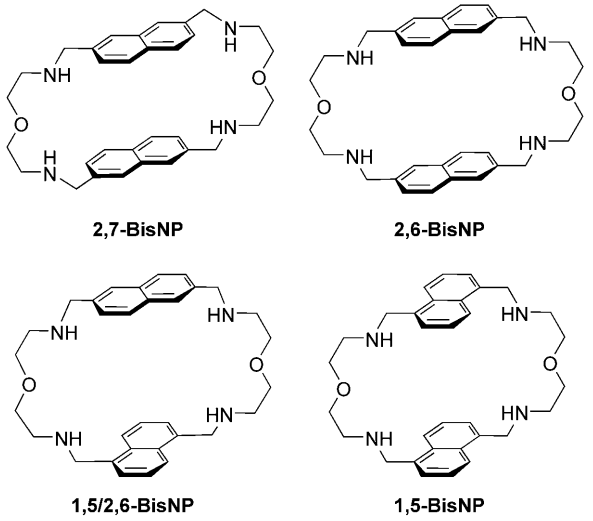


Figure 2. Results of thermal-denaturation experiments for the naphthalene derivatives. For the assignment of datasets and experimental details, see Figure 1.



Scheme 6. Schematic representation of the topology of the **BisNP** family of macrocycles.

bromide (**EB**), as observed by thermal denaturation experiments, is in agreement with the results of a recent independent study, which showed an absence of mismatch selectivity of **EB**.[27] Thiazole orange (**TO**) also induces thermal stabilization of all duplexes but to a smaller extent than the other intercalators, although it has been reported to have a higher affinity for duplex DNA than **EB**.[51]

The groove binders **DAPI** and **H33258** induce higher stabilization of all duplexes, without discrimination between matched and mismatched. The stabilization is particularly pronounced at a ligand per duplex ratio of $q=2$, and larger ΔT_m values are observed than those induced by intercalators. The absence of selective binding of these groove binders to mismatched duplexes, although expected, is in disagreement with studies reporting on binding of **DAPI** and **H33258** to mismatch-containing DNA duplexes.[38,39] Finally, the polyamine **SPM** also stabilizes all four duplexes but to a smaller extent than all of these minor-groove binders and intercalators, with the exception of **TO**.

Naphthalene derivatives: We previously identified the bisnaphthalene macrocycle **2,6-BisNP** as a promising mismatch-selective agent.[25] In the current work, we prepared and investigated an extended series of homo- and heterodimeric naphthalene derivatives, to establish the relationship between the structure of the macrocycle (substitution pattern of the naphthalene units, heteroatoms in the linking chains) and the mismatch-binding characteristics. The results of the duplex-DNA stabilization induced by the novel naphthalene derivatives (Figure 2, Scheme 6) show that all of the bisnaphthalene macrocycles (**2,6-** and **1,5-BisNP**; **2,7-BisNP**, **-S**, and **-N**; **1,5/2,6-BisNP**), the naphthalene–acridine heterodimer **2,7-NP/2,7-A**, and even the nonmacrocyclic bisnaphthalene **NP2** preferentially bind to the mismatch-containing duplexes, as indicated by the larger ΔT_m values with these than with the matched duplex ($\Delta T_m(TX) > \Delta T_m(TA)$). Interestingly, almost the same ΔT_m values are observed for the less stable **TT**-mismatched duplex with most of the compounds ($\Delta T_m(TT) \approx 9$–$11\,°C$); this result is indicative of similar binding affinities. On the other hand, the degree of selectivity, as represented by the difference in the thermal stabilization of well-matched (**TA**) and mismatch-containing duplexes, is strongly influenced by the structure of the ligands. Thus, no stabilization of the **TA** duplex ($\Delta T_m(TA) \leq 0$) is observed with macrocycles that have the 2,7-connectivity of the naphthalene units (**2,7-BisNP**, **-S**, and **-N**). Moreover, in these cases, the binding to the mismatch-containing duplexes is readily saturated at a 1:1 ligand-to-DNA ratio. Among the three derivatives, the **2,7-BisNP-S** shows a lower effect ($\Delta T_m(TT) \approx \Delta T_m(TC) \approx 6$–$7\,°C$). Thus, the presence of a sulfur atom in the linker does not afford any significant advantage; on the contrary, it has a penalty in terms of affinity.

Notably, all of the 2,7-substituted derivatives are more selective than the prototypal macrocycle with the 2,6 connectivity, that is, **2,6-BisNP**, which binds poorly but still significantly to the **TA** duplex ($\Delta T_m(TA) = 2$–$3\,°C$). Finally, the heterodimer **2,7-NP/2,7-A** also shows selective stabilization

of the mismatch-containing duplexes with a slight preference for the **TC** duplex, which may be related to the TC-mismatch selectivity of the bisacridine macrocycle **BisA** (Scheme 1).

Interestingly, macrocycle **1,5-BisNP**, although structurally very close to the 2,7 and 2,6 isomers, is much less selective for the mismatched duplexes, as indicated by the significant stabilization of the matched duplex, particularly pronounced with two equivalents of ligand (ΔT_m(TA)$\approx$7 °C at $q=2$). This provides evidence that this macrocycle, unlike the 2,7 and 2,6 isomers, exhibits a strong tendency to bind to fully paired DNA. It is noteworthy that the heterodimer **1,5/2,6-BisNP** exhibits a behavior that is intermediate between those of the "parent" homodimers **2,6-BisNP** and **1,5-BisNP** (indicated by thin lines in Figure 2), both in terms of affinity and selectivity. This observation may establish the "rule-of-thumb" that the DNA-binding properties of heterodimeric macrocycles may be estimated by knowing the properties of the homodimeric parents.

A similar decrease of the mismatch selectivity is observed in the case of the nonmacrocyclic bisnaphthalene derivative **NP2**, because this compound induces pronounced stabilization of the matched duplex (ΔT_m(TA)$\approx$6 °C at $q=2$). Comparison of this to the values obtained with its macrocyclic analogue **2,6-BisNP** allows us to confirm that the macrocyclic framework is indispensable for prevention of binding to the well-matched duplex. Finally, the heterodimer **2,7-NP/1,1′-FC**, which combines a naphthalene unit with a bulky ferrocene moiety, displays weak binding to all four duplexes. The weak DNA-binding affinity of this compound, which has the same overall tetracationic charge as the other macrocycles but a different shape, in combination with the absence of a preference for the mismatched structures, leads us to the conclude that, in the case of bisintercalator macrocycles, both aromatic units actively participate in the intercalation process and do not merely introduce steric bulk that prevents interaction with the well-matched duplexes.

Anthracene derivatives: Previously, we showed that the bisanthracene macrocycle **9,10-BisAN** gives different fluorescence responses when bound to TX-mismatched DNA and to well-matched DNA due to both binding selectivity and different binding modes.[26] Thus, we decided to include this compound in the present study, to serve as a reference for the bisanthracene analogues with different topology (**1,8-BisAN** and **1,8/9,10-BisAN**). The data of the thermal denaturation experiments (Figure 3) show that, in contrast to the moderate but still significant preference of macrocycle **9,10-BisAN** for mismatched duplexes, the isomer **1,8-BisAN** binds strongly the four duplexes without a clear discrimination (ΔT_m=17–22 °C at $q=2$). This stabilization profile, along with the very broad, multiphasic melting curves, is very similar to the ones observed with the groove binders **DAPI** and **H33258**, which are not mismatch-selective either (Figure 1). Interestingly, the heterodimer **1,8/9,10-BisAN** displays a smaller stabilization of the fully matched duplex while retaining a strong affinity for the mismatched duplexes, particularly **TC**. At the same time, the greatly increased ΔT_m values observed with two equivalents of this compound indicate a significant contribution of nonspecific binding. Finally, the hybrid anthracene–naphthalene macrocycle **2,7-NP/9,10-AN** shows preferential binding to the TT- and TC-mismatched duplexes with moderate binding to the **TA** control (ΔT_m(TT)$\approx\Delta T_m$(TC)$\approx$12 °C, ΔT_m(TA)=4 °C at $q=2$). The stabilization effect is almost saturated at a ligand-to-duplex ratio of 1:1 and, altogether, the profile is reminiscent of that of the bisnaphthalene series.

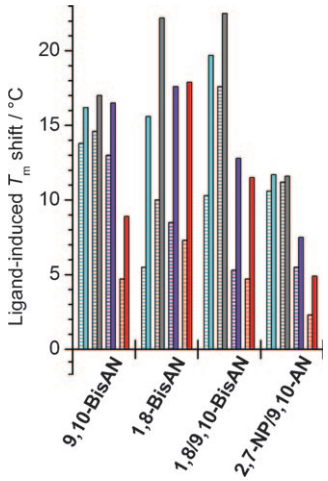
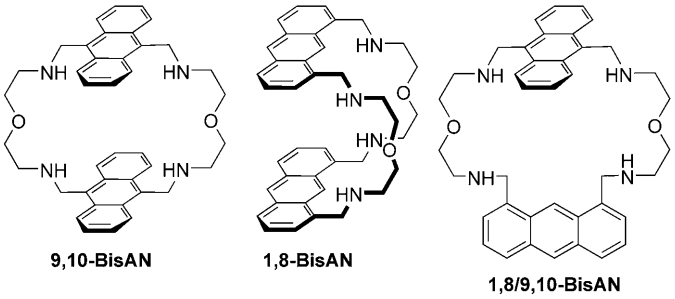


Figure 3. Results of thermal-denaturation experiments for the anthracene derivatives. For the assignment of datasets and experimental details, see Figure 1.



Scheme 7. Schematic representation of the topology of the **BisAN** family of macrocycles.

In summary, the anthracene units enhance the interaction with all forms of DNA, most likely due to their stronger π-stacking ability, as compared to the results with naphthalene rings. Additionally, the 1,8-substituted pattern imposes a particular conformation (Scheme 7) that may be more prone for insertion into the well-matched duplex, possibly by a groove-binding mode; this again provides evidence for the importance of the ligand topology. Finally, the introduction of a naphthalene unit drives the behavior back to that of the **BisNP** series (lower affinity but better mismatch selectivity).

Other macrocyclic compounds: After having established the preliminary relationships between the structure and DNA-

binding properties in the naphthalene and anthracene series of macrocycles, we turned our attention to other homodimeric macrocycles including various aromatic units, such as acridine, phenazine, phenanthroline, biphenyl, and bipyridine. The bisacridine macrocycle **2,7-BisA-N** (**BisA**) was the first mismatch-selective macrocycle reported,[21] so we anticipated that screening of other heteroaromatic derivatives could result in interesting new ligands. The results (Figure 4)

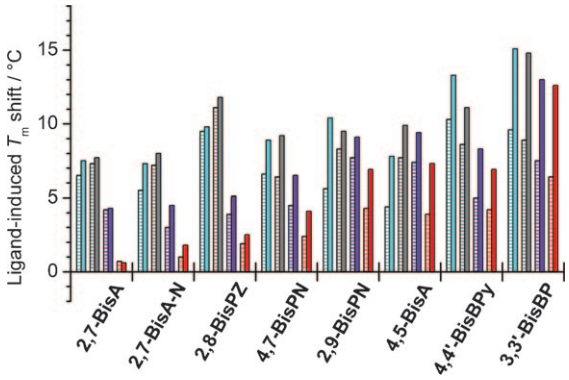


Figure 4. Results of thermal-denaturation experiments for the other macrocyclic compounds. For the assignment of datasets and experimental details, see Figure 1.

confirm the preferential binding of the macrocyclic bisacridines **2,7-BisA-N** and **2,7-BisA** to the mismatch-containing duplexes, with very similar behavior and, as previously observed,[25] with a lower affinity than their bisnaphthalene analogues. However, modification of the topology in the acridine series (in the case of **4,5-BisA**) results in increased binding to control duplex **TA**. The replacement of the acridine by other aromatic systems (**2,8-BisPZ**, **2,9-** and **4,7-BisPN**, **4,4′-BisBPy**) does not lead to significant improvement, because binding to the **TA** control is clearly more pronounced. Taken together, these results indicate that, as in the naphthalene and anthracene series, the substitution pattern of the aromatic residues, which determines the shape of the macrocycle, plays a crucial role in preventing binding to well-matched DNA and, thereby, determines the mismatch selectivity. On the other hand, the electronic structure of the aromatic units may play a role in the mismatch-binding strength, as illustrated by the significantly higher affinity of **2,8-BisPZ** than that of **2,7-BisA**. It should be also noted that the thermal denaturation studies reveal a slight preferential stabilization of **TC** duplexes by the bisacridine and bisphenazine macrocycles, whereas most bisnaphthalene derivatives clearly prefer the **TT** counterpart (Figure 2).

The thermal denaturation studies show even less mismatch selectivity of the structurally related derivatives **4,4′-BisBPy** and **3,3′-BisBP**, because these macrocycles, containing nonplanar flexible bipyridine and biphenyl units, strongly stabilize the matched duplex **TA** (ΔT_m(TA)$\approx$4–6 °C at $q=1$). Especially in the case of **3,3′-BisBP**, the large further increase in the ΔT_m value at $q=2$ and the broad, biphasic melting curves are typical of nonspecific binding to the four duplex structures, presumably by electrostatic-driven surface binding or accommodation in grooves.

Fluorescent-intercalator displacement (FID) titrations: Taken together, the thermal denaturation data indicate that the best candidates for TX mismatch recognition are the bisnaphthalene homodimers with the 2,7 connectivity, **2,7-BisNP** and **2,7-BisNP-N**, because these compounds did not stabilize the **TA** duplex at all, under our conditions. To confirm the selectivity of these optimized ligands, we investigated the binding to both **TT** and **TA** duplexes by using the FID method. The FID assay is a versatile method, which is used to investigate binding of ligands to various DNA structures and may provide apparent binding constants.[51,52] In this experiment, the DNA is fluorescently stained with a dye, typically ethidium bromide or thiazole orange, which is almost nonfluorescent in the absence of DNA but becomes fluorescent upon binding to DNA. Binding of ligands to the DNA–intercalator complex leads to partial displacement of the bound fluorophore, which is accompanied by a decrease in the fluorescence intensity. The fluorescence decrease may serve for evaluation of affinity in a series towards a given DNA structure. As seen in Figure 1, **EB** binds equally well to the mismatched and matched duplexes, which is an important criterion for choosing this intercalator as the fluorescent probe. Moreover, the fluorescence intensity of DNA-bound **EB** is not affected by the presence of mismatched base pairs in the DNA.[25,27]

In our experiments, the **TT** and **TA** duplexes were stained with **EB** and the displacement of the probe was calculated from the changes in fluorescence intensity upon addition of the ligands (see the Supporting Information). In the case of the duplex with a TT mismatch, addition of **2,7-BisNP** leads to the rapid displacement of the **EB** probe, which reaches a plateau upon addition of an increasing concentration of the ligand (Figure 5A). The shape of the curve clearly indicates a binding equilibrium that reaches a limiting value at 1–2 equivalents of ligand, a result that is fully consistent with the T_m data. On the other hand, very poor displacement of the probe from the fully paired **TA** control was observed. A totally different behavior was observed with the ligand **3,3′-BisBP** (Figure 5B), which readily displaced the probe from both duplexes, irrespective of the presence of a mismatch. Moreover, the same phenomena were observed when the experiments were repeated at 10-fold higher concentrations of DNA and **EB**, respectively (see the Supporting Information, Figure S1). Thus, the results of the FID titrations fully confirm the trend in selectivity drawn from the thermal denaturation experiments.

Discussion

We conclude that the **BisNP** compounds and, in particular, the 2,7-substituted derivatives, exhibit the best selectivity for the TX-mismatched duplexes over the fully paired duplex control. In addition, the **BisNP** series exhibits a strong pref-

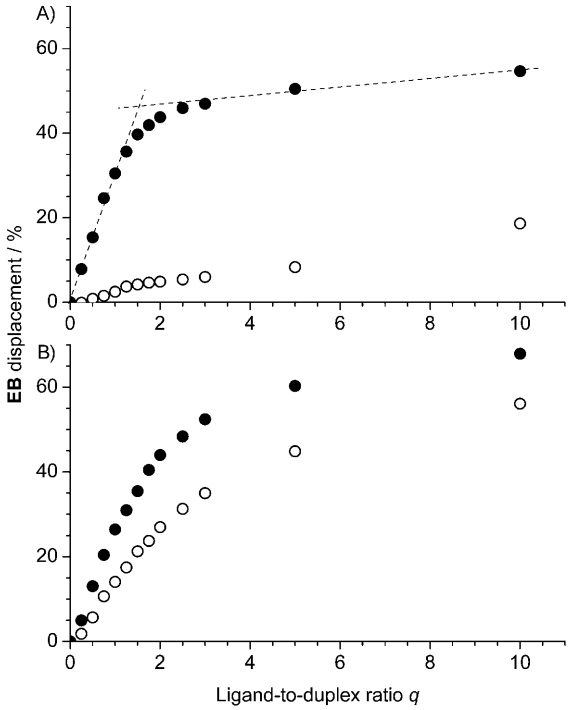


Figure 5. Displacement of **EB** upon titration of ligands **2,7-BisNP** (A) and **3,3′-BisBP** (B) to solutions of duplexes **TA** (○) and **TT** (●; $c=$ 0.1 μM) and **EB** ($c=0.4$ μM in cacodylate buffer, pH 6.0, $[Na^+]=110$ mM).

erence for TT and TC mismatches as compared to the TG mismatch; this result is consistent with the relative thermodynamic stability of the three mismatches, as shown previously. Although thermal stabilization measurements provide an indirect evaluation of affinity, an examination of the data enables us to conclude that the stabilization induced by the **2,7-BisNP** homodimers is mainly due to a single binding event occurring at the mismatch site present in the target duplex.

The replacement of the bicyclic naphthalene ring by tricyclic aromatic rings with a larger π surface (acridine, anthracene, phenazine, phenanthroline) does not give any tangible improvement in the recognition properties; in most cases, the corresponding homodimers do not show a significant increase in the stabilization of the mismatched duplexes and globally they exhibit a lower selectivity, as reflected by binding to the control duplex. The anthracene derivatives were an exception to this trend and showed higher ΔT_m values (16–20 °C for the **BisAN** derivatives versus 9–11 °C for the **BisNP** series for the **TT** duplex), but, clearly, these values result from several contributions, in particular, nonspecific binding at diverse sites.[26] The performance of the heterodimers possessing two different aromatic units (naphthalene–acridine, naphthalene–anthracene) demonstrates that inclusion of the **2,7-NP** unit leads to better selectivity. Furthermore, the introduction of flexible bicyclic units, which are less rigid and less prone to π-stacking interactions, totally abolishes the preferential mismatch binding; this is particularly obvious in the case of the biphenyl derivative (**3,3′-BisBP**). These observations suggest that π-stacking interactions with DNA bases are involved but might not be predominant in the interaction.

Finally and very importantly, the connectivity between the two linkers and the two aromatic units appears to be a key factor for both affinity and selectivity for the mismatched site. This underscores the crucial role played by the conformation of the ligand. It should be noted that the macrocycles presented herein are flexible molecules that, in principle, may adopt various conformations upon binding. However, it has been shown that the **BisA** and **BisNP** derivatives adopt a semiclosed conformation once bound to DNA or in complexes with planar aromatic substrates; these data were obtained in solution (NMR spectroscopy) and in the solid state (X-ray crystallography), respectively.[20,45] In addition, during the course of our study, an X-ray crystal structure of the bisphenanthroline compound (called **2,9-BisPN** herein) has been solved and shows that this molecule also adopts a semiclosed conformation in the solid state.[46] If these facts are taken together, it is reasonable to assume that all of the cyclic bisintercalators studied herein adopt a similar folded conformation in the free state in aqueous solution due to the constraints imposed by the two linkers and hydrophobic forces. On the other hand, the topology of the ligand and its internal molecular flexibility may vary greatly depending on the position of attachment of the two linkers on the aromatic platforms (as represented in Schemes 6 and 7), which, in turn, influences the ability of the ligand to interact with the DNA residues (bases and phosphates). This subtle influence is clearly illustrated by the **BisNP** series, because a change in the connectivity from the 2,6 to the 2,7 or 1,5 positions increases or decreases selectivity, respectively. To further support the existence of a folded conformation and to have insight into the flexibility of these macrocyclic systems, we performed molecular modeling studies for these three isomers. Initial stable conformations of **1,5-**, **2,6-**, and **2,7-BisNP** protonated at the nitrogen atoms[44] in the gas phase were generated (Figure 6A and Figures S2–S4 in the Supporting Information).[53] All geometries showed a stair-like structure with aromatic rings sliding away as far as possible. However, in molecular dynamics (MD) simulations with a water model (see the Supporting Information),[54,55] the three molecules showed a wide range of molecular motion over a magnitude of 30–40 kcal mol^{-1}. The lowest-energy conformations span from a semiopen structure in the case of **2,7-BisNP** (Figure 6B) to a semiclosed conformation for **2,6-** or **1,5-BisNP**, respectively (see the Supporting Information, Figures S2–S4). Notably, in the course of the simulation, **1,5-BisNP** spends more time in its semiopen form than **2,7-BisNP**, whereas **2,6-BisNP** shows a significant degree of distortion.[55] In no case was the completely closed conformation involving intramolecular stacking of the two aromatic units observed.

It can be hypothesized that, once bound to mismatched DNA, these compounds form a catenated complex, as seen previously for **BisA** in an apurinic site,[20b] to take advantage of the enhanced breathing motions of mispaired bases. The strength of the interaction and the stability of the resulting

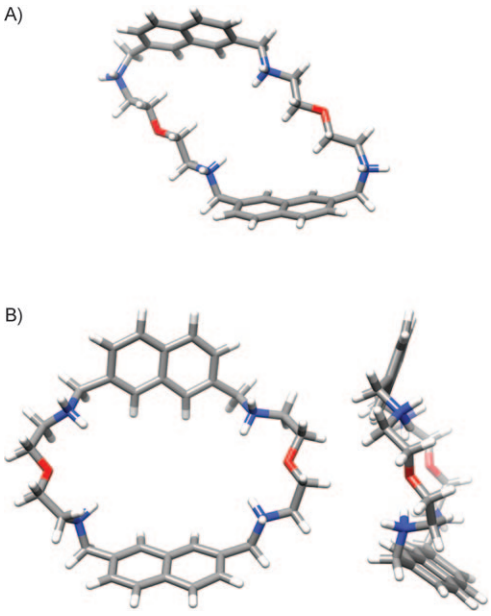


Figure 6. Geometries of **2,7-BisNP**$+4H^+$. A) Molecular mechanics optimization in the gas phase. B) Front and side views of the lowest energy conformation during a molecular dynamics simulation in a neutralized ($4Cl^-$) water box.

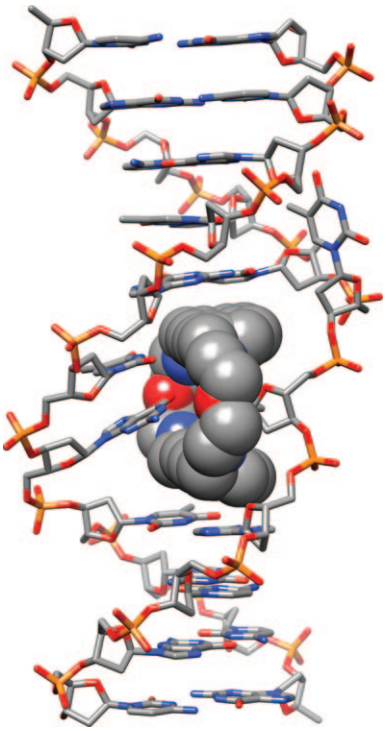


Figure 7. Structure of **2,7-BisNP** docked into an 11-mer duplex with a TT mismatch, d(CGCACT*CACGC):d(GCGTGTGTGCG). The structure of the oligonucleotide was derived from the NMR spectroscopy data of an abasic duplex[20b] by attachment of a thymine residue (T*); see the Supporting Information for details.

complex are highly dependent on the changes in entropy and enthalpy due to complexation. Thus, it may be assumed that the ligands, the conformation of which in the free state is similar to that when bound with mismatched DNA, have higher binding affinity due to smaller conformational entropy. Indeed, in the course of the MD simulation, a number of semiclosed structures were encountered within a reasonable range of energy (10–20 kcal mol^{-1}) compared with the lowest energy structures. To further support this hypothesis, the three **BisNP** isomers in several semiclosed forms were docked into an 11-mer duplex containing a TT mismatch.[56] The docking calculations gave very similar solutions for the three ligands (see the Supporting Information, Figure S5), in excellent agreement with the thermal denaturation data. An example of a docking solution with **2,7-BisNP** is represented in Figure 7.

In summary, on the grounds of the present data and on previous results indicating that binding of **2,7-BisA** at a TT mismatch induces extrahelical displacement of one thymine,[21] we can assume that a specific interaction is established between macrocycles of the **BisNP** type and TT- or TC-mismatched sites in DNA. Although the molecular modeling studies do not address the mismatch versus fully paired duplex selectivity, it can be hypothesized that the short distance between the two aromatic units is involved in the preference for the mispaired site, because a cyclic bisintercalator with long polyamide linkers has recently been shown to thread into intact DNA through opening of four Watson–Crick base pairs.[57] For this reason, it is unlikely that **1,5-BisNP** could intercalate between Watson–Crick base pairs and, thus, its higher propensity to bind fully paired DNA is attributable to a particular topology suitable for groove or surface binding. Studies are currently underway to experimentally determine the structure of the complexes between TX-mismatched duplexes and the best identified candidate **2,7-BisNP**.

Conclusion

By using the well-known [2+2] cyclocondensation for the synthesis of homodimeric macrocycles[43] and our own versatile two-step approach to the heterodimeric analogues, we prepared a collection of 20 bisintercalator-type macrocycles. These compounds display a large molecular diversity, and the heterodimeric scaffolds have been synthesized for the first time. This diversity allowed the fine-tuning of the binding behavior of the ligands with regard to thymine-mismatch-containing DNA duplexes and the establishment of comprehensive structure–property guides. The dramatic differences between the various series of macrocycles emphasize the strong influence of the size and topology of the macrocycle on mismatch recognition and suggest that a subtle equilibrium exists between the structural features in which selectivity originates. Finally, the novel compounds are highly water soluble and thus have great potential for use as modulators of mismatch-repair pathways or as molecular diagnostic tools.

Acknowledgements

We sincerely thank Dr. Jason Martin (Institut Curie) for proofreading the manuscript and Professor Heiko Ihmels (Universität Siegen, Germany) for a donation of 2,7-dimethylnaphthalene, and we gratefully acknowledge the generous financial support from the Centre National de la Recherche Scientifique.

[1] R. R. Iyer, A. Pluciennik, V. Burdett, P. L. Modrich, *Chem. Rev.* **2006**, *106*, 302–323.
[2] L. C. Young, J. B. Hays, V. A. Tron, S. E. Andrew, *J. Invest. Dermatol.* **2003**, *121*, 435–440.
[3] a) H. T. Lynch, T. Smyrk, J. F. Lynch, *Int. J. Cancer* **1996**, *69*, 38–43; b) S. Zhang, R. Lloyd, G. Bowden, B. W. Glickman, J. G. de Boer, *Environ. Mol. Mutagen.* **2002**, *40*, 243–250.
[4] Y. Pommier, *DNA Repair Modulators as Anticancer Agents*, ASC, New York, **2008**, pp. 128–133.
[5] a) G. Damia, M. D'Incalci, *Eur. J. Cancer* **2007**, *43*, 1791–1801; b) L. H. Hurley, *Nat. Rev. Cancer* **2002**, *2*, 188–200.
[6] a) J. R. Hart, O. Glebov, R. J. Ernst, I. R. Kirsch, J. K. Barton, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 15359–15363; b) C. A. Puckett, J. K. Barton, *J. Am. Chem. Soc.* **2007**, *129*, 46–47; c) C. A. Puckett, J. K. Barton, *Biochemistry* **2008**, *47*, 11711–11716; d) R. J. Ernst, H. Song, J. K. Barton, *J. Am. Chem. Soc.* **2009**, *131*, 2359–2366.
[7] a) B. A. Jackson, J. K. Barton, *J. Am. Chem. Soc.* **1997**, *119*, 12986–12987; b) B. A. Jackson, V. Y. Alekseyev, J. K. Barton, *Biochemistry* **1999**, *38*, 4655–4662; c) B. A. Jackson, J. K. Barton, *Biochemistry* **2000**, *39*, 6176–6182.
[8] a) H. Junicke, J. R. Hart, J. Kisko, O. Glebov, I. R. Kirsch, J. K. Barton, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 3737–3742; b) C. Cordier, V. C. Pierre, J. K. Barton, *J. Am. Chem. Soc.* **2007**, *129*, 12287–12295.
[9] a) B. M. Zeglis, J. A. Boland, J. K. Barton, *J. Am. Chem. Soc.* **2008**, *130*, 7530–7531; b) B. M. Zeglis, J. A. Boland, J. K. Barton, *Biochemistry* **2009**, *48*, 839–849.
[10] B. M. Zeglis, J. K. Barton, *Inorg. Chem.* **2008**, *47*, 6452–6487.
[11] a) K. Nakatani, S. Sando, H. Kumasawa, J. Kikuchi, I. Saito, *J. Am. Chem. Soc.* **2001**, *123*, 12650–12657; b) K. Nakatani, S. Sando, I. Saito, *Bioorg. Med. Chem.* **2001**, *9*, 2381–2385; c) T. Peng, T. Murase, Y. Goto, A. Kobori, K. Nakatani, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 259–262.
[12] S. Hagihara, H. Kumasawa, Y. Goto, G. Hayashi, A. Kobori, I. Saito, K. Nakatani, *Nucleic Acids Res.* **2004**, *32*, 278–286.
[13] A. Kobori, S. Horie, H. Suda, I. Saito, K. Nakatani, *J. Am. Chem. Soc.* **2004**, *126*, 557–562.
[14] K. Nakatani, S. Sando, I. Saito, *Nat. Biotechnol.* **2001**, *19*, 51–55.
[15] Y. Goto, H. Suda, A. Kobori, K. Nakatani, *Anal. Bioanal. Chem.* **2007**, *388*, 1165–1173.
[16] K. Nakatani, S. Horie, Y. Goto, A. Kobori, S. Hagihara, *Bioorg. Med. Chem.* **2006**, *14*, 5384–5388.
[17] a) X. L. Yang, R. B. Hubbard IV, M. Lee, Z. F. Tao, H. Sugiyama, A. H. Wang, *Nucleic Acids Res.* **1999**, *27*, 4183–4190; b) E. R. Lacy, K. K. Cox, W. D. Wilson, M. Lee, *Nucleic Acids Res.* **2002**, *30*, 1834–1841; c) E. R. Lacy, B. Nguyen, M. Le, K. K. Cox, C. O'Hare, J. A. Hartley, M. Lee, W. D. Wilson, *Nucleic Acids Res.* **2004**, *32*, 2000–2007.
[18] L. A. Frederico, T. A. Kunkel, B. R. Shaw, *Biochemistry* **1993**, *32*, 6523–6530.
[19] N. Peyret, P. A. Seneviratne, H. T. Allawi, J. SantaLucia, Jr., *Biochemistry* **1999**, *38*, 3468–3477.
[20] a) N. Berthet, J. Michon, J. Lhomme, M.-P. Teulade-Fichou, J.-P. Vigneron, J.-M. Lehn, *Chem. Eur. J.* **1999**, *5*, 3625–3630; b) M. Jourdan, J. Garcia, J. Lhomme, M.-P. Teulade-Fichou, J.-P. Vigneron, J.-M. Lehn, *Biochemistry* **1999**, *38*, 14205–14213.
[21] A. David, N. Bleimling, C. Beuck, J.-M. Lehn, E. Weinhold, M.-P. Teulade-Fichou, *ChemBioChem* **2003**, *4*, 1326–1331.
[22] a) R. J. Roberts, X. D. Cheng, *Annu. Rev. Biochem.* **1998**, *67*, 181–198; b) S. Klimasauskas, S. Kumar, R. J. Roberts, X. D. Cheng, *Cell* **1994**, *76*, 357–369.
[23] a) T. Peng, K. Nakatani, *Angew. Chem.* **2005**, *117*, 7446–7449; *Angew. Chem. Int. Ed.* **2005**, *44*, 7280–7283; b) K. Nakatani, S. Hagihara, Y. Goto, A. Kobori, M. Hagihara, G. Hayashi, M. Kyo, M. Nomura, M. Mishima, C. Kojima, *Nat. Chem. Biol.* **2005**, *1*, 39–43; c) Y. Oka, T. Peng, F. Takei, K. Nakatani, *Nucleic Acids Symp. Ser.* **2008**, *52*, 435–436.
[24] V. C. Pierre, J. T. Kaiser, J. K. Barton, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 429–434.
[25] M. Bahr, V. Gabelica, A. Granzhan, M.-P. Teulade-Fichou, E. Weinhold, *Nucleic Acids Res.* **2008**, *36*, 5000–5012.
[26] A. Granzhan, M.-P. Teulade-Fichou, *Chem. Eur. J.* **2009**, *15*, 1314–1318.
[27] M. H. Lim, H. Song, E. D. Olmon, E. E. Dervan, J. K. Barton, *Inorg. Chem.* **2009**, *48*, 5392–5397.
[28] J.-B. LePecq, C. Paoletti, *J. Mol. Biol.* **1967**, *27*, 87–106.
[29] a) D. Suh, J. B. Chaires, *Bioorg. Med. Chem.* **1995**, *3*, 723–728; b) E. C. Long, J. K. Barton, *Acc. Chem. Res.* **1990**, *23*, 271–273.
[30] H. W. Zimmermann, *Angew. Chem.* **1986**, *98*, 115–131; *Angew. Chem Int. Ed. Engl.* **1986**, *25*, 115–130.
[31] a) L. S. Lerman, *J. Mol. Biol.* **1961**, *3*, 18–30; b) M. Aslanoglu, *Anal. Sci.* **2006**, *22*, 439–443.
[32] a) L. G. Lee, C.-H. Chen, L. A. Chiu, *Cytometry* **1986**, *7*, 508–517; b) J. A. Bordelon, K. J. Feierabend, S. A. Siddiqui, L. L. Wright, J. T. Petty, *J. Phys. Chem. B* **2002**, *106*, 4838–4843.
[33] B. A. Armitage, *Top. Curr. Chem.* **2005**, *253*, 55–76.
[34] a) J. Bontemps, C. Houssier, E. Fredericq, *Nucleic Acids Res.* **1975**, *2*, 971–984; b) F. G. Loontiens, P. Regenfuss, A. Zechel, L. Dumortier, R. M. Clegg, *Biochemistry* **1990**, *29*, 9029–9039.
[35] a) W. D. Wilson, F. A. Tanious, H. J. Barton, R. L. Jones, K. Fox, R. L. Wydra, L. Strekowski, *Biochemistry* **1990**, *29*, 8452–8461; b) K. Jansen, B. Nordén, M. Kubista, *J. Am. Chem. Soc.* **1993**, *115*, 10527–10530.
[36] L. Dassonneville, F. Hamy, P. Colson, C. Houssier, C. Bailly, *Nucleic Acids Res.* **1997**, *25*, 4487–4492.
[37] J. Cho, R. R. Rando, *Nucleic Acids Res.* **2000**, *28*, 2158–2163.
[38] K. Yamashita, S. Sata, H. Takamiya, M. Takagi, S. Takenaka, *Chem. Lett.* **2001**, 680–681.
[39] E. Trotta, M. Paci, *Nucleic Acids Res.* **1998**, *26*, 4706–4713.
[40] a) R. F. Pasternack, E. J. Gibbs, J. J. Villafranca, *Biochemistry* **1983**, *22*, 2406–2414; b) A. B. Guliaev, N. B. Leontis, *Biochemistry* **1999**, *38*, 15425–15437; c) B. Jin, H. M. Lee, J. Ko, C. Kim, S. K. Kim, *J. Am. Chem. Soc.* **2005**, *127*, 2417–2424.
[41] L. A. Lipscomb, F. X. Zhou, S. R. Presnall, R. J. Woo, M. E. Peek, R. R. Plaskon, L. D. Williams, *Biochemistry* **1996**, *35*, 2818–2823.
[42] A. A. Ouameur, H.-A. Tajmir-Riahi, *J. Biol. Chem.* **2004**, *279*, 42041–42054.
[43] a) J. Jazwinski, J.-M. Lehn, R. Méric, J.-P. Vigneron, M. Cesario, J. Guilhem, C. Pascard, *Tetrahedron Lett.* **1987**, *28*, 3489–3492; b) N. E. Borisova, M. D. Reshetova, Y. A. Ustynyuk, *Chem. Rev.* **2007**, *107*, 46–79.
[44] a) M.-P. Teulade-Fichou, J.-P. Vigneron, J.-M. Lehn, *Supramol. Chem.* **1995**, *5*, 139–147; b) T. Paris, J.-P. Vigneron, J.-M. Lehn, M. Cesario, J. Guilheim, C. Pascard, *J. Inclusion Phenom. Macrocyclic Chem.* **1999**, *33*, 191–202.
[45] P. Cudic, J.-P. Vigneron, J.-M. Lehn, M. Cesario, T. Prangé, *Eur. J. Org. Chem.* **1999**, 2479–2484.
[46] C. Cruz, R. Delgado, M. G. B. Drew, V. Félix, *J. Org. Chem.* **2007**, *72*, 4023–4034.
[47] A. Dahan, T. Ashkenazi, V. Kuznetsov, S. Makievski, E. Drug, L. Fadeev, M. Bramson, S. Schokoroy, E. Rozenshine-Kemelmakher, M. Gozin, *J. Org. Chem.* **2007**, *72*, 2289–2296.
[48] Y.-S. Kim, K. M. Kim, R. Song, M. J. Jun, Y. Sohn, *J. Inorg. Biochem.* **2001**, *87*, 157–163.
[49] A. Granzhan, M.-P. Teulade-Fichou, *Tetrahedron* **2009**, *65*, 1349–1360.
[50] J.-L. Mergny, L. Lacroix, *Oligonucleotides* **2003**, *13*, 515–537.

[51] a) D. L. Boger, B. E. Fink, S. R. Brunette, W. C. Tse, M. P. Hedrick, *J. Am. Chem. Soc.* **2001**, *123*, 5878–5891; b) W. C. Tse, D. L. Boger, *Acc. Chem. Res.* **2004**, *37*, 61–69.

[52] a) A. J. Geall, I. S. Blagbrough, *J. Pharm. Biomed. Anal.* **2000**, *22*, 849–859; b) D. Monchaud, C. Allain, H. Bertrand, N. Smargiasso, F. Rosu, V. Gabelica, A. De Cian, J.-L. Mergny, M.-P. Teulade-Fichou, *Biochimie* **2008**, *90*, 1207–1223.

[53] Marvin 5.2.4, ChemAxon (http://www.chemaxon.com), Budapest, **2009**.

[54] Desmond Molecular Dynamics System, Version 2.2.6.2.3, D. E. Shaw Research, New York, **2009**.

[55] The video files showing the course of MD simulations are provided as web-only Supporting Information.

[56] GOLD 4.1: a) G. Jones, P. Willett, R. C. Glen, *J. Mol. Biol.* **1995**, *245*, 43–53; b) G. Jones, P. Willett, R. C. Glen, A. R. Leach, R. Taylor, *J. Mol. Biol.* **1997**, *267*, 727–748.

[57] Y. Chu, D. W. Hoffman, B. L. Iverson, *J. Am. Chem. Soc.* **2009**, *131*, 3499–3508.

Received: July 17, 2009
Published online: November 24, 2009

