DNA Structures

DOI: 10.1002/anie.200903194

Binaphthyl-DNA: Stacking and Fluorescence of a Nonplanar Aromatic Base Surrogate in DNA**

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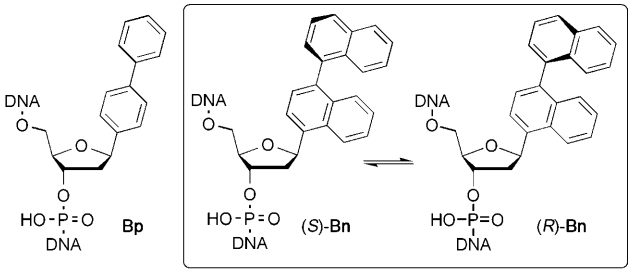
The replacement of canonical nucleobases with artificial aromatic surrogates allows the incorporation of new functions into the base stack of DNA. Arenes and heteroarenes have been introduced as shape mimics of natural bases and as artificial base pairs to help understand DNA–DNA and DNA–protein interactions.[1] Many base surrogates exhibit interesting fluorescence properties, which depend on stacking interactions with the environment.[1d] This has allowed the design of probes that report on the structure and function of enzymes and nucleic acids.[2] Recently, it has been recognized that oligomeric assemblies of fluorescent base surrogates offer the interesting opportunity to tune the optical properties through hybridization-controlled dye–dye interactions.[3]

Typical base surrogates are planar in order to facilitate π-stacking and hydrophobic interactions within the helical arrangement of DNA bases. For example, the stacking of pyrenes, perylenes, and phenanthrenes has been investigated in detail.[3a,i,j,4] The design paradigm has also been applied for the incorporation of planar polycyclic heterocycles such as cyanine and phenanthridinium dyes.[5,6]

To the best of our knowledge, the stacking of nonplanar units in DNA has not been described. We and Leumann et al. have explored the biphenyl "base" (**Bp** in Figure 1) as an intrinsically nonplanar polycycle.[2c,7] However, the stacking energy gained upon hybridization is sufficient to overcome the small barrier to rotation ($\Delta G \approx 10\ \mathrm{kJ\,mol^{-1}}$) resulting in the planarization of the biphenyl residue in the helical base stack.[8] Interestingly, multiply inserted biphenyl–biphenyl pairs have been found to stabilize DNA duplexes as a result of the zipperlike interstrand arrangement of biphenyl base pairs.[9] We herein demonstrate, perhaps surprisingly, that stabilization of the duplex structure can also be achieved when nonplanar base surrogates are stacked.

We studied the 1,1′-binaphthyl ring system, which comprises two naphthyl rings that on average are nearly orthogonal.[10] The rotation about the central bond is slow at 293 K in solution owing to the large barrier to rotation ($\Delta G \approx 100\ \mathrm{kJ\,mol^{-1}}$).[11] The optical properties of the 1,1′-binaphthyl chromophore depend upon the viscosity of the solvent.[10b] For this reason, one may envision using the 1,1′-binaphthyl chromophore as a new type of torsionally flexible dye in DNA.



5′-TAGTTCT**Bn**TGAGAAGGTG-3′: **1Bn**
5′-TAGTTCA**Bn**AGAGAAGGTG-3′: **2Bn**
5′-TAGTTCC**Bn**CGAGAAGGTG-3′: **3Bn**
5′-TAGTTCG**Bn**GGAGAAGGTG-3′: **4Bn**

5′-CGGCA**Bn**$_n$CGAGCGGC-3′: **5**: n=0, **5Bn**: n=1, **5Bn$_2$**: n=2, **5Bn$_3$**: n=3
5′-GCCGCTCG**Bn**$_m$TGCCG-3′: **5′**: m=0, **5′Bn**: m=1, **5′Bn$_2$**: m=2

Figure 1. Biphenyl-DNA (**Bp**), binaphthyl-DNA (**Bn**), and binaphthyl-modified oligonucleotides studied in this investigation. Note that the binaphthyl nucleoside exists in two interconverting diastereomeric forms.

The 4-linked 1,1′-binaphthyl ***C***-nucleoside was prepared by our recently published cuprate-glycosylation method.[12] The binaphthyl nucleoside was incorporated into oligonucleotides **1Bn–4Bn**, **5Bn$_n$**, and **5′Bn$_m$**. In the first group of oligonucleotides, **1Bn–4Bn**, only one binaphthyl base is incorporated in different nucleobase environments. The oligonucleotides **5Bn$_n$** are complementary to **5′Bn$_m$**, and the resulting duplexes contain various numbers of successive binaphthyl units. Several HPLC profiles showed two peaks (Figure S1 in the Supporting Information). Nevertheless, the HPLC analysis of a quantitative phosphodiesterase digest revealed only the five nucleoside components (Figure S2C,D in the Supporting Information). We assumed that the low rotation barrier of the naphthyl–naphthyl linkage causes the formation of diastereomeric mixtures in DNA. The mixture was fractionated by HPLC methods. However, HPLC analysis of each fraction showed, again, two peaks. This suggests that the rotation about the naphthyl–naphthyl linkage is not sufficiently hindered to prevent epimerization during isolation. This behavior is known from binaphthyl derivatives that lack substituents at the 2- and 2′-positions.[10b]

We examined the thermal stability of binaphthyl-containing oligonucleotide complexes by UV melting analysis (Table 1). The melting curves showed a single transition indicative of cooperative base pairing (Figure S3 in the Supporting Information). The replacement of the thymine base in the TA base pairs in **1T·1′A**, **2T·2′A**, **3T·3′A**, and **4T·4′A** by one binaphthyl base in duplexes **1Bn·1′A**, **2Bn·2′A**, **3Bn·3′A**, and **4BnT·4′A**, respectively, led to

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[**] This work was supported by Sigma-Proligo.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200903194.

Table 1: Thermal stability of binaphthyl-modified and unmodified duplexes.[a]

Duplex		X	Y	T_M [°C]
5′-TAGTTCT**X**TGAGAAGGTG-3′	**1Bn·1′A**	**Bn**	/	45.8
3′-ATCAAGA**Y**ACTCTTCCAC-5′	**1T·1′A**	T	/	54.6
5′-TAGTTCA**X**AGAGAAGGTG-3′	**2Bn·2′A**	**Bn**	/	47.0
3′-ATCAAGT**Y**TCTCTTCCAC-5′	**2T·2′A**	T	/	54.8
5′-TAGTTCC**X**CGAGAAGGTG-3′	**3Bn·3′A**	**Bn**	/	51.2
3′-ATCAAGG**Y**GCTCTTCCAC-5′	**3T·3′A**	T	/	59.9
5′-TAGTTCG**X**GGAGAAGGTG-3′	**4Bn·4′A**	**Bn**	/	54.6
3′-ATCAAGC**Y**CCTCTTCCAC-5′	**4T·4′A**	T	/	60.4
5′-CGGCACGAGCGGC-3′ 3′-GCCGTGCTCGCCG-5′	**5·5′**	/	/	64.8
5′-CGGCA**X**CGAGCGGC-3′ 3′-GCCGT-GCTCGCCG-5′	**5Bn·5′** **5T·5′** **5A·5′**	**Bn** T A	/ / /	58.7 55.5 57.8
5′-CGGCA**X**CGAGCGGC-3′ 3′-GCCGT**Y**GCTCGCCG-5′	**5Bn·5′Bn** **5T·5′T** **5A·5′A** **5T·5′A**	**Bn** T A T	**Bn** T A A	59.6 56.5 57.9 66.4
5′-CGGCA**XX**CGAGCGGC-3′ 3′-GCCGT **Y** GCTCGCCG-5′	**5Bn$_2$·5′Bn** **5T$_2$·5′T** **5A$_2$·5′A** **5T$_2$·5′A**	**Bn** T A T	**Bn** T A A	62.7 53.0 53.2 58.8
5′-CGGCA **X** CGAGCGGC-3′ 3′-GCCGT**YY**GCTCGCCG-5′	**5Bn·5′Bn$_2$** **5T·5′T$_2$** **5A·5′A$_2$** **5T·5′A$_2$**	**Bn** T A T	**Bn** T A A	62.8 52.5 51.6 58.7
5′-CGGCA**XX**CGAGCGGC-3′ 3′-GCCGT**YY**GCTCGCCG-5′	**5Bn$_2$·5′Bn$_2$** **5T$_2$·5′T$_2$** **5A$_2$·5′A$_2$** **5T$_2$·5′A$_2$**	**Bn** T A T	**Bn** T A A	67.4 52.2 51.3 66.0
5′-CGGCA**XXX**CGAGCGGC-3′ 3′-GCCGT **YY** GCTCGCCG-5′	**5Bn$_3$·5′Bn$_2$** **5T$_3$·5′T$_2$** **5A$_3$·5′A$_2$** **5T$_3$·5′A$_2$**	**Bn** T A T	**Bn** T A A	70.0 50.1 49.0 58.7

[a] $c = 1$ μM in 10 mM NaH_2PO_4, 0.1 M NaCl, pH 7.0.

decreases of the duplex stability by $\Delta T_M = 6$–9 °C. This significant destabilization appears plausible. Though intrastrand stacking of the inner naphthyl unit may partially compensate for the loss of hydrogen-bonding interactions, simultaneous intrahelical alignment of the adenine and the proximal naphthyl unit can probably occur only if one of the bases adopts a *syn* orientation (Figure 2A, Figures S4 and S5A in the Supporting Information). The outer naphthyl unit of the binaphthyl base in duplexes such as **1Bn**–**4Bn** will most likely protrude into the unfavorable aqueous environment in the major groove.

We next studied duplexes **5Bn$_n$·5′Bn$_m$** ($n = 0$–3, $m = 0$–2), which feature an increasing number of binaphthyl units (Table 1). The binaphthyl nucleotide in a bulge position (**5Bn·5′**, $n = 1$, $m = 0$, X = **Bn**) led to a duplex that was 6.1 °C less stable than the unmodified duplex **5·5′**. The introduction of the additional binaphthyl unit in **5Bn·5′Bn** conferred no further destabilization. Remarkably, the stability of the duplexes progressively increased as the number of successive binaphthyl residues increased. For example, the additional binaphthyl pair in the duplex **5Bn$_2$·5′Bn$_2$** provided an increase of duplex stability from $T_M = 59.6$ °C for the **5Bn·5′Bn** duplex to $T_M = 67.4$ °C for the **5Bn$_2$·5′Bn$_2$** duplex. To our surprise, duplex **5Bn$_3$·5′Bn$_2$**, which contains five successive binaphthyl bases, was even 5.2 °C more stable than unmodified duplex **5·5′**.

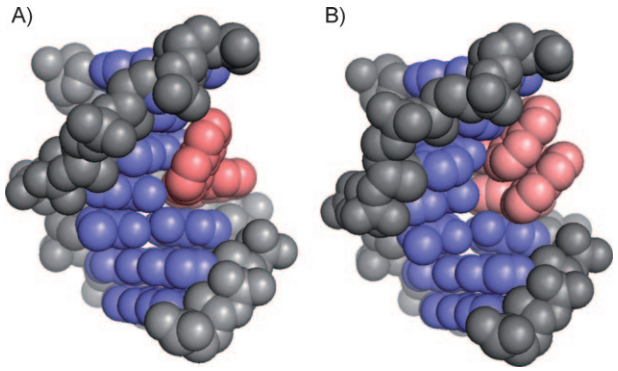

Figure 2. Space-filling representations of possible structures of oligonucleotide duplexes containing a) one binaphthyl residue (**Bn**) (in the *S* form, *syn* to deoxyribose) and b) two adjacent binaphthyl residues (upper **Bn** in the *S* form and *syn*, lower **Bn** in the *R* form and *anti* to deoxyribose). The sugar phosphate backbone is shown in gray, the nucleobases in blue, and binaphthyl in red. See the Supporting Information for details.

The pronounced decrease of duplex stability upon introduction of one binaphthyl pair and the significant stabilization of duplexes that contain two or more consecutive binaphthyl units is noteworthy. This behavior was not observed with the natural nucleobases thymine and adenine. The thymine–thymine (**5T·5′T**) and adenine–adenine pairs (**5A·5′A**) were less stabilizing than the corresponding binaphthyl–binaphthyl pair in **5Bn·5′Bn**. Each additional thymine or adenine residue in duplexes **5T$_n$·5′T$_m$** and **5A$_n$·5′A$_m$** ($n \geq 1$, $m \geq 1$) resulted in further destabilization. This is in stark contrast to the binaphthyl series, where each additional binaphthyl residue stabilized the duplex (Figure 3). Duplexes **5Bn$_3$·5′Bn$_2$**, which contain five successive binaphthyl bases, are 20 °C more stable than the thymine- and adenine-containing duplexes **5T$_3$·5′T$_2$** and **5A$_3$·5′A$_2$**. Interestingly, while one binaphthyl–binaphthyl pair (**5Bn·5′Bn**) was 6.8 °C less stable than an AT pair (**5T·5′A**), two succeeding binaphthyl pairs (**5Bn$_2$·5′Bn$_2$**) were 1.4 °C more stable than two succeeding AT pairs (**5T$_2$·5′A$_2$**). This suggests that two adjacent binaphthyl pairs stabilize duplex architecture, most likely through stacking interactions.

Increases of duplex stability upon the multiple incorporation of flexible aromatic base surrogates were probably described first by Leumann and co-workers.[7b,9] In biphenyl-modified DNA the two distal phenyl groups of a biphenyl–biphenyl pair were found to be stacked on top of each other; this arrangement may partly compensate for the energy cost of planarization of the biphenyl ring systems and the

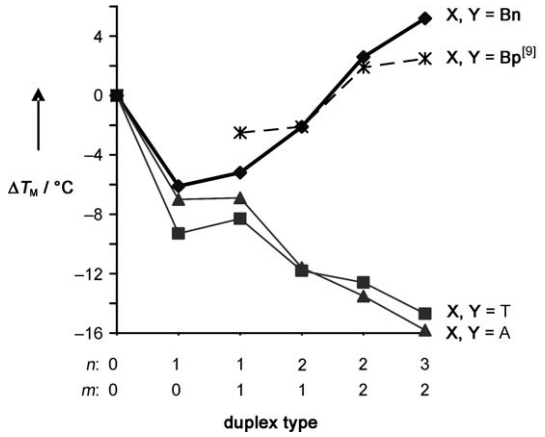

Figure 3. The influence of additional nucleotides X and Y on the stabilities of duplexes **5 X$_n$·5′Y$_m$** (X = **Bn**, T, or A; Y = **Bn**, T, or A; n = 0–3; m = 0–2). Data obtained for biphenyl-modified duplexes (dashed line)[9] is added for comparison.

perturbation of nucleobase–nucleobase stacking.[8a] By contrast, it is difficult to imagine planarization of the binaphthyl residue investigated in this study. It is, thus, unlikely that the distal naphthyl rings in **5 Bn·5′Bn** are in face-to-face contact. This explains why the binaphthyl–binaphthyl pair destabilized the duplex more efficiently ($\Delta T_M = -5.2\,°C$) than a biphenyl–biphenyl pair ($\Delta T_M = -2.5\,°C$).[9] However, additional binaphthyl units may result in intrastrand interactions between the distal naphthyl rings within the major groove (Figure 2B; Figure S5B in the Supporting Information).[13,3j] These interactions involve larger surfaces than the stacked phenyl rings in biphenyl base pairs, which may explain why the T_M increase upon introduction of one additional binaphthyl pair ($\Delta T_M = 7.8\,°C$) is higher than that upon introduction of one additional biphenyl pair ($\Delta T_M = 4.4\,°C$).[9] It is also feasible that the distal naphthyl units of two binaphthyl bases interact in an edge-to-face fashion (Figure S5C in the Supporting Information). Regardless of the exact mechanism involved, we assume that the torsional flexibility of the binaphthyl hinge facilitates stacking interactions which can occur at both the interior and the exterior of the DNA duplex.

The fluorescence properties also support the notion of binaphthyl–binaphthyl interactions in DNA. The oligonucleotides were excited at a wavelength of 305 nm. The fluorescence properties were characterized by means of the relative fluorescence I/I_B (I, I_B: fluorescence emission of binaphthyl-modified oligonucleotides and free 1,1′-binaphthyl, respectively). The investigation of oligonucleotides **1 Bn**–**4 Bn** revealed thymine and cytosine to be efficient quenchers of binaphthyl fluorescence (90–95% quenching, Table 2; see also Figure S8A in the Supporting Information). By comparison guanine and adenine were inefficient (ca. 50%) quenchers. Interestingly, the incorporation of a second or a third fluorophore in **5 Bn$_2$**, **5 Bn$_3$**, or **5′Bn$_2$** led to a strong enhancement of the fluorescence. For example, the oligonucleotide **5 Bn$_3$** ($I/I_B = 1.350$) was found to fluoresce with a 17-fold higher intensity than oligonucleotide **5 Bn** ($I/I_B = 0.078$). This behavior is in contrast to the recently observed decreases

Table 2: Fluorescence properties of binaphthyl-modified oligonucleotides.[a]

Single strands	I/I_B[b]	Double strands	I/I_B[b]
binaphthyl	1	–	–
1 Bn	0.055	**1 Bn·1′A**	0.099
2 Bn	0.531	**2 Bn·2′A**	0.217
3 Bn	0.091	**3 Bn·3′A**	0.050
4 Bn	0.474	**4 Bn·4′A**	0.075
5 Bn	0.078	**5 Bn·5′**	0.160
5 Bn$_2$	0.664	**5 Bn·5′Bn**	0.327
5 Bn$_3$	1.350	**5 Bn·5′Bn$_2$**	0.744
5′Bn	0.052	**5 Bn$_2$·5′Bn**	1.092
5′Bn$_2$	0.365	**5 Bn$_2$·5′Bn$_2$**	2.419
–	-	**5 Bn$_3$·5′Bn$_2$**	3.227

[a] $c = 1\,\mu M$ in 10 mM NaH_2PO_4, 0.1 M NaCl, pH 7.0, 20 °C. [b] Relative fluorescence based on the fluorescence intensity of 1,1′-binaphthyl at λ(emission) = 380 nm and λ(excition) = 305 nm.

of fluorescence upon multiple introduction of planar fluorophores such as pyrene and perylenes.[3h,14] We speculate that the first binaphthyl base serves as an insulator that protects the second and third binaphthyl fluorophore from quenching interactions with the pyrimidines.[15] This implies that the binaphthyl chromophore experiences only little self-quenching in this system.[16] Indeed, the experiments that involved two or more interacting binaphthyl units revealed enhancements of binaphthyl fluorescence upon hybridization (Table 2, see also Figure S8B in the Supporting Information). Duplexes **5 Bn$_n$·5′ Bn$_m$** fluoresced with 50–150% higher intensity than expected based on the sum of the fluorescence of the corresponding single strands.

The purpose of this investigation was to explore torsionally flexible, nonplanar base surrogates in DNA. At first glance, the observed stabilization of a DNA duplex upon successive introduction of multiple binaphthyl units may seem surprising. However, the ground-state potential energy curve of 1,1′-binaphthyl is flat in the region corresponding to a dihedral angle between 60° and 120°.[10b] Thus, the binaphthyl system may be well suited to adjust the two, flexibly linked aromatic units for stacking interactions which may involve both intrahelical and extrahelical partners. Of note, these interactions do not lead to the self-quenching of fluorescence as is frequently observed when planar aromatic base surrogates such as pyrenes are in contact.[3h,14] This behavior may be of interest for the design of oligonucleotide assemblies with light-harvesting properties.[17]

At present it remains unclear whether the DNA helix induces axial chirality of binaphthyl stacks. Preliminary modeling studies (Figure S5 in the Supporting Information) suggest that both the *R* and the *S* forms can be accommodated. Circular dichroism studies may be a useful means to probe the chirality of the binaphthyl systems. However, this would require modifications of the binaphthyl fluorophore in order to avoid overlap with the absorption of the nucleobases. The introduction of stable axial chirality through the incorporation of substituents in the 2- and 2′-positions would also provide interesting opportunities. The resulting three-dimensional chiral nucleobases could be useful tools in the fluorescence-based diagnosis of the handedness of nucleic

acid helices as well as in the construction of nucleotide-based nanostructures.[18]

Received: June 12, 2009
Published online: September 29, 2009

Keywords: binaphthyl · C-nucleosides · DNA · fluorescence · stacking interaction

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