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Local disruption of DNA-base stacking by bulky base surrogates

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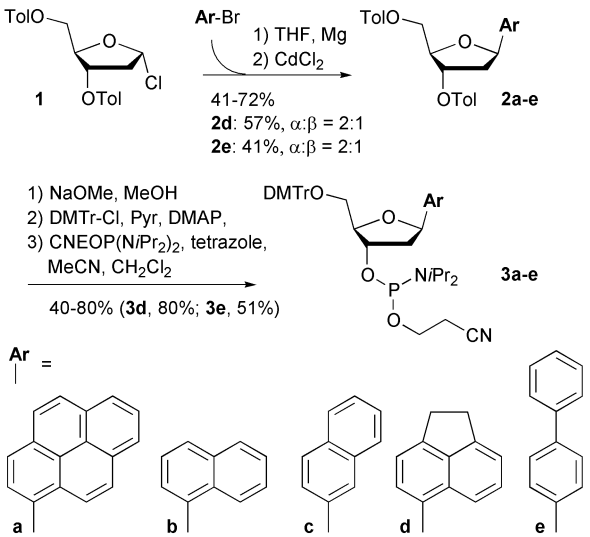
A novel biphenyl base surrogate disrupts 2-aminopurine base stacking while maintaining duplex integrity.

Cellular DNA is the subject of a multitude of chemical and enzymatic modification reactions. In order to gain access to their target structure, many DNA-processing enzymes require a local disruption of base stacking. An extreme form of base stacking disruption is the so-called 'base-flipping' whereby a nucleobase is rotated out of the DNA-helix and positioned in an extrahelical conformation.[1] This targeted disruption of base stacking has been observed for DNA-methyltransferases, DNA-glycosylases, endonucleases and is likely to occur in many other cases.[2] We became interested in mimicking the enzyme-mediated 'base-unstacking' and started a research program devoted to the conformational preorganisation of locally base-unstacked DNA, an endeavour ultimately aiming for the construction of enhanced binders of DNA-modifying enzymes.

It has been shown that the removal of one base of a Watson–Crick base pair can promote an unstacking of the remaining partner base.[3] However, these abasic sites confer a dramatic duplex destabilisation.[4] We hypothesised that a bulky base surrogate, incorporated in a DNA duplex should allow for a local unstacking of the opposing base without significant detriment to the global duplex stability. In order to test this hypothesis we introduced aromatic base analogues that lacked any hydrogen-bonding potential and were therefore devoid of maintaining Watson–Crick base pairing.[5]

Recently, the pyrenyl nucleotide **3a** and the naphthyl nucleotides **3b** and **3c** were synthesised and used in the synthesis of modified oligodeoxynucleotides (ODNs).[5] For the introduction of tetrahedral structural elements we chose to incorporate the acenaphthyl- and the biphenyl-nucleotides **3d** and **3e**. The synthesis of the known phosphoamidites **3a–c** and the new building blocks **3d** and **3e** required the formation of a *C*-glycosidic linkage and was accomplished according to a published protocol (Scheme 1).[5a] In the event of the *C*-glycosylation of **1**, it was essential to convert the aryl-Grignard reagents into less basic cadmium organic species in order to avoid the formation of glycals and furans. The removal of the toluoyl-protecting groups from **2a–e** and the subsequent regioselective introduction of the trityl-protecting group was followed by the reaction with a phosphitylating agent, which furnished the phosphoramidites **3a–e** in satisfying yields. The building blocks **3a–e** were used in the automated synthesis of oligonucleotides **4a–e** (Table 1). The couplings of the modified phosphoramidites proceeded with 98–99% yield. HPLC- and MALDI-TOF/MS analysis confirmed the identity and purity of the modified ODNs **5a–e**.

For an examination of the influence of the base surrogates in ODNs **4a–e** hybridisation studies were performed. To this end, modified oligonucleotides **4a–e** were hybridised with the ODN **5** which contained the base analogue 2-aminopurine (2AP).[6] 2AP forms a base-pair with thymidine and is virtually non-fluorescent when stacked within a DNA duplex but fluoresces upon disruption of base stacking.[7] In the duplexes **4·5** 2AP is placed opposite to the base surrogates and serves as a probe that can help in the analysis of stacking interactions.



Scheme 1 Synthesis of phosphoamidites containing bulky base surrogates. CNE = β-cyanoethyl, DMTr = 4,4′-dimethoxytrityl, Tol = toluoyl.

First, it was explored whether the *C*-glycosides **3a–e** were tolerated in a duplex. The melting curves showed sigmoidal behaviour which suggests that the binding of the modified oligonucleotides **4a–e** still followed a co-operative base-paring mechanism. Duplexes that contained the artificial base surrogates **b–e** showed similar melting temperatures (T_M = 58.0–59.1 °C). This even holds true for the biphenyl system in **4e**, which in contrast to the other modifications is by no means an entity that would be expected to stack. The pyrene residue has the largest hydrophobic surface area, and therefore yielded duplexes of high stability (T_M = 63.5 °C). A marked destabilisation was observed when the 2AP probe was paired with the mismatched G in **4g** (ΔT_M = −7.1 °C) or an abasic site

Table 1 Thermal denaturation and fluorescence data of **4·5**-duplexes

5′-CGG CA**X** CGA GCG GC-3′ **4a-g**
3′-GCC GTY GCT CGC CG-5′ **5**, Y=2AP

X =	T_M[a] (ΔT_M[b])/ °C	*Relative fluorescence*[c]
T, **4T**	63.2	0.09 (1.0)
1-pyrenyl, **4a**	63.5 (0.3)	0.11 (1.2)
1-naphthyl, **4b**	58.0 (−5.2)	0.15 (1.6)
2-naphthyl, **4c**	58.2 (−5.0)	0.20 (2.0)
acenaphthyl, **4d**	58.5 (−4.7)	0.13 (1.4)
4-biphenyl, **4e**	59.1 (−4.1)	0.54 (5.8)
abasic site, **4f**	51.9 (−11.3)	0.42 (4.6)
G, **4g**	56.1 (−7.1)	0.40 (4.3)

[a] Measured as denaturation curves at 1 μM DNA concentration in a buffered solution (100 mM NaCl, 10 mM NaH_2PO_4, pH7). [b] Relative to duplex **4T·5**. [c] Ratio between fluorescence of duplex **4·5** and the fluorescence of single stranded **5** (measured at 369 nm, conditions as specified in Fig. 1). The increase of the fluorescence of duplex **4·5** relative to the duplex **4T·5** is given in brackets.

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in **4f** (ΔT_M = −11.3 °C), which is known to support an unstacking of the opposing base.[3]

The fluorescence of ODN **5** was determined before and after hybridisation with the complementary strands **4T** and **4a**–**g**. Fig. 1A shows the steady-state fluorescence of oligonucleotide **5**. Upon hybridisation with the complementary strand **4T** a 2AP–T base-pair is formed. Expectedly, base stacking within the interior of duplex **4T·5** decreased the 2AP-fluorescence by a factor of 10.6. The fluorescence of duplexes **4a**–**e·5** was measured, in order to determine whether one of the base surrogates was able to increase the 2AP-fluorescence by base-unstacking (Fig. 1A). The emission profiles were characteristic for the 2AP fluorophore, with the exception of the pyrene containing duplex **4a·5** which was dominated by the pyrene fluorophore. The strongest fluorescence enhancement was observed with the biphenyl-ODN **4e**. The 2AP-emission was enhanced by a factor of 5.8 when compared to the A–T-containing duplex **4T·5**. Remarkably, the biphenyl-induced fluorescence enhancement exceeded the 4.6 fold and 4.3 fold increases that were observed when 2AP was paired against an abasic site or the mismatched base G (Fig. 1B). This suggests that the biphenyl residue is more efficient in promoting a local base stacking disruption than abasic residues or mismatched bases despite the fact that the latter modifications led to a larger global destabilisation as judged by the thermal stability of the duplexes (ΔT_M = −4.1 °C for **4e·5** and ΔT_M = −11.3 °C and −7.1 °C for **4f·5** and **4g·5**).

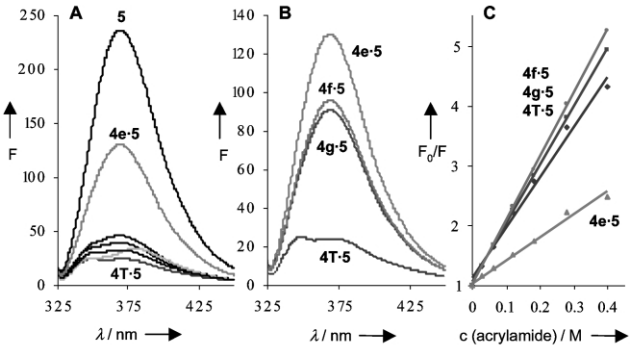

Fig. 1 Fluorescence emission spectra (calibrated on fluorescence of **5**) of A: **5**, **4e·5**, **4c·5**, **4b·5**, **4d·5**, **4a·5** and **4T·5** (order by fluorescence intensity) and of B: **4e·5**, **4f·5**, **4g·5** and **4T·5**. Excitation: 305 nm. C: Acrylamide quenching studies (Stern–Volmer plot) of 2AP in duplexes **4e·5**, **4f·5**, **4g·5** and **4T·5**. Buffer contained 15 mM $MgCl_2$.

In a minimum model the increase of the 2AP fluorescence can arise from two modes of base unstacking.[8] The 2AP-base can be unstacked but still reside in the interior of a locally distorted double helix. Alternatively, the 2AP-base could be pushed into an extrahelical conformation. Extrahelical 2AP but not innerhelical unstacked 2AP, would be susceptible to quenching by external reagents. In order to probe the environment of the 2AP-base, fluorescence quenching studies were performed. Fig. 1C shows Stern–Volmer plots in which the ratio of the fluorescence intensities in the absence (F_0) and the presence (F) of added quencher is plotted against the concentration of the quencher acrylamide. The slopes are proportional to the rate constant k_Q of bimolecular quenching and are hence a measure of the accessibility of the 2AP to the quencher.[3a] It became apparent that the biphenyl duplex **4e·5** is less sensitive to external quenching than the other duplexes [k_Q(**4f·5**)/k_Q(**4e·5**) = 2.8]. This suggests that in **4e·5** the conformational equilibrium is in favour of innerhelically unstacked 2AP when compared with duplexes that contain abasic sites or the mismatched G.

The bulky base surrogates in **4a**–**e** share no similarities with canonical nucleobases, neither by shape nor by polarity. Nevertheless, the melting curves indicated co-operative base pairing for all cases. It was surprising to note that the incorporation of the bulky biphenyl residue led to a small but significant increase of duplex stability when compared with duplexes **4b·5** and **4c·5** which contained intercalatable naphthyl rings. Recently, the incorporation of self-pairing or metal-coordinating bipyridyl residues was described.[9] It has to be noted that the biphenyl modification reported in here differs from the bipyridyl system since it spans two tilted planes rather than one.

The biphenyl residue in duplex **4e·5** proved to be most efficient in enhancing the fluorescence of an opposing 2AP-probe. Measurements with duplexes in which the 2AP-base was replaced by adenine (data not shown) excluded any hybridisation-induced biphenyl fluorescence. It is therefore justified to conclude that the increased fluorescence reflects 2AP-base unstacking. The studies outlined above represent the first example in which a local unstacking of the 2AP-base has been attempted by introducing non-natural base surrogates. Intuitively, a disruption of base stacking would be expected to correlate with duplex destabilisation. For example, it was shown that abasic sites destabilise stacking interactions and confer a dramatic duplex destabilisation.[3,4] However, the disruption of 2AP-base stacking was more effective in the biphenyl duplex **4e·5** than in abasic site and G containing duplexes **4f·5** and **4g·5**. Since the latter displayed lower duplex stabilities, it has to be concluded that a disruption of local base stacking can be achieved without destabilising the duplex significantly. When assessing the nature of the unstacked state it has to be noted that unstacked 2AP can adopt innerhelical and extrahelical conformations. The different susceptibilities to quenching reagents suggest that a control of the conformational equilibrium between innerhelical and extrahelical states could be possible. For example, the biphenyl base appears to shift the equilibrium in favour of innerhelically unstacked 2AP when compared with duplexes that contain an abasic site or the mismatched G-base.

It can be expected that a conformationally controlled disruption of local base stacking will render the target base more reactive towards enzymic modification reactions. We envision that the design of thermally stable duplex molecules with locally unstacked bases could allow for the construction of new biomolecular tools for interference with DNA-modifying enzymes. Studies towards the inhibition of DNA methyltransferases are in progress.

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