ORGANIC LETTERS

2006
Vol. 8, No. 19
4191−4194

Perylene-3,4:9,10-tetracarboxylic Acid Bisimide Dye as an Artificial DNA Base Surrogate

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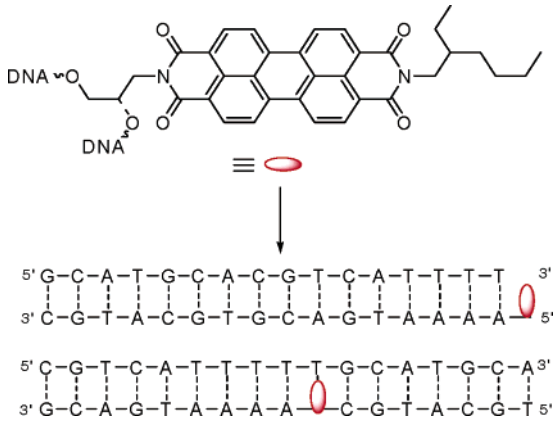
Received May 22, 2006

ABSTRACT



A phosphoramidite of the perylene bisimide dye was synthesized as a DNA building block that allows incorporation of this chromophore as an artificial nucleoside surrogate either at the 5′-terminus or at internal positions of duplex DNA. The internally incorporated perylene bisimide chromophore shows strong interactions with the DNA base stack; the 5′-terminally attached perylene bisimide is able to induce dimerization of two whole DNA duplexes.

Derivatives of perylene-3,4:9,10-tetracarboxylic acid bisimides ("perylene bisimides") have attracted significant research interest as versatile building blocks for the self-assembly of functional supramolecular architectures.[1] Due to the enormous chemical stability and the high quantum yields (up to 100%), perylene bisimides have also found consideration in academic and industrial research for highly fluorescent materials.[2] Perylene bisimides were modified with spermine[3] or other amines in order to yield positively charged species and to study their noncovalent DNA-binding interactions.[4] However, there are only a few examples for the covalent modification of oligonucleotides with perylene bisimides, although the chemical resistance and the unique fluorescence and self-assembling properties of this chromophore should be highly suitable for applications in DNA analytics and DNA-based nanotechnology.[5−9] In particular, perylene bi-

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10.1021/ol061246x CCC: $33.50 © 2006 American Chemical Society
Published on Web 08/18/2006

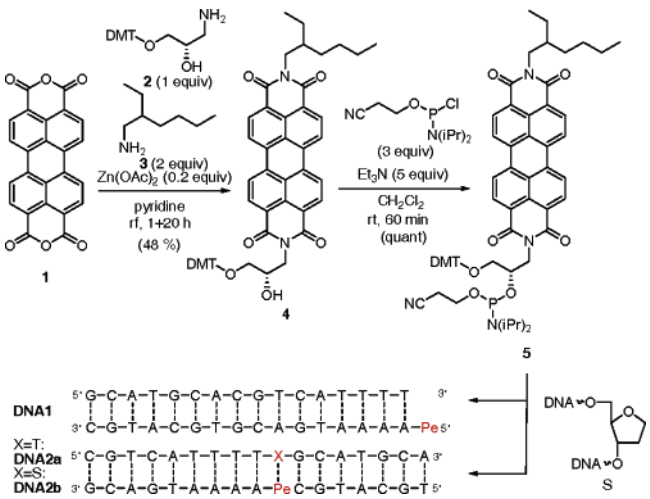
simide derivatives have been covalently attached to oligonucleotides for the DNA-directed assembly of the chromophores,[5] as thermophilic foldamers,[6] and as caps for the contruction of very stable DNA hairpin duplexes[7,8] and triplexes.[9] In contrast, the perylene dye without the carboxamide functionalities has been used widely for the fluorescent DNA and RNA labeling.[10,11]

We chose a new approach in order to study the stacking interactions of the perylene bisimide chromophore by incorporation as an artificial DNA base surrogate at specific sites in duplex DNA. The 2′-deoxyribofuranoside moiety was replaced by an acyclic linker system which is tethered to the nitrogen of one of the perylene bisimide functional groups. Recently, this synthetic approach has been used successfully by our group for the incorporation of ethidium[12] and indole[13] as DNA base surrogates for electron-transfer studies.[14] Avoiding the acid/base labile glycosidic bond of natural nucleosides, the application of a flexible acyclic linker as a nucleoside analogue allows the chromophore to intercalate perfectly[14] and provides a high chemical stability during the preparation of chromophore−DNA conjugates via automated phosphoramidite chemistry for biophysical and bioanalytical applications.[12]

Herein, we want to present briefly the synthetic work for the perylene bisimide nucleoside surrogate **4**, the DNA building block **5** and the corresponding perylene bisimide modified oligonucleotides. The duplexes **DNA1** and **DNA2a**/**DNA2b** have been chosen representatively to compare the interactions of the dye with the DNA base stack at the 5′-terminus and at an internal position. We examined preliminarily these modified DNA duplexes by methods of the optical spectroscopy and characterized the duplexes mainly by their UV/vis absorption and steady-state fluorescence.

Due to the low solubility of perylene bisimide derivatives in a variety of organic solvents the only suitable way to perform the synthesis of perylene bisimide-modified oligonucleotides in a sufficient yield was to prepare the corresponding DNA building block in the least possible number of subsequent reaction steps starting from 3,4:9,10-perylenetetracarboxylic anhydride (**1**). Hence, the first of the two reaction steps (Scheme 1) represents the preparation of the mixed bisimide **4** from two different amines. The enantiomerically pure (*S*)-aminopropane-2,3-diol (**2**) is the acyclic linker substitute for the 2′-deoxyribofuranoside and carries already the 4,4-dimethoxytrityl protecting group that is needed to perform the automated DNA phosphoramidite

Scheme 1. Synthesis of DNA Building Block **5** and Sequences of **DNA1** and **DNA2a**/**DNA2b**



chemistry.[12] The second amine should exhibit a similar reactivity as the amine **2** in order get a significant amount of the mixed bisimide derivative. We tried a variety of amines (methylamine, propylamine, *tert*-octylamine). Among these, the reaction of **1** with 2-ethylhexylamine (**3**) in the presence of **2** yielded nearly 50% of the desired mixed perylene bisimide derivative **4**. The coupling of the phosphiteamide group to the free hydroxy group of **4** was performed using standard procedures in CH_2Cl_2 yielding the phosphoramidite **5**.

Using the DNA building block **5**, the modified oligonucleotides of **DNA1** and **DNA2a**/**DNA2b** were prepared using the DNA synthesizer. To avoid precipitation of the perylene bisimide **5** during the automated DNA synthesis, the building block solution of **5** was prepared in CH_2Cl_2 and the coupling was carried out in an acetonitrile/CH_2Cl_2 mixture. The coupling time for **5** was extended from 1.6 min (standard) to 21 min. The modified single-stranded (ss) oligonucleotides **ssDNA1** and **ssDNA2** were quantified by their absorbance at 531 nm in DMSO ($\epsilon = 60\,250\ M^{-1}cm^{-1}$) and identified by ESI mass spectrometry. Duplexes were formed by heating of the modified oligonucleotides to 80 °C (10 min) in the presence of 1 equiv. of the corresponding complementary unmodified oligonucleotide strand, followed by slow cooling to rt.

DNA1 bears the perylene bisimide moiety (Pe) attached to the 5′-terminus of the oligonucleotide (Scheme 1). In contrast, the duplexes **DNA2a**/**DNA2b** contain the perylene bisimide chromophore at an internal position. **DNA2a** has a thymine (T) opposite to the perylene bisimide; in **DNA2b** an abasic site analogue (S) was incorporated into the counterstrand to allow an optimal intercalation of the perylene bisimide heterocycle.

First, we measured the absorption of the modified duplexes temperature-dependently in order to study the ground-state interactions of the perylene bisimide dye with the DNA base stack (Figure 1). In **DNA2b**, and similar in **DNA2a** (Figure S1), the dehybridization of the perylene bisimide chro-

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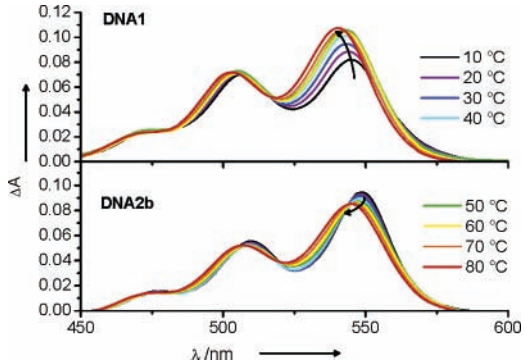

Figure 1. Temperature-dependent UV/vis absorption spectra of **DNA1** (top) and **DNA2b** (bottom), 2.5 μM in 10 mM Na-P_i buffer, pH 7.

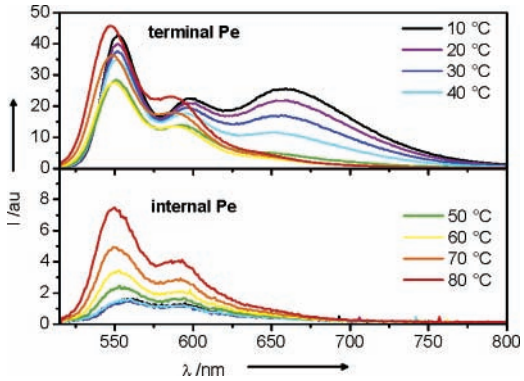

Figure 2. Temperature-dependent fluorescence spectra of **DNA1** (top) and **DNA2b** (bottom), 2.5 μM in 10 mM Na-P_i buffer, pH 7, excitation at 505 nm.

mophore above the melting temperature (T_m = 51 °C) results in small changes in the absorption, mainly a small hypsochromic shift from ~550 nm to 545 nm. Additionally, the 550 nm absorption band of **DNA1** shows a significant increase above the melting temperature. The latter result is remarkable and similar to the absorption properties of perylene bisimide-capped DNA hairpins that have been published recently by the group of Lewis et al.[8]

The melting temperatures of both duplexes, **DNA2a** and **DNA2b**, recorded at 260 nm (Figure S3, Supporting Information) are identical (T_m = 48 °C). This result indicates that the counterbase opposite to the intercalated perylene bisimide chromophore is in a bulged extrahelical position. Such a behavior has been previously observed for other modified DNA duplexes bearing chromophores as artificial DNA bases, e.g., in the case of ethidium- or indole-modified DNA[12,13] or thiazole orange-modified PNA−DNA hybrids.[15]

The hypsochromic shift of the absorption maxima that is observed for all three DNA duplexes at temperatures higher than the melting transition can be attributed to the partial destacking of the perylene bisimide chromophore with the adjacent DNA base pairs as a result of the duplex dehybridization. To support this interpretation, we compared representatively the absorption of **ssDNA2** in H_2O and in DMSO (Figure S2, Supporting Information). DMSO is known to disrupt the secondary structure of nucleic acids by interrupting the stacking interactions.[16] Therefore the absorption of the perylene bisimide in DMSO shifts hypsochromic by ca. 18 nm. The smaller shift from duplex **DNA2** to **ssDNA2** (Figure 1 and Figure S1, Supporting Information) of ca. 5 nm shows that a significant amount of stacking interactions of the perylene bisimide with the DNA bases is also present in the random-folded single-stranded oligonucleotides.

The emission properties of the duplexes **DNA1** and **DNA2a**/**DNAb** were studied temperature-dependently (Figure 2). Due to the small Stokes shift of the perylene bisimide dye the excitation wavelength for the steady-state fluorescence spectra was set to 505 nm in order to allow measuring the emission at wavelengths >515 nm. **DNA2a** (Figure S7, Supporting Information) and **DNA2b** show only very low emission quantities. A nearly complete quenching occurs in these duplexes below the melting temperature. Slightly higher emission is observed after dehybridization of the duplex at higher temperatures. Nevertheless, even in the single-stranded oligonucleotides that are present above the melting temperature the stacking interactions between the perylene bisimide chromophore and the adjacent DNA bases are strong enough for a significant fluorescence quenching. Based on redox potentials from the literature,[17] we assume that the observed fluorescence quenching is the result of a very efficient charge transfer process, meaning an injection of a positive charge into the DNA duplex yielding the corresponding perylene bisimide radical anion. We currently perform experiments to prove this hypothesis.

Very interesting is the observation that the fluorescence spectra of **DNA1** below the melting temperature exhibit an additional emission peak with a broad maximum at ~660 nm that represents the excimer-type fluorescence of the perylene bisimide dye that has been also observed in nanoaggregates of perylene bisimides.[18] It is remarkable that the excimer emission of **DNA1** vanishes at temperatures higher than the melting point of this duplex. Obviously, the intact secondary structure of the duplex is required for an efficient excimer formation between two perylene bisimide caps. The temperature dependence of the excimer-type emission behavior tracks well with the melting curve of **DNA1** at at 260 nm and at 540 nm as mentioned previously (see Figure 1 and Figure S4, Supporting Information). Moreover, the excimer formation of **DNA1** is clearly dependent on the duplex concentration in the sample (Figure 3). At higher concentration, a significantly higher ratio between excimer-type and monomer fluorescence (~660 nm

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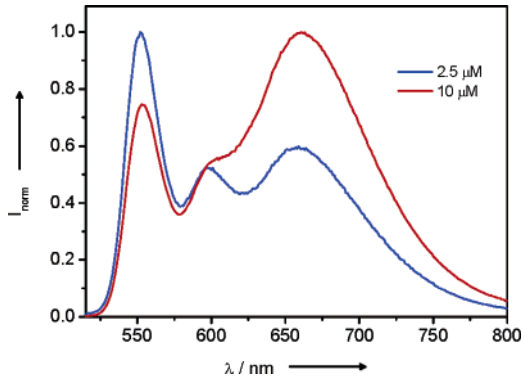

Figure 3. Normalized fluorescence spectra of **DNA1** at two different concentrations, in 10 mM Na-P_i buffer, pH 7, excitation at 505 nm.

vs ~550 nm) was recorded. Interestingly, at the higher concentration (10 μM) the excimer-type fluorescence of **DNA1** shows a similar thermal melting behavior (Figure S4, Supporting Information) as at low concentration (2.5 μM). With respect to the 5′-terminal position of the perylene bisimide dye in **DNA1**, our observations indicate a dimerization of two duplexes of **DNA1** via stacking of two perylene bisimide chromophores. This observation is supported by the CD spectrum of **DNA1** (Figure 4) that shows a strong band in the absorption range of the chromophore between 450 and 600 nm. This CD signal can be attributed to a an exciton coupling between two perylene bismide dyes. Similar observations have been reported for perylene bisimide-capped DNA hairpins.[8] It is important to note that **ssDNA1** cannot form such excimers and that two whole perylene bisimide-capped DNA duplexes are needed for the efficient dimerization. Hence, this type of perylene-capped oligonucleotides could be used in homogeneous DNA assays, for instance, for the sequence-selective detection of the complementary oligonucleotide by the excimer-type fluorescence. The internally perylene-modified oligonucleotides could be of potential use either as a electron acceptor[19] or electron hole donor for charge transport studies in DNA.

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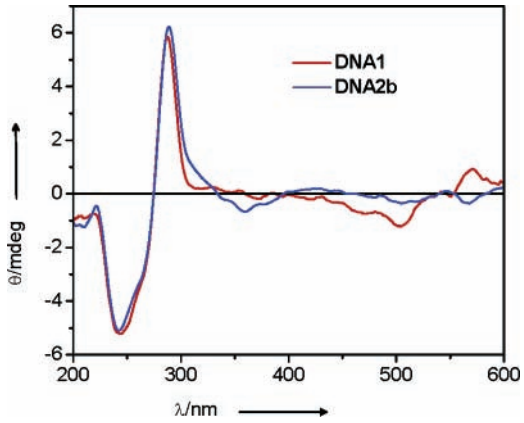

Figure 4. CD spectra of **DNA1** and **DNA2b**, 10 μM in 10 mM Na-P_i buffer, pH 7.

In conclusion, our approach represents a facile and fast access to DNA duplexes that have been modified with a perylene dye either at the 5′-terminal position or at internal positions. These modified oligonucleotides exhibit strong stacking interactions of the perylene bisimide chromophore with the DNA bases. The dimerization of **DNA1** indicates a high potential of this kind of DNA−perylene bisimide conjugates for the formation of ordered and thermally stable supramolecular DNA-based architectures for nanotechnological applications.

Acknowledgment. This work was supported by the Deutsche Forschungsgemeinschaft (Wa 1386/7), the Fonds der Chemischen Industrie, and the University of Regensburg. C.W. thanks the Technical University of Munich for a Ph.D. fellowship from 2003 until 2005.

Supporting Information Available: Experimental procedures and characterization for substances **4** and **5** and the modified DNA as mentioned in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

OL061246X