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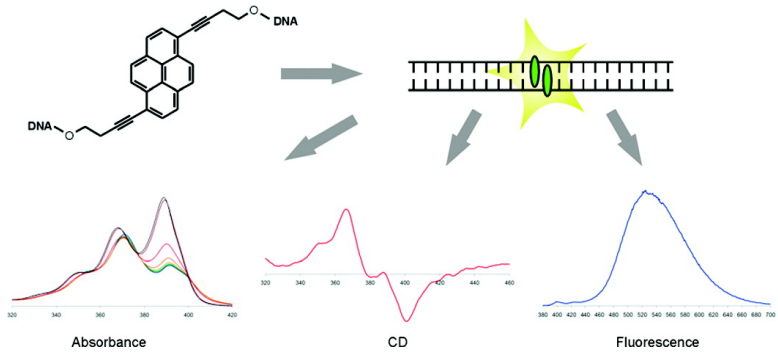

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Dialkynylpyrenes: Strongly Fluorescent, Environment-Sensitive DNA Building Blocks

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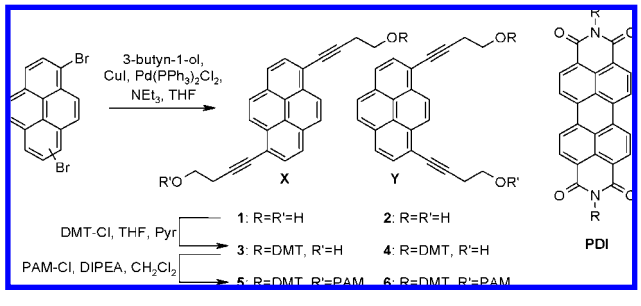
Perylenetetracarboxylic acid diimide (PDI; Scheme 1) represents a polyaromatic compound that has been extensively studied in the context of pigment research and optical as well as molecular electronic devices.[1−3] The compound shows a remarkable ability to self-organize into defined structures,[4−7] which has been applied to the construction of different DNA-based hybrids adopting stable hairpin and triplex structures.[8−13] Characteristic changes in the absorption vibronic band pattern are commonly used for monitoring PDI aggregation processes.[1,2,4−6,9−11,14,15] Relative orientation of multiple PDI units is assessable via characteristic CD signals and the occurrence of exciton coupled CD upon formation of twisted aggregates.[10,11] The fluorescence properties of PDI derivatives[1,2,16−18] prompted their use in the field of DNA-based diagnostics. Unfortunately however, in combination with DNA a dramatic loss of the quantum yield is observed.[8,10,12,19,20] In this study we demonstrate that dialkynyl pyrenes (DAP; 1,6- and 1,8-isomers) in conjugation with oligonucleotides (ONs) show very attractive properties similar to PDI−DNA hybrids, including a well-defined structural organization of hybrids and the possibility to use UV−vis and CD spectroscopy as signal readouts. Furthermore, in contrast to PDI−DNA conjugates, DAP−DNA conjugates are strongly fluorescent.

Extension of the pyrenyl system by two triple bonds was investigated for several reasons. First, advanced optical properties upon such extension were demonstrated for several classes of compounds, such as anthracene,[21] porphyrine,[22] and pyrene.[23−26] Moreover, extension of the π-system should increase the hydrophobic character, thus facilitating stacking interactions within the DNA conjugates and hybrids. Finally, it was interesting to see whether the introduction of the triple bond would still allow the pronounced self-organizing properties as in the case of pyrenedicarboxamide[27−34] or triazolyl derivatives[35] described previously.

Scheme 1. Preparation of Phosphoramidites **5** and **6**; Structure of PDI



A mixture of 1,6- and 1,8-dibromopyrene[35,36] was reacted with 3-butyn-1-ol under *Sonogashira* conditions, yielding a mixture of diols **1** and **2** of which pure isomeric fractions were obtained by column chromatography.[37] Reaction of the two isomers with DMT chloride furnished the monoprotected alcohols **3** and **4** which were converted to cyanoethyl phosphoramidites **5** and **6** by standard procedures (Scheme 1). Incorporation of the phosphoramidites into ONs **7**−**14** by solid-phase synthesis was straightforward (Table 1). It is noteworthy that, in contrast to the analogous PDI phosphoramidite,[20] **5** and **6** are readily soluble in acetonitrile, which significantly facilitates ON synthesis.

Table 1. Sequences of Oligonucleotides (ONs) Containing Dialkynylpyrene Building Blocks **X** and **Y** (for Structures See Scheme 1)

ON	sequence
7	5′-AGC TCG GTC A**X**C GAG AGT GCA-3′
8	3′-TCG AGC CAG T**X**G CTC TCA CGT-5′
9	5′-AGC TCG GTC **XX**C GAG AGT GCA-3′
10	3′-TCG AGC CAG **XX**G CTC TCA CGT-5′
11	5′-AGC TCG GTC A**Y**C GAG AGT GCA-3′
12	3′-TCG AGC CAG T**Y**G CTC TCA CGT-5′
13	5′-AGC TCG GTC **YY**C GAG AGT GCA-3′
14	3′-TCG AGC CAG **YY**G CTC TCA CGT-5′

UV−vis absorption spectra of the monomeric building blocks **1** and **2** were recorded in THF and water (Table 2 and Supporting Informaton, SI), revealing well-resolved vibronic bands in the 320−420 nm range. Comparison of absorption and fluorescence emission spectra of the corresponding ONs **7** and **11** showed nearly perfect mirror-image behavior as well as significant overlap of the most intense signals (SI), implying assignment of the longest wavelength absorption band to the $S_1 \leftarrow S_0$ transition; consequently, the vibronic bands were assigned to the (0→0) and (0→1) transition for the longest and second-longest wavelength band, respectively. In agreement with other alkynylpyrene derivatives,[38,39] the high absorptivities (Table 2) indicate that the $S_1 \leftarrow S_0$ transition is strongly allowed.

Table 2. UV−vis Absorption Data of Diols **1** and **2** (Concentration: 3 μM)

		$S_1^{v=0} \leftarrow S_0^{v=0}$		$S_1^{v=1} \leftarrow S_0^{v=0}$		
		λ_{max} (nm)	ε^a	λ_{max} (nm)	ε^a	ratio[b]
1	THF	386	8.6	364	5.0	1.7
	water[c]	384	6.2	363	4.4	1.4
2	THF	385	6.5	364	4.1	1.6
	water[c]	382	5.1	363	3.7	1.4

[a] $\times 10^4$ M^{-1} cm^{-1}. [b] Intensity ratio of the two vibronic bands. [c] Containing 1.5% THF, measured at 90 °C.

UV−vis spectra were recorded both for single strands and for hybrids containing either one or two pyrene units per strand. For ONs **7** and **11**, an absorption pattern similar to those of the monomeric building blocks with the maxima slightly shifted to longer wavelengths can be observed. The relative intensities of the absorption bands change, and signals are broadened when two pyrene units are present as in **9** and **13** (Figure 1; see SI for spectra

of the other ONs). Upon hybridization of **7** and **8**, an analogous intensity decrease of the longest wavelength band along with a red shift can be monitored by temperature-variable UV−vis spectroscopy (Figure 2; see SI for **11*****12**). The temperature-variable spectra very closely resemble the vibronic band pattern changes observed upon aggregation of PDI.[10,14] Hyperchromicity at 390 nm exceeds 100%, underlining the high sensitivity of the vibronic bands toward dialkynylpyrene aggregation.

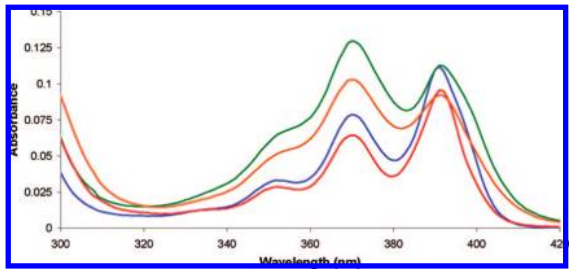


Figure 1. UV−vis spectra (300−420 nm region only) of ONs **7** (blue), **9** (green), **11** (red), and **13** (orange; ON concn: 1.5 μM; 10 mM phosphate buffer, pH 7.0, 100 mM NaCl).

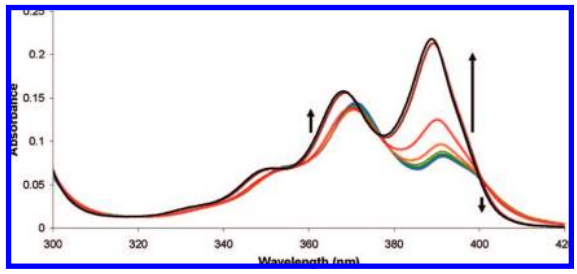


Figure 2. Temperature-dependent UV−vis spectrum of hybrid **7*****8** (10 °C intervals, 20 to 90 °C; arrows indicate rising temperature (ON concn: 1.5 μM each strand; 10 mM phosphate buffer, pH 7.0, 100 mM NaCl).

Thermal denaturation experiments showed distinct heating/cooling hysteresis for all hybrids (Figure 3), indicating a comparatively slow organization processes in the pyrene area. Due to the abovementioned high sensitivity of the vibronic band intensities, even small changes in the relative orientation of the pyrene moieties lead to significant differences in the absorption bands.

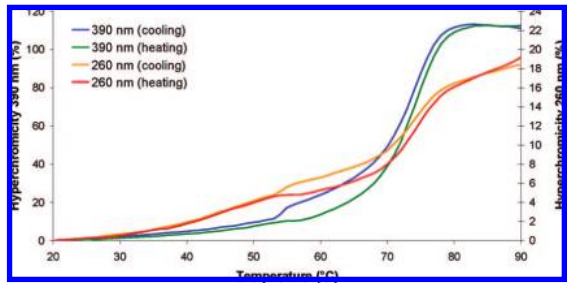


Figure 3. Heating and cooling curves of duplex **7*****8** (1.5 + 1.5 μM in 10 mM phosphate buffer pH 7.0, 100 mM NaCl).

The hysteretic behavior could be attributed to the pyrene units serving as points of interactions between the two strands. These interactions are primarily based on hydrophobic interactions and, thus, show different temperature dependence and dynamics than in the case of the formation of hydrogen bonds between the natural bases.[40] Alternatively, minor conformational changes in the natural DNA parts prior to melting of the duplex can lead to rearrangements in the pyrene area that are reflected in a change in absorbance, which, in turn, precludes the unambiguous determination of T_M values by means of UV−vis spectroscopy (see below).

CD measurements on single strands and hybrids gave insight into the ability of dialkynylpyrenes to self-organize within a DNA framework. For duplex **7*****8**, exciton coupling[41,42] leads to well-resolved signal splitting with extrema at 400 and 366 nm. Surprisingly, the signal shape almost perfectly matches that of two PDI units as described recently,[10,20] yet at a different wavelength (Figure 4, top). A similar pattern is found for **11*****12**. Even in single stranded ONs **9** and **13** exciton coupling is observable, while, for **10** and **14** unidirectional, induced CD signals are observed (SI). Remarkably, for duplex **13*****14**, which exhibits the highest quantum yield of all hybrids containing four pyrene units (see below), the CD spectrum is dominated by extraordinarily strong negative signals in the 350−400 nm range (Figure 4, bottom). These findings, along with the high fluorescence quantum yield, indicate a well-defined, helical structure in the pyrene region.[32,43]

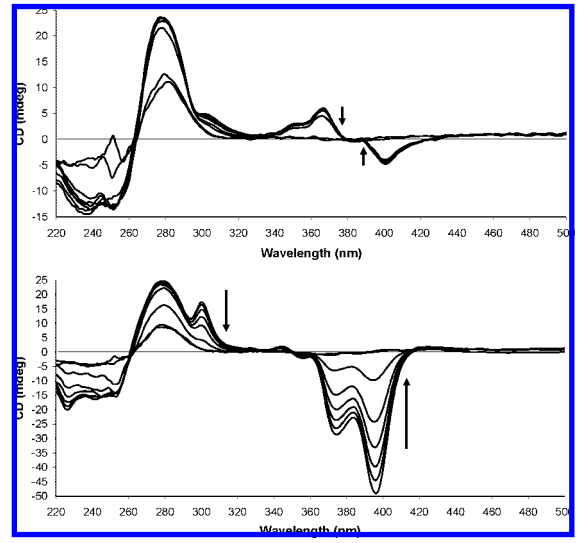


Figure 4. Temperature-dependent CD spectra of **7*****8** (top) and **13*****14** (bottom), recorded in 10 °C steps from 20 to 90 °C; direction of arrows indicates increasing temperature (ON concentration: 4.5 + 4.5 μM in 10 mM phosphate buffer pH 7.0, 100 mM NaCl).

As mentioned above, the excellent fluorescence properties of PDI in organic solvents are completely suppressed in DNA conjugates which require aqueous buffer systems representing a major drawback of PDI−DNA conjugates. Thus, the fluorescence properties of DAP-modified ONs are of fundamental interest. ONs containing one DAP fluorophore exhibit monomer emission spectra that are red-shifted by ~20 nm in comparison to unsubstituted pyrene. When two pyrene units are incorporated into one strand or when two strands each containing one unit are hybridized, the monomer fluorescence disappears completely in favor of a strong excimer signal with a maximum at ~525 nm (Figure 5 and SI).

For use as diagnostic tools, fluorescent tags are expected to exhibit reasonably high quantum yields and brightness.[44,45] Quantum yields were, therefore, determined for single stranded DAP−ONs and selected hybrids (Table 3). Remarkably, for both isomers quantum yields increase dramatically, when two single strands bearing one pyrene (**7**, **8**, **11** and **12**) hybridize, giving rise to interstrand excimer formation. It is noteworthy that excimer emission of DAP−DNA is not significantly influenced by adjacent bases, which allows for versatile applications of these tags.[46] In cases where intra- or interstrand excimers or exciplexes of two units can be formed, high quantum yields (>0.20) are found. The highest value of 0.39 is found for duplex **11*****12**. Considering furthermore the high absorptivitiy of **11*****12** (72 000 M^{-1} cm^{-1}, 370 nm; see SI for details), a remarkably high fluorescence brightness of up to

28 000 M^{-1} cm^{-1} is obtained. Thus, DAP−DNA conjugates exhibit high fluorescence quantum yields along with a general red shift, similar to the recently reported triazolylpyrene−DNA conjugates.[35]

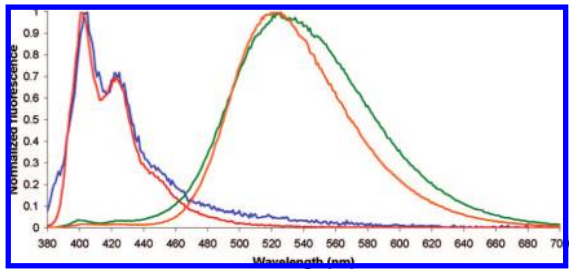


Figure 5. Normalized fluorescence spectra of **7** (blue), **11** (red), **7*8** (green), and **11*12** (orange; ON concn: 1.5 μM each strand; 10 mM phosphate buffer, pH 7.0, 100 mM NaCl; excitation wavelength: 370 nm).

Table 3. Fluorescence Quantum Yields of Single Strands and Duplexes (for Wavelengths see Supporting Information)

ON	Φ	ON	Φ	ONs	Φ	ONs	Φ
7	0.11	**11**	0.049	**7*8**	0.26	**11*12**	0.39
8	0.008	**12**	0.015	**7*12**	0.36	**11*8**	0.36
9	0.32	**13**	0.36	**9*10**	0.13	**13*14**	0.33
10	0.32	**14**	0.21	**9*14**	0.17	**13*10**	0.17

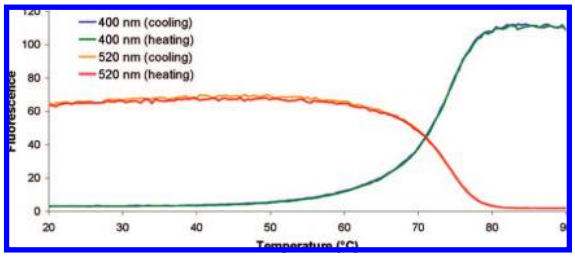


Figure 6. Heating/cooling curves of hybrid **7*8**, observed by fluorescence measurement at 400 nm (monomer) and 520 nm (excimer); excitation: 385 nm; 1.5 + 1.5 μM; 10 mM phosphate buffer pH 7.0, 100 mM NaCl.

The excimer signal serves as a readout for T_M determination which is not possible by UV−vis spectroscopy as mentioned above. This is demonstrated for hybrid **7*8**, which was also analyzed by UV−vis (Figure 3). With fluorescence detection, a T_M of 74 °C is obtained (Figure 6; see SI for **11*12** and unmodified duplex).

In conclusion, the synthesis of two isomeric dialkynylpyrene phosphoramidites and their incorporation into oligonucleotides have been demonstrated. The pyrene units show features that closely resemble those of the well-known perylene bisimide dye PDI with regard to its ability to self-organize within a DNA duplex. Thus, the dialkynylpyrenes open the possibility to gain information on structural details of DNA hybrids through characteristic absorption patterns and CD signals. Similar to PDI, the alkynyl pyrenes have high absorptivities in their S_0-S_1 transitions. In contrast to PDI, however, dialkynylpyrenes exhibit significant monomer as well as remarkably strong excimer fluorescence by formation of either intra- or interstrand excimers. Considering the relative ease of synthesis and the high quantum yields of the DNA conjugates, these building blocks represent promising candidates for applications in diagnostic tools like excimer-based molecular beacons, as well as for novel DNA-based materials with special optical properties.

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Supporting Information Available: Synthetic procedures, analytical details, additional melting curves, UV−vis, fluorescence and CD spectra. This information is available free of charge via the Internet at http://pubs.acs.org.

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