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Triazolylpyrenes: Synthesis, Fluorescence Properties, and Incorporation into DNA

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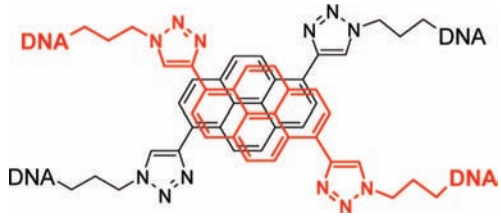
ABSTRACT



Synthesis of 1,6- and 1,8-triazolylpyrenes and their incorporation into oligonucleotides is described. In hybrids, triazolylpyrenes adopt interstrand stacking interactions. Exciton coupling is observed for the duplex containing a pair of the 1,6-isomer indicating a well-defined helical arrangement of the triazolylpyrene building blocks. Triazole substitution results in pronounced red-shifts of monomer as well as excimer fluorescence. Furthermore, quantum yields of the formed excimers are remarkably high.

Nucleic acids are of interest as nanometer-sized functional matter.[1,2] The highly reliable recognition pattern of the natural bases allows the well-designed construction of large assemblies. Introduction of unnatural building blocks into oligonucleotides greatly enhances the number of possible architectures and allows the design of DNA-like constructs which are highly diverse with regard to structural and functional properties.[3,4] The incorporation of simple, non-nucleosidic types of aromatic building blocks into oligonucleotides is intensively studied.[5–10] They are used as hairpin replacements[11–17] or as units for conformational control in DNA.[18–20] Recently, the helical arrangement of polyaromatic compounds within a DNA framework was reported.[21–24] In particular, extended stretches of achiral pyrene building blocks (**A**, Figure 1) were found to form double-helical self-assemblies.[22,25]

Due to their many interesting fluorescence properties, such as long lifetime, high quantum yield, and the possibility to form excimers, pyrene and its derivatives are of considerable interest for the development of sensors and diagnostic tools.[26–30] In addition, the photophysical properties of pyrene derivatives render them promising candidates for applications in materials sciences, e.g., as components in OLEDs.[31,32]

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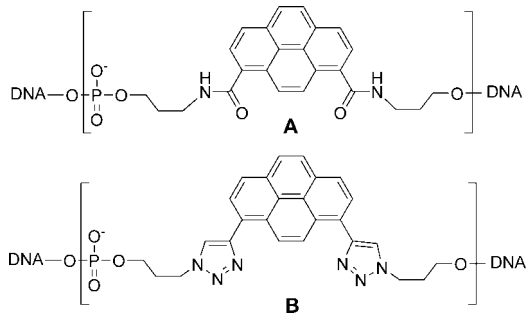

Figure 1. Carboxamide- (**A**) and triazolyl-substituted (**B**) pyrenes as building blocks in oligonucleotides.

As part of our efforts to develop new functional building blocks maintaining a high level of duplex organization, we have envisaged the synthesis of π-extended pyrene systems, such as the bistriazolyl derivative **B**. Besides their attractive electronic properties, triazoles are of particular interest in the present context, since they have been used as isosteric replacements of carboxamides.[33] However, except for one recent publication reporting the postmodification of oligonucleotides with azidopyrene,[34] no accounts on triazole substituted pyrenes exist in the literature. Here, we describe the synthesis and properties of bistriazolylpyrenes, as well as their effects on DNA hybrid stability and helical organization.

The synthesis of the target compounds is shown in Scheme 1. The known 1,8- or 1,6-isomeric diethynylpyrenes **1** and **2**[35] were treated with a 1:1 mixture of unprotected and 4,4′-dimethoxytrityl (DMT)-protected 1,3-azidopropanol, which are best prepared in situ from a mixture of the corresponding bromides.[36,37] After reaction at 50 °C for 15 h in the presence of cuprous iodide and sodium ascorbate, the bistriazolylpyrenes **3** and **4** were isolated after column chromatography. The Huisgen 1,3-dipolar cycloaddition is widely used for the preparation of 1,2,3-triazoles, in particular due to the finding that the presence of Cu(I) enhances rate as well as regioselectivity of the reaction between alkynes and azides.[38,39] The overall yields of 26 and 19% are appreciable since the method involves a two-step procedure and multiple reaction centers. The obtained alcohols were further converted into the phosphoramidites **5** and **6**. The two building blocks so obtained were subsequently incorporated into oligonucleotides (Table 1). Oligomers **7**−**12** were prepared by standard oligonucleotide synthesis, purified by reversed-phase HPLC and characterized by ESI-TOF MS (Supporting Information).

The properties of the triazolylpyrene-modified oligonucleotides were analyzed by thermal denaturation experiments as well as by UV−vis, fluorescence, and CD spectroscopy. Data were collected in two different buffer systems: Tris−HCl, which is commonly used in studies with DNA-based diagnostic tools, and phosphate buffer which is advised for temperature dependent measurements (Table 1). Hybrids **9*10** and **11*12** each contain an equal pair of the 1,6- or 1,8-bistriazolylpyrene isomers, while **9*12** and **11*10** are examples of hybrids with unequal building blocks. As shown in Table 1, replacement of an AT base pair with triazolylpyrenes generally results in a slight increase of hybrid stability.

Cooperativity of duplex formation and melting was confirmed by monitoring the hybridization process at different wavelengths, which showed that the stacking events in the DNA segments and in the pyrene region occur simultaneously.

As expected, single strands **9**−**12**, which contain one triazolylpyrene building block, show monomer fluorescence on excitation at 350 nm (Figure 2, top). The notable differences in fluorescence intensity can be ascribed on the one hand to the quenching effect of the neighboring bases[40] (single strands **10** and **12**) but obviously also to the regioisomeric substitution pattern of the pyrenes, since oligomer **9** containing a 1,6-triazolylpyrene shows a considerably higher fluorescence intensity than oligomer **11** having a 1,8-isomer. These differences are also reflected in the respective fluorescence quantum yields (Φ_f) given in Table 2. In contrast to the single strands, all hybrids show intensive excimer emission with a maximum intensity at 520 nm (Figure 2, bottom), which indicates an interstrand stacking arrangement of the triazolylpyrenes. In agreement with this, duplex melting is accompanied with an excimer-to-monomer change of the fluorescence signals (Figure 3). T_m values determined by fluorescence measurements (Sup-

Scheme 1. Synthesis of Bistriazolylpyrene Building Blocks

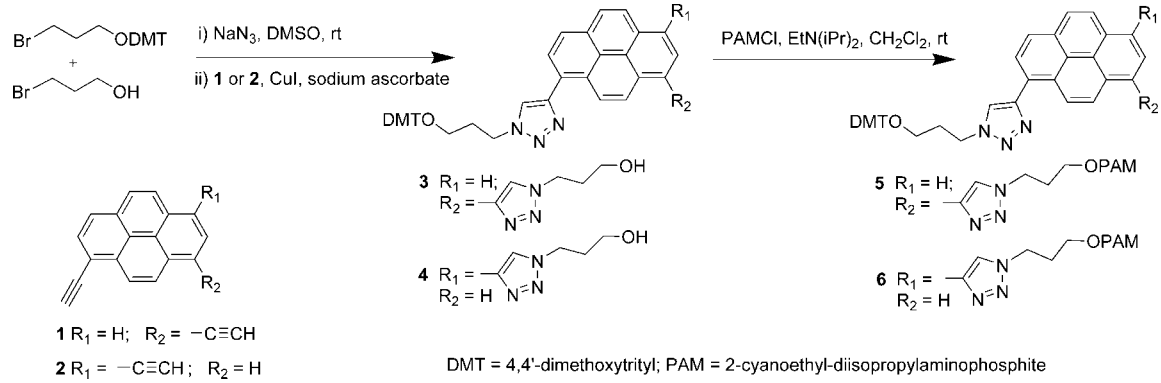

Table 1. Influence of Triazolylpyrenes on Duplex Stability[a]

	hybrid	T_m[b] (ΔT_m)[c] (°C)[d]	T_m[b] (ΔT_m)[c] (°C)[e]
7 **8**	5′AGCTCGGTCATCGAGAGTGCA 3′TCGAGCCAGTAGCTCTCACGT	70.1	71.2
9 **10**	5′AGCTCGGTCA**Y**$_{1.6}$CGAGAGTGCA 3′TCGAGCCAGT**Y**$_{1.6}$GCTCTCACGT	72.4 (2.3)	72.8 (1.6)
11 **12**	5′AGCTCGGTCA**Y**$_{1.8}$CGAGAGTGCA 3′TCGAGCCAGT**Y**$_{1.8}$GCTCTCACGT	72.0 (1.9)	71.5 (0.3)
9 **12**	5′AGCTCGGTCA**Y**$_{1.6}$CGAGAGTGCA 3′TCGAGCCAGT**Y**$_{1.8}$GCTCTCACGT	70.8 (0.7)	71.7 (0.5)
11 **10**	5′AGCTCGGTCA**Y**$_{1.8}$CGAGAGTGCA 3′TCGAGCCAGT**Y**$_{1.6}$GCTCTCACGT	69.3 (−0.8)	70.7 (−0.5)

[a] Conditions: 1.0 μM oligomer concentration; 100 mM NaCl. [b] T_m at 260 nm; [c] difference in T_m relative to duplex **7*8**. [d] 10 mM Tris−HCl buffer, pH 7.4. [e] 10 mM phosphate buffer, pH 7.0.

porting Information) correspond very well with the ones obtained from UV absorbance (Table 1). Furthermore, substitution with two triazole rings exhibits a distinct red-shift for monomer as well as excimer emissions (~20 and ~40 nm, respectively) compared to unsubstituted pyrene.

Sensitivity of pyrene fluorescence emission to the environment and conformational changes is of fundamental importance for the design of molecular probes. Quantum yields of pyrene, as well as other fluorescent dyes, are often significantly decreased in aqueous buffered systems.[40,41] In light of this, the high quantum yields of the triazolylpyrene-modified hybrids **9*10** and **11*12** (0.42 and 0.53, Table 2) are noteworthy. To the best of our knowledge, these are the highest excimer quantum yields reported so far for pyrene-containing DNA conjugates (see, e.g., refs 41 and 42).

Table 2. Emission Maxima and Quantum Yields (Φ_f) of Single Strands **9-12** and Hybrids **9*10** and **11*12** at 20 °C in Phosphate Buffer, pH 7.0 (Quinine Sulfate as Standard, Φ_f = 0.546)

	9	**10**	**11**	**12**	**9*10**	**11*12**
λ_{max} (nm)	406, 425	408, 429	406, 425	408, 429	520	520
Φ_f	0.27	0.05	0.09	0.06	0.42	0.53

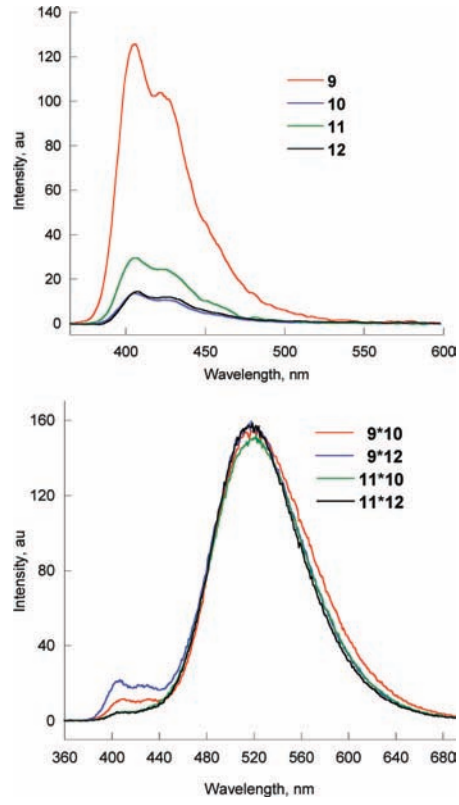

Figure 2. Emission spectra of single strands **9**−**12** (top) and hybrids **9*10**, **9*12**, **11*10**, and **11*12** (bottom). Conditions: oligomer concentration 1.0 μ M, 10 mM phosphate buffer, 100 mM NaCl, pH 7.0, 20 °C; excitation wavelength 350 nm.

Excitation spectra of triazolylpyrene-modified single strands and hybrids show distinct differences, which are in agreement with interstrand stacking of the triazolylpyrene units in the duplex: vibronic bands are better resolved and maxima are

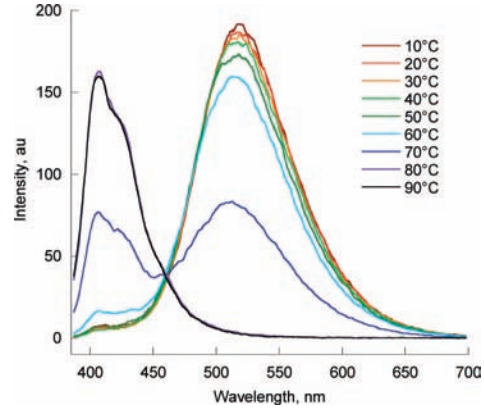

Figure 3. Temperature-dependent fluorescence spectrum of hybrid **11*12**: emission changes from excimer to monomer upon hybrid melting. For conditions, see Figure 2.

slightly blue-shifted in the hybrids (Supporting Information). Similar differences between single strands and hybrids are also observed in the UV−vis spectra (Supporting Information). These differences can be explained by a reduced flexibility of the triazolylpyrene rings within the duplex. Similar spectroscopic changes of the vibronic band intensities and shifts upon association/dissociation were reported for a perylene-modified DNA.[15]

Further insight on the structural details in the pyrene region was obtained by circular dichroism (CD) analysis. The CD spectrum of hybrid **9*10**, which contains a pair of the 1,6-isomer of bistriazolylpyrene, showed exciton coupling of the pyrene units, whereas only a chirally induced CD but no signal splitting could be detected for the hybrid **11*12** (Figure 4). Notably,

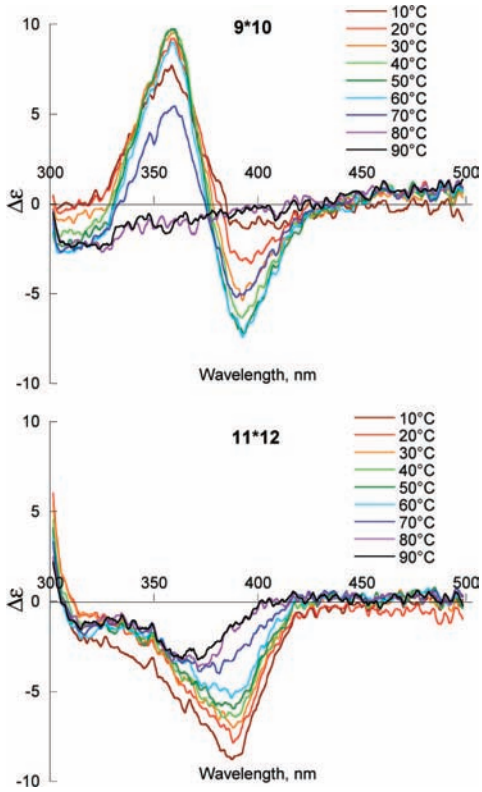

Figure 4. Temperature-dependent CD spectra of hybrids **9*10** (top) and **11*12** (bottom). Conditions: 5.0 μM oligomer concentration, 10 mM phosphate buffer, 100 mM NaCl, pH 7.0; $\Delta\epsilon$ (mol^{-1}•dm^3•cm^{-1}).

the duplex **9*10** also showed the highest melting temperature of all hybrids (see Table 1). One possible explanation is that the local flexibility in the pyrene region is significantly decreased in the hybrid **9*10** in comparison to hybrid **11*12**. Thus, the 1,6-isomer of bistriazolylpyrene would adopt a more rigid, twisted arrangement, whereas the 1,8-isomer leads to a more flexible local structure. Exciton-coupled CD spectra were reported previously for a system containing non-nucleosidic perylene building blocks,[43] as well as for oligonucleotides containing extended stretches of pyrenebisamides.[22] No helical organization was observed, however, for a single pyrene pair.[6] Thus, the present finding is the first example of a single pair of non-nucleosidic pyrenes with a rigid helical conformation in a duplex.

In conclusion, 1,6- and 1,8-triazolylpyrenes were synthesized from the respective diethynylpyrenes via alkyne azide "click" cycloaddition. The compounds were further transformed into phosphoramidites and incorporated into oligonucleotides. Replacement of a natural AT base pair with two triazolylpyrenes results in a slight stabilization of the duplex. Quantum yields of excimers formed on hybridization are in the range of 0.5, which is significantly higher than those of known pyrene-containing DNA hybrids. CD spectroscopy revealed exciton coupling for the duplex containing a pair or 1,6-isomers (hybrid **9*10**), indicating a well-defined helical arrangement of the triazolylpyrene building blocks. No exciton coupling was observed in hybrids containing the 1,8-isomer. Stability of hybrids, different interstrand organization, and attractive fluorescence properties render triazolylpyrenes promising candidates for use in DNA-based diagnostics and applications in materials sciences.

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Supporting Information Available: Experimental and analytical details; UV−vis, CD, and fluorescence spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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