# 1-Ethynylpyrene as a Tunable and Versatile Molecular Beacon for DNA

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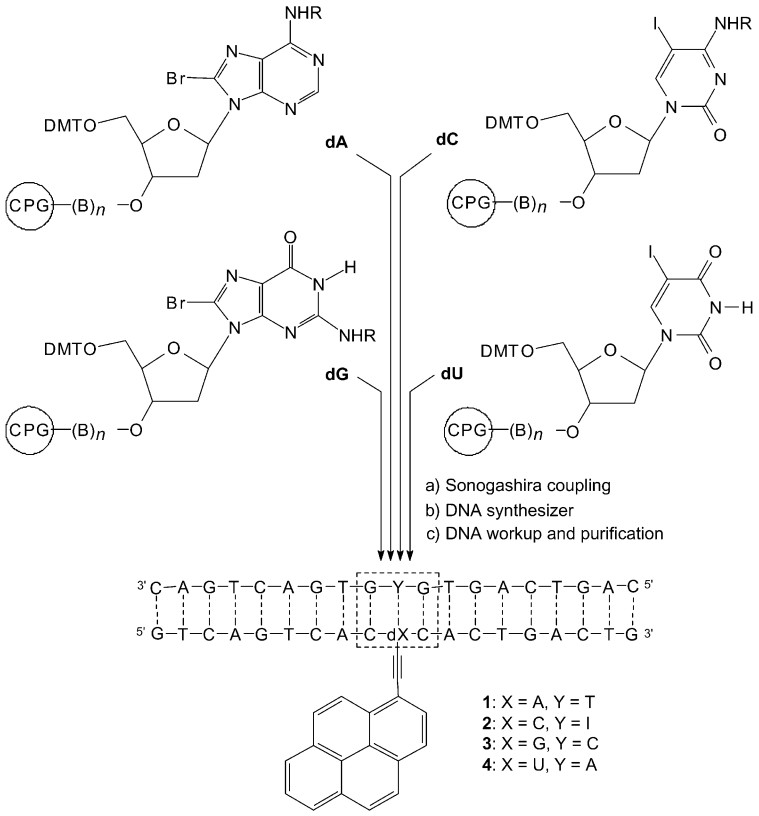
Fluorescent or luminescent probes that are sensitive to the local environment within DNA duplexes represent important tools for DNA hybridisation[1] and conformational changes caused by DNA–protein interactions,[1] or for the detection of physiologically important DNA base mismatches or lesions on DNA chips or microarrays.[2] As a consequence, there is a continuously increasing demand for new fluorophores that have a clear and specific range of spectral characteristics which are tunable to distinct excitation or emission wavelengths. One suitable and important way to create new emission properties is to attach chromophores covalently to natural DNA bases. Recently, we applied this modification strategy to the preparation of photoexcitable charge donors, which have been used for the investigation of DNA-mediated electron transport.[3,4]




***Scheme 1.*** *Schematic representation for the synthesis of the Py–C≡C–dX-modified DNA duplexes* ***1–4****. DMTO = dimethoxytrityl, B = DNA base, CPG = controlled pore glass, R = benzoyl or isobutyroyl, I = inosine.*

Herein, we report the properties of DNA duplexes bearing the 1-ethynylpyrene moiety (Py–C≡C) covalently attached to the bases dX = dA, dC, dG, or dU. Three structural features of these Py–C≡C–dX-modified DNA duplexes are important: i) a clear steric separation of the pyrene moiety from the DNA base stack due to the rigid ethynyl group, ii) a strong electronic coupling between the pyrene and the base moiety provided by the acetylene bridge and iii) a partial stacking of the base moiety as part of the delocalised Py–C≡C–dX chromophore. Moreover, the incorporation of the Py–C≡C–dX moiety could influence only the local conformation, but should not perturb the overall B-DNA duplex conformation.

The Py–C≡C–dX-modified oligonucleotides were synthesised by a semiautomated synthetic strategy with solid-phase Sonogashira-type cross-coupling conditions (Scheme 1).[4,5] It is important to note that a time-consuming synthesis of Py–C≡C–modified phosphoramidites[6] can be avoided because this modification protocol is based on commercially available DNA building blocks. First, the oligonucleotide was synthesised by following standard protocols on a DNA synthesiser up to the position of the Py–C≡C–dX unit. At this position, either 8-bromo-2′-deoxyadenosine, 2′-deoxy-5-iodocytidine, 8-bromo-2′-deoxyguanosine, or 2′-deoxy-5-iodouridine was inserted automatically without the final deprotection of the terminal 5′-OH group. Subsequently, the CPG vials were removed from the synthesiser and a Sonogashira-coupling reagent mixture containing $Pd(PPh_3)_4$ (60 mM), 1-ethynylpyrene (120 mM) and CuI (60 mM) in DMF/$Et_3N$ (3.5:1.5) was added to the CPG vials under dry conditions with syringes. After a coupling time of 3 h at room temperature, the CPGs were washed with different solvents, dried and attached to the DNA synthesiser to finish the synthesis automatically. Modification of the standard procedures for deprotection and cleavage of the oligonucleotides from the solid phase, or during workup was not necessary. The Py–C≡C–dX-modified oligonucleotides were purified by semi-preparative HPLC and identified by MALDI-TOF mass spectrometry. HPLC analysis of the unpurified oligonucleotides showed excellent coupling efficiencies of the Py–C≡C–unit to the oligonucleotides.

In the present work, a representative range of Py–C≡C–dX-modified duplexes **1**–**4** was prepared, that differ only by the base dX to which the pyrene modification group has been attached (Scheme 1). The base sequences of all duplexes **1**–**4** are the same and are based on modified duplexes we have used previously in electron-transport experiments.[3,4,7] Due to this experimental design, all observed spectroscopic differences between **1**–**4** can be attributed selectively to the different base

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pair dX–Y. In fact, the absorption properties of these Py–C≡C–dX-modified DNA duplexes depend remarkably on the attached base dX (Figure 1). The absorption spectra of the DNA

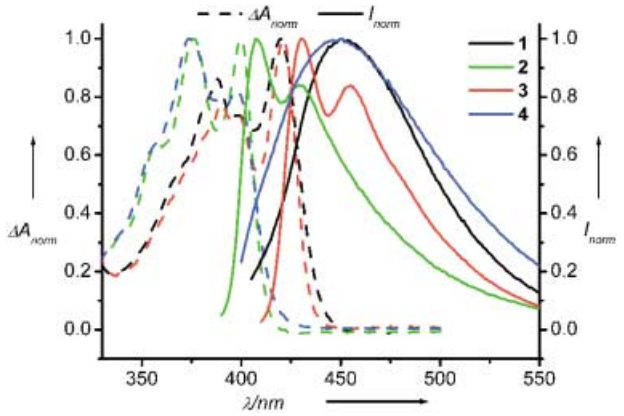


***Figure 1.*** *Normalised absorption and steady-state fluorescence spectra of DNA duplexes **1**–**4** in phosphate buffer (10 mM Na-P$_i$, pH 7.0), excitation at 385 nm (**1**), 376 nm (**2**), 391 nm (**3**), or 378 nm (**4**).*

duplexes **2** and **4** have their maxima at ~375 nm and ~400 nm. In comparison, the absorption of the DNA duplexes **1** and **3** is red-shifted significantly to ~390 nm and ~420 nm. Hence, the absorption properties can be attributed to two structurally different groups of duplexes. DNA **1** and **3** have the Py–C≡C– unit attached to purines (dA or dG), and DNA **2** and **4** have the Py–C≡C–unit attached to pyrimidines (dC or dU). The absorption difference between these two groups of duplexes could possibly be the result of different conformations of the Py–C≡C–dX-modified nucleosides (*anti* vs. *syn*).

The emission properties of the Py–C≡C–dX-modified DNA duplexes **1**–**4** vary significantly, too. However, in contrast to the absorption properties, the emission behaviour cannot be explained by simple structural features. In the Py–C≡C–dX group, the two chromophores are linked covalently by an acetylene bridge that provides the structural basis for a strong electronic coupling between them. A more careful look at the fluorescence spectra reveals that the emissions of the DNA duplexes **2** (dX = dC) and **3** (dX = dG) are typical for a pyrene structure with maxima at 408/430 nm or at 430/455 nm, respectively. This observation indicates a rather weak electronic coupling between the pyrene group and the heterocycles of the DNA bases dC or dG, although attachment of the pyrene group to the heterocycle of dG causes a significant red-shift of ~20 nm compared with dC. In contrast, the DNA duplexes **1** and **4** show broad and unstructured fluorescence bands with maxima at 447 nm and 452 nm, respectively. These emissions, which are different from that of pyrene, indicate that there is a strong electronic interaction between the chromophores in the Py–C≡C–dX moiety and show that a partial charge transfer takes place yielding intramolecular exciplexes containing both excited state and charge-separated state character.[8]

Based on this knowledge about the spectroscopic properties of DNAs **1**–**4**, their potential as molecular beacons for DNA analytics was elucidated in DNA hybridisation experiments. Accordingly, the UV/Vis absorption of the single-stranded (ss) Py–C≡C–dX-modified oligonucleotides **1**(ss)–**4**(ss) has been compared with the absorption of the corresponding DNA duplexes **1**–**4**. For all duplexes, a strong new absorption band appears at ~420 nm (**1** and **3**) or ~400 nm (**2** and **4**) as a result of the DNA-duplex formation (Figure 2). Hence, this absorption band

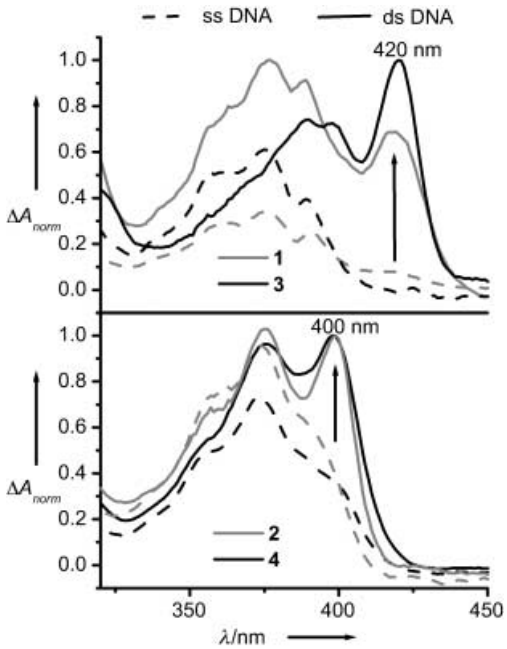


***Figure 2.*** *UV/Vis spectra of the single-stranded oligonucleotides **1**(ss)–**4**(ss) and DNA duplexes **1**–**4** (1.25 μM) in phosphate buffer (10 mM Na-P$_i$, pH 7.0).*

can be attributed to the ground-state interaction of the Py–C≡C–dX group with the adjacent base pairs (dC–dG). In order to rule out that such interactions also exist in the randomly folded single-stranded oligonucleotides, the absorption of **1**–**4** was measured temperature-dependently. Accordingly, the melting behaviour of the Py–C≡C–dX-DNA duplexes was recorded at 421 nm (**1** and **3**) or 400 nm (**2** and **4**; Figure 3 and

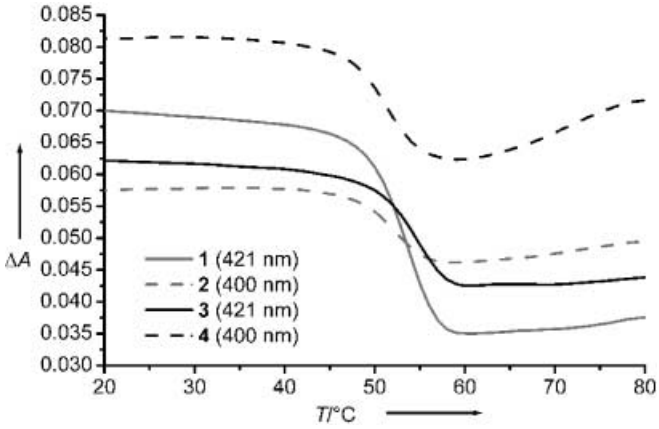


***Figure 3.*** *Melting temperatures ($T_m$) of the Py–C≡C–dX-modified DNA duplexes **1**–**4** (1.25 μM) in phosphate buffer (10 mM Na-P$_i$, 150 mM NaCl, pH 7).*

Table 1). The corresponding melting temperatures at these pyrene-specific wavelengths are ~10–15 °C lower than that of the whole DNA duplexes, which were recorded at 260 nm. This

***Table 1.*** *Melting temperatures ($T_m$) of the Py–C≡C–dX-modified DNA duplexes **1**–**4** (1.25 μM) in phosphate buffer (10 mM Na-$P_i$, 150 mM NaCl, pH 7).*

| DNA | $T_m$ (260 nm) | $T_m$ (400/421 nm) |
|---|---|---|
| **1** | 66 °C | 54 °C |
| **2** | 67 °C | 52 °C |
| **3** | 66 °C | 55 °C |
| **4** | 67 °C | 51 °C |

result shows that the local hybridisation at the Py–C≡C–dX-modification site breaks down at a lower temperature than the whole DNA duplex; this indicates a local structural perturbation. Possibly, such measurement of the local hybridisation in DNA by UV/Vis spectroscopy could be applied to the investigation of DNA–protein interactions, for example, to monitor base-flipping events of DNA damages in real-time.

The fluorescence properties of the Py–C≡C–dX-modified single-stranded oligonucleotides **1**(ss)–**4**(ss) show a remarkable difference from the spectra of the corresponding duplexes **1**–**4** (Figure 4). The fluorescence intensity increases by a factor up to 40 (in the case of **3**) if hybridisation with the complementary strand occurs. These fluorescence-intensity differences are highest when the DNA duplexes are excited at the wavelength of the ground-state interaction between the Py–C≡C–dX-group and the adjacent DNA bases, as described above (**1** and **3**: ~420 nm, **2** and **4**: ~400 nm).

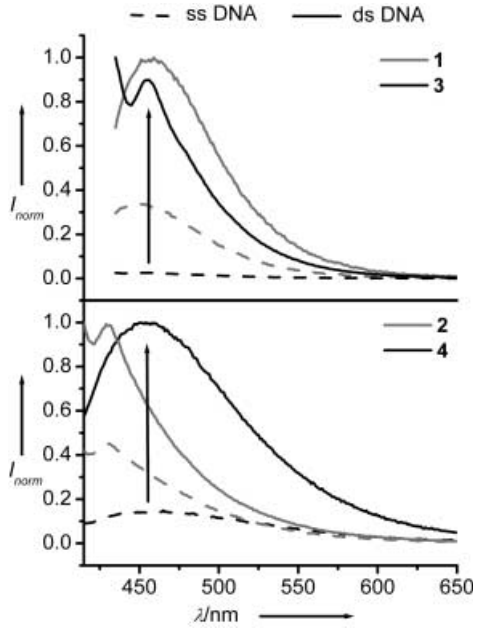


***Figure 4.*** *Steady-state fluorescence spectra of the single-stranded oligonucleotides **1**(ss)–**4**(ss) and DNA duplexes **1**–**4** (1.25 μM) in phosphate buffer (10 mM Na-$P_i$, pH 7.0), excitation at 422 nm (**1**), 400 nm (**2**), 422 nm (**3**), or 402 nm (**4**).*

In conclusion, it has been shown that the Py–C≡C– label could be introduced into oligonucleotides by using a solid-phase strategy based on commercially available phosphoramidites. The fluorescence and absorption properties of these Py–C≡C–dX-modified DNA duplexes can be tuned by variation of the attached nucleobase dX. Interestingly, the absorption and emission spectra of the Py–C≡C–dX-modified DNA duplexes overlay each other partially but not completely, which represents the prerequisite for FRET experiments. The DNA duplex hybridisation can be observed by both fluorescence and absorption spectroscopy. Hence, the Py–C≡C–dX group represents an important fluorescent label with different spectroscopic properties in single and double strands. Work is in progress to incorporate two different fluorescent probes by using only a single postsynthetic Sonogashira-type labelling reaction.

## Experimental Section

All experimental details about the preparation and spectroscopic characterisation of the DNA duplexes **1**–**4** are described in the Supporting Information; the results of MALDI-TOF-MS measurements and CD spectroscopy are also provided there.

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[1] For reviews see: a) C. Wojczewski, K. Stolze, J. W. Engels, *Synlett* **1999**, 1667–1678; b) M. E. Hawkins, *Cell Biochem. Biophys.* **2001**, *34*, 257–281; c) M. K. Johansson, R. M. Cook, *Chem. Eur. J.* **2003**, *9*, 3466–3471; d) T. T. Williams, J. K. Barton in *DNA and RNA Binders, Vol. 1* (Eds.: M. Demeunynck, C. Bailly, W. D. Wilson), Wiley-VCH, Weinheim, **2003**, pp. 146–177; e) S. Tyagi, F. R. Kramer, *Nat. Biotechnol.* **1996**, *13*, 303–309; f) S. Tyagi, S. A. E. Marras, F. R. Kramer, *Nat. Biotechnol.* **2000**, *18*, 1191–1196.

[2] For reviews see: a) C. M. Niemeyer, D. Blohm, *Angew. Chem.* **1999**, *111*, 3039–3043; *Angew. Chem. Int. Ed.* **1999**, *38*, 2865–2869; b) D. H. Blohm, A. Guiseppi-Elie, *Curr. Opin. Biotechnol.* **2001**, *12*, 41–47; c) M. C. Pirrung, *Angew. Chem.* **2002**, *114*, 1326–1341; *Angew. Chem. Int. Ed.* **1999**, *38*, 2865–2869; d) A. Jung, *Anal. Bioanal. Chem.* **2002**, *372*, 41–42.

[3] a) N. Amann, H.-A. Wagenknecht, *Synlett* **2002**, 687–691; b) N. Amann, E. Pandurski, T. Fiebig, H.-A. Wagenknecht, *Angew. Chem. Int. Ed.* **2002**, *41*, 2978–2980; *Angew. Chem.* **2002**, *114*, 3084–3087; c) N. Amann, E. Pandurski, T. Fiebig, H.-A. Wagenknecht, *Chem. Eur. J.* **2002**, *8*, 4877–4883; d) N. Amann, H.-A. Wagenknecht, *Tetrahedron Lett.* **2003**, *44*, 1685–1690; e) R. Huber, T. Fiebig, H.-A. Wagenknecht, *Chem. Commun.* **2003**, 1878–1879; f) E. Mayer, L. Valis, R. Huber, N. Amann, H.-A. Wagenknecht, *Synthesis* **2003**, 2335–2340.

[4] M. Rist, N. Amann, H.-A. Wagenknecht, *Eur. J. Org. Chem.* **2003**, 2498–2504.

[5] a) S. I. Khan, M. W. Grinstaff, *J. Am. Chem. Soc.* **1999**, *121*, 4704–4705; b) N. Minakawa, Y. Ono, A. Matsuda, *J. Am. Chem. Soc.* **2003**, *125*, 11 545–11 552.

[6] a) V. A. Korshun, I. A. Prokhorenko, S. V. Gontarev, M. V. Skorobogatyi, K. V. Balakin, E. V. Manasova, A. D. Malakhov, Y. A. Berlin, *Nucleosides Nucleotides* **1997**, *16*, 1461–1464; b) A. D. Malakhov, E. V. Malakhova, S. V.

  

Kuznitsova, I. V. Grechishnikova, I. A. Prokhorenko, M. V. Skorobogatyi, V. A. Korshun, Y. A. Berlin, *Russ. J. Bioorg. Chem.* **2000**, *26*, 34 – 44.

[7] Inosine (I) was used as the counterbase to Py–C≡C–dX to avoid fluorescence quenching as a result of a photoexcited oxidative charge-transfer process.

[8] a) T. Fiebig, K. Stock, S. Lochbrunner, E. Riedle, *Chem. Phys. Lett.* **2001**, *345*, 81 – 88; b) E. Pandurski, T. Fiebig, *Chem. Phys. Lett.* **2002**, *357*, 272 – 278; c) M. Raytchev, E. Pandurski, I. Buchvarov, C. Modrakowski, T. Fiebig, *J. Phys. Chem. A* **2003**, *107*, 4592 – 4600.

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