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Synthesis of Polysubstituted Pyrenes with Tuned Spectroscopic Properties for Two-Point Attachment

Vladimir L. Malinovskii[a] and Robert Häner*[a]

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Pyrene derivatives find widespread use as labels and building blocks in modified nucleic acids. To increase the variety of spectroscopic applications, a synthesis of differently substituted derivatives was developed. 1,8-Pyrenedicarboxylates bearing different substituents in the 3- and 6-position were synthesized. Absorption and steady-state fluorescence studies showed that the photophysical properties of the pyrene chromophore can be controlled by the nature of the substituents.

Introduction

Pyrene derivatives are increasingly gaining attention as valuable and versatile tools in various areas of chemical biology and materials research. Due to their richness in photophysical properties, pyrenes find use as microenvironment sensors,[1] as liquid crystals,[2] organic light-emitting diodes,[3] or as components of various types of oligomers, such as fluorescent polymers and dendrimers,[4] photoactive polypeptides[5] as well as in genetic probes.[6]

We recently reported the incorporation of non-nucleosidic polyaromatic building blocks into DNA.[7] Oligonucleotides containing such building blocks form DNA-like structures, which are stabilized by interstrand stacking interactions of the aromatic moieties. Among different aromatic compounds, pyrenes appear as most attractive for two reasons: (1) extended π-system, which brings favorable stacking properties, (2) pronounced sensitivity towards structural changes, which is reflected in changed photophysical properties.[8] Thus, the interstrand stacking arrangement of non-nucleosidic pyrenes gives rise to excimer formation,[9] which can be monitored by a characteristic band in the fluorescence spectrum.[8]

Structural changes of the pyrene core are likely to have an effect on both, stacking interactions and spectrophysical properties. To study this influence in more depth, we were facing the need of appropriately substituted, symmetrical derivatives. Somewhat surprisingly, we found that reports on the synthesis of such pyrene derivatives are almost completely absent. While many pyrenes bearing one functional group are commercially available and their incorporation into more complex system through a single covalent bond (*one-point attachment*) was intensively explored,[1–6,10,11] polysubstituted derivatives with two potential attachment sites are rarely described.[4c,12,13] Various types of linkers have been used for connecting pyrenes to DNA.[6,10,11,14,15] As part of our work, we have developed 1,8-pyrenedicarboxamide building blocks (Figure 1). The amide-type linkers used for incorporation of the pyrenes into DNA were chosen for the following reasons: (1) straightforward formation of the amide bonds from the appropriate acids or esters, (2) compatibility with the standard phosphoramidite method for DNA synthesis, (3) flexibility with regard to choice of the linker length, (4) negligible influence of the linker on the fluorescence properties of the pyrene chromophore. Herein, we report the synthesis of pyrenes with tuned photophysical properties that can be incorporated into oligonucleotides by a *two-point attachment*.

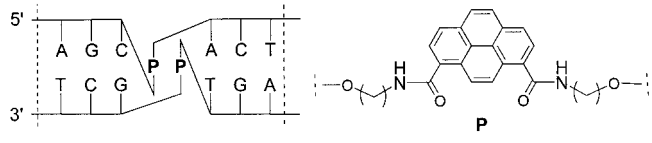

Figure 1. Illustration of a DNA duplex containing interstrand stacked, non-nucleosidic building blocks.

Results and Discussion

The general synthesis of the pyrene derivatives started from 1,8-pyrenedicarboxylic acid (**1**), which was prepared from pyrene as described in the literature[12] (Scheme 1). Diester **2** was obtained in high yield by treatment with methyl iodide in the presence of cesium fluoride. Compound **2** was subsequently investigated for its suitability to nitration and bromination. Nitro substition was of interest, since it would result in a change of the dipole moment and should, thus, lead to favourable stacking properties. Furthermore, the

[a] Department of Chemistry and Biochemistry, University of Bern,
Freiestrasse 3, 3012 Bern, Switzerland
E-mail: robert.haener@ioc.unibe.ch

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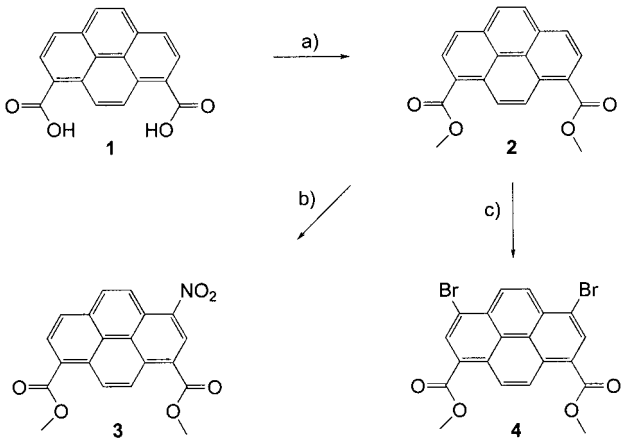
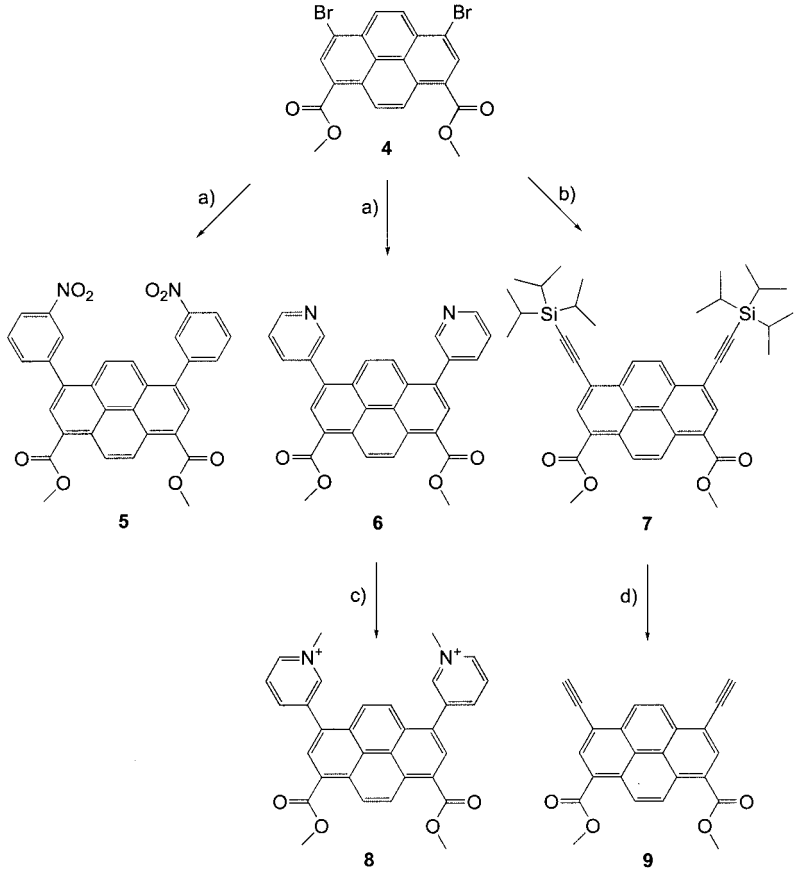
well-known property of aromatic nitro derivatives to act as fluorescence quenchers[16] is of interest in the context of using such building blocks for diagnostic applications. Likewise, bromo substitution should have a positive influence on the stacking properties and also result in an increased tendency for excimer formation. In addition, it opens the possibility of further modification by a wide range of transition metal based transformations of the obtained bromo derivatives.



Scheme 1. Synthesis of substituted pyrene derivatives. Reagents and conditions: (a) MeI, CsF, room temp.; 91%; (b) HNO_3/Ac_2O, 70 °C; 57%; (c) Br_2, nitrobenzene, 60 °C; 83%.

Among the many nitration methods known for pyrene nitration,[12,17] we found that the classical treatment with HNO_3/Ac_2O worked best. Thus, the mononitro derivative **3** was obtained in 57%. Conditions had to be optimized in order to reduce side reactions, due to oxidative cleavage of the pyrene core. Optimal temperature and HNO_3 concentration allowed to stop the reaction on the level of mononitration and, at the same time, decreased oxidative side reactions. Formation of a single isomer is a significant advantage because it avoids tedious separation steps.[18] Bromination of **2** with Br_2 in nitrobenzene[12,17a] proceeded very smoothly. Under optimized conditions, the dibromo compound **4** was obtained in a yield of 83% without the need of separation from byproducts.[19]

Modification of bromopyrenes by Suzuki- and Sonogashira-type couplings is under intensive use.[2b,20,21] Thus, compound **4** serves as an ideal intermediate on the way to further derivatives by a range of carbon–carbon bond-forming reactions.[22] As shown in Scheme 2, we chose a set of substitutions, which should influence the electronic properties either through extension of the π-system, variation of the dipole moment or by the introduction of



Scheme 2. Modification of dibromopyrene **4**. Reagents and conditions: (a) (3-nitrophenyl)boronic acid or 3-pyridyl pinacolyl boronate, $Pd(PPh_3)_4$, K_2CO_3, DMF, 100 °C; 70% (**5**) and 68% (**6**); (b) (triisopropylsilyl)acetylene, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, THF, reflux; 87%; (c) MeI, DMF, room temp.; 97%; (d) TBAF, THF, room temp.; 84%.

Table 1. Spectroscopic properties of pyrene derivatives.

Compound	Absorption[a]	Φ_f [b]	Fluorescence[c]	
	λ_{max} [nm] ($\log\varepsilon$ [$mol^{-1}dm^3\ cm^{-1}$])		diluted[d]	concd.[e]
2	245, 286 (4.53), 358 (4.48), 375 (4.45)	0.82	393, 414	438, 525
3	236, 289 (4.31), 385 (4.37), 405	0.02	410, 435	(*quenching*)
4	250, 293 (4.51), 373 (4.47), 400 (4.47)	0.09	419, 442	472
5	248, 295 (4.65), 375, 396 (4.56)	nd	(*quenching*)	(*quenching*)
6	248, 296 (4.43), 378, 397 (4.36)	0.50	425, 435	454
7	248, 293, 306 (4.72), 400 (4.54), 425 (4.64)	0.99	443, 470	480, 565
8	291, 386	nd	425	525
9	248, 300 (4.59), 388 (4.45), 410 (4.53)	0.34	425, 451	480, 570

[a] CH_2Cl_2 (shoulders not indicated). [b] Fluorescence quantum yield in cyclohexane at 20 °C, nd: not determined. [c] Solvent: CH_2Cl_2 for all compounds except **8** (H_2O). [d] Diluted conditions (range from 10^{-6} to 10^{-5} M). [e] Concentrated conditions (range from 10^{-3} M to saturated solutions).

charged substituents. All these factors are likely to affect the stacking behavior and spectroscopic properties of the modified pyrene building blocks. Suzuki coupling of dibromopyrene **4** with (3-nitrophenyl)boronic acid and 3-pyridylboronic acid pinacol ester, respectively, in DMF gave the corresponding tetrasubstituted pyrenes **5** and **6**. Similarly, compound **7** was obtained by Sonogashira coupling of **4** with (triisopropylsilyl)acetylene in THF/NEt_3. Treatment of **6** with MeI gave the dipyridinium derivative **8** in 97% yield. Finally, the diethynyl derivative **9** was obtained by deprotection of **7** with TBAF.

Next, the spectroscopic properties of the different compounds were investigated. Characteristic absorption and emission data of compounds **2**–**9** are summarized in Table 1.

Modification of the pyrene moiety with the different substituents resulted in considerable changes of fluorescence features. The emission spectra of a representative set of compounds is shown in Figure 2. As can be seen, a gradual redshift of monomer fluorescence in highly diluted solutions (10^{-5} M) is observed upon modification of the diester **2** with bromo or TIPS-ethynyl substituents (compounds **4** and **7**, respectively). On the other hand, the bis(nitrophenyl) derivative **5** exhibited almost no fluorescence. Since emission is very low even under high-dilution conditions, this can be attributed to intramolecular quenching of the pyrene fluorescence by the nitrophenyl substituents. In contrast, the mononitro derivative **3** shows moderate fluorescence emission in highly diluted solutions; quenching is observed only at concentrations $>10^{-3}$ M, where reduction of fluorescence intensity can be attributed to intermolecular quenching processes.

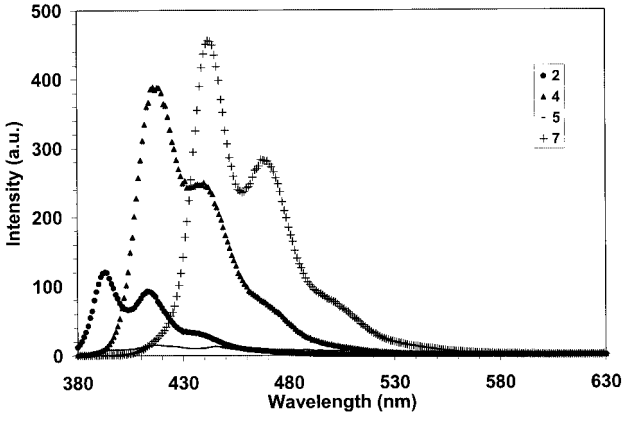


Figure 2. Representative fluorescence spectra (compounds **2**, **4**, **5** and **7**, CH_2Cl_2, 0.02 mm); excitation at 350 nm.

Additionally, the intermolecular quenching efficiency of the bis(nitrophenyl) compound **5** on fluorophor **7** was investigated. Figure 3 shows the effect of increasing concentrations of **5** on the emission intensity. As can be seen, already low concentrations of **5** lead to a drastic reduction of the fluorescence signal.

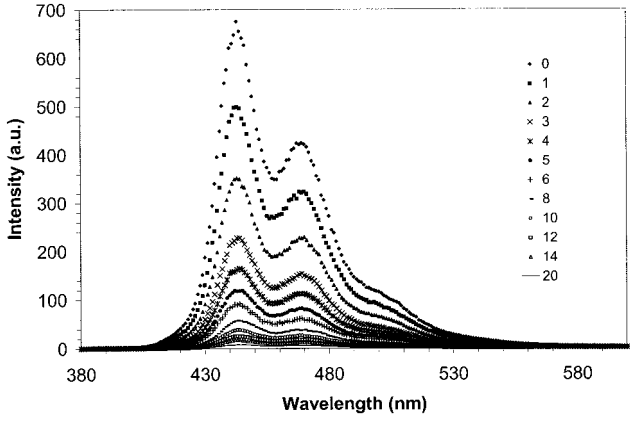


Figure 3. Quenching of fluorescence of pyrene **7** ($2\cdot10^{-5}$ M) by bis(nitrophenyl) pyrene **5** (1–20 equiv., shown on the right).

Conclusions

We have shown a general synthesis of electronically tuned pyrene derivatives. Products are synthesized from the readily accessible 1,8-pyrenedicarboxylic acid in good yields without the requirement of chromatographic purification. Absorption and steady-state fluorescence studies showed that the photophysical properties of the pyrene chromophore can be controlled by the nature of the substituents. Nitro derivatives were shown to quench fluorescence by intra- or intermolecular processes. While the obtained derivatives possess a rich potential for further modification, they

are also amenable to incorporation into oligonucleotides by *two-point attachment*.[9] Incorporation of these compounds into DNA, their effect on hybridization, as well as their spectroscopic properties are currently investigated.

Supporting Information (see also the footnote on the first page of this article): Full experimental procedures and characterization of compounds **2**–**9** as well as NMR and fluorescence spectra.

Acknowledgments

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