ORGANIC LETTERS

2012
Vol. 14, No. 20
5176–5179

2-Pyrenyl-DNA: Synthesis, Pairing, and Fluorescence Properties

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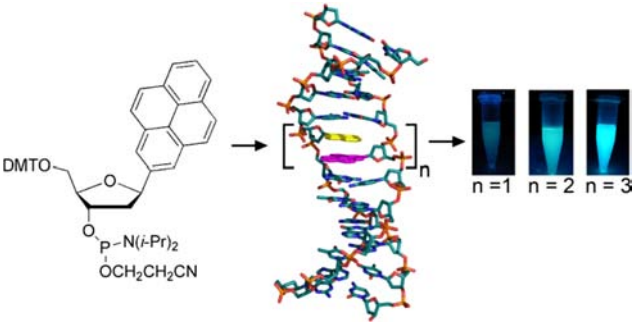
Received August 2, 2012

ABSTRACT



Multiple 2-pyrenyl-C-nucleosides were incorporated into the center of a DNA duplex resulting in stable pyrene self-recognition and excimer formation. This helical pyrene array may find use in DNA-mediated charge transfer and in the creation of DNA-based sensors.

Non-natural bases capable of forming Watson–Crick base pairs have been pivotal in understanding the structure-to-function relationship in nucleic acids and the intricate forces stabilizing the double helix.[1] Similarly, orthogonal bases and aromatic base replacements that lack hydrogen-bonding functional groups, and interact through edge-on or face-on stacking, have played an important role in understanding the recognition/processing by DNA polymerases and in attempting to expand the genetic code.[2] In this context, simple polycyclic aromatic hydrocarbons such as naphthalene, phenanthrene, and pyrene were introduced into DNA.[3] Pyrene was chosen to replace an entire adenine/thymine base pair, demonstrating for the first time that a Watson–Crick base pair may be replaced by an arene without a large decrease in thermal stability.[4]

Pyrene-functionalized oligonucleotides are of considerable interest in diagnostic applications, in creating functional DNA materials and DNA-multichromophore systems.[5] For example, pyrene's ability to form fluorescent excimers via $\pi-\pi$ interactions has led to the detection of nucleic acids, alkali metal ions, and proteins and has been used as reporters for esterases and lipases.[6] In a nonbiological context, pyrene is used as a photoexcitable electron donor to study charge transfer, in the construction of aromatic π-arrays, and in the formation of novel helical structures.[7]

Recently, we incorporated non-hydrogen bonding/non-shape complementary base surrogates such as bipyridine, biphenyl, and phenanthrene into the middle of a DNA duplex, and we discovered that they recognize each other

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10.1021/ol302150a
Published on Web 10/01/2012

through interstrand stacking interactions which results in a gradual increase in duplex stability.[8] We refer to this type of stacking interaction as the zipper-like recognition motif, confirmed by an NMR structure for a DNA duplex containing one biphenyl pair.[9]

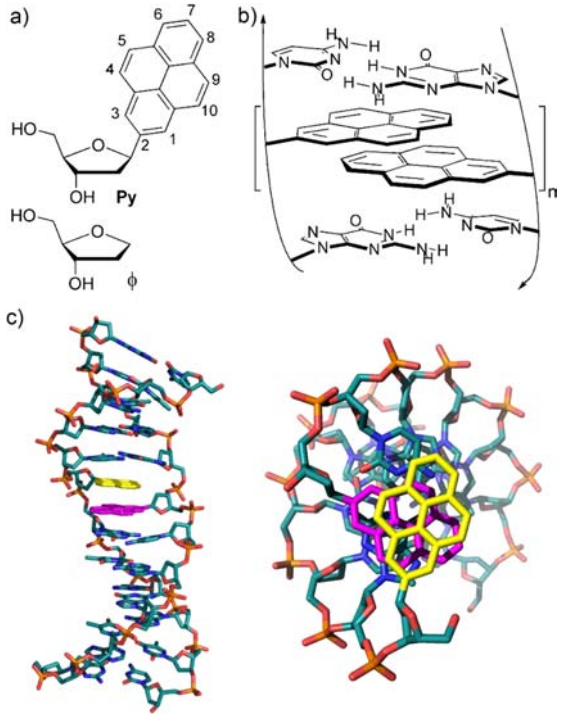
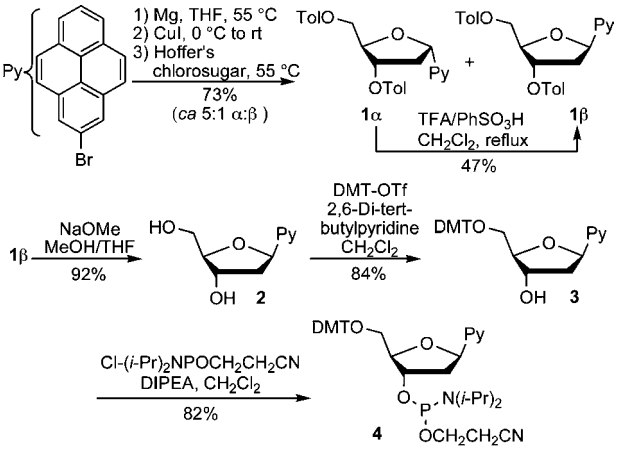


Figure 1. (a) Chemical structures of 2-pyrenyl deoxynucleoside (**Py**) and the abasic site (ϕ). (b) A cartoon of the zipper-like recognition motif highlighting pyrene interstrand stacking interactions; the vertical arrows represent a phosphodiester backbone. (c) Molecular model (HyperChem 8.0) of a duplex containing two interstrand stacked **Py** residues (left: side view, right: top view), pyrenes are shown in yellow and magenta.

We now report the hybridization properties and excimer formation of oligodeoxynucleotides containing a 2-pyrene-C-nucleoside as a non-natural base surrogate. Pyrene was attached at its 2-position to deoxyribose in order to maximize $\pi-\pi$ interactions through interstrand stacking interactions and minimize conformational isomerism around the C-glycosidic bond, as depicted in Figure 1.

Synthesis of 2-pyrene-C-nucleoside phosphoramidite **4** follows established routes in C-aryl-nucleoside preparation (Scheme 1).[10] Treatment of 2-bromopyrene with magnesium metal led to the formation of

Scheme 1. Synthesis of 2-Pyrene Deoxynucleoside Phosphoramidite **4**



2-pyrenylmagnesium bromide.[11] This was then transmetalated with copper iodide and reacted with Hoffer's chlorosugar (3,5-ditoluoyl-1-α-chloro-2-deoxy-D-ribose)[12] to give **1** as a mixture of α/β anomers. Next, acid-catalyzed epimerization[13] of the α-anomer gave the corresponding desired β-C-nucleoside. The 1′-β-configuration was verified by ^{1}H NMR NOE experiments. The toluoyl protecting groups were removed, and the 5′-hydroxyl group was protected with DMT-triflate/2,6-di-*tert*-butylpyridine[14] followed by phosphitylation using standard conditions. Phosphoramidite **4** (**Py**) along with the abasic site (ϕ) was incorporated into oligodeoxynucleotides using standard automated DNA synthesis. Oligodeoxynucleotides were purified by RP-HPLC and characterized by ESI-MS (see the Supporting Information).

Sequences containing one, two, or three consecutive **Py** residues were hybridized to the complementary sequence containing **Py** residues (duplexes 3−5 in Table 1) or ϕ residues (duplexes 6−11 in Table 1).

When a single **Py** residue was paired against an abasic site, as in duplex 6 or 9, a minor decrease in thermal stability was obtained (−0.4 and −1.1 °C compared to the unmodified sequence or −2.0 and −2.7 °C compared to the duplex containing an A:T base pair). These results are comparable to those obtained with a 1-pyrene deoxynucleoside (−1.6 or −2.2 °C compared to an A−T base pair)[4] demonstrating that both the 1-pyrenyl- and 2-pyrenyl-C-nucleotides can be paired against an abasic site without a large difference in thermal stability. Sequences containing two or three intrastacked **Py** residues paired against two or three abasic sites (duplexes 7, 8, 10, and 11) resulted in a gradual decrease in thermal stability reaching −9.8 and −10.3 °C. In contrast, duplexes containing interstrand stacked **Py** residues (duplexes 3, 4, and 5) were all more stable than the unmodified sequence. Duplex 5 is 9.6 °C

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Table 1. T_m Data (°C), Obtained from UV-Melting Curves at 260 nm, for Duplexes 1−12[a]

5′−d(GATGAC(**X**)$_n$GCTAG)
3′−d(CTACTG(**Y**)$_n$CGATC)

duplex	n	**X**	**Y**	T_m	ΔT_m[b]	ΔT_m[c]
1	0	–	–	48.0	–	–
2	1	T	A	49.6	+1.6	–
3	1	Py	Py	51.6	+3.6	+4.0/ +4.7
4	2	Py	Py	49.6	+1.6	+8.1/ +4.0
5	3	Py	Py	57.6	+9.6	+19.4/ +19.9
6	1	Py	ϕ	47.6	−0.4	–
7	2	Py	ϕ	41.5	−6.5	–
8	3	Py	ϕ	38.2	−9.8	–
9	1	ϕ	Py	46.9	−1.1	–
10	2	ϕ	Py	45.6	−2.4	–
11	3	ϕ	Py	37.7	−10.3	–
12	1	ϕ	ϕ	29.7	−18.3	–

[a] Conditions: 2.5 μM strand concentration in 10 mM NaH_2PO_4, 150 mM NaCl, pH 7.0. Estimated error in T_m = ± 0.5 °C. [b] ΔT_m is the diffrence in T_m relative to the duplex 1 lacking **X** and **Y** (n = 0). [c] ΔT_m is the diffrence in T_m between duplexes 3, 4, or 5 containing interstrand stacked pyrenes and the corresponding duplex containing intrastrand stacked pyrenes facing an abasic site ϕ (duplexes 6−8 and 9−11). The values are obtained by subtracting the T_m of duplex 3 from duplex 6 or 9, subtracting the T_m of duplex 4 from duplex 7 or 10, and subtracting the T_m of duplex 5 from duplex 8 or 11.

more stable than the unmodified sequence or 19.4 °C/ 19.9 °C more stable than duplex 8 or 11. These results confirm that interstrand stacking interactions play a dominant role in stabilizing a DNA duplex containing non-shape complementary/non-hydrogen bonding base pairs. Furthermore, these results differ from interstrand stacked pyrenes in DNA using non-nucleosidic linkers, in which stable hybrids were formed but with a minor gradual decrease in thermal stability compared to the unmodified sequence.[15]

To gain further insight into the hybridization behavior we measured the thermodynamic parameters of duplex formation, Table 2. Formation of duplex 3 (n = 1) was driven by a favorable entropic change ($\Delta\Delta S$ = +11.8 cal K^{-1} mol^{-1}) and an unfavorable change in enthalpy ($\Delta\Delta H$ = +2.9 kcal mol^{-1}) when compared to the unmodified sequence. Formation of duplex 4 (n = 2) was accompanied with an unfavorable change in entropy ($\Delta\Delta S$ = −2.0 cal K^{-1} mol^{-1}) that was compensated for by a favorable change in enthalpy ($\Delta\Delta H$ = −1.0 kcal mol^{-1}). A similar trend was observed for duplex 5. Subtraction of the enthalpies ($\Delta H_{n=3} - \Delta H_{n=1}$) compensates for the interaction of **Py** with the neighboring natural bases and revealed that duplex 5 is stabilized by −8.6 kcal mol^{-1}. This suggests that overall a duplex containing more than one **Py** base pair is enthalpically driven.

Table 2. Thermodynamic Parameters of Duplex Formation[a]

5′−d(GATGAC(**Py**)$_n$GCTAG)
3′−d(CTACTG(**Py**)$_n$CGATC)

no.	ΔH [kcal mol^{-1}]	$\Delta\Delta H$[b]	ΔS [cal K^{-1} mol^{-1}]	$\Delta\Delta S$[c]	$\Delta G^{298\ K}$ [kcal mol^{-1}]
0	−72.6	–	−199.2	–	−13.3
1	−69.7	+2.9	−187.4	+11.8	−13.8
2	−73.6	−1.0	−201.2	−2.0	−13.7
3	−78.3	−5.7	−209.5	−10.3	−15.9

[a] Enthalpies, entropies, and Gibb's free energies were obtained from van't Hoff plots (concentration−variation method). [b] $\Delta\Delta H = \Delta H_n - \Delta H_{n=0}$ and are in kcal mol^{-1}. [c] $\Delta\Delta S = \Delta S_n - \Delta S_{n=0}$ and are in cal K^{-1} mol^{-1}.

Next, the sequences were investigated for their ability to undergo excimer formation (Figure 2). Duplex 3 exhibited weak monomer emission at 378 and 398 nm and a broad structureless emission centered at 490 nm that is typical for pyrene excimer formation. Increasing the number of Py base pairs lead to a concomitant increase in excimer intensity in all duplexes (Figure 2a,b) and single strands (see Supporting Information). Surprisingly, duplex 5 (530 au) had an approximate 4- or 5-fold increase in excimer intensity when compared to duplex 8 (138 au) or 11 (107 au). It likely occurs because of a cooperative interaction of the **Py** residues and not simply from the sum of the excimer intensities originating from intramolecular excimer formation in the single strands. Similar behavior has been observed with pyrene substituted at the 2′-sugar position.[7b] Next, we measured the change in excimer intensity with increasing temperature and, as expected, found a gradual decrease in excimer emission. A shift from excimer emission to monomer emission was observed, in duplex 3, between 50 and 60 °C which is consistent with the obtained melting temperature (T_m = 51.6 °C). Duplex 5 also underwent a gradual decrease in excimer emission. However, even at 80 °C, which is well above the melting temperature, there was no monomer emission present and only weak excimer emission from intramolecular stacked pyrenes in the single strands was observed.

CD measurements were performed on both the **Py** containing duplexes and single strands in order to gain further insight into the structural preference (Figure 3). Duplex 3 exhibited a typical B-type conformation with almost equal positive (275 nm) and negative (250 nm) ellipticities zeroing at 263 nm (Figure 3a). At wavelengths above 300 nm, the CD spectra are biphasic due to exciton coupling between the pyrenes.[16] Interestingly, a negative-positive (∓) cotton effect for the pyrene band was observed in the case of duplex 3 which was inverted (±) in duplexes 7, 10 or the single strands. This change in sign can most likely be attributed to the change in distance and dihedral angle between the pyrenes.[17] The exciton coupling intensities

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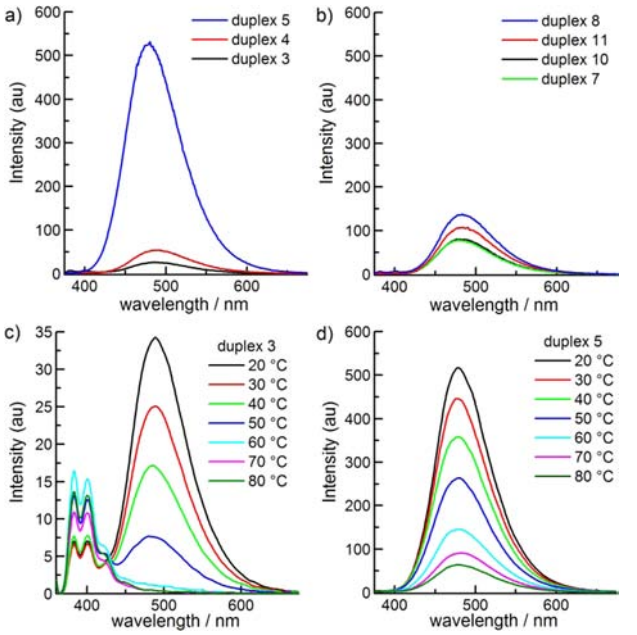

Figure 2. (a and b) Fluorescence spectra of duplexes 3, 4, 5 and 7, 8, 10, 11. (c and d) Temperature dependent fluorescence spectra of duplexes 3 and 5. Conditions: λ_{ex}, 350 nm; detector, 575 V; excitation slit, 5 nm; emission slit, 5 nm; same buffer and concentration as those in Table 1.

decrease with increasing temperature, and no exciton coupling occurred above the melting temperature (Figure 3b). The single strands in Figure 3c,e also maintained exciton coupling between the pyrenes with a positive Cotton effect (±) and approximately equal intensity at 330 and 350 nm. Increasing the number of Py base pairs (as observed for duplex 4 and 5) was accompanied by an increase in intensity for the positive peaks found at 345/330/277 nm and the negative peak at 237 nm. Overall, comparison of the unmodified duplex 1 with duplex 5 in the 200−300 nm region indicates that the accommodation of three **Py** base pairs is accompanied by a slight change in the DNA conformation.

In summary, the present results demonstrate that 2-pyrenyl containing DNA forms a duplex that is more stable than the unmodified duplex. The thermal stability, excimer formation, and CD spectroscopy support interstrand stacking as the stability determining factor. We are currently investigating if a similar behavior will be observed with DNA containing 1-pyrenyl or 4-pyrenyl base replacements.

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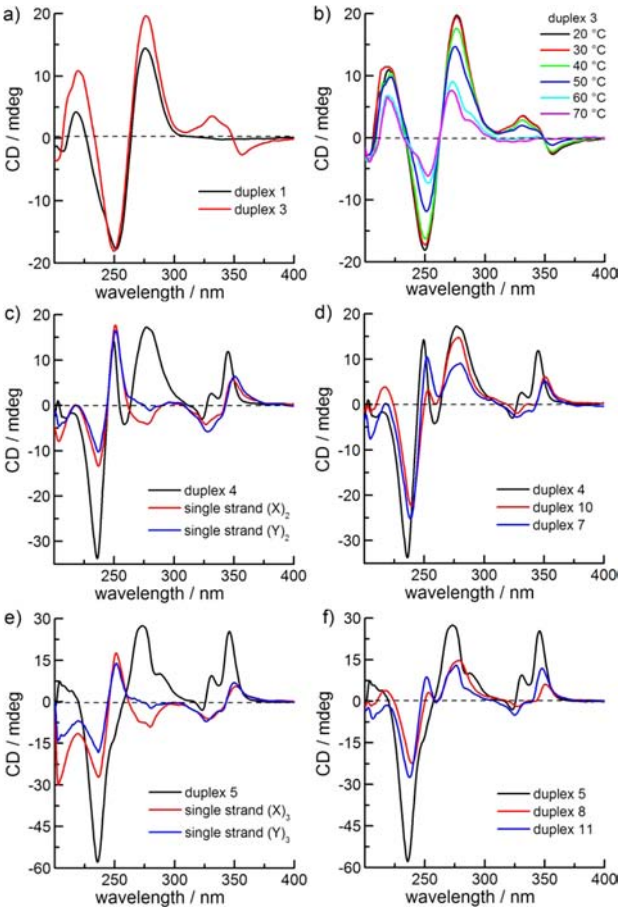

Figure 3. (a−f) CD spectra of the duplexes and single strands. Conditions: 5.0 μM strand concentration, same buffer as that in Table 1.

Since pyrene and substituted pyrenes can be used as photoexcitable electron donors, the present helical pyrene array may find use in studying DNA-mediated charge transfer. Such research is of importance in the design of aromatic π-arrays and in the construction of novel DNA-based sensors.

Acknowledgment. We acknowledge financial support from the Swiss National Science Foundation (Grant No. 200020-130373) and the EU for a Marie Curie Fellowship to F.W.

Supporting Information Available. Synthetic procedures and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.