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DNA containing phenanthroline- and phenanthrene-derived, non-nucleosidic base surrogates

Simon M. Langenegger and Robert Häner*

Department of Chemistry and Biochemistry, University of Bern, Freiestrasse 3, CH-3012 Bern, Switzerland

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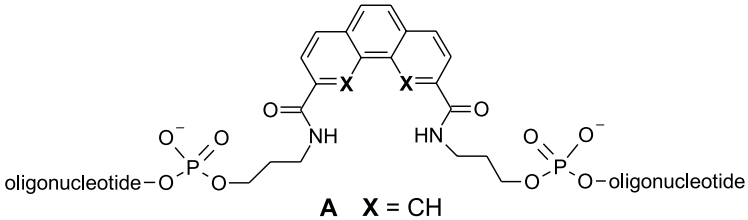
Abstract—Simple, non-nucleosidic phenanthroline- and phenanthrene derivatives have been synthesised and incorporated into oligodeoxynucleotides. Complementary strands containing the modified building blocks in opposite positions form stable hybrids. Thermal denaturation experiments show that the double strands containing the phenanthroline derivatives are more stable than the ones with the corresponding phenanthrenes. Furthermore, it was found that duplex stability is considerably decreased if the linkers of the modified building blocks are too short.

Besides their importance as the genetic material, nucleic acids are increasingly gaining interest as nanometer-sized, functional matter.[1–4] Due to the repetitive, well-defined arrangement of their building blocks, nucleic acids and related types of oligomers[5–8] are ideal objects for the designed construction of larger assemblies. Thus, they have been used for the spatially well-defined arrangement of gold nanoparticles[9,10] or for the generation of larger molecular assemblies and architectures.[11–14] Furthermore, they may find applications as molecular metal wires[15–19] and even as molecular computers[20,21] or machines.[22,23] The combination of nucleotides with non-natural building blocks enhances the number of possible constructs and their potential applications even further. Recently, we reported the synthesis and properties of a non-nucleosidic, phenanthrene-derived building block and its incorporation into double stranded DNA (Scheme 1, **A**).[24,25] This simple building block can serve as a base surrogate without destabilising the DNA duplex nor altering its overall B-DNA structure. Based on the data obtained, a model of interstrand-stacked phenanthrenes was proposed.[24] Since π-stacking interactions should be favoured by heteroaromatic derivatives,[26] we investigated the effect of the phenanthroline analogues (Scheme 1, **B**). Here, we report that the phenanthroline derivatives gives rise to more stable hybrids than the analogous phenanthrenes and that the length of the non-nucleosidic linker plays an important role.

The building blocks required for the synthesis of the modified oligonucleotides are shown in Scheme 2. The phenanthrene derivatives **1a–c** were prepared as



Scheme 1. Non-nucleosidic phenanthrene- and phenanthroline-derived building blocks.

Keywords: Phenanthrene; Phenanthroline; DNA; Duplex; Stacking.
* Corresponding author. Tel.: +41 31 631 4382; fax: +41 31 631 8057; e-mail: robert.haener@ioc.unibe.ch

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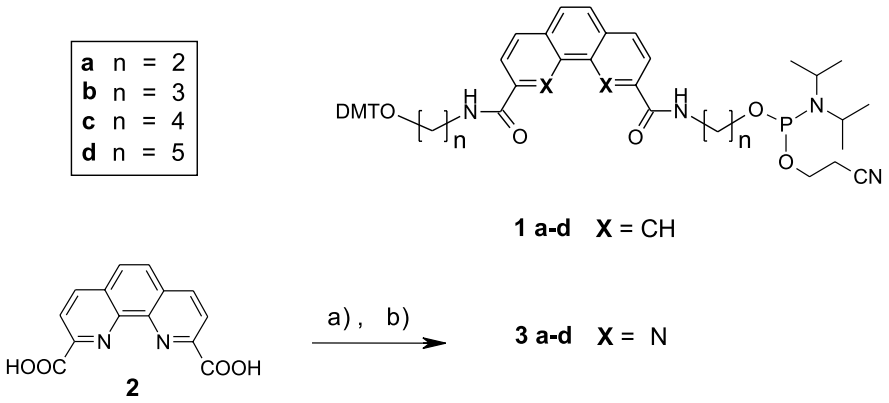


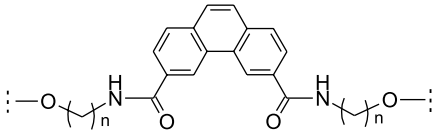
Scheme 2. Synthesis of the required phosphoramidites. Reagents and conditions: (a) $H_2N(CH_2)_nOH/H_2N(CH_2)_nODMT$ (1:1), Hünig's base, BOP; (b) 2-cyanoethyl diisopropylamidochloridophosphite, Hünig's base.

described previously.[25,27] In addition, **1d** was prepared according to the same procedures. Synthesis of the phenanthroline-derived compounds started from the known **2**.[28] Attachment of the different 4,4′-dimethoxytritylated linkers and subsequent phosphitylation yielded the phosphoramidite compounds **3a**–**d**.[29] Building blocks **2a**–**d** and **3a**–**d** were then used for the synthesis of the oligonucleotides shown in Table 1. Assembly of the oligomers involved standard automated oligonucleotide synthesis.[30,31] The crude oligomers were purified by reverse phase HPLC and their identity was verified by mass spectrometry. The oligonucleotides are sets of complementary 21mers containing a modified building block in the central position of each strand. **Pn** or **Qn**

Table 1. Influence of phenanthrene and phenanthroline nucleotide surrogates on the thermal stability of duplex DNA

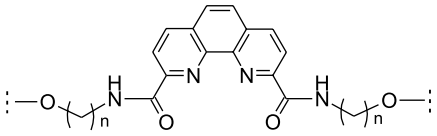
Entry	Duplex	T_m (°C)[a,b]	ΔT_m (°C)[c]
1	(5′) AGC TCG GTC A T C GAG AGT GCA (3′) TCG AGC CAG T A G CTC TCA CGT	68.0	—
2	(5′) AGC TCG GTC A **P2**C GAG AGT GCA (3′) TCG AGC CAG T **P2**G CTC TCA CGT	61.3	−6.7
3	(5′) AGC TCG GTC A **P3**C GAG AGT GCA (3′) TCG AGC CAG T **P3**G CTC TCA CGT	68.3	0.3[d]
4	(5′) AGC TCG GTC A **P4**C GAG AGT GCA (3′) TCG AGC CAG T **P4**G CTC TCA CGT	67.3	−0.7
5	(5′) AGC TCG GTC A **P5**C GAG AGT GCA (3′) TCG AGC CAG T **P5**G CTC TCA CGT	68.7	0.7
6	(5′) AGC TCG GTC A **Q2**C GAG AGT GCA (3′) TCG AGC CAG T **Q2**G CTC TCA CGT	65.6	−2.4
7	(5′) AGC TCG GTC A **Q3**C GAG AGT GCA (3′) TCG AGC CAG T **Q3**G CTC TCA CGT	71.1	3.1
8	(5′) AGC TCG GTC A **Q4**C GAG AGT GCA (3′) TCG AGC CAG T **Q4**G CTC TCA CGT	70.6	2.6
9	(5′) AGC TCG GTC A **Q5**C GAG AGT GCA (3′) TCG AGC CAG T **Q5**G CTC TCA CGT	70.2	2.2

Pn (phenanthrene)



P2,Q2: n = 2
P3,Q3: n = 3
P4,Q4: n = 4
P5,Q5: n = 5

Qn (phenanthroline)



[a] Conditions: Oligomer concentration 1.0 μM, 10 mM Tris–HCl, 100 mM NaCl, pH 7.4; temperature gradient: 0.5 °C/min.
[b] Melting temperatures were determined from the maximum of the first derivative of the melting curve (A_{260} against temperature); each T_m is the average of three independent experiments; exptl. error: ±0.5 °C.
[c] Difference in T_m relative to the control duplex (entry 1).
[d] Value taken from the literature.[24]

symbolise a phenanthrene or a phenanthroline, respectively, and n indicates the length of the alkyl linkers. For comparison, an unmodified reference duplex was synthesised possessing an AT-base pair in the central position.

The influence of the aromatic base surrogates on duplex stability was analysed by thermal denaturation experiments. The melting temperatures (T_m's, see Table 1) reveal that the phenanthrene-modified hybrids (entries 2–5) are consistently less stable than the corresponding hybrids containing phenanthroline and analogous linkers (entries 6–9). The difference in T_m's between the two series is in the range of 2–4 °C, always in favour of the phenanthroline-modified duplexes. Such a difference is comparable to the contribution of an AT-base pair to duplex stability. Furthermore, in both series the optimal linker length is reached with $n = 3$, that is, with propyl-type linkers. Duplex stability is rather insensitive to a further increase in the linker length. No statistically significant change (exptl. error: ±0.5 °C) is observed among the derivatives containing propyl, butyl or pentyl linkers in both series. In contrast, the stability is considerably diminished if the linker is too short. Thus, going from a propyl to an ethyl linker, the T_m is reduced by 7.0 and 5.5 °C in the phenanthrene and the phenanthroline series, respectively (cf. entries 2/3 and 6/7).

All oligomers investigated in the study showed a single, cooperative transition. Figure 1 shows the melting curve of the duplex containing phenanthrolines with three methylene groups in the linkers (i.e., **Q3**, see also entry 7 in Table 1). In addition, the circular dichroism spectra (CD) of the hybrids investigated are all in agreement with a B-form duplex. The CD spectrum of the duplex containing the **Q3** building blocks (see also Table 1, entry 7) is shown in Figure 2 as a representative example.

All the data obtained support a model of interstrand-stacked, non-nucleosidic building blocks in an otherwise regular B-DNA duplex. An illustration of interstrand-stacked phenanthrolines is shown in Figure 3. The flexible linkers connecting the polyaromatic hydrocarbons to the phosphate backbone of the nucleic acid do not compromise the overall stability of the hybrid nor do they substantially alter the overall B-DNA geometry. The increase in T_m, which is observed by going from phenanthrene to the corresponding phenanthroline building blocks is well in agreement with the expectation, since the higher dipole moment present in the heteroaromatic phenanthrolines should lead to stronger stacking interactions.[26]

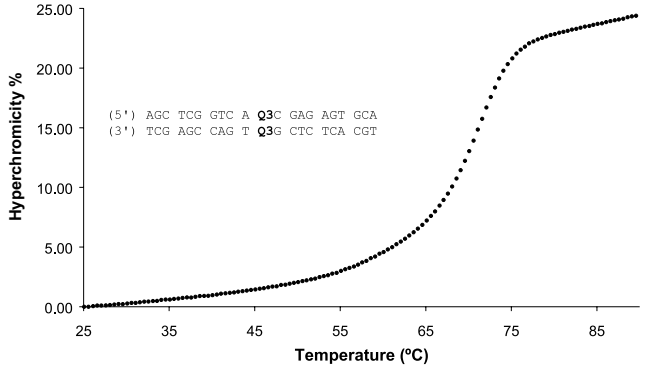


Figure 1. Representative thermal melting curve of a duplex containing two non-nucleosidic phenanthroline building blocks (**Q3**, see also Table 1, entry 7) in opposite positions.

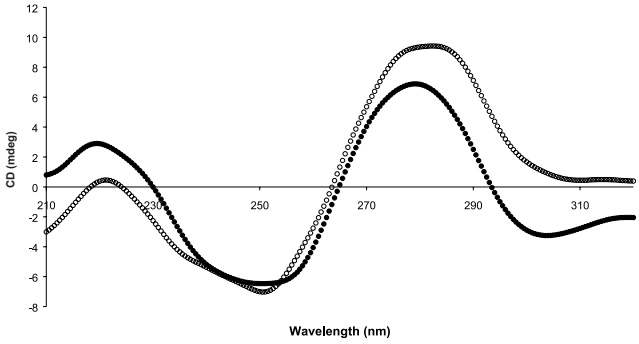


Figure 2. CD spectrum of the duplex containing two non-nucleosidic phenanthroline building blocks (●, **Q3**, see also Table 1, entry 7) in comparison to the unmodified duplex (○, Table 1, entry 1).

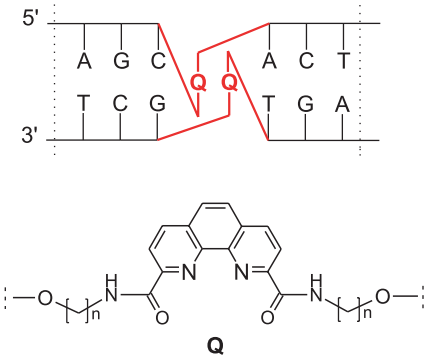


Figure 3. Illustration of a duplex containing interstrand-stacked phenanthrolines with flexible, non-nucleosidic linkers.

In conclusion, non-nucleosidic phenanthrene and phenanthroline building blocks are well tolerated in duplex DNA. In general, the phenanthroline derivatives give more stable hybrids than the corresponding phenanthrene analogs. The difference in T_m is most likely a result of the higher dipole moment of the heteroaromatic phenanthroline, compared to the phenanthrene, resulting in stronger interstrand stacking interactions. Finally, the stability of the hybrids is considerably decreased if the linkers of the building blocks are too short.

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29. Representative analytical data are given for compound **3b**: light-yellow foam. TLC (AcOEt–hexane 7:3 +2% Et_3N): R_f 0.28. ^1H NMR (300 MHz,$CDCl_3$): 1.10, 1.12 (2d, $J = 6.7$, 2*Me*CHN); 2.00 (m, $2CH_2CH_2CH_2$); 2.50 (t, $J = 6.4$, CH_2CN); 3.2–3.8 (m, $CH_2CH_2CH_2N$, $CH_2CH_2CH_2N'$, OCH_2CH_2CN, $2Me_2CHN$); 3.67 (s, 2MeO); 6.73 (d, 4 arom. H); 7.1–7.5 (m, 9 arom. H); 7.93 (s, 2 arom H); 8.44 (m, 2 arom. H); 8.59 (*m*, 2 arom. H, 2NH). ^{31}P NMR (162 MHz, $CDCl_3$): 147.78. HR-ESI-MS (pos. mode): 907.3943 ($[M+Na]^+$; calcd 907.3924).
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