Phenanthrene-Derived DNA Hairpin Mimics

by **Alfred Stutz**, **Simon M. Langenegger**, and **Robert Häner***

Department of Chemistry and Biochemistry, University of Bern, Freiestrasse 3, CH-3012 Bern
(phone: +41 31 631 4382; e-mail: robert.haener@ioc.unibe.ch)

Self-complementary oligodeoxynucleotides containing 3,6-disubstituted phenanthrenes adopt highly stable, hairpin-like structures. The thermodynamic stability of the hairpin mimics depends on the overall length of the phenanthrene building block. Hairpin loops composed of a phenanthrene-3,6-dicarboxamide and ethylene linkers were found to be optimal. The hairpin mimics are more stable than the analogous hairpins containing either a dT_4 or dA_4 tetraloop. Model studies indicate that the thermodynamic stability of the hairpin mimics is primarily due to aromatic stacking of the phenanthrene-3,6-dicarboxamide onto the adjoining base pair of the DNA duplex.

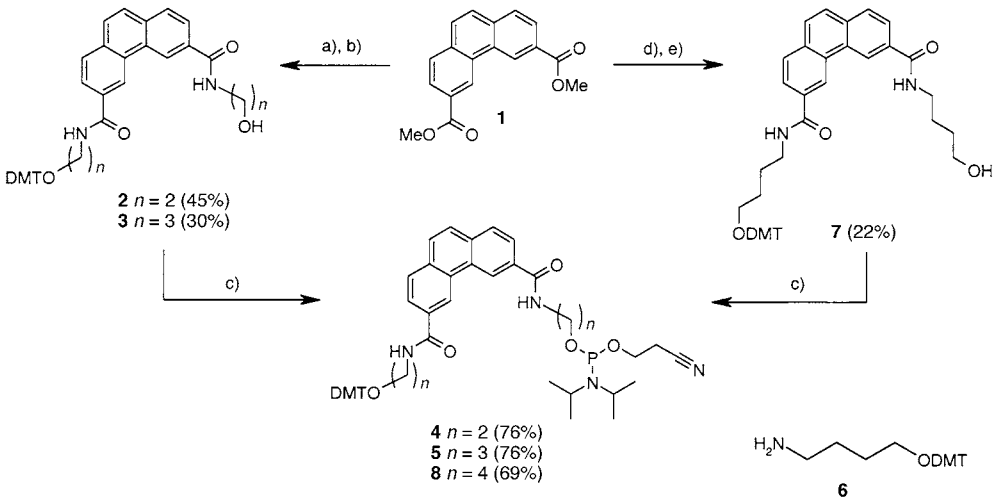
Introduction. – Hairpins belong to the most common and most important secondary-structure motives found in nucleic acids. [1] In functional RNA, they are essential elements for the formation of the correct three-dimensional structure [2–5]. In particular, hairpins containing a four-base loop (tetraloop) are emerging as a class of highly stable and structurally well-defined components of ribonucleic acids [1][6]. Hairpin formation is, although to a lesser extent, also observed in DNA. The requirements for the formation of DNA hairpins [7] and cruciform structures [8] in palindromic DNA sequences have been investigated in detail, and the involvement of such structures in the regulation of gene expression has been discussed [9][10]. Due to the importance of the hairpin motif, the replacement of the nucleotide loop by synthetic, non-nucleosidic linkers has been a topic of thorough investigation. Thus, the hairpin loop has been replaced with flexible, oligo(ethylene glycol) linkers in DNA [11] and RNA [12][13] as well as with more rigid aromatic derivatives [14–17]. In particular, (*E*)-stilbene-derived linkers led to a strong stabilization of the hairpin [15]. Structural analysis revealed a favorable stacking interaction between the stilbene and the adjacent base pair of he stem [18].

We have previously shown that phenanthrene-3,6-carboxamide-modified oligonucleotides form stable duplexes. Phenanthrene residues positioned in opposite sites can significantly enhance duplex stability, most likely *via* interstrand aromatic stacking [19]. In the course of this work, we found that self-complementary oligonucleotides connected through a phenanthrene-3,6-carboxamide-derived linker form stable, hairpin-like structures. We report here the synthesis of phenanthrene-3,6-dicarboxamide derivatives and their use as hairpin-loop mimics.

Results and Discussion. – *Preparation of Phenanthrene-Derived Building Blocks and Incorporation into Oligonucleotides.* Synthesis of the different phenanthrene-3,6-dicarboxamide phosphoramidite building blocks was carried out in analogy to a

previously reported procedure [19]. Thus, dimethyl phenanthrene-3,6-dicarboxylate (**1**) was converted into the corresponding *N*,*N*′-bis(2-hydroxyethyl) and *N*,*N*′-bis(3-hydroxypropyl)phenanthrene-3,6-carboxamides by reaction with the corresponding amino alcohols (*Scheme*). Subsequent treatment of the crude intermediates with DMT-Cl in pyridine resulted in a mixture of mono- and bis-protected derivatives. The desired mono(4,4′-dimethoxytrityl) compounds **2** and **3** were isolated by column chromatography and converted to the corresponding phosphoramidite building blocks **4** and **5**, respectively. Attempts to prepare the corresponding Bu-linked phosphoramidite in an analogous way failed. Reaction of dimethyl phenanthrene-3,6-dicarboxylate with 4-aminobutan-1-ol even under forced conditions (180°, 24 h) did not afford the desired *N*-(4-hydroxybutyl)phenanthrene-3,6-dicarboxamide. Therefore, diester **1** was hydrolyzed with aqueous NaOH. The crude sodium phenanthrene-3,6-dicarboxylate was activated with BOP and further reacted with a 1:1 mixture of 4-[(4,4′-dimethoxytrityl)oxy]butylamine (**6**) and 4-aminobutan-1-ol in DMF. This procedure yielded, albeit in a modest yield (22%), the desired 4,4′-monomethoxytritylated product **7**, which was converted into the phosphoramidite **8**. The three different phenanthrene-derived building blocks **4**, **5**, and **8** were incorporated into self-complementary oligonucleotides according to the standard phosphoramidite procedure [20][21]. To ensure a high incorporation yield of the modified building blocks, the coupling time was prolonged to 1.5 min in the respective cycles (see *Exper. Part*). Deprotection (conc. NH_3, 55°), followed by standard HPLC purification, yielded oligodeoxynucleotides **9** –

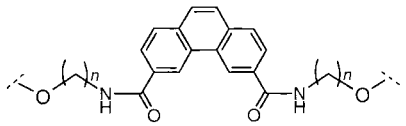
Scheme



a) 2-Aminoethanol ($n=2$) or 3-aminopropan-1-ol ($n=3$), 175°, 30 min. b) 4,4′-Dimethoxytrityl chloride (DMT-Cl), Py, r.t. c) $(^iPr_2N)_2P(OCH_2CH_2CN)$, *N*,*N*-Diisopropylammonium 1*H*-tetrazolide, CH_2Cl_2, r.t. 3 h. d) NaOH (2 equiv.), THF/EtOH/H_2O 1:1:1, reflux, 2 h. e) 4-(4,4′-Dimethoxytrityloxy)butanamine (**6**)/4-aminobutan-1-ol 1:1, $^iPr_2NEt$, [(benzotriazol-1-yl)oxy]tris(dimethylamino)phosphonium hexafluorophosphate (BOP), DMF, r.t., 1 h.

Table 1. T_m*-Values of Hairpin Sequences Containing a Phenanthrene Linker in Comparison with Their Counterparts Containing Either a* T_4*- or* A_4*-Hairpin Loop.* Conditions: oligonucleotides 2.5 μM, 100 mM NaCl, 10 mM *Tris*·HCl pH 7.5.

Hairpin		T_m [°C]
9	5′-GCA ATT GC -**P2**- GC AAT TGC-3′	79.2
10	5′-GCA ATT GC -**P3**- GC AAT TGC-3′	77.5
11	5′-GCA ATT GC -**P4**- GC AAT TGC-3′	76.7
12	5′-GCA ATT GC **TTTT** GC AAT TGC-3′	73.4
13	5′-GCA ATT GC **AAAA** GC AAT TGC-3′	72.5
14	5′-ATT GC -**P2**- GC AAT-3′	65.7
15	5′-ATT GC **TTTT** GC AAT-3′	60.0
16	5′-ATT GC **AAAA** GC AAT-3′	57.6



P2 $n=2$
P3 $n=3$
P4 $n=4$

11 and **14** (*Table 1*), along with the corresponding control oligodeoxynucleotides **12**, **13**, **15**, and **16**, containing dT_4 and dA_4 sequences instead of the phenanthrenes.

Investigation of Phenanthrene-Derived Hairpins. In their pioneering work, *Letsinger* and *Wu* [15], and *Lewis et al.* have demonstrated that (*Z*)- and (*E*)-stilbene linkers can give rise to stable hairpin analogues. In a series of publications, they showed that (*E*)-stilbene derivatives form more stable hairpin structures than the corresponding (*Z*)-isomers. The higher stability of the (*E*)-isomers was attributed to the difference of the spacer lengths between the (*Z*)- and the (*E*)-derivative [15][22]. This finding can, however, also be explained by the fact that (*Z*)-stilbene derivatives are considerably deviating from planarity and, thus, do not allow perfect stacking onto the duplex stem [23][24]. Phenanthrene represents a planar analogue of (*Z*)-stilbene, and should, therefore, result in more-favorable stacking effects. To investigate the suitability of phenanthrene-3,6-dicarboxamide linkers as hairpin mimics, the self-complementary oligonucleotides bearing Et, Pr, and Bu spacers (**9**, **10**, and **11**, resp.; see *Table 1*) were prepared, along with the corresponding control oligonucleotides containing either a dT_4 or dA_4 loop (**12** or **13**, resp.). Thermal-denaturation experiments were carried out at 100 mM NaCl, pH 7.5 (10 mM *Tris*-HCl) with a 2.5 μM oligonucleotide concentration. As can be seen from the T_m data in *Table 1*, all phenanthrene-linked oligonucleotides form a more-stable structure than the control oligonucleotides. Thermal-denaturation curves revealed a single, sharp transition for all oligonucleotides under the given experimental conditions (see *Fig. 1*). Several observations supported the conclusion that the observed transitions correspond to the melting of the respective hairpins. First, the experimental T_m values correspond well with the calculated transition temperatures [25] for the hairpins **12** (T_m calc: 70.1°, found: 73.4°) and **13** (T_m calc: 66.6°, found: 72.5°). Second, the T_m values were independent from the concentration of the oligonucleotides over a range from 1 to 5 μM (see *Table 2*), indicating a mono-molecular process. The hairpin mimic **9** containing the

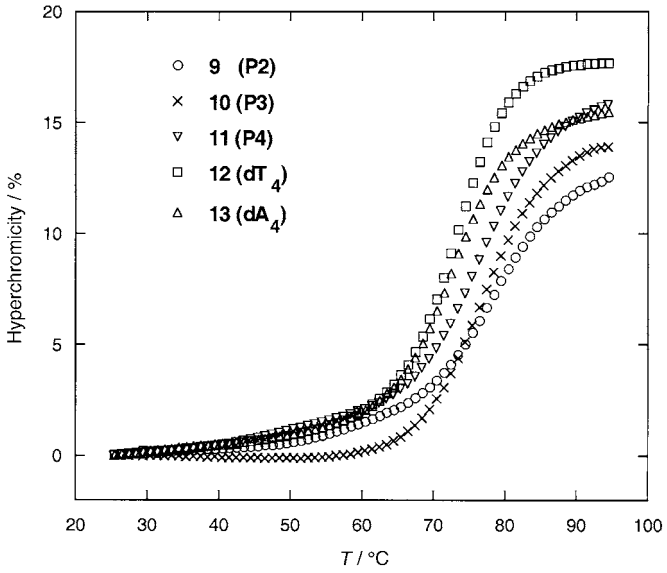


Fig. 1. *Melting curves of the hairpin mimics* **9**–**11** *in comparison with the control hairpins* **12** *and* **13**. Conditions: oligonucleotides 2.5 μM, 100 mM NaCl, 10 mM *Tris*·HCl, pH 7.5.

Table 2. T_m-*Values*[a]) *of* **9**–**13** *Measured at Different Oligomer Concentrations*

Hairpin	T_m [°C]		
	1 μM	2.5 μM	5 μM
9 (**P2**)	78.8	79.3	79.4
10 (**P3**)	77.6	77.2	77.0
11 (**P4**)	76.1	77.2	76.7
12 (**dT_4**)	73.0	73.2	73.8
13 (**dA_4**)	72.4	72.6	72.6

[a]) Conditions: 100 mM NaCl, 10 mM *Tris*·HCl pH 7.5.

Et-linked phenanthrene derivative **P2** showed the highest T_m (79.2°). The analogues **10** and **11** containing longer spacers had slightly lower T_m values (77.5° and 76.7° for **P3** and **P4**, resp.). A similar dependence of the stability of hairpin mimics on the linker length has been reported for stilbene-derived linkers. [18] The optimal phenanthrene building block, **P2**, was further incorporated into a shorter, self-complementary oligonucleotide (*Table 1*, oligonucleotide **14**) and compared to its dT_4 and dA_4 analogues (oligodeoxynucleotides **15** and **16**). Again, the phenanthrene-derived hairpin mimic showed a significantly higher thermodynamic stability ($\Delta T_m = 5.7°$ and 8.1°, resp.). Circular dichroism (CD) spectral analysis of the hairpin mimic **14** is in full agreement with an overall *B*-conformation (data not shown).

Structural Model of the Phenanthrene Hairpin Mimic. A model of the **P2**-containing hairpin **9** is shown in *Fig. 2*. The conformation represents a local-minimum structure

obtained with *Hyperchem* starting from *B* form DNA by using the amber force-field. According to this model, the concave side of the phenanthrene moiety is directed towards the minor groove of the *B* form helix. The distance between the phenanthrene and the adjacent GC base pair is *ca.* 3.3–3.4 Å, *i.e.*, well within the distance normally observed for base stacking in *B*-DNA. The increase of the transition temperature of the phenanthrene-modified hairpin analogues compared with their natural dA_4- or dT_4-containing counterparts can, therefore, be attributed to the optimal stacking interaction with the first base pair of the stem. The distance between the two phenanthrene-linked phosphates in the modelled structure of hairpin **9** is *ca.* 16.0 Å. This distance is shorter than the 17.8 Å found in a (*E*)-stilbene-derived hairpin structure [18], but lies well within the range of diametrical P–P distances (15.0 to 16.9 Å) observed in other, reported DNA hairpin structures [26][27]. This relatively large range of P–P distances can also serve as an explanation for the observation that the steady increase in the linker length from oligomers **9**–**11** (*Table 1*) does not affect the stability in a dramatic way. All these factors point towards the stacking effects as the major contribution to the stability of the hairpin-like structures.

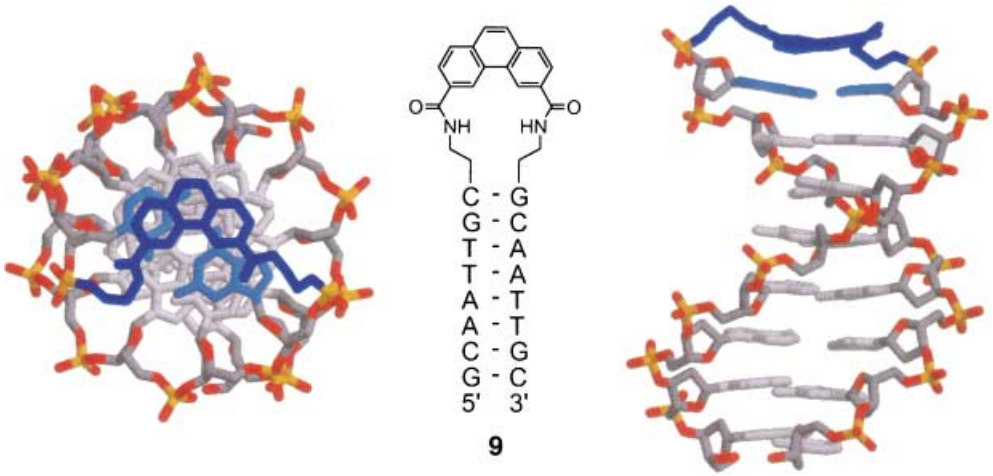


Fig. 2. Amber-*Minimized structure of hairpin* **9** *containing* **P2** (dark blue). *Left:* view along the helical axis; *right:* view perpendicular to the helical axis. The GC base pair adjacent to the phenanthrene is shown in light blue.

Conclusions. – Self-complementary oligodeoxynucleotides containing 3,6-disubstituted phenanthrenes adopt highly stable, hairpin-like structures. The thermodynamic stability of these hairpin mimics is higher than that of comparable hairpins with a dT_4 or dA_4 tetraloop. Spectroscopic analysis of the phenanthrene-modified hairpins shows no structural deviation from canonical *B* form DNA. Model studies, as well as the relative insensitivity of the structural stability towards the linker length, indicate that the thermodynamic stability of the hairpin mimics is primarily due to aromatic stacking of the phenanthrene-3,6-dicarboxamide onto the adjoining base pair of the DNA duplex.

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Experimental Part

General. Chemicals, solvents, and reagents for reactions were from *Acros*, *Aldrich*, or *Fluka*, and were of the highest quality available. Compound **1**, **3**, and **5** were prepared as described in [19]. (Cyanoethoxy)bis(dimethylamino)phosphine was prepared according to [28]. Solvents for extraction and chromatography were of technical grade and distilled prior to use. TLC: silica-gel *60* F_{254} glass plates (*Merck*); visualization by UV and/or *A*) by dipping in a soln. of anisaldehyde (10 ml), conc. H_2SO_4 (10 ml), and AcOH (2 ml) in EtOH (180 ml) or *B*) cerium(IV) sulfate (3 mM)/ammonium molybdate (250 mM) in aq. H_2SO_4 (10%), followed by heating. Flash column chromatography (CC): silica gel *60* (40–63 μm, 230–400 mesh, *Fluka*) at low pressure. The chromatography of acid-sensitive compounds was carried out with eluent containing 2% Et_3N. ^{1}H- and ^{13}C-NMR: *Bruker AC-300* or *Bruker AMX-400*, δ values in ppm (solvent signals as internal standards), J [Hz]; ^{31}P-NMR: *Bruker AMX-400*, δ values in ppm (85% H_3PO_4 as external standard). ESI-MS: *VG Platform* single quadrupole ESI mass spectrometer. HR-MALDI-MS: *IonSpec Ultima* FTMS mass spectrometer, 3,5-dihydroxybenzoic acid as matrix, pos. mode; Abbreviations: DMT: 4,4′-dimethoxytrityl; BOP: [(Benzotriazol-1-yl)oxy]tris-(dimethylamino)phosphonium hexafluorophosphate.

Synthesis of Phenanthrene-Derived Phosphoramidite Building Blocks. N-*{2-[(4,4′-Dimethoxytrityl)oxy]ethyl}*-N′-*(2-hydroxyethyl)phenanthrene-3,6-carboxamide* (**2**). *Dimethyl phenanthrene-3,6-dicarboxylate* (**1**; 1.38 g, 4.69 mmol) was dissolved in 2-aminoethanol (6.9 ml, 0.11 mol) and heated to 175° for 30 min. Then, the excess of 2-aminoethanol was removed under reduced pressure to give the crude *N*,*N*′-bis(2-hydroxyethyl)phenanthrene-3,6-dicarboxylate as a yellow solid (TLC (AcOEt/MeOH 9:1): R_f 0.24). At r.t., this intermediate was dissolved in dry pyridine (12 ml). Then, a soln. of DMT-Cl (1.52 g, 4.5 mmol) in pyridine (6 ml) was added over 1 h. The mixture was stirred for another 2 h, and then the solvent was removed under reduced pressure. The residue was taken up in CH_2Cl_2 and washed first with 10% citric acid and then with sat. aq. $NaHCO_3$ soln., dried ($MgSO_4$), and evaporated to give a highly viscous oil. The purification by CC (silica gel; AcOEt/hexane 8:2 → AcOEt (+1% NEt_3)) furnished **2** (1.391 g, 2.12 mmol, 45%) as colorless foam. TLC (AcOEt/hexane): R_f 0.29. UV (MeCN): 315 (15900), 309 (13800), 303 (16400), 288 (10100), 278 (sh, 13600), 258 (54500), 254 (52900), 251 (53800), 242 (49800), 237 (50000), 220 (36200). ^{1}H-NMR (400 MHz, $CDCl_3$): 2.43 (br. *s*, OH); 3.42 (*t*, J = 5.3, $ROCH_2$); 3.68 (*m*, CH_2OH); 3.73 (*s*, 2 MeO); 3.74 (*t*, J = 4.8, (partially hidden), CH_2N); 3.91 (*t*, J = 4.9, CH_2N'); 6.80 (*d*, J = 8.8, 4 arom. H); 7.12–7.82 (*m*, 17 arom. H); 8.80, 8.85 (2*s*, 2 CONH). ^{13}C-NMR (100 MHz, $CDCl_3$): 41.0, 43.7 (2*t*, CH_2N, CH_2N'); 55.6 (*q*, MeO); 62.5 (*t*, CH_2OH); 67.3 (*t*, CH_2OCAr_3); 86.7 (*s*, Ar_3C); 113.6, 122.0, 122.7, 124.8, 125.8, 127.3, 128.1, 128.2, 128.3, 128.5, 128.9, 129.0 (12*d*, arom. C); 129.7, 130.0 (2*s*, arom. C); 130.4 (*d*, arom. C); 132.65, 132.71, 134.01, 134.02 136.4, 145.2, 158.9 (7*s*, arom. C); 168.4, 169.2 (2*s*, CONH). HR-MALDI-MS: 677.2630 ($[M+Na]^+$; calc. 677.2622).

N-*{2-[(Diisopropylamino)(2-cyanoethyl)phosphinoxy]ethyl}*-N′-*{2-[(4,4′-dimethoxytrityl)oxy]ethyl}-phenanthrene-3,6-carboxamide* (**4**). At r.t., a soln. of **2** (1.29 g, 1.97 mmol) in CH_2Cl_2 (4 ml) was added slowly (over 30 min) to a suspension of diisopropylammonium 1*H*-tetrazolide (472 mg, 2.76 mmol) and (2-cyanoethyl)bis-(diisopropylamino)phosphine (713 mg, 2.36 mmol) in CH_2Cl_2 (6 ml). After 4 h at r.t., the mixture was diluted with CH_2Cl_2 and washed with sat. aq. $NaHCO_3$ soln. The org. layer was dried (K_2CO_3) and evaporated under reduced pressure. Purification of the resulting oil by CC (silica gel; AcOEt/hexane 2:3 → 3:2 (+2% Et_3N)) furnished **4** (1.284 g, 1.502 mmol, 76%) as colorless foam. TLC (AcOEt/hexane/NEt_3 5:4:1): R_f 0.36. UV (MeCN): 316 (15800), 310 (13600), 303 (16500), 288 (10000), 279 (sh., 13600), 258 (54600), 255 (52800), 251 (53700), 242 (49500), 237 (49900), 220 (36800). ^{1}H-NMR (400 MHz, $CDCl_3$): 1.19 (*d*, J = 6.5, 2 Me_2CHN); 2.62 (*t*, J = 6.0, CH_2CN); 3.4–4.0 (*m*, OCH_2CH_2CN, 2 Me_2CHN, NCH_2CH_2, $N'CH_2CH_2$); 3.75 (*s*, 2 MeO); 6.91 (*d*, J = 8.3, 4 arom. H); 6.9–8.1 (*m*, 17 arom. H); 9.23 (*s*, NH, N′H). ^{13}C-NMR (100 MHz, $CDCl_3$): 21.0 (*t*, J(C,P) = 5, CH_2CN); 25.0, 25.1 (2*q*, J(C,P) = 4, Me_2CH); 40.9 (*t*, CH_2N); 41.6 (*t*, J(C,P) = 7.0, $POCH_2CH_2N'$); 43.5 (*d*, J(C,P) = 12, Me_2CH); 55.6 (*q*, MeO); 58.9 (*t*, J(C,P) = 20, $POCH_2$); 62.7 (*t*, J(C,P) = 15, $POCH_2$); 62.9 (*t*, $DMTOCH_2$); 86.6 (*s*, Ar_3C); 113.6 (*d*, arom. C); 118.8 (*s*, CN); 122.5, 122.7, 125.3, 125.7, 127.2, 127.3, 128.3, 128.5, 128.6, 129.4, 129.5, 130.4 (12*d*, arom. C); 133.2, 133.3, 134.38, 134.43, 136.4, 145.3, 158.9 (7*s*, arom. C); 167.88, 168.00 (2 s, CONH). ^{31}P-NMR (216 MHz, $CDCl_3$): 149.53. HR-ESI-MS (pos. mode): 873.4220 ($[M+NH_4]^+$; calc. 873.4230).

4-[(4,4′-Dimethoxytrityl)oxy]butanamine (**6**). A mixture of 4-aminobutan-1-ol (1 g, 11.22 mmol) and phthalic anhydride (1.662 g, 11.22 mmol) was kept at 145° for 2 h. After cooling to r.t., the resulting yellow oil was dissolved in pyridine (20 ml), then DMT-Cl (4.06 g, 12 mmol) was added. After 2 h at r.t., the soln. was diluted with AcOEt/hexane 1:1 (100 ml), washed twice with sat. aq. $NaHCO_3$ soln., dried ($MgSO_4$), and evaporated. The remaining solid was dissolved in MeOH (40 ml) together with $NH_2NH_2 \cdot H_2O$ (2.5 g, 50 mmol). This soln. was stirred at 40° for 4 h. Then, the precipitate was filtered off, and the filtrate was reduced to *ca.*

10 ml. This soln. was diluted with CH_2CH_2 (150 ml), washed with 2M Na_2CO_3, dried ($MgSO_4$), and evaporated to give the crude product as a yellow oil. After purification by CC (20 g silica gel; $CH_2Cl_2 \rightarrow CH_2Cl_2 + 3\%$ MeOH (+5% NEt_3)), **6** (2.89 g, 7.4 mmol, 66%) was obtained as highly viscous, colorless oil. TLC (CH_2Cl_2/MeOH/NEt_3 8:1:1): R_f 0.6. UV (MeOH): 272 (8300), 256 (6700); 235 (17100), 221 (13900). ^{1}H-NMR (300 MHz, $CDCl_3$): 1.46 (br. *s*, NH_2); 1.55, 1.64 (2*m*, $NCH_2CH_2CH_2CH_2O$); 2.68 (*t*, *J* = 6.6, CH_2N); 3.07 (*t*, *J* = 6.5 CH_2O); 3.80 (*s*, 2 MeO); 6.83 (*d*, *J* = 8.8, 4 arom. H); 7.1–7.5 (*m*, 9 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 26.5, 27.7 (2*t*, $CH_2CH_2CH_2CH_2$); 40.4 (*t*, CH_2N); 54.3 (*q*, MeO); 62.1 (*t*, CH_2ODMT); 84.9 (*s*, Ar_3C); 112.1, 125.8, 126.9, 127.3, 127.5, 129.1 (6*d*, arom. C); 135.7, 144.4, 157.5 (3*s*, arom. C). HR-ESI-MS (pos. mode): 783.4390 ($[2M + H]^+$; calc. 783.4373).

N-*{4-[(4,4′-Dimethoxytrityl)oxy]butyl}*-N′-*(4-hydroxybutyl))phenanthrene-3,6-carboxamid* (**7**). At r.t., a suspension of **1** (0.5 g, 1.7 mmol) in EtOH/THF 1:1 (10 ml) was treated with 1M aq. NaOH (3.4 ml) and heated to reflux for 2 h. The solvent was then removed under reduced pressure, and the remaining solid was dried under high vacuum. The crude intermediate was suspended in DMF (8.5 ml)/^{i}Pr$_2$NEt (0.6 ml) and stirred for 30 min after the addition of BOP (827 mg, 1.87 mmol). Then, a soln. of **6** (800 mg, 2.04 mmol) and 4-aminobutan-1-ol (182 mg, 2.04 mmol) in CH_2Cl_2 (3 ml) was added, and the suspension was stirred for another h. The mixture was then diluted with AcOEt/hexane 1:1 (100 ml), washed with sat. aq. $NaHCO_3$ soln., dried ($MgSO_4$), and evaporated. The purification by CC (AcOEt) afforded **7** (269 mg, 0.378 mmol, 22%) as colorless foam. TLC (AcOEt/MeOH 95:5): R_f 0.54. UV (MeCN): 315 (14900), 309 (12700), 302 (15400), 292 (9600), 258 (51200), 254 (49900), 250 (51100), 241 (47800), 237 (48400) 220 (35900), 205 (58000). ^{1}H-NMR (300 MHz, $CDCl_3$): 1.75 (*m*, 2 $CH_2CH_2CH_2CH_2$); 3.13 (*t*, *J* = 5.5, DMTOCH_2); 3.52 (*m*, CH_2N, CH_2N', OH); 3.75 (*s*, 2 MeO); 3.76 (*m*, CH_2OH); 6.80 (*d*, *J* = 8.8, 4 arom. H); 7.00–7.92 (*m*, 17 arom. H); 8.87, 8.88 (2*s*, NH, N′H). ^{13}C-NMR(100 MHz, $CDCl_3$): 26.2, 26.7, 27.8, 30.1 (4*t*, $NCH_2CH_2CH_2$, $N'CH_2CH_2CH_2$); 40.3, 40.4 (2*t*, CH_2N, CH_2N'); 55.2 (*q*, 2 MeO); 62.5, 63.1 (2*t*, CH_2ODMT, CH_2OH); 85.9 (*s*, Ar_3C); 113.0, 121.4, 121.8, 124.7, 125.3, 126.6, 126.7, 127.5, 127.8, 128.1, 128.2, 128.5 (12*d*, arom. C); 129.4, 129.5 (2*s*, arom. C); 130.0 (*d*, arom. C); 132.3, 132.5, 133.4, 133.5, 136.5, 145.2, 158.3 (7*s*, arom. C); 167.9, 168.0 (2*s*, 2 CONH). HR-MALDI-MS: 733.3256 ($[M + Na]^+$; calc. 733.3248).

N-*{4-[(Diisopropylamino)(2-cyanoethyl)phosphinoxy]butyl}*-N′-*{4-[(4,4′-dimethoxytrityl)oxy]butyl}phenanthrene-3,6-carboxamide* (**8**). A soln. of **7** (370 mg, 0.52 mmol) in CH_2Cl_2 (2.5 ml) was added at r.t. over 30 min to a suspension of diisopropylammonium 1*H*-tetrazolide (125 mg, 0.73 mmol) and (2-cyanoethyl)bis(diisopropylamino)phosphine (188 mg, 0.63 mmol) in CH_2Cl_2 (3 ml). The mixture was stirred at r.t. for 4 h, then diluted with CH_2Cl_2 and washed with sat. aq. $NaHCO_3$ soln. The org. layer was dried (K_2CO_3), and the solvent was removed under reduced pressure. After CC (silica gel; AcOEt/hexane 2:3 → 3:2 (+2% Et_3N)), **8** (1.284 g, 1.502 mmol, 76%) was obtained as colorless foam. TLC (AcOEt/hexane/Et_3N 5:4:1): R_f 0.32. UV (MeCN): 315 (15700), 309 (13300), 303 (16200), 286 (10400), 278 (sh, 13600), 259 (55400), 255 (53500), 250 (54500), 241 (49500), 237 (49800) 220 (37100). ^{1}H-NMR (400 MHz, $CDCl_3$): 1.08, 1.09 (2*d*, *J* = 6.8, Me_2CH); 1.6–1.8 (*m*, $CH_2CH_2CH_2CH_2$); 2.54 (*t*, *J* = 6.3, CH_2CN); 3.06 (*t*, *J* = 6.0, CH_2ODMT); 3.4–3.8 (*m*, $P(OCH_2)_2$, CH_2N, CH_2N', $NCHMe_2$); 3.67 (*s*, 2 MeO); 6.6–8.0 (*m*, 21 arom. H); 5.05 (*s*, NH, N′H). ^{13}C-NMR (100 MHz, $CDCl_3$): 20.9 (*t*, *J*(C,P) = 8, CH_2CN); 25.0, 25.1 (2*q*, *J*(C,P) = 4, Me_2CH); 26.7, 27.2, 28.2 (3*t*, $CH_2CH_2CH_2CH_2$); 29.1 (*t*, *J*(C,P) = 7, $POCH_2CH_2$); 40.4, 40.7 (2*t*, CH_2N, CH_2N'); 43.4 (*d*, *J*(C,P) = 12, PN*C*H); 55.6 (*q*, MeO); 58.6 (*t*, *J*(C,P) = 20, $POCH_2CH_2CN$); 63.5 (*t*, CH_2ODMT); 63.6 (*t*, *J*(C,P) = 17, $POCH_2CH_2CH_2$); 86.3 (*s*, Ar_3C); 113.4 (*d*, arom. C); 118.5 (*s*, CN); 122.4, 122.5, 125.4, 125.6, 127.0, 128.2, 128.4, 128.5, 129.3, 130.4 (10*d*, arom. C); 133.3, 133.4, 134.2, 134.3, 136.9, 145.6, 158.7 (7*s*, arom. C); 167.96, 167.99 (2*s*, CONH). ^{31}P-NMR (216 MHz, $CDCl_3$): 148.68. HR-MALDI-MS: 933.4338 ($[M + Na]^+$; calc. 933.4327).

Synthesis and Analysis of Oligonucleotides. Standard nucleoside phosphoramidites were from *Chemgenes* (Ashland, MA). Oligonucleotides were synthesized on a *392 DNA/RNA Synthesizer* (*Applied Biosystems*) according to the standard phosphoramidite chemistry [20][21] on a 1.0-μmol scale ('trityl-off' mode). The coupling time was extended to 1.5 min for **4**, **5**, and **8**. Coupling efficiencies were >98% for all building blocks, including **4**, **5**, and **8**. The oligomers were detached and deprotected under standard conditions (conc. aq. NH_3, 55°, 16 h). The crude oligomers were purified by anion-exchange HPLC: *MonoQ HR 5/5* (*Pharmacia*); flow 1 ml/min; eluent *A:* 20 mM Na_3PO_4 in H_2O (pH 11.5); eluent *B:* 20 mM Na_3PO_4, 2M NaCl in H_2O (pH 11.5); elution at r.t.; detection at 260, 280, and 320 nm and desalted over *SepPak* cartridges (*Waters*, Milford, USA). All oligonucleotides were analyzed by ESI-MS. The masses were found to be within 0.0005% of the expected mass. The extinction coefficients at 260 nm (ε_{260}) were calculated with '*Biopolymer Calculator*' (http://paris.chem.yale.edu/extinct.html). For the analogues **P2**, **P3**, and **P4**, resp., an ε_{260} value of 49300 was estimated, according to the UV spectra of *N,N′*-bis(2-hydroxyethyl)phenanthrene-3,6-carboxamide (data not shown, measured in H_2O, 0.1M NaCl, 10 mM *Tris* · HCl pH 7.5.

Thermal-Denaturation Experiments. UV Melting curves were determined at 260 nm on a *Varian Cary 3e* spectrophotometer equipped with a *Peltier block* and *Varian WinUV* software. Unless otherwise indicated, the melting curves were measured at an oligomer concentration of 2.5 μM in 10 mM *Tris* · HCl, 100 mM NaCl, pH 7.5. A heating/cooling cycle in the temp. range of 0° – 95° or 20° – 95° was applied with a temp. gradient of 0.5°/min. All ramps were indicating equilibrium melting processes. T_m Values were defined as the maximum of the first derivative of the melting curve.

RNA-Folding Prediction and Modelling. Secondary-structure predictions and T_m calculations of **12** and **13** were obtained by using RNA *mfold* ([25], http://www.bioinfo.rpi.edu/applications/mfold/). The conformation of hairpin **9** (see *Fig. 2*) was optimized by the amber force field (*Hyperchem 7.0*, *Hypercube*, Waterloo, Ontario).

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