The Effect of a Non-nucleosidic Phenanthrene Building Block on DNA Duplex Stability

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Dedicated to Professor *Dieter Seebach* on the occasion of his 65th birthday

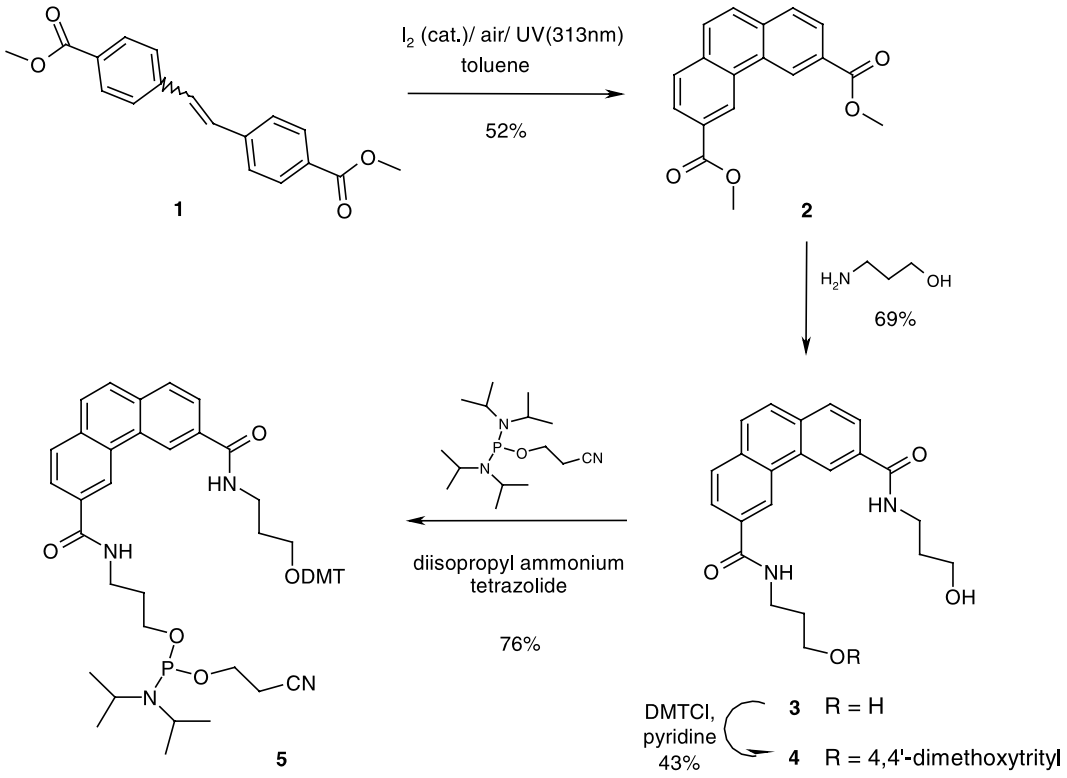
Oligonucleotides containing a phenanthrene-derived, non-nucleosidic building block with flexible linkers were synthesized. The effect of the phenanthrene moiety on duplex stability at different positions was investigated. Placement of two phenanthrene residues in opposite positions had a slightly positive effect on duplex stability. This positive effect was further increased, when two phenanthrene pairs were juxtaposed. In contrast, introduction of a single phenanthrene unit opposite to an adenosine or a thymidine led to a destabilization of the duplex. A model of a phenanthrene-modified duplex is proposed.

Introduction. – Modified oligonucleotides have found widespread applications as diagnostic and research tools. In addition, incorporation of different chemical modifications into oligonucleotides can lead to a better understanding of biological and chemical properties of nucleic acids. Chemical changes of sugar, base, or phosphate backbone have been a topic of intense research. Furthermore, combinations of different chemical modifications are well-known and have substantially contributed to innovation in the field of antisense technology [1][2]. The influence of nucleoside-linked aromatic and heteroaromatic base analogs on duplex stability and base pairing properties has been reported [3–6] and reviewed [7]. As part of our activities aimed at the development of non-nucleosidic DNA building blocks, we investigated the influence of a phenanthrene unit on duplex stability. Here, we report the synthesis and properties of oligonucleotides containing non-nucleosidic phenanthrene building blocks.

Results and Discussion. – Chemical synthesis of the phenanthrene phosphoramidite building block is shown in *Scheme 1*. The known dimethyl 4,4′-stilbenedicarboxylate **1** was prepared in analogy to the procedures reported in the literature [8]. Formation of the phenanthrene was achieved by oxidative photochemical cyclization of **1** [9]. The phenanthrenedicarboxylate **2** obtained was treated with 3-aminopropan-1-ol to afford the corresponding dicarboxamide **3**. Reaction of **3** with 4,4′-dimethoxytrityl chloride under controlled conditions (see *Exper. Part*) gave the mono-protected intermediate **4**, which was subsequently transformed into the phosphoramidite derivative **5**.

For control purposes, we synthesized an open-chain linker lacking the phenanthrene moiety. Thus, dimethyl malonate was treated with 3-aminopropan-1-ol to yield diamide **6**, which was directly transformed into the mono-protected **7**. Phosphitylation gave the desired phosphoramidite **8** (*Scheme 2*).

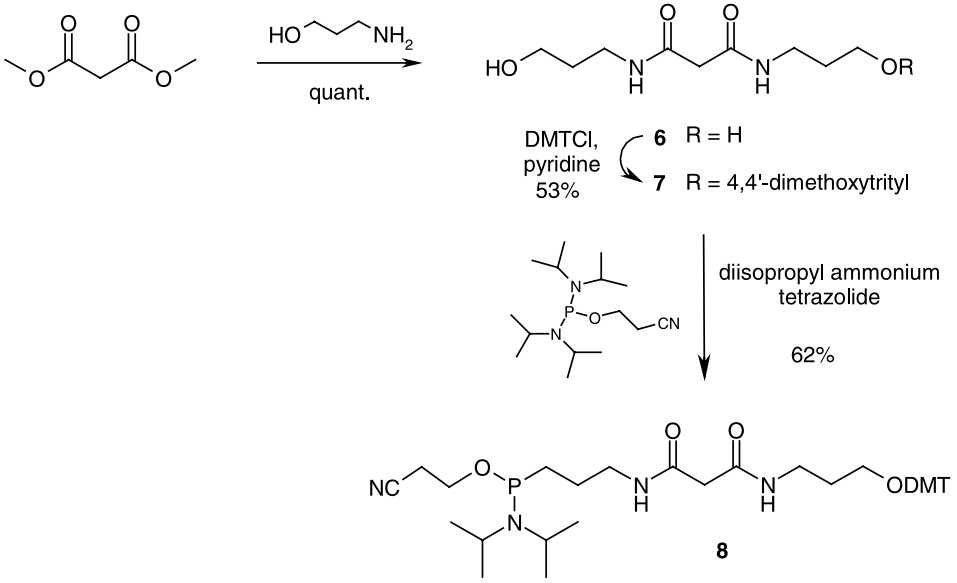
Scheme 1. *Synthesis of the Phenanthrenedicarboxamide-Derived Phosphoramidite Building Block* **5**



Building blocks **5** and **8** were incorporated into oligonucleotides in various positions. Assembly of the different oligonucleotides involved the standard phosphoramidite procedure [10][11]. To ensure a high incorporation yield of the modified building blocks, a longer coupling time was allowed in the respective cycles (see *Exper. Part*). Deprotection (conc. NH_3, 55°), followed by standard HPLC purification yielded oligonucleotides **9**–**20** (*Table 1*).

The effect of the building block **5** on duplex stability was analyzed by thermal denaturation experiments. As can be seen from *Table 1*, placement of a phenanthrene (**P**) opposite to a canonical base (thymine or adenine, *Entries 2* and *3*) results in a considerable destabilisation (ΔT_m −3.7 and −5.4°, respectively). On the other hand, no destabilization is observed when two phenanthrenes are placed at opposite sites in the duplex (ΔT_m +0.3°, *Entry 4*). Surprisingly, two pairs of juxtaposed phenanthrenes lead to a clear stabilization of the duplex (ΔT_m/mod +1.3°, *Entry 5*). Other combinations of **P-P** pairs, which are not juxtaposed (*Entries 6* and *7*) have a marginal effect. To better understand the contribution of the phenanthrene moiety to duplex formation, we synthesized oligonucleotides **19** and **20** containing the linker **L**, in which a simple CH_2 unit has replaced the phenanthrene. A strong decrease (ΔT_m −17.7°) in duplex stability was observed, when **L** was placed opposite to a natural base (adenine;

Scheme 2. *Synthesis of the Malonamide-Derived Phosphoramidite Building Block* **8**



see *Table 1*, *Entry 8*), which is in agreement with the data observed with a similar linker [12]. A duplex containing two **L**s in opposite positions (*Entry 9*) shows an even larger destabilization (ΔT_m −20.7°). On the other hand, placement of **P** opposite to **L** (*Entry 10*) partially reduces this destabilization (ΔT_m −12.3°). Thus, the phenanthrene moiety significantly contributes to duplex stability.

The data obtained can be interpreted as a consequence of favorable stacking effects of the phenanthrene residue within the duplex. This is supported by the fact that the duplex containing two **P-P** pairs (duplex **13**/**14**) shows a strongly increased hyperchromic effect compared to the unmodified duplex (duplex **9**/**10**; see *Fig. 1*). Circular dichroism (CD) spectral analysis of the duplex **13**/**14** is consistent with an overall *B*-conformation (data not shown).

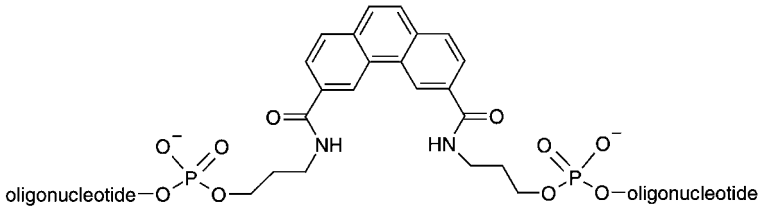
Finally, thermodynamic data were estimated from the thermal denaturation experiments (see *Table 2*). As can bee seen, the parameters obtained by analysis of the melting curves (curve-fitting method [13]) of the phenanthrene-modified duplex (*Entry 4*) are comparable to the unmodified duplex (*Entry 1*). Although the data obtained from the melting curves have to be interpreted with some caution (estimated error ±5%), they indicate an unfavorable reaction enthalpy for formation of the duplex containing two phenanthrenes (**P**), which is compensated by a favorable entropy term. On the other hand, the ΔH of formation of the duplex containing two linkers without phenanthrenes (**L**; *Entry 9*) is very unfavorable and cannot be offset by a favorable ΔS. The calculated $\Delta G^{25°}$ are reflecting the observed T_m values (*cf. Table 1*).

Based on the available data, we favor a model of a right-handed duplex in which two opposite aromatic phenanthrene units are stacked in an inter-strand fashion. Simple model considerations suggest that the relatively long and flexible linker arms enable a continuous duplex without loss of stacking interactions (see *Fig. 2*). Such a

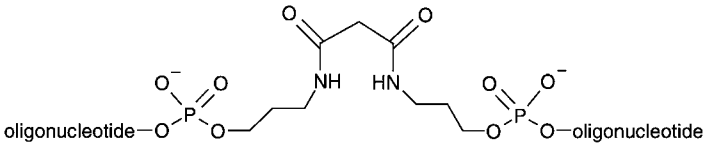
Table 1. *Hybridization Data* (T_m [°]) *of Different Oligonucleotide Duplexes Containing a Phenanthrene-Based Building Block* (**P**) *or a Linker without Aromatic Residue* (**L**). All measurements were carried out at pH 7.5 in 100 mM aqueous NaCl solution; for detailed conditions, see *Exper. Part.*

Entry	Oligonucleotide	Duplex	T_m [°C]	ΔT_m [°C]	ΔT_m/mod [°C]
1	**9**	5′ AGC TCG GTC ATC GAG AGT GCA	67.7		
	10	3′ TCG AGC CAG TAG CTC TCA CGT			
2	**9**	5′ AGC TCG GTC ATC GAG AGT GCA	64.0	– 3.7	– **3.7**
	11	3′ TCG AGC CAG T**P**G CTC TCA CGT			
3	**12**	5′ AGC TCG GTC A**P**C GAG AGT GCA	62.3	– 5.4	– **5.4**
	10	3′ TCG AGC CAG TAG CTC TCA CGT			
4	**12**	5′ AGC TCG GTC A**P**C GAG AGT GCA	68.0	0.3	**0.3**
	11	3′ TCG AGC CAG T**P**G CTC TCA CGT			
5	**13**	5′ AGC TCG GTC **PP**C GAG AGT GCA	70.3	2.6	**1.3**
	14	3′ TCG AGC CAG **PP**G CTC TCA CGT			
6	**15**	5′ AGC TCG GT**P** A**P**C GAG AGT GCA	67.3	– 0.4	– **0.2**
	16	3′ TCG AGC CA**P** T**P**G CTC TCA CGT			
7	**17**	5′ AGC TCG G**P**C A**P**C GAG AGT GCA	68.3	0.6	**0.3**
	18	3′ TCG AGC C**P**G T**P**G CTC TCA CGT			
8	**19**	5′ AGC TCG GTC A**L**C GAG AGT GCA	50.0	– 17.7	– **17.7**
	10	3′ TCG AGC CAG TAG CTC TCA CGT			
9	**19**	5′ AGC TCG GTC A**L**C GAG AGT GCA	47.0	– 20.7	– **20.7**
	20	3′ TCG AGC CAG T**L**G CTC TCA CGT			
10	**19**	5′ AGC TCG GTC A**L**C GAG AGT GCA	55.4	– 12.3	– **12.3**
	11	3′ TCG AGC CAG T**P**G CTC TCA CGT			

P =



L =



model of a continuous duplex is in agreement with the observed thermodynamic stability, the relatively strong hyperchromicity, and the shape of the melting curves, which shows a single, sharp transition indicating a highly cooperative melting process. Finally, it is worth mentioning that the phenanthrene building block **P** strongly prefers a **P** in opposite position compared to a canonical base (A or T). The relative destabilizations (ΔT_m –5.7 and –4.0°, see *Table 1*, *Entry 4* compared with *Entries 3* and *2*, resp.) are well comparable to *Watson-Crick* base-pair mismatches.

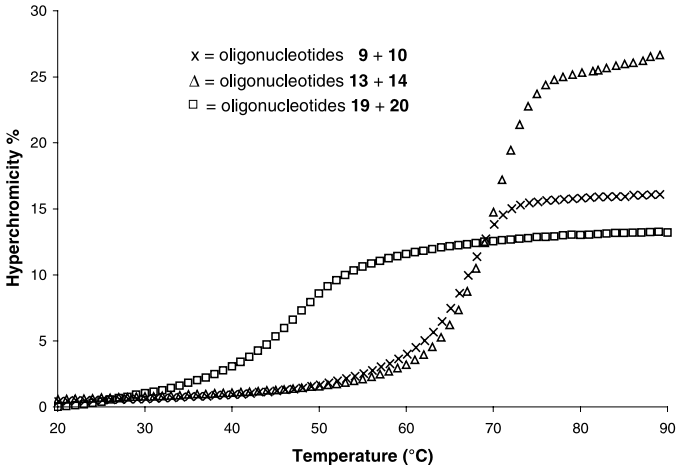


Fig. 1. T_m *Curves of three different duplexes.* × = Unmodified duplex; Δ = duplex containing two **PP** pairs; □ = duplex containing a linker **L** in each strand (*cf.* also *Table 1*).

Table 2. *Comparison of Thermodynamic Parameters Obtained from Experimental UV Melting Curves* (curve-fitting method, estimated error ±5%) [13]

Entry	Oligo-nucleotide	*Duplex*	ΔH [kcal mol^{-1}]	ΔS [cal mol^{-1} K^{-1}]	$\Delta G^{25°}$ [kcal mol^{-1}]
1	**9**	5′ AGC TCG GTC ATC GAG AGT GCA	– 129	– 351	– 24.3
	10	3′ TCG AGC CAG TAG CTC TCA CGT			
4	**12**	5′ AGC TCG GTC A**P**C GAG AGT GCA	– 117	– 314	– 23.4
	11	3′ TCG AGC CAG T**P**G CTC TCA CGT			
9	**19**	5′ AGC TCG GTC A**L**C GAG AGT GCA	– 63	– 167	– 13.2
	20	3′ TCG AGC CAG T**L**G CTC TCA CGT			

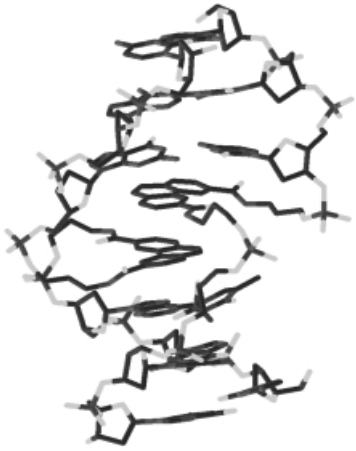


Fig. 2. *Illustration of a modified DNA duplex showing the possibility of inter-strand stacking of two phenanthrenes*

Conclusions. – Oligonucleotides containing non-nucleosidic phenanthrene building blocks (**P**) form stable duplexes. **P** strongly prefers **P** in opposite position compared to A or T. The data obtained are in agreement with a model of a continuous right-handed duplex with inter-strand stacked phenanthrenes.

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Experimental Part

General. Chemicals, solvents, and reagents for reactions were generally from *Acros*, *Aldrich*, or *Fluka*, and were of the highest quality available. (Cyanoethoxy)bis(*N,N*-dimethylamino)phosphine was prepared according to the procedure in [14]. Solvents for extraction and chromatography were of technical grade and distilled prior to use. TLC: silica-gel *60* F_{254} glass plates (*Merck*); visualisation by UV and/or *A*) by dipping in a soln. of H_2SO_4/H_2O/EtOH 14:4:1 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. In case of acid-sensitive compounds, the silica gel was pretreated with solvents containing 0.5–1% 4-methylmorpholine. ^{1}H- and ^{13}C-NMR: *Bruker AC-300*, δ values in ppm (solvents signals as internal standard), *J* [Hz]; ^{31}P-NMR: *Bruker AMX 400*, δ values in ppm (85% H_3PO_4 as external standard). EI-MS: *Autospec Q* (*Micromass*), ionization energy 70 eV; only selected peaks; LSI-MS: *Autospec Q* Cs^+ ion gun. ESI-MS: *VG Platform* single quadrupole ESI mass spectrometer. Oligonucleotides were synthesized on a *392 DNA/RNA Synthesizer* (*Applied Biosystems*) according to the standard phosphoramidite chemistry [10][11]. The nucleoside phosphoramidites were from *CHEMGENES* (Ashland, MA). The standard synthetic procedure ('trityl-off' mode) was used, and, only for the non-natural phosphoramidites, the coupling time was extended to 5 min. Coupling efficiencies for all building blocks including **5** and **8** were >98%. After standard detachment and deprotection (conc. NH_3, 55°, 16 h), the crude oligomers were purified by anion-exchange HPLC (*Macherey-Nagel, Nucleogen DEAE 60/7*) and desalted over *Sep-Pak* cartridges (*Waters*, Milford, USA). All oligonucleotides were analyzed by electrospray MS. The masses were found to be within 0.0005% of the expected mass. UV Melting curves were determined at 260 nm on a *Varian Cary 3e* spectrophotometer equipped with a *Peltier* block and *Varian WinUV* software. Complementary oligonucleotides were mixed to 1:1 stoichiometry, and the solns. were adjusted to a final duplex concentration of 0.5–0.7 μM in 0.1 mM Tris·HCl, 100 mM NaCl, pH 7.5. A heating-cooling-heating cycle in the temp. range of 0–90° or 20–90° 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. Thermodynamic date were determined directly from the melting curves by a curve-fitting procedure that is based on the two state model for a bimolecular non-self-complementary oligonucleotide pair [13]. Abbreviation: DMT: 4,4′-dimethoxytrityl.

Experiments Referring to Scheme 1. *Dimethyl Phenanthrene-3,6-dicarboxylate* (**2**) [9]. A soln. of 4.25 g (14.4 mmol) *dimethylstilbene-4,4′-dicarboxylate* (**1**) [8] in 3 l toluene, containing 0.42 g (1.6 mmol) I_2, was irradiated for 24 h using a medium-pressure Hg lamp (125 W) in a quartz tube, while O_2 was slowly bubbled through the mixture. After removal of the solvent under reduced pressure, the residue was taken up in CH_2Cl_2 and passed through a short column of silica gel. Concentration of the eluate followed by addition of MeOH precipitated a white solid, which, on recrystallization from CH_2Cl_2/MeOH mixture, gave **2** (2.18 g, 52% yield). White prisms. TLC (AcOEt/hexane): R_f 0.34. ^{1}H-NMR (300 MHz, $CDCl_3$): 4.07 (*s*, 6 H); 7.87 (*s*, 2 H); 7.96 (*d*, *J* = 8.4, 2 H); 8.26 (*d*, *J* = 8.4, 2 H); 9.50 (*s*, 2 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 52.5; 125.2; 127.1; 128.6; 128.8; 128.9; 130.0; 134.9; 167.3. EI-MS: 294 (100, $M^{+\cdot}$), 263(81), 176(79).

N,N′-*Bis(3-hydroxypropyl)phenanthrene-3,6-dicarboxamide* (**3**). A suspension of 3 g (10.2 mmol) of **2** in 15 ml of 3-aminopropanol was heated under reflux (160°) for 30 min. The mixture was acidified with 2M HCl and was extracted 4 × with 250 ml of CH_2Cl_2/i-PrOH (3:1). The org. phase was dried (Na_2SO_4) and evaporated to give 2.7 g (69%) of a white solid. ^{1}H-NMR (300 MHz, CD_3OD): 1.83 (*m*, 4 H); 3.50 (*t*, *J* = 7.0, 4 H); 3.62 (*t*, *J* = 6.2, 4 H); 7.82 (*m*, 2 H); 7.95 (*m*, 4 H); 9.33 (*s*, 2 H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 32.6; 36.8; 39.5; 122.3; 125.6; 127.9; 128.6; 129.5; 133.1; 133.3; 166.4. EI-MS: 380 (4, $M^{+\cdot}$), 362 (6), 344(95), 288(100).

N-*(3-[Bis(4-methoxyphenyl)(phenyl)methoxy]propyl)*-N′-*(3-hydroxypropyl)-phenanthrene-3,6-dicarboxamide* (**4**). A soln. of 2.7 g (7.1 mmol) of **3** in 71 ml of dry pyridine was placed under N_2. A soln. of 2.4 g

(7.1 mmol) of DMT chloride in 45 ml of dry pyridine was added slowly over 4 h at r.t. The mixture was stirred overnight, then 250 ml of AcOEt was added, and the mixture was washed 3 × with sat. aq. $NaHCO_3$ soln., dried (Na_2SO_4), and evaporated under reduced pressure. The resulting residue was purified by CC (silica gel; AcOEt, 0.5% 4-methylmorpholine). The fractions were combined, evaporated, and dried under high vacuum to furnish 2.1 g (43%) of **4**. White foam. TLC (AcOEt): R_f 0.32. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.67 (*t*, *J* = 5.1, 2 H); 1.98 (*t*, *J* = 5.8, 2 H); 3.34 (*t*, *J* = 5.3, 2 H), 3.67 (*s*, 6H); 3.72 (*m*, 4 H); 3.80 (*t*, *J* = 5.3, 2 H); 6.74 (*m*, 4 H) 7.2–8.9 (*m*, 15 H); 8.92 (*s*, 1 H); 9.02 (*s*, 1 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 29.2; 32.0, 37.9; 55.2; 60.4; 62.6; 86.7; 113.3; 121.6; 122.3; 124.6; 125.6; 126.9; 128.0; 128.2; 128.7; 128.9; 129.6; 129.8; 130.1; 132.6; 133.7; 133.8; 136.2; 144.8; 158.6; 168.0; 168.6. ESI-MS (pos.): 683 (20, $[M+H]^+$) , 303 (100, DMT).

N-*{3-[Bis(4-methoxyphenyl)(phenyl)methoxy]propyl}*-N′-*(3-{[bis(1-methylethyl)amino](2-cyanoethyl)-phosphinoxy}propyl)phenanthrene-3,6-carboxamide* (**5**). A suspension of 345 mg (2.01 mmol) of diisopropylammonium tetrazolide and 661 µl (1.84 mmol) (cyanoethoxy)bis(dimethylamino)phosphine in 5 ml of dry CH_2Cl_2, was placed under N_2; then a soln. of 1 g (1.46 mmol) **4** in 10 ml of dry CH_2Cl_2 was added slowly at r.t. After 45 min, 100 ml CH_2Cl_2 was added to the mixture, the mixture was washed with 100 ml of sat. aq. $NaHCO_3$ soln., dried (K_2CO_3), and evaporated under reduced pressure. The resulting oil was purified by CC (silica gel; AcOEt/hexane 1:1, 1% 4-methylmorpholine). The fractions were combined, evaporated, and dried under high vacuum to furnish 980 mg (76%) of **5**. White foam. TLC (AcOEt/hexane 1:1): R_f 0.21. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.08 (*m*, 12 H); 1.92 (*m*, 4 H); 2.53 (*m*, 2 H); 3.26 (*m*, 2 H); 3.50 (*m*, 2 H); 3.62 (*m*, 10 H); 3.72 (*m*, 2 H); 3.79 (*m*, 2 H); 6.90 (*m*, 4 H); 6.89 (*m*, 2 H); 7.1–7.4 (*m*, 7 H); 7.6–8.0 (*m*, 6 H); 9.06 (*s*, 1 H); 9.11 (*s*, 1 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 20.7; 24.7; 24.8; 29.4; 30.7; 38.3; 39.2; 43.1; 43.3; 55.2; 55.6; 58.3; 58.6; 62.3; 62.6; 113.3; 122.2; 122.6; 124.9; 125.3; 126.9; 128.0; 128.17; 128.3; 129.0; 130.1; 133.3; 134.0; 136.2; 158.6; 167.7. ^{31}P-NMR (161.9 MHz, $CDCl_3$): 149.3.

Experiments Referring to Scheme 2. N,N′-*Bis(3-hydroxypropyl)propanediamide* (**6**). A soln. of 1.73 ml (15.1 mmol) of dimethyl malonate in 6.8 ml of 3-aminopropan-1-ol was heated under reflux (160°) for 1 h; then, the solvent was removed by vacuum destillation, the white solid residue was pure **6** (3.3 g, quant.). ^{1}H-NMR (300 MHz, $CDCl_3$): 1.73 (*m*, 4 H); 3.20 (*s*, 2 H); 3.46 (*m*, 4 H); 3.67 (*t*, *J* = 5.6, 4 H) ^{13}C-NMR (75 MHz, (D_6)DMSO): 32.3; 35.9; 43.4; 58.4; 166.9. LSI-MS (DTT/DTE): 219 ($[M+H]^+$).

N-*{3-[Bis(4-methoxyphenyl)(phenyl)methoxy]propyl}*-N′-*(3-hydroxypropyl)propanediamide* (**7**). A soln. of 2.0 g (9.7 mmol) of **6** in 70 ml of dry pyridine was placed under N_2. A soln. of 3.3 g (7.1 mmol) of DMT chloride in 45 ml of dry pyridine was added slowly over 1 h at r.t. The mixture was stirred overnight, then 250 ml of AcOEt was added, and the mixture was washed 3 × with sat. aq. $NaHCO_3$ soln., then dried (Na_2SO_4), and evaporated under reduced pressure. The resulting residue was purified by CC (silica gel; AcOEt/MeOH 9:1, 0.5% 4-methylmorpholine). The fractions were combined, evaporated, and dried under high vacuum to furnish 1.71 g (35%) of **7**. Colorless oil. TLC (AcOEt/MeOH 9:1): R_f 0.24. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.69 (*m*, 2 H); 1.80 (*m*, 2 H); 3.05 (*s*, 2 H); 3.21 (*t*, *J* = 5.6, 2 H); 3.41 (*m*, 4 H); 3.61 (*t*, *J* = 5.6, 2 H); 3.80 (*s*, 6 H); 6.84 (*m*, 4 H); 7.2–7.4 (*m*, 9 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 29.3; 32.1; 36.3; 38.0; 42.8; 46.5; 55.3; 59.4; 61.5; 86.3; 113.3; 126.9; 127.9; 128.1; 130.0; 136.2; 145.0; 158.6; 167.5; 168.0. EI-MS: 520 (2, $M^{+\bullet}$), 303 (100, DMT).

N-*(3-{[Bis(1-methylethyl)amino](2-cyanoethoxy)phosphino}propyl)*-N′-*{3-[bis(4-methoxyphenyl)(phenyl)methoxy]propyl}phenanthrene-3,6-dicarboxamide* (**8**). A suspension of 461 mg (2.69 mmol) of diisopropylammonium tetrazolide and 880 µl (2.46 mmol) of (cyanoethoxy)bis(dimethylamino)phosphine in 8 ml of dry CH_2Cl_2 was placed under N_2; then, a soln. of 1 g (1.97 mmol) of **7** in 13.3 ml of dry CH_2Cl_2 was added slowly at r.t. After 3 h, 100 ml of CH_2Cl_2 was added to the mixture, which was washed with 100 ml of sat. aq. $NaHCO_3$ soln., dried (K_2CO_3), and evaporated under reduced pressure. The resulting oil was purified by CC (silica gel; AcOEt/hexane 2:1, 1% 4-methylmorpholine). The fractions were combined, evaporated, and dried under high vacuum to furnish 959 mg (69%) of **8**. Colorless oil. TLC (AcOEt/hexane 2:1): R_f 0.25. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.10 (*m*, 12 H); 1.72 (*m*, 4 H); 2.57 (*t*, *J* = 6.4, 2 H); 2.96 (*s*, 2 H); 3.10 (*t*, *J* = 5.7, 2 H), 3.3–3.6 (*m*, 6 H); 3.71 (*s*, 6 H); 6.76 (*m*, 4 H); 7.15–7.22 (*m*, 9 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 20.5; 24.7; 24.8; 29.3; 30.6; 30.7; 37.0; 37.9; 42.9; 43.0; 43.2; 55.3; 58.2; 58.5; 61.5; 61.7; 86.2; 113.2; 117.9; 126.9; 127.9; 128.1; 130.0; 136.2; 145.0; 158.5; 167.1; 167.4 ^{31}P-NMR (161.9 MHz, $CDCl_3$): 149.0.

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