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Synthesis of Cyclopentane Amide DNA (cpa-DNA) and Its Pairing Properties

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Received February 1, 2003

We recently reported on the synthesis and pairing properties of the DNA analogue bicyclo[3.2.1]-amide DNA (bca-DNA). In this analogue the nucleobases are attached via a linear, 4-bond amide-linker to a structurally preorganized sugar−phosphate backbone unit. To define the importance of the degree of structural rigidity of the bca-backbone unit on the pairing properties, we designed the structurally simpler cyclopentane amide DNA (cpa-DNA), in which the bicyclo[3.2.1]-scaffold was reduced to a cyclopentane unit while the base-linker was left unchanged. Here we present a synthetic route to the enantiomerically pure cpa-DNA monomers and the corresponding phosphoramidites containing the bases A and T, starting from a known, achiral precursor in 9 and 12 steps, respectively. Fully modified oligodeoxynucleotides were synthesized by standard solid-phase oligonucleotide chemistry, and their base-pairing properties with complementary oligonucleotides of the DNA-, RNA-, bca-DNA-, and cpa-DNA-backbones were assessed by UV melting curves and CD-spectroscopic methods. We found that cpa-oligoadenylates form duplexes with complementary DNA that are less stable by −2.7 °C/mod. compared to DNA. The corresponding cpa-oligothymidylates do not participate in complementary base-pairing with any of the investigated backbone systems except with its own (homo-duplex). As its congener bca-DNA, cpa-DNA seems to prefer left-handed helical duplex structures with DNA or with itself as indicated by the CD spectra.

Introduction

Oligonucleotide analogues are of considerable interest in antisense therapy to specifically inhibit the expression of disease-related genes[1] and in genomics to specify the hitherto unknown function of new proteins.[2] Carbohydrate-modified DNA analogues are of particular interest, as the replacement of deoxyribose units by synthetic constructs opens many degrees of freedom to tune the pairing properties of corresponding oligonucleotides.[3] In this context we recently focused on the concept of conformational restriction as a designer tool to enhance the functional performance of DNA analogues.[4]

One of these analogues is bicyclo[3.2.1]amide-DNA (bca-DNA, Figure 1), containing a backbone unit that is conformationally locked in a B-type geometry, to which the nucleobases are attached via a linear amide linker with extended distance between the base and the backbone units relative to DNA (4 instead of 3 bonds).[5] The main characteristics of this system as determined so far

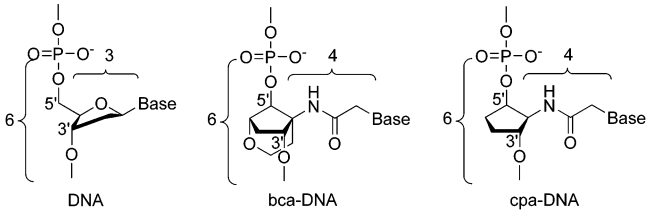

FIGURE 1. Representation of the chemical structures of DNA, bca-DNA, and cpa-DNA, highlighting the relative differences in the distance between base and backbone.

are a strong discrimination of RNA relative to DNA as the complementary pairing partner in random-base sequences, accompanied by a generally lower affinity relative to DNA. Strand association is constrained to antiparallel duplex formation, and Watson−Crick base-pairing is mismatch sensitive as in the case of DNA. An unexpected and interesting feature is the accommodation of bca-homo-adenine sequences in right- as well as left-handed helical structures, and their chirodegeneracy in complementary recognition of D- and L-RNA (both are complex partners in this sequence context).

To investigate the structure/property relationship of bca-DNA in more detail, we wished to evaluate the structurally simpler cyclopentane amide DNA (cpa-DNA, Figure 1), which as the only structural change lacks the pyranose ring. Here we report on the synthesis of the corresponding nucleoside building blocks containing the bases A and T, the synthesis of corresponding oligonucleotide analogues, as well as their pairing properties.

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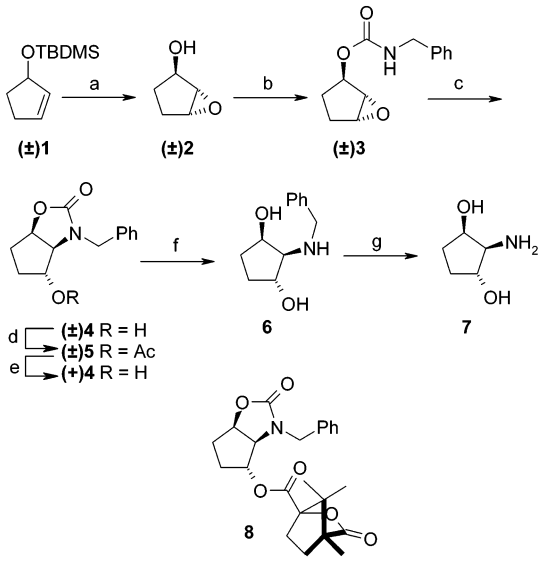
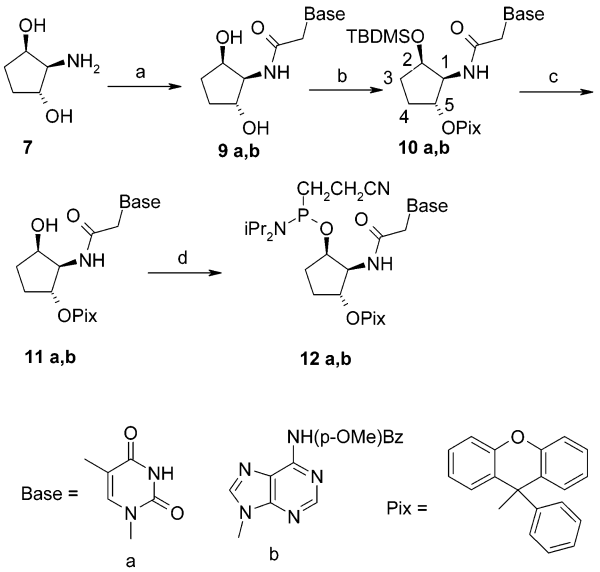
10.1021/jo034143q CCC: $25.00 © 2003 American Chemical Society
Published on Web 08/19/2003

SCHEME 1[a]



[a] Reagents and conditions: (a) (i) *m*-CPBA, CH_2Cl_2, rt, 42%; (ii) Bu_4NF, THF, rt, 93%. (b) BnNCO, toluene, 90 °C, 79%. (c) NaH, DMF, rt, 76%. (d) Ac_2O, DMAP, pyridine, rt, 97%. (e) PLE, phosphate buffer, pH 7, 30 °C, 31%. (f) 8 N KOH, 80 °C EtOH, 82%. (g) 10% Pd/C, H_2, MeOH, rt, 98%.

SCHEME 2[a]



[a] Reagents and conditions: (a) EDC, HOOC−CH_2−base, DMF, rt, 65% for **9a**, 66% for **9b**. (b) (i) 9-Chloro(9-phenyl)xanthene, pyridine/DMSO, rt; (ii) TBDMS−Cl, imidazole, DMF, rt; 33% for **10a**, 31% for **10b**. (c) Bu_4NF, THF, rt, 92% for **11a**, 98% for **11b**. (d) [(^{i}Pr$_2$N)(NCCH$_2$CH$_2$O)]PCl, ^{i}Pr$_2$NEt, THF, rt, 59% for **12a**, 63% for **12b**.

Result and Discussion

Synthesis of cpa-Monomers. 2-Amino-1,3-cyclopentanediol **7** constitutes the basic substructure that serves for the synthesis of the corresponding nucleoside building blocks. This unit, already known in racemic form,[6] was retrosynthetically related to epoxide **2** (Scheme 1).

Epoxidation of the racemic starting material (±)**1**[7] with *m*-CPBA led to the corresponding epoxides in a ratio of 3:1 (anti:syn) in 70% yield. Chromatographic separation of the anti isomer, followed by desilylation with Bu_4NF yielded (±)**2** in 93% yield, which was subsequently converted into the carbamate (±)**3** (79%) with benzyl isocyanate in toluene. The nitrogen in (±)**3** thus serves as the source of the amino function in **7**.

The intramolecular cyclization of (±)**3** was effected in analogy to a known procedure[8] by treatment with NaH in DMF and gave the cyclic carbamate (±)**4**, in 76% yield. To split (±)**4** into pure enantiomers we followed two strategies based on enzymatic kinetic resolution. First we tried a lipase-catalyzed esterification.[9] This enzyme is known to preferentially catalyze the esterification of *R* configured alcohols. However, with *pseudomonas fluorescens* lipase (PFL) no sufficient level of enantiomeric resolution could be obtained. The second approach was based on an esterase-catalyzed kinetic resolution[10] of (±)-**5** that was easily obtained from (±)**4** by standard acetylation. Both yield and enantioselectivity in the hydrolysis by *pig liver esterase* (PLE) proved to be superior. An enantiomeric excess of (+)**4** on the order of 93.0−99.5% (chiral GLC) at 31−37% conversion was observed. Carbamate (+)**4** was subsequently hydrolyzed to the phenylamine **6**, which was directly deprotected by hydrogenolysis to give the dihydroxyamine **7** in 80% yield over the two steps. Although it is known that PLE preferentially hydrolyzes *R* configured esters,[11] we preferred to rigorously confirm the absolute configuration of (+)**4**. This was done by converting (+)**4** into the corresponding (−)-camphanic acid ester **8**, and subjecting crystals of it to X-ray analysis. The X-ray data are in full agreement with the absolute configuration of (+)**4** given in Scheme 1 (see Supporting Information).

The direct and selective introduction of the base units into **7** with use of typical peptide coupling reagents (Scheme 2) needed some optimization. The use of *N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluorborate (TOTU) and ^{i}Pr$_2$NH in DMF led to a mixture of amides and esters. The same was observed when DCC in DMF was used in the presence of DMAP. The best results were obtained with EDC in DMF. Under these conditions, only the amino group in **7** reacted with the base units to give the desired amides in acceptable yields (66% of **9a**, and 65% of **9b**).

The next problem to be solved was the selective addressing of one of the two secondary hydroxyl groups in **9**. Direct pixylation of **9a** gave two regioisomers in a ∼4:1 ratio (^{1}H NMR) in 55% yield. The two regioisomers were not separable by column chromatography. However, after TBDMS protection of the remaining OH function, the isomers could be separated by column chromatography to yield pure **10a** as the major isomer in 33% yield over the two steps. After deprotection of the TBDMS

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TABLE 1. Yield after HPLC Purification (1.3 μmol scale), Sequence, and MS Data of cpa-Oligonucleotides 14 and 15 Synthesized and Used in the Present Study

sequence	$[M-1]^-$ (calcd)	ESI-MS (found)	yield OD^{260} (%)
13 $d(t^{cpa})_{10}$	3389.5	3390.1	2.7 (2.5)
14 $d(a^{cpa})_9$	3125.4	3125.7	2.3 (1.6)

TABLE 2. T_m Data (°C, 260 nm) of Duplexes[a]

	13 $d(t^{cpa})_{10}$	**15** $dT(t^{bca})_{10}$	**19** $d(T)_9$	**18** $d(T)_{10}$
14 $d(a^{cpa})_9$	15.5	<1[b]	~4	~4
17 $d(A)_{10}$	n.d.[c]	n.d.[c]	28.3	32.1
22 $r(A)_{10}$	n.d.[c]	n.d.[c]	20.1	24.5
16 $dT(a^{bca})_{10}$	n.d.[c]	21.6	19.1	23.6

[a] Total concentration of oligonucleotides: 5 mM in 10 mM Na-cacodylate, 1 M NaCl; pH 7 [b] Concentration= 4 μM. [c] n.d. = T_m not detectable.

group, the resulting compound **11a** (92%) was transformed into the phosphoramidite **12a** under standard conditions in 59% yield. The corresponding adenine-containing intermediate **10b** was obtained in the same way and was subsequently transformed into the phosphoramidite building block **12b** (63%). The position of the pixyl group at O−C(5) and the TBDMS group at O−C(2) in **10** was rigorously proven in the case of **10b** by NMR methods (see the Supporting Information). From $^1H-^{13}C$ COSY spectra, H−C(5) could be correlated with the quaternary sp^3 carbon of the pixyl group. Alternatively, a ^1H-NOE between H−C(2) and the Si−CH_3 of the TBDMS group etablished the TBDMS group to be attached at O−C(2). Thus the remaining task was to verify the configuration of the centers C(2) and C(5) relative to C(1). This was unambiguously done by NOE spectroscopy. A strong NOE between the amide NH and H−C(5) established the trans arrangement of substituents at C(1) and C(5). Alternatively, strong mutual NOEs between H−C(1) and H−C(2), carrying the TBDMS group, established the cis substitution pattern of C(1) and C(2).

Synthesis of cpa-Oligonucleotides. cpa-oligonucleotides **13** and **14** were prepared according to the standard protocols for automated solid-phase DNA synthesis on the 1.3-μmol scale (Table 1). As a solid support, commercially available universal solid support was used. The standard DNA synthesis cycle needed some adjustments. The coupling time was extended to 6 min and the standard activator tetrazole was replaced by the more active ethylthio-1*H*-tetrazole. With these conditions, coupling yields of 92−99% for cpa-T and 73−99% for cpa-A were obtained according to the trityl assay. Deprotection and detachment from solid support of crude oligomers was effected in concentrated NH_3:40% methylamine (1:1), 55 °C, 16 h. Under these conditions, byproducts arising from incomplete removal of the 3′-linker were observed by HPLC in nonnegligeable amounts. The oligonucleotides were purified by HPLC and characterized by ESI-MS (Table 1).

Duplex Formation Properties of cpa-DNA with Complementary DNA, bca-DNA, RNA, and L-RNA. To compare the pairing preferences of cpa-DNA with those of bca-DNA the following oligonucleotides **15**−**22**, prepared and investigated earlier, were included in the UV melting curve assays. The corresponding T_m data for duplex melting are summarized in Table 2. Figure 2 contains representative melting curves of selected duplexes.

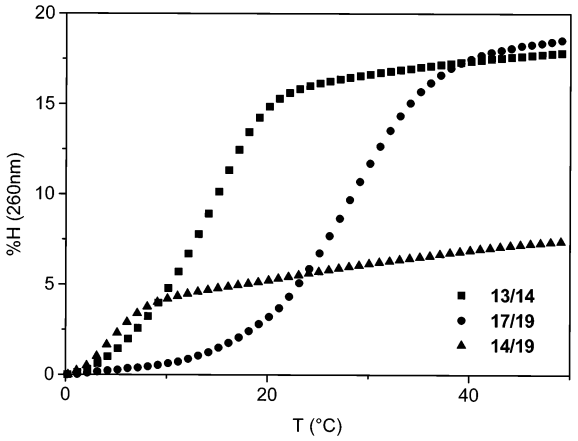

FIGURE 2. Representative UV melting curves of the duplexes **13**/**14**, **17**/**19**, and **14**/**19**. Total concentration of oligonucleotides: 5 μM, in 10 mM Na-cacodylate, 1 M NaCl; pH 7.0.

15: $dT(t^{bca})_{10}$ **16**: $dT(a^{bca})_{10}$ **17**: $d(A)_{10}$

18: $d(T)_{10}$ **19**: $d(T)_9$ **20**: $r^D(T)_{10}dT$

21: $r^L(T)_{10}dT$ **22**: $r(A)_{10}$

The T_m data show that the oligoadenylate **14** still forms a weak duplex with complementary DNA while the oligothymidylate **13** was unable to base-pair with any oligoadenylate sequence, except the one of the same backbone type. Given the T_m of 24 °C for the reference duplex $d(A)_9/d(T)_9$[12] we find a stronger duplex destabilization for the oligoadenylate with the cpa-backbone (−2.2 °C/mod.) as compared to the bca-backbone (−1.0 °C/mod.). In general, the pairing preference of the cpa-oligonucleotide correlates with that of the corresponding bca-backbone although on a more reduced level of affinity.

The pure cpa-DNA duplex shows also slightly reduced thermal stability compared to that of the pure bca-DNA duplex (−0.4 °C/mod.) and to the pure natural duplex (−0.9 °C/mod.). Interestingly, the two structurally related cpa- and bca-backbone types hardly communicate with each other within the oligoadenine/oligothymine sequence motif. The T_m data of both mixed-backbone, cpa/bca duplexes **13**/**16** and **14**/**15** are <1 °C, thus amounting to a loss in thermal stability of >2.4 °C/mod.

CD Spectra of cpa-DNA Single Strands and Duplexes. CD spectra were measured under the same conditions as for T_m analysis. The oligonucleotide cpa-(A_9) (**14**) seems to be preorganized in a left-handed helix as a single strand, as deduced from the enantiomorphic shape of its CD trace relative to that of $d(A_{10})$ (**17**) (Figure 3a). However, there are differences with respect to the CD trace of oligonucleotide **16**, carrying the bca-backbone. For this system, the single-strand structure and helicity is not clear. The CD curves of duplexes of **14** with complementary cpa-oligonucleotide **13**, and especially with complementary DNA **19**, are also reminiscent of left-handed duplex structures. This was concluded based on

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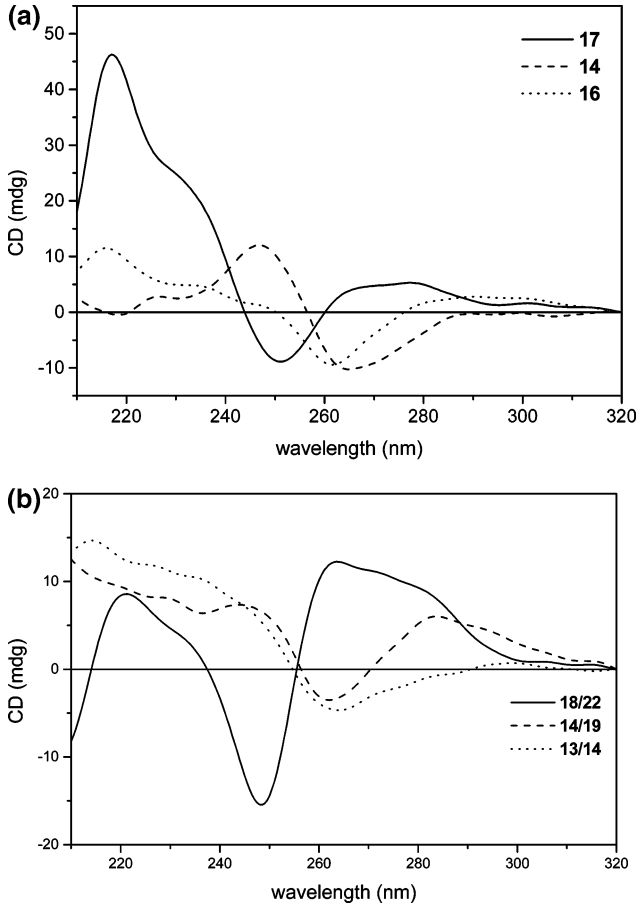

FIGURE 3. CD spectra of selected single strands (a) and duplexes (b). Experimental conditions as indicated in Table 2 (T = 5 °C).

TABLE 3. T_m Data of Selected Duplexes[a]

	duplex				
	14/20	**14/21**	**16/20**	**16/21**	**17/20**
T_m	7.0	19.1	30.7	20.1	37.0

[a] Experimental conditions as indicated in Table 2.

the inverted signs of maxima and minima of the bands at ca. 250 and 265 nm, relative to that of the duplex **18**/**22** (Figure 3b). Thus again, removal of the six-membered ring in bca-DNA, leading to cpa-DNA, does not cause substantial structural differences on the duplex level. It seems though that the cpa-DNA single strand **14** has a higher propensity for left-handed helix formation, compared to bca-DNA.

Pairing Properties of 15 with Complementary D- and L-RNA. As we have already reported, the bca-homoadenine oligonucleotide **16** displays chirodegenerate recognition properties in that it forms duplexes with both enantiomeric forms of complementary RNA (D-RNA **20** and L-RNA **21**). We therefore investigated whether the cpa-DNA series shares these properties too. Initially we studied the stability of duplexes by means of UV melting experiments (Table 3).

As shown in Table 3, the cpa-DNA oligonucleotide **14** pairs to both enantiomeric forms of complementary RNA, as is the case for bca-DNA. Interestingly, the cpa-DNA/L-RNA duplex is more stable than the cpa-DNA/D-RNA duplex by ca. 1.3 °C/base-pair. This is opposite to bca-DNA, which in the same sequence context prefers D-RNA over L-RNA by ca. 1.1 °C/base-pair. This may again be the consequence of the intrinsically left-handed, helical nature of the single stranded cpa-oligonucleotide.

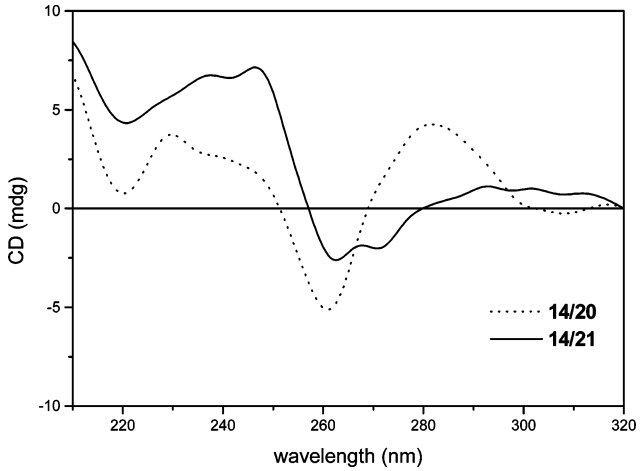

FIGURE 4. CD spectra (experimental conditions as indicated in Table 2, T = 5 °C) of cpa-DNA/RNA duplexes.

While in the case of bca-oligonucleotide **16** the CD spectra of the duplexes with D- and L-RNA virtually reflected enantiomorphic structures, the situation is less clear in the case of the duplexes of cpa-oligonucleotide **14** with D- and L-RNA (Figure 4). While the CD trace of the duplex with D-RNA (**14**/**20**) largely indicates a right-handed helix, that of the duplex with the L-RNA complement (**14**/**21**) is not enantiomorphic and may well reflect also a non-Watson−Crick base-paired structure. Thus the two related bca- and cpa-backbone systems deviate structurally from each other in this context. One explanation for this fact lies in the difference of the cyclopentane ring conformation in both systems.

Although the cpa-DNA backbone mimicks the D- (and not L-) ribose structure, the CD of their single strands indicate left-handed helical conformations. Recently, Urata et al. have reported the formation of a right-handed double helix by an L-oligodeoxyribonucleotide analogue in which the bases were fixed in the low *anti*-glycosyl conformation.[13] This is in contrast to the corresponding single strands, which according to CD spectroscopy occur in a left-handed helical arrangement. Thus the chirality of the deoxyribose unit does not imperatively determine the sense of helicity of a corresponding double helix. These observations are in full agreement with our results on the bca-DNA and the cpa-DNA analogue. In these cases it is not the glycosylic angle but the orientation of the base-linker unit that participates in defining the chiral sense of the double helix.

Structurally related but conformationally more flexible nucleoside analogues based on a serinol backbone unit to which the base thymine was connected in the same way as in cpa-DNA were incorporated into oligonucleotides in the past.[14] All oligonucleotides containing these modifications were strongly destabilizing when introduced in the center of their sequence. Thus a similar

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situation as for the cpa-DNA modification is encountered. Unfortunately there are no indications on the structural and pairing properties of fully modified oligonucleotide analogues of that backbone type. Thus, no conclusions on the effect of the five-membered-ring structure in the backbone unit of cpa-DNA can be drawn.

In summary, we presented the synthesis of the cpa-DNA monomers containing the bases adenine and thymine from the known compound **1** in 12 steps. Fully modified oligomers of this new DNA analogue were synthesized by standard oligonucleotide chemistry. Fully modified cpa-oligoadenylates form duplexes with complementary DNA that are less stable by −2.7 °C/mod. compared to DNA. The corresponding cpa-oligothymidylates do not participate in complementary base-pairing with any of the investigated backbone systems except with its own (homo-duplex). As its congener bca-DNA, cpa-DNA seems to prefer left-handed helical duplex structures with DNA or with itself as indicated by the CD spectra.

Thus the main effect of the removal of the six-membered ring in bca-DNA (→ cpa-DNA) is a reduced pairing affinity to complementary DNA, RNA, and bca-DNA, and a higher propensity of the single strand for left-handed helical structure formation.

Experimental Section

2,3-Epoxycyclopentanol ((±)2). To a solution of *m*-CPBA (54 g, 70% purity, 0.22 mol) in CH_2Cl_2 (150 mL) in an ice bath was added slowly a solution of (±)**1** (36.4 g, 0.18 mol) in CH_2Cl_2 (150 mL). The mixture was stirred overnight at room temperature. The resulting white precipitate was removed by filtration and the filtrate was extracted with 10% $Na_2S_2O_3$, followed by 5% NaOH, water, and brine. The organic phase was dried with Na_2SO_4 and evaporated and the residue was purified by CC (hexane/ethyl acetate 50:1, R_f 0.25 (anti isomer), 0.15 (syn isomer)). The desired anti isomer (16.1 g, 42%) was subsequently dissolved in THF (60 mL) and treated with $Bu_4NF{\cdot}3H_2O$ (36.8 g, 0.12 mol), and the mixture was stirred at room temperature overnight. After evaporation of THF, the residue was purified by column chromatography (hexane/ethyl acetate 1:2) to give (±)**2** (7.06 g, 93%) as a colorless oil. TLC (hexane/ethyl acetate 1:1) R_f 0.26. IR ($CHCl_3$, film) 3676 m, 3614 m, 3444 m, 3032−2860 m, 1436 w, 1394 w, 1316 w, 1232 s, 998 s, 918 w, 844 s, 800 m cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 4.36 (d, J = 5.1 Hz, 1H), 3.55 (br s, 1H), 3.42 (d, J = 2.2 Hz, 1H), 2.03−1.96, 1.90−1.79, 1.72 − 1.53 (3m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 71.3 (d), 58.5 (d), 56.8 (d), 29.2 (t), 24.8 (t). MS (EI^+) m/z 100 [M^+].

1-((*N*-Benzylcarbamoyl)oxy)-2,3-epoxycyclopentane ((±)3). To a solution of (±)**2** (4.6 g, 46 mmol) in toluene (40 mL) was added benzylisocyanate (8.4 mL, 69 mmol) at room temperature. The mixture was heated to 95 °C and stirred overnight. After evaporation of solvent, the residue was dissolved in ethyl acetate (50 mL) and washed with sat. $NaHCO_3$. The organic phase was dried over Na_2SO_4 and evaporated, and the residue was purified by CC (hexane/ethyl acetate 4:1) to give (±)**3** (8.51 g, 79%) as a white solid. TLC (CH_2Cl_2) R_f = 0.3. IR ($CHCl_3$, film) 3684 w, 3620 w, 3448 m, 3016−2873 s, 2400 m, 2254 w, 1802 m, 1724 s, 1514 s, 1228 s, 1047 s, 907 s, 846 m, 754 s cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 7.80 (t, J = 6.1 Hz, 1H), 7.33−7.20 (m, 5H), 5.00 (br s, 1H), 4.17 (d, J = 6.2 Hz, 2H), 3.60 (s, 1H), 3.49 (d, J = 2.2 Hz, 1H), 1.93−1.86, 1.76−1.65, 1.58−1.51 (3m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.7 (s), 138.2 (s), 128.7 (d), 128.5 (d), 127.5 (d), 127.4 (d), 127.2 (d), 74.5 (d), 56.7 (d), 56.4 (d), 45.1 (t), 26.7 (t), 25.2 (t). HRMS (ESI^+) m/z calcd for $C_{13}H_{15}NO_3$ 234.1130 [M + H]$^+$, found 234.1188 [M + H]$^+$.

3-Benzyl-4-hydroxy-hexahydro-cyclopentaoxazol-2-one ((±)4). To a suspension of 55% NaH (300 mg, 7.2 mmol) in DMF (45 mL) was added (±)**3** (300 mg, 1.29 mmol). This suspension was stirred at room temperature overnight, then cooled to 0 °C and slowly quenched with sat. NH_4Cl (50 mL). The aqueous phase was extracted with CH_2Cl_2 (4×), and the organic phase was dried over Na_2SO_4 and evaporated to remove as much DMF as possible. Purification by CC (hexane/ethyl acetate 1:1 → pure ethyl acetate) gave (±)**4** (228 mg, 76%) as a yellowish solid. TLC (hexane/ethyl acetate 1:1) R_f 0.22. IR ($CHCl_3$, film) 3684 m, 3620 m, 3448 br, 3020−2896 s, 2400 s, 1740 s, 1672 m, 1522 s, 1422 s, 1228 s, 1046 s, 929 s, 740 s cm^{-1}. NMR assignments from ^1H,^1H- and from ^1H,^{13}C-COSY: ^1H NMR (300 MHz, $CDCl_3$) δ 7.35−7.27 (m, 5H, Ar−H), 4.94 (t, J = 6.3 Hz, 1H, C(6a)−H), 4.65, 4.19 (2d, J = 15.1 Hz, 2H, CH_2Bn), 4.14 (d, J = 3.3 Hz, 1H, C(4)−H), 3.80 (d, J = 7.4 Hz, 1H, C(3a)−H), 2.19−2.00 (m, 2H, C(6)−H), 1.96 (br, 1H, OH), 1.91−1.78 (m, 1H, C(5)−H), 1.75−1.68 (m, 1H, C(5)−H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.3 (s, C(2)), 135.9 (s, C(ar)), 128.8 (d, C(ar)), 128.1 (d, C(ar)), 128.0 (d, C(ar)), 79.0 (d, C(6a)), 73.8 (d, C(4)), 66.9 (d, C(3a)), 47.3 (t, C(benzyl)), 30.9 (t, C(5)), 30.5 (t, C(5)). HRMS (ESI^+) m/z calcd for $C_{13}H_{15}NO_3$ 234.1130 [M + H]$^+$, found 234.1133 [M + H]$^+$.

Acetic Acid 3-Benzyl-2-oxo-hexahydro-cyclopentaoxazol-4-yl Ester ((±)5). To a solution of (±)**4** (500 mg, 2.15 mmol) in pyridine (20 mL) containing DMAP (39.3 mg, 320 μmol) was added acetic anhydride (310 μL, 3.23 mmol). The solution was stirred at room temperature for 6 h, then quenched with 140 mL of sat. $NaHCO_3$ (aq) and extracted with 3 × 100 mL of CH_2Cl_2, and then the organic phase was evaporated. The residual brown oil was purified by CC (hexane/ethyl acetate 3:1 → 2:1 → 1:1) to give (±)**5** (575 mg, 97%) as a colorless viscous oil that crystallized upon drying at HV. TLC (hexane/ethyl acetate 1:1) R_f 0.5. Mp 49−50 °C. IR (KBr) 2940 m, 1731 s, 1455 m, 759 s, 704 s. ^1H NMR (300 MHz, $CDCl_3$) δ 7.40−7.30 (m, 5H), 5.03 (d, J = 4.1 Hz, 1H), 4.90 (dd, J = 7.4, 7.4 Hz, 1H), 4.77, 4.25 (2d, J = 15.1 Hz, 2H), 3.83 (d, J = 7.0 Hz, 1H), 2.01 (s, 3H), 2.18−2.07, 2.00−1.86 (2m, 4H). ^{13}C NMR (75 MHz, benzene-d_6) 170.08 (s), 157.64 (s), 136.00 (s), 128.56 (d), 128.37 (d), 127.80 (d), 78.26 (d), 76.18 (d), 64.91 (d), 46.84 (t), 31.05 (t), 27.79 (t), 20.81 (q). HRMS (ESI^+) m/z calcd for $C_{15}H_{17}NO_4$ 276.1235 [M + H]$^+$, found 276.1229 [M + H]$^+$.

3-Benzyl-4-hydroxy-hexahydro-cyclopentaoxazol-2-one ((+)4). Ester (±)**5** (750 mg, 2.73 mmol) was suspended in 0.1 M phosphate buffer (35 mL, pH 7.0) and treated with 2.2 mg (210 U/mg) of pig liver esterase. The pH was kept constant at 7.0 by continuous addition of 1 N NaOH. The mixture was extracted with ethyl acetate (3 × 30 mL) and the organic phase was dried over Na_2SO_4. The solvent was evaporated and the residue purified by CC (hexane/ethyl acetate 1:1) to give (+)**4** (95 mg, 31%) as a white solid. ee >99% (chiral GLC). Mp 86−87 °C. $[\alpha]^{22}_D$ +37.5 (*c* 0.0032, MeOH).

***N*-Benzyl-2-aminocyclopentan-1,3-diol (6).** Alcohol (+)-**4** (1.35 g, 5.79 mmol) was dissolved in ethanol (20 mL), and 8 N KOH (15 mL) was added to this mixture. The mixture was stirred overnight under reflux. After cooling to room temperature, the mixture was extracted with ethyl acetate (3 × 50 mL). The organic phase was dried over Na_2SO_4, the solvent was evaporated, and the residue was purified by CC (CH_2Cl_2/MeOH/25% NH_4OH 9:1:0.1) to give **6** (979 mg, 82%) as a white solid. TLC (CH_2Cl_2/MeOH/25% NH_4OH 9:1:0.1) R_f 0.68. Mp 90−92 °C. $[\alpha]^{22}_D$ −50.5 (*c* 0.0019, MeOH). IR (KBr) 3386 s, 2907 s, 1584 w, 1044 m, 757 s. ^1H NMR (300 MHz, MeOD-d_3) δ 7.36−7.26 (m, 5H), 4.19 (dt, J = 8.07, 3.30 Hz, 1H), 4.06 (q, J = 6.86 Hz, 1H), 3.87 (2d, J = 12.8 Hz, 2H), 2.79 (t, J = 2.58 Hz, 1H), 2.25−1.97, 1.73−1.44 (2m, 4H). ^{13}C NMR (75 MHz,

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MeOD-d_3) δ 141.37 (s), 129.77 (d), 129.74 (d), 128.44 (d), 77.08 (d), 71.37 (d), 70.44 (d), 53.37 (t), 31.38 (t), 31.30 (t). HRMS (ESI$^+$) m/z calcd for $C_{12}H_{18}NO_2$ 208.1337 $[M + H]^+$, found 208.1321 $[M + H]^+$.

2-Aminocyclopentan-1,3-diol (7). To a solution of **6** (910 mg, 4.39 mmol) in ethanol (10 mL) in an autoclave was added 10% Pd/C (1.3 g). The autoclave was then charged with H_2 (10 atm) and the mixture was stirred overnight (~20 h) at room temperature. The reaction mixture was filtered over Celite, and the filtrate was evaporated to give pure **7** (503 mg, 98%) as a yellowish oil. TLC (CH_2Cl_2/MeOH/25% NH_4OH 9:1: 0.1) R_f 0.09. $[\alpha]^{22}_D$ −51.2 (*c* 0.0025, MeOH). ^{1}H NMR (300 MHz, MeOD-d_3) δ 4.09 (dt, J = 5.52, 2.94 Hz, 1H), 3.93 (q, J = 7.11 Hz, 1H), 2.80 (dd, J = 7.35, 5.16 Hz, 1H), 2.22−2.03, 1.69−1.43 (2m, 4H). ^{13}C NMR (75 MHz, MeOD-d_3) δ 78.50 (d), 73.31 (d), 64.03 (d), 31.09 (t), 30.90 (t). HRMS (ESI$^+$) m/z calcd for $C_5H_{12}NO_2$ 118.0868 $[M + H]^+$, found 118.0873 $[M + H]^+$.

***N*-[(2,5-Dihydroxycyclopentyl)-1-(thymin-1-yl)]-acetamide (9a).** To a solution of **7** (20 mg, 0.17 mmol) in DMF (2 mL) were added thymin-1-yl acetic acid (38 mg, 0.20 mmol) and EDC (49 mg, 0.25 mmol). The mixture was stirred at room temperature overnight. After evaporation of the solvent, the residue was purified by CC (CH_2Cl_2/MeOH 95:5 → 85:15) to give **9a** (31 mg, 66%) as a white solid. TLC (CH_2Cl_2/MeOH 85:15) R_f 0.44. Mp >210 °C dec. $[\alpha]^{22}_D$ −55.7 (*c* 0.0023, DMSO). IR (KBr) 3405 s, 3342 s, 1673 s, 1242 m, 1030 m. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.24 (s, 1H), 7.96 (d, J = 8.07 Hz), 7.37 (d, J = 1.08 Hz, 1H), 4.68, 4.62 (2d, J = 5.13, 3.69 Hz, 2H), 4.33 (s, 2H), 3.94 (m, 2H), 3.64 (dt, J = 7.32, 5.13 Hz, 1H), 2.01−1.85, 1.52−1.30 (2m, 4H), 1.74 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) 167.21 (s), 164.57 (s), 151.20 (s), 142.63 (d), 107.95 (s), 74.06 (d), 69.85 (d), 65.18 (d), 49.23 (t), 30.25 (t), 29.93 (t), 12.06 (q). HRMS (ESI$^+$) m/z calcd for $C_{12}H_{17}N_3O_5$ 284.1246 $[M + H]^+$, found 284.1241 $[M + H]^+$.

***N*-[(2,5-Dihydroxycyclopentyl)-1-(*N*^{6}-(*p*-methoxybenzoyl)adenin-1-yl)]-acetamide (9b).** To a solution of **7** (11 mg, 0.094 mmol) in DMF (0.5 mL) were added *N*^{6}-(*p*-methoxybenzoyl)adenin-1-yl acetic acid (32 mg, 0.103 mmol) and EDC (22 mg, 0.115 mmol). The mixture was stirred at room temperature overnight. After evaporation of the solvent, the residue was purified by CC (CH_2Cl_2/MeOH 95:5 → 88:12) to give **9b** (26 mg, 65%) as a white solid. TLC (CH_2Cl_2:MeOH 85:15) R_f 0.33. Mp >230 °C dec. $[\alpha]^{22}_D$ −28.8 (*c* 0.0017, DMSO-d_6). IR (KBr) 3282 s, 2938 m, 1702 s, 1246 m, 1177 m, 1093 m, 1033 m, 759 m.^{1}H NMR (300 MHz, DMSO-d_6) δ 10.99 (s, NH), 8.68 (s, 1H), 8.37 (s, 1H), 8.29 (d, J = 8.1 Hz, 1H), 8.03 (d, J = 8.5 Hz, 2H), 7.06 (d, J = 8.8 Hz, 2H), 5.03 (s, 2H), 4.78, 4.76 (2d, J = 3.7, 5.5 Hz, 2H), 3.98 (m, 2H), 3.84 (s, 3H), 3.68 (dd, J = 12.5, 7.7 Hz, 1H), 2.02−1.90, 1.52−1.50, 1.36−1.32 (3m, 4H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.38 (s), 165.05 (s), 162.69 (s), 152.77 (s), 151.50 (d), 150.38 (s), 145.67 (d), 130.71 (d), 125.77 (s), 125.03 (s), 113.86 (d), 74.05 (d), 69.83 (d), 61.66 (d), 55.66 (q), 45.34 (t), 30.22 (t), 29.92 (t). HRMS (ESI$^+$) m/z calcd for $C_{20}H_{23}N_6O_5$ 427.1729 $[M + H]^+$, found 427.1824 $[M + H]^+$.

***N*-[2-(*tert*-Butyldimethylsilyloxy)-5-(9-phenyl-xanthen-9-yloxy)-cyclopentyl-1-(thymin-1-yl)]-acetamide (10a).** To a solution of **9a** (50 mg. 0.18 mmol) in DMSO (0.2 mL) were added pyridine (0.8 mL) and 9-phenyl-xanthenyl chloride (25 mg, 0.09 mmol) at room temperature. The mixture was stirred for 5 h, and another portion of 9-phenyl-xanthen chloride (25 mg, 0.09 mmol) was added. After another 15 h, the reaction mixture was diluted with sat. $NaHCO_3$ and extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with water, dried over Na_2SO_4, and evaporated. CC (CH_2Cl_2/MeOH/TEA 94:5:1) of the residue gave the mixture of the monopixylated isomers (52 mg, ~4:1) as well as the dipixylated product (19 mg). The mixture of monopixylated isomers was subsequently dissolved in DMF (1 mL), and to this solution were added TBDMS−Cl (20 mg, 0.145 mmol) and imidazole (18 mg, 0.289 mmol) at room temperature. The mixture was stirred overnight followed by removal of the solvent. The residue was redissolved in ethyl acetate (20 mL) and extracted with sat. $NaHCO_3$ (aq) and brine. The organic phase was dried over Na_2SO_4 and evaporated. The residue was purified by CC (hexane/ethyl acetate 2:1 → 3:2) to give the major isomer **10a** (40 mg, 33% over 2 steps) as a white solid. TLC (hexane/ethly acetate 1:1) R_f 0.44 (major isomer), 0.32 (minor isomer). Data for **11a**: $[\alpha]^{22}_D$ +12.5 (*c* 0.002, MeOH). IR (KBr) 3423 m, 2950 w, 1697 s, 1448 m, 1242 m, 1066 m, 757 m. ^{1}H NMR (300 MHz, benzene-d_6) δ 10.24 (br s, 1H), 7.69 (d, J = 7.71 Hz, 2H), 7.41 (d, J = 5.88 Hz, 1H), 7.26−6.96 (m, 10H), 6.50 (s, 1H), 5.51 (d, J = 8.1 Hz, 1H), 4.39 (dt, J = 8.82, 4.41 Hz, 1H), 3.89 (s, 2H), 4.35−3.82 (m, 2H), 1.72 (s, 3H), 1.51−1.33, 1.27 (3m, 4H), 0.86 (s, 9H), −0.005, −0.144 (2s, 6H). ^{13}C NMR (75 MHz, benzene-d_6) δ 166.11 (s), 164.37 (s), 152.17 (s), 151.59 (s), 151.30 (s), 148.92 (s), 140.45 (d), 132.03 (d), 131.49 (d), 129.71 (d), 129.48 (d), 128.08 (d), 127.91 (d), 126.94 (d), 124.87 (s), 124.17 (s), 123.78 (d), 123.32 (d), 116.50 (d), 116.34 (d), 10.57 (s), 76.99 (d), 76.45 (s), 71.02 (d), 59.63 (d), 50.32 (t), 30.62 (t), 27.40 (t), 25.86 (q), 18.02 (s), 12.29 (q), −4.81 (q), −5.09 (q). HRMS (ESI$^-$) m/z calcd for $C_{37}H_{42}N_3O_6Si$ 652.8498 $[M - H]^-$, found 652.2836 $[M - H]^-$.

***N*-[2-(*tert*-Butyldimethylsilyloxy)-5-(9-phenyl-xanthen-9-yloxy)-cyclopentyl-1-(*N*^{6}-*p*-methoxybenzoyl)adenin-1-yl]-acetamide (10b).** To a solution of **9b** (80 mg 0.187 mmol) in DMSO (0.15 mL) were added pyridine (0.7 mL) and 9-phenyl-xanthenyl chloride (27 mg, 0.09 mmol) at room temperature. The mixture was stirred for 5 h. Then another portion of 9-phenyl-xanthenyl chloride (27 mg, 0.09 mmol) was added. After an additional 15 h, the reaction mixture was quenched with sat. $NaHCO_3$(aq) and extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with water, dried over Na_2SO_4, and evaporated. CC of the residue (CH_2Cl_2/MeOH/TEA 96.5:2.5:1 → 94.5:5:1) gave the mixture of monopixylated isomers (67 mg, ~3:1) that was subsequently dissolved in DMF (1 mL) and treated with TBDMSCl (30 mg, 0.196 mmol) and imidazole (26 mg, 0.392 mmol) at room temperature. This mixture was stirred at room temperature overnight. After removal of the solvent the residue was redissolved in ethyl acetate (20 mL) and washed with sat. $NaHCO_3$ (aq) and brine. The organic phase was dried over Na_2SO_4 and evaporated. The residue was purified by CC (hexane/ethyl acetate (+1% TEA) 1:1) to give the major isomer **10b** (46 mg, 31% over 2 steps) as a white solid. TLC (ethyl acetate) R_f 0.59 (major isomer), 0.42 (minor isomer). Data for **10b**: $[\alpha]^{22}_D$ +8.2 (*c* 0.0022, MeOH). IR (KBr) 3417 s, 2927 m, 1604 s, 1449 s, 1247 s, 1173 m, 759 m, 666 m. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.06 (s, 1H), 8.69 (s, 1H), 8.13 (s, 1H), 8.00 (d, J = 8.8 Hz, 2H), 7.43−6.97 (m, 15H), 5.20 (d, J = 8.1 Hz, 1H, N−H), 4.73, 4.57 (2d, J = 16.1 Hz, 2H), 4.06 (m, 1H, C(1)−H), 3.95 (t, J = 4.0, 1H, C(2)−H), 3.88 (s, 3H), 3.60 (q, J = 8.1 Hz, 1H, C(5)−H), 1.79, 1.26−1.11 (2m, 4H), 0.72 (s, 9H), −0.13, −0.20 (2s, 6H). ^{13}C NMR (75 MHz, benzene-d_6) δ 165.23 (s), 164.39 (s), 163.12 (s), 152.77 (s), 152.39 (s), 152.10 (d), 151.62 (s), 150.77 (s), 148.79 (s), 143.87 (d), 132.00 (d), 131.56 (d), 130.51 (d), 129.75 (d), 129.50 (d), 128.08 (d), 127.87 (d), 126.99 (d), 126.84 (s), 124.63 (s), 124.31 (s), 123.80 (d), 123.33 (d), 116.51 (d), 116.38 (d), 114.00 (d), 77.08 (d), 76.52 (s), 71.13 (d), 59.89 (d), 54.86 (q), 46.20 (t), 30.75 (t), 27.65 (t), 25.84 (q), 18.00 (s), −4.88 (q), −5.13 (q). HRMS (ESI$^-$) m/z calcd for $C_{45}H_{47}N_6O_6Si$ 795.3326 $[M - H]^-$, found 795.4184 $[M - H]^-$.

***N*-[2-Hydroxy-5-(9-phenyl-xanthen-9-yloxy)-cyclopentyl-1-(thymin-1-yl)]-acetamide (11a).** To a solution of **10a** (46 mg, 0.07 mmol) in THF (1 mL) was added $Bu_4NF{\cdot}3H_2O$ (45 mg, 0.14 mmol) at room temperature. The mixture was stirred overnight and the solvent removed. The residue was purified by CC (CH_2Cl_2/MeOH 19:1) to give **11a** (35 mg, 92%) as a white solid. TLC (CH_2Cl_2/MeOH 95:5) R_f 0.27. $[\alpha]^{22}_D$ +11.5 (*c* 0.0013, MeOH). IR (KBr) 3443 s, 1681 s, 1449 m, 1242 m. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.73 (s, 1H), 7.41−6.99 (m, 14H), 5.90 (d, J = 8.10 Hz, 1H), 4.19, 3.82 (2d, J = 15.8 Hz, 2H), 4.07 (dt, J = 14.3, 9.24 Hz, 1H), 3.97 (br, 1H), 3.58 (q, J = 7.84 Hz, 1H), 3.46 (br s, 1H), 1.86 (s, 3H), 1.33−1.11 (m, 4H). ^{13}C NMR (75 MHz,$CDCl_3$) δ 166.33 (s), 164.30 (s), 151.41 (s),

151.38 (s), 150.85 (s), 148.62 (s), 140.97 (d), 131.38 (d), 130.93 (d), 129.33 (d), 129.08 (d), 127.64 (d), 127.13 (d), 126.55 (d), 123.98 (s), 123.54 (s), 123.09 (d), 122.72 (d), 116.39 (d), 116.26 (d), 111.00 (s), 76.86 (d), 75.73 (s), 69.58 (d), 60.09 (d), 50.55 (t), 30.07 (t), 29.66 (t), 12.24 (q). HRMS (ESI$^-$) *m*/*z* calcd for $C_{31}H_{28}N_3O_6$ 538.1978 [M − H]$^-$, found 538.1960 [M − H]$^-$.

***N*-[2-Hydroxy-5-(9-phenyl-xanthen-9-yloxy)-cyclopentyl-1-(*N*^{6}-*p*-methoxybenzoyl)adenin-1-yl]-acetamide (11b).** To a solution of **10b** (38 mg, 0.0476 mmol) in THF (1 mL) was added $Bu_4NF{\cdot}3H_2O$ (30 mg, 0.0952 mmol) at room temperature. The mixture was stirred overnight and the solvent removed. The residue was purified by CC (CH_2Cl_2/MeOH/TEA 94:5:1) to give **11b** (32 mg, 98%) as a white solid. TLC (CH_2Cl_2/MeOH 95:5) R_f 0.45. $[\alpha]^{22}_D$ +16.3 (*c* 0.0019, MeOH). IR (KBr) 3390 s, 1674 m, 1602 s, 1456 s, 1246 s, 1174 m, 759 m. ^{1}H NMR (300 MHz, MeOD-d_3) δ 8.58 (s, 1H), 8.41 (s, 1H), 8.10 (d, J = 8.8 Hz, 2H), 7.37−7.07 (m, 15H), 4.99, 4.90 (2d, J = 16.9 Hz, 2H), 4.22 (dd, J = 8.1, 5.2 Hz, 1H), 3.99 (m, 1H), 3.92 (s, 3H), 1.97, 1.43−0.89 (2m, 4H). ^{13}C NMR (75 MHz, MeOD-d_3) δ 168.16 (s), 167.74 (s), 165.26 (s), 154.08 (s), 153.44 (d), 153.02 (s), 152.79 (s), 151.41 (s), 150.78 (s), 146.90 (d), 132.85 (d), 132.65 (d), 131.85 (d), 130.83 (d), 130.79 (d), 128.86 (d), 128.48 (d), 127.82 (d), 127.22 (s), 125.69 (s), 125.14 (s), 124.96 (d), 124.48 (s), 124.34 (d), 117.56 (d), 117.53 (d), 115.26 (d), 78.84 (d), 77.49 (s), 71.16 (d), 61.67 (d), 56.38 (q), 47.04 (t), 31.65 (t), 29.55 (t). HRMS (ESI$^-$) *m*/*z* calcd for $C_{39}H_{33}N_6O_6$ 681.2461 [M − H]$^-$, found 681.2459 [M − H]$^-$.

2′-(2-Cyanoethyl)-[1′-(2-(thymin-1-yl)-acetylamino)-3′-(9-phenyl-xanthen-9-yloxy)-cyclopentyl]-*N*,*N*′-diisopropylphosphoramidite (12a). To a solution of **11a** (25 mg, 0.046 mmol) in THF (1 mL) were added *N*,*N*′-diisopropylethylamine (48 μL, 0.276 mmol) and chloro-2-cyanoethoxydiisopropylaminophosphine (31 μL, 0.138 mmol) at room temperature. The reaction mixture was stirred at room temperature for 1 h, quenched with sat. $NaHCO_3$(aq), and extracted with ethyl acetate (3 × 20 mL). The combined organic phases were dried over Na_2SO_4 and evaporated and the residue was purified by CC (CH_2Cl_2/MeOH 50:1) to give **12a** (20 mg, 59%) as a white solid. TLC (ethyl acetate) R_f 0.66. ^{1}H NMR (300 MHz, benzene-d_6) δ 10.35, 10.00 (2s, 1H), 7.69 (t, J = 7.74 Hz, 2H), 7.49−6.70 (m, 10H), 6.75 (t, J = 8.10 Hz, 1H), 6.61, 6.48 (2d, J = 1.11 Hz, 1H), 5.87 (t, J = 8.46 Hz, 1H), 4.55 (m, 1H), 4.36−3.59 (m, 4H), 3.50−3.10 (m, 4H), 2.43−2.34, 2.21−2.12 (2m, 2H), 1.76, 1.74 (2s, 3H), 1.66−1.27 (m, 4H), 1.16−1.07 (m, 12H). ^{13}C NMR (75 MHz, benzene-d_6) δ 166.39 (s), 166.35 (s), 164.36 (s), 164.24 (s), 152.10 (s), 152.02 (s), 151.54 (s), 151.52 (s), 151.39 (s), 149.06 (s), 140.90 (d), 140.64 (d), 132.10 (d), 131.65 (d), 131.39 (d), 129.88 (d), 129.66 (d), 129.53 (d), 129.48 (d), 128.11 (d), 127.89 (d), 127.81 (d), 127.30 (s), 126.94 (d), 124.81 (s), 124.75 (s), 124.11 (d), 124.08 (s), 123.85 (d), 123.40 (d), 123.33 (d), 118.77 (s), 118.09 (s), 116.52 (d), 116.41 (d), 116.38 (d), 116.31 (d), 77.18 (d), 76.96 (d), 76.46 (s), 76.45 (s), 73.19 (dd, J_{C-P} = 15.7, Hz), 72.52 (dd, J_{C-P} = 9.10 Hz), 59.35 (dd, J_{C-P} = 5.46 Hz), 59.28 (dd, J_{C-P} = 3.64 Hz), 58.82 (t), 58.59 (t), 58.43 (t), 58.19 (t), 50.35 (t), 50.15 (t), 45.28 (s), 44.53 (s), 43.41 (d), 43.33 (t), 43.24 (t), 43.16 (t), 27.58 (t), 27.43(t), 24.70 (q), 24.67 (q), 24.60 (q), 24.57 (q), 24.51 (q), 24.38 (q), 24.67 (q), 20.43 (t), 20.34 (t), 20.16 (t), 20.09 (t), 12.35 (q). ^{31}P NMR (161.9 MHz, benzene-d_6) δ 148.69, 147.47. HRMS (ESI$^-$) *m*/*z* calcd for $C_{40}H_{45}N_5O_7P$ 738.3056 [M − H]$^-$, found 738.3047 [M − H]$^-$.

2′-(2-Cyanoethyl)-[1′-(2-(*N*^{6}-(*p*-methoxybenzoyl)adenin-1-yl)-acetylamino)-3′-(9-phenyl-xanthen-9-yloxy)-cyclopentyl]-*N*,*N*′-diisopropylphosphoramidite (12b). To a solution of **11b** (159 mg, 0.23 mmol) in THF (5 mL) were added *N*,*N*′-diisopropylethylamine (120 μL, 0.69 mmol) and chloro-2-cyanoethoxy-diisopropylaminophosphine (77 μL, 0.345 mmol) at room temperature. The reaction mixture was stirred for 1 h. The mixture was quenched with sat. $NaHCO_3$ (aq) and extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by CC (CH_2Cl_2/MeOH 100:1 → 2:0.5, 1% Et_3N) to give a yellowish viscous oil. The oil was redissolved in ethyl acetate (1 mL) and this solution was added dropwise to stirred hexane (40 mL). The resulting white precipitate was filtered off and washed with cold hexane. The precipitate was dried to give **12b** (130 mg, 63%) as a white solid. TLC (CH_2Cl_2/MeOH = 40:1): R_f 0.32. ^{1}H NMR (300 MHz, benzene-d^6): δ 10.03, 8.76 (2br s, 1H), 8.17, 8.08 (2s, 1H), 8.13−6.72 (m, 14H), 6.16, 6.10 (2d, J = 8.43, 8.82 Hz, 1H), 4.75−4.53 (m, 3H), 4.19−4.05, 3.91 (2m, 2H), 3.58−3.53, 3.20−3.07 (2m, 4H), 3.32 (s, 3H), 2.29−2.11 (m, 2H), 1.87−1.79, 1.67, 1.32−1.20 (3m, 4H), 1.12−0.86 (m, 12H). ^{13}C NMR (75 MHz, benzene-d_6) 165.84 (s), 165.69 (s), 165.03 (s), 164.94 (s), 163.24 (s), 152.57 (d), 151.93 (s), 151.85 (s), 151.57 (s), 151.55 (s), 150.70 (s), 149.10 (s), 148.99 (s), 144.57 (d), 144.34 (d), 132.10 (d), 132.05 (d), 131.74 (d), 131.63 (d), 130.79 (d), 130.71 (d), 129.94 (d), 129.80 (d), 129.53 (d), 129.42 (d), 128.08 (d), 127.79 (d), 126.98 (d), 124.55 (s), 124.17 (s), 124.09 (d), 123.65 (s), 123.50 (s), 123.36 (d), 123.22 (d), 118.83 (s), 118.30 (s), 116.47 (d), 116.44 (d), 116.41 (d), 116.36 (d), 114.04 (d), 77.30 (d), 76.51 (s), 76.49 (s), 73.55 (dd, J_{C-P} = 16.5 Hz), 72.58 (J_{C-P} = 9.75 Hz), 59.72 (dd, J_{C-P} = 3.75 Hz), 59.48 (dd, J_{C-P} = 4.50 Hz), 58.48 (t), 58.24 (t), 58.16 (t), 58.09 (t), 54.98 (q), 54.96 (q), 46.18 (t), 43.34 (d), 43.21 (d), 43.18 (d), 43.06 (d), 27.99 (t), 27.86 (t), 24.68 (q), 24.60 (q), 24.52 (q), 24.41 (q), 24.31 (q), 20.47 (t), 20.39 (t), 20.20 (t), 20.12 (t). ^{31}P NMR (161.9 MHz, benzene-d_6) δ 148.86, 147.56. HRMS (ESI$^-$) *m*/*z* calcd for $C_{48}H_{50}N_8O_7P$ 881.3540 [M − H]$^-$, found 881.4448 [M − H]$^-$.

Synthesis and Purification of Oligonucleotides. All oligonucleotides and analogues were synthesized on the 1.3-μmol scale on a Pharmacia Gene-Assembler Special DNA-synthesizer. Oligodeoxyribonucleotides were synthesized by phosphoramidite chemistry, using commercial building blocks (Glen Research) and reagents, and were deprotected and purified according to standard protocols. cpa-oligonucleotides **13** and **14** were assembled on universal CPG-support from CT-Gen, San José. The synthesis proceeded with the following changes to the synthetic cycle: The coupling time was extended to 6 min. Tetrazole (0.45 M in CH_3CN) was replaced by the more active 5-(ethylthio)-*1H*-tetrazole (0.25 M in CH_3-CN) as the activator. Coupling yields of **12a**,**b** were typically ∼95%. After removal of the last trityl group, oligonucleotides were detached from the support and deprotected by treatment with 25% NH_3/40%$MeNH_2$ (1:1) at 55 °C for 16 h. The crude oligonucleotides were purified by DEAE-HPLC (Nucleogen, Macherey-Nagel). Oligonucleotides were desalted over SEP-PAK C-18 cartridges (Waters). Purities of all modified oligonucleotides were controlled by RP-FPLC (PepRPC HR 5/5) and routinely characterized by ESI$^-$-TOF mass spectrometry (see Table 1 and Table S1 in the Supporting Information).

UV Melting Experiments and CD Spectra. Oligonucleotides were mixed to 1:1 stoichiometry using the UV extinction coefficients of natural oligodeoxynucleotides. UV melting curves were recorded on a Cary 3E UV/vis spectrophotometer (Varian) at 260 nm. Consecutive heating−cooling−heating cycles in the temperature intervall of 0−90 °C with a linear gradient of 0.2−0.5 °C/min were applied. Heating and cooling ramps were always superimposable indicating equilibrium conditions. CD spectra were measured on a JASCO J-715 spectrometer at the temperatures indicated.

Acknowledgment. The authors thank the Swiss National Science Foundation (grant no. 20-63582.00) for financial support of this project. The BENEFRI small molecule X-ray crystal service directed by Prof. H. Stöckli-Evans is thanked for providing the structure of compound **9**.

Supporting Information Available: Details on the synthesis and characterization of compound **8**, the X-ray structure of **8**, ^{1}H NMR spectra of compounds **2**−**12**, as well as 2D ^{1}H,^{13}C-COSY, and difference ^{1}H-NOE spectra of **10b**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO034143Q