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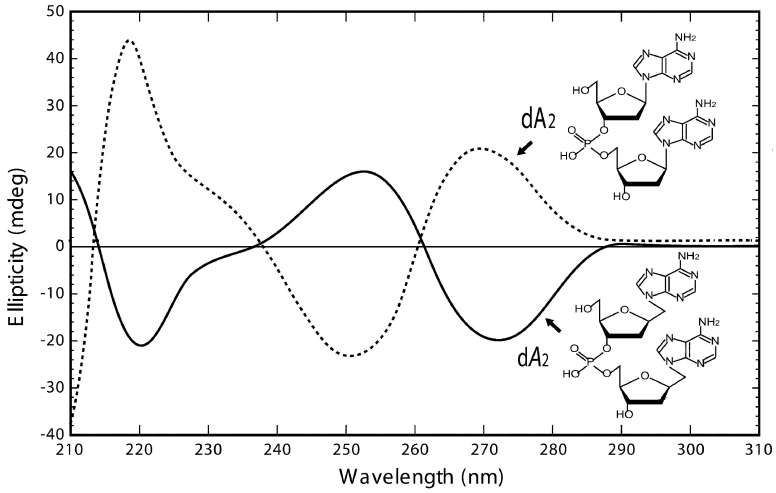

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Homo-*N*-oligonucleotides (N1/N9−C1′ Methylene Bridge Oligonucleotides): Nucleic Acids with Left-Handed Helicity

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Abstract: Oligonucleotides containing a methylene bridge between N1 or N9 of the heterocyclic base and C1′ of the pentofuranosyl ring (homo-*N*-oligonucleotides) were synthesized. Melting curves revealed that such homo-type oligomers could cross-pair with complementary homo-type or natural oligomers. Circular-dichroic studies provide evidence that the homo-type dimers have a left-handed stacked conformation and further suggest that single-stranded and double-stranded homo-type oligomers adopt a left-handed conformation, while duplexes with natural oligomers or nucleic acids form RNA-like right-handed helices. NMR spectroscopy (NOESY) provides supporting evidence for a left-handed stacked conformation of the homo-type dimer, while atomic force microscopy indicates a left-handed helical conformation of homo-type dsDNA. Homo-type dimers and oligomers showed high resistance to digestion by snake-venom and calf-spleen phosphodiesterases and nuclease S1.

Introduction

There has been substantial interest in the synthesis and applications of altered nucleic acids. We have investigated the properties of various modified nucleic acids for potential application to the development of therapeutic agents and research reagents. In such applications, it is important for the modified oligonucleotide to be resistant to nucleases yet to retain the ability to form duplexes with complementary natural nucleic acids. Many nucleic acid analogues have been synthesized with modifications to the base, sugar, and phosphate regions.[1−4] To our knowledge, however, no oligonucleotide analogues have been synthesized with an increase in the distance between the sugar and the base or in the flexibility of their linkage. Work in our laboratory on the development of altered nucleic acids has led to an interest in the effects of such modifications on the biochemical and physicochemical properties of nucleic acids and especially on their helical structure and handedness. In this latter regard, there has been discussion as to precisely why natural nucleic acids adopt a right-handed helical structure. Among the factors that are considered to determine the helical sense of nucleic acids is the chirality of the sugar, and it is known that nucleic acids with L-deoxyribose instead of the natural D-deoxyribose show left-handed helicity.[5,6] Another factor is the conformation about the glycosyl bond. For example, when the glycosyl torsion angles are restricted to a high anti or a low anti conformation, the helicity of the oligonucleotide can be reversed.[7−9]

We wondered what would be the effect on nucleic acid helicity of increasing the conformational flexibility in the region of the glycosyl bond. In one type of structure, a methylene group is inserted between N1 or N9 of the heterocyclic base and C1′ of the pentofuranosyl ring, a modification that is expected to introduce an extra degree of conformational flexibility compared to natural nucleic acids. The corresponding homo-*N*-nucleosides have previously been synthesized,[10−14] but the biochemical and physicochemical properties of the dimers or oligomers have not yet been reported.

In this paper we describe the manual synthesis of homo-*N*-nucleotide dimers and the automated synthesis of oligomers. We present the physicochemical properties of homo-type dimers and oligomers and propose a conformational model to explain their properties. CD and NMR evidence is presented that the dimers or oligomers have an inherent left-handed twist and that they form left-handed duplexes with complementary homo-type

(1) Eschenmoser, A.; Loewenthal, E. *Chem. Soc. Rev.* **1992**, *21*, 1−16.
(2) De Mesmaeker, A.; Altmann, K.-H.; Waldner, A.; Wendeborn, S. *Curr. Opin. Struct. Biol.* **1995**, *5*, 343−355.
(3) Matteucci, M. *Perspect. Drug Discovery Des.* **1996**, *4*, 1−16.
(4) Schöning, K.-U.; Scholz, P.; Guntha, S.; Wu, X.; Krishnamurthy, R.; Eschenmoser, A. *Science* **2000**, *290*, 1347−1351.
(5) Urata, H.; Shinohara, K.; Ogura, E.; Ueda, Y.; Akagi, M. *J. Am. Chem. Soc.* **1991**, *113*, 8174−8175.
(6) Hashimoto, Y.; Iwanami, N.; Fujimori, S.; Shudo, K. *J. Am. Chem. Soc.* **1993**, *115*, 9883−9887.
(7) Urata, H.; Miyagoshi, H.; Kumashiro, T.; Mori, K.; Shoji, K.; Akagi, M. *J. Am. Chem. Soc.* **2001**, *123*, 4845−4846.
(8) Uesugi, S.; Tezuka, T.; Ikehara, M. *J. Am. Chem. Soc.* **1976**, *98*, 969−973.
(9) Uesugi, S.; Yano, J.; Yano, E.; Ikehara, M. *J. Am. Chem. Soc.* **1977**, *99*, 2313−2323.
(10) Montgomery, J. A.; Hewson, K. *J. Heterocycl. Chem.* **1970**, *7*, 443−445.
(11) Hossain, N.; Blaton, N.; Peeters, O.; Rozenski, J.; Herdewijn, P. *Tetrahedron* **1996**, *52*, 5563−5578.
(12) Hossain, N.; Hendrix, C.; Lescrinier, E.; Van Aerschot, A.; Busson, R.; De Clercq, E.; Herdewijn, P. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1465−1468.
(13) Scremin, C. L.; Boal, J. H.; Wilk, A.; Phillips, L. R.; Beaucage, S. L. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 207−212.
(14) Boal, J. H.; Wilk, A.; Scremin, C. L.; Gray, G. N.; Phillips, L. R.; Beaucage, S. L. *J. Org. Chem.* **1996**, *61*, 8617−8626.

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Table 1. UV Spectral Characteristics of Monomers and Dimers[a]

compound	λ_{max}	λ_{min}	$\lambda_{max}/\lambda_{min}$
A	258.8	225.6	13.23
A	260.6	227.1	9.08
dA	259.7	224.9	12.46
d*A*	261.1	228.2	4.96
A_2	257.8	227.2	7.84
A_2	259.5	228.0	9.17
dA_2	257.7	226.3	9.06
dA_2	259.7	228.0	8.69

[a] The spectra were taken in 10 mM sodium phosphate, pH 7.0, containing 0.15 M NaCl. Italicized letters represent homo-type nucleotides.

oligomers but right-handed duplexes with natural nucleic acids. The left-handed nature of the homo-type duplex was confirmed by atomic force microscopy (AFM). As far as we know, this is the first report of a D-sugar nucleic acid without restricted glycosyl torsion angles that forms a left-handed helix under physiological conditions.

Results and Discussion

UV Spectra, Hyperchromicity, and Mixing Curves. The spectra of the homo-type monomers and dimers were almost identical to those of the corresponding natural compounds (Table 1). The homo-type compounds, however, displayed a small increase in λ_{max} (1.5−2.0 nm) and λ_{min} (0.8−3.5 nm) relative to the corresponding natural compounds.

Each of the four dimers A_2, A_2, dA_2, and dA_2, upon acidification, displayed an increase in absorbance at most wavelengths below ~300 nm (spectra not shown). (In this paper, an italicized "A" or "T" represents the homo-type nucleoside.) Each dimer also displayed a small decrease in λ_{max} (~0.8 nm) accompanying the increase in absorbance. The hyperchromicity values obtained were 8.15% for A_2, 7.42% for A_2, 10.26% for dA_2, and 6.05% for dA_2. The hyperchromicity value for A_2 (8.15%) obtained by the pH-shift method is less than the published value of 12%[15] obtained upon complete hydrolysis of the phosphodiester linkage, suggesting that there are residual stacking interactions between the bases even at low pH. However, dA_2 being resistant to enzymic degradation as described below, the pH-shift method of estimating hyperchromicity was used as a basis for comparing the four adenosine dimers. For both homo-type dimers, the hyperchromicity was less than that of the corresponding natural dimer: for A_2, it was ~9% less, and for dA_2, it was ~41% less.

The mixing curve for dA_2 and poly(dT) at 260 nm (not shown) in the presence of 1 M NaCl revealed a break at dA_2/poly(dT) ≈ 1:2, indicating the formation of a dA/2dT complex (the poly(dA)/2poly(dT) triple-helical complex forms in 1.0 M NaCl[15]). In contrast, the corresponding mixing curve for dA_2 and poly(dT) showed a break at dA_2/poly(dT) ≈ 1:1, indicating the formation of a 1:1 complex.

Melting Curves. Melting curves were taken for various nucleotide mixtures or self-complementary oligomers to determine the melting (transition) temperature, T_m, which can be taken as a measure of the strength of base-pairing (Table 2). The melting curve for A_2 and poly(U) in the presence of 1.0 M NaCl and 10 mM sodium phosphate, pH 7.0, was indistinguishable from that of poly(U) alone (which showed a weak meltinglike transition), whereas A_2 and poly(U) displayed a clear, cooperative hyperchromic effect characterized by a T_m of 13.0 °C. The melting curve for dA_2 and poly(U) under the same conditions also showed no transition. In contrast, the curve for dA_2 and poly(dT) (data not shown) displayed a clear, cooperative hyperchromic effect with a T_m of 6.3 °C. (Poly(dT) alone showed no meltinglike transition between 0 °C and 26 °C.) T_m for dA_2 and poly(dT) under these conditions was 19.5 °C.

Table 2. Melting Temperatures[a]

	T_m (°C)	
duplex	0.15 M NaCl	1 M NaCl
A_2 + poly(U)	—[b]	13.0
A_2 + poly(U)	−	NT[c]
dA_2 + poly(U)	−	12.5
dA_2 + poly(U)	−	NT
dA_2 + poly(dT)	−	19.5
dA_2 + poly(dT)	−	6.3
dA_{16} + poly(dT)	49.1	−
$dA_{15}dA$ + poly(dT)	25.1	−
$(dAdA)_8$ + poly(dT)	9.9	−
dT_{16} + poly(dA)	46.4	−
$dT_{15}dT$ + poly(dA)	NT	−
$(dTdT)_8$ + poly(dA)	NT	−
dA_{16} + dT_{16}	43.9	57.1
$dA_{15}dA$ + $dT_{15}dT$	39.7	−
dA_{50} + dT_{50}	65.8	80.4
dA_{50} + dT_{50}	62.3	67.1
$(AU)_8$	56.5	−
$(dAdT)_8$	44.0	−
$(dAdT)_7dAdT$	50.0	−
dA_{25} + poly(U)	46.7	−
$dA_7dA_{11}dA_7$ + poly(U)	37.8	−
dA_{25} + poly(U)	27.7	−

[a] T_m values were derived from melting curves for nucleotide mixtures recorded at 260 nm. Italicized letters represent homo-type nucleotides. The spectra were taken in 10 mM sodium phosphate, pH 7.0, containing 0.15 or 1.0 M NaCl. [b] Not determined. [c] No transition observed.

Whereas at 0 °C and in 1 M NaCl the natural dimer dA_2 formed a 1:2 complex with poly(dT), the corresponding homo-type adenosine dimer dA_2 formed a 1:1 complex. Therefore the T_m values characterizing the interaction of the two adenosine dimers with poly(dT) in the presence of 1 M NaCl cannot be directly compared. In contrast to the melting curve of A_2 and poly(U), that of A_2 and poly(U) provided no evidence for complex formation. The dimer dA_2 also showed no evidence of interaction with poly(U) but did interact weakly with poly(dT). An attempt was made to compare the effect of NaCl on T_m for both dA_2·poly(dT) and dA_2·poly(dT). The melting of dA_2·poly(dT) was not measured at NaCl concentrations above 0.3 M to keep well below the concentration of 1 M where this nucleotide mixture forms a triple-helical complex, and dA_2·poly(dT) melting could not be measured at NaCl concentrations below 0.5 M because of the difficulty of measuring T_m values close to 0 °C. There was, therefore, no common range of NaCl concentrations over which T_m could be compared.

Each of the three 16-mers dA_{16}, $(dAdA)_8$, and $dA_{15}dA$, when mixed with poly(dT) in the presence of 0.15 M NaCl, displayed sharp melting transitions ($T_{90} - T_{10}$, ~6 °C) and hyperchromicity values greater than 35% (see Table 2 for T_m values). The melting curve for the self-complementary $(dAdT)_7dAdT$ recorded at 0.15 M NaCl yielded a T_m of 50 °C and a broad melting range ($T_{90} - T_{10}$, 22 °C), while the corresponding natural 16-mer yielded a lower T_m of 44 °C and an even broader melting range ($T_{90} - T_{10}$, 44 °C; see Table 2). T_m for

(15) *Nucleic Acids*; Ikehara, M., Ed.; Asakura Press: Tokyo, 1979; pp 243−307.

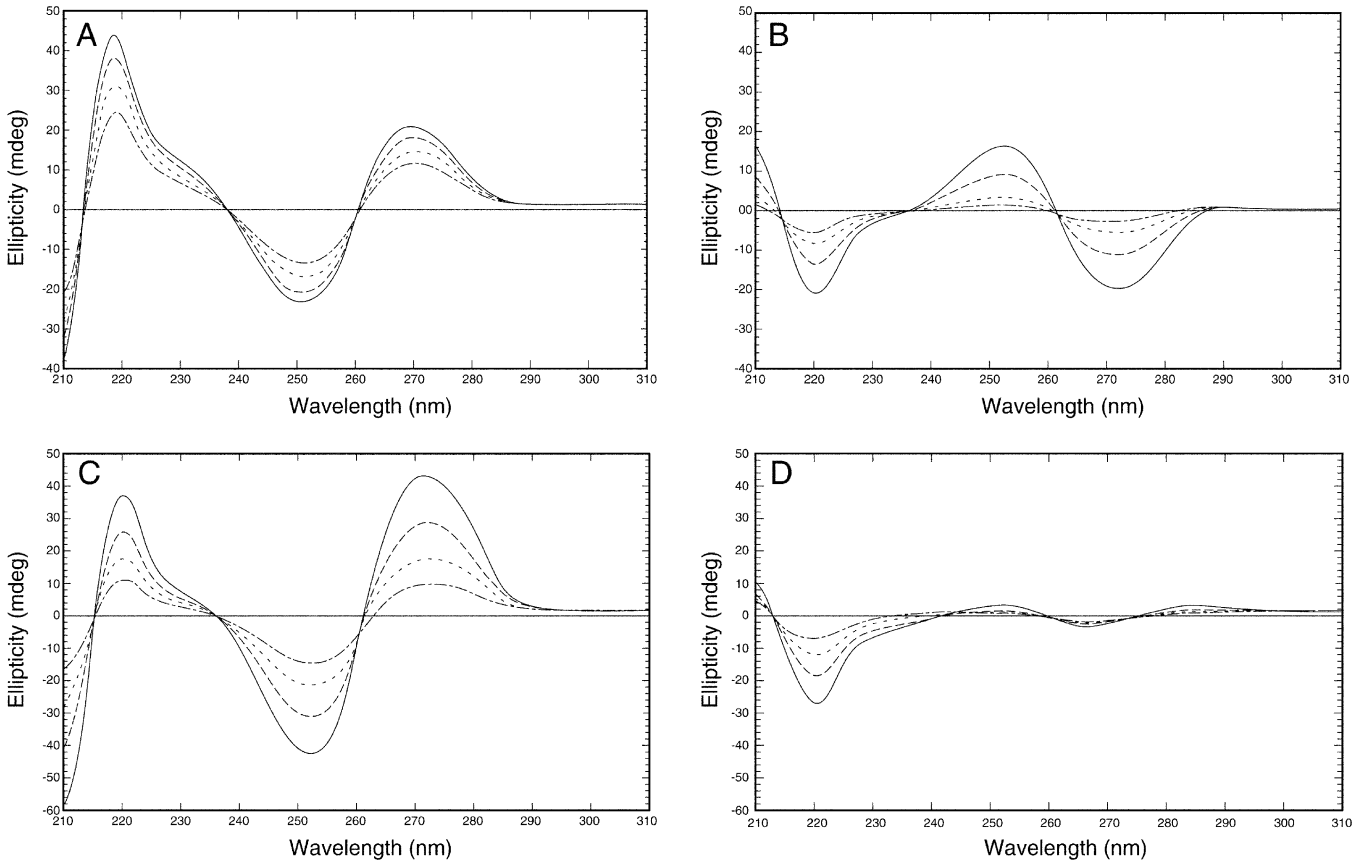


Figure 1. CD spectra of dA_2 (A) and dA_2 (B) at 0 °C (solid line), 18 °C (dashed line), 40 °C (dotted line), and 60 °C (dashed and dotted line) and of A_2 (C) and A_2 (D) at 0 °C (solid line), 20 °C (dashed line), 40 °C (dotted line), and 60 °C (dashed and dotted line). The base concentration was 0.1 mM.

(d*A*d*T*)$_7$d*A*dT increased from ∼43 °C in the absence of NaCl to ∼52 °C in 2.0 M NaCl (data not shown). The melting curves for complexes formed between poly(U) and a series of a 25-mers containing strings of natural residues of decreasing length sandwiched by homo-type residues yielded a monotonically decreasing series of T_m values from 47.8 °C for d*A*dA$_{23}$d*A*·poly(U) to 27.7 °C for the all-homo dA_{25}·poly(U).

When compared with the melting curve for (dAdT)$_8$, the corresponding curve for (d*A*d*T*)$_7$d*A*dT displayed a T_m value 6 °C higher (Table 2), as well as a narrower melting range and a higher hyperchromicity. The melting curves for the nonself-complementary complexes dA_{15}dA·dT_{15}dT and dA$_{16}$·dT$_{16}$ (Table 2) displayed the opposite trend: T_m for the homo-type complex was about 4 °C lower than that for the natural complex. These nonself-complementary complexes melted over a narrower range (about 13.5 °C) than did either the homo-type or natural self-complementary complexes (22.0 °C and 43.5 °C, respectively). The broader melting ranges of the self-complementary complexes may reflect the formation of intramolecular hairpin-type complexes (or a mixture of intramolecular and intermolecular complexes) and are consistent with a lower degree of cooperativity.

Circular Dichroism. The circular dichroic (CD) spectrum of dA_2 (Figure 1A) is similar to previously reported spectra,[16] with a positive maximum at 269.8 nm (θ_{obs}, 20.7 mdeg; this and subsequent values are reported for 0 °C), a negative maximum at 250.3 nm (θ_{obs}, −23.6 mdeg), a positive inflection point at ∼230 nm, and a positive maximum at ∼218.7 nm (θ_{obs}, 43.6 mdeg), as well as dichroic points at 260, 238, and 213 nm. In complete contrast, the spectrum of dA_2 (Figure 1B) is close to a mirror image about the $\theta = 0$ axis of that of the natural dimer, with a negative maximum at 271.5 nm (θ_{obs}, −19.4 mdeg), a positive maximum at 252.4 nm (θ_{obs}, 16.7 mdeg), a negative inflection point at ∼230 nm, and a negative maximum at 219.9 nm (θ_{obs}, −20.9 mdeg), though the relative intensities of the bands differ. The spectra taken at different temperatures also display good isodichroic points at 261 and 214.5 nm, as well as an "isodichroic region" at 234−239 nm. For both dimers, the intensity of the bands decreased as the temperature was raised from 0 °C to 60 °C. For dA_2, the decrease was approximately linear and amounted to 42−45%, depending on the band; but for dA_2, the decrease is better described by an exponential function and amounted to as much as 75−90%.

The spectra of A_2 (Figure 1C) are similar to previously reported spectra,[16] with a positive maximum at 271.3 nm (θ_{obs}, 43.2 mdeg), a negative maximum at ∼250 nm, a positive inflection point at ∼230 nm, and a negative maximum at 252.3 nm (θ_{obs}, −42.8 mdeg), as well as isodichroic regions at 260−261.5, 228.5−237, and 215−216 nm. In contrast, the spectrum of A_2 (Figure 1D) has an inverted CD band at 220.2 nm (θ_{obs}, −26.9 mdeg), a low-intensity inverted band at 251.3 nm (θ_{obs}, 3.1 mdeg), and an isodichroic point at 213.5 nm. The remainder of the spectrum consists of one or two low-intensity bands that are difficult to relate to the 270-nm band of A_2. For both

(16) Kang, H.; Chou, P.-J.; Johnson, W. C., Jr.; Weller, D.; Huang, S.-B.; Summerton, J. E. *Biopolymers* **1992**, *32*, 1351−1363.

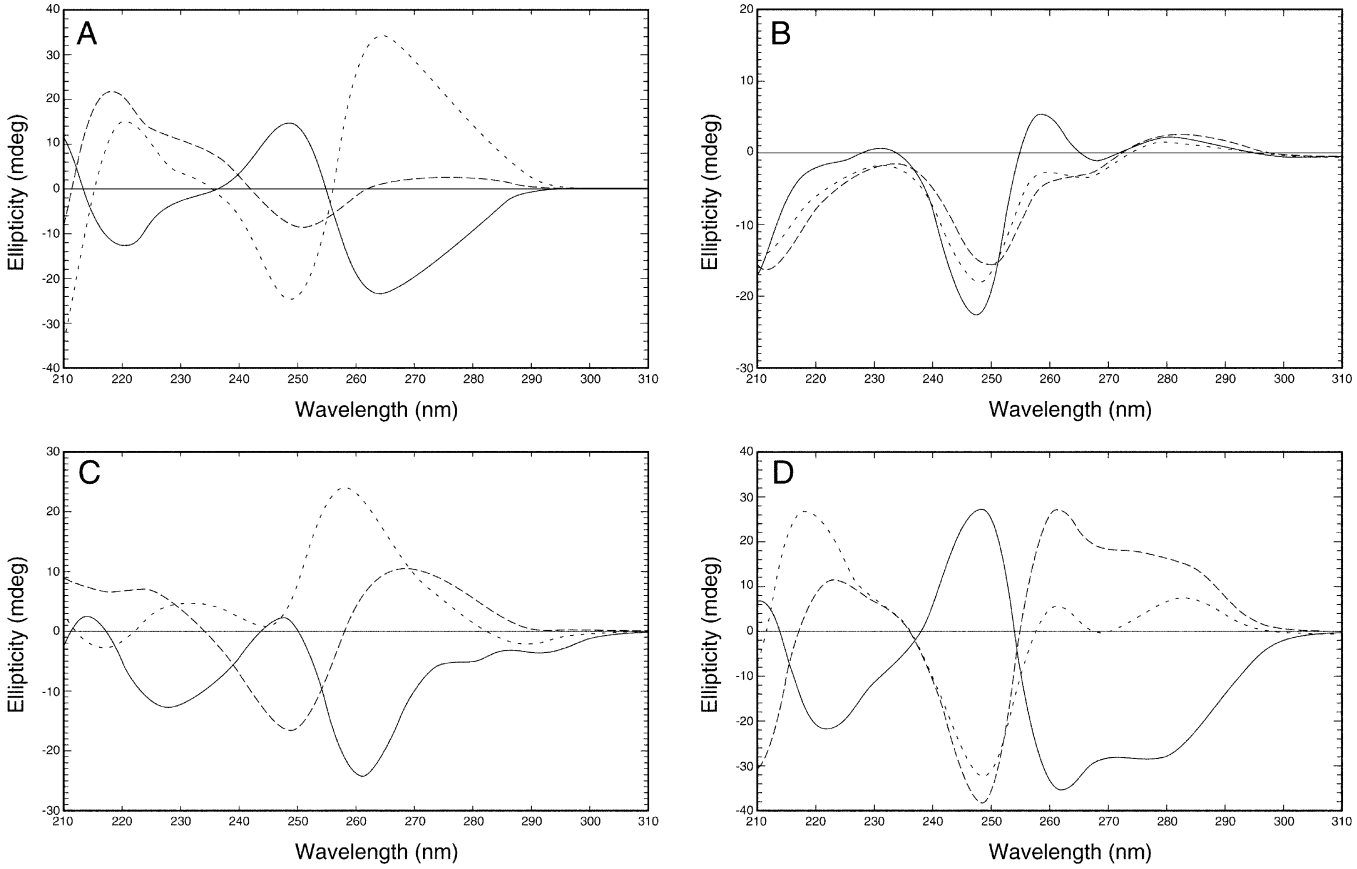


Figure 2. (A) CD spectra for dA_{15}dA (solid line), dA_{16} (dashed line), and poly(A) (dotted line) at 10 °C and a base concentration of 0.05 mM. (B) Spectra for dA_{15}dA·poly(dT) (solid line), dA_{16}·poly(dT) (dashed line), and (d*A*dA)$_8$·poly(dT) (dotted line), at 10 °C (natural oligomer) or 0 °C and a base concentration of 0.05 mM. (C) Spectra for (d*A*d*T*)$_7$d*A*dT (solid line), (dAdT)$_8$ (dashed line), and $(AU)_{12}$ (dotted line) at 10 °C and a base concentration of 0.1 mM. (D) Spectra for dA_{15}dA·dT_{15}dT (solid line), poly(A)·dT_{16} (dashed line), and dA_{16}·dT_{16} (dotted line) at 10 °C and a base concentration of 0.05 mM in each species.

compounds, the intensity of the major bands decreased nonlinearly as the temperature was raised from 0 °C to 60 °C.

The CD spectrum for dA_2, being an approximate reflection about the $\theta = 0$ axis of the spectrum for dA_2, can be interpreted as evidence for a left-handed stacked conformation, in contrast to the right-handed stacked conformation of dA_2. The good isodichroic points observed in the dA_2 spectra taken at various temperatures are consistent with a two-state model of the stacking−unstacking transition.[16] The spectrum for A_2, being clearly inverted in only one CD band, does not provide strong evidence for a left-handed stacked conformation. A left-handed stacked conformation of nucleotide dimers has previously been reported for dGdA at low pH[17] and for the conformationally restricted A°pA°, a dinucleoside monophosphate of *O*-cycloadenosine.[18] The apparently exponential decrease in the intensity of the CD bands of dA_2 as the temperature was increased suggests that its stacking conformation is less stable than that of dA_2.

The spectrum of dA_{15}dA is similar to that of dA_2 and strikingly resembles a mirror image about the $\theta = 0$ axis of that of natural poly(A) (Figure 2A). The spectra for d(*AA*)$_8$·poly(dT) and dA_{15}dA·poly(dT) display patterns similar to that of dA_{16}·poly(dT) (Figure 2B). The spectra of the self-complementary (d*A*d*T*)$_7$d*A*dT and (dAdT)$_8$ are very different, and in fact the spectrum of (d*A*d*T*)$_7$d*A*dT resembles a mirror image about the $\theta = 0$ axis of that of the RNA-type self-complementary oligomer $(AU)_{12}$ (Figure 2C). The spectra of dA_{15}dA·dT_{15}dT resemble a mirror image about the $\theta = 0$ axis of that of poly(A)·dT_{16} (Figure 2D).

The similarity of the CD spectra for the natural, alternating, and homo-type 16-mer complexes with poly(dT) (Figure 2B) provides evidence that (d*A*dA)$_8$·poly(dT) and dA_{15}dA·poly(dT) adopt a right-handed helical conformation similar to that of dA_{16}·poly(dT). Deviation spectra (not shown) suggest that the spectrum for d(*AA*)$_8$·poly(dT) is close to the average of the spectra for dA_{16}·poly(dT) and dA_{15}dA·poly(dT). The CD spectrum of (d*A*d*T*)$_7$d*A*dT is drastically different from that of (dAdT)$_8$ (Figure 2C). Though the two spectra are not precisely reflected about the $\theta = 0$ axis, the opposite sign of the major bands may reflect opposite helical twist (right-handed for the natural oligomer and left-handed for the homo-type). Our results suggest a closer structural resemblance, though with opposite helical twist, between the homo-type self-complementary oligodeoxyribonucleotide and RNA-type oligomers (Figure 2C). The spectra for nonself-complementary oligomer duplexes show a corresponding similarity between homo-type oligodeoxyribonucleotides and RNA/DNA hybrids, so that homo-type duplexes may resemble a left-handed version of A-type dsRNA or RNA/DNA hybrids.

(17) Dolinnaya, N. G.; Fresco, J. R. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 9242−9246.
(18) Ikehara, M.; Uesugi, S.; Yano, J. *Nature (London), New Biol.* **1972**, *240*, 16−17.

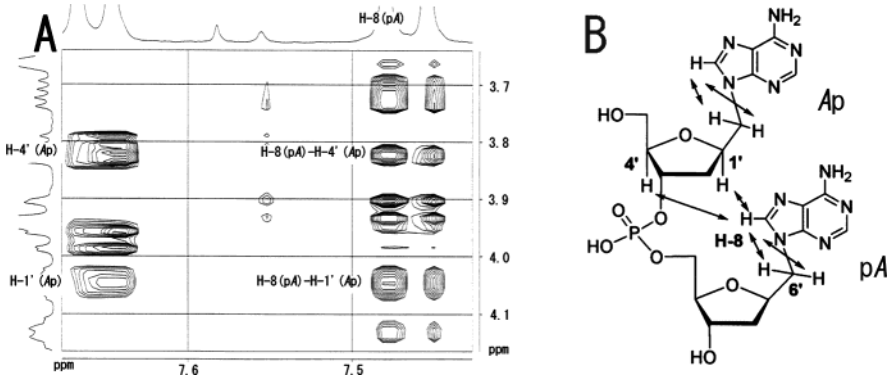


Figure 3. NMR (NOESY) analysis of dA_2. (A) Contour plot of part of the NOESY spectrum. (B) Schematic of the dimer showing the observed interresidue and intraresidue proton proximity relationships.

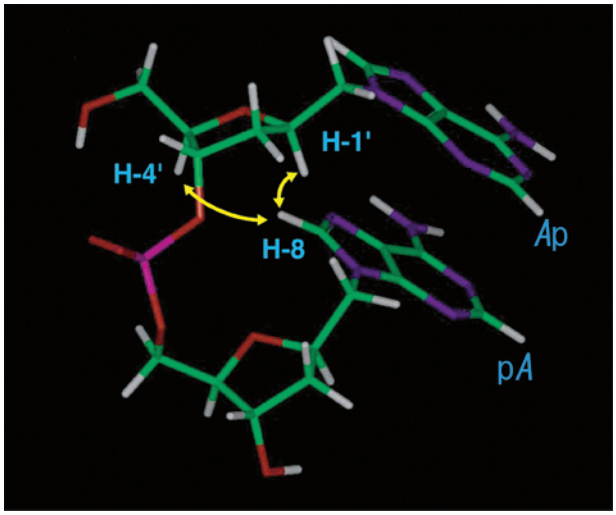


Figure 4. Tentative model of the conformation of dA_2 derived from NOESY measurements. Side view showing the interresidue proximity relationships of the H-8, H-1′, and H-4′ protons.

NOESY Spectra. To further investigate the base-stacking conformation of dA_2, we carried out a NOESY NMR study under the conditions used in the CD experiments. An NMR spectrum of dA_2 was acquired at 500 MHz. NOESY intraresidue cross-peaks included two between the H-8 proton of *A*p (the 3′-nucleotidyl unit) and the H-6′ and H-6″ protons of *A*p and two between the H-8 proton of p*A* (the 5′-nucleotidyl unit) and the H-6′ and H-6″ protons of p*A*. There were two strong interresidue cross-peaks, one between the H-8 proton of p*A* and the H-4′ proton of *A*p and one between the H-8 proton of p*A* and the H-1′ proton of *A*p (Figure 3). Weak interresidue cross-peaks were also observed between adenine base protons. Most other cross-peaks observed were intraresidue cross-peaks of pentofuranose. A hand-built model of the dimer constructed to fit the observed proximity relationships displayed a left-handed twist (Figure 4). The observed interresidue distances, which would be expected to be very much greater in a right-handed model of the dimer, corroborate the CD evidence for left-handedness.

Atomic-Force Microscopy (AFM). AFM has recently been applied to the investigation of biological macromolecules at the subnanometer level.[19−23] Because the technique is now sufficiently powerful to distinguish between right-handed and left-handed double-stranded DNA molecules,[19] we applied it to a comparison of natural (dA_{50}·dT_{50}) and homo-type (dA_{50}·dT_{50})

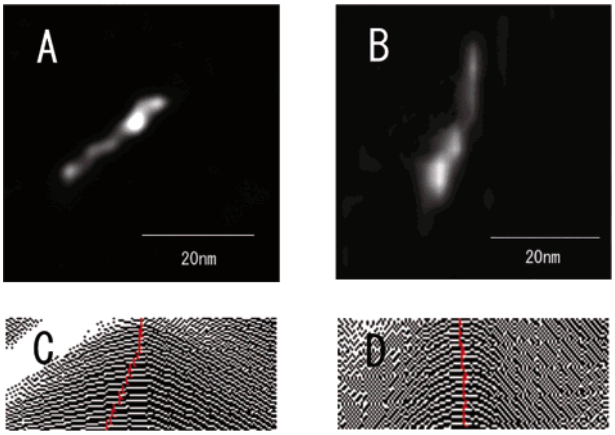


Figure 5. Atomic force microscopy of natural and homo-type 50-mers. (A) AFM image of dA_{50}·dT_{50}, (B) AFM image of dA_{50}·dT_{50}, (C) sectional image of dA_{50}·dT_{50}, and (D) sectional image of dA_{50}·dT_{50}.

dsDNA. The AFM images obtained revealed structures of opposite helicity (Figure 5A, B). A sectional image for natural dsDNA (Figure 5C) shows the expected shift of the points of maximal height to the right in the upper part, indicating a right-handed helix. In contrast, the sectional image for homo-type dsDNA shows a shift of the points of maximal height to the left in the upper part (Figure 5D), indicating a left-handed helix. These images provide further evidence for a left-handed helical conformation of homo-type dsDNA.

Nuclease Digestion. Snake venom phosphodiesterase is an exonuclease that cleaves DNA or RNA nonprocessively in the 3′→ 5′ direction to produce 5′-mononucleotides.[24] Venom phosphodiesterase readily digested dA_2 to yield 5′-dAMP and dA. By contrast, the enzyme did not detectably digest dA_2 (chromatograms not shown). Similarly, dA_{16} was largely digested by an hour's treatment with 0.36 U of the enzyme, whereas dA_{15}dA remained essentially intact under the same conditions, except for the cleavage of the single natural residue.

Calf-spleen phosphodiesterase is an exonuclease that hydrolyses single-stranded DNA or RNA in the 5′→3′ direction

(19) Mou, J.; Czajkowsky, D. M.; Zhang, Y.; Shao, Z. *FEBS Lett.* **1995**, *371*, 279−282.
(20) Tanigawa, M.; Okada, T. *Anal. Chim. Acta* **1998**, *365*, 19−25.
(21) Müller, D. J.; Fotiadis, D.; Engel, A. *FEBS Lett.* **1998**, *430*, 105−111.
(22) Oesterhelt, F.; Oesterhelt, D.; Pfeiffer, M.; Engel, A.; Gaub, H. E.; Müller, D. J. *Science* **2000**, *288*, 143−146.
(23) Tahirov, T. H.; Sato, K.; Ichikawa, E.; Sasaki, M.; Inoue, T.; Shiina, M.; Kimura, K.; Takata, S.; Fujikawa, A.; Morii, H.; Kumasaka, T.; Yamamoto, M.; Ishii, S.; Ogata, K. *Cell* **2002**, *108*, 57−70.
(24) Laskowski, M. In *The Enzymes. Vol. 4: Venom Exonuclease*, 3rd ed.; Boyer, P. S., Ed.; Academic Press: New York, 1971; pp 313−328.

to produce 3′-mononucleotides.[25] Its specificity is thus complementary to that of venom phosphodiesterase. The dimer dA_2 was completely digested after incubation for 1 h with 0.02 U of the enzyme. In contrast, dA_2 was only digested to the extent of ~3% under these conditions and even after an overnight incubation with 0.02 U almost 90% of the original dimer remained. The homo-type dimer was therefore digested at least 630 times more slowly than the natural dimer. Similarly, dA_{16} was 96% digested after overnight incubation with 0.02 U, whereas dA_{15}dA under these conditions was not digested by more than 20%. In other words, oligomers containing homo-type nucleotide residues were degraded several 100-fold more slowly by spleen phosphodiesterase than were the corresponding natural oligomers.

Nuclease S1 is a Zn^{2+}-dependent glycoprotein that acts both endonucleolytically and exonucleolytically on single-stranded nucleic acids, releasing 5′-terminal monophosphate products.[26] The 25-mers d*A*dA_{23}d*A*, dA_3dA_{19}dA_3, dA_5dA_{15}dA_5, dA_7dA_{11}dA_7, dA_9dA_7dA_9, and dA_{11}dA_3dA_{11} were all substantially digested by 1 U of the enzyme. The all-homo-type 25-mer dA_{25}, in contrast, showed not a trace of digestion by up to 10 U and, even at 100 U, showed only a trace of monomer. The 16-mer dA_{15}dA was virtually intact after overnight incubation in 66% fetal bovine serum, conditions that resulted in the complete digestion of dA_{16}. While all-homo-type deoxyadenosine oligomers were digested extremely slowly, if at all, by nuclease S1, oligomers containing at least three natural residues in an otherwise all-homo-type chain were susceptible to degradation.

Conclusions

We have synthesized adenine (both ribo- and deoxyribo-types) and thymine homo-*N*-nucleoside monomers and adenine homo-*N*-nucleotide dimers and shown that homo-*N*-nucleotide oligomers can be synthesized from the monomers by means of standard phosphoramidite chemistry on an automated DNA synthesizer. The glycosyl conformation of natural nucleic acids is one of the important factors determining their helicity.[7] In homo-type nucleic acids, the greater distance between sugar and base imposed by the inserted methylene group appears to confer an extra degree of conformational flexibility compared to the direct sugar-base link of natural nucleic acids. Such flexibility may explain the ability of homo-type oligomers to adopt a left-handed conformation in both the single-stranded and the double-stranded state while allowing a right-handed conformation in heteroduplexes with natural nucleic acids. A left-handed helical conformation has been observed for nucleic acids or nucleic acid analogues with alternating pyrimidine−purine sequences (at high salt concentrations; Z-DNA), with L-deoxyribose instead of D-deoxyribose as the sugar[27] and with the glycosyl torsion angle restricted to force a high anti conformation.[8,9] The modified nucleic acids described in the present paper, however, are to our knowledge the first reported example of D-sugar nucleic acids that form a left-handed double-helix under low salt conditions with no requirement for alternating pyrimidine−purine sequences and that have an unrestricted glycosyl torsion angle.

Experimental Section

General Methods. UV spectra, mixing curves, and melting curves were recorded with a Hitachi U-3200 double-beam spectrophotometer. Circular dichroic spectra were taken with a Jasco J-720 spectropolarimeter interfaced with an NEC PC-9801DX personal computer and fitted with a Jasco temperature controller. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 2000, Varian Unity Plus 300, or Bruker DRX 500 spectrometer, with tetramethylsilane or sodium 2,2-dimethyl-2-silapentane sulfonate as the internal standard. FAB mass spectra were recorded on a JEOL JMS-SX 102 mass spectrometer. High-pressure liquid chromatography (HPLC) was done on a Shimadzu system with an LC-6A liquid chromatograph coupled to an SPD-6A UV spectrophotometric detector operated from an SCL-6A system controller linked to a C-R3A data processor.

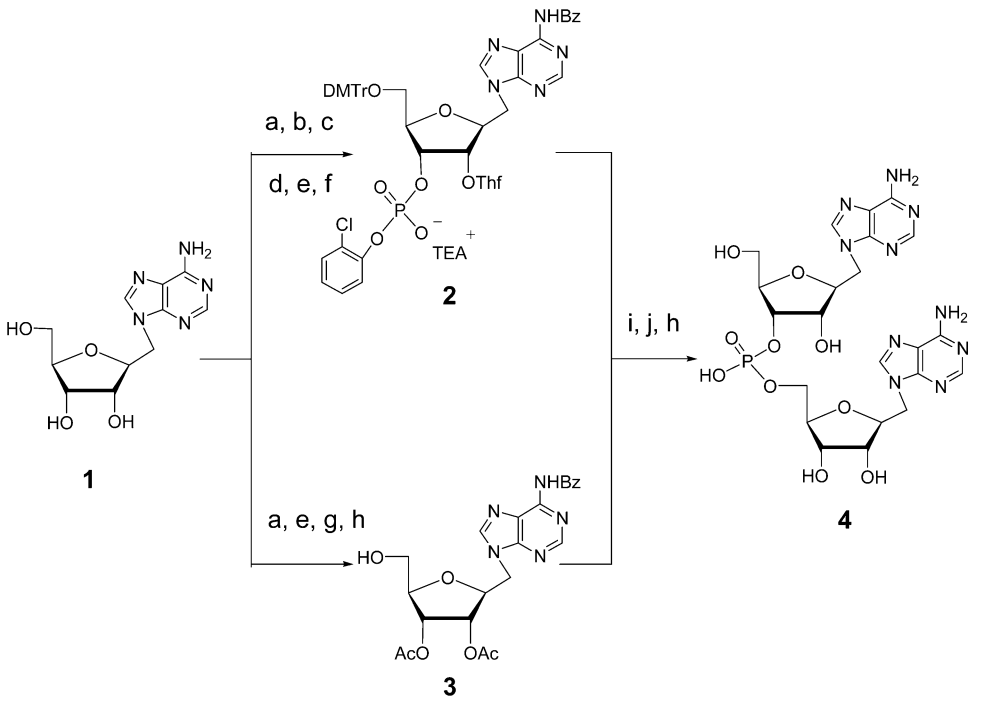
Thin-layer chromatography (TLC) was carried out on Merck silica gel 60 F_{254} precoated plates (Merck, Whitehouse Station, New Jersey). Analytical plates were 20 cm × 20 cm × 0.2 mm, and preparative plates were 20 cm × 20 cm × 2 mm. Column chromatography was performed on Wako silica gel C-300. We used published extinction coefficients[15] for adenosine and deoxyadenosine dimers and oligomers.

Materials. Phosphodiesterase from *Crotalus adamanteus* (oligonucleate 5′- nucleotidohydrolase; EC 3.1.4.1) was purchased from Koch-Light Ltd (now NBS Biologicals, Hatfield, Hertfordshire, U.K.), and phosphodiesterase from calf spleen (oligonucleate 3′-nucleotidohydrolase; EC 3.1.16.1) and nuclease S1 (EC 3.1.30.1) from *Aspergillus oryzae* were purchased from Boehringer (Mannheim, Germany). Sodium polyuridylate (poly(U); A_{280}/A_{260}, 0.35; A_{250}/A_{260}, 0.78; $S_{20,w}$, 6.0; 2.75 μmol/mg) was from Yamasa Corp. (Choshi, Chiba Prefecture, Japan). Unmodified phosphoramidite monomers, DNA solid supports, and other synthetic reagents were from Applied Biosystems (now PE Biosystems, Foster City, California). A long chain alkylamine controlled pore glass solid support (LCAA-CPG; 0.081 mmol amino groups/g) was from CPG Inc. (Lincoln Park, New Jersey).

Synthesis of Homo-*N*-dinucleoside Monophosphate **4** (Scheme 1**).** Preparation of compound **2**: To a suspension of **1** (916 mg, 3.26 mmol) in pyridine at 0 °C (15 mL) was added benzoyl chloride (2.75 g, 19.6 mmol). After 2 h, the reaction mixture was poured into chloroform (100 mL) containing ice (115 g) and sodium bicarbonate (9.0 g). The aqueous layer was extracted twice with chloroform, and the organic layers were combined and concentrated. The residue was dissolved in a mixture of ethanol (9.8 mL) and pyridine (6.5 mL), and the solution was treated with a mixture of 2 N NaOH (13 mL) and ethanol (13 mL) at room temperature for 30 min. The reaction mixture was neutralized with 2 N HCl (13 mL), concentrated, and applied to a reverse-phase column (ODS, 50 × 80 mm^2; Waters, Milford, Massachusetts), which was developed with a discontinuous methanol gradient (solvent system, methanol−water; step size, 5%) over 1000 mL. Appropriate fractions were combined and evaporated under reduced pressure to give 9-*N*-[(*β*-D-ribo-pentofuranosyl)methyl]-*N*^{6}-benzoyladenine (1.2 g, 95% yield). To a solution of this compound (160 mg, 0.42 mmol) and imidazole (113 mg, 1.66 mmol) in *N*,*N*-dimethylformamide (DMF; 1.1 mL), tetraisopropyldisiloxane dichloride (153 mg, 0.49 mmol) was added. The reaction mixture was stirred at room temperature for 3 h, poured into water, and extracted with dichloromethane. The organic layer was washed with cool dilute HCl and then with water and concentrated under reduced pressure. The residue was purified by preparative TLC with dichloromethane−methanol (95:5) as the solvent to give 9-*N*-[(3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-*β*-D-ribo-pentofuranosyl)methyl]-*N*^{6}-benzoyladenine (182 mg, 70% yield). This compound (150 mg, 0.24 mmol) was dissolved in dioxane, and then 2,3-dihydrofuran (336 mg, 4.8 mmol) was added in the presence of pyridinium *p*-toluene sulfonate (70 mg, 0.28 mmol) and

(25) Bernardi, A.; Bernardi, G. In *The Enzymes. Vol. 4: Spleen Acid Exonuclease*, 3rd ed.; Boyer, P. S., Ed.; Academic Press: New York, 1971; pp 329−336.
(26) Fraser, M. J.; Low, R. L. In *Nucleases: Fungal and Mitochondrial Nucleases*, 2nd ed.; Linn, S. M., Lloyd, R. S.; Roberts, R. J., Eds.; Cold Spring Harbor Laboratory Press: New York, 1993; pp 171−207.
(27) Urata, H.; Ogura, E.; Shinohara, K.; Ueda, Y.; Akagi, M. *Nucleic Acids Res.* **1992**, *20*, 3325−3332.

Scheme 1. Synthesis of Homo-*N*-diadenosine Monophosphate[a]



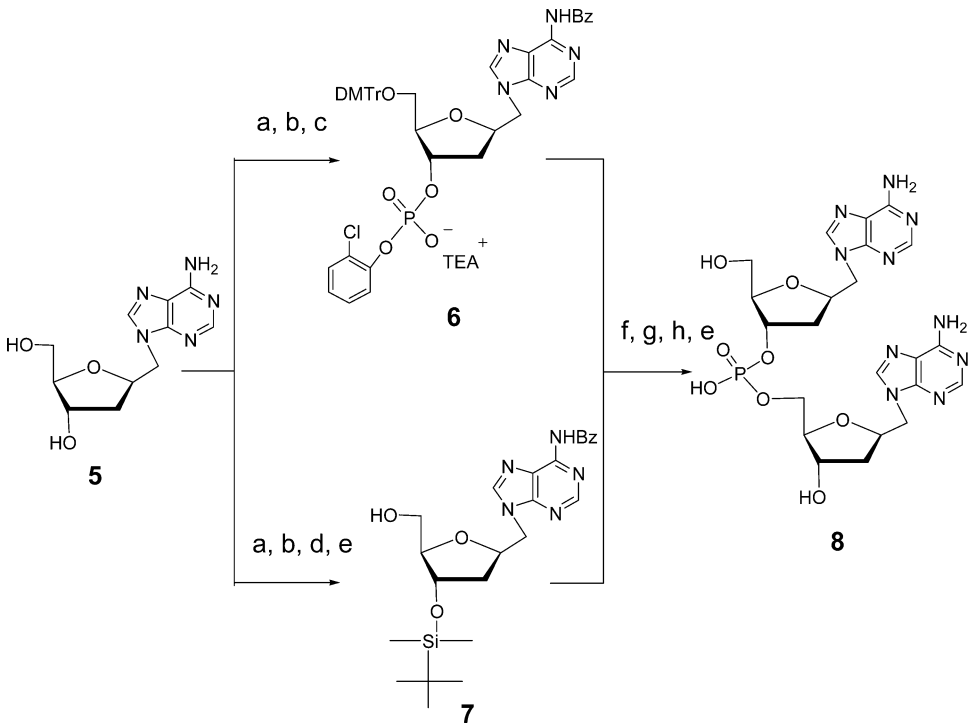
[a] Reagents and conditions: (a) (i) BzCl, pyridine, 0 °C, 2 h; (ii) 2 N NaOH, aq EtOH, THF, pyridine, rt, 30 min; (b) tetraisopropyldisiloxane dichloride, imidazole, DMF, rt, 3 h; (c) 2,3-dihydrofuran, pyridinium *p*-toluenesulfonate, dioxane, rt, 20 h; (d) *n*-Bu_4NF, THF, rt, 30 min; (e) DMTrCl, pyridine, rt, 2−5 h; (f) 2-chlorophenylphosphoditriazolidate, 1-methylimidazole, dioxane, rt, 2 h; (g) Ac_2O, pyridine, rt, 1 h; (h) 80% AcOH, rt, 1 h; (i) MSNT, pyridine, rt, 1 h; (j) concentrated NH_4OH, pyridine, rt, 2 days.

the solution was stirred at room temperature for 20 h. Pyridine (0.3 mL) and dichloromethane (10 mL) were added, and the mixture was washed with saturated sodium bicarbonate and water, after which the organic layer was dried with Na_2SO_4 and concentrated under reduced pressure. The residue was then dried by coevaporation with pyridine and toluene and treated with 0.5 M tetrabutylammonium fluoride (TBAF) in tetrahydrofuran solution (1.5 mL) at room temperature for 30 min. The solvent was removed under reduced pressure, and the residue was dissolved in 1.5 mL of pyridine−methanol (3:1, v/v). The resulting solution was passed through a cation exchange column (Dowex 50w × 2; 3 mL), washed with 10 mL of pyridine−methanol (3:1, v/v), and concentrated under reduced pressure. The residue was then purified by TLC with dichloromethane−methanol (94:6) as the solvent to give 9-*N*-[(2′-*O*-(tetrahydrofuranyl)-*β*-D-ribo-pentofuranosyl)methyl]-N^6-benzoyladenine (101 mg, 92% yield). This compound (100 mg, 0.22 mmol) was coevaporated with pyridine and dissolved in the same solvent (0.7 mL). To this solution, 4,4′-dimethoxytrityl chloride (DMTrCl; 89 mg, 0.26 mmol) was added. The reaction mixture was stirred at room temperature for 5 h, treated with methanol (5 drops) for 5 min at room temperature, and evaporated to dryness under reduced pressure. The residue was then dissolved in dichloromethane (10 mL), washed with water, and concentrated. The residue was purified by TLC with dichloromethane−methanol (95:5) containing 0.1% pyridine as the solvent to give 9-*N*-[(5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-(tetrahydrofuranyl)-*β*-D-ribo-pentofuranosyl)methyl]-N^6-benzoyladenine (107 mg, 64% yield). To 107 mg (0.14 mmol) of this compound, a solution of 2-chlorophenylphosphoditriazolidate in anhydrous dioxane (0.65 mL; 0.28 M stock-solution) and 1-methylimidazole (58 mg, 0.72 mmol) was added. After 2 h of stirring, 50% pyridine−water (1.0 mL) was added. The reaction mixture was then diluted with dichloromethane (10 mL) and washed with 0.1 M triethylammonium bicarbonate (pH 7, 10 mL). The organic layer was concentrated to give **2** (140 mg), which was used without further purification for the next step.

Preparation of compound **3**: 9-*N*-[(*β*-D-ribo-pentofuranosyl)methyl]-N^6-benzoyladenine (150 mg, 0.39 mmol) was coevaporated with pyridine and dissolved in the same solvent (1.2 mL). To this solution DMTrCl (172 mg, 0.51 mmol) was added. The reaction mixture was stirred at room temperature for 2 h, treated with methanol (5 drops) for 5 min at room temperature, and evaporated under reduced pressure. The residue was then dissolved in dichloromethane (10 mL), washed with water, and concentrated. The residue was purified by TLC with dichloromethane−methanol (93:7) containing 0.1% pyridine as the solvent, to give 9-*N*-[(5′-*O*-(4,4′-dimethoxytrityl)-*β*-D-ribo-pentofuranosyl)methyl]-N^6-benzoyladenine (202 mg, 74% yield). This compound (200 mg, 0.29 mmol) was dissolved in pyridine (1.0 mL), and after the addition of acetic anhydride (0.5 mL), the reaction mixture was stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure, the residue was treated with 80% acetic acid (4.5 mL) at room-temperature for 1 h, methanol (5 drops) was added, and the solvent again was evaporated under reduced pressure. The residue was purified by TLC with dichloromethane−methanol (93:7) to give 9-*N*-[(2′,3′-*O*-diacetyl-*β*-D-ribo-pentofuranosyl)methyl]-N^6-benzoyladenine (**3**; 136 mg, 65% yield).

Preparation of compound **4**: To a solution of **2** (135 mg, 0.14 mmol) and **3** (47 mg, 0.1 mmol) in pyridine (1.0 mL) 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole (MSNT; 83 mg, 0.28 mmol) was added, and the reaction mixture was stirred at room temperature for 1 h. Pyridine−water (50%; 1.0 mL) was added, the reaction mixture was diluted with dichloromethane (5 mL) and washed with water (5 mL), and the organic layer was dried with $MgSO_4$ and concentrated under reduced pressure. The residue was purified by TLC with dichloromethane−methanol (95:5) to give a fully protected dimer (131 mg, 94% yield). The protected dimer (131 mg, 0.094 mmol) was dissolved in pyridine (10 mL) and concentrated ammonium hydroxide (10 mL) and deprotection carried out at room temperature for 2 days. The solvent was then removed under reduced pressure, and the residue was chromatographed on a reverse-phase column (ODS, 10 × 100 mm^2; Waters) with a linear gradient from 0 to 40% acetonitrile in 0.05 M triethylammonium acetate, pH 7.0, over 400 mL. The main UV-absorbing fractions were combined and concentrated to dryness under reduced pressure. The purified DMTr-dimer was treated with 80% acetic acid at room temperature for 2 h, and after removal of the acid under

Scheme 2. Synthesis of Homo-*N*-deoxydiadenosine Monophosphate[a]



[a] Reagents and conditions: (a) (i) BzCl, pyridine, 0 °C, 1 h; (ii) 2 N NaOH, aq EtOH, THF, pyridine, rt, 30 min; (b) DMTrCl, pyridine, rt, 4 h; (c) 2-chlorophenylphosphoditriazolidate, dioxane, rt, 2 h; (d) *tert*-butyldimethylchlorosilane, imidazole, DMF, rt, 4 h; (e) 80% AcOH, rt, 2 h; (f) MSNT, pyridine, rt, 2 h; (g) *n*-Bu_4NF, THF, rt, 30 min; (h) concentrated NH_4OH, pyridine, rt, 2 days.

reduced pressure, the residue was dissolved in water (10 mL) and washed with ethyl acetate (2 × 10 mL). The aqueous solution was concentrated to give the dimer product, which was further purified by reverse-phase chromatography under the same conditions and then by anion-exchange chromatography on a Dowex column (Dowex 1 × 2, Cl^- form; 10 × 50 mm^2) with a linear gradient from 0 to 1 M NaCl over 200 mL. The main UV-absorbing fractions were combined, desalted on an ODS column, and freeze-dried to give **4** (36 mg, 59% yield). ^{1}H NMR (200 MHz, D_2O): δ 3.60−3.80 (m, 4H), 3.80−4.80 (m, 12H), 8.06 (bs, 2H), 8.14 (bs, 2H). FAB-MS: *m*/*z* 623 $(M-H)^-$. Anal. Calcd for $C_{22}H_{28}N_{10}PNa_2 \cdot 2.5H_2O$: C, 36.98; H, 4.66; N, 19.60. Found: C, 37.19; H, 4.72; N, 19.21.

Synthesis of Homo-*N*-deoxydinucleoside Monophosphate **8** (Scheme 2**).** Preparation of compound **6**: Compound **6** was prepared from **5** (133 mg, 0.50 mmol) essentially as described for **2** and used without further purification for the next step. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.88 (m, 1H, H-2′), 2.41 (m, 1H, H-2″), 3.15 (m, 2H, H-5′ and H-5″), 3.78 (s, 6H, −OMe), 4.28 (m, 2H, H-4′ and H-6′), 4.56 (m, 2H, H-3′ and H-6″), 5.00 (m, 1H, H-1′), 6.80 (m, 4H), 6.93 (m, 1H), 7.22 (m, 8H), 7.65 (m, 5H), 8.05 (m, 2H), 8.10 (s, 1H, H-2), 8.83 (s, 1H, H-8).

Preparation of compound **7**: To a solution of 9-*N*-[5′-*O*-(4,4′-dimethoxytrityl)-(2′-deoxy-β-D-ribo-pentofuranosyl)methyl]-N^6-benzoyladenine (80 mg, 0.11 mmol; prepared from **5**) and imidazole (41 mg, 0.55 mmol) in DMF (1.6 mL) *tert*-butyldimethylchlorosilane (54 mg, 0.33 mmol) was added, and the reaction mixture was stirred at room temperature for 4 h. Methanol (2 drops) was added, and the reaction mixture was concentrated and coevaporated with toluene. This material was deprotected with 80% acetic acid (5.5 mL) at room temperature for 2 h, and then methanol (2 drops) was added and the solvent was evaporated under reduced pressure. The residue was coevaporated with toluene and purified by TLC with dichloromethane−methanol (94:6) to give **7** (48 mg, 83% yield from DMTr derivative). ^{1}H NMR (200 MHz, D_2O): δ 0.06 (s, 6H), 0.86 (s, 9H), 1.86 (m, 1H, H-2′), 2.04 (m, 1H, H-2″), 3.50 (dd, J = 12, 4 Hz, 1H, H-5′), 3.66 (dd, J = 12, 3 Hz, 1H, H-5″), 3.78 (m, 1H, H-4′), 4.20−4.60 (m, 4H), 7.46−7.70 (m, 3H), 8.00−8.10 (m, 2H), 8.12 (s, 1H, H-2), 8.80 (s, 1H, H-8).

Preparation of compound **8**: To a solution of **6** (125 mg, 0.13 mmol) and **7** (48 mg, 0.1 mmol) in pyridine (1.0 mL) MSNT (77 mg, 0.26 mmol) was added, and the reaction mixture was stirred at room temperature for 2 h. Pyridine−water (50%; 1.0 mL) was added, the reaction mixture was diluted with dichloromethane (5 mL) and washed with water (5 mL), and the organic layer was dried with $MgSO_4$ and concentrated under reduced pressure. The residue was purified by TLC with dichloromethane−methanol (94:6) to give a fully protected dimer (109 mg, 83% yield). The protected dimer (75 mg, 0.056 mmol) was treated with 0.2 M TBAF in tetrahydrofuran solution (1 mL) at room temperature for 30 min, methanol (0.5 mL) was added, and the solvent was evaporated to dryness under reduced pressure. The residue was dissolved in methanol (2.0 mL) and concentrated ammonium hydroxide (20 mL), and deprotection was allowed to proceed at room temperature for 2 days, after which the solvent was removed under reduced pressure. This residue was chromatographed on a reverse-phase column (ODS, 10 × 100 mm^2; Waters) with a linear gradient from 0 to 35% acetonitrile in 0.1 M triethylammonium acetate, pH 7.0, over 400 mL. The main UV-absorbing fractions were combined and concentrated to dryness under reduced pressure. The purified DMTr-dimer was deprotected with 80% acetic acid at room temperature for 30 min, and after removal of the acid under reduced pressure, the residue was dissolved in water (10 mL) and washed with ethyl acetate (2 × 10 mL). The aqueous solution was concentrated to give the dimer product. The product was further purified by reverse-phase chromatography under the same conditions and then by anion-exchange chromatography on a Dowex column (Dowex 1 × 2, Cl− form; 10 × 50 mm^2) with a linear gradient from 0 to 0.5 M NaCl over 200 mL. The main UV-absorbing fractions were combined, desalted on an ODS column, and freeze-dried to give **8** (28 mg, 81% yield). ^{1}H NMR (500 MHz, D_2O): δ 1.84 (m, 2H, H-2′ (*A*p) and H-2′ (p*A*)), 2.05 (m, 1H, H-2″ (p*A*)), 2.21 (m, 1H, H-2″ (*A*p)), 3.58 (m, 2H, H-5′ and H-5″ (*A*p)), 3.76 (m, 2H, H-5′ and H-5″ (p*A*)), 4.01 (m, 1H, H-4′ (p*A*)), 4.06 (dd, J = 14.69, 7.28 Hz, 1H, H-6′ (p*A*)), 4.13 (m, 1H, H-6′ (*A*p)), 4.17 (bs, 1H, H-4′ (*A*p)), 4.25 (bs, 1H, H-6″ (p*A*)), 4.28 (bs, 1H, H-3′ (p*A*)), 4.33 (bs, 1H, H-6″ (*A*p)), 4.39 (m, 1H, H-1′ (*A*p)), 4.47 (m, 1H, H-1′ (p*A*)), 4.63 (bs, 1H, H-3′ (*A*p)),

7.80 (s, 1H, H-2 (p*A*)), 7.82 (s, 1H, H-8 (p*A*)), 7.99 (s, 1H, H-8 (*A*p)), 8.02 (s, 1H, H-2 (*A*p)). ^{13}C NMR (125 MHz, D_2O): δ 35.80 (C-2′, *A*p), 36.13 (C-2′, p*A*), 46.93 (C-6′, p*A*), 47.07 (C-6′, *A*p), 61.53 (C-5′, *A*p), 65.52 (C-5′, p*A*), 72.25 (C-3′, p*A*), 76.75 (C-1′, *A*p and p*A*), 76.79 (C-3′, *A*p), 85.10 (C-4′, p*A*), 85.80 (C-4′, *A*p), 117.16 (C-5, p*A*), 117.24 (C-5, *A*p), 142.07 (C-8, p*A*), 142.11 (C-8, *A*p), 147.65 (C-4, p*A*), 147.71 (C-4, *A*p), 151.22 (C-2, p*A*), 151.72 (C-2, *A*p), 154.33 (C-6, p*A*), 154.64 (C-6, *A*p). FAB-MS: *m*/*z* 591 $(M-H)^-$. Anal. Calcd for $C_{22}H_{28}N_8PNa_2 \cdot 4H_2O$: C, 37.27; H, 5.11; N, 19.74. Found: C, 36.94; H, 5.02; N, 19.82.

Preparation of Homo-*N*-Nucleoside Phosphoramidite. 9-*N*-[5′-*O*-(4,4′-Dimethoxytrityl)-2′-deoxy-3′-*O*-[(2-cyanoethoxy)-*N*,*N*-diisopropylaminophosphino]-β-D-ribo-pentofuranosyl]methyl]-N^6-benzoyladenine **9** and 1-*N*-[5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxy-3′-*O*-[(2-cyanoethoxy)-*N*,*N*-diisopropylaminophosphino]-β-D-ribo-pentofuranosyl]methyl]thymine **10** were prepared from the corresponding homo-*N*-nucleosides as described by Boal et al.[14]

Compound **9** ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.00−1.35 (m, 14H), 1.80 (m, 1H), 2.15 (m, 1H), 2.42 (t, J = 6.3 Hz 1H), 2.59 (t, J = 6.3 Hz, 1H), 3.05 (m, 2H), 3.60 (m, 2H), 3.78 (s, 6H), 4.10 (m, 1H), 4.30 (m, 1H), 4.40 (m, 1H), 4.53 (m, 1H), 4.60 (m, 1H), 6.80 (m, 4H), 7.25 (m, 7H), 7.60 (m, 3H), 8.03 (d, J = 6.9 Hz, 2H), 8.11 (d, J = 3.0 Hz, 1H), 8.82 (s, 1H), 9.04 (bs, 1H). FAB-MS: *m*/*z* 872 $(M + H)^+$.

Compound **10** ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.00−1.30 (m, 14H), 1.74 (s, 3H), 1.80 (m, 1H), 2.10 (m, 2H), 2.42 (t, J = 6 Hz, 1H), 2.58 (t, J = 6 Hz, 1H), 3.12 (m, 2H), 3.60 (m, 2H), 3.78 (s, 6H), 4.10 (m, 2H), 4.15 (m, 1H), 4.40 (m, 2H), 6.80 (m, 4H), 7.08 (m, 1H), 7.25 (m, 7H), 7.40 (m, 2H), 7.90 (bs, 1H). FAB-MS: *m*/*z* 757 $(M-H)^-$.

Preparation of Homo-*N*-Nucleoside-Derivatized CPG Support. To a solution of 9-*N*-[(5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxy-D-ribo-pentofuranosyl)methyl]-N^6-benzoyladenine (470 mg, 0.70 mmol) in dichloromethane (3 mL) were added 4-(dimethylamino)pyridine (123 mg, 1.05 mmol) and succinic anhydride (105 mg, 1.05 mmol). This mixture was stirred at room temperature for 3 h, dichloromethane was added, and the reaction mixture was washed with 0.5 M KH_2PO_4 (2 × 10 mL) and water (10 mL), after which the solvent was removed under reduced pressure. The residue (509 mg) was dissolved in DMF (5 mL), pentachlorophenol (190 mg, 0.71 mmol) and *N*,*N*′-dicyclohexylcarbodiimide (200 mg, 0.97 mmol) were added, and the reaction mixture was stirred at room temperature for a further 20 h. The precipitate that formed was removed by filtration and discarded, and the filtrate, containing the product, was concentrated under reduced pressure. The concentrated filtrate was dissolved in dichloromethane (10 mL) and precipitated by adding the solution to *n*-hexane (150 mL). The precipitate was collected by centrifugation for 5 min at 2000 rpm and dried in a desiccator under reduced pressure to give the 3′-succinyl ester (492 mg, 75% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.98 (m, 1H, H-2′), 2.16 (m, 1H, H-2″), 2.75 (t, J = 6.3 Hz, 2H), 3.02 (t, J = 6.3 Hz, 2H), 3.15 (dd, J = 10.2, 4.2 Hz, 1H, H-5′), 3.27 (dd, J = 10.2, 3.6 Hz, 1H, H-5″), 3.78 (s, 6H, −OMe), 4.08 (m, 1H, H-4′), 4.27 (dd, J = 14.1, 7.2 Hz, 1H, H-6′), 4.50 (m, 1H, H-1′), 4.63 (dd, J = 14.1, 2.4 Hz, 1H, H-6″), 5.35 (m, 1H, H-3′), 6.81 (m, 4H), 7.25 (m, 7H), 7.39 (m, 2H), 7.58 (m, 3H), 8.03 (m, 2H), 8.07 (s, 1H, H-2), 8.82 (s, 1H, H-8). FAB-MS: *m*/*z* 1018 $(M + H)^+$.

LCAA-CPG solid support (2 g, 0.16 mmol), succinyl ester (412 mg, 0.40 mmol), and triethylamine (0.055 mL, 0.44 mmol) were added to anhydrous DMF (10 mL) in a round-bottom flask and shaken at room temperature for 3 days. Unreacted amino groups on the support were acetylated with acetic anhydride/pyridine (1:3 v/v), after which the support was filtered, washed with pyridine and dichloromethane, and dried under reduced pressure. The nucleoside loading was determined by trityl analysis to be 39.5 μmol/g support.

Synthesis of Homo-*N*-nucleoside-containing Oligodeoxyribonucleotides. Oligodeoxyribonucleotides containing homo-*N*-nucleosides were synthesized on a 1-μmol scale by the cyanoethyl phosphoramidite method on an Applied Biosystems 380B solid-phase DNA synthesizer as described by Sinha et al.[28] Fully protected oligonucleotides containing a 5′-terminal DMTr group were deprotected in concentrated ammonium hydroxide at 55 °C overnight. The crude oligomers were purified by reverse-phase HPLC (ODS, 10 × 100 mm^2; Waters) at a flow rate of 2 mL/min with a linear gradient from 0 to 35% acetonitrile in 0.1 M triethylammonium acetate, pH 7.0, over 200 mL. The main UV-absorbing fractions were combined and concentrated under reduced pressure, and the terminal DMTr group was removed by treatment with 80% acetic acid at room temperature for 30 min. After removal of the acid under reduced pressure, the residue was dissolved in water (10 mL) and washed with ethyl acetate (2 × 10 mL), and the aqueous solution was concentrated to give the oligodeoxyribonucleotide product.

Hyperchromicity. The UV spectrum for each dimer (final concentration, 0.05 mM) was recorded in the presence of 10 mM sodium phosphate, pH 7.0, and 0.15 M NaCl in a total volume of 3 mL. Then 5 M HCl (60 μL) was added to the solution, and the spectrum was rerecorded. The percentage increase in absorbance at 260 nm upon acidification was calculated for each dimer, taking into account the dilution factor of 1.02. We also took the spectrum of each of the corresponding nucleosides A, *A*, dA, and d*A* at pH 7.0 and 1.0 to allow for changes in the absorbance of the bases as a function of pH. Each nucleoside displayed a decrease of ∼5% in A_{260} upon acidification (and also a small decrease in λ_{max} of ∼2.3 nm; data not shown). These absorbance decreases were assumed to have canceled part of the hyperchromic effect displayed by the dimers, so the absorbance increase observed upon acidification was corrected by the appropriate factor to obtain the hyperchromicity value.

Mixing Curves. Poly(dT) and dA_2 or dA_2 were mixed in various proportions to maintain a constant base concentration. Each nucleotide mixture contained, in a total volume of 3 mL, nucleotide dimer and/or polymer (total base-concentration, 0.1 mM), 1 M sodium chloride, and 10 mM sodium phosphate, pH 7.0. The mixture was scanned at 0 °C from 300 to 205 nm, and from each spectrum, A_{260} was determined and plotted against the proportion of poly(dT).

Melting Curves. Melting curves (absorbance−temperature profiles) were recorded in the presence of 10 mM sodium phosphate, pH 7.0, containing various concentrations of NaCl. Each nucleotide species was used at a base concentration of 0.05 mM to give a total base concentration of 0.1 mM. The absorbance was monitored at 260 nm as the temperature was increased from 0 °C at a rate of 0.5 °C/min. Before each melting curve was recorded, the nucleotide mixture was cooled through the melting transition to the starting temperature at a rate of not more than 1 °C/min. T_m values were determined as the temperature corresponding to half of the total absorbance increase, and the sharpness of the melting transition was characterized by the temperature range over which the absorbance went from 10 to 90% of the total increase ($T_{90} - T_{10}$).

CD Spectroscopy. The spectropolarimeter was calibrated with (+)-10-camphorsulfonic acid. Spectra were recorded in a 1-cm path length quartz cell at a speed of 50 nm/min, and the recorded spectra were digitized at 0.1-nm resolution and stored on a floppy disk. The bandwidth was 1 nm, and the response time, 0.25 s. For each determination, at least five spectra were accumulated, and the averaged spectrum was subjected to noise reduction.

NMR Spectroscopy (NOESY). Homo-*N*-deoxydiadenosine **8** was dissolved in D_2O containing 10 mM phosphate buffer and 0.15 M NaCl (the NaH_2PO_4/Na_2HPO_4 ratio was calculated to give pH 7.0 in H_2O and was not corrected for D_2O). NOESY spectra were acquired at 4 °C with suppression of the residual solvent peak by presaturation. Spectra were taken at mixing times of 0.2, 0.5, and 1 s, and the spectral width was 4006 Hz, with 2048 data points in t2, 128 increments in t1, and 16 scans per increment. The interresidue cross-peaks at the different mixing times were essentially the same. Data were zero-filled to 1024

(28) Sinha, N. D.; Biernat, J.; McManus, J.; Köster, H. *Nucleic Acids Res.* **1984**, *12*, 4539−4557.

data points in t1, apodized with Gaussian line-broadening and exponential line-narrowing, and analyzed with XWIN NMR software (Bruker).

Atomic Force Microscopy (AFM). Samples (250 μg/mL) were diluted with phosphate-buffered saline (PBS) to 0.5−1 μg/mL, made 10 mM in $MgCl_2$, and, after 5 min, anchored to a mica substrate, rinsed with 5 × 1 mL PBS, and dried in a vacuum oven at 40 °C for 10 min. AFM was carried out with a NanoScope IIIa multimode system (Digital Instruments, Santa Barbara, California) operated in the tapping mode in air at room temperature. The Super Sharp Silicon cantilevers (Nanosensors, Wetzlar-Blankenfeld, Germany) were 119 μm long with a spring constant of 36 Nm^{-1}. Typical resonant frequencies of the tip were 345 kHz. After an initial scan of a 1 μm × 1 μm area, the parameters were optimized for a scan of an 80 nm × 80 nm area, and 256 × 256 pixel images were collected at a rate of one scan line per second.

HPLC. Reverse-phase HPLC for enzyme-digestion experiments was carried out with a C_{18} Merck 50734 LiChrospher 100 RP-18 endcapped stainless steel cartridge (4 × 125 mm) maintained at 35 °C in a Shimadzu CTO-6A column oven. The HPLC buffer stock solution was 1 M triethylammonium acetate (TEAA), pH 7.0 (1 M in triethylamine, titrated to pH 7.0 with acetic acid), and the HPLC buffers were prepared by mixing the appropriate amounts of 1 M TEAA, pH 7.0, and acetonitrile with distilled deionized water. The column was operated at a flow rate of 1 mL/min, and the effluent was monitored at 260 nm.

Nuclease Digestion. Snake venom phosphodiesterase: Each oligomer was incubated at 37 °C in a reaction mixture containing 0.1 M Tris·HCl, pH 8.9, 0.1 M NaCl, and 14 mM $MgCl_2$ and either 0.036 U or 0.36 U of enzyme.[29] The final A_{260} for each oligomer was 4.0, and the final reaction volume was 0.1 mL. After 1 h, the reaction was quenched by the addition of ethylenediaminetetraacetic acid (EDTA; final concentration, 50 mM). For a zero time point, EDTA was added before the enzyme. A sample of each reaction mixture (20 μL) was analyzed by reverse-phase HPLC.

Calf spleen phosphodiesterase: Each oligomer was incubated at 37 °C in a reaction mixture containing 0.1 M ammonium succinate, pH 5.9, 1 mM EDTA, and either 0.001 U or 0.02 U of enzyme. The final A_{260} for each oligomer was 4.0, and the final reaction volume was 0.1 mL. The reaction was quenched by immersing the reaction tube in boiling water for at least 4 min, and a sample of each reaction mixture (20 μL) was subjected to reverse-phase HPLC.

Nuclease S1: Nuclease S1 digestion was carried out in 33 mM sodium acetate, pH 4.5, containing 50 mM NaCl and 0.03 mM $ZnSO_4$. The reaction mixture (50 μL), which contained 25-mer (final A_{260}, 4.0) and 0.1, 1.0, or 100 U of enzyme, or no enzyme, was incubated for 24 h at 37 °C. A sample of each reaction mixture (20 μL) was subjected to reverse-phase HPLC.

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(29) Higuchi, H.; Endo, T.; Kaji, A. *Biochemistry* **1990**, *29*, 8747−8753.