Hybridization properties of oligoarabinonucleotides

PAUL APOSTOLOS GIANNARIS AND MASAD JOSÉ DAMHA[1]

Department of Chemistry, McGill University, Montreal, QC H3A 2K6, Canada

Received September 8, 1993

This paper is dedicated to Professor John C. Polanyi on the occasion of his 65th birthday

PAUL APOSTOLOS GIANNARIS and MASAD JOSÉ DAMHA. Can. J. Chem. **72**, 909 (1994).

3′-Phosphoramidite derivatives of arabinocytidine, arabinoadenosine, and arabinouridine were prepared and used for the solid-phase synthesis of oligoarabinonucleotides (arabinonucleic acid, or ANA). Thermal denaturation analysis and gel mobility shift assays were used to investigate the interaction between ANA and complementary DNA and RNA. In general, the ANA/DNA and ANA/RNA complexes exhibited melting temperatures comparable to those of the corresponding DNA/DNA and DNA/RNA complexes. Thus the inversion of stereochemistry at the C2′ of ribonucleotides does not have a negative effect on interaction with natural sequences. In fact, in complexes with poly dT, oligoarabinoadenylates displayed greater hybridization affinity than oligoriboadenylates. In summary, we observed that (*i*) ara(Ap)$_7$A interacted favourably with poly rU and poly dT; (*ii*) ara(Cp)$_7$C formed a stable complex with poly rG; (*iii*) ara(Up)$_7$U did not bind with complementary poly rA; and (*iv*) the mixed oligoarabinonucleotide ara(UCU UCC CUC UCC C) formed complexes with complementary DNA and RNA. Hybridization was observed between the phosphorothioatelinked arabinoadenylate ara(Aps)$_7$A and poly rU and poly dT; however, this binding was weaker than that between the phosphorothioate-linked deoxyriboadenylate d(Aps)$_7$A and poly rU and poly dT. Both ara(Aps)$_7$A and its deoxy analog d(Aps)$_7$A displayed significant and similar resistance to digestion by snake venom phosphodiesterase.

PAUL APOSTOLOS GIANNARIS et MASAD JOSÉ DAMHA. Can. J. Chem. **72**, 909 (1994).

On a effectué la synthèse des dérivés 3′-phosphoramidite de l'arabinocytidine, de l'arabinoadénosine et de l'arabinouridine et on les a utilisés pour la synthèse en phase solide d'oligoarabinonucléotides (acide arabinonucléique, AAN). On a utilisé une analyse de dénaturation thermique et des essais de déplacements par mobilité sur gel pour étudier les interactions entre l'AAN et des ADN et ARN complémentaires. En général, les complexes AAN/ADN et AAN/ARN présentent des températures de fusion semblables à celles des complexes correspondants ADN/ADN et ADN/ARN. L'inversion de stéréochimie au niveau C2′ des ribonucléotides n'a donc pas d'effet négatif sur l'interaction avec les séquences naturelles. En fait, dans les complexes contenant des poly dT, les oligoarabinoadénylates présentent une affinité d'hybridation plus grande que celle des oligoriboadénylates. En résumé, on a observé que (*i*) l'ara(Ap)$_7$ interagit favorablement avec les poly rU et poly dT; (*ii*) que l'ara(Cp)$_7$C forme un complexe stable avec le poly rG; (*iii*) que l'ara(Up)$_7$U ne se lie pas avec le poly rA complémentaire et (*iv*) que l'oligoarabinonucléotide mixte ara(UCU UCC CUC UCC C) forme des complexes avec les ADN et ARN complémentaires. On a observé une hybridation entre l'arabinoadénylate ara(Aps)$_7$A lié au phosphorothioate et les poly rU et poly dT; toutefois, cette interaction est plus faible que celle entre le désoxyriboadénylate d(Aps)$_7$A lié au phosphorothioate et les poly rU et poly dT. L'ara(Aps)$_7$ ainsi que son analogue désoxy, d(Aps)$_7$A, présentent tous les deux une résistance importante et semblable à la digestion par la phosphodiestéase du venin de serpent.

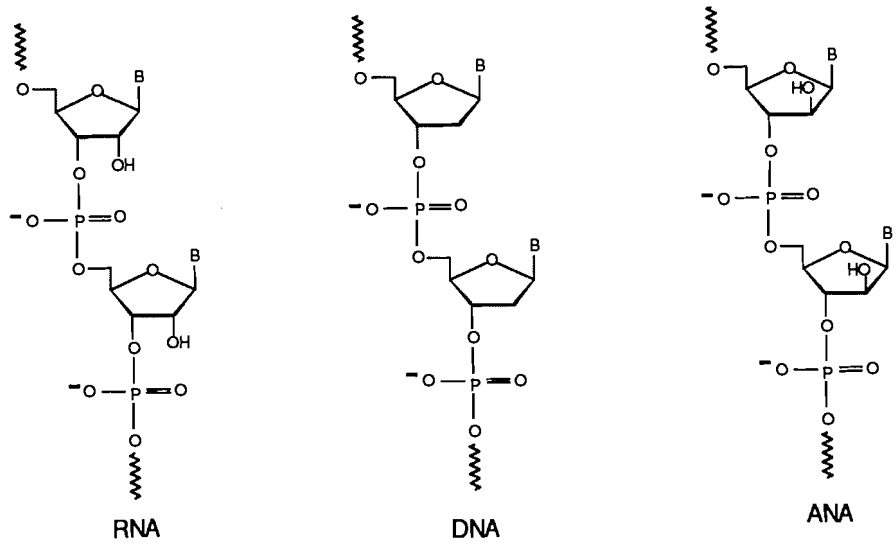
[Traduit par la rédaction]

Introduction

The antisense approach to gene regulation requires oligonucleotides that exhibit stability against degradation by cellular enzymes and the ability to hybridize complementary DNA or RNA targets with good affinity and specificity. Short oligodeoxyribonucleotides can function as antisense oligomers (1) although their utility is limited by nucleolytic degradation in vivo (2). Various modified oligomers including those bearing modified phosphodiester linkages (3), non-phosphate linkages (4), and 2′,5′- rather than 3′-5′-phosphodiester linkages (5) have been designed in an attempt to increase nuclease resistance without severely hindering the ability to hybridize target DNA or RNA (for reviews of this area, see ref. 6). In addition, oligomers incorporating modified nucleoside units including α-nucleosides (7), L-deoxyribo and L-ribonucleosides (8, 9), glyceronucleosides (10), 4′-thionucleosides (11), carbocyclic nucleosides (12), and 2′-O-alkyl nucleosides (13) have also been explored as possible antisense agents.

Oligonucleotides containing the sugar D-arabinose (arabinonucleic acids, or ANA) (Scheme 1) have not been investigated as antisense agents, except for one reported example of an oligomer of 1-α-D-arabinofuranosyl thymidine (14). This may be due in part to the inherent difficulties in ANA synthesis, which, as in RNA synthesis (15), necessitates the differential chemical protection of the 2′ and 3′ hydroxyl groups. In the original work by Wechter, oligoarabinonucleotide synthesis was carried out without 2′-OH protection, resulting in mixtures of 2′,5′- and 3′,5′-linked nucleotides (16). There had been two other major strategies in the synthesis of 3′,5′-linked arabinonucleotides. The first and most widely used strategy makes use of 5′,2′-O-protected arabinonucleotides as precursors, which upon phosphorylation of the remaining 3′-hydroxyl function affords a suitable building block for the synthesis of arabinonucleotides (17–19). The problem with this approach is that of selective protection of the 2′-hydroxyl function, a very difficult requirement because of its orientation towards the hindered β face of the sugar. In practice, this is accomplished by first masking the 5′- and 3′-OH groups with a disiloxane bridging group, then protecting the 2′-hydroxyl, and finally deblocking the disiloxane function so that 5′-trityl and 3′-phosphate moieties can be introduced (17, 18). The second strategy is limited to pyrimidine arabinonucleotides, as they require formation of O^2-2′-anhy-

[1]Author to whom correspondence may be addressed. Tel: (514) 398-7552. Fax: (514) 398-3797. E-Mail: Damha@omc.lan.mcgill.ca.



SCHEME 1

dronucleoside synthons, and this strategy is therefore rarely used (20). We thought that the best way to deal with the 2′-OH group was to block it only *after* the desired moieties of 5′- and 3′-positions had been introduced. This was readily achieved by sequential and regioselective tritylation (at 5′), phosphoramidation (at 3′), and acetylation (at 2′) of arabinonucleosides. The resulting 5′-tritylated arabinonucleoside 2′-*O*-acetyl-3′-*O*-phosphoramidites proved to be very useful synthons for the solid-phase synthesis of short arabinoadenylates and uridylates (21). Pfleiderer and co-workers have also made useful advances in using solid-phase phosphoramidite chemistry to make an all-β-D-arabinose "transfer ANA" molecule (22).

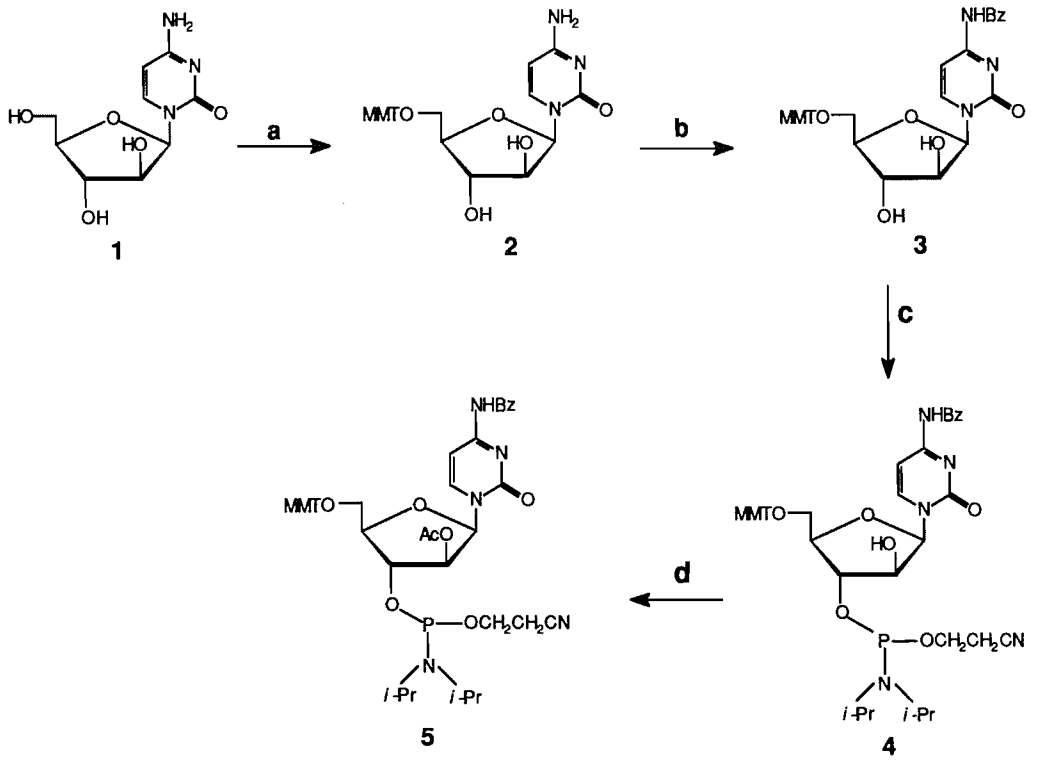
Arabinonucleosides have found application as antiviral (23) and antitumor (24) agents and, in particular, arabinofuranosyl cytosine (araC) has been found to be very effective in the treatment of leukemia (25). Although very little is known about the mechanism of action, its activity appears to be associated with its misincorporation into DNA (26). Mikita and Beardsley reported that the presence of araC at the 3′-terminus of a DNA primer strand results in a reduction in the rate of addition of the next nucleotide unit in vitro (27). Similarly, a single internucleotide araC insertion into an oligomer serving as the template strand hindered the function of certain polymerases, affecting, as a result, the process of replication in vitro (27). Whether these observations reflect differences in the structure of the complexes formed by normal DNA and those formed by oligomers containing araC is not clear. X-ray diffraction analysis of the self-complementary DNA oligomer d(CCAGGCaraCTGG) demonstrated that this oligomer forms a duplex with B-DNA conformation with only small conformational perturbations (28). However, molecular modeling studies have indicated that serious steric clashes may occur between the 2′-OH and neighboring atoms when araC is placed into the RNA strand of a DNA/RNA duplex (27). Altona and co-workers prepared self-complementary d(CGaraCTAGCG) to investigate the structural implications of an araC insertion into DNA by NMR analysis (29–31). They discovered that under certain conditions this oligomer exclusively adopts a hairpin structure whereas the unmodified DNA adopts a B-BNA type duplex. AraC incorporation into an alternating dC-dG sequence d(CGaraCGCG) has been shown to facilitate the B to Z conversion (32). Very little is known about the thermal stability of DNA chimeras containing 1-β-D-arabinonucleosides. A self-complementary DNA oligomer containing a single araC insert exhibited a somewhat lower melting temperature (T_m) (2°C/araC substitution) than the unmodified DNA (18) and no interaction was observed between a short oligomer of araU and poly rA (20*a*). In this work we prepared a series of 1-β-D-arabinonucleic acids and show, for the first time, that these oligomers can form stable complexes with complementary single-stranded DNA and RNA.

Results and discussion

Preparation of N^4*-benzoyl-5′-O-monomethoxytritylarabinocytidine-2′-O-acetyl-3′-O-N,N-diisopropyl-β-cyanoethylphosphoramidite* **5**

The title compound was prepared in good yield by acetylation of the 2′-OH *after* protection of the 4-amino and 5′- and 3′-hydroxyl groups (Scheme 2). Thus, 1-β-D-arabinofuranosylcytosine was first protected at the 5′-OH (monomethoxytrityl) and N^4 (benzoyl) positions, affording **3** in 60% combined yield. The protected arabinocytidine **3** was then phosphitylated with *N,N*-diisopropyl-β-cyanoethylphosphonamidic chloride, resulting in mainly **4** (77% yield). The free 2′-OH of **3** also reacted with the phophitylating reagent, although to a much lesser extent as indicated by additional very faint spots in the TLC plate. Compound **4** was produced as a pair of diastereoisomers that could be easily separated by silica gel column chromatography. The position of the phosphoramidite moiety on **4** was established by ^{13}C NMR. The spectrum clearly shows that the C4′, C3′, and C2′ resonances are coupled to phosphorus whereas that of C1′ is not, the two-bond $J(^{13}C3'–^{31}P)$ value being the greatest (Fig. 1). Furthermore, the greatest downfield shift in ^{13}C resonance of the arabinose sugar of **4** relative to the unphosphitylated derivative **3** was observed for C3′, identifying it as the carbon where the phosphoramidite moiety is attached. Subsequent 2′-O-acetylation of one diastereoisomer of **4** afforded the key activated monomer **5** in 87% yield.

Compound **5** was also obtained in a separate synthesis from ribocytidine. The key step in this approach was the inversion of the C2′-OH by strong heating with diphenyl carbonate – water in DMF (33) (Scheme 3). Under the reaction conditions, the initial 2′,3′-cyclic carbonate intermediate **7** reacts intramolecularly, forming 2,2′-cycloanhydride **8** which is subsequently



SCHEME 2

(a) MMT-Cl/pyridine, 22 h (79%); (b) (i) trimethylsilyl chloride/pyridine, 30 min; (ii) benzoyl chloride/pyridine, 2 h ; (iii) 29% ammonia, water, 0°C, 25 min (73%); (c) $(iPr_2N)P(OCH_2CH_2CN)Cl$/diisopropylethylamine/THF, 90 min (77%); (d) acetic anhydride/DMAP/diisopropylethylamine, 45 min (87%).

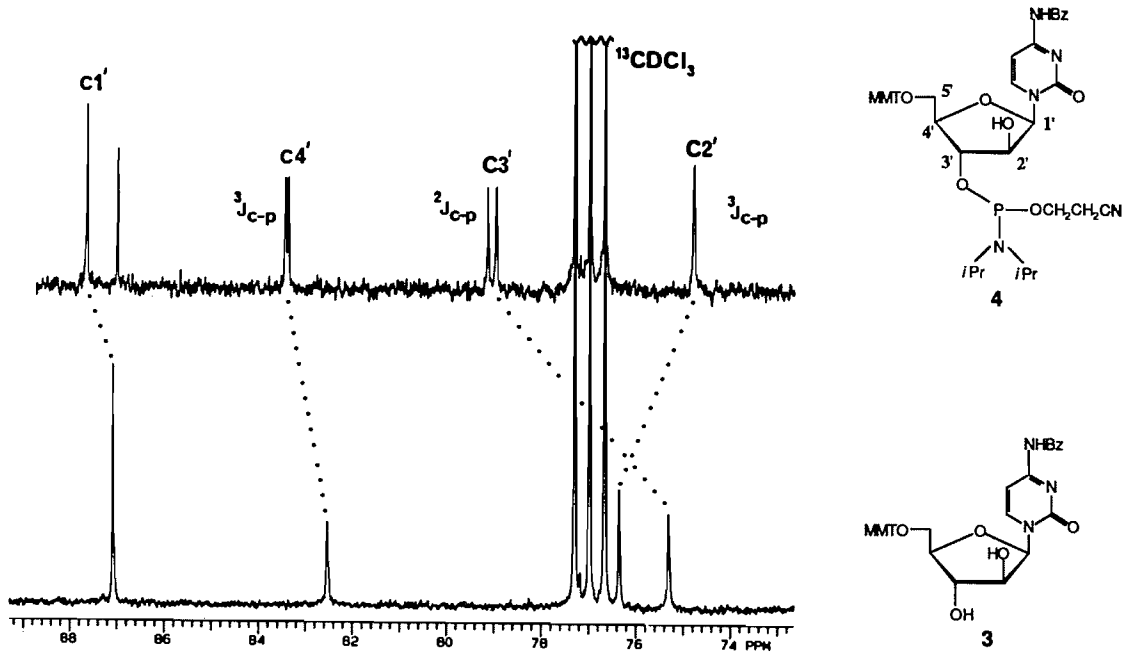
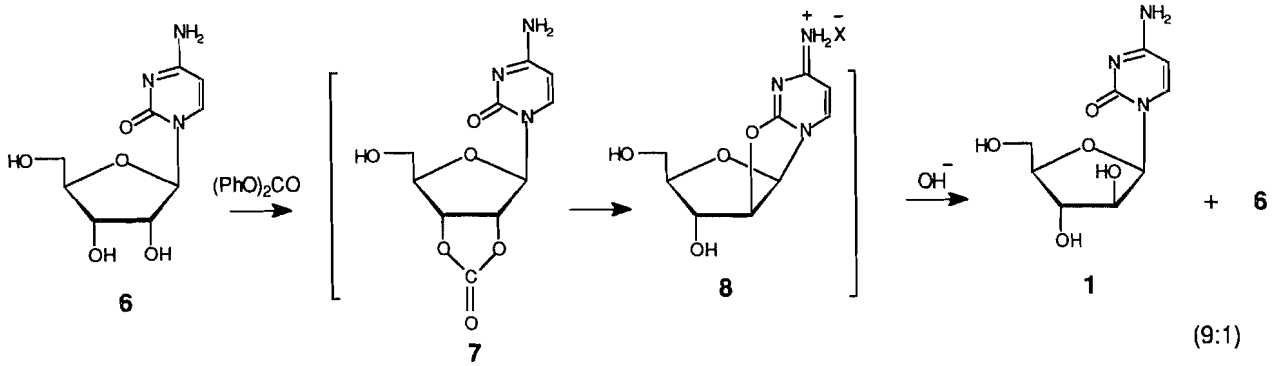


FIG. 1. ^{13}C NMR (DEPT, $CDCl_3$) spectra of arabinocytidine phosphoramidite **4** (one diastereoisomer) and arabinonucleoside **3**.

opened, producing a mixture of arabinocytidine and the starting material in a ratio of 9:1 (TLC, ethanol–water 1:1). These 2′ epimers could not be separated at this stage. They were, however, fully separated by silica gel chromatography after partial protection of the sugar (5′-MMT) and base (4-NH-Bz) moieties. Subsequent phosphitylation and acetylation of **3** afforded **5** in 65% combined yield.

Synthesis and characterization of oligoarabinonucleotides

Oligoarabinonucleotides were synthesized with an Applied Biosystems DNA/RNA synthesizer (model 381A) using amidite **5**, and analogous araU and araA 3′-*O*-(cyanoethyl)phosphoramidites (20). Oligomers were prepared on a 1.0 μmol scale employing "trityl on" synthesis cycle (60 s coupling time) and long-chain alkylamine controlled-pore glass (LCAA-CPG)



SCHEME 3

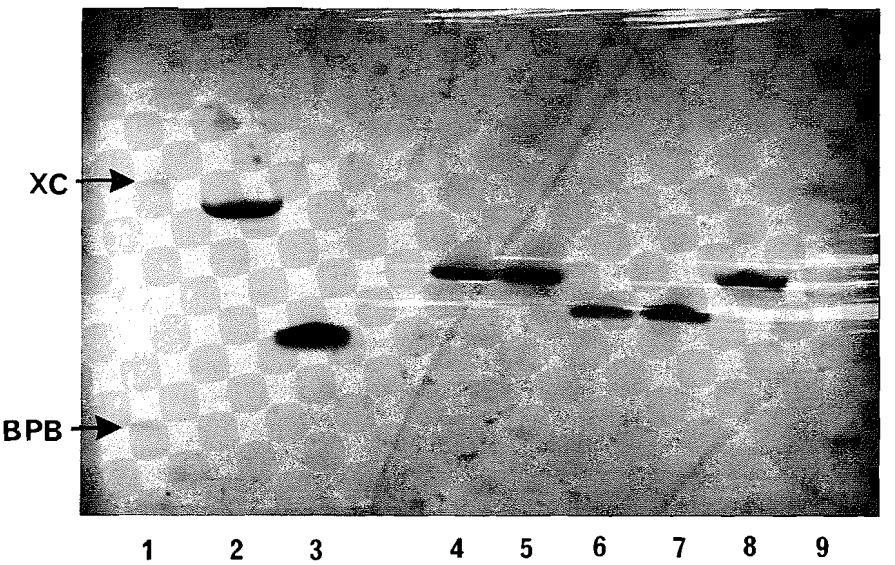


FIG. 2. Polyacrylamide gel electrophoresis (16%, denaturing) of oligoarabinonucleotides. Lanes: (1) xylene cyanol and bromophenol blue; (2) $d(Tp)_{14}T$; (3) $d(Tp)_4T$; (4) crude $ara(Cp)_9C$; (5) pure $ara(Cp)_9C$; (6) crude $ara(Cp)_7C$; (7) pure $ara(Cp)_7C$; (8) $ara(Cp)_9C$ treated with 0.1 N KOH for 24 h; (9) xylene cyanol and bromophenol blue.

bearing 3′-terminal arabinonucleosides. The coupling efficiency was routinely greater than 98% as monitored by the release of the MMT cation. RNA (34) and DNA (35) oligomers were prepared by standard means. Deprotection of sugar and phosphate moieties with concomitant release of oligomers from the glass support was accomplished by a single ammonia treatment (29% NH_4OH, 55°C, 8 h). The terminal trityl group was cleaved with trifluoroacetic acid during purification with a reversed phase OPC® cartridge (Applied Biosystem), and in the case of araC oligomers it was removed prior to purification by polyacrylamide gel electrophoresis (PAGE). Purity of ANA oligomers was verified by analytical gel electrophoresis and was determined to be greater than 95% (Fig. 2). No other bands that would indicate poor monomer coupling or branch formation were detected (21). The ANA, DNA, and RNA sequences prepared for this study are shown in Table 1.

The integrity of the oligoarabinonucleotides was established by chemical and enzymatic degradation with subsequent analysis of the nucleotide–nucleoside mixtures by polyacrylamide gel electrophoresis (PAGE). Oligoarabinonucleotides are expected to be resistant to base conditions since the *trans* relationship of the 2′-OH with respect to the 3′,5′-phosphodiester linkage prevents participation of the 2′-OH in intranucleotide cleavage. This stability has been previously observed for 3′,5′-linked dinu-

TABLE 1. Oligonucleotides prepared for binding studies

Oligonucleotide sequences	Designation
Normal oligomers	
5′-r(AAA AAA AA)	$r(Ap)_7A$
5′-r(UUU UUU UU)	$r(Up)_7U$
5′-r(CCC CCC CC)	$r(Cp)_7C$
5′-r(CCC CCC CCC CCC CC)	$r(Cp)_{14}C$
5′-d(AAA AAA AA)	$d(Ap)_7A$
5′-d(TTT TTT TT)	$d(Tp)_7T$
5′-d(CCC CCC CC)	$d(Cp)_7C$
5′-r(CCC UCU CCC UUC U)	r(U/C)-13-mer
5′-r(AGA AGG GAG AGG G)	r(A/G)-13-mer
5′-d(AGA AGG GAG AGG G)	d(A/G)-13-mer
Oligoarabinonucleotides	
5′-ara(AAA AAA AA)	$ara(Ap)_7A$
5′-ara(UUU UUU UU)	$ara(Up)_7U$
5′-ara(CCC CCC CC)	$ara(Cp)_7C$
5′-ara(CCC UCU CCC UUC U)	ara(U/C)-13-mer
Phosphorothioates	
5′-d(AsAsAs AsAsAs AsAs)	$d(Aps)_7A$
5′-ara(AsAsAs AsAsAs AsAs)	$ara(Aps)_7A$

TABLE 2. Binding of oligoarbinonucleotides with complementary DNA and RNA homopolymers[a]

Complex	T_m (%H) 100 mM NaCl	T_m (%H) 1.0 M NaCl
poly rU/ribo(Ap)$_7$A	23.0 (43)	57.6 (43)
ara(Ap)$_7$A	24.5 (25)	50.7 (28)
d(Ap)$_7$A	21.8 (29)	57.5 (30)
d(Aps)$_7$A	19.7 (29)	55.0 (30)
ara(Aps)$_7$A	12.1 (34)	44.1 (36)
poly dT/ribo(Ap)$_7$A	14.8 (25)	28.0 (38)
ara(Ap)$_7$A	25.5 (36)	52.6 (35)
d(Ap)$_7$A	27.0 (30)	65.0 (35)
d(Aps)$_7$A	19.6 (30)	57.7 (35)
ara(Aps)$_7$A	12.2 (12)	44.7 (35)
poly rA/ribo(Up)$_7$U		23.0 (30)
ara(Up)$_7$U		—
d(Up)$_7$U		25.0 (20)

[a]Equimolar mixtures on a per base basis were used. T_m's are ±0.5°C. Buffer: 10 mM sodium phosphate with [NaCl] as indicated. —, not observed. Hyperchromicity values (%H) are presented in parentheses relative to the initial temperature reading.

cleoside monophosphates of arabinocytidine (16), arabinouridine and arabinoadenosine (21), as well as for the trinucleotide ara-(ApApA) (17). The oligoarabinonucleotides prepared in this study were also found to be completely stable to treatment with 0.1 N KOH for 24 h (Fig. 2, compare lanes 2 and 4 with 7), whereas the corresponding oligoribonucleotides were completely degraded under these conditions (data not shown).

The arabinonucleotides displayed some stability against cleavage by snake venom phosphodiesterase (SVPDE). For example, only 5–10% hydrolysis of ara(Ap)$_7$A, ara(Cp)$_7$C, and ara(Up)$_7$U was detected during incubation of these oligomers with SVPDE at 37°C for 45 min, whereas oligoribonucleotides were completely degraded within 5 min under the same conditions (PAGE). The oligoarabinonucleotides, however, did not exhibit any significant stability, relative to ribonucleotides, when incubated with other nucleases. For example, treatment with the endonucleases Nuclease P1 and Ribonuclease S1, or the 5′-exonuclease calf spleen phosphodiesterase, resulted in degradation of both ara and ribo oligomers within 45 min at 37°C. Digestion with spleen phosphodiesterase served also to establish a 3′,5′-phosphodiester connectivity within the oligoarabinonucleotides, since 2′,5′-linked arabinonucleotides (16) and 2′-5′-linked oligoribonucleotides (5, 16) are not hydrolyzed by this enzyme.

The phosphorothioate-linked oligomers ara(Aps)$_7$A and d(Aps)$_7$A were characterized by incubation with SVPDE at 37°C for 16 h followed by analysis of the digest by HPLC. Under these conditions, less than 20% of ara(Aps)$_7$A and d(Aps)$_7$A was degraded whereas phosphodiester-linked d(Ap)$_7$A was completely degraded within 10 min. The increased stability of the phosphorothioate-linked oligomers d(Aps)$_7$A and ara(Aps)$_7$A, relative to d(Ap)$_7$A, is consistent with related work that shows that phosphorothioate-linked DNA (36) and phosphorothioate linked RNA (37) are more resistant to nuclease activity than normal DNA and RNA strands.

Interaction of oligoarabinonucleotides with complementary DNA and RNA

In the presence of poly dT and 100 mM NaCl, the oligoarabinoadenylate ara(Ap)$_7$A exhibits a melting temperature approximately equal to, or significantly higher than, the corresponding natural oligomers d(Ap)$_7$A and r(Ap)$_7$A (Table 2, Fig. 3). In addition, at 100 mM NaCl, the T_m of the complex formed between ara(Ap)$_7$A and poly rU was marginally higher than those values observed for the complexes d(Ap)$_7$A):(poly rU) and r(Ap)$_7$A):(poly rU) (Table 2, Fig. 3). The stoichiometry of complexes of ara(Ap)$_7$A and r(Ap)$_7$A with poly rU was determined in each case to be 2U:1A (triplex) from UV mixing curves (1 M NaCl at 20°C) (38).[2] All these results suggest that *the inversion of stereochemistry at the C2′ of ribonucleotides usually does not have a strong positive or negative effect on interaction with natural sequences, and that the 2′-OH group of ANA does not usually hinder the formation of ANA/DNA and ANA/RNA complexes.* In fact, the significantly higher T_m observed for complexes ara(Ap)$_7$A:poly dT and ara(Ap)$_7$A:poly rU relative to complexes r(Ap)$_7$A:poly dT and r(Ap)$_7$A:poly rU indicates that the 2′-OH of ANA exerts, in this case, a stabilizing effect.[3] Oligoribonucleotides containing modifications at the 2′-position, including 2′-*O*-methyl (13, 39), 2′-fluoro (40), 2′-*O*-allyl (41), 2′-*O*-ethyl (42), and 2′-*O*-propyl (42), generally exhibit higher T_m than their respective unmodified oligomers in the interaction with complementary RNA. This being the case, it would be of interest to determine whether the stabilizing effect exerted by 2′-substituents in ribonucleotides will also be evident for 2′-O-modified arabinonucleotides.

To study the effect of base sequence on complex stability, the interaction of oligoarabinonucleotide ara(5′-CCC UCU CCC UUC U-3′) (abbreviated ara(U/C)-13-mer) with complementary RNA (r(A/G)-13-mer) and DNA (d(A/G)-13-mer) was

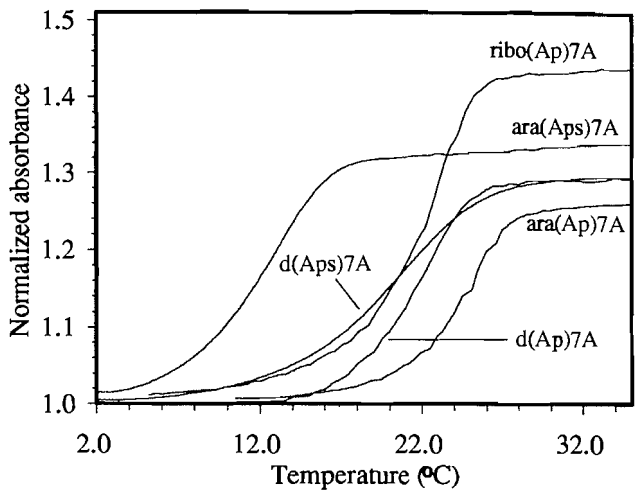


FIG. 3. Thermal denaturations of poly rU:oligomer complexes. Equimolar mixtures (per residue basis) of poly rU and each oligomer indicated below were prepared and denatured in 100 mM NaCl, 10 mM Na_2HPO_4, pH 7.0. Thermal denaturation curves for the mixtures of poly rU with phosphodiester-linked oligomers r(Ap)$_7$A, d(Ap)$_7$A, and ara(Ap)$_7$A as well as phosphorothioate-linked oligomers ara(Aps)$_7$A and d(Aps)$_7$A are shown normalized to their respective first temperature reading. T_m and %H values for these melts are tabulated in Table 2.

[2]1Py:1Pu mixtures were used in the hybridization assays of ara(Ap)$_7$A and poly dT and polyrU. We later determined that poly rU interacts with ara(Ap)$_7$A with a 2U:1A stoichiometry. We have also observed that mixtures of 1Py:1Pu and 2Py:1Pu (Pu = adenylates and Py = poly rU) give the same T_m values within experimental error.

[3]We make this statement with caution since ΔT_m may not reflect $\Delta\Delta G^0$.

TABLE 3. Binding of ara(U/C)-13-mer and ara(Cp)$_7$C

Oligonucleotide complex	T_m (%H)[a] CsCl 50 mM	T_m (%H) NaCl 200 mM
r(AG)-13-mer/r(U/C)-13-mer	65 (18)	75 (16)
/ara(U/C)-13-mer	48 (14)	53 (12)
/d(T/C)-13-mer	55 (16)	64 (15)
d(AG)-13-mer/r(U/C)-13-mer	41 (13)	50 (13)
/ara(U/C)-13-mer	11 (8)	18 (5)
/d(T/C)-13-mer	48 (16)	53 (16)
poly rG/ribo(Cp)$_7$C	73.2 (10)	77.5 (15)
ara(Cp)$_7$C	58.1 (4)	57.0 (5)
d(Cp)$_7$C	64.1 (4)	64.5 (5)
ara(Cp)$_{14}$C	85.0 (6)	83.4 (6)

[a] T_m are ±0.5°C for melts with poly rG, and ±1.0°C for all other melts (due to broad transitions). Hyperchromicity values (%H) are presented in parentheses relative to the absorbance reading at 20°C. Buffer: 10 mM sodium phosphate pH 7 with [CsCl] or [NaCl] as indicated. Equimolar mixtures (2.5 μmol in each strand) were used.

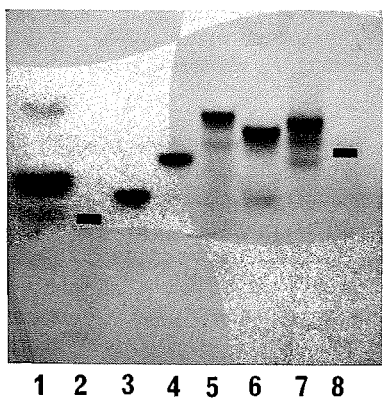


FIG. 4. Mobility shift assay of ara(U/C)-13-mer complex with RNA on non-denaturing PAGE (24%). Mixtures of approximate equimolar strand concentrations (2.0 μmol in each strand) in lanes 5–7. Single strands (ss) are shown in lanes 2–4, and 8. Lanes: (1) xylene cyanol and bromophenol blue; (2) ss r(U/C)-13mer; (3) ss d(T/C)-13-mer; (4) ss ara(U/C)-13-mer; (5) (r(U/C)-13-mer):(r(A/G)-13-mer); (6) (d(T/C)-13-mer):(r(A/G)-13-mer); (7) (ara(U/C)-13-mer):(r(A/G)-13-mer); (8) ss r(A/G)-13-mer. The mobility of the bands in lanes 2 and 8 have been highlighted for clarity. T_m values for these complexes are listed in Table 3.

investigated by UV thermal denaturation analysis (Table 3). All of the complexes listed in Table 3 showed similar broad cooperative transitions at 260 nm indicative of complex formation while the single strands, run on their own, exhibited only linear increases in absorbance.[4] In 200 mM NaCl, the ANA/RNA complex ara(U/C)-13-mer):(r(A/G)-13-mer) exhibited a T_m of 53°C, while the melting temperatures of the analogous RNA/RNA (T_m = 75°C) and DNA/RNA (T_m = 64°C) complexes were significantly higher. A mobility shift PAGE assay was used to confirm the formation of these complexes. Figure 4 clearly

[4] The only exception is r(A/G)-13-mer, which self-associates (ref. 5). The self-association of this oligomer can be largely eliminated by heating the sample to 90°C for 30 min before measurements. Longer heating times are not recommended as this may result in significant degradation.

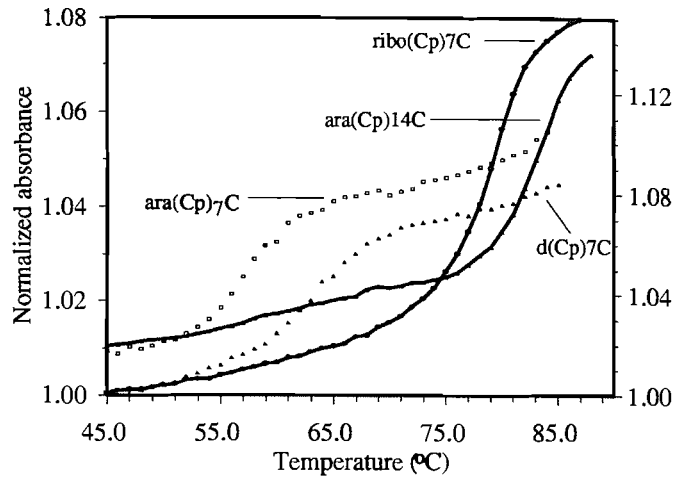


FIG. 5. Thermal denaturations for (oligomer):(poly rG) complexes in 200 mM NaCl, 10 mM Na_2HPO_4, pH 7.0. Curves for the interaction of r(Ap)$_7$A, d(Cp)$_7$C, ara(Cp)$_7$C, and ara(Cp)$_{14}$C are shown normalized to their respective absorbance readings at 20°C. Curves for complexes (poly rG):(ara(Cp)$_7$C) and (poly rG):(ara(Cp)$_{14}$C) are offset for clarity and the curve for (poly rG):(r(Cp)$_7$C) is plotted using the y-axis on the right part of the graph. T_m and %H values are tabulated in Table 3.

shows that an equimolar mixture of ara(U/C)-13-mer and r(A/G)-13-mer resulted in the formation of a new band migrating much more slowly than the component single strands (compare lane 7 to 4, and 8) and with very similar mobility to the slow bands in lanes 5 and 6, which represent the complexes (r(U/C)-13-mer):(r(A/G)-13-mer) and (d(A/G)-13-mer):(r(A/G)-13-mer). Hybridization was observed between ara(U/C)-13-mer and d(A/G)-13-mer (T_m = 18°C), although this was significantly weaker than that observed between r(U/C)-13-mer with d(A/G)-13-mer (T_m = 53°C).

Oligoarabinonucleotide ara(Cp)$_7$C forms a stable complex with poly rG in 200 mM NaCl, exhibiting a T_m of 57.0°C. This represents a reduction in T_m of 20° and 7.5° relative to, respectively, the control complexes (r(Cp)$_7$C):(poly rG) and (d(Cp)$_7$C):(poly rG) (Table 3, Fig. 5). The hyperchromic effect observed for (ara(Cp)$_7$C):(poly rG) (5 %H) was similar to that of (d(Cp)$_7$C):(poly rG) (5 %H) and significantly lower than that for the interaction between r(Cp)$_7$C with poly rG (15 %H). This observation may indicate that base stacking in (ara(Cp)$_7$C):(poly rG) is more closely related to that in (d(Cp)$_7$C):(poly rG) than in (r(Cp)$_7$C):(poly rG). As expected, a mixture of poly rG and a longer araC oligomer, namely ara(Cp)$_{14}$C, exhibited a higher T_m and %H relative to ara(Cp)$_7$C, confirming the interaction between araC and riboG residues.

In contrast to our previous finding that ara(Ap)$_7$A forms stable complexes with complementary RNA and DNA, we were unable to detect the formation of a complex between ara(Up)$_7$U and poly rA. There was no cooperative transition at 260 nm upon heating a mixture of ara(Up)$_7$U and poly rA (1 M NaCl), whereas under similar conditions we observed transitions for the melting of (r(Up)$_7$U):(poly rA) and (d(Tp)$_7$T):(poly rA) (Table 2). This observation is consistent with the work of Provenzale and Nagyvary, who showed that short oligomers of araU did not bind with poly rA under conditions where poly rU/poly rA formed (20*a*). It is possible that ara(Up)$_7$U binds to poly rA but the T_m value of the complex is too low to be detected.

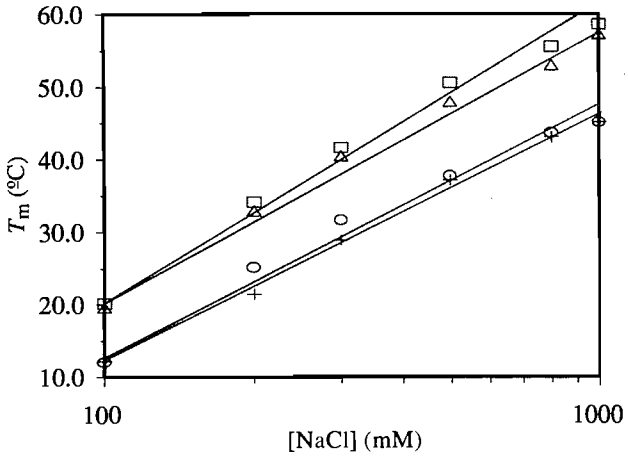


FIG. 6. Effect of increasing [NaCl] concentration on T_m for the complexes of phosphorothioate-linked oligoadenylates with poly dT and poly rU. Log [NaCl] versus T_m curves for the complexes (□) (poly dT):(d(Aps)$_7$A); (△) (poly rU):(d(Aps)$_7$A); (○) (poly rU):(ara(Aps)$_7$A); (+) (poly dT):(ara(Aps)$_7$A).

Interaction of the phosphorothioate ara(Aps)$_7$A with poly dT and poly rU

Replacement of the normal internucleotide linkage in ara(Ap)$_7$A with phosphorothioate linkages had a destabilizing effect on the complexes formed with poly rU and poly dT. For example, in 100 mM NaCl, the T_m of the complex (ara(Aps)$_7$A):(poly rU) was 12.4°C lower than that of the "all"-phosphodiester complex (ara(Ap)$_7$A):(poly rU) (Table 2). Similarly, ara(Aps)$_7$A gave a depressed T_m value when annealed to poly dT. It is clear from Fig. 4 and the data presented in Table 2 that the destabilizing effect resulting from phosphorothioate substitution in ara(Ap)$_7$A is significantly greater than in d(Ap)$_7$A. This destabilization was observed over a large range of ionic conditions (100 mM – 1 M NaCl) as illustrated in the plots of T_m vs. log [NaCl] (Fig. 6). Despite their enhanced nuclease stability, the weak hybridization affinity of phosphorothioate oligoarabinonucleotides may limit their use as antisense agents or as probes. However, we point out that the stability of complexes formed by phosphorothioate oligodeoxynucleotides is known to be sequence dependent.[5,6] Thus, to fully assess the binding potential of phosphorothioate ANA, oligomers of mixed base composition need to be investigated.

Conclusions

ANA oligomers can readily be prepared by the conventional solid-phase methodology employing phosphoramidite chemistry. Oligoarabinonucleotides were found to hybridize with complementary RNA and DNA exhibiting T_m's comparable to, and in some cases greater than, the corresponding unmodified oligomers. Although the ANA oligomers were susceptible to cleavage by certain nucleases, their stability under cellular conditions remains to be determined. Phosphorothioate-linked oligoarabinoadenylates exhibit characteristics normally observed for phosphorothioate DNA and RNA oligomers, for instance, significant stability against SVPDE cleavage and, in the case of ara(Aps)$_7$A, reduced T_m's when hybridized to complementary ssDNA and RNA relative to the unmodified ara(Ap)$_7$A. Our findings that ANA can form stable complexes with both ssDNA and RNA are very encouraging for those hoping to use sugar-modified antisense oligonucleotides as antisense agents or as probes. We are currently evaluating the effect of mixed base composition and 2′-modification (e.g., alkylation) on the ability of ANA to complex with both single- and double-stranded DNA and RNA.

[5]Generally, incorporation of phosphorothioate linkages at the 5′-side of a purine residue results in less destabilization of duplexes relative to when they are placed at the 5′-side of pyrimidines; this is particularly true in phosphorothioate d(AT) and d(GC) systems (43).

[6]Phosphorothiate substitution within (dTpT) is more destabilizing than substitution within (dCpC) oligomers (44).

Experimental

General procedures, chemicals, and reagents

THF was distilled from sodium benzophenone ketyl. Dimethylformamide (DMF), pyridine, and *N,N*-diisopropylethylamine were dried by stirring over CaH_2 followed by distillation under reduced pressure, and were stored over activated 4Å molecular sieves. Dichloromethane was distilled from P_2O_5. Acetonitrile was stirred in P_2O_5 and distilled from calcium hydride. Acetic anhydride was distilled and stored over magnesium turnings. Silylated ribonucleoside 3′-(cyanoethyl)phophoramidites were obtained from Dalton Chemical Laboratories, Inc. (Toronto, Ont.). DNA cyanoethyl phosphoramidite monomers, long-chain alkylamine controlled-pore glass (LCAA-CPG) bearing deoxyribonucleosides, tetraethylthiuram disulfide (TETD), and all other ancillary reagents for DNA/RNA/ANA synthesis were obtained from Applied Biosystems (Toronto, Ont.). LCAA-CPG bearing ribonucleosides or arabinonucleosides were prepared as previously described (45). *N*^{6}-benzoyl-5′-*O*-monomethoxytritylarabinoadenosinesine-2′-*O*-acetyl-3′-*O*-*N,N*-diisopropyl-β-cyanoethylphosphoramidite and 5′-*O*- monomethoxytrityllarabinouridine-2′-*O*-acetyl-3′-*O*-*N,N*-diisopropyl-β-cyanoethylphosphoramidites used in ANA synthesis were prepared according to Damha et al. (21). Cytidine, arabinocytidine, poly dT, poly rU, poly rG, and poly rA were used as obtained from Sigma Chemical Co (St. Louis, Mo.). Polyacrylamide gel electrophoresis reagents were obtained from Bio-Rad (Toronto, Ont.). HPLC grade solvents were purchased from Caledon (Toronto).

Preparation of N^4*-benzoyl-5′-*O*-monomethoxytritylarabinocytosine-2′-*O*-acetyl-3′-*O-N,N*-diisopropyl-β-cyanoethylphosphoramidite 5*

1. From 1-β-D-arabinofuranosylcytosine 1

Synthesis of 5′-O-monomethoxytritylarabinocytidine 2. Arabinocytidine **1** (18.0 mmol, 4.4 g) was coevaporated with pyridine (3 × 10 mL) and dissolved in dry pyridine (20 mL). Monomethoxytrityl chloride (19.8 mmol, 6.1 g) was added and the solution stirred at room temperature for 10 h, at which time a further 800 mg (0.1 equiv.) of monomethoxytrityl chloride was added and the mixture was allowed to stir for an additional 12 h. The mixture was evaporated to near dryness, dissolved in dichloromethane, and washed first with a solution of 5% sodium bicarbonate (100 mL) followed by brine (100 mL). The organic layer was dried over sodium sulfate and coevaporated with a solution of 10% dichloromethane in toluene (2 × 50 mL) to remove residual pyridine. This crude mixture was purified by silica gel chromatography by elution with dichloromethane–ethanol 85:15, affording 7.0 g (79% yield) of pure **2**. TLC (CH_2Cl_2/EtOH, 8:2) R_f 0.42; ($CHCl_3$/EtOH, 7:3) R_f 0.67. UV (95% EtOH/H_2O) λ_{max}: 230, 275 nm; λ_{min}: 224, 252 nm. ^{1}H NMR (acetone-d_6, 500 MHz) δ: 7.2–7.5 (m, 14, aryl trityl), 6.24 (d, 1, $J_{H1'-H2'}$ = 3.91 Hz, H1′), 4.34 (t, 1, H2′), 4.18 (t, 1, H3′), 4.07 (m, 1, H4′), 3.79 (s, 3, OCH_3), 3.45 (m, 2, H5′), 3.41 (m, 2, H5″).

N^4*-Benzoyl-5′-*O*-monomethoxytritylarabinocytidine 3.* Monomethoxytritylarabinocytidine **2** (10.9 mmol, 5.65 g) was coevaporated with pyridine (2 × 30 mL) and dissolved in pyridine (40 mL). Trimethylchlorosilane (54.8 mmol, 6.9 mL) was added to the solution over a period of 5 min and the mixture was stirred at ambient temperature (30 min) until all the starting material had reacted (TLC: dichlo-

romethane/ethanol 8:2). Benzoyl chloride (54.9 mmol, 6.4 mL) was added via syringe over a 5 min period. TLC analysis (dichloromethane/ethanol 8:2), 2 h later, indicated that less than 5% of non-benzoylated trisilylated product (R_f 0.76) remained. The reaction flask was immersed in an ice bath and water (5 mL) was added, followed by a solution of 29% ammonia (5 mL) 10 min later. After 15 min of stirring, the "thick" mixture was evaporated to near dryness, dissolved in dichloromethane (30 mL), and washed with water (30 mL). The organic layer was dried over sodium sulfate and the solvent was evaporated. The brown gum was coevaporated with toluene (2 × 30 mL) and purified twice by silica gel chromatography by elution with 5% ethanol–dichloromethane, affording 4.9 g (73% yield) of pure **3**. TLC: (CH_2Cl_2/EtOH 9:1) R_f 0.33; (EtOAc/EtOH, 9:1) R_f 0.63; (EtOAc) R_f = 0.13. UV (95% EtOH/H_2O, nm) λ_{max}: 230, 257, 307; λ_{min}: 224, 245, 286. ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 3.73 (s, 3, OCH_3), 3.45 (m, 2, H5′), 4.04 (m, 1, H4′), 4.31 (t, 1, H3′), 4.61 (t, 1, H2′), 6.21 (d, 1, $J_{H1'-H2'}$ = 4.85 Hz, H1′), 7.0–7.5 (m, 14, aryl trityl), 7.4–7.7 (m, 5, aryl benzoyl). MS (FAB-NBA) *m/e*: 620 (MH^+, 5.3%), 273 ($MMTr^+$, 100%), 228 (6.0%), 216 ($HCy^{Bz} + H^+$, 31.0%).

N^4-*Benzoyl-5′*-O-*monomethoxytritylarabinocytidine-3′*-O-N,N-*diiso-propyl*-β-cyanoethylphosphoramidite **4**. To a dry hypovial under argon was aded dry THF (30 mL), dry diisopropylethylamine (35.5 mmol, 6.2 mL), and the nucleoside derivative **3** (4.5 mmol, 8.8 g). *N,N*-Diisopropyl-β-cyanoethylphosphonamidic chloride (9.8 mmol, 2.2 mL) was added dropwise over 20 min. At the 90 min mark, ethyl acetate (prewashed with a solution of 5% sodium bicarbonate) (100 mL) was added and the mixture transferred to a separator funnel where it was washed with brine (4 × 100 mL) and dried over sodium sulfate. Removal of the solvent afforded 1.15 g of a white crystalline foam. TLC analysis (TLC: EtOAc/CH_2Cl_2/Et_3N 48:48:4) indicated a >95% completed reaction. The crude product was purified by silica gel chromatography by elution with CH_2Cl_2/hexanes/triethylamine 50:45:5, yielding both diastereoisomers of compound **4** in 77% combined yield. Physical properties of fast diastereoisomers: TLC (EtOAc/CH_2Cl_2/Et_3N, 48:48:4) R_f 0.62. ^{1}H NMR ($CDCl_3$, 100 MHz) δ: 8.12 (d, 1, H6, J_{H6-H5} = 2.4 Hz), 7.3–7.5 (H5, overlap with trityl), 6.14 (d, 1, $J_{H1'-H2'}$ = 4.98 Hz, H1′), 4.53 (2 × dd, 1, H2′, $J_{H2'-H3'} \cong J_{H1'-H2'}$ = 3.4 Hz), 4.29–4.34 (2 × dd, 1, H3′, $J_{H2'-H3'} \cong J_{H3'-H4'}$ = 3.9 Hz), 4.13–4.16 (m, 1, H4′), 3.4 (m, 2, H5′, H5″), 3.75 (s, 3, OCH_3), 1.17 (d, 6, i-Pr), 1.10 (d, 6, i-Pr). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 143.65 (s, C6), 113.2 (s, C5), 87.21 (s, C1′), 83.23 (d, C4′), 77.88 (d, C3′), 75.17 (d, C2′), 62.59 (s, C5′). ^{31}P NMR ($CDCl_3$, 80 MHz) δ: 150.60. Physical properties of slow diastereoisomer: TLC (EtOAc/CH_2Cl_2/Et_3N, 48:48:4) 0.48. ^{1}H NMR ($CDCl_3$, 100 MHz) δ: 8.10 (d, 1, H6, J_{H6-H5} = 2.3 Hz), 7.3–7.5 (H5, overlap with trityl), 6.25 (d, 1, $J_{H1'-H2'}$ = 4.03 Hz, H1′), 4.57 (2 × dd, 1, H2′, $J_{H2'-H3'} \cong J_{H1'-H2'}$ = 2.9 Hz), 4.30 (2 × dd, 1, H3′, $J_{H2'-H3'} \cong J_{H3'-H4'}$ = 3.5 Hz), 4.15 (m, 1, H4′), 3.4 (m, 2, H5′, H5″), 3.78 (s, 3, OCH_3), 1.13 (d, 6, i-Pr). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 86.98 (s, C1′), 83.42 (d, C4′), 78.95 (d, C3′), 74.79 (d, C2′), 62.90 (s, C5′). ^{31}P NMR ($CDCl_3$, 80 MHz) δ: 150.80. MS (FAB-NBA) *m/e*: 820 (MH^+, 6.9%), 602 ([MH^+ − $CNCH_2CH_2OP(OH)$(i-Pr)$_2$], 17.9%), 273 ($MMTr^+$, 100%), 216 (HCy^{Bz} − H^+, 33.9%), 201 ($CNCH_2CH_2OP$(i-Pr)$_2^+$, 46.3%).

N^4-*Benzoyl-5′*-O-*monomethoxytritylarabinocytosine-2′*-O-*acetyl-3′*-O-N,N-*diisopropyl*-β-cyanoethylphosphoramidine **5**. To a dry hypovial containing the slow diastereoisomer of **4** (1.1 mmol, 900 mg) and dimethylaminopyridine (0.4 mmol, 53 mg) were added dry THF (5 mL) and diisopropylethylamine (8.7 mmol, 1.5 mL). Acetic anhydride (4.4 mmol, 450 μL) was added over 1 min and the solution was stirred for 45 min since TLC analysis ((CH_2Cl_2/EtOAc/Et_3N, 1:1) R_f 0.63) indicated that all of the starting material had reacted. Ethyl acetate (prewashed with a solution of 5% sodium bicarbonate) (25 mL) was added and the mixture transferred to a separator funnel where it was washed with brine (5 × 100 mL) and dried over sodium sulfate. Removal of the solvent produced **5** as a white crystalline foam in 87% yield. TLC (EtOAc/CH_2Cl_2/Et_3N, 48:48:4) R_f 0.63. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.96 (d, 1, H6, J_{H6-H5} = 2.4 Hz), 7.3–7.5 (H5, overlap with trityl), 6.33 (d, 1, H1′, $J_{H1'-H2'}$ = 4.21 Hz), 5.45 (2 × dd, 1, H2′, $J_{H2'-H3'} \cong J_{H1'-H2'}$ = 3.2 Hz), 4.40 (2 × dd, 1, H3′, $J_{H2'-H3'} \cong J_{H3'-H4'}$ = 3.2 Hz), 4.25 (m, 1, H4′), 3.70 (s, 3, OCH_3), 3.42 (2 × dd, 1, H5″, $J_{H5'-H5''}$ = 10.2 Hz, $J_{5''-H4'}$ = 4.4 Hz), 3.36 (2 × dd, 1, H5′, $J_{H5'-H5''}$ = 10.2 Hz, $J_{H5'-H4'}$ = 5.6 Hz), 1.74 (s, 3, $COCH_3$). ^{31}P NMR ($CDCl_3$, 80 MHz) δ: 151.26. MS (FAB-NBA) *m/e*: 862 (MH^+, 1.6%), 644 ([MH^+ − $CNCH_2CH_2OP(OH)$(i-Pr)$_2$], 4.3%), 273 ($MMTr^+$, 100%), 216 ($HCy^{Bz} + H^+$, 14.5%), 201 ($CNCH_2CH_2OP$(i-Pr)$_2^+$, 14.0%).

2. *From cytidine*

A stirred mixture of cytidine (48.7 mmol, 11.8 g), diphenyl carbonate (73.4 mmol, 13.3 g), dimethylformamide (20 mL), and water (50 mmol, 0.9 mL) was heated at 150°C for 15 min and the resulting dark solution was poured, with stirring, into 120 mL of ether. The gummy yellow precipitate formed was washed with cold ether (3 × 50 mL). Two new compounds with a relative intensity of 9:1 were evident by TLC (isopropyl/ammonia/water, 55:35:10, R_f 0.66 and 0.56). This crude mixture was coevaporated (3 × 30 mL) and dissolved in dry pyridine (20 mL). Monomethoxytrityl chloride (31.2 mmol, 9.6 g) (1.3 equivalents based on 50% yield of **2**) was added and the solution stirred for 19 h at room temperature. Analysis by TLC showed the formation of two compounds (CH_2Cl_2/EtOH, 8:2, R_f 0.49 and 0.33) with a relative intensity of 1:9, respectively. The mixture was evaporated to near dryness, dissolved in dichloromethane, washed first with a solution of 5% sodium bicarbonate (100 mL) followed by brine (100 mL), and dried over sodium sulfate. Approximately one half of this mixture (5.8 mmol, 3.0 g), was coevaporated (2 × 30 mL) and dissolved in pyridine (40 mL). Trimethylchlorosilane (29.0 mmol, 3.6 mL) was added and the solution was stirred at ambient temperature for 20 min, at which time benzoyl chloride (29.5 mmol, 3.45 mL) was introduced via syringe. TLC analysis (CH_2Cl_2/EtOH 9:1), 2 h later, indicated that less than 5% of the starting material remained. The mixture was then cooled by immersing in an ice bath; water (5 mL) was added, followed 10 min later by a solution of 29% ammonia (5 mL). After 15 min of stirring, the "thick" mixture was evaporated to near dryness, dissolved in dichloromethane (30 mL), and washed with water (30 mL). The organic layer was dried over sodium sulfate and the solvent evaporated. The brown gum was coevaporated with toluene (2 × 30 mL) and purified twice by silica gel chromatography, eluting first with dichloromethane (750 mL) followed by 5% EtOH/CH_2Cl_2, and affording pure **3** in 26% yield from cytidine. Preparation of **5** from this point on was as described in the previous section.

Solid-phase synthesis and purification of oligonucleotides

Oligoribonucleotides were prepared and purified as previously described (34). DNA oligomers were prepared and purified by the "trityl on" method (35). ANA oligomers were prepared on a 1.0 μmol scale employing "trityl on" synthesis cycle (60 s coupling time). Deprotection of sugar and phosphate moieties with concomitant release of oligomers from the glass support was accomplished by a single ammonia treatment (55°C, 8 h). The terminal trityl group was cleaved with trifluoroacetic acid during purification with reversed phase OPC® cartridges (Applied Biosystems). Oligoarabinocytidines were purified by polyacrylamide gel electrophoresis (PAGE). Phosphorothioate-linked DNA and ANA were prepared using tetraethylthiuram disulfide (TETD) as the sulfurizing reagent. After each coupling the glass beads were exposed to TETD (instead of iodine–water) for 10 min and then washed *extensively* with dry acetonitrile. Phosphorothioate oligomers were deblocked and purified as for the normal (phosphate) sequences.

Polyacrylamide mobility shift assays

Experiments were carried out as previously described (5).

Enzyme resistance assay

Incubations of oligomers with nucleases and analysis of the digests by PAGE were carried out as described (21). Analysis of the SVPDE digests of ara(Aps)$_7$A and d(Aps)$_7$A by HPLC was carried out on a Waters instrument equipped with dual 501 pumps, a 480 UV detector, and a 740 Data module. A Whatman Patrisil ODS-2 (10 μm, 4.6 × 250 mm) column was used with a linear elution gradient 0–50% over 30 min (solvent A: 20 mM KH_2PO_4 pH 5.5; solvent B: methanol).

Melting temperature (T_m) measurements

Thermal denaturations were performed on a Varian–Cary 1 UV spectrophotometer equipped with a Peltier thermal unit. Equimolar mixtures (2.5 μmol in each strand) of complementary oligomers were allowed to equilibrate at 40°C for 20 min, followed by slow cooling (over 20–30 min) to the start temperature prior to starting (runs involving poly rG or poly rA were heated to 80°C for 20–30 min). Thermal denaturation curves were recorded at 1 min intervals at 260 nm while the temperature was ramped at a rate of 0.5°C/min. The curves were analyzed by the first derivative method to obtain T_m values. Hyperchromicity (*%H*) is reported with respect to the initial absorbance in each curve. T_m versus log NaCl data was obtained as follows: first denaturation was run in 100 mM NaCl; upon completion, the sample was cooled slowly to 40°C; an appropriate amount of NaCl crystals was added to increase the salt concentration to the next level, samples were then cooled to the start temperature, and denatured again. This process was repeated for all salt concentrations. No correction for volume was made. Stoichiometry was determined by the method of continuous variation (38) as previously described (5).

Acknowledgements

We gratefully acknowledge financial support from the Natural Sciences and Engineering Research Council of Canada (NSERCC), the Canadian Foundation for AIDS Research (CANFAR), McGill University, and the University of Toronto. M.J.D. acknowledges a post-graduate fellowship received from the Ministry of Colleges and Universities, Government of Ontario (John Polanyi Prize in Chemistry, 1989).

1. (*a*) P.C. Zamecnik and M.L. Stephenson. Proc. Natl. Acad. Sci. U.S.A. **75**, 280 (1978); (*b*) M.L. Stephenson and P.C. Zamecnik. Proc. Natl. Acad. Sci. U.S.A. **75**, 285 (1978); (*c*) P.C. Zamecnik, J. Goodchild, Y. Taguchi, and P.S. Sarin. Proc. Natl. Acad. Sci. U.S.A. **83**, 4143 (1986); (*d*) C.J. Markus-Sekura, A.M. Woerner, K. Shinozuka, G. Zon, and G.V. Quinnan. Nucleic Acids Res. **15**, 5749 (1987).
2. E. Wickstru. J. Biochem. Biophys. Methods, **13**, 97 (1986).
3. (*a*) P.S. Miller. *In* Oligodeoxynucleotides, antisense inhibitors of gene expression. *Edited by* J.S. Cohen. Macmillan, Houndsmills and London. 1989. pp. 79–95; (*b*) C.A. Stein and J.S. Cohen. *In* Oligodeoxynucleotides, antisense inhibitors of gene expression. *Edited by* J.S. Cohen. Macmillan, Houndsmills and London. 1989. pp. 97–117; (*c*) B. Froehler, P. Ng, and M. Matteucci. Nucleic Acids Res. **16**, 4831 (1988); (*d*) W.S. Marshall and M.H. Caruthers. Science, **259**, 1564 (1993).
4. (*a*) J. Cormier and K.K. Ogilvie. Nucleic Acids Res. **16**, 4583 (1988); (*b*) E.P. Stirchak, J.E. Summerton, and D.D. Weller. J. Org. Chem. **52**, 4202 (1987); (*c*) M. Matteucci. Nucleosides Nucleotides, **10**, 231 (1991); (*d*) B. Musicki and T.S. Widlanski. Tetrahedron Lett. **32**, 1267 (1991); (*e*) S.H. Kawai, D. Wang, P.A. Giannaris, M.J. Damha, and G. Just. Nucleic Acids Res. **21**, 1473 (1993); (*f*) B. Meng, S.H. Kawai, D. Wang, G. Just, P.A. Giannaris, and M.J. Damha. Angew. Chem. Int. Ed. Engl. **32**, 729 (1993).
5. P.A. Giannaris and M.J. Damha. Nucleic Acids Res. **21**, 4742 (1993).
6. (*a*) P.D. Cook. Anti-Cancer Drug Des. **6**, 585 (1991); (*b*) E. Ulhmann and A. Peyman. Chem. Rev. **90**, 543 (1990); (*c*) C. Hélène and J.-J. Toulmé. Biochim. Biophys. Acta, **99**, 1049 (990); (*d*) J.S. Cohen (*Editor*). Oligodeoxynucleotides, antisense inhibitors of gene expression. Macmillan, Houndsmills and London. 1989; (*e*) G. Zon. Pharm. Res. **5**, 539 (1988).
7. (*a*) F. Debart, B. Rayner, G. Degols, and J.-L. Imbach. Nucleic Acids Res. **20**, 1193 (1992); (*b*) F. Morvan, B. Rayner, J.-L. Imbach, S. Thenet, J.-R. Bertrand, J. Paoletti, C. Malvy, and C. Paoletti. Nucleic Acids Res. **15**, 3421 (1987).
8. (*a*) M.J. Damha, P.A. Giannaris, P. Marfey, and L.S. Reid. Tetrahedron Lett. **32**, 2573 (1991); (*b*) S. Fujimori and K. Shudo. J. Am. Chem. Soc. **112**, 7436 (1990); (*c*) M.L. Capobianco, A. Garbesi, F. Arcamone, B. Maschera, and G. Palu. Nucleic Acids Res. Symp. Ser. **24**, 2587 (1991).
9. G.W. Ashley. J. Am. Chem. Soc. **114**, 9731 (1992).
10. K.C. Schneider and S.A. Benner. J. Am. Chem. Soc. **112**, 453 (1990).
11. L. Bellon, J.-L. Barascut, G. Maury, G. Divita, R. Goody, and J.-L. Imbach. Nucleic Acids Res. **21**, 1587 (1993).
12. (*a*) M. Perbost, M. Lucas, and C. Charvis. Biochem. Biophys. Res. Commun. **165**, 742 (1989); (*b*) J. Sagi, A. Szemzo, J. Szecsi, and L. Otvos. Nucleic Acids Res. **18**, 2133 (1991).
13. B.S. Sproat, A.I. Lamond, B. Beijer, P. Neumer, and U. Ryder. Nucleic Acids Res. **17**, 3373 (1989); (*b*) E.A. Lesnik, C.J. Guinosso, A.M. Kawasaki, H. Sasmor, M. Zounes, L.L. Cummins, D.J. Ecker, P.D. Cook, and S.M. Freir. Biochemistry, **32**, 7832 (1993); (*c*) M. Cotten, B. Oberhauser, H. Brunear, A. Holzner, G. Issakides, C.R. Noe, G. Schaffner, E. Wagner, and M. Birnstiel. Nucleic Acids Res. **19**, 2629 (1991).
14. A.D. Adams, C.R. Petrie, and R.B. Meyer, Jr. Nucleic Acids Res. **19**, 3647 (1991).
15. (*a*) K.K. Ogilvie, A.L. Schifman, and C.L. Penney. Can. J. Chem. **57**, 2230 (1979); (*b*) K.K. Ogilvie, S.L. Beaucage, A.L. Schifman, N.Y. Theriault, and K.L. Sadana. Can. J. Chem. **56**, 2768 (1978); (*c*) R.T. Pon and K.K. Ogilvie. Nucleosides Nucleotides, **3**, 485 (1984); (*d*) N. Usman, K.K. Ogilvie, M.-Y. Jiang, and R.J. Cedergren. J. Am. Chem. Soc. **109**, 7845 (1987).
16. W.J. Wechter. J. Med. Chem. **10**, 762 (1967).
17. C. Gioeli, J.B. Chattopadhyaya, A.F. Drake, and B. Oberg. Chem. Scr. **19**, 13 (1982).
18. G.P. Beardsley, T. Mikita, M. Klaus, and A.L. Nussbaum. Nucleic Acids Res. **16**, 9165 (1988).
19. (*a*) K.K. Ogilvie, D.P.C. McGee, S.M. Boisvert, G.H. Hakimelahi, and Z.A. Proba. Can. J. Chem. **61**, 1204 (1983); (*b*) D.P.C. McGee. M.Sc. thesis, McGill University, Montreal, Canada (1981).
20. (*a*) G. Provenzale and J. Nagyvary. Biochemistry, **9**, 1744 (1970); (*b*) J. Nagyvary and G. Provenzale. Biochemistry, **8**, 4769 (1969); (*c*) K.K. Ogilvie and D. Iwacha. Can. J. Chem. **48**, 862 (1970); (*d*) G. Schramman. Angew. Chem. Int. Ed. Engl. **6**, 460 (1967).
21. M.J. Damha, N. Usman, and K.K. Ogilvie. Can. J. Chem. **67**, 831 (1989).
22. (*a*) M. Resmini and W. Pfleiderer. Helv. Chim. Acta, **76**, 158 (1993); (*b*) W. Pfleiderer. 10th Int. Roundtable: Nucleosides and Nucleotides, Park City, Utah, Sept 16, 1992.
23. (*a*) E.J. Reist, P.A. Sturm, R.Y. Pong, M.J. Tanga, and R.W. Sidwell. *In* Nucleotide analogues as antiviral agents. *Edited by* J.C. Martin. American Chemical Society, Washington, D.C. 1989. pp. 17–34; (*b*) J.L. Rideout, D.W. Henry, and L.M. Beacham III (*Editors*). Nucleosides, nucleotides and their biological activity. Academic Press, New York. 1983, and references therein.
24. R.S.K. Young and G.A. Fischer. Biochem. Biophys. Res. Commun. **32**, 23 (1968).
25. (*a*) M.J. Keating, K.B. McCredie, G.P. Bodey, T.L. Smith, E. Gehan, and E.J. Freieich. J. Am. Med. Assoc. **248**, 2481 (1982); (*b*) D.W. Kufe and D.R. Springs. Semin. Oncol. **12**, 34 (1985); (*c*) E. Frei III, J. Bickers, M. Lane, W. Leary, and R. Tally. Cancer Res. **29**, 1325 (1969).
26. (*a*) N.R. Cozzarelli. Annu. Rev. Biochem. **46**, 641 (1977); (*b*) A. Fridland. Biochemistry, **16**, 5308 (1977).
27. T. Mikita and G.P. Beardsley. Biochemistry, **27**, 4698 (1988).
28. (*a*) Y-G. Gao, G.A. van der Marel, J.H. van Boom, and A.H.-J. Wang. Biochemistry, **30**, 9922 (1991).
29. J.M.L. Pieters, E. de Vroom, G.A. van der Marel, J.H. van Boom, and C. Altona. Eur. J. Biochem. **184**, 415 (1989).
30. J.M.L. Pieters, E. de Vroom, G.A. van der Marel, J.H. van Boom, T.M.G. Koning, R. Kaptein, and C. Altona. Biochemistry, **29**, 788 (1990).
31. E. de Vroom, H.C.P.F. Roelen, C.P. Saris, T.N.W. Budding, G.A. van der Marel, and J.H. van Boom. Nucleic Acids Res. **16**, 2987 (1988).

32. M.-K. Teng, Y.-C. Liaw, G.A. van der Marel, J.H. van Boom, and A.H.-J. Wang. Biochemistry, **28**, 4923 (1989).
33. (*a*) J. Beránek, T.J. Delia, and P. Drašar. Collect. Czech. Chem. Commun. **42**, 1588 (1976); (*b*) K.K. Ogilvie. Carbohydrate Res. **24**, 210 (1972).
34. M.J. Damha and K.K. Ogilvie. *In* Protocols for oligonucleotide and analogs: synthesis and properties, methods in molecular biology. *Edited by* S. Agrawal. The Humana Press Inc., Totowas, N.J. 1993. pp. 81–114.
35. M.J. Gait (*Editor*). Oligonucleotide synthesis: a practical approach. IRL Press, Oxford. 1984.
36. C.A. Stein, C. Subasinghe, K. Shinozuka, and J.S. Cohen. Nucleic Acids Res. **16**, 3209 (1988).
37. (*a*) F. Morvan, B. Rayner, and J.-L. Imbach. Tetrahedron Lett. **49**, 7149 (1990); (*b*) S. Agrawal, J.Y. Tang, D. Sun, P.S. Sarin, and P.C. Zamecnik. Ann. N.Y. Acad. Sci. **660**, 3 (1992).
38. (*a*) P. Job. Anal. Chim. Acta, **9**, 113 (1928); (*b*) D.S. Pilch, C. Levenson, and R. Shafer. Proc. Natl. Acad. Sci. U.S.A. **87**, 1942 (1990).
39. (*a*) H. Inoue, Y. Hayase, M. Asaka, A. Imura, S. Iwai, K. Miura, and E. Ohtsuka. Nucleic Acids Res. **16**, 165 (1985).
40. A.M. Kawasaki, M.D. Casper, S.M. Freier, E.A. Lesnik, M.C. Zounes, L.L. Cummins, C. Gonzalez, and P.D. Cook. J. Med. Chem. **36**, 831 (1993).
41. A.M. Iribarren, B.S. Sproat, P. Neuner, I. Sulston, U. Ryder, and A.I. Lamond. Proc. Natl. Acad. Sci. U.S.A. **87**, 7747 (1990).
42. E.A. Lesnik, C.J. Guinosso, A.M. Kawasaki, H. Sasmor, M. Zounes, L.L. Cummins, D.J. Ecker, P.D. Cook, and S.M. Freier. Biochemistry, **32**, 7832 (1993).
43. (*a*) J.W. Suggs and D.A. Taylor. Nucleic Acids Res. **13**, 5707 (1985); (*b*) L. Kibler-Herzog, G. Zon, B. Uznaski, G. Whittier, and W.D. Wilson. Nucleic Acids Res. **19**, 2979 (1991); (*c*) L.J.P. Latimer, K. Hampel, and J.S. Lee. Nucleic Acids Res. **17**, 1549 (1989); (*d*) F. Eckstein and T.M. Jovin. Biochemistry, **22**, 4546 (1983); (*e*) T.M. Jovin, J.J. van de Sande, D.A. Zarling, D.J. Arndt-Jovin, F. Eickstein, H.H. Füldner, C. Greider, I. Grieger, E. Hamori, B. Kalisch, L.P. McIntosh, and M. Robert-Nicoud. Cold Springs Harbor Symp. Quant. Biol. **47**, 143 (1983).
44. C.A. Stein, C. Subasinghe, K. Shinozuka, and J.S. Cohen. Nucleic Acids Res. **16**, 3209 (1988).
45. M.J. Damha, P.A. Giannaris, and S.V. Zabarylo. Nucleic Acids. Res. **18**, 3813 (1990).