major interesting differences. These possibilities are under continuing investigation.

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**Supplementary Material Available:** Tables of bonding and thermal parameters, positional coordinates, and synthetic and spectroscopic details for the reported compounds (37 pages); tables of observed and calculated structure factors (41 pages). Ordering information is given on any current masthead page.

## Oligodeoxynucleotides That Contain 2′,5″ Linkages: Synthesis and Hybridization Properties

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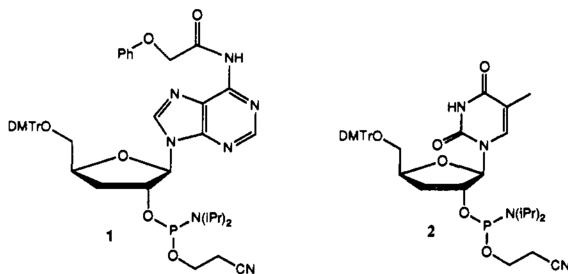
Would DNA be as useful a genetic material if the 3′-oxygen had been removed from RNA, forcing a 2′,5″ link? Divergent predictions have been offered,[1–6] but there was no experimental evidence on the properties of 2′,5″ DNA.[7,8] Our molecular modeling confirms that antiparallel Watson–Crick base pairing is possible, but in an open helix with poorer than normal base stacking. We have now prepared 16-mer oligodeoxynucleotides that contain one or more 2′,5″ linkages. Such linkages prove to destabilize normal 3′,5″ double helices. There is at best weak strand association, and that only at high salt concentrations, in isomeric DNA in which *all* links are 2′,5″.

We have described the synthesis of **1** and **2**.[9] Oligonucleotide synthesis was carried out on an Applied Biosystems Model 381A DNA synthesizer. The manufacturer's cycles and reagents proved satisfactory for coupling using phosphoramidites **1** and **2**. Since




the manufacturer's columns were used, all oligonucleotides, including those that contain only 2′,5″ linkages, have a (irrelevant) terminal 3″-hydroxyl. Deprotection, and purification by denaturing PAGE, followed standard protocols. Compounds **11**, **12**, **14**, and **16** were examined by electrospray mass spectroscopy and showed the expected negative molecular ions.

| LANE | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HOT OLIGO | | 12 | | | 14 | | | 15 | | | 14 | |
| COLD OLIGO | | 11 | | | 13 | | | 16 | | | 11 | |
| PMOL COLD | 0 | 1 | 10 | 0 | 1 | 10 | 0 | 1 | 10 | 0 | 1 | 10 |

**Figure 1.** Native polyacrylamide gel (20%, 1× TBE). Oligomers **12**, **14**, and **15** were 5′-end-labelled with $\gamma$-$^{32}$P ATP and T4 polynucleotide kinase. The labeled oligomer ("hot") (**12** lanes 1–3, **15** lanes 7–9, **14** lanes 4–6 and 10–12, <0.1 pmol each) was combined with unlabeled oligomer ("cold") (**11** lanes 2, 3, 11, and 12, **13** lanes 5 and 6, **16** lanes 8 and 9, 1 pmol in lanes 2, 5, 8, and 11, 10 pmol in lanes 3, 6, 9, and 12) in 6 mL of 5% glycerol/0.1 M load buffer, heated to 37 °C, slowly cooled to 0 °C, loaded, and run at 5 °C.

**Table I**[a]

| Nucleotide | | Sequence | | Tm |
|---|---|---|---|---|
| ANTI-16(**3**) | 3' | TCCGTACGTTCGAACA | 5' | |
| NORMAL-16(**4**) | 5' | AGGCATGCAAGCTTGT | 3' | 65.0 |
| (**5**) | | AGGCATGC***A***AGCTTGT | | 54.0 |
| (**6**) | | AGGC***A***TGCA***A***GCTTGT | | 52.9 |
| (**7**) | | AGGCATGC***AA***GCTTGT | | 53.7 |
| (**8**) | | AGGCA***T***GCAAGCTTGT | | 60.1 |
| (**9**) | | AGGCATG***T***AAGCTTGT | | 50.9 |
| (**10**) | | AGGCATGCCAGCTTGT | | 50.7 |
| (**11**) | 5' | ***AATAATAAATAATAA***T | 3' | |
| (**12**) | 3' | T***TATTATTTATTATTA*** | 5' | |
| (**13**) | 5' | AATAATAAATAATAAT | 3' | |
| (**14**) | 3' | TTATTATTTATTATTA | 5' | |
| (**15**) | 5' | ***AAAAAAAAAAAAAAA***T | 3' | |
| (**16**) | 3' | T***TTTTTTTTTTTTTTT*** | 5' | |
| (**17**) | 5' | ***AAAAAAAAAAAAAAA***A | 3' | |

[a] ***A*** and ***T*** designate the 2′,5′ isomer. Compounds **5–10** have the same sequence as oligomer **4** except where underlined. $T_m$'s were measured at oligomer concentrations of 5–10 μM in 1× SSC (150 mM sodium chloride, 15 mM sodium citrate, pH 7.29), to which $Na_3EDTA$ was added to 1 mM. Absorbance changes (260 nm) were monitored on a Cary 3 UV spectrometer, heating at 1 deg/min.

For $T_m$ studies,[10] equimolar concentrations (estimated from calculated extinction coefficients) of oligomer **3** and complements **4–10** were combined in 1× SSC (0.15 M NaCl, 15 mM sodium citrate). As shown in Table I, replacement of one 3′,5″ linkage (in **4**) by a 2′,5″ connection (in **5**) destabilizes the duplex with **3** nearly as much as a pyrimidine–pyrimidine mismatch in oligomer **10**. The potentially wobble-paired **9** is nevertheless destabilized. The subtlety of the effects of this substitution is clear from the lack of much extra destabilization in doubly altered **6** and **7** or the small effect seen in **8**.

When the fully 2′,5″-linked oligomer combinations **11** and **12** or **16** and **17** (1 μM each) are heated from 2 to 82 °C in 1× SSC, a steady increase in absorbance (260 nm) is observed but no cooperative transitions are detectable. Two increasingly deep cooperative transitions appear for **16**/**17** with 0.5 or 1.0 M NaCl, centered at 7 and 32 °C (0.5 M) and 8 and 35 °C (1.0 M), but none for **11**/**12**. Confirming the lack of association in **11**/**12**, the circular dichroism of the combination (5 μM each, 1 M NaCl, 15 mM sodium citrate, 19 °C) was identical to the summed CDs of the components; by contrast, **13**/**14** gave a large incremental CD under these conditions, as did **16**/**17** (2 μM each).

(1) (a) Lohrmann, R.; Orgel, L. E. *Tetrahedron* **1978**, *34*, 853–855. (b) Sulston, J.; Lohrmann, R.; Orgel, L. E.; Schneider-Bernloehr, H.; Weimann, B. J.; Miles, H. T. *J. Mol. Biol.* **1969**, *40*, 227–234.

(2) Usher, D. A.; McHale, A. H. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 1149–1153. Note that the high hydrolytic reactivity of the 2′,5″ linkage seen here occurred when it was part of a normal 3′,5″ oligonucleotide.

(3) (a) Srinivasan, A. R.; Olson, W. K. *Nucleic Acids Res.* **1986**, *14*, 5461–5479. (b) Dhingra, M. M.; Sarma, R. S. *Nature* **1978**, *272*, 798–801.

(4) Parthasarathy, R.; Malik, M.; Fridey, S. M. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 7292–7296.

(5) Anukanth, A.; Ponnuswamy, P. K. *Biopolymers* **1986**, *25*, 729–752.

(6) Krishnan, R.; Seshadri, T. P.; Viswamitra, M. A. *Nucleic Acids Res.* **1991**, *19*, 379–384.

(7) Hashimoto, H.; Switzer, C. *J. Am. Chem. Soc.*, in press. Note that the self-complementary oligomer proved to be monomolecular.

(8) Kierzek, R.; He, L.; Turner, D. H. *Nucleic Acids Res.* **1992**, *20*, 1685–1690.

(9) Rizzo, C.; Dougherty, J.; Breslow, R. *Tetrahedron Lett.*, in press.

(10) Puglisi, J. D.; Tinoco, I., Jr. In *Methods in Enzymology*; Dahlberg, J. E., Abelson, J. N., Eds.; Academic Press, Inc.: New York, 1989; Vol. 180, pp 304–325.

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A mobility shift assay, using native PAGE (Figure 1), strengthens the picture. As the figure shows, strand association at low salt is seen with the 3′,5″-linked **13**/**14** combination by retardation of labelled **14**, but no association can be detected for 2′,5″-mixed sequences **11**/**12**, for 2′,5″ homopolymers **15**/**16**, or for 2′,5″-**11** with 3′,5″-**14**.

Switzer reports[7] $T_m$ (lower than for normal DNA) evidence for association of $(3'\text{-deoxyA})_{12}$ with $(3'\text{-deoxyU})_{12}$ at high salt. Our $T_m$ studies confirm some association in our related **16**/**17**, but *only* at high salt. Recent studies on 2′,5″-linked RNA 6–10-mers also demonstrate weak strand association at high salt concentrations.[8] However, it is apparent from all this that, if duplex formation occurs with 3′-deoxy isomeric DNA, it is at best weaker than that for normal DNA and is seen only with high salt. This lack of strong association, especially under normal cellular conditions, may be the selective disadvantage that eliminated 2′,5″-linked nucleic acids from a role as genetic material.

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## Self-Association of 2′,5′-Linked Deoxynucleotides: Meta-DNA

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Both 3′,5′- and 2′,5′-linked nucleotides occur naturally,[1] but only nucleotides with the former linkage encode genetic information. In spite of this fact, activated mononucleotides generally give 2′,5′-linked nucleotides during abiotic oligomerization,[2,3] suggestive of a prebiotic bias toward this connectivity. Thus, the question presents itself as to whether the 2′,5′-internucleotide linkage arose first at the time of natural history during which chemical evolution is postulated and was later superseded by the 3′,5′-linkage during prebiotic or early biotic evolution. Such a scenario could be plausibly integrated into the currently held view that RNA may have served a central role in the origins of life.[4]

A successful genetic material must be capable of directing its reproduction. 3′,5′-Linked nucleic acids accomplish this through self-association by way of Watson–Crick base pairing. Whether 2′,5′-linked nucleotides can similarly self-associate has been the object of various hypotheses. These have been based upon studies of dinucleotides,[5] or theoretical calculations,[6] and lead to conflicting predictions. We report the synthesis of single-stranded DNA segments containing only "nongenomic" 2′,5′-internucleotide linkages, for which we propose the name "meta-DNA" (Figure 1), and find that they do self-associate.

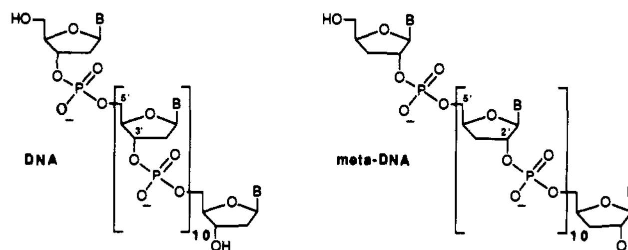


Figure 1.

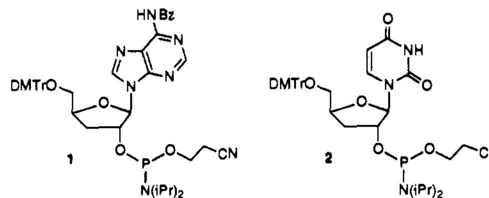


Figure 2.

We have synthesized three meta-DNA strands: a mutually complementary pair of 3′-deoxydodecanucleotides, meta-$dA_{12}$ and meta-$dU_{12}$, and one that was chosen to be self-complementary, meta-$d(AU)_6$.[7,8] The starting materials were phosphoramidites **1** and **2** (Figure 2), which were prepared by direct extensions of standard methods.[9] After deprotection, the oligomers were purified by high-performance liquid chromatography (HPLC),[10] and their composition was verified by laser desorption mass spectrometry and also by digestion to nucleoside monomers (snake venom phosphodiesterase in the presence of bacterial alkaline phosphatase),[11] followed by HPLC comparison with authentic monomer samples.

Self-association of meta-DNA dodecamers was assayed by examining their ultraviolet (UV) absorbance profiles versus temperature. Such profiles are well known to reveal hyperchromic effects due to the decrease in base-stacking interactions that occurs when genomic 3′,5′-linked DNA double helices dissociate to single strands.[12] These profiles and their first derivatives for the various dodecamers are reproduced in Figures 3 and 4.

As can be seen in Figure 3A, a mixture of meta-$dA_{12}$ and meta-$dU_{12}$ exhibits a dramatic hyperchromic change in UV absorbance between 5 and 25 °C, amounting to approximately 60% of the analogous effect observed with a mixture of genomic $dA_{12}$ and $dT_{12}$. This change is indicative of the dissociation of a meta-DNA complex. Analysis of the derivative curves for the absorbance profiles versus temperature (Figure 3B) allows the

(1) The 2′,5′-linkage is found in oligoadenylate messengers involved in the interferon-induced response to viral infection in eukaryotes, see: Adams, R. L. P.; Knowler, J. T.; Leader, D. P. *The Biochemistry of the Nucleic Acids*; Chapman and Hall: New York, 1986; pp 441–442.

(2) For oligomerizations in the absence of oligonucleotide templates, a situation where the bias toward a 2′,5′-linkage is generally acute and is obviously lacking any influence of an added template, see: (a) Lohrmann, R.; Orgel, L. E. *Tetrahedron* **1978**, *34*, 853. (b) Sawai, H. *J. Mol. Evol.* **1988**, *27*, 181.

(3) For reviews of oligomerizations in the presence of 3′,5′-linked RNA templates, see: (a) Joyce, G. F. *Cold Spring Harbor, Symposia on Quantitative Biology*; Cold Spring Harbor: New York, 1987; Vol. LII, pp 41–52. (b) Orgel, L. E. *J. Theor. Biol.* **1986**, *123*, 127.

(4) Reviewed in the following: Joyce, G. F. *Nature* **1989**, *338*, 217 and ref 3b.

(5) (a) Parthasarathy, R.; Malik, M.; Fridey, S. M. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 7292. (b) Dhingra, M. M.; Sarma, R. H. *Nature* **1978**, *272*, 798.

(6) (a) Srinivasan, A. R.; Olson, W. K. *Nucleic Acids Res.* **1986**, *14*, 5461. (b) Anukanth, A.; Ponnuswamy, P. K. *Biopolymers* **1986**, *25*, 729.

(7) Synthesis was performed on a controlled-pore glass support derivatized with the appropriate 5′-(dimethoxytrityl)-3′-deoxynucleoside using an Applied Biosystems 391 EP DNA synthesizer. For the derivatization procedure, see: Atkinson, T.; Smith, M. *Oligonucleotide Synthesis: A Practical Approach*; Gait, M. J., Ed.; IRL Press: Oxford, 1985; pp 47–49. DNA synthesis was performed on a 1-μmol scale.

(8) The following corresponding genomic 2′-deoxydodecanucleotides were also prepared as references in this work: $dA_{12}$, $dT_{12}$, and $d(AT)_6$.

(9) Phosphoramidites **1** and **2** were synthesized from 3′-dA and 5′-(dimethoxytrityl)-3′-dU, respectively, using the same procedures reported for the corresponding 2′-deoxynucleosides: Atkinson, T.; Smith, M. *Oligonucleotide Synthesis: A Practical Approach*; Gait, M. J., Ed.; IRL Press: Oxford 1985; pp 39–45. 3′-dA synthesis: Hansske, F.; Robins, M. J. *Tetrahedron Lett.* **1985**, *26*, 4295. 5′-(Dimethoxytrityl)-3′-dU synthesis: Ogilvie, K. K.; Hakimelahi, G. H.; Proba, Z. A.; Usman, N. *Tetrahedron Lett.* **1983**, *24*, 865. 3′-dA and 3′-dU (prepared by detritylation of its parent[10]) were spectroscopically identical with authentic samples purchased from Sigma Chemical Co.

(10) Deprotection (30% ammonium hydroxide in water, 55 °C, 15 h) and then reversed-phase HPLC purification was followed by detritylation (80% aqueous acetic acid).

(11) (a) Herdewijn, P.; Charubala, R.; Pfleiderer, W. *Helv. Chim. Acta* **1989**, *72*, 1729. (b) Eritja, R.; et al. *Nucleic Acids Res.* **1986**, *14*, 8135.

(12) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984; pp 143–146.

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