JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Subscriber access provided by ISTANBUL TEKNIK UNIV

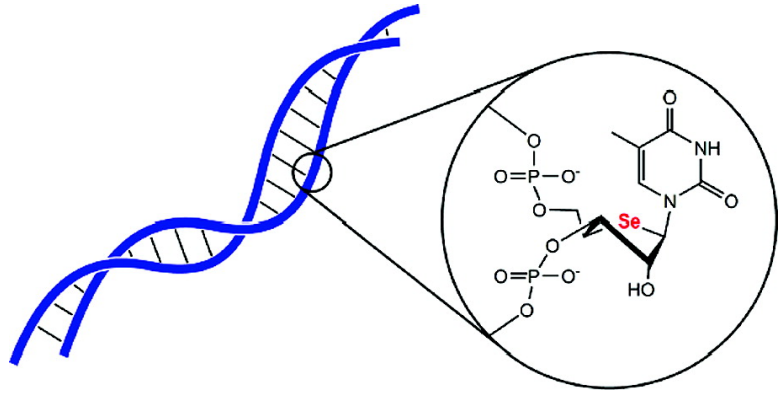
Communication

Synthesis and Biophysical Characterization of Oligonucleotides Containing a 4#-Selenonucleotide

Jonathan K. Watts, Blair D. Johnston, Kumarasamy Jayakanthan, Alexander S. Wahba, B. Mario Pinto, and Masad J. Damha

J. Am. Chem. Soc., **2008**, 130 (27), 8578-8579 • DOI: 10.1021/ja802205u • Publication Date (Web): 11 June 2008

Downloaded from http://pubs.acs.org on February 8, 2009



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

ACS Publications
High quality. High impact.

Journal of the American Chemical Society is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

J|A|C|S
COMMUNICATIONS

Published on Web 06/11/2008

Synthesis and Biophysical Characterization of Oligonucleotides Containing a 4′-Selenonucleotide

Jonathan K. Watts,[†] Blair D. Johnston,[‡] Kumarasamy Jayakanthan,[‡] Alexander S. Wahba,[†] B. Mario Pinto,*[,‡] and Masad J. Damha*[,†]

Department of Chemistry, McGill University, 801 Sherbrooke Street West, Montreal, QC, Canada H3A 2K6, and the Department of Chemistry, Simon Fraser University, Burnaby, BC, Canada V5A 1S6

Received March 26, 2008; E-mail: bpinto@sfu.ca; masad.damha@mcgill.ca

The replacement of the sugar ring (4′-) oxygen of nucleosides and nucleic acids by sulfur has been the subject of numerous studies. In the case of DNA and 2′-deoxy-2′-fluoroarabinonucleic acid (2′F-ANA), sulfur substitution causes a conformational switch to northern, RNA-like conformations that leads to interesting biological properties.[1,2] 4′-S-DNA can be amplified by PCR and can direct transcription in mammalian cells.[3] The conformational shift observed for 4′-S-FANA has made it useful for siRNA gene silencing.[2] 4′-S-RNA has also been applied to modification of aptamers and siRNA.[4]

It is of interest, therefore, to examine the biological properties of the corresponding selenium congeners. Selenium modification of the 4′-position of RNA has been one of the most challenging substitutions. We recently synthesized 4′-selenonucleosides containing adenine, cytosine, thymine, and uracil bases,[5] and after completion of the work became aware that two other groups had independently made the U and C analogues.[6] All three studies showed that 4′-selenoribonucleosides adopt southern, DNA-like conformations. This fact is surprising because of the presence of an α-face 2′-OH group. It would be even more surprising if this conformational preference were manifested in the context of oligonucleotides. For example, in the case of 4′-thio-DNA, a DNA-like conformation is observed for nucleosides,[7] but an RNA-like conformation is displayed by oligonucleotides.[1] For 4′-Se-RNA, a larger ring atom, together with the fact that the α-face 2′-OH group is incompatible with a B-form helix,[8] should similarly favor an RNA-like conformation. To explore this hypothesis, we have undertaken a study of the synthesis and biophysical properties of oligonucleotides containing 5-methyl-4′-selenouridine (abbreviated here for convenience as 4′-Se-rT) and report herein the first synthesis of this class of compounds.[9]

The phosphoramidite derivative of 4′-Se-rT was synthesized using standard procedures. Thus, the 5′-OH was selectively monomethoxytrityl-protected, then the 2′-OH was protected as a silyl ether using TBDMS-Cl and imidazole in DMF,[15] and the 3′-silylated byproduct was removed by chromatography. Finally, the 3′-phosphoramidite was synthesized by treatment with diisopropylamino-(2-cyanoethyl)-phosphoramidic chloride and diisopropylethylamine (DIPEA) in THF. Comparable results were obtained using either this isolated phosphoramidite or an in situ phosphitylation and coupling procedure. For the latter, 5′-MMT-2′-TBDMS-4′-Se-rT (30 μmol) was combined with 1 equiv diisopropylamino-(2-cyanoethyl)-phosphoramidic chloride and 1.16 equiv DIPEA in acetonitrile, and this reaction mixture was stirred for 2−12 h, then injected onto a solid-phase synthesis column containing a 5′-deprotected growing oligonucleotide, in the presence of 5-ethylthiotetrazole or 4,5-dicyanoimidazole activator. After 2−4 h, the column was returned to the DNA synthesizer for oxidation and addition of subsequent nucleotides. This procedure avoids the waste associated with priming the lines of the synthesizer as well as any loss during synthesis and purification of the phosphoramidite. Oligonucleotides were deprotected using methylamine (5 h, room temp) or 3:1 NH_4OH:EtOH (48 h, room temp) followed by tetrabutylammonium fluoride (1 M in THF, 24 h, room temp), and were purified by HPLC, as described in detail in the Supporting Information.

Coupling yields using either method were in the range of 10−40%, and the major byproduct in most of our syntheses was an oligonucleotide truncated immediately before the 4′-Se-rT insert (isolated in yields equal to or greater than those of the full-length product). The other major byproduct was of mass 260 amu greater than that of the truncated product, suggesting that the 4′-Se-rT product does break down after incorporation, at a later stage of the synthesis cycle.

Micura and co-workers observed a reversible oxidation of 2′-SeMe-modified nucleotides during solid-phase synthesis.[13] To avoid degradation of the selenoxide products, they included a reduction step (treatment with dithiothreitol in ethanol/water) after each iodine oxidation step. However, Huang's group did not observe such an oxidation.[12] We obtained similar results with or without a DTT reduction step of this type. A second potential source of breakdown products is the detritylation step, as observed by Carrasco et al. in the context of 2′-SeMe oligonucleotides.[12] Thus we kept TCA treatment steps to 100 s during the synthesis. Finally, it is conceivable that the breakdown could occur under the basic deprotection conditions, but no differences were observed between the conditions we tried.

Three self-complementary sequences were modified with 4′-Se-rT as well as dT, rT, and LNA-T controls (Table 1). We chose to modify one RNA 15mer (**A**), one B-form DNA sequence (the Dickerson-Drew dodecamer, **B**) and one 10mer DNA sequence which often crystallizes in the A-form when modified with an RNA insert (**C**).[16] In addition to these three sequences, a non-self-complementary DNA 10-mer (**D**) was also modified with a central 4′-Se-rT and the corresponding controls (Table 2).

Thermal denaturation studies of the four sequences revealed that the thermal affinity behavior of the 4′Se-RNA insert is usually between that of RNA and LNA (Tables 1−2). For A-form and hybrid duplexes (**A** and **D**:RNA), the progression in T_m was always dT < rT < 4′-Se-rT < LNA-T. As such, 4′-Se-rT inserts led to significant stabilization in these contexts.

In a B-form helix (**D**:DNA), both 4′-Se-rT and rT inserts led to slight destabilization ($\Delta T_m = -1.9$ °C), consistent with the hypothesis that 4′-Se-rT adopts an RNA-like conformation (Table 2). In contrast, LNA, whose high preorganization apparently outweighs the conformational differences, led to a moderate increase.

[†] McGill University.
[‡] Simon Fraser University.

10.1021/ja802205u CCC: $40.75 © 2008 American Chemical Society

Table 1. Sequences and T_m Values of Self-Complementary 4′-Se-rT-Modified Oligonucleotides and Controls.[a]

name	sequence (5′→3′)	T_m[b]	ΔT_m[c]	%H[d]
A1	GGACUGAtCAGUCCA	65.8		27.4
A2	GGACUGATCAGUCCA	68.4	+2.6	26.6
A3	GGACUGAXCAGUCCA	76.3	+10.5	15.7
A4	GGACUGALCAGUCCA	85.7	+19.9	26.3
B1	cgcgaattcgcg	53.2		8.0
B2	cgcgaaTtcgcg	67.6	+14.4	3.7
B3	cgcgaaXtcgcg	70.8	+17.6	4.9
B4	cgcgaaLtcgcg	63.0	+9.8	15.2
C1	gcgtatacgc	68.9		2.0[e]
C2	gcgtaTacgc	64.0	−4.9	4.5
C3	gcgtaXacgc	62.6	−6.3	5.3
C4	gcgtaLacgc	51.5	−17.4	16.5

[a] Legend: RNA, uppercase; dna, lowercase; 4′-Se-rT, X; LNA-T, L. [b] In °C. [c] Change in T_m relative to **A1**, **B1**, and **C1**, respectively. [d] Hyperchromicity percent: the increase in A_{260} upon melting. [e] A lower temperature transition was also observed for this sample only; this value represents only the hyperchromicity of the higher temperature transition.

Table 2. Sequences and T_m Values of Non-Self-Complementary 4′-Se-rT-Modified Oligonucleotides and Controls.[a]

		RNA target[b]			DNA target[c]		
name	sequence (5′→3′)	T_m[d]	ΔT_m[e]	%H	T_m[d]	ΔT_m[e]	%H
D1	ccattatagc	30.8	−	19.1	33.4	−	19.4
D2	ccatTatagc	31.2	+0.4	18.4	31.6	−1.9	15.2
D3	ccatXatagc	32.7	+1.9	14.5	31.6	−1.9	12.9
D4	ccatLatagc	38.8	+8.0	22.9	37.4	+4.0	19.7

[a] Legend as for Table 1. [b] Target strand: 5′-GCUAUAAUGG. [c] Target strand: 5′-gctataatgg. [d] In °C. [e] Change in T_m relative to **D1**.

For sequences **B1**−**B3** and **C1**−**C3**, the low hyperchromicity of melting (%H) implies that a hairpin structure is the main species present under the conditions of our T_m experiments. Thus, the fact that sequence **B** is stabilized by RNA and 4′-Se-RNA, while sequence **C** is destabilized, likely reflects the effect of these modifications on the loop structure. Much higher %H values for **B4** and **C4** suggest that an LNA insert, on the other hand, stabilizes the duplex structure of these sequences because of its very high binding affinity. We note that in both loops, 4′-Se-RNA behaves more like RNA than either DNA or LNA.

For sequence **A**, on the other hand, we have confirmed using native and denaturing PAGE that RNA and 4′-Se-RNA stabilize the duplex structure (Figure S1). Thus, the observed changes in T_m reflect binding affinity in an A-RNA duplex environment.

Two pieces of evidence suggest that despite its increased binding affinity, 4′-Se-RNA leads to a decrease in base stacking. The first is the lower hyperchromicity of melting observed for **A3** and **D3** (Tables 1−2), indicating that the bases are not as well-stacked for this species. The second is the CD spectrum of sequence **A3**, which follows much the same pattern as those of **A1** and **A2**, but with lower intensity for the 265 nm band, which is often associated with base stacking (Figure 1). The same order of CD band intensities is observed for sequence **D**, with both RNA and DNA target strands (Figure S2). The differences are smaller than for sequence **A**; however, perhaps because the greater flexibility of DNA allows it to adapt to the 4′-Se-rT structure with less reduction in base stacking, and also because sequence **D** contains only one insert per duplex instead of two.

In conclusion, the first oligonucleotides containing a 4′-seleno-ribonucleotide have been synthesized and characterized. In contrast to the DNA-like conformation observed for 4′-Se-rT nucleosides, a 4′-Se-rT insert in an oligonucleotide behaved more like RNA than DNA, both in terms of its thermal binding affinity and its effect on hairpin loop structure. 4′-Se-rT modification of A-RNA and hybrid duplexes led to increased binding affinity. Paradoxically, it also caused base destacking. Studies on the use of 4′-Se-RNA modifications in the phasing of X-ray crystallographic data, which will also allow confirmation of the RNA-like structure of 4′-Se-RNA, are underway.

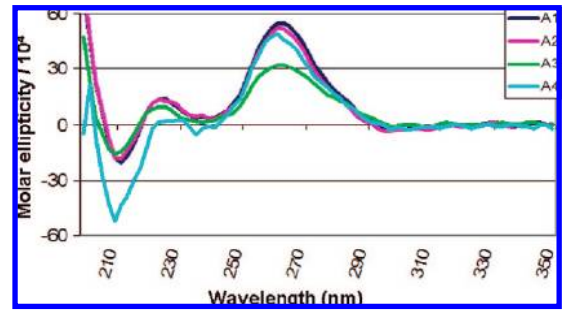


Figure 1. CD spectra of sequences **A1**−**A4**.

Acknowledgment. We are grateful to NSERC Canada for financial support in the form of grants (to B.M.P. and M.J.D.) and a postgraduate fellowship (to J.K.W.).

Supporting Information Available: Experimental details, MS data, CD spectra of **B**, **C**, and **D**, and PAGE showing that 4′-Se-rT stabilizes the double-stranded structure of sequence **A**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Inoue, N.; Minakawa, N.; Matsuda, A. *Nucleic Acids Res.* **2006**, *34*, 3476–3483.
(2) Watts, J. K.; Choubdar, N.; Sadalapure, K.; Robert, F.; Wahba, A. S.; Pelletier, J.; Pinto, B. M.; Damha, M. J. *Nucleic Acids Res.* **2007**, *35*, 1441–1451.
(3) Inoue, N.; Shionoya, A.; Minakawa, N.; Kawakami, A.; Ogawa, N.; Matsuda, A. *J. Am. Chem. Soc.* **2007**, *129*, 15424–15425.
(4) (a) Leydier, C.; Bellon, L.; Barascut, J. L.; Morvan, F.; Rayner, B.; Imbach, J. L. *Antisense Res. Dev* **1995**, *5*, 167–174. (b) Hoshika, S.; Minakawa, N.; Matsuda, A. *Nucleic Acids Res.* **2004**, *32*, 3815–3825. (c) Dande, P.; Prakash, T. P.; Sioufi, N.; Gaus, H.; Jarres, R.; Berdeja, A.; Swayze, E. E.; Griffey, R. H.; Bhat, B. *J. Med. Chem.* **2006**, *49*, 1624–1634. (d) Hoshika, S.; Minakawa, N.; Shionoya, A.; Imada, K.; Ogawa, N.; Matsuda, A. *ChemBioChem* **2007**, *8*, 2133–2138.
(5) Jayakanthan, K.; Johnston, B. D.; Pinto, B. M., *Carbohydr. Res.* **2008**, DOI: 10.1016/j.carres.2008.02.014.
(6) (a) Inagaki, Y.; Minakawa, N.; Matsuda, A. *Nucleic Acids Symp. Ser.* **2007**, *51*, 139–140. (b) Jeong, L. S.; Tosh, D. K.; Kim, H. O.; Wang, T.; Hou, X.; Yun, H. S.; Kwon, Y.; Lee, S. K.; Choi, J.; Zhao, L. X. *Org. Lett.* **2008**, *10*, 209–212.
(7) Koole, L. H.; Plavec, J.; Liu, H. Y.; Vincent, B. R.; Dyson, M. R.; Coe, P. L.; Walker, R. T.; Hardy, G. W.; Rahim, S. G.; Chattopadhyaya, J. *J. Am. Chem. Soc.* **1992**, *114*, 9936–9943.
(8) Tomita, K.-I.; Rich, A. *Nature* **1964**, *201*, 1160–1163.
(9) We note that selenium derivatization is becoming an increasingly important tool for X-ray crystallography of nucleic acids: modifications have included the incorporation of phosphosphoroselenoate,[10] 2′-selenomethyl,[11–13] and 5′-seleno[14] moieties.
(10) (a) Nemer, M. J.; Ogilvie, K. K. *Tetrahedron Lett.* **1980**, *21*, 4149–4152. (b) Wilds, C. J.; Pattanayek, R.; Pan, C.; Wawrzak, Z.; Egli, M. *J. Am. Chem. Soc.* **2002**, *124*, 14910–14916.
(11) (a) Du, Q.; Carrasco, N.; Teplova, M.; Wilds, C. J.; Egli, M.; Huang, Z. *J. Am. Chem. Soc.* **2002**, *124*, 24–25. (b) Teplova, M.; Wilds, C. J.; Wawrzak, Z.; Tereshko, V.; Du, Q.; Carrasco, N.; Huang, Z.; Egli, M. *Biochimie* **2002**, *84*, 849–858.
(12) Carrasco, N.; Buzin, Y.; Tyson, E.; Halpert, E.; Huang, Z. *Nucleic Acids Res.* **2004**, *32*, 1638–1646.
(13) (a) Hobartner, C.; Micura, R. *J. Am. Chem. Soc.* **2004**, *126*, 1141–1149. (b) Hobartner, C.; Rieder, R.; Kreutz, C.; Puffer, B.; Lang, K.; Polonskaia, A.; Serganov, A.; Micura, R. *J. Am. Chem. Soc.* **2005**, *127*, 12035–12045. (c) Moroder, H.; Kreutz, C.; Lang, K.; Serganov, A.; Micura, R. *J. Am. Chem. Soc.* **2006**, *128*, 9909–9918.
(14) Carrasco, N.; Ginsburg, D.; Du, Q.; Huang, Z. *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 1723–1734.
(15) Ogilvie, K. K.; Sadana, K. L.; Thompson, E. A.; Quilliam, M. A.; Westmore, J. B. *Tetrahedron Lett.* **1974**, *15*, 2861–2863.
(16) Lubini, P.; Zürcher, W.; Egli, M. *Chem. Biol.* **1994**, *1*, 39–45.

JA802205U