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Preparation, Characterization and Binding Properties of an Oligodeoxynucleotide Containing the 3'-Ribothioformacetal Phosphate Analog

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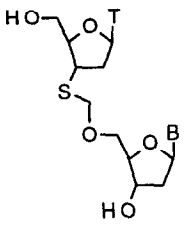
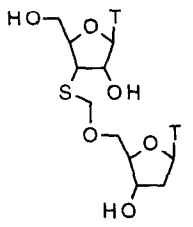
ABSTRACT: A thymine-thymine dimer containing the 3'-ribothioformacetal linkage **2** was prepared and incorporated into an oligodeoxynucleotide(ODN). The positions of 3'-ribothioformacetal **2** linkages were confirmed by partial and complete cleavage using $HgCl_2$. The ODN containing this synthon **2** binds to a complementary single stranded RNA with slightly less affinity as compared to the control ODN containing all 2'-deoxyribose nucleosides and phosphodiester linkages.

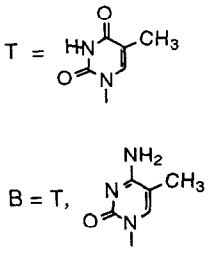
Oligodeoxynucleotide (ODN) analogs are of interest because of their potential ability to inhibit gene expression within cells.[1] We have recently described the synthesis and binding properties to complementary RNA of an ODN containing the 3'-thioformacetal **1** replacement of phosphodiester linkages.[2] The 3'-thioformacetal bearing ODNs demonstrated enhanced binding to a single stranded RNA target as compared to the control phosphodiester.[2] We now report the synthesis of the 2'-hydroxyl containing 3'-ribothioformacetal analog as a thymine-thymine dimer, **2**, its incorporation into an oligonucleotide and the resulting binding properties to the complementary RNA sequence.



1

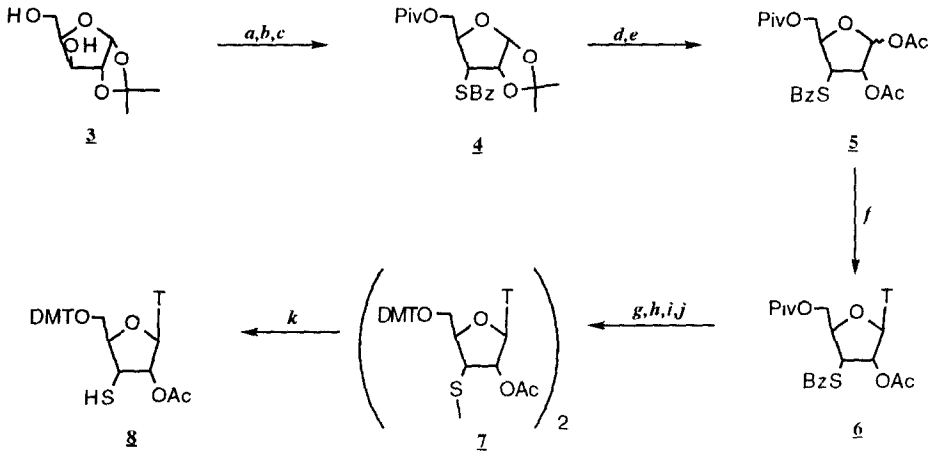


2



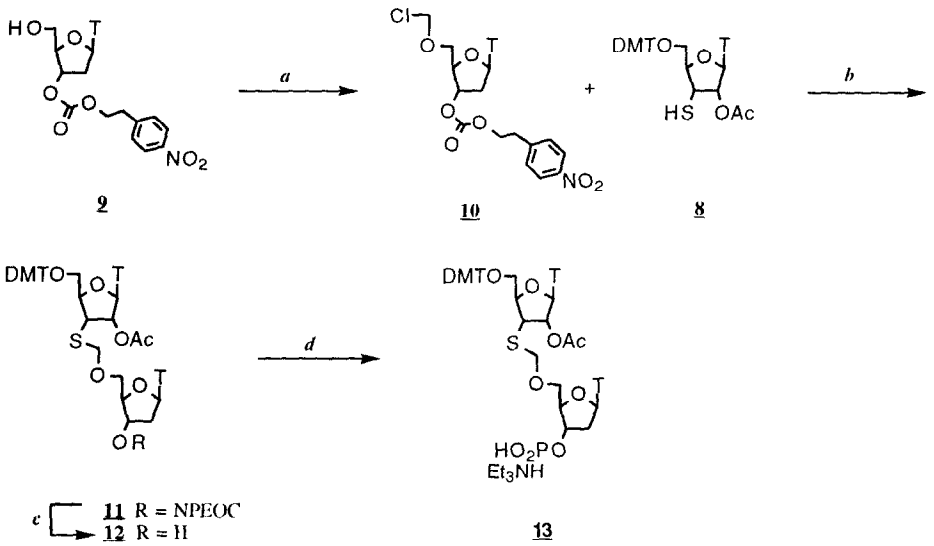
The synthesis (**Scheme 1**) started from commercial available 1,2-O-isopropylidene-α-D-xylofuranose **3**. The 5-position of **3** was selectively protected with trimethylacetyl chloride in pyridine. After activating the hydroxyl group at 3-position via trifluoromethylsulfonyl anhydride ($(TfO)_2O$) in pyridine, the thiobenzoyl was introduced to produce **4**. The isopropylidene group was removed with formic acid and the resulting hydroxyl groups were acetylated to yield compound **5**. Persilylated thymine was glycosylated with **5** by using TMS-triflate as the catalyst in acetonitrile to yield N-1-(2-O-acetyl-3-deoxy-5-O-pivaloyl-3-thiobenzoyl-β-D-ribofuranosyl)thymine (**6**).[3] Only the N-1-β-isomer, identified by ^{1}H-NMR, UV and MS analysis, was obtained. Deprotection of the acyl groups of **6** was followed by oxidation to the disulfide. Dimethoxytrityl and acetyl groups were then introduced into the 5'- and 2'-positions respectively to yield **7**.[4] The disulfide **7** was reduced *in situ* with sodium borohydride to **8** and used without purification.

Scheme 1:



a. Trimethylacetyl chloride, pyridine, 90%; *b*. $(TIO)_2O$, CH_2Cl_2, pyridine; *c*. thiobenzoic acid, NaH in DMF at 0°C, 60% for two steps; *d*. 75% formic acid at 50°C; *e*. Ac_2O, pyridine, 80% for two steps; *f*. perstlylated thymine (with Bis(trimethylsilyl) acetamide in acetonitrile), TMSOTf, acetonitrile at 70°C, 85%; *g*. NaOMe in MeOH; *h*. I_2 in pyridine; *i*. DMTCl in pyridine; *j*. Ac_2O in pyridine, 70% for four steps; *k*. $NaBH_4$ in EtOH (not isolated).

Scheme 2:



a. $(CH_2O)_n$, HCl in CH_2Cl_2 (not isolated); *b* DIPEA in CH_2Cl_2, 60%; *c*. DBU in CH_3CN, 80%; *d*. 2-chloro-4-H-1,3,2-benzodioxaphosphorin-4-one, pyridine,CH_2Cl_2, 75%

The p-nitrophenylethyloxycarbonyl (NPEOC) group was chosen to protect 3'-position of **9**[5] **(Scheme 2)**. This protecting group can be removed selectively by β elimination[5] namely DBU in acetonitrile at 20°C. Compound **9** was chloromethylated[2] to **10** at 0°C for 2 hours and evaporated. The resulting oil was dissolved in dichloromethane and added into the solution of **8** (1 equivalent based on **9**) in the presence of diisopropylethylamine (DIPEA) yielding the thymine-thymine dimer **11**. Deprotection of **11** produced the dimer **12**[6] which was converted to the H-phosphonate **13**.[7]

The sequences **P**, **R** and **T** shown in **Table 1** were synthesized via a H-phosphonate protocol.[8] Sequence **R** contained the thymine-thymine ribothioformacetal, **2**, at two positions as shown. Sequence **T** contained the previous reported thymine-thymine thioformacetal, **1**.[2]

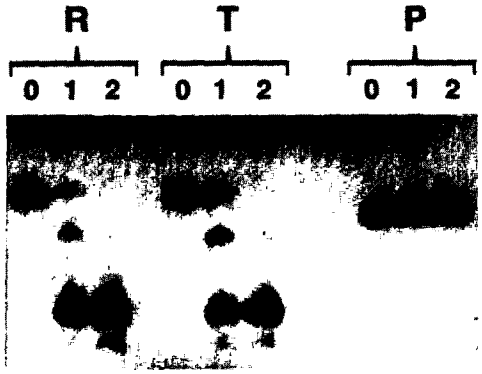
Table 1 Tm Analysis

		Tm°C	(ΔTm)
C. Complementary RNA	3' ApGpApGpApGpApGpApGpApApApApA 5		
P. Phosphodiester	5' d(TpCpTpCpTpCpTpCpTpCpTpTpTpTpT) 3'	62.5	
R. Ribothioformacetal	5' d(TpCpTpCpTpCpTpCpTpCpT*TpT*TpT) 3'	62.0	(-0.5)
T. Thioformacetal	5' d(TpCpTpCpTpCpTpCpTpCpT*TpT*TpT) 3'	63.0	(0.5)

* = modified linkage; p = phosphodiester bond; C = 5-Methyl C

The incorporation and the position of the ribothioformacetal (**2**) and thioformacetal (**1**) linkages were confirmed by partial and complete cleavage using $HgCl_2$ (**Figure 1**). Mercuric salts are known deprotection agents for the methylthiomethyl protecting group.[9] ODNs were 5'-end labeled with ^{32}P and treated with 1mM aqueous $HgCl_2$. Denaturing PAGE (polyacrylamide gel electrophoresis) analysis after $HgCl_2$ cleavage and β-mercaptoethanol quench showed two cleavages corresponding to the two modified linkages for **R** and **T**. The phosphodiester control **P** showed no cleavage under identical conditions. The ODNs were further characterized by digestion with nuclease and phosphatase and HPLC analysis of the monomers and dimers.[10] All ODNs showed the expected ratios.

Figure 1:



$HgCl_2$ Mapping conditions: The 5' labeled ODNs were treated with 1mM $HgCl_2$ in H_2O at 20°C (lane **0**: no treatment; lane **1**: 30 seconds and lane **2**: 15 minutes) and quenched with 10 mM β-mercaptoethanol.

The effect of the modification on hybridization affinity was assessed by thermal denaturation. The melting temperature(Tm) (**Table 1**) of analog/single stranded RNA duplexes was compared to duplex derived from the unmodified ODN **P**. The Tm of the ribothioformacetal duplex was slightly lower relative to the Tm's resulting from duplex derived from the thioformacetal and the control phosphodiester.

The synthesis of T*T dimer containing ribothioformacetal has been accomplished. The introduction of a hydroxyl group at the 2'-position has only a slight destablization effect on the duplex formation in this context. The 2'-OH is known to stablized glycosidic linkages to acidic conditions.[11] This ribo modification may find utility in thioformacetal containing oligonucleotide analogs which bear heterocycles particularly prone to deglycosylation such as adenine.

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REFERENCES AND NOTES:

1. Milligan, J. F.; Matteucci, M. D.; Martin, J. C. *J. Med. Chem.* **1993,** *36*, 1923.
2. Jones, R. J.; Lin, K.-Y.; Milligan, J. F.; Wadwani, S.; Matteucci, M. D. *J. Org. Chem.* **1993**, *58*, 2983.
3. Niedballa, U; Vorbruggen, H. *Angew. Chem.*, **1970**, *82*, 449.
4. Compound 7: ^{1}H-NMR δH(300MHz, $CDCl_3$) 9.96 (s, 1H, -NH); 7.73 (s, 1H, 6-H); 6.70-7.46 (m, 1 H, Ar-H); 6.04 (d, 1H, H-1'); 5.93 (d, 1H, H-2'); 3.82-3.97 (m, 2H, H-3' and H-4'); 3.78 (s, 6H, 2x-OCH_3); 3.54 (ddd, 2H, H-5'); 2.02 (s, 3H, -OAc); 1.65 (s, 3H, 5-CH_3). MS. required 1235.4, found M^+ 1234.8·
5. Himmelsbach, F.; Schultz, B. S.; Trichtinger, T.; Charubala, R.; Pfleiderer, W.; *Tetrahedron*, **1984**, *40*, 59.
6. Compound 12: ^{1}H-NMR δH(300MHz, $CDCl_3$) 9-10 (2 br.s, 2H, 2x-NH); 6.70-7.60 (m, 15 H, Ar-H and 2x6-H on T); 6.28 (t, 1H, H-1' of deoxyribo); 5.78 (d, 1H, H-1' of ribo); 5.72 (d, 1H, H-2' of ribo); 5.61 (q, 2H, -SCH_2O-); 4.40 (m, 1H, H-3' of deoxyribo); 3.78 (s, 6H, 2x-OCH_3); 3.30-4.20 (m, 7H, H-3', H-4', H-5' of ribo and H-4', H-5' of deoxyribo); 2.16-2.38 (m, 2H, H-2 of deoxyribo); 2.18 (s, 3H, -$COCH_3$); 1.94 (s, 3H, 5-CH_3 of deoxyribo T); 1.52 (s, 3H, 5-CH_3 of ribo T). MS: required 872.9, found M^+ 872.4.
7. Marugg, J. E.; Tromp, M.; Kuyl-Yeheskiely, E.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.*, **1986,** *27*, 2661.
8. Froehler, B. C.; Ng, P.; Matteucci, M. D. *Nucl. Acid Res.* ***1986,*** *14*, 5399.
9. Corey, E. J.; Bock, M. G. *Tetrahedron Lett.* **1975**, *16*, 3269.
10. Eadie, J. S.; McBride, L. J.; Huff, L. B.; Cartheart, R. *Anal. Biochem.* **1987,** *165*, 442.
11. Michelson, A. M. in *The Chemistry of Nucleosides and Nucleotides,* Academic Press, NY, **1963** 26.

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