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THE SYNTHESIS AND BINDING PROPERTIES OF OLIGONUCLEOTIDE ANALOGS CONTAINING DIASTEREOMERICALLY PURE CONFORMATIONALLY RESTRICTED ACETAL LINKAGES

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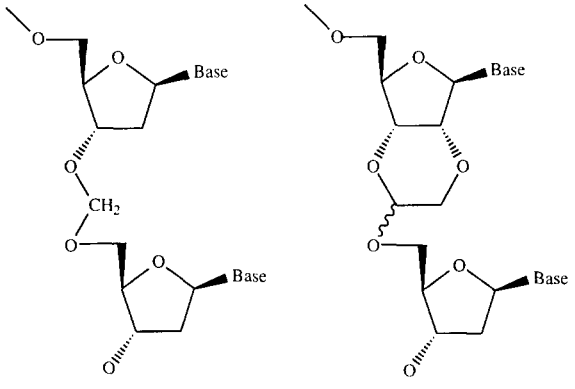
Abstract: The synthesis of 5,6-bicyclic thymine:thymine nucleoside containing a conformationally restricted dioxane acetal has been achieved from 2′-*O*-allyl 5-methyl uridine. The two diastereomers were separated and incorporated in a single position within an oligonucleotide (ON) sequence. The binding properties of these ONs when hybridized to complementary RNA and DNA were evaluated by thermal denaturation (Tm) analysis. Lower Tms for both diastereomers were obtained when compared to the corresponding control phosphodiester ON.

The covalent conformational preordering of a ligand to resemble its bound state is a central concept of ligand receptor design.[1,2] The classic crown ether work has spawned success in many structural recognition areas.[1,2] Attempts to preorder an ON to resemble its bound helical conformation have recently been reported.[3,4] Acetal internucleotide connections with ONs offer the opportunity to preorder key torsion angles of the backbone.

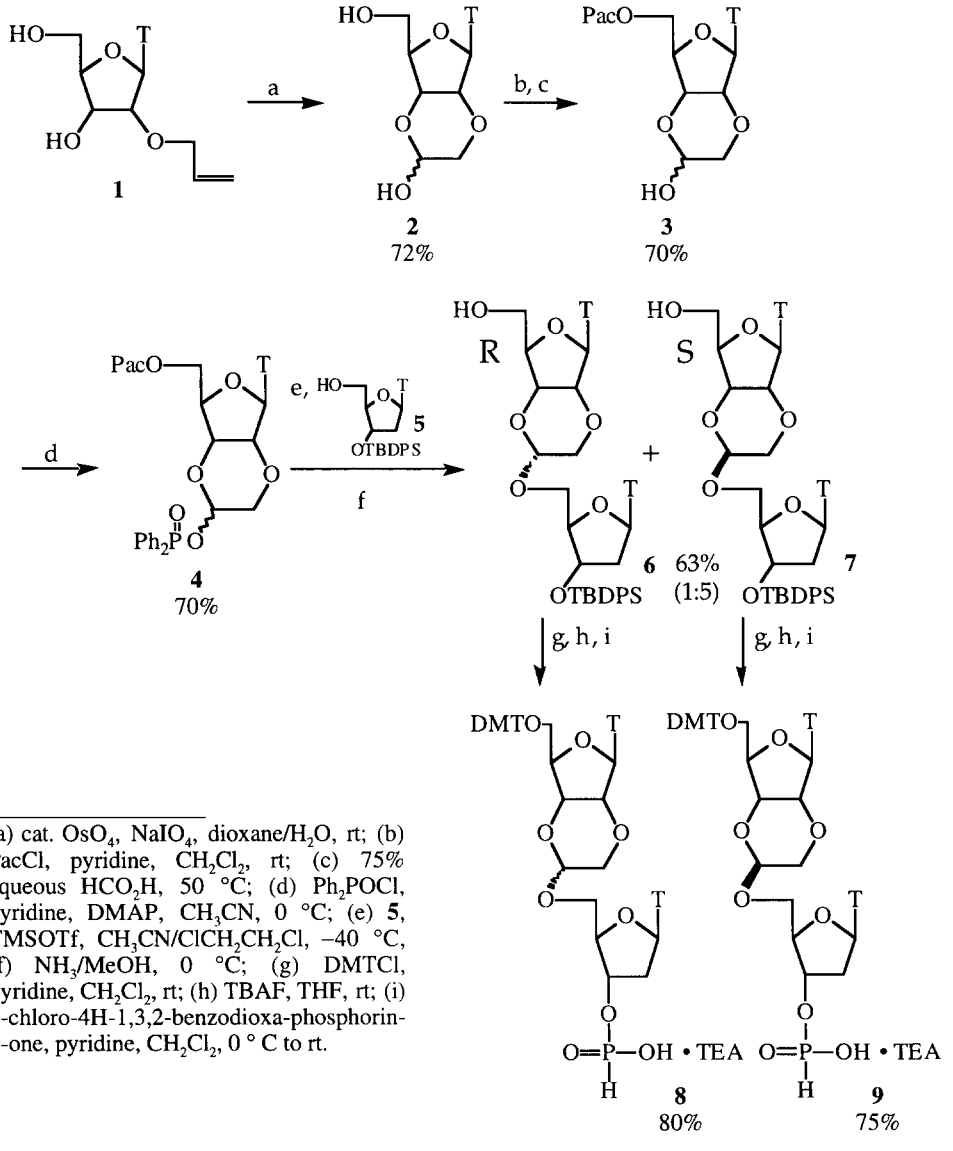
Figure 1



Formacetal **Dioxane Acetal**

The formacetal internucleotide linkage is a simple, neutral analog for phosphate.[5] The 2′ oxygen of ribose can be linked via a carbon spacer to the acetal carbon creating a connection reminiscent of polysaccharides. This dioxane acetal has been examined by modeling studies with the prediction that the R isomer could exist in the appropriate preordered conformation for formation of A-type helix with RNA.[6] A key feature of this restriction is that it should allow the ribose ring to assume its normal or 2′ exo "North" conformation. The synthesis of dimers bearing this linkage has been achieved directly from the 2′-*O*-allyl nucleoside derivatives. The binding postulate has been tested by synthesizing a thymine: thymine dimer, separating diastereomers, incorporating each into an ON, and measuring the ON's binding properties with complementary DNA and RNA.

Scheme 1. Synthesis of Dinucleosides 8 and 9



(a) cat. OsO_4, $NaIO_4$, dioxane/H_2O, rt; (b) PacCl, pyridine, CH_2Cl_2, rt; (c) 75% aqueous HCO_2H, 50 °C; (d) Ph_2POCl, pyridine, DMAP, CH_3CN, 0 °C; (e) **5**, TMSOTf, $CH_3CN/ClCH_2CH_2Cl$, −40 °C, (f) NH_3/MeOH, 0 °C; (g) DMTCl, pyridine, CH_2Cl_2, rt; (h) TBAF, THF, rt; (i) 2-chloro-4H-1,3,2-benzodioxa-phosphorin-4-one, pyridine, CH_2Cl_2, 0 ° C to rt.

2′-*O*-allyl-5-methyl uridine (**1**) was derived from thymine and ribose in 6 steps, as described in the literature.[7,8] Oxidation of **1** with catalytic $OsO_4/NaIO_4$ afforded hemiacetal **2** which was further transformed into the 5′-phenoxyacetyl (Pac) derivative **3** in 2 steps. Treatment of **3** with diphenylphosphinic chloride produced diphenylphosphinate **4**.[9] Coupling of **4** with 3′-*O*-*tert*-butyldiphenylsilyl thymidine **5** and selective removal of the

5′-*O*-Pac protecting group gave a 1:5 ratio of dinucleosides **6** and **7**, which were separated by flash silica gel chromatography.[10,11] Dimethoxytritylation and desilylation followed by phosphitylation[12] of **6** and **7** generated the corresponding H-phosphonates **8** and **9**, respectively. Compounds **8** and **9** were incorporated into 12mer ONs as T^{R}•T and T^{S}•T dimer blocks by standard solid-phase DNA chemistry using a H-phosphonate protocol.[13,14]

The binding properties of ONs with both single stranded RNA and DNA were evaluated by Tm analysis (Table 1). When hybridized to an RNA target, the Tm of the ON containing dimer **8** was lower than that of the phosphodiester control ON by 8 °C and no binding was observed for the ON containing dimer **9**. Similar results were also obtained for the corresponding DNA complement.

Table 1. Tm Analysis of ONs Against RNA and DNA
5′ $TC^{M}ATTTTTTC^{M}TT$

	Tm °C	
TT =	RNA	DNA
Phosphodiester control	37.0	39.5
3′, 5′ Formacetal	36.0	38.0
R Dioxane **8**	29.0	30.5
S Dioxane **9**	<25	<25

*C^{M} is 5-methyl-2′-deoxycytidine. Tm values were determined in a buffer solution of 140 mM KCl/5mM Na_2HPO_4/1 mM $MgCl_2$ at pH 7.2 and the concentration of all ONs was about 2 μM. Tm values are ±0.5 °C.

The poor binding affinity of the R isomer was not predicted by modeling studies.[6] Such studies did not take into account the conformational state of adjacent nucleosides. These 2′ deoxyribose nucleosides would be largely in the 2′ endo or "South" conformation. Consequently, the local conformation of the ribose ring would likely alternate between "North" and "South" conformations. Such rapid conformation switching has been shown to result in duplex instability relative to continuous "North" or "South" conformation.[15] This instability may be amplified in the current case given the rigidity of the analog ribose ring. Such conformationally restricted ONs bearing 2′-*O*-methyl substituents on all non-restricted nucleoside ribose positions could be expected to provide enhanced binding. Testing awaits further synthesis.

Acknowledgments

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10. Compound **8**: ^{1}H NMR δ (500 MHz, $CDCl_3$) 8.84 (s, 1H), 8.79 (s, 1H), 7.66 (m, 4H), 7.48–7.44 (m, 6H), 7.25 (s, 1H, ribo H6), 7.18 (s, 1H, deoxy H6), 6.41 (m, 1H, deoxy 1′), 5.92 (d, J = 8 Hz, 1H, ribo 1′), 4.74 (triplet, J = 6 Hz, 1H, dioxane 1), 4.38 (m, 1H, ribo 2′), 4.37 (m, 1H, deoxy 3′), 4.15 (m, 1H, ribo 3′), 4.09 (m, 1H, deoxy 4′), 4.05 (m, 1H, ribo 4′), 3.91 (dd, J = 11 Hz, J = 6 Hz, 1H, dioxane 2′), 3.78 (m, 1H, ribo 5″), 3.44 (m, 2H, deoxy 5″, 5′), 3.42 (m, 1H, ribo 5′), 3.28 (dd, J = 11 Hz, J = 6 Hz, 1H, dioxane 2), 2.34 (m, 1H, deoxy 2″), 1.99 (m, 1H, deoxy 2′), 1.90 (s, 3H, ribo 5-CH_3), 1.84 (s, 3H, deoxy 5-CH_3), 1.78 (s, br, 1H, ribo 5′-OH), 1.09 (s, 9H). The R configuration was determined by ROESY spectra.

11. Compound **9**: ^{1}H NMR δ (500 MHz, $CDCl_3$) 8.80 (s, 1H), 8.70 (s, 1H), 7.66 (m, 4H), 7.49-7.43 (m, 6H), 7.35 (s, 1H, ribo H6), 7.06 (s, 1H, deoxy H6), 6.35 (m, 1H, deoxy 1′), 5.58 (d, J = 8 Hz, 1H, ribo 1′), 4.46 (m, 1H, deoxy 3′), 4.31 (m, 1H, ribo 3′), 4.27 (m, 1H, ribo 4′), 4.23 (m, 1H, ribo 2′), 4.21 (triplet, J = 6 Hz, 1H, dioxane 1), 4.03 (m, 1H, deoxy 4′), 3.92 (m, 1H, ribo 5″), 3.87 (m, 1H, deoxy 5″), 3.73 (m, 1H, ribo 5′), 3.51 (dd, J = 11 Hz, J = 6 Hz, 1H, dioxane 2′), 3.46 (dd, J = 11 Hz, J = 6 Hz, 1H, dioxane 2), 3.10 (m, 1H, deoxy 5′), 2.63 (s, br, 1H, ribo-5′-OH), 2.46 (m, 1H, deoxy 2″), 2.01 (m, 1H, deoxy 2′) 1.89 (s, 3H, ribo 5-CH_3), 1.86 (s, 3H, deoxy 5-CH_3), 1.08 (s, 9H). The S configuration was determined by ROESY spectra.

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14. ON containing dimer **8** MALDI TOF MS: 3572.2 (calcd 3571.5 MH^+); ON containing dimer **9** MALDI TOF MS: 3572.2 (calcd 3571.5 MH^+).

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