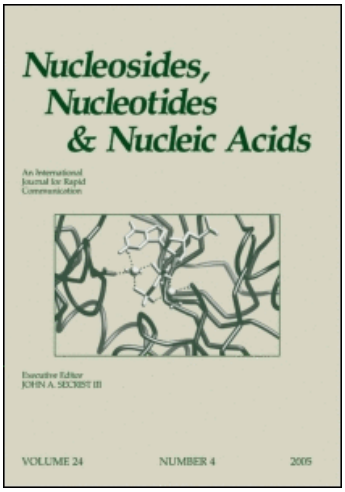

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Hybridization Properties of a DeoxyoIigonucleotide Containing Four Formacetal Linkages

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Hybridization Properties of a Deoxyoligonucleotide Containing Four Formacetal Linkages.

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Abstract: The deoxyoligonucleotide TCTCTCTCTCTTTT bearing four formacetal linkages has been synthesized. The hybridization properties of this compound with complementary RNA and single stranded DNA has been compared to control sequences bearing phosphodiester, phosphoramidate and methylphosphonate linkages. The formacetal oligomer hybridizes to RNA similarly to a diester and significantly better than the other neutral linkages.

Recently we reported the synthesis of a deoxyoligonucleotide bearing two formacetal linkages at the three prime end (1). This oligomer was characterized for binding to a complementary RNA sequence. We now report the synthesis of the sequence 5' TCTC*TC*TC*TC*TTTT where C is 5 methyl deoxycytidine and * is the formacetal linkage. Controls were prepared in which the modified linkage position * was the normal phosphodiester, methylphosphonate and methoxyethyl phosphoramidate. These four sequences which differ only in the backbone linkage at the starred positions were compared for their ability to hybridize to a complementary RNA and single stranded DNA sequences.

Synthesis: The diester control was synthesized in standard fashion (2). The formacetal, methyl phosphonate and amidate were synthesized in similar fashion, namely the construction of a dimer synthon bearing a dimethoxytrityl (DMT) group on the 5' 5-methyldeoxycytidine, the modified 3' 5' linkage and H-phosphonate on the 3' thymidine (Figure 1). These dimer synthons were then incorporated in the appropriate positions (2). The formacetal dimer was constructed as previously reported (1). The phosphoramidate (3) and methyl phosphonate (4) dimers were

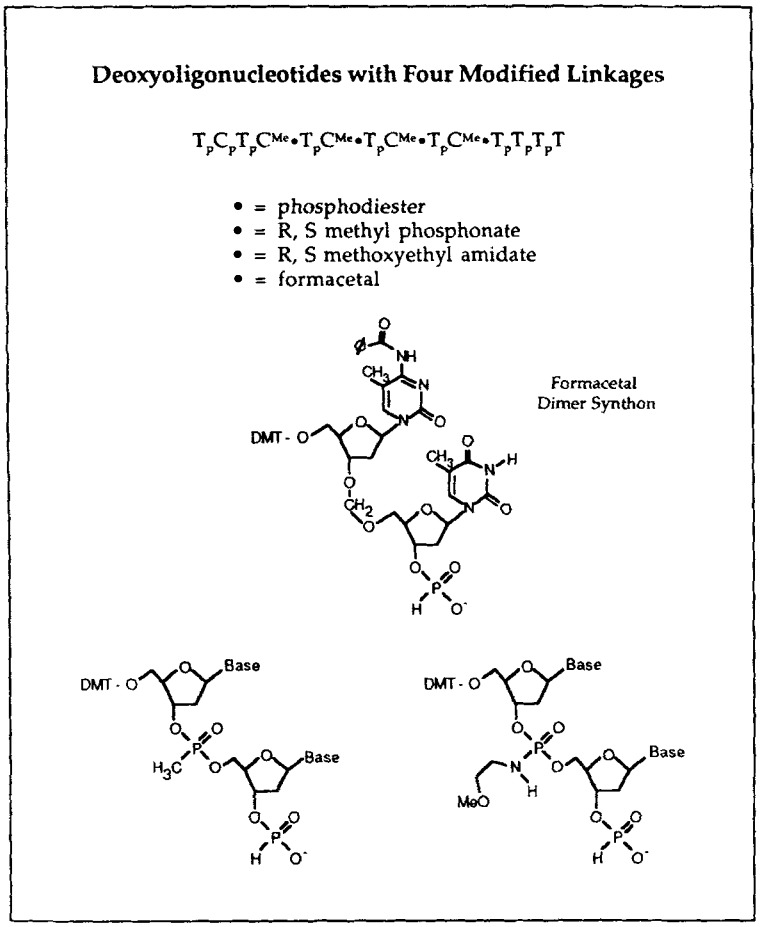

Figure 1

constructed using 5'DMT-N Benzoyl-5-methyldeoxycytidine and 3-t-butyldimethyl-silylthymidine using known chemistry (3, 4). All oligos were deblocked with concentrated NH_4OH for 16 hours at 20°C and purified by gel electrophoresis.

Hybridization to Complementary RNA and DNA: The complementary RNA was synthesized by T7 polymerase reaction on the appropriate primer template (5). Thermal denaturation comparisons were done by hybridizing the test oligos to the RNA or DNA and measuring the UV absorbation at temperatures from 20 to 80°C. The buffer contained salts designed to roughly mimic intracellular salt composition and concentration (140mM KCl, 10Mm NaCl, 5mM $MgCl_2$, 5mM NaH_2PO_4, pH 7). Sequences were used at a concentration of 2 micromolar. All compounds gave monophasic transitions and T_m values were assigned by finding the temperature at

Table 1

T_m of

$T_pC_pT_pC^{Me}\bullet T_pC^{Me}\bullet T_pC^{Me}\bullet T_pC^{Me}\bullet T_pT_pT_pT$

and Complementary RNA & DNA

Oligomer	ssRNA	ssDNA
Phosphodiester	60.0°C	49.5°C
Methyl Phosphonate	50.5	
Methoxyethyl Amidate	47.5	38.5
Formacetal	59.0	39.0

Buffer: 10 mM NaCl
140 mM KCl
5 mM Na_2PO_4
5 mM MgCl
pH 7

which the slope of the curve was maximum. The values are reported in Table 1. This data demonstrates that the formacetal is comparable to the diester in RNA binding and superior to both the methylphosphonate and phosphoramidate linkages. The formacetal compound binds to single stranded DNA with significantly less affinity as compared to the diester control.

The formacetal sequence binds to RNA with an affinity comparable to the diester control and significantly better than the methyl phosphonate and phosphoramidate. This is significant because oligonucleotides bearing neutral phosphate linkage replacements are of great interest because of their reported ability to cross cell membranes (7, 8, 9) and their nuclease resistance (10, 11). These properties are important for the eventual therapeutic applications of modified oligonucleotides in the control of gene expression.

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