Pergamon

Tetrahedron Letters, Vol. 35, No. 50, pp. 9315-9318, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)01992-4

Deoxyoligonucleotides Containing 2',5' Acetal Linkages: Synthesis and Hybridization Properties

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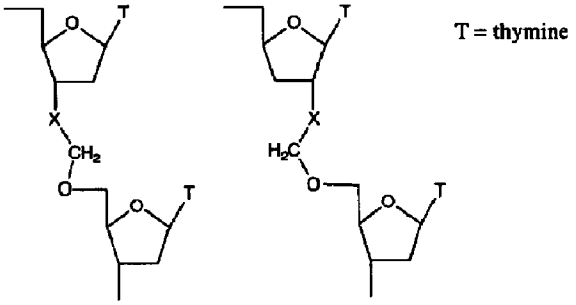
Abstract: Two 3'-deoxy-5-methyluridine-3'-deoxy-5-methyluridine dimers interconnected by 2',5' formacetal **3** and thioformacetal **4** were synthesized and incorporated into oligonucleotides. The 2',5' thioformacetal derivative resulted in substantial destabilization of duplex formation with ssRNA and ssDNA, the 2',5' formacetal ODN resulted in only modest destabilization of ssDNA and ssRNA as compared to the control 3',5' phosphodiester.

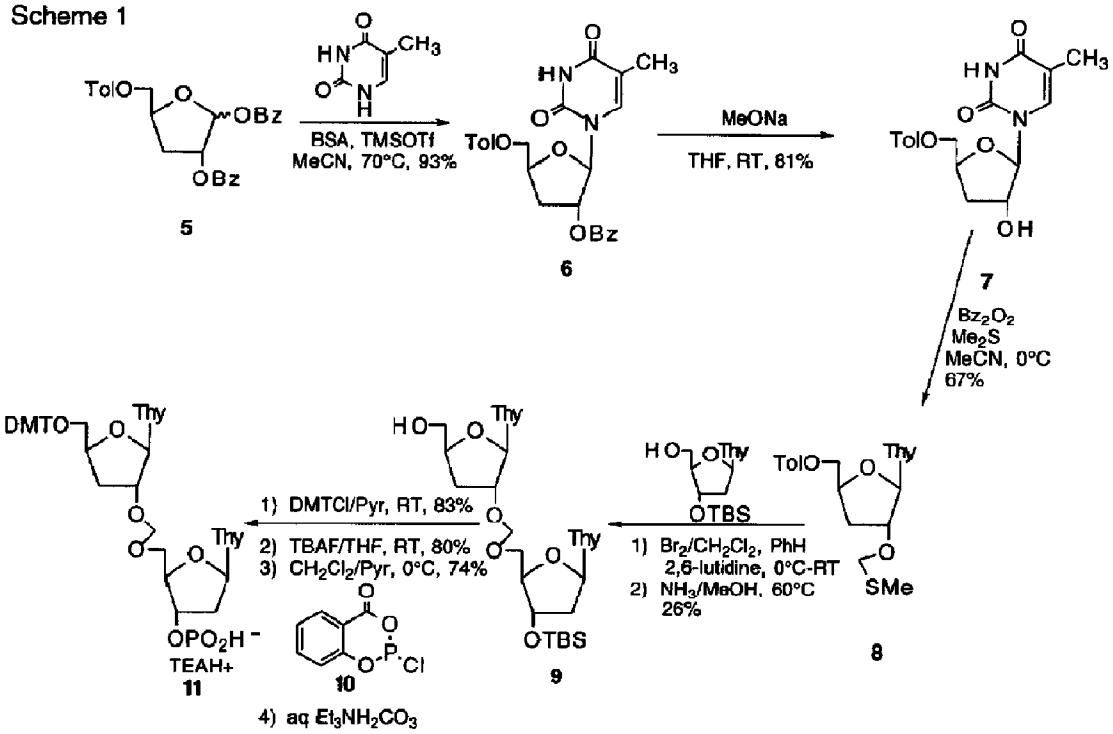
The 3',5' phosphodiester linkage in DNA and RNA serves as the basic scaffold for nucleic acid recognition.[1] Structural and evolutionary questions have resulted in the synthesis of 3'-deoxyoligonucleotides (ODN) bearing 2',5'-phosphodiester linkages and characterization of their hybridization properties with single stranded DNA.[2] Such hybrids have been shown to possess greatly diminished stability relative to unmodified DNA duplex. The 2',5' phosphodiester linkage in the context of oligonucleotides (ON) bearing 3' hydroxyls has been shown to confer selective hybridization properties to RNA over DNA, however, the RNA affinities are poorer than the native 3',5' connection.[3] The 2',5' connection in the realm of achiral phosphodiester replacements has not been explored.

Molecular modeling of A and B form helices suggest a shorter, smaller 2',5' linkage relative to a phosphodiester would result in favorable helix formation.[4] The synthesis and binding properties of the 3',5' formacetal,[5,6] a small neutral achiral 3',5' phosphate replacement **1** and its longer thio analog, the 3' thioformacetal **2** have been previously reported.[6] We now wish to report the synthesis of ODNs containing the formacetal **3** and the thioformacetal **4** as a 2',5' connection and their corresponding *in vitro* binding properties to single stranded RNA and single stranded DNA.

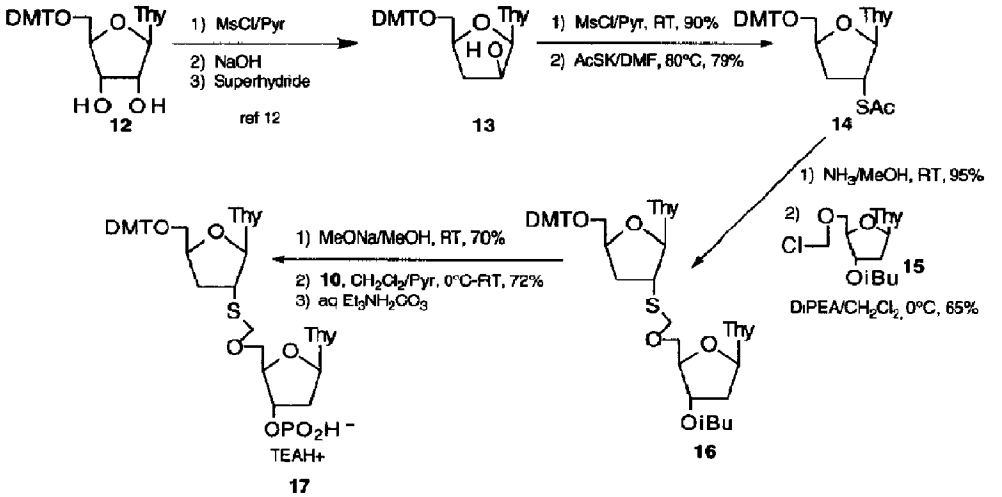


The modified backbones were synthesized within 5-methyluridine-5-methyluridine dimers (TT) which were in turn incorporated into ODNs as alternating linkages with phosphodiester. Scheme 1 shows the synthesis of the 2',5' formacetal T-T dimer. The 3'-deoxy sugar was synthesized according to a literature report[2a] to furnish compound **5** which was then condensed with thymine under Vorbruggen-type glycosylation conditions[7] to furnish the 3'-deoxy nucleoside **6**. Compound **6** was selectively deprotected at the 2'-position with sodium methoxide to furnish compound **7** which was treated with benzoyl peroxide/methyl sulfide[8] to form the methylthiomethyl ether **8**. **8** was then condensed[6] with 3'-t-butyldimethylsilyl-thymidine and the

Scheme 1



Scheme 2



4-methyl-benzoyl group was removed with methanolic NH_3 to yield the TT dimer **9**.[9] Substitution with a dimethoxytrityl ether (DMT) followed by desilylation of the 3'-TBS group with tetrabutylammonium fluoride (TBAF) and reaction with 2-chloro-4H-1,2,3-benzodioxaphosphorin-4-one[10] provided compound **11** which was suitably protected for ODN synthesis *via* the H-phosphonate method.[11]

The 2',5' thioformacetal synthesis is detailed in Scheme 2. 5'-DMT-5-methyluridine **12** was treated according to literature precedent[12] to afford the 3'-deoxy-ara-T compound **13**. Substitution of the 2'-hydroxyl group was effected with methanesulfonyl chloride followed by reaction with potassium thioacetate (AcSK) in DMF to yield compound **14**. The S-acetyl group was then removed with methanolic NH_3 and condensed with the chloromethyl ether **15**[6] to yield the T*T dimer **16**.[13] The 3'-isobutyryl group was removed and treated with **10** to furnish the T*T dimer **17**.

The dimers were incorporated into an ODN of the sequence 5' TC*TC*TC*TC*TC*T^TT^TT where C*=5-methyl-2'-deoxycytidine and T^T=2',5' or 3',5' modified thymidine dimers. 3',5' formacetal and 3',5' thioformacetal dimers[6] were synthesized as previously described and incorporated into the above sequence allowing for comparison in the hybridization experiments. All ODNs were characterized by digestion with nuclease and phosphatase enzymes[14] followed by HPLC analysis of the monomers and dimers and provided the expected nucleoside and dimer ratios. The binding to single stranded RNA and single stranded DNA was ascertained by thermal denaturation studies[15] (T_m) on the duplexes formed between the ODN and the complementary RNA or DNA (Table 1).

Table 1:

ODN	ssRNA(ΔT_m)*	ssDNA(ΔT_m)*	ΔT_m RNA-DNA
3',5' Phosphate	62.5°C	55°C	7.5°C
2',5' Phosphate	60.5°C (-2.0)	47°C (-8.0)	13.5°C
3',5' Formacetal **1**	62.0°C (-0.5)	54°C (-1.0)	8.0°C
3',5' Thioformacetal **2**	63.0°C (+0.5)	53°C (-2.0)	10.0°C
2',5' Formacetal **3**	61.5°C (-1.0)	53°C (-2.0)	8.5°C
2',5' Thioformacetal **4**	60.0°C (-2.5)	47.5°C (-7.5)	12.5°C

*T_m values ± 0.5°C

The 2',5' connection has previously been explored as a phosphodiester in an ODN and resulted in poor hybridization properties to complementary DNA.[2] The poor binding previously observed is confirmed in this study with a ΔTm of -8°C for only two 2',5' phosphodiester linkages. The interaction with a complementary RNA tells a different story with the destabilization being a modest -2°C. This "selectivity" for RNA is consistent with the previous observation in the 3' hydroxy series.[3, 16] The thioformacetal series shows a similar trend to the phosphodiester with the 2',5' connection resulting in particularly poor hybridization properties with a single strand DNA target (-7.5°C) and resulted in only a modest destabilization with RNA (-2.5°C). The 2' connection as compared to the 3' connection in both phosphodiester and the thioformacetal series results in destabilization of helices with the DNA helix being severely effected.

The 2',5' formacetal ODN results in comparable hybridization properties with DNA and RNA as compared to the 3',5' formacetal connection. The DNA binding properties show only modest destabilization relative to a 3',5' phosphodiester in both cases (-1.0°C for the 3',5' formacetal and -2°C for the 2',5' derivative). The RNA binding properties are nearly comparable to the 3',5' phosphodiester control with the 3',5' formacetal (-0.5°C) and 2',5' formacetal (-1.0°C) being almost identical. These results support the modeling hypothesis that the smaller formacetal linkage as a 2',5' connection results in favorable helix formation relative to the larger, longer 2',5' phosphodiester or 2',5' thioformacetal phosphodiester mimic.[4, 17]

The 2',5' formacetal may be of interest in ODN analogs designed to inhibit gene expression in a sequence specific manner (antisense RNA).[18] The presence of an electronegative 2' oxygen on the ribose sugar would be expected to stabilize the glycoside connection to heterocycles such as adenine.[19] Additionally, the 2',5' fxormacetal is synthesized directly from xylose, a very inexpensive sugar. Xylose has a 3' hydroxyl which

currently is deoxygenated to produce the 3' deoxyribose.[2a] The 3' hydroxy offers unexplored opportunities for modification toward the goal of enhanced biological activity.

Acknowledgments: Dr. Ruiming Zou for synthesis of 2',5' phosphodiester controls, Terry Terhorst for ODN synthesis, Shalini Wadwani and Theresa Huang for T_m measurements.

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9. ^{1}H NMR ($CDCl_3$) 300 Hz δ, Compound **9**: 9.5 (bs, 1H); 9.3 (bs, 1H); 7.70 (s, 1H); 7.54 (s, 1H); 6.27 (t, 1H); 5.82 (s, 1H); 5.12 (d, 1H); 4.83 (d, 1H); 4.42-4.38 (m, 3H); 4.13 (d, 1H); 4.15-3.97 (m, 1H); 3.81-3.75 (m, 3H); 2.27-2.18 (m, 2H); 1.99-1.87 (m, 8H); 0.91 (s, 9H); 0.08 (s, 6H).
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13. ^{1}H NMR ($CDCl_3$) 300 Hz δ, Compound **16**: 10.3 (bs, 1H); 10.04 (bs, 1H); 7.74 (s, 1H); 7.44 (s, 1H); 7.42-6.78 (m, 13H); 6.36 (t, 1H); 6.04 (d, 1H); 5.22 (m, 2H); 4.66 (d, 2H); 4.52 (m, 2H); 4.18-3.80 (m, 6H); 3.79 (s, 1H); 3.44 (ddd, 2H); 2.60-2.20 (m, 4H); 1.98 (s, 3H); 2.04-1.90 (m, 2H); 1.40 (s, 3H); 1.16 (m, 6H).
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15. T_m buffer was 140 mM KCl, 5mM Na_2HPO_4, 1mM $MgCl_2$, pH=7.2. The concentration of nucleic acid strands was 2 μM.
16. Molecular modeling studies suggest that in canonical A and B form helices, the 2',5' connection is pushed into the minor groove relative to the 3',5' connection. The phosphodiester linkage, therefore, would be tolerated more in the wide minor groove of A helix, relative to the narrow minor groove of B form helix.
17. This simplistic analysis does not incorporate the sugar pucker differences between the 2' and 3' connections and precise conformational assessment will require high field NMR analysis.
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(*Received in USA* 18 *August* 1994; *revised* 28 *September* 1994; *accepted* 7 *October* 1994)