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Preparation and Properties of a New Type of Acyclic, Achiral Nucleoside Analogue

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ABSTRACT

Preparation of the nucleoside analogues **1** and incorporation of **1**, B = T, in deoxyribooligonucleotides by the phosphoramidite method is described. A two-step deprotection procedure was developed to reduce cleavage of the modified allylic unit. The binding properties of the modified oligonucleotides towards complementary DNA and RNA has been evaluated by T_m measurements showing a ΔT_m of -2 to -6.5°C per modification. An oligonucleotide with two modifications at the 3′-end showed considerable resistance towards cleavage by a 3′-exonuclease. No antiviral activity against HIV-1 or HSV-1 was found for **1**, B = G or T, or for any of the trihydroxy derivatives **5**.

Key Words: Nucleoside analogues; Modified oligonucleotides; Antiviral agents.

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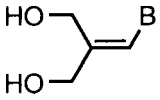
INTRODUCTION

Since the invention of Acyclovir and PNA, the search has been intensified for simple acyclic, preferable achiral nucleoside analogues as potential antisense monomers or antiviral compounds. We have initiated a study of one such type of compounds, **1**, *N*-1/9-[3-hydroxy-2-(hydroxymethyl)prop-1-enyl]nucleobases, which are acyclic, achiral analogues conformationally restricted by a C=C bond.



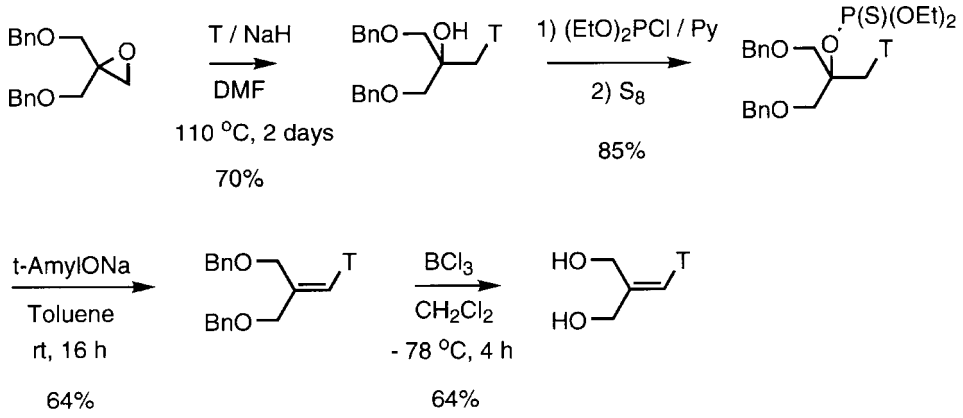
1, B = A, C, G, T, U

Model studies indicated that the nucleobase and the two hydroxy groups of **1** can be positioned close to the positions of the nucleobase and the 3′- and 5′-OH groups in ribonucleosides in the North conformation, as well as to the positions of the nucleobase and the 3′- and 5′-OH groups in deoxyribonucleosides in the South conformation. This is one of the prerequisites for good binding to RNA, resp. DNA.

PREPARATION

We have published the preparation of the thymine analogue,[1] and the A, G and U analogues can be obtained in a similar way. Only the G analogue had been described previously as a potential antiviral agent, without any biological data.[2] Our improved synthesis of the T analogue is shown in Sch. 1. The C analogue has to be prepared from the U analogue since in the last step of the procedure (Sch. 1), removal of the benzyl groups with BCl_3, the C analogue (**1**, $B = C^{Bz}$) decomposed.

The thymine analogue has been built into DNA oligonucleotides by phosphoramidite chemistry. Dimethoxytritylation of **1**, B = T, gave a mixture of the two mono-DMT derivatives **2a** and **2b** and the bis-DMT derivative **3** which were easily



Scheme 1.

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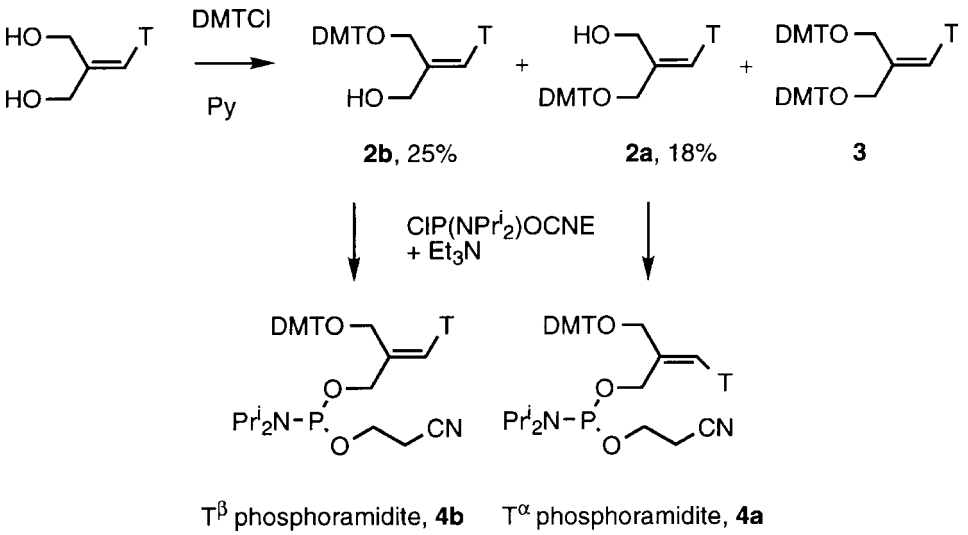

Scheme 2.

separated by column chromatography on silica. Standard phosphitylation of **2a** and **2b** gave the T^{α} and the T^{β} phosphoramidites **4a** and **4b** (Sch. 2).

The modified phosphoramidites were manually coupled with tetrazole activation using a LCAA-CPG support. After an aqueous wash, capping, and oxidation with Bu^tOOH, the DMT efficiency was 99–100%. However, the next coupling following a T^{β} unit proceeded with only 80–85% DMT-efficiency, irrespective of the type of amidite or the coupling time. The reason for this is at present unknown. Deprotection and cleavage from the support was performed by a two-step procedure: 1) neat, anhydrous diisopropylamine, 14 h at rt to remove the cyanoethyl groups;[3] 2) conc. aq. ammonia, 2 h at rt. If aq. ammonia alone was used the modified T unit was rapidly cleaved, probably by nucleophilic attack at the allylic position by NH_3 or OH^-. After removal of the cyanoethyl groups, the modified oligonucleotides were totally stable in water at pH 7 and less than 1% cleaved by conc. aq. ammonia after 24 h at rt. Several oligonucleotides containing one T^{α} or one or two T^{β} units (Table) were prepared, purified by ion exchange HPLC, and characterised by CE and ES-MS.

PROPERTIES

The modified oligonucleotides hybridised to DNA and RNA, although with reduced affinity (Table). The results for T^{β}, ΔT_m −2 to −6.5°C per modification, was better than that for T^{α}, as expected. A mismatch of C opposite T^{β} gave a large depression (ΔT_m –15.5°C) which indicates that the modified monomer discriminates well against mismatches.

The resistance of the modified unit towards 3′-exonucleolytic cleavage was evaluated on $dT_{11}T^{\beta}T^{\beta}T$. Under conditions where a dT_{14} was cleaved by SVP with a $t_{1/2}$

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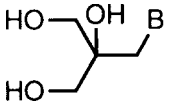
Table 1. Hybridization data (T_m,°C) for modified and unmodified oligodeoxyribonucleotides with DNA and RNA complements.[a]

	dA_{14}	ΔT_m[b]	rA_{14}	ΔT_m	rA_6CA_7	ΔT_m
dT_{14}	36.0		33.5		18.0	−15.5
$dT_7T^{\beta}T_6$	31.0	−5.0	28.0	−5.5	12.5	−15.5
$dT_7T^{\alpha}T_6$	26.0	−10.0	27.0	−6.5		
$dT_{11}T^{\beta}T^{\beta}T$	31.0	−2.5	29.0	−2.0		
	dGTGAGATGC	ΔT_m	rGTGAGATGC	ΔT_m		
dGCATCTCAC	39.0		41.0			
$dGCAT^{\beta}CT^{\beta}CAC$	28.0	−5.5	27.5	−6.5		

[a]T_m was determined by measuring absorbance at 260 nm against increasing temperature (0.5°C steps) on equimolar mixtures (3 μM in each strand) of modified oligomer and its complementary DNA or RNA strand in medium salt buffer (10 mM Na_2HPO_4, 100 mM NaCl, 0.1 mM EDTA, pH 7.0). T^{α} and T^{β} are explained in the text.
[b]Change in T_m per modification.

of 8 min, $dT_{11}T^{\beta}T^{\beta}T$ (apart from the first dT unit) was only ca. 35% cleaved after 2 h, i.e., $t_{1/2} > 3$ h.

Antiviral activities against HIV-1 and HSV-1 of **1**, B = G, T, and the saturated trihydroxy derivatives **5**, were evaluated. None of the compounds showed any significant effect at 30 or 300 μM.



5, B = A, C, G, T

CONCLUSION

The very simple acyclic, achiral nucleoside monomer **1**, B = T, is able to substitute a DNA monomer in oligonucleotides, albeit the binding to DNA and RNA complements is reduced. Work is in progress to optimise the coupling and deprotection procedures in order to prepare and study fully modified oligomers, and to examine possible reasons (less than optimal preorganised conformation, reduced solvation, or other factors) for the reduced binding properties. The hitherto examined nucleoside analogues had no antiviral effects towards HIV-1 or HSV-1.

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