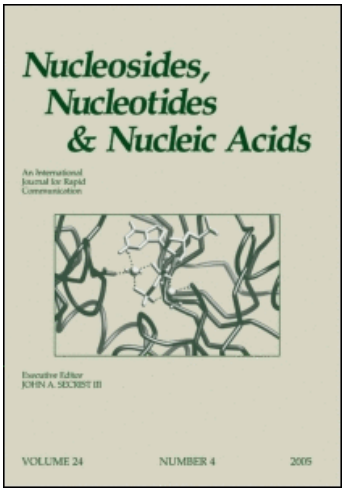

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INCREASED RNA AFFINITY OF HNA ANALOGUES BY INTRODUCING ALKOXY SUBSTITUENTS AT THE C-1 OR C-3 POSITION

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ABSTRACT

1,5-Anhydrohexitol nucleoside congeners with alkoxy substituents, were prepared, resulting in a further improvement of their RNA affinity and antisense potential.

The strong hybridizing potential of anhydrohexitol nucleic acids (HNA) by virtue of its pre-organisation by now is well documented (1–3), and some interesting biological antisense effects have been reported (inhibition of Ha-ras and ICAM-1 (4), and antimalarial activity (5)). To further augment the affinity for target RNA structures, two different roads can be explored by looking for analogues which either increase the conformational preorganisation of the monomeric structures, or which alternatively augment the hydration potential. In addition, cost and ease of synthesis need to be considered. Consequently, 3′-*O*-methylated altrohexitol analogues and 1′-*O*-methylglycosidic analogues were envisaged and incorporated into HNA sequences.

Synthesis of the 3′-*O*-methylated analogue followed the route previously described for preparation of the altrohexitol monomers (ANA) (6). Chemoselective methylation without temporary protection of the nucleobase gave the methylated nucleoside. Further modification yielded the desired phosphitylated building block **1***.

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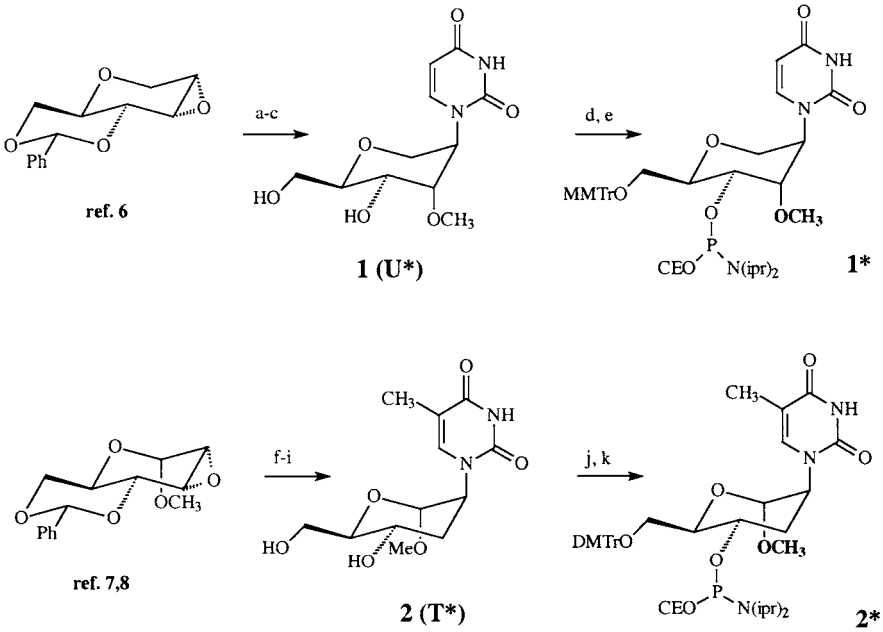
The 1′-*O*-methylglycosidic analogues were obtained starting from ubiquitous methyl glucopyranoside. Opening of the 2,3-*allo*-epoxide (7,8), was followed by deoxygenation of the 3-position. Removal of the benzylidene position is less straightforward because of the glycosidic linkage, but can be accomplished alternatively via hydrogenation in almost quantitative yield. Further modification yielded the desired phosphitylated building block **2***.

All oligos were assembled on a propanediol containing universal support, obviating the need of modified supports (9). With a coupling time of 3 minutes, coupling yields were consistently over 95% and higher. Electrospray ionization mass spectrometry (ESI-MS) in negative mode indicated all monoisotopic masses to be consistently within 0.5 Da of the calculated masses.



Scheme 1. a) 3.2 eq. uracil, 3 eq. NaH, DMF 120°C 24h (86%); b) 5 eq. NaH, 3 eq. CH_3I, THF, 7h 0°C (38%); c) 90% TFA (74%); d) MMTrCl, pyridine (89%); e) DIEA, CH_2Cl_2, $(iPr)_2N(CN)PCl$ (90%); f) 3 eq. thymine, 2.8 eq. NaH, DMF, 96 h, 120°C (71%); g) 2 eq. $CSCl_2$, 7 eq. DMAP, CH_2Cl_2 at −40°C followed by 4 eq. 2,4-$Cl_2C_6H_3OH$ at RT for 1 h (85%); h) 1.5 eq. Bu_3SnH, AIBN, toluene 80°C (85%); i) 10% TFA-MeOH 3 h (55%); alternatively H_2, Pd/C in MeOH-HOAc 98:2 for 18 h (90%); j) DMTrCl, pyridine (85%); k) DIEA, CH_2Cl_2, $(iPr)_2N(CN)PCl$ (67%).

When hybridised with complementary HNA, the introduction of the 3′-*O*-methylated uridine nucleoside analogue (**1**) into a HNA strand results in an increased thermal stability of the duplex compared to the unmodified HNA:HNA duplex ($\Delta T_m = +0.6°C$/modification) (entry **A** and **B**, Table 1). However, this increase is less pronounced than the increase in thermal stability obtained by modifying the nucleobase with a methyl substituent in the 5-position ($\Delta T_m = +1.1°C$/

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Table 1. Hybridisation Data for Hexameric Hexitol Sequences (6′→4′) with Incorporation of 3 Methylated Building Blocks **1** or **2**

	Sequence	HNA Complement	ANA Complement	RNA Complement	DNA Complement
A	**1** C **1** CC **1** (HNA)	52.4 (64)	58.8 (71)	31 (42)	no T_m
B	UCU CCU (HNA)	50.7 (61.2)	55#	30.5 (40)	no T_m
C	UCU CCU (ANA)	54	61.8 (71.2)	38.4 (47.6)	no T_m
D	TCT CCT (HNA)	54	60.6	39 (48)	no T_m
E	**2** C **2** CC **2** (HNA)	56.7	62.7	39.9 (49.5)	no T_m

T_m's in a buffer of 0.1 M NaCl and 20 mM phosphate, pH 7.4 with a duplex concentration of 4 μM. Numbers in brackets are T_m's in a high salt buffer (1.0 M NaCl). **1** denotes a 3′-*O*-methylated ANA monomer, **2** denotes a **1**′-*O*-methylated HNA monomer.
lit data incorrect (in ref. 12, the duplex was HNA-HNA, instead of HNA-ANA.

modification) (compare entry **A** and **D**). Hybridising the modified ONs with complementary RNA corroborates this pattern of thermal stabilisation of the duplexes. As expected, none of the hexitol based ONs hybridised with complementary DNA. Clearly, for this series the results indicate that a 5-methyl is more important than a 3′-*O*-methyl and that a methylation of the 3′- hydroxyl group in ANA is destabilising when pairing to an RNA sequence is envisaged.

For the hexitol analogue **2** comparison is more straightforward, and the thermal stabilisation is of the same order as for **1** when compared to HNA, as well in its pairing with hexitol oligonucleotides as with RNA sequences. Therefore, introduction of **2** seems to be slightly more favorable over addition of a HNA monomer.

Incorporation of respectively **1** or **2** into octameric (GCGUAGCG) HNA sequences and hybridisation with RNA, yielded an analogous picture with a small increase for introduction of **1** and a solid increase of 1.6°C in T_m for introduction of **2**. However, thermal unwinding of a self-complementary HNA duplex gave another pattern, where incorporation of **1** resulted in considerable stabilisation of the duplex ($\Delta T_m = +3$°C/modification) exceeding the stabilisation obtained for substitution of uracil for thymine ($\Delta T_m = +2.4$ °C/modification) (Table 2).

Table 2. Thermal Stability of Self Complementary HNA Sequences Containing 3′-*O*-methyl or 1′-*O*-methyl Modifications (**1** or **2**)

Sequence	T_m/°C	ΔT_m/°C[a]
GUGU ACAC	65.0	ref.
G **1** G **1** ACAC	76.7	+3
GTGT ACAC	74.5	+2.4/ref.
G **2** G **2** ACAC	76.9	+0.6

T_m's obtained in 0.1 M NaCl, 20 mM phosphate, pH 7.4 with an oligo concentration of 8 μM (4 μM of duplex).
[a] ΔT_m/modification.

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Generally, the ONs containing the 3′-*O*-methyl derivative (**1**) showed a small increase in thermal stability towards complementary sequences as compared to HNA, except in the case of a self-complementary sequence for which an increase in thermal stability of 3°C per modification was observed. Compared to ANA, however, the 3′-*O*-methylation caused a small decrease in thermal stability of duplexes between a modified ON and a complementary target, especially when targeting RNA. The methyl glycosidic analogues **2**, however, seem to be endowed with higher affinity for RNA in comparison with well-known HNA, while at the same time having economically more favorable monomers. These compounds therefore could have strong potential for antisense purposes and need to be evaluated further.

In addition, it proved possible to incorporate the new modified monomers into RNA without compromising their affinity for their respective RNA target.

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