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RNA Duplex Formation by Oligodeoxynucleotides Containing C-5 Alkyne and C-5 Thiazole Substituted Deoxyuridine Analogs

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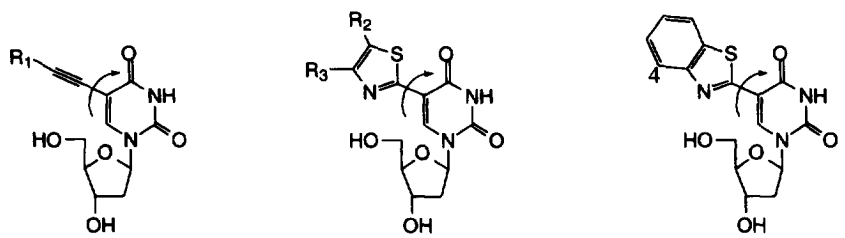
Abstract: The binding affinity of methyl substituted C-5 propyne and C-5 thiazole ODNs for RNA was assessed by thermal denaturation analysis (Tm). The results indicate that increased size of the alkyne substituent lead to decreased affinity, but certain methyl substitutions on the thiazole lead to higher affinity complexes with RNA. The increased affinity of methylthiazole ODNs to RNA was dependent on the position of the methyl substituent with 5-methylthiazole ODN (**2h**) exhibiting the highest Tm. The 5-methylthiazole group likely increases hydrophobic interactions with adjacent base pairs in the canonical double helix. Copyright © 1996 Elsevier Science Ltd

Antisense oligodeoxynucleotides (ODNs) containing C-5 propyne 2'-deoxyuridine (pdU, **1a**) are potent and specific inhibitors of gene expression.[1-5] The potent activity of these high affinity ODNs has resulted in precise demonstrations of antisense effects and, therefore, has provided a means for investigating the mechanisms of gene inhibition.[3-5] ODNs derived from C-5 thiazole dU (**1e**) form stable RNA complexes with stabilities comparable to pdU ODNs presumably by increased base stacking interactions with adjacent base pairs.[6] ODNs containing methyl substituted propyne dUs (**1b-1d**) and thiazole dUs (**1f-1h**) have been prepared and the binding affinity of these ODNs for RNA determined.



1a $R_1 = CH_3$
1b $= CH_2CH_3$
1c $= CH(CH_3)_2$
1d $= C(CH_3)_3$

1e $R_2 = R_3 = H$
1f $R_2 = R_3 = CH_3$
1g $R_2 = H$, $R_3 = CH_3$
1h $R_2 = CH_3$, $R_3 = H$

1i

Figure 1. C-5 Substituted Alkyne and Thiazole dU Analogs.

The C-5 alkynyl-dU analogs (**1a-1d**, Figure 1) were prepared by direct palladium (0) catalyzed coupling of the alkynes with 5-iodo-2'-deoxyuridine (IdU).[7] The C-5 thiazole-dU analogs (**1e-1i**, Figure 1) were synthesized by coupling 3',5' di-*O*-p-toluyl protected IdU with the tributylstannylthiazole derivatives in the presence of palladium (II) catalyst.[6] All dU analogs were incorporated into ODNs by automated DNA synthesis using the H-phosphonate approach.[8]

Table 1: Double Helix Tm Analysis of ODNs Containing C-5 Substituted dU Analogs[a]

RNA Target: 3' AGAGAGAGAGAAAAA 5'
ODN[b]: 5' TCTCTCTCTCUUUUU 3'

U	ODN	Tm (°C)	ΔTm (°C)	ΔTm/substitution (°C)
Thymidine	control	62.5	---	---
1a	**2a**	70.5	+8.0	+1.6
1b	**2b**	69.5	+7.0	+1.4
1c	**2c**	66.5	+4.0	+0.8
1d	**2d**	66.5	+4.0	+0.8
1e	**2e**	71.0	+8.5	+1.7
1f	**2f**	71.0	+8.5	+1.7
1g	**2g**	69.5	+7.0	+1.4
1h	**2h**	73.5	+11.0	+2.2
1i	**2i**	68.5	+6.0	+1.2

a) Tm values were measured at 260 nm in 140 mM KCl/5 mM Na_2HPO_4/1 mM $MgCl_2$ at pH 7.0 and the final concentration of ODNs was approximately 2μM.
b) U = C-5 substituted-dU ; C = 5-methyl-2'-deoxycytidine

The stabilities of the C-5 alkyne and thiazole dU ODN/RNA complexes were determined by Tm analysis[9] and all dU analogs stabilize the complex relative to the thymidine control ODN (Table 1). The propyne containing ODN (**2a**) and butyne containing ODN (**2b**) lead to very stable ODN/RNA duplexes (ΔTm = +1.6 and +1.4 °C/substitution, respectively). Increasing the steric bulk on the alkyne substituent resulted in decreased stability; the methylbutyne ODN (**2c**) and the dimethylbutyne ODN (**2d**) being less stable than the propyne ODN (**2a**) by 0.8°C/substitution. These results are consistent with the DNA/DNA duplex stabilities observed with ODN homopolymers containing increasing alkyl and alkynyl substitutions.[10] Substituted thiazole-dU containing ODNs (**2e-2i**) formed stable duplexes with RNA, but in contrast to the alkynes,

increased methyl substitution of the thiazole did not automatically result in decreased stability. The 5-methylthiazole ODN (**2h**) lead to the most stable duplex (ΔTm = +2.2°C/substitution) and the 4-methylthiazole ODN (**2g**) lead to a less stable duplex (ΔTm = +1.4°C/substitution). The stability of the dimethylthiazole (**2f**) ODN/RNA complex is between that of **2g** and **2h**, and equal to the unsubstituted thiazole ODN (**2e**). The C-5 benzthiazole containing ODN (**2i**) exhibited the lowest affinity (Tm = +1.2°C/substitution) among the thiazole dU substituted ODNs.

ODNs **2b-2d** represent sequential replacement of the hydrogens of propyne with methyl groups and the decreased Tm of the butyne ODN (**2b**), methylbutyne ODN (**2c**) and the dimethylbutyne ODN (**2f**) relative to the propyne ODN (**2a**) is likely due to unfavorable steric interactions of the additional methyl subtituents within the canonical double helix. The small decrease in Tm of the butyne ODN (**2b**) relative to the propyne ODN (**2a**) may be due to the single additional methyl substituent rotating into a conformation that minimizes these unfavorable steric interactions.

Thiazole dU containing ODNs bind to RNA with affinity comparable to propyne substituted ODNs, presumably because of the thiazole ring's ability to achieve coplanarity with the uracil base leading to increased base stacking interactions with adjacent base pairs.[6] Measurements of thiazole dipole moments indicate that the nitrogen atom is partially negative while the sulfur atom resides at the positive end of the dipole[11] and *ab initio* calculations indicate that the thiazole ring of **1e** probably exists preferentially in the coplanar conformation depicted in Figure 1 with the sulfur atom aligned with the O4 of uracil.[12] This conformation places the 5-methyl and the 4-methyl substituents in different locations in space. Molecular modeling of the ODN/RNA complexes[13] of **2g** and **2h** suggests that the 5-methyl group of **2h** resides in the center of the canonical double helix providing increased hydrophobic interactions with the thiazole ring of adjacent base pairs. Conversely, the 4-methyl substituent of **2g** creates an unfavorable steric interaction with the H2' and H3' of the sugar backbone of the adjacent base pair, and this is also true of the C4 of the benzthiazole substituted ODN (**2i**). The 4,5-dimethylthiazole ODN (**2f**) contains the favorable hydrophobic interaction of the 5-methyl substituent which is countered by the unfavorable steric interaction of the 4-methyl substituent resulting in an affinity intermediate between the two; and equal to the unsubstituted thiazole containing ODN (**3f**). The inability of the benzthiazole to reside in the center of the canonical double helix is reflected in the decreased Tm of benzthiazole containing ODN (**2i**) relative to the unsubstituted thiazole containing ODN (**2e**). This is in contrast to the high affinity ODNs containing large tricyclic pyrimidines which are positioned in the center of the canonical double helix and allow for increased stacking interactions with adjacent base pairs.[14]

The high Tm observed with **2h** , therefore, is consistent with the thiazole ring being in a preferred coplanar conformation with the uracil base and the 5-methyl substituent undergoing stacking interactions with adjacent base pairs.

In summary, the propyne containing ODN (**2a**) exhibited the highest affinity for RNA of all the alkynes tested and duplex stability decreased with increasing size of the alkyl substituent on the alkyne. The 5-methylthiazole ODN (**2h**) lead to the most stable duplex with RNA among the thiazole substituted ODNs. The Tm results indicate that the increased RNA affinity is due to increased hydrophobic interactions with adjacent base pairs in the center of the canonical double helix. The high binding affinities of these C-5 thiazole dU containing ODNs makes them potential agents for antisense gene inhibition.

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