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OLIGODEOXYNUCLEOTIDES CONTAINING 5-(1-PROPYNYL)-2'-DEOXYURIDINE FORMACETAL AND THIOFORMACETAL DIMER SYNTHONS

Kuei-Ying Lin, Jeffrey S. Pudlo, Robert J. Jones, Norbert Bischofberger, Mark D. Matteucci and Brian C. Froehler
Gilead Sciences, Inc., 353 Lakeside Drive, Foster City CA 94404

Abstract: Oligodeoxynucleotides containing the C-5 propyne 2'-deoxyuridine analog in conjunction with the formacetal and 3'-thioformacetal linkage are described. Thermal denaturation analysis demonstrates that these analogs have enhanced binding affinity to both single-strand RNA and DNA and double-strand DNA.

Oligodeoxynucleotides (ODNs) are potentially powerful reagents for the sequence specific inhibition of gene expression.[1] Recent studies have demonstrated that 5-(1-propynyl)-2'-deoxyuridine (pdU) and 5-(1-propynyl)-2'-deoxycytidine (pdC),[2] used in conjunction with the phosphorothioate linkage, are potent antisense inhibitors of gene expression.[3] It was also demonstrated that phosphorothioate ODNs do not permeate cells in tissue culture.[3] Backbone analogs are an important modification of ODNs to increase cellular permeability and much work has focused on the synthesis on neutral, achiral backbone analogs.[4-7] Among the most promising analogs are the acetals, namely the formacetal (**1**)[4,5] and the 3'-thioformacetal (**2**).[6] Reported herein are the combination of the C-5 propyne 2'-deoxyuridine with these two acetal linkages into dimer synthons (**3** and **4**) suitable for DNA synthesis (Figure 1). Due to the reactivity of the propyne moiety, synthesis of these acetal linked dimers required introduction of the propynyl group after acetal formation. ODNs derived from these analogs (**3** and **4**) demonstrate high binding affinity for single-strand RNA, DNA and double-helical DNA. The propyne and acetal modifications should render ODNs more hydrophobic and thus aid cellular permeability, therefore ODNs containing these analogs are potentially useful in therapeutic applications.

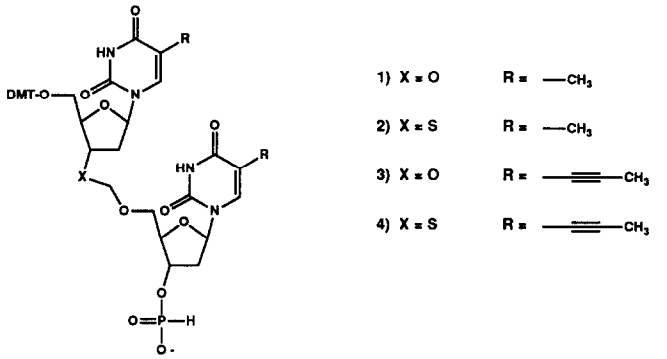
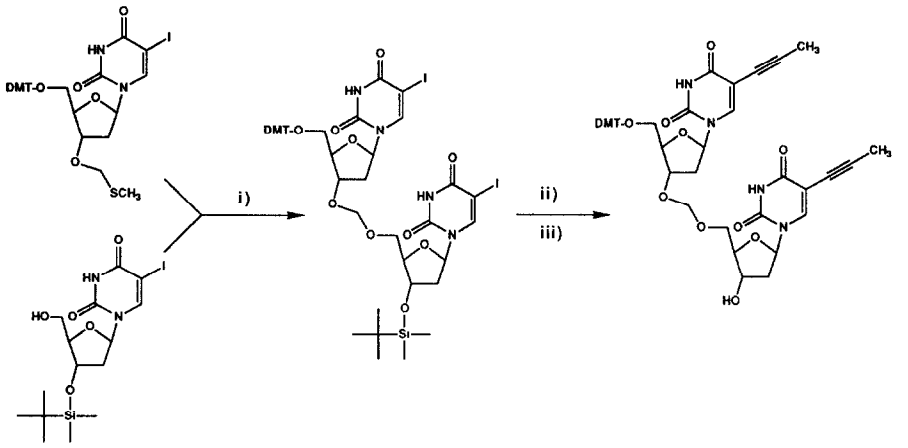
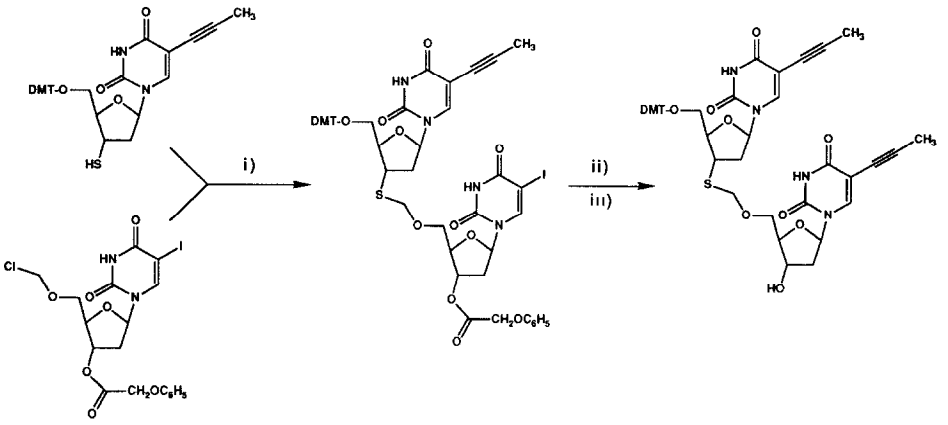


Figure 1: Structure of formacetal and 3'-thioformacetal dimer synthons.



Scheme I: i) Br_2 / 2,6-diethylpyridine / benzene / 4A molecular sieves; ii) Propyne / $(Ph_3P)_4Pd$ / CuI / Et_3N / DMF; iii) TBAF / THF

The formacetal dimer **1** has been synthesized by Br_2 activation of the 3'-methylthiomethyl ether of thymidine.[4,6] The C-5 propyne moiety of 2'-deoxyuridine is not stable to Br_2 activation and therefore the pdU-pdU formacetal dimer (**3**) was prepared from 5-iodo-5'-*O*-DMT-3'-*O*-[(methylthio)methyl]-2'-deoxyuridine [8] and 5-iodo-3'-*O*-(*tert*-butyldimethylsilyl)-2'-deoxyuridine (Scheme I). This yielded the 5-iodo-2'-deoxyuridine dimer and the propyne functionality was subsequently introduced at the C-5 position of both uracils with a single, high yield reaction (>90%).[9] Deprotection with TBAF and phosphitylation [10] yielded the H-phosphonate dimer **3**.

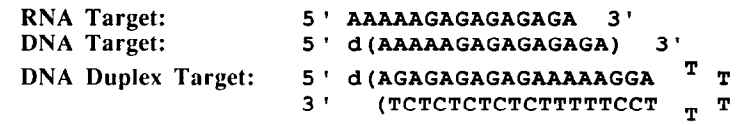


Scheme II: i) DIPEA / DMF; ii) NaOMe / MeOH; iii) Propyne / $(Ph_3P)_4Pd$ / CuI / Et_3N / DMF

The 3'-thioformacetal dimer **2** was synthesized from the 5'-chloromethyl ether of thymidine.[6] The C-5 propyne moiety was found to be modified by the reaction conditions required to prepare the chloromethyl ether (due to addition of HCl across the triple bond) and therefore the propyne was introduced after synthesis of the dimer. 5-iodo-3'-*O*-phenoxyacetyl-2'-deoxyuridine was converted to the cholоromethyl ether [6] and coupled with 5-(1-propynyl)-5'-*O*-DMT-3'-mercapto-2'-deoxyuridine [11] as described (Scheme II).[6] Following removal of the 3'-ester, the remaining 5-propynyl group was introduced in high yield [9] and phosphitylation [10] yielded the H-phosphonate dimer **4**.

These dimer synthons **1-4** were incorporated into two positions of an ODN (Figure 2) to assess the effect of these modifications on the thermal stability of the DNA-RNA duplex, DNA-DNA duplex and the triplex formed by these modified ODNs. The results shown in Table 1 demonstrate that these heterocyclic and backbone modifications are compatible with binding to target nucleic acid sequences. The effect of four pdU substitutions on the duplex and triplex is shown in Table 1 and an increase in Tm against all targets is observed (ODN **5** vs **8**). The inclusion of the formacetal linkage (dimer **1** or **3**) into the ODN (**6** or **9**) has little effect on the Tm and the propyne moiety appears to dominate the Tm of the ODN containing the pdU-pdU formacetal dimer (**3**, Table 1).

The 3'-thioformacetal linkage (dimer **2** or **4**) has a more substantial effect on the Tm of the ODN (**7** or **10**). The T-T 3'-thioformacetal (**2**) containing ODN (**7**) has no effect on the Tm of the DNA-RNA duplex, a destabilizing effect on the DNA-DNA duplex (ΔTm = -2.5 °C) and a stabilizing effect on the triplex (ΔTm = +2.6 °C). The pdU-pdU 3'-thioformacetal (**4**) containing ODN (**10**) has a strong stabilizing effect on binding to all targets (Table 1). The increase in Tm of ODN **10** bound to duplex DNA (triplex formation, ΔTm = +8.7 °C) appears to be additive for the two substitutions; substitution of T with pdU increases the Tm by 6.1 °C (ODN **8**) and substitution of the phosphodiester with the 3'-thioformacetal increases the Tm by 2.6 °C (ODN **7**). The increase in Tm of the DNA-RNA duplex, with ODN **10**, is synergistic (ΔTm = +9.0 °C); pdU substitution alone results in an increase in Tm of 6.5 °C (ODN **8**) and incorporation of the 3'-thioformacetal has no effect on the Tm (ODN **7**).



5) 5' TCTCTCTCTCTTTTT 3'
6) 5' TCTCTCTCTCT•TT•TT 3'
7) 5' TCTCTCTCTCT*TT*TT 3'
8) 5' TCTCTCTCTCUUUUT 3'
9) 5' TCTCTCTCTCU•UU•UT 3'
10) 5' TCTCTCTCTCU*UU*UT 3'

Figure 2: For ODNs **5-10** T is thymidine, C is 5-methyl-2'-deoxycytidine, and U is 5-(1-propynyl)-2'-deoxyuridine. All intermucleotide linkages are phosphodiester except for the following; • is the formacetal linkage (**1** or **3**) and * is the 3'-thioformacetal linkage (**2** or **4**)

Table 1: Duplex and Triplex Tm with Acetal Analogs

ODN	Duplex (RNA) [a]		Duplex (DNA) [a]		Triplex [b]	
	Tm (°C)	ΔTm (°C)	Tm (°C)	ΔTm (°C)	Tm (°C)	ΔTm (°C)
5. T-control	62.5	–	55.5	–	38.9	–
6. T-formacetal	62.0	-0.5	54.0	-1.5	nd	nd
7. T-thioformacetal	62.5	0	53.0	-2.5	41.5	+2.6
8. pdU-control	69.0	+6.5	59.5	+4.0	45.0	+6.1
9. pdU-formacetal	69.0	+6.5	59.5	+4.0	45.5	+6.6
10. pdU-thioformacetal	71.5	+9.0	61.5	+6.0	47.6	+8.7

a) Tm values were assessed in 140 mM KCl/5 mM Na_2HPO_4/1mM $MgCl_2$ at pH = 7.2 and the concentration of all oligonucleotides was ~2μM. b) Tm values assessed as in (a) except at pH = 6.6. Tm values are ± 0.5 °C.

In conclusion, ODNs containing 5-(1-propynyl)-2'-deoxyuridine formacetal and 3'-thioformacetal dinucleosides have been described. Tm analysis show that these heterocyclic and backbone modifications are compatible with each other and binding competent. The increase in Tm observed with the pdU-pdU 3'-thioformacetal modification on the stability of the DNA-RNA duplex is more than additive. The pdU-pdU 3'-thioformacetal is a promising analog for targeting single-strand RNA and double-strand DNA and may be useful in therapeutic applications.

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