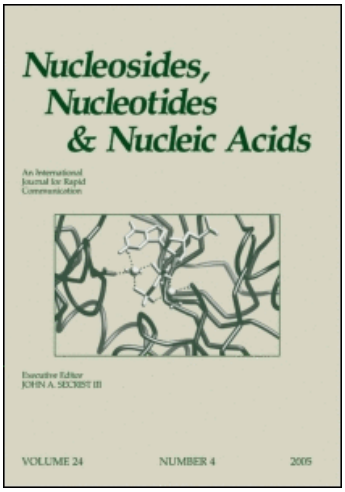

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Synthesis and Biophysical and Biological Evaluation of 2'-Modified Antisense Oligonucleotides

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SYNTHESIS AND BIOPHYSICAL AND BIOLOGICAL EVALUATION OF 2'-MODIFIED ANTISENSE OLIGONUCLEOTIDES

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ABSTRACT: A series of 2'-deoxy-2'-substituted adenosine modified oligonucleotides were synthesized and evaluated for their thermodynamic stability (Tms) and resistance to nuclease degradation in fetal calf serum.

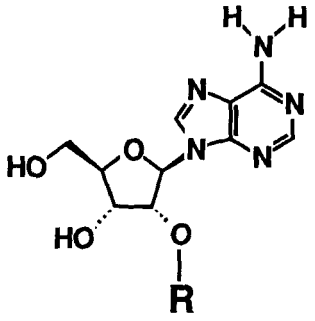
To date most efforts directed at enhancing the biological properties of antisense oligonuclotides have been focused on modifying the phosphorus atom of the DNA backbone.[1] In the studies reported herein, we have begun to investigate alternative sites for chemically modifying oligonucleotides. The modified oligonucleotides must be resistant to nucleases yet retain appropriate hybridization properties. Furthermore, we were particularly interested in placing functional groups on the nucleosides so that when the 2'-modified oligonucleotides hybridize with targeted RNA, the functionalities would reside in the minor groove formed by the heteroduplex and proximal to the 2'-hydroxyl group of the targeted RNA. This stems from our desire to effect catalytic cleavage of targeted RNA by a functional group attached to the oligonucleotide complement of the RNA. Other types of pendant groups, of a reactive and non-reactive nature, placed in the minor groove in this manner are also of interest.

Molecular modeling of 2'-O-modified adenine oligonucleotides was utilized to examine the space available in the minor groove of

TABLE 1 - THERMODYNAMIC STABILITY AND NUCLEASE RESISTANCE OF 2'-O-MODIFICATIONS

ISIS #	2'-O-MODIFICATON	Tm (ΔTm,°C)	T1/2 (hr)
ACC GAG GAT CAT GTC GTA CGC (21 mer, pos 1,5,8,11,18)			
1772	natural	67	
	P=S (1-20)	~57 (-10)	
1325	nonyl	51 (-16)	
1946	aminopropyl	61 (-6)	
CGA CTA TGC AAG TA'C (15 mer, position 14)			
1343	natural	53	1.2
1359	P=S (1-20)	46 (-7)	23
1322	nonyl	54 (+1)	5.8
1609	TBDM	52 (-1)	7.0
1947	aminopropyl	53 (0)	7.7
CGA CTA TGC AAA' A'A'C (15 mer, positions 12,13,14)			
1732	natural	52	1.0
	P=S (1-14)	~45 (-7)	20
1437	nonyl	49 (-3)	>64
1945	aminopropyl	54 (+2)	17.7

an "A" family of a mixed DNA-RNA duplex for functional groups of interest. The energy minimized structures of the lipophilic n-nonyl and t-butyldimethylsilyl (TBDM) groups reside in the minor groove whereas the aminoethyl-, propyl-, and butyl- groups curl back and appear to form an internal salt with the 3'-phosphate of the modified oligonucleotide. The imidazolylpropyl group in the 2'-position appears to span across the minor groove and towards the opposite 2'-hydroxyl of the RNA complement strand.



R = $-(CH_2)_8CH_3$ NONYL

R = $-(CH_2)_{2-4}NH_2$ AMINOALKYLS

R = $-Si(Me)_2C(Me)_3$ TBDM

R = $-(CH_2)_3-N$  IMIDAZOLYL PROPYL

CHART 1

TABLE 2 - SPECIFICITY OF 2'-O-MODIFICATIONS

X - STRAND 5'-CTC GTA CCA' TTC CGG TCC
Y - STRAND 3'-GAG CAU GGY' AAG GCC AGG (18 mer, 9-position)
Y = T (WATSON-CRICK COMPLEMENT)
Y = A, C, OR G (MISMATCH)
Y = NONE (BULGE LOOP)

ISIS #	2'-O-MODIFICATON	Tm (ΔTm, °C)
1583	natural	63 (0T, -9A,-6G, -14C, -12B)
1638	nonyl	58 (0T, -3A, -3G, -6C, -3B)
1645	TBDM	58 (0T, -4A, -4G, -2C, -4B)
1662	aminopropyl	60 (0T, -6A, -6G, -12C, -7B)

The novel 2'-substituted nucleosides were synthesized by direct coupling of the 2'-OH of protected adenosine with alkylhalides in the presence of sodium hydride in DMF. The 2'-substituted nucleosides, protected by standard procedures, were converted into their 3'-O-β-cyanoethyldiisopropylphosphoramidites which were inserted into specific positions in oligonucleotides via solid phase DNA synthesis. 2'-O-Modified adenosines, synthesized and inserted into sequence specific oligonucleotides, are depicted in CHART 1. A standard RNA synthesis cycle was utilized. The couplings of the modified nucleotides were greater than 94% as determined by

DMT cation evaluations. The oligonucleotides were purified by trityl-on HPLC. The structures of the modified oligonucleotides were confirmed by HPLC and NMR analysis of enzymatic degradations.

Thermodynamic stability of these novel structure classes, as determined by melting studies (Tms, TABLE 1), revealed that up to five pendant groups specifically placed within oligonucleotide sequences of 15 to 21 bases in length generally resulted in only a modest effect on Tms compared with the unmodified parent oligonucleotides. The effects on Tms were dependent on location in the sequence in that modifications placed near the 3'-end had less destablizing effects than 5'-end placements. Moreover, oligonucleotides containing 2'-modified adenosines placed near the 3'-end of the oligonucleotide sequences provided resistance to nuclease degradation in 10 % fetal calf serum of 6 to 64-fold greater than the unmodified sequences.

The specificity of the modified adenosines to Watson-Crick base pair with thymidine was determined by preparing a Y strand, an 18 mer with a T (Watson-Crick complement), A, G, C (mismatch), or the deletion of the nucleotide at the 9 position (budge loop). The X complement strand contains the 2'-modified adenosine at the 9th position the 2'-modified adenosine. Data in Table 2 indicate that the specificity of the modified adenosines were retained, ie. their Tm's were greatest when paired with T rather than the mismatch situations of A, G, and C.

The aminopropyladenosine modified oligonucleotides possess hybridization properties similar to unmodified parent oligonucleotides but were significantly enhanced relative to the phosphorothioates. Furthermore, aminopropyladenosine modified oligonuclotides are as resistant to nuclease degradation as the phosphorothioates. The data from these studies suggest that 2'-O-modified oligonuclotides represent a novel and pharmacological distinct class of antisense agents.

REFERENCES

1. Oligodeoxynucleotides: Antisense Inhibitors of Gene Expression. Edited Jack S. Cohen, CRC Press, Inc. Boca Raton, Florida, 1989; John Goodchild, Bioconjugate Chemistry, **1**, 165-187, 1990.