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2′-*O*-[2-[2-(*N*,*N*-Dimethylamino)ethoxy]ethyl] Modified Oligonucleotides: Symbiosis of Charge Interaction Factors and Stereoelectronic Effects‡

Marija Prhavc, Thazha P. Prakash, George Minasov, P. Dan Cook, Martin Egli,*,† and Muthiah Manoharan*

Department of Medicinal Chemistry, Isis Pharmaceuticals, Inc., Carlsbad, California 92008, Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School, Chicago, Illinois 60611, and Department of Biological Sciences, Vanderbilt University, Nashville, Tennessee 37235

mmanoharan@alnylam.com; martin.egli@vanderbilt.edu

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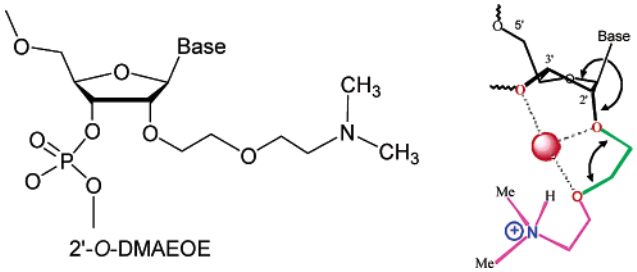
ABSTRACT



Oligonucleotides with a novel, 2′-*O*-[2-[2-(*N*,*N*-dimethylamino)ethoxy]ethyl] (2′-*O*-DMAEOE) modification have been synthesized. This modification, a cationic analogue of the 2′-*O*-(2-methoxyethyl) (2′-*O*-MOE) modification, exhibits high binding affinity to target RNA (but not to DNA) and exceptional resistance to nuclease degradation. Analysis of the crystal structure of a self-complementary oligonucleotide containing a single 2′-*O*-DMAEOE modification explains the importance of charge factors and gauche effects on the observed antisense properties. 2′-*O*-DMAEOE modified oligonucleotides are ideal candidates for antisense drugs.

To be effective, antisense oligonucleotides must have high binding affinity to the target RNA and high nuclease resistance.[1] They should also bind selectively to transport proteins and should be cell permeable *in vivo*. With a "gapmer" structure, where a deoxy region recruits RNase H and facilitates the cleavage of the mRNA duplex and a 2′-modified portion to enhance duplex stability,[2] 2′-*O*-modified oligonucleotides[3] have emerged as leading second-generation candidates for clinical applications. Among the 2′-modifications studied for antisense applications, two modification types stand out in terms of binding affinity to target RNA and nuclease resistance. These are the 2′-*O*-(2-methoxyethyl) (2′-*O*-MOE)[4] and 2′-*O*-(3-aminopropyl) (2′-*O*-AP) modifications and their homologues.[5] The 2′-*O*-MOE modification, due to additive gauche effects,[6] offers a 2 °C increase[7] in melting temperature (T_m) per modification as a phosphodiester (2′-*O*-MOE/PO) compared to the first-generation 2′-

‡ Dedicated to Professor Ernest L. Eliel.
† Vanderbilt University

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Table 1. ES-MS Analysis of Oligonucleotides with 2′-*O*-DMAEOE Modification and the Effect of 2′-*O*-DMAEOE and 2′-*O*-MOE Modifications on Duplex Stability with Complementary RNA[a]

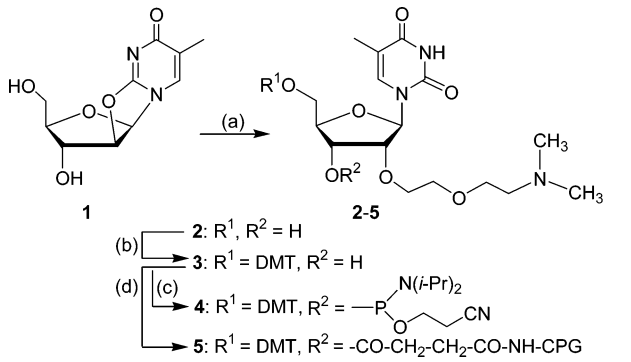
		ES MS				
no.	sequence	calcd	found	T_m,[c] °C	ΔT_m,[c] °C	ΔT_m[c]/unit
7	5′d(GCGTTTTTTTTTTGCG)3′ (parent)			48.3		
8	5′d(GCGT$^{▼}$T$^{▼}$T$^{▼}$T$^{▼}$T$^{▼}$T$^{▼}$T$^{▼}$T$^{▼}$GCG)3′			59.8	11.5	1.2
9	5′d(GCGT*T*T*T*T*T*T*T*T*T*GCG)3′	6186.83	6187.94	59.6	11.3	1.1
10	5′d(TCCAGGTGTCCGCATC)3′ (parent)			62.3		
11	5′d(T$^{▼}$CCAGGT$^{▼}$GT$^{▼}$CCGCAT$^{▼}$C)3′			66.7	4.4	1.1
12	5′d(T*CCAGGT*GT*CCGCAT*C)3′	5357.90	5354.54	65.5	3.2	0.8
13	5′ TTTTTTTTTTTTTTTT*T*T*T*3′	6542.20[b]	6542.62			
14	5′d(GCGTAT*ACGC)3′	3159.23	3158.23			

[a] T$^{▼}$ = 2′-*O*-MOE-5-methyluridine, T* = 2′-*O*-DMAEOE-5-methyluridine. [b] DMT-on. [c] T_m values were assessed in 100 mM NaCl, 10 mM phosphate buffer, 0.1 mM EDTA, pH 7, at 260 nm, and 4 μM oligonucleotides and 4 μM complementary length matched RNA. Standard deviation did not exceed ±0.5 °C.

deoxyphosphorothioate (2′-H/PS) compounds. This modification with a P═O linkage exhibits nuclease resistance (measured as the half-life of the full-length oligonucleotide, $t_{1/2}$) at approximately the same level as a 2′-deoxyphosphorothioate modification. The 2′-*O*-AP modification and its homologue 2′-*O*-DMAP exhibit exceptional nuclease resistance ($t_{1/2}$ 8-fold better than 2′-deoxyphosphorothioate compounds) due to the cationic alkyl chain, but have only moderate affinity for target RNA. To improve upon these modifications, we designed and synthesized the 2′-*O*-[2-[2-(*N*,*N*-dimethylamino)ethoxy]ethyl] (2′-*O*-DMAEOE) modification.[8] This modification combines the advantages of the gauche effect (as in 2′-*O*-MOE) and the charge effect (as in 2′-*O*-AP). Moreover, 2′-*O*-DMAEOE oligonucleotides can be expected to be more lipophilic than the 2′-*O*-MOE analogues, a property affecting protein binding and cellular permeation of oligonucleotides.

2′-*O*-DMAEOE−5-methyluridine-3′-phosphoramidite **4** and solid support **5** were synthesized as described in Scheme 1. Oligonucleotides **9**, **12**, **13**, and **14** (Table 1) were synthesized by using phosphoramidite **4** and solid support **5** and the standard phosphoramidites for incorporation of A, T, G, and C residues. Oxidation of the internucleosidic phosphite groups was carried out with 1-*S*-(+)-(10-camphorsulfonyl)oxaziridine.[9] (see Supporting Information for details.)

Scheme 1[a]



[a] Reagents and conditions: (a) BH_3·THF, 2-[2-(*N*,*N*-dimethylamino)ethoxy]ethanol, 150 °C. (b) DMTCl, Py, DMAP, rt. (c) *N*,*N*-Diisopropylammonium tetrazolide, (2-cyanoethyl)-*N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite, CH_3CN, rt. (d) (i) Succinic anhydride, NEt_3, $(CH_2Cl)_2$, DMAP, rt; (ii) 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU), DMF, CPG, rt.

Hybridization of the modified oligonucleotides **9** and **12** to complementary RNA and DNA was next studied. Oligonucleotides **9** and **12** demonstrated a duplex stabilization of 1.1 and 0.8 °C per modification as compared to the DNA analogue (Table 1) and 1.9 and 1.6 °C compared to DNA/P═S oligonucleotides.[10] There is no significant difference in T_m values between oligonucleotides modified with 2′-*O*-DMAEOE (**9** and **12**) and those modified with 2′-*O*-MOE (**8** and **11**). This suggests that the addition of the steric bulk in the 2′-*O*-DMAEOE modified oligonucleotides does not result in a substantial destabilization of hybridization with RNA. In contrast, hybridization of **9** with complementary DNA led to a duplex less stable than those formed with unmodified DNA oligonucleotides (0.42 °C destabilization per unit of modification). These findings suggest that preorganization of the 2′-*O*-DMAEOE oligonucleotide results in a preference for formation of a duplex with RNA.

To evaluate the stability of 2′-*O*-DMAEOE oligonucleotides against nucleases, a T_{19} P═O oligonucleotide **13** with

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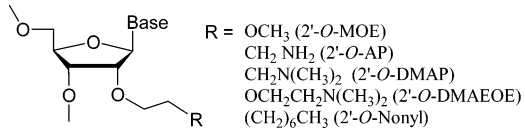

Figure 1. 2′-Modifications described in the text.

four 2′-*O*-DMAEOE modified residues at the 3′-end was synthesized and digested with snake venom phosphodiesterase (SVPD).[11] Figure 2 shows the relative 3′-exonuclease

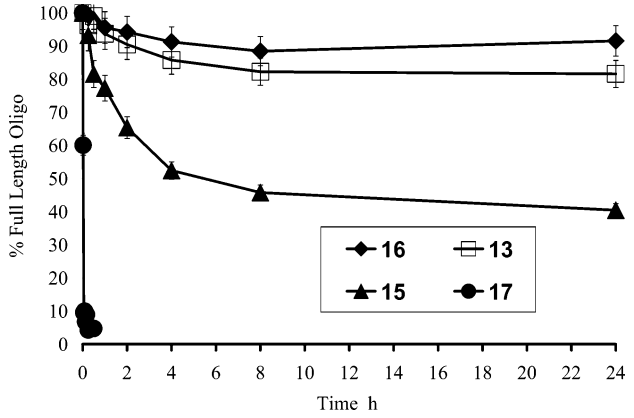

Figure 2. The disappearance of oligonucleotides **13**, **15**, **16**, and **17** in the presence of SVPD as a function of time. **15**−**16**: 5′-T_{15} T*$_4$-3′ (**15**, T* = 2′-*O*-MOE-5-methyluridine; **16**, T* = 2′-*O*-AP-5-methyluridine). **17**: T_{20}. 5′-^{32}P labeled oligonucleotides were digested with SVPD (5×10^{-3} U mL^{-1}) in 50 mM Tris-HCl buffer at pH 8.5, containing 72 mM NaCl and 14 mM $MgCl_2$ at 37 °C.

stability of the 2′-*O*-DMAEOE-modified oligonucleotide compared to DNA (oligonucleotide **17)**. The oligonucleotides modified with 2′-*O*-MOE (**15)** and 2′-*O*-AP (**16)** were also digested with SVPD (Figure 2). The 2′-*O*-DMAEOE modified oligonucleotide was much more stable to 3′-exonuclease mediated cleavage than a 2′-*O*-MOE oligonucleotide and showed nuclease stability similar to that of the oligonucleotide modified with 2′-*O*-AP[5c] (Figure 2).

The crystal structure of palindromic oligonucleotide **14** was determined to 1.6 Å resolution and refined to an *R*-factor of 19.2% (*R*-free = 22.4%; see Supporting Information for experimental details). Coordinates and structure factors have been deposited in the Protein Data Bank as 1NZG. The modified decamer duplex has a standard A-type geometry at all sugars, including the ribose moieties of 2′-*O*-modified residues, all adopting C3′-endo puckers. The torsion angles O2′−CA′−CB′−OC′ (atoms of 2′-*O*-substituents are denoted alphabetically) for both T*6 and T*16 display synclinal conformation, consistent with a gauche effect between O2′

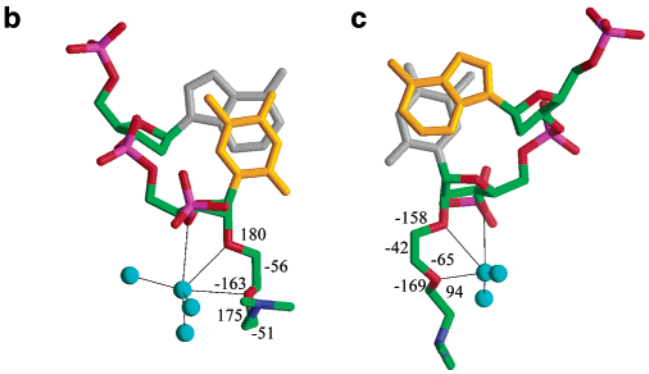

Figure 3. Conformation and hydration of 2′-*O*-DMAEOE substituents. (a) The sequence of the crystallized duplex, **14**, where T* = 2′-*O*-DMAEOE-5-methyluridine. The chemically modified base and the adenine immediately 5′ viewed along the normal to the top base (yellow); (b) $A_5pT^*_6$, (c) $A_{15}pT^*_{16}$. Atoms are green, red, blue, and magenta for carbon, oxygen, nitrogen, and phosphorus, respectively. Torsion angles around substituent bonds are included in degrees. Water molecules are shown as cyan spheres, and hydrogen bonds are drawn with thin solid lines.

and OC′ (Figure 3). The geometries of the 2′-*O*-DMAEOE ethoxy portions are very similar to those for 2′-*O*-MOE substituents in the crystal structures of a decamer duplex containing 2′-*O*-MOE 5-methyluridines.[6] The 2′-*O*-ethoxy moiety provides a binding site for a water molecule in 2′-*O*-MOE[6] and 2′-*O*-DMAEOE modifications. The water molecules form hydrogen bonds to the 3′- and the 2′-oxygen atoms as well as to OC′ of the substituent (Figure 3). The hydration motif found for 2′-*O*-MOE and 2′-*O*-DMAEOE residues presumably stabilizes their synclinal conformations. As in the case of the 2′-*O*-MOE modification, the enhanced RNA affinity and nuclease resistance provided by the 2′-*O*-DMAEOE modification is presumably due to the limited conformational flexibility of the substituent and to the formation of a water network that spans substituent, sugar, and phosphate groups.[6,12] Thus, the 2′-*O*-DMAEOE modification combines the benefits of 2′-*O*-MOE conformational preorganization with the superior nuclease resistance afforded by the positively charged 2′-*O*-AP modification.[5] The strategic placement of oxygen and nitrogen in the 2′-*O*-DMAEOE substituent provides another attractive feature. Usually, consecutive placement of cationic modifications such as 2′-*O*-AP or 2′-*O*-DMAP results in smaller increases in T_m than dispersed placement, presumably due to repulsion of adjacent cationic units.[5,13] The 2′-*O*-DMAEOE modification does not show this disadvantage; the gauche effect places the cationic group such that there is no repulsive destabilization when the residues are adjacent.

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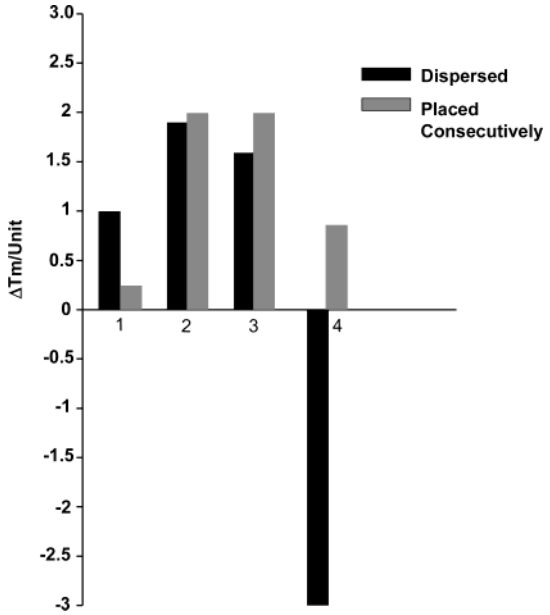

Figure 4. The binding affinity changes due to various 2′-modifications in comparison to 2′-deoxy oligonucleotide phosphorothioate; (1) 2′-*O*-AP, (2) 2′-*O*-MOE, (3) 2′-*O*-DMAEOE, and (4) 2′-*O*-nonyl.

That the stabilization is due to the gauche effect of the oxygen of the 2′-*O*-DMAEOE side chain is further confirmed by comparing it to a 2′-*O*-alkyl side chain lacking the intervening heteroatom. As shown in Figure 4, the 2′-*O*-nonyl substituent is highly destabilizing when dispersed throughout an oligonucleotide (as in sequence **11**, Table 1), but stabilizes the duplex when the modifications are adjacent (as in sequence **8**, Table 1).[14] The stabilization observed when modifications are adjacent in the case of 2′-*O*-nonyl is possibly due to a hydrophobic effect.[12] The 2′-*O*-DMAEOE modification displays advantages of both gauche effect and hydrophobic effect due to alkyl substituents.

In conclusion, we have synthesized novel 2′-*O*-DMAEOE modified oligonucleotides that combine the properties exhibited by the 2′-*O*-MOE and 2′-*O*-AP modifications. They showed binding affinity to complementary RNA similar to 2′-*O*-MOE modification and nuclease stability comparable to that of 2′-*O*-AP modified oligonucleotides. These properties make the 2′-*O*-DMAEOE modification an ideal candidate for further evaluation for antisense drug development and such efforts are in progress in our laboratory. The DMAEOE cytosine analogue has been synthesized by using standard conversion of 5-Me-U to 5-Me-C[15] and the purine analogues are being synthesized.

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Supporting Information Available: Experimental procedures, spectral data for compounds, synthesis of oligonucleotides and crystal data. This material is available free of charge via the Internet at http://pubs.acs.org.

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