Pergamon

Tetrahedron Letters 40 (1999) 661–664

TETRAHEDRON LETTERS

Synthesis, Hybridization, and Nuclease Resistance Properties of 2'-*O*-Aminooxyethyl (2'-*O*-AOE) Modified Oligonucleotides

Andrew M. Kawasaki,* Martin D. Casper, Thazha P. Prakash, Sheri Manalili, Henri Sasmor, Muthiah Manoharan, P. Dan Cook

Department of Medicinal Chemistry, Isis Pharmaceuticals,

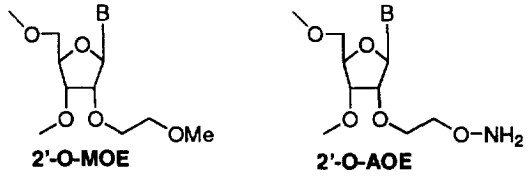
2292 Faraday Ave., Carlsbad, CA, 92008

Received 28 October 1998; accepted 19 November 1998

Abstract: The novel RNA mimic 2'-*O*-AOE has been incorporated into antisense oligonucleotides. This 2'-*O*-modification significantly enhances hybridization against target RNA, and furthermore, exhibits specificity for RNA over DNA. The nuclease resistance (SVPD) of 2'-*O*-AOE modified phosphodiester oligonucleotides is significantly higher than the unmodified DNA and comparable to the 2'-*O*-MOE oligonucleotides.

The paradigm of antisense oligonucleotides as therapeutic drugs requires that the oligonucleotide forms a stable duplex with target mRNA, prevents translation of message (most often via RNase H-mediated cleavage), and is resistant to nucleases.[1] Thus far, 2'-*O*-modified oligonucleotides as "gapmers"[2] (contiguous sequence of 2'-deoxy-phosphorothioate (P=S) oligonucleotide flanked on both 3'- and 5'- sides by modified oligonucleotides) have provided the most effective antisense molecules. The 2'-*O*-substituents provide enhancement of hybridization, the P=S linkages increase nuclease resistance, and the 2'-deoxy region activates the RNase H enzyme.[1] However, with experience we have found that sulfur-containing oligonucleotides can control the pharmacokinetic profile of the compounds, especially via enhanced oligonucleotide-protein binding. Thus, to modulate these parameters we aspire to control, albeit not necessarily eliminate, the sulfur content of the oligonucleotide while maintaining the desired attributes of an antisense drug. To date the 2'-*O*-methoxyethyl-modified oligonucleotide[3] (2'-*O*-MOE, Figure 1) used with the "gapmer"[2] technology has emerged as a leading candidate for clinical application. Although the 2'-*O*-MOE modification has desirable antisense qualities, its nuclease resistance within a phosphodiester (P=O) linkage could be further improved upon. Hence, we were led to investigate the pseudoisostere of MOE, the novel 2'-*O*-aminooxyethyl modification (2'-*O*-AOE, Figure 1).

Figure 1

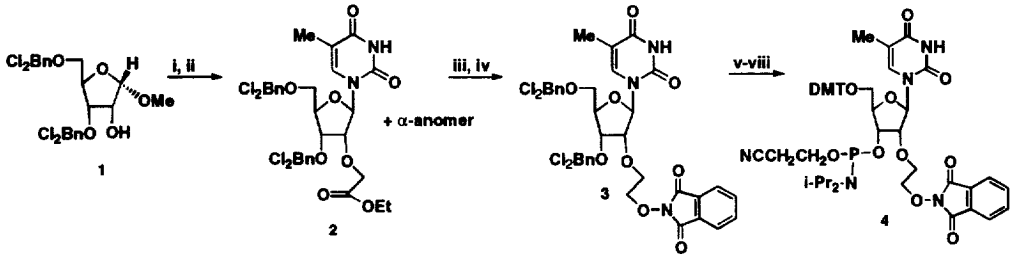


The hydroxylamino function present in this modification is observed in nature in the form of glycosylated antibiotics[4] and has also been synthetically incorporated into oligonucleotide backbones.[5] Among the unique properties of the hydroxylamino function are the unusual conformational preferences of the N-O bond[4,6] and

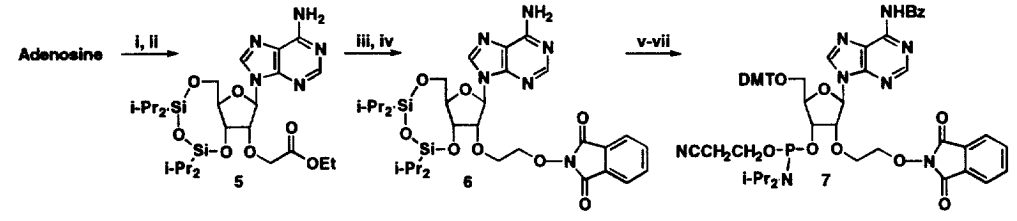
PII: S0040-4039(98)02498-8

the surprisingly low pK_a ($MeONH_2$, 4.2, MeONHMe, 4.75, $MeONMe_2$, 3.65).[7] We expect that the AOE modification will retain the putative "gauche effect"[8] within the side chain strengthening the bias of the furanose sugar toward the 3'-endo conformation. This preorganization is known to enhance hydridization with RNA.[1] Furthermore, the primary aminooxy group of *2'-O*-AOE nucleosides allows for further derivatization, e.g., into alkylaminooxy, oxime, etc. In this work we have successfully prepared the *2'-O*-AOE-5-methyluridine and -adenosine and incorporated the modified nucleosides into oligonucleotides. The *2'-O*-AOE modified oligonucleotides exhibit strong affinity to target RNA and nuclease resistance in snake venom phosphodiesterase (SVPD) assays.

Chemistry. The synthesis of *2'-O*-AOE-5-methyluridine was achieved via utilization of the selectively protected sugar **1**[3] (Scheme 1). Alkylation of compound **1** with ethyl 2-bromoacetate followed by glycosylation with thymine under Vorbrueggen[9] conditions afforded the protected nucleoside **2** in 44% along with the resolvable α-anomer. The activated ester **2** underwent facile reduction with $NaBH_4$ in good yield to give the alcohol which on subsequent Mitsunobu[10] reaction with N-hydroxyphthalimide provided **3** in good yield, thus incorporating a protected aminooxy function. Deprotection of the dichlorobenzyl groups followed by standard chemistries gave phosphoramidite **4** in modest yield.



Scheme 1. i: $BrCH_2CO_2Et$, NaH, DMF, 95%; ii: Thymine, BSA, CH_2ClCH_2Cl, TMS-OTrf, reflux, 44%; iii: $NaBH_4$, EtOH, 90%; iv: HONPhth, PPh_3, DEAD, 81%; v: BCl_3/CH_2Cl_2, -78° to RT; vi: aq $NaHCO_3$, -15-0°, 59%; vii: DMTCl, pyr, 68%; viii: $P(OCH_2CH_2CN)(N\text{-}iPr)_2$, 68%.



Scheme 2. i: NaH, $BrCH_2CO_2Et$, DMF; ii: $TIPDSCl_2$, pyr, 22%, 2 steps; iii: $NaBH_4$, EtOH (anh), 80%; iv: PhthNOH, PPh_3, DEAD, THF, 68%; v: BzCl, pyr, 48%; vi: HF, pyr, 86%; vii: DMTCl, pyr, 63%; vii: $P(OCH_2CH_2CN)(N\text{-}i\text{-}Pr_2)_2$, 55%.

The synthesis of *2'-O*-AOE-adenosine entailed a direct alkylation of adenosine with ethyl 2-bromoacetate to give the *2'-O*-alkylated nucleoside (Scheme 2) along with the putative 3'-O-isomer. The product mixture was not purified at this stage but was protected as the disiloxanylidene[11] derivative to provide the *2'-O*-ethylester **5** in 22% over two steps. Subsequent chemistries were analogous to that of the *2'-O*-AOE-5-methyluridine synthesis (Scheme 1) to afford moderate yields of the phosphoramidite **7**. Finally the modified

oligonucleotides were synthesized as phosphodiesters employing phosphoramidite chemistry and characterized (deprotection conditions were NH_4OH, 6 h, 55° C).

Hybridization.[12] The *2'-O*-AOE modified oligonucleotides showed impressive enhancement of T_m against RNA of 1.0 and 1.2° C/substitution (relative to DNA standard) when four or ten consecutive substitutions, respectively, were incorporated (Table I). In comparison to the *2'-O*-MOE modification, the stabilizing effects of ten *2'-O*-AOE substitutions were essentially equivalent. This translates to nearly +2.0 °C increase/modification when compared to the first generation phosphorothioate (P=S) drugs. Hybridization of the oligonucleotide with 10 substitutions against DNA resulted in a destabilized duplex (relative to DNA standard) as evidenced by the decrease in T_m of –0.94° C/substitution indicating the *2'-O*-AOE modified oligonucleotides display a high specificity toward RNA vs. DNA.

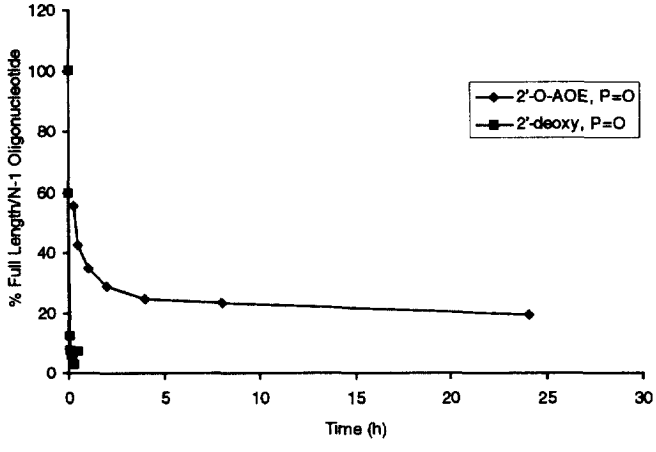
Table I. Effects of 2'-O-Aminooxyethyl (AOE) Modifications on DNA(antisense) • RNA(sense) Duplex Stability

Antisense sequence	subst	T_m (°C)	ΔT_m (°C)	ΔT_m (°C)/subst
CTC GTA CTT TTC CGG TCC		61.5		
CTC GTA CT*T* T*T*C CGG TCC	4	65.6	4.1	1.0
GCG TTT TTT TTT TGC G		48.2		
GCG T*T*T* T*T*T* T*T*T*T* T*GC G	10	60.0	11.9	1.2
GCG $T^{\S}T^{\S}T^{\S}$ $T^{\S}T^{\S}T^{\S}$ $T^{\S}T^{\S}T^{\S}$ $T^{\S}$GC G	10		12.1¥	1.2¥
GCG TTT TTT TTT TGC G†		53.5†		
GCG T*T*T* T*T*T* T*T*T*T* T*GC G†	10	44.0†	-9.4†	-0.94†

T* = 2'-O-AOE-5-methyluridine. $T^{\S}$ = 2'-O-MOE-5-methyluridine. ¥ Value from reference 3. † hybridized against DNA as sense strand.

Nuclease Resistance. To investigate the stability of the *2'-O*-AOE modified oligonucleotides against nucleases a 19-mer oligonucleotide was synthesized with a 3'-capping of four *2'-O*-AOE-5-methyluridines beginning with the 3'-penultimate position (terminal position not modified). In the SVPD assay the *2'-O*-AOE phosphodiester oligonucleotide in comparison with the unmodified phosphodiester exhibits a much increased stability (Figure 2), while in a head to head comparison with *2'-O*-MOE oligonucleotide, the *2'-O*-AOE oligonucleotide displays comparable stabilities (Figure 3) to the *2'-O*-MOE oligonucleotide, i.e., 50% and 40% of full length/N-1 oligonucleotide remains at 6 and 24 hours, respectively.

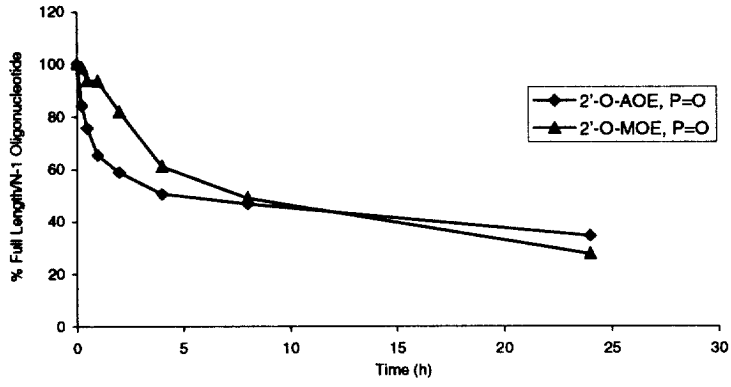
Figure 2. Relative Nuclease Resistance of 2'-O-AOE Oligo Capped with 4 Modified Nucleotides (SVPD Assay) TTT TTT TTT TTT TTT* T*T*T*T



Conclusions. In summary we have synthesized the novel 2'-*O*-AOE- 5-methyluridine and –adenosine nucleosides and successfully incorporated them into oligonucleotides. The novel 2'-*O*-modifications significantly enhance hybridization against RNA, and furthermore, exhibit specificity for RNA over DNA. Although the nuclease resistance (SVPD) of 2'-*O*-AOE modified oligonucleotides is comparable to that of 2'-*O*-MOE, the presently well-accepted antisense RNA mimetic, we desire further improvements. In this regard the primary aminooxy group of 2'-*O*-AOE nucleosides is currently being elaborated into other derivatives to optimize the properties of antisense molecules.

Acknowledgements. We thank our colleagues Steve Owens and Len Cummins for nuclease resistance studies and Elena Lesnik and Sue Freier for hybridization studies.

Figure 3. Relative Nuclease Resistance of 2'-O-AOE and 2'-O-MOE Oligos Capped with 4 Modified Nucleotides (SVPD Assay)
TTT TTT TTT TTT TTT* T*T*T*T



References

1) Cook, P. D. *Second Generation 2'-Modified Antisense Oligonucleotides*; Bristol, J. A., Ed.; Academic Press: New York, 1998; Vol. 33, pp 313-325.
2) Monia, B. P.; Lesnik, E. A.; Gonzalez, C.; Lima, W. F.; McGee, D.; Guinosso, C. J.; Kawasaki, A. M.; Cook, P. D.; Freier, S. M. *J. Biol. Chem.* **1993**, *268*, 14514-22.
3) Martin, P. *Helv. Chim. Acta* **1995**, *78*, 486-504.
4) Walker, S.; Gupta, V.; Kahne, D.; Gange, D. *J. Am. Chem. Soc.* **1994**, *116*, 3197-206.
5) Peoc'h, D.; Swayze, E. E.; Bhat, B.; Dimock, S.; Griffey, R.; Sanghvi, Y. S. *Nucleosides Nucleotides* **1997**, *16*, 959-962.
6) Mohan, V.; Griffey, R. H.; Davis, D. R. *Tetrahedron* **1995**, *51*, 6855-68.
7) Smith, P. A. S. *The Chemistry of Open-Chain Organic Nitrogen Compounds*; W. A. Benjamin, Inc: New York, 1966; Vol. II, pp 3, chapter 8.
8) Wolfe, S. *Accounts Chem. Res.* **1972**, *5*, 102-111.
9) Vorbrueggen, H.; Bennua, B. *Tetrahedron Lett.* **1978**, 1339-42.
10) Mitsunobu, O. *Synthesis* **1981**, 1-28.
11) Markiewicz, W. T. *J. Chem. Res. (S)* **1979**, 24-5.
12) Each sample contained 100 mM Na^+, 10 mM phosphate (pH 7), 0.1 mM EDTA, 4 μM modified oligonucleotide and 4 μM complementary, length matched RNA.