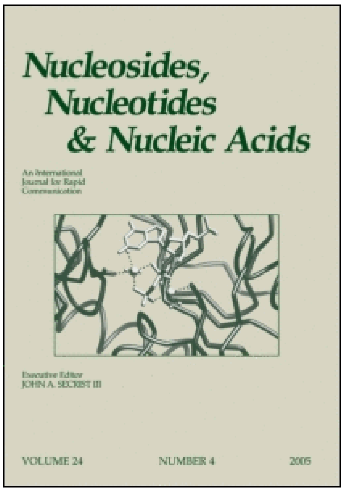

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ANTISENSE PROPERTIES OF 2'-*O*-DIMETHYLAMINOOXYETHYL (2'-*O*-DMAOE) OLIGONUCLEOTIDES

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ANTISENSE PROPERTIES OF 2′-*O*-DIMETHYLAMINOOXYETHYL (2′-*O*-DMAOE) OLIGONUCLEOTIDES

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ABSTRACT

Antisense oligonucleotides with 2′-*O*-{2-[*N*,*N*-dimethyl)aminooxy]ethyl} or (2′-*O*-DMAOE) modification were synthesized and evaluated for nuclease resistance and pharmacology both *in vitro* and *in vivo*. This modification exhibits very high nuclease resistance and efficacy in various biological (ICAM-1, C-*raf* and PKC-α) targets.

Antisense oligonucleotides are chemically modified in order to increase the binding affinity to target RNA, to enhance the nuclease resistance, to improve cellular absorption and/or modulate the protein binding of oligonucleotides (1). A number of modifications at 2′-position of the sugar have successfully met one or more of these goagls (2). 2′-*O*-Dimethylaminooxyethyl (2′-*O*-DMOE) (Fig. 1) is a new carbohydrate modification which exhibits high binding affinity towards target RNA and high nuclease resistance in a snake venom phosphodiesterase assay (3). Here we report the *in vivo* nuclease resistance, *in vitro* and *in vivo* pharmacology of 2′-*O*-DMAOE antisense oligonucleotides.

We synthesized (4) 2′-*O*-DMAOE phosphoramidites of all four nucleosides (A, T, C, G) with standard protecting groups for the exocyclic amino groups (Fig. 2). The modified nucleosides were also converted into their 3′-*O*-succinyl derivative and loaded on to amino alkyl controlled pore glass (CPG Fig. 2) using a standard

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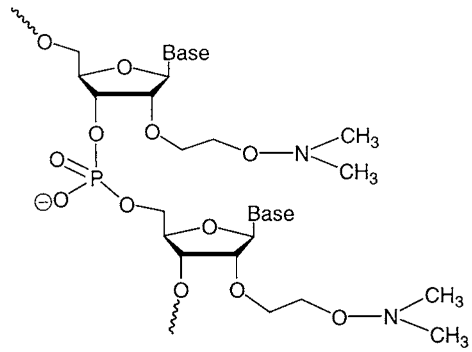
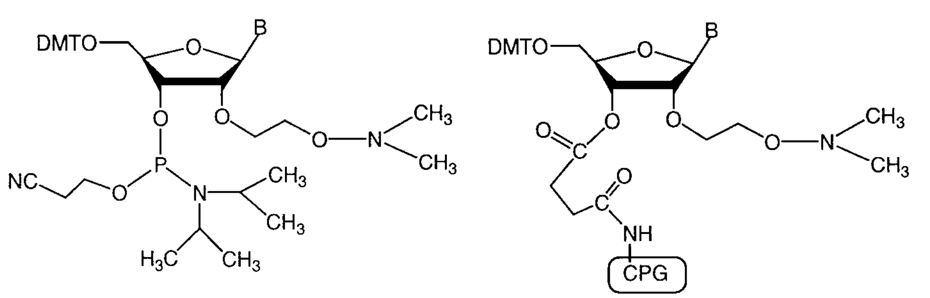


Figure 1.

synthetic procedure (5) to get functionalized solid supports in 55–60 μmol/g loading capacity. Modified oligonucleotides were synthesized with these building blocks.

Gapmer phosphorothioate oligonucleotides with five 2′-*O*-DMAOE modifications on 3′ and 5′ wings were synthesized for evaluation of *in vivo* stability (Table 1). A full phosphorothioate oligonucleotide **1**, a mixed backbone oligonucleotide **2** with phosphodiester at the wings, phosphorothioate at the gap were administered to BalbC mice by IP injection at a 50 mg/kg dose. After 24 h mice were sacrificed and the oligonucleotides were isolated from liver, kidney and spleen. The percentage of full length oligonucleotides in each organ was determined by CGE analysis. After 24 h almost 100% full length phosphorothioate oligonucleotide **1** was isolated from these organs whereas only 25–40% of mixed backbone oligonucleotide **2** was isolated.

Two fully modified oligonucleotides (**3** and **4**, Table 2) with 2′-*O*-DMAOE modifications targeted to the ICAM-1 mRNA (6) were analyzed for inhibition



B = ^{5Me}U, A^{bz}, ^{5Me}C^{bz}, G$_{ibu}$

bz = Benzoyl, ibu = isobutryl

Figure 2. 2′-*O*-DMAOE nucleoside phosphoramidite building blocks and functionalized CPGs.

Table 1. 2′-*O*-DMAOE Oligonucleotides Used for Evaluation of *In Vivo* Stability

No.	Sequence	ES MS Calcd	ES MS Found
1	5′A*sT*sG*sC*sA*sTsTsC†sTsGsC†sC†sC†sC†sC†sA*sA*sG* sG*sA* 3′	7494.7	7496.3
2	5′A*oT*oG*oC*oA*sTsTsC†sTsGsC†sC†sC†sC†sC†sA*oA*oG *oG*oA* 3′	7368.1	7367.9

A* = 2′-*O*-DMAOE A, C* = 2′-*O*-DMAOE ^{5Me}C, G* = 2′-*O*-DMAOEG.
T* = 2′-*O*-DMAOE ^{5Me}U, s = PS, o = PO, C† = ^{5Me}C.

of IL-1β stimulated ICAM-1 protein expression in HUVEC cells. These antisense oligonucleotides efficiently reduced the expression of ICAM-1 protein by a non-RNase H mediated antisense mechanism with an IC_{50} of 2 nM. These results demonstrate the high binding affinity of 2′-*O*-DMAOE modified oligonucleotides to the target mRNA. In another cell-based experiment gapmer oligonucleotide **5** reduced the expression of PKC-α mRNA (7) in human A549 ells with an IC_{50} of 50 nM. This oligonucleotide causes mRNA cleavage by recruitment of RNase H.

We have also examined the efficiency of 2′-*O*-DMAOE modified oligonulcoetide to modulate *in vivo* mRNA expression by RNase H mediated antisense mechanism. Gapmer oligonucleotides (**6** and **7**) with 2′-*O*-DMAOE modifications in the wings were synthesized to target C-*raf* mRNA. Female BALB/C mice were administered oligonucleotides at 3, 10, 25 and 50 mg/kg, once daily for three days. The mice were sacrificed and the tissue was harvested for analysis. Total mRNA was isolated. Quatitation (8) of the mRNA was done by Northern Blot and PhosphorImager analysis. Oligonucleotide **6** was a very potent inhibitor of the C-raf

Table 2. 2′-*O*-DMAOE antisense Oligonucleotides Used for Pharmacology

No.	Sequence	Target	ES MS Calcd	ES MS Found
3	5′ T*sC*sT*sG*sA*sG*sT*sA*sG*sC*sA*sG* sA*sG*sG*sA*sG*sC*sT*sC* 3′	ICAM-1	8605.9	8605.5
4	5′ T*oC*oT*oG*oA*oG*oT*oA*oG*oC*oA* oG*oA*oG*oG*oA*oG*oC*oT*oC*3′	ICAM-1	8300.6	8300.5
5	5′T*sT*sC*sT*sC*sGsC†sTsGsGsTsGsAsGsT* sT*sT*sC*sA▲ 3′	PKC-α	7146.4	7146.4
6	5′A*sT*sG*sC*sA*sTsTsC†sTsGsC†sC†sC†sC† sC†sA*sA*sG*sG*sA* 3′	C-*raf*	7496.1	7496.1
7	5′A*oT*oG*oC*oA*sTsTsC†sTsGsC†sC†sC† sC†sA*oA*oG*oG*oA* 3′	C-*raf*	7368.1	7367.9

A* = 2′-*O*-DMAOE A, C* = 2′-*O*-DMAOE ^{5Me}C, G* = 2′-*O*-DMAOEG,
T* = 2′-*O*-DMAOE ^{5Me}U, A▲ = 2′-*O*-MOE A, s = PS, o = PO, C† = ^{5Me}C.

mRNA expression. However mixed backbone oligonucleotide **7** was less effective in reducing the mRNA levels.

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