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Zwitterionic oligonucleotides with 2′-*O*-[3-(*N*,*N*-dimethylamino)propyl]-RNA modification: synthesis and properties

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Abstract

A novel 2′-modification, 2′-*O*-[3-(*N*,*N*-dimethylamino)propyl] or 2′-*O*-DMAP, has been incorporated into oligonucleotides and compared to the known 2′-*O*-(3-aminopropyl) or 2′-*O*-AP modification for antisense properties. The 2′-*O*-DMAP modified oligonucleotides exhibit very high nuclease resistance like the 2′-*O*-AP modification due to the 'charge effect' and maintain high binding affinity to target RNA relative to known modifications when a few 2′-*O*-DMAP residues are dispersed throughout the oligonucleotide.

Cationic oligonucleotides[1] (oligonucleotides with positively charged backbones) and zwitterionic oligonucleotides[2–4] (oligonucleotides with positively charged tethers) are interesting classes of nucleic acids because of their intrinsically favorable binding properties towards RNA and single-stranded as well as double-stranded DNA. High affinity binding is due to charge neutralization and potentially fast on-rates of hybridization. In addition, these oligonucleotides are expected to have good cellular permeation properties for potential therapeutic applications.

The 2′-position of the carbohydrate moiety has proven to be a prime position for oligonucleotide modifications for antisense technology. Many of these 2′-modifications exhibit high binding affinity to target RNA, enhanced chemical stability and nuclease resistance, as well as increased lipophilicity. All high binding affinity 2′-modifications have a $C_{3'}$-*endo* sugar pucker.[5] Among the 2′-modifications reported in the literature, the 2′-*O*-(3-aminopropyl) modification (Fig. 1), 2′-*O*-AP,[3] offers a 1.0°C increase in binding affinity/modification as a diester (2′-*O*-AP/P=O) compared to the first generation of 2′-deoxyphosphorothioate (2′-H/P=S) compounds when the positive charge is dispersed in the oligonucleotide chain. This modification as a phosphodiester linkage exhibits nuclease resistance (measured as the half-life of the full-length oligonucleotide, $t_{1/2}$) approximately 6 to 8 times better than a 2′-deoxyphosphorothioate modification. Thus, 2′-*O*-aminopropyl

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modified oligonucleotides[5] have shown the greatest nuclease resistance[4] of any modification tested thus far ($t_{1/2} > 24$ h in snake venom phosphodiesterase assays compared to 4 min for unmodified phosphodiester oligonucleotide). The superior nuclease resistance of the 2′-*O*-aminopropyl modification was attributed to the 'charge effect' present in the cationic side chain of the 2′-*O*-aminopropyl modified oligonucleotides, which interacts competitively with the metal ions involved in the phosphodiesterase activity of the nucleolytic enzymes.[3,4]

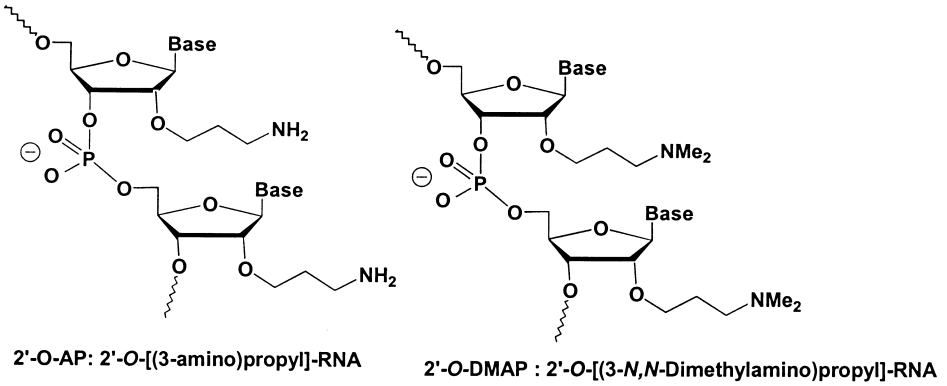

Figure 1.

We have now synthesized the dimethyl analog of the 2′-*O*-(3-aminopropyl), the 2′-*O*-DMAP modification (Fig. 1) and evaluated its antisense properties. Dialkylation of the amino group as in the 2′-*O*-DMAP modification would eliminate the need for a protecting group for the amino functionality in the synthesis of the 2′-*O*-aminopropyl modified oligonucleotides.[3] The protecting groups now used for 2′-*O*-aminopropyl modified oligonucleotides, phthalimido and trifluoroacetyl, have certain limitations in terms of monomer synthesis and oligonucleotide deprotection conditions.[6] Moreover, we expected the 2′-*O*-DMAP oligonucleotides to be more lipophilic than the 2′-*O*-AP modified oligonucleotides, a characteristic which could improve the cellular and protein binding properties and hence the biodistribution of the 2′-*O*-DMAP oligonucleotides compared to 2′-*O*-AP oligonucleotides.

We recently reported a novel and efficient scheme to synthesize the 2′-*O*-DMAP modified 5-methyluridine nucleoside **1** and the corresponding amidite **2**[7] involving alkylation of the 2′-*O*-position using a six-membered cyclic sulfate (1,3,2-dioxathiane 2,2-dioxide) followed by nucleophilic displacement with dimethylamine (Fig. 2). Treatment of *N*-3-benzyloxymethyl-5-methyluridine with NaH in DMF at –45°C followed by addition of the cyclic sulfate afforded the 2′-*O*-propylsulfate in 50% yield, with a selectivity for 2′ over 3′ alkylation of about 3:1. The sulfate was then displaced using dimethylamine as a nucleophile in an autoclave. Reductive cleavage of the *N*-3-benzyloxymethyl (BOM) protecting group using catalytic hydrogenation over a palladium hydroxide catalyst proceeded smoothly to give the 2′-modified nucleoside **1** in 67% yield. This compound was then protected at the 5′-hydroxyl with the dimethoxytrityl (DMT) group and then converted to the phosphoroamidite **2**.

The nucleoside **1** was loaded onto controlled pore glass (CPG) following the standard synthetic procedure[8] to obtain the functionalized CPG **3** with 53 μmol/g loading capacity. The amidite **2** was dissolved in anhydrous acetonitrile to 0.1 M and loaded onto an Expedite Nucleic Acid Synthesis system (Millipore 8909) to synthesize the oligonucleotides. The coupling efficiencies

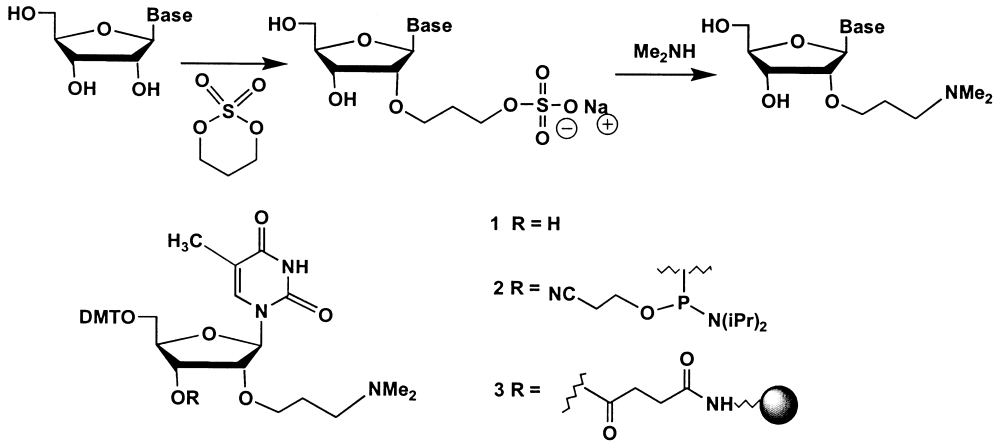

Figure 2.

were more than 98%. For the modified amidite **2**, the coupling time was extended to 10 min and coupling was repeated twice. Oxidation of the phosphite intermediate was carried out using CSO [1-*S*-(+)–(10-camphorsulfonyl) oxaziridine].[9] All other steps in the protocol supplied by Millipore were used without modification. After completion of the synthesis, the CPG was suspended in aqueous ammonia (30%) at room temperature for 2 h to cleave the oligonucleotide from the CPG. The CPG was filtered; the filtrate was then heated at 55°C for 6 h to effect the complete removal of the base-labile protecting groups. Ammonia was removed on a speed vac concentrator. The resulting product was purified by High Performance Liquid Chromatography (HPLC, Waters, C-4, 7.8×300 mm, A = 50 mM triethylammonium acetate (TEAAc), pH = 7, B = acetonitrile, 5 to 60% B in 55 min, flow 2.5 mL/min, λ = 260 nm). Detritylation with aqueous 80% acetic acid and evaporation followed by desalting by HPLC on a Waters C-4 column gave the 2′-modified oligonucleotides listed in Table 1, which were analyzed by HPLC, capillary gel electrophoresis (CGE) and mass spectrometry.

Table 1
Oligonucleotides containing the 2′-*O*-[3-(*N*,*N*-dimethylamino)propyl] modification

ISIS No.	Sequence	Mass Calculated	Mass Observed	HPLC Retention Time (min.[a])
111710	5' T*CC AGG T*GT* CCG CAT*C 3'	5239.36	5237.6	16.85
111709	5' CTC GTA CT*T* T*T*C CGG TCC 3'	5797.85	5796.80	17.58
111711	5' GCG T*T*T* T*T*T* T*T*T* T*GC G 3'	5887.34	5886.40	16.90
116168	5' TTT TTT TTT TTT TTT T*T*T* T* 3'	6124.09	6124.06	19.48

T* = 2'-*O*-[3-(*N*,*N*-dimethylamino)propyl] ^{5Me}U; [a]Waters C-4, 3.9x300 mm, solvent A=50 mm TEAAc, pH 7; Solvent B = CH_3CN; gradient 5-60% B in 55 min; flow rate 1.5 mL/min, λ = 260 nm.

When the modified oligonucleotides were hybridized to complementary RNA, the change in Tm was dependent on the placement of the modifications (Table 2). When the substitutions were not adjacent as in ISIS 111710, the modified oligonucleotide showed a +1.2°C increase in Tm for

Table 2
Tm values of 2′-*O*-[3-(*N*,*N*-dimethylamino)propyl] modified P=O oligonucleotides against RNA

ISIS No.	Sequence 5'to 3'	Tm °C	ΔTm °C	ΔTm/mod. °C
2221	TCC AGG TGT CCG CAT C	62.3		
111710	T*CC AGG T*GT* CCG CAT* C	67.2	4.72	1.2
3404	GCG TTTTTTTTT GCG	48.3		
111711	GCGT*T*T*T*T*T*T*T*T*T*GCG	46.5	-1.8	-0.18
2896	CTC GTA CTT TTC CGG TCC	61.8		
111709	CTC GTA CT*T* T*T*C CGG TCC	61.88	0.08	0.02

T* = 2'-*O*-[3-(*N*,*N*-dimethylamino)propyl]^{5Me}U. Tm measurement conditions are described in reference 11.

each substitution compared to unmodified DNA. This observed difference of a 1.2°C per modification translates to a nearly 2°C increase/modification when compared to the first generation of antisense compounds in clinical use, the 2′-deoxyphosphorothioate (2′-H/P=S) compounds.[10] However, when substitutions were placed adjacent to each other, the enhanced stability was lost (ISIS 111709). As more modifications were incorporated (ISIS 111711), destabilization was observed, perhaps owing to actual charge repulsion between adjacent cationic groups. This is in agreement with the reported hybridization profile of the 2′-*O*-aminopropyl and 2′-*O*-aminoethyl oligonucleotides.[3,6] The addition of steric bulk because of the two methyl groups does not cause additional destabilization relative to other 2′-*O*-amino modifications, however. In fact, there is stabilization of almost +0.6°C/modification in the 111710 sequence over the 2′-*O*-AP oligonucleotide with the same sequence and modification pattern. This difference might be due to the relative extents of hydration, which would be less for 2′-*O*-DMAP (one hydrogen bond with water in the protonated form) than 2′-*O*-AP (three hydrogen bonds with water in the protonated form).[4]

The 2′-*O*-DMAP end-capped oligonucleotide ISIS 116168 was digested with snake venom phosphodiesterase (SVPD) to evaluate the 3′-exonuclease resistance of this modification.[12] Fig. 3 shows the relative nuclease stability of the 2′-*O*-DMAP modified oligonucleotide compared to the

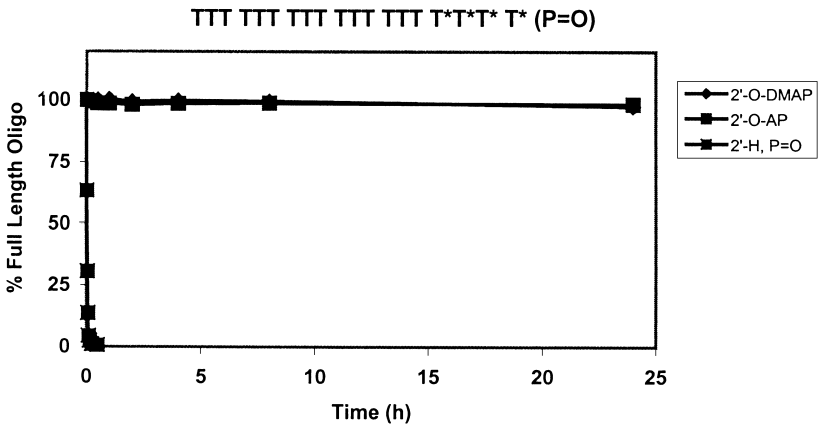

Figure 3. Nuclease resistance of 2′-H, 2′-*O*-AP and 2′-*O*-DMAP modified oligonucleotides

unmodified oligonucleotide and one with 2′-*O*-AP modifications. Incorporation of 2′-*O*-DMAP T residue provides very high exonuclease resistance with t $\frac{1}{2}$ > 24 h and it is similar to that of 2′-*O*-AP modification

High binding affinity to RNA, favorable lipophilicity, synthetic convenience, and extremely high nuclease resistance as diester (P=O) linkages all warrant further in vivo pharmacokinetic and biodistribution evaluation of 2′-*O*-DMAP modified oligomers in antisense and triplex-forming strategies. Furthermore, this modification is expected to improve the lifetime and efficacy of oligomers, having a favorable impact on the frequency of administration and on the oral absorption potential of therapeutic oligonucleotides.

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