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2′-*O*-[2-(Amino)-2-oxoethyl] Oligonucleotides

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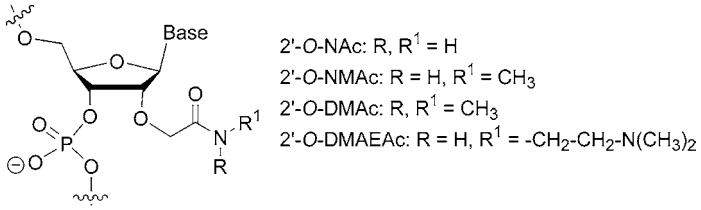
ABSTRACT



Oligonucleotides with novel modifications, 2′-*O*-[2-(amino)-2-oxoethyl] (2′-*O*-NAc), 2′-*O*-[2-(methylamino)-2-oxoethyl] (2′-*O*-NMAc), 2′-*O*-[2-(dimethylamino)-2-oxoethyl] (2′-*O*-DMAc), and 2′-*O*-[2-[[2-(dimethylamino)ethyl]amino]-2-oxoethyl] (2′-*O*-DMAEAc), have been synthesized. These modified oligonucleotides exhibit high binding affinity to complementary RNA (and not to DNA) and considerably enhance the nuclease stability of oligonucleotides with $t_{1/2}$ > 24 h.

Antisense oligonucleotides modified at the 2′-*O*-position of the sugar at one or both termini (gapmers)[1,2] have emerged as leading second-generation candidates for clinical applications. Chemical modifications such as 2′-*O*-methoxyethyl (2′-*O*-MOE) improve the metabolic stability, cellular absorption, and protein binding properties of oligonucleotides.[2]

To improve the antisense properties of 2′-*O*-MOE-modified oligonucleotides, we designed oligonucleotides with novel 2′-*O*-modifications, including 2′-*O*-[2-(methylamino)-2-oxoethyl] (2′-*O*-NMAc), 2′-*O*-[2-(dimethylamino)-2-oxoethyl] (2′-*O*-DMAc), and 2′-*O*-[2-[[2-(dimethylamino)ethyl]-amino]-2-oxoethyl] (2′-*O*-DMAEAc), as shown in Figure 1. Among them, the parent 2′-*O*-[2-(amino)-2-oxoethyl][3] (2′-*O*-NAc) modification has been synthesized and studied by Sproat et al. We postulated that the nucleosides containing

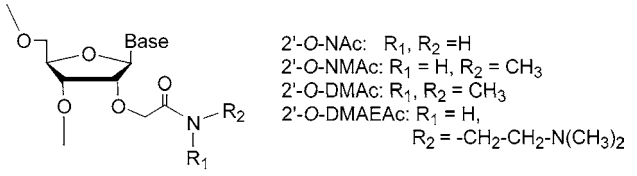


Figure 1.

these modifications should retain the "gauche effect"[4] and thus the $C_{3'}$-endo sugar pucker characteristic of the 2′-*O*-MOE nucleosides.[5] Consequently, oligonucleotides containing these modified nucleoside residues should display the enhanced hybridization to RNA targets observed with the 2′-*O*-MOE modified oligonucleotides. In addition, 2′-*O*-NMAc, 2′-*O*-DMAc, and 2′-*O*-DMEAc oligonucleotides should be more lipophilic than the corresponding 2′-*O*-MOE counterparts, and thus may have better protein binding and cellular permeation properties. Here we report the synthesis,

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(2) (a) Martin, P. *Helv. Chim. Acta* **1995**, *78*, 486-504 (b) Manoharan, M. *Biochim. Biophys. Acta* **1999**, *1489*, 117−130. (c) Monia, B. P.; Lesnik, E. A.; Gonzalez, C.; Lima, W. F.; Guinosso, C. J.; Kawasaki, A. M.; Cook, P. D.; Freier, S. M. *J. Biol. Chem.* **1993**, *268*, 14514−14522.

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(4) Wolfe, S. *Acc. Chem. Res.* **1972**, *5*, 102−111.

(5) Teplova, M.; Minasov, G.; Tereshko, V.; Inamati, G. B.; Cook, P. D.; Manoharan, M.; Egli, M. *Nat. Struct. Biol.* **1999**, *6*, 535−539.

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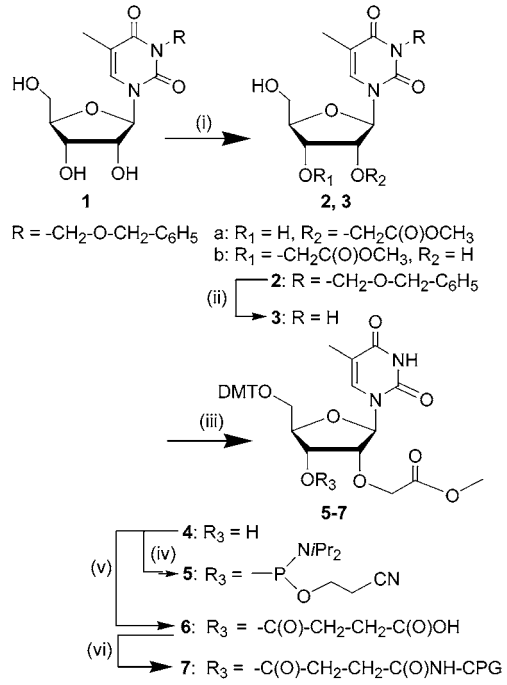
affinity toward target RNA, and nuclease stability of the 2′-*O*-NAc analogues.

The phosphoramidite **5** and the solid support **7** were synthesized as described in Scheme 1. Compound **1** was alkylated with methyl 2-bromoacetate to give 2′- and 3′-regio-isomers **2a** and **2b** in a ratio of 9:1.[6] The compound **2a** was isolated by silica gel chromatography and converted to **5** and **7** according to standard procedures.[7]

Scheme 1[a]



[a] Reagents and conditions: (i) Methyl 2-bromoacetate, NaH, DMF, −40 °C to room temperature. (ii) (a) H_2 30 psi, 10% Pd on carbon, MeOH; (b) triethylamine, MeOH. (iii) DMTCl, DMAP, Py. (iv) 2-Cyanoethyl-*N*,*N*-diisopropylaminochlorophosphite, diisopropylethylamine, CH_2Cl_2, rt. (v) succinic anhydride, 1,2-dichloroethane, DMAP, $(C_2H_5)_3N$, rt. (vi) 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluroniumtetrafluoroborate (TBTU), 4-methyl morpholine, DMF, aminoalkyl controlled pore glass (CPG), rt.

Table 1. Oligonucleotides with 2′-Modifications[a]

No	Sequences 5'-3'
8, 12, 16, 20, 27	d(tCC AGG tGt CCG CAt C)
9, 13, 17, 21, 28	d(GCG ttt ttt ttt tGC G)
10, 14, 18, 22, 29	d(CTC GTA Ctt ttC CGG TCC)
11, 15, 19, 23, 30	d(TTT TTT TTT TTT TTT ttt t)

[a] All oligonucleotides were phosphodiesters. **8**−**11**: t = 2′-*O*-(2-methoxy-2-oxoethyl)-5-methyluridine. **12**−**15**: t = 2′-*O*-DMAEAc-5-methyluridine. **16**−**19**: t = 2′-*O*-NMAc-5-methyluridine. **20**−**23**: t = 2′-*O*-NAc-5-methyluridine. **27**−**30**: t = 2′-*O*-DMAc-5-methyluridine.

solid support **7** was used for **11**. To avoid transamination during the treatment with amines, dC^{Ac} phosphoramidite was used for incorporation of the dC residues.[8] Oxidation of the internucleoside phosphite groups was carried out with 1-*S*-(+)-(10-camphorsulfonyl)oxaziridine.[9]

The 2′-*O*-modified oligonucleotides **12**−**23** were synthesized by using solid support-bound oligonucleotides **8**−**11** that contain the chemically reactive methyl ester function. Upon completion of the oligonucleotide synthesis, the modifications were introduced and the oligonucleotides were deprotected by treating the solid support-bound material with an appropriate amine as shown in Figure 2.[10] The solid support-bound oligonucleotides **8**−**11** were treated with 50% *N*,*N*-dimethylethylenediamine in EtOH (to yield **12**−**15,** Table 1), or 40% aqueous methylamine (to yield **16**−**19,** Table 1), or saturated methanolic ammonia (to yield **20**−**23,** Table 1). Reactions were allowed to proceed at room temperature for 24 h. Under these conditions, the methyl ester functions were converted to the corresponding amides in high yields (Figure 2). Simultaneously, the base and phosphate protecting groups were removed and the oligonucleotides were cleaved from solid support. The oligonucleotides bearing 2′-*O*-DMAEAc, 2′-*O*-NMAc, and 2′-*O*-NAc modifications (**12**−**15**, **16**−**19**, and **20**−**23**, respectively) were purified by reverse-phase HPLC and characterized by ES MS. The purity of the compounds **12**−**23** was assessed by HPLC and capillary gel electrophoresis.

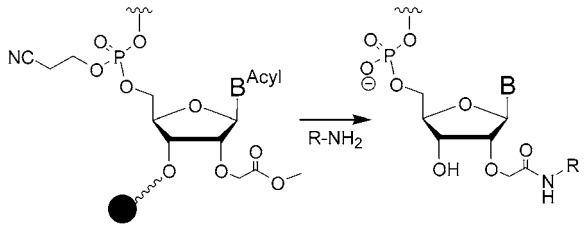


Figure 2.

In contrast to these results, treatment of the solid supports **8**−**11** with 40% aqueous dimethylamine for 3 days at room temperature gave only 50−60% of the desired oligonucleotides **27**−**30**. We attributed the low yield to the widely known, low reactivity of secondary amines toward ester functions. Furthermore, the deprotection at elevated temperature led to complex mixtures of products.

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(8) Reddy, M. P.; Farooqui, F.; Hanna, N. B. *Tetrahedron Lett* **1996**, *37*, 8691−8694.
(9) Manoharan, M.; Lu, Y.; Casper, M. D.; Just, G. *Org. Lett.* **2000**, *2*, 243−246. Although CSO has been used for oxidation of internucleosidic phosphite groups, other conventional oxidizing agents such as *tert*-butyl hydroperoxide in acetonitrile or iodine in tetrahydrofuran, pyridine, and water may also be used.
(10) (a) Keller, T. H.; Haner, R. *Helv. Chim. Acta* **1993**, *76*, 884−892. (b) Keller, T. H.; Haner, R. *Nucleic Acids Res.* **1993**, *21*, 4499−4505.

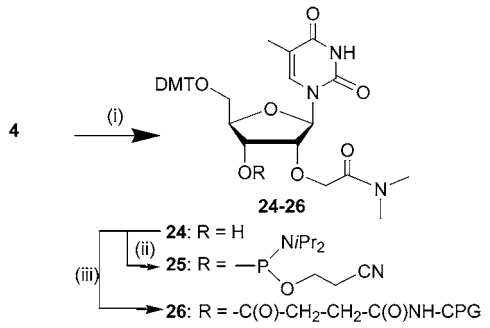
Table 2. T_m Data for the Oligonucleotides Hybridized to Complementary RNA[a]

	12, **16**, **20**, **27**		**13**, **17**, **21**, **28**		**14**, **18**, **22**, **29**	
t	T_m	ΔT_m/unit	T_m	ΔT_m/unit	T_m	ΔT_m/unit
thymidine	62.3		48.3		61.8	
2′-*O*-DMAEAc-5-methyluridine	67.8	1.4	50.0	0.2	62.1	0.1
2′-*O*-NMAc-5-methyluridine	69.2	1.7	60.0	1.2	66.0	1.0
2′-*O*-NAc-5-methyluridine	69.5	1.8	61.0	1.3	66.9	1.3
2′-*O*-DMAc-5-methyluridine	67.1	1.2	60.8	1.3	66.2	1.1

[a] Oligonucleotides **12**, **16**, **20**, **27**: 5′ d(tCC AGG tGt CCG CAt C) 3′. Oligonucleotides **13**, **17**, **21**, **28**: 5′ d(GCG ttt ttt ttt tGC G) 3′. Oligonucleotides **14**, **18**, **22**, **29**: 5′ d(CTC GTA Ctt ttC CGG TCC) 3′. T_m values were assessed in 100 mM Na^+, 10 mM phosphate, 0.1 mM EDTA, pH 7 at 260 nm, with 4 μM oligonucleotides and 4 μM complementary length matched RNA. Experimental error did not exceed ±0.5 °C.

In view of these facts, an alternative approach was taken for the synthesis of the 2′-*O*-DMAc modified oligonucleotides. The nucleoside **4** was treated with 2 M dimethylamine in THF for 18 h at room temperature to give **24** in 95% isolated yield (Scheme 2). This was then converted into a 3′-phosphoramidite **25** and a solid support **26** in a conventional manner.

Scheme 2 [a]



[a] Reagents and conditions: (i) 2 M dimethylamine in THF, rt. (ii) 2-Cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite, *N*,*N*-diisopropylammonium tetrazolide, CH_3CN, rt. (iii) (a) succinic anhydride, $ClCH_2CH_2Cl$, DMAP, $(C_2H_5)_3N$, rt; (b) TBTU, 4-methylmorpholine, DMF, amino alkyl CPG, rt.

The oligonucleotides **27**−**30** were assembled on the solid support as described above for **8**−**11** except that the phosphoramidite **25** and the solid support **26** were used instead of **5** and **7**. The solid support-bound material was deprotected with methylamine (4% solution) in aqueous ammonium hydroxide[8] at room temperature for 24 h to give **27**−**30** with no major side products observed by ES MS analysis of the crude material. The oligonucleotides obtained were purified and characterized as described above. In contrast, deprotection of **27**−**30** with concentrated aqueous ammonium hydroxide at elevated temperature (55 °C, 6 h) led to mixtures of the products where the dimethylamino group in the 2′-*O*-DMAc side chain was partially displaced with the amino group as evidenced by ES MS.

Hybridization of the modified oligonucleotides with complementary RNA (Table 2) was studied. The oligonucleotides with consecutive modifications, **17**, **18**, **21**, **22**, **28**, and **29**, demonstrated a duplex stabilization of 1.1−1.3 and 1.9−2.1 °C per modification as compared to the corresponding 2′-deoxy phosphodiester oligonucleotides (PO) and their phosphorothioate (PS) analogues,[11] respectively. Oligonucleotides **16**, **20**, and **27** with four dispersed modifications stabilized the duplex with RNA by 1.2−1.8 and 2−2.6 °C, compared to the corresponding PO and PS analogues, respectively.[11] These values are slightly better than the T_m enhancements observed for 2′-OMe and 2′-O-MOE modifications.

The oligonucleotides **12**, **13**, and **14** bearing cationic dimethylamino groups demonstrated a significant dependence of the melting temperatures (T_m) on the distance between the modified nucleoside residues. Compounds **13** and **14** with consecutively placed dimethylamino groups showed only a moderate increase in T_m (0.1−0.2 °C per modification compared to the PO control). In contrast, a T_m enhancement of 1.4 °C per modification was observed for oligonucleotide **12** where dimethylamino groups were dispersed. These observations are in agreement with the reported hybridization profile of oligonucleotides bearing 2′-*O*-aminopropyl and homologous groups.[12,1c]

Comparison of the T_m values of 2′-*O*-NAc oligonucleotides **20**−**22** with their 2′-*O*-NMAc (**16**−**18**) and 2′-*O*-DMAc analogues (**27**−**29**) demonstrated no significant difference in their thermal stability. This suggested that the addition of the steric bulk in the 2′-*O*-NMAc and 2′-*O*-DMAc oligonucleotides did not affect their hybridization affinity to RNA.

Specificity of hybridization of several 2′-modified oligonucleotides including 2′-*O*-MOE against complementary RNA reported in the literature was similar to that of unmodified DNA.[2a,11] We expect the specificity of hybridization of the novel 2′-modified oligonucleotides described in this Letter to be similar to that of unmodified DNA as well.

In contrast to results with complementary RNA, hybridization with the complementary DNA led to duplexes less stable than those formed with the corresponding PO controls (Table 3). These results demonstrated an RNA-selective hybridiza-

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Table 3. T_m Data for the Oligonucleotides[a] **13**, **17**, **21**, and **28** Hybridized to the Complementary DNA

t	T_m (°C)	ΔT_m (°C)	ΔT_m (°C)/ modification
thymidine (parent)	48.3		
2′-*O*-DMAEAc-5-methyluridine (**13**)	41.5	−6.8	−0.7
2′-*O*-NMAc-5-methyluridine (**17**)	39.9	−8.4	−0.8
2′-*O*-NAc-5-methyluridine (**21**)	41.4	−6.9	−0.7
2′-*O*-DMAc-5-methyluridine (**28**)	45.7	−2.6	−0.3

[a] 5′-d(GCGt$_{10}$GCG); T_m values were assessed in 100 mM Na^+, 10 mM phosphate, 0.1 mM EDTA, pH 7 at 260 nm, with 4 μM oligonucleotides and 4 μM complementary length matched DNA. Experimental error did not exceed ±0.5 °C.

tion of the 2′-*O*-NAc-, 2′-*O*-NMAc-, 2′-*O*-DMAc-, and 2′-*O*-DMAEAc-modified oligonucleotides.

To evaluate the metabolic stability of the novel oligonucleotides, digestion with snake venom phosphodiesterase (SVPD) was monitored as described previously.[13] The enzymatic hydrolysis of compounds **15**, **19**, **23**, and **30**, phosphodiester oligonucleotides capped at the 3′-terminus with four 2′-*O*-modified nucleoside residues, was evaluated. The plot of the time-dependent disappearance of the novel oligonucleotides and their 2′-deoxy and 2′-*O*-MOE analogues is shown in Figure 3. The enzymatic stability of the 2′-*O*-modified oligonucleotides studied decreased in the following order 2′-*O*-DMAEAc > 2′-*O*-NMAc > 2′-*O*-DMAc > 2′-*O*-NAc > 2′-*O*-MOE ≫ 2′-deoxy. All the novel modifications imparted significant stability. In particular, the resistance of the 2′-*O*-DMAEAc compound **15** against nucleolytic degradation is worth noticing.

In conclusion, we have synthesized three novel types of 2′-modified oligonucleotides that showed excellent binding affinity to complementary RNA and nuclease stability. Of the modified oligonucleotides described in this Letter, 2′-*O*-NMAc and 2′-*O*-DMAEAc oligonucleotides were the most synthetically feasible and have been selected for further biological evaluation.

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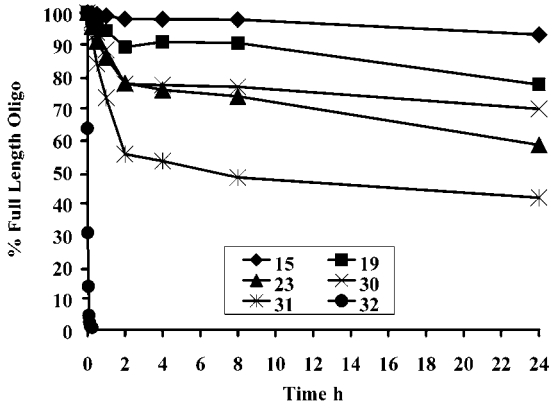

Figure 3. The disappearance of oligonucleotides **15**, **19**, **23**, **30**, **31**, and **32** in the presence of SVPD as a function of time. **31**: 5′-T_{15} t_4-3′ (t = 2′-*O*-MOE-5-methyluridine).; **32**: T_{20}. 5′-^{32}P labeled oligonucleotides were digested with SVPD (5×10^{-3} U mL^{-1}) in 50 mM Tris-HCl buffer at pH 8.5, containing 72 mM NaCl and 14 mM $MgCl_2$ at 37 °C.

Supporting Information Available: Experimental procedures and ^{1}H, ^{13}C NMR, and mass spectral data for compounds **2**−**4**, **6**, **24**, and **26** and ^{31}P NMR and mass spectral data for **5** and **25**; experimental procedure, ES MS data, and HPLC retention times for oligonucleotides **12**−**23** and **27**−**30**; procedures used for the T_m and nuclease studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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