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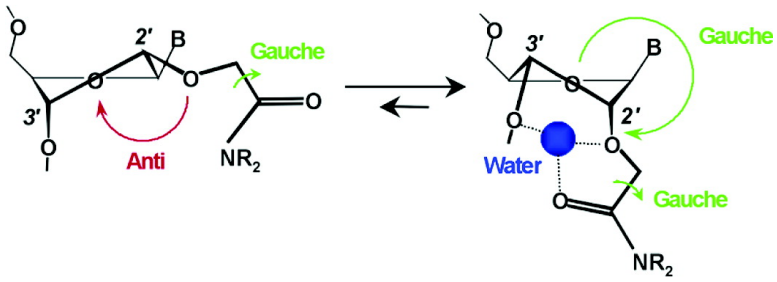

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Structural Rationalization of a Large Difference in RNA Affinity Despite a Small Difference in Chemistry between Two 2′-*O*-Modified Nucleic Acid Analogues

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Chemically modified antisense oligonucleotides (AONs) continue to be explored as anticancer, antiviral, and antiinflammatory agents. The first-generation DNA phosphorothioates (PS-DNA) are gradually being replaced by second- and third-generation modifications in the clinic.[1] Second-generation antisense nucleic acid analogues include those with 2′-*O*-modifications of the ribose. This type of modification has several advantages, among them ease of synthesis and increased RNA affinity, nuclease resistance, and cellular uptake.[2]

For example, duplexes between 2′-*O*-[2-(methoxy)ethyl]-RNA (MOE-RNA) and RNA exhibit stabilities that exceed those of duplexes between PS-DNA and RNA by about 2 °C per modification.[3] The nuclease resistance of MOE-RNA is comparable to that of PS-DNA.[3] The 2′-*O*-MOE substituent locks the ribose in a C3′-*endo* conformation and thus preorganizes the AON for the geometry preferred by the RNA target. In addition to the gauche effect between O4′ and O2′ in the ribose, a gauche effect between O2′ and the MOE methoxy-oxygen controls the conformation of the 2′-*O*-substituent. Crystal structures of 2′-*O*-modified oligonucleotides demonstrated that the MOE moiety improves the hydration of the sugar−phosphate backbone relative to DNA.[4]

2′-*O*-Substituents carrying a positive charge (i.e. 2′-*O*-(3-aminopropyl) or AP) confer superior nuclease resistance to AONs with one or more modifications at the 3′-end.[5] Such zwitterionic AONs may escape exonuclease degradation by displacing a catalyically important metal ion from the active site.[6] Recently, the beneficial features of the 2′-*O*-MOE and 2′-*O*-AP modifications with regard to RNA affinity and nuclease resistance, respectively, have been fused in the 2′-*O*-[2-{2-(*N*,*N*-dimethylamino)ethyloxy}ethyl] substituent,[7] and the sulfur analogue of MOE has been shown to improve cellular uptake.[8]

Two new RNA mimetics, 2′-*O*-[2-(methylamino)-2-oxoethyl]-RNA (2′-*O*-NMA-RNA)[9] and 2′-*O*-(*N*-methylcarbamate)-RNA (2′-*O*-NMC-RNA)[10] (Figure 1) exhibit strongly different effects on the RNA affinity of modified DNAs. Incorporation of 2′-*O*-NMA-T increased the stability of duplexes with RNA by between 1.8 and 2.5 °C (dispersed vs consecutive placement, respectively) per modified residue relative to the corresponding PS-DNAs.[9] Conversely, incorporation of 2′-*O*-NMC-T decreased the stability of duplexes with RNA by between 3.2 and 2.5 °C (dispersed vs consecutive placement, respectively) per modified residue relative to PS-DNA.[10] Molecular modeling studies suggested that the destabilization was in part due to a short contact between the NMC amino group and C1′ of thymidine.[10]

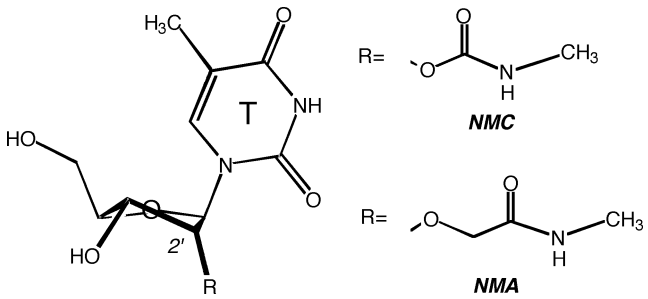

Figure 1. Structures of 2′-*O*-[2-(methylamino)-2-oxoethyl]- and 2′-*O*-(*N*-methylcarbamate)-modified thymidine (2′-*O*-NMA-T and 2′-*O*-NMC-T, respectively).

To better understand the underlying reasons for the different influences on stability of the 2′-*O*-NMA and 2′-*O*-NMC modifications, we have determined X-ray crystal structures of modified oligodeoxynucleotides with sequence GCGTAT*ACGC (T* = 2′-*O*-NMA-T/2′-*O*-NMC-T). The two decamers, referred to here as *NMA*-10mer and *NMC*-10mer, respectively, were synthesized and purified according to published procedures.[9,10] Crystals for both were grown using the nucleic acid mini-screen[11] (Hampton Research, Aliso Viejo, CA); hanging drops containing ca. 1 mM 10mer, 40 mM sodium cacodylate pH 7, 12 mM spermine•4HCl, 80 mM potassium chloride, and 10% 2-methyl-2,4-pentanediol (MPD) were equilibrated against a 1 mL reservoir of 35% MPD. Crystals of the *NMA*-10mer belong to space group $P2_1$ with cell constants $a = 27.23$ Å, $b = 44.97$ Å, $c = 44.42$ Å, and $\beta = 99.3°$, contain two duplexes per asymmetric unit (a.u.), and diffract to 1.30 Å resolution. Crystals of the *NMC*-10mer belong to space group $P2_12_12_1$ with cell constants $a = 24.60$ Å, $b = 44.88$ Å, $c = 46.31$ Å, contain one duplex per a.u., and diffract to 1.25 Å. Diffraction data were collected at 110 K on the 5-ID beam line of the DND-CAT synchrotron research center at the Advanced Photon Source, Argonne, IL ($\lambda = 0.96297$ Å). All data were integrated and merged in the DENZO/SCALEPACK suite.[12] Structure determination was accomplished with the programs CNS[13] (*NMC*-10mer) and EPMR[14] (*NMA*-10mer) using an A-form DNA duplex model. Initial refinement was carried out with CNS,[13] setting aside 5% randomly chosen reflections to calculate the *R*-free.[15] After rigid-body and several rounds of individual positional and isotropic-temperature factor refinements, the program SHELX-97[16] was used for anisotropic temperature factor refinement with all DNA atoms and most of the water molecules. The 2′-*O*-substituents were built into Fourier ($2F_o - F_c$) sum and ($F_o - F_c$) difference electron

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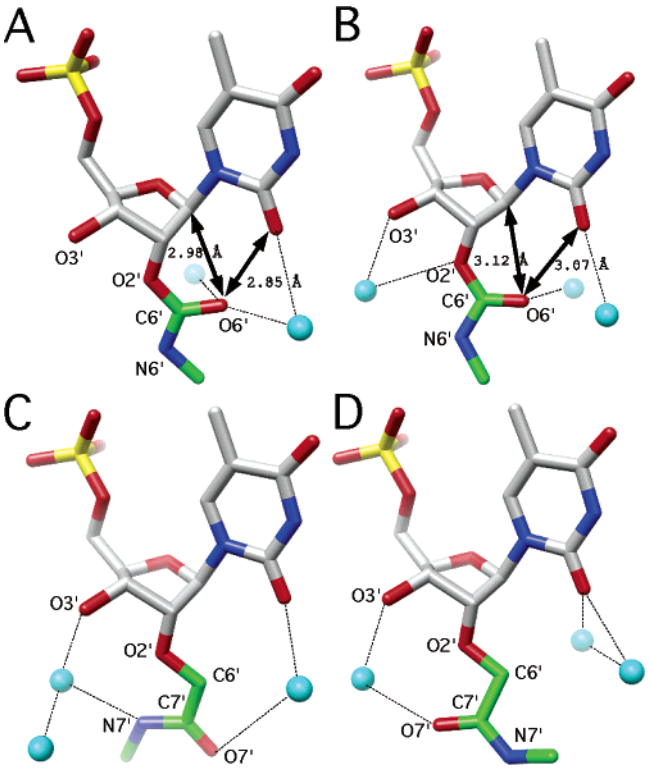

Figure 2. Conformations of 2′-*O*-NMA- and 2′-*O*-NMC-modified thymidines. (A) NMC-T6, (B) NMC-T106, (C) NMA-T206, (D) NMA-T306. Selected atoms are labeled, waters are cyan spheres, hydrogen bonds are dashed, and short contacts are highlighted with arrows.

density maps. Final values for *R*-work/*R*-free are 0.127/0.194 (*NMA*-10mer) and 0.153/0.221 (*NMC*-10mer). NMA and NMC coordinates have been deposited in the Protein Data Bank (www.rcsb.org; PDB entry codes 1XUX and 1XUW, respectively).

The *NMC*-10mer duplex and the two independent *NMA*-10mer duplexes adopt A-form geometries. The average values of helical rise and twist for the *NMC*-10mer duplex are 2.81 Å and 32.4°, respectively (all helical parameters are calculated with the program CURVES[17]). The corresponding values for the *NMA*-10mer duplexes are 2.77 Å and 31.6° (duplex 1) and 2.87 Å and 32.3° (duplex 2), respectively. The root-mean-square deviation (rmsd) based on all atoms between the *NMA*-10mer duplexes amounts to just 0.93 Å. All sugars in the three duplexes adopt C3′-*endo* conformation. Despite different space groups and somewhat different cell constants, the packing interactions in the two crystal structures are very similar. Terminal base pairs stack into the minor grooves of adjacent duplexes, such that the central portion of each duplex (harboring the 2′-*O*-substituents) remains largely unaffected by packing interactions. Residues of 10mer strands in the duplexes are numbered 1 to 10 and 101 to 110. Residues of the second duplex in the *NMA*-10mer structure are numbered 201 to 210 and 301 to 310. T6 and T106 are the modified residues in the *NMC*-10mer duplex and in the *NMA*-10mer structure the modified residues are T6, T106, T206, and T306 (Figure 2).

The torsion angles C3′-C2′-O2′-C6′ assume an *antiperiplanar* (*ap*) conformation (Figure 2). This orientation, together with the preferred trans conformation of the ester group, brings the keto oxygen of the 2′-*O*-NMC substituent in close contact with the O2 of thymine (Figure 2A,B). In addition to this unfavorable dipole−dipole interaction, there is a relatively short contact between O6′ and C1′. By comparison, the additional methylene group in the 2′-*O*-NMA substituent (C6′, Figure 2C,D) shifts the keto oxygen (O7′) away from the nucleobase, thus avoiding a steric clash. Independent of whether the keto oxygen assumes an *ap* (Figure 2C) or a *synclinal* (*sc*; Figure 2D) orientation relative to the 2′-oxygen, a water molecule is trapped between the 2′-*O*-NMA substituent and the phosphate group. This water molecule is hydrogen bonded to O3′, O2′ (slightly long) and either O7′ or N7′, and further waters provide a bridge to the phosphate. The *N*-methyl group of the 2′-*O*-NMC and 2′-*O*-NMA substituents exhibits a disordered orientation with both *ap* and *sc* conformations of the O6′-C6′-N6′-CH_3 (NMC) and O7′-C7′-N7′-CH_3 (NMA) torsions seen in the structures (i.e., Figure 2C,D).

In summary, the opposite effects on RNA affinity of the 2′-*O*-NMC and 2′-*O*-NMA modifications can be qualitatively rationalized by the following two structural observations: first, a steric clash and unfavorable electrostatics between NMC substituent and base and, second, formation of a stable water structure in the case of the 2′-*O*-NMA modification. The short contact seen for 2′-*O*-NMC-Ts is likely to occur also with purine residues, as O2 (Py) and N3 (Pu) occupy rather similar positions in space. The hydration pattern around 2′-*O*-NMA substituents is reminiscent of the water structure observed for 2′-*O*-MOE-RNA.[4]

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Supporting Information Available: Crystal data and refinement parameters and final electron density. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Crooke, S. T. In *Handbook of Experimental Pharmacology. Antisense Research and Application*; Crooke, S. T., Ed.; Springer: Berlin, 1998; Vol. 131, pp 1−50. (b) Filmore, D. *Modern Drug Discov.* **2004**, *June*, 49−50.
(2) (a) Cook, P. D. *Ann. Rep. Med. Chem.* **1998**, *33*, 313−325. (b) Manoharan, M. *Biochim. Biophys. Acta* **1999**, *1489*, 117−130. (c) Freier, S. M.; Altmann, K. H. *Nucleic Acids Res.* **1997**, *25*, 4429−4443.
(3) (a) Altmann, K. H.; Fabbro, D.; Dean, N. M.; Geiger, T.; Monia, B. P.; Muller, M.; Nicklin, P. *Biochem. Soc. Trans.* **1996**, *24*, 630−637. (b) Altmann, K.-H.; Dean, N. M.; Fabbro, D.; Freier, S. M.; Geiger, T.; Häner, R.; Hüsken, D.; Martin, P.; Monia, B. P.; Müller, M.; Natt, F.; Nicklin, P.; Phillips, J.; Pieles, U.; Sasmor, H.; Moser, H. E. *Chimia* **1996**, *50*, 168−176. (c) De Mesmaeker, A.; Häner, R.; Martin, P.; Moser, H. E. *Acc. Chem. Res.* **1995**, *28*, 366−374.
(4) (a) Tereshko, V.; Portmann, S.; Tay, E. C.; Martin, P.; Natt, F.; Altmann, K.-H.; Egli, M. *Biochemistry* **1998**, *37*, 10626−10634. (b) Teplova, M.; Minasov, G.; Tereshko, V.; Inamati, G. B.; Cook, P. D.; Manoharan, M.; Egli, M. *Nat. Struct. Biol.* **1999**, *6*, 535−539.
(5) Griffey, R. H.; Monia, B. P.; Cummins, L. L.; Freier, S.; Greig, M. J.; Guinosso, C. J.; Lesnik, E.; Manalili, S. M.; Mohan, V.; Owens, S.; Ross, B. R.; Sasmor, H.; Wancewicz, E.; Weiler, K.; Wheeler, P. D.; Cook, P. D. *J. Med. Chem.* **1996**, *39*, 5100−5109.
(6) Teplova, M.; Wallace, S. T.; Minasov, G.; Tereshko, V.; Symons, A.; Cook, P. D.; Manoharan, M.; Egli, M. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 14240−14245.
(7) Prhavc, M.; Prakash, T. P.; Minasov, G.; Egli, M.; Manoharan, M. *Org. Lett.* **2003**, *5*, 2017−2020.
(8) Prakash, T. P.; Manoharan, M.; Fraser, A. S.; Kawasaki, A. M.; Lesnik, E.; Sioufi, N.; Leeds, J. M.; Teplova, M.; Egli, M. *Biochemistry* **2002**, *41*, 11642−11648.
(9) Prakash, T. P.; Kawasaki, A. M.; Lersnik, E. A.; Owens, S. R.; Manoharan, M. *Org. Lett.* **2003**, *5*, 403−406.
(10) Prhavc, M.; Lesnik, E. A.; Mohan, V.; Manoharan. M.. *Tetrahedron Lett.* **2001**, *42*, 8777−8780.
(11) Berger, I.; Kang, C. H.; Sinha, N.; Wolters, M.; Rich, A. *Acta Crystallogr., Sect. D* **1996**, *52*, 465−468.
(12) Otwinowski, Z.; Minor, W. *Methods Enzymol.* **1997**, *276*, 307−326.
(13) Brünger, A. T.; Adams, P. D.; Clore, G. M.; DeLano, W. L.; Gros, P.; Grosse-Kunstleve, R. W.; Jiang, J. S.; Kuszewski, J.; Nilges, M.; Pannu, N. S.; Read, R. J.; Rice, L. M.; Simonson, T.; Warren, G. L. *Acta Crystallogr., Sect. D* **1998**, *54*, 905−921.
(14) Kissinger, C. R.; Gehlhaar, D. K.; Fogel, D. B. *Acta Crystallogr., Sect. D* **1999**, *55*, 484−491.
(15) Brünger, A. T. *Nature* **1992**, *355*, 472−475.
(16) Sheldrick, G. M.; Schneider, T. R. *Methods Enzymol.* **1997**, *277*, 319−343.
(17) Lavery, R.; Sklenar, H. *J. Biomol. Struct. Dyn.* **1988**, *6*, 63−91.

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