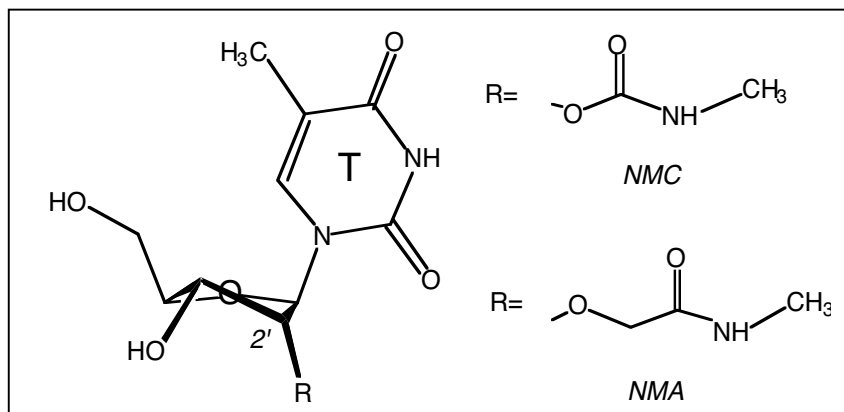


**Structural Rationalization of a Large Difference in RNA Affinity Despite of a Small Difference in Chemistry Between Two 2'-O-Modified Nucleic Acid Analogs.** Rekha Pattanayek<sup>1</sup>, Latsavongsakda Sethaphong<sup>1</sup>, Chongle Pan<sup>1</sup>, Marija Prhac<sup>2</sup>, Thazha P. Prakash<sup>2</sup>, Muthiah Manoharan<sup>3</sup>, Martin Egli<sup>1</sup>, <sup>1</sup>Dept. of Biochemistry, Vanderbilt Univ., Nashville, TN 37232, <sup>2</sup>Dept. of Medicinal Chemistry, Isis Pharmaceuticals Inc., Carlsbad, CA 92008; <sup>3</sup>Drug Discovery, Alnylam Pharmaceuticals Inc., Cambridge, MA 02142.



Chemically modified nucleic acids that can down-regulate protein synthesis by interfering with gene expression at the mRNA level are currently being evaluated as antisense compounds for potential therapeutic applications. An important criterion for the usefulness of a particular modification in an antisense oligonucleotide (AON) is whether it combines improved resistance with enhanced RNA affinity. Among the available sites for

chemical modification in nucleic acids, the ribose 2'-position has been found to be very valuable. Incorporation of 2'-O-(N-methylcarbamate)-modified thymidines (NMC; Figure) results in a reduction of the thermodynamic stability of NMC-DNA:RNA duplexes by ca. 2.5°C/nucleotide, while incorporation 2'-O-(N-methylacetamide)-modified thymidines (NMA; Figure) stabilizes NMA-DNA:RNA by ca. 2.5°C/nucleotide. To allow a direct comparison between the structural properties of the NMC and NMA modifications, the duplexes [d(GCGTAT\*ACGC)]<sub>2</sub> (T\*=NMC-T/NMA-T) were crystallized and their structures determined at 1.25 and 1.30 Å resolution, respectively. A comparison between the conformational properties of the NMC- and NMA-modified residues based on the crystal structures and the observed stability differences will be presented.