Chemical and biological consequences of locking the conformation of nucleosides at the two antipodal extremes of the pseudorotational cycle

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ABSTRACT
It is shown that in spite of the low energy barrier for the North (N)/South (S) equilibrium in nucleosides, enzymes show measurable differences in binding and/or catalytic activity between the two antipodal conformations.

INTRODUCTION
An unrestricted furanose ring can adopt a number of conformations which can be defined by the value of $P$ and $\nu_{\text{max}}$ in the pseudorotational cycle (Fig. 1). By convention, a phase angle $P = 0^\circ$ corresponds to a perfect $N$ conformation, while $P = 180^\circ$ equates with the $S$ antipode. When the sugar rings occupy $N$ and $S$ domains in polymeric structures (e.g. DNA and RNA) it leads, respectively, to two different categories of polymucleotide conformations, known as A- and B-type. Crystallographic data of either individual nucleosides, or those comprising DNA or RNA structures, reveal that the puckering modes of the furanose ring cluster near one of these two antipodal regions (Fig. 1). In solution, nonetheless, the sugar fluctuates rapidly between these two extremes because the energy barrier between the $N$ and $S$ states is very small. However, when a nucleoside(tide) binds to an organized macromolecule (i.e. an enzyme), measurable differences between $N$ and $S$ conformers in terms of energy of binding and/or catalytic activity can be detected. Nucleosides built on a bicyclo[3.1.0]hexane template can lock the conformation of the embedded cyclopentane ring into $N$ ($P = 342^\circ$) and $S$ ($P = 198^\circ$) domains situated at the extreme edge of these regions. Locked nucleosides built on this template have been used to probe the conformational preferences of a number of enzymes, and, as part of oligomeric structures, to impose a conformational bias that affects biological activity. Both chemical and biological changes associated with these locked conformations will be reviewed.

CHEMICAL CHANGES
The transition between $N$ and $S$ is accompanied by an inversion of ring pucker, which changes the axial and equatorial disposition of the substituents on the sugar ring.

Figure 1. Distribution of $P$ and $\nu_{\text{max}}$ (radius) for nucleosides in the Cambridge Structural Database.

During the synthesis of locked $N$ and $S$ versions of AZT, the azide group in $N$-AZT was introduced directly via anhydride formation followed by ring opening with $\text{NaN}_3$ (Scheme 1).

Scheme 1

In the case of $S$-AZT, the C2 oxygen was unable to reach its target electrophilic carbon, and prior inversion of configuration at C3' was required. Different results also occurred during DNA synthesis with phosphoramidite chemistry. The use of iodine for P(III) to P(V) oxidation led to strand cleavage only at the sites where the pseudosugar is...
N. The reason for this contrasting behavior lies in the restricted geometry of the bicyclo[3.1.0]hexane scaffold, which in the N case favors ejection of the excellent leaving group formed when iodine reacts with the phosphite. Under such conditions, displacement of the leaving group occurs via intramolecular attack of the base by a mechanism similar to that shown in Scheme 1. This situation was easily resolved by using t-BoOOH as the oxidant.3

**BILOGICAL CHANGES**

Bicyclo[3.1.0]hexane nucleosides have served as excellent tools to discern the conformational preferences of a number of enzymes discussed below.

**Adenosine Deaminase (ADA).** This enzyme catalyzes the irreversible hydrolytic deamination of adenosine. Because of the lack of the furanose O-atom, carbocyclic nucleosides are generally weak substrates of ADA. Setting the rate of deamination of the N analogue to 100, the relative rate of deamination of the S analogue was 1, while the relative rate for the flexible carbocyclic analogue, aristeromycin, was intermediate (58).4

**HIV Reverse Transcriptase (RT).** Reverse transcription by RT represents an essential step for retroviral replication where binding of the correct 2'-deoxynucleotide-5'-triphosphate complementary to the next position on the template is critical. Near the polymerase active site, the template resembles canonical A-type DNA with N puckered sugars. In agreement with this conformational preference, the potency and kinetics of RT inhibition by the S'-triphosphates of N-AZT and regular AZT were indistinguishable, whereas locked S-AZT failed to inhibit HIV RT.2

**Herpes Simplex Thymidine Kinase (HSV-tk).** The antiviral activity of all antitumor compounds depends on the effective phosphorylation catalyzed by HSV-tk. This enzyme phosphorylates both N- and S-thymidine providing the substrates for the ensuing cellular kinases to complete the pathway to the 5'-triphosphate metabolites (Table 1). Both N- and S-thymidine appear to be equivalent substrates for the first phosphorylation step. For the S conformer, the sluggishness of the first step is due the preferred syn disposition of the nucleobase as the elimination of this steric impediment restored preference for the S conformer. For the second step, also catalyzed by HSV-tk, the S conformer is definitely preferred as it is for the final cellular kinase.5

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>N-Thymidine</th>
<th>S-Thymidine</th>
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<tbody>
<tr>
<td>5'-monophosphate</td>
<td>76 ± 0.9</td>
<td>72 ± 5.4</td>
</tr>
<tr>
<td>5'-diphosphate</td>
<td>82 ± 6.3</td>
<td>378 ± 29</td>
</tr>
<tr>
<td>5'-triphosphate</td>
<td>197 ± 14.6</td>
<td>490 ± 67</td>
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Despite the robust phosphorylation of S-thymidine, the compound was inactive against HSV-infected cells, whereas N-thymidine showed potent activity. Experiments with radio-labeled drugs showed that only the 5'-triphosphate of N-thymidine was efficiently incorporated into DNA. This is further proof that, as for HIV-RT, polymerases have a clear preference for the N conformation.5

**DNA (cytosine-C5) methyl transferase.** Methylation of DNA is a post-replicative modification, which involves the addition of a methyl group to the 5-position of cytosine. Different protooncogenic pathways can up-regulate DNA methylation and aberrant methylation is commonly associated with cancer. Mammalian and bacterial DNA methylases operate by temporarily disrupting the base pairing (base flipping) of the target cytosine and consequently have an increase affinity for duplexes with abasic sugars at the target site. A synthetic ODN (5'-TGTCAGXGATGG-3') where X corresponds to an abasic N or S bicyclo[3.1.0]hexane pseudosugar produced duplexes of equal stability. However, inhibition of DNA methylation (IC50) of the bacterial enzyme (M.HhaI) for X = S was 14 nM, whereas no detectable activity for X = N was observed at < 75 nM. When X was the flexible abasic cyclopentane, the IC50 was 48 nM.6

**Adenosine Receptors.** For certain types of adenosine receptors, such as A3, N analogues have low nanomolar binding affinities in contrast to the isomeric S analogues, suggesting that the N conformation more closely resembles the receptor bound conformation.7

**CONCLUSION**

The examples shown here clearly demonstrate that differences in binding and catalytic activities reflect a definitive conformational preference by specific enzymes that perhaps is the result of evolutionary forces acting to achieve optimal catalytic efficiency by binding to a nucleoside(tide) with a precise sugar conformation.

**REFERENCES**