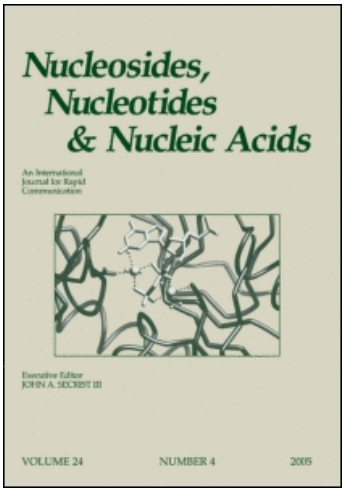

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Marquez, Victor E. , Russ, Pamela , Alonso, Randolph , Siddiqui, Maqbool A. , Shin, Kye-Jung , George, Clifford , Nicklaus, Marc C. , Dai, Fang and Ford Jr., Harry(1999) 'Conformationally Restricted Nucleosides. The Reaction of Adenosine Deaminase with Substrates Built on a Bicyclo[3.1.0]hexane Template', Nucleosides, Nucleotides and Nucleic Acids, 18: 4, 521 — 530
To link to this Article: DOI: 10.1080/15257779908041487
URL: http://dx.doi.org/10.1080/15257779908041487

NUCLEOSIDES & NUCLEOTIDES, 18(4&5), 521–530 (1999)

CONFORMATIONALLY RESTRICTED NUCLEOSIDES. THE REACTION OF ADENOSINE DEAMINASE WITH SUBSTRATES BUILT ON A BICYCLO[3.1.0]HEXANE TEMPLATE

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ABSTRACT. Adenosine deaminase (ADA) can discriminate between two distinct (North and South), conformationally rigid substrate conformers. (N)-methanocarba-2'dA (**4**) is deaminated 100 times faster than the antipodal (S)-methanocarba-2'dA (**5**), whereas a non-rigid analogue, aristeromycin (**6**), is deaminated at an intermediate rate. These results are in agreement with crystallographic data from ADA-ribonucleoside complexes showing the furanose ring of the bound purine in a C3'-*endo* (North) conformation. The data presented here suggests that **4** and **5** are useful probes to ascertain conformational preferences by purine metabolizing enzymes.

INTRODUCTION

One of the main obstacles in interpreting structure-activity correlations in nucleosides and nucleotides is the inherent flexibility of the complete ensemble comprising the sugar ring, the heterocyclic base, and side chain. The conformational preferences of each of these elements are interrelated and highly dependent on one another.[1] The conformation of the sugar ring can be described in terms of the pseudorotation phase angle *P* which is calculated from the endocyclic furanose torsion angles. The preferred ranges of *P* define two main puckering domains centered around C3'-*endo* (North, N) and C2'-*endo* (South, S).[2] These sugar puckering modes, in turn, favor a particular orientation of the glycosyl torsion angle χ (*syn* or *anti*), a factor that is also dependent on the nature of the base.[3] In a similar fashion, the three ranges of γ (+*sc*, -*sc*, and *ap*) for the side chain are not uniformly populated because their distribution is also dependent on the sugar pucker and the orientation of the base.[3]

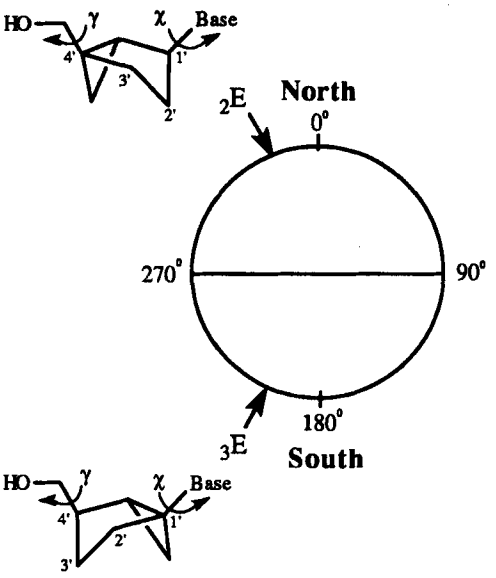


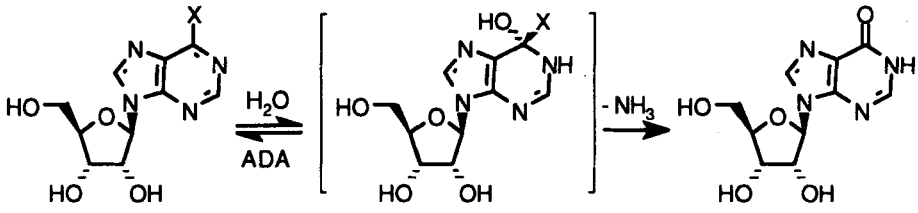
FIGURE 1. Pseudorotational cycle showing the rigid North and South conformations achieved with the bicyclo[3.3.0]hexane template.

When a nucleoside or a nucleotide binds to its target enzyme, a unique conformation of the entire ensemble is present at the active site. This conformation is the result of the molecule's conformational preference, combined with the changes induced by the enzyme for optimal fit. It is reasonable to speculate that enzymes have evolved to adjust to a particularly preferred conformation of the ligand. However, it is equally possible that within the allowable range of motions of the ligand ensemble, enzymes can also exert important changes in the conformation on the ligand.[4] It is for this reason that conformationally locked nucleosides, whose structural features in the solid state and in solution are identical, constitute important probes to determine the conformational preferences of enzymes for their nucleoside and nucleotide substrates.

To study this problem, we have proposed the use of methanocarba-nucleosides built on a rigid bicyclo[3.1.0]hexane template.[5,6] This carbocyclic template adopts a fixed conformation that mimics the ring pucker of a furanose ring in a specific N (C2'-*exo*, $_2$E) or S (C3'-*exo*, $_3$E) conformation depending on the relative disposition of the base and the hydroxymethyl group (FIG. 1).

We have already shown that rigid N and S conformers of methanocarba-AZT 5'-triphosphate bind differently to reverse transcriptase.[6] The conformationally locked $_2$E (N)

SCHEME 1



1, X = NH_2 (adenosine) ⟶ **1a**, Transition-state (X = NH_2) ⟶ **3** (inosine)
2, X = H (nebularine) ⟶ **2a**, Unproductive complex (X = H)

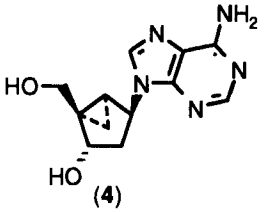
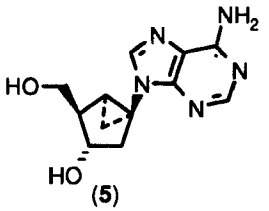
triphosphate inhibited HIV reverse transcriptase (RT) in a manner that was indistinguishable from that exhibited by AZT 5'-triphosphate, whereas the antipodal $_3$E (S) triphosphate inhibited the enzyme very poorly.[6] We hypothesize that this phenomenon of specificity shown by RT is general and applicable to other enzymes where differentiation between the two antipodal sugar conformations of their nucleoside or nucleotide substrates is possible. In this paper we demonstrate that adenosine deaminase (ADA) also shows a distinctive preference for the shape of its substrate.

SUBSTRATE PREFERENCE FOR ADENOSINE DEAMINASE (ADA)

ADA catalyzes the irreversible hydrolytic deamination of adenosine (**1**) to inosine (**3**) and ammonia (SCHEME 1).[7] In the most recent X-ray structure of ADA complexed with the inhibitor (6*S*)-hydroxyl-1,6-dihydropurine riboside (**2a**), Quiocho and Wang[8] found that the position of the base relative to the sugar moiety is in the *anti* conformation with χ in a narrow range of –106° to –111°. The orientation of the C4'-C5' bond (torsion angle γ) was *+sc*, and the furanose ring pucker was C3'-*endo* (N).[8] Not surprisingly, these parameters remained constant for other bound ligands such as (8*R*)-hydroxyl-2'-deoxycoformycin and 1-deazaadenosine.[8] The complex with (6*S*)-hydroxyl-1,6-dihydropurine riboside is interesting since this ligand represents the hydrated form of the common nucleoside nebularine (**2**) which is formed at the active site through the catalytic action of the enzyme.[9]

Based on the above observation, one would predict that (N)-methanocarba-2'dA (**4**)[10] is a better substrate for ADA than its antipodal rigid conformer, (S)-methanocarba-2'dA (**5**)[11] (TABLE 1). On the basis of ring puckering alone, the crystallographic pseudorotational parameters measured for the two molecules in the unit cell of (N)methanocarba-2'dA (**4**) are in complete agreement with the expected values for a rigid

TABLE 1. Conformational parameters of the X-ray structures of (N)-methanocarba-2'dA (**4**) and (S)-methanocarba-2'-dA (**5**).

Compound		*P*	χ	γ
 (**4**)	A	339.3° (N)	−167.6° (*anti*)	−177.6° (*ap*)
	B	342.8° (N)	−154.8° (*anti*)	+54.8° (+*sc*)
 (**5**)	A	200.2° (S)	−129.8° (*anti*)	−171.9° (*ap*)
	B	199.1° (S)	+50.9° (*syn*)	+54.0° (+*sc*)

pseudoboat bicylo[3.1.0]hexane template. Both molecules showed a nearly perfect $_2$E northern conformation [*P* = 339.25° (A) and *P* = 342.78° (B)].[10] The equivalent parameters for the two antipodal (S)-methanocarba-2'dA (**5**) molecules in the unit cell correlated, in turn, with a pure $_3$E southern conformation [*P* = 200° (A) and *P* = 199° (B)][12] (TABLE 1). Except for molecule B of the southern analogue, all torsion angles χ were in the *anti* range, while the less encumbered torsion angle γ alternated between the *ap* and +*sc* conformations as it is normally observed for ribonucleosides.

CONFORMATIONAL ANALYSIS

Although variable temperature ^{1}H NMR studies indicated that bicyclo[3.1.0]hexane nucleosides were conformationally rigid,[13] the integrity of the pseudoboat conformation —the hallmark of this system!— was tested throughout the entire conformational space of

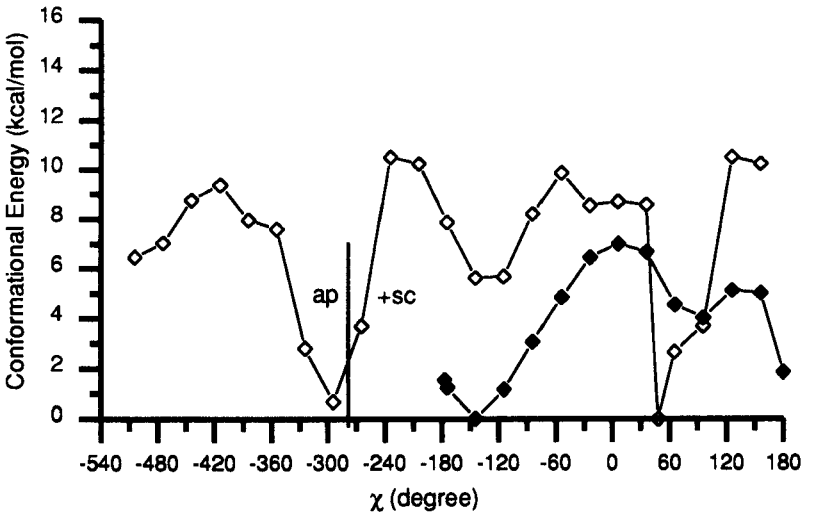


FIGURE 2. Plots of energy as a function of χ for *ab initio*-optimized structures of (N)-methanocarba-2'dA (**4**, filled diamonds) and (S)-methanocarba-2'dA (**5**, open diamonds).

the torsion angle χ (0° → 360°). The X-ray structures of **4**[10] and **5**[12] were imported into the molecular modeling program QUANTA (MSI, Inc.) and the missing hydrogen atoms were added. Following energy minimization using QUANTA's molecular mechanics program CHARMm, the structures were pre-optimized at the *ab initio* level using the program Spartan (Wavefunction, Inc.) at the HF/3-21G* level of theory. These pre-optimized geometries were re-optimized using the Density Functional Theory (DFT) method in the program Gaussian 94, Rev. D.3 (Gaussian, Inc.) at the B3LYP/6-31G(d) level of theory. These structures, which were assumed to be local or global energy minima, were then explored in a potential energy scan (PES) of the dihedral angle χ by rotating the base in equidistant angle steps. Each of the resulting geometries was optimized using the Gaussian DFT method at the same B3LYP/6-31G(d) level of theory for all internal coordinates, except χ.

For (N)-methanocarba-2'dA (**4**), the conformer used as the starting point for the calculations was molecule B in the crystal unit cell with O5' in the *+sc* conformation (TABLE 1). The angle step chosen for the PES was 3°, although only every tenth point is shown in FIG. 2. Because of the small increase in χ, eight optimization steps per conformation were usually sufficient to achieve convergence to a 10^{-4} kcal/mol level. As shown in FIG. 2, two distinct energy barriers of ca. 4 and 7 kcal/mol separate the most stable *anti* conformation ($\chi = -150°$) from the less stable *syn* conformation ($\chi = 90°$).

For (S)-methanocarba-2'dA (**5**), the conformer used as the starting point was molecule A in the crystal unit with χ in the *anti* range and γ as the *ap* rotamer. The angle step for the PES in this case was 30°. Because of the larger step size, 40 optimization steps per conformation were usually allowed; however, convergence to a 10^{-4} kcal/mol level was typically achieved in 20 steps or less.[14] In this case, it was the most sable *syn* conformation (χ = 60°) which appeared separated from the less stable *anti* conformation ($\chi \approx -150°$) by two almost equal energy barriers of ca. 11 kcal/mol (FIG. 2).

In both cases, the bicyclo[3.1.0]hexane system remained rigid as shown by the small variance of *P* and ν_{max}, while χ rotated from 0° → 360° (FIG. 3). Indeed, *P* showed maximal deviations of less than 12° from the ideal $_2$E (*P* =342°) and $_3$E (*P* =198°) conformations, and the puckering amplitude (ν_{max}) remained fairly constant (ca. 23°—33°) throughout the cycle. The torsion angle γ remained constant in the *+sc* conformation for (N)-methanocarba-2'dA (**4**, FIG. 3, top). For (S)-methanocarba-2'dA (**5**), however, the starting conformation with the *ap* rotamer (γ ca. ±180°) changed to *+sc* as the base rotated out of the *syn* conformation the first time ($\chi \approx -300° \rightarrow -240°$). It remained in the *+sc* range, only to revert back briefly to *ap* as χ moved from the *anti* conformation to the preferred *syn* ($\chi \approx 45° \rightarrow 90°$) conformation (FIG. 3 bottom).

In nucleosides, the rotation of the base is intrinsically associated with the sugar pucker which changes as χ rotates to relieve steric congestion. Therefore, the higher energy barriers for **4** and **5**, relative to nucleosides (ca. 1-2 kcal/mol),[3] are probably the result of the rigid bicyclo[3.1.0]hexane moiety which is unable to compensate for the unfavorable steric interactions generated during rotation of the base. It is for this reason that locking the sugar pucker disposes the rest of the ensemble to adopt a particular conformation.

BIOLOGICAL RESULTS

Determination of the relative rates of deamination was made spectrophotometrically at 50 μM substrate concentration (pH 7.4 and 37 °C) with calf intestine ADA. The enzymatic products for (N)-methanocarba-2'dA (**4**) and (S)-methanocarba-2'dA (**5**) showed λ_{max} values at 250.0 and 250.4 nm, respectively. At first glance, the difference between the rates of deamination of the carbocyclic substrates relative to adenosine appears to be related to the absence of the O4' oxygen (TABLE 2). However, this oxygen does not seem to play a critical role in the binding process since the X-ray structure of the inhibitor (6*S*)-hydroxyl-1,6-dihydropurine riboside (**2a**), bound to ADA, shows no involvement of this atom with the enzyme.[8] Therefore, the role of the O4' oxygen is probably to facilitate the critical hydration step by its inductive effect. Although we do not have direct chemical

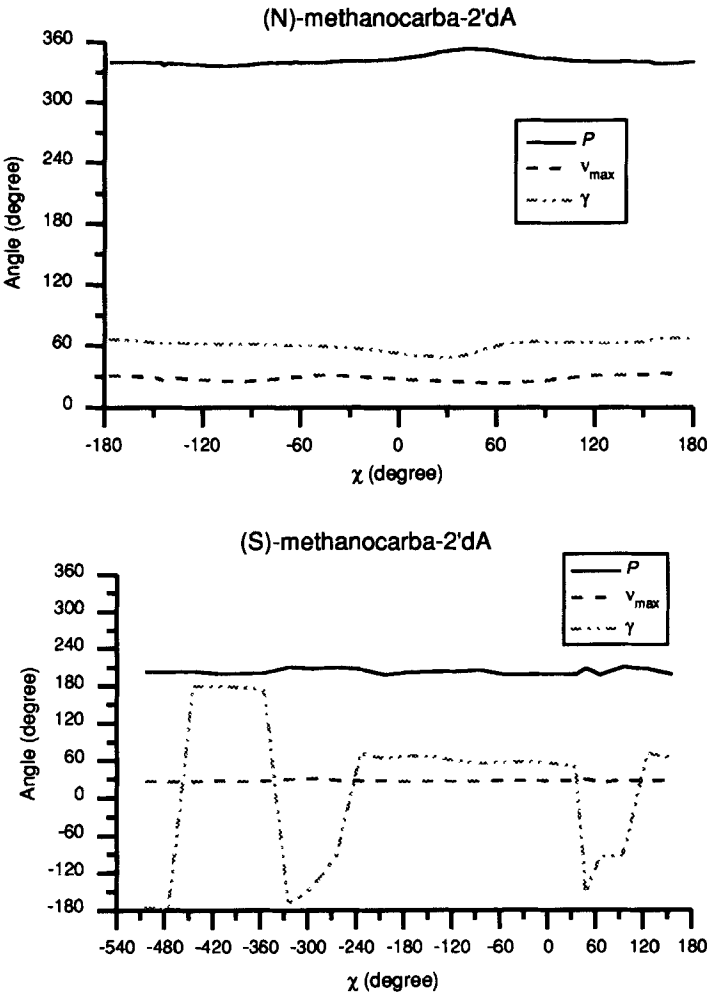
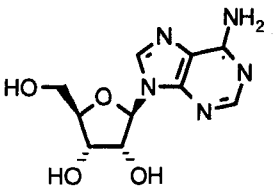
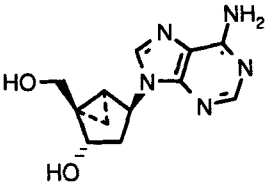
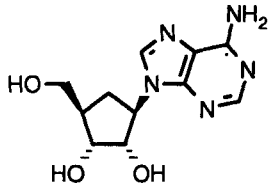
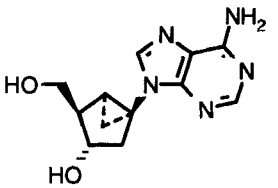


FIGURE 3. Plots of P, v_{max} and γ as a function of χ for (N)-methanocarba-2'dA (**4**, top) and (S)-methanocarba-2'dA (**5**, bottom).

proof for this effect in the purine series, we have shown that the O4' oxygen in pyrimidine nucleosides facilitates formation of a covalent hydrate relative to the equivalent carbocyclic analogue.[15] All things being equal for the three carbocyclic nucleosides (**4-6**), as far as the lack of the O4' oxygen is concerned, their differences as substrates for ADA could then be attributed to the differences in shape, and to the ease by which the enzyme is able to fit the substrates into the active pocket. The deduction made earlier regarding (N)-methanocarba-2'dA (**4**) as the best substrate amongst the carbocyclic analogues proved to be correct. We conclude that the nearly 100-fold difference in the rate of deamination

TABLE 2. Relative rates of deamination by ADA

Substrate	Relative Rate *vs* Adenosine	Relative Rate *vs* Aristeromycin
 adenosine (**1**)	100	—
 (N)-methanocarba-2'dA (**4**)	0.985	170
 aristeromycin (**6**)	0.577	100
 (S)-methanocarba-2'dA (**5**)	0.010	1.74

between (N)-methanocarba-2'-dA (**4**) and its southern antipode, (S)-methanocarba-2'dA (**5**), is due to the shape of the ensemble of **4** being nearly complementary to the binding cavity. Consistent with this argument, aristeromycin (**6**), which is a flexible molecule was deaminated at an intermediate rate.

CONCLUSION

We have shown that when ADA is presented with two conformationally rigid antipodal (N and S) substrate conformers, the enzyme deaminates the N conformer 100 times faster. This result is consistent with the X-ray structure of ADA complexed with the inhibitor (6*S*)-hydroxyl-1,6-dihydropurine riboside (**2a**), which shows the sugar moiety in a North (C3'-*endo*) conformation and the base in the *anti* form.[8] Crystallographic data for purine nucleosides show that a C3'-*endo* (N) conformation is associated with an *anti* glycosyl torsion angle, whereas a C2'-*endo* (S) conformation exhibitis an almost equal distribution between *syn* and *anti* forms.[3] This is exactly what one observes with the rigid bicyclo[3.1.0]hexane system. In the X-ray structure of (N)-methanocarba-2'dA (**4**) only the *anti* form is present, whereas the X-ray structure of (S)-methanocarba-2'dA (**5**) shows both *anti* and *syn* forms in the unit cell. (N)-methanocarba-2'dA (**4**) appears to meet the structural demands for best fit to ADA. The ring puckering is N, the torsion angle γ is most stable in the *+sc* conformation, and the rotation of χ required to move the base from the energy minimum conformation ($\chi = -150°$) to $-106°$ or $-111°$, as observed in the ADA complex with (6*S*)-hydroxyl-1,6-dihydropurine riboside (**2a**),[8] would require less than 1 kcal/mol (FIG. 2). Aristeromycin (**6**) is an intermediate substrate capable of fitting into the substrate cavity by its flexible nature. It exists as an O4'-*endo* conformer ($P = 89°$) in the solid state, but rapidly equilibrates in solution between $128° < P < 140°$, close to a C1'-*exo* ($_1$E) conformation,[16] At the other end of the spectrum, the conformationally rigid southern (S)-methanocarba-2'dA (**5**) cannot fit adequately into the active site and thus its rate of deamination is minimal. We additionally propose that the carbocyclic substrates are, in general, poorly deaminated relative to conventional nucleosides because of the loss of the inductive effect of the O4' oxygen which diminishes the formation of the critical hydrate intermediate. This means that more powerful substrates for ADA could be constructed on a North bicyclo[3.1.0]hexane template by facilitating hydration with electronegative substituents on the purine ring (i.e., F or CF_3 groups at C6).[17] These and other efforts are currently being pursued in our laboratory.

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