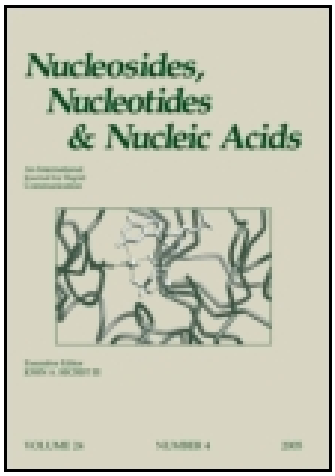

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Recent Advances in the Synthesis of Conformationally Locked Nucleosides and Their Success in Probing the Critical Question of Conformational Preferences by Their Biological Targets

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Recent Advances in the Synthesis of Conformationally Locked Nucleosides and Their Success in Probing the Critical Question of Conformational Preferences by Their Biological Targets

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ABSTRACT

The present work describes some recent approaches to the syntheses of three classes of locked-North nucleosides: β-D-ribo-, β-D-deoxyribo-, and β-D-dideoxyribonucleosides. The method developed for the latter class permitted access to a novel bicyclo[3.1.0]hexene-type nucleosides structurally similar to D4T and carbovir. A structural analysis and biological activities are discussed.

Key Words: Bicyclo[3.1.0]hexane and bicyclo[3.1.0]hexene; Conformationally locked carbocyclic nucleosides; Pseudorotational analysis; D4T and carbovir analogues; Intramolecular cyclopropanation; Lipase-catalyzed resolution.

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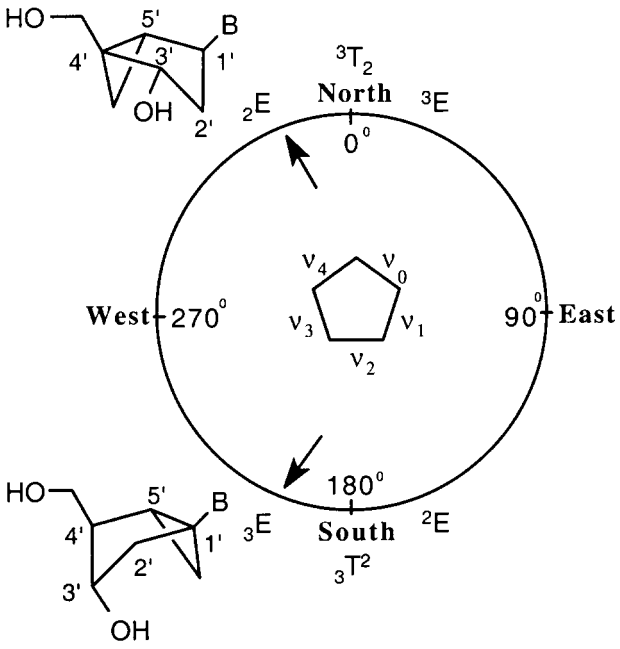

Figure 1. Fixed location of (N)- and (S)-methanocarba nucleosides in the pseudorotational cycle (nucleoside numbering).

INTRODUCTION

Conventional nucleosides or nucleotides equilibrate rapidly in solution between two extreme forms of ring pucker: (1) The North conformation with pseudorotational *P* values ranging between 342° to 18° ($_2E \rightarrow {}^3T_2 \rightarrow {}^3E$) and (2) the antipodal South conformation with values of *P* between 162° to 198° ($^2E \rightarrow {}^2T_3 \rightarrow {}_3E$) (Fig. 1).[1,2] Preference for any of these specific conformations in solution is determined by the interplay of important interactions such as anomeric and *gauche* effects.[2] However, when a nucleoside or nucleotide binds to its target enzyme, only one form is present at the active site. In order to query these enzymes for their conformational preference we have designed and synthesized conformationally locked nucleosides that reside strictly in the normal range of either North or South conformations.

The bicyclo[3.1.0]hexane scaffold provides a convenient way to lock the conformation of carbocyclic nucleosides which are known generically as a methanocarba (MC) nucleosides. Because of its exclusive pseudoboat conformation, a rigid North envelope ($_2E$) conformation can be constructed when the cyclopropane ring appears fused between carbons $C_{4'}$ and $C_{5'}$. Conversely, fusion of the cyclopropane ring between carbons $C_{1'}$ and $C_{5'}$ provides a rigid South ($_3E$) envelope conformation (Fig. 1).

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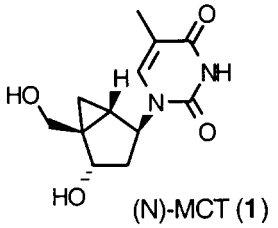
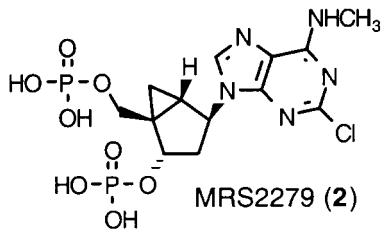
RESULTS AND DISCUSSION

The North Scaffold as a Promising Template for Drug Development

North- and South-MC nucleosides with the same nucleobase are very nicely discriminated by specific enzymes and thus function as target-selective agents. Some of the most important compounds with potential as drug candidates are in the North hemisphere. (N)-MC thymidine (**1**) is a very selective antiviral agent which has shown greater potency against HSV-1 and HSV-2 infection and less toxicity than the standard ganciclovir.[3,4] This compound is also effective in vivo against murine MC38 tumors transduced with the herpes thymidine kinase gene.[5] MRS2279 (**2**), a 3′,5′-biphosphate purine analogue from Dr. Jacobson's laboratory is a very potent and selective high affinity $P2Y_1$ receptor agonist which does not to block nucleotide signaling at most of the other known P2Y receptor subtypes.[6]



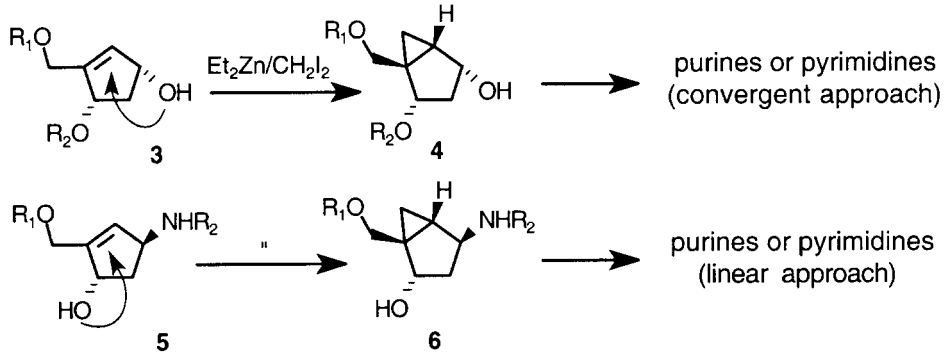
(N)-MCT (**1**)



MRS2279 (**2**)

SYNTHETIC METHODS

Most of the published methods for (N)-MC nucleosides rely on a critical hydroxyl-directed cyclopropanation step (Sch. 1) from the allylic position on a cyclopentenol precursor.[7–9] Although the cyclopropanation step works well, the synthesis of the starting carbocycle is quite laborious and requires expensive starting materials. The resulting pseudosugars can be converted to purine or pyrimidine (N)-MC nucleosides by convergent approaches (from **4**) or by linear approaches (from **6**).



Scheme 1.

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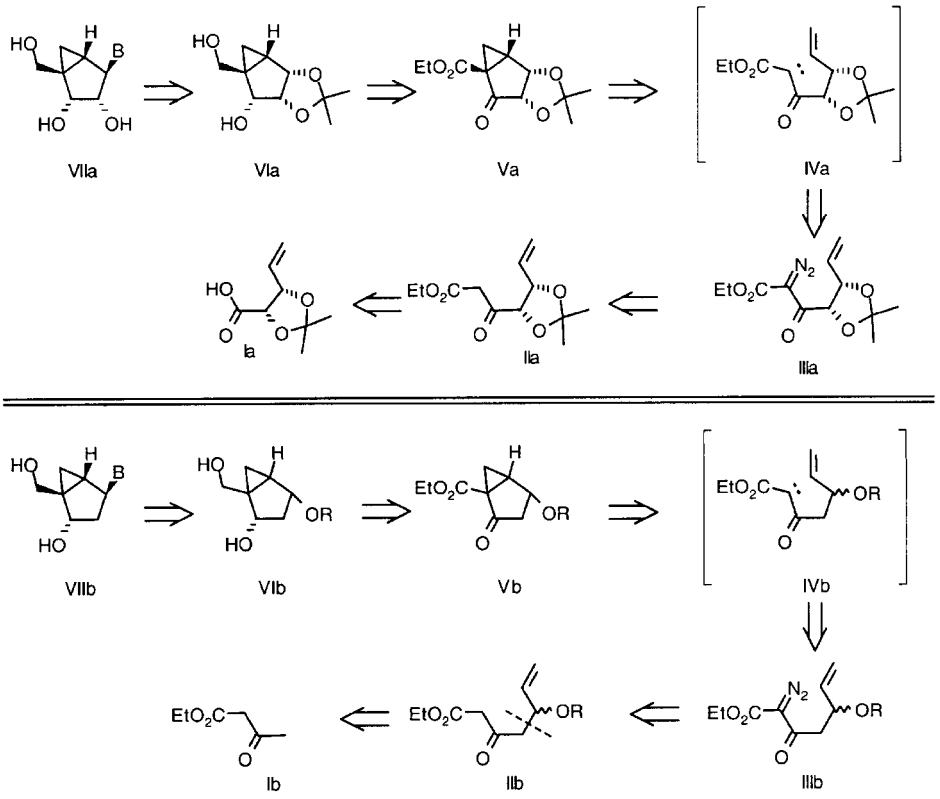

Scheme 2.

Normally, a convergent Mitsunobu coupling with the preformed nucleobase works better with purines. On the other hand, for pyrimidines, the formation of abundant *O*-alkylation products makes the linear approach the preferred route.

In the past couple of years we have developed some new approaches using more accessible and less expensive starting materials. At the core of these approaches lies a critical intramolecular cyclopropanation step from a carbene intermediate engendered from a diazo β-keto ester. The retrosynthetic analysis depicted in Sch. 2 illustrates the general strategy.

Steps Ia to VIIa describe the approach to ribo-like, (N)-MC nucleosides, whereas steps Ib to VIIb correspond to the deoxyribo series. The syntheses of the precursor diazo β-keto esters IIIa and IIIb are simple and straightforward, and after metal-catalyzed thermolysis the carbene intermediates IVa and IVb fold intramolecularly into the desired bicyclo[3.1.0]hexane scaffold. The top part in Sch. 2 depicts the desired β-D-like configuration, achieved via a stereospecific synthesis from a chiral precursor (Ia). For the bottom part, the process leading to intermediate VIb is achiral, and thus the racemic mixture is resolved at this stage via a lipase-catalyzed asymmetric acetylation.

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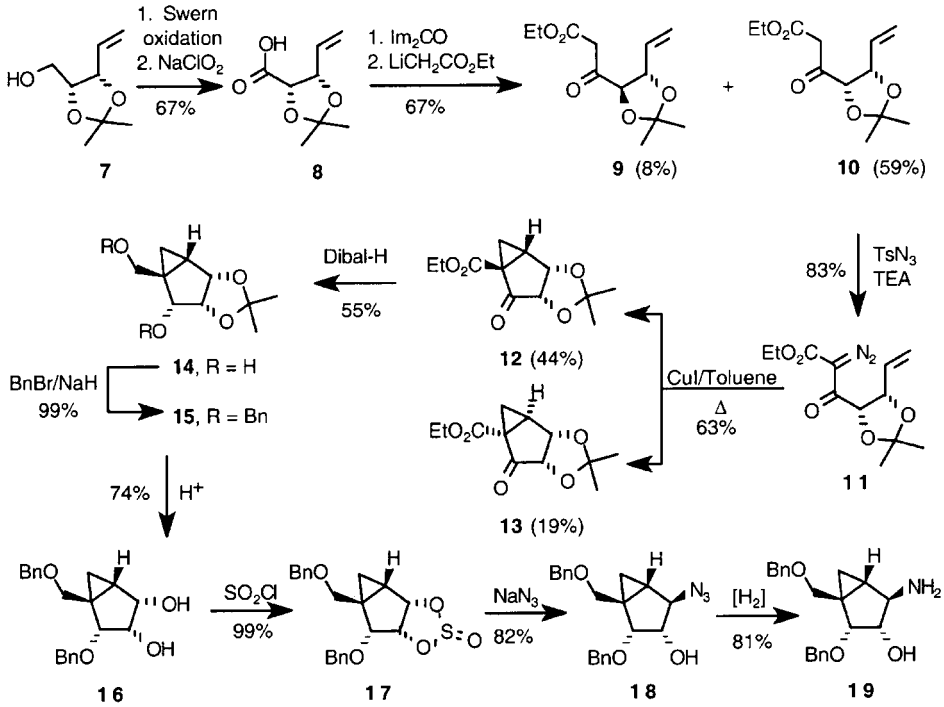
A. Enantiospecific Synthesis of Ribo-type, North-Methanocarba Nucleosides

Starting with inexpensive and plentiful D-isoascorbic acid, the chiral precursor **7**[10,11] was obtained in 4 steps and 60% overall yield (Sch. 3). After the stepwise oxidation of **7**, first under Swern conditions and then with sodium chlorite, Dieckmann condensation of the activated acid **8** with ethyl 2-lithioacetate afforded β-keto esters **9** (8%) and **10** (59%) as a mixture of separable diastereoisomers. Following diazo transfer with tosyl azide and triethylamine (TEA), β-keto ester **10** was converted to diazo compound **11**, which underwent metal-catalyzed thermolysis to generate the desired bicyclo[3.1.0]hexane scaffold via a carbenoid intermediate (IVa, Sch. 2). Comparisons with authentic compounds previously made from different sources,[12] confirmed that the major isomer, **12**, had the desired stereochemistry. Reduction of the carbonyl moiety gave diol **14**, which can be protected. In the case of benzyl-protected **15**, acid-catalyzed removal of the acetonide and reaction of **16** with thionyl chloride produced the cyclic sulfite **17**. This compound reacted well with sodium azide to give 82% of **18** and 10% of the alternative regioisomer. Azide **18** was efficiently reduced to amine **19** (overall yield from D-isoascorbic acid = 2%) which is a direct precursor of purine and pyrimidine analogues using well-known linear approaches.[12]



Scheme 3.

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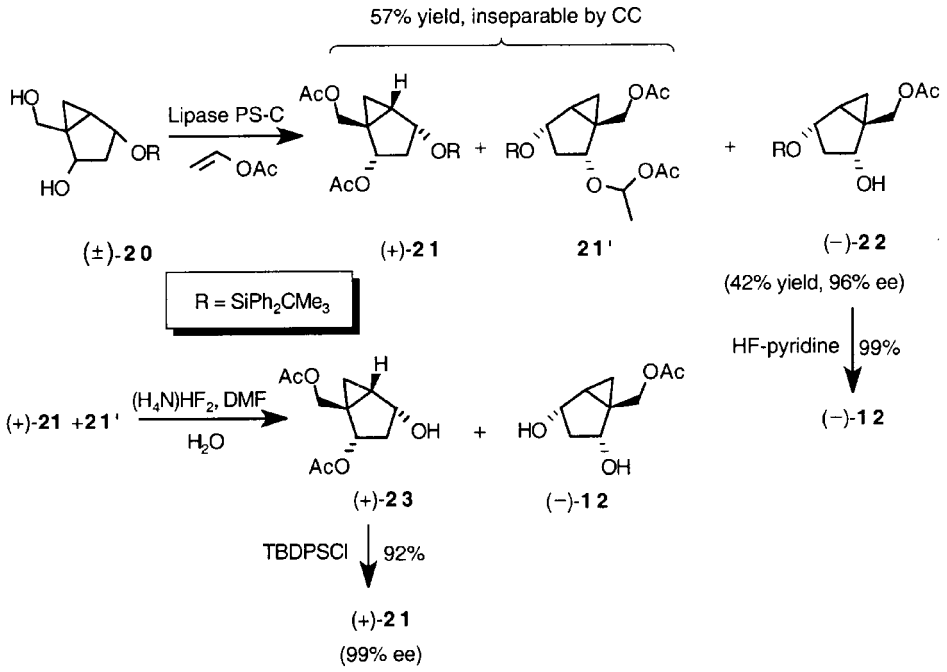
B. Chemico-Enzymatic Approaches to Deoxyribo-type, North-Methanocarba Nucleosides

In this case the process is basically achiral (Ib → VIIb, Sch. 2). A final resolution step using adenosine deaminase worked well only for purines, particulary for the guanosine analogue, which was obtained directly from the deamination of the 2,6-diaminopurine nucleoside.[13] To overcome this limitation, a resolution step prior to the incorporation of the nucleobase was recently accomplished via an economical lipase-catalyzed asymmetric acetylation performed on diol VIb (Sch. 2).[14]

Commercially available lipase PS-C in the presence of a large excess of vinyl acetate effectively discriminated between the enantiomers of *rac*-**20** (VIb, $R = SiPh_2CMe_3$, Schs. 2 and 4) producing diacetate **21** (57%) and monoacetate **22** (42%) after 64 h. Using chiral HPLC, the enantiomeric excess of monoacetate **22** was estimated to be 96% and, although the enantiomeric excess of **21** should have been over 98%, it contained 13% of an impurity (**21′**). Removal of this impurity was important since diacetate **21** corresponded to the enantiomer with the desired D-like configuration.[14] The structure of **21′** was that of an acetal arising from the reaction of the unrecognized enantiomer **22** with excess acetaldehyde—generated during the reaction—followed by further enzymatic acetylation. Treatment of contaminated **21** with ammonium hydrogenfluoride in aqueous DMF (50°C, overnight) gave an easily separable mixture of **23** and diol **12** in 79% and 11% yields, respec-



Scheme 4.

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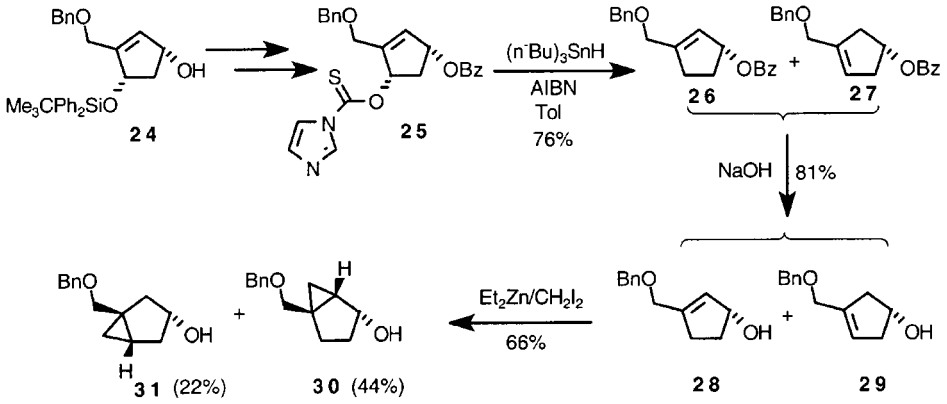
tively. The optical purity of **23**, was estimated to be 99% ee by HPLC after its conversion back to **21** with *tert*-butyldiphenylsilyl chloride.[14] Completing the synthesis of deoxyribo-type, (N)-MC nucleosides from diacetate **23** was simple and straightforward.

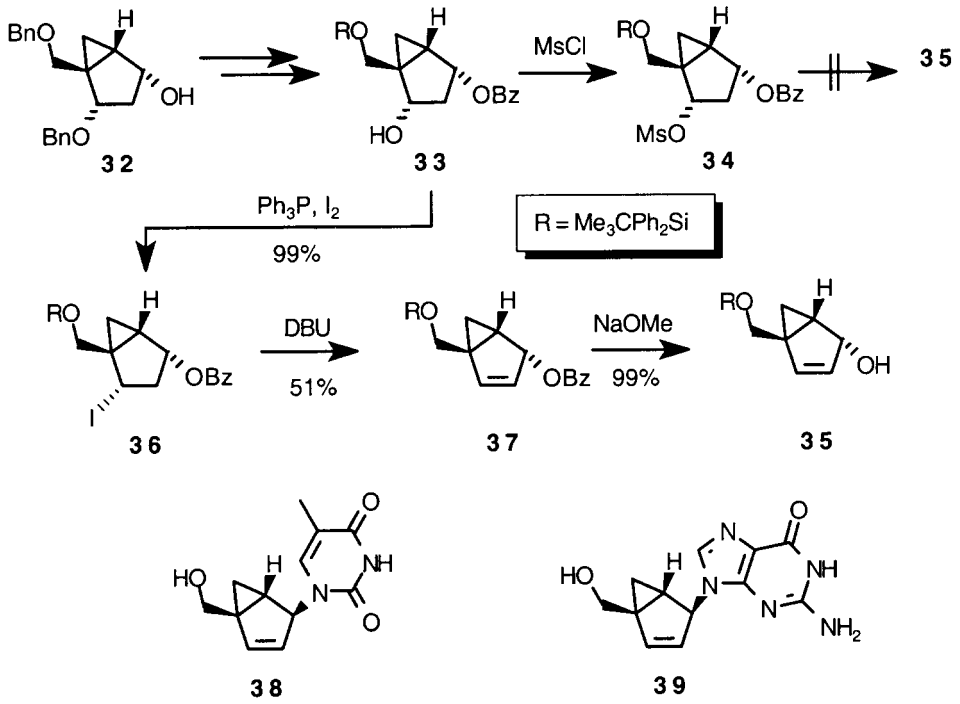
C. Enantiospecific Synthesis of Dideoxyribo-type and Dideoxydidehydroribo-type, North-Methanocarba Nucleosides. Synthesis of Analogues of D4T and Carbovir

The attempt to remove the secondary, $C_{3'}$-OH (nucleoside numbering) under radical deoxygenation conditions resulted in the destruction of the fused cyclopropane ring, which collapsed after formation of a radical intermediate at the adjacent carbon. To circumvent this problem, deoxygenation was performed prior to the formation of the bicyclo[3.1.0]hexane template (Sch. 5) starting from a compound readily available in our laboratory, such as **24**.[8] The corresponding thiocarbonylimidazolide (**25**) gave under radical deoxygenation conditions an inseparable mixture of cyclic allylic (**26**) and homoallylic (**27**) benzoate esters. The mixture of alcohols (**28** and **29**) obtained after hydrolysis of the benzoate esters was cyclopropanated to give isomeric bicyclo[3.1.0]hexanols, **30** and **31**, that were easily separable by column chromatography. With the exception of optical rotation, the spectral properties of **30** matched exactly those of its previously reported racemate (*rac*-**30**).[15] From compound **30**, purine nucleosides are readily accessible in a convergent manner by Mitsunobu coupling with the corresponding nucleobase. However, coupling of *rac*-**30** with pyrimidines, such as N^3-benzoylthymine, was reported to give rather low yields of the desired *N*-alkylated product.[15]

To improve the coupling yields for pyrimidine nucleosides we postulated that a bicyclo[3.1.0]hexenol, such as **35**, would react more readily (Sch. 6). The synthesis of this precursor required the elimination of the $C_{3'}$-OH (nucleoside sugar numbering) from a compound, such as **33**, which was easily obtained from **32**[9] in 3 steps.



Scheme 5.



Scheme 6.

When the secondary alcohol was converted to the mesylate ester **34** for a possible base-catalyzed E2 elimination, only the hydrolyzed product was obtained. Because of the constrained nature of the bicyclo[3.1.0]hexane template, a more favorable *anti*-elimination was sought by attempting to invert the configuration of the leaving group after treatment with I_2/Ph_3P/imidazole. However, the constrained nature of the system forced a double inversion to give instead the thermodynamic product **36** with the iodine axially disposed. Still, elimination occurred by refluxing **36** in toluene in the presence of DBU to produce the desired bicyclo[3.1.0]hexene pseudosugar **37** in 51% yield. Base-catalyzed hydrolysis of the benzoate ester afforded the key intermediate **35**. As predicted, coupling of **35** with N^3-benzoylthymine under Mitsunobu conditions gave the corresponding *N*-alkylated product as the major isomer in 43% yield. The final D4T analogue **38** was obtained after treatment with NH_4OH and final removal of the TBDPS group. A similar approach was used for the synthesis of the carbovir analogue **39**. Catalytic hydrogenation of **38** and **39** afforded the corresponding dideoxyribo-type analogues in excellent yields.

STRUCTURAL ANALYSIS AND STRUCTURE-ACTIVITY RELATIONSHIP

The use of bicyclo[3.1.0]hexane templates has already allowed us to determine conformational preference for enzymes such as adenosine deaminase,[9] HIV reverse

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Table 1. Pseudorotational parameters for **38**, D4T (**40**), and Carbovir (**41**[a] and **41**[b]). Numbers represent degrees (°).

	ν_0	ν_1	ν_2	ν_3	ν_4	P	ν_{max}	χ	γ
38	6.56	−5.83	2.67	1.68	−5.20	293.14	6.81	−119.12	56.17
40	−0.51	0.20	0.19	−0.49	0.60	72.08	0.61	−100.82	52.80
41[a]	−26.47	17.89	−1.09	−16.02	25.68	92.23	27.98	−86.24	−177.76
41[b]	−27.22	17.21	0.23	−17.56	26.98	89.55	28.91	−52.63	−82.90

transcriptase,[16] DNA (cytosine-C5) methyl transferase,[17] and several subtypes of adenosine receptors.[7] A common structural characteristic of D4T (**40**) and carbovir (**41**) is a double bond, a feature that imparts a certain level of rigidity to the five member ring. Thus, compounds **38** and **39** represent hybrid molecules that incorporate in their bicyclo[3.1.0]hexene template a similar double bond. Compound **38** provided adequate crystals for X-ray analysis. At first glance, a comparison between the X-ray structure of **38** and that of D4T (**40**) obtained from the Cambridge database revealed little differences. Indeed, the superposition of both structures shows a RMS deviation of only 0.039 Å. On the other hand, the pseudorotational parameters for these molecules are quite different, particularly with respect to the value of P (Table 1). Both structures are in the North hemisphere, but they are 140° apart from each other, separated from a perfect North ($P = 0°$) by an almost equal number of degrees (ca. 70°) towards the West and East, respectively. Also, the ring of D4T (**40**) is far more planar ($\nu_{max} = 0.61°$) with a mean deviation from planarity of only 0.0025 Å. The ring of **38** is more puckered ($\nu_{max} = 6.81°$) with a mean deviation from planarity of 0.025 Å.

Although we did not obtain a crystal structure of **39**, the rigid bicyclo[3.1.0]-hexene ring is expected to be similar to that of **38** with identical pseudorotational parameters. In contrast, the pseudorotational parameters for the two molecules present in the unit cell of a carbovir X-ray structure are quite different from those of **39** (Table 1). The main differences found are first the degree of flatness (see ν_{max}) which shows that the five-member ring of **38** (and by analogy that of **39**) is much flatter than that of carbovir. Secondly, the value of P places carbovir in an almost perfect East conformation, 157° away from **38** or **39**. These conformational differences may be associated with some unique biological activity for compounds **38** and **39**, which is currently under investigation.

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