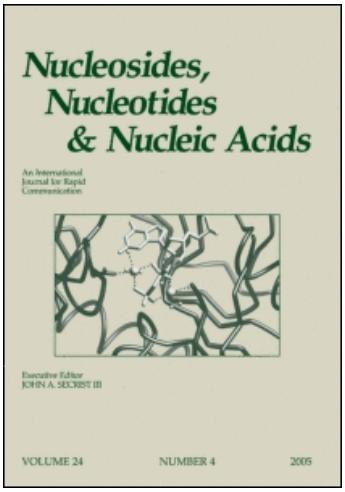


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Lessons from the Pseudorotational Cycle: Conformationally Rigid AZT Carbocyclic Nucleosides and Their Interaction with Reverse Transcriptase

Victor E. Marquez[a]; Abdallah Ezzitouni[a]; Pamela Russ[a]; Maqbool A. Siddiqui[a]; Harry Ford Jr[a]; Ron J. Feldman[b]; Hiroaki Mitsuya[b]; Clifford George[c]; Joseph J. Barchi Jr[a]
[a] Laboratory of Medicinal Chemistry, Division of Basic Sciences, Washington, DC [b] Experimental Retrovirology Section, Medicine Branch Division of Clinical Sciences National Cancer Institute, National Institutes of Health, Bethesda, MD [c] Laboratory for the Structure of Matter, Naval Research Laboratory, Washington

To cite this Article Marquez, Victor E. , Ezzitouni, Abdallah , Russ, Pamela , Siddiqui, Maqbool A. , Ford Jr, Harry , Feldman, Ron J. , Mitsuya, Hiroaki , George, Clifford and Barchi Jr, Joseph J.(1998) 'Lessons from the Pseudorotational Cycle: Conformationally Rigid AZT Carbocyclic Nucleosides and Their Interaction with Reverse Transcriptase', Nucleosides, Nucleotides and Nucleic Acids, 17: 9, 1881 — 1884
To link to this Article: DOI: 10.1080/07328319808004726
URL: http://dx.doi.org/10.1080/07328319808004726

NUCLEOSIDES & NUCLEOTIDES, 17(9-11), 1881-1884 (1998)

LESSONS FROM THE PSEUDOROTATIONAL CYCLE: CONFORMATIONALLY RIGID AZT CARBOCYCLIC NUCLEOSIDES AND THEIR INTERACTION WITH REVERSE TRANSCRIPTASE

Victor E. Marquez,*[a] Abdallah Ezzitouni,[a] Pamela Russ,[a] Maqbool A. Siddiqui,[a] Harry Ford, Jr.,[a] Ron J. Feldman,[b] Hiroaki Mitsuya,[b] Clifford George,[c] and Joseph J. Barchi, Jr.[a]

[a]*Laboratory of Medicinal Chemistry, Division of Basic Sciences, and* [b]*Experimental Retrovirology Section, Medicine Branch, Division of Clinical Sciences, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892 and* [c]*Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, DC 20375*

Abstract. Two conformationally locked *carba*-AZT nucleoside 5'-triphosphates built on a rigid bicyclo[3.1.0]hexane template showed exclusive Northern ($_2$E) and Southern ($_3$E) conformations, respectively. Inhibition of reverse transcriptase (RT) occurred selectively with the Northern *carba*-AZT triphosphate.

Introduction

The original underlying concept for the synthesis of carbocyclic nucleosides (*carba*-nucleosides) was to generate a stable C-N bond resistant to chemical and enzymatic hydrolysis, while at the same time causing minimal structural changes.[1] However, the absence of the 4'-oxygen represents a dramatic change in terms of stereoelectronic effects, and the structural changes ensuing its removal are quite significant. In *carba*-nucleosides, the anomeric effect, as well as important *gauche* interactions between the 4'-oxygen and any electronegative substituents (e.g., OH, N_3, F, etc.) occupying positions 2' and 3' are abolished. Normally, the combined effect of these important interactions drives the conformation of the sugar ring of nucleosides into two preferred forms of puckering as described in the pseudorotational cycle: 1) a Northern conformation approximating a 2'-*exo* ($_2$E) envelope pucker, and 2) a Southern conformation neighboring a 3'-*exo* ($_3$E) form.[2] In solution, the conformation of a nucleoside is represented by an equilibrium between these two extremes, and the direction of this equilibrium is often determined by the interplay of the above-mentioned forces. In the solid state, however, generally only one of the two typical solution conformations is present, and its selection is usually determined

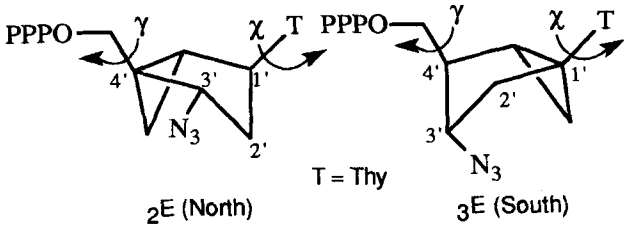


FIGURE 1. Conformationally locked forms of *carba*-AZT triphosphates

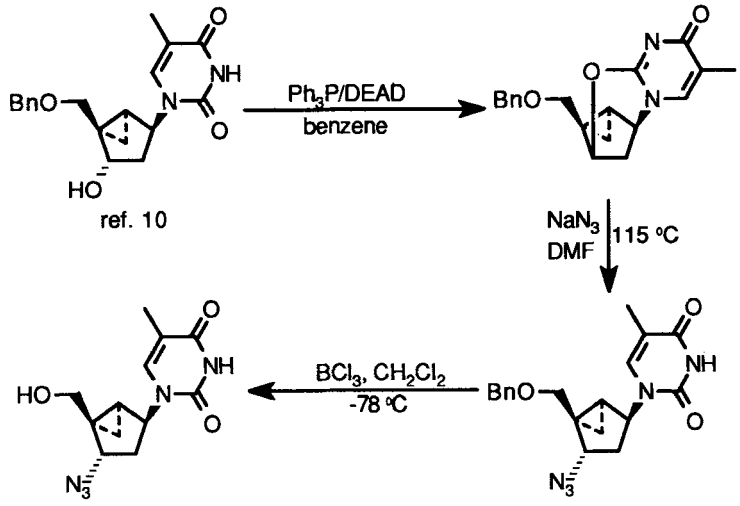
by specific crystal packing forces. Similarly, when a nucleoside or nucleotide binds to its target enzyme, only one form is typically present in the drug-receptor complex. Since the cyclopentane ring in conventional *carba*-nucleosides exists in an unusual 1'-*exo* form (as in *carba*-thymidine and *carba*-AZT),[3,4] we and others have constructed a new class of *carba*-nucleosides on a bicyclo[3.1.0]hexane template which produces conformationally locked forms of ring pucker that mimic the $_2$E (Northern) and $_3$E (Southern) conformation of conventional nucleosides.[5-10] For the present study, the two conformationally locked isomers of *carba*-AZT triphosphate (**1** and **2**) shown in FIGURE 1 were selected as synthetic targets.

It has been suggested that AZT's preference for an extreme $_3$E Southern conformation is responsible for its potent anti-HIV activity.[11] On the other hand, there is evidence that AZT-triphosphate binds to reverse transcriptase (RT) in a Northern conformation.[12] We decided to measure the affinity of RT for both conformationally locked $_2$E and $_3$E *carba*-AZT triphosphates using a recombinant enzyme and an enzyme isolated from several HIV-1 strains.

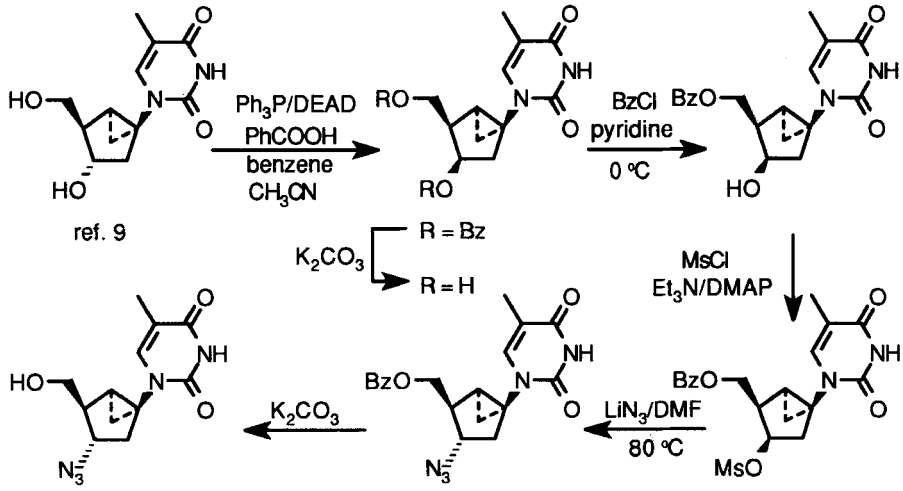
Synthesis

The two starting points for the synthesis of the azido compounds were the corresponding *carba*-thymidine analogues which have been previously reported.[9,10] In one case, the azido group was introduced from the corresponding anhydride intermediate formed under Mitsunobu reaction conditions (SCHEME 1). In the second case, standard Mitsunobu conditions were employed to invert the configuration of the secondary alcohol (SCHEME 2). Conversion of the secondary alcohol to the mesylate ester was followed by S_N2 displacement with NaN_3.

Synthesis of the corresponding 5'-triphosphates was performed using a "one pot" approach by reacting each *carba*-AZT nucleoside with phosphorus oxychloride and



SCHEME 1



SCHEME 2

subsequently treating the intermediates with tri-*n*-butylammonium pyrophosphate. Both 5'-triphosphates (**1** and **2**) were obtained as sodium salts.

Conformational Analysis

The X-ray structures of both Northern and Southern *carba*-AZT nucleosides confirmed their conformations as $_2$E (North) and $_3$E (South), exactly as illustrated in FIGURE 1 for the triphosphates **1** and **2**.[13] Analysis of coupling constants and NOEs also confirmed that the conformations present in solution were virtually identical to those observed in the solid state.[13] Therefore, it was anticipated that the conformation of each triphosphate would be maintained due to the rigid nature of the bicyclo[3.1.0]hexane system. Thus, triphosphate **1** was expected to exist as an $_2$E envelope with the base in the *anti* range (χ between –90 ° and 170°). In contrast, triphosphate **2** was expected to exist as an $_3$E envelope with the base constrained in the *syn* range (χ between 0° and 50°).

Biological Activity

Reverse transcriptase (RT) obtained either as a recombinant enzyme, or isolated from different HIV-1 strains, showed exclusive binding to the Northern *carba*-AZT triphosphate. The IC_{50} of ca. 1 nM was equal to that of AZT triphosphate used as a reference in the same experiment.

REFERENCES

1. Marquez, V. E.; Lim, M.-I. *Med. Res. Rev.*, **1986**, *1*, 1-40.
2. Plavec, J.; Tong, W.; Chattopadhyaya, J. *J. Am. Chem. Soc.* **1993**, *115*, 9734-9746.
3. Bodenteich, M.; Griengl, H. *Tetrahedron Lett.*, **1987**, *28*, 5311-5312.
4. Kálmán, A.; Koritsánszky, T.; Béres, J.; Sági, G. *Nucleosides Nucleotides* **1990**, *9* , 234-243.
5. Altmann, K.-H.; Kesselring, R.; Francotte, E.; Rihs, G. *Tetrahedron Lett.*, **1994**, *35*, 2331-2334.
6. Rodriguez, J. B.; Marquez, V. E.; Nicklaus, M. C.; Mitsuya, H.; Barchi, J. J., Jr. *J. Med. Chem.* **1994**, *37*, 3389
7. Altmann, K-H.; Imwinkelried, R.; Kesselring, R.; Rihs, G. *Tetrahedron Lett.* **1994**, *35*, 7625-7628.
8. Marquez, V. E.; Siddiqui, M. A.; Ezzitouni, A.; Russ, P.; Wang, J.; Wagner, R. W.; Matteucci, M. D. *J. Med. Chem.* **1996**, *39*, 3739-3747.
9. Ezzitouni, A.; Marquez, V. E. *J. Chem. Soc., Perkin Tans. 1*, **1997**, 1073-1078.
10. Ezzitouni, A.; Russ, P.; Marquez, V. E. *J. Org. Chem.*, **1997**, *62*, 4870-4873.
11. Van Roey, P.; Taylor, E. W.; Chu, C. K.; Schinazi, R. F. *Ann. N.Y. Acad. Sci.*, **1990**, *616*, 29-40.
12. Painter, G. R.; Aulabaugh, A. E.; Andrews, C. W. *Biochem. Biophys. Res. Commun.*, **1993**, *191*, 1166-1171.
13. Marquez, V. E.; Ezzitouni, A.; Russ, P.; Siddiqui, M. A.; Ford, H. Jr.; Fedlman, R.; Mitsuya, H.; George, C.; Barchi, J. Jr., Manuscript in preparation.