A Conformationally Locked Analogue of the Anti-HIV Agent Stavudine. An Important Correlation between Pseudorotation and Maximum Amplitude

Yongseok Choi,[†] Clifford George,[‡] Maria J. Comin,[†] Joseph J. Barchi, Jr.,[†] Hak Sung Kim,[§] Kenneth A. Jacobson,[§] Jan Balzarini,[∥] Hiroaki Mitsuya,[⊥] Paul L. Boyer,[⊗] Stephen H. Hughes,[⊗] and Victor E. Marquez*,[†]

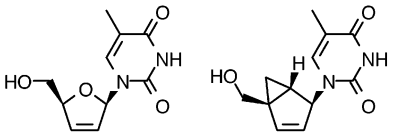
Laboratory of Medicinal Chemistry, Center for Cancer Research, NCI−Frederick, NIH, Frederick, Maryland 21702; Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, D.C. 20375; Molecular Recognition Section, Laboratory of Bioorganic Chemistry, National Institute of Diabetes, Digestive and Kidney Diseases, NIH, Bethesda, Maryland 20892, Katholieke Universiteit Leuven, Rega Institute, Minderbroedersstraat 10, B-3000 Leuven, Belgium; Experimental Retrovirology Section, Center for Cancer Research, NCI, NIH, Bethesda, Maryland 20892, and HIV Drug Resistance Program, Center for Cancer Research, NCI−Frederick, Frederick, Maryland 21702

Received March 12, 2003

The synthesis and biological evaluation of a bicyclo[3.1.0]hexene nucleoside designed as a conformational mimic of the anti-HIV agent stavudine (**1**, D4T) is described. The unsaturated methanocarbocyclic pseudosugar of *N*-MCD4T (**2**) was constructed from an iodo-substituted precursor by a DBU-catalyzed olefination reaction. Mitsunobu coupling with N^3-benzoylthymine afforded the desired target after deprotection. Both D4T and *N*-MCD4T are in the North (*N*) hemisphere of the pseudorotational cycle but 70° away from a perfect *N* ($P = 0°$) conformation toward the East and West hemispheres, respectively. Despite this large difference, the double bond reduces the puckering amplitude (ν_{max}) of *N*-MCD4T to 6.81°, and the superposition of both structures showed a RMS deviation of only 0.039 Å. The combined structural analysis of *P* and ν_{max} shows that while the value of *P* may differ substantially, the low ν_{max} resolves the differences and becomes the dominant pseudorotational parameter. *N*-MCD4T is active against HIV-1 and HIV-2 in CEM, MT-2, and MT-4 cells, and while it is somewhat less potent than D4T, it also appears to be less toxic. The triphosphate (*N*-MCD4TTP) inhibits HIV reverse transcriptase with a 10-fold higher IC_{50} than D4TTP. By virtue of its carbocyclic nature, *N*-MCD4T (**2**) is a more robust molecule stable to conditions that would cleave D4T.

Introduction

2′,3′-Dideoxynucleosides that target HIV-RT are important components in the highly active antiretroviral therapy (HAART) regimens used for the treatment of AIDS. Among the nucleoside analogues with significant clinical utility when used in this modality is stavudine (**1**, D4T).[1] An important structural characteristic of D4T is the presence of a double bond, a feature that renders the sugar ring nearly planar and imparts a high degree of rigidity to the molecule.



1 **2**

During the past few years, we have developed a group of structurally rigid methanocarba (MC) nucleosides built on a bicyclo[3.1.0]hexane template whose conformation appears locked into either a North (*N*) [$C_{2'}$-*exo* ($_2$E)] or South (*S*) [$C_{3'}$-*exo* ($_3$E)] pucker, ±18° away from the ideal *N* ($^3T_2$) and *S* ($_3T^2$) conformations of the pseudorotational cycle (Figure 1). These compounds have enabled us to probe the conformational preferences of various enzymes involved in nucleoside metabolism and nucleotide polymerization.[2−9] To impart a certain degree planarity to the bicyclo[3.1.0]hexane template to a level comparable to that of D4T, we have combined in a single structure, the two most salient features of these two classes of nucleoside analogues: a locked *N* conformation and a 2′,3′-double bond, two features that are embodied in the structure of *N*-MCD4T (**2**). In this paper we describe the synthesis and conformational analysis of *N*-MCD4T and compare its anti-HIV activity with that of D4T.

Results

Chemical Synthesis. For the synthesis of *N*-MCD4T (**2**) a convergent approach involving a Mitsunobu coupling of thymine with the 2,3-unsaturated pseudosugar **9** was envisaged. This important intermediate was to be obtained from the readily accessible 5-(benzyloxymethyl)-4-(benzyloxy)bicyclo[3.1.0]hexan-2-ol (**3**),[5] following a sequence designed to give the olefin after elimination of the C_4-OH (Scheme 1). The elimination was first attempted by converting **3** into **5** after three steps involving protection of the free hydroxyl group as a benzoate ester, removal of the two benzyl-protecting groups by catalytic transfer hydrogenation, and protection of the remaining primary alcohol as the *tert*-butyldiphenylsilyl (TBDPS) ether. These steps allowed

* Victor E. Marquez, Ph.D., Chief, Laboratory of Medicinal Chemistry, Center for Cancer Research, NCI-Frederick, 376 Boyles St., Bldg. 376, Rm. 104, Frederick, MD 21702-1201. Phone: (301) 846−5954(3). Fax: (301) 846-6033. E-mail: marquezv@dc37a.nci.nih.gov.

[†] Laboratory of Medicinal Chemistry, Center for Cancer Research, NCI.

[‡] Naval Research Laboratory.

[§] National Institute of Diabetes, Digestive and Kidney Diseases.

[∥] Katholieke Universiteit Leuven.

[⊥] Experimental Retrovirology Section, Center for Cancer Research, NCI.

[⊗] HIV Drug Resistance Program, Center for Cancer Research, NCI.

10.1021/jm030116g CCC: $25.00
Published on Web 06/20/2003

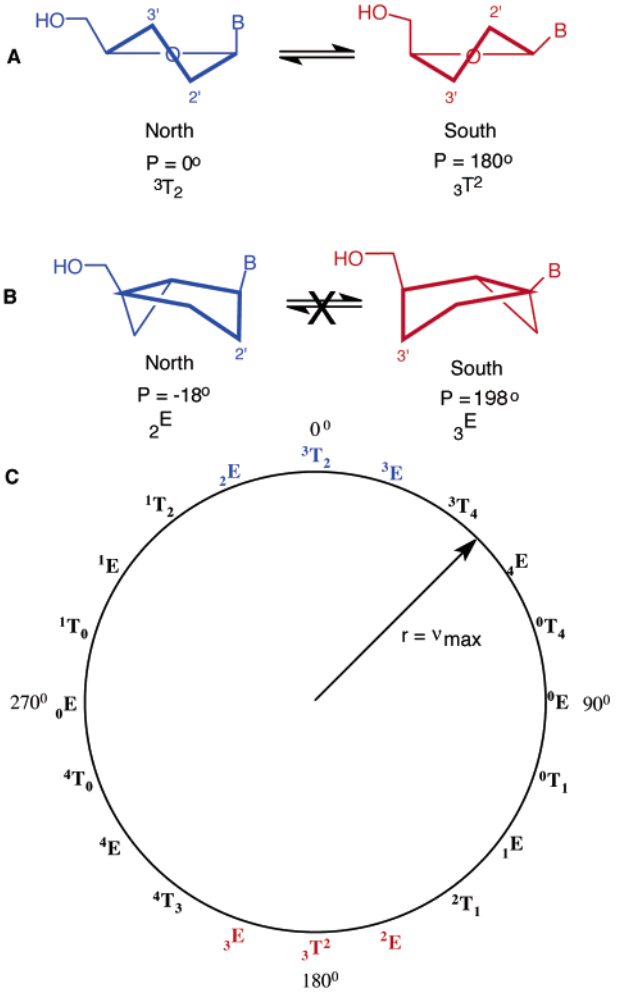
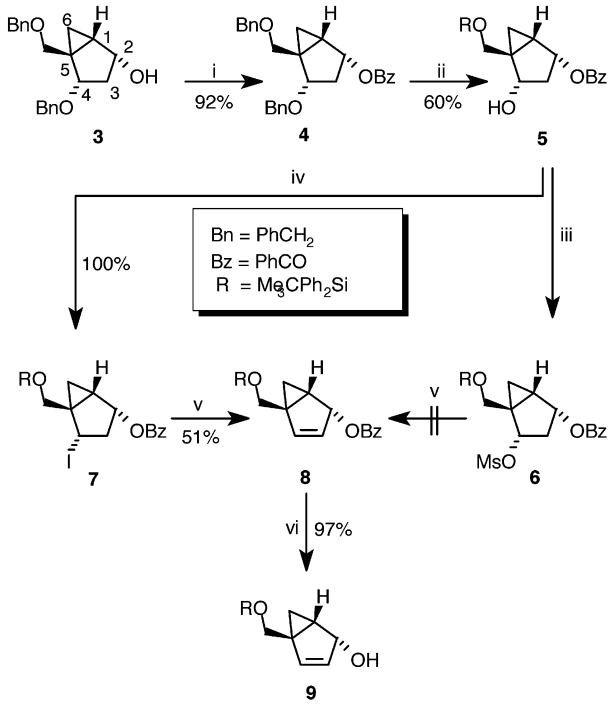


Figure 1. (A) Dynamic two-state equilibrium of North (*N*, *P* = 0°) and South (*S*, *P* = 180°) nucleoside pseudorotamers in solution. (B) Conformationally locked bicyclo[3.1.0]hexane nucleosides standing at the edge of the range for *N* ($_2$E−^{3}T$_2$−^{3}E) and *S* ($_3$E−^{2}T$_3$−^{2}E) conformations. (C) Pseudorotational cycle of the furanose ring in nucleosides showing alternating envelope (E) and twist (T) conformations every 18°. The radius of the circle corresponds to ν_{max}.

the functionalization of the remaining secondary alcohol in **5** as a methanesulfonyl ester (compound **6**) for a possible base-catalyzed E2 elimination reaction. Unfortunately, the rigidity of the bicyclo[3.1.0]hexane made this approach difficult because the disposition of the leaving group (OMs) and the two possible hydrogen atoms available for elimination was fixed in a gauche orientation that prohibited formation of the required antiperiplanar transition state (Figure 2A). Indeed, base-catalyzed elimination of **6** failed to give **8** under various conditions, including using DBU, and only the hydrolysis product was obtained. To overcome this obstacle, an inversion of stereochemistry at C_4 with iodine as the leaving group was attempted to fix this group anti relative to one of the two adjacent hydrogen atoms available for elimination (Figure 2B). The C_4-OH group was successfully replaced by iodine quantitatively after treatment with I_2/Ph_3/imidazole by a mechanism expected to proceed principally with inversion of configuration. Surprisingly, examination of the ^{1}H NMR spectrum of the product (**7**) did not show the H_4 proton signal as a doublet. With iodine in the inverted stereochemistry, the near 90° dihedral angle between H_4 and the H_3-exo hydrogen in the rigid bicyclo[3.1.0]hexane scaffold should have resulted in a $J_{4,3exo}$ coupling near zero. Instead, the H_4 signal appeared as a doublet of doublets with very similar coupling constant values, and an additional "W" coupling constant of ca. 1 Hz between H_4 and the H_6-exo proton located at the tip of the fused cyclopropane ring was also detected at 500 MHz (Figure 3). Since the bicyclo[3.1.0]hexane is a very predictable system in terms of coupling constants, we believe that

Scheme 1[a]



[a] Reagents and conditions: (i) BzCl, CH_2Cl_2/pyridine. (ii) (a) Pd Black, HCO_2H, MeOH; (b) Me_3CPh_2SiCl, imidazole, CH_2Cl_2. (iii) MsCl, CH_2Cl_2/Et_3N. (iv) Ph_3P, I_2, imidazole/toluene, 100 °C. (v) DBU/toluene, 100 °C. (vi) NaOMe/MeOH.

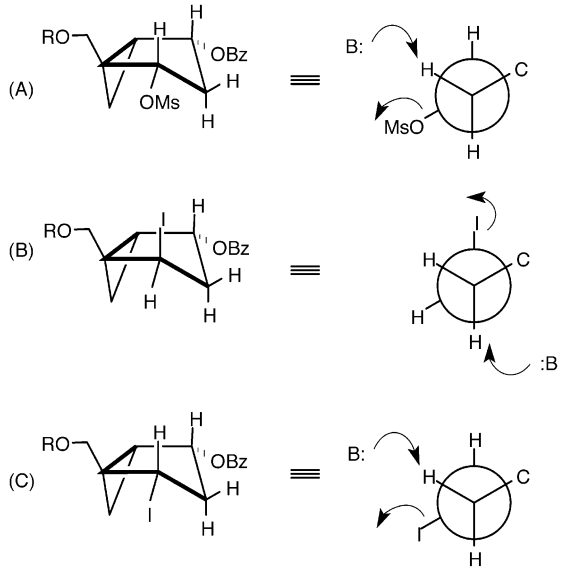


Figure 2. Relative disposition of leaving groups and hydrogens in a rigid bicyclo[3.1.0]hexane scaffold. (A) H and OMs groups in a gauche disposition. (B) H and stereochemically inverted iodine in an antiperiplanar disposition. (C) H and stereochemically retained iodine in a gauche disposition.

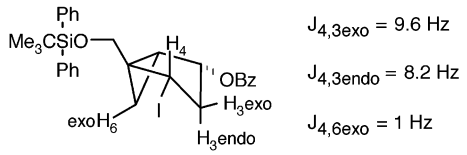
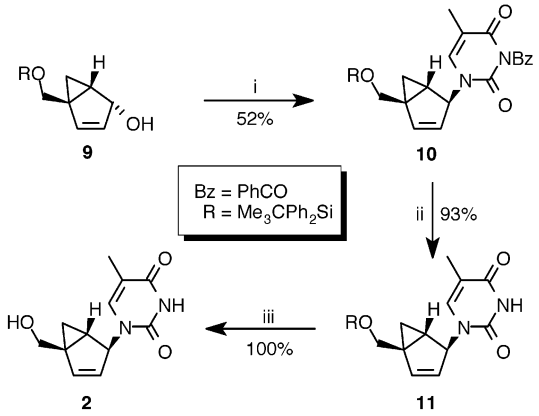


Figure 3. Coupling constants in compound **7** reflecting the retention of the iodine configuration at C_4 after reaction with I_2/Ph_3/imidazole.

Scheme 2[a]



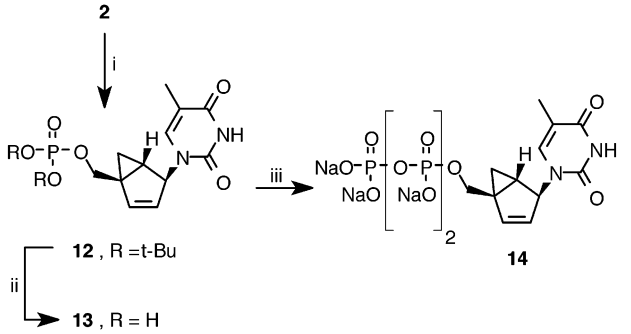
[a] Reagents and conditions: (i) Ph_3P, N^3-Bz-thymine, DEAD/THF. (ii) NH_4OH/MeOH. (iii) TBAF/THF.

the reaction gave the equatorially disposed product **7** resulting from a double inversion mechanism. Ordinarily, the reaction of I_2/Ph_3/imidazole is known to give mixtures of epimeric iodides with inverted and retained configurations—usually with the former product predominating—with the final outcome being determined by the thermodynamic stability of the products.[10] In the case of **7**, the more stable product is that in which the iodine is equatorial; hence, the configuration of the product remained unchanged after a double inversion mechanism. To complete the synthesis, compound **7** was refluxed in the presence of DBU to give the bicyclo[3.1.0]-hexene pseudosugar **8**. The optimized yield for this reaction was only 51%, possibly reflecting the less than ideal geometry (Figure 2C) that forces elimination via a two-step process (E1cB mechanism).[11] Hydrolysis of the benzoate ester moiety in **8** afforded the desired pseudosugar precursor **9**.

After the synthesis of **9**, coupling with N^3-benzoylthymine under Mitsunobu conditions gave the corresponding *N*-alkylated product **10** as the major isomer, reflecting the higher reactivity of the allylic alcohol in the bicyclo[3.1.0]hexen-2-ol system relative to the saturated bicyclo[3.1.0]hexan-2-ol where the *O*-alkylated products predominate (Scheme 2).[12] The target *N*-MCD4T (**2**) was obtained after treatment with ammonium hydroxide and removal of the TBDPS group with tetra-*n*-butylammonium fluoride (TBAF).

Phosphorylation of *N*-MCD4T (**2**) to the 5′-triphosphate (nucleoside numbering) was performed by a multistep approach following the method of Jacobson et al.[13] Thus, phosphytilation of **2** with di-*tert*-butyl *N*,*N*-diethylphosphoramidite followed by in situ oxidation afforded the corresponding di-*tert*-butyl phosphate **12** (Scheme 3). The di-*tert*-butyl groups were removed under acidic conditions, and following activation of the monoposhate with 1,1′-carbonyldiimidazole, the resulting intermediate phosphoroimidazolidate reacted with pyrophosphate to give the desired triphosphate (**14**, *N*-MCD4TTP).

Scheme 3[a]



[a] Reagents and conditions: (i) Di-*tert*-butyl *N*,*N*-diethylphosphoramidite, tetrazole/THF, 30 min, and then *m*-CPBA, −78 °C → room temperature. (ii) TFA/CH_2Cl_2. (iii) (a) 1,1′-carbonyldiimidazole, (b) tri-*n*-butylamine /MeOH, (c) tri-*n*-butylammonium pyrophosphate/DMF.

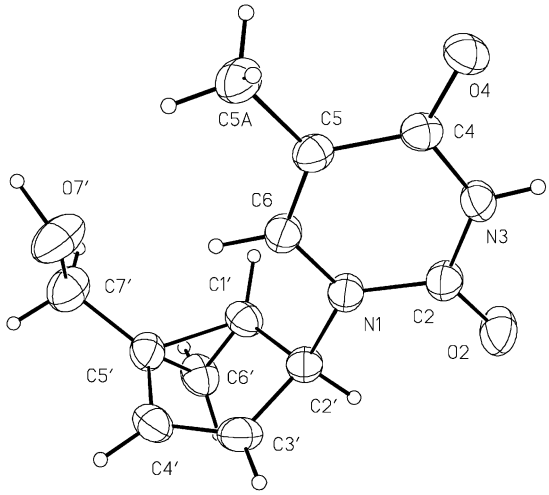


Figure 4. Displacement ellipsoid plot of **2** drawn at the 30% probability level.

X-ray Structures and Conformational Analysis. The newly synthesized bicyclo[3.1.0]hexene D4T analogue (*N*-MCD4T, **2**) provided adequate crystals for X-ray analysis, and the crystal structure (Figure 4) validated all of the spectral assignments. A comparison between the X-ray structure of *N*-MCD4T (**2**) and D4T (**1**) obtained from the Cambridge database (KONNOQ)[14] revealed minor differences, and the superposition of both structures (Figure 5) showed a RMS deviation of only 0.039 Å. In contrast, the pseudorotational parameters of these molecules are quite different, particularly with respect to the value of *P* (Table 1). Both D4T and *N*-MCD4T are in the *N* hemisphere; however, they are 140° apart from each other, separated by an almost equal number of degrees (ca. 70°) from a perfect *N* (P = 0°) pucker toward the East and West, respectively. In terms of ring puckering, the ring of D4T is more planar (ν_{max} = 0.61°) with a mean deviation from planarity of only 0.0025 Å. Relative to D4T, *N*-MCD4T appears slightly more puckered (ν_{max} = 6.81°) with a mean deviation from planarity of 0.025 Å. The reason for the structural similarity between D4T and *N*-MCD4T, despite their widely different *P* values, is that as ν_{max} → 0 the relevance of *P* diminishes. Pictorially, the radius

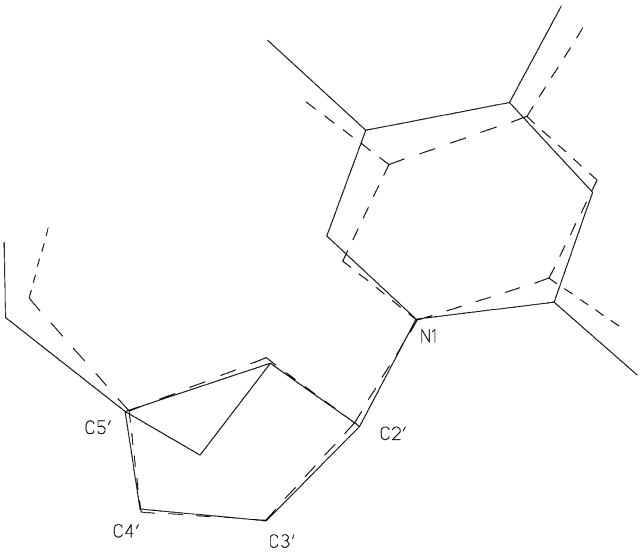


Figure 5. Least-squares fit of labeled atoms of compound **2** (solid line) to **1** (dashed lines). Hydrogen atoms have been omitted. Atom positions of both **1** and **2** are from experimental X-ray coordinates.

Table 1. Pseudorotational Parameters for D4T (**1**) and *N*-Mcd4t (**2**)[a,b]

	ν_0	ν_1	ν_2	ν_3	ν_4	P	ν_{max}	χ	γ
1	−0.51	0.20	0.19	−0.49	0.60	72.08	0.61	−100.82	52.80
2	6.56	−5.83	2.67	1.68	−5.20	293.14	6.81	−119.12	56.17

[a] $\nu_0-\nu_4$ represent the torsion angles of the five-membered ring. [b] Numbers represent degrees (deg).

Table 2. Anti-HIV Activity of D4T (**1**) and *N*-Mcd4t (**2**) in Human T-Lymphocyte (CEM) Cells

drug	virus	EC_{50}[a] (μM)	CC_{50}[b] (μM)
D4T	HIV-1 (III_B)	0.21 ± 0.08 ($n = 5$)	93 ± 37 ($n = 3$)
D4T	HIV-2 (ROD)	0.40 ± 0.14 ($n = 5$)	
N-MCD4T	HIV-1 (III_B)	0.96 ± 0.56 ($n = 4$)	>250 ($n = 3$)
N-MCD4T	HIV-2 (ROD)	1.45 ± 0.38 ($n = 4$)	

[a] Effective concentration required to protect CEM cells against the cytopathogenicity of HIV by 50%. [b] Cytostatic concentration required to inhibit CEM cell proliferation by 50%.

of the pseudorotational cycle in Figure 1 for *N*-MCD4T is 6.81 while the radius of the pseudorotational cycle for D4T is even smaller ($r = 0.61$). The radii of these two circles correspond to the value of ν_{max} (puckering amplitude), a value that in conventional nucleosides ranges from 0° to 45° depending on the extent of puckering. With radii of only 0.61 and 6.81, D4T and *N*-MCD4T are quite similar, making it likely that if all the intervening enzymes recognize them in a similar manner, the compounds would behave comparably against HIV.

Anti-HIV Activity. The relative anti-HIV activities of *N*-MCD4T and D4T varied according to the specific cell type and assay conditions. In human T-lymphocyte (CEM) cells, *N*-MCD4T was about 4-fold less potent than D4T against both HIV-1 and HIV-2 (Table 2) although it appears to be less toxic. In CEM/TK− cells *N*-MCD4T was ineffective ($EC_{50} > 250$ μM, $n = 2$), while D4T had an $EC_{50} = 27 \pm 5.8$ μM ($n = 2$). These results point to a critical role for cytosolic TK-1 in the activation of D4T, and more specifically for *N*-MCD4T, and also reflect the ability of D4T to be activated by alternative pathways. In MT-2 cells the anti-HIV activity of *N*-MCD4T was only ca. 3-fold less than that of D4T and in MT-4 it was about 10-fold less potent (Table 3). In PHA-PBM cells infected with HIV-1_{LAI} the potency of both drugs was nearly identical (Table 4).

Table 3. Anti-HIV (HIV-1_{LAI}) Activity of D4T and *N*-MCD4T in MT-4 and MT-2 Cells (average of five experiments)

drug	cell	EC_{50}[a] (μM)
AZT	MT-4	0.02 ± 0.004
AZT	MT-2	0.03 ± 0.01
D4T	MT-4	0.71 ± 0.26
D4T	MT-2	1.8 ± 0.7
N-MCD4T	MT-4	6.9 ± 2.3
N-MCD4T	MT-2	6.1 ± 1.2

[a] Effective concentration required to protect cells against the cytopathogenicity of HIV by 50% (duplicate experiments).

Table 4. Percent Inhibition of P24 Antigen Production by D4T and *N*-MCD4T in PHA-PBM Cells Exposed to HIV-1_{LAI}

drug	EC_{50} (μM)
D4T	0.04
N-MCD4T	0.06

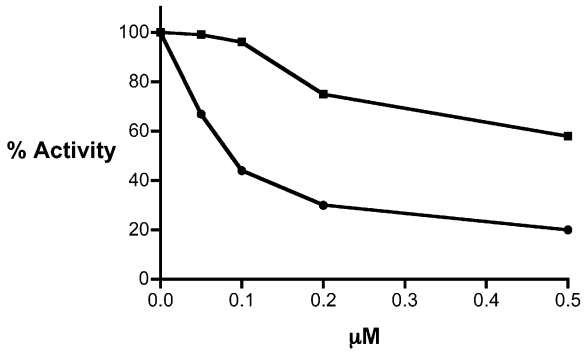
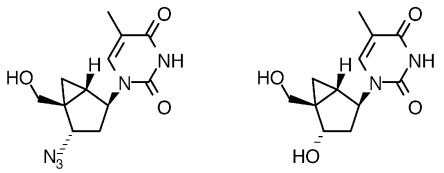


Figure 6. Inhibition of HIV RT by D4TTP (●) and *N*-MCD4TTP (■).

To determine the effect of ring pucker on the final target enzyme, reverse transcriptase (RT), the inhibition of RT by the triphosphates of D4T (D4TTP) and *N*-MCD4T (*N*-MCD4TTP, **14**) was determined (Figure 6). Under our experimental conditions, the IC_{50} for D4TTP was 0.08 μM while the IC_{50} for *N*-MCD4TTP was 0.65 μM reflecting a ca. 8-fold difference in potency favoring D4TTP. This result appears to be consistent with all the cell-based data.

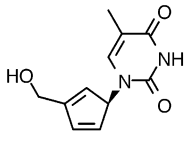
Discussion

The present work highlights the difference between the bicyclo[3.1.0]hexane platform for nucleoside analogues such as *N*-MCAZT (**15**) and *N*-MCT (**16**), and the



15

16



17

bicyclo[3.1.0]hexene platform of *N*-MCD4T (**2**). The subtle conformational change brought about by the double bond in the carbocyclic scaffold of *N*-MCD4T seems to allow this molecule to be recognized by the cellular kinases responsible for the formation of the critical triphosphate metabolite. In contrast, although the locked *N* form of the 5′-triphosphate of *N*-MCAZT was effectively incorporated by HIV RT, the unphosphorylated parent drug was ineffective in HIV-1 infected cells, possibly due to the failure of cellular kinases to phosphorylate it.[4] In a separate study, *N*-MCT (**16**) was shown to be effective only in HSV-1 and HSV-2 infected cells due to the adequate phosphorylation of the drug by the herpes-virus kinase.[15,16] Since HIV-1 does not encode a specific kinase, D4T and *N*-MCD4T must be activated by cellular kinases to be effective against HIV-1. Therefore, whatever advantages the flattening of D4T has for enzyme recognition, our data shows that flattening can be applied to a more exotic ring system not usually recognized by cellular kinases. The combined structural analysis of *P* and ν_{max} shows that while the value of *P* may differ substantially, the low ν_{max} diminishes the importance of these differences and becomes the dominant pseudorotational parameter. The important reduction of ca. 23° in the value of ν_{max} from a bicyclo[3.1.0]hexane system (ca 30°)[3] to a bicyclo[3.1.0]-hexene system (ca. 7°) is quite substantial. Taking into account that this value represents the radius of the pseudorotational cycle, its impact as a structural parameter is quite high. A fused cyclopropane ring can be considered equivalent to a double bond in terms of inducing planarity. The problem with a double bond, however, is that the resulting cyclopenta-2,4-dienyl system, such as in compound **17**, would be subject to rapid isomerization. Finally, it is tempting to speculate that the ca. 10-fold difference in ν_{max} between D4T and *N*-MCD4T is responsible for the 4−10-fold difference in potency seen in the various assays, including the polymerase assay with HIV RT. *N*-MCD4T, because it is a carbocyclic nucleoside, is expected to be a more robust molecule, stable to conditions in which D4T would be cleaved either chemically or enzymatically.[17,18] This enhanced stability, combined with an apparent lack of toxicity, makes *N*-MCD4T an attractive agent for further studies.

Experimental Section

General Procedures. All chemical reagents were commercially available. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Column chromatography was performed on silica gel 60, 230−400 mesh (E. Merck), and analytical TLC was performed on Analtech Uniplates silica gel GF. Routine IR and ^{1}H (400 MHz) and ^{13}C NMR (125 MHz) spectra were recorded using standard methods. Chemical shifts are reported in parts per million (δ) and signals are quoted as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Specific rotations were measured in a Perkin-Elmer model 241 polarimeter. Positive-ion fast-bombardment mass spectra (FABMS) were obtained on a VG 70-70E mass spectrometer at an accelerating voltage of 6 kV and a resolution of 2000. Glycerol was used as the sample matrix, and ionization was effected by a beam of xenon atoms. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA.

(1*S*,2*R*,4*S*,5*R*)-4-[Phenylmethoxy)-5-[(phenylmethoxy)-methyl]bicyclo[3.1.0]hex-2-yl Benzoate (4). A solution of **3** (2.05 g, 6.32 mmol) in methylene chloride (50 mL) containing pyridine (1.02 mL, 12.6 mmol) was maintained at 0 °C and treated dropwise with benzoyl chloride (0.81 mL, 6.95 mmol). After being stirred 3 h at room temperature, the reaction mixture was washed with dilute hydrochloric acid, saturated $NaHCO_3$, and brine. The organic layer was dried ($MgSO_4$) and filtered through a Celite pad. The filtrate was concentrated in vacuo, and the residue was purified on silica gel (hexanes/ethyl acetate, 15/1) to give **4** (2.50 g, 92%) as a colorless syrup: $[\alpha]^{22}$ +63.1° (*c* 0.59, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.96−7.16 (m, 15 H, Ph), 5.38−5.33 (m, 1 H, H-2), 4.56−4.39 (m, 4 H, 2 × Ph*CH*$_2$O), 4.28 (t, *J* = 8.1 Hz, 1 H, H-4), 3.83 (AB d, *J* = 10.3 Hz, 1 H, OC*H*H), 3.11 (AB d, *J* = 10.3 Hz, 1 H, OCH*H*), 2.51−2.43 (m, 1 H, H-3a), 1.77−1.73 (m, 1 H, H-3b), 1.44−1.37 (m, 1 H, H-1), 1.22 (irregular t, *J* = 5.1, 4.4 Hz, 1 H, H-6a), 0.573 (dd, *J* = 7.51, 6.0 Hz, 1 H, H-6b); FABMS (relative intensity) 429 (MH^+, 2.5); Anal. Calcd for $C_{28}H_{28}O_4$: C, 78.48; H, 6.59. Found: C, 78.27; H, 6.44.

(1*S*,2*R*,4*S*,5*R*)-5[(2,2-Dimethyl-1,1-diphenyl-1-silapropoxy)methyl]-4-hydroxybicyclo[3.1.0]hex-2-yl Benzoate (5). A solution of **4** (2.5 g, 5.83 mmol) in methanol (100 mL) was treated with Pd black (2.4 g) and formic acid (6.8 mL). The mixture was stirred at room-temperature overnight and filtered through a Celite pad. The filtrate was neutralized with triethylamine and concentrated in vacuo. The residue was purified on silica gel (hexanes/ethyl acetate, 1/2) to give the intermediate diol (1.05 g, 72.5%) as a colorless syrup. The diol (1.05 g, 4.23 mmol) was dissolved in methylene chloride (20 mL) containing imidazole (0.633 g, 9.31 mmol) and treated with a solution of *tert*-butylchlorodiphenylsilane (1.15 mL, 4.44 mmol) in methylene chloride (20 mL) at 0 °C. The mixture was stirred at 0 °C for 2 h and washed with water. The organic layer was dried ($MgSO_4$) and filtered through a Celite pad. The filtrate was concentrated in vacuo, and the residue was purified on silica gel (hexanes/ethyl acetate, 9/1) to give **5** (1.71 g, 81%) as a colorless syrup: $[\alpha]^{22}$ +20.8° (*c* 0.86, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.04−7.39 (m, 15 H, Ph), 5.41−5.36 (m, 1 H, H-2), 4.60 (t, *J* = 8.2 Hz, 1 H, H-4), 3.84 (AB d, *J* = 10.7 Hz, 1 H, *OCH*H), 3.68 (AB d, *J* = 10.7 Hz, 1 H, *OCHH*), 2.57−2.50 (m, 1 H, H-3a), 2.20 (br s, 1 H, O*H*), 1.71−1.67 (m, 1 H, H-3b), 1.49−1.41 (m, 1 H, H-1), 1.26 (irregular t, *J* = 4.67, 4.4 Hz, H-6a), 1.06 (s, 9 H, *t*-Bu), 0.51 (dd, *J* = 7.1, 6.2 Hz, 1 H, H-6b); FABMS (relative intensity) 487 (MH^+, 1.0); Anal. Calcd for $C_{30}H_{34}O_4Si$: C, 74.04; H, 7.04. Found: C, 74.08; H, 7.18.

(1*S*,2*R*,4*R*,5*R*)-5-[(2,2,-Dimethyl-1,1-diphenyl-1-silapropoxy)methyl]-4-iodobicyclo[3.1.0]hex-2-yl Benzoate (7). A solution of **5** (0.692 g, 1.42 mmol) in toluene (30 mL) was treated with triphenylphosphine (0.745 g, 2.84 mmol), imidazole (0.290 g, 4.26 mmol), and iodine (0.541 g, 2.13 mmol). The mixture was heated at 100 °C for 1 h under blanket of argon. After reaching room temperature, the mixture was poured into a saturated solution of $NaHCO_3$. Excess triphenylphosphine was destroyed by the addition of iodine until the iodine coloration persisted in the organic layer. The organic layer was washed twice with 5% (wt.) $Na_2S_2O_3$ and brine, dried over ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified on silica gel (hexanes/ethyl acetate, 8/1) to give **7** (0.847 g, 100%) as a colorless syrup: $[\alpha]^{23}$ +25.8° (*c* 0.50, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.98−7.31 (m, 15 H, Ph), 5.45−5.39 (m, 1 H, H-2), 4.63 (dd, *J* = 9.6, 8.2, Hz, 1 H, H-4), 4.40 (AB d, *J* = 11.1 Hz, 1 H, *OCH*H), 3.17 (AB d, *J* = 11.1 Hz, 1 H, *OCHH*), 2.85−2.78 (m, 1 H, H-3a), 2.05 (m, 1 H, H-1), 1.94−1.86 (m, 1 H, H-3b), 1.08 (s, 9 H, *t*-Bu), 0.86 (dd, *J* = 5.6, 4.5 Hz, 1 H, H-6a), 0.66 (irregular t, 1 H, H-6b); FABMS 597 (MH^+); Anal. Calcd for $C_{30}H_{33}IO_3Si$: C, 60.40; H, 5.58. Found: C, 60.27; H, 5.54.

(1*S*,2*R*,5*R*)-5-[(2,2-Dimethyl-1,1-diphenyl-1-silapropoxy)-methyl]bicyclo[3.1.0]hex-3-en-2-yl Benzoate (8). A mixture of **7** (1.38 g, 2.31 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 3.0 mL) in toluene (30 mL) was heated at 100 °C for 24 h under nitrogen gas. After reaching room temperature, water was added, and the organic layer was washed successively with saturated solutions of $NaHCO_3$, NH_4Cl, and NaCl. The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified on silica gel (hexanes/

ethyl acetate, 8/1) to give **8** (0.551 g, 51%) as a colorless syrup: $[\alpha]^{22}$ −36.8° (*c* 0.73, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.06−7.36 (m, 15 H, Ph), 6.18 (d, J = 5.5 Hz, 1 H, H-2), 6.12 (br d, J = 6.5 Hz, 1 H, H-4), 5.44 (br d, J = 5.9 Hz, 1 H, H-3), 3.83 (AB q, J = 5.4 Hz, 2 H, O*CH*$_2$), 1.86−1.81 (m, 1 H, H-1), 1.06 (s, 9 H, *t*-Bu), 0.86 (distorted t, 1 H, H-6a), 0.80 (dd, J = 8.2, 4.1 Hz, 1 H, H-6b); FABMS (relative intensity) 469 (MH^+, 0.9); Anal. Calcd for $C_{30}H_{32}O_3Si$: C, 76.88; H, 6.88. Found: C, 76.91; H, 6.97.

(1*S*,2*R*,5*R*)-5-[(2,2-Dimethyl-1,1-diphenyl-1-silapropoxy)-methyl]bicyclo[3.1.0]hex-3-en-2-ol (9). A solution of **8** (0.123 g, 0.263 mmol) in methanol (10 mL) was treated with 0.5 M NaOMe in methanol (0.58 mL) at 0 °C. The resulting solution was stirred at room-temperature overnight under nitrogen gas. After the mixture was concentrated in vacuo, the residue was purified on silica gel (hexanes/ethyl acetate, 8/1) to give **9** (0.093 g, 97%) as a colorless syrup: $[\alpha]^{22}$ −10.2° (*c* 1.07, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.60−7.28 (m, 10 H, Ph), 5.94 (dd, J = 5.5, 0.6 Hz, 1 H, H-4), 5.21 (dt, J = 5.5, 1.6 Hz, 1 H, H-3), 5.14−5.13 (dm, J = 6.4 Hz, 1 H, H-2), 3.72 (AB q, J = 10.9 Hz, 2 H, O*CH*$_2$), 1.52−1.47 (m, 1 H, H-1), 1.25 (br s, 1 H, O*H*), 0.97 (s, 9 H, *t*-Bu), 0.64 (dd, J = 8.2, 4.1 Hz, 1 H, H-6a), 0.80 (t, J = 4.1 Hz, 1 H, H-6b); FABMS (relative intensity) 347.1 ($MH^+ - H_2O$, 6.6), 363.1 ($MH^+ - H_2$, 6.2); Anal. Calcd for $C_{23}H_{28}O_2Si$: C, 75.78; H, 7.74. Found: C, 75.65; H, 7.81.

(1′*S*,2′*S*,5′*R*)-1-{5-[(2,2-Dimethyl-1,1-silapropoxy)methyl]-bicyclo[3.1.0]hex-3-en-2-yl}-5-methyl-3-(phenylcarbonyl)-1,3-dihydropyrimidine-2,4-dione (10). A solution of triphenylphosphine (0.893 g, 3.41 mmol) in anhydrous THF (10 mL) was treated dropwise with diethylazodicarboxylate (DEAD, 0.52 mL, 3.27 mmol) at 0 °C. After the mixture was stirred 30 min at 0 °C, a THF (30 mL) suspension of **9** (0.48 g, 1.32 mmol) and N^3-benzoylthymine (0.456 g, 1.98 mmol) was added. The mixture was stirred at 0 °C for 1 h and at room-temperature overnight. The next day all volatiles were removed in vacuo, and the residue was purified by silica gel chromatography (hexanes/ethyl acetate, 6/1) to give **10** (0.340 g, 45%) as a white foam: $[\alpha]^{23}$ +47.5° (*c* 0.16, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.89−7.30 (m, 15 H, Ph), 7.11 (br s, 1 H, H-6), 6.38 (d, J = 5.5 Hz, 1 H, H-4′), 5.41 (br s, 1 H, H-2′), 5.35−5.33 (m, 1 H, H-3′), 4.19 (AB d, J = 11.3 Hz, 1 H, *OCH*H), 3.46 (AB d, J = 11.3 Hz, 1 H, OCH*H*), 1.63 (m, 1 H, H-1′), 1.60 (d, $J \approx 1$ Hz, 3 H, *CH*$_3$), 1.04 (s, 9 H, *t*-Bu), 1.00 (dd, J = 8.5, 4.5 Hz, 1 H, H-6a′), 0.35 (irregular t, J = 4.5, 4.3 Hz, 1 H, H-6b′); FABMS (relative intensity) 577.1 (MH^+, 12.8); Anal. Calcd for $C_{35}H_{36}N_2O_4Si$: C, 72.89; H, 6.29; N, 4.86. Found: C, 72.90; H, 6.53; N, 4.65.

(1′*S*,2′*S*,5′*R*)-1-[5-(Hydroxymethyl)bicyclo[3.1.0]hex-3-en-2-yl]-5-methyl-1,3-dihydropyrimidine-2,4-dione (2). A solution of **10** (0.589 g, 1.02 mmol) in methanol (50 mL) was treated with concentrated NH_4OH (3.6 mL) and stirred at room temperature for 4 h. After all volatiles were removed in vacuo, the residue was purified by silica gel chromatography (hexanes/ethyl acetate, 2/1) to give **11** (0.45 g, 93%) as a white foam. This compound was immediately dissolved in anhydrous THF (20 mL) and treated with a solution of *n*-tetrabutylammonium fluoride (TBAF) in THF (1.1 mL, 1 M). The resulting mixture was stirred at room temperature for 1 h, and after concentrating in vacuo, the residue was purified by silica gel chromatography (chloroform/methanol, 24/1) to give **2** (0.223 g, 100%) as a white foam: mp 181−182 °C; [α] +170° (*c* 0.3, MeOH); ^{1}H NMR (CD_3OD) δ 7.28 (dd, J = 2.5, 1.2 Hz, 1 H, H-6), 6.42 (m, 1 H, H-4′), 5.36 (m, 2 H, H-2′, H-3′), 4.03 (AB d, J = 12.0 Hz, 1 H, OC*H*H), 3.30 (AB d, J = 12.0 Hz, 1 H, OCH*H*), 1.74 (d, J = 1.2 Hz, 3 H, *CH*$_3$), 1.60 (m, 1 H, H-1′), 1.06 (dd, J = 8.5, 4.3 Hz, 1 H, H-6a′), 0.36 (distorted t, 1 H, H-6b′); FABMS (relative intensity) 235 (MH^+, 100); Anal. Calcd for $C_{12}H_{14}N_2O_3 \cdot 0.25H_2O$: C, 60.37; H, 6.12; N, 11.73. Found: C, 60.70; H, 6.37; N, 11.35.

(1′*S*,2′*S*,5′*R*)-*tert*-Butyl{[4-(5-Methyl-2,4-dioxo-1,3-dihydropyrimidinyl)bicyclo[3.1.0]hex-3-en-2-enyl]methyl}phosphate (12). Neat di-*tert*-butyl diethylphosphoramidite (33 μL, 0.119 mmol) was added by syringe to a stirred solution of **2** (10.5 mg, 0.0448 mmol) in anhydrous THF (1.5 mL) at room temperature, followed by the addition of solid tetrazole (15.7 mg, 0.227 mmol). After the solution was stirred at room temperature for 30 min, the reaction mixture was cooled to −78 °C, and a solution of *m*-chloroperbenzoic acid (*m*-CPBA, 77%, 26.6 mg, ∼0.119 mmol) in CH_2Cl_2 (1 mL) was added rapidly. This step was carefully monitored by TLC to avoid overoxidation of the double bond. After oxidation, saturated solutions of $NaHCO_3$ (10 mL) and Na_2SO_3 (10 mL) were added. After being stirred for 10 min at room temperature, the aqueous layer was extracted with ethyl acetate (3 × 20 mL) and the combined organic extracts were washed with saturated $NaHCO_3$ (10 mL), dried (Na_2SO_4), filtered, and evaporated in vacuo. The residue was purified by column chromatography on silica gel with a step gradient from hexane/EtOAc, 1/1 to $CHCl_3/CH_3OH$, 10/1 to give **12** (18.5 mg, 97%) as an oil: ^{1}H NMR ($CDCl_3$) δ 8.37 (br s, 1 H, NH), 7.19 (d, J = 1.1 Hz, 1 H, H-6), 6.40 (m, 1 H, H-4′), 5.53 (m, 1 H, H-2′), 5.39 (m, 1 H, H-3′), 4.63 (dd, J = 11.5, 5.8, Hz, 1 H, OC*H*H), 3.69 (dd, J = 11.5, 6.6 Hz, 1 H, OCH*H*), 1.96 (d, J = 1.1 Hz, 3 H, C*H*$_3$), 1.74 (dd, J = 8.3, 4.4 Hz, 1 H, H-1′), 1.49 (s, 18 H, *t*-Bu), 1.23 (dd, J = 8.8, 4.7 Hz, 1 H, H-6′a), 0.59 (dd, J = 4.7, 4.4 Hz, 1 H, H-6′b). FABMS (negative-ion) 425 $[M^{2-} + H^+]^-$.

(1′*S*,2′*S*,5′*R*)-5-Methyl-1-{5-[(phosphoryloxy)methyl]-bicyclo[3.1.0]hex-3-en-2-yl}-1,3-dihydropyrimidine-2,4-dione (13). Compound **12** (18 mg, 0.042 mmol) was dissolved in a 5% solution of trifluoroacetic acid (TFA) in CH_2Cl_2 (3 mL). The solution was stirred at room temperature for 30 min, and the volatiles were removed in vacuo. The residue was dissolved in CH_2Cl_2 (3 mL) and evaporated four times to remove traces of TFA. The crude product **18** (13.5 mg) was used in the next step without further purification: ^{1}H NMR: (D_2O) δ 7.35 (m, 1 H), 6.52 (m, 1 H), 4.65−4.92 (m, 2 H), 4.53 (dd, J = 11.3, 6.0, 11.3 Hz, 1 H), 3.84 (dd, J = 11.3, 6.6 Hz, 1 H), 1.86 (d, J = 1.1 Hz, 3 H), 1.81−1.89 (m, 1 H), 1.29 (dd, J = 8.5, 4.7, Hz, 1 H), 0.62 (t, J = 4.7 Hz, 1 H). FABMS (negative-ion) 313 $[M^{2-} + H^+]^-$

(1′*S*,2′*S*,5′*R*)-5-Methyl-1-{5-[(triphosphoryloxy)methyl]-bicyclo[3.1.0]hex-3-en-2-yl}-1,3-dihydropyrimidine-2,4-dione, Tetraamonium Salt (14). A solution of crude **13** (13.5 mg) was dissolved in deionized water (3 mL) and treated with 1 M triethylammonium bicarbonate (0.1 mL). The mixture was lyophilized to give the triethylammonium salt which was dissolved in dimethylformamide (DMF, 2 mL) and treated with 1,1-carbonyldiimidazole (17.4 mg, 0.107 mmol) at room temperature. The resulting mixture was stirred for 4 h at the room temperature, and excess 1,1-carbonyldiimidazole was quenched by the addition of water (1 mL). After being stirred 30 min, all volatiles were removed with the aid of a nitrogen stream. The residue was dried under high vacuum, dissolved in anhydrous DMF (2 mL) and treated with tri-*n*-butylamine (200 uL, 0.84 mmol) and tri-*n*-butylammonium pyrophosphate (35 mg, 0.129 mmol). The reaction mixture was stirred for 4 days at room temperature. DMF was removed by a nitrogen stream, and the residue was purified by an ion-exchange column chromatography using Sephadex-DEAE A-25 resin with a linear gradient (0.01 to 0.5 M) of ammonium bicarbonate as the mobile phase to give **14** (14 mg, 61%): HPLC purity (>98%); ^{1}H NMR: (D_2O) δ 7.39 (m, 1 H), 6.60 (m, 1 H), 5.34−5.46 (m, 2 H), 4.43 (dd, J = 11.3, 6.0 Hz, 1 H), 4.00 (dd, J = 11.3, 6.6 Hz, 1 H), 1.89 (d, J = 1.1 Hz, 3 H), 1.85−1.94 (m, 1 H), 1.32 (dd, J = 8.8, 4.7 Hz, 1 H), 0.60 (dd, J = 4.7, 4.4 Hz, 1 H). ^{31}P NMR (D_2O) δ t (−21.95, −21.79, −21.63), d (−10.93, −10.77), d (−6.02, −5.87).

Single-Crystal X-ray Diffraction Analysis of 2. $C_{12}H_{14}N_2O_3$, FW = 234.25, orthorhombic space group $P2_12_12_1$, a = 5.6026(1), b = 8.0713(1), c = 24.8961(4) Å. V = 1171.03(3) Å^3, Z = 4, ρ_{calcd} = 1.329 mg mm^{-3}, λ(Cu Kα) = 1.54178 Å, μ = 0.801 mm^{-1}, F (000) = 496, T = 293 K. A colorless 0.60 × 0.05 × 0.02 mm crystal was used for data collection with a Bruker SMART[1] 6K CCD detector on a Platform goniometer. The Rigaku rotating Cu anode source was equipped with incident beam Gobel mirrors. Lattice parameters were determined using SAINT[19] from 3914 reflections within $6.8 < 2\theta < 133.8°$. The data collection range had a $\{(\sin\theta)/\lambda\}$max = 0.60. A set of 5983 reflections was collected in the ω scan mode. There

were 2021 unique reflections. Corrections were applied for Lorentz, polarization, and absorption effects. The structure was solved with SHELXTL[20] and refined with the aid of the SHELX97 system of programs. The full-matrix least-squares refinement on F^2 varied 161 parameters: atom coordinates and anisotropic thermal parameters for all non-H atoms. H atoms were included using a riding model, coordinate shifts of C applied to attached H atoms, C−H distances set to 0.96 to 0.93 Å, H angles idealized, U_{iso}(H) were set to 1.2 to 1.5 U_{eq}(C), except for those hydrogens involved in hydrogen bonding whose coordinates were refined. Final residuals were $R1 = 0.035$ for the 1792 observed data with $F_o > 4\sigma(F_o)$ and 0.040 for all data. Final difference Fourier excursions of 0.13 and −0.13 eÅ^{-3}.

Tables of coordinates, bond distances and bond angles, and anisotropic thermal parameters have been deposited with the Crystallographic Data Centre, Cambridge, CB2, and 1EW, England.

Antiviral Assays. Antiviral Activity in Human T-Lymphocyte (CEM) Cells. CEM cells (4.5×10^5 cells/mL) were suspended in fresh culture medium and infected with 100 $CCID_{50}$/mL of cell suspension of HIV-1 or HIV-2 (1 $CCID_{50}$ being the dose infective for 50% of cell cultures). One hundred microliters of the infected cell suspension was transferred to microplate wells, mixed with 100 μL of the appropriate dilutions of the test compounds (final concentrations of 200, 40, 8, 0.32 and 0.062 μg/mL), and further incubated at 37 °C. After 4 to 5 days, syncytia were counted by microscopic observation in CEM cell cultures. The 50% effective concentration (EC_{50}) corresponded to the compound concentration required to prevent syncytium formation by 50% in the virus-infected CEM cell cultures.

Antiviral Activity in MT-2 and MT-4 Cells. MT-4 cells (3×10^4) were exposed to 100 $TCID_{50}$ of HIV-1_{LAI} and were cultured in the presence of each compound. The EC_{50} values were determined using an MTT assay on day 5 of the cultures. All assays were conducted in duplicate. MT-2 cells (2×10^3) were exposed to 100 $TCID_{50}$ of HIV-1_{LAI} and were cultured in the presence of each compound. The EC_{50} values were determined using an MTT assay on day 7 of the cultures. All assays were conducted in duplicate.

Percent Inhibition of p24 Antigen in PHA-PMB Cells. PHA-PBM cells (1×10^6/mL) were exposed to 50 $TCID_{50}$ of HIV-1_{LAI} in the presence or absence of various concentrations of drugs in 10-fold serial dilutions in 96-well microculture plates. All assays were preformed in triplicate. The amounts of p24 antigen produced by the cells was determined after culturing for 7 days using a commercially available radioimmunoassay kit (DuPontyNEN). Drug concentrations that caused a 50% inhibition (EC_{50}) were determined by comparison with the p24 production level in drug-free control cell cultures.

Inhibition of Wild-Type HIV-1 Reverse Transcriptase (RT) by D4TTP and *N*-MCD4TTP. The assay was done in duplicate as previously described.[21] Briefly, for each sample, 0.25 μg of single-stranded M13mp18 DNA (New England Biolabs) was hybridized to 0.5 μL of 1.0 OD_{260}/mL −47 sequencing primer (New England Biolabs). The template-primer was suspended in a solution containing 100.0 μL of 25 mM Tris (pH 8.0), 75 mM KCl, 8.0 mM $MgCl_2$, 100.0 μg/mL bovine serum albumin (BSA), 10.0 mM 3-[(3-cholamidopropyl)dimethylammonio-]-1-propanesulfonate (CHAPS), 2.0 mM dithiothreitol, 10.0 μM each of dATP, dGTP, and dTTP, 5.0 μM dCTP, 2.0 μM [α-^{32}P] dCTP, and the indicated concentration of inhibitor. Extension was initiated by the addition of 1.0 μg of wild-type HIV-1 RT. The mixture was incubated for 30 min at 37 °C, and the reaction was halted by the addition of 3 mL of 10% trichloroacetic acid (TCA). Precipitated DNA was collected by suction filtration through Whatman GF/C glass filters. The amount of incorporated radioactivity was determined by liquid scintillation counting. The amount of incorporated radioactivity in the absence of inhibitor was considered 100% activity. The amount of radioactivity incorporated in the presence of inhibitor was normalized to this value.

References

(1) Cheer, S. M.; Goa, K. L. Stavudine once daily. *Drugs* **2002**, *62*, 2667−2674.

(2) Marquez, V. E.; Siddiqui, M. A.; Ezzitouni, A.; Russ, P. L.; Wang, J.; Wagner, R. W.; Matteucci, M. D. Nucleosides with a twist. Can fixed forms of sugar ring pucker influence biological activity in nucleosides and oligonucleotides? *J. Med. Chem.* **1996**, *39*, 3739−3747.

(3) Marquez, V. E.; Ezzitouni, A.; Siddiqui, M. A.; Russ, P. L.; Ikeda, H.; George, C. Conformational analysis of nucleosides constructed on a bicyclo[3.1.0]hexane template. Structure-antiviral activity for the northern and southern hemispheres of the pseudorotational cycle. *Nucleosides Nucleotides* **1997**, *16*, 1431−1434.

(4) Marquez, V. E.; Ezzitouni, A.; Russ, P.; Siddiqui, M. A.; Ford, H., Jr.; Feldman, R. J.; Mitsuya, H.; George, C.; Barchi, J. J., Jr. HIV-1 reverse transcriptase can discriminate between two conformationally locked carbocyclic AZT-triphosphate analogues. *J. Am. Chem. Soc.* **1998**, *120*, 2780−2789.

(5) Marquez, V. E.; Russ, P.; Alons, R.; Siddiqui, M. A.; Hernandez, S.; George, C.; Nicklaus, M. C.; Dai, F.; Ford, H., Jr. Synthesis of conformationally restricted carbocyclic nucleosides. The role of the O4′-oxygen in the key hydration step of adenosine deaminase. *Helv. Chim. Acta* **1999**, *82*, 2119−2129.

(6) Jacobson, K. A.; Ji, X.; Li, A.; Melman, N.; Siddiqui, M. A.; Shin, K. J.; Marquez, V. E.; Ravi, R. G. Methanocarba analogues of purine nucleosides as potent and selective adenosine receptor agonists. *J. Med. Chem.* **2000**, *43*, 2196−2203.

(7) Wang, P.; Nicklaus, M. C.; Marquez, V. E.; Brank, A. S.; Christman, J. K.; Banavali, N. K.; MacKerell, A. D., Jr. Use of oligodeoxyribonucleotides with conformationally constrained abasic sugar targets to probe the mechanism of base flipping by HhaI DNA (Cytosine C5)-methyltransferase. *J. Am. Chem. Soc.* **2000**, *122*, 12422−12434.

(8) Kim, H. S.; Ravi, R. G.; Marquez, V. E.; Maddileti, S.; Wihlborg, A.-K.; Erlinge, D.; Malsmjo, M.; Boyer, J. L.; Harden, T. K.; Jacobson, K. A. Methanocarba modifications of uracil and adenine nucleotides: High potency of northern ring conformation at $P2Y_1$, $P2Y_2$, $P2Y_4$, and $P2Y_{11}$ but not $P2Y_6$ receptors. *J. Med. Chem.* **2002**, *45*, 208−218.

(9) Zalah, L.; Huleihel, M.; Manor, E.; Konson, A.; Ford, H. Jr.; Marquez, V. E.; Johns, D. G.; Agbaria, R. Metabolic pathways of N-methanocarbathymidine, a novel antiviral agent in native and herpes simplex virus-infected Vero cells. *Antiviral Res.* **2002**, *55*, 63−75.

(10) Yu, D.; d′Alarcano, M. Synthesis of 3′-cyano-2′,3′-dideoxyadenosine and 2′,3′-dideoxy-3′-formyladenosine. *J. Org. Chem.* **1989**, *54*, 3240−3442.

(11) Smith, M. B.; March, J. *March's Advanced Organic Chemistry. Reactions, Mechanisms, and Structure*; John Wiley & Sons: New York, 2001, pp 1308−1309.

(12) Rodriguez, J. B.; Marquez, V. E.; Nicklaus, M. C.; Mitsuya, H.; Barchi, J. J., Jr. Conformationally locked nucleoside analogues. Synthesis of dideoxycarbocyclic nucleoside analogues structurally related to neplanocin C. *J. Med. Chem.* **1994**, *37*, 3389−3399.

(13) Kim, H. S.; Ravi, R. G.; Marquez, V. E.; Maddileti, S.; Wihlborg, A.-K.; Erlinge, D.; Malsmjo, M.; Boyer, J. L.; Harden, T. K.; Jacobson, K. A. Methanocarba modifications of uracil and adenine nucleotides: High potency of northern ring conformation at $P2Y_1$, $P2Y_2$, $P2Y_4$, and $P2Y_{11}$ but not $P2Y_6$ receptors. *J. Med. Chem.* **2002**, *45*, 208−218.

(14) Viterbo, D.; Milanesio, M.; Pomes-Hernandez, R.; Rodriguez-Tanty, C.; Colas-Gonzalez, I.; Sablon-Carrazana, M.; Duque-Rodriguez, *J. Acta Crystallogr., Sect. C* **2000**, *56*, 580.

(15) Zalah, L.; Huleihel, M.; Manor, E.; Konson, A.; Ford, H. Jr.; Marquez, V. E.; Johns, D. G.; Agbaria, R. Metabolic pathways of N-methanocarbathymidine, a novel antiviral agent in native and herpes simplex virus-infected Vero cells. *Antiviral Res.* **2002**, *55*, 63−75.

(16) Prota, A.; Vogt, J.; Perozzo, R.; Pilger, B.; Wurth, C.; Marquez, V. E.; Russ, P.; Schultz, G. E.; Folkers, G.; Scapozza, L. Kinetics and high resolution structure of HSV1 TK and the engineered Y101F mutant in complex with an antiviral drug with conformationally restricted sugar ring pucker. *Biochemistry* **2000**, *39*, 9597−9603.

(17) Balzarini, J.; Kang, G.-J.; Dalal, M.; Herdewijn, P.; DeClercq, E.; Broder, S. Johns, D. G. The anti-HLTV-III (anti-HIV) and cytotoxic activity of 2′, 3′-didehydro-2′,3′-dideoxyribonucleosides: A comparison with their parental 2′,3′-dideoxyribonucleosides. *Mol. Pharmacol.* **1987**, *32*, 162−167.

(18) Schinazi, R. F.; Peck, A.; Sommadossi, J. P.; Substrate specificity of *Escherichia coli* thymidine phosphorylase for pyrimidine

nucleosides with anti-human immunodeficiency virus activity. *Biochem. Pharmacol.* **1992**, *44*, 199−204.
(19) Bruker 1995 SMART and SAINT Data*Collection and Reduction Software for the SMART system*. Bruker-AXS, Madison, WI.
(20) Sheldrick, G. M. 1997. *SHELXTL* Version 5.1 Bruker Analytical X-ray Instruments, Madison, WI.
(21) Boyer, P. L.; Gao, H.-Q.; Clark, P. K.; Sarafianos, S. G.; Arnold, E.; Hughes, S. H. YADD mutants of human immunodeficiency virus type 1 and Moloney murine leukemia virus reverse transcriptase are resistant to lamivudine triphosphate (3TCTP) in vitro. *J. Virol.* **2001**, *75*, 6321−6328.

JM030116G