A Ring-Enlarged Oxetanocin A Analogue as an Inhibitor of HIV Infectivity

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Two ring-expanded analogues (compounds **2** and **3**) of the anti-HIV fermentation product oxetanocin A (**1**) were synthesized from commercially available diacetone D-glucose. Antiviral testing against HIV in ATH8 cells revealed that the ring-expanded analogue **2** possessed a similar activity profile as oxetanocin A. Neither compound, however, was capable of providing full protection to the cells against HIV infection. The isomeric ring-expanded analogue **3** was totally devoid of anti-HIV activity. Molecular modeling suggested that while oxetanocin A and compounds **2** and **3** share a large common substructure with the potent anti-HIV drug, dideoxyadenosine (ddA), the extra hydroxymethyl substituent may contribute negatively to the binding of these molecules to a critical enzyme. The negative contribution may be less important in oxetanocin and isomer **2** than in isomer **3**. From these studies it would appear that both oxetane and tetrahydrofuran rings are equivalent templates to support the adenine base in terms of anti-HIV activity.

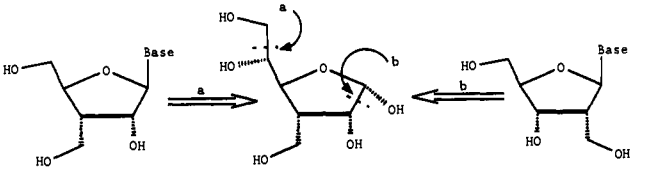
Oxetanocin A (**1**), a naturally occurring nucleoside recently isolated from the culture filtrates of *Bacillus megaterium*, has shown promising in vitro inhibitory activity against the human immunodeficiency virus (HIV),[1-3] the causative agent of AIDS. Although structurally distinct from the dideoxynucleosides known to be active against HIV,[4] it shares a common mechanism of action with these compounds due to the observed inhibition of HIV reverse transcriptase (RT) by its triphosphate anabolite.[5]

From the standpoint of structure vs activity, we were interested in ascertaining whether or not the presence of the four-membered oxetane ring constituted an absolute requirement for anti-HIV activity in this novel group of nucleosides. Oxetanocin A, as well as similar oxetanosyl N-glycosides with different base moieties, have also been reported to have important antiviral activities against HIV and other types of viruses.[3b,6] For example, the guanosine analogue (oxetanocin G) was found to be equivalent to 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (DHPG) against human cytomegalovirus (HCMV) in vitro.[7]

Several oxetanocin A analogues in which the 2′-substituent on the oxetane ring was modified or deleted have shown better anti-HIV activities than oxetanocin A itself,[5] and the replacement even of the oxetane ring by the isosteric cyclobutane ring produces carbocyclic analogues with anti-HIV activities comparable or superior to those of the parent compounds.[8] Moreover, the carbocyclic analogue of oxetanocin G, although comparable to acyclovir against the herpes viruses HSV-1 and HSV-2 in vitro, was clearly superior to this drug against the thymidine kinase deficient strains of the same viruses.[9,10]

In the present work we wanted to assess the importance of ring size in relation to anti-HIV activity by comparing oxetanocin A with the two isomeric ring-expanded analogues **2** and **3**. The molecular similarities between these compounds and oxetanocin A are illustrated by the dotted lines in structures **2** and **3**. These compounds can also be regarded as hydroxymethyl-substituted 2′,3′-dideoxyadenosines (hydroxymethyl-ddA's). The motivation for this work was twofold. First, the tetrahydrofuran ring is already known to be a very effective template for RT, as demonstrated by the excellent anti-HIV activity of various dideoxynucleosides.[4] Second, the furanose compounds ought to be synthetically more accessible than oxetane nucleosides and thus easier to modify in future structure–activity studies if activity were realized. As additional rationale, nucleosides containing a structurally related glycon, [2,3-dideoxy-3-(hydroxymethyl)-D-*erythro*-pentofuranoside], have been previously recognized as agents capable of selective incorporation at the terminal position of DNA.[11]

Scheme I



Chemistry

Retrosynthetically, the glycon portion of both target compounds could be derived from a common intermediate, such as the 3-deoxy-3-(hydroxymethyl)-D-allofuranose, through the selective cleavage of either vicinal diol functionality (Scheme I). This compound, protected as the 1,2:5,6-diisopropylidene derivative (**4**),[11-13] was obtained

† Laboratory of Medicinal Chemistry.
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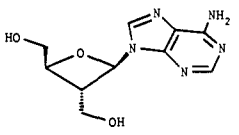
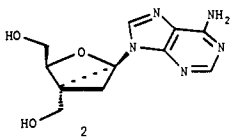
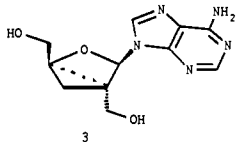


1 (Oxetanocin A)



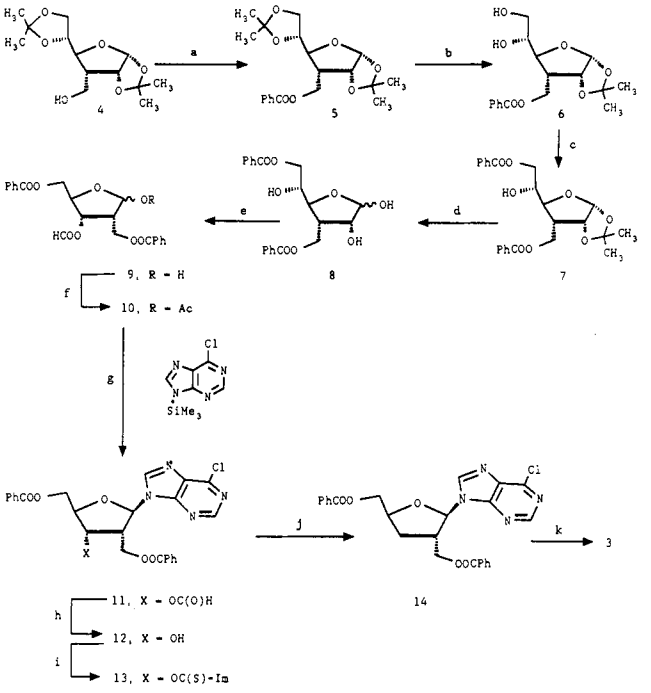
2



3

in three steps from commercially available 1,2:5,6-diisopropylidene-D-glucose. In a recently reported synthesis of oxetanocin A, the same allofuranose **4** was also employed as a synthetic precursor.[14] From this key intermediate, 2′-hydroxymethyl-ddA (**3**) was prepared as depicted in Scheme II. The introduction of a benzoyl protecting group at the primary alcohol function in **4**, followed by the selective removal of the 5,6-isopropylidene moiety, afforded intermediate diol **6**. The selective introduction of a second benzoyl moiety at the newly unmasked primary alcohol function in **6** gave the diester **7**, which was subsequently treated with BCl_3 to remove the remaining 1,2-isopropylidene group. This operation also generated the required vicinal diol functionality in **8** whose C-1 carbon was subsequently excised through the oxidative action of potassium periodate. Under such reactions conditions, the transiently generated aldehyde cyclized efficiently to the target sugar **9**. This compound was converted to the 1-*O*-acetate ester (**10**, mixture of anomers) which was coupled with silylated 6-chloropurine under Lewis acid catalysis[15] to give the desired β-N-9 isomer **11** in 66% yield. Only very small amounts of other isomers were detected (see Experimental Section). It is possible that the benzoate ester tethered to C-2 in structure **10** is capable of directing a preferred β-face attack.[16] This anchimeric assistance, however, appears to be less efficient than the one obtained through the participation of an ester function directly attached to C-2 (vide infra). Selective removal of the formate ester in **11** gave compound **12**. Deoxygenation of the secondary alcohol in **12**, following a typical two-step deoxygenation protocol,[17,18] afforded compound **14**. Compound **14** was converted to isomer **3** after treatment with hot saturated methanolic ammonia under pressure. The attachment of the sugar to the purine ring at N-9, as well as the desired β-stereochemistry of the adenine ring in compound **3**, were confirmed by the UV (λ_{max} 260 nm) and

Scheme II[a]



[a] (a) PhC(O)Cl, pyridine, 0 °C; (b) 0.06 N HCl/MeOH, 55 °C; (c) PhC(O)Cl, pyridine, −15 °C; (d) 1 M BCl_3, CH_2Cl_2; (e) KIO_4, $EtOH/H_2O$; (f) Ac_2O, pyridine, room temperature; (g) TMSOTf, CH_3CN; (h) concentrated NH_4OH, room temperature; (i) $(Im)_2C{=}S$, CH_2ClCH_2Cl, 70 °C; (j) *n*-Bu_3SnH, AIBN, toluene, Δ; (k) concentrated NH_3/MeOH, 100 °C, pressure bomb.

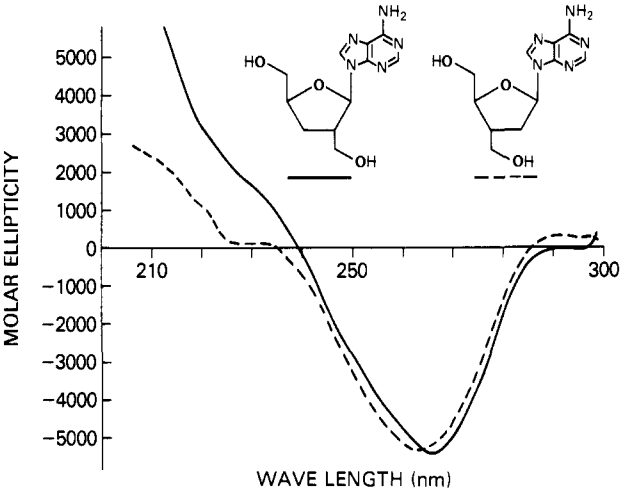


Figure 1. CD spectra of compounds **2** and **3** in methanol.

CD spectra (negative Cotton effect)[19] shown in Figure 1.

For the synthesis of 3′-hydroxymethyl-ddA (isomer **2**), intermediate **6** (Scheme II) was utilized as shown in Scheme III. Periodate oxidation of the vicinal diol in **6**, followed by in situ borohydride reduction of the generated aldehyde, afforded compound **15**. Protection of this molecule as the dibenzoate ester (compound **16**) was followed by removal of the isopropylidene group to give compound **17**. Compound **17** was readily converted to the diacetate **18**, which was used in the Lewis acid catalyzed coupling reaction with silylated 6-chloropurine.[15] The expected more efficient participation of the 2′-*O*-ester

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(15) Vorbruggen, H.; Krolikiewicz, K.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1234.
(16) The *O*-benzoate group branching out of C-2 in compound **10** is likely to control the stereochemistry via a cyclic six-membered 1,3 ortho ester ion. This is quite different from the results obtained in the coupling of 1-*O*-acetyl-D-oxetanose (1-*O*-acetyl-2-deoxy-2-(hydroxymethyl)-D-erythrooxetanose) with silylated adenine, where the α-anomer of oxetanocin A was the only product formed (see refs 14 and 27). In this case, participation of the *O*-benzoate group that branches out of C-4 was postulated to be the controlling factor in the stereochemistry. According to our results, a similar participation in **10** by the *O*-benzoate group that branches out of C-4 was not favored. These phenomena could be acounted for by differences in the thermodynamic stabilities of the intermediate bicyclic systems that are formed as transition states.
(17) Barton, D. H. R.; McCombie, S. W. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1574.
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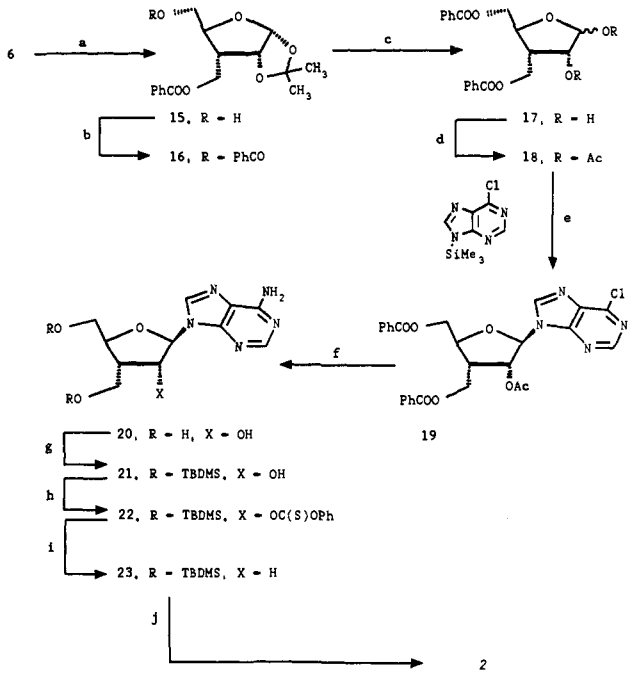
Scheme III[a]



[a] (a) i. $NaIO_4$, $EtOH/H_2O$. ii. $NaBH_4$; (b) PhC(O)Cl, pyridine, 0 °C; (c) Bio-Rad AG 50W-X8 (H^+), dioxane/H_2O; (d) Ac_2O, pyridine, room temperature; (e) TMSOTf, CH_3CN; (f) concentrated NH_3/MeOH, 100 °C, pressure bomb; (g) t-Bu$(Me)_2$SiCl, imidazole/DMF; (h) ClC(S)OPh, DMAP/CH_3CN; (i) n-Bu_3SnH, AIBN, toluene, Δ; (j) Bu_4NF/THF, room temperature.

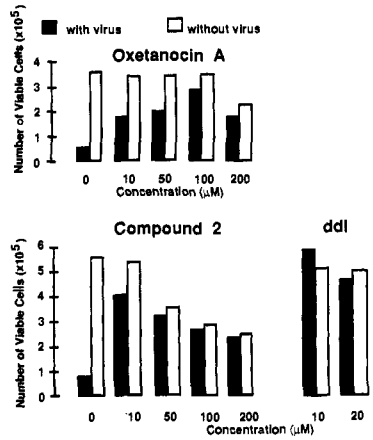


Figure 2. Protection of ATH8 cells from the cytopathogenic effects of HIV-1. Open bars are proportional to the number of viable cells on days 5–7 in the presence of the indicated concentration of drugs (toxicity control). The solid bars measure the same parameter when HIV-1 was incubated with the cells in the presence of drugs.

function in compound **18**, in directing the attack of the base from the β-side, was evident from the exclusive isolation (91% yield) of the desired β-N-9 isomer (compound **19**). Simultaneous removal of all three protective functions and conversion of the 6-chloropurine ring to adenine was accomplished through the action of hot concentrated methanolic ammonia under pressure. The two primary alcohol functions in the resulting nucleoside **20** were selectively protected as the corresponding *tert*-butyldimethylsilyl ethers, and the secondary alcohol was removed following a conventional two-step deoxygenation procedure,[20] giving compound **23**. Removal of the silyl ether functions in **23** afforded the second target isomer **2**. As

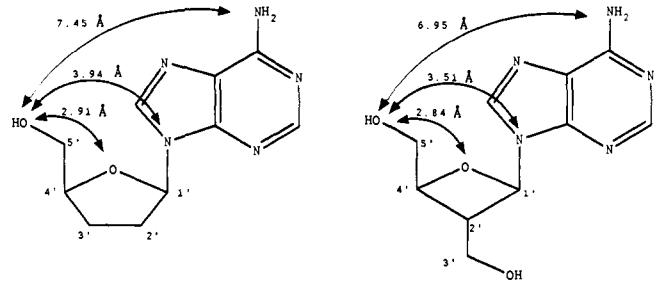


Figure 3. Important molecular dimensions in 2′,3′-dideoxyadenosine (ddA) and oxetanocin A.

before, the correct mode of attachment of the base was confirmed by the UV (λ_{max} 260 nm) and CD spectra (Figure 1).

Biological Results

A comparison of the protection afforded to HIV-infected ATH8 cells by oxetanocin A, the synthetic analogue **2**, and 2′,3′-dideoxyadenosine (ddI) is shown in Figure 2. Compound **2** and ddI were tested in the same experiment; however, since additional samples of oxetanocin A were unavailable, the data given for oxetanocin A correspond to an earlier test. It is our standard practice to use ddI as positive control since ddI was the drug of choice for clinical trials and it is also pharmacologically equivalent to ddA.[4] Although the first two compounds afforded significant protection from the cytopathogenic effects of HIV-1, neither achieved complete protection without toxicity. While oxetanocin A was less toxic and more protective at 100 μM, **2** appeared to be somewhat superior at 10 μM. The isomeric compound **3** was without activity as was compound **20**, the 2′-hydroxy analogue of compound **2**.

Molecular Modeling

The furanose ring in nucleosides and their analogues serves as a framework, or scaffolding, which properly maintains the position of the 5′-hydroxyl group with respect to the purine or pyrimidine ring system and so offers a substrate which is a geometrically good fit in the active site(s). A recent study, in which the solid-state conformation of nucleosides was correlated with their anti-HIV activity, concluded that compounds in which the 5′-hydroxyl group is at or near the correct position with respect to the base can function as adequate substrates for either the appropriate kinases or other important target enzymes.[21]

One of the simplest compounds that is known to possess anti-HIV activity is 2′,3′-dideoxyadenosine (ddA).[4] On the assumption that ddA does in fact have the appropriate geometry, its structure may be used as a reference template for modeling. This compound was modeled with use of Quanta (Polygen Corp.), and the structure with minimal potential energy was constructed. In the model, the torsion angle χ was +158.7°, which differs significantly from the value of +264.1° measured by X-ray diffraction.[22] This difference in χ corresponds to a small energy difference (less than 4 kcal), and in general, rotation of the purine ring about the glycosylic bond appears to be relatively free of hindrance. The furanose ring in ddA is puckered in a C-3′-exo conformation and this is reflected by the dimensions of the interatomic distances (in angstroms) shown in Figure 3.

(20) Robins, M. J.; Wilson, J. S.; Hansske, F. *J. Am. Chem. Soc.* **1983**, *105*, 4059.

(21) Van Roey, P.; Salerno, J. M.; Chu, C. K.; Schinazi, R. F. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 3929.

(22) Silverton, J. V.; Quinn, F. R.; Haugwitz, R. D.; Todaro, L. J. *Acta Crystallogr.* **1988**, *C44*, 321.

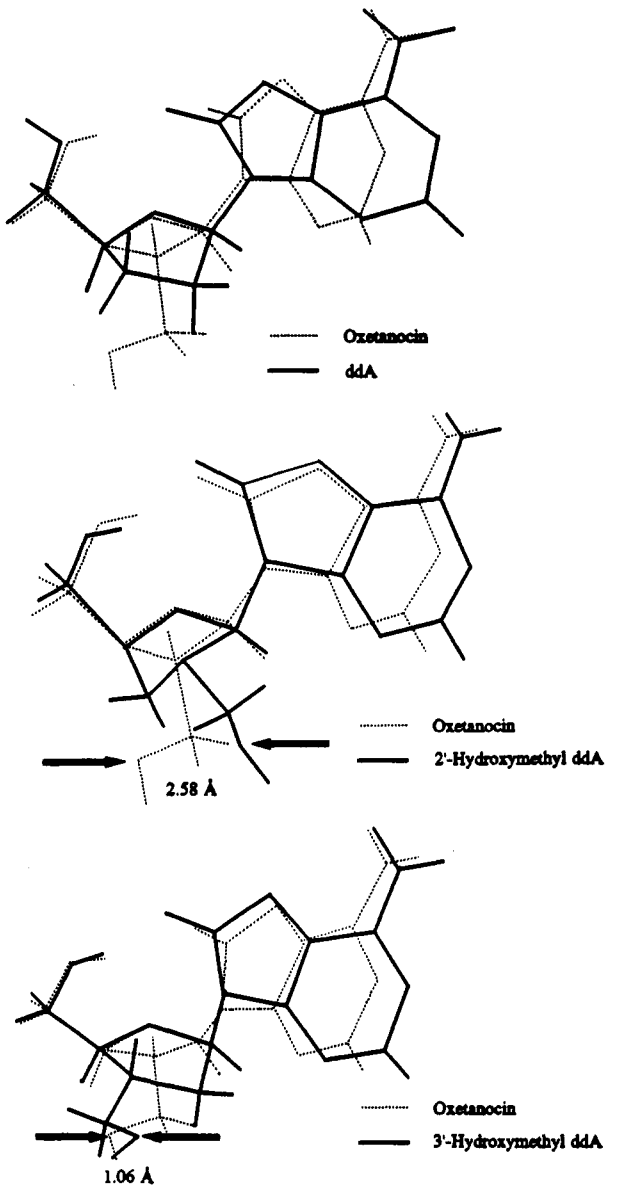


Figure 4. Superposition of oxetanocin A on 2',3'-dideoxyadenosine (ddA) and the two isomeric hydroxymethyl ddA analogues **2** and **3**. The distances (in Å) between the oxygen atoms on the extra hydroxymethyl side chain in **2** and **3** with respect to the equivalent oxygen atom in oxetanocin A are indicated.

A model of oxetanocin A (**1**) was also constructed and the conformation having the minimum potential energy was examined. In this molecule, the torsion angle χ was −169.3° which again was different from the value of −108,.3° measured by X-ray diffraction.[2] The equivalent interatomic distances for oxetanocin A are also shown in Figure 3.

When the geometric fit between ddA and oxetanocin A was examined, using as reference atoms the two purine nitrogens N-9 and N-6, the ring oxygens, and the oxygens of the hydroxymethyl chain at C-4', the root mean square (RMS) distance for this four-point fit was 0.221 Å. This shows the two molecules to have a large common substructure with essentially the same geometry and as a result, these two molecules can be largely superimposed, as shown in Figure 4.

The observation that both oxetanocin A and ddA have anti-HIV activity is consistent with the data derived from the models. The superior biological activity of ddA with respect to oxetanocin A suggests that although the upper part of oxetanocin A can lodge adequately into the binding site(s) used by ddA, the additional hydroxymethyl side chain of oxetanocin A may work against efficient binding to the site.

Table I. Interatomic Distances in Oxetanocin A and Related Compounds

compound	$O_{5'}$–$O_{4'}$, Å	$O_{5'}$–N_9, Å	$O_{5'}$–N_6, Å	χ, deg	fit to oxetanocin (RMS)
oxetanocin	2.84	3.51	6.95	−169.3	
3'-hydroxymethyl ddA	2.87	3.66	6.96	−179.5	0.069
2'-hydroxymethyl ddA	2.77	3.82	7.72	+160.5	0.353
ddA	2.91	3.94	7.45	+158.7	0.221

When the minimum energy conformational models of compounds **2** and **3** were examined, differences in the preferred mode of puckering of the sugar ring were observed. The sugar ring in 2'-hydroxymethyl-ddA (**3**) has a C-3'-exo conformation (south $^2T_3$), while that of 3'-hydroxymethyl-ddA (**2**) adopts the C-3'-endo conformation (north $^3T_2$). The preferred north $^3T_2$ for **2** is identical with that postulated[23] for the related nucleoside 9-(3'-*C*-methyl-β-D-xylofuranosyl)adenine, according to NMR studies conducted at variable temperatures. The geometric data given in Table I show that, for compounds **2** and **3**, the juxtaposition of the 5'-hydroxyl oxygens, the ring oxygens, and N-6 and N-9 gives, for each compound, a good fit to oxetanocin A. This is illustrated in Figure 4. The RMS for the fit of **2** to oxetanocin A (0.069 Å), compared to the corresponding value for compound **3** (0.353 Å), shows compound **2** to be a better fit to oxetanocin A.

The major difference between oxetanocin A and either of the hydroxymethyl-ddA isomers is in the disposition of the second hydroxymethyl group. In oxetanocin A, this side chain presumably does not intefere severely with the interaction of the compound with the ddA binding sites(s) and reasonable anti-HIV activity is observed. The models of **2** and **3** on the other hand show that the second hydroxymethyl side chain occupies extreme locations displaced (1 and 2.5 Å, respectively) to opposite sides of the position adopted by the hydroxymethyl side chain of oxetanocin A as can be seen in Figure 4. This, coupled with the biological data, suggests that in 3'-hydroxymethyl ddA (**2**) this side chain is in a position which does not interfere severely with the interaction at the ddA binding site(s). In the 2'-hydroxymethyl isomer (**3**), however, the hydroxymethyl group lies in a cleft between the two ring systems. This cleft, in the active compounds ddA, oxetanocin A, and 3'-hydroxymethyl-ddA, is empty, and it may well be a requirement of the active site(s) that it remain empty.[24]

The discovery of the anti-HIV activity of compound **2**, which appears to be pharmacologically similar to oxetanocin A, provides the basis for a new structure–activity study which should be guided by the results obtained here from the modeling experiments. The apparent equivalence of the tetrahydrofuran ring to the oxetane ring shall permit the use of chemistry that is simpler than the one employed in the syntheses of oxetanocin A.[14,25–29]

Experimental Section

General. All chemical reagents were commercially available. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Normal-phase column chromatography was performed on silica gel (silica gel 60, 230–400 mesh, E. Merck) and analytical TLC was performed on Analtech Uniplates silica gel GF with the solvents indicated for the individual experiments. The purity of the final target compounds

(23) Koole, L. H.; Buck, H. M.; Vial, J. M.; Chattopadhyaya, J. *Acta Chem. Scand.* **1989**, *43*, 665.

was evaluated by HPLC using an Altex column (5 μ ODS, 250 × 4.6 mm) with 15% MeOH in 0.1 M ammonium formate buffer (pH 5) as the mobile phase. The flow rate was 1 mL/min and the detector wavelength was set at 278 nm. Proton NMR spectra were recorded on a Varian XL-200 instrument. Proton chemical shifts are expressed as δ values with reference to Me_4Si. UV spectra were recorded in a Beckman Model 34 spectrophotometer. Circular dichroism spectra for compounds **2** and **3** were recorded as methanolic solutions (0.075 M) from 300 to 200 nm on a JASCO J500 spectropolarimeter in 1-cm cells at room temperature. Molar ellipticities were calculated according to the formula $100 \times \theta/[M] \times l$. Positive-ion fast atom bombardment (FAB) mass spectra were obtained on a VG 7070E mass spectrometer. Samples were dissolved in a glycerol matrix, and ionization was effected by a beam of xenon atoms derived by neutralizing xenon ions accelerated through 8.6 kV. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta GA, or by Galbraith Laboratories, Inc., Knoxville, TN.

3-Deoxy-3-(hydroxymethyl)-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (4). This compound was prepared in 64% yield following the published procedure of Engel et al.[13]

3-Deoxy-3-[(benzoyloxy)methyl]-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (5). A solution of **4** (1.45 g, 5.29 mmol) in anhydrous pyridine (20 mL) was cooled over ice and treated with benzoyl chloride (0.74 mL, 6.38 mmol) while the solution was magnetically stirred. After 2 min, pyridine hydrochloride began to precipitate from the reaction. The reaction was quenched with ethanol (5 mL) after TLC analysis (silica gel, EtOAc–hexane, 1:1) indicated that complete disappearance of starting materials (R_f = 0.36) and formation of the product (R_f = 0.66) had taken place. After removal of the solvents under vacuum, the residue was redissolved in EtOAc and chromatographed over silica gel (hexane–EtOAc, 3:2) to give 1.91 g (95%) of **5** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.32 (s, 6 H, 2 CH_3), 1.42 (s, 3 H, CH_3), 1.52 (s, 3 H, CH_3), 2.43 (m, 1 H, H-3), 3.80–3.97 (m, 2 H, CH_2OBz), 4.00–4.12 (m, 2 H, H-$6_{a,b}$), 4.48 (t, J = 10.0 Hz 1 H, H-4), 4.77 (dd, J = 10.0 Hz, J' = 5.0 Hz, H-5), 4.80 (t, J = 4.0 Hz, 1 H, H-2), 5.81 (d, J = 4.0 Hz, 1 H, H-1), 7.48 and 8.00 (m, 5 H, Ph); IR (neat) 2980, 2830, 1711, 1707, 1600, and 1285 cm^{-1}. Anal. ($C_{20}H_{26}O_7$) C, H.

5-*O*-Benzoyl-2-deoxy-2-[(benzoyloxy)methyl]-3-*O*-formyl-D-ribofuranose (9). Compound **5** (1.9 g, 5 mmol) was treated with a 1:1 mixture (150 mL) of 0.06 N aqueous HCl and methanol at 55 °C. TLC analysis on silica gel (CH_2Cl_2–acetone, 5:1) indicated that the reaction was complete after 1 h. The solution was cooled to room temperature, neutralized with solid $NaHCO_3$, and reduced to dryness under vacuum. The residue was extracted with chloroform (5 × 30 mL) and dried (Na_2SO_4). The combined extract was evaporated to dryness to give 1.47 g of **6** as a colorless oil. This material was immediately dissolved in a mixture of dry pyridine (15 mL) and anhydrous CH_2Cl_2 (15 mL) and cooled to −30 °C, and a solution of benzyol chloride (0.54 mL, 4.67 mmol) in anhydrous methylene chloride (10 mL) was added, dropwise, for a period of 10 min. After being stirred for 1.5 h, the mixture was concentrated to dryness and partitioned between chloroform and water. The organic phase was dried (Na_2SO_4) to give the dibenzoylated compound **7** as an oil. The crude oil was dissolved in methylene chloride (40 mL) and stirred with a 1 M methylene chloride solution (6 mL) of BCl_3 for 2 min. After quenching the reaction with water (10 mL), the organic layer was separated and washed successively with 5% aqueous $NaHCO_3$ solution (2 × 30 mL) and brine. The material was reduced to dryness in the presence of 20 g of silica gel and the resulting solid applied to a silica gel column. Elution of the compound with a 1:1 mixture of acetone and methylene chloride gave 0.95 g of **8** as a colorless oil. This oil was immediately dissolved in a 1:1 mixture (70 mL) of water and ethanol and treated at room temperature with KIO_4 (0.91 g) with stirring for 19 h. The precipitate formed was filtered off through a Celite pad and washed with additional ethanol. The combined filtrate and washings were evaporated to a small volume (15 mL) which was extracted with chloroform, dried (Na_2SO_4), and evaporated to dryness to give 0.85 g (43% from **5**) of **9** as a white solid. An analytical sample was obtained by recrystallization from EtOAc–hexane: mp 137–138 °C. Anal. ($C_{21}H_{20}O_8$) C, H.

5-*O*-Benzoyl-2-deoxy-2-[(benzoyloxy)methyl]-3-*O*-formyl-1-*O*-acetyl-D-ribofuranose (10). A solution of **9** (0.62 g, 1.55 mmol) in dry pyridine (16 mL) was treated with acetic anhydride (200 μL, 2.1 mmol) and the resultant mixture stirred at room temperature until the reaction was judged complete (20 h) by TLC (silica gel, hexane–EtOAc, 3:2). The pyridine was azeotropically removed under vacuum in the presence of methanol. The residue was then dissolved in EtOAc and washed with water and brine and concentrated at reduced pressure. After silica gel chromatography (hexane–EtOAc, 1:1), 0.47 g (70%) of **10** was obtained as a brownish syrup. ^{1}H NMR analysis indicated that the compound was obtained as a mixture of β-OAc/α-OAc anomers: δ 1.97 and 2.00 (s, 3 H, CH_3COO), 3.20 (m, 1 H, H-2), 4.60 (m, 5 H, H-4, 2 CH_2OBz), 5.78 (m, 1 H, H-3), 6.40 and 6.60 (doublets, J = 3.0 and J = 4.8 Hz, respectively, 1 H, H-1), 7.25 and 8.05 (m, 10 H, Ph). This compound was used without any further purification in the next step.

6-Chloro-9-[5-*O*-benzoyl-2-deoxy-2-[(benzoyloxy)methyl]-3-*O*-formyl-β-D-ribofuranosyl]-9*H*-purine (11). 6-Chloropurine (0.202 g, 1.3 mmol) was silylated with hexamethyldisilazane under reflux in the presence of a catalytic amount (ca. 0.005 g) of ammonium sulfate. After 30 min, the homogeneous solution was cooled and evaporated to dryness. A solution of the sugar **10** (0.47 g, 1.06 mmol) in dry acetonitrile was added to the solid silylated 6-chloropurine, followed by the addition of trimethylsilyl triflate (250 μL, ca. 1.3 mmol). The resultant mixture was stirred at room temperature for 20 min, concentrated to dryness in the presence of 7 g of silica gel, and loaded into a silica gel column. Elution with a mixture of hexane–EtOAc (3:2) and hexane–EtOAc (1:1) afforded 0.37 g (66%) of the desired product **11** as a white amorphous solid: ^{1}H NMR ($CDCl_3$) δ 4.06 (m, 1 H, H-2′), 4.53–4.82 (m, 5 H, H-4′, 2 CH_2OBz), 5.87 (d, J = 5.8 Hz, 1 H, H-3′), 6.39 (d, J = 8.8 Hz, 1 H, H-1′), 7.30–8.43 (m, 12 H, aromatics). Smaller amounts of the other possible isomers were isolated and tentatively identified as the β-N-7 isomer (H-1′ signal at δ 6.92, J = 9.0 Hz), α-N-9 isomer (H-1′ signal at δ 6.94, J = 7.0 Hz), and α-N-7 isomer (H-1′ signal at δ 7.22, J = 7.2 Hz). The desired compound **11** was used directly in the next step.

6-Chloro-9-[5-*O*-benzoyl-2-deoxy-2-[(benzoyloxy)methyl]-β-D-ribofuranosyl]-9*H*-purine (12). The fully protected nucleoside **11** (0.1 g) was dissolved in absolute ethanol (3 mL) and treated with 30 μL of concentrated ammonium hydroxide at room temperature for 1 h. The precipitate formed was collected (0.078 g, 81%) and dried. The compound was homogeneous on TLC (silica gel, hexane–EtOAc, 1:1) and it was further recrystallized from $CHCl_3$–hexane to give **12** as a white crystalline material: mp 187–188 °C; ^{1}H NMR ($CDCl_3$) δ 3.64 (m, 1 H, H-2′), 4.10 (br s, 1 H, OH), 4.47 (dd, J = 11.7 Hz, J' = 6.7 Hz, 1 H,

(24) One of the reviewers suggested that perhaps it would be more appropriate to use 2′-deoxyadenosine rather than ddA as a reference template. The possibility was raised that the 2′-hydroxymethyl substituent on oxetanocin A would actually mimic the 3′-hydroxyl of the natural nucleoside and that the 3′-hydroxymethyl substituent on ddA (compound **2**) may do the same. According to our modeling studies this appears to be correct. In the preferred south ($^2T_3$) conformation of 2′-deoxyadenosine, the 3′-hydroxyl group is in very close proximity to the hydroxyl group of the extra hydroxymethyl chains of the active compounds oxetanocin A (**1**) and 3′-hydroxymethyl-ddA (**2**). In the case of the inactive isomer, 2′-hydroxymethyl-ddA (**3**), the fit is less perfect, and after attempting to match the 3′-hydroxyl group of 2′-deoxyadenosine to the 2′-hydroxymethyl side chain of **3**, the side chain occupies the same cleft, between the purine and the tetrahydrofuran rings, which we suggest is required to be empty. The conclusion reached with both approaches is essentially the same; however, the recognition of 2′-deoxyadenosine as a good reference compound for oxetanocin A and related ring-enlarged analogues is very important.

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CH*H*OBz), 4.55 (m, 1 H, H-3′), 4.64 (distorted d, 2 H, H-5′$_{a,b}$), 4.76 (dd, J = 5.6 Hz, J' = 2.0 Hz, 1 H, H-4′), 4.92 (dd, J = 11.7 Hz, J' = 6.7 Hz, 1 H, C*H*HOBz), 6.37 (d, J = 8.4 Hz, 1 H, H-1′), 7.72 and 7.97 (m, 10 H, Ph), 8.32 (s, 1 H, H-2), 8.52 (s, 1 H, H-8). Anal. ($C_{25}H_{21}ClN_4O_6$) C, H, Cl, N.

6-Amino-9-[2,3-dideoxy-2-(hydroxymethyl)-β-D-ribofuranosyl]-9*H*-purine (3). Compound **12** (0.051 g, 0.1 mmol) was suspended in anhydrous 1,2-dichloroethane (7 mL) and stirred at 50 °C until the mixture was homogeneous. After the temperature was increased to 60 °C, 1,1′-thiocarbonyldiimidazole (0.027 g, 0.15 mmol) was added and stirring continued for 3 h. Residual starting material was detected by TLC (silica gel, hexane–EtOAc, 1:1) and the reaction was continued with an additional amount of 1,1′-thiocarbonyldiimidazole (0.035 g), for an extended reaction time (12 h) at a higher temperature (70 °C), to achieve completion. The reaction mixture was then poured on top of a silica gel column and the product eluted with hexane–EtOAc (1:1) to give **13** (0.049 g, 80%) as a syrup. This compound was immediately dissolved in anhydrous toluene (2 mL) together with azobis(isobutyronitrile) (AIBN, 0.010 g, 0.06 mmol) and the resultant solution was then immersed in an oil bath previously heated to 85 °C. Tri-*n*-butyltin hydride (45 μL, 0.17 mmol) was added to the reaction mixture and the reaction was judged complete after 5 min by TLC (silica gel, hexane–EtOAc, 1:1). After preparative TLC purification with the same solvent system, compound **14** (0.028 g, 72%) was obtained as a syrup: ^{1}H NMR ($CDCl_3$) δ 2.29 (m, 1 H, H-3′$_a$), 2.56 (m, 1 H, H-3′$_b$), 3.59 (m, 1 H, H-2′), 4.45–4.75 (m, 5 H, H-4′, 2 C*H*$_2$OBz), 6.24 (d, J = 4.8 Hz, 1 H, H-1′), 7.33–8.00 (m, 10 H, Ph), 8.33 (s, 1 H, H-2), 8.56 (s, 1 H, H-8). Compound **14** was then heated in a steel bomb under pressure for 2 days in the presence of concentrated methanolic ammonia. The product obtained was purified successively by silica gel column chromatography (silica gel, MeOH–EtOAc, gradient from 5% to 20% MeOH) and by reversed-phase column chromatography (J. T. Baker octadecyl 6-mL high-capacity disposable column, 15% aqueous methanol as eluant). The fractions collected were monitored by UV at 254 nm, and the compound collected under one peak was lyophilized to give 0.008 g (52%) of **3** as a white fluffy powder (99.04% pure by HPLC, k' = 4.27): UV (MeOH) λ_{max} 260 (log ε 4.08); ^{1}H NMR (D_2O) δ 2.15 (m, 2 H, H-3′$_{a,b}$), 2.99 (m, 1 H, H-2′), 3.60 (m, 4 H, 2 C*H*$_2$OH), 4.36 (m, 1 H, H-4′), 5.97 (d, J = 5.2 H, H-1′), 8.03 (s, 1 H, H-2), 8.20 (s, 1 H, H-8); MS (FAB, positive mode) m/z (rel intensity) 266 (MH^+, 62), 136 (b + 2 H, 100); high-resolution FAB MS m/z 266.1237 (MH^+, calcd 266.1253).

5-*O*-Benzoyl-3-deoxy-3-[(benzoyloxy)methyl]-1,2-di-*O*-acetyl-β-D-ribofuranose (18). Intermediate **6** (1.04 g 3 mmol) was dissolved in a mixture of ethanol (35 mL) and water (25 mL), and the solution was treated with $NaHCO_3$ (0.3 g) and a solution of $NaIO_4$ (0.687 g, 3.21 mmol) in water (13 mL). The resultant mixture was stirred at room temperature in the dark until the starting material was consumed (3 h) according to TLC analysis (silica gel, EtOAc). The reaction mixture was cooled (ice bath), and sodium borohydride (0.6 g) was added slowly. The resultant mixture was allowed to reach room temperature and stirring continued for 1 h. TLC analysis (silica gel, EtOAc) confirmed that complete reduction to alcohol **15** had taken place. Excess sodium borohydride was decomposed by the addition of 50% HOAc until a pH of 5 was reached, and the resulting brownish solution was titrated with 10% aqueous sodium bisulfite until it turned colorless. After the addition of brine, the organic product was extracted with $CHCl_3$ (5 × 50 mL) and dried ($MgSO_4$). Column chromatographic purification of the product (silica gel, CH_2Cl_2–EtOAc, from 1:1 to 2:3) afforded 0.89 g (84%) of **15** as an oil. This material was immediately dissolved in anhydrous pyridine (10 mL), cooled to 0 °C, and treated with benzoyl chloride (0.32 mL, 2.76 mmol) for 1 h with stirring. Methanol was added and the mixture concentrated to dryness. The residue was then partitioned between water and $CHCl_3$, and the organic layer was dried ($MgSO_4$) and then reduced to dryness to give crude **16**. This material was dissolved in a 1:1 mixture of water–dioxane (30 mL) and treated with a strong cation exchange resin (5 mL, Bio-Rad AG 50W-X8) at 85 °C for 36 h when TLC analysis (silica gel, EtOAC) indicated that the starting material had been consumed. The solid resin was removed by filtration and the filtrate was reduced in volume in the presence of a small amount of silica gel. Purification by column chromatography (silica gel, CH_2Cl_2–acetone, 1:1) afforded **17** as an oil. This oil was dissolved in anhydrous pyridine (10 mL) and treated with acetic anhydride (0.61 mL) at room temperature overnight. Excess methanol was added and the resulting solution was reduced to dryness. Purification by column chromatography (silica gel, hexane–EtOAc, from 4:1 to 1:1) afforded 1 g of **18** (71% from **6**) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.95 (s, 3 H, C*H*$_3$CO), 2.10 (s, 3 H, C*H*$_3$CO), 2.55 (m, 1 H, H-3), 4.40–4.80 (m, 5 H, H-4, H-5$_{a,b}$, C*H*$_2$OBz), 5.43 (d, J = 4.8 Hz, 1 H, H-2), 6.16 (s, 1 H, H-1), 7.40–7.60 (m, 6 H, Ph), 7.90–8.20 (m, 4 H, Ph). Anal. ($C_{24}H_{24}O_9$) C, H.

6-Chloro-9-[5-*O*-benzoyl-3-deoxy-3-[(benzoyloxy)methyl]-2-*O*-acetyl-β-D-ribofuranosyl]-9*H*-purine (19). 6-Chloropurine (0.395 g, 2.56 mmol) was silylated in HMDS (20 mL) at reflux in the presence of a catalytic amount of ammonium sulfate as performed for the synthesis of **11**. Compound **18** (0.576 g, 1.26 mmol), dissolved in anhydrous acetonitrile (6 mL), was added to the solid silylated base and the resultant mixture was stirred at room temperature for 15 min in the presence of trimethylsilyl triflate (0.24 mL, 1.26 mmol). The reaction was nearly complete after 5 min as judged by TLC analysis (silica gel, EtOAc–hexane, 1:1). The product was purified by column chromatography (silica gel, CH_2Cl_2–MeOH, 20:1) to give 0.630 g (91%) of **19** as a foam: ^{1}H NMR (C_6D_6) δ 2.68 (m, 1 H, H-3′), 4.15–4.70 (m, 5 H, H-4′, H-5′$_{a,b}$, C*H*$_2$OBz), 5.68 (d, J = 1.4 Hz, 1 H, H-1′), 6.00 (dd, J = 6 Hz, J' = 1.4 Hz, 1 H, H-2′), 7.12 (m, 6 H, Ph), 7.86 (s, 1 H, H-2), 8.10 (m, 4 H, Ph), 8.40 (s, 1 H, H-8). Anal. ($C_{27}H_{23}ClN_4O_7$) C, H, Cl, N.

6-Amino-9-[3-deoxy-3-(hydroxymethyl)-β-D-ribofuranosyl]-9*H*-purine (20). The protected nucleoside **19** (1.4 g, 2.54 mmol) was heated (100 °C) in a steel bomb under pressure in the presence of concentrated methanolic ammonia (100 mL) for 48 h. After cooling, the solvent was evaporated and the solid residue recrystallized from aqueous methanol to give 0.714 g (99%) of **20** as a white solid: mp 224–225 °C; ^{1}H NMR (D_2O) δ 2.56 (m, 1 H, H-3′), 3.60–3.92 (m, 4 H, 2 C*H*$_2$OH), 4.22 (m, 1 H, H-4′), 6.00 (d, J = 2.2 Hz, 1 H, H-1′), 8.29 (s, 1 H, H-2), 8.26 (s, 1 H, H-8); MS (FAB, positive mode) m/z (rel intensity) 282 (MH^+, 86) 136 (b + 2 H, 100). Anal. ($C_{11}H_{15}N_5O_4$) C, H, N.

6-Amino-9-[2,3-dideoxy-3-(hydroxymethyl)-β-D-ribofuranosyl]-9*H*-purine (2). A solution of imidazole (0.91 g, 13 mmol) and *tert*-butyldimethylchlorosilane (0.844 g, 5.6 mmol) in dimethylformamide (5 mL) was stirred at room temperature for 10 min. A mixture of **20** (0.72 g, 2.6 mmol) in dimethylformamide (5 mL) was added to the silylating agent solution and stirred at room temperature overnight. The reaction mixture was treated with methanol (5 mL) and concentrated in vacuo. The residue was treated with silica gel (12 g) and concentrated under vacuum. The residue was purified by column chromatography (silica gel, EtOAc–petroleum ether, gradient from 40% to 60% EtOAc) to give **21** (1.19 g, 90%) as a white solid which can be used directly for the next reaction. A suspension of **21** (0.45 g, 0.89 mmol) in dry acetonitrile (12 mL) was reacted with phenyl chlorothionoformate (0.15 mL, 1.08 mmol) and 4-(dimethylamino)pyridine (DMAP, 0.23 g, 1.89 mmol) at room temperature under nitrogen. After 16 h, the reaction mixture was reduced to dryness under vacuum and the residue was purified by column chromatography (silica gel, $CHCl_3$–Et_2O, gradient from 40% to 100% Et_2O) to give **22** (0.413 g, 72%) as a colorless oil. This material was immediately dissolved in toluene (15 mL) and heated at reflux under nitrogen in the presence of tri-*n*-butyltin hydride (0.40 mL, 1.49 mmol) and AIBN (0.016 g, 0.10 mmol) for 3 h. The reaction was cooled to room temperature and the toluene was evaporated under vacuum. The residue was purified by column chromatography (silica gel, EtOAc–$CHCl_3$, gradient from 20% to 100% EtOAc) to give **23** (0.237 g, 75%) as a white amorphous solid. A portion of this solid (0.040 g) was dissolved in anhydrous THF (2 mL) and treated with 320 μL of tetrabutylammonium fluoride (1 M solution in THF) with stirring at room temperature. After 5 min the reaction was concentrated to dryness and purified by column chromatography (silica gel, MeOH–EtOAc, gradient from 10% to 15% MeOH). The compound obtained was further purified by reverse-phase column chromatography (J. T. Baker octadecyl 6-mL high-capacity disposable column) using 15% methanol in water as eluant. The product-containing fractions were combined and lyophilized to give 0.014 g (66%) of **2** as a white solid which

was recrystallized from EtOH: mp 192-193 °C (99.10% pure by HPLC, $k' = 4.26$); UV (MeOH) λ_{max} 260 nm (log ϵ 4.08); ^{1}H NMR (D_2O) δ 2.40 (m, 1 H, H-3'), 2.60 (m, 2 H, H-2'$_{a,b}$), 3.70 (m, 4 H, 2 CH_2OH), 4.00 (m, 1 H, H-4'), 6.21 (dd, $J = 6.7$ Hz, $J' = 2.8$ H, 1 H, H-1'), 8.03 (s, 1 H, H-2), 8.21 (s, 1 H, H-8); MS (FAB, positive mode) m/z (rel intensity) 266 (MH^+, 63), 136 (b + 2 H, 100); high-resolution FAB MS, m/z 266.1266 (MH^+, calcd 266.1253). Anal. ($C_{11}H_{15}N_5O_3$) C, H, N.

Biological Procedures. HIV cytopathic effect assay was performed with ATH8 cells as previously described.[4] Briefly, 2 × 10^5 ATH8 cells were exposed to HTLV-III$_B$ virus (2000 virus particles/cell) for 45 min after treatment with polybrene, resuspended in 2 mL of culture medium containing interleukin 2 in the presence or absence of various concentrations of compounds, and incubated in culture tubes at 37 °C in 50% CO_2/95% air humidified atmosphere. Control cells were treated similarly but were not exposed to the virus. At various time points on days 5-7 of culture, the total viable cells were counted in a hemocytometer by the trypan blue dye exclusion method. As a minimum, all compounds were tested in duplicate dose-response experiments. The data in Figure 2 are from an experiment which is representative of the three performed.

Molecular Modeling. Models of A (1), 2',3'-dideoxyadenosine (ddA), 3'-hydroxymethyl-ddA (2), and 2'-hydroxymethyl-ddA (3) were developed with use of Quanta (Polygen Corp.). In each case, the two-dimensional structure was entered and a three-dimensional structure was calculated by the program. The potential energy of this three-dimensional structure was then minimized with use of 100 or 200 iterations of an adopted basis Newton-Raphson method. Optimum values of the important torsion angles χ, γ_1, and γ_2 were estimated in each case by means of a conformational search through 360° in 36 steps of 10° each. In the case of χ and γ_1, both angles were varied simultaneously and 36 × 36 conformations were examined for an energy minimum. Distance and angle measurements were made on the final minimized structures. Atomic coordinate data derived from X-ray diffraction analysis were taken from the original publications and entered into the program in Cambridge Crystal Database format. The structures of oxetanocin A and ddA that were based upon X-ray diffraction data were not modeled further but used only for purposes of comparison with the modeled structures.

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A New Class of HIV-1-Specific 6-Substituted Acyclouridine Derivatives: Synthesis and Anti-HIV-1 Activity of 5- or 6-Substituted Analogues of 1-[(2-Hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT)

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A series of novel acyclouridine derivatives substituted at both the C-5 and C-6 positions were synthesized for the purpose of improving the activity of a recently reported HIV-1-specific lead, 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT). Preparation of C-6 substituted derivatives was carried out based on the following three methods: (1) LDA (lithium diisopropylamide) lithiation of a thymine derivative (**4**) and subsequent reaction with electrophiles, (2) an addition-elimination reaction of HEPT or its 6-(phenylsulfinyl) derivative (**10**), or (3) palladium-catalyzed cross-coupling between a 6-iodo derivative (**16**) and terminal alkynes. Following the methods, 21 C-6 substituted analogues were synthesized. Among these, 6-(cyclohexylthio) (**8**), 6-phenoxy (**13**), and 6-benzyl (**27**) derivatives showed anti-HIV-1 (HTLV-III$_B$) activity with EC_{50} values of 8.2, 85, and 23 μM, respectively. Preparation of C-5 substituted derivatives was based on either LTMP (lithium 2,2,6,6-tetramethylpiperidide) lithiation of 6-(phenylthio)uracil derivative **37** or the above mentioned palladium-catalyzed cross-coupling of a 5-iodo-6-(phenylthio)uracil derivative (**38**). Following these methods, 11 C-5 substituted analogues were synthesized. Some 5-substituted derivatives (5-I, **44**; 5-CH=CPh_2, **49**; 5-CH=CHPh (*Z*), **54**; and 5-CH=CH_2, **55**) were more active than HEPT, but their selectivity indices (SI = CC_{50}/EC_{50}) were lower than that of HEPT. Compound **8** was also evaluated against another HIV-1 strain (HTLV-III$_{RF}$) and HIV-2 strains (LAV-2$_{ROD}$ and LAV-2$_{EHO}$). Only HTLV-III$_{RF}$ was as sensitive as HTLV-III$_B$.

Acquired immunodeficiency syndrome (AIDS) is a pandemic immunosuppressive disease caused by the depletion of helper T lymphocytes. The causative agent, termed human immunodeficiency virus type 1 (HIV-1), is a retrovirus. A similar retrovirus, HIV type 2 (HIV-2), also causes AIDS. Various compounds have been reported to inhibit the replication of HIV-1 in vitro.[1,2] A nucleoside analogue, 3'-azido-3'-deoxythymidine (AZT), is still the only drug approved for clinical use. Although a double-blind clinical trial has clearly demonstrated that AZT treatment prolongs the life of AIDS patients, serious side effects such as anemia and leukopenia are often associated with the long-term use of AZT.[3,4] Furthermore, clinical

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