Carbocyclic Analogues of the Potent Cytidine Deaminase Inhibitor 1-(β-D-Ribofuranosyl)-1,2-dihydropyrimidin-2-one (Zebularine)

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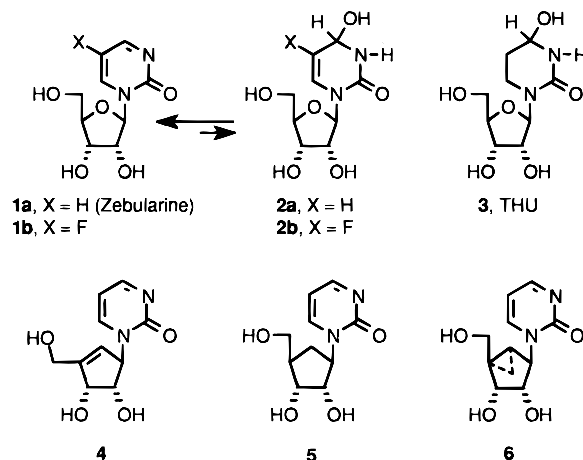
Three carbocylic analogues of the potent cytidine deaminase inhibitor (CDA) zebularine [1-(β-D-ribofuranosyl)-1,2-dihydropyrimidin-2-one, **1a**] were synthesized. The selected pseudosugar templates correspond, respectively, to the cyclopentenyl moiety of neplanocin A (compound **4**), the cyclopentyl moiety of aristeromycin (compound **5**), and a newly designed, rigid bicyclo-[3.1.0]hexane moiety (compound **6**). These three carba-nucleoside versions of zebularine were fashioned to overcome the inherent instability of the parent drug. Each target compound was approached differently using either convergent or linear approaches. The immediate precursor to the cyclopentenyl analogue **4** was obtained by a Mitsunobu coupling of pseudosugar **7** with 2-hydroxypyrimidine. The cyclopentyl analogue **5** was linearly constructed from carbocyclic amine **17**, and the final target **6** was similarly constructed from the carbobicyclic amine **27**. Of the three target compounds, only **5** showed a significant level of inhibition against human CDA, but it was 16 times less potent than zebularine (K_i = 38 μM vs $K_{i(apparent)}$ = 2.3 μM). Although these carbocyclic analogues appeared to be more stable than zebularine, replacement of the electronegative CO4′ oxygen for the less electronegative carbon in **4**−**6** presumably reduces the capacity of the pyrimidin-2(1*H*)-one ring to form a covalent hydrate, a step considered crucial for the compound to function as a transition-state inhibitor of the enzyme.

Introduction

Cytidine deaminase (CDA) is a widely distributed enzyme that catalyzes the hydrolytic deamination of cytosine nucleosides to the corresponding uracil nucleosides.[1,2] Clinically, the deamination of antileukemic nucleosides, such as cytosine arabinoside (ara-C) and 5-aza-2′-deoxycytidine (5-aza-CdR), results in loss of antitumor activity. Therefore, a strategy to prevent the inactivation of these drugs is to use them concurrently with CDA inhibitors.[2] Zebularine (**1a**)[3] and 5-fluorozebularine (**1b**)[3] are potent inhibitors of CDA which have been proposed as candidates for use in combination chemotherapy with ara-C or 5-aza-CdR.[4]

The crystal structures of the *E. coli* CDA complexed with zebularine (**1a**) and 5-fluorozebularine (**1b**) showed that these inhibitors bind at the active site as covalent hydrates at C4.[5] For zebularine, the K_i value of the single inhibitory diastereoisomer of **2a** was estimated to be 1.2×10^{-12} M when the equilibrium constant for the hydration of **1a** to **2a** was taken into account.[6] This value is roughly 8 orders of magnitude lower than the K_m value for cytidine.[6] By contrast, the fully reduced transition-state analogue tetrahydrouridine (**3**, THU) is a substantially weaker ligand compared to the hydrated forms of zebularine (**2a**) and 5-fluorozebularine (**2b**).[6,7] This result underscores the importance of the double bond in increasing the acidity of the OH proton in covalent hydrates **2a** and **2b**, both of which function as potent and tight-binding transition-state inhibitors of CDA.[5]



Unfortunately, the zebularines, and even THU, have some disadvantages in terms of stability. THU isomerizes in neutral (slow) and acidic (fast) aqueous solutions to a pyranoside form devoid of inhibitory activity.[8] Zebularine and 5-fluorozebularine, on the other hand, are extremely sensitive to basic conditions and they too follow a complex decomposition pathway that destroys biological activity.[9]

Carbocyclic nucleosides (carba-nucleosides) are generally more stable than conventional nucleosides to acidic

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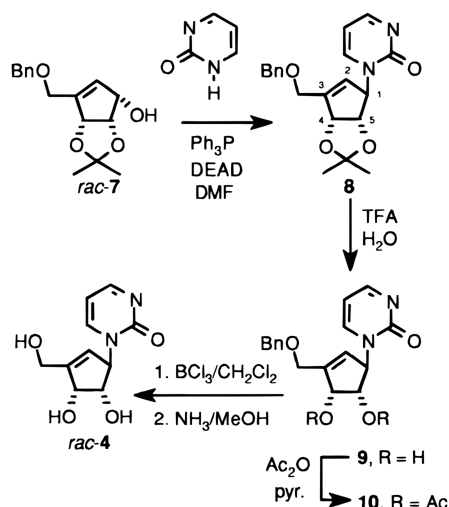
Scheme 1



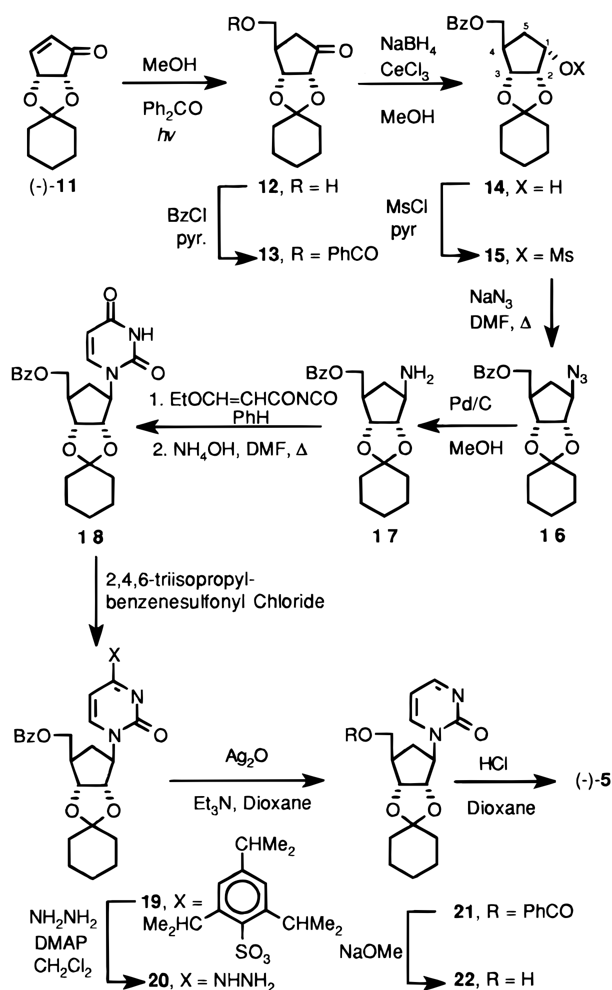
and basic conditions by virtue of a stable C–N bond.[10,11] Therefore, three carbocyclic pseudosugar templates present in some carbocyclic nucleosides were chosen to construct stable analogues of zebularine for evaluation as inhibitors of CDA. The cyclopentenyl moiety of neplanocin A,[12] the simpler cyclopentyl moiety of aristeromycin,[13] and the recently developed rigid bicyclo[3.1.0]hexane template[14] were selected to build three distinct carba-nucleoside versions (**4**–**6**) of zebularine.

Synthesis

The first target compound which contained the cyclopentenyl moiety of neplanocin A (cyclopentenyl zebularine, **4**) was synthesized as a racemate from the readily accessible carba-sugar precursor (±)-3-[(benzyloxy)methyl]-4,5-*O*-isopropylidene-2-cyclopenten-1-ol (**7**).[15] Mitsunobu coupling[16] of **7** with 2-hydroxypyrimidine afforded the desired *N*-alkylated product **8** in a modest yield (Scheme 1). Removal of the isopropylidene group with trifluoroacetic acid produced diol **9**, which was then converted to the diacetate **10**, prior to the removal of the benzyl ether group. Treatment of **10** with BCl_3, followed by acetate ammonolysis, gave the target racemic CPE-Z (*rac*-**4**). The lack of CDA inhibitory activity of racemic CPE-Z (vide infra) did not warrant synthesis of the single enantiomer corresponding to the natural configuration of neplanocin A.

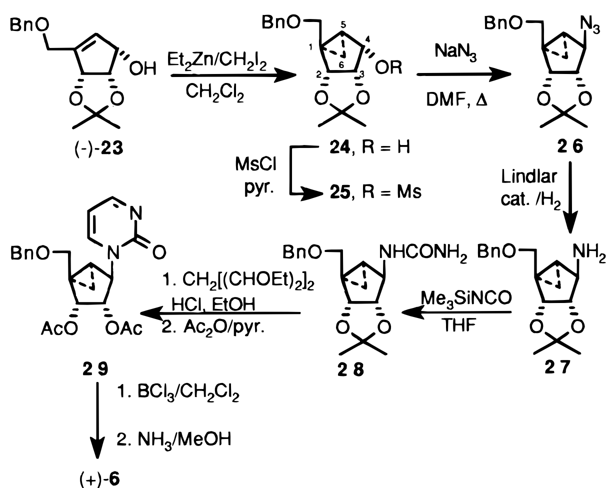
In principle, the cyclopentyl zebularine target **5** is just one reduction step away from **4**. However, regiospecific reduction of this double bond is not easily achievable,[17] plus the 2-oxopyrimidine ring is readily reduced under catalytic hydrogenation conditions.[8] Thus, we decided to prepare the chiral zebularine analogue **5** by constructing the pyrimidin-2(1*H*)-one ring from carbocyclic uridine via the corresponding 4-hydrazino intermediate (Scheme 2). This approach was developed earlier by Cech and Holy for the syntheses of pyrimidin-2(1*H*)-one nucleosides from uridine precursors.[18] The carbocyclic component of **5** was synthesized from the known (–)-2,3-(cyclohexylidenedioxy)-4-cyclopentenone (**11**), a useful chiral building block developed by Borchardt et al.[19] for the synthesis of carbocyclic nucleosides. From **11**, the requisite hydroxymethyl arm was introduced photochemically from the less hindered face of the molecule to give **12** as a single diastereoisomer. The resulting

Scheme 2



free hydroxyl group was then protected as benzoate ester to give **13**. Sodium borohydride reduction of **13**, again occurring from the less hindered side, produced a single alcohol product (**14**). NMR spectral analyses of these compounds, which differ from those reported by Borchardt et al. only in terms of the nature of the primary alcohol protecting group (i.e., benzoyl vs *tert*-butyl), were consistent with their data and confirmed the proposed structures.[19c] Alcohol **14** was converted to its methanesulfonate ester (mesylate **15**), which was subsequently displaced with NaN_3 to give the carbocyclic azide **16**. Reduction of the azido group to amine **17** enabled the construction of the uracil ring after condensation with 3-ethoxypropenoyl isocyanate, followed by base-catalyzed cyclization of the intermediate acryolyl urea to give compound **18**. Our approach to the 4-hydrazino intermediate **20**, which is required for the conversion of the uracil moiety to the pyrimidin-2(1*H*)-one ring, involved a slight variation from the original method of Cech and Holy.[18] In their approach, the leaving group X in structure **19** (Scheme 2) was a methylthio group. Instead, we decided to activate the C4-position as the 2,4,6-triisopropylbenzenesulfonate ester (compound **19**), which upon treatment with hydrazine afforded the 4-hydrazino intermediate **20**. Cleavage of the hydrazino group was accomplished with silver oxide in dioxane to give the antepenultimate intermediate **21**, accompanied by some uridine byproduct **18**.

Scheme 3



Tandem base and acid hydrolysis on **21** afforded the optically active target, cyclopentyl zebularine analogue (−)- **5**.

Since cyclopentyl zebularine [(−)-**5**] showed inhibitory activity against CDA (vide infra), we decided to synthesize the third target **6** also in optically pure form (Scheme 3). In agreement with previous reports, optically active cyclopentenol **23** underwent stereoselectve Simmons−Smith cyclopropanation to give alcohol **24** in excellent yield.[14f] At this stage, the synthetic approach followed was similar to the one used for the synthesis of (−)-**5**: (a) conversion of the secondary alcohol to the methanesulfonate ester (mesylate **25**), (b) nucleophilic displacement with NaN_3 to give the carbobicyclic azide **26**, and (c) reduction of the azido group to the carbobicyclic amine **27**. However, for the completion of the pyrimidin-2(1*H*)-one ring, we decided to follow a simpler method than the one of Cech and Holy.[18] This alternative method involved the condensation of a *β*-dialdehyde, or its equivalent synthon, with the corresponding carbobicyclic ureido nucleoside. This approach has been used successfully in the synthesis of pyrimidine-2(1*H*)-thione and 1-methylpyrimidin-2(1*H*)-one.[20,21] Thus, reaction of amine **27** with trimethylsilylisocyanate afforded cleanly, and in quantitative yield, the ureido-bicyclo[3.1.0]hexane intermediate **28**. This intermediate was cyclized in the presence of the complementary 3-carbon fragment, 1,1′,3,3′-tetraethoxypropane, to give the desired pyrimidin-2(1*H*)-one carba-nucleoside, which was converted to the corresponding diacetate **29** to aid in the isolation and purification. Although the overall conversion of **28** to **29** was only 40%, this approach to the pyrimidin-2(1*H*)-one ring was more efficient when compared to the previous method. Removal of the *O*-benzyl ether with BCl_3 and base-catalyzed hydrolysis of the acetate groups afforded the desired optically active target (+)-**6**.

The three different strategies used for the syntheses of these compounds deserve comment. It is known that Mitsunobu reactions with ambident anions, such as those from 2-oxopyridines[22] and 2-oxopyrimidines,[14a,c] give mixtures of *N*- and *O*-alkylated products. With the carbocyclic allylic alcohol **7**, the reaction gave predominantly the desired *N*-alkylated product **8** (the direct precursor of target **4**, Scheme 1). However, when either **14** or **24** were used, the reactions afforded exclusively the corresponding *O*-alkylated products (data not shown). For that reason, we were forced to use the longer linear approaches for the construction of the pyrimidin-2(1*H*)-one ring of targets **5** and **6** (Schemes 2 and 3). Chronologically, we started with the method of Cech and Holy,[18] which, while successful, was found to be lengthy and difficult. The alternate method involving the acid-catalyzed condensation of an *N*-substituted urea (i.e., **28**) with 1,1′,3,3′-tetraethoxypropane proved to be a more effective approach.[20,21]

Biological Results and Discussion

Compounds **4** and **6** showed no inhibition of mouse or human CDA at concentrations greater than 100 *μ*M. Compound **5**, however, did show inhibitory activity against human CDA, although it was 16-fold less potent than that of zebularine (K_i = 38 *μ*M versus $K_{i(apparent)}$ = 2.3 *μ*M). Although all the target compounds selected (**4**−**6**) are formal nucleoside analogues of zebularine by virtue of having a common pyrimidin-2(1*H*)-one ring, they failed to mimic zebularine in inhibiting CDA. For compound **4**, it can be argued that the flat cyclopentene ring does not allow the primary alcohol group, equivalent to the 5′-OH of ribose, to form an effective hydrogen bond with the enzyme.[5] Similarly, one can hypothesize that for **5** and **6** deviations from the ideal ribose conformation found in the zebularine−CDA complex[5] might be responsible for the lower potency of the former and inactivity of the latter. However, the most important lesson from this exercise is that just nucleoside mimicry is not enough, particularly when the enzymatic reaction involves a chemical transformation of the base, such as hydration. The replacement of an electronegative CO4′ oxygen for the less electronegative carbon undoubtedly resulted in a diminished inductive effect which changed the electronic environment of the pyrimidine ring and reduced its capacity to form a covalent hydrate. The transmision of information between the base and the sugar moiety has been well documented for the naturally occurring nucleosides, where the energetics for the protonation−deprotonation equilibrium are transmitted through the anomeric effect to drive the conformational North−South equilibrium of the sugar moieties.[23] In a case closer to the zebularines, which further supports the existence of a "cross-talk" between the ribose moiety and the heterocyclic base, we reported earlier that in sharp contrast with the known instability of 5-azacytidine in aqueous solution—which is rapidly converted to *N*-(formylamidino)-*N*′-*β*-D-ribofuranosyl urea and 1-*β*-D-ribofuranosyl-3-guanylurea after the formation of a covalent hydrate—the corresponding carbocyclic analogue was stable.[24] Therefore, while we have succeeded in synthesizing more stable analogues of zebularine (**4**−**6**), the corresponding biological activity was either reduced or lost because the capacity of zebularine to function as an effective CDA inhibitor is strictly associated with its ability to form a covalent hydrate of its pyrimidin-2(1*H*)-one aglycon. It is this requirement that is also responsible for the chemical transformation that initiates decomposition of the drug.

Experimental Section

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. Merk), and analytical TLC was performed on Analtech Uniplates silica gel GF. Infrared spectra were recorded on a Perkin-Elmer 1600 Series FTIR. Proton and ^{13}C NMR spectra were recorded on a Bruker AC-250 instrument at 250 and 62.9 MHz, respectively. Spectra were referenced to the solvent in which they were run (7.24 ppm for $CDCl_3$). Following the norm for reporting NMR data in nucleosides, the identity of protons and carbons on the pseudosugar ring (carbocyclic moiety) are indicated by numbers with primes. Specific rotations were measured in a Perkin-Elmer Model 241 polarimeter. Positive-ion fast-atom bombardment mass spectra (FABMS) were obtained on a VG 7070E 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.

***rel*-(1*R*,4*R*,5*S*)-1-[3-(Benzyloxy)methyl]-4,5-*O*-isopropylidene-2-cyclopenten-1-yl]pyrimidin-2(1*H*)-one (8).** A solution of **7**[15] (6.41 g, 23.2 mmol), triphenylphosphine (6.08 g, 23.2 mmol), and 2-hydroxypyrimidine (2.23 g, 23.2 mmol) in DMF (50 mL) was treated dropwise with diethyl azodicarboxylate (DEAD, 3.65 mL, 23.2 mmol). The resulting solution was stirred for 10 days at room temperature. The solvent was evaporated and the residue partitioned between water and EtOAc. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated to dryness. The oily residue revealed two major bands on TLC (silica gel, 5% MeOH in $CHCl_3$). The lower band (R_f = 0.27) corresponded to the desired product. The entire batch was purified by column chromatography with silica gel using, sequentially, 40% EtOAc in hexanes, $CHCl_3$, and 5% MeOH in $CHCl_3$ as eluants to give 3.94 g (48%) of **8**: mp 111–112 °C; ^{1}H NMR ($CDCl_3$) δ 1.34 (s, 3 H, CH_3), 1.44 (s, 3 H, CH_3), 4.26 (s, 2 H, $PhCH_2OCH_2$), 4.61 (s, 2H, $PhCH_2OCH_2$), 4.64 (d, 1H, J = 5.7 Hz, H-5′), 5.24 (d, 1 H, J = 5.7 Hz, H-4′), 5.45 (s, 1 H, H-1′), 5.65 (s, 1 H, H-2′), 6.30 (dd, 1 H, J = 6.6, 4.1 Hz, H-5), 7.35 (s, 5 H, Ph), 7.57 (dd, 1 H, J = 6.6, 2.8 Hz, H-4), 8.56 (m, 1 H, H-6); ^{13}C NMR ($CDCl_3$) δ 25.6, 27.1, 66.5, 70.4, 73.1, 83.5, 83.8, 103.9, 112.3, 121.8, 127.6, 128.3, 137.6, 145.1, 150.8, 155.7, 165.7. Anal. ($C_{20}H_{22}N_2O_4$) C, H, N.

***rel*-(1*R*,4*R*,5*S*)-1-[3-(Benzyloxy)methyl]-4,5-dihydroxy-2-cyclopenten-1-yl]pyrimidin-2(1*H*)-one (9).** A solution of **8** (0.565 g, 1.59 mmol) in a 1:1 mixture of water and CF_3COOH (6 mL) was stirred for 2 h at room temperature. The reaction mixture was diluted with 15 mL of water and extracted with CH_2Cl_2 (3 × 50 mL). The organic extract was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography on silica gel using 5–10% MeOH in CH_2Cl_2 as eluant to give 0.355 g (71%) of **9** as a clear oil: ^{1}H NMR (Me_2SO-d_6) δ 4.03 (t, 1 H, J = 5.5 Hz, H-5′), 4.12 (m, 2 H, $PhCH_2OCH_2$), 4.40 (d, 1 H, J = 5.5 Hz, H-4′), 4.55 (s, 2 H, $PhCH_2OCH_2$), 4.70–5.20 (br s, 2 H, OH), 5.44 (m, 1 H, H-1′), 5.69 (d, 1 H, J = 1.5 Hz, H-2′), 6.43 (dd, 1 H, J = 6.5, 4.1 Hz, H-5), 7.30–7.50 (m, 5 H, Ph), 7.90 (dd, 1 H, J = 6.5, 2.8 Hz, H-4), 8.52 (dd, 1 H, J = 3.9, 2.8 Hz, H-6); ^{13}C NMR (Me_2SO-d_6) δ 66.7, 68.6, 71.8, 72.5, 76.4, 104.2, 125.6, 127.5, 128.3, 138.4, 146.7, 147.4, 155.8, 165.6. Anal. ($C_{17}H_{18}N_2O_4$·$0.25H_2O$) C, H, N.

***rel*-(1*R*,4*R*,5*S*)-1-[3-[(Benzyloxy)methyl]-4,5-*O*-acetyl-2-cyclopenten-1-yl]pyrimidin-2(1*H*)-one (10).** A solution of **9** (0.497 g, 1.58 mmol) in pyridine (7 mL) was treated with acetic anhydride (0.50 mL, 5.30 mmol) and stirred at room temperature for 1.5 h. Methanol (30 mL) was added, and the solvents were evaporated. The residue was purified by column chromatography over silica gel with CH_2Cl_2 and 5% MeOH in CH_2Cl_2 as eluants to give 0.422 g (67%) of **10** as a clear oil: ^{1}H NMR ($CDCl_3$) δ 2.02 (s, 3 H, CH_3), 2.04 (s, 3 H, CH_3), 4.11 (m, 2 H, $PhCH_2OCH_2$), 4.55 (s, 2 H, $PhCH_2OCH_2$), 5.22 (t, 1 H, J = 6.0 Hz, H-5′), 5.90 (m, 2 H, H-2′, H-4′), 6.05 (m, 1 H, H-1′), 6.30 (dd, 1 H, J = 6.6, 4.0 Hz, H-5), 7.20–7.40 (m, 5 H, Ph), 7.50 (dd, 1 H, J = 6.6, 2.8 Hz, H-4), 8.55 (distorted t, 1 H, H-6); ^{13}C NMR (Me_2SO-d_6) δ 20.4, 20.5, 65.9, 67.7, 71.7, 73.7, 74.1, 104.4, 127.7, 128.3, 129.2, 138.2, 142.8, 147.5, 155.5, 166.3, 169.6, 170.0. Anal. ($C_{21}H_{22}N_2O_6$) C, H, N.

***rel*-(1*R*,4*R*,5*S*)-1-[3-(Hydroxymethyl)-4,5-dihydroxy-2-cyclopenten-1-yl]pyrimidin-2(1*H*)-one (4).** A stirred solution of **10** (0.147 g, 0.37 mmol) in CH_2Cl_2 (−78 °C) was treated with 1.85 mL of BCl_3 (1 M in CH_2Cl_2) for 2 h at −78 °C. Methanol (25 mL) was added and the reaction mixture was stirred for 40 min while allowing it to reach room temperature. The solvent was evaporated and the residue was purified by silica gel column chromatography with 5–25% methanol in CH_2Cl_2 to give 0.069 g (59%) of crude diacetate. This compound was immediately dissolved in saturated methanolic ammonia (25 mL) and stirred at 0 °C in a sealed vessel for 3 h. The solvent was evaporated to give a residue which was purified by silica gel column chromatography to give 0.051 g (100%) of **4**: mp 183–184 °C (EtOH–H_2O); ^{1}H NMR (Me_2SO-d_6) δ 3.95 (m, 1 H, H-5′), 4.09 (m, 2 H, CH_2OH), 4.37 (m, 1 H, H-4′), 4.89 (m, 2 H, OH), 5.08 (d, 1 H, J = 6.0 Hz, OH), 5.44 (br s, 1 H, H-1′), 5.54 (m, 1 H, H-2′), 6.42 (dd, 1 H, J = 6.6, 4.1 Hz, H-5), 7.87 (dd, 1 H, J = 6.6, 2.8 Hz, H-4), 8.51 (dd, 1 H, J = 4.0, 2.8 Hz, H-6); ^{1}H NMR (D_2O) δ 3.58 (d, 1 H, J = 5.6 Hz, H-5′), 3.67 (s, 2 H, CH_2OH), 4.05 (d, 1 H, J = 5.6 Hz, H-4′), 4.92 (br s, H-1′), 5.19 (s, 1 H, H-2′), 6.05 (m, 1 H, H-5), 7.35 (m, 1 H, H-4), 7.94 (m, 1 H, H-6); ^{13}C NMR (Me_2SO-d_6) δ 58.6, 68.1, 72.6, 76.8, 104.1, 122.9, 146.2, 152.1, 155.9, 165.5. Anal. ($C_{10}H_{12}N_2O_4$) C, H, N.

(2*R*,3*R*,4*R*)-4-Hydroxymethyl-2,3-(cyclohexylidenedioxy)-1-cyclopentanone (12). A solution of (−)-4,5-(cyclohexylidenedioxy)-2-cyclopentenone (**11**, 0.38 g, 1.96 mmol)[19] and benzophenone (0.14 g, 0.78 mmol) in MeOH (50 mL) was purged with argon for 60 min and then placed in a Rayonet Reactor RMR-400 equipped with one 350 nm lamp. The reaction mixture was photolyzed for 2 h. The mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography using hexane: EtOAc (1:1) as eluant to give **12** (0.22 g, 50%) as a colorless syrup: ^{1}H NMR ($CDCl_3$) δ 1.30–1.70 (m, 10 H, cyclohexyl), 2.15 (d, 1 H, J = 18.1 Hz, H-5$_a$), 2.55 (m, 1 H, H-4), 2.75 (dd, 1 H, J = 18.1, 9.2 Hz, H-5$_b$), 3.69 (dd, 1 H, J = 10.0, 3.7 Hz, C*H*HOH), 3.85 (dd, 1 H, J = 10.0, 3.2 Hz, CH*H*OH), 4.27 (d, 1 H, J = 5.4 Hz, H-3), 4.68 (d, 1 H, J = 5.4 Hz, H-2). Anal. ($C_{12}H_{18}O_4$) C, H.

(2*R*,3*R*,4*R*)-4-[(Benzoyloxy)methyl]-2,3-(cyclohexylidenedioxy)-1-cyclopentanone (13). A solution of **12** (0.88 g, 3.89 mmol) in pyridine (20 mL) was treated with benzoyl chloride (0.54 mL, 4.67 mmol) and stirred at room temperature for 3 h. The reaction mixture was reduced to dryness and the residue was dissolved in EtOAc (200 mL), washed with brine (100 mL), and dried ($MgSO_4$). The solvent was evaporated and the residue obtained (1.28 g) was used in the following reaction without further purification: ^{1}H NMR ($CDCl_3$) δ 1.30–1.75 (m, 10 H, cyclohexyl), 2.25 (d, 1 H, J = 18.1 Hz, H-5$_a$), 2.90 (m, 2 H, H-4, H-5$_b$), 4.30 (m, 2 H, H-3, C*H*HOCOPh), 4.52 (dd, 1 H, J = 10.0, 2.9 Hz, CH*H*OCOPh), 4.73 (d, 1 H, J = 5.3 Hz, H-2), 7.40–8.15 (m, 5 H, Ph).

(1*S*,2*S*,3*R*,4*R*)-4-[(Benzoyloxy)methyl]-2,3-(cyclohexylidenedioxy)cyclopentan-1-ol (14). A solution of **13** (1.28 g, 3.88 mmol) in MeOH (20 mL) was treated with $NaBH_4$ (0.15 g, 3.88 mmol) in the presence of $CeCl_3$·$7H_2O$ (1.45 g, 3.88 mmol) at 0 °C while being stirred for 5 min. The mixture was neutralized with glacial AcOH, and all volatiles were removed under reduced pressure. The residue was dissolved in EtOAc (200 mL), and the organic solution was washed with brine (100 mL), dried ($MgSO_4$), and evaporated to dryness. The residue was purified by silica gel column chromatography with hexane: EtOAc (4:1) as eluant to give **14** (1.00 g, 78% from **12**) as a colorless syrup: ^{1}H NMR ($CDCl_3$) δ 1.31–1.78 (m, 10 H, cyclohexyl), 1.80–2.05 (m, 2 H, H-5$_{a,b}$), 2.55 (m, 1 H, H-4), 4.12–4.40 (m, 3 H, H-1, H-2, H-3), 4.42–4.65 (m, 2 H, CH_2-OCOPh), 7.49–8.15 (m, 5 H, Ph). Anal. ($C_{19}H_{24}O_5$) C, H.

(1*S*,2*S*,3*R*,4*R*)-4-[(Benzoyloxy)methyl]-2,3-(cyclohexylidenedioxy)cyclopentan-1-ol Methanesulfonate (15). A stirred solution of **14** (1.07 g, 3.32 mmol) in pyridine (10 mL) was treated with methanesulfonyl chloride (0.39 mL, 4.98

mmol) at 0 °C and allowed to reach room temperature during the course of 2 h. All volatiles were removed under reduced pressure, and the residue was purified by silical gel column chromatography using CH_2Cl_2:MeOH (70:1) as eluant to give **15** (1.33 g, 100%) as a colorless syrup which was used in the following step without further purification: ^1H NMR ($CDCl_3$) δ 1.31−1.79 (m, 10 H, cyclohexyl), 2.01 (m, 1 H, H-5_a), 2.35 (m, 1 H, H-5_b), 2.59 (m, 1 H, H-4), 3.09 (s, 3 H, CH_3), 4.20 (dd, 1 H, J = 11.4, 6.0 Hz, C*H*HOCOPh), 4.31 (dd, 1 H, J = 11.4, 5.6 Hz, CH*H*OCOPh), 4.54 (d, 1 H, J = 5.7 Hz, H-3), 4.70 (t, 1 H, J = 5.5 Hz, H-2), 5.15 (m, 1 H, H-1), 7.40−8.02 (m, 5 H, Ph).

(1*R*,2*S*,3*R*,4*R*)-1-Azido-4-[(benzoyloxy)methyl]-2,3-(cyclohexylidenedioxy)cyclopentane (16). A stirred solution of **15** (1.36 g, 3.32 mmol) in DMF (20 mL) was treated with sodium azide (2.16 g, 33.2 mmol) at 0 °C. After the addition, the temperature was raised to 120 °C and the reaction was continued for 20 h. All volatiles were removed under reduced pressure, and the residue was dissolved in EtOAc (300 mL). The organic solution was washed with brine (100 mL), dried ($MgSO_4$) and evaporated. The residue was purified by silica gel column chromatography using hexane:EtOAc (8:1) as eluant to give **16** (0.93 g, 81%) as a colorless syrup: IR (neat) 2103 (N_3), 1720 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.32−1.71 (m, 10 H, cyclohexyl), 1.72 (m, 1 H, H-5_a), 2.35 (m, 1 H, H-5_b), 2.61 (m, 1 H, H-4), 4.11 (m, 1 H, H-1), 4.20−4.40 (m, 2 H, C*H*$_2$-OCOPh), 4.45 (br d, 1 H, J = 5.4 Hz, H-3), 4.55 (br d, 1 H, J = 5.9 Hz, H-2), 7.40−8.11 (m, 5 H, Ph). Anal. ($C_{19}H_{23}N_3O_4$) C, H, N.

(1*R*,2*S*,3*R*,4*R*)-1-Amino-4-[(benzyloxy)methyl]-2,3-(cyclohexylidenedioxy)cyclopentane (17). A stirred solution of **16** (1.93 g, 2.61 mmol) in MeOH (15 mL) was hydrogenated under a balloon in the presence of 10% Pd/C (0.15 g) for 20 h at room temperature. The mixture was filtered through a pad of Celite, and the solid cake was washed with MeOH. The filtrate was evaporated and the residue was dissolved in EtOAc (300 mL). The organic solution was washed with brine (100 mL), dried ($MgSO_4$), and evaporated. The residue was purified by silica gel column chromatography using CH_2Cl_2:MeOH (20:1) as eluant to give **17** (0.70 g, 81%) as a colorless syrup: IR (neat) 3376 (NH_2), 1719 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.30−1.70 (m, 11 H, H-5_a, cyclohexyl), 2.25 (m, 1 H, H-5_b), 2.47 (m, 1 H, H-4), 3.45 (m, 1 H, H-1), 4.21 (dd, 1H, J = 6.5, 3.2 Hz, H-3), 4.30−4.50 (m, 2 H, C*H*$_2$OCOPh), 4.55 (dd, 1 H, J = 6.5, 3.6 Hz, H-2), 7.35−8.09 (m, 5 H, Ph). Anal. ($C_{19}H_{25}NO_4$) C, H, N.

1-[(1′*R*,2′*S*,3′*R*,4′*R*)-4-[(benzoyloxy)methyl]-2,3-(cyclohexylidenedioxy)cyclopentan-1-yl]uracil (18). A solution of 3-ethoxypropenoyl isocyanate in benzene (20 mL), prepared according to the standard literature procedure[25] from 3-ethoxypropenoyl chloride (0.30 g, 2.23 mmol) and silver cyanate (0.62 g, 4.13 mmol), was added to a solution of **17** (0.28 g, 0.85 mmol) in benzene (20 mL), while the temperature was maintained at 10 °C. The pale yellow solution formed was stirred for 30 min at the same temperature and after that time the solvent was removed under reduced pressure. The residue was purified through a short silica gel column using CH_2Cl_2:MeOH (50:1) as eluant to give the corresponding acyclic pyrimidine precursor (0.30 g) as a colorless syrup. This intermediate was immediately dissolved in DMF (15 mL), treated with concentrated NH_4OH (20 mL), and heated to 110 °C for a period of 4 h, while ammonia gas was continuously bubbled into the reaction mixture. After cooling to room temperatue, water (20 mL) and CH_2Cl_2 (100 mL) were added, and the organic layer was washed with brine (30 mL) and dried ($MgSO_4$). The solution was evaporated and the residue was purified by silica gel column chromatography using CH_2Cl_2:MeOH (20:1) as eluant to give **18** (0.22 g, 60%) as a colorless white foam: UV (MeOH) λ_{max} 265.40 nm; ^1H NMR ($CDCl_3$) δ 1.20−1.75 (m, 10 H, cyclohexyl), 2.10−2.40 (m, 2 H, H-5′$_{a,b}$), 2.51 (m, 1 H, H-4′), 4.42 (m, 3 H, H-3′, C*H*$_2$OCOPh), 4.61 (t, 1 H, J = 6.4 Hz, H-2′), 4.83 (dd, 1 H, J = 6.9, 4.8 Hz, H-1′), 5.70 (dd, 1 H, J = 8.0, 2.0 Hz, H-5), 7.15 (d, 1 H, J = 8.0 Hz, H-6), 7.37−8.09 (m, 5 H, Ph), 9.25 (br s, 1 H, NH). Anal. ($C_{23}H_{26}N_2O_6$) C, H, N. A small amount of debenzoylated product was also isolated (0.06 g) and this was rebenzoylated after treatment with benzoyl chloride in pyridine to give additional **18**.

1-[(1′*R*,2′*S*,3′*R*,4′*R*)-4-(hydroxymethyl)-2,3-dihydroxycyclopentan-1-yl]pyrimidin-2(1*H*)-one (5). A stirred solution of **18** (0.20 g, 0.47 mmol) in CH_2Cl_2 (20 mL) was treated with Et_3N (0.26 mL, 1.88 mmol) and cooled to 5 °C. After the addition of 1,2,4-triisopropylbenzenesulfonyl chloride (0.14 g, 0.85 mmol) and DMAP (0.014 g, 0.12 mmol), the resulting solution was stirred for 20 h at room temperature. All volatiles were removed under reduced pressure, and the residue was purified through a short silica gel column using hexane:EtOAc (3:1) as eluant to give intermediate **19** as an amorphous solid: UV (MeOH) λ_{max} 281.0 nm; ^1H NMR ($CDCl_3$) δ 1.10−1.75 (m, 31 H, cyclohexyl, $CHMe_2$), 2.20−2.54 (m, 2 H, H-5′$_{a,b}$), 2.90 (m, 1 H, H-4′), 4.15−4.49 (m, 3 H, H-3′, C*H*$_2$OCOPh), 4.67 (t, 1 H, J = 6.3 Hz, H-2′), 5.01 (dd, 1 H, J = 6.9, 4.2 Hz, H-1′), 6.08 (d, 1 H, J = 7.10 Hz, H-5), 7.19 (s, 2 H, Ph), 7.38−8.09 (m, 6 H, H-6, Ph). This material (**19**, 0.26 g, 3.77 mmol) was dissolved in dioxane (20 mL) and stirred with anhydrous hydrazine (0.11 g, 3.77 mmol) for 1 h at room temperature. The solution was concentrated under reduced pressure to give the crude 4-hydrazino analogue **20** (0.20 g, 0.46 mmol), which was immediately dissolved in aqueous dioxane (20 mL dioxane, 0.4 mL H_2O) and treated with Ag_2O (0.32 g, 1.38 mmol) in the presence of Et_3N (0.13 mL, 0.92 mmol) for 4 h at reflux. The reaction mixture was filtered through a pad of Celite and washed with successively with EtOH (10 mL) and CH_2Cl_2 (20 mL). The filtrate was collected, evaporated to dryness, and purified by silica gel column chromatography using CH_2Cl_2:MeOH (50:1) as eluant to give, according to ^1H NMR analysis, a 1:1 mixture of the desired product **21** (0.12 g) and a uridine byproduct identical to **18**. The combined mixture was dissolved in MeOH (20 mL) and pyridine (0.4 mL) and stirred in the presence of NaOMe (0.5 M, 0.90 mL) for 20 h at room temperature. After neutralization of the reaction mixture with glacial AcOH, all volatiles were removed under reduced pressure. The residue was purified by silica gel column chromatography using CH_2Cl_2:MeOH (20:1) as eluant to give first the less polar product **22** (0.03 g), followed by the more polar uridine compound corresponding to debenzoylated **18** (0.05 g). Product **22** (0.03 g) was dissolved in dioxane (10 mL) and stirred at room temperature in the presence of 1 N HCl (1 mL) for 20 h. The solution was concentrated under reduced pressure and the residue was loaded on a short column of 50W-80 resin (H^+ form). After washing with water, compound **5** was eluted with 1 N NH_4OH. Reducing to dryness, redissolving in water, and freeze-drying yielded 0.02 g of **5** (19% from **18**) as a pale yellowish hygroscopic solid: UV (H_2O, pH 7) λ_{max} 307.0 nm (ϵ 2390); $[\alpha]^{25}_D$ −32.0 (c 1, MeOH); ^1H NMR ($CDCl_3$) δ 1.58 (m, 1 H, H-5′$_a$), 2.15 (m, 1 H, H-5′$_b$), 2.32 (m, 1 H, H-4′), 3.65 (m, 2 H, C*H*$_2$OH), 3.99 (dd, 1 H, J = 5.5, 3.5 Hz, H-3′), 4.38 (dd, 1 H, J = 8.7, 5.5 Hz, H-2′), 4.78 (m, 1 H, H-1′), 6.55 (dd, 1 H, J = 6.6, 4.3 Hz, H-5), 8.24 (dd, 1 H, J = 6.6, 2.6 Hz, H-4), 8.56 (dd, 1 H, J = 4.2, 2.6 Hz, H-6); ^{13}C NMR (CD_3OD) δ 28.9, 46.7, 64.5, 67.3, 73.7, 75.0, 106.4, 149.5, 158.5, 166.9; FAB MS m/z (relative intensity) 227 (MH^+, 35). Anal. ($C_{10}H_{14}N_2O_4 \cdot 1.6H_2O$) C, H, N.

(1*R*,2*R*,3*S*,4*S*,5*S*)-1-[(Benzyloxy)methyl]-2,3-*O*-isopropylidene-4-hydroxybicyclo[3.1.0]hexane (24). A stirred solution of **23**[15] (6.0 g, 21.66 mmol) in CH_2Cl_2 at 0 °C was treated with diethyl zinc (1 M hexane, 65 mL, 65 mmol) and CH_2I_2 (5.2 mL, 64.98 mmol). After 15 min, the mixture was allowed to reach room temperature and stirring continued for a total of 15 h. Aqueous saturated NH_4Cl was carefully added, and the resulting mixture was extracted with ether (500 mL), washed successively with saturated $NaHCO_3$ solution (100 mL) and brine (100 mL), dried ($MgSO_4$), and evaporated. The residue was purified by silica gel column chromatography using hexane:EtOAc (2:1) as eluant to give **24** (5.0 g, 79%) as a colorless syrup: ^1H NMR ($CDCl_3$) δ 0.61 (dd, 1 H, J = 8.6, 5.5 Hz, H-6_a), 1.17 (t, 1 H, J = 5.1 Hz, H-6_b), 1.29 (s, 3 H, CH_3), 1.52 (s, 3 H, CH_3), 1.69 (m, 1 H, H-5), 1.99 (br s, 1 H, OH), 3.17 (d, 1 H, J = 10.4 Hz, C*H*HOBn), 3.68 (d, 1 H, J =

10.4 Hz, CH*H*OBn), 4.55 (m, 4 H, PhC*H*$_2$O, H-3, H-4), 4.90 (br d, 1 H, $J = 5.9$ Hz, H-2), 7.25−7.39 (m, 5 H, Ph). Anal. ($C_{17}H_{22}O_4$) C, H.

(1*R*,2*R*,3*S*,4*R*,5*S*)-1-[(Benzyloxy)methyl]-2,3-*O*-isopropylidene-4-azidobicyclo[3.1.0]hexane (26). A stirred solution of **24** (0.74 g, 2.54 mmol) in pyridine (10 mL) was treated with methanesulfonyl chloride (0.30 mL, 3.81 mmol) at 0 °C for 2 h. The solution was then reduced to dryness, and the residue was dissolved in EtOAc (200 mL). The organic solution was washed with brine (100 mL), dried ($MgSO_4$), and evaporated to dryness under reduced pressure to give mesylate **25** (0.78 g). The crude mesylate was dissolved in DMF (15 mL) and reacted with NaN_3 (0.83 g, 12.7 mmol) at 100 °C for 18 h. After reaching room temperature, the reaction mixture was diluted with EtOAc (200 mL) and extracted with water (50 mL). The organic layer was washed with brine, dried ($MgSO_4$), and reduced to dryness. The residue was purified by silica gel column chromatography using hexane:EtOAc (5:1) as eluant to give **26** (0.70 g, 88%) as a colorless syrup: IR (neat) 2097 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.82 (ddd, 1 H, $J = 9.2$, 5.5, 1.3 Hz, H-6$_a$), 1.01 (t, 1 H, $J = 5.1$ Hz, H-6$_b$), 1.25 (s, 3 H, CH_3), 1.49 (s, 3 H, CH_3), 1.58 (m, 1 H, H-5), 3.35 (d, 1 H, $J = 10.5$ Hz, C*H*HOBn), 3.63 (d, 1 H, $J = 10.5$ Hz, CH*H*OBn), 3.83 (s, 1 H, H-4), 4.51 (dd, 1 H, $J = 7.2$, 1.3 Hz, H-2), 4.55 (s, 2 H, PhC*H*$_2$O), 5.02 (dd, 1 H, $J = 7.2$, 1.0 Hz, H-3), 7.25−7.39 (m, 5 H, Ph). Anal. ($C_{17}H_{21}O_3N_3$·0.4H_2O) C, H, N.

(1*R*,2*R*,3*S*,4*R*,5*S*)-1-[(Benzyloxy)methyl]-2,3-*O*-isopropylidene-4-aminobicyclo[3.1.0]hexane (27). A stirred solution of **26** (0.70 g, 2.22 mmol) in a mixture of MeOH (20 mL) and EtOAc (20 mL) was hydrogenated in a balloon in the presence of Lindlar's catalyst (0.14 g). After 3 h, the mixure was filtered and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography using $CHCl_3$:MeOH (10:1) as eluant to give **27** (0.67 g, 100%) as a colorless syrup: ^{1}H NMR ($CDCl_3$) δ 0.82 (ddd, 1 H, $J = 9.0$, 5.3, 1.2 Hz, H-6$_a$), 0.99 (t, 1 H, $J = 5.0$ Hz, H-6$_b$), 1.25 (s, 3 H, CH_3), 1.31 (ddd, 1 H, $J = 9.2$, 4.7, 1.3 Hz, H-5), 1.49 (s, 3 H, CH_3), 1.75 (br s, 1 H, NH_2), 3.26 (d, 1 H, $J = 10.3$ Hz, C*H*HOBn), 3.32 (s, 1 H, H-4), 3.72 (d, 1 H, $J = 10.3$ Hz, CH*H*OBn), 4.33 (dd, 1 H, $J = 7.1$, 1.33 Hz, H-2), 4.55 (AB d, 2 H, PhCH_2O), 5.05 (d, 1 H, $J = 7.1$, 0.8 Hz, H-3). Anal. ($C_{17}H_{23}O_3N$·0.2H_2O) C, H, N.

(1*R*,2*R*,3*S*,4*R*,5*S*)-1-[(Benzyloxy)methyl]-2,3-*O*-isopropylidene-4-ureidobicyclo[3.1.0]hexane (28). A stirred solution of **27** (0.20 g, 0.69 mmol) in THF (10 mL) was treated with trimethylsilyl isocyanate (0.24 mL, 1.52 mmol) at room temperature for 15 h. Although TLC revealed no change, the mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography using CH_2Cl_2:MeOH (15:1) as eluant to give **28** (0.23 g, 100%) as a colorless syrup: ^{1}H NMR ($CDCl_3$) δ 0.72 (dd, 1 H, $J = 8.6$, 5.9 Hz, H-6$_a$), 1.01 (t, 1 H, $J = 5.1$ Hz, H-6$_b$), 1.25 (s, 3 H, CH_3), 1.35 (dd, 1 H, $J = 8.9$, 4.0 Hz, H-5), 1.47 (s, 3 H, CH_3), 3.15 (d, 1 H, $J = 10.3$ Hz, C*H*HOBn), 3.81 (d, 1 H, $J = 10.3$ Hz, CH*H*OBn), 3.92 (br s, 2 H, NH_2), 4.43 (d, 1 H, $J = 6.9$ Hz, H-2), 4.44 (s, 1 H, H-4), 4.51 (AB q, 2 H $J = 11.9$ Hz, PhCH_2O), 5.01 (d, 1 H, $J = 6.9$, H-3) 5.18 (br s, 1 H, NH), 7.25−7.39 (m, 5 H, Ph). Anal. ($C_{18}H_{24}O_4N_2$·0.25H_2O) C, H, N.

(1′*R*,2′*R*,3′*S*,4′*R*,5′*S*)-1′-[(Benzyloxy)methyl]-2′,3′-di-*O*-acetyl-4′-(pyrimidin-2(1*H*)-on-1-yl)bicyclo[3.1.0]hexane (29). A stirred solution of **28** (0.24 g, 0.72 mmol) in EtOH (20 mL) was treated with concentrated HCl (0.3 mL) and heated at 45 °C for 20 min. After the addition of 0.72 mL of 1,1′,3,3′-tetraethoxypropane (1 M, EtOH), stirring was continued for 15 h at 45 °C. After volatiles were removed under reduced pressure, the residue was treated with acetic anhydride (0.5 mL) and pyridine (6 mL), and the resulting solution was stirred at room temperature for 15 h. The solution was reduced to dryness, dissolved in EtOAc (300 mL), washed with brine (100 mL), dried ($MgSO_4$), and evaporated. The residue was purified by silica gel column chromatography using CH_2Cl_2:MeOH (30:1) as eluant to give **29** (0.12 g, 40%) as a colorless syrup: UV (H_2O) λ_{max} 307 nm; ^{1}H NMR ($CDCl_3$) δ 0.92 (m, 1 H, H-6′$_a$), 1.47 (m, 2 H, H-6′$_b$, H-5′), 2.01 and 2.08 (s, 3 H, CH_3), 3.17 (d,1 H, $J = 10.1$ Hz, C*H*HOBn), 4.01 (d, 1 H, $J = 10.1$ Hz, CH*H*OBn), 4.55 (AB q, 2 H, $J = 11$ Hz, PhC*H*$_2$O), 5.10 (d, 1 H, $J = 7.1$ Hz, H-3′), 5.18 (s, 1 H, H-4′), 5.89 (m, 2 H, H-2′, H-5), 7.30−7.39 (m, 5 H, Ph), 8.48 (m, 2 H, H-4, H-6). Anal. ($C_{22}H_{24}O_6N_2$) C, H, N.

(1*R*,2*R*,3*S*,4*R*,5*S*)-1-(Hydroxymethyl)-2,3-dihydroxy-4-(pyrimidin-2-(1*H*)-on-1-yl)bicyclo[3.1.0]hexane (6). A stirred solution of **29** (0.07 g, 0.17 mmol) in CH_2Cl_2 (10 mL) was cooled to −78 °C and treated with a 1 M solution of BCl_3 in CH_2Cl_2 (1.2 mL). After 20 min, the mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography using CH_2Cl_2:MeOH (30:1) as eluant to give the intermediate diacetate (0.028 g), which was immediately treated with concentrated methanolic ammonia (10 mL) in a sealed vessel at 0 °C for 3 h. After removal of all the volatiles under reduced pressure, the residue was purified by silica gel column chromatography using the same solvent system to give **6** (0.018 g, 44%): UV (H_2O) λ_{max} 307.0 nm; $[\alpha]^{25}_D = +99.4°$ (c 0.17, MeOH); ^{1}H NMR (CD_3OD) δ 0.72 (m, 1 H, H-6′$_a$), 1.48 (m, 2 H, H-6′$_b$, H-5′), 3.27 (d, 1 H, $J = 11.7$ Hz, C*H*HOH), 3.82 (d, 1 H, $J = 10.8$ Hz, H-2′), 4.25 (d, 1 H, $J = 11.7$ Hz, CH*H*OH), 4.59 (d, 1 H, $J = 6.8$ Hz, H-3′), 4.85 (s, 1 H, H-4′), 6.56 (dd, 1 H, $J = 6.6$, 4.4 Hz, H-5), 8.55 (m, 1 H, H-4), 8.66 (dd, 1 H, $J = 6.6$, 2.6 Hz, H-6); ^{13}C NMR (CD_3OD) δ 11.96, 23.55, 38.42, 64.10, 67.23, 71.87, 77.19, 106.40, 148.39, 158.19, 166.94; FAB MS *m/z* (relative intensity) 239 (MH$^+$, 40). Anal. ($C_{11}H_{14}O_4N_2$) C, H, N.

Cytidine Deaminase Assay. Cytidine deaminase was measured either as already reported by following the decrease in absorbance at 282 nm that characterizes the conversion of cytidine to uridine[1,3,24] or by HPLC. For the HPLC method, the reaction mixtures (100 μL) contained 100 μM of inhibitor, various concentrations of cytidine ranging from 25 to 400 μM, and 2 μL of a human liver CDA preparation[1,26] in 10 mM Tris-HCl, pH 8.4. After a 10 min incubation at 37 °C, the reaction was terminated by heating to 95 °C for 1 min. The reaction mixtures were then centrifuged at 12 500 rpm for 2 min. The supernatant (50 μL) was analyzed by HPLC using a C18 reverse column (TOSOHAAS Bioseparation Specialists, 10 μm, 7.5 mm i.d. × 30 cm) with an eluant consisting of a mixture of 1 mL of 88% formic acid in water (4 L). Under isocratic conditions, at 1 mL/min, the retention time for cytidine was 4.66 min. Uridine, with a retention time of 5.80 min, coeluted with the inhibitor **5**. Cytidine deaminase was measured by following the decrease of the cytidine peak. Under these conditons, the calculated K_m for cytidine was 47 μM and the K_i for **5** was 38 μM.

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