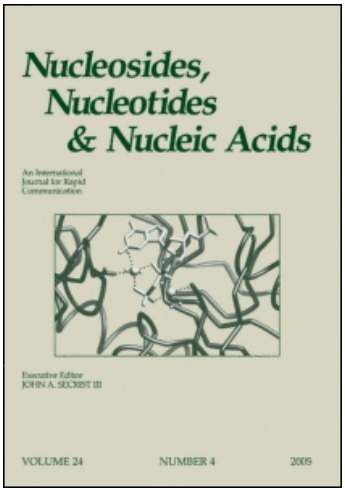

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Synthesis, Conformational Analysis, and Biological Activity of a Rigid Carbocyclic Analogue of 2'-Deoxy Aristeromycin Built on a Bicyclo[3.1.0]hexane Template

Maqbool A. Siddiqui[a]; Harry Ford Jr.[a]; Clifford George[b]; Victor E. Marquez[a]
[a] Laboratory of Medicinal Chemistry, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD [b] Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, DC

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SYNTHESIS, CONFORMATIONAL ANALYSIS, AND BIOLOGICAL ACTIVITY OF A RIGID CARBOCYCLIC ANALOGUE OF 2'-DEOXYARISTEROMYCIN BUILT ON A BICYCLO[3.1.0]HEXANE TEMPLATE#

Maqbool A. Siddiqui,[1] Harry Ford, Jr.,[1] Clifford George,[2] and Victor E. Marquez[1]*

[1]Laboratory of Medicinal Chemistry, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892 and [2]Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, DC 20375

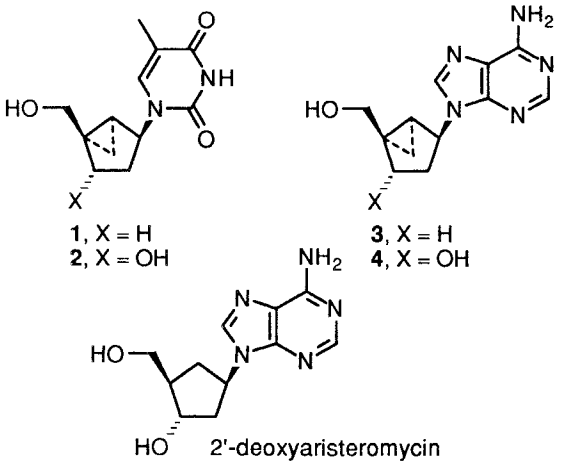
Abstract. A new chiral synthesis of the pseudosugar synthon (1R,2S,4R,5S)-1-[(benzyloxy)methyl]-2-*tert*-butyloxy-4-hydroxybicyclo[3.1.0]hexane (**12**) is reported. This compound was used as a template for the construction of carbocyclic nucleoside **4**, a conformationally rigid analogue of 2'-deoxyaristeromycin. The X-ray structure and ^1H NMR analysis confirmed the exclusive North [2'-*exo* ($_2$E)] conformation of **4** which is vastly different from that of other non-rigid carbocyclic nucleosides. Compound **4** showed good *in vitro* antiviral activity against human cytomegalovirus and EBV with minimal cytotoxicity.

Introduction.

Carbocyclic nucleosides have been synthesized mainly with the purpose of overcoming cleavage of the glycosyl bond, which can occur readily in conventional nucleosides by chemical or enzymatic means.[1] Although the expected similarity in bond lengths and bond angles between the tetrahydrofuran and cyclopentane rings allows carbocyclic analogues to behave, in some instances, as substrates or inhibitors of enzymes that activate and interconvert nucleosides and nucleotides in living cells, carbocyclic nucleosides are generally biologically less effective than their nucleoside counterparts.[2] In reality, the similarity between the tetrahydrofuran and cyclopentane ring is not all that perfect, specially when one considers that removal of the oxygen completely cancels the anomeric effect, as well as important *gauche* interactions between the furan oxygen and the 2'- and 3'-hydroxyl groups.[3] The interaction of these forces in nucleosides helps

#This manuscript is dedicated to Professor Yoshihisa Mizuno on the occasion of his 75th birthday.

determine the shape or conformation of the tetrahydrofuran ring, which generally adopts a form of ring puckering close to a 2'-*exo*/3'-*endo* ($^3T_2$, North, P = 0°) or 2'-*endo*/3'-*exo* ($^2T_3$, South, P = 180°) conformation as defined in the pseudorotational cycle.[4] In the solid state, usually one of these forms predominates, whereas in solution the two conformations appear to exist in a rapid dynamic equilibrium which may favor a specific conformer population.[2,5] In the absence of the aforementioned forces, the cyclopentane ring in carbocyclic nucleosides adopts instead an unusual 1'-*exo* ($_1$E, P = 126°) conformation that is relatively far from the typical North or South conformations observed in standard nucleosides.[6,7] Such a deviation from the conventional North or South conformations may explain, in part, the observed differences in biological potency between nucleosides and their carbocyclic counterparts.[2]



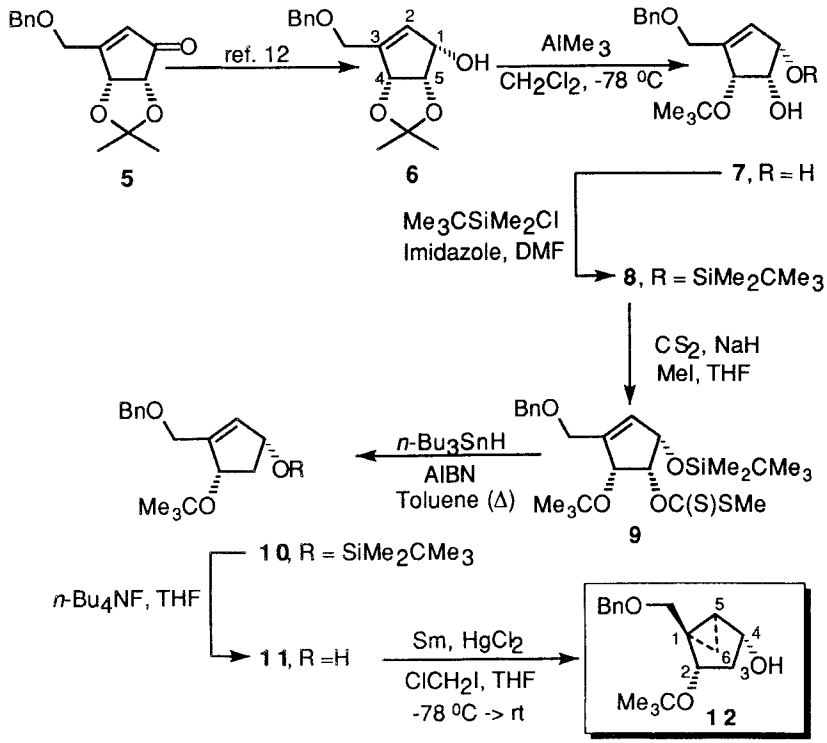
Recently, some new carbocyclic nucleosides built on a rigid bicyclo[3.1.0]hexane system have been shown to have ring conformations that mimic very well the North conformation of conventional nucleosides both in the solid state and in solution.[8-10] Indeed, the 3'-deoxy-*carba*-thymidine analogue **1**,[8,10] 2',3'-dideoxy-*carba*-adenosine **3**,[8,10] and *carba*-thymidine **2**[9] have all been shown to have rigid North 2'-*exo* ($_2$E, P = 342°) conformations very close to a 2'-*exo*/3'-*endo* form of ring pucker.[11] In the present work, the synthesis, conformational properties, and biological activity of a 2'-deoxy-*carba*-adenosine analogue (**4**) that can be regarded as a conformationally constrained 2'-deoxyaristeromycin analogue, are described. In addition, a new method for the construction of the bicyclo[3.1.0]hexane template is reported.

Chemistry.

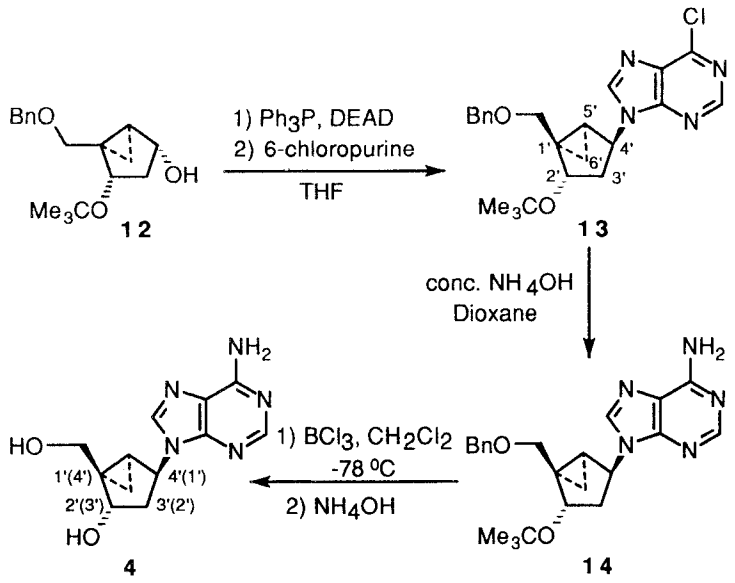
The new rigid pseudosugar template (1R,2S,4R,5S)-1-[(benzyloxy)methyl]-2-*tert*-butyloxy-4-hydroxybicyclo[3.1.0]hexane (**12**) was synthesized as illustrated in SCHEME 1. Synthesis of **12** represents a significant improvement over the previous syntheses[8-10] for two reasons: 1) compound **12** is chiral, so there is no need for optical resolution at the end of the synthesis, and 2) compound **12** represents a universal starting material for the synthesis of related carbocyclic 2'-deoxynucleoside analogues. The synthesis began with the known cyclopentenol **6** which was obtained from the sodium borohydride reduction of cyclopentenone **5** as reported previously.[12] Regioselective cleavage of the contiguous *O*-isopropylidenetriol system in **6** with trimethylaluminum[13] gave the corresponding carbocyclic 3-*tert*-butoxy-1,5-glycol **7**, which in the presence of *tert*-butyldimethylsilyl chloride reacted exclusively at the less hindered allylic alcohol position to give the protected intermediate **8**. From **8** a straightforward Barton's radical deoxygenation at C-5 proceeded uneventfully via the xanthate **9** in the presence of AIBN to give compound **10**. Lastly, deprotection of the silyl ether in **10** by fluoride ion unmasked the hydroxyl group (compound **11**) that was to direct the ensuing cyclopropanation to give exclusively the pivotal intermediate **12**. This compound was directly coupled to 6-chloropurine under Mitsunobu conditions[14] to give the protected carbocyclic nucleosides intermediate **13** (SCHEME 2). Following the aminolysis of **13** with ammonia, and the simultaneous removal of both benzyl and *tert*-butyl groups, the final target compound **4** was obtained (ribose numbering shown in parenthesis).

Conformational Analysis.

According to our previous ^{1}H NMR study performed on compound **3**,[10] the multiplicity of the pseudoanomeric proton signal for this class of carbocyclic nucleosides is very diagnostic. Indeed, the conformation of the bicyclo[3.1.0]hexane template, which is exclusively boat, causes the pseudoanomeric signal for these compounds to appear as a doublet, since two of the three dihedral angles involved approach 90°. This was also the case for compound **4** where the pseudoanomeric signal appeared at δ 4.80 (d, J = 6.7 Hz), with a similar multiplicity to the equivalent signal reported earlier for **3** (δ 4.90, d, J = 6.0 Hz).[10] A careful examination of these compounds with the aid of molecular models using the QUANTA/CHARMm program revealed that it would be very difficult for any other alternative to give the same multiplicity pattern for the pseudoanomeric proton. However, since we wanted to establish a direct correspondence between solid and solution conformations, suitable crystals of **4** were grown for X-ray analysis. Such an analysis



Scheme 1



Scheme 2

confirmed the identity of the structure as postulated by ^{1}H NMR, where the carbocyclic ring puckering corresponds to that of a 2'-*exo* ($_2$E) conformation close to the ideal 2'-*exo*/3'-*endo* ($^3T_2$) North conformation (FIGURE 1, molecule A).[15]

The unit cell of the crystal contained two nearly identical molecules (A and B) which differed only in terms of the value of the torsion angle γ that determines the orientation of the free primary hydroxyl group. The pseudorotational parameters calculated from the crystal structure were as follows: P = 339.25°, ν_{max} = 31.80°, χ = –167.6° (molecule A), and P = 342.78°, ν_{max} = 30.46°, χ = –154.8° (molecule B). These values are in perfect agreement with a 2'-*exo* ($_2$E) conformation with a theoretical value of P = 342°. The value of χ for both molecules corresponds to the characteristic *anti* orientation about the glycosyl bond. Also, the values of important dihedral angles that would determine the multiplicity of the pseudoanomeric proton signal in solution agree with the ^{1}H NMR data. The measured angles for H5'–C5'–C4'–H4' [–80.02° (molecule A) and –82.82° (molecule B)], H4'–C4'–C3'–H3'$_\beta$ [90.29° (molecule A) and 93.70° (molecule B)], and H4'–C4'–C3'–H3'$_\alpha$ [–30.90° (molecule A) and –27.10° (molecule B)] explain why a doublet is observed for the pseudoanomeric proton of **4**, and confirm that the compound has the identical conformation in solution as in the solid state.

Biological Activity.

Having forced the conformation of 2'-deoxyaristeromycin from 1'-*exo* ($_1$E) to 2'-*exo* ($_2$E), the question was to determine what kind of biological activity was associated with this change. One problem that one encounters with a rigid conformer — such as **4** — is that, as opposed to a normal nucleoside substrate, it cannot adapt to the demands of the receptor. Rigid analogues, therefore, although they may enjoy an entropic advantage, must fit perfectly to the binding site or else they would be totally excluded from it. The first step towards answering this questions was to test the compound as a substrate of one of the most common enzymes that operates on adenosine analogues, such as adenosine deaminase (ADA). Use of ADA was very selective during the deamination of racemic **3**, where only the enantiomer with a configuration equivalent to that of the "natural" nucleoside was deaminated by the enzyme.[10] Therefore, since the synthesis of **4** was chiral, the compound was expected to undergo complete deamination by ADA. Indeed, as seen in FIGURE 2, deamination of **4** to the hypoxanthine nucleoside was complete after 30 min with a $t_{1/2}$ of 3.2 min.

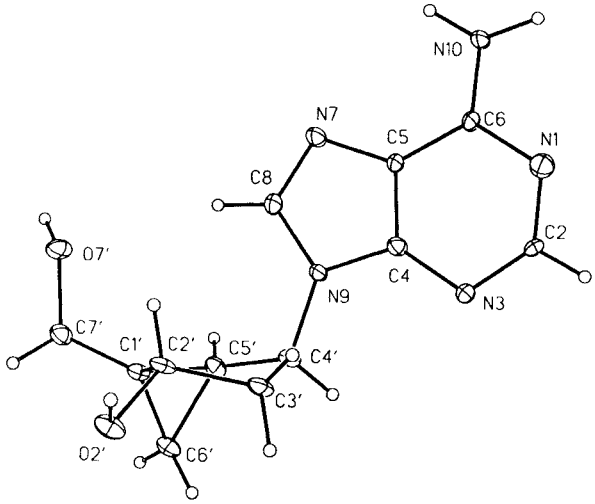


FIGURE 1. Perspective view of **4** (molecule A) as found in the crystal structure. C2' and C3' correspond, respectively, to C3' and C2' in a pentofuranose ring.

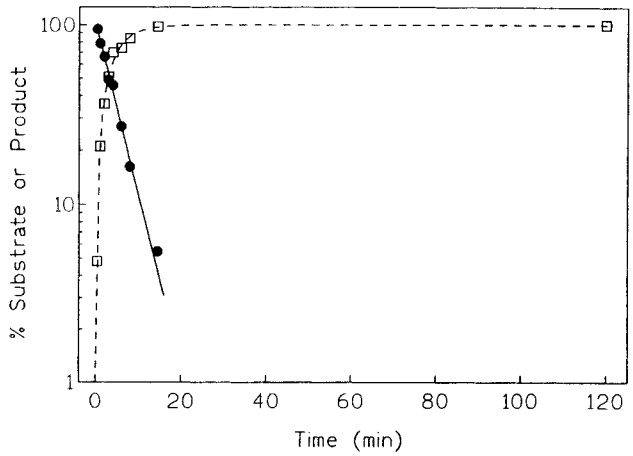


FIGURE 2. Substrate disappearance and product formation from the interaction of **4** with ADA [filled circles (substrate **4**) and open squares (hypoxanthine analogue)].

2'-Deoxyaristeromycin, the corresponding non-rigid analogue of **4**, was synthesized first in 1969[16] as a racemate and then in enantiomerically pure form in 1976.[17] To the best of our knowledge, no biological activity has ever been reported for this compound. We decided to evaluate **4** against some common viruses that most nucleoside analogues are invariably tested for antiviral activity. These included HSV-1, HSV-2, HCMV, and EBV.[18] The results on TABLE 1 indicate a weak level of activity against HSV-1 and, surprisingly, superior potency against HSV-2 which is contrary to the norm. Activity against HCMV was good (EC_{50} = 3.1 μg/mL) being only ten-times less potent than gancyclovir. However, if one considers that **4** was equally non-toxic to rapidly proliferating cells as to stationary cells, its antiviral selectivity is superior to that of gancyclovir which showed increased cytotoxicity towards rapidly dividing cells. Good activity against EBV (EC_{50} = 1.5 μg/mL) was also observed, which was only fifteen-fold weaker than that of acyclovir. These data are preliminary and additional studies are planned. However, since a number of adenosine analogues that are active against HCMV are also good inhibitors of the enzyme *S*-adenosylhomocysteine hydrolase (AdoHcy-ase), we investigated the properties of **4** against this enzyme. Activity against AdoHcy-ase was very low showing only 20% inhibition at 100 μM concentration.[19] This indicates that the antiviral activity of **4** is independent of its interaction with AdoHcy-ase and that the compound should be inactive against viruses that are sensitive to the inhibition of this enzyme.

In conclusion, we have synthesized a 2'-deoxycarbocyclic nucleoside analogue of 2'-deoxyaristeromycin with a rigid North conformation. Such a rigid conformation appears to preclude interaction with AdoHcy-ase, an enzyme that accepts as substrates various carbocyclic nucleoside analogues.[20] On the other hand, the rigid conformation appears to favor a selective interaction with target enzymes that are essential for the replication of HCMV and EBV viruses. Finally, although **4** is a carbocyclic mimic of 2'-deoxyadenosine, the conformational preference of the latter in solution is 2'-*endo*/3'-*exo* (South), opposite to that of **4**. In fact, **4** can be considered to be a 2'-deoxyribo mimic that conformationally looks more like a ribo mimic in a 2'-*exo*/3'-*endo* (North) conformation. It is possible, therefore, that if **4** is incorporated into a growing DNA chain, it could disrupt the conformation of the polymer where the sugar moieties are preferentially puckered 2'-*endo*/3'-*exo* (South) as in B-DNA.

EXPERIMENTAL

General.

All chemical reagents were commercially available. Melting points were determined on a Mel-Temp II apparatus, Laboratory Devices, USA, and are uncorrected. Column

TABLE 1. Antiviral Activity Spectrum

		Compound 4			Positive Control	
Virus[a]	Host Cell[b]	EC_{50}[c]	CC_{50}[d]	SI[e]	Drug[f]	EC_{50}[c]
HSV-1	HFF	72.0	>100	>1.4	ACV	0.8
HSV-2	HFF	13.9	>100	>7.2	ACV	4.0
HCMV	HFF	3.1	>100	>32.2	GCV	0.3
EBV	Daudi	1.5	>100	>66.7	ACV	0.1
—	Growing HFF	—	>100	—	ACV	40.0

[a]HSV-1 = herpes simplex type 1; HSV-2 = herpes simplex type 2; HCMV = human cytomegalovirus; EBV = Epstein-Barr virus. [b]HFF = human foreskin fibroblasts. [c]EC_{50} = inhibitory concentration in μg/mL required to reduce virus-induced cytopathogenicity (or viral capsid antigen for EBV) by 50%. [d]CC_{50} = cytotoxic concentration in μg/mL that produces 50% of cell death. [e]SI = selectivity index (CC_{50}/EC_{50}). [f]ACV = acyclovir; GCV = gancyclovir.

chromatography was performed on silica gel 60, 230-400 mesh (E. Merck), and analytical TLC was performed on Analtech Uniplates silica gel GF. Proton and ^{13}C-NMR spectra were recorder 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. 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. Uv spectra were recorded in a Shimadzu Model UV-2101PC spectrometer. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA.

Adenosine Deaminase Studies.

Adenosine deaminase (ADA) was purchased from Boehringer Mannheim (Lot # 12082828-41/Feb 94 (tag: MH 7-20) 2984 units/mL @ 25 °C. Compound **4** (50 μM) was incubated with 1.0 unit of ADA at 37 °C in 10 mM phosphate buffer, pH 7.1 in a volume

of 1 mL. At timed intervals, 50 μL aliquots of this solution were quenched with 450 μL of water containing the ADA inhibitor deoxycoformycin (4 μM). Hydrolysis kinetics was followed by HPLC with on-the-fly UV spectral characterization of all chromatographic components. HPLC conditions were as follows: pump: Waters 6000A; detector: Perkin-Elmer LC 235 Diode Array @ 260 nm, AUFS 0.05; column: Beckam/Altex 5 μ ODS, 250 x 4.6 mm; mobile phase: CH_3CN 7.5% in 0.01 M phosphate buffer, pH 7.0; flow rate: 1 mL/min. Curve fitting of the data to an exponential decay equation ($y = Aexp^{(-Bx)}$) for substrate disappearance and an exponential association equation ($y = A[1-exp^{(-Bx)}]$) for the inosine analogue product formation yielded the curves shown on in FIGURE 2. The hydrolysis rate constant (k/ADA) and $t^{1/2}$ of decay or formation were obtained using GraphPAD Inplot curve fitting program. Compound **4** (t_R = 9.9 min) was chromatographically pure under the isocratic conditions described, and the ADA hydrolysis product (t_R = 5.4 min) appeared as a less lipophilic peak.

X-ray analysis.

Crystal data for **4**•2H_2O: $C_{12}H_{15}N_5O_2$•2(H_2O), FW = 297.32, mp 259-261 °C (dec.). Triclinic, space group P1, a = 6.1740 (10), b = 8.270 (2), c = 14.760 (2) Å, α = 94.280 (10), β = 100.250 (10), γ = 102.240 (10)°, V = 719.8 (2) Å^3, Z = 2, D_c = 1.372 mg mm^{-3}, λ(Cu Kα) = 1.54178 Å, μ = 0.881 mm^{-1}, $F(000)$ = 316, T = 223 (2) °K. Final residuals were R = 0.0454 for 2040 reflections $I>2\sigma(I_0)$. A perspective view of the structure computed from the final relative atomic coordinates (TABLE 2) is shown in FIGURE 1.

Synthetic Chemistry.

(1S,4R,5S)-3-[(Benzyloxy)methyl]-4,5-*O*-isopropylidene-2-cyclopenten-1-ol (**6**).
This compound was prepared from **5** according to the procedure of Marquez, et al.[12]

(1S,4R,5S)-3-[(Benzyloxy)methyl]-4-*tert*-butyloxy-5-hydroxy-2-cyclopenten-1-ol (**7**).
A solution of **6** (0.61 g, 2.20 mmol) was stirred in anhydrous CH_2Cl_2 (25 mL) at –78 °C and treated with a solution of trimethylaluminum in toluene (2 M, 7.8 mL, 15.6 mmol). After the addition, the reaction was allowed to reach room temperature and stirring was continued for 18 h. The reaction mixture was cooled again to –78 °C and quenched with

TABLE 2. Atomic coordinates [x 10^4] and equivalent isotropic displacement parameters [$Å^2$ x 10^3] for **4**. U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.

	x	y	z	U (eq)
N(1A)	1128 (8)	−1508 (6)	7392 (3)	39 (1)
C(2A)	2613 (11)	−1764 (7)	6858 (4)	39 (2)
N(3A)	3765 (8)	−725 (6)	6382 (3)	33 (1)
C(4A)	3302 (9)	798 (7)	6487 (4)	28 (1)
C(5A)	1832 (9)	1258 (6)	7013 (4)	25 (1)
C(6A)	692 (9)	29 (6)	7470 (3)	28 (1)
N(7A)	1790 (8)	2926 (6)	6960 (3)	33 (1)
C(8A)	3244 (10)	3411 (7)	6423 (4)	35 (1)
N(9A)	4222 (7)	2185 (5)	6118 (3)	28 (1)
N(10A)	−804 (8)	265 (6)	7998 (3)	37 (1)
C(1'A)	6364 (8)	4839 (7)	4734 (4)	27 (1)
C(2'A)	4397 (9)	3576 (7)	4148 (4)	29 (1)
C(3'A)	4639 (9)	1887 (7)	4460 (4)	32 (1)
C(4'A)	5863 (9)	2277 (7)	5499 (4)	32 (1)
C(5'A)	7254 (9)	4027 (7)	5566 (4)	29 (1)
C(6'A)	8636 (9)	4392 (8)	4822 (4)	34 (1)
O(2'A)	4436 (7)	3645 (5)	3185 (2)	39 (1)
C(7'A)	6316 (9)	6642 (7)	4800 (4)	35 (1)
O(7'A)	4271 (6)	6845 (5)	5095 (3)	39 (1)
N(1B)	−2839 (8)	−6956 (6)	8507 (3)	36 (1)
C(2B)	−4437 (11)	−7472 (7)	8998 (4)	42 (2)
N(3B)	−5610 (8)	−6609 (6)	9421 (3)	39 (1)
C(4B)	−5066 (8)	−5000 (6)	9288 (3)	24 (1)
C(5B)	−3542 (8)	−4297 (6)	8772 (3)	24 (1)
C(6B)	−2301 (9)	−5341 (6)	8384 (3)	28 (1)
N(7B)	−3460 (7)	−2607 (5)	8759 (3)	29 (1)
C(8B)	−4919 (9)	−2352 (6)	9263 (4)	30 (1)
N(9B)	−5963 (7)	−3727 (5)	9601 (3)	25 (1)
N(10B)	−657 (7)	−4794 (6)	7929 (3)	29 (1)
C(1'B)	−7206 (9)	−1092 (6)	11070)4)	28 (1)
C(2'B)	−5581 (9)	−1982 (7)	11599 (3)	30 (1)
C(3'B)	−6449 (9)	−3844 (7)	11226 (4)	34 (1)
C(4'B)	−7610 (9)	−3841 (7)	10223 (4)	29 (1)
C(5'B)	−8516 (8)	−2270 (6)	10212 (3)	25 (1)
C(6'B)	−9689 (9)	−1893 (7)	10955 (4)	32 (1)
O(2'B)	−5327 (7)	−1680 (6)	12577 (3)	43 (1)
C(7'B)	−6393 (9)	754 (7)	11074 (4)	34 (1)
O(7'B)	−8114 (7)	1465 (5)	10584 (3)	42 (1)
O(1S)	8162 (7)	4833 (6)	2414 (3)	43 (1)
O(2S)	177 (7)	2296 (5)	2131 (3)	42 (1)
O(3S)	1297 (7)	7485 (5)	3518 (3)	46 (1)
O(4S)	−1117 (7)	−4 (6)	3346 (3)	51 (1)

an aqueous saturated solution of NH_4Cl (10 mL) (*caution: this is a very exothermic process and the addition of NH_4Cl should be done very slowly).* After reaching room temperature, the suspension was filtered and the solid cake was washed with $CHCl_3$ (25 mL). The filtrate was collected and extracted with $CHCl_3$ (3 x 50 mL), and the combined organic extract was washed with water (50 mL), dried (Na_2SO_4), and concentrated under vacuum. The crude product was purified by flash column chromatography over silica gel with a 0-50% gradient of EtOAc in hexane as eluant to give 0.349 g (54%) of the title compound **7** as a thick oil; ^1H NMR ($CDCl_3$) δ 7.20-7.40 (m, 5 H, Ph), 5.85 (br s, 1 H, H-2), 4.50 (m, 3 H, H-1, $PhCH_2O$), 4.42 (d, J = 5.4 Hz, 1 H, H-4), 4.15 (t, J = 5.4 Hz, 1 H, H-5), 4.06 (br s, 2 H, $PhCH_2OCH_2$), 1.25 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ 141.55, 137.96, 132.26, 128.38, 127.77, 127.69, 75.50, 74.17, 73.42, 72.76, 70.86, 66.11, 28.13. Anal. Calcd for $C_{17}H_{24}O_4$: C, 69.83; H, 8.27. Found: C, 69.57; H, 8.27.

(1S,4R,5S)-1-(*tert*-Butyldimethylsilyloxy)-3-[(Benzyloxy)methyl]-4-*tert*-butyloxy-5-hydroxy-2-cyclopentene (**8**).

A solution of **7** (8.04 g, 27.5 mmol) and imidazole (7.05 g, 103.55 mmol) in anhydrous DMF (80 mL) was treated with *tert*-butyldimethylsilyl chloride (6.70 g, 44.45 mmol). The mixture was stirred at room temperature under a blanket of argon for 40 min and quenched by the slow addition of water (100 mL). The reaction mixture was extracted with EtOAc (3 x 100 mL), and the combined organic extract was washed with brine (2 x 100 mL) and dried (Na_2SO_4). The solvent was evaporated and the product was purified by flash column chromatography over silica gel with a 0-10% gradient of EtOAc in hexane as eluant to give 9.77 (87.4%) of pure **8** as an oil; ^1H NMR ($CDCl_3$) δ 7.20-7.40 (m, 5 H, Ph), 5.75 (br s, 1 H, H-2), 4.50 (m, 3 H, H-1, $PhCH_2O$), 4.35 (d, J = 5.2 Hz, 1 H, H-4), 4.10 (m, 3 H, H-5, $PhCH_2OCH_2$), 1.25 (s, 9 H, $C(CH_3)_3$), 0.90 (s, 9 H, $SiC(CH_3)_3$), 0.1 (s, 6 H, $Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 143.20, 138.11, 130.21, 128.36, 127.79, 127.63, 74.54, 74.47, 73.76, 72.85, 72.45, 66.65, 28.29, 25.88, 18.30. 1.22. Anal. Calcd for $C_{23}H_{38}O_4Si \cdot 0.5H_2O$: C, 66.46; H,9.46. Found: C, 66.41; H,9.31.

(1S,4R,5S)-1-(*tert*-Butyldimethylsilyloxy)-3-[(Benzyloxy)methyl]-4-*tert*-butyloxy-5-[(methylthio)thiocarbonyloxy]-2-cyclopentene (**9**).

A solution of **8** (9.77 g, 24.02 mmol) in anhydrous THF (100 mL) was treated with carbon disulfide (10.2 mL, 168.8 mmol). The mixture was stirred at 0 °C for 5 min, and

NaH (80% suspension in oil, 2.2 g, 73.3 mmol) was added in portions. After the addition, the mixture was stirred at room temperature for 30 min. Methyl iodide (19.5 mL, 313.2 mmol) was added and after further stirring for 30 min, the reaction mixture was cooled to 0 °C, and excess NaH was destroyed by the slow addition of water (*caution: exothermic process*). The organic layer was separated and the aqueous layer was extracted with EtOAc (3 x 100 mL). The combined organic extract was dried (Na_2SO_4) and concentrated under vacuum. The crude product was purified by flash column chromatography over silica gel using a 0-5% gradient of EtOAc in hexane to give 9.83 g (82.4%) of pure **9** as an oil; ^{1}H NMR ($CDCl_3$) δ 7.20-7.40 (m, 5 H, Ph), 6.30 (t, J = 5.3 Hz, 1 H, H-5), 5.80 (br s, 1 H, H-2), 4.70 (d, J = 5.3 Hz, 1 H, H-4), 4.50 (m, 3 H, H-1, $PhCH_2O$), 4.15 (br s, 2 H, $PhCH_2OC\underline{H}_2$), 2.50 (s, 3 H, SCH_3), 1.20 (s, 9 H, $C(CH_3)_3$), 0.85 (s, 9 H, $SiC(CH_3)_3$), 0.00 and 0.01 (singlets, 6 H, $Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 216.09, 142.71, 138.03, 130.53, 128.38, 127.86, 127.69, 83.03, 74.51, 73.33, 72.97, 72.68, 66.21, 28.19, 26.00, 18.50, 18.03, 1.56. Anal. Calcd for $C_{25}H_{40}O_4S_2Si \cdot 0.25H_2O$: C, 59.90; H, 8.10, S, 12.77. Found: C, 59.84; H, 8.10; S, 12.72.

(1S,4R)-1-(*tert*-Butyldimethylsilyloxy)-3-[(Benzyloxy)methyl]-4-*tert*-butyloxy-2-cyclopentene (**10**).

A solution of **9** (9.82 g, 19.76 mmol) and azobis(isobutyronitrile) (AIBN, 2.04 g, 12.42 mmol) in anhydrous toluene (100 mL) was heated to ca. 50 °C under a blanket of argon, and treated slowly with tri-*n*-butyltin hydride (22 mL, 81.8 mmol). After the addition was complete, the mixture was heated (oil bath temp. 120 °C) for 1.5 h and then cooled to room temperature. The solvent was evaporated and the crude product was purified by flash column chromatography over silica gel with a gradient of 0-5% EtOAc in hexane to give **10** (5.94 g, 77%) as an oil; ^{1}H NMR ($CDCl_3$) δ 7.30-7.60 (m, 5 H, Ph), 5.75 (br s, 1 H, H-2), 4.60 (distorted triplet, 1 H, H-4), 4.50 (AB multiplet, 2 H, $PhCH_2O$), 4.41 (distorted triplet, 1 H, H-1), 4.10 (br s, 2 H, $PhCH_2OC\underline{H}_2$), 2.66 (dt, J = 13.2, 7.2 Hz, 1 H, H-5_a), 1.60 (dt, J = 13.2, 5.5 Hz, 1 H, H-5_b), 1.20 (s, 9 H, $C(CH_3)_3$), 0.90 (s, 9 H, $SiC(CH_3)_3$), 0.05 (s, 6 H, $Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 143.83, 132.31, 127.77, 127.53, 74.01, 73.43, 73.26, 72.77, 66.52, 45.97, 28.56, 25.89, 18.14, 1.14. Anal. Calcd for $C_{23}H_{38}O_3Si \cdot 0.5H_2O$: C, 69.12; H, 9.83. Found: C, 69.21; H, 9.71.

(1S,4R)-3-[(Benzyloxy)methyl]-4-*tert*-butyloxy-2-cyclopenten-1-ol (**11**).

A solution of **10** (4.82 g, 12.36 mmol) in anhydrous THF (80 mL) was treated with a solution of tetrabutylammonium fluoride in THF (1 M, 51 mL), and the resulting mixture

was stirred at room temperature overnight. The solvent was evaporated and the residue was treated with water and extracted with EtOAc (3 x 100 mL). The combined organic extract was washed with brine (2 x 100 mL) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography over silica gel using a gradient of 50-66% EtOAc in hexane to give **11** (3.152 g, 92%) as a clear oil; ^{1}H NMR ($CDCl_3$) δ 7.30-7.40 (m, 5 H, Ph), 5.90 (br s, 1 H, H-2), 4.60 (m, 1 H, H-4), 4.50 (AB multiplet, 2 H, $PhCH_2O$), 4.45 (m, 1 H, H-1), 4.10 (br s, 2 H, $PhCH_2OCH_2$), 2.70 (dt, J = 14.2, 7.2 Hz, 1 H, H-5_a), 1.95 (br s, 1 H, OH), 1.57 (dt, J = 14.2, 3.8 Hz, 1 H, H-5_b), 1.20 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ 145.49, 138.13, 132.39, 128.35, 127.76, 127.61, 74.44, 73.95, 73.48, 72.77, 66.42, 45.21, 28.48. Anal. Calcd for $C_{17}H_{24}O_3 \cdot 0.75H_2O$: C, 70.43; H, 8.86. Found: C, 70.62; H, 8.54.

(1R,2S,4R,5S)-1-[(Benzyloxy)methyl]-2-*tert*-butyloxy-4-hydroxybicyclo[3.1.0]hexane (**12**).

Samarium metal (2.30 g, 15.29 mmol) was placed in a flask and dried with a flame under a stream of argon. Anhydrous THF (10 mL) and a solution of mercuric chloride (0.40 g, 1.47 mmol) in 3 mL of THF were added and the mixture was stirred for 10 min prior to the addition of a solution of alcohol **11** (1.0 g, 3.61 mmol) in THF (10 mL). The reaction mixture was cooled to –78 °C and treated with chloroiodomethane (1.15 mL, 15.71 mmol). The resulting mixture was continuously stirred starting at –78 °C and allowed to reach ambient temperature during the course of the night. The following day, the reaction was quenched with a saturated solution of K_2CO_3 (50 mL) and extracted with ether (3 x 75 mL). The combined organic extract was washed with brine (2 x 75 mL), dried (Na_2SO_4), filtered and evaporated to dryness. The residue was purified by flash column chromatography over silica gel using a gradient of 0-50% EtOAc in hexane to give **12** (1.01 g, 96%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 7.30-7.40 (m, 5 H, Ph), 4.50 (AB q, J = 12 Hz, 2 H, $PhCH_2O$), 4.35 (m, 1 H, H-2), 4.25 (t, J = 8.1 H, 1 H, H-4), 3.92 (AB d, J = 10.2 Hz, 1 H, $PhCH_2OCHH$), 2.93 (AB d, J = 10.2 Hz, 1 H, $PhCH_2OCHH$), 2.18 (dt, J = 13.1, 7.6 Hz, 1 H, H-3_a), 1.50 (m, 2 H, H-3_b, OH), 1.15 (m, 11 H, H-5, H-6_{endo}, $C(CH_3)_3$), 0.42 (dd, J = 7.7, 5.6 Hz, 1 H, H-6_{exo}); ^{13}C NMR ($CDCl_3$) δ 128.34, 127.73, 127.58, 72.88, 71.27, 70.23, 69.33, 38.31, 33.20, 28.52, 26.52, 6.31. Anal. Calcd for $C_{18}H_{26}O_3 \cdot 0.25H_2O$: C, 73.31; H, 9.06. Found: C, 73.34; H, 8.98.

(1R,2S,4S,5S)-1-[(Benzyloxy)methyl]-2-*tert*-butyloxy-4-(6-chloro-9-purinyl)-bicyclo[3.1.0]hexane (**13**). A stirred solution of triphenyl phosphine (0.42 g, 1.60 mmol) in anhydrous THF (5 mL) was treated with diethyl azodicarboxylate (DEAD, 0.26 mL, 1.64 mmol) at room temperature. After 20 min, a suspension of 6-chloropurine (0.248 g, 1.60 mmol) in anhydrous THF (15 mL) was added and 20 min later the mixture became homogeneous. A solution of alcohol **12** (0.31 g, 1.06 mmol) in THF (10 mL) was then added and the resulting mixture was stirred at room temperature for 72 h. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography over silica gel using a gradient of 0-50% EtOAc in hexane to give **13** (0.263 g, 58%) as a solid, mp 112-113 °C; ^{1}H NMR ($CDCl_3$) δ 9.10 (s, 1 H, H-2), 8.70 (s, 1 H, H-8), 7.30-7.40 (m, 5 H, Ph), 5.19 (d, J = 5.9 Hz, 1 H, H-4'), 4.60 (m, 3 H, H-2', $PhCH_2O$), 4.05 (AB d, J = 9.9 Hz, 1 H, $PhCH_2OCHH$), 3.05 (AB d, J = 9.9 Hz, 1 H, $PhCH_2OCHH$), 1.85 (m, 2 H, H-3'$_{a,b}$), 1.55 (dd, J = 8.5, 3.8 Hz, 1 H, H-6'$_{endo}$), 1.05 (m, 10 H, H-5', $C(CH_3)_3$), 0.72 (dd, J = 8.2, 6.4 Hz, 1 H, H-6'$_{exo}$). Anal. Calcd for $C_{23}H_{27}ClN_4O_2$•0.5 H_2O: C, 63.36; H, 6.47; N, 12.85. Found: C, 63.34; H, 6.48; N, 12.88.

(1R,2S,4S,5S)-1-[(Benzyloxy)methyl]-2-*tert*-butyloxy-4-(6-amino-9-purinyl)-bicyclo[3.1.0]hexane (**14**). A solution of **13** (0.202 g, 0.47 mmol) in dioxan (15 mL) was treated with conc ammonium hydroxide (10 mL)and heated at 65 °C in a pressure bottle. After 14 h, the solvent was removed under reduced pressure and the crude product purified by flash column chromatography over silica gel using EtOAc to give **14** (0.146 g, 76%) as an oil; ^{1}H NMR ($CDCl_3$) δ 8.70 (s, 1 H, H-2), 8.30 (s, 1 H, H-8), 7.30-7.40 (m, 5 H, Ph), 6.20 (br s, 2 H, NH_2), 5.10 (d, J = 6.1 Hz, 1 H, H-4'), 4.60 (m, 3 H, H-2', $PhCH_2O$), 4.10 (AB d, J = 9.9 Hz, 1 H, $PhCH_2OCHH$), 3.10 (AB d, J = 9.9 Hz, 1 H, $PhCH_2OCHH$), 1.75 (m, 2 H, H-3'$_{a,b}$), 1.55 (m, 1 H, H-6'$_{endo}$), 1.05 (m, 10 H, H-5', $C(CH_3)_3$), 0.72 (m, 1 H, H-6'$_{exo}$); ^{13}C NMR δ 155.17, 152.08, 149.46, 139.79 137.93, 128.53, 127.83, 119.51, 73.37, 73.24, 71.04, 70.29, 54.48, 38.75, 33.53, 28.37, 24.30, 10.37. Anal. Calcd for $C_{23}H_{28}N_5O_2$•H_2O: C, 65.07; H, 7.12; N, 16.49. Found: C, 64.89; H, 6.85; N, 16.12.

(1R,2S,4S,5S)-1-Hydroxymethyl-2-hydroxy-4-(6-amino-9-purinyl)bicyclo[3.1.0]hexane (**4**).

A stirred solution of **14** (0.130 g; 0.319 mmol) in anhydrous CH_2Cl_2 (10 mL) and under a blanket of argon was cooled to –78 °C, and treated with a solution of BCl_3 (1 M/CH_2Cl_2,

3 mL). After 4 h, the reaction was quenched with MeOH (ca. 1 mL) and allowed to reach room temperature. The solvent was removed under reduced pressure and the residue was co-evaporated with additional MeOH (4 x 5 mL). The final residue was dissolved in MeOH (30 mL) and neutralized to pH 12 with 25% aqueous ammonium hydroxide. The volatiles were removed and the crude residue was purified by reverse phase chromatography (Baker octadecyl C-18) using a gradient of 0-20% MeOH in water to give **4** (0.058 g, 70%) as a solid, mp 259-261 °C (dec.); $[\alpha]_D^{25}$ –16.9 ° (c 0.13, DMF); ^{1}H NMR (D_2O) δ 8.20 (s, 1 H, H-2), 8.00 (s, 1 H, H-8), 4.80 (d, *J* = 6.7 Hz, 1 H, H-4'), 4.70 (t, *J* = 8.5 Hz, 1 H, H-2'), 4.00 (AB d, *J* = 12.3 Hz, 1 H, CHHOH), 3.31 (AB d, *J* = 12.3 Hz, 1 H, CHHOH), 2.01 (dd, *J* = 14.8, 7.9 Hz, 1 H, H-3'$_a$), 1.70 (m 2 H, H-3'$_b$, H-5'), 0.91 (dd, *J* = 5.7, 3.9 Hz, 1 H, H-6'$_{endo}$), 0.72 (distorted triplet, 1 H, H-6'$_{exo}$); ^{13}C NMR (D_2O/MeOH-d_6) δ 156.50, 153.23, 149.86,141.18, 120.10, 72.01, 63.57, 56.38, 37.70, 36.21, 26.50, 10.60; FAB MS *m/z* (relative intensity) 262 (MH+, 100), 136 (b + 2H, 58). Anal. Calcd for $C_{12}H_{15}N_5O_2 \cdot 0.33\ H_2O$: C, 53.93; H, 5.90; N, 26.21. Found: C, 53.78; H, 5.66; N, 26.04.

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