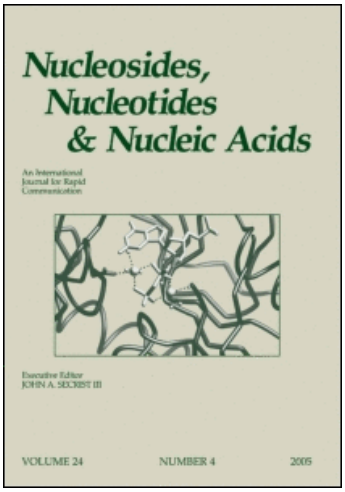

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Spin Labeled Nucleoside Analogues: 4'-Hydroxymorpholin-2'-Ylpurines and Pyrimidines

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SPIN LABELED NUCLEOSIDE ANALOGUES: 4'-HYDROXYMORPHOLIN-2'-YLPURINES AND PYRIMIDINES

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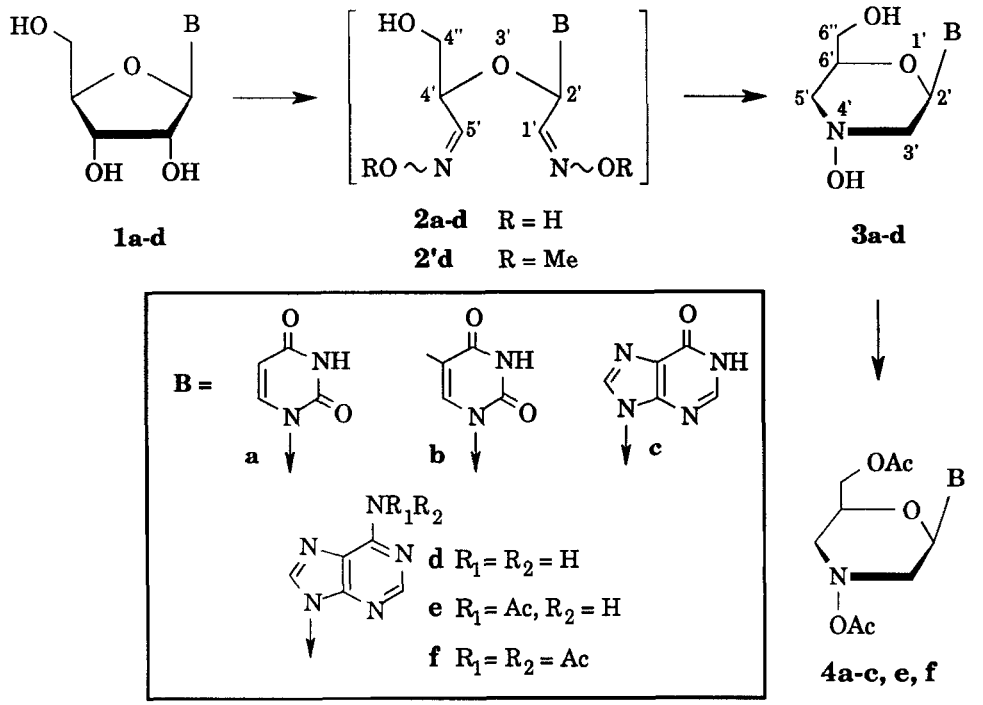
Abstract: Upon borane-pyridine reduction, a series of nucleoside dialdehyde dioximes **2** underwent cyclization to the corresponding 4'-hydroxymorpholin-2'-ylpurines or pyrimidines **3** from which the peracetyl derivatives **4** were prepared. At room temperature, compounds **3** and **4** exist as a mixture of invertomers in which the *4'S* (equatorial 4'-OH or 4'-OAc) predominates. A 14 kcal/mol, nitrogen inversion barrier was estimated from variable temperature experiments. N.O.E. and $^3J_{CH}$ measurements established the *anti* conformation of the base-"sugar" bond. Compounds **3** spontaneously oxidized to the corresponding aminoxyl free radicals, EPR spectra of which showed that they existed in a chair conformation.

Most generally, nucleoside analogues in which the ribose moiety has been replaced with a pyranosyl group do not bear interesting antiviral properties. A notable exception concerns the activity of such compounds against HIV virus strains.[1] On the other hand, spin labeled close analogues of sugars and nucleosides[2] constitute useful tools for the study of the structure and, hopefully, the pharmacological behavior of these biologically important molecules. We describe here the synthesis and conformational properties of 4'-hydroxymorpholin-2'-ylpurines and pyrimidines. These compounds are analogues of pyranosyl nucleosides in which the C-3'



Scheme 1

asymmetric carbon atom would be replaced with a nitrogen atom. Owing to the easiness of the nitrogen inversion, these compounds represent both C-3' epimers. For the sake of uniformity in numbering, the morpholine ring atoms will be primed regardless of the nature of the heterocyclic base which will be given priority over the morpholine ring in every case. Some of these results have been reported in a preliminary form.[3]

Nucleosides **1a-d** were converted to the corresponding dialdehydes *via* a classical[4] periodic oxidation and the dialdehydes treated with hydroxylamine to give the corresponding dioximes **2a-d** which were not, as a rule, isolated in a pure form but directly converted to the morpholine derivatives **3a-d** (Scheme 1).

The dioxime **2'd** was also prepared to serve as a model compound for configurational assignments in this series. The four possible geometric isomers of **2'd** were present in the equilibrium and their NMR data allowed an

TABLE 1. ^{1}H-NMR Data of **2'd**.

Configuration	Chemical shifts					Interproton couplings		
(% in mixture)	H-1'	H-2'	H-4'	H-5'	H-4"	$J_{1',2'}$	$J_{4',5'}$	$J_{4',4"}$
1'E,5'E (50)	7.80	6.38	3.95	7.32	3.59	5.0	7.0	7.5
					3.50			
1'E,5'Z (20)	7.82	6.35	4.59	6.78	3.60	5.0	6.0	7.0
					3.50			
1'Z,5'E (25)	7.32	6.70	3.94	7.32	3.58	5.0		
					3.50			
1'Z,5'Z (5)	7.32	6.69	4.59	6.78	3.58			
					3.50			

unequivocal attribution of their respective configurations (TABLE 1). The rule[5] stating that methine (α) protons of aldoximes are deshielded when *cis* to the OR group whereas the opposite applies to β protons, found a particularly clear application with compounds **2'd** where α protons (H-1' and H-5') are deshielded by *ca* 0.5 ppm when *cis* to OMe and β protons in the same situation shielded by *ca* 0.30 ppm (H-2') or 0.65 ppm (H-4'). This allowed an easy configurational assignment *(1'E,5'E)* in the case of the unique isolated isomer of **2d**. Acyclonucleosides **2d** and **2'd** which have been cited in a preliminary communication[6] showed some biological activity against rhinovirus RV3.

Upon reduction (BH_3-pyridine) compounds **2a-d** were cyclized to the morpholine derivatives **3a-d** in yields ranging from 58% to 83.5%. Except for **3c** which is a very hygroscopic compound for which no correct elementary analysis could be obtained, the other members of the series were high-melting solids. The structure of **3c** was established from its spectroscopic data and its acetylation to **4c**. Compounds **3a** and **3b** were also acetylated in good yields to **4a** and **4b** respectively. Acetylation of **3d** gave a mixture of **4e** and **4f** from which the stable **4e** was isolated in pure form. The *N,N*-diacetyl

TABLE 2. ^{1}H-NMR Data (200 MHz) of Compounds **3** and **4**: Chemical Shifts (δ ppm).

Compd	t (°C)	H-2'	H-3'$_{ax}$	H-3'$_{eq}$	H-5'$_{ax}$	H-5'$_{eq}$	H-6'	Ha,b-6"	Others
3a	80[a]	5.72	2.59	3.09	2.42	3.12	3.85	3.49	8.20 (NH), 7.62 (H-6) 5.59 (H-5)
3b	60[b]	6.42	3.02	3.69	2.98	3.61	4.32	3.99	7.60 (H-6), 1.89 (CH_3)
3c	100[a]	5.91	3.15	3.35	2.57	3.17	4.00	3.48	8.07 (NH), 8.03 (H-2) 8.03 (H-8)
3d	80[a]	5.94	3.20	3.60	2.57	3.18	3.99	3.50	8.22 (H-2), 8.18 (H-8) 8.14 (NOH), 6.79 (NH_2), 4.46 (CH_2OH)
4a	55[c]	6.03	2.76	3.61	2.73	3.61	4.28	4.22	8.52 (NH), 7.40 (H-6) 6.03 (H-5), 2.10, 2.12 (OAc)
4b[d]	55[e]	5.59	3.01	3.49	2.81	3.01	4.25	4.21	9.86 (NH), 7.86 (H-6), 2.06, 2.03 (OAc), 1.87 (CH_3)
(4'R)-**4b**[d]	-40[e]	6.22	3.48	3.40	3.03	3.33	4.57	3.95-4.20	10.05 (NH), 7.78 (H-6), 1.76 (Me)
(4'S)-**4b**[d]	-40[e]	5.88	2.96	3.55	2.64	3.39	4.02-4.20	4.02-4.20	10.05 (NH), 7.77 (H-6), 1.77 (Me)
4c	100[a]	6.01	3.56	3.62	2.89	3.42	4.28	4.12 4.18	12.4 (NH), 8.17 (H-2), 7.98 (H-8), 2.02, 2.08 (OAc)
4e	55[c]	6.28	4.49	3.92	3.01	3.59	4.48	4.34	8.77 (H-2), 8.47 (NH), 8.21 (H-8), 2.78 (NAc), 2.20 (2xOAc)
4f	55[c]	6.27	3.41	3.88	2.94	3.53	4.40	4.25	8.98 (H-2), 8.29 (H-8), 2.39 (2xNAc), 2.11 (2xOAc)

a. DMSO-d_6. b. Pyridine-d_5. c. $CDCl_3$. d. 400 MHz. e. $(CD_3)_2CO$.

TABLE 3. ^{1}H-NMR of Compounds **3** and **4** (Same Conditions as in TABLE 2). Coupling Constants (J in Hz).

Compd	$J_{2',3'ax}$	$J_{2',3'eq}$	$J_{5'ax,6'}$	$J_{5'eq,6'}$	$J_{3'ax,3'eq}$	$J_{5'ax,5'eq}$	$J_{5'ax,3'eq}$	$J_{6',6"a}$	$J_{6',6"b}$
3a	10.5	2.5	11.0	~2.0	10.5	11.0	~1.0	5.0	5.0
3b	10.0	~2.0	11.0	2.0	11.0	11.0	~1.0	4.5	4.5
3c	10.5	2.8	11.0	2.0	10.5	11.0	1.0	5.0	5.0
3d	10.5	2.3	11.0	1.0	10.5	11.0	1.0	5.0	5.0
4a	10.5	2.3	11.0	1.0	10.5	11.0	1.0	3.5	3.5
4b	10.0	2.0	10.5	1.5	10.5	10.5	1.0	-	-
(4'R)-**4b**	10.0	2.7	11.5	~2.0	14.0	14.5	-	7.0	
(4'S)-**4b**	10.0	2.0	10.0	~2.0	10.0	10.0	~2.0		
4c	9.5	3.5	11.0	2.0	11.0	11.0	1.0	6.0	4.0
4e	10.5	2.2	11.0	1.5	10.5	11.0	1.5	5.0	5.0
4f	10.5	2.7	11.0	1.5	10.5	11.0	1.0	5.0	5.0

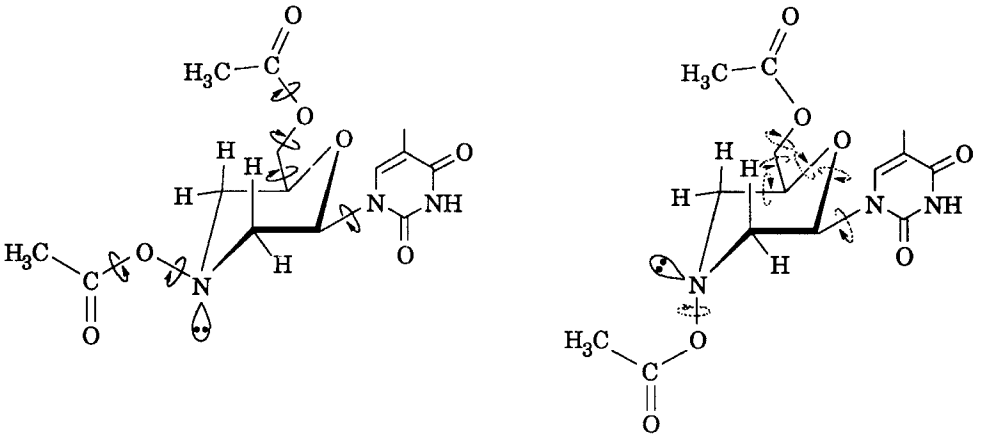
derivative **4f** in the presence of traces of moisture slowly lost one acetyl group to give **4e**.

^{1}H-NMR data of compounds **3** and **4** are collected in TABLES 2 and 3, ^{13}C-NMR data in TABLE 4. At room temperature, the spectra were poorly resolved and the NMR had to be measured at temperatures higher than 55 °C to obtain well-resolved time-averaged spectra. Variable temperature NMR experiments run on **4b** indicated, using the Gutowsky's approximation,[7] a $\Delta G^{\ddagger}$ value of 14 kcal/mol at 288 K for the intertransformation of two forms. At -40 °C, the 400 MHz spectrum of **4b** showed a 3:1 mixture of two species both in the same $^1C_{4'}$ conformation as shown by the NMR coupling constants (TABLE 3). In fact the same chair form is exclusive for all **3** and **4** compounds independently of the temperature. The phenomenon corresponding to the 14 kcal/mol energy barrier is clearly an inversion of the nitrogen atom and the two frozen forms of **4b** correspond to invertomers. The attribution of the configuration at N-4' (*4'S* for the more abundant, equatorial, *4'R* for the minor, axial, invertomers) were based on two convergent sets of observations. It has long been known that the presence of a lone pair syn- or antiperiplanar

TABLE 4. ^{13}C-NMR Data (δ in ppm) of Compounds **3a-d** (50.3 MHz) and **4b** (100.6 MHz).

	3a	**3b**	**3c**	**3d**	**4b**
t (°C)	100	60	100	80	50
solvent	DMSO-d_6	DMSO-d_6	DMSO-d_6	DMSO-d_6	$(CD_3)_2CO$
$C_{2'}$	77.17	77.02	77.10	76.98	78.61
$C_{3'}$	59.12	59.26	59.43	59.52	58.51
$C_{5'}$	57.71	57.91	57.77	58.02	56.88
$C_{6'}$	73.99	74.13	73.85	73.93	72.75
$C_{6''}$	61.63	61.73	61.64	61.75	64.78
C_2	149.38	149.67	145.22	152.25	150.97
C_4	162.00	163.11	147.83	148.79	164.04
C_5	101.31	109.19	123.66	118.42	111.50
C_6	139.91	135.87	155.73	155.62	136.46
C_8	-	-	137.47	138.63	-
Others	-	11.42 (CH_3)	-	-	168.67, 170.81 ($COCH_3$), 19.46, 20.61 ($COCH_3$)
$J_{C,H}$ (Hz)					C_6-H_6 181 C_6-CH_3 6 C_6-$H_{2'}$ 4

to a C-H bond of a vicinal methylene group impart a small positive increment to the value of the $^2J_{CH2}$ thus decreasing its absolute value.[8] The major invertomer (Scheme 2) can on this basis be assigned the *(4'S)* configuration (equatorial). This invertomer is the most stable in all the series, as shown by the time-averaged values of these couplings closer to 10 than to 14 Hertz (TABLE 3). The other set of observations in favor of this assignment consists in the effect of the N-4' configuration of **4b** upon pertinent chemical shifts (TABLE 2). Whereas the chemical shifts of the equatorial protons are almost unaffected by a change in configuration, H-2' and H-6' are deshielded when in 1,3-diaxial orientation relative to the 4'-acetoxy group (*4'R* configuration).



Scheme 2

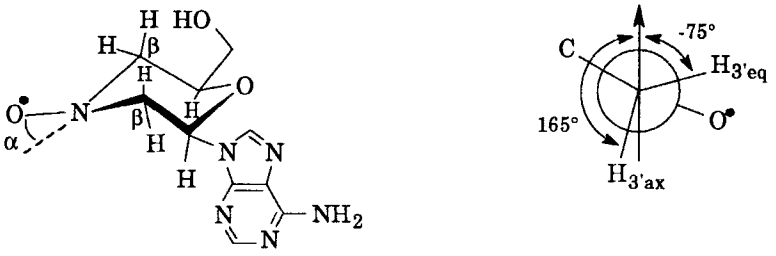
On the other hand, axial H-3' and H-5' are shielded when antiperiplanar to the lone pair on nitrogen (*4'S* configuration) through electron transfer from the lone pair to the antiperiplanar H-C σ* orbital.[9]

A Monte Carlo conformational search was performed on **4b** using the MacroModel 3.5 software[10] in which were introduced the MM2 parameters developed[11] from *ab initio* studies for the N(sp^3)-O(sp^3) bond. The search was conducted using the chloroform solvation option and the solvent accessible surface area was analytically recomputed at each optimization step. The N(sp^3)-O(sp^3)-C(sp^2) bending and C(sp^3)-N(sp^3)-O(sp^3)-C(sp^2) torsional parameters being unavailable, the corresponding default parameters, respectively N-O-Y and X-N-O-Y were used. The torsional angles marked with solid curved arrows in Scheme 2 were varied and 2000 conformations were generated for each invertomer. Other Monte Carlo experiments were performed (3000 structures for each invertomer) varying another set of torsional angles (dotted curved arrows in Scheme 2) to also explore the conformational behavior of the morpholine ring itself. Regardless of the starting invertomer, the most stable form found was a $^{1'}C_{4'}$ chair with an axial 4'-acetoxy group (*4'R* configuration). The *4'S* invertomer (equatorial) in the same chair form was higher in energy by 0.3-0.4 kcal/mol. The alternate chair ($^{4'}C_{1'}$) and the best boat ($^{1',4'}B$) were less stable by about 4.5

kcal/mol and 4.0 kcal/mol respectively. Using the available but inadequate parameters developed for the $C(sp^3)N(sp^3)O(sp^3)C(sp^3)$ group instead of the default parameters did not provide significant modification with MacroModel version 3.5 but with MacroModel version 3.1 in these same conditions the equatorial *(4'S)* invertomer was found more stable than its axial counterpart by 0.2 kcal/mol, this discrepancy coming probably from the slight difference in the treatment of solvation between the two versions of this software. We are developing the MM2 $N(sp^3)$-$O(sp^3)$-$C(sp^2)$ and $C(sp^3)$-$N(sp^3)$-$O(sp^3)$-$C(sp^2)$ parameters but it is doubtful that this improvement should change significantly the MacroModel 3.5 predicted relative stabilities of the two invertomers. Moreover semi-empirical quantum mechanical techniques (AM1 and PM3, PRECISE, MMOK) also predicted the axial invertomer of **4b** to be more stable than the equatorial one by about 2.5-3.0 kcal/mol.

To study the conformational features of the C-2'-N-1 bond, we measured the heteronuclear coupling constant between H-2' and C-6 using the INEPT procedure.[12] The time-averaged value of 4.0 Hz was obtained. Either the classical relationship $[^3J_{CH} = 4.26 - 1.00.\cos\theta + 3.56.\cos(2\theta)]$[13] theoretically established for propane but shown to be generally applicable even for H-C-N-C dihedral angles[14] or a more recent one[15] $[^3J_{CH} = 7.66.\cos^2\theta - 0.9.\cos\theta - 0.02]$ led to the same set of four possible torsional angles: ± 40°, ± 130-135°. N.O.E. experiments on **4b** showed an important population transfer between H-6 and $H\text{-}3'_{ax}$, but none between H-6 and either $H\text{-}3'_{eq}$ or H-2' thus excluding the two *syn* conformations ($\theta = \pm 40°$). Among the two possible *anti* conformations, that corresponding to a C-6-N-1-C-2'-H-2' torsional angle of -130-135° (χ_{CN}[16] between +70 and +75°) is the less probable for two reasons. In this case, the distances $H\text{-}6\text{-}H\text{-}3'_{ax}$, $H\text{-}6\text{-}H\text{-}3'_{eq}$ should be *ca* 2.0 and 2.5 Å respectively, whereas the distances H-2'-H-5' and $H\text{-}2'\text{-}H\text{-}3'_{eq}$ are of *ca* 2.5 Å. The measured $H\text{-}6\text{-}H\text{-}3'_{ax}$ N.O.E. interaction was much weaker than the ones for which the interproton distance was known to be 2.5 Å. The second argument is that AM1 and PM3 computations (PRECISE MMOK) indicated a preferred C-6-N-1-C-2'-H-2' torsional angle of *ca* 150° closer to the conformation we favor: a conformer with a C-6-N-1-C-2'-H-2' torsional angle of 130-135°, thus an *anti* form corresponding to a χ_{CN} value of 165-170°.

Compounds **3** oxidized spontaneously in solution to the corresponding aminoxyl free radicals, EPR spectra of which showed large hyperfine coupling



Scheme 3

constants (*ca* 16 G) with nitrogen and two hydrogen atoms (H-3'$_{ax}$, H-5'$_{ax}$) and two small (*ca* 3.5 G) hyperfine coupling constants with the neighbouring equatorial protons (H-3'$_{eq}$, H-5'$_{eq}$). In a theoretical study of the conformational dependence of β hyperfine coupling constants in aminoxyls,[17] compound **3d** was used as an example. The β hyperfine couplings depend both on the out-of-plane deformation of the aminoxyl group (α angle of Scheme 3) and on the dihedral angle between the H_β-C_β-N plane and the plane including the C_β-N bond and an axis passing through the nitrogen atom and perpendicular to the C_β-N-$C_{\beta'}$ plane. As the a_N value depends on the α angle, the hyperfine coupling constants measured for **3d** in DMSO at 25 °C [a_N = 16.5 G, $a_{H3'ax}$ = $a_{H5'ax}$ = 16.7 G, $a_{H3'eq}$ = $a_{H5'eq}$ = 3.5 G, $a_{H2'}$ = $a_{H6'}$ = 0.8 G] indicated a $^{1'}C_{4'}$ chair (Scheme 3) with an α value of 20°.

Compounds **3** and **4** were submitted to antibacterial, anticancer and antiviral testings following described procedures.[18] None of them showed any significant activity against *Neurospora crassa, E coli, B subtilis,* SV40, herpes simplex virus type 1 (KOS) and type 2 (G), vaccinia virus, Coxsackie B4 virus, Sindbis virus, parainfluenza virus type 3, reovirus type 1, polio virus type 1 and human immunodeficiency virus type 1 and 2. None of these compounds showed any notable cytotoxicity against MK (mouse kidney), CV1, L1210 and Molt-4 cells (maximum non-cytotoxic concentration > 250 μM).

EXPERIMENTAL

General methods.[19] Starting materials **1a,c,d** were commercial (FLUKA). The known[20] compound **1b** was prepared from 1,2,3,5-tetra-O-

acetyl-β-D-ribofuranose and thymine following Vorbrüggen's method,[21] followed by deacetylation (NH_3/MeOH, 2 days). The 400 MHz proton NMR spectra and the INEPT experiments have been performed using a BRUKER AMX 400 spectrometer.

Preparation of dioximes 2a-d. - These compounds were obtained from the corresponding dialdehydes[4] using classical methods. To a solution of $NH_2OH.HCl$ (5 mmol) and a base NaOAc (5 mmol) or K_2CO_3 (2.5 mmol) in 10:1 MeOH/H_2O (20 mL) a solution of the dialdehyde (1 mmol) in MeOH (5 mL) was added. After 14 h at 25°, the solvents were removed by evaporation and the residue was submitted to a column chromatography.

***(2R,4S)*-1-(1,5-Bis-*N*-hydroxyimino-4-hydroxymethyl-3-oxa-pent-2-yl)uracil (2a).** - A column chromatography (7:3 AcOEt/MeOH) gave **2a** (77%, R_F 0.69) as a mixture of geometrical isomers: white foam, ν_{max}^{KBr} 3700-3200 (NH, OH), 2900 (CH), 1680 (C=O), and 1620 (C=C) cm^{-1}. MS: *m/z* (%) 112 (100), 69 (63), 98 (8), 57 (7), 149 (6), 199 (1), and 256 (1, $M^{\cdot+}$ - OH).

***(2R,4S)*-1-(1,5-Bis-*N*-hydroxyimino-4-hydroxymethyl-3-oxa-pent-2-yl)thymine (2b).** - A column chromatography (4:1 $CHCl_3$/MeOH) gave **2b** (89.5%, R_F 0.16) as a mixture of geometrical isomers: λ_{max}^{EtOH} 204 nm (ε 13985), 264 (8588); ν_{max}^{KBr} 3500-3200 (NH, OH), 2930 (CH), 1691 (C=O) cm^{-1}. MS: *m/z* (%) 55 (100), 126 (57, thymine), 88 (35), 70 (17), 161 (12, $M^{\cdot+}$ - thymine), 182 (7), 205 (1.5), 220 (0.5), 242 (0.23), 256 (0.12, $M^{\cdot+}$ - CH_2OH), 269 (0.17, $M^{\cdot+}$ - OH), and 287 (0.13, $M^{\cdot+}$ + 1).

***(2R,4S)*-9-(1,5-Bis-*N*-hydroxyimino-4-hydroxymethyl-3-oxa-pent-2-yl)hypoxanthine (2c).** - A column chromatography (7:3 AcOEt/MeOH) gave **2c** (90%, R_F 0.07) as a mixture of geometrical isomers: amorphous beige powder, ν_{max}^{KBr} 3500-3200 (OH), 2680 (CH), 1670 (C=O), 1580, 1520 (C=N) cm^{-1}. ^{1}H-NMR (DMSO-d_6) of the *(E-E)*-isomer: δ 12.4 (*b s*, 1 H, NH), 11.0-11.7 (*b ss*, 2 H, 2N~OH), 8.7 (*s*, 1 H, H-8), 8.10 (*s*, 1 H, H-2), 7.82 (*d*, 1 H, $J_{1',2'}$ ~ 5 Hz, H-1'), 7.30 (*d*, 1 H, $J_{4',5'}$ ~ 7.5 Hz, H-5'), 6.75 (*d*, 1 H, H-2'), 6.30 (*d*, 1 H, H-4'), 4.90 (*b s*, 1 H, CH_2OH), and 3.90 (*m*, Hab-4''). MS: *m/z* (%) 112 (100), 69 (65), 161 (44, $M^{\cdot+}$ -hypoxanthine), 169 (32), 153 (30), 73 (17), 88 (15), 98 (8), 125 (5), 237 (2), and 259 (1).

***(2R,4S)*-9-(1,5-Bis-*N*-hydroxyimino-4-hydroxymethyl-3-oxa-pent-2-yl)adenine (2d).** - A column chromatography (2:1 *i*-Pr_2O/MeOH) gave **2d** as a mixture of geometrical isomers (total yield 80%, R_F 0.4). A pure sample of

the *(1'E,5'E)* isomer was obtained by crystallization 2:1 *i*-Pr_2O/MeOH. Its properties are the following: mp 184-185 °C; $[\alpha]_D^{24}$ +42.0° (*c* 1.0, MeOH); R_F 0.4 (2:1 $Me_2CH)_2O$/MeOH); λ_{max}^{EtOH} 213 nm (ε 12650) and 260 (13700); ν_{max}^{KBr} 3400-3210 (OH, NH), 2920 (C-H), 1655 (C=NOH), and 1580-1485 (C=C, C=N aromatic) cm^{-1}. ^{1}H-NMR (CD_3OD): δ 8.48, 8.40 (2 *s*, 2 H, H-2, H-8), 7.98 (*d*, 1 H, $J_{1',2'}$ = 5.0 Hz, H-1'), 7.54 (*d*, 1 H, $J_{4',5'}$ = 7.4 Hz, H-5'), 6.62 (*d*, 1 H, H-2'), 4.14 (*ddd*, 1 H, $J_{4',4"a}$ = 2.5 Hz, $J_{4',4"b}$ = 4.0 Hz, H-4'), 3.83 (*dd*, 1 H, $J_{4"a,4"b}$ = 10.0 Hz, Hb-4"), and 3.75 (*dd*, 1 H, Ha-4"). MS: *m/z* (%) 135 (100), 116 (4.7), 88 (2.1), 164 (1.9), 192 (1.9), 148 (1.6), 108 (1.5), 189 (0.5), 207 (0.4), and 279 (0.1).

Anal. Calcd for $C_{10}H_{13}N_7O_4$ (295.26): C, 40.68; H, 4.44; N, 33.21. Found: C, 40.79; H, 4.66; N, 33.18.

(2*R*,4*S*)-9-(4-Hydroxymethyl-1,5-bis-*N*-methoxyimino-3-oxa-pent-2-yl)adenine (2'd). - To a solution of sodium acetate (2g, 24.4 mmol) and methoxyamine hydrochloride (2.21 g, 26.5 mmol) in water (180 mL), the corresponding dialdehyde (1.5 g, 5.66 mmol) was added and the reaction mixture stirred at 45 °C for 1 h. The solvents, the excess of methoxyamine and acetic acid were removed by evaporation (35 °C, 5 h, 0.5 mm Hg). Column chromatography (2:1 $(Me_2CH)_2O$/MeOH) of the residue afforded a mixture of the four geometrical isomers of **2'd** (1.29 g, 75%): R_F 0.48 (2:1 $(Me_2CH)_2O$/MeOH); λ_{max}^{EtOH} 205 nm (ε 2500) and 258 (10800); ν_{max}^{KBr} 3350-3220 (OH, NH), 2960 (C-H), 1640 (C=NOMe), and 1605-1480 (C=C, C=C aromatic) cm^{-1}. MS: *m/z* (%) 78 (100), 135 (53), 189 (50), 205 (40), 102 (19.7), 221 (18.2), 323 (15.2, M^{+}), 77 (15.1), 136 (12.1), and 108 (7).

Anal. Calcd for $C_{12}H_{17}N_7O_4$ (323.31): C, 44.58; H, 5.30; N, 30.33. Found: C, 44.53; H, 5.13; N, 30.18.

Preparation of compounds 3a-d. - To a solution of one of the dioximes **2a-d** (0.7 mmol) in MeOH (10 mL), BH_3.Py complex (0.21 mL, 2.1 mmol) was added and the pH kept at 2-2.5 by continuous addition of methanolic 6M HCl. After the pH was stabilized at 2.5 without further addition of HCl, the reaction mixture was stirred for another 1 h, and the pH was adjusted to 7-8 (with 10% aqueous NaOH saturated with NaCl). The solution was then concentrated, and the residue was washed with CH_2Cl_2 (2x5 mL) to remove the excess of borane complex, then submitted to a column chromatography to give **3a-d**, respectively.

***(2R,4RS,6S)*-1-(4-Hydroxy-6-hydroxymethylmorpholin-2-yl)uracil (3a).** - A column chromatography (9:1 AcOEt/MeOH) gave **3a** (136 mg, 80%, R_F 0.30), white powder: mp 223-226 °C (decomp.); $[\alpha]_D^{24}$ +27.8° (*c* 1.14, H_2O); λ_{max}^{EtOH} 206 nm (ε 3880) and 260 (5790); ν_{max}^{KBr} 3420 (NH), 3413 (N-OH), 3200 (OH), 1715, 1694 C=O), and 1629 (C=C) cm^{-1}. MS: m/z (%) 131 (100, $M^{\cdot+}$ - uracil), 57 (98), 70 (72), 114 (63, uracil), 95 (35), 139 (16), 226 (4.4, $M^{\cdot+}$ - OH), 182 (2.8), 166 (2.8), and 243 (0.4, $M^{\cdot+}$)

Anal. Calcd for $C_9H_{13}N_3O_5$ (243.22): C, 44.45; H, 5.39; N, 17.28. Found: C, 44.32; H, 5.33; N, 16.99.

***(2R,4RS,6S)*-1-(4-Hydroxy-6-hydroxymethylmorpholin-2-yl)thymine (3b).** - A column chromatography (9:1 AcOEt/MeOH) gave **3b** (156 mg, 83.5%, R_F 0.7): mp 220-221 °C (transition at 113-116 °C); $[\alpha]_D^{20}$ +32.9° (*c* 0.55, MeOH); λ_{max}^{EtOH} 207 nm (ε 3312), and 264 (3724; ν_{max}^{KBr} 3408 (NH), 3119, 3090 (OH), 1707, 1682 (C=O), and 1660 (C=C) cm^{-1}. MS: *m/z* (%) 55 (100), 131 (61, $M^{\cdot+}$ - thymine), 114 (49), 70 (48), 84 (23, MeC(=NH)C=C=O), 126 (23, thymine), 167 (2), 180 (2), 240 (3.2, $M^{\cdot+}$ - OH), and 257 (0.5 $M^{\cdot+}$).

Anal. Calcd for $C_{10}H_{15}N_3O_5$ x 1/2 H_2O (266.26): C, 45.10; H, 6.02; N, 15.78. Found: C, 45.10; H, 5.83; N, 15.90.

***(2R,4RS,6S)*-9-(4-Hydroxy-6-hydroxymethylmorpholin-2-yl)hypoxanthine (3c).** - A column chromatography (7:3 Me_2CO/EtOH) gave **3c** (109 mg, 58%, R_F 0.34) as a very hygroscopic compound: mp 167-172 °C (195° decomp.); $[\alpha]_D^{20}$ -26° (*c* 1.04, H_2O); λ_{max}^{H2O} 194 nm (ε 24215) and 248 (10 876); ν_{max}^{KBr} 3500-3200 (NH, OH), 2920, 2820 (CH), 1685 (C=O), 1580 C=C), and 1500 (C=N) cm^{-1}. MS: *m/z* (%) 136 (100, hypoxanthine), 54 (90), 115 (25), 81 (25), 95 (12), 131 (3, $M^{\cdot+}$ - hypoxanthine), 149 (2), 220 (6, $M^{\cdot+}$ - OH-CH_2OH), 250 (6, $M^{\cdot+}$ - OH), and 267 (2, $M^{\cdot+}$).

***(2R,4RS,6S)*-9-(4-Hydroxy-6-hydroxymethylmorpholin-2-yl)adenine (3d).** - A column chromatography (7:3 AcOEt/MeOH) gave **3d** (145 mg, 78%, R_F 0.3): mp 258-260 °C (decomp.); $[\alpha]_D^{22}$ -4.5° (*c* 1.0 0.1 M HCl); λ_{max}^{H2O} 207 nm (ε 23786) and 258 (16626); ν_{max}^{KBr} 3325 (NH), 3200, 3090 (OH), 2847 (CH), 1678 (C=C), 1607 (C=N) cm^{-1}. MS: *m/z* (%) 135 (100, adenine), 108 (35), 54 (23), 164 (22), 219 (15, $M^{\cdot+}$ - OH-CH_2OH), 249 (3, $M^{\cdot+}$ - OH), and 266 (3, $M^{\cdot+}$).

Anal. Calcd for $C_{10}H_{14}N_6O_3$ (266.26): C, 45.11; H, 5.30; N, 31.56. Found: C, 45.10; H, 5.02; N, 31.31.

Acetylation of 3a-d compounds. - **3a-d** (1 mmol) were treated 16 h at 20 °C with a mixture of Ac_2O (3 mL) and pyridine (10 mL). After usual workup, the products **4a-d** were purified using column chromatography.

***(2R,4RS,6S)*-1-(4-Acetoxy-6-acetoxymethylmorpholin-2-yl)uracil (4a).** - A column chromatography (19:1 CH_2Cl_2/MeOH) gave **4a** (265 mg, 81%, R_F 0.32): mp 141-143 °C; $[\alpha]_D^{23}$ +6.8° (*c* 1.1, MeOH); λ_{max}^{EtOH} 206 nm (ε 10057) and 260 (13020); ν_{max}^{KBr} 3200 (NH), 3100-3020 (CH), 1755, 1740, 1700, 1680 (C=O), and 1620 (C=C) cm^{-1}. MS: *m/z* (%) 95 (100), 60 (82, OAc), 70 (77), 157 (66), 208 (38, $M^{\cdot+}$ - 2xOAc), 112 (36, uracil), 261 (12, $M^{\cdot+}$ OAc), and 285 (2, $M^{\cdot+}$ - Ac).

Anal. Calcd for $C_{13}H_{17}N_3O_7$ (327.30): C, 47.71; H, 5.24; N, 12.84. Found: C, 47.56; H, 5.20; N, 12.74.

***(2R,4RS,6S)*-1-(4-Acetoxy-6-acetoxymethylmorpholin-2-yl)thymine (4b).** - A column chromatography (19:1 CH_2Cl_2/MeOH) gave **4b** (314 mg, 92%, R_F 0.44): white foam, $[\alpha]_D^{26}$ +9.16° (*c* 0.05, $CHCl_3$); λ_{max}^{EtOH} 207 nm (ε 8782) and 263 (8435); ν_{max}^{KBr} 3400 (NH), 1760, 1740, and 1690 (C=O) cm^{-1}. MS: *m/z* (%) 68 (100), 55 (82), 96 (52), 156 (44), 127 (25, thymine), 173 (27), 222 (9, $M^{\cdot+}$ - 2xOAc), 240 (0.9, $M^{\cdot+}$ - OAc-Ac), and 282 (2, $M^{\cdot+}$ - OAc).

Anal. Calcd for $C_{14}H_{19}N_3O_7$ (341.32): C, 49.27; H, 5.61; N, 12.31. Found: C, 49.06; H, 5.79; N, 12.01.

***(2R,4RS,6S)*-9-(4-Acetoxy-6-acetoxymethylmorpholin-2-yl)hypoxanthine (4c).** - A column chromatography (9:1 CH_2Cl_2/MeOH) gave **4c** (292 mg, 83%, R_F 0.4): mp 211-215 °C; $[\alpha]_D^{20.5}$ -21° (*c* 1.0, 1:1 MeOH/H_2O); λ_{max}^{EtOH} 203 nm (ε 15332) and 243 (10464); ν_{max}^{KBr} 3440 (NH), 1730, 1680 (C=O), 1580, and 1540 (C=N) cm^{-1}. MS: *m/z* (%) 45 (100), 60 (55, OAc), 95 (38), 137 (35, hypoxanthine), 157 (13), 231 (5.5, $M^{\cdot+}$ - 2xOAc), 292 (1.5, $M^{\cdot+}$ - OAc), and 309 (0.6, $M^{\cdot+}$ - Ac).

Anal. Calcd for $C_{14}H_{17}N_5O_6$ (351.32): C, 47.86; H, 4.88; N, 19.93. Found: C, 47.58; H, 4.86; N, 19.71.

***(2R,4RS,6S)*-6-Acetamido-9-(4-acetoxy-6-acetoxymethylmorpholin-2-yl)purine (4e).** - A column chromatography of the product of acetylation of **3d** gave **4e** (263 mg, 67%, R_F 0.3) and **4f** (65 mg, 15%, R_F 0.42). Compound **4f** slowly decomposed on silica gel. Properties of **4e** are the following: white solid, mp 145-146 °C; $[\alpha]_D^{21}$ -27.8° (*c* 1.1, MeOH); λ_{max}^{EtOH} 210 nm (ε 23082), and 270 (19211); ν_{max}^{KBr} 3320 (NH), 3060 (CH), 1755, 1730, 1710 (C=O), 1600, and

1580 (C=N) cm^{-1}. MS: *m/z* (%) 178 (100, *N*-acetyl-adenine), 135 (78, adenine), 333 (61, $M^{\cdot+}$ - OAc), 95 (32), 206 (28), 60 (18, OAc), 157 (17), 273 (16, $M^{\cdot+}$ - 2xOAc), 95 (32), 206 (28), 60 (18, OAc), 157 (17), 273 (16, $M^{\cdot+}$ - 2xOAc), 231 (14), and 393 (0.5, $M^{\cdot+}$).

Anal. Calcd for $C_{16}H_{20}N_6O_6$ (392.37): C, 48.98; H, 5.14; N, 21.42. Found: C, 48.76; H, 5.07; N, 21.21.

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