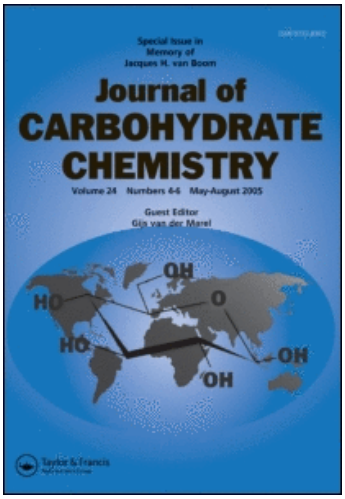

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Synthesis and Anti-HIV Activity of Further Examples of 1-[3-Deoxy-3-(*N*-hydroxyamino)-β-d-*threo*-(and β-d-*erythro*)-pentofuranosyl]thymine Derivatives[1]

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SYNTHESIS AND ANTI-HIV ACTIVITY OF FURTHER EXAMPLES OF 1-[3-DEOXY-3-(*N*-HYDROXYAMINO)-β-D-*THREO*- (AND β-D-*ERYTHRO*)-PENTOFURANOSYL]THYMINE DERIVATIVES[1]

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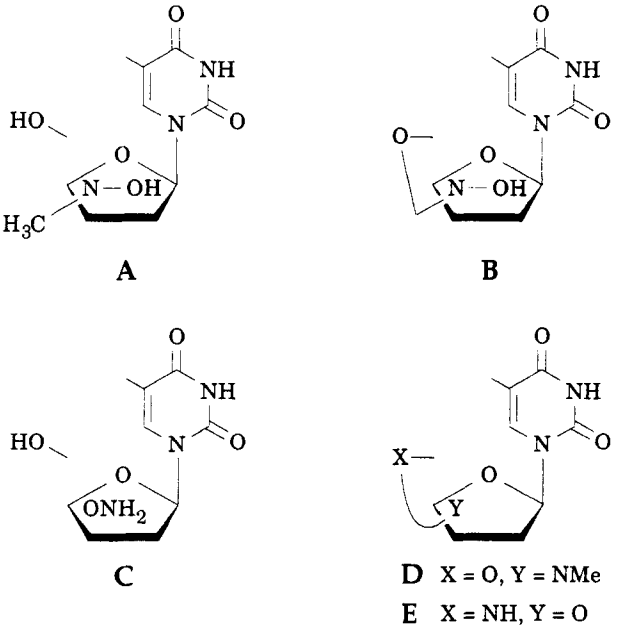
ABSTRACT

Upon sodium cyanoborohydride reduction followed by de-*O*-silylation, the *O*-methyloxime and *N*-benzylnitrone of 5'-TBDMS-3'-ketothymidine gave resolvable epimeric mixtures of 1-[2,3-dideoxy-3-(*N*-methoxyamino)-β-D-*threo*- and β-D-*erythro*-pentofuranosyl]thymine and 1-[3-(*N*-benzyl-*N*-hydroxyamino)-2,3-dideoxy-β-D-*threo*- and β-D-*erythro*-pentofuranosyl]thymine respectively. These compounds were inactive against HIV. On the other hand, 1-[2,3-dideoxy-3-(*N*-hydroxyamino)-5-*O*-TBDMS-β-D-*threo*-pentofuranosyl]thymine, upon treatment with acetone, then de-*O*-silylation, gave the bicyclonucleoside analogue **15**, slightly more active against HIV *in vitro* than DDI.

INTRODUCTION

We have shown[1,2,3] that thymidine analogues belonging to the β-D-*threo* series and having a nitrogen-bearing group at the 3' position as **A** and its bicyclic congener **B** (Scheme 1), could exhibit an anti-HIV activity in the range of that of DDI (2',3'-dideoxyinosine) recently introduced into clinical use. On the other hand, structurally close analogues of **A** and **B**, **C** and **D-E** respectively, were devoid of anti-HIV activity.[4] In order to assess the structure- (anti-HIV) activity

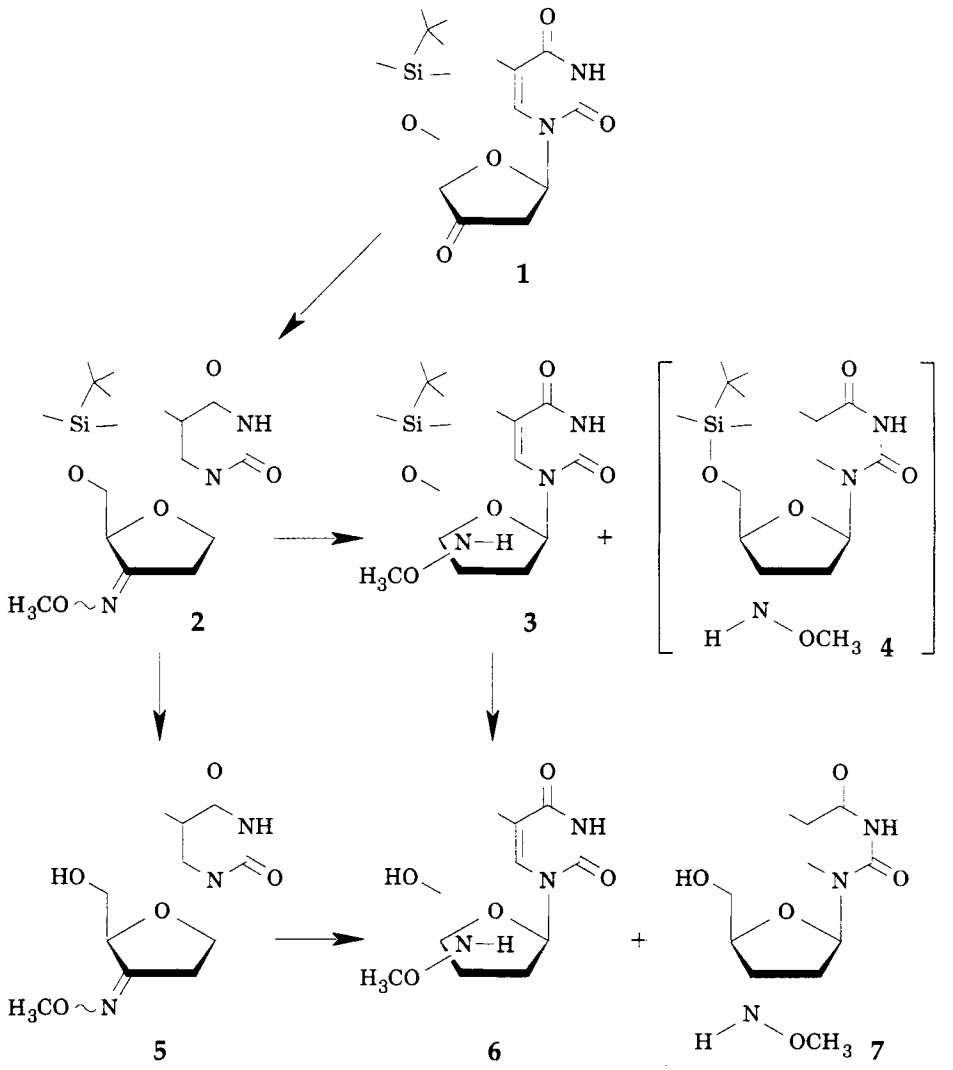
relationships in these series, we prepared further examples of these β-D-*threo* nucleosides, one of which exhibited an anti-HIV activity superior to that of DDI.



Scheme 1

RESULTS AND DISCUSSION

Blocked 3'-ketothymidine **1**[3] treated with *O*-methylhydroxylamine yielded the corresponding *O*-methyloxime **2** (92%) as a 3:1 *E*/*Z* mixture. Treated with sodium cyanoborohydride in 100% acetic acid, **2** underwent a reduction without de-*O*-silylation leading stereoselectively to the major β-D-*threo* compound **3**, the β-D-*erythro* epimer **4** not being isolated in a pure form, but its structure assigned from its NMR spectrum. On the other hand, when the reduction was performed in methanolic hydrochloric acid at pH 2 the de-*O*-silylation of **2** to **5** preceded its stereoselective reduction to a 3:1 resolvable mixture of **6** and **7** (Scheme 2). From the NMR data collected in Tables 1 and 2, it is clear that compound **4** having both values of $J_{3',4'}$ and $J_{2'\alpha,3'}$ of ca. 3 Hz, hence H-3' *trans* to H-4' and Hα-2', belongs to the β-D-*erythro* series, thus **3** to the β-D-*threo* series. Upon treatment of **1** with *N*-benzylhydroxylamine, the unstable nitrone **8** was formed and immediately reduced (sodium cyanoborohydride) to a 3:1 mixture of **9** and **10** (combined yield 67% from **1**).



Scheme 2

After resolution of the mixture, **9** and **10** were de-*O*-silylated to **11** and **12** respectively (Scheme 3). The configurations of **9-12** were established from their NMR data as indicated for **3** and **4**.

The *erythro* configuration of compounds **4**, **7**, **10**, and **12** is further supported by the fact that the populations of their γ^+ conformers,[5] expressed in percentages, are superior to 47%[6] (85, 85, 90, and 80% respectively) whereas percentages of respectively 34, 40, and 30% are found for the *threo* compounds **3**, **6**, and **11**.

Table 1. ^1H NMR chemical shifts ($CDCl_3$, at 25 °C, 200 MHz, δ in ppm) of nucleoside analogues

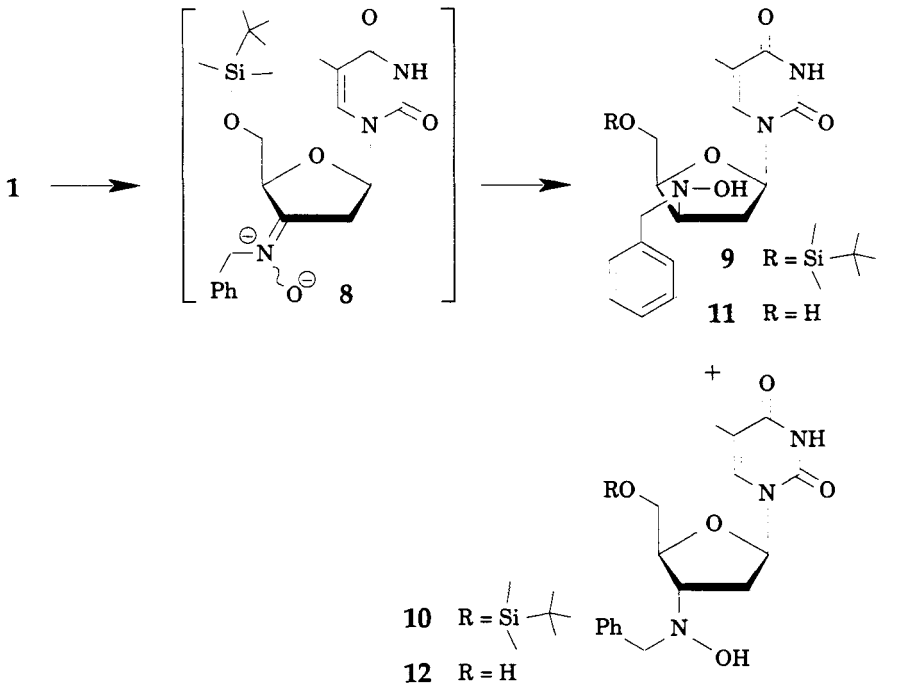
Cmpd	$H_{1'}$	$H_{2'\beta}$	$H_{2'\alpha}$	$H_{3'}$	$H_{4'}$	$H_{5'a}$	$H_{5'b}$
***(E)*-2**	6.31	2.43	3.35		4.59	3.91	4.08
***(Z)*-2**	6.31	2.62	3.05		4.81	3.92	4.20
3	6.21	2.00	2.54	3.89	4.09	3.91	3.98
4	6.29	2.01	2.30	3.76	4.04	3.79	3.97
***(E)*-5**	6.30	2.78	3.27		4.59	~4.00	~4.00
***(Z)*-5**	6.22	2.90	3.08		4.83	?	?
6	6.13	1.95	2.60	~3.95	4.11	~3.95	~3.95
7	6.13	~2.25	2.33	3.83	4.05	3.81	3.96
9[a]	6.10	2.27	2.47	3.60	~4.10	~4.00	~4.15
	6.49	2.57	2.63	3.78	~4.40	4.29	~4.40
10	6.31	1.90	2.78	3.60	4.31	3.76	3.98
11[b]	6.01	2.18	2.39	3.56	4.00	3.80	3.80
12[b]	6.12	1.92	~3.50	~3.50	4.10	~3.50	~3.50
14	6.21	2.66	2.82	5.02	4.02	3.82	3.93
15[b]	6.01	2.17	2.42	3.21	4.03	3.78	3.88

a. C_5D_5N at 80 °C. b. $(CD_3)_2SO$.

Table 2. ^1H NMR interproton couplings ($CDCl_3$, 25 °C, 200 MHz, J in Hz) of nucleosides analogues

Cmpd	$J_{1',2'\beta}$	$J_{1',2'\alpha}$	$J_{2'\alpha,2'\beta}$	$J_{2'\beta,3'}$	$J_{2'\alpha,3'}$	$J_{3',4'}$	$J_{4',5'a}$	$J_{4',5'b}$
***(E)*-2**	8.0	6.8	18.0				2.5	2.0
***(Z)*-2**	9.0	6.0	16.0					2.0
3	5.5	7.0	14.0	5.5	6.5	~5.0	5.5	4.0
4	8.0	6.0	13.5	8.0	3.0	~3.0	2.5	2.5
***(E)*-5**	7.0	7.0	19.0				?	?
***(Z)*-5**	8.5	6.5	16.5				?	?
6	5.5	7.0	14.5	5.5	7.0	~5.0	~5.0	~5.0
7	6.7	6.7	13.5	7.0	6.0	4.5	3.0	2.5
9[a]	7.0	7.0	13.0	7.5	8.0	~7.0	?	?
	7.0	7.0	13.0	7.0	7.0	7.0	6.0	?
10	7.5	6.5	13.5	8.0	3.0	3.0	3.0	2.0
11[b]	7.0	7.0	13.3	8.0	7.0	7.0	5.5	5.5
12[b]	6.5	6.5	13.5	9.0	?	~3.0	~3.0	~3.0
14	7.0	7.0	14.5	8.5	3.0	5.5	8.5	4.0
15	2.0	8.0	14.0	0.5	5.0	4.0	0.5	2.5

a. C_5D_5N at 80 °C. b. $(CD_3)_2SO$.



Scheme 3

None of these 3'-amino-2',3'-dideoxythymidine derivatives (**3**, **4**, **6**, **7**, **10-12**) existed in a pure conformational form and this situation even rendered difficult the assignment of the H-2' signals to either the 2'α (on the α side) or the 2'β protons. In an attempt to solve this conformational problem, we resorted to two different "computational" procedures, the NOE experiments giving no reliable answer in these cases. The first procedure consisted in applying the old Altona and Sundaralingam's approximation[7] stating that the nucleosides consist in mixtures of N ($^3_2$T) and S ($^2_3$T) forms. Time-averaged coupling constants were calculated for a number of representative mixtures using a simple Karplus type equation[7] (^3J = 10.6 $\cos^2\theta$ - 1.2 $\cos\theta$) neglecting the influence of the electronegativity of neighbour groups upon the coupling constants. The percentages of N and S forms were chosen to give the better possible fit with the experimental values. Results concerning compounds **6** and **7** are collected in Table 3.

The second approach consisted in molecular dynamics experiments carried out using the MacroModel 3.1 software[8] and its included MM2 force field in which were introduced the MM2 parameters developed[9] from ab initio

Table 3. Comparison of experimental and estimated coupling constants of compounds **6** and **7**

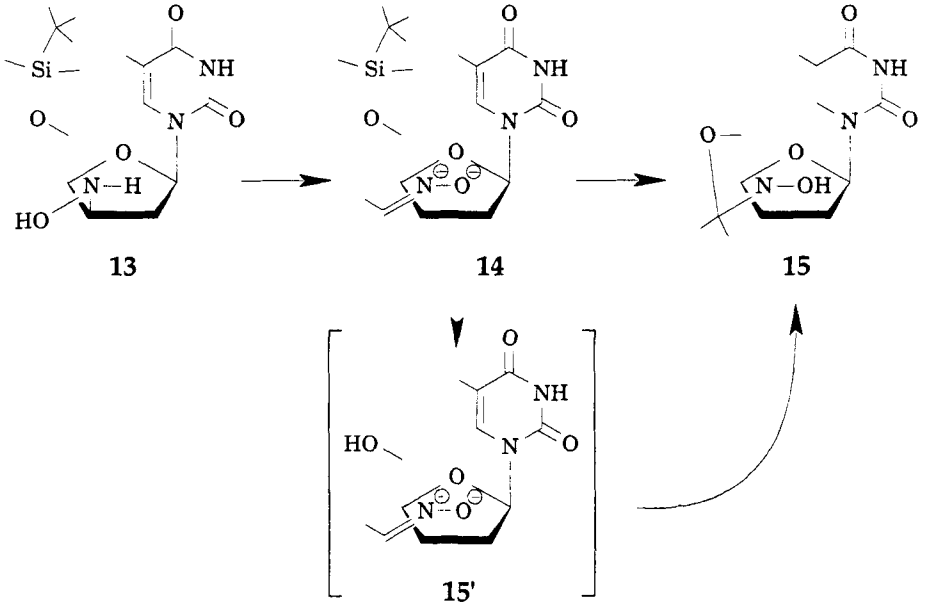
$^3J_{i,j}$	$\mathbf{6}_{exp}$	$\mathbf{6}_{est}$[a,b]	$\mathbf{6}_{MD}$[c]	$\mathbf{6}_{MDS}$[d]	$\mathbf{7}_{exp}$	$\mathbf{7}_{est}$[a,e]	$\mathbf{7}_{MD}$[c]	$\mathbf{7}_{MDS}$[d]
$J_{1',2'\alpha}$	7.0	7.0	4.0	3.9	6.7	7.1	4.8	5.2
$J_{1',2'\beta}$	5.5	5.0	9.6	9.8	6.7	7.1	7.8	7.6
$J_{2'\alpha,3'}$	7.0	4.9	6.5	6.4	6.0	3.3	3.9	4.5
$J_{2'\beta,3'}$	5.5	5.3	9.3	9.4	7.0	6.5	5.2	6.0
$J_{3',4'}$	~5.0	6.0	6.8	6.8	4.5	2.7	4.3	5.7

a. Using the Altona and Sundaralingam's N $\rightleftharpoons$ S approximation. b. 50% N, 50% S. c. From molecular dynamics simulations in vacuum. d. From molecular dynamics simulations in chloroform. e. 30% N, 70% S.

studies for the N(sp^3)-O(sp^3) bond. Both 3'-epimers were built and, for each of them, both invertomers at the 3' nitrogen atom, the separate treatment of any possible invertomer being made necessary by the fact that the MM2 force field explicitly takes into account lone pairs. Each of the four generated configurations were separately submitted to a 60 ps molecular dynamics simulation (1500 °K) with 2 fs time steps without prior equilibration or heating period, in order to generate in each case 500 conformers. Each pool of conformers was submitted to a multiconformer search to minimize their energies using successively the Polak-Ribiere conjugate gradient method, then the truncated Newton-Raphson method and to select the unique conformers. The four sets of conformers were merged into two sets of β-D-*threo* (**6**) and β-D-*erythro* (**7**) configurations respectively, and their temperature-averaged coupling constants[10] (25 °C) computed using a QCPE program,[11] slightly modified to insure its compatibility with MacroModel's output files. The same procedure was carried out using the Still's continuum solvation model[12] for chloroform included in the MacroModel software, the results being also collected in Table 3. Data of Table 3 indicate that implementation of the solvation model does not notably modify the computed ^3J couplings. The same observation applies when examining the conformer populations found using molecular dynamics. For compound **6** a major ^2E conformer (63% in chloroform, 77% in vacuum) was found, the other most populated conformers (ca.10%) corresponding also to structures close to ^2E in such a way that the temperature-averaged values approximate those of this major species. For compound **7**, the major conformer

($^0T_4$) represented only 15% of the mixture, and the temperature-averaged values differed notably from those of this major conformer. None of the theoretical estimations of the coupling constants led to a perfect prediction of the experimental values but the N ⇌ S method performed better for **6**, while the molecular dynamics led to better results for **7**.

Treated with acetone, **13**[3] led to an equilibrium mixture of **13** and **14** (59% percent conversion, 33% isolated yield). The ^1H NMR of **14** (Tables 1 and 2) showed the expected deshielding of $H_{3'}$ while the value of $^3J_{2'\alpha,3'}$ was decreased owing to the electronegativity of the nitrone function. Upon de-*O*-silylation, the nitrone **14** underwent an almost quantitative (92% isolated yield) cyclization to **15** (Scheme 4), ^1H NMR data of which indicate that it adopts the same conformation of its perhydrooxazine ring (intermediate between a $^{O5'}C_{3'}$ chair and a $^{O,N}F$ flattened[13] chair) as its parent compound **B**.[3]



Scheme 4

Upon spontaneous oxidation in the air, compounds bearing a *N*-hydroxy group, gave the corresponding aminoxyl radicals, representative examples of which were submitted to EPR spectroscopy (Table 4). For all aminoxyls the values of g and a_N were in the expected range.[14,15] For the *N*-benzyl derivatives **9** and **10**, the sum of the hyperfine coupling constants concerning the methylene group were 17.4 and 17.2 G respectively. Even if these values are slightly outside

Table 4. EPR data of aminoxyls formed upon oxidation of *N*-hydroxyamines.[a]

Compd	t/°C	g	a_N	$a^{\beta}_{H_{CH2}}$	$a^{\beta}_{H_{CH}}$	a^{γ}_{H}
9	120	2.0058	14.5	9.5	3.1	1.15
				7.9		2x0.8
10	100	2.0060	15.0	8.6	3.4	3x0.5
				8.6		
15	50	2.0062	15.3		20.8	2x0.6
						7x0.3

a. In diglyme solutions, a in G.

the typical range[16] (18-19.5 G) corresponding to a molecular population distributed between the two conformers in which one methylenic C-H bond lies in the plane of the aminoxyl group, these conformers should be predominant. The small methinic couplings with $H_{3'}$ (3.1 and 3.4 G) indicate for the major conformer a structure where the $C_{3'}$-$H_{3'}$ also resides in the aminoxyl plane. For compound **15**, the only usable a_H hyperfine coupling ($a_{H3'}$ = 20.8 G) is very close to that measured for its analog B,[3] which points toward **15** existing in the same flattened chair conformation as its diamagnetic precursor. In all these EPR spectra, small long-range couplings were found, which could eventually be used to estimate the number of γ hydrogen atoms.

Compounds **6**, **7**, **11**, **12**, and **15** have been submitted to the NCI in vitro anti-AIDS Drug Discovery Program of the US National Cancer Institute. Following a described procedure,[17] only **15** was scored as active (EC_{50} 3 10^{-6} M, IC_{50} > 2 10^{-4} M). It is more promising that compounds **A** and **B** scored as moderately active in the same test system. In fact, **15** is slightly more active than DDI, the last nucleoside introduced in anti-AIDS therapy. More thorough biological studies on **15** and some congeners are being undertaken, as well as 3D-QSAR studies in an attempt to elucidate the mechanism of action of this novel type of anti-HIV nucleoside.

EXPERIMENTAL

General methods. See ref. 18.

(*E* and *Z*)-1-[5-*O*-*tert*-Butyldimethylsilyl-2,3-dideoxy-3-*N*-methoxyimino)-β-D-*glycero*-pentofuranosyl]thymine (2). To a solution of **1** (1 g, 2.84

mmol) in MeOH (20 mL), pyridine (3 mL) was added then *O*-methylhydroxylamine hydrochloride (0.31 g, 3.71 mmol). The reaction mixture was stirred overnight at room temperature. After solvent evaporation, the residue was extracted with EtOAc (100 mL), washed with H_2O (10 mL) and saturated NaCl solution (10 mL), then the organic phase was dried (Na_2SO_4), filtered, concentrated and submitted to a column chromatography (3:2 EtOAc/petroleum ether) to give **2** (1 g, 92%) as a mixture of isomers (3:1 *E*/*Z*). Syrup; R_F 0.45 (*E*), 0.39 (*Z*) (3:2 EtOAc/petroleum ether); $[\alpha]_D^{20}$ +66.3° (*c* 0.9, $CHCl_3$); λ_{max}^{EtOH} 208 nm (ε 14500) and 265 (9550); ν_{max}^{KBr} 3460 (NH), 3040, 2920, 2840 (CH), 1700-1680 (C=O), and 1460 (C=N) cm^{-1}. ^1H NMR ($CDCl_3$) δ 9.01 (*bs*, 1 H, NH), 7.72 (*q*, $J_{H\text{-}6,Me}$ = 1.5 Hz, H-6 *Z*), 7.60 (*q*, H-6 *E*), 3.90 (*s*, N-OMe *E*), 3.89 (*s*, N-OMe *Z*), 1.94 (*d*, 3 H, Me-5), 0.91 (*s*, 9 H, CMe_3), 0.11 (*s*, $SiMe_2$ *Z*), and 0.09 (*s*, $SiMe_2$ *E*). MS: *m*/*z* (%) 200 (100), 73 (88), 89 (83), 117 (62, CMe_3 - $SiMe_2$·), 126 (18, thymine), 258 (8, $M^{\cdot+}$ - thymine), 326 (3, $M^{\cdot+}$ - CMe_3), 353 (1, $M^{\cdot+}$ -2Me), and 383 (0.8, $M^{\cdot+}$).

Anal. Calcd for $C_{17}H_{29}N_3O_5Si$ (383.52): C, 53.24; H, 7.62; N, 10.96. Found: C, 53.25; H, 7.60; N, 10.69.

1-[5-*O*-*tert*-Butyldimethylsilyl-2-3-dideoxy-3-(*N*-methoxyamino)-β-D-*threo*-pentofuranosyl]thymine (3). To a solution of **2** (0.64 g, 1.67 mmol) in acetic acid (15 mL) at room temperature, $NaBH_3CN$ (0.32 g, 5.09 mmol) was added. After 3 h, the conversion of the starting material was very poor (~10%). Another portion of $NaBH_3CN$ (10.32 g, 5.09 mmol) was added and after 2 h, the reaction mixture was concentrated, the solvent coevaporated 5 times with toluene, and the residue dissolved in EtOAc (100 mL), washed (2x10 mL aqueous saturated $NaHCO_3$, then 10 mL saturated NaCl solution), dried (Na_2SO_4), concentrated, then submitted to a column chromatography (3:2 EtOAc/petroleum ether) which gave **2** (0.45 g unconverted starting material) **3** (0.1 g, 15.5%), and **4** (~30 mg, 4.7%): **3** is a white solid, mp 75.6-76.3 °C; R_F 0.28 (3:2 EtOAc/petroleum ether); $[\alpha]_D^{23}$ -18.7° (*c* 0.4, $CHCl_3$); λ_{max}^{EtOH} 213 nm (ε 6400) and 267 (10900); ν_{max}^{KBr} 3240 (NH), 3020, 2940, 2900 (CH), 1690, 1650 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.20 (*bs*, 1 H, NH-thymine), 7.84 (*q*, 1 H, $J_{6,Me}$ = 1.5 Hz, H-6), 6.10 (*d*, 1 H, $J_{NH,3'}$ = 6.5 Hz, NH), 3.52 (*s*, 3 H, OMe), 1.96 (*d*, 3 H, Me), 0.96 (*s*, 9 H, Me_3C), 0.14 (*s*, 6H, $SiMe_2$). MS: *m*/*z* (%) 89 (100), 73 (64), 101 (49), 57 (20, Me_3C), 126 (10, thymine), 228 (7.6), 117 (6.5, Me_3CSiMe_2) 253 (1.2), 385 (0.5, $M^{\cdot+}$).

Anal. Calcd for $C_{17}H_{31}N_3O_5Si$ (385.54): C, 52.96; H, 8.10; N, 10.90. Found: C, 52.76; H, 7.97; N, 10.88.

1-[5-*O*-*tert*-Butyldimethylsilyl-2,3-dideoxy-3-(*N*-methoxyamino)-β-D-*erythro*-pentofuranosyl]thymine (4). Obtained as described for **3**, but not purified. R_F 0.25 (3:2 EtOAc/petroleum ether). ^1H NMR ($CDCl_3$ δ 9.00 (*bs*, 1 H, NH-

thymine), 7.58 (*q*, 1 H, $J_{6,Me}$ = 1.5 Hz, H-6), 3.58 (*s*, 3 H, OMe), 1.91 (*d*, 3 H, Me), 0.92 (*s*, 9 H, Me_3C), and 0.11 (s, 6 H, $SiMe_2$).

(*E* and *Z*)-1-[2,3-Dideoxy-3-(*N*-methoxyimino)-β-D-*glycero*-pentofuranosyl]thymine (5). To a solution of **2** (0.64 g, 1.67 mmol) in MeOH (50 mL) at room temperature, $NaBH_3CN$ (1.15 g, 18.37 mmol) was added, and the pH was brought to ~2 with 6M HCl/MeOH. After 2 h no more starting material was detectable (TLC). The pH was adjusted to 7 with 10% NaOH solution (saturated with NaCl), the solution was concentrated to dryness, and the residue, submitted to a column chromatography (19:1 CH_2Cl_2/MeOH), gave 0.22 g (49%) of **5** (R_F 0.19), 0.12 g (8.8%) of **6** (R_F 0.15), and 0.04 g (26.5%) of **7** (R_F 0.12). Compound **5**, a white solid, was a 4:1 mixture of *E*/*Z* isomers: mp 166-167.6 °C, $[\alpha]_D^{24}$ +37.8° (*c* 0.94, $CHCl_3$); λ_{max}^{EtOH} 208 nm (ε 27100) and 265 (20700); ν_{max}^{KBr} 3460 (NH), 3120, 3040, 2900 (CH), 1690, 1650 (C=O), and 1460 (C=N) cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.90 (*bs*, 1 H, NH), 7.59 (*q*, H-6 *Z*), 7.40 (*q*, $J_{6,Me}$ = 1.5 Hz, H-6 *E*), 3.94 (*s*, N-OMe *E*), 3.90 (s, N-OMe Z), 2.52 (*bs*, 1 H, OH), and 1.93 (*d*, 1 H, Me). MS: *m*/*z* (%) 56 (100), 144 (70, $M^{\cdot+}$ - thymine), 113 (47), 126 (45, thymine), 135 (22.5), 239 (8.4, $M^{\cdot+}$ - OMe), 208 (3.5), and 178 (1.9, $M^{\cdot+}$ + 1).

Anal. Calcd for $C_{11}H_{15}N_3O_5$ (269.26): C, 49.07; H, 5.62; N, 15.61. Found: C, 48.83; H, 5.57; N, 15.47.

1-[2,3-Dideoxy-3-(*N*-methoxyamino)-β-D-*threo*-pentofuranosyl]thymine (6). Prepared as described for **5**: white solid, mp 89.9-90.6 °C; $[\alpha]_D^{26}$ +30.6° (*c* 0.87, $CHCl_3$); λ_{max}^{EtOH} 209 nm (ε 17160) and 267 (18280); ν_{max}^{KBr} 3460 (NH), 3380 (OH), 3140, 3000, 2910 (CH), 1700, 1650 (C=O), and 1070 (O-C) cm^{-1}. ^1H NMR ($CDCl_3$) δ 9.43 (*bs*, 1 H, NH), 7.80 (*q*, 1 H, $J_{6,Me}$ = 1 Hz, H-6), 5.89 (~*d*, 1 H, $J_{3',NH}$ = 4 Hz, NH-thymine), 3.58 (*s*, 3 H, OMe), 3.13 (*bs*, 1 H, OH), and 1.93 (*d*, 3 H, Me). MS: *m*/*z* (%) 102 (100), 99 (76), 127 (50, thymine), 69 (60), 47 (58), 145 (30, $M^{\cdot+}$ - thymine), 114 (29), 210 (3.1), and 271 (2.3, $M^{\cdot+}$).

Anal. Calcd for $C_{11}H_{17}N_3O_5$ (271.28): C, 48.70; H, 6.32; N, 15.49. Found: C, 48.62; H, 6.32; N, 15.57.

1-[2,3-Dideoxy-3-(*N*-methoxyamino)-β-D-*erythro*-pentofuranosyl]thymine (7). Obtained as described for **5**: amorphous powder; ν_{max}^{KBr} 3900 (NH, OH), 3040, 2900, 2800 (CH), 1680-1640 (C=O), and 1090 (C-O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 9.31 (*bs*, 1 H, NH-thymine), 7.47 (*q*, 1 H, $J_{6,Me}$ = 1 Hz, H-6), 5.70 (*bs*, 1 H, NH), 3.57 (*s*, 3 H, OMe), 3.05 (*bs*, 1 H, OH), and 1.91 (*d*, 3 H, Me).

Anal. Calcd for $C_{11}H_{17}N_3O_5$ (271.28): C, 48.70; H, 6.32; N, 15.49. Found: C, 48.32; H, 6.52; N, 15.07.

1-[3-(*N*-Benzyl-*N*-hydroxyamino)-5-*O*-*tert*-butyldimethylsilyl-2,3-dideoxy-β-D-*threo*-pentofuranosyl]thymine (9). To a solution of *N*-benzyl-

hydroxylamine hydrochloride (3.8 g, 23.8 mmol) in MeOH (50 mL), pyridine (2.4 mL, 29.7 mmol) was added under N_2, at room temperature, then immediately a crude solution of **1** (2.1 g, 5.96 mmol) in MeOH (5 mL). The color of the solution changed from light-brown to greenish in 10-20 min. The reaction was monitored by TLC. When all the starting material was consumed, $NaBH_3CN$ (1.45 g, 85%, 17.8 mmol) was added in one portion. After ~2 h stirring at 20 °C, the reaction mixture was concentrated, partitioned between EtOAc (200 mL) and H_2O (15 mL). The organic phase was dried (Na_2SO_4), concentrated, and submitted to a column chromatography (19:1 CH_2Cl_2/MeOH) to give **9** (1.38 g) and **10** (0.46 g) (cumulated yield 67%). **9** was a white crystalline powder: mp 115-116 °C; R_F (19:1 $CHCl_2$/MeOH); $[\alpha]_D^{20}$ +39° (*c* 1, $CHCl_3$); λ_{max}^{EtOH} 206 nm (ε 18200), and 267 (9600); ν_{max}^{KBr} 3390 (NH, OH), 3100, 2954 (CH), 1690-1670 (C=O) cm^{-1}. ^1H NMR (C_5D_5N, 80 °C) δ 9.13 (*bs*, 1 H, NH), 8.08 (*q*, 1 H, $J_{6,Me}$ = 1 Hz, H-6), 7.30 (*m*, 3 H, Ph), 7.52 (*m*, 2 H, Ph), 5.00-4.10 (*bs*, 1 H, N-OH), 4.08 and 3.90 (*AB*, 2 H, CH_2-Ph), 1.98 (*d*, 3 H, Me), 0.97 (*s*, 9 H, CMe_3), and 0.12 (*s*, 2 H, $SiMe_2$). ^1H NMR ($CDCl_3$) δ 8.50 (*bs*, 1 H, NH), 7.67 (*q*, 1 H, H-6), 7.35 (*m*, 5 H, Ph), 6.61 (*bs*, 1 H, N-OH), 4.00 and 3.71 (*AB*, CH_2-Ph), 1.90 (*d*, 3 H, Me), 0.92 (*s*, 9 H, CMe_3), and 0.13 (*s*, 6 H, $SiMe_2$). MS: *m/z* (%) 91 (100, benzyl·), 73 (29), 81 (22), 160 (10.5), 145 (6.8), 117 (6, Me_3CSiMe_2), 127 (3.3, thymine), 286 (1), 329 (0.4, $M^{\cdot+}$ - $OSiMe_2CMe_3$), and 404 (0.2, $M^{\cdot+}$ - CMe_3).

Anal. Calcd for $C_{23}H_{35}N_3O_5Si$ (461.64): C, 59.84; H, 7.64; N, 9.10. Found: C, 59.82; H, 7.65; N, 9.15.

1-[3-(*N*-Benzyl-*N*-hydroxyamino)-5-*O*-*tert*-butyldimethylsilyl-2,3-dideoxy-β-D-*erythro*-pentofuranosyl]thymine (10). Prepared as described for **9**, **10** was obtained as a white solid: mp 117-118 °C; R_F 0.29 (19:1 CH_2Cl_2/MeOH); $[\alpha]_D^{21}$ +26° (*c* 1, $CHCl_3$); λ_{max}^{EtOH} 206 nm (ε 20900), and 266 (11300); ν_{max}^{KBr} 3440 (NH), 3230 (OH), 3040, 2955, 2857 (CH), 1680, 1662 (C=O), and 1639 (C=C) cm^{-1}. ^1H NMR ($CDCl_3$) δ ~8.50 (*bs*, 1 H, NH), 7.57 (*q*, 1 H, $J_{6,Me}$ = 1 Hz, H-6), 7.42 (*m*, 5 H, Ph), 6.10 (*bs*, 1 H, N-OH), 3.92 and 3.80 (*AB*, 2 H, CH_2-Ph), 1.91 (*d*, 3 H, Me), 0.92 (*s*, 9 H, CMe_3), and 0.09 (*s*, 6 H, $SiMe_2$). MS: *m/z* (%) 91 (100, benzyl) 73 (28), 81 (30), 145 (8), 117 (4, Me_3CSiMe_2), 127 (2.5, thymine), 286 (0.9), 335 (0.4, $M^{\cdot+}$ - thymine) 404 (0.15, $M^{\cdot+}$ - CMe_3).

Anal. Calcd for $C_{23}H_{35}N_3O_5Si$ (461.64): C, 59.84; H, 7.64; N, 9.10. Found: C, 59.79; H, 7.62; N, 9.07.

1-[3-(*N*-Benzyl-*N*-hydroxyamino)-2,3-dideoxy-β-D-*threo*-pentofuranosyl]thymine (11). A mixture of **9** (0.3 g, 0.65 mmol) and 80% acetic acid (6 mL) was stirred 16 h at 20 °C, then the reaction mixture was concentrated and, after removal of the last traces of acetic acid by codistillation with toluene, submitted

to a column chromatography (9:1 CH_2Cl_2/MeOH) to give **11** (0.2 g, 87%): mp 97.1-98.4 °C; R_F 0.37 (9:1 CH_2Cl_2/MeOH); $[\alpha]_D^{23}$ +31° (*c* 1, $CHCl_3$); λ_{max}^{EtOH} 208 nm (ε 5200) and 266 (3000); ν_{max}^{KBr} 3400 (NH), 3196 (OH), 3063, 2960, 2880 (CH), and 1680 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6) δ 11.26 (*bs*, 1 H, NH); 7.91 (*bs*, 2 H, H-6 + N-OH), 7.30 (*m*, 5 H, Ph), 4.67 (*t*, 1 H, OH), 3.78 and 3.62 (*AB*, 1 H, CH_2-Ph), and 1.77 (*d*, 3 H, Me). MS: *m/z* (%) 91 (100, benzyl), 69 (47), 81 (24), 127 (6.8, thymine), 160 (4.8), 180 (2.4), 205 (1.6), 221 (1, $M^{\cdot+}$ - thymine), 286 (0.6), 239 (0.4), 328 (0.16, $M^{\cdot+}$ - H_2O), and 347 (0.06, $M^{\cdot+}$).

Anal. Calcd for $C_{17}H_{21}N_3O_5$ (347.37): C, 58.78; H, 6.09; N, 12.10. Found: C, 58.51; H, 6.35; N, 11.88.

1-[3-(*N*-Benzyl-*N*-hydroxyamino)-2,3-dideoxy-β-D-*erythro*-pentofuranosyl]thymine (12). 0.2 g of **10** was desilylated as described for **9** to give **12** (0.125 g, 83%): mp 99.6-100 °C; R_F 0.52 (9:1 CH_2Cl_2/MeOH); $[\alpha]_D^{26}$ +13° (*c* 1.1 MeOH); λ_{max}^{MeOH} 207 nm (ε 1940) and 267 (1020); ν_{max}^{KBr} 3390 (NH), 3301 (OH), 3060, 2940 (CH), and 1687 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6) δ 11.25 (*bs*, 1 H, NH), 8.02 (*q*, 1 H, $J_{6,Me}$ = 1 Hz, H-6), 7.30 (*m*, 5 H, Ph), 7.78 (*bs*, 1 H, N-OH), 5.30 (*t*, 1 H, OH), 3.17 (*s*, 2 H, CH_2-Ph), and 1.79 (*d*, 3 H, Me). MS: *m/z* (%) 91 (100, benzyl), 106 (36), 146 (28), 126 (14, thymine), 204 (17), 180 (7), 221 (1.8, $M^{\cdot+}$ - thymine), 286 (1.6), and 328 (1.2, $M^{\cdot+}$ - H_2O).

Anal. Calcd for $C_{17}H_{21}N_3O_5$ (347.37): C, 58.78; H, 6.09; N, 12.10. Found: C, 58.50; H, 6.22; N, 11.91.

1-[5-*O*-*tert*-Butyldimethylsilyl-2,3-dideoxy-3-(prop-2-ylidenamino)-β-D-*threo*-pentofuranosyl]thymine 3'-*N*-oxide (14). A solution of **13** (0.3 g, 0.81 mmol) and acetone (8 mL) was left for 2 days at room temperature. The reaction mixture was concentrated to dryness and the crude product was submitted to a column chromatography (17:3 Et_2O/MeOH) to give 0.13 g of starting material (43%) and 0.12 g of **14** (33%) as a white crystalline powder: mp 92.3-92.7 °C; R_F 0.3 (17:3 Et_2O/MeOH); $[\alpha]_D^{25}$ +44.9° (*c* 0.4, $CHCl_3$); λ_{max}^{EtOH} 221 nm (ε 5180) and 259 (14700); ν_{max}^{KBr} 3400 (NH), 3040, 2940, 2840 (CH), 1700 and 1680 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 9.46 (*bs*, 1 H, NH), 8.23 (*q*, 1 H, $J_{6,Me}$ = 1 Hz, H-6), 2.21 (*s*, 6 H, Me_2C=), 1.89 (*d*, 3 H, Me), 0.88 (*s*, 9 H, Me_3C), and 0.06 (*s*, 6 H, $SiMe_2$). MS: *m/z* (%) 73 (100), 21 (74), 56 (64, Me_3C), 112 (34), 213 (31), 127 (19.5, thymine) 354 (7, $M^{\cdot+}$ - Me_3C), 281 (2), and 412 (1.3, $M^{\cdot+}$).

Anal. Calcd for $C_{19}H_{33}N_3O_5Si$ (411.58): C, 55.45; H, 8.08; N, 10.21. Found: C, 55.12; H, 8.38; N, 9.76.

1-[2,3-Dideoxy-3-(*N*-hydroxyamino)-3-*N*,5-*O*-(isopropano)-β-D-*threo*-pentofuranosyl]thymine (15). A solution of **14** (0.12 g, 0.29 mmol) in 80% acetic acid (10 mL) was stirred overnight at room temperature. The reaction mixture

was concentrated and the last traces of acetic acid removed by codistillation with toluene. The reaction mixture submitted to a column chromatography (7:3 $CHCl_3$/acetone) gave **15** (0.08 g, 92%): mp 184-184.2 °C; R_F 0.2 (7:3 $CHCl_3$/acetone); $[\alpha]_D^{24}$ -157.9° (*c* 0.82, $CHCl_3$); λ_{max}^{EtOH} 209 nm (ε 8400) and 268 (8200); ν_{max}^{KBr} 3410 (NH), 3360 (OH), 3020, 2970, 2820 (CH), and 1700-1640 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6) δ 11.16 (*bs*, 1 H, NH), 8.22 (*bs*, 2 H, H-6 + N-OH), 1.78 (*d*, 3 H, Me), and 1.26 and 1.30 (*2s*, 2x3 H, Me_2C). MS: *m/z* (%) 81 (100), 69 (98), 127 (62, thymine), 41 (38), 99 (35), 171 (17, $M^{\cdot+}$ - thymine), 239 (12), 152 (7), 196 (2), and 298 (1, $M^{\cdot+}$ +1).

Anal. Calcd for $C_{13}H_{19}N_3O_5$ (297.31): C, 52.52; H, 6.44; N, 14.13. Found: C, 52.05; H, 6.54; N, 13.64.

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