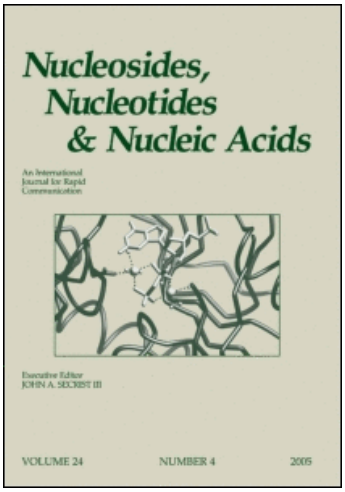

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SYNTHESIS AND ANTI-HIV ACTIVITY OF THYMIDINE ANALOGUES BEARING A 4'-CYANOVINYL GROUP AND SOME DERIVATIVES THEREOF

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SYNTHESIS AND ANTI-HIV ACTIVITY OF THYMIDINE ANALOGUES BEARING A 4′-CYANOVINYL GROUP AND SOME DERIVATIVES THEREOF

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ABSTRACT

Treatment of 3′-*O*-*tert*-butyldimethylsilyl-2′,5′-dideoxy-5′-oxothymidine (**4**) with potassium or magnesium nitromethanide afforded in good yield the resolvable epimeric mixture of the expected blocked nitronucleosides **5** which upon dehydration led to the corresponding *E*-nitroenofuranosylthymidine **6**. Nucleophilic attack of cyanide onto the nitrovinyl group led to a nucleoside analogue bearing a terminal 1-cyanovinyl group (**7**), a soft electrophilic group which, upon reaction with benzeneselenol, underwent a conjugate addition to the phenylselenonucleoside derivative **9**. All these compounds, eventually de-*O*-silylated, were subject of *in vitro* biological testing, some exhibiting interesting cytotoxic or antiviral properties.

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INTRODUCTION

The 1-cyanovinyl group behaves as a soft electrophile. We have shown (1) that its electrophilicity depends on the structure of the sugar or nucleoside on which it is fixed and that this property is correlated with the antiviral and cytotoxic properties of the molecule bearing this group. A number of the simple sugar derivatives tested bore high biological activities either cytotoxic or antiviral (2) whereas the corresponding modified uridines (3) were almost devoid of activity. This led us to extend our interests toward deoxynucleosides and to prepare thymidine analogues bearing this electrophilic group.

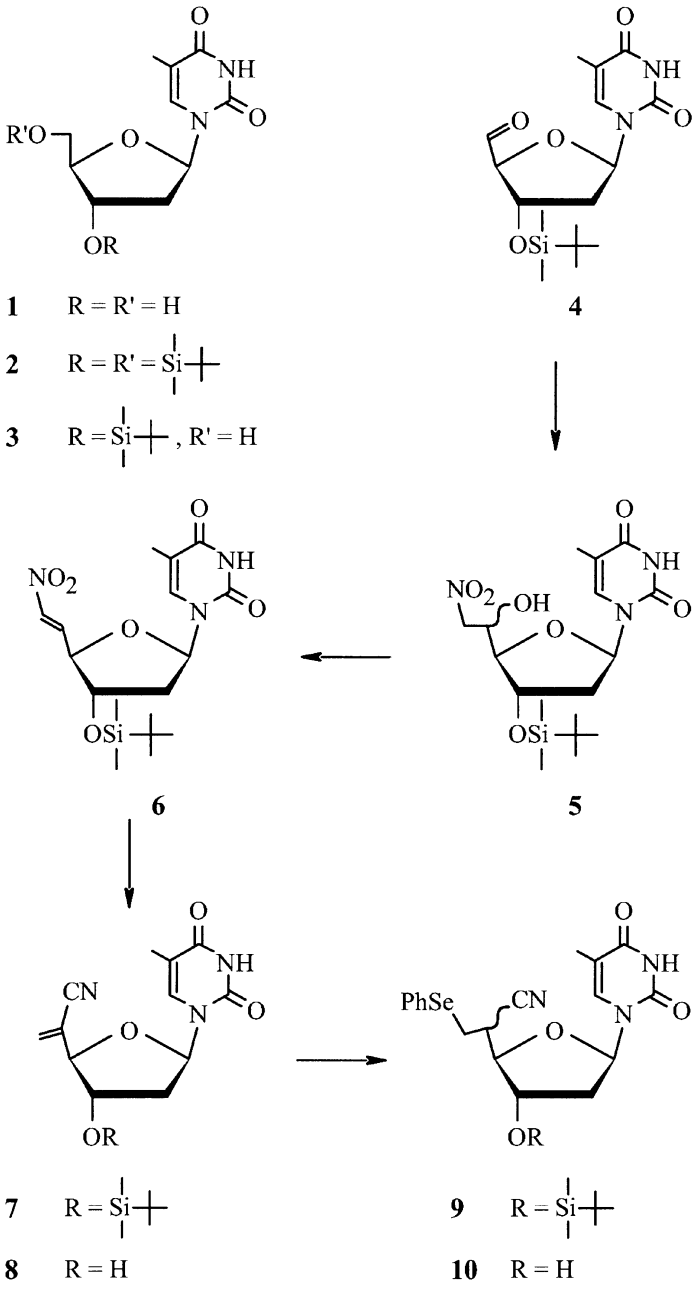
RESULTS AND DISCUSSION

Compounds **2**–**3** (Scheme 1) are known but as they constitute very important starting materials and since we considerably improved the yields, we will briefly describe the new procedures used.

Ogilvie's team (4) using *tert*-butylchlorodimethylsilane (TBDMSCl) and imidazole for the di-*O*-silylation and 80% acetic acid for the mono-de-*O*-silylation, prepared **3** in overall yield of 50% from **1**. More recently, Wang and Middleton (5) using slightly different conditions, obtained yields ranging from 55 to 65%. We ran the di-*O*-silylation at higher temperature with TBDMSCl in pyridine and the partial hydrolysis with dilute trifluoroacetic acid obtaining a 82% overall yield from **1** to **3**.

Compound **3** was almost quantitatively (97%) oxidized to **4** using the Dess-Martin periodinane reagent following the procedure described by Samano and Robins (6). Oxidation using chromic anhydride-pyridine led to a much lower yield (70%) (7) whereas the dicyclohexylcarbodiimide/pyridine/dimethylsulfoxide/trifluroacetic procedure afforded a 84% yield (5).

The aldehyde **4** was submitted to a Henry's reaction (8) to afford **5** as an epimeric mixture. We have shown that aldehydosugars adopt a preferred conformation where the carbonyl group almost eclipses the vicinal C-O single bond (9) but that the attack by sodium nitromethylide does not proceed from the most accessible face, but from the opposite face, a preliminary rotation of the carbonyl group allowing the incipient negative charge on the oxygen atom to be directed away from the vicinal oxygen atom (9,10). This corresponds to a Felkin-Anh's stereochemical approach (11) and leads to the D-*ribo* epimer as the major compound. On the contrary, in the presence of a dication like magnesium, the two oxygen atoms are locked together by chelation and the issue of the reaction is that of a Cram-Kopecky's (12) trajectory. Some results are shown in Table 1. The two epimers **5a** (β-D-*ribo*) and **5b** (α-L-*lyxo*) have been isolated. Their configurations have been assigned from the results of previous work (9,10). Dehydration ($NaOAc/Ac_2O$) of a mixture of **5a** and **5b** afforded the *E*-nitroenose derivative **6** ($J_{5',6'} = 13.5\,Hz$)



Scheme 1.

Table 1. Epimeric Distribution of the Henry's Reaction (**4** → **5**) in Different Reaction Conditions (Quantities Relative to 1 mmol of **4**)

$MeNO_2$	t-BuOK (mmol)	MeMgCl (mmol)	t (°C)	Yield (%)	D-*ribo*/L-*lyxo*
3	1.3	0	− 20	67	2:1
3	0	3	0	71	1:3
4	0	1.3	0	65	1:3
3	0	1.3	− 78 → 0	68	1:6

in 76% yield, the L-*lyxo* epimer reacting approximately 15 times faster than its epimer.

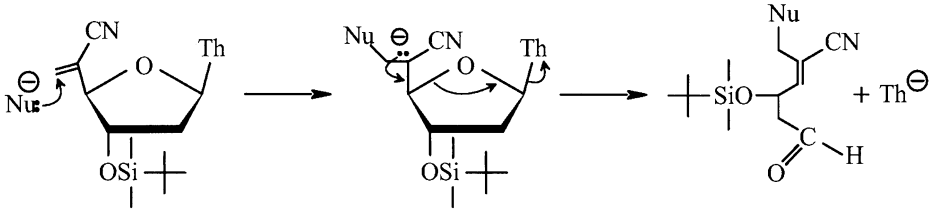
The first examples of sugar derivatives bearing a 1-cyanovinyl group have been described by Paulsen (13) then extended to a large variety of sugars and nucleosides (2,3,10). Treatment of **6** with potassium cyanide in buffered phase-transfer conditions led in 85% yield to **7** which was de-*O*-silylated to **8** (94%).

Compounds **7** and **8** are soft electrophiles but as the thyminyl group bears nucleofugal properties, a number of nucleophilic attacks, particularly those using phosphorous nucleophiles which were feasible in other series (9), led to a loss of the thyminyl group probably via the following mechanism (Scheme 2).

The particularly soft phenylselenol nucleophile was added to **7** to yield an unresolvable mixture of the two epimers of **9** (79%) which was de-*O*-silylated to **10** (93%).

The nucleoside analogues have been tested for the CC_{50} (cytotoxic concentration) and IC_{50} (cytostatic concentration) on CEM cells (Table 2) as described in the experimental section. Antiproliferative activities of each compound against human (NCI-H460, HT29, Caki-1, HL60) or murine (L1210) cancer cell lines and normal cell lines (MRC5) were compared to evaluate their selectivity (Table 2).

Compound **2**, *O*-silylated at two positions, exhibited more pronounced cytotoxic and cytostatic properties against CEM cells, than its analogue **3**



Scheme 2.

Table 2. Cytotoxic ($CC_{50}/\mu M$) and Cytostatic ($IC_{50}/\mu M$) Activities of Modified Thymidines

Compound Cell line	**2**	**3**	**5a**	**5b**	**6**	**7**	**8**	**9**	**10**[a]
CEM/CC_{50})	6.1	> 50	> 50	> 50	5.4	1.2	14.6	11.6	22
CEM (IC_{50})	5.1	> 50	30.5	35.5	3.6	0.82	4.9	3.7	10.6
MRC5 (CC_{50})	4	> 100	> 100	> 100	26	3.4	31.5	28.3	> 100
L1210 (CC_{50})	nd	nd	12	17	2	1	10	2.3	3.5
NCI-H460 (CC_{50})	2.8	> 100	> 100	69	26	2.6	24	5.4	18.5
HT29 (CC_{50})	2	> 100	> 100	> 100	24	5.7	41	8.7	50.5
Caki-1 (CC_{50})	11	> 100	> 100	> 100	30	33	47.5	27	100
HL-60 (CC_{50})	3	100	42.5	45.5	23.5	3.2	22	4.2	30.6

[a] 2:1 Epimeric mixture.

only silylated at the 3′ position. These cytotoxic and antiproliferative (cytostatic) properties of TBDMS groups on nucleoside analogues have already been described (14). However, **2** did not show any significant difference between IC_{50} and CC_{50} on CEM cell lines and between CC_{50} for non-tumoral (MRC5) and tumoral cells. The two epimers **5a** and **5b** obtained from **4** by a Henry's reaction had very similar cellular activities except on the NCI-H460 cell lines for which the α-L-*lyxo* epimer (**5b**) exhibited a better activity than the β-D-*ribo*-one (**5a**). When a mixture of **5a** and **5b** was dehydrated to afford **6**, an improvement of activities on the cancer cell lines was observed. This was especially the case with the murine lymphocytic leukemia (L1210) for which the *in vitro* selectivity index (ratio between CC_{50} for non-tumoral (MRC5) and tumoral cells), was high (13). The most cytostatic nucleoside of this study was compound **7**. However, the *in vitro* selectivity index observed was low considering the antiproliferative effect on the non-tumoral cell line ($CC_{50} = 3.4\,\mu M$). A decrease of all activities was observed when **7** was de-*O*-silylated into **8**. An interesting selectivity index on L1210 (12) was restored with compound **9**. The 3′-*O*-desilylation of **9** to afford **10** brought about a decrease of activity similar to the one observed between compounds **7** and **8**. However **10** retained the antiproliferative properties of **9** on L1210 cells with a remarkable selectivity index (> 28).

The compounds were also evaluated against a panel of viruses in cell culture. None of the compounds showed significant activity at subtoxic concentrations against herpes simplex virus type 1 and type 2, vaccinia virus and vesicular stomatitis virus in E_6SM cell cultures; vesicular stomatitis virus, Coxsackie virus B4 and respiratory syncytial virus in HeLa cell cultures; and parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4 and Punta Toro virus in Vero cell cultures. However, whereas **9** showed some marginal inhibitory activity against varicella-zoster virus (strains YS and YS/R) (EC_{50}: 3.5 μM; CC_{50}: 16 μM), compounds **5a**, **6**, **7**, **5b**, **9** and **10** were endowed with anti-cytomegalovirus activity (strains AD-169 and Davis) at

Table 3. Anti-Human Cytomegalovirus Activity of Test Compounds in Human Embryonic Lung Fibroblasts

Compound	Anti-CMV Activity EC_{50} (μM)[a]		Cytotoxicity/Cytostatic Activity	
	AD-169	Davis	Cell Morphology[b] (MCC)	Cell Growth[c] (IC_{50})
5a	14	4	50	110
5b	17	16	50	92
6	20	10	50	42
7	43	31	200	35
8	> 200	> 200	> 200	161
9	3.1	3.1	20	16
10[d]	50	38	200	30

[a] Inhibitory concentration required to reduce virus plaque formation by 50%. Virus input was 100 plaque forming units (PFU).
[b] Minimum cytotoxic concentration required to cause a microscopically detectable alteration in cell morphology.
[c] Cytostatic concentration required to reduce HEL cell growth by 50%.
[d] 2:1 Epimeric mixture.

subtoxic concentrations (Table 3). The highest selectivity (ratio of CC_{50}/EC_{50}) was performed by **5a** (8–27). With regard to their anti-HIV activity, **9** and **10** inhibited HIV-1- and HIV-2-induced cytopathicity in CEM cell cultures at 2.5–10 μM; and 3.5–5.3 μM, respectively, whereas their cytostatic concentrations were 17 and 34 μM when measured in the same assay. This resulted in a selectivity index of 6–10 for **10**.

EXPERIMENTAL

General Synthetic Methods

Melting points (uncorrected) were determined under microscope with a Mettler FP52 melting-point apparatus. Thin layer chromatographies (TLC) were performed on silica gel HF_{254} (Merck) with detection by UV light. Column chromatography was conducted on silica gel 60 (0.04–0.2 mm, Merck). IR spectra were recorded on a Perkin-Elmer Model 1600 spectrometer and UV spectra were measured on a Kontron Uvicon 810 spetrophotometer. NMR spectra were recorded at 20°C on Bruker WP 200 SY (^1H 200 MHz) or Varian Gemini (^1H 300 MHz) spectrometers (chemical shifts in ppm from TMS;δunits). Optical rotations were measured with a Schmidt-Haensch polarimeter. Mass spectra (EIMS, 70 eV) were recorded on a VG-70-70E spectrometer.

Antiproliferative Activity

Antiproliferative activities of the compounds expressed at CC_{50} were determined after 3 days using five cancer cell lines in exponential grow rate [L1210 (murine lymphocytic leukemia, ATCC # CCL-219), NCI-H460 (human lung large cell carcinoma, ATCC # HTB-177), HT29 (human colon adenocarcinoma ATCC # HTB-38), Caki-1 (human kidney carcinoma, ATCC # HTB-46) and HL60 (human promyelocytic leukemia, ATCC # CCL-240)]. A normal cell line [MRC5 (human foetal lung fibroblast, ATCC # CCL-171)] was used as control. With MRC5 cells, antiproliferative effects of each compound were determined using the MTT assay as previously described by Park et al[15]. Briefly, the MTT test is based on the enzymatic reduction of the tetrazolium salt MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] in living, metabolically active cells, but not dead cells. The reaction product, a purple colored formazan soluble in dimethylsulfoxide, is measured colorimetrically at 550 nm, using a multiwell plate reader. Measurements are, as a rule, duplicated. These results are issued from two distinct manipulations. Appropriate positive controls were included CC_{50} (cytotoxic concentration) and IC_{50} (cytostatic concentration) of each compound were determined on the human T lymphoblastoid cell line CEM (ATCC # CL-119) after 4 days of culture at exponential rate. At the end of this period, cells were incubated with propidium iodide that stained only dead cells. The distinction between cytotoxic and cytostatic effects of the compound was made by using a flow cytometry cell analyzer (Facscalibur Becton Dickinson). The total cell number and ratio between living and dead cells were representative of the cytostatic and cytotoxic properties of the compounds respectively. Measurements are generally duplicated.

Antiviral Assays

The antiviral assays, other than HIV, were based on inhibition of virus-induced cytopathicity in either E_6SM [herpes simplex virus type 1 (HSV-1), HSV-2, vaccinia virus (VV)], HEL (VZV, CMV) or Vero (VSV, respiratory syncytial virus, Coxsackie virus, parainfluenza-3 virus, Sindbis virus, Punta Toro virus, reovirus-1) cell cultures, following previously established procedures. Briefly, confluent cell cultures in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus, 1 $CCID_{50}$ being the virus dose required to infect 50% of the cell cultures. After a 1 h virus adsorption period, residual virus was removed, and the cell cultures were incubated in the presence of varying concentrations (400, 200, 100, ...μg/mL) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds.

Inhibition of HIV-1-Induced Cytopathicity in CEM Cell Cultures

The methodology of the anti-HIV assays has been described previously. Briefly, human CEM ($\sim 3 \times 10^5$ cells ml^{-1}) cells were infected with 100 $CCID_{50}$ of HIV-1 (III_B) or HIV-2 (ROD)/ml and seeded in 200 µl wells of a microtiter plate, containing appropriate dilutions of the test compounds. After 4 days of incubation at 37°C, virus-induced CEM giant cell formation was examined microscopically.

3′,5′-Bis-(*O*-*tert*-butyldimethylsilyl)-2′-deoxythymidine (2). To a solution of thymidine (24.22 g, 0.100 mol) in anhydrous pyridine (200 mL), *ter*-butylchlorodimethylsilane (37.67 g, 0.250 mol) was added. The reaction mixture was stirred at room temperature for 1 h, at 60–70°C for 22 h, then cooled to room temperature. The excess of silylating agent was destroyed by addition under stirring of water (10 mL, 15 min). Solvents were evaporated and coevaporated with toluene (2×100 mL), the residue dissolved in ice-cold EtOAc (400 mL), washed successively with a cold 1 M HCl solution (200 mL), with cold water (2×100 mL), saturated $NaHCO_3$ aqueous solution, and aqueous saturated sodium chloride solution (100 mL), then dried (Na_2SO_4), and concentrated. The residue was crystallized in a mixture of EtOAc (5 mL) and hexane (65 mL) giving **2** (39.18 g) as a white solid. A second fraction of **2** (4.06 g) was obtained after recrystallization in hexane (15 mL). Cumulated yield: 43.23 g (92%).

3′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxythymidine (3). To a solution of **2** (44.2 g, 93.9 mmol) in CH_2Cl_2 (510 mL) a mixture of trifluoroacetic acid and water (10:1, v/v, 51 mL) was added and the reaction mixture was stirred at room temperature for 45 min, then in an ice bath for 30 min. The pale yellow solution was diluted with cold CH_2Cl_2 (100 mL), washed with ice-cold water (450 mL), saturated $NaHCO_3$ (200 mL) and NaCl (150 mL) aqueous solutions, dried (Na_2SO_4), concentrated, and submitted to a column chromatography (6:1 → 4:1 CH_2Cl_2/acetone) to afford **3** (29.6 g, 89%).

1-[3-*O*-(*tert*-Butyldimethylsilyl)-2-deoxy-β-D-*erythro*-pentodialdo-1,4-furanosyl]thymine (4). 3 (7.13 g, 20.0 mmol) was oxidized with Dess-Martin periodinane reagent (12.72 g, 30.0 mmol) following the procedure described by Samano and Robins (6) affording crude **4** (6,85 g, 97%) as a white solid which was used for the next step without further purification.

1-[3-*O*-(*tert*-Butyldimethylsilyl)-2,6-dideoxy-6-nitro-β-D-*ribo* and α-L-*lyxo*-hexofuranosyl]thymine (5a,b). *Method A:* Under argon atmosphere, methylmagnesium chloride in THF (6.57 mL of a 2.97 M solution, 19.5 mmol) was added at 0°C to a stirred solution of nitromethane (2.44 mL, 45.0 mmol) in anhydrous THF (75 mL) for 10 min, and the stirring was continued at that

temperature for an additional 10 min. After cooling to $-78\,^{\circ}C$, a solution of **4** (5.31 g, 15.0 mmol) in dry THF (25 mL) was added in 15 min and the temperature was allowed to rise to $-23\,^{\circ}C$ in 3 h. The reaction mixture was kept at that temperature for 18 h, at 0 °C for 9 h, diluted with ice-cold EtOAc (300 mL), washed successively with a cold 1 M HCl solution (100 mL), water (2×100 mL), saturated aqueous NaCl (100 mL), dried (Na_2SO_4), evaporated, and purified by column chromatography (3:2 hexane/EtOAc). The two diastereoisomers were partially separated to afford the less polar product **5a** (0.24 g), the slower moving isomer **5b** (0.47 g), both as white foams, and a mixture of the two isomers (3.50 g), total yield (68%). The ratio of **5a** to **5b** was 1:6 (from the ^1H NMR spectra of the crude product): **5a** (β-D-*ribo* isomer), mp 67.9–69.1 °C; R_F 0.59 (1:3 hexane/EtOAc); $[\alpha]_D^{26}$ +12.7° (*c* 1.0, $CHCl_3$); ν_{max}^{KBr} 3414 and 3204 (OH, NH), 2955, 2928, and 2859 (CH), 1693 (C = O), 1555 (NO_2(as)), 1471, 1382 (NO_2(s)), 1125, 1048, and 833 cm^{-1}. ^1H NMR (C_6D_6, 300 MHz): δ10.20 (*s*, 1 H, NH), 6.56 (*q* 1 H, $J_{5,Me\text{-}6}$ 1.0 Hz, H-6), 5.63 (*dd*, 1 H, $J_{1',2'a}$ 6.5 Hz, $J_{1',2'b}$ 7.3 Hz, H-1′), 4.66 (*bd*, 1 H, $J_{5',5'\text{-}OH}$ 6.3 Hz, 5′-OH), 4.56 (*quint.*, 1 H, $J_{2'a,3'}$ 3.1 Hz, $J_{2'b,3'}$ 6.4 Hz, $J_{3',4'}$ 2.4 Hz, H-3′), 4.41 (*dd*, 1 H, $J_{6'a,6'b}$ 17.5 Hz, $J_{5',6'b}$ 8.9 Hz, Hb-6′), 4.40 (*m*, 1 H, $J_{5',6'a}$ 8.3 Hz, $J_{4',5'} \sim 1$ Hz, H-5′), 3.96 (*dd*, 1 H, Ha-6′), 3.69 (*d*, 1 H, $J_{3',4'}$ 2.4 Hz, H-4′), 2.39 (*ddd*, 1 H, $J_{2'a,2'b}$ 13.4 Hz, Hb-2′), 1.82 (*ddd*, 1 H, Ha-2′), 1.69 (*d*, 3 H, Me-5), 0.91 (*s*, 9 H, CMe_3), 0.04 and 0.03 (2 *s*, 6 H, $SiMe_2$). EIMS: *m/z* (%) 171 (100), 75 (99), 117 (52), 201 (17), 295 (4), and 358 (2, $M^{\cdot+}$ - *t*-Bu).

Anal. Calcd for $C_{17}H_{29}N_{3O}7Si$ (415.52): C, 49.14; H, 7.03; N, 10.11. Found: C, 49.25; H, 7.12; N, 9.96.

5b (α-L-*lyxo* isomer) mp 69.7–71.1 °C; R_F 0.59 (1:3 hexane/EtOAc), $[\alpha]_D^{27}$ +21.7° (*c* 1.0, $CHCl_3$); ν_{max}^{KBr} 3400 and 3200 (OH, NH), 2955, 2928, and 2858 (CH), 1689 (C = O), 1556 (NO_2(as)), 1470, 1379 (NO_2(s)), 1114, 1065, and 836 cm^{-1}. ^1H NMR (C_6D_6, 300 MHz, 60 °C): δ9.10 (*bs*, 1 H, NH), 6.24 (q, 1 H, $J_{Me\text{-}5,6}$ 1.2 Hz, H-6), 5.49 (*t*, 1 H, $J_{1',2'a} \sim J_{1',2'b}$ 6.9 Hz, H-1′), 4,46 (*quint*., 1 H, $J_{2a',3'}$ 3.3 Hz, $J_{2'b,3'}$ 6.6 Hz, $J_{3',4'}$ 3.0 Hz, H-3′), 4.31 (*m*, 1 H, $J_{4',5'}$ 6.0 Hz, $J_{5',6'a}$ 8.1 Hz, $J_{5',6'b}$ 3.9 Hz, H-5′), 4.14 (*dd*, 1 H, $J_{6'a,6'b}$ 13.2 Hz, Hb-6′), 4.08 (*dd*, 1 H, Ha-6′), 3.54 (*dd*, 1 H, H-4′), 3.35 (*bs*, 1 H, 5′-OH), 2.27 (*quint*., 1 H, $J_{2'a,2'b}$ 13.5 Hz, Hb-2′), 1.77 (*ddd*, 1 H, Ha-2′), 1.64 (*d*, 3 H, Me-5), 0.89 (*s*, 9 H, CMe_3), 0.03 and 0.01 (2 *s*, 6 H, $SiMe_2$). EIMS: *m/z*(%) 171 (100), 75 (81), 117 (49), 201 (10), 295 (3), and 340 (1, 358 (1, $M^{\cdot+}$ - *t*-Bu).

Anal. Calcd for $C_{17}H_{29}N_3O_7Si$ (415.52): C, 49.14; H, 7.03; N, 10.11. Found: C, 49.16; H, 7.16; N, 9.88.

Method B: To a solution of nitromethane (2.75 mL, 45.0 mmol) in *ter*-butanol (45 mL), potassium *tert*-butoxide in *tert*-butanol (19.5 mL of a 1.0 M solution, 19.5 mmol) was added in 10 min and the mixture, containing the precipated potassium salt, was stirred for 5 min, then **4** (5.21 g, 15.0 mmol) was added in one portion. The reaction mixture was stirred at ambient temperature for 60 min, quenched with a saturated aqueous solution of NH_4Cl (50 mL) then diluted with EtOAc (200 mL) and water (15 mL).

The layers were separated, the organic phase was washed successively with water (50 mL), a saturated aqueous solution of NaCl (50 mL), dried (Na_2SO_4) and concentrated under reduced pressure. Purification by column chromatography (3:2 hexane/EtOAc) afforded **5ab** (4,17 g, 67%) containing the faster and slower moving products in a 2:1 ratio (^{1}H NMR spectra).

1-[*(E)*-3-*O*-(*tert*-Butyldimethylsilyl)-2,5,6-trideoxy-6-nitro-β-D-*erythro*-hex-5-enofuranosyl]thymine (6). **5a,b** (4.67 g, 11.2 mmol) was dissolved at 0°C in acetic anhydride (28.0 mL) and sodium acetate (5.62 g, 68.5 mmol) was added. The mixture was stirred at 0°C for 30 min and at room temperature for 7.5 h and the solvent was evaporated (oil pump, bath 35–40°C). The residue was suspended in EtOAc (240 mL), washed with a mixture of saturated aqueous $NaHCO_3$ and NaCl solutions (50/50 mL), with saturated NaCl (50 mL), dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (2:1 hexane/EtOAc) to give compound **6** (3.38 g, 76%). An analytical sample was obtained by recrystallization from hexane/EtOAc, mp 135.1–136.9°C; R_F 0.23 (1:1 hexane/EtOAc); $[\alpha]_D^{24}$+55.1° (*c* 1.0, $CHCl_3$); ν_{max}^{KBr} 3186 (NH), 2957, 2927, and 2858 (CH), 1705 and 1691 (C=O), 1531 (NO_2(as)), 1471, 1352 (NO_2(s)), 1119, 1056, and 837 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz): δ9.40 (*s*, 1 H, NH), 7.31 (*dd*, 1 H, $J_{5',6'}$ 13.5 Hz, $J_{4',5'}$ 4 Hz, H-5′), 7.17 (*dd*, 1 H, $J_{4',6'}$ 1 Hz, H-6′), 7.02 (*q*, 1H, $J_{5\text{-Me},6}$ 1.2 Hz, H-6), 6.20 (*t*, 1 H, $J_{1',2'a} \sim J_{1',2'b} \sim 6.7$ Hz, H-1′), 4.50–4.33 (*m*, 2H, $J_{3',4'}$; 5.5 Hz, $J_{2'a,3'}$ 6.4 Hz, $J_{2'b,3'}$ 5.0 Hz, H-3′ and H-4′), 2.50–2.23 (*m*, 2 H, $J_{2'a,2'b}$ 13.6 Hz, Hb-2′ and Ha-2′), 1.97 (*d*, 3 H, 5-Me), 0.91 (*s*, 9 H, CMe_3), 0.12 (*s*, 6 H, $SiMe_2$). EIMS: *m/z* (%) 73 (100), 201 (23), 183 (14), 295 (10), 340 (8, $M^{\cdot+}$ - *t*-Bu).

Anal. Calcd for $C_{17}H_{27}N_3O_6Si$ (397.51): C, 51.37; H, 6.85; N, 10.57. Found: C, 51.13; H, 6.84; N, 10.50.

1-[3-*O*-*tert*-Butyldimethylsilyl)-2,5-dideoxy-5-*C*-methylene-β-D-*erythro*-hexofuranosyluronononitrile]thymine (7). A solution of **6** (600 mg, 1.5 mmol) in CH_2Cl_2 (30 mL) was added to a NaOAc/AcOH buffer solution prepared from NaOAc (2.46 g, 30 mmol), water (7.5 mL), AcOH (1.72 mL, 30 mmol) and *n*-Bu_4NHSO_4 (100 mg, 0.3 mmol). To the vigorously stirred biphasic mixture, KCN (1.37 g, 21.0 mmol) was added followed by the addition of further small quantities of KCN (after 2.5 h, 0.20 g, 3.0 mmol; after 5.5 h, 0.19 g, 3.0 mmol; after 25.5 h, 0.10, 1.5 mmol) where the pH changed from 6.0 to 7.0. The reaction mixture was stirred during an additional 3 h, then diluted with water (10 mL). The organic layer was separated, washed with water (10 mL), dried (Na_2SO_4) and concentrated. The crude product was purified by column chromatography (8:1 CH_2Cl_2/EtOAc) to give **7** (480 mg, 85%), mp 44.9–47.1°C; R_F 0.16 (8:1 CH_2Cl_2/EtOAc); $[\alpha]_D^{25}$+31.3° (*c* 1.0, $CHCl_3$);ν_{max}^{KBr} 3186 (NH), 3056, 3028, and 2856 (CH), 2227 (CN), 1697 (C=O), 1472, 1254, 1065, and 837 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz):δ9.51

(*s*, 1 H, NH), 7.41 (*q*, 1 H, $J_{5\text{-Me},6}$, 1 Hz, H-6), 6.37 (*t*, 1 H, $J_{1',2'a} \sim J_{1',2'b} \sim 6.5$ Hz, H-1′), 6.12 and 6.08 (2 *s*, 2 H, $H_2C=C_{5'}$), 4.40 (*dt*, 1 H, $J_{3',4'}$ 4.5 Hz, $J_{2'b,3'}$ 4.5 Hz, $J_{2'a,3'}$ 6.5 Hz, H-3′), 4.23 (*d*, 1 H, H-4′), 2.37 (*ddd*, 1 H, $J_{2'a,2'b}$ 14 Hz, Hb-2′), 2.25 (*dt*, 1 H, Ha-2′), 1.95 (*d*, 3 H, 5-Me), 0.89 (*s*, 9 H, CMe_3), 0.09 and 0.08 (2 *s*, 6 H, $SiMe_2$). EIMS: *m/z* (%) 320 (100, $M^{\cdot+}$ - *t*-Bu), 168 (78), 73 (76), 252 (22), and 377 (0.2, $M^{\cdot+}$).

Anal. calcd for $C_{18}H_{27}N_3O_4Si$ (377.52): C, 57.27; H, 7.21; N, 11.13. Found: C, 56.92; H, 7.29; N, 10.87.

1-[2,5-Dideoxy-5-*C*-methylene-β-D-*erythro*-hexofuranosyluronononitrile]-thymine (8). A solution of **7** (570 mg, 1.50 mmol) in a mixture of trifluoroacetic acid and water (10:1, 12.5 mL) was stirred at room temperature for 5 h, evaporated and coevaporated with toluene (3 × 15 mL). The crude product was purified by column chromatography (7:3 CH_2Cl_2/acetone) to give compound **8** (370 mg, 94%), mp 145.1–148.1°C; R_F 0.18 (2:1 CH_2Cl_2/acetone); $\alpha_D^{28} + 26.8°$ (*c* 1.0, MeOH); ν_{max}^{KBr} 3372 and 3223 (OH and NH), 2224 (CN), 1721 and 1656 (C=O), 1477, 1272, and 1055 cm^{-1}. ^1H NMR (DMSO-d_6, 200 MHz, 40°C): δ11.00 (*s*, 1 H, NH), 7.42 (*q*, 1 H, $J_{5\text{-Me},6}$ 1 Hz, H-6), 6.27 (*d*, 1 H, $J_{4',Ha}$ 0.3 Hz, **Ha**HbC=$C_{5'}$), 6.23 (*d*, 1 H, $J_{4',Hb}$ 0.7 Hz, Ha**Hb**C=$C_{5'}$), 6.23 (*t*, 1 H, $J_{1',2'a} \sim J_{1',2'b} \sim 6.6$ Hz, H-1′), 5.58 (*d*, 1 H, $J_{3',4'}$ 5 Hz, H-4′), 4.35 (*m*, 1 H, $J_{2'a,3'}$ 5.0 Hz, $J_{2'b,3'}$ 6.9 Hz, $J_{3',3'\text{-OH}} \sim 5$ Hz, H-3′), 4.27 (*d*, 1 H, 3′-OH), 2.35 (*quint*., 1 H, $J_{2'a,2'b}$ 13.5 Hz, Hb-2′), 2.19 (*ddd*, 1 H, Ha-2′), and 1.78 (*d*, 3 H, 5-Me). EIMS: *m/z* (%) 126 (100, 55 (81), 94 (71), 138 (46), 263 (9, $M^{\cdot+}$).

Anal. Calcd for $C_{12}H_{13}N_3O_4$ (263.26): C, 54.75; H, 4.98; N, 15.96. Found: C, 54.59; H, 4.93; N, 15.89.

1-[3-*O*-(*tert*-Butyldimethylsilyl)-2,5-dideoxy-5-*C*-phenylselenomethyl-β-D-*ribo*- and α-L-*lyxo*-hexofuranosyluronononitrile]thymine (9a,b). A mixture of **7** (566 mg, 1.5 mmol), benzeneselenol (0.95 mL, 9.0 mmol), and triethylamine (2.09 μL, 0.15 mmol) was stirred at room temperature under argon atmosphere for 1 h, and diluted with EtOAc (5 mL) and hexane (6 mL). Air was bubbled through the solution during 1 h and the mixture was submitted to column chromatography (13:7 hexane/EtOAc to give **9a,b** (630 mg, 79%) as a colorless solid, mp 44.6–46.5°C; R_F 0.18 (3:2 hexane/EtOAc); ν_{max}^{KBr} 3186 (NH), 3054 (CH arom.), 2955, 2927, and 2855 (CH alph.), 2244 (CN), 1695 (C=O), 1472, 1363, 1258, 1226, 1084, and 836 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz), the ratio of the two isomers was about 2:1, major component: δ9.23 (*s*, 1 H, NH), 7.68–7.20 (*m*, 6 H, Ph and H-6), 6.37 (*t*, 1 H, $J_{1',2'a} \sim J_{1',2'b} \sim 6.7$ Hz, H-1′), 4.32 (*dt*, 1 H, $J_{2'a,3'}$ 5 Hz, $J_{2'b,3'}$ 6.5 Hz, $J_{3',4'}$ 5 Hz, H-3′), 3.98 (*dd*, 1 H, $J_{4',5'}$ 2 Hz, H-4′), 3.27 (*dd*, 1 H, $J_{5''a,5''b}$ 13 Hz, $J_{5',5''b}$ 7.7 Hz, Hb-5″), 3.12 (*dd*, 1 H, $J_{5',5''a}$ 8 Hz, Ha-5″), 2.88 (*td*, 1 H, H-5′), 2.45-2.13 (*m*, 2 H, Ha-2′, Hb-2′), 1.95 (*d*, 3 H, $J_{Me\text{-}5,6}$ 1 Hz, Me-5), 0.84 (*s*, 9 H, CMe_3), 0.04 and −0.02 (2 *s*, 6 H, $SiMe_2$). Minor isomer: δ9.26 (*s*, 1 H, NH),

7.68–7.20 (*m*, 6 H, Ph and H-6), 6.25 (*t*, 1 H, $J_{1',2'a} \sim J_{1',2'b} \sim 7.0$ Hz, H-1′), 4.50 (*quint*., 1 H, $J_{2'a,3'}$ 3.5 Hz, $J_{2'b,3'}$ 6.5 Hz, $J_{3',4'}$ 3.8 Hz, H-3′), 4.05 (*dd*, 1 H, $J_{4',5'}$ 5 Hz, H-4′), 3.30–3.00 (*m*, 3 H, H_2C-5″ and H-5′), 2.45–2.13 (*m*, 2 H, Ha-2′, Hb-2′), 1.92 (*d*, 3 H, $J_{Me\text{-}5,6}$ 1 Hz, Me-5), 0.90 (*s*, 9 H, CMe_3), 0.13 and 0.11 (2 *s*, 6 H, $SiMe_2$). EIMS: *m/z* (%) 73 (100), 478 (50, $M^{\cdot+}+1$ - *t*-Bu), 168 (37), 320 (23, $M^{\cdot+}$ - *t*-Bu - PhSeH), 201 (22), 352 (5), and 410 (3).

Anal. Calcd for $C_{24}H_{33}N_3O_4SeSi$ (534.59): C, 53.92; H, 6.22; N, 7.86. Found: C, 54.02; H, 6.30; N, 7.73.

1-(2,5-dideoxy-5-*C*-phenylselenomethyl-β-D-*ribo* and α-L-*lyxo*-hexo-furanosylurononitrile]thymine (10a,b). Compound **10a,b** was prepared from **9a,b** (630 mg, 1.18 mmol) as described for **8**. The crude product was purified by column chromatography (3:1 CH_2Cl_2/acetone) to give **10a,b** (460 mg, 93%) as a pale yellow solid. Mp 66.3–67.4°C; R_F 0.16 (3:1 CH_2Cl_2/acetone); ν_{max}^{KBr} 3430 and 3200 (OH and NH), 3052 (CH arom.), 2924 (CH aliph.), 2243 (CN), 1690 (C = O), 1476, 1273, 1076, and 739 cm^{-1}. ^1H NMR (DMSO-d_6, 300 MHz), the ratio of the two isomers was about 2:1; major component: δ11.16 (*s*, 1 H, NH), 7.42 (*q*, 1 H, $J_{Me\text{-}5,6}$ 1 Hz, H-6), 7.60–7.20 (*m*, 5 H, PhSe), 6.18 (*t*, 1 H, $J_{1',2'a} \sim J_{1',2'b}$ 6.8 Hz, H-1′), 5.54 (*d*, 1 H, $J_{3',3'}$-OH 4.5 Hz, 3′-OH), 4.26 (*m*, 1 H, $J_{2'a,3'}$ 5.1 Hz, $J_{2'b,3'}$ 6.5 Hz, $J_{3',4'}$ 4.5 Hz, H-3′), 3.84 (*t*, 1 H, $J_{4',5'}$ 4.5 Hz, H-4′), 3.42 (*m*, 1 H, $J_{5',5''a}$ 10.0 Hz, $J_{5',5''b}$ 5.2 Hz, H-5′), 3.31 (*dd*, 1 H, $J_{5''a,5''b}$ 14 Hz, Hb-5″), 3.22 (*dd*, 1 H, Ha-5″), 2.26 (*m*, 1 H, $J_{2'a,2'b}$ 14 Hz, Hb-2′), 2.13 (*ddd*, 1 H, Ha-2′), and 1.75 (*d*, 3 H, Me-5); minor isomer: δ11.16 (*s*, 1 H, NH), 7.42 (*q*, 1 H, $J_{Me\text{-}5,6}$ 1 Hz, H-6), 7.60–7.20 (*m*, 5 H, PhSe), 6.19 (*dd*, 1 H, $J_{1',2'a}$ 6.3 Hz, $J_{1',2'b}$ 7.8 Hz, H-1′), 5.59 (*d*, 1 H, $J_{3',3'\text{-}OH}$ 4.8 Hz, 3′-OH), 4.37 (*m*, 1 H, $J_{2'a,3'}$ 3.2 Hz, $J_{2'b,3'}$ 6.6 Hz, $J_{3',4'}$ 3.3 Hz, H-3′), 3.89 (*dd*, 1 H, $J_{4',5'}$ 6.9 Hz, H-4′), 3.50 (*m*, 1 H, $J_{5',5''a}$ 10.2 Hz, $J_{5'.5''b}$ 4.5 Hz, H-5′), 3.29 (*dd*, 1 H, $J_{5''a,5''b}$ 14 Hz, Hb-5″), 3.18 (*dd*, 1 H, Ha-5″), 2.33 (*dd*, 1 H, $J_{2'a,2'b}$ 14 Hz, Hb-2′), 2.08 (*ddd*, 1 H, Ha-2′), 1.75 (*d*, 3 H, Me-5). EIMS *m/z* (%) 78 (100), 126 (73), 158 (57), 55 (53), 314 (3), 251 (2), and 421 (1, $M^{\cdot+}+1$).

Anal. Calcd for $C_{18}H_{19}N_3O_4Se$ (420.33): C, 51.44; H, 4.56; N, 10.00. Found: C, 51.74; H, 4.90; N, 9.74.

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