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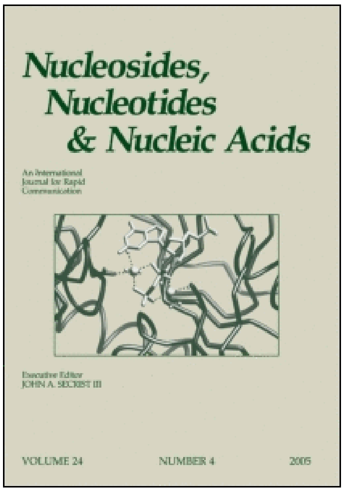

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New Acyclonucleosides: Synthesis and Anti-HIV Activity

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NEW ACYCLONUCLEOSIDES: SYNTHESIS AND ANTI-HIV ACTIVITY

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▫ *The synthesis of new acyclic nucleosides is described. These syntheses were accomplished by various methods: glycosylation, selective or total deprotection, oxidation/reduction, chlorination or azidation of hydroxyl groups. The compounds were characterized with NMR, mass and IR spectroscopy. Antiviral properties of these compounds were evaluated on HIV-1 infected cell lines.*

Keywords Acyclonucleoside, Selective Deprotection, Azidation, Chlorination

INTRODUCTION

For several years, there has been an intensive search for drugs effective in the chemotherapy of viral infections by, e.g., HIV, herpes simplex or cytomegalovirus.[1−4] Most of these drugs are analogues of naturally accuring nucleosides.[5]

The introduction of acyclovir[6] as a clinically useful antiviral drug has spurred interest in acyclic nucleosides as potential chemotherapeutic agents. Consequently, many different families of nucleoside analogues and acyclonucleosides[7,8] have been synthesized and tested in order to enhance biological activity and selectivity, or to lower their toxicity.

Continuing with our work in this field,[9,10] we report here the synthesis of azido or chloro acyclonucleosides with N-3 modified thymine, to determine the influence of the azido, chloro or carboxymethyl groups on anti-HIV activity.

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RESULTS AND DISCUSSION

Chemistry

The starting material 1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)thymine (**1**) was prepared using the classical method of Vorbrüggen.[11,12] Thymine was silylated in dry 1,2-dichloroethane with TMSC1 (0.8 equiv.) and HMDS (0.8 equiv.). The reaction was continued by addition of a solution of 1-*O*-acetyl-2,3,5-tribenzoylribofuranose (1 equiv.) and a Lewis acid in 1,2-dichloroethane. Two equivalents of $SnCl_4$ are needed for the reaction because of the inhibition due to excess of silylation reagents in solution. Compound **1** was obtained in 91% yield. Alkylation of thymine with *tert*-butyl bromoacetate in the presence of potassium carbonate in *N*,*N*-dimethylformamide gave compound **2** in quantitative yield (Scheme 1). The *tert*-butyl ester was selected because it is stable in presence of ammonia, which was later used for deprotection of hydroxyl groups.

The next step was the selective or total deprotection of sugar moiety (Scheme 2). The removal of benzoyl groups from **1** to give the unprotected compound **3** proceeded readily with sodium methoxide in 98% yield. Using the same method for total deprotection of **2** gave a mixture of two products. Structural elucidation of these compounds indicated that one of them is the expected product **4** (50%) and the second one **4′** (24%) results from total deprotection and transesterification. Compound **2** was then debenzoylated using an excess of ammonia in methanol (88.9%).

For selective deprotection of compounds **1** and **2**, we have found that secondary hydroxyl groups can be efficiently and selectively generated from their benzoylated forms by controlling the amount of NH_3, reaction time, and temperature. The best results were obtained at $-15°C$ using 25 equivalents of ammonia per benzoyl protected secondary hydroxyl group. The reaction was checked by TLC and stopped by evaporation, as soon as the trihydroxyl derivatives showed up. This method requires no particular precaution and implies an easy work up (concentration of the reaction mixture). Compounds **5** and **6** were obtained after purification in 68% and 66.6% yields, respectively.

The 2′,3′-seconucleosides **7–10** retain the carbon framework of ribose nucleosides and chirality of the anomeric carbon, yet allow for greater flexibility than the furanose moiety. The synthetic route chosen for these analogues was periodate oxidation and reduction of obtained dialdehyde with borohydride

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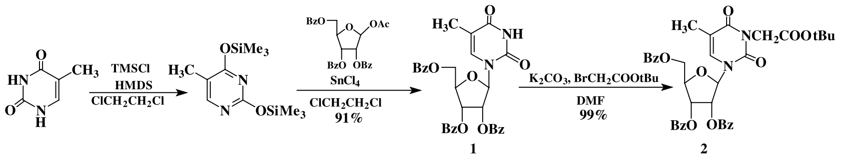
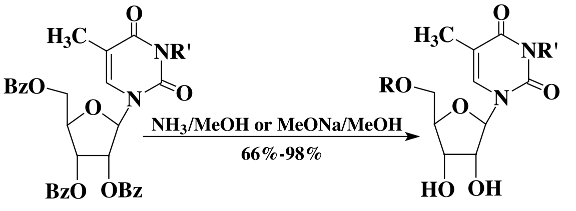


SCHEME 1 Glycosylation/N-alkylation.



1 R' = H
2 R' = $CH_2COOtBu$

3 R = R' = H
4 R = H, R' = CH_2CO_2tBu
4' R = H, R' = CH_2CO_2Me
5 R = Bz, R' = H
6 R = Bz, R' = CH_2CO_2tBu

SCHEME 2 Selective or total deprotection of hydroxyl groups.

(Scheme 3). This transformation was carried out in one step, using periodate and borohydride bound resins.[13–18] The final products **7–10** were easily recovered after filtration of the reaction mixture followed by evaporation of the filtrate and purification.

The introduction of an azide function was achieved in two steps. The first one is mesylation of acyclonucleosides in the presence of mesyl chloride in anhydrous pyridine at 0°C. The crude mixture was used in second step without purification. Azidation was carried out in DMF at 100°C in the presence of NaN_3. After purification, compounds **11–14** were obtained in 47.7–61.6% overall yield, comparable to literature values (Scheme 4).

Chlorination of compound **7** with an excess of thionyl chloride in dimethylformamide, for 3 days at room temperature gave compound **15** in lower yield (30%). The use of pyridine as a solvent leads to product **15** in acceptable yield (60.4%). This reaction was applied to compounds **8–10** to give corresponding chlorinated compounds **16–18** (Scheme 5).

The deprotection of 5′-benzoyl group was realized by methanolic ammonia solution (7 M), after 4 days, compounds **13** and **14** gives **19** and **20** in 70% and 80% yield, respectively (Scheme 6).

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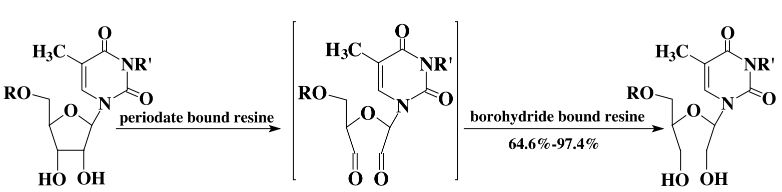
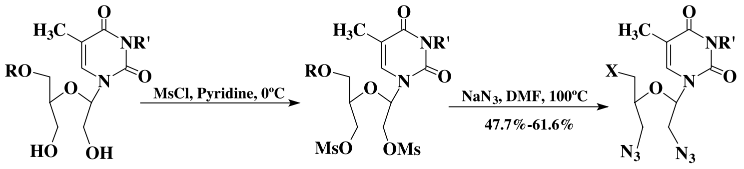


3 R = H, R' = H
4 R = H, R' = CH_2CO_2tBu
5 R = Bz, R' = H
6 R = Bz, R' = CH_2CO_2tBu

7 R = H, R' = H
8 R = H, R' = CH_2CO_2tBu
9 R = Bz, R' = H
10 R = Bz, R' = CH_2CO_2tBu

SCHEME 3 Reaction of oxidation/reduction.



7 R = H, R'= H
8 R = H, R'= CH_2CO_2tBu
9 R = Bz, R'= H
10 R = Bz, R'= CH_2CO_2tBu

R = Ms, R'= H
R = Ms, R'= CH_2CO_2tBu
R = Bz, R'= H
R = Bz, R'= CH_2CO_2tBu

11 X = N_3, R'= H
12 X = N_3, R'= CH_2CO_2tBu
13 X = OBz, R'= H
14 X = OBz, R'= CH_2CO_2tBu

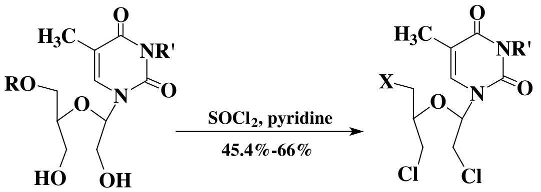
SCHEME 4 Preparation of azido compounds.

Deprotection of compound **17** with methanolic ammonia gives a product resulting from debenzoylation of primary hydroxyl group and intramolecular cyclization. Mass spectroscopy of compound **21** presents two peaks representing MH^+ (261) and MNH_4^+ (278). ^{1}H NMR confirms the structure and shows the presence of two compounds. The diastereoselectivity of the cyclization was 63/37, but we have not determined the relative configurations (Scheme 7). The same cyclization was observed (by mass spectroscopy) when the reaction of deprotection of compound **18** was realized.

Finally, compounds **8**, **12**, **16**, and **20** were treated with TFA giving the desired derivatives **23** in quantitative yield, **24**, **25**, and **26** in 87.6%, 83% and 52% yield respectively (Scheme 8).

Biological Evaluation

The synthesized compounds **7**, **11**, **15**, **19**, **22**, **23**, **24**, and **25** were tested for their in vitro inhibitory effects on the replication of RNA virus (HIV-1). The anti HIV 1 activity was tested on CEM-SS and MT_4 infected respectively with HIV-1 LAI and HIV IIIB according to protocols described previously.[19] In the case of CEM-SS cells, the production of virus was measured by quantification of reverse transcriptase activity associated with the releasing of virus particles in the culture supernatant; for MT4 cells, the assay was based on the virus induced



7 R = H, R'= H
8 R = H, R'= CH_2CO_2tBu
9 R = Bz, R'= H
10 R = Bz, R'= CH_2CO_2tBu

15 X = Cl, R'= H
16 X = Cl, R'= CH_2CO_2tBu
17 X = OBz, R'= H
18 X = OBz, R'= CH_2CO_2tBu

SCHEME 5 Chlorination of acyclonucleosides.

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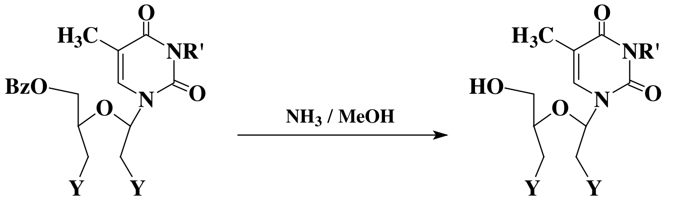

13 $Y = N_3$, R'= H
14 $Y = N_3$, R'= CH_2CO_2tBu

19 $Y = N_3$, R'= H
20 $Y = N_3$, R'= CH_2CO_2tBu

SCHEME 6 Deprotection of primary hydroxyl group.

cytopathogenicity. Cell viability was evaluated by measuring the activity of mitochondrial electron transport by the MTT assay.[20] The antiviral activity is expressed as EC_{50}, the concentration of the compound necessary to reduce virus replication by 50% and was derived from the computer-generated median effect plot of the dose-effect data.[21] The cytotoxicity is expressed as CC_{50}, the concentration of drug needed to reduce the viability of uninfected cells by 50%. The results are summarized in Table 1. For infected CEM-SS cells, the selectivity index (SI = ratio CC_{50}/EC_{50}) is low and close to 2, indicating that the compound have no specific antiviral activity. Similar results can be derived from the MT4 assay as no protection against the virus induced cytopathic effect was observed in presence of the drug.

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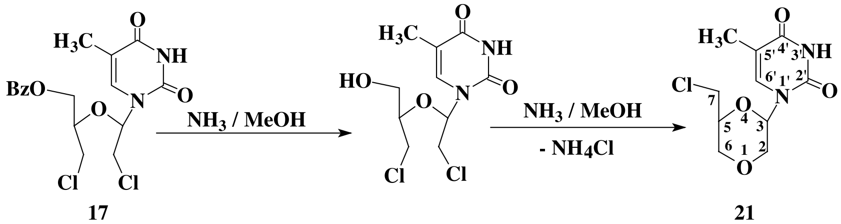
EXPERIMENTAL SECTION

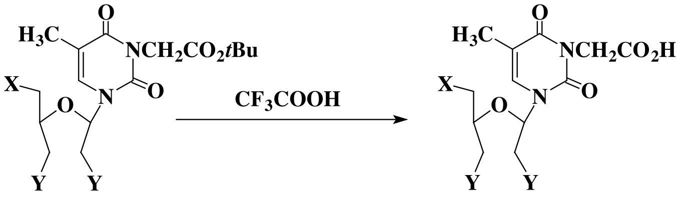
All the solvents and chemicals were commercially available and, unless otherwise stated, were used as received. DMF and $ClCH_2CH_2Cl$ were distilled twice over P_2O_5 and over CaH_2 just before use. Reactions were monitored by thin-layer chromatography (TLC) on precoated 0.2 mm silica gel 60 F_{254} (Merck) plates and visualized in several ways: with an ultraviolet light source at 254 nm, by spraying with sulfuric acid (6N) and heating to 200°C. Silica gel (Merck Kieselgel 60, 15–40 μm) was used for flash chromatography. ^1H NMR spectra were recorded at 400.13 MHz with a Brüker DPX spectrometer. Chemical shifts (δ) are expressed in ppm with Me_4Si as internal standard (δ = 0). Data are reported as follows: chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; q, quintet; m,



17

21

SCHEME 7 Intramolecular cyclization.



8 X = Y = OH
12 X = Y = N_3
16 X = Y = Cl
20 X = OH, Y = N_3

22 X = Y = OH
23 X = Y = N_3
24 X = Y = Cl
25 X = OH, Y = N_3

SCHEME 8 Deprotection of carboxyl group.

multiplet and br, broad), coupling constants (Hz) and assignment. Melting points (mp) were determined with a Kofler block and are uncorrected. Rotatory dispersions were measured with a Jasco (DIP-370) polarimeter in a 1 dm quartz cell at 22°C. IR spectra were recorded on a Perkin Elmer 1310 grating spectrophotometer and are reported in wave number (cm^{-1}). Chemical-impact mass spectra (CI) was recorded with a Kratos MS 580 mass spectrometer at the laboratoire de Chimie Organique Structurale of the Université Pierre et Marie Curie (Paris VI).

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)thymine (1). To thymine (4 mmol, 0.504 g) in dry dichloroethane (20 mL) was added (0.676 mL, 0.8 eq) HMDS and (0.3 mL, 0.8 eq) TMSC1. The reaction mixture was heated at 80°C for 3 h. After cooling to room temperature a solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (2.016 g, 1 eq) in dichloroethane (20 mL) was added followed by $SnCl_4$ (0.932 mL, 2 eq). The solution was stirred at room temperature until TLC indicated the completion of the reaction (2 h). The reaction mixture was quenched by $NaHCO_3$ saturated water solution and extracted with chloroform.

TABLE 1 Antiviral Effects of Derivatives of Acyclonucleosides

	CEM-SS		MT-4	
Compound	EC_{50}[a]	CC_{50}[b]	EC_{50}[a]	CC_{50}[b]
7	$>1.10^{-1}$	$>1.10^{-1}$	$>1.10^{-1}$	$>1.10^{-1}$
11	$>1.10^{-1}$	$>1.10^{-1}$	$>1.10^{-1}$	$>1.10^{-1}$
15	$>CC_{50}$	7.610^{-2}	$>CC_{50}$	1.10^{-1}
19	$>1.10^{-1}$	$>1.10^{-1}$	$>CC_{50}$	7.810^{-2}
22	$>1.10^{-1}$	$>1.10^{-1}$	$>1.10^{-1}$	$>1.10^{-1}$
23	$>1.10^{-1}$	$>1.10^{-1}$	$>1.10^{-1}$	$>1.10^{-1}$
24	$>1.10^{-1}$	$>1.10^{-1}$	$>1.10^{-1}$	$>1.10^{-1}$
25	$>1.10^{-1}$	$>1.10^{-1}$	$>1.10^{-1}$	$>1.10^{-1}$

[a] 50% Effective concentration (mg/mL) or concentration required to inhibition the replication of HIV-1 by 50%.

[b] 50% Cytotoxic concentration (mg/mL) or concentration required to reduce the viability of uninfected cells by 50%.

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The chloroform solution was dried over $MgSO_4$ and the solvent was removed by evaporation under reduced pressure. The crude product was purified by chromatography on silica gel (elution with a gradient of dichloromethane/ethanol). Compound **1** was isolated as a white solid (2.07 g, 91%). R_f = 0.53 (CH_2Cl_2/EtOH, 97/3, v/v); mp = 94°C; $[\alpha]_D = -21.1°$ (c = 1, $CHCl_3$); IR: 3186 (N–H), 3065 (C–H), 1724 (C = O benzoyl), 1694 (C = O thym), 1603 (C = C), 1266 (C–O–C); ^1H NMR ($CDCl_3$) δ 8.8 (s, 1H, N–H), 8.2–7.35 (m, 15H, Ar), 7.16 (q, 1H, $J_{H6,CH3}$ = 1.0 Hz, H-6), 6.43 (d, 1H, $J_{1',2'}$ = 6.2 Hz, H-1′), 5.91 (dd, 1H, $J_{3',2'}$ = 6.2 Hz, $J_{3',4'}$ = 3.7 Hz, H-3′), 5.76 (t, 1H, H-2′), 4.88 (dd, 1H, $J_{5'a,5'b}$ = 12.1 Hz, $J_{5'a,4'}$ = 2.5 Hz, H-5′a), 4.70 (m, 1H, H-4′), 4.65 (dd, 1H, $J_{5'b,4'}$ = 3.4 Hz, H-5′b), 1.60 (d, 3H, $J_{CH3,H6}$ = 1.0 Hz, CH_3).

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-3-*tert*-butoxycarbonylmethylthymine (2). To a solution of 1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)thymine (300 mg, 0.526 mmol) in DMF (10 mL) was added K_2CO_3 (725 mg, 10 eq), after ten minutes *tert*-butyl bromoacetate (0.155 mL, 2 eq) was added and the reaction mixture was stirred at room temperature for two hours. The solution was filtered and the K_2CO_3 washed with dichloroethane. The crude product was subjected to preparative TLC using CH_2Cl_2/EtOH (99.5/0.5, v/v) yielding **2** (356 mg, 99%) as a sirup. R_f = 0.56 (CH_2Cl_2/EtOH, 97/3, v/v); $[\alpha]_D = -63.6°$ (c = 0.91, $CHCl_3$); IR: 2973 (C–H), 1728 (C = O benzoyl), 1694 (C = O thym), 1667 (C = O CO_2tBu), 1599 (C = C), 1266 (C–O–C); ^1H NMR ($CDCl_3$) δ 8.2–7.34 (m, 15H, Ar), 7.20 (brs, 1H, H-6), 6.45 (d, 1H, $J_{1',2'}$ = 6.0 Hz, H-1′), 5.93 (dd, 1H, $J_{3',2'}$ = 6.0 Hz, $J_{3',4'}$ = 4.2 Hz, H-3′), 5.75 (t, 1H, H-2′), 4.88 (dd, 1H, $J_{5'a,5'b}$ = 12.2 Hz, $J_{5'a,4'}$ = 2.4 Hz, H-5′a), 4.70 (m, 1H, H-4′), 4.64 (dd, 1H, $J_{5'b,4'}$ = 3.3 Hz, H-5′b), 4.58 (d, 1H, J = 16.5, CH_2), 4.52 (d, 1H, J = 16.5, CH_2), 1.64 (brs, 3H, CH_3), 1.42 (s, 9H, tBu).

1-(β-D-Ribofuranosyl)thymine (3). 1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)thymine (**1**) (500 mg, 0.877 mmol) was dissolved in a 7 M solution of ammonia in methanol (19 mL, 150 eq). After 4 days the solution was evaporated to dryness. The crude product was subjected to preparative TLC (CH_2Cl_2/EtOH, 70/30, v/v) yielding 98% (222 mg). R_f = 0.54 (CH_2Cl_2/EtOH, 90/10, v/v); $[\alpha]_D = -12.9°$ (c = 0.43, CH_3OH); IR: 3365 (O–H), 2933 (C–H), 1694 (C = O thym); ^1H NMR ($CDCl_3$) δ 7.83 (q, 1H, $J_{H6,CH3}$ = 1.0 Hz, H-6), 5.89 (d, 1H, $J_{1',2'}$ = 4.4 Hz, H-1′), 4.19 (dd, 1H, $J_{2'3'}$ = 5.3 Hz, H-2′), 4.15 (dd, 1H, $J_{3',4'}$ = 4.0 Hz, H-3′), 3.88 (ddd, 1H, $J_{5'a,4'}$ = 2.7 Hz, $J_{5'b,4'}$ = 3.0 Hz, H-4′), 3.84 (dd, 1H, $J_{5'a,5'b}$ = 12.2 Hz, H-5′a), 3.73 (dd, 1H, H-5′b), 1.87 (d, 3H, $J_{CH3,H6}$ = 1.0 Hz, CH_3).

3-*tert*-Butoxycarbonylmethyl-1-(β-D-ribofuranosyl)thymine (4). Compound **4** was prepared according to the procedure described for **3** starting from **2** (110 mg, 0.16 mmol). Yield 88.9% (53 mg); R_f = 0.54 (CH_2Cl_2/EtOH, 90/10, v/v); IR: 3379 (O–H), 2936 (C–H), 1703 (C = O thym), 1661 (C = O

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CO_2tBu), 1636 (C = C), 1240 (C–O–C); ^1H NMR (CD_3OD) δ 7.95 (d, 1H, $J_{H6,CH3}$ = 1.0 Hz, H-6), 5.90 (d, 1H, $J_{1',2'}$ = 4.4 Hz, H-1′), 4.55 (s, 2H, CH_2), 4.35 (t, 1H, $J_{3',2'}$ = $J_{3',4'}$ = 4.6 Hz, H-3′), 4.26 (t, 1H, H-2′), 4.00 (ddd, 1H, $J_{4',5'a}$ = 2.6 Hz, $J_{4',5'b}$ = 2.9 Hz, H-4′), 3.87 (dd, 1H, $J_{5'a,5'b}$ = 12.2 Hz, H-5′a), 3.75 (dd, 1H, H-5′b), 1.91 (d, 3H, $J_{CH3,H6}$ = 1.0 Hz, CH_3), 1.46 (s, 9H, tBu).

1-(5-*O*-Benzoyl-β-D-ribofuranosyl)thymine (5). A solution of 1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)thymine (499 mg, 0.875 mmol) in methanol (15 mL) and 6.25 mL of solution of ammonia in methanol (7 M) was stirred at −15°C. After 7 h the solution was evaporated to dryness in a cold bath. The crude product was purified using preparative TLC (CH_2Cl_2/EtOH, 97/3, v/v) pure **5** was recovered in 68% yield (215 mg). R_f = 0.21 (CH_2Cl_2/EtOH, 97/3, v/v); IR: 3360 (O–H), 2953 (C–H), 1710 (C = O benzoyl), 1670 (C = O thym), 1265 (C–O–C); ^1H NMR (CD_3OD) δ 8.10–7.40 (m, 5H, Ar), 7.35 (d, 1H, $J_{H6,CH3}$ = 1.0 Hz, H-6), 5.88 (d, 1H, $J_{1', 2'}$ = 4.4 Hz, H-1′), 4.71 (dd, 1H, $J_{5'a,5'b}$ = 12.2 Hz, $J_{5'a,4'}$ = 2.9 Hz, H-5′a), 4.51 (dd, 1H, $J_{5'b,4'}$ = 4.0 Hz, H-5′b), 4.27 (m, 1H, H-4′), 4.27 (m, 1H, H-3′), 4.26 (t, 1H, $J_{2',3'}$ = 4,4 Hz, H-2′), 1.59 (d, 3H, $J_{CH3,H6}$ = 1.0, CH_3).

1-(5-*O*-Benzoyl-β-D-ribofuranosyl)-3-*tert*-butoxycarbonylmethylthymine (6). Compound **6** was prepared the same way as that described for **5** starting from **2** (200 mg, 0.29 mmol). Yield 66.6% (92 mg); R_f = 0.50 (CH_2Cl_2/EtOH, 97/3, v/v); IR: 3365 (O–H), 2947 (C–H), 1711 (C = O benzoyl), 1665 (C = O thym), 1660 (C = O CO_2tBu), 1271 (C–O–C); ^1H NMR (CD_3OD) δ 8.05 (dd, 2H, J = 7.4 Hz, J = 1.0 Hz, Ar), 7.63 (brt, 1H, J = 7.4 Hz, Ar), 7.50 (t, 2H, J = 7.4 Hz, Ar), 7.44 (d, 1H, $J_{H6,CH3}$ = 1.0 Hz, H-6), 5.83 (d, 1H, $J_{1',2'}$ = 4.0 Hz, H-1′), 4.73 (dd, 1H, $J_{5'a,5'b}$ = 12.4 Hz, $J_{5'a,4'}$ = 2.4 Hz, H-5′a), 4.54 (s, 2H, CH_2), 4.52 (dd, 1H, $J_{5'b,4'}$ = 4.0 Hz, H-5′b), 4.42 (m, 1H, H-4′), 4.32 (t, 1H, $J_{3',4'}$ = $J_{3',2'}$ = 5.0 Hz, H-3′), 4.27 (dd, 1H, H-2′), 1.68 (d, 3H, $J_{CH3,H6}$ = 1.0 Hz, CH_3), 1.45 (s, 9H, tBu).

1-[1-(1,3-Dihydroxyisopropoxy)-2-hydroxyethyl]thymine (7). To a solution of **3** (430 mg, 1.666 mmol) in methanol (38 mL) were added 1.88 g each of periodate and borohydride resins. The mixture was stirred at room temperature until reaction was complete (4 days). The intermediate dialdehyde had a higher R_f and the final diol had a lower R_f than the starting compound. The resin beds were filtered and washed with methanol. The methanol filtrate was evaporated under reduced pressure to obtain the desired acyclic product in 64.6% yield (280 mg). R_f = 0.58 (CH_2Cl_2/EtOH, 90/10, v/v); IR: 3395 (O–H), 2955 (C–H), 1673 (C = O thym), 1269 (C–O–C); ^1H NMR (CD_3OD) δ 7.6 (d, 1H, $J_{H6,CH3}$ = 1.1 Hz, H-6), 5.96 (t, 1H, $J_{1',2'}$ = 5.5 Hz, H-1′), 3.79 (dd, 1H, $J_{2'a,2'b}$ = 11.9 Hz, H-2′a), 3.70 (dd, 1H, H-2′b), 3.69 (m, 1H, H-3′a), 3.68 (dd, 1H, $J_{3'a,3'b}$ = 12.5 Hz, $J_{3'4'}$ = 6.3 Hz, H-3′b), 3.58 (dd, 1H, $J_{5'a,5'b}$ = 12.0 Hz, $J_{5'a,4'}$ = 5.4 Hz, H-5′a), 3.57 (m, 1H, H-4′), 3.56 (dd, 1H, $J_{5'b,4'}$ = 5.3 Hz, H-5′b), 1.91 (d, 3H, $J_{CH3,H6}$ = 1.1 Hz, CH_3).

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Compounds **8**, **9** and **10** were prepared according to the procedure described for **7** starting from **4** (102 mg, 0.274 mmol), **5** (250 mg, 0.69 mmol), and **6** (92 mg, 0.193 mmol).

1-[1-(1,3-Dihydroxyisopropoxy)-2-hydroxyethyl]-3-*tert*-butoxycarbonylmethylthymine (8). Yield: 91.7% (94 mg); R_f = 0.55 (CH_2Cl_2/EtOH, 90/10, v/v); IR: 3420 (O–H), 2927 (C–H), 1700 (C = O thym), 1666 (C = O CO_2tBu), 1251 (C–O–C); ^{1}H NMR (CD_3OD) δ: 7.65 (q, 1H, $J_{H6,CH3}$ = 1.0 Hz, H-6), 5.97 (t, 1H, $J_{1',2'}$ = 5.4 Hz, H-1′), 4.56 (s, 2H, CH_2), 3.77 (dd, 1H, $J_{2'a,2'b}$ = 11.9 Hz, $J_{2'a,1'}$ = 5.4 Hz, H-2′a), 3.74 (dd, 1H, $J_{5'a,5'b}$ = 11.6 Hz, $J_{5'a,4'}$ = 3.9 Hz, H-5′a), 3.71 (m, 1H, H-4′), 3.69 (dd, 1H, $J_{2'b,1'}$ = 5.4 Hz, H-2′b), 3.63 (dd, 1H, $J_{3'a,3'b}$ = 11.9 Hz, $J_{3'a,4'}$ = 5.0 Hz, H-3′a), 3.56 (dd, 1H, $J_{3'b,4'}$ = 2.2 Hz, H-3′b), 3.55 (m, 1H, H-5′b), 1.93 (d, 3H, $J_{CH3,H6}$ = 1.0 Hz, CH_3), 1.46 (s, 9H, tBu).

1-[1-(1-*O*-Benzoylmethyl-2-hydroxyethoxy)-2-hydroxyethyl]thymine (9). Yield: 79.6% (200 mg), R_f = 0.50 (CH_2Cl_2/EtOH, 90/10, v/v); IR: 3397 (O–H), 2935 (C–H), 1720 (C = O benzoyl), 1698 (C = O thym), 1270 (C–O–C); ^{1}H NMR (CD_3OD) δ: 8.10–7.40 (m, 5H, Ar), 7.36 (d, 1H, $J_{H6,CH3}$ = 1.0 Hz, H-6), 5.95 (t, 1H, $J_{1',2'}$ = 5.7 Hz, H-1′), 4.00 (m, 1H, H-4′), 3.80 (dd, 1H, $J_{5'a,5'b}$ = 12.0 Hz, $J_{5'a,4'}$ = 4.8 Hz, H-5′a), 3.78 (dd, 1H, $J_{2'a,2'b}$ = 12.0 Hz, H-2′a), 3.74 (dd, 1H, $J_{5'b,4'}$ = 5.3 Hz, H-5′b), 3.68 (dd, 1H, $J_{3'a,3'b}$ = 12.2 Hz, $J_{3'a,4'}$ = 2.2 Hz, H-3′a), 3.67 (dd, 1H, $J_{3'b,4'}$ = 4.0 Hz, H-3′b), 3.65 (dd, 1H, H-2′b), 1.46 (d, 3H, $J_{CH3,H6}$ = 1.0 Hz, CH_3).

1-[1-(1-*O*-Benzoylmethyl-2-hydroxyethoxy)-2-hydroxyethyl]-3-*tert*-butoxycarbonyl methylthymine (10). Yield: 97.4% (90 mg); R_f = 0.63 (CH_2Cl_2/EtOH, 95/5, v/v); IR: 3399 (O–H), 2930 (C–H), 1725 (C = O benzoyl), 1699 (C = O thym), 1661 (C = O CO_2tBu), 1271 (C–O–C); ^{1}H NMR (CD_3OD) δ: 7.95–7.41 (m, 5H, Ar), 7.4 (d, 1H, $J_{H6,CH3}$ = 1.0 Hz, H-6), 5.99 (t, 1H, $J_{1',2'}$ = 5.6 Hz, H-1′), 4.50 (s, 2H, CH_2), 4.42 (m, 1H, H-5′a), 4.30 (dd, 1H, $J_{5'a,5'b}$ = 12.0 Hz, $J_{5'b,4'}$ = 2.7 Hz, H-5′b), 4.02 (m, 1H, H-4′), 3.81 (dd, 1H, $J_{2'a,2'b}$ = 12.0 Hz, H-2′a), 3.78 (dd, 1H, $J_{3'a,3'b}$ = 11.9 Hz, $J_{3'a,4'}$ = 4.8 Hz, H-3′a), 3.75 (dd, 1H, $J_{3'b,4'}$ = 5.4 Hz, H-3′b), 3.67 (dd, 1H, H-2′b), 1.58 (d, 3H, $J_{CH3,H6}$ = 1.0 Hz, CH_3), 1.46 (s, 9H, tBu).

1-[1-(1,3-Azidoisopropoxy)-2-azidoethyl]thymine (11). To a solution of compound **7** (329 mg, 1.26 mmol) in a pyridine (4.5 mL) at 0°C were added 12 equivalents of mesyl chloride (0.860 mL). After two h, the solution was quenched with the addition of a $NaHCO_3$ saturated water solution and extracted with $CHCl_3$. The organic layer was dried ($MgSO_4$), filtered, and the solvent removed. The resulting product (262 mg, 0.53 mmol) was dissolved in DMF (5 mL). This solution was immersed in an oil bath at 100°C and sodium azide (524 mg, 15 eq) was added. After 1 h, the product was extracted with 3 × 20 mL of chloroform and

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extracts were evaporated. The crude product was purified using preparative TLC (CH_2Cl_2/EtOH, 98/2, v/v) yielding 211 mg (50%). R_f = 0.56 (CH_2Cl_2/EtOH, 98/2, v/v); $[\alpha]_D$ = 50.1° (c = 0.33, $CHCl_3$); IR: 2103 (N_3), 1693 (C = O thym), 1651 (C = C), 1265 (C–O–C); ^{1}H NMR ($CDCl_3$) δ: 9.12 (s, 1H, H-3), 7.27 (brs, 1H, H-6), 6.07 (t, 1H, $J_{1',2'}$ = 5.5, H-1′), 3.77 (m, 1H, H-4′), 3.63 (dd, 1H, $J_{2'a,2'b}$ = 13.2 Hz, H-2′a), 3.61 (dd, 1H, $J_{3'a,3'b}$ = 13.2 Hz, $J_{3'a,4'}$ = 3.0 Hz, H-3′a), 3.51 (dd, 1H, H-2′b), 3.46 (dd, 1H, $J_{3'b,4'}$ = 5.4 Hz, H-3′b), 3.41 (dd, 1H, $J_{5'a,5'b}$ = 13.2 Hz, $J_{5'a,4'}$ = 7.0 Hz, H-5′a), 3.35 (dd, 1H, $J_{5'b,4'}$ = 4 Hz, H-5′b), 1.97 (brs, 3H, CH_3); MS (DCI/NH_3) m/z 358 (M Na^+).

Compounds **12**, **13** and **14** were prepared according to the procedure described for **15** starting from **8** (635 mg, 1.69 mmol), **9** (146 mg, 0.40 mmol), and **10** (119 mg, 0.249 mmol), respectively.

1-[1-(1,3-Azidoisopropoxy)-2-azidoethyl]-3-*tert*-butoxycarbonylmethylthymine (12). Yield: 47.7% (364 mg); R_f = 0.74 (CH_2Cl_2/EtOH, 98/2, v/v); $[\alpha]_D$ = 94° (c = 1.23, $CHCl_3$); IR: 2979 (C–H), 2104 (N_3), 1707 (C = O thym), 1666 (C = O CO_2tBu), 1651 (C = C), 1258 (C–O–C); ^{1}H NMR (CD_3OD) δ: 7.59 (d, 1H, $J_{H6,CH3}$ = 1.0 Hz, H-6), 6.10 (t, 1H, $J_{1',2'}$ = 5.8 Hz, H-1′), 4.58 (s, 2H, CH_2), 3.83 (m, 1H, H-4′), 3.75 (dd, 1H, $J_{2'a,2'b}$ = 13.1 Hz, H-2′a), 3.65 (dd, 1H, $J_{3'a,3'b}$ = 13.2 Hz, $J_{3'a,4'}$ = 4.2 Hz, H-3′a), 3.58 (dd, 1H, H-2′b), 3.48 (dd, 1H, $J_{3'b,4'}$ = 5.5 Hz, H-3′b), 3.41 (m, 2H, H-5′a, H-5′b), 1.98 (d, 3H, $J_{CH3,H6}$ = 1.0 Hz, CH_3), 1.47 (s, 9H, tBu); MS (DCI/NH_3) m/z 472 (MNa^+).

1-[1-(1-*O*-Benzoylmethyl-2-azidoethoxy)-2-azidoethyl]thymine (13). Yield: 48% (80 mg); R_f = 0.70 (CH_2Cl_2/EtOH, 98/2, v/v); IR: 2940 (C–H), 2108 (N_3), 1725 (C = O benzoyl), 1705 (C = O thym), 1650 (C = C), 1268 (C–O–C); ^{1}H NMR ($CDCl_3$) δ: 9.10 (s, 1H, H-3), 8.07–7.30 (m, 5H, Ar), 7.14 (q, 1H, $J_{H6,CH3}$ = 1.0 Hz, H-6), 6.08 (dd, 1H, $J_{1',2'a}$ = 5.8 Hz, $J_{1',2'b}$ = 5.1 Hz, H-1′), 4.38 (m, 1H, H-5′a), 4.37 (dd, 1H, $J_{5'a,5'b}$ = 12.5 Hz, $J_{5'b,4'}$ = 4.8 Hz, H-5′b), 4.08 (m, 1H, H-4′), 3.70 (dd, 1H, $J_{3'a,3b'}$ = 12.8 Hz, $J_{3'a,4'}$ = 5.0 Hz, H-3′a), 3.59 (dd, 1H, $J_{3'b,4'}$ = 4.1 Hz, H-3′b), 3.58 (dd, 1H, $J_{2'a,2'b}$ = 12.8 Hz, H-2′a), 3.48 (dd, 1H, H-2′b), 1.61 (d, 3H, $J_{CH3,H6}$ = 1.0 Hz, CH_3).

1-[1-(1-*O*-Benzoylmethyl-2-azidoethoxy)-2-azidoethyl]-3-*tert*-butoxycarbonylmethyl thymine (14). Yield: 61.6% (81 mg); R_f = 0.50 (CH_2Cl_2/EtOH, 99/1, v/v); IR: 2925 (C–H), 2107 (N_3), 1728 (C = O benzoyl), 1708 (C = O thym), 1670 (C = O CO_2tBu), 1651 (C = C), 1271 (C–O–C); ^{1}H NMR (CD_3OD) δ: 7.93–7.26 (m, 5H, Ar), 7.15 (q, 1H, $J_{H6,CH3}$ = 1.1 Hz, H-6), 6.11 (dd, 1H, $J_{1',2'a}$ = 6.4 Hz, $J_{1',2'b}$ = 4.7 Hz, H-1′), 4.54 (d, 1H, J = 16.5, CH_2), 4.50 (d, IH, J = 16.5, CH_2), 4.42 (dd, 1H, $J_{5'a,5'b}$ = 12.2 Hz, $J_{5'a,4'}$ = 6.6 Hz, H-5′a), 4.32 (dd, 1H, $J_{5'b,4'}$ = 3.28 Hz, H-5′b), 4.10 (m, 1H, H-4′), 3.70 (dd, 1H, $J_{3a,3'b}$ = 13.1, $J_{3'a,4'}$ = 4.4 Hz, H-3′a); 3.58 (dd, 1H, $J_{3'b,4'}$ = 5.8 Hz, H-3′b), 3.57 (dd, 1H, $J_{2'a,2'b}$ = 13.1 Hz, H-2′a), 3.46 (dd, 1H, H-2′b), 1.61 (d, 3H, $J_{CH3,H6}$ = 1.0 Hz, CH_3), 1.46 (s, 9H, tBu); MS (DCI/NH_3) m/z 551 (MNa^+).

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1-[1-(1,3-Chloroisopropoxy)-2-chloroethyl]thymine (15). Compound **7** (103 mg, 0.396 mmol) was stirred with pyridine (2 mL) under inert atmosphere. Four equivalents of SOCl$_2$ (0.33 mL) were added at 0°C. After 10 min the mixture was stirred at room temperature for 3 days, then MeOH was added and the solution was kept for 10 min at room temperature and concentrated. The crude product was purified by preparatives TLC using CH$_2$Cl$_2$/EtOH (96/04, v/v), compound **15** was obtained as yellow syrup (87 mg, 60.4%). R$_f$ = 0.5 (CH$_2$Cl$_2$/EtOH, 98/2, v/v); IR: 3185 (N–H), 3043 (C–H), 1688 (C = O thym), 1650 (C = C), 1260 (C–O–C); ^{1}H NMR (CD$_3$OD) δ: 9.06(s, 1H, H-3), 7.21 (q, 1H, $J_{H6,CH3}$ = 1.1 Hz, H-6), 6.09 (d, 1H, $J_{1',2'}$ = 6.4 Hz, H-1′), 3.98 (m, 1H, H-4′), 3.78 (dd, 1H, $J_{2'a,2'b}$ = 11.9 Hz, H-2′a), 3.66 (dd, 1H, H-2′b), 3.65 (dd, 1H, $J_{5'a,5'b}$ = 11.9 Hz, $J_{5'a,4'}$ = 4.3 Hz, H-5′a), 3.64 (dd, 1H, $J_{5'b,4'}$ = 5.5 Hz, H-5′b), 3.31 (m, 1H, H-3′a), 3.60 (dd, 1H, $J_{3'a,3'b}$ = 12 Hz, $J_{3'b,4'}$ = 6.2 Hz, H-3′b), 1.97 (d, 3H, $J_{CH3,H6}$ = 1, CH$_3$). MS (DCI/NH$_3$) m/z 315 (MH$^+$), 317 (MH$^+$), 332 (MNH$_4^+$), 334 (MNH$_4^+$).

Compounds **16**, **17** and **18** were prepared the same way as that described for **15** starting from **8** (103 mg, 0.275 mmol), **9** (206 mg, 0.566 mmol), and **10** (90 mg, 0.188 mmol), respectively.

1-[1-(1,3-Chloroisopropoxy)-2-chloroethyl]-3-*tert*-butoxycarbonylmethylthymine (16). Yield: 66% (78 mg), R$_f$ = 0.68 (CH$_2$Cl$_2$/EtOH, 98/2, v/v); IR: 2930 (C–H), 1708 (C = O thym), 1670 (C = O CO$_2$tBu), 1650 (C = C), 1255 (C–O–C); ^{1}H NMR (CD$_3$OD) δ: 7.23 (brs, 1H, H-6), 6.10 (t, 1H, $J_{1',2'}$ = 6.1 Hz, H-1′), 4.57 (s, 2H, CH$_2$), 3.97 (m, 1H, H-4′), 3.77 (dd, 1H, $J_{2'a,2'b}$ = 12.0 Hz, H-2′a), 3.76 (dd, 1H, $J_{3'a,3'b}$ = 12.2 Hz, $J_{3'a,4'}$ = 5.5 Hz, H-3′a), 3.72 (dd, 1H, $J_{3'b,4'}$ = 5.0 Hz, H-3′b), 3.67 (dd, 1H, H-2′b), 3.63 (dd, 1H, $J_{5'a,5'b}$ = 11.9 Hz, $J_{5'a,4'}$ = 1.3 Hz, H-5′a), 3.61 (dd, 1H, $J_{5'b,4'}$ = 5.7 Hz, H-5′b), 1.98 (brs, 3H, CH$_3$), 1.46 (s, 9H, tBu); MS (DCI/NH$_3$) 451 (MNa$^+$), 453 (MNa$^+$).

1-[1-(1-*O*-Benzoylmethyl-2-chloroethoxy)-2-chloroethyl]thymine (17). Yield: 60% (136 mg), R$_f$ = 0.60 (CH$_2$Cl$_2$/EtOH, 98/2, v/v); IR: 2950 (C–H), 1730 (C = O benzoyl), 1699 (C = O thym), 1650 (C = C), 1275 (C–O–C); ^{1}H NMR (CDCl$_3$) δ: 8.67 (s, 1H, H-3), 8.0–7.3 (m, 5H, Ar), 7.08 (q, 1H, $J_{H6,CH3}$ = 1.0 Hz, H-6), 6.2 (t, 1H, $J_{1',2'}$ = 6.1 Hz, H-1′), 4.46 (dd, 1H, $J_{5'a,5'b}$ = 12.1 Hz $J_{5'a,4'}$ = 6.0 Hz, H-5′a), 4.43 (dd, 1H, $J_{5'b,4'}$ = 4.0 Hz, H-5′b), 4.19 (m, 1H, H-4′), 3.80 (dd, 1H, $J_{3'a,3'b}$ = 12.2 Hz, $J_{3'a,4'}$ = 5.5 Hz, H-3′a), 3.79 (dd, 1H, $J_{2'a,2'b}$ = 12.0 Hz, H-2′a), 3.75 (dd, 1H, $J_{3'b,4'}$ = 5.2 Hz, H-3′b), 3.63 (dd, 1H, H-2′b), 1.61 (d, 3H, $J_{CH3,H6}$ = 1.0 Hz, CH$_3$).

1-[1-(1-*O*-Benzoylniethyl-2-chloroethoxy)-2-chloroethyl]-3-*tert*-butoxycarbonylmethyl thymine (18). Yield 45.4% (44 mg), R$_f$ = 0.52 (CH$_2$Cl$_2$/EtOH, 98/2, v/v); IR: 2955 (C–H), 1725 (C = O benzoyl), 1699 (C = O thym), 1666 (C = O CO$_2$tBu), 1651 (C = C), 1270 (C–O–C); ^{1}H NMR (CD$_3$OD) δ: 8.0–7.4 (m, 5H, Ar), 7.10 (q, 1H, $J_{H6,CH3}$ = 1.0 Hz, H-6), 6.15 (t, 1H,

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$J_{1',2'}$ = 6.0 Hz, H-1′), 4.52 (s, 2H, CH_2), 4.48 (dd, 1H, $J_{5'a,5'b}$ = 12.1 Hz, $J_{5'a,4'}$ = 7.0 Hz, H-5′a), 4.39 (dd, 1H, $J_{5'b,4'}$ = 3.2 Hz, H-5′b), 4.18 (m, 1H, H-4′), 3.79 (dd, 1H, $J_{3'a,3'b}$ = 12.0 Hz, $J_{3'a,4'}$ = 5.5 Hz, H-3′a), 3.77 (m, 1H, H-3′b), 3.73 (dd, 1H, $J_{2'a,2'b}$ = 11.9 Hz, H-2′a), 3.64 (dd, 1H, H-2′b), 1.60 (d, 3H, $J_{CH3,H6}$ = 1.0 Hz, CH_3), 1.45 (s, 9H, tBu).

1-[1-(1-*O*-Hydroxymethyl-2-azidoethoxy)-2-azidoethyl]thymine (19). Compound **13** (60 mg, 0.145 mmol) was dissolved in 3 mL of methanol and a solution of ammonia in methanol 7M (2 mL, 100 eq). After 4 days, the solution was evaporated and the crude product was purified using preparatives TLC (CH_2Cl_2/EtOH, 92/8, v/v). The expected compound was isolated in 69% yield (31 mg). R_f = 0.40 (CH_2Cl_2/EtOH, 95/5, v/v); IR: 3300 (O–H), 2923 (C–H), 2105 (N_3), 1690 (C = O thym), 1650 (C = C), 1263 (C–O–C); ^{1}H NMR (CD_3OD) δ: 7.57 (q, 1H, $J_{H6,CH3}$ = 1.1 Hz, H-6), 6.04 (t, 1H, $J_{1',2'}$ = 6.0 Hz, H-1′), 3.72 (dd, 1H, $J_{2'a,2'b}$ = 13.1 Hz, H-2′a), 3.67 (m, 1H, H-4′), 3.66 (dd, 1H, $J_{5'a,4'}$ = 12.8 Hz, $J_{5'a,4'}$ = 3.8 Hz, H-5′a), 3.62 (dd, 1H, $J_{3'a,3'b}$ = 13.0 Hz, $J_{3'a,4'}$ = 3.8 Hz, H-3′a), 3.55 (dd, 1H, $J_{3'b,4'}$ = 5.8 Hz, H-3′b), 3.51 (dd, 1H, $J_{5'b,4'}$ = 5.5 Hz, H-5′b), 3.43 (dd, 1H, H-2′b), 1.90 (d, 3H, $J_{CH3,H6}$ = 1.1 Hz, CH_3); MS (DCI/NH_3) m/z 311 (MH^+), 328 (MNH_4^+).

Compound **20** was prepared according to the procedure described for **19** starting from **14** (116 mg, 0.22 mmol).

1-[1-(1-Hydroxymethyl-2-azidoethoxy)-2-azidoethyl]-3-*tert*-butoxycarbonylmethyl thymine (20). Yield: 79.3% (74 mg), R_f = 0.61 (CH_2Cl_2/EtOH, 98/2, v/v), IR: 3300 (O–H), 2923 (C–H), 2105 (N_3), 1690 (C = O thym), 1666 (C = O CO_2tBu), 1650 (C = C), 1255 (C–O–C); ^{1}H NMR (CD_3OD) δ: 7.64 (q, 1H, $J_{H6,CH3}$ = 1.0 Hz, H-6), 6.07 (t, 1H, $J_{1',2'}$ = 5.9 Hz, H-1′), 4.55 (s, 2H, CH_2), 3.73 (dd, 1H, $J_{2'a,2'b}$ = 13.0 Hz, $J_{2'a,1'}$ = 5.9 Hz, H-2′a), 3.69 (m, 1H, H-5′a), 3.69 (m, 1H, H-4′), 3.63 (dd, 1H, $J_{3'a,3'b}$ = 13.2 Hz, $J_{3'a,4'}$ = 3.9 Hz, H-3′a), 3.55 (dd, 1H, $J_{5'a,5'b}$ = 12.4 Hz, $J_{5'b,4'}$ = 3.4 Hz, H-5′b), 3.51 (dd, 1H, $J_{3'b,4'}$ = 5.3 Hz, H-3′b), 3.46 (dd, 1H, H-2′b), 1.94 (d, 3H, $J_{CH3,H6}$ = 1.0 Hz, CH_3), 1.46 (s, 9H, tBu).

5-(Chloromethyl)-3-(thymin-1-yl)-1,4-dioxane (21). Compound **17** (30 mg, 0.075 mmol) was dissolved in 2 mL of methanol and a solution of ammonia in methanol 7 M (1 mL, 7 mmol). After 4 days, the solution was evaporated and the crude product was purified using preparatives TLC (CH_2Cl_2/EtOH, 98/2, V/V). The dioxane nucleoside **21** was isolated in 82% (16 mg). R_f = 0.50 (CH_2Cl_2/EtOH, 98/2, v/v), IR: 3200 (N–H), 3000 (C–H), 1690 (C = O thym), 1650 (C = C), 1260 (C–O–C); ^{1}H NMR (CD_3OD), *isomer* 1 = δ: 7.86 (q, 1H, $J_{H6',CH3}$ = 1.1 Hz, H-6′), 6.12 (dd, 1H, $J_{3,2b}$ = 2.3 Hz, $J_{3,2a}$ = 6.1 Hz, H-3), 4.85 (dd, 1H, $J_{2a,2b}$ = 10.5, H-2a), 4.68 (dd, 1H, H-2b), 4.12 (m, 1H, H-5), 3.71 (dd, 1H, $J_{6a,5}$ = 3.8 Hz, $J_{6a,6b}$ = 11.7 Hz, H-6a), 3.70 (dd, $J_{6b,5}$ = 3.4 Hz, H-6b), 3.62 (dd, 1H, $J_{7a,5}$ = 3.1 Hz, $J_{7a,7b}$ = 11.8 Hz, H-7a), 3.61 (dd, $J_{7b,5}$ = 3.1 Hz, H-7b), 1.95 (d, 1H, CH_3). *isomer*

2 = δ: 7.79 (q, 1H, $J_{H6',CH3}$ = 1.1 Hz, H-6′), 6.10 (dd, 1H, $J_{3,2b}$ = 2.3 Hz, $J_{3,2a}$ = 6.0 Hz, H-3), 4.80 (dd, 1H, $J_{2a,2b}$ = 10.4, H-2a), 4.70 (dd, 1H, H-2b), 4.10 (m, 1H, H-5), 3.74 (dd, 1H, $J_{6a,5}$ = 3.4 Hz, $J_{6a,6b}$ = 11.6 Hz, H-6a), 3.68 (dd, $J_{6b,5}$ = 3.3 Hz, H-6b), 3.60 (dd, 1H, $J_{7a,5}$ = 3.6 Hz, $J_{7a,7b}$ = 11.8 Hz, H-7a), 3.58 (dd, $J_{7b,5}$ = 2.1 Hz, H-7b), 1.95 (d, 1H, CH_3); MS (DCI/NH_3) m/z 261 (MH^+), 278 (MNH_4^+).

1-[1-(1,3-Dihydroxyisopropoxy)-2-hydroxyethyl]-3-carboxymethylthymine (22). This compound was prepared by the reaction of **8** (31 mg, 0.08 mmol) with TFA (1 mL) at room temperature for 2 h. After purification by preparative TLC using butanol/water/acetic acid (50/25/25, v/v/v), the product **21** was isolated in 98.3% yield (25 mg). R_f = 0.25 (CH_2Cl_2/EtOH, 90/10, v/v); IR: 3384 (O–H), 2935 (C–H), 1696 (C = O thymine), 1698 (C = O CO_2H), 1635 (C = C). 1255 (C–O–C); ^{1}H NMR (CD_3OD) δ: 7.65 (q, 1H, $J_{H6,CH3}$ = 1.1 Hz, H-6), 5.97 (t, 1H, $J_{1',2'}$ = 5.4 Hz, H-1′), 4.67 (d, 1H, J = 16.9 Hz, CH_2), 4.62 (d, 1H, J = 16.9 Hz, CH_2), 3.77 (dd, 1H, $J_{2'a,2'b}$ = 11.9 Hz, H-2′a), 3.70 (dd, 1H, H-2′b), 3.68 (m, 1H, H-5′a), 3.68 (m, 1H, H-5′b), 3.64 (m, 1H, H-4′), 3.55 (m, 1H, H-3′a), 3.55 (dd, 1H, $J_{3'a,3'b}$ = 11.4 Hz, $J_{3'b,4'}$ = 4.6 Hz, H-3′b), 1.93 (d, 3H, $J_{CH3,H6}$ = 1.1 Hz, CH_3).

Compounds **24**, **25** and **26** were prepared in a manner similar to that described for **23** starting from **12** (68 mg, 0.151 mmol), **16** (25 mg, 0.058 mmol), and **20** (40 mg, 0.094 mmol), respectively.

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1-[1-(1,3-Azidoisopropoxy)-2-azidoethyl]-3-carboxymethylthymine (23). Yield: 87.6% (52 mg), R_f = 0.18 (CH_2Cl_2/EtOH, 98/2, v/v); IR: 3300 (O–H), 2930 (C–H), 2104 (N_3), 1702 (C = O thym), 1667 (C = O CO_2H), 1644 (C = C), 1288 (C–O–C); ^{1}H NMR (CD_3OD) δ: 7.34 (s, 1H, H-6), 6.07 (t, 1H, $J_{1',2'}$ = 5.8 Hz, H-1′), 4.60 (s, 2H, CH_2), 3.80 (m, 1H, H-4′), 3.70 (dd, 1H, $J_{2'a,2'b}$ = 13.0 Hz, H-2′a), 3.60 (dd, 1H, $J_{3'a,3'b}$ = 13.1 Hz, $J_{3'a,4'}$ = 4.0 Hz, H-3′a), 3.50 (dd, 1H, H-2′b), 3.45 (dd, 1H, $J_{3'b,4'}$ = 5.5 Hz, H-3′b), 3.38 (m, 2H, H-5′a, H-5′b), 1.97 (s, 3H, CH_3); MS (DCI/NH_3) m/z 416 (MNa^+), 432 (MK^+).

1-[1-(1,3-Chloroisopropoxy)-2-chloroethyl]-3-carboxymethylthymine (24). Yield: 83.1% (18 mg), R_f = 0.25 (CH_2Cl_2/EtOH, 98/2, v/v); IR: 3310 (O–H), 2927 (C–H), 1704 (C = O thym), 1667 (C = O CO_2H), 1649 (C = C), 1255 (C–O–C); ^{1}H NMR (CD_3OD) δ: 7.63 (q, 1H, $J_{H6,CH3}$ = 1.2 Hz, H-6), 6.14 (dd, 1H, $J_{1',2'a}$ = 6.0 Hz, $J_{1',2'b}$ = 6.7 Hz, H-1′), 4.68 (d, 1H, J = 16.9 Hz, CH_2), 4.61 (d, 1H, J = 16.9 Hz, CH_2), 4.07 (1H, m, H-4′), 3.93 (dd, 1H, $J_{2'a,2'b}$ = 11.9 Hz, H-2′a), 3.88 (dd, 1H, H-2′b), 3.83 (dd, 1H, $J_{5'a,5'b}$ = 11.9 Hz, $J_{5'a,4'}$ = 5.1 Hz, H-5′a), 3.79 (dd, 1H, $J_{5'b,4'}$ = 4.9 Hz, H-5′b), 3.68 (dd, 1H, $J_{3'a,3'b}$ = 12.1 Hz, $J_{3'a,4'}$ = 4.4 Hz, H-3′a), 3.63 (dd, 1H, $J_{3'b,4'}$ = 6.0 Hz, H-3′b), 1.95 (d, 3H, $J_{CH3,H6}$ = 1.2 Hz, CH_3); MS (DCI/NH_3) m/z 373 (MH^+), 375 (MH^+), 390 (MNH_4^+), 392 (MNH_4^+).

1-[1-(-1-Hydroxymethyl-2-azidoethoxy)-2-azidoethyl]-3-carboxymethylthymine (25). Yield: 52% (18 mg); R_f = 0.40 (CH_2Cl_2/EtOH, 98/2, v/v); IR: 3250 (O–H), 2923 (C–H), 2105 (N_3), 1703 (C = O thym), 1670 (C = O CO_2H), 1650 (C = C), 1265 (C–O–C); ^{1}H NMR (CD_3OD) δ: 7.56 (brs, 1H, H-6), 6.07 (t, 1H, $J_{1',2'}$ = 4.2 Hz, H-1′), 3.70 (m, 1H, H-5′a), 3.69 (m, 1H, H-4′), 3.68 (dd, 1H, $J_{2'a,2'b}$ = 12.5 Hz, H-2′a), 3.56 (dd, 1H, $J_{3'a,3'b}$ = 13.5 Hz, $J_{3'a,4'}$ = 3.0 Hz, H-3′a), 3.54 (m, 1H, H-5′b), 3.50 (m, 1H, H-2′b), 3.46 (dd, 1H, $J_{3'b,4'}$ = 4.8 Hz, H-3′b), 3.38 (s, 2H, CH_2), 1.97 (brs, 3H, CH_3); MS (DCI/NH_3) m/z 369 (MH^+), 386 (MNH_4^+).

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