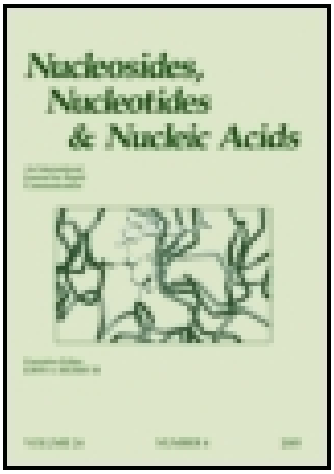

Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:
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Synthesis and Antiviral Evaluation of Azt Analogues with A Spacer Arm Between Glucidic and Base Moieties. Part II

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Published online: 19 Jun 2007.

To cite this article: Vincent Roy , Rachida Zerrouki , Pierre Krausz , Géraldine Laumond & Anne Marie Aubertin (2007) Synthesis and Antiviral Evaluation of Azt Analogues with A Spacer Arm Between Glucidic and Base Moieties. Part II, Nucleosides, Nucleotides and Nucleic Acids, 26:5, 413-421, DOI: 10.1080/15257770701426153

To link to this article: http://dx.doi.org/10.1080/15257770701426153

Nucleosides, Nucleotides, and Nucleic Acids, 26:413–421, 2007

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770701426153

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SYNTHESIS AND ANTIVIRAL EVALUATION OF AZT ANALOGUES WITH A SPACER ARM BETWEEN GLUCIDIC AND BASE MOIETIES. PART II

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□ *This article describes the synthesis of a series of AZT analogues bearing an acyclic chain between the sugar and the base moieties is described. These new compounds were readily obtained using microwave irradiation. The compounds were characterized by ^1H NMR and IR spectroscopy. Antiviral (HIV-1) properties of these compounds were examined.*

Keywords Nucleoside; HIV; microwave; spacer arm

INTRODUCTION

In the continuity of our program to find new anti-HIV nucleosides,[1–4] we were interested in the preparation of drugs in which thymine is linked to ribofuranose by an aliphatic chain. Spacer arms have been used in antisense oligonucleoside synthesis for preventing the enzymatic degradation of DNA by nucleases.[5] On the other hand, it is well known that increasing the length of the spacer arm linkage would thus enhance bending of DNA, leading to base pair opening where the bases then become susceptible to attack by reactive groups.[6] Taking AZT as a model molecule,[7] we prepared a new family of AZT analogues **8a, b, c** (Scheme 1) whose lack of 3′-hydroxyl function should stop DNA elongation, and we studied

Received 10 July 2006; accepted 15 February 2007.
Financial support from the "Conseil Régional du Limousin" is gratefully acknowledged. The authors are grateful to Dr. Michel Guilloton for useful comments on the manuscript.
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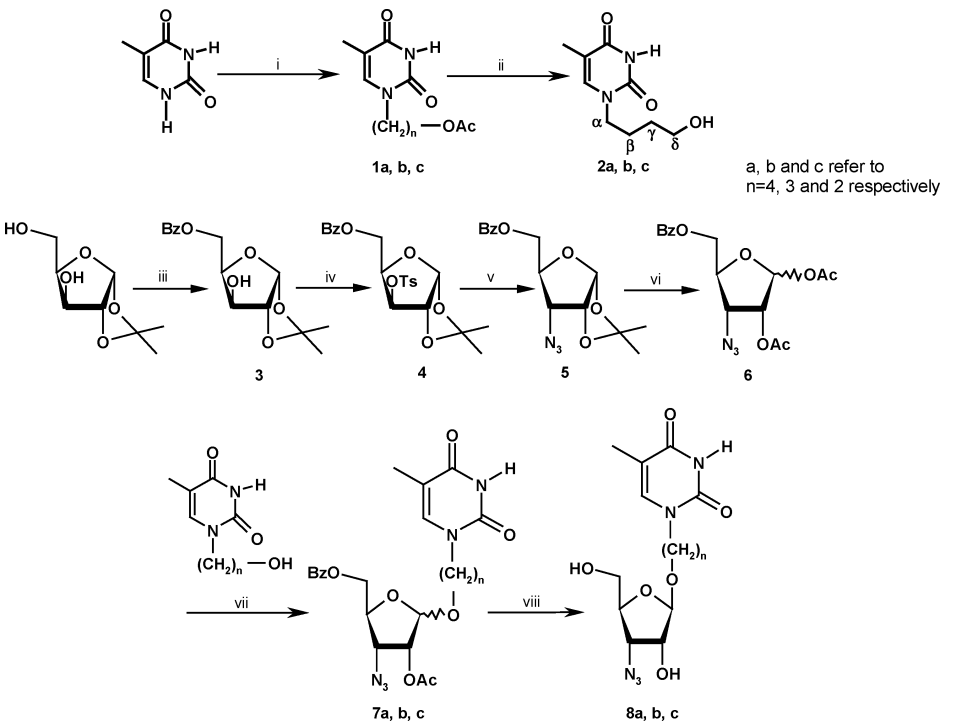

SCHEME 1 i) NaH, X-$(CH_2)_n$-OAc, DMF, MW (4 minutes). ii) KOH/Al_2O_3, MW (2 minutes). iii) BzCl, toluene/pyridine, 50°C. iv) TsCl, pyridine. v) LiN_3, DMF, 110°C. vi) a)CF_3COOH 60% b) Ac_2O, AcONa, MW (6 minutes). vii) $SnCl_4$, CH_3CN. viii) NH_3, MeOH/CH_2Cl_2.

the influence of the spacer arm and, more precisely, its length on the inactivation of the human immunodeficiency virus (HIV-1).

RESULTS AND DISCUSSION

N-1 substituted thymine (**2a, b, c)** derivatives were synthesised according to the microwave irradiation method described in our preceding article.[1]

The reaction of 1,2-*O*-isopropylidene-α-D-xylofuranose with benzoyl chloride in solvent mix (toluene/pyridine; 4/1) gave **3** in 83% yield.[8] Compound **4** was obtained from **3** in the presence of tosyl chloride in pyridine, in 74% yield. The azidation reaction was first realized according to Gautier et al.[9] in reflux DMF in presence of NaN_3 during 4 days, but with no success. We also used the Jeon et al. method[10] in reflux DMF with NaN_3 and 18-crown-6. In this case we did not observe any improvement. Compound **5** was finally obtained[7] in DMF at 110°C in presence of 10 equiv. of LiN_3[11] during 5 days. After purification, we obtained **5** in 51% yield. The structure of **5** was confirmed by spectroscopic data. IR spectrum displays an azido band at 2107 cm^{-1} and ^1H NMR indicates the disappear-

ance of the tosyl group and the presence of a benzoyl group. Treatment of compound **5** with 60% trifluoroacetic acid[12] gave in quantitative yield 1,2-dihydroxyl compound**,** which was used without further purification after evaporation of solvent. Acetylation reaction, was achieved under microwave irradiation during 6 minutes in presence of acetic anhydride and 1.1 equiv. of sodium acetate.[13] After purification, we obtained **6** in 77.6% yield on 2 steps and a ratio β/α of 85/15 was determined by ^{1}H NMR. We observed a ratio β/α of 1/1 after microwave activation in presence of acetic anhydride without sodium acetate.

Syntheses of **7a, b, c** were performed by coupling alkylated thymine **2 a, b, c** via Hanessian's procedure[14] ($SnCl_4$/acetonitrile). After purification, compounds **7a, b, c** were obtained in 74, 72, and 76% yields, respectively; anomeric ratios (β/α = 85/15 for these three compounds) were determined by ^{1}H NMR. We eliminate the α anomer by preparative thin layer chromatography on silica gel. Compounds **8a, b, c** were obtained after removal of the protecting acetyl and benzoyl groups by 7N methanolic NH_3. IR spectrum displays a hydroxyl band at 3400 cm^{-1} and ^{1}H NMR indicates the disappearance of the acetyl and benzoyl groups.

BIOLOGICAL EVALUATION

The synthesized compounds **8a, b, c** were evaluated for their in vitro inhibitory effects on the replication of RNA virus (HIV-1) (Table 1). The anti HIV-1 activity was tested on CEM-SS and MT_4 cell lines infected respectively with HIV-1 LAI and HIV-IIIB according to protocols described previously.[15] In the case of CEM-SS cells, the production of virus was measured by quantification of reverse transcriptase activity associated with the release of virus particles in the culture supernatant; for MT4 cells the assay was based on the virus induced cytopathogenicity. Cell viability was evaluated by measuring the activity of mitochondrial electron transport by the MTT assay.[16] The antiviral activity was expressed as EC_{50}, the

TABLE 1 Biological evaluation

CEM-SS		MT-4	
EC_{50}[a]	CC_{50}[b]	EC_{50}	CC_{50}
8a $>2.10^{-1}$ mg/mL	$>2.10^{-1}$ mg/mL	$>2.10^{-1}$ mg/mL	$1.9.10^{-1}$ mg/mL
8b $>1.10^{-1}$ mg/mL	$>1.10^{-1}$ mg/mL	$>1.10^{-1}$ mg/mL	$>1.10^{-1}$ mg/mL
8c $>1.10^{-1}$ mg/mL	$>1.10^{-1}$ mg/mL	$>1.10^{-1}$ mg/mL	$>1.10^{-1}$ mg/mL

[a]50% effective concentration (mg/mL) or concentration required to inhibit HIV-1 replication by 50%.

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

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.[17] Cytotoxicity was 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 compounds have no specific antiviral activity. Similar results were obtained with the MT4 assay which showed that the drugs did not bring any sensible protection against the virus-induced cytopathic effect (Table 1).

EXPERIMENTAL SECTION

All the solvents and chemicals were commercially available and, unless otherwise stated, were used as received. CH_2Cl_2 and CH_3CN 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 (6 N) and heating to 200°C. Silica gel (Merck Kieselgel 60, 15–40 μm) was used for flash chromatography. Microwave irradiations were performed by the means of a monomode reactor (Synthewave 402 from Prolabo) with focused waves. ^{1}H NMR spectra were recorded at 400.13 MHz with a Bruker spectrometer. Chemical shifts (δ) are expressed in ppm with Me_4Si as an internal standard ($\delta = 0$). Data are reported as follows: chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; quin, quintet; m, multiplet, and br, broad), coupling constants (Hz), and assignment. Melting points (m.p.) 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}).

5-O-Benzoyl-1,2-O-isopropylidene-α-D-xylofuranose (3). To a solution of 1,2-O-isopropylidene-α-D-xylofuranose (1 g, 5,26 mmol) in 10 mL of an anhydrous solvent mix: toluene/pyridine (4/1), we added dropwise with stirring and cooling 0.644 mL of benzoyl chloride (1.1 eq.) in 1 mL of toluene over 2.5 hours. The mixture was left to stand for 12 hours at room temperature and then diluted with water (15 mL). The solution was extracted with $CHCl_3$ (3 × 30 mL). The organic layer was dried ($MgSO_4$), filtered, and the solvent removed. The product was purified using flash chromatography with an elution gradient of $CHCl_3$/petroleum ether and was recovered in 83% yield as a viscous oil (1.283 g). $[\alpha]_D^{22} = +8.49$ (c 2.15; $CHCl_3$), $R_f = 0.53$ ($CHCl_3$/EtOH; 94/6; V/V), IR: 3479 (OH), 2987 (CH), 1698 (C=O), 1602 (C=C aromatic), 1452 (C-C), 1275 (C-O-C), RMN ^{1}H ($CDCl_3$, δ): 5.96 (d, 1H, $J_{1,2} = 3.6$ Hz, H_1), 4.79 (dd, 1H, $J_{5a,4} = 9.3$ Hz, $J_{5a,\,5b} = 12.9$ Hz, H_{5a}), 4.59 (t, 1H, $J = 3.6$ Hz, H_2), 4.40 (dd, 1H, $J_{5b,4} =$

4.9 Hz, $J_{5b,5a}$ = 12.9 Hz, H_{5b}), 4.37 (m, 1H, H_4), 4.18 (dd, 1H, $J_{3,4}$ = 2.9 Hz, H_3), *isopropylidene*: 1.50 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), *benzoyl*: 8.05 (dd, 2H, J = 1.0 Hz, J = 7.9 Hz), 7.59 (tt, 1H, J = 1.0 Hz, J = 7.5 Hz), 7.45 (br t, 2H, J = 7.9 Hz).

5-O-Benzoyl-1,2-O-isopropylidene-3-O-paratoluenesulfonyl-α-D-xylofuranose (4). To a solution of 2,228 g of **3** (7.578 mmol) in 15 mL of anhydrous pyridine, we added 3.59 g of tosyl chloride (2.5 eq.). The reaction mixture was stirred under argon during 15 hours at 50 C. After reaction completion the solution was quenched with the addition of a saturated $NaHCO_3$ solution and extracted with CH_2Cl_2 (3 × 20 mL). The organic layer was washed with 20 mL of water and dried ($MgSO_4$), filtered, and the solvent removed. The product was purified using flash chromatography with an elution gradient of AcOEt/petroleum ether and was recovered in 74% yield as a white solid (3.846 g). T_f = 94°C, $[\alpha]_D^{22}$ =—68.18 (c 0.8; $CHCl_3$), R_f = 0.43 ($CHCl_3$/EP; 9/1; V/V), IR: 2989 (CH), 1724 (C=O ester), 1599 (C=C aromatic), 1452 (C-C), 1273 (C-O-C), RMN ^{1}H ($CDCl_3$, δ): 5.98 (d, 1H, $J_{1,2}$ = 3.7 Hz, H_1), 4.94 (d, 1H, $J_{3,4}$ = 2.9 Hz, H_3), 4.82 (d, 1H, $J_{2,1}$ = 3.7 Hz, H_2), 4.54 (dt, 1H, $J_{4,3}$ = 2.9 Hz, $J_{4,5}$ = 6.3 Hz, H_4), 4.40 (dd, 1H, $J_{5a,4}$ = 6.3 Hz, $J_{5a,5b}$ = 11.4 Hz, H_{5a}), 4.27 (dd, 1H, $J_{5b,4}$ = 6.3 Hz, $J_{5b,5a}$ = 11.4 Hz, H_{5b}), *isopropylidene*: 1.55 (s, 3H, CH_3), 1.50 (s, 3H, CH_3), *benzoyl*: 7.91 (dd, 2H, J = 1.0 Hz, J = 7.9 Hz), 7.56 (tt, 1H, J = 1.0 Hz, J = 7.5 Hz), 7.42 (br t, 2H, J = 7.9 Hz), *tosyl*: 7.77 (d, 2H, J = 8.2 Hz), 7.22 (d, 2H, J = 8.2 Hz), 2.27 (s, 3H, CH_3).

3-Azido-5-O-benzoyl-3-deoxy-1,2-O-isopropylidene-α-D-xylofuranose (5). To a solution of 0.865 mg of 5-*O*-benzoyl-1,2-*O*-isopropylidene-3-*O*-paratoluenesulfonyl-α-D-xylofuranose (1.994 mmol) in 10 mL of anhydrous DMF, we added 0.977 g of lithium azide (10 eq.). The mixture was stirred under argon during 5 days at 110°C and was then poured into solvent mix: H_2 O/ $CHCl_3$. The solution was extracted with $CHCl_3$ (3 × 30 mL). The organic layer was washed with water (2 × 20 mL) and dried ($MgSO_4$), filtered, and the solvent removed. The product was purified using flash chromatography with an elution gradient of $CHCl_3$/petroleum ether and was recovered in 51% yield as white solid (324 mg). T_f = 79°C, $[\alpha]_D^{22}$ = +108.13 (c 3.0; $CHCl_3$), R_f = 0.52 (AcOEt/EP; 1/3; V/V), IR: 2987 (CH), 2107 (N_3), 1727 (C=O ester), 1601 (C=C aromatic), 1451 (C-C), 1272 (C-O-C), MS (IC): m/z = 337 (MNH_4^+), m/z = 320 (MH^+) m/z = 292 (MH^+- N_2), m/z = 279 (MNH_4^+- C_3H_6O), m/z = 262 (MH^+- C_3H_6O), RMN ^{1}H ($CDCl_3$, δ): 5.85 (d, 1H, $J_{1,2}$ = 3.7 Hz, H_1), 4.79 (dd, 1H, $J_{2,1}$ = 3.7 Hz, $J_{3,2}$ = 4.4 Hz, H_2), 4.68 (dd, 1H, $J_{5a,4}$ = 3.2 Hz, $J_{5a,5b}$ = 12.3 Hz, H_{5a}), 4.48 (dd, 1H, $J_{5b,4}$ = 4.4 Hz, $J_{5b,5a}$ = 12.3 Hz, H_{5b}), 4.39 (ddd, 1H, $J_{4,5a}$ = 3.2 Hz, $J_{4,5b}$ = 4.4 Hz, $J_{4,3}$ = 9.6 Hz, H_4), 3.46 (dd, 1H, $J_{3,2}$ = 4.4 Hz, $J_{3,4}$ = 9.6 Hz, H_3), *isopropylidene*:1.61 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), *benzoyl*: 8.05 (dd, 2H, J = 1.3 Hz, J = 8.4 Hz), 7.58 (tt, 1H, J = 1.3 Hz, J = 7.4 Hz), 7.45 (br t, 2H, J = 7.9 Hz).

1,2-di-O-Acetyl-3-azido-5-O-benzoyl-3-deoxy-D-ribofuranose (6). To a solution of 0.3 g of 3-azido-5-*O*-benzoyl-3-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranose we added 9 mL of TFA at 60%. The mixture was stirred over 2.5 hours and evaporated to dryness. The residue was stirred with 0.085 g of sodium acetate (1.1 eq.) and 0.532 mL of acetic anhydride (6 eq.) under microwave irradiation over 6 minutes (P: 48 W). We added 20 mL of CH_2Cl_2 and the organic phase was washed with a saturated $NaHCO_3$ solution. The aqueous phase was extracted with CH_2Cl_2 (2 × 30 mL) and the organic layer was washed with water (2 × 20 mL) and dried ($MgSO_4$), filtered, and the solvent removed. The product was purified using flash chromatography with an elution gradient of AcOEt/petroleum ether and was recovered in 77.6% yield on two steps as a viscous oil (265 mg) with (β/α; 85/15). R_f = 0.58 (AcOEt/EP; 1/2; V/V), IR: 2113 (N_3), 1752 (C=O ester), 1601 (C=C), 1272 (C-O-C), RMN ^{1}H ($CDCl_3$, δ): *anomer* β: 6.16 (br s, 1H, H_1), 5.37 (d, 1H, $J_{2,3}$ = 4.7 Hz, H_2), 4.67 (dd, 1H, $J_{5a,4}$ = 3.8 Hz, $J_{5a,5b}$ = 12.2 Hz, H_{5a}), 4.60 (dd, 1H, $J_{5b,4}$ = 3.8 Hz, $J_{5b,5a}$ = 12.2 Hz, H_{5b}), 4.37 (q, 1H, J = 3.8 Hz, H_4), 4.22 (dd, 1H, $J_{3,2}$ = 4.7 Hz, $J_{3,4}$ = 3.8 Hz, H_3), *anomer* α: 6.46 (d, 1H, $J_{1,2}$ = 4.5 Hz, H_1), 5.29 (dd, 1H,, $J_{2,1}$ = 4.5 Hz, $J_{2,3}$ = 7.6 Hz, H_2), 4.54 (dd, 1H, $J_{5a,4}$ = 4.8 Hz, $J_{5a,5b}$ = 13.1 Hz, H_{5a}), 4.44–4.48 (m, 2H, H_4 and H_{5a}), 4.47 (dd, 1H, $J_{5b,4}$ = 4.6 Hz, $J_{5b,5a}$ = 12.2 Hz, H_{5b}), 4.19 (dd, 1H, $J_{3,4}$ = 3.9 Hz, $J_{3,2}$ = 7.6 Hz, H_3), *acetyle:* 2.19 (s, 3H, CH_3), 1.92 (s, 3H, CH_3), *benzoyl*: 8.08 (dd, 2H, J = 1.2 Hz, J = 7.9 Hz), 7.59 (tt, 1H, J = 1.2 Hz, J = 7.9 Hz), 7.46 (t, 2H, J = 7.9 Hz).

1-(4-(2-O-Acetyl-3-azido-5-O-benzoyl-3-deoxy-D-ribofuranosyloxy)butyl)-thymine (7a). To a dry system 1,2-di-*O*-acetyl-3-azido-5-*O*-benzoyl-3-deoxy-D-ribofuranose (130 mg, 0.358 mmol) in 5 mL of acetonitrile were added 1-(4-hydroxybutyl)-thymine (71 mg, 1 eq.) and tin (IV) chloride (0.083 mL; 2 eq.). The reaction mixture was kept for 2 hours at room temperature and the solution was quenched with the addition of a saturated $NaHCO_3$ solution and extracted with CH_2Cl_2 (3 × 15 mL). The organic layer was dried ($MgSO_4$), filtered, and the solvent removed. The product was purified using flash chromatography with an elution gradient of CH_2Cl_2/MeOH and was recovered in 74% yield (132 mg). R_f = 0.5 (CH_2Cl_2/MeOH; 95/5; V/V), IR: 3193 (NH), 2111 (N_3), 2929 (CH alkyl), 1685 (C=O Thym), 1272 (C-O-C), RMN ^{1}H ($CDCl_3$, δ): *thymine:* 8.29 (s, 1H, NH), 6.93 (q, 1H, $J_{6,CH3}$ = 1.1 Hz, H_6), 1.91 (d, 3H, $J_{CH3,6}$ = 1.1 Hz, CH_3), *N-alkyl*: 3.72 (dt, 1H, $J_{\alpha,\beta}$ = 6.4 Hz, $J_{\alpha,\alpha}$ = 9.8 Hz, H_α), 3.62 (t, 2H, $J_{\delta,,\gamma}$ = 7.4 Hz, H_δ), 3.39 (dt, 1H, $J_{\alpha,\beta}$ = 6.4 Hz, $J_{\alpha,\alpha}$ = 9.8 Hz, H_α), 1.65 (m, 2H, H_β ou Hγ), 1.48 (m, 2H, H_β ou Hγ), *ose*: 5.25 (d, 1H, $J_{2',3'}$ = 4.6 Hz, $H_{2'}$), 4.98 (br s, 1H, $H_{1'}$), 4.60 (dd, 1H, $J_{5'a,4'}$ = 4.4 Hz, $J_{5'a,5'b}$ = 11.8 Hz, $H_{5'a}$), 4.42 (dd, 1H, J = 4.4 Hz, J = 11.8 Hz, $H_{5'b}$), 4.35 (dt, 1H, $J_{4',5'}$ = 4.4 Hz, $J_{4',3'}$ = 8.1 Hz, $H_{4'}$), 4.16 (dd, 1H, $J_{3',2'}$ = 4.6 Hz, $J_{3',4'}$ = 8.1 Hz, $H_{3'}$), *benzoyl*: 8.07 (dd, 2H, J = 1.3 Hz, J = 8.4 Hz), 7.59 (tt, 1H, J = 1.3 Hz, J = 7.4 Hz), 7.45 (br t, 2 H, J = 7.9 Hz), *acetyl*: 1.60 (s, 3H, CH_3).

1-(3-(2-O-Acetyl-3-azido-5-O-benzoyl-3-deoxy-D-ribofuranosyloxy) propyl)-thymine (7b). Compound **7b** was prepared according to the procedure described for **7 a** starting from 1-(3-hydroxypropyl)-thymine (64 mg, 0.349 mmol) and **6** (127 mg, 1 eq.). Yield: 72% (122 mg), $R_f = 0.53$ (CH_2Cl_2/ MeOH; 95/5; V/V), IR: 3184 (NH), 2929 (CH alkyl), 2109 (N_3), 1682 (C=O Thym), 1282 (C-O-C), RMN ^{1}H ($CDCl_3$, δ): *thymine:* 8.27 (s, 1H, NH), 6.93 (q, 1H, $J_{6,CH3} = 1.1$ Hz, H_6), 1.91 (d, 3H, $J_{CH3,6} = 1.1$ Hz, CH_3), *N-alkyl*: 3.83 (dt, 1H, $J_{\alpha,\beta} = 5.1$ Hz, $J_{\alpha,\alpha} = 9.8$ Hz, H_α), 3.73 (t, 2H, $J_{\gamma,\beta} = 7.4$ Hz, H_γ), 3.46 (dt, 1H, $J_{\alpha,\beta} = 4.9$ Hz, $J_{\alpha,\alpha} = 9.8$ Hz, H_α), 1.67 (m, 2H, H_β), *ose*: 5.24 (d, 1H, $J_{2',3'} = 4.6$ Hz, $H_{2'}$), 4.98 (br s, 1H, $H_{1'}$), 4.60 (dd, 1H, $J_{5'a,4'} = 4.4$ Hz, $J_{5'a,5'b} = 11.8$ Hz, $H_{5'a}$), 4.41 (dd, 1H, $J = 4.4$ Hz, $J = 11.8$ Hz, $H_{5'b}$), 4.35 (dt, 1H, $J_{4',5'} = 4.4$ Hz, $J_{4',3'} = 8.1$ Hz, $H_{4'}$), 4.16 (dd, 1H, $J_{3',2'} = 4.6$ Hz, $J_{3',4'} = 8.1$ Hz, $H_{3'}$), *benzoyl*: 8.07 (dd, 2H, $J = 1.2$ Hz, $J = 8.4$ Hz), 7.59 (tt, 1H, $J = 1{,}2$ Hz, $J = 7.4$ Hz), 7.46 (br t, 2H, $J = 7.8$ Hz), *acetyl*: 1.60 (s, 3H, CH_3).

1-(3-(2-O-Acetyl-3-azido-5-O-benzoyl-3-deoxy-D-ribofuranosyloxy)ethyl)-thymine (7c). Compound **7c** was prepared according to the procedure described for **7a** starting from 1-(3-hydroxyethyl)-thymine (80 mg, 0.468 mmol) and **6** (170 mg, 1 eq.). Yield: 76% (168 mg), $R_f = 0.55$ (CH_2Cl_2/ MeOH; 95/5; V/V), IR: 3189 (NH), 2925 (CH alkyl), 2109 (N_3), 1689 (C=O Thym), 1272 (C-O-C), RMN ^{1}H ($CDCl_3$, δ): *thymine:* 8.37 (s, 1H, NH), 6.93 (q, 1H, $J_{6,CH3} = 1.1$ Hz, H_6), 1.91 (d, 3H, $J_{CH3,6} = 1.1$ Hz, CH_3), *N-alkyl*: 3.84 (m, 2H, H_α and H_β), 3.64 (ddd, 1H, $J_{\alpha,\beta} = 2.9$ Hz, $J_{\alpha,\beta} = 4.6$ Hz, $J = 10.1$ Hz, H_α or H_β), 3.58 (ddd, 1H, $J_{\alpha,\beta} = 2.9$ Hz, $J_{\alpha,\beta} = 4.3$ Hz, $J = 10.3$ Hz, H_α or H_β), *ose*: 5.23 (d, 1H, $J_{2',3'} = 4.8$ Hz, $H_{2'}$), 4.98 (br s, 1H, $H_{1'}$), 4.61 (dd, 1H, $J_{5'a,4'} = 3.7$ Hz, $J_{5'a5'b} = 11.7$ Hz, $H_{5'a}$), 4.40 (dd, 1H, $J_{5'b4'} = 4.9$ Hz, $J_{5'a5'b} = 11.7$ Hz, $H_{5'b}$), 4.35 (m,1H, $H_{4'}$), 4.12 (dd, 1H, $J_{3',2'} = 4.8$ Hz, $J_{3',4'} = 7.6$ Hz, $H_{3'}$), *benzoyl*: 8.06 (dd, 2H, $J = 1.2$ Hz, $J = 8.4$ Hz), 7.60 (tt, 1H, $J = 1.2$ Hz, $J = 7.4$ Hz), 7.47 (br t, 2H, $J = 7.8$ Hz), *acetyl*: 1.88 (s, 3H, CH_3).

1-(4-(3-Azido-3-deoxy-β-D-ribofuranosyloxy)butyl)-thymine (8a). Compound **7a** (130 mg, 0.26 mmol) was stirred with methanolic ammonia (7 N) (50 eq., 1.85 mL) in 3 mL of solvent mix: methanol/CH_2Cl_2 (3/1) at room temperature during 48 hours. The solvent was removed under reduced pressure and the crude residue was purified by preparative thin layer chromatography on silica gel (CH_2Cl_2/ EtOH) to yield compound **8 a** as a viscous oil in 70% (63 mg), $[\alpha]_D^{22} = -15.897^\circ$ (c 0.26; CH_3OH), $R_f = 0.46$ (CH_2Cl_2/MeOH; 9/1;V/V), IR: 3406 (OH), 2107 (N_3), 3021 (CH ar.), 2946 (CH alkyl), 1676 (C=O Thym), 1451 (C-C), 1273 (C-O-C), RMN ^{1}H (CD_3OD, δ): *thymine:* 7.42 (q, 1H, $J_{6,CH3} = 0.9$ Hz, H_6), 1.86 (d, 3H, $J_{CH3,6} = 0.9$ Hz, CH_3), *N-alkyl*: 3.74–3.76 (m, 1H, H_α), 3.65 (t, 2H, $J_{\delta,\gamma} = 7.6$ Hz, H_δ), 3.41 (dt, 1H, $J_{\alpha,\beta} = 6.2$ Hz, $J_{\alpha,\alpha} = 9.6$ Hz, H_α), 1.73 (quin, 2H, $J = 7.6$ Hz, Hγ), 1.58 (tt, 2H, $J_{\beta,\alpha} = 6.2$ Hz, $J_{\beta,\gamma} = 7.6$ Hz, H_β), *ose*: 4.86 (br s, 1H, $H_{1'}$), 4.12 (br d, 1H, $J_{2',3'} = 4.6$ Hz, $H_{2'}$), 4.06 (dt, 1H, $J_{4',5'} = 5.5$

Hz, $J_{4',3'} = 7.7$ Hz, $H_{4'}$), 3.78 (dd, 1H, $J_{3',2'} = 4.6$ Hz, $J_{3',4'} = 7.7$ Hz, $H_{3'}$), 3.66 (dd, 1H, $J_{5'a,4'} = 5.5$ Hz, $J_{5'a,5'b} = 12.3$ Hz, $H_{5'a}$), 3.60 (dd, 1H, $J_{5'b,4'} =$ 5,5 Hz, $J_{5'b,5'a} = 12,3$ Hz, $H_{5'b}$), RMN ^{13} C (CD_3OD): *thymine*: 167.06 (C-4), 153.13 (C-2), 111.29 (C-5), 143.32 (C-6), 12.32 (CH3); *ose*: 108.91 (C-1′), 82.39 (C-4′), 77.27 (C-2′), 64.74 (C-5′), 63.77 (C-3′); *N-alkyl*: 68.37 (C-α), 49.26 (C-δ), 27.63 (C-β), 27.08 (C-γ).

1-(3-(3-Azido-3-deoxy-β -D-ribofuranosyloxy)propyl)-thymine (8b). Compound **8b** was prepared according to the procedure described for **8 a** starting from **7b** (100 mg, 0.205 mmol) and stirred with 1.47 mL of methanolic ammonia (7 N) (50 eq.). Yield: 68% (45 mg), $[\alpha]_D^{22} = +9.933°$ (c 0.4; CH_3OH), $R_f = 0.57$ (CH_2Cl_2/MeOH; 9/1;V/V), IR: 3363 (OH), 2931 (CH alkyl), 2108 (N_3), 1678 (C=O Thym), 1473 (C-C), 1259 (C-O-C), RMN ^{1}H (CD_3OD, δ): *thymine:* 7.43 (q, 1H, $J_{6,CH3} = 1.1$ Hz, H_6), 1.87 (d, 3H, $J_{CH3,6} = 1.1$ Hz, CH_3), *N-alkyl*: 3.76–3.80 (m,1H, H_α), 3.81 (t, 2H, $J_{\gamma,\beta} = 6.3$ Hz, Hγ), 3.44 (dt, 1H, $J_{\alpha,\beta} = 5.8$ Hz, $J_{\alpha,\alpha} = 10.2$ Hz, H_α), 1.92 (quin, 2H, $J = 6.3$ Hz, H_β); *ose*: 4.84 (br s, 1H, $H_{1'}$), 4.12 (br d, 1H, $J_{2',3'} = 4.6$ Hz, $H_{2'}$), 4.06 (ddd, 1H, $J_{4',5} = 4.3$ Hz, $J_{4',5'} = 5.3$ Hz, $J_{4',3'} = 7.8$ Hz, $H_{4'}$), 3.78–3.80 (m, 1H, $H_{3'}$), 3.68 (dd, 1H, $J_{5'a,4'} = 4.3$ Hz, $J_{5'a,5'b} = 11.8$ Hz, $H_{5'a}$), 3.60 (dd, 1H, $J_{5'b,4'} = 5.3$ Hz, $J_{5'b,5'a} = 11.8$ Hz, $H_{5'b}$), RMN ^{13} C (CD_3OD): *thymine*: 167.11 (C-4), 153.13 (C-2), 111.09 (C-5), 143.72 (C-6), 12.32 (CH3); ose: 108.98 (C-1′), 82.44 (C-4′), 77.25 (C-2′), 64.40 (C-5′), 63.46 (C-3′); *N-alkyl*: 63.11 (C-α), 47.25 (C-γ), 29.97 (C-β).

1-(2-(3-Azido-3-deoxy-β-D-ribofuranosyloxy)ethyl)-thymine (8c). Compound **8c** was prepared according to the procedure described for **8 a** starting from **7 c** (57 mg, 0.12 mmol) and stirred with 0.86 mL of methanolic ammonia (7 N) (50 eq.). Yield: 77% (29 mg), $[\alpha]_D^{22} = +22.75°$ (c 0.4; CH_3OH), $R_f = 0.43$ (CH_2Cl_2/MeOH; 9/1;V/V), IR: 3350 (OH), 2937 (CH alkyl), 2109 (N_3), 1683 (C=O Thym), 1472 (C-C), 1259 (C-O-C), RMN ^{1}H (CD_3OD, δ): *thymine:* 7.41 (q, 1H, $J_{6,CH3} = 1.1$ Hz, H_6), 1.86 (d, 3H, $J_{CH3,6} =$ 1.1 Hz, CH_3), *N-alkyl:* 3.82–3.97 (m, 3H, H_β and H_α), 3.60 (m,1H, H_α); *ose*: 4.86 (br s, 1H, $H_{1'}$), 4.11 (br d, 1H, $J_{2',3'} = 4.6$ Hz, $H_{2'}$), 4.05 (ddd, 1H, $J_{4',5} =$ 4.6 Hz, $J_{4',5'} = 5.3$ Hz, $J_{4',3'} = 7.8$ Hz, $H_{4'}$), 3.72 (dd, 1H, $J_{3',2'} = 4.6$ Hz, $J_{3',4'} =$ 7.8 Hz, $H_{3'}$), 3.62 (dd, 1H, $J_{5'a,4'} = 5.3$ Hz, $J_{5'a,5'b} = 11.8$ Hz, $H_{5'a}$), 3.60 (dd, 1H, $J_{5'b,4'} = 4.6$ Hz, $J_{5'b,5'a} = 11.8$ Hz, $H_{5'b}$), RMN ^{13} C (CD_3OD): *thymine*: 167.11 (C-4), 153.15 (C-2), 110.74 (C-5), 144.15 (C-6), 12.28 (CH_3); *ose*: 108.83 (C-1′), 828.62 (C-4′), 77.27 (C-2′), 64.32 (C-5′), 63.46 (C-3′); *N-alkyl*: 66.50 (C-α) and (C-β).

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