SYNTHESIS OF β-D-RIBONUCLEOSIDES DERIVED FROM DIPYRAZOLO[3,4-*b*:3',4'-*d*]PYRIDIN-3-ONE SYSTEM

Alice M. R. Bernardino[a], ChristianeM. Nogueira[a], Carla M. de O. Lepesch[a], Claudia R. B. Gomes[a], Francis J. Schmitz[c], Gilberto A. Romeiro[a], Helena de S. Pereira[b], Isabel C. de P. P. Frughlhetti[b], Mara R. P. de Oliveira[a], Maria C. B. V. de Souza[a]*, Marietta Y. W. T. Lee[d], Salma A. Chaves[a] and Vitor F. Ferreira[a].

[a]Departamento de Quimica Orgânica, Instituto de Quimica, Universidade Federal Fluminense (UFF), Outeiro de São João Batista s/n°, Niterói, CEP 24020-150, R. J., Brasil
[b]Departamento de Biologia Celular e Molecular, Instituto de Biologia, Universidade Federal Fluminense (UFF), Outeiro de São João Batista s/n°, Niterói, CEP 24020-150, R. J., Brasil.
[c]Department of Chemistry and Biochemistry, University of Oklahoma, Norman, OK 73019, USA
[d]Department of Medicine, University of Miami, School of Medicine, Miami, Fl 33101, USA

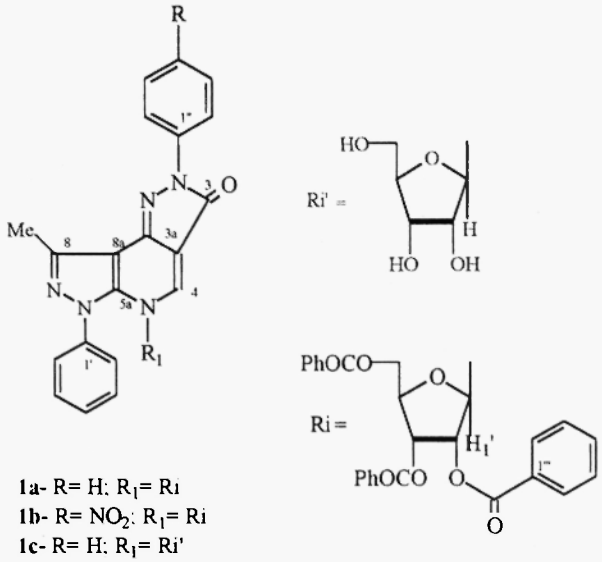
Abstract

The new ribonucleosides 8-methyl-2,6-diphenyl-5-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-2*H*,6*H*-dipyrazolo[3,4-b:3',4'-d]pyridin-3(5*H*)-one (1a) and 8-methyl-2-(p-nitrophenyl)-6-phenyl-5-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-2*H*,6*H*-dipyrazolo[3,4-b:3',4'-d]pyridin-3(5*H*)-one (1b) were prepared from stannic chloride-catalyzed ribosylations of the corresponding heterocycles 6a and 6b. Attempts of de-O-benzoylation of 1a and 1b failed to give the free ribosides 1c and 1d. The effect of compounds 1a, 1b, 6a and 6b on the catalytic activities of reverse transcriptase from recombinant immunodeficiency virus type 1 was evaluated. Compounds 1b and 6b inhibited the RNA dependent DNA polymerase activity of the enzyme by more than 50% at a concentration of 70 μM. In contrast, human placental DNA polymerase activities α and ε were unaffected by this concentration. The structures of the nucleosides are supported by 1D and 2D-nmr techniques (nOdes, HMBC and HMQC experiments).

Results and Discussion

The number of agents available for the treatment of viral infections has increased dramatically during the past decade.[1] Several currently approved antiviral drugs such as acyclovir, ganciclovir, ribavirin, AZT, ddI, ddC and d4T were obtained by synthetic methods. Most of the currently licensed antiviral substances and many of the drugs used in anticancer chemotherapy are nucleoside analogues.[2-4]

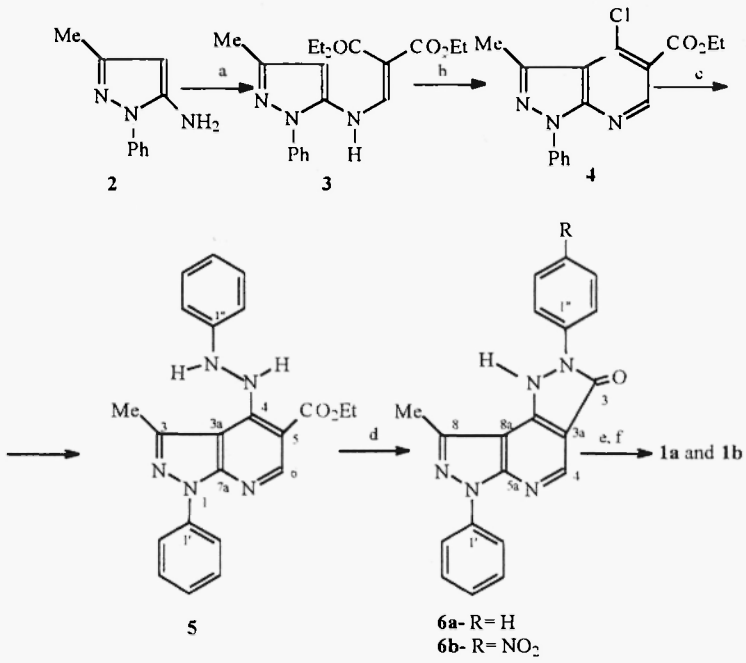
As part of an ongoing program for the synthesis of potential antiviral agents[5] one decided to investigate the preparation of new ribonucleosides having dipyrazolopyridine moieties (1).



1a- R= H; R_1= Ri
1b- R= NO_2; R_1= Ri
1c- R= H; R_1= Ri'
1d- R= NO_2; R_1= Ri'

The synthesis of these compounds was accomplished according to the reaction sequence outlined in Scheme I.

SCHEME 1

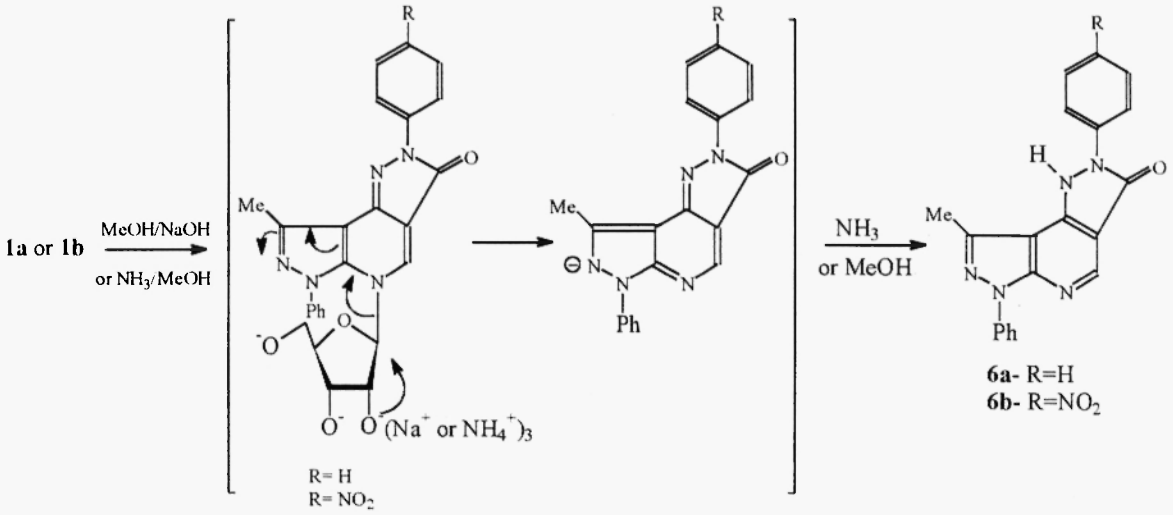


a) $EtOCH(CO_2Et)_2$ b) $POCl_3$ c) $C_6H_5NHNH_2$ or p-NO_2-$C_6H_4NHNH_2$
d) Glacial AcOH e) HMDS/TMSCl f) $SnCl_4$/1-O-acetyl-2,3,5-tri-O-benzoyl-D-ribofuranose

Reaction of phenylhydrazine with β-aminocrotononitrile[6] gave 5-amino-3-methyl-1-phenylpyrazole (2). Condensation of this 5-aminopyrazole with diethyl ethoxymethylenemalonate provided the enamine derivative 3, which was cyclized in refluxing phosphorus oxychloride to afford 5-carbethoxy-4-chloro-3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine (4).[7] Treatment of 4 with phenylhydrazine in refluxing ethanol led to N-phenyl-N'-(5-carbethoxy-3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridin-4-yl)hydrazine (5) (40% yield). When 4 was allowed to react with p-nitrophenylhydrazine, 8-methyl-2-(p-nitrophenyl)-6-phenyl-2*H*,6*H*-dipyrazolo[3,4-*b*:3',4'-*d*]pyridin-3(1*H*)-one (6b) was isolated in 38% yield. Ring closure of 5 in boiling glacial acetic acid provided 8-methyl-2,6-diphenyl-2*H*,6*H*-dipyrazolo[3,4-*b*:3',4'-*d*]pyridin-3(1*H*-)one (6a) in 93% yield.[7] Glycosylation of the silylated nitrogenated bases 6a and 6b was accomplished by a treatment with 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose in presence of anhydrous stannic chloride[8] providing the desired monoribofuranosyl derivatives 8-methyl-2,6-diphenyl-5-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-2*H*,6*H*-dipyrazolo[3,4-*b*:3',4'-*d*]pyridin-3(5*H*)-one (1a) in 26% yield, and 8-methyl-2-(p-nitrophenyl)-6-phenyl-5-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-2*H*,6*H*-dipyrazolo[3, 4-*b*:3',4'-*d*]pyridin-3(5*H*)one (1b) in 35% yield. The low yields obtained were probably due to steric hindrance effect from N5 -phenyl substituent.

Attempts of de-O-benzoylation of 1a and 1b, using methanolic sodium hydroxide[9] or methanolic amonium solutions [9] were unsuccessful leading to decomposition of the nucleosides back to the nitrogenated bases. Comparing these results with some unpublished work one can speculate that pyrazolic ring is responsible for this problem since this moeity turns the nitrogenated bases a good leaving group as indicated in the Scheme 2.

SCHEME 2



The structures of 1a and 1b are supported by HMBC and HMQC experiments. The position of glycosylation was established on the basis of crosspeak between C4 and the anomeric proton H1' ($^3J_{CH}$) in the HMBC spectrum, clearly indicating that the ribosylation occurred at N5. The anomeric configuration of compounds was assigned by one-dimensional nuclear Overhauser effect difference spectroscopy (nOeds).[10] Saturation of H1' (riboside) resulted in nuclear Overhauser enhancements of the H4' signals (1.6% and 2.3% for 1a and 1b, respectively) establishing β-configuration.

Biological Studies

Compounds 1a, 1b, 6a and 6b were evaluated for antiviral efficacy against RNA dependent DNA polymerase activity of the HIV-1 reverse transcriptase. To evaluate the potential toxicity of these substances their interactions with cellular polymerases alfa and epsilon, involved in human chromosomal DNA replication, were analyzed. The polymerization reactions for HIV RT contained 50 mM Tris-HCl (pH 7.8), 6 mM $MgCl_2$, 1mM dithiotreitol, 50 mM KCl, 1 mg/ml bovine serum albumin, 5μM dTTP, 20 μCi/ml of ^{3}HdTTP (47 Ci/mmol) and 0.26 OD/ml of oligo(rA)p(dT) templete primer (from Pharmacia). The reaction mixture was incubated at 37° for 30 minutes and the reaction was stopped by adding ice cold 5% TCA containing 20 mM sodium pyrophosphate. The precipitates were collected on Whatman GF/C filters and washed. Radioactivity was determinated by liquid scintillation. DNA polymerases alfa and epsilon activities were measured as previously

described.[11, 12] Results shown in Table 1 indicate that, with increasing amount of substances 1b and 6b, DNA polymerase activity of HIV-1 RT was gradually inhibited. Derivatives 1a and 6a, in which the nitro group was absent, proved little effect in inhibiting polymerase activity (data not shown), suggesting involvement of this group in blocking the enzymatic activity. On the other hand, all substances were ineffective in inhibiting polymerase activity of cellular DNA polymerase (data not shown).

Table 1: Effects of substances 1b, and 6b on HIV Reverse Transcriptase activity

	% polymerase inhibition of RT at			
Derivatives	25μM	50μM	70μM	100μM
1b	0	30	50	65
6b	20	52	70	75

Experimental

General- Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. The ir spectra were recorded on a Perkin-Elmer 1420 spectrometer as potassium bromide pellets and frequencies are expressed in cm^{-1}. Ultraviolet (uv) spectra were obtained on a Schimadzu spectrophotometer; λ are in nm and ε in mol^{-1} cm^{-1}. Mass spectra were obtained using VG Autospec and VG-ZAB-E spectrometers. Thin layer chromatography was carried out on prelayered silica gel plates (Sigma) or was performed with silica gel 60F-254 (Riedel de Häen), and ultraviolet light was used for visualization. The ^{1}H and ^{13}C nmr spectra were acquired on Bruker AC-300 and Varian VXR-500 instruments, in the solvents specified. Chemical shifts are reported in ppm (δ) and J values in Hz.

Proton and carbon spectra were typically obtained at ambient temperature except the HMBC spectrum for 6b which was carried out at 57° C. The two dimensional experiments were acquired using standard Varian Associates automated programs for data acquisition and processing. A Nalorac inverse detection probe was used for HMQC and HMBC experiments. Inverse detected HMBC experiments were carried out using mixing times of 83 milliseconds (J = 6 Hz) and 55 milliseconds (J = 9 Hz) with 512 t_1 increments of 122 microseconds for a spectral width of 4097.5 Hz. Nuclear Overhauser experiments were carried out at 299.94 MHz with a spectral window of 2848 Hz with a pulse flip angle of 50°, acquisition time of 1.472 seconds and 10.0 seconds relaxation delay using gated decoupling.

5-Carbethoxy-4-chloro-3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine (4). A stirred mixture of α-carbethoxy-β-(3-methyl-1-phenyl-5-pyrazolylamino)-ethyl-acrylate (3) (19.0 g, 0.055 mol) and phosphorus oxychloride (46 ml) was refluxed for 5 hours and then excess phosphorus oxychloride was removed under reduced pressure. The resulting viscous material was diluted with water, the pH was adjusted to 8 with 10%

sodium hydroxide solution. The precipitate was collected by filtration and recrystallized from ethanol to yield 10.39 g (60%) of 4, mp 110° C.[7]

N-Phenyl-N'-(5-carbethoxy-3-methyl-1-phenyl-1H-pyrazolo[3,4-*b*]pyridin-4-yl)hydrazine (5). A stirred mixture of 5-carbethoxy-4-chloro-3-methyl-1-phenyl-1H-pyrazolo[3,4-*b*] pyridine (4) (0.88 g, 0.0028 mol), phenylhydrazine (0.27 ml, 0.0027 mol) and 5 ml of ethanol was refluxed for 4 hours. After cooling , the resulting solid was separated by filtration and recrystallized from ethanol to afford 0.42 g (40%) of 5, mp 181-182° C.[7]

8-Methyl-2,6-diphenyl-2*H*,6*H*-dipyrazolo[3,4-*b*:3',4'-*d*]pyridin-3(1*H*)-one (6a). A stirred solution of N-phenyl-N'-(5-carbethoxy-3-methyl-1-phenyl-1*H*-pyrazolo[3,4-b]pyridin-4-yl)hydrazine (5) (0.59 g, 1.52 mmoles) in 10 ml of glacial acetic acid was refluxed for 4 hours. After cooling, the precipitate was collected by filtration, washed with water, and air dried to give 0.48 g (93%) of 6a as yellow crystals, mp>300° C decomp [7]; ir (potassium bromide) 1625 (C=O), uv: λ (ethanol) 348 (ε 5,613), 292 (ε 25,672); ms: m/z (relative intensity) 341 (M^+, 100), 207 (10), 77 (32); ^{1}H-nmr (DMSO-d_6, 300.13 MHz) δ 8.68 (s, 1H, H4), 8.10 (d, 2H, J = 8.0 Hz, H2' or H2"), 7.95 (d, 2H, J = 8.0 Hz, H2' or H2"), 7.60-7.50 (m, 4H, H3' and H3"), 7.41 (t, 1H, H4' or H4"), 7.30 (t, 1H, H4' or H4"), 2.75 (s, 3H, methyl); ^{13}C-nmr (DMSO-d_6, 75.4 MHz) δ 159.1 (C3), 144.6 (C4), 143.6 (C8b or C5a), 138.6 (C1' or C1"), 137.6 (C1' or C1"), 129.0 (C3' or C3"), 128.8 (C3' or C3"), 126.4 (C4' or C4"), 125.1 (C4' or C4"), 121.8 (C2' or C2"), 120.1 (C2' or C2"), 107.5 (C3a), 102.0 (C8a).

8-Methyl-(2-p-nitrophenyl)-6-phenyl-2*H*,6*H*-dipyrazolo[3,4-*b*:3',4'-*d*]pyridin-3(1*H*)-one (6b). A stirred mixture of 5-carbethoxy-4-chloro-3-methyl-1-phenyl-1H-pyrazolo[3,4-b] pyridine (4) (1.50 g, 0.0047 mol), p-nitrophenylhydrazine (0.78g, 0.0051 mol) and 10 ml of ethanol was refluxed for 57 hours. After cooling, the resulting solid was collected by filtration and was refluxed in glacial acetic acid for 4 hours to give 0.69 g (38%) of 6b as orange crystals, mp 295-298° C; ir (potassium bromide): 1640 (C=O), 1570 and 1380 (NO_2); uv: λ (ethanol) 351 (ε 5,769), 269 (3,718); high resolution ms: calcd for $C_{20}H_{14}N_6O_3$ 386.1127. Found m/z 386.1124; ^{1}H-nmr (DMSO-d_6, 300.13 MHz) δ 8.50 (s, 1H, H4), 8.38 (d, 2H., J = 7.0 Hz, H2"), 8.35 (d, 2H, J = 7.0 Hz, H3"), 7.85 (d, 2H, J = 6.0 Hz, H2'), 7.60 (t, 2H, J = 6.0 Hz, H3'), 7.45 (t, 1H, J = 6.0 Hz, H4') , 2.75 (s, 3H, methyl); ^{13}C-nmr (DMSO-d_6, 75.4 MHz) δ 160.8 (C3), 143.9 (C5a or C8b), 143.6 (C5a or C8b or C1", or C4"), 142.5 (C5a or C8b or C1", or C4"), 140.2 (C4), 137.4 (C1'), 128.9 (C3'), 127.3 (C4'), 124.2 (C3"), 122.6 (C2'), 117.7 (C2"), 107.4 (C3a), 102.3 (C8a).

8-Methyl-2,6-diphenyl-5-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-2*H*,6*H*-dipyrazolo[3,4-*b*:3',4'-*d*]pyridin-3(5*H*)-one (1a). A stirred solution of 8-methyl-2,6-diphenyl-2*H*,6*H*-dipyrazolo[3,4-*b*:3',4'-*d*]pyridin-3(1*H*)-one (6a) (0.68 g, 0.002 mol), hexamethyldisilazane (11.2 ml, 0.053 mol) and chlorotrimethylsilane (0.5 ml, 0.004 mol) was refluxed under nitrogen, for two hours. Excess reagent was removed under reduced pressure. A solution of 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (0.857 g, 0.0017 mol) in 16 ml of 1,2-dichloroethane was added to the resulting solid and the solution was stirred at room temperature for 5 minutes. A solution of anhydrous stannic chloride (0.24 ml, 0.0021 mol) in 2 ml of 1,2-dichloroethane was added, slowly,

with stirring. After 1 hour, the reaction mixture was diluted with dichloromethane (30 ml), filtered and washed with a saturated sodium bicarbonate solution (3 x 25 ml). After drying over anhydrous magnesium sulfate, the solvent was removed under reduced pressure to leave an orange oil which was purified by preparative chromatography on silica gel plates (20 x 20 cm; dichloromethane:ethyl acetate, 9:1) to give 0.347 g (26%) of 1c as a yellow solid, mp 125-126° C; ir (potassium bromide): 1726 (OC=O), 1650 (C=O); uv: λ (chloroform) 411 (ε 4,780), 283 (ε 50,750); high resolution ms (FAB) m/z : calcd for $C_{46}H_{36}N_5O_8$ (M+1) 786.2564. Found 786.2584; ^{1}H-nmr ($CDCl_3$, 300.13 MHz) nitrogenated base δ 8.48 (s, 1H, H4), 8.25 (d, 2H, H2"), 7.60-7.25 (m, 16H, H2', H3', H4', H3"), 7.18 (t, 1H, H4"), 2.75 (s, 3H, methyl), riboside δ 8.12 (d, 2H, J = 8.6 Hz, H2'"), 7.74 (d, 2H, J = 8.6 Hz, H2'"), 7.66 (d, 2H, J = 8.6 Hz, H2'"), 7.60-7.25 (m, 16H, H3'", H4'"), 6.04 (d, 1H, J = 4.0 Hz, H1'), 5.75-5.65 (m, 2H, H2' and H3'), 4.82 (dd, 1H, J = 12.0 and 4.0 Hz, H5' or H5"), 4.65 (dd, 1H, J = 12.0 and 3.0 Hz, H5' or H5"), 4.42-4.38 (m, 1H, H4'); ^{13}C-nmr ($CDCl_3$, 75.4 MHz) nitrogenated base δ 160.8 (C3), 145.3 (C8), 140.6 (C8b), 139.2 (C5a), 133.6 (C1"), 132.7 (C4), 128.6 (C3"), 124.6 (C4"), 119.6 (C2"), 114.3 (C3a), 105.0 (C8a), riboside δ 166.1 (C2'-O-C=O), 164.6 (C3'-O-C=O), 164.5 (C5'-O-C=O), 88.4 (C1'), 80.0 (C4'), 73.7 (C2'), 70.6 (C3'), 63.1 (C5'), nitrogenated base and riboside δ 133.9, 133.8, 133.4, 132.7, 130.0, 129.7, 129.5, 129.1, 128.5, 128.4, 127.0 (aromatic protonated carbons of type C2'"-C4'" and C2'-C4'), 133.2, 133.1, 129.4, 128.2 (aromatic quaternary carbons of type C1'" and C1').

8-Methyl-(2-p-nitrophenyl)-6-phenyl-5-(2,3,5-tri-O-benzoyl-β-D-ribofuranos-yl)-2*H*,6*H*-dipyr-azolo[3,4-*b*:3',4'-*d*]pyridin-3(5*H*)-one (1b). Compound 1b was obtained as described for 1a, using 0.331g (0.85 mmol) of 8-methyl-(2-p-nitrophenyl)-6-phenyl-2*H*,6*H*-dipyrazolo[3,4-*b*:3',4'-*d*]pyridin-3(1*H*)-one (6b), 5 ml (0.024 mol) of hexamethyldisilazane, 0.21 ml (0.0017 mol) of chlorotrimethylsilane, a solution of 0.364 g (0.72 mmol) of 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose in 7 ml of 1,2-dichloroethane, and a solution of 0.12 ml (0.001 mol) of stannic chloride in 1 ml of 1,2-dichloroethane. The nucleoside 1d was isolated as an orange solid (0.209 g, 35%), mp 132-135° C; ir (potassium bromide) 1725 (OC=O), 1650 (C=O), uv: λ (chloroform) 362 (ε 15,111), 270 (ε 14,256); high resolution ms (FAB) m/z: calcd for $C_{46}H_{35}N_6O_{10}$ (M+1) 831.2415. Found 831.2432; ^{1}H-nmr ($CDCl_3$, 499.84 MHz) nitrogenated base δ 8.46 (d, 2H, J = 9.0 Hz, H2"), 8.42 (s, 1H, H4), 8.31 (d, 2H, J = 9.0 Hz, H3"), 7.60-7.30 (m, 14H, H2', H3', H4'), 2.70 (s, 3H, methyl), riboside δ 8.12 (d, 2H, J= 8.0 Hz, H2'"), 7.72 (d, 2H, J = 8.0 Hz, H2'"), 7.65 (d, 2H, J = 8.0 Hz, H2'"), 7.60-7.30 (m, 14H, H3'", H4'"), 6.07 (d, 1H, J = 5.0 Hz, H1'), 5.70-5.60 (m, 2H, H2' and H3'), 4.84 (dd, 1H, J = 13.0 and 3.0 Hz, H5' or H5"), 4.67 (dd, 1H, J = 13.0 and 4.0 Hz, H5' or H5"), 4.45-4.40 (m, 1H, H4'); ^{13}C-nmr ($CDCl_3$, 125.70 MHz) nitrogenated base δ 161.71 (C3), 145.44 (C8), 144.80, 143.38, 141.90 (quaternary carbons of type C8b or C5a or C1" or C4"), 139.19 (C1" or C4"), 133.65 (C4), 124.76 (C3"), 118.30 (C2"), 113.40 (C3a), 104.85 (C8a) , riboside δ 166.11 (C2'-O-C=O), 164.71 (C3'-O-C=O), 164.53 (C5'-O-C=O), 88.52 (C1'), 80.16 (C4'), 73.86 (C2'), 70.49 (C3'), 63.03 (C5'), nitrogenated base and riboside δ 138.92, 134.12, 133.93, 133.73, 130.25,

129.78, 129.74, 129.63, 129.61, 128.84, 128.76, 128.71, 128.61, 128.55, 128.47, 128.40, 128.36, 128.31, 128.09, 127.87, 127.02 (aromatic carbons of type C1'''-C4''' and C1'-C4').

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