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Synthesis of Novel Nucleosides of 4-Oxoquinoline-3-carboxylic Acid Analogues

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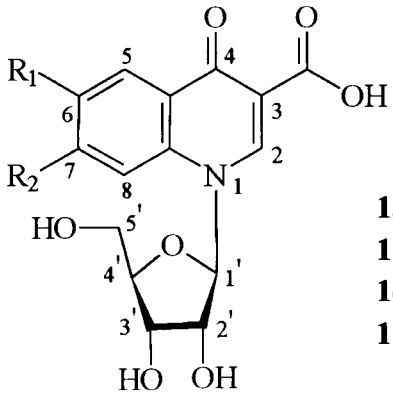
ABSTRACT: *A series of new ribonucleosides* **1a–d** *having 4-oxoquinoline-3-carboxylic acid substituted with a chloro or bromo atom in the aromatic ring, as the nitrogen base, was synthesized and examined for anti-HIV activity. Compounds* **1a** *and* **1c** *showed a modest inhibition activity on HIV-1 reverse transcriptase, inhibiting 10% of the enzyme activity at the concentration of 100* μM. © 1999 John Wiley & Sons, Inc. Heteroatom Chem 10: 197–202, 1999

INTRODUCTION

It is well known that natural and synthetic nucleoside analogues are potent chemotherapeutic agents for the treatment of HIV [1–3] infections. These observations prompted us to synthesize a series of new nucleosides **1a–d** having 4-oxoquinoline-3-carboxylic acid substituted with a chloro or bromo atom in the aromatic ring of the nitrogen base. These substances were evaluated regarding their antiviral activity.



1a- R_1= H, R_2= Cl
1b- R_1= Cl, R_2= H,
1c- R_1= H, R_2= Br
1d- R_1= Br, R_2= H

The synthetic route used for preparing compounds **1a–d** was by the reaction sequence outlined in Scheme 1.

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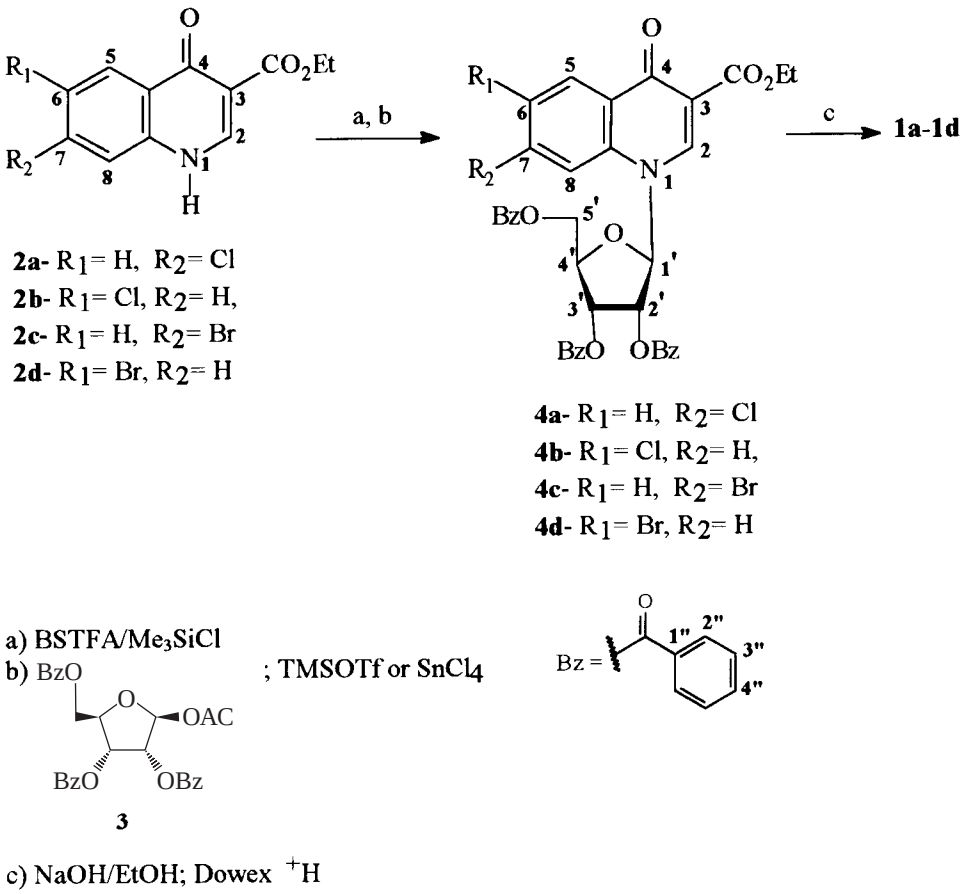

SCHEME 1 Synthetic route used for preparing nucleosides **1a–d**.

RESULTS

4-Oxoquinolines **2a–d** were prepared by known procedures [4,5] and were silylated by a slightly modified procedure that makes use of bis-(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% of trimethylchlorosilane [6]. The reactions were carried out using acetonitrile as solvent at 60–70°C, affording the corresponding trimethylsilyl derivatives that were immediately condensed with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose (**3**), in a one-pot reaction under trimethylsilyl trifluoromethanesulfonate (TMSOTf) or stannic chloride catalysis [7–9]. This procedure has given acceptable yields of the corresponding protected nucleosides: ethyl 7-chloro-1,4-dihydro-4-oxo-1-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)quinoline-3-carboxylate (**4a**), ethyl 6-chloro-1,4-dihydro-4-oxo-1-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)quinoline-3-carboxylate (**4b**), ethyl 7-bromo-1,4-dihydro-4-oxo-1-(2,3,5-tri-*O*-benzoyl-*β*-D)-ribofuranosyl) quinoline-3-carboxylate (**4c**) and ethyl 6-bromo-1,4-dihydro-4-oxo-1-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)quinoline-3-carboxylate (**4d**).

Removal of the protecting benzoyl groups was achieved by treatment with ethanolic sodium hydroxide solution at 50–60°C, producing the free nucleosides 7-chloro-1,4-dihydro-4-oxo-1-(*β*-D-ribofuranosyl) quinoline-3-carboxylic acid (**1a**), 6-chloro-1,4-dihydro-4-oxo-1-(*β*-D-ribofuranosyl)quinoline-3-carboxylic acid (**1b**), 7-bromo-1,4-dihydro-4-oxo-1-(*β*-D-ribofuranosyl)quinoline-3-carboxylic acid (**1c**), 6-bromo-1,4-dihydro-4-oxo-1-(*β*-D-ribofuranosyl)quinoline-3-carboxylic acid (**1d**) in 75%, 68%, 77% and 70% yields, respectively.

The structures of the nucleosides were supported by ^{1}H and ^{13}C NMR spectroscopy [^{1}H (Tables 1 and 2), ^{13}C (Tables 1 and 2), DEPT, ^{1}H-^{1}H-COSY, HETCOR], UV, and by FAB-HR analysis.

The stereochemistry of the anomeric carbon of nucleosides **4a–d** was assigned, by use of one-dimensional nuclear Overhauser effect difference spectroscopy. Irradiation of H1′ (riboside) resulted in n.O.e.s of H4′ signals (**4a**: 2.24%, **4b**: 3.44%, **4c**: 3.45%, **4d**:

TABLE 1 Proton (299.94 MHz) and Carbon (75.0 MHz) Chemical Shift Assignments for **4a–4d** ($CDCl_3$)

	4a		***4b***		***4c***		***4d***	
C/H	δ (^{13}C)	δ (^{1}H); (*J* Hz)	δ (^{13}C)	δ (^{1}H); (*J* Hz)	δ (^{13}C)	δ (^{1}H); (*J* Hz)	δ (^{13}C)	δ (^{1}H); (*J* Hz)
2	142.6	8.99 (s)	143.0	8.97 (s)	142.4	8.98 (s)	142.9	8.97 (s)
3	112.6	—	112.1	—	112.7	—	112.2	—
4	173.5	—	173.0	—	173.6	—	172.8	—
4a	126.8	—	131.7 or 128.6	—	127.5 or 127.1	—	*a*	—
5	129.7, 129.6 or 129.5	8.45 (d, 8.7)	127.5	8.46 (d, 2.4)	129.7	8.36 (d, 8.7)	130.6	8.64 (d, 2.1)
6	125.7	7.38 (dd, 8.7, 1.8)	131.7 or 128.6	—	128.6, 128.5 or 128.4	7.51 (dd, 8.6, 1.8)	119.4	—
7	139.2 or 139.1	—	132.7	7.46 (dd, 9.0, 2.4)	127.5 or 127.1	—	135.5	7.63–7.54 (m)
8	114.9	7.76 (d, 1.5)	116.5	7.64–7.55 (m)	117.8	7.93 (d, 1.8)	116.7	7.63–7.54 (m)
8a	139.2 or 139.1	—	136.7	—	139.3	—	137.1	—
1′	89.2	6.55 (d, 5.1)	90.3	6.48 (d, 5.1)	89.1	6.57 (d, 5.7)	90.3	6.46 (d, 5.1)
2′	75.0	5.95–5.88 (m)	74.2	6.00 (t, 5.1)	75.1	5.91 (t, 5.7)	74.1	6.02 (t, 5.4)
3′	71.3	5.95–5.88 (m)	70.7	5.91 (t, 5.4)	71.3	5.95–5.90 (m)	70.7	5.93 (t, 5.4)
4′	81.7	4.96 (q, 3.3)	81.0	4.96–4.94 (m)	81.7	4.96 (q, 3.0)	81.0	4.97–4.92 (m)
5′	63.6	4.88 (d, 2.7)	63.3	4.87 (dd, 12.5, 2.7)	63.6	4.88 (d, 3.0)	63.3	4.88 (dd, 11.7, 2.7)
1″	128.8, 128.2 or 127.7	—	128.8, 128.1, 127.7	—	128.8, 128.2, 127.7	—	*a*	—
2″	129.7, 129.6 or 129.5	8.08 (dd, 2H, 9.0, 1.5) and 7.99–7.94 (m, 4H)	129.7, 129.6, 129.5	8.09 (dd, 2H, 8.1, 1.2) and 7.96–7.91 (m, 4H)	129.6, 129.5	8.08 (dd, 2H, 8.7, 1.5) and 7.99–7.94 (m, 4H)	129.7, 129.6, 129.5	8.08 (d, 2H, 7.8, 1.5) and 7.95–7.91 (m, 4H)
3″	128.6, 128.5, 128.4	7.47–7.40 (m)	128.6, 128.5	7.44–7.36 (m)	128.6, 128.5 or 128.4	7.43–7.37 (m)	128.6, 128.4	7.47–7.36 (m)
4″	134.0, 133.8, 133.5	7.63,7.55 (m)	134.0, 133.8, 133.5	7.64–7.55 (m)	134.0, 133.8, 133.5	7.63–7.54 (m)	134.0, 133.8, 133.5	7.63–7.54 (m)
O-C=0	164.0	—	164.2	—	164.0	—	164.0	—
C1″-C=O	165.9	—	165.9	—	166.0	—	165.8	—
C1″-C=O	165.0	—	165.0	—	165.1	—	164.9	—
C1″-C=O	164.5	—	164.5	—	164.5	—	164.5	—
CH_2CH_3	60.7	4.24–4.01 (m)	60.7	4.26–4.03 (m)	60.7	4.24–4.01 (m)	60.8	4.26–4.01 (m)
CH_2CH_3	14.0	1.22 (t, 7.2)	14.1	1.27–1.21 (m)	14.0	1.22 (t, 7.2)	14.1	1.26 (t, 7.2)

[a]129.8, 128.5, 128.1, or 127.8.

TABLE 2 Proton (299.94 MHz) and Carbon (75.0 MHz) Chemical Shift Assignments for **1a–1d** (DMSO-d_6)

	1a		***1b***		***1c***		***1d***	
C/H	δ (^{13}C)	δ (^{1}H); (*J* Hz)	δ (^{13}C)	δ (^{1}H); (*J* Hz)	δ (^{13}C)	δ (^{1}H); (*J* Hz)	δ (^{13}C)	δ (^{1}H); (*J* Hz)
2	144.7	9.60 (s)	144.2	9.63 (s)	144.6	9.58 (s)	144.6	9.63 (s)
3	108.4	—	108.2	—	108.0	—	108.6	—
4	177.7	—	176.9	—	177.8	—	177.3	—
4a	124.1	—	131.4 or 126.7	—	124.3	—	127.2	—
5	128.0	8.50 (d, 8.7)	124.7	8.43 (s)	127.9	8.41 (d, 8.7)	128.3	8.58 (d, 2.4)
6	126.8	7.85 (dd, 8.7, 1.8)	131.4 or 126.7	—	129.6	7.97 (dd, 8.7, 1.5)	119.8	—
7	139.2	—	134.0	8.17 (s)	128.4	—	137.0	8.27 (dd, 9.0, 2.4)
8	117.6	8.23 (d, 1.8)	120.2	8.17 (s)	120.4	8.36 (d, 1.5)	120.6	8.10 (d, 9.3)
8a	139.8	—	137.6	—	139.8	—	138.3	—
1′	93.3	6.35 (d, 3.0)	93.3	6.34 (d, 2.7)	93.2	6.35 (d. 3.3)	93.6	6.34 (d, 2.4)
2′	75.3	4.36–4.30 (m)	75.2	4.33–4.31 (m)	75.4	4.36–4.30 (m)	75.5	4.33–4.29 (m)
3′	69.2	4.18–4.12 (m)	69.0	4.18–4.14 (m)	69.6	4.18–4.13 (m)	69.3	4.18–4.12 (m)
4′	85.7	4.28–4.24 (m)	85.5	4.27–4.24 (m)	85.7	4.27–4.24 (m)	85.7	4.26–4.24 (m)
5′a	60.0	4.01–3.94 (m)	59.8	4.00–3.96 (m)	60.0	3.99–3.93 (m)	60.1	4.05–3.94 (m)
5′b	60.0	3.85–3.76 (m)	59.8	3.82–3.78 (m)	60.0	3.83–3.77 (m)	60.1	3.84–3.76 (m)
2′OH	—	6.08 (d, 5.7)	—	6.05 (d, 5.4)	—	6.07 (d, 5.7)	—	6.04 (d, 5.1)
3′OH	—	5.46 (d, 5.7)	—	5.45 (d, 5.7)	—	5.47 (d, 6.0)	—	5.45 (d, 6.0)
4′OH	—	5.40 (t, 4.5)	—	5.40 (t, 4.5)	—	5.39 (t, 4.5)	—	5.42 (t. 4.2)
COOH	165.5	14.95 (s)	165.3	14.82 (s)	165.6	14.94 (s)	165.8	14.82

3.79%) establishing the β-configuration [10]. In addition, the H2 signal was also increased by 8.02% for (**4a**), 5.12% (**4b**), 9.20% (**4c**), and 3.99% (**4d**), confirming the position of rybosylation as N1.

ANTIVIRAL ACTIVITY

The inhibitory effect of 4-oxoquinoline ribosides **1a–d** was evaluated on the reverse transcriptase (RT) polymerase activity using recombinant enzyme of HIV-1. The sequence that expressed RT HIV-1 was introduced into an *Escherichia coli* expression plasmid pUC12N [12]. This recombinant RT was composed of 66 kDa protein. The bacteria containing this plasmid were grown for 12–16 hours with shaking at 37°C and were collected by centrifugation for 10 minutes at 10,000 rpm. The pellet was washed once with cold 100 mM NaCl, 20 mM Tris chloride, 1 mM EDTA, final pH 7.4. Bacteria were then disrupted in 0.2 mM NaCl, 20% (vol/vol) glycerol, 1% Triton X-100, 1 mM EDTA, 2 mM dithiotreitol, 25 mM Tris chloride, pH 8.0. The lysates were kept at 4°C for 30 minutes, and the insoluble material was removed by centrifugation at 10,000 rpm. The supernatant was collected and passed over Sephadex G-25 columns at 4°C pre-equilibrated with 0.2 M NaCl, 2 mM dithiotreitol, 0.2% Triton X-100, 20% glycerol, 25 mM Tris chloride, pH 7.4. After loading, the columns were washed with the same pre-equilibration buffer, and the fractions were assayed both for reverse transcriptase activity and for protein concentration. The polymerization reactions (50 μL) contained 50 mM Tris HCl (pH 7.8), 6 mM $MgCl_2$, 1 mM dithiothreitol, 50 mM KCl, 20 μM dTTP, 10 μM of [^{3}H] dTTP (47 Ci/mmol), and 150 μg/mL poly(rA)·oligo(dT) template primer (Pharmacia) and 1 U of enzyme. The reaction mixture was incubated at 37°C for 30 minutes, and the incubation was stopped by adding ice-cold 5% trichloroacetic acid (TCA) containing 20 mM of sodium pyrophosphate. The precipitates were collected on Whatman GF/C filters and washed with sodium phosphate 0.1 M. The incorporated triphosphate was measured by assaying for ^{3}H in a liquid scintillation counter. One unit of enzyme is defined as the amount of enzyme that incorporates 1 pmol of dTTP in 30 minutes at 37°C under standard assay conditions. The nucleosides **1a** and **1c** did show a modest inhibition activity on HIV-1 reverse transcriptase, inhibiting 10% of the enzyme activity at the concentration of 100 μM.

EXPERIMENTAL

Melting points were determined on a Fisher–Johns melting-point apparatus and were uncorrected. The IR spectra were recorded on a Perkin-Elmer 1420 spectrometer as potassium bromide pellets, and frequencies were expressed in cm^{-1}. Ultraviolet (UV) spectra were recorded on a Schimadzu spectrophotometer; λ in nm and ε in $mole^{-1}\ cm^{-1}$. Mass spectra were obtained using VG Autospec and VG-ZAB-E spectrometers. NMR spectra were acquired on a Var-

ian Unity Plus-300 instrument at 299.94 MHz (^{1}H) and 75.0 MHz (^{13}C) in specified solvents. Chemical shifts are reported as (δ) relative to tetramethylsilane as an internal standard.

Proton and carbon spectra were typically obtained at room temperature. The two-dimensional experiments were carried out using standard Varian Associates automated programs for data acquisition and processing. Nuclear Overhauser experiments were carried out at 299.94 Hz, with a spectral window of 4000 Hz with 45° pulse width, acquisition time of 3.744 and 5.0 seconds relaxation delay using gated decoupling.

General Procedure for Ethyl 7-Chloro-1,4-dihydro-4-oxo-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)quinoline-3-carboxylate (**4a**)*, Ethyl 6-Chloro-1,4-dihydro-4-oxo-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)quinoline-3-carboxylate* (**4b**)*, Ethyl 7-Bromo-1,4-dihydro-4-oxo-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl) Quinoline-3-carboxylate* (**4c**) *and Ethyl 6-bromo-1,4-dihydro-4-oxo-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)quinoline-3-carboxylate* (**4d**)

A stirred solution of 4-oxoquinoline **2a–d** (4 mmoles), anhydrous acetonitrile (5 mL), and BSTFA (3.0 mL, 11.2 mmoles) containing 1% of Me_3SiCl was heated at 60–70°C, under nitrogen, for 3 hours (compounds **2a** and **2c**) or 5 hours (compounds **2b** and **2d**). The resulting mixture was allowed to cool to room temperature, and a solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose (2.020 g, 4.0 mmoles) in 15 mL of acetonitrile was added, followed by dropwise addition of TMSOTf (0.4 mL, 2.09 mmoles). After having been stirred for 3 hours, at room temperature, the solution was poured into ice-cold water (20 g) and neutralized with saturated aqueous sodium bicarbonate solution. The resulting mixture was extracted with methylene chloride (3 × 20 mL), and the combined organic layers were washed with water (3 × 20 mL) and dried over anhydrous magnesium sulfate. The solvent was removed under reduced pressure to produce **4a–4d**:

4a: 2.34 g (84%) white crystals, recrystallized from ethanol, mp 162°C; UV λ_{max} ($CHCl_3$) 328 (ε 16,589), 242 (ε 49,102); IR 1715 (C=O), 1260 (C–O); HRMS (FAB) *m/z* calcd for $C_{38}H_{30}NO_{10}{}^{37}Cl$ $(M+H)^+$ 698.1607, found 698.1599; ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$), see Table 1.

4b: 2.06 g (74%), white crystals, recrystallized from ethanol, mp 163°C; UV λ_{max} ($CHCl_3$) 323 (ε 14,954), 242 (ε 46,762); IR 1720 (C=O), 1260 (C–O); HRMS (FAB) *m/z*: calcd for $C_{38}H_{30}NO_{10}{}^{37}Cl$ $(M+H)^+$ 698.1607, found 698.1593; ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$), see Table 1.

4c: 2.40 g (81%), white crystals, recrystallized from ethanol, mp 167°C, UV λ_{max} ($CHCl_3$) 329 (ε 16,427), 243 (ε 46,869); IR 1715 (C=O), 1260 (C–O); HRMS (FAB) *m/z* calcd for $C_{38}H_{30}NO_{10}{}^{81}Br$ $(M+H)^+$ 742.1111, found 742.1123; ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$), see Table 1.

4d: 2.19 g (74%), white crystals recrystallized from ethanol, mp 160°C; UV λ_{max} ($CHCl_3$) 323 (ε 11,129), 242 (ε 35,905); IR 1720 (C=O), 1260 (C–O); HRMS (FAB) *m/z* calcd for $C_{38}H_{30}NO_{10}{}^{81}Br$ $(M+H)^+$ 742.1111, found 742.1143; ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$), see Table 1.

General Procedure for Preparing 7-Chloro-1,4-dihydro-4-oxo-1-(β-D-ribofuranosyl)quinoline-3-carboxylic Acid (**1a**)*, 6-Chloro-1,4-dihydro-4-oxo-1-(β-D-ribofuranosyl)quinoline-3-carboxylic Acid* (**1b**)*, 7-Bromo-1,4-dihydro-4-oxo-1-(β-D-ribofuranosyl)quinoline-3-carboxylic Acid* (**1c**)*, 6-Bromo-1,4-dihydro-4-oxo-1-(β-D-ribofuranosyl)quinoline-3-carboxylic Acid* (**1d**)

A mixture of the protected nucleosides **4a–d** (0.67 mmol) in 0.5 N methanolic sodium hydroxide (40 mL) was stirred at 50°C (**4a**: 10 h, **4b**: 16 h, **4c**: 12 h, **4d**: 14 h). The resulting solution obtained was neutralized with Dowex 50 H^+. After filtration and evaporation, the solid was partitioned between water and ethyl ether. The aqueous solution was evaporated under reduced pressure to give **1a–d**:

1a: 176.4 mg (74%), white crystals, mp 192°C; UV λ_{max} (CH_3OH) 316 (ε 10,778), 254 (ε 23,764); IR 3660–3000 (OH), 1710 (C=0); HRMS (FAB) *m/z*: calcd for $C_{15}H_{14}NO_7{}^{37}Cl$ $(M+H)^+$ 358.0507; found 358.0518; ^{1}H-NMR (DMSO-d_6) and ^{13}C-NMR (DMSO-d_6), see Table 2.

1b: 162.1 mg, (68%), white crystals, mp 202°C; UV λ_{max} (CH_3OH) 323 (ε 10,955), 254 (ε 33,610); IR 3620–2900 (OH), 1710 (C=O); HRMS (FAB) *m/z*: calcd for $C_{15}H_{14}NO_7{}^{37}Cl$ $(M+H)^+$ 358.0506; found 358.0508; ^{1}H-NMR (DMSO-d_6), and ^{13}C-NMR (DMSO-d_6), see Table 2.

1c: 206.5 mg, (77%), white crystals, mp 194°C; UV λ_{max} (CH_3OH) 317 (ε 9472), 263 (ε 25,748); IR 3630–3100 (OH), 1710 (C=O); HRMS (FAB) *m/z*: calcd for $C_{15}H_{14}NO_7{}^{81}Br$ $(M+H)^+$ 402.0011; found 402.0068; ^{1}H-NMR (DMSO-d_6) and ^{13}C-NMR (DMSO-d_6), see Table 2.

1d: 187.7 mg (70%), white crystals, mp 189°C; UV λ_{max} (CH_3OH) 325 (ε 10,440), 219 (ε 32,840); IR 3620–3000 (OH), 1720 (C=O); HRMS (FAB) *m/z*: calcd for $C_{15}H_{14}NO_7{}^{81}Br$ $(M+H)^+$ 402.0011; found

402.0020; ^{1}H-NMR (DMSO-d_6) and ^{13}C-NMR (DMSO-d_6), see Table 2.

REFERENCES

[1] Périgaud, C.; Gosslin, G.; Imbach, J. L. Nucleosides Nucleotides 1992; 11, 903.
[2] Vera, V. M. Prog Drug Res 1997; 48, 195.
[3] de Clercq, E. Clin Microbiol Rev 1995; 8, 200.
[4] Price, C.; Robert, R. M. J Am Chem Soc 1946; 68, 1204.
[5] Duffin, G. F.; Kendall, J. D. J Am Chem Soc 1948; 893.
[6] da Matta, A. D.; Bernadino, A. M. R.; Romeiro, G. A.; de Oliveira, M. R. P.; de Souza, M. C. B. V.; Ferreira, V. F. Nucleosides Nucleotides 1996; 15, 889.
[7] Niedballa, U.; Vorbrüggen, H. J Org Chem 1974; 39, 3654.
[8] Niedballa, U.; Vorbrüggen, H. J Org Chem 1974; 39, 3668.
[9] de la Cruz, A.; Elguero, J.; Goya, P.; Martinez, A.; Clercq, E. De J Chem Soc Perkin Trans 1993; I, 845.
[10] Rosemeyer, H.; Tóth, G.; Seela, F. Nucleosides Nucleotides 1989; 8, 587.
[11] Hizi, A.; McGill, C.; Hughes, S. H. Proc Natl Acad Sci USA 1988; 85, 1218.