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Synthesis and Anti-Hepatitis B Virus Activity of Glucosylated 2-*O*-Ethyluracils

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Summary. 2-*O*-Ethyluracils were silylated with *HMDS* and condensed in the presence of *TMS*-triflate with β-D-glucose pentaacetate to give the corresponding β-nucleosides. Alternatively, these could be synthesized by nucleoside coupling of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide with the sodium salts of 2-*O*-ethyluracils, which were deprotected with saturated ammonia in methanol. 6′-*O*-Tosylate nucleoside derivatives were prepared by treating of the latter with tosyl chloride in anhydrous pyridine. The compounds thus obtained were treated with sodium azide in anhydrous *DMF* to afford the corresponding 6′-azido nucleoside derivatives, which can also be prepared by treatment with sodium azide in the presence of carbon tetrabromide and triphenylphosphine in anhydrous *DMF*. Nucleophilic displacement of the 6′-tosyloxy group by morpholine gave 6′-deoxy-6′-morpholino nucleosides. The reduction of the azido group of the 6′-azido nucleosides using triphenylphosphine in pyridine afforded the 6′-amino analogues. Glucosylated 2-*O*-ethyluracils showed moderate activity against HBV.

Keywords. Nucleosides, convergent synthesis of; 2-*O*-Ethyluracils; Human immunodeficiency virus; Glycosides; Oligonucleotides.

Introduction

Hepatitis B virus (HBV) causes acute and chronic hepatitis, which affects nearly 300 million people worldwide [1]. Chronic infection with HBV has been associated with a high risk for the development of primary hepatocellular carcinoma [2]. In such carriers vaccination is not an effective therapy and alpha interferon has demonstrated some promise [3]. Effective antiviral therapy against HBV infection has not been fully developed. Studies have been hampered by the extremely narrow host range and limited access to experimental culture systems. Nucleoside analogues and, particularly, the unnatural L-configuration have emerged as potential anti-HBV agents with more promising pharmacological and toxicological profiles [4] than their D-counterparts. Thus, 2′-fluoro-5-methyl-β-L-arabino furanosyluracil (β-L-*FMAU*) is considered as a clinical candidate for treatment of chronic HBV

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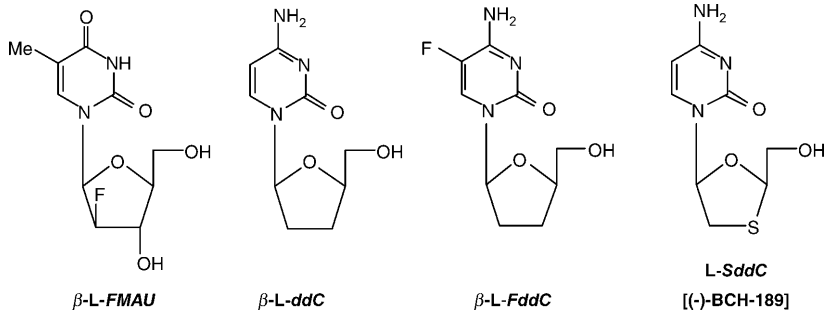


Fig. 1. Potential anti-HBV agents

infections and is undergoing preclinical toxicology studies [5]. The 2′,3′-dideoxy-β-L-cytidine (β-L-*ddC*) and its 5-fluoro analogue (β-L-*FddC*) demonstrated equally potent activity against HBV *in vitro*, having the same ED_{50} value of 0.01 μM. The unusual group of nucleosides such as L-*SddC* [(−)-BCH-189] in which the 3′-CH_2 group has been replaced by a hetero-atom [6] exhibits potent anti-HBV and HIV activity *in vitro*. In this direction, we were successful in synthesizing a series of 2-*O*-alkylated 2-uracil nucleoside derivatives [7, 8] in order to find new antiviral agents.

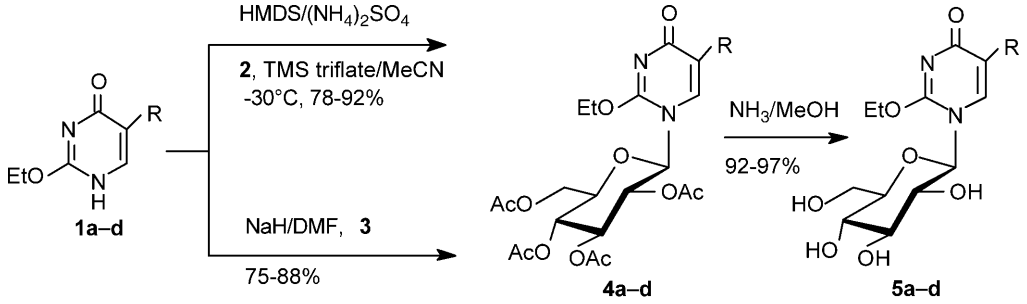
Results and Discussion

Synthesis

The 2-*O*-ethyluracils (**1a**–**d**) were prepared as described by *Hilbert* and *Jansen* [9]. Silylation of the nucleobase with 1,1,1,3,3,3-hexamethyldisilazane (*HMDS*) was carried out according to *Vorbrüggen et al.* [10] by refluxing the nucleobase in *HMDS* in the presence of catalytic amounts of ammonium sulfate. Coupling of β-D-glucose pentaacetate (**2**) with the silylated derivatives of **1a**–**d** was carried out under the *Vorbrüggen* conditions [11] to afford the β-anomers **4a**–**d**. Alternatively, **4a**–**d** could be synthesized by treatment of 2-*O*-ethyluracils (**1a**–**d**) with NaH in *DMF* at 70–80°C followed by addition of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**3**). In all cases the yields were in the range of 75–92% after purification by column chromatography on silica gel with ether:petroleum ether (1:1, v/v). Treatment of the nucleoside derivatives **4a**–**d** with saturated ammonia in methanol resulted in complete deprotection of the hydroxyl groups and the resulting products were purified by column chromatography on silica gel using 5–10% MeOH in $CHCl_3$ as eluent to give **5a**–**d** in 92–97% yields (Scheme 1).

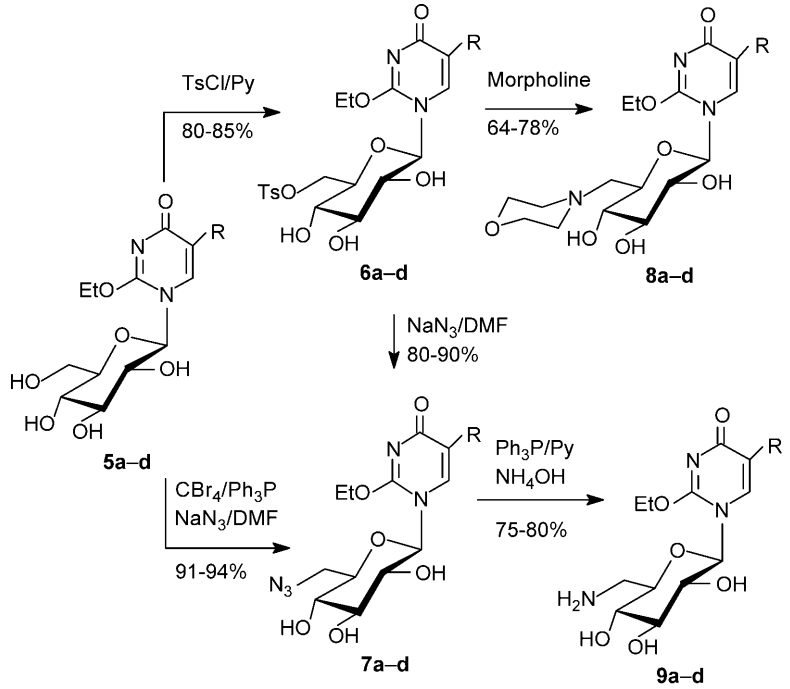
The 6′-*O*-tosylate nucleoside derivatives **6a**–**d** were prepared in 80–85% yields by treating of **5a**–**d** with tosyl chloride in dry pyridine at 0°C, which were treated with sodium azide in anhydrous *DMF* at 80°C to afford the corresponding 6′-azido derivatives **7a**–**d** in 80–90% yields. Compounds **5a**–**d** were allowed to react with sodium azide in the presence of carbon tetrabromide and triphenylphosphine in anhydrous *DMF* at room temperature to give **7a**–**d** in 91–94% yields.

Nucleophilic displacement of the 6′-tosyloxy group in **6a**–**d** by morpholine at reflux temperature gave 6′-deoxy-6′-morpholino nucleosides **8a**–**d** in 64–78% yields.



1, 4, 5	R
a	H
b	Me
c	Br
d	I

Scheme 1



5–9	R
a	H
b	Me
c	Br
d	I

Scheme 2

Table 1. Inhibition of HBV replication by selected compounds

Compd.	Inhibition/%	Cytotoxicity/%
5a	60.11	02.00
5b	66.50	02.50
5c	70.50	08.00
5d	72.13	09.01
7a	65.15	07.00
7b	78.07	01.22
7c	30.11	03.50
7d	29.50	03.50
8a	78.11	01.25
8b	79.33	02.01
8c	26.30	04.15
8d	68.33	10.00
9a	69.00	11.00
9b	25.00	04.00
9c	80.15	01.99
9d	23.18	04.50

Following the procedures reported in Ref. [12] on the utility of the azido group as a synthon for a terminal amino group in an oligonucleotide, we reduced the azido group of compounds **7a**–**d** using triphenylphosphine in pyridine and obtained the 6′-amino analogues **9a**–**d** in 75–80% yields (Scheme 2).

Testing

Preliminary viral screening against HBV indicated that compounds **5a**, **5b**, **7b**, **8a**, **8b**, and **9c** were found to be active against HBV replication with $IC_{50} = 83$, 83, 80, and 88 μM and $CC_{50} > 90$, 93, 88, and 95 μM. Compounds **7c**, **7d**, **8c**, **9b**, and **9d** showed moderate viral replication inhibition and low cytotoxicity, while compounds **5c**, **5d**, **7a**, **8d**, and **9a** showed high inhibition with high cytotoxicity (Table 1).

Conclusions

The glucosylated 2-*O*-ethyluracils can be synthesized either by condensation of the silylated bases with β-D-glucose pentaacetate or by alkylation of the sodium salt of the base derivatives with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide. 5′-Azido and 5′-amino derivatives were also synthesized in order to increase the number of tested compounds screened for anti-HBV activity.

Experimental

Pyridine was distilled from CaH_2 and stored over molecular sieves. Other solvents were purified according to the standard procedures. *TLC* was performed on plastic plates Silica Gel 60 F_{254} (E. Merck, layer thickness 0.2 mm). The detection was achieved by treatment with a solution of

15% H_2SO_4 in methanol and heating at 150°C. Concentrations were performed on a rotary evaporator at a temperature below 40°C. ^1H and ^{13}C NMR spectra were recorded on a Bruker AC 250 FT NMR spectrometer at 250 MHz for ^1H NMR and 62.9 MHz for ^{13}C NMR with *TMS* as an internal standard. EIMS and FABMS spectra were recorded with a Finnigen MAT 312/AMD. Infrared spectra were recorded in KBr pellets on a Pye Unicam SP-1025 spectrometer. The microanalyses were performed at the microanalytical unit, Universität Konstanz, Germany. Viral screening against HBV was conducted at the National Liver Institute, Menoufia University, Egypt.

General Procedure for Preparation of **4a–d**

Method A. A mixture of 2-*O*-ethyluracil derivatives **1a–d** (5 mmol), 60 mg of $(NH_4)_2SO_4$ and 40 cm^3 of 1,1,1,3,3,3-hexamethyldisilazane was refluxed (140°C) overnight. The obtained clear solution was cooled and the solvent was removed *in vacuo*. The resulting residue was dissolved in 15 cm^3 of anhydrous acetonitrile and a solution of 1.25 g of β-D-glucose pentaacetate (**2**) (3.2 mmol) in 15 cm^3 of anhydrous acetonitrile was added while stirring. The mixture was cooled to -30°C and a solution of 0.75 cm^3 of *TMS* triflate (3.9 mmol) in 5 cm^3 of anhydrous acetonitrile was added dropwise during 5 minutes. The mixture was stirred for 1 h at -30°C and then at room temperature for 1 h. The mixture was diluted with 200 cm^3 of CH_2Cl_2, washed with 150 cm^3 of a cold sat. aq. $NaHCO_3$ solution, 3×100 cm^3 of cold H_2O and dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was chromatographed on silica gel with ether:petroleum ether (1:1, v/v) to afford **4a–d** in 78–92% yields.

Method B. 2-*O*-Ethyluracil derivatives **1a–d** (5 mmol) were suspended in 25 cm^3 of anhydrous *DMF* at room temperature. To this suspension 0.26 g of NaH (50%, 5 mmol) were added and the mixture was stirred at 70–80°C for 0.5 h and cooled to room temperature. 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**3**) (2.26 g, 5.5 mmol) was added, the mixture was stirred at room temperature for 3 h until the starting material was consumed (*TLC*) and then filtered. The residue resulting from evaporation of the filtrate under reduced pressure was purified by silica gel column chromatography with ether:petroleum ether (1:1, v/v) to afford **4a–d**.

1-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-2-ethoxypyrimidin-4(1H)-one
(**4a**, $C_{20}H_{26}N_2O_{11}$)

Yield: Method A 1.35 g (90%), Method B 1.99 g (85%), foam; ^1H NMR (*DMSO-d*$_6$): $\delta = 1.40$ (t, $J = 6.9$ Hz, CH_3), 2.02, 2.03, 2.04, 2.07 (4s, 4$COCH_3$), 4.09 (dd, $J_{6',6''} = 2.0$, $J_{5',6'} = 12.2$ Hz, 6′-H), 4.22 (m, 5′-H), 4.32–4.42 (m, 6′-H, OCH_2), 5.06 (t, $J_{3',4'} = 9.5$ Hz, 4′-H), 5.16 (t, $J_{2',3'} = 9.2$ Hz, 2′-H), 5.58 (t, $J_{2',3'} = 9.3$ Hz, 3′-H), 6.44 (d, $J_{1',2'} = 8.0$ Hz, 1′-H), 6.66 (d, $J = 5.5$ Hz, 5-H), 8.47 (d, $J = 5.5$ Hz, 6-H) ppm; ^{13}C NMR (*DMSO-d*$_6$): $\delta = 14.05$ (CH_3), 20.08, 20.19 (4CO*C*H_3), 61.39 (C-6′), 62.95 (OCH_2), 67.78 (C-2′), 70.19 (C-3′), 71.18 (C-4′), 71.82 (C-5′), 92.66 (C-1′), 101.32 (C-5), 160.81 (C-6), 164.19 (C-2), 168.57 (C-4), 168.88, 169.12, 169.35, 169.74 (4*C*OCH_3) ppm; FABMS (*DMSO* + 3-nitrobenzyl alcohol): $m/z = 471$ ($M + H^+$).

1-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-2-ethoxy-5-methylpyrimidin-4(1H)-one
(**4b**, $C_{21}H_{28}N_2O_{11}$)

Yield: Method A 1.42 g (92%), Method B 2.07 g (88%), foam; ^1H NMR (*DMSO-d*$_6$): $\delta = 1.42$ (t, $J = 7.0$ Hz, CH_3), 1.77 (s, CH_3), 2.02, 2.03, 2.05, 2.07 (4s, 4$COCH_3$), 4.07 (dd, $J_{6',6''} = 2.0$, $J_{5'}{}_{,6'} = 12.1$ Hz, 6′-H), 4.25 (m, 5′-H), 4.35 (m, 6′-H), 4.53 (q, $J = 7.1$ Hz, OCH_2), 5.05 (t, $J_{3',4'} = 9.5$ Hz, 4′-H), 5.17 (t, $J_{2',3'} = 9.2$ Hz, 2′-H), 5.55 (t, $J_{2',3'} = 9.6$ Hz, 3′-H), 6.40 (d, $J_{1',2'} = 8.0$ Hz, 1′-H), 8.37 (s, 6-H) ppm; ^{13}C NMR (*DMSO-d*$_6$): $\delta = 13.65$ (CH_3), 14.05 (CH_3), 20.09, 20.19 (4CO*C*H_3), 61.35 (C-6′), 65.15 (OCH_2), 67.79 (C-2′), 70.16 (C-3′), 71.13 (C-4′), 71.80

(C-5′), 92.63 (C-1′), 117.99 (C-5), 134.95 (C-6), 160.19 (C-2), 167.17 (C-4), 168.83, 169.13, 169.30, 169.76 (4*C*OCH$_3$) ppm; FABMS (*DMSO* + 3-nitrobenzyl alcohol): $m/z = 485$ ($M + H^+$).

1-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-5-bromo-2-ethoxypyrimidin-4(1H)-one (**4c**, $C_{20}H_{25}BrN_2O_{11}$)

Yield: Method A 1.38 g (79%), Method B 2.05 g (75%), foam; ^{1}H NMR (*DMSO-d$_6$*): $\delta = 1.38$ (t, $J = 6.9$ Hz, CH$_3$), 2.02, 2.03, 2.04, 2.07 (4s, 4COCH$_3$), 4.06 (dd, $J_{6',6''} = 2.0$, $J_{5',6'} = 12.2$ Hz, 6′-H), 4.20 (m, 5′-H), 4.38 (m, 6′-H), 4.54 (q, $J = 7.0$ Hz, OCH$_2$), 5.09 (t, $J_{3',4'} = 9.5$ Hz, 4′-H), 5.19 (t, $J_{2',3'} = 9.0$ Hz, 2′-H), 5.53 (t, $J_{2',3'} = 9.1$ Hz, 3′-H), 6.47 (d, $J_{1',2'} = 8.1$ Hz, 1′-H), 8.37 (s, 6-H) ppm; ^{13}C NMR (*DMSO-d$_6$*): $\delta = 14.06$ (CH$_3$), 20.08, 20.20 (4CO*C*H$_3$), 61.36 (C-6′), 65.25 (OCH$_2$), 67.75 (C-2′), 70.22 (C-3′), 71.19 (C-4′), 71.87 (C-5′), 92.73 (C-1′), 139.30 (C-5), 159.31 (C-6), 165.59 (C-2), 168.87 (C-4), 168.86, 169.19, 169.34, 169.77 (4*C*OCH$_3$) ppm; FABMS (*DMSO* + 3-nitrobenzyl alcohol): $m/z = 549$, 551 ($M + H^+$).

1-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-2-ethoxy-5-iodo-pyrimidin-4(1H)-one (**4d**, $C_{20}H_{25}IN_2O_{11}$)

Yield: Method A 1.48 g (78%), Method B 2.29 g (77%), foam; ^{1}H NMR (*DMSO-d$_6$*): $\delta = 1.40$ (t, $J = 6.9$ Hz, CH$_3$), 2.02, 2.03, 2.04, 2.09 (4s, 4COCH$_3$), 4.04 (dd, $J_{6',6''} = 2.1$, $J_{5',6'}$ 12.1 Hz, 6′-H), 4.20 (m, 5′-H), 4.41 (m, 6′-H), 4.58 (q, $J = 7.0$ Hz, OCH$_2$), 5.01 (t, $J_{3',4'} = 9.5$ Hz, 4′-H), 5.11 (t, $J_{2',3'} = 9.2$ Hz, 2′-H), 5.58 (t, $J_{2',3'} = 9.3$ Hz, 3′-H), 6.48 (d, $J_{1',2'} = 8.0$ Hz, 1′-H), 8.37 (s, 6-H) ppm; ^{13}C NMR (*DMSO-d$_6$*): $\delta = 14.06$ (CH$_3$), 20.08, 20.17 (4CO*C*H$_3$), 61.41 (C-6′), 65.25 (OCH$_2$), 67.71 (C-2′), 70.23 (C-3′), 71.15 (C-4′), 71.79 (C-5′), 92.61 (C-1′), 138.19 (C-5), 156.21 (C-6), 165.99 (C-2), 168.87 (C-4), 168.89, 169.15, 169.35, 169.75 (4*C*OCH$_3$) ppm; FABMS (*DMSO* + 3-nitrobenzyl alcohol): $m/z = 597$ ($M + H^+$).

General Procedure for Preparation of **5a–d**

A saturated solution of ammonia in methanol (25 cm^3) was added dropwise while stirring to a solution of 0.5 g of **4a–d** in 5 cm^3 of methanol at 0°C. The mixture was stirred at room temperature for 2 h and the solvent was removed *in vacuo*. The residue was chromatographed on silica gel with 5–10% MeOH in $CHCl_3$ as eluent to give **5a–d**.

1-D-Glucopyranosyl-2-ethoxypyrimidin-4(1H)-one (**5a**, $C_{12}H_{18}N_2O_7$)

Yield: 0.30 g (95%); mp 168–170°C; ^{1}H NMR (*DMSO-d$_6$*): $\delta = 1.31$ (t, $J = 6.9$ Hz, CH$_3$), 3.26–3.31 (m, 3′-H, 4′-H, 5′-H, 6′-H), 3.48 (m, 6′-H), 3.64 (m, 2′-H), 4.31 (q, $J = 6.9$ Hz, OCH$_2$), 4.51 (t, $J = 5.7$ Hz, 6′-OH), 4.99 (d, $J = 5.1$ Hz, 4′-OH), 5.08 (d, $J = 3.8$ Hz, 3′-OH), 5.31 (d, $J = 4.6$ Hz, 2′-OH), 5.71 (d, $J_{1',2'} = 7.5$ Hz, 1′-H), 6.58 (d, $J = 5.5$ Hz, 5-H), 8.35 (d, $J = 5.5$ Hz, 6-H) ppm; ^{13}C NMR (*DMSO-d$_6$*): $\delta = 14.14$ (CH$_3$), 60.45 (C-6′), 62.73 (OCH$_2$), 69.44 (C-4′), 72.54 (C-2′), 76.51 (C-5′), 77.57 (C-3′), 96.17 (C-1′), 101.59 (C-5), 160.12 (C-6), 164.30 (C-2), 169.70 (C-4) ppm; MS: $m/z = 302$ (M^+).

1-D-Glucopyranosyl-2-ethoxy-5-methylpyrimidin-4(1H)-one (**5b**, $C_{13}H_{20}N_2O_7$)

Yield: 0.32 g (97%); mp 210–213°C; ^{1}H NMR (*DMSO-d$_6$*): $\delta = 1.39$ (t, $J = 7.0$ Hz, CH$_3$), 1.75 (s, CH$_3$), 3.29–3.33 (m, 3′-H, 4′-H, 5′-H, 6′-H), 3.45 (m, 6′-H), 3.60 (m, 2′-H), 4.30 (q, $J = 6.9$ Hz,

OCH_2), 4.54 (brs, 6′-OH), 5.01 (brs, 4′-OH), 5.12 (brs, 3′-OH), 5.33 (brs, 2′-OH), 5.73 (d, $J_{1',2'} = 7.3$ Hz, 1′-H), 8.27 (s, 6-H) ppm; ^{13}C NMR (*DMSO-d_6*): $\delta = 13.50$ (CH_3), 14.05 (CH_3), 60.43 (C-6′), 62.79 (OCH_2), 69.55 (C-4′), 72.52 (C-2′), 76.58 (C-5′), 77.67 (C-3′), 96.27 (C-1′), 117.98 (C-5), 134.90 (C-6), 161.00 (C-2), 167.04 (C-4) ppm; MS: $m/z = 316$ (M^+).

1-D-Glucopyranosyl-5-bromo-2-ethoxypyrimidin-4(1H)-one (**5c**, $C_{12}H_{17}BrN_2O_7$)

Yield: 0.32 g (93%); mp 188–190°C; ^{1}H NMR (*DMSO-d_6*): $\delta = 1.39$ (t, $J = 6.9$ Hz, CH_3), 3.16–3.28 (m, 3′-H, 4′-H, 5′-H, 6′-H), 3.44 (m, 6′-H), 3.61 (m, 2′-H), 4.30 (q, $J = 6.9$ Hz, OCH_2), 4.51 (brs, 6′-OH), 4.92 (brs, 4′-OH), 5.05 (brs, 3′-OH), 5.26 (brs, 2′-OH), 5.68 (d, $J_{1',2'} = 7.4$ Hz, 1′-H), 8.30 (s, 6-H) ppm; ^{13}C NMR (*DMSO-d_6*): $\delta = 14.01$ (CH_3), 60.41 (C-6′), 62.71 (OCH_2), 69.48 (C-4′), 72.52 (C-2′), 76.56 (C-5′), 77.60 (C-3′), 96.19 (C-1′), 139.25 (C-5), 159.40 (C-6), 166.39 (C-2), 168.79 (C-4) ppm; MS: $m/z = 380$, 382 (M^+).

1-D-Glucopyranosyl-2-ethoxy-5-iodopyrimidin-4(1H)-one (**5d**, $C_{12}H_{17}IN_2O_7$)

Yield: 0.33 g (92%); mp 223–225°C; ^{1}H NMR (*DMSO-d_6*): $\delta = 1.37$ (t, $J = 6.9$ Hz, CH_3), 3.22–3.37 (m, 3′-H, 4′-H, 5′-H, 6′-H), 3.67 (m, 6′-H), 3.70 (m, 2′-H), 4.36 (q, $J = 6.9$ Hz, OCH_2), 4.88–5.01 (m, 2′-OH, 3′-OH, 4′-OH, 6′-OH), 5.67 (d, $J_{1',2'} = 7.0$ Hz, 1′-H), 8.30 (s, 6-H) ppm; ^{13}C NMR (*DMSO-d_6*): $\delta = 14.08$ (CH_3), 60.47 (C-6′), 62.76 (OCH_2), 69.50 (C-4′), 72.55 (C-2′), 76.56 (C-5′), 77.66 (C-3′), 96.27 (C-1′), 138.29 (C-5), 156.19 (C-6), 165.90 (C-2), 168.75 (C-4) ppm; MS: $m/z = 302$ (M^+).

General Procedure for Preparation of **6a–d**

p-Toluenesulfonyl chloride (1.71 g, 10 mmol) was added to an ice-cooled solution of 10 mmol of **5a–d** in 50 cm^3 of anhydrous pyridine and left to stand overnight at 4°C. The pyridine was removed *in vacuo* and the residue was purified by column chromatography using 10% MeOH in $CHCl_3$ as eluent to give **6a–d**.

1-(6-O-p-Tolylsulfonyl-β-D-glucopyranosyl)-2-ethoxypyrimidin-4(1H)-one
(**6a**, $C_{19}H_{24}N_2O_9S$)

Yield: 3.74 g (82%); mp 189–190°C; ^{1}H NMR (*DMSO-d_6*): $\delta = 1.31$ (t, $J = 6.9$ Hz, CH_3), 2.49 (s, CH_3), 3.30–3.40 (m, 3′-H, 4′-H, 5′-H), 3.60 (m, 6′-H), 3.68 (m, 2′-H), 4.39 (q, $J = 6.9$ Hz, OCH_2), 5.02–5.12 (m, 2′-OH, 3′-OH, 4′-OH), 5.66 (d, $J_{1',2'} = 7.0$ Hz, 1′-H), 6.55 (d, $J = 5.5$ Hz, 5-H), 7.39 (d, $J = 7.8$ Hz, H_{arom}), 7.78 (d, $J = 7.8$ Hz, H_{arom}), 8.33 (d, $J = 5.5$ Hz, 6-H) ppm; ^{13}C NMR (*DMSO-d_6*): $\delta = 14.10$ (CH_3), 26.26 (CH_3), 62.75 (OCH_2), 65.40 (C-6′), 69.33 (C-4′), 72.64 (C-2′), 76.44 (C-5′), 77.51 (C-3′), 96.10 (C-1′), 101.63 (C-5), 127.88, 129.80, 132.50, 145.00 (C_{arom}), 160.06 (C-6), 164.38 (C-2), 169.71 (C-4) ppm; FABMS (*DMSO* + 3-nitrobenzyl alcohol): $m/z = 457$ ($M + H^+$).

1-(6-O-p-Tolylsulfonyl-β-D-glucopyranosyl)-2-ethoxy-5-methylpyrimidin-4(1H)-one
(**6b**, $C_{20}H_{26}N_2O_9S$)

Yield: 3.99 g (85%); mp 190–193°C; ^{1}H NMR (*DMSO-d_6*): $\delta = 1.41$ (t, $J = 7.0$ Hz, CH_3), 1.75 (s, CH_3), 2.45 (s, CH_3), 3.39–3.43 (m, 3′-H, 4′-H, 5′-H), 3.65 (m, 6′-H), 3.64 (m, 2′-H), 4.32 (q, $J = 6.9$ Hz, OCH_2), 5.00 (brs, 4′-OH), 5.10 (brs, 3′-OH), 5.23 (brs, 2′-OH), 5.79 (d, $J_{1',2'} = 7.0$ Hz, 1′-H), 7.33 (d, $J = 7.8$ Hz, H_{arom}), 7.81 (d, $J = 7.8$ Hz, H_{arom}), 8.20 (s, 6-H) ppm; ^{13}C NMR (*DMSO-d_6*): $\delta = 13.43$ (CH_3), 14.01 (CH_3), 26.21 (CH_3), 62.70 (OCH_2), 66.03 (C-6′), 69.50 (C-4′), 72.44 (C-2′), 76.63 (C-5′), 77.57 (C-3′), 96.33 (C-1′), 117.93 (C-5), 127.80, 129.80, 132.53, 145.12 (C_{arom}), 134.92 (C-6), 161.09 (C-2), 167.24 (C-4) ppm; FABMS (*DMSO* + 3-nitrobenzyl alcohol): $m/z = 471$ ($M + H^+$).

1-(6-O-p-Tolylsulfonyl-β-D-glucopyranosyl)-5-bromo-2-ethoxypyrimidin-4(1H)-one
(**6c**, $C_{19}H_{23}BrN_2O_9S$)

Yield: 4.33 g (81%); mp 178–180°C; ^{1}H NMR (*DMSO-d*$_6$): $\delta = 1.44$ (t, $J = 6.9$ Hz, CH_3), 2.51 (s, CH_3), 3.26–3.37 (m, 3′-H, 4′-H, 5′-H), 3.64 (m, 6′-H), 3.51 (m, 2′-H), 4.41 (q, $J = 6.9$ Hz, OCH_2), 4.99–5.09 (m, 2′-OH, 3′-OH, 4′-OH), 5.75 (d, $J_{1',2'} = 7.2$ Hz, 1′-H), 7.30 (d, $J = 7.8$ Hz, H_{arom}), 7.80 (d, $J = 7.8$ Hz, H_{arom}), 8.35 (s, 6-H) ppm; ^{13}C NMR (*DMSO-d*$_6$): $\delta = 14.01$ (CH_3), 26.20 (CH_3), 62.76 (OCH_2), 65.81 (C-6′), 69.40 (C-4′), 72.56 (C-2′), 76.66 (C-5′), 77.69 (C-3′), 96.34 (C-1′), 127.81, 129.77, 132.55, 145.19 (C_{arom}), 139.32 (C-5), 159.88 (C-6), 166.45 (C-2), 168.83 (C-4) ppm; FABMS (*DMSO* + 3-nitrobenzyl alcohol): $m/z = 535$, 637 ($M + H^+$).

1-(6-O-p-Tolylsulfonyl-β-D-glucopyranosyl)-2-ethoxy-5-iodopyrimidin-4(1H)-one
(**6d**, $C_{19}H_{23}IN_2O_9S$)

Yield: 4.65 g (80%); mp 199–203°C; ^{1}H NMR (*DMSO-d*$_6$): $\delta = 1.38$ (t, $J = 6.9$ Hz, CH_3), 2.50 (s, CH_3), 3.25–3.36 (m, 3′-H, 4′-H, 5′-H), 3.73 (m, 6′-H), 3.73 (m, 2′-H), 4.41 (q, $J = 6.9$ Hz, OCH_2), 4.98–5.11 (m, 2′-OH, 3′-OH, 4′-OH), 5.71 (d, $J_{1',2'} = 7.0$ Hz, 1′-H), 7.35 (d, $J = 7.8$ Hz, H_{arom}), 7.77 (d, $J = 7.8$ Hz, H_{arom}), 8.30 (s, 6-H) ppm; ^{13}C NMR (*DMSO-d*$_6$): $\delta = 14.05$ (CH_3), 26.30 (CH_3), 62.77 (OCH_2), 65.45 (C-6′), 69.59 (C-4′), 72.66 (C-2′), 76.77 (C-5′), 77.96 (C-3′), 96.43 (C-1′), 127.77, 129.87, 132.59, 145.23 (C_{arom}), 138.37 (C-5), 156.34 (C-6), 165.90 (C-2), 168.70 (C-4) ppm; FABMS (*DMSO* + 3-nitrobenzyl alcohol): $m/z = 583$ ($M + H^+$).

General Procedure for Preparation of **7a–d**

Method A. Carbon tetrabromide (0.97 g, 3 mmol) was added to a mixture of 2 mmol of **5a–d**, 0.68 g of triphenylphosphine (2.6 mmol) and 0.39 g of NaN_3 (6 mmol) in 10 cm^3 of anhydrous *DMF*. The mixture was stirred at room temperature for 48 h, was quenched by addition of 5 cm^3 of methanol, and the solvent was removed *in vacuo*. The residual oil was applied to a column of silica and eluted with 6% MeOH in $CHCl_3$ to give **7a–d**.

Method B. A mixture of 5 mmol of **6a–d** and 0.32 g of NaN_3 (5 mmol) in 25 cm^3 of anhydrous *DMF* was heated for 2 h at 80°C (*TLC*). The solvent was removed under reduced pressure and the residue was triturated with ice–H_2O. A white precipitate was collected by filtration, washed with ice–H_2O and ether and dried. The products were crystallized from absolute ethanol to give **7a–d**.

1-(6-Azido-6-deoxy-β-D-glucopyranosyl)-2-ethoxypyrimidin-4(1H)-one
(**7a**, $C_{12}H_{17}N_5O_6$)

Yield: Method A 0.60 g (92%), Method B 1.39 g (85%); mp 193–195°C; IR (KBr): $\bar{\nu} = 3415$ (OH), 2119 (CN_3) cm^{-1}; FABMS (*DMSO* + 3-nitrobenzyl alcohol): $m/z = 328$ ($M + H^+$).

1-(6-Azido-6-deoxy-β-D-glucopyranosyl)-2-ethyl-5-methylpyrimidin-4(1H)-one
(**7b**, $C_{13}H_{19}N_5O_6$)

Yield: Method A 0.64 g (94%), Method B 1.53 g (90%); mp 205–207°C; IR (KBr): $\bar{\nu} = 3430$ (OH), 2127 (CN_3) cm^{-1}; FABMS (*DMSO* + 3-nitrobenzyl alcohol): $m/z = 342$ ($M + H^+$).

1-(6-Azido-6-deoxy-β-D-glucopyranosyl)-5-bromo-2-ethoxypyrimidin-4(1H)-one
(**7c**, $C_{12}H_{16}BrN_5O_6$)

Yield: Method A 0.75 g (93%), Method B 1.68 g (83%); mp 266–268°C; IR (KBr): $\bar{\nu} = 3423$ (OH), 2129 (CN_3) cm^{-1}; FABMS (*DMSO* + 3-nitrobenzyl alcohol): $m/z = 406$, 408 ($M + H^+$).

1-(6-Azido-6-deoxy-β-D-glucopyranosyl)-2-ethoxy-5-iodopyrimidin-4(1H)-one
(**7d**, $C_{12}H_{16}IN_5O_6$)

Yield: Method A 0.82 g (91%), Method B 1.80 g (80%); mp 279–282°C; IR (KBr): $\bar{\nu} = 3410$ (OH), 2125 (CN_3) cm^{-1}; FABMS (*DMSO* + 3-nitrobenzyl alcohol): $m/z = 454$ ($M + H^+$).

General Procedure for Preparation of **8a–d**

A solution of 1 mmol of **6a–d** in 4.35 cm^3 of morpholine (50 mmol) was stirred under reflux for 13 h. The solvent was removed *in vacuo* and the residue was chromatographed on silica with 5% MeOH in $CHCl_3$ to remove the impurities and then with 10–15% MeOH in $CHCl_3$ as eluent to afford **8a–d**.

1-(6-Deoxy-6-morpholino-β-D-glucopyranosyl)-2-ethoxypyrimidin-4(1H)-one
(**8a**, $C_{16}H_{25}N_3O_7$)

Yield: 0.28 g (75%), foam; ^{1}H NMR (*DMSO-d*$_6$): $\delta = 1.33$ (t, $J = 6.9$ Hz, CH_3), 2.55 (m, 6′-H), 2.80 (m, $2NCH_2$), 3.39 (m, 5′-H), 3.60 (m, 2′-H), 3.70–3.80 (m, 3′-H, 4′-H, $2OCH_2$), 4.30 (q, $J = 6.9$ Hz, OCH_2), 4.70 (brs, 3OH), 5.40 (d, $J_{1',2'} = 7.0$ Hz, 1′-H), 6.60 (d, $J = 5.5$ Hz, 5-H), 8.33 (d, $J = 5.5$ Hz, 6-H) ppm; ^{13}C NMR (*DMSO-d*$_6$): $\delta = 14.10$ (CH_3), 54.30 ($2NCH_2$), 61.25 (C-6′), 62.73 (OCH_2), 66.60 ($2OCH_2$), 66.70 (C-5′), 69.84 (C-4′), 73.56 (C-2′), 76.97 (C-3′), 96.90 (C-1′), 101.67 (C-5), 160.43 (C-6), 164.31 (C-2), 169.60 (C-4) ppm; MS: $m/z = 371$ (M^+).

1-(6-Deoxy-6-morpholino-β-D-glucopyranosyl)-2-ethoxy-5-methylpyrimidin-4(1H)-one
(**8b**, $C_{17}H_{27}N_3O_7$)

Yield: 0.30 g (78%); mp 145–147°C; ^{1}H NMR (*DMSO-d*$_6$): $\delta = 1.42$ (t, $J = 6.9$ Hz, CH_3), 1.79 (s, CH_3), 2.65 (m, 6′-H), 2.77 (m, $2NCH_2$), 3.43 (m, 5′-H), 3.56 (m, 2′-H), 3.70–3.85 (m, 3′-H, 4′-H, $2OCH_2$), 4.36 (q, $J = 6.9$ Hz, OCH_2), 4.77 (brs, 3OH), 5.44 (d, $J_{1',2'} = 7.0$ Hz, 1′-H), 8.30 (s, 6-H) ppm; ^{13}C NMR (*DMSO-d*$_6$): $\delta = 13.56$ (CH_3), 14.11 (CH_3), 54.36 ($2NCH_2$), 61.45 (C-6′), 62.77 (OCH_2), 66.80 ($2OCH_2$), 66.66 (C-5′), 69.74 (C-4′), 73.50 (C-2′), 76.99 (C-3′), 96.96 (C-1′), 118.05 (C-5), 135.11 (C-6), 161.11 (C-2), 166.90 (C-4) ppm; MS: $m/z = 385$ (M^+).

1-(6-Deoxy-6-morpholino-β-D-glucopyranosyl)-5-bromo-2-ethoxypyrimidin-4(1H)-one
(**8c**, $C_{16}H_{24}BrN_3O_7$)

Yield: 0.35 g (78%), yellow foam; ^{1}H NMR (*DMSO-d*$_6$): $\delta = 1.42$ (t, $J = 6.9$ Hz, CH_3), 2.61 (m, 6′-H), 2.67 (m, $2NCH_2$), 3.51 (m, 5′-H), 3.50 (m, 2′-H), 3.70–3.84 (m, 3′-H, 4′-H, $2OCH_2$), 4.33 (q, $J = 6.9$ Hz, OCH_2), 4.79 (brs, 3OH), 5.54 (d, $J_{1',2'} = 7.0$ Hz, 1′-H), 8.31 (s, 6-H) ppm; ^{13}C NMR (*DMSO-d*$_6$): $\delta = 14.06$ (CH_3), 54.42 ($2NCH_2$), 61.67 (C-6′), 62.92 (OCH_2), 67.23 ($2OCH_2$), 67.66 (C-5′), 69.70 (C-4′), 73.44 (C-2′), 76.87 (C-3′), 96.99 (C-1′), 139.30 (C-5), 159.43 (C-6), 166.43 (C-2), 168.60 (C-4) ppm; MS: $m/z = 450$, 452 (M^+).

1-(6-Deoxy-6-morpholino-β-D-glucopyranosyl)-2-ethoxy-5-iodopyrimidin-4(1H)-one
(**8d**, $C_{16}H_{24}IN_3O_7$)

Yield: 0.38 g (78%); mp 212–214°C; ^{1}H NMR (*DMSO-d*$_6$): $\delta = 1.37$ (t, $J = 6.9$ Hz, CH_3), 2.63 (m, 6′-H), 2.59 (m, $2NCH_2$), 3.48 (m, 5′-H), 3.59 (m, 2′-H), 3.73–3.89 (m, 3′-H, 4′-H, $2OCH_2$), 4.41 (q, $J = 6.9$ Hz, OCH_2), 4.72 (brs, 3OH), 5.74 (d, $J_{1',2'} = 7.0$ Hz, 1′-H), 8.30 (s, 6-H) ppm; ^{13}C NMR (*DMSO-d*$_6$): $\delta = 14.04$ (CH_3), 54.41 ($2NCH_2$), 61.40 (C-6′), 62.63 (OCH_2), 66.77 ($2OCH_2$, C-5′), 69.78 (C-4′), 73.62 (C-2′), 76.89 (C-3′), 96.97 (C-1′), 138.30 (C-5), 155.99 (C-6), 166.09 (C-2), 168.80 (C-4) ppm; FABMS (*DMSO* + 3-nitrobenzyl alcohol): $m/z = 498$ ($M + H^+$).

General Procedure for Preparation of **9a–d**

The azido derivatives **7a–d** (1 mmol) and 0.43 g of triphenylphosphine (1.65 mmol) were dissolved in 30 cm^3 of pyridine and stirred at room temperature for 1 h. Concentrated NH_4OH (25%) was then added and the solution was stirred for 2 h. The solvent was removed *in vacuo*, 5 cm^3 of H_2O were added, and triphenylphosphine and triphenylphosphine oxide were removed by filtration. The filtrate was extracted with benzene and with ether to remove the residual triphenylphosphine and then concentrated to dryness. Recrystallization of the solid residue from absolute ethanol gave **9a–d**.

1-(6-Amino-6-deoxy-β-D-glucopyranosyl)-2-ethoxypyrimidin-4(1H)-one
(**9a**, $C_{12}H_{19}N_3O_6$)

Yield: 0.23 g (78%); mp 115–117°C; IR (KBr): $\bar{\nu} = 3455$, 3415 (NH_2) cm^{-1}; FABMS (*DMSO* + 3-nitrobenzyl alcohol): $m/z = 302$ ($M + H^+$).

1-(6-Amino-6-deoxy-β-D-glucopyranosyl)-2-ethoxy-5-methylpyrimidin-4(1H)-one
(**9b**, $C_{13}H_{21}N_3O_6$)

Yield: 0.25 g (80%); mp 170–171°C; IR (KBr): $\bar{\nu} = 3460$, 3419 (NH_2) cm^{-1}; FABMS (*DMSO* + 3-nitrobenzyl alcohol): $m/z = 316$ ($M + H^+$).

1-(6-Amino-6-deoxy-β-D-glucopyranosyl)-5-bromo-2-ethoxypyrimidin-4(1H)-one
(**9c**, $C_{12}H_{18}BrN_3O_6$)

Yield: 0.28 g (76%); mp 193–195°C; IR (KBr): $\bar{\nu} = 3470$, 3435 (NH_2) cm^{-1}; FABMS (*DMSO* + 3-nitrobenzyl alcohol): $m/z = 380$, 382 ($M + H^+$).

1-(6-Amino-6-deoxy-β-D-glucopyranosyl)-2-ethoxy-5-iodopyrimidin-4(1H)-one
(**9d**, $C_{12}H_{18}IN_3O_6$)

Yield: 0.32 g (75%); mp 271–275°C; IR (KBr): $\bar{\nu} = 3450$, 3425 (NH_2) cm^{-1}; FABMS (*DMSO* + 3-nitrobenzyl alcohol): $m/z = 428$ ($M + H^+$).

Antiviral Assays

The hepatoplastoma cell line (Hep G2-2.2.15) was used to evaluate the antiviral effect of the tested compounds against HBV [13]. The cells were incubated in growth medium (RPMI-1640, 10% heat-inactivated fetal-calf serum (FCS)) and antibiotics at 37°C, 5% CO_2 with and without tested compounds. The average production HBV virion *DNA* from cell cultures with addition of different concentration of a tested compound was expressed relatively to HBV virion *DNA* in cultures without the tested compound. Quantitative analysis of HBV-*DNA* was done using a semiquantitative nested PCR followed by DIG PCR ELISA as previously described [14]. The cytotoxic effect of the compounds was assessed by culturing the Hep G2-2.2.15 cells in the presence of compounds as described for the viability of their cells and they were analyzed using a MTT-assay.

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