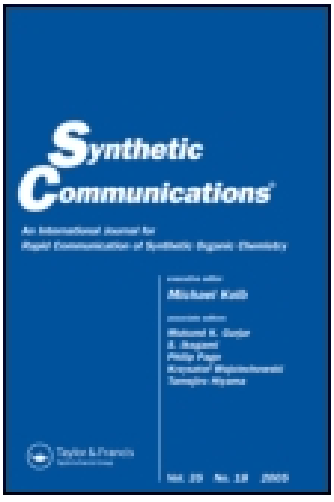


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# Synthesis of New 5-Substituted Pyrimidine Acyclonucleosides

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# Synthesis of New 5-Substituted Pyrimidine Acyclonucleosides

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**Abstract:** Acyclonucleosides of pyrimidine were prepared by condensing appropriately silylated 5-substituted pyrimidines with an acyclic side chain in the form of an acetylated haloalkoxyalcohol and subsequent removal of the protecting acetyl group in base. Also, acyclonucleosides with a 1-ethoxymethyl side chain have been investigated. The activity against hepatitis B virus (HBV) has been tested.

**Keywords:** Pyrimidine acyclonucleosides, substituted pyrimidines

The synthesis and biological evaluation of modified acyclonucleosides have been very active research areas for a number of years. Several pyrimidine acyclonucleosides are presently known as potent antiviral agents.[1–5] Among them, acyclic nucleoside of the 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio) thymine (HEPT) type has shown high selectivity toward HIV-1,[6,7] and it was found that replacement of the sulfur atom with a methylene group resulted in a new series of potent HEPT analogues, of which MKC-442 (6-benzyl-1-ethoxymethyl-5-isopropyluracil) was found to be extremely potent.[8,9] Therefore, a series of new pyrimidine acyclonucleosides are still of great interest as potential new active compounds with less prominent side effects.

We have previously reported the condensation between methyl 2-deoxy-3,5-di-*O*-toluoyl-*D*-pentofuranoside and silylated pyrimidines gave anomeric mixtures of 2′-deoxynucleosides, which can be envisaged as formal analogues of nucleic acids components.[10] As a continuation of our interest in this area,

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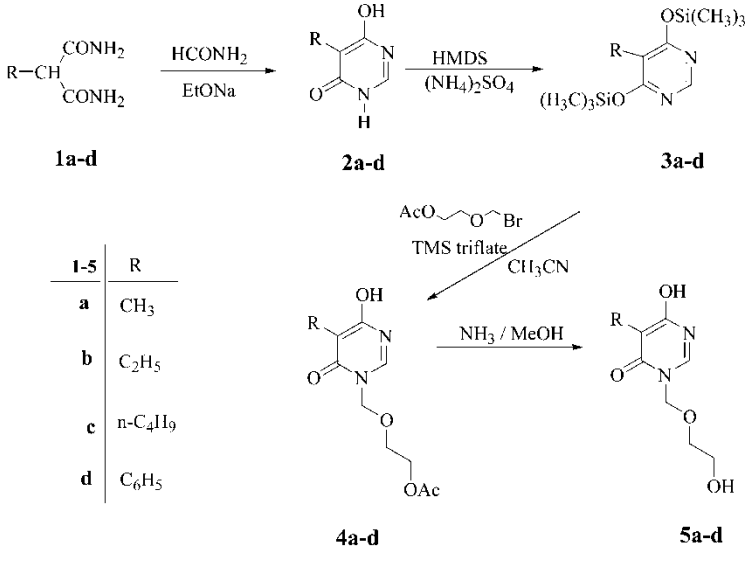
we now report the synthesis and anti-HBV activity of a new series of pyrimidine acyclonucleosides in which the carbohydrate moiety has been replaced by an acyclic side chain[11]

## RESULTS AND DISCUSSION

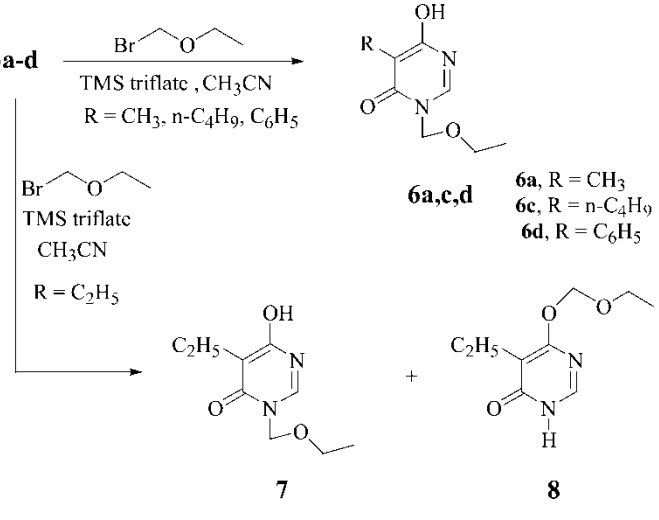
The starting 4-hydroxy-6(1H)-pyrimidinones **2a–d** have been prepared on condensation of malondiamide derivatives **1a–d** with formamide in the presence of sodium ethoxide by the method of Hull.[12] Silylation of **2a–d** with 1,1,1,3,3,3-hexamethyldisilazane (HMDS) in the presence of a catalytic amount of ammonium sulfate was performed according to Wittenburg[13] to give the derivatives **3a–d**. The latter were condensed with 2-acetoxyethoxy methyl bromide[14] using the trimethylsilyl trifluoromethanesulfonate (TMS triflate) using method of Vorbrüggen et al.[15] in anhydrous acetonitrile to give acyclonucleosides **(4a–d)** in 65–76% yield. After their chromatographic purification, treatment of these nucleosides with methanolic ammonia at room temperature for 24 h, followed by chromatographic purification, resulted in complete deprotection of the hydroxyl group, and the corresponding 1-(2-hydroxyethoxymethyl)pyrimidine derivatives **(5a–d)** were obtained in 82–90% yield (Scheme 1). The structures of the new compounds were determined on the basis of their elemental microanalyses and spectral data, which are in agreement with their proposed structures




*Scheme 1.*

Similarly, condensation of bromomethyl ethyl ether with the silylated pyrimidines **3a–b**, using TMS triflate as the Lewis acid catalyst, gives the alkylated products **6–8**. As reported in Scheme 2, *N*-alkylated products **6a,c,d** were obtained, on condensation of bromomethyl ethyl ether with **3a,c,d**, as the sole products in fairly good yields. However, in the case of alkylation of 5-ethylpyrimidine derivative **3b**, column chromatographic purification of the reaction mixture gave two products. The less polar product appeared to be the required *N*-alkylated product (30% yield). The $^{1}H$ NMR spectrum of the more polar product (25% yield) showed the presence of ethoxymethyl moiety, which shifted downfield compared with that of *N*-alkylated product **7**. The methylene singlet signal appeared at $\delta$ 5.49 ppm for the product **8**, compared to $\delta$ 5.24 ppm for *N*-alkylated product **7**. In addition, the $^{13}C$ NMR spectrum showed the chemical shift at C-1 of acyclic part of **7** at $\delta$ 74.38 ppm and that of compound **8**at $\delta$ 90.28 ppm. This indicated that the alkylation of **3b** occurred on the nitrogen atom to give *N*-alkylated product **7** and on the oxygen atom to give *O*-alkylated product **8**, respectively. The FAB-mass spectra of **7** and **8** showed the same protonated positive molecular ion peak $(M+H)^{+}$ at m/z 199. An attempt to synthesize the acyclonucleosides **4–7** by simple alkylation of the sodium salt of the pyrimidines **2a–d** with the appropriate halomethyl ethers according to the method of Sasaki et al.[16] was unsuccesful.

Preliminary viral screening against HBV indicated that compounds **4a** and **4c** showed moderate viral replication inhibition and low cytotoxicity. Compounds **5a**, **5d**, and **6a** showed high inhibition with moderate cytotixicity,




*Scheme 2.*

*Table 1.* Inhibition of HBV replication by selected compounds

| Compound (10 μM) | Inhibition (%) | | | Cytotoxicity (%) |
|---|---|---|---|---|
| | 1 week | 2 weeks | 3 weeks | |
| **4a** | 24.6 | 18.3 | 11.1 | 4.8 |
| **4c** | 26.7 | 14.6 | 9.3 | 4.6 |
| **5a** | 80.5 | 78.7 | 72.3 | 15.2 |
| **5d** | 78.7 | 75.2 | 70.4 | 13.0 |
| **6a** | 80.6 | 77.5 | 70.6 | 14.1 |
| **6c** | 79.5 | 75.4 | 73.5 | 4.2 |
| **6d** | 80.4 | 78.5 | 71.1 | 4.8 |
| **7** | 85.8 | 83.4 | 80.5 | 2.5 |
| **8** | 75.2 | 80.5 | 70.2 | 3.4 |

and compounds **6c**, **6d**, **7**, and **8** showed high inhibition with low cytotoxicity (Table 1).

## EXPERIMENTAL

Melting points were determined on a Boetius melting-point apparatus and are uncorrected. Elemental analysis were carried out in the Microanalytical Laboratories of the Faculty of Science, Cairo University. $^{1}H$ and $^{13}C$ NMR spectra were recorded on a Bruker AC 250 FT-NMR spectrometer at 250 MHz for $^{1}H$ NMR and 62.9 MHz for $^{13}C$ NMR. Mass spectra were recorded using fast atom bombardment (FAB) on a Kratos Ms 50-spectrometer. Analytical TLC plates 60 $F_{254}$ and silica gel (0.040–0.063 mm) were purchased from Merck. Anhydrous $CH_3CN$ was distilled from $P_2O_5$, followed by distillation from CaH. All other solvents were used after distillation and drying. Viral screening against HBV has been done at National Liver Institute, Monoufiya University, Shebien El-Koam, Egypt.

### General Procedure for the Preparation of Compounds (2a–c)

A mixture of methyl malondiamide **1a** or ethyl malondiamide **1b** and/or n-butyl malondiamide **1c** (10 mmol), formamide (15 mmol), and sodium ethoxide [prepared by dissolving 0.92 g (40 mmol) of sodium metal in 30 ml of ethanol] was refluxed for 12 h. The mixture was evaporated to dryness under reduced pressure, and the obtained residue was dissolved in water and acidified with 4M HCl. The solid product was filtered off, dried, and recrystallized from the proper solvent to afford **2a, 2b**, and **2c**, respectively, as white prisms in 71–83% yields.

## Data

**4-Hydroxy-5-methylpyrimidin-6(1*H*)-one (2a)**. White prisms from methanol, (yield 83%): mp >320°C (reported[17] yield 52%, mp >330°C). $^1$H NMR (DMSO-$d_6$), ($\delta$ ppm): 1.77 (s, 3H, $CH_3$), 7.93 (s, 1H, C2-H), 11.63 (sb, 1H, OH), 12.01 (sb, 1H, NH). $^{13}$C NMR (DMSO-$d_6$), ($\delta$ ppm): 7.68 ($CH_3$), 97.98 (C5), 146.30 (C2), 163.07, 164.38 (C4 and C6). Anal. cacld. for $C_5H_6N_2O_2$: C, 47.62; H, 4.80; N, 22.21. Found: C, 47.85; H, 5.12; N, 21.90.

**5-Ethyl-4-hydroxypyrimidin-6(1*H*)-one (2b).** White prisms from methanol, (yield 71%): mp >320°C. $^1$H NMR (DMSO-$d_6$), ($\delta$ ppm): 0.97 (t, 3H, $J =$ Hz, $C\underline{H}_3CH_2$), 2.31 (q, 2H, $J = 7.3$ Hz, $C\underline{H}_2CH_3$), 7.92 (s, 1H, C2-H), 11.61 (sb, 1H, OH), 11.90 (sb, 1H, NH). $^{13}$C NMR (DMSO-$d_6$), ($\delta$ ppm) 12.45 ($CH_3$), 15.49 ($CH_2$), 104.26 (C5), 146.58 (C2), 163.01, 164.06 (C4 and C6). Anal. calcd. for $C_6H_8N_2O_2$: C, 51.42; H, 5.75; N, 19.99. Found: C, 51.12; H, 5.80; N, 20.25.

**5-Butyl-4-hydroxypyrimidin-6(1*H*)-one (2c).** White prisms from ethanol, (yield 77%): mp 288–290°C (reported[18] yield 39%, mp 282°C). $^1$H NMR (DMSO-$d_6$), ($\delta$ ppm): 0.87 (t, 3H, $J = 7.0$ Hz, $CH_3$), 1.23–1.44 (m, 4H, $CH_3C\underline{H}_2C\underline{H}_2CH_2$), 2.25–2.31 (t, 2H, $J = 7.2$ Hz, $CH_3CH_2CH_2C\underline{H}_2$), 7.89 (s, 1H, C2-H), 11.60 (sb, 1H, OH), 12.20 (sb, 1H, NH). $^{13}$C NMR (DMSO-$d_6$), ($\delta$ ppm): 13.83 ($CH_3$), 21.81, 22.11, 29.70 ($3 \times CH_2$), 102.88 (C5), 146.51 (C2), 162.34, 164.28 (C4 and C6). Anal. calcd. for $C_8H_{12}N_2O_2$: C, 57.13; H, 7.19; N, 16.66. Found: C, 57.43; H, 7.54; N, 16.47.

**4-Hydroxy-5-phenylpyrimidin-6(1*H*)-one (2d)**

This compound was prepared according to ref. 12.

## General Procedure for the Preparation of Compounds (4a–d)

A mixture of pyrimidine derivatives **2a–d** (10 mmol), 1,1,1,3,3,3-hexamethyldisilazane (HMDS) (30 ml), and ammonium sulfate (30 mg) was heated under reflux for 6 h. The mixture was concentrated at room temperature under reduced pressure to obtain the silylated derivatives **3a–d** as pale yellow oil. Anhydrous MeCN (20 ml) was added, and the solution was stirred at −25°C. TMS triflate (2.35 g, 10.5 mmol) was added to the mixture followed by the dropwise addition of the 2-acetoxyethoxy methyl bromide (20 mmol) in anhydrous MeCN (5 ml). The reaction mixture was stirred at −25°C for 2 h and then at room temperature for 1 h. The reaction mixture was diluted with 200 ml of $CH_2Cl_2$ and washed with ice-cold saturated aqueous $NaHCO_3$ ($4 \times 100$ ml). The organic phase was separated, washed with cold water ($4 \times 100$ ml), dried over $Na_2SO_4$, and evaporated to obtain

the crude products, which were chromatographed on a silica-gel column using methanol/chloroform (5–10%) as eluent.

## Data

**1-[(2-Acetoxyethoxy)methyl]-4-hydroxy-5-methylpyrimidin-6(1*H*)-one (4a).** White solid (yield 68%), mp 104–106°C. $^1$H NMR (DMSO-$d_6$), ($\delta$ ppm): 1.79 (s, 3H, $CH_3$), 1.98 (s, 3H, $CH_3CO$), 3.63–3.75 (m, 2H, $CH_2$), 4.07–4.11 (m, 2H, $CH_2$), 5.28 (s, 2H, $NCH_2O$), 8.27 (s, 1H, C2-H), 11.35 (sb, 1H, OH). $^{13}$C NMR (DMSO-$d_6$), ($\delta$ ppm): 8.05 ($CH_3$), 20.46 ($\underline{C}H_3CO$), 62.83 ($CH_2$), 67.02 ($CH_2$), 74.71 ($NCH_2O$), 97.25 (C5), 149.88 (C2), 162.32 (C4), 164.85 (C6), 170.16 ($\underline{C}OCH3$). FAB MS: m/z (%) 243 (M + 1, 100), 154 (36). Anal. cacld. for $C_{10}H_{14}N_2O_5$: C, 49.58; H, 5.82; N, 11.56. Found: C, 49.32; H, 6.10; N, 11.78.

**1-[(2-Acetoxyethoxy)methyl]-4-hydroxy-5-ethylpyrimidin-6(1*H*)-one (4b).** White solid (yield 65%), mp 106–108°C,. $^1$H NMR ($CDCl_3$), ($\delta$ ppm): 1.12 (t, 3H, $J = 7.3$ Hz, $C\underline{H}_3CH_2$), 2.08 (s, 3H, $CH_3CO$), 2.51 (q, 2H, $J = 7.3$ Hz, $C\underline{H}_2CH_3$), 3.85–3.89 (m, 2H, $CH_2$), 4.20–4.24 (m, 2H, $CH_2$), 5.26 (s, 2H, $NCH_2O$), 8.07 (s, 1H, C2-H), 11.32 (sb, 1H, OH). $^{13}$C NMR ($CDCl_3$), ($\delta$ ppm): 11.92 ($CH_3$), 16.15 ($CH_2$), 20.56 ($\underline{C}H_3CO$), 62.63 ($CH_2$), 68.22 ($CH_2$), 75.46 ($NCH_2O$), 106.31 (C5), 147.42 (C2), 162.29 (C4), 164.08 (C6), 170.60 ($CH_3CO$). FAB MS: m/z (%) 257 (M + 1, 100), 154 (47). Anal. calcd. for $C_{11}H_{16}N_2O_5$: C, 51.56; H, 6.29; N, 10.93. Found: C, 51.85; H, 6.50; N, 11.22.

**1-[(2-Acetoxyethoxy)methyl]-4-hydroxy-5-n-butylpyrimidin-6(1*H*)-one (4c).** White solid (yield 65%), mp 78–80°C,. $^1$H NMR ($CDCl_3$), ($\delta$ ppm): 0.86–0.91 (t, 3H, $J = 7.0$ Hz, $C\underline{H}_3CH_2CH_2$), 1.21–1.41 (m, 4H, $CH_3C\underline{H}_2C\underline{H}_2CH_2$), 1.96 (s, 3H, $C\underline{H}_3CO$), 2.23–2.30 (t, 2H, $J = 6.8$ Hz, $CH_3CH_2CH_2C\underline{H}_2$), 3.64–3.73 (m, 2H, $CH_2$), 4.12–4.23 (m, 2H, $CH_2$), 5.24 (s, 2H, $NCH_2O$), 8.26(s, 1H, C2-H), 11.25 (sb, 1H, OH). $^{13}$C NMR ($CDCl_3$), ($\delta$ ppm): 13.66 ($CH_3$), 20.43 ($\underline{C}H_3CO$), 22.01, 22.15, 28.46 (3 × $CH_2$), 62.81 (CH2), 68.11 ($CH_2$), 74.37 ($NCH_2O$), 103.88 (C5), 146.43 (C2), 161.73, 163.64 (C6), 170.35 ($\underline{C}OCH3$). FAB MS: m/z (%) 285 (M + 1, 100), 154 (32). Anal. calcd. for $C_{13}H_{20}N_2O_5$: C, 54.92; H, 7.09; N, 9.85. Found. C, 55.13; H, 7.21, N, 10,12.

**1-[(2-Acetoxyethoxy)methyl]-4-hydroxy-5-phenylpyrimidin-6(1*H*)-one (4d).** White solid (yield 76%), mp 136–138°C,. $^1$H NMR ($CDCl_3$), ($\delta$ ppm): 2.02 (s, 3H, $CH_3$), 3.81–3.85 (m, 2H, $CH_2$), 4.15–4.18 (m, 2H, $CH_2$), 5.33 (s, 2H, $NCH_2O$), 7.30–7.63 (m, 5H, Ar-H), 8.04 (s, 1H, $C_2$-H), 11.46 (sb, 1H, OH). $^{13}$C NMR ($CDCl_3$), ($\delta$ ppm): 20.56 ($CH_3$), 62.78 ($CH_2$), 68.45 ($CH_2$), 75.81 ($NCH_2O$), 104.63 (C5), 127.52, 127.80, 130.09, 130.49 (Ar-C), 148.68 (C2), 161.31 (C4), 163.84 (C6), 170.73 ($\underline{C}OCH_3$). FAB MS: m/z

(%) 305 (M + 1, 100), 189 (32), 154 (58). Anal. calcd. for $C_{15}H_{16}N_2O_5$: C, 59.21; H, 5.30; N, 9.21. Found: C, 59.52; H, 5.11; N, 9.50.

## General Procedure for the Preparation of (5a–d)

A mixture of **4a**, **4b**, **4c**, and/or **4d** (5 mmol), methanol (25 ml), and concentrated aqueous ammonia (25%, 25 ml) was stirred at room temperature for 24 h. The solvent was evaporated under reduced pressure, and the residue was purified on a silica-gel column using 5–10% methanol in diethyl ether to give **5a–d** in 82–90% yields.

## Data

**4-Hydroxy-1-[(2-hydroxyethoxy)methyl]-5-methylpyrimidin-6(1*H*)-one (5a).** Colorless oil (yield 87%). $^1$H NMR (DMSO-$d_6$), ($\delta$ ppm): 1.77 (s, 3H, $CH_3$), 3.54–3.61 (m, 2H, $CH_2$), 3.75–3.84 (m, 2H, $CH_2$), 4.53 (sb, 1H, OH), 5.16 (s, 2H, $NCH_2O$), 8.07 (s, 1H, C2-H), 11.26 (sb, 1H, OH). $^{13}$C NMR (DMSO-$d_6$), ($\delta$ ppm): 8.04 ($CH_3$), 61.78 ($CH_2$), 68.04 ($CH_2$), 72.62 ($NCH_2O$), 98.01 (C5), 147.86 (C2), 161.23 (C4), 163.58 (C6). Anal. calcd. for $C_8H_{12}N_2O_4$: C, 48.00; H, 6.04; N, 13.99. Found: 48.32; H, 5.95; N, 14.12.

**5-Ethyl-4-hydroxy-1-[(2-hydroxyethoxy)methyl]pyrimidin-6(1*H*)-one (5b).** Colorless oil (yield 85%). $^1$H NMR (DMSO-$d_6$), ($\delta$ ppm): 1.11 (t, 3H, $J = 7.1$, $C\underline{H}_3CH_2$), 2.44 (q, 2H, $J = 7.3$ Hz, $C\underline{H}_2CH_3$), 3.75–3.79 (m, 2H, $CH_2$), 4.11–4.17 (m, 2H, $CH_2$), 4.61 (sb, 1H, OH), 5.24 (s, 2H, $NCH_2O$), 7.96 (s, 1H, C2-H), 11.27 (sb, 1H, OH). $^{13}$C NMR (DMSO-$d_6$) ($\delta$ ppm): 11.75 ($CH_3$), 15.45 ($CH_2$), 61.62 ($CH_2$), 66.34 ($CH_2$), 73.34 ($NCH_2O$), 105.41 (C5), 146.44 (C2), 162.48 (C4), 164.03 (C6). Anal. calcd. for $C_9H_{14}N_2O_4$: C, 50.46; H, 6.59; N, 13.08. Found: C, 50.13; H, 6.50; N, 13.34.

**5-Butyl-4-hydroxy-1-[(2-hydroxyethoxy)methyl]pyrimidin-6(1*H*)-one (5c).** Colorless oil (yield 82%). $^1$H NMR (DMSO-$d_6$), ($\delta$ ppm) 0.85–0.89 (t, 3H, $J = 7.2$ Hz, $CH_3$), 1.11–1.13 (m, 4H, $2 \times CH_2$), 2.11–2.19 (t, 2H, $J = 7.3$ Hz, $CH_2$), 3.44–3.51 (m, 2H, $CH_2$), 4.07–4.11 (m, 2H, $CH_2$), 4.62 (sb, 1H, OH), 5.21 (s, 2H, $NCH_2O$), 7.97 (s, 1H, C2-H), 11.23 (sb,1H, OH). $^{13}$C NMR (DMSO-$d_6$), ($\delta$ ppm): 13.54 ($CH_3$), 22.21, 22.43, 29.21 ($3 \times CH_2$), 62.75 ($CH_2$), 68.46 ($CH_2$), 72.44 ($NCH_2O$), 102.65 (C5), 144.56 (C2), 161.76 (C4), 164.03 (C6). Anal. calcd. for $C_{11}H_{18}N_2O_4$: C, 54.43; H, 7.49; N, 11.56. Found: C, 54.57; H, 7.22; N, 11.12.

**4-Hydroxy-1-[(2-hydroxyethoxy)methyl]-5-phenylpyrimidin-6(1*H*)-one (5d).** Colorless oil (yield 90%). $^1$H NMR (DMSO-$d_6$), ($\delta$ ppm): 3.41–3.47 (m, 2H,

$CH_2$), 3.58–3.62 (m, 2H, $CH_2$), 4.63 (sb, 1H, OH), 5.11 (s, 2H, $NCH_2O$), 7.21–7.32 (m, 5H, Ar-H), 7.91 (s, 1H, C2-H), 11.23 (sb, 1H, OH). $^{13}C$ NMR (DMSO-$d_6$) ($\delta$ ppm) 60.06 ($CH_2$), 69.19 ($CH_2$), 71.58 ($NCH_2O$), 104.22 (C5), 126.52, 127.60, 130.20, 130.41 (Ar-C), 147.62 (C2), 161.41 (C4), 163.72 (C6). Anal. calcd. for $C_{13}H_{14}N_2O_4$: C, 59.54; H, 5.38; N, 10.68. Found: C, 59.33; H, 5.51, N, 10.44.

## General Procedure for the Preparation of Compounds (6–8)

A solution of chloromethyl ethyl ether (1.86 g, 20 mmol) in anhydrous MeCN (10 ml) was added to a stirred solution of the silylated pyrimidines **3a–d** in anhydrous MeCN at −25°C. The stirring was continued for 2 h at −25°C and for 1 h at room temperature, then the reaction mixture worked up as previously described. The compounds were purified by silica-gel column chromatography with methanol in chloroform (0–5%). *N*-Alkylated products **6a–c** were obtained as the sole products in 70–75% yield. *N*-Alkylated product **7** and its *O*-alkylated counterpart **8** mixture were obtained and separated. Fractions with *N*-alkylated product **7** were eluated faster than their *O*-alkylated counterpart **8**.

## Data

**1-(Ethoxymethyl)-4-hydroxy-5-methylpyrimidin-6(1*H*)-one (6a).** White solid (yield 73%), mp 156–158°C. $^1H$ NMR (DMSO-$d_6$), ($\delta$ ppm): 1.11 (t, 3H, $J = 7.0$ Hz, $CH_3$), 1.79 (s, 3H, $CH_3$), 3.53 (q, 2H, $J = 7.0$ Hz, $CH_2$), 5.24 (s, 2H, $NCH_2O$), 8.26 (s, 1H, C2-H), 11.42 (sb, 1H, OH). $^{13}C$ NMR (DMSO-$d_6$), ($\delta$ ppm): 8.07 ($CH_3$), 14.76 ($CH_3$), 64.11 ($CH_2$), 74.40 ($NCH_2O$), 97.24 (C5), 148.83 (C2), 162.28 (C4), 164.79 (C6). FAB MS: m/z (%). 185 (M + 1, 100). 154 (16). Anal. calcd. for $C_8H_{12}N_2O_3$: C, 52.17; H, 6.57; N, 15.21. Found: C, 52.43; H, 6.25; N, 15.11.

**5-Butyl-1-(ethoxymethyl)-4-hydroxypyrimidin-6(1*H*)-one (6c).** White solid (yield 70%), mp 98–100°C $^1H$ NMR (DMSO-$d_6$) ($\delta$ pmm): 0.85–0.90 (t, 3H, $J = 7.0$ Hz, $C\underline{H}_3CH_2CH_2CH_2$), 1.08–1.13 (t, 3H, $J = 7.0$ Hz, $C\underline{H}_3CH_2O$), 1.25–1.38 (m, 4 H, 2 × $CH_2$), 2.28–2.34 (t, 2H, $J = 7.1$ Hz, $CH_2$), 3.49–3.58 (q, 2H, $J = 6.8$ Hz, $OCH_2CH_3$), 5.24 (s, 2H, $NCH_2O$), 8.25 (s,1H, C2-H), 11.27 (sb, 1H, OH). $^{13}C$ NMR (DMSO-$d_6$) ($\delta$ ppm): 13.76 ($CH_3$), 14.78 ($CH_3$), 22.04, 22.16, 29.58 (3 × $CH_2$), 64.16 ($O\underline{C}H_2CH_3$), 74.36 ($NCH_2O$), 102.03 (C5), 149.05 (C2), 162.03 (C4), 164.78 (C6). FAB MS: m/z (%) 227 (M + 1, 100). Anal. calcd. for $C_{11}H_{18}N_2O_3$: C, 58.39; H, 8.02; N, 12.38. Found: C, 58.15; H, 8.35; N, 12.11.

**1-(Ethoxymethyl)-4-hydroxy-5-phenylpyrimidin-6(1*H*)-one (6d).** White solid (yield 75%), mp 218–220°C. $^1$H NMR (DMSO-$d_6$), ($\delta$ ppm): 1.12 (t, 3H, $J = 3.6$ Hz, $CH_3$), 3.58 (q, $J = 7.0$ Hz, $CH_2$), 5.28 (s, 2H, $NCH_2O$), 7.21–7.49 (m, 5H, Ar-H), 8.44 (s, 1H, C2-H), 11.33 (s, 1H, OH). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 14.80 ($CH_3$), 64.35 ($CH_2$), 74.73 ($NCH_2O$), 101.91 (C5), 126.22, 127.16, 130.33, 132.59 (Ar-C), 150.33 (C2), 160.66 (C4), 164.21 (C6). FAB MS: m/z (%) 247 (M + 1, 100), 189 (10), 176 (20). Anal. calcd. for $C_{13}H_{14}N_2O_3$: C, 63.40; H, 5.73; N, 11.38. Found: C, 63.70; H, 5.54; N, 11.53.

**1-(Ethoxymethyl)-5-ethyl-4-hydroxypyrimidin-6(1*H*)-one (7).** White solid (yield 30%), mp 160–162°C. $^1$H NMR (DMSO-$d_6$), ($\delta$ ppm): 0.97 (t, 3H, $J = 7.3$ Hz, $CH_3$), 1.11 (t, 3H, $J = 7.0$ Hz, $CH_3$), 2.32 (q, 2H, $J = 7.4$ Hz, $CH_2$), 3.54 (q, 2H, $J = 7.0$ Hz, $CH_2$), 5.24 (s, 1H, $NCH_2O$), 8.25 (s, 1H, C2-H), 11.33 (sb, 1H, OH). $^{13}$C NMR (DMSO-$d_6$), ($\delta$ ppm): 12.31 ($CH_3$), 14.78 ($CH_3$), 15.87 ($CH_2$), 64.17 ($CH_2$), 74.38 ($NCH_2O$), 103.36 (C5), 149.09 (C2), 161.86 (C4), 164.56 (C6). FAB MS: m/z (%) 199 (M + 1, 100), 169 (16), 154 (20). Anal. calcd. for $C_9H_{14}N_2O_3$: C, 54.53; H, 7.12; N, 14.13. Found: C, 54.60; H, 7.42; N, 14.45.

**4-(Ethoxymethoxy)-5-ethylpyrimidin-6(1*H*)-one (8).** White solid (yield 30%), mp 118–120°C. $^1$H NMR (DMSO-$d_6$), ($\delta$ ppm): 0.99 (t, 3H, $J = 7.3$ Hz, $CH_3$), 1.13 (t, 3H, $J = 7.0$ Hz, $CH_3$), 2.35 (q, 2H, $J = 7.2$ Hz, $CH_2$), 3.67 (q, 2H, $J = 7.0$ Hz, $CH_2$), 5.49 (s, 1H, $OCH_2O$), 8.01 (s, 1H, C2-H). $^{13}$C NMR (DMSO-$d_6$), ($\delta$ ppm): 12.38 ($CH_3$), 14.90 ($CH_3$), 15.51 ($CH_2$), 64.41 ($CH_2$), 90.28 ($OCH_2O$), 108.05 (C5), 146.84 (C2), 162.82 (C4), 163.63 (C6). FAB MS: m/z (%) 199 (M + 1, 100). Anal. calcd. for $C_9H_{14}N_2O_3$: C, 54.53; H, 7.12; N, 14.13. Found: C, 54.85; H, 7.25; N, 14.50.

## BIOLOGICAL ACTIVITY STUDIES

The required cell line was made by transfection of Hep G2-cells with a plasmid containing multiple tandem copies of HBV genome (subtype ayw).[19] The 2.2.15 cell line was maintained in RPMI-1640 (Glutamax) culture media containing 100 IU/ml nystatin +380 μg/ml G418 (geneticin). The transfected Hep G2-2.2.15 cell line was kept in tissue culture flask at 37°C + 5% $CO_2$. Maintenance media were added to the cell culture together with the tested compounds (final concentration = 10 μM). The supernatant liquid was collected after 1, 2, and/or 3 weeks by asbiration of the media from culture flask and washing the cells twice by PBS. Ten percent versene/trypsin was added, and the cells were incubated for 1 min at 37°C.[20] The DNA replication was estimated by the PCR (polymerase chain reaction) technique. The PCR reaction mixture contained 14 μL of extracted supernatant, 4 mmol/L of $MgCl_2$, 10 μmol/L of DIG-11-dUTP,

190 μmol/L of dTTP, 200 μmol/L of dATP, dGTP, 1,5 U Taq polymerase, 20 mmol/L of HCl (pH 8.4), 50 mmol/L of KCl, 1 μmol/L of HCID-1 primer (5′GGAAAGAAGTCAGAAGGCA3′), and 1 μmol/L of HCID-2 (5′TTGGGGGAGGAGATTAGGTT3′), in a total volume of 50 μL. PCR reaction conditions were 32 cycles of 1 min at 94°C, 30 s at 58°C, and 30 s at 72°C + 3 s for each cycle in a thermal circler as described in literature.[21] The percentage inhibition could be calculated by the relation between the plank experiment (containing maintenance media without the tested compounds) and the results obtained after the periods. The percentage cytotoxicity could be estimated by the relation between the number of the living and dead cells after 3 weeks as counted by the Haemocytometer.



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