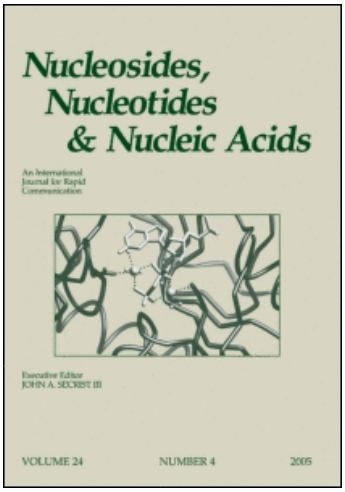

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Synthesis of 2-Bromomethyl-3-Hydroxy-2-Hydroxymethyl-Propyl Pyrimidine and Theophylline Nucleosides Under Microwave Irradiation. Evaluation of Their Activity Against Hepatitis B Virus

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SYNTHESIS OF 2-BROMOMETHYL-3-HYDROXY-2-HYDROXYMETHYL-PROPYL PYRIMIDINE AND THEOPHYLLINE NUCLEOSIDES UNDER MICROWAVE IRRADIATION. EVALUATION OF THEIR ACTIVITY AGAINST HEPATITIS B VIRUS

E. S. H. El Ashry, N. Rashed, A. Abdel-Rahman, L. F. Awad, and H. A. Rasheed □ *Department of Chemistry, Faculty of Science, Alexandria University, Alexandria, Egypt*

□ *Alkylation of 2-methylthiopyrimidin-4(1H)-one (**1a**) and its 5(6)-alkyl derivatives **1b–d** as well as theophylline (**7**) with 2,2-*bis*(bromomethyl)-1,3-diacetoxypropane (**2**) under microwave irradiation gave the corresponding acyclonucleosides 1-[(3-acetoxy-2-acetoxymethyl-2-bromomethyl)prop-1-*yl*]-2-methyl-thio pyrmidin-4(1*H*)-ones **3a–d** and 7-[(3-acetoxy-2-acetoxymethyl-2-bromomethyl) prop-1-*yl*]theophylline (**8**), which upon further irradiation gave the double-headed acyclonucleosides 1,1′-[(2,2-diacetoxymethyl)-1,3-propylidene]-*bis*[(2-(methylthio)-pyrimidin-4(1*H*)-ones] **4a–c**, and 7,7′-[(2,2-diacetoxymethyl)-1,3-propylidene]-*bis*(theophylline) (**9**). The deacetylated derivatives were obtained by the action of sodium methoxide. The activity of deacetylated nucleosides against Hepatitis B virus was evaluated. Compound **5b** showed moderate inhibition activity against HBV with mild cytotoxicity.*

Keywords Acyclonucleosides; Double-headed acyclonucleosides; Theophylline; Microwave alkylation reactions; Hepatitis B virus

INTRODUCTION

Acyclic nucleosides[1–4] and their chemotherapeutic value as well as denderimers have attracted the attention toward the synthesis of various analogues with variant chemical modifications. Development has progressed in the synthesis of acyclic nucleosides whose glycons and aglycons are modified.[5–7] Acyclic nucleosides of the tetra*seco* type showed

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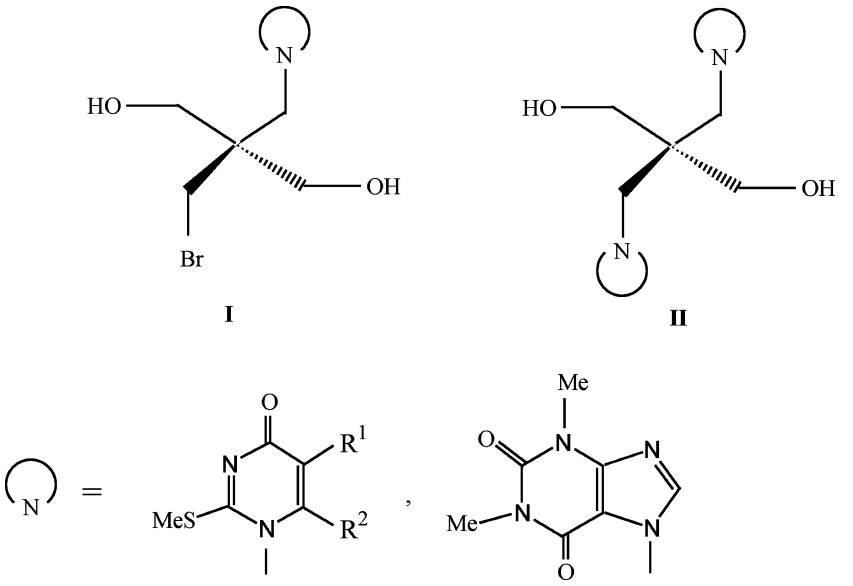


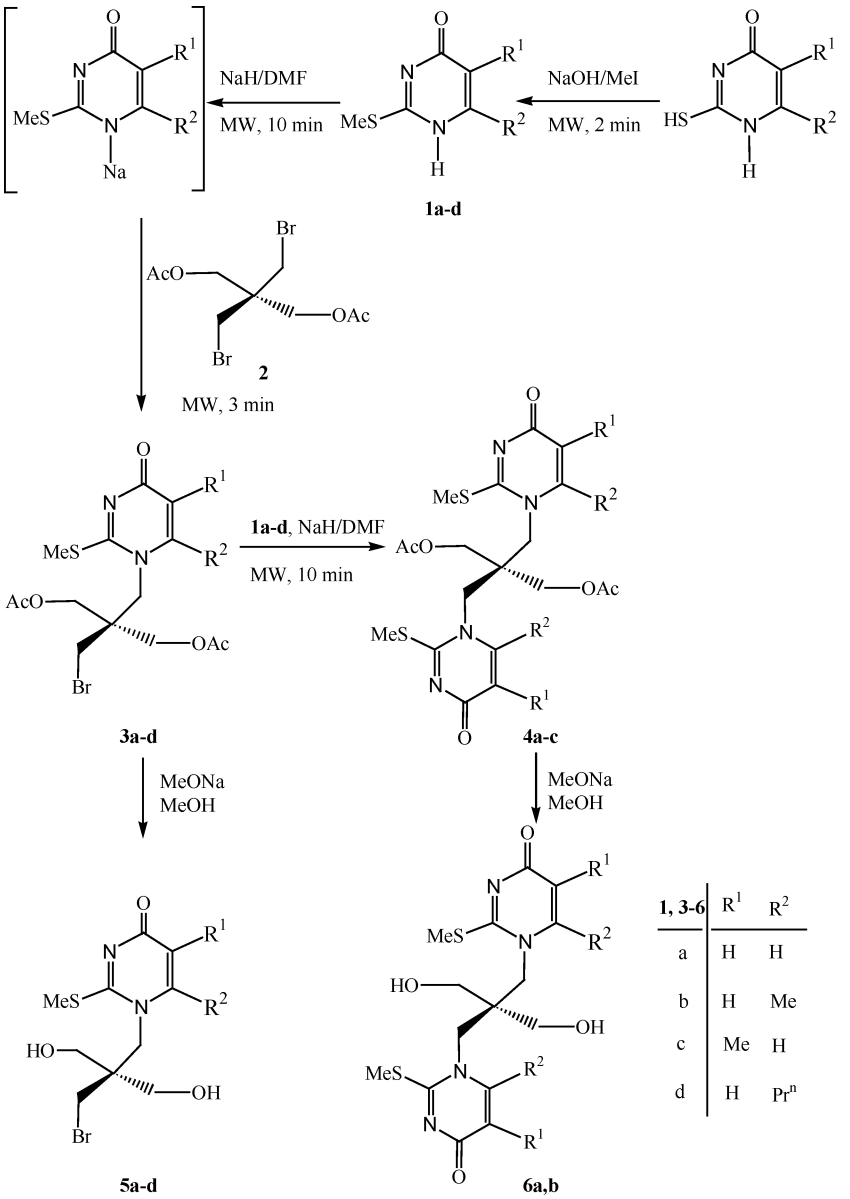
FIGURE 1 Target acyclonucleosides.

interesting antiviral activities.[8–12] Although many acyclonucleoside analogues have been synthesized and their activity against Hepatitis B[8,9] virus were evaluated, an urgent need for new classes of compounds with high activity and low toxicity is still required. Thus, we report here a fast and simple procedure for the alkylation of the modified pyrimidine bases and theophylline with the objective of synthesizing branched chain tetra*seco* nucleosides of type I and II (Figure 1).

RESULTS AND DISCUSSION

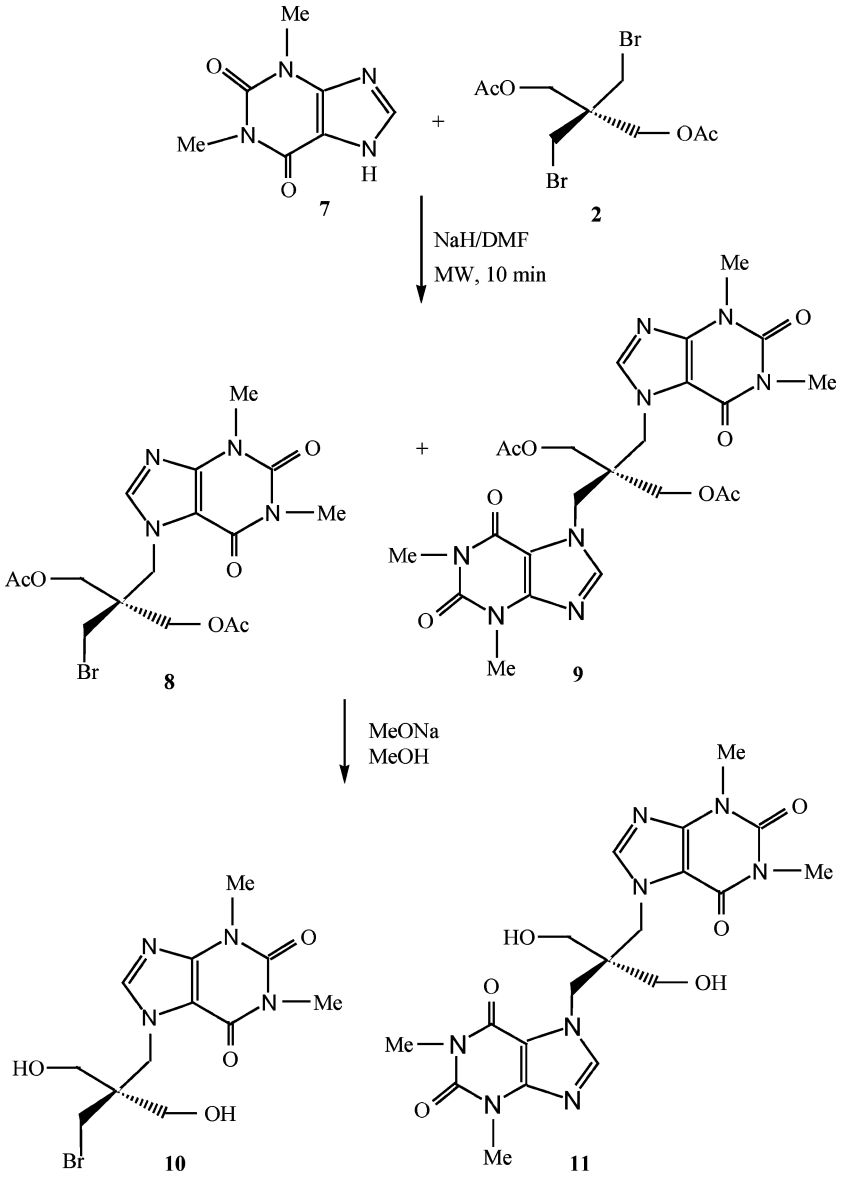
Microwave irradiation is now widely approved as a safe, convenient, and economical way for small-scale work in organic synthesis.[13–19] Recent reports demonstrated that a variety of organic reactions have been conducted efficiently by using selected organic solvents; other reports described accelerated organic reactions in nonaqueous media. The acceleration in reaction rates is ascribed to elevated temperatures and high pressure reached rapidly in a microwave oven. The application of microwave irradiation in the synthesis of heterocyclic compounds were reviewed.[18,19] Continuing our work[18–23] on the use of microwave irradiation in organic synthesis, we have selected this technique for the synthesis of our targets. Thus, the starting methylthiopyrimidinones **1a–d** were synthesized in better yields and shorter times than using conventional heating, by methylation of substituted 2-thiouracils with methyl iodide in presence of sodium hydroxide under microwave irradiation for 2 min. When the alkylation of **1a** with

2,2-*bis*(bromomethyl)-1,3-diacetoxypropane (**2**) (2:1 molar equivalent) under conventional heating was done, the product **3a** was formed in a very poor yield even upon prolongation of the time of heating and much of the base was recovered unchanged. On the other hand, when the reaction of 2.5 molar equivalents of **1a–d** with 1 molar equivalent of **2** was carried out under the microwave irradiation, it gave within 3 min the monoacyclo- nucleosides **3a–d** as the main products (Scheme 1). When the irradiation was continued

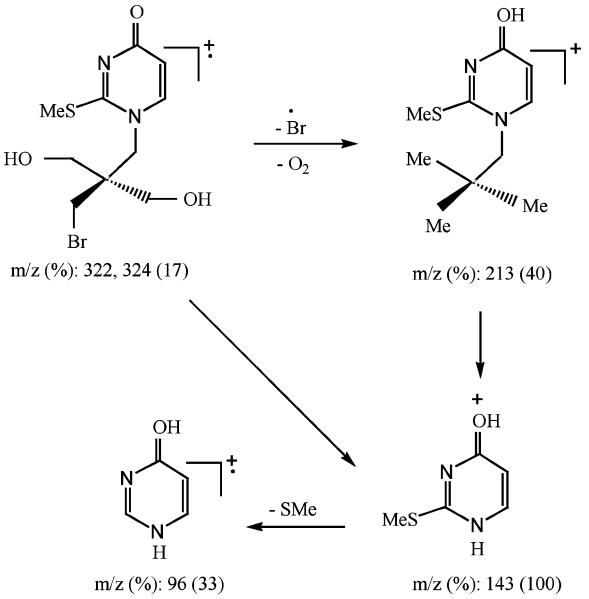


SCHEME 1

for 10 min, the double-headed acyclonucleosides **4a–c** were formed in addition to the monoacyclonucleoside **3**. Chromatographic purification of the reaction mixture on silica gel column afforded **3a–d** in 35–61% yield and **4a–c** in 22–26% yield. The ^{1}H NMR spectra for compounds **3a–d** confirmed the presence of two acetyl and four methylene groups in addition to the protons of the pyrimidine ring. Deacetylation of **3a–d** with sodium methoxide in methanol gave the corresponding acyclonucleoside analogues

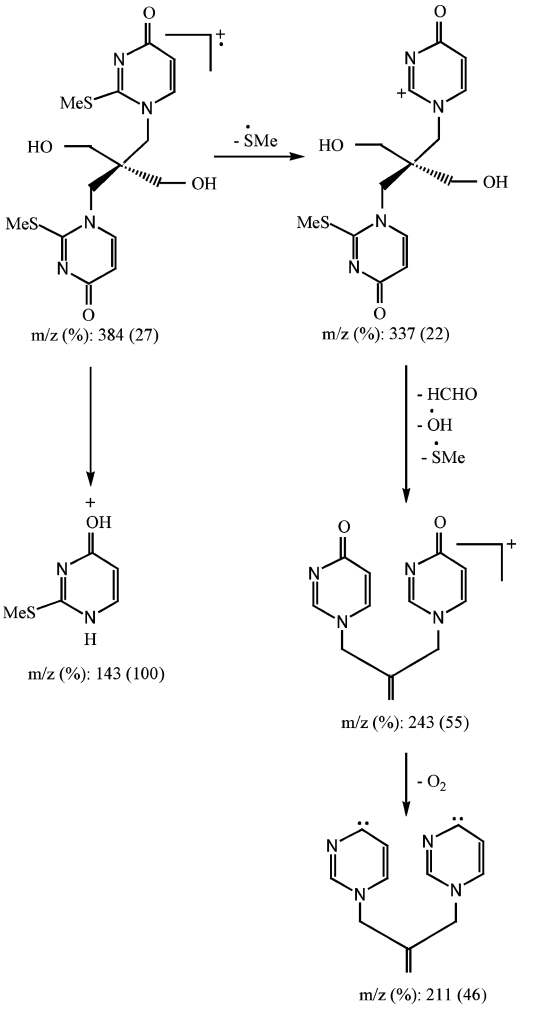


SCHEME 2



SCHEME 3

5a–d in 76–96% yield. The possible susceptibility of the methylthio group to nucleophilic substitution reaction by the methoxide ion to give the respective methoxy derivatives was not observed. This can be due to the electron donating effect of the alkyl substituent group on C-5 or C-6 which retard the nucleophilic substitution reaction.[23] The ^{1}H NMR data were in satisfactory agreement with the assigned structure **5a–d**, where it showed the absence of the two acetyl groups and the presence of the thiomethyl group in addition to the other protons of the deacetylated products. Mass spectra of **5a** and **5c** confirmed the presence of the bromine atom by showing their molecular ion peaks at m/z 322, 324 and 336, 338, respectively (Scheme 3) and their base peaks at m/z 143, 157 corresponding to the respective pyrimidinonium ions. The structure of the double-headed nucleosides **4a–c** and the symmetry in the molecule was deduced based on their ^{1}H NMR spectra, which showed four methylene protons as two singlets at δ 4.26, 4.39 and δ 4.47, 4.57 ppm, the two acetyl and the two thiomethyl protons as two singlets at δ 2.05, 2.09 and δ 2.52, 2.56, respectively, in addition to the protons of the pyrimidine base. The mass spectrum of **4b** confirmed that two pyrimidine bases were alkylated with **2** to give the respective bisacyclonucleoside. Although the formation of such double-headed acyclic nucleoside was successful for a variety of substituents on the pyrimidine ring, the 2-methylthio-6-(n-propyl)-pyrimidin–4(1*H*)-one (**1d**) failed to form the respective acyclonucleoside **4d**. This may be attributed to the bulkness of the substituent on C-6, which hindered the nucleophilic attack of the N-1 in **1d**, on the C-Br in **2** to form the C–N bond. Treatment



SCHEME 4

of **4a,b** with sodium methoxide in methanol furnished the deacetylated products **6a,b**. The structures of **6** were established and confirmed by their spectral data (MS and ^{1}H NMR). The mass spectrum of **6a** revealed a peak at m/z 384 and 143 corresponding to the molecular ion and base peaks, respectively (Scheme 4). The ^{1}H NMR spectrum showed the absence of acetyl groups and presence of two hydroxyl groups in addition to the other protons of the base and the methylene groups.

On the other hand, when a mixture of theophylline (**7**) and 2,2-*bis*(bromomethyl)-1,3-diacetoxypropane (**2**) was irradiated in the microwave oven for 10 min, a mixture of mono- and ditheophyllinyl acyclonucleosides **8** and **9**, respectively, was obtained whose ratio was found to be varied based on the ratio of the reactants. Thus, increasing the amount of

TABLE 1 Inhibitory Concentration (IC_{50}) Cytotoxic Effect (CC_{50}) and Selective Index (SI) of Compounds **4**, **5**, and **11**

Compound	IC_{50} (μM)	CC_{50} (μM)	SI
Lamivudine	<0.1	>100	>1000
4a	1.9	>100	>52.6
4b	3.3	>100	>30.3
4c	7.5	>100	>13.3
5a	3.5	>100	>28.6
5b	0.2	>100	>454.5
5c	12.5	>100	>8.0
5d	11.7	>100	>8.5
11	7.7	>100	13.0

the theophylline led to a higher ratio of the double headed analogue. The two products could be readily separated by column chromatography. The ^{1}H NMR spectrum of **8** showed the presence of two acetyl, four methylene, and two N-methyl groups in addition to the aromatic methine proton at 7.70 ppm. Both of the four methylene and four N-methyl groups in compound **9** were observed as two singlets at 4.27, 4.49 and 3.35, 3.54 ppm, respectively, indicating the symmetry in the structure. Deprotection of **8** and **9** with sodium methoxide in methanol gave the deprotected derivative **10** and **11**, respectively (Scheme 2). ^{1}H NMR spectra of **10** and **11** showed the absence of the acetyl groups that present in their precursors at 2.08 and 2.15 ppm and the presence of broad signals at 4.92 and 5.09 ppm, respectively, corresponding to their hydroxyl groups.

The results of the viral screening against HBV of selected compounds indicated that compound **5b** showed moderate viral replication inhibition and mild cytotoxicity with selective index >454.5. On the other hand, the effective concentration of compounds **4a**, **4b**, **4c**, **5a**, **5c**, **5d**, **11** was 10.0 μM, which showed very low inhibition and high cytotoxicity with selective index >52.6, >30.3, >13.3, >28.6, >8.0, >8.5, >13.0, respectively.

EXPERIMENTAL

Melting points were determined with Mel-Temp apparatus and are uncorrected. All solvents were distilled and dried before using. IR spectra were recorded for compounds in a matrix of KBr with Unicam SP1025 Spectrophotometer. The NMR spectra were recorded on a Bruker AC 300 MHz spectrometers. Chemical shifts were reported in δ scale (ppm) relative to TMS as a standard and the coupling constants J values are given in Hz. EI mass spectra were recorded on a Varian MAT 311A spectrometer. TLC was performed on 60 F_{254} precoated plastic plates silica gel (Merck). Column chromatography was performed on silica gel (Baker, 30–60 μm). Irradiation was done in a domestic microwave oven EM-230M (800 watt

TABLE 2 Results of Inhibition of HBV Replication by Compounds **4**, **5**, and **11**

Compound	Dreug conc. (μM)	HBV DNA in supernatant	Hep G2 viable cells
Lamivudine	1.0	0.25	1.03
	10.0	0.18	1.01
	100.0	0.15	1.07
4a	1.0	0.22	0.16
	10.0	0.20	0.12
	100.0	0.19	0.08
4b	1.0	0.15	0.06
	10.0	0.14	0.05
	100.0	0.14	0.04
4c	1.0	0.22	0.06
	10.0	0.14	0.05
	100.0	0.11	0.03
5a	1.0	0.42	1.01
	10.0	0.40	1.01
	100.0	0.40	1.01
5b	1.0	0.65	1.50
	10.0	0.50	1.40
	100.0	0.45	1.30
5c	1.0	0.29	1.00
	10.0	0.28	0.07
	100.0	0.25	0.06
5d	1.0	0.12	0.08
	10.0	0.11	0.07
	100.0	0.10	0.07
11	1.0	0.44	2.01
	10.0	0.40	1.90
	100.0	0.38	1.80

output power). Microanalyses were performed in the unit of Microanalysis at Faculty of Science, Cairo University.

*2-Methylthio-5(6)-substituted Pyrimidin-4(1*H*)-ones (1a–d): General Procedure.* A solution of substituted 2-thiouracils (0.01 mol) and sodium hydroxide (0.4 g, 0.01 mol) in water (10 mL) and ethanol (20 mL) was treated with methyl iodide (5 mL, 0.07 mol) and irradiated by MW for 2 min in a closed Teflon vessel. The solid was washed thoroughly with water, dried, and crystallized from ethanol (yield 88–92%); mps. are identical with the literature.[24]

*2,2-*bis*(bromomethyl)-1,3-diacetoxypropane (**2**).* To a stirred solution of 2,2-*bis*(bromomethyl)-propane-1,3-diol (10 g, 38 mmol) in dry pyridine (10 mL) and acetic anhydride (10 mL) was added and stirring was continued for 2 h. The reaction mixture was left overnight at 0°C. Crushed ice was added to the reaction mixture and the product that separated out was collected, washed with water, dried, and crystallized from ethanol (11.0 g, 82% yield), mp 85–87°C (Slobodin and Shokhor,[25] mp 84–86°C).

*Reaction of 2,2-*bis*(bromomethyl)-1,3-diacetoxypropane (**2**) with 2-methylthio-5(6)-substituted Pyrimidin-4(1*H*)-ones (**1a–d**).* (a) General procedure under microwave: To a stirred solution of compounds **1a–d** (6.25 mmol) in dry DMF (5 mL), sodium hydride (0.15 g, 6.25 mmol) was added. After complete evolution of hydrogen gas, the mixture was irradiated by MW for 10 min in a closed Teflon vessel. 2,2-*bis*(Bromomethyl)-1,3-diacetoxypropane (**2**, 0.86 g, 2.5 mmol) was then added and the mixture was subjected to microwave irradiation for 3 min TLC, using petroleum ether:ethyl acetate (5:1), showed spots corresponding to the reactants and acyclomononucleosides **3a–d**. The irradiation was continued for another 7 min, whereby a new spot was observed corresponding to the double-headed acyclonucleosides **4a–c**. The reaction mixture was evaporated to dryness and the residue was chromatographed on a silica gel column with petroleum ether:ethyl acetate (15:1) to give compounds **3a–d**. Further elution gave compounds **4a–c**. (b) Conventional procedure: A solution of **1a** (0.71 g, 5 mmol) in dry DMF (10 mL) and sodium hydride (0.12 g, 5 mmol) was heated to complete evolution of hydrogen gas at 80°C for 1 h. Compound **2** (0.86 g, 2.5 mmol) was then added and the reaction mixture was stirred for 8 h at 80°C, poured onto cold water (20 mL), and extracted with ethyl acetate. The organic layers were collected, dried, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel using petroleum ether:ethyl acetate (15:1) to give **3a** (0.1 g, 10% yield) and the recovered base (0.52 g).

*1-[(3-Acetoxy-2-acetoxymethyl-2- bromomethyl)prop-1-yl]-2-methylthiopyrmidin-4(1H)-one (**3a**).* Colorless syrup (35% yield); R_f = 0.41(5:1 petroleum ether:ethyl acetate); ^{1}H NMR ($CDCl_3$, 300 MHz), δ_H: 2.07 (s, 6 H, 2 OAc), 2.55 (s, 3 H, SMe), 3.56 (s, 2 H, CH_2Br), 4.22 (s, 4 H, 2 CH_2O), 4.44 (s, 2 H, CH_2N), 6.41 (d, 1 H, *J* 5.6 Hz, H-5), 8.26 (d, 1 H, *J* 5.6 Hz, H-6). Anal. calcd. for $C_{14}H_{19}BrN_2O_5S$: C, 41.29; H, 4.70; N, 6.88. Found: C, 41.03; H, 4.99; N, 6.98%.

*1-[(3-Acetoxy-2-acetoxymethyl-2-bromomethyl)prop-1-yl]-6-methyl-2-methylthiopyrimidin-4(1H)-one (**3b**).* Colorless syrup (44% yield); R_f = 0.24 (5:1 petroleumether:ethyl acetate); ^{1}H NMR ($CDCl_3$, 300 MHz), δ_H: 2.07 (s, 6 H, 2 OAc), 2.37 (s, 3 H, Me), 2.55 (s, 3 H, SMe), 3.55 (s, 2 H, CH_2Br), 4.21 (s, 4 H, $2CH_2O$), 4.43 (s, 2 H, CH_2N), 6.25 (s, 1 H, H-5). Anal. calcd. for $C_{15}H_{21}BrN_2O_5S$: C, 42.76; H, 5.02; N, 6.65. Found: C, 42.92; H, 5.33; N, 6.74%.

*1-[(3-Acetoxy-2-acetoxymethyl-2-bromomethyl)prop-1-yl]-5-methyl-2-methylthiopyrimidin-4(1H)-one (**3c**).* Colorless syrup (46% yield); R_f = 0.28 (5:1 petroleum ether:ethyl acetate); ^{1}H NMR ($CDCl_3$, 300 MHz), δ_H: 2.07 (s, 6 H, 2 OAc,), 2.43 (s, 3 H, Me), 2.54 (s, 3 H, SMe), 3.58 (s, 2 H, CH_2Br), 4.23 (s, 4 H, 2 CH_2O), 4.44 (s, 2 H, CH_2N), 8.09 (s, 1H, H-6). Anal. calcd. for $C_{15}H_{21}BrN_2O_5S$: C, 42.76; H, 5.02; N, 6.65. Found: C, 42.49; H, 5.32; N, 7.03%.

*1-[(3-Acetoxy-2-acetoxymethyl-2-bromomethyl)prop-1-yl]-2-methylthio-6-n-propylpyrimidin-4(1*H*)-one (**3d**).* Colorless syrup (61% yield); R_f = 0.24 (5:1 petroleum ether:ethyl acetate); ^{1}H NMR ($CDCl_3$, 300 MHz), δ_H: 0.88 (t, 3 H, J = 7.2 Hz, Me-pr), 1.65 (m, 2 H, CH_2-pr), 2.00 (s, 6 H, 2 OAc), 2.47 (s, 3 H, SMe), 2.51 (t, 2 H, CH_2- pr), 3.45 (s, 2 H, CH_2Br), 4.14 (s, 4 H, 2 CH_2O), 4.35 (s, 2 H, CH_2N), 6.15 (s, 1 H, H-5). Anal. calcd. for $C_{17}H_{25}BrN_2O_5S$: C, 45.44; H, 5.61; N, 6.23. Found: C, 45.88; H, 5.34; N, 6.00%.

1,1′-[(2,2-Diacetoxymethyl)-1,3-propylidene]-bis*[(2-methylthio)pyrimidin-4(1*H*)-one] (**4a**).* Colorless syrup (26% yield); R_f = 0.14 (5:1 petroleum ether:ethyl acetate); ^{1}H NMR ($CDCl_3$, 300 MHz), δ_H: 2.09 (s, 6 H, 2 OAc), 2.56 (s, 6 H, 2 SMe), 4.39 (s, 4 H, 2 CH_2O), 4.57 (s, 4 H, 2 CH_2N), 6.42 (d, 2 H, J 5.7 Hz, 2 H-5), 8.26 (d, 2 H, J 5.7, 2 H-6). Anal. calcd. for $C_{19}H_{24}N_4O_6S_2$: C, 48.70; H, 5.16; N, 11.96. Found: C, 49.08; H, 5.54; N, 12.06%.

1,1′-[(2,2-Diacetoxymethyl)1,3-propylidene]-bis*[6-methyl-2-(methylthio)pyrimidin-4(1*H*)-one] (**4b**).* Colorless syrup (23% yield); R_f = 0.11 (5:1 petroleum ether:ethyl acetate); ^{1}H NMR ($CDCl_3$, 300 MHz), δ_H: 2.05 (s, 6 H, 2 OAc), 2.35 (s, 6 H, 2 Me), 2.54 (s, 6 H, 2 SMe), 4.26 (s, 4 H, 2 CH_2O), 4.47 (s, 4 H, 2 CH_2N), 6.23 (s, 2 H, 2 H-5); MS EI, m/z (%) : 496 (M^+, 12), 449 (M^+-SMe, 16), 341 [M^+-(MeSH, AcOH, SMe), 99], 267 [M^+-(2MeSH, AcOH, 2Me, Ac), 55], 157 (6-methyl-2-methylthio-pyrimidin-4(1*H*)-onium ion, 100). Anal. calcd. for $C_{21}H_{28}N_4O_6S_2$: C, 50.79; H, 5.68; N, 11.28. Found: C, 50.93; H, 5.48; N, 11.44%.

1,1′-[(2,2-Diacetoxymethyl)-1,3-propylidene]-bis*[5-methyl-2-(methylthio)- pyrimidin-4(1*H*)-one] (**4c**).* Colorless syrup (22% yield); R_f = 0.12 (5:1 petroleum ether:ethyl acetate); ^{1}H NMR ($CDCl_3$, 300 MHz), δ_H: 2.05 (s, 12 H, 2 Me, 2 OAc), 2.52 (s, 6 H, 2 SMe), 4.30 (s, 4 H, 2 CH_2O), 4.51 (s, 4 H, 2 CH_2N), 8.07 (s, 2 H, 2 H-6). Anal. calcd. for $C_{21}H_{28}N_4O_6S_2$: C, 50.79; H, 5.68; N, 11.28. Found: C, 50.93; H, 5.79; N, 11.59%.

*Deacetylation of compounds **3a–d** and **4a,b**: General procedure.* A solution of compounds **3a–d** or **4a,b** (0.2 mmol) in dry methanol (3 mL) was treated with sodium methoxide (0.15 mol of sodium in 3 mL dry methanol) with stirring at room temperature for 2 h. The solution was neutralized by Amberlite IR-120(H^+) resin. The mixture was filtered, washed with methanol, and the combined filtrate was evaporated and crystallized from ethanol to afford **5a–d** and **6a**,**b**, respectively.

*1-[(2-Bromomethyl-3-hydroxy-2-hydroxymethyl)prop-1-yl]-2-methylthio-pyrimidin-4(1*H*)-one (**5a**).* Colorless crystals (76% yield); mp. 120–122°C; ^{1}H NMR (CD_3OD+D_2O, 300 MHz), δ_H: 2.55 (s, 3 H, SMe), 3.59 (s, 2 H, CH_2Br), 3.67 (s, 4 H, 2 CH_2O), 4.41 (s, 2 H, CH_2N), 6.56 (d, 1 H, J 5.7 Hz, H-5), 8.23 (d, 1 H, J 5.7 Hz, H-6); MS EI, m/z (%): 322, 324 (M^+,17), 213[M^+-(Br, 2Me), 40], 143 (2-methylthiopyrimidin-4(1*H*)-onium ion, 100). Anal. calcd.

for $C_{10}H_{15}BrN_2O_3S$: C, 37.16; H, 4.68; N, 8.67. Found: C, 37.45; H, 4.96; N, 8.55%.

*1-[(2-Bromomethyl-3-hydroxy-2-hydroxymethyl)prop-1-yl]-6-methyl-2-methylthiopyrimidin-4(1*H*)-one (**5b**).* Colorless syrup (96% yield); ^{1}H NMR (DMSO-d_6, 300 MHz), δ_H: 2.04 (s, 3 H, Me), 2.50 (s, 3 H, SMe), 3.46, 3.48 (2 d, 4 H, J = 5.2, 5.5 Hz, 2 CH_2O), 3.58 (s, 2 H, CH_2Br), 4.26 (s, 2 H, CH_2N), 4.80 (t, 2 H, 2 OH), 6.83 (s, 1 H, H-5). Anal. calcd. for $C_{11}H_{17}BrN_2O_3S$: C, 39.18; H, 5.08; N, 8.31. Found: C, 39.42; H, 5.08; N, 8.58%.

*1-[(2-Bromomethyl-3-hydroxy-2-hydroxymethyl)prop-1-yl]-5-meth- yl-2-methylthiopyrimidin-4(1*H*)-one (**5c**).* Colorless syrup (84% yield); ^{1}H NMR (CD_3OD+D_2O, 300 MHz), δ_H: 2.13 (s, 3 H, Me), 2.56 (s, 3 H, SMe), 3.64 (s, 2 H, CH_2Br), 3.71 (s, 4 H, 2 CH_2O), 4.44 (s, 2 H, CH_2N), 8.09 (s, 1 H, H-6); MS EI: m/z (%): 336, 338 (M^+, 50), 157 (5-methyl-2-methylthiopyrimidin-4(1*H*)-onium ion, 100). Anal. calcd. For $C_{11}H_{17}$ Br N_2 O_3S: C, 39.18; H, 5.08; N, 8.31. Found: C, 39.44; H, 5.25; N, 8.11%.

*1-[(2-Bromomethyl-3-hydroxy-2-hydroxymethyl)prop-1-yl]-2-methylthio-6-(n)propylpyrimidin-4(1*H*)-one (**5d**).* Colorless syrup (77% yield); ^{1}H NMR (DMSO-d_6, 300 MHz), δ_H: 0.89 (t, 3 H, J = 7.3 Hz, Me-pr), 1.71 (m, 2 H, CH_2-pr), 2.52 (s, 3 H, SMe), 2.55 (t, 2 H, CH_2-pr), 3.90 (s, 2 H, CH_2Br), 4.47 (2 d, 4 H, J = 5.8, 5.9 Hz, 2 CH_2O), 4.55 (s, 2 H, CH_2N), 5.35 (br t, 2 H, 2 OH), 6.53 (s, 1 H, H-5). Anal. calcd. for $C_{13}H_{21}BrN_2O_3S$: C, 42.74; H, 5.79; N, 7.67. Found: C, 42.60; H, 5.33; N, 7.42%.

*1,1′-[(2,2-Dihydroxymethyl)-1,3-propylidene]-bis[(2-methylthio)pyrimidin-4(1*H*)-one] (**6a**).* Colorless syrup (87% yield); ^{1}H NMR (DMSO-d_6, 300 MHz), δ_H: 2.50 (s, 6 H, 2 SMe), 3.47 (s, 4 H, 2 CH_2O), 4.49 (s, 4 H, 2 CH_2N), 5.06 (br s, 2 H, 2 OH), 6.65 (d, 2 H, J 5.7 Hz, 2 H-5), 8.35 (d, 2 H, J 5.7 Hz, 2 H-6); MS EI: m/z (%): 384 (M^+, 27), 143 (2-methylthio-pyrimidin-4(1*H*)-onium ion, 100), Anal. calcd. for $C_{15}H_{20}N_4O_4S_2$: C, 46.86; H, 5.24; N, 14.57 Found: C, 47.24; H, 5.69; N, 14.62%.

*1,1′-[(2,2-Dihydroxymethyl)-1,3-propylidene]-bis[6-methyl-2-(methylthio) pyrimidin-4(1*H*)-one] (**6b**).* Colorless syrup (96% yield); ^{1}H NMR (DMSO-d_6, 300 MHz), δ_H: 2.29 (s, 6 H, 2 Me), 2.50 (s, 6 H, 2 SMe), 4.21 (s, 4 H, 2 CH_2O), 4.46 (s, 4 H, 2 CH_2N), 5.02 (br t, 2 H, 2 OH), 6.47 (s, 2 H, 2 H-5). Anal. Calcd. for $C_{17}H_{24}N_4O_4S_2$: C, 49.50; H, 5.86; N, 13.58. Found: C, 49.21; H, 6.21; N, 13.87%.

*7-[(3-Acetoxy-2-acetoxymethyl-2-bromomethyl)prop-1-yl)theophylline (8) and 7,7′-[(2,2-diacetoxymethyl)-1,3-propylidene]-bis(theophylline) (**9**).* To a stirred suspension of **7** (0.45 g, 2.5 mmol) in dry DMF (5 mL), NaH (0.06 g, 2.5 mmol) was added. After complete evolution of hydrogen gas the mixture was irradiated by MW for 10 min in a closed Teflon vessel, then treated with compound 2 (0.346 g, 1 mmol), and the reaction mixture was processed as for **1a–d**. Column chromatography gave compound **8** as colorless syrup (47% yield); TLC ($CHCl_3$-MeOH 8:2): R_f = 0.51; ^{1}H NMR ($CDCl_3$, 300 MHz), δ_H: 2.08

(s, 6 H, 2 OAc), 3.39 (s, 3 H, NMe), 3.48 (s, 2 H, CH_2Br), 3.59 (s, 3 H, NMe), 4.18 (s, 4 H, 2 CH_2O), 4.65 (s, 2 H, CH_2N), 7.70 (s, 1 H, H-8). Anal. calcd. for $C_{16}H_{21}BrN_4O_6$: C, 43.16; H, 4.75; N, 12.58. Found: C, 43.42; H, 5.03; N, 12.94%. Further elution gave compound **9** as colorless syrup (22% yield); TLC ($CHCl_3$-MeOH 8:2): $R_f = 0.38$; ^{1}H NMR ($CDCl_3$, 300 MHz), δ_H: 2.15 (s, 6 H, 2 OAc), 3.35 (s, 6 H, 2 NMe), 3.54 (s, 6 H, 2 NMe), 4.27 (s, 4 H, 2 CH_2O), 4.49 (s,4 H, 2 CH_2N), 7.86 (s, 2 H, 2 H-8). Anal. calcd. for $C_{23}H_{28}N_8O_8$: C, 50.73; H, 5.18; N, 20.58. Found: C, 50.95; H, 5.54; N, 20.86%.

*7-[(2-Bromomethyl-3-hydroxy-2-hydroxymethyl)prop-1-yl]theophylline (**10**).* Deacetylation of compound **8** was carried out as in the general method to afford **10** as colorless syrup (83% yield); ^{1}H NMR (DMSO-d_6, 300 MHz), δ_H: 3.27 (s, 3 H, NMe), 3.33 (br s, 4 H, 2 CH_2O), 3.40 (s, 3 H, NMe), 3.57 (s, 2 H, CH_2Br), 4.44 (s, 2 H, CH_2N), 4.92 (br t, 2 H, 2 OH), 7.92(s, 1 H, H-8). Anal. calcd. for $C_{12}H_{17}$ BrN_4O_4: C, 39.90; H, 4.74; N, 15.51. Found: C, 40.22; H, 5.10; N, 15.92%.

*7,7′-[(2,2-Dihydroxymethyl)-1,3-propylidene]*bis*(theophylline) (**11**).* Compound **9** (0.1 g) was deacetylated as above to afford **11** as colorless syrup (76% yield); ^{1}H NMR (DMSO-d_6, 300 MHz), δ_H: 3.29 (s, 6 H, 2 NMe), 3.62 (s, 6 H, 2 NMe), 4.21 (br s, 4 H, 2 CH_2O), 4.39 (s, 4 H, 2 CH_2N), 5.09 (br t, 2 H, 2 OH), 7.96 (s, 2 H, 2 H-8). Anal. calcd. for $C_{19}H_{24}$ N_8O_6: C, 49.56; H, 5.25; N, 24.34. Found: C, 50.01; H, 4.77; N, 24.36%.

BIOLOGICAL ACTIVITY STUDIES

Preparation and Culture of Hep G2 2.2.15 Cells

The required cell line was made by transfection of Hep G2-cells with a plasmid containing multiple tandem copies of HBV genome (subtype ayw).[26] The Hep G2-2.2.15 cell line was maintained in RPMI-1640 (Glutamax) culture media containing 100 IU/mL nystatin and 380 μg/mL G418 (geneticin). The transferred Hep G2–2.2.15 cell line was kept in tissue culture flask at 37°C + 5% CO_2. Subcultures were set up after a week by aspiration of the media from culture flask and washing the cells twice by PBS. A 10% versene/trypsin was added and the cells were incubated for 1 min at 37°C. The drug Lamivudine, which is a potent selective inhibitor of HBV replication, has been used as a standard for the comparative studies.

PCR-ELLISA

The PCR reaction mixture contained 14 μL extracted supernatant, 4 mmol/L $MgCl_2$, 10 μmol/L DIG-11-dUTP, 190 μmol/L dTTP, 200 μmol/L dATP, dGTP, dCTP, 1.5 U Taq polymerase, 20 mmol/L HCI (pH 8.4), 50 mmol/L KCI, 1 μmol/L HCID-1 primer (5′ GGA AAG AAG TCA

GAA GGC A3′), and 1 μmol/L HCID-2 (5′TTG GGG GAG GAG ATT AGG TT3), in total volume 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 the literature.[27]

Cytotoxicity Assay

A colorimetric assay for living cells utilized the colorless substrate 3-(3,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) that is modified to a colored product by any living cells, but not by dead cells or tissue culture medium is the attractive candidate for this purpose. The cytotoxic effect of the compounds was accessed by culturing the Hep G2–2.2.15 cells in the presence of compounds using a MTT-assay.[28]

Calculation of IC_{50}, CC_{50}, and SI

The 50% inhibitory concentration of antiviral drugs (IC_{50}) was determined by interpolation from the plots of amount of DNA copies versus antiviral drug concentration. The 50% cytotoxic effect (CC_{50}) was calculated from the average viability of the cells with concentration of drugs. The selective index (SI) could be calculated as CC_{50}/IC_{50}.

REFERENCES

1. El Ashry, E.S.H.; El Kilany, Y. Acyclonucleosides: Part 1. *seco*-Nucleosides. *Advances in Heterocyclic Chemistry* **1996**, 67, 391–438.
2. El Ashry, E.S.H.; El Kilany, Y. Acyclonucleosides: Part 2. *diseco*-Nucleosides. *Advances in Heterocyclic Chemistry* **1997**, 68, 1–88.
3. El Ashry, E.S.H.; El Kilany, Y. Acyclonucleosides: Part 3. Tri-, Tetra-, and Pentaseco-Nucleosides. *Advances in Heterocyclic Chemistry* **1998**, 69, 129–215.
4. Chu, C.K.; Cutler, S.J. Chemistry and Antiviral Activities of Acyclonucleosides. *Journal of Heterocyclic Chemistry* **1986**, 23, 289–319.
5. (a) De Clercq, E. Development of Resistance of Human Immunodeficiency Virus (HIV) to Anti-HIV Agents: How to Prevent the Problem? *International Journal of Antimicrobiol Agents* **1997** 9, 21–36. (b) Mellors, J.W.; Rinaldo, Jr. C. R.; Gupta, P.; White, R.M.; Todd, J.A.; Kingsley, L.A. Prognosis in HIV-1 Infection Predicted by the Quantity of Virus in Plasma, *Science*, **1996** 272, 1167–1170. (c) Pai, S.B.; Liu, S.-H.; Zhu, Y.-L.; Chu, C.K.; Cheng, Y.C. Inhibition of Hepatitis B Virus by a Novel L-Nucleoside, 2′-Fluoro-5-Methyl-β-L-Arabino-Furanosyl Uracil. *Antimicrobial Agents in Chemotherapy* **1996** 40, 380–386. (d) Condreay, L.D.; Jansen, R.W.; Powdrill, T.F.; Johnson, L.C.; Selleseth, D.W.; Paff, M.T.; Daluge, S.M.; Painter, G.R.; Furman, P.A.; Ellis, M.N.; Averett, D.R. Evaluation of the Potent Anti-Hepatitis B Virus Agent (-) *cis*-5-Fluoro-1-[2-(Hydroxymethyl)-1,3-Oxathiolan-5-yl]Cytosine in a Novel In Vivo Model, *Antimicrobial Agents in Chemotherapy*, **1994** 38, 616–619. (e) Tsai, C.-C.; Follis, K.E.; Sabo, A.; Beck, T.W.; Grant, R.F.; Bischofberger, N.; Benveniste, R.; Black, R.E. Prevention of SIV Infection in Macaques by (R)-9-(2-Phosphonylmethoxypropyl)Adenine. *Science*, **1995** 270, 1197–1199.
6. (a) Colcacino, J.M.; Malcolm, S.K.; Jaskunas, S.R. Effect of Fialuridine on Replication of Mitochondrial DNA in CEM Cells and in Human Hepatoblastoma Cells in Culture, *Antimicrobial Agents in Chemotherapy*, **1994** 38, 1997–2002. (b) Chu, C.K.; Ma, T.W.; Shanmuganathan, K.; Wang, C.; Xiang, Y.; Pai, S.B.; Yao, G.-Q.; Sommadossi, J.-P.; Cheng, Y.-C. 1995. Use of 2′-Fluoro-5-Methyl-β-L-Arabinofuranosyluracil as a Novel Antiviral Agent for Hepatitis B Virus and Epstein-Barr Virus,

Antimicrobial Agents in Chemotherapy 39, 979–981. (c) Lin, T.S., Lou, M.Z., Pai, S.B., Dutschuman, G.E.; Cheng, Y.-C. Synthesis and Biological Evaluation of 2′,3′-Dideoxy-L-Pyrimidine Nucleosides as Potential Antiviral Agents against Human Immunodeficiency Virus (HIV) and Hepatitis B Virus (HBV). *Journal of Medicinal Chemistry* **1994** 37, 798–803. (d) Doong, S.-L.; Tsai, G.-H.; Shinazi, R.F.; Liotta, D.C.; Cheng, Y.-C. Inhibition of the Replication of Hepatitis B Virus In Vitro by 2′,3′-Dideoxy-3′-Thiacytidine and Related Analogues. *Proceedings of the National Academy of Sciences USA*, **1991** 88, 8495–8499. (e) Furman, P.A.; Davis, M.; Liotta, D.C.; Paff, M.; Frick, L.W.; Nelson, D.J.; Dornsife, R.E.; Warster, J.A.; Wilson, L.J.; Fyfe, J.A.; Tuttle, J.V.; Miller, W.H.; Condreay, L.; Averett, D.R.; Shinazi, R.F.; Painter, G.R. The Anti-Hepatitis B Virus Activities, Cytotoxicities, and Anabolic Profiles of the (−) and (+) Enantiomers of *cis*-5-Fluoro-1-[2-(Hydroxymethyl)-1,3-Oxathiolan-5-yl]Cytosine. *Antimicrobial Agents in Chemotherapy* **1992** 36, 2686–2692.

7. (a) Naesens, L.; Snoeck, R.; Andrei, G.; Balzarini, J.; Neyts, J.; De Clercq, E. HPMPC (Cidofovir), PMEA (Adefovir) and Related Acyclic Nucleoside Phosphonate Analogs: A Review of Their Pharmacology and Clinical Potential in the Treatment of Viral Infections. *Antiviral & Chemistry Chemotherapy* **1997** 1–23. (b) De Clercq, E. Broad-Spectrum Anti-DNA Virus and Anti-Retrovirus Activity of Phosphonylmethoxyalkylpurines and Pyrimidines. *Biochemical Pharmacology* **1991** 42, 963–972. (c) Beach, J.W.; Jeong, L.S.; Alves, A.J.; Pohl, D.; Kim, H.O.; Chang, C.-N.; Doong, S.-L.; Schinazi, R.F.; Cheng, Y.-C.; Chu, C.K. Synthesis of Enantiomerically Pure (2′R,5′S)-(−)-1-[2-(Hydroxy-methyl)Oxathiolan-5-yl]Cytosine as a Potent Antiviral Agent against Hapatitis B Virus (HBV) and Human Immunodeficiency Virus (HIV). *Journal of Organic Chemistry* **1992** 57, 2217–2219.
8. El Ashry, E.S.H.; Abdel Rahman, A.A.-H.; Rashed, N.; Rasheed, H.A. Homoacylovir Analogues of Unnatural Bases and Their Activity against Hepatitis B Virus. *Pharmazie* **1999**, 54, 893–897.
9. El Ashry, E.S.H.; Abdel Rahman, A.A.-H.; Rashed, N.; Rasheed, H.A. Synthesis and Anti-Hepatitis B Virus Activity of Some 2,3-Dihydroxyprop-1-yl Unnatural Hetaryls. *Archiv Pharmazie Pharmaceutical Medicinal Chemistry* **1999**, 332, 327–330.
10. Awad, L.; El Ashry, E.S. Synthesis and Conformational Analysis of seco *C*-Nucleosides and Their diseco Double-Headed Analogues of the 1,2,4-Triazole,1,2,4-Triazolo [3,4-*b*] 1,3,4-Thiadiazole. *Carbohydrate Research* **1998**, 312, 9–22.
11. Rashed, N.; Abdel Hamid, H.; Ramadan, E.; El Ashry, E.S.H. Acyclo *C*-Nucleoside Analogs. Regioselective Annellation of a Triazole Ring to 5-Methyl-1,2,4-Triazino[5,6-*b*]Indole and Formation of Certain 3-Polyhydroxyalkyl Derivatives. *Nucleosides Nucleotides* **1998**, 17, 1373–1384.
12. Hamed, A.; Abo-amaym, E.R.; El Ashry, E.S.H. Synthesis of Acyclo *C*-Nucleosides of Phenanthro[9,10-*e*][1,2,4]Triazino[3,4-*c*][1,2,4] Triazoles, and Their Precursors. *Nucleosides Nucleotides* **1998**, 17, 1385–1407.
13. Gedye, R.N.; Smith, F.E.; Westaway, K.C. The Rapid Synthesis of Organic Compounds in Microwave Ovens. *Canadian Journal of Chemistry* **1988**, 66, 17–26.
14. Abramovitch, R.A. Applications of Microwave Energy in Organic Chemistry. *Organic Preparation Procedures International* **1991**, 23, 683–711.
15. Gedye, R.; Smith, F.; Westaway, K.; Ali, H.; Baldisera, L.; Laberge, L.; Rousell, J. The Use of Microwave Ovens for Rapid Organic Synthesis. *Tetrahedron Letters* **1986**, 27, 279–282.
16. Giguere, R.J.; Bray, T.L.; Duncan, S.M.; Majetich, G. Applications of Commercial Microwave Ovens to Organic Synthesis. *Tetrahedron Letters* **1986**, 27, 4945–4948.
17. Bogdal, D.; Pielichowski, J.; Jaskot, K. A Rapid Williamson Synthesis under Microwave Irradiation in Dry Medium. *Organic Preparation Procedures International* **1998**, 30, 427–432.
18. El Ashry, E.S.; Ramadan, E.; Kassem, A.A.; Haggar, M. Microwave Irradiation for Accelerating Organic Reactions. Part 1: Three, Four and Five Membered Heterocycles. *Advances in Heterocyclic Chemistry* **2005**, 88, 1–110.
19. El Ashry, E.S.H.; Kassem, A.A.; Ramadan, E. Microwave Irradiation for Accelerating Organic Reactions. Part 2: Six, Seven Fused and Spiro Heterocyclic Ring Systems. *Advances in Heterocyclic Chemistry* **2006**, 90, 1–127.
20. Abdel-Rahman, A.-H.; El Ashry, E.S.H. Efficient Synthesis of 5-Hydroxymethyl Pyrimidines and Their Nucleosides Using Microwave Irradiation *Synlett* **2002**, 2043–2044.
21. Abdel Hamid, H.M.; Ramadan, E.; Hagar, M.; El Ashry, E.S.H. Synthesis of Aryloxyacetic Acids, Esters and Hadrazides Assisted by Microwave Irradiation. *Synthetic Communications* **2004**, 34, 377–382.
22. (a) El Ashry, E.S.H.; Ramadan, E.; Abdel Hamid, H.M.; Hagar, M. Microwave Irradiation for Accelerating Each Step for the Synthesis of 1,2,4-Triazino[5,6-*b*] Indole-3-Thiols and Their Derivatives from Isatin and 5-Chloroisatin. *Synlett* 723–725. (b) El Ashry, E.S. H., Ramadan, E., Abdel Hamid, H.,

Haggar, M. Microwave Irradiation for Enhancing the Synthesis of Quinoline Derivatives from Isatin, *Synthetic Communications*, **2005** 35, 2243–2250.

23. (a) El Ashry, E.S.H.; Ramadan, E.; Abdel Hamid, H.M.; Hagar, M. Synthesis of Azalactones, Phenylpyruvic Acid and 1,2,4-Triazine Derivatives under Microwave Irradiation, *Letters in Organic Chemistry*, 2, 415–418. (b) Abdel Rahman, A.A.-H., Magdy, A.; Zahran, M.A.; Abdel Megied, A.E.-S.; Pedersen, E.B.; and Nielsen, C.; 1996. Synthesis S^2-Alkyl-2-Thiouridines, *Synthesis*, **2004**. 237–241.
24. Brown, T.H.; Blakemore, R.C.; Durant, G.J.; Emmett, J.C.; Ganellin, C.R.; Parsons, M.E.; Rawlings, D.A.; Walker, T.F. Isocytosine H_2-Receptor Histamine Antagonists I. Oxmetidine and Related Compounds. *European Journal of Medicinal Chemistry* **1998**, 23, 53–62.
25. Slobodin, Y.M.; Shokhor, I.N. Structure of Gustavson's Hydrocarbon II. Stepwise Synthesis of Spiropentane. *Journal of General Chemistry* **1951**, 21, 2005–2011.
26. Sells, M.A.; Chen, M.L.; Acs, G. Production of Hepatitis B Virus Particles in Hep G2 Cells Transfected with Cloned Hepatitis B Virus DNA. *Proceedings of the National Academy of Sciences USA* **1987**, 84, 1005–1009.
27. Korba, B.E.; Gerin, J.L. Use of a Standardized Cell Culture Assay to Assess Activities of Nucleoside Analogs against Hepatitis B Virus. *Antiviral Research* **1992**, 19, 55–70.
28. Fouad, T.; Nielsen, C.; Brunn, L.; Pederson, E.B. Use of Standardization Cell Culture Assay to Assess Activities of Some Potent Anti-HIV Nucleoside Analogues against Hepatitis B Virus Replication. *Sc. J. Az. Med. Fac.*(GIRLS) **1998**, 19, 1173–1187.