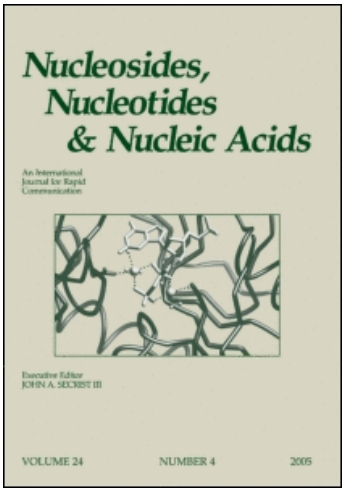


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis and Anti-HBV Activity of Thiouracils Linked via S and N-1 to the 5-Position of Methyl β-D-Ribofuranoside

Adel A. -H. Abdel-Rahman[a]; Ahmed E. -S. Abdel-Megied[a]; Adel E. -S. Goda[a]; Ibrahim F. Zeid[a]; El Sayed H. El Ashry[b]
[a] Department of Chemistry, Faculty of Science, Menoufia University, Shebin El-Koam, Egypt [b] Department of Chemistry, Faculty of Science, Alexandria University, Alexandria, Egypt

Online publication date: 27 October 2003

To cite this Article Abdel-Rahman, Adel A. -H. , Abdel-Megied, Ahmed E. -S. , Goda, Adel E. -S. , Zeid, Ibrahim F. and Ashry, El Sayed H. El(2003) 'Synthesis and Anti-HBV Activity of Thiouracils Linked via S and N-1 to the 5-Position of Methyl β-D-Ribofuranoside', Nucleosides, Nucleotides and Nucleic Acids, 22: 11, 2027 — 2038
To link to this Article: DOI: 10.1081/NCN-120026404
URL: http://dx.doi.org/10.1081/NCN-120026404

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 22, No. 11, pp. 2027–2038, 2003

Synthesis and Anti-HBV Activity of Thiouracils Linked via S and N-1 to the 5-Position of Methyl β-D-Ribofuranoside

Adel A.-H. Abdel-Rahman,[1] Ahmed E.-S. Abdel-Megied,[1] Adel E.-S. Goda,[1] Ibrahim F. Zeid,[1] and El Sayed H. El Ashry[2,*]

[1]Department of Chemistry, Faculty of Science, Menoufia University, Shebin El-Koam, Egypt
[2]Department of Chemistry, Faculty of Science, Alexandria University, Alexandria, Egypt

ABSTRACT

Reverse nucleoside derivatives of 2-(methylsulfanyl)uracils **6a-d** were prepared by treating of the sodium salt of 2-(methylsulfanyl)uracils (**5a-d**) with methyl 2,3-*O*-isopropylidene-5-*O*-*p*-toluenesulfonyl-β-D-ribofuranoside (**2**). The alkylation of 2-thiouracils **4a-d** with methyl 5-deoxy-5-iodo-2,3-*O*-isopropylidene-D-ribofuranoside (**3**) afforded the corresponding *S*-ribofuranoside derivatives **8a-d**. Deisopropylidenation of **6a-d** and **8a-d** afforded the corresponding deprotected derivatives **7a-d** and **9a-d**, respectively. The Anti-HBV activity of selected compounds was studied.

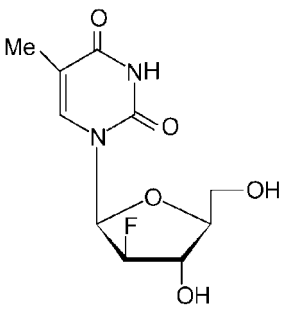
Key Words: Thiouracil; Ribofuranoside; Nucleoside.

*Correspondence: El Sayed H. El Ashry, Department of Chemistry, Faculty of Science, Alexandria University, Alexandria, Egypt; Fax: 20-3-4271360; E-mail: Eelashry@link.net; eelashry60@hotmail.com.

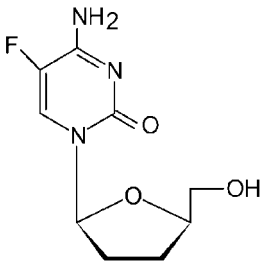
DOI: 10.1081/NCN-120026404
1525-7770 (Print); 1532-2335 (Online)

INTRODUCTION

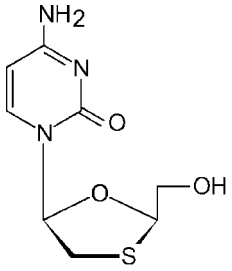
The wide prevalence of Hepatitis B virus (HBV) infection and the lack of an ideal drug to treat the virus, has a great degree of prominence. Vaccination is not an effective therapy in chronic infections that ended by cirrhosis of the liver and/or hepatocellular carcinoma. In this respect alpha interferon has demonstrated some promise.[1] 2′-Fluoro-5-methyl-β-L-arabino furanosyluracil (β-L-FMAU) is considered as a clinical candidate for treatment of chronic HBV infections.[2] 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. The unusual group of nucleosides such as L-SddC [(-)-BCH-189] in which the 3′-CH_2 group has been replaced by a heteroatom [3] exhibits potent anti-HBV and HIV activity in vitro.



β-L-FMAU



β-L-FddC



L-SddC
[(-)-BCH-189]

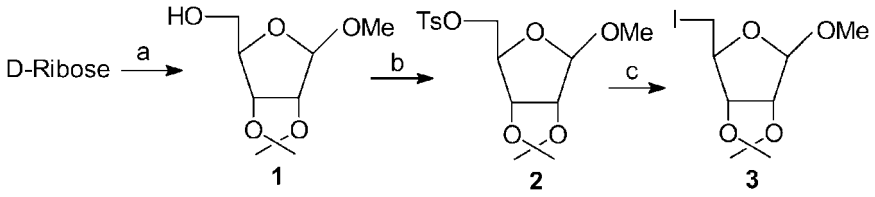
Thiated pyrimidinones, and their nucleosides are of considerable biological importance as components of the tRNA of various microorganism, yeasts and mammalian cells[4] and the corresponding nucleosides such as 4-thiouridine and 5-fluoro-4-thio-2′-deoxyuridine exhibit antineoplastic properties.[5] 2′3′-Dideoxy-3′-fluoro-4-thiopyrimidine[6] has potent and selective inhibition of the retrovirus HIV. Towards the development of nucleosides that exhibit antiviral activity, a number of *S*-alkylated 2-thiouracil[7,8] and acyclic nucleoside[9,10] analogues were synthesized.

The present work deals with the synthesis and biological activity of thiouracil analogs linked via their S or N-1 to the primary carbon atom (C-5) of methyl β-D-ribofuranoside. Such compounds which are known as isonucleosides or reversed nucleosides could be promising anti-HBV agents and/or metabolized into the active compounds after cell uptake.

RESULTS AND DISCUSSION

The preparation of methyl 2,3-*O*-isopropylidene-D-ribofuranoside (**1**) has attracted much attention give whether it is a mixture of anomers or has a β configuration.[11] The procedure for obtaining **1** has been slightly modified by treating a solution of dry D-ribose in dry acetone with 2,2-dimethoxypropane and dry methanol saturated with hydrogen chloride followed by neutralization with pyridine

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016



Scheme 1. a) Acetone/2,2-dimethoxypropane, MeOH/HCl; b) TsCl/Py; c) NaI/DMF.

to give **1** in 75% yield as a colorless oil. Treatment of **1** with *p*-toluenesulfonyl chloride in pyridine at room temperature afforded **2** in 80% yield (Sch. 1).[12]

The NMR spectra of methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside (**1**)[11] and methyl 2,3-*O*-isopropylidene-5-*O*-*p*-toluenesulfonyl-β-D-ribofuranoside (**2**)[12] showed conclusively, on the basis of the singlet signal for H-1, a justification for the assignment of the β configuration of these compounds.

Methyl 5-deoxy-5-iodo-2,3-*O*-isopropylidene-D-ribofuranoside (**3**)[13] was prepared by treating of **2** with sodium iodide in DMF at reflux temperature to afford **3** in 90 % yield [lit.[13] 76%].

Methyl 5-deoxy-5-[2-(methylsulfanyl)uracil-1-yl]-2,3-*O*-isopropylidene-β-D-ribofuranosides (**6a-d**) were prepared in 70–84% yields according to a slightly modified procedure of Holy[14] by the displacement of the tosyloxy group in **2** with sodium derivatives of **5a-d** (Sch. 2).[15]

Deprotection of the nucleosides **6a-d** with 80% acetic acid at reflux temperature gave the corresponding methyl 5-deoxy-5-[2-(methylsulfanyl)uracil-1-yl]-β-D-ribofuranosides (**7a-d**) in 68–75% yields. The structures were confirmed by studying ^{1}H and ^{13}C NMR spectra.

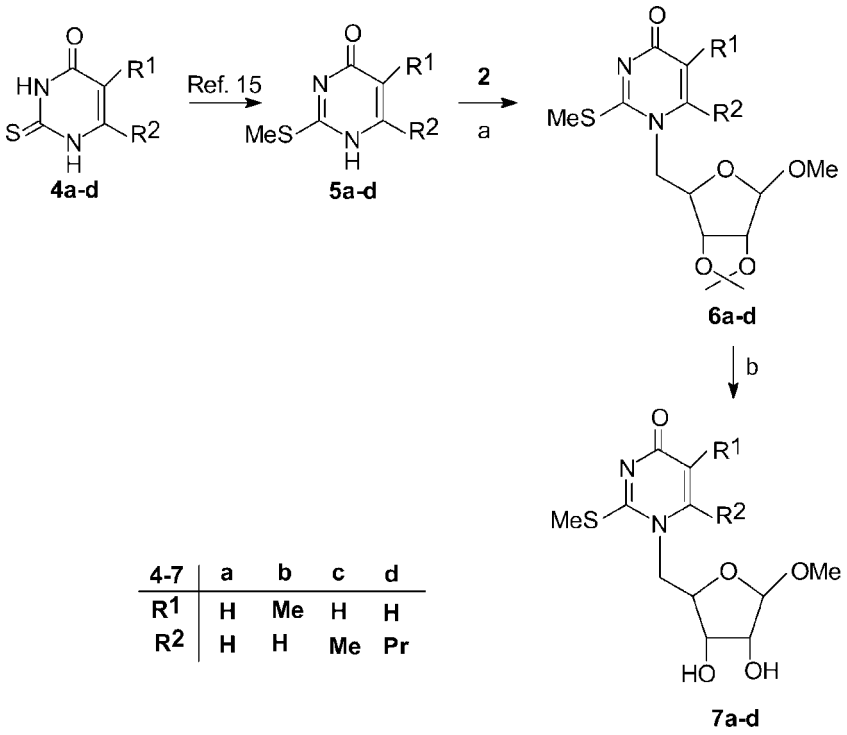
On the other hand, the alkylation of substituted 2-thiouracils **4a-d** with methyl 5-deoxy-5-iodo-2,3-*O*-isopropylidene-β-D-ribofuranoside (**3**)[13] in presence of sodium hydroxide in water and ethanol in a similar manner to that used for the alkylation of thiouracils[14] to afford the *S*-alkylated derivatives **8a-d** in 78–85% yields (Sch. 3). Their structures were established by ^{1}H NMR analysis which showed the chemical shifts of *S*-CH_2 in the range δ 3.13–3.22 in accordance with reported[15] for other analogues.

Deprotection of **8a-d** with 80% acetic acid at reflux temperature afforded the corresponding deprotected derivatives **9a-d** in 70–75% yields. Their ^{1}H NMR spectra showed the disappearance of the isopropylidene group in all cases.

Preliminary viral screening against HBV indicated that compound **7b** was found to be active against HBV replication with $IC_{50} = 90\,\mu M$ and $CC_{50} > 100\,\mu M$. Compounds **7a**, **7c**, **7d**, **9c** and **9d** showed moderate viral replication inhibition and low cytotoxicity, while compounds **9a** and **9b** showed high inhibition with high cytotoxicity (Table 1).

EXPERIMENTAL

All m.p.'s are uncorrected. Pyridine was distilled from CaH_2 and stored over molecular sieves. Other solvents were purified according to the standard procedures.

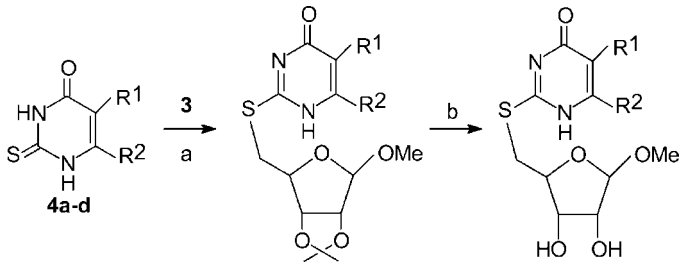


Scheme 2. a) NaH/DMF; b) 80% AcOH, reflux.

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. 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

Table 1. Inhibition of HBV replication by selected compounds.

	Inhibition (%)			
Compound	1 Week	2 Weeks	3 Weeks	Cytotoxicity (%)
7a	25.30	14.20	08.40	03.30
7b	90.04	81.10	70.70	01.05
7c	15.80	18.60	−10.90	03.10
7d	30.50	25.90	17.70	05.50
9a	88.50	87.90	71.30	06.10
9b	86.60	85.90	73.30	05.30
9c	38.60	30.60	13.40	04.10
9d	27.70	25.30	−03.80	04.00



4,8,9	a	b	c	d
R^1	H	Me	H	H
R^2	H	H	Me	Pr

Scheme 3. a) NaOH/EtOH/H_2O; b) 80% AcOH, reflux.

NMR with TMS as an internal standard. EI MS spectra were recorded with Finnigen MAT 312/AMD. The microanalyses were performed at the microanalytical unit, University Konstanz, Germany. Viral screening against HBV was conducted at the National Liver Institute, Menoufia University, Egypt.

Methyl 2,3-*O*-Isopropylidene-β-D-ribofuranoside (1). A solution of 20.0 g (133.3 mmol) of dry D-ribose, in 400 mL of dry acetone, 40 mL of 2,2-dimethoxypropane and 80 mL of methanol containing 8 mL of methanol saturated with hydrogen chloride was stirred for 5 h at 0°C. The reaction mixture was stirred at 25°C for overnight. The resulting orange solution was neutralized with pyridine and evaporated to a yellow oil. This oil was partitioned between 200 mL of water and 100 mL of ether. The water layer was extracted twice with 100 mL portions of ether, and the combined ether extracts were dried over Na_2SO_4. Evaporation under reduced pressure yielded a pale yellow oil which was distilled at 0.3 mm and 80°C to give 25 g, 75% [lit.[11] 70%] of a colorless oil.

Methyl 2,3-*O*-Isopropylidene-5-*O*-*p*-toluenesulfonyl-β-D-ribofuranoside (2). *p*-Toluenesulfonyl chloride (9.52 g, 50 mmol) was added in small portions to a stirred cold solution of **1** (10 g, 49 mmol) in dry pyridine (100 mL). The reaction mixture was stirred for overnight at room temperature. The solvent was evaporated and coevaporated with toluene (3×20 mL). The residue was dissolved in water (100 mL) and extracted with dichloromethane (3×50 mL). The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to give **2** (14.04 g, 80%). M.p. 82–84°C [lit.[12] 83–84°C]. ^{1}H NMR ($CDCl_3$): $\delta = 1.28$ (s, 3 H, CH_3), 1.44 (s, 3 H, CH_3), 2.45 (s, 3 H, CH_3), 3.22 (s, 3 H, OMe), 4.01 (d, 2 H, $J_{4,5} = 6.5$ Hz, H-5), 4.31 (d, 1 H, $J_{3,4} = 7.0$ Hz, H-4), 4.53 (d, 1 H, $J_{2,3} = 5.9$ Hz, H-3), 4.60 (d, 1 H, $J_{2,3} = 5.9$ Hz, H-2), 4.92 (s, 1 H, H-1), 7.36 (d, 2 H, $J = 7.9$ Hz, Ar-H), 7.80 (d, 2 H, $J = 8.3$ Hz, Ar-H). ^{13}C NMR ($CDCl_3$): $\delta = 21.5$ (CH_3), 24.7 (CH_3), 26.2 (CH_3), 54.9 (OMe), 69.2 (C-5), 81.3 (C-4), 83.5 (C-3), 84.8 (C-2), 109.4 (C-1), 112.6 ($C\,Me_2$), 127.9, 129.9, 132.7, 145.0 (Ar-C).

Methyl 5-Deoxy-5-iodo-2,3-*O*-isopropylidene-D-ribofuranoside (3). A stirred solution of the tosyl derivative **2** (5 g, 14 mmol) in 50 mL of dry DMF was refluxed with dry sodium iodide 2.37 g (15.5 mmol) for 40 min. The mixture was then cooled and filtered from inorganic precipitate. The latter was washed with ether (3×50 mL) and the combined filtrates were evaporated under reduced pressure in 80°C bath to a volume of about 15 mL. To this solution was added 200 mL of water and the mixture was extracted with ether (5×20 mL). The combined extracts were dried over Na_2SO_4, filtered and evaporated under reduced pressure in an 80–90°C bath to give 3.95 g, 90% [lit. (13) 76%] as a colorless liquid. ^{1}H NMR ($CDCl_3$): $\delta = 1.32$ (s, 3 H, CH_3), 1.48 (s, 3 H, CH_3), 3.16 (dd, 1 H, $J_{4',5a} = 6.1$Hz, $J_{5a,5b} = 9.9$ Hz, H-5_a), 3.28 (dd, 1 H, $J_{4,5} = 6.1$ Hz, H-5_b), 3.36 (s, 3 H, OMe), 4.46 (d, 1 H, $J_{3,4} = 6.1$, Hz, H-4), 4.63 (d, 1 H, $J_{2,3} = 5.8$ Hz, H-3), 4.77 (d, 1 H, $J_{2,3} = 5.8$ Hz, H-2), 5.05 (s, 1 H, H-1). ^{13}C NMR ($CDCl_3$): $\delta = 6.6$ (C-5), 25.0 (CH_3), 24.4 (CH_3), 55.2 (OMe), 83.0 (C-4), 85.3 (C-3), 87.4 (C-2), 109.7 (C-1), 112.6 (CMe$_2$).

General Procedure for the Preparation of 6a-d. To a stirred suspension of 2-(methylsulfanyl)uracils (**5a-d**) (5 mmol) in dry DMF (20 mL) was added NaH (0.12 g, 5 mmol) and after almost complete evolving of hydrogen, the mixture was heated to 80°C for 1 h. Then compound **2** (1.79 g, 5 mmol) was added, the reaction mixture was stirred for additional 10–12 h (TLC) at 80°C, cooled to room temperature and filtered. The mixture was evaporated to dryness at reduced pressure and purified with silica gel column chromatography using 2% MeOH in $CHCl_3$ to give **6a-d** in 70–84% yields.

Methyl 5-Deoxy-5-[2-(methylsulfanyl)uracil-1-yl]-2,3-*O*-isopropylidene-β-D-ribofuranosides (6a). Yield = 1.23 g, 75% as a white foam. ^{1}H NMR ($CDCl_3$): $\delta = 1.32$ (s, 3 H, CH_3), 1.48 (s, 3 H, CH_3), 2.53 (s, 3 H, SCH_3), 3.31 (s, 3 H, OMe), 4.38–4.41 (m, 2 H, H-5′), 4.46–4.52 (m, 1 H, H-4′), 4.64 (d, 1 H, $J_{2',3'} = 5.9$ Hz, Hz, H-3′), 4.77 (d, 1 H, $J_{2',3'} = 5.9$ Hz, H-2′), 5.00 (s, 1 H, H-1′), 6.44 (d, 1 H, $J_{5,6} = 5.0$ Hz, H-5), 8.23 (d, 1 H, $J_{5,6} = 4.9$ Hz, H-6). ^{13}C NMR ($CDCl_3$): $\delta = 13.6$ (SCH_3), 24.6 (CH_3), 26.1 (CH_3), 54.4 (OMe), 66.1 (C-5′), 81.5 (C-4′), 83.8 (C-3′), 84.8 (C-2′), 103.3 (C-1′), 109.0 (C-5), 112.6 (CMe$_2$), 157.1 (C-6), 167.7 (C-2), 171.7 (C-4). EI-MS: (positive mode); m/z: 328 [M^+]. Anal. Calcd for $C_{14}H_{20}N_2O_5S$ (328.38): C 51.20, H 6.13, N 8.53. Found: C 51.00, H 6.15, N 8.48.

Methyl 5-Deoxy-5-[5-methyl-2-(methylsulfanyl)uracil-1-yl]-2,3-*O*-isopropylidene-β-D-ribofuranosides (6b). Yield = 1.43 g, 84% as a white foam. ^{1}H NMR ($CDCl_3$): $\delta = 1.33$ (s, 3 H, CH_3), 1.50 (s, 3 H, CH_3), 2.09 (s, 3 H, CH_3), 2.52 (s, 3 H, SCH_3), 3.32 (s, 3 H, OMe), 4.43 (d, 2 H, $J_{4',5'} = 6.3$ Hz, H-5′), 4.54 (d, 1 H, $J_{3',4'} = 6.5$ Hz, H-4′), 4.64 (d, 1 H, $J_{2',3'} = 5.9$ Hz, H-3′), 4.77 (d, 1 H, $J_{2',3'} = 5.9$ Hz, H-2′), 5.01 (s, 1 H, H-1′), 8.07 (s, 1 H, H-6). ^{13}C NMR ($CDCl_3$): $\delta = 11.7$ (CH_3), 13.6 (SCH_3), 24.6 (CH_3), 26.1 (CH_3), 54.3 (OMe), 66.1 (C-5′), 81.5 (C-4′), 83.9 (C-3′), 84.9 (C-2′), 109.1 (C-1′), 112.1 (C-5), 112.5 (CMe$_2$), 156.5 (C-6), 166.3 (C-2), 168.5 (C-4). EI-MS: (positive mode); m/z: 342 [M^+]. Anal. Calcd for $C_{15}H_{22}N_2O_5S$ (342.40): C 52.61, H 6.47, N 8.18. Found: C 52.50, H 6.36, N 7.99.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

Methyl 5-Deoxy-5-[6-methyl-2-(methylsulfanyl)uracil-1-yl]-2,3-*O*-isopropylidene-β-D-ribofuranosides (6c). Yield = 1.37 g, 80% as a white foam. ^{1}H NMR ($CDCl_3$): δ = 1.33 (s, 3 H, CH_3), 1.49 (s, 3 H, CH_3), 2.36 (s, 3 H, CH_3), 2.53 (s, 3 H, SCH_3), 3.32 (s, 3 H, OMe), 4.32–4.38 (m, 2 H, H-5′), 4.45–4.54 (m, 1 H, H-4′), 4.63 (d, 1 H, $J_{2',3'}$ = 5.9 Hz, H-3′), 4.75 (d, 1 H, $J_{2',3'}$ = 5.8 Hz, H-2′), 5.01 (s, 1 H, H-1′), 6.27 (s, 1 H, H-5). ^{13}C NMR ($CDCl_3$): δ = 13.4 (SCH_3), 23.2 (CH_3), 24.5 (CH_3), 25.9 (CH_3), 54.2 (OMe), 65.8 (C-5′), 81.4 (C-4′), 83.8 (C-3′), 84.7 (C-2′), 101.5 (C-1′), 108.9 (C-5), 112.0 (*C*Me_2), 167.4 (C-6), 168.2 (C-2), 170.9 (C-4). EI-MS: (positive mode); *m/z*: 342 [M^+]. Anal. Calcd for $C_{15}H_{22}N_2O_5S$ (342.40): C 52.61, H 6.47, N 8.18. Found: C 52.52, H 6.40, N 8.02.

Methyl 5-Deoxy-5-[2-(methylsulfanyl)-6-propyluracil-1-yl]-2,3-*O*-isopropylidene-β-D-ribofuranosides (6d). Yield = 1.29 g, 70% as a white foam. ^{1}H NMR ($CDCl_3$): δ = 0.95 (t, 3 H, *J* = 7.4 Hz, CH_3), 1.33 (s, 3 H, CH_3), 1.50 (s, 3 H, CH_3), 1.63–1.75 (m, 2 H, CH_2), 2.53 (s, 3 H, SCH_3), 2.55–2.58 (m, 2 H, CH_2), 3.32 (s, 3 H, OMe), 4.36–4.40 (m, 2 H, H-5′), 4.49–4.52 (m, 1 H, H-4′), 4.63 (d, 1 H, $J_{2',3'}$ = 5.9 Hz, H-3′), 4.76 (d, 1 H, $J_{2',3'}$ = 5.9 Hz, H-2′), 5.01 (s, 1 H, H-1′), 6.26 (s, 1 H, H-5). ^{13}C NMR ($CDCl_3$): δ = 13.7 (CH_3), 14.0 (SCH_3), 21.7 (CH_2), 25.0 (CH_3), 26.4 (CH_3), 39.4 (CH_2), 54.8 (OMe), 66.2 (C-5′), 81.9 (C-4′), 84.3 (C-3′), 85.2 (C-2′), 101.5 (C-1′), 109.3 (C-5), 112.6 (*C*Me_2), 168.7 (C-6), 171.2 (C-2), 171.7 (C-4). EI-MS: (positive mode); *m/z*: 370 [M^+]. Anal. Calcd for $C_{17}H_{26}N_2O_5S$ (370.46): C 55.11, H 7.07, N 7.56. Found: C 54.97, H 6.95, N 7.47.

General Procedure for the Preparation of 7a-d. Compounds **6a-d** (0.25 g) were refluxed in 80% aqueous acetic acid (10 mL) for 2 h. The solvents were removed in vacuo. Water (4 × 5 mL) and then EtOH (3 × 5 mL) were coevaporated from the remaining residue. The residue was purified by column chromatography using 5% MeOH in $CHCl_3$ to give **7a-d** in 68-75% yields.

Methyl 5-Deoxy-5-[2-(methylsulfanyl)uracil-1-yl]-β-D-ribofuranoside (7a). Yield = 0.16 g, 73%. M.p. = 160°C. ^{1}H NMR ($CDCl_3$): δ = 2.50 (s, 3 H, SCH_3), 3.37 (s, 3 H, OMe), 4.41–4.51 (m, 3 H, H-4′, H-5′), 4.66–4.78 (m, 2 H, H-2′, H-3′), 5.05 (s, 1 H, H-1′), 5.23 (brs, 1 H, 3′-OH), 5.58 (brs, 1 H, 2′-OH), 6.48 (d, 1 H, $J_{5,6}$ = 5.0 Hz, H-5), 8.22 (d, 1 H, $J_{5,6}$ = 5.0 Hz, H-6). ^{13}C NMR ($CDCl_3$): δ = 13.4 (SCH_3), 54.5 (OMe), 66.2 (C-5′), 81.5 (C-4′), 84.1 (C-3′), 86.6 (C-2′), 104.3 (C-1′), 108.0 (C-5), 158.1 (C-6), 168.1 (C-2), 172.1 (C-4). EI-MS: (positive mode); *m/z*: 288 [M^+]. Anal. Calcd for $C_{11}H_{16}N_2O_5S$ (288.31): C 45.82, H 5.59, N 9.71. Found: C 45.63, H 5.45, N 9.59.

Methyl 5-Deoxy-5-[5-methyl-2-(methylsulfanyl)uracil-1-yl]-β-D-ribofuranosides (7b). Yield = 0.17 g, 75%. M.p. = 171°C. ^{1}H NMR ($CDCl_3$): δ = 2.10 (s, 3 H, CH_3), 2.51 (s, 3 H, SCH_3), 3.35 (s, 3 H, OMe), 4.46–4.58 (m, 3 H, H-4′, H-5′), 4.70 (d, 1 H, $J_{2',3'}$ = 5.9 Hz, H-3′), 4.80 (d, 1 H, $J_{2',3'}$ = 5.9 Hz, H-2′), 5.01 (s, 1 H, H-1′), 5.30 (brs, 1 H, 3′-OH), 5.35 (brs, 1 H, 2′-OH), 8.00 (s, 1 H, H-6). ^{13}C NMR ($CDCl_3$): δ = 11.7 (CH_3), 13.5 (SCH_3), 54.3 (OMe), 66.2 (C-5′), 81.5 (C-4′), 83.4 (C-3′), 84.5 (C-2′), 108.9 (C-1′), 111.0 (C-5), 155.5 (C-6), 167.0 (C-2), 169.0 (C-4).

EI-MS: (positive mode); m/z: 302 [M^+]. Anal. Calcd for $C_{12}H_{18}N_2O_5S$ (302.34): C 47.67, H 6.00, N 9.26. Found: C 47.50, H 5.91, N 9.12.

Methyl 5-Deoxy-5-[6-methyl-2-(methylsulfanyl)uracil-1-yl]-β-D-ribofuranosides (7c). Yield = 0.15 g, 70%. M.p. = 143°C. ^{1}H NMR ($CDCl_3$): δ = 2.30 (s, 3 H, CH_3), 2.52 (s, 3 H, SCH_3), 3.35 (s, 3 H, OMe), 4.40–4.58 (m, 3 H, H-4′, H-5′), 4.68 (d, 1 H, $J_{2',3'}$ = 5.9 Hz, H-3′), 4.72 (d, 1 H, $J_{2',3'}$ = 5.8 Hz, H-2′), 5.05 (s, 1 H, H-1′), 5.29 (brs, 1 H, 3′-OH), 5.59 (brs, 1 H, 2′-OH), 6.30 (s, 1 H, H-5). ^{13}C NMR ($CDCl_3$): δ = 13.4 (SCH_3), 25.9 (CH_3), 54.2 (OMe), 65.8 (C-5′), 81.0 (C-4′), 83.2 (C-3′), 84.5 (C-2′), 102.0 (C-1′), 107.8 (C-5), 168.2 (C-6), 169.1 (C-2), 171.0 (C-4). EI-MS: (positive mode); m/z: 302 [M^+]. Anal. Calcd for $C_{12}H_{18}N_2O_5S$ (302.34): C 47.67, H 6.00, N 9.26. Found: C 47.47, H 5.89, N 9.15.

Methyl 5-Deoxy-5-[2-(methylsulfanyl)-6-propyluracil-1-yl]-β-D-ribofuranosides (7d). Yield = 0.15 g, 68%. M.p. = 166°C. ^{1}H NMR ($CDCl_3$): δ = 0.90 (t, 3 H, J = 7.4 Hz, CH_3), 1.60–1.74 (m, 2 H, CH_2), 2.50 (s, 3 H, SCH_3), 2.65–2.73 (m, 2 H, CH_2), 3.35 (s, 3 H, OMe), 4.40–4.50 (m, 3 H, H-4′, H-5′), 4.68 (d, 1 H, $J_{2',3'}$ = 5.9 Hz, H-3′), 4.78 (d, 1 H, $J_{2',3'}$ = 5.9 Hz, H-2′), 5.06 (s, 1 H, H-1′), 5.31 (brs, 1 H, 3′-OH), 5.63 (brs, 1 H, 2′-OH), 6.30 (s, 1 H, H-5). ^{13}C NMR ($CDCl_3$): δ = 13.7 (CH_3), 13.9 (SCH_3), 21.8 (CH_2), 39.3 (CH_2), 54.5 (OMe), 66.5 (C-5′), 81.8 (C-4′), 83.8 (C-3′), 84.5 (C-2′), 102.0 (C-1′), 108.3 (C-5), 169.0 (C-6), 172.1 (C-2), 172.4 (C-4). EI-MS: (positive mode); m/z: 330 [M^+]. Anal. Calcd for $C_{14}H_{22}N_2O_5S$ (330.39): C 50.89, H 6.71, N 8.47. Found: C 50.67, H 6.60, N 8.33.

General Procedure for the Preparation of 8a-d. A solution of substituted 2-thiouracils (**4a-d**) (10 mmol), **3** (3.14 g, 10 mmol) and sodium hydroxide (0.4 g, 10 mmol) in water (20 mL) and ethanol (40 mL) was stirred at 60°C for 1 h. The mixture was allowed to cool. The solvents were removed under reduced pressure. The residue was purified by column chromatography using 3% MeOH in $CHCl_3$ to give **8a-d** in 78–85% yields.

Methyl 5-Deoxy-2,3-*O*-isopropylidene-5-(uracil-2-yl)-thio-β-D-ribofuranosides (8a). Yield = 2.57 g, 82% as a white foam. ^{1}H NMR ($CDCl_3$): δ = 1.33 (s, 3 H, CH_3), 1.50 (s, 3 H, CH_3), 3.13–3.20 (m, 2 H, H-5′), 3.32 (s, 3 H, OMe), 4.46–4.55 (m, 1 H, H-4′), 4.62 (d, 1 H, $J_{2',3'}$ = 5.9 Hz, H-3′), 4.78 (d, 1 H, $J_{2',3'}$ = 5.9 Hz, H-2′), 5.00 (s, 1 H, H-1′), 6.40 (d, 1 H, $J_{5,6}$ = 5.0 Hz, H-5), 8.22 (d, 1 H, $J_{5,6}$ = 4.9 Hz, H-6). EI-MS: (positive mode); m/z: 314 [M^+]. Anal. Calcd for $C_{13}H_{18}N_2O_5S$ (314.35): C 49.67, H 5.77, N 8.91. Found: C 49.50, H 5.81, N 8.99.

Methyl 5-Deoxy-2,3-*O*-isopropylidene-5-(5-methyluracil-2-yl)-thio-β-D-ribofuranosides (8b). Yield = 2.78 g, 85% as a white foam. ^{1}H NMR ($CDCl_3$): δ = 1.31 (s, 3 H, CH_3), 1.50 (s, 3 H, CH_3), 2.05 (s, 3 H, CH_3), 3.14–3.20 (m, 2 H, H-5′), 3.32 (s, 3 H, OMe), 4.54 (m, 1 H, H-4′), 4.69 (d, 1 H, $J_{2',3'}$ = 5.9 Hz, H-3′), 4.81 (d, 1 H, $J_{2',3'}$ = 5.9 Hz, H-2′), 5.01 (s, 1 H, H-1′), 8.05 (s, 1 H, H-6). EI-MS: (positive mode); m/z: 328 [M^+]. Anal. Calcd for $C_{14}H_{20}N_2O_5S$ (328.38): C 51.20, H 6.13, N 8.53. Found: C 51.00, H 6.09, N 8.41.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

Methyl 5-Deoxy-2,3-*O*-isopropylidene-5-(6-methyluracil-2-yl)-thio-β-D-ribofuranosides (8c). Yield = 2.62 g, 80% as a white foam. ^{1}H NMR ($CDCl_3$): δ = 1.31 (s, 3 H, CH_3), 1.50 (s, 3 H, CH_3), 2.30 (s, 3 H, CH_3), 3.13–3.19 (m, 2 H, H-5′), 3.30 (s, 3 H, OMe), 4.45–4.55 (m, 1 H, H-4′), 4.65 (d, 1 H, $J_{2',3'}$ = 5.9 Hz, H-3′), 4.78 (d, 1 H, $J_{2',3'}$ = 5.8 Hz, H-2′), 5.00 (s, 1 H, H-1′), 6.25 (s, 1 H, H-5). EI-MS: (positive mode); *m/z*: 328 [M^+]. Anal. Calcd for $C_{14}H_{20}N_2O_5S$ (328.38): C 51.20, H 6.13, N 8.53. Found: C 51.07, H 6.18, N 8.61.

Methyl 5-Deoxy-2,3-*O*-isopropylidene-5-(6-propyluracil-2-yl)-thio-β-D-ribofuranosides (8d). Yield = 2.81 g, 78% as a white foam. ^{1}H NMR ($CDCl_3$): δ = 0.95 (t, 3 H, J = 7.5 Hz, CH_3), 1.33 (s, 3 H, CH_3), 1.50 (s, 3 H, CH_3), 1.66–1.75 (m, 2 H, CH_2), 2.55–2.65 (m, 2 H, CH_2), 3.15–3.22 (m, 2 H, H-5′), 3.31 (s, 3 H, OMe), 4.49–4.57 (m, 1 H, H-4′), 4.65 (d, 1 H, $J_{2',3'}$ = 5.9 Hz, H-3′), 4.78 (d, 1 H, $J_{2',3'}$ = 5.9 Hz, H-2′), 5.01 (s, 1 H, H-1′), 6.25 (s, 1 H, H-5). EI-MS: (positive mode); *m/z*: 360 [M^+]. Anal. Calcd for $C_{16}H_{24}N_2O_5S$ (360.43): C 53.31, H 6.71, N 7.77. Found: C 53.11, H 6.67, N 7.63.

General Procedure for the Preparation of 9a-d. Compounds **8a-d** (0.25 g) were refluxed in 80% aqueous acetic acid (10 mL) for 2 h. The solvents were removed in vacuo and were coevaporated with water (4 × 5 mL) and EtOH (3 × 5 mL). The residue was purified by column chromatography using 6% MeOH in $CHCl_3$ to give **9a-d** in 70–75% yields.

Methyl 5-Deoxy-5-(uracil-2-yl)-thio-β-D-ribofuranosides (9a). Yield = 0.15 g, 72%. M.p. = 110°C. ^{1}H NMR ($CDCl_3$): δ = 3.22–3.30 (m, 2 H, H-5′), 3.35 (s, 3 H, OMe), 4.40–4.50 (m, 1 H, H-4′), 4.66–4.76 (m, 2 H, H-2′, H-3′), 5.05 (s, 1 H, H-1′), 5.25 (brs, 1 H, 3′-OH), 5.60 (brs, 1 H, 2′-OH), 6.40 (d, 1 H, $J_{5,6}$ = 5.0 Hz, H-5), 8.20 (d, 1 H, $J_{5,6}$ = 5.0 Hz, H-6). EI-MS: (positive mode); *m/z*: 274 [M^+]. Anal. Calcd for $C_{10}H_{14}N_2O_5S$ (274.29): C 43.78, H 5.14, N 10.21. Found: C 43.61, H 5.03, N 10.11.

Methyl 5-Deoxy-5-(5-methyluracil-2-yl)-thio-β-D-ribofuranosides (9b). Yield = 0.16 g, 75%. M.p. = 152°C. ^{1}H NMR ($CDCl_3$): δ = 2.11 (s, 3 H, CH_3), 3.12–3.18 (m, 2 H, H-5′), 3.31 (s, 3 H, OMe), 4.46–4.56 (m, 1 H, H-4′), 4.75 (d, 1 H, $J_{2',3'}$ = 5.9 Hz, H-3′), 4.86 (d, 1 H, $J_{2',3'}$ = 5.9 Hz, H-2′), 5.01 (s, 1 H, H-1′), (brs, 1 H, 3′-OH), 5.62 (brs, 1 H, 2′-OH), 8.02 (s, 1 H, H-6). EI-MS: (positive mode); *m/z*: 288 [M^+]. Anal. Calcd for $C_{11}H_{16}N_2O_5S$ (288.31): C 45.82, H 5.59, N 9.71. Found: C 45.70, H 5.61, N 9.80.

Methyl 5-Deoxy-5-(6-methyluracil-2-yl)-thio-β-D-ribofuranosides (9c). Yield = 0.15 g, 71%. M.p. = 123°C. ^{1}H NMR ($CDCl_3$): δ = 2.35 (s, 3 H, CH_3), 3.20–3.30 (m, 2 H, H-5′), 3.31 (s, 3 H, OMe), 4.40–4.50 (m, 1 H, H-4′), 4.62 (d, 1 H, $J_{2',3'}$ = 5.9 Hz, H-3′), 4.71 (d, 1 H, $J_{2',3'}$ = 5.8 Hz, H-2′), 5.01 (s, 1 H, H-1′), (brs, 1 H, 3′-OH), 5.58 (brs, 1 H, 2′-OH), 6.27 (s, 1 H, H-5). EI-MS: (positive mode); *m/z*: 288 [M^+]. Anal. Calcd for $C_{11}H_{16}N_2O_5S$ (288.31): C 45.82, H 5.59, N 9.71. Found: C 45.75, H 5.41, N 9.60.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Methyl 5-Deoxy-5-(6-propyluracil-2-yl)-thio-β-D-ribofuranosides (9d). Yield = 0.15 g, 70% as awhite foam. ^{1}H NMR ($CDCl_3$): $\delta = 0.98$ (t, 3 H, $J = 7.3$ Hz, CH_3), 1.60–1.75 (m, 2 H, CH_2), 2.50–2.64 (m, 2 H, CH_2), 3.20–3.28 (m, 2 H, H-5′), 3.31 (s, 3 H, OMe), 4.40–4.53 (m, 1 H, H-4′), 4.63 (d, 1 H, $J_{2',3'} = 5.9$ Hz, H-3′), 4.73 (d, 1 H, $J_{2',3'} = 5.9$ Hz, H-2′), 5.02 (s, 1 H, H-1′), 5.30 (brs, 1 H, 3′-OH), 5.60 (brs, 1 H, 2′-OH), 6.31 (s, 1 H, H-5). EI-MS: (positive mode); m/z: 316 [M^+]. Anal. Calcd for $C_{13}H_{20}N_2O_5S$ (316.39): C 49.35, H 6.37, N 8.85. Found: C 49.28, H 6.26, N 8.64.

Biological Activity Studies. The hepatoplastoma cell line (Hep G2-2.2.15) was used to evaluate the antiviral effect of the tested compounds against HBV.[16] 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. Quantition of HBV-DNA was done using a semiquantitative nested PCR followed by DIG PCR ELISA as previously described.[17] The cytotoxic effect of the compounds was accessed by culturing the Hep G2-2.2.15 cells in the presence of compounds as described for the viability of their cells were analyzed using a MTT-assay.

ACKNOWLEDGMENTS

The authors thank DANIDA for the support of this work at Menoufia University, through the project "Development of New Drugs Against Hepatitis" and Prof. Dr. R. R. Schmidt for making available the spectral measurements at Konstanz University.

REFERENCES

1. 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-arabinofuranosyl uracil. Antimicrob. Agents Chemother. **1996**, *40*, 380.
2. Ma, T.; Lin, J.-S.; Newton, M.G.; Cheng, Y.-C.; Chu, C.K. Synthesis ans anti-hepatitis B virus activity of 9-(2-deoxy-2-fluoro-β-L-arabinofuranosyl)purine nucleosides. J. Med. Chem. **1997**, *40*, 2750.
3. 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-hydroxymethyloxathiolan-5-yl)cytosine as a potent antiviral agent against hepatitis B virus (HBV) and human immunodeficiency virus (HIV). J. Org. Chem. **1992**, *57*, 2217; Jeong, L.S.; Alves, A.J.; Carrigan, S.W.; Kim, H.O.; Beach, C.-W.; Chu, C.K. An efficient synthesis of enantiomerically pure (+)-(2S, 5R)-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-

yl]cytosine[(+)-BCH-189] from D-galactose. Tetrahedron Lett. **1992**, *33*, 595; Coates, J.A.V.; Cammack, N.S.; Jenkinson, H.J.; Mutton, I.M.; Pearson, B.A.; Storer, R.; Cameron, J.M.; Penn, C.R. The separated enantiomers of 2′-deoxy-3′-thiacytidine (BCH 189) both inhibit human immunodeficiency virus replication in vitro. Antimicrob. Agents Chemother. **1992**, *36*, 202; Grove, K.L.; Guo, X.; Liu, S.-H.; Gao, Z.; Chu, C.K.; Cheng, Y.-C. Anticancer activity of β-L-dioxolane-cytidine, a novel nucleoside analogue with the unnatural L configuration. Cancer Res. **1995**, *55*, 3008.

4. Ajitkumar, P.; Cherayil, J.D. Thionucleosides in transfer ribonucleic acid: Diversity, structure, biosynthesis and function. Chem. Rev. **1988**, *52*, 103.
5. Kulikowski, T.; Bretmer, M.; Dzik, J.M.; Zielinski, Z.; Ciesla, J.; Roda, W.; Vilpo, J.A.; Shugar, D. Synthesis and antimetabolic properties of 4,5-substituted pyrimidine 2′-deoxynucleosides and their 5′-monophosphates. Nuc. Acid. Res. Symp. Ser. **1987**, *18*, 61.
6. Matthes, E.; Lehmann, C.; Von Janta-Lipinski, M.; Scholz, D. Inhibition of HIV-replication by 3′-fluoro-modified nucleosides with low cytotoxicity. Biochim. Biophys. Res. Commun. **1989**, *165*, 488.
7. Zahran, M.A.; Abdel-Megeid, A.E.-S.; Abdel-Rahman, A.A.-H.; El-Emam, A.; Pedersen, E.B.; Nielsen, C. Synthesis of 2-alkylthio analogues of AZT and their activity against HIV-1. Heterocycles **1995**, *41*, 2507; Zahran, M.A.; Abdel-Megied, A.E.-S.; Abdel-Rahman, A.A.-H.; Sofan, M.A.; Pedersen, E.B.; Nielsen, C. Synthesis of 3′-deoxy-3′-fluoro and 3′-amino nucleosides from 2-methylthiopyrimidin-4(1H)-ones. Monatsh. Chem. **1996**, *127*, 979; Abdel-Rahman, A.A.-H.; Zahran, M.A.; Abdel-Megied, A.E.-S.; Pedersen, E.B.; Nielsen, C. Synthesis of S^2-alkyl-2-thiouridines. Synthesis **1996**, *2*, 237.
8. Abdel-Rahman, A.A.-H. Synthesis of glucosylated 2-methylthiouracils as new antiviral agents. Afinidad **1997**, *468*, 135; Synthesis of some 2-methylthiouracil nucleosides and its 5-halo analogues of 2-acetamido-2-deoxy-D-glucose. Pharmazie **2001**, *56*, 773; Abdel-Rahman, A.A.-H.; Abdel-Bary, H.M.; Pedersen, E.B.; Nielsen, C. Synthesis of 4-methylthio analogues of FLT and AZT and their evaluation against HIV. Arch. Pharm. **1995**, *328*, 67; Abdel-Rahman, A.A.-H.; Abdel-Aal, M.T. Synthesis of (S)-1-(2′,3′-dihydroxypropyl)-2-alkylthiouracils as new antiviral agents. Pharmazie **1998**, *53*, 377; Zeid, I.F.; Abdel-Rahman, A.A.-H.; Abdel-Megied, A.E.-S.; El-Etrawy, A.S. Synthesis of new thiolated acyclonucleosides with potential anti-HBV activity. Nucleosides & Nucleotides **1999**, *18*, 95; Abdel-Rahman, A.A.-H.; Abdel-Aal, M.T. Synthesis of 2′,3′-dideoxynucleosides from 2-(methylsulfanyl)uracils. J. Chem. Res. (S) **2002**, 251, (M) **2002**, 601.
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. Arch. Pharm. **1999**, *332*, 327; Homoacyclovir analogues of unnatural bases and their activity against hepatitis B virus. Pharmazie **1999**, *54*, 893.
10. El Ashry, E.S.H.; El Kilany, Y. Acyclonucleosides: Part 1. *seco*-Nucleosides. Adv. Heterocycl. Chem. **1996**, *67*, 391; Acyclonucleosides: Part 2. *diseco*-Nucleosides **1997**, *68*, 1; Acyclonucleosides: Part 3. *tri-, tetra-, and pentaseco*-Nucleosides **1998**, *69*, 129.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

11. Reist, E.J.; Spencer, R.R.; Wain, M.E.; Junga, I.G.; Goodman, L.; Baker, B.R. Potential anticancer agents. LVII. Synthesis of alkylating agents derived from 6-amino-6-deoxy-D-glucose and 5-amino-5-deoxy-D-ribose. J. Org. Chem. **1961**, *26*, 2821; Baker, R.; Fletcher, H.G. 2,3,5-Tri-O-benzyl-D-ribosyl and -L-arabinosyl bromides. J. Org. Chem. **1961**, *26*, 4605; Montogomery, J.A.; Hewson, K. The synthesis of homoribose. J. Org. Chem. **1964**, *29*, 3436.
12. Leonard, N.J.; Carraway, K.L. 5-Amino-5-deoxyribose derivatives. Synthesis and use in preparation of "reversed" nucleosides. J. Heterocyclic Chem. **1966**, *3*, 485.
13. Kissman, H.M.; Baker, B.R. The synthesis of certain 5-deoxy-D-ribofuranosides. J. Am. Chem. Soc. **1957**, *79*, 5534.
14. Brown, T.H.; Blakemore, R.C.; Emmett, G.J.; Robin, G.C.; Parsons, M.E.; Anthony, D.A.; Walker, T.F. Isocytosine H2-receptor histamine antagonists. II. Synthesis and evaluation of biological activity at histamine H1- and H2-receptors of 5-(heterocyclyl)methylisocytosines. Eur. J. Med. Chem. **1988**, *23*, 53.
15. Doong, S.L.; Tasi, C.H.; Schinazi, 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. Proc. Natl. Acad. Sci. USA **1991**, *88*, 8495.
16. Korba, B.E.; Gerin, J.L. A cell culture assay for compounds which inhibit hepatitis B virus replication. Antiviral Res. **1992**, *19*, 55.
17. Fouad, T.; Nielsen, C.; Brunn, L.; Pedersen, E.B. Use of standardization cell culture assay to assess activities of some potent anti-HIV nucleosides analogues against hepatitis B virus replication. Sc. J. Az. Med. Fac. (GIRLS) **1998**, *19*, 1173.

Received September 5, 2002
Accepted August 5, 2003

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016