Notes

Does the Anti-Hepatitis B Virus Activity of (+)-5′-Noraristeromycin Exist in Its 4′-Epimer and 4′-Deoxygenated Derivatives?

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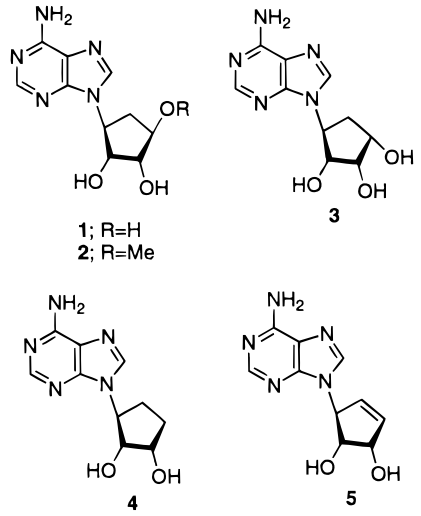
Received January 21, 1998

To begin an exploration of the structural parameters responsible for the activity of (+)-5′-noraristeromycin toward hepatitis B virus (HBV), three derivatives varied at the C-4′ position have been prepared and evaluated. The syntheses began with a Mitsunobu coupling reaction of an appropriate cyclopentanol with 6-chloropurine. The products of these reactions were synthetically altered by standard ammonolysis and deprotection procedures to give the desired products. Evaluation of the new derivatives indicated that removal of the C-4′ hydroxyl of (+)-5′-noraristeromycin increased its potency toward HBV by approximately 10-fold.

We recently reported[1] significant activity for (+)-5′-noraristeromycin (**1**) (Chart 1) toward hepatitis B virus (HBV). In exploring derivatives related to **1** that could possess more potent activity, the methoxy derivative **2** was pursued,[2] which indicated that the 4′-hydroxyl hydrogen of **1** was necessary for its anti-HBV properties. We then chose to determine the role of the stereochemical configuration of the C-4′ hydroxyl group on the HBV properties of **1** as well as the importance of the 4′-hydroxyl itself on the activity. For these purposes, the epimer **3**, the deoxy **4**, and the alkene **5** were chosen as candidate compounds. The results of this study are described here.

Chart 1. Historical Structures and Target Compounds



Chemistry

The preparation of target compound **3** (Scheme 1) began with the Mitsunobu coupling of the chiral acetate **6**[3] with 6-chloropurine. This reaction is known to proceed with inversion of configuration[4] and, consequently, led to **7**.[5] Glycolization of **7** to **8** was followed by ammonolysis to provide **3**.

Scheme 2 shows the synthetic routes used to realize **4** and **5**. This pathway also began with a Mitsunobu coupling using the protected glycol cyclopentenol **9**[6] and 6-chloropurine. Ammonolysis of the product of this reaction (**10**) followed by deprotection with Dowex 50 × 8 acidic resin yielded **5**.[7] Catalytic hydrogenation of **5** resulted in **4**.[7]

Results

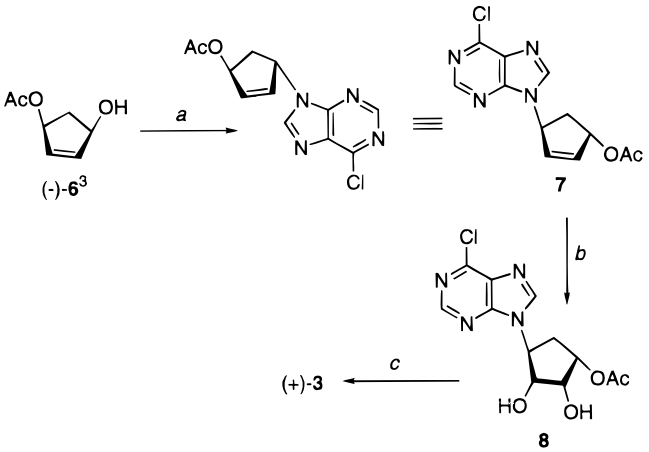
The anti-HBV data for compound **3** compared to **1** (Table 1) verified the importance of the original stereochemical configuration of the C-4′ hydroxyl group in **1**. However, removal of the C-4′ hydroxyl from **1** (as in **4**) showed a 7−10-fold increase in anti-HBV activity. Presence of a double bond in **4** (that is, **5**) resulted in a slight decrease in potency, but with an accompanying decrease in toxicity. While none of the derivatives described herein are as effective an anti-HBV agent as is 3TC (Table 1), the results for **4** and **5** suggest them as prototype compounds from which new entities should be designed and studied.

Experimental Section

General. Melting points were recorded on a Meltemp II melting point apparatus and are uncorrected. Combustion analyses were performed by M−H−W Laboratories, Phoenix, AZ. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 250 spectrometer (operated at 250 and 62.5 MHz, respectively), all referenced to internal tetramethylsilane (TMS) at 0.0 ppm. The spin multiplicities are indicated by the symbols s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad).

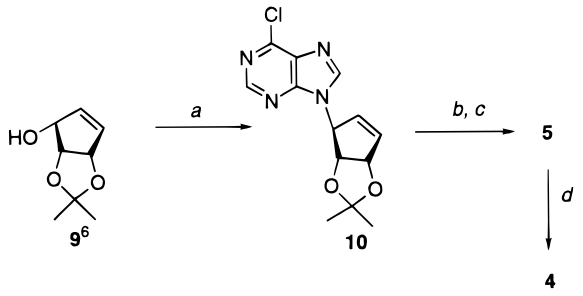
S0022-2623(98)00038-7 CCC: $15.00
Published on Web 05/01/1998

Scheme 1[a]



[a] Reaction conditions: (a) 6-chloropurine, PPh_3, DIAD, THF; (b) OsO_4/60% aq 4-methylmorpholine *N*-oxide in THF; (c) NH_3 in MeOH, 120 °C, 2 days.

Scheme 2[a]



[a] Reaction conditions: (a) 6-chloropurine, PPh_3, DIAD, THF; (b) NH_3 in MeOH, 120 °C, 2 days; (c) Dowex 50 × 8 acidic resin, MeOH; (d) PtO_2, MeOH, H_2, 25 psi.

Table 1. Inhibition of Hepatitis B Virus by 5′-Noraristeromycin Derivatives[a]

compd	CC_{50} (μM)	EC_{50} (μM)	EC_{90} (μM)	SI(CC_{50}/EC_{90})
1	446 ± 20	1.4 ± 0.1	9.6 ± 0.8	46
2	>1000	>10	>10	
3	1883 ± 101	>10	>10	
4	93 ± 7.4	0.120 ± 0.016	0.978 ± 0.077	95
5	325 ± 17	0.145 ± 0.015	1.4 ± 0.2	232
3TC	1884 ± 123	0.070 ± 0.008	0.209 ± 0.018	9014

[a] For details, see the Experimental Section.

The optical rotations were obtained on a Perkin-Elmer 241 polarimeter. Reactions were monitored by thin-layer chromatography (TLC) using 0.25-mm Whatman Diamond silica gel 60-F_{254} precoated plates with visualization by irradiation with a Mineralight UVGL-25 lamp. Column chromatography was performed on Whatman silica, 230−400 mesh, 60 Å, and elution with the indicated solvent system. Yields refer to chromatographically and spectroscopically (^{1}H and ^{13}C NMR) homogeneous materials.

(1*S*,2*R*,3*R*,4*S*)-Acetic Acid 4-(6-Chloro-9*H*-purin-9-yl)-2,3-dihydroxycyclopentyl Ester (8). A stirring, chilled (−10 °C) suspension of 6-chloropurine (5.0 g, 32.35 mmol) and Ph_3P (8.49 g, 32.35 mmol) in dry THF (100 mL) was treated dropwise with diisopropyl azodicarboxylate (DIAD) (6.54 g, 32.35 mmol), and the solution stirred at that temperature for 10 min, after which the ice bath was removed and the reaction stirred for an additional 15 min. To this was then added a solution of **6**[3] (5.06 g, 35.6 mmol) in dry THF (50 mL), and the reaction mixture stirred at room temperature for 2 h, followed by stirring at 55 °C for 2 days. The solvent was evaporated under reduced pressure, and the residue was purified via column chromatography eluting with EtOAc/MeOH (9:1) to give 10.0 g of **7** contaminated with triphenylphosphine oxide as a yellow solid which was used directly in the next step without further characterization.

To the impure **7** (10.0 g) in THF/H_2O (11:1, 240 mL) were added 4-methylmorpholine *N*-oxide (10 mL) and OsO_4 (150 mg), and the solution stirred at room temperature overnight. The solvents were removed under reduced pressure, the residue was coevaporated with toluene (2 × 50 mL) and then acetone (50 mL), and the residue was purified by column chromatography eluting with CH_2Cl_2/MeOH (95:5) to afford 2.75 g of **8** (13% from **6**) as a white solid: mp 193−194 °C; ^{1}H NMR (DMSO-d_6) δ 2.0 (s, 3H), 2.15 (m, 1H), 2.90 (dt, 1H), 4.12 (m, 2H), 5.17 (br, 1H), 5.23 (m, 1H), 5.34 (m, 1H), 5.37 (t, 1H), 8.05 (s, 1H), 8.79 (br, 2H); ^{13}C NMR (DMSO-d_6) δ 20.9, 33.4, 53.5, 71.5, 75.6, 77.4, 130.7, 147.2, 148.7, 151.2, 152.2, 170.3. Calcd for $C_{12}H_{13}Cl\,N_4O_4$ (C, H, N).

(1*S*,2*R*,3*R*,4*S*)-4-(6-Amino-9*H*-purin-9-yl)cyclopentane-1,2,3-triol ((+)-3). A solution of **8** (2.75 g, 8.76 mmol) in saturated methanolic ammonia was sealed in a steel vessel and heated at 100 °C for 2 days. The solvent was removed under reduced pressure, the residue was triturated with H_2O and filtered, and the solvent was removed to give 0.85 g of **9** (39%) as a pale-gray solid: mp 235−236 °C; $[\alpha]^{23}_D$ +15.3 ° (*c* 0.59, DMSO); ^{1}H NMR (DMSO-d_6) δ 2.01 (t, 1H), 2.46 (m, 1H), 3.83 (br, 1H), 4.11 (m, 2H), 5.06 (m, 4H), 7.18 (br, 2H), 8.12 (s, 1H), 8.17 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 37.0, 52.6, 71.6, 73.9, 78.7, 118.3, 140.3, 149.6, 151.9, 155.8. Calcd for $C_{10}H_{13}N_5O_3$ (C, H, N).

(1′*S*,2′*R*,3′*S*)-9-[2′,3′-(Isopropylidenedioxy)cyclopent-4′-en-1′-yl]-6-chloro-9*H*-purine (10). To a stirring, chilled (−10 °C) suspension of 6-chloropurine (5.66 g, 36.6 mmol) and Ph_3P (9.63 g, 36.7 mmol) in dry THF (100 mL) was added dropwise DIAD (7.40 g, 36.6 mmol), and the solution stirred for 10 min, at which point the ice bath was removed and the reaction stirred for an additional 15 min. To this was added a solution of **9**[6] (5.20 g, 36.6 mmol) in dry THF (150 mL), and the reaction mixture stirred at room temperature for 2 h, followed by stirring at 55 °C for 2 days. The solvent was removed under reduced pressure and the residue purified via column chromatography, eluting with EtOAc, followed by EtOAc/MeOH (15:1). Fractions containing product were combined and evaporated to give 2.35 g (24%) of **10** as a white crystalline solid: mp 128−129 °C; ^{1}H NMR ($CDCl_3$) δ 1.27 (s, 3H), 1.51 (s, 3H), 4.74 (d, 1H), 5.55 (d, 1H), 5.72 (s, 1H), 5.99 (dd, 1H), 6.42 (d, 1H), 8.01 (s, 1H), 8.78 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 21.9, 25.7, 27.3, 66.2, 83.7, 84.7, 112.7, 128.6, 132.1, 139.1, 143.2, 151.3, 152.2. Calcd for $C_{13}H_{13}Cl\,N_4O_3$ (C, H, N).

(1′*S*,2′*R*,3′*S*)-1′-(6-Amino-9*H*-purin-9-yl)-2′,3′-dihydroxycyclopent-4′-ene (5). A solution of **10** (2.3 g, 7.86 mmol) in saturated methanolic ammonia (150 mL) was sealed in a steel vessel and heated at 110 °C overnight. The solvent was evaporated, the residue was dissolved in MeOH (100 mL), and Dowex 50 × 8 resin beads were added. The mixture was refluxed for 1 h, and the solvent was removed. The residue was loaded onto a Dowex resin column, and the product was eluted with concentrated NH_4OH. The fractions containing product were combined and evaporated under reduced pressure. The resultant residue was purified via column chromatography on silica gel eluting with EtOAc and then EtOAc/MeOH (9:1). Fractions containing the desired product were combined and evaporated, followed by recrystallization in MeOH to afford 1.30 g (85%) of **5** as a white solid: mp 180−181 °C (lit.[7c] mp 175−176 °C); $[\alpha]^{23}_D$ +166.3° (*c* 0.15, MeOH) (lit.[7c] $[\alpha]^{23}_D$ −170.0° (*c* 1.0, H_2O)); ^{1}H NMR (DMSO-d_6) δ 4.33 (q, 1H), 4.56 (br, 1H), 4.95 (d, 1H), 5.10 (d, 1H), 5.39 (d, 1H), 5.99 (dd, 1H), 6.13 (m, 1H), 7.19 (s, 2H), 8.08 (s, 1H), 8.12 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 64.6, 72.5, 76.1, 119.1, 132.3, 135.9, 139.7, 149.6, 152.2, 155.9. Calcd for $C_{10}H_{11}N_5O_2$ (C, H, N).

(1′*S*,2′*R*,3′*S*)-1′-(6-Amino-9*H*-purin-9-yl)-2′,3′-dihydroxycyclopentane (4). To a solution of **5** (1.0 g, 4.29 mmol) in MeOH (50 mL) was added PtO_2 (0.20 g), and the mixture was placed under H_2 and shaken overnight at 25 psi. The mixture was filtered over a Celite pad, the pad rinsed with MeOH, and the filtrate evaporated under reduced pressure. The residue

was then purified via column chromatography eluting with CH_2Cl_2/MeOH (10:1) (**Note:** TLC showed no difference from starting material, but NMR clearly shows the correct product). Fractions containing product were combined and evaporated to afford 0.58 g (58%) of **4** as a white crystalline solid: mp 214−215 °C (lit.[7b] mp 218 °C); $[\alpha]^{23}_D$ +62.1° (*c* 0.28, MeOH) (lit.[7b] $[\alpha]^{23}_D$ −50.0° (*c* 0.80, H_2O)); ^{1}H NMR (DMSO-d_6) δ 1.63 (m, 1H), 1.95−2.25 (m, 3H), 4.01 (br, 1H), 4.41 (m, 1H), 4.68 (m, 2H), 4.96 (d, 1H), 7.16 (s, 2H), 8.11 (s, 1H), 8.18 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 25.7, 28.8, 59.5, 70.5, 76.1, 119.4, 140.4, 149.6, 151.9, 155.9. Calcd for $C_{10}H_{13}N_5O_2$ (C, H, N).

Anti-HBV. Antiviral analyses for the inhibition of HBV replication were performed on confluent cultures of the chronically HBV-producing human hepatoblastoma cell line, 2.2.15, as previously described.[8] Cells were treated with nine consecutive daily doses (medium removed daily) of the agents. Antiviral activity was assessed by dot blot hybridization analysis for reductions in the levels of extracellular HBV virion DNA and cytotoxicity by uptake of neutral red dye.[8] In these analyses, reductions in virion production of less than 3-fold are routinely not statistically significant.[8]

Acknowledgment. This research was supported by funds from the Department of Health and Human Services U19-AI31718 (S.W.S.) and also Contract NO1-AI-45159 (B.K.) between the National Institute of Allergy and Infectious Diseases and Georgetown University, and this is greatly appreciated. We are also grateful to Dr. James Leahy of the University of California at Berkley for the optical rotation data.

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JM980038A