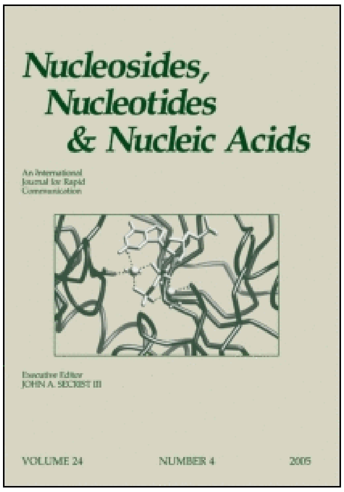

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Design and Synthesis of Acyclic Nucleoside Analogs with Chlorinated Imidazo[1,2-*a*]pyridine Bases

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ABSTRACT

A series of acyclic C-nucleoside analogs of 2,6-dichloro- and 2,6,7-trichloroimidazo[1,2-*a*]pyridine were synthesized and tested for antiviral activity. The appropriate hydroxymethyl-substituted heterocycles were treated successively with thionyl chloride, an appropriate nucleophile, then diisopropylethylamine to obtain the desired acyclic nucleoside analogs. These compounds were evaluated for activity against human cytomegalovirus and herpes simplex virus, type 1. Two of the dichloro analogs, but none of the trichloro analogs demonstrated slight antiviral activity (IC_{50}'s = 20–45 μM) at non-cytotoxic concentrations.

Key Words: Antiviral; Nucleoside analogs; Imidazo[1,2-*a*]pyridine.

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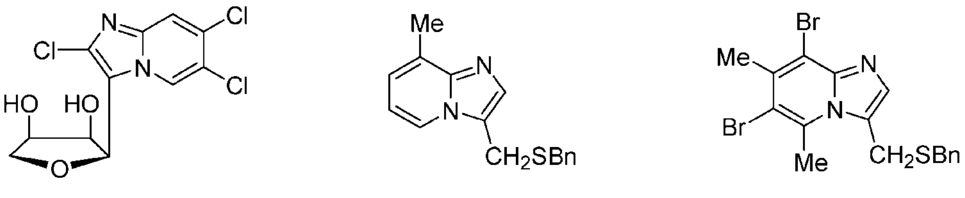
INTRODUCTION

Human cytomegalovirus (HCMV) is an opportunistic infection which is endemic in both the industrialized and developing worlds.[1] Although healthy persons seldom exhibit HCMV-related pathologies, immunocompromised individuals can show a wide array of symptoms including retinitis and gastritis in AIDS patients, organ rejection in transplant recipients, and birth defects in neonates.[2] The four currently licenced HCMV treatments, ganciclovir (GCV),[3] cidofovir,[4] foscarnet,[5] and fomivirsen,[6] all suffer from limitations including toxicity and poor bioavailability. The search for compounds with good antiviral activity and fewer limitations has led our laboratory to synthesize a number of different nucleoside analogs, including 2,5,6-trichloro-1-(β-D-ribofuranosyl)benzimidazole (TCRB).[7] TCRB, however, was found to be unstable in vivo due to glycosidic bond cleavage.[8] Subsequent investigations in our laboratory have focused on the synthesis of compounds which maintain the activity of TCRB, but are less susceptible to glycosidic bond cleavage.

One possible modification includes the use of C-nucleosides whose C-C glycosidic bonds are resistant to the cleavage found for nucleosides with the usual C-N glycosidic bond. One such compound is the nucleoside 2,6,7-trichloro-3-(α-D-erythrofuranosyl)imidazo[1,2-*a*]pyridine (**1**) which has been shown to be active against HCMV at non-cytotoxic concentrations.[9] Gueiffier et al. have reported the synthesis of several acyclic imidazo[1,2-*a*]pyridine nucleosides analogs including 8-methyl-3-benzylthiomethylimidazo[1,2-*a*]pyridine (**2**) and 6,8-dibromo-5,7-dimethyl-3-benzylthiomethylimidazo[1,2-*a*]pyridine (**3**),[10–14] which have demonstrated excellent antiviral activity (Fig. 1). Based on the above compounds, we initiated the synthesis of some acyclic nucleoside analogs of some polychlorinated imidazo[1,2-*a*]pyridines to determine if this approach would provide compounds with good antiviral activity. We now describe the synthesis and antiviral activity of a series of acyclic nucleoside analogs using 2,6-dichloro- and 2,6,7-trichloroimidazo[1,2-*a*] pyridine bases.

RESULTS AND DISCUSSION

Synthesis of the desired acyclic nucleoside analogs started from the known compounds 2,6-dichloro-3-formyl- and 2,6,7-trichloro-3-formylimidazo[1,2-*a*]pyridine[15]



1 **2** **3**

Figure 1. Imidazo[1,2-*a*]pyridines with demonstrated efficacy against HCMV.

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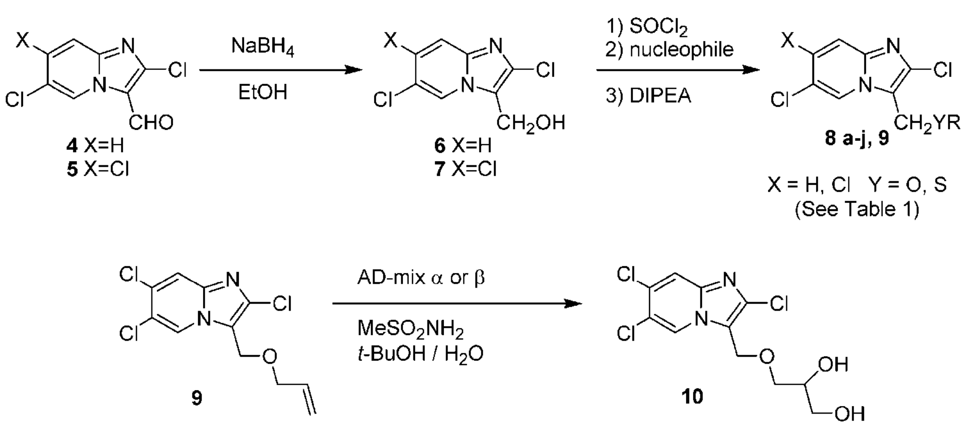


Scheme 1. Synthesis of acyclic imidazo[1,2-*a*]pyridine nucleosides.

(**4** and **5**, Sch. 1). Compounds **4** and **5** were reduced with sodium borohydride in absolute ethanol to provide the 3-hydroxymethyl derivatives **6** and **7**.

Following a procedure similar to that of Gueiffier et al., the hydroxymethyl substituted imidazo[1,2-*a*]pyridines **6** and **7** were treated first with thionyl chloride, then with an appropriate nucleophile followed by diisopropylethylamine. After heating the mixture at reflux temperature, and subsequent aqueous work-up, the acyclic nucleosides were isolated in moderate yield.

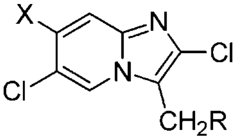
To synthesize the desired polyhydroxylated derivative 2,6,7-trichloro-3-[2,3-dihydroxy(propoxymethyl)]imidazo[1,2-*a*]pyridine (**10**), AD-mix α and β[16] were used to dihydroxylate the allyl ether 2,6,7-trichloro-3-(prop-2-enoxymethyl)imidazo[1,2-*a*]pyridine **9**. Because the dihydroxylation using AD-mix α and β should produce +**10** and −**10** in different ratios; any difference in antiviral or cytotoxic activity could be attributed to the enrichment of the active compound in one of the product pools. However, since neither of the product mixtures from either AD-mix α or from AD-mix β demonstrated any significant antiviral activity, the separation and characterization was not pursued further.

The synthesized compounds were assayed for antiviral activity against both HCMV and HSV-1 (Table 1). Despite the structural similarities to already known trichlorinated imidazo[1,2-*a*]pyridine nucleosides, and the acyclic imidazo[1,2-*a*]pyridine nucleoside analogs synthesized by Gueiffier, none of the compounds synthesized in this study showed significant antiviral activity. Surprisingly, it was two of the dichlorinated analogs rather than the trichlorinated analogs that displayed any amount of antiviral activity.

Experimental

General Procedures. Melting points were taken on a Mel-Temp apparatus, and are uncorrected. NMR spectra were obtained on a Bruker DPX300 or Bruker DRX500 spectrometer; chemical shift values were determined relative to an internal

Table 1. Antiviral activity of acyclic imidazo[1,2-*a*]pyridine nucleosides.



			50% Inhibitory concentration (μM)			
			Antiviral		Cytotoxicity	
No.	X	R	HCMV plaque[a]	HSV-1 ELISA[b]	Visual[c]	Growth[c]
8a	H	$OCH_2CH_2CH_2OH$	100	60	100	>100[d]
8b	H	SCH_2CH_2OH	>100	>100	>100	>100
8c	H	SCH_2CH_3	22	20	100	>100
8d	H	$OCH_2CH{=}CH_2$	18	45	100	>100
8e	Cl	OCH_2CH_2OH	100	>100	100	>100
8f	Cl	$OCH_2CH_2CH_2OH$	100	>100	100	>100
8g	Cl	SCH_2CH_2OH	>100	60	>100	>100
8h	Cl	SBn	100	>100	100	>100
8i	Cl	SCH_2CH_3	>100	>100	>100	>100
8j	Cl	OBn	100	>100	100	>100
±10	Cl	$OCH_2CH(OH)CH_2OH$	>100	>100	>100	>100
TCRB[e]			2.9	102	238	210
GCV[f]			7.4	3.5	>100	>100

[a] Plaque reduction assays were performed in duplicate wells as described in the text.

[b] Compounds were assayed by ELISA in quadruplicate wells.

[c] Visual cytotoxicity was scored on HFF cells at the time of HCMV plaque enumeration; inhibition of KB cell growth was determined in triplicate wells as described in the text.

[d] >100 indicates an IC_{50} greater than the highest concentration tested.

[e] Data for TCRB published previously as compound 9 in Ref.[7].

[f] Averages from 108, 33, and 3 experiments, respectively using GCV.

tetramethylsilane standard. Mass spectrometry was performed at the University of Michigan Department of Chemistry facility. Elemental analysis was performed at the University of Michigan Chemistry Department elemental analysis facility. Thin layer chromatography was performed on silica gel GHLF plates from Analtech (Newark, DE). Chromatograms were visualized under UV light at 254 nm. Column chromatography was performed on Silica gel 60 (230–400 mesh, E. Merck, Darmstadt, Germany) with the specified column size and solvent system. Evaporation of all solvents was accomplished under reduced pressure, at less than 40°C. All solvents were dried prior to use according to known procedures.[17] All reagents were obtained from commercial sources or were synthesized from literature procedures, and were used without further purification.

2,6-Dichloro-3-hydroxymethylimidazo[1,2-*a*]pyridine (6). 2,6-Dichloro-3-formylimidazo[1,2-*a*]pyridine[15] (**4**, 18.18 g, 85 mmol) was dissolved in 250 mL of absolute ethanol to which was added sodium borohydride (3.25 g, 86 mmol). The solution was stirred at room temperature for 1 h, then 25 mL of water and 5 mL of acetic acid were added with stirring. The solution was poured into 1 L of water, and the solvent was evaporated to approx. 750 mL. The aqueous suspension was allowed to stand at 4°C for 16 hours. The resulting solids were collected by vacuum filtration, rinsed with 150 mL of cold water, and the vacuum was maintained for an additional 1 h. The product was then dried in a vacuum oven (60°C, 12 mmHg) for 24 h to yield 15.6 g (85%) of **6** as a tan crystalline solid: mp 182–184°C; R_f 0.4 (1:1 hex:EtOAc); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.34 (d, 1H), 7.62 (d, 1H), 4.43 (dd, 1H), 5.41 (t, 1H, D_2O exch.), 4.79 (s, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6): δ 140.97, 133.41, 126.54, 123.30, 120.20, 119.88, 117.08, 50.91.

2,6,7-Trichloro-3-hydroxymethylimidazo[1,2-*a*]pyridine (7). 2,6,7-Trichloro-3-formylimidazo[1,2-*a*]pyridine[15] (**5**, 7.98 g, 32 mmol) was treated as above to provide 6.72 g (84%) of **7** as a white crystalline solid: mp 220–222°C; R_f 0.5 (1:1 hex:EtOAc); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.84 (s, 1H), 8.02 (s, 1H), 5.44 (t, 1H, D_2O exch.), 4.78 (s, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6): δ 140.95, 134.10, 129.42, 124.74, 120.38, 118.62, 116.31, 50.83.

2,6-Dichloro-3-[3-hydroxy(propoxymethyl)]imidazo[1,2-*a*]pyridine (8a). 2,6-Dichloro-3-hydroxymethylimidazo[1,2-*a*]pyridine (**6**, 0.45 g, 2.1 mmol) was dissolved in 20 mL of thionyl chloride and the resulting solution was heated at reflux temperature for 1 h, after which it was cooled to room temperature and evaporated to dryness. The residual oil was dissolved in 5 mL of 1,3-propanediol and the solution was stirred at room temperature for 10 minutes. Dry THF (20 mL) and diisopropylethylamine (5 mL) were then added and the resulting solution was heated at reflux temperature for 16 h. The solution was cooled to room temperature and evaporated to dryness, then the residue was suspended in 5% aqueous Na_2CO_3 (50 mL) and extracted twice with EtOAc (2 × 50 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered and evaporated to yield a yellow solid. The solid was recrystallized from hot CH_2Cl_2 to yield 0.15 g (26%) of **8a** as a pale yellow crystalline solid: mp 117–120°C; R_f 0.2 (1:1 hex:EtOAc); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.64 (d, 1H), 7.64 (d, 1H), 7.46 (dd, 1H), 4.80 (s, 2H), 3.52 (m, 2H), 4.5–3.8 (b, 1H, D_2O exch.), 3.42 (m, 2H) 1.65 (m, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6): δ 141.34, 134.91, 127.01, 123.24, 120.27, 117.20, 117.15, 66.56, 59.24, 57.61, 32.48. HRMS m/z calcd. for $C_{11}H_{12}Cl_2N_2O_2$ 274.0276, found 274.0275. Anal calcd for $C_{11}H_{12}Cl_2N_2O_2 \bullet 1/5\ CH_2Cl_2$: C, 46.05; H, 4.28; N, 9.59. Found: C, 46.75; H, 4.42; N, 9.64.

2,6-Dichloro-3-[2-hydroxy(ethylthiomethyl)]imidazo[1,2-*a*]pyridine (8b). 2,6-Dichloro-3-hydroxymethylimidazo[1,2-*a*]pyridine (**6**, 0.40 g, 1.8 mmol) was treated as above, except that 2-mercaptoethanol was used as the nucleophile. The crude product was subjected to column chromatography (40 × 350 mm) with 6:1 hex:EtOAc to yield 0.16 g (31%) of **8b** as a pale yellow solid: mp 210–212°C; R_f 0.2 (1:1 hex:EtOAc); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.73 (d, 1H), 7.62 (d, 1H), 7.43 (dd, 1H), 4.78 (s, 1H, D_2O exch.), 4.20 (s, 2H), 3.48 (t, 2H), 2.48 (m, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6): δ 141.09, 133.99, 126.47, 123.42, 119.97, 117.20, 116.71, 60.38, 33.47, 22.78. HRMS m/z calcd. for $C_{10}H_{10}Cl_2N_2OS$ 275.9891, found 275.9892. Anal calcd for $C_{10}H_{10}Cl_2N_2OS$: C, 43.33; H, 3.64; N, 10.11. Found: C, 43.25; H, 3.65; N, 10.00.

2,6-Dichloro-3-(ethylthiomethyl)imidazo[1,2-*a*]pyridine (8c). 2,6-Dichloro-3-hydroxymethylimidazo[1,2-*a*]pyridine (**6**, 0.50 g, 2.3 mmol) was treated as above except that ethanethiol was used as the nucleophile. The crude product was recrystallized from hot hexane/EtOAc to yield 0.26 g (43%) of **8c** as a pale yellow crystalline solid: mp 121–122°C; R_f 0.5 (3:1 hex:EtOAc); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.73 (d, 1H), 7.64 (d, 1H), 7.45 (dd, 1H), 4.20 (s, 2H). 2.39 (q, 2H), 1.15 (t, 3H). ^{13}C-NMR (125 MHz, DMSO-d_6): δ 141.06, 133.97, 126.44, 123.36, 119.94, 117.20, 116.65, 24.52, 22.15, 14.50. HRMS m/z calcd. for $C_{10}H_{10}Cl_2N_2S$ 259.9942, found 259.9951. Anal calcd for $C_{10}H_{10}Cl_2N_2S$: C, 45.99; H, 3.86; N, 10.73. Found: C, 45.60; H, 4.06; N, 10.67.

2,6-Dichloro-3-(prop-2-enoxymethyl)imidazo[1,2-*a*]pyridine (8d). 2,6-Dichloro-3-hydroxymethylimidazo[1,2-*a*]pyridine (**6**, 0.43 g, 2.0 mmol) was treated as above except that allyl alcohol was used as the nucleophile. The crude product was recrystallized from boiling hexane to yield 0.34 g (67%) of **8d** as a white crystalline solid: mp 94–95°C; R_f 0.3 (6:1 hex:EtOAc); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.67 (d, 1H), 7.65 (d, 1H), 7.48 (dd, 1H), 5.90 (m, 1H), 5.27 (dd, 1H), 5.16 (dd, 1H), 4.83 (s, 1H), 4.03 (d, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6): δ 142.08, 135.75, 135.34, 127.75, 123.92, 120.99, 117.90, 117.72, 117.67, 70.74, 59.24. HRMS m/z calcd. for $C_{11}H_{10}Cl_2N_2O$ 256.0170, found 256.0181. Anal calcd for $C_{11}H_{10}Cl_2N_2O$: C, 51.38; H, 3.92; N, 10.90. Found: C, 51.35; H, 4.20; N, 11.07.

2,6,7-Trichloro-3-[2-hydroxy(ethoxymethyl)]imidazo[1,2-*a*]pyridine (8e). 2,6,7-Trichloro-3-hydroxymethylimidazo[1,2-*a*]pyridine (**7**, 0.40 g, 1.6 mmol) was treated as above except that ethylene glycol was used as the nucleophile. The crude product was recrystallized from EtOH/water to yield 0.15 g (32%) of **8e** as a white crystalline solid: mp 165–167°C; R_f 0.3 (1:1 hex:EtOAc); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.91 (s, 1H), 8.07 (s, 1H), 4.84 (s, 2H), 4.71 (t, 1H), 3.50 (m, 4H). ^{13}C-NMR (125 MHz, DMSO-d_6): δ 141.43, 135.50, 129.98, 125.04, 119.02, 117.36, 116.45, 71.35, 60.04, 59.41. HRMS m/z calcd. for $C_{10}H_9Cl_3N_2O_2$ 293.9730, found 293.9727. Anal calcd for $C_{10}H_9Cl_3N_2O_2$: C, 40.64; H, 3.07; N, 9.48. Found: C, 40.64; H, 3.21; N, 9.20.

2,6,7-Trichloro-3-[3-hydroxy(propoxymethyl)]imidazo[1,2-*a*]pyridine (8f). 2,6,7-Trichloro-3-hydroxymethylimidazo[1,2-*a*]pyridine (**7**, 0.50 g, 2.0 mmol) was treated as above except that 1,3-propanediol was used as the nucleophile. The crude product was recrystallized from boiling EtOAc to yield 0.30 g (48%) of **8f** as a white crystalline solid: mp 142–143°C; R_f 0.2 (1:1 hex:EtOAc); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.87 (s, 1H), 8.06 (s, 1H), 4.80 (s, 2H), 4.5–4.2 (b, 1H, D_2O exch.), 3.51 (m, 2H), 3.42 (m, 2H), 1.64 (m, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6): δ 142.04, 136.27, 130.57, 125.40, 119.71, 118.00, 117.14, 67.23, 59.83, 58.27, 33.13. HRMS m/z calcd. for $C_{11}H_{11}Cl_3N_2O_2$ 307.9886, found 307.9889. Anal calcd for $C_{11}H_{11}Cl_3N_2O_2$: C, 42.68; H, 3.58; N, 9.05. Found: C, 42.57; H, 3.74; N, 8.79.

2,6,7-Trichloro-3-[2-hydroxy(ethylthiomethyl)]imidazo[1,2-*a*]pyridine (8g). 2,6,7-Trichloro-3-hydroxymethylimidazo[1,2-*a*]pyridine (**7**, 0.50 g, 2.0 mmol) was treated as above except that 2-mercaptoethanol was used as the nucleophile. The crude product was recrystallized from EtOAc/hexane to yield 0.34 g (55%) of **8g** as a white crystalline solid: mp 176–179°C; R_f 0.3 (1:1 hex:EtOAc); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.97 (s, 1H), 8.06 (s, 1H), 4.83 (s, 1H, D_2O exch.), 4.21 (s, 2H), 3.48 (m, 2H), 2.51 (m, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6): δ 141.08, 134.66, 129.41, 124.86, 118.72, 117.01, 116.47, 60.36, 33.46, 22.73. HRMS m/z calcd. for $C_{10}H_9Cl_3N_2OS$ 309.9501, found 309.9504. Anal calcd for $C_{10}H_9Cl_3N_2OS$: C, 38.54; H, 2.91; N, 8.99. Found: C, 38.71; H, 3.13; N, 8.98.

2,6,7-Trichloro-3-(benzylthiomethyl)imidazo[1,2-*a*]pyridine (8h). 2,6,7-Trichloro-3-hydroxymethylimidazo[1,2-*a*]pyridine (**7**, 0.40 g, 1.6 mmol) was treated as above except that benzyl mercaptan was used as the nucleophile. The crude product was recrystallized from EtOH/water to yield 0.27 g (47%) of **8h** as a pale yellow crystalline solid: mp 149–152°C; R_f 0.8 (1:1 hex:EtOAc); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.02 (s, 1H), 7.58 (s, 1H), 7.22 (m, 5H), 3.79 (s, 2H), 3.64 (s, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 141.92, 137.39, 136.63, 131.17, 128.68, 128.64, 127.37, 123.17, 120.58, 116.82, 115.15, 36.18, 23.61. HRMS m/z calcd. for $C_{15}H_{11}Cl_3N_2S$ 355.9708, found 355.9706. Anal calcd for $C_{15}H_{11}Cl_3N_2S$: C, 50.37; H, 3.10; N, 7.83. Found: C, 50.49; H, 3.38; N, 7.68.

2,6,7-Trichloro-3-(ethylthiomethyl)imidazo[1,2-*a*]pyridine (8i). 2,6,7-Trichloro-3-hydroxymethylimidazo[1,2-*a*]pyridine (**7**, 0.50 g, 2.0 mmol) was treated as above except that ethanethiol was used as the nucleophile. The crude product was recrystallized from hot hexane/EtOAc to yield 0.49 g (29%) of **8i** as a pale yellow crystalline solid: mp 186–188°C; R_f 0.6 (2:1 hex:EtOAc); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.96 (s, 1H), 8.06 (s, 1H), 4.19 (s, 2H), 2.38 (q, 2H), 1.15 (t, 3H). ^{13}C-NMR (125 MHz, DMSO-d_6): δ 141.06, 134.64, 129.38, 124.81, 118.72, 116.96, 116.50, 24.49, 22.12, 14.47. HRMS m/z calcd. for $C_{10}H_9Cl_3N_2S$ 293.9552, found 293.9565. Anal calcd for $C_{10}H_9Cl_3N_2S$: C, 40.63; H, 3.07; N, 9.48. Found: C, 40.38; H, 3.14; N, 9.12.

2,6,7-Trichloro-3-(benzyloxymethyl)imidazo[1,2-*a*]pyridine (8j). 2,6,7-Trichloro-3-hydroxymethylimidazo[1,2-*a*]pyridine (**7**, 0.50 g, 2.0 mmol) was treated as above except that benzyl alcohol was used as the nucleophile. The crude product was recrystallized from hot hexane/EtOAc to yield 0.40 g (47%) of **8j** as a white crystalline solid: mp 149–152°C; R_f 0.8 (1:1 hex:EtOAc); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.20 (s, 1H), 7.64 (s, 1H), 7.33 (m, 5H), 4.81 (s, 2H), 4.51 (s, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 142.30, 137.42, 137.16, 131.73, 128.77, 128.57, 128.37, 123.46, 120.98, 116.84, 116.28, 72.23, 59.65. HRMS m/z calcd. for $C_{15}H_{11}Cl_3N_2O$ 339.9937, found 339.9924. Anal calcd for $C_{15}H_{11}Cl_3N_2O$: C, 52.74; H, 3.25; N, 8.20. Found: C, 52.88; H, 3.42; N, 8.10.

2,6,7-Trichloro-3-(prop-2-enoxymethyl)imidazo[1,2-*a*]pyridine (9). 2,6,7-Trichloro-3-hydroxymethylimidazo[1,2-*a*]pyridine (**7**, 1.08 g, 4.3 mmol) was treated as above except that allyl alcohol was used as the nucleophile. The crude product was recrystallized from boiling hexane to yield 0.86 g (69%) of **9** as a white crystalline solid: mp 91–92°C; R_f 0.6 (1:1 hex:EtOAc); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.32 (s, 1H), 7.68 (s, 1H), 5.89 (m, 1H), 5.29 (m, 2H), 4.80 (s, 2H), 3.99 (d, 2H).

^{13}C-NMR (125 MHz, DMSO-d_6): δ 142.36, 137.33, 133.68, 131.77, 126.08, 123.47, 121.06, 118.77, 116.94, 116.32, 71.00, 59.70. HRMS m/z calcd. for $C_{11}H_9Cl_3N_2O$ 289.9780, found 289.9786. Anal calcd for $C_{11}H_9Cl_3N_2O$: C, 45.31; H, 3.11; N, 9.61. Found: C, 45.31; H, 3.23; N, 9.34.

2,6,7-Trichloro-3-[2,3-dihydroxy(propoxymethyl)]imidazo[1,2-*a*]pyridine (±10). 2,6,7-Trichloro-3-(prop-2-enoxymethyl)imidazo[1,2-*a*]pyridine (**9**, 0.44 g, 1.5 mmol) was dissolved in a mixture of *t*-BuOH (15 mL) and water (15 mL). To this stirred solution was added AD-mix α (2.2 g) and methanesulfonamide (0.15 g, 1.6 mmol). The resulting biphasic suspension was stirred rapidly at room temperature for 16 h, then quenched with solid sodium bisulfite until the yellow-orange color was discharged. Brine (20 mL) was added and the suspension was extracted with EtOAc (2 × 20 mL). The combined organic extracts were dried over $MgSO_4$, filtered and evaporated to dryness. The residue was subjected to column chromatography (40 × 350 mm) on silica gel with 10% MeOH/$CHCl_3$. Product-containing fractions were pooled and evaporated to provide 0.39 g (80%) of **±10** as a white powder: mp 175–176°C; R_f 0.4 (10% MeOH/$CHCl_3$); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.89 (s, 1H), 8.06 (s, 1H), 4.83 (d, 2H), 4.8–4.6 (b, 1H, D_2O exch.), 4.6–4.4 (b, 1H, D_2O exch.), 3.60 (t, 1H), 3.50 (dd, 1H), 3.37 (dd, 1H), 3.30 (d, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6): δ 141.40, 135.33, 129.93, 125.03, 118.96, 117.30, 116.40, 71.54, 70.38, 62.85, 59.76. HRMS m/z calcd. for $C_{11}H_{11}Cl_3N_2O_3$ 323.9835, found 323.9825. Anal calcd for $C_{11}H_{11}Cl_3N_2O_3$: C, 40.58; H, 3.41; N, 8.60. Found: C, 40.59; H, 3.67; N, 8.51.

AD-mix β provided a 71% yield of **±10** under similar reaction conditions: mp 175–176°C; R_f 0.4 (10% MeOH/$CHCl_3$); ^{1}H-NMR and ^{13}C-NMR spectra were identical to those obtained above.

Biological Evaluation

Cell Culture Procedures. The routine growth and passage of KB, BSC-1, and HFF cells was performed in monolayer cultures using minimal essential medium (MEM) with either Hanks salts [MEM(H)] or Earle salts [MEM(E)] supplemented with 10% calf serum or 10% fetal bovine serum (HFF cells). The sodium bicarbonate concentration was varied to meet the buffering capacity required. Cells were passaged at 1:2 to 1:10 dilutions according to conventional procedures by using 0.05% trypsin plus 0.02% EDTA in a HEPES buffered salt solution.[18]

Virological Procedures. The Towne strain, plaque-purified isolate P_o, of HCMV was kindly provided by Dr. Mark Stinski, University of Iowa. The KOS strain of HSV-1 was used in most experiments and was provided by Dr. Sandra K. Weller, University of Connecticut. Stock HCMV was prepared by infecting HFF cells at a multiplicity of infection (m.o.i.) of <0.01 plaque-forming units (p.f.u.) per cell as detailed previously.[19] High titer HSV-1 stocks were prepared by infecting KB cells at an m.o.i. of <0.1 also as detailed previously.[19] Virus titers were determined using monolayer cultures of HFF cells for HCMV and monolayer cultures of BSC-1 cells for HSV-1 as described earlier.[20] Briefly, HFF or BSC-1 cells were planted as described above in 96-well cluster dishes and incubated overnight at 37°C. The next day cultures were inoculated with HCMV or HSV-1 and serially diluted 1:3 across the remaining eleven columns of the 96-well plate. After virus adsorption the inoculum was replaced with fresh medium and cultures were incubated for seven days for HCMV, two or three days for HSV-1. Plaques were enumerated under 20-fold magnification in wells having the dilution which gave 5 to 20 plaques per well. Virus titers were calculated according to the following formula: Titer (p.f.u./mL) = number of plaques $\times 5 \times 3^n$; where n represents the n^{th} dilution of the virus used to infect the well in which plaques were enumerated.

HCMV Plaque Reduction Assay. HFF cells in 24-well cluster dishes were infected with approximately 100 p.f.u. of HCMV per cm^2 cell sheet using the procedures detailed above. Following virus adsorption, the compounds, prepared as 10 mg/mL stock solutions in DMSO were diluted with growth medium and were added to duplicate wells in four to eight selected concentrations. After incubation at 37°C for 7–10 days, cell sheets were fixed, stained with crystal violet and microscopic plaques enumerated as described above. Drug effects were calculated as a percentage of reduction in number of plaques in the presence of each drug concentration compared to the number observed in the absence of drug.

HSV-1 ELISA. An ELISA was employed[21] to detect HSV-1. Ninety-six-well cluster dishes were planted with 10,000 BSC-1 cells per well in 200 μL per well of MEM(E) plus 10% calf serum. After overnight incubation at 37°C, selected drug concentrations in quadruplicate and HSV-1 at a concentration of 100 p.f.u./well were added. Following a 3-day incubation at 37°C, medium was removed, plates were blocked, rinsed, and horse radish peroxidase conjugated rabbit anti-HSV-1 antibody was added. Following removal of the antibody containing solution, plates were rinsed, and then developed by adding 150 μL per well of a solution of tetramethylbenzidine as substrate. The reaction was stopped with H_2SO_4 and absorbance was read at 450 and 570 nm. Drug effects were calculated as a percentage of the reduction in absorbance in the presence of each drug concentration compared to absorbance obtained with virus in the absence of drug.

Cytotoxicity Assays. Two different assays were used for routine cytotoxicity testing. (i) Cytotoxicity produced in stationary HFF cells was determined by microscopic inspection of cells not affected by the virus used in plaque assays.[19] (ii) The effect of compounds during two population doublings of KB cells was determined by crystal violet staining and spectrophotometric quantitation of dye eluted from stained cells as described earlier.[22] Briefly, 96-well cluster dishes were planted with KB cells at 3000–5000 cells per well. After overnight incubation at 37°C, test compound was added in quadruplicate at six to eight concentrations. Plates were incubated at 37°C for 48 h in a CO_2 incubator, rinsed, fixed with 95% ethanol, and stained with 0.1% crystal violet. Acidified ethanol was added and plates read at 570 nm in a spectrophotometer designed to read 96-well ELISA assay plates.

Data Analysis. Dose response relationships were used to quantitate drug effects by linear regression of the percent inhibition of parameters derived in the preceding assays against $\log_{10}$ drug concentrations. Fifty percent inhibitory concentrations

(IC_{50}'s) were calculated from the linear portions of the regression lines. Samples containing positive controls (acyclovir for HSV-1, GCV for HCMV, and 2-acetylpyridine thiosemicarbazone for cytotoxicity) were used in all assays.

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