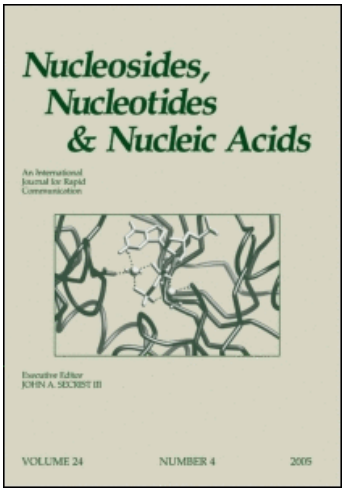


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis and Antiviral Activity of Novel Aza-acyclonucleosides

Stephen C. Bergmeier[a]; Susan L. Fundy[a]; John C. Dracht[b]
[a] Division of Medicinal Chemistry and the Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio [b] Department of Biologic and Materials Sciences, College of Dentistry, University of Michigan, Ann Arbor, Michigan

To cite this Article Bergmeier, Stephen C. , Fundy, Susan L. and Dracht, John C.(1999) 'Synthesis and Antiviral Activity of Novel Aza-acyclonucleosides', Nucleosides, Nucleotides and Nucleic Acids, 18: 2, 227 — 238
To link to this Article: DOI: 10.1080/15257779908043070
URL: http://dx.doi.org/10.1080/15257779908043070

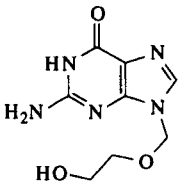
NUCLEOSIDES & NUCLEOTIDES, 18(2), 227-238 (1999)

SYNTHESIS AND ANTIVIRAL ACTIVITY OF NOVEL AZA-ACYCLONUCLEOSIDES

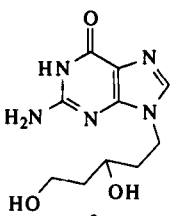
Stephen C. Bergmeier,* Susan L. Fundy and John C. Drach†
Division of Medicinal Chemistry and the Comprehensive Cancer Center, 500 W. 12th Ave., The Ohio State University, Columbus, Ohio 43210-1290 and †Department of Biologic and Materials Sciences, College of Dentistry, University of Michigan, Ann Arbor, Michigan 48109-1078

ABSTRACT. We have prepared a series of novel aza-acyclonucleosides as potential antiviral agents. These compounds were prepared from diethanolamine and the desired purine or pyrimidine base via a Mitsunobu coupling. No antiviral activity was observed against either HSV-1 or HCMV.

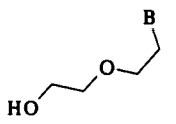
The discovery of acyclovir (**1**) as a potent antiviral agent,[1] prompted further design and evaluation of acyclonucleosides as antiviral agents. This vast group of compounds, with varied molecular structures, was recently reviewed.[2] Despite the antiviral activity of the *diseco*-nucleoside (**2**), very few examples of this type of acyclonucleoside have been prepared and evaluated.[3] One pertinent example, the 2-(hydroxyethoxy)ethyl acyclonucleosides (**3**) showed only minor activity against HSV-1. This activity was limited to the guanine derivative.[4] Only a few examples of acyclonucleosides containing basic amines either in or on the acyclic chain have been reported.[5] In order to help address



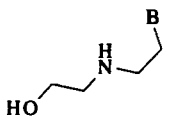
1, acyclovir



2

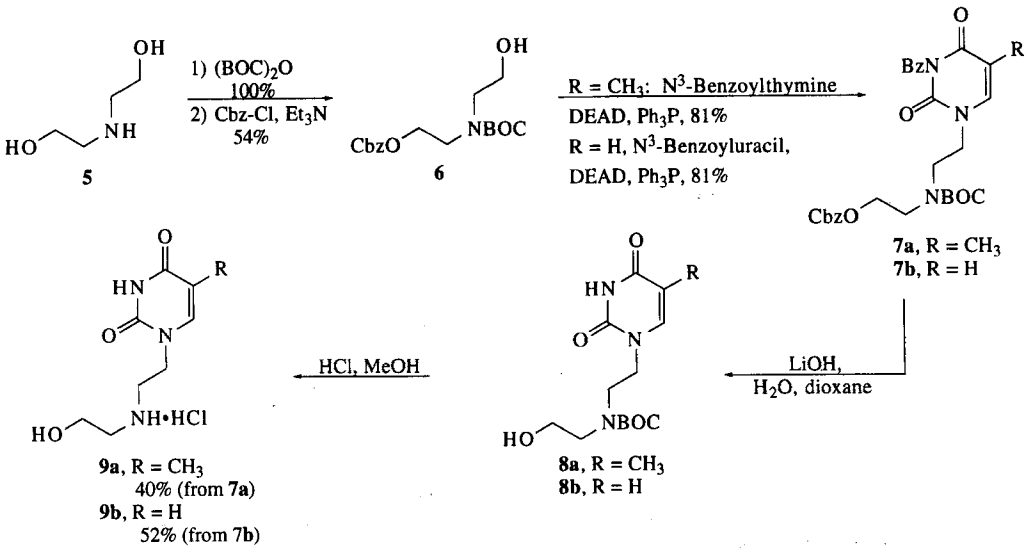


3, B = purine or pyrimidine



4, B = purine or pyrimidine

FIG. 1: Examples of acyclonucleosides

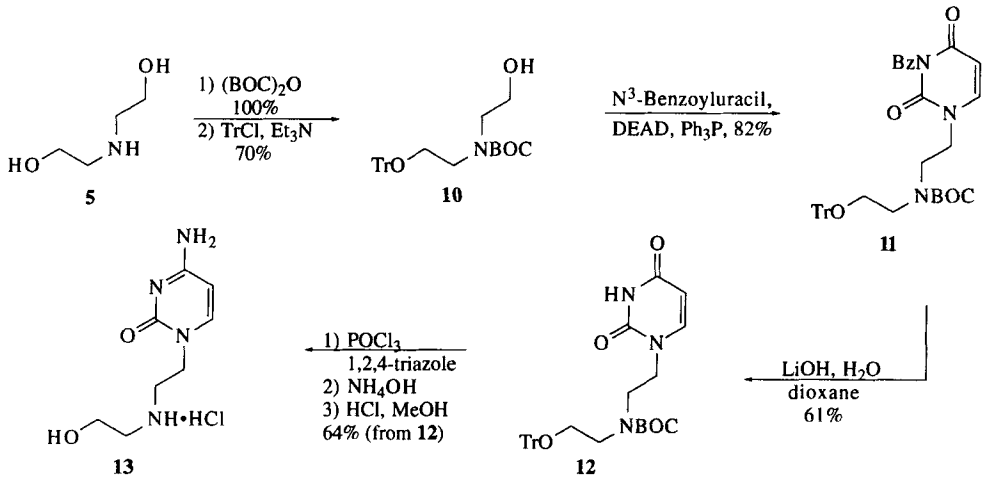


SCHEME 1

the apparent lack of information about amine containing acyclonucleosides, we set out to prepare *diseco* -nucleoside analogs of the general type (**4**) with a basic nitrogen in the acyclosugar moiety. We report here the synthesis and antiviral evaluation of this new class of acyclonucleosides.

The thymine and uracil derivatives **9a** and **9b** were prepared by the method shown in scheme 1. Previously, we reported the preparation of **7a** as part of a program aimed at the preparation of a new type of peptide nucleic acid,[6] and found that diethanolamine was an ideal starting material for the preparation of nucleoside analogs such as (**4**). Protection of the nitrogen of diethanolamine as the BOC derivative in quantitative yield, followed by monoprotection of the diol as the benzylcarbonate provided **6** in 54% yield. Mitsunobu reaction of **6** with either N^3-benzoylthymine[7] or N^3-benzoyluracil[7] gave **7a** and **7b** in good yield.[8] Hydrolysis of the N^3-benzoyl group along with concomitant removal of the benzylcarbonate provided either **8a** or **8b** which when treated with HCl[9] gave the desired aza-acyclonucleosides **9a** and **9b**.

We initially planned to utilize **8b** for a preparation of the cytidine analog **13**. However, all attempts to directly convert **8b** to **13** failed, most likely due to the free hydroxyl group. We attempted to reprotect the alcohol, both as the THP and TBS derivatives, but the insolubility of **8b** did not permit such transformations. Thus, we were forced to start with a different diethanolamine derivative. We chose to install a trityl group on the terminal hydroxyl group since concurrent removal with the BOC group seemed



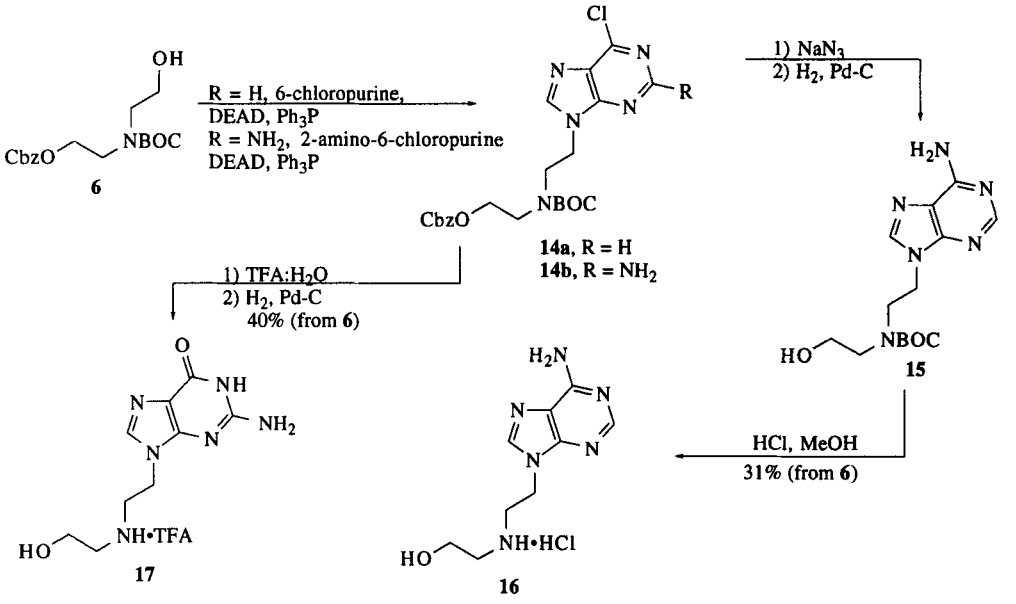
SCHEME 2

probable. As shown in scheme 2, treatment of diethanolamine with BOC_2O gave the *N*-BOC derivative in quantitative yield. Tritylation then gave **10** in 70% yield. Mitsunobu coupling of **10** with N^3-benzoyluracil gave an excellent yield of **11**. Hydrolysis of the N^3-benzoyl group provided **12**. The uracil derivative was then readily converted to the cytidine derivative with $POCl_3$/1,2,4 triazole followed by NH_3.[10] Finally, concomitant removal of the BOC and trityl groups gave **13** in good yield.

The preparation of the purine derivatives was relatively straightforward but purification of the intermediates proved difficult and thus crude material was taken directly on to the final products. As before, Mitsunobu coupling of the purine base with **6** provided **14a** or **14b**. Both of these compounds proved very difficult to separate from triphenylphosphine oxide generated in the Mitsunobu coupling. The chloropurine derivative **14a** was simply treated with sodium azide in DMF to provide the corresponding azidopurine. Reduction to the amine (H_2, 10% Pd-C) followed by removal of the BOC group provided **16** in good overall yield.

In a similar fashion, hydrolysis of **14b** followed by hydrogenolysis provided the guanine derivative **17**.

The acyclonucleosides were tested for activity against herpes simplex virus type 1 (HSV-1) and human cytomegalovirus (HCMV) and for cytotoxicity.[11] Compounds **9a**, **9b**, **13**, **16** and **17** were not active at concentrations up to 100 μM against HCMV in the plaque assay nor against HSV-1 in an ELISA. Neither were the compounds cytotoxic to stationary human foreskin fibroblasts or growing KB cells at the highest concentration tested, 100 μM.



SCHEME 3

In summary, we have prepared a new class of *diseco* -nucleosides containing a basic nitrogen in the side chain. These compound were evaluated against both HCMV and HSV-1, but were inactive. The lack of antiviral activity suggests that the compounds were not substrates for HSV-1 deoxypyrimidine kinase nor for HCMV UL97 kinase and therefore could not be converted to active triphosphates. Alternatively, these compounds may have limited uptake into the cells or were not inhibitory as their triphosphates against the polymerase.

EXPERIMENTAL

^1H and ^{13}C spectra were recorded on a Bruker AF 250, Bruker AF 270 or a Bruker DRX 400 model spectrometer. Chemical shifts are reported in ppm relative to trimethylsilane. Melting points were taken using a Thomas Hoover capillary melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was performed on Whatman pre-coated silica gel F_{254} aluminum foils. Purification of the reaction products was carried out by flash column chromatography using a glass column dry packed with silica gel (230-400 mesh ASTM) according to the method of Still[12]. Visualization was accomplished with UV light and/or ninhydrin solution followed by heating. Exact mass measurements recorded in the electron impact (EI) mode were determined at The Ohio State

University Chemical Instrument Center with a Kratos MS-30 mass spectrometer. UV spectra were recorded on a Beckman DU-40 spectrometer. Combustion analyses were performed at Quantitative Technologies, Inc., Whitehouse, New Jersey. Infrared spectra were obtained on salt plates in CCl_4 or as a KBr pellet, using a Nicolet Protégé 460 model spectrometer. Peaks are reported in cm^{-1}. THF was distilled from sodium and benzophenone. CH_3CN was distilled from phosphorus pentoxide before use. CH_2Cl_2 was distilled from CaH_2. Et_3N, iPr_2NEt, and pyridine were distilled from CaH_2 and stored over KOH pellets. Benzoyl chloride was distilled under vacuum just before use. Acetyl chloride and EtOAc were distilled under N_2 before use. All reactions were carried out under an N_2 atmosphere unless otherwise specified.

***N*-BOC diethanolamine.** A solution of diethanolamine (4.8 g, 45.4 mmol) in THF (45.4 mL) was cooled to 0°C. To this solution, di-*tert* -butyl dicarbonate (9.9 g, 45.4 mmol) was added. The reaction was stirred at rt for 24 h. Rotary evaporation of the solvent followed by column chromatography (EtOAc) gave 9.3 g of *N*-BOC diethanolamine (100%) as an oil: ^1H NMR ($CDCl_3$) δ 1.46 (s, 9H), 3.43 (t, 4H), 3.76 (t, 4H). ^{13}C NMR ($CDCl_3$) δ 28.02, 51.58, 61.04, 79.72, 155.93. IR (CCl_4) 1669, 2977, 3385 cm^{-1}. HRMS calculated for $C_9H_{19}NO_4$ was 205.1315, found 205.1319.

***O*-benzyloxycarbonyl-*N*-*tert*-butoxycarbonyl diethanolamine (6).** *N*-BOC diethanolamine (5.0 g, 24.4 mmol), DMAP (300 mg, 2.43 mmol), and Et_3N (3.4 mL, 24.36 mmol) were dissolved in CH_2Cl_2 (49 mL). The mixture was cooled to 0 °C and benzylchloroformate (3.5 mL, 24.4 mmol) was slowly added. The reaction was stirred at room temperature for 18 h. The reaction was washed with brine, extracted with CH_2Cl_2, dried over $MgSO_4$, filtered and concentrated. Chromatography (25% EtOAc/hexanes) gave 4.44 g of **6** (54%) as an oil: ^1H NMR ($CDCl_3$) δ 1.43 (s, 9H), 3.39 (m, 2H), 3.51 (m, 2H), 3.66 (m, 2H), 4.25 (m, 2H), 5.17 (s, 2H), 7.34 (m, 5H). ^{13}C NMR ($CDCl_3$) δ 27.85, 46.98, 50.46, 60.79, 65.81, 69.12, 79.71, 127.74, 129.10, 134.88, 154.58, 155.51. IR (CCl_4) 1693, 1747, 2975, 3450 cm^{-1}. Anal. Calcd. for $C_{17}H_{25}NO_6$: C, 60.16; H, 7.42; N, 4.13. Found: C, 59.82; H, 7.44; N, 4.03.

1-(5-*O*-benzyloxycarbonyl-3-aza-(*N*-*tert*-butoxycarbonyl)-pentyl)-N^3-benzoyl thymine (7a). A solution of **6** (4.3 g, 12.6 mmol), N^3-benzoyl thymine[7] (2.9 g, 12.6 mmol), and Ph_3P (4.3 g, 16.4 mmol) in THF (79 mL) was cooled to 0 °C and diethylazodicarboxylate (2.6 mL, 16.4 mmol) was slowly added. The reaction was stirred at rt 2.5 h. Concentration followed by chromatography (first in 30% iPrOH/hexanes then 1% MeOH/$CHCl_3$) gave 5.62 g of **7a** (81%) as an oil: ^1H NMR ($CDCl_3$) δ 1.39 (s, 9 H), 1.77 (s, 3 H), 3.45 (m, 4H), 3.74 (m, 2H), 4.22 (m, 2H), 5.13 (s, 2H), 7.03 (s, 1H),

7.32 (m, 5H), 7.42 (t, 2H), 7.57 (t, 1H), 7.85 (m, 1H), 8.08 (d, 1H). ^{13}C NMR ($CDCl_3$) δ 11.94, 28.08, 46.25, 47.29, 65.86, 69.64, 80.54, 109.73, 128.14, 128.42, 128.82, 130.36, 131.74, 134.58, 134.92, 140.53, 149.71, 154.68, 155.14, 163.06, 169.05. IR (CCl_4) 1651, 1694, 1747, 2977,3482 cm^{-1}. UV (EtOH) λ_{max} = 251.0 FABMS (M+H) calculated for $C_{29}H_{33}N_3O_8$ was 551.2377, found 552.2360.

1-(5-*O*-benzyloxycarbonyl-3-aza-(*N*-*tert*-butoxycarbonyl)-pentyl)-N^3-benzoyl uracil (7b). A solution of **6** (1.34 g, 3.9 mmol), N^3-benzoyl uracil[7] (920 mg, 4.3 mmol), and PPh_3 (1.3 g, 5.1 mmol) in THF (24 mL) was cooled to 0 °C and diethylazodicarboxylate (0.80 mL 5.1 mmol) was slowly added. The reaction was stirred at room temperature for 1.5 h. Concentration followed by chromatography (first in 30% iPrOH/hexanes then 5% MeOH/$CHCl_3$) gave 1.78 g of **7b** (81 %) as an oil: ^1H NMR ($CDCl_3$) δ 1.43 (s, 9H), 3.47 (m, 4H), 3.82 (m, 2H), 4.22 (m, 2H), 5.14 (s, 2H), 5.63 (d, 1H), 7.19 (d, 1H), 7.34 (m, 5H), 7.45 (t, 2H), 7.60 (t, 1H), 7.92 (m, 1H), 8.09 (d, 1H). ^{13}C NMR ($CDCl_3$) δ 28.26, 46.38, 47.66, 47.94, 66.02, 69.83, 80.90, 101.46, 128.60, 130.00, 130.65, 131.56, 131.92, 134.88, 144.35, 149.80, 154.74, 155.35, 162.53, 168.96. IR (CCl_4) 1664, 1701, 1747, 2976, 3067 cm^{-1}. UV(EtOH) λ_{max} = 252.5 FABMS (M+Na) calculated for $C_{28}H_{31}N_3O_8$•Na was 560.2010, found 560.2022.

1-(5-hydroxy-3-aza-pentyl)-thymine hydrochloride (9a). A solution of **7a** (1.1g, 2.0 mmol) in dioxane (15 mL) was slowly added to a 0 °C solution of LiOH (1.3 mg, 31.8 mmol) in H_2O (15 mL). The reaction was warmed to rt and stirred for 18 h. The reaction was acidified to pH 4 with conc. H_3PO_4, diluted with H_2O, extracted with CH_2Cl_2 and dried over $MgSO_4$ to give crude **8a**. The crude mixture was filtered through silica gel using 4% MeOH/$CHCl_3$: ^1H NMR (DMSO-d_6) δ 1.23 (d, 9H), 1.69 (s, 3H), 3.18 (m, 2H), 3.45 (m, 4H), 3.74 (m, 2H), 4.78 (m, 1H), 7.35 (d, 1H). Crude **8a** was dissolved in 22.2 mL of a 1M solution of HCl in EtOAc (prepared by placing acetyl chloride (1.4 mL, 20 mmol) in dry EtOAc (20 mL) followed by addition of anhydrous MeOH (0.8 mL, 20 mmol)) and stirred for 24 h. The solution was concentrated and recrystalization from EtOH gave 199 mg of **9a** (40%) as a white solid: mp 205-207°C ^1H NMR (D_2O) δ 1.68, (s, 3H), 3.06 (t, 2H), 3.29 (t, 2H), 3.68 (t, 2H), 4.00 (t, 2H), 7.42 (s, 1H). ^{13}C NMR (D_2O) δ 11.46, 30.38, 45.13, 46.54, 56.69, 111.70, 142.47, 153.04, 167.11. IR (KBr) 1680, 1708, 3102, 3376 cm^{-1}. UV (H_2O) λ_{max} = 268.0 Anal. Calcd. for $C_9H_{15}N_3O_3$•HCl•1/4 H_2O: C,42.53; H, 6.54; N, 16.53. Found: C, 42.87; H, 6.31; N, 16.26.

1-(5-hydroxy-3-aza-pentyl)-uracil hydrochloride (9b). A solution of **7b** (1.7 g, 3.2 mmol) in dioxane (24 mL) was slowly added to a 0 °C solution of LiOH (2.1 g, 50.8

mmol) in H_2O (24 mL). The reaction was warmed to rt and stirred for 18 h. The reaction was acidified to pH 4 with conc. H_3PO_4, diluted with H_2O (50 mL), extracted with CH_2Cl_2 and dried over $MgSO_4$ to give crude **8b**. The crude mixture was filtered through silica gel using 10% MeOH/$CHCl_3$: ^1H NMR (D_2O) δ 1.17 (d, 9H), 3.21 (t, 2H), 3.49 (m, 4H), 3.75 (m, 2H), 5.62 (m, 1H), 7.40 (d, 1H). Crude **8b** was dissolved in 18.9 mL of a 1M solution of HCl in MeOH (prepared by placing acetyl chloride (1.2 mL, 17 mmol) in anhydrous MeOH (17.7 mL)) and stirred for 24 h. The solution was concentrated and recrystalization from EtOH gave 392 mg of **9b** (52%) as a white solid: mp 210-212°C ^1H NMR (D_2O) δ 3.06 (t, 2H), 3.29 (t, 2H), 3.68 (t, 2H), 3.99 (t, 2H), 5.68 (d, 1H), 7.46 (d, 1H). ^{13}C NMR (D_2O) δ 48.30, 49.25, 52.49, 59.51, 105.33, 149.62, 155.77, 169.58. IR (KBr) 1614, 1698, 2953, 3176, 3255 cm^{-1}. UV (H_2O) λ_{max} = 263.0 Anal. Calcd. for $C_8H_{13}N_3O_3$•HCl: C, 40.77; H, 5.99; N, 17.83. Found C, 40.81; H, 5.88; N, 17.64.

***O*-triphenylmethyl-*N*-*tert*-butoxycarbonyl diethanolamine (10).** To a 0 °C solution of *N*-BOC diethanolamine (4.1 g, 20.0 mmol), DMAP (240 mg, 2.0 mmol) and Et_3N (2.8 mL, 20.0 mmol) in CH_2Cl_2 (40 mL) was added trityl chloride (5.6 g, 20.0 mmol). The reaction was warmed to rt and stirred for 24 h. The reaction was washed with brine and concentrated. Chromatography (30% EtOAc/ hexanes) gave 3.7 g of **10** (70%, based on recovered starting material) as an oil: ^1H NMR ($CDCl_3$) δ 1.42 (m, 9H), 3.24 (m, 2H), 3.49 (m, 5H), 3.72 (m, 2H), 7.23 (m, 10H), 7.45 (m, 5H). ^{13}C ($CDCl_3$) δ 28.38, 29.68, 48.93, 51.51, 62.42, 80.17, 127.05, 127.82, 128.62, 143.87. IR (CCl_4) 1693, 2927, 3441 cm^{-1}. FABMS (M+Na) calculated for $C_{28}H_{33}NO_4$•Na was 470.2309, found 470.2316.

1-(5-*O*-triphenylmethyl-3-aza-(*N*-*tert*-butoxycarbonyl)-N^3-benzoyl uracil (11). A solution of **10** (2.3 g, 5.1 mmol), N^3-benzoyl uracil[7] (1.1g, 5.1 mmol), and PPh_3 (1.7 g, 6.6 mmol) in THF (22 mL) was cooled to 0 °C and diethylazodicarboxylate (1.0 mL, 6.6 mmol) was slowly added. The reaction was warmed to rt and stirred for 3 h. Concentration followed by chromatography (35% EtOAc/ hexanes) gave 2.69 g of **11** (82 %) as an oil: ^1H NMR ($CDCl_3$) δ 1.41 (m, 9H), 3.22 (m, 2H), 3.43 (m, 2H), 3.62 (m, 2H), 3.81 (m, 2H), 5.55 (d, 1H), 7.30 (m, 19H),7.62 (m, 1H), 8.14 (d, 1H). ^{13}C NMR ($CDCl_3$) δ 14.25, 28.27, 46.12, 47.96, 48.56, 61.89, 82.29, 80.26, 87.05, 101.16, 127.05, 127.79, 129.44, 129.88, 130.57, 131.70, 132.03, 134.66, 143.53, 144.58, 149.74, 155.53, 156.58, 162.48, 168.92. IR (CCl_4) 1667, 1748, 2975, 3443 cm^{-1}. UV (EtOH) λ_{max} = 269.5. FABMS (M+Na) calculated for $C_{39}H_{39}N_3O_6$•Na was 668.2847, found 668.2757.

1-(5-*O*-triphenylmethyl-3-aza-(*N*-*tert*-butoxycarbonyl)-uracil (12). A solution of **11** (2.7 g, 4.2 mmol) in dioxane (32 mL) was slowly added to a 0 °C solution of LiOH (2.8 g, 65.7 mmol) in H_2O (32 mL). The reaction was warmed to rt and stirred for 18 h. The reaction was acidified to pH 4 with conc. H_3PO_4, diluted with H_2O (50 mL), extracted with EtOAc and dried over $MgSO_4$. Chromatography (3% MeOH/ $CHCl_3$) gave 1.38 g of **12** (61%) as an oil. ^1H NMR ($CDCl_3$) δ 1.38 (d, 9H), 3.35 (m, 5H), 3.64 (m, 2H), 3.88 (m, 2H), 5.61 (t, 1H), 7.14 (d, 1H), 7.35 (m, 15 H). ^{13}C NMR ($CDCl_3$) δ 28.29, 46.79, 48.04, 48.78, 62.32, 80.32, 87.04, 101.69, 127.04, 127.84, 128.55, 143.75, 144.84, 150.79, 155.41, 163.69. IR(CCl_4) 1686, 3057, 3195 cm^{-1}. UV(EtOH) λ_{max} = 266.5. FABMS (M+Na) calculated for $C_{32}H_{35}N_3O_5$•Na was 564.2477, found 564.2483.

1-(5-hydroxy-3-aza-pentyl)-cytosine hydrochloride (13). To a 0 °C suspension of 1,2,4-triazole (0.21 g, 2.97 mmol) in CH_3CN (1.2 mL) was added $POCl_3$ (0.10mL, 1.0 mmol). After stirring for 5 min, Et_3N (0.47 mL, 3.4 mmol) was added. After stirring for an additional 1 h, the reaction was warmed to rt and **12** (0.11 g, 0.20 mmol) was added. The reaction was stirred for 5 h, then filtered, diluted with EtOAc (16 mL) and washed with saturated aq. $NaHCO_3$ solution (8 mL), brine (8 mL), dried over $MgSO_4$ and concentrated. The crude material was dissolved in dioxane (1.2 mL) and conc. NH_4OH (1.2 mL) and stirred 18 h. The solution was concentrated and the residue was dissolved in 1.9 mL of a 1M solution of HCl in EtOAc (prepared by placing acetyl chloride (0.12 mL, 1.7 mmol) in dry EtOAc (1.7 mL) followed by addition of anhydrous MeOH (0.07 mL, 1.7 mmol)) and stirred for 24 h. Recrystalization from ethanol gave 30 mg of **13** (64%) as a white solid: mp 219-241°C (decomposes). ^1H NMR (D_2O) δ 3.09 (m, 2H), 3.34 (m, 2H), 3.71 (m, 2H), 4.07 (m, 2H), 5.99 (d, 1H), 7.65 (d, 1H). ^{13}C NMR (D_2O) δ 46.10, 46.31, 49.71, 56.66, 95.42, 149.19, 150.29, 160.07. IR (KBr) 1681.22, 1723.79, 3336.97, 3519.35 cm^{-1}. UV (H_2O) λ_{max} = 273.0. FABMS (M+H) calculated for $C_8H_{14}N_4O_2$ was 198.1118, found 199.1256.

9-(5-hydroxy-3-aza-pentyl)-adenine hydrochloride (16). A suspension of **6** (1.7 g, 5 mmol), 6-chloropurine (0.77 g, 5 mmol) and Ph_3P (1.7 g, 6.5 mmol) in THF (30 mL) was treated with diethylazodicarboxylate (1.02 mL, 6.5 mmol) at which point everything went into solution. This mixture was stirred for 24 h and then concentrated. The residue was dissolved in $CHCl_3$ and filtered through a plug of silica gel (2.5 cm x 5 cm) eluting with $CHCl_3$. This material was concentrated, dissolved in DMF (8 mL), treated with sodium azide (0.65 g, 10 mmol) and heated to 70 °C for 3 h. The solution was concentrated and the residue dissolved in EtOAc and washed with H_2O (3 x). The solution

was dried (Na_2SO_4) and concentrated. To the residue was added 10% Pd-C (1 g), EtOH:EtOAc (3:1, 15 mL) and the reaction stirred under an atmosphere of H_2 (balloon) for 12 h. The reaction was filtered through celite and concentrated. The residue was dissolved in 55.6 mL of a 1M solution of HCl in MeOH (prepared by adding acetyl chloride (3.6 mL, 50 mmol) to anhydrous MeOH (52 mL)) and stirred for 24 h. The solution was concentrated and 400 mg of **16** (31%)was isolated as a white solid: mp decomposes 200-247 °C; ^1H NMR (D_2O) δ 3.15 (t, 2H), 3.58 (t, 2H), 3.71 (t, 2H), 4.59 (m, 2H), 8.23 (s, 1H), 8.33 (s, 1H). ^{13}C NMR (D_2O) δ 41.02, 46.51, 49.66, 56.59, 118.54, 144.71, 145.26, 149.17, 150.38. IR (KBr) 1596.65, 1685.14, 2930.97, 3220.28 cm^{-1}. UV (H_2O) λ_{max} = 259.5. FABMS (M+H) calculated for $C_9H_{14}N_6O$ was 222.1231, found 223.1307

9-(5-hydroxy-3-aza-pentyl)-guanine triflouroacetate (17). A suspension of **6** (1.7 g, 5 mmol), 2-amino-6-chloropurine (0.85 g, 5 mmol) and Ph_3P (1.7 g, 6.5 mmol) in THF (30 mL) was treated with diethylazodicarboxylate (1.02 mL, 6.5 mmol). This mixture was stirred for 24 h and then concentrated. The residue was dissolved in $CHCl_3$ and filtered through a plug of silica gel (2.5 cm x 5 cm) eluting with $CHCl_3$. This solution was concentrated and dissolved in TFA:H_2O (3:1, 15 mL) and stirred for 48 h. This reaction was concentrated and the residue triturated with Et_2O to obtain a white solid which was immediately dissolved in MeOH (30 mL). To this solution was added 10% Pd-C (1 g) and the reaction stirred under an atmosphere of H_2 (balloon) for 18 h. The reaction was filtered through celite and concentrated to yield 705 mg of **17** (40%) as a white solid: mp 209-210 °C; ^1H NMR (D_2O) δ 3.13 (t, 2H), 3.45 (t, 2H), 3.74 (t, 2H), 4.27 (t, 2H), 7.54 (s, 1H). ^{13}C NMR (D_2O) δ 40.43, 46.42, 48.49, 56.34, 115.37, 139.15, 151.01, 153.26, 158.06. IR (KBr) 1682, 3124, 3393 cm^{-1}. UV (H_2O) λ_{max} = 251.0 Anal. Calcd. for $C_9H_{14}N_6O_2 \cdot C_2F_3HO_2 \cdot 1/2\ H_2O$: C, 36.54; H, 4.46; N, 23.26. Found C, 36.64; H, 4.32; N, 23.55.

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.

Virological procedures. The Towne strain, plaque-purified isolate Po, 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.[11] High titer HSV-1 stocks were prepared by infecting KB cells at an m.o.i. of <0.1 also as detailed previously.[11] 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.[13]

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, compounds dissolved in growth medium were added to duplicate wells in four to eight selected concentrations. After incubation at 37 °C for 7 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[14] to detect HSV-1. Ninety-six-well cluster dishes were planted with 10,000 BSC-1 cells per well in 200 mL 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 mL per well of a solution of tetramethylbenzidine as substrate. The reaction was stopped with H2SO4 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: (i) Cytotoxicity produced in stationary HFF cells was determined by microscopic inspection of cells not affected by the virus used in plaque assays.[11] (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.[15]

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 hours in a CO2 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. No dose-response relationships were observed in any of the assays for any compound because no activity was detected at concentrations up to 100 μM. Samples containing positive controls (acyclovir for HSV-1, ganciclovir for HCMV, and 2-acetylpyridine thiosemicarbazone for cytotoxicity) were used in all assays and gave the expected activities.

ACKNOWLEDGMENTS

We thank Julie M. Breitenbach and Roger G. Ptak for expert performance of antiviral and cytotoxicity assays. We gratefully acknowledge support of this project by the American Cancer Society, Ohio Division, Inc. This work was supported in part by grant P30CA16058, National Cancer Institute, Bethesda, MD and by University of Michigan research funds.

REFERENCES

1. Schaeffer, H.J.; Beauchamp, L.; de Miranda, P.; Elion, G.B.; Bauer, D.J.; Collins, P. *Nature (London)* **1978**, *272*, 583-585.
2. (a) Acyclonucleosides: Part 1. *seco*-Nucleosides. El Ashry, E.S.H.; El Kilany Adv. Het. Chem., Katritzky, A.R.; Ed. Academic Press: San Diego, 1997, Vol 67, p 391. (b) Acyclonucleosides: Part 2. *diseco*-Nucleosides. El Ashry, E.S.H.; El Kilany Adv. Het. Chem., Katritzky, A.R.; Ed. Academic Press: San Diego, 1997, Vol 68, p 1. (c) Acyclonucleosides: Part 3. *tri-*, *tetra-*, and *pentaseco*-Nucleosides. El Ashry, E.S.H.; El Kilany Adv. Het. Chem., Katritzky, A.R.; Ed. Academic Press: San Diego, 1997, Vol 69, p 129. (d) Chu, C.K.; Cutler, S.J. *J. Het. Chem.* **1986**, *23*, 289-319.
3. (a) Shirokova, E.A.; Tarussova, N.B.; Shipitisin, A.V.; Semizarov, D.G.; Krayevsky, A.A. *J. Med. Chem.* **1994**, *37*, 3739-3848. (b) Legraverend, M.; Boumchita, H.; Zerial, A.; Huel, C.; Lemaitre, M.; Bisagni, E. *J. Med. Chem.* **1990**, *33*, 2476-2480.

4. Van Aerschot, A.; Zhigang,N.; Rozenski, J.; Claes, P.; DeCleercq, E.; Herdewijn, P. *Nucleosides Nucleotides* **1994**, *13*, 1791-1800.
5. (a) Dvorakova, H.; Masojidkova, M.; Holy, A.; Balzarini, J.; Andrei, G.; Snoeck, R.; De Clercq, E. *J. Med. Chem.* **1996**, *39*, 3263-3268. (b) Genevois-Borella, A.; Florent, J.-C.; Monneret, C.; Grierson, D.S. *Tetrahedron Lett.* **1990**, *31*, 4879-4882. (c) Ogilvie, K.K.; Nguyen-Ba, N.; Gillen, M.F.; Radatus, B.K.; Cheriyan, U.O.; Hanna, H.R.; Smith, K.O.; Galloway, K.S. *Can. J. Chem.* **1984**, *62*, 241-252. (d) Liu, M.-C.; Kuzmich, S.; Lin, T.-S. *Tetrahedron Lett.* **1984**, *25*, 613-616. (e) Kelley, J.L.; Krochmal, M.P.; Schaeffer, H.J. *J. Med. Chem.* **1981**, *24*, 1528-1531.
6. Bergmeier, S.C.; Fundy, S.L. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 3135-3138.
7. (a) Procedure taken from: Cruickshank, K.A.; Jiricny, J.; Reese, C.B. *Tetrahedron Lett.* **1984**, *25*, 681-684. (b) Spectral data: Csuk, R.; Eversmann, L. *Tetrahedron* **1998**, *54*, 6445-6456.
8. Harnden, M.R.; Parkin, A.; Parratt, M.J.; Perkins, R.M. *J. Med. Chem.* **1993**, *36*, 1343-1355.
9. Gibson, F.S.; Bergmeier, S.C.; Rapoport, H. *J. Org. Chem.* **1994**, *59*, 3216-3218.
10. Pérez-Pérez, M.-J.; Rosenski, J.; Busson, R.; Herdewijn, P. *J. Org. Chem.* **1995**, *60*, 1531-1537.
11. Turk, S.R.; Shipman, Jr., C.; Nassiri, R.; Genzlinger, G.; Krawczyk, S.H.; Townsend, L.B.; Drach, J.C. *Antim. Ag. Ch.*. **1987**, *31*, 544-550.
12. Still, C.W.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *50*, 2923-2925.
13. Prichard, M.N.; Turk, S.R.; Coleman, L.A.; Engelhardt, S.L.; Shipman, Jr., C.J.; Drach, J.C. *J.Virol. Met.* **1990**, *28*, 101-106.
14. Prichard, M.N.; Shipman, C.J. *Antivir. Res.* **1990**, *14*, 181-206.
15. Prichard, M.N.; Prichard, L.E.; Baguley, W.A.; Nassiri, M.R.; Shipman, Jr, C.J. *Antim. Ag. Ch.* **1991**, *35*, 1060-1065.

received 8/17/97
accepted 11/23/98