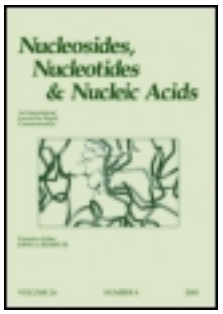


Nucleosides, Nucleotides and Nucleic Acids

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# Synthesis of 4-Substituted Imidazo[4,5-d][1,2,3]triazine (2-Azapurine)nucleosides

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NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 19(1&2), 39–68 (2000)

# SYNTHESIS OF 4-SUBSTITUTED IMIDAZO[4,5-*d*][1,2,3]TRIAZINE (2-AZAPURINE)NUCLEOSIDES

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*Dedicated to the memory of Dr. Gertrude B. Elion*

**ABSTRACT** Several methods for functionalization of the 4-position of imidazo[4,5-*d*][1,2,3]triazin-4-one were investigated. These investigations were successful and led to the preparation of 4-amino, 4-triazol-1-yl, 4-methoxy, 4-methylthio, 4-methylamino, 4-thio, 4-nitrobenzyl, and 4-unsubstituted 9-(β-D-ribofuranosyl)-imidazo-[4,5-*d*][1,2,3]triazine (2-azapurine ribosides). The 4-unsubstituted compound (**19**) was slightly active against HCMV in plaque and yield reduction experiments and was not cytotoxic at 100 μM. The methylamino (**15**), hydrazino (**16**), and *p*-nitrobenzylthio (**20**) were inactive against HCMV but slightly cytotoxic. The thiomethyl-substituted analog (**21**) was the most active with activity comparable to ganciclovir but with greater cytotoxicity. We conclude that even though none of the tested compounds had antiviral activity superior to ganciclovir, the new synthetic methods will provide a route to more interesting compounds.

## INTRODUCTION

Although imidazo[4,5-*d*][1,2,3]triazine (2-azapurine) nucleosides possess significant biological activities,[1,2] there is a remarkable absence in the literature of a successful method for the introduction of substituents into the 4-position of preformed

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7-substituted imidazo[4,5-*d*][1,2,3]triazine nucleosides. One manifestation of this paucity is that of all the 7-glycosylimidazo[4,5-*d*][1,2,3]triazine derivatives reported,[3,8] there are only three distinct substituents in the 4-position; the 4-oxo, 4-amino, and 4-methylthio groups. These compounds were obtained by either the direct nitrous acid ring closure of an appropriately substituted ortho-aminoimidazole carboxamide, or by glycosylation of the preformed heterocycle.

However, a few examples of chemical functional group interconversions at the 4-position of a preformed 5,7-unsubstituted imidazo[4,5-*d*][1,2,3]triazine have been reported. For example, the 4-chloro and 4-methylthio substituents have been introduced via chlorination,[8] or methylation,[9] respectively, of the 4-thio derivative. A subsequent displacement of the 4-chloro or the 4-methylthio moiety with dimethylamine has furnished the 4-dimethylamino derivative.[9,10] The only report of any functional group interconversion at the 4-position of a 7-glycosylimidazo[4,5-*d*][1,2,3]triazine is the enzymatic hydrolytic deamination by adenosine deaminase[4] of the 4-amino derivatives (2-aza-adenosine, 2-aza-arabinoadenosine, or 2'-deoxy-2-aza-adenosine) to afford the corresponding 4-keto derivatives (2-azainosines). All attempts by these workers to effect a chemical deamination of these 2-azaadenosine derivatives were reported to be unsuccessful.

Thus, methods had not been developed for the transformation of the 4-oxo moiety of an imidazo[4,5-*d*][1,2,3]triazine nucleoside into any other functional group at the time we began this investigation. 4-Methylthio-7-(β-D-ribofuranosyl)imidazo[4,5-*d*][1,2,3]-triazine[8] should be amenable to a nucleophilic attack at the 4-position. However, we have found that the preferred position for nucleophilic attack by amines and hydroxide anion is the 6-position, leading to an opening of the imidazole ring. Thus, 4-methylthio-7-(β-D-ribofuranosyl)imidazo[4,5-*d*][1,2,3]triazine did not appear to be a useful intermediate for the synthesis of various 4-substituted-7-(β-D-ribofuranosyl)imidazo[4,5-*d*][1,2,3]-triazines.[11]

However, several 4-substituted amino-7-(2-fluorobenzyl)imidazo[4,5-*d*][1,2,3]-triazines have been synthesized[12a] by reacting the dimethylaminopyridinium salt of 7-

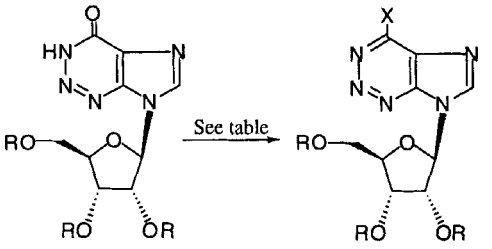
(2-fluorobenzyl)imidazo[4,5-*d*][1,2,3]triazin-4-one, prepared *in situ,* with primary amines. Before the publication of these results[12a], we reported[12b] the results of similar studies with imidazo[4,5-*d*][1,2,3]triazine nucleosides. Herein, we report the synthesis and initial antiviral testing of some 4-substituted imidazo[4,5-*d*][1,2,3]triazine nucleosides and some intermediates from which other 4-substituted derivatives can be prepared.



## RESULTS AND DISCUSSION

We initated studies to develop methodologies for a functionalization of the 4-oxo moiety of the readily available 7-(β-D-ribofuranosyl)imidazo[4,5-*d*][1,2,3]triazin-4-one (**1**, 2-azainosine). Our initital study found that **2** was unreactive towards phosphorous oxychloride and triethylamine in chloroform at room temperature, as determined by TLC. We then investigated the reactivity of **2** towards phosphorous oxychloride and triethylamine in chloroform at reflux temperature. To our surprise, we obtained $N^4$,$N^4$-diethyl-2-azaadenosine (**4**, table 1, entry a), and not the desired 4-chloro derivative, when the reaction mixture was heated at reflux temperature. The formation of this product most likely results from N-dealkylation of a 4-N,N,N-triethylammonium intermediate. No reaction occurred at room temperature when we attempted a similar reaction with DBU as the base, although decomposition occurred at higher temperatures. In an effort to isolate the 4-chloro derivative or a pyridinium type intermediate[13], we elected to use a base which would catalyze the reaction of **2** with phosphorous oxychloride at a lower temperature. A previous report[12b] from our laboratory prompted us to explore the analogous reaction of **2** with phosphorous oxychloride and 4-(dimethylamino)pyridine, a well known catalyst of acylation reactions.[3,8] In contrast to the previous experiment with triethylamine or DBU, **2** was reacted with phosphorous oxychloride in the presence of 4-(dimethylamino)pyridine (4-DMAP) at 0 °C to afford, presumably, the N,N-(dimethylamino)pyridinium salt. We expected that *in situ* treatment of this mixture with methanolic ammonia would afford 2-azaadenosine (**14**). However, we isolated the 4-methoxy derivative **5**, (table 1, entry b) after quenching the reaction with methanolic ammonia for 1 h at 0 °C. By extending the reaction time to 24 h, 2-azaadenosine (**14**) was obtained. Alternatively, when compound **2**

**TABLE 1.** Synthesis of Ribosyl Protected 4-Substituted 7-(β-D-Ribofuranosyl)-imidazo[4,5-*d*][1,2,3]triazines.




**1:** R = H
**2:** R = Ac
**3:** R = TBS

**4-11**

| Entry | Starting Material | CONDITIONS | PRODUCT | R | X | Yield (%) |
|---|---|---|---|---|---|---|
| a | **2** | $POCl_3$, $Et_3N$, 5 °C- r.t. | **4** | Ac | $N(Et)_2$ | 49 |
| b | **2** | 1) $POCl_3$, DMAP 2) MeOH/$NH_3$ | **5** | Ac | MeO | 27 |
| c | **2** | $POCl_3$, $Et_3N$, 1,2,4-triazole | **6** | Ac | * | 30 |
| d | **3** | 1) $POCl_3$, DMAP 2) MeOH/$NH_3$ | **7** | TBS | MeO | 51 |
| e | **3** | 1) $POCl_3$, DMAP 2) MeOH/NaOMe | **7** | TBS | MeO | 29 |
| f | **3** | 1) $POCl_3$, pyridine 2) MeOH/$NH_3$ | **8** | TBS | $NH_2$ | 59 |
| g | **3** | 1) $POCl_3$, pyridine 2) $MeNH_2$ | **8** | TBS | $NH_2$ | 45 |
| h | **3** | 1) $POCl_3$, DMAP 2) $MeNH_2$ | **9** | TBS | NHMe | 24 |
| i | **3** | 1) $POCl_3$, pyridine 2) MeOH/NaOMe | **7** | TBS | MeO | 41 |
| j | **3** | $POCl_3$, $Et_3N$, 1,2,4-triazole | **10** | TBS | * | 31 |
| k | **3** | Lawesson's Reagent | **11** | TBS | SH | 35 |
| l | **3** | 1) $POCl_3$, pyridine 2) $H_2S$ | **11** | TBS | SH | 42 |

* 

was reacted with a premixed suspension of phosphorous oxychloride, 1,2,4-triazole and triethylamine, a 30% yield of 7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-4-(1,2,4-triazol-1-yl)imidazo[4,5-*d*][1,2,3]triazine (**6**, table 1, entry c) was obtained.

The acetyl protecting groups were advantageous in this approach towards the synthesis of 2-azaadenosine, because a nucleophilic displacement and deprotection could be accomplished in a single step. However, by using the base stable *t*-butyldimethylsilyl (TBS) protecting group,[16] we would have more freedom to explore various reactions of the 2-azahypoxanthine moiety. Therefore, the reaction of 2-azainosine[5] (**1**) with *t*-butyldimethylsilyl chloride, in DMF, in the presence of imidazole afforded the tri-*O*-t-butyldimethylsilyl derivative **3**, in a 77% yield, after chromatography. We subsequently established that the product obtained by partitioning the reaction mixture between water and ethyl acetate was suitably pure for our purposes. This method provides essentially a quantitative conversion of the free nucleoside **1** to the protected nucleoside **3**. Compound **3** was reacted with phosphorous oxychloride and 4-(dimethylamino)pyridine in ethanol-free chloroform and then quenched with methanolic ammonia to afford **7** (table 1, entry d) in a 51% yield.

Since compound **7** was found to be a useful intermediate (*vida infra*), we continued our search for a better synthesis of **7**. Phosphorous oxychloride was added to a solution of **3** and DMAP and this was followed by quenching with a solution of methanolic sodium methoxide (table 1, entry e) to give compound **7**. This reaction was slower than the reaction with methanolic ammonia and furnished a lower yield of compound **7**.

We then studied the reaction of **3** with phosphorous oxychloride in the presence of pyridine. The reaction of **3** under these conditions was sluggish with a reaction time of 3 h being neccessary for the complete consumption of starting material, as determined by TLC. More importantly, the addition of methanolic ammonia (table 1, entry f) or methylamine (table 1, entry g) (Zincke reaction[12]) to the reaction mixture yielded the 2-azaadenosine derivative **8** instead of the 4-methoxy derivative **7**. When DMAP was used as the base in this reaction, compound **9** was obtained since no Zincke reaction occurred (table 1, entry h). Treatment of **3** with $POCl_3$ and pyridine followed by quenching with methanolic

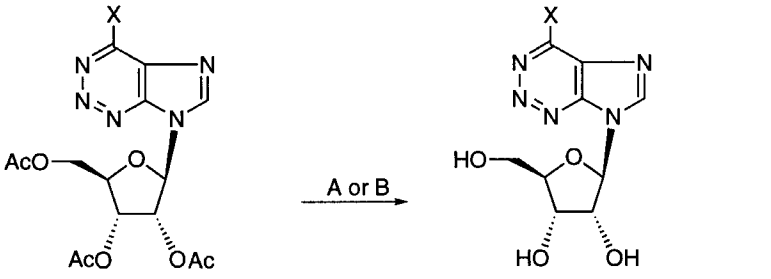
sodium methoxide gave the 4-methoxy derivative **7** (table 1, entry i) with no evidence for the formation of compound **14**. However, the 4-methoxy derivative **7** (table 1, entry i) was contaminated with impurities which could not be readily removed by silica gel chromatography. Finally, compound **3** was treated with a premixed suspension of phosphorous oxychloride, 1,2,4-triazole and triethylamine to give a 31% yield of the intermediate **10** (table 1, entry j).

We then initated studies designed to furnish the previously unreported 4-thio derivative **17**. We explored the reaction of **3** with phosphorous pentasulphide in various solvent systems, but were unable to isolate any of the desired product from these reactions. However, the reaction of **3** with Lawesson's reagent[17] furnished a smooth conversion of **3** to **11**, albeit in low yield (table 1, entry k). This reaction proceeded in either toluene or 1,2-dichloroethane, however, the reaction in 1,2-dichloroethane proceeded at a lower temperature and in better yield. Alternatively, phosphorous oxychloride was added to a solution of **3** in pyridine, and then hydrogen sulphide was bubbled through the reaction mixture to give **11** in a reasonable yield (table 1, entry l).

Deprotection of compound **4** with methanolic ammonia gave the previously unreported compound **12** (Scheme 1). When we attempted to deprotect compound **5** with methanolic ammonia, 2-azaadenosine (**14**) was isolated as a pure solid instead of the expected 4-methoxy-7-(β-D-ribofuranosyl)imidazo[4,5-*d*][1,2,3]triazine (**13**). However, a successful deblocking of **5** was accomplished with sodium carbonate in methanol to yield the free nucleoside **13**. Treatment of compound **6** and **13**, individually, with methanolic ammonia gave a 35% and 70% yield of 2-azaadenosine (**14**), respectively.

Deprotection of the 4-methoxy derivative **7** was accomplished using tetra-*n*-butylammonium fluoride[16] in THF to afford the free nucleoside (**13**, Scheme 2) in a 53% yield. This compound was identical to **13** obtained from a removal of the acyl groups of **5**. Compounds **8** and **9** were deprotected in a similar fashion to give compounds **14** and **15**, respectively. The attempted deblocking of **10** with TBAF was unsuccessful, and only decomposition occurred. However, treatment of either **7** or **10** with methanolic ammonia gave compound **8**. To explore the reaction of **13** with

**SCHEME 1**

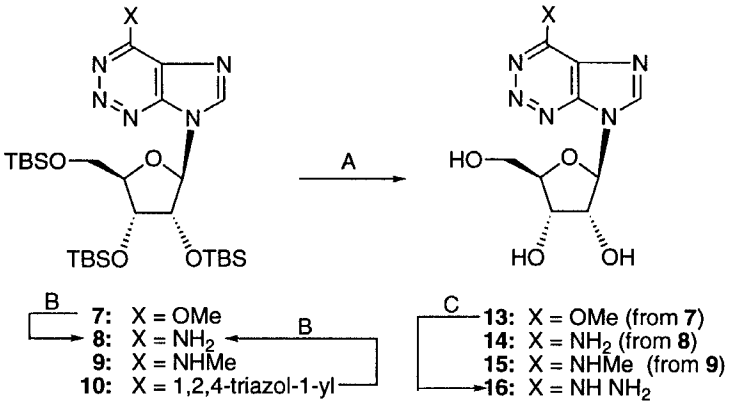



**4:** X = $N(Et)_2$
**5:** X = OMe
**6:** X = 1,2,4-triazol-1-yl

**12:** X = $N(Et)_2$ (from **4**)
**13:** X = OMe (from **5**)
**14:** X = $NH_2$ (from **5** or **6**)

A) $NH_3$/MeOH; B) $Na_2CO_3$, MeOH

**SCHEME 2**




**7:** X = OMe
**8:** X = $NH_2$
**9:** X = NHMe
**10:** X = 1,2,4-triazol-1-yl

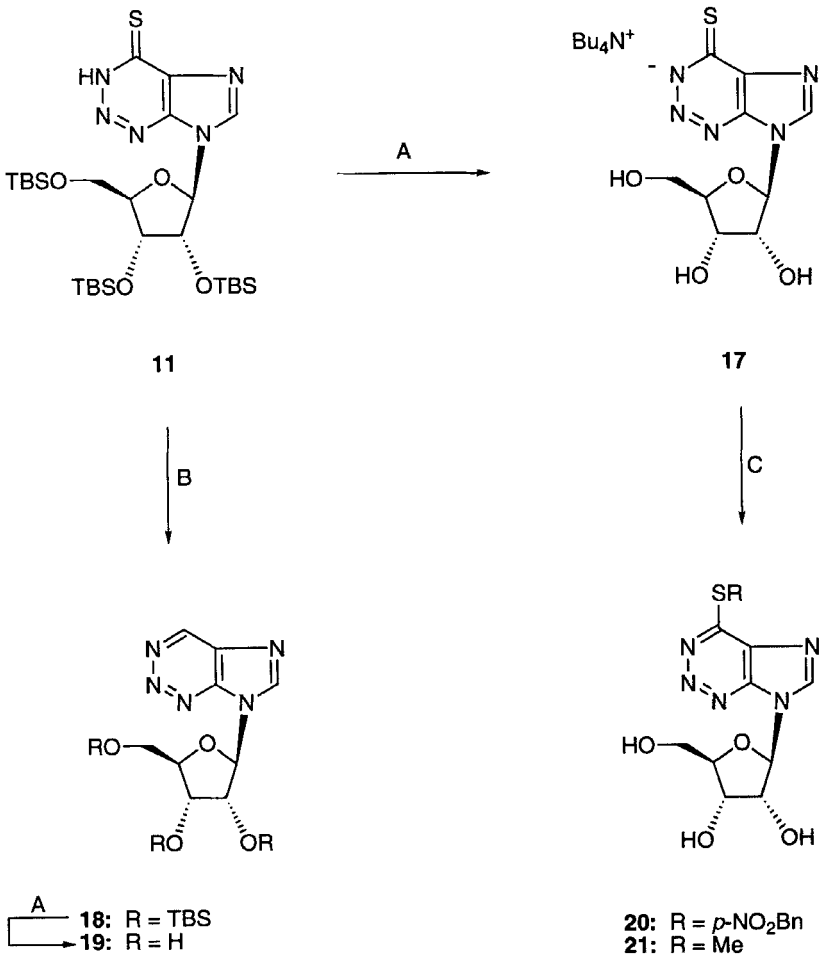
**13:** X = OMe (from **7**)
**14:** X = $NH_2$ (from **8**)
**15:** X = NHMe (from **9**)
**16:** X = NH $NH_2$

A) TBAF, THF; B) $NH_3$/MeOH; C) $NH_2NH_2$, MeOH.

other nucleophiles, we treated **13** with hydrazine hydrate in methanol at room temperature for 8 hours and obtained a 66% yield of **16**, a structural analog of 6-hydrazino-9-(β-D-ribofuranosyl) purine.

Deprotection of compound **11**, (Scheme 3) with tetra-*n*-butylammonium fluoride, afforded the free nucleoside as the tetra-*n*-butylammonium salt **17**. This salt (**17**) was

**SCHEME 3**




A) TBAF, THF; B) Raney Ni, EtOH, r.t.; C) MeI, MeOH, or *p*-$NO_2$BnCl, MeOH.

alkylated, individually, with 4-nitrobenzyl chloride and with methyl iodide to yield the novel 4-nitrobenzylthio derivative **20** and the known[8] 4-methylthio derivative **21**.

Since 2-azanebularine (**19**) had not been reported in the literature, we synthesized **19** by a dethiation of the protected 4-thio derivative **11** with Raney nickel. The reaction proceded smoothly in ethanol to yield the protected 2-azanebularine derivative **18**. A removal of the protecting groups from **18** with tetra-*n*-butylammonium fluoride was

conducted at a low temperature since the product (**19**) decomposes rather easily. A 49% yield of 2-azanebularine (**19**) was obtained if the deblocking was conducted at 0 °C, and the product was immediately isolated.

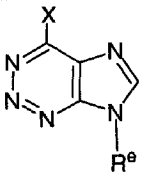
Therefore, we have accomplished the synthesis of several novel 4-substituted imidazo[4,5-*d*][1,2,3]triazine nucleoside derivatives which until now have been unreported. The finding that the 4-methoxy group of compound **13** can be readily displaced with amine nucleophiles furnishes a convenient method for the synthesis of other N4-alkylamino-2-azaadenosine derivatives.

Several of the target compounds were tested for activity against human cytomegalovirus (HCMV) and for cytotoxicity in the cells used to propagate the virus [human foreskin fibroblasts, (HFF's)]. The unsubstituted compound **19** was slightly active against HCMV in plaque and yield reduction experiments and was not cytotoxic to stationary HFF's at the highest concentration tested (Table 2). The substituted analogs **15**, **16,** and **20** were inactive against HCMV but slightly cytotoxic. The thiomethyl-substituted analog **21** was the most active with activity comparable to ganciclovir albeit with greater cytotoxicity (Table 2). We conclude that even though none of the tested compounds had antiviral activity superior to ganciclovir, the new synthetic methods will provide a route to more interesting compounds.

## EXPERIMENTAL

Melting points were taken on a Thomas-Hoover apparatus and are uncorrected. The silica gel used for chromatography[19] was silica gel 60 230-400 mesh (E. Merck, Darmstadt, West Germany), Thin Layer Chromatography (TLC) was performed on pre-scored SilicAR 7GF plates (Analtech, Newark, DE, USA). Compounds were visualized by illuminating under UV light (254 nm) and by spraying with 10-20% methanolic sulfuric acid followed by charring. The following solvent system designations are used throughout the manuscript: solvent system A: ethyl acetate; solvent system B: ethyl acetate/cyclohexane 1:2, v/v; solvent system C: ethyl acetate/cyclohexane 1:1, v/v; solvent system D: chloroform/ethanol 9:1, v/v; solvent system E: chloroform/methanol 4:1, v/v; solvent system F: chloroform/methanol 8:1, v/v; solvent system G: ethyl acetate/methanol

**TABLE 2.** Antiviral Activity and Cytotoxicity of Some Imidazo[4,5-*d*][1,2,3]triazine Ribonucleosides



| compound no. | substituent X | 50 or 90% inhibitory concentration (μM) antiviral activity[a] HCMV plaque | HCMV yield | cytotoxicity[b] HFF |
|---|---|---|---|---|
| **19** | H | 40 | 15 | >100 |
| **15** | $NHCH_3$ | >100 | | 70 |
| **16** | $NHNH_2$ | >100 | | 70 |
| **20** | $S\text{-}CH_2\text{-}C_6H_4\text{-}p\text{-}NO_2$ | >100 | | >100 |
| **21** | $SCH_3$ | 16 | 0.3 | 100 |
| ganciclovir | | 7.7[c] | 1.8[d] | >100[c] |

[a]Plaque and yield reduction assays were performed in duplicate wells as described in the text. Ninety percent inhibitory concentrations presented for data from yield assays. [b]Visual cytotoxicity scored on HFF cells at time of HCMV plaque enumeration. [c]Average of 88 experiments in which DHPG was used as a positive control. [d]Average from two to four separate experiments. [e]β-D-ribofuranosyl.

9:1, v/v; solvent system H: methanol/water 2:3, v/v; solvent system I: methanol/water 2:1, v/v; solvent system J: methanol/water 1:3, v/v; solvent system K: ethyl acetate/cyclohexane 1:9, v/v; solvent system L: ethyl acetate/cyclohexane 1:18, v/v; solvent system M: ethyl acetate/cyclohexane 1:3, v/v; solvent system N: chloroform/methanol 16:1, v/v; solvent system O: ethyl acetate/cyclohexane 1:6, v/v; solvent system P: methanol/water 3:1, v/v;

solvent system Q: chloroform/methanol 32:1, v/v; solvent system R: ethyl acetate/hexanes 1:5, v/v; solvent system S: ethyl acetate/hexane 3:7, v/v; solvent system T: chloroform/methanol 49:1, v/v. All evaporations were carried out using a rotary evaporator connected to a water aspirator pump. The water bath temperature was maintained between 40 and 50 °C unless otherwise stated. Determination of UV spectra was performed on a Hewlet-Packard 8450-A UVNIS spectrophotometer. IR spectra were obtained on a Nicolet 5 DXB FT spectrophotometer. $^{1}$H-NMR spectra were obtained on a BRUKER 300 MHz or 270 MHz instrument. $^{13}$C-NMR spectra were obtained on a BRUKER 360 or 300 instrument operating at either 90 MHZ or 75 MHZ, respectively. The chemical shifts are expressed in parts per millions relative to the standard chemical shift of the solvent system DMSO-$d_6$ ($\delta$ = 2.50 (1 H), 39.5 ($^{13}$C)). Where neccessary, deuterium exchange, and homonuclear decoupling experiments were used to obtain proton shift assignments. Combustion analysis were performed either by the University of Michigan, Ann Arbor or MHW, Phoenix, Az.

**7-[2,3,5-Tri-O-(t-butyldimethylsilyl)-β-D-ribofuranosyl]imidazo[4,5-*d*]-[1,2,3]triazin-4-one (3).** A solution of 7-(β-D-ribofuranosy)imidazo[4,5-*d*]-[1,2,3]triazin-4-one[5], (**1**, 2.52 g, 9.34 mmol), imidazole (5.1 g, 75 mmol) and *t*-butyldimethylsilyl chloride (5.65 g, 37.5 mmol) in DMF (50 mL) was stirred at room temperature for 2 h. At that time, an additional portion of imidazole (2.6 g, 38 mmol) and *t*-butyldimethylsilyl chloride (2.8 g, 18.6 mmol) were added. After 3 h of stirring, the mixture was evaporated under reduced pressure and the resulting syrup was partitioned between ethyl acetate (150 mL) and ice water (150 mL). The organic layer was washed with ice water (3 x 150 mL), and brine (100 mL), dried over sodium sulphate, filtered, and evaporated to a sticky foam which was kept under reduced pressure at room temperature for 16 h. This foam was purified by silica gel chromatography (4.5 cm x 21 cm, eluting with cyclohexane then solvent system B then solvent system C, $R_f$ = 0.79 in solvent system A) to give a foam (4.4 g, 77%): $^{1}$H NMR (DMSO-$d_6$): $\delta$ 15.21 (bs, 1H), 8.67 (s ,1H), 6.08 (d, 1H, J = 5.4 Hz), 4.79 (m, 1H), 4.35 (m, 1H), 4.04 (m, 1H), 3.96 (m, 1H), 3.76 (m, 1H), 0.90, 0.87,0.73 (3s, 27H), 0.11, 0.09, 0.05, -0.06, -0.30 (5s, 18H),

$^{13}C$ NMR (DMSO-$d_6$): δ 154.1 (s, 5, C-4), 144.7 (s, q, C-7a), 142.0 (d, d, C-6), 126.7 (s, d, C-4a), 88.1 (d, m, C-1'), 85.1 (d, m, C-4'), 75.2 (d, m, C-2'), 71.4 (d, m, C-1'), 61.9 (t, m, C-5'), 25.6, 25.5, 25.3 (3s, 3m, *t*-Bu-$CH_3$), 17.8, 17.5,17.3 (3s, 3q, t-Bu-C), -4.8, -5.1, -5.6, -5.7, -5.8 (5s, Sm); UV [$\lambda_{max}$nm (log ε)]: methanol 284 (3.69), 207 (4.23); pH 1 300 (3.92), 214 (4.29); pH 11 299 (3.90), 248 (3.81), 228 (3.87). *Anal.* Calcd. for $C_{27}H_{53}N_5O_5Si_3$: C, 52.99; H, 8.72; N, 11.44. Found: C, 53.01; H, 8.79; N, 11.37.

**4-(N,N-Diethylamino)-7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazo-[4,5-*d*][1,2,3]triazine (4).** Phosphorous oxychloride (0.23 g, 1.52 mmol) was added dropwise to a solution of 7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazo[4,5,*d*]-[1,2,3]triazin-4-one[5] (**2**, 0.5 g, 1.27 mmol) and triethylamine (0.9 g, 8.9 mmol) in chloroform (25 mL), which had been cooled in an ice-bath to *ca.* 5 °C. After the addition was complete, the ice-bath was removed, and the mixture was stirred for 30 min at room temperature. The reaction mixture was then heated at reflux temperature for 30 min. After cooling to room temperature, ice (25 cc) was added, and the mixture was washed with water (2 x 25 mL and brine (25 mL). The organic layer was dried over sodium sulphate, filtered, and the filtrate was evaporated to yield a dark syrup which was purified by silica gel chromatography (3 cm x 10 cm, eluting with solvent system A, $R_f$ = 0.55 in solvent system A) to give a yellow syrup which was crystallized from solvent system H (10 mL) to yield **4** as fine needles (0.28 g, 49%): mp 128-129 °C; $^1H$ NMR (DMSO-$d_6$): δ 8.67 (s, 1H), 6.38 (d, 1H, $J$ = 5.1 Hz), 6.07 (m, 1H), 5.68 (m, 1H), 4.45 (m, 1H), 4.41 (m, 1H), 4.26 (m, 1H), 4.0 (m, 4H), 2.12, 2.03, 2.02, (3s, 9H), 1.24 (t, 6H); UV [$\lambda_{max}$nm (log ε]: methanol, 322 (3.82), 269 (4.11), 214 (4.10); pH 1, 323 (3.57), 267 (4.12), 219 (4.09); pH 11, 324 (3.79), 271 (4.11), 233 (3.81); IR (KBr): 2984, 1752, 1602, 1365, 1223, 1145, 1027 $cm^{-1}$; *Anal.* Calcd. for $C_{19}H_{26}N_6O_7$: C, 50.66; H, 5.81; N, 18.66. Found: C, 50.83; H, 5.81; N, 18.70.

**4-Methoxy-7-(2,3,5-tri-*O*-acetyl-β-ribofuranosyl)imidazo[4,5-*d*][1,2,3]-triazine (5).** A cold (0 °C), stirred solution of **2**, (1.0 g, 2.54 mmol) and 4-(dimethylamino)pyridine (2.23 g, 18.3 mmol) in chloroform (50 mL) was treated dropwise

with phosphorous oxychloride (0.46 g, 3.0 mmol). After the addition was complete (*ca.* 10 min), the ice-bath was removed and the mixture was allowed to stir at room temperature for 10 min. The reaction mixture was again cooled to 0 °C, and an additional quantity of phosphorous oxychloride (0.46 g, 3.0 mmol) was added dropwise. After this addition was complete, the mixture was stirred for 5 min and methanolic ammonia (50%, v/v, 5 mL) was added over a 5 min period. The mixture was stirred for 1 h, at 0 °C, and then evaporated to dryness. The residue was dissolved in solvent system A (100 mL), and the solution was washed with water (4 x 100 mL), and brine (100 mL). The organic layer was then dried over sodium sulphate, filtered, and the filtrate was concentrated to a volume of 5 mL. This solution was purified by silica gel chromatography (3 cm x 10 cm, eluting with solvent system A, $R_f$ 0.51 in solvent system A) and crystallized from solvent system L (6 mL) to afford **5** as a crystalline product (0.38 g, 27%): mp 146-147 °C; $^1$H NMR (DMSO-$d_6$): δ 8.94 (s, 1H), 6.49 (d, 1H, J =5.1 Hz), 6.08 (m, 1H), 5.71 (m, 1H), 4.46-4.42 (2m, 2H), 4.30 (s, 3H), 4.26 (m, 1H), 2.12, 2.03, 2.01, (3s, 9H); UV [$\lambda_{max}$nm (log e)]: methanol, 264 (3.78), 249 (3.86), 205 (4.24); pH 1, 267 (3.78), 249 (3.84), 205 (4.29); pH 11, 267 (3.78), 249 (3.83); IR (KBr): 3078, 1757, 1611, 1365, 1251, 1211, 1081, 1044 cm$^{-1}$; *Anal.* Calcd. for $C_{16}H_{19}N_5O_8$: C, 46.95; H, 4.67; N, 17.11. Found: C, 46.99; H, 4.85; N, 17.21.

**4-(1,2,4-Triazol-1-yl)-7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazo-[4,5-*d*][1,2,3]triazine (6).** A stirred solution of 1,2,4-triazole (3.10 g, 45.0 mmoles), acetonitrile (40 mL) and phosphorous oxychloride (0.93 mL, 10.0 mmoles) was cooled to 0 °C in an ice bath. Triethylamine (6.3 mL, 45.0 mmoles) was then added and the reaction was stirred for 1 h. At that time **2** (791 mg, 2.0 mmoles) was added in one portion and the suspension was stirred for 3.5 h. The reaction mixture was filtered through a bed of Celite, and the solvent was removed under reduced pressure. The resultant residue was partitioned between ethyl acetate (75 mL) and water (75 mL), and the organic layer was washed with an additional portion of water (75 mL) and brine (50 mL). The organic layer was dried ($MgSO_4$), filtered and the filtrate was evaporated to dryness under reduced pressure. The residue was purified by silica gel chromatography (3 cm x 10

cm, eluting with solvent system T, $R_f$ = 0.63 in solvent system D) and the resultant product was recrystallized from ethyl acetate/ hexanes to yield 271 mg (30%) of **6** as a crystalline solid: mp 205-206 °C; $^1$H NMR (DMSO-$d_6$): δ 9.85 (s, 1H), 9.30 (s, 1H), 8.60 (s, 1H), 6.63 (m, 1H), 6.12 (m, 1H), 5.77 (m, 1H), 4.5 (m, 2H), 4.33 (m, 1H), 2.14, 2.08, 2.05 (s, 3H each); UV [$\lambda_{max}$nm (log ε)]: methanol, 273 (4.15), 220 (4.35), 221 (trough); pH 1, 272 (4.20), 219 (4.46); pH 11, 281 (3.91), 242 (3.98); *Anal.* Calcd. for $C_{17}H_{18}N_8O_7$: C, 45.74; H, 4.06; N, 25.10. Found: C, 45.83; H, 4.05; N, 25.43.

**4-Methoxy-7-(2,3,5-tri-*O*-(*t*-butyldimethylsilyl)-β-D-ribofuranosyl)-imidazo[4,5-*d*][1,2,3]triazine (7). METHOD A:** A solution of phosphorous oxychloride (0.45 g, 2.9 mmol) in chloroform (2 mL) was added to a stirred cold (ice-bath) solution of **3** (1.5 g, 2.46 mmol) and 4-(dimethylamino)pyridine (1.45 g, 11.88 mmol) in chloroform over a 10 min period. After the addition was complete, the green solution was allowed to stir for 5 min and then treated, dropwise, with methanolic ammonia (5 drops, saturated at 0 °C). The solution turned orange and was stirred for 5 min and then methanol (5 mL) was added. The ice-bath was removed, and after 10 min, an additional portion of methanolic ammonia was added (5 mL). The mixture was heated at reflux temperature for 1 h and subsequently evaporated to afford a thick slurry. This slurry was co-evaporated with cyclohexane (20 mL) and the resulting residue was suspended in cyclohexane (25 mL). The suspension was filtered, and the filter cake was washed with additional cyclohexane (20 mL). The combined filtrates were then evaporated to dryness, and the residue purified by silica gel chromatography (3 cm x 10 cm, eluting with solvent system B, $R_f$ = 0.84 in solvent system A) to give compound **7** as a foam (0.789, 51%): $^1$H NMR (DMSO-$d_6$): δ 8.98 (s, 1H), 6.18 (d, 1H, *J*=5.3 Hz), 4.95 (m, 1H), 4.41 (m, 1H), 4.29 (s, 3H), 4.06 (m, 2H), 3.78 (m, 1H), 0.91, 0.86, 0.72 (3s, 27H), 0.13, 0.10, 0.051-0.76, -0.34 (5s, 18H); UV [$\lambda_{max}$nm (log ε)]: methanol, 266(sh) (3.72), 250 (3.79), 206 (4.27); pH 1, 266(sh) (4.02), 252 (4.18), 208 (4.47); pH 11, 270 (sh) (3.89), 252 (3.96); *Anal.* Calcd. for $C_{28}H_{55}N_5O_5Si_3$: C, 53.72; H, 8.85; N, 11.19. Found: C, 53.97; H, 8.72; N, 11.11.

**METHOD B:** Phosphorous oxychloride (0.66 g, 4.3 mmol) was added to a stirred solution of **3** (1.25 g, 2.00 mmol) and 4-(dimethylamino)pyridine (2.1 g, 17.23 mmol) in

chloroform (40 mL) over a 1 min period. The dark green solution was stirred for 15 min, and then methanol (2 mL) and sodium methoxide (2 mL, 1.0 M in methanol) was added. The mixture was stirred for 16 h, and then evapoorated to dryness. The resultant residue was partitioned between aqueous acetic acid (50 mL, 10%, v/v) and ethyl acetate (100 mL). The organic layer was washed with water (50 mL), saturated sodium bicarbonate solution (50 mL), brine (50 mL), dried over sodium sulphate, filtered and the filtrate was evaporated to dryness. The residue was purified by silica gel chromatography (3 x 10 cm, eluting with solvent system C) to give a foam (0.37g, 29%). This foam was identical by $^1$H-NMR spectrum and TLC (solvent system A) to the product obtained in Method A.

**METHOD C:** Phosphorous oxychloride was added in two portions (0.33 g, 2.2 mmol) to a stirred solution of **3** (0.50 g, 0.82 mmol) in pyridine (20 mL). The mixture was stirred for 3 h, and then methanol (1 mL}, and sodium methoxide (1 mL, 1.0 M in methanol) was added. After allowing the mixture to stir for 16 h, the solution was evaporated to a thick syrup which was partitioned between ethyl acetate (50 mL) and 50% saturated brine (50 mL). The organic layer was washed with brine (2 x 50 mL), dried over sodium sulphate, filtered and the filtrate was evaporated to dryness. The residue was purified by silica gel chromatography (3 cm x 10 cm, eluting with solvent system B) to give a brown foam (0.21 g, 41%), which was identical by $^1$H-NMR and TLC analysis (solvent system A) to the product obtained in Method A and B. Starting material (50 mg, 10%) was also recovered from the column.

**4-Amino-7-[2,3,5-tri-*O*-(*t*-butyldimethylsilyl)-β-D-ribofuranosyl]imidazo-[4,5-*d*][1,2,3]triazine (8).**

**METHOD A:** A solution of **7** (2.0 g, 3.2 mmol) in methanolic ammonia (50 mL, saturated at 0 °C) was kept in a sealed pressure bottle at room temperature for 72 h. The solution was then evaporated to a thick syrup which was dissolved in methanol (20 mL) and the product was allowed to crystallize at 4 °C for 18 h to afford 1.2 g of product. The mother liquor was then evaporated to a syrup and once again was taken up in methanol (5 mL) and allowed to stand at 4 °C for 48 h to afford an additional 0.14 g of product, for a total yield of 1.34 g of **8** (69%): mp 224-225 °C (decomp.); $^1$H NMR (DMSO-$d_6$): δ 8.67 (s ,1H), 7.86 (bs, 2H, $D_20$ exchangeable), 6.07 (d, 1H, $J$ = 5.9 Hz), 4.96 (m, 1H), 4.37

(m,1H), 4.02 (m, 2H), 3.77 (m, 1H), 0.91, 0.87, 0.71, (3s, 27H), .0.13, 0.11, 0.063, 0.058, -0.10, -0.36 (6s, 18H); UV [$\lambda_{max}$nm (log ε)]: methanol, 298 (3.80), 256 (3.92) 208 (4.23); pH 1, 302 (4.02), 257 (4.12), 209 (4.40); pH 11, 302 (3.98), 256 (4.08), 229 (4.12); IR (KBr): 2955, 2931, 2858, 1687, 1651, 1473, 1258, 1078, 838, 779 $cm^{-1}$; TLC: $R_f$ = 0.49 solvent system C; *Anal.* Calcd. for $C_{27}H_{54}N_6O_4Si_3$: C, 53.08; H, 8.90; N, 13.75. Found: C, 53.11; H, 9.00; N, 13.74.

**METHOD B:** Phosphorous oxychloride (0.68 9, 4.4 mmol) was added to a stirred solution of **3** (1.24 g, 2.03 mmol) in pyridine (25 mL). After stirring for 75 min the solution was warmed to *ca.* 70 °C on a steam bath. The solution was allowed to cool to room temperature over the next 15 min, and then treated with methanolic ammonia (5 mL, 50%, v/v). The flask containing the mixture was sealed, and the reaction was allowed to stand for 16 h. At that time, the solution was evaporated to yield a paste which was partitioned between chloroform (150 mL) and aqueous acetic acid (10%, 150 mL). To break-up the ensuing emulsion, brine (150 mL) was added, followed by acetone (5 mL), and the mixture was vacuum filtered through a bed of Celite. The organic layer was washed with water (2 x 150 mL), saturated sodium bicarbonate solution (50 mL), brine (50 mL), dried over sodium sulphate, filtered and the filtrate was evaporated to dryness. The residue was purified by silica gel chromatography (2.5 cm x 6 cm, eluting with solvent system B, Rf = 0.49 in solvent system C), the resultant product was co-evaporated with methanol (3 x 10 mL), and then crystallized from methanol to yield 0.725 g (59%) of a tan solid (mp 226-227 °C, decomp.) which was identical by TLC and $^1$H-NMR analysis to **8** obtained in Method A.

**METHOD C:** Phosphorous oxychloride (2.64 g, 17.25 mmol) was added to a stirred solution of **3**, (3.76, 6.14 mmol) in pyridine (50 mL). The solution became warm, after stirring for 45 min, and a solution of methylamine in methanol (25 mL, 1:1, v/v) was then added. The mixture was stirred for 75 min, and then evaporated to dryness. The residue was purified by silica gel chromatography (4 cm x 25 cm, eluting with solvent system C, $R_f$ = 0.49 in solvent system C), and the resultant residue was co-evaporated with methanol (25 mL), and crystallized from methanol (15 mL) to yield **8** as a solid (1.7 g, 45%): mp

225-226 °C; identical by $^1$H-NMR and TLC analysis to the products obtained from Methods A and B.

**METHOD D:** A solution of **10**, (100 mg, 0.15 mmol) in methanolic ammonia (10 mL, saturated at 0 °C) was kept in a pressure bottle at room temperature for 72 h. The solution was then evaporated to a thick syrup which was purified by column choromatography as in Method C (58 mg, 63%) and the resultant solid (**8**) was shown to be identical by $^1$H-NMR and TLC analysis to the products obtained from Methods A, B and C.

**4-Methylamino-7-[2,3,5-tri-*O*-(*t*-butyldimethylsilyl)-β-D-ribofuranosyl]-imidazo[4,5-*d*][1,2,3]triazine (9).** A solution of phosphorous oxychloride (0.33 g, 2.1 mmol) in chloroform (5 mL) was added to a stirred, ice-cold solution of **2**, (0.6 g, 1.46 mmol) and 4-(dimethylamino)pyridine (1.0 g, 8.2 mmol) in chloroform (10 mL). The cold solution was stirred for 20 min, and then methylamine gas (3.2 g, 103 mmol) was bubbled into the solution. The ice-bath was removed, and the cold solution was stirred for an additional 2 h. The solvent was evaporated under reduced pressure, and the residue was partitioned between ethyl acetate (50 mL) and 5% aqueous acetic acid (50 mL). The organic layer was then washed with 5% aqueous acetic acid (50 mL), water (50 mL), brine (50 mL), saturated sodium bicarbonate solution (50 mL), brine (50 mL), dried over $Na_2SO_4$, and then evaporated to dryness. The residue was purified by silica gel chromatography (22 cm x 300 cm, eluting with solvent system Q, $R_f$ = 0.58 in solvent system N) to give **9** as a foam (0.22 g, 24%): $^1$H NMR (DMSO-$d_6$): δ 8.67 (s, 1H), 8.24 (q, 1H, $D_2O$ exchangeable), 6.08 (d, 1H, $J$ = 5.9 Hz), 4.97 (m, 1H), 4.36 (m, 1H), 4.02 (m, 1H), 3.77 (m, 1H), 3.07 (bs, 3H), 0.92, 0.87, 0.71, (3s, 27H), .0.13, 0.11, 0.07, 0.06, -0.10, -0.37 (6s, 18H); UV [$\lambda_{max}$nm (log ε)]: methanol, 309 (4.08), 260 (4.30) 210 (4.47); pH 1, 311 (4.24), 263 (4.40), 209 (4.64); pH 11, 309 (4.16), 262 (4.32), 232 (4.30); IR (KBr): 2957, 2931, 2898, 2859, 1641, 1474, 1464, 1257, 1221, 1162, 1130, 1108, 1070, 1045, 1002, 969, 939, 837, 779, 674 cm$^1$; *Anal.* Calcd. for $C_{28}H_{56}N_6O_4Si_3$: C, 53.81; H, 9.02; N, 13.45. Found: C, 53.99; H, 9.30; N, 13.22. Further elution of the column furnished a fraction, which upon evaporation, yielded a foam

(40 mg) which was identified on the basis of TLC (solvent system C) and $^1$H NMR as a mixture of starting material and the 4-amino derivative **8** in the approximate ratio of 1:1. **

**4-(1,2,4-Triazol-1-yl)-7-[2,3,5-tri-*O*-(*t*-butyldimethylsilyl)-β-D-ribofuranosyl]imidazo[4,5-*d*][1,2,3]triazine (10).** A stirred solution of 1,2,4-triazole (3.10 g, 45.0 mmoles) and phosphorous oxychloride (0.93 mL, 10.0 mmoles) in acetonitrile (40 mL) was cooled to 0 °C in an ice bath. Triethylamine (6.3 mL, 45.0 mmoles) was then added and the reaction was stirred for 1 h. At that time, compound **3** (1.22 g, 1.99 mmol) was added in one portion and the mixture was stirred for 3.5 h. The reaction was filtered through a bed of Celite, and the solvent was removed under reduced pressure. The resultant residue was partitioned between ethyl acetate (75 mL) and water (75 mL), and then washed with an additional portion of water (75 mL) and brine (50 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent system was evaporated under reduced pressure. The residue was then purified by twice performing silica gel chromatography (3 cm x 10 cm, eluting with solvent system R, then solvent system S *each time*, $R_f$ = 0.41 in solvent system S) to give 411 mg (31%) of **10** as a yellow foam: $^1$H NMR (DMSO-$d_6$): δ 9.86 (s, 1H), 9.29 (s, 1H), 8.60 (s, 1H), 6.30 (d, 1H, $J$ = 12.6 Hz), 4.94 ($m_1$ 1H); 4.47 (m, 1H); 4.1 (m, 2H); 3.8 (m, 2H), 0.90, 0.89, 0.79 (9s, 27H), 0.14, 0.11, 0.09, -0.18 (s, 18H); UV [$\lambda_{max}$nm (log ε)]: methanol, 272 (4.13), 221 (4.38); pH 1, 284 (4.29), 224 (4.41); pH 11, 275 (4.11), 234 (4.15); *Anal* Calcd. for $C_{29}H_{54}N_8O_4Si_3$: C, 52.53; H, 8.21; N, 16.90. Found: C, 52.84; H, 8.09; N, 16.76.

**7-[2,3,5-Tri-*O*-(*t*-butyldimethylsilyl)-β-D-ribofuranosyl]imidazo[4,5-*d*]-[1,2,3]triazine-4-thione (11).**

**METHOD A:** *p*-Methoxyphenylthionophosphine sulphide dimer (4.0 g, 9.9 mmol) was added to a solution of **3** (5.0 g, 8.17 mmol) in toluene (100 mL) and the suspension was stirred at 75 °C under an argon atmosphere for 1 h. An additional portion of *p*-methoxyphenylthionophosphine sulphide dimer (1.0 g, 2.5 mmol) was then added and the heating and stirring was continued for 30 min. The resulting dark solution was then evaporated, the residue was dissolved in ethyl acetate (200 mL) and the solution was

washed with saturated sodium bicarbonate solution (2 x 10 mL), and brine (100 mL). The organic layer was dried over sodium sulphate, filtered and the filtrate was evaporated to dryness. The residue was taken up in methanol (100 mL), and the pH of the solution was adjusted to pH = 5 with Dowex 50X $H^+$ resin. The resin was removed by filtration, and the resin bed was washed with methanol (3 x 20 mL). The combined filtrates were evaporated to yield a foam, which was then dissolved in solvent system M (50 mL). The solution was filtered, and then purified by silica gel chromatography (4 cm x 26 cm, eluting with cyclohexane then solvent system M, $R_f$ = 0.43 in solvent system N). The resultant residue was again purified by silica gel chromatography (4 cm x 26 cm, eluting with solvent system 0, $R_f$ = 0.43 in solvent system N) to give (1.8 g, 35%) of **11** which was impure as determined by TLC analysis. An analytical sample was obtained by recrystallizing a portion (0.20 g) from cyclohexane (10 mL) to obtain a yellow crystalline product (0.19 g) which had retained some solvent: mp 153-154 °C (decomp.); $^1H$ NMR (DMSO-$d_6$): δ 13.20 (bs, 1H, $D_20$ exchangeable), 8.58 (s 1 H), 6.17 (d, 1H), 4.46 (m, 1H), 4.26 (m, 1H), 4.16 (m, 1H), 4.01 (m, 1H), 3.79 (m, 1H), 0.94, 0.91, 0.80, (3s, 27H); .0.15, 0.13, 0.08, 0.72, -0.02, -0.16 (6s, 18H); UV [$\lambda_{max}$nm (log ε)]: methanol, 316 (4.31), 205 (4.25); pH 1,315 (4.32), 210 (4.47); pH 11,306 (4.05), 233 (4.01); IR (KBr): 2954, 2931, 2859, 1574, 1384, 1257, 1169, 1144, 1115, 837, 778 $cm^{-1}$; *Anal.* Calcd. for $C_{27}H_{53}N_5O_4Si_3S$ (5/6 $C_6H_{12}$): C, 55.05; H, 9.09; N, 10.03. Found: C, 54.87; H, 8.98; N, 10.03.

**METHOD B:** Phosphorous oxychloride (0.1 mL, 1 mmol) was added to a solution of **3** (0.15 g, 0.245 mmol) in pyridine (15 mL). The mixture was stirred for 1 h at room temperature, and then hydrogen sulphide (g) was bubbled through the solution for 15 min. The mixture was then partitioned between ethyl acetate (25 mL) and aqueous acetic acid (10%, 75 mL). The organic layer was washed with water (50 mL), saturated aqueous sodium bicarbonate solution (50 mL), and then brine (50 mL). The solution was dried ($Na_2SO_4$), filtered and evaporated to dryness. The residue was purified by silica gel chromatography (2.5 cm x 70 cm, eluting with solvent system C, $R_f$ = 0.43 in solvent

system N) and the resultant solid was crystallized from cyclohexane to afford 65 mg (42%) of **11** which was identical to the product obtained in Method A: mp 153-154 °C (decomp.).

**4-(N,N-Diethylamino)-7-(β-D-ribofuranosyl)imidazo[4,5-*d*][1,2,3]triazine, (12).** A solution of **4** (0.21 g, 0.47 mmol) in methanol (5 mL) was treated with sodium carbonate (0.2 g, 1.9 mmol). The heterogenous mixture was stirred for 2 h, and then filtered. The pH of the filtrate was adjusted to pH = 6 with Dowex 50 x $H^+$ resin. The resin was removed by filtration, and the resin bed was washed with methanol (10 mL). The combined filtrates were evaporated, and the residue was purified by silica gel chromatography (2 cm x 2 cm, eluting with solvent system F, $R_f$ = 0.39 in solvent system G). The product was evaporated and co-evaporated with ethyl acetate (10 mL) to afford a foam (36 mg, 24%). Drying at 80 °C over phosphorous pentoxide afforded **12** as a hard glass: mp 133-135 °C; $^1$H NMR (DMSO-$d_6$): δ 8.64 (s, 1H), 6.04 (d, 1H, $J$ = 5.7Hz), 5.63 (d, 1H, $D_2O$ exchangeable), 5.36 (d, 1H, $D_2O$ exchangeable), 5.34 (t, 1H, $D_2O$ exchangeable), 4.61 (m, 1H), 4.17 (m, 1H), 4.0 (m, 5H); 3.70-3.60 (2m, 2H, H-5'ab); 1.21 (t, 6H, $CH_3$); UV [$\lambda_{max}$nm (log ε)] methanol, 321 (3.66), 270 (3.92), 213 (3.95); pH 1, 319 (3.34), 266 (4.01), 225 (3.99); pH 11, 271 (3.96), 233 (3.69); IR (KBr): 3290, 3131, 2927, 1606, 1417, 1311, 1131, 1091, 861 cm$^{-1}$; *Anal.* Calcd. for $C_{13}H_{20}N_6O_4$: C, 48.14; H, 6.21; N, 25.91. Found: C, 48.22; H, 6 25; N, 25.76.

**4-Methoxy-7-(β-D-ribofuranosyl)imidazo[4,5-*d*][1,2,3]triazine (13).**

**METHOD A:** A suspension of **5** (0.2 g, 0.49 mmol) and sodium carbonate (0.2 g, 1.9 mmol) in methanol (5 mL) was stirred for 1 h. At that time the pH of the solution was adjusted to pH = 6 with Dowex 50 x $H^+$ resin and the mixture was filtered. The resin bed was washed with methanol (3 x 10 mL), and the combined filtrates were evaporated to dryness. The residue was dissolved in hot ethanol *(ca.* 80 °C, 25 mL), the solution was filtered, and then evaporated to dryness. The solid residue was treated with ethanol (5 mL) and the slurry was filtered to give **13** (0.075 g, 54%): mp 189-190 °C (decomp.); $^1$H NMR (DMSO-$d_6$): δ 9.01 (s, 1H), 6.18 (d, 1H, $J$ = 5.0 Hz), 5.63 (d, 1H, $D_2O$ exchangeable), 5.27 (d, 1H, $D_2O$ exchangeable), 5.09 (t, 1H, $D_2O$ exchangeable), 4.65 (m, 1H), 4.29 (s, 3H), 4.22 (m, 1H), 4.01 (m, 1H), 3.72 (m, 1H), 3.59 (m, 1H);

UV [$\lambda_{max}$nm (log ε)]: methanol, 268 (3.73), 250 (3.80), 205 (4.27); pH 1,267 (3.76), 250 (3.80), 205 (4.30); pH 11,268 (3.70), 250 (3.73); IR (KBr): 3482, 3384, 3208, 3110, 1612, 1502, 1453, 1430, 1415, 1363, 1292, 1128, 1058, 948, 891, 871, 946 $cm^{-1}$; TLC: $R_f$ = 0.32 solvent system G; *Anal.* Calcd. for $C_{10}H_{13}N_5O_5$: C, 42.41; H, 4.62; N, 24.73. Found: C, 42.66; H, 4.89; N, 24.74.

**METHOD B**: Tetra-*n*-butylammonium fluoride (25 mL, 1.0 M solution in THF) was added to a solution of **7** (5.25 g, 8.39 mmol) in THF (50 mL). The solution was stirred for 1 h and then methanol (10 mL) was added, and the mixture was evaporated to dryness. The residue was purified by silica gel chromatography (7 cm x 4 cm, eluting with solvent system D, $R_f$ = 0.32 in solvent system G) and the resultant product was evaporated to dryness and then crystallized from ethanol (25 mL) at 4 °C to afford a pink solid (1.05 g, 51%): mp 187-188 °C (dec). This solid was found to be identical by $^1$H-NMR and TLC analysis with **13** described in Method A. An additional 0.5 g of material could be obtained by chromatography of the mother liquor on a silica column (4 cm x 40 cm) using solvent system D. This furnished a total yield of 1.55 g (65%).

**4-Amino-7-(β-D-ribofuranosyl)imidazo[4,5-*d*][1,2,3]triazine (2-Azaadenosine, 14).**

**METHOD A:** A solution of **5** (0.1 g, 0.24 mmol) in methanolic ammonia (2 mL, 50%, v/v) was stirred in a sealed flask for 72 h. The crystalline mass which had separated was collected by filtration to yield the pure product (0.036 g, 55%): mp 236-238 °C; mp lit[8] 240-241 °C; *Anal.* Calcd. for $C_9H_{12}N_6O_4$: C, 40.30; H, 4.51; N, 31.33. Found: C, 40.15; H, 4.65; N, 31.13.

**METHOD B:** A solution of **8** (2.1 g, 3.4 mmol) in THF (25 mL) was treated with a tetra-*n*-butylammonium fluoride (13 mL, 1.0 M solution in THF). After 30 min, the reaction mixture was applied directly to a silica gel column (4.5 cm x 7 cm), and purified by elution with solvent system E. The resultant material was crystallized from ethanol (20 mL) to afford 0.74 g (81%) of **14** which was identical by TLC and $^1$H NMR to the products obtained in Method A.

**METHOD C:** A solution of **6** (100 mg, 0.22 mmoles) in methanolic ammonia (5 mL, saturated at 0 °C) was kept in a sealed flask for 72 h. After treatment with ethyl acetate, the crystallized material was collected by filtration to yield **14** (21 mg, 35%) which was identical by TLC and $^1$H NMR to the products obtained in Methods A and B.

**4-Methylamino-7-(β-D-ribofuranosyl)imidazo[4,5-*d*][1,2,3]triazine (15).**

Tetra-*n*-butylammonium fluoride (4 mL, 1.0 M solution in THF) was added to a solution of **9** (0.84 g, 1.3 mmol) in THF (25 mL). The mixture was stirred for 30 min, and then an additional portion of tetra-*n*-butylammonium fluoride (1 mL, 1.0 M in THF) was added. After 5 min, the mixture was evaporated, and the resultant residue was purified by column chromatography (2.5 cm x 30 cm, eluting with solvent system E, $R_f$ = 0.41 in solvent system E). The product was crystallized from methanol (10 mL) to yield a yellow solid (0.125 9, 34%): mp 138-140 °C; $^1$H NMR (DMSO-$d_6$): δ 8.70 (s, 1H), 8.23 (q, 1H, $D_2O$ exchangeable), 6.06 (d, 1H, $J$ = 5.6 Hz), 5.57 (d, 1H, $D_2O$ exchangeable), 5.25 (d, 1H, $D_2O$ exchangeable), 5.19 (t, 1H, $D_2O$ exchangeable), 4.65 (m, 1H), 4.19 (m, 1H), 4.00 (m, 1H), 3.71 (m, 1H), 359 (m, 1H), 3.09 (s, 3H); UV [$\lambda_{max}$nm (log ε)]: methanol, 309 (3.83), 261 (3.97), 210 (4.17); pH 1, 314 (3.38), 256 (3.94), 223 (3.98); pH 11, 310 (3.85), 261 (4.00), 227 (4.02); IR (KBr): 3391, 1651, 1384, 1129, 1105, 1089, 1063, 1105, 1089, 1047, 821, 645 cm$^{-1}$; TLC: *Anal.* Calcd. for $C_{10}H_{14}N_6O_4$ (0.5 $H_2O$): C, 41.24; H, 5.18; N, 28.86. Found: C, 41.59; H, 5.43; N, 28.70.

**4-Hydrazino-7-(β-D-ribofuranosyl)imidazo[4,5-*d*][1,2,3]triazine (16).**

A suspension of **13** (0.30 g, 1.06 mmol) in methanol (5 mL) was treated with hydrazine hydrate (0.10 mL) and the mixture was stirred for 2 h. An additional portion of hydrazine hydrate was then added (0.15 mL), and stirring was continued for 6 h. The reaction mixture was then diluted with methanol (10 mL) and stirred for an additional 8 h. The suspension was then chilled via an ice-bath and after 0.5 h, the precipitated product was collected by filtration under positive argon pressure to yield 0.21 g of product (66%) which was dried under reduced pressure at 80 °C: mp> 200 °C (decomp.); $^1$H NMR (DMSO-$d_6$): δ 9.30 (bs, 1H, $D_2O$ exchangeable), 8.69 (s, 1H), 6.05 (d, 1H, J= 5.1 Hz), 5.57 (d, 1H, $D_2O$ exchangeable), 5.26 (d, 1H, $D_2O$ exchangeable), 5.20 (t, 1H, $D_2O$ exchangeable),

4.83 (bs, 2H, $D_20$ exchangeable), 4.65 (m, 1H), 4.18 (m, 1H), 3.99 (m, 1H) 3.70 (m, 1H), 3.58 (m, 1H); UV [$\lambda_{max}$nm (log ε)]: methanol, 265 (4.21), 206 (4.40); pH 1, 280 (410), 256 (4.24), 210 (4.55); pH 11, 303 (4.17), 287(4.17), 264(4.19), 232 (4.20); IR (KBr): 3325, 2931, 1644, 1440, 1419, 1384, 1335, 1103, 1053, 646 $cm^{-1}$ TLC: $R_f$ = 0.13 solvent system E; *Anal.* Calcd. for $C_9H_{13}N_7O_4$ (0.5 $H_20$) (0.25 MeOH): C, 37.00; H, 5.03; N, 32.66. Found: C, 37.06; H, 5.08; N, 32.60.

**7-(β-D-Ribofuranosyl)imidazo[4,5-*d*][1,2,3]triazine-4-thione, tetra-*n*-butyl-ammonium salt (17).** Tetra-*n*-butylammonium fluoride (2.2 mL, 1.0 M solution in THF) was added to a solution of **11** (0.52 g, 0.83 mmol) in THF (15 mL). After stirring the solution for 1 h at room temperature methanol was added (2 mL), followed by silica gel (3 g). The slurry was then evaporated under reduced pressure until a free-flowing powder had formed. Column chromatography of the powder (25 cm x 300 cm, eluting with solvent system E, $R_f$ = 0.2 in solvent system N) gave a syrup which crystallized upon the addition of ethanol (5 mL) to afford **17** (0.15 g, 34%): mp 154-155 °C; $^1H$ NMR (DMSO-$d_6$): δ 8.35 (s ,1H), 5.89 (d, 1H, J= 6.2 Hz), 5.49 (bs, 2H, $D_20$ exchangeable), 5.17 (bs, 1H, $D_20$ exchangeable), 4.61 (m, 1H), 4.15 ($m_1$ 1H), 3.96 (m, 1H), 3.66 (m, 1H), 3.54 (m, 1H), 3.15 (m, 8H), 1.53 (m, 8H), 1~28 (m, 8H), 0.91 (t, 12H); UV [$\lambda_{max}$nm (log ε)]: methanol, 315 (4.02), 205 (4.08); pH 1, 314 (4.18), 232 (3.77), 206 (4.16); pH 11,306 (3.87), 232 (3.77); IR (KBr): 3208, 3105, 2962, 2875, 1546, 1383, 1193, 125, 1074, 1059, 969 $cm^{-1}$; *Anal.* Calcd. for $C_{25}H_{46}N_6O_4$ S: C, 57.01; H, 8.79; 15.96. Found: C, 56.98; H, 8.77; N, 15.86.

**7-[2,3,5-Tri-O-(t-butyldimethylsilyl)-β-D-ribofuranosyl]imidazo[4,5-*d*]-[1,2,3]triazine (18).** Under argon, Raney nickel (24 g, weighed wet with ethanol) was added to a solution of **11** (2.0 g, 2.9 mmol) in ethanol (50 mL) over a period of 1 h. The suspension was stirred for an additional 10 min, after the last portion of Raney nickel was added, and the mixture was filtered through Celite. The Celite bed was washed with ethanol (5 x 10 mL) and the combined filtrates were evaporated to dryness. The residue was purified by silica gel chromatography (3 cm x 3 cm, eluting with solvent system J, $R_f$= 0.51 in solvent system J) to give a dark syrup which solidified upon standing at room

temperature (0.4 g, 23%). This solid was purified by silica gel chromatography (3 cm x 9 cm, eluting with solvent system 0, Rf = 0.69 in solvent system N) and the resultant residue was co-evaporated with methanol (2 x 5 mL). The residue was then dissolved in solvent system P (20 mL), treated with Norit and the suspension was filtered. A solid separated from the solution over the next 72 h at room temperature. The solid was collected by filtration to yield 0.19 g of **18** as long needles. The mother liquor was allowed to stand at 4 °C for 1 week and an additional 0.1 g of product was obtained for a total yield of 0.29 g (17%): mp 139-140 °C; $^1$H NMR (DMSO-$d_6$): δ 9.77 (s, 1H), 9.18 (s, 1H), 6.22 (d, 1H, $J$ =4.8 Hz)) 4.99 (m, 1H), 4.47 (m, 1H), 4.07 (bs, 1H) 4.02 (m, 1H), 3.80 (m, 1H), 0.92, 0.85, 0.73, (3s, 27H), 0.14, 0.11, 0.05, 0.04, -0.05, -0.18 (6s, 18H); UV [$\lambda_{max}$nm (log ε)]: methanol, 251 (4.00), 206 (4.57); pH 1, 254 (4.47), 214 (4.74); pH 11, 253 (4.26), 232 (4.22); IR (KBr): 3107, 3043, 2957, 2931, 2896, 2859, 1588, 1488, 1473, 1385, 1318, 1260, 1168, 1095, 1086, 838, 778 cm$^{-1}$; *Anal.* Calcd. for $C_{27}H_{53}N_5O_4Si_3$: C, 54.42; H, 8.95; N, 11.75. Found: C, 54.52; H, 8.77; N, 11.91.

**7-(β-D-Ribofuranosyl)imidazo[4,5-*d*][1,2,3]triazine (19).**

Tetra-*n*-butylammonium fluoride (7.3 mL, 1.0 M in THF) was added dropwise to a stirred, cold (ice-bath) solution of **18** (1.4 g, 2.35 mmol) in THF (20 mL). The solution was stirred with continued cooling for 15 min, then an additional portion of tetra-*n*-butylammonium fluoride (1.0 mL, 1.0 M in THF) was added. After an additional 10 min of stirring, the cold solution was applied directly to a silica gel column (5 cm x 15 cm) which had been packed in solvent system D. Column chromatography (eluting with solvent system D, then solvent system E) gave a residue ($R_f$ = 0.45 in solvent system E) which crystallized upon the addition of methanol (15 mL) to afford **19** as white needles (290 mg, 49%): mp 178-179 °C (decomp.); $^1$H NMR (DMSO-$d_6$): δ 9.75 (s, 1H), 9.21 (s, 1H), 6.23 (d, 1H, $J$ =4.9 Hz), 5.66 (d, 1H, $D_2O$ exchangeable), 5.28 (d, 1H, $D_2O$ exchangeable), 5.10 (t, 1H, $D_2O$ exchangeable), 4.70 (m, 1H), 4.24 (m, 1H), 4.03 (m, 1H), 3.75 (m, 1H), 3.63 (m, 1H); UV [$\lambda_{max}$nm (log ε)]: methanol, 251 (3.69), 208 (4.25); pH 1, 297 (3.86), 248 (3.62), 207 (4.06); pH 11, 310 (3.24), 250 (3.70); IR (KBr): 3504, 3334, 3160, 3103, 3037, 2955, 2935, 2902, 1590, 1492, 1425, 1411, 1385, 1341,

1208, 1191, 1126, 1114, 1097, 1055, 1023, 793, 640, 617 $cm^{-1}$; *Anal.* Calcd. for $C_9H_{11}N_5O_4$: C, 42.69; H, 4.38; N, 27.66. Found: C, 42.47; H, 4.28; N, 27.51.

**7-(β-D-Ribofuranosyl)-4-(4-nitrobenzylthio)imidazo[4,5-d][1,2,3]triazine (20).** *p*-Nitrobenzyl chloride (0.18 g, 1.05 mmol) was added to a solution of **17** (0.50 g, 0.95 mmol) in methanol (15 mL), and the mixture was stirred for 2 h. At that time, an additional portion of *p*-nitrobenzyl chloride (0.05 g, 0.03 mmol) was added, and stirring was continued for an additional 1 h. The mixture was then evaporated and the resulting syrup was purified by silica gel chromatography (3 cm x 15 cm, solvent system G, $R_f$ = 0.72 solvent system E) to give a pink syrup. This syrup crystallized upon the addition of methanol (25 mL) to afford **20** (0.33 g, 82%): mp 160-161 °C; $^1H$ NMR (DMSO-$d_6$): δ 9.06 (s, 1H), 6.17 (d, 1H, $J$ = 4.8 Hz), 5.64 (d, 1H, $D_20$ exchangeable), 5.27 (d, 1H, $D_20$ exchangeable), 5.09 (t, 1H, $D_20$ exchangeable), 4.64 (m, 1H), 4.22 (m, 1H), 4.01 (m, 1H), 3.61 (m, 1H), 3.57 (m, 1H), 2.83 (s, 3H); UV [$\lambda_{max}$nm (log ε)]: methanol, 282 (4.24), 231 (4.12), 206 (4.39); pH 1, 287 (4.26), 205 (4.43); pH 11, 286 (4.26), 235 (4.10); IR (KBr): 3333, 3239, 3148, 3118, 3089, 2942, 2883, 1572, 1537, 1468, 1387, 1350, 1201, 1133, 1109, 1083, 1066, 1018, 1000, 954, 839, 807, 711, 647, 563 $cm^{-1}$; *Anal.* Calcd. for $C_{16}H_{16}N_6O_6$ S: C, 45.71; H, 3.83; N, 19.99. Found: C, 45.71; H, 4.08; N, 20.07.

**4-Methylthio-7-(β-D-ribofuranosyl)imidazo[4,5-*d*][1,2,3]triazine (21).** Iodomethane (0.062 mL, 1.0 mmol) was added to a solution of **17** (0.53 *g,* 1.0 mmol) in methanol (5 mL). After stirring the solution for 1 h, the mixture was evaporated to a thick slurry. The solid was collected by filtration, and washed with ice-cold methanol (3 x 5 mL) to afford **21** (230 mg, 77%): mp 175-176 °C; $^1H$ NMR (DMSO-$d_6$): δ 9.06 (s ,1H), 6.17 (d, 1H, *J=4.8* Hz), 5.64 (d, 1H, $D_20$ exchangeable), 5.27 (d, 1H, $D_20$ exchangeable), 5.09 (1, $1H_1$ $D_20$ exchangeable), 4.64 (m, 1H), 4.22 (m, 1H), 4.01 (m, 1H,), 3.61 (m, 1H), 3.57 (m, 1 H), 2.83 (s, 3H); UV [$\lambda_{max}$nm (log ε)]: methanol, 300 (3.95), 280 (3.96), 232 (4.03), 206 (4.14); pH 1, 305 (3.96), 282 (3.98), 233 (4.05), 205 (4.21); pH 11, 305 (3.97), 282 (3.99), 234 (4.05); IR (KBr): 3332, 3093, 2942, 2916, 2866, 1570, 1478, 1428, 1409, 1325, 1314, 1214, 1142, 1116, 1098, 1084, 1060,

1031, 991, 948, 866, 811, 763, 645, 618 $cm^{-1}$; TLC: $R_f$ = 0.65 solvent system E; *Anal.* Calcd. for $C_{10}H_{13}N_5O_4$ S: C, 40.10; H, 4.37; N, 23.40. Found: C, 40.20; H, 4.46; N, 23.36.

**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.[20]

**Virological procedures.** The Towne strain, plaque-purified isolate $P_0$ of HCMV was kindly provided by Dr. Mark Stinski, University of Iowa. 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.[21] 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.[22] Briefly, HFF 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 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. 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 x 5 x $3^n$; where n represents the nth 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, 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.

**HCMV yield assay.** HFF cells were planted as described above in 96-well cluster dishes, incubated overnight, medium removed and the cultures were inoculated with HCMV at a m.o.i. of 0.5 to 1 p.f.u. per cell as reported elsewhere.[22] After virus adsorption, inoculum was replaced with 0.2 mL of fresh medium containing test compounds. The first row of 12 wells was left undisturbed and served as virus controls. Each well in the second row received an additional 0.1 mL of medium with test compound at three times the desired final concentration. The contents of the 12 wells were mixed by repeated pipetting and then serially diluted 1:3 along the remaining wells. In this manner, six compounds could be tested in duplicate on a single plate with concentrations from 100 μM to 0.14 μM. Plates were incubated at 37 °C for seven days, subjected to one cycle of freezing and thawing; aliquots from each of the eight wells of a given column were transferred to the first column of a fresh 96-well monolayer culture of HFF cells. Contents were mixed and serially diluted 1:3 across the remaining eleven columns of the secondary plate. Each column of the original primary plate was diluted across a separate plate in this manner. Cultures were incubated, plaques were enumerated, and titers calculated as described above.

**Cytotoxicity assay.** Cytotoxicity produced in stationary HFF cells was determined by microscopic inspection of cells not affected by the virus used in plaque assays.[21]

**Data Analysis.** Dose response relationships were used to quantify drug effects by linearly regressing the percent inhibition of parameters derived in the preceding assays (except for yield experiments) against log drug concentrations. For yield experiments, the log of viral titer was plotted against the log drug concentration. Fifty percent inhibitory concentrations ($IC_{50}$'s) and ninety percent inhibitory concentrations ($IC_{90}$'s, yield experiments) were calculated from the linear portions of the regression lines.

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