Design, Synthesis and Antiviral Activity of Novel 4,5-Disubstituted 7-(β-D-Ribofuranosyl)pyrrolo[2,3-*d*][1,2,3]triazines and the Novel 3-Amino-5-methyl-1-(β-D-ribofuranosyl)- and 3-Amino-5-methyl-1-(2-deoxy-β-D-ribofuranosyl)-1,5-dihydro-1,4,5,6,7,8-hexaazaacenaphthylene as Analogues of Triciribine

Michael T. Migawa,[†,||] John C. Drach,[§] and Leroy B. Townsend[†,‡,*]

Department of Chemistry, College of Literature, Sciences and Arts, Department of Medicinal Chemistry, College of Pharmacy, and Department of Biological and Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, Michigan 48109

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The synthesis of several heterocyclic analogues of the biologically important nucleoside antibiotic toyocamycin and the tricyclic nucleoside triciribine (TCN) were prepared along with their 2′-deoxy counterparts. Coupling of 2-nitropyrrole-3,4-dicarboxamide (**15**) under a variety of conditions with α-chloro-2-deoxy-3,4-*di*-*O*-toluoyl-D-ribofuranose (**16a**) gave mixtures of the α and β anomers. A coupling of **15** with 1-chloro-2,3,5-tri-*O*-benzoyl-D-ribofuranose (**18**) gave exclusively the β anomer. Individually, the two pyrrole nucleosides were treated with Pd/C, H_2 to reduce the nitro groups and cyclized with nitrous acid, and the corresponding 4-position was functionalized as a triazoyl derivative. Nucleophillic displacement was carried out with ammonia to give a mixture of 4-amino-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-[1,2,3]triazine-5-carbonitrile (**26**) and 2-amino-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrole-3,4-dicarbonitrile (**27**), the latter being formed via a retro-Diels−Alder reaction. The subsequent addition of hydrogen sulfide, water, methanol, hydroxylamine, cyanamide, hydrazine and methylhydrazine to the 5-cyano group was carried out to give the corresponding analogues. In the case of methyl hydrazine, subsequent treatment with NaOMe in methanol gave the title hexaazaacenaphthylenes. Biological evaluation of the compounds established that the pyrrole (**17β**, **19**−**21**) and most of the pyrrolotriazine (**22**, **24**, **28**, **32**−**34**) nucleosides were inactive or weakly active against human cytomegalovirus (HCMV) and herpes simplex virus type 1 (HSV-1). In contrast **29** and **31** were active against one or both of these viruses but activity was poorly separated from cytotoxicity. In contrast, the 2-aza analogue of sangivamycin (**30**) was active against HCMV and HSV-1 but this apparent activity was most likely due to its high cytotoxicity. The tricyclic nucleoside **12**, was active against its target virus, human immunodeficiency virus type 1 (HIV-1), but this activity was not well separated from cytotoxicity.

Introduction

For several years we have been involved in the synthesis of structural analogues of the naturally occurring purine nucleosides, adenosine and guanosine.[1] Several of these analogues have been synthesized and subsequently demonstrated to be useful chemotherapeutic agents. These discoveries by our group and others have contributed to the vast number of known purine ribo- and 2-deoxyribonucleoside analogues.[2] While modifications of the sugar moiety, the heterocylic base and the exocyclic substituents have all been reported, our major research interests have focused on modifications of the purine ring per se. The preparation of these base-modified nucleosides are synthetically challenging and can result in the discovery of new classes of chemotherapeutic agents, potentially with novel biological moles of action.[3,4]

While many different types of heterocyclic modifications are possible, most modifications have been limited to 5:6 fused heterocycles containing only carbon and nitrogen. Of these 5:6 fused nitrogenous heterocycles, three basic categories have evolved, i.e., (1) the substitution of carbon for nitrogen (the deazapurines), (2) the substitution of nitrogen for carbon (the azapurines), and (3) the transposition of carbon and nitrogen (the aza-deazapurines).

One class of deazapurine nucleosides that has received extensive attention are the pyrrolo[2,3-*d*]pyrimidines.[2,5] Significant interest in the synthesis of pyrrolo-[2,3-*d*]pyrimidines was generated by the isolation and biological activity of the naturally occurring nucleoside antibiotics, tubercidin (**1**), toyocamycin (**2**) and sangivamycin (**3**). Subsequently, further interest was prompted by the total synthesis of these naturally occurring compounds.

Tubercidin (**1**) (Figure 1) inhibits the growth of several strains of bacteria, and both tubercidin and several

* To whom correspondence should be addressed. E-mail: ltownsen@umich.edu.
† Department of Chemistry.
‡ Department of Medicinal Chemistry.
§ Department of Biological and Materials Sciences.
|| Current address: IBIS Therapeutics, A Division of ISIS Pharmaceuticals, Inc., 2292 Faraday Avenue, Carlsbad, CA 92008.

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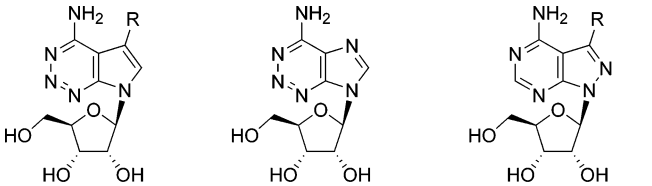
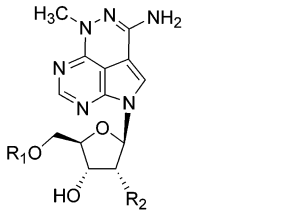


1 R=H
2 R=CN
3 R=CONH$_2$

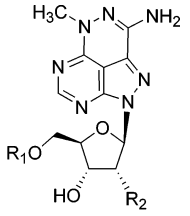
4

5 R=H
6 R=CN
7 R=CONH$_2$

Figure 1.



8 R_1=H, R_2=OH (TCN)
9 R_1=PO_3^{2-}, R_2=OH (TCN-P)
10 R_1 = H, R_2 = H (2-deoxy-TCN)



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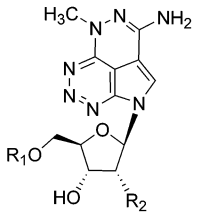

12 R_2=OH (2-aza-TCN)
13 R_2=H (2-deoxy-2-aza-TCN)

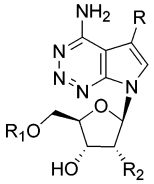

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Figure 2.

5-substituted derivatives inhibit both DNA and RNA viruses albeit at concentrations that inhibit DNA, RNA, and protein synthesis in mice and human cell lines.[6,7] Toyocamycin (**2**) is a known antineoplastic antibiotic with specific antitumor activity.[8,9] Sangivamycin (**3**) is active against L1210 leukemia, P338 leukemia and Lewis lung carcinoma and has been in clinical trials against colon cancer, gall bladder cancer and acute myelogenous leukemia in humans.[10,11]

An example of the second type of purine modifications includes the 2-azapurines. Interest in this area was generated when some 2-azapurine ribonucleoside analogues demonstrated antitumor activity, e.g. 2-azaadenosine (**4**) exhibits cytotoxicity five times greater than its 8-azapurine counterpart against human epidermoid carcinoma cells in vitro.[12]

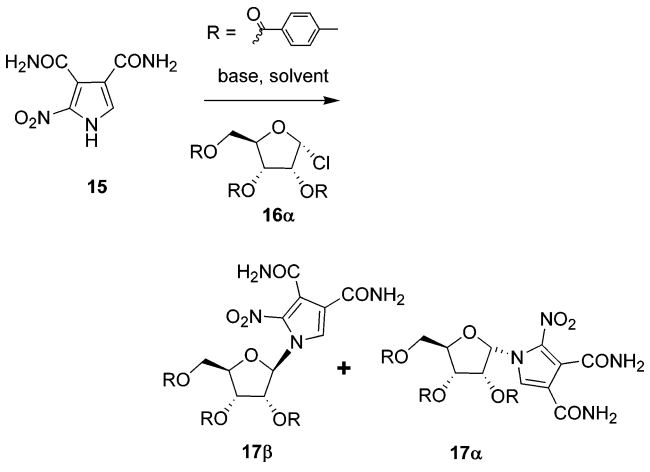
The third major class of purine modifications is the aza-deazapurines, e.g. the pyrazolo[3,4-*d*]pyrimidines, which have demonstrated antitumor activity. The adenosine analogue (4-APP ribofuranoside, **5**) was found to possess modest antiproliferative properties. However, the introduction of certain substituents at the three position of **5** (i.e., **6** and **7**) led to a dramatic increase in the cytotoxicity.[13]

Structurally related to the above bicyclic purine nucleoside analogues are the tricyclic nucleoside TCN[14] (**8**, triciribine) (Figure 2) and 8-amino-6-methyl-2-β-D-ribofuranosyl-1,2,3,5,6,7-hexaazaacenaphthalene[15] (7-Aza-TCN, **11**). TCN was the first to be prepared, and it was subsequently shown to exhibit potent antitumor activity.[16] The water-soluble 5′-monophosphate derivative, TCN-P (**9**), was in phase-II clinical trials as an anticancer agent.[17] More recently, TCN and TCN-P have been shown to inhibit HIV (IC_{50} = 60 nM)[4,18,19] and act by a unique mechanism.[20] Interestingly, 2-deoxy-TCN (**10**)[4] and other recently prepared sugar-modified analogues[19,21] were inactive against HIV-1. Although a structure−activity relationship has been built for TCN analogues modified[22,23] at the exocyclic positions of the TCN aglycone, only one analogue of TCN with a modified heterocyclic ring has previously been reported, 8-amino-6-methyl-2-(β-D-ribofuranosyl)-1,2,3,5,6,7-hexaazaacenaphthalene (7-Aza-TCN, **11**). However, compound **11** was shown to be inactive against HIV and noncytotoxic[15] despite being structurally related to compounds **6**, **7** and **8**. In view of the above structure−activity relationship studies we proposed to synthesize the target compound 3-amino-5-methyl-1-(β-D-ribofuranosyl)-1,5-dihydro-1,4,5,6,7,8-hexaazaacenaphthalene (**12**) and the 2-deoxy analogue **13**, which are structurally related to **2**−**4** and **8**.

A subsequent literature search revealed that the 1,4,5,6,7,8-hexaazaacenaphthalene ring system has never been reported. Additionally, it was of considerable interest that the entire class of aza-deazapurine nucleoside analogues, the pyrrolo[2,3-*d*][1,2,3]triazine nucleosides, had not been reported in the literature either. These facts prompted us to initiate studies designed to develop a route into pyrrolo[2,3-*d*][1,2,3]triazine nucleosides which could be expanded to include the synthesis of certain 1,4,5,6,7,8-hexaazaacenaphthalene nucleosides as well.

Only two pyrrolo[2,3-*d*][1,2,3]triazines heterocycles have been reported[24,25] in the literature. Since the synthetic route for the preparation of these pyrrolo-[2,3-*d*][1,2,3]triazine heterocycles is very limited in regards to exocyclic functional groups, we developed a synthetic route that provided 7-benzylpyrrolo[2,3-*d*]-[1,2,3]triazines which was amenable toward functional group modifications at the C-4, C-5 and C-6 positions.[26] In this previous work we demonstrated that the intramolecular diazo-coupling of 1-benzyl-2-aminopyrrole-3,4-dicarboxamide gave 7-benzyl-5-carboxamidopyrrolo-[2,3-*d*][1,2,3]triazin-4-one. However, we were unable to expand this methodology to include the β-D-ribofuranosyl- and 2-deoxy-β-D-ribofuranosyl- derivatives of the pyrrolo[2,3-*d*][1,2,3]triazine ring system since we were unable to obtain the 1-glycosyl-2-aminopyrrole-3,4-dicarboxamides that would be required for the synthesis of the 7-glycosyl-pyrrolo[2,3-*d*][1,2,3]triazines. To prepare the β-D-ribofuranosyl- and 2-deoxy-β-D-ribofuranosyl derivatives of the pyrrolo[2,3-*d*][1,2,3]triazine ring system we needed the appropriate N^1-unsubstituted-2-aminopyrrole-3-carboxamide derivative which could be coupled to an appropriately substituted sugar derivative and then converted into the desired 7-(β-D-ribofuranosyl)- and 7-(2-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*][1,2,3]-triazine derivatives. We have developed an efficient synthesis of 2-aminopyrrole-3,4-dicarboxamide.[27] We now report on the synthetic efforts undertaken toward the glycosylation of 2-aminopyrrole-3,4-dicarboxamide and its precursors and their conversion into the title compounds.

Table 1



entry	base	sovent	temp (°C)	time (h)	α:β	yield (%)
1	NaH	CH_3CN	25	20	5:3	74
2	KOH	CH_3CN	25	20	5:4	60
3	NaH	1:4 DMF/THF	25	20	5:3	77
4	NaH	CH_3CN/BSA	25	1	1:4	98
5	NaH	CH_3CN/BSA	0 then 25	8	1:6	98

Results and Discussion

Chemical. Initial glycosylation attempts were carried out on 2-nitro-3,4-dicarboxamidepyrrole (**15**) with 3,5-di-*O*-toluoyl-2-deoxy-α-D-ribofuranosyl chloride (**16α**, Table 1). Compound **16α** is readily available by literature methods[28] and is a stable crystalline solid.[29] In contrast, most ribosyl chlorides are difficult to obtain as a stable solid, and must usually be prepared fresh, just prior to use.[30,31] Therefore, it was convenient to develop the sodium-salt methodology with this 2-deoxyribofuranosyl chloride rather than its ribofuranosyl congener.

The standard sodium salt procedure[32] for the preparation of pyrrole nucleosides is carried out by deprotonation of the heterocycle with sodium hydride in acetonitrile followed by treatment with the chlorosugar **16α**. This reaction proceeds very rapidly with a complete Walden inversion and results in the exclusive formation of a β-nucleoside. ^{1}H NMR studies[33] of **16α** has demonstrated that anomerization of this sugar is a function of both time and solvent polarity; the β-anomer, **16*β***, is formed more quickly in more polar solvents than in less polar solvents. (In CH_2Cl_2, typically 80% of **16α** remains after 40 min at 25 °C, and 70% remains after 120 min) Moreover, **16*β*** has been demonstrated to react much faster than its α-congener. Consequently, relatively slow reactions, such as the standard Hilbert-Johnson nucleoside synthesis,[33] react with **16α** to give predominately the α-product. Conversely, relatively rapid reactions, such as sodium salt reactions, react with **16α** to give predominately the β-product.[34]

It was predicted that **16α** would react with the pyrrole **15** under the sodium salt method to give exclusively the β-anomer. However, as shown in Table 1, the reaction of **15** under the reported conditions[35] (entry 1) gave a 5:3, α/β product ratio. Since both **15** and its corresponding salt are insoluble in acetonitrile, the reaction rate is reduced and the α-anomer predominates.

Moreover, phase transfer conditions[36] (entry 2) gave similar results, probably also due to the solubility affecting the reaction rate. Changing the solvent to a more polar mixture of THF/DMF (entry 3) did not effect a substantial change in the anomeric ratio; however, in this case both the pyrrole **15** and its sodium salt were soluble. Either an increased rate of anomerization of the chlorosugar **16α** or a slow reaction rate could account for these results.

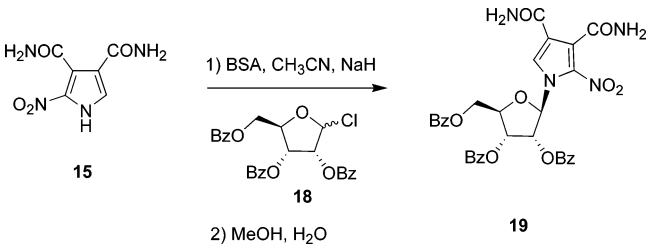
We then tried a rather novel procedure where the pyrrole **15** was first silylated with BSA to form an unidentified silylated intermediate and then treated sequentially with sodium hydride and the chlorosugar **16α** (entry 4). After heating the mixture in aqueous methanol to effect a desilylation, a 98% yield of 2-nitro-1-[3,5-di-*O*-(*p*-toluoyl)-2-deoxy-α-D-ribofuranosyl]-pyrrole-3,4-dicarboxamide (**17α**) and 2-nitro-1-[3,5-di-*O*-(*p*-toluoyl)-2-deoxy-β-D-ribofuranosyl]pyrrole-3,4-dicarboxamide (**17*β***) was obtained in a 1:4 α:β ratio, respectively. We then found (entry 5) that by cooling the silylated sodium salt of pyrrole **15** before adding the chlorosugar (**16α**) and then allowing the reaction to warm to ambient temperature gave, after workup, a 98% yield of nucleosides **17α** and **17*β*** in a 1:6 α:β ratio, respectively. Flash chromatography of the α,β-mixtures was only partially successful in the separation of anomers. However, fractional crystallization, in carbon tetrachloride and methanol, of the fractions enriched in the β-anomer gave pure **17*β***. A small sample of compound **17α** was obtained by flash chromatography.

As an additional explanation for our increase in stereoselectivity when silylated pyrrole **15** is used, it cannot be ruled out that the unsilylated 2-nitropyrrole initially reacts with the α-anomer to give an ester of the expected β-configuration. An intramolecular rearrangement would then give the α-glycoside. When the pyrrole is silylated, the nitro functionality would be unavailable to participate in the glycosylation and the esterification−rearrangement mechanism would be unavailable.

The anomeric configuration of each anomer was determined by ^{1}H NMR. It has been observed that the ^{1}H NMR resonance of the anomeric proton of a α-2-deoxyribonucleoside appears as a quartet with a peak-width-at-half-height ($W_{1/2}$) of 10.4 ± 0.4 Hz. Additionally the peak for the α-anomeric proton resonance appears downfield relative to the β-anomeric proton. The ^{1}H NMR peak for the resonance of the anomeric proton of compound **17α** was observed as a doublet ($J_{H1,H2a}$ = O) centered at 6.8 ppm. The $W_{1/2}$ was calculated to be 10.5 Hz. Conversely, the ^{1}H NMR resonance of the anomeric proton of a β-2′-deoxyribonucleoside is usually observed as a 'pseudo-triplet' with a $W_{1/2}$ of 13.7 ± 0.5 Hz. Compound **17*β*** was in agreement with these observations and a 'pseudo-triplet' centered at 0.1 ppm upfield from the ^{1}H NMR resonance of the anomeric proton of **17α** was observed. The $W_{1/2}$ was calculated to be 13.5 Hz.

The preparation of β-D-ribofuranosyl derivatives by the sodium salt method generally uses 2,3,5-tri-*O*-acyl-D-ribofuranosyl halides.[30] Neighboring group participation of the 2′-acyl group to form an acyloxonium cation results in the exclusive formation of a β-nucleoside. In several cases, an attack at the acyloxonium ion by the heterocycle has been reported to give products substituted at the 2′-carbonyl carbon.[34]

Scheme 1



Therefore, we elected to use the procedure we had developed for the preparation of the 2-deoxy derivative in order to circumvent the possibility of substitution at the 2′-carbonyl carbon. 2-Nitropyrrole-3,4-dicarboxamide (**15**) was silylated with BSA to form an unidentified silylated intermediate and then treated sequentially with sodium hydride and freshly prepared 2,3,5-(tri-*O*-benzoyl)-D-ribofuranosyl chloride.[37] When gylcosylation was complete, the product was heated in aqueous methanol to effect a desilylation and furnished a 70% yield of 2-nitro-1-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)-pyrrole-3,4-dicarboxamide (**19**). As expected, the *β*-anomer was the only anomer formed and no attack at the 2′-acylcarbonyl carbon by the sodium salt of pyrrole **15** was observed.

Finally, a reduction of the 2-nitro groups of **19** and **17***β* was accomplished by hydrogenation over 10% palladium on carbon in THF/ethanol to give 2-amino-1-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)pyrrole-3,4-dicarboxamide (**20**) and 2-amino-1-[3,5-di-*O*-(*p*-toluoyl)-2-deoxy-*β*-D-ribofuranosyl]pyrrole-3,4-dicarboxamide (**21**), respectively (Scheme 2). This demonstrated the feasibility of accessing the 2-aminopyrrole 2-deoxyribosides and ribosides by the coupling of an appropriately substituted carbohydrate with 2-nitropyrrole-3,4-dicarboxamide followed by a catalytic hydrogenation reaction. Studies were then initiated to develop a synthesis of the 1,2,3-triazine portion of the 4-amino-1-(*β*-D-ribofuranosyl)-pyrrolo[2,3-*d*][1,2,3]triazines by the diazo-coupling conditions that were used successfully for the preparation of the *N*-benzyl derivative.[26] These initial attempts were unsuccessful due to the insolubility of the pyrrole ribonucleoside **20** in HCl (aq) at −35 °C and resulted in the starting material being recovered. Moreover, allowing the reaction to warm to higher temperatures resulted in decomposition of the starting material.

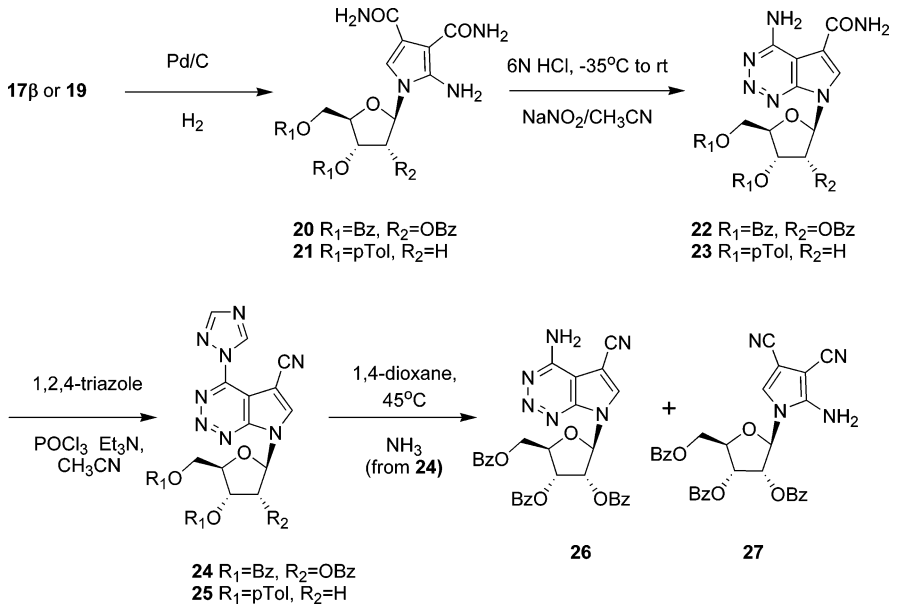
Subsequently, it was found that the use of acetonitrile as a cosolvent soubilized **20** at the required low temperatures and 5-carboxamido-1-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)pyrrolo[2,3-*d*][1,2,3]triazin-4-one (**22**) was obtained in a good yield. Likewise, treatment of the 2′-deoxyribonucleoside **21** with sodium nitrite in HCl (aq) and acetonitrile at −35 °C afforded 1-(3,5-di-*O*-*p*-tolouyl-2-deoxy-*β*-D-ribofuranosyl)pyrrolo[2,3-*d*][1,2,3]-triazin-4-one-5-carboxamide (**23**). These compounds were the first examples of pyrrolo[2,3-*d*][1,2,3]triazine nucleosides. The treatment of compound **22** and **23** with a premixed suspension of $POCl_3$, 1,2,4-triazole and triethylamine afforded 4-(triazol-1-yl)-1-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)pyrrolo[2,3-*d*][1,2,3]triazine-5-carbonitrile (**24**) and 4-(triazol-1-yl)-1-[3,5-di-*O*-(*p*-toluoyl)-2-deoxy-*β*-D-ribofuranosyl]pyrrolo[2,3-*d*][1,2,3]triazine-5-carbonitrile (**25**), respectively. These intermediates were useful in the preparation of other N-4 and N-5 substituted pyrrolo[2,3-*d*][1,2,3]triazine ribonucleosides.

We next initiated studies designed to develop a method for the nucleophillic displacement of the triazole substituent of **24** with ammonia in an effort to prepare 4-amino derivatives related to toyocamycin (**2**) and sangivamycin (**3**). Nucleophilic displacement reactions of nucleoside **24** in liquid ammonia were unsuccessful, probably due to decomposition. Subsequently, ammonia dissolved in several different solvents was used, and this strategy proved to be successful. Subjecting nucleoside **24** to commercially available 0.5 M ammonia in 1,4-dioxane (Aldrich) and acetonitrile at 45 °C gave an approximate 1.4:1 mixture of 4-amino-1-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)pyrrolo[2,3-*d*][1,2,3]triazine-5-carbonitrile (**26**) and 2-amino-1-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)pyrrole-3,4-dicarbonitrile (**27**)**.** A similar reaction has been previously reported[26] from our laboratory. Recently we have reported[26] that 7-benzyl-4-(1,2,4-triazol-1-yl)pyrrolo[2,3-*d*][1,2,3]triazine-5-carbonitrile undergoes a thermal elimination of nitrogen in the presence of NH_3/CH_3CN to give a mixture of 4-amino-7-benzylpyrrolo[2,3-*d*][1,2,3]triazine-5-carbonitrile and 2-amino-1-benzylpyrrole-3,4-dicarbonitrile. Two ^{15}N labeling studies have suggested that this reaction may proceed by a retro-Diels−Alder reaction involving the elimination of N-2 and N-3 from the imino tautomer of compound **26**.[38] Deprotection of **26** with sodium carbonate in ethanol, followed by a work up with acetic acid, gave ethyl 4-amino-1-(*β*-D-ribofuranosyl)pyrrolo[2,3-*d*][1,2,3]triazine-5-carboxylate (**33**). This compound (**33**) is most likely formed by the hydrolysis of an intermediate ethyl imidate. It was found that nucleoside **26** could be deprotected under acyl transfer conditions. Treatment of **26** with a catalytic amount of potassium cyanide in methanol gave methyl 4-amino-1-(*β*-D-ribofuranosyl)-pyrrolo[2,3-*d*][1,2,3]triazine-5-formimidate (**28**).

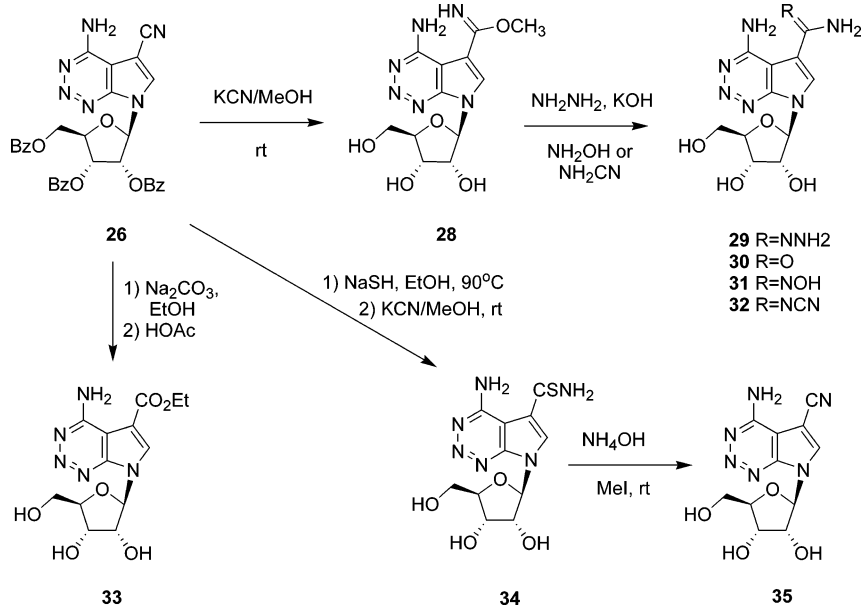
Attempts to prepare the 5-thioamide analogue by treating the methyl imidate **28** with sodium hydrogensulfide produced a complex mixture of products. In direct contrast, a variety of other nucleophiles produced a clean reaction with the methyl imidate **28** (Scheme 3). Treatment of **28** with hydrazine monohydrate in H_2O at reflux temperature afforded the carboxamidrazone, 4-amino-1-(*β*-D-ribofuranosyl)pyrrolo[2,3-*d*][1,2,3]triazine-5-carboxamidrazone (**29**), in good yield. The target compound, 4-amino-1-(*β*-D-ribofuranosyl)pyrrolo[2,3-*d*]-[1,2,3]triazine-5-carboxamide (2-azasangivamycin, **30**), was obtained by the treatment of **28** with an aqueous solution of sodium hydroxide. The carboxamidoxime, 4-amino-1-(*β*-D-ribofuranosyl)pyrrolo[2,3-*d*][1,2,3]triazine-5-carboxamidoxime (**31**) and the cyanoamidine, 4-amino-1-(*β*-D-ribofuranosyl)pyrrolo[2,3-*d*][1,2,3]triazine-5-*N*-cyanoamidine (**32**), were prepared by heating **28** in an aqueous solution of hydroxylamine or cyanamide, respectively.

Since we were unable to prepare 2-azathiosangivamycin directly from **28**, we opted to modify our synthetic route. The thioamide **34** was prepared by heating a mixture of compound **26** in a solution of sodium hydrogensufide, generated in situ, and methanol in a sealed tube. Deprotection of this benzoylated thioamide intermediate could then be accomplished using potassium cyanide in methanol to give 4-amino-1-(*β*-D-ribofuranosyl)pyrrolo[2,3-*d*][1,2,3]triazine-5-thiocarboxamide

Scheme 2



Scheme 3

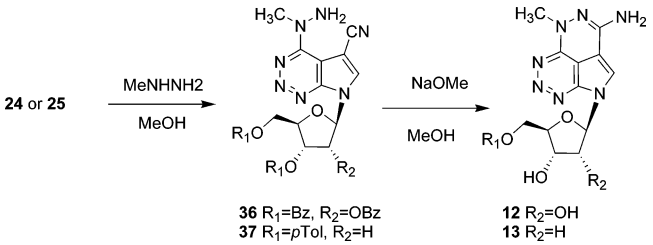


(2-azathiosangivamycin, **34**), which was isolated as a mono hydrate.

The final pyrrolopyrimidine analogue target, 2-azatoyocamycin, was then prepared from 2-azathiosangivamycin. Treatment of **34** with excess methyl iodide in ammonium hydroxide gave the desired 4-amino-1-(β-D-ribofuranosyl)pyrrolo[2,3-*d*][1,2,3]triazine-5-carbonitrile (2-azatoyocamycin, **35**). This reaction presumably proceeds through a methylation of the thione group followed by an elimination.

We next turned our attention to the preparation of 2-aza-TCN and 2-deoxy-2-aza-TCN. A displacement of the triazole group of nucleoside **24** and **25** with methylhydrazine in methanol gave 4-(1-methylhydrazino)-1-(β-D-ribofuranosyl)pyrrolo[2,3-*d*][1,2,3]triazine-5-carbonitrile (**36**) and 4-(1-methylhydrazino)-1-[3,5-di-*O*-(*p*-toluoyl)-2-deoxy-β-D-ribofuranosyl]pyrrolo[2,3-*d*][1,2,3]-triazine-5-carbonitrile (**37**), respectively (Scheme 4). It is interesting to note that very high regioselectivity and

Scheme 4



chemoselectivity was achieved and neither the 2-methylhydrazino adduct was formed nor did any deacylation occur. Treatment of compound **36** or **37** with sodium methoxide in methanol effected a removal of the acyl groups and a concomitant cyclization to afford 3-amino-5-methyl-1-(β-D-ribofuranosyl)-1,5-dihydro-1,4,5,6,7,8-hexaazaacenaphthalene (**12**) and 3-amino-5-methyl-1-(2-deoxy-β-D-ribofuranosyl)-1,5-dihydro-1,4,5,6,7,8-hexaazaacenaphthalene (**13**), respectively. These are the

Table 2. Antiviral Activity and Cytotoxicity of Pyrrole, Pyrrolotriazine and Tricyclic Nucleosides

		50% inhibitory concentratin (μM)[a,b]					
		HCMV		HSV-1	HIV-1	HFF	KB
compound	heterocycle	plaque	yield[c]	ELISA	RT/CPE	visual	growth
17*β*	pyrrole	32	–	>100	–	32	
19	pyrrole	32	–	>100	–	32	
20	pyrrole	>10[d]	–	>10[d]	–	>10[d]	>10[d]
21	pyrrole	12	–	>100	–	32	100
22	pyrrolotriazine	>10[d]	–	>10[d]	–	>10[d]	>10[d]
24	pyrrolotriazine	>10[d]	–	>10[d]	–	>10[d]	>10[d]
28	pyrrolotriazine	32	–	>100	–	21	20
29	pyrrolotriazine	32	1.0	80	–	32	3
30	pyrrolotriazine	0.3	0.4	2.5	–	0.3	0.08
31	pyrrolotriazine	<2.0[e]	–	0.9	–	6.6[e]	1.5
32	pyrrolotriazine	>100[e]	–	>100	–	>100[e]	5
33	pyrrolotriazine	>100	–	>100	–	>100	80
34	pyrrolotriazine	8.5	–	40	–	32	2
36	pyrrolotriazine	>10[d]	–	>10[d]	–	>10[d]	>10[d]
12	tricyclic	14	–	>100	2.2/0.6	10	2.5
8	tricyclic (triciribine, TCN)	2.5[f]	–	23	0.04/–[f] 0.02[g]	100[e]	100[e]

[a] Antiviral activity was determined using a plaque assay in duplicate for HCMV and an ELISA in quadruplicate for HSV-1. Two different assays were used for HIV-1: The amount of reverse transcriptase (RT) activity in culture supernatants was measured in triplicate and viral cytopathogenic effect (CPE) was scored by visual inspection of duplicate cultures, all as described in the text. Ganciclovir was used as a positive control in all HCMV assays, acyclovir was used in all HSV-1 assays, and zidovudine (AZT) was used in the HIV-1 assays. [b] Visual cytotoxicity was scored on uninfected HFF cells not affected by virus in HCMV plaque assays. Inhibition of KB cell growth was measured as described in the text in quadruplicate assays; 2-acetylpyridine thiosemicarbazone was used as a positive control. [c] 90% inhibitory concentration. [d] Limit of solubility. [e] Average of duplicate experiments. [f] Data previously presented in ref 23. [g] Data derived by syncytial plaque assay, presented previously in ref 4.

first reported examples of nucleosides containing the 1,4,5,6,7,8-hexaazanaphthalene ring system.

Biological. The target compound 2-aza-TCN (**12**) and several precursor nucleosides were evaluated for activity against two herpesviruses (human cytomegalovirus (HCMV) and herpes simplex virus type 1 (HSV-1). The pyrrole nucleosides (**17**, **19**−**21**) and most of the pyrrolotriazine nucleosides (**22**, **24**, **28**, **32**−**34**) were inactive or only weakly active against HCMV and HSV-1 (Table 2). Compounds such as **21** or **34**, that had modest activity against HCMV, were cytotoxic at concentrations of only two to three times their antiviral concentrations.

Compounds **29** and **31** were active against one or both of these viruses but their activity was poorly separated from cytotoxicity. In contrast, the 2-aza analogue of sangivamycin (**30**) appeared to be very active against HCMV and HSV-1 but this activity was most likely due to its high cytotoxicity (Table 2).

2-Aza-TCN, **12**, had only weak activity against HCMV (most likely related to its cytotoxicity) and no activity against HSV-1. In contrast, it was active against human immunodeficiency virus type 1 (HIV-1) in two different assays (Table 2). But this activity was observed only at concentrations near those that produced cytotoxicity in HFF (human diploid fibroblasts) and KB (human cancer) cells. Thus in comparison to triciribine (TCN), this new TCN analogue (**12**) was less active against HIV-1 and more cytotoxic (Table 2).

To more thoroughly examine the cytotoxicity of key compounds, the effects of sangivamycin (**3**), 2-aza-sangivamycin (**30**) and 2-aza-TCN (**12**) on the growth of CEM-SS cells was examined. This cell line was chosen because HIV-1 was propagated in it thus a direct comparison of anti-HIV activity to cytotoxicity is most valid. The data in Figure 3 show that as reported several times previously,[8] sangivamycin was highly toxic, inhibiting cell growth at 0.01 to 0.1 μM. In contrast, its 2-aza analogue (**30**) was approximately 10-fold less growth inhibitory. These effects were comparable to those on HFF cells but somewhat less than on KB cells (Table 2). The 2-aza analogue of TCN (**12**) was the least growth inhibitory, approximately 100-less so than sangivamycin. However, even though it was less cytotoxic, it's activity against HIV-1 occurred at concentrations only a few fold lower than its growth inhibitory concentrations. This is significantly less favorable than TCN itself, leading us to conclude that 2-aza-TCN is less promising as an anti-HIV compound than is TCN.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Silica gel, SilicAR 40−63 μm 230−400 mesh (Mallinckrodt) was used for chromatography. Flash column chromatography refers to the chromatography technique described by Still (*J. Org. Chem*. **1978**, *43*, 2923−2925). (X% EtOAc/Hex, Y cm × Z cm) means the solvent system that is used as the eluent, the diameter of the column (Y) and the height of silica gel (Z). Solvent systems are expressed as a percentage of the more polar component with respect to total volume (v/v%). Thin-layer chromatography (TLC) was performed on prescored SilicAR 7GF plates (Analtech, Newark, DE). Compounds were visualized by illuminating with UV light (254 nm) or by spraying with 10% methanolic sulfuric acid followed by charring on a hot plate. Evaporations were carried out under reduced pressure (water aspirator) with the bath temperature not exceeding 50 C, unless specified otherwise. The ^{1}H (300, 360, or 500 MHz) ^{13}C (75, 90, or 125 MHz) and ^{15}N (50 MHz) NMR spectra were recorded on Bruker instruments. The chemical shifts are expressed in parts per millions relative to the standard chemical shift of the solvent for DMSO-d_6; 2.50 ppm (^{1}H NMR), 39.50 ppm (^{13}C NMR), and relative to tetramethylsilane as an internal standard for $CDCl_3$ (^{1}H NMR), and relative to the standard chemical shift of the solvent for ^{13}C NMR (77.0 ppm). Chemical shifts for ^{15}N NMR spectra are recorded in ppm relative to 2% ^{15}N-benzamide, 0.2% Cr(Acac)$_3$ in DMSO-d_6 as an external standard (100.0 ppm). ^{1}H NMR assignments reported were made by homonuclear decoupling experiments, or COSY experiments, or assigned by analogy. ^{13}C NMR assignments were made by *J*-modulated decoupling, DEPT-

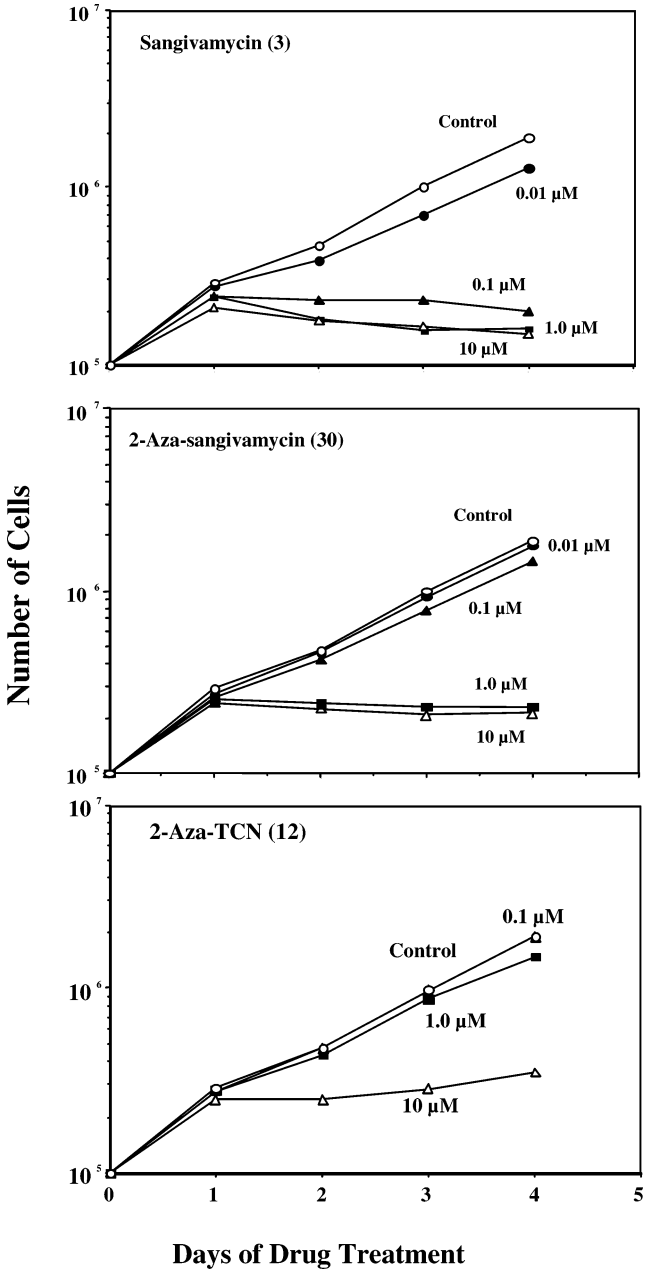


Figure 3. Effect of selected compounds on the growth of CEM-SS cells. Cells were planted in the presence of the indicated concentrations of compounds, harvested on the days shown and enumerated by Coulter counter.

135, DEPT-90 or selective decoupling of fully coupled spectra, or assigned by analogy. UV spectra were recorded on a Hewlet-Packard 8450-A UV/VIS spectrophotometer. IR spectra were recorded on a Nicolet 5 DXB FT spectrophotometer. Mass spectroscopy and elemental analyses were performed by the University of Michigan Chemistry Department or by MHW Laboratories, Phoenix, AZ.

2-Nitro-1-[3,5-di-*O*-(*p*-toluoyl)-2-deoxy-*β*-D-ribofuranosyl]pyrrole-3,4-dicarboxamide (17*β*) and 2-Nitro-1-[3,5-di-*O*-(*p*-toluoyl)-2-deoxy-α-D-ribofuranosyl]pyrrole-3,4-dicarboxamide (17α). Bis(trimethylsilyl)acetamide (0.5 mL, 2.02 mmol) was added to a stirred mixture of 2-nitropyrrole-3,4-dicarboxamide[27] (**15**, 100 mg, 0.505 mmol) in CH_3CN (10 mL) and stirred at room temperature under argon. After 20 min, a solution had formed and NaH (60% dispersion in mineral oil, 30 mg, 0.75 mmol) was added in one portion. After 15 min, the reaction mixture was cooled to 0 °C (ice bath) and then 3,5-di-*O*-(*p*-toluoyl)-2-deoxy-α-D-ribofuranosyl chloride[28,33] (**16α**, 266 mg, 0.94 nmmol) was added in one portion. After 25 min at 0 °C, the reaction was allowed to proceed at room temperature for 8 h. At that time the solvent was evaporated under reduced pressure until a syrup was obtained. 90% MeOH (aq) (15 mL) was added, and the reaction was then heated on a steam bath for 10 min. At that time, the whole reaction mixture was partitioned between H_2O and EtOAc (20 mL/20 mL), the organic layer was separated and washed with brine (10 mL), dried (Na_2SO_4) and filtered and the filtrate was evaporated under reduced pressure. The resultant syrup was subjected to flash chromatography (2% MeOH/$CHCl_3$ then 6% MeOH/$CHCl_3$, 2 cm × 25 cm). The product, R_f = 0.32 (10%MeOH/$CHCl_3$), was collected to give a fraction containing 21 mg of **17** with an ratio of 1:0.6, and a 255 mg fraction containing **17** with an ratio of 1:8.7, as determined by integration of the ^{1}H NMR resonances for the anomeric protons. Recrystallization of 250 mg of this latter fraction in CCl_4/MeOH gave 142 mg (57% recovery) of pure **17*β***.

17*β*: mp 198−199 °C; R_f = 0.32 (10%MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.06 (s, 1H, H-5), 7.92 (bd, 3H, collapses to d, 2H upon addition of D_2O, $CONH_2$, Ph), 7.83 (d 2H, j = 8.0 Hz, Ph), 7.63 (bs, 1H, D_2O exchangeable, $CONH_2$), 7.53 (bs, 1H, D_2O exchangeable, $CONH_2$), 7.37 (d, 2H, J = 8.0 Hz, Ph), 7.30 (d, 2 H, J = 8.0 Hz, Ph), 7.3 (bs, 1 H, D_2O exchangeable, $CONH_2$), 6.72 (t, 1 H, J = 6.2 Hz, H-1′), 5.59 (m, 1 H, H-3′), 4.68−4.54 (m, 3 H, H-4′, H-5′), 2.96−2.88 (m, 1 H, H2a′), 2.73−2.66 (m, 1 H, H-2b′), 2.40 (s, 3 H, CH_3), 2.37 (s, 3 H, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): 165.5, 165.2, 164.3, 162.6, 144.2, 143.9, 133.2, 129.5, 129.4, 129.3, 126.5, 126.4, 124.7, 124.2, 117.4, 88.2, 82.3, 74.0, 63.9; Anal. ($C_{27}H_{26}N_4O_9$): C, H, N. **17α**: A small sample of compound **17α** was obtained as an oil by repeated flash chromatography (2% MeOH/$CHCl_3$ then 6% MeOH/$CHCl_3$, 2 cm × 25 cm): R_f = 0.34 (10%MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.14 (s, 1 H, H-5), 7.94 (d, 2 H, J = 7.9 Hz, Ph), 7.76 (bs, 1 H, D_2O exchangeable, $CONH_2$), 7.63 (d, 2 H, j = 7.9 Hz, Ph, Ph), 7.50 (bs, 1 H, D_2O exchangeable, $CONH_2$), 7.53 (bs, 1 H, D_2O exchangeable, $CONH_2$), 7.38 (d, 2 H, J = 7.9 Hz, Ph), 7.23 (d, 2 H, J = 7.4 Hz, Ph), 6.80 (d, 2 H, J = 6.1 Hz, H-1′), 5.61 (m, 1 H, H-3′), 5.2 (m, 1 H, H-5′), 4.5 (m, 2 H, H-4′), 3.15−3.09 (m, 1 H, H2a′), 2.53−2.47 (m, 1 H, H-2b′), 2.40 (s, 3 H, CH_3), 2.35 (s, 3 H, CH_3); HRMS (CI,NH_3) Calcd. for (M + H) $C_{27}H_{27}N_9$, predicted: 551.0778, Found: 551.0795.

2-Nitro-1-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)pyrrole-3,4-dicarboxamide (19). Compound **19** was prepared in a 69% yield from bis(trimethylsilyl)acetamide (10.0 mL, 40.6 mmol), 2-nitropyrrole-3,4-dicarboxamide (**15**, 2.01 g, 10.0 mmol), NaH (60% dispersion in mineral oil, 487 mg, 13.2 mmol) and 2,3,5-(tri-*O*-benzoyl)-D-ribofuranosyl chloride[30] (**18**, 6.3 g, 13.2 mmol) in CH_3CN (100 mL) as described for compound **17**: mp 125 °C (sinters); R_f = 0.46 (10%MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.1−7.4 (m, 20H, collapses to 18H upon adddition of H_2O, Ph, H-5, $CONH_2$), 6.81 (d, 2H, J = 1.8 Hz, H-1′), 5.96 (m, 1H, H-2′), 5.79 (m, 1H, H-3′), 4.96 (m, 1H, H-4′), 4.79 (m, 2H, H-5); Anal. ($C_{32}H_{26}N_4O_{11}$):C, H, N.

2-Amino-1-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)pyrrole-3,4-dicarboxamide (20). Compound **20** was prepared in a quantitative yield by hydrogenation of compound **19** (2.04 g, 3.17 mmol) with 10% palladium on carbon (95 mg) and H_2 (40 psi) in EtOH/THF (75 mL/35 mL) as described for compound **21**. An analytical sample was prepared by recystallization of 500 mg of **20** with a mixture of EtOH/EtOAc and drying under reduced pressure at 78 °C for 24 h to give 263 mg (53% recovery) of pure **20**: mp 230−232 °C; R_f = 0.62 (10%MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.75 (bs, 1 H, D_2O exchangeable, $CONH_2$), 8.0−7.9 (m, 6 H, Ph), 7.7−7.6 (m, 4 H, collapses to 3 H upon addition of D_2O, $CONH_2$, Ph), 7.5−7.4 (m, 7 H, pH, H-5), 7.21 (bs, 1 H, D_2O exchangeable, $CONH_2$), 6.75 (bs, 1 H, D_2O exchangeable, $CONH_2$), 6.64 (bs, 1 H, D_2O exchangeable, $CONH_2$), 6.31 (d, 2 H, J = 4.4 Hz, H-1′), 5.9−5.8 (m, 2 H, H-2′, H-3′), 4.8−4.7 (m, 1 H, H-4′), 4.7 (m, 2 H, H-5); *Anal. (*$C_{32}H_{28}N_4O_9$): C, H, N.

2-Amino-1-[3,5-di-*O*-(*p*-toluoyl)-2-deoxy-*β*-D-ribofuranosyl]pyrrole-3,4-dicarboxamide (21). A solution of **17*β***

(439 mg, 0.80 mmol) and 10% palladium on carbon (10%, 143 mg) was hydrogenated at 40 psi in MeOH (60 mL) and EtOAc (20 mL) for 18 h. At that time, the solution was filtered through Celite and the filtrate was evaporated under reduced pressure to dryness. Recrystallization of the solid from EtOAc/hexanes and drying under reduced pressure at 78 °C for 24 h gave 292 mg (70%) of **21**: mp 119−120 °C (sinters); $R_f = 0.45$ (10%MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.79 (bs, 1 H, D_2O exchangeable, $CONH_2$), 7.96 (d, 2 H, J = 8.0 Hz, Ph), 7.87 (d, 2 H, J = 8.1 Hz, Ph), 7.63 (bs, 3 H, D_2O exchangeable, $CONH_2$, NH_2), 7.61 (bs, 1 H, D_2O exchangeable, $CONH_2$), 7.14 (bs, 1 H, D_2O exchangeable, $CONH_2$), 6.16 (t, 1 H, J = 6.7 Hz, H-1′), 5.64 (m, 1 H, H-3′), 4.53−4.47 (m, 3 H, H-4′, H-5′), 2.65 (m, 2 H, H2′), 2.40 (s, 3 H, CH_3), 2.37 (s, 3 H, CH_3); Anal. ($C_{27}H_{28}N_4O_7$): C, H, N.

5-Carboxamido-1-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)pyrrolo[2,3-*d*][1,2,3]triazin-4-one (22). A stirred mixture of **20** (6.12 g, 9.99 mmol) and CH_3CN (200 mL) was cooled to −40 °C (dry ice/CH_3CN). 6 N HCl (100 mL, pre-cooled to 0 °C) was added to the cold, stirred mixture. At that time, a solution of $NaNO_2$ (1.26 g, 18.3 mmol in 10 mL of H_2O) was added dropwise over a period of 10 min, while maintaining the cold bath between −30 °C and −40 °C. The reaction was then allowed to stir at room temperature until an internal temperature of 0 °C was reached. The reaction mixture was poured onto H_2O/EtOAc (1:1, 500 mL), the organic layer was separated, dried (Na_2SO_4) and filtered and the filtrate was evaporated to dryness. The resultant red solid was purified by flash chromatography (3% MeOH/$CHCl_3$, 5 cm × 20 cm), and the fractions with R_f = 0.78 (10% MeOH/$CHCl_3$) were evaporated to 50 mL. Hexanes (250 mL) was then added to the solution with vigorous stirring. The precipitate which had formed was collected by vacuum filtration and dried under reduced pressure at 78 °C for 48 h to give 4.45 g (71%) of **22** as a white solid:**:** mp 185−187 C (decomp); R_f = 0.78 (10% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 15.24 (bs, 1H, D_2O exchangeable, NH), 9.16 (bs, 1H, D_2O exchangeable, $CONH_2$), 8.53 (s, 1 H, C-6), 8.0−7.9 (m, 6H, Ph), 7.6 (m, 4H, collapses to 3H upon addition of D_2O, $CONH_2$, Ph), 7.5−7.4 (m, 6 H, Ph), 6.87 (d, 1 H, J = 4.8 Hz, H-1′), 6.30 (m, 1 H, H-2′), 6.11 (m, 1 H, H-3′), 4.92 (m, 1 H, H-4′), 4.76 (m, 2 H, H-5); Anal. ($C_{32}H_{25}N_5O_9$): C, H, N.

5-Carboxamido-1-[3,5-di-*O*-(*p*-toluoyl)-2-deoxy-*β*-D-ribofuranosyl]pyrrolo[2,3-*d*][1,2,3]triazin-4-one (23). A stirred mixture of **20** (1.37 g, 2.63 mmol) and CH_3CN (32 mL) was cooled to −40 °C (dry ice/CH_3CN). 6 N HCl (16 mL, pre-cooled to 0 °C) was added to the cold, stirred mixture, and a solution of $NaNO_2$ (316 mg, 5.26 mmol in 1 mL of H_2O) was added dropwise over a period of 5 min, while maintaining the bath between −30 °C and −40 °C. The reaction was allowed to stir at room temperature until an internal temperature of 0 °C was reached. The reaction mixture was poured onto H_2O/EtOAc (1:1, 200 mL). At that time, a precipitate had formed between the aqueous and organic layers. Collection of this precipitate by filtration and drying under reduced pressure at 50 °C gave 945 mg (68%) of **23** as a white solid. An additional 70 mg (5%) of **23** could be obtained after the organic layer was separated, dried (Na_2SO_4) and filtered, and the filtrate was evaporated to dryness: mp 209−210 °C; R_f = 0.75 (10% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 15.36 (bs, 1H, D_2O exchangeable, NH), 9.16 (bs, 1H, D_2O exchangeable, $CONH_2$), 8.41 (s, 1 H, C-6), 7.97 (m, 2 H, Ph), 7.85 (m, 2 H, Ph), 7.61 (bs, 1H, D_2O exchangeable, $CONH_2$), 7.37 (m, 2 H, Ph), 7.28 (m, 2 H, Ph), 6.86 (t, 1 H, J = 6.7 Hz, H-1′), 5.74 (m, 1 H, H-3′), 4.62−4.56 (m, 3 H, H-4′, H-5′), 3.20−2.82 (m, 2 H, H-2′); Anal. ($C_{27}H_{25}N_5O_7$): C, H, N.

4-(Triazol-1-yl)-1-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)-pyrrolo[2,3-*d*][1,2,3]triazine-5-carbonitrile (24). A suspension of 1,2,4-triazole (7.9 g, 115 mmol) in CH_3CN (100 mL), stirred under argon, was treated with phosphorus oxychloride (2.4 mL, 26 mmol), and the white suspension was cooled to 0 °C. Triethylamine (16 mL, 115 mmol) was then added, and the mixture was allowed to stir at 0 °C for 1 h. Compound **22** (2.00 g, 3.21 mmol) was then added in one portion**.** The reaction mixture was allowed to stir for 1.5 h at room temperature and then filtered through Celite. The Celite was washed with CH_3CN (100 mL), and the combined fitrate and washings were evaporated under reduced pressure until a thick oil was obtained. This oil was dissolved in $CHCl_3$ (200 mL), washed with H_2O (100 mL), dried ($MgSO_4$) and filtered, and the filtrate was evaporated under reduced pressure to give a brown foam. This was purified by flash chromatography (15% EtOAc/toluene, 5 cm × 20 cm), and the product, R_f = 0.31 (20% EtOAc/toluene, 5 cm × 20 cm), was evaporated to dryness. Sequential addition of $CHCl_3$ (10 mL) followed by hexanes (100 mL) gave a precipitate which was collected by filtration and dried under reduced pressure at 70 °C for 24 h to give 1.23 g (58%) of **24** as a white solid: mp 120 °C; R_f = 0.0.31 (20% EtOAc/toluene); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.88 (s, 1 H), 9.48 (s, 1 H), 8.75 (s, 1 H), 8.0−7.9 (m, 6 H, Ph), 7.7−7.6 (m, 3 H, Ph), 7.5−7.4 (m, 6 H, Ph), 7.08 (d, 1 H, J = 4.1 Hz, H-1′), 6.40 (m, 1 H, H-2′), 6.23 (m, 1 H, H-3′), 5.0 (m, 1 H, H-4′), 4.9−4.8 (m, 2 H, H-5); Anal. ($C_{34}H_{24}N_8O_7$): C, H, N.

4-(Triazol-1-yl)-1-[3,5-di-*O*-(*p*-toluoyl)-2-deoxy-*β*-D-ribofuranosyl]-pyrrolo[2,3-*d*][1,2,3]triazine-5-carbonitrile (25). A stirred suspension of 1,2,4-triazole (1.64 g, 0.66 mmol) in CH_3CN (30 mL), under argon, was treated with phosphorus oxychloride (0.49 mL, 5.3 mmol), the white suspension was cooled to 0 C. Triethylamine (3.32 mL, 23.8 mmol) was added and the mixture was allowed to stir at 0 °C for 1 h, at which time compound **23** (350 mg, 0.659 mmol) was added in one portion. The reaction mixture was stirred for 5 h at room temperature and filtered through Celite, and the filter cake was washed with CH_3CN (20 mL). The filtrate and washing were evaporated under reduced pressure, and the oily residue was purified by flash chromatography (30% EtOAc/hexanes then 50% EtOAc hexanes, 2 cm × 25 cm). The resultant solid, 180 mg (48%) R_f = 0.44 (50% EtOAc/hexanes), was recrystallized from EtOAc/hexanes and dried under reduced pressure at 78 C for 24 h to give 87 mg (48% recovery) of **25** as white crystals: mp 205−206 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.88 (s, 1H), 9.38 (s, 1H), 8.63 (s, 1H), 7.97 (d, 1 H, J = 7.9 Hz, Ph), 7.75 (d, 1 H, J = 8.0 Hz, Ph), 7.37 (d, 1 H, J = 7.8 Hz, Ph), 7.21 (d, 1 H, J = 7.8 Hz, Ph), 7.02 (t, 1 H, J = 6.4 Hz, H-1′), 5.86 (m, 1 H), 4.7−4.6 (m, 3 H), 3.3−3.2 (m, 2 H), 2.40 and 2.30 (s, 3 H, CH_3, CH_3); Anal. ($C_{27}H_{25}N_5O_7$): C, H, N.

4-Amino-1-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)pyrrolo[2,3-*d*][1,2,3]triazine-5-carbonitrile (26) and 2-Amino-1-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)pyrrole-3,4-dicarbonitrile (27). A sealed vessel containing Compound **24** (100 mg, 0.15 mmol), NH_3 (Aldrich, 0.5 M in 1,4-dioxane, 4 mL) and CH_3CN (6 mL) was sealed and stirred at 45 °C for 7 days. At that time the solvent was evaporated to a yellow oil which was dissolved in EtOAc (20 mL) and washed successively with H_2O (20 mL) and brine (20 mL). The aqueous layers were back extracted with EtOAc (20 mL), and the combined organic layers were dried (Na_2SO_4), filtered and evaporated under reduced pressure to give a yellow oil which was purified by flash chromatography (30% EtOAc/hexanes then 40% EtOAc/hexanes then 50% EtOAc/hexanes, 2 cm × 20 cm). The appropriate fractions, R_f = see below, were evaporated to dryness and then dried under reduced pressure at room temperature for 48 h.

Pyrrolotriazine (26): Yield 45 mg (49%): mp > 105 °C (decomp); R_f = 0.39 (50% EtOAc/toluene); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.85 (s, 1 H, C-6), 8.0−7.9 (m, 6 H, Ph), 7.7−7.6 (m, 3 H, Ph), 7.58 (bs, 2 H, D_2O exchangeable, NH_2), 7.5−7.4 (m, 6 H, Ph), 6.81 (d, 1 H, J = 4.2 Hz, H-1′), 6.34 (m, 1 H, H-2′), 6.22 (m, 1 H, H-3′), 4.93 (m, 1 H, H-4′), 4.8−4.7 (m, 2 H, H-5); IR (KBr) 3459−2856, 2229 (CN), 1731, 1635 cm^{-1}; MS (CI,NH_3) 594 (M + H − N_2); HRMS (CI,NH_3) Calcd. for (M + H) $C_{32}H_{25}N_6O_7$, predicted: 605.1785, found: 605.1791. Anal. ($C_{32}H_{24}N_6O_7$): C, H, N.

Pyrrole (27): Yield 32 mg (36%): mp 200−201 °C; R_f = 0.61 (50% EtOAc/toluene); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.0−7.8 (m, 6 H, Ph), 7.7−7.6 (m, 4 H, Ph, C-5), 7.6−7.4 (m, 6 H, Ph), 6.93 (bs, 2 H, D_2O exchangeable, NH_2), 6.30 (d, 1 H, J = 3.9 Hz, H-1′), 5.87 (m, 2 H, H-2′, H-3′), 4.8−4.7 (m, 3 H, H-4′,

H-5′); IR (KBr) 3406−2944, 2230 (CN), 1718 cm^{-1}; Anal. ($C_{32}H_{24}N_4O_7$): C, H, N.

Methyl 4-Amino-1-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-[1,2,3]triazine-5-formimidate (28). Compound **26** (810 mg), KCN (64 mg, 0.99 mmol) and MeOH (30 mL) were stirred at room temperature for 5 h. At that time, the reaction was cooled to −10 °C, and the resultant precipitate was collected by filtration and washed with diethyl ether (2 × 30 mL). Drying the collected solid under reduced pressure at 60 °C for 48 h gave 295 mg (68%) of **28**, which was pure as determined by ^{1}H NMR. An analytical sample was prepared by suspending 100 mg of **28** in CH_2Cl_2 (15 mL), heating at reflux temperature for 10 min and then collecting the solid by filtration. Drying as before gave 96 mg (96% recovery) of pure **28** as a white solid**:** mp 187−189 °C (eff.); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.87 (d, 1 H, J = 3.4 Hz, D_2O exchangeable, NH_2), 9.87 (s, 1 H, D_2O exchangeable, NH), 8.23 (s, 1 H, C-6), 7.83 (d, 1 H, J = 3.3 Hz, D_2O exchangeable, NH_2), 6.25 (d, 1 H, J = 6.0 Hz, H-1′), 5.48 (d, 1 H, D_2O exchangeable, OH), 5.2 (m, 3 H, D_2O exchangeable, OH × 2), 4.4 (m, 1 H, H-2′), 4.1 (m, 1 H, H-3′), 4.0 (m, 1 H, H-4′), 3.7−3.6 (m, 2 H, H-5′); UV [λ_{max} (ε)] (PH1) 313 (5100), 268 (7600), 238 (11 600); (EtOH) 321 (5500), 274 (8300); (pH11) 320 (5900), 273 (7900), 238 (7100);. Anal. ($C_{12}H_{16}N_6O_5$): C, H, N.

4-Amino-1-(β-D-ribofuranosyl)pyrrolo[2,3-*d*][1,2,3]-triazine-5-carboxamidrazone (29). Compound **28** (150 mg, 0.46 mmol), hydrazine monohydrate (0.44 mL, 9.3 mmol) and methanol (3 mL) were heated on a steam bath for 25 min, and then H_2O was slowly added until a clear solution had formed. Heating was continued for 10 min, and then the reaction was allowed to stand at room temperature so that crystallization could commence. After 20 h at room temperature, the precipitate was collected by filtration, washed with cold diethyl ether (20 mL) and dried under reduced pressure at 60 °C for 2 days to give 134 mg (89%) of **29**. This solid was recrystallized from H_2O and then dried under reduced pressure over P_2O_5 at 78 °C for 24 h to give 111 mg (74% yield, 83% recovery) of pure **29**: mp > 240 °C (decomp); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.80 (bs, 1 H, D_2O exchangeable, NH_2), 8.05 (s, 1 H, C-6), 7.66 (bs, 1 H, D_2O exchangeable, NH_2), 6.22 (d, 1 H, J = 6.0, H-1′), 5.87 (bs, 1 H, D_2O exchangeable), 5.48 (d, 1 H, D_2O exchangeable), 5.23 (d, 1 H, D_2O exchangeable), 5.1−5.0 (m, 3 H, D_2O exchangeable), 4.40 (m, 1 H, H-2′), 4.12 (m, 1 H, H-3′), 3.94 (m, 1 H, H-4′), 3.7−3.6 (m, 2 H, H-5′); IR (KBr) 3397−3198, 1638, 1465 cm^{-1}; UV [λ_{max} (ε)] (PH1) 312 (5800), 268 (8500); (EtOH) 323 (1600), 282 (2900); (pH11) 321 (6500), 276 (11 700), 228 (7100);. HRMS (CI, methane) Calcd. for (M + H) $C_{11}H_{18}N_8O_4$, predicted: 325.1373, Found: 325.1377.

4-Amino-1-(β-D-ribofuranosyl)pyrrolo[2,3-*d*][1,2,3]-triazine-5-carboxamide (2-AzaSangivamycin, 30). A 1.25 N solution of NaOH (8 mL) and compound **28** (200 mg, 0.62 mmol) were stirred at room temperature for 1.5 h. At that time, the reaction was allowed to stand for 15 min at room temperature. The precipitate was collected by filtration and washed with diethyl ether (20 mL). Drying this solid under reduced pressure at 60 °C for 24 h gave 128 mg (67%) of **30** as a white solid. Recrystallization from H_2O and drying under reduced pressure at 78 °C for 48 h gave 126 mg (64%) of pure **30** as a hemihydrate: mp > 270 °C (decomp); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.5−7.5 (bs, 2 H, D_2O exchangeable, NH_2), 8.43 (s, 1 H, C-6), 8.13 (bs, 1 H, D_2O exchangeable, $CONH_2$), 7.64 (bs, 1 H, D_2O exchangeable, $CONH_2$), 6.24 (d, 1 H, J = 5.6, H-1′), 5.58 (d, 1 H, D_2O exchangeable, OH), 5.30 (d, 1 H, D_2O exchangeable, OH), 5.06 (t, 1 H, D_2O exchangeable, 5′-OH), 4.40 (m, 1 H, H-2′), 4.13 (m, 1 H, H-3′), 3.98 (m, 1 H, H-4′), 3.7−3.6 (m, 2 H, H-5′); Anal. ($C_{11}H_{14}N_6O_5 \cdot H_2O$): C, H, N.

4-Amino-1-(β-D-ribofuranosyl)pyrrolo[2,3-*d*][1,2,3]-triazine-5-carboxamidoxime (31). Compound **28**, 135 mg, 0.42 mmol), hydroxylamine (50% aqueous solution, 500 mg) and H_2O (4 mL) were heated on a steam bath for 1 h. The reaction was then allowed to stand at room temperature so that crystallization could commence. After 20 h at room temperature, the precipitate was collected by filtration, washed with cold diethyl ether (20 mL) and dried under reduced pressure at 60 °C for 2 days. This solid was recrystallized from H_2O and then dried under reduced pressure at 78 °C for 24 h to give 125 mg (92%) of pure **31**: mp 258−260 °C (eff.); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.82 (bs, 1 H, D_2O exchangeable), 9.23 (bs, 1 H, D_2O exchangeable), 8.18 (s, 1 H, C-6), 7.84 (bs, 1 H, D_2O exchangeable), 6.24 (d, 1 H, J = 5.7, H-1′), 6.16 (bs, 1 H, D_2O exchangeable), 5.51 (d, 1 H, D_2O exchangeable), 5.26 (d, 1 H, D_2O exchangeable), 5.06 (d, 1 H, D_2O exchangeable), 4.39 (m, 1 H, H-2′), 4.13 (m, 1 H, H-3′), 3.95 (m, 1 H, H-4′), 3.7−3.5 (m, 2 H, H-5′); Anal. ($C_{11}H_{15}N_7O_5$): C, H, N.

4-Amino-1-(β-D-ribofuranosyl)pyrrolo[2,3-*d*][1,2,3]-triazine-5-*N*-cyanoamidine (32). Compound **28** (146 mg, 0.45 mmol), cyanamide (95 mg, 2.25 mmol) and H_2O (5 mL) were heated on a steam bath for 1 h. The reaction was then allowed to stand at room temperature so that crystallization could commence. After 20 h at room temperature, the precipitate was collected by filtration, washed with cold diethyl ether (20 mL) and dried under reduced pressure at 60 °C for 2 days. This solid was recrystallized from H_2O and then dried under reduced pressure at 78 °C for 24 h to give 77 mg (51%) of pure **32**: mp > 220 °C (decomp); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.22 (bs, 1 H, D_2O exchangeable), 8.67 (bs, 3 H, collapses to [s, 1 H] upon addition of D_2O), 8.23 (bs, 1 H, D_2O exchangeable), 6.29 (d, 1 H, J = 5.4, H-1′), 5.60 (d, 1 H, D_2O exchangeable), 5.29 (d, 1 H, D_2O exchangeable), 5.02 (t, 1 H, D_2O exchangeable), 4.4 (m, 1 H, H-2′), 4.1 (m, 1 H, H-3′), 4.0 (m, 1 H, H-4′), 3.7−3.6 (m, 2 H, H-5′); Anal. ($C_{12}H_{14}N_8O_4 \cdot 0.5$ H_2O): C, H, N.

Ethyl 4-Amino-1-(β-D-ribofuranosyl)pyrrolo[2,3-*d*][1,2,3]-triazine-5-carboxylate (33). Compound **26** (500 mg, 0.83 mmol), Na_2CO_3 (701 mg, 6.61 mmol) and EtOH/H_2O (10:1, 33 mL) were stirred at room temperature for 52 h. At that time, AcOH (glacial, 3 mL) was added and the solution was evaporated under reduced pressure until a volume of 10 mL was obtained. H_2O (50 mL) was then added, the clear mixture was extracted with hexanes (3 × 50 mL) and the aqueous layer was evaporated under reduced pressure at 60 °C to 1/2 of its original volume. The resultant aqueous suspension was heated on a steam bath until a clear solution was observed. Cooling to room temperature produced white crystals which were collected by filtration and then dried under reduced pressure at 78 °C for 48 h to give 175 mg (62%) of **33**: mp 130−131 °C (sinters); R_f = 0. 16 (10% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.63 (s, 1 H, C-6), 8.1 (bs, 1 H, D_2O exchangeable, $CONH_2$), 7.7 (bs, 1 H, D_2O exchangeable, $CONH_2$), 6.28 (d, 1 H, J = 5.3 Hz, H-1′), 5.57 (bs, 1 H, D_2O exchangeable, OH), 5.3−5.2 (m, 3 H, D_2O exchangeable, OH × 2), 4.43 (m, 2 H, H-2′), 4.34 (q, 2 H, CH_2), 4.15 (m, 1 H, H-3′), 4.00 (m, 1 H, H-4′), 3.8−3.6 (m, 2 H, H-5′), 1.33 (t, 3 H, CH_3); IR (KBr) 3416−3152, 1690, 1662 cm^{-1}; UV [λ_{max} (ε)] (PH1) 310 (6500), 265 (1 0000), 229 (13 000); (EtOH) 311 (6500), 276 (10 000); (pH11) 313 (8000), 273 (10 000);. Anal. ($C_{13}H_{17}N_5O_6$): C, H, N.

4-Amino-1-(β-D-ribofuranosyl)pyrrolo[2,3-*d*][1,2,3]-triazine-5-thiocarboxamide (2-Azathiosangivamycin, 34). H_2S (g) was bubbled into a solution of MeOH (70 mL) and NaOMe (358 mg, 6.62 mmol), held at 0 °C in an ice bath. After 30 min, the solution was added to a pressure tube containing Compound **26** (400 mg, 0.66 mmol). The tube was sealed and heated at 90 °C for 1 h. At that time, the reaction was cooled to room temperature, the pH was adjusted to 7 with 1 N HCl and the solvent was evaporated under reduced pressure until a yellow oil was obtained. H_2O was added to the oil, and the precipitate was collected by filtration, washed with cold H_2O (20 mL) and dried under reduced pressure at 70 °C for 48 h to give 336 mg (79%) of crude 4-amino-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*][1,2,3]triazine-5-thiocarboxamide, R_f = 0.60 (10% MeOH/$CHCl_3$). The crude benzoylated compound (259 mg, 0.41 mmol), KCN (78 mg, 1.2 mmol) and (MeOH 15 mL) were stirred at room temperature for 4 h. At that time, the reaction mixture was evaporated to dryness and purified by preparative TLC (Silica, 4 × 500 μm plates, eluting with 20% MeOH/EtOAc, 30% MeOH/EtOAc, then 50% MeOH/EtOAc). The most prevalent band was eluted off of the plates

with 30% EtOAc/MeOH and the solution was filtered through Celite. The filtrate was evaporated to dryness, dissolved in a minimal amount of H_2O and then freeze-dried for 48 h to give 76 mg (54%) of **34** as a light yellow solid: mp > 180 °C (decomp); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.86 (bs, 1 H, D_2O exchangeable, $CSNH_2$), 9.70 (bs, 1 H, D_2O exchangeable, $CSNH_2$), 8.3 (bs, 3 H, collapses to [s, 1H] upon addition of D_2O, NH_2, C-6), 6.30 (d, 1 H, J = 4.2, H-1′), 5.58 (d, 1 H, D_2O exchangeable), 5.29 (d, 1 H, D_2O exchangeable), 5.11 (m, 3 H, D_2O exchangeable), 4.41 (m, 1 H, H-2′), 4.15 (m, 1 H, H-3′), 3.97 (m, 1 H, H-4′), 3.7−3.6 (m, 2 H, H-5′); Anal. ($C_{11}H_{14}N_6O_4S\cdot H_2O$): C, H, N.

4-Amino-1-(β-D-ribofuranosyl)pyrrolo[2,3-*d*][1,2,3]-triazine-5-carbonitrile (2-Azatoyocamycin, 35). A stirred mixture of 4-amino-1-β-D-ribofuranosyl)pyrrolo[2,3-*d*][1,2,3]-triazine-5-thiocarboxamide (2-azathiosangivamycin) (**34**, 0.203 mmol) in H_2O (1.5 mL), CH_3CN (1.5 mL) and NH_4OH (0.5 mL) was treated with MeI (0.14, 1.18 mmol) and stirred for 1 h at room temperature. At that time, the reaction was evaporated and coevaporated with toluene. The resulting yellow solid was recrystallized from EtOH to give 20 mg (34%) of **35** as a tan solid mp > 180 °C (decomp); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.79 (s, 1 H), 7.49 (bs, 2 H, D_2O exchangeable, NH_2), 6.25 (d, 1 H, J = 4.9, H-1′), 6.62 (d, 1 H, D_2O exchangeable), 5.27 (d, 1 H, D_2O exchangeable), 5.19 (t, 3 H, D_2O exchangeable), 4.42 (m, 1 H, H-2′), 4.13 (m, 1 H, H-3′), 3.99 (m, 1 H, H-4′), 3.7−3.6 (m, 2 H, H-5′); IR (KBr) 3161, 2066 (CN), 1636 cm^{-1}; HRMS (CI, NH_3) Calcd. for (M + H) $C_{11}H_{13}N_6O_4$, predicted: 293.0998, Found: 293.1008.

4-(1-Methylhydrazino)-1-(β-D-ribofuranosyl)pyrrolo-[2,3-*d*][1,2,3]triazine-5-carbonitrile (36). A solution of **24** (1.0 g, 1.52 mmol), methylhydrazine (2.0 mL, 38 mmol) and MeOH (45 mL) were stirred at room temperature. After 3 h, the reaction mixture was cooled to 0 °C, and the precipitate was collected by filtration. This solid was washed with cold MeOH (5 mL) and dried under reduced pressure at 50 °C for 24 h to give 789 mg (82%) of **36**, which was sufficiently pure to be used in subsequent reactions. An analytical sample was prepared by subjecting 195 mg of **36** to flash chromatography (20% EtOAc/toluene then 40% EtOAc/toluene, 2 cm × 20 cm), and collecting the major spot, R_f = 0.09 (20% EtOAc/toluene). $CHCl_3$ (5 mL) and hexanes (50 mL) were added sequentially to the solid, and the solid was collected by filtration and dried as before to give 163 mg (84% recovery) of pure **36**: mp 204−206 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.75 (s, 1 H, C-6), 8.0−7.9 (m, 6 H, Ph), 7.6 (m, 3 H, Ph), 7.5−7.4 (m, 6 H, Ph), 6.79 (d, 1 H, J = 3.8, H-1′), 6.29 (m, 1 H, H-2′), 6.17 (m, 1 H, H-3′), 5.43 (S, 2 H, D_2O exchangeable, NH_2), 4.92 (m, 1 H, H-4′), 4.8 (m, 2 H, H-5′), 3.51 (S, 3 H, CH_3); Anal. ($C_{33}H_{27}N_7O_7$): C, H, N.

3-Amino-5-methyl-1-(β-D-ribofuranosyl)-1,5-dihydro-1,4,5,6,7,8-hexaazaacenaphthalene (12). NaOMe (228 mg, 4.22 mmol) was added to a stirred solution **36** (594 mg, 0.94 mmol) in MeOH (35 mL) at room temperature. After stirring for 1 h, the reaction was heated at reflux temperature for 17 h at which time a yellow precipitate was observed. The reaction mixture was cooled to room temperature, and the precipitate was collected by filtration and dried under reduced pressure at 60 °C for 24 h. This solid was suspended in CH_2Cl_2 (40 mL) and held at reflux temperature for 20 min. At that time, the suspension of yellow powder was cooled to room temperature, and the yellow powder was collected by filtration and dried under reduced pressure at 78 °C for 48 h to give 260 mg (86%) of **12**: mp 268−270 °C (eff.); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.40 (s, 1 H, H-2), 6.56 (s, 1 H, D_2O exchangeable, NH_2), 6.01 (d, 1 H, J = 6.2, H-1′), 5.63 (bs, 1 H, D_2O exchangeable, 5′-OH), 5.35 (bs, 1 H, D_2O exchangeable, 3′-OH), 5.20 (bs, 1 H, D_2O exchangeable, 2′-OH), 4.56 (m, 1 H, H-2′), 4.14 (m, 1 H, H-3′), 4.00 (m, 1 H, H-4′), 3.7−3.5 (m, 2 H, H-5′), 3.49 (s, 3 H, CH_3); Anal. ($C_{12}H_{15}N_7O_4$): C, H, N.

3-Amino-5-methyl-1-(2-deoxy-β-D-ribofuranosyl)-1,5-dihydro-1,4,5,6,7,8-hexaazaacenaphthalene (13). A stirred solution of **25** (158 mg, 0.280 mmol) in MeOH (8 mL) was treated with methylhydrazine (0.37 mL, 7.0 mmol) at room temperature. After 2 h, the reaction mixture was evaporated to dryness and then purified by filtering through a layer of silica gel (3% MeOH/$CHCl_3$, 2 cm × 2 cm) to obtain 145 mg (95%) of crude **37**. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.73 (s, 1H), 7.97 (d, 1 H, J = 8.0 Hz, Ph), 7.7.87 (d, 1 H, J = 8.2 Hz, Ph), 7.38 (d, 1 H, J = 8.1 Hz, Ph), 7.30 (d, 1 H, J = 8.0 Hz, Ph, 6.87 (t, 1 H, J = 6.9 Hz, H-1′), 5.78 (bs, 2 H, D_2O exchangeable, NH_2), 5.40 (m, 1 H), 5.65−4.56 (m, 3 H), 3.1−2.8 (m, 2 H), and 2.37 (s, 3 H, CH_3, CH_3); This crude yellow oil (**37**) was dissolved in MeOH (10 mL), NaOMe (65 mg, 1.21 mmol) was added, and the solution was stirred at room temperature for 1 h. At that time, the yellow solution was heated at reflux temperature for 16 h, cooled to room temperature and evaporated under reduced pressure to dryness. The solid was treated with EtOAc (15 mL), filtered and purified by preparative TLC (SiO_2, 2000 μm, 20 cm × 20 cm, eluting with 30% MeOH/EtOAc). The bright yellow band was collected to give 45 mg (53%) of **13** as a yellow solid. An analytical sample was prepared by recrystallization from MeOH and drying under reduced pressure at 78 °C for 2 days to give 20 mg (44% recovery) of **13**: mp > 230 °C (decomp); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.39 (s, 1 H), 6.54 (s, 2 H, D_2O exchangeable, NH_2), 6.48 (t, 1 H, J = 6.7 Hz, H-1′), 5.39 (d, 1 H, D_2O exchangeable, 3′-OH), 5.08 (t, 1 H, D_2O exchangeable, 5′-OH), 4.43 (m, 1 H), 3.91 (m, 1 H), 3.6−3.5 (m, 2 H), 3.52 (s, 3 H, CH_3), 2.8−2.3 (m, 2 H); IR (KBr) 3418−3079, 1695, 1636, 1474 cm^{-1}; HRMS (EI, 70 eV) Calcd. for (M+) $C_{12}H_{15}N_7O_3$, predicted: 305.1236, Found: 305.1238.

In Vitro Antiviral Studies. Cell Culture Procedures. The routine growth and passage of KB, HFF and BSC-1 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 or fetal calf serum as detailed previously.[39] 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. CEM cells were passaged twice weekly at 1:10 dilutions using RPMI 1640 with 10% fetal calf serum.[4]

HIV-1 Assay. The HIV strain III_B producer cell line H9III_B was obtained through the courtesy of Dr. R. C. Gallo. HIV strain III_B was propagated in CEM-SS cells as described previously by Kucera et al.[4,40] To evaluate the activities of compounds in cells acutely infected with HIV, two different assays were used. In one, reverse transcriptase (RT) was employed as a marker for HIV-1. CEM-SS cells were infected at a multiplicity of infection of approximately 0.001 plaque forming units (pfu). per cell with strain III_B of HIV-1 in a minimal volume of stock virus in growth medium. Cultures were incubated at 37 °C for 2 h to permit virus adsorption and then diluted to 5 × 10^5 cells per mL with RPMI 1640 containing 10% fetal bovine serum. One-tenth milliliter was then added to each well of a 96-well cluster dish which had been pretreated with poly-L-lysine. Fresh medium (0.1 mL with 10% fetal bovine serum) containing test compounds in twice the desired final concentration was added to triplicate wells at seven concentrations ranging from 100 to 0.14 μM. After 6 days incubation, supernatant samples were taken and the amount of RT activity was measured by the incorporation of [^{3}H]dTTP into acid insoluble material using the assay described by White et al.[41] In the other assay, viral cytopathology was determined microscopically at 30-fold magnification in cells infected with HIV-1 infected as described above.

HCMV Assays. The Towne strain, plaque-purified isolate P_o, of HCMV was kindly provided by Dr. Mark Stinski, University of Iowa. Both plaque and yield reduction assays were used. In the former, HFF cells in 24-well cluster dishes were infected with approximately 100 plaque forming units (pfu) of HCMV per well using the procedures detailed earlier.[39] Following virus adsorption, compounds dissolved in MEM(E) containing 0.5% methylcellulose and 5% fetal calf serum were added to duplicate wells in four to eight selected concentrations. After incubation at 37 °C for 8−10 days, cell sheets were

fixed and stained with crystal violet, and microscopic plaques were enumerated. 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. Protocols for HCMV yield-reduction experiments have been previously described.[42] Briefly, monolayer cultures of HFF cells in 96-well culture dishes (Costar, Cambridge, MA) were infected at a multiplicity of infection (MOI) of 0.5, 0.05, or 0.005 PFU/cell and incubated in the presence of the test compounds for 6 to 7 days. Following one cycle of freezing at −76 °C and thawing at 37 °C, the resulting lysates were diluted, and the amount of infectious virus was quantified on new cultures of HFF cells.

HSV-1 ELISA. An enzyme-linked immunosorbent assay (ELISA)[43] was employed to detect HSV-1. Briefly, 96-well cluster dishes were planted with 10 000 BSC-1 cells per well in 200 L per well of MEM(E) plus 10% calf serum. After overnight incubation at 37 °C, selected drug concentrations in triplicate and HSV-1 (KOS strain kindly provided by Dr. Sandra K. Weller, University of Connecticut) at a concentration of 100 pfu/well were added. Following a 3-day incubation at 37 °C, medium was removed, plates were blocked and rinsed and horseradish peroxidase conjugated rabbit anti-HSV-1 antibody was added. Following removal of the antibody containing solution, plates were rinsed and then developed by adding 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. Several different assays were used to explore the cytotoxicity of selected compounds using methods we have detailed previously. (i) Cytotoxicity produced in stationary HFF cells and in CEM-SS cells was determined by microscopic inspection of cells not affected by the virus used in the respective assays.[39] (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.[44] Briefly, 96-well cluster dishes were planted with KB cells at 5000 cells per well. After overnight incubation at 37 °C, test compound was added in triplicate at 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 were read at 570 nm in a spectrophotometer designed to read 96-well ELISA assay plates. (iii) The effect of compounds on the growth of CEM-SS cells was performed in six-well cluster dishes. Cells were planted at 100 000 cells per well and incubated for 18−24 h. Growth medium was removed and replaced with medium containing 0 to 10 μM in duplicate of one of compounds shown in Figure 1. Daily for 4 days, cells were harvested by standard techniques and enumerated in a Coulter Counter.

Data Analysis. Dose−response relationships for all the foregoing assays except HCMV yield assays were constructed by linearly regressing the percent inhibition of parameters derived in the preceding sections against log drug concentrations. Fifty-percent inhibitory (IC_{50}) concentrations were interpolated from the regression lines. For HCMV yield assays, log/log dose−response curves were constructed by linear regression and 90% inhibitory (IC_{90}) concentrations were interpolated from the regression lines. Samples containing positive controls [acyclovir, ganciclovir and zidovudine (AZT), respectively] for HSV-1, HCMV and HIV were used in all assays.

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Supporting Information Available: Elemental analyses data. This material is available free of charge via the Internet at http://pubs.acs.org.

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