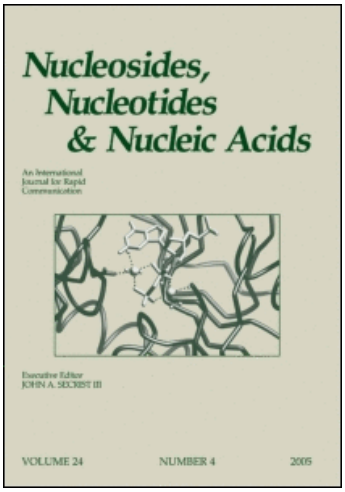

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Synthesis and Antiviral Activity of 2-Substituted Analogs of Triciribine[#]

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ABSTRACT

Triciribine (TCN) and triciribine monophosphate (TCN-P) have antiviral and antineoplastic activity at low or submicromolar concentrations. In an effort to improve and better understand this activity, we have conducted a structure-activity relationship study to explore the effect of substitutions at the 2-position of triciribine. 2-Methyl-(2-Me-TCN), 2-ethyl-(2-Et-TCN), 2-phenyl-(2-Ph-TCN), 2-chloro-(2-Cl-TCN), and 2-aminotriciribine(2-NH_2-TCN) were designed and synthesized to determine the effects of substitutions at the 2-position which change the steric, electronic, and hydrophobic properties of TCN, while maintaining the integrity of the tricyclic ring system. These compounds were evaluated for activity against human immunodeficiency virus (HIV-1), herpes simplex virus type 1 (HSV-1), and human cytomegalovirus (HCMV) and were found to be either less active than TCN and TCN-P or inactive at the highest concentrations

[#]In honor and celebration of the 70th birthday of Professor Leroy B. Townsend.

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tested, 100 μM. We conclude that substitutions at the 2-position of triciribine adversely affect the antiviral activity most likely because these analogs are not phosphorylated to active metabolites.

Key Words: Triciribine; Triciribine monophosphate; 2-Substituted analogs; HIV; HSV-1; HCMV; Pyrrolo[2,3-*d*]pyrimidine nucleosides.

INTRODUCTION

Triciribine (TCN) is a tricyclic nucleoside that was first synthesized by Schram and Townsend in 1971.[1] Initial testing of triciribine and the water soluble prodrug, triciribine-5′-monophosphate[2] (TCN-P) against murine leukemic L1210 cells revealed their potential as antineoplastic agents. This discovery led to extensive in vitro[3–17] and in vivo[18–22] studies of TCN and TCN-P as novel antineoplastic agents. Phase I clinical trials were completed with TCN-P[23–29] and TCN-P was advanced to phase II studies as a potential antineoplastic agent.[27,29–32]

Early studies revealed that TCN is converted intracellularly to TCN-P by adenosine kinase.[4,6,7] Phosphorylation is essential for antineoplastic activity as demonstrated by the absence of growth inhibition when adenosine kinase deficient cells were treated with TCN.[3,11,12] The activity of TCN-P also requires adenosine kinase because extracellular TCN-P is a charged species and does not cross the cell membrane and therefore must be dephosphorylated to TCN by extracellular phosphatases or cellular ecto-5′-nucleotidases and rephosphorylated to TCN-P by an intracellular adenosine kinase.[7,12] Unlike other nucleoside analogs, TCN is metabolized only to the monophosphate and not to the di- or triphosphate forms.[9] Furthermore, no incorporation of TCN into nucleic acids has been observed.[16] TCN-P inhibits both DNA and protein synthesis,[15,16] but the exact mechanism is unknown.

More recently, we have found that TCN and TCN-P are selective and potent inhibitors of HIV-1 and HIV-2 in acutely and persistently infected cells.[33] These studies also found no cross resistance to TCN or TCN-P in AZT- or TIBO-resistant HIV strains[33] suggesting that TCN and TCN-P have a different mode of action than AZT and TIBO. Furthermore, cytotoxicity such as that observed in murine L1210 cells appears to be highly cell line specific[3] and was not observed in human cell lines used to propagate HIV and human cytomegalovirus (HCMV).[33] Even though TCN was not very cytotoxic in these cell lines, it had to be phosphorylated to TCN-P to be active against HIV-1.[34] Studies on the antiviral mechanism of action of TCN are currently underway.

The earliest analogs of TCN that were synthesized and evaluated were the 7-azaTCN analogs,[35–39] which were found[37] to be virtually inactive due to their inability to be phosphorylated. This prompted us to initiate specific structure activity relationship studies to further investigate the structural requirements and mode of action of TCN and TCN-P. In a series of studies designed to specifically explore the structural requirements for the sugar moiety at the N-8 position of TCN for biological activity, our first study explored the requirements for rigidity of the ribosyl moiety. That study established that a disruption of the rigidity of the ribosyl moiety adversely affected phosphorylation and therefore biological activity.[40] Our second

study explored the hydroxyl requirements of the ribosyl moiety at the N-8 position of TCN and established that specific dehydroxylation of the ribosyl moiety also adversely affected phosphorylation and therefore biological activity of the deoxy TCN derivative.[41]

This manuscript describes the first study in our series of investigations designed to explore the tolerance and requirements for maintaining biological activity of TCN when substitutions occur directly on the tricyclic ring system of TCN. We have synthesized and evaluated new analogs with substitutions at the 2-position of TCN (e.g., 2-methyl-(2-Me-TCN), 2-ethyl-(2-Et-TCN), 2-phenyl-(2-Ph-TCN), 2-chloro-(2-Cl-TCN), and 2-aminotriciribine (2-NH_2-TCN)) (Fig. 1). These compounds were selected since they would change the steric, electronic, and hydrophobic properties of TCN, while maintaining the integrity of the tricyclic ring system. Based on this rationale, we synthesized these analogs for the following reasons: (i) a methyl or an ethyl group at the 2-position, as representative aliphatic groups, to increase steric bulk and hydrophobicity while having only a slight electron donating effect; (ii) a phenyl group at the 2-position as a representative aromatic group to increase steric bulk and hydrophobicity while having only a slight electron withdrawing effect; (iii) a chloro group at the 2-position as a representative electron-withdrawing

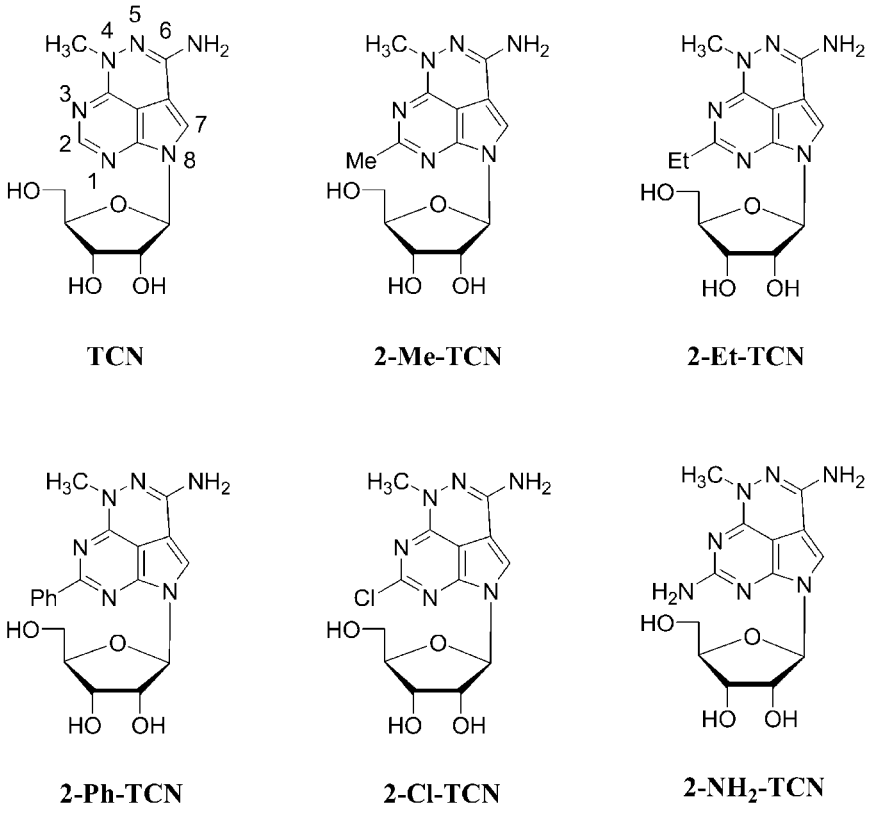

Figure 1. 2-Substituted analogs of triciribine (TCN).

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group to increase the electron withdrawing effect as well as steric bulk and hydrophobicity; and (iv) an amino group at the 2-position as a representative electron donating group to increase the electron donating effect as well as decrease hydrophobicity. The 2-amino group can also invoke hydrogen bonding if a hydrogen acceptor is available at the binding site of the target molecule. Biological analysis of these analogs has provided some important insights into the tolerance and requirements of these substitutions on the tricyclic ring system of TCN with regards to phosphorylation and in vitro activity against selected viruses.

RESULTS AND DISCUSSION

6-Amino-2,4-dimethyl-8-(β-**D**-ribofuranosyl)pyrrolo[4,3,2-*de*]pyrimido[4,5-*c*]pyridazine (2-methyltriciribine, 2-Me-TCN) was synthesized, as illustrated in Sch. 1, from 2-amino-5-bromo-3,4-dicyanopyrrole[42] (1) in 8.5% overall yield. Heating **1** with triethylorthoacetate in acetonitrile at reflux temperature for 1 h and sealing the isolated product in a steel reaction vessel with ethanolic ammonia at 105°C for 6 h afforded **2** in 78% yield. Silylation of compound **2** with 2 equivalents of *N,O*-bis (trimethylsilyl)acetamide (BSA) in dry acetonitrile under argon at room temperature and then glycosylation with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**TBAR**) and 3 equivalents of trimethylsilyl trifluoromethanesulfonate (TMSOTf) at 80°C for 3 h gave an 88% yield of compound **3**. Deprotection of **3** with methanolic ammonia and reprotection of the intermediate **4** with acetic anhydride in pyridine afforded the intermediate **5**, a less hydrophobic and more manageable intermediate than compound **3**. Debromination of **5** with 10% palladium on charcoal under 50 psi of hydrogen gas gave the intermediate **6** which was diazotized with sodium nitrite in aqueous acetic acid at 60°C over 4 h to give the intermediate **7**. Compound **7** was treated with phosphorous oxychloride ($POCl_3$) at reflux temperature for 0.5 h to obtain the intermediate **8**. The reaction of **8** with methylhydrazine in ethanol gave the intermediate **9** which was ring closed in ethanol, under acidic conditions, at reflux temperature to afford the intermediate **10**. Compound **10** was deprotected with methanolic ammonia to give 2-methyltriciribine (**2-Me-TCN**).

6-Amino-2-ethyl-4-methyl-8-(β-D-ribofuranosyl)pyrrolo[4,3,2-*de*]pyrimido[4,5-*c*]-pyridazine (2-ethyltriciribine, 2-Et-TCN) was synthesized, as illustrated in Sch. 2, from 2-amino-5-bromo-3,4-dicyanopyrrole (**1**) by a different synthetic route than the one used for **2-Me-TCN** in order to avoid the same deprotection and reprotection steps needed to convert compound **3** to compound **5**. After heating **1** with triethylorthopropionate in acetonitrile at reflux temperature for 1 h, the isolated product was placed in a steel reaction vessel with ethanolic ammonia. The vessel was sealed and the mixture heated at 105°C for 6 h to afford compound **11** in 94% yield. Crude 6-bromo-5-cyano-2-ethylpyrrolo[2,3-*d*]pyrimidin-4-one (**12**) was obtained in 90% yield by diazotizing compound **11** with sodium nitrite in aqueous acetic acid at 100°C for 13 h. Chlorination of compound **12** with phosphorous oxychloride at reflux temperature for 1 h gave an 81% yield of crude **13**. Silylation of compound **13** with 3 equivalents of BSA in dry acetonitrile under argon at reflux temperature was followed by treatment with 1 equivalent of **TBAR** and 0.5 equivalents of **TMSOTf** in dichloroethane (DCE), under argon, at 70°C for 18 h to afford

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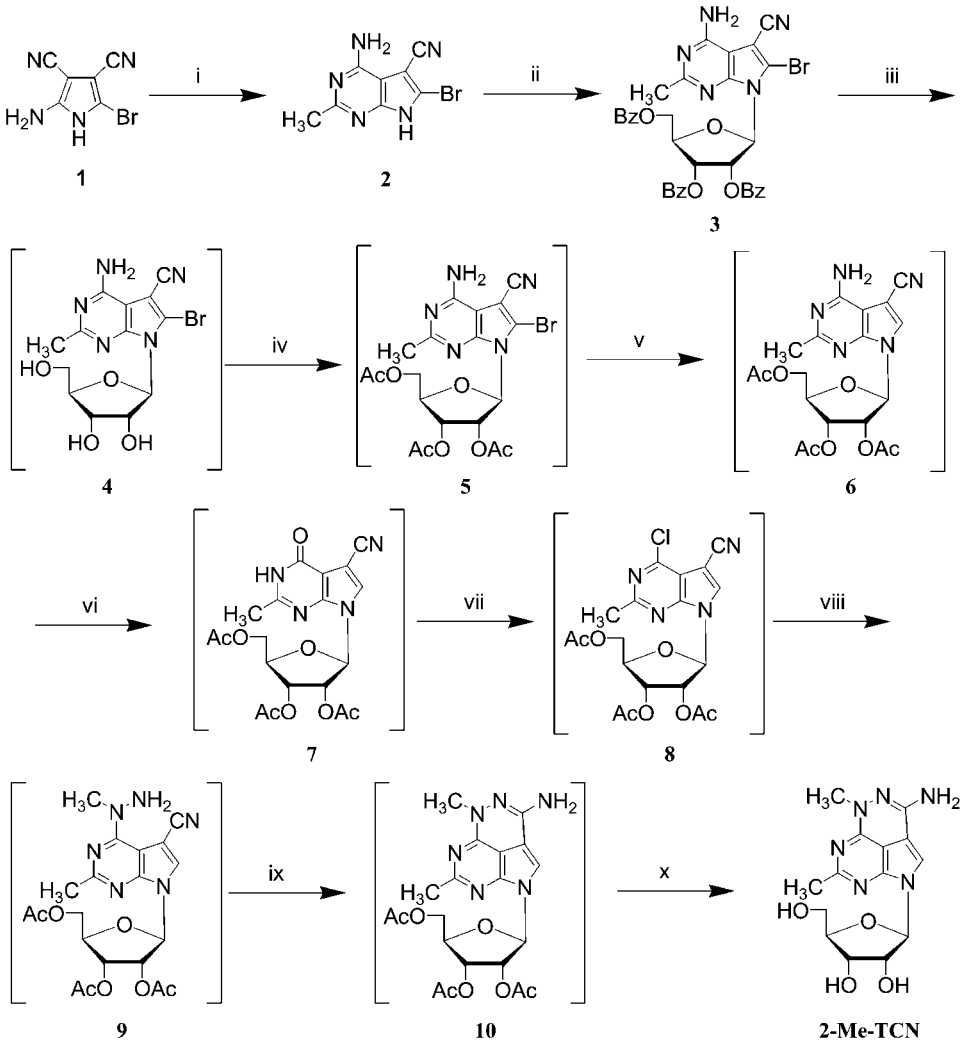
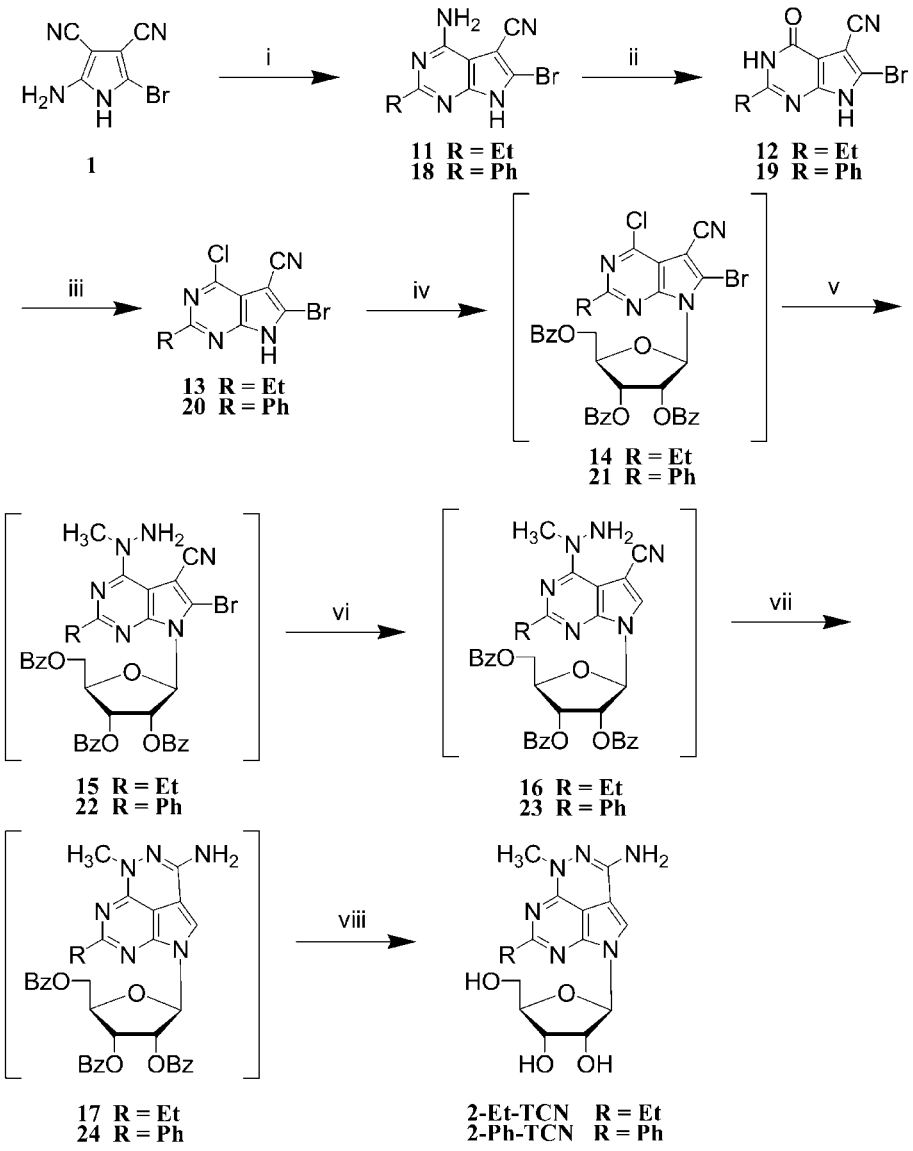


Scheme 1. Synthesis of 2-methyltriciribine (2-Me-TCN). Reagents: i) $(EtO)_3CCH_3$, CH_3CN, (NH_3-EtOH); ii) BSA, CH_3CN then TBAR, TMSOTf, DCE; iii) NH_3, MeOH; iv) Ac_2O, pyridine; v) 10% Pd-C, H_2, EtOH, EtOAc, 1N NH_4OH; vi) $NaNO_2$, H_2O, AcOH; vii) $POCl_3$; viii) NH_2NHCH_3, EtOH; ix) HCl, EtOH; x) NH_3, MeOH.

compound **14**. Treatment of compound **14** with methylhydrazine in ethanol at room temperature for 1 h afforded **15**. Debromination was accomplished by heating a mixture of compound **15**, ammonium formate, and 10% palladium on charcoal in ethanol at reflux temperature for 4 h to give compound **16**. Ring annulation of **16** was accomplished in ethanol at reflux temperature for 16 h, under acidic conditions, to give **17**. Deprotection of **17** with methanolic ammonia afforded 2-ethyltriciribine (**2-Et-TCN**) in a 6% overall yield from compound **1**.



Scheme 2. Synthesis of 2-ethyl (2-Et-TCN) and 2-phenyltriciribine (2-Ph-TCN). Reagents: i) $(EtO)_3CR$, CH_3CN then NH_3, EtOH; ii) $NaNO_2$, AcOH, H_2O; iii) $POCl_3$; iv) BSA, CH_3CN then TBAR, TMSOTf, DCE; v) NH_2NHCH_3, EtOH, $CHCl_3$; vi) HCO_2NH_4, 10% Pd-C, EtOH; vii) conc. HCl, EtOH, reflux; viii) NH_3, MeOH.

6-Amino-4-methyl-2-phenyl-8-(β-D-ribofuranosyl)pyrrolo[4,3,2-*de*]pyrimido[4,5-*c*]pyridazine (2-phenyltriciribine, **2-Ph-TCN**) (Sch. 2) was prepared in 23% overall yield from 2-amino-5-bromo-3,4-dicyanopyrrole (**1**) and triethylorthobenzoate by essentially the same procedure as described above for the preparation of **2-Et-TCN**.

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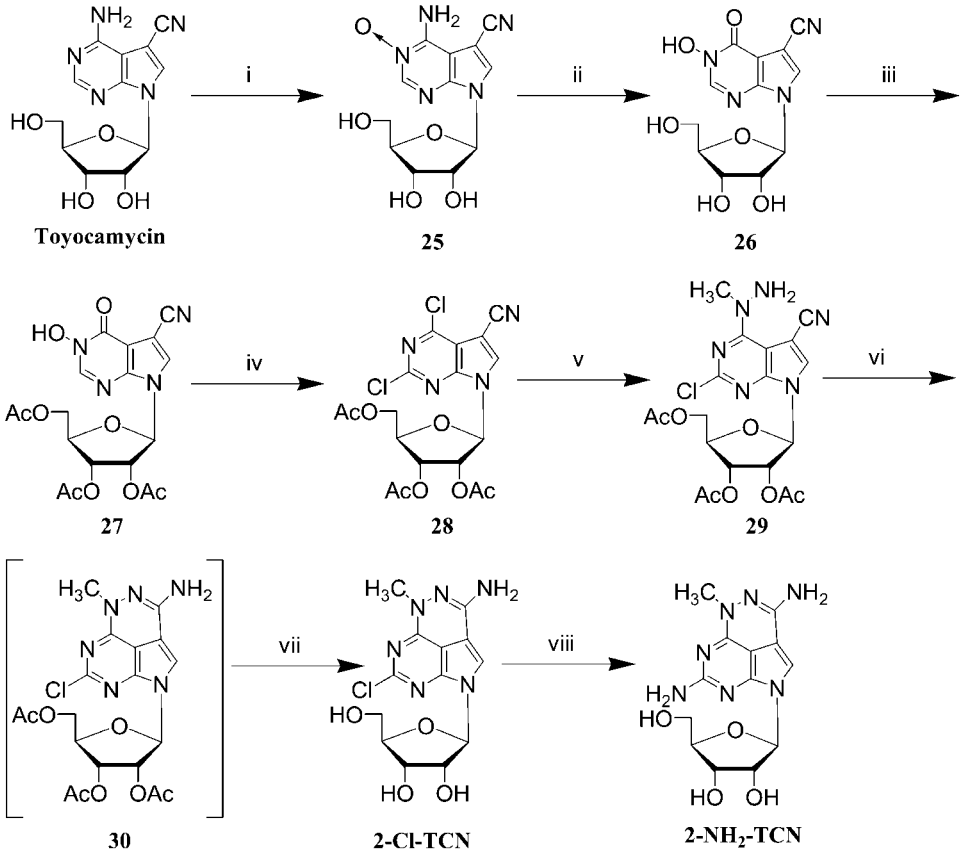
6-Amino-2-chloro-4-methyl-8-(β-D-ribofuranosyl)pyrrolo[4,3,2-*de*]pyrimido[4,5-*c*]-pyridazine (2-chlorotriciribine, **2-Cl-TCN**) and 2,6-diamino-4-methyl-8-(β-D-ribo-furanosyl)pyrrolo[4,3,2-*de*]pyrimido[4,5-*c*]pyridazine (2-aminotriciribine, **2-NH_2-TCN**) (Sch. 3) were synthesized from 5-cyano-2,4-dichloro-7-(2,3,5-tri-*O*-acetyl-β-D-ribo-furanosyl)pyrrolo[2,3-*d*]pyrimidine[43] (**28**). Compound **28** was synthesized from toyocamycin, as previously described,[43] by first forming the 3-*N*-oxide (**25**) with meta-chloroperbenzoic acid in acetic acid and then diazotizing the 4-amino group with sodium nitrite in *N,N*-dimethylformamide, acetic acid, and water to give compound **26**. Selective protection of the ribosyl hydroxyl groups (**27**) was accomplished with acetic anhydride in pyridine and chlorination of the 2- and 4-positions was achieved with phosphorous oxychloride at reflux temperature to give compound **28**. Treatment of compound **28** with methylhydrazine in ethanol at room temperature for 1 h afforded **29**. Ring annulation of **29** was accomplished in ethanol, under acidic conditions, at reflux temperature for 3 h to give compound **30** and subsequent deprotection with methanolic ammonia afforded 2-chlorotriciribine



Scheme 3. Synthesis of 2-chloro (2-Cl-TCN) and 2-aminotriciribine (2-NH_2-TCN). Reagents: i) mCPBA, HOAc; ii) $NaNO_2$, DMF, H_2O, AcOH; iii) Ac_2O, pyridine; iv) $POCl_3$; v) NH_2NHCH_3, EtOH; vi) conc. HCl, EtOH, reflux; vii) NH_3, MeOH; viii) NH_3(l), 105°C.

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(**2-Cl-TCN**) in 50% yield from compound **28**. Treatment of **2-Cl-TCN** with liquid ammonia sealed in a steel reaction vessel at 105°C for 7 days afforded 2-aminotriciribine (**2-NH_2-TCN**) in 77% yield.

These TCN analogs (2-Me-TCN, 2-Et-TCN, 2-Ph-TCN and 2-Cl-TCN) were evaluated in assays that use growth inhibition of human cells as indicators of cytotoxicity. 2-Me-TCN and 2-Et-TCN were not cytotoxic to KB cells, human foreskin fibroblasts (HFF) or CEM-SS cells (Table 1). Only 2-Cl- and 2-NH_2-TCN showed marginal cytotoxicity ($IC_{50} = 33$ and $60\,\mu M$, respectively) toward KB cells and only 2-Ph-TCN showed marginal cytotoxicity in both HFF cells ($IC_{50} = 21\,\mu M$) and CEM-SS cells ($IC_{50} = 15\,\mu M$).

The six target compounds also were evaluated for activity against three viruses. Activity against HIV-1 was measured in an assay that employs reverse transcriptase (RT) in culture supernatants as a marker for HIV-1. For 2-Me-TCN and 2-Et-TCN, it was found that after six days of incubation with CEM-SS cells acutely infected with HIV, the amount of RT activity in the culture supernatant was equal to that of control (Table 1) thereby establishing that these two analogs have no activity against HIV. Under the same conditions, 2-Ph-TCN and 2-Cl-TCN were active at an IC_{50} of $3\,\mu M$ and $25\,\mu M$, respectively, suggesting that these two analogs have activity against HIV, however, at a fraction of the activity of TCN ($IC_{50} = 0.04\,\mu M$) and TCN-P ($IC_{50} = 0.04\,\mu M$). 2-NH_2-TCN has yet to be tested.

The 2-substituted TCN analogs were also evaluated against HSV-1 in an ELISA and HCMV in a plaque assay. The replication of HSV-1 was equal to that of control for all the analogs tested, even at concentrations as high as $100\,\mu M$ (Table 1). Only

Table 1. Cytotoxicity and antiviral activity of 2-substituted TCN analogs.

	50% Inhibitory concentration (μM)					
	Cytotoxicity in human cells[a]			Antiviral activity[b]		
Compound	KB growth	HFF visual	CEM-SS visual	HIV-1 RT	HSV-1 ELISA	HCMV plaque
TCN	> 100	100	> 100	0.04	23	2.5
TCN-P	10	19	> 1.0	0.04	20	0.8
2-Me-TCN	> 100	> 100	> 100	> 100	> 100	> 100
2-Et-TCN	> 100	> 100	> 100	> 100	> 100	> 100
2-Ph-TCN	> 100	21	15	3	> 100	32
2-Cl-TCN	33	> 100	> 100	25	> 100	100
2-NH_2-TCN	60	> 100	NT[c]	NT[c]	> 100	> 100

[a]Inhibition of KB cell growth was measured in triplicate as described in the text. Visual cytotoxicity was scored on uninfected HFF and CEM-SS cells used in HCMV plaque and HIV RT assays.

[b]Antiviral activity was determined using an ELISA assay in quadruplicate for HSV-1, a plaque assay in duplicate for HCMV and the amount of reverse transcriptase (RT) activity in culture supernatants in triplicate for HIV-1 as described in the text.

[c]NT = Not tested.

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2-Ph-TCN was found to have marginal activity ($IC_{50} = 32\,\mu M$) against HCMV, which is less active than TCN ($IC_{50} = 2.5\,\mu M$) and TCN-P ($IC_{50} = 0.8\,\mu M$).

The phosphorylation of two analogs was determined and compared to that of TCN. After a 24-h incubation of uninfected CEM-SS cells with 100 μM 2-Me-TCN, a 5 μM intracellular concentration of a phosphorylated product was detected. After a similar incubation with 10 μM 2-Ph-TCN, no intracellular product was found. In contrast, incubation with 10 μM TCN for 5 or 12 h gave intracellular concentrations of 175–285 μM TCN-P.

We conclude that a substitution at the 2-position of TCN leads to a loss in antiviral activity. Limited data with two compounds suggest that the lack of activity is due to low phosphorylation of the analogs to an active metabolite.

EXPERIMENTAL

General Procedures. Reaction mixtures were evaporated at temperatures less than 60°C under reduced pressure (water aspirator) using a Buchi R-151 rotary evaporator. Melting points (uncorrected) were obtained on a Laboratory Devises Mel-Temp melting point apparatus. Thin layer chromatography used Analtech GHLF SiO_2 prescored plates. Developed TLC plates were visualized under ultraviolet light (254 nm). E. Merck silica gel (230–400 mesh) was used for gravity or flash column chromatography. Proton magnetic resonance (^1H NMR) spectra were obtained with a Brucker Avance DPX 300 or DRX 500 spectrometer (solutions in $CDCl_3$ or DMSO-d_6) with the chemical shifts reported in parts per million downfield from tetramethylsilane as the internal standard. UV spectra were obtained with a Kontron UVIKON 860 ultraviolet spectrometer. Elemental analysis were performed by the Analytical Laboratory, Department of Chemistry, University of Michigan, Ann Arbor, MI.

4-Amino-6-bromo-5-cyano-2-methylpyrrolo[2,3-*d*]pyrimidine (2). 2-Amino-5-bromo-3,4-dicyanopyrrole[42] (**1**) (8.44 g, 40 mmol) was suspended in dry acetonitrile (150 mL). Triethylorthoacetate (11 mL, 9.73 g, 60 mmol) was added and the mixture was heated at reflux temperature for 1 h. The dark brown solution was cooled to room temperature and filtered through filter paper. The acetonitrile was removed on a rotoevaporator to afford a brown solid. The brown solid was transferred to a steel reaction vessel, which was cooled to 0°C in ice water. Ethanolic ammonia (200 mL), saturated at 0°C, was added to the reaction vessel. The vessel was then sealed and heated at 105°C in an oil bath for 6 h. The vessel was cooled to 0°C, in ice water and the dark solution poured into a 500 mL erlenmeyer flask. The solution was treated with decolorizing carbon (5.0 g) and vacuum filtered through a pad of Celite. The Celite pad was washed with ethanol (100 mL) and the ethanol was removed from the solution using a rotoevaporator. The resulting solid was suspended in methanol (150 mL) and then dissolved with 28–30% ammonium hydroxide (40 mL). The solution was again treated with decolorizing carbon (5.0 g) and vacuum filtered through a pad of Celite. The filtrate was slowly treated with acetic acid (80 mL) to precipitate a white solid. This suspension was allowed to stand at −5°C for 8 h and the solid collected by filtration. The white solid was suspended

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in methanol (200 mL), with stirring, at reflux temperature for 1 h, cooled to 5°C, and filtered. The white solid was dried at 120°C in a vacuum oven at reduced pressure (water aspirator) for 4 h to yield 7.86 g (78%) of **2**. R_f 0.58 (9:1 chloroform: methanol), mp 340°C dec.; ^{1}H-NMR (DMSO-d_6) δ 13.73 (1H, bs, NH), 7.34 (2H, bs, NH_2), 2.43 (3H, s, CH_3); Anal. Calcd. For $C_8H_6BrN_5$: C, 38.10; H, 2.38; N, 27.78. Found: C, 38.23; H, 2.57; N, 27.44.

4-Amino-6-bromo-5-cyano-2-methyl-7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (3). *N*,*O*-Bis(trimethylsilyl)acetamide (BSA, 4.1 g, 2.9 mL, 20 mmol) was added to a stirred suspension of **2** (2.52 g, 10 mmol) in dry acetonitrile (100 mL) at room temperature under argon. After 15 min, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (5.0 g, 10 mmol) was added along with trimethylsilyl trifluoromethanesulfonate (TMSOTf, 6.7 g, 5.4 mL, 30 mmol). The suspension was stirred at room temperature for 10 min, during which time the suspension became a clear yellow solution. Under argon, the solution was heated at 80°C for 3 h and then cooled. The solution was diluted with ethyl acetate (100 mL) and then added to a saturated aqueous solution of sodium bicarbonate (100 mL) at room temperature. The aqueous layer was separated and discarded and the organic layer was washed with brine (100 mL) and dried over magnesium sulfate. After filtration, the solvent was removed and the residue was dissolved in chloroform and eluted from a silica gel column (7 cm (d) × 9 cm (h)) using a solvent system of 1:9 ethyl acetate:chloroform. Fractions of 100 mL were collected and the UV containing fractions (TLC, 1:4 ethyl acetate:chloroform) with an R_f value 0.31 were combined and evaporated to dryness. The residue was dissolved in dichloromethane (100 mL) and evaporated. The residue was dried in a vacuum oven at 65°C for 12 h to obtain 6.1 g (88% yield) of pure **3**. mp 196–198°C; $R_f = 0.31$ (1:4 ethyl acetate:chloroform); ^{1}H NMR ($CDCl_3$) δ 2.60 (s, 3H), 4.71 (1H, m), 4.84 (2H, m), 5.56 (2H, bs), 6.32 (1H, d), 6.61 (1H, m), 6.66 (1H, m), 7.39 (6H, m), 7.56 (3H, m), 7.97 (6H, m). Anal. Calcd for: $C_{34}H_{26}BrN_5O_7$: C, 58.62; H, 3.74; N, 10.06. Found: C, 58.52; H, 3.91; N, 9.96.

6-Amino-2,4-dimethyl-8-(β-D-ribofuranosyl)pyrrolo[4,3,2-*de*]pyrimido[4,5-*c*]pyridazine (2-Methyltriciribine, 2-Me-TCN). Saturated methanolic ammonia (300 mL), at 0°C, was added to a pressure bottle containing **3** (6.1 g, 8.76 mmol). The bottle was sealed and the reaction mixture was allowed to stir at room temperature for 18 h. The reaction vessel was cooled to 0°C, opened, and the solvent was evaporated to dryness. TLC showed the disappearance of starting material and the appearance of a new spot at $R_f = 0.37$ (9:1 chloroform:methanol). Without further purification, the residue, presumed to be compound **4** was dissolved in a mixture of acetic anhydride (4.48 g, 4.35 mL, 43.8 mmol) and pyridine (150 mL) and the mixture was stirred for 6 h, under argon. TLC showed the disappearance of starting material and the appearance of a new spot at $R_f = 0.87$ (9:1 chloroform:methanol). The reaction mixture was evaporated, under vacuum, at 60°C and the residue was coevaporated with toluene (3 × 50 mL). The residue, presumed to be compound **5**, was dissolved in a mixture of ethyl acetate (100 mL), ethanol (100 mL), and 1N ammonium hydroxide (9 mL). Palladium on charcoal (10%, 300 mg) was added and the mixture was placed

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under 50 psi of hydrogen gas and shaken on a Parr hydrogenator for 12 h at room temperature. TLC showed the disappearance of starting material and the appearance of a new spot at $R_f = 0.79$ (9:1 chloroform:methanol). The palladium on charcoal was removed by filtration through Celite and the solvent was removed under vacuum. Without further purification, water (200 mL) was added to the residue, presumed to be compound **6**, and the mixture was heated to 60°C before glacial acetic acid (100 mL) was added. Sodium nitrite (9.0 g, 130 mmol) was added, in six portions, over a period of 1 h. After 3 h, TLC showed the disappearance of starting material and the appearance of a new spot at $R_f = 0.60$ (9:1 chloroform:methanol). The solvent was removed and the light yellow solid was extracted with ethyl acetate (150 mL) and a saturated solution of sodium bicarbonate (90 mL). The aqueous layer was washed with ethyl acetate (2×50 mL) and then discarded. The ethyl acetate layers were combined and dried over magnesium sulfate. The magnesium sulfate was removed by filtration and the ethyl acetate was evaporated under vacuum to give 2.6 g (69% yield from **3**) of crude product, presumed to be compound **7**. Without further purification or characterization, **7** (2.6 g, 4.6 mmol) was dissolved in phosphorous oxychloride (50 mL) and heated at reflux temperature for 0.5 h. The cooled solution was poured over ice water and shaken vigorously until all the phosphorous oxychloride was destroyed, leaving a precipitate which was presumed to be compound **8** suspended in the water. TLC of the precipitate showed the disappearance of starting material and the appearance of a new spot at $R_f = 0.95$ (9:1 chloroform: methanol). The precipitate was collected by filtration and air dried for 2 h. Without further purification or characterization, methylhydrazine (0.276 g, 0.2 mL, 6 mmol) was added to a solution of **8** in a mixture of ethanol (80 mL) and chloroform (40 mL) and stirred at room temperature for 2 h. TLC showed the disappearance of starting material and the appearance of a new spot at $R_f = 0.80$ (9:1 chloroform: methanol). The solvent was removed under vacuum and the yellow residue, presumed to be compound **9**, was dissolved in a mixture of ethanol (60 mL) and 1 drop of conc. HCl. The solution was heated at reflux temperature for 3 h, at which time TLC showed the disappearance of starting material and the appearance of a new spot at $R_f = 0.66$ (9:1 chloroform:methanol). The solvent was removed under vacuum and the residue **10** was dissolved in methanol (20 mL) and transferred to a glass pressure bottle. Methanolic ammonia (180 mL), saturated at 0°C, was added and the pressure bottle was sealed. The sealed reaction vessel was warmed to room temperature and the mixture was stirred for 18 h. The solvent was removed under vacuum and the residue was adsorbed onto silica gel and applied to the top of a silica gel column (3 cm (d) × 12 cm (h)). The residue was eluted from the column using a mixture of chloroform:methanol (19:1) as the eluting solvent system. Fractions of 10 mL were collected and the UV containing fractions (TLC, 9:1 chloroform:methanol) with R_f values of 0.18 were combined and evaporated to dryness. The residue was dried at 60°C in a vacuum oven to obtain 0.36 g (18% yield from **7**) of 2-methyltriciribine (2-Me-TCN). $R_f = 0.18$ (9:1 chloroform:methanol); mp 211–212°C dec; ^{1}H NMR (DMSO-d_6) δ 6.98 (1H, s, H-7), 6.22 (2H, s, NH_2), 6.02 (1H, d, OH), 5.76 (1H, d, H-1'), 5.36 (1H, d, OH), 5.18 (1H, t, OH), 4.51 (1H, m), 4.10 (1H, m), 3.98 (1H, m), 3.63 (2H, m), 3.33 (3H, s, NCH_3), 2.35 (3H, s, CH_3). Anal. Calcd for: $C_{14}H_{18}N_6O_4 \cdot 0.50\ CH_3OH$: C, 49.71; H, 5.71; N, 24.00. Found: C, 49.76; H, 5.69; N, 23.80.

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4-Amino-6-bromo-5-cyano-2-ethylpyrrolo[2,3-*d*]pyrimidine (11). 2-Amino-5-bromo-3,4-dicyanopyrrole[42] (**1**) (8.44 g, 40 mmol) was suspended in dry acetonitrile (150 mL). Triethylorthopropionate (12 mL, 10.58 g, 60 mmol) was added and the mixture was heated at reflux temperature for 1 h. The dark brown solution was cooled to room temperature and filtered through filter paper. The acetonitrile was removed on a rotoevaporator to afford a brown solid. The brown solid was transferred to a steel reaction vessel which was cooled to 0°C, in ice water. Ethanolic ammonia (200 mL), saturated at 0°C, was added to the reaction vessel. The vessel was then sealed and heated at 105°C in an oil bath for 6 h. The vessel was cooled to 0°C, in ice water and the dark solution poured into a 500 mL erlenmeyer flask. The solution was treated with decolorizing carbon (5.0 g) and vacuum filtered through a pad of Celite. The Celite pad was washed with ethanol (100 mL) and the ethanol was removed from the solution using a rotoevaporator. The resulting solid was suspended in methanol (150 mL) and then dissolved with 28–30% ammonium hydroxide (40 mL). The solution was again treated with decolorizing carbon (5.0 g) and vacuum filtered through a pad of Celite. The filtrate was slowly treated with acetic acid (80 mL) to precipitate a white solid. This suspension was allowed to stand at −5°C for 8 h and the solid collected by filtration. The white solid was suspended in methanol (200 mL), with stirring, at reflux temperature for 1 h, cooled to 5°C, and filtered. The white solid was dried at 120°C in a vacuum oven at reduced pressure (water aspirator) for 4 h to yield 8.62 g (81%) of **11**. R_f 0.63 (9:1 chloroform:methanol), mp 327–329°C dec.; ^{1}H-NMR (DMSO-d_6) δ 13.73 (1H, bs, NH), 7.27 (2H, bs, NH_2), 2.67 (2H, q, CH_2), 1.24 (3H, t, CH_3); Anal. Calcd. For $C_9H_8BrN_5$: C, 40.60; H, 3.01; N, 26.32. Found: C, 40.83; H, 3.04; N, 26.04.

6-Amino-2-ethyl-4-methyl-8-(β-D-ribofuranosyl)pyrrolo[4,3,2-*de*]pyrimido[4,5-*c*]-pyridazine (2-Ethyltriciribine, 2-Et-TCN). Compound **11** (2.13 g, 8 mmol) was suspended in a mixture of water (100 mL) and glacial acetic acid (50 mL) and heated to 100°C. Aqueous sodium nitrite (6.9 g, 100 mmol in 100 mL water) was added dropwise to the suspension over a period of 1 h and then the mixture was allowed to stir at 100°C for another 12 h. The reaction mixture was cooled to room temperature and then stored at 5°C for 4 h. The precipitate was collected by vacuum filtration, resuspended in water, and heated at reflux temperature for 3 h. The reaction mixture was again cooled to room temperature and then stored at 5°C for 4 h. TLC of the precipitate showed the disappearance of starting material and the appearance of a new spot at $R_f = 0.44$ (9:1 chloroform:methanol). The precipitate, presumed to be compound **12**, was collected by vacuum filtration and dried at 80°C for 12 h in a vacuum oven (crude yield 1.92 g (90%)). Compound **12** (0.80 g, 3 mmol) was suspended in phosphorous oxychloride (50 mL) and heated at reflux temperature for 1 h. The reaction mixture was cooled to room temperature and then poured over ice water (300 mL). The mixture was vigorously stirred and the temperature maintained at 0°C until all of the phosphorous oxychloride was destroyed. The suspension was extracted with ethyl acetate (3 × 100 mL). The ethyl acetate extracts were combined and washed with a saturated solution of sodium bicarbonate (2 × 100 mL) and dried over magnesium sulfate. The solvent was removed and the yellow residue, compound **13** [crude yield 0.70 g (81%); ^{1}H-NMR (DMSO-d_6) δ

2.90 (q, CH_2), 1.23 (t, CH_3)], was dried at 70°C for 12 h in a vacuum oven and used without further purification. *N,O*-Bis(trimethylsilyl)acetamide (BSA, 1.34 g, 0.95 mL, 6.6 mmol) was added to a stirred suspension of **13** (0.63 g, 2.2 mmol) in dry acetonitrile (50 mL) and then heated at reflux temperature for 20 min, under argon. The reaction mixture was cooled to room temperature and the solvent was removed. The residue and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (1.12 g, 2.2 mmol) were dissolved in dry dichloroethane (DCE) (50 mL), under argon, and cooled to 0°C in an ice bath. Trimethylsilyl trifluoromethanesulfonate (TMSOTf, 0.25 g, 0.2 mL, 1.1 mmol) was added to the reaction mixture and the reaction mixture was stirred at room temperature for 1 h and at 70°C for 18 h. TLC of the reaction mixture showed the disappearance of starting material and the appearance of a new spot at $R_f = 0.78$ (1:1 ethyl acetate:hexane). The reaction mixture was cooled to room temperature, diluted with dichloromethane (60 mL), and poured over an ice-cold solution saturated with sodium bicarbonate (100 mL). The aqueous layer was separated and discarded and the organic layer was washed with water (50 mL × 2), brine (50 mL) and dried over magnesium sulfate. After filtration, the solvent was removed and the residue, presumed to be **14**, was suspended in ethanol (50 mL) and then treated with methylhydrazine (0.14 g, 3 mmol, 0.1 mL). The reaction mixture was allowed to stir for 1 h, at which time a TLC of the reaction mixture showed the disappearance of starting material and the appearance of a new spot at $R_f = 0.52$ (1:1 ethyl acetate:hexane). The solvent was removed under vacuum and the residue, presumed to be **15**, ammonium formate (2.0 g, 30 mmol) and 10% palladium on activated charcoal (140 mg) were heated in ethanol (100 mL) at reflux temperature for 4 h. TLC of the reaction mixture showed the disappearance of starting material and the appearance of a new spot at $R_f = 0.49$ (1:1 ethyl acetate:hexane). The hot reaction mixture was filtered through Celite and washed with hot ethanol (100 mL). The solvent was removed and the residue was dissolved in chloroform, applied to a silica gel column (5 cm (d) × 5 cm (h)) and eluted from the column using a solvent system of 1:1 ethyl acetate:hexane. Fractions of 100 mL were collected and the UV containing fractions (TLC, 1:1 ethyl acetate:hexane) with R_f values of 0.49 were combined and evaporated to dryness. The residue was dissolved in dichloromethane (100 mL) and evaporated. The residue, presumed to be **16**, was dissolved in a mixture of ethanol (100 mL) and 1 drop of conc. HCl. The solution was heated at reflux temperature for 16 h, at which time TLC showed the disappearance of starting material and the appearance of a new spot at $R_f = 0.37$ (1:1 ethyl acetate:hexane). The solvent was removed under vacuum and the residue, presumed to be **17**, was dissolved in methanol (20 mL) and transferred to a glass pressure bottle. Methanolic ammonia (180 mL), saturated at 0°C, was added to the pressure bottle and sealed. The sealed reaction vessel was warmed to room temperature and the mixture was stirred for 24 h. The solvent was removed under vacuum and the residue was adsorbed onto silica gel and applied to the top of a silica gel column (3 cm (d) × 12 cm (h)). The residue was eluted from the column using a mixture of chloroform:methanol (9:1) as the eluting solvent system. Fractions of 10 mL were collected and the UV containing fractions (TLC, 9:1 chloroform:methanol) with R_f values of 0.18 were combined and evaporated to dryness. The residue was heated at reflux temperature in dichloromethane (50 mL) for 2 h, cooled to room temperature, collected by vacuum filtration, and dried at 70°C in a vacuum oven to

obtain 0.07 g (10% yield from **13**) of 2-ethyltriciribine (2-Et-TCN). $R_f = 0.18$ (9:1 chloroform:methanol); mp 130–132°C dec; ^{1}H NMR (DMSO-d_6) δ 6.98 (1H, s, H-7), 6.21 (2H, s, NH_2), 5.93 (1H, d, OH), 5.75 (1H, d, H-1′), 5.37 (1H, d, OH), 5.19 (1H, t, OH), 4.52 (1H, q), 4.08 (1H, m), 3.97 (1H, m), 3.63 (1H, m), 3.55 (1H, m), 3.40 (3H, s, NCH_3), 2.60 (2H, q, CH_2), 1.21 (3H, t, CH_3). Anal. Calcd for: $C_{15}H_{20}N_6O_4 \cdot 1.0\ H_2O$: C, 49.18; H, 6.01; N, 22.95. Found: C, 49.26; H, 5.77; N, 22.57.

4-Amino-6-bromo-5-cyano-2-phenylpyrrolo[2,3-*d*]pyrimidine (18). 2-Amino-5-bromo-3,4-dicyanopyrrole[42] (**1**) (8.44 g, 40 mmol) was suspended in dry acetonitrile (150 mL). Triethylorthobenzoate (13.6 mL, 13.46 g, 60 mmol) was added and the mixture was heated at reflux temperature for 1 h. The dark brown solution was cooled to room temperature and filtered through filter paper. The acetonitrile was removed on a rotoevaporator to afford a brown solid. The brown solid was transferred to a steel reaction vessel which was cooled to 0°C, in ice water. Ethanolic ammonia (200 mL), saturated at 0°C, was added to the reaction vessel. The vessel was then sealed and heated at 105°C in an oil bath for 6 h. The vessel was cooled to 0°C, in ice water and the dark solution poured into a 500 mL erlenmeyer flask. The solution was treated with decolorizing carbon (5.0 g) and vacuum filtered through a pad of Celite. The Celite pad was washed with ethanol (100 mL) and the ethanol was removed from the solution using a rotoevaporator. The resulting solid was suspended in methanol (150 mL) and then dissolved with 28–30% ammonium hydroxide (40 mL). The solution was again treated with decolorizing carbon (5.0 g) and vacuum filtered through a pad of Celite. The filtrate was slowly treated with acetic acid (80 mL) to precipitate a white solid. This suspension was allowed to stand at −5°C for 8 h and the solid collected by filtration. The white solid was suspended in water (200 mL), with stirring, at reflux temperature for 1 h, cooled to 5°C, and filtered. The white solid was dried at 120°C in a vacuum oven at reduced pressure (water aspirator) for 4 h to yield 11.30 g (90%) of **18**. R_f 0.79 (9:1 chloroform: methanol), mp 284–285°C dec.; ^{1}H-NMR (DMSO-d_6) δ 13.70 (1H, bs, NH), 8.31 (2H, m,), 7.48 (3H, m), 6.92 (2H, bs, NH_2); Anal. Calcd. For $C_{13}H_8BrN_5$: C, 49.68; H, 2.55; N, 22.29. Found: C, 49.75; H, 2.77; N, 22.29.

6-Amino-4-methyl-2-phenyl-8-(β-D-ribofuranosyl)pyrrolo[4,3,2-*de*]pyrimido[4,5-*c*]-pyridazine (2-Phenyltriciribine, 2-Ph-TCN). Compound **18** (1.88 g, 6 mmol) was suspended in glacial acetic acid (150 mL) and heated at 60°C. Aqueous sodium nitrite (20.7 g, 300 mmol in 120 mL water) was added in 40 mL portions to the suspension over a period of 3 h and then the mixture was allowed to stir at 60°C for another 48 h. The reaction mixture was cooled to 0°C and the precipitate was collected by vacuum filtration and washed with water (3 × 50 mL). TLC of the precipitate showed the disappearance of starting material and the appearance of a new spot at $R_f = 0.26$ (19:1 chloroform:methanol). The precipitate, 6-bromo-5-cyano-2-phenylpyrrolo[2,3-*d*]pyrimidin-4-one (**19**) [crude yield 1.80 g (96%); ^{1}H-NMR (DMSO-d_6) δ 13.91 (1H, s, NH), 12.65 (1H, s, NH), 8.08 (2H, m), 7.56 (3H, m)], was collected by vacuum filtration and dried at 80°C for 18 h in a vacuum oven. Compound **19** (1.80 g, 6 mmol)

was suspended in phosphorous oxychloride (40 mL) and heated at reflux temperature for 1.5 h. The reaction mixture was cooled to room temperature and then poured over ice water (200 mL). The mixture was vigorously stirred and the temperature was maintained at 0°C until all of the phosphorous oxychloride was destroyed. The precipitate, 6-bromo-4-chloro-5-cyano-2-phenylpyrrolo[2,3-*d*]pyrimidine (**20**) [crude yield 2.05 g (100%); $R_f = 0.23$ (19:1 chloroform:methanol); ^{1}H-NMR (DMSO-d_6) δ 13.90 (1H, s, NH), 8.33 (1H, m), 8.07 (1H, m), 7.54 (3H, m)], was collected by vacuum filtration and dried at 80°C for 48 h in a vacuum oven. Without further purification, *N,O*-bis(trimethylsilyl)acetamide (BSA, 1.62 g, 1.15 mL, 8 mmol) was added to a stirred suspension of compound **20** (1.33 g, 4 mmol) in dry acetonitrile (100 mL) and heated at reflux temperature for 30 min, under argon. The reaction mixture was cooled to room temperature and the solvent was removed. The residue and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (2.04 g, 4 mmol) were dissolved in dry dichloroethane (DCE) (100 mL), under argon, and cooled to 0°C in an ice bath. Trimethylsilyl trifluoromethanesulfonate (TMSOTf, 0.63 g, 0.5 mL, 2.75 mmol) was added to the reaction mixture and the reaction mixture was stirred at room temperature for 20 min and at 80°C for 18 h. TLC of the reaction mixture showed the disappearance of starting material and the appearance of a new spot at $R_f = 0.27$ (1:3 ethyl acetate:hexane). The reaction mixture was cooled to room temperature, diluted with dichloromethane (100 mL), and poured over an ice-cold solution saturated with sodium bicarbonate (100 mL). The aqueous layer was separated and discarded and the organic layer was washed with water (2×50 mL), brine (50 mL) and dried over magnesium sulfate. After filtration, the solvent was removed and the residue, presumed to be compound **21** [crude yield = 2.6 g (83%)], was dissolved in a mixture of ethanol (100 mL) and chloroform (50 mL) and then treated with methylhydrazine (0.31 g, 6.6 mmol, 0.35 mL). The reaction mixture was allowed to stir for 2 h, at which time TLC of the reaction mixture showed the disappearance of starting material and the appearance of a new spot at $R_f = 0.60$ (1:1 ethyl acetate:hexane). The solvent was removed under vacuum and the residue, presumed to be **22**, ammonium formate (2.1 g, 33 mmol) and 10% palladium on activated charcoal (260 mg) were heated in ethanol (100 mL) at reflux temperature for 4 h. TLC of the reaction mixture showed the disappearance of starting material and the appearance of a new spot at $R_f = 0.55$ (1:1 ethyl acetate:hexane). The hot reaction mixture was filtered through Celite and washed with hot ethanol (100 mL). The solvent was removed and the residue was dissolved in chloroform, applied to a silica gel column (5 cm (d) × 5 cm (h)) and eluted from the column using a solvent system of 1:1 ethyl acetate:hexane. Fractions of 100 mL were collected and the UV containing fractions (TLC, 1:1 ethyl acetate:hexane) with R_f values of 0.55 were combined and evaporated to dryness. The residue was dissolved in dichloromethane (100 mL) and evaporated. The residue, presumed to be **23** [crude yield = 1.45 g (62% yield from **21**)], was dissolved in a mixture of ethanol (100 mL) and 1 drop of conc. HCl. The solution was heated at reflux temperature for 16 h, at which time TLC showed the disappearance of starting material and the appearance of a new spot at $R_f = 0.41$ (1:1 ethyl acetate:hexane). The solvent was removed under vacuum and the residue, presumed to be **24**, was dissolved in methanol (20 mL) and transferred to a glass pressure bottle. Methanolic ammonia (200 mL), saturated at 0°C, was added to the pressure bottle and sealed. The sealed reaction

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vessel was warmed to room temperature and the mixture was stirred for 18 h. The solvent was removed under vacuum and the residue was adsorbed onto silica gel and applied to the top of a silica gel column (3 cm (d) × 12 cm (h)). The residue was eluted from the column using a mixture of chloroform:methanol (9:1) as the eluting solvent system. Fractions of 10 mL were collected and the UV containing fractions (TLC, 9:1 chloroform:methanol) with R_f values of 0.15 were combined and evaporated to dryness. The residue was heated at reflux temperature in dichloromethane (50 mL) for 20 min, cooled to room temperature, collected by vacuum filtration, and dried at 70°C in a vacuum oven for 18 h to obtain 0.42 g (52% yield from **23**) of 2-phenyltriciribine (**2-Ph-TCN**). $R_f = 0.15$ (9:1 chloroform:methanol); mp 186°C dec; ^{1}H NMR (DMSO-d_6) δ 8.35 (2H, m), 7.44 (3H, m), 7.12 (1H, s, H-7′), 6.27 (2H, s, NH_2), 5.96 (1H, d, H-1′), 5.47 (1H, d, OH), 5.27 (1H, d, OH), 5.09 (1H, t, OH), 4.59 (1H, m), 4.17 (1H, m), 3.97 (1H, m), 3.63 (2H, m), 3.35 (3H, s, NCH_3). Anal. Calcd for: $C_{19}H_{20}N_6O_4$: C, 57.58; H, 5.05; N, 21.21. Found: C, 57.23; H, 5.20; N, 20.83.

6-Amino-2-chloro-4-methyl-8-(β-D-ribofuranosyl)pyrrolo[4,3,2-*de*]pyrimido[4,5-*c*]-pyridazine (2-Chlorotriciribine, 2-Cl-TCN). 5-Cyano-2,4-dichloro-7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine[43] (**28**) (1.2 g, 2.55 mmol), synthesized from toyocamycin as previously described (Sch. 3), was dissolved in ethanol (50 mL) and then treated with methylhydrazine (0.23 g, 5.1 mmol, 0.27 mL). The reaction mixture was allowed to stir for 1 h, at which time TLC of the reaction mixture showed the disappearance of starting material and the appearance of a new spot at $R_f = 0.82$ (9:1 chloroform:methanol). The solvent was removed under vacuum to obtain a yellow solid, presumed to be **29** [crude yield = 1.10 g, (90%)]. Intermediate **29** (0.55 g, 1.1 mmol) was dissolved in ethanol (100 mL) and 1 drop of conc. HCl. The solution was heated at reflux temperature for 2 h, at which time TLC showed the disappearance of starting material and the appearance of a new spot at $R_f = 0.67$ (9:1 chloroform:methanol). The solvent was removed under vacuum and the residue, presumed to be **30**, was dissolved in methanol (20 mL) and transferred to a glass pressure bottle. Methanolic ammonia (180 mL), saturated at 0°C, was added to the pressure bottle and sealed. The sealed reaction vessel was warmed to room temperature and the mixture was stirred for 24 h. The solvent was removed under vacuum and the residue was adsorbed onto silica gel and applied to the top of a silica gel column (3 cm (d) × 12 cm (h)). The residue was eluted from the column using a mixture of chloroform:methanol (9:1) as the eluting solvent system. Fractions of 10 mL were collected and the UV containing fractions (TLC, 9:1 chloroform:methanol) with R_f values of 0.14 were combined and evaporated to dryness. The residue was heated at reflux temperature in dichloromethane (50 mL) for 0.5 h, cooled to room temperature, collected by vacuum filtration, and dried at 100°C in a vacuum oven to obtain 0.22 g (55% yield from **29**) of 2-chlorotriciribine (2-Cl-TCN). $R_f = 0.14$ (9:1 chloroform:methanol); mp 205–207°C dec; ^{1}H NMR (DMSO-d_6) δ 7.13 (1H, s, H-7), 6.46 (2H, s, NH_2), 5.78 (1H, d, H-1′), 5.43 (1H, d, OH), 5.22 (1H, d, OH), 5.10 (1H, t, OH), 4.37 (1H, q), 4.05 (1H, m), 3.93 (1H, m), 3.60 (2H, m), 3.53 (3H, s, NCH_3). Anal. Calcd for: $C_{13}H_{15}ClN_6O_4 \cdot 0.2\ CH_2Cl_2$: C, 42.67; H, 4.14; N, 22.24. Found: C, 42.83; H, 4.36; N, 22.23.

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2,6-Diamino-4-methyl-8-(β-D-ribofuranosyl)pyrrolo[4,3,2-*de*]pyrimido[4,5-*c*]pyridazine (2-Aminotriciribine, 2-NH_2-TCN). 2-Chlorotriciribine (**2-Cl-TCN**) (0.25 g, 0.7 mmol) was added to a steel reaction vessel and cooled to −70°C in a bath of isopropanol and dry ice. Liquid ammonia (20 mL) was added and the reaction vessel was sealed and heated at 100°C for 7 days. The reaction vessel was cooled to −70°C in a bath of isopropanol and dry ice and then opened. The liquid ammonia was allowed to evaporate and the residue was adsorbed onto silica gel and applied to the top of a silica gel column (3 cm (d) × 9 cm (h)). The residue was eluted from the column using a mixture of chloroform:methanol (9:1) as the eluting solvent system. Fractions of 10 mL were collected and the UV containing fractions (TLC, 9:1 chloroform:methanol) with R_f values of 0.10 (9:1 chloroform:methanol) were combined and evaporated to dryness. The residue was heated at reflux temperature in dichloromethane (50 mL) for 2 h, cooled to room temperature, collected by vacuum filtration, and dried at 80°C in a vacuum oven to obtain 0.18 g (77% yield) of 2-aminotriciribine (**2-NH_2**-TCN). $R_f = 0.10$ (9:1 chloroform:methanol); mp 240°C dec; ^{1}H NMR (DMSO-d_6) δ 6.72 (1H, s, H-7), 6.27 (2H, s, NH_2), 5.92 (2H, s, NH_2), 5.75 (2H, m, H-1′ and OH), 5.36 (1H, d, OH), 5.11 (1H, d, OH), 4.38 (1H, m), 4.03 (1H, m), 3.95 (1H, m), 3.59 (2H, m), 3.50 (3H, s, NCH_3). Anal. Calcd for: $C_{13}H_{17}N_7O_4 \cdot 0.5\ H_2O$: C, 45.35; H, 5.23; N, 28.48. Found: C, 45.61; H, 5.13; N, 28.20.

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.[44] 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.

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.[33,45] To evaluate the activities of compounds in cells acutely infected with HIV, 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 (p.f.u.). 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 mL was then added to each well of a 96-well cluster dish which had been pre-treated 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 μM 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.[46]

HCMV Plaque Assay. The Towne strain, plaque-purified isolate P_o, of HCMV was kindly provided by Dr. Mark Stinski, University of Iowa. HFF cells in 24-well cluster dishes were infected with approximately 100 plaque forming units (p.f.u.) of HCMV per well using the procedures detailed earlier.[44] 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 8–10 days, cell sheets were fixed, stained with crystal violet and microscopic plaques 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.

HSV-1 ELISA. An enzyme-linked immunosorbent assay (ELISA)[47] was employed to detect HSV-1. Briefly, 96-well cluster dishes were planted with 10,000 BSC-1 cells per well in 200 μL per well of MEM(E) plus 10% calf serum. After overnight incubation at 37°C, selected drug concentrations in triplicate and HSV-1 (KOS strain kindly provided by Dr. Sandra K. Weller, University of Connecticut) at a concentration of 100 p.f.u./well were added. Following a 3-day incubation at 37°C, medium was removed, plates were blocked, rinsed, and horse radish peroxidase conjugated rabbit anti-HSV-1 antibody was added. Following removal of the antibody containing solution, plates were rinsed, and then developed by adding a solution of tetramethylbenzidine as substrate. The reaction was terminated 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.[44] (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.[48] 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 read at 570 nm in a spectrophotometer designed to read 96-well ELISA assay plates.

Data Analysis. Dose-response relationships 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 calculated 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.

Extraction Procedures for Chromatographic Analysis. The extraction procedures used to obtain the aqueous phase of supernatant were previously described.[40] Briefly, CEM-SS cells, grown in suspension with 100 μM concentrations of compounds for 24 h, were harvested by centrifugation at room temp. After harvest,

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the resulting cell pellet was resuspended in cold PBS, again centrifuged, and the resulting cell pellet resuspended by vortexing in 0.4 mL of 0.6 N TCA (4°C) and kept on ice for 15 min. This suspension was microfuged for 30 s at 12,000 g (4°C) and the supernatant carefully removed to an Eppendorf tube. Volume was measured and an equal amount of ice cold Freon containing 0.5 M TOA (trioctylamine) was added to the tube. The mixture was vortexed for 5 or more sec then microfuged for 30 s at 12,0000 g (4°C). The lower phase was removed by aspiration and discarded. The aqueous phase was frozen at −76°C until HPLC analysis.

Reverse Phase HPLC Analysis. HPLC analysis used was previously described.[49] Briefly, HPLC was performed on a Spectra-physics chromatography system consisting of a model SP8800 ternary pump, a SP8500 dynamic mixer, a SP8780 autosampler and a SP8490 variable wavelength detector. Peaks were integrated on a model SP4270 integrator System and data management was achieved using a Compaq model 386 computer with WINner 386 software (Spectra-physics). Ion pair reverse-phase chromatography was performed on a 3.9 × 300 mm μ Bondapak C18 column (Waters). The solvent was 5 mM tetrabutylammonium hydroxide (TBA), 5% methanol pH adjusted to 2.5 with formic acid. Separation of nucleoside monphosphates was carried out at a flow rate of 1 mL/min with dual wavelength detection set at 254 and 290 nm.

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REFERENCES

1. Schram, K.H.; Townsend, L.B. The synthesis of 6-amino-4-methyl-8-(β-D-ribofuranosyl)(4-H, 8-H)pyrrolo[4,3,2-de]pyrimido[4,5-c]pyridazine, a new tricyclic nucleoside. Tetrahedron Lett. **1971**, *49*, 4757–4760.
2. Townsend, L.B.; Lewis, A.F.; Roti Roti, L.W. Synthesis of 6-Amino-4-methyl-8-(β-D-ribofuranosyl)pyrrolo[4,3,2-de]pyrimido[4,5-c]pyridazine-5′-phosphate as a Novel Compound and Its Utility Against L-1210 Mouse Leukemia. US Patent 4, 123, 524, October 31, 1978.
3. Plagemann, P.G. Transport, phosphorylation, and toxicity of a tricyclic nucleoside in cultured Novikoff rat hepatoma cells and other cell lines and release of its monophosphate by the cells. J. Natl. Cancer Inst. **1976**, *57*, 1283–1295.
4. Roti Roti, L.W.; Roti Roti, J.L.; Townsend, L.B. Studies on the mechanism of cytotoxicity of tricyclic nucleoside NSC#154020 in L1210 cells. Proc. Am. Assoc. Cancer Res. **1978**, *19*, 40.

5. Bennett, L.L., Jr.; Smithers, D.; Hill, D.L.; Rose, L.M.; Alexander, J.A. Biochemical properties of the nucleoside of 3-amino-1,5-dihydro-5-methyl-1,4,5,6,8-pentaazaacenaphthylene (NSC-154020). Biochem. Pharmacol. **1978**, *27*, 233–241.
6. Schweinsberg, P.D.; Smith, R.G.; Loo, T.L. Identification of the metabolites of an antitumor tricyclic nucleoside (NSC-154020). Biochem. Pharmacol. **1981**, *30*, 2521–2526.
7. Wotring, L.L.; Townsend, L.B.; Crabtree, G.W.; Parks, R.E., Jr. A possible role for ecto-5′-nucleotidase in cytotoxicity and intracellular nucleotide formation from the tricyclic nucleoside-5′-phosphate (TCN-P). Proc. Am. Assoc. Cancer Res. **1981**, *22*, 257.
8. Bennett, L.L., Jr.; Allan, P.W.; Chang, C.H. Phosphorylation of ''tricyclic nucleoside'' by adenosine kinases from L1210 cells and HEp-2 cells. Biochem. Pharmacol. **1983**, *32*, 2601–2602.
9. Wotring, L.L.; Passiatore, J.E.; Roti Roti, J.L.; Hudson, J.L.; Townsend, L.B. Effects of the tricyclic nucleoside 6-amino-4-methyl-8-(β-D- ribofuranosyl)pyrrolo[4,3,2-de]pyrimido[4,5-c]pyridazine on the viability and cell cycle distribution of L1210 cells in vitro. Cancer Res. **1985**, *45*, 6355–6361.
10. Moore, E.C.; Hurlbert, J.M.; Ravel, J.M.; Massia, S. Mechanisms of inhibition of protein and purine biosynthesis by tricyclic nucleoside phosphate (TCN-P, triciribine phosphate, NSC 280594). Proc. Am. Assoc. Cancer Res. **1986**, *27*, 303.
11. Behrens, B.C.; Hamilton, T.C.; Louie, K.G.; Grotzinger, K.R.; McKoy, W.M.; Tsuruo, T.; Young, R.C.; Ozols, R.F. Activity of tricyclic nucleoside 5′-phosphate in model systems of human ovarian cancer. Invest. New Drugs **1986**, *4*, 295–304.
12. Wotring, L.L.; Crabtree, G.W.; Edwards, N.L.; Parks, R.E., Jr.; Townsend, L.B. Mechanism of activation of triciribine phosphate (TCN-P) as a prodrug form of TCN. Cancer Treat Rep. **1986**, *70*, 491–497.
13. Wotring, L.L.; Roti Roti, J.L.; Hudson, J.L.; Passiatore, J.E.; Borysko, K.Z.; Newcomb, R.D.; Townsend, L.B. Triciribine (TCN), a novel tricyclic adenosine analog with anticancer activity. Nucleosides Nucleotides **1987**, *6*, 95–109.
14. Wotring, L.L.; Townsend, L.B.; Mandell, K.L.; Borysko, K.Z. Metabolism of triciribine in L1210 cells. Proc. Am. Assoc. Cancer Res. **1989**, *30*, 590.
15. Moore, E.C.; Hurlbert, R.B.; Boss, G.R.; Massia, S.P. Inhibition of two enzymes in de novo purine nucleotide synthesis by triciribine phosphate (TCN-P). Biochem. Pharmacol. **1989**, *38*, 4045–4051.
16. Wotring, L.L.; Townsend, L.B.; Jones, L.M.; Borysko, K.Z.; Gildersleeve, D.L.; Parker, W.B. Dual mechanisms of inhibition of DNA synthesis by triciribine. Cancer Res. **1990**, *50*, 4891–4899.
17. Moore, E.C.; Hurlbert, R.B.; Massia, S.P. Inhibition of CCRF-CEM human leukemic lymphoblasts by triciribine (tricyclic nucleoside, TCN, NSC-154020). Accumulation of drug in cells and comparison of effects on viability, protein synthesis and purine synthesis. Biochem. Pharmacol. **1989**, *38*, 4037–4044.
18. Basseches, P.J.; Durski, A.; Powis, G. High-performance liquid chromatographic assay of the antineoplastic agent tricyclic nucleoside 5′-phosphate and its disposition in rabbit. J. Chromatogr. **1982**, *233*, 227–234.

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19. Basseches, P.J.; Powis, G. Metabolism and disposition of 3-amino-1,5-dihydro-5-methyl-1-β-D-ribofuranosyl-1,4,5,6,8-pentaazaacenaphthylene in the rat. Cancer Res. **1984**, *44*, 3672–3678.
20. Liu, C.; Kimler, B.F.; Evans, R.G.; Morantz, R.A. Intracerebral chemotherapy in the 9L rat brain tumor model. Proc. Ann. Meet. Am. Assoc. Cancer Res. **1992**, *33*, A2654.
21. Schilcher, R.B.; Young, J.D.; Baker, L.H. Reversed-phase high-performance liquid chromatographic determination of tricyclic nucleoside and tricyclic nucleoside 5′-phosphate in biological specimens. J. Chromatogr. **1985**, *337*, 55–62.
22. Townsend, L.B.; Milne, G.H. Synthesis, chemical reactivity, and chemotherapeutic activity of certain selenonucleosides and nucleosides related to the pyrrolo[2,3-*d*]-pyrimidine nucleoside antibiotics. Ann. N.Y. Acad. Sci. **1975**, *255*, 91–103.
23. Mittelman, A.; Casper, E.S.; Godwin, T.A.; Cassidy, C.; Young, C.W. Phase I study of tricyclic nucleoside phosphate. Cancer Treat Rep. **1983**, *67*, 159–162.
24. Cobb, W.R.; Bogden, A.E.; Reich, S.D.; Griffin, T.W.; Kelton, D.E.; LePage, D.J. Activity of two phase I drugs, homoharringtonine and tricyclic nucleotide, against surgical explants of human tumors in the 6-day subrenal capsule assay. Cancer Treat. Rep. **1983**, *67*, 173–178.
25. Crabtree, G.W.; Spremulli, E.N.; Weiman, M.C.; Smith, D.E.; Salvatore, J.R.; Cummings, F.J.; Calabresi, P. Phase I clinical and pharmacokinetic study of tricyclic nucleoside 5′-monophosphate (TCN-P; NSC 280594). Proc. Am. Soc. Clin. Oncol. **1983**, *2*, 33.
26. Feun, L.G.; Savaraj, N.; Bodey, G.P.; Lu, K.; Yap, B.S.; Ajani, J.A.; Burgess, M.A.; Benjamin, R.S.; McKelvey, E.; Krakoff, I. Phase I study of tricyclic nucleoside phosphate using a five-day continuous infusion schedule. Cancer Res. **1984**, *44*, 3608–3612.
27. Powis, G.; Basseches, P.J.; Kroschel, D.M.; Richardson, R.L.; O'Connell, M.J.; Kvols, L.K. Disposition of tricyclic nucleoside-5′-monophosphate in blood and plasma of patients during phase I and II clinical trials. Cancer Treat Rep. **1986**, *70*, 359–362.
28. Schilcher, R.B.; Haas, C.D.; Samson, M.K.; Young, J.D.; Baker, L.H. Phase I evaluation and clinical pharmacology of tricyclic nucleoside 5′-phosphate using a weekly intravenous regimen. Cancer Res. **1986**, *46*, 3147–3151.
29. Hoffman, K.; Holmes, F.A.; Fraschini, G.; Esparza, L.; Frye, D.; Raber, M.N.; Newman, R.A.; Hortobagyi, G.N. Phase I-II study: Triciribine (tricyclic nucleoside phosphate) for metastatic breast cancer. Cancer Chemother. Pharmacol. **1996**, *37*, 254–258.
30. O'Connell, M.J.; Rubin, J.; Hahn, R.G.; Kvols, L.K.; Moertel, C.G. Phase II clinical trial of tricyclic nucleoside phosphate for advanced colorectal cancer. Cancer Treat Rep. **1987**, *71*, 333–334.
31. Lyss, A.P.; Morrell, L.E.; Perry, M.C. Triciribine phosphate (TCN-P) in advanced non-small cell lung cancer (NSCLC): Interim analysis of toxicity. Proc. Am. Soc. Clin. Oncol. **1991**, *10*, 120.
32. Feun, L.G.; Blessing, J.A.; Barrett, R.J.; Hanjani, P. A phase II trial of tricyclic nucleoside phosphate in patients with advanced squamous cell carcinoma of

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the cervix. A gynecologic oncology group studyAm. J. Clin. Oncol. **1993**, *16*, 506–508.
33. Kucera, L.S.; Iyer, N.P.; Puckett, S.H.; Buckheit, R.W., Jr.; Westbrook, L.; Toyer, B.R.; White, E.L.; Germany-Decker, J.M.; Shannon, W.M.; Chen, R.C.; Nassiri, M.R.; Shipman, C., Jr.; Townsend, L.B.; Drach, J.C. Activity of triciribine and triciribine-5′-monophosphate against human immunodeficiency virus types 1 and 2. AIDS Res. Hum. Retroviruses **1993**, *9*, 307–314.
34. Ptak, R.G.; Borysko, K.Z.; Porcari, A.R.; Buthod, J.L.; Holland, L.E.; Shipman, C., Jr.; Townsend, L.B.; Drach, J.C. Phosphorylation of triciribine is necessary for activity against HIV-1. AIDS Res. Human Retroviruses **1998**, *14*, 1315–1322.
35. Townsend, L.B.; Kawasaki, A.M.; VanSickle, A.P.; Wotring, L.L. The synthesis and biological activity of certain pentaazaacenaphthylenes, hexaazaacenaphthylenes and their corresponding nucleosides. Nucleic Acids Symp. Ser. **1986**, *17*, 41–44.
36. Kawasaki, A.M.; Townsend, L.B. An unusual reductive ring-opening of the 1,2,3,5,6,7-hexaazaacenaphthylene ring system. Tetrahedron Lett. **1989**, *30*, 3287–3290.
37. VanSickle, A.; Kawasaki, A.M.; Townsend, L.B. The Synthesis of 8-amino-6-N-methyl-1,2,3,5,6,7-hexaazaacenaphthylene. Heterocycles **1990**, *30*, 963–969.
38. Kawasaki, A.M.; Wotring, L.L.; Townsend, L.B. Synthesis and cytotoxicity studies of 8-amino-6-methyl-2-β-D-ribofuranosyl-1,2,3,5,6,7-hexaazaacenaphthylene (7-aza-TCN) and the corresponding 2′-deoxy- and arabinonucleoside analogues. J. Med. Chem. **1990**, *33*, 3170–3176.
39. Kawasaki, A.M.; Townsend, L.B. Synthesis of 8-amino-4-methylthio-6-methyl-2-(β-D-ribofuranosyl)-2,6-dihydro-1,2,3,5,6,7-hexaazaacenaphthylene and an unusual reductive ring-opening of the 1,2,3,5,6,7-hexaazaacenaphthylene ring system. J. Heterocycl. Chem. **1991**, *28*, 1–5.
40. Porcari, A.R.; Borysko, K.Z.; Ptak, R.G.; Breitenbach, J.M.; Wotring, L.L.; Drach, J.C.; Townsend, L.B. Acyclic sugar analogs of triciribine: Correlation of antiviral and antineoplastic activity with intracellular phosphorylation. Nucleosides & Nucleotides **1999**, *18*, 2475–2497.
41. Porcari, A.R.; Borysko, K.Z.; Ptak, R.G.; Breitenbach, J.M.; Vittori, S.; Wotring, L.L.; Drach, J.C.; Townsend, L.B. Deoxy sugar analogs of triciribine: Correlation of antiviral and antineoplastic activity with intracellular phosphorylation. J. Med. Chem. **2000**, *43*, 2438–2448.
42. Swayze, E.E.; Hinkley, J.M.; Townsend, L.B. The improved preparation of a versatile synthon for the synthesis of pyrrolo[2,3-*d*]pyrimidines. In *Nucleic Acid Chemistry*; Townsend, L.B., Tipson, R.S., Eds.; Wiley-Interscience: New York, 1991; Vol. 4, 16–17.
43. Cheng, C.S.; Hinshaw, B.C.; Panzica, R.P.; Townsend, L.B. Synthesis of 2-amino-5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidin-4-one. An important precursor for the synthesis of nucleoside Q and Q_0. J. Am. Chem. Soc. **1976**, *98*, 7870–7872.
44. Turk, S.R.; Shipman, C., Jr.; Nassiri, M.R.; Genzlinger, G.; Krawczyk, S.H.; Townsend, L.B.; Drach, J.C. Pyrrolo[2,3-*d*]pyrimidine nucleosides as inhibitors of human cytomegalovirus. Antimicrob. Agents Chemother. **1987**, *31*, 544–550.

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45. Kucera, L.S.; Iyer, N.; Leake, E.; Raben, A.; Modest, E.J.; Daniel, L.W.; Piantadosi, C. Novel membrane-interactive ether lipid analogs that inhibit infectious HIV-1 production and induce defective virus formation. AIDS Res. Hum. Retroviruses **1990**, *6*, 491–501.
46. White, E.L.; Buckheit, R.W.; Ross, L.J.; Germany, J.M.; Andries, K.; Pauwels, R.; Janssen, P.A.J.; Shannon, W.M.; Chirigos, M.A. A TIBO derivative, R82913, is a potent inhibitor of HIV-1 reverse transcriptase with heteropolymer templates. Antiviral Res. **1991**, *16*, 257–266.
47. Prichard, M.N.; Shipman, C., Jr. A three-dimensional model to analyze drug-drug interactions. Antiviral Res. **1990**, *14*, 181–205.
48. Prichard, M.N.; Prichard, L.E.; Baguley, W.A.; Nassiri, M.R.; Shipman, C., Jr. Three-dimensional analysis of the synergistic cytotoxicity of ganciclovir and zidovudine. Antimicrob. Agents Chemother. **1991**, *35*, 1060–1065.
49. Walseth, T.F.; Graff, G.; Moos, M.C., Jr.; Goldberg, N.D. Separation of 5′-ribonucleoside monophosphates by ion-pair reverse-phase high-performance liquid chromatography. Analytical Biochemistry **1980**, *107* (1), 240–245.

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