Synthesis and Cytokine Modulation Properties of Pyrrolo[2,3-*d*]-4-pyrimidone Nucleosides

Guangyi Wang,*,† Robert C. Tam,‡ Esmir Gunic,† Jinfa Du,† Josie Bard,‡ and Bharati Pai‡

Chemistry and Immunology Laboratories, ICN Pharmaceuticals, Inc., Costa Mesa, California, 92626

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A series of pyrrolo[2,3-*d*]pyrimidone nucleosides were synthesized and evaluated for their ability to enhance Type 2 cytokines and to suppress Type 1 cytokines in human T cells activated in vitro. Compounds **16b**, **16c**, **16d**, **18c**, and **19b** induced substantial enhancement of IL-4 (a Type 2 cytokine) levels while three compounds (**16b**, **16c**, and **16f**) showed significant suppression of IFNγ (a Type 1 cytokine) levels. The results revealed a strict structural requirement for the nucleoside-mediated enhancement of IL-4. Modifications of the ribofuranose moiety of the nucleosides either abolished or dramatically reduced the activity. Both the 5′-hydroxy and 5-carboxamidine are crucial for the activity. Of the few nucleoside analogues that demonstrated enhancement on Type 2 cytokine production, 7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone-5-carboxamidine (**16c**) showed a dramatic enhancement (>200%) of IL-4 levels and a significant enhancement (36%) of IL-5 levels. Moreover, this compound showed substantial suppression of the Type 1 cytokines, IFNγ (42%), IL-2 (54%), and TNFα (55%). Similarly, compound **16b** showed a substantial enhancement of IL-4 (46%) and suppression of IL-2 (35%), IFNγ (30%), and TNFα (26%). To our knowledge, these are the first nucleoside analogues that induce a Type 2 cytokine bias in T cells. The cytokine modulation property of **16c** and **16b** merits the therapeutic evaluation of these compounds in treating diseases in which immunopathology is associated with polarized Type 1 cytokine responses.

Introduction

Small molecule compounds have displayed promising activity as nonspecific modulators of immune responses.[1] Certain purine analogues and purine nucleoside analogues have also shown immunostimulatory properties such as 7-allyl-8-oxoguanosine, which can enhance murine NK activity,[2,3] and 6-substituted purine amino acids, which stimulate cytotoxic T lymphocytes.[4] Acyclonucleoside phosphonates PMEG, (*R*)-PMPA, and (*S*)-PMPA were shown to stimulate the secretion of tumor necrosis factor-α (TNFα) and interleukin 10 (IL-10) and to enhance interferon-γ (IFNγ)-induced nitric oxide.[5] Recently, N^1-(β-D-ribofuranosyl)triazole-4-carboxamide (ribavirin) demonstrated a stimulatory effect on the secretion of Type 1 cytokines (IL-2, TNFα, IFNγ) and a suppressive effect on the secretion of Type 2 cytokines (IL-4, IL-5, IL-10).[6,7] As Type 1 cytokines contribute to active host defense against intracellular pathogens, such as viruses, and Type 2 cytokines can antagonize Type 1 responses,[8] the induction of a Type 1 cytokine bias by ribavirin would be favorable for eliminating viruses through the enhancement of antiviral immunity. As both these acyclonucleoside phosphonates and ribavirin have broad spectrum antiviral activities, these data support the view that the antiviral activity of these agents could be partly attributed to their immunomodulatory effects.

Although Type 1 cytokines provide benefit in host defense against pathogens, strongly polarized Type 1 cytokine responses can promote different immunopathological reactions including experimental autoimmune uveoretinitis, experimental autoimmune encephalitis, insulin dependent diabetes mellitus, contact dermatitis, and some chronic inflammatory disorders, such as rheumatoid arthritis and Crohn's disease.[9] Therefore, an immunomodulatory agent which biases endogenous cytokine responses toward a Type 2 profile could antagonize the polarized Type 1 cytokine responses and would have significant therapeutic potential in treating these autoimmune diseases.

There are currently many immunomodulatory strategies to induce a Type 2 cytokine bias which have shown efficacy in clinical evaluation. These include monoclonal antibodies (mAb) to Type 1 cytokines, recombinant Type 2 cytokines, and soluble Type 1 cytokine receptors.[10−15] However, certain problems limit their clinical use. Although preliminary results are encouraging, in most cases chronic treatment is necessary due to relapse. However, continued treatment with mAbs can lead to development of antibodies against the monoclonal antibodies or anti-ds DNA autoantibodies similar to those observed in systemic lupus erythematosus,[16] thereby limiting their usefulness. "Humanized" monoclonal antibodies have been developed which apparently reduce the risk of an induced immune response to these mAbs. However, these are still under development, and in addition these new mAbs, along with cytokines, remain large proteins and therefore may have difficulty reaching their target sites. Soluble receptors for TNFα

* Corresponding author: Guangyi Wang, Chemistry Laboratory, ICN Pharmaceuticals, 3300 Hyland Avenue, Costa Mesa, CA 92626. Telephone: (714) 545-0100, ext. 4165. Fax: (714) 668-3141. E-mail: gwang@icnpharm.com.
† Chemistry Laboratory.
‡ Immunology Laboratory.

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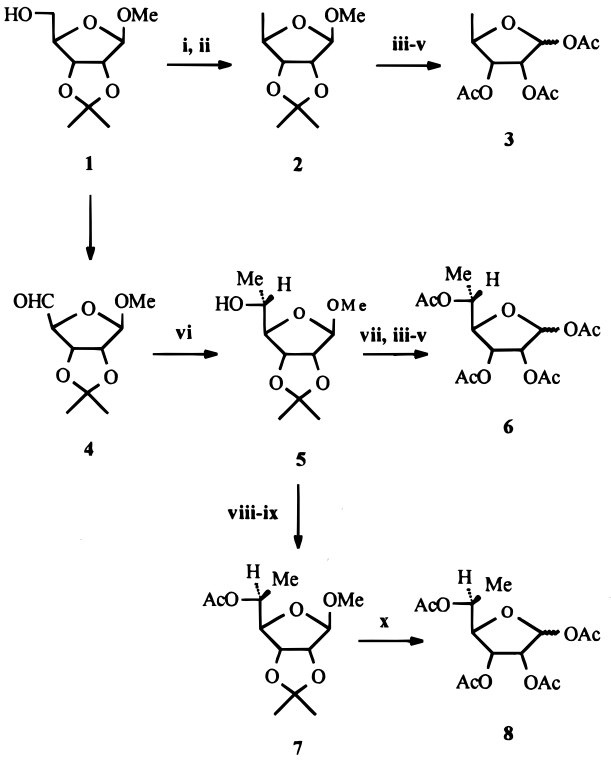
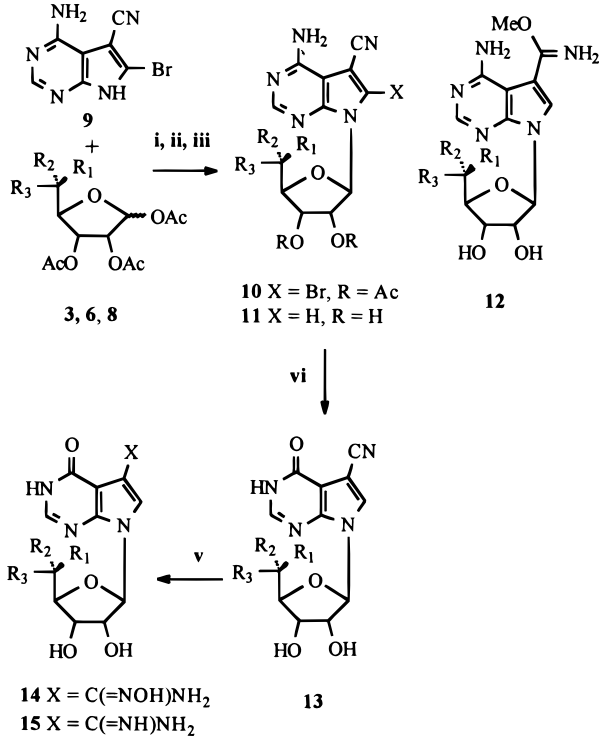
Scheme 1[a]



[a] Reagents and conditions: (i) TsCl, pyridine, rt, 91%; (ii) $LiAlH_4$, ether, reflux, 83%; (iii) TFA−H_2O (9:1), −10 °C; (iv) Ac_2O, pyridine, rt; (v) Ac_2O, AcOH, H_2SO_4, rt, 33% for **3** (three steps), 68% for **6** (three steps); (vi) MeMgBr, ether, −5 °C; (vii) same as iv, 55% (two steps); (viii) MsCl, pyridine; (ix) NaOAc, DMF; 125 °C, 52% (two steps); (x) same as v, 70%.

Scheme 2[a]



10-15a $R_1 = R_2 = R_3 = H$,
10b $R_1 = H$, $R_2 = Me$, $R_3 = OAc$; **11-15b** $R_1 = H$, $R_2 = Me$, $R_3 = OH$
10c $R_1 = Me$, $R_2 = H$, $R_3 = OAc$; **11-15c** $R_1 = Me$, $R_2 = H$, $R_3 = OH$

[a] Reagents and conditions: (i) 1. HMDS, xylene, $(NH_4)_2SO_4$, reflux, 2. TMSOTf, $ClCH_2CH_2Cl$, reflux; (ii) H_2, 10% Pd/C, Et_3N, 1,4-dioxane; (iii) 1. NH_3, MeOH, 2. NaOAc, DMF, 130 °C, 43−50% (three steps); (iv) $NaNO_2$, AcOH−H_2O, 70 °C, 68−70%; (v) $NH_2OH{\cdot}HCl$, K_2CO_3, EtOH, reflux, 64−83%.

can inhibit specific ligand function,[17] but they are limited by their short half-lives in plasma.[18]

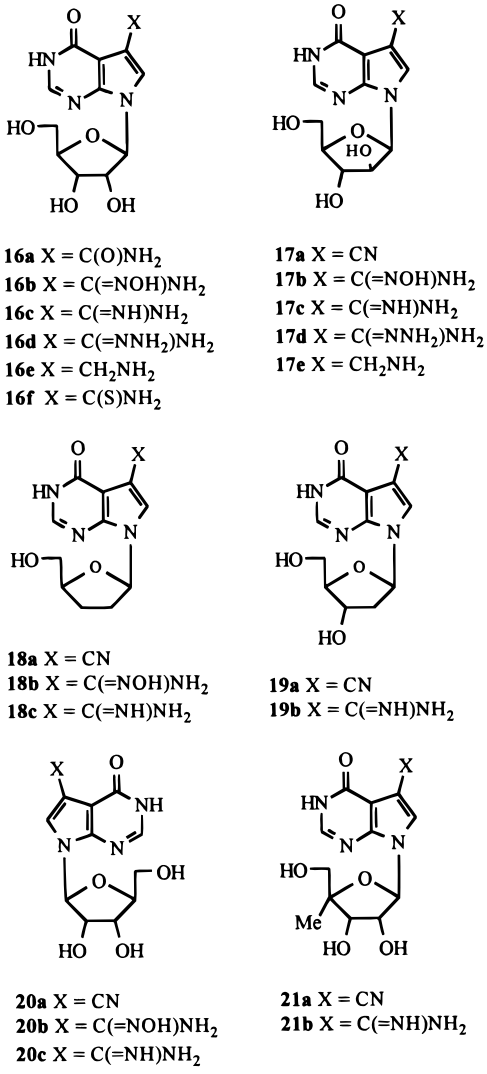
Since the development of nucleoside analogues which induce a Type 2 cytokine bias may alleviate some of the limitations of the macromolecules currently under development, we have investigated purine nucleoside analogues for induction of a Type 2 cytokine bias. In this article, we present synthesis and cytokine modulation properties of one class of purine nucleoside analogues, pyrrolo[2,3-*d*]-4-pyrimidone nucleosides.

Chemistry

Synthesis of 5′-deoxy- and 5′-*C*-methylpyrrolo[2,3-*d*]-pyrimidone nucleosides is shown in Schemes 1 and 2. Compound **1**[19] was converted to a 5-*O*-tosyl derivative, which was reduced with lithium aluminum hydride to the 5-deoxy derivative **2**[20] in good yield. Compound **2** was converted to **3** in a moderate yield by a sequence of reactions: selective deprotection of the isopropylidene with trifluoroacetic acid; acetylation at O2 and O3 in pyridine, and anomeric acetylation in the presence of sulfuric acid. The aldehyde **4**,[21] prepared from **1**, was reacted with methylmagnesium bromide to yield **5**[22] and its 5(*S*)-*C*-methyl isomer (*R*/*S* ratio ∼3:1) in good yield. The mixture was acetylated and separated to give 5-*O*-acetyl-2,3-isopropylidene-1-*O*,5(*R*)-*C*-dimethylribofuranose, which was subjected to the same sequence of reactions as **2** to give **6** in good yield. Compound **5** was converted to a mesylate, which was subjected to a nucleophilic substitution with acetate to give **7**. Compound **8** was obtained from **7** in one step by treatment with acetic anhydride and acetic acid in the presence of sulfuric acid. However, the ease with which the reaction was conducted was overshadowed by the poor quality of the product as some impurities could not be removed by chromatography. Therefore, the sequential reactions as described for preparation of **3** and **6** should be a better choice.

Condensation reactions of **3**, **6**, and **8** with silylated 6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine **9**[23] were conducted according to a published procedure for preparation of toyocamycin[24] with minor modifications. The resulting products **10a**−**c** were subjected to removal of bromine by hydrogenation and the subsequent deacetylation with ammonia to give **11a**−**c**, respectively, which were invariably accompanied with a significant amount (20−40%) of **12a**−**c**. However, **12a**−**c** could be readily converted to **11a**−**c** by heating in DMF in the presence of sodium acetate. Overall, **11a**−**c** were obtained in satisfactory yields. Although compounds **11a**[25] and **11b**,**c**[26] were prepared previously by different synthetic routes, the present route is probably more convenient. Compounds **11a**−**c** were treated with sodium nitrite to give **13a**−**c**, respectively, which were converted to **14a**−**c** in good yields by treatment with hydroxyamine.[27] Compound **15a** was obtained by heating **13a** in ammonia-saturated methanol in a steel bomb. Compounds **15b**−**c** were prepared, respectively, in good yields from **14b**−**c** by hydrogenation over Raney nickel.[27]

Chart 1



Compounds **16a**−**d**,**f** shown in Chart 1were prepared according to previously published procedures.[27] Compound **16e** was obtained by hydrogenation of 5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone[27] over palladium hydroxide. Compounds **17a**−**e** were prepared from arabinotoyocamycin[28] by procedures similar to those described for **13**−**15** and **16d**−**e**. Compounds **18a**−**c** were obtained from 2′,3′-dideoxytoyocamycin[29] by procedures similar to those used for the preparation of **13**−**15**. Compounds **19a**−**b** were prepared from 2′-deoxytoyocamycin[30] by the same procedures as used for **13a** and **15a**, respectively. Compounds **20a**−**c** were obtained by procedures similar to those used for **13**−**15** from L-toyocamycin, which was prepared by the same procedure as used for D-toyocamycin[24] except that 1-*O*-acetyl-tri-2,3,5-*O*-benzoyl-β-L-ribofuranose was used. Compounds **21a**−**b** were obtained from 4′-*C*-methyl-toyocamycin[31] by the same procedure as used for **13a** and **15a**.

Results and Discussion

We examined a series of pyrrolo[2,3-*d*]-4-pyrimidone nucleosides for their ability to induce a Type 2 cytokine profile. Following polyclonal activation of human T cells with 10 ng of phorbol-myristate-acetate and 0.5 μg of ionomycin and concomitant treatment with nucleosides for 48 h, cell-free supernatants were assayed for the presence of the Type 2 cytokines, IL-4 and IL-5, and of the Type 1 cytokines, IFNγ, IL-2, and TNFα. Limitations based on the yield of T cells practically isolated from a single donor meant that we were not able to assay all 26 pyrrolo[2,3-*d*]-4-pyrimidone nucleosides at six concentrations in quadruplicate using T cells from one donor. Therefore we assessed these compounds in the following three-step manner.

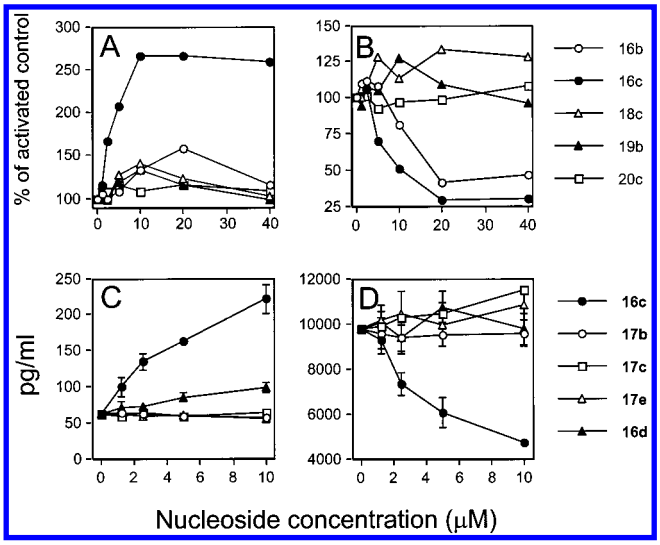


Figure 1. The dose response effect of selected pyrrolo[2,3-*d*]-4-pyrimidone nucleosides at 0−40 μM (panel A and panel B) or at 0−10 μM (panel C and panel D) on PMA/ION-stimulated T cell expression of the Type 2 cytokine, IL-4 (A and C), and the Type 1 cytokine, IFNγ (B and D). T cell-derived cytokine levels from a representative of three (A and B) or five (C and D) human donors were determined in cell-free supernatants by ELISA. Data for A and B are shown as percentage of activated control. This was calculated as shown in the Experimental Section. Activated levels of IL-4 and IFNγ were 62 and 10670 pg/mL, respectively. Data for C and D are shown as the mean levels (pg/mL ± standard deviation) of PMA/ION-induced IL-4 and IFNγ secretion. Resting levels were <30 pg/mL for all cytokines tested.

First we performed preliminary dose-range finding studies to determine the active range for the effect of all compounds on IL-4 (Type 2) and IFNγ (Type 1) production in a dose range of 1.25−40 μM. Figure 1A (IL-4) and Figure 1B (IFNγ) show the dose response effects of active compounds **16b**, **16c**, **18c**, and **19b** and a representative inactive compound **20c**. A peak activity for enhancement of IL-4 was observed at 10 μM for compounds **16c**, **18c**, and **19b** and at 20 μM for **16b** after which the activity leveled off or declined at concentrations up to 40 μM (Figure 1A). The peak activity of **16c** for suppression of IFNγ levels was observed at 20 μM. Compound **19b** showed elevated IFNγ levels at 10 μM, whereas compound **16b** showed a suppressive effect. Compound **20c** did not show any activity for IL-4 enhancement or IFNγ suppression throughout the concentration range, 1.25−40 μM. No polarization to a Type 2 cytokine profile was observed with the other compounds at this dose range. From these data we found that the peak activity, if any, was observed at 10−20 μM, after which inhibitory responses (Type 1 cytokines) and enhanced responses (Type 2 cytokines) reached a plateau or declined.

Table 1. Effect of Pyrrolo[2,3-*d*]pyrimidone Nucleosides (10 μM) on PMA/ION-Stimulated T Cell Expression of the Type 2 Cytokine, IL-4, or the Type 1 Cytokine, IFNγ

treatment[a]	IL-4	IFNγ	treatment[a]	IL-4	IFNγ	treatment[a]	IL-4	IFNγ
PMA/ION	162 ± 40	10384 ± 2234	PMA/ION	162 ± 40	10384 ± 2234	PMA/ION	162 ± 40	10384 ± 2234
+ **13a**	147 ± 4	9137 ± 3115	+ **16a**	183 ± 58	9137 ± 2076	+ **17e**	142 ± 49	10799 ± 1246
+ **13b**	160 ± 32	13291 ± 4569	+ **16b**	236 ± 46*	7269 ± 1369*	+ **18b**	152 ± 42	11214 ± 1038
+ **13c**	146 ± 20	10903 ± 831	+ **16c**	557 ± 134*	5919 ± 83*	+ **18c**	234 ± 46*	13291 ± 2284
+ **14a**	149 ± 26	10591 ± 3738	+ **16d**	207 ± 54	11941 ± 2076	+ **19a**	194 ± 56	11941 ± 4362
+ **14b**	186 ± 16	12253 ± 3323	+ **16e**	125 ± 87	9345 ± 3530	+ **19b**	258 ± 56*	14745 ± 1038
+ **14c**	162 ± 59	11422 ± 2076	+ **16f**	176 ± 44	7476 ± 2076	+ **20b**	142 ± 29	9657 ± 2492
+ **15a**	152 ± 31	13914 ± 2076	+ **17b**	132 ± 23	10384 ± 3737	+ **21a**	147 ± 7	9034 ± 2907
+ **15b**	159 ± 81	12149 ± 1454	+ **17c**	131 ± 22	7788 ± 4153	+ **21b**	144 ± 20	9553 ± 2908
+ **15c**	146 ± 75	10695 ± 2493	+ **17d**	117 ± 72	9864 ± 2492			

[a] T cell-derived cytokine levels from five individual human donors were determined in cell-free supernatants by ELISA. Compound numbers are shown in bold. Data are shown collectively as the mean cytokine concentration (pg/mL ± standard deviation) for all cytokines. Resting levels were <30 pg/mL for all cytokines. *p < 0.0001 when compared to activated control.

Table 2. Effect of Selected Pyrrolo[2,3-*d*]pyrimidone Nucleosides (10 μM) on PMA/ION-Stimulated T Cell Expression of the Type 2 Cytokines, IL-4 and IL-5, or the Type 1 Cytokines, IL-2, IFNγ, and TNFα

	Type 2 cytokines[b]		Type 1 cytokines[c]		
treatment[a]	IL-4	IL-5	IL-2	IFNγ	TNFα
PMA/ION	162 ± 40	93 ± 62	27712 ± 7192	10384 ± 2234	2152 ± 725
+ **16a**	183 ± 18	67 ± 31	24386 ± 4434	9137 ± 2076	2173 ± 474
+ **16b**	236 ± 46*	73 ± 32	18012 ± 1663*	7269 ± 1869*	1592 ± 30*
+ **16c**	557 ±134*	127 ± 12*	12747 ± 4542*	5919 ± 83*	968 ± 374*
+ **16f**	176 ± 44	59 ± 26	23000 ± 8313	7476 ± 2076	2216 ± 388

[a] T cell-derived cytokine levels from five individual human donors were determined in cell-free supernatants by ELISA. Compound numbers are shown in bold. The mean absolute level (pg/mL ± standard deviation) of PMA/ION-induced cytokine secretion is shown for IL-4, IL-5, IL-2, IFNγ, and TNFα. Resting levels were <30 pg/mL for all cytokines. [b] Enhancement of Type 2 cytokines, *p < 0.001 when compared to activated control. [c] Inhibition of Type 1 cytokines, *p < 0.001 when compared to activated control.

We then proceeded to focus on the range of peak activity by comparing the effects of all compounds on IL-4 (Type 2) and IFNγ (Type 1) production but now assayed in triplicate (to permit calculation of a standard deviation) at a limited dose range (1.25−10 μM). The dose response effect, as shown by mean absolute values (pg/mL ± standard deviation), of five representative compounds (**16c**, **16d**, **17b**, **17c**, **17e**) on activation-induced IL-4 and IFNγ levels is shown in Figure 1C (IL-4) and Figure 1D (IFNγ). In activated T cells from a representative of five human donors, it was observed that there was a dose dependent increase in IL-4 levels in response to both **16c** and **16d** in the dose range 1.25−10 μM. No dose response with regard to IL-4 levels was seen with the other three compounds in the same dose range (Figure 1C). A negative dose response in IFN-γ levels was observed with **16c**, but not with **16d** and the other three compounds in the same dose range (Figure 1D). Collectively the dose response data from Figure 1 show that, in representative donors at the specified dose ranges, compound **16c** was the most potent enhancer of IL-4 and suppressor of IFNγ of all 26 nucleosides tested. Significant IL-4 enhancement was also observed with compounds **16b**, **16d**, **18c**, and **19b**. Moderate IFNγ elevation was observed with compounds **18c** and **19b**.

Finally we compared the mean activity (pg/mL ± standard deviation) of all compounds at the approximate peak concentration (10 μM) in T cells from five human donors (Table 1) and for an expanded repertoire of cytokines (Table 2). A concentration of 10 μM is 5 times lower than the lowest effective dose of the acyclonucleoside phosphonates on cytokine production in macrophages.[5] This lower concentration also supports our hypothesis of a more specific immune modulation. The data in Table 1 show that of the 26 pyrrolo[2,3-*d*]-4-pyrimidone nucleosides tested, **16c** dramatically enhanced IL-4 levels, more than 2-fold over the activated control. Much less dramatic but still substantial IL-4 enhancement was seen with **16b** (46%), **16d** (28%), **18c** (45%), and **19b** (59%). In addition to the elevation of IL-4 levels, **16b** and **16c**, as well as **16f**, suppressed the activated control levels of IFNγ by more than 25%, with **16c** being the most potent (43%). In contrast, some of these nucleosides such as **13b** (28% above activated control), **15a** (34% above activated control), **18c** (28% above activated control), and **19b** (42% above activated control) demonstrated substantial elevation of IFNγ levels. It is noteworthy that the standard deviations for the stimulated cytokine levels in T lymphocytes in the presence or absence of compound are quite high. This reflects the variability in both the donor-to-donor response of T cells to the stimulant (PMA/ION) and the effect of compounds on activated cytokine responses. Collectively, these data show that **16b** and **16c** could induce a Type 2 cytokine bias whereas **16d**, **18c**, and **19b** appear to be Type 2 cytokine stimulants but not Type 1 cytokine suppressants.

Table 2 shows the effects of a few selected nucleosides on the production of two Type 2 (IL-4, IL-5) and three Type 1 (IL-2, IFN-γ, TNFα) cytokines. In addition to the enhancement of IL-4 and suppression of IFNγ levels, **16c** also enhanced IL-5 levels and suppressed IL-2 and TNFα levels substantially, which indicates that the induction of a Type 2 cytokine bias by **16c** is likely a general phenomenon. Like **16c**, compound **16b** also suppressed all three Type 1 cytokines substantially though it enhanced IL-4, but not IL-5, levels.

On the basis of these observations we believe that the within-donor comparison of all compounds at an expanded dose response range (1.25−40 μM), and at a limited dose response (1.25−10 μM) in representative donors, coupled with strong cumulative data in several donors at the approximate peak active concentration (10

μM) provide the basis for a SAR analysis of the pyrrido-[2,3-*d*]-4-pyrimidone nucleosides tested.

Of the compounds tested at a concentration of 10 μM, five compounds (**16b**, **16c**, **16d**, **18c**, and **19b**) demonstrated substantial but varied elevation of IL-4 levels in activated human T cells. One structural feature common to all five compounds is an intact 5′-hydroxy, although **16b**, **16c**, and **16d** have the intact ribose as the sugar moiety while **18c** and **19b** have 2′,3′-dideoxyribose and 2′-deoxyribose, respectively. Also common to these five compounds is a polar, positively charged (at physiological condition) carboxamidino functional group (carboxamidine, carboxamidoxime, or carboxamidrazon) at the C5 position of the nucleoside base. Both structural features mentioned above are required for the IL-4 enhancement, which can be concluded from the following observations. Compounds **14a**−**c**, **15a**−**c**, and **21b**, which contain either a carboxamidoxime or a carboxamidine group at the C5 position but are modified at either the C5′ or C4′ position, are completely devoid of the IL-4 enhancement. Compounds **16a**, **16e**, and **16f**, which have the intact ribose but have at the C5 neither of the aforementioned three carboxamidino functional groups, did not show any significant elevation of IL-4 levels. Compounds **17b**, **17c**, and **17d**, which contain carboxamidoxime, carboxamidine, and carboxamidrazon, respectively, and an arabinose sugar moiety, are also devoid of the IL-4 enhancement. Also as mentioned earlier, **18c** and **19b**, which have carboxamidine at the C5 but whose ribose moieties are modified at the C2′ and C3′ positions, have much less activity when compared to **16c**. These results indicated that the intact ribose moiety was crucial to the high activity of **16c**, which implicated that a phosphorylation by ribonucleoside kinases could be involved.

The mechanism by which active members of the pyrrido[2,3-*d*]pyrimidone nucleosides induce a Type 2 cytokine bias is not known at present, but it is the subject of further study. It is noteworthy that this class of compounds can be categorized as inosine analogues. Indeed inosine itself can inhibit inflammatory (Type 1) cytokine production and protect against inflammatory responses in vivo.[32] Several drugs which are used in the treatment of autoimmune and inflammatory diseases, including adenosine kinase inhibitors,[33] methotrexate,[34] sulfasalazine,[35] and aspirin,[36] may exert their beneficial effects by releasing adenosine. As adenosine is readily metabolized to inosine, one can postulate that a common antiinflammatory pathway is influenced by inosine. It was also concluded that inosine inhibits the inflammatory cytokine production in immunostimmulated mouse macrophages and spleen cells by a posttranscriptional mechanism.[32]

For the IL-4 enhancement by the pyrrolo[2,3-*d*]-pyrimidone nucleosides, we surmise based upon the strict structural requirements mentioned above that these nucleosides were probably phosphorylated by cellular ribonucleoside kinases and functioned either at the monophosphate level or at the triphosphate level. The active pyrrolo[2,3-*d*]pyrimidone nucleoside analogues causing IL-4 enhancement in this article seems to act somewhat differently from inosine. The enhancement of IL-4 levels is more likely due to the elevated level of the IL-4 mRNA which was observed when the activated T cells were treated with **16c**.[37] Compared to the posttranscriptional mechanism of inosine, the IL-4 enhancement by these pyrrido[2,3-*d*]pyrimidone nucleosides is more likely at pretranscriptional level. It is difficult at this time for us to postulate a mechanism of action. However, a signal transduction pathway requiring the phosphorylated pyrrolo[2,3-*d*]pyrimidone nucleosides may be one of possible mechanisms. The future work on pyrrolo[2,3-*d*]-4-pyrimidone nucleoside 5′-monophosphates, the 3′,5′-cyclic monophosphates, the triphosphates, and their prodrugs may provide some clues to the hypothesis.

Conclusion

We have synthesized and evaluated a series of pyrrolo[2,3-*d*]-4-pyrimidone nucleosides as enhancers of Type 2 cytokine (IL-4, IL-5) production and suppressors of Type 1 cytokine (IFNγ, IL-2, TNFα) production in human T cells activated in vitro. Among those nucleoside analogues (**16b, 16c**, **16d**, **18c**, and **19b**) which showed stimulatory effects on IL-4 production, 7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone-5-carboxamidine (**16c**) demonstrated a dramatic enhancement (344%). Moreover, this compound showed a significant enhancement of IL-5 and a substantial suppression of IL-2, IFNγ, and TNFα levels. To our knowledge, this is the first nucleoside analogue which could induce such a Type 2 cytokine bias. Also of note is **16b**, which significantly enhanced IL-4 and suppressed IL-2, IFNγ, and TNFα levels. Owing to their cytokine modulation properties, **16c** and **16b** merit the therapeutic evaluation in treating diseases in which immunopathology is associated with polarized Type 1 cytokine responses. These include autoimmune diseases such as multiple sclerosis and rheumatoid arthritis. Work is in progress toward elucidation of the mechanism of action by which these compounds induce the effects on Type 2 and Type 1 cytokine levels. The results of such studies should shed more light on the future design of more effective immunomodulators.

Experimental Section

(A) Chemistry. ^{1}H NMR spectra were obtained on a Varian Mercury 300 spectrometer, and tetramethylsilane was used as the internal standard. Elemental analysis was conducted by NuMega Resonance, Inc., San Diego, CA. Melting points were measured on a capillary melting point measurement apparatus and are uncorrected. Anhydrous solvents were purchased from Aldrich or Fluka without further treatment unless noted. Thin-layer chromatography plates and silica gel for flash chromatography were supplied by ICN Biomedicals. Solvent ratios are based on volume in case that solvent mixture was used.

A usual workup procedure was used for most of the reactions in the Experimental Section: The mixture was diluted with ethyl acetate (or methylene chloride), washed sequentially with water (or brine), dilute sodium bicarbonate, and water, dried (Na_2SO_4), and concentrated to dryness at reduced pressure.

5-Deoxy-2,3-*O*-isopropylidene-1-*O*-methyl-β-D-ribofuranose (2). A solution of **1**[19] (15.0 g, 73.45 mmol) and *p*-toluenesulfonyl chloride (24.5 g, 128.53 mmol) in anhydrous pyridine (250 mL) was stirred at room temperature overnight and quenched with methanol (5.0 mL). The resulting mixture was stirred for 30 min and concentrated. After the usual workup, the residue was chromatographed on silica (EtOAc/hexanes, 1:3) to give 24.0 g (91%) of 2,3-*O*-isopropylidene-1-*O*-methyl-5-*O*-tosyl-β-D-ribofuranose (a tosylate) as a colorless solid.

To a stirred suspension of $LiAlH_4$ (7.45 g, 196.4 mmol) in anhydrous diethyl ether (400 mL) was added the tosylate (22.0 g, 61.38 mmol) in diethyl ether/toluene (2.5:1, 200 mL). The resulting mixture was refluxed overnight, cooled, and quenched with ethyl acetate and then with water. The usual workup and subsequent chromatography on silica (EtOAc/hexanes, 1:3) gave 9.64 g (83%) of **2** as a colorless liquid: ^{1}H NMR ($CDCl_3$) δ 4.93 (s, 1H, H-1), 4.63 (d, J = 6.0 Hz, 1H), 4.50 (d, J = 6.0 Hz, 1H), 4.34 (q, J = 6.9 Hz, 1H, H-4), 3.20 (s, 3H, OMe), 1.47 (s, 3H, Me), 1.30 (s, 3H, Me), 1.28 (d, J = 6.9 Hz, 3H, 5-Me).

5-Deoxy-1,2,3-tri-*O*-acetyl-β-D-ribofuranose (3). A solution of **2** (9.0 g, 47.8 mmol) in 80 mL of TFA/water (9:1) stood at −10 °C for 1 h and then was concentrated to dryness in vacuo at low temperature (below 0 °C). The residue was dissolved in toluene/methanol and then concentrated. Chromatography on silica (EtOAc/hexanes, 3:1) gave 2.94 g of 5-deoxy-1-*O*-methylribofuranose as a syrup, which was dissolved in acetic anhydride (7.4 mL) and pyridine (30 mL). The resulting solution stood at room temperature overnight and was concentrated. The usual workup and subsequent chromatography on silica (EtOAc/hexanes, 1:3) gave 3.83 g of 5-deoxy-2,3-di-*O*-acetyl-1-*O*-methylribofuranose, which was dissolved in a mixture of acetic acid (36 mL) and acetic anhydride (4 mL). The resulting solution was cooled with ice, and concentrated sulfuric acid (350 μL, 6.6 mmol) in acetic acid (4 mL) was added. The mixture stood at room temperature for 2 h and was worked up by the usual procedure. Chromatography on silica (EtOAc/hexanes, 1:3) gave 4.03 g (33%, 3 steps) of **3** (α/β ratio, 1:3) as a colorless syrup. The ^{1}H NMR data of the β-anomer are identical to those reported:[20] ^{1}H NMR (α-anomer, $CDCl_3$) δ 6.36 (d, J = 4.5 Hz, 1H, H-1), 5.25 (dd, J = 6.9, 4.5 Hz, 1H, H-2), 4.95 (dd, J = 6.9, 3.9 Hz, 1H, H-3), 4.33 (m, 1H, H-4), 2.12, 2.11, 2.07 (m, 9H, 3OAc), 1.36 (d, J = 6.6 Hz, 1H, Me).

5(*R*)-*C*-Methyl-1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (6). To a stirred solution of **4**[21] (5.1 g, 25.22 mmol) in diethyl ether (25 mL) at −40 °C under argon was added methylmagnesium bromide (3.0 M in diethyl ether, 400 mL). The mixture was allowed to warm to −5 °C, stirred for 3 h at this temperature, and quenched with ethanol. The resulting mixture was washed sequentially with dilute aqueous acetic acid, water, and dilute sodium bicarbonate, dried (Na_2SO_4), and concentrated to give 4.75 g of the crude **5** (ratio of the 5(*R*)/5(*S*) isomer, 3:1).

A solution of **5** (the crude, 4.0 g, 18.3 mmol) and acetic anhydride (5.0 mL) in pyridine (40 mL) stood at room temperature overnight and then was quenched with ethanol (2.5 mL). The resulting mixture was stirred for 2 h and then worked up. Chromatography on silica (8% EtOAc in hexanes) gave 3.07 g of 5-*O*-acetyl-1-*O*,5(*R*)-*C*-dimethyl-2,3-*O*-isopropylideneribofuranose and 0.62 g of the 5(*S*)-*C*-methyl isomer.

5-*O*-Acetyl-1-*O*,5(*R*)-*C*-dimethyl-2,3-*O*-isopropylideneribofuranose (2.50 g, 9.60 mmol) was subjected to the same treatment as described for **2** and gave 2.04 g (68%, 3 steps) of **6** (α/β ratio, ~2:1) as a colorless syrup: ^{1}H NMR (β-anomer, $CDCl_3$) δ 6.14 (d, J = 0.9 Hz, 1H, H-1), 5.43 (dd, J = 6.6, 5.1 Hz, 1H, H-3), 5.31 (dd, J = 5.1, 1.2 Hz, 1H, H-2), 5.04 (m, 1H, H-5), 4.18 (dd, J = 6.6, 4.5 Hz, 1H, H-4), 2.11, 2.08, 2.06, 2.05 (4s, 12H, 4OAc), 1.25 (d, J = 6.6 Hz, 1H, Me); ^{1}H NMR (α-anomer, $CDCl_3$) δ 6.38 (d, J = 4.5 Hz, 1H, H-1), 5.43 (dd, J = 6.9, 2.7 Hz, 1H, H-2), 5.18 (dd, J = 6.6, 4.5 Hz, 1H, H-3), 5.06 (m, 1H, H-5), 4.24 (t, J = 3.0 Hz, 1H, H-4), 2.13, 2.11, 2.062, 2.056 (4s, 12H, 4OAc), 1.27 (d, J = 6.6 Hz, 1H, Me).

5(*S*)-*C*-Methyl-1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (8). A solution of **5** (contaminated by a small amount of the 5(*S*)-isomer, 7.24 g, 33.17 mmol) and methanesulfonyl chloride (3.1 mL, 39.92 mmol) in anhydrous pyridine (50 mL) stood at room temperature for 1 h, was cooled to 0 °C, and was quenched with water (1.0 mL). The usual workup and subsequent chromatography on silica (30% EtOAc in hexanes) gave 8.62 g of 1-*O*-5(*R*)-*C*-dimethyl-2,3-*O*-isopropylidene-5-*O*-mesyl-β-D-ribofuranose as a colorless syrup, which was dissolved in anhydrous DMF (350 mL). Anhydrous sodium acetate (3.5 g, 42.5 mmol) was added, and the resulting mixture was heated at 125 °C under argon for 4 days. Solvent was evaporated and the residue chromatographed on silica (EtOAc/hexanes, 1:3) to give 1.2 g of the intact mesylate and 3.96 g of **7** as a colorless solid.

To a solution of **7** (3.96 g, 15.21 mmol) in acetic acid (65 mL) and acetic anhydride (6.5 mL) at 5 °C was added concentrated sulfuric acid (1.3 mL, 24.4 mmol). The resulting solution was stirred at room temperature for 18 h, poured onto ice (250 g), and extracted with ethyl acetate. After the usual workup, the residue was chromatographed on silica (30% EtOAc in hexanes) to give 3.56 g of **8** (contaminated by some impurities) as a colorless syrup. A small amount of pure sample was obtained from a careful chromatography: ^{1}H NMR (β-anomer, $CDCl_3$) δ 6.12 (s, 1H, H-1), 5.27−5.33 (m, 2H, H-2, H3), 5.01 (m, 1H, H-5), 4.16 (m, 1H, H-4), 2.12, 2.10, 2.07, 2.06 (4s, 12H, 4OAc), 1.25 (d, J = 6.6 Hz, 1H, Me); ^{1}H NMR (α-anomer, $CDCl_3$) δ 6.42 (d, J = 4.5 Hz, 1H, H-1), 5.22 (dd, J = 6.6, 4.5 Hz, 1H, H-2), 5.14 (dd, J = 6.6, 3.0 Hz, 1H, H-3), 5.08 (m, 1H, H-5), 4.27 (t, J = 3.0 Hz, 1H), 2.12, 2.11, 2.08, 2.06 (4s, 12H, 4OAc), 1.29 (d, J = 6.6 Hz, 1H, Me).

4-Amino-5-cyano-7-(5-deoxy-β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine (11a). A mixture of 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine **9**[23] (2.20 g, 9.22 mmol), HMDS (220 mL), anhydrous *m*-xylene (70 mL), and ammonium sulfate (100 mg) was refluxed under argon overnight, concentrated to dryness, and dried under vacuum for 30 min. The residue together with **3** (2.00 g, 7.68 mmol) was dissolved in anhydrous 1,2-dichloroethane (240 mL), the resulting solution was cooled to 0 °C, and TMSOTf (3.78 mL) in 1,2-dichloroethane (10 mL) was added. The resulting solution was stirred at room temperature for 30 min, refluxed for 18 h under argon, and then poured into ice−water (200 mL) containing sodium bicarbonate (5 g). The precipitate was filtered, and the organic layer was dried (Na_2SO_4) and concentrated. Chromatography on silica (EtOAc/hexanes, 1:1) gave 1.72 g (51%) of **10a**.

A mixture of **10a** (1.40 g, 3.19 mmol) and 10% Pd/C (350 mg) in anhydrous 1,4-dioxane (160 mL) containing triethylamine (1.6 mL) was shaken in a hydrogenation apparatus (25 psi hydrogen) at room temperature for 4 h. The catalysts were filtered, and the filtrate was concentrated to dryness. The residue was chromatographed on silica (EtOAc/hexanes, 3:2 to 1:0), and the purified product was dissolved in ammonia-saturated methanol (200 mL). The resulting solution stood at ambient temperature overnight and then concentrated to dryness. The residue containing **11a** and **12a** (approximately 1:1) was heated in DMF (50 mL) at 130 °C for 1.5 h in the presence of sodium acetate (50 mg). Solvent was evaporated, and the residue was chromatographed on silica (5% MeOH in EtOAc) to give 750 mg (85%) of **11a** which has identical ^{1}H NMR data (in C_5D_5N) to those reported.[25] Total yield was 43% (three steps): ^{1}H NMR of **11a** in DMSO-d_6 δ 8.39. 8.21 (2s, 2H, H-2, H-6), 6.88 (s, 2H, NH_2), 6.02 (d, J = 5.1 Hz, 1H, H-1′), 5.45 (d, J = 5.7 Hz, 1H, OH), 5.18 (d, J = 5.4 Hz, 1H, OH), 4.39 (dd, J = 10.2, 5.1 Hz, 1H), 3.94 (m, 1H, H-4′), 3.86 (dd, J = 9.9, 5.1 Hz, 1H), 1.28 (d, J = 6.3 Hz, 3H, 5′-Me).

4-Amino-5-cyano-7-(5(*R*)-*C*-methyl-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (11b) was prepared from **6** by the same procedure as described for **11a**. Colorless solid. Yield: 50% (three steps). Compound **11b** has ^{1}H NMR data identical to those reported.[26]

4-Amino-5-cyano-7-(5(*S*)-*C*-methyl-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (11c) was prepared from **8** by the same procedure as described for **11a**. Colorless solid. Yield: 45% (three steps). Compound **11c** has ^{1}H NMR data identical to those reported.[26]

5-Cyano-7-(5-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone (13a). To a stirred solution of **11a** (660 mg, 2.40 mmol) in water (45 mL) and acetic acid (3 mL) at 50 °C was added in portions sodium nitrite (1.20 g, 17.39 mmol), and the resulting mixture was stirred at 70 °C for 1 h. Solvent was evaporated, and the residue was chromatographed on silica (5% MeOH in EtOAc) to give 460 mg (69%) of **13a**, which was recrystallized from methanol as a colorless solid: mp 241−242 °C; ^{1}H NMR (DMSO-d_6) δ 12.48 (br, 1H, NH), 8.28, 8.08

(2s, 2H, H-2, H-6), 5.96 (d, J = 4.5 Hz, 1H, H-1′), 5.48 (br, 1H, OH), 5.20 (br, 1H, OH), 4.34 (m, 1H), 4.26 (m, 1H), 3.94 (m, 1H, H-4′), 3.84 (m, 1H), 1.28 (d, J = 6.3 Hz, 3H, Me). Anal. ($C_{12}H_{12}N_4O_4$) C, H, N.

5-Cyano-7-(5(*R*)-*C*-methyl-β-D-ribofuranosyl)pyrrolo-[2,3-*d*]-4-pyrimidone (13b) was prepared from **11b** by the same procedure as described for **13a**. Yield: 68%. Colorless solid (recrystallized from water): mp 236−237 °C; ^{1}H NMR (DMSO-d_6) δ 12.51 (s, br, 1H, NH), 8.34, 8.08 (2s, 2H, H-2, H-6), 5.98 (d, J = 6.6 Hz, 1H, H-1′), 5.41 (d, J = 6.3 Hz, 1H), 5.19 (d, J = 3.9 Hz, 1H, OH), 5.11 (d, J = 4.5 Hz, 1H, OH), 4.31 (dd, J = 10.8, 5.4 Hz, 1H), 4.14 (m, 1H), 3.80 (m, 1H, H-5′), 3.68 (m, 1H), 1.07 (d, J = 6.3 Hz, 3H, 5′-Me). Anal. ($C_{13}H_{14}N_4O_5$) C, H, N.

5-Cyano-7-(5(*S*)-*C*-methyl-β-D-ribofuranosyl)pyrrolo-[2,3-*d*]-4-pyrimidone (13c) was prepared from **11c** by the same procedure as described for **13a**. Yield: 70%. Colorless solid: ^{1}H NMR (DMSO-d_6) δ 12.5 (s, br, 1H, NH), 8.38, 8.08 (2s, 2H, H-2, H-6), 6.01 (d, J = 5.1 Hz, 1H, H-1′), 5.47 (br, 1H, OH), 5.15 (br, 1H, OH), 5.07 (d, J = 5.4 Hz, 1H, OH), 4.24 (m, 1H), 4.06 (m, 1H), 3.73−3.86 (m, 2H, H-4′, H-5′), 1.13 (d, J = 6.3 Hz, 3H, 5′-Me).

7-(5-Deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone-5-carboxamidoxime (14a). A mixture of **13a** (300 mg, 1.09 mmol), hydroxylamine hydrochloride (226 mg, 3.26 mmol), and potassium carbonate (225 mg, 1.63 mmol) in ethanol (40 mL) was refluxed under argon overnight. The precipitate was filtered and washed with warm ethanol. The filtrate was concentrated, and the residue was chromatographed on silica (MeOH/$CHCl_3$, 1:3) to give 270 mg (87%) of **14a** as a colorless solid (recrystallizaed from methanol): ^{1}H NMR (DMSO-d_6) δ 12.4 (br, 1H, NH), 9.16 (br, 1H, NOH), 8.01, 7.45 (2s, 2H, H-2, H-6), 6.75 (s, br, 2H, NH_2), 6.00 (d, J = 5.1 Hz, 1H, H-1′), 5.40 (br, 1H, OH), 5.18 (br, 1H, OH), 4.32 (m, 1H), 3.93 (m, 1H, H-4′), 3.78 (m, 1H), 1.27 (d, J = 6.6 Hz, 3H, Me).

7-(5(*R*)-*C*-Methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone-5-carboxamidoxime (14b) was prepared from **13b** by the same procedure as described for **14a**. Yield: 83%. Colorless solid (recrystallized from water): ^{1}H NMR (DMSO-d_6) δ 12.45 (s, 1H, NH), 9.16 (s, 1H, NOH), 8.01, 7.67 (2s, 2H, H-2, H-6), 6.75 (br, 2H, NH_2), 6.03 (d, J = 7.5 Hz, 1H, H-1′), 5.31 (d, J = 6.9 Hz, 1H, OH), 5.14 (d, J = 4.2 Hz, 1H, OH), 5.04 (d, J = 4.5 Hz, 1H, OH), 4.31 (dd, J = 12.3, 6.9 Hz, 1H), 4.10 (m, 1H), 3.66−3.80 (m, 2H), 1.07 (d, J = 6.6 Hz, 3H, Me). Anal. ($C_{13}H_{17}N_5O_6\cdot H_2O$) C, H, N.

7-(5(*S*)-*C*-Methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone-5-carboxamidoxime (14c) was prepared from **13c** by the same procedure as described for **14a**. Yield: 64%. Colorless solid (recrystallized from water): ^{1}H NMR (DMSO-d_6) δ 12.43 (s, 1H, NH), 9.19 (s, 1H, NOH), 8.01, 7.80 (2s, 2H, H-2, H-6), 6.75 (br, 2H, NH_2), 6.08 (d, J = 6.3 Hz, 1H, H-1′), 5.33 (d, J = 6.6 Hz, 1H, OH), 5.10 (d, J = 4.5 Hz, 1H, OH), 5.02 (d, J = 4.8 Hz, 1H), 4.28 (q, J = 10.4, 6.0 Hz, 1H), 4.03 (m, 1H), 3.72−3.84 (m, 2H), 1.08 (d, J = 6.3 Hz, 3H, Me). Anal. ($C_{13}H_{17}N_5O_6\cdot 0.5H_2O$) C, H, N.

7-(5-Deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone-5-carboxamidine (15a). A solution of **14a** (120 mg, 0.43 mmol) in ammonia-saturated methanol (120 mL) was heated in a steel bomb at 125 °C for 64 h. After removal of solvent, the residue was chromatographed on silica (5% aqueous ammonia (30%) in MeOH) to give a brownish solid, which was dissolved in hot water and decolored with charcoal to give 71 mg (56%) of **15a** as a colorless solid: mp 257−259 °C (dec, water/methanol); ^{1}H NMR (DMSO-d_6) δ 11.2 (br, 1H, NH), 8.82 (br, 2H, NH_2), 8.17, 7.96 (2s, 2H, H-2, H-6), 6.03 (d, J = 3.9 Hz, 1H, H-1′), 5.57 (br, 1H, OH), 5.22 (br, 1H, OH), 4.16 (t, J = 4.2 Hz, 1H), 3.95 (m, H, H-4′), 3.84 (t, J = 5.4 Hz, 1H), 1.31 (d, J = 6.0 Hz, 3H, Me).

7-(5(*R*)-*C*-Methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone-5-carboxamidine hydrochloride (15b). A mixture of **14b** (120 mg, 0.35 mmol), ammonium chloride (21 mg, 0.39 mmol), and Raney nickel (Aldrich, 200 mg, wet) in water (70 mL) was shaken in a hydrogenation apparatus (50 psi hydrogen) at room temperature for 18 h. Catalysts were filtered, and the filtrate was concentrated. Crystallization from water/methanol gave 90 mg (72%) of **15a** as a colorless solid: ^{1}H NMR (DMSO-d_6) δ 10.42 (br, 2H, NH_2), 8.63 (br, 3H, H-6, NH_2), 8.14 (s, 1H, H-2), 5.98 (d, J = 7.2 Hz, 1H, H-1′), 5.18−5.54 (m, br, 3H, 3OH), 4.35 (t, J = 5.1 Hz, 1H), 4.17 (m, 1H), 3.70−3.84 (m, 2H), 1.07 (d, J = 6.3 Hz, 3H, Me).

7-(5(*S*)-*C*-Methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone-5-carboxamidine hydrochloride (15c) was prepared from **14c** by the same procedure as described for **15b**. Yield: 60%. Colorless solid (water/methanol): ^{1}H NMR (DMSO-d_6) δ 10.10 (s, 2H, NH_2), 8.78 (br, 2H, NH_2), 8.75 (s, 1H, H-6), 8.25 (s, 1H, H-2), 6.07 (d, J = 5.7 Hz, 1H, H-1′), 5.53 (d, J = 6.0 Hz,1H, OH), 5.25 (d, J = 5.1 Hz, 1H, OH), 4.82 (d, J = 5.7 Hz, 1H, OH), 4.26 (dd, J = 10.4, 5.7 Hz, 1H), 4.09 (m, 1H), 3.72−3.83 (m, 2H), 1.12 (d, J = 6.3 Hz, 3H, Me). Anal. ($C_{13}H_{18}ClN_5O_5\cdot H_2O$) C, H, N.

5-Aminomethyl-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone (16e). A mixture of 5-cyano-7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]-4-pyrimidone[27] (120 mg, 0.41 mmol) and 20% palladium hydroxide (Aldrich, 120 mg) in water (100 mL) was shaken in a hydrogenation apparatus (50 psi hydrogen) at room temperature for 15 h. Catalysts were filtered, and solvent was evaporated. Recrystallization from water yielded 80 mg (65%) of **16e** as a colorless solid: mp >250 °C (dec); ^{1}H NMR (DMSO-d_6) δ 7.89 (s, 1H, H-2), 7.15 (s, 1H, H-6), 5.95 (d, J = 6.6 Hz, 1H, H-1′), 5.0−5.5 (br, 3H, 3OH), 4.28 (t, J = 5.7 Hz, 1H), 4.03 (t, J = 3.0 Hz, 1H), 3.84 (dd, J = 7.2, 3.6 Hz, 1H), 3.71 (s, 2H, CH_2), 3.45−3.60 (m, 2H, H5′).

5-Cyano-7-(β-D-arabinofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone (17a) was prepared from arabinotoyocamycin[28] by the same procedure as described for **13a**. Yield: 79%. Slightly brownish solid (recrystallized from water): mp 314 °C (dec); ^{1}H NMR (DMSO-d_6) δ 12.46 (br, 1H, NH), 8.14 (s, 1H, H-2), 8.05 (d, J = 2.4 hz, 1H, H-6), 6.33 (d, J = 5.4 Hz, 1H, H-1′), 5.56 (d, J = 6.0 Hz, 1H, OH), 5.54 (d, J = 5.4 Hz, 1H, OH), 5.15 (t, J = 5.4 Hz, 1H, OH), 4.13 (dd, J = 10.5, 5.1 Hz, 1H), 4.04 (dd, J = 9.6, 5.1 Hz, 1H), 3.77 (dd, J = 9.0, 5.1 Hz, 1H), 3.66 (m, 2H, H-5′). Anal. ($C_{12}H_{12}N_4O_5$) C, H, N.

7-(β-D-Arabinofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone-5-carboxamidoxime (17b) was prepared from **17a** by the same procedure as described for **14a**. Yield: 72%. Slightly brownish solid (recrystallized from water): ^{1}H NMR (DMSO-d_6) δ 12.40 (br, 1H, NH), 9.10 (s, 1H, NOH), 7.99−7.62 (2s, 2H, H-2, H-6), 6.75 (br, 2H, NH_2), 6.39 (d, J = 4.2 Hz, 1H, H-1′), 5.53 (br, 2H, 2OH), 5,07 (br, 1H, OH), 4.05 (m, 2H), 3.75 (dd, J = 9.0, 4.8 Hz, 1H), 3.60 (m, 2H, H-5′). Anal. ($C_{12}H_{15}N_5O_6$) C, H, N.

7-(β-D-Arabinofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone-5-carboxamidine hydrochloride (17c) was prepared from **17b** by the same procedure as described for **15b**. Yield: 58%. Slightly brownish solid (recrystallized from water): mp 232−234 °C (dec); ^{1}H NMR (DMSO-d_6) δ 13.05 (br, 1H, NH), 10.15 (br, 2H, NH_2), 8.71 (s, 2H, NH_2), 8.61 (s, 1H, H-6), 8.23 (s, 1H, H-2), 6.44 (d, J = 4.8 Hz, 1H, H-1′), 5.62 (m, 2H, 2OH), 5,09 (t, 1H, OH), 4.03−4.16 (m, 2H), 3.83 (m, 1H), 3.68 (m, 2H, H-5′). Anal. ($C_{12}H_{16}ClN_5O_5\cdot H_2O$) C, H, N.

7-(β-D-Arabinofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone-5-carboxamidrazone hydrochloride (17d). A solution of **17a** (300 mg) in methanol was slowly saturated with dry HCl at 10 °C for 2 h and then stood at ambient temperature for 6 h and was concentrated to dryness. Crystallization from methanol gave 272 mg (73%) of methyl 7-(β-D-arabinofuranosyl)-pyrrolo[2,3-*d*]-4-pyrimidone-5-formimidate hydrochloride as a colorless solid, which was converted to **17d** by the same procedure for preparation of **16d**.[27] Yield: 52%. Slightly brownish solid (recrystallized from water): ^{1}H NMR (DMSO-d_6) δ 12.2 (br, NH), 9.31 (br, NH_2), 8.47, 8.21 (2s, 2H, H-2, H-6), 6.43 (d, J = 5.1 Hz, 1H, H-1′), 4.70−5.80 (br, 5H, NH_2, 3OH), 4.13 (dd, J = 9.0, 4.5 Hz, 1H), 4.05 (m, 1H), 3.82 (dd, J = 10.8, 6.3 Hz, 1H), 3.60−3.78 (m, 2H, H-5′). Anal. ($C_{12}H_{16}N_6O_5\cdot HCl$) C, H, N.

5-Aminomethyl-7-(β-D-arabinofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone (17e) was prepared from **17a** by the same procedure as described for **16e**. Yield: 74%. Colorless solid (recrystallized from MeOH): mp 218−200 °C (dec); ^{1}H NMR

(DMSO-d_6) δ 7.85, 7.08 (2s, 2H, H-2, H-6), 6.31 (d, J = 4.2 Hz, 1H, H-1′), 5.48 (br, 4H, NH_2, 2OH), 5.05 (br, 1H, OH), 4.02 (m, 2H), 3.70 (s, 2H, CH_2), 3.52−3.69 (m, 3H).

7-(2,3-Dideoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone-5-carboxamidoxime (18b). 2′,3′-Dideoxytoyocamycin[29] was converted to **18a** by the same procedure as described for **13a**. Yield: 73%. Colorless solid (recrystallized from water): ^{1}H NMR (DMSO-d_6) δ 12.45 (br, 1H, NH), 8.34, 8.06 (2s, 2H, H-2, H-6), 6.31 (dd, J = 6.9, 2.7 Hz, 1H, H-1′), 5.03 (t, J = 5.4 Hz, 1H, OH), 4.10 (m, 1H, H-4′), 3.48−3.69 (m, 2H, H-5′), 2.13−2.50 (m, 2H), 1.97 (m, 2H).

Compound **18b** was prepared from **18a** by the same procedure as described for **14a**. Yield: 64%. Colorless solid (recrystallized from water): mp 194 °C (dec); ^{1}H NMR (DMSO-d_6) δ 12.35 (br, 1H, NH), 9.14 (s, 1H, NOH), 8.00, 7.66 (2s, 2H, H-2, H-6), 6.75 (br, 2H, NH_2), 6.30 (dd, J = 6.6, 3.9 Hz, 1H, H− 1′), 4.94 (t, J = 5.4 Hz, 1H, OH), 4.06 (m, 1H, H-4′), 3.44−3.62 (m, 2H, H-5′), 2.35−2.48 (m, 1H), 1.87−2.31 (m, 2H). Anal. ($C_{12}H_{15}N_5O_4$) C, H, N.

7-(2,3-Dideoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone-5-carboxamidine hydrochloride (18c) was prepared from **18b** by the same procedure as described for **15b**. Yield: 86%. Colorless solid (recrystallized from water): mp 259 °C (dec); ^{1}H NMR (DMSO-d_6) δ 13.0 (br, 1H, NH) 10.18 (br, 2H, NH_2), 8.81 (s, 1H, H-6), 8.81 (s, br, 3H, NH_2, H-6), 8.21 (s, 1H, H2), 6.37 (dd, J = 6.9, 3.0 Hz, 1H, H-1′), 4.96 (t, br, 1H, OH), 4.09 (m, 1H, H-4′), 3.56 (m, 2H, H-5′), 2.42−2.56 (m, 1H), 2.17−2.58 (m, 1H), 2.02 (m, 2H). The sample for elemental analysis was recrystallized from aqueous sodium carbonate solution and then from water. Anal. ($C_{12}H_{15}N_5O_3\cdot 0.5H_2O$) C, H, N.

5-Cyano-7-(2-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone (19a) was prepared from 2′-deoxytoyocamycin[30] by the same procedure as described for **13a**. Yield: 47%. Colorless solid (recrystallized from water): mp 235−236 °C (dec); ^{1}H NMR (DMSO-d_6) δ 12.49 (s, 1H, NH), 8.30, 8.08 (2s, 2H, H-2, H-6), 6.43 (t, J = 6.6 Hz, 1H, H-1′), 5.34 (d, J = 4.2 Hz, 1H, OH), 5.01 (t, J = 5.4 Hz, 1H, OH), 4.33 (m, 1H, H-3′), 3.83 (dd, J = 7.2, 4.2 Hz, H-4′), 3.46−3.62 (m, 2H, H-5′), 2.22−2.47 (m, 2H, H2′). Anal. ($C_{12}H_{12}N_4O_4$) C, H, N.

7-(2-Deoxy-β-D-Ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone-5-carboxamidine (19b) was prepared from **19a** by the same procedure as described for **15a**. Yield: 36%. Colorless solid (recrystallized from water): mp 251 °C (dec); ^{1}H NMR (DMSO-d_6) δ 11.1 (br, 2H, NH), 8.99 (s, 2H, NH_2), 8.23, 7.96 (2s, 2H, H-2, H-6), 6.43 (t, J = 6.9 Hz, 1H, H-1′), 5.35 (br, 1H, OH), 5.18 (br, 1H, OH), 4.35 (m, 1H, H-3′), 3.84 (m, H-4′), 3.53 (m, 2H, H-5′), 2.21−2.44 (m, 2H, H-2′). Anal. ($C_{12}H_{15}N_5O_4\cdot H_2O$) C, H, N.

5-Cyano-7-(β-L-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone (20a). The starting material, β-L-toyocamycin, was prepared according to the same procedure described for β-D-toyocamycin.[24] Colorless solid (recrystallized from water): mp 242−243 °C (dec); ^{1}H NMR (DMSO-d_6) δ 8.44, 8.21 (2s, 2H, H-2, H-6), 6.91 (br, 2H, NH_2), 6.05 (d, J = 5.7 Hz, 1H, H-1′), 5.47 (d, J = 6.0 Hz, 1H, OH), 5.17 (m, 2H, 2OH), 4.36 (dd, J = 11.1, 5.7 Hz, 1H), 4.09 (dd, J = 8.7, 4.8 Hz, 1H), 3.92 (dd, J = 7.2, 3.6 Hz, 1H), 3.51−3.69 (m, 2H, H-5′). Anal. ($C_{12}H_{13}N_5O_4$) C, H, N.

β-L-Toyocamycin was converted to **20a** by the same procedure as described for **13a**. Yield: 94%. Colorless solid (recrystallized from water): mp >250 °C (dec); ^{1}H NMR (DMSO-d_6) δ 12.51 (br, 1H, NH), 8.34, 8.09 (2s, 2H, H-2, H-6), 6.01 (d, J = 5.4 Hz, 1H, H-1′), 5.50 (d, J = 6.0 Hz, 1H, OH), 5.23 (d, J = 4.5 Hz, 1H, OH), 5.12 (t, J = 5.4 Hz, 1H, OH), 4.29 (dd, J = 10.5, 5.4 Hz, 1H), 4.07 (dd, J = 8.4, 4.8 Hz, 1H), 3.91 (dd, J = 7.2, 3.6 Hz, 1H), 3.51−3.68 (m, 2H, H-5′). Anal. ($C_{12}H_{12}N_4O_5$) C, H, N.

7-(β-L-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone-5-carboxamidoxime (20b) was prepared from **20a** by the same procedure as described for **14a**. Yield: 96%. Colorless solid (recrystallized from water): mp 187−188 °C (dec); ^{1}H NMR (DMSO-d_6) δ 12.4 (br, 1H, NH) 9.16 (br, 1H, NOH), 8.02, 7.71 (2s, 2H, H-2, H-6), 6.75 (br, 2H, NH_2), 6.07 (d, J = 6.0 Hz, 1H, H-1′), 5.40 (br, d, 1H, OH), 5.17 (br, d, 1H, OH), 5.06 (br, t, 1H, OH), 4.29 (m, 1H), 4.05 (d, J = 4.5 Hz, 1H), 3.89 (m, 1H), 3.49−3.62 (m, 2H, H-5′). Anal. ($C_{12}H_{15}N_5O_6\cdot H_2O$) C, H, N.

7-(β-L-Ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone-5-carboxamidine (20c) was prepared from **20b** by the same procedure as described for **15b**. After reaction was complete, the filtrate was concentrated to a small volume and was made alkaline (pH 9) with sodium carbonate. The precipitate was filtered and recrystallized from water to give 114 mg (74%) of **20c** as a colorless solid: mp 271 °C (dec); ^{1}H NMR (DMSO-d_6) δ 11.1 (br, 2H, NH), 8.81 (br, 2H, NH_2), 8.21, 7.95 (2s, 2H, H-2, H-6), 5.91 (d, J = 6.0 Hz, 1H, H-1′), 5.51 (br, 2H, 2OH), 5.25 (br, 1H, OH), 4.40 (t, J = 5.7 Hz, 1H), 4.07 (m, 1H), 3.93 (m, 1H), 3.49−3.66 (m, 2H, H-5′). Anal. ($C_{12}H_{15}N_5O_5$) C, H, N.

5-Cyano-7-(4-*C*-methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone (21a) was prepared from 4′-*C*-methyltoyocamycin[31] by the same procedure as described for **13a**. Yield: 83%. Colorless solid (recrystallized from methanol): mp 221−222 °C; ^{1}H NMR (DMSO-d_6) δ 12.48 (s, 1H, NH), 8.34, 8.07 (2s, 2H, H-2, H-6), 5.99 (d, J = 6.9 Hz, 1H, H-1′), 5.35 (d, J = 6.6 Hz, 1H, OH), 5.19 (m, 2H, 2OH), 4.2 (m, 1H, H-2′), 3.99 (t, J = 4.8 Hz, H-3′), 3.40−3.48 (m, 2H, H-5′), 1.12 (s, 3H, Me). Anal. ($C_{13}H_{14}N_4O_5$) C, H, N.

7-(2-Deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone-5-carboxamidine (21b) was prepared from **21a** by the same procedure as described for **15a**. Yield: 43%. Colorless solid (recrystallized from water/methanol): mp 235 °C (dec); ^{1}H NMR (DMSO-d_6) δ 11.2 (br, 2H, NH), 8.64 (s, 2H, NH_2), 8.20, 7.93 (2s, 2H, H-2, H-6), 5.83 (d, J = 7.8 Hz, 1H, H-1′), 5.37 (br, 1H, OH), 5.17 (br, 2H, OH), 4.66 (dd, J = 7.2, 5.4 Hz, 1H, H-2′), 4.02 (d, J = 5.1 Hz, 1H, H-3′), 3.29−3.50 (m, 2H, H-5′), 1.14 (s, 3H, Me). Anal. ($C_{13}H_{18}ClN_5O_5\cdot H_2O$) C, H, N.

(B) Biological Tests. Preparation of Human T Cells and Activation in Vitro. Peripheral blood mononuclear cells were isolated from healthy donors by density gradient centrifugation followed by T cell enrichment using Lymphokwik (One Lambda, Canoga Park, CA). Contaminating monocytes were removed by adherence to plastic. Purified T cells were >99% $CD2^+$, <1% $HLA\text{-}DR^+$, and <5% $CD25^+$ and were maintained in RPMI-AP5 (RPMI-1640 medium containing 5% autologous plasma, 1% L-glutamine, 1% penicillin/streptomycin, and 0.05% 2-mercaptoethanol). For determination of cytokine protein levels, T cells (0.2×10^6 cells in a volume of 0.2 mL) were activated by the addition of 2 ng of phorbol myristate acetate plus 0.1 mg of ionomycin (PMA-ION, both from Calbiochem, San Diego, CA) and incubated in 96-well plates in the presence of 0 or 10 μM of various test nucleosides for 48 h at 37 °C. Following activation, supernatants were analyzed for cell-derived cytokine production.

Extracellular Cytokine Analyses. Human cytokine levels were determined in cell supernatants, following appropriate dilution, using ELISA kits specific for IL-2, TNFα, IFNγ, IL-4 (Biosource, Camarillo, CA) and IL-5 (Endogen, Woburn, MA). All ELISA results were expressed as pg/mL. Data are shown as the percentage of activated control calculated as the ratio of activated T cell cytokine level in the presence of test nucleoside over the cytokine level of untreated activated T cells × 100%. Zero effect on cytokine levels by test nucleosides would give a percentage of activated control value of 100%.

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