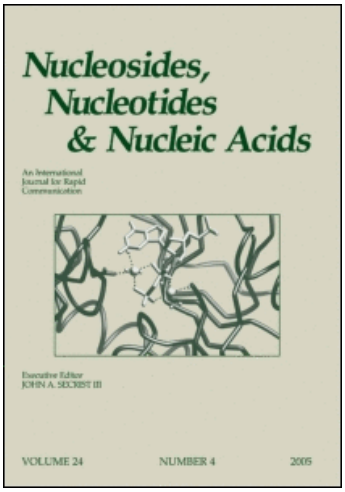

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis, Antiproliferative, and Antiviral Evaluation of Certain Acyclic 6-Substituted Pyrrolo[2,3-*D*]-pyrimidine Nucleoside Analogs Related to Sangivamycin and Toyocamycin

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To cite this Article Swayze, Eric E. , Shannon, William M. , Buckheit, Robert W. , Wotring, Linda L. , Drach, John C. and Townsend, Leroy B.(1992) 'Synthesis, Antiproliferative, and Antiviral Evaluation of Certain Acyclic 6-Substituted Pyrrolo[2,3-*D*]-pyrimidine Nucleoside Analogs Related to Sangivamycin and Toyocamycin', Nucleosides, Nucleotides and Nucleic Acids, 11: 8, 1507 — 1527
To link to this Article: DOI: 10.1080/07328319208021192
URL: http://dx.doi.org/10.1080/07328319208021192

NUCLEOSIDES & NUCLEOTIDES, 11(8), 1507-1527 (1992)

SYNTHESIS, ANTIPROLIFERATIVE, AND ANTIVIRAL EVALUATION OF CERTAIN ACYCLIC 6-SUBSTITUTED PYRROLO[2,3-*D*]-PYRIMIDINE NUCLEOSIDE ANALOGS RELATED TO SANGIVAMYCIN AND TOYOCAMYCIN

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ABSTRACT

A number of 6-substituted 7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine and 7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidine derivatives related to the nucleoside antibiotics toyocamycin and sangivamycin were prepared and tested for their biological activity. Treatment of 2-amino-5-bromo-3,4-dicyanopyrrole (**2**) with triethylorthoformate, followed by alkylation *via* the sodium salt method with either 2-(acetoxyethoxy)methyl bromide or (1,3-diacetoxy-2-propoxy)methyl bromide, furnished the corresponding N-substituted pyrroles **3a** and **3b**. These compounds were then smoothly converted to the requisite deprotected 4-amino-6-bromopyrrolo[2,3-*d*]-pyrimidine-5-carbonitriles **5a** and **5b** (toyocamycin analogs) by methanolic ammonia. The 6-amino-derivatives were obtained by a displacement of the bromo group with liquid ammonia. Conventional functional group transformations involving the 5-cyano group furnished the 5-carboxamide (sangivamycin) and 5-thioamide analogs. Compounds substituted at the 7-position with a ribosyl moiety were active against human cytomegalovirus (HCMV) at micromolar concentrations, but the apparent activity was not selective. The 7-ribosyl compounds also had no activity against human immunodeficiency virus (HIV), though they were all cytotoxic. The new compounds were also evaluated against HCMV, herpes simplex virus type I (HSV-1), HIV, and also for their ability to inhibit the growth of L1210 murine leukemic cells *in vitro*. None of these compounds with (2-hydroxyethoxy)methyl substituents or 7-(1,3-dihydroxy-2-propoxy)methyl substituent at N-7 showed significant cytotoxicity toward L1210, or toward uninfected human foreskin fibroblasts (HFF cells), and KB cells. Nor were they cytotoxic in human lines CEM or MT2. Only compound **4a** was found to be active against HCMV, having an IC_{50} of 32 μM.

Human cytomegalovirus (HCMV) is a member of the herpes family of viruses and typically infects immunocompromised individuals, such as those taking

immunosuppressive drugs or undergoing chemotherapy.[1] A more recent concern has been the large incidence of HCMV infections among patients infected with human immunodeficiency virus (HIV),[2] which is the causative agent of AIDS. Furthermore, it has been discovered that HCMV can stimulate HIV gene expression, which could enhance the consequences of the HIV infection.[3] The interrelationship between these two viruses indicates that simultaneous chemotherapy of both viral infections would be more beneficial than treatment for either infection alone. The drug of choice for the treatment of HCMV infections is 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (DHPG, ganciclovir),[1,4] which is active against several herpes viruses.[4,5] However, in both animal and human studies, ganciclovir has toxic effects,[6] including suppression of bone marrow.[7] This is especially problematic in AIDS patients since zidovudine (AZT), the only agent approved for the treatment of AIDS, is also myelosuppressive. The combined hematologic toxic effects of these two drugs precludes their simultaneous use,[8] and therefore coincident treatment of both HIV and HCMV infections is not currently possible. The severity of untreated HCMV infections and the toxicity of ganciclovir make the development of a more potent and less toxic drug for HCMV infections a high priority.

Prior work in our laboratory identified analogs of the pyrrolo[2,3-*d*]pyrimidine nucleoside antibiotics as possible anti-HCMV agents.[9-10] In a previous study, a series of derivatives including the ribosyl nucleosides tubercidin, toyocamycin, and sangivamycin were found to be active against HCMV at concentrations below 1 μM but were cytotoxic at similar concentrations.[9] Tubercidin and sangivamycin are known to be by phosphorylated by the cellular enzyme adenosine kinase, leading to cytotoxicity in uninfected cells as well as anti-viral activity.[11] Thus, it is possible that the other adenosine-type pyrrolo[2,3-*d*]-pyrimidine nucleosides studied[9] were phosphorylated by adenosine kinase as well. The activity of these compounds is thought to be due to incorporation of the phosphorylated nucleoside into RNA.[12] Their high toxicity, along with the observation that antiviral activity was not separated from cytotoxicity suggests that the adenosine type pyrrolo-[2,3-*d*]pyrimidine nucleosides are phosphorylated by cellular kinases.

In contrast, the highly selective antiviral agents 9-[(2-hydroxyethoxy)methyl]-guanine (ACV, acyclovir) and ganciclovir are preferentially phosphorylated by viral thymidine kinase in herpes simplex infected cells,[13] resulting in relatively low toxicity to uninfected cells. Based upon this rationale, we prepared several acyclic analogs of the nucleoside antibiotics tubercidin, toyocamycin, and sangivamycin to determine if they might be activated preferentially in infected cells, leading to selective antiviral activity.[10, 14-17] In one study, we found that acyclic 5-halogenated tubercidin analogs were active against HCMV and HSV-1.[10, 14,17] In contrast, we found to our surprise that the 5-CN (toyocamycin) and 5-$CONH_2$ (sangivamycin) acyclic analogs were not active against

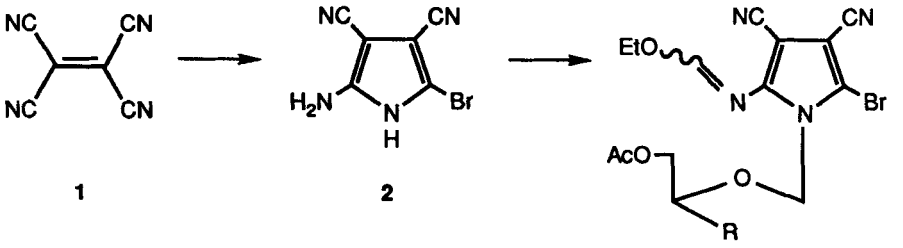
herpesviruses even though both the 2'-deoxy and arabinosyl analogs of toyocamycin, sangivamycin and thiosangivamycin were active antivirals.[9,18] One exception to this observation was our finding that acyclic 5-thiocarboxamides (thiosangivamycin analogs) were active against HCMV. Compounds with 7-(dihydroxypropoxy)methyl- (DHPM) and 7-(hydroxyethoxy)methyl- (HEM) substituents had 50% inhibitory concentrations of 8 μM[15] and 11μM,[16] respectively, with minimal visual cytotoxicity.

These considerations prompted us to extend our work with pyrrolo[2,3-*d*]-pyrimidines and to explore the effect of substitution at the 6-position. Initially, the known 6-Br and 6-NH_2 analogs of toyocamycin, sangivamycin and thiosangivamycin (**6a-f**)[19a] were evaluated for activity against HCMV and HSV-1. The high toxicity of these compounds toward both infected and uninfected cells (reported herein) encouraged us to prepare the corresponding acyclic analogs in order to determine if replacement of the ribosyl moiety with an acyclic side chain would enhance their selectivity as antiviral agents. In light of a recent report[20] which identified the anti-HIV activity of similar acyclic pyrrolo[2,3-*d*]pyrimidine nucleosides, we have also evaluated the 6-substituted toyocamycin derivatives **6a** and **6b** as well as the new acyclic nucleosides for activity against HIV.

CHEMISTRY

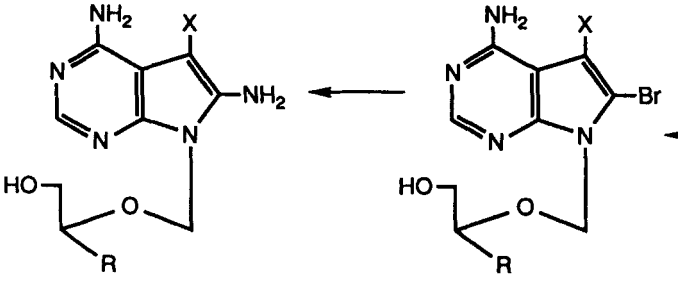
The previous route used in the synthesis of acyclic pyrrolo[2,3-*d*]pyrimidine nucleosides involved alkylation of 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine *via* the sodium salt method to yield a mixture of isomers which could not be seperated until after removal of the bromine moiety.[15,16] Since we desired functionalization of the 6-position, including the previously unisolated 6-bromo compounds, this method was unsuitable. A careful survey of the literature led us to consider the method of Ramasamy,[21] which fixes the site of alkylation at the desired location by first alkylating a pyrrole precursor. Annulation to the requisite pyrrolo[2,3-*d*]pyrimidine is then accomplished *via* treatment with methanolic ammonia. Using a modification[22] of Middleton's procedure,[23] 2-amino-5-bromo-3,4-dicyanopyrrole (**2**) was prepared from tetracyanoethylene (**1**) on a large scale in good yield. Treatment of this pyrrole with triethylorthoformate in refluxing acetonitrile afforded the ethoxymethylene derivative of **2** in quantitative yield. Alkylation of this derivative **2** with either (2-acetoxyethoxy)methyl bromide[24] or (1,3-diacetoxy-2-propoxy)methyl bromide[25] using the sodium salt method afforded the desired N-substituted pyrroles **3a** and **3b** in good yield.

Treatment of compounds **3a** and **3b** with methanolic ammonia at room temperature effected a ring annulation with a concurrent removal of the acetyl protecting groups to afford the acyclic pyrrolo[2,3-*d*]pyrimidines **5a** and **5b** in high yield. Treatment of compounds **5a** and **5b** with hydrogen peroxide in aqueous ammonia gave the desired



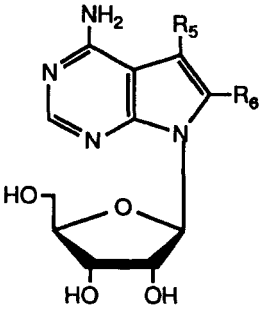
1 2

3a, R = H
3b, R = CH_2OAc



4a, R = H, X = CN
4b, R = CH_2OH, X = CN
4c, R = H, X = $CONH_2$
4d, R = CH_2OH, X = $CONH_2$
4e, R = H, X = $CSNH_2$
4f, R = CH_2OH, X = $CSNH_2$

5a, R = H, X = CN
5b, R = CH_2OH, X = CN
5c, R = H, X = $CONH_2$
5d, R = CH_2OH, X = $CONH_2$



6a, R_5 = CN, R_6 = Br
6b, R_5 = CN, R_6 = NH_2
6c, R_5 = $CONH_2$, R_6 = Br
6d, R_5 = $CONH_2$, R_6 = NH_2
6e, R_5 = $CSNH_2$, R_6 = NH_2

ELEMENTAL ANALYSES

Compound	Molecular Formula	Calcd/Found C	H	N
3a	$C_{14}H_5N_4O_4Br$	43.88	3.95	14.62
		43.71	4.09	14.80
3b	$C_{17}H_{19}N_4O_6Br$	44.85	4.21	12.31
		45.00	4.37	12.48
4a	$C_{10}H_{12}N_6O_2$	48.38	4.87	33.86
		48.15	4.87	33.89
4b	$C_{11}H_{14}N_6O_3$	47.47	5.07	30.21
		47.56	5.13	29.96
4c	$C_{10}H_{14}N_6O_3$	45.10	5.30	31.57
		45.22	5.20	31.59
4d	$C_{11}H_{16}N_6O_4$	44.59	5.44	28.37
		44.39	5.69	28.10
4e	$C_{10}H_{14}N_6O_2S$	42.54	5.00	29.78
		42.42	5.02	29.85
4f	$C_{11}H_{16}N_6O_3S$	42.29	5.16	26.91
		42.46	4.98	27.01
5a	$C_{10}H_{10}N_5O_2Br$	38.48	3.23	22.45
		38.62	3.35	22.55
5b	$C_{11}H_{12}N_5O_3Br$	38.62	3.54	20.48
		38.45	3.71	20.39
5c	$C_{10}H_{12}N_5O_3Br$	36.38	3.66	21.22
		36.34	3.89	21.09
5d	$C_{11}H_{14}N_5O_4Br$	36.68	3.92	19.45
		36.83	3.92	19.58

acyclic 4-amino-6-bromopyrrolo[2,3-*d*]pyrimidine-5-carboxamide nucleosides **5c** and **5d**, while displacement of the 6-bromine moiety occurred readily upon the reaction of **5a** and **5b** with liquid ammonia at 80 °C in a sealed vessel to yield the 6-amino derivatives **4a** and **4b**.

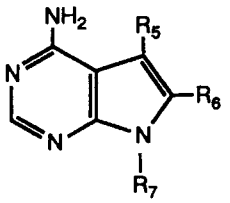
Unfortunately these 6-amino-5-cyano compounds were resistant to attack of the nitrile by hydrogen peroxide, presumably due to deactivation of the cyano group toward nucleophilic attack by the neighboring amino moiety. An alternate route to the desired 6-amino-5-carboxamides **4c** and **4d** would be the nucleophilic displacement of the bromine moiety at the 6-position of compounds **5c** and **5d**. However, treatment of these compounds with liquid ammonia at up to 130 °C afforded only starting material. Interestingly, it was previously found by our group[19a] that liquid ammonia at 120 °C did convert the ribonucleoside **6c** into the 6-amino-ribonucleoside **6d**. Our failure to obtain compounds **4c** and **4d** in a similar manner could be explained by the increased electron withdrawing capacity of a ribosyl moiety[19b] when compared to the alkoxymethyl substituents employed in this study. Earlier work on the ribonucleosides by our group revealed a possible route to the desired acyclic 4,6-diaminopyrrolo[2,3-*d*]pyrimidine-5-carboxamide nucleosides. An attempt to convert the 6-amino-5-cyano compound **6b** into the corresponding carboxamide oxime using hydroxylamine resulted in the unexpected formation of the carboxamide **6d**.[19a] Using this reaction as a model, we found that treatment of compounds **4a** and **4b** with hydroxylamine generated in situ from hydroxylamine hydrochloride and base in aqueous ethanol yielded the requisite carboxamides **4c** and **4d**.

The acyclic 4,6-diaminopyrrolo[2,3-*d*]pyrimidine-5-thiocarboxamide nucleosides **4e** and **4f** were prepared in moderate yields by the reaction of nitriles **4a** and **4b** with sodium hydrogensulfide generated *in situ* from methanolic sodium methoxide and hydrogen sulfide at 120 °C in a steel reaction vessel. We made several attempts to prepare the 6-bromo-5-thiocarboxamide analogs, all were unsuccessful. Treatment of **5c** with both P_4S_{10} and Lawesson's reagent gave an intractable mixture of products, as did a similar reaction with the mono *t*-butyl-dimethylsilyl derivative of **5c**. Direct treatment of **5a** with the conditions used for the synthesis of compound **4e** gave an intractable mixture of products, even at lower temperatures. Additionally, treatment of **5a** with diethylamine and hydrogen sulfide in dimethylformamide gave no isolatable products.

BIOLOGICAL RESULTS AND DISCUSSION

***In Vitro* Antiproliferative Evaluations.** The potential of these compounds as antitumor agents was evaluated by determining whether they inhibited the growth of L1210 murine leukemic cells *in vitro*. As shown in Table I, none of these acyclic 4-amino-5,6-disubstituted pyrrolo[2,3-*d*]pyrimidine nucleosides caused any significant inhibition.

TABLE I. Cytotoxicity of 6-Substituted Pyrrolo[2,3-*d*]pyrimidine Nucleosides Against L1210 Murine Leukemic Cells *In Vitro*



compound no.	R_5	R_6	R_7	growth rate,[a] % of control	IC_{50}, μM[b]
5a	CN	Br	HEM[c]	78	>100
5b	CN	Br	DHPM[c]	91	---
6a[d]	CN	Br	ribosyl	30	0.50
4a	CN	NH_2	HEM	88	>100
4b	CN	NH_2	DHPM	84	>100
6b[d]	CN	NH_2	ribosyl	31	0.26
5c	$CONH_2$	Br	HEM	92	---
5d	$CONH_2$	Br	DHPM	92	---
6c[d]	$CONH_2$	Br	ribosyl	26	0.80
4c	$CONH_2$	NH_2	HEM	92	---
4d	$CONH_2$	NH_2	DHPM	87	>100
6d[d]	$CONH_2$	NH_2	ribosyl	7	3.8
4e	$CSNH_2$	NH_2	HEM	92	---
4f	$CSNH_2$	NH_2	DHPM	91	---
6e[d]	$CSNH_2$	NH_2	ribosyl	22	0.27

[a]The effect of each compound at 100 μM on the growth rate of L1210 cells. [b]IC_{50} is the concentration required to decrease the growth rate to half of the control rate. A dash indicates no significant inhibition of cell growth at 100 μM. [c]Abbrevations used: HEM for (hydroxyethoxy)methyl, DHPM for (dihydroxypropoxy)methyl. [d]Synthesis of compounds **6a-e** has been reported previously.[19]

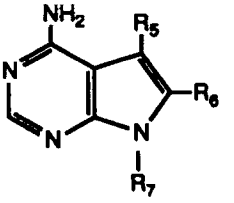
The lack of cytotoxicity of these compounds was not surprising, since replacement of the ribosyl moiety of pyrrolo[2,3-*d*]pyrimidine nucleosides with acyclic moieties has led to a loss of cytotoxicity in previous studies.[15,16] Thus, these data further support the generalization that acyclic pyrrolo[2,3-*d*]pyrimidine adenosine analogs, with a 5-CN, $CONH_2$ or $CSNH_2$ substituent have a decreased cytotoxic activity in comparision with the corresponding ribonucleosides.

Antiviral Activity. Several 6-substituted analogs of toyocamycin, sangivamycin, and thiosangivamycin were evaluated for activity against HCMV, HSV-1, and HIV (Tables II and III). Cytotoxicity of each compound was determined by several techniques in both growing and stationary cells. Examination for activity against HCMV and HSV-1 (Table II) revealed that substitution at position seven with a ribosyl moiety uniformly conferred good activity (IC_{50} <5 μM). This activity, however was coupled with cytotoxicity toward uninfected cells, establishing that there was no specific antiviral activity and precluding the use of the ribosyl compounds, *per se*, as anti-herpes agents. In contrast, the acyclic compounds were found to be devoid of antiviral activity against HCMV and HSV-1 with the exception of compound **4a**, which possessed slight activity against HCMV (IC_{50} = 32 μM). Additional evaluation against HCMV in yield reduction assays also showed slight activity (IC_{90} = 100 μM). All the other acyclics were inactive in yield reduction assays at the highest concentration tested (100 μM). The results with **4e** and **4f** were particularly disappointing because we hypothesized that these 6-substituted thioamide compounds would possess selective anti-HCMV activity, analogous to that previously reported for the 6-unsubstituted analogs.[15,16]

The lack of activity against HCMV and HSV-1 and low cytotoxicity of the acyclic nucleoside analogs may be due to inability of viral and cellular kinases to phosphorylate them. In contrast, the ribosyl derivatives show high toxicity to both infected and uninfected cells, and are presumably phosphorylated by cellular kinases. It has been reported that HCMV does not induce a viral thymidine kinase[26] but may induce a novel nucleoside kinase capable of phophorylating nucleosides.[27] The slight selective activity of compound **4a** against HCMV lends support to the theory that a unique mechanism is operating in HCMV infected cells, and may contribute to understanding more about the structural requirements necesary for nucleoside analogs to possess activity.

The new acyclic nucleosides, as well as some previously prepared ribosyl nucleosides also were tested for anti-HIV activity. As expected, the compounds bearing a ribosyl substituent (**6a** and **6b**, Table III) were extremely toxic toward uninfected cells, precluding their use as anti-HIV agents. Replacement of the ribosyl moiety with an acyclic side chain resulted in complete loss of both activity and cytotoxicity. It has been recently reported[20] that 4-amino-5-cyano-6-(methylthio)-7-[(1,3-dihydroxy-2-propoxy)methyl]-

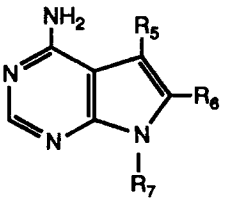
TABLE II. Antiviral Activity and Cytotoxicity of 6-Substituted Pyrrolo[2,3-*d*]pyrimidines



compound no.	R_5	R_6	R_7	50% inhibitory concentration (μM) antiviral activity[a] HCMV	HSV-1	cytotoxicity[b] visual	growth
5a	CN	Br	HEM[c]	>100[d]	>100	100	---
5b	CN	Br	DHPM[c]	>100	>100	100	---
6a	CN	Br	ribosyl	4	1.5	2.5	
4a	CN	NH_2	HEM	32	>100	>100	---
4b	CN	NH_2	DHPM	>100	>100	>100	---
6b	CN	NH_2	ribosyl	0.5		0.2	0.3
5c	$CONH_2$	Br	HEM	>100	>100	100	---
5d	$CONH_2$	Br	DHPM	>100	>100	100	---
6c	$CONH_2$	Br	ribosyl	3		10	3.6
4c	$CONH_2$	NH_2	HEM	>100	>100	>100	---
4d	$CONH_2$	NH_2	DHPM	>100		>100	---
6d	$CONH_2$	NH_2	ribosyl	3		7	6.8
4e	$CSNH_2$	NH_2	HEM	>100	>100	>100	---
4f	$CSNH_2$	NH_2	DHPM	>100	>100	>100	---
6e	$CSNH_2$	NH_2	ribosyl	0.4		0.6	<0.1
acyclovir				110[e]	3.4	>100	---
ganciclovir				8.2[f]	4.5	>100[f]	---

[a]Plaque assays and enzyme immunoassays (EIA) were performed as described in the text. Plaque assays were for HCMV, EIA for HSV-1. [b]Visual cytotoxicity was scored on HFF cells at time of HCMV plaque enumeration. Inhibition of KB cell growth was determined by labeled precursor incorporation as described in the text. [c]Abbrevations used: HEM for (hydroxyethoxy)methyl, DHPM for (dihydroxypropoxy)methyl. [d]>100 indicates IC_{50} not reached at the noted (highest) concentration tested. [e]Average concentration derived from at least three experiments. [f]Average of 81 experiments.

TABLE III. Activity Against HIV and Cytotoxicity of 6-Substituted Pyrrolo[2,3-*d*]-pyrimidine Nucleosides



compound no.	R_5	R_6	R_7	50% inhibitory concentration (μM) antiviral activity[a] HIV	cytotoxicity[b] MT2 cells	cytotoxicity[b] CEM cells
5a	CN	Br	HEM[c]	[d]	207	>320
5b	CN	Br	DHPM[c]	300[e]	>300	>300
6a	CN	Br	ribosyl	[d]	0.7	1.2
4a	CN	NH_2	HEM	>400[e]	>400	>400
4b	CN	NH_2	DHPM	>360[e]	>360	254
6b	CN	NH_2	ribosyl	[d]	0.1	---
5c	$CONH_2$	Br	HEM	>300[e]	>300	>300
5d	$CONH_2$	Br	DHPM	>280[e]	>280	>280
4c	$CONH_2$	NH_2	HEM	>380[e]	>380	>380
4d	$CONH_2$	NH_2	DHPM	>340[e]	>340	>340
4e	$CSNH_2$	NH_2	HEM	>350[e]	>350	>350
4f	$CSNH_2$	NH_2	DHPM	>320[e]	>320	>320
zidovudine (AZT)				0.002f	>39[f]	>39[f]

[a]Assays in MT2 and CEM cells as described in the text. [b]Cytotoxicity by XTT colorimetric assay as described in the text. [c]Abbrevations used: HEM for (hydroxyethoxy)methyl, DPHM for (dihydroxypropoxy)methyl. [d]Inactive against HIV at all concentrations tested up to 100 μg/ml. [e]Inactive and non-toxic at highest concentration tested (shown in table). [f]Average of four or more experiments.

pyrrolo[2,3-*d*]pyrimidine is active against HIV, having an IC_{50} of 20 μM. This compound differs from **4b** and **5b** only in that it bears a methylthio group at the six-position in place of an amino or bromine moiety, respectively. It is interesting that such a seemingly small variation in structure would result in complete loss of activity. The lack of anti-HIV activity found for our 6-substituted acyclic pyrrolo[2,3-*d*]pyrimidines, combined with the modest activity seen for several other acyclic nucleosides,[20] may help us understand the structure-activity relationships of anti-HIV compounds.

EXPERIMENTAL SECTION

Chemistry

General Procedures. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Thin-layer chromatography (TLC) was run on silica gel 60F-254 (Analtech, Inc.). Ultraviolet spectra were recorded on a Hewlett-Packard 8450 A spectrophotometer. Infrared (IR) spectra were taken on a Nicolet 5DXB infrared spectrometer. Nuclear magnetic resonance (NMR) spectra were determined at 270 MHz with a BRUKER WP 270 SY. The chemical shift values are expressed in δ values (parts per million) relative to the standard chemical shift of the solvent DMSO-d_6 or $CDCl_3$. Elemental analysis were performed by M-H-W Laboratories, Phoenix, AZ. E. Merck silica gel (230-400 mesh) was used for chromatography. Detection of components on TLC was made by UV light absorption at 254 nm. Evaporations were carried out under reduced pressure (water aspirator) with the bath temperature below 40 °C, unless specified otherwise.

General Procedure for the Preparation of 1-Substituted Pyrroles 3a and 3b. A mixture of 2-amino-5-bromo-3,4-dicyanopyrrole[22] (**2**, 4.40 g, 20.9 mmol) and triethylorthoformate (6.19 g, 41.8 mmol) in dry acetonitrile (150 mL) under argon was heated to reflux for 2 h, cooled to room temperature, and then evaporated to dryness. The crude solid was repeatedly treated with toluene (200 mL), then concentrated under reduced pressure. This process was repeated until a dry powder was obtained. The resulting solid was dissolved in dry acetonitrile (150 mL) and treated with sodium hydride (60% w/w, 1.00 g, 25.1 mmol) at room temperature. The solution was stirred for 0.5 h, then either (2-acetoxy-ethoxy)methylbromide (4.12 g, 20.9 mmol) or (1,3-diacetoxy-2-propoxy)methyl bromide (5.62 g, 20.9 mmol) was added dropwise. After another 0.5 h at room temperature, sodium acetate (0.17 g, 2.1 mmol) then acetic acid (0.1 mL, 2.1 mmol) was added. The mixture was filtered and the precipitate washed with acetonitrile. The filtrate and washings were concentrated to an oil, then diluted with chloroform and filtered through a 500 mL fritted funnel containing silica gel. The silica was washed with chloroform until no additional product was detected by TLC. The combined washings were concentrated to yield an off white oily solid, which was triturated with hexane, filtered, and recrystallized from methanol to yield pure **3a** or **3b**.

4-(Ethoxymethylene)imino-6-bromo-1-[2-acetoxyethoxy)methyl]-pyrrole-3,4-dicarbonitrile (3a). Yield 55%; mp 102-103 °C; TLC (5% MeOH in $CHCl_3$): $R_f = 0.72$; FTIR (KBr) ν_{max} 2227, 1741, 1725, 1613, 1243, 1137, 1111, 1057, 637 cm^{-1}; 1H NMR ($CDCl_3$): δ 8.39 (1H, s), 5.39 (2H, s), 4.37 (2H, qt), 4.16 (2H, t), 3.66 (2H, t), 2.03 (3H, s), 1.40 (3H, t). Anal. ($C_{14}H_5N_4O_4Br$). C, H, N.

4-(Ethoxymethylene)imino-6-bromo-1-[(1,3-diacetoxy-2-propoxy)methyl]pyrrole-3,4-dicarbonitrile (3b). Yield 78%; mp 106-107 °C; TLC (5% MeOH in $CHCl_3$): $R_f = 0.67$; UV λ_{max} (ε mM): (pH 1) 260 (7.6); (EtOH) 273 (11); (pH 11) 226 (13), 280 (11); FTIR (KBr) ν_{max} 2227, 1737, 1636, 1267, 1253, 1109, 1070, 1042, 837 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.54 (1H, s), 5.41 (2H, s), 4.38 (2H, qt), 4.12-3.98 (5H, m), 1.97 (6H, s), 1.34 (3H, t). Anal. ($C_{17}H_{19}N_4O_6Br$). C, H, N.

General Procedure for the Preparation of 7-Substituted 4-Amino-6-bromopyrrolo[2,3-*d*]pyrimidine-5-carbonitriles 5a and 5b. To 100 mL of saturated methanolic ammonia in a pressure bottle at -78 °C was added **3a** or **3b** (6 mmol). The bottle was sealed and the solution stirred for 24 h at room temperature. The resulting suspension was cooled to -78 °C and then filtered to yield pure **5a** or **5b**.

4-Amino-6-bromo-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]-pyrimidine-5-carbonitrile (5a). Yield 89%; mp 235-236 °C (d); TLC (40:9:1, EtOAc/MeOH/H_2O) $R_f = 0.71$; UV λ_{max} (ε mM): (pH 1) 230 (15), 282 (16); (EtOH) 219 (19), 285 (16); (pH 11) 229 (11), 285 (16); FTIR (KBr) ν_{max} 3569, 3366, 3329, 3116, 2942, 2225, 1666, 1649, 1596, 1364, 1306, 1115, 1051, 792 cm^{-1}; ^1H NMR (DMSO-d_6): δ 8.24 (1H, s), 6.70 (2H, s), 5.62 (2H, s), 4.66 (1H, t), 3.49-3.43 (4H, m). Anal. ($C_{10}H_{10}N_5O_2Br$). C, H, N.

4-Amino-6-bromo-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo-[2,3-*d*]pyrimidine-5-carbonitrile (5b). Yield 94%; mp 231-232 °C (d); TLC (40:9:1, EtOAc/MeOH/H_2O) $R_f = 0.62$; UV λ_{max} (ε mM): (pH 1) 230 (15), 282 (15.7); (EtOH) 219 (19), 285 (16); (pH 11) 228 (12), 285 (17); FTIR (KBr) ν_{max} 3340, 3065, 2233, 1665, 1602, 1391, 1312, 1102, 1061, 1037 cm^{-1}; ^1H NMR (DMSO-d_6): δ 8.23 (1H, s), 6.96 (2H, s), 5.69 (2H, s), 4.57 (2H, t), 3.60 (1H, m), 3.44-3.21 (4H, m). Anal. ($C_{11}H_{12}N_5O_3Br$). C, H, N.

General Procedure for the Preparation of 7-Substituted 4-Amino-6-bromopyrrolo[2,3-*d*]pyrimidine-5-carboxamides 5c and 5d. To **5a** or **5b** (6.3 mmol) in 100 mL 28% aqueous NH_4OH was added 10 mL of aqueous hydrogen peroxide (30% w/w). The mixture was stirred for 2 h at room temperature, then allowed to stand at 5 °C for 18 h. The resulting solid was filtered and washed with generous portions of cold water to yield crude product, which was recrystallized from ethanol/water to give pure **5c** or **5d**.

4-Amino-6-bromo-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]-pyrimidine-5-carboxamide (5c). Yield 81%; mp 236-237 °C (d); TLC (40:9:1, EtOAc/MeOH/H_2O) R_f = 0.44; UV λ_{max} nm (ε mM): (pH 1) 280 (19); (EtOH) 286 (13); (pH 11) 285 (15); FTIR (KBr) ν_{max} 3363, 3160, 1595, 1480, 1419, 1274, 1114, 1052, 841, 652, 565 cm^{-1}; ^1H NMR (DMSO-d_6): δ 8.13 (1H, s). 7.95 (1H, bs), 7.67-7.59 (3H, bs), 5.62 (2H, s), 4.66 (1H, t), 3.48-3.43 (4H, m). Anal. ($C_{10}H_{12}N_5O_3Br$). C, H, N.

4-Amino-6-bromo-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo-[2,3-*d*]pyrimidine-5-carboxamide (5d). Yield 56%; mp 208-209 °C; TLC (40:9:1, EtOAc/MeOH/H_2O) R_f = 0.30); UV λ_{max} nm (ε mM): (pH 1) 281 (20); (EtOH) 288 (15); (pH 11) 284 (16); FTIR (KBr) ν_{max} 3339, 3128, 1645, 1602, 1275 cm^{-1}; ^1H NMR (DMSO-d_6): δ 8.13 (1H, s), 7.95 (1H, bs), 7.67 (2H, bs), 7.56 (1H, bs), 5.70 (2H, s), 4.57 (2H, t), 3.62 (1H, m), 3.47-3.23 (4H, m). Anal. ($C_{11}H_{14}N_5O_4Br$). C, H, N.

General Procedure for the Preparation of 7-Substituted 4,6-Diaminopyrrolo[2,3-*d*]pyrimidine-5-carbonitriles 4a and 4b. A steel vessel containing **5a** (2.18 g, 7.0 mmol) was charged with 70 mL of liquid ammonia. The vessel was sealed and heated to 85 °C for 16 h. The ammonia was allowed to evaporate, and the residue was triturated with boiling methanol, then allowed to stand at 5 °C for 18 h. The resulting solid was filtered to yield **4a** or **4b**, which was pure by NMR and used in subsequent steps. A portion of this solid was recrystallized from ethanol/water for analysis.

4,6-Diamino-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (4a). Yield 94%; mp 255-257 °C (d); TLC (40:9:1, EtOAc/MeOH/H_2O) R_f = 0.50); UV λ_{max} nm (ε mM): (pH 1) 293 (12); (EtOH) 291 (18); (pH 11) 290 (17); FTIR (KBr) ν_{max} 3474, 3435, 3317, 3122, 2196, 1656, 1609, 1567, 1434, 1119, 1055, 649 cm^{-1}; ^1H NMR (DMSO-d_6): δ 8.00 (1H, s), 7.22 (2H, s), 6.11 (2H, s), 5.45 (2H, s), 4.65 (1H, bs), 3.44 (4H, bs). Anal. ($C_{10}H_{12}N_6O_2$). C, H, N.

4,6-Diamino-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]-pyrimidine-5-carbonitrile (4b). Yield 96%; mp 226-227 °C; TLC (40:9:1, EtOAc/MeOH/H_2O) R_f = 0.42; UV λ_{max} nm (ε mM): (pH 1) 294 (14); (EtOH) 291 (17); (pH 11) 291 (19); FTIR (KBr) ν_{max} 3255, 2200, 1659, 1595, 1574, 1441, 1060 cm^{-1}; ^1H NMR (DMSO-d_6): δ 8.00 (1H, s), 7.17 (2H, s), 6.12 (2H, s), 5.52 (2H, s), 4.70 (2H, t), 3.57-3.25 (5H, m). Anal. ($C_{11}H_{14}N_6O_3$). C, H, N.

4,6-Diamino-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (4c). To 40 mL of absolute ethanol was added sodium methoxide (330 mg, 6.0 mmol), then hydroxylamine hydrochloride (347 mg, 5.0 mmol). After stirring for 0.25 h, **4a** (248 mg, 1.0 mmol) and 10 mL water were added, and the solution was stirred

and heated at reflux for 9 h. The solvent was removed, and the resulting solid dissolved in warm water, then stored at 5 °C to yield 203 mg (76%) of **4c**. Recrystallization from ethanol/water gave needles (brown tinge): mp 210-211 °C (d); TLC (40:9:1, EtOAc/MeOH/H_2O) R_f = 0.22 ; UV λ_{max} nm (ε mM): (pH 1) 293 (16); (EtOH) 291 (18); (pH 11) 292 (19); FTIR (KBr) ν_{max} 3381, 3177, 1595, 1476, 1441, 1110, 1061 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.93 (1H, s), 7.43 (2H, s), 6.70 (2H, s), 6.22 (2H, s), 5.51 (2H, s), 4.65 (1H, bs), 3.45 (4H, bs). Anal. ($C_{10}H_{14}N_6O_3$). C, H, N.

4,6-Diamino-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]-pyrimidine-5-carboxamide (4d). To 40 mL of absolute ethanol was added sodium methoxide (330 mg, 6.0 mmol), then hydroxylamine hydrochloride (347 mg, 5.0 mmol). After stirring for 0.25 h, **4b** (278 mg, 1.0 mmol) and 10 mL of water were added and the solution was stirred and heated at reflux for 9 h. The solvent was removed, and the resulting solid dissolved in warm water, then stored at 5 °C to yield 164 mg (57%) of **4d** as its monohydrochloride salt. This solid was taken up in water and passed through a 1 cm column containing 3 g Dowex 1 ($^-$OH form). Concentration under reduced pressure and recrystallization from ethanol/water gave **4d** as flakes (brown tinge): mp 214-215 °C (d); TLC (40:9:1, EtOAc/MeOH/H_2O) R_f = 0.15; UV λ_{max} nm (ε mM): (pH 1) 294 (16); (EtOH) 291 (18); (pH 11) 293 (19); FTIR (KBr) ν_{max} 3402, 3184, 2917, 1609, 1462, 1061 639 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.93 (1H, s), 7.47 (2H, s), 6.99 (2H, s), 6.20 (2H, s), 5.57 (2H, s), 4.73 (2H, bs), 3.58-3.43 (5H, m). Anal. ($C_{11}H_{16}N_6O_4$). C, H, N.

4,6-Diamino-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine-5-thiocarboxamide (4e). Sodium methoxide (354 mg, 6.44 mmol) in 60 mL of anhydrous methanol was saturated with hydrogen sulfide at room temperature. This solution was added to **4a** (800 mg, 6.44 mmol), then heated to 120 °C in a sealed vessel for 16 h. Acetic acid (0.5 mL) was added to the room temperature solution and the solvent removed under reduced pressure. The resulting greenish solid was triturated with hot water, kept at 5 °C for 18 h, then filtered and dried to yield 673 mg (74%) of **4e**. This solid was dissolved in boiling ethanol/water, treated with decolorizing carbon, filtered and cooled to yield 430 mg of crystalline **4e**: mp 213-215 °C (d); TLC (40:9:1, EtOAc/MeOH/H_2O) R_f = 0.52l; UV λ_{max} nm (ε mM): (pH 1) 278 (12), 368 (10); (EtOH) 273 (13), 360 (9.1); (pH 11) 270 (13), 361 (9.6); FTIR (KBr) ν_{max} 3276, 1609, 1448, 1050, 878 cm^{-1}; ^1H NMR (DMSO-d_6): δ 8.15 (2H, bs), 8.06 (1H, s), 8.00 (2H, bs), 6.39 (2H, s), 5.52 (2H, s), 4.68 (1H, bs), 3.47 (4H, bs). Anal. ($C_{10}H_{14}N_6O_2S$). C, H, N.

4,6-Diamino-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]-pyrimidine-5-thiocarboxamide (4f). Sodium methoxide (110 mg, 2.00 mmol) in 20 mL of anhydrous methanol was saturated with hydrogen sulfide at room temperature. This solution was added to **4b** (278 mg, 1.00 mmol), then heated to 120 °C in a sealed vessel for 16 h.The room temperature solution was neutralized to pH 7 with 1N HCl, and the solvent removed under reduced pressure. The resulting greenish solid was triturated with hot water, kept at 5 °C for 18 h, then filtered and cooled to yield 149 mg (48%) of crystalline **4f**: mp 219-220 °C (d); TLC (40:9:1, EtOAc/MeOH/H_2O) Rf = 0.40; UV λ_{max} nm (ε mM): (pH 1) 277 (13), 367 (11); (EtOH) 273 (14), 361 (9.7); (pH 11) 270 (13), 361 (9.5); FTIR (KBr) ν_{max} 3150, 1631, 1595, 1455, 1040, 871 cm^{-1}; ^1H NMR (DMSO-d_6): δ 8.15 (2H, bs), 8.06 (1H, s), 7.94 (2H, bs), 6.38 (2H, s), 5.59 (2H, s), 4.73 (2H, t), 3.59 (1H, m) 3.53-3.28 (4H, m). Anal. ($C_{11}H_{16}N_6O_3S$). C, H, N.

BIOLOGICAL EVALUATIONS

***In Vitro* Antiproliferative Studies.** The *in vitro* cytotoxicity against L1210 was evaluated as described previously.[28] L1210 cells were grown in static suspension culture using Fischer's medium for leukemic cells of mice, with 10% horseserum (Hyclone Laboratories) and without antibiotics The growth rate was calculated from determinations of cell number at 0, 48, and 96 h in the presence of various concentrations of the test compound. Growth rate was defined as the slope of the semilogarithmic plot of cell number against time for the treated culture as a percent of the slope for the control culture. This parameter was determined experimentally by calculating the ratio of the population doubling time (T_d) of control cells to the T_d of treated cells. When the growth rate decreased during the experiment, the rate reported was that between 48 and 96 h. The IC_{50} was defined as the concentration required to decrease the growth rate to 50% of the control.

***In Vitro* Antiviral Evaluation. (a) Cells and Viruses.** KB cells, an established human cell line derived from an epidermoid oral carcinoma, were routinely grown in minimal essential medium (MEM) with Hank salts [MEM(H)] supplemented with 5% fetal bovine serum. Diploid human foreskin fibroblasts (HFF cells) were grown in MEM with Earle's salts [MEM(E)] supplemented with 10% fetal bovine serum. Cells were passaged according to conventional procedures as detailed previously.[9] A plaque-purified isolate, P_O, of the Towne strain of HCMV was used and was a gift of Dr. M. F. Stinski, University of Iowa. The S-148 strain of HSV-1 was provided by Dr. T. W. Schafer of Schering Corporation. Stock preparations of HCMV and HSV-1 were prepared and titered as described elsewhere.[9] The HTLV-IIIB strain of HIV-1 was propagated in the human T-lymphocyte cell line, H9 as detailed elsewhere.[29] The virus inoculum consisted of supernatant fluids from H9-IIIB producer cultures.

(b) Antiviral Assays for Herpesviruses. HCMV plaque reduction experiments were performed using monolayer cultures of HFF cells by a procedure similar to that referenced above[9] for titration of HCMV, with the exceptions that the virus inoculum (0.2 mL) contained approximately 50 PFU of HCMV and the compounds to be assayed were dissolved in the overlay medium. Protocols for the HCMV yield reduction assay have been described previously.[30] HSV-1 was assayed using an enzyme immunoassay described by Prichard and Shipman.[31]

(c) Antiviral Assay for HIV.

(1) *Drug Dilution and Addition to Plates.* Drugs were dissolved in DMSO at 40 mg/mL or in sterile deionized water at 2 mg/mL, unless otherwise specified. Drug dilutions were made in medium. Drugs in DMSO were initially diluted 1/200; drugs in water diluted 1/10. Subsequent dilutions were made in log or 0.5 log series. Each dilution was added to plates in the amount of 100 µl/well. Drugs were tested in triplicate wells per dilution with infected cells, and in duplicate wells per dilution with uninfected cells for evaluation of cytotoxicity. After addition of cells to plates (see following section), the highest drug concentration was 100 µg/mL, except for control experiments with zidovudine where 10 µg/mL was the highest concentration used. The highest DMSO concentration was 0.25%.

(2) *Infection and Distribution of Cells to Microtiter Plates.* A viable cell count (trypan blue) was performed on the cells to be used. The desired total number of polybrene treated cells was placed in a 50 mL conical centrifuge tube (sterile, disposable), and virus was added to give a MOI of 0.03 $TCID_{50}$/cell on MT-2 cells and approximately 0.12 $TCID_{50}$/cell on CEM cells. Fresh medium was added to adjust the cell density to 1 x 10^5 cells/mL, and the virus-cell suspension was incubated at 37 °C for 1-2 hrs until ready for plating. Uninfected cells were prepared in the same manner but without the addition of virus. Cell pellets were collected by low speed centrifugation and supernates were discarded. Infected and uninfected cells were resuspended in appropriate volume of medium and added to plates in the amount of 100 µL/well to give a starting cell number of 1 x 10^4 cells/well. Plates were incubated for 7 days in a humidified atmosphere of 5% CO_2 in air.

(3) *Quantitation of Viral Cytopathic Effect (CPE) and Drug Activity.* On day 7 post-infection, the viable cells were measured with a tetrazolium salt, XTT added to the test plates. A solution was used to dissolve the XTT formazan produced. The optical density value is a function of the amount of formazan produced which is proportional to the number of viable cells. Plates were read at a wave-length of 570 nm on a V_{max} plate reader (Molecular Devices). The percent inhibition or CPE per drug concentration was measured as test over control and expressed in percent.

(d) Cytotoxicity Assays. Their basic tests for cellular cytotoxicity were routinely employed for compounds examined in antiviral assays. Cytotoxicity produced in HFF cells was estimated by visual scoring of cells not affected by virus infection in the plaque reduction assays described above. Drug-induced cytopathology was estimated at 35- and 60-fold magnificaiton and scored on a zero to four plus basis on the day of staining for plaque enumeration. Cytotoxicity in KB cells was determined by measuring the effects of compounds on the incorporation of tritiated thymidine, uridine and leucine into acid precipitable material. Following data analysis (see below), data for the precursors were averaged and the average presented. Details of the procedure are in reference 9. Cytotoxicity in CEM and MT2 cells was determined colorimetrically as described above in the HIV Assay except uninfected cells were used.

(e) Data Analysis. Dose-response relationships were constructed by linear regression analysis of the percent inhibition of parameters derived in the preceeding sections against log of drug concentration. Fifty-percent inhibitory (IC_{50}) concentrations were calculated from the regression lines. Samples containing positive controls (acyclovir, gancyclovir, zidovudine) were used in all assays. Results from sets of assays were rejected if inhibition by the positive control deviated from its mean response by more than 1.5 standard deviations.

Acknowledgement. The authors are indebted to Mary A. Walters, Scott K. Eberhardt, Gail Genzlinger, Lisa A. Coleman, Allison C. Westerman, Sandra L. Engelhardt and Edward D. Kreske for expert technical assistance. We also thank Jack M. Hinkley for large scale preparations of starting materials as well as Mrs. Rae L. Herrst for her expert preparation of the manuscript. This work was supported with Federal Funds from the Department of Health and Human Services under contracts NO1-AI-42554, NO1-AI-72641 and in part by a research grant #CH-312 from the American Cancer Society. Studies also were supported by the University of Michigan/Southern Research Institute National Cooperative Drug Discovery Group funded by grant U01-AI25739. Linda L. Wotring was supported in part by a grant from the Office of the Vice President for Research, the University of Michigan.

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Received 2/21/92
Accepted 5/14/92