Synthesis, Antiproliferative and Antiviral Activity of Imidazo[4,5-*d*]isothiazole Nucleosides as 5:5 Fused Analogs of Nebularine and 6-Methylpurine Ribonucleoside

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A series of imidazo[4,5-*d*]isothiazole nucleosides related to the antibiotic nebularine and the highly cytotoxic 6-methyl-9-β-D-ribofuranosylpurine have been synthesized from the corresponding heterocycles. The sodium salt glycosylation of the imidazo[4,5-*d*]isothiazoles proceeded smoothly, giving mixtures of N-4 and N-6 regioisomers in generally good yields. The protected derivatives were deblocked using standard conditions to afford the desired imidazo[4,5-*d*]-isothiazole nucleosides, usually as crystalline solids. None of the new nucleosides or heterocycles displayed selective activity against human cytomegalovirus (HCMV) or herpes simplex virus type 1 (HSV-1). The N-6 glycosylated imidazo[4,5-*d*]isothiazoles were completely inactive up to the highest concentration tested. The N-6 glycosylated imidazo[4,5-*d*]isothiazoles also were inactive in antiproliferative and cytotoxicity assays, except for 3-methyl-6-β-D-ribofuranosylimidazo[4,5-*d*]isothiazole (**15a**) and 5-(benzylthio)-6-(2-deoxy-β-D-ribofuranosyl)imidazo[4,5-*d*]-isothiazole (**5e**), which showed moderate inhibition of L1210 cell growth. However, the heterocycles and several of the N-4 glycosylated derivatives were toxic to HFF, KB and L1210 cells; compounds with 5-benzylthio substituents were the most cytotoxic agents in this series.

Introduction

Purine nucleosides and their analogs have been extensively investigated as antitumor and antiviral agents.[1−14] While these studies have included compounds with a variety of ring modifications and substituents, most have contained a fused 6:5 carbon−nitrogen heterocyclic system analogous to the purine ring, for example, 9-β-D-ribofuranosylpurine (nebularine) (**I**, Figure 1)[6] and 6-methyl-9-β-D-ribofuranosylpurine (**II**).[18] As part of our program to prepare a series of novel purine nucleoside analogs, we were interested in the replacement of the C2−N3 grouping of the purine ring with a single sulfur atom. The sulfur atom has been proposed to be analogous to a −CH=CH− grouping because of its steric and electronic properties.[15] For example thiophene could be viewed as an analog of benzene, and 5-amino-2-β-D-ribofuranosyl-1,2,4-thiadiazol-3-one might be considered an analog of cytidine.[16] We have extended this analogy and envisioned imidazo-[4,5-*d*]isothiazoles as analogs of purines having the −(N=CH)− group in the pyrimidine ring of purines replaced with a bivalent sulfur atom. We have recently prepared[19] a series of imidazo[4,5-*d*]isothiazoles and shown that they retain similar spatial characteristics relative to the parent purines[17,18] via a modeling study, which was later confirmed by an X-ray crystal structure.[19] These considerations suggest that the nucleosides of imidazo[4,5-*d*]isothiazoles should be good steric mimics of the corresponding purine nucleosides, but with very different electronic character, which may lead to some interesting biological properties.

A series of imidazo[4,5-*d*]isothiazole nucleosides (**III**), including the analog **15a** of 6-methylpurine (**II**), has been prepared by coupling the previously described imidazo[4,5-*d*]isothiazoles[19,22,23] with the appropriate sugar derivatives. In addition to the ribosyl nucleosides, the 2′-deoxyribosyl and arabinosyl derivatives were prepared. All compounds were tested for activity against two herpes viruses and for effects on the proliferation of one normal and two cancer cell lines.

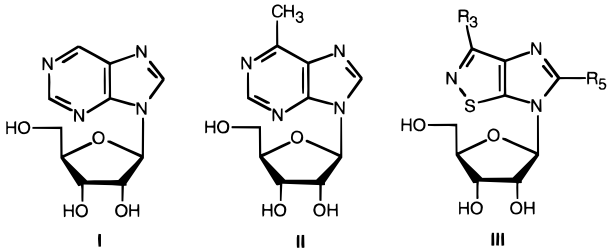

Figure 1. Substituted 6-(β-D-ribofuranosyl)imidazo[4,5-*d*]-isothiazoles (**III**) as analogs of nebularine (**I**) and 6-methylpurine ribonucleoside (**II**).

Chemistry

The sodium salt glycosylation method[20,21,24] was used to prepare the N-4 and N-6 2′-deoxyribosyl derivatives of the variously substituted imidazo[4,5-*d*]isothiazoles, analogously to the imidazo[4,5-*d*]isothiazoles from 3-methyl-5-(methylthio)imidazo[4,5-*d*]isothiazole (**1b**)[19] (Scheme 1). Reaction of the *in situ* generated sodium salt of compounds **1a**−**e**[19] with 2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl chloride (**2**)[25] at room temperature gave the corresponding N-6 and N-4 β-nucleosides **3** and **4** in 63−88% overall yields, with no evidence of the formation of the corresponding α-anomers seen in the ^{1}H NMR spectra. The regiospecificity of the reaction appeared to be governed by steric principles, as heterocycles bearing both 3- and 5-substituents gave primarily N-6 nucleosides (for example, **3b**:**4b** = 7:2) while the less hindered derivatives afforded

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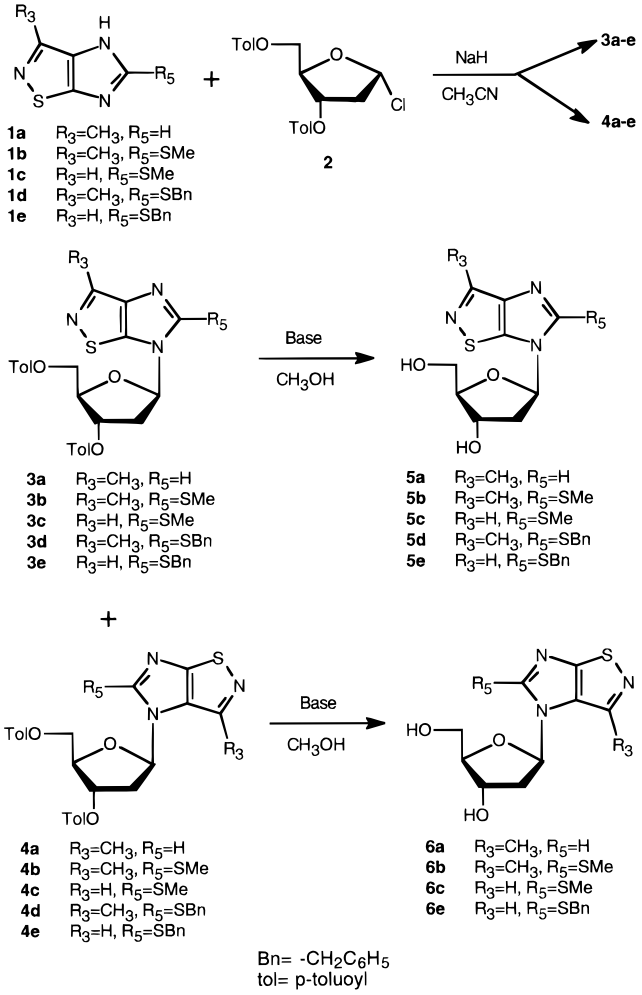
Scheme 1. Synthesis of (2′-Deoxyribofuranosyl)imidazo[4,5-*d*]isothiazoles



1a R_3=CH_3, R_5=H
1b R_3=CH_3, R_5=SMe
1c R_3=H, R_5=SMe
1d R_3=CH_3, R_5=SBn
1e R_3=H, R_5=SBn

2

3a R_3=CH_3, R_5=H
3b R_3=CH_3, R_5=SMe
3c R_3=H, R_5=SMe
3d R_3=CH_3, R_5=SBn
3e R_3=H, R_5=SBn

5a R_3=CH_3, R_5=H
5b R_3=CH_3, R_5=SMe
5c R_3=H, R_5=SMe
5d R_3=CH_3, R_5=SBn
5e R_3=H, R_5=SBn

4a R_3=CH_3, R_5=H
4b R_3=CH_3, R_5=SMe
4c R_3=H, R_5=SMe
4d R_3=CH_3, R_5=SBn
4e R_3=H, R_5=SBn

6a R_3=CH_3, R_5=H
6b R_3=CH_3, R_5=SMe
6c R_3=H, R_5=SMe
6e R_3=H, R_5=SBn

the N-4 and N-6 products in an approximately 1:1 ratio (for example **3a**:**4a** = **3c**:**4c** = 1:1). The N-4 and N-6 regioisomers were readily separated by flash column chromatography to give the protected nucleosides, and the blocking groups were then removed via methanolysis to afford the desired nucleosides **5a**−**e** and **6a**−**c**,**e** as crystalline solids.

The site of glycosylation was determined by a comparison of the UV spectra of compounds **5** and **6** with the spectra of the corresponding alkylated compounds prepared previously.[19] The N-6 glycosylated 5-unsubstituted compound **5a** had a UV spectrum which compared well with that of the corresponding 6-methyl derivative prepared by a direct synthesis, showing a strong absorption with a λ_{max} at 208 nm and medium absorption having a λ_{max} of 248 nm This was readily discernible from the pattern shown by **6a**, which included a strong absorption at a λ_{max} of 246 nm and a distinctive shoulder in the 250 nm region. Assignment of the 5-substituted derivatives **5b**−**e** and **6b**,**c**,**e** was accomplished in a similar manner. The UV spectra of the previously prepared 3,4-dimethyl-5-(methylthio)-imidazo[4,5-*d*]isothiazole displays two distinct peaks at 215 and 276 nm and a shoulder at 258 nm. In contrast, 3,6-dimethyl-5-(methylthio)imidazo[4,5-*d*]isothiazole shows an absorption with a λ_{max} at 219 and 270 nm, with no shoulder evident. These patterns were nearly identical to those shown by the 5-substituted compounds (series **b**−**e**). It was also possible to make a tentative assignment of the site of glycosylation before deprotection by observing the TLC mobility (ethyl acetate/hexane) of each isomer formed, since in all cases the N-4 isomer had a larger R_f value than the corresponding N-6 isomer.

The carbon-13 NMR spectra[26] of the N-6 and N-4 derivatives also showed a distinctly different pattern in the chemical shifts of the four imidazo[4,5-*d*]isothiazole carbon atoms, which compared well with that of the corresponding *N*-methyl derivatives. It can be seen from Table 1 that the bridgehead carbons of N-4 derivatives are shifted dramatically with respect to the corresponding N-6 isomers. The C3a signals of the N-4 compounds show a large upfield shift of 12−14 ppm, while the C6a resonances are shifted downfield 15−18 ppm, relative to the corresponding N-6 derivatives. The other imidazo[4,5-*d*]isothiazole ^{13}C resonances exhibit smaller shifts. The N-4 isomers also show a 3−5 ppm upfield shift of the C3 signal and 1−3 ppm downfield shift of the C5 resonance, relative to the N-6 isomers. This pattern is consistent with that shown by the 4-methyl compounds, which show an upfield shift of 10 ppm for C3a and a downfield shift of 10 ppm for C6a, with respect to the 6-methyl analog. Furthermore, this pattern is also consistent with that shown by the related purine ring system. For example, 7-methylpurine (analogous to a 4-methylimidazo[4,5-*d*]isothiazole) shows an upfield shift of 8 ppm and a downfield shift of 9 ppm for the corresponding bridgehead carbon signals, relative to 9-methylpurine (analogous to a 6-methylimidazo-[4,5-*d*]isothiazole).[26] This distinctive pattern of carbon-13 chemical shifts shown by N-substituted imidazo-[4,5-*d*]isothiazoles provides a simple means for the assignment of the regiochemistry of substituted imidazo-[4,5-*d*]isothiazole nucleosides.

The individual assignments of the imidazo[4,5-*d*]-isothiazole carbon atoms for N-6 substituted derivatives were made by observing the fully coupled and partially decoupled ^{13}C NMR spectra. The 3- and 5-unsubstituted derivatives have nonquaternary ring carbons, which could readily be assigned due to the large one-bond coupling (J_{CH}). The bridgehead and other quaternary ^{13}C resonances were readily assigned by observing the partially decoupled spectra. For example, irradiating at the frequency of the resonance due to the 3-methyl peak of **5b** in the ^1H NMR spectrum decouples the C3 and C3a resonances, which appear as sharp singlets at δ 152.1 and 147.3 ppm, respectively. The C3 carbon is assigned to the most downfield resonance on the basis of its chemical shift in relation to the 3-unsubstituted derivatives. The introduction of a methyl group causes the expected large downfield shift (∼9 ppm) of the C3 carbon, while the downfield shift of the C3a is only 1−2 ppm. Furthermore, the absolute intensity of the C3 signal is larger than that of the C3a resonance, due to the NOE enhancement provided by decoupling the adjacent methyl protons. The C6a shows a three-bond coupling to the anomeric proton ($^3J_{CH}$ = 5 Hz) and appears as a doublet at δ 147.1 ppm. The C5 appears as a multiplet at δ 151.6 ppm, due to the presence of two three-bond couplings. Assignment of the other N-6 derivatives was accomplished in a similar manner, or by analogy to these assignments.

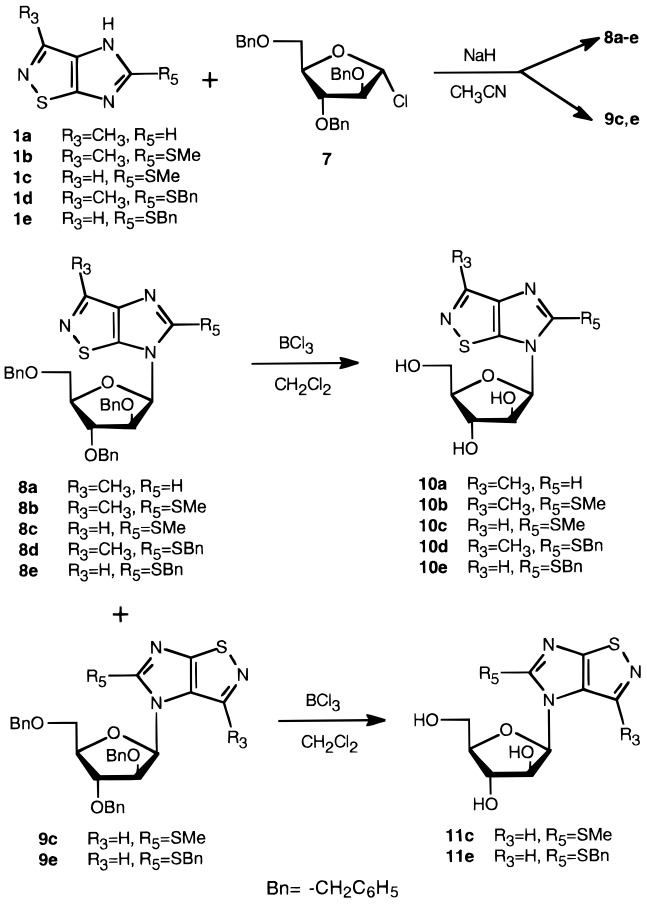
The carbon-13 resonances for the N-4 isomers were assigned on the basis of their chemical shifts and also

by observation of the fully coupled and partially decoupled ^{13}C spectra. The C6a carbon of N-4 imidazo[4,5-*d*]isothiazoles is uncoupled and appears as a sharp singlet as the most downfield resonance at δ 163 ppm. Irradiation at the frequency of the 3-methyl signal in the ^1H NMR spectrum of **6b** decouples the two- and three-bond couplings to the C3 and C3a carbons, respectively. Therefore, C3 appears as a singlet with high relative intensity (due to NOE enhancement from decoupling), while C3a appears as a doublet ($^3J_{CH} = 5$ Hz) at the most upfield resonance of 135−136 ppm, due to a three-bond coupling from the anomeric proton. The signal corresponding to C5 is complex, due to multiple couplings, and appears significantly downfield of the C3a signal. The C3 resonance shows either a large one-bond coupling for the 3-unsubstituted derivatives or a small ($^3J_{CH} = 7$ Hz for **6b**) two-bond coupling for the 3-methyl derivatives in the fully coupled ^{13}C spectrum. The large differences in the chemical shifts of the carbon-13 resonances for the N-4 isomers readily allows the assignment of a new derivative via the application of standard carbon-13 substituent effect chemical shift rules onto an analogous N-4 imidazo[4,5-*d*]isothiazole.

The anomeric configuration was assigned as β by an inspection of the ^1H NMR spectra, which showed the anomeric proton as a pseudotriplet for the 5-unsubstituted compounds **5a** and **6a**. When a substituent was present at the 5-position (**5b**−**e** and **6b**,**c**,**e**), the pseudotriplet was not observed, and the resonance assigned to the anomeric proton appeared as a doublet of doublets with a peak width of 15−17 Hz. These observations are consistent with the spectra observed for similar 8-unsubstituted and 8-substituted 2′-deoxyribofuranosylpurine nucleosides, and led to the assignment of these compounds as β-nucleosides.[27] Furthermore, the starting halogenose is known to have the α-configuration in the solid state[28] and has previously led to the exclusive formation of β-anomers utilizing the sodium salt glycosylation procedure at room temperature,[29,30] presumably due to a direct Walden inversion at the C1 carbon.

The arabinofuranosides **10** and **11** (Scheme 2) were also prepared using the sodium salt glycosylation method, employing 2,3,5-tri-*O*-benzyl-α-D-arabinofuranosyl chloride (**7**)[31] as the requisite halogenose. As found for the corresponding 2-deoxyribofuranosyl synthon, the regiospecificity of the reaction appeared to be governed by steric parameters. However, the halogenose **7** is more hindered than the corresponding 2-deoxyhalogenose **2**, and the reaction therefore proceeded with more selectivity for the N-6 isomers. The N-6 isomers **8a**−**e** were isolated in 46−68% yield after chromatography to remove traces of the minor N-4 isomer (4−30%), as well as unreacted starting material (11−17%). Only when no 3-substituent was present was a significant quantity of the N-4 isomers **9c** and **9e** isolated (24% and 30%, respectively). The increased steric requirements of the arabinofuranosyl synthon were also evident by slower reaction rates. Although homogeneous by TLC, a small amount of the α-anomer was evident for products **8** (3−5%), **9c** (20%), and **9e** (7%) upon examination of the ^1H NMR spectrum. In all cases, the anomeric proton of the major component appeared downfield of that of the minor component. Thus, the configuration of the major product was assigned as β (C1′−C2′ *cis*) and the minor product as α (C1′−C2′ *trans*), since this pattern of chemical shifts is indicative of β-anomers.[27] That the major product is the β-anomer is expected, since the starting halogenose is predominantly the α-anomer, and the gycosylation is presumed to occur via an S_N2 mechanism.

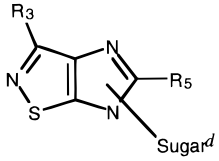
Scheme 2. Synthesis of Arabinofuranosylimidazo[4,5-*d*]isothiazoles



The blocking groups were removed via treatment with boron trichloride in dichloromethane (−78 °C to 0 °C) to afford the nucleosides **10a**−**e**, **11c**, and **11e**. The workup of this reaction proved critical, as these nucleosides were quite sensitive to a cleavage of the sugar moiety by acidic conditions. If the quenched acidic reaction mixture was not completely neutralized at 0 °C, the desired nucleoside was converted to the corresponding heterocycle. The nucleoside derivative **10a** was obtained in crystalline form and in a nearly quantitative yield. However the 5-substituted compounds proved more difficult to crystallize and were subject to decomposition. The yields of pure **10b**−**e**, **11c**, and **11e** were therefore modest, despite the apparent clean conversion of the protected nucleosides into a single product (by TLC and ^1H NMR of the crude product). In several cases, the nucleosides could not be induced to crystallize and were isolated as hard foams by lyophilization. Despite the presence of traces of the corresponding α-anomer before deprotection, only a single anomer could be detected by ^1H NMR after the products had been isolated by chromatography or crystallization.

The site of glycosylation was determined by examination of UV and ^{13}C NMR spectra in a similar manner as for the 2′-deoxy derivatives **5** and **6**. The assignments for the imidazo[4,5-*d*]isothiazole carbon-13 resonances

Table 1. Heteroaromatic Carbon-13 Resonances of Imidazo[4,5-*d*]isothiazole Nucleosides



compd no.	substituent R_3	substituent R_5	sugar[d]	chemical shift (ppm)[a] C3	C3a	C6a	C5
			Deoxy				
5a	CH_3	H	N-6	153.3	148.8	146.0	145.0[b]
6a	CH_3	H	N-4	148.4	134.9	163.6	144.5[b]
5b	CH_3	SCH_3	N-6	152.1	147.3	147.1[b]	151.6[b]
6b	CH_3	SCH_3	N-4	147.3[b]	135.2[b]	163.1[b]	154.2[b]
5c	H	SCH_3	N-6	143.7[b]	148.9	147.9	152.4
6c	H	SCH_3	N-4	140.0[b]	136.6	163.2	154.9
5d	CH_3	$SCH_2C_6H_5$	N-6	152.3	147.3	146.9	149.4
5e	H	$SCH_2C_6H_5$	N-6	143.5[b]	148.6	147.4	150.0
6e	H	$SCH_2C_6H_5$	N-4	140.2[b]	136.4	163.0	153.0
			Arabino				
10a	CH_3	H	N-6	152.0[b]	148.2[b]	148.4[b]	145.3[b]
10b	CH_3	SCH_3	N-6	151.1	147.0	150.1[b]	151.4[b]
10c	H	SCH_3	N-6	143.0	148.6	150.9	152.5
11c	H	SCH_3	N-4	141.6	138.8	161.8	153.5
10d	CH_3	$SCH_2C_6H_5$	N-6	151.3	147.0	149.9[c]	149.5[c]
10e	H	$SCH_2C_6H_5$	N-6	143.2	148.6	150.7[c]	150.6[c]
11e	H	$SCH_2C_6H_5$	N-4	141.7	138.6	161.7	151.8
			Ribo				
15a	CH_3	H	N-6	153.2	148.9	146.0	145.3
16a	CH_3	H	N-4	148.2	134.7	163.5	144.5
15b	CH_3	SCH_3	N-6	152.0	147.5	147.2	152.8
15c	H	SCH_3	N-6	143.7	149.2	148.0	153.7
16c	H	SCH_3	N-4	140.2	136.7	163.3	155.9
15d	CH_3	$SCH_2C_6H_5$	N-6	152.2	147.5	147.1	150.8

[a] Assignments by analogy and from chemical shifts unless otherwise specified. [b] Unequivocally assigned from fully or partially coupled spectra. [c] Assignment uncertain. [d] Abbreviation used: deoxy = 2′-deoxy-β-D-*erythro*-pentofuranosyl; arabino = β-D-arabinofuranosyl; ribo = β-D-ribofuranosyl.

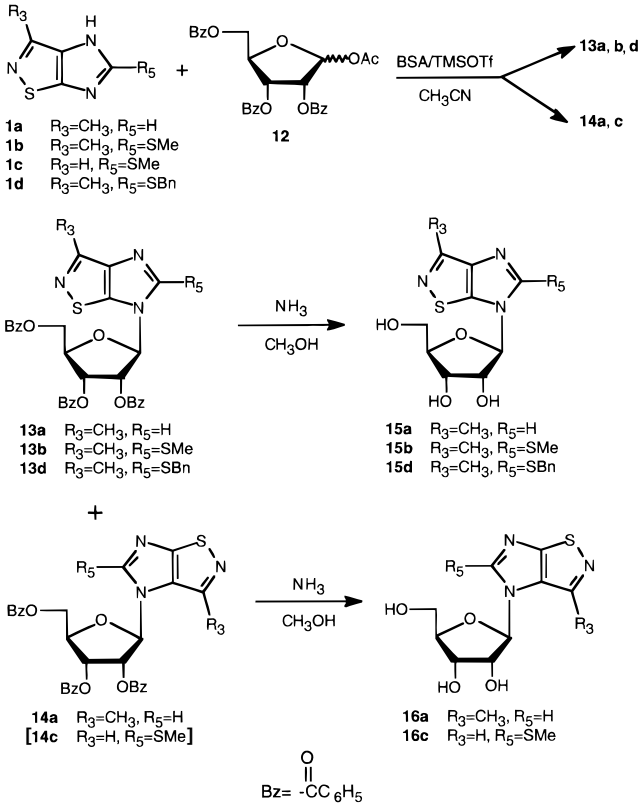
(Table 1) were determined by analogy of the chemical shifts to the corresponding 2′-deoxy derivatives and by selective decoupling experiments. For example, decoupling the 3-methyl proton signal of **10a** showed C3 as a singlet, C3a as a doublet ($^3J_{CH}$ = 12 Hz), C6a as a pseudotriplet ($^3J_{CH}$ = 5 Hz), and C5 as a doublet of doublets (J_{CH} = 166 Hz, $^3J_{CH}$ = 3 Hz). For a few of the compounds, the chemical shifts of a pair of quaternary carbons were extremely close together, and the assignments could not be made definitively based on observed trends. Selective decoupling experiments were not performed, due to a lack of sufficient material.

The patterns of chemical shifts and couplings seen for the imidazo[4,5-*d*]isothiazole carbon atoms in the β-D-arabinofuranosyl series generally follow the same trends seen for the corresponding 2′-deoxy nucleosides. This similarity readily allows the assignment of regioisomers and is expected since the carbohydrate portion of the molecule is relatively distant from the imidazo[4,5-*d*]isothiazole carbon atoms. A characteristic of these β-D-arabinofuranosyl nucleosides is a small (~3 ppm) downfield shift of the C6a signal. This is presumably due to the anisotropic effect of the 2′-hydroxyl moiety, which could be relatively close to the C6a carbon, since it is in the *cis* orientation with respect to the heterocycle.

We next prepared several β-D-ribofuranosylimidazo[4,5-*d*]isothiazole derivatives via condensation of the silylated heterocycles with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**12**, Scheme 3) in the presence of a Lewis acid catalyst. In this manner, reaction of 3-methylimidazo[4,5-*d*]isothiazole (**1a**) with **12** under modified[32] Vorbruggen[33] glycosylation conditions gave the nucleosides **13a** and **14a** as single anomers in 37% and 23% yields, respectively, after a separation of regioisomers by column chromatography. It was preferable to use (trimethylsilyl)trifluoromethanesulfonate (TMSOTf) as the catalyst, since use of the commonly employed tin tetrachloride ($SnCl_4$) gave low yields and required longer reaction times. This may be due to the ability of $SnCl_4$ to complex with the isothiazole sulfur atom, leading to a reduction in reactivity. Despite the high temperature (80 °C) necessary for completion of the reaction, some regioselectivity is evident, with formation of the N-6 isomer being preferred. Deprotection with methanolic ammonia provided the 6-methyl-9-β-D-ribofuranosylpurine analogs **15a** and **16a** as crystalline solids in nearly quantitative yields.

Unfortunately, this reaction was not as successful with the 5-substituted imidazo[4,5-*d*]isothiazoles **1b**–**d**, giving variable yields of nucleosides, along with deep red decomposition products. Use of $SnCl_4$ as the catalyst, and/or change of solvent to 1,2-dichloroethane, did not lead to the formation of the deep red decomposition products but resulted in the formation of little or no nucleoside material, even upon extended reaction times and elevated temperatures. The reaction of compounds **1b** and **1d** with **12** at 50 °C in the presence of TMSOTf under similar conditions gave the corresponding N-6 nucleosides **13b** and **13d** as the only isolable products in 52% and 21% yields, respectively. The formation of a single regioisomer is presumably due

Scheme 3. Synthesis of Ribofuranosylimidazo[4,5-*d*]isothiazoles

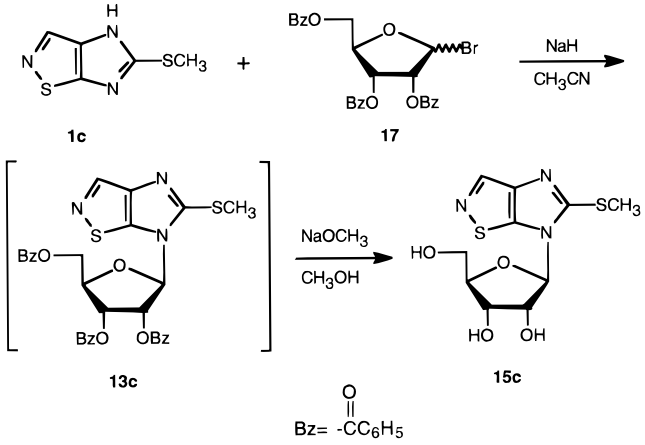


to the steric demands of the 5-alkylthio moiety, which prevents a reaction at the sterically more crowded N-4 position. Deprotection with methanolic ammonia gave good yields of the 6-β-D-ribofuranosylimidazo[4,5-*d*]-isothiazoles **15b** and **15d**. Reaction of the imidazo[4,5-*d*]isothiazole **1c** under similar conditions gave a complex mixture, from which only one nucleoside product was formed. However, this nucleoside could not be separated from several highly colored decomposition products, even after careful column chromatography. The impure, darkly colored crude product was therefore deblocked with methanolic ammonia, and the crude nucleoside was chromatographed, which provided **16c** as a syrup. This material was then crystallized to provide the N-4 isomer **16c** in 11% overall yield from the corresponding heterocycle.

Since the N-6 isomer (**13c**) was not obtained using silyl glycosylation conditions, we utilized the sodium salt method, employing 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl bromide (**17**, Scheme 4)[34] as the glycosylation reactant. This reaction proved to be extremely sluggish, even at elevated temperatures, and required a large excess of halogenose to go to completion. The lower reactivity of **17** relative to the halogenoses **2** and **7** is presumably due to the participation of the 2-acyloxy group in the reaction. After extensive column chromatography to separate the nucleoside products from unreacted sugar products, the desired nucleoside **13c** was obtained in low yield. The crude material was deblocked with sodium methoxide in methanol to provide the desired nucleoside **15c** in 11% yield from **1c** after chromatography and crystallization.

The site of glycosylation was determined by examination of UV and ^{13}C NMR spectra in a manner similar to that previously described for the 2′-deoxy and arabinosyl derivatives. The carbon-13 assignments for the imidazo[4,5-*d*]isothiazole carbon resonances are shown in Table 1. As expected, the general patterns of chemical shifts and couplings seen for the imidazo[4,5-*d*]-isothiazole carbon atoms in the β-D-ribofuranosyl series are similar to those of the corresponding 2′-deoxy nucleosides, which readily allows the unequivocal assignment of the N-4 and N-6 regioisomers.

Scheme 4. Synthesis of an N-4 Ribofuranosylimidazo[4,5-*d*]isothiazole

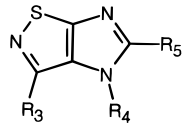


Compounds **15a**−**d**, **16a**, and **16c** were determined to have the β-configuration by a conversion of a small sample of each compound to the corresponding 2′,3′-*O*-isopropylidine. This was readily accomplished by treatment of each nucleoside derivative (~5 mg) with perchloric acid (1 drop) in acetone (0.5 mL) for 1 h at room temperature. After the reaction was quenched with dilute aqueous sodium bicarbonate, followed by extraction with chloroform, a nearly quantitative yield of the corresponding 2′,3′-*O*-isopropylidine was obtained. Examination of the ^{1}H NMR spectra ($CDCl_3$) of these crude products showed a separation of 0.22, 0.26, 0.26, 0.25, 0.24, and 0.27 ppm between the isopropylidine methyl peaks of the 2′,3′-*O*-isopropylidine nucleosides derived from **15a**, **15b**, **15c**, **15d**, **16a**, and **16c**, respectively. This separation (>0.15 ppm) is characteristic of β-D-ribofuranosyl nucleosides.[35] The exclusive formation of β-anomers is expected due to the role of the neighboring acyloxy group in the reaction, which promotes the formation of *trans* nucleosides.[36]

Biology

The five variously substituted imidazo[4,5-*d*]isothiazoles **1a**−**e** and certain of their ribosyl, deoxyribosyl, and arabinosyl derivatives (**5a**−**e**, **6a**−**c**,**e**, **10a**−**e**, **11a**,**c**,**e**, **15a**−**d**, and **16a**,**c**) were evaluated *in vitro* for effects on cell proliferation and for activity against herpes simplex virus type 1 (HSV-1) and human cytomegalovirus (HCMV). The heterocyles **1a**−**e** inhibited proliferation of all the cell lines studied (Table 2). The most potent was the 5-benzylthio derivative **1e** with IC_{50} = 0.3 and 0.2 μM for L1210 and KB cells, respectively. Compound **1e** also strongly inhibited proliferation of several other human tumor cell lines. In the presence of 1 μM **1e**, growth of G-361 cells was 7%, HT-29 cells 6%, and ZR-75-1 cells 15% of control. In the presence of 10 μM, growth of G-361 cells was 2%, HT-29 cells 0%, and ZR-75-1 cells 3% of control. All of the N-4 glycosylated imidazo[4,5-*d*]isothiazoles except **6a** were mod-

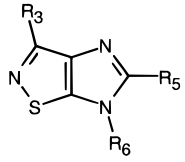
Table 2. Antiproliferative and Antiviral Activity of Certain Imidazo[4,5-*d*]isothiazole Heterocycles and N-4 Nucleosides



	substituent			50% inhibitory concentration (μM)				
compd no.	R_3	R_5	R_4[a]	L1210[b]	KB[c]	HFF[d]	HCMV[e]	HSV-1[f]
1a[g]	CH_3	H	H	17	20	32	32	45
1b	CH_3	SCH_3	H	13	60	26	18	60
1c	H	SCH_3	H	7	15	32	32	45
1d	CH_3	$SCH_2C_6H_5$	H	7	9	10	21	45
1e	H	$SCH_2C_6H_5$	H	0.3	0.2	14	21	15
6a	CH_3	H	deoxy	78	>100[h]	>100	>100	>100
6b	CH_3	SCH_3	deoxy	33	30	32	32	60
6c	H	SCH_3	deoxy	20	6	15	22	10
6e	H	$SCH_2C_6H_5$	deoxy	2.2	3	2.1	3.2	15
11c	H	SCH_3	arabino	11	15	21	32	25
11e	H	$SCH_2C_6H_5$	arabino	1.5	3	2.1	3.2	>100
16a	CH_3	H	ribo	100	>100	32	32	70
16c	H	SCH_3	ribo	8.6	15	21	32	20

[a] Abbreviation used: deoxy, 2′-deoxy-β-D-*erythro*-pentofuranosyl; arabino, β-D-arabinofuranosyl; ribo, β-D-ribofuranosyl. [b] Inhibition of the growth of murine L1210 cells. The 50% inhibitory concentration is the concentration required to decrease growth rate to half of the control rate; average of two or three experiments. [c] Inhibition of KB cell growth was determined as described in the text in quadruplicate assays. [d] Visual cytotoxicity scored on HFF cells at the time of plaque enumeration. [e] Plaque reduction assay in HFF cells; average of duplicate experiments. [f] Compounds were assayed by ELISA in quadruplicate wells. [g] Compounds **1a**,**b** reported in refs 19b and 23. [h] At 100 μM, the highest concentration evaluated, 0−50% inhibition was observed.

Table 3. Antiproliferative and Antiviral Activity of N-6 Imidazo[4,5-*d*]isothiazole Nucleosides



	substituent			50% inhibitory concentration (μM)				
compd no.	R_3	R_5	R_6[a]	L1210[b]	KB[c]	HFF[d]	HCMV[e]	HSV-1[f]
5a	CH_3	H	deoxy	>100[h]	>100	>100	>100	>100
5b	CH_3	SCH_3	deoxy	>100	>100	>100	>100	>100
5c	H	SCH_3	deoxy	>100	>100	>100	>100	>100
5d	CH_3	$SCH_2C_6H_5$	deoxy	>100	>100	>100	>100	>100
5e	H	$SCH_2C_6H_5$	deoxy	8	>100	>100	>100	>100
10a	CH_3	H	arabino	>100	>100	>100	>100	>100
10b	CH_3	SCH_3	arabino	>100	>100	>100	>100	>100
10c	H	SCH_3	arabino	>100	>100	>100	>100	100
10d	CH_3	$SCH_2C_6H_5$	arabino	>100	>100	>100	>100	>100
10e	H	$SCH_2C_6H_5$	arabino	>100	>100	>100	>100	>100
15a	CH_3	H	ribo	10	90	150	>100	>100
15b	CH_3	SCH_3	ribo	>100	>100	>100	>100	>100
15c	H	SCH_3	ribo	>100	>100	>100	>100	>100
15d	CH_3	$SCH_2C_6H_5$	ribo	>100	>100	>100	>100	>100

[a] [−f,h]See legend to Table 2 for footnotes.

erately toxic to human foreskin fibroblasts (HFF cells) in a visual assay and also inhibited the growth of KB and L1210 cells (Table 2). This toxicity was greatest for the 5-benzylthio derivatives **6e** and **11e** and was seen in both normal (HFF cells) and tumor (L1210 and KB) cells. The lack of or low activity for the 5-unsubstituted compounds **6a** and **16a** suggested that the presence of a 5-alkylthio or a 5-arylthio moiety may be important for the cytotoxic properties of this series. The 5-benzylthio substituent conferred the highest potency, as exemplified by **1e**, **6e**, and **11e**. Activity, or lack thereof, against herpes simplex virus type 1 (HSV-1) and human cytomegalovirus (HCMV) paralleled cytotoxicity to L1210, KB, and HFF cells. Similarly, the heterocycles **1a**−**e** inhibited HCMV and HSV-1 to approximately the same extent that they produced cytotoxicity (Table 2), indicating that neither they nor the N-4 nucleosides have specific antiviral activity.

In contrast, most of the N-6 glycosylated imidazo[4,5-*d*]isothiazoles (Table 3) were inactive in the L1210 assay as well as both antiviral assays and their respective cytotoxicity controls. The two exceptions to this generalization were that the 3-unsubstituted 5-(benzylthio)-6-(2-deoxy-β-D-ribofuranosyl) derivative **5e**, and the 5-unsubstituted 3-methyl 6-β-D-ribofuranosyl derivative **15a**, were moderately cytotoxic to L1210 cells. The latter compound is particularly interesting in this regard since it is the analog of 6-methyl-9-β-D-ribofuranosylpurine (**II**), which is extremely cytotoxic (IC_{50} = 4 nM).[18] Thus, replacing the purine ring system with the imidazo[4,5-*d*]isothiazole ring system led to a major decrease (≥1000-fold) in cytotoxic potency. The structures of 6-methyl-9-β-D-ribofuranosylpurine (**II**) and **15a** overlap closely, as shown by modeling studies and X-ray crystal data, implying that steric alterations are probably not responsible for the differing activity. The

fusion of two five-membered rings is expected to lead to an increased electron density of the overall ring system, and their differing biological activity is probably related to the profoundly different electronic characteristics of these imidazo[4,5-*d*]isothiazoles.

Experimental Section

General. Unless otherwise noted, general methods and experimental procedures were the same as described previously.[19] In all cases, the N-4 derivatives tested exhibited no separation between antiviral activity and toxicity toward uninfected cells.

4-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-3-methylimidazo[4,5-*d*]isothiazole (4a) and 6-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-3-methylimidazo[4,5-*d*]isothiazole (3a). 3-Methylimidazo[4,5-*d*]isothiazole (**1a**, 0.28 g, 2 mmol) was dissolved in dry acetonitrile (20 mL). Sodium hydride (80% w/w dispersion in mineral oil, 90 mg, 3 mmol) was added in one portion, and the resulting suspension stirred at room temperature for 1 h. 2-Deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl chloride (**2**, 0.97 g, 2.5 mmol) was added in one portion, and stirring was continued for 2.5 h at room temperature. Saturated ammonium chloride (2 mL) was added, and the mixture was partitioned between ethyl acetate (60 mL) and water (20 mL). The aqueous layer was washed with ethyl acetate, and the combined organics were washed with brine, dried over magnesium sulfate, filtered, and concentrated to a syrup. Chromatography (70% ethyl acetate/hexane, 4 × 20 cm) afforded **4a** (0.28 g, 29%) as a syrup which was homogeneous by TLC and used without further purification: R_f 0.61 (70% ethyl acetate/hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (s, 1H, 5-H), 7.94 (d, 2H), 7.82 (d, 2H), 7.29 (d, 2H), 7.22 (d, 2H), 6.38 (t, J = 6.6 Hz, 1H, H-1′), 5.70 (m, 1H), 4.71−4.59 (m, 3H), 2.95−2.75 (m, 2H), 2.67 (s, 3H), 2.45 (s, 3H), 2.40 (s, 3H). Continued elution of the column provided 0.32 g (33%) of **3a**, which was homogeneous by TLC and used without further purification: R_f 0.29 (70% ethyl acetate/hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (d, 2H), 7.85 (s, 1H, 5-H), 7.84 (d, 2H), 7.28 (d, 2H), 7.20 (d, 2H), 6.26 (t, J = 6.7 Hz, 1H, H-1′), 5.71 (m, 1H), 4.70−4.54 (m, 3H), 2.82−2.75 (m, 2H), 2.59 (s, 3H), 2.44 (s, 3H), 2.40 (s, 3H).

6-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-3-methylimidazo[4,5-*d*]isothiazole (5a). To a solution of sodium methoxide (20 mg) in methanol (20 mL) was added **3a** (0.32 g, 0.66 mmol), and the suspension was stirred 18 h at room temperature. The resulting solution was diluted with water (10 mL) and then adjusted to pH 7 with 1 N HCl. The solution was concentrated and then chromatographed (10% methanol/chloroform, 2 × 15 cm) to provide 0.14 g (82%) of **5a**: mp 148−149 °C; $[\alpha]_D$ −12.1° (*c* 1.0, methanol); R_f 0.26 (10% methanol/chloroform); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.22 (s, 1H), 6.26 (t, J = 6.7 Hz, 1H, H-1′), 5.40 (d, 1H, D_2O exchangeable), 4.94 (t, 1H, D_2O exchangeable), 4.30 (m, 1H), 3.87 (m, 1H), 3.47 (m, 2H), 2.48 (s, 3H), 2.31 (m, 2H); ^{13}C NMR (90 MHz, DMSO-d_6) δ 153.32 (C3), 148.79 (C3a), 145.99 (C6a), 145.04 (C5), 87.74, 85.75, 70.82, 61.88, 40.21−38.82 (C-2′ hidden by solvent peak), 16.28; UV λ_{max} (methanol) 208 (16 300), 248 (5560) nm. Anal. Calcd for $C_{10}H_{13}N_3O_3S$: C, H, N.

4-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-3-methylimidazo[4,5-*d*]isothiazole (6a). To a solution of sodium methoxide (20 mg) in methanol (10 mL) was added **4a** (0.28 g, 0.57 mmol), and the suspension was stirred 18 h at room temperature. The resulting solution was diluted with an equal volume of water and then adjusted to pH 7 with 1 N HCl. The solution was concentrated and then chromatographed (10% methanol/chloroform to 20% methanol chloroform, 2 × 15 cm) to provide 0.11 g (76%) of **6a**. A sample was crystallized from water for analysis: mp 155−156 °C; $[\alpha]_D$ −35.2° (*c* 0.9, methanol); R_f 0.26 (10% methanol/chloroform); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.41 (s, 1H), 6.33 (t, J = 6.4 Hz, 1H, H-1′), 5.38 (d, 1H, D_2O exchangeable), 4.94 (t, 1H, D_2O exchangeable), 4.35 (m, 1H), 3.87 (dd, 1H), 3.49 (m, 2H), 2.60 (s, 3H), 2.66−2.31 (m, 2H); ^{13}C NMR (90 MHz, DMSO-d_6) δ 163.51 (C6a), 148.37 (C3), 144.48 (C5), 134.86 (C5), 87.89, 85.05, 70.05, 61.18, 40.10, 18.11; UV λ_{max} (methanol) 207 (4170), 246 (9250), 250 (shoulder) nm. Anal. Calcd for $C_{10}H_{13}N_3O_3S \cdot 0.4H_2O$: C, H, N.

4-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-3-methyl-5-(methylthio)imidazo[4,5-*d*]isothiazole (4b) and 6-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-3-methyl-5-(methylthio)imidazo[4,5-*d*]isothiazole (3b). 3-Methyl-5-(methylthio)imidazo[4,5-*d*]isothiazole (**1b**, 926 mg, 5 mmol) was dissolved in warm, dry acetonitrile (50 mL). Sodium hydride (80% w/w dispersion in mineral oil, 180 mg, 6 mmol) was added in one portion, and the resulting suspension was stirred at room temperature for 1 h. The suspension was cooled with an ice water bath, and 2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl chloride (**2**, 2.33 g, 6 mmol) was added in one portion. The cold bath was removed, and stirring was continued for 3 h at room temperature. Ethyl acetate (50 mL) was added, and the suspension was filtered through a thin pad (2 cm) of silica, which was washed with ethyl acetate until no more product eluted (by TLC). The combined filtrate washings were concentrated to a brown syrup, which was chromatographed (30% ethyl acetate/hexane, 5 × 20 cm). Fractions containing the faster moving minor product were pooled and concentrated to afford 0.52 g (19%) of crude **4b** as a foam after drying at 0.1 mmHg. This material contained a slightly faster moving impurity by TLC, but was of significant purity for use in further reactions: R_f 0.67 (50% ethyl acetate/hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00−7.87 (m, 4H), 7.30−7.19 (m, 4H), 6.30 (dd, 1H, H-1′), 5.71−5.64 (m, 1H), 4.89−4.65 (m, 2H), 4.55−4.49 (m, 1H), 2.96−2.50 (m, 2H), 2.69 (s, 3H), 2.62 (s, 3H), 2.44 (s, 3H), 2.40 (s, 3H). Fractions containing the slower moving major product were pooled and concentrated to afford 1.74 g (65%) **3b** as colorless crystals: mp 126−127 °C; R_f 0.45 (50% ethyl acetate/hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00−7.82 (m, 4H), 7.33−7.18 (m, 4H), 6.31 (dd, 1H, H-1′), 5.70−5.65 (m, 1H), 4.71−4.58 (m, 3H), 2.82−2.50 (m, 2H), 2.74 (s, 3H), 2.58 (s, 3H), 2.44 (s, 3H), 2.39 (s, 3H). Anal. Calcd for $C_{27}H_{27}N_3O_5S_2$: C, H, N.

6-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-3-methyl-5-(methylthio)imidazo[4,5-*d*]isothiazole (5b). To a solution of **3b** (538 mg, 1 mmol) in methanol (10 mL) was added 0.3 mL of Dowex-1 ($^-$OH) washed with methanol. The resulting suspension was stirred at room temperature for 24 h and then filtered. The resin was washed well with methanol, and the filtrates were concentrated. The residue was dissolved in ethyl acetate and then partitioned between hexane (20 mL) and water (20 mL). The precipitated solid was dissolved by heating on a steam bath, and the aqueous phase was removed and allowed to cool slowly to 0 °C. The resulting solid was collected and dried to yield 198 mg (66%, mp 151−153 °C) of **5b**. A second crop provided an additional 56 mg (19%, mp 150−151 °C), giving a combined yield of 85%: $[\alpha]_D$ −0.8° (*c* 1.0, methanol); R_f 0.44 (10% methanol/chloroform); ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.12 (dd, 1H, $^1/_2$ height peak width = 15 Hz), 5.44 (d, 1H, D_2O exchangeable), 4.92 (t, 1H, D_2O exchangeable), 4.28 (m, 1H), 3.88 (m, 1H), 3.48 (m, 2H), 2.67 (s, 3H), 2.47 (s, 3H), 2.40−2.15 (m, 2H); ^{13}C NMR (90 MHz, DMSO-d_6) δ 152.06 (C3), 151.60 (C5), 147.29 (C3a), 147.09 (C6a), 87.53, 84.74, 70.59, 61.64, 39.30, 15.88, 15.32; UV λ_{max} (methanol) 221 (15 470), 269 (7720) nm. Anal. Calcd for $C_{11}H_{15}N_3O_3S$: C, H, N.

4-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-3-methyl-5-(methylthio)imidazo[4,5-*d*]isothiazole (6b). To a solution of **4b** (500 mg, 0.93 mmol) in methanol (10 mL) was added 0.3 mL of Dowex-1 ($^-$OH) washed with methanol. The resulting suspension was stirred at room temperature for 24 h and then filtered. The resin was washed well with methanol, and the filtrates were concentrated. The residue was dissolved in ethyl acetate and then partitioned between hexane (20 mL) and water (20 mL). The precipitated solid was dissolved by heating on a steam bath, and the aqueous phase was removed and allowed to cool slowly to 0 °C. The resulting solid was collected and dried to yield 213 mg (76%) of **6b**: mp 68−70 °C; $[\alpha]_D$ +21.0° (*c* 1.1, methanol); R_f 0.43 (10% methanol/chloroform); ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.11 (dd, 1H, $^1/_2$ height peak width = 17 Hz), 5.40 (d, 1H, D_2O exchangeable), 4.95 (t, 1H,

D_2O exchangeable), 4.27 (m, 1H), 3.77 (m, 1H), 3.66 (m, 2H), 2.70 (s, 3H), 2.58 (s, 3H), 2.41 (m, 1H), 2.21 (m, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.12, (C6a) 154.18 (C5), 147.26 (C3), 135.24 (C3a), 86.95, 84.11, 69.39, 60.85, 40.43, 19.17, 15.08; UV λ_{max} (methanol) 258 (12 660), 277 (14 360) nm. Anal. Calcd for $C_{11}H_{15}N_3O_3S$: C, H, N.

4-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-5-(methylthio)imidazo[4,5-*d*]isothiazole (4c) and 6-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-5-(methylthio)imidazo[4,5-*d*]isothiazole (3c). 5-(Methylthio)-imidazo[4,5-*d*]isothiazole (**1c**, 0.21 g, 1.24 mmol) was dissolved in warm, dry acetonitrile (20 mL). Sodium hydride (80% w/w dispersion in mineral oil, 45 mg, 1.49 mmol) was added in one portion, and the resulting suspension was stirred at room temperature for 1 h. 2-Deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl chloride (**2**, 0.54 g, 1.38 mmol) was added in one portion, and stirring was continued for 2 h at room temperature. Saturated aqueous ammonium chloride (1 mL) was added, and the mixture was partitioned between ethyl acetate (30 mL) and water (15 mL). The aqueous layer was washed with ethyl acetate (2 × 10 mL), and the combined organic phases were washed with brine, dried over magnesium sulfate, filtered, and concentrated. The resulting syrup was chromatographed (35% ethyl acetate/hexane to 50% ethyl acetate/hexane, 4 × 15 cm). Fractions containing the faster moving minor product were pooled and concentrated to afford 0.32 g (49%) of crude **4c** as an oil after drying at 0.1 mmHg: R_f 0.80 (50% ethyl acetate/hexane); ^1H NMR (300 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.96 (d, 2H), 7.82 (d, 2H), 7.29 (d, 2H), 7.20 (d, 2H), 6.24 (dd, 1H, H-1′), 5.70 (m, 1H), 4.70−4.55 (m, 3H), 2.90−2.65 (m, 1H), 2.77 (s, 3H), 2.50−2.30 (m, 1H), 2.47 (s, 3H), 2.42 (s, 3H). Fractions containing the slower moving major product were pooled and concentrated to afford 0.33 g (50%) of **3c**, which was used without further purification: R_f 0.48 (50% ethyl acetate/hexane); ^1H NMR (300 MHz, $CDCl_3$) δ 8.39 (s, 1H), 7.96 (d, 2H), 7.84 (d, 2H), 7.29 (d, 2H), 7.84 (d, 2H), 6.28 (dd, 1H, H-1′), 5.67 (m, 1H), 4.68−4.62 (m, 3H), 2.85−2.60 (m, 2H), 2.75 (s, 3H), 2.45 (s, 3H), 2.39 (s, 3H).

6-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-5-(methylthio)imidazo[4,5-*d*]isothiazole (5c). To a solution of **3c** (0.29 g, 0.55 mmol) in methanol (10 mL) was added 0.3 mL of Dowex-1 ($^-$OH) washed with methanol. The resulting suspension was stirred at room temperature for 48 h and then filtered. The resin was washed well with methanol, and the filtrates were concentrated. The residue was dissolved in ethyl acetate and then partitioned between hexane (20 mL) and water (20 mL). The precipitated solid was dissolved by heating on a steam bath, and the aqueous phase was removed and allowed to cool slowly to 0 °C. The resulting solid was collected and dried to yield 72 mg (46%) of **5c** after two crops: mp 131.5−132 °C; $[\alpha]_D$ +1.4° (*c* 0.6, methanol); R_f 0.54 (10% methanol/chloroform); ^1H NMR (360 MHz, DMSO-d_6) δ 8.55 (s, 1H), 6.13 (dd, 1H, $^1/_2$ height peak width = 15.0 Hz), 5.47 (d, 1H, D_2O exchangeable), 4.95 (t, 1H, D_2O exchangeable), 4.31 (m, 1H), 3.90 (m, 1H), 3.51 (m, 2H), 2.68 (s, 3H), 2.40−2.20 (m, 2H); ^{13}C NMR (90 MHz, DMSO-d_6) δ 152.41 (C5), 148.88 (C3a), 147.90 (C6a), 143.70 (C3), 87.87, 85.02, 70.88, 61.76, 39.41, 15.22; UV λ_{max} (methanol) 221 (11 140), 272 (5490) nm. Anal. Calcd for $C_{10}H_{13}N_3O_3S_2$: C, H, N.

4-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-5-(methylthio)imidazo[4,5-*d*]isothiazole (6c). To a solution of **4c** (0.29 g, 0.55 mmol) in methanol (10 mL) was added 0.3 mL of Dowex-1 ($^-$OH) washed with methanol. The resulting suspension was stirred at room temperature for 48 h and then filtered. The resin was washed well with methanol, and the filtrates were concentrated. The residue was dissolved in ethyl acetate and then partitioned between hexane (20 mL) and water (20 mL). The precipitated solid was dissolved by heating on a steam bath, and the aqueous phase was removed and allowed to cool slowly to 0 °C. The resulting solid was collected and dried to yield 43 mg (27%) of **6c** after two crops: mp 156−157 °C; $[\alpha]_D$ −5.3° (*c* 0.7, methanol); R_f 0.51 (10% methanol/chloroform); ^1H NMR (360 MHz, DMSO-d_6) δ 8.68 (s, 1H), 6.09 (dd, 1H, $^1/_2$ height peak width = 16.0 Hz), 5.40 (d, 1H, D_2O exchangeable), 5.03 (t, 1H, D_2O exchangeable), 4.36 (m, 1H), 3.89 (m, 1H), 3.58 (t, 2H), 2.72 (s, 3H), 2.55−2.25 (m, 2H); ^{13}C NMR (90 MHz, DMSO-d_6) δ 163.17 (C6a), 154.88 (C5), 140.00 (C3), 136.57 (C3a), 87.87, 85.09, 70.59, 61.40, 39.70 (partially obscured by solvent peak), 14.99; UV λ_{max} (methanol) 220 (4740), 255 (9860), 279 (12 080) nm. Anal. Calcd for $C_{10}H_{13}N_3O_3S_2$: C, H, N.

4-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-3-methyl-5-[(phenylmethyl)thio]imidazo[4,5-*d*]isothiazole (4d) and 6-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-3-methyl-5-[(phenylmethyl)thio]imidazo[4,5-*d*]isothiazole (3d). 3-Methyl-5-[(phenylmethyl)thio]-imidazo[4,5-*d*]isothiazole (**1d**, 0.30 g, 1.15 mmol) was dissolved in warm, dry acetonitrile (12 mL). Sodium hydride (80% w/w dispersion in mineral oil, 42 mg, 1.38 mmol) was added in one portion, and the resulting suspension stirred at room temperature for 2 h. 2-Deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl chloride (**2**, 0.54 g, 1.38 mmol) was added in one portion, and stirring was continued for 3 h at room temperature. Ethyl acetate (20 mL) was added, and the suspension was filtered through a thin pad (2 cm) of silica, which was washed with ethyl acetate until no more product eluted (by TLC). The combined filtrate washings were concentrated to a brown syrup, which was chromatographed (30% ethyl acetate/hexane, 4 × 15 cm). Fractions containing the faster moving minor product were pooled and concentrated to afford 0.16 g (23%) of crude **4d** as an oil after drying at 0.1 mmHg: R_f 0.46 (30% ethyl acetate/hexane); ^1H NMR (300 MHz, $CDCl_3$) δ 8.05−7.80 (m, 4H), 7.40−7.05 (m, 9H), 6.24 (dd, 1H, H-1′), 5.59 (m, 1H), 4.85−4.40 (m, 5H), 2.75−2.20 (m, 2H), 2.60 (s, 3H), 2.46 (s, 3H), 2.39 (s, 3H). Fractions containing the slower moving major product were pooled and concentrated to afford 0.59 g (70%) of solid **3d**, which was used without further purification: R_f 0.31 (30% ethyl acetate/hexane); ^1H NMR (300 MHz, $CDCl_3$) δ 7.94 (d, 2H), 7.85 (d, 2H), 7.35−7.15 (m, 9H), 6.12 (dd, 1H, H-1′), 5.56 (m, 1H), 4.65−4.30 (m, 5H), 2.69−2.25 (m, 2H), 2.62 (s, 3H), 2.45 (s, 3H), 2.39 (s, 3H).

6-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-3-methyl-5-[(phenylmethyl)thio]imidazo[4,5-*d*]isothiazole (5d). A suspension of **3d** (0.45 g, 0.73 mmol) in methanolic ammonia (15 mL) was stirred 48 h at room temperature. The resulting solution was concentrated, and the residue was chromatographed (80% ethyl acetate/hexane, 2 × 15 cm) to provide 0.26 g of a clear glass. Crystallization from methanol/water afforded 0.24 g (85%) of **5d** hydrate: mp 90−91 °C; $[\alpha]_D$ +84.8° (*c* 1.1, methanol); R_f 0.58 (10% methanol/chloroform); ^1H NMR (360 MHz, DMSO-d_6) δ 7.38−7.20 (m, 5H), 6.09 (dd, 1H, $^1/_2$ height peak width = 15.5 Hz), 5.41 (d, 1H, D_2O exchangeable), 4.92 (t, 1H, D_2O exchangeable), 4.62 (s, 2H), 4.25 (m, 1H), 3.85 (m, 1H), 3.55−3.40 (m, 2H), 2.49 (s, 3H), 2.21−2.05 (m, 2H); ^{13}C NMR (90 MHz, DMSO-d_6) δ 152.30 (C3), 149.44 (C5), 147.34 (C3a), 146.87 (C6a), 136.98, 128.94, 128.54, 127.53, 87.76, 85.11, 70.78, 61.77, 39.35, 37.65, 16.29; UV λ_{max} (methanol) 220 (20 590), 272 (8800) nm. Anal. Calcd for $C_{17}H_{19}N_3O_3S_2 \cdot H_2O$: C, H, N.

4-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-5-[(phenylmethyl)thio]imidazo[4,5-*d*]isothiazole (4e) and 6-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-5-[(phenylmethyl)thio]imidazo[4,5-*d*]isothiazole (3e). 5-[(Phenylmethyl)thio]imidazo[4,5-*d*]isothiazole (**1e**, 90 mg, 0.33 mmol) was dissolved in warm, dry acetonitrile (5 mL). Sodium hydride (80% w/w dispersion in mineral oil, 12 mg, 0.39 mmol) was added in one portion, and the resulting suspension was stirred at room temperature for 1 h. 2-Deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl chloride (152 mg, 0.39 mmol) was added in one portion, and stirring was continued for 3 h at room temperature. The mixture was filtered through a thin pad of silica, which was then washed with ethyl acetate until no more product eluted (by TLC). The filtrate and washings were combined and concentrated, and the resulting syrup was chromatographed (30% ethyl acetate/hexane, 2 × 15 cm). Fractions containing the faster moving minor product were pooled and concentrated to afford 90 mg (45%) of crystalline **4e**: mp 115−116 °C; R_f 0.51 (30% ethyl acetate/hexane); ^1H NMR (270 MHz, $CDCl_3$) δ 8.37 (s, 1H), 7.94 (d, 2H), 7.82 (d, 2H), 7.35−7.18 (m, 5H), 6.17 (dd, 1H, H-1′), 5.62 (m, 1H), 4.70−4.45 (m, 5H), 2.75−2.35 (m, 2H), 2.44 (s, 3H), 2.40 (s, 3H). Fractions containing the slower moving

major product were pooled and concentrated to afford 91 mg (46%) of crystalline **3e**, which was used without further purification: mp 118−121 °C; R_f 0.34 (30% ethyl acetate/hexane); ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.46 (s, 1H), 7.96 (d, 2H), 7.84 (d, 2H), 7.35−7.17 (m, 5H), 6.17 (t, 1H, H-1′), 5.61 (m, 1H), 4.65−4.40 (m, 5H), 2.50−2.34 (m, 2H), 2.46 (s, 3H), 2.41 (s, 3H).

6-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-5-[(phenylmethyl)thio]imidazo[4,5-*d*]isothiazole (5e). To a solution of sodium methoxide (20 mg) in methanol (20 mL) was added **3e** (0.16 g, 0.27 mmol), and the suspension was stirred 24 h at room temperature. The resulting solution was diluted with an equal volume of water and then adjusted to pH 6 with 1 N HCl. The solution was concentrated, chromatographed (5% methanol/chloroform, 2 × 15 cm), and then crystallized from toluene/ethanol to provide 81 mg (83%) of **5e**: mp 99−102 °C; $[\alpha]_D$ +69.0° (*c* 0.7, methanol); R_f 0.24 (5% methanol/chloroform); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.60 (s, 1H), 7.40−7.20 (m, 5H), 6.10 (t, J = 6.8 Hz, 1H, H-1′), 5.42 (d, 1H, D_2O exchangeable), 4.94 (t, 1H, D_2O exchangeable), 4.27 (s, 2H), 4.27 (m, 1H), 3.87 (m, 1H), 3.49 (m, 2H), 2.25−2.05 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 149.96 (C5), 148.56 (C3a), 147.43 (C6a), 143.49 (C5), 136.67, 128.44, 128.09, 127.07, 87.60, 84.90, 70.58, 61.56, 39.33, 37.27; UV λ_{max} (methanol) 218 (18 290), 275 (7690) nm. Anal. Calcd for $C_{16}H_{17}N_3O_3S_2 \cdot {}^1/_8H_2O$: C, H, N.

4-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-5-[(phenylmethyl)thio]imidazo[4,5-*d*]isothiazole (6e). To a solution of sodium methoxide (20 mg) in methanol (20 mL) was added **1a** (0.16 g, 0.27 mmol), and the suspension was stirred 24 h at room temperature. The resulting solution was diluted with an equal volume of water and then adjusted to pH 6 with 1 N HCl. The solution was concentrated, chromatographed (5−10% methanol/chloroform, 2 × 15 cm), and then crystallized from petroleum ether/methanol to provide 94 mg (94%) of **6e**: mp 144−145 °C; $[\alpha]_D$ +44.6° (*c* 1.0, methanol); R_f 0.18 (5% methanol/chloroform); ^{1}H NMR (360 MHz, DMSO-d_6) δ 8.70 (s, 1H), 7.43−7.24 (m, 5H), 6.08 (dd, 1H, $^1/_2$ height peak width = 16.0 Hz), 5.37 (d, D_2O exchangeable), 5.03 (t, D_2O exchangeable), 4.56 (s, 2H), 4.33 (m, 1H), 3.86 (m, 1H), 3.57 (t, 2H), 2.38 (m, 1H), 2.14 (m, 1H); ^{13}C NMR (90 MHz, DMSO-d_6) δ 163.02 (C6a), 153.01 (C5), 140.17 (C3), 136.93, 136.41 (C5), 128.90, 128.51, 127.52, 87.86, 85.18, 70.53, 61.35, 39.73, 36.75; UV λ_{max} (methanol) 256 (12 000), 282 (14 620) nm. Anal. Calcd for $C_{16}H_{17}N_3O_3S_2$: C, H, N.

3-Methyl-6-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-imidazo[4,5-*d*]isothiazole (8a). Sodium hydride (80% w/w, 90 mg, 3 mmol) was added in one portion to a solution of **1a** (278 mg, 2 mmol) in dry acetonitrile (20 mL), and the resulting suspension was stirred for 1 h at room temperature. A solution of 2,3,5-tri-*O*-benzyl-α-D-arabinofuranosyl chloride (**7**, 3 mmol) in dichloromethane (6 mL) was added via cannula, and the mixture was stirred for 48 h at room temperature. Saturated ammonium chloride (2 mL) was added, and the suspension was partitioned between water (20 mL) and ethyl acetate (60 mL). The organic layer was washed with water and brine and then dried over magnesium sulfate. The solvent was removed, and the residue was chromatographed (50% ethyl acetate/hexane to 70% ethyl acetate/hexane). Fractions containing the faster moving product (R_f 0.54, 50% ethyl acetate/hexane) were pooled and concentrated to afford 90 mg (8%) of the N-4 isomer. Fractions containing the major product were combined to provide 539 mg (50%) of **8a**: R_f 0.25 (50% ethyl acetate/hexane); ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.74 (s, 1H), 7.40−6.95 (m, 15H), 5.92 (d, J = 4.0 Hz, 1H, H-1′), 4.54−4.05 (m, 9H), 3.62 (m, 2H), 2.62 (s, 3H).

6-β-D-Arabinofuranosyl-3-methylimidazo[4,5-*d*]isothiazole (10a). To a solution of **8a** (0.45 g, 0.84 mmol) in dry dichloromethane (5 mL) at −78 °C was added boron trichloride (1.0 M in dichloromethane, 8.4 mL) dropwise over 0.25 h. The solution was warmed to 0 °C via an ice bath and then stirred for 2 h. The reaction mixture was cooled to −78 °C and quenched via the dropwise addition of methanol (5 mL), and the vigorously stirred solution was then neutralized to pH 7 at 0 °C with an ice cold solution of 1 M sodium hydroxide (23−25 mL). The organic layer was removed, and the aqueous phase was washed with hexane and then lyophilized. The resulting crude solid was chromatographed (20% methanol/chloroform, 2 × 15 cm) to provide 0.23 g (100%) of **10a**: mp 175−176 °C; $[\alpha]_D$ −13.0° (*c* 1.0, methanol); R_f 0.20 (10% methanol/chloroform); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.18 (s, 1H), 6.09 (d, J = 4.1 Hz, 1H, H-1′), 5.98 (d, D_2O exchangeable), 5.57 (d, 1H, D_2O exchangeable), 4.98 (t, D_2O exchangeable), 4.16 (m, 1H), 3.98 (dd, 1H), 3.70−3.50 (m, 2H), 2.45 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 151.99 (C3), 148.43 (C6a), 148.15 (C3a), 145.31 (C5), 86.32, 84.81, 76.84, 75.75, 61.42, 16.04; UV λ_{max} (methanol) 210 (15 820), 249 (5670) nm. Anal. Calcd for $C_{10}H_{13}N_3O_4S$: C, H, N.

3-Methyl-5-methylthio-6-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)imidazo[4,5-*d*]isothiazole (8b). 3-Methyl-5-(methylthio)imidazo[4,5-*d*]isothiazole (**1b**, 926 mg, 5 mmol) was dissolved in warm, dry acetonitrile (50 mL). Sodium hydride (80% w/w dispersion in mineral oil, 180 mg, 6 mmol) was added in one portion, and the resulting suspension was stirred at room temperature for 1 h. 2,3,5-Tri-*O*-benzyl-α-D-arabinofuranosyl chloride (**7**, 2.63 g, 6 mmol) in 10 mL of dichloromethane was added via cannula, and stirring was continued for 24 h at room temperature. Saturated aqueous ammonium chloride (5 mL) was added, and the suspension was concentrated to a small volume. Water (50 mL) was added, and the mixture was extracted with ethyl acetate (50 mL + 2 × 10 mL). The combined organics were washed with brine (20 mL), dried over magnesium sulfate, filtered, and concentrated to a brown syrup. This residue was chromatographed (chloroform to 2% methanol/chloroform, 5 × 20 cm) to separate **8b** (R_f 0.61, 2% methanol/chloroform), which was contaminated with a small amount of the N-4 isomer (R_f 0.74), from unreacted starting material (0.16 g, 17%). Fractions containing the faster moving products were pooled, concentrated, and chromatographed again (30% ethyl acetate/hexane, 5 × 20 cm) to afford the faster moving N-4 isomer (0.12 g, 4%) as an oily solid containing an inseparable mixture of anomers (α/β = 1.2:1). Continued elution of the column gave **8b** (2.00 g, 68%) as a syrup containing an inseparable mixture of anomers (α/β = 1:25) after drying at 0.1 mmHg: R_f 0.61 (2% methanol/chloroform); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36−6.90 (m, 13H), 6.92 (m, 2H), 6.10 (d, J = 4.0 Hz, 1H, H-1′ of β-anomer), 6.04 (d, J = 3.4 Hz, 0.04H, H-1′ of α-anomer), 4.61−4.45 (m, 4H), 4.47−4.20 (m, 3H), 4.14 (m, 1H), 4.04 (m, 1H), 3.75-3.58 (m, 2H), 2.66 (s, 3H), 2.60 (s, 3H).

6-β-D-Arabinofuranosyl-3-methyl-5-(methylthio)imidazo[4,5-*d*]isothiazole (10b). To a solution of **8b** (2.0 g, 3.4 mmol) in dry dichloromethane (15 mL) at −78 °C was added boron trichloride (1.0 M in dichloromethane, 34 mL) dropwise over 0.5 h. The solution was warmed to 0 °C via an ice bath and then stirred for 2 h. The reaction mixture was cooled to −78 °C and quenched via the dropwise addition of methanol (17 mL), and the vigorously stirred solution was then neutralized to pH 7 at 0 °C with an ice cold solution of saturated sodium bicarbonate (ca. 80 mL). Chloroform (50 mL) was added, and the organic layer was extracted with water (2 × 50 mL). The combined aqueous layers were allowed to stand overnight at 5 °C, and the resulting solid was removed by filtration and then washed with ethanol. This process was repeated, and the filtrate was concentrated onto 8 g of silica gel and then chromatographed (10% methanol/chloroform, 4 × 20 cm) to yield a clear glass, which was homogeneous by TLC. Attempts to crystallize this material from a variety of solvents failed and led to decomposition of some product. Chromatography (90:9:1 ethyl acetate/methanol/water, 4 × 20 cm) provided a glassy residue, which was dissolved in water at room temperature and then lyophilized to afford 0.49 g (44%) of **10b** as the hemihydrate: mp 154−156 °C; $[\alpha]_D$ −9.6° (*c* 1.0, methanol); R_f 0.52 (90:9:1 ethyl acetate/methanol/water); ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.97 (d, J = 3.6 Hz, 1H, H-1′), 5.89 (br s, 1H, D_2O exchangeable), 5.57 (br s, 1H, D_2O exchangeable), 4.95 (br s, 1H, D_2O exchangeable), 4.14 (m, 1H), 3.99 (m, 1H), 3.82 (m, 1H), 3.62 (m, 2H), 2.67 (s, 3H), 2.44 (s, 3H); ^{13}C NMR (90 MHz, DMSO-d_6) δ 151.44 (C5), 151.13 (C3), 150.06 (C6a), 147.01 (C3a), 86.15, 85.57, 76.56, 76.24, 61.62, 16.38, 15.58; UV λ_{max} (methanol) 221 (16 680), 269 (8450) nm. Anal. Calcd for $C_{11}H_{15}N_3O_4S_2 \cdot {}^1/_2H_2O$: C, H, N.

5-(Methylthio)-4-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)imidazo[4,5-*d*]isothiazole (9c) and 5-(Methylthio)-6-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)imidazo[4,5-*d*]-isothiazole (8c). 5-(Methylthio)imidazo[4,5-*d*]isothiazole (**1c**, 650 mg, 3.8 mmol) was dissolved in warm, dry acetonitrile (75 mL). Sodium hydride (80% w/w dispersion in mineral oil, 137 mg, 4.56 mmol) was added in one portion, and the resulting suspension was stirred at room temperature for 1 h. 2,3,5-Tri-*O*-benzyl-α-D-arabinofuranosyl chloride (**7**, 5 mmol) in 11 mL of dichloromethane was added via cannula, and stirring was continued for 48 h at room temperature. Saturated aqueous ammonium chloride (5 mL) was added, and the suspension was concentrated to a small volume and then partitioned between water (40 mL) and ethyl acetate (40 mL). The aqueous phase was extracted with ethyl acetate (2 × 20 mL) and the combined organics were washed with brine (20 mL), dried over magnesium sulfate, filtered, and concentrated to a brown syrup. This residue was chromatographed (chloroform to 2% methanol/chloroform, 5 × 20 cm) to afford nucleoside material and unreacted starting material (79 mg, 12%). The combined nucleoside products were chromatographed (30% ethyl acetate, 5 × 20 cm) to afford 0.53 g (24%) of **9c** as an inseparable mixture of anomers (α/β = 1:5): R_f 0.84 (50% ethyl acetate/hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.28 (s, 1H), 7.38−7.18 (m, 13H), 6.79 (m, 2H), 6.05 (d, J = 3.2 Hz, 1H, H-1′ of β-anomer), 5.98 (d, J = 4.1 Hz, 0.19H, H-1′ of α-anomer), 4.60−3.60 (m, 11H), 2.73 (s, 3H). Continued elution of the column with 50% ethyl acetate/hexane gave **8c** (1.09 g, 50%) as a syrup containing an inseparable mixture of anomers (α/β = 1:30): R_f 0.58 (50% ethyl acetate/hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.39 (s, 1H), 7.38−7.15 (m, 13H), 6.89 (m, 2H), 6.06 (d, J = 3.9 Hz, 1H, H-1′ of β-anomer), 6.00 (d, J = 3.1 Hz, 1H, H-1′ of α-anomer), 4.53 (m, 4H), 4.30−4.03 (m, 5H), 3.68 (m, 2H), 2.69 (s, 3H).

6-β-D-Arabinofuranosyl-5-(methylthio)imidazo[4,5-*d*]-isothiazole (10c). To a solution of **8c** (1.09 g, 1.90 mmol) in dry dichloromethane (9 mL) at −78 °C was added boron trichloride (1.0 M in dichloromethane, 19 mL) dropwise over 0.5 h. The solution was warmed to 0 °C via an ice bath and then stirred for 2 h. The reaction mixture was cooled to −78 °C, the reaction was quenched via the dropwise addition of methanol (10 mL), and the vigorously stirred solution was then neutralized to pH 7 at 0 °C with an ice cold solution of saturated sodium bicarbonate. Chloroform (30 mL) was added, and the organic layer was extracted with water (2 × 30 mL). The aqueous layer was washed with ether, concentrated, slurried with 2:1 ethanol/ethyl acetate, and filtered. The filtrates were concentrated and chromatographed (15% methanol/chloroform, 4 × 20 cm) to yield a clear glass, which was taken up in methanol, diluted with water, and lyophilized to yield 0.33 g (58%) of **10c** hemihydrate. This material was very hygroscopic, and a portion was recrystallized from methanol to provide anhydrous **10c**: mp 138−140 °C; $[\alpha]_D$ −3.7° (*c* 1.2, methanol); R_f 0.27 (10% methanol/chloroform); ^{1}H NMR (360 MHz, DMSO-d_6) δ 8.46 (s, 1H), 6.00 (d, J = 5.2 Hz, 1H, H-1′), 5.97 (d, 1H, D_2O exchangeable), 5.64 (d, 1H, D_2O exchangeable), 5.03 (t, 1H, D_2O exchangeable), 4.16 (m, 1H), 4.01 (m, 1H), 3.83 (m, 1H), 3.62 (m, 2H), 2.68 (s, 3H); ^{13}C NMR (90 MHz, DMSO-d_6) δ 152.46 (C5), 150.87 (C6a), 148.63 (C3a), 142.99 (C3), 86.05, 85.67, 76.66, 76.26, 61.60, 15.38; UV λ_{max} (methanol) 222 (13 450), 272 (7040) nm. Anal. Calcd for $C_{10}H_{13}N_3O_4S_2$: C, H, N.

4-β-D-Arabinofuranosyl-5-(methylthio)imidazo[4,5-*d*]-isothiazole (11c). To a solution of **9c** (0.53 g, 0.93 mmol) in dry dichloromethane (5 mL) at −78 °C was added boron trichloride (1.0 M in dichloromethane, 9.4 mL) dropwise over 0.5 h. The solution was warmed to 0 °C via an ice bath and then stirred for 2 h. The reaction mixture was cooled to −78 °C, the reaction was quenched via the dropwise addition of methanol (5 mL), and the vigorously stirred solution was then neutralized to pH 7 at 0 °C with an ice cold solution of saturated sodium bicarbonate. Chloroform (15 mL) was added, and the organic layer was extracted with water (2 × 15 mL). The aqueous layer was washed with ether, concentrated, slurried with 2:1 ethanol/ethyl acetate, and filtered. The filtrates were concentrated and then chromatographed (15% methanol/chloroform, 2 × 20 cm). Fractions containing product were concentrated and recrystallized from warm water to yield 0.11 g (39%) of **11c**: mp 187−189 °C dec; $[\alpha]_D$ +12.9° (*c* 1.0, methanol); R_f 0.38 (10% methanol/chloroform); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.48 (s, 1H), 5.97 (d, J = 4.1 Hz, 1H, H-1′), 5.60 (d, 1H, D_2O exchangeable), 5.59 (d, 1H, D_2O exchangeable), 5.15 (t, 1H, D_2O exchangeable), 4.10−4.01 (m, 2H), 3.84 (m, 1H), 3.70 (m, 2H), 2.72 (s, 3H); ^{13}C NMR (360 MHz, DMSO-d_6) δ 161.82 (C6a), 153.46 (C5), 141.57 (C3), 138.81 (C3a), 87.02, 84.97, 76.20, 75.82, 60.82, 15.11; UV λ_{max} (methanol) 221 (6130), 255 (10 950), 278 (12 940) nm. Anal. Calcd for $C_{10}H_{13}N_3O_4S_2$: C, H, N.

3-Methyl-5-[(phenylmethyl)thio]-6-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)imidazo[4,5-*d*]isothiazole (8d). Sodium hydride (80% w/w, 90 mg, 3 mmol) was added in one portion to a solution of **1d** (653 mg, 2.5 mmol) in dry acetonitrile (25 mL), and the resulting suspension was stirred for 1 h at room temperature. A solution of 2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl chloride (**7**, 3 mmol) in dichloromethane (11 mL) was added via cannula, and the mixture was stirred for 72 h at room temperature. Saturated ammonium chloride (2.5 mL) was added, and the mixture was concentrated to a small volume and then partitioned between water (25 mL) and ethyl acetate (25 mL). The organic layer was washed with brine, dried over magnesium sulfate, and filtered. The solvent was removed, and the residue was chromatographed (chloroform to 2% methanol/chloroform, 4 × 20 cm) to afford nucleoside material, followed by unreacted starting material (74 mg, 11%). The nucleoside material was chromatographed again (30% ethyl acetate/hexane, 4 × 20 cm), and fractions containing the major product were combined to provide 1.09 g (66%) of **8d**: R_f 0.51 (2% methanol/chloroform); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35−7.15 (m, 13H), 6.89 (m, 2H), 6.00 (d, J = 3.9 Hz, 1H, H-1′), 4.59−4.35 (m, 6H), 4.20−4.11 (m, 3H), 3.98 (m, 1H), 3.94 (m, 1H), 3.63 (m, 2H), 2.62 (s, 3H).

6-β-D-Arabinofuranosyl-3-methyl-5-[(phenylmethyl)-thio]imidazo[4,5-*d*]isothiazole (10d). To a solution of **8d** (1.09 g, 1.64 mmol) in dry dichloromethane (8 mL) at −78 °C was added boron trichloride (1.0 M in dichloromethane, 16 mL) dropwise over 0.25 h. The solution was warmed to 0 °C via an ice bath and then stirred for 2 h. The reaction mixture was cooled to −78 °C, the reaction was quenched via the dropwise addition of methanol (8 mL), and the vigorously stirred solution was then neutralized to pH 7 at 0 °C with an ice cold solution of saturated sodium bicarbonate. The mixture was extracted with chloroform (3 × 25 mL) and then ethyl acetate (3 × 25 mL). The combined organic layers were washed with brine, concentrated, and then chromatographed (5% methanol/chloroform to 10% methanol/chloroform, 4 × 20 cm). Fractions containing product were concentrated and chromatographed again (325:9:1 ethyl acetate/methanol/water) to yield 0.42 g (65%) of a clear foam, which was homogeneous by TLC. Attempts to crystallize this material from a variety of solvents failed and led to decomposition of some product. Chromatography (5% methanol/chloroform to 10% methanol/chloroform, 4 × 20 cm) provided a glassy residue, which was dissolved in ethanol, diluted with toluene, and concentrated afford 0.37 g (51%) of **10d** as a hydroscopic foam, which retained 0.5 mol of toluene: $[\alpha]_D$ −3.1° (*c* 0.9, methanol); R_f 0.42 (10% methanol/chloroform); ^{1}H NMR (360 MHz, DMSO-d_6) δ 8.32 (s, 1H), 7.42−7.24 (m, 5H), 5.96 (d, J = 3.7 Hz, 1H, H-1′), 5.92 (d, 1H, D_2O exchangeable), 5.58 (d, 1H, D_2O exchangeable), 5.00 (t, 1H, D_2O exchangeable), 4.47 (m, 2H), 4.07 (m, 1H), 3.97 (m, 1H), 3.79 (m, 1H), 3.60 (m, 2H), 2.47 (s, 3H); ^{13}C NMR (90 MHz, DMSO-d_6) δ 151.33 (C3), 149.87 (C6a or C5), 149.41 (C5 or C6a), 147.03 (C3a), 136.85, 128.94, 128.48, 127.47, 86.18, 85.52, 76.59, 76.22, 61.59, 37.54, 11.31; UV λ_{max} (methanol) 221 (18 710), 271 (8740) nm. Anal. Calcd for $C_{17}H_{19}N_3O_4S_2 \cdot {}^1/_2C_6H_5CH_3$: C, H, N.

5-[(Phenylmethyl)thio]-4-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)imidazo[4,5-*d*]isothiazole (9e) and 5-[(Phenylmethyl)thio]-6-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)imidazo[4,5-*d*]isothiazole (8e). 5-(Methylthio)-imidazo[4,5-*d*]isothiazole (**1e**, 247 mg, 1 mmol) was dissolved

in warm, dry acetonitrile (20 mL). Sodium hydride (80% w/w dispersion in mineral oil, 36 mg, 1.2 mmol) was added in one portion, and the resulting suspension stirred at room temperature for 1 h. 2,3,5-Tri-*O*-benzyl-α-D-arabinofuranosyl chloride (**7**, 1.3 mmol) in 5 mL dichloromethane was added via cannula, and stirring was continued for 48 h at room temperature. Saturated aqueous ammonium chloride (1 mL) was added, and the suspension was partitioned between water and ethyl acetate. The organic phase was washed with brine, dried over magnesium sulfate, filtered, and concentrated to a syrup. This residue was chromatographed (20% ethyl acetate/hexane, 4 × 15 cm) to afford 198 mg (30%) of **9e** as an inseparable mixture of anomers (α/β = 1:5): R_f 0.75 (30% ethyl acetate/hexane); ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.27 (s, 1H), 7.45−7.02 (m, 18H), 6.73 (m, 2H), 6.03 (d, J = 3.1 Hz, 1H, H-1′ of β-anomer), 5.98 (d, J = 3.3 Hz, 0.25H, H-1′ of α-anomer), 4.65−3.45 (m, 13H). Continued elution of the column gave **8e** (297 mg, 46%) as a syrup containing an inseparable mixture of anomers (α/β = 1:13): R_f 0.60 (30% ethyl acetate/hexane); ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.42 (s, 1H), 7.45−7.07 (m, 18H), 6.86 (m, 2H), 6.01 (s, 1H, H-1′ of β-anomer), 5.95 (s, 0.08H, H-1′ of α-anomer), 4.65−3.55 (m, 13H).

6-β-D-Arabinofuranosyl-5-[(phenylmethyl)thio]imidazo-[4,5-*d*]isothiazole (10e). To a solution of **8e** (297 mg, 0.46 mmol) in dry dichloromethane (5 mL) at −78 °C was added boron trichloride (1.0 M in dichloromethane, 4.6 mL) dropwise over 0.25 h. The solution was warmed to 0 °C via an ice bath and then stirred for 2 h. The reaction mixture was cooled to −78 °C and quenched via the dropwise addition of methanol (5 mL), and the vigorously stirred solution was then neutralized to pH 7 at 0 °C with an ice cold solution of 1 M sodium hydroxide (ca. 13 mL). The organic layer was removed, and the aqueous phase was extracted with chloroform (3 × 10 mL) and then with ethyl acetate (2 × 10 mL). The combined organics were dried over magnesium sulfate, filtered, concentrated, and chromatographed (5% methanol/chloroform, 2 × 15 cm) to provide a residue which was taken up in methanol, diluted with water, and lyophilized to afford 117 mg (67%) of **10e** as a hydroscopic foam: $[\alpha]_D$ +4.8° (*c* 1.0, methanol); R_f 0.44 (10% methanol/chloroform); ^{1}H NMR (360 MHz, DMSO-d_6) δ 8.51 (s, 1H), 7.44−7.24 (m, 5H), 6.00 (d, 1H, D_2O exchangeable), 5.96 (d, J = 3.7 Hz, 1H, H-1′), 5.61 (d, 1H, D_2O exchangeable), 5.02 (t, 1H, D_2O exchangeable), 4.50 (m, 2H), 4.10 (m, 1H), 3.98 (m, 1H), 3.81 (m, 1H), 3.62 (m, 2H); ^{13}C NMR (90 MHz, DMSO-d_6) δ 150.73 (C6a or C5), 150.63 (C5 or C6a), 148.61 (C3a), 143.22 (C3), 136.99, 128.97, 128.51, 127.49, 86.11, 85.66, 76.72, 76.26, 61.59, 37.18; UV λ_{max} (methanol) 219 (19 551), 274 (8490) nm. Anal. Calcd for $C_{16}H_{17}N_3O_4S_2$: C, H, N.

4-β-D-Arabinofuranosyl-5-[(phenylmethyl)thio]imidazo-[4,5-*d*]isothiazole (11e). To a solution of **9e** (198 mg, 0.30 mmol) in dry dichloromethane (5 mL) at −78 °C was added boron trichloride (1.0 M in dichloromethane, 3.0 mL) dropwise over 0.25 h. The solution was warmed to 0 °C via an ice bath and then stirred for 2 h. The reaction mixture was cooled to −78 °C, quenched via the dropwise addition of methanol (5 mL), and the vigorously stirred solution was then neutralized to pH 7 at 0 °C with an ice cold solution of 1 M sodium hydroxide (ca. 9 mL). The organic layer was removed, and the aqueous phase was extracted with chloroform (3 × 10 mL) and then with ethyl acetate (2 × 10 mL). The combined organics were dried over magnesium sulfate, filtered, concentrated, and chromatographed (5% methanol/chloroform, 2 × 15 cm) to provide a residue which was taken up in methanol, diluted with water, and lyophilized to afford 32 mg (28%) of **11e** hemihydrate as a hygroscopic foam: $[\alpha]_D$ −28.4° (*c* 0.4, methanol); R_f 0.40 (10% methanol/chloroform); ^{1}H NMR (360 MHz, DMSO-d_6) δ 8.50 (s, 1H), 7.45−7.24 (m, 5H), 5.97 (d, J = 4.1 Hz, 1H, H-1′), 5.61 (d, 1H, D_2O exchangeable), 5.57 (d, 1H, D_2O exchangeable), 5.15 (t, 1H, D_2O exchangeable), 4.57 (q, 2H), 4.02 (m, 2H), 3.82 (m, 1H), 3.70 (m, 2H); ^{13}C NMR (90 MHz, DMSO-d_6) δ 161.68 (C6a), 151.76 (C5), 141.72 (C3), 138.63 (C3a), 136.87, 128.93, 128.51, 127.52, 87.05, 84.90, 76.30, 75.75, 60.75, 36.75; UV λ_{max} (methanol) 256 (10 480), 281 (12 560) nm. Anal. Calcd for $C_{16}H_{17}N_3O_4S_2 \cdot {}^1/_2H_2O$: C, H, N.

3-Methyl-4-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)imidazo[4,5-*d*]isothiazole (14a) and 3-Methyl-6-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)imidazo[4,5-*d*]isothiazole (13a). 3-Methylimidazo[4,5-*d*]isothiazole (**1a**, 0.20 g, 1.4 mmol) was suspended in dry acetonitrile (15 mL), to which was added *N*,*O*-bis(trimethylsilyl)acetamide (0.37 mL, 1.5 mmol) in one portion. The suspension was heated to 80 °C for 0.5 h, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**12**, 0.72 g, 1.4 mmol) was added in one portion, and then (trimethylsilyl)-trifluoromethanesulfonate (0.30 mL, 1.6 mmol) was added dropwise over 5 min. The solution was stirred for 1.25 h at 80 °C, allowed to cool to room temperature, and then poured into ice cold dichloromethane (15 mL) and saturated aqueous sodium bicarbonate (15 mL). The aqueous layer was washed with dichloromethane (15 mL), and the combined organic phases were dried over magnesium sulfate, filtered, and concentrated. The residue was chromatographed (50% ethyl acetate/hexane, 4 × 20 cm), and fractions containing the faster moving product were concentrated to yield 0.22 g (26%) of **14a** as a foam: R_f 0.49 (50% ethyl acetate/hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08−7.90 (m, 6H), 7.62−7.36 (m, 10H), 6.36 (d, J = 5.5 Hz, 1H, H-1′), 6.02 (t, 1H), 5.94 (m, 1H), 4.91−4.65 (m, 3H), 2.68 (s, 3H). Fractions containing the slower moving product were combined, and concentrated to yield 0.31 g (37%) of **13a** as a foam: R_f 0.26 (50% ethyl acetate/hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08−7.93 (m, 6H), 7.60−7.39 (m, 10H), 6.23 (d, J = 4.7 Hz, 1H, H-1′), 5.88−5.82 (m, 2H), 4.91−4.66 (m, 3H), 2.59 (s, 3H).

3-Methyl-6-β-D-ribofuranosylimidazo[4,5-*d*]isothiazole (15a). A suspension of **13a** (309 mg, 0.53 mmol) in 15 mL of methanolic ammonia was stirred at room temperature in a pressure tube for 48 h. The solvent was removed, and the residue was taken up in water (15 mL) and extracted with chloroform (2 × 5 mL) and then hexane (10 mL). The aqueous phase was lyophilized, and the resulting solid was chromatographed (15% 9:1 methanol−water/ethyl acetate, 2 × 15 cm) to afford 135 mg (94%) of **15a** (mp 159−161 °C) which was homogeneous on TLC and contained no impurities in the ^{1}H NMR spectrum. A sample was recrystallized from methanol for analysis: mp 171−172.5 °C; $[\alpha]_D$ −59.2° (*c* 1.0, methanol); R_f 0.27 (10% methanol/chloroform); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.22 (s, 1H), 5.77 (d, J = 6.6 Hz, 1H, H-1′), 5.55 (d, 1H, D_2O exchangeable), 5.31 (d, 1H, D_2O exchangeable), 5.04 (t, 1H, D_2O exchangeable), 4.16 (dd, 1H), 4.03 (m, 1H), 3.98 (m, 1H), 3.55 (m, 2H), 2.50 (s, 3H); ^{13}C NMR (90 MHz, DMSO-d_6) δ 153.19 (C3), 148.90 (C3a), 146.00 (C6a), 145.31 (C5), 89.00, 86.01, 73.93, 70.81, 61.64, 16.26; UV λ_{max} (methanol) 209 (16 420), 249 (6730) nm. Anal. Calcd for $C_{10}H_{13}N_3O_4S$: C, H, N.

3-Methyl-4-β-D-ribofuranosylimidazo[4,5-*d*]isothiazole (16a). A suspension of **14a** (192 mg, 0.33 mmol) in 10 mL of methanolic ammonia was stirred at room temperature in a pressure tube for 48 h. The solvent was removed, and the residue was taken up in water (15 mL) and extracted with chloroform (2 × 5 mL) and then hexane (10 mL). The aqueous phase was lyophilized, and the resulting solid was chromatographed (15% 9:1 methanol−water/ethyl acetate, 2 × 15 cm) to afford 73 mg (82%) of **16a** (mp 150−151 °C) which was homogeneous on TLC and contained no impurities in the ^{1}H NMR spectrum. A sample was recrystallized from methanol for analysis: mp 157−158 °C; $[\alpha]_D$ −11.7° (*c* 0.8, methanol); R_f 0.21 (10% methanol/chloroform); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.45 (s, 1H), 5.90 (d, J = 5.2 Hz, 1H, H-1′), 5.61 (d, 1H, D_2O exchangeable), 5.26 (d, 1H, D_2O exchangeable), 5.09 (t, 1H, D_2O exchangeable), 4.24 (dd, 1H), 4.09 (dd, 1H), 3.97 (dd, 1H), 3.63 (m, 2H), 2.61 (s, 3H); ^{13}C NMR (90 MHz, DMSO-d_6) δ 163.50 (C6a), 148.23 (C3), 144.51 (C5), 134.73 (C3a), 89.29, 85.54, 75.27, 69.64, 60.75, 18.14; UV λ_{max} (methanol) 207 (4860), 246 (10 000), 252 (shoulder) nm. Anal. Calcd for $C_{10}H_{13}N_3O_4S$: C, H, N.

3-Methyl-5-(methylthio)-6-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)imidazo[4,5-*d*]isothiazole (13b). 3-Methyl-5-(methylthio)imidazo[4,5-*d*]isothiazole (**1b**, 0.56 g, 3 mmol) was suspended in dry acetonitrile (15 mL) to which was added *N*,*O*-bis(trimethylsilyl)acetamide (0.78 mL, 3.15 mmol) in one portion. The suspension was heated to 80 °C for 0.5 h, 1-*O*-

acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**12**, 1.51 g, 3 mmol) was added in one portion, and then the mixture was allowed to cool to 50 °C over 0.5 h. (Trimethylsilyl)trifluoromethanesulfonate (0.61 mL, 3.15 mmol) was added dropwise over 5 min, and the solution was stirred for 0.75 h at 50 °C. The dark reaction mixture was allowed to cool to room temperature and then poured into ice cold dichloromethane (35 mL) and saturated aqueous sodium bicarbonate (35 mL). The aqueous layer was washed with dichloromethane (35 mL), and the combined organic phases were dried over magnesium sulfate, filtered and concentrated. The residue was chromatographed (5 × 15 cm, chloroform to 1% methanol/chloroform), and fractions containing product were concentrated to yield 1.15 g (61%) of crude **13b** as a syrup, which was used without further purification: R_f 0.60 (2% methanol/chloroform); ^{1}H NMR (360 MHz, $CDCl_3$) δ 8.12 (d, 2H), 7.99 (d, 2H), 7.96 (d, 2H), 7.48−7.35 (m, 9H), 6.42 (d, J = 6.5 Hz, 1H, H-1′), 5.93 (dd, 1H), 5.80 (t, 1H), 4.88−4.68 (m, 3H), 2.71 (s, 3H), 2.56 (s, 3H).

3-Methyl-5-(methylthio)-6-β-D-ribofuranosylimidazo-[4,5-*d*]isothiazole (15b). A suspension of **13b** (0.98 g, 1.5 mmol) in 40 mL of methanolic ammonia was stirred at room temperature in a pressure tube for 24 h. The solvent was removed, and the residue was partitioned between water (40 mL) and chloroform (20 mL). The organic layer was removed, and the aqueous layer was washed with chloroform (3 × 20 mL). The aqueous phase was concentrated in vacuo, and the residue was recrystallized from methanol to yield 0.30 g of **15b** (61%): mp 119−121 °C; $[\alpha]_D$ −34.5° (*c* 1.0, methanol); R_f 0.31 (10% methanol/chloroform); ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.70 (d, 1H, D_2O exchangeable), 5.62 (d, J = 6.3 Hz, 1H, H-1′), 5.38 (d, 1H, D_2O exchangeable), 5.05 (t, 1H, D_2O exchangeable), 4.18 (dd, 1H), 4.01 (m, 1H), 3.96 (m, 1H), 3.55−3.48 (m, 2H), 2.67 (s, 3H), 2.48 (s, 3H); ^{13}C NMR (90 MHz, DMSO-d_6) δ 152.78 (C5), 152.03 (C3), 147.54 (C3a), 147.19 (C6a), 87.91, 86.02, 73.62, 70.82, 61.70, 16.32, 15.71; UV λ_{max} (methanol) 221 (15 600), 268 (7700) nm. Anal. Calcd for $C_{11}H_{15}N_3O_4S_2 \cdot {}^1/_2H_2O$: C, H, N.

5-(Methylthio)-4-β-D-ribofuranosylimidazo[4,5-*d*]isothiazole (16c). 5-(Methylthio)imidazo[4,5-*d*]isothiazole (**1c**, 684 mg, 4 mmol) was suspended in dry acetonitrile (40 mL), to which was added *N*,*O*-bis(trimethylsilyl)acetamide (1.04 mL, 4.2 mmol) in one portion. The suspension was heated to 80 °C for 0.5 h, and the resulting solution was cooled to 50 °C. 1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**12**, 2.02 g, 4 mmol) was added in one portion, and then (trimethylsilyl)-trifluoromethanesulfonate (0.81 mL, 4.2 mmol) was added dropwise over 5 min. The solution was stirred for 1 h at 50 °C and then heated to 80 °C for 0.5 h. The resulting dark solution was poured into ethyl acetate (50 mL) and saturated aqueous sodium bicarbonate (50 mL). The organic phase was removed, dried over magnesium sulfate, filtered, and concentrated. The dark red oil was chromatographed (30% ethyl acetate/hexane to 50% ethyl acetate/hexane, 4 × 20 cm), and fractions containing a product having an R_f 0.30 were pooled and concentrated to yield a dark oil. The crude material was stirred in 10 mL of methanolic ammonia at room temperature in a pressure bottle for 48 h. The dark solution was concentrated to a gum and then chromatographed (10% methanol/chloroform, 2 × 15 cm) to afford an oil which crystallized upon the addition of water to yield 136 mg (11%) of **16c** (mp 167−172 °C). A portion was recrystallized from methanol (80% recovery) for analysis: mp 167−172 °C dec; $[\alpha]_D$ −42.3° (*c* 1.2, methanol); R_f 0.30 (10% methanol/chloroform); ^{1}H NMR (360 MHz, DMSO-d_6) δ 8.72 (s, 1H), 5.65 (d, J = 7.3 Hz, 1H, H-1′), 5.54 (d, 1H, D_2O exchangeable), 5.30 (d, 1H, D_2O exchangeable), 5.18 (t, 2H, D_2O exchangeable), 4.37 (dd, 1H), 4.09 (m, 1H), 3.98 (m, 1H), 3.62 (m, 2H), 2.72 (s, 3H); ^{13}C NMR (90 MHz, DMSO-d_6) δ 163.29, (C6a) 155.92 (C5), 140.20 (C3), 136.66 (C3a), 88.18, 86.28, 73.61, 70.41, 61.37, 15.25; UV λ_{max} (methanol) 222 (4430), 255 (10 550), 279 (12 640) nm. Anal. Calcd for $C_{10}H_{13}N_3O_4S_2$: C, H, N.

3-Methyl-5-[(phenylmethyl)thio]-6-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)imidazo[4,5-*d*]isothiazole (13d). 3-Methyl-5-[(phenylmethyl)thio]imidazo[4,5-*d*]isothiazole (**1d**, 527 mg, 2 mmol) was suspended in dry acetonitrile (20 mL), to which was added *N*,*O*-bis(trimethylsilyl)acetamide (0.52 mL, 2.1 mmol) in one portion. The suspension was heated to 80 °C for 0.5 h, and the resulting solution was cooled to 50 °C. 1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**12**, 1.02 g, 2 mmol) was added in one portion, and then (trimethylsilyl)trifluoromethanesulfonate (0.43 mL, 2.2 mmol) was added dropwise over 5 min. The solution was stirred for 1.5 h at 50 °C, and the resulting dark red solution was poured into dichloromethane (50 mL) and saturated aqueous sodium bicarbonate (50 mL). The aqueous layer was washed with dichloromethane (25 mL), and the combined organic phases were dried over magnesium sulfate, filtered, and concentrated. The dark red oil was chromatographed (30% ethyl acetate/hexane, 4 × 20 cm) to yield an oil which crystallized upon the addition of ethanol. This crude product was recrystallized from ethanol to yield 0.30 g (21%) of **13d**: mp 146.5−147 °C; R_f 0.36 (30% ethyl acetate/hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (dd, 2H), 7.94 (m, 4H), 7.59−7.22 (m, 14H), 6.30 (d, 6.6H, H-1′), 5.89 (dd, 1H), 5.73 (t, 1H), 4.84−4.63 (m, 3H), 4.44 (dd, 2H), 2.59 (s, 3H). Anal. Calcd for $C_{38}H_{31}N_3O_7S_2$: C, H, N.

3-Methyl-5-[(phenylmethyl)thio]-6-β-D-ribofuranosylimidazo[4,5-*d*]isothiazole (15d). A suspension of **13d** (280 mg, 0.40 mmol) in 10 mL of methanolic ammonia was stirred at room temperature in a pressure tube for 48 h. The solvent was removed and the residue chromatographed (5% methanol/chloroform to 10% methanol/chloroform, 2 × 15 cm) to yield a glass. This residue was dissolved in a small amount of methanol, diluted with excess water, and then lyophilized to yield 0.16 g (98%) of **15d**: mp 108.5−110 °C; $[\alpha]_D$ −72.6° (*c* 1.2, methanol); R_f 0.34 (10% methanol/chloroform); ^{1}H NMR (360 MHz, DMSO-d_6) δ 7.44−7.26 (m, 5H), 5.74 (d, J = 6.84 Hz, 1H, H-1′), 5.61 (d, 1H, D_2O exchangeable), 5.37 (d, 1H, D_2O exchangeable), 5.04 (t, 2H, D_2O exchangeable), 4.49 (s, 2H), 4.17 (m, 1H), 4.00 (m, 1H), 3.95 (m, 1H), 3.53 (m, 2H), 2.50 (s, 3H); ^{13}C NMR (90 MHz, DMSO-d_6) δ 152.19 (C3), 150.84 (C5), 147.54 (C3a), 147.08 (C6a), 136.84, 129.03, 128.51, 127.51, 87.98, 86.04, 73.67, 70.79, 61.67, 37.44, 16.29; UV λ_{max} (methanol) 221 (21 730), 271 (9660) nm. Anal. Calcd for $C_{17}H_{19}N_3O_4S_2 \cdot H_2O$: C, H, N.

5-(Methylthio)-6-β-D-ribofuranosylimidazo[4,5-*d*]isothiazole (15c). Sodium hydride (80% w/w in mineral oil, 47 mg, 1.56 mmol) was added in one portion to a solution of **1c** in dry acetonitrile at room temperature. The mixture was stirred at room temperature for 1 h, and a solution of 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl bromide (**17**, 6.25 mmol) in 5 mL of acetonitrile was added dropwise over 5 m. The mixture was stirred for 2 h at room temperature, and then poured into water (30 mL) and ethyl acetate (50 mL). The organic phase was washed with saturated sodium bicarbonate and brine, dried over magnesium sulfate, filtered, and concentrated. The residue was chromatographed (chloroform to 1% methanol/chloroform, 4 × 20 cm) to afford a large amount of sugar byproducts, followed by nucleoside material. The crude residue was dissolved in methanol (15 mL) containing sodium methoxide (20 mg) and stirred 3 h at room temperature. The solution was adjusted to pH 5 with 1 N HCl, concentrated, and chromatographed (2 × 15 cm, 10% methanol/chloroform), and the residue was recrystallized from methanol to yield 43 mg (11%) of **15c**: mp 151−152 °C; $[\alpha]_D$ −28.8° (*c* 1.4, methanol); R_f 0.18 (10% methanol/chloroform); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.56 (s, 1H), 5.70 (d, J = 6.9 Hz, 1H, H-1′), 5.61 (d, 1H, D_2O exchangeable), 5.36 (d, 1H, D_2O exchangeable), 5.05 (t, 2H, D_2O exchangeable), 4.23 (m, 1H), 4.03−3.97 (m, 2H), 3.55 (m, 2H), 2.67 (s, 3H); ^{13}C NMR (90 MHz, DMSO-d_6) δ 153.69 (C5), 149.20 (C3a), 148.00 (C6a), 143.70 (C3), 87.88, 86.11, 73.58, 70.82, 61.66, 15.48; UV λ_{max} (methanol) 221 (12 130), 273 (6050) nm. Anal. Calcd for $C_{10}H_{13}N_3O_4S_2$: C, H, N.

***In Vitro* Antiproliferative Studies.** The *in vitro* cytotoxicity against L1210 was determined in an L1210 cell growth assay described previously.[37] L1210 murine leukemic cells (kindly provided by J. R. Bertino, Memorial Sloan Kettering Center, New York, NY 10021) were grown in Fischer's medium supplemented with 10% heat inactivated (56 °C, 30 min) horse

serum and were subcultured by serial dilution. Growth rates in the presence of selected concentrations of the test compound were calculated from determinations of the number of cells at 0, 24, 48, 72, and 96 h. The growth rate was calculated as the slope of a semilogarithmic plot of these cell numbers against time for the treated culture as a percent of the slope obtained for control cultures. The IC_{50} was defined as the concentration required to decrease the growth rate to 50% of that of the untreated control cells (population doubling time = 12 h).

The G-361 human melanoma, HT-29 human colon carcinoma, and ZR-75-1 human breast carcinoma cell lines (all from the ATCC, Rockville, MD 20852-1776) were grown in RPMI 1640 medium supplemented with 5% fetal bovine serum and were subcultured using 0.5 g trypsin, 0.2g EDTA/L. Inhibition of cell proliferation was assayed as described previously.[38] The cells were plated at the following densities, compounds were added the following day, and growth was assayed on the indicated number of days after plating: G-361, 1000 cells/well, 6 days; HT-29, 2000 cells/well, 5 days; ZR-75-1, 5000 cells/well, 6 days. The results were expressed as percent of control optical density (sulforhodamine B) on the assay day.

Cell Culture Procedures for Antiviral Studies. The routine growth and passage of KB, BSC-1, and HFF cells was performed in monolayer cultures using minimal essential medium (MEM) with either Hanks salts [MEM(H)] or Earle salts [MEM(E)] supplemented with 10% calf serum or 10% fetal bovine serum (HFF cells). The sodium bicarbonate concentration was varied to meet the buffering capacity required. Cells were passaged at 1:2 to 1:10 dilutions according to conventional procedures by using 0.05% trypsin plus 0.02% EDTA in a HEPES-buffered salt solution.

Virological Procedures. The Towne strain, plaque-purified isolate P_o, of HCMV was kindly provided by Dr. Mark Stinski, University of Iowa. The KOS strain of HSV-1 was used in most experiments and was provided by Dr. Sandra K. Weller, University of Connecticut. Stock HCMV was prepared by infecting HFF cells at a multiplicity of infection (moi) of <0.01 plaque-forming units (pfu) per cell as detailed previously.[39] High-titer HSV-1 stocks were prepared by infecting KB cells at an moi of <0.1 also as detailed previously.[39] Virus titers were determined using monolayer cultures of HFF cells for HCMV and monolayer cultures of BSC-1 cells for HSV-1 as described earlier.[40] Briefly, HFF or BSC-1 cells were planted as described above in 96-well cluster dishes and incubated overnight at 37 °C. The next day cultures were inoculated with 0.2 mL of HCMV or HSV-1 stock suspension and serially diluted 1:3 across the remaining 11 columns of the 96-well plate. After virus adsorption the inoculum was replaced with fresh medium, and cultures were incubated for 7 days for HCMV, 2 or 3 days for HSV-1. Plaques were enumerated under 20-fold magnification in wells having the dilution which gave 5−20 plaques per well. Virus titers were calculated according to the following formula: Titer (pfu/mL) = number of plaques $\times 5 \times 3^n$; where n represents the nth dilution of the virus used to infect the well in which plaques were enumerated.

HCMV Plaque Reduction Assay. HFF cells in 24-well cluster dishes were infected with approximately 100 pfu of HCMV per cm^2 cell sheet using the procedures detailed above.[39] Following virus adsorption, compounds dissolved in growth medium were added to duplicate wells in four to eight selected concentrations. After incubation at 37 °C for 7 days, cell sheets were fixed and stained with crystal violet, and microscopic plaques were enumerated as described above. Drug effects were calculated as a percentage of reduction in number of plaques in the presence of each drug concentration compared to the number observed in the absence of drug.

HSV-1 ELISA. An ELISA developed by us[41] was employed to detect HSV-1. Ninety-six-well cluster dishes were planted with 10 000 BSC-1 cells per well in 200 μL per well of MEM-(E) plus 10% calf serum. After overnight incubation at 37 °C, selected drug concentrations in quadruplicate and HSV-1 at a concentration of 100 pfu/well were added. Following a 3-day incubation at 37 °C, medium was removed, plates were blocked and rinsed, and horseradish peroxidase conjugated rabbit anti-HSV-1 antibody was added. Following removal of the antibody containing solution, plates were rinsed and then developed by adding 150 μL per well of a solution of tetramethylbenzidine as substrate. The reaction was stopped with H_2SO_4, and absorbance was read at 450 and 570 nm. Drug effects were calculated as a percentage of the reduction in absorbance in the presence of each drug concentration compared to absorbance obtained with virus in the absence of drug.

Cytotoxicity Assays in Antiviral Studies. Two different assays were used to explore cytotoxicity of selected compounds using methods we have detailed previously. (i) Cytotoxicity produced in stationary HFF cells was determined by microscopic inspection of cells not affected by the virus used in plaque assays.[38] (ii) The effect of compounds during two population doublings of KB cells was determined by crystal violet staining and spectrophotometric quantitation of dye eluted from stained cells as described earlier.[42] Briefly, 96-well cluster dishes were planted with KB cells at 3000−5000 cells per well. After overnight incubation at 37 °C, test compounds were added in quadruplicate at six to eight concentrations. Plates were incubated at 37 °C for 48 h in a CO_2 incubator, rinsed, fixed with 95% ethanol, and stained with 0.1% crystal violet. Acidified ethanol was added, and absorbance of solutions in individual wells was read at 570 nm in a spectrophotometer designed to read 96-well plates.

Data Analysis. Dose−response relationships were constructed by linearly regressing the percent inhibition of parameters derived in the preceding sections against log drug concentrations. Fifty-percent inhibitory (IC_{50}) concentrations were calculated from the regression lines. Samples containing positive controls (acyclovir for HSV-1, ganciclovir for HCMV, and 2-acetylpyridine thiosemicarbazone for cytotoxicity) were used in all assays.

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References

(1) Brown, G. B.; Weliky, V. S. The Synthesis of 9-β-D-Ribofuranosylpurine and the Identity of Nebularine. *J. Biol. Chem.* **1953**, *204*, 1019−1024.
(2) Giner-Sorolla, A.; Medrek, L.; Bendich, A. Synthesis and Biological Activity of 9-β-D-Ribofuranosyl-6-hydroxylaminopurine. *J. Med. Chem.* **1966**, *9*, 143−144.
(3) Elion, G. B.; Burgi, E.; Hitchings, G. H. Studies on Condensed Pyrimidine Systems. IX. The Synthesis of Some 6-Substituted Purines. *J. Am. Chem. Soc.* **1952**, *74*, 411−414.
(4) Schabel, F. M., Jr.; Montgomery, J. A.; Skipper, H. E.; Laster, W. R., Jr.; Thomson, J. R. Experimental Evaluation of Potential Anticancer Agents I. Quantitative Therapeutic Evaluation of Certain Purine Analogs. *Cancer Res.* **1961**, *21*, 690−699.
(5) Montgomery, J. A.; Schabel, F. M., Jr.; Skipper, H. E. Experimental Evaluation of Potential Anticancer Agents IX. The Ribonucleosides and Ribonucleotides of Two Purine Antagonists. *Cancer Res.* **1962**, *22*, 504−509.
(6) Bennett, L. L., Jr.; Vail, M. H.; Chumley, S.; Montgomery, J. A. Activity of Adenosine Analogs Against a Cell Culture Line Resistant to 2-Fluoroadenine. *Biochem. Pharmacol.* **1966**, *15*, 1719−1728.
(7) Montgomery, J. A.; Clayton, S. J.; Fitzgibbon, W. E., Jr. The Ribonucleosides of 4-Aminopyrazolo[3,4-*d*]pyrimidine. *J. Heterocycl. Chem.* **1964**, *1*, 215−216.
(8) Mizuno, Y.; Ikehara, M.; Watanabe, K. A.; Suzaki, S.; Itoh, T. Synthetic Studies of Potential Antimetabolites. IX. The Anomeric Configuration of Tubercidin. *J. Org. Chem.* **1963**, *28*, 3329−3331.
(9) Tolman, R. L.; Robins, R. K.; Townsend, L. B. Pyrrolopyrimidine Nucleosides III. The Total Synthesis of Toyocamycin, Sangivamycin, Tubercidin, and Related Derivatives. *J. Am. Chem. Soc.* **1969**, *91*, 2102−2108.

(10) Townsend, L. B.; Drach, J. C.; Wotring, L. L.; Vittori, S.; Pudlo, J. S.; Swayze, E. E.; Gupta, P.; Maruyama, T.; Saxena, N.; Coleman, L. A.; Westerman, A. C.; Spurr, J.; Nassiri, M. R.; Turk, S. R.; Krawczyk, S. H. Design, Synthesis, and Studies on the Structure Activity Relationships of Certain Pyrrolo[2,3-*d*]-pyrimidine Nucleosides and Structurally Related Analogs as Potential Antineoplastic and Antiviral Agents. *Il Farmaco* **1991**, *46*, 113−139.

(11) Namikoshi, M.; Carmichael, W. W.; Sakai, R.; Jares-Erijman, E. A.; Kaup, A. M.; Rinehart, K. L. 9-Deazaadenosine and Its 5′-α-D-Glucopyranoside Isolated from the Cyanobacterium anabaena affinis Strain VS-1. *J. Am. Chem. Soc.* **1993**, *115*, 2504−2505.

(12) Koyama, G.; Maeda, K.; Umezawa, H.; Iitaka, Y. The Structural Studies of Formycin and Formycin B. *Tetrahedron Lett.* **1966**, 597−602.

(13) Robins, R. K.; Townsend, L. B.; Cassidy, F.; Gerster, J. F.; Lewis, A. F.; Miller, R. L. Structure of the Nucleoside Antibiotics Formycin, Formycin B and Laurusin (1). *J. Heterocycl. Chem.* **1966**, *3*, 110−114.

(14) Shimada, N; Yagisawa, N.; Naganawa, H.; Takita, T.; Hamada, M.; Takeuchi, T.; Umezawa, H. Oxanosine, a Novel Nucleoside From Actinomycetes. *J. Antibiot.* **1981**, *34*, 1216−1218.

(15) Parkanyi, C. Recent Progress in the Quantum Chemistry of Organic Sulfur Compounds. *Mech. React. Sulfur Compd.* **1969**, *4*, 69−94.

(16) Chwang, T. L.; Nemec, J.; Welch, A. D. Synthesis and Characterization of 5-Amino-2-(β-D-ribofuranosyl)-1,2,4-thiadiazoline-3-one, a Sulfur-Containing Analog of Cytidine. *J. Carbohydr. Nucleosides Nucleotides* **1980**, *7*, 159−166.

(17) Gordon, M. P.; Weliky, V. S.; Brown, G. B. A Study of the Action of Acid and Alkali on Certain Purines and Purine Nucleosides. *J. Am. Chem. Soc.* **1957**, *79*, 3245−3250.

(18) Montgomery, J. A.; Hewson, K. Analogs of 6-Methyl-9-β-D-ribofuranosylpurine. *J. Med. Chem.* **1968**, *11*, 48−52.

(19) (a) Swayze, E. E. The Synthesis of Several Members of the New Imidazo[4,5-*d*]isothiazole Ring System: Novel 5:5 Fused Analogs of Purines and Purine Nucleosides. Ph.D. Thesis, 1994, The University of Michigan, Ann Arbor, MI. (b) Swayze, E. E.; Townsend, L. B. The Synthesis of Substituted Imidazo[4,5-*d*]-isothiazoles via the Ring Annulation of Isothiazole Diamines: An Investigation of the Chemical, Physical, and Biological Properties of Several Novel 5:5 Fused Analogs of the Purine Ring System. *J. Org. Chem.* **1995**, *60*, 6309−6317.

(20) Srivastava, P. C.; Robins, R. K.; Meyer, R. B. Synthesis and Properties of Purine Nucleosides and Nucleotides. In *Chemistry of Nucleosides and Nucleotides*; Townsend, L. B., Ed.; Plenum Press: New York, 1988; Vol. 1, pp 113−281.

(21) Revankar, G. R.; Robins, R. K. The Synthesis and Chemistry of Heterocyclic Analogues of Purine Nucleosides and Nucleotides. In *Chemistry of Nucleosides and Nucleotides*; Townsend, L. B., Ed.; Plenum Press: New York, 1991; Vol. 2, pp 161−398.

(22) Swayze, E. E.; Townsend, L. B. The Synthesis of Several Imidazo[4,5-*d*]isothiazoles; Derivatives of a New Ring System. 206th American Chemical Society National Meeting (Org #351), August 22−27, 1993, Chicago, IL.

(23) Swayze, E. E.; Townsend, L. B. The Synthesis of Several Imidazo[4,5-*d*]isothiazoles; Derivatives of a New Ring System. *J. Heterocycl. Chem.* **1993**, *30*, 1379−1383.

(24) Kazimierczuk, Z.; Cottam, H. B.; Revankar, G. R.; Robins, R. K. Synthesis of 2′-Deoxyadenosine, and Related 2′-Deoxynucleosides via a Novel Direct Stereospecific Sodium Salt Glycosylation Procedure. *J. Am. Chem. Soc.* **1984**, *106*, 6379−6382.

(25) Hoffer, M. α-Thymidin. *Chem. Ber.* **1960**, *93*, 2777−2781.

(26) Chenon, M.-T.; Pugmire, R. J.; Grant, D. M.; Panzica, R. P.; Townsend, L. B. Carbon-13 Magnetic Resonance. XXV. A Basic Set of Parameters for the Investigation of Tautomerism in Purines Established from Carbon-13 Magnetic Resonance Studies Using Certain Purines and Pyrrolo[2,3-*d*]pyrimidines. *J. Am. Chem. Soc.* **1975**, *97*, 4627−4636.

(27) Townsend, L. B. Nuclear Magnetic Resonance Spectroscopy in the Study of Nucleic Acid Components and Certain Related Derivatives. In *Synthetic Procedures in Nucleic Acid Chemistry*; Zorbach, W. W., Tipson, R. S., Eds.; John Wiley & Sons: New York, 1972; Vol. 2, pp 323−340.

(28) Bhattacharya, A. K.; Ness, R. K.; Fletcher, H. G., Jr. 2-Deoxy-D-*erythro*-pentose. IX. Some Relationships Among the Rotations of Acylated Aldopentoses, Aldopentosyl Halides, and Anhydropentitols. *J. Org. Chem.* **1963**, *28*, 428−435.

(29) Ramasamy, K.; Imamura, N.; Robins, R. K.; Revankar, G. R. A Facile and Improved Synthesis of Tubercidin and Certain Related Pyrrolo[2,3-*d*]pyrimidine Nucleosides by the Stereospecific Sodium Salt Glycosylation Procedure. *J. Heterocycl. Chem.* **1988**, *25*, 1893−1898.

(30) Ramasamy, K.; Robins, R. K.; Revankar, G. R. Direct Synthesis of Pyrrole Nucleosides by the Stereospecific Sodium Salt Glycosylation Procedure. *J. Heterocycl. Chem.* **1987**, *24*, 863−868.

(31) Glaudemans, C. P. J.; Fletcher, H. G., Jr. Syntheses with Partially Benzylated Sugars. III. A Simple Pathway to a "*cis*-Nucleoside," 9-β-D-Arabinofuranosyladenine (Spongoadenosine). *J. Org. Chem.* **1963**, *28*, 3004−3006.

(32) Pudlo, J. S.; Townsend, L. B. A New and Novel Approach Towards the Synthesis of 3′-Deoxy-3′-hydroxymethyl Ribofuranosides. *Tetrahedron Lett.* **1990**, *22*, 3101−3104.

(33) Vorbruggen, H.; Krolikiewicz, K.; Bennua, B. Nucleoside Synthesis with Trimethylsilyl Triflate and Perchlorate as Catalysts. *Chem. Ber.* **1981**, *114*, 1234−1255.

(34) Stevens, J. D.; Ness, R. K.; Fletcher, H. G., Jr. Syntheses with Partially Benzylated Sugars. XI. Studies on the Synthesis of the Anomeric 5,6-Dimethyl-1-D-ribofuranosylbenzimidazoles (Ribazoles). Comparison of the Condensation of 2,3,5-Tri-*O*-benzoyl-D-ribofuranosyl Bromide and 2,3,5-Tri-*O*-benzyl-D-ribofuranosyl Chloride with 5,6-Dimethylbenzimidazole. *J. Org. Chem.* **1968**, *33*, 1806−1810.

(35) Tapiero, C.; Imbach, J.-L. The Determination of Anomeric Configuration of D-Ribofuranosyl Nucleosides by Proton Magnetic Resonance Spectroscopy. In *Nucleic Acid Chemistry. Improved and New Synthetic Procedures, Methods, and Techniques*; Townsend, L. B., Tipson, R. S., Eds.; John Wiley & Sons: New York, 1978; Part 2, pp 1055−1059.

(36) Baker, B. R. In *Ciba Foundation Symposium on the Chemistry and Biology of Purines*; Wolstenholme, G. E., O'Connor, C. M., Eds.; Little, Brown and Co.: Boston, 1957; p 120.

(37) Wotring, L. L.; Townsend, L. B. Study of the Cytotoxicity and Metabolism of 4-amino-3-carboxamido-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine Using Inhibitors of Adenosine Kinase and Adenosine Deaminase. *Cancer Res.* **1979**, *39*, 3018−3023.

(38) Meade, E. A.; Wotring, L. L.; Drach, J. C.; Townsend, L. B. Synthesis, Antiproliferative, and Antiviral Activity of 4-Amino-1-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyridazin-7(6*H*)-one and Related Derivatives. *J. Med. Chem.* **1993**, *36*, 3834−3842.

(39) Turk, S. R.; Shipman, C., Jr.; Nassiri, M. R.; Genzingler, G.; Krawczyk, S. H.; Townsend, L. B.; Drach, J. C. Pyrrolo[2,3-*d*]-pyrimidine Nucleosides as Inhibitors of Human Cytomegalovirus. *Antimicrob. Agents Chemother.* **1987**, *31*, 544−550.

(40) Prichard, M. N.; Turk, S. R.; Coleman, L. A.; Engelhardt, S. L.; Shipman, C., Jr.; Drach, J. C. A Microtiter Virus Yield Reduction Assay for the Evaluation of Antiviral Compounds Against Human Cytomegalovirus and Herpes Simplex Virus, *J. Virol. Methods* **1990**, *28*, 101−106.

(41) Prichard, M. N.; Shipman, C., Jr. A Three-dimensional Model to Analyze Drug-drug Interactions. *Antiviral Res.* **1990**, *14*, 181−206.

(42) Prichard, M. N.; Prichard, L. E.; Baguley, W. A.; Nassiri, M. R.; Shipman, C., Jr. Three-dimensional Analysis of the Synergistic Cytotoxicity of Ganciclovir and Zidovudine. *Antimicrob. Agents Chemother.* **1991**, *35*, 1060−1065.

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