Synthesis and Anti-HIV Activity of 4′-Azido- and 4′-Methoxynucleosides[1]

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A series of nucleosides were synthesized in which the 4′-hydrogen was substituted with either an azido or a methoxy group. The key steps in the syntheses of the 4′-azido analogues were the stereo- and regioselective addition of iodine azide to a 4′-unsaturated nucleoside precursor followed by an oxidatively assisted displacement of the 5′-iodo group. The 4′-methoxynucleosides were made via epoxidation of 4′-unsaturated nucleosides with in situ epoxide opening by methanol. Reaction-mechanism considerations, empirical conformation rules, NMR-based conformational calculations, and NOE experiments suggest that the 4′-azidonucleosides prefer a 3′-endo (N-type) conformation of the furanose moiety. When evaluated for their inhibitory effect on HIV in A3.01 cell culture, all the 4′-azido-2′-deoxy-β-D-nucleosides exhibited potent activity. IC_{50}'s ranged from 0.80 μM for 4′-azido-2′-deoxyuridine (**6c**) to 0.003 μM for 4′-azido-2′-deoxyguanosine (**6e**). Cytotoxicity was detected at 50–1500 times the IC_{50}'s in this series. The 4′-methoxy-2′-deoxy-β-D-nucleosides were 2–3 orders of magnitude less active and less toxic than their azido counterparts. Modifications at the 2′- or 3′-position of the 4′-substituted-2′-deoxynucleosides tended to diminish activity. Further evaluation of 4′-azidothymidine (**6a**) in H9, PBL, and MT-2 cells infected with HIV demonstrated a similar inhibitory profile to that of AZT. However, 4′-azidothymidine (**6a**) retained its activity against HIV mutants which were resistant to AZT.

Nucleoside analogues occupy a prominent position among inhibitors of human immunodeficiency virus. Currently, 3′-azido-3′-deoxythymidine (AZT) and dideoxyinosine (ddI) are the only drugs approved for the treatment of AIDS, while several other nucleoside analogues are in clinical development.[2] Fertile though the nucleoside field has been, these compounds are associated with serious toxicity. AZT has a deleterious effect on bone marrow progenitor cells,[3] which can lead to anemia and neutropenia.[4] The use of dideoxycytidine (ddC) and of didehydrodideoxythymidine (d4T) is limited by associated painful sensory–motor peripheral neuropathy.[2,5] Dideoxyinosine also shares this complication as well as causing acute pancreatitis[6,7] and hepatotoxicity[8] in some cases.

Another concern has been the emergence of resistant HIV strains in patients undergoing treatment with nucleosides. AZT-resistant variants have been isolated from patients on prolonged AZT therapy.[9] It has been estimated that resistance develops consistently within 18 months of the start of AZT treatment.[10] More recently, HIV strains having decreased sensitivity to ddI have been isolated from patients receiving ddI for more than 6 months.[11] These ddI-resistant strains were also shown to be resistant to ddC. In another study, clinical HIV isolates resistant to AZT displayed marked resistance to d4T.[12] It appears, then, that some cross resistance is inevitable among this class of similar nucleoside structures.

An important criterion for the design of any new nucleoside drug would, therefore, be a distinct dissimilarity of structure to the current family of dideoxynucleosides. It would be hoped that virus variants elicited by any of the other nucleoside drugs would not be cross resistant to such a compound. Furthermore, the adverse side effects uniquely associated with dideoxynucleosides, e.g. peripheral neuropathy and hematopoietic toxicity, might be absent.

Our investigations of novel nucleosides having potential anti-HIV activity have led us to nucleosides substituted at the 4′-position. Prior references to modification at this position have been infrequent. Verheyden et al. described the first synthesis of nucleocidin, a naturally occurring nucleoside antibiotic which contains a fluorine at the 4′-position.[13] Subsequently, the syntheses, via similar

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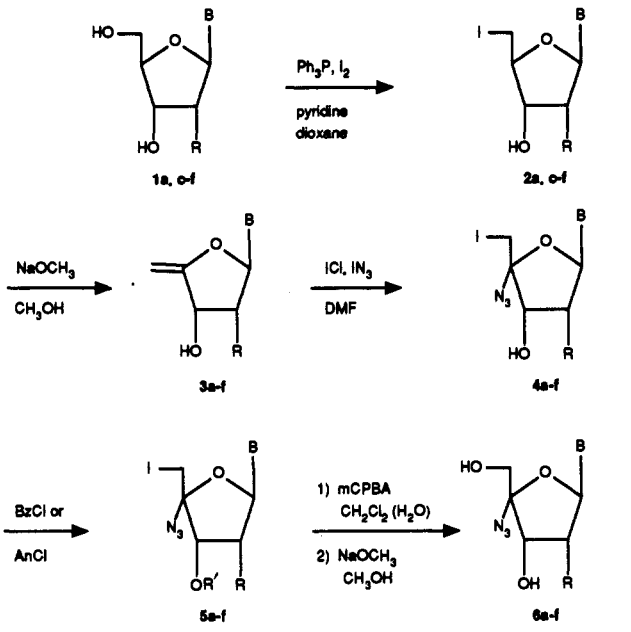
Scheme I



Compd.	B	R	R'
1-4, 6a	thymine	H	
1-4, 6b	uracil	OH	
1-4, 6c	uracil	H	
1-4, 6d	adenine	H	
1-4, 6e	guanine	H	
1-4, 6f	5-chlorouracil	H	
5a	thymine	H	An
5b	uracil	OBz	Bz
5c	uracil	H	An
5d	N^6,N^6-dibenzoyladenine	H	Bz
5e	N^2-benzoylguanine	H	Bz
5f	5-chlorouracil	H	An

chemistry, of ribonucleosides substituted at 4' with fluorine[14,15] as well as with methoxy[15,16,17] and azido groups[18] were described. More recently, other laboratories have reported the syntheses of uridine derivatives substituted at 4' with alkoxy[19] or fluorine.[20]

We now report the syntheses and biological evaluations of 4'-azido-2'-deoxynucleosides and 4'-methoxy-2'-deoxynucleosides which have potent in vitro anti-HIV activity. These compounds are unique because they are, thus far, the only nucleoside analogues which retain the 3'-hydroxy group and are exceptionally effective inhibitors of HIV. Furthermore, the 4'-azidonucleosides described herein are the only potent nucleoside inhibitors of HIV having an azido group in other than a 3'α-position.

Chemistry

The synthesis of the 4'-azidonucleosides was based on a strategy developed previously in our laboratories.[18] However, some improvements were realized over the earlier methodology, and a new method of 5'-iodine displacement was developed.

The general route to the 4'-azido analogues is illustrated in Scheme I. Selective iodination at 5' was found to be most efficient when the unprotected nucleoside was treated with triphenylphosphine, iodine, and either pyridine or imidazole[21] in dioxane. Yields ranged from 29% to 59%.

The dehydrohalogenation of 5'-halonucleosides has been well-described.[22] In the present work, we have found that either DBN[23] or sodium methoxide[24] will readily generate the 4'-olefin. Higher yields and easier purifications were usually obtained using sodium methoxide, however. Some of the 5'-iodo derivatives such as compounds **2a**[25,26] and **2c**[27] and the 4',5'-didehydro derivatives **3a**,[23,28] **3b**,[23] and **3d**[29] are known in the literature.

A key step in the synthesis was the addition of iodine azide to the 4'-double bond (compounds **3a–f**) in a regio- and stereospecific manner. Prior work from our laboratory[18] utilized iodine azide generated in situ from iodine monochloride and sodium azide to add to N^4-benzoyl-5'-deoxy-4',5'-didehydrocytidine. In this preparation, iodine added preferentially to the β-face of the double bond to give an iodonium ion intermediate which was opened on the α-side, at 4', by azide ion. Thus, 4'-azido-N^4-benzoyl-5'-deoxy-5'-iodocytidine was isolated in a 60%

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yield while only a slight amount of the 4′-epimer was formed.

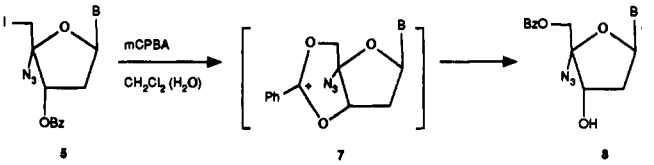
Having now extended this reaction to a series of 4′-unsaturated nucleosides, we have found that iodine azide addition usually proceeds in nearly quantitative yield with exceptionally high regio- and stereospecificity. NMR analysis of the IN_3 addition reactions of 4′-unsaturated-2′-deoxynucleosides indicated a 9:1 or higher ratio of the desired β-D-erythro isomer to the α-L-threo. No regioisomers were detected. The isolated yields could be very good, as exemplified by iodine azide addition to 2′,5′-dideoxy-4′,5′-didehydrouridine (**3c**), which afforded 4′-azido-2′,5′-dideoxy-5′-iodouridine (**4c**) in a 91% yield. The purine 2′-deoxyribosides reacted similarly to the pyrimidines. On the other hand, the stereospecificity of the iodine azide addition deteriorated when the 3′-hydroxy group was absent. For example, the addition to the 4′,5′-unsaturated derivative of 3′-deoxythymidine and 2′,3′-dideoxyadenosine gave 1:1 mixtures of the 4′-azido substituted D- and L-glycero epimers, albeit in satisfactory combined yields.

The regio- and stereochemical assignments of the iodine azide addition products were based on their ^{13}C NMR spectra. Evidence that the iodine was on the 5′-carbon was provided by the ^{13}C–^{1}H coupling of $C_{5'}$. These couplings were found to be 152–154 Hz, which is in the range characteristic of a proton geminal to an iodine (ca. 152 Hz),[30] not geminal to an azide (ca. 142 Hz).[31] The configuration at 4′ was assigned on the basis of the chemical shift of $C_{5'}$. It has been shown[17] that when $C_{5'}$ and $O_{3'}$ are trans disposed, the $^{13}C_{5'}$ signal is downfield relative to the $^{13}C_{5'}$ signal when $C_{5'}$ and $O_{3'}$ are cis. Thus, when 4′-epimeric pairs were available, the assignments were straightforward. In the cases in which both epimers were not obtained or the 3′-hydroxy group was absent, NOE experiments verified the configuration at 4′.

The conversion of the 5′-iodide to a 5′-hydroxyl caused the most difficulty. The diminished reactivity of a 5′-iodide when 4′ bears an electron-withdrawing substituent has been documented. For instance, in the case of 5′-deoxy-4′-fluoro-5′-iodo-2′,3′-*O*-isopropylideneadenosine,[13] only azide ion under forcing conditions would displace the 5′-iodide. The 5′-azido product was then converted to the 5′-alcohol by photolysis to an imine which was hydrolyzed to an aldehyde and reduced. In other examples in which an azido or methoxy group was present at 4′, the 5′-iodide could be displaced with benzoate ion only after prolonged heating in polar, aprotic solvents. The 5′-ester was obtained in low yield, but simple saponification furnished the 5′-alcohol.[15,16,18]

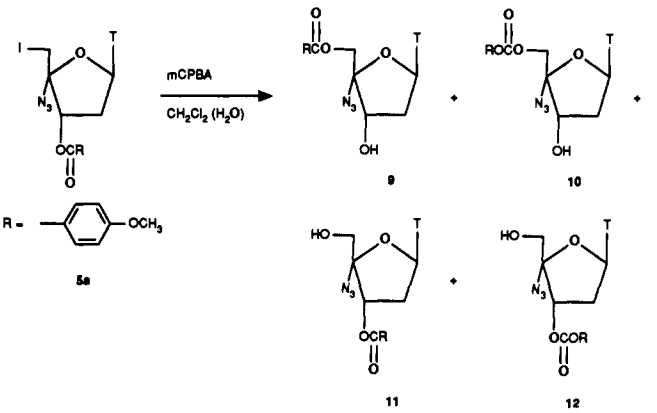
We first attempted the iodide displacement of 4′-azido-5′-deoxy-5′-iodothymidine using oxygen nucleophiles. Both lithium and silver carboxylates in a variety of solvents led only to degradation. Common products of these reactions, obtained in low yield, were the 4′-olefins **3**. In order to stabilize the nucleoside, the 3′-benzoate was made and then the displacement trials were repeated. Again, no 5′-esterified product could be detected in the complex melanges. Additionally, silver nitrate, silver perchlorate,[32] and bis(tributyltin) oxide with silver nitrate[33] would not effect hydrolysis at 5′.

Scheme II



5 7 8

Scheme III



5a 9 10 11 12

Having failed with a litany of nucleophiles, it was felt that efforts should be directed toward improving the leaving ability of the iodide. Such activation of an iodide can be achieved via oxidation to the corresponding hypervalent iodide.[34,35] If water is present during oxidation, hydrolysis will take place in situ.[35] It was found that when 4′-azido-3′-*O*-benzoyl-5′-deoxy-5′-iodonucleosides **5** were treated with *m*-chloroperbenzoic acid in water-saturated dichloromethane, a number of products were generated. Surprisingly, a major component of the product mixtures was 5′-*O*-benzoyl-3′-hydroxyl analogue **8** (Scheme II), obtained in yields ranging from 10% to 30%. This product must form via a 3′,5′-cyclic benzoxonium ion (**7**) which then undergoes hydrolysis. Such participations or migrations of 3′,5′-trans-oriented acyl groups are, to our knowledge, unprecedented.

Intrigued by the occurrence of this 3′,5′-trans interaction, we investigated the reaction further. When the oxidative hydrolysis was performed on the same substrates, but lacking the benzoate at 3′, no reaction at 5′ was detected and only glycosidic cleavage occurred. Likewise, 3′-deoxynucleosides would not undergo 5′-iodide displacement under these conditions. On the other hand, when the 3′-*O*-benzoyl group was replaced with a 3′-*O*-(*p*-methoxybenzoyl) group, enhancing the electron density of the acyl carbonyl, the yield of the 5′-esterified product increased as much as 2-fold.

The product mixture from the oxidative hydrolysis of the 3′-*O*-(*p*-methoxybenzoyl) derivative of 4′-azido-5′-deoxy-5′-iodothymidine (**5a**) was examined in greater detail and is depicted in Scheme III. At least 12 products were

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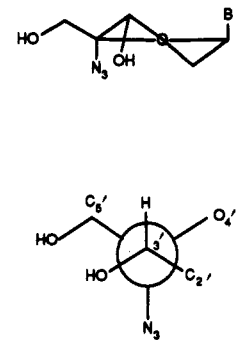


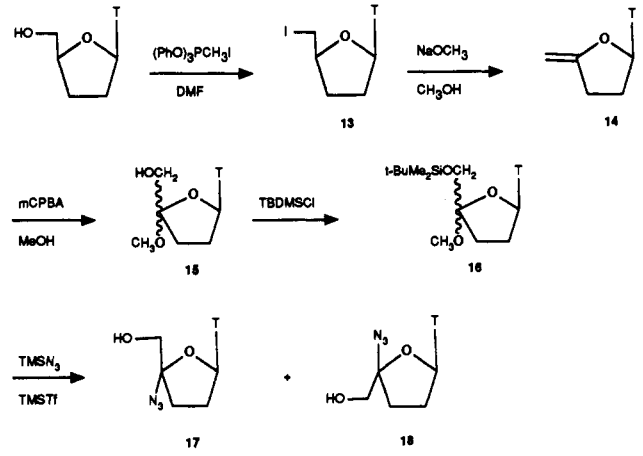
Figure 1.

visible by thin-layer chromatography. The major ones were identified by NMR as 5'-*O*-(*p*-methoxybenzoyl) derivative **9** (20% yield), 5'-*O*-[(*p*-methoxyphenoxy)carbonyl] derivative **10** (13% yield), 3'-*O*-(*p*-methoxybenzoyl) derivative **11** (7% yield), and 3'-*O*-[(*p*-methoxyphenoxy)carbonyl] derivative **12** (5% yield). Unreacted starting material accounted for 5%, while the rest of the mixture was approximately evenly distributed among at least six other products and some thymine. The two carbonate derivatives **10** and **12** undoubtedly arise via a Baeyer–Villiger side reaction of the 3',5'-cyclic *p*-methoxybenzoxonium ion intermediate with excess peracid.[36]

All of these major reaction products could be hydrolyzed to the desired deprotected nucleoside **6**. Therefore, the most expeditious route was to simply treat the crude oxidation mixture with base and isolate the final product from a now less complex mixture. The isolated yields of the deprotected 4'-azidonucleosides ranged from 35% to 71% over the two steps, except for 4'-azido-2'-deoxyguanosine, where difficulties in purification gave a lower yield.

The unusually close proximity of the 3'- and 5'-hydroxy groups, which enables the formation of a cyclic acyloxonium intermediate, must be a consequence of the azido group at 4'. It is likely that, due to secondary orbital interactions,[37] the azide at 4' would preferentially adopt a pseudoaxial orientation. This orientation would also be in agreement with observations that electronegative substituents on the sugar moiety of nucleosides prefer being axial.[38,39] With the azide pseudoaxial, the hydroxy group at 3' would then be forced into a pseudoequitorial position in order to minimize eclipsing the azide. The result would be an extreme 3'-endo conformation of the furanose ring with the 3'-hydroxy and the 5'-(hydroxymethyl) groups both pseudoequatorial and in close proximity. Figure 1 illustrates schematically the proposed 3'-endo furanose conformation of the 4'-azido-2'-deoxynucleosides. The accompanying Newman projection along the C_3–$C_{4'}$ bond depicts the gauche relationship between the 3'-hydroxy and

Scheme IV



4'-azido groups and the resulting syn-periplanar conformation of the 3'- and 5'-hydroxy groups.

Further evidence that the 4'-azidonucleosides prefer a 3'-endo conformation is provided by the detailed analysis of the ^1H NMR spectrum of 4'-azidothymidine. In D_2O, the vicinal proton couplings of 1', 2', and 3' were used to calculate torsional angles using modified Karplus relations. These angles best describe a 3'-endo furanose conformation.[40] Furthermore, difference NOE spectra of the 4'-azidonucleosides showed a strong enhancement of the pyrimidine 6-H when the 3'-H was irradiated. This, also, would be consistent with a 3'-endo conformation.[41]

As mentioned above, the oxidative displacement of the 5'-iodide was not successful in the 3'-deoxy series. As shown in Scheme IV, an alternate method was developed for the synthesis of 4'-azido-3'-deoxythymidine (**17**) which did not utilize a 4'-azido-5'-iodo intermediate. 3',5'-Dideoxy-4',5'-didehydrothymidine (**14**) was first prepared from 3'-deoxythymidine by iodination at 5' followed by base-catalyzed dehydrohalogenation. Treating **14** at 0 °C in methanol with *m*-chloroperbenzoic acid afforded 4'-methoxy-3'-deoxythymidine (**15**) as a mixture of D- and L-isomers in the ratio of 1:3, respectively. This purified, but unresolved, mixture was protected at 5' as the *tert*-butyldimethylsilyl ether and then the azide was introduced at 4' by reaction with excess azidotrimethylsilane in the presence of trimethylsilyl triflate.[42] Concomitantly, the 5'-silyl ether was cleaved and a 37% yield of 4'-azido-3'-deoxythymidine (**17**) and a 25% yield of its 4'-epimer **18** were isolated.

Some other analogues were derived from the 4'-azido products (Scheme V). 4'-Azido-2'-deoxycytidine was made from 4'-azido-2'-deoxyuridine following methodology developed by Reese.[43] Thus, uridine derivative **6c** was acetylated and converted to the 4-triazolide, which upon treatment with ammonium hydroxide furnished cytidine analogue **21**. 4'-Azido-2'-deoxyinosine (**22**) was made by

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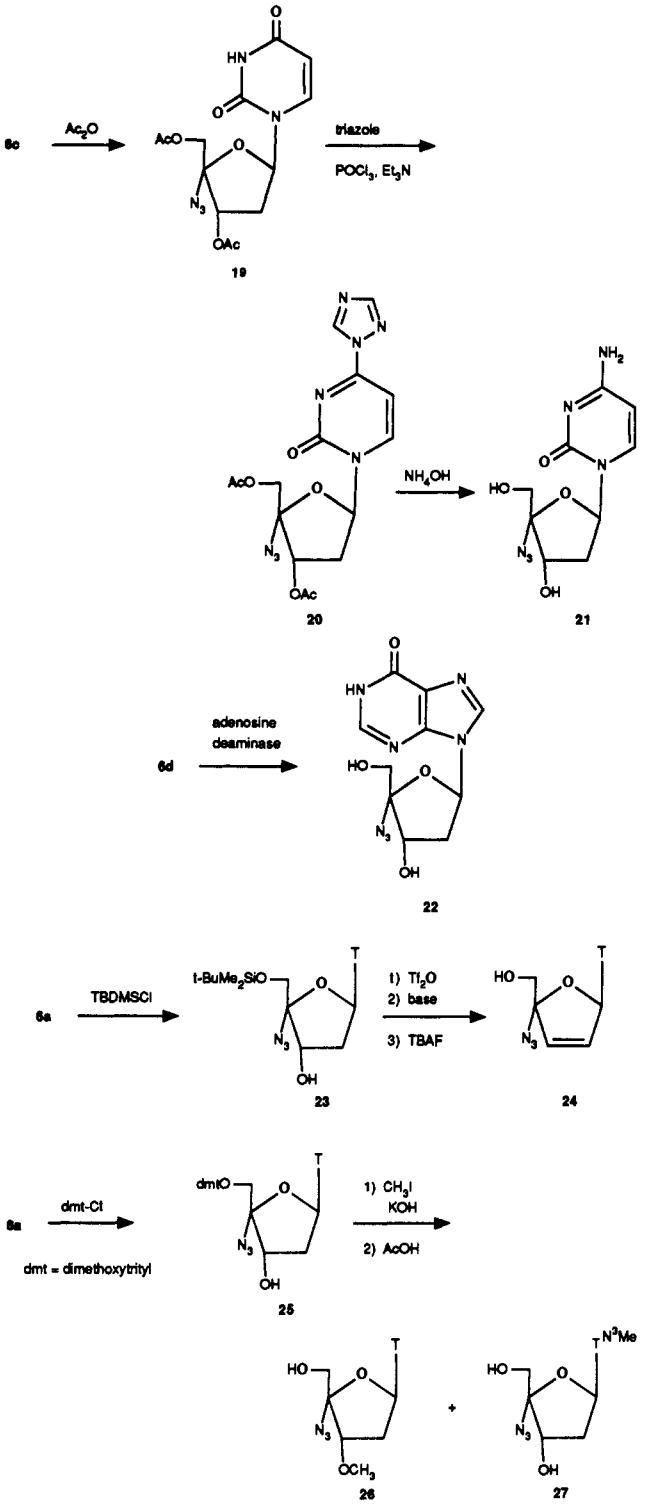
Scheme V



enzymatic deamination of 4′-azido-2′-deoxyadenosine using a procedure described by Herdewijn.[44] 4′-Azido-3′-deoxy-2′,3′-didehydrothymidine (**24**) was synthesized from 4′-azidothymidine via silylation at 5′ followed by trifluoromethanesulfonation at 3′ and then elimination of the sulfonate ester and desilylation. 4′-Azido-3′-*O*-methylthymidine (**26**) was isolated in low yield after treatment of 4′-azido-5′-*O*-(dimethoxytrityl)thymidine (**25**) with methyl iodide and potassium hydroxide[45] followed by deprotection with aqueous acetic acid. The major product of this reaction was 4′-azido-N^3-methylthymidine (**27**).

The synthesis of 4′-azido-3′-deoxythymidine (**17**), described above, utilized the 4′-methoxy analogue as an intermediate. The simple procedure developed to insert a methoxy group at 4′ was also carried out on other substrates as shown in Scheme VI. The key step in this procedure is the transient formation of a 4′,5′-epoxide which is opened immediately by the methanol solvent. An initial concern was that the presence of a hydroxy group at 3′ would direct the epoxidation exclusively to the α-face.[46] This would lead to the wrong 4′-methoxy epimer when the epoxide is opened. Therefore, the synthesis of 2′-deoxy-4′-methoxyadenosine (**31**) began with the 4′-unsaturated-2′-deoxyadenosine derivative **3d** in which the 3′-hydroxyl was masked as a silyl ether (**28**). Epoxidation of **28** followed by in situ opening with methanol yielded a 4:1 mixture of the β-D-erythro (**29**) to α-L-threo (**29epi**) 4′-methoxy epimers (some oxidation at N^1 of **28** also occurred). When the same reaction was carried out on unprotected 4′-unsaturated-2′-deoxyadenosine (**3d**) the proportion of the β-D-erythro isomer diminished, but the overall yield nearly doubled. The net result was a slightly greater yield of the desired isomer when the 3′-hydroxy remained unprotected. Chromatographic separation of the epimers was much easier, though, when the silyl ether was present. Therefore, in the case of 2′-deoxyadenosine, 3′-silylation was ultimately the best course.

In the case of the other 2′-deoxynucleosides, however, it was found that the protection of the 3′-hydroxy group provided no advantage. The most expeditious preparation of 4′-methoxythymidine (**32**) and 2′-deoxy-4′-methoxyguanosine (**33**) was simply to treat the unprotected 4′-unsaturated precursor with *m*-chloroperbenzoic acid in methanol. Mixtures of the 4′-epimers were obtained and were separated by chromatography. 3′-Deoxy-3′-fluoro-4′-methoxythymidine (**37**) was also made via this route starting from 4′,5′-didehydro-3′,5′-dideoxy-3′-fluorothymidine (**36**). The unsaturated nucleoside **36** has been previously described,[47] but in this case, it was prepared differently using the iodination/elimination procedure as described above. 4′-Methoxy derivatives of other nucleosides have been prepared previously using a somewhat longer synthesis which required the difficult transformation of 5′-iodide to 5′-hydroxyl.[15,17]

The assignment of configuration at 4′ of the 4′-methoxynucleosides **31–33** was made following the ^{13}C NMR rules used to establish the 4′-azido-5′-iodonucleoside structures. In every case the $^{13}C_{5'}$ signal of the erythro epimer appeared downfield of that of the threo. The 3′-fluoro-4′-methoxy-3′-deoxythymidine (**37**) configuration was proven by NOE difference experiments which displayed an interaction between 1′-H and the 4′-OCH_3 and between 6-H and 5′-H.

Lastly, 1-(4-azido-2-deoxy-α-L-*threo*-pentofuranosyl)-thymine, the 4′-epimer of 4′-azidothymidine (**6a**), was desired for both physical and biological comparison with

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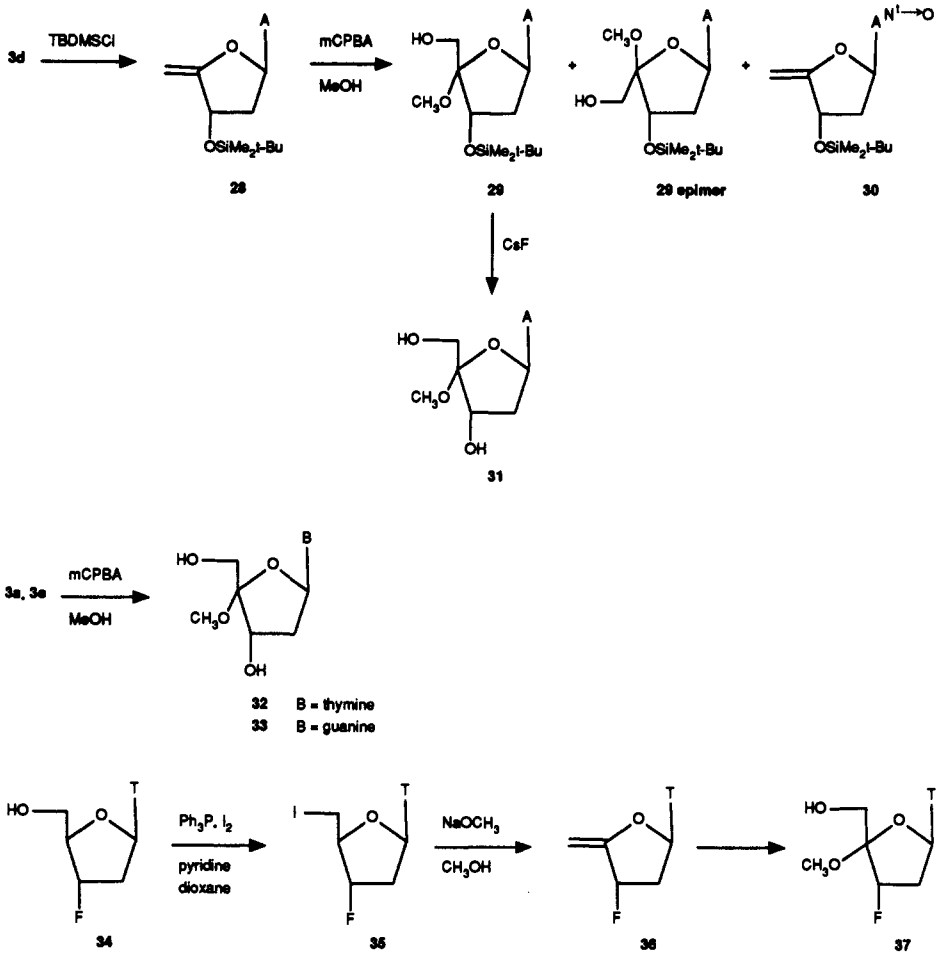
Scheme VI



4'-azidothymidine (**6a**). A kilogram-scale preparation of **6a** provided crystallization mother liquors containing trace amounts of the 4'-epimer. Using preparative HPLC, the 4'-epimer of **6a** was isolated from these residues in sufficient quantity for testing purposes.

Antiviral Activity and Discussion

The in vitro anti-HIV activities of the 4'-substituted nucleosides are shown in Table I. Compounds were tested for their inhibition of the cytopathogenicity of LAV (III_B) in CD^{4+} T-cells (A3.01). AZT was included as a control. Estimates of toxicity were obtained by determining the 25% and 100% cytotoxic concentrations for mock-infected cells.

From the results, some general trends in the structure–activity relationships can be seen. First, the three compounds (**6a** 4'-epimer, **32** 4'-epimer, and **18**) having the unnatural (α-L) configuration at 4' were all inactive. Also, the two ribosides **6b** and 4'-azidocytidine had weak and no activity, respectively. On the other hand, all of the 4'-azido-2'-deoxy-β-D-nucleosides were potent inhibitors of HIV. IC_{50}'s ranged from 0.80 μM for 4'-azido-2'-deoxyuridine (**6c**) to 0.003 μM for 4'-azido-2'-deoxyguanosine (**6e**). AZT had an IC_{50} equal to 0.01 μM under the same conditions. The 4'-methoxy-2'-deoxynucleosides **31–33** were also inhibitors, but were 2–3 orders of magnitude less active than their azido counterparts.

The effect of modification at the 3'-position is well-illustrated in compounds **17**, **24**, and **26**. Compared to 4'-azidothymidine (**6a**), which has an IC_{50} of 0.01 μM, removal of the 3'-hydroxy group (compound **17**) results in complete loss of activity, as does dehydration to form the 2',3'-unsaturated analogue **24**. Methylation of the 3'-hydroxy group (compound **26**) lowers the activity 2000-fold. In the 4'-methoxy series, the replacement of the 3'-hydroxyl of 4'-methoxythymidine (**32**) with fluorine (compound **37**) made little difference in activity.

Table I. Inhibitory Effects of Compounds on the Cytopathogenicity of LAV(III_B) in A3.01 Cells and Cellular Toxicity[a]

compd	IC_{50},[b] μM	CC_{25},[c] μM	CC_{100},[d] μM	SI[e]
6a	0.01	8	200	800
αL-6a	NA[f]	>200	>200	
6b	73	>200	>200	>2.74
6c	0.8	200	>200	250
6d	0.13	50	>50	385
6e	0.003	0.21	1.9	70
6f	0.056	1000	>1000	17900
17	NA	22	200	
18	NA	11.1	33.3	
21	0.004	0.21	1.9	53
22	0.65	<22	200	<34
24	NA	2.5	7.4	
26	20.4	>200	>200	>9.8
31	15.5	1000	>1000	64.5
32	8.49	>200	>200	>23.6
αL-32	NA	>200	>200	
33	0.1	22	67	220
37	3.2	400	>400	125
4'-azidocytidine[18]	NA	8.2	74.1	
AZT	0.01	825	3300	82,500

[a] Values shown are the result of a single determination. See the Experimental Section for details. [b] Concentration of the compound which reduced virus levels by 50% compared to a control culture. [c] Concentration of the compound at which 25% of the cells were destroyed. [d] Lowest concentration at which complete destruction of the cells was observed. [e] Selectivity index = CC_{25}/IC_{50}. [f] Not active up to the concentration causing cytotoxicity.

Table II. In Vitro Antiviral Activity of 4'-Azidothymidine and AZT[a]

		4'-azidothymidine				AZT			
virus strain	cell	IC_{50},[b] μM	CC_{25},[c] μM	CC_{100},[d] μM	SI[e]	IC_{50}, μM	CC_{25}, μM	CC_{100}, μM	SI
HIV-1 LAV	A3.01	0.01	8	200	800	0.01	825	3300	82500
	H9	0.04	25	667	625	0.03	44	1200	1467
	PBL	0.02	2.5	>200	125	0.08	4	>500	50
	MT-2	0.40	56	>500	140	0.16	56	>500	350
G 762-3 (pre-AZT)	MT-2	0.43				0.22			
G 691-2 (post-AZT)	MT-2	0.34				10			
H 112-2 (pre-AZT)	MT-2	0.40				0.20			
G 910-6p2 (post-AZT)	MT-2	0.52				31			

[a] Values shown are the result of a single determination. See the Experimental Section for details. [b] Concentration of the compound which reduced virus levels by 50% compared to a control culture. [c] Concentration of the compound at which 25% of the cells were destroyed. [d] Lowest concentration at which complete destruction of the cells was observed. [e] Selectivity index = CC_{25}/IC_{50}.

When toxicity is taken into account, the most selective compounds were the 4'-azido derivatives of thymidine (**6a**), 2'-deoxyadenosine (**6d**), and 5-chloro-2'-deoxyuridine (**6f**). The last compound follows a trend previously observed[48] for many 5-chloro derivatives of anti-HIV pyrimidine nucleosides in that this substitution causes an increase in selectivity due to a diminution of cytotoxicity rather than an increase in antiviral potency. Because of its high potency and low bone marrow toxicity (data not shown), we chose to evaluate 4'-azidothymidine in other cell lines and against AZT-resistant clinical isolates of HIV. As shown in Table II, 4'-azidothymidine was equipotent with AZT when tested against HIV-1 LAV infected peripheral blood lymphocytes (PBL), H9 cells, and MT-2 cells. The selectivity indices (SI) of 4'-azidothymidine and AZT in these cell lines were also similar, in contrast to the determination in A3.01 cells, where AZT was 100-fold more selective. The lower SI of 4'-azidothymidine in the A3.01 cells is due entirely to its higher toxicity. This exceptional toxicity cannot be explained at this time, but may be due to a unique sensitivity of the cellular DNA polymerases in this cell line to inhibition by 4'-azidothymidine triphosphate. 4'-Azidothymidine was also tested against HIV clinical isolates resistant to AZT. In the two cases examined, 4'-azidothymidine remained equally effective before and after AZT resistance had developed.

It has become almost axiomatic that nucleosides must be devoid of the 3'-hydroxy group to be considered as potential inhibitors of HIV.[49] Thus, the antiviral activity of the 4'-substituted-2'-deoxynucleosides may be considered surprising. In fact, we have shown that in this series, removal of the 3'-hydroxy is deleterious to activity. Furthermore, preliminary investigations into the mechanism of action of 4'-azidothymidine demonstrated that in spite of the presence of a 3'-hydroxy group, 4'-azidothymidine can still act as a DNA chain terminator.[50]

A second conundrum concerns the conformation of the sugar moiety of the 4'-substituted nucleosides. As was mentioned in the Chemistry discussion, the presence of the electron-withdrawing 4'-substituent appears to induce a preference for a 3'-*endo*-ribose conformation. This being true, these compounds are among a minority of nucleoside analogues which are both potent inhibitors of HIV and favor this sugar conformation. Most other nucleoside inhibitors prefer a C_3-exo or C_2-endo sugar conformation.[39,51] Therefore, it might be concluded that the conformation of the furanose moiety is not, in itself, a primary determinant of anti-HIV activity.

The results presented here suggest new avenues to explore in the design of anti-HIV nucleosides. Certainly, the 4'-position has been undeservedly underrepresented among points of nucleoside modification and may yet offer further benefits to antiviral activity. Likewise, manipulation of the sugar conformation via the judicous selection of substituents may help to achieve desirable biological properties. Lastly, the deletion of the 3'-hydroxy group should not be considered a sine qua non for anti-HIV activity.

Experimental Section

General Methods. Nuclear magnetic resonance spectra were recorded on a Bruker WM-300 (^{1}H NMR, 300 MHz) spectrometer, and chemical shifts are reported in parts per million downfield from internal tetramethylsilane. Coupling constants are reported for final products only. Mass spectra (MS) were recorded on Finnigan MAT CH7 and MAT 311A spectrometers operating in the direct inlet mode. UV spectra were recorded on a Hewlett-Packard 8450A spectrophotometer. Elemental analyses were obtained by Syntex Analytical Research. Column chromatography utilized 70–230 mesh silica gel 60 from E. Merck, while preparative thin-layer chromatography was performed on 20 × 20 × 0.1 cm silica gel GF plates from Analtech. Melting points were determined on a hot-stage microscope and are corrected. **Caution:** *The explosive potential of azido compounds must be recognized even though we have not encountered problems during these small-scale preparations.*

5'-Deoxy-5'-iodothymidine (2a). Triphenylphosphine (1.57 g, 6.0 mM) and iodine (1.52 g, 6.0 mM) were added to a suspension of thymidine (0.968 g, 4.0 mM) in dioxane (20 mL) containing pyridine (0.65 mL, 8.0 mM). After stirring the mixture for 7 h at room temperature, methanol (1.0 mL) was added, and then the solvents were removed by evaporation. A solution of the

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residue in ethyl acetate was washed successively with H_2O, 10% aqueous sodium thiosulfate solution, and brine. After drying ($MgSO_4$), the EtOAc solution was concentrated in vacuo to a syrup which was taken up in hot EtOH and filtered. The filtrate was concentrated to a volume of 15 mL. On cooling, 0.806 g (57%) of **2a** crystallized out: mp 173–174 °C (lit.[25] mp 172–173 °C).

1-(2,5-Dideoxy-β-D-*glycero*-pent-4-enofuranosyl)thymine (3a). A 1 N solution of sodium methoxide in MeOH (0.85 mL) was added to a suspension of **2a** (100 mg, 0.284 mM) in MeOH. The solution was heated at reflux for 16 h, cooled to room temperature, and neutralized by the addition of glacial acetic acid. After removal of solvents by evaporation, the residue was crystallized from ethanol to give 58 mg (91%) of **3a**: mp 207–208 °C (lit.[23] mp 208–210 °C).

4'-Azido-5'-deoxy-5'-iodothymidine (4a). Iodine monochloride (10.8 g, 67 mM) was added, under nitrogen, to a stirred suspension of sodium azide (8.70 g, 134 mM) in DMF (60 mL) at room temperature. The mixture warmed slightly. After 20 min, a solution of **3a** (6.00 g, 26.8 mM) in DMF (600 mL) was added dropwise over 30 min. Stirring was continued for an additional hour and then saturated aqueous sodium bicarbonate was added (200 mL) followed by enough saturated aqueous sodium thiosulfate solution to render the mixture colorless. The mixture was filtered and the filtrate was evaporated to an oil. A solution of the oil in H_2O (300 mL) was extracted four times with EtOAc (250 mL portions). The combined organic extracts were dried ($MgSO_4$), and the solvent was removed by evaporation to give 11 g (100%) of **4a** as a viscous syrup of sufficient purity for use in subsequent reactions. (The ratio of **4a** and its 4'-epimer in this material was ~20:1.) An analytical sample of **4a** was prepared by chromatographing a portion of the above product on a silica gel plate using 5% MeOH in CH_2Cl_2 as eluent: UV λ_{max} (EtOH) 264 nm (ε 9990); ^{1}H NMR (DMSO-d_6) δ 11.40 (br s, 1 H, NH), 7.49 (d, 1 H, 6), 6.34 (t, 1 H, 1'), 6.16 (d, 1 H, OH), 4.60 (m, 1 H, 3'), 3.68 (d, 1 H, 5'), 3.63 (d, 1 H, 5'), 2.52 (m, 1 H, 2'), 2.30 (m, 1 H, 2'), 1.81 (d, 3 H, CH_3); ^{13}C NMR (DMSO-d_6) δ 97.7 (4'), 9.3 (5'). Anal. ($C_{10}H_{12}IN_5O_4$) C, H, N.

4'-Azido-5'-deoxy-5'-iodo-3'-*O*-(4-methoxybenzoyl)thymidine (5a). A solution of **4a** (11 g, 28 mM) and *p*-anisoyl chloride (5.3 g, 31 mM) in pyridine (100 mL) was kept at room temperature for 16 h. Methanol (10 mL) was added and the solution was concentrated by evaporation to a syrup. The syrup was chromatographed on a column of silica gel (800 g) using 2% CH_3OH in CH_2Cl_2 as eluent. **5a** (13 g, 95%) was isolated as a foam which was crystallized from EtOH: mp 153–154 °C; UV λ_{max} (EtOH) 262 nm (ε 29 600); ^{1}H NMR ($CDCl_3$) δ 8.87 (br s, 1 H, NH), 8.04 (m, 2 H, Ph), 7.44 (d, 1 H, 6), 6.96 (m, 2 H, Ph), 6.51 (dd, 1 H, 1'), 5.78 (dd, 1 H, 3'), 3.89 (s, 3 H, OCH_3), 3.81 (d, 1 H, 5'), 3.75 (d, 1 H, 5'), 2.69 (m, 2 H, 2'), 1.99 (d, 3 H, CH_3). Anal. ($C_{18}H_{18}IN_5O_6$) C, H, N.

4'-Azidothymidine (6a). A solution of **5a** (420 mg, 0.80 mM) and 85% *m*-chloroperbenzoic acid (650 mg, 3.2 mM) in water-saturated dichloromethane (10 mL) was stirred at room temperature for 3.5 h. The reaction mixture, now containing a precipitate, was diluted with EtOAc (40 mL) and then washed with dilute sodium metabisulfite solution followed by saturated sodium bicarbonate solution. The ethyl acetate phase was dried ($MgSO_4$) and evaporated to a foam. To a solution of the foam in methanol (8.0 mL) was added 1 N sodium methoxide in methanol (1.6 mL). After 30 min at room temperature, the reaction was neutralized with Dowex 50 (H^+) resin, filtered, and concentrated in vacuo to ~0.5 mL. The concentrated mixture was chromatographed on silica gel plates using 10% MeOH in CH_2Cl_2 containing 1% concentrated NH_4OH as eluent. The major band was isolated and crystallized from ethanol to give 80 mg (35%) of **6a**: mp 175–176 °C; UV λ_{max} (H_2O) 266 nm (ε 9830); ^{1}H NMR (DMSO-d_6) δ 11.33 (br s, 1 H, NH), 7.59 (d, J = 1 Hz, 1 H, 6), 6.32 (dd, J = 7, 5.5 Hz, 1 H, 1'), 5.74 (d, J = 6 Hz, 1 H, OH), 5.56 (t, J = 6 Hz, 1 H, OH), 4.47 (dd, J = 7, 12 Hz, 1 H, 3'), 3.66 (d, J = 6, 12 Hz, 1 H, 5'), 3.59 (dd, J = 6, 12 Hz, 1 H, 5'), 2.31 (m, 2 H, 2'), 1.79 (d, J = 1 Hz, 3 H, CH_3); IR (KBr) 2120 cm^{-1} (N_3). Anal. ($C_{10}H_{13}N_5O_5$) C, H, N.

1-(4-Azido-2-deoxy-α-L-*threo*-pentofuranosyl)thymine (6a 4'-Epimer). Reverse-phase HPLC analysis (C-18-Bondapak, 25% MeOH in H_2O) of the mother liquor of **6a** from a large-scale preparation showed a peak with a slightly longer retention time (10.5 vs 7.5 min for **6a**). Sufficient material of this byproduct for complete characterization was isolated by semipreparative reverse-phase HPLC on a Whatman Partisil 10 ODS-3 column (28% MeOH in H_2O). Crystallization from MeOH/EtOAc/hexane gave rhombic plates: mp 162–164 °C; UV λ_{max} (MeOH) 265 nm (ε 8300); ^{1}H NMR (DMSO-d_6) δ 11.41 (br s, 1 H, NH), 7.47 (d, J = 1 Hz, 1 H, 6), 6.51 (dd, J = 9, 6 Hz, 1 H, 1'), 5.68 (d, J = 6 Hz, 1 H, OH), 5.29 (t, J = 6 Hz, 1 H, OH), 4.07 (t, J = 5 Hz, 1 H, 3'), 3.78 (dd, J = 11, 6 Hz, 1 H, 5'), 3.74 (dd, J = 11, 6 Hz, 1 H, 5'), 2.52 (m, 1 H, 2'), 2.17 (dd, J = 14, 6 Hz, 1 H, 2'), 1.82 (d, J = 1 Hz, 3 H, CH_3); no NOE enhancements seen between 6-H or 5-CH_3 and 5'-CH_2; ^{13}C NMR (DMSO-d_6) δ 109.6 (4'), 61.5 (5'); IR (KBr) 2130 cm^{-1} (N_3); $[\alpha]_D$ +200.4° (c = 0.011, MeOH). Anal. ($C_{10}H_{13}N_5O_5$) C, H, N.

4'-Azido-5'-deoxy-5'-iodouridine (4b). In a manner similar to that described for **4a**, **3b**[23] was converted to **4b** in 65% yield. The amorphous product was used in subsequent reactions without further purification: ^{1}H NMR (DMSO-d_6) δ 11.47 (br s, 1 H, NH), 7.74 (d, 1 H, 6), 6.25 (d, 1 H, OH), 6.07 (d, 1 H, 1'), 5.72 (d, 1 H, 5), 5.69 (d, 1 H, OH), 4.47 (m, 1 H, 2'), 4.28 (t, 1 H, 3'), 3.62 (d, 1 H, 5'), 3.52 (d, 1 H, 5'); ^{13}C NMR (DMSO-d_6) δ 96.6 (4'), 11.1 (5').

4'-Azido-2',3'-di-*O*-benzoyl-5'-deoxy-5'-iodouridine (5b). Except for the substitution of benzoyl chloride (2.2 equiv) for *p*-anisoyl chloride, **5b** was obtained from **4b** in a manner similar to that described for the preparation of **5a**. Crystallization from EtOAc/hexane afforded **5b** in 45% yield: mp 120–122 °C; UV λ_{max} (MeOH) 256 (sh) (ε 13 700), 231 nm (ε 27 600); ^{1}H NMR (DMSO-d_6) δ 11.64 (br s, 1 H, NH), 7.90 (m, 4 H, Ph), 7.89 (d, 1 H, 6), 7.65 (m, 2 H, Ph), 7.46 (m, 4 H, Ph), 6.30 (d, 1 H, 1'), 6.16 (d, 1 H, 3'), 6.05 (m, 1 H, 2'), 5.74 (d, 1 H, 5), 3.86 (d, 1 H, 5'), 3.79 (d, 1 H, 5'). Anal. ($C_{23}H_{18}IN_5O_7$) C, H, N.

4'-Azidouridine (6b). In a manner similar to that described for **6a**, **5b** was converted to **6b** in 35% yield. **6b** was isolated as an amorphous solid: UV λ_{max} (MeOH) 260 nm (ε 10 700); ^{1}H NMR (DMSO-d_6) δ 11.40 (br s, 1 H, NH), 7.82 (d, J = 8 Hz, 1 H, 6), 6.08 (d, J = 6.4 Hz, 1 H, 1'), 5.82 (d, J = 6 Hz, 1 H, OH), 5.71 (d, J = 8 Hz, 1 H, 5), 5.65 (t, J = 5.7 Hz, 1 H, OH), 5.60 (d, J = 6.6 Hz, 1 H, OH), 4.25 (q, J = 6.4, 5, 6.6 Hz, 1 H, 2'), 4.17 (t, J = 5, 6 Hz, 1 H, 3'), 3.48 (dd, J = 12, 5.7 Hz, 1 H, 5'), 3.41 (dd, J = 12, 5.7 Hz, 1 H, 5'); IR (KBr) 2130 cm^{-1} (N_3). Anal. ($C_9H_{11}N_5O_6 \cdot {}^1/_4CH_3OH \cdot {}^1/_4H_2O$) C, H, N.

2',5'-Dideoxy-5'-iodouridine (2c). In a manner similar to that described for **2a**, 2'-deoxyuridine was converted to **2c** in 59% yield and was crystallized from EtOH: mp 160–161 °C (lit.[27] mp 154–155 °C).

1-(2,5-Dideoxy-β-D-*glycero*-pent-4-enofuranosyl)uracil (3c). In a manner similar to that described for **3a**, **2c** was converted to **3c** in 65% yield: mp 148–149 °C (EtOH); UV λ_{max} (H_2O) 261 nm (ε 9820); ^{1}H NMR (DMSO-d_6) δ 11.38 (br s, 1 H, NH), 7.53 (d, 1 H, 6), 6.39 (t, 1 H, 1'), 5.63 (d, 1 H, 5), 5.55 (d, 1 H, OH), 4.71 (m, 1 H, 3'), 4.30 (t, 1 H, 5'), 4.15 (dd, 1 H, 5'), 2.40 (m, 1 H, 2'), 2.21 (m, 1 H, 2'). Anal. ($C_9H_{10}N_2O_4$) C, H, N.

4'-Azido-2',5'-dideoxy-5'-iodouridine (4c). In a manner similar to that described for **4a**, **3c** was converted to **4c** and isolated as a foam in 91% yield: UV λ_{max} (EtOH) 259 nm (ε 10 300); ^{1}H NMR (DMSO-d_6) δ 11.42 (br s, 1 H, NH), 7.67 (d, 1 H, 6), 6.32 (t, 1 H, 1'), 6.17 (d, 1 H, OH), 5.67 (d, 1 H, 5), 4.58 (m, 1 H, 3'), 3.67 (d, 1 H, 5'), 3.62 (d, 1 H, 5'), 2.54 (m, 1 H, 2'), 2.33 (m, 1 H, 2'); ^{13}C NMR (DMSO-d_6) δ 97.8 (4'), 9.24 (5'). Anal. ($C_9H_{10}IN_5O_4$) C, H, N.

4'-Azido-2',5'-dideoxy-5'-iodo-3'-*O*-(4-methoxybenzoyl)uridine (5c). In a manner similar to that described for **5a**, **4c** was converted to **5c**. However, chromatography was not necessary because after evaporation of the pyridine, addition of EtOH caused the precipitation of nearly pure product in 66% yield: mp 223–225 °C (EtOH); UV λ_{max} (EtOH) 260 nm (ε 30 200); ^{1}H NMR (DMSO-d_6) δ 11.51 (br s, 1 H, NH), 8.03 (d, 2 H, Ph), 7.81 (d, 1 H, 6), 7.08 (d, 2 H, Ph), 6.56 (dd, 1 H, 1'), 5.76 (d, 1 H, 5), 5.39 (dd, 1 H, 3'), 3.86 (s, 3 H, OCH_3), 3.84 (d, 1 H, 5'), 3.71 (d, 1 H, 5'), 2.98 (m, 1 H, 2'), 2.62 (m, 1 H, 2'). Anal. ($C_{17}H_{16}IN_5O_6$) C, H, N.

4'-Azido-2'-deoxyuridine (6c). In a manner similar to that described for **6a**, **5c** was converted to **6c** in 60% yield: mp 272–273 °C (H_2O/EtOH); UV λ_{max} (H_2O) 260 nm (ε 9810); ^{1}H NMR (DMSO-d_6) δ 11.37 (br s, 1 H, NH), 7.77 (d, J = 8 Hz, 1 H, 6),

6.31 (t, J = 6 Hz, 1 H, 1'), 5.81 (br s, 1 H, OH), 5.65 (d, J = 8 Hz, 1 H, 5), 5.62 (br s, 1 H, OH), 4.45 (m, 1 H, 3'), 3.65 (d, J = 12 Hz, 1 H, 5'), 3.58 (d, J = 12 Hz, 1 H, 5'), 2.30 (m, 2 H, 2'); IR (KBr) 2126 cm^{-1} (N_3). Anal. ($C_9H_{11}N_5O_5 \cdot {}^1/_2H_2O$) C, H, N.

2',5'-Dideoxy-5'-iodoadenosine (2d). In a manner similar to that described for **2a**, 2'-deoxyadenosine was converted to **2d** in 29% yield: mp 163–168 °C (MeOH/EtOAc/hexane): UV λ_{max} (MeOH) 259 nm (ϵ 14 800); ^{1}H NMR (DMSO-d_6) δ 8.34 (s, 1 H, 8), 8.15 (s, 1 H, 2), 7.26 (br s, 2 H, NH_2), 6.39 (t, 1 H, 1'), 5.54 (d, 1 H, OH), 4.43 (m, 1 H, 3'), 3.97 (m, 1 H, 4'), 3.57 (dd, 1 H, 5'), 3.43 (dd, 1 H, 5'), 2.99 (m, 1 H, 2'), 2.32 (m, 1 H, 2'). Anal. ($C_{10}H_{12}IN_5O_2$) C, H, N.

9-(2,5-Dideoxy-β-D-*glycero*-pent-4-enofuranosyl)adenine (3d). In a manner similar to that described for **3a**, **2d** was converted to **3d** in 78% yield: mp 162–164 °C (acetone) (lit.[29] mp 165-166 °C), ^{1}H NMR (DMSO-d_6) δ 8.31 (s, 1 H, 8), 8.16 (s, 1 H, 2), 7.30 (br s, 2 H, NH_2), 6.60 (m, 1 H, 1'), 5.63 (d, 1 H, OH), 5.01 (m, 1 H, 3'), 4.25 (m, 1 H, 5'), 4.15 (m, 1 H, 5'), 3.00 (m, 1 H, 2'), 2.40 (m, 1 H, 2'). Anal. ($C_{10}H_{11}N_5O_2 \cdot {}^1/_2H_2O$) C, H, N.

4'-Azido-2',5'-dideoxy-5'-iodo-N^6,N^6,$O^{3'}$-tribenzoyladenosine (5d). In a manner similar to that described for **4a**, **3d** was converted to **4d**. **4d** was isolated as a partially purified syrup and was immediately benzoylated using 8 equiv of benzoyl chloride and catalytic DMAP in pyridine. After chromatography on a silica gel column (40% EtOAc in hexane), **5d** was isolated as a foam in 77% overall yield: UV λ_{max} (MeOH) 235 (ϵ 30 700), 250 (sh) (ϵ 27 150), 273 nm (21 100); ^{1}H NMR ($CDCl_3$) δ 8.71 (s, 1 H, 8), 8.34 (s, 1 H, 2), 8.12 (m, 2 H, Ph), 7.86 (m, 4 H, Ph), 7.64 (m, 1 H, Ph), 7.50 (m, 4 H, Ph), 7.37 (m, 4 H, Ph), 6.63 (dd, 1 H, 1'), 6.20 (t, 1 H, 3'), 3.79 (s, 2 H, 5'), 3.43 (m, 1 H, 2'), 2.94 (m, 1 H, 2'); ^{13}C NMR ($CDCl_3$) δ 97.3 (4'), 6.5 (5'). Anal. ($C_{31}H_{23}IN_8O_5$) C, H, N.

4'-Azido-2'-deoxyadenosine (6d). In a manner similar to that described for **6a**, **5d** was converted to **6d** in 48% yield. Precipitation from MeOH/EtOAc/hexane furnished **6d** as an amorphous solid: UV λ_{max} (0.1 N HCl) 257 nm (ϵ 14 300); ^{1}H NMR (DMSO-d_6) δ 8.32 (s, 1 H, 8), 8.15 (s, 1 H, 2), 7.32 (br s, 2 H, NH_2), 6.52 (dd, J = 7, 5 Hz, 1 H, 1'), 5.80 (d, J = 6 Hz, 1 H, OH), 5.62 (t, J = 6 Hz, 1 H, OH), 4.74 (m, 1 H, 3'), 3.71 (dd, J = 12, 6 Hz, 1 H, 5'), 3.56 (dd, J = 12, 6 Hz, 1 H, 5'), 2.83 (m, 1 H, 2'), 2.47 (m, 1 H, 2'); IR (KBr) 2131 cm^{-1} (N_3); MS (M^+) 292.1034, calcd for $C_{10}H_{12}N_8O_3$ 292.1032. Anal. ($C_{10}H_{12}N_8O_3 \cdot {}^1/_3CH_3OH \cdot {}^1/_2H_2O$) C, H, N.

2',5'-Dideoxy-5'-iodoguanosine (2e). In a manner similar to that described for **2a**, 2'-deoxyguanosine was converted to **2e** in 45% yield: mp 175–180 °C dec (H_2O); UV λ_{max} (MeOH) 255 nm (ϵ 14 600); ^{1}H NMR (DMSO-d_6) δ 10.62 (s, 1 H, NH), 7.92 (s, 1 H, 8), 6.46 (br s, 2 H, NH_2), 6.15 (t, 1 H, 1'), 5.50 (br s, 1 H, OH), 4.33 (m, 1 H, 3'), 3.91 (m, 1 H, 4'), 3.49 (dd, 1 H, 5'), 3.39 (dd, 1 H, 5'), 2.75 (m, 1 H, 2'), 2.24 (m, 1 H, 2'). Anal. ($C_{10}H_{12}IN_5O_3 \cdot {}^1/_2H_2O$) C, H, N.

9-(2,5-Dideoxy-β-D-*glycero*-pent-4-enofuranosyl)guanine (3e). In a manner similar to that described for **3a**, **2e** was converted to **3e** in 94% yield: mp >300 °C dec (H_2O); UV λ_{max} (MeOH) 254 nm (ϵ 12 400); ^{1}H NMR (DMSO-d_6) δ 10.72 (br s, 1 H, NH), 7.87 (s, 1 H, 8), 6.54 (br s, 2 H, NH_2), 6.37 (t, 1 H, 1'), 5.59 (br s, 1 H, OH), 4.88 (m, 1 H, 3'), 4.24 (d, 1 H, 5'), 4.14 (d, 1 H, 5'), 2.80 (m 1 H, 2'), 2.34 (m, 1 H, 2'). Anal. ($C_{10}H_{11}N_5O_3 \cdot {}^3/_4H_2O$) C, H, N.

4'-Azido-2',5'-dideoxy-5'-iodoguanosine (4e). In a manner similar to that described for **4a**, **3e** was converted to **4e** in 15% yield: mp 178–182 °C dec (EtOH); UV λ_{max} (MeOH) 256 nm (ϵ 17 300); ^{1}H NMR (DMSO-d_6) δ 10.69 (br s, 1 H, NH), 7.99 (s, 1 H, 8), 6.54 (br s, 2 H, NH_2), 6.34 (t, 1 H, 1'), 6.21 (br s, 1 H, OH), 4.73 (t, 1 H, 3'), 3.74 (d, 1 H, 5'), 3.59 (d, 1 H, 5'), 2.93 (m, 1 H, 2'), 2.49 (m, 1 H, 2'); ^{13}C NMR (DMSO-d_6) δ 96.5 (4'), 8.4 (5'). Anal. ($C_{10}H_{11}IN_8O_3 \cdot {}^1/_2EtOH$) C, H, N.

4'-Azido-N^2,$O^{3'}$-dibenzoyl-2',5'-dideoxy-5'-iodoguanosine (5e). With the exception that 3 equiv of benzoyl chloride was substituted for the p-anisoyl chloride, **4e** was converted to **5e** in a manner similar to that described for the preparation of **5a**. After crystallization from MeOH, **5e** was obtained in 63% yield: mp 136–139 °C; UV λ_{max} (MeOH) 233 nm (ϵ 26 200); ^{1}H NMR ($CDCl_3$) δ 12.13 (br s, 1 H, NH), 9.42 (br s, 1 H, NH), 8.16 (dd, 4 H, Ph), 7.85 (s, 1 H, 8), 7.51–7.76 (m, 6 H, Ph), 6.86 (dd, 1 H, 1'), 6.33 (dd, 1 H, 3'), 3.68 (d, 1 H, 5'), 3.62 (d, 1 H, 5'), 3.35 (m, 1 H, 2'), 2.91 (m, 1 H, 2'). Anal. ($C_{24}H_{19}IN_8O_5$) C, H, N.

4'-Azido-2'-deoxyguanosine (6e). In a manner similar to that described for **6a**, **5e** was converted to **6e**. Purification on a silica gel column using the upper phase of a 5.5:1.5:3 mixture of EtOAc/n-PrOH/H_2O as eluent followed by precipitation from hot chloroform furnished **6e** as a powder in 18% yield: mp 250 °C dec; UV λ_{max} (0.1 N NaOH) 266 nm (ϵ 9460), λ_{max} (0.1 N HCl) 258 nm (ϵ 9790); ^{1}H NMR (DMSO-d_6) δ 10.72 (br s, 1 H, NH), 7.90 (s, 1 H, 8), 6.55 (br s, 2 H, NH_2), 6.29 (dd, J = 7.3, 5 Hz, 1 H, 1'), 5.76 (br d, J = 5 Hz, 1 H, OH), 5.46 (br t, J = 6 Hz, 1 H, OH), 4.63 (m, 1 H, 3'), 3.66 (dd, J = 11.5, 5 Hz, 1 H, 5'), 3.57 (dd, J = 11.5, 5 Hz, 1 H, 5'), 2.65 (m, 1 H, 2'), 2.43 (m, 1 H, 2'); IR (KBr) 2120 cm^{-1} (N_3); MS (LSIMS) $(M + H)^+$ 309.1063, calcd for $C_{10}H_{12}N_8O_4$ 309.1062.

5-Chloro-2',5'-dideoxy-5'-iodouridine (2f). In a manner similar to that described for **2a**, 5-chloro-2'-deoxyuridine (Sigma) was converted to **2f** in 70% yield: mp 175 °C dec (EtOH); UV λ_{max} (EtOH) 276 nm (ϵ 9180); ^{1}H NMR (DMSO-d_6) δ 10.91 (br s, 1 H, NH), 8.03 (s, 1 H, 6), 6.16 (dd, 1 H, 1'), 5.49 (br d, 1 H, OH), 4.16 (m, 1 H, 3'), 3.84 (m, 1 H, 4'), 3.55 (dd, 1 H, 5'), 3.43 (dd, 1 H, 5'), 2.35 (m, 1 H, 2'), 2.12 (ddd, 1 H, 2'). Anal. ($C_9H_{10}ClIN_2O_4$) C, H, N.

5-Chloro-1-(2,5-dideoxy-β-D-*glycero*-pent-4-enofuranosyl)uracil (3f). In a manner similar to that for **3a**, **2f** was converted to **3f** in 67% yield: mp 170–171 °C (EtOH); UV λ_{max} (EtOH) 276 nm (ϵ 9820); ^{1}H NMR (DMSO-d_6) δ 11.94 (br s, 1 H, NH), 7.96 (s, 1 H, 6), 6.36 (t, 1 H, 1'), 5.54 (d, 1 H, OH), 4.69 (m, 1 H, 3'), 4.32 (d, 1 H, 5'), 4.17 (d, 1 H, 5'), 2.51 (m, 1 H, 2'), 2.18 (m, 1 H, 2'). Anal. ($C_9H_9ClN_2O_4$) C, H, N.

4'-Azido-5-chloro-2',5'-dideoxy-5'-iodo-3'-*O*-(4-methoxybenzoyl)uridine (5f). In a manner similar to that described for **4a**, **3f** was converted to **4f**, which was isolated as a partially purified syrup and was immediately anisoylated using the procedure of **5a**. After crystallization from acetone, **5f** was obtained in 65% yield over the two steps: mp 166–168 °C; UV λ_{max} (EtOH) 266 nm (ϵ 26 100); ^{1}H NMR ($CDCl_3$) δ 8.67 (br s, 1 H, NH), 8.01 (m 2 H, Ph), 7.89 (s, 1 H, 6), 6.94 (m, 2 H, Ph), 6.44 (dd, 1 H, 1'), 5.74 (dd, 1 H, 3'), 3.87 (s, 3 H, OCH_3), 3.79 (d, 1 H, 5'), 3.74 (d, 1 H, 5'), 2.76 (m, 1 H, 2'), 2.64 (m, 1 H, 2'); ^{13}C NMR ($CDCl_3$) δ 96.6 (4'), 6.64 (5'). Anal. ($C_{17}H_{15}ClIN_5O_6 \cdot {}^2/_3(CH_3)_2CO$) C, H, N.

4'-Azido-5-chloro-2'-deoxyuridine (6f). In a manner similar to that described for **6a**, **5f** was converted to **6f** in 55% yield: mp 153–154 °C (EtOH/hexane); UV λ_{max} (H_2O) 276 nm (ϵ 9060); ^{1}H NMR (DMSO-d_6) δ 11.87 (br s, 1 H, NH), 8.21 (s, 1 H, 6), 6.26 (dd, J = 5, 1.5 Hz, 1 H, 1'), 5.78 (d, J = 6 Hz, 1 H, OH), 5.72 (t, J = 5 Hz, 1 H, OH), 4.46 (m, 1 H, 3'), 3.63 (m, 2 H, 5'), 2.24–2.45 (m, 2 H, 2'); IR (KBr) 2120 cm^{-1} (N_3). Anal. ($C_9H_{10}ClN_5O_5$) C, H, N.

3',5'-Dideoxy-5'-iodothymidine (13). Methyltriphenoxyphosphonium iodide (1.05 g, 2.32 mM) was added to a 0 °C solution of 3'-deoxythymidine (500 mg, 2.21 mM) in THF (20 mL). The mixture was brought to room temperature and stirred for 2 h. After dilution with $CHCl_3$, the solution was washed with aqueous sodium thiosulfate, followed by brine. The organic phase was dried ($MgSO_4$), and the solvents were evaporated. The residue was purified on a silica gel column (4% → 5% MeOH in CH_2Cl_2) to give a syrup which crystallized from EtOAc/hexane to afford 572 mg (77%) of **13**: mp 166–167 °C; UV λ_{max} (MeOH) 266 nm (ϵ 8930); ^{1}H NMR ($CDCl_3$) δ 8.82 (br s, 1 H, NH), 7.48 (d, 1 H, 6), 6.13 (dd, 1 H, 1'), 3.99 (m, 1 H, 4'), 3.48 (dd, 1 H, 5'), 3.42 (dd, 1 H, 5'), 1.82–2.45 (m, 4 H, 2',3'), 1.96 (d, 3 H, CH_3). Anal. ($C_{10}H_{13}IN_2O_3$) C, H, N.

(*R*)-1-(5-Methylene-2-tetrahydrofuranyl)thymine (14). In a manner similar to that described for **3a**, **13** was converted to **14** in 60% yield: mp 149–153 °C (EtOAc/hexane); ^{1}H NMR[52] (DMSO-d_6) δ 11.37 (br s, 1 H, NH), 7.37 (d, 1 H, 6), 6.33 (dd, 1 H, 1'), 4.24 (dd, 1 H, 5'), 3.93 (dd, 1 H, 5'), 2.08–2.85 (m 4 H, 2',3'), 1.80 (d, 3 H, CH_3). Anal. ($C_{10}H_{12}N_2O_3 \cdot {}^1/_4H_2O$) C, H, N.

1-(2,3-Dideoxy-4-methoxy-*glycero*-pentofuranosyl)thymine (15). Potassium bicarbonate (36 mg, 0.36 mM) was added

(52) In order to maintain consistency with the other products, peak assignments are numbered following conventional nucleoside numbering.

to a solution of 14 (30 mg, 0.144 mM) in MeOH (1.0 mL). While under nitrogen, the mixture was cooled in an ice bath and then ~98% *m*-chloroperbenzoic acid (37 mg, 0.214 mM) was added in one portion with stirring. After 3 min, the reaction was quenched by the addition of saturated sodium bicarbonate solution (1.0 mL) and 10% aqueous sodium thiosulfate (1.0 mL). The resulting mixture was extracted twice with 1:3 EtOAc/$CHCl_3$. The extracts were washed with brine, dried ($MgSO_4$), and concentrated in vacuo. Purification of the residue on silica gel plates using 5% MeOH in $CHCl_3$ as eluent afforded 8 mg (22%) of **15** as a syrup. **15** consisted of a mixture of 4'-epimers and was used immediately in subsequent reactions.

1-(2,3-Dideoxy-5-*O*-*tert*-butyldimethylsilyl-4-methoxy-*glycero*-pentofuranosyl)thymine (16). A solution of **15** (19 mg, 74 μM), *tert*-butyldimethylsilyl chloride (31 mg, 0.21 mM), and imidazole (31 mg, 0.45 mM) in DMF (2.0 mL) was kept at room temperature for 3 h. The reaction was quenched with 2 drops of MeOH, diluted with saturated sodium bicarbonate, and extracted with EtOAc. The extracts were washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified on a silica gel plate (4% MeOH in $CHCl_3$) to give 24 mg (89%) of **16** as a syrup. The ^1H NMR spectrum showed a 1:3 mixture of β-D- and α-L-isomers: ^1H NMR ($CDCl_3$) δ 8.60 (br s, 1 H, NH_L), 8.55 (br s, 1 H, NH_D), 7.46 (d, 1 H, 6_L), 7.44 (d, 1 H, 6_D), 6.40 (dd, 1 H, $1'_D$), 6.30 (dd, 1 H, $1'_L$), 3.93 (d, 1 H, $5'_L$), 3.81 (d, 1 H, $5'_D$), 3.68 (d, 1 H, $5'_L$), 3.63 (d, 1 H, $5'_D$), 3.36 (s, 3 H, OCH_{3D}), 3.31 (s, 3 H, OCH_{3L}), 1.82–2.65 (m, 8 H, $2'_L$, $2'_D$, $3'_L$, $3'_D$), 1.95 (d, 3 H, CH_{3D}), 1.93 (d, 3 H, CH_{3L}); irradiation at 4'-OCH_3 of the major (α-L) isomer causes an enhancement of the 5-CH_3 in the difference NOE spectrum; MS 339 $(M - OCH_3)^+$.

4'-Azido-3'-deoxythymidine (17) and 1-(4-Azido-2,3-dideoxy-α-L-*glycero*-pentofuranosyl)thymine (18). Azidotrimethylsilane (65 μl, 480 μM) and trimethylsilyl trifluoromethanesulfonate (9 μl, 47 μM) were added to a solution of the 4'-epimeric mixture **16** (23 mg, 62 μM) in CH_2Cl_2 (2.0 mL). After stirring for 72 h at room temperature, the reaction was diluted with 1:3 EtOAc/$CHCl_3$ (50 mL) and washed with saturated sodium bicarbonate and then with brine. The organic solution was dried ($MgSO_4$), and the solvents were removed in vacuo. The residue was purified on a silica gel plate using water-saturated EtOAc as eluent. Two major bands were isolated. The less polar isolate was **17**, which crystallized from EtOAc/hexane (5 mg, 30%): mp 55–60 °C dec; UV λ_{max} (MeOH) 266 nm (ε 9200); ^1H NMR ($CDCl_3$) δ 8.97 (br s, 1 H, NH), 7.34 (d, J = 1 Hz, 1 H, 6), 6.31 (dd, J = 8, 4 Hz, 1 H, 1'), 4.00 (br d, J = 12 Hz, 1 H, 5'), 3.81 (br d, J = 12 Hz, 1 H, 5'), 2.88 (br s, 1 H, OH), 2.65 (m, 1 H, 2'), 2.45 (m, 1 H, 3'), 2.20 (m, 1 H, 2'), 1.93 (m, 1 H, 3'), 1.92 (d, J = 1 Hz, 3 H, CH_3); irradiation at 5'-H causes an enhancement of 6-H and 5-CH_3 in the difference NOE spectrum; IR ($CHCl_3$) 2120 cm^{-1} (N_3); MS (M^+) 267.0967, calcd for $C_{10}H_{13}N_5O_4$ 267.0968.

The more polar isolate was the 4'-epimer **18**, which was crystallized from EtOAc/hexane (3 mg, 18%): mp 129–131 °C; UV λ_{max} (MeOH) 266 nm (ε 8800); ^1H NMR ($CDCl_3$) δ 8.78 (br s, 1 H, NH), 7.34 (d, J = 1 Hz, 1 H, 6), 6.45 (dd, J = 8, 6 Hz, 1 H, 1'), 3.91 (br d, J = 12 Hz, 1 H, 5'), 3.74 (br d, J = 12 Hz, 1 H, 5'), 2.46 (m, 1 H, 2'), 2.31 (m, 1 H, 3'), 2.19 (m, 1 H, 2'), 2.04 (m, 1 H, 3'), 1.97 (d, J = 1 Hz, 3 H, CH_3); no NOE was observed between 5'-H and 6-H or 5-CH_3; IR ($CHCl_3$) 2120 cm^{-1} (N_3); MS (M^+) 267.0970 calcd for $C_{10}H_{13}N_5O_4$, 267.0968.

3',5'-Di-*O*-acetyl-4'-azido-2'-deoxyuridine (19). A solution of **6c** (269 mg, 1.00 mM) and acetic anhydride (1.0 mL, 11 mM) in dry pyridine (6 mL) was kept at room temperature for 4 h. After evaporation of the solvent, the residue was chromatographed on silica gel plates using 5% MeOH in CH_2Cl_2 as eluent. The major band was isolated and crystallized from EtOH to give 334 mg (95%) of **19**: mp 136–137 °C; UV λ_{max} (EtOH) 257 nm (ε 9780); ^1H NMR ($CDCl_3$) δ 8.63 (br s, 1 H, NH), 7.39 (d, 1 H, 6), 6.38 (dd, 1 H, 1'), 5.80 (d, 1 H, 5), 5.43 (dd, 1 H, 3'), 4.40 (s, 2 H, 5'), 2.62 (m, 1 H, 2'), 2.52 (m, 1 H, 2'), 2.64 (s, 3 H, OAc), 2.62 (s, 3 H, OAc). Anal. ($C_{13}H_{15}N_5O_7$) C, H, N.

4'-Azido-2'-deoxycytidine (21). According to the procedure of Reese,[43] **19** (300 mg, 0.85 mM) was first converted to 4-triazolyl derivative **20**. Following a preparative thin-layer silica gel chromatographic purification (8% MeOH in CH_2Cl_2), **20** was treated with 1:2 dioxane/concentrated NH_4OH (room temperature, 16 h). After removal of solvents, the residue was purified by chromatography on silica gel plates using the organic phase of a 5.5:1.5:3 mixture of EtOAc/*n*-PrOH/H_2O as eluent. **21** (160 mg, 70%) was isolated as a foam: UV λ_{max} (0.1 N HCl) 278 nm (ε 12100), λ_{max} (0.1 N NaOH) 270 nm (ε 8710); ^1H NMR (DMSO-d_6) δ 7.72 (d, J = 7 Hz, 1 H, 6), 7.23 (br s, 1 H, NH), 7.14 (br s, 1 H, NH), 6.33 (dd, J = 7, 5 Hz, 1 H, 1'), 5.75 (d, J = 7 Hz, 1 H, 5), 5.70 (d, J = 5.5 Hz, 1 H, OH), 5.53 (t, J = 6 Hz, 1 H, OH), 4.34 (t, J = 5 Hz, 1 H, 3'), 3.65 (dd, J = 12, 6 Hz, 1 H, 5'), 3.58 (dd, J = 12, 6 Hz, 1 H, 5'), 2.25 (m, 2 H, 2'); IR (KBr) 2123 cm^{-1} (N_3). Anal. ($C_9H_{12}N_6O_4 \cdot {}^1/_2H_2O$) C, H, N.

4'-Azido-2'-deoxyinosine (22). Following the procedure of Herdewijn,[44] **6d** (146 mg, 0.50 mM) was hydrolyzed using adenosine aminohydrolase. After chromatography on silica gel plates (20% MeOH in CH_2Cl_2), **22** was crystallized from MeOH/EtOAc/hexane to furnish 68 mg (46%): mp 150–153 °C; UV λ_{max} (MeOH) 245 (ε 11000), 249 (ε 10900), 272 nm (sh) (ε 3980); ^1H NMR (DMSO-d_6) δ 11.68 (br s, 1 H, NH), 8.30 (s, 1 H, 8), 8.08 (s, 1 H, 2), 6.48 (dd, J = 7, 5 Hz, 1 H, 1'), 5.80 (d, J = 5 Hz, 1 H, OH), 5.47 (t, J = 5 Hz, 1 H, OH), 4.69 (m, 1 H, 3'), 3.69 (dd, J = 12, 5 Hz, 1 H, 5'), 3.58 (dd, J = 12, 5 Hz, 1 H, 5'), 2.76 (m, 1 H, 2'), 2.50 (m, 1 H, 2'); IR (KBr) 2115 cm^{-1} (N_3). Anal. ($C_{10}H_{11}N_7O_4$) C, H, N.

4'-Azido-5'-*O*-(*tert*-butyldimethylsilyl)thymidine (23). A solution of **6a** (200 mg, 0.71 mM), *tert*-butyldimethylchlorosilane (120 mg, 0.71 mM), and imidazole (110 mg, 1.6 mM) in DMF (1.0 mL) was kept for 1 h at 37 °C. After evaporation of the solvent, the residue was chromatographed on silica gel plates using 10% MeOH in CH_2Cl_2 as eluent. Crystallization from EtOAc afforded 240 mg (84%) of **23**: mp 169–170 °C; UV λ_{max} 265 nm (ε 9670); ^1H NMR ($CDCl_3$) δ 8.53 (br s, 1 H, NH), 7.30 (s, 1 H, 6), 6.43 (dd, 1 H, 1'), 4.47 (ddd, 1 H, 3'), 3.97 (d, 1 H, 5'), 3.85 (d, 1 H, 5'), 2.31–2.50 (m, 2 H, 2'), 2.30 (d, 1 H, OH), 1.93 (d, 3 H, CH_3). Anal. ($C_{16}H_{27}N_5O_5Si$) C, H, N.

1-(4-Azido-2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)thymine (24). Trifluoromethanesulfonic anhydride (40 μL, 0.24 mM) was slowly added to a 0 °C solution of **23** (80 mg, 0.20 mM) in CH_2Cl_2 (1.0 mL) containing pyridine (20 μL, 0.24 mM). The solution was brought to room temperature and left for 1 h before mixing with an equal volume of H_2O. The organic phase was isolated and evaporated to a colorless, viscous syrup, which was dissolved in DMF (1.0 mL). Lithium benzoate (128 mg, 1.0 mM) was added and the mixture was stirred at room temperature for 5.5 h, at which time TLC (5% MeOH in CH_2Cl_2) showed that the triflate had been consumed and the more polar olefin was present. After dilution with an equal volume of ethyl acetate, the solution was washed with brine and evaporated to a foam. The foam was dissolved in a 0.5 M solution of tetrabutylammonium fluoride in THF (0.8 mL, 0.4 mM) and kept for 30 min at room temperature. Dry Dowex 50 (H^+) resin (80 mg) was added, and after a few minutes of stirring, the filtered solution was applied onto a silica gel plate and eluted with 5% MeOH in CH_2Cl_2. The major band corresponded to **24** and was isolated as 12 mg (23%) of foam: UV λ_{max} (EtOH) 264 nm (ε 9480); ^1H NMR (DMSO-d_6) δ 11.5 (br s, 1 H, NH), 7.46 (s, 1 H, 6), 7.06 (s, 1 H, 1'), 6.43 (m, 2 H, 2',3'), 5.60 (br t, J = 6 Hz, 1 H, OH), 3.52–3.65 (m, 2 H, 5'), 1.73 (s, 3 H, CH_3); IR (KBr) 2116 cm^{-1} (N_3). Anal. ($C_{10}H_{11}N_5O_4 \cdot {}^1/_4H_2O$) C, H, N.

4'-Azido-3'-*O*-methylthymidine (26). A solution of **6a** (1.0 g, 3.53 mM), 4,4'-dimethoxytrityl chloride (1.43 g, 4.22 mM), and DMAP (30 mg, 0.24 mM) in pyridine (10 mL) was kept at room temperature for 7 h. The mixture was diluted with H_2O and extracted twice with CH_2Cl_2. The extracts were combined, washed with brine, dried ($MgSO_4$), and evaporated to 2.6 g of a syrup. The syrup was purified on a silica gel column (5% MeOH in CH_2Cl_2) to afford 1.99 g (96%) of 4'-azido-5'-*O*-(dimethoxytrityl)thymidine (**25**) as a yellow solid. Following the procedure of Hampton,[45] **25** was treated with methyl iodide and KOH in a mixture of benzene and dioxane. After workup, the crude methylation mixture was treated with 80% acetic acid for 5 h at room temperature. Following removal of the solvents in vacuo, the residue was chromatographed on silica gel plates using 5% MeOH in CH_2Cl_2 as eluent. Three products were isolated. The most polar was starting material **6a**, recovered in 25% yield. The product of intermediate polarity was 4'-azido-N^3-methylthymidine (**27**), isolated as 0.29 g (28%) of amorphous solid: UV λ_{max} (MeOH) 265 nm (ε 8540); ^1H NMR (DMSO-d_6) δ 7.67 (d, J = 1

Hz, 1 H, 6), 6.36 (dd, J = 7, 5 Hz, 1 H, 1'), 5.76 (d, J = 6 Hz, 1 H, OH), 5.59 (t, J = 6 Hz, 1 H, OH), 4.47 (dt, J = 7 Hz, 1 H, 3'), 3.68 (dd, J = 6, 12 Hz, 1 H, 5'), 3.60 (dd, J = 6, 12 Hz, 1 H, 5'), 3.17 (s, 3 H, NCH_3), 2.22–2.41 (m, 2 H, 2'), 1.84 (d, J = 1 Hz, 3 H, CH_3); IR ($CHCl_3$) 2135 cm^{-1} (N_3); MS (M^+) 297.1069, calcd for $C_{11}H_{15}N_5O_5$ 297.1073.

The least polar isolate was the desired 3'-*O*-methyl derivative **26**, isolated as 0.15 g (14%) of an amorphous solid: UV λ_{max} (MeOH) 265 nm (ϵ 7630); ^1H NMR (DMSO-d_6) δ 11.37 (s, 1 H, NH), 7.57 (d, J = 1 Hz, 1 H, 6), 6.30 (dd, J = 7, 5 Hz, 1 H, 1'), 5.66 (t, J = 6 Hz, 1 H, OH), 4.26 (t, J = 7 Hz, 1 H, 3'), 3.67 (dd, J = 12, 6 Hz, 1 H, 5'), 3.61 (dd, J = 12, 6 Hz, 1 H, 5'), 3.38 (s, 3 H, OCH_3), 2.47 (m, 1 H, 2'), 2.33 (m, 1 H, 2'), 1.79 (d, J = 1 Hz, 3 H, CH_3); MS (M^+) 297.1076, calcd for $C_{11}H_{15}N_5O_5$ 297.1073.

9-[3-*O*-(*tert*-Butyldimethylsilyl)-2,5-dideoxy-β-D-*glycero*-pent-4-enofuranosyl]adenine (28). A solution of **3d** (233 mg, 1.00 mM), *tert*-butyldimethylchlorosilane (710 mg, 4.70 mM), and imidazole (615 mg, 9.04 mM) in DMF (5 mL) was stirred at room temperature for 75 min. The reaction was quenched with MeOH (0.5 mL), diluted with saturated sodium bicarbonate (100 mL), and extracted with EtOAc. The extract was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was chromatographed on silica gel plates using 80% EtOAc in hexane as eluent. Crystallization from EtOAc/hexane afforded 173 mg (50%) of **28**: mp 162–163 °C; UV λ_{max} (MeOH) 258 nm (ϵ 15800); ^1H NMR ($CDCl_3$) δ 8.36 (s, 1 H, 8), 7.90 (s, 1 H, 2), 6.55 (dd, 1 H, 1'), 5.62 (br s, 2 H, NH_2), 5.10 (dd, 1 H, 3'), 4.51 (m, 1 H, 5'), 4.24 (m, 1 H, 5'), 2.92 (m, 1 H, 2'), 2.52 (m, 1 H, 2'), 0.94 (s, 9 H, *t*-Bu), 0.16 (s, 3 H, CH_3), 0.15 (s, 3 H, CH_3). Anal. ($C_{16}H_{25}N_5O_2Si$) C, H, N.

3'-*O*-(*tert*-Butyldimethylsilyl)-2'-deoxy-4'-methoxyadenosine (29). To a stirred solution of **28** (170 mg, 0.49 mM) in MeOH (5 mL) was added sodium bicarbonate (108 mg, 1.28 mM) and 85% *m*-chloroperbenzoic acid (200 mg, 0.98 mM). After 30 min at room temperature the mixture was diluted with EtOAc (50 mL) and washed with 5% sodium thiosulfate solution and then with saturated sodium bicarbonate solution. The washings were back-extracted with EtOAc. The organic solutions were combined, dried ($MgSO_4$), and concentrated in vacuo. The residue was chromatographed on silica gel plates using 10% MeOH in $CHCl_3$ as eluent. Two bands were isolated. The less polar one was crystallized from EtOAc/hexane to give 8 mg (4%) of a 6:1 mixture of **29** and its 4'-epimer: mp 203–204 °C; UV λ_{max} (MeOH) 259 nm (ϵ 14400); ^1H NMR (DMSO) δ 8.30 (s, 1 H, 8), 8.13 (s, 1 H, 2), 7.29 (br s, 2 H, NH_2), 6.44 (dd, 1 H, 1'), 5.17 (dd, 1 H, OH), 4.90 (t, 1 H, 3'), 3.58 (dd, 1 H, 5'), 3.41 (dd, 1 H, 5'), 3.36 (s, 3 H, OCH_3), 2.74 (m, 1 H, 2'), 2.46 (m, 1 H, 2'), 0.90 (s, 9 H, *t*-Bu), 0.11 (s, 6 H, Me_2). MS (M^+) 395.1994, calcd for $C_{17}H_{29}N_5O_4Si$ 395.1989.

The more polar band was **9-[3-*O*-(*tert*-butyldimethylsilyl)-2,5-dideoxy-β-D-*glycero*-pent-4-enofuranosyl]adenine *N*^{1}-oxide (30)**, isolated as 14 mg (8%) of an amorphous solid: UV λ_{max} (MeOH) 233 (ϵ 38900), 262 (ϵ 7680), 302 nm (ϵ 2310); ^1H NMR (DMSO) δ 8.64 (s, 1 H, 2 or 8), 8.51 (s, 1 H, 2 or 8), 6.60 (dd, 1 H, 1'), 5.18 (dd, 1 H, 3'), 4.31 (t, 1 H, 5'), 4.13 (d, 1 H, 5'), 3.03 (m, 1 H, 2'), 2.43 (m, 1 H, 2'), 0.91 (s, 9 H, *t*-Bu), 0.16 (s, 6 H, Me_2); MS (M^+) 363.1729, calcd for $C_{16}H_{15}N_5O_3Si$ 363.1727.

2'-Deoxy-4'-methoxyadenosine (31). A solution of **29** (27 mg, 70 μM) and cesium fluoride (25 mg, 160 μM) in DMF (2 mL) was stirred for 3 h at room temperature. Saturated ammonium chloride solution (4 drops) was added, and then the solvents were removed in vacuo. The residue was applied onto a silica gel plate and eluted with 12% MeOH in $CHCl_3$, affording 10 mg (52%) of **31** after crystallization from MeOH/EtOAc/hexane: mp 106–108 °C; UV λ_{max} (0.1 N HCl) 258 nm, λ_{max} (0.1 N NaOH) 260 nm; ^1H NMR (DMSO-d_6) δ 8.30 (s, 1 H, 8), 8.14 (s, 1 H, 2), 7.27 (br s, 2 H, NH_2), 6.37 (dd, J = 8, 3 Hz, 1 H, 1'), 5.10 (t, J = 6 Hz, 1 H, OH), 4.96 (d, J = 7 Hz, 1 H, OH), 4.70 (m, 1 H, 3'), 3.63 (dd, J = 11, 6 Hz, 1 H, 5'), 3.50 (dd, J = 11, 6 Hz, 1 H, 5'), 3.35 (s, 3 H, OCH_3), 2.60 (m, 1 H, 2'), 2.47 (m, 1 H, 2'); ^{13}C NMR (DMSO-d_6) δ 106.7 (4'), 59.9 (5'); MS (M^+) 281.1126, calcd for $C_{11}H_{15}N_5O_4$ 281.1124.

4'-Methoxythymidine (32). To a stirred suspension of **3a** (525 mg, 2.34 mM) in CH_3OH (50 mL) was added 80% *m*-chloroperbenzoic acid (606 mg, 2.81 mM). After 1 h, the reaction was quenched with saturated sodium sulfite solution (0.2 mL), and then the solvents were removed in vacuo. The residue was chromatographed on a silica gel column using the upper phase of a 7.5:0.75:2.0 EtOAc/1-propanol/H_2O mixture as eluent. The product (360 mg) was isolated as a mixture of 4'-epimers. The epimers were separated by semipreparative HPLC on a Whatman Partisil 10 ODS-3 column (18% CH_3OH in H_2O). First to be eluted was **32**, which was isolated as 118 mg (19%) of foam: UV λ_{max} (MeOH) 266 nm (ϵ 9290); ^1H NMR (DMSO-d_6) δ 11.28 (br s, 1 H, NH), 7.67 (d, J = 1.2 Hz, 1 H, 6), 6.13 (dd, J = 3.6, 7.4 Hz, 1 H, 1'), 5.19 (t, J = 5.6 Hz, 1 H, OH), 4.89 (d, J = 7 Hz, 1 H, OH), 4.43 (m, 2 H, 3'), 3.66 (dd, J = 5.4, 11.7 Hz, 1 H, 5'), 3.48 (dd, J = 5.8, 11.7 Hz, 1 H, 5'), 3.30 (s, 1 H, OCH_3), 2.22 (m, 2 H, 2'), 1.77 (d, J = 1.2 Hz, 3 H, CH_3); no NOE was observed between 4'-OCH_3 and 6-H; MS (M^+) 272.1005, calcd for $C_{11}H_{16}N_2O_6$ 272.1008.

The second product eluted from the column was 1-(2-deoxy-4-methoxy-α-L-*threo*-pentofuranosyl)thymine, the 4'-epimer of **32**, which crystallized on evaporation of the appropriate fractions to give 87 mg (14%): mp 160–161 °C; UV λ_{max} (MeOH) 265 nm (ϵ 6680); ^1H NMR (DMSO-d_6) δ 11.30 (br s, 1 H, NH), 7.30 (s, 1 H, 6), 6.48 (t, J = 7 Hz, 1 H, 1'), 5.38 (d, J = 4.6 Hz, 1 H, OH), 4.64 (t, J = 6 Hz, 1 H, OH), 4.10 (t, J ≈ 4.6 Hz, 1 H, 3'), 3.61 (m, 2 H, 5'), 3.25 (s, 3 H, OCH_3), 2.33 (m, 1 H, 2'), 2.12 (m, 1 H, 2'), 1.81 (s, 3 H, CH_3); irradiation at 4'-OCH_3 causes an enhancement of 6-H in the difference NOE spectrum. Anal. ($C_{11}H_{16}N_2O_6$) C, H, N.

2'-Deoxy-4'-methoxyguanosine (33). To a vigorously stirred suspension of **3e** (249 mg, 1.00 mM) and sodium bicarbonate (277 mg, 3.30 mM) in MeOH (10 mL) and CH_2Cl_2 (10 mL) was added 80% *m*-chloroperbenzoic acid (324 mg, 1.50 mM). After 16 h at room temperature, sodium thiosulfate (150 mg) was added, and the mixture was stirred for another 10 min and then filtered. The filtrate was concentrated in vacuo and the residue was triturated with hot acetonitrile. The resulting solid was purified by HPLC on a Whatman Partisil 10 ODS-3 column (10% → 30% MeOH in H_2O). From the crude mixture, containing many products, was obtained 3 mg (1%) of pure **33**: mp 117–127 °C; ^1H NMR (DMSO-d_6) δ 10.67 (br s, 1 H, NH), 7.87 (s, 1 H, 8), 6.50 (br s, 2 H, NH_2), 6.12 (dd, J = 3.8, 6.6 Hz, 1 H, 1'), 5.02 (t, J = 5.6 Hz, 1 H, OH), 4.91 (d, J = 6.6 Hz, 1 H, OH), 4.58 (m, 1 H, 3'), 3.61 (dd, J = 5.6, 12 Hz, 1 H, 5'), 3.51 (dd, J = 5.8, 12 Hz, 1 H, 5'), 3.32 (s, 3 H, OCH_3), 2.34–2.49 (m, 2 H, 2'); ^{13}C NMR (DMSO-d_6) δ 106.3 (4'), 59.6 (5'); MS (LSIMS) (M + H)$^+$ 298.1150, calcd for $C_{11}H_{15}N_5O_5$ 298.1147.

1-(3-Fluoro-2,3,5-trideoxy-β-D-*glycero*-pent-4-enofuranosyl)thymine (36). In a manner similar to that of **2a**, 3'-deoxy-3'-fluorothymidine[53] was converted to 3',5'-dideoxy-3'-fluoro-5'-iodothymidine (**35**). Despite recrystallization from toluene, **35** remained contaminated with triphenylphosphine oxide. Nevertheless, this material was treated with sodium methoxide, as in the preparation of **3a**, to give **36** in an overall yield of 77% after crystallization from EtOH: mp 167–168 °C (lit.[44] mp 161.5–163 °C). Anal. ($C_{10}H_{11}FN_2O_3$) C, H, N.

3'-Deoxy-3'-fluoro-4'-methoxythymidine (37). To a stirred solution of **36** (400 mg, 1.77 mM) in CH_3OH (40 mL) were added sodium bicarbonate (178 mg, 2.44 mM) and 85% *m*-chloroperbenzoic acid (496 mg, 2.44 mM). After 5 h and again after 10 h, additional sodium bicarbonate (79 mg, 0.94 mM) and *m*-chloroperbenzoic acid (200 mg, 0.99 mM) were added. Five hours after the second addition, the reaction mixture was diluted with CH_2Cl_2 (170 mL) and washed with saturated $NaHCO_3$. The aqueous washings were back-extracted twice with EtOAc. All organic extracts were combined, dried ($MgSO_4$), and concentrated in vacuo to a syrup which was purified on a silica gel column (5% MeOH in CH_2Cl_2), affording 124 mg (27%) of amorphous **37**: UV λ_{max} (MeOH) 265 nm (ϵ 7560); ^1H NMR (DMSO-d_6) δ 11.35 (br s, 1 H, NH), 7.56 (d, J = 1 Hz, 6), 6.26 (t, J = 6 Hz, 1 H, 1'), 5.37 (dt, J = 53, 7 Hz, 1 H, 3'), 5.30 (t, J = 6 Hz, 1 H, OH), 3.60 (m, 2 H, 5'), 3.27 (s, 3 H, OCH_3), 2.50 (m, 2 H, 2'), 1.78 (d, J = 1 Hz, 3 H, CH_3); MS (M^+) 274.0961, calcd for $C_{11}H_{15}FN_2O_5$ 274.0965; a

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difference NOE spectrum of the 3'-O-(*tert*-butyldimethylsilyl) derivative of **37** yielded enhancements at 6-H and 5-CH_3 when 5'-H was irradiated and at 1'-H when 4'-OCH_3 was irradiated.

Biological Methods. The CD^{4+} T cell lines A3.01, H9, and MT-2, as well as the lab virus strain HIV-1 LAV and the clinical isolates G762-3 (pre-AZT treatment), G691-2 (post AZT treatment), H112-2 (pre-AZT treatment), and G910-6p2 (post-AZT treatment), were obtained from the AIDS Research and Reference Reagent Program, AIDS Program, National Institute of Allergy and Infectious Diseases (NIAID), NIH. PBL were obtained from the Stanford Blood Bank, Stanford University, Stanford, CA. The cells were infected with the virus for 3 h at 37 °C in 5% CO_2 in air. Cells were mock-infected at the same time to be used to detect cytotoxicity and to serve as cell controls. After the 3-h infection incubation, the cells were washed to remove unadsorbed virus. Test compounds were serially diluted 3-fold in 96-well plates. AZT was diluted 5-fold. The infected and uninfected cells were added to appropriate wells of the plate. For A3.01, H9, and PBL cells, the plates were incubated for 7 days with a change of medium and compound on day 4. At the end of the 7-day incubation period the cells were evaluated for cytotoxicity by visual inspection. Cytotoxicity was graded subjectively using cell morphology and cell death as criteria. For MT-2 cells, the plates were incubated for 4 days and cytotoxicity was evaluated by trypan blue dye exclusion with a hemocytometer. CC_{25} indicates the concentration with which about 25% cell destruction was observed. CC_{100} accordingly is the concentration at which complete destruction of the cell layer was observed. Virus levels were determined by two methods: For A3.01, H9, and PBL cells, a reverse transcriptase assay[54] was carried out on the cell supernatants. For MT-2 cell, p24 core antigen levels were determined with the Du Pont p24 antigen test kit. IC_{50} is the concentration of the test substance which reduced virus levels by 50% compared with control cultures. SI, the selectivity index, is the ratio of CC_{25}/IC_{50}.

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Inhibitors of Human Purine Nucleoside Phosphorylase. Synthesis and Biological Activities of 8-Amino-3-benzylhypoxanthine and Related Analogues

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A series of 3-substituted hypoxanthines (**6-10**, **14-17**) and related analogues (**22**, **23**) have been synthesized as inhibitors of purine nucleoside phosphorylase (PNP), which may conceivably act as T-cell-selective immunosuppressive agents with potential utility in autoimmune disorders such as rheumatoid arthritis, in organ transplantations, and in T-cell leukemias. The compounds were evaluated for their PNP activity by a radiochemical assay and also for their cytotoxic effects on a T-lymphoblastoid cell line (MOLT-4). Appropriate substitutions on 3-benzylhypoxanthine (**7a**) (IC_{50} in PNP assay, 112 μM; IC_{50} in MOLT-4 assay, 204.2 μM) increase potency: 8-amino (**17a**; 42.6, 65.2), 2-hydroxy (**9a**; 13.4, 28.6), 2-amino (**10a**; 11.4, 29.1), and 2,8-diamino (**16a**; 5.0, 11.9). Variation of the 3-aryl substituents of **16a** as in **16b-d** has thus far failed to further increase potency. Replacement of the 6-oxygen function in **7a** with the analoguous nitrogen or sulfur functions, as in **22a** and **23a**, resulted in little change in activity. Other variations including the increase of the 3-aliphatic chain length as in **6h** and **7h** (n = 2), the substitution of the phenyl ring with electron-withdrawing groups as in **7e-g**, and replacement of the 2-hydrogen with methylthio as in **8a** and **14a** resulted in decrease of activity. The values for **16a-d** represent moderate but significant activities, as compared to the most active inhibitor presently known, 8-amino-9-thienylguanine (1c; 0.17, 0.82). 2,8-Diamino-3-substituted hypoxanthines (**16a-d**) represent a novel structural type hitherto unreported in the literature, and efficient methodologies for their synthesis were developed in the present studies. The formation of the aminoimidazole moiety occurred through a base-catalyzed 1,5-($O \rightarrow N$)-carbamimidoyl rearrangement (**13** to **14**, **20** to **16**).

Introduction

Human purine nucleoside phosphorylase (PNP) (EC 2.4.2.1), an essential enzyme of the purine salvage pathway, catalyzes a reversible, phosphorolytic cleavage of ribo- and deoxyribonucleosides of guanine and hypoxanthine.[1] Genetic deficiency of PNP is generally associated with severe selective impairment in T-, but not B-lymphocyte function.[2] Conceivably, inhibitors of PNP should similarly induce a selective suppression of T-cell-mediated immunity.[3-5]

T-cell-selective immunosuppressive agents are potentially useful in the treatment of autoimmune disorders such as rheumatoid arthritis (RA), in the prevention of rejection in bone marrow or organ transplants, and in the treatment of T-cell leukemias.[3-6] One of these, cyclosporine, is now an accepted prophylactic medication in allogenic organ transplants[6a] and has demonstrated efficacy in clinical studies for the treatment of RA.[6b,c] The implication of T cells in the pathogenesis of RA is also supported by the success of lymphoid irradiation,[7a] lymphapheresis,[7b] or

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