Synthesis and Anti-HIV Activity of Different Sugar-Modified Pyrimidine and Purine Nucleosides

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A series of base-modified pyrimidine 3′-azido-2′,3′-dideoxynucleosides and 3′-substituted purine and pyrimidine 2′,3′-dideoxynucleosides have been synthesized and evaluated for their inhibitory activity against human immunodeficiency virus (HIV) replication in MT-4 cells. The following pyrimidine derivatives emerged as the most potent and/or selective inhibitors of HIV-induced cytopathogenicity (in order of decreasing selectivity: 3′-azido-3′-deoxythymidine (AZT), 3′-azido-2′,3′-dideoxyuridine (AzddUrd), 3′-azido-2′,3′-dideoxy-5-methylcytidine (AzddMeCyd), 3′-fluoro-ddUrd (FddUrd), 3′-fluoro-ddThd (FddThd), the N^4-hydroxylated derivative of AzddMeCyd and the N^4-methylated derivative of AzddMeCyd. Among the purine 2′,3′-dideoxynucleosides, 3′-azido-2′,3′-dideoxyguanosine (AzddGuo), 3′-fluoro-ddGuo (FddGuo), and 3′-fluoro-2,6-diaminopurine 2′,3′-dideoxynucleoside (FddDAPR) were the most selective inhibitors of HIV replication.

Since the discovery of the human immunodeficiency virus (HIV) as the causative agent of the acquired immunodeficiency syndrome,[1,2] there has been a growing interest in compounds that can block the replication of retroviruses. Considering the importance of the virus-encoded reverse transcriptase in the replication of HIV, this enzyme can be considered as an attractive target for the design of new effective chemotherapeutic agents.[3] Indeed, the activity of 3′-azido-3′-deoxythymidine (**1a**), which is currently the only licensed drug for the treatment of AIDS patients,[4] can be partly attributed to an inhibition of the reverse transcriptase, following its conversion to the 5′-triphosphate metabolite.[5,6] Since the discovery of AZT as an antiretroviral agent, the synthesis and biological activity of other interesting 3′-azido-2′,3′-dideoxynucleosides have been described.[7-11] 2′,3′-Dideoxynucleosides,[12-15] especially 2′,3′-dideoxycytidine,[12] 2′,3′-dideoxyadenosine,[12] 2′,3′-dideoxy-2,6-diaminopurine ribonucleoside,[13] 2′,3′-dideoxy-5-fluorocytidine,[14] and 2′,3′-dideoxythymidine,[15] selectively suppress the replication of HIV in different cell models in vitro. They are converted by different metabolic pathways to their 5′-triphosphate metabolites.[16-24] Other active compounds, i.e. 2′,3′-unsaturated nucleosides and also some 3′-fluoro-2′,3′-dideoxynucleosides,[10,27,28] were recently discovered in our[10,15,23,24] and other laboratories,[25,26] and all these compounds look promising as anti-HIV agents.

As an extension of our work on sugar-modified 2′,3′-dideoxyadenosines, we have now tested the adenosine analogues **2a**, **2c**, **2e**, **2f**, **4a**, and **5**. Also, a new method

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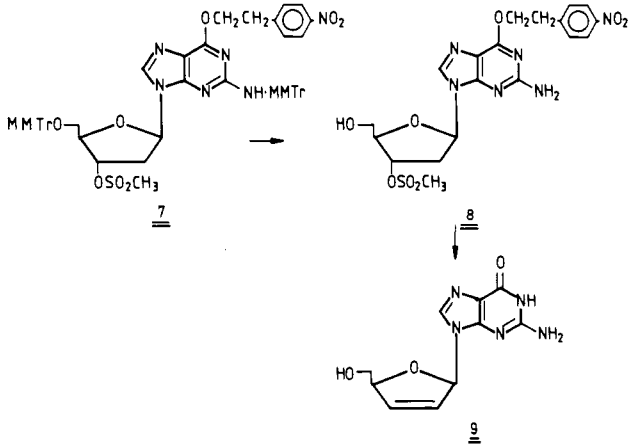
Scheme I

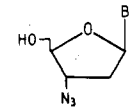


of synthesis for 3′-azido-2′,3′-dideoxyadenosine (**1b**) and 3′-azido-2′,3′-dideoxyguanosine (**1c**) is described. Until very recently,[8] no report appeared on the synthesis and activity of the 2′,3′-unsaturated analogue of 2′,3′-dideoxyguanosine. To the best of our knowledge, only a protected analogue of 2′,3′-dideoxy-2′,3′-didehydroguanosine has been reported in the literature,[29] but the authors apparently did not succeed in deprotection. 2′,3′-Didehydro-2′,3′-dideoxyguanosine (**9**) was mentioned as an intermediate in the synthesis of 2′,3′-dideoxyguanosine by Sanger et al.[30] Their synthesis started with N^2-isobutyryl-5′-*O*-(monomethoxytrityl)-2′-deoxyguanosine. However, this unsaturated guanosine derivative was not isolated nor characterized and its yield was very low.

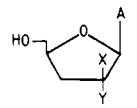
A structure–activity analysis is provided for several base-modified pyrimidine 3′-azido-2′,3′-dideoxynucleosides (**1a**, **1d**, **1e**, **1f**, **1g**) and compared with the previously described compounds **1h** and 1i.[10] Although some of these compounds (**1d**, **1e**, **1h**) were previously described by Lin et al.,[9] these authors only evaluated them against the replication of murine Moloney leukemia virus, as a model for measuring anti-HIV activity. However, 3′-azido-2′,3′-dideoxyuridine (**1d**) was first recognized as an anti-HIV agent by Schinazi et al.[31] Also, the activities of three 3′-fluoro-2′,3′-dideoxynucleosides (**3a**, **3b**, **3c**), three nucleotides with a 2-deoxy-β-D-*threo*-pentofuranose moiety (**11a**, **11b**, **11c**), and two new 3′-C-substituted 2′,3′-dideoxynucleosides [1-(3-cyano- and 3-ethynyl-3-deoxy-β-D-arabinofuranosyl)thymine (**6a** and **6b**)] are described. Since the introduction of a sulfur-containing group into an organic compound increases its lipophilicity as well as its ability to cross the blood–brain barrier (a prerequisite for an efficient anti-HIV drug), different 3′-substituted sulfur-containing nucleosides (**15b**, **15d**, **15e**, **15f**) were also synthesized.

Chemistry

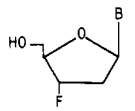
The synthesis of 2′,3′-didehydro-2′,3′-dideoxyguanosine (**9**) starts with 2′-deoxyguanosine following a reaction scheme that is similar to that described by McCarthy et al.[32] for the synthesis of 2′,3′-didehydro-2′,3′-dideoxy-



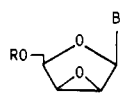
1a: B = thymin-1-yl (T)
b: B = adenin-9-yl (A)
c: B = guanin-9-yl (G)
d: B = uracil-1-yl (U)
e: B = cytosin-1-yl (C)
f: B = N^4-methylcytosin-1-yl
g: B = 4-(hydroxyamino)-5-methyl-1,2-dihydro-2-pyrimidin-1-yl
h: B = 5-methylcytosin-1-yl
i: B = N^4,5-dimethylcytosin-1-yl



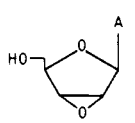
	X	Y
2a:	Cl	H
b:	H	Cl
c:	H	Br
d:	Br	H
e:	OH	H
f:	H	OH
g:	H	OMs
h:	OMs	H



3a: B = guanin-9-yl
b: B = 2,6-diaminopurin-9-yl
c: B = uracil-1-yl
d: B = thymin-1-yl



4a: B = adenin-9-yl; R = H
b: B = thymin-1-yl; R = Tr



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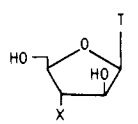

6a: X = CN
b: X = C≡CH

MMTr, monomethoxytrityl; T, trityl; Ts, tosyl; Ms, mesyl; Bz, benzoyl; Ac, acetyl

adenosine. Reaction of deoxyguanosine with 3 equiv of monomethoxytrityl chloride in dimethylformamide containing triethylamine (3.2 equiv) at room temperature afforded N^2,5′-*O*-bis(monomethoxytrityl)-2′-deoxyguanosine in a yield of 90%. This reaction is similar to the synthesis of the dimethoxytrityl derivative, as described by the Khorana group.[33] Mesylation of the 3′-hydroxyl group without protection of the amide function gave side reactions as could be expected. Therefore we protected the amide function with the (*p*-nitrophenyl)ethyl protecting group[34] before mesylation of the 3′-hydroxyl group. This reaction did not give detectable amounts of the N^3,3′-cyclonucleosides. The main difficulty in this reaction is the purification of the compound, which is, after two column chromatographic purifications, still contaminated with 1,2-bis(ethoxycarbonyl)hydrazine. Therefore we preferred to mesylate the 3′-hydroxyl group, to afford in one step the substituted 2′-deoxyguanosine derivative **7**. Deprotection of the acid labile groups with acetic acid (80%) at room temperature afforded **8**. The total yield for the synthesis of **8** from tritylated 2′-deoxyguanosine was 61%. A double elimination reaction with potassium *tert*-butylate in dimethyl sulfoxide or sodium methanolate

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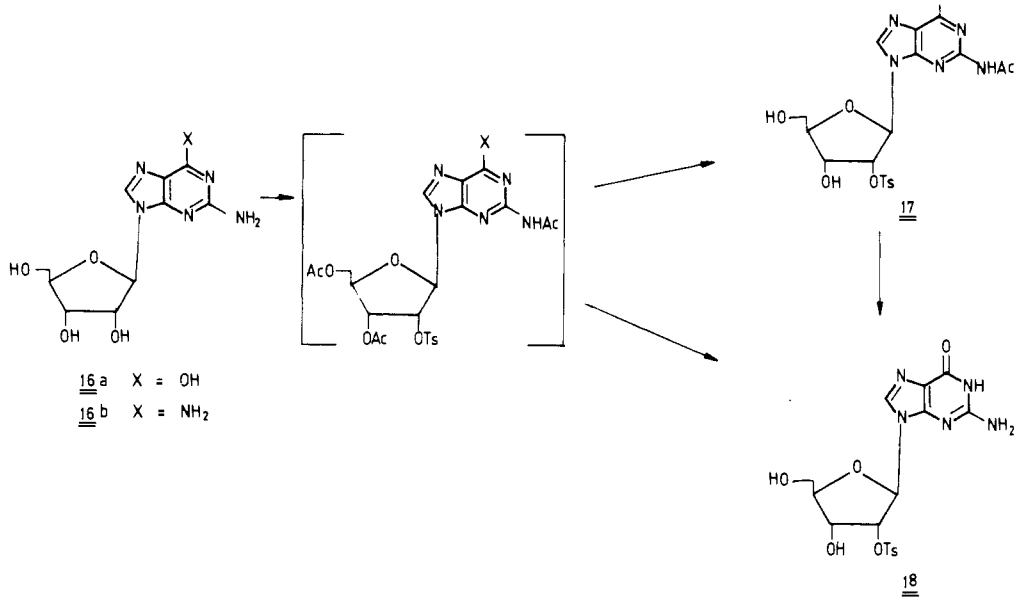
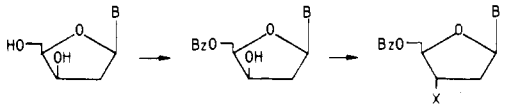
Scheme II



in dimethylformamide gave 2′,3′-didehydro-2′,3′-dideoxyguanosine in 78% yield (Scheme I).

Reaction of 9-(2-deoxy-β-D-*threo*-pentofuranosyl)adenine (**11a**)[40] with benzoyl chloride in pyridine gave the 5′-*o*-benzoylated compound **12a**. When this compound was added to a mixture of triphenylphosphine, carbon tetrabromide, and lithium azide in dimethylformamide[35a,b] and kept for 24 h at room temperature, 5′-*O*-benzoyl-3′-azido-2′,3′-dideoxyadenosine (**13a**) was isolated in 68% yield.



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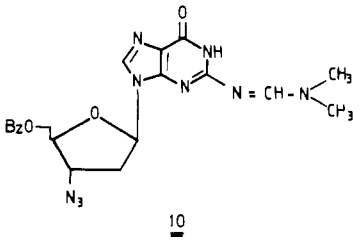
11a, B = adenin-9-yl; 11b, 2,6-diaminopurin-9-yl; 11c, B = guanin-9-yl; 12a, B = adenin-9-yl; 12b, B = guanin-9-yl; 13a, B = adenin-9-yl, X = N_3; 13b, B = guanin-9-yl, X = F; 13c, B = guanin-9-yl, X = N_3

Debenzoylation following the usual procedure afforded 3′-azido-2′,3′-dideoxyadenosine (**1b**). During the reaction with lithium azide, a side compound with higher mobility on TLC was formed which disappeared during the workup procedure. This labile intermediate was assumed to be the N^6-(dimethylamino)methylene derivative and the structure was proven for the guanine analogue. Hata et al. reported that this method for the introduction of an azido group was not amenable for N^2-benzoylguanosine.[35a] We repeated the reaction on 9-(5-*O*-benzoyl-2-deoxy-β-D-*threo*-pentofuranosyl)guanine (**11c**) and obtained the same results as with the adenine analogue. In this case, the reaction mixture, after addition of methanol, was evaporated and directly applied onto a silica column. The intermediate N^2-[(dimethylamino)methylene]-5′-*O*-benzoyl-3′-azido-2′,3′-dideoxyguanosine (**10**) could be isolated together



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with 5′-*O*-benzoyl-3′-azido-2′,3′-dideoxyguanosine (**13c**). Both protecting groups were removed with ammonia in methanol. It is well known that such amidine protecting groups are very labile.[36] The isolated 3′-azido-2′,3′-dideoxyguanosine (**1c**) was identical with the compound described earlier by Imazawa et al.[37] This reaction with lithium azide, carbon tetrabromide, and triphenylphosphine is somewhat similar to the reaction with (diethylamido)sulfur trifluoride (DAST). No base protection is needed because the reagent provides a transient protection. The labile intermediate is easily destroyed during the work-up procedure.

As described in our recent publication,[11] the regiospecific tosylation in the 2′-position of 2,6-diamino-9-β-D-ribofuranosylpurine (**16b**) by the Wagner–Moffatt[38] procedure went to completion, though only a fraction of the compound could be isolated by crystallization directly from the reaction mixture. The same problem was encountered with guanosine. Therefore, 2′-*O*-tosylguanosine, obtained from the reaction of guanosine with dibutyltin oxide and *p*-toluenesulfonyl chloride, was peracetylated with acetic anhydride in pyridine, freed from triethylammonium chloride by washing with water, and deacetylated with methanol–ammonia to afford 2′-*O*-tosylguanosine (**18**) in 61% yield (Scheme II). An alternative synthesis starts from 2,6-diaminopurine 9-β-D-ribofuranoside. The reaction mixture, after 2′-O-tosylation,[11] was also peracetylated, washed with water, and deacetylated with ammonia in methanol. This reaction sequence yielded 2′-*O*-tosyl-N^2-acetyl-2,6-diamino-9-β-D-ribofuranosylpurine (**17**) because of the higher stability of the N^2-acetyl group compared with the N^6-acetyl group.[39] Deamination of **17** with

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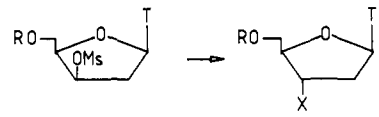
nitrous acid gave 2′-*O*-tosyl-N^2-acetylguanosine (**18**), which was readily deacetylated under the same experimental conditions as described above. The products of both syntheses were completely identical. The reaction with lithium triethylborohydride, as described by Robins,[40] gave 9-(2-deoxy-β-D-*threo*-pentofuranosyl)guanine (**11c**) in good yield. In this case, the anion-exchange resin was used in the acetate form and the compound was eluted with 5% aqueous acetic acid. The use of Dowex 1 X-2 ($^-$OH) and elution with water, methanol, or ammonia did not elute **11c** quantitatively from the column. The hydride shift reaction was also carried out on the 5′-*O*,N^2-bis-trityl derivative of **18**. However, in this case only 20% of the rearranged compound could be isolated. The other isolated compound is N^2-tritylguanine, probably arising from β-elimination of the intermediate 3′-keto derivative.

The 5′-*O*-benzoyl derivative of **11c** was synthesized by reaction of **11c** with benzoic anhydride in dimethylformamide in the presence of 4-(dimethylamino)pyridine and triethylamine. This procedure is similar to the Matsuda procedure for the synthesis of guanosine peracetylated in the carbohydrate moiety.[41] Reaction of **12b** with DAST was carried out in dichloromethane, which is not an ideal solvent for this reaction because of the poor solubility of the starting material. 3′-Fluoro-2′,3′-dideoxyguanosine (**3a**) was obtained after debenzoylation of **13b** in the usual way.

When 2′-*O*-mesylcordycepin[27] (**2g**) was treated with lithium chloride in dimethylformamide at 100 °C, the 2′-chloro compound **2a** was formed. ^{1}H NMR of **2a** shows a $J_{1',2'}$ value of 6.15 Hz. The same reaction conducted with lithium bromide was also stopped after 72 h though some starting material was still present. The reaction mixture contained only one other nucleoside compound, which had a greater mobility on TLC ($CHCl_3$–MeOH, 90:10) than the starting material. This compound had a $J_{1',2'}$ value of 3.1 Hz and was identified as 9-(2-bromo-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)adenine (**2c**). Because of the greater leaving group capability of a bromo substituent, the intermediate with the *threo* configuration (**2d**) underwent fast exchange in the reaction condition with external lithium bromide. Identification of both compounds was made possible by comparison with the reaction products obtained by the reaction of 9-(3-deoxy-2-*O*-mesyl-β-D-*threo*-pentofuranosyl)adenine[27] (**2h**) with lithium chloride and lithium bromide. In the case of the bromo analogue, only one nucleoside was produced which has the same mobility on TLC and the same UV and ^{1}H NMR spectrum ($J_{1',2'}$ = 3.1 Hz) as **2c**. The reaction with lithium chloride gave a compound with a different R_f value on TLC [$CHCl_3$–MeOH (85:25) 0.70 for **2b** compared to 0.57 for **2a**] and with a different ^{1}H NMR spectrum ($J_{1',2'}$ = 2.9 Hz compared to 6.15 Hz for **2a**). These values correspond very well with those found for 9-(2-azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)adenine ($J_{1',2'}$ = 3.1 Hz) and 9-(2-azido-2,3-dideoxy-β-D-*threo*-pentofuranosyl)adenine ($J_{1',2'}$ = 6.1 Hz).[27]

3′-(Acetylthio)-2′,3′-dideoxythymidine (**15b**) was synthesized from 1-(5-*O*-trityl-3-*O*-mesyl-2-deoxy-β-D-*threo*-pentofuranosyl)thymine (**14a**)[42] by a nucleophilic displacement with potassium thioacetate in dimethylformamide followed by detritylation with 80% aqueous acetic acid. The intermediate 5′-*O*-trityl-3′-(acetylthio)-2′,3′-dideoxythymidine (**15a**) was deacetylated with ammonia in methanol. The crystalline precipitate that was collected appeared to be the dimer of 5′-*O*-trityl-3′-mercapto-2′,3′-dideoxythymidine (**15c**) because of the absence of other exchangeable protons as N–H on ^{1}H NMR and because of the inability to alkylate the thiol group with ethyl iodide in methanol containing sodium methanolate. Detritylation with acetic acid afforded the dimer of 3′-mercapto-2′,3′-dideoxythymidine (**15d**).[43] The mass spectrum of this compound showed no molecular ion peak because of the easy cleavage of the disulfide bond: 258 was found as the highest signal. Chemical ionization with ammonia or isobutane did not give more information (259 as highest peak). Reaction of 1-(3-*O*-mesyl-2-deoxy-β-D-*threo*-pentofuranosyl)thymine[42] (**14b**) with *p*-thiocresol in a mixture of dimethylformamide and ethanol in the presence of sodium ethanolate afforded 3′-(*p*-tolylthio)-2′,3′-dideoxythymidine (**15e**). 5′-*O*-(Monomethoxytrityl)-2′-deoxythymidine was treated with phenoxythioformyl chloride in acetonitrile in the presence of 4-(dimethylamino)pyridine, as described by Robins et al.[44] for their synthesis of 2′-deoxynucleosides. This compound was detritylated with 2% of *p*-toluenesulfonic acid in a mixture of $CHCl_3$–MeOH (4:1) to afford **15f**.



14a: R = Tr
b: R = H

15a: R = Tr, X = $SC(O)CH_3$
b: R = H, X = $SC(O)CH_3$
c: R = Tr, X = S-)$_2$
d: R = H, X = S-)$_2$
e: R = H, X = $SC_6H_4CH_3$
f: R = H, X = $OC(S)OC_6H_6$

The synthesis of 3′-azido-2′,3′-dideoxythymidine (**1a**) was previously described by Horwitz et al.[42] The same modus operandi was followed here except that 1-(5-*O*-trityl-3-*O*-mesyl-β-D-*threo*-pentofuranosyl)thymine (**14a**) was first detritylated before the azido group was introduced. This reaction sequence gave a better yield. 3′-Azido-2′,3′-dideoxyuridine (**1d**) was prepared by the same method as described by Lin et al.[45] except that the monomethoxytrityl group was used instead of the trityl group to protect the 5′-hydroxyl group. In the Experimental Section, our physical data for this compound are given because they differ somewhat from those described by Lin and Mancini.[45] The data are in agreement with those published by Krenitsky et al.[46] 5′-*O*-Trityl-3′-azido-2′,3′-dideoxyuridine[45] and 5′-*O*-trityl-3′-azido-2′,3′-dideoxythymidine[42] were used as starting materials for the synthesis of **1f** and **1g**, respectively. This reaction involves the intermediary conversion of the 4-carbonyl function into a good leaving group[47] followed by a substitution reaction with methylamine or hydroxylamine. Detritylation with acetic acid afforded the 4-modified analogues **1f** and **1g**; **1f** was purified by crystallization after conversion to the hydrochloride salt.

1-(5-*O*-Trityl-2,3-epoxy-β-D-lyxofuranosyl)thymine (**4b**) prepared according to the method of Codington and Fox[48] was treated with the ethylenediamine complex of ethy-

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Table I. Comparative Potency and Selectivity of 2′,3′-Dideoxynucleoside Analogues as Inhibitors of HIV Replication in MT-4 Cells

compd	ED_{50},[a] μM	CD_{50},[b] μM	SI[c] (ratio CD_{50}/ED_{50})
1a	0.004[d]	20[d]	5000[d]
1b	5.0[e]	10[e]	2[e]
1c	2.8[f]	165[f]	59[f]
1d	0.36[d]	244[d]	677[d]
1e	3.1	34.1	11
1f	605	>1000	>1.6
1g	1.5	92	61
1h	1.8	>1000	555
1i	17.3	>1000	>58
2a	>500	>500	
2c	>500	>500	
2e	>500	>500	
2f	>50	45	<0.5
3a	2.4[f]	237[f]	96[f]
3b	4.5[f]	360[f]	80[f]
3c	0.04[f]	16[f]	400[f]
3d	0.001[d]	0.197[d]	197[d]
4a	>100	>100	
5	>500	>500	
6a	>500	>500	
6b	>500	>500	
9	>5	11	>2.2
11a	>100	>100	
11b	>125[f]	>625[f]	
11c	158	>500	3.1
15b	27	29	1.1
15d	16	34	2.1
15e	>100	116	<1.2
15f	>250	>250	

[a] Effective dose of compound, achieving 50% protection of MT-4 cells against the cytopathic effect of HIV. [b] Cytotoxic dose of compound, required to reduce the viability of normal uninfected MT-4 cells by 50%. [c] Selectivity index: ratio CD_{50}/ED_{50}. [d] Reference 56. [e] Reference 10. [f] Reference 11.

nyllithium following the prescription of Ashwell et al.[49] This reaction resulted in a clean conversion to the 3′-ethynyl derivative which was isolated in 74% yield after detritylation. In the same article[49] the reaction was described of 1-(5-*O*-trityl-2,3-epoxy-β-D-lyxofuranosyl)uracil with sodium cyanide in dimethyl sulfoxide at 47 °C in 22% yield. Similar results were obtained when the thymin-1-yl analogue was reacted with tetraethylammonium cyanide as nucleophile in dimethylformamide at room temperature (26% yield). The use of other solvents such as acetonitrile or dimethyl sulfoxide gave lower yields.

Previously described methods have been used for the synthesis of 3′-azido-2′,3′-dideoxy-5-methylcytidine[10,50] (**1h**), 1-(3-azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)-4-(methylamino)-5-methyl-2(1*H*)-pyrimidinone[10] (**1i**), 9-(2,3-epoxy-β-D-lyxofuranosyl)adenine[51] (**4a**), 9-(2,3-epoxy-β-D-ribofuranosyl)adenine[53,54] (**5**), 9-(2-deoxy-β-D-*threo*-pentofuranosyl)adenine[40] (**11a**), 9-(3-deoxy-β-D-*threo*-pentofuranosyl)adenine[40] (**2e**), 9-(2-deoxy-β-D-*threo*-pentofuranosyl)-2,6-diaminopurine[11] (**11b**), 3′-fluoro-2′,3′-dideoxyuridine[55] (**3c**), 9-(3-fluoro-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)-2,6-diaminopurine[55] (**3b**). 3′-Azido-2′,3′-dideoxycytidine was a gift from Dr. D. G. Johns (National Institutes of Health, Bethesda, MD).

Antiviral Activity

The anti-HIV activity and cytotoxicity of the 2′,3′-dideoxynucleoside analogues are shown in Table I. Among the 3′-azido derivatives, compound **1a** (AZT) turned out to be the most selective anti-HIV agent, followed by **1d** (AzddUrd), **1h** (AzddMeCyd), **1g** (the N^4-hydroxylated derivative of **1h**), **1c** (AzddGuo), and **1i** (the N^4-methylated derivative of **1h**). AzddCyd (**1e**) showed a relatively poor selectivity index. The N^4-methylated derivative of **1e** (compound **1f**) was virtually devoid of anti-HIV activity, while AzddAdo (**1b**) was only protective at cytotoxic concentrations. Methylation of the 4-amino group of AzddCyd and AzddMeCyd substantially decreased the anti-HIV activity of the compounds.

Since the AzddCyd derivatives may be converted to the corresponding 5′-mono-, di-, and triphosphates by several cellular kinases (i.e. dCyd kinase, dCMP/CMP kinase, nucleoside diphosphate kinase), while being subject to deamination by Cyd/dCyd deaminase at the nucleoside level or dCMP deaminase at the nucleoside 5′-monophosphate level, complex metabolic interactions seem to be involved in the conversion of these compounds to their intracellularly active forms. In this perspective it should be clarified if, and to what extent, deamination of AzddCyd and AzddMeCyd to AzddUrd and AZT, respectively, is a prerequisite for their anti-HIV activity.

Among the 3′-halo-substituted 2′,3′-dideoxynucleoside derivatives, the pyrimidine nucleosides FddUrd (**3c**) and FddThd (**3d**) proved clearly more active and selective than the purine nucleosides FddGuo (**3a**) and FddDAPR (**3b**). The 2′-chloro- (**2a**) and 2′-bromo- (**2c**) substituted ddAdo derivatives were devoid of any anti-HIV activity (ED_{50} = >500 μM). None of the other 3′-substituted ddThd or araT derivatives [i.e. 3′-acetylthio- (**15b**), the dimers of 3′-mercapto- (**15d**), 3′-(tolylthio)- (**15e**), and 3′-*O*-[phenoxy(thiocarbonyl)- (**15f**) ddThd, and 3′-cyano- (**6a**) and 3′-ethynyl- (**6b**) araT] showed any selectivity and/or antiviral activity. Nor were any of the purine 2′-deoxyxylosides (**11a**, **11b**, **11c**) or 2′,3′-epoxides of ddAdo (**4a**, **5**), 3′-deoxyadenosine (cordycepin, **2f**) or 3′-deoxy-araA (**2e**) endowed with substantial anti-HIV activity.

Experimental Section

Melting points were determined with a Büchi-Tottoli apparatus and are uncorrected. Infrared spectra were recorded with a Perkin-Elmer 257 spectrophotometer on samples in potassium bromide disks at 1.5%. Ultraviolet spectra were recorded with a Beckman UV 5230 spectrophotometer. The ^1H NMR and ^{13}C NMR spectra were determined with a JEOL FX 90Q spectrometer with tetramethylsilane as internal standard (s = singlet, d = doublet, t = triplet; m = multiplet, br = broad signal). Electron-impact mass spectra (70 eV) were recorded on a AEI-MS12 mass spectrometer. Isobutane or ammonia chemical ionization mass spectra (70 eV) were obtained on a KRATOS-MS50 mass spectrometer. R, base; S, sugar. Precoated Merck silica gel F254 plates were used for TLC. Column chromatography was performed on Merck silica gel (0.063–0.200 mm). Anhydrous dimethyl sulfoxide was obtained by distillation in vacuo after drying with calcium hydride; water was removed from *N*,*N*-dimethylformamide by distillation with benzene followed by distillation in vacuo. Potassium *tert*-butylate was sublimated immediately before use. Pyridine was dried by distillation after it has been stored on potassium hydroxide. Dichloromethane was dried with calcium

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chloride and distilled. Tetrahydrofuran was refluxed for 10 h on lithium aluminum hydride and distilled.

9-(5-*O*-Benzoyl-2-deoxy-β-D-*threo*-pentofuranosyl)adenine (12a). A solution of 0.48 mL (4.12 mmol) of benzoyl chloride in 10 mL of pyridine was added dropwise over a period of 30 min to a cooled solution (ice bath) of 1 g (3.75 mmol) of 9-(2-deoxy-β-D-*threo*-pentofuranosyl)adenine (**11a**) in 50 mL of pyridine–dimethylformamide (4:1). The reaction mixture was kept overnight at 4 °C, evaporated, and purified by column chromatography ($CHCl_3$–MeOH, 96:4), giving 1.02 g (2.87 mmol, 77%) of the 5′-O-benzoylated compound which crystallizes upon addition of MeOH: mp 210–212 °C; UV (MeOH) λ_{max} 259 nm (ε 15800); MS, *m/e* 355 (M^+); ^1H NMR (DMSO-d_6) δ 2.18–2.57 and 2.64–3.26 (2 × m, H-2′, H-2″), 4.20–4.70 (m, H-3′, H-4′, H-5′, H-5″), 6.34 (2 H, 3′-OH and H-1′, H-1′ as a dd), 7.48–7.69 and 7.88–8.04 (2 × m, benzoyl), 8.17 and 8.38 (2 × s, H-8 and H-2).

3′-Azido-2′,3′-dideoxyadenosine (1b). 9-(5-*O*-Benzoyl-2-deoxy-β-D-*threo*-pentofuranosyl)adenine (**12a**) (300 mg, 0.85 mmol) was added to a mixture of 445 mg (1.7 mmol) of triphenylphosphine, 564 mg (1.7 mmol) of carbon tetrabromide, and 490 mg (10 mmol) of lithium azide in 10 mL of dimethylformamide. After 24 h at room temperature, MeOH (1 mL) was added, the solvent was reduced to 5 mL by evaporating, and the residue was partitioned between EtOAc (50 mL) and H_2O (50 mL). The H_2O layer was extracted once with EtOAc (50 mL) and the combined organic layer was dried, evaporated, and purified by column chromatography [(1) CH_2Cl_2–MeOH 99:1, (2) CH_2Cl_2–MeOH 97:3)], yielding 220 mg (0.58 mmol, 68%) of **13a**: ^1H NMR ($CDCl_3$) δ 2.43–2.79 (m, 1 H, H-2′), 3.02–3.40 (m, 1 H, H-2″), 4.27 (m, 1 H, H-4′), 4.62 (m, 2 H, H-5′, H-5″), 4.75 (m, 1 H, H-3′), 6.18 (br s, 2 H, NH_2), 6.30 (dd, 1 H, *J* = 5.05 and 6.8 Hz, H-1′), 7.47 and 7.96 (2 × m, phenyl), 7.91 and 8.27 (2 × s, 2 × 1 H, H-8 and H-2). This compound was debenzoylated overnight with methanol saturated with ammonia (10 mL). After evaporation of the solvent, the title compound (**1b**) was purified by column chromatography ($CHCl_3$–MeOH, 95:5) and crystallized from MeOH: 150 mg (0.51 mmol, 88% yield); mp 189–190 °C.

3′-Azido-2′,3′-dideoxyguanosine (1c). A solution of 740 mg (2 mmol) of 9-(5-*O*-benzoyl-2-deoxy-β-D-*threo*-pentofuranosyl)-guanine, 1.1 g (4.2 mmol) of triphenylphosphine, 1.4 g (4.2 mmol) of carbon tetrabromide, and 600 mg (12.2 mmol) of lithium azide was kept at room temperature for 24 h. After addition of 2 mL of MeOH and evaporation of the solvent, the reaction mixture was applied on a silica column and eluted with $CHCl_3$–MeOH 90:10. The first nucleoside that was eluted was identified as N^2-[(dimethylamino)methylene]-5′-*O*-benzoyl-3′-azido-2′,3′-dideoxyguanosine (220 mg, 0.49 mmol): MS, *m/e* 451 (M^+); IR (KBr) 2110 cm^{-1} (N_3); UV (MeOH) λ_{max} 303 nm (ε 17000); ^1H NMR (DMSO-d_6) δ 2.40–2.60 (H-2′), 3.06 and 3.18 (2 × s, $(CH_3)_2N$), H-2″ is hidden by the two methyl peaks, 4.26 (m, H-4′), 4.58 (m, H-5′, H-5″), 4.92 (m, H-3′), 6.38 (t, H-1′), 7.50–7.80 (m) and 7.90–8.10 (m) (benzoyl), 8.12 (s), 8.66 (s) (N=CHN and H-8); ^{13}C NMR (DMSO-d_6) δ 35.8 and 34.7 [$N(CH_3)_2$], 60.9, 63.8, 81.2, 82.7, 120.1, 128.7, 129.2, 133.5, 137.9, 149.5, 157.3, 157.6, 157.9, 165.5. The second compound [5′-*O*-benzoyl-3′-azido-2′,3′-dideoxyguanosine: IR 2110 cm^{-1} (N_3)], 300 mg (0.76 mmol) (total yield 62%), was further identified after debenzoylation with methanol saturated with ammonia overnight and purification by column chromatography ($CHCl_3$–MeOH 90:10). The compound was crystallized as described by Imazawa et al. (160 mg, 0.53 mmol, 70%). This compound has the same mobility on TLC as the product of Eckstein: mp 230 °C (darkening); UV (MeOH) λ_{max} 253 nm (ε 13900) 270 (sh); IR (KBr) 2110 (N_3) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.22–2.87 (m, H-2′, H-2″), 3.59 (m, 2 H, H-5′, H-5″), 3.88 (m, H-4′), 4.57 (m, H-3′), 5.11 (t, 5′-OH), 6.07 (t, H-1′, *J* = 6.3 Hz), 6.54 (br s, NH_2), 7.94 (s, H-8), 10.6 (br s, NH).

3′-Azido-2′,3′-dideoxyuridine (1d): mp (MeOH) 168–169 °C (lit.[45] mp 161–163 °C; lit.[46] mp 166.5–168.5 °C); IR (KBr) 2100 (N_3) cm^{-1}; UV (MeOH) λ_{max} 259 nm (ε 10600); ^1H NMR (DMSO-d_6) δ 2.35 (t, 2 H, H-2′, H-2″), 3.62 (m, 2 H, H-5′, H-5″), 3.84 (m, 1 H, H-4′), 4.36 (m, 1 H, H-3′), 5.64 (d, *J* = 8.1 Hz, H-5), 6.08 (t, *J* = 6.5 Hz, H-1′), 7.84 (d, 1 H, H-6). Anal. ($C_9H_{11}N_5O_4$) C, H, N.

1-(3-Azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)-4-(methylamino)-2(1*H*)-pyrimidinone (1f). A mixture of 0.99 g (2 mmol) of 5′-*O*-trityl-3′-azido-2′,3′-dideoxyuridine,[45] 0.7 mL of phosphorus oxychloride, and 2 mL of *N*-methylimidazole in pyridine (50 mL) was stirred for 30 min at room temperature after which 10 mL of a solution of methylamine (40%) in H_2O was added. The reaction mixture was stirred for another 30 min at room temperature, the organic solvent was evaporated, and the residue was extracted twice with $CHCl_3$ (100 mL). The organic layer was dried and evaporated. Detritylation was performed with 80% of acetic acid for 15 min at 100 °C. Evaporation and coevaporation with toluene leaves an oil, which was purified by column chromatography ($CHCl_3$–MeOH 9:1). The free base was dissolved in MeOH and a solution of 1 N hydrochloric acid in MeOH was added until pH 2 (indicator paper). The solvent was evaporated and the title compound was crystallized from MeOH–Et_2O. A second crystallization, after treatment with charcoal in MeOH, was needed to become an analytically pure compound: mp 164 °C dec; IR (KBr) 2100 (N_3) cm^{-1}; UV (H_2O) λ_{max} 273 nm (ε 12500); ^1H NMR (D_2O–ref DSSA) δ 2.56 (t, 2 H, H-2′, H-2″), 3.10 (s, 3 H, CH_3), 3.86 (m, 2 H, H-5′, H-5″), 4.13 (m, 1 H, H-4′), 4.31 (m, 1 H, H-3′), 6.17 (t, *J* = 6.15 Hz, H-1′), 6.20 (d, *J* = 7.9 Hz, H-5), 7.95 (d, H-6). Anal. ($C_{10}H_{15}N_6O_3Cl$) C, H, N.

1-(3-Azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)-4-(hydroxyamino)-5-methyl-2(1*H*)-pyrimidinone (1g). A mixture of 1.4 mL of phosphorus oxychloride, 4 mL of *N*-methylimidazole, and 2.04 g of 5′-*O*-trityl-3′-azido-2′,3′-dideoxythymidine[42] in 50 mL of pyridine was stirred for 30 min at room temperature. A solution of hydroxylamine hydrochloride (3 g) in H_2O (50 mL), containing 6 mL of triethylamine, was added to the reaction mixture and stirring was continued for 7 h at room temperature. The mixture was concentrated, extracted twice with $CHCl_3$ (100 mL), dried, and evaporated. The residual oil was heated at 100 °C in 80% of acetic acid for 20 min, evaporated, coevaporated with toluene, and purified by column chromatography ($CHCl_3$–MeOH 97:3): 790 mg (2.8 mmol, 70% yield); mp ($CHCl_3$–Et_2O 1:3) 139.5–140 °C; IR (KBr) 2100 (N_3) cm^{-1}; UV (MeOH) λ_{max} 271 nm (ε 8400), 234 (13400); ^1H NMR (DMSO-d_6) δ 1.76 (d, 3 H, CH_3), 2.14 (m, 2 H, H-2′, H-2″), 3.56 (m, 2 H, H-5′, H-5″), 3.76 (m, 1 H, H-4′), 4.36 (m, 1 H, H-3′), 5.09 (t, 1 H, 5′-OH), 6.07 (t, *J* = 6.8 Hz, H-1′), 6.90 (d, *J* = 1.3 Hz, H-6), 9.32 (br s, 1 H, NOH), 10.13 (br s, 1 H, NH); ^{13}C NMR (DMSO-d_6) δ 12.7 (CH_3), 35.3 (C-2), 60.8 and 61.3 (C-3 and C-5), 82.7 and 83.4 (C-1 and C-4), 106.5 (C-5), 126.2 (C-6), 143.7 (C-4), 149.1 (C-2). Anal. ($C_{10}H_{14}N_6O_4$) C, H, N.

9-(2-Chloro-2,3-dideoxy-β-D-*threo*-pentofuranosyl)adenine (2a). A 329-mg (1 mmol) portion of 3′-*O*-mesylcordycepin[27] (**2g**) and 425 mg (10 mmol) of lithium chloride in 10 mL of dimethylformamide were heated for 72 h at 100 °C. After evaporation of the solvent, the reaction mixture was applied on a XAD-2 column eluting first with H_2O and then with H_2O–MeOH (1:1). The UV-absorbing fractions were collected, evaporated, and purified by column chromatography on silica gel ($CHCl_3$–MeOH 9:1). The title compound was crystallized from MeOH–Et_2O: 100 mg (0.37 mmol, 37% yield); mp 244 °C; MS, *m/e* 269 (M^+); UV (MeOH) λ_{max} 259 nm (ε 15700); ^1H NMR (DMSO-d_6) δ 2.50 (m, 2 H, H-3′, H-3″), 3.72 (m, 2 H, H-5′, H-5″), 4.15 (m, 1 H, H-4′), 4.88–5.30 (m, 2 H, H-2′ and 5′-OH), 6.42 (d, 1 H, *J* = 6.15 Hz, H-1′), 7.26 (br s, 2 H, NH_2), 8.15 and 8.45 (2 × s, 2 × 1 H, H-8 and H-2). Anal. ($C_{10}H_{12}N_5O_2Cl$) C, H, N.

9-(2-Bromo-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)-adenine (2c). A mixture of 329 mg (1 mmol) of 3′-*O*-mesylcordycepin[27] (**2g**) and 870 mg of lithium bromide in 10 mL of dimethylformamide was heated for 72 h at 100 °C. The solvent was evaporated and the residue was purified by column chromatography. The title compound was crystallized from MeOH–Et_2O, yielding 110 mg (0.35 mmol, 35%): mp 232 °C dec; MS, *m/e* 313 (M^+); UV (MeOH) λ_{max} 259 nm (ε 15700); ^1H NMR (DMSO-d_6) δ 2.20–2.96 (m, H-3′, H-3″), 3.66 (m, 2 H, H-5′, H-5″), 4.44 (m, 1 H, H-4′), 5.04 (m, H-2′), 5.24 (t, *J* = 5.7 Hz, 5′-OH), 6.33 (d, *J* = 3.1 Hz, H-1′), 7.30 (br s, 2 H, NH_2), 8.15 and 8.39 (2 × s, 2 × 1 H, H-8 and H-2). Anal. ($C_{10}H_{12}N_5O_2Br$) C, H, N.

N^2-Acetyl-2′-*O*-tosyl-2,6-diamino-9-β-D-ribofuranosylpurine (17). The reaction was carried out as described for the synthesis of 2′-*O*-tosyl-2,6-diamino-9-β-D-ribofuranosylpurine on a 10-mmol scale.[11] The H_2O layer, obtained after the Et_2O wash, was evaporated, coevaporated with pyridine, and treated overnight at room temperature with 100 mL of a mixture of pyridine–acetic

anhydride (5:1). The reaction mixture was evaporated, dissolved in $CHCl_3$ (250 mL), washed with 10% sodium bicarbonate (2 × 250 mL) and H_2O (250 mL), dried, and evaporated. Traces of pyridine were removed by coevaporation with toluene. The residual oil was treated overnight at room temperature with MeOH saturated with ammonia at 0 °C (250 mL). After evaporation of the solvent, the title compound was purified by column chromatography ($CHCl_3$–MeOH 9:1): 4.11 g (8.6 mmol, 86% yield); MS, *m/e* 478 (M^+); UV (MeOH) λ_{max} 272 nm (ϵ 12600); ^1H NMR (DMSO-d_6) δ 2.21 (s, CH_3CO), 2.29 (s, $CH_3C_6H_4$), 3.65 (m, H-5′, H-5″), 4.03 (m, H-4′), 4.37 (m, H-3′), 5.55 (dd, J = 5.3 and 7.0 Hz, H-2′), 6.00 (d, J = 7.0 Hz, H-1′), 7.02 (d) and 7.44 (d) (J = 8.35 Hz, aromatic H), 7.30 (br s, NH_2), 8.03 (s, H-8).

2′-*O*-Tosylguanosine (18). A. N^2-Acetyl-2′-*O*-tosyl-2,6-diamino-9-β-D-ribofuranosylpurine (2.87 g, 6 mmol) was dissolved in 200 mL of H_2O (the mixture was heated for complete solubilization). To the cooled solution, 7.2 g of sodium nitrite and 7.2 mL of acetic acid were added and the mixture was stirred overnight at room temperature. After neutralization of the reaction mixture with 2 N NaOH, the solvent was evaporated and the residue was filtered through a path of silica gel, eluting with $CHCl_3$–MeOH (85:15). The solvent was evaporated and the residue was treated overnight at room temperature with methanol saturated with ammonia. Evaporation of the solvent yielded a solute, which was crystallized from MeOH: 1.55 g (3.55 mmol, 59%); mp 242–243 °C dec; MS, *m/e* 437 (M^+), 151 (B + H^+), 131 (S-tosyl-H); UV (MeOH) λ_{max} 254 nm (ϵ 14900); ^1H NMR (DMSO-d_6) δ 2.31 (s, CH_3), 3.57 (m, H-5′, H-5″), 3.97 (m, H-4′), 4.27 (m, H-3′), 5.19 (OH), 5.36 (dd, J = 5.3 and 7.0 Hz, H-2′), 5.83 (d, J = 7.0 Hz, H-1′), 5.93 (OH), 6.37 (br s, NH_2), 7.12 (d) and 7.46 (d) (aromatic H, J = 8.0 Hz), 7.70 (s, H-8).

B. A suspension of 28.3 g (100 mmol) of guanosine (dried overnight in vacuo at 80 °C on P_2O_5) and 49.8 g (200 mmol) of dibutyltin oxide in a mixture of 750 mL of anhydrous MeOH and 600 mL of dimethylformamide was stirred for 4 days at room temperature and refluxed for 3 h. The reaction mixture was cooled in an ice bath, and 210 mL of triethylamine and 286 g (1.5 mmol) of tosyl chloride were added. After stirring for 15 min, the clear solution was evaporated, coevaporated with pyridine, and dissolved in a mixture of pyridine–acetic anhydride (10:1, 1 L). The reaction mixture was kept overnight at room temperature and 100 mL of MeOH was added. The reaction was stirred for 1 h, evaporated, and diluted with $CHCl_3$ (1 L). The suspension was filtered, and the filtrate was washed with H_2O (3 × 200 mL), dried, and evaporated. The residue was treated with ammonia in methanol (800 mL) overnight at room temperature and evaporated. When the residue was diluted with $CHCl_3$–MeOH (9:1), a crystalline precipitate occurs, which was isolated and washed with MeOH and Et_2O. The mother liquor was further purified by column chromatography ($CHCl_3$–MeOH 85:15); total yield 26.8 g (61.4 mmol, 61%).

9-(2-Deoxy-β-D-*threo*-pentofuranosyl)guanine (11c). One hundred milliliters of a 1 M solution of lithium triethylborohydride in tetrahydrofuran was added to a solution of 4.37 g (10 mmol) of 2′-*O*-tosylguanosine in 70 mL of dimethyl sulfoxide. The reaction mixture was kept at room temperature overnight, H_2O (5 mL) was added, and the reaction mixture was concentrated on a rotary evaporator to a viscous oil. The yellow oil was applied on a Dowex 1 X-2 (AcO^-) column (30 cm × 3 cm) which was eluted first with H_2O (1 L) and then with 5% of acetic acid in H_2O. The UV-absorbing fractions were pooled and evaporated to give 2.65 g (9.3 mmol, 93%) of the title compound: UV (MeOH) λ_{max} 254 nm (ϵ 13500); ^1H NMR (DMSO-d_6) δ 2.20 (m, 1 H, H-2′), 2.77 (m, 1 H, H-2″), 3.75 (m, 2 H, H-5′, H-5″), 3.92 (m, 1 H, H-4′), 4.40 (m, 1 H, H-3′), 6.10 (dd, 1 H, J = 2.0 and 8.1 Hz, H-1′), 6.64 (br s, 2 H, NH_2), 8.06 (s, 1 H, H-8). Anal. ($C_{10}H_{13}N_5O_4 \cdot H_2O$) C, H, N.

9-(5-*O*-Benzoyl-2-deoxy-β-D-*threo*-pentofuranosyl)guanine (12b). To a solution of 2.67 g (10 mmol) of 9-(2-deoxy-β-D-*threo*-pentofuranosyl)guanine, dried by coevaporation with dimethylformamide, in 150 mL of anhydrous dimethylformamide (by heating to 60 °C and cooled to room temperature) were added 5 mL of triethylamine and 50 mg of 4-dimethylaminopyridine. A solution of 2.5 g (11 mmol) of benzoic anhydride in 25 mL of anhydrous dimethylformamide was added dropwise to this solution over a period of 2 h with stirring. The reaction mixture was stored for 5 h at room temperature and for 60 h at 4 °C. The solvent was evaporated and the mixture was purified by column chromatography on silica ($CHCl_3$–MeOH 9:1) and crystallized from H_2O–tetrahydrofuran: 2.26 g (6.1 mmol, 61%); mp 253 °C (darkening); UV (MeOH) λ_{max} 251 nm (ϵ 14100); ^1H NMR (DMSO-d_6) δ 2.1–3.0 (m, 2 H, H-2′, H-2″), 4.13–4.80 (m, 4 H, H-3′, H-4′, H-5′, H-5″), 5.75 (d, 1 H, 3′-OH), 6.13 (dd, 1 H, J = 2.4 and 8.6 Hz, H-1′), 6.51 (br s, 2 H, NH_2), 7.40–7.70 (m) and 7.90–8.10 (m) (phenyl), 8.03 (s, 1 H, H-8).

3′-Fluoro-2′,3′-dideoxyguanosine (3a). To a suspension of 740 mg (2 mmol) of 9-(5-*O*-benzoyl-2-deoxy-β-D-*threo*-pentofuranosyl)guanine in 100 mL of dichloromethane was added 1 mL of DAST and the mixture was stirred for 2 h at room temperature. Sodium bicarbonate (1 g) was added, the solvent was evaporated, and the mixture was purified by column chromatography ($CHCl_3$–MeOH 9:1), giving 250 mg (0.7 mmol, 35%) of 5′-*O*-benzoyl-3′-fluoro-2′,3′-dideoxyguanosine (**13b**): ^1H NMR (DMSO-d_6) δ 2.40–3.30 (m, H-2′, H-2″), 4.53 (m, H-4′, H-5′, H-5″), 5.66 (m, $J_{3',F}$ = 53 Hz, H-3′), 6.25 (dd, J = 6.12 and 7.5 Hz, H-1′), 6.53 (br s, NH_2), 7.40–7.73 (m, phenyl), 7.82–8.14 (m, phenyl and H-8). The compound was further identified after debenzoylation with 10 mL of methanol saturated with ammonia at room temperature overnight followed by evaporation of the solvent. A crystalline precipitate [20 mg from 115 mg (0.31 mmol) starting material] was collected and the mother liquor was further purified by column chromatography ($CHCl_3$–MeOH 8:2) which gave another 40 mg of crystalline material: total yield 0.223 mmol, 72%: mp 255 °C (darkening); UV (MeOH) λ_{max} 253 nm (ϵ 14600); ^1H NMR (DMSO-d_6) δ a multiplet centered around 2.80 (H-2′, H-2″), 3.57 (m, H-5′, H-5″), 4.16 (m, $J_{4',F}$ = 27.0 Hz, H-4′), 5.11 (5′-OH), 5.39 (m, $J_{3',F}$ = 53.0 Hz, H-3′), 6.17 (dd, H-1′), 6.50 (br s, NH_2), 7.94 (s, H-8); ^{13}C NMR (DMSO-d_6) δ 37.0 (d, J = 21.0 Hz, C-2′), 60.95 (d, J = 11.0 Hz, C-5′), 82.7 (s, C-1′), 85.6 (d, J = 22.6 Hz, H-4′), 95.0 (d, J = 173.3 Hz, C-3′), 116.8 (C-5), 135.3 (C-8), 151.0 (C-4), 153.8 (C-2), 156.7 (C-6). Anal. ($C_{10}H_{12}N_5O_3F \cdot {}^1/_2H_2O$) C, H, N.

1-(3-Cyano-3-deoxy-β-D-arabinofuranosyl)thymine (6a). A solution of 4.85 mg (10 mmol) of 1-(5-*O*-trityl-2,3-epoxy-β-D-lyxofuranosyl)thymine[48] (**4b**) and 7.8 g (50 mmol) of tetraethylammonium cyanide in 100 mL of anhydrous dimethylformamide was stirred for 2 days at room temperature. The solvent was evaporated. The oily residue was diluted with H_2O (200 mL), neutralized with acetic acid, and extracted twice with $CHCl_3$ (2 × 200 mL). The combined organic layer was dried, evaporated, and purified by column chromatography to give 770 mg (1.3 mmol, 26% yield) of 1-(3-cyano-3-deoxy-5-*O*-trityl-β-D-arabinofuranosyl)thymine: UV (MeOH) λ_{max} 264 nm; ^1H NMR ($CDCl_3$) δ 1.62 (s, CH_3), 3.10–3.68 (m, H-3′, H-5′, H-5″), 4.16 (m, H-4′), 4.97 (dd, H-2′, $J_{1',2'}$ = 4.8 Hz, $J_{2',3'}$ = 5.7 Hz), 6.16 (d, H-1′), 7.40 (m, trityl and H-6). This compound was detritylated with 80% of acetic acid at 100 °C for 20 min. After evaporation and coevaporation with toluene, the title compound was purified by column chromatography ($CHCl_3$–MeOH 95:5) and crystallized from MeOH: 312 mg (1.17 mmol, 90%); mp 243 °C; IR (KBr) 2250 (CN) cm^{-1}; UV (MeOH) λ_{max} 267 nm (ϵ 10500); ^1H NMR (DMSO-d_6) δ 1.77 (d, CH_3), 3.20 (m, H-3′), 3.65 (m, H-5′, H-5″), 4.12 (m, H-4′), 4.80 (dd, J = 6.3 and 7.9 Hz, H-2′), 6.10 (d, J = 6.3 Hz, H-1′), 7.55 (d, H-6). Anal. ($C_{11}H_{13}N_3O_5$) C, H, N.

1-(3-Deoxy-3-ethynyl-β-D-arabinofuranosyl)thymine (6b). The reaction was carried out on a 5-mmol amount and the modus operandi was that described by Ashwell et al.[49] The yield of crystalline material was 74%. The only difference is that the 5′-O-tritylated compound was purified by column chromatography with $CHCl_3$–MeOH (97:3) as eluent and the detritylated compound with $CHCl_3$–MeOH (95:5). **6b**: mp 200 °C; MS, *m/e* 266 (M^+); UV (MeOH) λ_{max} 267 nm (ϵ 10400); IR (KBr) 2120 (C≡CH) (weak) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.74 (s, CH_3), 2.91 (m, H-3′), 3.16 (d, J = 2.2 Hz, C≡CH), 3.60–3.96 (m, H-4′, H-5′, H-5″), 4.42 (m, H-2′), 5.24 (5′-OH), 5.86–6.12 (m, 2′-OH and H-1′, $J_{1',2'}$ = 6.15 Hz), 7.68 (s, H-6), 11.2 (br s, NH). Anal. ($C_{12}H_{14}N_2O_5$) C, H, N.

6-*O*-[(*p*-Nitrophenyl)ethyl]-3′-*O*-mesyl-2′-deoxyguanosine (8). To a mixture of N^2,5′-*O*-bis(monomethoxytrityl)-2′-deoxyguanosine[33] (5 mmol, 4.06 g), triphenylphosphine (2.96 g, 7.5 mmol), and (*p*-nitrophenyl)ethanol (1.88 g, 7.5 mmol) in 150 mL of dichloromethane was added 1.8 mL (7.5 mmol) of diethylazadicarboxylate and the reaction was stirred for 5 h at room

temperature. The reaction mixture was evaporated and purified by column chromatography [(1) $CHCl_3$, (2) $CHCl_3$–MeOH 99.5:0.5]. However, the pooled fractions still contain bis(ethoxycarbonyl)hydrazine as revealed by ^1H NMR. Therefore, the residue, after evaporation of the solvent, was dissolved in pyridine (100 mL), containing 1.2 mL (15 mmol) of methanesulfonyl chloride, and the mixture was kept overnight at room temperature. After addition of MeOH (5 mL), the solvent was evaporated and the residue was partitioned between $CHCl_3$ (150 mL) and H_2O (150 mL). The organic layer was washed with H_2O (2 × 150 mL), dried, evaporated, and coevaporated with toluene. TLC ($CHCl_3$–MeOH 99:1) revealed one compound (UV detection), which was purified by column chromatography with $CHCl_3$ as eluent, 5′-*O*,N^2-bis(monomethoxytrityl)-6-*O*-[(*p*-nitrophenyl)ethyl]-3′-*O*-mesyl-2′-deoxyguanosine (**7**): UV (MeOH) λ_{max} 261 nm (ε 25 000), 282 (21 800); ^1H NMR ($CDCl_3$) δ 2.64 (m, H-2′, H-2″), 2.97 (s, CH_3SO_2), 3.16 (t, $CH_2C_6H_4$), 3.38 (m, H-5′, H-5″), 3.73 and 3.75 (2 × s, 2 × CH_3O), 4.29 (m, H-4′), 4.45 (t, CH_2O), 5.30 (m, H-3′), 6.10 (br t, H-1′), 6.23 (br s, NH), 6.65, 6.74, and 6.84 (ortho H of *p*-methoxyphenyl), 7.2 (m, trityl and d of (*p*-nitrophenyl)ethyl), 7.68 (s, H-8), 8.08 (d, (*p*-nitrophenyl)ethyl). This compound was treated with 80% of acetic acid (100 mL) for 6 h at room temperature, and after evaporation of the solvent, it was purified by column chromatography ($CHCl_3$–MeOH 97.5:2.5, $CHCl_3$–MeOH 95:5) and crystallized from MeOH. The total yield is 1.48 g (3.05 mmol, 61%). **8**: mp 177–179 °C; UV (MeOH) λ_{max} 282 nm (ε 18 700), 252 (15 700); ^1H NMR (DMSO-d_6) δ H-2′ and H-2″ are partially hidden by DMSO, the multiplet is centered at 2.80 ppm, 3.26 (t, $CH_2C_6H_4$), 3.32 (s, 3 H, CH_3SO_2), 3.62 (m, 2 H, H-5′, H-5″), 4.19 (m, 1 H, H-4′), 4.69 (t, 2 H, CH_2O), 5.25 (br t, 5′-OH), 5.36 (m, 1 H, H-3′), 6.25 (dd, 1 H, J = 5.7 and 8.3 Hz, H-1′), 6.49 (2 H, NH_2), 7.62 (d) and 8.17 (d) (2 × 2 H, phenyl), 8.10 (s, 1 H, H-8). Anal. ($C_{19}H_{22}N_6O_8S$) C, H, N.

2′,3′-Dideoxy-2′,3′-didehydroguanosine (9). A 494-mg (1 mmol) portion of **8** was added to a solution of 340 mg (3 mmol) of potassium *tert*-butylate in anhydrous dimethyl sulfoxide (10 mL). The reaction mixture was stirred for 1 h at room temperature, poured into MeOH (100 mL), and neutralized with Amberlite IRC 50 (H^+). After filtration of the resin and evaporation of MeOH, the residue was triturated two times with Et_2O (250 mL) and decanted (to remove dimethyl sulfoxide). The residue was applied on a XAD-2 (300–1000 mesh) column, which was washed with H_2O and eluted with H_2O–MeOH (65:35). The UV-positive fractions were collected and evaporated to dryness: 195 mg (0.78 mmol, 78% yield). The product was dried in vacuo at room temperature for 2 days. The reaction in dimethylformamide with sodium methanolate followed the same procedure except that the solvent (DMF) was evaporated in vacuo. **9**: UV (H_2O) λ_{max} 250 nm (ε 15 400), 270 (sh); UV (0.1 N NaOH) λ_{max} 266 nm (ε 13 500), 257 (13 100); ^1H NMR (DMSO-d_6) δ 3.56 (m, 2 H, H-5′, H-5″), 4.88 (m, 1 H, H-4′), 4.91 (t, 1 H, 5′-OH), 6.07 (dm, 1 H, H-3′), 6.43 (dt, 1 H, H-2′), 6.64 (NH_2), 6.72 (m, 1 H, H-1′), 7.70 (s, 1 H, H-8), 10.8 (br s, 1 H, NH); ^{13}C NMR (DMSO-d_6) δ 63.0 (C-5′), 87.3 and 87.9 (C-1′ and C-4′), 116.6 (C-5), 125.5 (C-3′), 134.3 (C-2′), 135.1 (C-8), 151.0 (C-4), 154.4 (C-2), 157.6 (C-6). Anal. ($C_{10}H_{11}N_5O_3 \cdot H_2O$) C, H, N.

3′-(Acetylthio)-2′,3′-dideoxythymidine (15b). A solution of 565 mg (1 mmol) of 1-(5-*O*-trityl-3-*O*-mesyl-2-deoxy-β-D-*threo*-pentofuranosyl)thymine[42] and 456 mg (4 mmol) of potassium thioacetate in 10 mL of dimethylformamide was heated for 2 h at 100 °C. After the mixture was cooled to room temperature, the solvent was evaporated, and the residue was dissolved in $CHCl_3$ (100 mL) and washed two times with 100 mL of H_2O. The organic layer was dried and purified by column chromatography ($CHCl_3$). ^1H NMR shows the presence of an acetyl group and the reversed configuration at C-3′: ^1H NMR ($CDCl_3$) δ 1.48 (d, 3 H, CH_3), 2.31 (s, 3 H, CH_3CO), 2.20–2.68 (m, H-2′, H-2″), 3.44 (m, 2 H, H-5′, H-5″), 3.92–4.40 (m, 2 H, H-3′, H-4′), 6.23 (dd, 1 H, J = 5.8 and 6.2 Hz, H-1′), 7.20–7.56 (m, trityl), 7.63 (d, 1 H, H-6), 9.64 (br s, 1 H, NH). The product was detritylated by heating for 15 min at 100 °C in 80% of acetic acid and purified by column chromatography [(1) $CHCl_3$, (2) $CHCl_3$–MeOH 97:3)]: 200 mg (66%, 0.66 mmol) as a foam. We were not able to crystallize this compound. **15b**: UV (EtOH) λ_{max} 266 nm (ε 9900); ^1H NMR ($CDCl_3$) δ 1.92 (d, 3 H, CH_3), 2.38 (s, 3 H, CH_3), 2.50 (m, H-2′, H-2″), 3.34 (br t, 5′-OH), 3.94 (m, 4 H, H-3′, H-4′, H-5′, H-5″), 6.14 (dd, 1 H, J = 4.4 and 6.4 Hz, H-1′), 7.81 (d, J = 1.3 Hz, H-6), 9.57 (br s, NH). Anal. ($C_{12}H_{16}N_2O_5S$) C, H, N.

3′-(*p*-Tolylthio)-2′,3′-dideoxythymidine (15e). To a solution of 1.44 g (4.5 mmol) of 1-(3-*O*-mesyl-2-deoxy-β-D-*threo*-pentofuranosyl)thymine[42] and 5.6 g (45 mmol) of thiocresol in 25 mL of dimethylformamide was added 25 mL of EtOH containing 1.4 g of sodium ethanolate (20 mmol). The mixture was stirred for 2 h at 80 °C, evaporated, and diluted with $CHCl_3$ (100 mL) and H_2O (100 mL). The emulsion was neutralized with 1 N HCl, and the $CHCl_3$ layer was dried, evaporated, and treated with acetic acid (80%) for 20 min at 100 °C. After evaporation and column chromatography ($CHCl_3$–MeOH 98:2), the title compound was isolated as an oil in 52% yield (856 mg). We were not able to crystallize the compound. **15e**: MS, m/e 348 (M^+); UV (MeOH) λ_{max} 264 nm (ε 10 500); ^1H NMR ($CDCl_3$) δ 1.85 (s, 3 H, CH_3), 2.32 (s, 3 H, CH_3), 2.44 (m, H-2′, H-2″), 3.60–4.08 (m, H-3′, H-4′, H-5′, H-5″), 6.08 (t, J = 6.1 Hz, H-1′), 7.15 (d) and 7.38 (d) (J = 8.3 Hz, aromatic H), 7.60 (s, 1 H, H-6). Anal. ($C_{17}H_{20}N_2O_4S \cdot 0.15CHCl_3$) C, H, N.

3′-*O*-[Phenoxy(thiocarbonyl)]-2′-deoxythymidine (15f). 5′-*O*-(Monomethoxytrityl)-3′-*O*-[phenoxy(thiocarbonyl)]-2′-deoxythymidine (650 mg, 1 mmol), prepared from 5′-*O*-(monomethoxytrityl)-2′-deoxythymidine according to the procedure of Robins,[41] was dissolved in 10 mL of $CHCl_3$–MeOH (4:1) containing 2% of *p*-toluenesulfonic acid and kept at room temperature for 15 min. The reaction mixture was neutralized with 0.5 N NaOH, evaporated, and purified by column chromatography ($CHCl_3$–MeOH 95:5), yielding 280 mg (0.74 mmol, 74%) of 3′-*O*-[phenoxy(thiocarbonyl)]-2′-deoxythymidine: MS, m/e 127 ($B + 2H^+$), 126 ($B + H^+$), 253 (carbohydrate part); UV (MeOH) λ_{max} 264 nm (ε 10 500); ^1H NMR (DMSO-d_6) δ 1.79 (s, CH_3), 2.50 (m, H-2′, H-2″), 3.73 (m, H-5′, H-5″), 4.27 (m, H-4′), 5.31 (5′-OH), 5.73 (m, H-3′), 6.25 (t, J = 7 Hz, H-1′), 7.14–7.62 (m, phenyl), 7.78 (s, H-6). Anal. ($C_{17}H_{18}N_2O_6S$) C, H, N.

Antiviral Test Procedures. The HTLV-III_B strain of HIV was used throughout all experiments. The virus was prepared from the culture supernatant of a persistently HTLV-III_B-infected HUT-78 cell line. The antiviral assays were based on the protection of HIV-infected MT-4 cells against virus-induced cytopathogenicity and run in parallel with the cytotoxicity assays aimed at establishing the toxicity of the compounds for uninfected MT-4 cells. These assay procedures have been described previously.[57]

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Registry No. 1a, 30516-87-1; **1b**, 66323-44-2; **1c**, 66323-46-4; **1d**, 84472-85-5; **1e**, 84472-89-9; **1f**, 115913-78-5; **1g**, 115913-79-6; **1h**, 87190-79-2; **1i**, 108895-46-1; **2a**, 115913-80-9; **2c**, 115941-55-4; **2e**, 6998-75-0; **2f**, 73-03-0; **2g**, 110143-00-5; **3a**, 92562-88-4; **3b**, 114753-53-6; **3c**, 41107-56-6; **3d**, 25526-93-6; **4g**, 40110-98-3; **4b**, 115913-84-3; **5**, 2627-64-7; **6a**, 115913-83-2; **6b**, 115913-85-4; **7**, 115913-87-6; **8**, 115913-86-5; **9**, 53766-80-6; **10**, 115913-76-3; **11a**, 13276-53-4; **11b**, 116002-29-0; **11c**, 116002-28-9; **12a**, 115913-73-0; **12b**, 115913-75-2; **13a**, 115913-74-1; **13b**, 115913-82-1; **13c**, 115913-77-4; **14a**, 104218-44-2; **14b**, 94919-65-0; **15b**, 115913-88-7;

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15d, 107601-08-1; 15e, 115913-89-8; 15f, 115913-90-1; 16a, 118-00-3; 17, 115913-81-0; 18, 69370-84-9; 4-$(NO_2)C_6H_4CH_2CH_2OH$, 100-27-6; 4-MeC_6H_4SH, 106-45-6; 5′-O-trityl-3′-azido-2′,3′-dideoxyuridine, 84472-84-4; 5′-O-trityl-3′-azido-2′,3′-dideoxythymidine, 29706-84-1; 1-(3-cyano-3-deoxy-5-O-trityl-β-D-arabinofuranosyl)thymine, 115941-56-5; N^2,5′-O-bis(monomethoxytrityl)-2′-deoxyguanosine, 84870-95-1; 5′-O-(monomethoxytrityl)-3′-O-[phenoxy(thiocarbonyl)]-2′-deoxythymidine, 115913-91-2.

2(1*H*)-Quinolinones with Cardiac Stimulant Activity. 1. Synthesis and Biological Activities of (Six-Membered Heteroaryl)-Substituted Derivatives

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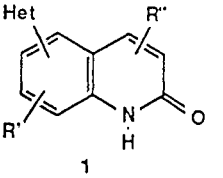
A series of (six-membered heteroaryl)-substituted 2(1*H*)-quinolinones (1) was synthesized, and structure–activity relationships for cardiac stimulant activity were determined. Most compounds were prepared by acidic hydrolysis of a heteroaryl-2-methoxyquinoline obtained by palladium-catalyzed cross-coupling methodology. Direct reaction of a pyridinylzinc reagent with a 6-haloquinolinone also proved successful. In anesthetized dogs, 6-pyridin-3-yl-2(1*H*)-quinolinone (**3**; 50 μg/kg) displayed greater inotropic activity (percentage increase in d*P*/d*t* max) than positional isomers (**2**, **4–6**), and potency was maintained with either mono- (**13**, **15**) or di- (**16**) alkylpyridinyl substituents. Introduction of a 4- (**24**) or 7- (**25**) methyl group into **3** reduced inotropic activity, whereas the 8-isomer (**26**) proved to be the most potent member of the series. Compound **26** and the 2,6-dimethylpyridinyl analogue (**27**) were approximately 6 and 3 times more potent than milrinone. Several quinolinones displayed positive inotropic activity (decrease in QA interval) in conscious dogs after oral administration (1 mg/kg), and **26**, **27** were again the most potent members of the series. Compound **27** (0.25, 0.5, 1.0 mg/kg po) demonstrated dose-related cardiac stimulant activity, which was maintained for at least 4 h. No changes in heart rate were observed. Compounds **3**, **4**, **26**, and **27** also selectively stimulated the force of contraction, rather than heart rate, in the dog heart–lung preparation. For a 50% increase in d*P*/d*t* max with **27**, heart rate changed by less than 10 beats/min. In norepinephrine contracted rabbit femoral artery and saphenous vein, **27** produced dose related (5×10^{-7} to 5×10^{-4} M) vasorelaxant activity. The combined cardiac stimulant and vasodilator properties displayed by **27**, coupled with a lack of effect on heart rate, should be beneficial for the treatment of congestive heart failure.

Congestive heart failure (CHF) is a major health problem[1] of increasing incidence, due to an aging population and improved treatment of other cardiovascular disorders.[2] Current therapy for CHF relies heavily on digitalis, diuretics, and vasodilators, but annual mortality rates between 30 and 50% are still commonly observed.[3] Consequently, there is strong clinical demand for improved agents, particularly those that correct the major hemodynamic derangements characteristic of CHF.[4] Over the last few years, a variety of novel inodilator agents have been described,[5] and clinical evaluation of several of these drugs is currently in progress.[6-8] Although these compounds belong to several diverse chemical series,[5] two common structural features appear to be important for inodilator activity—a cyclic carboxamide function and an appropriately positioned heteroaromatic system. These individual pharmacophores can also be expressed in the 2(1*H*)-quinolinone system (**1**) where a high degree of



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conformational constraint allows an accurate assessment of the optimum location of the carboxamide and heterocyclic moieties. Moreover, since the overall topography of series **1** is subtly different from milrinone and CI-930, for example, then a modified pharmacological/pharmacodynamic profile might also be expected. This paper describes our initial studies with a novel series of 2-(1*H*)-quinolinones substituted with various six-membered heteroaryl systems.[9] These compounds display marked cardiac stimulant activity, with little effect on heart rate, and may be useful for the treatment of CHF.

Chemistry. The heteroaryl 2-methoxyquinoline intermediates (Table III) required for the preparation of most of the various mono- and disubstituted 2(1*H*)-quinolinones listed in Tables I and II were synthesized following routes A, B, and C outlined in Scheme I.[10] Thus,

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