Synthesis and Antiviral Activity of Several 2,5′-Anhydro Analogues of 3′-Azido-3′-deoxythymidine, 3′-Azido-2′,3′-dideoxyuridine, 3′-Azido-2′,3′-dideoxy-5-halouridines, and 3′-Deoxythymidine against Human Immunodeficiency Virus and Rauscher-Murine Leukemia Virus

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Several 2,5′-anhydro analogues of 3′-azido-3′-deoxythymidine (AZT), 3′-azido-2′,3′-dideoxyuridine (AZU), 3′-azido-2′,3′-dideoxy-5-bromouridine, 3′-azido-2′,3′-dideoxy-5-iodouridine, and 3′-deoxythymidine and the 3′-azido derivative of 5-methyl-2′-deoxyisocytidine have been synthesized for evaluation as potential anti-HIV (human immunodeficiency virus) agents. These 2,5′-anhydro derivatives, compounds **13–17**, demonstrated significant anti-HIV-1 activity with IC_{50} values of 0.56, 4.95, 26.5, 27.1, and 48 μM, respectively. Compared to that of the parent compounds AZT and AZU, the respective 2,5′-anhydro analogues, compounds **13** and **14**, were somewhat less active. Whereas AZT was cytotoxic with a $TCID_{50}$ of 29 μM, the toxicity of the 2,5′-anhydro derivative of AZT, compound **13**, was reduced considerably to a $TCID_{50}$ value of >100 μM. The 2,5′-anhydro analogue of 5-methyl-2′-deoxyisocytidine also demonstrated anti-HIV-1 activity with an IC_{50} value of 12 μM. These compounds were also evaluated against Rauscher-Murine leukemia virus (R-MuLV) in cell culture. Among them, AZT, 3′-azido-2′,3′-dideoxy-5-iodouridine, 3′-azido-2′,3′-dideoxy-5-bromouridine, and 2,5′-anhydro-3′-azido-3′-deoxythymidine (**13**) were found to be most active, with IC_{50} values of 0.023, 0.21, 0.23, and 0.27 μM, respectively.

3′-Azido-3′-deoxythymidine (AZT, Zidovudine) was first synthesized by Horwitz et al.[2] and later by a modification of this procedure by Lin and Prusoff.[3] 3′-Azido-2′,3′-dideoxyuridine (**2**, AZU) was first synthesized by Lin[4] and, subsequently, Schinazi et al.[5] reported that AZU (CS-87) possesses significant anti-HIV activity. References to many biochemical and clinical studies of AZT have been presented by Lin et al.[6] and Hirsch.[7]

AZT has been reported to be of marked benefit in the therapy of acquired immunodeficiency syndrome (AIDS), but it is significantly toxic to many individuals receiving this drug.[8,9] To circumvent this problem, various 3′-azido analogues of pyrimidine deoxyribonucleosides were synthesized and a number of them were found to be active against HIV-1 in cell culture; however, none were more active than AZT.[6]

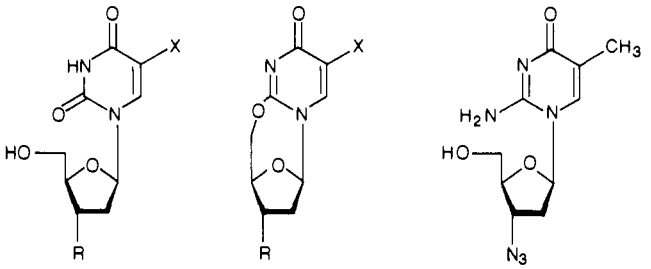
In an attempt to increase the antiviral activity and decrease the cytotoxicity of AZT, AZU, 3′-azido-2′,3′-dideoxy-5-bromouridine (**3**, 3′-N_3-BUdR), 3′-azido-2′,3′-dideoxy-5-iodouridine (**4**, 3′-N_3-IUdR), and 3′-deoxythymidine (**5**), we synthesized the corresponding 2,5′-anhydro derivatives of these compounds, as well as the 3′-azido analogue of 5-methyl-2′-deoxyisocytidine.

The present report describes the syntheses of these compounds and some of their physical properties, as well as their activity against HIV-1 and R-MuLV in cell culture.

Chemistry

The 2,5′-anhydro analogues of AZT, AZU, 3′-azido-2′,3′-dideoxy-5-bromouridine, 3′-azido-2′,3′-dideoxy-5-iodouridine, and 3′-deoxythymidine (compounds **13–17**) and the 3′-azido derivative of 5-methyl-2′-deoxyisocytidine (compound **18**) (Chart I) have been synthesized for evaluation as potential anti-HIV agents. The starting parent compounds, **1–5** were prepared by the methodology previously described.[2-4,10-12] Our approach for the syntheses of the desired compounds **13–18** is outlined in Scheme I. Tosylation of compounds **1–5** with *p*-toluenesulfonyl chloride in anhydrous pyridine at room temperature for 1–3 days gave the corresponding tosylates **6–10**. Compounds **6** and **7** were then reacted with NaI in 2-butanone under reflux for 6 h[13] to produce the 5′-iodo derivatives **11** and **12**. Treatment of compounds **11** and **12** with silver acetate[14] in refluxing MeCN for 1 h afforded the desired

Chart I. Structure Formulas of Various 2,5′-Anhydro-3′-azido Analogues of Pyrimidine Deoxyribonucleosides and the 3′-Azido Derivative of 5-Methyl-2′-deoxyisocytidine



1: X = CH_3, R = N_3
2: X = H, R = N_3
3: X = Br, R = N_3
4: X = I, R = N_3
5: X = CH_3, R = H

13: X = CH_3, R = N_3
14: X = H, R = N_3
15: X = Br, R = N_3
16: X = I, R = N_3
17: X = CH_3, R = H

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(2) Horwitz, J. P.; Chua, J.; Noel, M. *J. Org. Chem.* **1964**, *29*, 2076.
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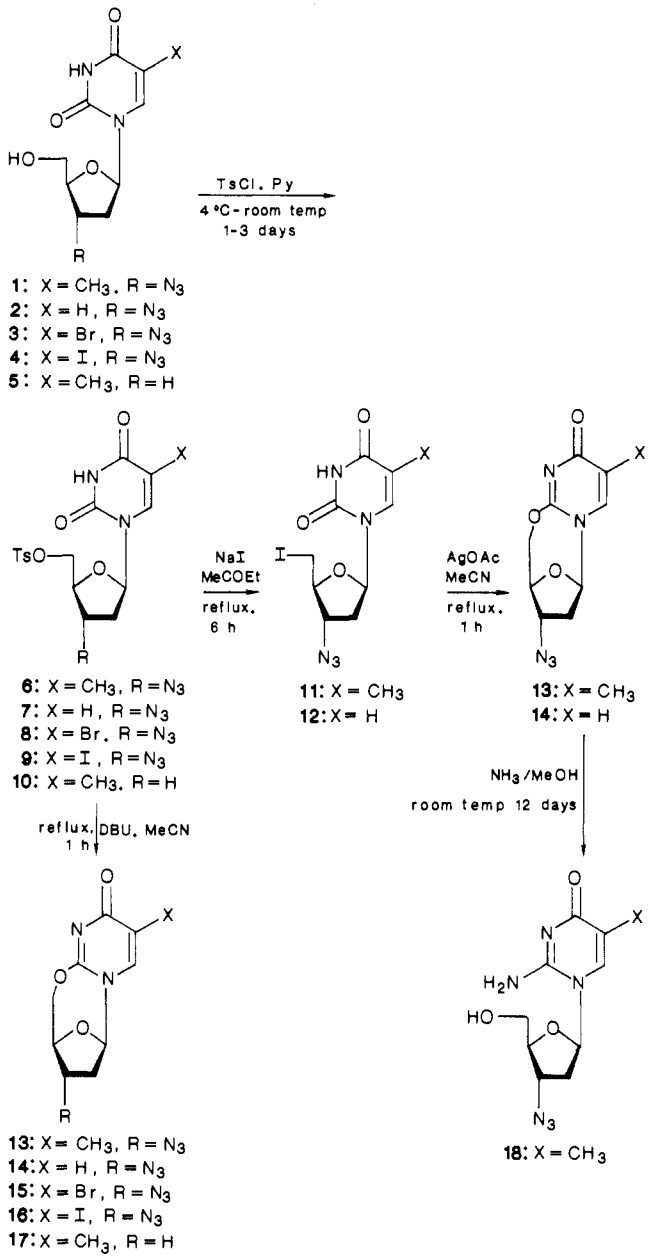
Scheme I



2,5′-anhydro analogues **13** and **14**. The reaction time was critical, as prolonged heating resulted in decreased yield of the products. Ammonalysis of compound **13** with methanolic ammonia in a pressure bottle at room temperature for 12 days yielded the 3′-azido derivative of 5-methyl-2′-deoxyisocytidine, compound **18**. The 2,5′-anhydro derivatives **13–17** were also synthesized by treating the tosylates **6–10** with 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU)[15] in refluxing MeCN for 1 h. This one-step reaction was easier and more convenient; therefore, it was the reaction of choice. These compounds were characterized by NMR, UV, MS, and IR spectra and elemental analysis as described in the Experimental Section.

Antiviral Activity

The compounds were evaluated against HIV-1, and the antivirial activity was expressed as the concentration (μM) that inhibits viral replication by 50%. In general, the parent compounds AZT (**1**), AZU (**2**), 3′-azido-2′,3′-dideoxy-5-bromouridine (**3**), and 3′-azido-2′,3′-dideoxy-5-iodouridine (**4**) were approximately 1.8–6 times more active against HIV-1 than their corresponding 2,5′-anhydro derivatives (Table I). The 2,5′-anhydro analogues of AZT, AZU, 3′-azido-2′,3′-dideoxy-5-bromouridine, and 3′-azido-2′,3′-dideoxy-5-iodouridine, compounds **13–16**, have significant antiviral activity with respective IC_{50} values of 0.56, 4.95, 28, and 27.1 μM. The 2,5′-anhydro-2′,3′-dideoxythymidine (**17**) was approximately 126 times less active against HIV-1 in vitro than the parent compound 2′,3′-dideoxythymidine (**5**, d2T), with respective IC_{50} values of 0.38 and 48 μM. 5-Methyl-3′-azido-2′,3′-dideoxyisocytidine (**18**) also demonstrated anti-HIV-1 activity, with an IC_{50} of 12 μM (Table I). The host-cell cytotoxicity of the 2,5′-anhydro derivative of AZT, compound **13**, had a $TCID_{50}$ of >100 μM, whereas the parent compound, AZT, was cytotoxic with a $TCID_{50}$ of 29 μM. The 2,5′-anhydro derivatives, compounds **15** and **16**, had $TCID_{50}$ values of 168 and 194 μM, respectively, and hence were somewhat less cytotoxic than the parent compounds, which had $TCID_{50}$ values of 127 and 153 μM.

Table I. Effect of Various 2,5′-Anhydro Nucleoside Analogues on the Replication of HIV-1 and R-MuLV in Vitro

	HIV-1			R-MuLV		
compd	IC_{50}[a]	$TCID_{50}$[b]	$TCID_{50}/IC_{50}$	IC_{50}[a]	$TCID_{50}$[b]	$TCID_{50}/IC_{50}$
1	0.1	29	290	0.023	>400	17391
2	2.76	166	60	138	>395	3
3	8.73	127	15	0.23	>301	1309
4	12.6	153	12	0.21	>264	1257
5	0.38	240	631	>442	>442	1
13	0.56	>100	179	0.27	>400	1481
14	4.95	>100	20	nd[c]	nd[c]	nd[c]
15	28	168	6	16.1	>319	20
16	27.1	194	7	17.7	>277	16
17	48	346	7	>480	>480	1
18	12	470	39	114	>376	3

[a] The IC_{50} values represent the drug concentration (in μM) required to inhibit 50% of viral replication. [b] The $TCID_{50}$ values represent the drug concentration (in μM) required to inhibit 50% of host cell replication. [c] Nd: not determined.

The anti-HIV-1 activity of these compounds follow the order AZT (**1**) > 2′,3′-dideoxythymidine (**5**) > 2,5′-anhydro-3′-azido-3′-deoxythymidine (**13**) > AZU > 2,5′-anhydro-3′-azido-2′,3′-dideoxyuridine (**14**) > 3′-azido-2′,3′-dideoxy-5-bromouridine (**3**) > 5-methyl-3′-azido-2′,3′-dideoxyisocytidine (**18**) ≃ 3′-azido-2′,3′-dideoxy-5-iodouridine (**4**) > 2,5′-anhydro-3′-azido-2′,3′-dideoxy-5-bromouridine (**15**) ≃ 2,5′-anhydro-3′-azido-2′,3′-dideoxy-5-iodouridine (**16**) > 2,5′-anhydro-2′,3′-dideoxythymidine (**17**).

These compounds were also evaluated against Rauscher–Murine leukemia virus (R-MuLV) in vitro. Among them, AZT, 3′-azido-2′,3′-dideoxy-5-iodouridine (**4**), 3′-azido-2′,3′-dideoxy-5-bromouridine, and 2,5′-anhydro-3′-azido-3′-deoxythymidine (**13**) were found to be most active, with IC_{50} values of 0.023, 0.21, 0.23, and 0.27 μM, respectively. These findings are summarized in Table I.

Stability Determinations and Discussion

The stabilities of those 2,5′-anhydro nucleoside derivatives (**13–17**) which displayed antiviral activity (Table I), as well as compound **18**, were determined at pH 7.5 and 2.0 (Table II). This was carried out in order to determine if the biological activity of this class of compounds was due to the anhydro compound itself, or whether hydrolysis of the 2,5′-anhydro linkage occurred in solution to yield the

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Table II. Stability of Various 2,5'-Anhydro Nucleoside Analogues at pH 7.4 and 2.0

compd	half-life ($t_{1/2}$) at 37 °C	
	pH 7.4	pH 2.0
13	>75 h (0)[a]	29.5 min (7.5)[a]
14	120 h (nd)[b]	11.3 min (18)[a]
15	60.5 h (3.5)[a]	14.3 min (3.5)[a]
16	95 h (3.8)[a]	12 min (3.8)[a]
17	84.3 h (nd)[b]	13.7 s (nd)[b]
18	>168 h (nd)[b]	140 min (nd)[b]

[a] The number in the parentheses indicates amount of parent compound formed at $t_{1/2}$ and is expressed as the percentage of the initial anhydro compound present. [b] nd refers to not detectable, when the limits of detection correspond to a peak size that is 0.5% of that of the anhydro compound initially present.

parent compound which, as previously shown, has antiviral activity.[6,10] The 2,5'-anhydro nucleosides were incubated in PBS (pH 7.4) or 0.01 N HCl (pH 2.0) and the amount of anhydro compound remaining, as well as the amount of parent compound and the pyrimidine base generated, was determined by reverse-phase HPLC as described in the Experimental Section. All of the compounds tested are stable at neutral pH, with $t_{1/2}$ values ranging from 60.5 h for 2,5'-anhydro-3'-azido-2',3'-dideoxy-5-bromouridine (**15**) to >168 h for 5-methyl-3'-azido-2',3'-dideoxyisocytidine (**18**). At low pH, however, the compounds displayed shorter and more variable half-lives, ranging from 13.7 s for 2,5'-anhydro-2',3'-dideoxythymidine (**17**) to 140 min for 5-methyl-3'-azido-2',3'-dideoxyisocytidine (**18**). Pyrimidine base formation was not detected from any of the compounds. Moreover, the anhydro compounds decomposed to yield 7.5% or less of the parent compound after 1 half-life at either neutral or acidic pH, except for 18% of 2,5'-anhydro-3'-azido-2',3'-dideoxyuridine (**14**), which was recovered as 3'-azido-2',3'-dideoxyuridine (**2**).

Further evidence to suggest that the conversion of the 2,5'-anhydro nucleosides to the parent (nonanhydro) compounds need not be required for biological activity is provided by the recent studies of Simpson et al.,[16] who investigated the effect of these compounds on mitochondrial DNA synthesis. At 25 μM, AZT inhibited the uptake of [^{3}H]dATP into mitochondrial DNA by 51%, whereas the 2,5'-anhydro analogue of AZT (**13**) inhibited DNA synthesis by only 5%. However, at a concentration of 25 μM, both An-N_3-IUdR (**16**) and An-N_3-BUdR (**15**) inhibited mitochondrial DNA synthesis more strongly than did the parent compounds (100% and 90% inhibition versus 12% and 5% for the respective parent compounds **4** and **3**). Whereas the lower activity of An-N_3-TdR relative to AZT could involve a partial conversion to AZT, the markedly greater activity of the halogenated anhydro compounds cannot be so explained. These compounds would not be expected to show greater biological activity if such activity was solely dependent upon conversion of an inactive prodrug to an active (parent) species. The molecular basis for the inhibitory activity of the halogenated anhydro nucleosides is the subject of current investigations by this laboratory.

These data suggest that the basis for the antiviral activity of the 2,5'-anhydro nucleoside analogues does not depend on a conversion of an "inactive" (anhydro) prodrug to yield an active (parent) species, but rather the anhydro compounds appear to be active per se. However, an intracellular cleavage of the 2,5'-anhydro linkage or other metabolic conversions cannot be ruled out and will be the subject of subsequent investigations.

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Experimental Section

Melting points were determined with a Thomas-Hoover Unimelt apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 500 MHz on a Brucker WM-500 spectrometer with Me_4Si as the internal reference. The UV spectra were recorded on a Beckman-25 spectrophotometer. IR spectra were taken on the Perkin-Elmer 21 spectrophotometer. The mass spectra (at 70 eV) were provided by Yale University Chemical Instrumentation Center. TLC was performed on EM precoated silica gel sheets containing a fluorescent indicator. Elemental analyses were carried out by the Baron Consulting Co., Orange, CT. Where analyses are indicated only by symbols of the elements, the analytical results obtained for those elements were within ±0.4% of the theoretical values.

3'-Azido-5'-*O*-(*p*-tolylsulfonyl)-3'-deoxythymidine (6). To a solution of 3'-azido-3'-deoxythymidine (**1**, 1 g, 3.8 mmol) in dry pyridine (10 mL) at 0 °C (ice bath) with stirring was added *p*-toluenesulfonyl chloride (1.5 g, 6 mmol). The reaction mixture was stirred overnight at room temperature and then slowly poured into 300 mL of ice–water with vigorous stirring. The resultant precipitate was collected by filtration, washed with ice–water, and dissolved in CH_2Cl_2 (30 mL). This solution was washed with water and the organic layer was separated with a separation funnel. The CH_2Cl_2 solution was then dried with anhydrous Na_2SO_4, evaporated to a small volume, passed through a short silica gel column, and eluted with more CH_2Cl_2. The solvent was evaporated to dryness in vacuo. The residue was crystallized from EtOH to yield 1.2 g (74%) of product: mp 123–125 °C (lit.[3] mp 126–128 °C); UV (MeOH) λ_{max} 264 nm (ϵ 10480), λ_{min} 240 nm; UV (0.01 N HCl) λ_{max} 267 nm (ϵ 10130), λ_{min} 244 nm; UV (0.01 N NaOH) λ_{max} 266 nm (ϵ 8370), λ_{min} 247; NMR (Me_2SO-d_6) δ 1.76 (s, 3 H, 5-CH_3), 2.27–2.37 (m, 1 H, 2'-H_A), 2.38–2.43 (m, 4 H, $C_6H_4CH_3$ and 2'-H_B), 3.92–3.94 (m, 1 H, 4'-H), 4.21–4.25 (m, 1 H, 5'-H_A), 4.29–4.32 (m, 1 H, 5'-H_B), 4.38–4.41 (m, 1 H, 3'-H), 6.06 (t, 1 H, 1'-H), 7.38 (s, 1 H, 6-H) 7.46 (d, 2 H, phenyl-H_α), 7.80 (d, 2 H, phenyl-H_β), 11.34 (s, 1 H, 3-NH, D_2O exchangeable).

Compounds **7–10** were basically prepared by the same methodology as described for the synthesis of compound **6** except, as noted below, with variation in reaction temperatures, time, and purification procedures.

3'-Azido-5'-*O*-(*p*-tolylsulfonyl)-2',3'-dideoxyuridine (7). The temperature and time for this reaction were at 4 °C and overnight, respectively. The product was purified by silica gel column chromatography (CH_2Cl_2–MeOH, 8:0.5, v/v) to afford 0.61 g (76%) of yellow amorphous solid: IR (film) 4.80 μm (azido); UV (MeOH) λ_{max} 262 nm (ϵ 11500), λ_{min} 245 nm; UV (0.01 N HCl) λ_{max} 262 nm (ϵ 10500), λ_{min} 243 nm; UV (0.01 N NaOH) λ_{max} 262 nm (ϵ 8570), λ_{min} 246 nm; NMR (Me_2SO-d_6) δ 2.28–2.37 (m, 2 H, 2'-H), 2.41 (s, 3 H, $C_6H_4CH_3$), 3.92–3.95 (m, 1 H, 4'-H), 4.21–4.29 (m, 2 H, 5'-H), 4.34–4.36 (m, 1 H, 3'-H) 5.57 (d, 1 H, 5-H), 6.00 (t, 1 H, 1'-H), 7.45–7.48 (m, 3 H, 6-H, and phenyl-H_α) 7.79 (d, 2 H, phenyl-H_β), 11.3 (s, 1 H, 3-NH, D_2O exchangeable); MS *m/e* 408 (M^+ + 1), 296 (M^+ – $C_4H_3NO_2$, uracil-1-yl). Anal. ($C_{16}H_{17}N_5O_6S$) C, H; N: calcd, 17.19; found, 16.62.

3'-Azido-5'-*O*-(*p*-tolylsulfonyl)-2',3'-dideoxy-5-bromouridine (8). The reaction mixture was kept at room temperature and followed by TLC (CH_2Cl_2–MeOH, 8:0.5, v/v, R_f 0.5) for 3 days until the reaction was complete. The product was isolated by silica gel column chromatography (CH_2Cl_2–MeOH, 8:0.5, v/v) and crystallized from EtOH to produce 0.28 g (67%): mp 141–143 °C; IR (KBr) 4.80 μm (azido); UV (MeOH) λ_{max} 274 nm (ϵ 8440), λ_{min} 244 nm; UV (0.01 N HCl) λ_{max} 276 nm (ϵ 8650), λ_{min} 247 nm; UV (0.01 N NaOH) λ_{max} 275 nm (ϵ 6180), λ_{min} 250 nm; NMR (Me_2SO-d_6) δ 2.29–2.39 (m, 1 H, 2'-H_A), 2.46 (s, 3 H, $C_6H_4CH_3$), 2.48–2.51 (m, 1 H, 2'-H_B), 3.92–3.95 (m, 1 H, 4'-H), 4.25–4.35 (m, 2 H, 5'-H), 4.36–4.38 (m, 1 H, 3'-H), 5.98 (s, 1 H, 1'-H), 7.45 (d, 2 H, phenyl-H_α), 7.78 (d, 2 H, phenyl-H_β), 7.94 (s, 1 H, 6-H), 11.9 (s, 1 H, 3-NH, D_2O exchangeable); MS *m/e* 488 (M^+ + 2). Anal. ($C_{16}H_{16}BrN_5O_6S$) C, H, N.

3'-Azido-5'-*O*-(*p*-tolylsulfonyl)-2',3'-dideoxy-5-iodouridine (9). The reaction mixture was stirred at room temperature for 2 days, followed by TLC (CH_2Cl_2–MeOH, 8:0.5, v/v, R_f 0.54). The desired compound was isolated by silica gel column chromatography and crystallized from EtOH to yield 0.34 g (49%): mp

151–153 °C; IR (KBr) 4.80 μm (azido); UV (MeOH) λ_{max} 284 nm (ε 6640), λ_{min} 246 nm; UV (0.01 N HCl) λ_{max} 289 nm (ε 6830), λ_{min} 249 nm; UV (0.01 N NaOH) λ_{max} 275 nm (ε 5920), λ_{min} 252 nm; NMR (Me_2SO-d_6) δ 2.25–2.39 (m, 1 H, 2′-H_A), 2.40 (s, 3 H, $C_6H_4CH_3$), 2.46–2.49 (m, 1 H, 2′-H_B), 3.91–3.94 (m, 1 H, 4′-H), 4.24–4.34 (m, 2 H, 5′-H), 4.35–4.39 (m, 1 H, 3′-H), 5.96 (t, 1 H, 1′-H), 7.45 (d, 2 H, phenyl-H_α), 7.79 (d, 2 H, phenyl-H_β), 7.93 (s, 1 H, 6-H), 11.7 (s, 1 H, 3-NH, D_2O exchangeable); MS *m/e* 534 (M^+ + 1), 298 (M^+ + 1-$C_4H_2IN_2O_2$, 5-iodouracil-1-yl). Anal. ($C_{16}H_{16}IN_5O_6S$) C, H, N.

3′-Deoxy-5′-*O*-(*p*-tolylsulfonyl)thymidine (10). The reaction solution was stirred at room temperature for 40 h, followed by TLC (CH_2Cl_2–MeOH, 8:1, v/v, R_f 0.42). The title compound was isolated by silica gel column chromatography (CH_2Cl_2–MeOH, 8:1, v/v) and crystallized from EtOH to yield 0.22 g (38%) of white needles as product: mp 130–131 °C; UV (MeOH) λ_{max} 270 nm (ε 8360), λ_{min} 242 nm; UV (0.01 N HCl) λ_{max} 268 nm (ε 6840), λ_{min} 244 nm; UV (0.01 N NaOH) λ_{max} 267 nm (ε 7710), λ_{min} 245 nm; NMR (Me_2SO-d_6) δ 1.75 (s, 3 H, 5-CH_3), 1.79–1.81 (m, 1 H, 2′-H_A), 1.87–1.98 (m, 2 H, 3′-H), 2.20–2.39 (m, 1 H, 2′-H_B) 2.48 (s, 3 H, $C_6H_4CH_3$), 4.13–4.18 (m, 2 H, 5′-H_A and 5′-H_B) 4.25–4.27 (m, 1 H, 4′-H), 5.96 (dd, 1 H, 1′-H), 7.35 (s, 1 H, 6-H), 7.45 (d, 2 H, phenyl-H_α) 7.77 (d, 2 H, phenyl-H_β), 11.3 (s, 1 H, 3-NH, D_2O exchangeable); MS *m/e* 381 (M^+ + 1). Anal. ($C_{17}H_{20}N_2O_6S$) C, H, N.

3′-Azido-5′-iodo-3′,5′-dideoxythymidine (11). A mixture of tosylate **6** (0.19 g, 0.5 mmol) and NaI (0.36 g, 2.45 mmol) in 2-butanone (20 mL) was refluxed for 6 h. The sodium *p*-toluenesulfonate salt that formed during the reaction was removed by filtration, and the filtrate was evaporated to dryness under reduced pressure. The resulting residue was dissolved in CH_2Cl_2 and washed with water. The organic layer was separated, dried with anhydrous Na_2SO_4, and passed through a charcoal pad. The CH_2Cl_2 solution was evaporated to dryness in vacuo to afford an oily residue, which was further dried under vacuum overnight. The oily product weighed 1.5 g (85%): R_f 0.53 (CH_2Cl_2–Me_2CO, 8:1.5, v/v); UV (MeOH) λ_{max} 265 nm (ε 11 280), λ_{min} 234 nm; UV (0.01 N HCl) λ_{max} 266 nm (ε 10 510), λ_{min} 234 nm; UV (0.01 N NaOH) λ_{max} 267 nm (ε 9950), λ_{min} 244 nm; NMR (Me_2SO-d_6) δ 1.78 (s, 3 H, 5-CH_3), 2.28–2.33 (m, 1 H, 2′-H_A), 2.47–2.54 (m, 1 H, 2′-H_B), 3.41–3.45 (m, 1 H, 5′-H_A) 3.52–3.56 (m, 1 H, 5′-H_B), 3.83–3.87 (m, 1 H, 4′-H), 4.33–4.36 (m, 1 H, 3′-H), 6.12 (t, 1 H, 1′-H), 7.52 (s, 1 H, 6-H) 11.4 (s, 1 H, 3-NH, D_2O exchangeable); MS *m/e* 378 (M^+ + 1), 127 (thymine + 1). Anal. ($C_{10}H_{12}IN_5O_3$) C, H, N.

3′-Azido-5′-iodo-2′,3′,5′-trideoxyuridine (12). A mixture of compound **7** (0.5 g, 1.2 mmol) and NaI (0.5 g, 3.3 mmol) in 18 mL of 2-butanone was refluxed for 6 h. The precipitate, formed during the reaction, was removed by filtration, and the filtrate was evaporated to dryness in vacuo. The residue was purified by silica gel column chromatography (CH_2Cl_2–Me_2CO, 8:5, v/v) to give 0.35 g (79%) of amorphous product: R_f 0.45 (CH_2Cl_2–Me_2CO, 8:1.5, v/v); IR (film) 4.80 μm (azido); UV (MeOH) λ_{max} 260 nm (ε 11 070), λ_{min} 228 nm; UV (0.01 N HCl) λ_{max} 262 nm (ε 10 990), λ_{min} 230 nm; UV (0.01 N NaOH) λ_{max} 261 nm (ε 8740), λ_{min} 240 nm; NMR (Me_2SO-d_6) δ 2.31–2.56 (m, 2 H, 2′-H), 3.42–3.55 (m, 2 H, 5′-H_A and 5′-H_B), 3.86–3.89 (m, 1 H, 4′-H), 4.30–4.34 (m, 1 H, 3′-H), 5.66 (d, 1 H, 5-H), 6.11 (t, 1 H, 1′-H), 7.68 (d, 1 H, 6-H), 11.7 (s, 1 H, 3-NH, D_2O exchangeable); MS *m/e* 364 (M^+ + 1). Anal. ($C_9H_{10}IN_5O_3$) C, H, N.

2,5′-Anhydro-3′-azido-3′,5′-dideoxythymidine (13). Method A. A mixture of the 5′-iodo derivative, compound **11** (0.31 g, 0.83 mmol), and silver acetate (0.39 g, 2.34 mmol) in MeCN (25 mL) was refluxed for 1 h and monitored by TLC. The reaction time was critical since prolonged heating resulted in a decrease of the desired product yield. After filtration of the reaction mixture through a Celite pad to remove any insoluble material, the filtrate was evaporated to dryness in vacuo. The crude product was purified by silica gel column chromatography (CH_2Cl_2–MeOH, 8:1, v/v, R_f 0.61) and then crystallized from EtOH to yield 0.12 g (58%): mp 224–225 °C dec; IR (KBr) 4.80 μm (azido); UV (MeOH) λ_{max} 245 nm (ε 12 130), λ_{min} 218 nm; UV (0.01 N HCl) λ_{max} 251 nm (ε 10 850), λ_{min} 218 nm; UV (0.01 N NaOH) λ_{max} 250 nm (ε 12 010), λ_{min} 217 nm; NMR (Me_2SO-d_6) δ 1.78 (s, 3 H, 5-CH_3), 2.44–2.49 (m, 1 H, 2′-H_A), 2.60–2.66 (m, 1 H, 2′-H_B), 4.08–4.11 (m, 1 H, 4′-H), 4.52–4.62 (m, 3 H, 3′-H, 5′-H_A, and 5′-H_B), 5.04 (t, 1 H, 1′-H), 7.77 (s, 1 H, 6-H); MS *m/e* 250 (M^+ + 1). Anal. ($C_{10}H_{11}N_5O_3$) C, H, N.

Method B. After dissolution of compound **6** (1.07 g, 2.54 mmol) in MeCN (70 mL), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 0.7 g, 4.6 mmol) was added to the solution. Monitoring by TLC, the reaction mixture was refluxed for 1 h. The hot solution was immediately filtered through a Celite pad, and the filtrate was evaporated to dryness. The residue was purified by silica gel (95 g) column chromatography (CH_2Cl_2–MeOH, 8:1, v/v, R_f 0.61) and crystallized from EtOH to produce 0.39 g (60%) of product: mp and all spectroscopic data were identical with those obtained in method A.

2,5′-Anhydro-3′-azido-2′,3′-dideoxyuridine (14). This compound was prepared from compounds **12** and **7** by method A and method B, respectively, as described in the synthesis of compound **13**: yield, 0.24 g (67%); mp 203–204 °C dec; IR (KBr) 4.83 μm (azido); UV (MeOH) λ_{max} 239.5 nm (ε 14 970), λ_{min} 216 nm; UV (0.01 N HCl) λ_{max} 239 nm (ε 14 160), λ_{min} 210 nm; UV (0.01 N NaOH) λ_{max} 240 nm (ε 14 910), λ_{min} 215 nm; NMR (Me_2SO-d_6) δ 2.46–2.64 (m, 1 H, 2′-H_A), 2.66–2.73 (m, 1 H, 2′-H_B), 4.18 (d, 1 H, 4′-H), 4.58–4.60 (m, 2 H, 5′-H_A and 5′-H_B), 4.62–4.67 (m, 1 H, 3′-H), 5.92 (d, 1 H, 5-H), 6.13 (d, 1 H, 1′-H), 7.88 (d, 1 H, 6-H); MS *m/e* 236 (M+ + 1). Anal. ($C_9H_9N_5O_3$) C, H, N.

Compounds **15–17** were synthesized from the respective tosylate derivatives (**8–10**) by the same methodology as described for the synthesis of compound **13** using method B:

2,5′-Anhydro-3′-azido-2′,3′-dideoxy-5-bromouridine (15): yield, 0.13 g (49%); mp 206–208 °C dec; R_f 0.3 (CH_2Cl_2–MeOH, 8:0.4, v/v); IR (KBr) 4.80 μm (azido); UV (MeOH) λ_{max} 246 nm (ε 7350), λ_{min} 220; UV (0.01 N HCl) λ_{max} 259 nm (ε 7310), λ_{min} 224 nm; UV (0.01 N NaOH) λ_{max} 256 nm (ε 7820), λ_{min} 220 nm; NMR (Me_2SO-d_6) δ 2.41–2.49 (m, 1 H, 2′-H_A), 2.70–2.73 (m, 1 H, 2′-H_B), 4.21–4.23 (m, 1 H, 4′-H), 4.55–4.58 (m, 2 H, 5′-H_A and 5′-H_B), 4.58–4.65 (m, 1 H, 3′-H), 6.10 (d, 1 H, 1′-H), 8.46 (s, 1 H, 6-H); MS *m/e* 315 (M^+ + 1). Anal. ($C_9H_8BrN_5O_3$) C, H, N.

2,5′-Anhydro-3′-azido-2′,3′-dideoxy-5-iodouridine (16): yield, 0.14 g (45%); mp 205–207 °C dec; R_f 0.44 (CH_2Cl_2–MeOH, 8:0.4, v/v); IR (KBr) 4.80 μm (azido); UV (MeOH) λ_{max} 261 nm (ε 7650), λ_{min} 228 nm; UV (0.01 N HCl) λ_{max} 264 nm (ε 8280), λ_{min} 231 nm; UV (0.01 N NaOH) λ_{max} 264 nm (ε 8740), λ_{min} 231 nm; NMR (Me_2SO-d_6) δ 2.39–2.49 (m, 1 H, 2′-H_A), 2.65–2.70 (m, 1 H, 2′-H_B), 4.18–4.21 (m, 1 H, 4′-H), 4.54–4.57 (m, 2 H, 5′-H_A and 5′-H_B), 4.58–4.63 (m, 1 H, 3′-H), 6.10 (t, 1 H, 1′-H), 8.46 (s, 1 H, 6-H); MS *m/e* 361 (M^+). Anal. ($C_9H_8IN_5O_3$) C, H, N.

2,5′-Anhydro-3′-deoxythymidine (17): yield, 0.1 g (50%); mp 202–203 °C; R_f 0.42 (CH_2Cl_2–MeOH, 8:1, v/v); UV (MeOH) λ_{max} 247 nm (ε 8990), λ_{min} 215 nm; UV (0.01 N HCl) λ_{max} 260 nm (ε 6410), λ_{min} 237 nm; UV (0.01 N NaOH) λ_{max} 248 nm (ε 10 140); λ_{min} 219 nm; NMR (Me_2SO-d_6) δ 1.99 (s, 3 H, 5-CH_3), 2.01–2.09 (m, 3 H, 2′-H_A, 3′-H_A, and 3′-H_B), 2.40–2.46 (m, 1 H, 2′-H_B), 4.06–4.08 (m, 1 H, 5′-H_A), 4.32–4.34 (m, 1 H, 5′-H_B), 4.64 (d, 1 H, 4′-H), 5.86 (d, 1 H, 1′-H), 7.79 (s, 1 H, 6-H); MS *m/e* 208 (M^+). Anal. ($C_{10}H_{12}N_2O_3$) C, H, N.

5-Methyl-3′-azido-2′,3′-dideoxyisocytidine (18). Compound **13** (0.25 g, 0.99 mmol) was dissolved in saturated methanolic ammonia (110 mL) and kept in a pressure bottle at room temperature for 12 days. The solvent was then evaporated to dryness in vacuo. After dissolving in a small amount of CH_2Cl_2, the residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH, 8:1, v/v, R_f 0.68) to yield 0.19 g (71%) of product as an amorphous powder: IR (KRr) 4.80 μm (azido); UV (0.01 N HCl) λ_{max} 261 nm (ε 7780), λ_{min} 244 nm; UV (0.01 N NaOH) λ_{max} 260 nm (ε 6500), λ_{min} 250 nm; NMR (Me_2SO-d_6), δ 1.72 (s, 3 H, 5-CH_3), 2.31–2.43 (m, 2 H, 2′-H) 3.59–3.79 (m, 2 H, 5′-H), 3.80–3.82 (m, 1 H, 4′-H), 4.42–4.44 (m, 1 H, 3′-H), 5.32 (s, 1 H, 5′-OH, D_2O exchangeable), 5.84 (t, 1 H, 1′-H), 6.87 (s, 2 H, 2-NH_2, D_2O exchangeable), 7.52 (s, 1 H, 6-H); MS *m/e* 267 (M^+ + 1). Anal. ($C_{10}H_{14}O_3N_6$) C, H, N.

Antiviral Test Procedures. The antiviral assay utilized the CEM-F cell line. The virus was prepared from a culture supernatant of HIV-infected CEM-F cells. Briefly, CEM-F cells were seeded at 1.5×10^5 cells/mL and infected with approximately 30 $TCID_{50}$ (50% tissue culture infectious dose) of HIV. The cells were then incubated for 45 min at 37 °C. The test compounds, in culture medium, were added at various concentrations. Six days after incubation at 37 °C, the antiviral activity was evaluated

in the culture supernatant for p25gag viral protein by an antigen capture assay (ELISA). The antiviral activity was expressed as the dose which reduced by 50% (IC_{50} in μM) the amount of p25gag viral protein in the supernatant fluid of the infected–treated cells as compared to the infected–untreated cells. Cytotoxicity was expressed as the dose which inhibited the replication of the host cell by 50% ($TCID_{50}$).

These compounds were also evaluated against Rauscher–Murine leukemia virus (R-MuLV) in cell culture by similar methodology as previously described.[10]

Procedures for Stability Determinations. The stability of each compound was determined by incubating a 2 mg/mL solution in either phosphate-buffered saline (pH 7.4) or 0.01 N HCl (pH 2) at 37 °C. At various times, a 20-μL aliquot of the incubation was analyzed on an 8 mm × 10 cm μBondapak C-18 Radial-Pak Column (Waters), by employing a linear gradient of 0–30% CH_3CN into 0.01 M $(NH_4)OOCCH_3$ (pH 5.5) at 3.0 mL/min over 30 min. The column effluent was monitored at 254 nm with an Altex Model 153 UV detector. The chromatographic peaks were integrated on a Model 621 Data Master System (Gilson Electronics) and the $t_{1/2}$ was determined from a plot of peak area vs time of incubation. Peaks were identified on the basis of comparison with retention times of the 2,5′-anhydro compounds, parent (nonanhydro) compounds, and the respective pyrimidine bases summarized as follows:

compd	t_R, min	compd	t_R, min
1	16.3	5-bromouracil	6.1
13	13.1	4	20.1
thymine	5.9	16	16.8
2	13.1	5-iodouracil	7.6
14	9.2	5	11.8
uracil	3.1	17	10.7
3	18.3	18	12.8
15	14.3		

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On the Structure Selectivity Problem in Drug Design. A Comparative Study of Benzylpyrimidine Inhibition of Vertebrate and Bacterial Dihydrofolate Reductase via Molecular Graphics and Quantitative Structure–Activity Relationships

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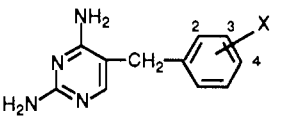
Quantitative structure–activity relationships (QSAR) have been derived for the action of 68 5-(substituted benzyl)-2,4-diaminopyrimidines on dihydrofolate reductase (DHFR) from *Lactobacillus casei* and chicken liver. The QSAR are analyzed with respect to the stereographics models of the active sites of the enzymes and found to be in good agreement. Using these QSAR equations, we have attempted to design new trimethoprim-type antifolates having higher selectivity for the bacterial enzyme. The general problem of developing selective inhibitors is discussed.

DHFR is a ubiquitous enzyme that is present in bacteria, protozoa, plants, and mammals. It catalyzes the reduction of dihydrofolate to tetrahydrofolate in the presence of the coenzyme NADPH. In its tetrahydro form this vitamin performs many vital functions, most of which involve the transfer of single-carbon units necessary for nucleic acid and certain amino acid synthesis. Inhibition of this enzyme results in cessation of DNA synthesis and eventually cell death.[1] DHFR inhibitors such as methotrexate and trimethoprim are used extensively as antineoplastic and antibacterial agents, respectively.[2] Trimethoprim is a potent and selective inhibitor of bacterial DHFRs as opposed to the mammalian enzymes.[3,4] Its toxicity and selectivity have provided the impetus for further study of the binding of the (2,4-diaminobenzyl)pyrimidine nucleus to DHFRs from various species.

In continuing our study of the comparative inhibition of dihydrofolate reductase (DHFR) from various sources by substituted (2,4-diaminobenzyl)pyrimidines (I) via



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molecular graphics and quantitative structure–activity relationships (QSAR), new data are presented on the inhibition of the enzyme from *Lactobacillus casei*.[5-7] The structure–activity relationships of binding of this class of

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