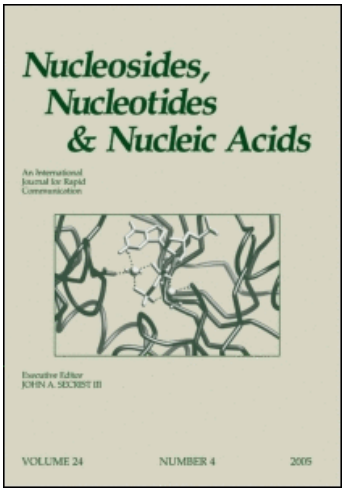

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NEW AZT CONJUGATES AS POTENT ANTI-HIV AGENTS

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□ *In an attempt to discover anti-HIV agents with much reduced cytotoxicity from the currently available HIV-reverse transcriptase inhibitors, AZT conjugates of cholanic acids, 2-imidazolidone-4-carboxylic acid and its derivatives, and N,N′-disubstituted 5-hydroxy-tetrahydropyrimidin-2-ones have been synthesized and their anti-HIV profiles determined with CEM-SS cell line. The AZT conjugates with 2-imidazolidone-4-carboxylic acid and 2-pyrrolidone-5-carboxylic acid through an ester linkage, and with N,N′-diphenyl-5-hydroxy-tetrahydropyrimidin-2-one through a succinate tether showed significantly higher therapeutic indexes than AZT while they also retained or enhanced AZT's anti-HIV activity. Thus, structural features that favor the desired therapeutic profile of the conjugates appear to include a five-membered ring cyclic urea or lactam, and six-membered ring cyclic urea with N,N′-diphenyl substitution.*

Keywords AZT; Prodrug; Steroid; 2-Imidazolidone-4-carboxylic acid; 2-Pyrrolidone-5-carboxylic acid; Tetrahydropyrimidinone; Anti-HIV

INTRODUCTION

Azidothymidine (AZT), the first clinically approved agent for treatment of acquired immunodeficiency syndrome (AIDS), acts by inhibiting the reverse transcriptase of human immunodeficiency virus (HIV),[1–3] Although AZT has attained a mainstay status in the treatment of AIDS, its serious dose-related side effects have necessitated ever growing research to find safer and more effective analogs,[4] One promising approach to improve the therapeutic index has been the prodrug strategy. This approach relies mostly on derivatization at the 5′-O of AZT. The derivative is cleaved by metabolism to gradually release AZT. Since AZT has a short plasma half-life due in part to glucuronidation in the liver at the 5′-OH[5] and has a poor cellular penetrating capability,[6] the derivatization could increase drug

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stability against metabolic destruction and improve its cellular bioavailability, thus reducing dose-related toxicity. One important class of AZT prodrugs has been carboxylic esters derived from acids such as bicyclams attached to an acid group,[7] retinoic acid,[8] 1,4-dihydronicotinic acid,[8] and steroid acids.[9] Another class is phosphoesters, some of which are derived from salicyl alcohols,[10] ether lipids,[11] or lipophilic glycosides.[12] More recently, a new class of AZT prodrugs, 5′-O-carbonates of AZT, has been presented.[13] In our design of new steroid-AZT conjugates, four rationales were employed: 1) Biologically inactive steroid acids obtained on the basis of the antedrug concept[14] are devoid of systemic toxicity. 2) The acids are designed to improve lipophilicity and bioavailability of the resulting prodrugs. 3) Protection of the 5′-OH could increase drug half-life in vivo. 4) Binding of steroid conjugates to transcortin could further improve metabolic stability.[15] In our previous study,[16] three AZT conjugates **4–6** from steroid acids **1–3** (Figure 1) were shown to have either no anti-HIV activity (conjugate **4**), or reduced activity (conjugates **5** and **6**). The inactive conjugate, however, showed very strong anti-growth activity against a number of cancer cell lines. The unusual profile of the conjugate **4** prompted us to study AZT conjugates from a few more cholanic acid derivatives. Steroid acids **7–9** (Figure 1) were therefore used to conjugate with AZT to form prodrugs **10–12** (Figure 1) for comparison.

In another front of our prodrug study, we proposed to conjugate AZT with certain HIV-protease inhibitors. This is of the same idea as the "double drugs," a new class of prodrug form of an HIV protease inhibitor conjugated with a reverse transcriptase inhibitor by a spontaneously cleavable linker.[17] Not only could such conjugates achieve some of the envisaged advantages of

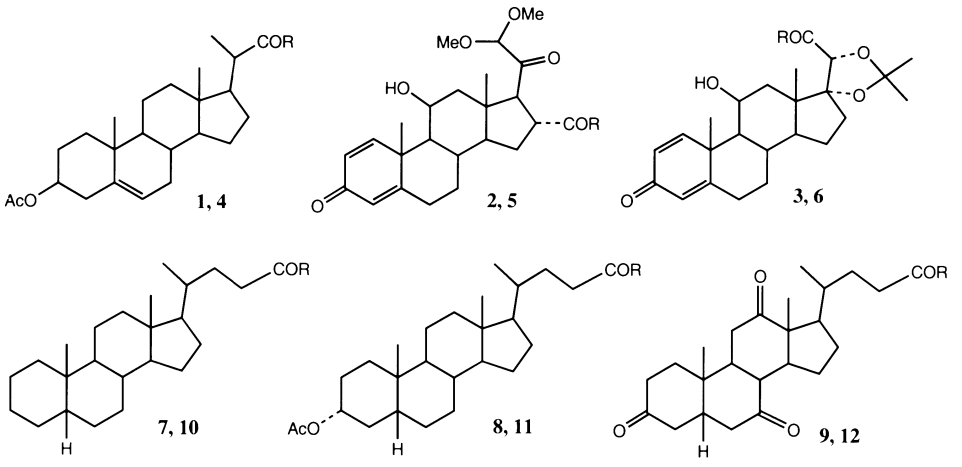


FIGURE 1

the AZT-steroid acid conjugates, they could also deliver a synergistic anti-HIV effect by having simultaneous attacks at two different stages of the viral life cycle. Of special interest to us was the cyclic urea HIV-protease inhibitor **13** (Figure 2), which gave very potent anti-HIV activity.[18] As an alcohol, compound **13** can not directly form an ester with AZT. But with a tether of succinate, it could form a diester conjugate with AZT. Succinic acid, which is released upon hydrolytic cleavage of the diester prodrug, is not expected to introduce serious toxic side effects since it is an endogenous compound in the body. Thus a conjugate between AZT and the cyclic urea appeared to be an interesting target. But before the acquisition of the cyclic urea **13** which requires a long synthetic procedure, a model study to conjugate AZT with cyclic urea acid **14**, and cyclic urea alcohols **15** and **16** (Figure 2) was undertaken to establish the conjugation methodology. Acid **14** is commercially available, while alcohols **15** and **16** were thought to be accessible through simple synthetic preparations. Two of the model conjugates were made, subjected to anti-HIV bioassay, and gave some surprising results. While AZT-**16** conjugate through a succinate tether showed lower anti-HIV activity, and a slightly better therapeutic index than AZT, AZT-**14** ester conjugate gave higher anti-HIV activity, and much higher therapeutic index than AZT. The better than expected results in the latter conjugate prompted us to investigate AZT conjugates with derivatives of cyclic urea acid **14**, and new targets were designed from acids **17–22** (Figure 3). This study describes both the synthesis and anti-HIV bioassay

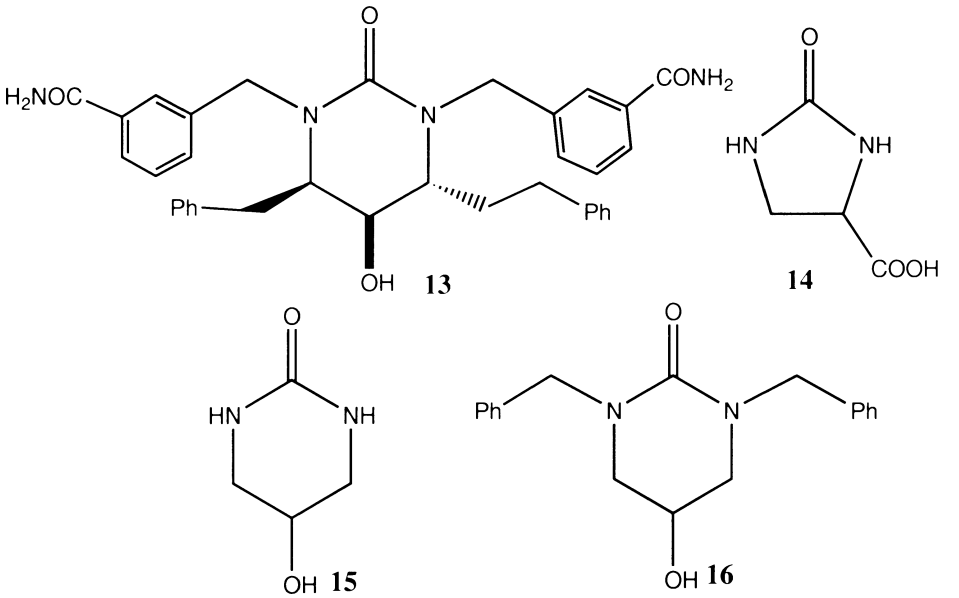
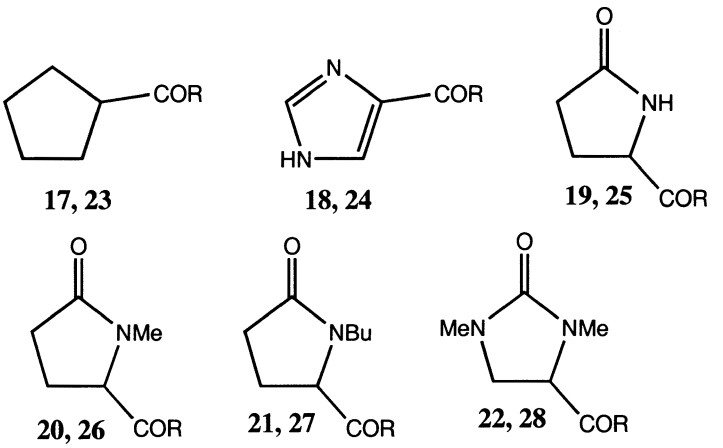


FIGURE 2



17-22: R=OH; **23-28**: R=AZT

FIGURE 3

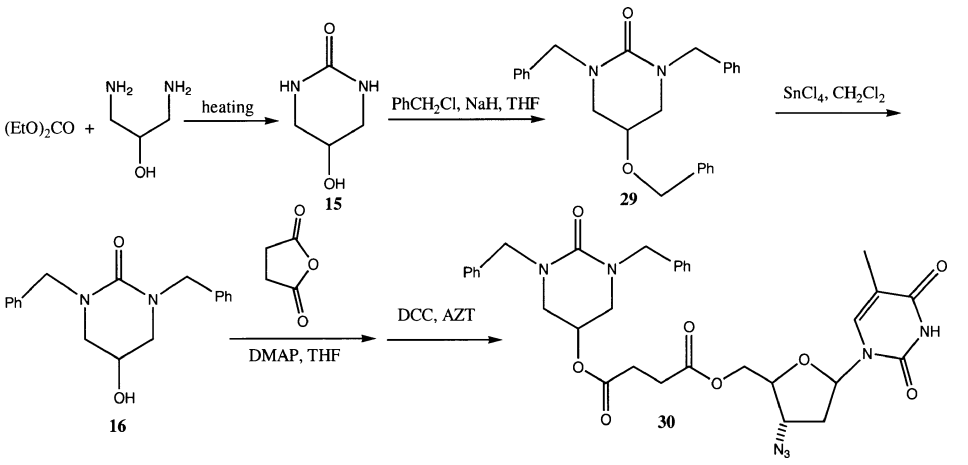
results of these new AZT conjugates. Another AZT conjugate, which was prepared from N,N′-diphenyl-5-hydroxytetrahydropyrimidin-2-one during our attempts to synthesize the tetrahydropyrimidin-2-ones, is also discussed here.

RESULTS AND DISCUSSIONS

Chemistry

A standard procedure for ester formation between AZT and an acid was used in the preparation of the conjugates from the three cholanic acids **7–9** and the conjugate from cyclopentane-carboxylic acid **17**. Good yields were obtained (73–89%) for these four targets.

Preparation of AZT/N,N′-dibenzyltetrahydropyrimidin-2-one conjugate **30** is shown in Scheme 1. The first intermediate **15** was obtained by a procedure modified from the literature.[19] Instead of a sealed tube reaction at higher temperatures as reported, the two reagents (ethyl carbonate and 1,3-diamino-2-propanol) were simply heated to reflux under a reflux condenser. The yield of our reaction (39%), however, was lower than the literature result (75%). The second intermediate N,N′-dibenzyl-5-O-benzyltetrahydropyrimidin-2-one **29** (Scheme 1) was designed so that a selective O-benzyl cleavage would yield the desired N,N′-dibenzyl-5-hydroxytetrahydropyrimidinone **16**. While tribenzylation of **15** proceeded smoothly with benzyl chloride and sodium hydride to give **29**, a selective debenzylation at the O turned out to be more difficult. Initial attempts to selectively remove the benzyl group with catalytic hydrogenation (Pd/C)

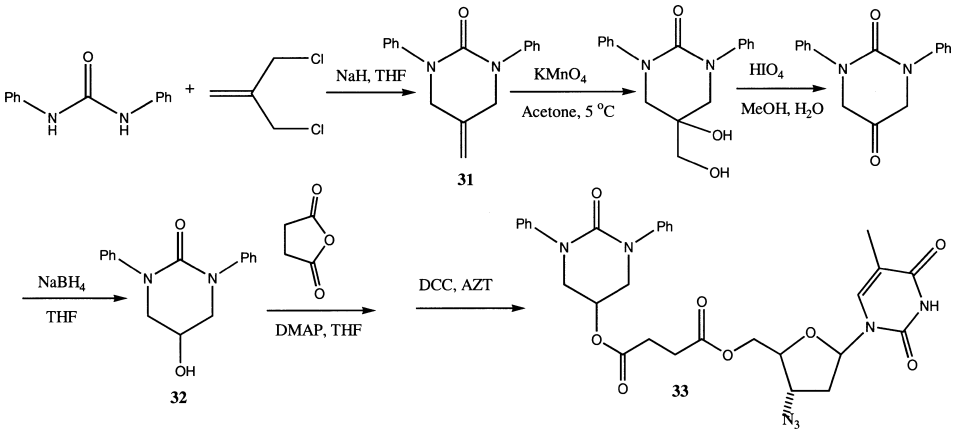


SCHEME 1

failed. What was obtained as a result appeared to be N-benzyl-5-hydroxytetrahydropyrimidin-2-one. Thus a literature procedure with $SnCl_4$[20] was tried, and it did selectively cleave O-benzyl to give the desired alcohol **16**, though very slowly. Conjugation of AZT with **16** went without much complication. As shown in the last two steps of Scheme 1, succinic anhydride was condensed with **16** to form an acid tether, which was then condensed with AZT in the presence of DCC to yield the diester conjugate **30** (45%).

Although successful for the N,N′-dibenzyl derivative, the procedure of Scheme 1 could not produce the corresponding N,N′-dialkyl derivatives, mainly because of difficulties in cleavage of the O-alkyl bond. Therefore, an alternative procedure was designed to obtain other N,N′-disubstituted derivatives.

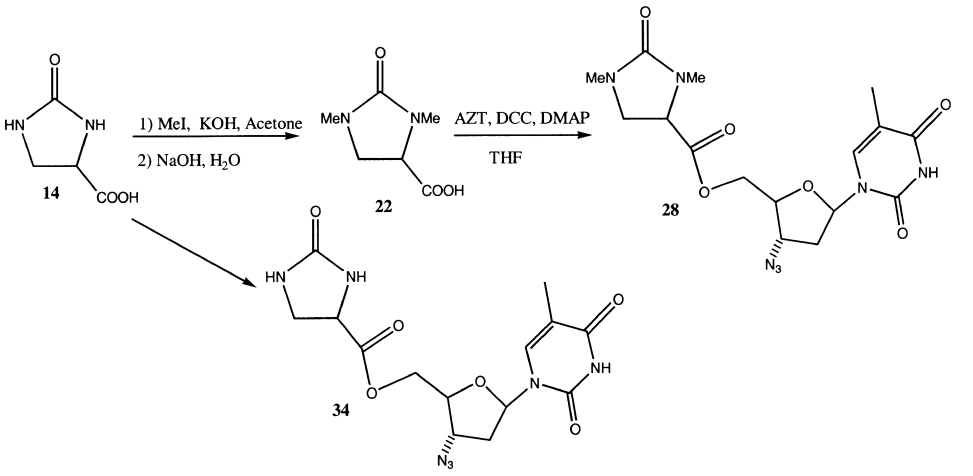
Scheme 2 gives another route to access AZT/N,N′-disubstituted tetrahydropyrimidinone conjugates. It was envisaged that 3-chloro-2-chloromethylpropene should condense, in the presence of a base, with N,N′-disubstituted urea to form a six-membered ring with a methylene substitution at the C-5. When N,N′-dimethyl or N,N′-dibutyl urea was used, unfortunately, very low yield of the desired product was detected, and many side products made purification difficult. Carbanilide, however, turned out to be quite different. The diphenyl urea condensed with 3-chloro-2-chloromethylpropene in the presence of NaH to give N,N′-diphenyl-5-methylenetetrahydropyrimidin-2-one **31** in good yield (83%) without difficulty in isolation. The next intermediate would be alcohol **32**, which could have been most efficiently obtained by ozonolysis (O_3) and reduction ($NaBH_4$). However, due to a lack of the ozonolysis apparatus in our laboratory, a longer procedure was used to convert **31** to **32** (Scheme 2). Potassium permanganate treatment at low temperatures turned **31** to a diol, which was oxidized by periodic acid to the ketone,



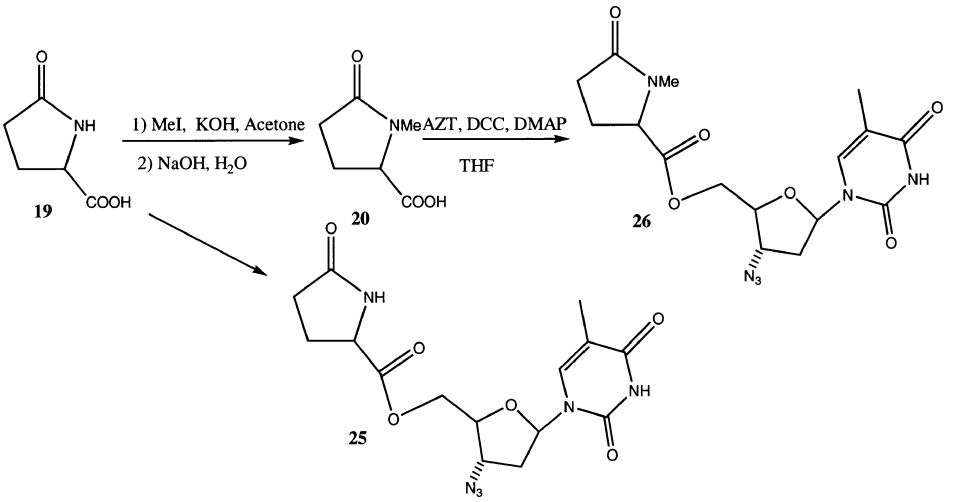
SCHEME 2

and then reduced to alcohol **32**. Conjugation of AZT with **32** proceeded to form **33** just as smoothly as in the synthesis of conjugate **30**.

Scheme 3–5 each provide a three step procedure to convert either 2-imidazolidone-4-carboxylic acid (racemic) or 2-pyrrolidone-5-carboxylic acid (racemic) to their corresponding AZT conjugates with N,N′-dimethylation, N-methylation, or N-butylation. N,N′-Dimethylation and N-methylation (Schemes 3 and 4) were achieved by treating the imidazolidone and pyrrolidone with methyl iodide and potassium hydroxide in refluxing acetone. N-Butylation (Scheme 5), on the other hand, was achieved by treatment of the pyrrolidone with butyl bromide and potassium hydroxide in DMSO. Recovery of the acid group was through a treatment with aqueous sodium



SCHEME 3



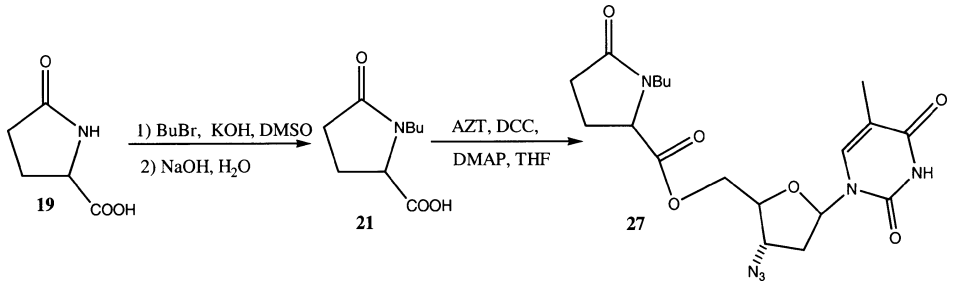
SCHEME 4

hydroxide, while ester formation between the resulting acid and AZT proceeded under the standard conditions to yield conjugates **28**, **26**, and **27**.

Synthesis of AZT conjugates with 2-imidazolidone-4-carboxylic acid (racemic), 2-pyrrolidone-5-carboxylic acid (racemic), and 4-imidazolecarboxylic acid required a slight modification of the standard procedure, mainly in the purification. This was necessary since the resulting products were significantly more polar than AZT, while all of the other conjugates mentioned above were less polar than AZT. The polarity issue necessitated the use of methanol in the column separation of conjugates **34**, **25**, and **24**, while all the other conjugates were separated with either benzene/acetone or EtOAc/hexanes system.

Biology

The ester conjugates were subjected to in vitro anti-HIV bioassays with CD4-expressing lymphocytes (CEM-SS cell line). Drug efficacy (EC_{50}) was obtained by adding HIV and the conjugate to the cell to measure protection



SCHEME 5

TABLE 1 Anti-HIV Activity and Cytotoxicity in CEM-SS Cell

Agent	EC_{50} (M)	IC_{50} (M)	TI_{50} (IC_{50}/EC_{50})
10	4.2×10^{-8}	3.7×10^{-5}	8.8×10^{2}
11	8.9×10^{-8}	$>1.0 \times 10^{-4}$	$>1.1 \times 10^{3}$
12	3.1×10^{-7}	$>1.0 \times 10^{-4}$	$>3.2 \times 10^{2}$
30	7.4×10^{-8}	9.9×10^{-5}	1.3×10^{3}
33	1.6×10^{-8}	8.9×10^{-4}	5.6×10^{4}
34	7.6×10^{-9}	$>2.0 \times 10^{-4}$	$>2.6 \times 10^{4}$
28	1.0×10^{-7}	$>1.0 \times 10^{-4}$	$>1.0 \times 10^{3}$
25	1.8×10^{-8}	$>2.0 \times 10^{-4}$	$>1.1 \times 10^{4}$
26	8.1×10^{-8}	$>1.0 \times 10^{-4}$	$>1.2 \times 10^{3}$
27	8.5×10^{-8}	$>1.0 \times 10^{-4}$	$>1.2 \times 10^{3}$
23	4.0×10^{-8}	$>1.0 \times 10^{-4}$	$>2.5 \times 10^{3}$
24	3.1×10^{-7}	$>1.0 \times 10^{-4}$	$>3.2 \times 10^{2}$
32	Inactive		
14	Inactive		
19	Inactive		
AZT	1.8×10^{-8}[a]	1.8×10^{-5}[c]	1.0×10^{3}[b]

[a] EC_{50} data of AZT obtained by the NCI at the same time as the other agents.

[b] TI_{50} data of AZT cited from an earlier NCI paper. See reference 21.

[c] IC_{50} value derived from the EC_{50} and TI_{50} values given. For a rationale, see reference 22.

of the drug against HIV-induced cell death. Cytotoxicity (IC_{50}) data was obtained from adding only the conjugate to the cell to measure cell death caused by the agent. Therapeutic index (TI) is the ratio IC_{50}/EC_{50}. As summarized in Table 1, the three steroid acid conjugates **10–12** all showed anti-HIV activity, which is different from conjugate **4**, which gave no activity in our previous study.[16] Also worthy of notice is the decreased cytotoxicity of these three conjugates compared with conjugate **4**. They gave IC_{50} values of 3.7×10^{-5}, $>1.0 \times 10^{-4}$, and $>1.0 \times 10^{-4}$, while conjugate **4** showed a value of 4.7×10^{-6}. When a comparison is made with AZT, it is clear that these conjugates (**10–12**) were weaker anti-HIV agents than AZT (EC_{50} values of 4.2×10^{-8}, 8.9×10^{-8}, and 3.1×10^{-7} vs. 1.8×10^{-8}). However, **11** had a somewhat higher TI value than AZT (IC_{50} value of $>1.1 \times 10^{3}$ vs. 1.0×10^{3}).

The two AZT-tetrahydropyrimidinone conjugates **30** and **33** gave quite different results. While the dibenzyl derivative **30** showed significantly lower anti-HIV activity than AZT (EC_{50} of 7.4×10^{-8} vs. 1.8×10^{-8}) and similar TI value to that of AZT (1.3×10^{3} vs. 1.0×10^{3}), the diphenyl derivative **33** gave similar anti-HIV activity to AZT, but significantly higher TI value (5.6×10^{4} vs. 1.0×10^{3}), and thus significantly lower cytotoxicity than AZT.

The AZT conjugate of 2-imidazolidone-4-carboxylic acid **34** gave very strong anti-HIV activity and significantly higher TI than AZT. This acid has a five-membered ring with a urea group in the ring. The derivatives (Scheme 3) designed to compare with this acid all have a five-membered ring, with either no functional group in the ring (**17**), two nitrogens just as the urea but no

carbonyl (**18**), a lactam group (**19**), or the lactam/urea with their nitrogens alkylated (**20–22**). Table 1 shows that except for conjugate **25**, which was derived from acid **19**, all the AZT conjugates from these derivatives (**17–22**) gave significantly lower anti-HIV activity. Conjugate **25** exhibited the same EC_{50} value as AZT, but much higher TI and thus IC_{50}. It appears that a nonsubstituted amide or urea group in the five-membered ring is necessary for potent anti-HIV activity.

The question of whether the acid component or the other alcohol tethered to AZT by a succinate group contributed to the anti-HIV activity of the three potent conjugates **34**, **25**, and **33** has been answered by the data for these components **14**, **19**, and **32** as shown in the table. These three compounds all failed to give any anti-HIV activity, thus they could not have contributed to the activity after the breakage of the conjugates. The active compound after the breakage must be AZT, and only AZT.

EXPERIMENTAL/CHEMISTRY

The steroid acids **7–9** were purchased from Steraloids, Newport, RI. 2-Pyrrolidone-5-carboxylic acid and solvents were bought from Fisher Scientific, Fair Lawn, NJ. All the other reagents were ordered from Aldrich Chemical, Milwaukee, WI. NMR spectra were obtained with a Bruker HX-300 spectrometer and the chemical shifts reported in parts per million (ppm) down field from tetramethylsilane as an internal standard. Keys: s (singlet), d (doublet), t (triplet), q (quartet). Elemental analysis was carried out by Galbraith Laboratories, Knoxville, TN. Melting points were determined on a Thomas Hoover Capillary Melting Point Apparatus and are uncorrected.

A standard procedure for the preparation of **10–12** and **23** is described as follows. A mixture of the acid (1.6 mmol), AZT (0.82 mmol), DCC (1.6 mmol), and DMAP (0.06 mmol) in dry THF (5 mL) was stirred under a rubber septum for 3 days. The mixture was then filtered, and the solid thoroughly washed with EtOAc. The combined organic solution was condensed, and the resulting residue chromatographed (3:1 benzene/acetone) to yield the major product.

5β-Cholanic Acid, 3′-Azido-3′-deoxythymidin-5′-yl Ester (10). White solid (73%). ^{1}H NMR ($CDCl_3$, 270 MHz) δ: 8.92 (1H, br. s, NH), 7.23 (1H, s, H-6″), 6.12 (1H, t, J = 6.3 Hz, H-1′), 4.34 (2H, d of AB-quartet, $\Delta\nu = 24.0$ Hz, $J_{AB} = 12.2$ Hz, $J_A = 4.4$ Hz, $J_B = 3.9$ Hz, H-5′), 4.18 (1H, m), 4.09 (1H, m), 1.94 (3H, s, Me of thymidine), 0.92 (3H, d, J = 6 Hz, Me of steroid), 0.91 (3H, s, Me of steroid), 0.64 (3H, s, Me of steroid). ^{13}C NMR ($CDCl_3$, 68 MHz): 173.51, 163.34, 149.96, 135.11, 111.25, 85.62, 81.95, 63.25, 60.84, 56.71, 56.04, 43.82, 42.87, 40.64, 40.37, 37.68, 36.00, 35.42, 35.36, 31.14, 31.02, 28.24, 27.57, 27.29, 27.11, 26.62, 24.27, 21.40, 20.91, 18.34, 12.60, 12.11. Analysis Calcd for $C_{34}H_{51}N_5O_5$: C66.97, H8.43, N11.49. Found: C66.83, H8.51, N11.38.

5β-Cholanic Acid-3,7,12-trione, 3′-Azido-3′-deoxythymidin-5′-yl Ester (12). White solid (78%). ^{1}H NMR ($CDCl_3$, 270 MHz) δ: 9.09 (1H, br. s, NH), 7.23 (1H, d, J = 1.0 Hz, H-6″), 6.09 (1H, t, J = 6.3 Hz, H-1′), 4.34 (2H, d of AB-quartet, $\Delta\nu = 25.4$ Hz, $J_{AB} = 12.2$ Hz, $J_A = 4.4$ Hz, $J_B = 3.9$ Hz, H-5′), 4.20 (1H, m), 4.08 (1H, m), 1.93 (3H, s, Me of thymidine), 1.39 (3H, s, Me of steroid), 1.06 (3H, s, Me of steroid), 0.85 (3H, d, J = 6.8 Hz, Me of steroid). ^{13}C NMR ($CDCl_3$, 68 MHz): 211.73, 208.80, 208.44, 173.33, 163.43, 149.96, 135.26, 111.22, 85.71, 81.86, 63.22, 60.72, 56.90, 51.79, 48.98, 46.81, 45.56, 44.95, 42.78, 38.60, 37.59, 36.43, 36.00, 35.45, 35.30, 31.17, 30.29, 27.60, 25.09, 21.88, 18.61, 12.57, 11.80. Analysis Calcd for $C_{34}H_{45}N_5O_8$: C62.66, H6.96, N10.75. Found: C62.72, H6.87, N10.63.

3α-Acetyloxy-5β-cholanic Acid, 3′-Azido-3′-deoxythymidin-5′-yl Ester (11). White solid (89%). ^{1}H NMR ($CDCl_3$, 270 MHz) δ: 9.34 (1H, br. s, NH), 7.23 (1H, s, H-6″), 6.11 (1H, t, J = 6.3 Hz, H-1′), 4.70 (1H, m, H-3), 4.33 (2H, d of AB-quartet, $\Delta\nu = 24.4$ Hz, $J_{AB} = 12.2$ Hz, $J_A = 4.4$ Hz, $J_B = 3.4$ Hz, H-5′), 4.17 (1H, m), 4.08 (1H, m), 2.01 (3H, s, Ac), 1.93 (3H, s, Me of thymidine), 0.91 (3H, s, Me of steroid), 0.90 (3H, d, J = 6.5 Hz, Me of steroid), 0.63 (3H, s, Me of steroid). Analysis Calcld for $C_{36}H_{53}N_5O_7$: C64.74, H8.00, N10.49. Found: C64.58, H8.21, N10.31.

Cyclopentanecarboxylic Acid, 3′-Azido-3′-deoxythymidin-5′-yl Ester (23). Wax-like solid (83%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 9.820 (1H, br. s, NH), 7.206 (1H, s, H-6″), 6.103 (1H, t, J = 6.3 Hz, H-1′), 4.299 (2H, d of AB-quartet, $\Delta\nu = 26.8$ Hz, $J_{AB} = 12.1$ Hz, $J_A = 4.3$ Hz, $J_B = 3.6$ Hz, H-5′), 4.160 (1H, m), 4.050 (1H, m), 2.727 (1H, m), 2.450 (1H, m), 2.277 (1H, m), 1.879 (3H, s, Me), 1.46–1.95 (8H, m, cyclopentane). ^{13}C NMR ($CDCl_3$, 75 MHz): 176.07, 163.92, 150.24, 135.07, 111.21, 85.26, 81.77, 63.19, 60.64, 43.56, 37.53, 30.00, 25.66, 12.47. Analysis Calcd for $C_{16}H_{21}N_5O_5$: C52.89, H5.83, N19.27. Found: C52.95, H5.93, N19.04.

5-Hydroxytetrahydropyrimidin-2-one (15). This compound has been reported in the literature,[19] based on which we have developed a modified synthetic procedure. Thus, a mixture of 1,3-diamino-2-propanol (24 g, 266 mmol) and ethyl carbonate (17 g, 144 mmol) was heated to reflux under a reflux condenser for 8 h. More ethyl carbonate (14 g, 115 mmol) was added, and reflux continued for 2 days. After being cooled to room temperature, the reaction mixture was treated with methanol/acetone (2:1, 25 mL) to induce crystal formation. Filtration separated the solid product, which was washed with methanol/acetone (1:1) to yield 8.5 g of the desired compound. The mother liquor was condensed, and then treated with methanol/acetone (1:1) to give more white solid (3.5 g). A total of 12 g of 5-hydroxy-tetrahydropyrimidin-2-one was isolated (39%).

N,N′-Dibenzyl-5-O-benzyltetrahydropyrimidin-2-one (29). A mixture of 5-hydroxy-tetrahydropyrimidin-2-one (3 g, 25 mmol), benzyl chloride (15 g, 120 mmol) and sodium hydride (4.8 g, 60%, 120 mmol) in anhydrous DMF (35 mL) was heated to 60°C for 2 days. The mixture was then poured into 3% aqueous HCl (550 mL) and extracted with ethyl acetate/hexanes (1:1, 2 × 250 mL). The combined organic solution was washed with water (80 mL) and then dried over sodium sulfate. Filtration and condensation gave a brown oil, which slowly yielded crystals upon standing. Removal of the liquid and washing with ethyl acetate/petroleum ether yielded large crystals (3.44 g). The mother liquor was chromatographed (1:1 EtOAc/hexanes) to give more desired product (3.25 g). A total of 6.69 g of N,N′-dibenzyl-5-O-benzyltetrahydropyrimidin-2-one (67%) was obtained. M.P. 76–77°C. ^{1}H NMR ($CDCl_3$, 270 MHz) δ: 7.15–7.35 (15H, m, aromatic), 4.60 (4 H, AB-quartet, CH_2Ph), 4.314 (2H, s, OCH_2), 3.74 (1H, m, H-5), 3.19–3.37 (4H, m, H-4 & H-6). Analysis Calcd for $C_{25}H_{26}N_2O_2$: C77.69, H6.78, N7.25. Found: C78.05, H6.98, N7.15.

N,N′-Dibenzyl-5-hydroxytetrahydropyrimidin-2-one (16). To a solution of N,N′-dibenzyl-5-O-benzyltetrahydropyrimidin-2-one (2.2 g, 5.7 mmol) in dichloromethane (70 mL) was added, via a syringe, $SnCl_4$ (12 g, 45 mmol). The mixture was stirred under a nitrogen atmosphere for 2 weeks, and then quenched with water (70 mL). The resulting mixture was extracted with dichloromethane (2 × 100 mL), and the combined extracts dried over sodium sulfate. Rotary evaporation of the dried solution and flash chromatography (1:1 and 2:1 ethyl acetate/hexanes) isolated N,N′-dibenzyl-5-hydroxytetrahydropyrimidin-2-one as a white solid (1.15 g, 68%) after vacuum drying. ^{1}H NMR ($CDCl_3$, 270 MHz) δ: 7.25–7.37 (10H, m, aromatic), 4.61 (4H, AB-quartet, NCH_2Ph), 4.02 (1H, m, H-5), 3.35 (2H, dd, J = 11.7, 3.4 Hz, H-4 & H-6), 3.15 (2H, dd, J = 11.7, 3.9 Hz, H-4 & H-6). ^{13}C NMR ($CDCl_3$, 75 MHz): 155.76, 137.97, 128.61, 127.99, 127.34, 61.86, 51.64, 51.53. Analysis Calcd for $C_{18}H_{20}N_2O_2$: C72.95, H6.80, N9.45. Found: C73.25, H6.97, N9.19.

N,N′-Dibenzyltetrahydropyrimidin-2-on-5-yl 3′-Diazo-3′-deoxythymidin-5′4-yl Succinate (30). The 5-hydroxypyrimidinone (**16**) (200 mg, 0.67 mmol), succinic anhydride (130 mg, 1.3 mmol), and DMAP (100 mg, 0.8 mmol) were stirred in dry DMF (2.5 mL) under nitrogen for 7.5 h. DCC (270 mg, 1.3 mmol) was then added, followed by addition of AZT (290 mg, 1.1 mmol) 10 min later. Stirring continued for another 11 h and the mixture was poured in aqueous HCl (130 mL, 0.3%). The aqueous suspension was extracted with 1:1 ethyl acetate/hexanes (400 mL), and the organic layer washed with water (50 mL). Rotary evaporation and flash chromatography (4:1 EtOAc/hexanes and EtOAc) isolated the succinate (195 mg, 45%) as a white solid after vacuum evaporation. ^{1}H NMR ($CDCl_3$, 270 MHz)

δ: 8.42 (1H, br. s, NH), 7.18–7.37 (11H, m, aromatic & H-6″), 6.05 (1H, t, J = 6.3 Hz, H-1′), 5.05 (1H, m, H-5), 4.63 (2H, AB-quartet, $\Delta\nu = 28.8$ Hz, $J_{AB} = 14.6$ Hz, CH_2Ph), 4.62 (2H, AB-quartet, $\Delta\nu = 57.1$ Hz, $J_{AB} = 15.1$ Hz, CH_2Ph), 4.45 (1H, dd, J = 12.2, 5.4 Hz, H-5′), 4.30 (1H, dd, J = 12.2, 3.9 Hz, H-5′), 4.21 (1H, m, H-4′), 4.01 (1H, m, H-3′), 3.41–3.50 (2H, m, H-4 & H-6), 3.18–3.27 (2H, m, H-4 & H-6), 2.37–2.59 (6H, m, CH_2CH_2, H-2′), 1.88 (3H, d, J = 1.0 Hz, Me). ^{13}C NMR ($CDCl_3$, 68 MHz): 171.59, 171.25, 163.34, 155.40, 149.96, 137.89, 135.51, 128.48, 128.02, 127.29, 111.22, 85.77, 81.77, 64.66, 63.31, 60.47, 51.37, 48.56, 37.34, 28.88, 28.70, 12.44. Analysis Calcd for $C_{32}H_{35}N_7O_8$: C59.53, H5.46, N15.18. Found: C59.90, H5.70, N14.85.

N,N′-Diphenyl-5-methylenetetrahydropyrimidin-2-one (31). To a suspension of carbanilide (10 g, 47 mmol) and NaH oil suspension (60%, 4.9 g, 122 mmol) in freshly distilled THF (150 mL) was added 3-chloro-2-chloromethylpropene (6.5 g, 52 mmol). The resulting mixture was heated to a slow reflux under a reflux condenser equipped with a drying tube ($CaSO_4$). Heating was stopped after 12 h. The cooled solution was quenched with aqueous NH_4Cl (saturated, 130 mL), and then condensed via rotary evaporation to remove THF. The remaining aqueous solution and slightly yellow solid was extracted with EtOAc/hexanes (3:1, 2 × 300 mL). The combined organic solution was washed with water (2 × 70 mL) and dried over Na_2SO_4. Condensation of the dried solution to a volume of about 30 mL resulted in precipitation, which, after a wash with petroleum ether, gave 10.3 g of white solid (83%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.14–7.37 (10H, m, aromatic), 5.127 (2H, m, methylene), 4.377 (4H, t, J = 1.2 Hz, H-4 & H-6). ^{13}C NMR ($CDCl_3$, 75 MHz): 155.43, 142.97, 136.14, 128.57, 125.37, 125.18, 111.11, 53.60.

N,N′-Diphenyl-5-hydroxytetrahydropyrimidin-2-one (32). To an acetone solution (20 mL) of N,N′-diphenyl-5-methylenetetrahydropyrimidin-2-one (630 mg, 2.3 mmol) was added through a dropping funnel 25 mL of an aqueous $KMnO_4$ (500 mg, 3 mmol) during a period of 10 min at 0°C. The resulting mixture was stirred at the same temperature for 10 min and then acidified with aqueous HCl (50 mL, 3%). Condensation via rotary evaporation removed acetone, which was followed by extraction with EtOAc (3 × 200 mL). The combined organic solution was condensed to give an oil. The oil was dissolved in methanol (60 mL), and 12 mL of aqueous HIO_4 solution (700 mg, 3.0 mmol) added. The mixture was stirred for 4 h, and then condensed via rotary evaporation to remove methanol. The remaining aqueous mixture was extracted with EtOAc/hexanes (2:1, 250 mL), and the organic layer washed with saturated aqueous $NaHCO_3$ (30 mL) and water (30 mL). The washed solution was dried over sodium sulfate, condensed, and further dried under vacuum. The resulting residue was dissolved in dry THF

(10 mL) and treated with $NaBH_4$ (200 mg, 5.4 mmol). The resulting mixture was stirred for 20 min and then poured into 2% aqueous NH_4Cl (50 mL). Rotary evaporation removed THF, and the remaining aqueous mixture was extracted with EtOAc (3 × 100 mL). The combined organic solution was condensed, and the remaining residue chromatographed (3:1 EtOAc/hexanes) to yield N,N′-diphenyl-5-hydroxytetrahydropyrimidin-2-one as a white solid (268 mg, 42%).

N,N′-Diphenyltetrahydropyrimidin-2-on-5-yl 3′-Diazo-3′-deoxythymidin-5′-yl Succinate (33). A mixture of N,N′-diphenyl-5-hydroxytetrahydro–pyrimidin-2-one (215 mg, 0.8 mmol), succinic anhydride (156 mg, 1.56 mmol), and DMAP (114 mg, 0.93 mmol) in dry THF (8 mL) was stirred under nitrogen for 10 h. DCC (275 mg, 1.3 mmol) and AZT (320 mg, 1.2 mmol) were then added. The resultant mixture was stirred under nitrogen for 30 hours. The solution was condensed and its residue chromatographed (3:1 and 2:1 benzene/acetone) to yield the desired succinate as a white solid (192 mg, 39%). ^{1}H NMR ($CDCl_3$, 270 MHz) δ: 9.13 (1H, br s, NH), 7.37–7.11 (11H, m, phenyls and vinyl of thymine), 6.04 (1H, t, J = 6.1 Hz, H-1′), 5.39 (1H, m, H-5), 4.28 (2H, d of AB-quartet, $\Delta\nu = 37.1$ Hz, $J_{AB} = 11.7$ Hz, $J_A = 4.4$ Hz, $J_B = 4.2$ Hz, H-5′), 4.10–3.82 (6H, m), 2.79–2.67 (4H, m, CH2CH2), 2.26 (2H, m, H-2′), 1.80 (3H, s, Me). Analysis Calcd for $C_{30}H_{31}N_7O_8$: C58.34, H5.06, N15.88. Found: C58.02, H5.24, N15.53.

N,N′-Dimethyl-2-imidazolidone-4-carboxylic Acid (22). A mixture of 2-imidazolidone-4-carboxylic acid (1.5 g, 11.5 mmol), KOH pellets (3.0 g, 87%, 46 mmol) and methyl iodide (8.5 g, 57 mmol) in acetone (20 mL) was stirred at room temperature for 40 min, and then heated to reflux under a reflux condenser for 2 days. The mixture was filtered, and the solid washed with acetone. The combined acetone solution was condensed to remove acetone, and then mixed with aqueous HCl (3%, 40 mL). The aqueous mixture was extracted with EtOAc (4 × 100 mL). The combined organic solution was condensed and then subjected to flash chromatography (EtOAc) to isolate the major product as a brownish oil. The oil was added to an aqueous NaOH (3%, 30 mL), and stirred for 5 min. Aqueous HCl (3%, 40 mL) was added, and the acidified mixture extracted with EtOAc (3 × 100 mL). The combined organic solution was washed with water (30 mL) and then dried over sodium sulfate. The solution was filtered, condensed, and dried under vacuum to yield a brownish solid (370 mg, 20%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 6.096 (1H, br. s, COOH), 4.091 (1H, dd, J = 10.01, 5.96 Hz, H-4), 3.6228 (1H, dd, J = 10.01, 9.18 Hz, H-5), 3.4478 (1H, dd, J = 9.18, 5.96 Hz, H-5), 2.898 (3H, s, Me), 2.807 (3H, s, Me).

N,N′-Dimethyl-2-imidazolidone-4-carboxylic Acid, 3′-Diazo-3′-deoxythymidin-5′-yl Ester (28). A mixture of N,N′-dimethyl-2-imidazolidone-4-carboxylic acid (300 mg, 1.9 mmol), DCC (400 mg, 1.94 mmol), DMAP

(14 mg, 0.1 mmol) and AZT (297 mg, 1.1 mmol) was stirred in freshly distilled THF (4 mL) under a rubber septum for 2 days. The resultant mixture was filtered, and the solid washed with acetone. The combined organic solution was condensed, and the residue chromatographed (EtOAc and 2:1 EtOAc/acetone) to yield the main product as a yellow solid (440 mg, 57%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 10.195 (1H, s, NH), 7.160 (1H, s, H-6″), 6.010 (1H, t, J = 6.6 Hz, H-1′), 4.448 (2H, m, H-5′), 4.328 (1H, m, H-4′), 4.136 (1H, dd, J = 9.5, 5.7 Hz, H-4), 4.055 (1H, m, H-3′), 3.599 (1H, t, J = 9.3 Hz, H-5), 3.389 (1H, dd, J = 9.2, 5.7 Hz, H-5), 2.866 (3H, s, NMe), 2.773 (3H, s, NMe), 2.43–2.65 (2H, m, H-2′), 1.925 (3H, s, CMe). ^{13}C NMR ($CDCl_3$, 75 MHz): 170.19, 163.99, 160.46, 150.12, 136.31, 111.15, 86.51, 81.13, 64.28, 60.60, 56.97, 47.94, 36.65, 30.96, 30.27, 12.27. Analysis Calcd for $C_{16}H_{21}N_7O_6$: C47.17, H5.20, N24.07. Found: C46.91, H5.36, N23.82.

N-Methyl-2-pyrrolidone-5-carboxylic Acid (20). This acid was synthesized from 2-pyrrolidone-5-carboxylic acid using the same procedure as that for N,N′dimethyl-2-imidazolidone-4-carboxylic acid, and a white solid (25%) obtained. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 13.04 (1H, br. s, COOH), 4.096 (1H, m, H-5), 2.677 (3H, s, Me), 2.12–2.34 (3H, m), 1.903 (1H, m). ^{13}C NMR (DMSO-d6, 75 MHz): 174.35, 173.52, 60.76, 28.93, 28.37, 22.25.

N-Methyl-2-pyrrolidone-5-carboxylic Acid, 3′-Diazo-3′-deoxythymidin-5′-yl Ester (26). This ester was prepared from the acid and AZT using the same procedure as that for N,N′-dimethyl-imidazolidone-4-carboxylic acid, 3′-azido-3′-deoxythymidin-5′-yl ester. A yield of 65% was achieved (slightly yellow solid). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 9.679 (1H, s, NH), 7.102 (1H, s, H-6″), 5.965 (1H, m, H-1′), 4.410 (2H, m, H-5′), 4.255 (1H, m), 4.170 (1H, m), 4.011 (1H, m), 2.842 (3H, s, NMe), 2.30–2.58 (5H, m), 2.084 (1H, m), 1.924 (3H, s, CMe). ^{13}C NMR ($CDCl_3$, 75 MHz): 175.16, 171.38, 163.91, 150.15, 136.31, 111.40, 86.62, 81.21, 64.23, 61.35, 60.59, 36.83, 29.07, 28.88, 22.64, 12.37. Analysis Calcd for $C_{16}H_{20}N_6O_6$: C48.98, H5.14, N21.42. Found: C48.82, H5.35, N21.23.

N-Butyl-2-pyrrolidone-5-carboxylic Acid (21). A mixture of 2-pyrrolidone-5-carboxylic acid (2.0 g, 15.5 mmol), KOH pellets (3.9 g, 85%, 59 mmol), and butyl bromide (10.0 g, 72.5 mmol) in DMSO (10 mL) was stirred for 6 h and then heated to a slow reflux under a reflux condenser for 2 days. After being cooled down to room temperature, the mixture was acidified with aqueous HCl (3%, 190 mL), and then extracted with EtOAc/hexanes (3:2, 4 × 150 mL). The combined organic solution was condensed and the residue chromatographed (1:1 and 2:1 EtOAc/hexanes) to give the major nonpolar product as a yellow oil. The oil was added to an aqueous NaOH (3%, 15 mL), and stirred for 5 min. More water (50 mL) was then added,

followed by extraction with EtOAc (3 × 80 mL).The combined organic solution was washed with water (20 mL), and dried over Na_2SO_4. Condensation of the EtOAc solution followed by vacuum evaporation yielded a brownish oil (520 mg, 18%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 5.707 (1H, br. s, COOH), 4.242 (1H, dd, J = 9.0, 3.0 Hz, H-5), 3.733 (1H, m, NCH_2), 2.959 (1H, m, NCH_2), 2.12–2.65 (4H, m), 1.497 (2H, m, CH_2 in butyl), 1.303 (2H, m, CH_2 in butyl), 0.919 (3H, t, J = 7.3 Hz, Me).

N-Butyl-2-pyrrolidone-5-carboxylic Acid, 3′-Azido-3′-deoxythymidin-5′-yl Ester (27). This ester was prepared from the acid and AZT using the same procedure as that for N,N′-dimethyl-2-imidazolidone-4-carboxylic acid, 3′-azido-3′-deoxythymidin-5′-yl ester. The product was obtained as a slightly yellow thick oil (82%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 9.896 (1H, br. s, NH), 7.143 (1H, s, H-6″), 6.022 (1H, t, J = 6.4 Hz, H-1′), 4.417 (2H, d of AB-quartet, $\Delta\nu$ = 29.0 Hz, J = 11.9, 6.0 Hz, H-5′), 4.22–4.32 (2H, m), 4.029 (1H, m), 3.710 (1H, m, NCH_2), 2.888 (1H, m, NCH_2), 2.26–2.61 (5H, m), 2.105 (1H, m), 1.949 (3H, s, Me), 1.469 (2H, m, CH_2 in butyl), 1.306 (2H, m, CH_2 in butyl), 0.909 (3H, t, J = 7.2 Hz, Me in butyl). ^{13}C NMR (CDCl3, 75 MHz): 175.14, 171.71, 163.88, 150.16, 136.05, 111.48, 86.26, 81.11, 64.20, 60.57, 59.43, 41.51, 36.75, 29.36, 29.09, 23.03, 19.95, 13.59, 12.35. Analysis Calcd for $C_{19}H_{26}N_6O_6$: C52.53, H6.03, N19.34. Found: C52.29, H6.31, N19.08.

2-Imidazolidone-4-carboxylic Acid, 3′-Azido-3′-deoxythymidin-5′-yl Ester (34). A mixture of 2-imidazolidone-4-carboxylic acid (500 mg, 3.8 mmol), DCC (790 mg, 3.8 mmol), DMAP (25 mg, 0.2 mmol) and AZT (600 mg, 2.2 mmol) in dry THF (10 mL) was stirred under a rubber septum for 3 days. The resulting suspension was filtered, and the solid washed with EtOAc (100 mL). The combined THF-EtOAc solution was condensed, and the residue chromatographed (EtOAc/MeOH 10:1) to yield the polar product as a white solid (1.2 g, 82%). ^{1}H NMR (DMSO-d6, 300 MHz) δ: 11.353 (1H, s, H-3″), 7.394 (1H, d, J = 1.1 Hz, H-6″), 6.765 (1H, s, NH of imidazolidone), 6.376 (1H, s, NH of imidazolidone), 6.105 (1H, t, J = 6.8 Hz, H-1′), 4.495 (1H, m), 4.26–4.35 (3H, m), 3.996 (1H, m), 3.593 (1H, t, J = 9.1 Hz, H-5), 3.345 (1H, m), 2.429 (1H, m, H-2′), 2.304 (1H, m, H-2′), 1.784 (3H, d, J = 1.1 Hz, Me). ^{13}C NMR (DMSO-d6, 75 MHz): 172.07, 163.73, 162.81, 150.44, 135.98, 110.18, 83.63, 80.46, 64.19, 60.09, 53.36, 43.06, 35.51, 12.13. Analysis Calcd for $C_{14}H_{17}N_7O_6$: C44.33, H4.52, N25.85. Found: C44.44, H4.68, N25.82.

2-Pyrrolidone-5-carboxylic Acid, 3′-Azido-3′-deoxythymidin-5′-yl Ester (25). It was synthesized by the same procedure as that for 2-imidazolidone-4-carboxylic acid, 3′-azido-3′-deoxythymidin-5′4-yl ester. A white solid (85%) was obtained. ^{1}H NMR ($CDCl_3$, 270 MHz) δ: 10.30 (1H, br. s, NH of thymidine), 7.71 (1H, s, NH of pyrrolidone), 7.10 (1H, s, H-6″), 5.85 (1H, t,

J = 6.2 Hz, H-1′), 4.23–4.61 (4H, m), 4.01 (1H, m), 2.13–2.75 (6H, m), 1.89 (3H, s, Me). ^{13}C NMR ($CDCl_3$, 68 MHz): 178.77, 171.71, 164.04, 150.33, 137.00, 111.16, 87.54, 81.52, 63.65, 60.32, 55.71, 36.61, 29.25, 24.60, 12.23. Analysis Calcd for $C_{15}H_{18}N_6O_6$: C47.62, H4.80, N22.21. Found: C47.35, H5.12, N21.89.

4-Imidazolecarboxylic Acid, 3′-Azido-3′-deoxythymidin-5′-yl Ester (24). The procedure for the synthesis of 2-imidazolidone-5-caboxylic acid, 3′-azido-3′-deoxythymidin-5′-yl ester was used to yield a white solid (40%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 10.075 (1H, br. s, NH of thymidine), 8.281 (1H, t, J = 0.8 Hz, imidazole), 7.643 (1H, t, J = 1.4 Hz, imidazole), 7.109 (1H, dd, J = 1.6, 0.8 Hz, imidazole), 7.042 (1H, d, J = 1.1 Hz, H-6″), 5.878 (1H, dd, J = 7.7, 4.8 Hz, H-1′), 4.674 (2H, d of AB-quartet, $\Delta\nu$ = 21.3 Hz, J_{AB} = 12.0 Hz, J_A = 3.5 Hz, J_B = 4.9 Hz, H-5′), 4.509 (1H, m, H-4′), 4.086 (1H, m, H-3′), 2.700 (1H, m, H-2′), 2.524 (1H, m, H-2′), 1.886 (3H, d, J = 1.1 Hz, Me). Analysis Calcd for $C_{14}H_{15}N_7O_5$: C46.54, H4.18, N27.14. Found: C46.23, H4.39, N26.88.

BIOLOGICAL ASSAYS

Anti-HIV assay was carried out with CD4 expressing lymphocytes (CEM-SS cell line) through the following standard procedure. Agent is dissolved dimethyl sulfoxide and diluted 1:100 in cell culture medium before preparing serial half-log10 dilutions. CD4 expressing lymphocytes are added and after a brief interval HIV-1 is added, resulting in a 1:200 final dilution of the compound. Uninfected cells with the compound serve as a toxicity control, and infected and uninfected cells without the compound serve as basic controls. Cultures are incubated at 37°C in a 5% carbon dioxide atmosphere for 6 days. The tetrazolium salt, XXT, is added to all wells, and cultures are incubated to allow formazan color development be visible viable cells. Individual wells are analyzed spectraphotometrically to quantitate formazan production, and in addition are viewed microscopically for detection of viable cells and confirmation of protective activity. Drug-treated virus-infected cells are compared with drug-treated non-infected cells and with other appropriate controls (untreated infected and untreated non-infected cells, drug-containing wells without cells, etc.) on the same plate. Data are reviewed in comparison with other tests done at the same time and a determination about activity is made.

CONCLUSION

We have synthesized three AZT conjugates with cholanic acids, two conjugates with N,N′-disubstituted tetrahydropyrimidinones, seven AZT prodrugs from 2-imidazolidone-4-carboxylic acid and six of its derivatives.

Anti-HIV and cytotoxicity bioassay with CEM-SS cell line shows that three of these conjugates, AZT conjugates with 2-imidazolidone-4-carboxylic acid, 2-pyrrolidone-5-carboxylic acid, and N,N′-diphenyl-5-hydroxy-tetrahydropyrimidin-2-one gave anti-HIV activities similar to or greater than AZT, and they exhibited much lower cytotoxic effects and thus much greater TI values than AZT. Since the compounds other than AZT used to synthesize the conjugates are inactive against HIV, the anti-HIV activity after the breakage of the conjugates could only have come from AZT. Structural features that favor the desired therapeutic profile of the conjugates appear to include a five-membered ring cyclic urea or lactam, and six-membered ring cyclic urea with N,N′-diphenyl substitution. Thus a good stride has been made towards our project goals with the three conjugates **34**, **25**, and **33**.

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22. Since AZT has been extensively studied in the past 20 years or so, it is the NCI's policy that when it is used as a standard compound in anti-HIV screening assays, only its EC_{50} value is determined. The IC_{50} and TI_{50} values of AZT, as is suggested by the NCI, should be obtained from previously published articles such as reference 21.