Synthesis and Evaluation of "AZT-HEPT", "AZT-Pyridinone", and "ddC-HEPT" Conjugates as Inhibitors of HIV Reverse Transcriptase[1]

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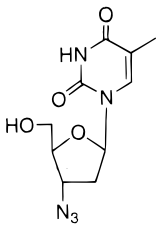
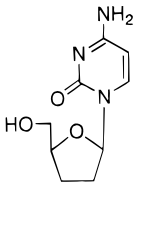
To test the concept that HIV reverse transcriptase could be effectively inhibited by "mixed site inhibitors", a series of seven conjugates containing both a nucleoside analogue component (AZT **1**, ddC **2**) and a nonnucleoside type inhibitor (HEPT analogue **12**, pyridinone **27**) were synthesized and evaluated for their ability to block HIV replication. The (N-3 and C-5)AZT-HEPT conjugates **15**, **22**, and **23** displayed 2−5 μM anti-HIV activity, but they had no effect on the replication of HIV-2 or the HIV-1 strain with the Y181C mutation. The (C-5)AZT-pyridinone conjugates **34**−**37** were found to be inactive. In marked contrast, the ddC-HEPT molecule **26** displayed the same potency (EC_{50} = 0.45 μM) against HIV-1 (wild type and the Y181C nevirapine-resistant strain) and HIV-2 in cell culture. No synergistic effect was observed for these bis-substrate inhibitors, suggesting that the two individual inhibitor components in these molecules do not bind simultaneously in their respective sites. Interestingly, however, the results indicate that the AZT-HEPT conjugates and the ddC-HEPT derivative **26** inhibit reverse transcriptase (RT) in an opposite manner. One explanation for this difference is that the former compounds interact preferentially with the hydrophobic pocket in RT, whereas **26** (after supposed triphosphorylation) inhibits RT through binding in the catalytic site.

Introduction

The human immunodeficiency virus type 1 (HIV-1) polymerase, reverse transcriptase (RT), catalyzes the multistep synthesis of a double-strand DNA copy of the viral RNA genome, for subsequent integration into the host cell DNA.[2] As this enzyme is specific to HIV, the development of RT inhibitors has been, and remains, a key strategy in the fight against AIDS. To date, five 2′,3′-dideoxynucleoside analogues, zidovudine (AZT, **1**), didanosine (ddI), zalcitabine (ddC, **2**), stavudine (d4T), and lamivudine (3TC), have been approved for the control of HIV infection.[3] Although drug resistance

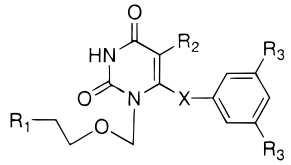


AZT (**1**)



ddC (**2**)

emerges rapidly to these nucleoside based inhibitors (NRTIs),[4,5] their use in combination therapy together with a protease inhibitor has proven to be a highly effective means to maintain the viral charge at remarkably low levels.[6] However, adverse side effects become increasingly important on the long term in the existing therapy regimes, due to a large extent to the substantial doses of protease inhibitors that are required. Increasing attention is thus been directed toward the use of nonnucleoside inhibitors of reverse transcriptase (NNRTIs)[7,8] in the combination approach. Unlike nucleoside analogues, compounds of this structurally diverse class of molecules inhibit RT in a noncompetitive manner through binding in a hydrophobic pocket which is located proximal to the catalytic site.[7,9,10] Nevirapine (BI-R6-587, Viramune), the BHAP derivative (delavirdine, Rescriptor), and the benzoxazinone DMP-266 (efavirenz, Sustiva) are the first molecules of this family to be approved for use in clinic,[11] but other NNRTIs including the analogue **4** (I-EBU, MKC-442)[12] of HEPT **3** and loviride (α-APA, R89439)[13] also show considerable promise. Rapid onset of drug resistance is also observed for the NNRTIs when employed individually, but their very pronounced selectivity toward inhibition of HIV-1 RT and their low toxicities make them attractive candidates in the combination therapy approach.[5,14]



HEPT (**3**) : R_1 = OH, R_2 = CH_3, R_3 = H, X = S

MKC-442 (**4**) : R_1 = H, R_2 = $CH(CH_3)_2$, R_3 = CH_3, X = CH_2

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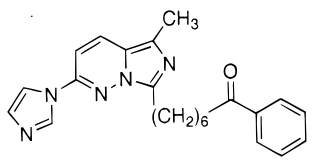
Pertinent in this context is the finding that a synergistic inhibition of HIV replication is observed in vitro and in clinical trials for certain combinations of nucleoside and nonnucleoside RT inhibitors,[14,15] [nevirapine/AZT/ddI,[16] Merck's pyridinone L-697,661/AZT,[17] delavirdine/AZT/ddI,[18] and MKC-442/AZT/ddI[12,19]]. Furthermore, in the presence of several agents, the occurrence of new mutations may lead to suppresion of others that emerge for each agent when used alone or to phenotypical conversion of resistance to sensitivity. For example, the Y181C mutation,[20] or the L100I mutation,[21] in an AZT resistance background (T215Y) significantly suppressed (phenotypic) resistance to AZT. Thus the rationale for NRTI-NNRTI combination therapy includes the possibility that a synergistic drug activity will lead to greater efficacy and that the emergence of resistance to individual agents will be forestalled.[22]

From a mechanistic viewpoint it has also been suggested that using a combination of both types of RT inhibitors would be advantageous. Indeed, kinetic studies indicated that binding of the NNRTIs in the hydrophobic pocket is translated by a lower rate of incorporation of the dNTPs, but without interfering significantly with nucleotide binding.[23−25] This apparent cooperative interaction between the NNRTI-binding pocket and the substrate-binding site led Spence et al.[23] and Rittinger et al.[24] to postulate that an inhibitor combining the functionalities of a nonnucleoside inhibitor and a nucleotide analogue could bind very tightly and specifically to the HIV-RT and could be effective in the treatment of AIDS. Furthermore, calculations on the additivity of binding energies for multicomponent systems suggest that the affinity of RT for a bis-substrate type molecule may be greater than that for the separate components.[26] These concepts give genesis to an alternative approach to combination therapy using "mixed site inhibitors", i.e., single molecules which bind simultaneously in both regions of RT.

Such hybrid compounds were also advocated on the basis of early crystallographic data.[27] However, in the light of further structural studies it appears that, although the distance between the catalytic site and the hydrophobic binding pocket is relatively small (10−15 Å),[28−30] the two sites are distinct. Thus, from these static structures no obvious channel was discerned whereby a single molecule could fit into both sites. However, as RT is a particularly flexible and dynamic molecule[29,31,32] and in view of its capacity to accommodate, or interact, with a wide range of NNRTIs, including those with elongated structures such as the imidazo[1,5-*b*]pyridazine **5**,[33] it cannot yet be excluded that inhibitors will be found which either do or, of equal interest, act as if they bind in both the catalytic and hydrophobic sites.



(5)

On the basis of this premise we initiated a program several years back to construct and evaluate the anti-HIV activity of a series of mixed inhibitors containing, at one extremity, a known nucleoside analogue inhibitor and, at the other end of a functionalized tethering arm, a nonnucleoside RT inhibitor.[34,36,37] In the preliminary stage of this investigation particular attention has been directed to the construction of NRTI-NNRTI conjugates of AZT **1** and ddC **2** with compound **13**, a HEPT analogue (Scheme 1), and the pyridinone **27** issuing from our laboratories (Scheme 4).[38]

In the seven mixed inhibitors that were synthesized (compounds **15**, **22**, **23**, **26**, and **34**−**37**; Schemes 1−4), the tethering arm was varied in length and composition, containing between 14 and 20 atoms. The connection to AZT was made via the N-3 and C-5 positions, and to ddC through a bond to the C-4 amino group. The choice of the point of connection to the nucleoside inhibitor component was based upon the observation that the N-4 substituted ddC analogues maintain some activity[39] and that derivatives of AZT bearing a variety of N-3 side chains display good to potent anti-HIV-1 activity.[40] It was also anticipated that the presence of the elongated C-5 substituent in the pyrimidine ring of AZT would not interfer with the potential of this molecule to participate in Watson−Crick type hydrogen-bonding interactions.[41] For the nonnucleoside component in these conjugates, little was known concerning the introduction of N-substituents on the amino group in the pyridinone **27**. However, it has been demonstrated that a variety of substituents can replace the terminal hydroxyl group in the N-1 side chain in HEPT **3**.[42−44]

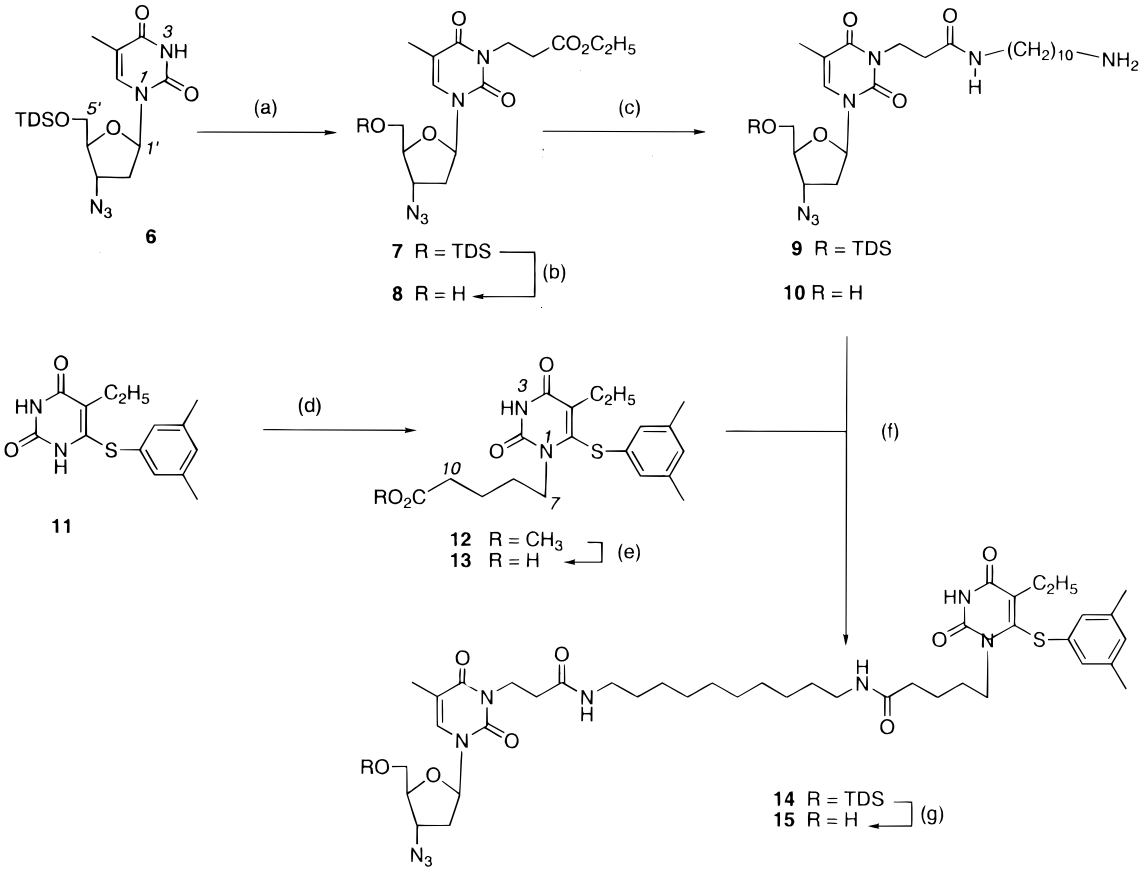
Evaluation of the HIV activity of these new bis-substrate inhibitors revealed that, whereas the AZT-pyridinone compounds **34**−**37** were devoid of any significant activity, the (N-3)AZT-HEPT conjugate **15** and the two (C-5)AZT-HEPT systems **22** and **23** displayed anti-HIV-1 activity at micromolar concentrations. Particularily remarkable, the ddC-HEPT compound **26** proved to be a potent inhibitor of both HIV-1 and HIV-2 RT ($EC_{50} \approx 0.45$ μM).

Chemistry

To construct the AZT-HEPT type conjugate **15** (Scheme 1), 5′-*O*-(thexyldimethylsilyl) protected AZT **6** was converted to the N-3 substituted compound **7** through reaction with ethyl acrylate in the presence of DBU (84%).[40,45] Reaction of this ester derivative with a large excess of 1,10-diaminodecane[46] in methanol at room temperature gave **9** in 94% yield, and the corresponding reaction of 5′-*O*-deprotected **8** provided amine **10** (63%).

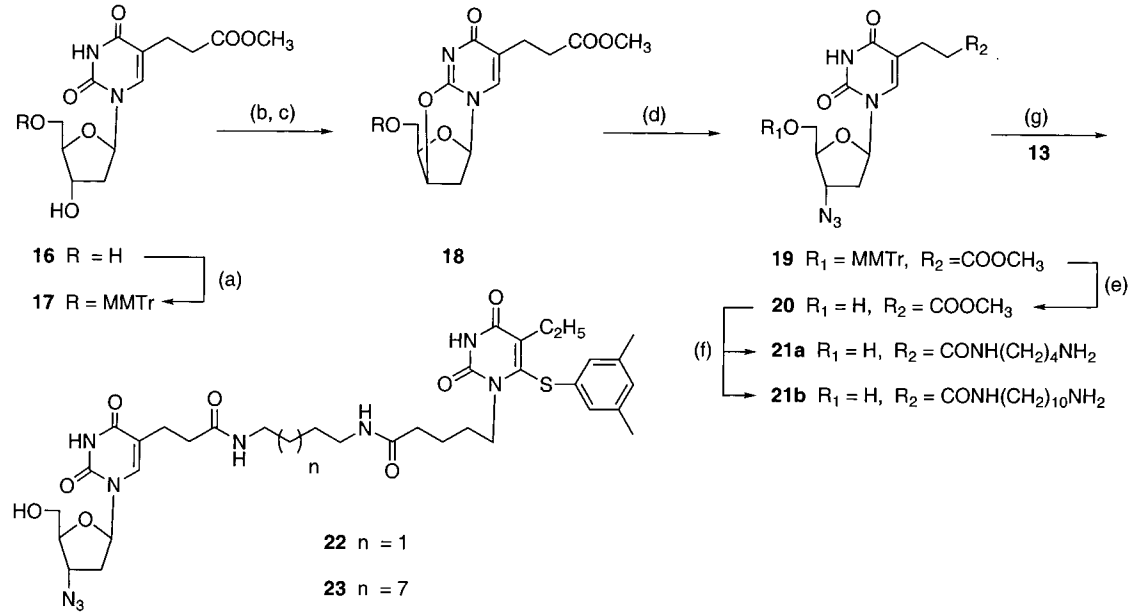
The HEPT component **13**, with an acid function at the end of the N-1 side chain, was then prepared by reaction of the available pyrimidine base **11**[44] with methyl 5-bromovalerate in the presence of potassium carbonate (DMF, 60 °C, 5 h). In this process the N-1 alkylation product[47,48] **12** was obtained (15−20% yield) along with three bisalkylated compounds. Attempts to improve the yield of the monoalkylation using Cs_2CO_3 as the base or phase transfer conditions[49] failed (KOtBu-THF or K_2CO_3-DMF, 18-crown-6). Saponification of **12** afforded the desired carboxylic derivative **13**. Condensation of the 5′-*O*-protected amine **9** with acid **13** using *N*-methylmorpholine and 2-chloro-4,6-dimethoxy-1,3,5-triazine[50] (CDMT) as the coupling reagent then gave compound **14** in 60% yield. This intermediate was

Scheme 1. Synthesis of N-3 Substituted AZT-HEPT Conjugate **15**[a]



[a] Reagents: (a) DBU, DMF, CH_2=$CHCOOC_2H_5$ (84%); (b) Dowex 50X2, EtOH (86%); (c) $NH_2(CH_2)_{10}NH_2$, MeOH, 20 °C, 24 h then 40 °C; 54 h for **9** (94%) and 50 °C, 4 days for **10** (63%); (d) $Br(CH_2)_4COOCH_3$, K_2CO_3, DMF, 60 °C (19%); (e) 1 N NaOH, acetone (94%); (f) CDMT, NMM, DMF, then **13** (60%); (g) Dowex 50X2, MeOH (85%).

Scheme 2. Synthesis of C-5 Substituted AZT-HEPT Conjugates **22** and **23**[a]

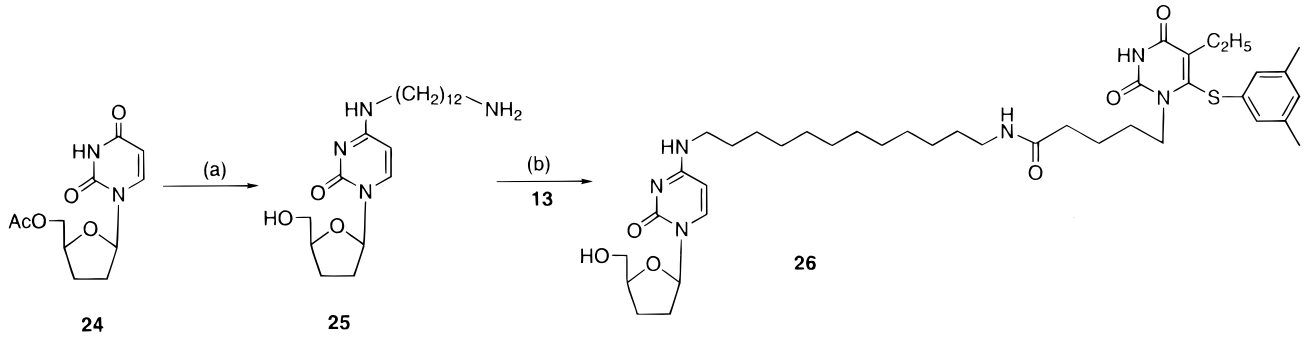


[a] Reagents: (a) MMTrCl, pyridine (50%); (b) MsCl, pyridine; (c) DBU, THF, reflux (86%); (d) NaN_3, DMF, 120 °C (85%); (e) Dowex 50X2, MeOH (89%); (f) $NH_2(CH_2)_nNH_2$ (n = 4, 10), MeOH, 4−5 days (**21a**: 76%; **21b**: 74%); (g) CDMT, NMM, DMF, then **13** (**22**: 57%; **23**: 57%).

converted to **15** by treatment with Dowex 50X2 (H^+) resin in MeOH (85%).

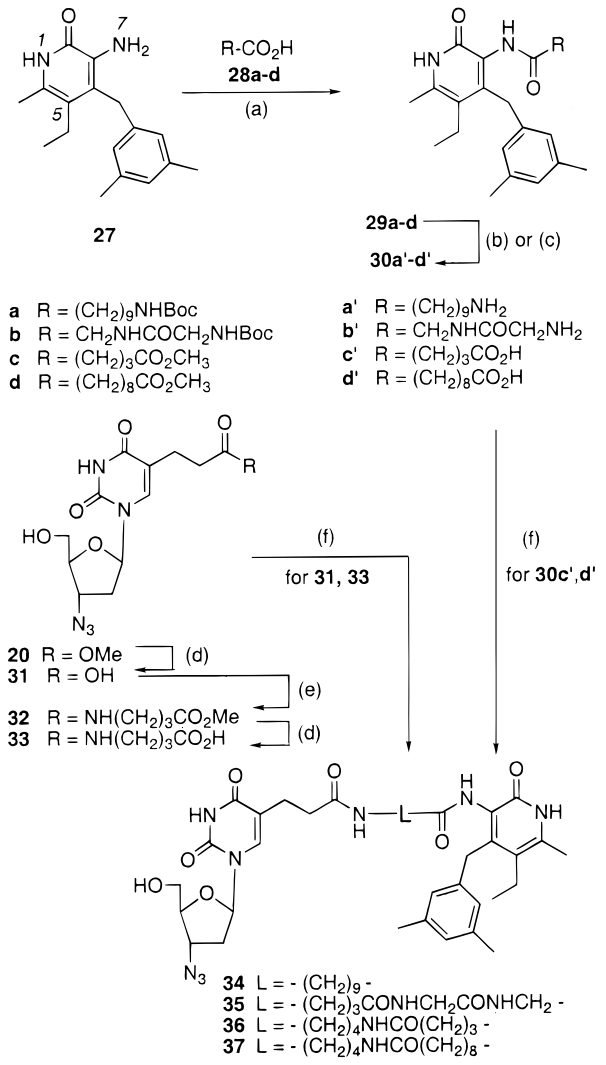
To prepare the C-5 substituted AZT-HEPT conjugates **22** and **23** (Scheme 2), the known methyl ester[41] **16** was converted into its 5′-*O*-methoxytrityl (MMTr) derivative **17**, and this intermediate was treated[51] with MsCl followed by DBU to give the 2,3′-anhydronucleoside **18** (86%). Reaction of this compound with sodium azide in

Scheme 3. Synthesis of N-4-Substituted ddC-HEPT Conjugate **26**[a]



[a] Reagents: (a) TPSCI, Et_3N, DMAP, CH_2Cl_2, then $NH_2(CH_2)_{12}NH_2$ (33%); (b) CDMT, NMM, DMF, then **13** (69%).

Scheme 4. Synthesis of C-5 Substituted AZT-Pyridinone Conjugates **34**−**37**[a]



[a] Reagents: (a) DCC, HOBT, CH_2Cl_2, 0 °C, 30 min, then NMM and **27**, 0 °C to 20 °C, 15 h (**29a**: 45%; **29b**: 72.5%; **29c**: 75%; **29d**: 77%); (b) for derivatives **29a** and **29b**. HCl conc., EtOAc, 20 °C, 10 to 30 min (**30a′**: 60%; **30b′**: 73.5%); (c) for derivatives **29c** and **29d**: 4 N NaOH, MeOH/THF (2/3), 20 °C, 5 min to 1 h 30, then HCl (**30c′**: 74%; **30d′**: 97%); (d) 2 N NaOH, then Dowex 50X2 (**31**: 95%; **33**: 73%); (e) CDMT, NMM, DMF, then NMM and HCl·$H_2N(CH_2)_3CO_2Me$ (76%); (f) **31**, **33**, **30c′**, or **30d′**, CDMT, NMM, DMF, 0 °C to 20 °C, then respectively **30a′**, **30b′**, or **21a**, 0 °C to 20 °C, 20 h (**34**: 65%; **35**: 75%; **36**: 57%; **37**: 63%).

hot DMF effected ring opening with introduction of the 3′ azido function to give **19**. Removal of the methoxytrityl group (Dowex 50X2 resin) then gave AZT analogue **20** in 34% overall yield from **16**. Treatment of this ester with a large excess of the requisite diamine in MeOH at room temperature for 4−5 days produced amino compounds **21a** and **21b** in 75% yield. Subsequent CMDT mediated coupling of **21a** or **21b** with HEPT derivative **13** gave, in 57% yield, the corresponding conjugates **22** and **23**.

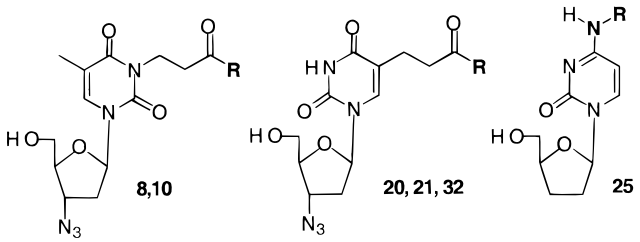
The ddC component in the mixed inhibitor **26** (Scheme 3) was prepared in a one-pot procedure[39] (33% yield), involving reaction of 5′-*O*-acetyl-2′,3′-dideoxyuridine **24** with triisopropylbenzenesulfonyl (TPS) chloride, followed by treatment with 1,12-diaminododecane (5 equiv). Coupling of amino derivative **25** with **13** using CMDT provided **26** in good yield.

For the construction of conjugates containing the pyridinone motif[38] **27** (Scheme 4), the primary amino group in this molecule was condensed[52] with the amino acids **28a** and **28b** and with the ester acids **28c** and **28d** under DCC/HOBT conditions[53] (45−77%). The derived amides **29a**,**b** were then N-deprotected[54] to give amines **30a′**,**b′**, and the esters **29c**,**d** were treated with aqueous base to give the corresponding acids **30c**,**d**. The bis-substrate inhibitors **34** and **35** were then obtained via amide bond forming reactions involving pyridinones **30a′**/**30b′** and the AZT derivatives **31** and **33**. Compound **31** was prepared from **20** by ester hydrolysis. Reaction of this carboxylic compound with methyl-γ-aminobutyrate[55] (CDMT), followed by aqueous NaOH, gave **33**. In similar amide bond forming reactions, conjugates **36** and **37** were prepared from pyridinones **30c′**/**30d′** and the amino nucleoside analogues **21a** (75%).

Results and Discussion

The bis-substrate molecules (N-3)AZT-HEPT **15**, (C-5)AZT-HEPT **22** and **23**, ddC-HEPT **26**, and (C-5)AZT-pyridinones **34**−**37**, as well as their NNRTI [HEPT analogue **12** and pyridinones **29a**−**d**, **30a′**−**d′**] and NRTI [(N-3)AZT **8** and **10**, (C-5)AZT **20**, **21a**, **21b**, **32**, and ddC **25**] precursors with different lateral side chains, were evaluated in vitro for their ability to block replication of HIV-1 in two human T-4 lymphoblastoid cell lines, CEM-SS and MT-4. As the data in Tables 1 and 3 show, all active compounds proved to be more potent in the CEM model system. Furthermore, the relative trend in activities, with respect to the reference products [AZT, ddC, and **12**], remained essentially the same in the cell lines studied. For this reason, the

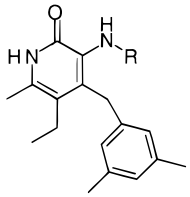
Table 1. Structure and Antiviral Activity of AZT and ddC Derivatives against HIV-1 and HIV-2[a]



		CEM-SS HIV-1 LAI		MT-4 HIV-1 IIIB		PBM HIV-1 IIIB		PBM HIV-2 D194
compd	R	EC_{50} (μM)[b]	CC_{50} (μM)[c]	EC_{50} (μM)[b]	CC_{50} (μM)[c]	EC_{50} (μM)[b]	CC_{50} (μM)[c]	EC_{50} (μM)[b]
8	OC_2H_5	0.8	>100	4.3	>100	2.1	>100	8
10	$NH(CH_2)_{10}NH_2$	42	60	>100	>100	40	80	>100
20	OCH_3	>100	>100	>100	>100	>100	>100	>100
21a	$NH(CH_2)_4NH_2$	>100	>100	>100	>100	>100	>100	>100
21b	$NH(CH_2)_{10}NH_2$	20	38	>100	60	75	77	68
32	$NH(CH_2)_3COOCH_3$	>100	>100	>100	75	>100	>100	>100
25	$(CH_2)_{12}NH_2$	7.8	48	~100	>100	2.9	61	4.7
AZT		0.002	>100	0.02	>100	0.001	75	0.006
ddC		0.031	34	2.4	>100	0.016	10	0.07

[a] See the Experimental Section for the assay method. The values are means of duplicate experiments. [b] Effective concentration of compound required to achieve 50% inhibition of HIV-1 multiplication in infected cells. The symbol (>) indicates that the EC_{50} was not reached at the highest concentration tested. [c] Cytotoxic concentration required to reduce by 50% the viability of noninfected treated cells.

Table 2. Inhibition of HIV LAI Replication (CEM-SS cells) by Pyridinones **27**, **29**, **30** and Heterodimers **34**−**37**[a]



compd	R	EC_{50} (μM)[b]	CC_{50} (μM)[c]
27	H	0.017	>10
29a	$CO(CH_2)_9NHBoc$	>10	>10
30a′	$CO(CH_2)_9NH_2$	58	>66
29b	$COCH_2NHCOCH_2NHBoc$	3.2	>100
30b′	$COCH_2NHCOCH_2NH_2$	0.72	>100
29c	$CO(CH_2)_3COOCH_3$	24	>100
30c′	$CO(CH_2)_3COOH$	4.5	28
29d	$CO(CH_2)_8COOCH_3$	>10	>10
30d′	$CO(CH_2)_8COOH$	70	>10
34	$CO(CH_2)_9NHCO(CH_2)_2$-AZT	>10	>25
35	$COCH_2NHCOCH_2NHCO$-$(CH_2)_3NHCO(CH_2)_2$-AZT	>100	26
36	$CO(CH_2)_3CONH$-$(CH_2)_4NHCO(CH_2)_2$-AZT	25	>250
37	$CO(CH_2)_8CONH$-$(CH_2)_4NHCO(CH_2)_2$-AZT	33	35

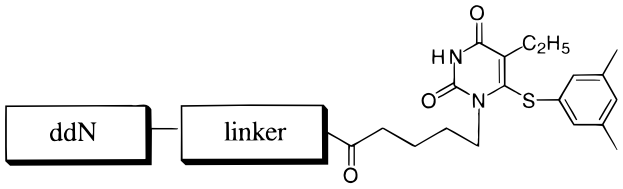
[a−c] See corresponding footnotes of Table 1.

discussion will focus on the results obtained in the CEM cell system. For the more active compounds, RT inhibition was also evaluated in peripheral blood mononuclear (PBM) cells infected with both HIV-1 IIIB and HIV-2 D194 strains.

Central to the idea of constructing bis-substrate inhibitors was to determine to what extent the individual NRTI and NNRTI components in each molecule retain their capacity to inhibit HIV-1 RT once the lateral side chains were introduced at the predetermined positions. As the data in Table 1 shows, relative to AZT ($EC_{50} = 0.002\ \mu M$) the presence of the ethyl 3-propionate motif at N-3 in the AZT analogue **8** results in a 400-fold decrease in activity. However, in agreement with previous findings for AZT analogues[40] bearing short functionalized side chains at N-3, this compound still remained active at submicromolar concentrations ($EC_{50} = 0.8\ \mu M$). Increasing the length of the side chain linker through condensation with diaminodecane to give **10** produced a further decrease in potency ($EC_{50} = 42\ \mu M$).

Modification of the C-5 position of AZT through conversion to **20** resulted in essentially a complete loss of activity. A comparable loss in RT inhibition potency was observed for the chain extended ester **32** and amine **21a**. These results are in agreement with a report by Chu et al.[56] However, it is noteworthy that the amine derivative **21b**, with a 15-atom chain, retained partial antiviral activity ($EC_{50} = 20\ \mu M$). This effect, as for isomer **10**, was accompanied by an increase in cytotoxicity.

Table 3. Antiviral Activity of Conjugates "HEPT"-ddN against Selected HIV Strains[a]



			CEM-SS HIV-1 LAI		MT-4 HIV-1 IIIB		PBM HIV-1 IIIB		PBM HIV-2 D194	CEM-SS HIV-1 (181C)[d]
compd	ddN	linker	EC_{50} (μM)[b]	CC_{50} (μM)[c]	EC_{50} (μM)[b]	CC_{50} (μM)[c]	EC_{50m} (μM)[b]	CC_{50} (μM)[c]	EC_{50} (μM)[b]	EC_{50} (μM)[b]
15	AZT N-3	$(CH_2)_2CONH(CH_2)_{10}NH$	2.8	>25	>10	>10	5.5	>25	>25	23
22	AZT C-5	$(CH_2)_2CONH(CH_2)_4NH$	3.3	>55	>10	>10	4.3	>50	>50	>50
23	AZT C-5	$(CH_2)_2CONH(CH_2)_{10}NH$	1.7	>25	>10	~10	5.1	>25	>25	>25
26	ddC N-4	$NH(CH_2)_{12}NH$	0.43	>25	6.2	>10	0.46	>10	0.43	0.53
AZT			0.002	>100	0.02	>100	0.001	75	0.006	0.004
ddC			0.031	34	2.4	>100	0.016	10	0.07	ND[e]
12			0.025	>33	0.33	>10	0.18	>33	>33	>33

[a–c] See corresponding footnotes of Table 1. [d] Nevirapine-resistant. [e] Not determined.

Among the nucleoside analogues bearing a long side chain substituent, the best activity was observed for the N-4 functionalized derivative of ddC, compound **25** (EC_{50} = 7.8 μM). This ddC analogue similarily displayed micromolar activity in the PBM cell systems infected with HIV-1 and HIV-2.

Concerning the NNRTI components, evaluation of a series of N-3 functionalized analogues of pyridinone **27** (Table 2) revealed that amino derivative **30b′** with an additional Gly-Gly dipeptide fragment is active at submicromolar concentrations (EC_{50} = 0.72 μM). Similarily, the N-Boc-Gly-Gly derivative **29b** and analogue **30c′** with a terminal carboxylic acid group displayed micromolar activity. For **29b** and **30b′**, their activity appears to be unrelated to their cytotoxic properties (SI >31−140).

The HEPT analogue **12** was found (Table 3) to display significant anti-HIV-1 activity in CEM cells (EC_{50} = 0.025 μM) and in PBM (HIV-1 IIIB) cells (EC_{50} = 0.18 μM).[57,58] Indeed, in the CEM model, this molecule was 80-fold more potent than HEPT itself.[44] As expected for an NNRTI, compound **12** was devoid of any anti-HIV-2 activity.[59]

Looking next at the bis-substrate inhibitors, although the activities observed for the substituted pyridinones were encouraging, the conjugates **34**−**37** obtained by coupling of **30a′**−**d′** to the C-5 substituted AZT derivatives **21a**, **31**, and **33** were inactive. In contrast, the (N-3)AZT-HEPT conjugate **15** and, in particular, the two (C-5)AZT-HEPT bis-substrate inhibitors **22** and **23** were all equipotent, displaying 2−5 μM activity in both the CEM and PBMC (HIV-1 IIIB) models. As for the NNRTI component **12**, none of these conjugates were endowed with anti-HIV-2 activity.

Relative to the AZT-HEPT inhibitors **15**, **22**, and **23** and the N-4 substituted derivative of ddC **25**, a 10-fold increase in activity was observed for the mixed inhibitor ddC-HEPT **26**. This trend was also found in PBM (HIV-1 IIIB) cells. Most important, however, was the marked difference in the capacity of the AZT-HEPT and ddC-HEPT systems to inhibit HIV-2 replication. Thus, whereas compounds **15**, **22**, and **23** had no effect against HIV-2, the ddC-HEPT conjugate **26** was active at submicromolar concentrations against this HIV strain (EC_{50} = 0.43 μM).

Overall, the activities observed for the conjugates **15**, **22**, **23**, and **26** were inferior to the values for the parent nucleoside analogue and the NNRTI components. However, this observation alone cannot be taken as evidence against a synergistic inhibition of RT by these molecules. Indeed, the differences in activities may result in differences in cell membrane permeability and/or different levels of phosphorylation of the nucleoside subunit.

In determining whether the four bis-substrate molecules which display activity actually inhibit RT through simultaneous interaction with the hydrophobic and catalytic sites, several points and observations must be considered. First, a common feature for each of the four active bis-substrate molecules is the presence of the HEPT component. One could conclude, therefore, that this entity is essential for activity. However, this is clearly not the case for compound **26**, since NNRTIs are highly selective toward inhibition of HIV-1 RT, and this molecule is equipotent in its capacity to block RT function in the cell lines infected respectively with HIV-1 and HIV-2. One possible interpretation for this result is that the ddC component alone contributes to the activity observed, being successfully converted to its triphosphate derivative[60,61] and binding in the catalytic site. Following on this idea, it is interesting that, although compound **26** is less active than ddC, it is at least 10 times more active than its precursor **25** with the 12-dodecylamine side chain on the C-4 amino group.

Looking next at the (N-3)AZT-HEPT inhibitor **15**, it has previously been shown that N-3 substituted AZT analogues maintain their capacity to inhibit HIV replication.[40] In this context, good levels of activity were observed for compound **8**. However, compound **10** with the longer N-3 substituent was significantly less active than conjugate **15**. Similarily, the two (C-5)AZT-HEPT conjugates are distinctly more potent than their C-5 substituted AZT precursors. These observations, together with the fact that none of the three bis-substrate molecules inhibited HIV-2 replication at the highest concentration tested (>25 μM), would tend to rule out

the participation of the nucleoside component in the capacity of these molecules to inhibit RT.

To clarify further these questions, the four conjugates were evaluated, in CEM-SS cells, against replication of a nevirapine-resistant HIV-1 strain (Y181C). Compared to the wild type virus, the activities for the AZT-HEPT derivatives **15**, **22**, and **23** and the HEPT analogue **12** were reduced by at least 8-fold. In contrast, compound **26** showed identical activity against both the wild type and HIV-1/Y181C strains. These results confirmed that it is the HEPT component that is essential for antiviral activity with the AZT-HEPT conjugates and, once again, that it is the nucleoside component which confers activity to **26**.[62]

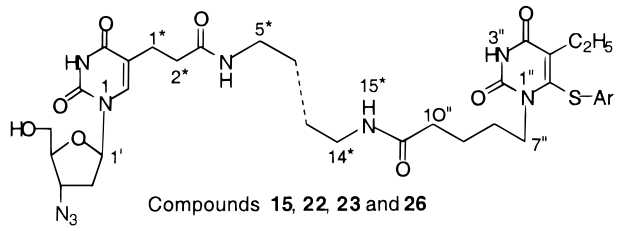
From this analysis we conclude that our molecules are not binding to RT so as to produce a synergistic effect. It is remarkable, however, that these relatively large systems do inhibit RT with a pronounced level of activity. Concerning the interaction of the AZT-HEPT conjugates with RT-1, it may be that these systems bind in the hydrophobic pocket in such a fashion that the side chain and the AZT component sit outside the site, interacting with the surrounding medium and perhaps also binding weakly to neighboring residues. This view is supported by crystal structure data which clearly show that the N-1 side chain of HEPT is directed toward the exterior of the NNRTI-binding pocket.[35,63] Given the structural similarity between HEPT and pyridinone **27**, it is conceivable that the lack of activity for the AZT-pyridinone conjugates is a consequence of a wrong positioning of the tethering arm on the NNRTI component.[64]

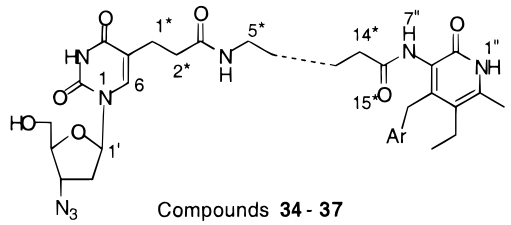
Finally, one key reason compounds **15**, **22**, **23**, and **26** appear to inhibit RT through interaction of only one component in their bis-substrate structures may be that the tethering arm is poorly adapted to permit the nucleoside and the NNRTI motifs to communicate simultaneously with their respective sites. This point is being pursued in further investigation, as is a study of the triphosphorylation of the ddC-HEPT conjugate **26** and the capacity of the desired triphosphate to inhibit the isolated enzyme. This latter study is pertinent in view of recent reports that show that certain bis-(hetero)aromatic compounds are effective RT-1 and RT-2 inhibitors.[65] Indeed there is evidence[66] to suggest that such systems inhibit RT through a mechanism which targets neither the catalytic site nor the hydrophobic pocket.

Experimental Section

Chemistry. Melting points were determined using an Electrothermal 9200 apparatus and were uncorrected. UV spectra were determined on a Varian-Cary/3E spectrophotometer. IR spectra were obtained with a Perkin-Elmer 1710 spectrophotometer. ^{1}H NMR spectra were recorded at 294 K in the given solvents on a Bruker AM-300 spectrometer using the hydrogenated residue of the deuterated solvents ($CHCl_3$, δ = 7.25 ppm, CH_3OH, δ = 3.30 ppm, and DMSO, δ = 2.54 ppm) as internal standards. Chemical shifts were reported as δ values in parts per million units, downfield from TMS. The splitting pattern abbreviations are as follows: s = singulet, d = doublet, dd = doublet of doublet, dt = doublet of triplet, t = triplet, q = quartet, br = broad, m = multiplet. Coupling constants (J) are given in hertz (Hz). Chemical ionization (CI) and electrospray ionization (ESI) mass spectra (MS) were recorded on a Nermag R10-10-C spectrometer. High-resolution mass spectra (HRMS) were obtained on a JEOL-700 spectrometer. Elemental analyses were performed by the "Service de Microanalyses du CNRS" (Vernaison-Lyon, France). The thin-layer chromatographic analyses were performed using precoated silica gel (Merck, $60F_{254}$) plates, and the spots were examined with UV light and phosphomolybdic acid spray. Column chromatographies were carried out on Merck silica gel (230−240 mesh). In all coupling experiments using DCC, the glassware was dried in an oven for a 24 h period before use and the reactants were dried over calcium chloride under vacuum at room temperature during 12 h.

^{1}H NMR numbering for nucleosides, pyridinones derivatives, and HEPT derivatives is as shown in Schemes 1 and 4; for heterodimers it is as shown below, i.e., usual for the nucleosidic entity, * for linker's atoms, and ′ ′ for NNRTI moiety.





3′-Azido-3′-deoxy-3-[2-(ethoxycarbonyl)ethyl]-5′-*O*-(thexyldimethylsilyl)thymidine (7). To a solution of **6**[40] (220 mg, 0.54 mmol) in DMF (5 mL) at room temperature under argon was added DBU (165 μL, 1.11 mmol) followed, after 1 h, by ethylacrylate (120 μL, 1.10 mmol). After the mixture was stirred for 20 h, H_2O was added, and the solution was extracted with diethyl ether (20 mL × 3). The organic layer was washed with brine, dried, concentrated under reduced pressure, and chromatographed (heptane/EtOAc: 6/4) to give **7** as a colorless oil (230 mg, 84% yield): $[\alpha]^{20}_D$ +39° (*c* 0.75, $CHCl_3$); IR ($CHCl_3$) ν 2108, 1737, 1646 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.17 (d, 6H, J = 2 Hz, $(CH_3)_2Si$), 0.89 (m, 12H, $(CH_3)_2C$ × 2), 1.25 (t, 3H, J = 7 Hz, CH_3CH_2), 1.65 (m, 1H, $(CH_3)_2CH$), 1.93 (s, 3H, CH_3), 2.21 (m, 1H, CH-2′a), 2.44 (m, 1H, CH-2′b), 2.64 (t, 2H, J = 7.5 Hz, CH_2-8), 3.80 (m, 1H, CH-5′a), 3.92 (m, 1H, CH-5′b), 3.95 (m, 1H, CH-4′), 4.14 (q, 2H, J = 7 Hz, CH_3CH_2), 4.20−4.27 (m, 3H, CH-3′, CH_2-7), 6.21 (t, 1H, J = 6.5 Hz, CH-1′), 7.37 (s, 1H, CH-6); MS (CI−NH_3) *m*/*z* 527 (M + NH_4)$^+$, 510 (M + H)$^+$. Anal. ($C_{23}H_{39}N_5O_6Si$) C, H, N.

3′-Azido-3′-deoxy-3-[2-(ethoxycarbonyl)ethyl]thymidine (8). Dowex 50X2−200 ion-exchange resin (500 mg) was added to a solution of **7** (203 mg, 0.40 mmol) in EtOH (30 mL). After 16 h, the resin was removed by filtration and washed throughly with EtOH, and the filtrate was evaporated. After flash chromatography (CH_2Cl_2 to CH_2Cl_2/MeOH: 95/5) compound **8** was obtained as a colorless oil (125 mg, 86% yield): $[\alpha]^{20}_D$ +41° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) ν 3440, 2108, 1729, 1705, 1672, 1647 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.25 (t, 3H, J = 7 Hz, CH_3CH_2), 1.93 (s, 3H, CH_3), 2.40 (m, 1H, CH-2′a), 2.56 (m, 1H, CH-2′b), 2.63 (m, 3H, CH_2-8, OH), 3.81 (m, 1H, CH-5′a), 3.95−4.00 (m, 2H, CH-4′, CH-5′b), 4.13 (q, 2H, J = 7 Hz, CH_3CH_2), 4.24 (t, 2H, J = 7.5 Hz, CH_2-7), 4.41 (m, 1H, CH-3′), 6.03 (t, 1H, J = 6.5 Hz, CH-1′), 7.36 (s, 1H, CH-6); MS (CI−NH_3) *m*/*z* 368 (M + H)$^+$. Anal. ($C_{15}H_{21}N_5O_6$) C, H, N.

3-[[(10-Aminodecyl)amino]-3-oxopropyl]-3′-azido-3′-deoxy-5′-*O*-(thexyldimethylsilyl)thymidine (9). A solution of ethyl ester **7** (153 mg, 0.30 mmol) and 1,10-diaminodecane (525 mg, 3.05 mmol) in MeOH (5 mL) was stirred at room

temperature for 24 h, then at 40 °C for 50 h. After concentration, the crude product was purified by flash chromatography (CH_2Cl_2/MeOH/Et_3N: 10/0.5/0.5) to give **9** (180 mg, 94% yield) as a foam: ^{1}H NMR (DMSO-d_6) δ 0.13 (d, 6H, J = 2.5 Hz, $(CH_3)_2Si$), 0.86 (m, 12H, $(CH_3)_2C \times 2$), 1.18−1.37 (m, 16H, CH_2-13 to CH_2-19, NH_2), 1.47 (m, 2H, CH_2-12), 1.60 (m, 1H, $(CH_3)_2CH$), 1.84 (s, 3H, CH_3), 2.28−2.38 (m, 4H, CH_2-2′, CH_2-20), 2.69 (t, 2H, J = 7.5 Hz, CH_2-8), 2.99 (m, 2H, CH_2-11), 3.79 (m, 2H, CH_2-5′), 3.87 (m, 1H, CH-4′), 4.01 (t, 2H, J = 7.5 Hz, CH_2-7), 4.38 (m, 1H, CH-3′), 6.13 (t, 1H, J = 6.5 Hz, CH-1′), 7.52 (s, 1H, CH-6), 7.91 (t, 1H, J = 5.5 Hz, NH-10); MS (CI−NH_3) m/z 636 $(M + H)^+$.

3-[[(10-Aminodecyl)amino]-3-oxopropyl]-3′-azido-3′-deoxythymidine (10). Compound **10** was prepared from nucleoside **8** (60 mg, 0.163 mmol) as described for amine **9**. After the mixture was stirred at 50 °C for 4 days and concentration, the residue was rinsed twice with EtOAc then purified by flash chromatography (CH_2Cl_2/MeOH/Et_3N: 20/1/1) to give **10** (53 mg, 63% yield) as a pale yellow solid: ^{1}H NMR (CD_3OD) δ 1.25−1.40 (m, 14H, CH_2-13 to CH_2-19), 1.40−1.50 (m, 4H, CH_2-12, NH_2), 1.90 (s, 3H, CH_3), 2.38 (m, 2H, CH_2-2′), 2.43 (m, 2H, CH_2-20), 2.65 (t, 2H, J = 7 Hz, CH_2-8), 3.11 (t, 2H, J = 7 Hz, CH_2-11), 3.74 (dd, 1H, J = 12, 3 Hz, CH-5′a), 3.84 (dd, 1H, J = 12, 3 Hz, CH-5′b), 3.91 (m, 1H, CH-4′), 4.18 (t, 2H, J = 7 Hz, CH_2-7), 4.33 (dd, 1H, J = 11, 6 Hz, CH-3′), 6.17 (t, 1H, J = 6 Hz, CH-1′), 7.84 (s, 1H, CH-6); MS (CI−NH_3) m/z 494 $(M + H)^+$; HRMS (CI, CH_4) m/z 494.3069 [$(M + H)^+$ calcd for $C_{23}H_{40}N_7O_5$: 494.3091].

6-[(3,5-Dimethylphenyl)thio]-5-ethyl-1-[4-(methoxycarbonyl)butyl]uracil (12). To a solution of **11**[44] (1.00 g, 3.62 mmol) in dry DMF (20 mL) were added K_2CO_3 (0.50 g, 3.62 mmol) and methyl 5-bromovalerate (520 μL, 3.63 mmol). The reaction mixture was heated at 60 °C for 5 h. Then, H_2O (50 mL) was added, and the solution was extracted with EtOAc (50 mL). The organic layer was washed with brine, dried, concentrated under reduced pressure, and chromatographed (cyclohexane/EtOAc: 65/35) to give **12** as a white solid which was recrystallized in EtOAc (270 mg, 19% yield): mp 130 °C; IR ($CHCl_3$) ν 3391, 1735, 1675 cm^{-1}; UV (MeOH) λ_{max} 282 (ϵ = 15.000) nm; UV (0.1 N NaOH) λ_{max} 284 (ϵ = 15.000) nm; ^{1}H NMR ($CDCl_3$) δ 1.03 (t, 3H, J = 7 Hz, CH_2CH_3), 1.40−1.70 (m, 4H, CH_2-8, CH_2-9), 2.23 (m, 2H, CH_2-10), 2.28 (s, 6H, Ar-CH_3), 2.70 (q, 2H, J = 7 Hz, CH_2CH_3), 3.65 (s, 3H, OCH_3), 3.93 (m, 2H, CH_2-7), 6.78 (s, 2H, Ar-H), 6.90 (s, 1H, Ar-H), 10.10 (br s, 1H, NH-3); MS (CI−NH_3) m/z 408 $(M + NH_4)^+$, 391 $(M + H)^+$. Anal. ($C_{20}H_{26}N_2O_4S$) C, H, N.

1-(4-Carboxybutyl)-6-[(3,5-dimethylphenyl)thio]-5-ethyluracil (13). To a cold mixture of **12** (320 mg, 0.82 mmol) in acetone (4 mL), 1 N NaOH (2.5 mL) was added dropwise. After the mixture was stirred for 3 h at room temperature, acetone was removed in vacuo, and the product was precipitated from the solution by slowly adding 1 N HCl, then was filtered, washed with cold water, and dried to give **13** as a white solid which was recrystallized in MeOH (290 mg, 94% yield): mp 157−158 °C; IR (KBr) ν 342, 3003, 1705, 1667 cm^{-1}; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 1.03 (t, 3H, J = 7 Hz, CH_2CH_3), 1.35−1.65 (m, 4H, CH_2-8, CH_2-9), 2.20 (m, 2H, CH_2-10), 2.28 (s, 6H, Ar-CH_3), 2.67 (q, 2H, J = 7 Hz, CH_2CH_3), 3.93 (m, 1H, CH_2-7), 6.77 (s, 2H, Ar-H), 6.87 (s, 1H, Ar-H), 10.50 (br, 2H, NH-3, COOH).

2′-Deoxy-5-[2-(methoxycarbonyl)ethyl]-5′-*O*-(4-methoxytrityl)uridine (17). A solution of nucleoside **16**[41] (4.19 g, 13.34 mmol) and 4-methoxytrityl chloride (7.71 g, 23.81 mmol) in dry pyridine (300 mL) was stirred for 20 h under argon. The reaction was quenched with MeOH (5 mL), and the mixture was concentrated to dryness. The residue was dissolved in CH_2Cl_2 and washed twice with water, and the organic layer was dried and concentrated. Flash chromatography (EtOAc/cyclohexane/Et_3N: 9/1/0.5) provided **17** as a foam (3.94 g, 50% yield): ^{1}H NMR ($CDCl_3$) δ 1.70 (br s, 1H, OH), 2.10−2.50 (m, 6H, CH_2-2′, CH_2-7, CH_2-8), 3.44 (m, 2H, CH_2-5′), 3.58 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 4.04 (m, 1H, CH-4′), 4.55 (m, 1H, CH-3′), 6.36 (t, 1H, J = 7 Hz, CH-1′), 6.85 (d, 2H, J = 9 Hz, Ar), 7.20−7.35 (m, 10H, Ph), 7.41 (d, 2H, J = 9 Hz, Ar), 7.51 (s, 1H, CH-6), 8.65 (br s, 1H, NH); MS (CI−NH_3) m/z 587 $(M + H)^+$.

2,3′-Anhydro-2′-deoxy-5-[2-(methoxycarbonyl)ethyl]-5′-*O*-(4-methoxytrityl)uridine (18). At 0 °C, methanesulfonyl chloride (775 μL, 10.00 mmol) was added dropwise, under an argon atmosphere, to a solution of nucleoside **17** (3.91 g, 6.67 mmol) in dry pyridine (40 mL). The resulting mixture was stirred at room temperature for 22 h, then quenched at 0 °C with water (10 mL). After evaporation, the residue was taken up in CH_2Cl_2 and washed twice with water, and the organic layer was dried and concentrated to give a foam (4.40 g). To the crude mesylate derivative dissolved in dry THF (100 mL) was added DBU (1 mL, 6.68 mmol). After being stirred at reflux for 5 h, the mixture was diluted with cold H_2O (100 mL) and extracted with EtOAc (3 × 100 mL). The combined organic phase was dried, evaporated under reduced pressure, and chromatographed (CH_2Cl_2/MeOH/Et_3N: 98/2/1, then 96/4/1) to give **18** as a foam (3.26 g, 86% yield): $[\alpha]^{20}_D$ −6° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.40 (m, 1H, CH-2′a), 2.54 (d, 1H, J = 13 Hz, CH-2′b), 2.55−2.70 (m, 4H, CH_2-7, CH_2-8), 3.34 (m, 2H, CH_2-5′), 3.63 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 4.24 (m, 1H, CH-4′), 5.14 (br s, 1H, CH-3′), 5.48 (d, 1H, J = 3.5 Hz, CH-1′), 6.80 (d, 2H, J = 8 Hz, Ar), 7.05 (s, 1H, CH-6), 7.15−7.30 (m, 10H, Ph), 7.41 (d, 2H, J = 8 Hz, Ar); MS (CI−NH_3) m/z 569 $(M + H)^+$.

3′-Azido-2′,3′-dideoxy-5-[2-(methoxycarbonyl)ethyl]-5′-*O*-(4-methoxytrityl)uridine (19). Compound **18** (3.41 g, 6.00 mmol) was heated at 120 °C with sodium azide (1.56 g, 24.00 mmol) in dry DMF (300 mL) for 20 h. After cooling, the mixture was poured into ice−water and extracted with EtOAc (3 × 100 mL). The combined phase was dried, evaporated under reduced pressure, and chromatographed (CH_2Cl_2/MeOH/Et_3N: 99/1/1, then 97/3/1) to give **19** as a foam (3.12 g, 85% yield): $[\alpha]^{20}_D$ +15° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) ν 3392, 2107, 1710, 1688 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.20−2.50 (m, 6H, CH_2-2′, CH_2-7, CH_2-8), 3.38 (dd, 1H, J = 11, 3 Hz, CH-5′a), 3.53 (dd, 1H, J = 11, 3 Hz, CH-5′b), 3.59 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.98 (dd, 1H, J = 7.5, 3.5 Hz, CH-4′), 4.32 (m, 1H, CH-3′), 6.23 (t, 1H, J = 6.5 Hz, CH-1′), 6.88 (d, 2H, J = 8 Hz, Ar), 7.30−7.50 (m, 12H, Ar), 7.58 (s, 1H, CH-6), 8.95 (br s, 1H, NH).

3′-Azido-2′,3′-dideoxy-5-[2-(methoxycarbonyl)ethyl]-uridine (20). Dowex 50X2−200 mesh ion-exchange resin (2.0 g) was added to a solution of **19** (1.60 g, 2.60 mmol) in MeOH (50 mL). After 3 h, the resin was removed by filtration and washed thoroughly with MeOH, and the filtrate was evaporated. After flash chromatography (CH_2Cl_2/MeOH: 97/3) and recrystallization from diethyl ether, **20** (790 mg, 89% yield) was obtained as a white solid: mp 80−81 °C; $[\alpha]^{20}_D$ +45° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) ν 3520, 3392, 2108, 1710, 1688 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.45 (t, 2H, J = 6 Hz, CH_2-8), 2.55−2.70 (m, 4H, CH_2-2′, CH_2 -7), 3.40 (br s, 1H, OH), 3.67 (s, 3H, OCH_3), 3.80 (d, 1H, J = 12 Hz, CH-5′a), 3.97 (m, 1H, CH-4′), 4.02 (d, 1H, J = 12 Hz, CH-5′b), 4.42 (dd, 1H, J = 11, 6 Hz, CH-3′), 6.20 (t, 1H, J = 6.5 Hz, CH-1′), 7.72 (s, 1H, CH-6), 9.10 (br s, 1H, NH); MS (CI−NH_3) m/z 340 $(M + H)^+$. Anal. ($C_{13}H_{17}N_5O_6$) C, H, N.

5-[[(4-Aminobutyl)amino]-3-oxopropyl]-3′-azido-2′,3′-dideoxyuridine (21a). A solution of nucleoside **20** (180 mg, 0.53 mmol) and 1,4-diaminobutane (490 mg, 5.55 mmol) in MeOH (5 mL) was stirred under argon for 4 days. After repeated evaporations with toluene, the crude product was rinsed three times with EtOAc to give **21a** as a white, hygroscopic, fluffy solid (160 mg, 76% yield): ^{1}H NMR (DMSO-d_6) δ 1.25−1.35 (m, 6H, CH_2-12, CH_2-13, NH_2), 2.20−2.45 (m, 6H, CH_2-2′, CH_2-7, CH_2-8), 3.00 (m, 2H, CH_2-11), 3.61 (m, 2H, CH_2-5′), 3.83 (m, 1H, CH-4′), 4.40 (dd, 1H, J = 11, 5 Hz, CH-3′), 6.09 (t, 1H, J = 6.5 Hz, CH-1′), 7.63 (s, 1H, CH-6), 7.82 (t, 1H, J = 5 Hz, NH-10); MS (CI−NH_3) m/z 396 $(M + H)^+$.

5-[[(10-Aminodecyl)amino]-3-oxopropyl]-3′-azido-2′,3′-dideoxyuridine (21b). A solution of nucleoside **20** (150 mg, 0.44 mmol) and 1,10-diaminodecane (760 mg, 4.41 mmol) in MeOH (4 mL) was stirred under argon for 5 days. After evaporation, the crude product was rinsed three times with

EtOAc. The residual solid was purified by flash chromatography (CH_2Cl_2/MeOH/Et_3N: 15/5/1) to give **21b** as a white, hygroscopic solid (156 mg, 74% yield): ^{1}H NMR (DMSO-d_6) δ 1.20−1.30 (m, 14H, CH_2-13 to CH_2-19), 1.30−1.40 (m, 4H, CH_2-12, NH_2), 2.20−2.45 (m, 6H, CH_2-2′, CH_2-7, CH_2-8), 2.57 (m, 2H, CH_2-20), 3.00 (m, 2H, CH_2-11), 3.63 (m, 2H, CH_2-5′), 3.84 (m, 1H, CH-4′), 4.42 (m, 1H, CH-3′), 6.10 (t, 1H, J = 6.5 Hz, CH-1′), 7.64 (s, 1H, CH-6), 7.83 (t, 1H, J = 5 Hz, NH-10); MS (CI−NH_3) m/z 480 (M + H)$^+$; HRMS (CI, CH_4) m/z 480.2910 [(M + H)$^+$ calcd for $C_{22}H_{38}N_7O_5$: 480.2934].

4-*N*-(12-Aminododecyl)-2′,3′-dideoxycytidine (25). Compound **24**[39,67] (250 mg, 0.98 mmol) was dried by coevaporating twice with anhydrous pyridine and was dissolved in anhydrous dichloromethane (15 mL). Triethylamine (35 μL, 2.51 mmol) was added, then (2,4,6-triisopropylphenyl)sulfonyl chloride (600 mg, 1.98 mmol) and 4-(dimethylamino)pyridine (25 mg, 0.02 mmol). The reaction was stirred for 3 h under argon at room temperature, and 1,12-diaminododecane (1.25 g, 6.23 mmol) was added. After being stirred for 2 h, the mixture was evaporated under reduced pressure, and the residue was purified by flash chromatography (CH_2Cl_2/MeOH/Et_3N: 18/2/1 then 15/5/1) to give, after trituration with EtOAc, 128 mg (33% yield) of **25** as a foam: ^{1}H NMR (DMSO-d_6) δ 1.15−1.30 (m, 20H, CH_2 × 9, NH_2), 1.46 (m, 2H, CH_2-9), 1.75−1.85 (m, 3H, CH-2′a, CH_2-3′), 2.23 (m, 1H, CH-2′b), 2.50 (CH_2-19 with DMSO), 3.20 (dt, 2H, J = 6, 6 Hz, CH_2-8), 3.53 (dd, 1H, J = 12, 4 Hz, CH-5′a), 3.66 (dd, 1H, J = 12, 3.5 Hz, CH-5′b), 3.99 (m, 1H, CH-4′), 5.69 (d, 1H, J = 7.5 Hz, CH-5), 5.92 (dd, 1H, J = 6.5, 3 Hz, CH-1′), 7.65 (t, 1H, J = 5.5 Hz, NH), 7.82 (d, 1H, J = 7.5 Hz, CH-6). MS (CI−NH_3) m/z 395 (M + H)$^+$; HRMS (CI, CH_4) m/z 395.3013 [(M + H)$^+$ calcd for $C_{21}H_{39}N_4O_3$: 395.3022].

General Procedure for the Synthesis of the Pyridinone Analogues 29a−d. *N*,*N*′-Dicyclohexylcarbodiimide (7.0 equiv) was added to the cooled mixture (0 °C) of hydroxybenzotriazole (7.0 equiv) and acid[52] **28a**, **28b**, **28c**, or **28d** (7.0 equiv) in anhydrous CH_2Cl_2. An exothermic reaction and formation of a white precipitate were observed. The mixture was stirred at 0 °C under argon for 35 min. The solution of the pyridinone **27**,[38] in CH_2Cl_2, was added dropwise. After 5 min, *N*-methylmorpholine (7.7 equiv) was added. The mixture was stirred at 0 °C for 3 h and at room temperature for 16 h. *N*,*N*′-Dicyclohexylurea was filtered off. The solvent was removed in vacuo, and the crude was taken in EtOAc. The organic layer was washed twice with an aqueous solution of sodium bicarbonate, once with aqueous solution of citric acid, with water and brine. The organic layer was dried and concentrated under reduced pressure. The residue was chromatographed as indicated.

3-[10-(*N*-*tert*-Butoxycarbonyl)aminodecanoyl]amino-4-(3,5-dimethylbenzyl)-5-ethyl-6-methylpyridin-2(*1H*)-one (29a). From 10-(*N*-*tert*-butoxycarbonyl)amino decanoic acid **28a** (1.49 g, 5.18 mmol) and pyridinone analogue **27** (0.20 g, 0.74 mmol) was obtained compound **29a** as a yellow light solid (0.18 g, 45% yield) after flash chromatography (CH_2Cl_2/EtOH: 1/0 to 95/5): TLC (CH_2Cl_2/EtOH: 95/5) R_f 0.29; mp 148−149 °C; ^{1}H NMR ($CDCl_3$) δ 0.96 (t, 3H, J = 7 Hz, CH_2CH_3), 1.25 (br s, 10H, CH_2-11 to CH_2-15), 1.43 (s, 9H, Boc), 1.57−1.65 (m, 4H, CH_2-10, CH_2-16), 2.22 (s, 6H, Ar-CH_3), 2.30 (q, 2H, J = 7 Hz, CH_2CH_3), 2.44 (s, 3H, CH_3-6), 2.44−2.55 (m, 2H, CH_2-9), 3.08 (t, 2H, J = 7 Hz, CH_2-17), 3.98 (s, 2H, CH_2-Ar), 4.50 (br s, 1H, NHBoc), 6.57 (s, 2H, Ar-H), 6.81 (s, 1H, Ar-H), 7.55 (br s, 1H, NH). Anal. ($C_{32}H_{49}N_3O_4$·0.4H_2O) C, H, N, O.

3-[*N*-(*tert*-Butoxycarbonyl)glycylglycyl]amino-4-(3,5-dimethylbenzyl)-5-ethyl-6-methylpyridin-2(*1H*)-one (29b). From *N*-(*tert*-butoxycarbonyl)glycylglycine **28b** (2.76 g, 11.91 mmol) and pyridinone analogue **27** (0.46 g, 1.70 mmol) was obtained compound **29b** as a white solid (0.60 g, 72.5% yield) after flash chromatography (CH_2Cl_2/EtOH: 1/0 to 9/1): TLC (CH_2Cl_2/EtOH: 9/1) R_f 0.21; mp 187−188 °C; ^{1}H NMR ($CDCl_3$) δ 0.84 (t, 3H, J = 6 Hz, CH_2CH_3), 1.37 (s, 9H, Boc), 2.19 (s, 6H, Ar-CH_3), 2.24 (s, 3H, CH_3-6), 2.32 (q, 2H, J = 7 Hz, CH_2CH_3), 3.75 (br s, 2H, CH_2-9), 3.83 (s, 2H, CH_2Ar), 3.96 (br s, 2H, CH_2-12), 5.58 (br s, 1H, NHBoc), 6.62 (s, 2H, Ar-H), 6.75 (s, 1H, Ar-H), 7.65 (br s, 1H, NH), 8.34 (br s, 1H, NH), 12.65 (br s, 1H, NH-1). Anal. ($C_{26}H_{36}N_4O_5$·H_2O) C, H.

4-(3,5-Dimethylbenzyl)-5-ethyl-3-(4-methoxycarbonylbutanoyl)amino-6-methylpyridin-2(*1H*)-one (29c). From monomethylglutarate **28c** (0.76 g, 5.18 mmol) and pyridinone analogue **27** (0.20 g, 0.74 mmol) was obtained compound **29c** as a white solid (0.22 g, 75% yield) after flash chromatography (CH_2Cl_2/EtOH: 1/0 to 95/5) TLC (CH_2Cl_2/EtOH: 95/5) R_f 0.08; mp 205−206 °C; ^{1}H NMR ($CDCl_3$) δ 0.95 (t, 3H, J = 7 Hz, CH_2CH_3), 1.85−2.05 (m, 2H, CH_2-10), 2.23 (s, 6H, Ar-CH_3), 2.37 (s, 3H, CH_3-6), 2.41−2.47 (m, 6H, CH_2-9, CH_2-11,CH_2-CH_3), 3.63 (s, 3H, COOCH_3), 3.92 (s, 2H, CH_2Ar), 6.59 (s, 2H, Ar-H), 6.81 (s, 1H, Ar-H), 7.15 (br s, 1H, NH), 12.50 (br s, 1H, NH-1). Anal. ($C_{23}H_{30}N_2O_4$·0.25H_2O) C, H.

4-(3,5-Dimethylbenzyl)-5-ethyl-3-(9-methoxycarbonylnonanoyl)amino-6-methylpyridin-2(1*H*)-one (29d). From sebacic acid monomethylester **28d** (1.12 g, 5.18 mmol) and pyridinone analogue **27** (0.20 g, 0.74 mmol) was obtained compound **29d** as a white solid (0.27 g, 77% yield) after flash chromatography (CH_2Cl_2/EtOH: 1/0 to 95/5): TLC (CH_2Cl_2/EtOH: 95/5) R_f 0.11; mp 161−162 °C; ^{1}H NMR ($CDCl_3$) δ 0.94 (t, 3H, J = 7 Hz, CH_2CH_3), 1.25 (br s, 8H, CH_2-11 to CH_2-14), 1.50−1.77 (m, 4H, CH_2-10, CH_2-15), 2.22 (s, 6H, Ar-CH_3), 2.34 (s, 3H, CH_3-6), 2.27−2.50 (m, 6H, CH_2-9, CH_2-16, CH_2CH_3), 3.64 (s, 2H, COOCH_3), 3.92 (s, 2H, CH_2Ar), 6.57 (s, 2H, Ar-H), 6.80 (s, 1H, Ar-H), 7.01 (br s, 1H, NH), 12.25 (br s, 1H, NH-1). Anal. ($C_{28}H_{40}N_2O_4$) C, H, N, O.

General Procedure for the Synthesis of the Pyridinone Analogues 30a′, 30b′. The N-protected pyridinones **29a** and **29b** were dissolved in EtOAc. Concentrated hydrochloric acid was added, and the mixture was stirred at room temperature for 20 to 30 min, basified by addition of concentrated ammonia. The solvent was removed in vacuo, and water was added. The obtained precipitate was filtered off, washed with small quantities of water, and dried over calcium chloride under vacuum.

3-(10-Aminodecanoyl)amino-4-(3,5-dimethylbenzyl)-5-ethyl-6-methylpyridin-2(*1H*)-one (30a′). Compound **29a** (0.15 g, 0.28 mmol) was deprotected with 0.64 mL of concentrated hydrochloric acid to give compound **30a′** as a yellow solid (73 mg, 60% yield): TLC (CH_2Cl_2/EtOH: 8/2) R_f 0.08; mp 239−240 °C; ^{1}H NMR ($CDCl_3$ and CD_3OD) δ 0.69 (t, 3H, J = 7 Hz, CH_2CH_3), 1.05 (br s, 10H, CH_2-11 to CH_2-15), 1.37 (s, 4H, CH_2-10, CH_2-16), 1.99 (s, 6H, Ar-CH_3), 2.06 (s, 3H, CH_3-6), 2.03−2.10 (m, 2H, CH_2-9), 2.12 (q, 2H, J = 7.5 Hz, CH_2-CH_3), 2.64 (t, 2H, J = 7 Hz, CH_2-17), 3.61 (s, 2H, CH_2Ar), 6.39 (s, 2H, Ar-H), 6.57 (s, 1H, Ar-H). Anal. ($C_{27}H_{41}N_3O_4$·0.4H_2O) C, H, O.

4-(3,5-Dimethylbenzyl)-5-ethyl-3-(*N*-glycylglycyl)amino-6-methylpyridin-2(1*H*)-one (30b′). Compound **29b** (0.20 g, 0.41 mmol) was deprotected with 0.95 mL of concentrated hydrochloric acid to give compound **30b′** as a white solid (59 mg, 73.5% yield): TLC (CH_2Cl_2/EtOH: 9/1) R_f 0.29; mp 154−155 °C; ^{1}H NMR (DMSO-d_6) δ 0.80 (t, 3H, J = 7 Hz, CH_2CH_3), 2.20 (s, 3H, CH_3-6), 2.23 (s, 6H, Ar-CH_3), 2.27 (q, 2H, J = 7 Hz, CH_2CH_3), 3.63 (s, 2H, CH_2-9), 3.75 (s, 2H, CH_2Ar), 3.98 (d, 2H, J = 5 Hz, CH_2-12), 6.76 (s, 2H, Ar-H), 6.82 (s, 1H, Ar-H), 8.06 (br s, 2H, NH_2), 8.69 (tl, 1H, J = 5 Hz, NH), 9.18 (s, 1H, NH), 11.68 (br s, 1H, NH-1). Anal. ($C_{21}H_{28}N_4O_3$·1.25H_2O) C, H.

General Procedure for the Synthesis of the Pyridinone Analogues 30c′, 30d′. An aqueous solution of 4 N sodium hydroxide was added to the suspension of pyridinone **29c** or **29d** in a mixture of MeOH/THF (1/1.5). The solid was immediately dissolved. The mixture was stirred at room temperature for 5 min. The solvents were removed in vacuo, and water was added. The aqueous layer thus obtained was acidified by addition of an aqueous solution of 3 N hydrochloric acid. The precipitate was filtered off, washed with water, and dried over calcium chloride under vacuum at room temperature.

3-(4-Carboxybutanoyl)amino-4-(3,5-dimethylbenzyl)-5-ethyl-6-methylpyridin-2(*1H*)-one (30c′). Compound **29c**

(98 mg, 0.25 mmol) was deprotected with 0.60 mL of aqueous solution of 4 N sodium hydroxide to give compound **30c′** as a white solid (70 mg, 74% yield): TLC (CH_2Cl_2/EtOH: 9/1) R_f 0.07; mp 234−235 °C; ^{1}H NMR (DMSO-d_6) δ 0.81 (t, 3H, J = 7 Hz, CH_2CH_3), 1.69−1.86 (m, 2H, CH_2-10), 2.19 (s, 3H, CH_3-6), 2.23 (s, 6H, Ar-CH_3), 2.23−2.39 (m, 6H, CH_2-9, CH_2-11,CH_2-CH_3), 3.74 (s, 2H, CH_2Ar), 6.76 (s, 2H, Ar-H), 6.81 (s, 1H, Ar-H), 8.97 (s, 1H, CO_2H), 11.74 (br s, 1H, NH-1). Anal. ($C_{22}H_{28}N_2O_4$) C, H, N.

3-(9-Carboxynonanoyl)amino-4-(3,5-dimethylbenzyl)-5-ethyl-6-methylpyridin-2(*1H*)-one (30d′). Compound **29d** (200 mg, 0.43 mmol) was deprotected with 1.0 mL of aqueous solution of 4 N sodium hydroxide to give compound **30d′** as a white solid (188 mg, 97% yield): TLC (CH_2Cl_2/EtOH: 9/1) R_f 0.14; mp 134−135 °C; ^{1}H NMR (DMSO-d_6) δ 0.81 (t, 3H, J = 7 Hz, CH_2CH_3), 1.20 (br s, 8H, CH_2-11 to CH_2-14), 1.44−1.64 (m, 4H, CH_2-10, CH_2-15), 2.15 (s, 3H, CH_3-6), 2.22 (s, 6H, Ar-CH_3), 2.15−2.36 (m, 6H, CH_2-9, CH_2-16,CH_2CH_3), 3.74 (s, 2H, CH_2Ar), 6.76 (s, 2H, Ar-H), 6.81 (s, 1H, Ar-H), 8.94 (s, 1H, CO_2H), 11.75 (br s, 1H, NH-1). Anal. ($C_{27}H_{38}N_2O_4$·0.25H_2O) C, H, N.

3′-Azido-5-(2-carboxyethyl)-2′,3′-dideoxyuridine (31). To a solution of methyl ester **20** (403.0 mg, 1.18 mmol) in MeOH (5 mL) was added 2 mL of 2 N NaOH dropwise at 0 °C. After being stirred for 3 h at room temperature, the mixture was neutralized with Dowex 50X2−200 mesh ion-exchange resin. After filtration and evaporation, the residue obtained was rinsed with CH_2Cl_2 and dissolved in MeOH, and the product **31** (365 mg, 95% yield) precipitated by adding diethyl ether: TLC (EtOAc/MeOH: 8/2) R_f 0.35; mp 170−172 °C; $[\alpha]^{20}_D$ +35° (*c* 1.1, MeOH); IR (KBr) 3600−3200, 2109, 1703, 1665 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.25−2.40 (m, 6H, CH_2-2′, CH_2-7, CH_2-8), 3.55−3.65 (m, 2H, CH_2-5′), 3.83 (m, 1H, CH-4′), 4.41 (m, 1H, CH-3′), 6.09 (m, 1H, CH-1′), 7.71 (s, 1H, CH-6), 11.20 (br, 2H, NH, COOH).

3′-Azido-2′,3′-dideoxy-5-[[[3-(methoxycarbonyl)propyl]-amino]-3-oxopropyl]uridine (32). To a cooled solution of 130 mg (0.40 mmol) of **31** and 60 μL (0.54 mmol) of *N*-methylmorpholine (NMM) in DMF (3 mL) was added 2-chloro-4,6-dimethoxy-1,3,5-triazine (70 mg, 0.40 mmol). After the mixture was stirred for 3 h at 0 °C, NMM (60 μL, 0.54 mmol) and methyl-γ-aminobutyrate hydrochloride[55] (63 mg, 0.41 mmol) were added successively. After 20 h, the solvent was removed in vacuo, and the residue was purified by flash chromatography (EtOAc/MeOH: 94/4) to give **32** as a white solid (130 mg, 76% yield): mp (EtOAc) 128−130 °C; $[\alpha]^{20}_D$ +27.5° (*c* 1.0, MeOH); IR (KBr) 3406, 3322, 1735, 1710, 1676, 1658 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.64 (m, 2H, CH_2-12), 2.25−2.45 (m, 8H, CH_2-2′, CH_2-7, CH_2-8, CH_2-13), 3.04 (m, 2H, CH_2-11), 3.58 (s, 3H, OCH_3), 3.60 (m, 2H, CH_2-5′), 3.84 (m, 1H, CH-4′), 4.41 (dd, 1H, J = 11.5, 5.5. Hz, CH-3′), 5.20 (br s, 1H, OH), 6.09 (t, 1H, J = 6 Hz, CH-1′), 7.64 (s, 1H, CH-6), 7.86 (t, 1H, J = 5.5 Hz, NH-10), 11.37 (br s, 1H, NH-3); MS (CI−NH_3) *m*/*z* 425 (M + H)$^+$. Anal. ($C_{17}H_{24}N_6O_7$) C, H, N.

3′-Azido-5-[[(3-carboxypropyl)amino]-3-oxopropyl]-2′,3′-dideoxyuridine (33). To a solution of methylester **32** (100 mg, 0.23 mmol) in MeOH (4 mL) was added 0.5 mL of 2 N NaOH at 0 °C dropwise. After being stirred for 4 h at room temperature, the mixture was neutralized with Dowex 50X2−200 ion-exchange resin. After filtration and concentration, the residue obtained was rinsed with CH_2Cl_2, then recrystallized in MeOH to give **33** as a white solid (71 mg, 73% yield): TLC (EtOAc/MeOH/AcOH: 9/1/0.02) R_f 0.16; mp 155−156 °C; $[\alpha]^{20}_D$ +30° (*c* 1.0, MeOH); IR (KBr) 3405, 3353, 2123, 1703, 1683, 1642 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.60 (m, 2H, CH_2-12), 2.15−2.45 (m, 8H, CH_2-2′, CH_2-7, CH_2-8, CH_2-13), 3.03 (m, 2H, CH_2-11), 3.61 (m, 2H, CH_2-5′), 3.81 (m, 1H, CH-4′), 4.40 (dd, 1H, J = 11.5, 5.5 Hz, CH-3′), 5.20 (br s, 1H, OH), 6.08 (t, 1H, J = 6.5 Hz, CH-1′), 7.63 (s, 1H, CH-6), 7.84 (t, 1H, J = 5.5 Hz, NH-10), 11.35 (br s, 1H, NH-3), 12.10 (br, 1H, COOH).

General Procedure for the Synthesis of Heterodimers 14, 22, 23, 26, 34−37. To a cooled solution of carboxylic acid compound **13**, **31**, **33**, **30c′**, or **30d′** (1.0 equiv) and *N*-methylmorpholine (1.5 equiv) in dry DMF was added 2-chloro-4,6-dimethoxy-1,3,5-triazine (1.1 equiv). After the mixture was stirred for 3 h at 0 °C, a solution was added, in DMF, of the amino derivative **9**, **21a**, **21b**, **25**, **30a′**, or **30b′** (≈1.0 equiv). After 20 h, the solvent was removed in vacuo, and the residue was chromatographed.

3′-Azido-3-[*N*-15-[6-(3,5-dimethylphenylthio)-5-ethyl-1-(5-oxopentyl)uracil]-4,15-diaza-3-oxopentadecanyl]-3′-deoxy-5′-*O*-(thexyldimethylsilyl)thymidine (14). From the nucleoside **9** (160 mg, 0.252 mmol) and HEPT analogue **13** (95 mg, 0.250 mmol) was obtained compound **14** as a white solid (150 mg, 60% yield) after flash chromatography (EtOAc/MeOH: 98/2): mp 93−95 °C; ^{1}H NMR (DMSO-d_6) δ 0.13 (d, 6H, J = 2.5 Hz, $(CH_3)_2$Si), 0.84−0.94 (m, 15H, $(CH_3)_2$C × 2, CH_2CH_3), 1.24 (br s, 12H, CH_2-7* to CH_2-12*), 1.30−1.45 (m, 8H, CH_2-6*, CH_2-13*, CH_2-8″, CH_2-9″), 1.60 (m, 1H, $(CH_3)_2CH$), 1.84 (s, 3H, CH_3-5), 1.99 (m, 2H, CH_2-10″), 2.25 (s, 6H, Ar-CH_3), 2.25−2.40 (m, 4H, CH_2-2′, CH_2-2*), 2.50 (CH_2CH_3 with DMSO), 2.99 (m, 4H, CH_2-5*, CH_2-14*), 3.75−3.90 (m, 5H, CH-4′, CH_2-5′, CH_2-7″), 4.00 (m, 2H, CH_2-1*), 4.37 (m, 1H, CH-3′), 6.12 (t, 1H, J = 6 Hz, CH-1′), 6.91 (s, 2H, Ar-H), 6.93 (s, 1H, Ar-H), 7.20 (t, 1H, J = 5.5 Hz, NHCO), 7.51 (s, 1H, CH-6), 7.88 (t, 1H, J = 5.5 Hz, NHCO), 11.60 (br s, 1H, NH-3″); MS (ESI) *m*/*z* 874.5 (M + Na)$^+$, 852.5 (M + H)$^+$.

3′-Azido-2′,3′-dideoxy-5-[*N*-9-[6-(3,5-dimethylphenylthio)-5-ethyl-1-(5-oxopentyl)uracil]-4,9-diaza-3-oxononanyl]-uridine (22). From the nucleoside **21a** (54 mg, 0.144 mmol) and HEPT analogue **13** (58 mg, 0.146 mmol) was obtained compound **22** as a white solid (62 mg, 57%) after flash chromatography (EtOAc/MeOH: 90/10) and recrystallization from EtOAc/MeOH: mp 120−123 °C; ^{1}H NMR (DMSO-d_6) δ 0.91 (t, 3H, J = 7.5 Hz, CH_2CH_3), 1.30−1.45 (m, 8H, CH_2-12*, CH_2-13*, CH_2-8″, CH_2-9″), 1.98 (m, 2H, CH_2-10″), 2.20−2.40 (m, 10H, CH_2-2′, CH_2-2*, Ar-CH_3), 2.42 (m, 2H, CH_2-1*), 2.50 (CH_2CH_3 with DMSO), 3.00 (m, 4H, CH_2-5*, CH_2-8*), 3.63 (m, 2H, CH_2-5′), 3.83 (m, 3H, CH-4′, CH_2-7″), 4.41 (m, 1H, CH-3′), 5.25 (br s, 1H, OH), 6.10 (m, 1H, CH-1′), 6.90 (s, 2H, Ar-H), 6.92 (s, 1H, Ar-H), 7.63 (s, 1H, CH-6), 7.72 (br s, 1H, NHCO), 7.79 (br s, 1H, NHCO), 11.35 (br s, 1H, NH), 11.60 (br s, 1H, NH); MS (ESI) *m*/*z* 751 (M + Na)$^+$. Anal. ($C_{35}H_{47}N_9O_8S$) C, H, N.

3′-Azido-2′,3′-dideoxy-5-[*N*-15-[6-(3,5-dimethylphenylthio)-5-ethyl-1-(5-oxopentyl)uracil]-4,15-diaza-3-oxopentadecanyl]uridine (23). From the nucleoside **21b** (71 mg, 0.148 mmol) and HEPT analogue **13** (58 mg, 0.146 mmol) was obtained compound **23** as a white solid (71 mg, 57% yield) after flash chromatography (EtOAc/MeOH: 95/5) and recrystallization from EtOAc: mp 98−100 °C; ^{1}H NMR (DMSO-d_6) δ 0.90 (t, 3H, J = 7.5 Hz, CH_2CH_3), 1.20 (br s, 12H, CH_2-7* to CH_2-12*), 1.35−1.45 (m, 8H, CH_2-6*, CH_2-13*, CH_2-8″, CH_2-9″), 1.97 (m, 2H, CH_2-10″), 2.20−2.35 (m, 10H, CH_2-2′, CH_2-2*, Ar-CH_3), 2.40 (m, 2H, CH_2-1*), 2.50 (CH_2CH_3 with DMSO), 2.98 (m, 4H, CH_2-5*, CH_2-14*), 3.61 (m, 2H, CH_2-5′), 3.80 (m, 3H, CH-4′, CH_2-7″), 4.40 (m, 1H, CH-3′), 5.23 (br s, 1H, OH), 6.08 (t, 1H, J = 6.5 Hz, CH-1′), 6.89 (s, 2H, Ar-H), 6.91 (s, 1H, Ar-H), 7.61 (s, 1H, CH-6), 7.67 (t, 1H, J = 5.5 Hz, NHCO), 7.77 (t, 1H, J = 5.5 Hz, NHCO), 11.35 (br s, 1H, NH), 11.60 (br s, 1H, NH); MS (ESI) *m*/*z* 860 (M + Na)$^+$, 838 (M + H)$^+$. Anal. ($C_{41}H_{59}N_9O_8S$) C, H, N.

2′,3′-Dideoxy-4-*N*-[*N*-13-[6-(3,5-dimethylphenylthio)-5-ethyl-1-(5-oxopentyl)uracil]-13-azadodecyl]cytidine (26). From the nucleoside **25** (74 mg, 0.180 mmol) and HEPT analogue **13** (68 mg, 0.181 mmol) was obtained compound **26** as a white solid (98 mg, 69% yield): mp 116−118 °C; ^{1}H NMR (DMSO-d_6) δ 0.92 (t, 3H, J = 7.5 Hz, CH_2CH_3), 1.25 (m, 16H, CH_2-3* to CH_2-10*), 1.30−1.50 (m, 8H, CH_2-2*, CH_2-11*, CH_2-8″, CH_2-9″), 1.75−1.90 (m, 3H, CH-2′a, CH_2-3′), 1.98 (m, 2H, CH_2-10″), 2.20−2.25 (m, 7H, CH-2′b, Ar-CH_3), 2.50 (CH_2CH_3 with DMSO), 2.99 (m, 2H, CH_2-12*), 3.19 (dt, 2H, J = 6.5, 6.5 Hz, CH_2-1*), 3.54 (dd, 1H, J = 12, 4 Hz, CH-5′a), 3.66 (d, 1H, J = 12 Hz, CH-5′b), 3.82 (m, 2H, CH_2-7″), 4.02 (m, 1H, CH-4′), 5.00 (br s, 1H, OH), 5.70 (d, 1H, J = 7.5 Hz, CH-5), 5.93 (dd, 1H, J = 6.5, 3 Hz, CH-1′), 6.90 (s, 2H, Ar-H), 6.93 (s, 1H, Ar-H), 7.61 (t, 1H, J = 5.5 Hz, NH), 7.72 (t, 1H, J = 5.5 Hz, NHCO), 7.84 (d, 1H, J = 7.5 Hz, CH-6), 11.60 (br s, 1H, NH-

3″); MS (ESI) *m*/*z* 775.5 $(M + Na)^+$, 753.5 $(M + H)^+$. Anal. ($C_{40}H_{60}N_6O_6S$) C, H, N.

3′-Azido-2′,3′-dideoxy-5-[*N*-15-[4-(3,5-dimethylbenzyl)-5-ethyl-6-methyl-2-oxo-(*1H*)-pyridin-3-yl]-4,15-diaza-3,14-dioxopentadecanyl]uridine (34). From the nucleoside **31** (22 mg, 0.067 mmol) and pyridinone analogue **30a′** (25 mg, 0.058 mmol) was obtained compound **34** as a white solid (28 mg, 65% yield) after flash chromatography (EtOAc/MeOH: 85/15): mp 136−138 °C; ^{1}H NMR (DMSO-d_6) δ 0.78 (t, 3H, J = 7 Hz, CH_2CH_3), 1.10−1.30 (m, 10H, CH_2-7* to CH_2-11*), 1.30−1.45 (m, 2H, CH_2-12*), 1.45−1.55 (m, 2H, CH_2-6*), 2.15−2.40 (m, 17H, CH_2-2′, CH_2-2*, CH_2-13*, CH_3-6″, CH_2CH_3, Ar-CH_3), 2.40−2.45 (m, 2H, CH_2-1*), 2.98−3.05 (m, 2H, CH_2-5*), 3.60−3.65 (m, 2H, CH_2-5′), 3.71 (s, 2H, CH_2Ar), 3.84 (m, 1H, CH-4′), 4.43 (m, 1H, CH-3′), 5.26 (br s, 1H, OH), 6.10 (t, 1H, J = 6.5 Hz, CH-1′), 6.73 (s, 2H, Ar-H), 6.77 (s, 1H, Ar-H), 7.68 (s, 1H, CH-6), 7.79 (t, 1H, J = 5.5 Hz, NH-4*), 8.92 (br s, 1H, NH-7″), 11.35 (br s, 1H, NH), 11.60 (br s, 1H, NH); MS (ESI) *m*/*z* 769 $(M + Na)^+$, 747 $(M + H)^+$. Anal. ($C_{39}H_{54}N_8O_7$) C, H, N.

3′-Azido-2′,3′-dideoxy-5-[*N*-15-[4-(3,5-dimethylbenzyl)-5-ethyl-6-methyl-2-oxo-(*1H*)-pyridin-3-yl]-4,9,12,15-tetraaza-3,8,11,14-tetraoxopentadecanyl]uridine (35). From the nucleoside **33** (60 mg, 0.146 mmol) and pyridinone analogue **30b′** (58 mg, 0.151 mmol) was obtained compound **35** as an amorphous white solid (85 mg, 75% yield) after flash chromatography (CH_2Cl_2/MeOH: 8/2): ^{1}H NMR (DMSO-d_6) δ 0.75 (t, 3H, J = 7 Hz, CH_2CH_3), 1.57−1.62 (m, 2H, CH_2-6*), 2.09−2.35 (m, 17H, CH_2-2′, CH_2-2*, CH_2-7*, CH_3-6″, CH_2CH_3, Ar-CH_3), 2.39−2.42 (m, 2H, CH_2-1*), 3.00−3.05 (m, 2H, CH_2-5*), 3.61 (m, 2H, CH_2-5′), 3.68−3.72 (m, 4H, $COCH_2NH$, CH_2-Ar), 3.80−3.84 (m, 3H, CH-4′, $COCH_2NH$), 4.41 (m, 1H, CH-3′), 5.32 (t, 1H, J = 5 Hz, OH), 6.08 (t, 1H, J = 6.5 Hz, CH-1′), 6.72 (s, 2H, Ar-H), 6.77 (s, 1H, Ar-H), 7.66 (s, 1H, CH-6), 7.92 (t, 1H, J = 5 Hz, NHCO), 8.13 (m, 2H, NHCO × 2), 8.99 (br s, 1H, NH-7″), 11.30 (br s, 1H, NH), 11.65 (br s, 1H, NH); MS (ESI) *m*/*z* 799 $(M + Na)^+$. Anal. ($C_{37}H_{48}N_{10}O_9 \cdot 2H_2O$) C, H, N.

3′-Azido-2′,3′-dideoxy-5-[*N*-15-[4-(3,5-dimethylbenzyl)-5-ethyl-6-methyl-2-oxo-(*1H*)-pyridin-3-yl]-4,9,15-triaza-3,-10,14-trioxopentadecanyl]uridine (36). From the nucleoside **21a** (51 mg, 0.13 mmol) and pyridinone analogue **30c′** (50 mg, 0.13 mmol) was obtained compound **36** as a yellow light solid (56 mg, 57% yield) after flash chromatography (CH_2Cl_2/EtOH: 1/0 to 8/2): TLC (CH_2Cl_2/EtOH: 8/2) R_f 0.55; mp 170−171 °C; ^{1}H NMR (CD_3OD) δ 0.88 (t, 3H, J = 7 Hz, CH_2CH_3), 1.40−1.50 (m, 4H, CH_2-6*, CH_2-7*), 1.85−1.95 (m, 2H, CH_2-12*), 2.20 (s, 6H, Ar-CH_3), 2.28 (s, 3H, CH_3-6″), 2.23−2.38 (m, 12H, CH_2-1*, CH_2-2*, CH_2-11*, CH_2-13*, CH_2-2′, CH_2CH_3), 2.57 (t, 2H, J = 6 Hz, CH_2-8*), 3.00−3.20 (m, 4H, CH_2-5*, CH_2-Ar), 3.84−3.90 (m, 3H, CH_2-5′, CH-4′), 4.30 (m, 1H, CH-3′), 6.15 (t, 1H, J = 6 Hz, CH-1′), 6.65 (s, 2H, Ar-H), 6.77 (s, 1H, Ar-H), 7.77 (s, 1H, CH-6). Anal. ($C_{38}H_{51}N_9O_8 \cdot 2H_2O$) C, H, N.

3′-Azido-2′,3′-dideoxy-5-[*N*-20-[4-(3,5-dimethylbenzyl)-5-ethyl-6-methyl-2-oxo-(*1H*)-pyridin-3-yl]-4,9,20-triaza-3,-10,19-trioxoicosanyl]uridine (37). From the nucleoside **21a** (43 mg, 0.11 mmol) and pyridinone analogue **30d′** (50 mg, 0.11 mmol), compound **37** was obtained as a white solid (57 mg, 63% yield) after flash chromatography (CH_2Cl_2/EtOH: 1/0 to 8/2): TLC (CH_2Cl_2/EtOH: 9/1) R_f 0.47; mp 164−165 °C; ^{1}H NMR (CD_3OD) δ 0.88 (t, 3H, J = 7 Hz, CH_2CH_3), 1.13−1.30 (m, 8H, CH_2-13*, CH_2-14*, CH_2-15*, CH_2-16*), 1.45−1.50 (m, 4H, CH_2-6*, CH_2-7*), 1.50−1.57 (m, 4H, CH_2-12*, CH_2-17*), 2.13 (t, 2H, J = 8 Hz, CH_2-2*) 2.21 (s, 6H, Ar-CH_3), 2.29 (s, 3H, CH_3-6″), 2.32−2.41 (m, 10H, CH_2-1*, CH_2-11*, CH_2-18*, CH_2-2′, CH_2CH_3), 2.98 (s, 2H, CH_2Ar), 3.00−3.15 (m, 4H, CH_2-5*, CH_2-8*), 3.75−3.92 (m, 3H, CH_2-5′, CH-4′), 4.35 (q, 1H, J = 5 Hz, CH-3′), 6.16 (t, 1H, J = 6 Hz, CH-1′), 6.68 (s, 2H, Ar-H), 6.79 (s, 1H, Ar-H), 7.78 (s, 1H, CH-6), 7.97 (br s, 1H, NH). Anal. ($C_{43}H_{61}N_9O_8 \cdot 0.5H_2O$) C, H, N, O.

3′-Azido-3-[*N*-15-[6-(3,5-dimethylphenylthio)-5-ethyl-1-(5-oxopentyl)uracil]-4,15-diaza-3-oxopentadecanyl]-3′-deoxythymidine (15). Dowex 50X2−200 ion-exchange resin (150 mg) was added to a solution of **14** (100 mg, 0.101 mmol) in MeOH (4 mL). After 16 h, the resin was removed by filtration and washed throughly with MeOH, and the filtrate was evaporated. After flash chromatography (CH_2Cl_2/MeOH: 95/5) and trituration with diethyl ether, **15** was obtained as a white solid (73 mg, 85% yield): mp 148−150 °C; ^{1}H NMR (DMSO-d_6) δ 0.90 (t, 3H, J = 7 Hz, CH_2CH_3), 1.22 (br s, 12H, CH_2-7* to CH_2-12*), 1.30−1.50 (m, 8H, CH_2-6*, CH_2-13*, CH_2-8″, CH_2-9″), 1.82 (s, 3H, CH_3-5), 1.96 (m, 2H, CH_2-10″), 2.24 (s, 6H, Ar-CH_3), 2.25−2.40 (m, 4H, CH_2-2′, CH_2-2*), 2.50 (CH_2-CH_3 with DMSO), 2.97 (m, 4H, CH_2-5*, CH_2-14*), 3.63 (m, 2H, CH_2-5′), 3.83 (m, 3H, CH-4′, CH_2-7″), 3.98 (m, 2H, CH_2-1*), 4.40 (m, 1H, CH-3′), 5.25 (br s, 1H, OH), 6.21 (t, 1H, J = 6 Hz, CH-1′), 6.89 (s, 2H, Ar-H), 6.92 (s, 1H, Ar-H), 7.70 (t, 1H, J = 5.5 Hz, NHCO), 7.75 (s, 1H, CH-6), 7.85 (t, 1H, J = 5.5 Hz, NHCO), 11.50 (br s, 1H, NH-3″); MS (ESI) *m*/*z* 874 $(M + Na)^+$, 852 $(M + H)^+$. Anal. ($C_{42}H_{61}N_9O_8S$) C, H, N.

Antiviral Assay Procedures. The effects of the compounds on the replication of HIV-1 were evaluated (see Tables 1−3), as previously described[68] in CEM-SS cells and MT-4 cells. In brief, the determination of anti HIV-1 IIIB activity in MT-4 cells was based on a reduction of the virus induced cytopathogenicity; the cell viability was measured by the mitochondrial dehydrogenase activity, an enzyme reducing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromine (MTT assay) into formazan (formazan production was measured by reading the optical density at 540 nm).[69] The virus production by HIV-1 LAI or nevirapine-resistant HIV-1 CEM-SS infected cells was measured by the reverse transcriptase activity associated with virions released in culture supernatants. MT-4 and CEM-SS cells were incubated, respectively, with 50 or 100 $TCID_{50}$ of the different virus; after a 30 min adsorption, free virus was eliminated by washes and cells were then cultured in the presence of different concentrations of compounds for 5 days, the time at which virus multiplication was determinated. The 50% inhibition of virus multiplication (EC_{50}) was derived from a computer-generated median effect plot of the dose−effect data.[70] In the same experiment, cytotoxicity of the molecules was evaluated on uninfected cells using the MTT assay. The 50% cytotoxic concentration (CC_{50}) was defined as the concentration of drugs which reduced the cell viability by 50% and was calculated using the program aforementionated. Preparation of PBMC and infection with HIV-1 IIIB or HIV-2 D 194 have already been reported.[68] CEM-SS cells were obtained from Peter Nara and nevirapine-resistant HIV-1(N119, point mutation at RT codon 181) from D. Richman through the AIDS Research and References Reagent Program, Division of AIDS, NIAID, NIH.

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References

(1) Presented in part: Pontikis, R.; Note, R.; Dechaux, E.; Guillaumel, J.; Aubertin, A.-M.; Grierson, D. S.; Monneret, C. "Mixed inhibitors" of HIV-reverse transcriptase: synthesis and antiviral activity. XIII International Round Table "Nucleosides, Nucleotides and Their Biological Applications", Sept 6−10, 1998, Montpellier, France; *Nucleosides Nucleotides* **1999**, *18*, 707−708.

(2) (a) For a review, see: Jacobo-Molina, A.; Arnold, E. HIV Reverse transcriptase structure−function relationships. *Biochemistry* **1991**, *30*, 6351−6361. (b) Goff, S. P. Retroviral reverse transcriptase: synthesis, structure and function. *J. Acquired Immune Defic. Syndr.* **1990**, *3*, 817−831.

(3) (a) Balzarini, J.; De Clercq, E. Analysis of inhibition of retroviral reverse transcriptase. *Methods Enzymol.* **1996**, *275*, 472−502. (b) Lipsky, J. J. Antiretroviral drugs for AIDS. *Lancet* **1996**, *348*, 800−803.

(4) Arts, E. J.; Wainberg, M. A. Mechanisms of nucleoside analogue antiviral activity and resistance during human immunodeficiency virus reverse transcription. *Antimicrob. Agents Chemother.* **1996**, *40*, 527−540.

(5) De Clercq, E. Development of resistance of human immunodeficiency virus (HIV) to anti-HIV agents: how to prevent the problem? *Int. J. Antimicrob. Agents* **1997**, *9*, 21−36.
(6) Flexner, C. HIV-protease inhibitors. *N. Engl. J. Med*. **1998**, *338*, 1281−1292.
(7) De Clercq, E. The role of nonnucleoside reverse transcriptase inhibitors (NNRTIs) in the therapy of HIV-1 infection. *Antiviral Res*. **1998**, *38*, 153−179.
(8) Proudfoot, J. R. Nonnucleoside inhibitors of HIV-1 reverse transcriptase. *Exp. Opin. Ther. Patents* **1998**, *8*, 971−982.
(9) Ren, J.; Esnouf, R.; Garman, E.; Somers, D.; Ross, C.; Kirby, I.; Keeling, J.; Darby, G.; Jones, Y.; Stuart, D.; Stammers, D. High-resolution structures of HIV-1 RT from four RT-inhibitor complexes. *Nature Struct. Biol*. **1995**, *2*, 293−302.
(10) Ding, J.; Das, K.; Moereels, H.; Koymans, L.; Andries, K.; Janssen, P. A. J.; Hughes, S. H.; Arnold, E. Structure of HIV-1 RT/TIBO R 86183 complex reveals similarity in the binding of diverse nonnucleoside inhibitors. *Nature Struct. Biol*. **1995**, *2*, 407−415.
(11) Holtzer, C. D.; Coleman, R. L. Use of nonnucleoside reverse-transcriptase inhibitors. *Am. J. Health-Syst. Pharm*. **1998**, *55*, 283−287.
(12) Piras, G.; Nakade, K.; Yuasa, S.; Baba, M. Three-drug combination of MKC-442, lamivudine and zidovudine in vitro: potential approach towards effective chemotherapy against HIV-1. *AIDS* **1997**, *11*, 469−475.
(13) Members of the Loviride Collaborative Study Group, Staszewski, S.; Miller, V.; Kober, A.; Colebunders, R.; Vandercam, B.; Delescluse, J.; Clumeck, N.; Van Wanzeele, F.; De Brabander, M.; De Creé, J.; Moeremans, M, Andries, K.; Boucher, C.; Stoffels, P.; Janssen, P. A. J. Evaluation of the efficacy and tolerance of R 018893, R 089439 (loviride) and placebo in asymptomatic HIV-1 infected patients. *Antiviral Ther*. **1996**, *1*, 42−50.
(14) De Clercq, E. Nonnucleoside reverse transcriptase inhibitors (NNRTIs) for the treatment of human immunodeficiency virus type 1 (HIV-1) infections: strategies to overcome drug resistance development. *Med. Res. Rev*. **1996**, *16*, 125−157.
(15) Johnson, V. A. Combination therapy for HIV-1 infection-overview: preclinical and clinical analysis of antiretroviral combinations. *Antiviral Res*. **1996**, *29*, 35−39.
(16) D'Aquila, R. T.; Hughes, M. D.; Johnson, V. A.; Fischl, M. A.; Sommadossi, J.-P.; Liou, S.-H.; Timpone, J.; Myers, M.; Basgoz, N.; Niu, M.; Hirsch, M. S. and the National Institute of Allergy and Infections Diseases AIDS Clinical Trials Group Protocol 241 Investigators. Nevirapine, zidovudine and didanosine compared with zidovudine and didanosine in patients with HIV-1 infection. *Ann. Int. Med*. **1996**, *124*, 1019−1030.
(17) (a) Goldman, M. E.; Nunberg, J. H.; O'Brien, J. A.; Quintero, J. C.; Schleif, W. A.; Freund, K. F.; Gaul, S. L.; Saari, W. S.; Wai, J. S.; Hoffman, J. M.; Anderson, P. S.; Hupe, D. J.; Emini, E. A.; Stern, A. M. Pyridinone derivatives: specific human immunodeficiency virus type 1 reverse transcriptase inhibitors with antiviral activity. *Proc. Natl. Acad. Sci. U.S.A*. **1991**, 88, 6863−6867. (b) Schooley, R. T.; Campbell, T. B.; Kuritzkes, D. R.; Blaschke, T.; Stein, D. S.; Rosandich, M. E.; Phair, J.; Pottage, J. C.; Messari, F.; Collier, A.; Kahn, J.; Ray, G.; Curtis, S.; Bakhtiari, M.; Kessler, H.; Coombs, R.; Corey, L.; Volberding, P.; Calandra, G.; Massari, F. Phase I study of combination therapy with L-697,661 and zidovudine. *J. Acquired Immune Defic. Syndr. Hum. Retrovirol*. **1996**, *12*, 363−370.
(18) (a) Chong, K.-T.; Pagano, P. J. Inhibition of human immunodeficiency virus type 1 infection in vitro by combination of delavirdine, zidovudine and didanosine. *Antiviral Res*. **1997**, *34*, 51−63. (b) Davey, R. T., Jr.; Chaitt, D. G.; Reed, G. F.; Freimuth, W. W.; Herpin, B. R.; Metcalf, J. A.; Eastman, P. S.; Falloon, J.; Kovacs, J. A.; Polis, M. A.; Walker, R. E.; Masur, H.; Boyle, J.; Coleman, S.; Cox, S. R.; Wathen, L.; Daenzer, C. L.; Lane, H. C. Randomized, controlled phaseI/II trial of combination therapy with delavirdine (U-90152S) and conventional nucleosides in human immunodeficiency virus type 1-infected patients. *Antimicrob. Agents Chemother*. **1996**, *40*, 1657−1664.
(19) Brennan, T. M.; Taylor, D. L.; Bridges, C. G.; Leyda, J. P.; Tyms, A. S. The inhibition of human immunodeficiency virus type 1 infection in vitro by a nonnucleoside reverse transcriptase inhibitor MKC-442, alone and in combination with other anti-HIV compounds. *Antiviral Res*. **1995**, *26*, 173−187.
(20) Larder, B. A. 3′-Azido-3′-deoxythymidine resistance suppressed by a mutation conferring human immunodeficiency virus type 1 resistance to nonnucleoside reverse transcriptase inhibitors. *Antimicrob. Agents Chemother*. **1992**, *36*, 2664−2669.
(21) Byrnes, V. W.; Emini, E. A.; Schleif, W. A.; Condra, J. H.; Schneider, C. L.; Long, W. J.; Wolfgang, J. A.; Graham, D. J.; Gotlib, L.; Schlabach, A. J.; Wolanski, B. S.; Blahy, O. M.; Quintero, J. C.; Rhodes, A.; Roth, E.; Titus, D. L.; Sardana, V. V. Susceptibilities of human immunodeficiency virus type 1 enzyme and viral variants expressing multiple resistance-engendering amino acid substitutions to reverse transcriptase inhibitors. *Antimicrob. Agents Chemother*. **1994**, *38*, 1404−1407.
(22) Vandamme, A.-M.; Van Vaerenbergh, K.; De Clercq, E. Anti-human immunodeficiency virus drug combination strategies. *Antiviral Chem. Chemother*. **1998**, *9*, 187−203.
(23) Spence, R. A.; Kati, W. M.; Anderson, K. S.; Johnson, K. A. Mechanism of inhibition of HIV-1 reverse transcriptase by nonnucleoside inhibitors. *Science* **1995**, *267*, 988−993.
(24) Rittinger, K.; Divita, G.; Goody, R. S. Human immunodeficiency virus reverse transcriptase substrate-induced conformational changes and the mechanism of inhibition by nonnucleoside inhibitors *Proc. Natl. Acad. Sci. U.S.A*. **1995**, *92*, 8046−8049.
(25) Gu, Z.; Quan, Y.; Li, Z.; Arts, E. J.; Wainberg, M. A. Effects of nonnucleoside inhibitors of human immunodeficiency virus type 1 in cell-free recombinant reverse transcriptase assays. *J. Biol. Chem*. **1995**, *270*, 31046−31051.
(26) (a) Shuker, S. B.; Hajduk, P. J.; Meadows, R. P.; Fesik, S. W. Discovering high-affinity ligands for proteins: SAR by NMR. *Science* **1996**, *274*, 1531−1534. (b) Jencks, W. P. On the attribution and additivity of binding energies. *Proc. Natl. Acad. Sci. U.S.A*. **1981**, *78*, 4046−4050.
(27) Nanni, R. G.; Ding, J.; Jacobo-Molina, A.; Hughes, S. H.; Arnold, E. Review of HIV-1 reverse transcriptase three-dimensional structure: implications for drug design. *Perspect. Drug Discov. Des*. **1993**, *1*, 129−150.
(28) Kohlstaedt, L. A.; Wang, J.; Friedman, J. M.; Rice, P. A.; Steitz, T. A. Crystal structure at 3.5 Å resolution of HIV-1 reverse transcriptase complexed with an inhibitor. *Science* **1992**, *256*, 1783−1790.
(29) Kroeger-Smith, M. B.; Rouzer, C. A.; Taneyhill, L. A.; Smith, N. A.; Hughes, S. H.; Boyer, P. L.; Janssen, P. A. J.; Moereels, H.; Koymans, L.; Arnold, E.; Ding, J.; Das, K.; Zhang, W.; Michejda, C.; Smith, R. H., Jr. Molecular modeling studies of HIV-1 reverse transcriptase nonnucleoside inhibitors: total energy of complexation as a predictor of drug placement and activity. *Protein Sci*. **1995**, *4*, 2203−2222.
(30) Mager, P. P. A check on rational drug design: molecular simulation of the allosteric inhibition of HIV-1 reverse transcriptase. *Med. Res. Rev*. **1997**, *17*, 235−276.
(31) Esnouf, R.; Ren, J.; Ross, C.; Jones, Y.; Stammers, D.; Stuart, D. Mechanism of inhibition of HIV-1 reverse transcriptase by nonnucleoside inhibitors. *Nature Struct. Biol*. **1995**, *2*, 303−308.
(32) Rodgers, D. W.; Gambling, S. J.; Harris, B. A.; Ray, S.; Culp, J. S.; Hellmig, B.; Woolf, D. J.; Debouck, C.; Harrison, S. C. The structure of unliganded reverse transcriptase from the human immunodeficiency virus type 1. *Proc. Natl. Acad. Sci. U.S.A*. **1995**, *92*, 1222−1226.
(33) Livermore, D. G. H.; Bethell, R. C.; Cammack, N.; Hancock, A. P.; Hann, M. M.; Green, D. V. S.; Lamont, R. B.; Noble, S. H.; Orr, D. C.; Payne, J. J.; Ramsay, M. V. J.; Shingler, A. H.; Smith, C.; Storer, R.; Williamson, C.; Willson, T. Synthesis and anti-HIV-1 activity of a series of imidazo[1,5-*b*]pyridazines. *J. Med. Chem*. **1993**, *36*, 3784−3794.
(34) Velázquez, S.; Alvarez, R.; San-Félix, A.; Jimeno, M.-L.; De Clercq, E.; Balzarini, J.; Camarasa, M.-J. Synthesis and anti-HIV activity of [AZT]-[TSAO-T] and [AZT]-[HEPT] dimers as potential multifunctional inhibitors of HIV-1 reverse transcriptase. *J. Med. Chem*. **1995**, *38*, 1641−1649. These authors constructed dimers of AZT with either TSAO-T or HEPT, the two inhibitor components being connected via the N-3 position of the thymine bases. The abscence of activity for the [AZT]-[HEPT] conjugates can now be rationalized from crystal structure data.[35] Indeed, one key feature of the RT-HEPT complex is a H-bond between N-3 of the inhibitors and the carbonyl oxygen of Lys 101. Interuption of this stabilizing interaction would be expected to result in a significant loss of activity.
(35) Hopkins, A. L.; Ren, J.; Esnouf, R. M.; Willcox, B. E.; Jones, Y.; Ross, C.; Miyasaka, T.; Walker, R. T.; Tanaka, H.; Stammers, D. K.; Stuart, D. I. Complexes of HIV-1 reverse transcriptase with inhibitors of the HEPT series reveal conformational changes relevant to the design of potent nonnucleoside inhibitors. *J. Med. Chem*. **1996**, *39*, 1589−1600.
(36) Renoud-Grappin, M.; Fossey, C.; Fontaine, G.; Ladurée, D.; Aubertin, A.-M.; Kirn, A. Imidazo[1,5-*b*]pyridazine-d4T conjugates: synthesis and anti-human immunodeficiency virus evaluation. *Antiviral Chem. Chemother*. **1998**, *9*, 205−223.
(37) Chamorro, C.; Camarasa, M.-J.; Pérez-Pérez, M.-J.; De Clercq, E.; Balzarini, J.; San Félix, A. An approach towards the synthesis of potential metal-chelating TSAO-T derivatives as bidentate inhibitors of human immunodeficiency virus type 1 reverse transcriptase. *Antiviral Chem. Chemother*. **1998**, *9*, 413−423.
(38) (a) Dollé, V.; Nguyen, C.-H.; Bisagni, E. Studies towards 4-C−Alkylation of Pyridin-2(*1H*)-one derivatives. *Tetrahedron* **1997**, *53*, 12505−12524. (b) Dollé, V.; Fan, E.; Nguyen, C.-H.; Aubertin, A.-M.; Kirn, A.; Andreola, M. L.; Jamieson, G.; Tarrago-Litvak,

L.; Bisagni, E. A new series of pyridinone derivatives as potent nonnucleoside human immunodeficiency virus type 1 specific reverse transcriptase inhibitors. *J. Med. Chem.* **1995**, *38*, 4679−4686. (c) Dollé, V.; Aubertin, A.-M.; Ludwig, O.; Nguyen, C.-H.; Bisagni, E.; Legraverend, M. Synthesis of new nonnucleoside inhibitors of HIV-1. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 173−178.

(39) Dechaux, E.; Pontikis, R.; Monneret, C. Efficient synthesis of N-4 alkyl derivatives of 2′,3′-dideoxycytidine (ddC). *Nucleosides Nucleotides* **1999**, *18*, 1−4. Anti HIV-1 activity of the *4-N*-butylddC: ≈50 μM.

(40) Adams, D. R.; Perez, C.; Maillard, M.; Florent, J.-C.; Evers, M.; Hénin, Y.; Litvak, S.; Litvak, L.; Monneret, C.; Grierson, D. S. Preparation and anti-HIV activity of N-3-substituted thymidine nucleoside analogues. *J. Med. Chem.* **1997**, *40*, 1550−1558.

(41) Dreyer, G. B.; Dervan, P. B. Sequence-specific cleavage of single-stranded DNA: oligodeoxynucleotide-EDTA.Fe(II). *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 968−972.

(42) Tanaka, H.; Takashima, H.; Ubasawa, M.; Sekiya, K.; Nitta, I.; Baba, M.; Shigeta, S.; Walker, R. T.; De Clercq, E.; Miyasaka T. Synthesis and antiviral activity of deoxy analogues of 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT) as potent and selective anti-HIV-1 agents. *J. Med. Chem.* **1992**, *35*, 4713−4719.

(43) Kim, D.-K.; Gam, J.; Kim, Y.-W.; Lim, J.; Kim, H.-T.; Kim, K. H. Synthesis and anti-HIV-1 activity of a series of 1-alkoxy-5-alkyl-6-(arylthio)uracils. *J. Med. Chem.* **1997**, *40*, 2363−2373.

(44) Pontikis R.; Benhida, R.; Aubertin, A.-M.; Grierson, D. S.; Monneret, C. Synthesis and anti-HIV activity of novel N-1 side chain-modified analogues of 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT). *J. Med. Chem.* **1997**, *40*, 1845−1854.

(45) Peshakova, L.; Kalcheva, V.; Madzhova, L. Interaction of thymine and uracils with some α,*β*-unsaturated esters. *Izv. Khim.* **1991**, *24*, 91−95.

(46) Taylor, M. J.; Dervan, P. B. Kinetic Analysis of sequence-specific alkylation of DNA by pyrimidine oligodeoxyribonucleotide-directed triple-helix formation. *Bioconjugate Chem.* **1997**, *8*, 354−364.

(47) The site of alkylation of **12** was established as N-1 by UV spectroscopy[48] [λ_{max} (MeOH) = 282 nM; λ_{max} (0.1 N NaOH) = 284 nm]. On the other hand, reaction of **11** with methyl 3-bromopropionate afforded solely the N-3 alkylated product which showed a bathochromic shift (19 nM) of the UV spectra in alkaline medium; this derivative is devoid of activity against HIV-1.

(48) (a) Shugar, D.; Fox, J. J. Spectrophotometric studies of nucleic acid derivatives and related compounds as a function of pH. I Pyrimidines. *Biochim. Biophys. Acta* **1952**, *9*, 199−218. (b) Shapiro, R.; Kang, S. Buffer-catalyzed tautomerism of uracil monoanion. *Biochim. Biophys. Acta* **1971**, *232*, 1−4.

(49) Lazrek, H. B.; Taourirte, M.; Barascut, J.-L.; Imbach, J.-L. Solid−liquid phase-transfert catalysis I: Studies of the N-alkylation of purines and pyrimidines. *Nucleosides Nucleotides* **1991**, *10*, 1285−1293.

(50) Kaminski, Z. J. 2-Chloro-4,6-dimethoxy-1,3,5-triazine. A new coupling reagent for peptide synthesis. *Synthesis* **1987**, 917−920.

(51) Maillard, M.; Faraj, A.; Frappier, F.; Florent, J. C.; Grierson, D. S.; Monneret, C. Synthesis of 3′-substituted-2′,3′-dideoxynucleoside analogues as potential anti-AIDS drugs. *Tetrahedron Lett.* **1989**, *30*, 1955−1958.

(52) Carboxylic acids **28b**,**c**,**d** are commercially available. Treatment of 10-aminodecanoic acid with *tert*-butyl dicarbonate and triethylamine in dichloromethane afforded N-protected derivative **28a** (72% yield) along with some dimeric compound.

(53) Thaisrivongs, S.; Pals, D. T.; Turner, S. R.; Kroll, L. T. Conformationally constrained renin inhibitory peptides: *γ*-lactam-bridged dipeptide isostere as conformational restriction. *J. Med. Chem.* **1988**, *31*, 1369−1376.

(54) Stahl, G. L.; Walter, R.; Smith, C. W. General procedure for the synthesis of mono-N-acylated 1,6-diaminohexanes. *J. Org. Chem.* **1978**, *43*, 2285−2286.

(55) (a) Methyl-*γ*-aminobutyrate hydrochloride was prepared by treatment of *γ*-aminobutyric acid with thionyl chloride in methanol; mp (ethanol, diethyl ether) 122−124 °C; lit.[55b] 121.5−122.5 °C. (b) Garmaise, D. L.; Schwartz, R.; McKay, A. F. Amino acids. VI. Preparation and chemistry of *ω*-carbalkoxyalkyl isothiocyanates. *J. Am. Chem. Soc.* **1958**, *80*, 3332−3335.

(56) Chu, C. K.; Schinazi, R. F.; Ahn, M. K.; Ullas, G. V.; Gu, Z. P. Structure−activity relationships of pyrimidine nucleosides as antiviral agents for human immunodeficiency virus type 1 in peripheral blood mononuclear cells. *J. Med. Chem.* **1989**, *32*, 612−617.

(57) Recently a MKC-442 analogue having the same *ϵ*-functionalized acyclic chain was reported[58] (EC_{50} = 38 μm vs HIV-1 IIIB in MT-4 cells).

(58) Tanaka, H.; Walker, R. T.; Hopkins, A. L.; Ren, J.; Jones, E. Y.; Fujimoto, K.; Hayashi, M.; Miyasaka, T.; Baba, M.; Stammers, D. K.; Stuart, D. I. Allosteric inhibitors against HIV-1 reverse transcriptase: design and synthesis of MKC-442 analogues having an *ω*-functionalized acyclic structure. *Antiviral Chem. Chemother.* **1998**, *9*, 325−332.

(59) Baba, M.; De Clercq, E.; Iida, S.; Tanaka, H.; Nitta, I.; Ubasawa, M.; Takashima, H.; Sekiya, K.; Umezu, K.; Nakashima, H.; Shigeta, S.; Walker, R. T.; Miyasaka, T. Anti-human immunodeficiency virus type 1 activities and pharmacokinetics of novel 6-substituted acyclouridine derivatives. *Antimicrob. Agents Chemother.* **1990**, *34*, 2358−2363.

(60) Discrepancy in inhibitory effects, between AZT-containing conjugates and derivative **26** elaborated from ddC, can be explained by differences in cellular metabolism, since AZT and ddC show different paterns of intracellular phosphorylation, due to the more or less specificity of the involved kinases.[61]

(61) Eriksson, S.; Kierdaszuk, B.; Munch-Petersen, B.; Öberg, B.; Johansson, N. G. Comparison of the substrate specificities of human thymidine kinase 1 and 2 and deoxycytidine kinase toward antiviral and cytostatic nucleoside analogues. *Biochem. Biophys. Res. Commun.* **1991**, *176*, 586−592.

(62) Conjugates combining in their stucture d4T and imidazo[1,5-*d*]-pyridazine were endowed with similar RT inhibition mode.[36]

(63) Esnouf, R. M.; Ren, J.; Hopkins, A. L.; Ross, C. K.; Jones, E. Y.; Stammers, D. K.; Stuart, D. I. Unique features in the structure of the complex between HIV-1 reverse transcriptase and the bis-(heteroaryl)piperazine (BHAP) U-90152 explain resistance mutations for this nonnucleoside inhibitor. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 3984−3989.

(64) Tanaka, H.; Baba, M.; Hayakawa, H.; Sakamaki, T.; Miyasaka T.; Ubasawa, M.; Takashima, H.; Sekiya, K.; Nitta, I.; Shigeta, S.; Walker, R. T.; Balzarini, J.; De Clercq, E. A new class of HIV-1 specific 6-substituted acyclouridine derivatives: synthesis and anti-HIV-1 activity of 5- or 6-substituted analogues of 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT). *J. Med. Chem.* **1991**, *34*, 349−357.

(65) Milton, J.; Slater, M. J.; Bird, A. J.; Spinks, D.; Scott, G.; Price, C. E.; Downing, S.; Green, D. V. S.; Madar, S.; Bethell, R.; Stammers, D. K. Biaryl acids: novel nonnucleoside inhibitors of HIV reverse transcriptase types 1 and 2. *Bioorg. Med. Chem. Lett.*, **1998**, *8*, 2623−2628.

(66) Lingham, R. B.; Hsu, A. H. M.; O'Brien, J. A.; Sigmund, J. M.; Sanchez, M.; Gagliardi, M. M.; Heimbuch, B. K.; Genilloud, O.; Martin, I.; Diez, M. T.; Hirsch, C. F.; Zink, D. L.; Liesch, J. M.; Koch, G. E.; Gartner, S. E.; Garrity, G. M.; Tsou, N. N.; Salituro, G. M. Quinoxapeptins: novel chromodepsipeptide inhibitors of HIV-1 and HIV-2 reverse transcriptase. I. The producing organism and biological activity. *J. Antibiot.* **1996**, *49*, 253−259.

(67) Robins, M. J.; Wilson J. S.; Madej, D.; Low, N. H.; Hansske, F.; Wnuk, S. F. Nucleic acid related compounds. 88. Efficient conversions of ribonucleosides into their 2′,3′-anhydro, 2′ (and 3′)-deoxy, 2′,3′-didehydro-2′,3′-dideoxy, and 2′,3′-dideoxynucleoside analogues. *J. Org. Chem.* **1995**, *60*, 7902−7908.

(68) Moog, C.; Wick, A.; Le Ber, P.; Kirn, A.; Aubertin, A.-M. Bicyclic imidazo derivatives, a new class of highly selective inhibitors for the human immunodeficiency virus type 1. *Antiviral Res.* **1994**, *24*, 275−288.

(69) Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55−63.

(70) Chou, J.; Chou, T.-C. Dose−effect analysis with microcomputers: quantitation of ED_{50}, LD_{50}, synergism, antagonism, low-dose risk, receptor binding and enzyme kinetics. *Computer software for Apple II series and IBM-PC and instruction manual*; Elsevier-Biosoft, Elsevier Science Publishers: Cambridge, U.K., 1985; pp 19−28.

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