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5-Modified-2′-dU and 2′-dC as Mutagenic Anti HIV-1 Proliferation Agents: Synthesis and Activity

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With the goal of limiting HIV-1 proliferation by increasing the mutation rate of the viral genome, we synthesized a series of pyrimidine nucleoside analogues modified in position 5 of the aglycone moiety but unmodified on the sugar part. The synthetic strategies allow us to prepare the targeted compounds directly from commercially available nucleosides. All compounds were tested for their ability to reduce HIV-1 proliferation in cell culture. Two of them (5-hydroxymethyl-2′-dU (**1c**) and 5-hydroxymethyl-2′-dC (**2c**)) displayed a moderate antiviral activity in single passage experiments. The same two compounds plus two additional ones (5-carbamoyl-2′-dU (**1a**) and 5-carbamoylmethyl-2′-dU (**1b**)) were potent inhibitors of HIV-1 RT activity in serial passage assays, in which they induced a progressive loss of HIV-1 replication. In addition, viruses collected after seven passages in the presence of **1c** and **2c** replicated very poorly after withdrawal of these compounds, consistent with the accumulation of deleterious mutations in the HIV-1 genome.

Introduction

According to the Joint United Nations Programme on HIV/AIDS (UNAIDS), in 2007 more than 33 million people were living with human immunodeficiency virus type 1 (HIV-1[a]), the etiological agent of acquired immunodeficiency syndrome (AIDS).

Since the isolation of HIV-1 in 1983,[1,2] important efforts have been expended in developing vaccinal approaches and antiviral chemotherapies. Whereas the first strategy has not yet afforded sufficient positive results for use in human treatments, the second one has provided a panel of 25 drugs approved by the U.S. Food and Drug Administration (FDA) for controlling HIV replication. Among them, a fusion inhibitor[3] and a co-receptor inhibitor[4] prevent the viral particles from entering the cells. Twelve molecules target the viral reverse transcriptase (RT), a key enzyme in the retroviral life cycle that catalyzes the conversion of genomic RNA into double-stranded proviral DNA.[5] One drug targets the viral integrase,[6] which is responsible for proviral DNA integration into cellular DNA. Ten other drugs are protease inhibitors[7] that affect maturation of HIV particles by inhibiting Gag and Gag-Pol precursors processing. As the pool of anti-HIV drugs was expanding, it rapidly appeared that the emergence and selection of mutations conferring resistance to these drugs were a major concern in AIDS treatments.[8,9]

The rapid emergence of resistance mutations is linked to the genetic diversity of HIV-1 virions, which is due to the low fidelity of HIV-1 RT, enhanced by the lack of intrinsic proofreading activity, the high level of HIV replication, and the high rate of RT-mediated recombination.[10] As a result, combinations of drugs targeting the same or different viral targets have been used for more than a decade to control HIV-1 replication. Highly active antiretroviral therapies (HAART)[11] that associate at least three antiviral drugs highly reduced mortality rates. Under HAART, viral loads become undetectable for several years, but the virus is never eliminated from its host, and it is now widely admitted that a cure of AIDS will require a strategy different from the existing ones. In addition, new inhibitors that are active against HIV-1 multiresistant strains must be developed.

Ten years ago, Loeb et al.[12] suggested that it should be possible to push the HIV-1 exceptionally high mutation rate from a virus self-defense system into a tool for virus destruction. This idea was based on the work of Chemistry Nobel Prize Laureate Manfred Eigen, who described the properties of viral populations with high mutation rates existing as "quasispecies". Eigen's theory predicted that a small increase in the mutation rate may result in a dramatic loss of viability.[13] This mechanism is called "lethal mutagenesis" and is by definition a process that pushes viral populations to extinction. The mutation rate in RNA viruses and retroviruses is so high that a slight increase (1.1- to 2.8-fold for RNA viruses such as vesicular stomatitis virus or poliovirus[14] and 13-fold for spleen necrosis virus[15]) in the mutation frequency can drive the viral population to exceed the error threshold for

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[a] Abbreviations: AIDS, acquired immunodeficiency syndrome; FDA, Food and Drug Administration; HIV-1, human immunodeficiency virus type 1; HAART, highly active antiretroviral therapy; RT, reverse transcriptase; NRTI, nucleoside reverse transcriptase inhibitors; CC_{50}, 50% cytotoxic concentration; IC_{50}, 50% inhibitory concentration; $TCID_{50}$, 50% tissue culture infective dose; TMEDA, tetramethylethylenediamine.

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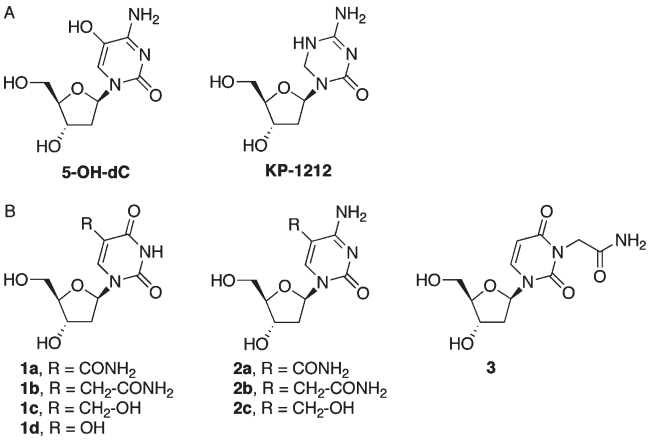
Chart 1. Structures of Modified Pyrimidine Nucleoside Analogues Known for Their Mutagenic Properties (A) and the Ones Prepared and Evaluated in This Study (B)



viability.[14,16] In comparison, DNA viruses or bacteria can accept an 80000-fold increase in mutation frequency[17] without damage to their viability. Even if there is not much theory underpinning this concept, empirical evidence broadly supports it. Chemical mutagens have been used to slightly increase error rates and dramatically reduce viral titers of several viruses such as vesicular stomatitis virus,[14] poliovirus 1,[18] foot-and-mouth disease virus,[19] and hepatitis C virus.[20]

In the case of HIV-1, Loeb et al. confirmed this hypothesis using 5-hydroxy-2′-deoxycytidine (5-OH-dC, Chart 1).[12] Once transformed by cellular kinases into 5-OH-dCTP, this nucleotide analogue was incorporated by HIV-1 RT opposite the template guanosine and mispaired with deoxyadenosine during subsequent replication of the altered DNA, resulting in an increase of G to A substitutions. They found that a 2-fold increase in the mutation rate of the HIV genome after exposure to 5-OH-dC had a large effect on viral lethality. More recently, Koronis Pharmaceuticals developed another mutagenic nucleoside,[21−23] KP-1212 (Chart 1). Following incorporation of KP-1212 monophosphate (administrated as a prodrug, KP-1461) and several rounds of replication, the accumulation of mutations exceeded the crucial threshold beyond which HIV-1 viability is highly reduced, leading to viral collapse. As for 5-OH-dC, only a few mutations were needed to induce a large effect on virus proliferation. KP-1212 reached phase 2a trials for clinical development before being suspended in 2008 in order to conduct new preclinical evaluation studies.

The mechanism of action of viral decay accelerators introducing mutations in the viral genome is potentially harmful for mitochondrial and/or genomic DNA, and this risk has to be kept in mind when developing new mutagenic nucleoside analogues. For KP-1212, clinical studies have demonstrated that it induced only moderate adverse effects. Moreover, genotoxicity assays conducted with KP-1212 revealed very little mutagenic activity comparable to approved NRTIs.[23] It was demonstrated that human mitochondrial DNA polymerase γ (hDNA pol γ) efficiently incorporated KP-1212-TP whereas mouse DNA polymerase β (mDNA pol β) did not.[24] On the other hand, KP-1212-MP can be efficiently excised by the exonuclease activity of hDNA pol γ, limiting by this way errors in host mitochondrial genome.[23] Finally, mismatches that are present in double-stranded DNA can be corrected by cellular repair enzymes, while those present in RNA/DNA hybrids, which are the replication intermediates of HIV-1 RNA, cannot. Mutagenic nucleoside could be advantageous when used in combination with chain terminator nucleoside analogues. Studies showed that HIV strains resistant to chain terminators exhibited no cross-resistance toward KP-1461 and that viruses cultured in the presence of KP-1461 displayed increased sensitivity to AZT.[23]

Within the framework of our research, we designed several nucleoside analogues that are modified only on the aglycone moiety in order to remain as potential substrates of HIV-1 RT after anabolysation into triphosphates, to base-pair with at least two natural nucleosides, and finally to increase the error rate in the proviral DNA.[25,26] Ambiguous hydrogen-bonding properties can be obtained by affecting several factors such as ionization, rotation, or tautomerization of the base moiety. For instance, a simple rotation of the exocyclic carbamoyl group of the pseudobase of ribavirin (1,2,4-triazole-3-carboxamide) results in two distinct hydrogen-bonding configurations that pair with either uracil or cytosine. Numerous investigators have demonstrated the mutagenic properties of this broad-spectrum antiviral nucleoside.[18,27−32] Hydroxylated pyrimidine nucleosides are another class of ambiguous nucleosides. The presence of a hydroxyl group on the C-5 position shifts the tautomeric equilibrium toward the unpreferred or rare form, leading to mispairing and potentially to mutagenesis. 5-OH-dC was found active against HIV-1[12] probably because the hydroxyl group increases the imino tautomer frequency, allowing base-pairing with guanosine as well as adenosine.[33]

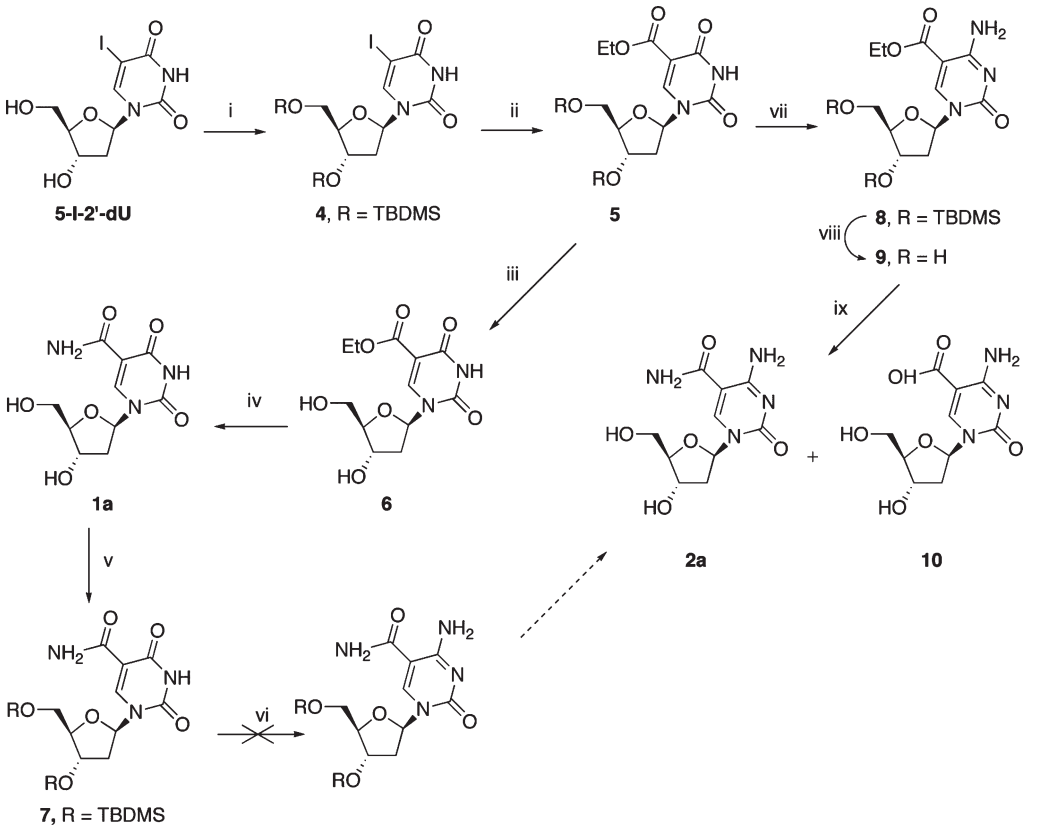
In this paper, we report the synthesis of pyrimidine nucleoside analogues modified on position 5 by a carbamoyl, carbamoylmethyl, hydroxyl, or hydroxymethyl group (Chart 1) as well as their anti-HIV-1 activity, showing that some of them reduce viral replication in cell culture.

Chemistry

The carbamoylnucleoside derivatives **1a**,**b**, **2a**,**b**, and **3** are unpublished or poorly described and characterized in literature. On the other hand, hydroxylated nucleosides **1c**, **2c**, and **1d** are known because they were isolated as DNA oxidative damage products.[34,35] For the latter compounds, some syntheses were published as well as their ability to form mismatches with natural nucleosides[36−40] but no data were found concerning their use as mutagenic nucleosides for antiviral purposes. Consequently, we focused our efforts on developing simple and rapid methods to achieve our targeted compounds starting from commercially available 2′-deoxynucleosides such as 2′-deoxyuridine or its 5-halogenated derivatives.

The synthesis of compounds **1a** and **2a** is described in Scheme 1 and starts with the commercially available 5-iodo-2′-deoxyuridine. Classical silylation of 5-iodo-2′-deoxyuridine was realized with *tert*-butyldimethylsilyl chloride (TBDMSCl) in the presence of imidazole in dimethylformamide (DMF) to give the 3′,5′-di-O-protected compound **4**. This silylated nucleoside **4** was first deprotonated on the N-3 position with sodium hydride in THF to give the sodium salt of **4**, which was treated with *sec*-butyllithium (*sec*-BuLi) in presence of tetramethylethylenediamine (TMEDA) for iodine/lithium exchange. The mix sodium/lithium intermediate was finally reacted in situ with ethyl chloroformate to give the C-5 adduct **5** in 67% yield. Protecting position N-3 by forming the sodium salt allowed an optimum selectivity for the anion formation on position C-5.[41] The derivative **5** was deprotected from its silyl

Scheme 1. Synthesis of Compounds **1a** and **2a**[a]



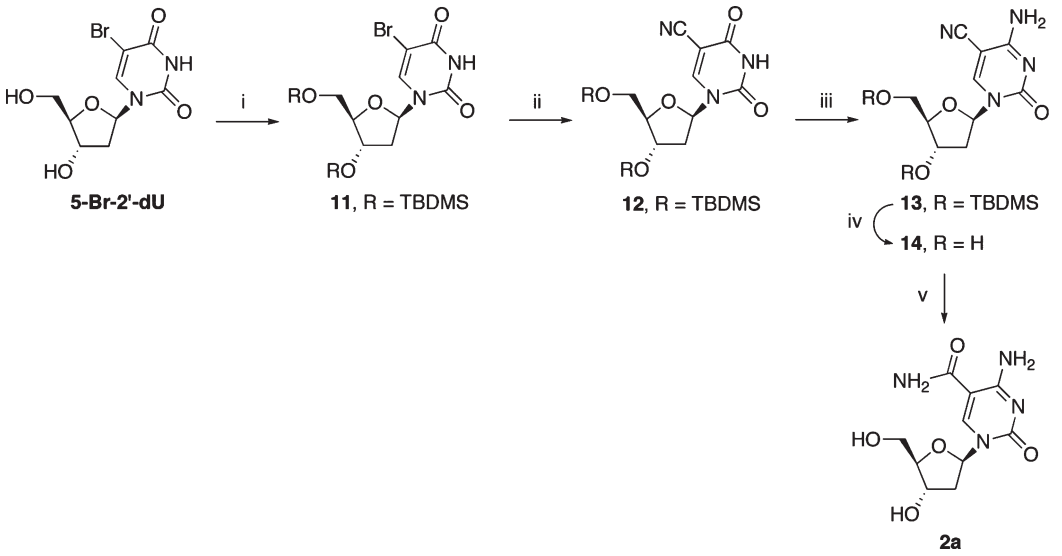
[a] Reagents and conditions: (i) TBDMSCl, imidazole, DMF, room temperature, 60 h, quantitative; (ii) NaH, THF, room temperature, 30 min; then *sec*-BuLi, TMEDA, −78 °C, 15 min; then ClCOOEt, −78 °C, 75 min, 67%; (iii) TBAF·$3H_2O$, THF, room temperature, 3 h, 81%; (iv) NH_4OH, pyridine, 80 °C, 48 h, 50%; (v) TBDMSCl, imidazole, DMF, room temperature, 60 h, 55%; (vi) TPSCl, DMAP, Et_3N, CH_3CN, room temperature, 20 h; then NH_4OH 25%, room temperature, 3 h, degradation; (vii) TPSCl, DMAP, Et_3N, CH_3CN, room temperature, 21 h; then NH_4OH 25%, room temperature, 4 h, 70%; (viii) TBAF·.$3H_2O$, THF, room temperature, 20 h, 67%; (ix) 25% NH_4OH, pyridine, 80 °C, 48 h, 20% (**2a**) and 40% (**10**).

groups by treatment with tetrabutylammonium fluoride trihydrate (TBAF·$3H_2O$) in THF. The resulting deprotected compound **6** was reacted with aqueous ammonia in pyridine to give compound **1a** in a total of four steps and a 27% overall yield as illustrated in Scheme 1. The same synthetic route but using 5-methoxycarbonyl nucleoside as the intermediate also afforded compound **1a** but in lower yield (16%). The conversion of the intermediate to compound **2a** was then considered. However, after protection of the hydroxyls with silyl groups, attempts to convert compound **7** into the cytidine analogue with 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl) in the presence of dimethylaminopyridine (DMAP) and triethylamine in acetonitrile followed by ammonolysis with NH_4OH at room temperature[42] failed because of complete degradation of the starting material. On the other hand, conversion of compound **5** into the cytidine derivative **8** using the same procedure was efficient (70% yield). At this stage, the silylated protecting groups of **8** were eliminated and treatment of compound **9** with ammonia in pyridine in a sealed tube at 80 °C gave the target nucleoside derivative **2a** in 20% yield along with 5-carboxyl-2′-deoxycytidine **10** (40%).

Alternatively, **2a** can be prepared with a better overall yield, 14% instead of 6%, starting from 5-bromo-2′-deoxyuridine (Scheme 2). The hydroxyl groups of 5-Br-2′-dU were protected to give compound **11** as described for 5-iodo-2′-dU. Cyanation at position 5 was inspired from the methodology of "double addition elimination" developed by Inoue et al.[43] In the first step, the reaction of 5-Br-2′-dU with 2 equiv of potassium cyanide in the presence of 18-crown-6 ether in DMF at room temperature gave the intermediate 3′,5′-di-*O*-acetyl-6-cyano-2′-dU. The "one pot" reaction with an additional equivalent of potassium cyanide and 18-crown-6 ether under thermal treatment led to the addition of a second cyano group (at position 5) and the elimination of the 6-cyano group affording the more stable 3′,5′-di-*O*-acetyl-5-cyano-2′-dU. Performing the first step of the reaction at higher temperature and/or higher KCN concentrations decreased the reaction yield. This strategy led to only a partial conversion of the 6-cyano into the 5-cyano nucleoside and also to the removal of some protecting groups. 5-Cyano-2′-dU derivative **12** was reacted under the conditions described above for the preparation of **8** to give the intermediate **13**. Removal of the silylated protecting groups by TBAF·$3H_2O$ in THF followed by hydrolysis of the cyano group of **14** with a 1 M sodium hydroxide solution afforded the target compound **2a** in 14% global yield.

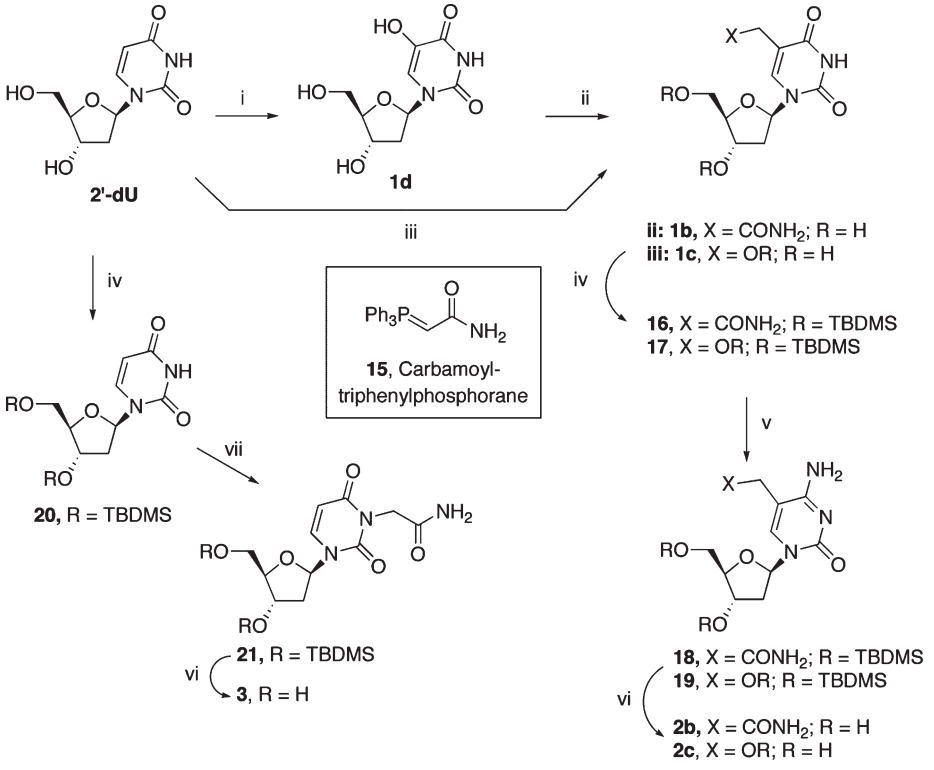
The preparation of compounds **1b**, **1d**, and **2b** started with 2′-deoxyuridine (Scheme 3). 5-OH-2′-dU **1d** was obtained from 2′-dU in one step and 54% yield by reaction with bromide in water followed by in situ treatment with pyridine.[40] Wittig coupling on **1d** with carbamoyltriphenylphosphorane[44] **15** in 1,4-dioxane gave compound **1b** in 38% total yield.[45] After silyl protection of the hydroxyl groups of **1b**, conversion of the 4-carbonyl function of **16** to the corresponding amino group was achieved following the procedure of activation/substitution as previously described for the preparation of compound

Scheme 2. Synthesis of Compound **2a**[a]



[a] Reagents and conditions: (i) TBDMSCl, imidazole, DMF, room temperature, 60 h, 96%; (ii) KCN, 18-crown-6, DMF, room temperature, 60 h; then KCN, 18-crown-6, DMF, 80 °C, 9 h, 65%; (iii) TPSCl, DMAP, Et_3N, CH_3CN, room temperature, 21 h; then NH_4OH (27%), room temperature, 90 min, 73%; (iv) TBAF·$3H_2O$, THF, room temperature, 4 h, 64%; (v) 1 M NaOH, room temperature, 4 h, 49%.

Scheme 3. Synthesis of Compounds **1b**, **1c**, **1d**, **2b**, **2c**, and **3**[a]



[a] Reagents and conditions: (i) Br_2, H_2O; then pyridine, 0 °C, 30 min, and room temperature, 24 h, 54%; (ii) **15**, 1,4-dioxane, 100 °C, 4 h, 71%; (iii) H_2CO, Et_3N, H_2O, 100 °C, 48 h, 40%; (iv) TBDMSCl, imidazole, DMF, room temperature, 60 h, 91% (**16**), 69% (**17**) and 90% (**20**); (v) TPSCl, DMAP, Et_3N, CH_3CN, room temperature, 21 h; then NH_4OH (27%), room temperature, 90 min, 61% (**18**) and 2 h, 51% (**19**); (vi) TBAF·$3H_2O$, THF, room temperature, 2 h, 83% (**2b**), 54% (**2c**), and 65% (**3**); (vii) NaH, THF, room temperature, 30 min; then 2-chloroacetamide, THF, 0 °C to room temperature, 3 h, 72%.

8. Removal of TBDMS protecting groups by TBAF·$3H_2O$ in THF afforded the 5-carbamoylmethyl-2′-dC, **2b** (46% in three steps).

The synthesis of compounds **1c** and **2c** was realized straightforwardly as shown in Scheme 3. 2′-dU was reacted with formaldehyde in the presence of triethylamine and water to furnish compound **1c** in 40% yield according to the methodology described by LaFrancois et al. for the preparation of isotopically labeled compounds.[40] The synthetic strategy described in Scheme 1 applied to compound **1c** afforded the corresponding cytidine analogue **2c** in 19% overall yield.

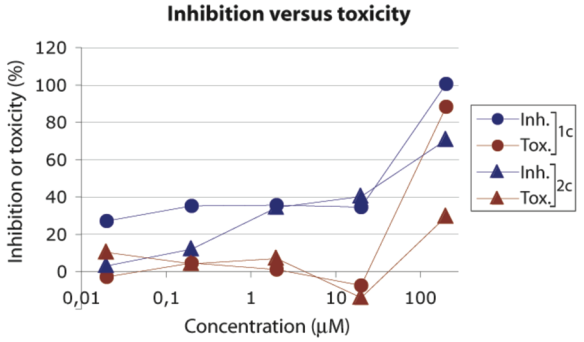


Figure 1. Viral inhibition of HIV-1 LAI (Inh.) and cellular toxicity (Tox.) curves for compounds **1c** and **2c** in CEM-SS cells in single passage experiments.

Table 1. Anti-HIV-1 Activity (IC_{50}) and Cytotoxicity (CC_{50}) of 5-Modified 2′-dU and 2′-dC Derivatives in Cell Cultures on Limited Time (Single Passage, 5 Days)

	HIV-1 LAI/CEM-SS		HIV-1 IIIB/MT4	
	IC_{50} (μM)[a]	CC_{50} (μM)[b]	IC_{50} (μM)[a]	CC_{50} (μM)[b]
1a	> 200	> 200	> 200	> 200
1b	> 200	> 200	> 200	> 200
1c	30	120	> 130	130
1d	> 200	> 200	> 200	> 200
2a	> 200	> 200	> 200	> 200
2b	> 200	> 200	> 200	> 200
2c	24	> 200	> 200	> 200
3	> 200	> 200	> 200	> 200
AZT	0.0024	> 1	0.018	> 1

[a] IC_{50}: 50% inhibitory concentration, which is the concentration needed to inhibit 50% virus replication in vitro. [b] CC_{50}: 50% cytotoxic concentration, which is the concentration required to cause 50% death of uninfected cells.

The final target compound **3** was obtained in three steps as shown in Scheme 3. Silylation of 2′-dU gave compound **20**, which was treated successively with NaH in THF and 2-chloroacetamide to give the N-alkylated derivative **21**. Removal of the silyl protecting groups under standard conditions afforded compound **3** in 42% global yield.

^{1}H and ^{13}C NMR, MS, UV, and elemental analysis afforded analytical data in harmony with the proposed structures of the above compounds.

Results and Discussion

5-Modified pyrimidine nucleoside derivatives were synthesized in order to test their ability to reduce HIV-1 replication by lethal mutagenesis. Their activity on HIV-1 replication was first measured by quantification of (i) the inhibition of virus-induced cytopathogenicity in infected MT-4 cells and (ii) the reverse transcriptase (RT) activity associated with virus particles released from infected CEM-SS cells. The 50% cytotoxic concentration (CC_{50}) was evaluated in parallel to the 50% inhibitory concentration (IC_{50}). The dose−effect curves (illustrated for compounds **1c** and **2c** in Figure 1, red curves) were plotted with the computer-generated median effect to give the CC_{50} values (Table 1, CC_{50} entries). In both cell types the large majority of molecules did not induce toxicity (CC_{50} > 200 μM, the higher concentration tested). Only compound **1c** was found to be toxic for cells with a CC_{50} of 120 and 130 μM in CEM-SS and MT-4 cells, respectively. In parallel, we determined the inhibitory concentrations (IC_{50}) for each compound by measuring the RT activity in

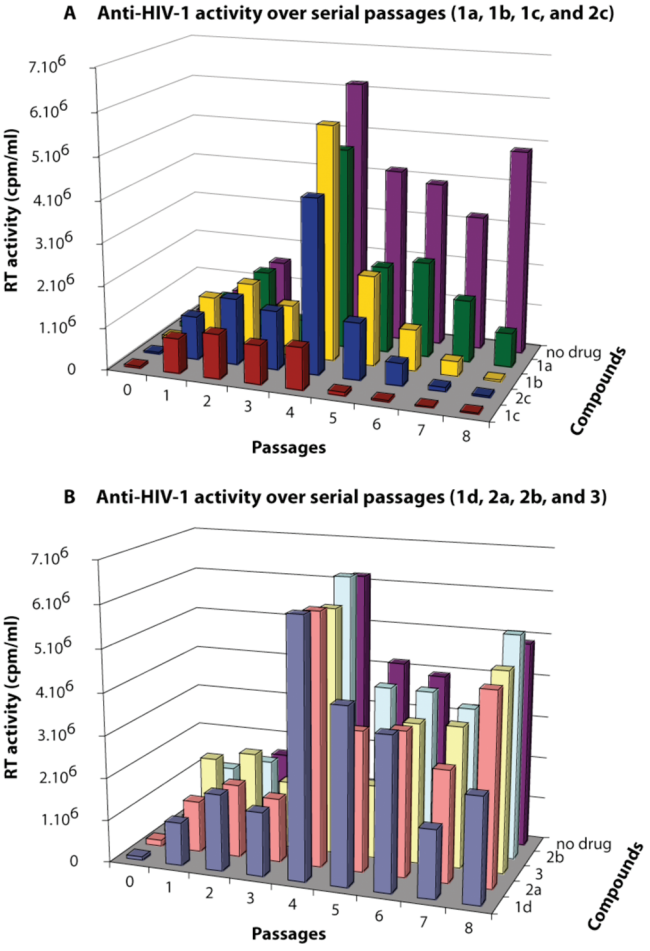


Figure 2. RT activity detected in supernatant over serial passage experiments of HIV-1 LAI in CEM-SS cells. Panel A shows active molecules (**1a**, **1b**, **1c**, and **2c**), whereas panel B concerns inactive compounds (**1d**, **2a**, **2b**, and **3**). The concentration of all compounds is 200 μM except for **1c** (100 μM).

supernatant of cell cultures. The dose−effect curves (illustrated for compounds **1c** and **2c** in Figure 1, blue curves) were plotted to afford the IC_{50} values (Table 1, IC_{50} entries). Two molecules, **1c** and **2c**, displayed a moderate effect on HIV-1 replication in CEM-SS cells (IC_{50} = 30 and 24 μM, respectively). However, none of these compounds were found to exhibit anti-HIV activity on MT-4 cells.

In order to evaluate the potential mutagenic effect of our compounds, serial passage experiments were performed in CEM-SS cells infected with HIV-1 LAI. The CC_{50} data allow us to determine the concentrations of our compounds to be used in this assay. For nontoxic compounds (CC_{50} > 200 μM) the infected cells were treated with a 200 μM solution, but for compound **1c** (CC_{50} = 120 μM) a 100 μM solution was used in order to limit cellular mortality. Figure 2 shows the effect of our compounds on HIV replication over eight sequential passages. An identical volume of supernatant from the control and the treated cultures was transferred to uninfected cells at each passage. In control cultures (Figure 2, purple bars), we observed a regular increase of RT titer until passage 4 that was most likely a consequence of the low multiplicity of infection used for the initial infection (passage 0). For the last four passages, RT titer showed considerable fluctuations but kept high values. In contrast, virus replication followed a

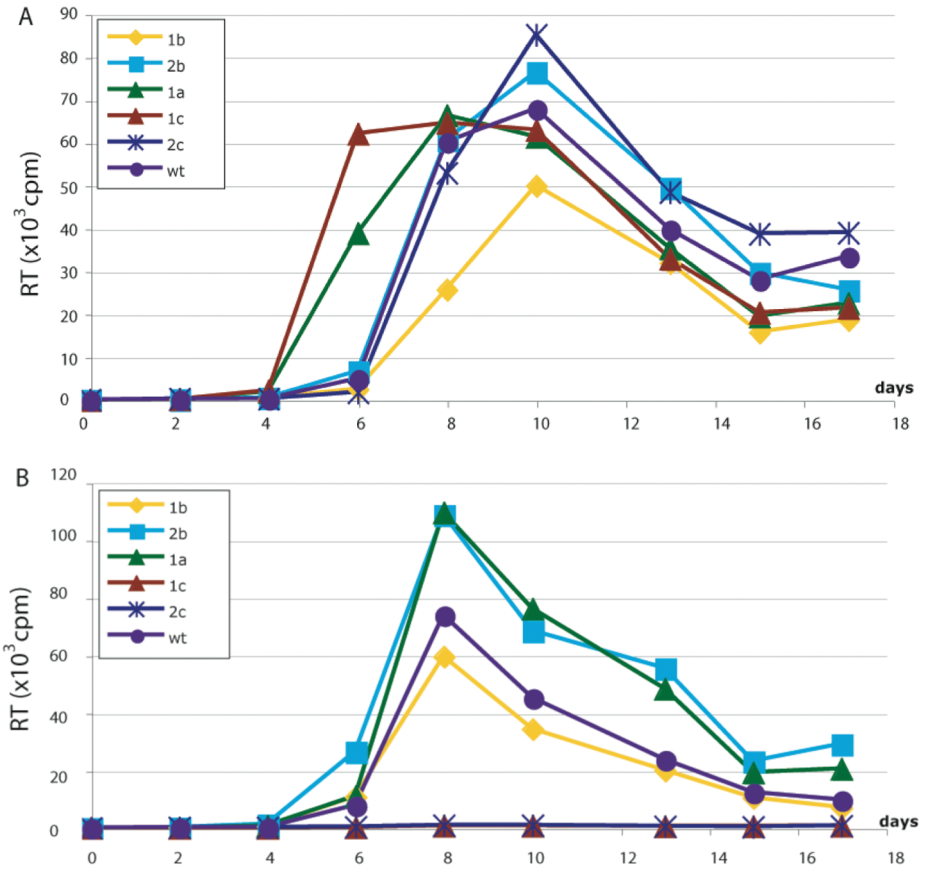


Figure 3. Viral replication kinetics. CEM-SS cells were infected with a normalized amount of viruses (equivalent to 25 cpm/mL of RT counts) issued from passage 3 (A) or 7 (B). Supernatant RT was quantitated over 17 days.

different trend in several cultures treated with our nucleoside analogues (Figure 2A).

In four of the eight treated cultures (Figure 2A, **1a**, **1b**, **1c**, and **2c**), while RT activity was almost equivalent to control values until passage 4, RT titer considerably and gradually decreased from passage 5 to 8. RT titers in the supernatant were almost undetectable at passage 8 for compounds **1b** and **1c**. The lower HIV-1 replication at passage 4 in cultures treated with compound **1c** might be due to a higher activity of this compound or/and to its toxicity. The other four compounds (Figure 2B, **1d**, **2a**, **2b**, and **3**) did not show any significant difference with the untreated control in limiting replication of HIV-1 after passage 4.

The results obtained with **1a**, **1b**, **1c**, and **2c** are consistent with lethal mutagenesis resulting from a progressive accumulation of mutations in the HIV genome due to the incorporation of mutagenic analogues during repetitive infection of host cells.

In order to mimic the in vivo situation, we chose to infect untreated and treated cultures with identical volumes of supernatant rather than with identical amounts of virus. However, by doing so, we cannot exclude that the progressive decrease in HIV-1 titers observed with compounds **1a**, **1b**, **1c**, and **2c** was due to partial inhibition of the virus replication at each passage, although the increase of viral replication during the first four passages seems to argue against this possibility. In order to distinguish between lethal mutagenesis and partial inhibition due to other mechanisms, we used a normalized amount of viruses (based on RT activity) produced in the presence or absence of nucleoside analogues at the end of passages 3 and 7 to infect fresh CEM-SS cells in the absence of drug. If our compounds induce lethal mutagenesis, then the mutations accumulated in the viral genome after a number of serial passages should exceed the error threshold and dramatically reduce viral fitness even after the nucleoside analogue that produced these mutations has been removed. Alternatively, if our compounds partially inhibit viral replication by other mechanisms, their effects should disappear after they have been removed.

Viruses collected after three passages in the absence of nucleoside analogue or in the presence of **1a**, **1b**, **1c**, **2b**, or **2c** all replicated efficiently (Figure 3A). This result was expected, as these compounds showed no effect on HIV-1 replication after three serial passages (Figure 2). At the opposite, viruses collected after seven passages in the presence of compounds **1c** and **2c** hardly replicated at all in the absence of nucleoside analogue (Figure 3B). Thus, this experiment unambiguously demonstrates that **1c** and **2c** induce lethal mutagenesis of HIV-1. However, viruses collected after seven passages in the presence of **1a** and **1b** replicated as efficiently as viruses passaged in the absence of nucleoside analogues (Figure 3B), even though these compounds were active in the serial passage assay (Figure 2A). Thus, **1a** and **1b** do not induce lethal mutagenesis, even though they partially inhibit viral replication. The mechanism of action of these compounds is presently unknown. Finally, viruses collected after seven passages in the presence of **2b** also replicated as efficiently as viruses passaged in the absence of nucleoside analogues (Figure 3B). This result was expected, since this compound was also inactive in the two previous assays (Table 1 and Figure 2).

The first relationship between the structure of our compounds and their effects on HIV replication over several

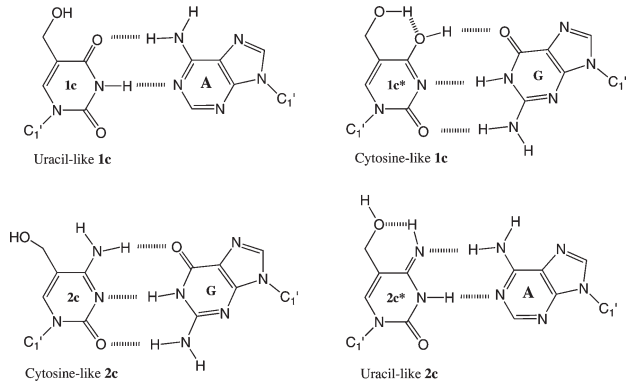


Figure 4. Base-pairing schemes involving "predominant" (left) and "rare" (∗, right) tautomers for compounds **1c** and **2c**.

passages reveals three features. (1) Only modification of position 5 by a hydroxymethyl group (compounds **1c** and **2c**) confers an anti HIV-1 mutagenic activity, albeit compound **1c** shows significant cytotoxicity whereas compound **2c** does not at the highest concentration tested. (2) Surprisingly, modification of position 5 of dU by a hydroxyl group (compound **1d**) has no effect, whereas this modification has a positive effect on the anti-HIV activity in the dC series (5-OH-dC described by Loeb et al.[12]). (3) Introduction of carbamoyl or carbamoylmethyl modification in both series does not induce mutagenic activity, even if in the dU series compounds **1a** and **1b** reduce HIV proliferation over several passages.

It is difficult to identify the origin of the lack of mutagenic activity of nucleoside analogues because it may be directly linked to intrinsic molecule inactivity but also to several indirect effects such as a poor phosphorylation or a lack of recognition and incorporation by the viral polymerase. However, the difference between compounds **1d** and 5-OH-dC anti-HIV activity can be related to the stabilization of ionized forms or rare tautomers through the hydroxyl group inductive and fields effects. La Francois et al. established a linear correlation between the σ_m Hammet values for a substituent and the pK observed in the dU and dC series.[35] Reducing σ_m (from electron-attracting to electron-donating groups) increases pK_a in the dU series and pK_b in the dC series, thus increasing the affinity of N-3 for a proton. Moreover a ^{1}H NMR study of a broad series of C-5 substituted 2′-deoxyuridine concluded that electron-withdrawing groups cause a downfield shift of H-6, whereas electron-donating groups induce an upfield shift.[46] As a result, the effect of C-5-substituents can be estimated through the value of the H-6 displacement. For dU and compound **1d** these values are in accordance with the ones previously published.[35] They indicate that the hydroxyl modification induces an electron donating effect (7.33 ppm for **1d** compared to 7.71 ppm for 2′-dU). Whereas this electron donating effect may enhance the frequency of the imino form relative to the amino tautomer in dC series, at the opposite, the stabilization of the keto tautomer in dU series is unfavorable to multibase pairing and mutagenicity.

Concerning the activity observed with compounds **1c** and **2c**, it cannot be assigned to the substituent electronic effects, since the methylene group between the hydroxyl and the heterocyclic ring reduces dramatically the spread of the OH effect as confirmed by the ^{1}H NMR H-6 displacements (7.73 and 7.75 ppm compared to 7.71 and 7.70 ppm for dU and dC, respectively). Alternatively, intramolecular hydrogen bonding may stabilize the rare tautomers **1c*** and **2c*** as illustrated in Figure 4 and allow base pairing of **1c** and **2c** with either A or G. The hydroxymethyl group might also be directly involved in intermolecular base-pairing, stabilizing noncanonical base-pairs and favoring mutagenicity.[37] However, additional experiments will have to be conducted to test these hypotheses.

Conclusion

Among the dU and dC analogues we synthesized and tested in this study, two displayed anti-HIV-1 activity in single passage assays. The same two compounds were the most efficient in the serial passage assay, and viruses collected after seven consecutive passages in the presence of these nucleoside analogues failed to replicate after withdrawal of the drugs. The progressive loss of HIV-1 replication is consistent with deleterious mutagenesis of HIV-1 induced by these two compounds. The data presented here are also consistent with a detailed analysis of the viral RT gene before the first and after the eighth passage (El Safadi, Paillart, et al., manuscript in preparation). The two mutagenic compounds are characterized by a hydroxymethyl substituent at position C-5 of dU and dC. Intramolecular hydrogen bonding or inter-residue hydrogen bonding between these groups and neighboring bases in DNA strand might favor misincorporation of these residues and explain the observed mutagenic activity. Two dU analogues with carbamoyl and carbamoylmethyl groups at position C-5 inhibited HIV-1 replication during serial passages, but this was the result of partial inhibition of viral replication at each passage rather than accumulation of deleterious mutations. Additional biochemical studies would be required to elucidate the mechanism of action of these two dU analogues.

Experimental Section

Materials and Methods. All chemicals are of pa purity and purchased from Sigma. Organic solvents were dried and distilled when necessary. Pyridine and triethylamine were distilled over KOH. CH_2Cl_2 was distilled over P_2O_5. MeOH and THF were distilled over Na in the presence of benzophenone for THF. Flash chromatography was performed on silica gel 60 (40−63 μM) from Merck. Thin layer chromatography was performed on Merck 60F_{254} coated plates. UV spectra were recorded on a Nanodrop ND-100 spectrophotometer. pH was measured on a Schott CG-825 apparatus. Proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR) were recorded at 300 MHz on a Bruker Advance spectrometer. Chemical shifts were reported in ppm relative to tetramethylsilane (TMS), and signals are given as follows: s (singlet); d (doublet); t (triplet); m (multiplet). Mass spectra were recorded on Bruker Daltonics MicroTOF LC spectrometer using positive electron spray ionization (ESI) mode. Elemental analyses were performed with an Elementar VARIO ELIII CHN analyzer, and the purity of the target compounds was more than 95%.

3′,5′-Di-*O*-*tert*-butyldimethylsilyl-5-iodo-2′-deoxyuridine (4). To a solution of 5-iodo-2′-deoxyuridine (0.765 g, 2.16 mmol) in dry DMF (4.5 mL) *tert*-butyldimethylsilyl chloride (2.10 g, 14 mmol) and imidazole (1.40 g, 20 mmol) were added. The clear solution was stirred at room temperature for 60 h. Water (40 mL) was added, the aqueous layer was extracted with EtOAc (3 × 50 mL), and the combined organic layers were washed with brine (3 × 50 mL), dried over Na_2SO_4, and concentrated to dryness. The oily residue was purified by silica gel column chromatography (0−3% MeOH in CH_2Cl_2) to give pure compound **4** (1.24 g, 99%) as a white foam. ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 0.1−0.2 (m, 12H, CH_3−Si); 0.8−1.0 (2s, 18H,

^{t}Bu-Si); 2.1−2.3 (m, 2H, CH_2-2′ and 2″); 3.7−3.9 (m, 3H, CH-4′, CH_2-5′ and 5″); 4.3−4.4 (m, 1H, CH-3′); 6.09 (t, 1H, CH-1′, $^3J_{H1'\text{-}H2'} = {}^3J_{H1'\text{-}H2''} = 7.2$ Hz); 7.97 (s, 1H, CH-6); 11.74 (s, 1H, NH-3). ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: −5.07 and −4.40 (4C, CH_3-Si); 18.08 (2C, $\underline{C}(CH_3)_3$); 26.08 and 26.30 (6C, C-($\underline{C}H_3)_3$); 40.34 (C-2′); 63.05 (C-5′); 70.40 (C-3′); 72.37 (C-1′); 84.95 (C-4′); 87.85 (C-5); 144.40 (C-6); 150.46 (C-2); 160.84 (C-4). MS (ESI, pos) m/z: 581.4 ($M + H^+$).

3′,5′-Di-*O*-*tert*-butyldimethylsilyl-5-ethylcarboxylate-2′-deoxyuridine (5). To a solution of compound **4** (0.880 g, 1.51 mmol) in freshly distilled THF (3 mL) was slowly added a suspension of sodium hydride (0.160 g, 4 mmol) in freshly distilled THF (6 mL). The reaction mixture was stirred at room temperature for 30 min, then cooled to −78 °C. *N*,*N*′-Tetramethylethylenediamine (TMEDA, 0.5 mL, 3 mmol) was added followed by the dropwise addition of a 1.3 M solution of *sec*-BuLi in cyclohexane (2.7 mL, 3.51 mmol). The reaction mixture was stirred for 15 min. Then freshly distilled ethyl chloroformate (2 mL, 21.1 mmol) was slowly added, and the solution was stirred for an additional 75 min at −78 °C. Reaction was quenched at low temperature by adding water (5 mL). The aqueous layer was extracted by EtOAc (4 × 20 mL), and combined organic layers were washed with brine (3 × 20 mL), dried over Na_2SO_4, and concentrated to dryness under reduced pressure. The solid material was purified by silica gel chromatography (10−25% EtOAc in petroleum ether) to give pure compound **5** (0.530 g, 67%) as a white foam. ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 0.0−0.1 (m, 12H, CH_3-Si); 0.8−0.9 (2s, 18H, ^{t}Bu-Si); 1.23 (dd, 3H, $\underline{CH_3}$-CH_2-O, $^3J_1 = 6.9$ Hz, $^3J_2 = 7.3$ Hz); 2.2−2.3 (m, 2H, CH_2-2′ and 2″); 3.7−3.8 (m, 2H, CH_3-$\underline{CH_2}$); 3.9−4.0 (m, 1H, CH_2-5′); 4.1−4.3 (m, 2H, CH-4′ and $\underline{CH_2}$-5″); 4.3−4.4 (m, 1H, CH-3′); 6.03 (t, 1H, CH-1′, $^3J_{H1'\text{-}H2'} = {}^3J_{H1'\text{-}H2''} = 6.6$ Hz); 8.31 (s, 1H, CH-6); 11.60 (s, 1H, NH-3). ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: −5.52 and −4.63 (4C, CH_3-Si); 13.7 ($\underline{CH_3}$-CH_2-O); 18.11 and 18.33 (2C, $\underline{C}(CH_3)_3$); 25.09 and 25.91 (6C, C($\underline{CH_3}$)$_3$); 42.18 (C-2′); 60,8 (CH_3-$\underline{CH_2}$-O); 63.14 (C-5′); 72.72 (C-3′); 86.99 (C-1′); 88.89 (C-4′); 105.04 (C-5); 147.36 (C-6); 149.18 (C-2); 158.99 (C-4); 163.22 (CO-ester). MS (ESI, pos) m/z: 527.6 ($M + H^+$).

5-Ethylcarboxylate-2′-deoxyuridine (6). To a solution of compound **5** (0.500 g, 0.95 mmol) in THF (8 mL), TBAF·3H$_2$O (0.900 g, 2.85 mmol) was added. The solution was stirred at room temperature for 3 h. Solvent was removed under reduced pressure and the brown oily residue was purified by silica gel column chromatography (0−10% MeOH in CH_2Cl_2), affording pure compound **6** (0.230 g, 81%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 1.24 (t, 3H, $\underline{CH_3}$-CH_2-O, $^3J = 6.9$ Hz); 2.1−2.3 (m, 2H, CH_2-2′ and 2″); 3.5−3.7 (m, 2H, CH_2-5′ and 5″); 3.8−3.9 (m, 1H, CH-4′); 4.17 (q, 2H, CH_3-$\underline{CH_2}$, $^3J = 6.9$ Hz); 4.2−4.3 (m, 1H, CH-3′); 5.0−5.1 (br s, 1H, OH-5′); 5.2−5.3 (br s, 1H, OH-3′); 6.12 (dd, 1H, CH-1′, $^3J_{H1'\text{-}H2'} = 6.5$, $^3J_{H1'\text{-}H2''} = 6.2$ Hz); 8.79 (s, 1H, CH-6); 11.53 (s, 1H, NH-3). ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: 13.9 ($\underline{CH_3}$-CH_2-O); 39.28 (C-2′); 60,6 (CH_3-$\underline{CH_2}$-O); 60.35 (C-5′); 69.45 (C-3′); 82.18 (C-1′); 89.56 (C-4′); 102.26 (C-5); 149.32 (C-2); 151.84 (C-6); 159.77 (C-4); 164.14 (CO-ester). MS (ESI, pos) m/z: 322.3 ($M + Na^+$).

5-Carbamoyl-2′-deoxyuridine (1a). A solution of compound **6** (0.220 g, 0.74 mmol) in a pyridine/25% ammonia mixture (5 mL, 1/4) was heated at 80 °C in a sealed tube for 48 h. After the mixture was cooled to room temperature, solvents were removed under vacuum and the crude residue was coevaporated twice with water and dissolved in methanol for crystallization. Pure compound **1a** was obtained as a white solid (0.099 g, 50%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 2.0−2.1 (m, 2H, CH_2-2′ and 2″); 3.5−3.6 (m, 2H, CH_2-5′ and 5″); 3.7−3.8 (m, 1H, CH-4′); 4.2−4.3 (m, 1H, CH-3′); 5.00 (t, 1H, OH-5′, $^3J_{OH5'\text{-}H5'} = {}^3J_{OH5'\text{-}H5''} = 5.1$ Hz); 5.28 (d, 1H, OH-3′, $^3J_{OH3'\text{-}H3'} = 4.4$); 6.13 (dd, 1H, CH-1′, $^3J_{H1'\text{-}H2'} = 6.9$ Hz, $^3J_{H1'\text{-}H2''} = 6.6$ Hz); 7.52 and 8.12 (2s, 2H, NH2); 8.66 (s, 1H, CH-6); 11.85 (s, 1H, NH-3). ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: 33.58 (C-2′); 61,79 (C-5′); 70.79 (C-3′); 84.38 (C-1′); 87.70 (C-4′); 109.36 (C-5); 138.69 (C-6); 150.87 (C-2); 163.69 (C-4); 171.76 (CO-amide). MS (ESI, pos) m/z: 294.1 ($M + Na^+$). UV (H_2O, 30 μM): $\lambda_{max} = 276$ nm, $\varepsilon = 10\,400$ mol^{-1}·L·cm^{-1}. Anal. ($C_{10}H_{13}N_3O_6$) C, H, N.

3′,5′-Di-*O*-*tert*-butyldimethylsilyl-5-ethylcarboxylate-2′-deoxycytidine (8). A solution of compound **5** (0.840 g, 1.59 mmol) in anhydrous acetonitrile (25 mL) was treated with freshly distilled triethylamine (0.7 mL, 5.03 mmol), DMAP (0.590 g, 4.88 mmol), and triisopropylbenzenesulfonyl chloride (TPSCl, 1.46 g, 4.43 mmol). The yellow solution rapidly turned red. After the mixture was stirred for 21 h at room temperature, a 25% aqueous solution of ammonium hydroxide (35 mL) was added. The reaction mixture was slightly decolorized, and stirring was maintained for 1.5 h. Solvents were removed under vacuum, and the brown residue was partitioned between water (50 mL) and EtOAc (50 mL). The aqueous layer was extracted with EtOAc (3 × 50 mL), and the combined organic layers were washed with a saturated solution of $NaHCO_3$ (2 × 50 mL) and brine (2 × 50 mL). The organic layer was dried over Na_2SO_4 and concentrated to dryness under reduced pressure. Purification by silica gel column chromatography (0−5% MeOH in CH_2Cl_2) gave pure **8** (0.59 g, 70%) as a white foam. ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 0.06 and 0.09 (2s, 12H, CH_3-Si); 0.84 and 0.89 (2s, 18H, ^{t}Bu-Si); 1.28 (t, 3H, $\underline{CH_3}$-CH_2, $^3J_{CH3\text{-}CH2} = 7.3$ Hz) 2.1−2.2 (m, 1H, CH_2-2′); 2.5−2.6 (m, 1H, CH_2-2″); 3.7−3.8 (m, 2H, CH_2-5′ and 5″); 3.9−4.0 (m, 1H, CH-4′); 4.2−4.3 (m, 2H, CH_3-$\underline{CH_2}$); 4.3−4.4 (m, 1H, CH-3′); 6.16 (t, 1H, CH-1′, $^3J_{H1'\text{-}H2'} = {}^3J_{H1'\text{-}H2''} = 6.2$ Hz); 7.66 and 7.99 (2s, 2H, NH_2); 8.45 (s, 1H, CH-6). ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: −5.56 and −4.74 (CH_3−Si); 15.26 ($\underline{CH_3}$-CH_2); 18.53 and 18.87 ($\underline{C}$-(CH_3)$_3$); 24.96 and 25.84 (C-($\underline{CH_3}$)$_3$); 43.76 (C-2′); 63.38 and 63.59 (CH_3-$\underline{CH_2}$ and C-5′); 72.97 (C-3′); 87.26 (C-1′); 90.34 (C-4′); 104.06 (C-5); 150.85 (C-6); 156.35 (C-2); 164.95 (CO-ester); 168.58 (C-4). MS (ESI, pos) m/z: 550.43 ($M + Na^+$).

5-Ethylcarboxylate-2′-deoxycytidine (9). To a solution of compound **8** (0.580 g, 1.1 mmol) in THF (8 mL) was added TBAF·3H$_2$O (1.20 g, 3.8 mmol). The reaction mixture was stirred at room temperature for 16 h. Then solvent was removed and the crude material was purified by silica gel chromatography (2−10% MeOH in CH_2Cl_2) to give pure **9** (0.22 g, 67%) as a white solid. ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 1.30 (t, 3H, $\underline{CH_3}$-CH_2, $^3J_{CH3\text{-}CH2} = 6.9$ Hz) 2.0−2.1 (m, 1H, CH_2-2′); 2.2−2.4 (m, 1H, CH_2-2″); 3.6−3.7 (m, 2H, CH_2-5′ and 5″); 3.8−3.9 (m, 1H, CH-4′); 4.1−4.2 (m, 3H, CH_3-$\underline{CH_2}$ and CH-3′); 5.05 (br s, 1H, OH-5′); 5.26 (br s, 1H, OH-3′); 6.08 (t, 1H, CH-1′, $^3J_{H1'\text{-}H2'} = {}^3J_{H1'\text{-}H2''} = 5.9$ Hz); 7.65 and 7.95 (2s, 2H, NH_2); 8.96 (s, 1H, CH-6). ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: 14.96 ($\underline{CH_3}$-CH_2); 43.46 (C-2′); 61.57 and 61.68 (CH_3-$\underline{CH_2}$ and C-5′); 69.54 (C-3′); 84.25 (C-1′); 86.95 (C-4′); 104.32 (C-5); 149.64 (C-6); 155.38 (C-2); 164.09 (CO-ester); 167.98 (C-4). MS (ESI, pos) m/z: 300.40 ($M + H^+$).

5-Carbamoyl-2′-deoxycytidine (2a). A solution of compound **9** (0.189 g, 0.63 mmol) in a mixture pyridine−25% ammonia (1−3 mL) was heated to 80 °C in a sealed tube for 48 h. Solvents were eliminated under reduced pressure, and crude material was coevaporated once with water. The resulting white powder was purified by silica gel chromatography (10−40% MeOH in CH_2Cl_2) and pure fractions were pooled, concentrated, and filtrated through PTFE membrane Minisart SRP 15 (0.20 μm, Sartorius) unit to give pure compound **2a** (0.034 g, 20%) as a white powder. ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 2.1−2.2 (m, 2H, CH_2-2′ and 2″); 3.5−3.7 (m, 2H, CH_2-5′ and 5″); 3.7−3.9 (m, 1H, CH-4′); 4.1−4.3 (m, 1H, CH-3′); 5.08 (t, 1H, OH-5′, $^3J_{OH5'\text{-}H5'} = {}^3J_{OH5'\text{-}H5''} = 5.4$ Hz); 5.22 (d, 1H, OH-3′, $^3J_{OH3'\text{-}H3'} = 4.2$ Hz); 6.11 (t, 1H, CH-1′, $^3J_{H1'\text{-}H2'} = {}^3J_{H1'\text{-}H2''} = 6.5$ Hz); 7.3−7.6 (m, 2H, NH_2-4); 7.72 and 8.32 (2s, 2H, NH_2-amide); 8.41 (s, 1H, CH-6). ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: 40.20 (C-2′); 61.60 (C-5′); 70.79 (C-3′); 88.78 (C-1′); 89.36 (C-4′); 99.07 (C-5); 144.75 (C-6); 156.06 (C-2); 163.90 (C-4); 169.22 ($CONH_2$). MS (ESI, pos) m/z: 293.21 ($M + Na^+$). UV (H_2O, 30 μM): $\lambda_{max} = 284$ nm, $\varepsilon = 13\,500$ mol^{-1}·L·cm^{-1}. Anal. ($C_{10}H_{14}N_4O_5$) C, H, N.

5-Carboxyl-2′-deoxycytidine (10). Compound **10** (0.069 g, 40%) was obtained as a side product during the transformation of ethyl carboxylate **9** into carboxamide derivative **2a**. ^{1}H NMR (D_2O, 300 MHz) δ ppm: 2.1−2.2 (m, 1H, CH_2-2′); 2.2−2.4 (m, 1H, CH_2-2″); 3.6−3.8 (m, 2H, CH_2-5′ and 5″); 3.9−4.0 (m, 1H, CH-4′); 4.2−4.4 (m, 1H, CH-3′); 6.11 (t, 1H, CH-1′, $^3J_{H1'\text{-}H2'} = {}^3J_{H1'\text{-}H2''} = 6.6$ Hz); 8.36 (s, 1H, CH-6). ^{13}C NMR (D_2O, 75 MHz) δ ppm: 39.63 (C-2′); 61.21 (C-5′); 70.45 (C-3′); 86.40 (C-1′); 86.77 (C-4′); 103.08 (C-5); 146.25 (C-6); 156.67 (C-2); 164.83 (C-4); 170.66 (COOH). MS (ESI, pos) m/z: 272.5 ($M + H^+$).

Alternative to the Preparation of Compound 2a. 3′,5′-Di-*O*-*tert*-butyldimethylsilyl-5-bromo-2′-deoxyuridine (11). To a solution of 5-bromo-2′-deoxyuridine (3.99 g, 12.98 mmol) in dry DMF (26 mL), *tert*-butyldimethylsilyl chloride (11.80 g, 77.88 mmol) and imidazole (7.00 g, 103.8 mmol) were added. The clear solution was stirred at room temperature for 60 h and treated as described for compound **4**. Pure compound **11** (6.69 g, 96%) was obtained as a white foam. ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 0.1−0.2 (m, 12H, CH_3−Si); 0.88 and 0.91 (2s, 18H, ^{t}Bu-Si); 2.1−2.3 (m, 2H, CH_2-2′ and 2″); 3.7−4.0 (m, 3H, CH-4′, CH_2-5′ and 5″); 4.3−4.4 (m, 1H, CH-3′); 6.10 (dd, 1H, CH-1′, $^3J_{H1'\text{-}H2'} = 6.5$ Hz, $^3J_{H1'\text{-}H2''} = 6.9$ Hz); 7.99 (s, 1H, CH-6); 11.86 (s, 1H, NH-3). ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: −4.50 and −4.36 (4C, CH_3-Si); 18.10 (2C, $\underline{C}(CH_3)_3$); 26.07 and 26.32 (6C, $C(\underline{CH_3})_3$); 40.34 (C-2′); 62.38 (C-5′); 70.68 (C-3′); 86.15 (C-1′); 87.62 (C-4′); 88.06 (C-5); 149.26 (C-6); 149.37 (C-2); 160.54 (C-4). MS (ESI, pos) m/z: 534.68 ($M + H^+$).

3′,5′-Di-*O*-*tert*-butyldimethylsilyl-5-cyano-2′-deoxyuridine (12). To a solution of compound **11** (3.60 g, 6.73 mmol) in dry DMF (14 mL) were added KCN (0.880 g, 13.13 mmol) and 18-crown-6 (3.20 g, 12.12 mmol). The clear solution was stirred at room temperature for 60 h. Then additional amounts of KCN (0.15 g, 2.24 mmol) and 18-crown-6 (0.43 g, 1.63 mmol) were added, and the solution was heated at 85 °C for 9 h. The orange reaction mixture was allowed to cool to room temperature, diluted with a EtOAc/H_2O (20 mL, v/v, 50/50) mixture, and quickly neutralized with a 1 M HCl solution. *Caution*: This step should be done under a well-ventilated fume hood, and neutralization should be conducted with care to avoid acidification of the solution and the concomitant formation of the highly toxic HCN gas. Layers were separated, and the aqueous layer was extracted with AcOEt (4 × 50 mL). The combined extracts were washed with brine (2 × 50 mL), dried over Na_2SO_4, and concentrated to dryness. The residue was purified by silica gel column chromatography (0−3% MeOH in CH_2Cl_2) to give **12** (2.10 g, 65%) as a white foam. ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 0.1−0.2 (m, 12H, CH_3−Si); 0.88 and 0.91 (2s, 18H, ^{t}Bu-Si); 2.1−2.4 (m, 2H, CH_2-2′ and 2″); 3.7−3.8 (m, 1H, CH_2-5′); 3.8−4.0 (m, 2H, CH-4′, CH_2-5″); 4.3−4.4 (m, 1H, CH-3′); 6.02 (dd, 1H, CH-1′, $^3J_{H1'\text{-}H2'} = 6.0$ Hz, $^3J_{H1'\text{-}H2''} = 6.1$ Hz); 8.44 (s, 1H, CH-6); 12.07 (s, 1H, NH-3). ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: −4.65 and −4.42 (4C, CH_3−Si); 18.11 (2C, $\underline{C}(CH_3)_3$); 26.09 and 26.34 (6C, $C(\underline{CH_3})_3$); 40.32 (C-2′); 62.77 (C-5′); 70.54 (C-3′); 82.95 (C-1′); 85.37 (C-4′); 88.06 (C-5); 115.53 (CN); 150.28 (C-2); 150.95 (C-6); 160.73 (C-4). MS (ESI, pos) m/z: 481.52 ($M + H^+$).

3′,5′-Di-*O*-*tert*-butyldimethylsilyl-5-cyano-2′-deoxycytidine (13). A solution of compound **12** (1.90 g, 3.95 mmol) in anhydrous acetonitrile (50 mL) was treated with freshly distilled triethylamine (2.2 mL, 8.6 mmol), DMAP (2.01 g, 16.6 mmol), and tri-isopropylbenzenesulfonyl chloride (TPSCl, 5.51 g, 18.2 mmol), followed by ammonolysis as for compound **8**. The residue was purified by silica gel chromatography (0−10% MeOH in CH_2Cl_2) to give **13** (1.37 g, 72%) as a white foam. ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 0.0−0.1 (m, 12H, CH_3−Si); 0.88 and 0.90 (2s, 18H, ^{t}Bu-Si); 2.1−2.3 (m, 2H, CH_2-2′ and 2″); 3.7−3.9 (m, 3H, CH-4′, CH_2-5′ and 5″); 4.3−4.4 (m, 1H, CH-3′); 6.02 (t, 1H, CH-1′, $^3J_{H1'\text{-}H2'} = {}^3J_{H1'\text{-}H2''} = 6.4$ Hz); 7.55 and 8.03 (2s, 2H, NH_2); 8.40 (s, 1H, CH-6). ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: −5.52 and −4.64 (CH_3-Si); 18.11 and 18.35 ($\underline{C}$-$(CH_3)_3$); 25.06 and 25.92 (C-$(\underline{CH_3})_3$); 41.97 (C-2′); 62.76 (C-5′); 72.72 (C-3′); 86.59 (C-1′); 88.86 (C-4′); 106.02 (C-5); 115.42 (CN); 150.73 (C-6); 154.21 (C-2); 164.08 (C-4). MS (ESI, pos) m/z: 480.48 ($M + H^+$).

5-Cyano-2′-deoxycytidine (14). Removal of silyl protecting groups from compound **13** (0.950 g, 1.98 mmol) was achieved by reaction with TBAF·3H_2O (2.19 g, 6.93 mmol) in THF (15 mL) as described for compound **5**. Purification by silica gel column chromatography (0−12% MeOH in CH_2Cl_2) and crystallization from MeOH afforded compound **14** (0.32 g, 64%) as a pale-yellow solid. ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 2.0−2.1 (m, 1H, CH_2-2′); 2.1−2.3 (m, 1H, CH_2-2″); 3.5−3.7 (m, 2H, CH_2-5′ and 5″); 3.7−3.8 (m, 1H, CH-4′); 4.1−4.2 (m, 1H, CH-3′); 5.5−5.8 (m, 2H, OH-3′ and OH-5′); 6.01 (t, 1H, CH-1′, $^3J_{H1'\text{-}H2'} = {}^3J_{H1'\text{-}H2''} = 6.0$ Hz); 7.2−7.6 (2 br s, 2H, NH_2); 8.71 (s, 1H, CH-6). ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: 41.42 (C-2′); 60.89 (C-5′); 79.15 (C-3′); 86.71 (C-1′); 88.20 (C-4′); 106.38 (C-5); 115.34 (CN); 150.83 (C-6); 153.03 (C-2); 162.82 (C-4). MS (ESI, pos) m/z: 275.19 ($M + Na^+$). UV (H_2O, 30 μM): $\lambda_{max} = 281$ nm, $\varepsilon = 9300$ $mol^{-1}\cdot L\cdot cm^{-1}$. Results are in accordance with ^{1}H NMR and UV data published by Lahoud et al.[47]

5-Carbamoyl-2′-deoxycytidine (2a). Compound **14** (0.210 g, 0.83 mmol) in water (60 mL) was treated with a 1 M NaOH solution (6 mL). After 4 h of being stirred at room temperature, the reaction mixture was neutralized with Dowex 50WX8 hydrogen form resin. Purification by silica gel column chromatography (10−30% MeOH in CH_2Cl_2) followed by filtration through PTFE membrane Minisart SRP 15 (0.20 μm, Sartorius) unit afforded pure compound **2a** (0.11 g, 49%) as a white foam. All characteristics are in accordance with the ones previously described.

5-Hydroxy-2′-deoxyuridine (1d). Starting from 2′-deoxyuridine (3.47 g, 15.2 mmol), 5-hydroxy-2′-deoxyuridine **1d** (2.00 g, 54%) was prepared as previously described by La Francois et al.[40] ^{1}H NMR and MS data are in accordance with the data previously published. ^{1}H NMR (D_2O, 300 MHz) δ ppm: 2.0−2.1 (m, 2H, CH_2-2′ and 2″); 3.5−3.6 (m, 2H, CH_2-5′ and 5″); 3.7−3.8 (m, 1H, CH-4′); 4.2−4.3 (m, 1H, CH-3′); 5.00 (dd, 1H, OH-5′, $^3J_{OH5'\text{-}H5'} = 5.1$ Hz, $^3J_{OH5'\text{-}H5''} = 4.8$ Hz); 5.22 (d, 1H, OH-3′, $^3J_{OH3'\text{-}H3'} = 4.0$ Hz); 6.19 (dd, 1H, CH-1′, $^3J_{H1'\text{-}H2'} = 6.6$ Hz, $^3J_{H1'\text{-}H2''} = 7.3$ Hz); 7.33 (s, 1H, CH-6); 8.67 (s, 1H, OH-5); 11.43 (s,1H, NH-3). ^{13}C NMR (D_2O, 75 MHz) δ ppm: 41.43 (C-2′); 62.65 (C-5′); 71.54 (C-3′); 86.06 (C-1′); 88.93 (C-4′); 123.33 (C-6); 132.65 (C-5); 151.85 (C-2); 162.07 (C-4). MS (ESI, pos) m/z: 267.36 ($M + Na^+$). UV (H_2O, 30 μM): $\lambda_{max} = 278$ nm, $\varepsilon = 7600$ $mol^{-1}\cdot L\cdot cm^{-1}$. Anal. ($C_9H_{12}N_2O_6$) C, H, N.

Carbamoyltriphenylphosphorane (15) was prepared as described by Trippett et al.[44] in 66% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 1.92 (s, 1H, CH vinyl); 7.2−7.7 (m, 15H, CH phenyl). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 128.45 (CH vinyl); 128.60 (9C, CH-m and CH-p phenyl); 132.00 and 132.13 (6C, CH-o aromatic); 133.19 (3C, C aromatic); 172.50 (CO amide). ^{31}P NMR ($CDCl_3$, 300 MHz) δ ppm: 3.04 (1P).

5-Carbamoylmethyl-2′-deoxyuridine (1b). To a solution of 5-hydroxy-2′-deoxyuridine **1d** (1.65 g, 6.76 mmol) in 1,4-dioxane (37 mL) was added carbamoyltriphenylphosphorane **15** (4.30 g, 13.48 mmol). The yellow solution was stirred for 4 h at 100 °C. Solvent was eliminated under reduced pressure and the crude material was crystallized from methanol to give pure compound **1b** (1.22 g, 71%) as a white solid. ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 2.0−2.1 (m, 2H, CH_2-2′ and 2″); 3.03 (s, 2H, C5-$\underline{CH_2}$); 3.5−3.6 (m, 2H, CH_2-5′ and 5″); 3.7−3.8 (m, 1H, CH-4′); 4.2−4.3 (m, 1H, CH-3′); 4.99 (dd, 1H, OH-5′, $^3J_{OH5'\text{-}H5'} = 5.2$ Hz, $^3J_{OH5'\text{-}H5''} = 5.1$ Hz); 5.24 (d, 1H, OH-3′, $^3J_{OH3'\text{-}H3'} = 4.2$ Hz); 6.17 (dd, 1H, CH-1′, $^3J_{H1'\text{-}H2'} = 6.7$ Hz, $^3J_{H1'\text{-}H2''} = 7.0$ Hz); 6.85 and 7.28 (2s, 2H, NH_2); 7.73 (s, 1H, CH-6); 11.31 (s, 1H, NH-3). ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: 32.98 (C5-$\underline{CH_2}$); 37.86 (C-2′); 61.03 (C-5′); 70.38 (C-3′); 85.22 (C-1′); 86.64 (C-4′); 108.79 (C-5); 140.09 (C-6); 151.43 (C-2); 165.69 (C-4); 175.46 (CO-amide). MS (ESI, pos) m/z: 308.34 ($M + Na^+$). UV (H_2O, 30 μM): $\lambda_{max} = 266$ nm, $\varepsilon = 7500$ $mol^{-1}\cdot L\cdot cm^{-1}$. Anal. ($C_{11}H_{15}N_3O_6$) C, H, N.

3′,5′-Di-*O*-*tert*-butyldimethylsilyl-5-carbamoylmethyl-2′-deoxyuridine (16). To a solution of compound **1b** (0.90 g, 3.33 mmol) in dry DMF (7 mL) were added imidazole (1.90 g, 27.9 mmol) and *tert*-butyldimethylsilyl chloride (3.09 g, 20.5 mmol). The reaction mixture was stirred at room temperature for 60 h. Water (50 mL) was added, and the aqueous layer was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine (3 × 50 mL), dried over Na_2SO_4, and concentrated under vacuum. The oily residue was purified by silica gel chromatography (0−3% MeOH in CH_2Cl_2) to give pure compound **16** (1.55 g, 91%) as a white foam. ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 0.08 and 0.09 (2s, 12H, $\underline{CH_3}$-Si); 0.89 and 0.90 (2s, 18H, $^t\underline{Bu}$-Si); 2.1−2.2 (m, 2H, CH_2-2′ and 2″); 3.02 (s, 2H, C5-$\underline{CH_2}$); 3.7−3.8 (m, 2H, CH_2-5′ and 5″); 3.8−3.9 (m, 1H, CH-4′); 4.3−4.4 (m, 1H, CH-3′); 6.16 (dd, 1H, CH-1′, $^3J_{H1'\text{-}H2'}$ = 6.3 Hz, $^3J_{H1'\text{-}H2''}$ = 7.5 Hz); 6.85 and 7.28 (2s, 2H, NH_2); 7.50 (s, 1H, CH-6); 11.37 (s, 1H, NH-3). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: −5.04 and −4.75 (4C, $\underline{CH_3}$-Si); 18.05 and 18.35 (2C, $(CH_3)_3\underline{C}$-Si); 25.73 and 25.88 (6C, $(\underline{CH_3})_3$C-Si); 33.08 (C5-$\underline{CH_2}$); 42.18 (C-2′); 63.07 (C-5′); 72.84 (C-3′); 86.94 (C-1′); 88.94 (C-4′); 109.07 (C-5); 139.42 (C-6); 151.13 (C-2); 164.56 (C-4); 172.48 (CO-amide). MS (ESI, pos) *m*/*z*: 513.5 ($M + H^+$).

3′,5′-Di-*O*-*tert*-butyldimethylsilyl-5-carbamoylmethyl-2′-deoxycytidine (18). To a solution of compound **16** (1.50 g, 2.92 mmol) in anhydrous acetonitrile (50 mL) were added DMAP (1.10 g, 9.1 mmol), freshly distilled triethylamine (1.22 mL, 8.8 mmol), and triisopropylbenzenesulfonyl chloride (2.75 g, 9.1 mmol). The solution was stirred at room temperature for 21 h before a 25% solution of ammonium hydroxide in water (70 mL) was added. After the mixture was stirred for an additional 1.5 h at room temperature, solvents were removed under vacuum. The crude material was dissolved in a water/EtOAc (100 mL, 1/1) mixture. Layers were separated, and the aqueous was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with a saturated solution of $NaHCO_3$ (3 × 50 mL) and then with brine (3 × 50 mL), dried over Na_2SO_4, and concentrated to dryness. A purification by silica gel chromatography afforded compound **18** (0.91 g, 61%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 0.07 and 0.08 (2s, 12H, $\underline{CH_3}$-Si); 0.88 and 0.89 (2s, 18H, $^t\underline{Bu}$-Si); 2.0−2.2 (m, 2H, CH_2-2′ and 2″); 3.05 (s, 2H, C5-$\underline{CH_2}$); 3.6−3.8 (m, 2H, CH_2-5′ and 5″); 3.8−4.0 (m, 1H, CH-4′); 4.4−4.5 (m, 1H, CH-3′); 6.18 (dd, 1H, CH-1′, $^3J_{H1'\text{-}H2'}$ = 6.2 Hz, $^3J_{H1'\text{-}H2''}$ = 7.4 Hz); 6.97 (br s, 2H, NH_2-4); 7.02 and 7.36 (2s, 2H, NH_2-amide); 7.41 (s, 1H, CH-6).

5-Carbamoylmethyl-2′-deoxycytidine (2b). To a solution of compound **18** (0.870 g, 1.70 mmol) in THF (20 mL) was added TBAF·$3H_2O$ (2.68 g, 8.5 mmol). The solution was stirred at room temperature for 2 h. Solvent was removed under reduced pressure, and the crude material was purified by silica gel column chromatography (0−20% MeOH in CH_2Cl_2). Pure fractions were pooled, concentrated, and filtrated through PTFE membrane Minisart SRP 15 (0.20 μm, Sartorius) unit. Compound **2b** (0.40 g, 83% yield) was obtained by crystallization from absolute ethanol. ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 1.9−2.1 (2 m, 2H, CH_2-2′ and 2″); 3.08 (s, 2H, C5-$\underline{CH_2}$); 3.5−3.6 (m, 2H, CH_2-5′ and 5″); 3.7−3.8 (m, 1H, CH-4′); 4.1−4.3 (m, 1H, CH-3′); 4.95 (t, 1H, OH-5′, $^3J_{OH5'\text{-}H5'}$ = $^3J_{OH5'\text{-}H5''}$ = 5.5 Hz); 5.20 (d, 1H, OH-3′, $^3J_{OH3'\text{-}H3'}$ = 4.2 Hz); 6.15 (dd, 1H, CH-1′, $^3J_{H1'\text{-}H2'}$ = 6.5 Hz, $^3J_{H1'\text{-}H2''}$ = 7.0 Hz); 6.6−6.9 (m, 1H, NH_2-4); 7.04 (s, 1H, NH_2-amide); 7.1−7.3 (m, 1H, NH_2-4); 7.39 (s, 1H, NH_2-amide); 7.61 (s, 1H, CH-6). ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: 30.43 (C5-$\underline{CH_2}$); 39.35 (C-2′); 60.96 (C-5′); 70.18 (C-3′); 86.19 (C-1′); 86.55 (C-4′); 101.79 (C-5); 139.58 (C-6); 157.02 (C-2); 166.28 (C-4); 174.88 (CO-amide). MS (ESI, pos) *m*/*z*: 307.43 ($M + Na^+$). UV (H_2O, 30 μM): λ_{max} = 277 nm, ε = 7800 $mol^{-1}\cdot L\cdot cm^{-1}$. Anal. ($C_{11}H_{16}N_4O_5$) C, H, N.

5-Hydroxymethyl-2′-deoxyuridine (1c). Compound **1c** was obtained from commercially available 2′-deoxyuridine.[40] 2′-Deoxyuridine (1.0 g, 4.39 mmol) in water (10 mL) was reacted with triethylamine (8 mL) and 37% aqueous formaldehyde (20 mL) at reflux for 48 h to give, after silica gel chromatography (4−14% MeOH in CH_2Cl_2), filtration through PTFE membrane Minisart SRP 15 (0.20 μm, Sartorius) unit, and crystallization from ethanol, compound **1c** (0.45 g, 40%). The ^{1}H NMR data were identical with the ones previously published.[40] ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 2.0−2.1 (m, 2H, CH_2-2′ and 2″); 3.5−3.6 (m, 2H, CH_2-5′ and 5″); 3.8−3.9 (m, 1H, CH-4′); 4.1−4.2 (m, 2H, $\underline{CH_2}$-OH); 4.2−4.3 (m, 1H, CH-3′); 4.9−5.0 (m, 2H, OH-5′ and 5-CH_2-$\underline{OH}$); 5.2−5.3 (m, 1H, OH-3′); 6.22 (dd, 1H, CH-1′, $^3J_{H1'\text{-}H2'}$ = 6.4 Hz, $^3J_{H1'\text{-}H2''}$ = 7.0 Hz); 7.73 (s, 1H, CH-6); 11.31 (s, 1H, NH-3). ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: 45.96 (C-2′); 61.82 (C5-$\underline{CH_2}$); 61.90 (C-5′); 70.99 (C-3′); 84.35 (C-1′); 87.74 (C-4′); 114.71 (C-5); 137.21 (C-6); 150.80 (C-2); 163.08 (C-4). MS (ESI, pos) *m*/*z*: 281.54 ($M + Na^+$). UV (H_2O, 30 μM): λ_{max} = 264 nm, ε = 8600 $mol^{-1}\cdot L\cdot cm^{-1}$. Anal. ($C_{10}H_{14}N_2O_6$) C, H, N.

5-Hydroxymethyl-2′-deoxycytidine (2c). Compound **2c** was prepared following the strategy used to convert compound **1b** into **2b** and described by LaFrancois et al. for the preparation of labeled nucleoside analogues of compound **2c**.[40] 5-Methylhydroxy-2′-deoxyuridine **1c** (1.80 g, 7 mmol), TBDMSCl (10.5 g, 70 mmol), and imidazole (4.80 g, 70 mmol) in dry DMF (14 mL) were stirred for 60 h at room temperature. Purification by silica gel column chromatography (5−15% AcOEt in petroleum ether) afforded compound **17** as a white foam (2.9 g, 69%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 0.1−0.2 (m, 18H, 6 × CH_3-Si); 0.8−1.0 (m, 27H, 3$(CH_3)_3$); 2.0−2.1 (m, 2H, CH_2-2′ and 2″); 3.6−3.7 (m, 2H, CH_2-5′ and 5″); 3.7−3.8 (m, 1H, CH-4′); 4.3−4.4 (m, 3H, $\underline{CH_2}$-O and CH-3′); 6.17 (dd, 1H, CH-1′, $^3J_{H1'\text{-}H2'}$ = 6.4 Hz, $^3J_{H1'\text{-}H2''}$ = 7.0 Hz); 7.43 (s, 1H, CH-6); 11.34 (s, 1H, NH-3).

Compound **17** (2.90 g, 4.83 mmol), DMAP (1.75 g, 14.5 mmol), Et_3N (2 mL, 14.5 mmol), and triisopropylbenzenesulfonyl chloride (4.60 g, 15.2 mmol) in anhydrous acetonitrile (83 mL) were reacted for 21 h at room temperature. A 25% ammonia solution (115 mL) was added, and the reaction was pursued for 4 h at room temperature. Extractions with EtOAc and purification by silica gel column chromatography (0−2.5% MeOH in CH_2Cl_2) afforded compound **19** (1.49 g, 51%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 0.0−0.1 (m, 18H, 6 × CH_3−Si); 0.8−0.9 (m, 27H, 3$(CH_3)_3$); 1.9−2.2 (2 m, 1H, CH_2-2′ and 2″); 3.6−3.8 (m, 3H, CH-4′, CH_2-5′ and 5″); 4.3−4.4 (m, 1H, CH-3′); 4.4−4.5 (m, 1H, $\underline{CH_2}$-O); 6.18 (dd, 1H, CH-1′, $^3J_{H1'\text{-}H2'}$ = 5.9 Hz, $^3J_{H1'\text{-}H2''}$ = 7.9 Hz); 6.5−6.6 and 7.4−7.5 (2 m, 2H, NH_2); 7.47 (s, 1H, CH-6).

Treatment of **19** (1.48 g, 2.47 mmol) with TBAF·$3H_2O$ (7.78 g, 24.7 mmol) in THF (18 mL) followed by a purification by silica gel column chromatography (10−30% MeOH in CH_2Cl_2), a filtration through PTFE membrane Minisart SRP 15 (0.20 μm, Sartorius) unit, and a crystallization from ethanol afforded compound **2c** (0.34 g, 54%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 1.9−2.1 (2 m, 2H, CH_2-2′ and 2″); 3.5−3.6 (m, 2H, CH_2-5′ and 5″); 3.7−3.8 (m, 1H, CH-4′); 4.1−4.2 (m, 3H, $\underline{CH_2}$-OH and CH-3′); 4.9−5.0 (m, 2H, OH-5′ and 5-CH_2-$\underline{OH}$); 5.20 (d, 1H, OH-3′, $^3J_{H3'-OH}$ = 4.1 Hz); 6.16 (dd, 1H, CH-1′, $^3J_{H1'\text{-}H2'}$ = 6.6 Hz, $^3J_{H1'\text{-}H2''}$ = 6.9 Hz); 6.6−6.7 and 7.3−7.4 (2 m, 2H, NH_2); 7.75 (s, 1H, CH-6). ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: 40.74 (C-2′); 57.93 (C5-$\underline{CH_2}$); 61.85 (C-5′); 70.90 (C-3′); 85.15 (C-1′); 87.58 (C-4′); 106.38 (C-5); 139.38 (C-6); 155.42 (C-2); 164.84 (C-4). MS (ESI, pos) *m*/*z*: 280.21 ($M + Na^+$). UV (H_2O, 30 μM): λ_{max} = 274 nm, ε = 8700 $mol^{-1}\cdot L\cdot cm^{-1}$. Anal. ($C_{10}H_{15}N_3O_5$) C, H, N. Data are in accordance with the ones published for the same compound isolated after oxidation of 5-methyl-2′-deoxycytidine by menadione.[48]

3′,5′-Di-*O*-*tert*-butyldimethylsilyl-2′-deoxyuridine (20). As described for **4**, 2′-deoxyuridine (0.950 g, 4.17 mmol) was treated with TBDMSCl (3.78 g, 25 mmol) and imidazole (2.23 g, 33.3 mmol) in DMF (8 mL) at room temperature for 60 h to give, after purification by silica gel chromatography, compound **20**

(1.7 g, 90% yield). ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 0.0−0.1 (m, 12H, CH_3-Si); 0.89 and 0.90 (2s, 18H, ^{t}Bu-Si); 2.1−2.3 (m, 2H, CH_2-2′ and 2″); 3.6−3.9 (m, 3H, CH-4′, CH_2-5′ and 5″); 4.3−4.4 (m, 1H, CH-3′); 5.54 (d, 1H, CH-5, $^3J_{H5-H6}$ = 8.0 Hz); 6.13 (t, 1H, CH-1′, $^3J_{H1'-H2'}$ = $^3J_{H1'-H2''}$ = 6.5 Hz); 7.71 (d, 1H, CH-6, $^3J_{H5-H6}$ = 8.0 Hz); 11.34 (s, 1H, NH-3). ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: −5.05 and −4.36 (4C, CH_3−Si); 18.06 (2C, $\underline{C}(CH_3)_3$); 26.10 and 26.27 (6C, $C(\underline{C}H_3)_3$); 40.65 (C-2′); 62.86 (C-5′); 70.84 (C-3′); 73.58 (C-1′); 85.48 (C-4′); 103.28 (C-5); 137.92 (C-6); 150.64 (C-2); 163.36 (C-4). MS (ESI, pos) m/z: 457.85 ($M + H^+$).

3′,5′-Di-*O*-*tert*-butyldimethylsilyl-3-*N*-carbamoylmethyl-2′-deoxyuridine (21). To a solution of compound **20** (1.60 g, 3.50 mmol) in freshly distilled THF (10 mL) was slowly added a suspension of sodium hydride in freshly distilled THF (0.280 g, 7 mmol, 10 mL). The mixture was stirred for 30 min at room temperature, then cooled to 0 °C before adding the chloroacetamide in suspension in freshly distilled THF (3.70 g, 40 mmol, 30 mL). The reaction mixture was allowed to warm to room temperature and stirred for an additional 3 h before the reaction was stopped by the addition of water (10 mL). The aqueous layer was extracted with EtOAc (4 × 50 mL), and the combined organic layer was washed with brine (3 × 50 mL), dried with Na_2SO_4, and concentrated to dryness. Purification by silica gel column chromatography (0−5% MeOH in CH_2Cl_2) afforded compound **21** (1.29 g, 72% yield). ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 0.85 and 0.10 (2s, 12H, $\underline{CH_3}$-Si); 0.89 and 0.90 (2s, 18H, ^{t}Bu-Si); 2.1−2.3 (m, 2H, CH_2-2′ and 2″); 3.7−3.9 (m, 3H, CH-4′, $\underline{C}H_2$-5′ and 5″); 4.33 (s, 2H, N3-$\underline{CH_2}$); 4.3−4.4 (m, 1H, CH-3′); 5.76 (d, 1H, CH-5, $^3J_{H5-H6}$ = 8.1 Hz); 6.16 (dd, 1H, CH-1′, $^3J_{H1'-H2'}$ = 6.4 Hz, $^3J_{H1'-H2''}$ = 6.5 Hz); 7.11 and 7.55 (2s, 2H, NH_2); 7.79 (d, 1H, CH-6, $^3J_{H5-H6}$ = 8.1 Hz). ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: −4.14 and −4.60 (4C, CH_3−Si); 18.01 and 18.46 (2C, $\underline{C}(CH_3)_3$); 26.00 and 26.23 (6C, $C(\underline{C}H_3)_3$); 40.34 (C-2′); 42,40 (N3-$\underline{C}H_2$); 62.76 (C-5′); 71.67 (C-3′); 83.57 (C-1′); 87.20 (C-4′); 101.36 (C-5); 139.26 (C-6); 150.91 (C-2); 162.10 (C-4); 168,72 (CO amide). MS (ESI, pos) m/z: 514.81 ($M + H^+$).

3-*N*-Carbamoylmethyl-2′-deoxyuridine (3). Elimination of silyl protecting groups from **21** (1.26 g, 2.45 mmol) by the procedure previously described for compound **2b** afforded the target compound **3** (0.450 g, 65% yield). ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 2.0−2.2 (m, 2H, CH_2-2′ and 2″); 3.5−3.6 (m, 2H, CH_2-5′ and 5″); 3.7−3.9 (m, 1H, CH-4′); 4.2−4.3 (m, 1H, CH-3′); 4.33 (s, 2H, N3-$\underline{CH_2}$); 5.09 (t, 1H, OH-5′, $^3J_{H5'-OH}$ = $^3J_{H5''-OH}$ = 5.0 Hz); 5.32 (d, 1H, OH-3′, $^3J_{H3'-OH}$ = 3.9 Hz); 5.78 (d, 1H, CH-5, $^3J_{H5-H6}$ = 8.4 Hz); 6.15 (t, 1H, CH-1′, $^3J_{H1'-H2'}$ = $^3J_{H1'-H2''}$ = 6.7 Hz); 7.09 and 7.57 (2s, 2H, NH_2); 7.93 (d, 1H, CH-6, $^3J_{H5-H6}$ = 8.4 Hz). ^{13}C NMR (DMSO-d_6, 75 MHz) δ ppm: 40.31 (C-2′); 42,86 (N3-$\underline{C}H_2$); 61.64 (C-5′); 70.73 (C-3′); 85.63 (C-1′); 87.96 (C-4′); 101.29 (C-5); 139.66 (C-6); 150.95 (C-2); 162.21 (C-4); 168,81 (CO amide). MS (ESI, pos) m/z: 308.36 ($M + Na^+$). UV (H_2O, 30 μM): λ_{max} = 263 nm, ε = 7900 $mol^{-1} \cdot L \cdot cm^{-1}$. Anal. ($C_{11}H_{15}N_3O_6$) C, H, N.

Anti-HIV Activity. The cell cultures of MT-4 and CEM-SS cells were maintained at 37 °C in a 5% CO_2 atmosphere in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS) containing penicillin, streptomycin, and glutamine. MT-4 cells were infected with HIV-1 IIIB isolate at 5 $TCID_{50}$ (tissue culture infective dose) and CEM-SS cells with HIV-1 LAI isolate at 20 $TCID_{50}$. These viruses are two closely related isolates differing by their cytopathogenicity. After 30 min of adsorption, the infected cells were washed to remove unadsorbed virus particles and resuspended in RPMI at the final concentration of 10^5 cells/mL, in the presence of various dilutions of the test compounds. After 5 days, virus production was determined by measuring RT activity in culture medium as already described.[49] The 50% inhibitory concentration (IC_{50}) was derived from the computer-generated median effect plot of the dose−effect data.[50] AZT was used as a positive control (IC_{50} = 2.4 nM). The entire experiment was repeated twice.

Cell Toxicity. The cytotoxicity of the drugs was evaluated in parallel by incubating uninfected cells in the presence of ranging concentrations of antiviral products. Cell viability was measured by means of a colorimetric reaction based on the capacity of mitochondrial dehydrogenase of living cells to reduce 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) into formazan, the quantity of which was determined by the optical density at 540 nm.[51] The 50% cytotoxic concentration (CC_{50}) was calculated with the program used for the determination of IC_{50}.

Serial Passage Experiments. Stock preparations of HIV-1 LAI was added at a multiplicity of 0.01−100 μL aliquots of RPMI medium containing 2.5 × 10^5 CEM-SS cells. After 4 h at 37 °C, the infected cells were washed twice with PBS (without $MgCl_2$) and resuspended in 1 mL of medium with or without the different deoxynucleoside analogues (200 μM, except compound **1c**, 5CH_2OH-2′dU, which was used at 100 μM) in a 48-well plate. After 4 days, the indicated amount of supernatant was transferred to fresh CEM-SS cells. This procedure was iterated for eight cycles; aliquots of both cells and supernatants were frozen at each passage. Virus production was monitored by measuring RT activity in culture supernatants as described above. All experiments were carried out in duplicate.

Viral Replication Kinetics. The kinetics of viral replication was determined with CEM-SS cells. CEM-SS cells (2.5 × 10^5) were infected with a normalized low dose (equivalent to 25 cpm/mL RT activity) of HIV-1 viruses issued from passages 1, 3, and 7 for 1 h at 37 °C. Cells were washed out twice with PBS and incubated in 1 mL of RPMI at 37 °C in a 48-well plate in the absence of nucleoside analogues. Virus growth was measured over 17 days by RT activity in culture supernatant as described above. The culture medium was changed at days 2, 4, 6, 8, 10, 13, and 15.

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Supporting Information Available: Elemental analysis data for target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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