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Synthesis and Biological Evaluation of Fatty Acyl Ester Derivatives of (−)-2′,3′-Dideoxy-3′-thiacytidine

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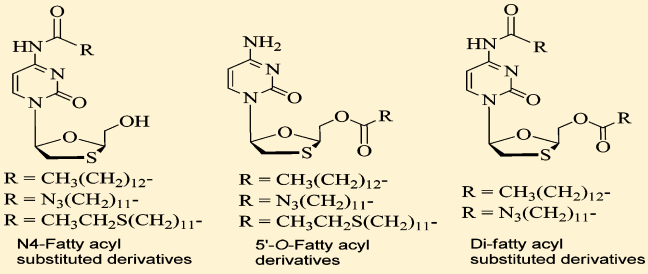
Supporting Information

ABSTRACT: A number of fatty acyl derivatives of (−)-2′,3′-dideoxy-3′-thiacytidine (lamivudine, 3TC, **1**) were synthesized and evaluated for their anti-HIV activity. The monosubstituted 5′-*O*-fatty acyl derivatives of 3TC (EC_{50} = 0.2−2.3 μM) were more potent than the corresponding monosubstituted N_4-fatty acyl (EC_{50} = 0.4−29.4 μM) and 5′-*O*-N_4-disubstituted (EC_{50} = 72.6 to >154.0 μM) derivatives of the nucleoside. 5′-*O*-Myristoyl (**16**) and 5′-*O*-12-azidododecanoyl derivatives (**17**) were found to be the most potent compounds (EC_{50} = 0.2−0.9 μM) exhibiting at least 16−36-fold higher anti-HIV activity against cell-free virus than **1** (EC_{50} = 11.4−32.7 μM). The EC_{90} values for **16** against B-subtype and C-subtype clinical isolates were several folds lower than those of **1**. The cellular uptake studies confirmed that compound **16** accumulated intracellularly after 1 h of incubation with CCRF-CEM cells and underwent intracellular hydrolysis. 5′-*O*-Fatty acyl derivatives of **1** showed significantly higher anti-HIV activity than the corresponding physical mixtures against the B-subtype virus.



INTRODUCTION

Lamivudine [(−)-2′,3′-dideoxy-3′-thiacytidine, 3TC, **1**] is a reverse transcriptase inhibitor known to block human immunodeficiency virus-1 (HIV-1) and hepatitis B virus replication (HBV).[1] This anti-HIV drug is commonly used in combination with two or more other anti-HIV drugs in Highly Active Antiretroviral Therapy (HAART) programs for the treatment of HIV infection.[2] Compound **1** has been shown to have a higher therapeutic index than 3′-azido-2′,3′-dideoxythymidine (AZT) and 3′-fluoro-2′,3′-dideoxythymidine (FLT) and possesses anti-HIV activity against AZT-resistant virus.[3] Although **1** has potent activity against wild-type HIV, over time, mutations in HIV reverse transcriptase have generated multiple strains of 3TC-resistant viruses.[4−7] It is therefore important to continue developing new and effective RT inhibitors.

Myristoyl-CoA:protein *N*-myristoyltransferase (NMT) is a crucial enzyme involved in catalyzing the myristoylation of several proteins involved in the life cycle of HIV (e.g., capsid protein p17, $Pr160^{gag\text{-}pol}$, $Pr55^{gag}$, and $p27^{nef}$). At *N*-terminal glycine, viral proteins are covalently attached to myristic acid in the presence of NMT, making proteins more hydrophobic. In turn, this *N*-myristoylation improves protein−protein and protein−membrane interactions.[8] For example, after *N*-myristoylation, p17 protein localizes itself toward the cell membrane, where a new virus is produced.[9]

Several fatty acid analogues of myristic acid have been shown to inhibit NMT.[10] HIV-1 replication can be inhibited by heteroatom-containing analogues of myristic acid without cellular toxicity.[11,12] Several fatty acids, such as 2-methoxydodecanoic acid, 4-oxatetradecanoic acid, and 12-thioethyldodecanoic acid, reduce HIV-1 replication in acutely infected T-lymphocytes. For example, 12-thioethyldodecanoic acid was moderately active (EC_{50} = 9.4 μM) against HIV-infected $CD4^+$ T lymphocytes.[13]

We previously reported the synthesis and evaluation of several 5′-*O*-fatty acyl esters of AZT, FLT, and 2′,3′-didehydro-2′,3′-dideoxythymidine (d4T) for inhibition of HIV-1.[14−16] These conjugates were designed to achieve anti-HIV activity through inhibition of reverse transcriptase and NMT by nucleosides and fatty acids.

Herein, we report the synthesis of fatty acyl derivatives of **1**, their cellular uptake profiles, and the characterization of their antiviral activity against lab-adapted HIV-1 and wild-type and mutant clinical isolates. Three fatty acids, myristic acid, 12-azidododecanoic acid, and 12-thioethyldodecanoic acid, were conjugated at 5′-*O*- and N_4-position of the nucleoside. Selection of the fatty acids was based on the anti-HIV activity of the previously reported corresponding fatty acyl derivatives of FLT and d4T.[14−16] We hypothesized that the attachment of the

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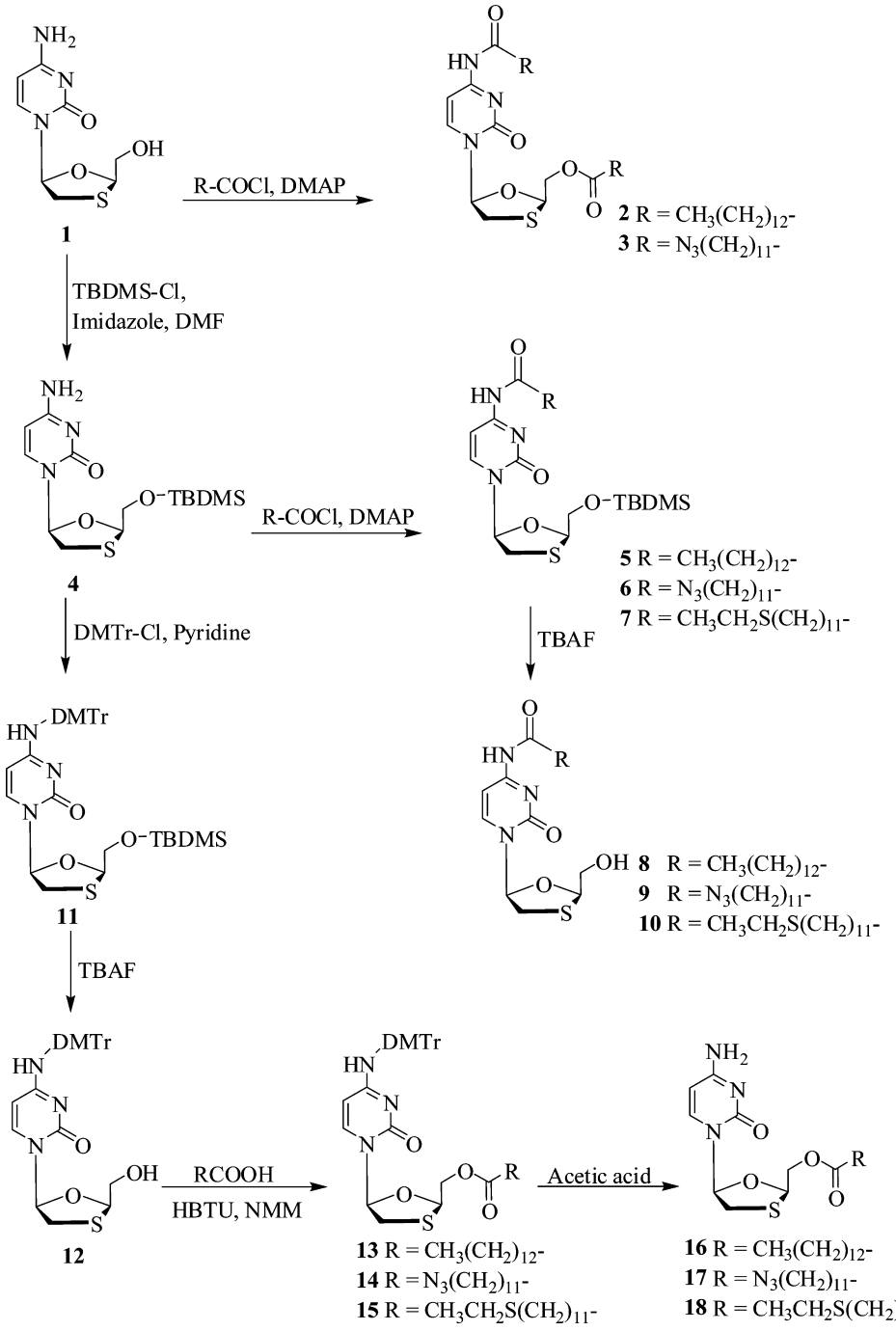
Scheme 1. Synthesis of Fatty Acyl Ester Derivatives of Compound 1



nucleoside to the long chain fatty acid analogues would enhance compound **1** lipophilicity and thus its cellular uptake. Furthermore, once the ester conjugate entered the cells, it would be hydrolyzed by esterases to yield two active species: the nucleoside analogue and the fatty acid targeting RT and NMT enzymes, respectively. The results of this study demonstrate that conjugation of fatty acids with **1** yields anti-HIV agents having enhanced lipophilicity, higher cellular uptake, increased potency, and broader spectrum of antiviral activity. Microbicides are topically applied agents that prevent or reduce transmission of infectious disease, in particular HIV/AIDS. Thus, a broad-based microbicide with an extended range of activity against HIV would be highly advantageous. Fatty acyl conjugates of **1** were envisioned and designed to be used as topical anti-HIV microbicides.

RESULTS AND DISCUSSION

Chemistry. *Fatty Acyl Ester Derivatives of Compound 1.* 5′-Hydroxyl and/or 4-amino positions were substituted with the fatty acids to synthesize three classes of compounds: two 5′,N_4-disubstituted (**2** and **3**), three N_4-monosubstituted (**8**–**10**), and three 5′-*O*-esters (**16**–**18**) of compound **1** (Scheme 1). Three fatty acids myristic acid, 12-azidododecanoic acid, and 12-thioethyldodecanoic acid were used for the conjugation with **1**.

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5′,N_4-Disubstituted derivatives (**2** and **3**) were synthesized by reacting **1** with the appropriate fatty acyl chloride in the presence of 4-dimethylaminopyridine (DMAP) as a base. N_4-Substituted derivatives (**8**−**10**) were synthesized by first selectively protecting the 5′-hydroxyl group with *tert*-butyldimethylsilyl chloride (TBDMS-Cl) in the presence of imidazole as a base to afford **4**. 5′-*O*-TBDMS-protected nucleoside (**4**) was further reacted with the fatty acyl chloride followed by deprotection of TBDMS to afford N_4-substituted derivatives (**8**−**10**).

5′-*O*-Fatty acyl derivatives of the nucleoside (**16**−**18**) were synthesized by first protecting the 4-amino group of **4** with 4,4′-dimethoxytrityl (DMTr) protecting group in the presence of 4,4′-dimethoxytrityl chloride (DMTr-Cl) and pyridine to afford **11**. The TBDMS group was then removed from the 5′-*O*-position by treatment of **11** with tetrabutylammonium fluoride (TBAF) to yield the N_4-DMTr derivative (**12**). Compound **12** was then reacted with the fatty acids in the presence of 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and *N*,*N*-diisopropylethylamine (DIPEA) followed by DMTr deprotection with acetic acid to afford 5′-*O*-(fatty acyl) ester derivatives of the nucleoside (**16**−**18**).

The attachment of the long chain fatty acid analogues to **1** enhanced their lipophilicity as shown by calculated partition coefficients (Log P) (Table 1). All compounds were initially synthesized at 100 mg scale and were tested to determine their anti-HIV activities and cytotoxicity profiles. Compounds **8**, **16**, and **17** were then synthesized in a larger scale (5 g) for further biological evaluations. The compounds were first purified by using silica gel column chromatography and then semi-preparative HPLC. The purity of final products (>95%) was confirmed by analytical HPLC.

Table 1. *In Vitro* Anti-HIV Activity of Fatty Acyl Derivatives of Compound 1 against the Lab-Adapted Virus

		anti-HIV activity		
	cytotoxicity[a]	X4[c]	R5[d]	
compd	EC(50)[b] (μM)	EC(50) (μM)	EC(50) (μM)	Log P (calcd)[e]
1	>436.6	32.7	11.4	0.06
2	>154.0	>154.0	135.1	ND[f]
3	>148.0	148.0	72.6	ND
8	>227.5	10.9	0.7	5.05
9	>221.0	29.4	3.8	4.26
10	>212.0	5.3	0.4	4.14
16	>227.7	0.5	0.2	5.79
17	>221.1	0.9	0.7	5.00
18	>212.2	2.3	0.2	4.88
DMSO	>100	>100	>100	
C-2[g]	>25	0.02	0.4	

[a]Cytotoxicity assay (MTS). [b]50% effective concentration. [c]Single-round infection assay (lymphocytotropic strain). [d]Single-round infection assay (monocytotropic strain). [e]Calculated partition coefficient using ChemBioDraw Ultra 12.0. [f]Not determined. [g]Assay control (Dextran sulfate (50 KDa)).

5(6)-Carboxyfluorescein Derivatives of 1. Compound **1** was attached to 5(6)-carboxyfluorescein (FAM) using β-alanine and 12-aminododecanoic acid as linkers. First, N_4-DMTr protected nucleoside (**12**) was reacted with the corresponding Fmoc-amino acid in the presence of HBTU and DIPEA. Second, *N*-Fmoc deprotection to the free amino group was achieved in the presence of piperidine. Finally, FAM was attached to the free amino group in the presence of HBTU and DIPEA, followed by DMTr deprotection to afford the 5(6)-carboxyfluorescein derivatives of the nucleoside (**23** and **24**, Scheme 2). These compounds were used to determine the cellular uptake profile of the fatty acyl esters of **1**. The nucleoside attached to FAM through β-alanine (**23**) was used as a control compound **1** analogue. The nucleoside attached to FAM through 12-aminododecanoyl (**24**) was used as an analogue of 5′-*O*-(12-azidododecanoyl)-2′,3′-dideoxy-3′-thiacytidine (**17**) and other 5′-*O*-fatty acyl ester derivatives of compound **1**.

Biological Activities. *Anti-HIV Activities of Fatty Acyl-Nucleoside Conjugates.* The conjugates of compound **1** were tested for cytotoxicity and anti-HIV activity against lab-adapted and various strains of multidrug resistant viruses. Table 1 illustrates the anti-HIV-1 activity of the fatty acyl ester derivatives of the nucleoside in comparison with parent compound **1** against the cell-free (X4 and R5) virus. The data provide structure−activity relationships for different fatty acyl ester derivatives of **1** by comparing N_4-substituted, 5′-*O*-substituted, and 5′-*O*,N_4-disubstituted compounds. The anti-HIV activity of fatty acyl derivatives of the nucleoside was dependent on the site of esterification. 5′-*O*,N_4-Disubstituted derivatives of the nucleoside (**2** and **3**) displayed significantly less activity (EC_{50} = 72.6 to >154.0 μM) than the other monosubstituted fatty acyl derivatives (EC_{50} = 0.2−29.4 μM) and compound **1** alone (EC_{50} = 11.4−32.7 μM). In general, minimal cytotoxicity was observed (EC_{50} > 148 μM) for synthesized 5′-*O*,N_4-, N_4-, and 5′-*O* substituted fatty acyl esters of **1**.

All the N_4- or 5′-*O*-monosubstituted derivatives of the nucleoside (**8**−**10** and **16**−**18**) exhibited a higher potency than **1** against cell-free HIV-1. 5′-*O*-Monosubstituted ester derivatives (**16**−**18**) were the most potent compounds (EC_{50} = 0.2−2.3 μM) among all the derivatives as determined by viral inhibition single-round infection assays. 5′-*O*-Myristoyl analogue (**16**) exhibited the highest anti-HIV activity (EC_{50} = 0.2−0.5 μM). Compound **8** (EC_{50} = 0.7 μM) was the most potent analogue against cell-associated HIV-1 showing approximately 115-fold higher antiviral activity than **1** (EC_{50} = 80.3 μM) (data not shown).

Anti-HIV Activities of 5′-O-Myristoyl Analogue (16) against Clinical Isolates and Multi-Drug Resistant HIV. On the basis of the observed high antiviral activity against X4 and R5 lab-adapted virus, **16** was further tested against clinical isolates of HIV, and antiviral activity results were compared with those of compound **1** (Table 2). The IC_{90} values for **16** against the B-subtype virus and C-subtype virus were approximately 20- and 11-fold lower than those of **1**, respectively. When tested against drug resistant virus, the IC_{90} values of **16** against NNRTI and NRTI-resistant viruses were 9 and 38 times lower than those of **1**. Improved IC_{50} and IC_{90} values directly increased the therapeutic index of **16**. Both **1** and **16** were poorly active against B-MDR strain of HIV 4755-5, containing multiple NRTI-resistant mutations. These results indicate that conjugate **16** can be used against NNRTI and NRTI-resistant viruses, significantly improving the anti-HIV profile of **1**.

Table 3 illustrates the anti-HIV-1 activity of 5′-*O*-ester conjugates **16**−**18** and their corresponding physical mixtures against the B-subtype virus. Physical mixtures were prepared by mixing the fatty acid with **1** in similar equimolar ratios to that of the corresponding conjugates. The physical mixtures of the corresponding fatty acids and **1** showed significantly less anti-

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Scheme 2. Synthesis of 5′-Carboxyfluorescein Nucleoside Derivatives 23 and 24 through Different Linkers

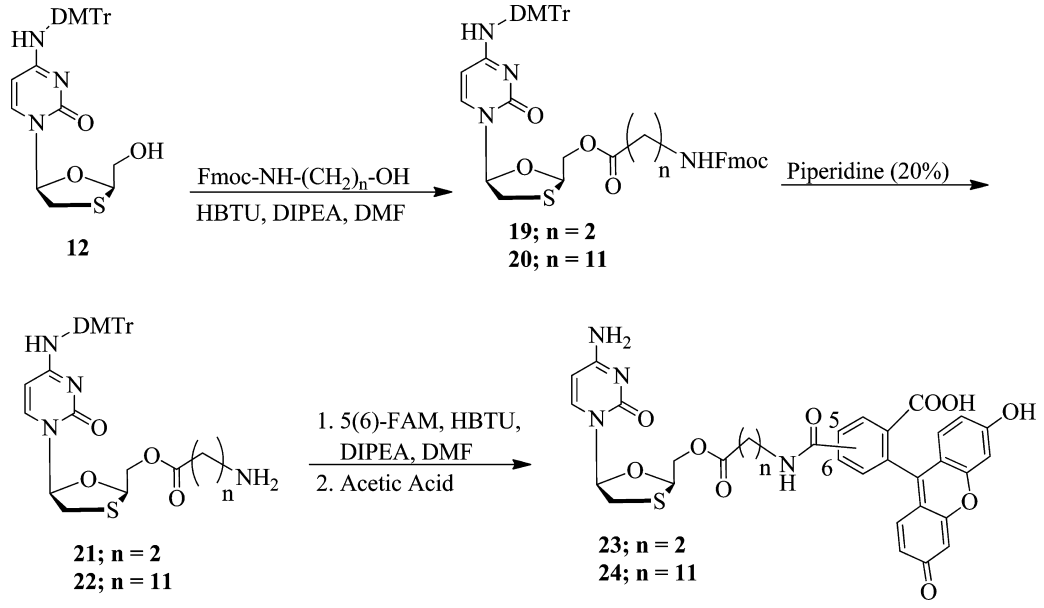

Table 2. Anti-HIV Evaluation against Clinical Isolates and MDR Viruses[a]

compd	virus	clade/ resistance	IC_{50} (nM)	IC_{90} (nM)	CC_{50} (nM)
1	94US3393IN	B	87.3	305.6	>13,097
	98USMSC5016	C	21.8	305.6	>13,097
	A-17	B-NNRTI	92.1	282.9	>13,097
	71361-1	B-NRTI	812.0	9,622	>13,097
	4755-5	B-MDR	>13,097	>13,097	>13,097
16	94US3393IN	B	4.2	15.0	>6,830
	98USMSC5016	C	1.9	27.5	>6,830
	A-17	B-NNRTI	2.8	31.1	>6,830
	71361-1	B-NRTI	43.3	252.7	>6,830
	4755-5	B-MDR	5,086.0	>6,830	>6,830

[a] IC_{50}, 50% of the maximal inhibitory concentration; IC_{90}, 90% of the maximal inhibitory concentration; CC_{50}, 50% cytotoxic concentration.

Table 3. Anti-HIV Activity of 5′-*O*-Fatty Acyl Derivatives of 1 and the Corresponding Physical Mixtures against Clinical Isolate (94US3393IN, Clade B) Virus[a]

composition	IC_{50} (nM)	IC_{90} (nM)	CC_{50} (nM)
5′-*O*-(myristoyl)-3TC (**16**)	4.2	15.0	>6,830
1 + myristic acid	123.8	435.2	>21,869
5′-*O*-(12-azidododecanoyl)-3TC (**17**)	14.9	134.7	>22,113
1 + 12-azidododecanoic acid	9,871.8	18,431.4	>21,266
5′-*O*-(12-thioethyldodecanoyl)-3TC (**18**)	14.7	78.3	>21,222
1 + 12-thioethydodecanoic acid	257.5	609.1	>20,440

[a] IC_{50}, 50% of the maximal inhibitory concentration; IC_{90}, 90% of the maximal inhibitory concentration; CC_{50}, 50% cytotoxic concentration.

HIV activity than the 5′-*O*-ester conjugates **16**–**18**. For example, 5′-*O*-myristoyl analogue (**16**) (IC_{50} = 4.2 nM) exhibited 29-fold higher activity than the physical mixture of **1** and myristic acid (EC_{50} = 123.8 nM). In general, the physical mixtures in equimolar ratio exhibited 8–137-fold lower anti-HIV activity than the corresponding ester conjugates, confirming the cellular uptake of the conjugate and intracellular hydrolysis and release of **1** and the fatty acid contribution to the higher anti-HIV profile of the ester conjugates compared to the physical mixtures.

In summary, structure–function analysis revealed that the anti-HIV activity of fatty acyl substituted derivatives of nucleosides was clearly dependent on the nature of the nucleoside and fatty acid analogue. The conjugation of RT inhibiting nucleoside analogues with selected long chain fatty acids (NMT inhibitors) exerted a synergistic anti-HIV effect. Fatty acyl derivatives **8**, **16**, and **17** exhibited better anti-HIV profiles than **1**, with **16** being the most potent.

Cellular Uptake Studies. The presence of long chain fatty acid at the 5′-position enhanced the lipophilicity of **1** as shown by calculated Log P values (Table 1). The rate of cellular uptake and the intracellular hydrolysis of lipophilic conjugates to the parent nucleoside and fatty acids determine the overall anti-HIV activities of the conjugates. An indirect evidence of intracellular hydrolysis is that monosubstituted 5′-*O*-fatty acyl ester derivatives of the nucleoside (**16**–**18**, EC_{50} = 0.2–2.3 μM) were more potent than the corresponding monosubstituted amide N_4-fatty acyl (**8**–**10**, EC_{50} = 0.4–29.4 μM) and 5′-*O*-N_4-disubstituted derivatives (**2** and **3**, EC_{50} = 72.6 to >154.0 μM). Amide derivatives are known to be more stable than the corresponding ester derivatives toward hydrolytic enzymes. The lower anti-HIV activities of more stable amide derivatives compared to the 5′-*O*-fatty acyl ester derivatives of **1** reflect that intracellular hydrolysis is critical for generating higher potency.

Cellular studies were performed to understand the uptake profile of 5′-*O*-fatty acyl derivatives in comparison with **1** and to confirm intracellular hydrolysis. Cellular uptake of compound **16** was monitored in human T lymphoblastoid cells (CCRF-CEM, ATCC No. CCL-119). The growing cells (1×10^6) were incubated with compound **16** (50 μM) for 1–72 h. The cellular accumulation of compound **16** was monitored with HPLC analysis and detection at 265 nm after cellular lysis. The cellular analysis confirmed that compound **16** was accumulated intracellularly after 1 h of incubation (analytical HPLC profile, Supporting Information). Cellular hydrolysis was observed after 1 h of incubation of compound **16** (50 μM) with the main peak

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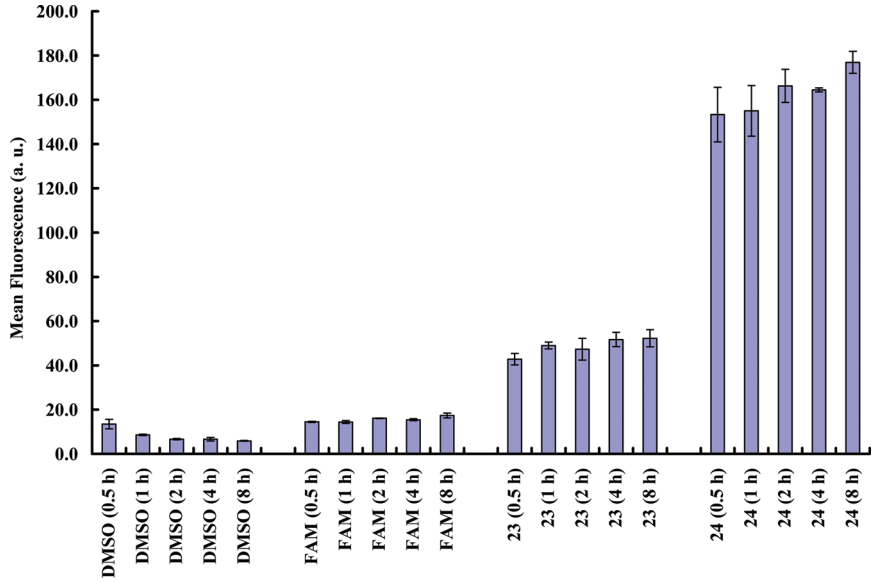

Figure 1. Cellular uptake of 5(6)-carboxyfluorescein nucleoside derivatives **23** and **24** (10 μM) along with FAM and DMSO as controls at different time intervals.

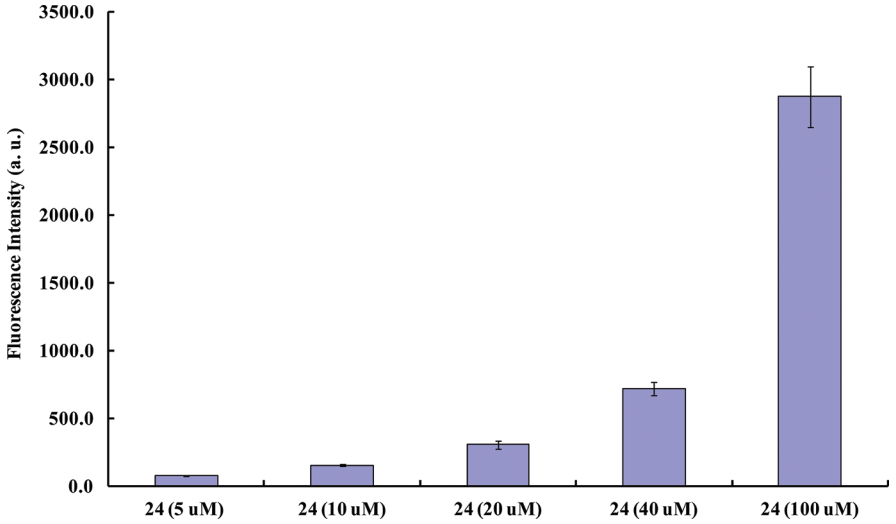

Figure 2. Cellular uptake of 5(6)-carboxyfluorescein nucleoside derivative **24** at different concentrations.

appearing at 15.3 min. Compound **1** and potential phosphorylated products peaks were detected at 1.7−2.7 min in the HPLC profile and overlapped with cell extract peaks, making this method challenging for comparative studies with the parent analogue. Thus, 5(6)-carboxyfluorescein (FAM) derivatives of the nucleoside were synthesized to quantify and visualize the cellular uptake of the fatty acyl conjugates compared to parent compound **1** more accurately.

The nucleoside attached to FAM through β-alanine (**23**) (Log P = 1.51) was used as the control compound **1** analogue. The nucleoside attached to FAM through 12-aminododecanoic acid (**24**) was used as a lipophilic analogue of 5′-*O*-(12-azidododecanoyl)-2′,3′-dideoxy-3′-thiacytidine (**16**) (Log P = 5.42) and other fatty acid ester analogues of compound **1**. Both **23** and **24** remained stable in culture medium after incubation up to 24 h as shown by comparison of the analytical HPLC profile with background cell culture medium without the compound (Supporting Information), ruling out the extracellular hydrolysis of the compounds. The CCRF-CEM cells were used for the study and were grown to 70% confluency in the culture media. The cells were incubated with the fluorescein-substituted conjugates (**23** and **24**) for different time periods, at different concentrations, and in the presence or absence of trypsin (Figures 1−3). DMSO and FAM were used as controls. The cells were analyzed by flow cytometry (FACSCalibur: Becton Dickinson) using FITC channel and CellQuest software. The data presented are based on the mean fluorescence signal for 10000 cells. All the assays were carried out in triplicate.

In the time response study, the cells were first incubated with 10 μM of the compounds for varying amounts of time (0.5 h, 1 h, 2 h, 4 h, and 8 h; Figure 1). Compound **24** exhibited 3−6-fold higher cellular uptake than **23** and FAM alone. The results clearly indicate that the presence of a long chain enhances the cellular uptake of compound **1**, presumably by increasing lipophilicity. The continuous incubation of cells with compounds for up to 8 h did not show significant differences in uptake, suggesting that cellular uptake was not time

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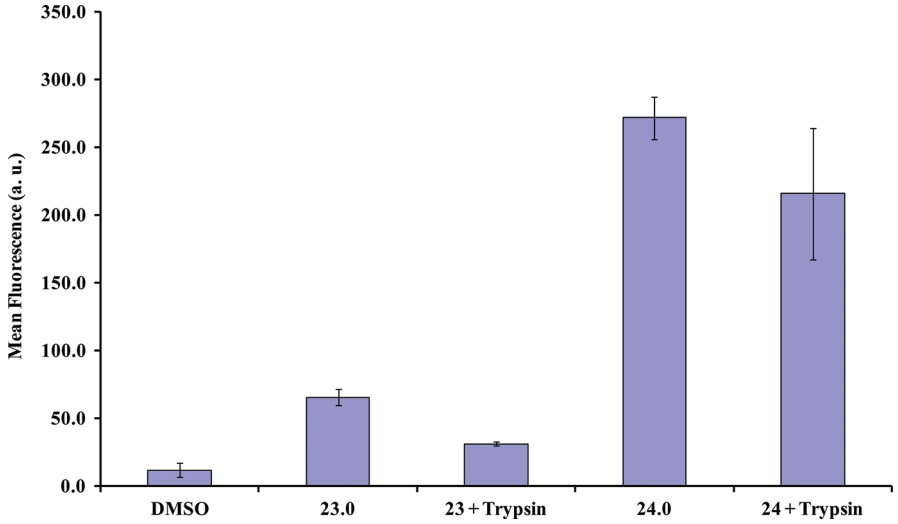

Figure 3. Cellular uptake studies of **23** and **24** (10 μM) with DMSO as controls with and without treatment with trypsin.

dependent and that most of the fatty acyl ester derivative is absorbed into the cells within the first 30 min.

The cells were then incubated with different concentrations (5, 10, 20, 40, and 100 μM) of the carboxyfluorescein derivative **24** for 1 h (Figure 2). The data suggest that cellular uptake was concentration dependent.

To confirm that the enhanced uptake of the 5(6)-carboxyfluorescein derivative **24** was not due to compound absorption onto the cell membrane surface, cells were incubated with 10 μM of DMSO, **23**, and **24** for 1 h, and then half of the cells were treated with trypsin for 5 min to wash the adsorbed molecules (if any) from the cell membrane. The comparison of the data between trypsin-treated and untreated cells indicates that only a small amount of the fluorescence was due to the cell surface absorption. Cellular uptake of the trypsin-treated cells with **24** was approximately 7 times higher than that of **23** (Figure 3). However, trypsin-untreated cells incubated with **24** showed only 4-fold higher cellular uptake than **23**. These results suggest that the higher cellular uptake of **24** is not due to artificial uptake and/or absorption to the cell membrane.

Cell Viability Study. Cell viability studies were performed to analyze the effect of FAM, **23**, and **24** on live cells. CCRF-CEM cells were incubated with 10 μM of compounds and mixed with trypan blue (0.1%) to identify the dead cells. The percentage of viability was calculated using Cellometer Auto T.4 (Nexcelom Bioscience). It was observed that at least 80% of the cells were viable in the presence of the compounds during a 24 h incubation period (Figure S12, Supporting Information). Differences in cell viability, therefore, did not account for the observed differences in cellular uptake.

Real Time Fluorescence Microscopy in Live CCRF-CEM Cells. CCRF-CEM cells were incubated with 10 μM DMSO, FAM, **23**, and **24** for 1 h and were imaged using a light microscope (ZEISS Axioplan 2) equipped with transmitted light microscopy, differential-interference contrast, and an Achroplan 40× objective. Cells showed no significant fluorescence when incubated with DMSO, FAM, and **23**. However, cells incubated with **24** showed fluorescence (Figure S13, Supporting Information). The results further confirm higher cellular uptake of **24**, a fatty acyl derivative of compound **1**, in comparison to **23** and FAM alone. Conceptually, these data indicate that the fatty acyl derivatives of nucleosides have better cellular uptake than their parent nucleosides.

CONCLUSIONS

Several 5′-O-substituted or N_4-substituted fatty acyl derivatives of **1** were synthesized, and their anti-HIV activity and cellular uptake were evaluated. In general, the conjugation of selected fatty acids with RT inhibiting nucleoside analogues resulted in better anti-HIV profiles due to improved cellular uptake and possibly intracellular hydrolysis of the conjugates yielding two parent inhibitors targeting RT and NMT, two enzymes involved in the life cycle of HIV-1. Although other fatty acyl ester derivatives of other nucleosides were previously reported by us,[14−16] the 5′-*O*-ester conjugates of **1** exhibited significantly higher anti-HIV activity than the parent analogue and the corresponding physical mixtures, improved cellular uptake, and demonstrated higher barrier to drug resistance.

Among all the ester derivatives, **8**, **16**, and **17** were found to have better anti-HIV activity than **1** and the other fatty acyl derivatives examined. For example, compound **16**, was at least 57-fold more potent than **1**. The data indicate that conjugation of **1** with myristic acid analogues is effective in achieving higher anti-HIV activity possibly by improving the cellular uptake of **1**.

The presence of a long chain fatty acid at the 5′-position enhanced the lipophilicity of **1** and its cellular uptake as shown by calculated Log P values and cellular uptake studies of compound **16** and 5′-carboxyfluoroscein derivatives of the nucleoside containing short chain (**23**) and long chain (**24**) alkyl ester groups, respectively. Cellular uptake and intracellular hydrolysis of ester conjugate **16** was demonstrated in analytical HPLC analysis. FACS experiments in CCRF-CEM cells showed that **24** had significantly higher cellular uptake than **23**. Fluorescence microscopy of cells incubated with these compounds further confirmed these results. Increased inhibition by fatty acyl ester derivatives of **1** may be due to their increased rate of uptake and subsequent intracellular hydrolysis yielding two antiviral agents with different targets, the parent nucleoside and the fatty acid analogue. The potential dual mechanism of action of the compounds increased the barrier to resistance. The 5′-*O*-ester conjugates exhibited significantly higher anti-HIV activity than the corresponding physical mixtures. These data provide the basis for a new and rational

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design of potent and safe anti-HIV agents as potential topical anti-HIV microbicides using **1** as the parent nucleoside.

EXPERIMENTAL SECTION

Materials. Compound **1** was purchased from EuroAsia Trans Continental (Bombay, India). 12-Bromododecanoic acid was purchased from Sigma Aldrich Chemical Co. 5(6)-Carboxyfluorescein (FAM) was purchased from Novabiochem. All the other reagents including solvents were purchased from Fisher Scientific. The final products were purified on a PhenomenexGemini 10 μm ODS reversed-phase column (2.1 × 25 cm) with a Hitachi HPLC system using a gradient system at a constant flow rate of 17 mL/min (Table 4).

Table 4. HPLC Method Used for the Purification of the Final Compounds

time (min)	water concentration A (%)	acetonitrile concentration B (%)	flow rate (mL/min)
0.00	100.0	0.0	1.0
1.0	100.0	0.0	17.0
45.0	0.0	100.0	17.0
55.0	0.0	100.0	17.0
59.0	100.0	0.0	17.0
60.0	100.0	0.0	1.0

The purity of the compounds was confirmed by using a Hitachi analytical HPLC system on a C18 column (Grace Allsphere ODS 2−3 μm, 150 × 4.6 mm) using a gradient system (water/acetonitrile 30:70 v/v) at constant flow rate of 1 mL/min with UV detection at 265 nm. The purity of final products (>95%) was confirmed by analytical HPLC. The chemical structures of final products were characterized by nuclear magnetic resonance spectrometry (^{1}H NMR and ^{13}C NMR) determined on a Bruker NMR spectrometer (400 MHz) and confirmed by a high-resolution PE Biosystems Mariner API time-of-flight electrospray mass spectrometer. Chemical shifts are reported in parts per millions (ppm). For cellular uptake studies, cells were analyzed by flow cytometry (FACSCalibur: Becton Dickinson) using FITC channel and CellQuest software. Cell-viability studies were conducted using Cellometer Auto T.4 (Nexcelom Biosciences). The real time microscopy in live CCRF-CEM cell line with or without compounds were imaged using a ZEISS Axioplan 2 light microscope equipped with transmitted light microscopy with a differential-interference contrast method and an Achroplan 40× objective.

Chemistry. *(−)-N_4,5′-(Ditetradecanoyl)-2′,3′-dideoxy-3′-thiacytidine (**2**) and (−)-N_4,5′-di(12-azidododecanoyl)-2′,3′-dideoxy-3′-thiacytidine (**3**).* In general, a reaction mixture consisting of the appropriate fatty acid (1.0 mmol), oxalyl chloride (100 μL, 1.2 mmol), and anhydrous benzene (18 mL) was stirred at room temperature (25 °C) for 1 h. The obtained yellow solution was evaporated to dryness under reduced pressure to prepare the acid chloride. Lamivudine (**1**, 100 mg, 0.44 mmol) and 4-dimethylaminopyridine (DMAP, 160 mg, 1.3 mmol) were dissolved in dry benzene (20 mL). The freshly prepared acid chloride (1.1 mmol) from the reaction of the fatty acid with oxalyl chloride was added to the mixture. The reaction mixture was refluxed at 100 °C for 4 h. After the completion of reaction, the reaction mixture was cooled down to room temperature and neutralized with 5% sodium bicarbonate solution. The benzene layer was separated, and the aqueous layer was extracted with dichloromethane (3× 100 mL). The organic layer was separated and mixed with the benzene layer and concentrated at reduced pressure. The residue was purified with silica gel column chromatography using dichloromethane and methanol (0−1%) as eluents.

*(−)-N_4,5′-(Ditetradecanoyl)-2′,3′-dideoxy-3′-thiacytidine (**2**).* Yield (155 mg, 53%). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.90−9.40 (br s, 1H, N*H*), 8.16 (d, J = 7.5 Hz, 1H, H-6), 7.48 (d, J = 7.5 Hz, 1H, H-5), 6.34 (dd, J = 3.2 and 5.1 Hz 1H, H-1′), 5.39−5.43 (m, 1H, H-4′), 4.65 (dd, J = 12.5 and 4.8 Hz, 1H, H-5″), 4.45 (dd, J = 12.5 and 3.0 Hz, 1H, H-5′), 3.64 (dd, J = 5.1 and 12.6 Hz, 1H, H-2″), 3.29 (dd, J = 3.2 and 12.6 Hz, 1H, H-2′), 2.47 (t, J = 7.6 Hz, 2H, CH_2CONH), 2.40 (t, J = 7.6 Hz, 2H, CH_2COO), 1.60−1.75 (m, 4H, CH_2CH_2CO), 1.20−1.40 (br m, 40H, methylene protons), 0.89 (t, J = 6.7 Hz, 6H, CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 175.06 (CONH), 173.39 (COO), 163.21 (C-4), 154.89 (C-2 C═O), 145.87 (C-6), 95.59 (C-5), 88.70 (C-1′), 87.75 (C-4′), 62.49 (C-5′), 38.31 (C-2′), 37.00 (CH_2CONH), 34.27, 32.00, 29.73, 29.63, 29.47, 29.43, 29.26, 29.16, 25.14, 22.77 (methylene carbons), 14.48 (CH_3). HR-MS (ESI-TOF) (m/z): $C_{36}H_{63}N_3O_5S$, calcd, 649.9675; found, 650.1885 [M + H]$^+$.

*(−)-N_4,5′-Di(12-azidododecanoyl)-2′,3′-dideoxy-3′-thiacytidine (**3**).* Yield (170 mg, 55%). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.90−9.12 (br s, 1H, N*H*), 8.18 (d, J = 7.2 Hz, 1H, H-6), 7.49 (d, J = 7.2 Hz, 1H, H-5), 6.36 (m, 1H, H-1′), 5.43 (dd, J = 4.9 and 3.0 Hz, 1H, H-4′), 4.67 (dd, J = 12.5 and 4.9 Hz, 1H, H-5″), 4.47 (dd, J = 12.5 and 3.0 Hz, 1H, H-5′), 3.64 (dd, J = 12.6 and 5.4 Hz, 1H, H-2″), 3.20−3.34 (m, 5H, H-2′, CH_2N_3), 2.49 (t, J = 7.2 Hz, 2H, CH_2CO), 2.42 (t, J = 7.2 Hz, 2H, CH_2CO), 1.55−1.79 (m, 8H, −CH_2CH_2CO, $CH_2CH_2N_3$), 1.20−1.45 (br m, 28H, methylene protons). ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 179.51 (CONH), 174.52 (COO), 163.20 (C-4), 146.10 (C-6), 96.50 (C-5), 88.60 (C-1′), 83.84 (C-4′), 64.82 (C-5′), 63.06, 51.87 (CH_2N_3), 39.43 (C-2′), 37.96 (CH_2CONH), 34.80 (CH_2COO), 34.47, 29.84, 29.78, 29.63, 29.53, 29.47, 29.23, 29.20, 27.10, 25.14 (methylene carbons). HR-MS (ESI-TOF) (m/z): $C_{32}H_{53}N_9O_5S$, calcd, 675.8855; found, 676.6085 [M + H]$^+$.

*(−)-5′-O-(t-Butyldimethylsilyl)-2′,3′-dideoxy-3′-thiacytidine (**4**).* Compound **1** (1.09 mmol, 250 mg), *tert*-butyldimethylsilyl chloride (500 mg, 3.27 mmol), and imidazole (230 mg, 3.27 mmol) were dissolved in dry DMF (10 mL), and the reaction mixture was stirred for 18 h at room temperature. The solvent was concentrated at reduced pressure, and the residue was purified with silica gel column chromatography using dichloromethane and methanol (0−5%) as eluents to yield **4** (350 mg, 95%).

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.21 (d, J = 7.6 Hz, 1H, H-6), 6.32 (dd, J = 5.2 and 2.8 Hz, 1H, H-1′), 6.02 (d, J = 7.6 Hz, 1H, H-5), 5.26 (t, J = 3.0 Hz, 1H, H-4′), 4.18 (dd, J = 11.8 and 3.0 Hz, 1H, H-5″), 3.96 (dd, J = 11.8 and 3.0 Hz, 1H, H-5′), 3.54 (dd, J = 12.5 and 5.2 Hz, 1H, H-2″), 3.19 (dd, J = 12.5 and 2.8 Hz, 1H, H-2′), 0.95 (s, 9H, $(CH_3)_3$C), 0.15 (s, 6H, CH_3Si). HR-MS (ESI-TOF) (m/z): $C_{14}H_{25}N_3O_3SSi$, calcd, 343.1386; found, 344.3933 [M + H]$^+$, 686.4590 [2M + H]$^+$.

*(−)-5′-O-(t-Butyldimethylsilyl)-N_4(tetradecanoyl)-2′,3′-dideoxy-3′-thiacytidine (**5**), (−)-5′-O-(t-butyldimethylsilyl)-N_4(12-azidododecanoyl)-2′,3′-dideoxy-3′-thiacytidine (**6**), and (−)-5′-O-(t-butyldimethylsilyl)-N_4(12-thioethyldodecanoyl)-2′,3′-dideoxy-3′-thiacytidine (**7**).* Compound **4** (140 mg, 0.4 mmol) and DMAP (70 mg, 0.6 mmol) were dissolved in dry benzene (10 mL). The corresponding acid chloride (0.48 mmol) (prepared as described above) was added dropwise, and the reaction mixture was refluxed for 4 h at 100 °C. The reaction mixture was cooled down to room temperature and neutralized with saturated sodium bicarbonate solution (100 mL). The benzene layer was separated, and the aqueous layer was extracted with dichloromethane (3 × 100 mL). The organic layer was separated and mixed with the benzene layer and concentrated at reduced pressure. The residue was purified with silica gel column chromatography using dichloromethane and methanol (0−1%) as eluents to afford **5**−**7**.

*(−)-5′-O-(t-Butyldimethylsilyl)-N_4(tetradecanoyl)-2′,3′-dideoxy-3′-thiacytidine (**5**).* Yield (110 mg, 50%). ^{1}H NMR (400 MHz, CD_3OD, δ ppm): 8.12 (d, J = 7.5 Hz, 1H, H-6), 6.24 (dd, J = 5.3, 3.5 Hz, 1H, H-1′), 5.81 (d, J = 7.5 Hz, 1H, H-5), 5.26 (t, J = 3.3 Hz, 1H, H-4′), 4.09 (dd, J = 11.7 and 3.3 Hz, 1H, H-5″), 3.96 (dd, J = 11.7 and 3.3 Hz, 1H, H-5′), 3.49 (dd, J = 12.2 and 5.3 Hz, 1H, H-2″), 3.11 (dd, J = 12.2 and 3.5 Hz, 1H, H-2′), 2.24 (t, J = 7.4 Hz, 2H, CH_2CO), 1.56 (t, J = 7.1 Hz, 2H, CH_2CH_2CO), 1.20−1.35 (br m, 20H, methylene protons), 0.91 (s, 9H, $(CH_3)_3$C), 0.86 (t, J = 6.8 Hz, 3H, CH_3), 0.11 (s, 6H, CH_3Si). HR-MS (ESI-TOF) (m/z): $C_{28}H_{51}N_3O_4SSi$, calcd, 553.8727; found, 554.1020 [M + H]$^+$.

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*(−)-5′-O-(t-Butyldimethylsilyl)-N$_4$(12-azidododecanoyl)-2′,3′-dideoxy-3′-thiacytidine (**6**).* Yield (110 mg, 50%). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.40−8.69 (br s, 2H, N*H* and *H*-6), 7.44 (d, *J* = 7.3 Hz, 1H, H-5), 6.37−6.41 (m, 1H, H-1′), 5.33 (t, *J* = 2.5 Hz, 1H, H-4′), 4.27 (dd, *J* = 11.9 and 2.5 Hz, 1H, H-5″), 4.01 (dd, *J* = 11.9 and 2.5 Hz, 1H, H-5′), 3.64 (dd, *J* = 12.7 and 5.2 Hz, 1H, H-2″), 3.27−3.30 (m, 3H, H-2′, CH_2N_3), 2.47 (s, 2H, CH_2CO), 1.60−1.82 (m, 4H, CH_2CH_2CO, $CH_2CH_2N_3$), 1.28−1.45 (br m, 14H, methylene protons), 0.99 (s, 9H, $(CH_3)_3C$), 0.18 (s, 6H, CH_3Si). HR-MS (ESI-TOF) (*m*/*z*): $C_{26}H_{46}N_6O_4SSi$, calcd, 556.8317; found, 567.0023 $[M + H]^+$.

*(−)-5′-O-(t-Butyldimethylsilyl)-N$_4$(12-thioethyldodecanoyl)-2′,3′-dideoxy-3′-thiacytidine (**7**).* Yield (110 mg, 50%). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 9.29−9.41 (br s, 1H, N*H*), 8.60 (d, *J* = 7.5, 1H, H-6), 7.46 (d, *J* = 7.5 Hz, 1H, H-5), 6.37 (dd, *J* = 5.2 and 2.3 Hz, 1H, H-1′), 5.32 (t, *J* = 2.2 Hz, 1H, H-4′), 4.68 (dd, *J* = 11.9 and 2.2 Hz, 1H, H-5″), 4.00 (dd, *J* = 11.9 and 2.2 Hz, 1H, H-5′), 3.63 (dd, *J* = 12.7, 5.2 Hz, 1H, H-2″), 3.26−3.33 (m, 1H, H-2′), 2.53−2.62 (m, 4H, CH_2SCH_2), 2.39 (t, *J* = 7.5 Hz, 2H, CH_2COO), 1.52−1.83 (m, 4H, CH_2CH_2COO and SCH_2CH_2), 1.21−1.50 (br m, 17H, methylene protons, CH_3CH_2S), 0.99 (s, 9H, $(CH_3)_3C$), 0.18 (s, 6H, CH_3Si). HR-MS (ESI-TOF) (*m*/*z*): $C_{28}H_{51}N_3O_4S_2Si$, calcd, 585.3077; found, 585.8926 $[M + H]^+$, 607.8040 $[M + Na]^+$.

*(−)-N$_4$(Tetradecanoyl)-2′,3′-dideoxy-3′-thiacytidine (**8**), (−)-N$_4$(12-azidododecanoyl)-2′,3′-dideoxy-3′-thiacytidine (**9**), and (−)-N$_4$(12-thioethyldodecanoyl)-2′,3′-dideoxy-3′-thiacytidine (**10**).* Tetrabutylammonium fluoride (1.5 mL, 1M) was added to **5**−**7** (150 mg), and the reaction mixture was stirred at room temperature for 3 h. The solvent was concentrated at reduced pressure, and the residue was purified with silica gel column chromatography using dichloromethane and methanol (0−1%) as eluents to afford **8**−**10**.

*(−)-N$_4$(Tetradecanoyl)-2′,3′-dideoxy-3′-thiacytidine (**8**).* $[\alpha]_D^{20}$ −26.7 (*c* 0.15 g/100 mL, MeOH/AcCN (1:1 v/v)). Yield (55 mg, 65%). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.85 (s, 1H, *NH*), 8.38 (d, *J* = 7.5 Hz, 1H, H-6), 7.23 (d, *J* = 7.5 Hz, 1H, H-5), 6.21 (dd, *J* = 5.2 and 3.2 Hz, 1H, H-1′), 5.26 (t, *J* = 4.1 Hz, 1H, H-4′), 3.79−3.89 (m, 2H, H-5″ and H-5′), 3.56 (dd, *J* = 12.3 and 5.2 Hz, 1H, H-2″), 3.20 (dd, *J* = 12.3 and 3.2 Hz, 1H, H-2′), 2.38 (t, *J* = 7.3 Hz, 2H, CH_2CO), 1.53 (t, *J* = 6.5 Hz, 2H, CH_2CH_2CO), 1.15−1.35 (s, 20H, methylene protons), 0.89 (t, *J* = 6.7 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 174.80 (*C*OO), 163.41 (C-4), 155.11 (C-2 C═O), 146.17 (C-6), 95.8 (C-5), 88.90 (C-1′), 87.94 (C-4′), 62.70 (C-5′), 38.51 (C-2′), 37.16 (CH_2CONH), 32.16, 29.92, 29.88, 29.73, 29.57, 29.30, 25.28, 22.96 (methylene carbons), 14.77 (CH_3). HR-MS (ESI-TOF) (*m*/*z*): $C_{22}H_{37}N_3O_4S$, calcd, 439.6119; found, 440.3352 $[M + H]^+$, 462.2543 $[M + Na]^+$, 878.1789 $[2M + H]^+$, 900.0877 $[2M + Na]^+$.

*(−)-N$_4$(12-Azidododecanoyl)-2′,3′-dideoxy-3′-thiacytidine (**9**).* Yield (50 mg, 60%). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.45−8.47 (br s, 2H, N*H*, H-6), 7.49 (d, *J* = 7.5 Hz, 1H, H-5), 6.38 (dd, *J* = 5.3 and 3.4 Hz, 1H, H-1′), 5.41 (t, *J* = 3.2 Hz, 1H, H-4′), 4.21 (dd, *J* = 12.7 and 3.2 Hz, 1H, H-5″), 4.01 (dd, *J* = 12.7 and 3.2 Hz, 1H, H-5′), 3.68 (dd, *J* = 12.5 and 5.3 Hz, 1H, H-2″), 3.27−3.31 (m, 3H, H-2′, CH_2N_3), 2.48 (t, *J* = 6.8 Hz, 2H, CH_2CO), 1.58−1.68 (m, 4H, CH_2CH_2CO, $CH_2CH_2N_3$), 1.25−1.45 (br m, 14H, methylene protons). ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 173.65 (*C*OO), 162.40 (C-4), 155.04 (C-2 C═O), 145.47 (C-6), 96.28 (C-5), 88.32 (C-1′), 88.23 (C-4′), 62.99 (C-5′), 51.67 (CH_2N_3), 39.18 (C-2′), 38.05 (CH_2CONH), 29.61, 29.54, 29.45, 29.31, 29.19, 29.02, 26.89, 25.03 (methylene carbons). HR-MS (ESI-TOF) (*m*/*z*): $C_{20}H_{32}N_6O_4S$, calcd, 452.5709; found, 453.2421 $[M + H]^+$, 903.9628 $[2M + H]^+$.

*(−)-N$_4$(12-Thioethyldodecanoyl)-2′,3′-dideoxy-3′-thiacytidine (**10**).* Yield (50 mg, 50%). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.58 (s, 1H, N*H*), 8.02−8.07 (br s, 1H, H-6), 6.26−6.30 (br s, 1H, H-5), 6.13−6.17 (m, 1H, H-1′), 5.35 (d, *J* = 2.8 Hz, 1H, H-4′), 4.59−4.72 (m, 1H, H-5″), 4.35−4.45 (m, 1H, H-5′), 3.53−3.65 (m, 1H, H-2″), 3.20−3.32 (m, 1H, H-2′), 2.45−2.75 (m, 4H, CH_2SCH_2), 2.33−2.43 (m, 2H, CH_2COO), 1.53−1.70 (m, 4H, SCH_2CH_2, CH_2CH_2CO), 1.20−1.40 (br m, 17H, methylene protons, CH_3CH_2S). ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 174.08 (*C*OO), 163.02 (C-4), 155.44 (C-2 *C*═O), 145.85 (C-6), 96.73 (C-5), 88.61 (C-1′), 88.48 (C-4′), 63.14 (C-5′), 39.38 (C-2′), 38.10 (CH_2CONH), 34.83, 32.06, 26.31−30.10, 25.32 (methylene carbons), 15.23 (CH_3). HR-MS (ESI-TOF) (*m*/*z*): $C_{22}H_{37}N_3O_4S_2$, calcd, 471.6769; found, 472.1656 $[M + H]^+$, 941.7961 $[2M + H]^+$.

*(−)-5′-O-(t-Butyldimethylsilyl)-N$_4$-(4,4′-dimethoxytrityl)-2′,3′-dideoxy-3′-thiacytidine (**11**).* Compound **4** (600 mg, 1.75 mmol) was dissolved in dry pyridine (10 mL). A solution of 4,4′-dimethoxytrityl chloride (DMTr-Cl, 1.4 mg, 4.4 mmol) in 10 mL of pyridine was added to the reaction mixture dropwise at 0 °C. The reaction mixture was stirred for 30 min. The temperature was raised to room temperature, and stirring was continued overnight. The reaction mixture was neutralized with saturated sodium bicarbonate solution (500 mL) and was extracted with dichloromethane (3 × 200 mL). The organic layer was separated and concentrated *in vacuo*. The residue was purified with silica gel column chromatography using dichloromethane and methanol (0−1%) as eluents to yield **11** (1.05 g, 90%).

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.81 (d, *J* = 7.7 Hz, 1H, H-6), 7.74 (dd, *J* = 5.7 and 3.3 Hz, 1H, DMTr proton), 7.55 (dd, *J* = 5.7 and 3.2 Hz, 1H, DMTr proton), 7.12−7.33 (m, 7H, DMTr protons), 6.82−6.88 (m, 4H, DMTr protons), 6.33 (dd, *J* = 5.2 and 3.0 Hz, 1H, H-1′), 5.17−5.22 (m, 1H, H-4′), 5.03 (d, *J* = 7.7 Hz, 1H, H-5), 4.36−4.40 (m, 1H, H-5″), 4.06 (dd, *J* = 11.8 and 3.1 Hz, 1H, H-5′), 3.81 (s, 6H, DMTr-OCH_3), 3.52 (dd, *J* = 12.2 and 5.2 Hz, 1H, H-2″), 3.17 (dd, *J* = 12.2 and 3.0 Hz, 1H, H-2′), 0.80 (s, 9H, $(CH_3)_3C$), 0.06 (s, 6H, CH_3Si). HR-MS (ESI-TOF) (*m*/*z*): $C_{35}H_{43}N_3O_5SSi$, calcd, 645.8835; found, 686.4624 $(M + K)^+$, 1289.3671 $(2M + H)^+$.

*(−)-N$_4$-(4,4′-Dimethoxytrityl)-2′,3′-dideoxy-3′-thiacytidine (**12**).* Compound **11** (1 g, 1.55 mmol) was dissolved in tetrabutylammonium fluoride (4.65 mL, 1 M, 4.65 mmol,) and stirred for 3 h. The reaction mixture was concentrated at reduced pressure, and the residue was purified with silica gel column chromatography using dichloromethane (2% triethylamine) and methanol (0−1%) as eluents to yield **12** (820 mg, 90%).

^{1}H NMR (400 MHz, CD_3OD, δ ppm): 8.53 (d, *J* = 7.8 Hz, 1H, H-6), 7.32−7.34 (m, 2H, DMTr protons), 7.17−7.23 (m, 6H, DMTr protons), 7.11−7.13 (m, 1H, DMTr protons), 6.75−7.78 (m, 4H, DMTr protons), 6.22 (dd, *J* = 5.3 and 2.5 Hz, 1H, H-1′), 6.01 (d, *J* = 7.8 Hz, 1H, H-5), 5.25 (t, *J* = 3.2, 1H, H-4′), 3.99 (dd, *J* = 12.8 and 3.2 Hz, 1H, H-5″), 3.84 (dd, *J* = 12.8 and 3.2 Hz, 1H, H-5′), 3.69 (s, 6H, DMTr-OCH_3), 3.51 (dd, *J* = 12.6 and 5.3 Hz, 1H, H-2″), 3.27 (dd, *J* = 12.6 and 2.5 Hz, 1H, H-2′). HR-MS (ESI-TOF) (*m*/*z*): $C_{29}H_{29}N_3O_5S$, calcd, 531.1828; found, 531.92 $[M + H]^+$, 632.6916 $[M + TEA)]^+$, 1061.1780 $[2M + 1]^+$.

*(−)-N$_4$-(4,4′-Dimethoxytrityl)-5′-O-(tetradecanoyl)-2′,3′-dideoxy-3′-thiacytidine (**13**), (−)-5′-O-(12-azidododecanoyl)-N$_4$-(4,4′-dimethoxytrityl)-2′,3′-dideoxy-3′-thiacytidine (**14**), and (−)-N$_4$-(4,4′-dimethoxytrityl)-5′-O-(12-thioethyldodecanoyl)-2′,3′-dideoxy-3′-thiacytidine (**15**).* Compound **12** (150 mg, 0.30 mmol), the corresponding fatty acid (0.60 mmol), and HBTU (250 mg, 0.65 mmol) were dissolved in dry DMF (10 mL). Diisopropylethylamine (DIPEA, 2 mL, 15 mmol) was added to the reaction mixture, and stirring was continued overnight at room temperature. The reaction mixture was concentrated at reduced pressure, and the residue was purified with silica gel column chromatography using dichloromethane (2% triethylamine) as eluents to afford **13**−**15**. These compounds were used directly for subsequent deprotection reactions.

*(−)-N$_4$-(4,4′-Dimethoxytrityl)-5′-O-(tetradecanoyl)-2′,3′-dideoxy-3′-thiacytidine (**13**).* Yield (100 mg, 50%). ^{1}H NMR (400 MHz, CD_3OD, δ ppm) 8.62 (d, *J* = 7.8 Hz, 1H, H-6), 7.40−7.43 (m, 2H, DMTr protons), 7.26−7.30 (m, 6H, DMTr protons), 7.18−7.22 (m, 1H, DMTr proton), 6.85 (d, *J* = 8.9 Hz, 4H, DMTr protons), 6.28−6.32 (m, 1H, H-1′), 6.08 (d, *J* = 7.8 Hz, 1H, H-5), 5.34 (t, *J* = 3.1 Hz, 1H, H-4′), 4.07 (dd, *J* = 12.8 and 3.1 Hz, 1H, H-5″), 3.92 (dd, *J* = 12.8 and 3.1 Hz, 1H, H-5′), 3.78 (s, 6H, DMTr-OCH_3), 3.60 (dd, *J* = 5.3 and 12.3 Hz, 1H, H-2″), 3.36 (d, *J* = 12.3 Hz, 1H, H-2′), 2.29 (t, *J* = 7.3 Hz, 2H, CH_2CO), 1.60 (t, *J* = 6.8 Hz, 2H, CH_2CH_2CO), 1.20−1.42 (br m, 20H, methylene protons), 0.91 (s, *J* = 6.6 Hz, 3H, CH_3). HR-MS (ESI-TOF) (*m*/*z*): $C_{43}H_{55}N_3O_6S$, calcd, 741.3812; found, 742.35 $[M + H]^+$, 843.4629 $[M + TEA]^+$, 1483.7079 $[2M + H]^+$.

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*(−)-5′-O-(Tetradecanoyl)-2′,3′-dideoxy-3′-thiacytidine (**16**), (−)-5′-O-(12-azidododecanoyl)-2′,3′-dideoxy-3′-thiacytidine (**17**), and (−)-5′-O-(12-thioethyldodecanoyl)-2′,3′-dideoxy-3′-thiacytidine (**18**).* Acetic acid (AcOH, 80%, 10 mL) was added to compounds **13**−**15** (0.25 mmol). The reaction mixture was heated at 80 °C for 30 min. The reaction mixture was concentrated at reduced pressure, and the residue was purified with silica gel column chromatography using dichloromethane as the eluent to afford **16**−**18**.

*(−)-5′-O-(Tetradecanoyl)-2′,3′-dideoxy-3′-thiacytidine (**16**).* $[\alpha]_D^{20}$ −34.6 (*c* 0.61 g/100 mL, MeOH/AcCN (3:1 v/v)). Yield (50 mg, 65%). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.72 (d, *J* = 7.5 Hz, 1H, H-6), 6.30 (t, *J* = 4.7 Hz, 1H, H-1′), 5.97 (d, *J* = 7.5 Hz, 1H, H-5), 5.32 (dd, *J* = 3.5 and 5.5 Hz, 1H, H-4′), 4.51 (dd, *J* = 12.1 and 5.5 Hz, 1H, H-5″), 4.35 (dd, *J* = 12.1 and 3.5 Hz, 1H, H-5′), 3.52 (dd, *J* = 12.0 and 5.3 Hz, 1H, H-2″), 3.09 (dd, *J* = 12.0 and 4.2 Hz, 1H, H-2′), 2.34 (t, *J* = 7.6 Hz, 2H, CH_2CO), 1.49−1.72 (m, 2H, CH_2CH_2CO), 1.18−1.42 (br m, 20H, methylene protons), 0.87 (s, *J* = 6.7 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 173.39 (COO), 165.98 (C-4), 155.64 (C-2 C═O), 140.73 (C-6), 94.96 (C-5), 87.79 (C-1′), 83.21 (C-4′), 64.48 (C-5′), 38.22 (C-2′), 34.24 (CH_2COO), 32.10, 29.87, 29.84, 29.79, 29.65, 29.54, 29.45, 29.31, 25.03, 22.88 (methylene carbons), 14.33 (CH_3). HR-MS (ESI-TOF) (*m*/*z*): $C_{22}H_{37}N_3O_4S$, calcd, 439.2505; found, 440.2835 $[M + H]^+$.

*(−)-5′-O-(12-Azidododecanoyl)-2′,3′-dideoxy-3′-thiacytidine (**17**).* $[\alpha]_D^{20}$ −55.0 (*c* −0.42 g/100 mL, MeOH). Yield (50 mg, 65%). ^{1}H NMR (400 MHz, CD_3OD, δ ppm): 7.71 (d, *J* = 7.5 Hz, 1H, H-6), 6.29 (dd, *J* = 5.2 and 3.8 Hz, 1H, H-1′), 6.01 (d, *J* = 7.5 Hz, 1H, H-5), 5.33 (dd, *J* = 3.2 and 5.4 Hz, 1H, H-4′), 4.51 (dd, *J* = 12.2 and 5.4 Hz, 1H, H-5″), 4.35 (dd, *J* = 12.2 and 3.2 Hz, 1H, H-5′), 3.52 (dd, *J* = 12.0 and 5.2 Hz, 1H, H-2″), 3.21−3.28 (m, 2H, CH_2N_3), 3.08 (dd, *J* = 12.0 and 3.8 Hz, 1H, H-2′), 2.34 (t, *J* = 7.5 Hz, 2H, CH_2CO), 1.54−1.64 (m, 4H, CH_2CH_2CO, $CH_2CH_2N_3$), 1.22−1.38 (br m, 14H, methylene protons). ^{13}C NMR (CD_3OD, 100 MHz, δ ppm): 173.40 (COO), 164.96 (C-4), 154.96 (C-2 C═O), 140.98 (C-6), 95.31 (C-5), 87.64 (C-1′), 83.51 (C-4′), 64.32 (C-5′), 51.64 (CH_2N_3), 38.20 (C-2′), 34.19 (CH_2CO), 29.88, 29.71, 29.60, 29.54, 29.39, 29.29, 29.24, 28.98, 26.86, 24.98, (methylene carbons). HR-MS (ESI-TOF) (*m*/*z*): $C_{20}H_{32}N_6O_4S$, calcd, 452.2206; found, 453.1729 $[M + H]^+$.

*(−)-5′-O-(12-Thioethyldodecanoyl)-2′,3′-dideoxy-3′-thiacytidine (**18**).* Yield (50 mg, 65%). ^{1}H NMR (400 MHz, CD_3OD, δ ppm): 7.64 (d, *J* = 7.4 Hz, 1H, H-6), 6.29−6.35 (m, 1H, H-1′), 5.91 (d, *J* = 7.4 Hz, 1H, H-5), 5.30−5.34 (m, 1H, H-4′), 4.49 (dd, *J* = 12.0 and 5.7 Hz, 1H, H-5″), 4.35 (dd, *J* = 12.0 and 3.2 Hz, 1H, H-5′), 3.50 (dd, *J* = 12.0 and 5.3 Hz, 1H, H-2″), 3.04 (dd, *J* = 12.0 and 4.6 Hz, 1H, H-2′), 2.44−2.63 (m, 4H, CH_2SCH_2), 2.34 (t, *J* = 7.4 Hz, 2H, CH_2CO), 1.50−1.79 (m, 4H, SCH_2CH_2, CH_2CH_2CO), 1.18−1.48 (br m, 17H, methylene protons). ^{13}C NMR (CD_3OD, 100 MHz, δ ppm): 173.36 (COO), 166.01 (C-4), 155.60 (C-2 C═O), 140.49 (C-6), 95.35 (C-5), 87.73 (C-1′), 83.03 (C-4′), 64.55 (C-5′), 38.04 (C-2′), 34.20 (CH_2COO), 31.82, 29.80, 29.67, 29.58, 29.41, 29.62, 29.11, 28.90, 25.00, 22.88 (methylene carbons), 14.33 (CH_3). HR-MS (ESI-TOF) (*m*/*z*): $C_{22}H_{37}N_3O_4S_2$, calcd, 471.2225; found, 472.2418 $[M + H]^+$, 941.9318 $[2M + H]^+$.

*(−)-5′-O-(12(N-Fmoc-aminododecanoyl)-N$_4$-(4,4′-dimethoxytrityl)-2′,3′-dideoxy-3′-thiacytidine (**19**) and (−)-5′-O-(3(N-Fmoc-aminopropanoyl)-N$_4$-(4,4′-dimethoxytrityl)-2′,3′-dideoxy-3′-thiacytidine (**20**).* Compound **12** (320 mg, 0.60 mmol), the corresponding Fmoc-amino acid (1.2 mmoL), and HBTU (500 mg, 1.3 mmol) were dissolved in a mixture of dry DMF (10 mL) and DIPEA (2 mL, 15 mmol). The reaction mixture was stirred overnight at room temperature. The reaction mixture was concentrated and dried under reduced pressure to afford crude 5′-*O*-Fmoc-amino acid derivatives of N_4-DMTr-2′,3′-dideoxy-3′-thiacytidine, **19** and **20**.

*(−)-5′-O-(12(N-Fmoc-aminododecanoyl)-N$_4$-(4,4′-dimethoxytrityl)-2′,3′-dideoxy-3′-thiacytidine (**19**).* HR-MS (ESI-TOF) (*m*/*z*): $C_{47}H_{44}N_4O_8S$, calcd, 824.288; found, 825.2218 $[M + H]^+$, 1650.0664 $[M + H]^+$.

*(−)-5′-O-(12(N-Fmoc-aminododecanoyl)-N$_4$-(4,4′-dimethoxytrityl)-2′,3′-dideoxy-3′-thiacytidine (**19**).* HR-MS (ESI-TOF) (*m*/*z*): $C_{56}H_{62}N_4O_8S$, calcd, 950.4288; found, 951.8527 $[M + H]^+$.

*(−)-5′-O-(3-Aminopropanoyl)-N$_4$-(4,4′-dimethoxytrityl)-2′,3′-dideoxy-3′-thiacytidine (**21**) and (−)-5′-O-(12-aminododecanoyl)-N$_4$-(4,4′-dimethoxytrityl)-2′,3′-dideoxy-3′-thiacytidine (**22**).* The crude products were dissolved in THF (10 mL). To the reaction mixture was added piperidine (6 μL, 0.06 mmol) and 1-octanethiol (10 mmol solution in THF, 0.6 mL, 6 mmol). The reaction mixture was allowed to stir for 1 h at room temperature. The reaction solution was concentrated at reduced pressure. The residue was purified with reversed phase HPLC using C_{18} column and water/acetonitrile as solvents as described above to yield **21** and **22**.

*(−)-5′-O-(3-Aminopropanoyl)-N$_4$-(4,4′-dimethoxytrityl)-2′,3′-dideoxy-3′-thiacytidine (**21**).* Overall yield (200 mg, 55%). HR-MS (ESI-TOF) (*m*/*z*): $C_{32}H_{34}N_4O_6S$, calcd, 602.2199; found, 603.1806 $[M + H]^+$, 1205.0313 $[2M + H]^+$.

*(−)-5′-O-(12-aminododecanoyl)-N$_4$-(4,4′-dimethoxytrityl)-2′,3′-dideoxy-3′-thiacytidine (**22**).* Overall yield = 210 mg, 52%). HR-MS (ESI-TOF) (*m*/*z*): $C_{41}H_{52}N_4O_6S$, calcd, 728.3608; found, 729.2265 $[M + H]^+$, 1458.1201 $[2M + H]^+$.

*General Procedure for the Synthesis of 5′-O-(5(6)-Carboxyfluorescein) Derivatives **23** and **24**.* A mixture of 5(6)-carboxyfluoroscein (430 mg, 1.15 mmol), the corresponding N_4-DMTr-5′-*O*-aminoacyl derivative of lamivudine (**21** or **22**, 0.29 mmoL), and HBTU (440 mg, 1.15 mmol) was dissolved in a mixture of dry DMF (10 mL) and DIPEA (2 mL, 15 mmol) and stirred overnight at room temperature. The reaction mixture was concentrated and dried under vacuum. Acetic acid (80%, 10 mL) was added to the reaction mixture and was heated at 80 °C for 30 min. The final compounds were purified with reversed phase HPLC using C_{18} column and using water/acetonitrile as solvents as described above.

*(−)-5′-O-(3-(N(5(6)-Carboxyfluorescein)aminopropanoyl)-2′,3′-dideoxy-3′-thiacytidine (**23**).* Yield (40 mg, 20%). ^{1}H NMR (400 MHz, CD_3CN + D_2O, δ ppm) 8.41 (s, 0.5H, FAM−Ar-H, 5 or 6 isomer), 8.11 and 8.12 (two d, *J* = 8.0 Hz, 1H, H-6), 8.00−8.05 (m, 1H, FAM−Ar-H, 5 or 6 isomer), 7.93 (d, *J* = 8.0 Hz, 0.5H, FAM−Ar-H, 5 or 6 isomer), 7.53 (s, 0.5H, FAM−Ar-H, 5 or 6 isomer), 7.29 (d, *J* = 8.0 Hz, 0.5H, FAM−Ar-H, 5 or 6 isomer), 6.78−6.90 (m, 4H, FAM−Ar-H), 6.71 (dd, *J* = 2.4 and 8.9 Hz, 2H, FAM−Ar-H, 5 or 6 isomer), 6.10 and 6.17 (two d, *J* = 8.0, 2H, H-1′, H-5), 5.36−5.42 and 5.25−5.31 (two m, 1H, H-4′), 4.55 (dd, *J* = 12.6 and 4.4 Hz, 1H, H-5″), 4.32 (dd, *J* = 12.6 and 2.9 Hz, 1H, H-5′), 3.45−3.69 (m, 3H, H-2″ and CH_2NH), 3.13−3.21 (m, 1H, H-2′), 2.72 and 2.61 (two t, *J* = 6.5 Hz, 2H, CH_2CO). ^{13}C NMR (CD_3CN + D_2O, 100 MHz, δ ppm): 172.49, 172.32 (COO), 168.80 (CONH), 167.24, 167.11 (COO-FAM), 160.71 (Ar-*C*-FAM), 159.78, 159.72 (C-4), 154.66 (C-2 C═O), 147.62, 147.55 (Ar-*C*-FAM), 144.50, 144.39 (C-6), 136.44, 133.91, 130.70, 129.60, 128.39, 115.06, 111.93, 102.87 (FAM-*C*), 94.32, 94.26 (C-5), 87.38, 87.21 (C-1′), 85.17, 84.98 (C-4′), 63.93, 63.81 (C-5′), 49.09 (CH_2NH_2), 37.98, 37.87 (C-2′), 36.15, 36.06 (CH_2COO). HR-MS (ESI-TOF) (*m*/*z*): $C_{32}H_{26}N_4O_{10}S$, calcd, 658.137; found, 330.2546 $[M + 2H]^{2+}$, 659.2739 $[M + H]^+$, 1317.2294 $[2M + H]^+$.

*(−)-5′-O-(12-(N(5(6)-Carboxyfluorescein)aminododecanoyl)-2′,3′-dideoxy-3′-thiacytidine (**24**).* Yield (30 mg, 16%). ^{1}H NMR (400 MHz, CD_3CN + D_2O, δ ppm) 8.28−8.35 (m, 0.5H, FAM−Ar-H, 5 or 6 isomer), 8.11 and 8.12 (two d, *J* = 8.0 Hz, 1H, H-6), 7.96−8.04 (m, 1.5H, FAM−Ar-H), 7.52 (s, 0.5H, FAM−Ar-H, 5 or 6 isomer), 7.25 (d, *J* = 8.0 Hz, 0.5H, FAM−Ar-H, 5 or 6 isomer), 6.68−6.74 (m, 2H, FAM−Ar-H), 6.59−6.67 (m, 2H, FAM−Ar-H), 6.56 (dd, *J* = 2.3 and 8.8 Hz, 2H, FAM−Ar-H), 6.13−6.22 (m, 1H, H-1′), 6.08 and 6.09 (two d, *J* = 8.0, 2H, H-5), 5.33−5.40 (m, 1H, H-4′), 4.50 (dd, *J* = 12.5 and 4.8 Hz, 1H, H-5″), 4.38 (dd, *J* = 12.5 and 3.1 Hz, 1H, H-5′), 3.54 (dd, *J* = 5.5 and 12.6 Hz, 1H, H-2″), 3.34 (m, 3H, H-2′ and CH_2NH), 2.25−2.34 (m, 2H, CH_2COO), 1.40−1.60 (m, 4H, CH_2CH_2CO, CH_2CH_2NH), 1.11−1.25 (br m, 14H, methylene protons). ^{13}C NMR (CD_3CN + D_2O, 100 MHz, δ ppm): 174.02 (COO), 170.02 (CONH), 167.21 (COO-FAM), 160.84 (C-4), 153.67 (C-2 C═O), 148.19, 145.23 (Ar-*C*-FAM), 142.19 (C-6), 137.63, 135.24, 130.52, 130.39, 130.10, 128.11, 126.38, 125.50, 124.85, 123.85, 113.80, 110.95, 103.48 (FAM-*C*), 94.73 (C-5), 88.12 (C-1′), 85.72 (C-4′), 64.39 (C-5′), 49.69 (CH_2NH_2), 38.55 (C-2′), 34.54 (CH_2COO), 30.17, 30.13,

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30.07, 30.01, 29.94, 29.87, 29.80, 29.71, 29.59 (methylene carbons), 27.58 ($CH_2CH_2NH_2$), 25.54 (CH_2CH_2COO). HR-MS (ESI-TOF) (m/z): $C_{41}H_{44}N_4O_{10}S$, calcd, 784.2778; found, 393.0862 $[M + 2H]^{2+}$, 784.9019 $[M]^+$, 1569.4510 $[2M + H]^+$.

Anti-HIV Assays. The anti-HIV activity of the compounds was evaluated according to the previously reported procedure.[14,17−19] Compound anti-HIV activity was evaluated in single-round (MAGI) infection assays using X4 (IIIB) and R5 (BaL) HIV-1 and P4R5 cells expressing CD4 and coreceptors. In summary, P4R5MAGI cells were cultured at a density of 1.2×10^4 cells/well in a 96 well plate approximately 18 h prior to infection. Cells were incubated for 2 h at 37 °C with purified, cell-free HIV-1 laboratory strains IIIB or BaL (Advanced Biotechnologies, Inc., Columbia, MD) in the absence or presence of each agent. After 2 h, cells were washed, cultured for an additional 46 h, and subsequently assayed for HIV-1 infection using the Galacto-Star β-Galactosidase Reporter Gene Assay System for Mammalian Cells (Applied Biosystems, Bedford, MA). Reductions in infection were calculated as a percentage relative to the level of infection in the absence of agents, and 50% inhibitory concentrations (EC_{50}) were derived from regression analysis. Each compound concentration was tested in triplicate wells. Cell toxicity was evaluated using the same experimental design but without the addition of virus. The impact of compounds on cell viability was assessed using an MTT (reduction of tetrazolium salts) assay (Invitrogen, Carlsbad, CA).

For the assessment of compounds against wild-type (WT; R5; clones = 94US3393IN [B subtype] and 98USMSC5016 [C subtype]) and drug resistant (clones = 4755-5, 71361-1, and A17) HIV-1 clinical isolates, PHA-P stimulated cells from at least two normal donors were pooled, diluted in fresh media, and plated in the interior wells of a 96 well round-bottom microplate. Each plate contains virus/cell control wells (cells + virus), experimental wells (drug + cells + virus), and compound control wells (drug + media, no cells, necessary for MTS monitoring of cytotoxicity). Test drug dilutions were prepared in microtiter tubes, and each concentration was placed in appropriate wells. Following addition of the drug dilutions to the PBMCs, a predetermined dilution of virus stock was then placed in each test well (final MOI ≅ 0.1). Since HIV-1 is not cytopathic to PBMCs, the same assay plate can be used for both antiviral efficacy and cytotoxicity measurements. Compounds were incubated with the virus and cells in a 96 well format for 6 h. The cells were then washed by removing 75% of the medium (150 μL) and replacing with 150 μL of fresh (no drug) medium. The plates were then centrifuged (~200g) for 10 min, after which 150 μL of medium was removed, and an additional 150 μL of fresh medium was added to each well and further incubated for 6 days or until peak reverse transcriptase (RT) activity was detected. A microtiter plate-based RT reaction was utilized.[20] Incorporated radioactivity (counts per minute, CPM) was quantified using standard liquid scintillation techniques. Compound IC_{50} (50%, inhibition of virus replication) was calculated using statistical software and regression analysis.

*Cellular Uptake of Compound **16**.* Cellular uptake and accumulation of compound **16** was evaluated in CCRF-CEM cells by analytical HPLC studies. CCRF-CEM cells were grown in 75 cm^2 culture flasks with serum free RPMI medium to ~70−80% confluency (1×10^6 cells/mL). The medium was replaced with RPMI medium containing compound **16** (50 μM). The cells were incubated at 37 °C for 1−72 h. After incubation for the indicated time, the cells were collected by centrifugation. The medium was removed carefully by decantation, and the cell pallets were washed with ice-cold PBS to remove any medium. The cell pallets were thoroughly extracted with equal volume of methanol, chloroform, and isopropanol mixture (4:3:1 v/v/v) and filtered through 0.2 μm filters. Compound **16** in cell lysates was detected by analytical HPLC analysis (20 μL injection) at 265 nm using a gradient of water (0.1% TFA)/acetonitrile (0.1% TFA).

Cellular Uptake Study of Fluorescence-Labeled Nucleoside Analogues. All of the stock solutions for compounds FAM, **23**, and **24** were prepared in DMSO. The human T lymphoblastoid cells CCRF-CEM (ATCC No. CCL-119) were grown on 25 cm^2 cell culture flasks with RPMI-1640 medium containing 10% fetal bovine serum. Upon reaching about 70% confluency, the cells were treated as described below and incubated for 1 h or longer at 37 °C.

*Cellular Uptake of FAM, **23** and **24** at Different Time Points.* When the cells reached about 70% confluency, FAM, **23**, or **24** (1 mL, 20 μM) in RMPI-1640 medium was added to 1 mL of cells to make the final concentration 10 μM. The cells were incubated for 0.5, 1, 2, 4, and 8 h at 37 °C. Then, the flow cytometry assays were performed as described below.

Cellular Uptake of 24 at Different Concentrations. When the cells reached about 70% confluency, 1 mL of graded concentrations (0, 10, 20, 40, 80, and 200 μM in RMPI-1640) of **24** was added to 1 mL of cells to make the final concentration 0, 5, 10, 20, 40, and 100 μM. The cells were incubated for 1 h at 37 °C. Then, the flow cytometry assays were performed as described in the General Information.

*Cellular Uptake of **23** and **24** with Trypsin Treatment.* When the cells reached about 70% confluency, FAM, **23**, or **24** (1 mL, 20 μM) in RMPI-1640 medium was added to 1 mL of cells to make the final concentration 10 μM. The cells were incubated for 0.5, 1, 2, 4, and 8 h at 37 °C. The cells used were incubated with 0.25% trypsin/0.53 mM EDTA for 5 min before washing with PBS (pH 7.4).

Flow Cytometry. The cells were washed twice with PBS (pH 7.4) at 2000 rpm for 5 min. Then the cells were analyzed by flow cytometry (FACS Calibur: Becton Dickinson) using FITC channel and CellQuest software. The data presented are based on the mean fluorescence signal for 10000 cells collected. All the assays were done in triplicate.

Cell Viability Assay. When the cells reached about 70% confluency, the cells were incubated with a solution of CCRF-CEM cell alone or 10 μM FAM, **23**, or **24** for 24 h at 37 °C. Then, 20 μL of the cells from each flask was treated with 2 μL of trypan blue (0.1%) for 1 min. The cells were then transferred to a Cellometer counting slide and analyzed using Cellometer Auto T.4 (Nexcelom Bioscience). All the assays were performed in triplicate.

*Stability of Compounds **23** and **24** in Culture Media.* Compounds **23** and **24** were dissolved in cell culture media to make a final concentration of 10 μM. The incubation was continued in culture media for 24 h at 37 °C. The stability of compounds was determined with a Hitachi analytical HPLC system using a C18 Shimadzu Premier 3 μm column (150 cm ×4.6 mm) using Method B at a flow rate of 1 mL/min with detection at 265 nm. Dimethyl sulfoxide and FAM were used as controls.

Real Time Fluorescence Microscopy in the Live CCRF-CEM Cell Line. The cellular uptake studies and intracellular localization of CCRE-CEM cell alone, or incubated with **23** and **24** were imaged using a ZEISS Axioplan 2 light microscope equipped with transmitted light microscopy with a differential-interference contrast method and an Achroplan 40× objective. The human T lymphoblastoid cells CCRF-CEM (ATCC No. CCL-119) were grown on 60 mm Petri Dishes with RPMI-1640 medium containing 10% fetal bovine serum. Upon reaching about 70% confluency, the cells were incubated with a solution of 10 μM of **23** and **24** for 1 h at 37 °C. They were then observed under the fluorescent microscope under bright field and FITC channels (480/520 nm).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Analytical HPLC methods; cellular uptake analysis of compound **16** by analytical HPLC; stability of compound **24** in cell culture medium; cell viability study of **23** and **24** in CCRF-CEM cells; real time fluorescence microscopy of **23** and **24** in live CCRF-CEM cell line; and ^1H NMR and ^{13}C NMR of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The views expressed by the authors do not necessarily reflect the views of USAID or CONRAD.

The authors declare no competing financial interest.

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ABBREVIATIONS USED

AZT, 3′-azido-2′,3′-dideoxythymidine; d4T, 2′,3′-didehydro-2′,3′-dideoxythymidine; FLT, 3′-fluoro-2′,3′-dideoxythymidine; DIPEA, *N*,*N*-diisopropylethylamine; DMAP, 4-dimethylaminopyridine; DMF, *N*,*N*-dimethylformamide; DMTr, 4,4′-dimethoxytrityl; FTC, (−)-5-fluoro-2′,3′-dideoxy-3′-thiacytidine; HAART, highly active antiretroviral therapy; HBTU, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HBV, hepatitis B virus; NMM, *N*-methylmorpholine; NRTI, nucleoside reverse transcriptase inhibitors; TBAF, tetrabutylammonium fluoride; TBDMS-Cl, *tert*-butyldimethylsilyl chloride; 3TC, (−)-2′,3′-dideoxy-3′-thiacytidine; TFA, trifluoroacetic acid

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