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Synthesis and Anti-HIV Activities of Glutamate and Peptide Conjugates of Nucleoside Reverse Transcriptase Inhibitors

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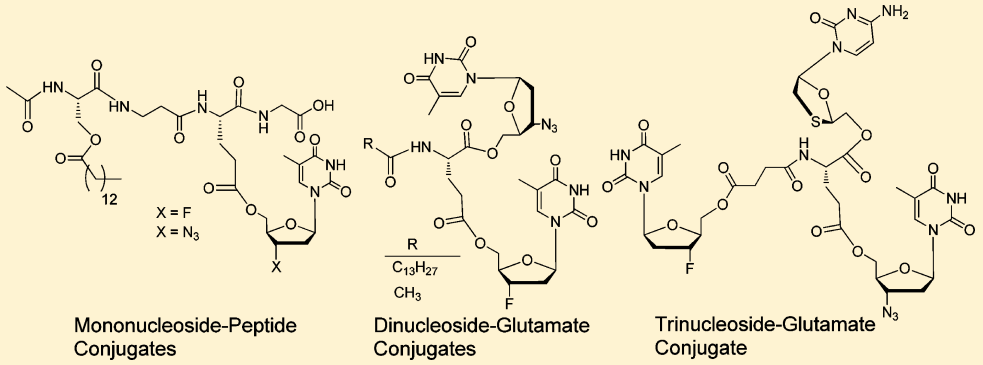
Supporting Information



ABSTRACT: Mono-, di-, and trinucleoside conjugates of glutamate or peptide scaffolds containing nucleoside reverse transcriptase inhibitors were synthesized. Among dinucleoside glutamate ester derivatives, *N*-myristoylated derivatives showed significantly higher anti-HIV activity than the corresponding *N*-acetylated conjugates against cell-free virus. Myristoyl-Glu(3TC)-FLT (**46**, EC_{50} = 0.3−0.6 μM) and myristoyl-Glu(FTC)-FLT (**47**, EC_{50} = 0.1−0.4 μM) derivatives were the most active glutamate−dinucleoside conjugates. A trinucleoside glutamate derivative containing AZT, FLT, and 3TC (**34**, EC_{50} = 0.9−1.4 μM) exhibited higher anti-HIV activity than AZT and 3TC against cell-free virus. Compound **34** also exhibited higher anti-HIV activity against multidrug (IC_{50} = 5.9 nM) and NNRTI (IC_{50} = 12.9 nM) resistant viruses than parent nucleosides. The physical mixture containing FLT−succinate, AZT, 3TC, and glutamic acid exhibited 115-fold less activity against cell associated virus (EC_{50} = 91.9 μM) when compared to **34** (EC_{50} = 0.8 μM). Other conjugates showed less or comparable potency to that of the corresponding physical mixtures.

INTRODUCTION

The introduction of highly active antiretroviral therapy (HAART) in the mid-1990s resulted in a significant decrease of morbidity and mortality in the human immunodeficiency virus-1 (HIV-1)-infected patient population. A combination of nucleoside reverse transcriptase inhibitors (NRTIs) is used in HAART to reduce the patient's viral load. Each nucleoside analogue, however, has different cellular uptake rates and pharmacokinetics and poor patient compliance during long-term use of combination therapy and emergence of drug resistance compromise the sustained efficacy of HAART.

NRTIs succumb to newly developed resistant virus. For example, (−)-2′,3′-dideoxy-3′-thiacytidine (3TC) is a nucleoside analogue commonly used in the treatment of both HIV-1 and hepatitis B virus (HBV).[1] Although 3TC has potent activity against wild type HIV, a single point mutation at methionine 184 residue (M184) results in 3TC-resistant mutant virus (M184 V/I).[2−4] Cytidine deamination in 3TC and the generation of steric hindrance at amino acid 184 residue have been proposed as reasons for development of resistance.[2,3] Similar to HIV, mutation at Met552 with Val and Ile (M552 V/I) results in development of 3TC resistant HBV strains.[5] Furthermore, nucleoside analogues are polar and have limited cellular uptake. Thus, designing new lipophilic conjugates containing different NRTIs on a multivalent scaffold may have major advantages such as broad-spectrum activity against drug-resistant HIV, dose simplicity, and simultaneous delivery of several NRTIs to the HIV-infected cells.

Diversity in the structure and physicochemical properties of peptides allow their applications as scaffolds and for targeted drug delivery. Peptides with different chain lengths have been used as spacers to deliver active drugs through lysosomal hydrolysis.[6−8] Peptides have also been used as linkers to deliver

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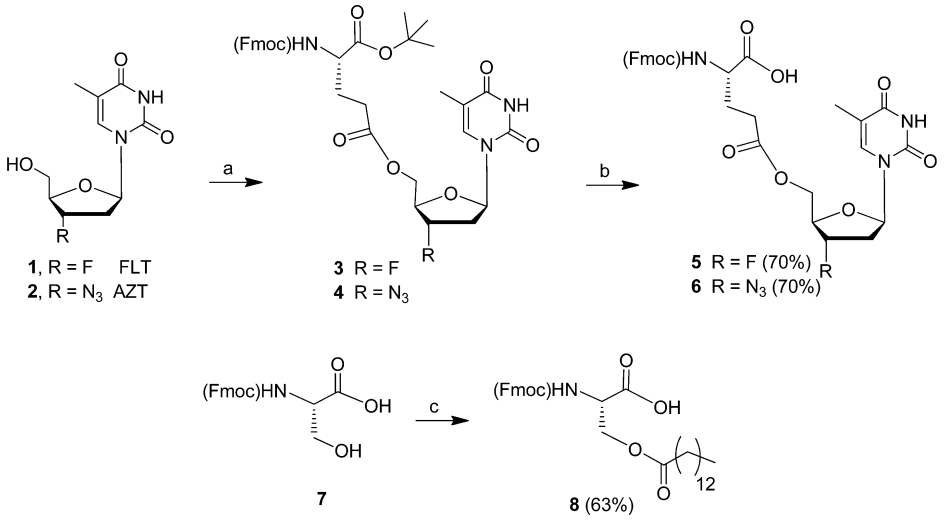
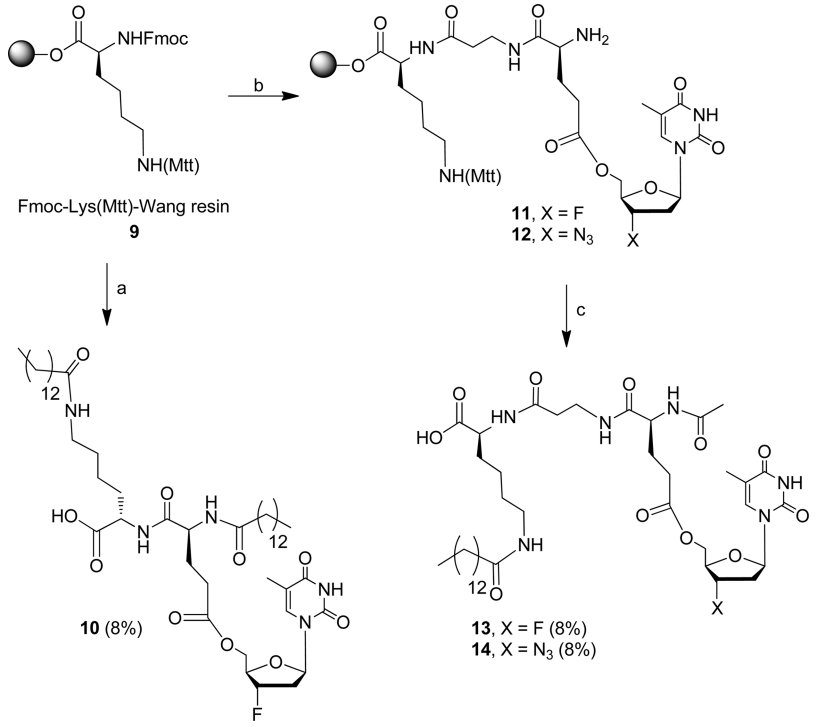
Scheme 1. Synthesis of Fmoc-Glu(FLT)-OH (5), Fmoc-Glu(AZT)-OH (6), and Fmoc-Ser(OMy)-OH (8) as Building Blocks[a]



[a]Reagents and conditions: (a) Fmoc-Glu(OH)OtBu, HBTU, DIPEA, DMF; (b) TFA/H_2O (95:5 v/v); (c) myristoyl chloride, DIPEA, DMF.

Scheme 2. Solid-Phase Synthesis of Dipeptide My-Glu(FLT)-Lys(My)-OH (10) and Tripeptide−Nucleoside Conjugates Ac-Glu(FLT)-βAla-Lys(My)-OH (13) and Ac-Glu(AZT)-βAla-Lys(My)-OH (14)[a]



[a]Reagents and conditions: (a) (1) piperidine/DMF (20%), (2) HBTU, **5**, NMM, (3) piperidine/DMF (20%), (4) TFA/DCM (5:95 v/v), (5) myristic anhydride, DIPEA, DMF, (6) TFA/H_2O/anisole (95:2.5:2.5 v/v/v); (b) (1) piperidine/DMF (20%), (2) HBTU, Fmoc-βAla-OH, NMM, (3) piperidine/DMF (20%), (4) HBTU, **5** or **6**, NMM, (5) piperidine/DMF (20%); (c) (1) DIPEA, acetic anhydride, (2) TFA/DCM (5:95 v/v), (3) myristic anhydride, DIPEA, DMF, (4) TFA/H_2O/anisole (95:2.5:2.5 v/v/v).

drugs at desired sites where they undergo site-specific enzymatic hydrolysis releasing the bioactive compounds. Chau et al. reported a specific peptide sequence of matrix metalloproteinase, an enzyme overexpressed in cancer cells for delivering anticancer drugs, such as methotrexate, directly to the cancer cells.[9−11]

Peptide esters have also been shown to improve the bioavailability of active drugs. For example, peptide prodrugs of lopinavir showed higher oral bioavailability than lopinavir.[12] Peptide conjugates of 5-aminolevulinic acid showed improved pharmacological response compared to that of the parent analogue as a result of better cellular uptake.[13]

Furthermore, peptide derivatives have been shown to produce direct pharmacological activity against some targeted enzymes. Peptide-based drugs, ramipril, enalapril, and captopril, were developed as angiotensin converting enzyme inhibitors.[14] Enfuvirtide is an approved peptide-based anti-HIV drug which blocks gp41 and prevent HIV-1 cell entry.[15] HIV protease

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inhibitors, such as lopinavir and saquinavir, are also peptide-based drugs.[12,16]

Herein, we report the synthesis and anti-HIV evaluation of mono-, di-, and trinucleoside conjugates of NRTIs substituted on a glutamate or a multivalent peptide scaffold containing a glutamic acid. *N*-Myristoylated and *N*-acetylated peptide-mononucleoside conjugates contained one myristoyl group attached to the side chains of glutamic acid and were synthesized by using Fmoc solid-phase peptide synthesis. The mononucleoside conjugates were used as control for comparative studies with di- and trinucleosides. *N*-Myristoylated and *N*-acetylated dinucleoside peptide ester derivatives included different nucleosides attached on carboxylic acid groups and were synthesized by using multistep solution phase methods. A glutamic acid conjugate with three different nucleosides was synthesized as a trinucleoside ester conjugate. The nucleosides included 3′-azido-2′,3′-deoxythymidine (AZT), 3′-fluoro-2′,3′-deoxythymidine (FLT), 2′,3′-dideoxy-3′-thiacytidine (3TC), and (−)-2′,3′-dideoxy-5-fluoro-3′-thiacytidine (FTC).

Nucleoside−glutamate scaffold conjugates were designed with the expectation that attachment of more than one nucleoside analogue to the scaffold would generate a substituted multivalent conjugate capable of delivering different nucleosides to the HIV-target cells. Myristic acid was attached to the scaffolds to improve the conjugate's lipophilicity and cellular uptake. Several fatty acyl esters of nucleoside analogues have been previously synthesized to improve their lipophilicity and cellular uptake,[17−19] as shown with fatty acyl esters of 2′,3′-didehydro-2′,3′-dideoxythymidine (stavudine, d4T).[17] It is expected that once the conjugate enters the cells, it will be hydrolyzed by esterase and/or peptidases, releasing the parent nucleoside analogues. Simultaneous intracellular release of different nucleosides would help to increase the barrier to viral resistance. Combined multidrug conjugates could also bear the benefits of synergistic anti-HIV effects, broader-spectrum activity, simpler dosing, and improved pharmacokinetic properties.

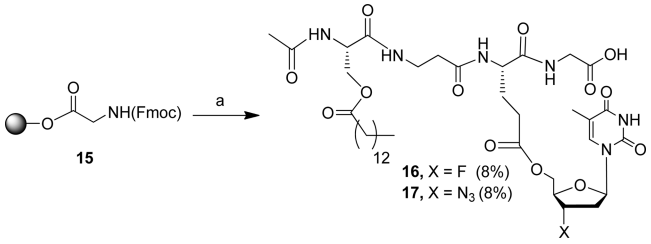
RESULTS AND DISCUSSION

Chemistry. *Synthesis of Myristoylated Mononucleoside Dipeptide, Tripeptide, and Tetrapeptide Conjugates.* Dipeptide (**10**), tripeptide (**13**, **14**), and tetrapeptide (**16**, **17**) nucleoside conjugates containing one nucleoside and at least one myristoyl moiety attached in the peptide side chain were synthesized in three steps: (i) Fmoc-protected building block synthesis (Scheme 1), (ii) peptide assembly on the resin, and (iii) deprotection followed by acylation of the free amino groups (Schemes 2 and 3).

Fmoc-Glu(nucleoside)-OH building blocks (**5** or **6**, nucleoside = FLT or AZT) were synthesized by the reaction of Fmoc-Glu(OH)-*t*Bu with the corresponding nucleoside (AZT or FLT) in the presence of 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and *N*,*N*-diisopropylethylamine (DIPEA), followed by the deprotection of *t*Bu group with TFA (Scheme 1). Fmoc-Ser(OMy)OH (**8**), a fatty acyl-substituted building block, was synthesized by the reaction of Fmoc-Ser(OH)-OH (**7**) with myristoyl chloride in the presence of DIPEA (Scheme 1).

Dipeptide-nucleoside conjugate My-Glu(FLT)-Lys(My)-OH (**10**) was synthesized by assembling Fmoc-protected building block, Fmoc-Glu(FLT)-OH (**5**), on Fmoc-Lys(Mtt)-Wang resin **9** by using Fmoc solid-phase peptide synthesis strategy

Scheme 3. Synthesis of Tetrapeptide−Nucleoside Conjugates of FLT and AZT 16 and 17[a]



[a]Reagents and conditions: (a) (1) piperidine/DMF (20%), (2) HBTU, **5** or **6**, NMM, (3) piperidine/DMF (20%), (4) HBTU, Fmoc-*β*Ala-OH, NMM, (5) piperidine/DMF (20%), (6) HBTU, **8**, NMM, (7) piperidine/DMF (20%), (8) DIPEA, acetic anhydride, (9) TFA/anisole/water (95:2.5:2.5 v/v/v).

at room temperature. Lysine side chain protecting group 4-methyltrityl (Mtt) was removed by adding TFA/DCM (5:95 v/v) to the resin NH_2-Glu(FLT)-Lys(Mtt)-Wang resin. The free amino groups were myristoylated by using myristic anhydride. The peptide was cleaved from the resin by using TFA/H_2O/anisole (95:2.5:2.5 v/v/v) to yield dimyristoylated FLT−dipeptide conjugate **10** (Scheme 2).

For the synthesis of tripeptide-nucleoside conjugates **13** and **14** containing a myristoylated lysine group, the peptide was assembled on Fmoc-Lys(Mtt)-Wang resin by Fmoc solid-phase peptide synthesis strategy at room temperature using Fmoc-protected amino acids [Fmoc-*β*-Ala-OH and Fmoc-Glu-(nucleoside)-OH (**5** or **6**)], followed by acetylation. After acidic removal of Mtt group, the amino group of lysine side chain was myristoylated using myristic anhydride in the presence of DIPEA as a base to afford mysristoylated tripeptide derivatives Ac-Glu(FLT)-*β*Ala-Lys(My)-OH (**13**) and Ac-Glu(AZT)-*β*Ala-Lys(My)-OH (**14**) (Scheme 2).

The synthesis of tetrapeptide−nucleoside conjugates containing a myristoylated serine residue is depicted in Scheme 3. Deprotection of Fmoc-Gly-Wang resin (**15**) in the presence of piperidine, coupling reactions with building blocks, **5** or **6**, Fmoc-*β*-Ala-OH, and **8**, respectively, in the presence of HBTU, followed by cleavage in the presence of TFA/anisole/water (95:2.5:2.5 v/v/v), afforded tetrapeptide−nucleoside conjugates Ac-Ser(My)-*β*Ala-Glu(FLT)Gly-OH (**16**) and Ac-Ser(My)-*β*Ala-Glu(AZT)Gly-OH (**17**) (Scheme 3).

Synthesis of Dinucleoside and Trinucleoside Glutamate Ester Derivatives Functionalized with Acetyl or Myristoyl Moiety. The synthesis of glutamate esters containing two nucleosides (AZT-FLT, FLT-3TC, AZT-3TC) with acetyl or myristic acid (Scheme 4) and glutamate esters containing three nucleosides (AZT-FLT-3TC) (Scheme 5) was accomplished by solution phase synthesis.

Dinucleoside−glutamate conjugates were synthesized by the reaction of an appropriate building block, such as **5** or **6** containing a free *α*-carboxylic acid, with other nucleosides, such as dimethoxytrityl (DMTr)-protected 3TC (DMTr-3TC) or AZT, in the presence of HBTU and DIPEA to afford **18**, **22**, or **23**. The synthesis of DMTr-3TC has been previously reported by us.[20] In the next step, Fmoc deprotection was accomplished in the presence of piperidine and *n*-octanethiol to afford **19**, **24**, or **25**. *n*-Octanethiol was used in excess as a scavenger because the conjugates were not stable in the presence of piperidine or other bases like DMAP. The use of piperidine in ratios more than 0.1 equiv or increasing cleavage reaction time resulted in

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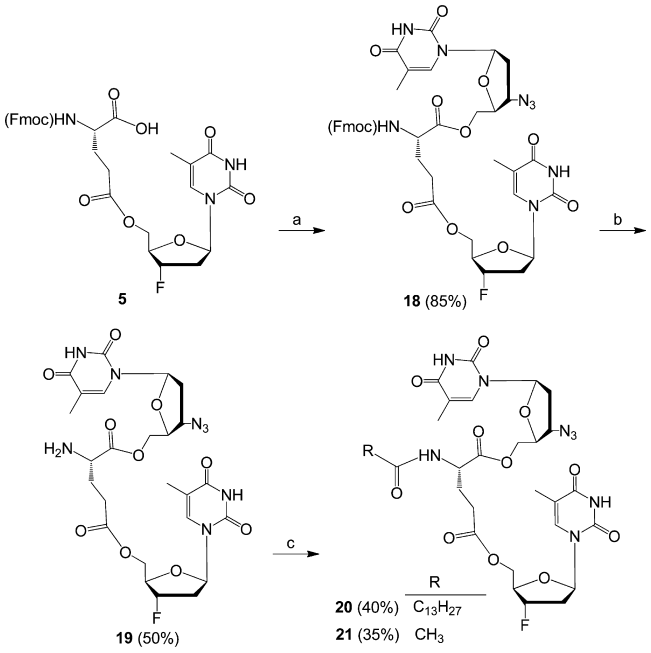
Scheme 4. Synthesis of Glutamic Acid Esters of Dinucleosides (AZT and FLT) (20 and 21)[a]



[a]Reagents and conditions: (a) AZT, HBTU, DIPEA, DMF; (b) piperidine, *n*-octanethiol; (c) $[RCO]_2O$, DIPEA, DMF.

the loss of the nucleoside from side chain of glutamate at δ-carboxylic acid. After Fmoc deprotection, free *N*-terminal was reacted with myristic anhydride or acetic anhydride in the presence of DIPEA to afford **20**, **21**, **26**, **28**, **30**, and **32**. The deprotection of DMTr group in **26**, **28**, **30**, and **32** was accomplished in the presence of acetic acid. This strategy afforded the dinucleoside glutamate esters containing two different nucleosides with acetyl or myristic group My-AZT-FLT (**20**), Ac-AZT-FLT (**21**) (Scheme 4), My-FLT-3TC (**27**), Ac-FLT-3TC (**29**), My-AZT-3TC (**31**), and Ac-AZT-3TC (**33**) (Scheme 5). Myristoyl and acetyl capping at *N*-terminal was carried out to provide peptides with high and low lipophilicity, respectively.

A trinucleoside−glutamate conjugate containing three different nucleosides (FLT, 3TC, AZT) was synthesized (**34**, Scheme 5) from DMTr protected 3TC-AZT conjugate **25**. In compound **34**, 3TC, AZT, and FLT were attached to the *C*-terminal, the side chain carboxylate of glutamate, and the *N*-terminal through a linker, respectively. A glutamate conjugate containing two different nucleosides, 3TC and AZT, at α and δ-carboxylic acids (NH_2-Glu(AZT)-3TC-DMTr) (**25**) was reacted first with FLT−succinate in the presence of DIPEA, followed by DMTr cleavage with acetic acid to afford a trinucleoside−glutamate conjugate **34** (Scheme 5).

DMTr-protected FTC (**39**) was synthesized according to the previously reported synthesis for DMTr-protected 3TC (**35**).[20] FTC was reacted with *tert*-butyldimethylsilyl chloride and imidazole in dry DMF to yield 5′-protected TBDMs-FTC derivative **37**. Subsequent reaction with DMTr-Cl in the presence of pyridine afforded 4-amino DMTr-protected derivative **38**. Removal of 5′-TBDMS group in the presence of tetrabutylammonium fluoride afforded DMTr-protected FTC (**39**) (Scheme 6).

Myristoylated dinucleoside−glutamate conjugates, My-FLT-3TC (**46**) and My-FLT-FTC (**47**), were synthesized starting from *t*-butyl protected glutamic acid. Glu(O*t*Bu)-OH (**40**) was reacted with myristic anhydride in the presence of DIPEA to generate My-Glu(OtBu)-OH (**41**). Conjugation of **41** with FLT in the presence of HBTU, DIPEA, and HOBt afforded My-Glu(OtBu)-OFLT (**42**). HOBt was used to protect the racemization of glutamic acid. Deprotection of *t*Bu in **42** was accomplished in the presence of TFA/DCM (95:5 v/v) to yield My-Glu(OH)-OFLT (**43**) (Scheme 7).

Reaction of DMTr protected 3TC and FTC (**35** and **39**) with **43** in the presence of DIPEA, HBTU, and HOBt, followed by cleavage of DMTr protecting group with acetic acid/TFA (98:2 v/v) mixture, afforded myristoylated dinucleoside−glutamate conjugates, **46** and **47** conjugates, in 58−64% yield (Scheme 7).

Anti-HIV Activity Evaluation. The anti-HIV activity and cytotoxicity of glutamate and peptide conjugates of nucleosides were primarily evaluated as previously reported[17,21−23] using X4 (IIIB) and R5 (BaL) HIV-1 and P4R5 cells expressing CD4 and HIV-1 coreceptors, stably transfected with β-galactosidase reporter gene under the control of HIV-1 LTR. The impact of compounds on cell viability was assessed using an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, reduction of tetrazolium salt) assay. Compound antiviral activity was evaluated using a single-round infection assay.[24] Tables 1−3 illustrate the anti-HIV-1 activity of selected compounds and their corresponding physical mixtures against cell-free lab-adapted virus. In general, minimal cytotoxicity was observed (EC_{50} > 100 μM) for all conjugates. A selected conjugate was further evaluated against wild-type and mutant HIV-1 clinical isolates in a multiround infection assay.

Anti-HIV Activities of Myristoylated Mononucleoside Dipeptide, Tripeptide, and Tetrapeptide Conjugates. The mononucleoside−peptide conjugates were used as control for comparative studies with di- and trinucleosides. As shown in Table 1, dipeptide−nucleoside (**10**), tripeptide−nucleoside (**13**), and tetrapeptide−nucleoside (**16**, **17**) conjugates containing one nucleoside and one myristoyl moiety displayed EC_{50} values ranging from 6.9 to 86.4 μM against cell-free virus and were not active against cell associated virus (EC_{50} > 100 μM; data not shown). Myristoylated peptide−AZT conjugate **17** (EC_{50} = 63.1−86.4 μM) was approximately 6 times less active than AZT (EC_{50} = 10.8−14.2 μM). Dimyristoylated peptide−FLT conjugate **10** (EC_{50} = 26.8−29.1 μM) was 34- to 73-fold less active than FLT (EC_{50} = 0.4−0.8 μM). Monomyristoylated peptide−FLT conjugates **13** and **16** (EC_{50} = 6.9−11.1 μM) were nearly 14−17 times less active than FLT. Reduced anti-HIV activity could be due to low cellular uptake of compounds containing peptide backbones or limited intracellular hydrolysis to parent nucleosides under assay conditions. The attachment of myristic acid did not improve the anti-HIV profile of the nucleosides, suggesting that increasing the lipophilicity of the mononucleoside−peptide conjugates was not an effective approach to enhancing the anti-HIV activity of the NRTIs.

Anti-HIV Activities of Dinucleoside and Trinucleoside Glutamate Ester Derivatives Functionalized with Acetyl or Myristoyl Moiety. Anti-HIV activity of glutamate esters of two and three different nucleosides was evaluated and compared with that of mononucleoside−peptide conjugates, the corresponding physical mixtures of nucleosides with or without myristic acid, and with glutamate ester **43** containing one nucleoside (FLT) (Tables 2 and 3). The data showed improved anti-HIV activity against cell-free virus for dinucleoside and

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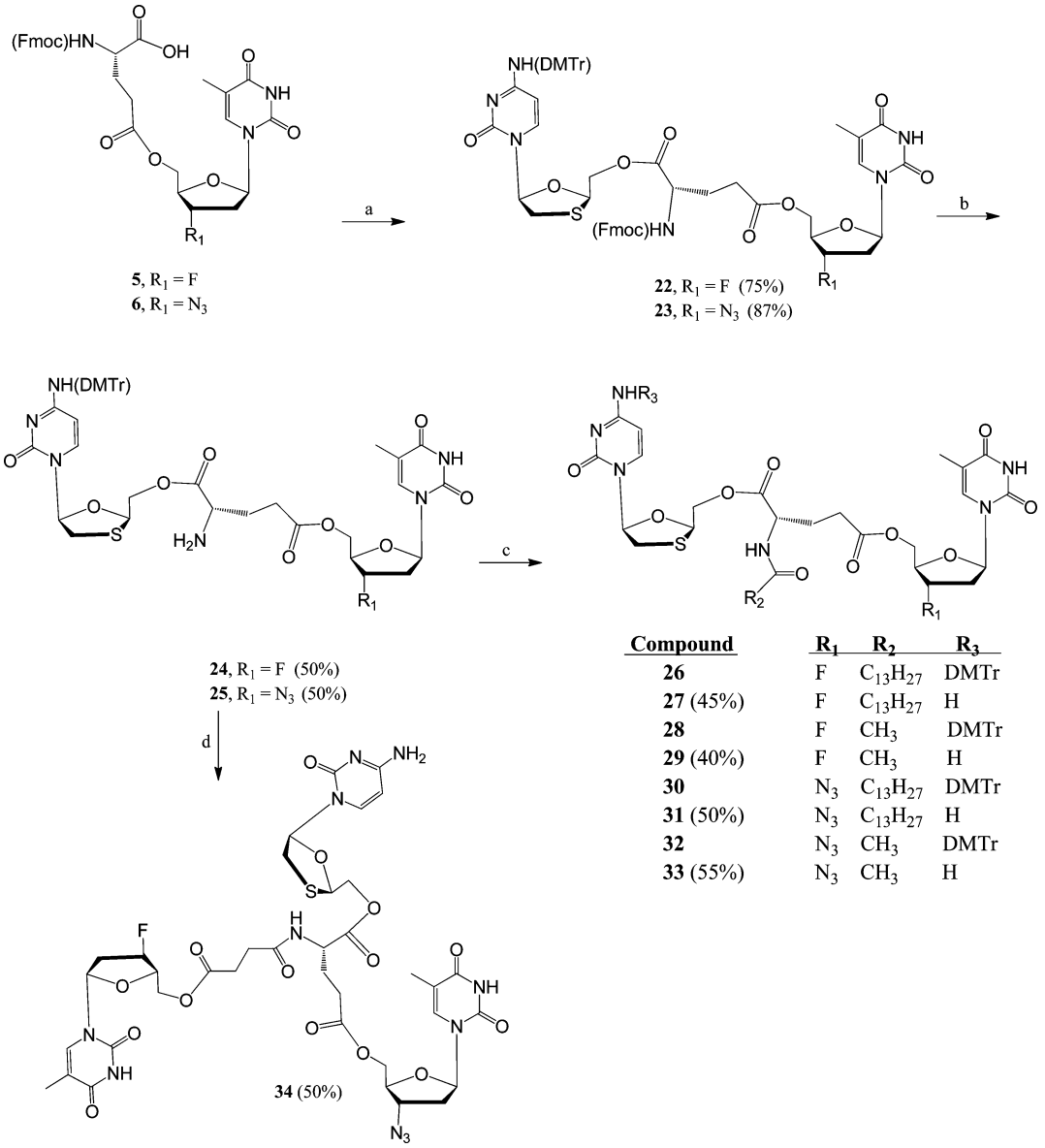
Scheme 5. Synthesis of Glutamic Acid Esters of Dinucleosides and Trinucleosides (AZT, FLT, and 3TC) (26–34)[a]



Compound	R_1	R_2	R_3
26	F	$C_{13}H_{27}$	DMTr
27 (45%)	F	$C_{13}H_{27}$	H
28	F	CH_3	DMTr
29 (40%)	F	CH_3	H
30	N_3	$C_{13}H_{27}$	DMTr
31 (50%)	N_3	$C_{13}H_{27}$	H
32	N_3	CH_3	DMTr
33 (55%)	N_3	CH_3	H

[a]Reagents and conditions: (a) 3TC-DMTr, HBTU, DIPEA, DMF; (b) piperidine, octanethiol; (c) (1) $[R_2CO]_2O$, DIPEA, DMF, (2) acetic acid; (d) (1) FLT-succinate, HBTU, DIPEA, DMF, (2) acetic acid.

trinucleoside compounds (EC_{50} = 0.1–17.0 μM, Table 2) versus that of mononucleoside–peptide conjugates (EC_{50} = 6.9–86.4 μM, Table 1) against cell-free virus. Furthermore, conjugates containing myristic acid showed superior anti-HIV activity compared to the corresponding compounds without the fatty acid, possibly due to improved lipophilicity and cellular uptake. For example, glutamate ester of FLT and 3TC with myristic acid (**27** and **46**) (EC_{50} = 0.3–2.7 μM) exhibited a 5- to 41-fold higher anti-HIV activity against cell-free virus than the corresponding conjugate without myristic acid (**29**) (EC_{50} = 12.3–17.0 μM). Similarly, improved anti-HIV activity of lipophilic *N*-myristoylated conjugates **20** and **31** was observed versus the corresponding *N*-acetylated derivatives **21** and **33**, respectively. These data suggest the potential contribution of higher lipophilicity in improving cellular uptake and generating compounds with better anti-HIV profile. These data are in consistence with our previously reported data exhibiting significantly higher anti-HIV profile and/or cellular uptake of several of lipophilic fatty acyl derivatives of d4T,[17] AZT,[18] and FLT,[19] when compared to those of the parent nucleosides. Fatty acid nucleoside derivatives possess dramatically different physicochemical properties from the parent nucleoside compounds. The compounds are lipophilic and are expected to enhance drug penetration through membranes.

Among all glutamate esters with two nucleosides, compound **47**, containing conjugated FLT and FTC, was the most potent anti-HIV agent (EC_{50} = 0.1–0.4 μM) and was superior to the individual parent nucleosides FLT and FTC with EC_{50} values of 0.4–2.0 μM. The anti-HIV activity of the remaining glutamate esters containing two nucleosides was higher or comparable to that of 3TC and AZT but less than that of FLT.

A myristoylated glutamate ester of FLT (**43**, EC_{50} = 6.3–7.7 μM) was also evaluated for antiviral activity. Addition of AZT as a second nucleoside (**20**, EC_{50} = 2.4–3.6 μM) improved the activity of the conjugate by 2.1- to 2.6-fold (versus that of **43**). Addition of 3TC as the second nucleoside (**27**, EC_{50} = 2.0–2.7

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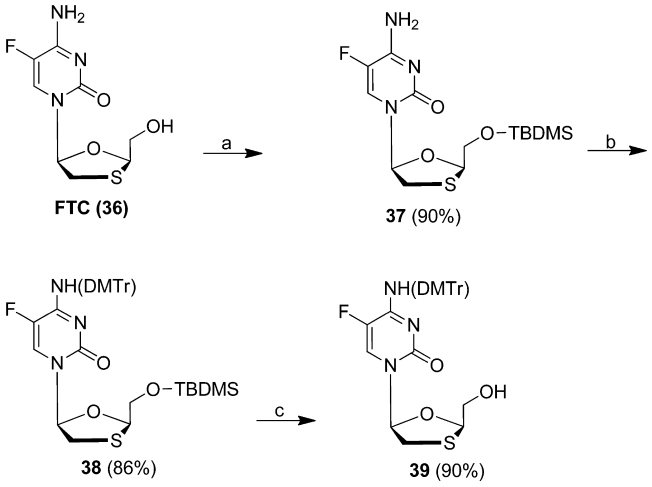
Scheme 6. Synthesis of FTC-DMTr (39)[a]



[a]Reagents and conditions: (a) TBDMS-Cl, imidazole, DMF; (b) DMTr-Cl, pyridine; (c) TBAF.

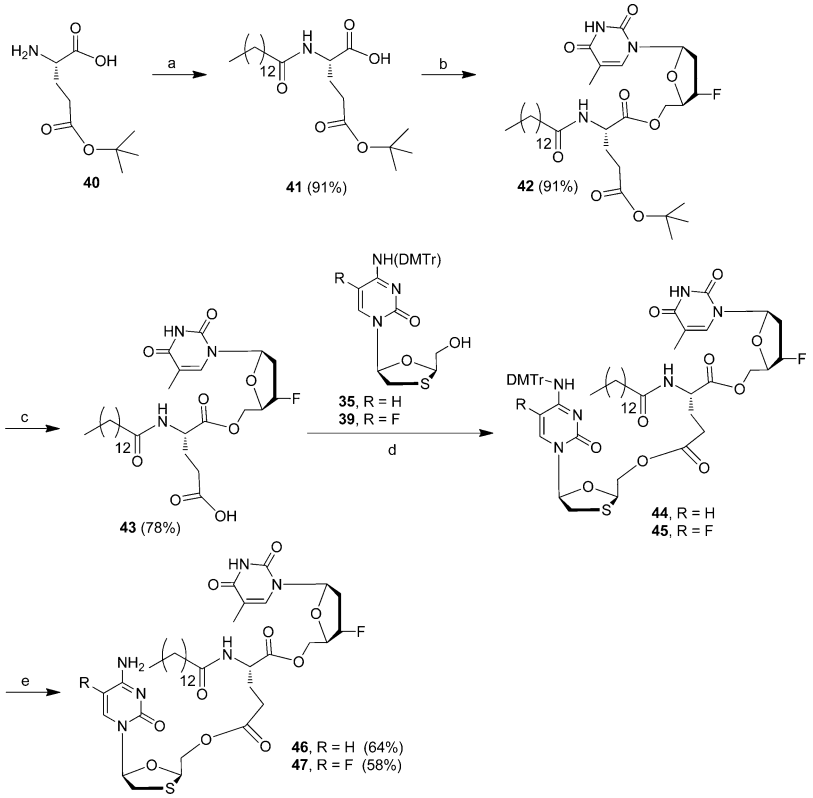
μM) enhanced anti-HIV activity approximately 3-fold when compared to that of **43**. Compound **47** with FTC as the second nucleoside exhibited significantly (EC_{50} = 0.1−0.4 μM) higher anti-HIV activity by 16- to 77-fold. Improved antiviral activity suggests synergistic or additive contribution of the second nucleoside to the overall anti-HIV activity. Because AZT and 3TC (EC_{50} = 10.8−32.7 μM) have lower activity against HIV compared to FTC (EC_{50} = 0.8−2.0 μM), their cooperative effect in **20** and **27** was less than that observed in **47**.

Position of nucleoside on glutamic acid also played an important role in antiviral activity as observed in compounds **27** versus **46** with glutamate esters of FLT and 3TC. Because FLT is more potent than 3TC, the substitution of FLT in these derivatives can be considered more crucial for anti-HIV activity. Compound **46** (EC_{50} = 0.3−0.6 μM) with FLT substituted at the C-terminal of glutamic acid displayed 3- to 9-fold higher antiviral activity than **27** (EC_{50} = 2.0−2.7 μM) with FLT substituted at the side chain of glutamate ester, possibly due to higher intracellular release of FLT from **46** than from **27**.

A glutamate derivative with three different nucleosides (**34**, FLT-AZT-3TC) showed higher anti-HIV activity (EC_{50} = 0.9−1.4 μM) against cell-free virus than the conjugates containing two of the corresponding nucleosides [**21** (FLT-AZT), **29** (FLT-3TC), and **33** (AZT-3TC)] (Table 2). Compound **34** showed nearly 10−12- and 8−36-fold higher anti-HIV activity than AZT and 3TC, respectively, but slightly less activity than FLT. Although compound **34** exhibited less anti-HIV activity than FLT against cell-free virus, the conjugate showed significantly higher anti-HIV activity (EC_{50} = 0.8 μM) against cell associated virus when compared with FLT, AZT, 3TC, and other glutamate esters with two nucleosides (data not shown). These data suggest that the incorporation of the third nucleoside using a glutamate as a scaffold could generate conjugates with high anti-HIV activity against both cell-free and cell-associated virus.

Physical mixtures were prepared by mixing glutamic acid with the nucleosides with or without myristic acid in similar equimolar ratios to that of the corresponding conjugates. Table 3 depicts the anti-HIV activity of synthesized glutamate−

Scheme 7. Synthesis of Glutamic Acid Esters of Nucleosides (FLT, FTC, and 3TC) (46 and 47)[a]



[a]Reagents and conditions: (a) $(C_{13}H_{27}CO)_2O$, DIPEA, DMF; (b) FLT, DIPEA, HBTU, HOBt, DMF; (c) TFA/DCM (95:5 v/v); (d) DIPEA, HBTU, HOBt, DMF; (e) acetic acid/TFA (98:2 v/v).

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Table 1. Anti-HIV1 Activity of Peptide−Nucleoside Conjugates

compd	chemical name	cytotoxicity[a] EC_{50}[b] (μM)	antiviral ativity X4[c] EC_{50} (μM)	antiviral ativity R5[d] EC_{50} (μM)
FLT	3′-fluoro-2′,3′-dideoxythymidine	>400	0.8	0.4
AZT	3′-azido-2′,3′-dideoxythymidine	>374	10.8	14.2
10	myristoyl-Glu(FLT)-Lys(Myristoyl)-OH	>108	26.8	29.1
13	Ac-Glu(FLT)-β-Ala-Lys(myristoyl)-OH	>121	10.6	6.9
16	Ac-Ser(myristoyl)-β-Ala-Glu(FLT)-Gly-OH	>119	11.1	6.9
17	Ac-Ser(myristoyl)-β-Ala-Glu(AZT)-Gly-OH	>116	63.1	86.4
DMSO	dimethyl sulfoxide	>1000	>1000	>1000
positive control[e]	dextran sulfate (50 KDa)	>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]Assay control.

Table 2. Anti-HIV Activity of Nucleoside−Glutamate Derivatives with or without Myristoyl Moiety

compd	chemical name	cytotoxicity[a] EC_{50}[b] (μM)	antiviral activity (X4)[c] EC_{50} (μM)	antiviral activity (R5)[d] EC_{50} (μM)
FLT	3′-fluoro-2′,3′-dideoxythymidine	>400	0.8	0.4
AZT	3′-azido-2′,3′-dideoxythymidine	>374	10.8	14.2
3TC	(−)-2′,3′-dideoxy-3′-thiacytidine	>436	32.7	11.3
FTC	(−)-5-fluoro-2′,3′-dideoxy-3′-thiacytidine	>404	2.0	0.8
20	mysristoyl-Glu(FLT)-AZT	>120	2.4	3.6
21	acetyl-Glu(FLT)-AZT	>151	13.5	13.1
27	mysristoyl-Glu(FLT)-3TC	>126	2.0	2.7
29	acetyl-Glu(FLT)-3TC	>160	17.0	12.3
31	myristoyl-Glu(AZT)-3TC	>122	6.0	6.5
33	acetyl-Glu(AZT)-3TC	>154	12.0	13.4
34	FLT-succinate-Glu(AZT)-3TC	>107	0.9	1.4
43	myristoyl-Glu(OH)-FLT	>51	6.3	7.7
46	myristoyl-Glu(3TC)-FLT	>38	0.6	0.3
47	myristoyl-Glu(FTC)-FLT	>37	0.4	0.1
DMSO	dimethyl sulfoxide	>1000	>1000	>1000
positive control[e]	dextran sulfate (50 KDa)	>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]Assay control.

nucleoside conjugates when compared with the corresponding physical mixtures in μM. For example, the activity of **34** was compared to the corresponding physical mixtures either with or without myristic acid (**56** and **55**, Table 3). The physical mixture containing FLT, AZT, 3TC, and glutamic acid (**55**) showed comparable activity to conjugate **34** against cell-free virus. However, compound **55** (EC_{50} = 30.5 μM) exhibited 38-fold lower activity than **34** (EC_{50} = 0.8 μM) against cell-associated virus. Replacing FLT in **55** with FLT−succinate in a physical mixture (**54**) (corresponding to conjugate **34**) resulted in reduced anti-HIV activity by 2-fold against X4 cell free virus and 115-fold against cell associated virus (EC_{50} = 91.9 μM) when compared to **34** (EC_{50} = 0.8 μM). This reduction in the activity might be due to slow hydrolysis of FLT−succinate to FLT, the molecule responsible for anti-HIV activity.

In general, the physical mixtures of nucleosides and glutamic acid with myristic acid showed higher or comparable potency to that of other corresponding conjugates, possibly because of the presence of completely free nucleosides in the mixtures. For example, the physical mixture of FLT, AZT, glutamic acid, and myristic acid in equimolar ratio (**48**) exhibited 8−18-fold higher active than its corresponding conjugate **20** against cell-free virus. The physical mixture of FLT, 3TC, glutamic acid, and myristic acid in equimolar ratio (**50**) showed comparable activity with corresponding conjugate **46**, but was 5−14-fold more potent than conjugate **27**. These data were expected because the physical mixtures contain free nucleosides that can be immediately converted to active triphosphosphates after cellular uptake. On the other hand, the corresponding lipophilic conjugates need to undergo intracellular hydrolysis to parent free nucleosides. The rate of regeneration of the nucleoside from the conjugate is dependent upon the rate of hydrolysis of the conjugate by esterase. Although the conjugates displayed comparable or lower anti-HIV activity than the corresponding physical mixture, it is expected that the lipophilic compounds may have a prolonged effect possibly due to the sustained intracellular hydrolysis to parent nucleosides.

Furthermore, physical mixtures containing myristic acid generally exhibited 2−4-fold higher anti-HIV activity (see **49** vs **48**, **51** vs **50**, and **55** vs **56**) than that of compounds without myristic acid. Addition of myristic acid in equivalent ratio in physical mixture **56** improved the activity against cell-free virus by 3−6-fold in comparison with **34** and the physical mixture **55**. These data suggest that the presence of myristic acid plays an important role in improving the anti-HIV activity of the physical mixtures. These data are consistent with previously reported results indicating that medium chain fatty acids enhance the permeability and cellular uptake of other compounds and can be used as absorption enhancers.[25] These data were also consistent with those obtained for the

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Table 3. Anti-HIV Activity of Nucleoside−Glutamate Derivatives with or without Myristoyl Moiety and the Corresponding Physical Mixtures

compd	composition	cytotoxicity[a] EC_{50}[b] (μM)	antiviral activity (X4)[c] EC_{50} (μM)	antiviral activity (R5)[d] EC_{50} (μM)
20	myristoyl-Glu(FLT)-AZT	>120	2.4	3.6
48	FLT + AZT + glutamic acid + myristic acid	>113	0.3	0.2
21	acetyl-Glu(FLT)-AZT	>151	13.5	13.1
49	FLT + AZT + glutamic acid	>152	1.5	1.1
27	myristoyl-Glu(FLT)-3TC	>126	2.0	2.7
46	myristoyl-Glu(3TC)-FLT	>38	0.6	0.3
50	FLT + 3TC + glutamic acid + myristic acid	>118	0.4	0.2
29	acetyl-Glu(FLT)-3TC	>160	17.0	12.3
51	FLT + 3TC + glutamic acid	>161	1.0	1.3
31	myristoyl-Glu(AZT)-3TC	>122	6.0	6.5
52	AZT + 3TC + glutamic acid + myristic acid	>115	2.2	1.0
33	acetyl-Glu(AZT)-3TC	>154	12.0	13.4
53	AZT + 3TC + glutamic acid	>155	2.6	1.4
34	FLT-succinate-Glu(AZT)-3TC	>107	0.9	1.4
54	FLT-succinate + AZT + 3TC + glutamic acid	>101	1.8	1.6
55	FLT + AZT + 3TC + glutamic acid	113	0.9	0.6
56	FLT + AZT + 3TC + glutamic acid + myristic acid	>90	0.3	0.2
DMSO	dimethyl sulfoxide	>1000	>1000	>1000
positive control[e]	dextran sulfate (50 KDa)	>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]Assay control.

conjugates containing two nucleosides and myristic acid (**20**, **27**, **29**, and **31**) as described above. These results indicate that the addition of myristic acid to **34** may further improve the anti-HIV activity against cell-free virus.

*Anti-HIV Activities of Glutamate−Trinucleoside Conjugate **34** (FLT-AZT-3TC) Against Clinical Isolates and Multidrug Resistant HIV.* Because glutamate−trinucleoside conjugate **34** was found to be consistently effective against both X4 and R5 lab-adapted viruses, it was further evaluated against four clinical isolates of HIV-1 (two wild type isolates from clades B and C, and two resistant mutants) (Table 4). The anti-HIV activity of **34** was compared with that of its precursors, AZT, FLT, and 3TC. Conjugate **34** showed high antiviral activity against the multidrug resistant virus 4755-5 (IC_{50} = 5.9 nM) and its activity was 2219, 411, and 18 times better than that of 3TC (IC_{50} >13097 nM), AZT (IC_{50} = 2425 nM), and FLT (IC_{50} = 107.3 nM), respectively. Compound **34** also exhibited higher antiviral activity (IC_{50} = 12.9 nM) against virus A17 bearing NNRTI-resistant mutations when compared with parent nucleosides 3TC (IC_{50} = 92.1 nM), FLT (IC_{50} = 26.0 nM), and AZT (IC_{50} = 21.7 nM). However, the antiviral activity of **34** against the wild-type isolates from B and C subtypes was weaker than FLT. These results indicate that conjugate **34** can be used against MDR and NNRTI-resistant strains, improving the anti-HIV profile of the parent nucleosides.

Table 4. Anti-HIV Evaluation against Wild-Type Clinical Isolates and Resistant Viruses[a]

compd	virus	clade/resistance	IC_{50} (nM)	IC_{90} (nM)
3TC	94US3393IN	B-wild type	87.3	305.6
	98USMSC5016	C-wild type	21.8	305.6
	A-17 MDR	B-NNRTI	92.1	282.9
	4755-5 MDR	B-MDR	>13097	>13097
FLT	94US3393IN	B-wild type	2.0	20.4
	98USMSC5016	C-wild type	2.0	12.3
	A-17 MDR	B-NNRTI	26.0	87.2
	4755-5 MDR	B-MDR	107.3	1065
AZT	94US3393IN	B-wild type	2.4	21.3
	98USMSC5016	C-wild type	1.95	34.2
	A-17 MDR	B-NNRTI	21.7	152.3
	4755-5 MDR	B-MDR	2425	10657
34	94US3393IN	B-wild type	26.7	160.6
	98USMSC5016	C-wild type	22.7	84.5
	A-17 MDR	B-NNRTI	12.9	28.3
	4755-5 MDR	B-MDR	5.9	46.0

[a]Multiround infection assay using HIV-1 clinical isolates (wild type and NNRTI and multidrug (MDR) resistant mutants) and peripheral blood mononuclear cells.

CONCLUSIONS

Dinucleoside and trinucleoside conjugates of multivalent scaffolds (e.g., glutamic acid and peptides) were synthesized with the goal of improving anti-HIV activity by simultaneously delivering different nucleosides to infected or HIV-naïve target cells. Improved activity may be associated with increased potency, broader antiviral spectrum, and higher barrier to drug resistance. Peptide conjugates with one nucleoside and myristic acid did not improve the anti-HIV activity of their parent nucleosides. Addition of two myristic acids was not beneficial either, and the antiviral activity was reduced drastically.

On the other hand, the glutamate esters containing two nucleosides exhibited higher anti-HIV activity than conjugates substituted with one nucleoside only and other peptide analogues. The presence of a myristic acid in the glutamate conjugates and their corresponding physical mixtures improved anti-HIV activity. Myristoylated glutamate conjugates **46** (EC_{50} = 0.3−0.6 μM, containing FLT and 3TC) and **47** (EC_{50} = 0.1−

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0.4 μM, containing FLT and FTC) were the most active glutamate conjugates.

Among all the tested compounds, a trinucleoside glutamate derivative containing AZT, FLT, and 3TC (**34**, EC_{50} = 0.9−1.4 μM) exhibited higher anti-HIV activity than AZT and 3TC against cell-free virus. Conjugate **34** was also more active against multidrug resistant and NNRTI-resistant HIV clinical isolates when compared to 3TC, FLT, and AZT. Furthermore, compound **34** was more potent against cell-associated virus when compared with the corresponding physical mixtures.

Compounds **46**, **47**, and **34** showed better antiviral profiles than their parent nucleosides, suggesting that multinucleoside conjugation on a glutamate scaffold may be considered for the development of new anti-HIV drugs for therapeutic and preventive purposes.

EXPERIMENTAL SECTION

Materials and Methods. PS3 automated peptide synthesizer (Rainin Instrument Co., Oakland, CA) was used to synthesize peptides. In general, all peptides were synthesized by the solid-phase synthesis strategy employing *N*-(9-fluorenyl)methoxycarbonyl (Fmoc)-based chemistry and Fmoc-L-amino acid building blocks. HBTU and *N*-methylmorpholine (NMM) in *N*,*N*-dimethylformamide (DMF) were used as coupling and activating reagents, respectively. Wang resin, Fmoc-amino acid Wang resins, coupling reagents, Fmoc-protected amino acids (Fmoc-Glu-OtBu, Fmoc-Ser-OH, Fmoc-Lys(Mtt)-OH, Fmoc-β-Ala-OH, Fmoc-Gly-OH), and HBTU were purchased from Novabiochem. 3TC (Lamivudine), AZT (Zidovudine), FTC (Emtricitabine), and FLT (Alovudine) were purchased from Euro Asia Tran Continental (Bombay, India). DIPEA and all the other reagents including solvents were purchased from Fisher Scientific.

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). Chemical shifts are reported in parts per millions (ppm). The chemical structures of final products were confirmed by a high-resolution PE Biosystems Mariner API time-of-flight electrospray mass spectrometer or Biosystems QStar Elite time-of-flight electrospray mass spectrometer. The purity of final products (>95%) was confirmed by analytical HPLC. The analytical HPLC was performed on a Hitachi analytical HPLC system using a C18 Shimadzu Premier 3 μm column (150 cm × 4.6 mm) using two different systems at a flow rate of 1 mL/min with detection at 265 nm.

Synthesis of Myristoylated Mononucleoside Dipeptide, Tripeptide, and Tetrapeptide Conjugates. Glutamate ester conjugates of AZT, FLT, and myristic acid were synthesized employing a PS3 automated peptide synthesizer and Fmoc solid-phase peptide synthesis using Fmoc-L-amino acid building blocks. The glutamate−nucleoside conjugates were assembled on Wang resin solid support at room temperature. The building blocks, **5**, **6**, and **8**, were synthesized from Fmoc-Glu(OH)-O*t*Bu and Fmoc-Ser-OH, respectively, as described below:

Fmoc-Glu(3′-fluoro-2′,3′-dideoxythymidine-5′-yl)-OH (5) and Fmoc-Glu(3′-azido-2′,3′-dideoxythymidine-5′-yl)-OH (6). Fmoc-Glu(OH)O*t*Bu (5 g, 11.8 mmol), **1** or **2**, 14.1 mmol), and HBTU (6.7 g, 17.6 mmol) were dissolved in DMF (25 mL). DIPEA (5 mL, 38 mmol) was added to the solution, and the reaction mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure to yield crude Fmoc-Glu(3′-fluoro-2′,3′-dideoxythymidine-5′-yl)O*t*Bu (**3**) or Fmoc-Glu(3′-azido-2′,3′-dideoxythymidine-5′-yl)O*t*Bu (**4**).

3: HR-MS (ESI-TOF) (*m*/*z*): $C_{34}H_{38}FN_3O_9$, calcd 651.2592; found 652.1312 $[M + H]^+$; 1303.2873 $[2 M + H]^+$. **4**: HR-MS (ESI-TOF) (*m*/*z*): $C_{34}H_{38}N_6O_9$, calcd 674.2700; found 697.0440 $[M + Na]^+$.

Trifluoroacetic acid/water (TFA/water, 95:5 v/v, 20 mL) was added to the reaction mixture containing **3** or **4**. The reaction mixture was stirred for 1 h to remove *t*-butyl protecting group at *C*-terminal. TFA was removed at reduced pressure, and the residue was purified with HPLC using a C_{18} column and water/acetonitrile as solvents as described above to yield **5** and **6**.

Fmoc-Glu(3′-fluoro-2′,3′-dideoxythymidine-5′-yl)-OH (5). Overall yield (5.0 g, 70%). ^{1}H NMR (400 MHz, CD_3OD, δ ppm): 7.78 (d, *J* = 7.4 Hz, 2H, Fmoc Ar-H), 7.60−7.69 (m, 2H, Fmoc Ar-H), 7.35−7.42 (m, 3H, H-6, Fmoc Ar-H), 7.31 (dt, *J* = 7.4 and 3.3 Hz, 2H, Fmoc Ar-H), 6.23 (dd, *J* = 6.4 and 9.3 Hz, 1H, H-1′), 5.27 (dd, *J* = 5.9 and 53.3 Hz, 1H, H-3′), 4.34−4.51 (m, 5H, H-4′, H-5″, Glu C*H*(α), and Fmoc NHCOOC*H*$_2$), 4.28 (dd, *J* = 5.9 and 9.1 Hz, 1H, H-5′), 4.22 (t, *J* = 6.7 Hz, 1H, Fmoc NHCOOCH$_2$C*H*), 2.10−2.58 (m, 6H, Glu C*H*$_2$C*H*$_2$COO (β and γ methylene), H-2″, and H-2′), 1.87 (s, 3H, C*H*$_3$). HR-MS (ESI-TOF) (*m*/*z*): $C_{30}H_{30}FN_3O_9$, calcd 595.1966; found 596.1989 $[M + H]^+$, 1191.4610 $[2 M + H]^+$.

Fmoc-Glu(3′-azido-2′,3′-dideoxythymidine-5′-yl)-OH (6). Overall yield (5.2 g, 70%). ^{1}H NMR (400 MHz, CD_3OD, δ ppm): 7.78 (d, *J* = 7.4 Hz, 2H, Fmoc Ar-H), 7.35−7.70 (m, 2H, Fmoc Ar-H), 7.44 (s, 1H, H-6), 7.37 (t, *J* = 7.4 Hz, 2H, Fmoc Ar-H), 7.30 (t, *J* = 7.4 Hz, 2H, Fmoc Ar-H), 6.10 (t, *J* = 6.4 Hz, 1H, H-1′), 4.15−4.45 (m, 7H, H-3′, H-5′, H-5″, Glu-α-C*H*, Fmoc NHCOOC*H*$_2$C*H*), 4.05 (dd, *J* = 4.8 and 9.0 Hz, 1H, H-4′), 2.52 (t, *J* = 7.2 Hz, 2H, Glu-C*H*$_2$COO (β-methylene), 2.32−2.48 (m, 2H, Glu-C*H*$_2$CH$_2$COO (γ-methylene)), 2.14−2.30 (m, 1H, H-2′), 1.90−2.05 (m, 1H, H-2″), 1.85 (s, 3H, 5-C*H*$_3$). ^{13}C NMR (CD_3OD, 100 MHz, δ ppm): 175.27 (*C*OOH), 173.97 (*C*OOAZT), 162.39 (C-4 *C*═O), 152.25 (C-2 *C*═O), 145.40, 142.73 (Fmoc Ar-C), 137.85 (C-6), 128.95, 128.33, 126.42, 121.08 (Fmoc Ar-C), 112.02 (C-5), 86.70 (C-1′), 83.176 (C-4′), 68.16 (*C*H$_2$OCONH), 64.88 (C-5′), 62.15 (Glu *C*H(α), 54.57 (C-3′), 37.88 (C-2′), 31.47 (Glu γ-*C*H$_2$), 27.99 (Glu β-*C*H$_2$), 12.73 (5-*C*H$_3$). HR-MS (ESI-TOF) (*m*/*z*): $C_{30}H_{30}N_6O_9$, calcd 618.2074; found 619.2564 $[M + H]^+$.

Fmoc-Ser(O-myristoyl)-OH (8). Compound **7** (5 g, 15.3 mmol) and DIPEA (10 mL, 75 mmol) were dissolved in DMF (20 mL). Myristoyl chloride (10 g, 23 mmol) was added to the solution. The reaction mixture was stirred for overnight. The solvent was removed at reduced pressure, and the residue was purified using silica gel column chromatography using dichloromethane (DCM)/methanol (0−5%) as eluents. The compound was eluted at 5% methanol in DCM to yield **8** (5.3 g, 63%).

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.70 (d, *J* = 7.4 Hz, 2H, Fmoc Ar-H), 7.53 (d, *J* = 6.9 Hz, 2H, Fmoc Ar-H), 7.34 (t, *J* = 7.4 Hz, 2H, Fmoc Ar-H), 7.25 (t, *J* = 7.4 Hz, 2H, Fmoc Ar-H), 5.50 (d, *J* = 8.0 Hz, serine C*H*(α)), 4.65−4.71 (m, 1H, serine C*H*″ (β)), 4.45 (dd, *J* = 3.9 and 11.4 Hz, 1H, serine C*H*′ (β)), 4.32−4.39 (m, 2H, Fmoc NHCOOC*H*$_2$), 4.17 (t, *J* = 6.9 Hz, 1H, Fmoc NHCOOCH$_2$C*H*), 2.26 (t, *J* = 7.5 Hz, 2H, C*H*$_2$COO), 1.54 (t, *J* = 6.3 Hz, 2H, C*H*$_2$CH$_2$COO), 1.13−1.25 (br m, 20H, methylene protons), 0.80 (t, *J* = 6.6 Hz, 3H, C*H*$_3$). HR-MS (ESI-TOF) (*m*/*z*): $C_{32}H_{43}NO_6$, calcd 537.3090; found 538.2673 $[M + H]^+$, 1075.5442 $[2 M + H]^+$.

***N*-Myristoyl-Glu(3′-fluoro-2′,3′-dideoxythymidine-5′-yl)-Lys-(myristoyl)OH [My-Glu(FLT)-Lys(My)-OH (10)].** Resin **9** (300 mg, 0.45 mmol/g) was swelled in DMF for 30 min. Compound **5** (320 mg, 0.54 mmol), HBTU (200 mg, 0.54 mmol), and NMM (0.54 mmol) were added to the swelled resin suspension in DMF. The mixture was shaken overnight at room temperature. The resin was filtered and washed two times with DMF (10 mL). Fmoc deprotection was carried out using piperidine in DMF (20%, 10 mL). The resin was washed with DMF (3 × 10 mL). To the resin was added TFA/DCM mixture (5:95 v/v, 10 mL) to remove methyltrityl protecting group (Mtt) at lysine side chain. The mixture was shaken for 1 h at room temperature. The resin was washed with DCM (3 × 10 mL) and DMF (10 mL) and swelled in DMF (10 mL). Myristic anhydride (100 mg, 1.08 mmol) and DIPEA (2 mL, 15 mmol) were added to the swelled resin. The mixture was shaken for 2 h at room temperature. The resin was washed with DMF (2 × 10 mL). A mixture of TFA/anisole/water (95:2.5:2.5 v/v/v, 10 mL) was added to the resin, and the mixture was shaken for 1 h. After filtration, the solution was concentrated and dried under reduced pressure. The crude peptide conjugates were purified with reversed phase HPLC using a C_{18} column and water/acetonitrile

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as solvents as described above and were lyophilized to yield **10** (10 mg, 8.0%).

HR-MS (ESI-TOF) (m/z): $C_{49}H_{84}FN_5O_{10}$, calcd 921.6202; found 922.9989 $[M + H]^+$, 1845.9558 $[2\ M + H]^+$.

***N*-Acetyl-Glu(3′-fluoro-2′,3′-dideoxythymidine-5′-yl)-β-Ala-Lys(myristoyl)-OH [Ac-Glu(FLT)-βAla-Lys(My)-OH (13)] and *N*-Acetyl-Glu(3′-azido-2′,3′-dideoxythymidine-5′-yl)-βAla-Lys-(myristoyl)-OH [Ac-Glu(AZT)-βAla-Lys(My)-OH (14)].** The peptide was assembled on Fmoc-Lys(Mtt)-Wang resin (600 mg, 0.45 mmol/g) by Fmoc solid-phase peptide synthesis strategy at room temperature using Fmoc protected amino acids [Fmoc-β-Ala-OH (C + 1) and Fmoc-Glu(nucleoside)-OH (C + 2) (**5** or **6**, 1.08 mmol)]. HBTU (1.08 mmol) and NMM (1.08 mmol) in DMF were used as coupling and activating reagents, respectively. Fmoc deprotection at each step was carried out using piperidine in DMF (20%). NH_2-Glu(FLT)-βAla-Lys(Mtt)-Wang resin (**11**) or NH_2-Glu(AZT)-βAla-Lys(Mtt)-Wang resin (**12**) was transferred to the reaction vessel and swelled in DMF (2 mL) for 30 min. Acetic anhydride (2 mL) and DIPEA (2 mL, 15 mmol) were added to the mixture. The reaction was shaken at room temperature for 30 min to cap the *N*-terminal with acetyl group. *N*-Acetylated resin was washed with DMF (2 × 10 mL). TFA/DCM (5%, 10 mL) to the resin. The mixture was shaken for 1 h at room temperature. The resin was washed with DCM (3 × 10 mL) and DMF (10 mL) and swelled in DMF (10 mL). Free amino group at lysine side chain was further myristoylated by adding myristic anhydride (100 mg, 1.08 mmol) and DIPEA (2 mL, 15 mmol) to the swelled resin. The mixture was shaken for 2 h at room temperature. The resin was washed with DMF (3 × 10 mL). A mixture of TFA/anisole/water (95:2.5:2.5 v/v/v, 10 mL) was added to the resin, and the mixture was shaken for 1 h. After filtration, the solution was concentrated and dried under reduced pressure. The crude peptide conjugates were purified with reversed phase HPLC using a C_{18} column and water/acetonitrile as solvents as described above and were lyophilized to yield **13** and **14**.

13: Overall yield (20 mg, 8%). ^{1}H NMR (400 MHz, CD_3OD, δ ppm): 7.48 (s, 1H, FLT H-6), 6.28 (dd, J = 5.7 and 8.8 Hz, 1H, FLT H-1′), 5.29 (dd, J = 53.6 and 4.8 Hz, 1H, FLT H-3′), 4.20−4.70 (m, 6H, FLT H-4′, FLT H-5′, FLT H-5″, Lys COC*H*NH (CH-α), Glu COC*H*NH (CH-α)], 3.40−3.50 (m, 2H, β-Ala CH_2NH), 3.17 (t, J = 6.7 Hz, 2H, Lys CH_2-NHCO), 2.20−2.60 (m, 6H, FLT H-2′, FLT H-2″, β-Ala CH_2COO, Glu CH_2CH_2COO), 2.17 (t, J = 7.6 Hz, 3H, myristate CH_2COO), 1.94−2.05 (m, 3H, Ac CH_3), 1.88 (s, 3H, FLT 5-CH_3), 1.20−1.70 (m, 20H, myristate methylene and Lys methylene protons), 0.90 (t, J = 6.6 Hz, 3H, myristate CH_3). HR-MS (ESI-TOF) (m/z): $C_{40}H_{65}FN_6O_{11}$, calcd 824.4695; found 825.6640 $[M + H]^+$, 1651.2282 $[2\ M + H]^+$.

14: Overall yield (20 mg, 8%). HR-MS (ESI-TOF) (m/z): $C_{40}H_{65}N_9O_{11}$, calcd 847.4804; found 848.9452 $[M + H]^+$.

***N*-Acetyl-Ser(myristoyl)-β-Ala-Glu(3′-fluoro-2′,3′-dideoxythymidine-5′-yl)-Gly-OH [Ac-Ser(My)-βAla-Glu(FLT)Gly-OH (16)] and *N*-Acetyl-Ser(myristoyl)-β-Ala-Glu(3′-azido-2′,3′-dideoxythymidine-5′-yl)-Gly-OH [Ac-Ser(My)-βAla-Glu(AZT)Gly-OH (17)].** The peptides were assembled on resin **15** (600 mg, 0.45 mmol/g) by Fmoc solid-phase peptide synthesis strategy using Fmoc protected amino acids (**5** or **6**) (C + 1), Fmoc-β-Ala-OH (C + 2), and **8** (C + 3), each 1.08 mmol]. HBTU (1.08 mmol) and NMM (1.08 mmol) in DMF were used as coupling and activating reagents, respectively. Fmoc deprotection at each step was carried out using piperidine in DMF (20%). NH_2-Ser(My)-βAla-Glu(FLT)Gly-Wang resin or NH_2-Ser(My)-βAla-Glu(AZT)-Gly-Wang resin was transferred to the reaction vessel and swelled in DMF (2 mL) for 30 min. Acetic anhydride (2 mL) and DIPEA (2 mL, 15 mmol) were added to the mixture. The reaction was shaken at room temperature for 30 min to cap *N*-terminal amino group with acetyl group. *N*-Acetylated resins were washed with DMF (2 × 10 mL). A mixture of TFA/anisole/water (95:2.5:2.5 v/v/v, 10 mL) was added to the resins. The mixtures were shaken for 1 h to cleave the peptides. After filtration, the solution was concentrated and dried under reduced pressure. The crude peptide conjugates were purified with reversed phase HPLC using a C_{18} column and water/acetonitrile as solvents as described above and were lyophilized to yield **16** and **17**.

16: Overall yield (20 mg, 8%). ^{1}H NMR (400 MHz, CD_3OD, δ ppm): 7.48 (s, 1H, FLT H-6), 6.28 (dd, J = 5.6 and 8.8 Hz, 1H, FLT H-1′), 5.29 (dd, J = 53.5 and 4.4 Hz, 1H, FLT H-3′), 4.58−4.66 [m, 1H, Ser COC*H*NH (CH-α)], 4.22−4.50 (m, 5H, FLT H-4′, FLT H-5″, and Glu COC*H*NH (CH-α), Ser CH_2O (CH-β)], 3.98 (dd, J = 3.9 and 17.8 Hz, 1H, FLT H-5′), 3.72−3.92 [m, 2H, Gly COCH_2NH (CH-α)], 3.38−3.58 (m, 2H, β-Ala CH_2NH), 2.24−2.64 (m, 8H, FLT H-2′, FLT H-2″, β-Ala CH_2COO, Glu CH_2CH_2COO), 2.16 (t, J = 7.2 Hz, 3H, myristate CH_2COO), 1.94−2.08 (m, 3H, Ac CH_3), 1.88 (s, 3H, FLT 5-CH_3), 1.52−1.66 (m, 2H, myristate CH_2CH$_2$COO), 1.22−1.38 (br m, 20H, methylene protons), 0.90 (t, J = 6.3 Hz, 3H, myristate CH_3). HR-MS (ESI-TOF) (m/z): $C_{39}H_{61}FN_6O_{13}$, calcd 840.4281; found 841.3842 $[M + H]^+$.

17: Overall yield (20 mg, 8%). ^{1}H NMR (400 MHz, CD_3OD, δ ppm): 7.48 (s, 1H, H-6 AZT), 6.14 (t, J = 6.6 Hz, 1H, AZT H-1′), 4.20−4.70 [m, 6H, AZT H-5′, AZT H-5″, AZT H-3′, Ser COC*H*NH (CH-α), Ser CH_2O (CH-β), Glu COC*H*NH, (CH-α)], 4.07 (dd, J = 4.9 and 8.9 Hz, 1H, AZT H-4′), 3.84−4.05 [m, 2H, Gly COCH_2NH (CH-α)], 3.44 (dd, J = 6.6 and 12.5 Hz, 2H, β-Ala CH_2NH), 2.10−2.66 (m, 10H, FLT H-2′, FLT H-2″, β-Ala CH_2COO, Glu CH_2CH_2COO, myristate CH_2COO), 1.92−2.09 (m, 3H, acetyl CH_3), 1.89 (s, 3H, 5-CH_3-AZT), 1.52−1.72 (m, 2H, myristate-CH_2CH$_2$COO), 1.24−1.50 (br m, 20H, methylene protons), 0.90 (t, J = 6.5 Hz, 3H, myristate CH_3). HR-MS (ESI-TOF) (m/z): $C_{39}H_{61}N_9O_{13}$, calcd 863.4389; found 864.9022 $[M + H]^+$, 1729.8398 $[2\ M + H]^+$.

Synthesis of Dinucleoside and Trinucleoside Glutamate Ester Derivatives Functionalized with Acetyl or Myristoyl Moiety. Glutamate−nucleoside conjugates containing more than one nucleoside with or without myristoyl group were synthesized by using solution phase synthesis.

Fmoc-Glu(OFLT)-OAZT (18). Compound **5** (500 mg, 0.84 mmol), AZT (269 mg, 1 mmol), and HBTU (650 mg 1.7 mmol) were dissolved in DMF (10 mL). DIPEA (5 mL, 37 mmol) was added to the solution, and the reaction mixture was stirred overnight. The solvent was removed, and the residue was dried under reduced pressure. The residue was purified with reversed phase HPLC using a C_{18} column and water/acetonitrile as solvents as described above and was lyophilized to yield **18** (620 mg, 85%).

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.24 (s, 1H, FLT N*H*), 9.09 (s, 1H, AZT N*H*), 7.75 (d, J = 7.2 Hz, 1H, Fmoc Ar-H), 7.57 (d, J = 3.6 Hz, 1H, Fmoc Ar-H), 7.38 (t, J = 7.2 Hz, Fmoc Ar-H), 7.29 (t, J = 7.2 Hz, 2H, Fmoc Ar-H), 7.15 (s, 1H, FLT H-6), 7.07 (s, 1H, AZT H-6), 6.14 (t, J = 8.1 Hz, 1H, FLT H-1′), 5.80 (t, J = 6.9 Hz, 1H, AZT H-1′), 5.17 (dd, J = 2.0 and 55.6 Hz, 1H, FLT H-3′), 4.23−4.60 (m, 6H, AZT H-5′, AZT H-5″, AZT H-3′, FLT H-4′, FLT H-5′, and FLT H-5″), 4.19 (t, J = 6.7 Hz, 1H, Glu HN-C*H*-COO CH(α)), 3.95−4.05 (m, 1H, AZT H-4′), 2.16−2.69 (m, 8H, AZT H-2′, AZT H-2″, FLT H-2′, FLT H-2″, and Glu CH_2CH_2COO), 1.88 (br s, 6H, FLT 5-CH_3 and AZT 5-CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 172.3, 171.4 (FLT *C*OO, AZT *C*OO), 163.85, 163.72 (FLT C-4 *C*═O, AZT C-4 *C*═O), 156.23 (Fmoc O*C*ONH), 150.10, 149.98 (AZT C-2 *C*═O and FLT C-2 *C*═O), 143.74, 143.55, 141.31 (Fmoc Ar-C), 137.23, 135.88 (AZT C-6, FLT C-6), 127.88, 127.10, 125.05, 125.00, 120.08, (Fmoc Ar-C), 111.37, 111.28 (AZT C-5, FLT C-5), 93.23 (J = 177.8 Hz, FLT C-3′), 87.82 (FLT C-1′), 86.53 (AZT C-1′), 82.21 (J = 26.3 Hz, FLT C-4′), 81.72 (AZT C-4′), 68.00 (Fmoc *C*H$_2$-OCONH), 63.82 (FLT C-5′), 60.29 (AZT C-5′), 53.48, 53.31 (AZT C-3′, CH(α)), 47.04 (Fmoc *C*H-CH$_2$-OCONH), 37.58 (J = 20.1 Hz, FLT C-2′), 36.94 (AZT C-2′), 29.72 (Glu γ-CH$_2$), 25.60 (Glu β-CH$_2$), 12.57, 12.43 (AZT 5-CH$_3$, FLT 5-CH$_3$). HR-MS (ESI-TOF) (m/z): $C_{40}H_{41}FN_8O_{12}$, calcd 844.2828; found 845.0241 $[M + H]^+$.

NH_2-Glu(OFLT)-OAZT (19). Compound **18** (610 mg, 0.72 mmol) was dissolved in THF (10 mL). Piperidine (7.18 μL, 0.072 mmol) and 1-octanethiol (7.3 mmol, 10 mM solution in THF, 0.73 mL) were added to the reaction mixture. The mixture was stirred at room temperature for 1 h. The solvent was removed, and the residue was dried under reduced pressure. The residue purified with reversed phase HPLC using a C_{18} column and water/acetonitrile as solvents as described above and lyophilized to yield **19** (225 mg, 50%).

^{1}H NMR (400 MHz, CD_3OD, δ ppm): 7.44 (s, 1H, FLT H-6), 7.38 (s, 1H, AZT H-6), 6.22 (dd, J = 8.1 Hz, 1H, FLT H-1′), 6.02 (t, J = 6.9 Hz, 1H, AZT H-1′), 5.23 (dd, J = 2.0 and 55.6 Hz, 1H, FLT H-3′), 4.20−4.55 (m, 6H, AZT H-5′, AZT H-5″, AZT H-3′, FLT H-4′, FLT H-5′, and FLT H-5″), 4.15 (t, J = 6.7 Hz, 1H, Glu HN-C*H*-COO CH(α)), 3.98−4.04 (m, 1H, AZT H-4′), 2.16−2.69 (m, 8H, AZT H-2′, AZT H-2″, FLT H-2′, FLT H-2″, CH_2CH_2COO), 1.87 (br s, 6H, FLT 5-CH_3, AZT 5-CH_3). ^{13}C NMR (CD_3OD, 100 MHz, δ ppm): 173.29, 170.17 (FLT *C*OO, AZT *C*OO), 166.40, 163.72 (FLT C-4 *C*═O, AZT C-4 *C*═O), 152.33, 152.19 (AZT C-2 *C*═O, FLT C-2 *C*═O), 139.13, 137.89 (AZT C-6, FLT C-6), 114.03, 112.08 (AZT C-5, FLT C-5), 95.07 (J = 176.7 Hz, FLT C-3′), 88.12 (FLT C-1′), 87.34 (AZT C-1′), 83.86 (J = 27.3 Hz, FLT C-4′), 82.72 (AZT C-4′), 66.90 (FLT C-5′), 61.99 (AZT C-5′), 53.23, 52.28 (AZT C-3′, CH(α)), 38.26 (J = 21.2 Hz, FLT C-2′), 37.10 (AZT C-2′), 30.14 (Glu γ-CH_2), 26.63 (Glu β-CH_2), 12.73, 12.53 (AZT 5-CH_3, FLT 5-CH_3). HR-MS (ESI-TOF) (m/z): $C_{25}H_{31}FN_8O_{10}$, calcd 622.2147; found 622.9532 [M + H]$^+$, 1244.9406 [2 M + H]$^+$.

***N*-Myristoyl-Glu(OFLT)-OAZT (20).** Compound **19** (75 mg, 0.12 mmol) and myristic anhydride (100 mg, 0.24 mmol) were dissolved in DMF (10 mL). DIPEA (5 mL, 37 mmol) was added to the solution. The mixture was stirred for 2 h at room temperature. The solvent was removed under reduced pressure. The residue was purified with reversed phase HPLC using a C_{18} column and water/acetonitrile as solvents as described above and was lyophilized to yield **20** (40 mg, 40%).

^{1}H NMR (400 MHz, CD_3OD, δ ppm): 7.45, 7.44 (two s, 2H, AZT H-6, FLT H-6), 6.23 (dd, J = 8.9 and 5.6 Hz, 1H, FLT H-1′), 6.09 (t, J = 6.7 Hz, 1H, AZT H-1′), 5.24 (dd, J = 5.0 and 53.6 Hz, 1H, FLT H-3′), 4.32−4.48 (m, 6H, AZT H-5′, AZT H-5″, AZT H-3′, FLT H-4′, FLT H-5′, and FLT H-5″), 4.22 (dd, J = 3.8 and 11.4 Hz, 1H, Glu HN-C*H*-COO CH(α)), 4.06 (dd, J = 4.8 and 8.6 Hz, 1H, AZT H-4′), 2.15−2.60 (m, 10H, AZT H-2′, AZT H-2″, FLT H-2′, FLT H-2″, myristate CH_2COO, Glu CH_2CH_2COO), 1.87 (s, 6H, FLT 5-CH_3, AZT 5-CH_3), 1.58 (t, J = 6.6 Hz, 2H, CH_2CH_2COO), 1.23−1.33 (br m, 20H, methylene protons), 0.87 (t, J = 6.7 Hz, 3H, 5-CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 173.78, 172.37, 171.56 (FLT *C*OO, AZT *C*OO, *C*ONH), 164.01 (FLT C-4 *C*═O and AZT C-4 *C*═O), 150.27 (AZT C-2 *C*═O, FLT C-2 *C*═O), 137.14, 135.77 (AZT C-6, FLT C-6), 111.37 (AZT C-5, FLT C-5), 93.27 (J = 179.4 Hz, FLT C-3′), 87.56 (FLT C-1′), 86.32 (AZT C-1′), 82.19 (J = 26.6 Hz, FLT C-4′), 81.68 (AZT C-4′), 63.83 (FLT C-5′), 60.33 (AZT C-5′), 51.48, 51.35 (AZT C-3′, CH(α)), 37.59 (J = 20.8 Hz, FLT C-2′), 36.96 (AZT C-2′), 36.39 (CH_2COO), 34.14, (Glu γ-CH_2), 31.93 (Glu β-CH_2), 30.04, 29.90, 29.83, 29.66, 29.60, 29.51, 29.46, 29.36, 29.31, 29.16, 27.27 25.59, 24.97, 22.70 (methylene carbons), 14.15 (My-CH_3) 12.73, 12.53 (AZT 5-CH_3, FLT 5-CH_3). HR-MS (ESI-TOF) (m/z): $C_{39}H_{57}FN_8O_{11}$, calcd 832.4131; found 832.8583 [M]$^+$, 1665.8057 [2 M + H]$^+$.

***N*-Acetyl-Glu(OFLT)-OAZT (21).** Compound **19** (75 mg, 0.12 mmol) was dissolved in DMF (10 mL). DIPEA (5 mL, 37 mmol) and acetic anhydride (2 mL, 20 mmol) were added to the solution. The reaction mixture was stirred for 2 h at room temperature. The solvent was removed under reduced pressure, and the residue was purified with reversed phase HPLC using a C_{18} column and water/acetonitrile as solvents as described above and was lyophilized to yield **21** (30 mg, 35%).

^{1}H NMR (400 MHz, CD_3OD, δ ppm): 7.45, 7.43 (two s, 2H, AZT H-6, FLT H-6), 6.23 (dd, J = 8.8 and 5.6 Hz, 1H, FLT H-1′), 6.09 (t, J = 6.6 Hz, 1H, AZT H-1′), 5.24 (dd, J = 5.0 and 53.6 Hz, 1H, FLT H-3′), 4.33−4.47 (m, 6H, AZT H-5′, AZT H-5″, AZT H-3′, FLT H-4′, FLT H-5′, FLT H-5″), 4.22 (dd, J = 3.2 and 11.0 Hz, 1H, Glu HN-C*H*-COO CH(α)), 4.07 (dd, J = 4.6 and 8.8 Hz, 1H, AZT H-4′), 2.11−2.61 (m, 8H, AZT H-2′, AZT H-2″, FLT H-2′, FLT H-2″, and Glu CH_2CH_2COO), 1.96 (s, 3H, acetyl CH_3), 1.87 (s, 6H, FLT 5-CH_3, AZT 5-CH_3). ^{13}C NMR (CD_3OD, 100 MHz, δ ppm): 172.17, 172.10, 171.40 (FLT *C*OO, AZT *C*OO, *C*ONH), 164.93, 164.82 (FLT C-4 *C*═O, AZT C-4 *C*═O), 150.93, 150.73 (AZT C-2 *C*═O, FLT C-2 *C*═O), 136.57, 136.07 (AZT C-6, FLT C-6), 110.55 (AZT C-5, FLT C-5), 93.69 (J = 176.6 Hz, FLT C-3′), 85.80 (FLT C-1′), 85.51 (AZT C-1′), 82.42 (J = 26.0 Hz, FLT C-4′), 81.55 (AZT C-4′), 64.31 (FLT C-5′), 60.94 (AZT C-5′), 51.75 (AZT C-3′, CH(α)), 36.96 (J = 20.6 Hz, FLT C-2′), 36.14 (AZT C-2′), 29.39 (Glu γ-CH_2), 26.04 (Glu β-CH_2), 20.88 (acetyl CH_3), 12.73, 12.53 (AZT 5-CH_3, FLT 5-CH_3). HR-MS (ESI-TOF) (m/z): $C_{27}H_{33}FN_8O_{11}$, calcd 664.2253; found 664.9298 [M + H]$^+$, 1328.8401 [2 M + H]$^+$.

Fmoc-Glu(OFLT)-O(3TC(DMTr)) (22). (−)-N_4-(4,4′-Dimethoxytrityl)-2′,3′-dideoxy-3′-thiacytidine (DMTr-3TC) was synthesized by using a previously reported method.[20] Compound **5** (500 mg, 0.84 mmol), DMTr-3TC (535 mg, 1 mmol), and HBTU (650 mg 1.7 mmol) were dissolved in DMF (10 mL). DIPEA (5 mL, 37 mmol) was added to the solution, and the reaction mixture was stirred overnight at room temperature. The solvent was removed, and the residue was dried under reduced pressure. The residue was purified with reversed phase HPLC using a C_{18} column and water/acetonitrile as solvents as described above and was lyophilized to yield **22** (820 mg, 75%).

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.99 (s, 1H, FLT N*H*), 7.74 (d, J = 7.5 Hz, 1H, Fmoc Ar-H), 7.55 (d, J = 7.6 Hz, 1H, 3TC H-6), 7.38 (t, J = 7.5 Hz, Fmoc Ar-H), 7.06−7.33 (m, 13H, DMTr Ar-H, Fmoc Ar-H, and FLT H-6), 6.76−6.87 (m, 4H, DMTr Ar-H protons), 6.34 (m, 1H, 3TC H-1′), 6.14−6.22 (m, 1H, FLT H-1′), 5.64 (d, J = 7.6 Hz, 1H, 3TC H-5), 5.05−5.27 (m, 2H, FLT H-3′ and 3TC H-4′), 4.24−4.45 (m, 8H, 3TC H-5′, 3TC H-5″, Fmoc NHCOOCH_2C*H*, FLT H-4′, FLT H-5′, FLT H-5″), 4.17 (t, J = 6.7 Hz, 1H, Glu HN-C*H*-COO CH(α)), 3.75 and 3.78 (two s, 6H, DMTr CH_3O), 3.46 (dd, J = 11.8 and 5.3 Hz, 1H, 3TC H-2″), 2.97 (dd, J = 11.8 and 5.3 Hz, 1H, 3TC H-2′), 1.97−2.67 (m, 6H, FLT H-2′, FLT H-2″, and Glu CH_2CH_2COO), 1.86 (s, 3H, 5-CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 172.00 (FLT *C*OO), 171.25 (3TC *C*OO), 165.06 (3TC C-4), 163.50 (FLT C-4 *C*═O), 158.69 (DMTr Ar-*C*-OCH$_3$), 156.00 (3TC C-2 *C*═O), 150.09 (FLT C-2 *C*═O), 144.24 (3TC C-6), 135.38 (FLT C-6), 129.91, 128.52, 128.37, 127.82, 127.53, 127.11, 125.00, 120.08, 113.60 (Fmoc Ar-C and DMTr Ar-C), 111.41 (FLT C-5), 95.17 (3TC C-5), 93.20 (J = 178.3 Hz, FLT C-3′), 87.64 (3TC C-1′), 86.01 (FLT C-1′), 83.16 (3TC C-4′), 82.16 (J = 26.9 Hz, FLT C-4′), 81.69 (DMTr Ph_3*C*-NH), 70.34 (Fmoc *C*H$_2$-OCONH), 67.15 (FLT C-5′), 65.76 (3TC C-5′), 55.28 (DMTr O*C*H$_3$), 53.40 (*C*H(α)), 47.04 (Fmoc *C*H-CH$_2$-OCONH), 38.16 (FLT C-2′), 37.36 (3TC C-2′), 29.86, (Glu γ-CH_2), 27.16 (Glu β-CH_2), 12.64 (FLT 5-CH_3). HR-MS (ESI-TOF) (m/z): $C_{59}H_{57}FN_6O_{13}S$, calcd 1108.3688; found 1109.4804 [M + H]$^+$, 1131.4395 [M + Na]$^+$, 1147.4739 [M + K]$^+$, 2218.5704 [2 M + H]$^+$.

Fmoc-Glu(OAZT)-O(3TC(DMTr)) (23). Compound **6** (520 mg, 0.84 mmol), DMTr-3TC (535 mg, 1 mmol), and HBTU (650 mg 1.7 mmol) were dissolved DMF (10 mL). DIPEA (5 mL, 37 mmol) was added to the solution, and the reaction mixture was stirred overnight at room temperature. The solvent was removed, and the residue was dried under reduced pressure. The residue was purified with reversed phase HPLC using a C_{18} column and water/acetonitrile as solvents as described above and was lyophilized to yield **23** (840 mg, 87%).

HR-MS (ESI-TOF) (m/z): $C_{59}H_{57}N_9O_{13}S$, calcd 1131.3797; found 1132.3485 [M + H]$^+$, 2265.1887 [2 M + H]$^+$.

NH_2-Glu(OFLT)-O(3TC(DMTr)) (24). Compound **22** (800 mg, 0.72 mmol) was dissolved in THF (10 mL). Piperidine (7.2 μL, 0.072 mmol) and 1-octanethiol (7.3 mmol, 10 mM solution in THF, 0.73 mL) were added to the reaction mixture. The mixture was stirred for 1 h at room temperature. The solvent was removed, and the residue was dried under reduced pressure. The residue was purified with reversed phase HPLC using a C_{18} column and water/acetonitrile as solvents as described above and lyophilized to yield **24** (300 mg, 50%).

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.44 (d, J = 7.8 Hz, 1H, 3TC H-6), 7.25−7.33 (m, 4H, DMTr Ar-H), 7.18−7.21 (m, 2H, DMTr Ar-H and FLT H-6), 7.12 (d, J = 8.8 Hz, 4H, DMTr Ar-H), 6.84 (d, J = 8.8 Hz, 4H, DMTr Ar-H), 6.20 (dd, J = 5.7 and 8.6 Hz, 1H, FLT H-1′), 6.13 (t, J = 5.2 Hz, 1H, 3TC H-1′), 5.33 (dd, J = 4.0 and 6.0 Hz, 1H, 3TC H-4′), 5.10−5.31 (m, 2H, FLT H-3′ and 3TC H-5), 4.28−4.45 (m, 4H, 3TC H-5′, 3TC H-5″, FLT H-5′, and FLT H-5″), 4.18−4.26 (m, 1H, FLT H-4′), 4.07 (t, J = 6.6 Hz, 1H, HN-C*H*(CH_2)-COO (CH(α)), 3.77 (s, 6H, DMTr-CH_3O), 3.41 (dd, J = 11.9 and 5.2 Hz,

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1H, 3TC H-2″), 3.05 (dd, J = 11.9 and 5.2 Hz, 1H, 3TC H-2′), 2.10−2.66 (m, 6H, FLT H-2′, FLT H-2″, and Glu CH_2CH_2COO), 1.85 (s, 3H, 5-CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 172.56 (FLT COO), 169.13 (3TC COO), 165.55 (3TC C-4, FLT C-4 C═O), 159.68 (DMTr Ar-C-OCH_3), 159.16 (3TC C-2 C═O), 151.51 (FLT C-2 C═O), 145.56 (3TC C-6), 136.96 (FLT C-6), 130.77, 130.72, 129.30, 129.09 128.86, 128.41, 127.35, 114.33, 113.70 (DMTr Ar-C), 111.90 (FLT C-5), 96.74 (3TC C-5), 94.11 (J = 177.9 Hz, FLT C-3′), 88.58 (3TC C-1′), 86.72 (FLT C-1′), 82.92 (J = 26.6 Hz, FLT C-4′), 82.78 (3TC C-4′), 71.72 (DMTr Ph_3C-NH), 67.15 (FLT C-5′), 64.53 (3TC C-5′), 55.66 (DMTr OCH_3), 52.36 (CH(α)), 37.85 (J = 21.1 Hz, FLT C-2′), 37.27 (3TC C-2′), 29.70, (Glu γ-CH_2), 25.80 (Glu β-CH_2), 12.73 (FLT 5-CH_3). HR-MS (ESI-TOF) (m/z): $C_{44}H_{47}FN_6O_{11}S$, calcd 886.3008; found 887.2456 $[M + H]^+$, 1774.5611 $[2 M + H]^+$.

NH_2-Glu(OAZT)-O(3TC(DMTr)) (25). Compound **23** (815 mg, 0.72 mmol) was dissolved in THF (10 mL). Piperidine (7.2 μL, 0.072 mmol) and 1-octanethiol (7.3 mmol, 10 mM solution in THF, 0.73 mL) were added to the reaction mixture. The mixture was stirred for 1 h at room temperature. The solvent was removed under reduced pressure, and the residue was purified with HPLC using a C_{18} column and water/acetonitrile as solvents as described above to yield **25** (325 mg, 50%).

^1H NMR (400 MHz, $CDCl_3$, δ ppm): 8.04 (d, J = 7.8 Hz, 1H, 3TC H-6), 7.02−7.47 (m, 10H, DMTr Ar-H and AZT H-6), 6.82 (d, J = 8.8 Hz, 4H, DMTr Ar-H), 6.18−6.35 (m, 1H, 3TC H-1′), 6.15 (d, J = 7.8 Hz, 1H, 3TC H-5), 6.07 (t, J = 6.5 Hz, 1H, AZT H-1′), 5.30−5.55 (m, 1H, 3TC H-4′), 4.66 (dd, J = 6.4 and 12.1 Hz, 1H, 3TC H-5′), 4.57 (dd, J = 3.2 and 12.1 Hz, 1H, 3TC H-5″), 4.26−4.46 (m, 3H, AZT H-3′, AZT H-5′, and AZT H-5″), 4.21 (t, J = 6.6 Hz, 1H, Glu HN-CH-COO CH(α)), 3.93−4.07 (m, 1H, AZT H-4′), 3.75 (s, 6H, DMTr CH_3O), 3.63−3.70 (m, 1H, 3TC H-2″), 3.75 (dd, J = 12.3 and 5.5 Hz, 1H, 3TC H-2′), 2.67 (t, J = 7.0 Hz, 2H, CH_2COO), 2.07−2.56 (m, 4H, AZT H-2′, AZT H-2″, Glu $CHCH_2CH_2COO$), 1.85 (s, 3H, AZT 5-CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 173.20 (AZT COO), 169.95 (3TC COO), 166.25 (3TC C-4, AZT C-4 C═O), 161.54 (DMTr Ar-C-OCH_3), 160.03 (3TC, C-2 C═O), 148.70 (AZT C-2 C═O), 145.48 (3TC C-6), 138.34 (AZT C-6), 137.37, 131.25, 129.32, 128.7, 127.69, 114.00 (DMTr Ar-C), 111.86 (AZT C-5), 95.32 (3TC C-5), 88.88 (AZT C-1′), 87.15 (3TC C-1′), 84.02 (AZT C-4′), 82.93 (3TC C-4′), 67.30 (AZT C-5′), 65.16 (3TC C-5′), 62.06 (DMTr OCH_3), 55.69 (AZT C-3′), 53.03 (CH(α)), 37.48 (AZT C-2′), 37.38 (3TC C-2′), 30.15 (Glu γ-CH_2), 26.53 (Glu β-CH_2), 12.54 (AZT 5-CH_3). HR-MS (ESI-TOF) (m/z): $C_{44}H_{47}N_9O_{11}S$, calcd 909.3116; found 910.4154 $[M + H]^+$, 1821.5154 $[2 M + H]^+$.

***N*-Myristoyl-Glu(OFLT)-O(3TC(DMTr)) (26).** Compound **24** (100 mg, 0.12 mmol) and myristic anhydride (100 mg, 0.24 mmol) were dissolved in DMF (10 mL). DIPEA (5 mL, 37 mmol) was added to the solution. The mixture was stirred for 2 h at room temperature. The solvent was removed under reduced pressure, and the residue was purified with HPLC using a C_{18} column and water/acetonitrile as solvents as described above to yield **26**.

HR-MS (ESI-TOF) (m/z): $C_{58}H_{73}FN_6O_{12}S$, calcd 1096.4991; found 1097.2874 $[M + H]^+$, 2193.8167 $[2 M + H]^+$.

***N*-Myristoyl-Glu(OFLT)-O3TC (27).** Compound **26** was dissolved in acetic acid (80% in water, 10 mL) and was heated at 80 °C for 30 min to remove DMTr protection. Acetic acid was removed under reduced pressure, and the residue was purified with reversed phase HPLC using a C_{18} column and water/acetonitrile as solvents as described above and was lyophilized to yield **27** (40 mg, 45%).

^1H NMR (400 MHz, CD_3OD, δ ppm): 8.13 (d, J = 7.8 Hz, 1H, 3TC H-6), 7.45 (s, 1H, FLT H-6), 6.28 (dd, J = 3.6 and 5.4 Hz, 1H, FLT H-1′), 6.22 (dd, J = 5.7 and 8.8 Hz, 1H, 3TC H-1′), 6.17 (d, J = 7.8 Hz, 1H, 3TC H-5), 5.43 (dd, J = 3.2 and 4.9 Hz, 1H, 3TC H-4′), 5.25 (dd, J = 5.2 and 53.7 Hz, 1H, FLT H-3′), 4.59 (dd, J = 4.9 and 12.4 Hz, 1H, 3TC H-5″), 4.31−4.55 (m, 4H, 3TC H-5′, FLT H-5′, FLT H-5″, FLT H-4′), 4.27 (dd, J = 3.0 and 10.8 Hz, 1H, Glu HN-CH-COO CH(α)), 3.54−3.65 (m, 2H, 3TC H-2′ and H-2″), 2.11−2.65 (m, 8H, FLT H-2′, FLT H-2″, myristate CH_2COO, and Glu CH_2CH_2COO), 1.86 (s, 3H, 5-CH_3), 1.58 (t, J = 6.8 Hz, 2H, CH_2CH_2COOH), 1.20−1.35 (br m, 20H, methylene protons), 0.85 (t, J = 6.6 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 175.92, 172.97 (FLT COO, 3TC COO), 172.13 (CONH), 165.61 (3TC C-4), 160.80 (FLT C-4 C═O), 151.57 (3TC C-2 C═O), 147.96 (FLT C-2 C═O), 145.21 (3TC C-6), 136.97 (FLT C-6), 111.34 (FLT C-5), 97.92 (3TC C-5), 93.27 (J = 179.4 Hz, FLT C-3′), 87.99 (3TC C-1′), 86.32 (FLT C-1′), 82.19 (J = 26.6 Hz, FLT C-4′), 81.68 (3TC C-4′), 64.96 (FLT C-5′), 64.14 (3TC C-5′), 54.23 (CH(α)), 37.59 (J = 20.8 Hz, FLT C-2′), 36.96 (3TC C-2′), 36.40 (CH_2COO), 31.93, 26.34, 30.34, 30.21, 30.09, 29.91, 29.76, 29.69, 26.80, 23.16 (methylene carbons, Glu γ-CH_2, Glu β-CH_2), 13.88 (myristate CH_3), 12.10 (FLT 5-CH_3). HR-MS (ESI-TOF) (m/z): $C_{37}H_{55}FN_6O_{10}S$, calcd 794.3684; found 795.1608 $[M + H]^+$, 1590.3674 $[2 M + H]^+$.

***N*-Acetyl-Glu(OFLT)-O(3TC(DMTr)) (28).** Compound **24** (100 mg, 0.12 mmol) and acetic anhydride (2 mL, 20 mmol) were dissolved in DMF (10 mL). DIPEA (5 mL, 37 mmol) was added to the solution. The reaction mixture was stirred for 2 h at room temperature. The solvent was removed under reduced pressure, and the residue was purified with HPLC using a C_{18} column and water/acetonitrile as solvents as described above to yield **28**.

HR-MS (ESI-TOF) (m/z): $C_{46}H_{49}FN_6O_{12}S$, calcd 928.3113; found 929.1423 $[M + H]^+$.

***N*-Acetyl-Glu(OFLT)-O3TC (29).** Compound **28** was dissolved in acetic acid (80% in water, 10 mL) and was heated at 80 °C for 30 min to remove DMTr protection. Acetic acid was removed under reduced pressure, and the residue was purified with reversed phase HPLC using a C_{18} column and water/acetonitrile as solvents as described above and was lyophilized to yield **29** (30 mg, 40%).

^1H NMR (400 MHz, CD_3OD, δ ppm): 8.13 (d, J = 7.8 Hz, 1H, 3TC H-6), 7.46 (s, 1H, FLT H-6), 6.28 (dd, J = 3.7 and 5.4 Hz, 1H, FLT H-1′), 6.23 (dd, J = 5.7 and 8.8 Hz, 1H, 3TC H-1′), 6.16 (d, J = 7.8 Hz, 1H, 3TC H-5), 5.44 (dd, J = 3.1 and 4.9 Hz, 1H, 3TC H-4′), 5.25 (dd, J = 5.2 and 53.7 Hz, 1H, FLT H-3′), 4.60 (dd, J = 4.9 and 12.4 Hz, 1H, 3TC H-5″), 4.32−4.55 (m, 4H, 3TC H-5′, FLT H-5′, FLT H-5″, FLT H-4′), 4.26 (dd, J = 3.3 and 11.4 Hz, 1H, Glu HN-CH-COO CH(α)), 3.71 (dd, J = 5.7 and 12.6 Hz, 1H, 3TC H-2″), 3.58 (dd, J = 5.7 and 12.6 Hz, 1H, 3TC H-2′), 2.11−2.63 (m, 6H, FLT H-2′, FLT H-2″, and Glu CH_2CH_2COO), 1.96 (s, 3H, acetyl CH_3), 1.86 (s, 3H, FLT 5-CH_3). ^{13}C NMR (CD_3OD, 100 MHz, δ ppm): 173.71, 173.65 (FLT COO, 3TC COO), 172.86 (CONH), 161.46 (3TC C-4), 161.21 (3TC C-2 C═O), 152.33 (FLT C-2 C═O), 146.00 (3TC C-6), 137.69 (FLT C-6), 112.06 (FLT C-5), 95.23 (J = 176.4 Hz, FLT C-3′), 95.14 (3TC C-5), 88.73 (3TC C-1′), 87.13 (FLT C-1′), 85.55 (3TC C-4′), 84.03 (J = 26.0 Hz, FLT C-4′), 65.69 (FLT C-5′), 64.99 (3TC C-5′), 53.03 (CH(α)), 38.64 (FLT C-2′), 38.42 (3TC C-2′), 31.04 (Glu γ-CH_2), 27.55 (Glu β-CH_2), 22.48 (acetyl CH_3), 12.78 (FLT 5-CH_3). HR-MS (ESI-TOF) (m/z): $C_{25}H_{31}FN_6O_{10}S$, calcd 626.1806; found 627.6073 $[M + H]^+$, 1254.9359 $[2 M + H]^+$.

***N*-Myristoyl-Glu(AZT)-3TC-DMTr (30).** Compound **25** (100 mg, 0.12 mmol) and myristic anhydride (100 mg, 0.24 mmol) were dissolved in DMF (10 mL). DIPEA (5 mL, 37 mmol) was added to the solution. The mixture was stirred for 2 h at room temperature. The solvent was removed under reduced pressure, and the residue was purified with HPLC using a C_{18} column and water/acetonitrile as solvents as described above to yield **30**.

HR-MS (ESI-TOF) (m/z): $C_{58}H_{73}N_9O_{12}S$, calcd 1119.5099; found 1120.3216 $[M + H]^+$.

***N*-Myristoyl-Glu(OAZT)-O3TC (31).** Acetic acid (80% in water, 10 mL) was added to compound **30**. The mixture was heated at 80 °C for 30 min to remove DMTr protection. Acetic acid was removed under reduced pressure, and the residue was purified with reversed phase HPLC using a C_{18} column and water/acetonitrile as solvents as described above to yield **30** (35 mg, 50%). ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 7.91 (d, J = 7.8 Hz, 1H, 3TC H-6), 7.17 (s, 1H, AZT H-6), 6.20 (dd, J = 3.7 and 5.2 Hz, 1H, 3TC H-1′), 6.14 (d, J = 7.8 Hz, 1H, 3TC H-5), 5.94 (t, J = 6.5 Hz, 1H, AZT H-1′), 5.31 (dd, J = 4.3 and 3.6 Hz, 1H, 3TC H-4′), 4.32−4.58 (m, 4H, 3TC H-5′, 3TC H-5″, AZT H-3′), 4.21−4.29 (m, 3H, AZT H-5′, AZT H-5″, Glu HN-CH-COO (CH(α)), 3.94 (dd, J = 3.7 and 5.1 Hz, 1H, AZT H-4′), 3.47 (dd, J = 5.2 and 12.6 Hz, 1H, 3TC H-2″), 3.13 (dd, J = 3.7 and 12.6

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Hz, 1H, 3TC H-2′), 2.07−2.51 (m, 8H, AZT H-2′, AZT H-2″, myristate CH_2COO, and Glu CH_2CH_2COO), 1.81 (s, 3H, AZT 5-CH_3), 1.40−1.58 (m, 2H, CH_2CH_2COOH), 1.04−1.27 (br m, 20H, methylene protons), 0.78 (t, J = 7.0 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 175.92, 172.79 (AZT COO, 3TC COO), 171.70 (CONH), 164.82 (3TC C-4), 160.74 (AZT C-4 C═O), 150.67 (3TC C-2 C═O), 148.15 (AZT C-2 C═O), 143.44 (3TC C-6), 136.83 (AZT C-6), 111.32 (AZT C-5), 94.90 (3TC C-5), 87.07 (AZT C-1′), 86.45 (3TC C-1′), 84.35 (AZT C-4′), 81.86 (3TC C-4′), 64.38 (AZT C-5′), 63.75 (3TC C-5′), 60.80 (AZT C-3′), 51.54 (CH(α)), 38.22 (AZT C-2′), 37.05 (CH_2COO), 36.24 (3TC C-2′), 32.11, 30.11, 29.99, 29.83, 29.55, 29.47, 29.40, 25.80 (methylene carbons), 26.77 (Glu γ-CH_2), 22.85 (Glu β-CH_2), 14.19 (myristate CH_3), 12.40 (AZT 5-CH_3). HR-MS (ESI-TOF) (m/z): $C_{37}H_{55}N_9O_{10}S$, calcd 817.3793; found 818.2535 [M + H]$^+$, 1636.0917 [2 M + H]$^+$.

***N*-Acetyl-Glu(OAZT)-O3TC-DMTr (32).** To compound **25** (110 mg, 0.12 mmol) in DMF (10 mL) was added DIPEA (5 mL, 37 mmol) and acetic anhydride (2 mL, 20 mmol). The reaction mixture was stirred for 2 h at room temperature. The solvent was removed under reduced pressure, and the residue was purified with HPLC using a C_{18} column and water/acetonitrile as solvents as described above to yield **32**.

HR-MS (ESI-TOF) (m/z): $C_{46}H_{49}N_9O_{12}S$, calcd 951.3221; found 952.2062 [M + H]$^+$, 1903.9995 [2 M + H]$^+$.

***N*-Acetyl-Glu(OAZT)-O3TC (33).** Acetic acid (80% in water, 10 mL) was added to compound **32**. The reaction mixture was heated at 80 °C to remove DMTr protection. Acetic acid was removed under reduced pressure, and the residue was purified with reversed phase HPLC using a C_{18} column and water/acetonitrile as solvents as described above to yield **33** (35 mg, 55%).

^{1}H NMR (400 MHz, CD_3OD, δ ppm): 8.02 (d, J = 7.9 Hz, 1H, 3TC H-6), 7.33 (s, 1H, FLT H-6), 6.20−6.30 (m, 1H, 3TC H-1′), 6.03−6.16 (m, 2H, AZT H-1′, 3TC H-5), 5.42 (t, J = 3.7 Hz, 1H, 3TC H-4′), 4.43−4.62 (m, 3H, 3TC H-5″, 3TC H-5′, AZT H-3′), 4.25−4.35 (m, 3H, AZT H-5′, AZT H-5″, Glu HN-C*H*-COO (CH(α)), 4.02 (dd, J = 4.5 and 9.1 Hz, 1H, AZT H-4′), 3.57 (dd, J = 5.5 and 12.4 Hz, 1H, 3TC H-2″), 3.24 (dd, J = 3.4 and 12.4 Hz, 1H, 3TC H-2′), 2.11−2.53 (m, 6H, AZT H-2′, AZT H-2″, Glu CH_2CH_2COO), 1.96 (s, 3H, acetyl CH_3), 1.87 (s, 3H, 5-CH_3). ^{13}C NMR (CD_3OD, 100 MHz, δ ppm): 173.22, 172.50 (AZT COO, 3TC COO), 172.24 (CONH), 165.41 (3TC C-4), 161.32 (AZT C-4 C═O), 151.49 (3TC C-2 C═O), 148.53 (AZT C-2 C═O), 144.96 (3TC C-6), 137.32 (AZT C-6), 111.56 (AZT C-5), 95.03 (3TC C-5), 87.92 (AZT C-1′), 86.03 (3TC C-1′), 84.92 (AZT C-4′), 82.40 (3TC C-4′), 65.19 (AZT C-5′), 64.42 (3TC C-5′), 61.36 (AZT C-3′), 52.36 (CH(α)), 38.29 (AZT C-2′), 37.36 (3TC C-2′), 30.65 (Glu γ-CH_2), 27.06 (Glu β-CH_2), 22.60 (acetyl CH_3), 12.60 (AZT 5-CH_3). HR-MS (ESI-TOF) (m/z): $C_{25}H_{31}N_9O_{10}S$, calcd 649.1915; found 650.1956 [M + H]$^+$, 692.1986 [M + Na]$^+$.

FLT-Succ-Glu(OAZT)-O3TC (34). Compound **25** (110 mg, 0.12 mmol), DIPEA (5 mL, 37 mmol), and FLT−succinate (100 mg, 0.30 mmol) were dissolved in DMF (10 mL). The reaction mixture was stirred for 2 h at room temperature. The solvent was removed under reduced pressure. Acetic acid (80% in water, 10 mL) was added to the residue. The reaction mixture was heated at 80 °C to remove DMTr protection. Acetic acid was removed under reduced pressure, and the residue was purified with HPLC using a C_{18} column and water/acetonitrile as a solvent as described above to yield **34** (55 mg, 50%).

^{1}H NMR (400 MHz, CD_3CN, δ ppm): δ 10.7 (s, 1H, N*H*), 9.59 (s, 2H, N*H*), 7.97 (d, J = 7.6 Hz, 1H, 3TC H-6), 7.77 (s, 1H, N*H*), 7.36 (s, 1H, FLT H-6), 7.29 (s, 1H, AZT H-6), 6.96 (d, 1H, N*H*), 6.17−6.26 (m, 2H, 3TC H-1′ and FLT H-1′), 6.14 (d, J = 7.6 Hz, 1H, 3TC H-5), 6.06 (t, J = 6.3 Hz, 1H, AZT H-1′), 5.40 (t, J = 3.6 Hz, 1H, 3TC H-4′), 5.24 (dd, J = 4.8 and 53.7 Hz, 1H, FLT H-3′), 4.16−4.59 (m, 9H, 3TC H-5′, 3TC H-5″, FLT H-5′, FLT H-5″, FLT H-4′, AZT H-5′, AZT H-5″, AZT H-3′, Glu HN-C*H*-COO (CH(α)), 4.00 (dd, J = 6.6 Hz, 1H, AZT H-4′), 3.56 (dd, J = 11.9 and 5.2 Hz, 1H, 3TC H-2″), 3.24 (dd, J = 11.9 and 5.2 Hz, 1H, 3TC H-2′), 2.05−2.66 (m, 12H, succinate OOCCH_2CH_2CON, FLT H-2′, FLT H-2″, AZT H-2′, AZT H-2″, and Glu CH_2CH_2COO), 1.83 (s, 6H, FLT 5-CH_3 and AZT 5-CH_3). ^{13}C NMR (CD_3CN, 100 MHz, δ ppm): 174.50, 173.95, 172.95 (3TC COO, FLT COO, AZT COO), 166.38 (3TC C-4), 161.62 (AZT C-4 C═O and FLT C-4 C═O), 152.38, 152.27, 148.86 (AZT C-2, FLT C-2, 3TC C-2 C═O), 145.83, 138.25, 137.50 (FLT C-6, AZT C-6, 3TC C-6), 112.22, 112.04 (FLT C-5, AZT C-5), 95.43 (J = 176.5 Hz, FLT C-3′), 96.42 (3TC C-5), 88.78 (3TC C-1′), 86.85 (FLT C-1′), 86.81 (AZT C-1′), 86.81 (3TC C-4′), 84.11 (J = 26.0 Hz, FLT C-4′), 83.16 (AZT C-4′), 65.93, 65.05, 64.99 (3TC C-5′, FLT C-5′, AZT C-5′), 62.20 (AZT C-3′), 52.64 (CH(α)), 38.79 (3TC C-2′), 38.48 (J = 19.0 Hz, FLT C-2′), 37.76 (AZT C-2′), 31.86, 31.06 (succinate two CH_2 groups), 30.24 (Glu γ-CH_2), 27.71 (Glu β-CH_2), 12.89, 12.75 (AZT 5-CH_3 and FLT 5-CH_3). HR-MS (ESI-TOF) (m/z): $C_{37}H_{44}FN_{11}O_{15}S$, calcd 933.2723; found 934.2864 [M + H]$^+$, 1867.6791 [2 M + H]$^+$.

(−)-5′-*O*-(*t*-Butyldimethylsilyl)-5-fluoro-2′,3′-dideoxy-3′-thiacytidine (5′-TBDMS FTC, 37). FTC (500 mg, 2.18 mmol), *tert*-butyldimethylsilyl chloride (1 g, 6.54 mmol), and imidazole (440 mg, 6.54 mmol) were dissolved in dry DMF (10 mL), and the reaction mixture was stirred for 18 h at room temperature. The solvent was concentrated under reduced pressure, and the residue was purified with silica gel column chromatography using dichloromethane and methanol (0−5%) as eluents to yield **37** (700 mg, 90%).

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.50−9.20 (br s, 2H, NH_2), 8.17 (d, J = 6.4 Hz, 1H, H-6), 6.25 (dd, J = 5.2 and 2.4 Hz, 1H, H-1′), 5.17−5.21 (t, J = 2.4 Hz, 1H, H-4′), 4.15 (dd, J = 11.8 and 2.4 Hz, 1H, H-5″), 3.90 (dd, J = 11.8 and 2.4 Hz, 1H, H-5′), 3.47 (dd, J = 12.4 and 5.2 Hz, 1H, H-2″), 3.14 (dd, J = 12.4 and 2.4 Hz, 1H, H-2′), 0.90 (s, 9H, $(CH_3)_3$C), 0.10 (s, 6H, CH_3Si). ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 158.24 (J = 14.5 Hz, C-4), 153.73 (C-2 C═O), 136.32 (J = 240.7 Hz, C-5), 126.04 (J = 32.7 Hz, C-6), 88.22 (C-1′), 87.30 (C-4′), 63.66 (C-5′), 39.11 (C-2′), 25.85, 18.56 ($(CH_3)_3$C-Si), −5.47, −5.49 (CH_3-Si). HR-MS (ESI-TOF) (m/z): $C_{14}H_{25}FN_3O_3SSi$, calcd 361.1292; found 362.4160 [M + H]$^+$, 723.8098 [2 M + H]$^+$.

(−)-5′-*O*-(*t*-Butyldimethylsilyl)-*N*$_4$-(4,4′-dimethoxytrityl)-5-fluoro-2′,3′-dideoxy-3′-thiacytidine (5′-TBDMS-N4-DMTr-FTC, 38). Compound **37** (600 mg, 1.75 mmol) was dissolved in dry pyridine (10 mL). A solution of DMTr-Cl (700 mg, 1.2 equiv) 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 for overnight. The reaction mixture was neutralized with saturated sodium bicarbonate solution (100 mL) and was extracted with dichloromethane (3 × 100 mL). The organic layer was separated and concentrated under reduced pressure. The residue was purified with silica gel column chromatography using dichloromethane and methanol (0−1%) as eluents to yield **38** (1.0 g, 86%).

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.73−9.40 (br s, 1H, N*H*), 8.49 (d, J = 6.0, 1H, H-6), 7.27 (d, J = 3.6 Hz, 5H, DMTr protons), 7.17 (d, J = 8.7 Hz, 4H, DMTr protons), 6.83 (d, J = 8.7 Hz, 4H, DMTr protons), 6.26−6.29 (br s, 1H, H-1′), 5.23−5.25 (br s, 1H, H-4′), 4.25 (dd, J = 10.2 and 1.8 Hz, 1H, H-5″), 3.94 (d, J = 10.2 Hz, 1H, H-5′), 3.80 (s, 6H, DMTr-OCH_3), 3.53 (dd, J = 12.2 Hz, J = 4.6 Hz, 1H, H-2″), 3.24 (d, J = 12.2 Hz, 1H, H-2′), 0.94 (s, 9H, $(CH_3)_3$C), 0.15 (s, 6H, CH_3Si). ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 158.63 (C-4), 155.85 (C-2 C═O), 151.47, 147.33 (DMTr-*C*), 139.46 (C-5), 129.14, 127.86, 127.77 (DMTr-*C*), 127.09 (C-6), 113.17 (DMTr-*C*), 89.14 (C-1′), 87.26 (C-4′), 81.44 (DMTr-C-NH), 63.22 (C-5′), 55.26 (DMTr-OCH_3), 39.59 (C-2′), 25.86 (CH_3-C), 18.66 ((CH_3)$_3$C-Si), −5.46, −5.50 (CH_3-Si). HR-MS (ESI-TOF) (m/z): $C_{35}H_{43}FN_3O_5SSi$, calcd 663.2598; found 663.9596 [M + H]$^+$, 765.0250 [M + TEA]$^+$.

(−)-*N*$_4$-(4,4′-Dimethoxytrityl)-5-fluoro-2′,3′-dideoxy-3′-thiacytidine (DMTr-FTC, 39). Compound **38** (1 g, 1.55 mmol) was dissolved in 1 M solution of tetrabutylammonium fluoride (4.5 mL, 1M, 3 equiv) and stirred for 3 h. The reaction mixture was concentrated under reduced pressure, and the residue was purified with silica gel column chromatography using dichloromethane (2% triethylamine) and methanol (2%) as eluents to yield **39** (750 mg, 90%).

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.83−7.96 (m, 1H, H-6), 7.10−7.35 (m, 9H, DMTr protons), 6.80 (s, 4H, DMTr protons),

6.42−6.46 (br s, 1H, O*H*), 6.15−6.19 (br s, 1H, H-1′), 5.14−5.18 (br s, 1H, H-4′), 4.06−4.20 (m, 1H, H-5″), 3.93 (d, J = 12.5 Hz, 1H, H-5′), 3.78 (s, 6H, DMTr-OCH_3), 3.34−3.50 (m, 1H, H-2″), 2.98−3.15 (m, 1H, H-2′). HR-MS (ESI-TOF) (m/z): $C_{29}H_{29}FN_3O_5S$, calcd 549.1734; found 550.5078 $[M + H]^+$, 651.6923 $[M+TEA]^+$, 1122.0138 $[2\ M + Na]^+$.

***N*-Myristoyl-Glu(O*t*Bu)-OH (41).** DIPEA (390 mg, 3 mmol) was added to the mixture of gamma-*tert*-butoxy-glutamic acid (**40**, 203 mg, 1 mmol) and myristic anhydride (525 mg, 1.2 mmol) in dry DMF (10 mL) at room temperature. The reaction mixture was stirred for 4 h. After completion of the reaction, the solvent was removed under reduced pressure. Water (40 mL) was added, and the mixture was extracted with dichloromethane (3 × 10 mL). The organic layer was dried over $MgSO_4$ (anhydrous) and concentrated under reduced pressure. The crude residue was purified on silica gel column chromatography eluted with DCM and methanol (0−10%) to give **41** (375 mg, 91%).

^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.98 (s, 1H, COO*H*), 6.51 (d, J = 8.0 Hz, 1H, N*H*), 4.52 (q, J = 4.0 Hz, 1H, −C*H*COOH), 2.33−2.16 (m, 4H, −CH_2CH_2COOC−), 1.58 (t, J = 4.0 Hz, −CH_2CONH−), 1.39 (s, 9H, −C(CH_3)$_3$), 1.20 (s, 22H, −(CH_2)$_{11}$), 0.83 (t, J = 8.0 Hz, 3H, −CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 174.01 (COOH), 172.75 (COOC), 163.06 (CONH), 80.89 (COOC−), 51.97 (NHCH−), 36.80 (−CH_2COOC), 36.67 (−CH_2CONH−), 32.05, 31.78, 29.81, 29.78, 29.62, 29.48, 29.39, 28.16, 27.39, 25.73, 22.82 ((CH_3)$_3$, methylene −CH_2−), 14.26 (CH_3). HR-MS (ESI-TOF) (m/z) (negative mode): $C_{23}H_{43}NO_5$, calcd 413.3140; found 412.280 $[M - 1]^-$.

***N*-Myristoyl-Glu(O*t*Bu)-OFLT (42).** DIPEA (390 mg, 3 mmol) was added to the mixture of **41** (413 mg, 1 mmol), FLT (244 mg, 1 mmol), and HBTU (1.13 g, 3 mmol) in dry DMF (30 mL). The mixture was stirred at room temperature for 6 h. After completion of reaction, the solvent was removed under reduced pressure. Water (40 mL) was added and extracted with dichloromethane (3 × 20 mL). The organic layer was dried over Na_2SO_4 (anhydrous) and concentrated under reduced pressure. The crude residue was purified on silica gel column chromatography eluted with dichloromethane and methanol (0−10%) to give **42** (581 mg, 91%).

HR-MS (ESI-TOF) (m/z): $C_{33}H_{54}FN_3O_8$, calcd 639.3895; found 640.2220 $[M + 1]^+$.

***N*-Myristoyl-Glu(OH)-OFLT (43).** Compound **42** (500 mg, 0.86 mmol) was treated with TFA/DCM (95%, 20 mL). The reaction mixture was stirred for 2 h at room temperature. After completion of the reaction, TFA/DCM was removed under reduced pressure. The crude product was dissolved in DCM and purified on silica gel column eluted with DCM and methanol (0−10%) to give **43** (355 mg, 78%).

^{1}H NMR ($CDCl_3$, 500 MHz, δ ppm): 10.37 (s, 1H, COO*H*), 8.05 (br s, 1H, CON*H*), 7.41 (s, 1H, FLT C-6), 6.46−6.28 (m, 1 H, FLT H-1′), 5.22 (d, J = 53.4 Hz, 1H, FLT H-3′), 4.65−4.25 (m, 4H, FLT H-4′, FLT H-5′, −C*H*COOH), 2.84−2.56 (m, 1H, FLT H-2′), 2.49−2.40 (m, 3H, Glu γ CH_2-COO and FLT H-2″), 2.27−2.16 (m, 2H, Glu β −CH_2-CH_2-COO), 1.86 (s, 3H, FLT 5-CH_3), 1.63−1.56 (m, −CH_2CONH−), 1.24 (br s, 22H, −(CH_2)$_{11}$), 0.87 (t, J = 8.0 Hz, 3H, −CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz, δ ppm): 177.29 (COOH), 174.66 (COOC), 171.65 (CONH), 165.84 (FLT C-4), 149.81 (FLT C-2), 136.87 (FLT C-6), 111.22 (FLT C-5), 93.95 (d, J = 177.5 Hz, FLT C-3′), 85.98 (FLT C-1′), 82.32 (FLT C-4′), 82.10, 64.86 (FLT C-5′), 51.66 (NHCH−), 38.21, 36.28 (FLT C-2′), 31.90 (Glu γ-CH_2), 29.85, 29.67, 29.63, 29.60, 29.46, 29.33, 29.28, 29.21, 27.53, 25.59, 22.67 (methylene −CH_2− and Glu β-CH_2), 14.10 (CH_3), 12.20 (FLT C5 CH_3). HR-MS (ESI-TOF) (m/z): $C_{29}H_{46}FN_3O_8$, calcd 583.32; found 584.186 $[M + 1]^+$.

General Method for the Synthesis of *N*-Myristoyl-Glu(O3TC-DMTr)-OFLT (44) and *N*-Myristoyl-Glu(OFTC-DMTr)-OFLT (45). DIPEA (260 mg, 2 mmol) was added to the mixture of **3** (200 mg, 0.34 mmol), 3TC-DMTr/FTC-DMTr (0.34 mmol), and HBTU (758 mg, 2 mmol) in dry DMF (40 mL) at room temperature. The mixture was stirred for 6 h. After completion of the reaction, the solvent was removed under reduced pressure. Water (40 mL) was added, and the mixture was extracted with DCM (3 × 20 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude products were carried forward for the next reaction.

44: HR-MS (ESI-TOF) (m/z): $C_{58}H_{73}FN_6O_{12}S$, calcd 1096.4990; found 1097.2520 $[M + 1]^+$.

45: HR-MS (ESI-TOF) (m/z): $C_{58}H_{72}F_2N_6O_{12}S$, calcd 1114.4897; found 1115.2175 $[M + 1]^+$.

General Method for the Synthesis of *N*-Myristoyl-Glu-(O3TC)-OFLT (46) and *N*-Myristoyl-Glu(OFTC)-OFLT (47). Compounds **44** and **45** were treated with a mixture of acetic acid/TFA (98:2 v/v, 20 mL). The reaction mixture was stirred for 1 h at room temperature. After completion of the reaction, acetic acid/TFA was removed under reduced pressure. The crude products were dissolved in DCM and purified on a silica gel column eluted with DCM and methanol (0−10%) to afford **46** and **47**, respectively.

46: Overall yield 174 mg, 64%. ^{1}H NMR ($CDCl_3$, 500 MHz, δ ppm): 8.49 (s, 1H, FLT-NH), 7.99 (s, 1H, 3TC-C6), 7.18 (s, 1H, FLT H-6), 6.67−6.62 (m, 1H, FLT H-1′), 6.18 (s, 1H, 3TC H-1′), 6.08−6.0 (br m, 1H, 3TC H-4′), 5.31 (s, 1H, FLT H-4′), 5.20 (d, J = 57.7 Hz, 1H, FLT H-3′), 4.58−4.28 (m, 5H, 3TC H-5′, FLT H-5′ and Glu α −C*H*-COOFLT), 3.49 (br m, 1H, 3TC H-2′), 3.33 (br s, 1H, 3TC H-2″), 2.58−2.31 (br m, 3H, FLT H-2′, Glu γ-CH_2), 2.19−1.16 (br m, 2H, Glu β −CH_2CH$_2$-COOFTC), 1.94 (br s, 1H, FLT H-2″), 1.83 (s, 3H, FLT 5-CH_3), 1.53 (t, J = 7.2 Hz, 2H, −CH_2-CONH−), 1.22 (br s, 22H, methylene protons −(CH_2)$_{11}$), 0.85 (t, J = 8 Hz, 3H, −CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 174.96, 172.43 (COO3TC, COOFLT), 171.59 (CONH), 164.75, 163.65 (FLT C-4), 160.45 (3TC C-4), 150.66 (FLT C-2), 147.47 (3TC C-2), 143.51 (3TC C-6), 136.77 (FLT C-6), 114.45 (3TC C-5), 111.56 (FLT C-5), 94.73 (FLT C-3′), 87.28 (3TC C-1′), 86.72 (FLT C-1′), 84.94 (3TC C-4′), 82.30 (d, J = 25.0 Hz, FLT C-4′), 64.29 (FLT C-5′), 63.80 (3TC C-5′), 51.81 (Glu α NH-CH−), 37.23 (3TC C-2′), 36.32 (−CH_2−CONH−), 32.06 (FLT C-2′), 29.84, 29.82, 29.80, 29.66, 29.50, 29.40, 26.94, 25.82, 22.83 (methylene carbons, Glu β-CH_2 and Glu γ-CH_2), 14.27 (CH_3), 12.43 (FLT 5-CH_3). HR-MS (ESI-TOF) (m/z): $C_{37}H_{55}FN_6O_{10}S$, calcd 794.3680; found 795.1980 $[M + 1]^+$.

47: Overall yield 161 mg, 58%. ^{1}H NMR ($CDCl_3$ + CD_3OD, 500 MHz, δ ppm): 8.08 (d, J = 8.1 Hz, 1H, FTC H-6), 7.25 (s, 1H, FLT H-6), 6.75 (dd, J = 15.4 Hz, J = 7.7 Hz, 1H, FLT H-1′), 6.24 (br s, 1 H, FTC H-1′), 6.21−6.09 (m, 1H, FTC H-4′), 5.34 (t, J = 2.9 Hz, 1 H, FLT H-4′), 5.28−5.13 (m, 1H, FLT H-3′), 4.65−4.36 (m, 5H, FTC H-5′, FLT H-5′ and Glu α-C*H*-COOFLT), 3.55 (dd, J = 12 Hz, J = 5.2 Hz, 1H, FTC H-2′), 3.26−3.22 (m, 1H, FTC H-2″), 2.35−2.41 (br m, 6H, FLT H-2′, Glu γ-CH_2, myristate CH_2CH$_2$COO), 2.10−1.96 (m, 2H, Glu β −CH_2CH_2COOFTC), 1.86 (s, 3H, FLT 5-CH_3), 1.57 (t, J = 7.2 Hz, 2H, −CH_2CONH−), 1.22 (s, 20H, methylene protons −(CH_2)$_{10}$), 0.85 (t, J = 8.0 Hz, 3H, −CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 174.38, 172.41 (COOFTC, COOFLT), 171.79 (CONH), 164.22 (FLT C-4), 156.46 (FTC C-4), 150.56 (FLT C-2), 150.49 (FTC C-2), 136.36 (FLT C-6), 136.08 (FTC C-6), 126.85 (d, J = 33.0 Hz, FTC C-5), 111.66 (FLT C-5), 93.12 (d, J = 179.65 Hz, FLT C3′), 87.37 (FTC C-1′), 86.95 (FLT C-1′), 86.13 (FTC C-4′), 82.18 (d, J = 27.3 Hz, FLT C-4′), 64.21 (FLT C-5′), 63.39 (FTC C-5′), 51.59 (Glu NHCH−), 39.06 (FTC C2′), 36.41 (−CH_2CONH−), 32.03 (FLT C-2′), 29.94, 29.80, 29.76, 29.63, 29.47, 29.40, 25.71, 22.80 (methylene −CH_2−), 27.06 (Glu β −CH_2CCOOFTC), 14.23 (−CH_3), 12.49 (FLT 5-CH_3). HR-MS (ESI-TOF) (m/z): $C_{37}H_{54}F_2N_6O_{10}S$, calcd 812.3590; found 813.1642 $[M + 1]^+$.

Anti-HIV Assays. The anti-HIV activity of the compounds was evaluated according to the previously reported procedure[17,21−23] 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). Dextran sulfate, a polyanionic HIV entry inhibitor,[27,28] was used as assay control. 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 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. Pooling PBMCs from more than one donor is used to minimize the variability observed between individual donors, which results from quantitative and qualitative differences in HIV infection and overall response to the PHA and IL-2 of primary lymphocyte populations. 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). Because 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 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 (∼200*g*) 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.[26] 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.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

HPLC purification method, characterization of compounds, analytical HPLC methods, ^{1}H NMR, ^{13}C NMR, and/or analytical HPLC of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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; DMF, *N*,*N*-dimethylformamide; Fmoc, fluorenylmethyloxycarbonyl; FTC, (−)-5-fluoro-2′,3′-dideoxy-3′-thiacytidine; HAART, highly active antiretroviral therapy; HBTU, 1,1,3,3-tetramethyluronium hexafluorophosphate; HBV, hepatitis B virus; HOBt, hydroxybenzotriazole; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NMM, *N*-methylmorpholine; Mtt, 4-methyltrityl; NRTI, nucleoside reverse transcriptase inhibitors; 3TC, (−)-2′,3′-dideoxy-3′-thiacytidine; TFA, trifluoroacetic acid

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