Synthesis and Evaluation of Double-Prodrugs against HIV. Conjugation of D4T with 6-Benzyl-1-(ethoxymethyl)-5-isopropyluracil (MKC-442, Emivirine)-Type Reverse Transcriptase Inhibitors via the SATE Prodrug Approach

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This paper reports the synthesis and the antiviral activities of new double-prodrugs against HIV based on the known mixed SATE (*S*-acyl-2-thioethyl) prodrug approach. The monophosphate of the nucleoside reverse transcriptase inhibitor (NRTI) d4T was masked with one SATE group and one aromatic group through which a nonnucleoside reverse transcriptase inhibitor (NNRTI) was linked. Double-prodrug **1** was a hybrid between d4T monophosphate and the known NNRTI MKC-442, which were linked through a labile *p*-hydroxybenzoyl protection group in the N-3 position of MKC-442. Double-prodrugs **2** and **3** were conjugates between d4T monophosphate and the new NNRTIs **15** and **19** linked through a stable phenolic linker that was a part of the N-1 substituents of the NNRTIs. The double-prodrugs **1**, **2**, and **3** all had good activities against wild-type HIV-1, Y181C mutant, and also against a HIV-2 strain that was resistant to NNRTIs.

Introduction

One of the main targets for the inhibition of the human immunodeficiency virus (HIV) is the viral enzyme reverse transcriptase (RT). There are two major types of drugs against RT. The nucleoside reverse transcriptase inhibitors (NRTIs)[1] are phosphorylated inside the cell to give the corresponding triphosphates that act as substrate analogues toward RT and can be incorporated into the growing DNA chain. However, NRTIs do not contain the 3′-hydroxy group, which is essential for continuation of the DNA chain, and therefore they serve as chain terminators resulting in nonfunctional viral DNA. The nonnucleoside reverse transcriptase inhibitors (NNRTIs)[2] are allosteric inhibitors of HIV-1 RT. They bind to a hydrophobic pocket close to but distinct from the active site. Binding of the inhibitor alters the conformation of the active site and inactivates the enzyme. As this pocket is only present in HIV-1 RT, the inhibitors are very specific against HIV-1 and do not inhibit HIV-2 RT or cellular polymerases.

One disadvantage of the NRTIs is the need for triphosphorylation inside the cell. To circumvent this problem, a number of prodrugs of NRTIs have been developed.[3] In principle, the first phosphate is added chemically to the nucleoside, but as a molecule containing a charged phosphate cannot cross the cell membrane, the phosphate group is masked with different protecting groups. Ideally the protecting groups assist transportation into the cell followed by release of the nucleotide inside the cell. Two phosphorylation steps are then necessary before the molecule can be recognized as a substrate for RT.

The SATE (*S*-acyl-2-thioethyl) prodrugs were first introduced in 1993 by Imbach et al.[4] The SATE group was developed as a carboxyesterase labile protection group for the ddU (2′,3′-dideoxyuridine) nucleotide. The bis-SATE prodrugs, with two SATE groups masking the phosphate, have been synthesized for the NRTIs AZT (3′-azido-3′-deoxythymidine)[5] and d4T (3′-deoxy-2′,3′-didehydrothymidine).[6] Remarkable results were obtained especially for d4T, where the bis-SATE prodrug displayed nearly the same activity in CEM cells lacking thymidine-kinase (TK^- cells) as in normal CEM cells. Also, mixed SATE prodrugs have been synthesized with one SATE group and one aromatic moiety masking the phosphate. This prodrug of AZT was still active although less so as compared to the bis-SATE prodrug.[7] The activity of the d4T mixed SATE prodrug was reported to be in the submicromolar range, without stating any exact value.[8]

In our laboratories, we have for some time worked with analogues of the NNRTI MKC-442 (Figure 1).[9] One series of analogues contained unsaturated and aromatic moieties as part of the N-1 substituent (Figure 1) and displayed nanomolar activities against wild-type HIV-1.[10,11]

In this paper, we introduce the double prodrug concept based on the known "mixed SATE pronucleotides" incorporating a SATE chain and an aryl substituent as biolabile phosphate protection groups.[7] Such constructs have demonstrated their ability to deliver selectively inside infected cells the corresponding mononucleotide after esterase activation. D4T was chosen as the NRTI

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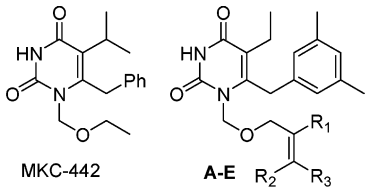


Compd	R_1	R_2	R_3
A	H	H	H
B	H	CH_3	CH_3
C	CH_3	H	H
D	H	H	Ph
E	CH_3	H	Ph

Figure 1. Structures of MKC-442 and analogues **A**−**E** having activities against HIV-1 (wt) in the nanomolar range.

as the first phosphorylation is the rate-limiting step for conversion of d4T to the corresponding triphosphate,[12] whereas d4T monophosphate (d4TMP) is easily converted to the di- and triphosphate.[13] Through the aromatic moiety of the mixed SATE prodrug of d4T, we link a NNRTI resulting in a conjugate that combines two types of RT inhibitors. These are usually given in combination treatments, and by combining them the NNRTI will assist the cellular uptake of the hydrophilic nucleotide and inside the cell the two fragments will be released to act at two different sites of RT. Furthermore, it is believed that cellular uptake of both compounds might prevent or reduce the building up of reservoirs with virus mutants.

The target molecule **1** (Figure 2) consists of MKC-442 linked to the double-prodrug through a labile *p*-hydroxybenzoyl group in the N-3 position. The target molecules **2** and **3** are linked through a more stable phenolic linker that is part of the N-1 substituent of new MKC-442 analogues **15** and **19**, which resemble the inhibitors previously reported (Figure 1).[10,11]

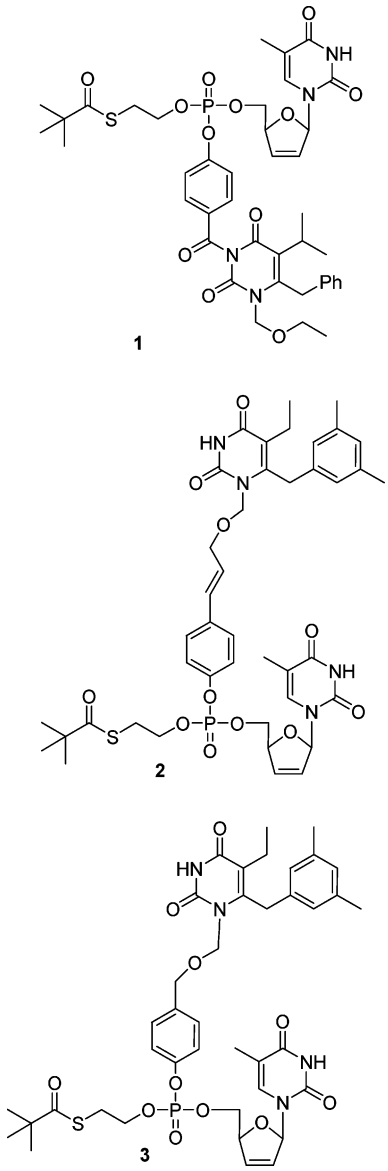


Figure 2. Structures of the target double-prodrugs **1**−**3** as conjugates of a NRTI (d4T) and a NNRTI (MKC-442; **15** or **19**).

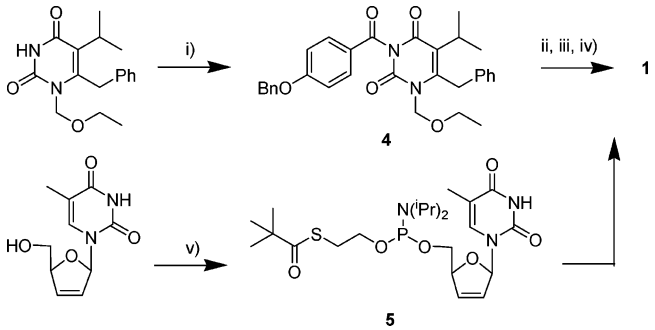
Results and Discussion

Chemistry. For the synthesis of target compound **1** (Figure 2), MKC-442 was protected in the N-3 position with a *p*-benzyloxybenzoyl group. The benzyl function could easily be removed at a later stage to liberate the phenolic function necessary for double-prodrug formation.

As such, *p*-benzyloxybenzoic acid was converted into the corresponding acid chloride using thionyl chloride and a catalytical amount of DMF[14] and reacted directly with MKC-442[15] in pyridine using diisopropyl ethylamine (DIPEA) as a base to give the benzyl protected product **4** in 86% yield (Scheme 1). Compound **4** was easily debenzylated using H_2 and Pd/C,[16] but TLC analysis showed decomposition of the compound upon standing. Compound **4** was therefore deprotected on the needed scale prior to every reaction.

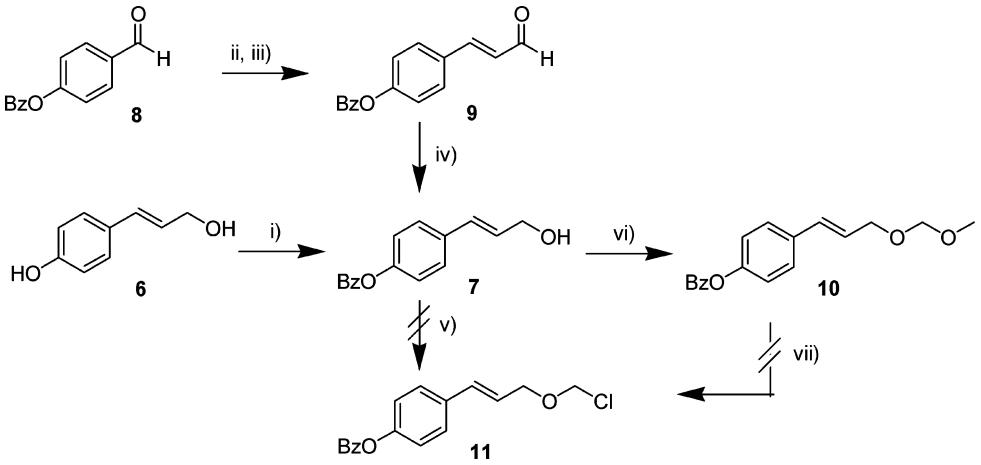
(*S*-Pivaloyl-2-thioethyl)-*N*,*N*-bis(diisopropylamino)-phosphine was prepared according to literature procedures,[17] noting that the compound was unstable to any trace of acid. It was therefore necessary to perform reactions, TLC analysis and column chromatography, using an excess of triethylamine (TEA) in all cases.[18] The bisamidite was successfully reacted with the debenzylated analogue of compound **4** using diisopropylamine (DIA) as a base and tetrazole as an activating

Scheme 1[a]



[a] (i) *p*-Benzyloxybenzoic acid chloride, DIPEA, pyridine, 86%; (ii) H_2, Pd/C; (iii) **5**, 1*H*-tetrazole, CH_3CN; (iv) *tert*-butylhydroperoxide, 38% (three steps); (v) (*S*-pivaloyl-2-thioethyl)-*N*,*N*-bis-(diisopropylamino)phosphine, DIA, 1*H*-tetrazole, CH_3CN, 94%.

Scheme 2[a]



[a] (i) Bz_2O, NaOH, H_2O, IPA, 58%; (ii) [1,3]dioxalan-2-ylmethyl-triphenyl-phosphonium bromide, TDA-1, K_2CO_3; (iii) HCl, H_2O, 68%; (iv) $NaBH_4$, KH_2PO_4, 78%; (v) CH_2O, HCl (g); (vi) $ClCH_2OCH_3$, DIPEA, CH_2Cl_2, 97%; (vii) BCl_3.

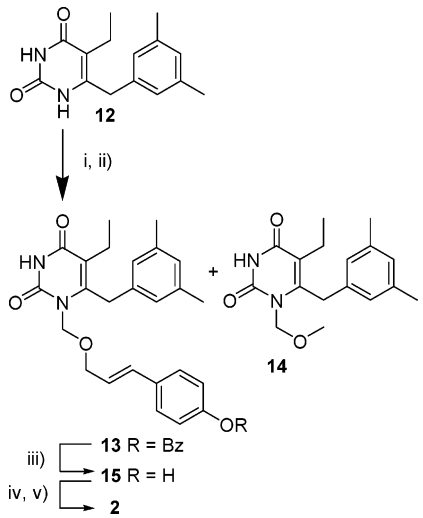
agent. Further reaction of the formed amidite with d4T[19] followed by in-situ oxidation by iodine gave a complex mixture according to TLC analysis, and the ^{31}P NMR spectra did not show the presence of the expected product. Instead, (*S*-pivaloyl-2-thioethyl)-*N*,*N*-bis(diisopropylamino)phosphine was reacted with d4T under the same conditions as above to give the amidite **5** in 94% yield. Compound **4** was deprotected using H_2 and Pd/C, and the product was reacted with the amidite **5** in CH_3CN using 1*H*-tetrazole as an activating agent, followed by oxidation with *tert*-butylhydroperoxide to give the target compound **1** as a mixture of two diastereomers in 38% overall yield for the three steps (Scheme 1).

Target compound **2** (Figure 2) was a double-prodrug between d4T and a new NNRTI resembling structure **D** depicted in Figure 1. The phenolic moiety, necessary for double-prodrug formation, was incorporated as a part of the linker in the N-1 position of the NNRTI. The benzoyl group was in this case chosen as the protection group for the phenolic functionality, as hydrogenolysis of a benzyl group would not be possible due to the presence of the double bond in the linker.

For the synthesis of the N-1 substituent, the alcohol **6** was synthesized according to literature procedures by ethyl esterification of *p*-hydroxycinnamic acid[20] followed by DIBAL reduction of the ester group.[21] Compound **6** was benzoylated using benzoic anhydride and NaOH in a mixture of H_2O and isopropyl alcohol (IPA) to give the monoprotected compound **7** in 58% yield.[22] Alternatively, compound **7** was synthesized from the aldehyde **8**,[23] which was subjected to the Wittig conditions described by Daubresse et al.[24] using (1,3-dioxolan-2-yl-methyl)-triphenylphosphonium bromide and TDA-1 (tris[2-(2-methoxyethoxy)ethyl]amine) as a catalyst. After hydrolysis of the dioxolane, the aldehyde **9** was obtained in 68% yield as the pure *trans*-isomer. Compound **9** was reduced with $NaBH_4$ and KH_2PO_4 to give the alcohol **7** in 78% yield.[24]

Attempts were made to synthesize the chloromethyl ether **11**, which would be the ideal compound for N-1 alkylation. However, reaction of alcohol **7** with formaldehyde and HCl(g), a procedure that has previously been used to convert allylic alcohols to the corresponding chloromethyl ethers,[25] only produced the product where

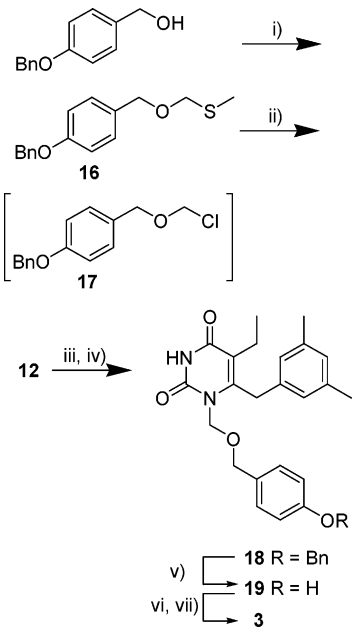
Scheme 3[a]



[a] (i) BSA, $CHCl_3$; (ii) **10**, TMS-triflate, 38%; (iii) NH_3/MeOH, 78%; (iv) **5**, 1*H*-tetrazole; (v) *tert*-butylhydroperoxide, 62%.

the hydroxyl group of compound **7** was replaced directly by a chloride. Goff et al.[26] obtained similar results when reacting cinnamylic alcohols with formaldehyde and HCl(g). Instead, they developed a new method for the synthesis of this type of chloromethyl ethers where the corresponding MOM-ether was cleaved with BCl_3 to give the cinnamyl chloromethyl ether. Indeed, the procedure for cinnamyl alcohol was reproduced to give the crude chloromethyl ether, which upon reaction with the silylated base **12** (see structure in Scheme 3) gave the desired N-1 cinnamyloxymethyl uracil in 45% yield. Encouraged by these results, the MOM-ether **10** was synthesized from alcohol **7** by reaction with chloromethylmethyl ether and DIPEA as the base to give compound **10** in 97% yield (Scheme 2). It should be noted that, due to decomposition of the starting material, NaH could not be used as the base for this reaction. Subjecting the MOM-ether **10** to BCl_3 followed by reaction with the silylated base did not produce the desired product. Only starting material **10** was reisolated, and again we observed the formation of the undesired chloride directly attached at the allylic position.

Scheme 4[a]



[a] (i) NaH, NaI, $ClCH_2SMe$, DME, 70%; (ii) SO_2Cl_2, CH_2Cl_2, −78 °C; (iii) BSA, $CHCl_3$; (iv) **17**, CsI, 78%; (v) H_2, $Pd(OH)_2$/C, 81%; (vi) (*S*-pivaloyl-2-thioethyl)-*N*,*N*-bis(diisopropylamino)phosphine, 1*H*-tetrazole; (vii) *tert*-butylhydroperoxide, 60%.

As we were not successful in the synthesis of compound **11**, we instead turned to the MOM-ether **10**, which in principle could function as a reactant for N-1 alkylation. 6-(3,5-Dimethylbenzyl)-5-ethyl-1*H*-pyrimidine-2,4-dione (**12**)[15] was silylated using BSA (bis(trimethylsilyl)acetamide) and reacted with compound **10** and trimethylsilyl trifluoromethanesulfonate[27] (TMS-triflate) (Scheme 3). A mixture of the desired compound **13** and the expected byproducts 6-(3,5-dimethylbenzyl)-5-ethyl-1-methoxymethyl-1*H*-pyrimidine-2,4-dione (**14**) and the alcohol **7** was formed, necessitating tedious column chromatography to separate the compounds. A pure fraction containing compound **13** was isolated as well as a mixed fraction of compound **13** and **14**. The total yield of compound **13** was estimated to be 38%. The benzoyl protection group was removed using NH_3/MeOH to give compound **15** in 78% yield. At this stage, compound **15** could easily be separated from the byproduct **14** by column chromatography. Compound **15** was reacted with the amidite **5** using 1*H*-tetrazole as an activating agent followed by oxidation with *tert*-butylhydroperoxide. The target compound **2** was isolated in 62% yield (Scheme 3).

Target compound **3** was a conjugate between d4T and a new NNRTI that also contained an aromatic moiety as part of the N-1 substituent. This could be used as a tool to incorporate the phenolic moiety necessary for double-prodrug formation. The structure of the NNRTI **19** (Scheme 4) resembled that of TNK-651 (6-benzyl-1-(benzyloxymethyl)-5-isopropyluracil) that has previously been reported to have activities similar to those of MKC-442.[28]

In this case, the benzyl group was a suitable protection group as there were no reducible functionalities present in the molecule. For the synthesis of the N-1 substituent, benzyloxybenzyl alcohol was reacted with chloromethyl methyl sulfide in the presence of NaH and NaI in dimethoxyethane (DME) to give the product **16** in 70% yield. The methyl sulfide group was then cleaved using sulfuryl chloride at −78 °C to give the chloromethylated compound **17**.[29] As this compound was assumed not to be stable, the cold solution was added directly to a solution of the silylated base **12**, which led to formation of the desired product **18** in 78% yield. The phenolic benzyl group was removed selectively by hydrogenolysis using $Pd(OH)_2$/C as a catalyst to give compound **19** in 81% yield. Compound **19** was reacted with (*S*-pivaloyl-2-thioethyl)-*N*,*N*-bis(diisopropylamino)-phosphine using 1*H*-tetrazole as an activating agent followed by *tert*-butylhydroperoxide oxidation to give the target compound **3** in 60% yield (Scheme 4).

Table 1. Antiviral Activity and Cytotoxicity Data of Novel Double-Prodrugs (Compounds **1**−**3**) and MKC-442 Analogues (Compounds **13**, **15**, **18**, **19**) in MT-4 Cells[a] (MKC-442 (6-Benzyl-1-ethoxymethyl-5-isopropyluracil) and d4T (3′-Deoxy-2′,3′-didehydrothymidine) Are Used for Comparison)

	EC_{50} (μM)[b] − MT-4 cells			
compound	IIIB[d]	N119[e]	HIV-2	CC_{50} (μM)[c]
d4T	0.5	1.8	0.5	>100
MKC-442	0.03	79	>10	>100
1	0.03	2.7	ND[f]	35
13	0.005	1	ND[f]	35
15	0.02	2.4	>10	35
2	0.04	3.3	ND[f]	35
18	0.03	0.3	ND[f]	12
19	0.003	0.5	>10	38
3	0.003	0.4	4	35

[a] See the Experimental Section for a description of the assay. [b] EC_{50}: Inhibitory concentration of compound required to achieve 50% inhibition of HIV multiplication in MT-4-infected cells. [c] CC_{50}: Cytotoxic concentration of compound required to reduce the viability of normal uninfected MT-4 cells by 50%. [d] Wild-type HIV-1 strain. [e] The strain N119 harbors the Y181C mutation that usually causes resistance toward this type of NNRTI. [f] Not determined.

Biological Results and Discussion

For the biological evaluation of the double-prodrugs, we set up a test system with three different HIV strains. Strain HTLV-IIIB was the wild-type HIV-1 normally used for our test experiments. The strain N119 was also HIV-1 but harbors the mutation Y181C in RT, a mutation that renders the virus resistant to the NNRTI MKC-442 and most analogues of this compound. However, the analogues **A**−**E** (Figure 1) actually displayed activities against this strain at submicromolar levels.[10,11] The last strain was a HIV-2 wild-type strain, which does not contain the hydrophobic pocket in RT that is the usual binding site for NNRTIs. This strain is therefore not affected by NNRTIs.

The initial tests were performed in MT-4 cells using the MTT method for quantification[30] (Table 1). In these tests, d4T showed the expected activities against all three strains. Also, MKC-442 showed the expected activities as the compound was active against wild-type HIV-1 (IIIB) and had no activity against the mutated strain N119 and HIV-2. The double-prodrug **1** was active against IIIB and also against N119. This activity must stem from the d4T part of the molecule; however, based on these results, it was not clear if the active component was d4T or d4T monophosphate.

The precursors **13** and **15** for double-prodrug **2** both had good activities against IIIB and were also active

Table 2. Antiviral Activity and Cytotoxicity Data of Novel Double-Prodrugs (Compounds **1**−**3**) and MKC-442 Analogues (Compounds **15**, **19**) in CEM Wild Type (wt) or Thymidine-Kinase-Deficient CEM (TK$^-$) Cells[a] (MKC-442 (6-Benzyl-1-ethoxymethyl-5-isopropyluracil) and d4T (3′-Deoxy-2′,3′-didehydrothymidine) Are Used for Comparison)

	EC_{50} (μM)[b] − CEM wt			EC_{50} (μM)[b] − CEM TK$^-$			CC_{50} (μM)[c]
compound	IIIB[d]	N119[e]	HIV-2	IIIB	N119	HIV-2	CEM wt/TK$^-$
d4T	0.3	0.8	0.1	>10	>10	>10	>100
MKC-442	0.03	>100	>10	0.03	>10	>10	>100
1	0.03	0.3	0.02	0.006	0.3	3	32
15	0.002	0.4	>10	0.006	0.2	>10	32
2	0.01	0.3	0.1	0.005	0.2	4	32
19	ND[f]	ND[f]	>10	ND[f]	ND[f]	>10	32
3	ND[f]	ND[f]	0.1	ND[f]	ND[f]	2	32

[a] See the Experimental Section for a description of the assay. [b] EC_{50}: Inhibitory concentration of compound required to achieve 50% inhibition of HIV multiplication in MT-4-infected cells. [c] CC_{50}: Cytotoxic concentration of compound required to reduce the viability of normal uninfected MT-4 cells by 50%. [d] Wild-type HIV-1 strain. [e] The strain N119 harbors theY181C mutation that usually causes resistance toward this type of NNRTI. [f] Not determined.

against the mutant N119. However, as expected, compound **15** showed no activity against HIV-2. The double-prodrug **2** was also active against both IIIB and N119. Based on these results, it was not possible to determine which part of the molecule induced the activity.

The precursors **18** and **19** for double-prodrug **3** displayed good activities against both IIIB and N119, whereas compound **19** was not active against HIV-2 as expected. Again, the double-prodrug **3** was active against IIIB and N119, but it was not possible based on these data to determine from which part of the molecule the activities stemmed. Interestingly, double-prodrug **3** had some activity against HIV-2, which must stem from either d4T or d4T monophosphate (Table 1).

As seen from examination of the results listed in Table 1, the novel compound **1** was more cytotoxic for MT-4 cells as d4T and MKC-442, respectively. This result is in accord with unpublished results from our group where an N-3 benzoylated derivative of MKC-442 was found more cytotoxic than MKC-442 even though it was hydrolyzed (within 10 h) to MKC-442 and benzoic acid in the medium used for biological testing (RPMI 1640) with $CC_{50} = 31$ μM.

To obtain more knowledge about the properties of the double-prodrugs, we extended the test system to include two different cell types. One cell type was wild-type CEM cells (CEM wt), whereas the other type (CEM TK$^-$) lacked thymidine-kinase (TK) and therefore the ability to carry out the first phosphorylation of d4T. Using this cell type made it possible to determine if d4T was released in the cell as d4T or as the desired d4T-monophosphate.

Indeed, when d4T was tested (using the ELISA method[31] for quantification, Table 2), the expected activity against all three strains in CEM wt cells was observed. When tested in (TK)-deficient CEM cells, all activity was lost (EC_{50} > 10 μM). MKC-442 was active against IIIB in both cell types and lost all activity against both N119 and HIV-2 in both cell types. Double-prodrug **1** was active against all three strains in CEM cells. Based on the results for d4T and MKC-442, the activity against N119 and HIV-2 must stem from the d4T part of the molecule. Interestingly, in CEM TK$^-$ cells, good to excellent activities were observed against all three strains (for HIV-2 the ED_{50}-values in three independent tests were 3-3-4 μM for compound **1**, 3-4-5 μM for compound **2**, and 3-2-2 μM for compound **3**). As d4T displayed no activity by itself (the ED_{50}-values in three independent tests were all above 10 μM), we could conclude that d4T-monophosphate was indeed released inside the cell (Table 2).

However, there were two possible routes for the degradation of double-prodrug **1** (Figure 3). One possibility was that MKC-442 was hydrolyzed off outside the cell giving a new type of prodrug **1a** and MKC-442 (route A). These two compounds were transported into the cell where prodrug **1a** was hydrolyzed to give d4T-monophosphate. The other possibility (route B) was transportation of the double-prodrug into the cell, followed by hydrolysis in the desired way, releasing d4T-monophosphate and MKC-442.

The stability of double-prodrug **1** was tested by incubating the compound with the media used for the biological tests and checking the presence of **1** by LC-MS analysis at certain time intervals. The results showed the presence of compound **1** at time 0 and after 20 min. After 40 min, the compound was hardly detected, and in the subsequent analysis, after 4 h the compound was not detected.

The results indicate that route A might be the major route for the breakdown of the double-prodrug **1**. However, we have no information on the time-scale for cellular uptake, which means that a combination of the two routes could also apply.

The precursor **15** for double-prodrug **2** showed excellent activities against IIIB and good activities against the mutant N119 in both cell lines. As expected, no activity was observed against HIV-2. For double-prodrug **2**, good activities were observed in CEM wt cells against all three virus types. The activity against HIV-2 must stem from the d4T part of the molecule. In (TK)-deficient CEM cells, good to excellent activities were observed against IIIB and N119 (Table 2). These activities could in principle stem from **15** that was separated from the double-prodrug outside the cell. However, some activity against HIV-2 was observed for double-prodrug **2**, indicating that d4T-monophosphate was indeed released inside the cell. This conclusion was supported by stability studies that showed the presence of double-prodrug **2** even after 6 days in the test media.

Similar results were obtained for double-prodrug **3** that was also present after 5 days in the media. Even though no activity of the precursor **19** was found against HIV-2, double-prodrug **3** was active in both cell lines, showing that d4T-monophosphate was indeed released inside the cell.

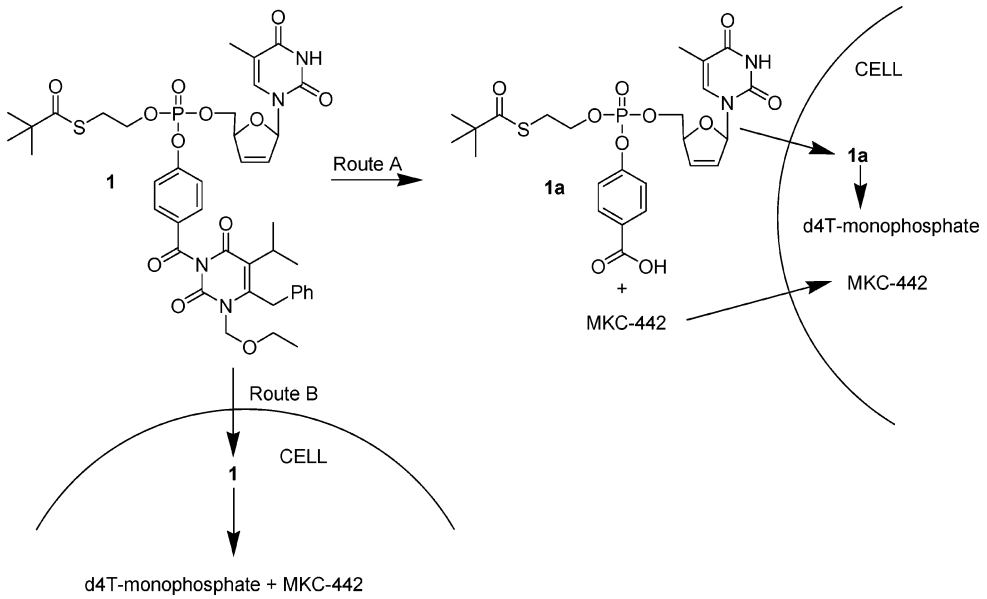


Figure 3. Possible routes for degradation of double-prodrug **1**. Route A: MKC-442 is hydrolyzed off outside the cell followed by transportation into the cell of MKC-442 and prodrug **1a**. Route B: Double-prodrug **1** is transported into the cell and then hydrolyzed into the active components.

Conclusions

In summary, double-prodrugs based on the SATE prodrug approach have been developed. Double-prodrug **1** was a hybrid between d4T and the known NNRTI MKC-442 linked through a cleavable *p*-hydroxybenzoyl protection group in the N-3 position of MKC-442. The double-prodrug had interesting activities against three virus types in both CEM wt and CEM TK^- cells, showing that d4T monophosphate was indeed released inside the cell. However, it was not possible to conclude if the double-prodrug entered the cell as the intact molecule or if it was hydrolyzed partly outside the cell to give the new prodrug **1a** and MKC-442, which were then individually transported into the cell.

Double-prodrugs **2** and **3** were hybrids between d4T and the new NNRTIs **15** and **19** linked through the phenol containing N-1 substituents of the NNRTIs. The precursors **15** and **19** both showed excellent activities against IIIB and even good activities against the mutant N119, results that correlated well with the activities previously reported for similar molecules.[10,11] Both double-prodrugs **2** and **3** were active against HIV-2 in TK-deficient CEM cells, proving release of d4T monophosphate inside the cell. As the possible hydrolysis pathway of this type of double-prodrug was simple as compared to double-prodrug **1** and stability studies proved the compounds to be stable in the test media for a minimum of 5 days, we conclude that the double-prodrugs were transported into the cells and hydrolyzed in the desired way to give d4T monophosphate and the NNRTIs **15** or **19**.

Experimental Section

NMR spectra were recorded on a Varian Gemini 2000 NMR spectrometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C with tetramethylsilane as an internal standard. ^{31}P NMR spectra were recorded at 121.5 MHz using 85% H_3PO_4 as an external standard. Chemical shifts are reported in parts per million (δ), and signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). MALDI mass spectra were recorded on a 4.7 T Ultima (IonSpec, Irvine, CA) Fourier transform ion cyclotron resonance (FTICR) mass spectrometer. LC-HRMS analysis was performed on a Waters 2795 system with a Waters 996 photodiode array (PDA) detector using a Waters Symmetry C_{18} Column (2.1 × 50 mm, 3.5 μm) coupled with a Micromass LCT apparatus with an AP-ESI probe.using Leu-Enkephalin (556.2771 g/mol) for calibration (0−95% ACN, 10 min, 0.2 mL/min). Melting points were determined on a Büchi melting point apparatus. Elemental analysis were performed at Mikroanalytisk afdeling, University of Copenhagen, Copenhagen, Denmark; found values agreed favorably with the calculated ones. The progress of reactions was monitored via TLC (analytical silica gel plates 60 F_{254}). Merck silica gel (0.040−0.063 mm) was used for column chromatography. Solvents for chromatography were bought as HPLC grade or distilled prior to use. Reactions were in general carried out under a N_2 or Ar atmosphere. Pyridine was dried over KOH. CH_3CN was dried over 3 Å sieves. CH_2Cl_2 was dried over 4 Å sieves.

6-Benzyl-3-(4-benzyloxy-benzoyl)-1-ethoxymethyl-5-isopropyl-1*H*-pyrimidine-2,4-dione (4). 4-Benzyloxy-benzoic acid (2.01 g, 8.8 mmol) was dissolved in $SOCl_2$ (30 mL), and a few drops of DMF were added. The mixture was refluxed overnight. After the mixture was cooled to room temperature, the solvent was evaporated under reduced pressure and the crude 4-benzyloxy-benzoyl chloride was used without further purification.

DIPEA (1.55 g, 12 mmol) was dissolved in dry pyridine (50 mL). 6-Benzyl-1-ethoxymethyl-5-isopropyl-1*H*-pyrimidine-2,4-dione (MKC-442, 1.21 g, 4 mmol) was added, the solution was cooled to 0 °C, and the crude 4-benzyloxy-benzoyl chloride was added. The solution was stirred at room temperature overnight, and then the reaction was quenched with H_2O (5 mL) and the solvent was evaporated under reduced pressure. The residue was redissolved in CH_2Cl_2 (200 mL). The organic phase was washed with H_2O (2 × 100 mL), dried ($MgSO_4$), and evaporated under reduced pressure. The product was purified by silica column chromatography (50 → 70% CH_2Cl_2 in cyclohexane). Yield: 1.77 g (86%), white foam; R_f = 0.21 (CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 1.18 (3H, t, J = 7.0 Hz, CH_2CH_3), 1.29 (6H, d, J = 6.7 Hz, $CH(CH_3)_2$), 2.90 (1H, heptet, J = 6.9 Hz, $CH(CH_3)_2$), 3.62 (2H, q, J = 7.0 Hz, CH_2CH_3), 4.24 (2H, s, CH_2Ph), 5.15 (4H, s, $PhCH_2O$, NCH_2O), 7.04 (2H, d, J = 9.0 Hz, H-aryl), 7.15−7.43 (10H, m, H-aryl), 7.90 (2H, d, J = 9.0 Hz, H-aryl). ^{13}C NMR ($CDCl_3$): δ 15.01 (CH_2CH_3), 20.37 ($CH(CH_3)_2$), 28.54 ($CH(CH_3)_2$), 33.59 (CH_2Ph), 65.31 (OCH_2-

CH_3), 70.30, 73.15 (NCH_2O, OCH_2Ph), 115.38, 119.61, 124.54, 127.25, 127.34, 127.39, 128.31, 128.71, 129.28, 132.89, 135.17, 135.80, 148.43, 150.81, 161.33, 164.20, 167.91 (C-aryl, CO). MS (MALDI, *m/z*) = 535 ($M + Na^+$).

2,2-Dimethyl-thiopropionic Acid *S*-(2-{Diisopropylamino-[5-(5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-2,5-dihydro-furan-2-ylmethoxy]-phosphanyloxy}-ethyl) Ester (5). 1-(5-Hydroxymethyl-2,5-dihydro-furan-2-yl)-5-methyl-1*H*-pyrimidine-2,4-dione (d4T, 0.15 g, 0.67 mmol) was dissolved in dry CH_3CN (5 mL) containing 3 Å activated sieves (0.6 g). The solution was cooled to 0 °C, and a solution of (*S*-pivaloyl-2-thioethyl)-*N*,*N*-bis(diisopropylamino)phosphine (0.40 g, 1.02 mmol) and DIA (0.14 g, 1.4 mmol) in dry CH_3CN (3 mL) was added. A solution of 1*H*-tetrazole (3.0 mL, 0.45 M in CH_3CN, 1.4 mmol) was then added. The mixture was stirred for 2 h and then diluted with acid-free EtOAc (20 mL). The organic phase was washed with brine (2 × 20 mL) and H_2O (2 × 20 mL), dried ($MgSO_4$), and evaporated under reduced pressure. The product was purified by silica column chromatography (cyclohexane/EtOAc/TEA, 50:49:1), yielding the title compound (0.33 g, 94%) as a clear glace; $R_f = 0.56$ (1% TEA/EtOAc, prerun). ^{31}P NMR ($CDCl_3$/pyridine-d_5/H_3PO_4 external): δ 147.99, 149.85. MS (MALDI, *m/z*) = 522 ($M + Li^+$).

2,2-Dimethyl-thiopropionic Acid *S*-(2-{[4-(4-Benzyl-3-ethoxymethyl-5-isopropyl-2,6-dioxo-3,6-dihydro-2*H*-pyrimidine-1-carbonyl)-phenoxy]-[5-(5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-2,5-dihydro-furan-2-ylmethoxy]-phosphoryloxy}-ethyl) Ester (1). 6-Benzyl-3-(4-benzyloxy-benzoyl)-1-ethoxymethyl-5-isopropyl-1*H*-pyrimidine-2,4-dione (4, 0.22 g, 0.43 mmol) and Pd−C (10%, 0.046 g, 0.043 mmol Pd) were suspended in MeOH (25 mL). The atmosphere was changed for N_2 and then H_2. The suspension was stirred 1.5 h at room temperature and then filtered through Celite. The solvent was evaporated under reduced pressure, and the crude compound was dried in vacuo. To the dried compound was added 3 Å activated sieves (0.5 g) and dry CH_3CN (4 mL). A solution of 1*H*-tetrazole (1.9 mL, 0.45 M in CH_3CN, 0.86 mmol) was added and then dropwise a solution of the amidite (5, 0.34 g, 0.66 mmol) in dry CH_3CN (4 mL). The mixture was stirred for 2 h at room temperature followed by addition of a solution of *tert*-butyl hydroperoxide (0.15 mL, 5.7 M in decane (anhydrous, over 4 Å sieves), 0.86 mmol). The mixture was stirred another 1 h at room temperature and then diluted with CH_2Cl_2 (20 mL). The organic phase was washed with 10% aqueous $Na_2S_2O_3$ (2 × 20 mL) and H_2O (2 × 20 mL), dried ($MgSO_4$), and evaporated under reduced pressure. The product was purified by silica column chromatography (0 → 2% EtOH in CH_2Cl_2), yielding the title compound (0.14 g, 38%) as a light yellow foam; R_f = 0.34 (EtOH/CH_2Cl_2, 5:95). ^1H NMR ($CDCl_3$): δ 1.15−1.32 (18H, m, OCH_2CH_3, $CH(CH_3)_2$, $C(CH_3)_3$), 1.85, 1.89 (3H, 2 × d, J = 1.2 Hz, CH_3-d4T), 2.91 (1H, heptet, J = 7.0 Hz, $CH(CH_3)_2$), 3.10−3.20 (2H, m, SCH_2), 3.60−3.69 (2H, m, OCH_2CH_3), 4.16−4.48 (6H, m, SCH_2CH_2, CH_2Ph, $POCH_2$), 5.00−5.06 (1H, m, CH_2CHO), 5.10−5.22 (2H, m, NCH_2O), 5.88 (0.5H, dt, J = 1.9 Hz, 5.6 Hz, CH=CHCHN), 5.93 (0.5H, dt, J = 1.9 Hz, 6.0 Hz, CH=CHCHN), 6.28−6.35 (1H, m, CH_2CHCH=CH), 6.91−6.95 (0.5H, m, OCHN), 6.98−7.01 (0.5H, m, OCHN), 7.10−7.43, 7.91−7.98 (10H, 2 × m, H-aryl, H-6), 8.48 (0.5H, s, NH), 8.69 (0.5H, s, NH). ^{31}P NMR ($CDCl_3$/H_3PO_4 external): δ −7.08, −6.12. MS (MALDI, *m/z*) calcd 875.2698 ($M + Na^+$), found 875.2734 ($M + Na^+$).

Benzoic Acid 4-*trans*-(3-Hydroxy-propenyl)-phenyl Ester (7) from *trans*-4-(3-Hydroxy-propenyl)-phenol (6). *trans*-4-(3-Hydroxy-propenyl)-phenol (**6**, 0.11 g, 0.73 mmol) was dissolved in isopropyl alcohol (IPA, 5 mL), containing NaOH (0.080 g, 2 mmol) dissolved in H_2O (1 mL). Benzoic anhydride (0.45 g, 2 mmol) was added, and the solution was stirred at room temperature for 0.5 h and then the solvent was evaporated under reduced pressure. The residue was partitioned between EtOAc (25 mL) and H_2O (25 mL). The organic phase was washed with brine (3 × 25 mL), dried ($MgSO_4$), and evaporated under reduced pressure. The product was purified by silica column chromatography (0 → 2% EtOH in CH_2Cl_2), yielding the title compound (0.11 g, 58%) as white needles; mp 125−127 °C (EtOAc/PE (60−80 °C)); R_f = 0.38 (EtOH/CH_2Cl_2, 5:95). ^1H NMR ($CDCl_3$): δ 1.67 (1H, brs, OH), 4.33 (2H, d, J = 5.1 Hz, CH_2), 6.34 (1H, td, J = 5.6 Hz, 16 Hz, $CHCH_2$), 6.63 (1H, d, J = 15.9 Hz, CH-aryl), 7.15−7.21 (2H, m, H-aryl), 7.40−7.55 (2H, m, H-aryl), 7.60−7.67 (1H, m, H-aryl), 8.17−0.22 (2H, m, H-aryl). ^{13}C NMR ($CDCl_3$): δ 63.58 (CH_2), 121.81, 127.44, 128.55, 128.79, 129.43, 130.04, 130.14, 133.60, 134.53, 150.34 (C-aryl, CH=CH), 165.13 (CO). MS (MALDI, *m/z*) calcd 277.0835 ($M + Na^+$), found 277.0837 ($M + Na^+$).

Benzoic Acid *trans*-4-(3-Oxo-propenyl)-phenyl Ester (9). TDA-1 (1.06 g, 4.67 mmol) was dissolved in a two-phase system consisting of a saturated aqueous solution of K_2CO_3 (30 mL) and CH_2Cl_2 (30 mL). The solution was stirred vigorously, and [1,3]dioxalan-2-ylmethyl-triphenyl-phosphonium bromide (2.91 g, 6.78 mmol) and benzoic acid 4-formyl-phenyl ester (**8**, 1.02 g, 4.52 mmol) were added. The mixture was stirred at room temperature overnight. The phases were then separated, and the aqueous phase was extracted with CH_2Cl_2 (2 × 30 mL). HCl (10% aqueous solution, 30 mL) was added to the combined organic fractions, and this was stirred overnight at room temperature. The aqueous layer was diluted with H_2O (30 mL), and the phases were separated and the aqueous phase extracted with CH_2Cl_2 (2 × 30 mL). The combined organic layers were washed with saturated aqueous solution of $NaHCO_3$ (2 × 30 mL) and brine (2 × 30 mL), dried ($MgSO_4$), and evaporated under reduced pressure. The product was purified by silica column chromatography (CH_2Cl_2), yielding the title compound (0.78 g, 68%) as white needles; mp 103−105 °C (EtOAc/PE (60−80 °C)); R_f = 0.49 (EtOAc/CH_2Cl_2, 1:9). ^1H NMR ($CDCl_3$): δ 6.71 (1H, dd, J = 7.8 Hz, 15.9 Hz, $CHCHO$), 7.29−7.34, 7.50−7.56, 7.63−7.70, 8.18−8.23 (10H, m, H-aryl, CH-aryl), 9.72 (1H, d, J = 7.4 Hz, CHO). ^{13}C NMR ($CDCl_3$): δ 122.56, 128.66, 129.07, 129.74, 130.23, 131.76, 133.90, 151.52, 153.14 (C-aryl, CH=CH), 164.76 (COO), 193.49 (CHO). MS (MALDI, *m/z*) = 253 ($M + H^+$).

Benzoic Acid *trans*-4-(3-Hydroxy-propenyl)-phenyl Ester (7) from Benzoic Acid *trans*-4-(3-Oxo-propenyl)-phenyl Ester (9). Benzoic acid *trans*-4-(3-oxo-propenyl)-phenyl ester (**9**, 2.19 g, 8.68 mmol) and KH_2PO_4 (11.81 g, 86.80 mmol) were dissolved in MeOH (140 mL) and cooled to 0 °C. $NaBH_4$ (0.82 g, 21.70 mmol) was added in portions. The mixture was stirred at 0 °C for 1 h, and then the reaction was quenched by careful addition of H_2O (50 mL). The reaction mixture was acidified to pH = 3 with aqueous solution of HCl (4 M). The MeOH phase was evaporated under reduced pressure, and the remaining aqueous phase was extracted with CH_2Cl_2 (3 × 100 mL). The organic phase was washed with H_2O (2 × 100 mL), dried ($MgSO_4$), and evaporated under reduced pressure. The product was purified by silica column chromatography (2 → 3% EtOH in CH_2Cl_2), yielding the title compound (1.73 g, 78%); data were consistent with those reported above.

Benzoic Acid *trans*-4-(3-Methoxymethoxy-propenyl)-phenyl Ester (10). Benzoic acid *trans*-4-(3-hydroxy-propenyl)-phenyl ester (**7**, 0.25 g, 1 mmol) was dissolved in dry CH_2Cl_2 (5 mL). The solution was cooled to 0 °C, and DIPEA (0.23 g, 1.8 mmol) and chloro-methoxy-methane (0.12 g, 1.5 mmol) were added. The solution was stirred overnight at room temperature. The solvent was evaporated, and the product was purified by silica column chromatography (cyclohexane/EtOAc, 3:1), yielding the title compound (0.29 g, 97%) as a white solid; mp 74−75 °C (cyclohexane); R_f = 0.53 (cyclohexane/EtOAc, 1:1). ^1H NMR ($CDCl_3$): δ 3.41 (3H, s, CH_3), 4.24 (2H, d, J = 6.2 Hz, $CHCH_2$), 4.71 (2H, s, OCH_2O), 6.28 (1H, td, J = 6.1 Hz, 16.0 Hz, $CHCH_2$), 6.65 (1H, d, J = 15.9 Hz, CH-aryl), 7.18 (2H, d, J = 8.4 Hz, H-aryl), 7.41−7.68 (5H, m, H-aryl), 8.20 (2H, d, J = 7.5 Hz, H-aryl). ^{13}C NMR ($CDCl_3$): δ 55.33 (CH_3), 67.74 ($CHCH_2$), 95.65 (OCH_2O), 121.80, 125.87, 127.48, 128.56, 129.48, 130.15, 131.55, 133.59, 134.53, 150.39 (C-aryl, CH=CH), 165.10 (CO). MS (MALDI, *m/z*) calcd 321.1097 ($M + Na^+$), found 321.1090 ($M + Na^+$).

Benzoic Acid *trans*-4-{3-[6-(3,5-Dimethyl-benzyl)-5-ethyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-ylmethoxy]-

propenyl}-phenyl Ester (13). *N*,*O*-Bis(trimethylsilyl)acetamide (BSA, 1.70 g, 8.38 mmol) was dissolved in dry CH_3CN (25 mL). 6-(3,5-Dimethylbenzyl)-5-ethyl-1*H*-pyrimidine-2,4-dione (12, 0.87 g, 3.35 mmol) was added, and the mixture was stirred 10 min until a clear solution was obtained. The solution was cooled to −45 °C,and TMS triflate (0.82 g, 3.69 mmol) was added followed by addition of benzoic acid 4-(3-methoxymethoxypropenyl)-phenyl ester (10, 2.00 g, 6.70 mmol) dissolved in dry CH_3CN (10 mL). The mixture was stirred at room temperature overnight, and then the reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (25 mL). The solvent was evaporated under reduced pressure, and the residue was dissolved in CH_2Cl_2 (150 mL). The organic phase was washed with H_2O (100 mL), brine (100 mL), and H_2O (100 mL), dried ($MgSO_4$), and evaporated under reduced pressure. The product was partly purified by silica column chromatography first giving 0.36 g of the desired product 13 (cyclohexane/EtOAc, 1:1) and then purification of mixed fractions yielding 0.71 g of a mixture between 13 and the byproduct 6-(3,5-dimethylbenzyl)-5-ethyl-1-methoxymethyl-1*H*-pyrimidine-2,4-dione 14 (chromatographed on silica gel with cyclohexane/acetone, 3:1). From the ^{1}H NMR spectra, it was determined that the desired product 13 constituted 0.31 g of this mixture. Total yield: 0.67 g (38%) as white foam (pure fraction); R_f = 0.38 (cyclohexane/EtOAc, 1:1). ^{1}H NMR ($CDCl_3$): δ 1.08 (3H, t, J = 7.4 Hz, CH_2CH_3), 2.27 (6H, s, 2 × CH_3-aryl), 2.48 (2H, q, J = 7.5 Hz, CH_2CH_3), 4.11 (2H, s, CH_2-aryl), 4.29 (2H, d, J = 6.2 Hz, OCH_2CH), 5.20 (2H, s, NCH_2O), 6.21 (1H, td, J = 6.2 Hz, 15.9 Hz, $CHCH_2$), 6.65 (1H, d, J = 16.0 Hz, CH-aryl), 6.71 (2H, s, H-aryl), 6.90 (1H, s, H-aryl), 7.15−7.21 (2H, m, H-aryl), 7.40−7.67 (5H, m, H-aryl), 8.17−8.23 (2H, m, H-aryl), 9.25 (1H, s, NH). ^{13}C NMR ($CDCl_3$): δ 13.76 (CH_2CH_3), 19.20 (CH_2CH_3), 21.27 (2 × CH_3-aryl), 33.36 (CH_2-aryl), 70.14 (OCH_2CH), 72.49, (NCH_2O), 116.87 (C-5), 121.83, 124.94, 124.99, 127.56, 128.55, 128.99, 129.44, 130.14, 132.38, 133.59, 134.21, 134.86, 138.90, 149.30, 150.54, 151.90 (C-aryl, CH=CH, C-2, C-6), 163.20, 165.05 (CO, C-4). MS (MALDI, *m/z*) calcd 547.2203 ($M + Na^+$), found 547.2208 ($M + Na^+$).

6-(3,5-Dimethylbenzyl)-5-ethyl-1-[*trans*-3-(4-hydroxyphenyl)-allyloxymethyl]-1*H*-pyrimidine-2,4-dione (15). Benzoic acid *trans*-4-{3-[6-(3,5-dimethylbenzyl)-5-ethyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-ylmethoxy]-propenyl}-phenyl ester (**13**, 0.32 g, 0.61 mmol) was dissolved in a saturated solution of NH_3 in MeOH (10 mL). The solution was stirred overnight at room temperature, and then the solvent was evaporated under reduced pressure. The residue was redissolved in CH_2Cl_2 (50 mL), and the organic phase was washed with H_2O (25 mL), dried ($MgSO_4$), and evaporated under reduced pressure. The product was purified by silica column chromatography (EtOH/CH_2Cl_2, 2:98), yielding the title compound (0.20 g, 78%) as white foam; R_f = 0.24 (EtOH/CH_2Cl_2, 5:95). ^{1}H NMR ($CDCl_3$): δ 1.06 (3H, t, J = 7.4 Hz, CH_2CH_3), 2.26 (6H, s, 2 × CH_3-aryl), 2.46 (2H, q, J = 7.3 Hz, CH_2CH_3), 4.10 (2H, s, CH_2-aryl), 4.24 (2H, d, J = 6.2 Hz, OCH_2CH), 5.18 (2H, s, NCH_2O), 6.05 (1H, td, J = 6.4 Hz, 15.8 Hz, $CHCH_2$), 6.53 (1H, d, J = 15.6 Hz, CH-aryl), 6.70 (2H, s, H-aryl), 6.79 (2H, d, J = 8.9 Hz, H-aryl), 6.89 (1H, s, H-aryl), 7.22 (2H, d, J = 8.6 Hz, H-aryl), 9.23 (1H, s, NH). ^{13}C NMR ($CDCl_3$): δ 13.74 (CH_2CH_3), 19.19 (CH_2CH_3), 21.25 (2 × CH_3-aryl), 33.35 (CH_2-aryl), 70.53 (OCH_2CH), 72.47 (NCH_2O), 115.56 (C-aryl), 116.86 (C-5), 122.00 (C-aryl), 125.01 (CH-aryl), 127.36, 127.95, 128.86, 133.31, 134.83 (C-aryl), 138.91 (CH_2CH), 149.59 (C-2), 151.91, 155.96 (C-aryl, C-6), 163.34 (C-4). MS (MALDI, *m/z*) = 443 ($M + Na^+$).

2,2-Dimethyl-thiopropionic Acid *S*-(2-*trans*-{[4-{3-[6-(3,5-Dimethylbenzyl)-5-ethyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-ylmethoxy]-propenyl}-phenoxy]-[5-(5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-2,5-dihydro-furan-2-ylmethoxy]-phosphoryloxy}-ethyl) Ester (2). 6-(3,5-Dimethylbenzyl)-5-ethyl-1-[*trans*-3-(4-hydroxy-phenyl)-allyloxymethyl]-1*H*-pyrimidine-2,4-dione (15, 0.100 g, 0.24 mmol) was dissolved in dry CH_3CN (2.5 mL) in a flame-dried bottle containing 3 Å molecular sieves (0.25 g). 1*H*-Tetrazole solution (1.06 mL, 0.45 M in CH_3CN, 0.48 mmol) was added, and then dropwise a solution of the amidite (5, 0.184 g, 0.36 mmol) in dry CH_3CN (2.5 mL) was added. The mixture was stirred at room temperature for 2.5 h followed by addition of a solution of *tert*-butyl hydroperoxide (0.084 mL, 5.7 M in decane (anhydrous, over 4 Å sieves), 0.48 mmol). The mixture was stirred 1 h and then diluted with CH_2Cl_2 (25 mL). The organic phase was washed with a saturated aqueous solution of $Na_2S_2O_3$ (2 × 20 mL) and H_2O (2 × 20 mL), dried ($MgSO_4$), and evaporated under reduced pressure. The product was purified by silica column chromatography (EtOH/CH_2Cl_2, 3:97), yielding the title compound (0.126 g, 62%) as white foam, R_f = 0.21 (EtOH/CH_2Cl_2, 5:95). ^{1}H NMR ($CDCl_3$): δ 1.04−1.12 (3H, m, CH_2CH_3), 1.22, 1.23 (9H, 2 × s, $C(CH_3)_3$), 1.81 (3H, s, CH_3–d4T), 2.27 (6H, s, 2 × $ArCH_3$), 2.40−2.55 (2H, m, CH_2CH_3), 3.10−3.20 (2H, m, SCH_2), 4.00−4.47 (8H, m, OCH_2-CH, $POCH_2$, CH_2-aryl, SCH_2CH_2), 4.98−5.45 (3H, m, CH_2CHO, NCH_2O), 5.82 (1H, m, CH=C*H*CHN), 6.08−6.20 (1H, m, CH_2CH=CH), 6.27−6.34 (1H, m, C*H*=CHCHN), 6.54, 6.56 (1H, 2 × d, J = 15.9 Hz, CH_2CH=C*H*), 6.71 (2H, s, H-aryl), 6.89 (1H, s, H-aryl), 6.97−7.32 (6H, m, OCHN, 4 × H-aryl, H-6 (d4T)), 9.14, 9.26, 9.41, 9.44 (2H, 4 × s, 2 × NH). ^{31}P NMR ($CDCl_3$/H_3PO_4 external): δ −6.26, −5.36. MS (MALDI, *m/z*) = 873 ($M + Na^+$).

1-Benzyloxy-4-methylsulfanylmethoxymethyl-benzene (16). NaH (0.80 g, 60% suspension in mineral oil, 20 mmol) was suspended in dimethoxyethane (DME, 10 mL). The suspension was cooled to 0 °C, and 4-benzyloxybenzyl alcohol (2.14 g, 10 mmol) was added, followed by addition of NaI (1.50 g, 10 mmol) and chloromethyl methyl sulfide (0.97 g, 10 mmol). The reaction was stirred 1.5 h at 0 °C and then 4 h at room temperature. The reaction was quenched by addition of H_2O (20 mL), and the aqueous phase was extracted with Et_2O (3 × 50 mL). The organic phase was dried (K_2CO_3) and evaporated. The product was purified by silica column chromatography (10 → 20% Et_2O in PE (60−80 °C)), yielding the title compound (1.91 g, 70%) as a clear oil; R_f = 0.32 (20% Et_2O/PE (60−80 °C)). ^{1}H NMR ($CDCl_3$): δ 2.17 (3H, s, SCH_3), 4.55 (2H, s, OCH_2S), 4.66 (2H, 2, aryl-CH_2O), 5.06 (2H, s, aryl-CH_2O-aryl), 6.92−6.98, 7.24−7.45 (9H, m, H-aryl). ^{13}C NMR ($CDCl_3$): δ 13.87 (CH_3S), 69.01 (aryl-CH_2O-aryl), 69.99 (aryl-CH_2O), 74.07 (OCH_2S), 114.82, 127.40, 127.93, 128.55, 129.78, 136.92, 158.50 (C-aryl). MS (MALDI, *m/z*) = 297 ($M + Na^+$).

1-(4-Benzyloxy-benzyloxymethyl)-6-(3,5-dimethylbenzyl)-5-ethyl-1*H*-pyrimidine-2,4-dione (18). 1-Benzyloxy-4-methylsulfanylmethoxymethyl-benzene (**16**, 0.127 g, 0.46 mmol) was dissolved in dry CH_2Cl_2 (2 mL) and cooled to −78 °C. Sulfuryl chloride (0.062 g, 0.46 mmol) was dissolved in CH_2Cl_2 (2 mL) and added dropwise to the cold solution. The solution was stirred 30 min at −78 °C. BSA (0.157 g, 0.77 mmol) was dissolved in dry $CHCl_3$ (2 mL), and 6-(3,5-dimethylbenzyl)-5-ethyl-1*H*-pyrimidine-2,4-dione (**12**, 0.080 g, 0.31 mmol) was added and the mixture was stirred 10 min to give a clear solution. CsI (0.081 g, 0.31 mmol) was added, and then the cold solution from above was transferred. The reaction mixture was stirred at room temperature overnight. The reaction was quenched by the addition of a saturated aqueous solution of $NaHCO_3$ (20 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 20 mL), and the combined organic phases were dried ($MgSO_4$) and evaporated under reduced pressure. The product was purified by silica column chromatography (EtOAc/PE (60−80 °C), 2:1), yielding the title compound (0.117 g, 78%) as a white foam; R_f = 0.36 (EtOAc/PE (60−80 °C), 1:1). ^{1}H NMR ($CDCl_3$): δ 1.07 (3H, t, J = 7.3 Hz, CH_2CH_3), 2.26 (6H, s, 2 × CH_3), 2.46 (2H, q, J = 7.3 Hz, CH_2CH_3), 4.05 (2H, s, aryl-CH_2-aryl), 4.58 (2H, s, OCH_2-aryl), 5.06 (2H, s, OCH_2-aryl), 5.17 (2H, s, NCH_2O), 6.63 (2H, s, H-aryl), 6.88 (1H, s, H-aryl), 6.95 (2H, d, J = 8.0 Hz, H-aryl), 7.22−7.46 (7H, m, H-aryl), 9.18 (1H, s, NH). ^{13}C NMR ($CDCl_3$): δ 13.74 (CH_2CH_3), 19.14 (CH_2CH_3), 21.24 (2 × CH_3), 33.21 (CH_2-aryl), 70.00 (OCH_2-aryl), 71.28 (OCH_2-aryl), 72.53 (NCH_2O), 114.79 (C-aryl), 116.72 (C-5), 124.97, 127.41, 127.94, 128.55, 128.91, 129.47, 129.65, 134.83, 136.83, 138.81 (C-aryl), 149.29, 151.84 (C-2, C-6), 158.64 (C-aryl), 163.18 (C4). MS (MALDI, *m/z*) = 507 ($M + Na^+$).

6-(3,5-Dimethyl-benzyl)-5-ethyl-1-(4-hydroxy-benzyloxymethyl)-1*H*-pyrimidine-2,4-dione (19). 1-(4-Benzyloxybenzyloxymethyl)-6-(3,5-dimethylbenzyl)-5-ethyl-1*H*-pyrimidine-2,4-dione (**18**, 1.00 g, 2.06 mmol) and catalyst (0.15 g, 20 wt % Pd (dry basis) on carbon, wet) were suspended in CH_2Cl_2/MeOH (50 mL, 1:1). The atmosphere was exchanged for H_2, and the mixture was stirred 1.5 h at room temperature. Another portion of catalyst was added (0.07 g, 20 wt % Pd (dry basis) on carbon, wet), and the mixture was stirred under H_2 overnight. Another portion of catalyst (0.07 g, 20 wt % Pd (dry basis) on carbon, wet) was added, and the reaction mixture was stirred another 2 h under H_2. The mixture was then filtered through Celite that was washed with additional portions of MeOH and CH_2Cl_2. The combined filtrates were evaporated under reduced pressure, and the product was purified by silica column chromatography (EtOAc/PE (60−80 °C), 1:1), yielding the title compound (0.659 g, 81%) as a white foam; R_f = 0.36 (EtOAc/PE (60−80 °C), 1:1). ^{1}H NMR ($CDCl_3$): δ 1.05 (3H, t, J = 7.4 Hz, CH_2CH_3), 2.26 (6H, s, 2 × CH_3), 2.44 (2H, q, J = 7.5 Hz, CH_2CH_3), 4.05 (2H, s, aryl-CH_2-aryl), 4.55 (2H, s, OCH_2-aryl), 5.17 (2H, s, NCH_2O), 6.63 (2H, s, H-aryl), 6.81 (2H, d, J = 8.5 Hz, H-aryl), 6.87 (1H, s, H-aryl), 7.17 (2H, d, J = 8.5 Hz, H-aryl), 9.50 (1H, s, NH). ^{13}C NMR ($CDCl_3$): δ 13.71 (CH_2CH_3), 19.14 (CH_2CH_3), 21.25 (2 × CH_3), 33.21 (CH_2-aryl), 71.61 (OCH_2-aryl), 72.68 (NCH_2O), 115.48 (C-aryl), 116.80 (C5), 125.00, 128.96, 129.30, 129.58, 134.78, 138.86 (C-aryl), 149.66, 151.96 (C-2, C-6), 155.99 (C-aryl), 163.60 (C-4). MS (MALDI, *m/z*) = 417 ($M + Na^+$).

2,2-Dimethyl-thiopropionic Acid *S*-(2-{{4-[6-(3,5-Dimethylbenzyl)-5-ethyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-ylmethoxymethyl]-phenoxy}-[5-(5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-2,5-dihydro-furan-2-ylmethoxy]-phosphoryloxy}-ethyl) Ester (3). 6-(3,5-Dimethylbenzyl)-5-ethyl-1-(4-hydroxy-benzyloxymethyl)-1*H*-pyrimidine-2, 4-dione (19, 0.094 g, 0.24 mmol) was dissolved in dry CH_3CN (2.5 mL) in a flame-dried bottle containing 3 Å molecular sieves (0.25 g). 1*H*-Tetrazole solution (1.06 mL, 0.45 M in CH_3CN, 0.48 mmol) was added, and then dropwise was added a solution of the amidite (5, 0.184 g, 0.36 mmol) in dry CH_3CN (2.5 mL). The mixture was stirred at room temperature for 2.5 h followed by addition of a solution of *tert*-butyl hydroperoxide (0.084 mL, 5.7 M in decane (anhydrous, over 4 Å sieves), 0.48 mmol). The mixture was stirred 1 h and then diluted with CH_2Cl_2 (25 mL). The organic phase was washed with a saturated aqueous solution of $Na_2S_2O_3$ (2 × 20 mL) and H_2O (2 × 20 mL), dried ($MgSO_4$), and evaporated under reduced pressure. The product was purified by silica column chromatography (EtOH/CH_2Cl_2, 3:97), yielding the title compound (0.118 g, 60%) as a white foam; R_f = 0.16 (EtOH/CH_2Cl_2, 5:95). ^{1}H NMR ($CDCl_3$): δ 1.07 (3H, t, J = 7.5 Hz, CH_2CH_3), 1.22, 1.23 (9H, 2 × s, $C(CH_3)_3$), 1.79, 1.86 (3H, 2 × d, J = 1.1 Hz, CH_3-d4T), 2.27 (6H, s, 2 × CH_3-aryl), 2.46 (2H, q, J = 7.4 Hz, CH_2CH_3), 3.09−3.18 (2H, m, SCH_2), 4.08 (2H, s, aryl-CH_2-aryl), 4.14−4.25 (2H, SCH_2CH_2O), 4.34−4.41 (2H, m, OCH_2-CHO), 4.61 (2H, s, OCH_2-aryl), 4.99−5.06 (1H, m, CH_2CHO), 5.12−5.28 (2H, m, NCH_2O), 5.86−5.96 (1H, m, CH=C*H*CHN), 6.29−6.36 (1H, m, C*H*=CHCHN), 6.68 (2H, s, H-aryl), 6.89 (1H, s, H-aryl), 7.00−7.08 (1H, m, OCHN), 7.11−7.32 (5H, m, H-aryl), 9.00−9.10 (1H, m, NH), 9.24−9.35 (1H, m, NH). ^{31}P NMR ($CDCl_3$/H_3PO_4 external): δ −5.92, −5.21. MS (MALDI, *m/z*) calcd 847.2748 ($M + Na^+$), found 847.2730 ($M + Na^+$).

Virus and Cells. The HIV-1 strains HTLV-IIIB (wild type) and N119 (NNRTI resistant Y181C), as well as the HIV-2 strain (wild-type SBL 6669), were propagated in H9 cells at 37 °C, 5% CO_2, using RPMI 1640 with 10% heat-inactivated fetal calf serum (FCS) and antibiotics (growth medium). Culture supernatant was filtered (0.45 nm), aliquotted, and stored at −80 °C until use. The HIV strains were obtained from the NIH AIDS Research and Reference Program and NIBSC centralized facility for AIDS reagents. CEM wt and CEM TK$^-$ cells were kindly provided by Dr. Staffan Eriksson, Uppsala, Sweden.

Inhibition of HIV-1 Replication. Compounds were examined for possible antiviral activity against HIV-1 and HIV-2 using MT-4, CEM, and CEM TK$^-$ cells as target cells. Cells were incubated with virus (0.005 MOI) and growth medium containing the test dilutions of compound for 6 days in parallel with virus-infected and uninfected control cultures without compound added. Expression of HIV in MT-4 cultures was indirectly quantified using the MTT assay as previously described.[30] Expression of HIV in CEM and CEM TK$^-$ cultures was quantified by HIV-1 and HIV-2 antigen detection ELISA.[31] Compounds mediating less than 30% reduction of HIV expression were considered without biological activity. Compounds were tested in parallel for cytotoxic effect in uninfected cultures containing the test dilutions of compound as described above. A 30% inhibition of cell growth relative to control cultures was considered significant. The 50% inhibitory concentration (IC_{50}) and the 50% cytotoxic concentration (CC_{50}) were determined by interpolation from the plots of percent inhibition versus concentration of compound.

Stability Studies. Double-prodrugs **1**, **2**, or **3** (1.5 mg) were dissolved in DMSO (15 mL), and 30 μL of this solution was added to growth medium to obtain a concentration of 0.117 mM. Samples (50 μL) were taken at certain time intervals and analyzed directly by LC-MS.

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

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