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Synthesis and Biological Evaluation of Prodrug-Type Anti-HIV Agents: Ester Conjugates of Carboxylic Acid-Containing Dipeptide HIV Protease Inhibitors and a Reverse Transcriptase Inhibitor

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Abstract—On the basis of substrate transition-state mimic concept of HIV protease, a series of small-sized dipeptide inhibitors containing hydrophilic carboxyl group were designed and synthesized. These dipeptide inhibitors showed good HIV protease inhibitory activity, but their anti-HIV activity was poor. The low antiviral activities of these inhibitors were probably due to their inadequate cell membrane permeability caused by the presence of a free carboxylic acid in the inhibitors. Based on the prodrug concept as well as the combination of two different classes of anti-HIV agents, conjugates of HIV protease inhibitors with a nucleoside reverse transcriptase inhibitor were synthesized. Some of these conjugates exhibited excellent antiviral activity compared with that of individual inhibitors. The synergistic enhancement of anti-HIV activities of these conjugates may be due to their ability to penetrate into the target cell and subsequent regeneration of two different classes of anti-HIV agents in the cytoplasm.

Introduction

The identification of human immunodeficiency virus type-1 (HIV-1) as the causative agent of acquired immunodeficiency syndrome (AIDS) has prompted an intense research effort to find effective therapies for this disease. Advances in research of HIV have postulated that the virally encoded reverse transcriptase (RT) and aspartic protease are essential for the proliferation of HIV, and inhibitors of these enzymes are effective targets of the chemotherapy of AIDS.[1] The discovery of clinically effective HIV protease (PR) inhibitors and RT inhibitors in the recent past has significantly improved the lifestyle of HIV infected patients.[2] Recently, to obtain a sustained benefit from antiviral therapy, combination of RT inhibitors and PR inhibitors has become the standard clinical practice, which contributes to prevent emergence of drug resistant virus and reduce the side effects of chemotherapy.[3]

Previously, we developed a series of substrate-based peptidomimetic HIV PR inhibitors containing an unnatural amino acid allophenylnorstatine (Apns = (2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid) with a hydroxymethylcarbonyl (HMC) isostere as a transition-state mimic.[4] Among them, a tripeptide KNI-272 (Fig. 1) showed potent HIV-1 PR inhibitory activity ($K_i = 5.5$ pM) and highly potent in vitro antiviral activity with low cytotoxicity.[5,6] Although KNI-272 showed a good oral bioavailability in human clinical trials, the plasma half-life was short.[5c] The structure of KNI-272 complexed with HIV-1 protease showed that the HMC group in KNI-272 interacted excellently with the aspartic acid carboxyl groups of the HIV PR active site and the solution, crystalline and complex structures of KNI-272 were similar except for the P3 moiety.[7] Therefore, we considered that the P2–P2′ moieties might be the core group for enzyme inhibition, and studied small dipeptide-based inhibitors as advantageous compounds.[8–10] To reduce the size, we removed the P3 moiety and substituted P2 with a hydrophilic carboxyl group (Fig. 1). The resulting inhibitors exhibited good enzyme inhibitory activity in spite of absence of the P3 moiety, but their anti-HIV activities were poor.[8,9] The poor antiviral activities were probably due to the free carboxyl group which was supposed to be inappropriate for the penetration across the cell membrane.[11,12]

A useful approach to overcome this problem is to transform the PR inhibitor into a prodrug by esterification of

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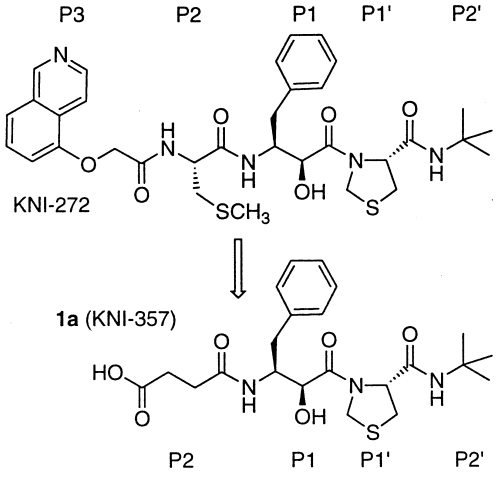


Figure 1. Structures of tripeptide inhibitor, KNI-272, and small-sized dipeptide inhibitor, **1a** (KNI-357).

the carboxyl group with an alcohol component.[13,14] In general, a prodrug is prepared by chemical modification of the functional group of a biologically active molecule by a pharmaceutically inactive carrier, and when administered in vivo will liberate the parent molecule.[15,16] When effective anti-HIV agents such as nucleoside RT inhibitors were used for masking a free carboxylic acid of HIV PR inhibitors, the following advantages were envisaged (Fig. 2): (1) The undesirable physicochemical properties such as low membrane permeability would be improved. (2) Hybrid-type prodrugs can exhibit synergistic anti-HIV activity from two different anti-HIV mechanisms. Once the prodrug escapes extracellular hydrolysis, it would enter the target cell wherein the intracellular hydrolysis regenerates the inhibitors, which can act on two separate targets. (3) Synergistic anti-HIV activity will make it possible to reduce the drug dosage, resulting in suppression of side effects. (4) The conjugation of HIV PR inhibitors with nucleoside-based RT inhibitors such as 3′-azido-3′-deoxythymidine (AZT)[17] may facilitate the penetration through the biological membrane mediated by the nucleoside transporters.[18] In addition, the cell membrane has affinity for nucleosides.[19]

Based on these premises, we directly esterified the carboxyl group of HIV PR inhibitors (**1a**–**1k**, Scheme 1) with a nucleoside RT inhibitor, AZT. The resulting conjugates (**2a**–**2k**, Tables 1 and 2) were tested for their antiviral activities, and some of them exhibited a synergistic enhancement of anti-HIV activity.[20,21] In this paper, we describe the synthesis and biological activity of dipeptide HIV PR inhibitors containing free carboxyl group and the prodrug-type conjugates of dipeptide HIV PR inhibitors with a nucleoside RT inhibitor, AZT, by direct esterification. The pharmacodynamic properties such as the stability of the conjugates in aqueous medium, rat plasma and human serum were also evaluated.

Chemistry

The syntheses of small-sized dipeptide HIV protease inhibitors **1a**–**1k** are summarized in Scheme 1. Dipeptide *tert*-butylamide derivatives **3a** (Apns-Thz-NHBu^t ·HCl; Thz = L-thioproline = L-1,3-thiazolidine-4-carboxylic acid; Bu^t = *tert*-butyl), **3b** (Apns-Dmt-NHBu^t·HCl; Dmt = L-dimethylthioproline = L-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid), and 2-methylbenzylamide derivatives **3c** (Apns-Thz-NH-(2-methyl)Bzl·HCl) and **3d** (Apns-Dmt-NH-(2-methyl)Bzl·HCl) were prepared according to the methods described previously.[10] Dipeptide derivatives **3a**–**3d** were treated with the corresponding carboxylic anhydrides in the presence of triethylamine to afford the PR inhibitors **1a**–**1k** in good yield.

The synthetic procedures of AZT-half esters **4a**–**4d** are shown in Scheme 2, which were slightly modified from the reported methods.[22] AZT was treated with the corresponding succinic anhydride or glutaric anhydride in *N,N*-dimethylformamide (DMF) in the presence of dimethylaminopyridine (DMAP) to afford corresponding AZT-half esters **4a**–**4d** in good yield. Compound **4b** was obtained as a mixture of AZT-(2*S*,3*R*)-dimethylhemisuccinate and AZT-(2*R*,3*S*)-dimethylhemisuccinate because *meso*-2,3-dimethylsuccinic acid was used for the preparation of the anhydride. From the consideration of regioselectivity, AZT-2,2-dimethylhemisuccinate (**4e**)

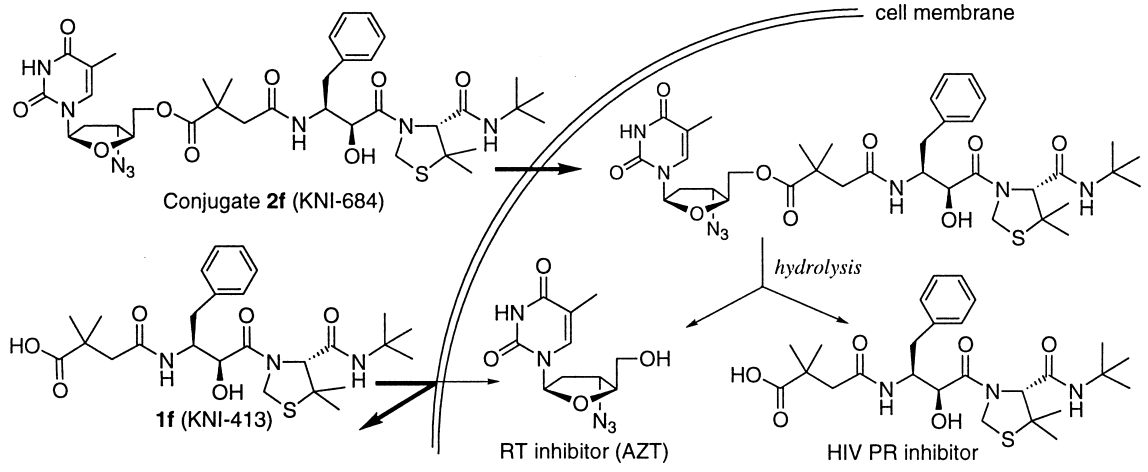
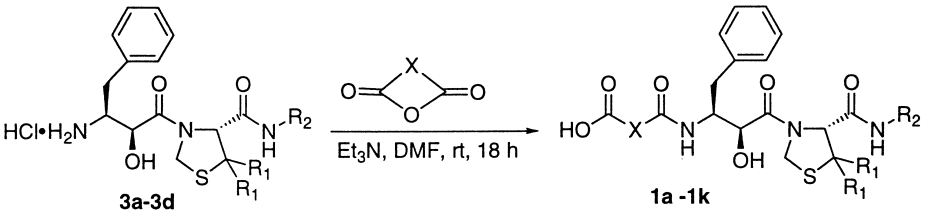


Figure 2. Design and proposed mechanism of conjugates of HIV PR inhibitor with RT inhibitor.



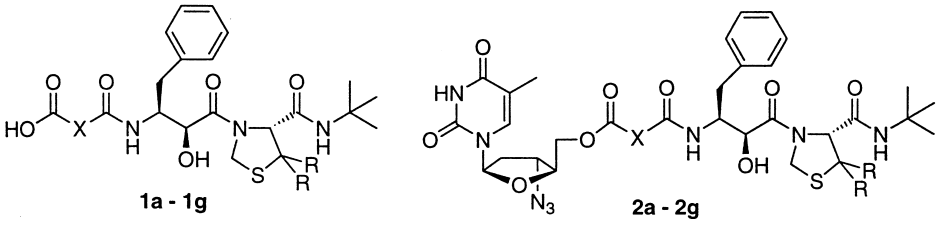
3a: R_1=H, R_2=*tert*-butyl
3b: R_1=Me, R_2=*tert*-butyl
3c: R_1=H, R_2=2-methylbenzyl
3d: R_1=Me, R_2=2-methylbenzyl

Compound	X	R_1	R_2
1a (KNI-357)	-CH_2CH_2-	H	*tert*-butyl
1b (KNI-391)	-$C(Me)_2CH_2$-	H	*tert*-butyl
1c (KNI-547)	-CH(Me)CH(Me)- *	H	*tert*-butyl
1d (KNI-417)	-$CH_2CH_2CH_2$-	H	*tert*-butyl
1e (KNI-418)	-$CH_2C(Me)_2CH_2$-	H	*tert*-butyl
1f (KNI-413)	-$C(Me)_2CH_2$-	Me	*tert*-butyl
1g (KNI-549)	-CH(Me)CH(Me)- *	Me	*tert*-butyl
1h (KNI-689)	-$C(Me)_2CH_2$-	H	2-methylbenzyl
1i (KNI-690)	-CH(Me)CH(Me)- *	H	2-methylbenzyl
1j (KNI-852)	-$C(Me)_2CH_2$-	Me	2-methylbenzyl
1k (KNI-691)	-CH(Me)CH(Me)- *	Me	2-methylbenzyl

* mixture of (2*R*, 3*S*) and (2*S*, 3*R*).

Scheme 1.

Table 1. Biological activity of HIV protease inhibitors and prodrug-type anti-HIV agents

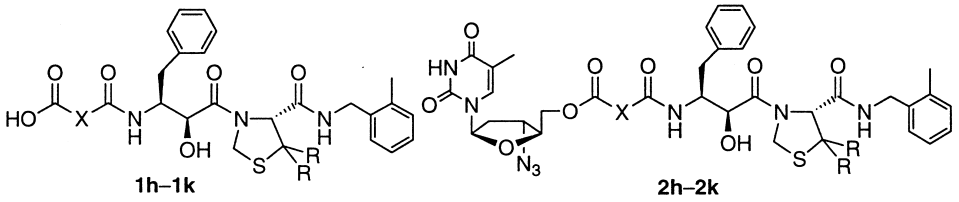


Compound	X	R	Inhibition of HIV protease[a] (%)	EC_{50} HIV-1_{IIIB}/		Relative potency
				MT-4 (μM)	CEM-SS (nM)	
1a (KNI-357)	-CH_2CH_2-	H	74	N.D.[b]		
1b (KNI-391)	-$C(Me)_2CH_2$-	H	93 (20)	>2000		
1c (KNI-547)	-CH(Me)CH(Me)-[c]	H	97 (32)	>2000		
1d (KNI-417)	-$CH_2CH_2CH_2$-	H	82 (6)	N.D.[b]		
1e (KNI-418)	-$CH_2C(Me)_2CH_2$-	H	50	N.D.[b]		
1f (KNI-413)	-$C(Me)_2CH_2$-	Me	99 (76)	52		
1g (KNI-549)	-CH(Me)CH(Me)-[c]	Me	99 (78)	225		
2a (KNI-679)	-CH_2CH_2-	H	14		42	3.0
2b (KNI-680)	-$C(Me)_2CH_2$-	H	9		27	4.7
2c (KNI-681)	-CH(Me)CH(Me)-[c]	H	26		27	4.7
2d (KNI-682)	-$CH_2CH_2CH_2$-	H	12		192	0.7
2e (KNI-683)	-$CH_2C(Me)_2CH_2$-	H	12		4682	0.03
2f (KNI-684)	-$C(Me)_2CH_2$-	Me	41		19	6.6
2g (KNI-685)	-CH(Me)CH(Me)-[c]	Me	69		90	1.4
AZT	—	—	—		126	1.0
KNI-272	—	—	100 (96)	0.57	40	

[a]% of inhibition in the presence of 5 μM or 50 nM (in parentheses) of inhibitors.
[b]Not determined.
[c]Mixture of (2*R*,3*S*) and (2*S*,3*R*).

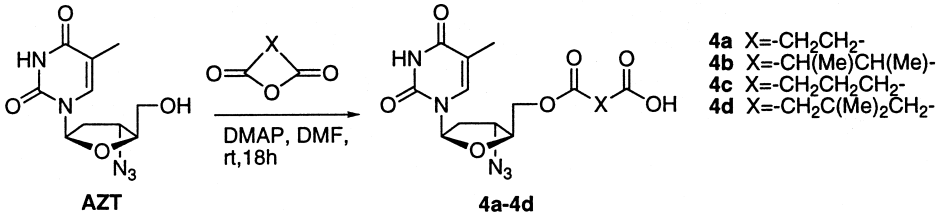
was synthesized as shown in Scheme 3. Treatment of 2,2-dimethylsuccinic anhydride with *p*-methoxybenzyl alcohol (MBzl-OH) in THF:ether (1:2) in the presence of dicyclohexylamine (DCHA) at 4 °C afforded the β,β-dimethyl-*p*-methoxybenzyl ester (**5**). HPLC and NMR data indicated that the resulting half-ester (**5**) contained 20% of regioisomer (α,α-dimethyl-*p*-methoxybenzyl ester). Compound **5** was used for the next reaction

Table 2. Biological activity of HIV protease inhibitors and prodrug-type anti-HIV agents

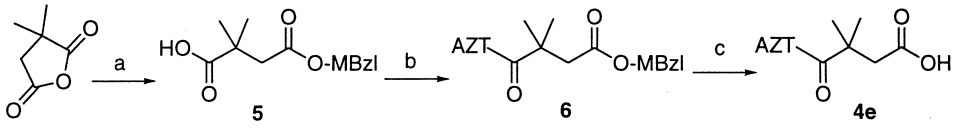


Compound	X	R	Inhibition of HIV protease[a] (%)	EC_{50} HIV-1_{IIIB}/ CEM-SS (nM)	Relative potency
1h (KNl-689)	-$C(Me)_2CH_2$-	H	87	N.D.[b]	
1i (KNI-690)	-CH(Me)CH(Me)-[c]	H	88	N.D.[b]	
1j (KNI-852)	-$C(Me)_2CH_2$-	Me	100 (78)	inactive	
1k (KNI-691)	-CH(Me)CH(Me)-[c]	Me	100 (76)	N.D.[b]	
2h (KNI-692)	-$C(Me)_2CH_2$-	H	11	3.8	2.9
2i (KNI-693)	-CH(Me)CH(Me)-[c]	H	57	8.9	1.2
2j (KNI-694)	-$C(Me)_2CH_2$-	Me	83	0.24	46
2k (KNI-695)	-CH(Me)CH(Me)-[c]	Me	96 (39)	7.3	1.5
AZT	—	—	—	11	1.0

[a]% of inhibition in the presence of 5 μM or 50 nM (in parentheses) of inhibitors.
[b]Not determined.
[c]Mixture of (2*R*,3*S*) and (2*S*,3*R*).



Scheme 2.



Scheme 3. Reagents and conditions: (a) *p*-methoxybenzyl alcohol, DCHA, THF:ether (1:2), 4 °C, 18 h; (b) AZT, DCC, DMAP, DMF, rt, 36 h; (c) anisole, TFA, rt, 1 h.

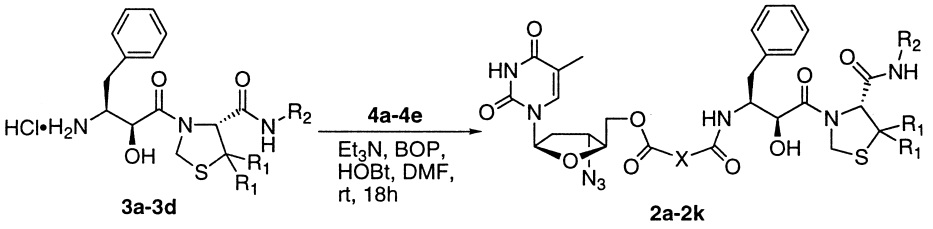
without further purification. Condensation of half-ester (**5**) and AZT by use of *N,N*′-dicyclohexylcarbodiimide (DCC) in the presence of DMAP afforded compound **6**. Finally, the *p*-methoxybenzyl group of compound **6** was removed by treatment with trifluoroacetic acid (TFA) in the presence of anisole. The resulting AZT-2,2-dimethylhemisuccinate (**4e**) contained 12% of regioisomer, and was used for the subsequent reaction without further purification.

Scheme 4 illustrates the procedure to synthesize the conjugates of dipeptide HIV PR inhibitors with AZT. Condensation of AZT-half esters **4a**–**4e** and the dipeptide derivatives **3a**–**3d** by use of benzotriazol-1-yloxy-tris-(dimethylamino)phosphoniumhexafluorophosphate/1-hydroxybenzotriazole (BOP/HOBt) in the presence of triethylamine afforded compounds **2a**–**2k**. Scheme 5 illustrates another synthetic route for preparation of compounds **2b**, **2f**, **2h**, **2j** from the consideration of regio-selectivity of succinyl moieties. Condensation of dipeptide inhibitors **1b**, **1f**, **1h**, **1j** with AZT by use of DCC in the presence of DMAP afforded the objective compounds **2b**, **2f**, **2h**, **2j**. However, in this synthetic strategy, the undesirable intramolecular cyclization predominantly occurred, resulting in the succinimide derivatives such as **7** (Fig. 3), and the yield of the desired product was low (< 10%).

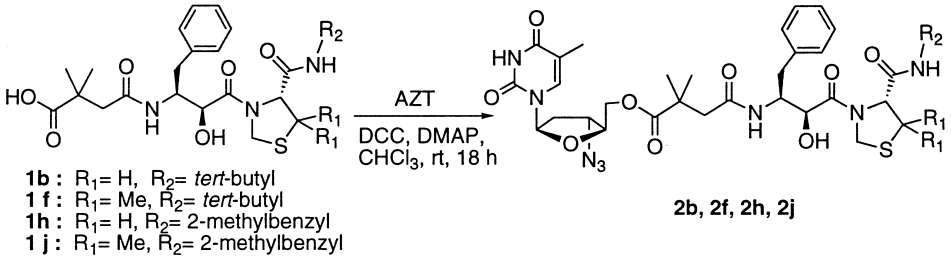
Biological Evaluation and Discussion

Small dipeptide-based HIV PR inhibitors containing carboxyl group

From the solution, crystalline, and complex structures of KNI-272, the P2–P2′ moiety was revealed to be the core group for the inhibition of HIV PR. Therefore, in order to reduce the size, the P3 moiety of KNI-272 was not incorporated, and the P2 position had a hydrophilic carboxyl group. The resulting compound **1a** (KNI-357) showed PR inhibitory activity (5 μM inhibition = 74%) in



Scheme 4.



Scheme 5.

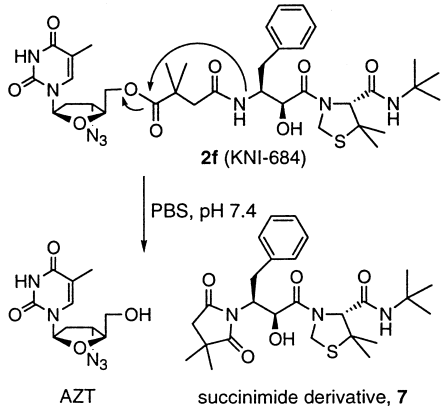


Figure 3. Succinimide formation of conjugate **2f** (KNI-684).

spite of the decrease of the recognition site by size reduction. This result encouraged us to continue the structure–activity relationship (SAR) study of various analogues of compound **1a** (KNI-357), and the outcome of this study is shown in Table 1. From the SAR of HIV PR inhibitors, bulky and lipophilic amino acids such as Val were preferred for P2 site. Therefore, we introduced the alkyl side chain to the succinyl or glutaryl moiety. The resulting compound **1b** (KNI-391), which has a β,β-dimethylsuccinyl group at P2 site as a valine mimic, showed better enzyme inhibitory activity (5 μM inhibition = 93%, 50 nM inhibition = 20%) than compound **1a**. The α,β-dimethylsuccinyl derivative **1c** (KNI-547) also exhibited good inhibition (5 μM inhibition = 97%, 50 nM inhibition = 32%). In previous studies, we found that the methyl substitution at the 5 position of the L-1,3-thiazolidine-4-carboxylic acid (Thz) at the P1′ position remarkably enhanced the protease inhibitory activity, and hence we replaced the Thz of **1b** and **1c** by the L-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid (Dmt). The resulting compounds **1f** (KNI-413) and **1g** (KNI-549) remarkably enhanced enzyme inhibitory activities (50 nM inhibition = 76 and 78%, respectively). These potent enzyme inhibitory activities were due to the conformational constraint and hydrophobicity of the bulky dimethyl group. Compounds **1d** and **1e**, with an additional methylene group in the P2 succinyl group of **1a** and **1b**, did not significantly enhance enzyme inhibitory activity (5 μM inhibition = 82 and 50%, respectively). Recently, Mimoto et al. reported that replacement of the *tert*-butylamide moiety at P2′ position by 2-methylbenzylamide moiety significantly enhanced the HIV PR inhibitory activity.[10] These results prompted us to replace the P2′ moiety of compounds **1b**, **1c**, **1f** and **1g**. However, the enzyme inhibitory activities of the resulting compounds **1h**–**1k** were not enhanced (Table 2).

Next, we determined the antiviral activities of potent HIV PR inhibitors in cell culture against HIV-1 strain IIIB in MT-4 cells. In spite of good HIV PR inhibitory activity, compounds **1b** and **1c** did not show the antiviral activity (Table 1). Compounds **1f** and **1g**, which showed more potent enzyme inhibitory activities than **1b** and **1c**, showed poor antiviral activities (EC_{50} = 52 and 225 μM, respectively).

Hybrid-type prodrugs conjugating dipeptide HIV PR inhibitors with AZT by direct esterification

The antiviral efficacy of inhibitors depends not only on the enzyme inhibitory activity, but also on their intracellular concentration, which is closely related to the cell membrane permeability.[23–25] It is known that drugs containing free carboxylic acid group often have reduced membrane permeability as a result of unfavorable physicochemical properties.[11,12] Small dipeptide-based inhibitors with free carboxylic acid at P2 site were supposed to be unsuitable for the penetration across the cell membrane. Therefore, we considered that a prodrug approach involving conversion of a free carboxylic acid into an ester would be effective for increasing the anti-HIV activity. Moreover, adoption of a nucleoside RT inhibitor as the

alcohol component was expected to enhance the anti-HIV activities from the viewpoint of membrane permeability.

At first, we employed ethanol for masking of a free carboxylic acid, but the ethyl ester of HIV PR inhibitor did not show increased anti-HIV activity. This could probably be due to the physicochemical properties not being suitable for the penetration across the cell membrane or the ethyl ester was not hydrolyzed intracellularly. Then, we selected the nucleoside RT inhibitor, AZT, as an alcohol component, and esterified the carboxyl groups of the HIV PR inhibitors **1a**–**1k**. The resulting hybrid-type prodrugs **2a**–**2k** were studied for their HIV PR inhibitory activity and anti-HIV activity, and the results are shown in Tables 1 and 2, respectively. The conversion of the inhibitors to their conjugates decreased the enzyme inhibition in all cases (**2a**–**2k**). These results showed that AZT is not suitable as P3 ligand and the hydrogen of carboxylic acid may be important for the interaction with the enzyme.

The antiviral activities of these conjugates (**2a**–**2k**) were determined using HIV-1_{IIIB}/CEM-SS assay system. The EC_{50} values of AZT cited in Tables 1 and 2 were not consistent as these experiments were carried out at different times and also due to the different population of the spontaneous variant in wild type CEM cell line used (as reported by Tornevik et al.[26]). In order to evaluate the enhancement of the anti-HIV activity, we employed the relative potency based on the EC_{50} of AZT. All the conjugates except for compounds **2d** and **2e** showed potent anti-HIV activities with low cytotoxicity (therapeutic index >70). Compounds **2g**, **2i** and **2k** showed similar antiviral activities with AZT. Compound **2f** (KNI-684) showed excellent anti-HIV activity ($EC_{50}=19$ nM), which was 6.6 times more potent than that of AZT and 2.1 times that of KNI-272. The equimolar mixture of the parent HIV PR inhibitor **1f** (KNI-413) and AZT exhibited the same antiviral activity as AZT. Among these conjugates, compound **2j** (KNI-694) showed extremely potent antiviral activity that is 46 times more than that of AZT. The major consideration in this 'double-drug' strategy is focused not only on transportation across the cell membrane, but also on its reversion to the parent compounds in the cytoplasm to act on their respective targets.[27–29] These results suggested that conjugated compounds act according to the above proposed mechanism (Fig. 2).

Stability study of the conjugates

The in vitro stability of the conjugates was examined in water, phosphate buffered saline (PBS, pH 7.4), rat plasma and human serum. The degradation behavior of the conjugates in PBS (pH 7.4, at 37 °C) and water (at room temperature) was determined by HPLC, and the half-lives of the compounds are summarized in Table 3. In PBS (pH 7.4), the conjugate **2f** (KNI-684) containing succinyl group in the P2 moiety gradually disintegrated to AZT and succinimide derivative **7** by intramolecular cyclization (Fig. 3).[22b,29] Succinimide derivative **7** was isolated from the fraction of HPLC, and characterized by mass spectrometry and NMR. Furthermore, compound **7** was synthesized, and its identity was established by comparison of HPLC profile and mass spectrum. Similar cyclization phenomena were observed for other conjugates. As expected from the energetically favorable cyclization to the five-membered ring,[22b,29] the prodrugs containing succinyl group decomposed at a faster rate than the glutaryl derivatives (**2d**, **2e**). The side chain of the succinyl derivatives influenced the disintegration rates. In the PBS (pH 7.4), compound **2a** lacking methyl group in succinyl moiety was stable ($t_{1/2}=33$ h), whereas compounds (**2b**, **2f** and **2j**) containing dimethylsuccinyl moiety easily released AZT by succinimide formation with relatively short half-lives (3.2, 2.9 and 3.8 h respectively). The HIV PR inhibitory activities of the succinimide derivatives were very poor (5 μM inhibition; $<5\%$). Nevertheless, the conjugates **2f** and **2j** showed excellent anti-HIV activities. The remarkable antiviral activity of the conjugated compounds may be due to their penetration into the cell and later splitting into two different classes of anti-HIV agents, HIV PR inhibitor and AZT (Fig. 2).

Table 3. Kinetic data for the degradation of conjugates

Compound	Solvent	Temperature (°C)	$t_{1/2}$ (h)
2a	PBS, pH 7.4	37	33
2b	PBS, pH 7.4	37	3.2
2d	PBS, pH 7.4	37	>96
2e	PBS, pH 7.4	37	>96
2f	PBS, pH 7.4	37	2.9
2j	PBS, pH 7.4	37	3.8
2f	Distilled water	rt	>96
2j	Distilled water	rt	>96

The concept involved in the design of these hybrid-type prodrugs was to regenerate the parent compounds intracellularly by esterase-mediated hydrolysis. Therefore, we examined the stability of compounds **2f** and **2j** in the presence of an esterase. Porcine liver esterase (Sigma, E.C. 3.1.1.1) was used in our studies as this esterase has been widely used in similar studies and also the results correlated well with those of plasma studies.[30–32] Consequently, both compounds **2f** and **2j** exhibited resistance to hydrolysis with the esterase, and the disintegration profiles were almost the same in the case of PBS (pH 7.4). Since the antiviral efficacy of conjugates depends on so many factors such as enzyme inhibition, cell membrane permeability, extracellular stability and intracellular disintegration, correlation between them is very complicated.

Furthermore, in order to evaluate the susceptibility of the conjugates to hydrolysis under physiological conditions, compounds **2f** and **2j** were incubated at 37 °C in rat plasma and human serum. We used human serum for susceptibility study because the results were known to correlate well with those of plasma studies.[33] In rat plasma, both **2f** and **2j** decomposed rapidly and their half-lives were 16 and 3 min, respectively (Fig. 4). On the other hand, both the prodrugs were stable in the human serum even after 1 h incubation. It is well known that enzymes such as esterase are highly efficient in rat

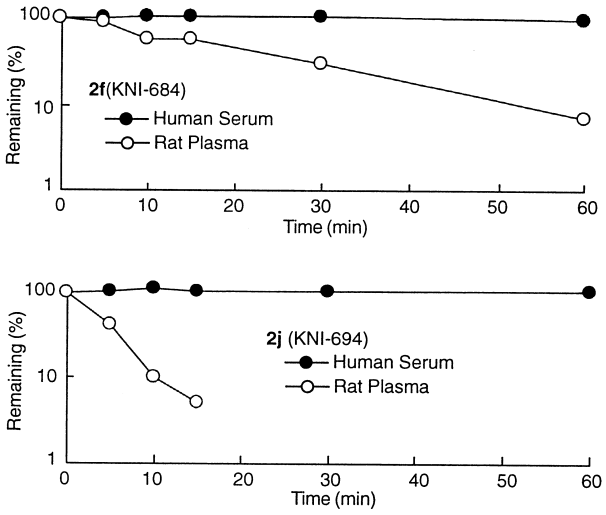


Figure 4. Stability of **2f** (KNI-684) and **2j** (KNI-694) in rat plasma and human serum.

plasma.[34] The short half-lives of the prodrugs in rat plasma and the sufficient stability in human serum suggested that further pharmacokinetic study in rat was inappropriate.

Conclusion

In this study, a series of small dipeptide-based HIV PR inhibitors containing free carboxylic acid in P2 site were designed and synthesized on the basis of the substrate transition-state mimic concept. These inhibitors exhibited good enzyme inhibitory activity, but the anti-HIV activity was poor. Since it was supposed that the free carboxylic acid was not suitable for the penetration across the cell membrane, we considered converting these inhibitors into a prodrug. Direct esterification of carboxyl group of the HIV PR inhibitors with 5′-hydroxyl group of the nucleoside RT inhibitor gave remarkably potent anti-HIV agents. The antiviral activities of the conjugates of two different anti-HIV agents were considerably potent compared with that of the individual components. Based on the prodrug concept as well as conjugation of two different classes of anti-HIV agents, we have developed potent anti-HIV agents.

Experimental

HIV PR inhibition

HIV PR inhibitory activity of the test compounds was determined based on the inhibition of cleavage of the HIV PR substrate (SQNYPIV) by using recombinant HIV-1 PR (NY5-type sequence) as previously reported.[10]

Antiviral activity

Antiviral activity of test compounds was determined based on inhibition of HIV-1 IIIB-induced cytopathic effect in CEM-SS cells in vitro as previously reported.[10]

Stability of conjugates in PBS

The chemical stability of the conjugates was determined in phosphate buffered saline (PBS), pH 7.4. To 12 mL of PBS (pH 7.4) was added 120 μL of conjugate solution (0.5 mM in dimethylsulfoxide) and the mixture was incubated at 37 °C in a water bath. At different points of time ($T = 0$, 1, 2, 4, 8, 24, 48, 72, 96 h), 1 mL of the samples was withdrawn and directly analyzed by HPLC. HPLC was performed using a C18 reverse phase column (4.6 × 150 mm; YMC Pack ODS AM302) with binary solvent system: linear gradient of CH_3CN (10–80%, 30 min) in 0.1% aqueous TFA at a flow rate of 0.9 mL/min, detected at UV 230 nm.

Stability of conjugates in the presence of esterase

Porcine liver esterase (PLE; carboxylic-ester hydrolase; EC 3.1.1.1; E-2884) was obtained from Sigma as a suspension in a 3.2 M $(NH_4)_2SO_4$ solution (pH 8). 3.2 μL of this suspension (containing 3750 units of enzyme per mL) was diluted with 12 mL of phosphate buffered saline (PBS, pH 7.4). In this buffer–PLE solution, the stability of the conjugates was determined in a similar manner as described in the section of stability in PBS.

Stability of conjugates in rat plasma and human serum

To 1.8 mL of rat plasma or human serum was added 0.2 mL of a solution of conjugates (10 μg/mL in CH_3OH:CH_3CN = 1:1, v/v) and the mixture was incubated at 37 °C. At different points of time ($T = 5$, 10, 15, 30, 60 min), 0.1 mL of the samples was withdrawn and added to 400 μL of CH_3CN:CH_3COOH (9:1, v/v). The samples were centrifuged (15,000 rpm, 10 min) and 400 μL of the supernatants was evaporated (30 °C, 1.5 h). The residues were dissolved in 200 μL of 30% acetonitrile and 100 μL of the samples was analyzed by HPLC. HPLC was performed using a C18 reverse phase column (4.6×250 mm; GL-science Inertsil ODS-3) with binary solvent system: acetonitrile:0.01 M phosphate buffer, pH 7.0 (1:1, v/v), at a flow rate of 1.0 mL/min, detected at UV 265 nm.

Chemistry

In general, reagents and solvents were used as purchased without further purification. Column chromatography was performed on Merck 107734 silica gel 60 (70–230 mesh). TLC was performed using Merck silica gel $60F_{254}$ precoated plates. Melting points were measured on a Yanagimoto micro melting apparatus without correction. Analytical HPLC was performed using a C18 reverse phase column (4.6×150 mm; YMC Pack ODS AM302) with binary solvent system: linear gradient of CH_3CN (20–80%, 30 min) in 0.1% aqueous TFA at a flow rate of 0.9 mL/min, detected at UV 230 nm. Preparative HPLC was carried out on a C18 reverse phase column (20×250 mm; YMC Pack ODS SH343-5) with binary solvent system: linear gradient of CH_3CN in 0.1% aqueous TFA at a flow rate of 5.0 mL/min, detected at UV 230 nm. ^1H NMR spectra were obtained on a Varian 400 or JEOL 270 MHz spectrometer with TMS as an internal standard. FAB–MS was

performed on a JEOL JMS-SX102A spectrometer equipped with the JMA-DA7000 data system. Optical rotation was determined with a Horiba SEPA-300 polarimeter.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-(succinyl)amino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (succinyl-Apns-Thz-NHBu^t, 1a, KNI-357). To the solution of *tert*-butylamide derivative **3a** (200 mg, 0.55 mmol) in DMF were added succinic anhydride (55 mg, 0.605 mmol) and triethylamine (76 μL, 0.55 mmol), and stirred for 18 h at rt. After removal of the solvent in vacuo, the residue was dissolved in EtOAc (30 mL), washed with 10% citric acid and saturated NaCl, dried over anhydrous Na_2SO_4, and concentrated in vacuo. Purification of the product by preparative HPLC and reprecipitation from *n*-hexane gave 219 mg of the title compound as a white solid. Yield 88%: mp 211–212 °C; $[\alpha]_D^{23}$ −46.6° (c = 0.57, MeOH); ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.01 (br s, 1H), 8.09 (d, J = 8.2 Hz, 1H), 7.65 (s, 1H), 7.35 (d, J = 7.3 Hz, 2H), 7.24–7.10 (m, 3H), 5.30 (d, J = 6.6 Hz, 1H), 4.95 (d, J = 9.6 Hz, 1H), 4.77 (m, 1H), 4.64 (d, J = 9.6 Hz, 1H), 4.47 (m, 1H), 4.09 (s, 1H), 3.38–3.55 (m, 1H, partially covered by H_2O peaks), 2.99 (dd, J = 11.6, 6.6 Hz, 1H), 2.60–2.68 (m, 2H), 2.21–2.35 (m, 4H), 1.26 (s, 9H); MS (FAB) m/z = 466 $[M+H]^+$. Anal. calcd for $C_{22}H_{31}N_3O_6S$: C, 56.75; H, 6.71; N, 9.03. Found: C, 56.51; H, 6.75; N, 9.03.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-(3,3-dimethylsuccinyl)amino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (3,3-dimethylsuccinyl-Apns-Thz-NHBu^t, 1b, KNI-391). Compound **1b** was prepared from 2,2-dimethylsuccinic anhydride and compound **3a** in a manner similar to that described for compound **1a**. Yield 86%: mp 99–102; $[\alpha]_D^{23}$ −37.4° (c = 0.50, MeOH); ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.00 (s, 1H), 8.09 (d, J = 8.3 Hz, 1H), 7.72 (s, 1H), 7.36 (d, J = 7.3 Hz, 2H), 7.22–7.12 (m, 3H), 5.04 (d, J = 6.9 Hz, 1H), 4.94 (d, J = 9.2 Hz, 1H), 4.77 (t, J = 6.9 Hz, 1H), 4.67 (d, J = 9.6 Hz, 1H), 4.34–4.45 (m, 1H), 4.11 (m, 1H), 3.30–3.24 (m, 1H, partially covered by H_2O peaks), 2.99 (dd, J = 11.6, 6.6 Hz, 1H), 2.65–2.57 (m, 2H), 2.35 (d, J = 14.2 Hz, 1H), 2.22 (d, J = 14.2 Hz, 1H), 1.26 (s, 9H), 0.96 (s, 3H), 0.86 (s, 3H); HRMS (FAB): m/z 494.2327 for $[M+H]^+$ (calcd 494.2325 for $C_{24}H_{36}N_3O_6S$).

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-(2,3-dimethylsuccinyl)amino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (2,3-dimethylsuccinyl-Apns-Thz-NHBu^t, 1c, KNI-547). To a solution of *meso*-2,3-dimethylsuccinic acid (176 mg, 1.2 mmol) in DMF was added DCC (248 mg, 1.2 mmol) and stirred for 3 h at rt. After *N,N′*-dicyclohexylurea was removed by filtration, compound **3a** (438 mg, 1.09 mmol) and triethylamine (151 μL, 1.09 mmol) were added to the solution, and stirred for 18 h at rt. After removal of the solvent in vacuo, the residue was dissolved in EtOAc (30 mL), washed with 10% citric acid and saturated NaCl, dried over anhydrous Na_2SO_4, and concentrated in vacuo. Purification of the product by preparative HPLC and reprecipitation from *n*-hexane gave 467 mg of the title compound as a mixture of 2*R*,3*S*-dimethylsuccinyl derivative and 2*S*,3*R*-dimethylsuccinyl derivative, as a white solid in 86% yield: mp 229–231 °C; 2 peaks on HPLC (Rt = 17.16 and 17.48 min for the two diastereomers); ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.16 (br s, 1H), 8.26–8.17 (m, 1H), 7.70 (s, 1H), 7.38–7.36 (m, 2H), 7.20–7.10 (m, 3H), 5.58 (d, J = 7.9 Hz, 1H), 5.13–5.03 (m, 1H), 4.96–4.94 (m, 1H), 4.81–4.67 (m, 1H), 4.47–4.37 (m, 1H), 4.13–4.02 (br m, 1H), 3.40–3.32 (m, 1H, partially covered by H_2O peaks), 2.99 (dd, J = 11.6, 6.6 Hz, 1H), 2.73–2.63 (m, 2H), 2.39–2.31 (m, 1H), 2.18–2.15 (m, 1H), 1.26 (s, 9H), 1.05–0.91 (m, 3H), 0.71 (d, J = 6.3 Hz, 1.5H), 0.44 (d, J = 6.6 Hz, 1.5H); MS (FAB): m/z = 494 $[M+H]^+$. Anal. calcd for $C_{24}H_{35}N_3O_6S$: C, 58.40; H, 7.15; N, 8.51. Found: C, 58.94; H, 7.54; N, 8.23.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-(glutaryl)amino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (glutaryl-Apns-Thz-NHBu^t, 1d, KNI-417). Compound **1d** was prepared from glutaric anhydride and compound **3a** in a manner similar to that described for compound **1a**. Yield 80%: mp 161–163 °C ; $[\alpha]_D^{22}$ −47.5° (c = 0.57, MeOH); ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.01 (br s, 1H), 8.07 (d, J = 8.3 Hz, 1H), 7.69 (s, 1H), 7.35 (d, J = 6.9 Hz, 2H), 7.23–7.09 (m, 3H), 5.21 (br s, 1H), 4.97 (d, J = 9.6 Hz, 1H), 4.77 (m, 1H), 4.66 (d, J = 9.2 Hz, 1H), 4.46 (m, 1H), 4.11 (br s, 1H), 3.36–3.29 (m, 1H, partially covered by H_2O peaks), 2.99 (dd, J = 11.9, 6.6 Hz, 1H), 2.66–2.58 (m, 2H), 2.08–1.98 (m, 4H), 1.63–1.55 (m, 2H), 1.26 (s, 9H); MS (FAB): m/z = 480 $[M+H]^+$. Anal. calcd for $C_{23}H_{33}N_3O_6S$: C, 56.50; H, 7.01; N, 8.60. Found: C, 56.43; H, 6.90; N, 8.58.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-(3,3-dimethlyglutaryl)amino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (3,3-dimethylglutaryl-Apns-Thz-NHBu^t, 1e, KNI-418). Compound **1e** was prepared from 3,3-dimethylglutaric anhydride and compound **3a** in a manner similar to that described for compound **1a**. Yield 80%: mp 74–75 °C; $[\alpha]_D^{22}$ −36.0° (c = 0.56, MeOH); ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.13 (br s, 1H), 8.09 (d, J = 8.3 Hz, 1H), 7.71 (s, 1H), 7.36 (d, J = 7.3 Hz, 2H), 7.22–7.09 (m, 3H), 5.27 (br s, 1H), 4.98 (d, J = 9.2 Hz, 1H), 4.78 (m, 1H), 4.70 (d, J = 9.6 Hz, 1H), 4.50–4.48 (m, 1H), 4.17 (br s, 1H), 3.34–3.30 (m, 1H, partially covered by H_2O peaks), 3.00 (dd, J = 11.9, 6.6 Hz, 1H), 2.66–2.58 (m, 2H), 2.13–2.02 (m, 4H), 1.26 (s, 9H), 0.85 (s, 3H), 0.78 (s, 3H); HRMS (FAB): m/z 508.2488 for $[M+H]^+$ (calcd 508.2481 for $C_{25}H_{38}N_3O_6S$).

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-(3,3-dimethlysuccinyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (3,3-dimethylsuccinyl-Apns-Dmt-NHBu^t, 1f, KNI-413). Compound **1f** was prepared from 2,2-dimethylsuccinic anhydride and compound **3b** in a manner similar to that described for compound **1a**. Yield 89%: mp 98–101 °C; $[\alpha]_D^{24}$ +35.4° (c = 0.55, MeOH); ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.00 (br s, 1H), 8.09 (d, J = 8.3 Hz, 1H), 7.69 (s, 1H), 7.35 (d, J = 6.9 Hz, 2H), 7.22–7.07 (m, 3H), 5.57 (d, J = 7.9 Hz, 1H), 5.08 (d, J = 9.2 Hz, 1H), 5.04 (s, 1H), 4.86 (d, J = 9.2 Hz, 1H), 4.52 (s, 1H), 4.42 (dd, J = 7.3, 2.0 Hz, 1H), 4.14–4.03 (br m, 1H), 2.76–2.56 (m, 1H), 2.35 (d, J = 14.2 Hz, 1H), 2.22 (d, J = 14.2 Hz, 1H), 1.49 (s, 3H), 1.40 (s, 3H), 1.27 (s, 9H), 0.94 (s, 3H), 0.85 (s, 3H);

HRMS (FAB): m/z 522.2644 for $[M+H]^+$ (calcd 522.2638 for $C_{26}H_{40}N_3O_6S$).

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-(2,3-dimethlysuccinyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (2,3-dimethylsuccinyl-Apns-Dmt-NHBu^t, 1g, KNI-549). Compound **1g** was prepared from *meso*-2,3-dimethylsuccinic acid and compound **3b** in a manner similar to that described for compound **1c**. Yield 81%; mp 113–115 °C; 2 peaks on HPLC (Rt = 19.90 and 20.19 min for the two diastereomers); ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.23 (br s, 1H), 8.24–8.16 (m, 1H), 7.65 (s, 1H), 7.37–7.34 (m, 2H), 7.26–7.10 (m, 3H), 5.57 (d, J = 7.9 Hz, 1H), 5.17–5.06 (m, 1H), 4.92–4.87 (m, 1H), 4.44–4.36 (m, 1H), 4.20–4.07 (br m, 1H), 2.73–2.61 (m, 2H), 2.40–2.25 (m, 1H), 2.17–2.10 (m, 1H), 1.49 (s, 3H), 1.40 (s, 3H), 1.27 (s, 9H), 0.96–0.91 (m, 3H), 0.69 (d, J = 6.3 Hz, 1.5 H), 0.43 (d, J = 6.9 Hz, 1.5H); MS (FAB): m/z 522 $[M+H]^+$. Anal. calcd for $C_{26}H_{39}N_3O_6S\cdot 2/3\ H_2O$: C, 58.51; H, 7.62; N, 7.87. Found: C, 58.56; H, 7.63; N, 7.35.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-(3,3-dimethlysuccinyl)amino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (3,3-dimethylsuccinyl-Apns-Thz-NH-(2-Me)Bzl, 1h, KNI-689). Compound **1h** was prepared from 2,2-dimethylsuccinic anhydride and compound **3c** in a manner similar to that described for compound **1a**. Yield 90%; mp 103–104 °C; $[\alpha]_D^{27}$ −8.8° (c = 0.40, MeOH); ^{1}H NMR (270 MHz, DMSO-d_6) δ 8.40 (t, J = 5.6 Hz, 1H), 8.02 (d, J = 8.6 Hz, 1H), 7.30–7.12 (m, 9H), 5.43 (d, J = 6.0 Hz, 1H), 4.96 (d, J = 9.6 Hz, 1H), 4.85 (t, J = 6.6 Hz, 1H), 4.73 (d, J = 9.6 Hz, 1H), 4.43–4.36 (m, 1H), 4.33–4.25 (m, 2H), 4.22–4.16 (br m, 1H), 3.37 (dd, J = 11.6, 7.3 Hz, 1H), 3.07 (dd, J = 11.6, 5.9 Hz, 1H), 2.72–2.55 (m, 2H), 2.25 (s, 3H), 2.09 (s, 2H), 0.96 (s, 3H), 0.88 (s, 3H); HRMS (FAB): m/z 542.2321 for $[M+H]^+$ (calcd 542.2325 for $C_{28}H_{36}N_3O_6S$).

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-(2,3-dimethlysuccinyl)amino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (2,3-dimethylsuccinyl-Apns-Thz-NH-(2-Me)Bzl, 1i, KNI-690). Compound **1i** was prepared from *meso*-2,3-dimethylsuccinic acid and compound **3c** in a manner similar to that described for compound **1c**. Yield 89%; mp 99–101 °C; two peaks on HPLC (Rt = 19.03 min and 19.36 min for the two diastereomers); ^{1}H NMR (270 MHz, DMSO-d_6) δ 8.48–8.37 (m, 1H), 8.16 (d, J = 9.2 Hz, 0.5H), 8.12 (d, J = 9.2 Hz, 0.5 H), 7.31–7.10 (m, 9H), 5.01–4.96 (m, 1H), 4.92–4.83 (m, 1H), 4.80–4.72 (m, 1H), 4.43–4.32 (m, 2H), 4.26–4.14 (m, 2H), 3.40–3.31 (m, 1H), 3.10–3.04 (m, 1H), 2.74–2.58 (m, 2H), 2.35–2.31 (m, 1H), 2.25 (s, 3H), 2.18–2.15 (m, 1H), 0.95 (d, J = 6.6 Hz, 1.5H), 0.92 (d, J = 7.6 Hz, 1.5H), 0.72 (d, J = 5.9 Hz, 1.5H), 0.47 (d, J = 6.6 Hz, 1.5H); HRMS (FAB): m/z 542.2319 for $[M+H]^+$ (calcd 542.2325 for $C_{28}H_{36}N_3O_6S$).

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-(3,3-dimethlysuccinyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (3,3-dimethylsuccinyl-Apns-Dmt-NH-(2-Me)Bzl, 1j, KNI-852). Compound **1j** was prepared from 2,2-dimethylsuccinic anhydride and compound **3d** in a manner similar to that described for compound **1a**. Yield 92%; mp 100–102 °C; $[\alpha]_D^{21}$ +42.4° (c = 0.40, MeOH); ^{1}H NMR (270 MHz, DMSO-d_6) δ 8.41–8.37 (m, 1H), 8.04–8.01 (m, 1H), 7.32–7.10 (m, 9H), 5.07 (d, J = 9.2 Hz, 1H), 4.91 (d, J = 9.2 Hz, 1H), 4.51 (s, 1H), 4.46–4.31 (m, 2H), 4.21–4.14 (m, 2H), 2.71–2.54 (m, 2H), 2.26 (s, 3H), 2.24 (s, 2H), 1.50 (s, 3H), 1.36 (s, 3H), 0.96 (s, 3H), 0.87 (s, 3H); HRMS (FAB): m/z 570.2643 for $[M+H]^+$ (calcd 570.2638 for $C_{30}H_{40}N_3O_6S$).

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-(2,3-dimethlysuccinyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (2,3-dimethylsuccinyl-Apns-Dmt-NH-(2-Me)Bzl, 1k, KNI-691). Compound **1k** was prepared from *meso*-2,3-dimethysuccinic acid and compound **3d** in a manner similar to that described for compound **1c**. Yield 94%; mp 108–110 °C; two peaks on HPLC (Rt = 19.07 and 19.31 min for the two diastereomers); ^{1}H NMR (270 MHz, DMSO-d_6) δ 8.38–8.36 (m, 1H), 8.19–8.12 (m, 1H), 7.30–7.13 (m, 9H), 5.16–5.09 (m, 1H), 4.97–4.92 (m, 1H), 4.51 (s, 0.5H), 4.48 (s, 0.5H), 4.44–4.36 (m, 2H), 4.20–4.14 (m, 2H, partially covered by H_2O peaks), 2.78–2.60 (m, 2H), 2.41–2.33 (m, 1H), 2.28 (s, 3H), 2.19–2.09 (m, 1H), 0.95 (d, J = 6.6 Hz, 1.5H), 0.92 (d, J = 8.3 Hz, 1.5H), 0.71 (d, J = 6.3 Hz, 1.5H), 0.47 (d, J = 6.6 Hz, 1.5H); HRMS (FAB): m/z 570.2632 for $[M+H]^+$ (calcd 570.2638 for $C_{30}H_{40}N_3O_6S$).

3′-Azido-3′-deoxythymidine 5′-hemisuccinate (4a).[22] To a solution of AZT (267 mg, 1.00 mmol) in DMF (3 mL) were added succinic anhydride (120 mg, 1.2 mmol) and DMAP (24 mg, 0.2 mmol) at 0 °C, and the mixture was stirred at rt for 18 h. After the solvent was removed by evaporation, the residue was dissolved in EtOAc (30 mL) and washed with saturated NaCl. The organic layer was extracted with saturated $NaHCO_3$. The aqueous phase was acidified with citric acid to pH 3–4, and extracted with EtOAc (30 mL). The organic layer was washed with saturated NaCl, dried over anhydrous Na_2SO_4 and evaporated in vacuo. The residue was obtained as a colorless oil (350 mg, 95%) and was used without further purification in the next step: TLC R_f 0.48 ($CHCl_3$:MeOH:H_2O = 8:3:1, v/v); single peak on HPLC, Rt 9.69 min; ^{1}H NMR (270 MHz, DMSO-d_6) δ 11.34 (s, 1H), 7.45 (s, 1H), 6.15–6.10 (m, 1H), 4.48–4.41 (m, 1H), 4.33–4.21 (m, 2H), 3.99–3.94 (m, 1H), 2.57–2.52 (m, 2H), 2.45–2.30 (m, 4H), 1.80 (s, 3H); HRMS (FAB): m/z 368.1198 for $[M+H]^+$ (calcd 368.1206 for $C_{14}H_{18}N_5O_7$).

3′-Azido-3′-deoxythymidine 5′-2,3-dimethylhemisuccinate (4b). To a solution of 88 mg (0.6 mmol) of *meso*-2,3-dimethylsuccinic acid in DMF was added 103 mg (0.5 mmol) of DCC at 0 °C, and the mixture was stirred at rt for 5 h. After *N*,*N*′-dicyclohexylurea was removed by filtration, AZT (134 mg, 0.5 mmol) and DMAP (6.1 mg, 0.1 mmol) were added at 0 °C, and the solution was stirred at rt for 18 h. After the solvent was removed by evaporation, the residue was dissolved in EtOAc (30 mL), washed with saturated NaCl, and extracted with saturated $NaHCO_3$. The aqueous phase was acidified with citric acid to pH 3–4, and extracted with EtOAc (30 mL). The

organic layer was washed with saturated NaCl, dried over anhydrous Na_2SO_4 and evaporated in vacuo. The residue was obtained as colorless oil (150 mg, 76%) as a mixture of 2*R*,3*S*-dimethylsuccinate and 2*S*,3*R*-dimethylsuccinate and was used without further purification in the next step: TLC R_f 0.62 ($CHCl_3$:MeOH:H_2O = 8:3:1, v/v); single peak on HPLC, Rt 14.10 min; ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.38 (s, 1H), 11.36 (s, 1H), 7.44 (d, J = 1.2 Hz, 1H), 6.15–6.10 (m, 1H), 4.49–4.42 (m, 1H), 4.27–4.21 (m, 2H), 3.99–3.96 (m, 1H), 2.76–2.55 (m, 2H), 2.46–2.31 (m, 2H), 1.80 (s, 3H), 1.12–1.02 (m, 6H); HRMS (FAB): m/z 396.1513 for $[M+H]^+$ (calcd 396.1519 for $C_{16}H_{22}N_5O_7$).

3′ - Azido - 3′ - deoxythymidine 5′ - hemiglutarate (4c).[22b] Compound **4c** was prepared from glutaric anhydride in a manner similar to that described for compound **4a**. Compound **4c** was obtained as colorless oil (yield 82%) and used without further purification in the next step: TLC R_f 0.51 ($CHCl_3$:MeOH:H_2O = 8:3:1, v/v); single peak on HPLC, Rt 11.32 min; ^{1}H NMR (270 MHz, DMSO-d_6) δ 11.36 (s, 1H), 7.45 (d, J = 1.2 Hz, 1H), 6.13 (t, J = 6.5 Hz, 1H), 4.50–4.43 (m, 1H), 4.30–4.20 (m, 2H), 4.01–3.95 (m, 1H), 2.45–2.30 (m, 4H), 2.26 (t, J = 7.4 Hz, 2H), 1.80 (s, 3H), 1.75 (t, J = 7.3 Hz, 2H); HRMS (FAB): m/z 382.1368 for $[M+H]^+$ (calcd 382.1363 for $C_{15}H_{20}N_5O_7$).

3′-Azido-3′-deoxythymidine 5′-3,3-dimethylhemiglutarate (4d). Compound **4d** was prepared from 3,3-dimethylglutaric anhydride in a manner similar to that described for compound **4a**. Compound **4d** was obtained as colorless oil (yield 73%) and used without further purification in the next step: TLC R_f 0.57 ($CHCl_3$:MeOH:H_2O = 8:3:1, v/v); single peak on HPLC, Rt 15.19 min; ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.07 (s, 1H), 11.36 (s, 1H), 7.45 (d, J = 1.0 Hz, 1H), 6.13 (t, J = 6.5 Hz, 1H), 4.48–4.42 (m, 1H), 4.32–4.19 (m, 1H), 4.01–3.96 (m, 1H), 2.46 (d, J = 5.4 Hz, 2H), 2.41–2.33 (m, 2H), 2.29 (s, 2H), 1.79 (s, 3H), 1.05 (s, 6H); HRMS (FAB): m/z 410.1682 for $[M+H]^+$ (calcd 410.1676 for $C_{17}H_{24}N_5O_7$).

***p*-Methoxybenzyl-3,3-dimethylhemisuccinate (5).** To a solution of 2,2-dimethylsuccinic anhydride (1.0 g, 7.80 mmol) in THF:ether (1:2, 6 mL) were added *p*-methoxybenzyl alcohol (0.81 mL, 6.50 mmol) and dicyclohexylamine (1.55 mL, 7.80 mmol) at 0 °C, and the mixture was stirred at 4 °C for 18 h. After the solvent was removed in vacuo, the residue was dissolved in EtOAc (50 mL), washed with 10% citric acid and saturated NaCl, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The product was obtained (2.0 g, 97%, as colorless oil) as a mixture of two diastereomers: *p*-methoxybenzyl-3,3-dimethylsuccinate (**5**) and *p*-methoxybenzyl-2,2-dimethylsuccinate. The ratios of the two diastereomers were estimated by HPLC. The desired isomer (**5**) was predominantly obtained (4:1). The product was directly used for the next step without further purification: TLC R_f 0.45 and 0.42 for the two diastereomers ($CHCl_3$:MeOH = 10:1, v/v); 2 peaks on HPLC, Rt = 19.90 and 20.34 min (1:4) for the two diastereomers; HRMS(FAB) m/z 266.1158 for $[M]^+$ (calcd 266.1154 for $C_{14}H_{18}O_5$).

AZT-[3,3-dimethylsuccinyl-*p*-methoxybenzoate] (6). To a solution of compound **5** (1.04 g, 3.9 mmol) in DMF (10 mL) were added AZT (1.04 g, 4.0 mmol), DCC (0.81 g, 4.0 mmol) and DMAP (47.6 mg, 0.4 mmol) at 0 °C and the mixture was stirred for 36 h at rt. After filtration, the filtrate was concentrated in vacuo, and the residue was extracted with EtOAc. The organic layer was washed with 10% citric acid, 5% $NaHCO_3$ and saturated NaCl, dried over anhydrous Na_2SO_4, and concentrated in vacuo to give **6**. The crude product was purified by silica gel column chromatography with $CHCl_3$–MeOH and compound **6** was obtained (1.22 g, 60.7%, as colorless oil) as a mixture of diastereomers. The product was directly used for the next step without further purification: TLC R_f 0.54 ($CHCl_3$:MeOH = 10:1, v/v); single peak on HPLC, Rt = 21.64 min; HRMS (FAB) m/z 516.2103 for $[M+H]^+$ (calcd 516.2094 for $C_{24}H_{30}N_5O_8$).

3′-Azido-3′-deoxythymidine 5′-2,2-dimethylhemisuccinate (4e). To compound **6** (1.21 g, 2.37 mmol) was added anisole (0.515 mL, 2.6 mmol) and the mixture was stirred in trifluoroacetic acid (4 mL) for 1 h at rt. After removal of the TFA in vacuo, the residue was dissolved in EtOAc (30 mL) and washed with brine. The organic layer was extracted with saturated $NaHCO_3$. The aqueous layer was acidified with citric acid until pH 4, and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The product **4e** was obtained (160 mg, 31%, colorless oil) as a mixture of two diastereomers: 3′-azido-3′-deoxythymidine 5′-2,2-dimethylhemisuccinate (**4e**) and 3′-azido-3′-deoxythymidine 5′-3,3-dimethylhemisuccinate. The ratios of the two diastereomers were estimated by HPLC. The desired isomer (**4e**) was predominantly obtained (4:1). The product was used for the next step without further purification: TLC R_f 0.37 and 0.32 for the two diastereomers ($CHCl_3$:MeOH = 10:1, v/v); two peaks on HPLC, Rt = 11.04 and 11.43 min (1:4) for the two diastereomers; HRMS (FAB): m/z 396.1526 for $[M]^+$ (calcd 396.1519 for $C_{16}H_{22}N_5O_7$).

{(2*S*,5*R*)-3-Azido-5-[5-methyl-2,4-dioxo-3,4-dihydro-1(2H)-pyrimidinyl]tetrahydro-2-furanyl}methyl 4-[((1*S*,2*S*)-1-benzyl-3-{(4*R*)-4-[(*tert*-butylamino)carbonyl]-1,3-thiazolidin-3-yl}-2-hydroxy-3-oxopropyl)amino]-4-oxobutanoate (AZT-succinyl-Apns-Thz-NHBu^t, 2a, KNI-679). To the stirred solution of AZT-hemisuccinate **4a** (170 mg, 0.46 mmol) in DMF were added intermediate **3a** (154 mg, 0.42 mmol), HOBt (77 mg, 0.5 mmol), triethylamine (128.8 μL, 0.92 mmol) and BOP (205 mg, 0.46 mmol), and the solution was stirred for 18 h. After removal of the solvent in vacuo, the residue was dissolved in EtOAc (30 mL), washed with 5% $NaHCO_3$, 10% citric acid and saturated NaCl, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was further purified by preparative HPLC and reprecipitated from *n*-hexane to give 166 mg (75%) of **2a** as a white solid. Mp 105–106 °C; $[\alpha]_D^{24}$ −22.6° (c = 0.49, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.36 (s, 1H), 8.22 (d, J = 8.1 Hz, 1H), 7.70 (s, 1H), 7.43 (d, J = 1.3 Hz, 1H), 7.21 (d, J = 7.1 Hz, 2H), 7.18–7.11 (m, 3H), 6.12 (t, 1H), 4.92 (d, J = 9.5 Hz, 1H), 4.76 (t, J = 7.0 Hz, 1H), 4.63 (d,

$J=9.5$ Hz, 1H), 4.46–4.40 (m, 2H), 4.30 (dd, $J=12.9$, 4.8 Hz, 1H), 4.19–4.15 (m, 1H), 4.10–4.18 (m, 1H), 3.96–3.93 (m, 1H), 3.35–3.30 (m, 1H), 3.00–2.96 (m, 1H), 2.68–2.56 (m, 2H), 2.46–2.26 (m, 6H), 1.71 (s, 3H), 1.25 (s, 9H); MS (FAB): m/z 715 $[M+H]^+$. Anal. calcd for $C_{32}H_{42}N_8O_9S\cdot CF_3COOH$: C, 49.27; H, 5.23; N, 13.52. Found: C, 49.50; H, 5.48; N, 13.77.

AZT-[3,3-dimethylsuccinyl-Apns-Thz-NHBu^t] (2b, KNI-680). Compound **2b** was prepared via two routes. As in Scheme 4: compound **2b** was prepared from AZT-2,2-dimethylhemisuccinate **4e** and compound **3a** in a manner similar to that described for compound **2a** in 60% yield. As in Scheme 5: to a solution of dipeptide inhibitor **1b** (200 mg, 0.41 mmol) in $CHCl_3$ were added AZT (130 mg, 0.49 mmol), DMAP (25 mg, 0.20 mmol) and DCC (92 mg, 0.45 mmol), and the solution was stirred for 18 h at rt. After filtration, the filtrate was concentrated in vacuo, and the residue was dissolved in EtOAc, washed with 10% citric acid, 5% $NaHCO_3$ and saturated NaCl, dried over anhydrous Na_2SO_4, and concentrated in vacuo. Purification of the product by preparative HPLC and reprecipitation from *n*-hexane gave 12 mg of the title compound as a white solid. Yield 8%; mp 83–84 °C; $[\alpha]_D^{22}$ −98.9° ($c=0.41$, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.36 (s, 1H), 7.71 (s, 1H), 7.60 (d, $J=8.2$ Hz, 1H), 7.45 (s, 1H), 7.37 (d, $J=7.3$ Hz, 2H), 7.21–7.10 (m, 3H), 6.15–6.11 (m, 3H), 5.20 (s, 1H), 4.89 (d, $J=9.3$ Hz, 1H), 4.77 (m, 1H), 4.66 (d, $J=9.3$ Hz, 1H), 4.43–4.35 (m, 2H), 4.27–4.23 (m, 1H), 4.14–4.10 (m, 2H), 3.93 (dd, $J=9.5$, 4.8 Hz, 1H), 3.32 (dd, $J=11.5$, 7.5 Hz, 1H), 3.01–2.96 (m, 1H), 2.80–2.59 (m, 2H), 2.47 (s, 2H), 2.43–2.28 (m, 2H), 2.47 (s, 2H), 2.43–2.28 (m, 2H), 1.72 (s, 3H), 1.26 (s, 9H), 1.16 (s, 3H), 1.01 (s, 3H); HRMS (FAB): m/z 743.3176 for $[M+H]^+$ (calcd 743.3176 for $C_{34}H_{47}N_8O_9S$).

AZT-[2,3-dimethylsuccinyl-Apns-Thz-NHBu^t] (2c, KNI-681). Compound **2c** was prepared from AZT-2,3-dimethylhemisuccinate **4b** and compound **3a** in a manner similar to that described for compound **2a**, and obtained as a mixture of 2*R*,3*S*-dimethylsuccinyl derivative and 2*S*,3*R*-dimethylsuccinyl derivative. Yield 80%: mp 102–103 °C; two peaks on HPLC (Rt = 21.38 min and 21.75 min for the two diastereomers); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.37 (s, 1H), 8.24 (d, $J=7.8$ Hz, 0.5 H), 8.22 (d, $J=7.7$ Hz, 0.5H), 7.72–7.71 (m, 0.5H), 7.69 (s, 0.5 H), 7.43 (d, $J=1.0$ Hz, 0.5H), 7.42 (d, $J=0.9$ Hz, 0.5 H), 7.36 (d, $J=7.9$ Hz, 2H), 7.22–7.07 (m, 3H), 6.15–6.10 (m, 1H), 4.96 (m, 1H), 4.77 (t, $J=7.0$ Hz, 1H), 4.71 (d, $J=9.3$ Hz, 0.5H), 4.67 (d, $J=9.5$ Hz, 0.5H), 4.45–4.41 (m, 2H), 4.28–4.23 (m, 2H), 4.20–4.05 (m, 1H), 3.99–3.94 (m, 1H), 3.35–3.29 (m, 1H), 2.99 (dd, $J=116$, 6.3 Hz, 1H0, 2.70–2.55 (m, 2H), 2.46–2.28 (m, 4H), 1.77 (d, $J=0.9$ Hz, 1.5H), 1.76 (s, 1.5H), 1.26 (s, 4.5H), 1.25 (s, 4.5H), 0.99 (d, $J=6.0$ Hz, 1.5H), 0.91 (d, $J=6.6$ Hz, 1.5H), 0.68 (d, $J=6.0$ Hz, 1.5H), 0.47 (d, $J=6.6$ Hz, 1.5H); MS (FAB): $m/z=743$ $[M+H]^+$. Anal. calcd for $C_{34}H_{46}N_8O_9S\cdot CF_3COOH\cdot 3H_2O$: C, 47.47; H, 5.86; N, 12.30. Found: C, 47.04; H, 5.40; N, 12.04.

AZT-[glutaryl-Apns-Thz-NHBu^t] (2d, KNI-682). Compound **2d** was prepared from AZT-hemiglutarate **4c** and compound **3a** in a manner similar to that described for compound **2a**. Yield 85%: mp 100–101 °C; $[\alpha]_D^{21}$ −23.2° ($c=0.40$, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.37 (s, 1H), 8.10 (d, $J=8.4$ Hz, 1H), 7.70 (s, 1H), 7.44 (d, $J=1.3$ Hz, 1H), 7.34 (d, $J=7.1$ Hz, 2H), 7.20–7.08 (m, 3H), 6.13 (m, 1H), 4.96 (d, $J=9.5$ Hz, 1H), 4.76 (m, 1H), 4.65 (d, $J=9.3$ Hz, 1H), 4.47–4.43 (m, 2H, 4.27–4.20 (m, 2H), 4.14–4.09 (m, 2), 3.99–3.95 (m, 1H), 3.35–3.30 (m, 1H), 2.99 (dd, $J=11.7$, 6.6 Hz, 1H), 2.68–2.54 (m, 2H), 2.46–2.41 (m, 1H), 2.37–2.30 (m, 1H), 2.16–2.12 (m, 2H), 2.06–2.03 (m, 2H), 1.77 (d, $J=0.9$ Hz, 3H), 1.65–1.58 (m, 2H), 1.26 (s, 9H); MS (FAB): m/z 729 $[M+H]^+$. Anal. calcd for $C_{33}H_{44}N_8O_9S\cdot CF_3COOH\cdot H_2O$: C, 48.83; H, 5.50; N, 13.02. Found: C, 49.18; H, 5.43; N, 12.83.

AZT-[3,3-dimethylglutaryl-Apns-Thz-NHBu^t] (2e, KNI-683). Compound **2e** was prepared from AZT-3,3-dimethylhemiglutarate **4d** and compound **3a** in a manner similar to that described for compound **2a**. Yield 85%: mp 90–92 °C; $[\alpha]_D^{21}$ −10.1° ($c=0.51$, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.35 (s, 1H), 8.06 (d, $J=8.2$ Hz, 1H), 7.71 (s, 1H), 7.46 (d, $J=0.9$ Hz, 1H), 7.36 (d, $J=7.3$ Hz, 2H), 7.21–7.15 (m, 2H), 7.09 (t, $J=7.3$ Hz, 1H), 6.15 (t, $J=6.5$ Hz, 1H), 4.93 (d, $J=9.5$ Hz, 1H), 4.76 (t, $J=7.0$ Hz, 1H), 4.67 (d, $J=9.3$ Hz, 1H), 4.48–4.44 (m, 2H), 4.30 (dd, $J=12.1$, 4.9 Hz, 1H), 4.17–4.13 (m, 2H), 3.95 (dd, $J=9.2$, 5.1 Hz, 1H), 3.35–3.30 (m, 1H), 2.96 (dd, $J=11.7$, 6.6 Hz, 1H), 2.67–2.57 (m, 2H), 2.47–2.40 (m, 1H), 2.36–2.29 (m, 1H), 2.27–2.03 (m, 4H), 1.75 (s, 3H), 1.25 (s, 9H), 0.87 (s, 3H), 0.69 (s, 3H); MS (FAB): m/z 757 $[M+H]^+$. Anal. calcd for $C_{35}H_{48}N_8O_9S\cdot CF_3COOH$: C, 51.03; H, 5.67; N, 12.87. Found: C, 51.18; H, 6.06; N, 12.38.

AZT-[3,3-dimethylsuccinyl-Apns-Dmt-NHBu^t] (2f, KNI-684). Compound **2f** was prepared via two routes. As in Scheme 4: compound **2f** was prepared from AZT-2,2-dimethylhemisuccinate **4e** and compound **3b** in a manner similar to that described for compound **2a** in 74% yield. As in Scheme 5: compound **2f** was prepared from AZT and dipeptide inhibitor **1f** in a manner similar to that described for compound **2b** in 8.9% yield: mp 95–97 °C; $[\alpha]_D^{22}$ −56.5° ($c=0.50$, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.36 (s, 1H), 7.67 (s, 1H), 7.57 (d, $J=$ 8.4 Hz, 1H), 7.45 (d, $J=1.1$ Hz, 1H), 7.36 (d, $J=7.1$ Hz, 2H), 7.22–7.16 (m, 2H), 7.12 (t, $J=7.3$ Hz, 1H), 6.14–6.11 (m, 1H), 5.05 (d, $J=9.0$ Hz, 1H), 4.82 (d, $J=9.0$ Hz, 1H), 4.51 (s, 1H), 4.42–4.37 (m, 2H), 4.25–4.21 (m, 1H), 4.14–4.10 (m, 2H), 3.93 (dd, $J=9.5$, 5.1 Hz, 1H), 2.78–2.72 (m, 1H), 2.64–2.60 (m, 1H), 2.46 (s, 2H), 2.45–2.38 (m, 1H), 2.34–2.28 (m, 1H), 1.74 (d, $J=0.9$ Hz, 3H), 1.48 (s, 3H), 1.39 (s, 3H), 1.27 (s, 9H), 1.15 (s, 3H), 1.00 (s, 3H); HRMS (FAB): m/z 771.3508 for $[M+H]^+$ (calcd 771.3488 for $C_{36}H_{51}N_8O_9S$).

AZT-[2,3-dimethylsuccinyl-Apns-Dmt-NHBu^t] (2g, KNI-685). Compound **2g** was prepared from AZT-2,3-dimethylhemisuccinate **4b** and compound **3b** in a manner similar to that described for compound **2a**, and obtained as a mixture of 2*R*,3*S*-dimethylsuccinyl derivative and 2*S*,3*R*-dimethylsuccinyl derivative. Yield 46%: mp 110–112 °C; 2 peaks on HPLC (Rt = 23.66 and

24.00 min for the two diastereomers); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.38 (s, 1H), 8.25–8.21 (m, 1H), 7.68 (s, 0.5H), 7.67 (s, 0.5H), 7.43 (d, J = 1.1 Hz, 0.5H), 7.41 (d, J = 1.1 Hz, 0.5H), 7.35 (d, J = 7.9 Hz, 2H), 7.25–7.07 (m, 3H), 6.14–6.10 (m, 1H), 5.13 (d, J = 8.9 Hz, 0.5H), 5.08 (d, J = 8.9 Hz, 0.5H), 4.90 (d, J = 8.9 Hz, 0.5H), 4.88 (d, J = 8.9 Hz, 0.5H), 4.52 (s, 0.5H), 4.50 (s, 0.5H), 4.45–4.37 (m, 2H), 4.28–4.23 (m, 2H), 4.19–4.08 (m, 1H), 3.99–3.93 (m, 1H), 2.72–2.56 (m, 2H), 2.46–2.28 (m, 4H), 1.77 (d, J = 1.0 Hz, 1.5H), 1.75 (d, J = 1.0 Hz, 1.5 H), 1.49 (s, 1.5H), 1.48 (s, 1.5H), 1.40 (s, 3H), 1.27 (s, 4.5H), 1.26 (s, 4.5H), 0.98 (d, J = 6.2 Hz, 1.5H), 0.91 (d, J = 6.6 Hz, 1.5H), 0.67 (d, J = 6.2 Hz, 1.5H), 0.45 (d, J = 6.6 Hz, 1.5H); HRMS (FAB): m/z 771.3504 for $[M+H]^+$ (calcd 771.3488 for $C_{36}H_{51}N_8O_9S$).

AZT-[3,3-dimethylsuccinyl-Apns-Thz-NH-(2-Me)Bzl] (2h, KNI-692). Compound **2h** was prepared via two routes. As in Scheme 4: compound **2h** was prepared from AZT-2,2-dimethylhemisuccinate **4e** and compound **3c** in a manner similar to that described for compound **2a** in 23% yield. As in Scheme 5: compound **2h** was prepared from AZT and dipeptide inhibitor **1h** in a manner similar to that described for compound **2b**. Yield 3.8%: mp 102–103 °C; $[\alpha]_D^{21}$ −4.8° (c = 0.15, MeOH); 22% yield; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.36 (s, 1H), 8.40 (m, 1H), 7.56 (d, J = 8.2 Hz, 1H), 7.45 (d, J = 1.1 Hz, 1H), 7.31–7.25 (m, 3H), 7.20–7.10 (m, 6H), 6.13 (t, J = 6.5 Hz, 1H), 4.92 (d, J = 9.2 Hz, 1H), 4.84 (m, 1H), 4.71 (d, J = 9.5 Hz, 1H), 4.43–4.33 (m, 3H), 4.26–4.11 (m, 4H), 3.94 (dd, J = 9.3, 5.3 Hz, 1H), 3.36 (dd, J = 11.7, 7.5 Hz, 1H), 3.07 (dd, J = 11.7, 5.9 Hz, 1H), 2.81–2.65 (m, 2H), 2.46 (s, 2H), 2.44–2.28 (m, 2H), 2.25 (s, 3H), 1.74 (s, 3H), 1.15 (s, 3H), 1.01 (s, 3H); HRMS (FAB): m/z 813.3015 for $[M+Na]^+$ (calcd 813.3006 for $C_{38}H_{46}N_8O_9SNa$).

AZT-[2,3-dimethylsuccinyl-Apns-Thz-NH-(2-Me)Bzl] (2i, KNI-693). Compound **2i** was prepared from AZT-2,3-dimethylhemisuccinate **4b** and compound **3c** in a manner similar to that described for compound **2a**, and obtained as a mixture of 2*R*,3*S*-dimethylsuccinyl derivative and 2*S*,3*R*-dimethylsuccinyl derivative. Yield 41%: mp 114–115 °C; 2 peaks on HPLC (Rt = 21.15 min and 21.44 min for the two diastereomers); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.37 (s, 1H), 8.43–8.37 (m, 1H), 8.17 (d, J = 8.2 Hz, 1H), 7.42 (m, 1H), 7.29–7.26 (m, 3H), 7.23–7.07 (m, 6H), 6.12 (t, J = 6.6 Hz, 0.5H), 6.11 (t, J = 6.6 Hz, 0.5H), 4.99 (d, J = 9.2 Hz, 0.5H), 4.97 (d, J = 9.2 Hz, 0.5H), 4.87–4.82 (m, 1H), 4.77 (d, J = 9.5 Hz, 0.5H), 4.73 (d, J = 9.5 Hz, 0.5H), 4.44–4.31 (m, 3H), 4.26–4.12 (m, 4H), 3.99–3.93 (m, 1H), 3.39–3.32 (m, 0.5H, partially covered by DMSO peaks), 3.09–3.05 (m, 0.5H), 2.77–2.56 (m, 2H), 2.46–2.29 (m, 4H), 2.26 (s, 1.5H), 2.25 (s, 1.5H), 1.77 (s, 1.5H), 1.75 (d, J = 0.9 Hz, 1.5H), 0.99 (d, J = 6.2 Hz, 1.5H), 0.91 (d, J = 6.4 Hz, 1.5H), 0.69 (d, J = 6.0 Hz, 1.5H), 0.49 (d, J = 6.6 Hz, 1.5H); MS (FAB): m/z = 791 $[M+H]^+$. Anal. calcd for $C_{38}H_{46}N_8O_9S\cdot CF_3COOH$: C, 53.09; H, 5.24; N, 12.38. Found: C, 52.98; H, 5.49; N, 12.14.

AZT-[3,3-dimethylsuccinyl-Apns-Dmt-NH-(2-Me)Bzl] (2j, KNI-694). Compound **2j** was prepared via two routes. As in Scheme 4: compound **2j** was prepared from AZT-2,2-dimethylhemisuccinate **4e** and compound **3d** in a manner similar to that described for compound **2a** in 18% yield. As in Scheme 5: compound **2j** was prepared from AZT and dipeptide inhibitor **1j** in a manner similar to that described for compound **2b**. Yield 7.5%: mp 107–108 °C; $[\alpha]_D^{19}$ +38.3° (c = 0.06, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.36 (s, 1H), 8.39 (m, 1H), 7.56 (d, J = 8.4 Hz, 1H), 7.45 (d, J = 1.1 Hz, 1H), 7.33–7.29 (m, 3H), 7.20–7.10 (m, 6H), 6.12 (m, 1H), 5.04 (d, J = 9.3 Hz, 1H), 4.88 (d, J = 9.3 Hz, 1H), 4.50 (s, 1H), 4.45–4.36 (m, 3H), 4.24–4.11 (m, 4H), 3.94 (dd, J = 9.5, 5.3 Hz, 1H), 2.80–2.64 (m, 2H), 2.47 (s, 2H), 2.43–2.38 (m, 1H), 2.34–2.29 (m, 1H), 2.27 (s, 3H), 1.75 (d, J = 0.7 Hz, 3H), 1.50 (s, 3H), 1.35 (s, 3H), 1.14 (s, 3H), 1.02 (s, 3H); HRMS (FAB): m/z 841.3312 for $[M+Na]^+$ (calcd 841.3319 for $C_{40}H_{50}N_8O_9SNa$). Anal. calcd for $C_{40}H_{50}N_8O_9S\cdot CF_3COOH\cdot 1.5H_2O$: C, 52.55; H, 5.67; N, 11.67. Found: C, 52.54; H, 5.58; N, 11.07.

AZT-[2,3-dimethylsuccinyl-Apns-Dmt-NH-(2-Me)Bzl] (2k, KNI-695). Compound **2k** was prepared from AZT-2,3-dimethylhemisuccinate **4b** and compound **3d** in a manner similar to that described for compound **2a**, and obtained as a mixture of 2*R*,3*S*-dimethylsuccinyl derivative and 2*S*,3*R*-dimethylsuccinyl derivative. Yield 68%: mp 116–118 °C; two peaks on HPLC (Rt = 25.36 and 25.75 min for the two diastereomers); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.37 (s, 1H), 8.40–8.35 (m, 1H), 8.18 (d, J = 8.8 Hz, 1H), 7.43 (d, J = 6.3 Hz, 0.5H), 7.42 (d, J = 6.3 Hz, 0.5H), 7.32–7.27 (m, 3H), 7.22–7.07 (m, 6H), 6.12 (t, J = 6.8 Hz, 0.5H), 6.11 (t, J = 6.8 Hz, 0.5H), 5.14 (d, J = 9.1 Hz, 0.5H), 5.09 (d, J = 9.1 Hz, 0.5H), 4.95 (d, J = 9.0 Hz, 0.5 H), 4.93 (d, J = 9.0 Hz, 0.5H), 4.50 (s, 0.5H), 4.47 (s, 0.5H), 4.46–4.36 (m, 3H), 4.26–4.10 (m, 4H), 3.99–3.93 (m, 1H), 2.77–2.55 (m, 2H), 2.46–2.29 (m, 4H), 2.27 (s, 1.5H), 2.26 (s, 1.5H), 1.77 (d, J = 0.9 Hz, 1.5H), 1.76 (d, J = 0.9 Hz, 1.5H), 1.50 (s, 1.5H), 1.49 (s, 1.5H), 1.35 (s, 1.5H), 1.34 (s, 1.5H), 0.98 (d, J = 6.2 Hz, 1.5H), 0.90 (d, J = 6.4 Hz, 1.5H), 0.68 (d, J = 6.2 Hz, 1.5H), 0.48 (d, J = 6.5 Hz, 1.5H); MS (FAB): m/z = 819 $[M+H]^+$. Anal. calcd for $C_{40}H_{50}N_8O_9S\cdot CF_3$ COOH: C, 54.07; H, 5.51; N, 12.01. Found: C, 53.95; H, 5.70; N, 11.73.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-(succinimidyl)-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (7). To the solution of compound **1f** (50 mg, 0.10 mmol) in DMF was added *N*-ethyl-*N*′-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC·HCl) (20 mg, 0.11 mmol) and the mixture was stirred for 18 h at rt. After removal of the solvent in vacuo, the residue was dissolved in EtOAc, washed with 10% citric acid, 5% $NaHCO_3$ and saturated NaCl, dried over anhydrous Na_2SO_4, and concentrated in vacuo. Purification of the product by preparative HPLC and reprecipitation from *n*-hexane gave 20 mg of the title compound as a white solid. Yield 43%: mp 93–94 °C; TLC R_f 0.67 ($CHCl_3$:MeOH = 10:1, v/v); ^{1}H NMR (270 MHz, DMSO-d_6) δ 7.53 (s, 1H), 7.25–7.13 (m, 3H), 7.08–7.04 (m, 2H), 4.95–4.89 (m, 2H), 4.70 (m, 1H), 4.56 (m, 1H), 4.34 (s, 1H), 3.27–3.21 (m, 1H), 3.10–3.00 (m, 1H), 2.33 (m, 2H), 1.42 (s, 3H), 1.30 (s, 3H), 1.19 (s, 9H), 0.94 (s, 6H);

HRMS (FAB): m/z 504.2538 for $[M+H]^+$ (calcd 504.2532 for $C_{26}H_{38}N_3O_5S$).

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References and Notes

1. (a) Kohl, N. E.; Emini, E. A.; Schleif, W. A.; Davis, L. J.; Heimbach, J. C.; Dixon, R. A. F.; Scolnick, E. M.; Sigal, I. S. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 4686. (b) Norbeck, D. W.; Kempf, D. J. *Annu. Rep. Med. Chem.* **1991**, *26*, 141. (c) Huff, J. R. *J. Med. Chem.* **1991**, *34*, 2305. (d) Martin, J. A. *Antiviral Res.* **1992**, *17*, 265. (e) Schinazi, R. F. *Perspect. Drug Discovery Des.* **1993**, *1*, 151. (f) Thaisrivaongs, S. *Annu. Rep. Med. Chem.* **1994**, *29*, 133. (g) Kempf, D. J. *Perspect. Drug Discovery Des.* **1994**, *2*, 427. (h) Darke, P. L.; Huff, J. R. *Adv. Pharmacol.* **1995**, *25*, 399. (i) De Clercq, E. *J. Med. Chem.* **1995**, *38*, 2491. (j) West, M. L.; Fairlie, D. P. *Trends Pharmacol. Sci.* **1995**, *16*, 67. (k) Helm, K. *Biol. Chem.* **1996**, *377*, 765. (l) Kempf, D. J.; Sharm, H. L. *Curr. Pharm. Des.* **1996**, *2*, 225.

2. (a) Pollard, R. B. *Pharmacotherapy* **1994**, *14*, 21S. (b) Ho, D. D.; Neumann, A. U.; Perelson, A. S.; Chen, W.; Leonard, J. M.; Markowitz, M. *Nature* **1995**, *373*, 123. (c) Vacca, J. P.; Condra, J. H. *Drug Discovery Today* **1997**, *2*, 261. (d) De Clercq, E. *Clin. Microbiol. Rev.* **1997**, *10*, 674. (e) Molla, A.; Granneman, G. R.; Sun, E.; Kempf, D. J. *Antiviral Res.* **1998**, *39*, 1.

3. (a) Craig, J. C.; Whittaker, L.; Duncan, I. B.; Roberts, N. *Antiviral Chem. Chemother.* **1994**, *5*, 380. (b) Molla, A.; Japour, A. *Curr. Opin. Infect. Dis.* **1997**, *10*, 491. (c) Gulick, R.; Mellors, J.; Havlir, D.; Eron, J.; Gonzalez, C.; McMahon, D.; Richman, D.; Valentine, F.; Jonas, L.; Meibohm, A.; Emini, E.; Chodakewitz, J. *New Engl. J. Med.* **1997**, *337*, 734. (d) Mathez, D.; Bagnarelli, P.; Gorin, I.; Katalama, C.; Pialoux, G.; Saimot, G.; Tubiana, P.; De Truchis, P.; Chauvin, J.-P.; Mills, R.; Rode, R.; Clementi, M.; Leibowitch, J. *Antiviral Ther.* **1997**, *2*, 175. (e) Autran, B.; Carcelain, G.; Li, T. S.; Blanc, C.; Mathez, D.; Tubiana, R.; Katalaba, C.; Debre, P.; Leibowitch, J. *Science* **1998**, *277*, 112. (f) Vandamme, A. M.; Van Vaerenbergh, K.; De Clercq, E. *Antiviral Chem. Chemother.* **1998**, *9*, 187.

4. (a) Mimoto, T.; Imai, J.; Tanaka, S.; Hattori, N.; Takahashi, O.; Kisanuki, S.; Nagano, Y.; Shintani, M.; Hayashi, H.; Sakikawa, H.; Akaji, K.; Kiso, Y. *Chem. Pharm. Bull.* **1991**, *39*, 2465. (b) Mimoto, T.; Imai, J.; Tanaka, S.; Hattori, N.; Kisanuki, S.; Akaji, K.; Kiso, Y. *Chem. Pharm. Bull.* **1991**, *39*, 3088.

5. (a) Mimoto, T.; Imai, J.; Kisanuki, S.; Enomoto, H.; Hattori, N.; Akaji, K.; Kiso, Y. *Chem. Pharm. Bull.* **1992**, *40*, 2251. (b) Kageyama, S.; Mimoto, T.; Murakawa, Y.; Nomizu, M.; Ford, H. Jr.; Shirasaka, T.; Gulnik, S.; Erickson, J.; Takada, K.; Hayashi, H.; Broder, S.; Kiso, Y.; Mitsuya, H. *Antimicrob. Agents Chemother.* **1993**, *37*, 810. (c) Mimoto, T.; Hattori, N.; Takaku, H.; Kisanuki, S.; Fukazawa, T.; Terashima, K.; Kato, R.; Nojima, S.; Misawa, S.; Ueno, T.; Imai, J.; Emonoto, H.; Tanaka, S.; Sakikawa, H.; Shintani, M. Hayashi, H.; Kiso, Y. *Chem. Pharm. Bull.* **2000**, *48*, 1310.

6. Kiso, Y. *Biopolymers* **1996**, *40*, 235.

7. (a) Baldwin, E. T.; Bhat, T. N.; Gulnik, S.; Liu, B.; Topol, I. A.; Kiso, Y.; Mimoto, T.; Mitsuya, H.; Erickson, J. W. *Structure* **1995**, *3*, 581. (b) Wang, Y. X.; Freedberg, D. I.; Yamazaki, T.; Wingfield, P. T.; Stahl, S. J.; Kaufman, J. D.; Kiso, Y.; Torchia, D. A. *Biochemistry* **1996**, *35*, 9945. (c) Wang, Y. X.; Freedberg, D. I.; Wingfield, R. T.; Stahl, S. J.; Kaufman, J. D.; Kiso, Y.; Bhat, T. N.; Erickson, J. W.; Torchia, D. A. *J. Am. Chem. Soc.* **1996**, *118*, 12287. (d) Ohno, Y.; Kiso, Y.; Kobayashi, Y. *Bioorg. Med. Chem.* **1996**, *4*, 1565.

8. Kiso, Y.; Matsumoto, H.; Mizumoto, S.; Kimura, T.; Fujiwara, Y.; Akaji, K. *Biopolymers* **1999**, *51*, 59.

9. (a) Kiso, Y.; Yamaguchi, S.; Matsumoto, H.; Mimoto, T.; Kato, R.; Nojima, S.; Takaku, H.; Fukazawa, T.; Kimura, T.; Akaji, K. *Arch. Pharm. Pharm. Med. Chem.* **1998**, *331*, 87. (b) Mitoguchi, T.; Nakata, S.; Enomoto, H.; Kimura, T.; Akaji, K.; Hattori, N.; Kato, R.; Mimoto, T.; Kiso, Y. In *Peptide Chemistry 1995*; Nishi, N., Ed.; Protein Res. Found.: Osaka, 1996; pp 373–376.

10. Mimoto, T.; Kato, R.; Takaku, H.; Nojima, S.; Terashima, K.; Misawa, S.; Fukazawa, T.; Ueno, T.; Sato, H.; Shintani, M.; Kiso, Y.; Hayashi, H. *J. Med. Chem.* **1999**, *42*, 1789.

11. Bundgaard, H. In *Bioreversible Carriers in Drug Design: Theory and Application*; Roche, E. B., Ed.; Pergamon Press: New York, 1987; pp 13–94.

12. (a) Iley, J.; Moreira, R.; Calheiros, T.; Mendes, E. *Pharm. Res.* **1997**, *14*, 1634. (b) Moreira, R.; Mendes, E.; Calheiros, T.; Bacelo, M. J.; Iley, J. *Tetrahedron Lett.* **1994**, *35*, 7107.

13. Daehne, W. V.; Frederiksen, E.; Gundersen, E.; Lund, F.; March, P.; Petersen, H. J.; Roholt, K.; Tybring, L.; Godtfredsen, W. O. *J. Med. Chem.* **1970**, *13*, 607.

14. Ulm, E. H.; Hichens, M.; Gomez, H. J.; Till, A. E.; Hamd, E.; Vassil, T. C.; Biollaz, J.; Brunner, H. R.; Schelling, J. L. *Br. J. Pharmacol.* **1982**, *14*, 357.

15. Sinkula, A. A. In *Medicinal Chemistry*; Mathieeu, J., Ed.; Elsevier: Amsterdam, 1977; pp 125–696.

16. Wermuth, C. G.; Gaignault, J.-C.; Marchandeau, C. In *The Practice of Medicinal Chemistry*, Wermuth, C. G., Ed.; Academic Press: London, 1996; p 671.

17. Mitsuya, H.; Weinhold, K.; Furman, P. A.; Clair, M. H. S.; Lehman, S. N.; Gallo, R. C.; Bolognesi, D.; Barry, D. W.; Broder, S. *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 7096.

18. (a) Huang, Q.-Q.; Yao, S. Y. M.; Ritzel, M. W. L.; Paterson, A. R. P.; Cass, C. E.; Young, J. D. *J. Biol. Chem.* **1994**, *269*, 17757. (b) Chan, T. C. K.; Shaffer, L.; Redmond, R.; Pennington, K. L. *Biochem. Pharmacol.* **1993**, *46*, 273. (c) Domin, B. A.; Mahony, W. B.; Zimmerman, T. P. *Biochem. Pharmacol.* **1993**, *46*, 725.

19. For reviews of nucleoside transporters and receptors, see: (a) Cass, C. E.; Young, J. D.; Baldwin, S. A. *Biochem. Cell Biol.* **1998**, *76*, 761. (b) Ralevic, V.; Burnstock, G. *Pharmacol. Rev.* **1998**, *50*, 413.

20. Kimura, T.; Matsumoto, H.; Matsuda, T.; Hamawaki, T.; Akaji, K.; Kiso, Y. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 803.

21. Kiso, Y.; Matsumoto, H.; Yamaguchi, S.; Kimura, T. *Letters in Peptide Science* **1999**, *6*, 275.

22. (a) Tadayoni, B. M.; Friden, P. M.; Walus, L. R.; Musso, G. F. *Bioconjugate Chem.* **1993**, *4*, 139. (b) Tamamura, H.; Ishihara, T.; Oyake, H.; Imai, M.; Otaka, A.; Ibuka, T.; Arakaki, R.; Nakashima, H.; Murakami, T.; Waki, M.; Matsumoto, A.; Yamamoto, N.; Fujii, N. *J. Chem. Soc., Perkin Trans. 1* **1998**, 495.

23. Bilello, J. A.; Bilello, P. A.; Stellrecht, K.; Leonard, J.; Norbeck, D. W.; Kempf, D. J.; Robins, T.; Drusano, G. L. *Antimicrob. Agents Chemother.* **1996**, *40*, 1491.

24. Nascimbeni, M.; Lamotte, C.; Peytavin, G.; Farinotti, R.; Clavel, F. *Antimicrob. Agents Chemother*. **1999**, *43*, 2629.
25. Aggarwal, S. K.; Gogu, S. R.; Rangan, S. R. S.; Agrawal, K. C. *J. Med. Chem*. **1990**, *33*, 1505.
26. Tornevik, Y.; Ullman, B.; Balzarini, J.; Wahren, B.; Eriksson, S. *Biochem. Pharmacol*. **1995**, *49*, 829.
27. Camplo, M.; Niddam, V.; Barthelemy, P.; Faury, P.; Mourier, N.; Simon, V.; Charvet, A. S.; Trabaud, C.; Graciet, J. C.; Chermann, J. C.; Kraus, J. L. *Eur. J. Med. Chem*. **1995**, *30*, 789.
28. Calenbergh, S. V.; Nair, V. *Nucleosides Nucleotides* **1999**, *18*, 737.
29. Matsumoto, H.; Hamawaki, T.; Ota, H.; Kimura, T.; Goto, T.; Sano, K.; Hayashi, Y.; Kiso, Y. *Bioorg. Med. Chem. Lett*. **2000**, *10*, 1227.
30. Amsberry, K. L.; Gerstenberger, A. L.; Borchardt, R. T. *Pharm. Res*. **1991**, *8*, 455.
31. Bundgaard, H.; Klixbull, U.; Falch, E. *Int. J. Pharmaceut*. **1986**, *29*, 19.
32. Siddiqui, A.; McGuigan, C.; Ballatore, C.; Srinivasan, S.; De Clercq, E.; Balzarini, J. *Bioorg. Med Chem. Lett*. **2000**, *10*, 381.
33. Anderson, B. D.; Conradi, R. A.; Spilman, C. H.; Forbes, A. D. *J. Pharm. Sci*. **1985**, *74*, 382.
34. Welch, R. M.; Brown, A.; Ravitch, J.; Dahl, R. *Clin. Pharmacol. Ther*. **1995**, *58*, 132.