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Water-soluble prodrugs of dipeptide HIV protease inhibitors based on $O \rightarrow N$ intramolecular acyl migration: design, synthesis and kinetic study

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Abstract—To improve the low water-solubility of HIV protease inhibitors, we synthesized water-soluble prodrugs of KNI-727, a potent small-sized dipeptide-type HIV-1 protease inhibitor consisting of an Apns-Dmt core (Apns; allophenylnorstatine, Dmt; (*R*)-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid) as inhibitory machinery. These prodrugs contained an *O*-acyl peptidomimetic structure with an ionized amino group leading to an increase in water-solubility, and were designed to regenerate the corresponding parent drugs based on the $O \rightarrow N$ intramolecular acyl migration reaction via a five-membered ring intermediate at the α-hydroxy-β-amino acid residue, that is Apns. The synthetic prodrug **3a** improved the water-solubility (13 mg/mL) more than 8000-fold in comparison with the parent compound, which is the practically acceptable value as water-soluble drug. Furthermore, to understand the structural effects of the *O*-acyl moiety on the migration rate, we evaluated several phenylacetyl-type and benzoyl-type prodrugs. These prodrugs were stable as an HCl salt and in a strongly acidic solution corresponding to gastric juice (pH 2.0), and could be converted to the parent compounds promptly under aqueous conditions from slightly acidic to basic pH at 37 °C.

1. Introduction

Human immunodeficiency virus type-1 (HIV-1) encodes an aspartic protease that is responsible for the processing of viral precursor proteins such as gag and gag-pol polyproteins to form mature structural proteins and functional enzymes required in the production of infective viral particles.[1–3] Therefore, HIV-1 protease (HIV-1 PR) has been an attractive target for the design of inhibitors for effective antiviral therapy. Recently, many potent protease inhibitors that bind to the active site of HIV-1 PR have been developed based on the strategy of a substrate transition-state mimic, and several compounds are clinically used in combination therapies for AIDS.[4–8] However, the low water-solubility of these anti-AIDS drugs is a serious problem[9–11] causing undesirable pharmaceutical properties such as erratic oral absorption and poor oral bioavailability. Mimoto et al. reported the potent tripeptide-type HIV-1 PR inhibitors KNI-272[12–14] (**2a**) and KNI-279[12] (**2b**), and small-sized dipeptide-type inhibitor KNI-727[15–19] (**4a**) (Fig. 1). These inhibitors contain an allophenylnorstatine [Apns: (2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid] residue as a hydroxymethylcarbonyl (HMC) isostere derived from a natural scissile amino acid sequence 'Phe-Pro' based on the concept of a substrate-transition state mimic.[12–20] However, these peptidomimetics as well as other HIV-1 PR inhibitors such as ritonavir[21,22] and amprenavir[23] exhibited significantly low water-solubility in physiological media.

To overcome this low water-solubility, one effective strategy is to convert the water-insoluble parent drugs into hydrophilic prodrugs by covalently attaching appropriate solubilizing moieties such as phosphates,[24,25] sugars[26,27] and amines,[28,29] which can eliminate the parent drugs enzymatically or chemically under physiological conditions. Thaisrivongs et al. reported phosphate-type water-soluble prodrugs[10] of HIV-1 PR inhibitors. We recently reported distinct water-soluble prodrugs[30] derived from HIV-1 PR

Keywords: HIV-1 protease; O→N; Intramolecular acyl migration; HIV protease inhibitor; Water-soluble prodrug.
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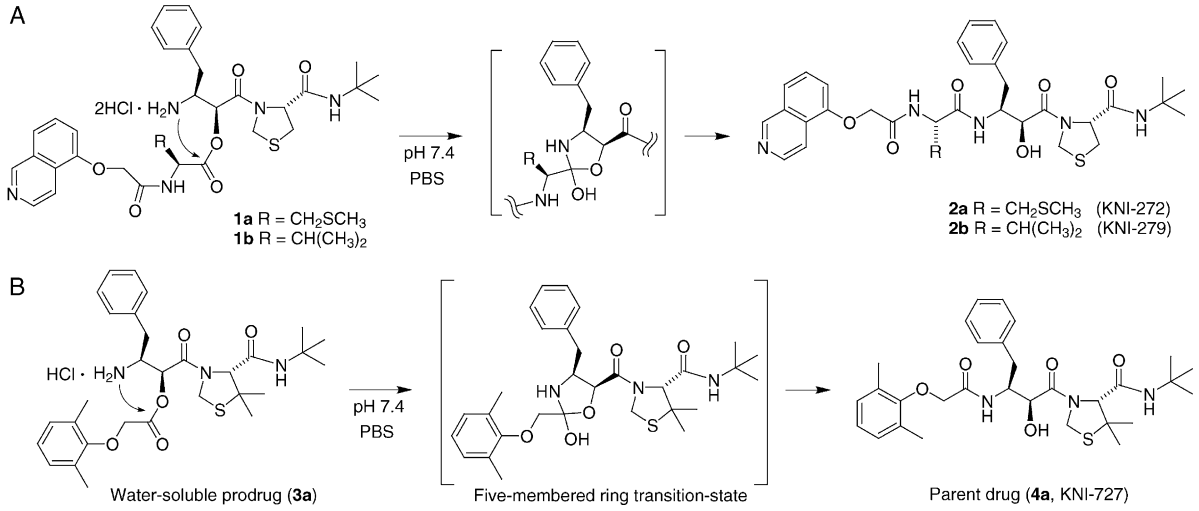


Figure 1. Design of prodrugs based on the *O*→*N* acyl migration: (A) prodrugs of KNI-272 and-279; (B) prodrug of KNI-727.

inhibitors, which contain an Apns core structure. These prodrugs were designed to enable a chemical regeneration of the parent compound by adoption of a unique pH sensitive self-cleavable linker.

On the other hand, it is well known that an acyl migration reaction is observed between adjacent amino and hydroxyl groups, and the formation of *O*-acylpeptides occurs when peptides containing β-hydroxy-α-amino acids such as serine and threonine residues are exposed to strong acids.[31,32] The solubility of these *O*-acylpeptides in aqueous media is generally increased by the newly produced amino group, and the reverse reaction to the peptides can be achieved by a pH shift to weak basic conditions in aqueous media.[33]

By introducing this pH-dependent reversible group, Hurley et al.[34] produced prodrugs of peptidomimetic inhibitors of renin, which is an aspartic protease responsible for the regulation of blood pressure in the cardiovascular system. Using a similar approach, we independently obtained water-soluble prodrugs (**1a** and **1b**) of KNI-272 (**2a**) and KNI-279 (**2b**), respectively, based on *O*→*N* intramolecular acyl migration reaction.[17,35–38]

In the present study, the same strategy was adapted to a potent small-sized dipeptide HIV-1 PR inhibitor, KNI-727 (**4a**, HIV-1 PR inhibitory activity: 96% at 50 nM, K_i value: 1.4 nM) and its analogues (**4b–f**),[15] and a series of prodrugs **3a–f** (Table 1) were designed and synthesized to increase water-solubility. Then, the water-solubility and ability of the *O*→*N* intramolecular acyl migration reaction of these prodrugs to regenerate the parent compounds were studied to better understand the properties of these compounds as prodrugs. Furthermore, since a proper range of migration rates is necessary for higher gastrointestinal absorption, one of our interests is to understand the structural effects of *O*-acyl groups on the migration reaction. We previously evaluated[38] that the phenylacetyl-type and benzoyl-type prodrugs **5** and **6a–e** (Fig. 2), corresponding to the phenoxyacetyl-type prodrugs **3a–f**, and found steric and

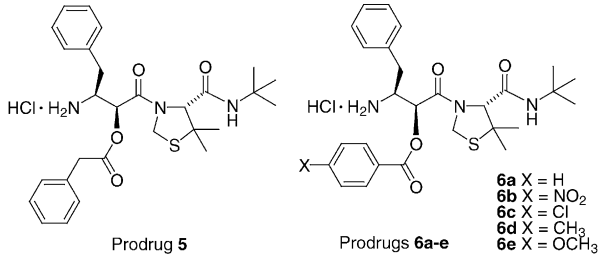


Figure 2. Structures of prodrugs **5** and **6a–e**.

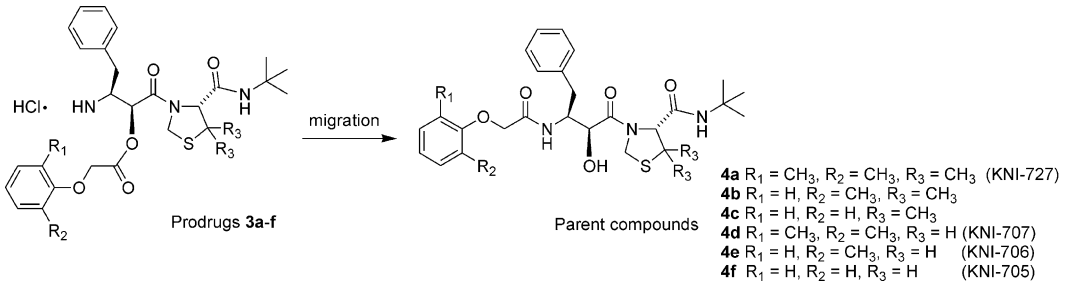
electrostatic effects of the *O*-acyl groups on the migration rate of those prodrugs (**3c**, **5** and **6a–e**). In this paper, we provide more details. The prodrugs had practical water-solubility and were stable in a strongly acidic solution (pH 1.0) corresponding to gastric juice, but the parent compounds were rapidly regenerated under physiological conditions (pH 7.4). These prodrugs were converted to the parent compounds without the formation of any by-products, thereby simplifying preclinical and clinical trials and providing an advantage over other prodrugs which contain spontaneously[25,30] or enzymatically[24,28] cleavable water-soluble moieties or linkers.

2. Chemistry

The synthesis of prodrugs **3a–f**, **5** and **6a–e** is shown in Scheme 1. Phenoxyacetic acids **8a–c**,[15] phenylacetic acid **9** or benzoic acids **10a–e** were introduced into the starting materials **7a**,**b**[12,15,37] with *N*,*N*′-dicyclohexylcarbodiimide (DCC) in the presence of a catalytic amount of DMAP to afford *O*-acyl dipeptides **11a–f**, **12** and **13a–e**. The deprotection of **11a–f**, **12** and **13a–e** with 4 N HCl-dioxane gave the desired prodrugs **3a–f**, **5**, **6a–e** as hydrochloride salts.

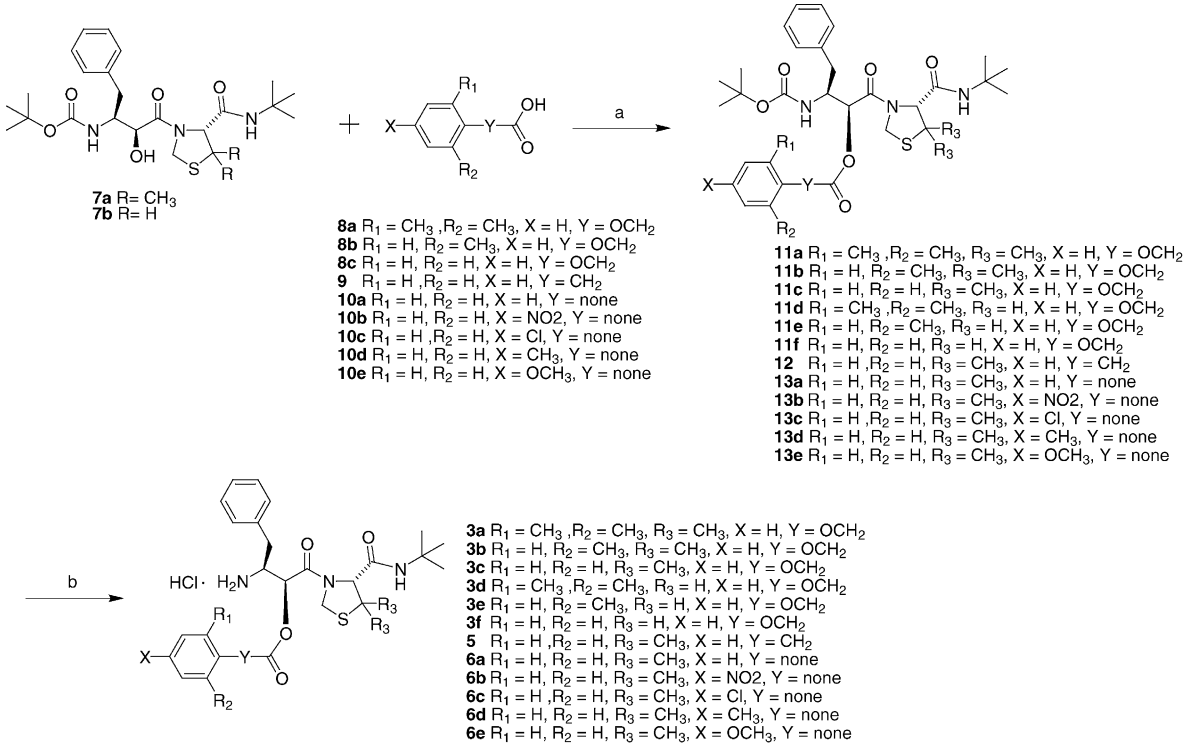
The known parent compounds **4a** and **4d–f** were synthesized in the manner reported by Mimoto et al.[15] The parent compounds **4b**, **4c**, **15** and **16a–e** were also synthesized in a similar manner (Scheme 2), namely, the

Table 1. Water-solubility and $t_{1/2}$ values of prodrugs



Prodrug	R_1	R_2	R_3	Solubility (mg/mL)		Ratio of solubility (Prodrug/Parent drug)	$t_{1/2}$[a] (min)		
				Prodrug	Parent drug		pH 7.4	pH 5.5	pH 4.9
3a	CH_3	CH_3	CH_3	13	0.0015 (**4a**)	8667	<1	4.5	24.8
3b	H	CH_3	CH_3	14	0.018 (**4b**)	778	<1	2.6	20.8
3c	H	H	CH_3	21	0.047 (**4c**)	447	<1	3.2	24.0
3d	CH_3	CH_3	H	15	0.023 (**4d**)	652	<1	4.2	23.8
3e	H	CH_3	H	15	0.042 (**4e**)	357	<1	2.4	19.6
3f	H	H	H	32	0.123 (**4f**)	260	<1	2.9	21.2
1a[b]	prodrug of KNI-272 (**2a**)			—	—	—	<1	7	28
1b[b]	prodrug of KNI-279 (**2b**)			—	—	—	7.7	290	820

[a] $t_{1/2}$ is the time required for 50% release of parent drugs at 37 °C in PBS (pH7.4).
[b] From ref 37.



Scheme 1. Reagents: (a) DCC, DMAP, CH_2Cl_2, rt; (b) 4 N-HCl/dioxane, rt.

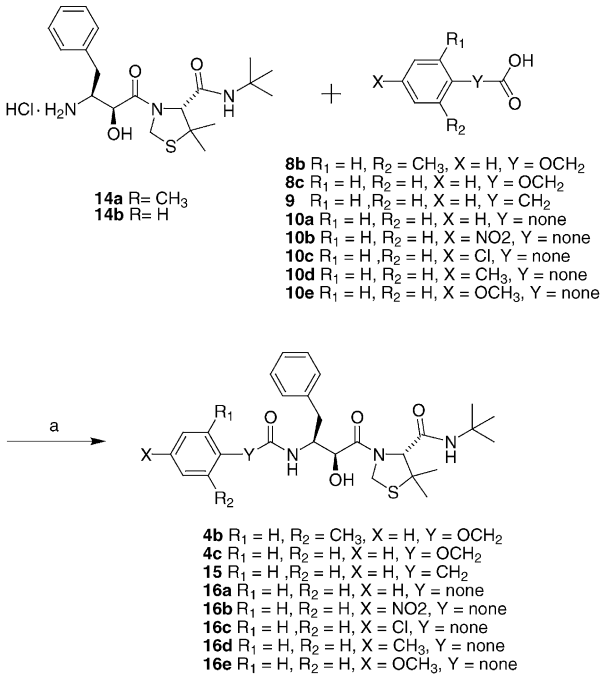
starting materials **14a**,**b**[15] and phenoxyacetic acids **8b**,**c**, phenylacetic acid **9** or benzoic acids **10a**–**e** were coupled with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide·HCl (EDC·HCl) in the presence of HOBt[37–41] to afford the parent compounds **4b**, **4c**, **15** and **16a**–**e**.

3. Results and discussion

3.1. Water-solubility of prodrugs

The water-solubility of synthetic prodrugs **3a**–**f** was determined and compared to that of their parent compounds

(Table 1). The water solubility of the parent drugs used was dependent on their hydrophobicity, and varied from 0.0015 mg/mL for the most hydrophobic compound **4a** to 0.123 mg/mL for the most hydrophilic compound **4f**. Although a similar pattern of water-solubility to the parent drugs was observed in the prodrugs, their values were very high with relatively similar values ranging from 4.3 to 32 mg/mL. The water-solubility of prodrug **3a** showed the highest gain in comparison with the corresponding parent compound **4a**. The gain seemed to be larger when the parent compound was more hydrophobic. These results suggest that utilizing the *O*-acyl peptidomimetic to obtain an ionized amino group as a water-solubilizing unit is a useful strategy for increasing the water-solubility of hydrophobic drugs.



Scheme 2. Reagents: (a) EDC·HCl, HOBt, Et_3N, DMF.

3.2. *O*→*N* intramolecular acyl migration introduction of KNI-727-type prodrugs

To examine the *O*→*N* acyl migration reaction of prodrugs, the KNI-727-type prodrugs **3a–f** were dissolved in phosphate-buffered saline (PBS) with a pH value ranging from 4.9 to 7.4 and incubated at 37 °C. The migration reaction was monitored by HPLC. Typical HPLC charts for **3a** in PBS (pH 5.5) are shown in Figure 3. **3b–f** exhibited similar HPLC profiles (data not shown), and indicating that prodrugs **3a–f** were converted quantitatively to the corresponding parent compounds **4a–f**.

The time course of the migration reaction in prodrug **3a** is shown in Figure 4. The migration reaction of prodrugs **3a** was very fast ($t_{1/2} < 1$ min) under physiological conditions (pH 7.4). In an acidic aqueous buffer of pH 4.9 or 5.5, a slower migration reaction was observed than in neutral or basic aqueous buffers. This is due to the decrease in nucleophilicity of the amino group under acidic conditions. In comparison with the previously reported $t_{1/2}$ values[37] of prodrugs **1a** and **1b**, prodrugs **1a** and **3a** showed almost the same $t_{1/2}$ values of less than 1 min, and prodrug **1b** showed the value of 7 min at pH 7.4 (Table 1). However, at pH 5.5, prodrug **3a** had a smaller $t_{1/2}$ value (4.5 min) than prodrug **1a** ($t_{1/2} = 7$ min) and **1b** ($t_{1/2} = 290$ min), indicating that in terms of the migration rate, these prodrugs ranked in the order of **3a** ≥ **1a** ≫ **1b** (Fig. 5). These results could be explained by the steric effect of the *O*-acyl groups, since prodrug **1b**, which showed slow migration, has a bulky isopropyl group, prodrug **1a**, which showed an intermediate rate of migration, has a methylthiomethyl group as a α-substituent at the *O*-acyl group, and prodrug **3a**, which

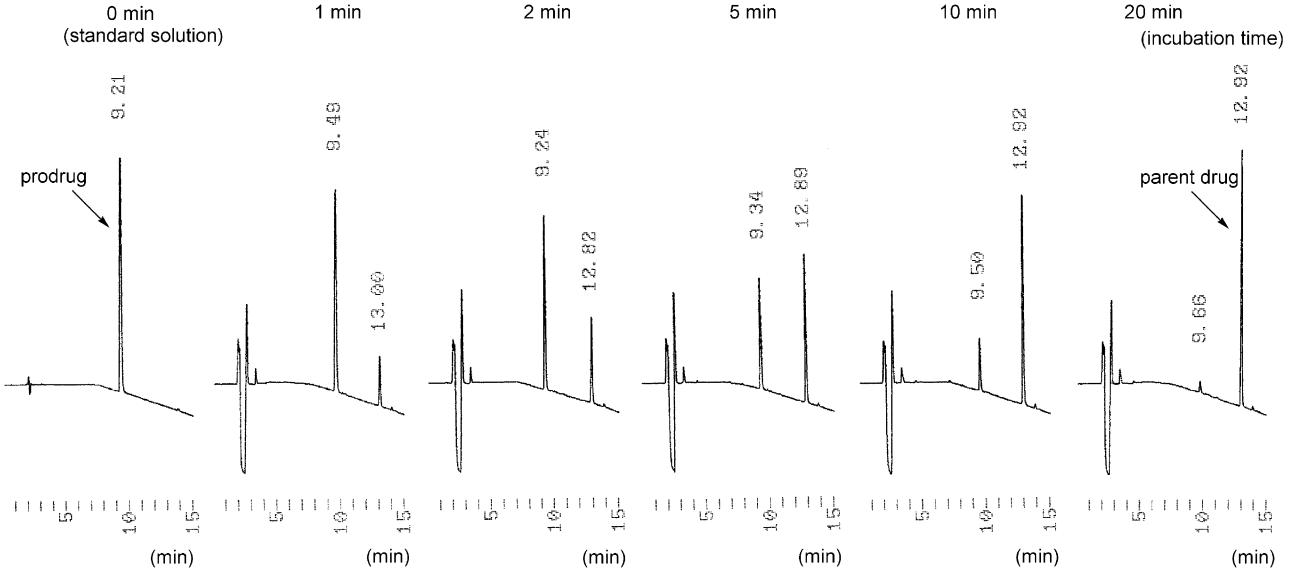


Figure 3. HPLC profiles of the *O*→*N* migration reaction of prodrug **3a** in PBS (pH 5.5) at 37 °C. The peak with a retention time of about 9 min corresponds to prodrug **3a** and a peak with a retention time of about 13 min corresponds to parent compound **4a**.

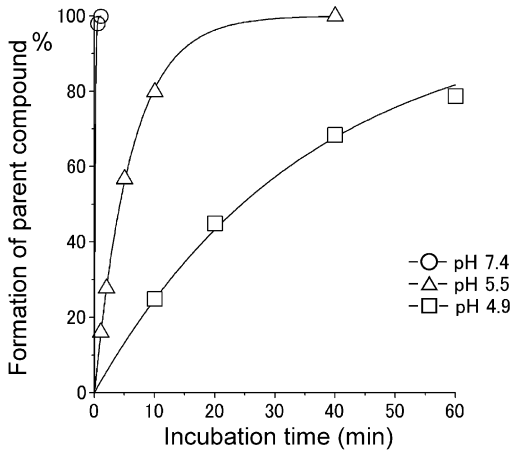


Figure 4. Migration reaction of prodrug **3a** under various pH conditions.

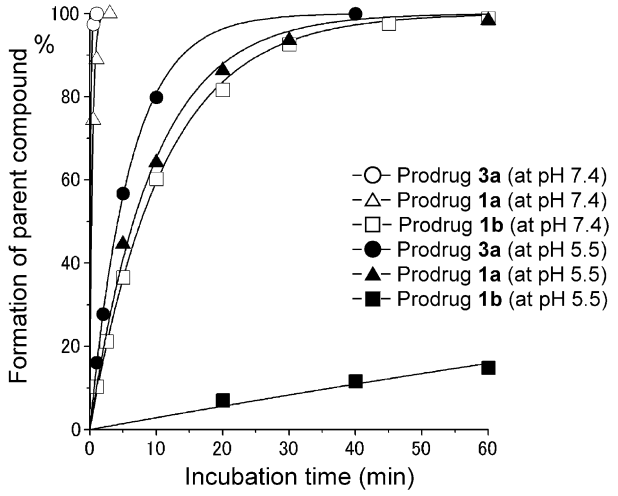


Figure 5. Comparison with the migration rates of prodrugs **3a** and **1**.

showed fast migration, has a non-branched α-substituent suggesting that the α-substituent at the *O*-acyl group is important to modulate the migration rate.

Next, to understand the structure-migration rate relationship, we compared the synthetic prodrugs **3a–f**. However, the $t_{1/2}$ values of the prodrugs **3a–f** at pH 7.4 were so small that the migration rates could not be compared. Therefore, the time course of the migration reaction at pH 5.5, shown in Figure 6, was used for the comparison. We anticipated that **3a** and **3d**, which had the most bulky *O*-acyl group (2,6-dimethylphenoxyacetyl; $R_1 = CH_3$, $R_2 = CH_3$), would show the slowest migration due to steric hindrance, and **3c** and **3f**, which had the least bulky *O*-acyl group (phenoxyacetyl; $R_1 = H$, $R_2 = H$), would show the fastest migration. Predictably, **3a** and **3d** showed the slowest migration. However, **3b** and **3e**, having a 2-methylphenoxyacetyl group ($R_1 = H$, $R_2 = CH_3$), showed the fastest migration among the prodrugs in fact. This result suggested that the introduction of one methyl group to the benzene ring of the *O*-acyl group probably restricted the conformation of the molecule to favor migration rather than steric hindrance, leading to the acceleration of the migration.

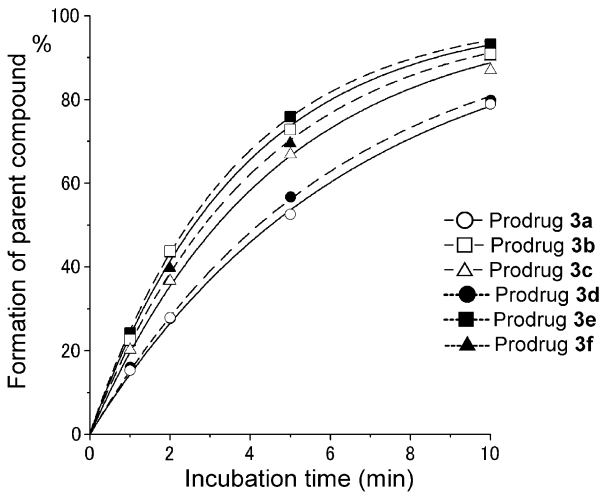


Figure 6. Time course of the migration reaction of prodrugs **3a–f** in PBS (pH 5.5).

Prodrugs **3a**, **3b** and **3c** ($R_3 = CH_3$) migrated slightly slower than prodrugs **3d**, **3e** and **3f** ($R_3 = H$), respectively, suggesting that the steric effect at the R_3 position was not so important to the migration rate. This could be explained by the fact that the R_3-substituent was too far from the reaction center of migration to afford a steric effect.

From these results, it is suggested that the derivatization of the compounds at a position away from the migration point such as the P1′ site will be tolerated, and the strategy of producing water-soluble prodrugs using the *O*→*N* acyl migration reaction is applicable as a general approach to increasing the water solubility of HIV-1 PR inhibitors based on the Apns-Dmt and Apns-Thz [Thz; (*R*)-1,3-thiazolidine-4-carboxylic acid] core structures.

3.3. Effect of the acyl groups on the *O*→ *N* acyl migration of prodrugs

The evaluation of KNI-727-type prodrugs **3a–f** suggested that the structure of the *O*-acyl moiety was important to the migration rate. One of our interests is to understand the structural effects of *O*-acyl groups on the migration rate. In this paper, we provide more detail on the steric and electrostatic effects of *O*-acyl groups on the migration of prodrugs (**3c**, **5** and **6a–e**) found in our previous study.[38] Firstly, we focused on the effect of the position of the phenyl ring. Prodrug **3c**, having the phenoxyacetyl group ($pK_a = 3.2$),[42] showed a fast migration. Prodrug **5**, having the phenylacetyl group ($pK_a = 4.31$),[42] was slightly slower than prodrug **3c**. This was probably due to the weaker electron-withdrawing effect of the phenylacetyl group. However, a remarkably slow migration was observed for prodrug **6a**. Since benzoic acid in **6a** has a similar pK_a value ($pK_a = 4.20$)[42] to phenylacetic acid in **5**, the slower migration suggested that the steric effect of the phenyl ring significantly influences the *O*→*N* acyl migration rate only in cases where the phenyl ring is connected to the carbonyl carbon directly. Secondly, because the observed slow migration of the benzoyl group was appropriate considering the electrostatic effect, we evaluated

prodrugs **6a**–**e**, which contained *p*-substituted benzoyl groups. On the introduction of an electron-withdrawing group, such as a nitro (**6b**) group or a chlorine atom (**6c**), an accelerated migration was observed, while on the introduction of an electron-donating group, such as methyl (**6d**) or methoxy (**6e**) groups, the migration rate was decelerated. Hammett plots[43] of the migration rate constants of prodrugs **6a**–**e**, against the standard reference reaction (ionization of *p*-substituted benzoic acids), gave a linear free energy relationship ($\rho = 1.31$, $r = 0.9982$, see ref 38). This suggests that the migration reaction proceeds under a single mechanism or the same rate-limiting step,[43] and the migration rate of these prodrugs depends only on the electrostatic effect under a constant steric effect.

4. Conclusion

Using the strategy applied to produce prodrugs **1a** and **1b** of KNI-272 (**2a**) and -279 (**2b**) based on the *O*→*N* acyl migration reaction, water-soluble prodrugs **3a**–**f** of an HIV-1 PR inhibitor, KNI-727 (**4a**), and its analogues were synthesized, and the ability of these compounds to act water-soluble prodrugs was evaluated. Prodrugs **3a**–**f** exhibited high solubility in aqueous media and were stable under strong acidic conditions (pH 2.0) similar to gastric juice. However, **3a**–**f** could be converted rapidly to the corresponding parent compounds under physiological conditions (pH 7.4). Considering that they are stably maintained in the stomach as a solution, and then begin to be converted under the intestinal neutral conditions to the hydrophobic parent drugs suitable for absorption by the intestinal brush border membrane, these prodrugs are useful as orally administered water-soluble agents. However, we found the migration of the benzoyl-type prodrugs to be remarkably slow. To understand the steric and electrostatic effects of the *O*-acyl moiety on the migration rate, we examined several types of prodrugs with a phenylacetic or benzoyl group. The phenyl group was predominantly affected when it was directly connected to the carbonyl carbon (i.e., the benzoyl group). In the *p*-substituted benzoyl-type prodrugs, Hammett plots of migration rate constants gave a linear free energy relationship, and the *O*→*N* acyl migration rate was predicted precisely. This strategy would be applicable to other HIV-1 PR inhibitors,[12–20] which contain Apns as a substrate transition-state mimic or other drugs which contain α-hydroxy-β-amino structures, and this study may contribute to the efficient design of prodrugs providing appropriate gastrointestinal absorption.

5. Experimental

Reagents and solvents used were purchased from Wako Pure Chemical Ind., Ltd. (Osaka, Japan), Nacalai Tesque (Kyoto, Japan) and Watanabe Chem. Ind., Ltd. (Hiroshima, Japan) without further purification. TLC was performed using Merck Silica gel 60 F_{254} precoated plates. Column chromatography was performed on Merck 107734 silica gel 60 (70–230 mesh). Melting points were measured on a Yanagimoto micro melting apparatus. Analytical HPLC was performed using a C18 reverse phase column (4.6×150 mm; YMC Pack ODS AM302) with a binary solvent system: a linear gradient of CH_3CN in 0.1% aqueous TFA with a flow rate of 1.0 mL/min, and detection at 230 nm. ^{1}H NMR spectra were obtained on a JEOL AL300 spectrometer with TMS as an internal standard. FAB-MS was performed on a JEOL JMS-SX102A spectrometer equipped with the JMA-DA7000 data system.

5.1. (1*S*,2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-1-({(4*R*)-4-[(*tert*-butylamino)carbonyl]-5,5-dimethyl-1,3-thiazolan-3-yl}carbonyl)-3-phenylpropyl 2-(2,6-dimethylphenoxy)-acetate [Boc-Apns(2,6-dimethylphenoxyacetyl)-Dmt-NHBu^t, 11a]

To a solution of Boc-Apns-Dmt-NHBu^t (**7a**)[12,15,37] (200 mg, 0.41 mmol) and 2,6-dimethylphenoxyacetic acid (**8a**)[15] (144 mg, 0.80 mmol) in dichloromethane (5 mL) were added DMAP (10 mg, 0.08 mmol) and DCC (208 mg, 1.00 mmol) at 0 °C and the mixture was stirred overnight at room temperature. After removal of the solvent in vacuo, the residue was dissolved in EtOAc, washed sequentially with 5% citric acid, 5% $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$, and concentrated in vacuo. The residue was applied to a silica gel column (2.5×10 cm) and eluted with hexane–EtOAc (3:1) to give 260 mg (98%) of the title compound **11a** as a white solid: mp 82–84 °C; $[\alpha]_D^{23} = -9.12$ (*c* 0.34, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.34 (s, 9H), 1.35–1.45 (m, 9H), 1.45–1.72 (m, 6H), 2.29 (s, 6H), 2.83–3.14 (m, 2H), 4.08–4.30 (m, 1H), 4.41–4.68 (m, 2H), 4.75–5.10 (m, 2H), 5.50 (s, 1H), 5.61 (s, 1H), 6.89–7.05 (m, 3H), 7.10–7.38 (m, 6H); MS (FAB): *m*/*z* 656 $[M+H]^+$. Anal. calcd for $C_{35}H_{49}N_3O_7S$: H, 7.53; C, 64.10; N, 6.41. Found: H, 7.54; C, 64.88; N, 6.20.

5.2. (1*S*,2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-1-({(4*R*)-4-[(*tert*-butylamino)carbonyl]-5,5-dimethyl-1,3-thiazolan-3-yl}carbonyl)-3-phenylpropyl 2-(2-methylphenoxy)acetate [Boc-Apns(2-methylphenoxyacetyl)-Dmt-NHBu^t, 11b]

Compound **11b** was prepared from Boc-Apns-Dmt-NHBu^t (**7a**) (200 mg, 0.41 mmol) and 2-methylphenoxyacetic acid (**8b**)[15] (135 mg, 0.81 mmol) according to the procedure described for compound **11a**. Yield 220 mg (85%); mp 72–75 °C; $[\alpha]_D^{26} = -6.23$ (*c* 0.53, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 (s, 9H), 1.36 (s, 9H), 1.42–1.60 (m, 6H), 2.30 (s, 3H), 2.75 (dd, $J = 11.0$, 14.0 Hz, 1H), 2.97 (dd, $J = 4.0$, 14.0 Hz, 1H), 3.98–4.30 (m, 2H), 4.70–5.98 (m, 5H), 5.40–5.70 (m, 2H), 6.68–6.85 (m, 1H), 6.88–7.05 (m, 2H), 7.10–7.40 (m, 7H); MS (FAB): *m*/*z* 642 $[M+H]^+$. Anal. calcd for $C_{34}H_{47}N_3O_7S$: H, 7.38; C, 63.63; N, 6.55. Found: H, 7.53; C, 63.37; N, 6.66.

5.3. (1*S*,2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-1-({(4*R*)-4-[(*tert*-butylamino)carbonyl]-5,5-dimethyl-1,3-thiazolan-3-yl}carbonyl)-3-phenylpropyl 2-phenoxyacetate [Boc-Apns(phenoxyacetyl)-Dmt-NHBu^t, 11c]

Compound **11c** was prepared from Boc-Apns-Dmt-NHBu^t (**7a**) (200 mg, 0.41 mmol) and phenoxyacetic acid (**8c**)[15] (123 mg, 0.81 mmol) according to the procedure described for compound **11a**. Yield 214 mg (84%); mp 72–76 °C; $[\alpha]_D^{26} = -4.29$ (*c* 0.57, MeOH); ^{1}H NMR

(300 MHz, $CDCl_3$) δ 1.33 (s, 9H), 1.37 (s, 9H), 1.42–1.58 (m, 6H), 2.76 (dd, $J = 10.0$, 14.0 Hz, 1H), 2.96 (dd, $J = 2.3$, 14.0 Hz, 1H), 3.98–4.30 (m, 2H), 4.51 (br.s, 1H), 4.70–4.95 (m, 6H), 5.40–5.70 (m, 2H), 6.90–7.08 (m, 4H), 7.18–7.40 (m, 8H); MS (FAB): m/z 628 $[M+H]^+$. Anal. calcd for $C_{33}H_{45}N_3O_7S$: H, 7.22; C, 63.13; N, 6.69. Found: H, 7.42; C, 62.96; N, 6.63.

5.4. (1*S*,2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-1-({(4*R*)-4-[(*tert*-butylamino)carbonyl]-1,3-thiazolan-3-yl}carbonyl)-3-phenylpropyl 2-(2,6-dimethylphenoxy)acetate [Boc-Apns(2,6-dimethylphenoxyacetyl)-Thz-NHBu^t, 11d]

Compound **11d** was prepared from Boc-Apns-Thz-NHBu^t (**7b**)[12,15,37] (200 mg, 0.43 mmol) and 2,6-dimethylphenoxyacetic acid (**8a**) (155 mg, 0.86 mmol) according to the procedure described for compound **11a**. Yield 230 mg (86%); mp 73–76 °C; $[\alpha]_D^{23} = -80.20$ (c 0.50, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32 (s, 9H), 1.37 (s, 9H), 2.31 (s, 6H), 2.72–3.02 (m, 2H), 3.12 (dd, $J = 6.8$, 11.3 Hz, 1H), 3.5 (dd, $J = 4.5$, 11.3 Hz, 1H), 4.05–4.28 (m, 1H), 4.33–4.76 (m, 3H), 4.83 (dd, $J = 4.5$, 6.8 Hz, 1H), 4.87–5.03 (m, 2H), 5.59 and 6.30 (br.s×2, total 1H), 6.88–7.10 (m, 3H), 7.10–7.43 (m, 6H); MS (FAB): m/z 628 $[M+H]^+$. Anal. calcd for $C_{33}H_{45}N_3O_7S \cdot 1/4H_2O$: H, 7.22; C, 63.13; N, 6.69. Found: H, 7.50; C, 62.89; N, 6.84.

5.5. (1*S*,2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-1-({(4*R*)-4-[(*tert*-butylamino)carbonyl]-1,3-thiazolan-3-yl}carbonyl)-3-phenylpropyl 2-(2-methylphenoxy)acetate [Boc-Apns(2-methylphenoxyacetyl)-Thz-NHBu^t, 11e]

Compound **11e** was prepared from Boc-Apns-Thz-NHBu^t (**7b**) (200 mg, 0.43 mmol) and 2-methylphenoxyacetic acid (**8b**) (143 mg, 0.86 mmol) according to the procedure described for compound **11a**. Yield 241 mg (91%); mp 72–75 °C; $[\alpha]_D^{23} = -69.20$ (c 0.57, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (s, 9H), 1.37 (s, 9H), 2.31 (s, 3H), 2.74 (dd, $J = 10.3$, 14.3 Hz, 1H), 2.88 (dd, $J = 3.6$, 14.3 Hz, 1H), 3.10 (dd, $J = 6.9$, 11.1 Hz, 1H), 3.47 (dd, $J = 4.8$, 11.1 Hz, 1H), 3.98-4.22 (m, 1H), 4.32–4.72 (m, 2H), 4.72–5.01 (m, 4H), 5.53 and 6.29 (br.s×2, total 1H), 6.75 (d, $J = 8.1$ Hz, 1H), 6.92 (d, $J = 7.4$ Hz, 1H), 7.10–7.38 (m, 8H); MS (FAB): m/z 614 $[M+H]^+$. Anal. calcd for $C_{32}H_{43}N_3O_7S$: H, 7.06; C, 62.62; N, 6.85. Found: H, 7.30; C, 62.79; N, 7.02.

5.6. (1*S*,2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-1-({(4*R*)-4-[(*tert*-butylamino)carbonyl]-1,3-thiazolan-3-yl}carbonyl)-3-phenylpropyl 2-phenoxyacetate [Boc-Apns(phenoxyacetyl)-Thz-NHBu^t, 11f]

Compound **11f** was prepared from Boc-Apns-Thz-NHBu^t (**7b**) (200 mg, 0.43 mmol) and phenoxyacetic acid (**8c**) (125 mg, 0.86 mmol) according to the procedure described for compound **11a**. Yield 215 mg (83%); mp 73–76 °C; $[\alpha]_D^{23} = -89.76$ (c 0.43, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (s, 9H), 1.37 (s, 9H), 2.78 (dd, $J = 10.0$, 14.0 Hz, 1H), 2.87 (dd, $J = 4.0$, 14.0 Hz, 1H), 3.09 (dd, $J = 6.8$, 11.6 Hz, 1H), 3.47 (dd, $J = 5.0$, 11.6 Hz, 1H), 3.98–4.20 (m, 1H), 4.30–4.53 (m, 1H), 4.60–4.92 (m, 5H), 5.54 and 6.27 (br.s×2, total 1H), 6.88–7.40 (m, 11H); MS (FAB): m/z 600 $[M+H]^+$. Anal. calcd for $C_{31}H_{41}N_3O_7S$: H, 6.89; C, 62.08; N, 7.01. Found: H, 7.10; C, 61.89; N, 6.93.

5.7. (1*S*,2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-1-({(4*R*)-4-[(*tert*-butylamino)carbonyl]-5,5-dimethyl-1,3-thiazolan-3-yl}carbonyl)-3-phenylpropyl 2-phenylacetate [Boc-Apns(phenylacetyl)-Dmt-NHBu^t, 12]

Compound **12** was prepared from Boc-Apns-Dmt-NHBu^t (**7a**) (200 mg, 0.41 mmol) and phenylacetic acid (**9**) (110 mg, 0.81 mmol) according to the procedure described for compound **11a**. Yield 228 mg (92%); mp 102–105 °C; $[\alpha]_D^{26} = -35.51$ (c 0.54, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.10–1.40 (m, 18H), 1.44 (s, 3H), 1.49 (s, 3H), 2.86 (dd, $J = 9.3$, 14.1 Hz, 1H), 2.96 (dd, $J = 5.9$, 14.1 Hz, 1H), 3.67-3.82 (m, 2H), 4.12–4.28 (m, 1H), 4.66–4.86 (m, 2H), 4.96 (d, $J = 7.5$ Hz, 1H), 5.29–5.44 (m, 1H), 5.61 (br.s, 1H), 7.10–7.48 (m, 10H); MS (FAB): m/z 612 $[M+H]^+$. Anal. calcd for $C_{33}H_{45}N_3O_6S$: H, 7.41; C, 64.79; N, 6.87. Found: H, 7.39; C, 64.57; N, 7.21.

5.8. (1*S*,2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-1-({(4*R*)-4-[(*tert*-butylamino)carbonyl]-5,5-dimethyl-1,3-thiazolan-3-yl}carbonyl)-3-phenylpropyl benzoate [Boc-Apns(benzoyl)-Dmt-NHBu^t, 13a]

Compound **13a** was prepared from Boc-Apns-Dmt-NHBu^t (**7a**) (200 mg, 0.41 mmol) and benzoic acid (**10a**) (99 mg, 0.81 mmol) according to the procedure described for compound **11a**. Yield 249 mg (74%); mp 195–198 °C; $[\alpha]_D^{23} = -20.19$ (c 0.52, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.34 (s, 18H), 1.37 (s, 3H), 1.51 (s, 3H), 3.01 (dd, $J = 9.3$, 13.9 Hz, 1H), 3.11 (dd, $J = 5.4$, 13.9 Hz, 1H), 4.31 (s, 1H), 4.35–4.50 (m, 1H), 4.85 (d, $J = 8.9$ Hz, 1H), 5.03 (d, $J = 8.9$ Hz, 1H), 5.22 (d, $J = 8.1$ Hz, 1H), 5.53 (d, $J = 3.9$ Hz, 1H), 5.94 (br.s, 1H), 7.13–7.38 (m, 5H), 7.44 (t, $J = 8.1$ Hz, 2H), 7.59 (t, $J = 7.5$ Hz, 1H), 8.04 (d, $J = 7.2$ Hz, 2H); HRMS (FAB): m/z 598.2955 for $[M+H]^+$ (calcd 598.2951 for $C_{32}H_{44}N_3O_6S$). Anal. calcd for $C_{32}H_{43}N_3O_6S$: H, 7.25; C, 64.30; N, 7.25. Found: H, 7.34; C, 64.20; N, 7.36.

5.9. (1*S*,2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-1-({(4*R*)-4-[(*tert*-butylamino)carbonyl]-5,5-dimethyl-1,3-thiazolan-3-yl}carbonyl)-3-phenylpropyl 4-nitrobenzoate [Boc-Apns(*p*-nitrobenzoyl)-Dmt-NHBu^t, 13b]

Compound **13b** was prepared from Boc-Apns-Dmt-NHBu^t (**7a**) (200 mg, 0.41 mmol) and *p*-nitrobenzoic acid (**10b**) (135 mg, 0.81 mmol) according to the procedure described for compound **11a**. Yield 260 mg (99%); mp 109–113 °C; $[\alpha]_D^{26} = -30.84$ (c 0.53, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30–1.45 (m, 18H), 1.45–1.68 (m, 6H), 3.06 (dd, $J = 8.7$, 13.5 Hz, 1H), 3.12 (dd, $J = 6.0$, 13.5 Hz, 1H), 4.24 (s, 1H), 4.38–4.52 (m, 1H), 4.83 (d, $J = 9.0$ Hz, 1H), 5.00 (d, $J = 9.0$ Hz, 1H), 5.26 (d, $J = 8.1$ Hz, 1H), 5.57 (d, $J = 3.6$ Hz, 1H), 5.61 (br.s, 1H), 7.18–7.42 (m, 5H), 8.17 (d, $J = 8.7$ Hz, 2H), 8.28 (d, $J = 8.7$ Hz, 2H); HRMS (FAB): m/z 643.2816 for $[M+H]^+$ (calcd 643.2802 for $C_{32}H_{43}N_4O_8S$). Anal. calcd for $C_{32}H_{42}N_4O_8S$: H, 6.59; C, 59.8; N, 8.72. Found: H, 6.69; C, 59.51; N, 8.89.

5.10. (1*S*,2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-1-({(4*R*)-4-[(*tert*-butylamino)carbonyl]-5,5-dimethyl-1,3-thiazolan-3-yl}carbonyl)-3-phenylpropyl 4-chlorobenzoate [Boc-Apns(*p*-chlorobenzoyl)-Dmt-NHBu^t, 13c]

Compound **13c** was prepared from Boc-Apns-Dmt-NHBu^t (**7a**) (200 mg, 0.41 mmol) and *p*-chlorobenzoic acid (**10c**) (127 mg, 0.81 mmol) according to the procedure described for compound **11a**. Yield 257 mg (99%); mp. 99–102 °C; $[\alpha]_D^{26} = -32.21$ (*c* 0.51, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (s, 18H), 1.38–1.62 (m, 6H), 3.02 (dd, *J* = 9.3, 14.6 Hz, 1H), 3.11 (dd, *J* = 6.8, 14.6 Hz, 1H), 4.27 (s, 1H), 4.35–4.49 (m, 1H), 4.81 (d, *J* = 9.0 Hz, 1H), 5.01 (d, *J* = 9.0 Hz, 1H), 5.25 (d, *J* = 8.1 Hz, 1H), 5.51 (d, *J* = 4.2 Hz, 1H), 5.76 (br.s, 1H), 7.18–7.38 (m, 5H), 7.47 (d, *J* = 8.7 Hz, 2H), 7.95 (d, *J* = 8.7 Hz, 2H); HRMS (FAB): *m*/*z* 632.2555 for $[M+H]^+$ (calcd 632.2561 for $C_{32}H_{43}ClN_3O_6S$). Anal. calcd for $C_{32}H_{42}ClN_3O_6S$: H, 6.70; C, 60.79; N, 6.65. Found: H, 6.65; C, 60.79; N, 6.55.

5.11. (1*S*,2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-1-({(4*R*)-4-[(*tert*-butylamino)carbonyl]-5,5-dimethyl-1,3-thiazolan-3-yl}carbonyl)-3-phenylpropyl 4-methylbenzoate [Boc-Apns(*p*-toluoyl)-Dmt-NHBu^t, 13d]

Compound **13d** was prepared from Boc-Apns-Dmt-NHBu^t (**7a**) (200 mg, 0.41 mmol) and *p*-toluic acid (**10d**) (110 mg, 0.81 mmol) according to the procedure described for compound **11a**. Yield 228 mg (92%); mp 103–106 °C; $[\alpha]_D^{26} = -32.35$ (*c* 0.51, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.34 (s, 18H), 1.38 (s, 3H), 1.51 (s, 3H), 2.42 (s, 3H), 3.02 (dd, *J* = 9.3, 14.2 Hz, 1H), 3.11 (dd, *J* = 5.7, 14.2 Hz, 1H), 4.30 (s, 1H), 4.33–4.50 (m, 1H), 4.82 (d, *J* = 9.3 Hz, 1H), 4.82 (d, *J* = 9.3 Hz, 1H), 5.02 (d, *J* = 9.0 Hz, 1H), 5.22 (d, *J* = 7.8 Hz, 1H), 5.49 (d, *J* = 4.2 Hz, 1H), 5.93 (br.s, 1H), 7.13–7.48 (m, 7H), 7.92 (d, *J* = 8.1 Hz, 2H); HRMS (FAB): *m*/*z* 612.3099 for $[M+H]^+$ (calcd 612.3107 for $C_{33}H_{46}N_3O_6S$). Anal. calcd for $C_{33}H_{45}N_3O_6S$: H, 7.41; C, 64.79; N, 6.87. Found: H, 7.30; C, 64.29; N, 7.41.

5.12. (1*S*,2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-1-({(4*R*)-4-[(*tert*-butylamino)carbonyl]-5,5-dimethyl-1,3-thiazolan-3-yl}carbonyl)-3-phenylpropyl 4-methoxybenzoate [Boc-Apns(*p*-anisoyl)-Dmt-NHBu^t, 13e]

Compound **13e** was prepared from Boc-Apns-Dmt-NHBu^t (**7a**) (200 mg, 0.41 mmol) and *p*-anisic acid (**10e**) (123 mg, 0.81 mmol) according to the procedure described for compound **11a**. Yield 253 mg (99%); mp 95–99 °C; $[\alpha]_D^{27} = -40.28$ (*c* 0.51, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.34 (s, 18H), 1.38–1.45 (m, 3H), 1.51 (s, 3H), 3.01 (dd, *J* = 9.0, 13.8 Hz, 1H), 3.10 (dd, *J* = 6.0, 13.8 Hz, 1H), 3.87 (s, 3H), 4.30 (s, 1H), 4.35–4.50 (m, 1H), 4.81 (d, *J* = 9.0 Hz, 1H), 5.02 (d, *J* = 9.0 Hz, 1H), 5.23 (d, *J* = 8.7 Hz, 1H), 5.47 (d, *J* = 4.2 Hz, 1H), 5.91 (br.s, 1H), 6.91 (d, *J* = 8.7 Hz, 2H), 7.12–7.43 (m, 5H), 8.02 (d, *J* = 8.7 Hz, 2H); HRMS (FAB): *m*/*z* 628.3052 for $[M+H]^+$ (calcd 628.3056 for $C_{33}H_{46}N_3O_7S$). Anal. calcd for $C_{33}H_{45}N_3O_7S$: H, 7.22; C, 63.13; N, 6.69. Found: H, 7.19; C, 62.89; N, 7.12.

5.13. ((1*S*,2*S*)-1-Benzyl-3-{(4*R*)-4-[(*tert*-butylamino)carbonyl]-5,5-dimethyl-1,3-thiazolan-3-yl}-2-{[2-(2,6-dimethylphenoxy)acetyl]oxy}-3-oxopropyl)ammonium chloride [Apns(2,6-dimethylphenoxyacetyl)-Dmt-NHBu^t·HCl, 3a]

To compound **11a** (125 mg, 0.20 mmol) were added anisole (100 μL) and 4 N-HCl/dioxane (2 mL), and the mixture was stirred for 2 h at room temperature. After the reaction mixture was concentrated in vacuo, hexane was added to give 105 mg (95%) of the title compound **3a** as a white solid: mp 126–127 °C; $[\alpha]_D^{25} = -6.88$ (*c* 0.47, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27 (s, 9H), 1.42 (s, 3H), 1.53 (s, 3H), 2.25 (s, 6H), 2.87 (dd, *J* = 9.6, 14.0 Hz, 1H), 3.02 (dd, *J* = 2.8, 14.0 Hz, 1H), 3.60–3.92 (m, 1H), 4.42–4.73 (m, 3H), 4.94 (d, *J* = 8.8 Hz, 1H), 5.04 (d, *J* = 8.8 Hz, 1H), 5.75 (br.s, 1H), 6.88–7.10 (m, 3H), 7.18–7.48 (m, 5H), 7.85 (br.s, 1H), 8.45 (br s, 3H); MS (FAB): *m*/*z* 556 $[M+H]^+$. Anal. calcd for $C_{30}H_{41}N_3O_5S{\cdot}HCl{\cdot}2H_2O$: H, 7.08; C, 57.54; N, 6.71. Found: H, 6.94; C, 57.80; N, 6.46.

5.14. ((1*S*,2*S*)-1-Benzyl-3-{(4*R*)-4-[(*tert*-butylamino)carbonyl]-5,5-dimethyl-1,3-thiazolan-3-yl}-2-{[2-(2-methylphenoxy)acetyl]oxy}-3-oxopropyl)ammonium chloride [Apns(2-methylphenoxyacetyl)-Dmt-NHBu^t·HCl, 3b]

Compound **3b** was prepared from **11b** (125 mg, 0.20 mmol) according to the procedure described for compound **3a**. Yield 80 mg (73%); mp 148–154 °C; $[\alpha]_D^{26} = -29.43$ (*c* 0.53, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27 (s, 9H), 1.40 (s, 3H), 1.49 (s, 3H), 2.21 (s, 3H), 2.90 (dd, *J* = 9.9, 13.9 Hz, 1H), 3.02 (d, *J* = 13.9 Hz, 1H), 3.38–3.90 (m, 1H), 4.49 (br.s, 1H), 4.81–5.08 (m, 4H), 5.75 (br.s, 1H), 6.80–6.97 (m, 2H), 7.08–7.49 (m, 7H), 7.85 (br.s, 1H), 8.51 (br.s, 3H); MS (FAB): *m*/*z* 542 $[M+H]^+$. Anal. calcd for $C_{29}H_{39}N_3O_5S$ ·HCl: H, 7.10; C, 58.42; N, 7.05. Found: H, 6.97; C, 58.24; N, 7.02.

5.15. {(1*S*,2*S*)-1-Benzyl-3-{(4*R*)-4-[(*tert*-butylamino)carbonyl]-5,5-dimethyl-1,3-thiazolan-3-yl}-3-oxo-2-[(2-phenoxyacetyl)oxy]propyl}ammonium chloride [H-Apns(phenoxyacetyl)-Dmt-NHBu^t·HCl, 3c]

Compound **3c** was prepared from **11c** (114 mg, 0.18 mmol) according to the procedure described for compound **3a**. Yield 100 mg (98%); mp 141–145 °C; $[\alpha]_D^{26} = -22.97$ (*c* 0.67, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.27 (s, 9H), 1.40 (s, 3H), 1.48 (s, 3H), 2.89 (dd, *J* = 9.9, 13.8 Hz, 1H), 3.02 (dd, *J* = 2.4, 13.8 Hz, 1H), 3.52–3.88 (m, 1H), 4.50 (br.s, 1H), 4.80-5.08 (m, 4H), 5.75 (br.s, 1H), 6.90–7.06 (m, 7H), 7.18–7.48 (m, 3H), 7.85 (br.s, 1H), 8.53 (br.s, 3H); MS (FAB): *m*/*z* 528 $[M+H]^+$. Anal. calcd for $C_{28}H_{37}N_3O_5S$ ·HCl: H, 6.79; C, 59.61; N, 7.45. Found: H, 7.02; C, 59.90; N, 7.15.

5.16. ((1*S*,2*S*)-1-Benzyl-3-{(4*R*)-4-[(*tert*-butylamino)carbonyl]-1,3-thiazolan-3-yl}-2-{[2-(2,6-dimethylphenoxy)acetyl]oxy}-3-oxopropyl)ammonium chloride [Apns-(2,6-dimethylphenoxyacetyl)-Thz-NHBu^t·HCl, 3d]

Compound **3d** was prepared from **11d** (125 mg, 0.20 mmol) according to the procedure described for compound **3a**. Yield 105 mg (95%); mp 134–137 °C;

$[\alpha]_D^{21} = -62.40$ (*c* 0.25, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (s, 9H), 2.25 (s, 6H), 3.78–3.08 (m, 3H), 3.44 (dd, *J* = 7.5, 11.7 Hz, 1H), 3.70–3.92 (br.s, 1H), 4.32–4.82 (m, 4H), 5.02 (m, 1H), 5.88 (br.s, 1H), 6.88–7.10 (m, 3H), 7.18–7.48 (m, 6H), 7.94 (br.s, 1H), 8.50 (br.s, 3H); MS (FAB): *m*/*z* 528 $[M+H]^+$. Anal. calcd for $C_{28}H_{37}N_3O_5S \cdot HCl \cdot 1/2H_2O$: H, 6.79; C, 59.61; N, 7.45. Found: H, 6.86; C, 58.68; N, 7.33.

5.17. ((1*S*,2*S*)-1-Benzyl-3-{(4*R*)-4-[(*tert*-butylamino)-carbonyl]-1,3-thiazolan-3-yl}-2-{[2-(2-methylphenoxy)-acetyl]oxy}-3-oxopropyl)ammonium chloride [Apns(2-methylphenoxyacetyl)-Thz-NHBu^t·HCl, 3e]

Compound **3e** was prepared from **11e** (125 mg, 0.20 mmol) according to the procedure described for compound **3a**. Yield 68 mg (62%); mp 120–122 °C; $[\alpha]_D^{21} = -13.73$ (*c* 0.15, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (s, 9H), 2.21 (s, 3H), 2.88 (dd, *J* = 10.1, 14.5 Hz, 1H), 2.98 (dd, *J* = 6.4, 11.7 Hz, 1H), 3.02 (dd, *J* = 2.6, 14.5 Hz, 1H), 3.40 (dd, *J* = 7.5, 11.7 Hz, 1H), 3.81 (br.s, 1H), 3.62-3.80 (m, 2H), 3.87-5.09 (m, 3H), 5.84 (br.s, 1H), 6.80–6.94 (m, 7H), 7.03–7.20 (m, 2H), 7.20–7.60 (m, 5H), 7.93 (br.s, 1H), 8.53 (br.s, 1H); MS (FAB): *m*/*z* 514 $[M+H]^+$. Anal. calcd for $C_{27}H_{35}N_3O_5S \cdot HCl$: H, 6.60; C, 58.95; N, 7.53. Found: H, 6.78; C, 58.53; N, 7.53.

5.18. {(1*S*,2*S*)-1-Benzyl-3-{(4*R*)-4-[(*tert*-butylamino)-carbonyl]-1,3-thiazolan-3-yl}-3-oxo-2-[(2-phenoxyacetyl)-oxy]propyl}ammonium chloride [Apns(phenoxyacetyl)-Thz-NHBu^t·HCl, 3f]

Compound **3f** was prepared from **11f** (114 mg, 0.19 mmol) according to the procedure described for compound **3a**. Yield 98 mg (96%); mp 114–117 °C; $[\alpha]_D^{21} = -7.27$ (*c* 0.05, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.27 (s, 9H), 2.89 (dd, *J* = 10.0, 14.4 Hz, 1H), 3.00 (dd, *J* = 6.0, 11.9 Hz, 1H), 3.04 (dd, *J* = 3.2, 14.4 Hz, 1H), 3.40 (dd, *J* = 7.4, 11.9 Hz, 1H), 3.80–3.86 (m, 1H), 4.73 (d, *J* = 9.5 Hz, 1H), 4.78 (t, *J* = 7.4 Hz, 1H), 4.90 (d, *J* = 17.0 Hz, 1H), 4.98 (d, *J* = 17.0 Hz, 1H), 4.95 (d, *J* = 9.5 Hz, 1H), 5.81 (d, *J* = 3.0 Hz, 1H), 6.86–7.08 (m, 3H), 7.20–7.50 (m, 7H), 7.92 (br.s, 1H), 8.45 (br.s, 3H); MS (FAB): *m*/*z* 500 $[M+H]^+$. Anal. calcd for $C_{26}H_{33}N_3O_5S \cdot HCl$: H, 6.39; C, 58.25; N, 7.84. Found: H, 6.51; C, 57.99; N, 7.81.

5.19. {(1*S*,2*S*)-1-Benzyl-3-{(4*R*)-4-[(*tert*-butylamino)-carbonyl]-5,5-dimethyl-1,3-thiazolan-3-yl}-3-oxo-2-[(2-phenylacetyl)oxy]propyl}ammonium chloride [Apns(phenylacetyl)-Dmt-NHBu^t·HCl, 5]

Compound **5** was prepared from **12** (150 mg, 0.24 mmol) according to the procedure described for compound **3a**. Yield 133 mg (99%); mp 73–75 °C; $[\alpha]_D^{27} = -30.83$ (*c* 0.55, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (s, 9H), 1.40–1.60 (m, 6H), 3.02–3.22 (m, 2H), 3.52–3.85 (m, 3H), 3.94 (s, 1H), 4.33 (s, 1H), 4.76 (d, *J* = 8.4 Hz, 1H), 4.99 (d, *J* = 8.4 Hz, 1H), 5.27 (s, 1H), 6.04 (s, 1H), 7.18–7.55 (m, 10H), 8.87 (br.s, 2H); Ms (FAB): *m*/*z* 512 $[M+H]^+$. Anal. calcd for $C_{28}H_{38}ClN_3O_4S \cdot H_2O$: H, 7.12; C, 59.40; N, 7.42. Found: H, 7.20; C, 59.49; N, 7.35.

5.20. (1*S*,2*S*)-2-Ammonio-1-({(4*R*)-4-[(*tert*-butylamino)-carbonyl]-5,5-dimethyl-1,3-thiazolan-3-yl}carbonyl)-3-phenylpropyl benzoate chloride [Apns(benzoyl)-Dmt-NHBu^t·HCl, 6a]

Compound **6a** was prepared from **13a** (150 mg, 0.24 mmol) according to the procedure described for compound **3a**. Yield 128 mg (95%); mp 121–123 °C; $[\alpha]_D^{27} = -28.70$ (*c* 0.54, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32 (s, 9H), 1.45 (s, 3H), 1.54 (s, 3H), 3.34 (dd, *J* = 7.4, 13.4 Hz, 1H), 3.77 (dd, *J* = 7.5, 13.4 Hz, 1H), 3.87–4.12 (m, 1H), 4.29 (s, 1H), 5.19 (d, *J* = 8.7 Hz, 1H), 5.43 (d, *J* = 8.7 Hz, 1H), 5.53 (d, *J* = 7.5 Hz, 1H), 5.72–6.07 (m, 1H), 7.14–7.48 (m, 7H), 7.55 (t, *J* = 7.2 Hz, 1H), 7.83 (d, *J* = 7.5 Hz, 2H), 8.50–9.40 (br.s, 1H); Ms(FAB): *m*/*z* 498 $[M+H]^+$. Anal. calcd for $C_{27}H_{36}ClN_3O_4S \cdot 1/2H_2O$: H, 6.87; C, 59.90; N, 7.74. Found: H, 7.14; C, 59.90; N, 7.55.

5.21. (1*S*,2*S*)-2-Ammonio-1-({(4*R*)-4-[(*tert*-butylamino)-carbonyl]-5,5-dimethyl-1,3-thiazolan-3-yl}carbonyl)-3-phenylpropyl 4-nitrobenzoate chloride [Apns(*p*-nitro-benzoyl)-Dmt-NHBu^t·HCl, 6b]

Compound **6b** was prepared from **13b** (150 mg, 0.23 mmol) according to the procedure described for compound **3a**. Yield 130 mg (96%); mp 160–161 °C; $[\alpha]_D^{23} = -35.77$ (*c* 0.56, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26 (s, 9H), 1.43 (s, 3H), 1.54 (s, 3H), 3.08 (dd, *J* = 9.9, 14.7 Hz, 1H), 3.14 (d, *J* = 14.7 Hz, 1H), 3.90–4.05 (m, 1H), 4.52 (s, 1H), 5.00 (d, *J* = 8.4 Hz, 1H), 5.12 (d, *J* = 8.4 Hz, 1H), 6.01 (s, 1H), 7.22–7.57 (m, 5H), 7.82 (s, 1H), 8.28–8.43 (m, 4H), 8.58 (br.s, 2H); Ms(FAB): *m*/*z* 543 $[M+Na]^+$. Anal. calcd for $C_{27}H_{35}ClN_4O_6S$: H, 6.09; C, 56.00; N, 9.67. Found: H, 6.34; C, 55.71; N, 9.39.

5.22. (1*S*,2*S*)-2-Ammonio-1-({(4*R*)-4-[(*tert*-butylamino)-carbonyl]-5,5-dimethyl-1,3-thiazolan-3-yl}carbonyl)-3-phenylpropyl 4-chlorobenzoate chloride [Apns(*p*-chloro-benzoyl)-Dmt-NHBu^t·HCl, 6c]

Compound **6c** was prepared from **13c** (150 mg, 0.24 mmol) according to the procedure described for compound **3a**. Yield 124 mg (92%); mp 92–95 °C; $[\alpha]_D^{27} = -30.38$ (*c* 0.52, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26 (s, 9H), 1.43 (s, 3H), 1.53 (s, 3H), 3.02 (dd, *J* = 9.0, 15.9 Hz, 1H), 3.14 (d, *J* = 15.9 Hz, 1H), 3.88–4.02 (m, 1H), 4.50 (s, 1H), 4.98 (d, *J* = 8.7 Hz, 1H), 5.11 (d, *J* = 8.7 Hz, 1H), 5.93 (s, 1H), 7.22–7.55 (m, 6H), 7.65 (d, *J* = 8.7 Hz, 2H), 7.82 (s, 1H), 8.09 (d, *J* = 8.7 Hz, 2H), 8.54 (br.s, 2H); MS (FAB): *m*/*z* 532 $[M+H]^+$. Anal. calcd for $C_{27}H_{35}Cl_2N_3O_4S \cdot 1/4H_2O$: H, 6.24; C, 56.59; N, 7.33. Found: H, 6.42; C, 56.71; N, 7.09.

5.23. (1*S*,2*S*)-2-Ammonio-1-({(4*R*)-4-[(*tert*-butylamino)-carbonyl]-5,5-dimethyl-1,3-thiazolan-3-yl}carbonyl)-3-phenylpropyl 4-methylbenzoate chloride [Apns(*p*-toluoyl)-Dmt-NHBu^t·HCl, 6d]

Compound **6d** was prepared from **13d** (150 mg, 0.25 mmol) according to the procedure described for compound **3a**. Yield 134 mg (99%); mp 98–102 °C

$[\alpha]_D^{27} = -31.54$ (*c* 0.56, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26 (s, 9H), 1.42 (s, 3H), 1.53 (s, 3H), 2.41 (s, 3H), 3.05 (dd, *J* = 9.9, 14.1 Hz, 1H), 3.15 (d, *J* = 14.1 Hz, 1H), 3.77–3.98 (m, 1H), 4.50 (s, 1H), 4.99 (d, *J* = 8.5 Hz, 1H), 5.13 (d, *J* = 8.5 Hz, 1H), 5.89 (br.s, 1H), 7.18–7.52 (m, 7H), 7.82 (s, 1H), 7.95 (d, *J* = 8.1 Hz, 2H), 8.57 (br.s, 2H); MS (FAB): *m*/*z* 512 $[M+H]^+$. Anal. Calcd. for $C_{28}H_{38}ClN_3O_4S{\cdot}1/2H_2O$: H, 7.06; C, 60.36; N, 7.54. Found: H, 7.02; C, 60.23; N, 7.80.

5.24. (1*S*,2*S*)-2-Ammonio-1-({(4*R*)-4-[(*tert*-butylamino)-carbonyl]-5,5-dimethyl-1,3-thiazolan-3-yl}carbonyl)-3-phenylpropyl 4-methoxybenzoate chloride [Apns(*p*-anisoyl)-Dmt-NHBu^t·HCl, 6e]

Compound **6e** was prepared from **13e** (150 mg, 0.24 mmol) according to the procedure described for compound **3a**. Yield 120 mg (89%); mp 72–76 °C; $[\alpha]_D^{27} = -34.18$ (*c* 0.54, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26 (s, 9H), 1.42 (s, 3H), 1.52 (s, 3H), 3.04 (dd, *J* = 9.3, 14.1 Hz, 1H), 3.15 (d, *J* = 14.1 Hz, 1H), 3.87 (s, 3H), 4.50 (s, 1H), 4.92–5.04 (m, 1H), 5.06–5.18 (m, 1H), 5.78–5.93 (m, 1H), 7.07 (d, *J* = 8.7 Hz, 2H), 7.22-7.53 (m, 5H), 7.75–7.85 (m, 1H), 8.02 (d, *J* = 8.7 Hz, 2H), 8.58 (br.s, 2H); Ms(FAB): *m*/*z* 528 $[M+H]^+$. Anal. calcd for $C_{28}H_{38}ClN_3O_5S{\cdot}1/2H_2O$: H, 6.86; C, 58.68; N, 7.33. Found: H, 6.91; C, 58.55; N, 7.33.

5.25. *N*4-(*tert*-Butyl)-(4*R*)-3-((2*S*,3*S*)-2-hydroxy-3-{[2-(2-methylphenoxy)acetyl]amino}-4-phenylbutanoyl)-5,5-dimethyl-1,3-thiazolane-4-carboxamide (2-methylphenoxyacetyl-Apns-Dmt-NHBu^t, 4b)

To a solution of Apns-Dmt-NHBu^t·HCl (**14**)[12,15] (100 mg, 0.23 mmol) and 2,6-dimethylphenoxyacetic acid (**8b**) (43 mg, 0.26 mmol) in DMF (5 mL) were added HOBt (38 mg, 0.25 mmol), EDC·HCl (48 mg, 0.25 mmol) and Et_3N (23 mg, 0.23 mmol) stepwise at 0 °C and the mixture was stirred overnight at room temperature. After removal of the solvent in vacuo, the residue was dissolved in EtOAc, washed sequentially with 5% citric acid, 5% $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$, and concentrated in vacuo. The residue was applied to a silica gel column (1.5×15 cm) and eluted with hexane–EtOAc (1:1) to give 116 mg (92%) of the title compound **4b** as a white solid: mp 73–75 °C; $[\alpha]_D^{25} = -19.2$ (*c* 0.25, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (s, 9H), 1.53 (s, 3H), 1.59 (s, 3H), 2.25 (s, 3H), 2.68–2.91 (m, 2H), 3.73 (dd, *J* = 6.9, 17.7 Hz, 1H), 4.18–4.38 (m, 2H), 4.40–4.55 (m, 2H), 4.72–5.20 (m, 3H), 6.58–6.70 (m, 2H), 6.88–7.02 (m, 2H), 7.02–7.32 (m, 7H); MS (FAB): *m*/*z* 542 $[M+H]^+$. Anal. calcd for $C_{29}H_{39}N_3O_5S$: H, 7.26; C, 64.30; N, 7.76. Found: H, 7.37; C, 64.01; N, 7.84.

5.26. *N*4-(*tert*-Butyl)-(4*R*)-3-{(2*S*,3*S*)-2-hydroxy-3-[(2-phenoxyacetyl)amino]-4-phenylbutanoyl}-5,5-dimethyl-1,3-thiazolane-4-carboxamide (phenoxyacetyl-Apns-Dmt-NHBu^t, 4c)

Compound **4c** was prepared from Apns-Dmt-NHBu^t·HCl (**14**) (100 mg, 0.23 mmol) and phenoxyacetic acid (**8c**) (40 mg, 0.26 mmol) according to the procedure described for compound **4b**. Yield 109 mg (88%); mp 71-75 °C; $[\alpha]_D^{26} = -2.64$ (*c* 0.50, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.34 (s, 9H), 1.49–1.66 (m, 6H), 2.68–2.82 (m, 2H), 3.76 (dd, *J* = 6.8, 17.6 Hz, 1H), 4.18–4.52 (m, 4H), 4.69–5.18 (m, 3H), 6.60 (br.s, 1H), 6.78–6.90 (m, 2H), 6.91–7.12 (m, 3H), 7.12–7.39 (m, 7H); MS (FAB): *m*/*z* 528 $[M+H]^+$. Anal. calcd for $C_{28}H_{37}N_3O_5S$: H, 7.07; C, 63.73; N, 7.96. Found: H, 7.14; C, 63.42; N, 7.91.

5.27. *N*4-(*tert*-Butyl)-(4*R*)-3-{(2*S*,3*S*)-2-hydroxy-4-phenyl-3-[(2-phenylacetyl)amino]butanoyl}-5,5-dimethyl-1,3-thiazolane-4-carboxamide (phenylacetyl-Apns-Dmt-NHBu^t, 15)

Compound **15** was prepared from Apns-Dmt-NHBu^t·HCl (**14**) (45 mg, 0.11 mmol) and phenylacetic acid (**9**) (19 mg, 0.14 mmol) according to the procedure described for compound **4b**. Yield 49 mg (84%); mp 79–82 °C; $[\alpha]_D^{24} = -13.73$ (*c* 0.51, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27 (s, 6H), 1.30 (s, 3H), 1.51 (s, 3H), 1.57 (s, 3H), 2.51–2.80 (m, 2H), 3.47 (s, 2H), 4.06–4.20 (m, 1H), 4.37 (s, 1H), 4.63–5.18 (m, 3H), 5.58–6.15 (m, 1H), 6.66 (s, 1H), 6.85–7.12 (m, 4H), 7.12–7.40 (m, 6H); HRMS (FAB): *m*/*z* 512.2576 for $[M+H]^+$ (calcd 512.2583 for $C_{28}H_{38}N_3O_4S$). Anal. calcd for $C_{28}H_{37}N_3O_4S$: H, 7.29; C, 65.72; N, 8.21. Found: H, 7.42; C, 65.43; N, 8.17.

5.28. *N*4-(*tert*-Butyl)-(4*R*)-3-[(2*S*,3*S*)-3-(benzoylamino)-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolane-4-carboxamide (benzoyl-Apns-Dmt-NHBu^t, 16a)

Compound **16a** was prepared from Apns-Dmt-NHBu^t·HCl (**14**) (45 mg, 0.11 mmol) and benzoic acid (**10a**) (17 mg, 0.14 mmol) according to the procedure described for compound **4b**. Yield 50 mg (88%); mp 96–99 °C; $[\alpha]_D^{24} = -30.59$ (*c* 0.51, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26–1.40 (m, 9H), 1.49–1.71 (m, 6H), 2.91 (dd, *J* = 5.1, 15.0 Hz, 1H), 3.00 (dd, *J* = 9.3, 15.0 Hz, 1H), 4.31–4.48 (m, 1H), 4.52 (s, 1H), 4.59–4.71 (m, 1H), 4.78–4.88 (m, 1H), 4.89–5.00 (m, 1H), 5.02–5.20 (m, 1H), 6.62–7.00 (m, 1H), 7.08–7.32 (m, 5H), 7.32–7.44 (m, 2H), 7.44–7.58 (m, 1H), 7.62 (d, *J* = 7.5 Hz, 1H), 7.66 (d, *J* = 1.5, 6.9 Hz, 1H); Ms(FAB): *m*/*z* 498 $[M+H]^+$. Anal. calcd for $C_{27}H_{35}N_3O_4S{\cdot}1/3H_2O$: H, 7.14; C, 64.39; N, 8.34. Found: H, 7.26; C, 64.30; N, 8.67.

5.29. *N*4-(*tert*-Butyl)-(4*R*)-3-{(2*S*,3*S*)-2-hydroxy-3-[(4-nitrobenzoyl)amino]-4-phenylbutanoyl}-5,5-dimethyl-1,3-thiazolane-4-carboxamide (*p*-nitrobenzoyl-Apns-Dmt-NHBu^t, 16b)

Compound **16b** was prepared from Apns-Dmt-NHBu^t·HCl (**14**) (45 mg, 0.11 mmol) and *p*-nitrobenzoic acid (**10b**) (23 mg, 0.14 mmol) according to the procedure described for compound **4b**. Yield 55 mg (88%); mp 108–112 °C; $[\alpha]_D^{25} = -40.97$ (*c* 0.55, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32 (s, 9H), 1.48–1.64 (m, 6H), 2.84–3.08 (m, 2H), 4.35 (s, 1H), 4.40–4.45 (m, 1H), 4.60–4.72 (m, 1H), 4.73–4.83 (m, 1H), 4.84–4.98 (m, 1H), 5.12 (d, *J* = 9.3 Hz, 1H), 7.11–7.37 (m, 5H), 7.76 (d,

J = 8.7 Hz, 1H), 7.83 (d, J = 8.7 Hz, 1H), 8.20 (d, J = 8.7 Hz, 1H), 8.25 (d, J = 8.7 Hz, 1H); HRMS (FAB): m/z 543.2281 for $[M+H]^+$ (calcd 543.2277 for $C_{27}H_{35}N_4O_6S$). Anal. calcd for $C_{27}H_{34}N_4O_6S\cdot 1/3H_2O$: H, 6.42; C, 58.47; N, 10.10. Found: H, 6.37; C, 58.40; N, 10.21.

5.30. *N*4-(*tert*-Butyl)-(4*R*)-3-{(2S,3S)-3-[(4-chlorobenzoyl)amino] - 2 - hydroxy - 4 - phenylbutanoyl} - 5,5 - dimethyl-1,3-thiazolane-4-carboxamide (*p*-chlorobenzoyl-Apns-Dmt-NHBu^t, 16c)

Compound **16c** was prepared from Apns-Dmt-NHBu^t·HCl (**14**) (45 mg, 0.11 mmol) and *p*-chlorobenzoic acid (**10c**) (22 mg, 0.14 mmol) according to the procedure described for compound **4b**. Yield 52 mg (86%); mp 103–106 °C; $[\alpha]_D^{25} = -37.57$ (c 0.54, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22–1.40 (m, 9H), 1.47–1.67 (m, 6H), 2.89 (dd, J = 7.5, 15.0 Hz, 1H), 2.98 (dd, J = 9.6, 15.0 Hz, 1H), 4.30–4.45 (m, 1H), 4.49 (s, 1H), 4.57–4.70 (m, 1H), 4.74–4.88 (m, 1H), 4.89–5.00 (m, 1H), 5.15 (d, J = 9.9 Hz, 1H), 6.60–7.02 (m, 1H), 7.10–7.32 (m, 5H), 7.33 (d, J = 8.7 Hz, 1H), 7.37 (d, J = 8.4 Hz, 1H), 7.54 (d, J = 8.7 Hz, 1H), 7.60 (d, J = 8.4 Hz, 1H); MS(FAB): m/z 532 $[M+H]^+$. Anal. calcd for $C_{27}H_{34}ClN_3O_4S\cdot 1/2H_2O$: H, 6.52; C, 59.93; N, 7.77. Found: H, 6.47; C, 59.95; N, 7.98.

5.31. *N*4-(*tert*-Butyl)-(4*R*)-3-{(2*S*,3*S*)-2-hydroxy-3-[(4 - methylbenzoyl)amino] - 4 - phenylbutanoyl} - 5,5 - dimethyl-1,3-thiazolane-4-carboxamide (*p*-toluoyl-Apns-Dmt-NHBu^t, 16d)

Compound **16d** was prepared from Apns-Dmt-NHBu^t·HCl (**14**) (45 mg, 0.11 mmol) and *p*-toluic acid (**10d**) (19 mg, 0.14 mmol) according to the procedure described for compound **4b**. Yield 49 mg (84%); mp 101–105 °C; $[\alpha]_D^{25} = -37.45$ (c 0.51, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22–1.40 (m, 9H), 1.48–1.63 (m, 6H), 2.30–2.44 (m, 3H), 2.89 (dd, J = 8.4, 14.7 Hz, 1H), 2.98 (dd, J = 8.7, 14.7 Hz, 1H), 4.33–4.47 (m, 1H), 4.51 (s, 1H), 4.59–4.70 (m, 1H), 4.75–4.89 (m, 1H), 4.90–5.00 (m, 1H), 5.07–5.22 (m, 1H), 6.73–7.00 (m, 1H), 7.10–7.24 (m, 5H), 7.24–7.34 (m, 2H), 7.51 (d, J = 8.1 Hz, 1H), 7.55 (d, J = 8.1 Hz, 1H); Ms(FAB): m/z 512 $[M+H]^+$. Anal. calcd for $C_{28}H_{37}N_3O_4S$: H, 7.29; C, 65.72; N, 8.21. Found: H, 7.36; C, 65.41; N, 8.07.

5.32. *N*4-(*tert*-Butyl)-(4*R*)-3-{(2*S*,3*S*)-2-hydroxy-3-[(4 - methoxybenzoyl)amino]-4-phenylbutanoyl}-5,5-dimethyl-1,3-thiazolane-4-carboxamide (*p*-anisoyl-Apns-Dmt-NHBu^t, 16e)

Compound **16e** was prepared from Apns-Dmt-NHBu^t·HCl (**14**) (45 mg, 0.11 mmol) and *p*-anisic acid (**10e**) (21 mg, 0.14 mmol) according to the procedure described for compound **4b**. Yield 51 mg (85%); mp 96–100 °C; $[\alpha]_D^{25} = -38.99$ (c 0.61, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24–1.40 (m, 9H), 1.48–1.70 (m, 6H), 2.88 (dd, J = 6.6, 15.0 Hz, 1H), 2.97 (dd, J = 8.7, 15.0 Hz, 1H), 3.55–3.78 (m, 1H), 3.82 (s, 3H), 4.30–4.43 (m, 1H), 4.51 (s, 1H), 4.58–4.68 (m, 1H), 4.77–4.87 (m, 1H), 4.89–5.00 (m, 1H), 5.06–5.22 (m, 1H), 6.49–6.82 (m, 1H), 6.82–7.00 (m, 3H), 7.12–7.22 (m, 3H), 7.22–7.33 (m, 2H), 7.61 (t, J = 9.0 Hz, 2H); Ms(FAB): m/z 528 $[M+H]^+$. Anal. calcd for $C_{28}H_{37}N_3O_5S\cdot 1/2H_2O$: H, 7.14; C, 62.66; N, 7.83. Found: H, 6.94; C, 62.62; N, 7.68.

5.33. Water-solubility of prodrugs and parent compounds

The water-solubility of the prodrugs and parent compounds was determined by HPLC analysis. HPLC was performed using a C18 reverse phase column (YMC AM-302 ODS; 4×150 mm) and a binary solvent system at a flow rate of 1 mL/min with detection at UV 230 nm. An excessive amount of the prodrug or the parent drug was suspended in pure water under supersonification for 15 min at rt, and subjected to centrifugal membrane filtration (pore size, 0.45 μm). Water-solubility was calculated from the peak area of the solution compared with the standard solution (1 mg/mL in MeOH) of the prodrug or parent drug. The conversion of prodrugs to the parent drugs was not observed during the experiment, since the compound solution was slightly acidic due to an HCl salt.

5.34. Determination of the reaction rate of *O*→*N* acyl migration of synthetic prodrugs

The migration rate of prodrugs **3a–f**, **5** and **6a–e** was determined by RP-HPLC. First, 5 μL of a methanol solution (1 mg/mL) of the prodrug was poured into 500 μL of PBS buffer (pH 7.4, 5.5, 4.9) and incubated at 37 °C. At different points in time, 200 μL of the mixture was then analyzed directly by HPLC. HPLC was performed using a C18 reverse phase column (YMC AM-302 ODS; 4×150 mm) and a binary solvent system at a flow rate of 1 mL/min, with detection at UV 230 nm. Prodrugs **3a–f**, **5** and **6a–e** were flowed out with a linear gradient of MeCN 40–100% (15min.) in 0.1% aqueous TFA. The half-life of the prodrugs was calculated from the peak areas at each incubation time.

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References and notes

1. 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. U.S.A.* **1988**, *85*, 4686.
2. Seelmeier, S.; Schmidt, H.; Turk, V.; von der Helm, K. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 6612.
3. Debouck, C. *AIDS R. H.* **1992**, *8*, 153.
4. Huff, J. R. *J. Med. Chem.* **1991**, *34*, 2305.
5. De Clercq, E. *J. Med. Chem.* **1995**, *38*, 2491.
6. Kiso, Y. *Biopolymers* **1996**, *40*, 235.
7. Wei, X.; Ghosh, S. K.; Taylor, M. E.; Johnson, V. A.; Emini, E. A.; Deutsh, P.; Lifson, J. D.; Bonhoeffer, S.; Nowak, M. A.; Hahn, B. H.; Saag, M. S.; Shaw, G. M. *Nature* **1995**, *273*, 117.
8. Richman, D. D. *Nature* **1995**, *374*, 494.

9. Rosenberg, S. H.; Woods, K. W.; Sham, H. L.; Kleinert, H. D.; Martin, D. L.; Stein, H.; Cohen, J.; Egan, D. A.; Bopp, B.; Merits, I.; Garren, K. W.; Hoffman, D. J.; Plattner, J. J. *J. Med. Chem.* **1990**, *33*, 1962.
10. Chong, K.-T.; Ruwart, M. J.; Hinshaw, R. R.; Wilkinson, K. F.; Rush, B. D.; Yancey, M. F.; Strohbach, J. W.; Thaisrivongs, S. *J. Med. Chem.* **1993**, *36*, 2575.
11. Thaisrivongs, S.; Strohbach, J. W. *Biopolymers* **1999**, *51*, 51.
12. Mimoto, T.; Hattori, N.; Takaku, H.; Kisanuki, S.; Fukazawa, T.; Kato, R.; Nojima, S.; Misawa, S.; Ueno, T.; Imai, J.; Enomoto, H.; Tanaka, S.; Shikikawa, H.; Shintani, M.; Hayashi, H.; Kiso, Y. *Chem. Pharm. Bull.* **2000**, *48*, 1310.
13. 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. Agent Chemother.* **1993**, *37*, 810.
14. Mimoto, T.; Imai, J.; Kisanuki, S.; Enomoto, H.; Hattori, N.; Akaji, K.; Kiso, Y. *Chem. Pharm. Bull.* **1992**, *40*, 2251.
15. (a) 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. (b) HIV-1 PR inhibitory activities: KNI-707 (**4d**): 56% inhibition at 50 nM, 95% inhibition at 5 μM (K_i value: 21.7 nM), KNI-706 (**4e**): 82% inhibition at 5 μM, KNI-705 (**4f**): 48% inhibition at 5 μM.
16. Kiso, Y.; Matsumoto, H.; Mizumoto, S.; Kimura, T.; Fujiwara, Y.; Akaji, K. *Biopolymers* **1999**, *51*, 59.
17. Kiso, Y.; Matsumoto, H.; Yamaguchi, S.; Kimura, T. *Lett. Peptide Sci.* **1999**, *6*, 275.
18. Mimoto, T.; Imai, J.; Tanaka, S.; Hattori, N.; Takahashi, O.; Kisanuki, S.; Nagano, Y.; Shintani, M.; Hayashi, H.; Shikikawa, H.; Akaji, K.; Kiso, Y. *Chem. Pharm. Bull.* **1991**, *39*, 2465.
19. Mimoto, T.; Imai, J.; Tanaka, S.; Hattori, N.; Kisanuki, S.; Akaji, K.; Kiso, Y. *Chem. Pharm. Bull.* **1991**, *39*, 3088.
20. Kiso, Y.; Yamaguchi, S.; Matsumoto, H.; Mimoto, T.; Kato, R.; Nojoma, S.; Takaku, H.; Fukazawa, T.; Kimura, T.; Akaji, K. *Arch. Pharm. Pharm. Med. Chem.* **1998**, *331*, 87.
21. Kempf, D. J.; Sham, H. L.; Marsh, K. C.; Flentge, C. A.; Betebenner, D.; Green, B. E.; McDonald, E.; Vasavanonda, S.; Saldivar, A.; Wideburg, N. E.; Kati, W. M.; Ruiz, L.; Zhao, C.; Fino, L.; Patterson, J.; Molla, A.; Plattner, J. J.; Norbeck, D. W. *J. Med. Chem.* **1998**, *41*, 602.
22. Kempf, D. J.; Marsh, K. C.; Denissen, J. F.; McDonald, E.; Vasavanonda, S.; Flentge, C. A.; Green, B. E.; Fino, L.; Park, C. H.; Kong, X.-P.; Wideburg, N. E.; Saldivar, A.; Ruiz, L.; Kati, W. M.; Sham, H. L.; Robins, T.; Stewart, K. D.; Hsu, A.; Plattner, J. J.; Leonard, J. M.; Norbeck, D. W. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 2484.
23. Kim, E. E.; Baker, C. T.; Dwyer, M. D.; Murcko, M. A.; Rao, B. G.; Tung, R. D.; Navia, M. A. *J. Am. Chem. Soc.* **1995**, *117*, 1181.
24. Mathé, C.; Périgaud, C.; Gosselin, G.; Imbach, L.-L. *J. Org. Chem.* **1998**, *63*, 8547.
25. Nicolaou, M. G.; Yuan, C.-S.; Borchardt, R. T. *J. Org. Chem.* **1996**, *61*, 8636.
26. Truelove, J. E.; Hussain, A. A.; Kostenbauder, H. B. *J. Pharm. Sci.* **1980**, *69*, 231.
27. Friend, D. R.; Chang, G. W. *J. Med. Chem.* **1984**, *27*, 261.
28. Takata, J.; Karube, Y.; Nagata, Y.; Matsushita, Y. *J. Pharm. Sci.* **1995**, *84*, 96.
29. Pochopin, N. L.; Charman, W. N.; Stella, V. J. *Int. J. Pharm.* **1995**, *121*, 157.
30. (a) Matsumoto, H.; Sohma, Y.; Kimura, T.; Hayashi, Y.; Kiso, Y. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 605. (b) Sohma, Y.; Hayashi, Y.; Ito, T.; Matsumoto, H.; Kimura, T.; Kiso, Y. *J. Med. Chem.* **2003**, *46*, 4124.
31. Wakamiya, T.; Tarumi, Y.; Shiba, T. *Bull. Chem. Soc. Jpn.* **1974**, *47*, 2686.
32. Oliyai, R.; Siahaan, T. J.; Stella, V. J. *Pharm. Res.* **1995**, *12*, 323.
33. Oliyai, R.; Stella, V. *J. Bioorg. Med. Chem. Lett.* **1995**, *5*, 2735.
34. Hurley, T. R.; Colson, C. E.; Hicks, G.; Ryan, M. J. *J. Med. Chem.* **1993**, *36*, 1496.
35. Kimura, T.; Ohtake, J.; Nakata, S.; Enomoto, H.; Moriwaki, H.; Akaji, K.; Kiso, Y. In Ohno, M., Ed.; *Peptide Chemistry* 1994; Protein Research Foundation: Osaka, 1995; p 157.
36. Kiso, Y., Kimura, T., Ohtake, J., Nakata, S., Enomoto, H., Moriwaki, H., Nakatani, M., Akaji, K. *Peptides: Chemistry, Structure and Biology (Proceedings of the Fourteenth American Peptide Symposium*); Mayflower Scientific: UK; p 157.
37. Hamada, Y.; Ohtake, J.; Sohma, Y.; Kimura, T.; Hayashi, Y.; Kiso, Y. *Bioorg. Med. Chem.* **2002**, *10*, 4155.
38. Hamada, Y.; Matsumoto, H.; Kimura, T.; Hayashi, Y.; Kiso, Y. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2727.
39. Horiki, K. *Tetrahedron Lett.* **1977**, *22*, 1897.
40. König, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788.
41. (a) Katagi, T.; Kataoka, H. *Heterocycles* **1987**, *26*, 2109. (b) Kataoka, H.; Katagi, T. *Chem. Pharm. Bull.* **1989**, *37*, 3166.
42. Lide, D. R. *Handbook of Chemistry and Physics*, 82nd ed..; CRC: Boca Raton, 1982; p 46.
43. Hammett, L. P. *Physical Organic Chemistry: Reaction Rates, Equilibra, and Mechanisms*, 2nd ed.; McGraw-Hill: New York, 1970; p 347.