Water-Soluble Prodrugs of the Human Immunodeficiency Virus Protease Inhibitors Lopinavir and Ritonavir

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We studied the synthesis, cleavage rates, and oral administration of prodrugs of the HIV protease inhibitors (PIs) lopinavir and ritonavir. Phosphate esters attached directly to the central hydroxyl groups of these PIs did not demonstrate enzyme-mediated cleavage in vitro and did not provide measurable plasma levels of the parent drugs in vivo. However, oxymethylphosphate (OMP) and oxyethylphosphate (OEP) prodrugs provided improved rates of cleavage, high levels of aqueous solubility, and high plasma levels of the parent drugs when dosed orally in rats and dogs. Dosing unformulated capsules containing the solid prodrugs led to plasma levels equivalent to those observed for dosing formulated solutions of the parent drugs. A direct synthetic process for the preparation of OMP and OEP prodrugs was developed, and the improved synthetic method may be applicable to the preparation of analogous soluble prodrugs of other drug classes with limited solubility.

Introduction

HIV protease inhibitors (PIs[a]) are relatively lipophilic molecules and are poorly soluble in water. When dosed orally, insoluble drugs are not efficiently absorbed from the solid state and require specialized formulations to enhance their solubility in the gastrointestinal tract. Consequently, HIV PIs have been associated with high pill counts and large capsule sizes. Prodrugs that enhance aqueous solubility could reduce the pill burden for these important therapies, impacting patient compliance and quality of life. Prodrugs have been widely used by medicinal chemists to mask the properties of drug molecules to allow for absorption and distribution to the targeted sites of action.[1] Several prodrug approaches for HIV PIs have been reported. Phosphate esters, which undergo cleavage by phosphatase in vivo to provide the parent drug, have been employed to provide improved aqueous solubility, since they are highly ionized at physiological pH.[2] In fact, fosamprenavir (Figure 1), a phosphate ester prodrug with a lower pill burden, is the currently marketed form of amprenavir (APV).[3] Other approaches for water-soluble prodrugs of HIV PIs have employed self-cleavable spacers and oxygen to nitrogen acyl migration.[4,5] In this report, we present the synthesis, cleavage rates, and oral pharmacokinetics (PK) of prodrugs of the HIV PIs, lopinavir (LPV) and ritonavir (RTV), shown in Figure 1.[6] LPV is coformulated with RTV to enhance its pharmacokinetic profile and is widely used in highly active antiretroviral therapy (HAART) in combination with two nucleoside reverse transcriptase inhibitors. LPV and RTV also have poor aqueous solubilities, and the original formulation for LPV/RTV required three soft-gelatin capsules (SGC) taken twice daily. We explored water-soluble prodrugs of LPV and RTV with the goal of achieving equivalent pharmacokinetics with a lower pill burden.

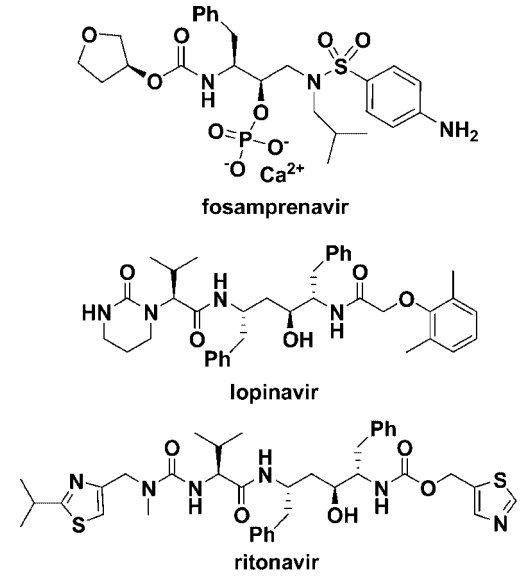

Figure 1. Structures of fosamprenavir, lopinavir, and ritonavir.

Results and Discussion

Initially, we investigated phosphate esters attached to the central hydroxyl groups of LPV and RTV. The prodrugs were prepared under the standard conditions using the phosphoramidite method (Scheme 1). Deprotection of the phosphate triesters was accomplished by hydrogenolysis with Pd on carbon for LPV and by treatment with trimethylsilyl bromide for RTV. A pharmacokinetic study of phosphate **1** was conducted in rats at 5 mg/kg. Dosed intravenously, **1** had a short half-life (0.23 h) and a high clearance rate (2.58 L/(h·kg)). Dosed orally, plasma concentrations of **1** or RTV were not detected in any of the plasma samples (out to 3 h after dosing). The pharmacokinetic results for **1** are in contrast to those observed with

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[a] Abbreviations: PI, protease inhibitor; OMP, oxymethylphosphate; OEP, oxyethylphosphate; APV, amprenavir; PK, pharmacokinetic; LPV, lopinavir; RTV, ritonavir; HAART, highly active antiretroviral therapy; CIAP, calf intestinal alkaline phosphatase; MTM, methylthiomethyl; OBP, oxybutylphosphate; OIBP, oxyisobutylphosphate; EC, enteric coating.

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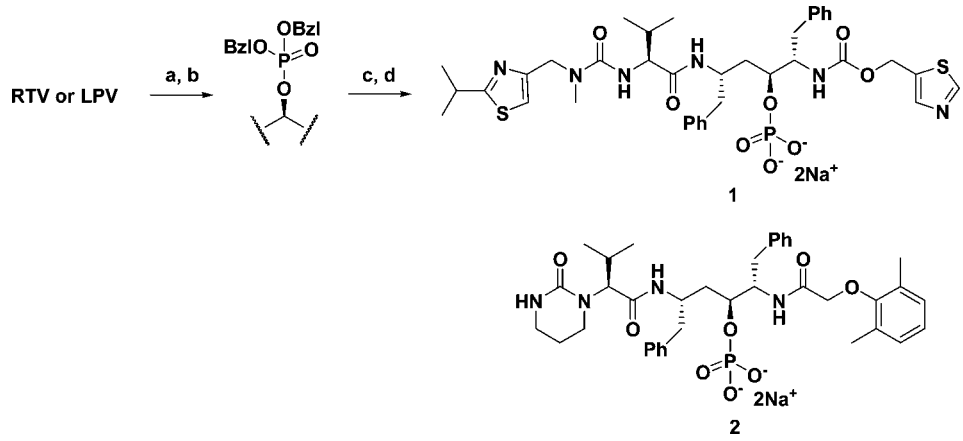
Scheme 1. Synthesis of LPV and RTV Phosphates[a]



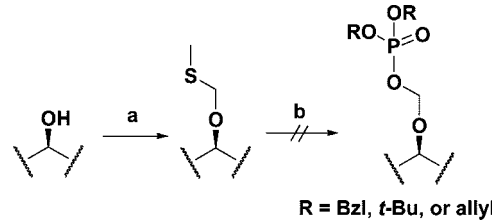
[a] Reagents and conditions: (a) $(BzlO)_2PN(Et)_2$, 1*H*-tetrazole; (b) mCPBA; (c) $Pd(OH)_2$ on C, H_2 for LPV or trimethylsilyl bromide for RTV; (d) Na_2CO_3.

Table 1. In Vitro Cleavage of Phosphates

compd	prodrug	CIAP $t_{1/2}$ (min)[a]
1	RTV-phosphate	no dephos[b]
2	LPV-phosphate	no dephos[b]
3	RTV-OMP	7.0
4	RTV-OEP	14.8
5	RTV-OBP	31.7
6	RTV-OIBP	>30
7	LPV-OMP	13.9
8	LPV-OEP	20.4

[a] Calf intestinal alkaline phosphatase. [b] No dephosporylation was observed.

Scheme 2. Attempted Synthesis of Homologated Phosphates[a]



[a] Reagents and conditions: (a) Me_2S, $(PhCO)_2O_2$; (b) $(RO)_2P(O)OH$, *N*-iodosuccinimide.

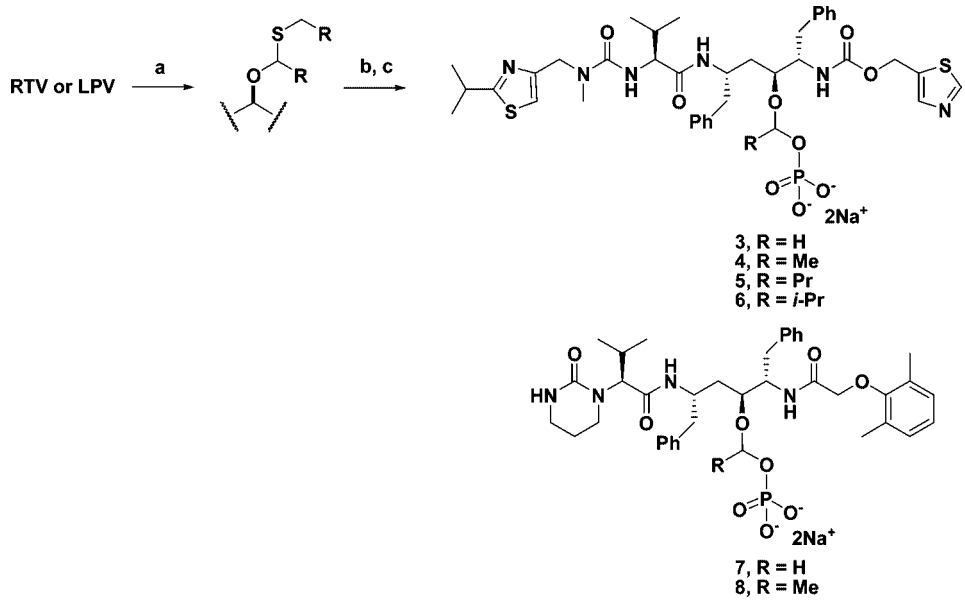
fosamprenavir, which provided systemic levels of APV when dosed orally in rats.[3] In order to understand the in vivo results, the rates of dephosphorylation of the prodrugs were evaluated in vitro using calf intestinal alkaline phosphatase (CIAP). As shown in Table 1, no dephosphorylation could be detected for phosphate prodrug **1** or **2**, while fosamprenavir demonstrated a half-life of 13.2 min in this assay. As a possible explanation for this observation, the peptidomimetic cores of APV and RTV differ significantly in structure. The core of APV and thus fosamprenavir (Figure 1) is an (*R*)-hydroxyethylsulfonamide dipeptide isostere,[3] whereas the core of LPV and RTV is a pseudo-C_2-symmetric dipeptide isostere with an *S* stereochemical configuration at the central hydroxyl group.[6] Thus, the structural features in the vicinity of the phosphate ester differ both in stereochemistry and in P2′ amide structure (carboxamide for LPV and RTV vs sulfonamide for fosamprenavir), and the accessibility of the phosphate ester by the phosphatase enzyme might differ significantly.

One potential solution to this problem was to move the phosphate ester cleavage site away from the peptidomimetic core through a formaldehyde acetal linkage, which would release parent drug and formaldehyde upon phosphate ester cleavage.[7] Attempts to synthesize such oxymethylphosphate (OMP) prodrugs from the methylthiomethyl (MTM) ethers of pseudo-C_2-symmetric HIV PI cores (Scheme 2) using the chemistry employed for the synthesis of paclitaxel prodrugs (reaction with protected phosphate diesters in the presence of *N*-iodosuccinimide)[8] failed to give the desired phosphate triester products. This was most likely due to high instability of the oxymethyl-linked phosphate triesters attached to these cores, although the triesters could not be isolated to test this hypothesis. We discovered, in an attempt to avoid the phosphate triesters, that treatment of the MTM ethers with crystalline phosphoric acid (orthophosphoric acid)[9] in the presence of *N*-iodosuccinimide, followed by treatment with sodium carbonate, gave the OMP prodrugs **3** (RTV-OMP) and **7** (LPV-OMP) directly (Scheme 3). The reaction was conducted in the presence of 4 Å molecular sieves to scavenge water, and workup involved filtration to remove the sieves, treatment with sodium thiosulfate to remove any iodine or *N*-iodosuccinimide, and finally treatment with Na_2CO_3 to give the sodium salts. Final products were purified by elution on reversed-phase HPLC. We discovered that this methodology was also applicable to the synthesis of homologated prodrugs with alkyl branching on the methylene group. For example, oxyethylphosphate (OEP) prodrugs **4** (RTV-OEP) and **8** (LPV-OEP) were synthesized starting from ethyl sulfide in place of methyl sulfide.[10] The thioether intermediates also underwent clean conversion to the OEP prodrugs using crystalline phosphoric acid in the presence of *N*-iodosuccinimide. The prodrugs were obtained as a mixture of diastereomers at the branching carbon. By use of the same methods, oxybutylphosphate **5** (RTV-OBP) and oxyisobutylphosphate **6** (RTV-OIBP) prodrugs were prepared. Upon cleavage by phosphatase, the OEP, OBP, and OIBP phosphates release acetaldehyde, butyraldehyde, and isobutyraldehyde, respectively, instead of formaldehyde that is released by the OMP prodrugs. Although the amount of formaldehyde generated from OMP prodrugs would be small, formaldehyde is a probable human carcinogen.[11]

In contrast to prodrugs **1** and **2**, which showed no dephosphorylation in the CIAP assay, the half-lives of OMP prodrugs **3** and **7** were 7 and 14 min, respectively, values similar to those observed for fosamprenavir in this assay (Table 1). The OEP prodrugs **4** and **8** had 2-fold longer half-lives compared to the OMP prodrugs. Slower rates of cleavage were also observed for **5** and **6**.

The OMP and OEP prodrugs were completely soluble in water at 5 mg/mL, whereas the parent drugs had low aqueous solubilities (Table 2). We examined the pH-dependent chemical

Scheme 3. Synthesis of Homologated Phosphates Using Phosphoric Acid[a]



[a] Reagents and conditions: (a) RCH_2SCH_2R, $(PhCO)_2O_2$; (b) H_3PO_4, *N*-iodosuccinimide; (c) Na_2CO_3.

Table 2. Aqueous Solubility Data[a]

compd	solubility (μg/mL)
LPV	7
RTV	3
3, RTV-OMP	>5000
4, RTV-OEP	>5000
7, LPV-OMP	>5000
8, LPV-OEP	>5000

[a] Unbuffered at 25 °C by the shake flask method.

Table 3. pH-Dependent Chemical Stability of Prodrugs at 25 °C

	$t_{1/2}$			
compd	pH 2	pH 5	pH 7.4	pH 9
3, RTV-OMP	8.6 h	NA[a]	NA[a]	NA[a]
7, LPV-OMP	7.8 h	NA[a]	NA[a]	NA[a]
4, RTV-OEP	<15 min	76 min	12 days	>100 days
8, LPV-OEP	<15 min	133 min	19 days	>100 days

[a] NA = not available.

stability of the prodrugs, as shown in Table 3. The OMP prodrugs **3** and **7** demonstrated half-lives of 8 h at pH 2, a value similar to the gastric pH in humans. The OEP prodrugs **4** and **8**, however, showed pH-dependent stability and rapidly degraded with conversion to parent at low pH and were markedly more stable at higher pH values. The short half-lives at pH 2 for the OEP prodrugs could result in acid mediated removal of the prodrug moiety in the stomach, resulting in precipitation of parent drug. As a result, protection from stomach acid through enteric coating (EC) might be necessary for the OEP prodrugs in order to realize the benefit of their high aqueous solubilities.

The pharmacokinetic properties of the prodrugs were initially characterized in rats (Table 4). Since LPV has low bioavailability in rats when dosed alone due to rapid metabolism by cytochrome P450 3A isoenzymes (CYP3A), LPV prodrugs were codosed with RTV, which potently inhibits CYP3A and enhances LPV plasma concentrations.[12] While dosing of the parent compounds required the use of formulations containing ethanol and propylene glycol, the high solubility of the prodrugs allowed for dosing as solutions in 5% dextrose in water. RTV prodrugs **3**, **4**, and **5** produced RTV plasma exposures that were 82−87% lower than the AUC obtained after administration of

Table 4. Oral PK in Rats at 5 mg/kg

		parent plasma level	
entry	compd	C_{max} [a]	AUC [b]
1	RTV	1.36	3.53
2	LPV[c]	1.55	9.28
3	**3**, RTV-OMP	0.13	0.46
4	**4**, RTV-OEP	0.26	0.63
5	**5**, RTV-OBP	0.13	0.45
6	**7**, LPV-OMP[c]	2.27	19.65
7	**8**, LPV-OEP[c]	2.19	9.53

[a] μg/mL. [b] μg·h/mL. [c] Codosed with 5 mg/kg RTV.

the parent (entries 3, 4, and 5 vs entry 1). In contrast, dosing LPV-OMP **7** with RTV in rats led to plasma levels of LPV that were 2-fold higher than dosing of the parent LPV/RTV (cf. entries 2 and 6). Similarly, LPV-OEP **8** gave comparable LPV levels to those observed for dosing the parent (cf. entries 2 and 7). Observed plasma concentrations of the prodrugs were low (C_{max} = 0.02 μg/mL for **3**, C_{max} = 0.14 μg/mL for **7**), suggesting efficient release of parent in the intestine prior to absorption.

The pharmacokinetic properties of the prodrugs were studied more extensively in dogs (Table 5), the preferred species for evaluation of RTV formulations for human study.[13] Unlike the lower bioavailability of RTV-OMP **3** in rats, oral dosing of **3** provided nearly identical RTV plasma levels to dosing parent in dogs (cf. entries 1 and 2). Codosing LPV-OMP **7** with parent RTV provided LPV plasma exposure levels nearly identical to those obtained after dosing parent LPV (cf. entries 3 and 4). Importantly, administration of a mixture of the solid RTV-OMP **3** and LPV-OMP **7** in unformulated capsules resulted in plasma levels of both LPV and RTV comparable to codosing of the parents (cf. entries 3 and 5). This finding indicates that, as expected, dissolution of the soluble prodrugs in vivo is efficient. This result also demonstrates that prodrugs of each parent compound can be used together in combination and undergo efficient dephosphorylation.

The gastric pH of fasted dogs is variable and can reach high values.[3,13] Therefore, in order to simulate the lower gastric pH environment found in humans, dogs were pretreated with histamine to stimulate the release of gastric acid.[13] Histamine

Table 5. Oral PK in Dogs at 5 mg/kg[a]

				LPV level		RTV level	
entry	LPV form	RTV form	dosage form	C_{max}[b]	AUC[c]	C_{max}[b]	AUC[c]
1		parent	solution[d]			3.58	8.06
2		**3**, RTV-OMP	solution[d]			3.73	8.14
3	parent	parent	solution[d]	2.50	11.26	1.32	2.19
4	**7**, LPV-OMP	parent	solution[d]	2.36	12.71	2.13	4.43
5	**7**, LPV-OMP	**3**, RTV-OMP	capsule[e]	2.62	16.35	2.74	5.74
6[f]	**7**, LPV-OMP	**3**, RTV-OMP	capsule[e]	3.90	14.42	1.34	2.71
7[f]	**7**, LPV-OMP	**4**, RTV-OEP	capsule[e]	1.76	6.09	0.64	1.26
8[f]	**7**, LPV-OMP	**4**, RTV-OEP	EC capsule[g]	2.98	13.08	0.76	2.14
9[f]	**7**, LPV-OMP	**5**, RTV-OBP	capsule[e]	2.35	9.31	0.35	0.77
10[f]	**7**, LPV-OMP	**6**, RTV-OIBP	capsule[e]	1.47	5.63	0.30	0.58
11[f]	**8**, LPV-OEP	**4**, RTV-OEP	capsule[e]	1.60	5.82	0.61	1.27
12[f]	**8**, LPV-OEP	**4**, RTV-OEP	EC capsule[g]	3.14	13.61	1.64	3.84

[a] Prodrugs were dosed at the molar equivalent of 5 mg/kg of the parent. [b] μg/mL. [c] μg·h/mL. [d] RTV and LPV were dissolved in 5% dextrose containing 20% ethanol, 30% propylene glycol, with 2 equiv of methanesulfonic acid; **3** and **7** were dissolved in 5% dextrose. [e] Unformulated. [f] Dogs were pretreated with histamine prior to dosing. [g] EC = enteric coating that dissolves at pH 5.5.

pretreatment had no effect on the LPV plasma levels observed for codosed OMP prodrugs **3** and **7** (cf. entries 5 and 6), consistent with their stability at low pH. However, codosing of LPV-OMP **7** with RTV-OEP **4** after histamine pretreatment resulted in low plasma exposure levels of both LPV and RTV (entry 7). As described earlier, the OEP prodrugs decompose to parent under low pH conditions and this degradation could result in precipitation of the drugs, resulting in lower absorption. In order to test this hypothesis, LPV-OMP **7** and RTV-OEP **4** were codosed in enteric coated capsules (coating dissolves at pH 5.5), and improved parent exposure levels resulted (cf. entries 7 and 8). Coadministration of LPV-OMP **7** with RTV-OBP **5** or RTV-OIBP **6** in histamine pretreated dogs provided similarly low plasma levels to those observed for coadministration with RTV-OEP **4** (entries 9 and 10). It is likely that **5** and **6** share similar pH dependent stability profiles with **4**, leading to precipitation of parent in the gut. Codosing of two OEP produgs **8** and **4** together also resulted in low plasma levels of both parent drugs (entry 11). Once again, enteric coating improved the observed levels of the parent drugs and the levels were comparable to those observed for dosing the parents (cf. entries 3 and 12).

Conclusions

Phosphate esters attached directly to the central hydroxyl groups of LPV and RTV were not cleaved by phosphatase in vitro and were ineffective for delivery of parent drugs in vivo, in contrast to the HIV PI phosphate prodrug amprenavir. However, the OMP and OEP prodrugs of LPV and RTV provided dramatically improved aqueous solubilities compared to the parent molecules, demonstrated rapid enzyme-mediated cleavage in vitro, and produced high plasma levels of the parent drugs in vivo (rats and dogs). We demonstrated that dosing unformulated capsules in dogs containing the solid OMP and OEP prodrugs led to plasma levels equivalent to those observed for dosing formulated solutions of the parent drugs. Because of their high aqueous solubilities, it is hoped that using OMP and OEP prodrugs of LPV and RTV will lead to a lower pill burden by reducing the need for solubility enhancing excipients. We developed a direct method for the synthesis of these prodrugs, avoiding unstable phosphate triester intermediates by using phosphoric acid in place of the protected phosphate diesters. This improved synthetic method was useful for the preparation of methylene-linked as well as branched alkyl-linked phosphate ester prodrugs and may be applicable for the preparation of analogous soluble prodrugs of other drug classes with limited solubility.

Experimental Section

Methods. Calf Intestinal Alkaline Phosphatase Assay. The prodrugs (30 mM) in sodium phosphate (50 mM, pH 7.4) were incubated with CIAP (0.000 125 units/μL) in Tris buffer at pH 8.0 (400 μL, 10 mM) at 37 °C for 30 min. The samples were quenched with 20 μL of 5 M NaCl and 160 μL of 50% MeOH and 50% acetonitrile, allowed to stand on ice for 30 min, and then centrifuged at 10000 rpm for 10 min @ 4 °C. The supernatant was analyzed by HPLC or LC/MS/MS.

Pharmacokinetic Analysis. All prodrugs were formulated as 5 mg/mL solutions in 5% dextrose in water, and ritonavir for codosing was formulated as a 5 mg/mL solution in 5% dextrose containing 20% ethanol, 30% propylene glycol, and 2 equiv of methanesulfonic acid. Sprague−Dawley-derived rats (male, 0.25−0.35 kg, $n = 3$) and beagle dogs (male and female, 8−12 kg, $n = 3$) received prodrug doses equivalent to 5 mg/kg of body weight doses of the parent (5 mg equiv/kg) by oral gavage, with or without a prior 5 mg/kg dose of ritonavir by oral gavage. Alternatively, solid prodrugs or mixtures of solid prodrugs were added to capsules and dosed orally. Approximately 30 min prior to drug administration, some dogs received a 100 μg/kg subcutaneous dose of histamine. Plasma samples were analyzed by reversed-phase HPLC with an internal standard.

Solubility. Approximately 5 mg of each compound was weighed into 2 mL glass vials (in triplicate). One milliliter of distilled, deionized water purified by a Milli Q filtration system was added, and the samples were vortex mixed and sonicated. Vials were wrapped in aluminum foil to protect them from light and equilibrated by tumbling in a water bath maintained at 25 °C for 1 day. Samples were centrifuged, filtered, and prepared for HPLC assay by dilution to ensure concentrations within the standard curve. Samples were assayed by HPLC, and the compounds were detected using a UV detector set at 215 nm.

Prodrug Stability. Rapidly degrading samples were equilibrated at 25 °C and directly assayed by HPLC. The peak area vs time data were fit to a first order kinetic expression. More stable samples were subjected to equilibration at elevated temperatures (40−90 °C) for up to 7 days, and a first order kinetic model was used to predict stability at 25 °C.

Chemistry. Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. Anhydrous solvents were obtained from Aldrich (Milwaukee, WI) and used directly. All reactions involving air- or moisture-sensitive reagents were performed under a nitrogen or argon atmosphere. All final compounds were purified to >95% purity as determined by high-performance liquid chromatography (HPLC). Analytical HPLC analysis was performed on a Waters Alliance HPLC system and Waters Empower 2 software using a linear gradient (16 min)

starting with 5% acetonitrile in 1 mM NH_4OAc (pH 8) and ending with acetonitrile on a Waters XTerra RP18 column (5 μm, 4.6 mm $\times$ 150 mm, flow rate = 1 mL/min) with UV detection at 220 and 254 nm (RTV t_R = 10.90 min and LPV t_R = 11.0 min). Silica gel chromatography was performed using either glass columns packed with silica gel 60 (230−400 mesh) or prepacked silica gel cartridges (Biotage, 36−63 μm). Preparative reversed-phase HPLC was conducted on a Waters 600 system using Waters Nova-Pak C18 (6 μm, 60 Å) or Biotage KP-C18-HS cartridges. NMR spectra were determined with Bruker ARX 300 MHz or Varian Unity INOVA 500 MHz spectrometers. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane internal standard. Integrations of diastereomeric mixtures are approximate. Low-resolution mass spectral (MS) ESI data were determined on a Thermo-Finnigan SSQ7000 instrument. Combustion analysis was performed by Quantitative Technologies Inc. (Whitehouse, NJ), and data were within 0.4% of calculated values.

Disodium N^1-((1*S*,3*S*,4*S*)-1-Benzyl-5-phenyl-3-(phosphonatooxy)-4-{[(1,3-thiazol-5-ylmethoxy)carbonyl]amino}pentyl)-N^2-{[[(2-isopropyl-1,3-thiazol-4-yl)methyl](methyl)amino]carbonyl}-L-valinamide (1). A solution of RTV (6.0 g, 8.32 mmol), dibenzyl *N*,*N*-diethylphosphoramidite (3.96 g, 12.5 mmol), and 1*H*-tetrazole (2.63 g, 37.5 mmol) in THF (100 mL) was stirred at room temperature for 4 h. The mixture was cooled to −45 °C, followed by dropwise addition of a solution of *m*-chloroperoxybenzoic acid (7.2 g, 41.7 mmol) in dichloromethane (100 mL). The mixture was warmed to room temperature and stirred for 1 h. A 10% solution of $Na_2S_2O_3$ (100 mL) was added, and the mixture was stirred for 30 min. The reaction mixture was extracted with ethyl acetate and washed with 10% $Na_2S_2O_3$ and then saturated $NaHCO_3$. The organic phase was dried over $MgSO_4$, filtered, and evaporated. The residue was purified by chromatography on silica gel, eluting with 2% methanol in dichloromethane containing 0.05% NH_4OH, to give the phosphate triester (6.2 g, 76% yield). ^{1}H NMR (300 MHz, CD_3OD) δ ppm 0.81 (t, J = 7.12 Hz, 6H), 1.36 (d, J = 7.12 Hz, 6H), 1.74−2.01 (m, 3H), 2.53−2.82 (m, 4H), 2.95 (s, 3H), 3.90−4.10 (m, 1H), 4.31−4.58 (m, 5H), 5.04−5.12 (m, 4H), 5.16 (s, 2H), 6.07 (d, J = 8.48 Hz, 1H), 7.05−7.20 (m, 12H), 7.31−7.44 (m, 8H), 7.79 (s, 1H), 8.93 (s, 1H). MS (ESI) *m*/*z* 981.6 $(M + H)^+$. To a solution of the product from the first step (6.2 g, 6.32 mmol) in dichloromethane (200 mL) at 0 °C was added trimethylsilyl bromide (3.87 g, 25.3 mmol) via syringe, and the mixture was stirred at 0 °C for 1 h. The solvent was evaporated, and the residue was triturated with water (50 mL), followed by evaporation under reduced pressure. The residue was purified by chromatography (C18), eluting with 20% acetonitrile in water (0.1% trifluoroacetic acid) and then with 40% acetonitrile in water (0.1% trifluoroacetic acid) to give the pure acid (1.21 g, 24%). The disodium salt was formed by treating 1.21 g of the purified acid in acetonitrile (75 mL) with a solution of $NaHCO_3$ (0.254 g) in water (50 mL). After the mixture was stirred for 15 min the solvent was evaporated to give **1**. ^{1}H NMR (300 MHz, DMSO-d_6), δ ppm 0.66 (d, J = 6.1 Hz, 3H), 0.75 (d, J = 6.1 Hz, 3H), 1.26 (d, J = 6.8 Hz, 6H), 1.45−1.61 (m, 2H), 1.80−1.93 (m, 1H), 2.59−2.65 (m, 1H), 2.86 (s, 3H), 3.14−3.23 (m, 2H), 3.82 (t, J = 9.0 Hz, 1H), 3.94−4.07 (m, 2H), 4.35−4.52 (m, 2H), 5.07 (d, J = 12.9 Hz, 2H), 5.23 (d, J = 12.9 Hz, 1H), 6.99−7.19 (m, 10H), 7.25 (s, 1H), 7.83 (s, 1H), 9.03 (s, 1H). MS (ESI) *m*/*z* 801.4 $(M + H)^+$. Analytical HPLC t_R = 7.89 min.

Disodium (1*S*,3*S*)-1-((1*S*)-1-{[(2,6-Dimethylphenoxy)acetyl]amino}-2-phenylethyl)-3-{[(2*S*)-3-methyl-2-(2-oxotetrahydropyrimidin-1(2*H*)-yl)butanoyl]amino}-4-phenylbutylphosphate (2). A solution of LPV (0.250 g, 0.40 mmol), dibenzyl diethylphosphoramidite (0.28 mL, 0.94 mmol), and 1*H*-tetrazole (0.14 g, 1.99 mmol) in THF (4.0 mL) was stirred at room temperature for 68 h. Dichloromethane (4.0 mL) was added, and the mixture was cooled to −45 °C, followed by addition of *m*-chloroperoxybenzoic acid (0.089 g, 0.52 mmol). After being stirred for 30 min at −45 °C, the mixture was diluted with ethyl acetate and washed twice with 10% Na_2CO_3 and then with brine. The organic phase was dried over $MgSO_4$, filtered, and evaporated. The residue was purified by chromatography on silica gel, eluting with 33% ethyl acetate in chloroform and then with 5% methanol in ethyl acetate to give the phosphate triester (0.324 g, 90% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.73 (d, J = 6.62 Hz, 3H), 0.77 (d, J = 6.25 Hz, 3H), 1.21−1.40 (m, 1H), 1.48−1.62 (m, 1H), 1.68−1.81 (m, 1H), 1.81−1.93 (m, 1H), 1.95−2.08 (m, 1H), 2.13 (s, 6H), 2.53−2.77 (m, 4H), 2.81−2.93 (m, 2H), 2.94−3.06 (m, 2H), 4.04−4.18 (m, 2H), 4.35 (d, J = 11.03 Hz, 2H), 4.46−4.59 (m, 1H), 4.78 (t, J = 9.93 Hz, 1H), 5.11 (dd, J = 7.91, 5.70 Hz, 4H), 6.30 (s, 1H), 6.86−6.96 (m, 1H), 6.97−7.05 (m, 2H), 7.08−7.26 (m, 12H), 7.30−7.45 (m, 10H), 7.60 (d, J = 5.52 Hz, 1H), 7.63 (d, J = 5.15 Hz, 1H). MS (ESI) *m*/*z* 889.0 $(M + H)^+$. To a solution of the product from the first step (0.320 g, 0.36 mmol) in a mixture of ethyl acetate (1.8 mL) and methanol (1.8 mL) was added $Pd(OH)_2$ on carbon (0.100 g, 20% by wt Pd), and the mixture was stirred under an atmosphere of hydrogen (balloon pressure) for 16 h. The mixture was filtered through Celite, and the solvent was evaporated. Methanol and water were added, and the pH was adjusted to 9 by addition of 10% Na_2CO_3 solution. The sample was purified by chromatography (C18), eluting with a gradient of 0−100% methanol in water to give **2** (0.215 g, 79% yield). ^{1}H NMR (300 MHz, CD_3OD), δ ppm 0.86 (d, J = 6.6 Hz, 3H), 0.99 (d, J = 6.6 Hz, 3H), 1.55−1.77 (m, 3H), 2.03−2.23 (m, 2H), 2.10 (s, 6H), 2.71 (d, J = 7.4 Hz, 2H), 2.90−3.00 (m, 2H), 3.06−3.19 (m, 4H), 3.94 (q, J = 14.3 Hz, 2H), 4.36−4.45 (m, 1H), 4.46 (d, J = 11.0 Hz, 1H), 4.48−4.57 (m, 1H), 4.67−4.70 (m, 1H), 6.87−6.97 (m, 3H), 7.08−7.24 (m, 8H), 7.30−7.32 (m, 2H). MS (ESI) *m*/*z* 709.0 $(M + H)^+$, 731.0 $(M + Na)^+$. Anal. ($C_{37}H_{47}N_4Na_2O_8P \cdot 1.8H_2O$) C, H, N. Analytical HPLC t_R = 8.28 min.

General Procedure for Preparation of Compounds 3−8. Preparation of Disodium N^1-((1*S*,3*S*,4*S*)-1-Benzyl-5-phenyl-3-[(phosphonatooxy)methoxy]-4-{[(1,3-thiazol-5-ylmethoxy)carbonyl]amino}pentyl)N^2-{[[(2-isopropyl-1,3-thiazol-4-yl)methyl](methyl)amino]carbonyl}-L-valinamide (3). To a solution of RTV (5.0 g, 6.9 mmol) and methyl sulfide (4.1 mL, 55.8 mmol) in acetonitrile (35 mL) at 0 °C was added benzoyl peroxide (6.7 g, 27.7 mmol) in four equal portions over 20 min, and the mixture was stirred at 0 °C for 1 h and then at room temperature for 1 h. The mixture was diluted with ethyl acetate and washed with 10% Na_2CO_3 and then brine. The organic phase was dried over $MgSO_4$, filtered, and evaporated. The residue was purified by chromatography on silica gel, eluting with a gradient of 33−100% ethyl acetate in chloroform to give the MTM ether (4.56 g, 84% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.78 (d, J = 6.62 Hz, 3H), 0.79 (d, J = 6.62 Hz, 3H), 1.30 (d, J = 6.62 Hz, 6H), 1.46−1.66 (m, 2H), 1.82−2.02 (m, 1H), 2.14 (s, 3H), 2.57−2.77 (m, 4H), 2.88 (s, 3H), 3.11−3.29 (m, 1H), 3.62 (t, J = 5.88 Hz, 1H), 3.93 (t, J = 7.91 Hz, 1H), 4.00−4.11 (m, 1H), 4.20 (s, 1H), 4.37−4.55 (m, 2H), 4.58−4.76 (m, 2H), 5.04−5.21 (m, 2H), 6.07 (d, J = 8.46 Hz, 1H), 7.03−7.28 (m, 10H), 7.74 (d, J = 8.82 Hz, 1H), 7.84 (s, 1H), 9.05 (s, 1H). MS (ESI) *m*/*z* 781.3 $(M + H)^+$. To a solution containing the thioether from the first step (4.56 g, 5.8 mmol), phosphoric acid (4.0 g, 40.8 mmol), and molecular sieves (4 Å, 18 g) in THF (60 mL) at 0 °C was added *N*-iodosuccinimide (2.0 g, 8.9 mmol), and the mixture was stirred at room temperature for 1 h. The reaction mixture was filtered through Celite, and the solids were washed with methanol. The filtrate was treated with 1 M $Na_2S_2O_3$ until it was colorless and adjusted to pH 10 by addition of solid Na_2CO_3, and the precipitate was removed by filtration. The filtrate was concentrated under reduced pressure, and the residue was purified by chromatography (C18), eluting with a gradient of 0−100% methanol in water to give **3** (2.64 g, 52% yield). ^{1}H NMR (300 MHz, CD_3OD), δ ppm 0.81 (d, J = 7.0 Hz, 3H), 0.86 (d, J = 7.0 Hz, 3H), 1.35 (d, J = 7.0 Hz, 6H), 1.64−1.73 (m, 1H), 1.89−2.03 (m, 2H), 2.60−2.90 (m, 4H), 2.98 (s, 3H), 3.24−3.28 (m, 1H), 3.63−3.67 (m, 1H), 4.02−4.09 (m, 2H), 4.20−4.30 (m, 1H), 4.46−4.64 (m, 2H), 4.95 (dd, J = 5.3, 10.5 Hz, 1H), 5.10 (q, J = 12.5 Hz, 2H), 5.10−5.15 (m, 1H), 7.07−7.21 (m, 11H), 7.77 (s, 1H), 8.93 (s, 1H). MS (ESI) *m*/*z* 831.3 $(M + H)^+$, 853.2 $(M + Na)^+$. Anal. ($C_{38}H_{49}N_6Na_2O_9PS_2 \cdot 3.1H_2O$) C, H, N. Analytical HPLC t_R = 8.56 min.

Disodium N^1-((1*S*,3*S*,4*S*)-1-Benzyl-5-phenyl-3-[1-(phosphonatooxy)ethoxy]-4-{[(1,3-thiazol-5-ylmethoxy)carbonyl]amino}pentyl)-N^2-{[[(2-isopropyl-1,3-thiazol-4-yl)methyl](methyl)amino]carbonyl}-L-valinamide (4). The thioether was prepared by the general procedure from RTV (0.50 g, 0.69 mmol), ethyl sulfide (1.9 mL, 17.6 mmol), and benzoyl peroxide (0.84 g, 3.47 mmol) as a mixture of diastereomers (about 1:1) (0.42 g, 75% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.69−0.85 (m, 6H), 1.09−1.23 (m, 3H), 1.30 (d, J = 6.99 Hz, 6H), 1.46, 1.48 (2s, 3H), 1.50−1.62 (m, 2H), 1.86−2.09 (m, 1H), 2.54−2.84 (m, 6H), 2.87 (s, 3H). 3.12−3.29 (m, 1H), 3.57−3.73 (m, 1H), 3.87−4.12 (m, 2H), 4.13−4.29 (m, 1H), 4.35−4.60 (m, 2H), 4.67−4.85 (m, 1H), 5.03−5.31 (m, 2H), 6.07 (d, J = 8.46 Hz, 2H), 7.08−7.31 (m, 10H), 7.65 (d, J = 8.82 Hz, 1H), 7.79 (d, J = 8.82 Hz, 1H), 7.84, 7.86 (2s, 1H) 9.03, 9.05 (2s, 1 H). MS (ESI) m/z 809.1 $(M + H)^+$. The thioether (0.15 g, 0.19 mmol) was treated with phosphoric acid (0.090 g, 0.92 mmol) and *N*-iodosuccinimide (0.084 g, 0.37 mmol) in DMF (4.5 mL) using the general procedure to give **4** as a mixture of diastereomers (0.080 g, 48% yield). ^{1}H NMR (300 MHz, CD_3OD) δ ppm 0.84−0.99 (m, 6 H), 1.33−1.39 (m, 9H), 1.44−1.71 (m, 1H), 1.95−2.11 (m, 1.5H), 2.20−2.35 (m, 0.5H), 2.55−2.97 (m, 4H), 3.01 (s, 3H), 3.22−3.27 (m, 1H), 3.74−3.84 (m, 0.5H), 3.90−4.02 (m, 1.5H), 4.07−4.16 (m, 1.5H), 4.21−4.31 (m, 0.5H), 4.42−4.63 (m, 2H), 4.91−4.96 (m, 1H), 5.07−5.12 (m, 1H), 5.31−5.38 (m, 0.5H), 5.42−5.48 (m, 0.5H), 6.94−7.25 (m, 11H), 7.65−7.73 (m, 1H), 8.90−8.92 (m, 1H). MS (ESI) m/z 867.1 $(M + Na)^+$. Anal. ($C_{39}H_{51}N_6Na_2O_9PS_2 \cdot 4.8H_2O$) C, H, N. Analytical HPLC t_R = 8.53 min.

Disodium N^1-((1*S*,3*S*,4*S*)-1-Benzyl-5-phenyl-3-[1-(phosphonatooxy)butoxy]-4-{[(1,3-thiazol-5-ylmethoxy)carbonyl]amino}pentyl)-N^2-{[[(2-isopropyl-1,3-thiazol-4-yl)methyl](methyl)amino]carbonyl}-L-valinamide (5). The thioether was prepared by the general procedure from RTV (3.0 g, 4.2 mmol), butyl sulfide (18.0 mL, 103.1 mmol), and benzoyl peroxide (2.0 g, 8.3 mmol) as a mixture of diastereomers (about 1:1) (2.43 g, 68% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.65−0.96 (m, 12H), 1.30, 1.31 (2d, J = 6.99 Hz, 6H), 1.33−2.05 (m, 6H), 2.53−2.83 (m, 6H), 2.87 (s, 3H), 3.15−3.29 (m, 1H), 3.64−3.78 (m, 1H), 3.86−4.28 (m, 3H), 4.37−4.48 (m, 2H), 4.61 (t, J = 6.43 Hz, 1H), 5.04−5.37 (m, 2H), 6.07 (d, J = 8.46 Hz, 1H), 6.99 (d, J = 9.19 Hz, 1H), 7.09−7.29 (m, 10H), 7.35 (d, J = 8.82 Hz, 1H), 7.63 (d, J = 8.46 Hz, 1H), 7.80 (d, J = 8.82 Hz, 1H), 7.84, 7.87 (2s, 1H), 7.94 (d, J = 6.99 Hz, 1H), 9.03, 9.05 (2s, 1 H). MS (ESI) m/z 865.3 $(M + H)^+$. The thioether (1.2 g, 1.4 mmol) was treated with phosphoric acid (0.85 g, 8.7 mmol) and *N*-iodosuccinimide (0.406 g, 1.8 mmol) in DMF (28 mL) using the general procedure to give **5** (0.605 g, 47% yield). ^{1}H NMR (300 MHz, CD_3OD) δ ppm 0.88−0.98 (m, 9H), 1.33−1.36 (m, 6H), 1.44−2.24 (m, 7H), 2.56−2.85 (m, 4H), 2.88−2.98 (m, 1H), 3.01 (s, 3H), 3.90−4.03 (m, 1H), 4.03−4.16 (m, 2H), 4.18−4.26 (m, 0.5H), 4.29−4.39 (m, 0.5H), 4.46−4.65 (m, 2H), 4.93−5.02 (m, 1H), 5.08−5.14 (m, 1H), 5.22−5.30 (m, 1H), 6.97−7.25 (m, 11H), 7.65−7.74 (m, 1H), 8.91−8.92 (m, 1H). MS (ESI) m/z 895.4 $(M + Na)^+$. Anal. ($C_{41}H_{55}N_6Na_2O_9PS_2 \cdot 1.7H_2O$) C, H, N. Analytical HPLC t_R = 8.92 min.

Disodium N^1-((1*S*,3*S*,4*S*)-1-Benzyl-3-[2-methyl-1-(phosphonatooxy)propoxy]-5-phenyl-4-{[(1,3-thiazol-5-ylmethoxy)carbonyl]amino}pentyl)-N^2-{[[(2-isopropyl-1,3-thiazol-4-yl)methyl](methyl)amino]carbonyl}-L-valinamide (6). The thioether was prepared using the general procedure from RTV (1.0 g, 1.38 mmol), diisobutyl sulfide (6.2 mL, 35.5 mmol), and benzoyl peroxide (2.0 g, 8.3 mmol) as a mixture of diastereomers (about 1:1) (0.890 g, 75% yield). ^{1}H NMR (300 MHz, CD_3OD) δ ppm 0.83−1.07 (m, 12H), 1.10, 1.11 (2d, J = 6.80 Hz, 12H), 1.36, 1.37 (2d, J = 6.99 Hz, 6H), 1.57−1.85 (m, 3H), 1.91−2.24 (m, 3H), 2.42−2.89 (m, 9H), 2.90−3.04 (m, 1H), 2.96 (s, 3H), 2.97 (s, 3H), 3.24−3.35 (m, 1H), 3.82−4.23 (m, 3H), 4.27−4.61 (m, 4H), 5.10−5.33 (m, 2H), 6.95−7.32 (m, 10H), 7.78, 7.81 (2s, 1H), 8.91, 8.92 (2s, 1H). MS (ESI) m/z 865.2 $(M + H)^+$. The thioether (0.888 g, 1.03 mmol) was treated with phosphoric acid (0.50 g, 5.1 mmol) and *N*-iodosuccinimide (0.46 g, 2.0 mmol) in DMF (20 mL) using the general procedure to give **6** (0.49 g, 52% yield). ^{1}H NMR (300 MHz, CD_3OD), δ ppm 0.86−1.03 (m, 12H), 1.33−1.36 (m, 6H), 1.44−1.75 (m, 1H), 1.85−2.27 (m, 3H), 2.54−2.9 (m, 5H), 3.01 (s, 3H), 3.78−4.38 (m, 4H), 4.46−4.68 (m, 2H), 4.91−5.15 (m, 3H), 6.97−7.26 (m, 11H), 7.64−7.73 (m, 1H), 8.91−8.92 (m, 1H). MS (ESI) m/z 895.0 $(M + Na)^+$. Anal. ($C_{41}H_{55}N_6Na_2O_9PS_2 \cdot 2.6H_2O$) C, H, N. Analytical HPLC t_R = 8.91 min.

Disodium [((1*S*,3*S*)-1-((1*S*)-1-{[(2,6-Dimethylphenoxy)acetyl]amino}-2-phenylethyl)-3-{[(2*S*)-3-methyl-2-(2-oxotetrahydropyrimidin-1(2*H*)-yl)butanoyl]amino}-4-phenylbutyl)oxy]methylphosphate (7). To a solution LPV (3.0 g, 4.8 mmol), DMSO (18 mL), and acetic acid (3.6 mL) at room temperature was added acetic anhydride (23 mL), and the mixture was stirred for 48 h at room temperature. The reaction was quenched with ice, and 10% Na_2CO_3 was added to adjust the pH to 7. The mixture was extracted with ethyl acetate and washed with 10% Na_2CO_3 and then brine. The organic was dried over Na_2SO_4, filtered, and evaporated to give the crude product, which was purified by chromatography on silica gel, eluting with a gradient of 25−100% ethyl acetate in dichloromethane to give the thioether (2.1 g, 64% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.83 (d, J = 6.62 Hz, 3H), 0.88 (d, J = 6.62 Hz, 3H), 1.46−1.59 (m, 1H), 1.68−1.82 (m, 3H), 2.14 (s, 6H), 2.22 (s, 3H), 2.59 (dd, J = 13.79, 9.38 Hz, 1H), 2.73−2.93 (m, 3H), 2.94−3.23 (m, 4H), 3.78 (t, J = 6.25 Hz, 1H), 4.00−4.15 (m, 2H), 4.13−4.24 (m, 1H), 4.46−4.58 (m, 2H), 4.60−4.77 (m, 2H), 4.83−5.00 (m, 1H), 6.40 (s, 1H), 6.85−7.04 (m, 3H), 7.10−7.39 (m, 10H). MS (ESI) m/z 689.4 $(M + H)^+$. The thioether (1.73 g, 2.5 mmol) was treated with phosphoric acid (1.23 g, 12.6 mmol) and *N*-iodosuccinimide (1.13 g, 5.0 mmol) using the general procedure to give **7** (1.19 g, 60% yield). ^{1}H NMR (300 MHz, CD_3OD), δ ppm 0.84 (d, J = 6.6 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H), 1.54−1.77 (m, 3H), 1.98−2.19 (m, 2H), 2.12 (s, 6H), 2.64−2.78 (m, 2H), 2.87−2.95 (m, 2H), 3.04−3.23 (m, 4H), 3.80 (dd, J = 3.4, 10.3 Hz, 1H), 3.96−4.07 (m, 2H), 4.33 (d, J = 11.0 Hz, 1H), 4.41−4.50 (m, 1H), 4.71 (dd, J = 4.0, 10.6 Hz, 1H), 5.09 (dd, J = 5.5, 8.1 Hz, 1H), 5.15 (dd, J = 5.5, 8.8 Hz, 1H), 6.87−6.98 (m, 3H), 7.08−7.25 (m, 8H), 7.31−7.33 (m, 2H). MS (ESI) m/z 739.5 $(M + H)^+$, 761.4 $(M + Na)^+$. Anal. ($C_{38}H_{49}N_4Na_2O_9P \cdot 3.9H_2O$) C, H, N. Analytical HPLC t_R = 8.19 min.

Disodium 1-[((1*S*,3*S*)-1-((1*S*)-1-{[(2,6-Dimethylphenoxy)acetyl]amino}-2-phenylethyl)-3-{[(2*S*)-3-methyl-2-(2-oxotetrahydropyrimidin-1(2*H*)-yl)butanoyl]amino}-4-phenylbutyl)oxy]ethylphosphate (8). The thioether was prepared using the general procedure from LPV (0.50 g, 0.80 mmol), ethyl sulfide (2.1 mL, 19.5 mmol), and benzoyl peroxide (1.16 g, 4.8 mmol) as a mixture of diastereomers (about 1.6:1) (0.36 g, 61% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.76 (d, J = 6.62 Hz, 3H), 0.82, 0.90 (2d, J = 6.25 Hz, 3H), 1.18, 1.19 (2t, J = 7.35 Hz, 3H), 1.36−1.78 (m, 3H), 1.46, 1.52 (2d, J = 6.25 Hz, 3H), 1.97−2.11 (m, 1H), 2.14, 2.15 (2s, 6H), 2.53−2.96 (m, 7H), 2.98−3.07 (m, 2H), 3.61 (t, J = 5.70 Hz, 1H), 3.78 (d, J = 9.19 Hz, 1H), 4.06−4.13 (m, 2H), 4.17−4.32 (m, 1H), 4.32−4.40 (m, 1H) 4.44−4.68 (m, 1H), 4.79−4.96 (m, 1H), 6.27−6.36 (m, 1H), 6.88−6.97 (m, 1H), 6.99−7.04 (m, 2H), 7.08−7.30 (m, 8H), 7.45−7.57 (m, 1H), 7.59−7.68 (m, 1H), 7.89−7.98 (m, 1H). MS (ESI) m/z 717.3 $(M + H)^+$. The thioether (0.15 g, 0.21 mmol) was treated with phosphoric acid (0.082 g, 0.84 mmol) and *N*-iodosuccinimide (0.094 g, 0.42 mmol) in DMF (4.5 mL) using the general procedure to give **8** as a mixture of diastereomers (0.066 g, 39% yield). ^{1}H NMR (300 MHz, CD_3OD) δ ppm 0.85 (d, J = 6.6 Hz, 1.5H), 0.86 (d, J = 6.6 Hz, 1.5H), 0.94 (d, J = 6.6 Hz, 1.5H), 0.96 (d, J = 6.6 Hz, 1.5H), 1.45 (d, J = 5.1 Hz, 1.5H), 1.46 (d, J = 5.1 Hz, 1.5H), 1.50−1.74 (m, 3H), 1.88−1.98 (m, 0.5H), 2.09−2.25 (m, 1.5H), 2.09 (s, 3H), 2.13 (s, 3H), 2.74−2.77 (m, 2H), 2.85−3.19 (m, 6H), 3.92−4.03 (m, 2.5H), 4.16−4.21 (m, 0.5H), 4.23 (d, J = 11.0 Hz, 0.5H), 4.35 (d, J = 11.0 Hz, 0.5H), 4.40−4.54 (m, 1H), 4.63−4.70 (m, 1H), 5.39−5.50 (m, 1H), 6.88−6.99 (m, 3H), 7.07−7.30 (m, 10H). MS (ESI) m/z 775.3 $(M + Na)^+$. Anal. ($C_{39}H_{51}N_4Na_2O_9P \cdot 0.8H_2O$) C, H, N. Analytical HPLC t_R = 8.73 min.

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