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Tyrosine-Based 1-(*S*)-[3-Hydroxy-2-(phosphonomethoxy)propyl]cytosine and -adenine ((*S*)-HPMPC and (*S*)-HPMPA) Prodrugs: Synthesis, Stability, Antiviral Activity, and in Vivo Transport Studies

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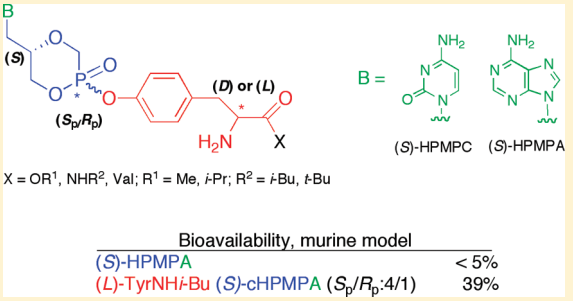
Supporting Information

ABSTRACT: Eight novel single amino acid (**6**−**11**) and dipeptide (**12**, **13**) tyrosine P−O esters of cyclic cidofovir ((*S*)-cHPMPC, **4**) and its cyclic adenine analogue ((*S*)-cHPMPA, **3**) were synthesized and evaluated as prodrugs. In vitro IC_{50} values for the prodrugs (<0.1−50 μM) vs vaccinia, cowpox, human cytomegalovirus, and herpes simplex type 1 virus were compared to those for the parent drugs ((*S*)-HPMPC, **2**; (*S*)-HPMPA, **1**; IC_{50} 0.3−35 μM); there was no cytoxicity with KB or HFF cells at ≤100 μM. The prodrugs exhibited a wide range of half-lives in rat intestinal homogenate at pH 6.5 (<30−1732 min) with differences of 3−10× between phostonate diastereomers. The tyrosine alkylamide derivatives of **3** and **4** were the most stable. (L)-Tyr-NH-*i*-Bu cHPMPA (**11**) was converted in rat or mouse plasma solely to two active metabolites and had significantly enhanced oral bioavailability vs parent drug **1** in a mouse model (39% vs <5%).



INTRODUCTION

Acyclic nucleoside phosphonates (ANPs)[1] are an important class of compounds endowed with antiviral activity against several DNA viruses and retroviruses. The prototype member, (*S*)-9-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine ((*S*)-HPMPA, **1**),[2] was followed by several generations of newer ANPs, some of which are currently in clinical use (cidofovir ((*S*)-HPMPC), **2**,[3] adefovir dipivoxil,[2] and tenofovir disoproxil fumarate[4]) (Figure 1). In their triphosphate-like form, these compounds act as competitive inhibitors or alternative substrates for viral DNA polymerases and, if incorporated into DNA, act as chain terminators, thus preventing further chain elongation.[5]

Certain virulent infectious diseases against which much of the contemporary population lacks immunity, notably smallpox, are of current concern because of ease of dissemination, contagiousness, and high mortality rate, thereby stimulating interest in developing antiviral drugs.[6] An oral drug formulation that could be widely used and rapidly distributed would be especially valuable. ANPs of the 3-hydroxy-2-(phosphonomethoxy)propyl (HPMP) series, which includes both **1** and **2**, are active against poxviruses and also polyoma-, papilloma-, adeno-, and herpesviruses.[1] However, because of the highly polar character of the phosphonic acid group, which is ionized at physiological pH, these drugs have inadequate oral bioavailability.[7]

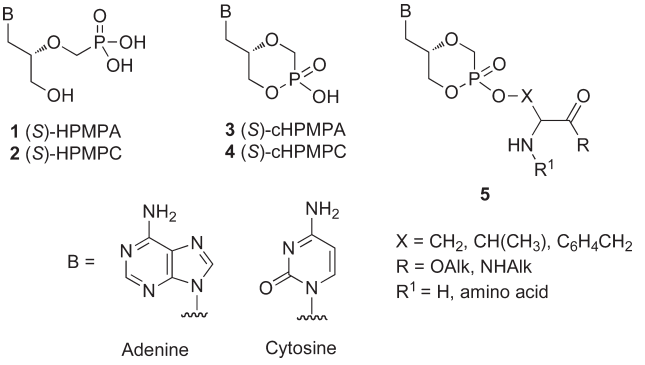


Figure 1. Structures of (*S*)-HPMPA (**1**), (*S*)-HPMPC (**2**), and their cyclic forms **3** and **4** and general structure of the amino acid/dipeptide ester prodrugs **5**. Stereochemistry at the phostonate phosphorus and amino acid α-carbon is omitted.

Several traditional and new prodrug approaches to increase the lipophilicity of polar molecules, allowing them to passively diffuse via the transcellular route, have been utilized to overcome this limitation

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Table 1. Comparison of Calculated log *D* Values for Serine- and Tyrosine-Based (*S*)-cHPMPC and (*S*)-cHPMPA Derivatives with the Values of Parent 1 and 2

	ClogD			ClogD	
serine-based compd	pH 6.5	pH 7.4	tyrosine-based compd	pH 6.5	pH 7.4
Ser-OMe cHPMPC	−1.97	−1.73	Tyr-OMe cHPMPC	−0.41	0.04
Ser-O-*i*-Pr cHPMPC	−1.21	−0.97	Tyr-O-*i*-Pr cHPMPC	0.35	0.80
Val-Ser-OMe cHPMPC	−3.30	−2.45	Val-Tyr-OMe cHPMPC	−1.45	−0.60
Ser-OMe cHPMPA	−1.99	−1.65	Tyr-OMe cHPMPA	−0.33	0.15
Ser-O-*i*-Pr cHPMPA	−0.84	−0.81	Tyr-O-*i*-Pr cHPMPA	0.43	0.91
Val-Ser-OMe cHPMPA	−3.22	−2.35	Val-Tyr-OMe cHPMPA	−1.36	−0.49
2	−4.60	−4.70	**1**	−4.51	−4.59

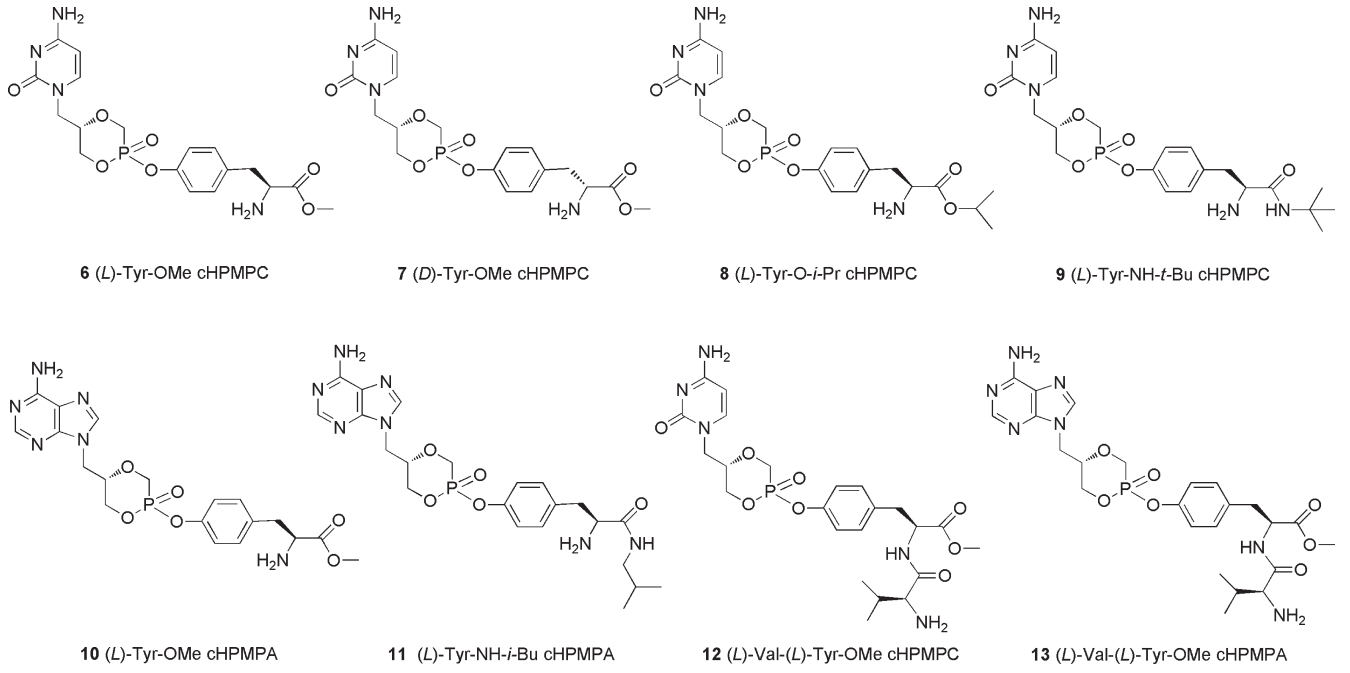

Figure 2. Structures of tyrosine ester (**6**−**11**) and Val-Tyr ester (**12**, **13**) prodrugs of **1** and **2**.

of nucleotide analogues.[8,9] Acyloxyalkyl[9] (pivaloyloxymethyl, POM), alkyloxyalkyl,[10] *S*-acylthioethyl[11] (SATE, aryl, cyclic 1-aryl-1, 3-propanyl, and cyclosaligenyl phophonate esters), and various phosphonamidate derivatives of ANPs[8,9] have been previously examined. Long chain alkoxyalkyl esters of **1** and **2** have shown high bioavailability and good efficacy in a murine pox infection model.[12,13] Among them, hexadecyloxypropyl-HPMPC (HDP-HPMPC, CMX001) is currently under development as an oral drug for treatment of human cytomegalovirus (HCMV) and smallpox virus infections.[12] However, HDP-HPMPC recently failed in an in vivo efficacy trial (monkeypox model), likely because of metabolic differences between rodents and monkeys.[14] Toxicity evaluations of long-chain derivatives of both **1** and **2** also revealed increased cytotoxicity compared to the parent drugs.[15] In addition, the low aqueous solubility of these very lipophilic prodrugs may limit their application.[16]

We have proposed a different approach to the development of orally bioavailable **1** and **2**, namely, by conjugating the cyclic form of an ANP (Figure 1, structures **3** and **4**) with a dipeptide or an amino acid, using the side chain of an appropriate amino acid, such as Ser, to create an ester linkage to mask the remaining phosphonic acid hydroxyl group (Figure 1, general structure **5**). The cyclic derivative may afford improved pharmacological and antiviral parameters relative to the parent drug.[7] The attachment of a nontoxic peptidomimetic promoiety offers the potential for decreasing the polarity to enhance transcellular absorption, while retaining sufficient aqueous solubility, and the advantage of a functionally diverse promoiety, permitting its PK properties to be "tuned". Previously, we reported the synthesis and biological evaluation of several phosphono dipeptide ester prodrugs of **4** with the dipeptide attached via the side chain hydroxyl group of an (L)-Ser,[17,18] as well as single amino acid (Val, Phe) prodrugs coupled by an ethylene glycol linkage.[19] Transport studies in an in vivo murine model displayed an 8-fold increase in oral bioavailability of total **2** species for (L)-Val-(L)-Ser-OMe cHPMPC relative to **2** or its cyclic form **4**.[18] Because drugs with peptide-like structures have been reported to be recognized by the peptide-specific intestinal transporter PEPT1,[20] we explored the possibility of an active transport mediated by this transporter for several serine-based dipeptide and single amino acid prodrugs of **1** and **2**.[21,22] This investigation revealed that Val-Ser-OMe cHPMPC stereoisomers were tightly bound but not transported by hPEPT1 expressed in the model system. Several single amino acid prodrugs of both **1** and

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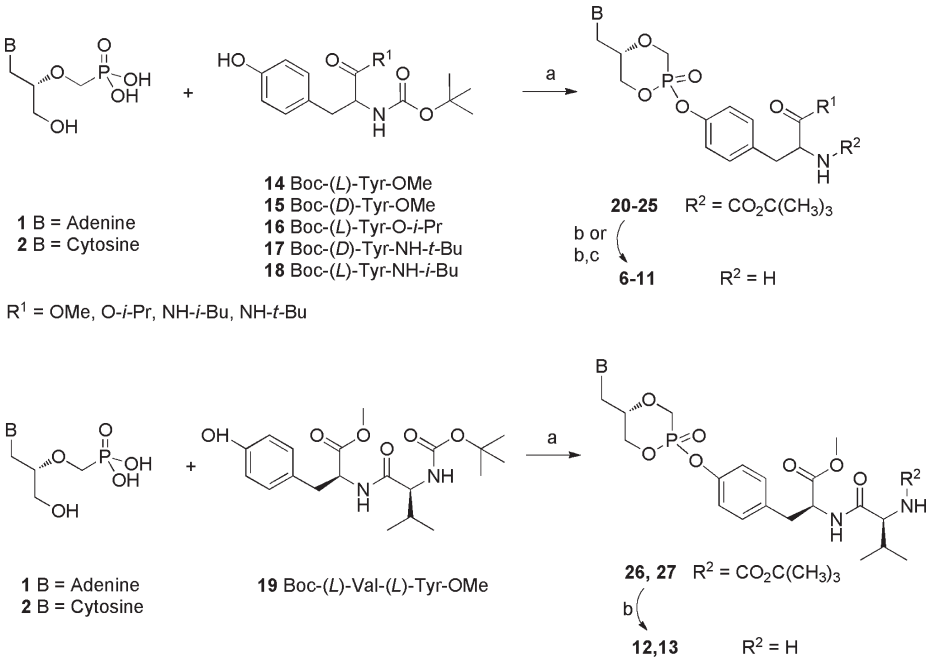
Scheme 1. Synthesis of the Prodrugs 6−13[a]



[a] Reagents and conditions: (a) PyBOP, DIEA, DMF, 40 °C; (b) TFA, CH_2Cl_2, room temp; (c) 0.1 M HCl/MeOH soln, −20 °C.

2 also showed no evidence of transport in this model, suggesting that an alternative transport mechanism operates for these prodrugs.[21,22]

An important goal in prodrug design is to retain a suitable balance between biological stability of the prodrug and efficient conversion to the active parent drug and/or metabolite in vivo. In a preliminary communication, we reported the prodrugs derived from different amino acid ester-linked promoieties (serine, threonine, and tyrosine) with the enhanced chemical stability of the tyrosine-based **1** and **2** prodrugs compared to the serine analogues at the physiological pH.[23] A comparison of the calculated log *D* for equivalent serine and tyrosine prodrugs of **1** and **2** predicts higher lipophilicity of the tyrosine series even without introducing lipophilic alkyl groups (Table 1).

In the present study, we explore the potential of the tyrosine promoiety and, in particular, its further modification at one or more of its functional sites (e.g., NH_2; CO_2H; α-C, P stereochemistry) on the properties of potential prodrugs of **1** and **2**. We report the synthesis of a series of tyrosine-based single amino acid (**6**−**11**) and dipeptide (**12**, **13**) P−O ester conjugates of **1** and **2** (Figure 2), together with their biological evaluation, their chemical and enzymatic activation, and transport studies to assess bioavailability and stereochemical aspects of their synthesis.

RESULTS AND DISCUSSION

Chemistry. The tyrosine-based **1** and **2** prodrugs **6**−**13** were synthesized as outlined in Scheme 1. Commercially unavailable Boc-protected tyrosine esters (**15**, **16**) and amides (**17**, **18**) were synthesized according to literature methods.[24,25] Dipeptide **19** was prepared in good yield by a standard coupling procedure using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)/*N*-hydroxybenzotriazole (HOBt) in CH_2Cl_2.[26] Conjugation of either **1** or **2** with amino acids **14**−**18** or dipeptide **19** was performed in dimethylformamide (DMF) using diisopropylethylamine (DIEA) as the base and benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) as coupling reagent at 35−40 °C, as previously described.[18]

The reaction was monitored by ^{31}P NMR and stopped when **3** or **4**, which is formed initially, was no longer present. After solvent removal under vacuum, the residue was purified using column chromatography, yielding the Boc-protected conjugates (**20**−**27**) in moderate to good yields (27−76%) as mixtures of two phostonate diastereomers. In the next step, the *tert*-butoxycarbonyl group was removed using trifluoroacetic acid (TFA) in dichloromethane (CH_2Cl_2). The derived product was purified by column chromatography, using methanol−dichloromethane as eluent, to which a small amount of TFA (0.5%, v/v) was added. The prodrugs **6**−**13** were precipitated from methanol by addition of diethyl ether (Et_2O), giving the final products as TFA salts in 22−76% yield. The TFA salts of exemplary compounds **6**, **10**, and **11** were quantitatively converted into their respective hydrochloric acid salts using 0.1 M HCl methanolic solution at −20 °C (the diastereomers ratio did not change (^{31}P NMR)).

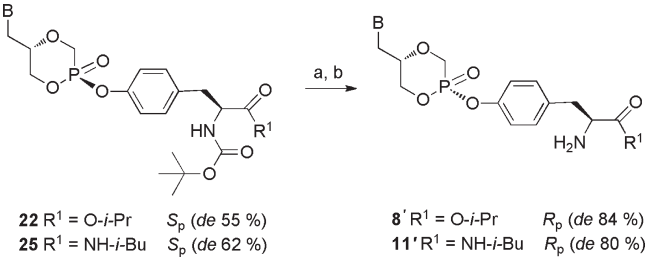
1 and **2** are chiral compounds, exerting their antiviral effect as the *S*-enantiomers. A dipeptide or amino acid promoiety, when conjugated to **3** or **4** via a phosphoester bond, produces a new chiral center at the phosphorus atom, leading to two different diastereomers (S_p and R_p) of the resulting prodrug, with two distinct ^{31}P NMR signals that facilitate monitoring the individual stereoisomers in the synthetic mixture. Our preliminary results on serine-based amino acid and dipeptide prodrugs of **1** and **2**[23,27] as well as literature data on stability of the aryl esters of cyclic cidofovir[28] demonstrated significant difference in the stabilities of these types of diastereomers, with the diastereomer having an upfield chemical shift generally having a longer half-life (either in buffers or tissue homogenates).[18,27] Thus far we were unable to obtain an amino acid/dipeptide prodrug crystal suitable for X-ray analysis in order to determine the absolute configuration

of the diastereomers.[18] However, using X-ray crystallography, we recently established the configuration of the phosphorus chiral center in each diastereomer of two model compounds: the phenyl esters of **3** and **4**, which can be considered as structural models for tyrosine-based prodrugs. In this study, it was found that the more upfield ^{31}P NMR chemical shift corresponded to an (*R*)-configuration at the phosphorus atom.[29]

To ascertain diastereomer difference in in vitro antiviral potency and in vivo transport assays, separation or else control of the diastereomeric excess (de) in the mixture was required. The separation of the diastereomers by RP-HPLC procedure, previously reported by us for serine-based dipeptide ester prodrugs of **4**,[18] proved to be a time-consuming procedure allowing preparation of diastereomers only in small scale. The conversion of one diastereomer of an aryl ester of **4** into the other was accomplished previously via incubation of a mixture enriched in one diastereomer with a catalytic amount of the substituted sodium phenoxide.[28] For our tyrosine-based prodrugs we developed a modified transesterification procedure that can convert the less stable diastereomer (S_p) primarily formed in the PyBOP coupling procedure substantially into the more stable one (R_p). Thus, treatment of **22** or **25** (S_p, de ≈ 55−62%) with cesium carbonate (Cs_2CO_3) in the presence of a catalytic amount of the appropriate amino acid in DMF provided a mixture highly enriched in the R_p diastereomer (equilibrium ratio by ^{31}P NMR, $S_p/R_p \approx 1:9$). After removal of inorganic salt by filtration, evaporation, and addition of TFA, prodrugs (**8′** or **11′**) containing the R_p isomer in de ≈ 80−84% could be isolated (Scheme 2). The TFA salt of compound **11′** could be coverted into the corresponding hydrochloric acid salt as described above.

Interestingly, enrichment of the R_p isomer in the (L)-Val-(L)-Tyr-OMe cHPMPA (**13**) mixture from S_p (de ≈ 88%) to a ratio of isomers of approximately 1:1 was observed during the TFA deprotection step.

Scheme 2. Synthesis of Compounds 8′ and 11′ Enriched with R_p Diastereomers[a]



[a] Reagents and conditions: (a) Cs_2CO_3, DMF, 0.2 equiv of **16** or **18**, room temp; (b) TFA, CH_2Cl_2, room temp.

Prodrugs **6**−**13**, **8′**, **11′** were characterized by NMR spectroscopy (^{1}H, ^{13}C, and ^{31}P). A complete chemical shift assignment of all proton and carbon signals was made based on their coupling constants with the help of additional NMR experiments (DEPT and HSQC). The structures of **6**−**13**, **8′**, **11′** were confirmed by high resolution mass spectrometry, and the purity was analyzed by HPLC.

^{1}H NMR spectroscopy was previously shown to be of immense value in characterizing the chair or boat/twist conformations of individual conformers and in analysis of conformational equilibria.[30−32] A NMR analysis of each diastereomer of the **3** and **4** phenyl ester model compounds, in particular, inspection of their $^3J_{HH}$, $^3J_{HP}$, and $^2J_{HP}$ values,[29] has demonstrated that the R_p diastereomer is predominantly in a chair conformation with nucleobase and phenyl substituent occupying *equatorial* and *axial* positions, respectively, whereas the S_p isomer was present in solution as equilibrium conformers. Analysis of the ^{1}H NMR chemical shifts and H−P coupling constants of compounds **6**−**13** (enriched with S_p isomer) and of **8′**, **11′** (enriched with R_p isomer) and comparison to the results for the model

Table 3. Half-Lives for the Prodrugs 6−13 in Buffer (pH 6.5 and pH 7.4) and in Rat Intestinal Homogenate (pH 6.5) at 37 °C

		half-life $t_{1/2}$ (min)		
		buffer		intestinal homogenate
compd	isomer	pH 6.5	pH 7.4	pH 6.5
(L)-Tyr-OMe cHPMPC (**6**)	R_p	256	121	<30
	S_p	56	19	<30
(D)-Tyr-OMe cHPMPC (**7**)	R_p	385	126	210
	S_p	91	21	72
(L)-Tyr-O-*i*-Pr cHPMPC (**8**)	R_p	770	239	<30
	S_p	121	22	<30
(D)-Tyr-NH-*t*-Bu cHPMPC (**9**)	R_p	1732	630	1732
	S_p	210	60	231
(L)-Tyr-OMe cHPMPA (**10**)	R_p	330	nd[a]	<30
	S_p	55	nd[a]	<30
(L)-Tyr-NH-*i*-Bu cHPMPA (**11**)	R_p	770	231	771
	S_p	122	26	114
(L)-Val-(L)-Tyr-OMe	R_p	346	61	<30
cHPMPA (**12**)	S_p	67	12	<30
(L)-Val-(L)-Tyr-OMe	R_p	346	66	<30
cHPMPC (**13**)	S_p	95	15	<30

[a] nd = not determined.

Table 2. Comparison of the Chemical Shifts and Coupling Constants of 11 to Those of a Model Phenyl Ester[29] (CD_3OD, 400 MHz)

		chemical shift (coupling constant, Hz),				
compd	isomer	CH_aH_bO	CH_aH_bO	CH_aH_bP	CH_aH_bP	CHO
(L)-TyrNH-*i*-Bu cHPMPA (**11**, **11′**)	S_p	4.76 (12.1, 12.1, 2.7)	4.54 (11.7, 7.4)	4.49 (15.2, 7.0)	4.23 (14.9, 4.3)	4.42
	R_p	4.63 (17.6, 11.7, 2.0)	4.48 (11.3, 11.3, 0.9)	4.35 (15.2, 11.2)	4.10 (15.3, 1.2)	4.38
Ph cHPMPA[29]	S_p	4.71 (12.1, 12.1, 2.8)	4.50 (nd)	4.46 (15.0, 7.0)	4.15 (14.8, 4.0)	4.32
	R_p	4.61 (17.6, 11.6, 2.9)	4.42 (11.6. 11.6, 1.2)	(nd)	4.10 (15.3, 1.4)	nd[a]

[a] nd = not defined.

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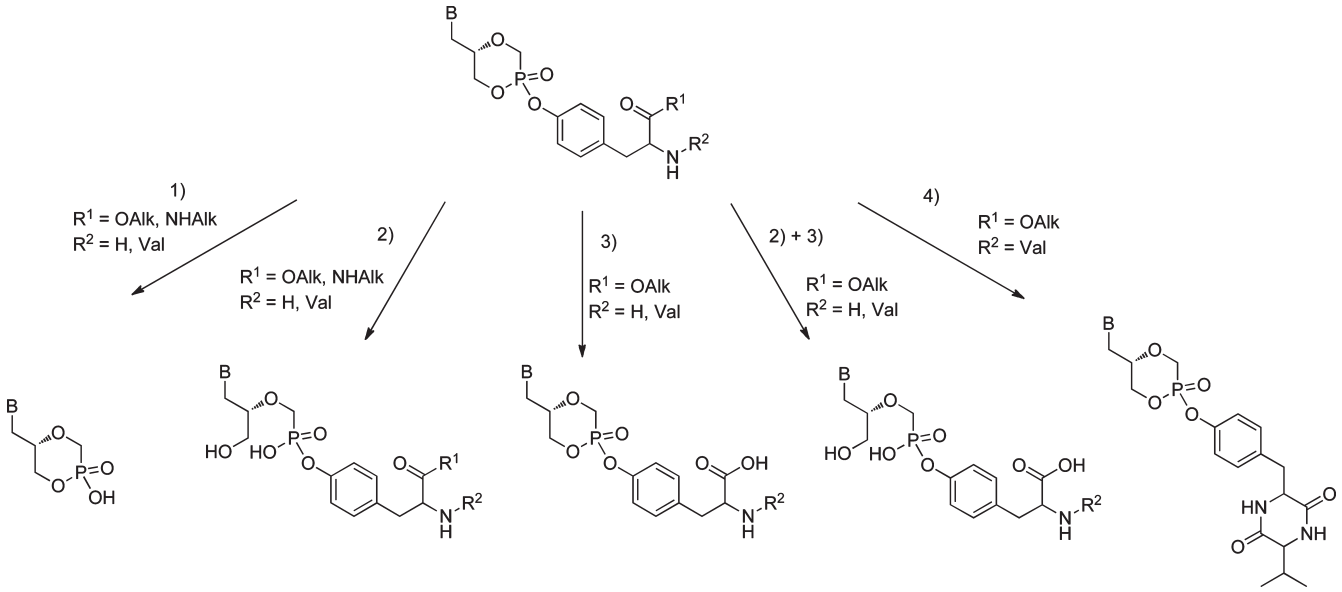
Scheme 3. Methabolic Pathways Observed for Prodrugs 6−13 in Buffers and Intestinal Homogenate



compounds suggest that the tyrosine-based prodrug diastereomers behave in the solution similarly to the respective diastereomers of the phenyl ester model compounds (Table 2).[29]

Stability and Hydrolysis Studies. The ideal prodrug should combine sufficient ruggedness to withstand the rigors of the gastrointestinal environment with physiochemical and biological properties permitting good absorption and efficient conversion to the active parent compound once absorbed. Previous studies[18] showed that the serine-based dipeptide cyclic cidofovir conjugates were relatively stable under acidic conditions and slowly hydrolyzed at neutral pH, whereas when exposed to cellular and tissue homogenates, they were rapidly metabolized.

The chemical and enzymatic stabilities and possible metabolic pathways of the prodrugs **6**−**13** were studied using liquid chromatography−mass spectrometry (LC−MS) analysis. All compounds were chemically robust under acidic conditions. Chemical stability was also determined by evaluating the hydrolysis rates of the prodrugs in 200 mM phosphate buffer at physiologically relevant pH (6.5 and 7.4) at 37 °C. To assess the enzymatic stability, the rate of disappearance of each prodrug was determined in rat intestinal homogenate (pH 6.5) at 37 °C. The chemical and enzymatic hydrolyses of each prodrug followed pseudo-first-order kinetics over several half-lives ($t_{1/2}$). Half-lives of the compounds **6**−**13** are reported in Table 3. The tyrosine-based prodrugs of **1** and **2** proved to be chemically more stable than serine-based compounds[18,23] because of impossibility of β-elimination pathway in the aryl esters. A significant difference in stability was also observed between the two diastereomers (about 3- to 10-fold) of compounds **6**−**13**. Similar differences in stabilities of diastereomers were reported previously for salicylate and aryl ester prodrugs of **2** (5.4- to 9.4-fold).[28] In general, the prodrugs **6**−**8**, **10**, **12**, **13** were less stable in intestinal homogenates compared to buffer solutions, indicating enzymatic degradation of the prodrugs.

Compounds **6**, **7**, **10**, **12**, **13**, which have a methyl carboxylate group in common, demonstrated relatively short half-lives in buffers (pH 6.5 and 7.4). Changing the methyl ester to isopropyl (compound **8**) enhanced the chemical stability of this compound, likely because of increased resistance of the isopropyl group to nucleophilic attack. However, the enzymatic stability of all the (L)-tyrosine esters was relatively low (<30 min). Increased enzymatic stability of the prodrugs was observed when a (D)-amino acid was substituted for the (L)-amino acid component of the promoiety structure (compound **7**). However, the most promising chemical and enzymatic stabilities among the compounds tested were observed for the tyrosine amide prodrugs **9** and **11** (Table 3).

LC−MS analysis of the chemically and enzymatically hydrolyzed tyrosine-linked prodrugs **6**−**13** identified several metabolic and hydrolysis pathways (Scheme 3): (1) cleavage of the exocyclic P−O bond with release of the cyclic form of the parent drug; (2) cleavage of the endocyclic P−O bond with formation of acyclic form of prodrug; (3) hydrolysis of the carboxylate ester group (for **6**−**8**, **10**, **12**, **13**); (4) cyclization of the dipeptide promoiety to form a diketopiperazine ring (DKP) (for **12**, **13** only). The formation of a product obtained by both pathways 2 and 3 was also observed for compounds **6**−**8**, **10**, **12**, **13**. The single amino acid **3** and **4** conjugates had the simpler hydrolysis pathway compared to the dipeptide derivatives, with the formation of fewer metabolites. Because of the stability of the alkyl amide moiety, LC−MS analysis of the degradation products of (L)-Tyr-NH-*i*-Bu cHPMPA **11** showed the formation of only two metabolites formed by pathways 1 and 2 in intestinal homogenate or in buffers: the cyclic form of the parent drug (**3**) and the acyclic form of the prodrug (L)-Tyr-NH-*i*-Bu HPMPA (**28**). A similar result was observed for (D)-Tyr-NH-*t*-Bu cHPMPC (**9**).

Stability studies of **11** performed in rat or mice plasma also demonstrated formation of only two metabolites, (L)-Tyr-NH-*i*-Bu HPMPA (**28**) and (*S*)-cHPMPA (**3**). The half-lives of the two diastereomers in rat plasma differed nearly 10-fold: 23 (S_p) and 213 (R_p) min.

As with salicylate and aryl ester prodrugs of **2**,[28] the product distribution for prodrugs **9** and **11** can be explained by nonselective hydrolytic attack at phosphorus opposite the exocyclic or endocyclic P−O bonds to form a trigonal bipyramid intermediate that is transformed subsequently to the respective products. The ratio of exocyclic to endocyclic P−O bond

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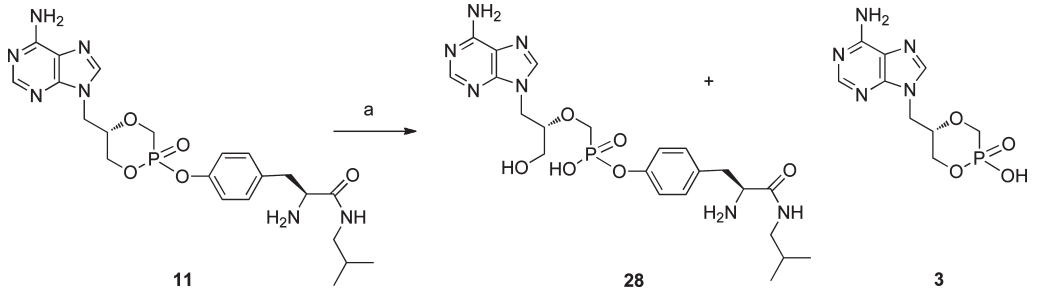
Scheme 4. Synthesis of (L)-Tyr-NH-*i*-Bu HPMPA (28) and (*S*)-cHPMPA (3)[a]



[a] Reagents and conditions: (a) aqueous 14.8 M NH_4OH, 38 °C.

Table 4. In Vitro Antiviral Activity and Cytotoxicity of Tyrosine-Based Prodrugs 6−13, 8′, 11′, and 28

		antiviral activity[a] IC_{50} (μM)				cytotoxicity[b] IC_{50} (μM)	
compd	salt	cowpox	vaccinia	HSV-1	HCMV	KB	HFF
(L)-Tyr-OMe cHPMPC (**6**)	HCl	50	4	25	0.23	>100[c]	>100
(D)-Tyr-OMe cHPMPC (**7**)	TFA	35	25	35	0.2	>100	>100
(L)-Tyr-O-*i*-Pr cHPMPC (**8**)	TFA	40	30	20	0.12	>100	>100
(L)-Tyr-O-*i*-Pr cHPMPC (**8′**)	TFA	10	8	3	0.22	>100	>100
(D)-Tyr-NH-*t*-Bu cHPMPC (**9**)	TFA	20	10	1.5	<0.1	>100	>100
(L)-Tyr-OMe cHPMPA (**10**)	TFA	7	3	25	0.45	>100	>100
(L)-Tyr-OMe cHPMPA (**10**)	HCl	3.5	3	35	0.45	>100	>100
(L)-Tyr-NH-*i*-Bu cHPMPA (**11**)	TFA	7	4.5	50	0.3	>100	>100
(L)-Tyr-NH-*i*-Bu cHPMPA (**11**)	HCl	2	1.5	35	1.3	>100	100
(L)-Tyr-NH-*i*-Bu cHPMPA (**11′**)	TFA	2.5	1	nd[d]	0.55	>100	100
(L)-Val-(L)-Tyr-OMe cHPMPC (**12**)	TFA	30	20	15	<0.1	>100	>100
(L)-Val-(L)-Tyr-OMe cHPMPA (**13**)	TFA	8	4	35	0.3	>100	>100
(L)-Tyr-NH-*i*-Bu HPMPA (**28**)	AcOH	3	0.5	45	0.29	>100	100
(*S*)-HPMPA (**1**)		4	2	35	0.41	>100	100
(*S*)-HPMPC (**2**)		30	20	nd[d]	0.28	>100	100
(*S*)-cHPMPA (**3**)		0.6	1	30	1.3	>100	100
(*S*)-cHPMPC (**4**)		30	40	50	0.25	>100	>100
acyclovir		nd[d]	nd[d]	0.6	nd[d]	nd[d]	
ganciclovir		nd[d]	nd[d]	nd[d]	0.14	nd[d]	>100
3L[e]						3	nd[d]

[a] Plaque reduction assays were used for all viruses and performed in duplicate as described in the text with an average variation of ~10%. [b] Visual cytotoxicity was scored on HFF cells at the time of HCMV plaque enumeration in duplicate wells. Inhibition of KB cell growth was determined in triplicate wells as described in the text. [c] >100 indicates an IC_{50} greater than the highest concentration tested, 100 μM. [d] nd = not determined. [e] 2-Acetylpyridine thiosemicarbazone used as a positive control for cytotoxicity.

cleavage for compound **11** reached from 1:1 to 1:2.3, depending on the pH and the diastereomeric content of the compound.

Because **3** and the acyclic prodrug derivative **28** were the major metabolites of **11**, it was necessary to test their antiviral activity and thus to obtain synthetic sample. Acyclic (L)-Tyr-NH-*i*-Bu HPMPA prodrug **28** and **3** were prepared by treatment of **11** with aqueous ammonia solution. Both products were further isolated by preparative RP-HPLC (Scheme 4).

Antiviral Activity. The parent drugs **1**−**4**, the prodrugs **6**−**13**, **8′**, **11′**, and two metabolites of **11** were evaluated in vitro against selected DNA viruses, including two poxviruses (vaccinia and cowpox) and two herpesviruses (HSV-1 and HCMV). Acyclovir (ACV) and ganciclovir (GCV) were used as positive controls for HSV-1 and HCMV assays, respectively. All the compounds also were tested for cytoxicity in two human cell lines, a human carcinoma of the nasopharynx (KB cells) and normal human fibroblasts (HFF). In our hands, the adenine-based compounds (*S*)-HPMPA (**1**) and its cyclic homologue **3** were approximately 10 times more active against the poxviruses than were cytosine-based (*S*)-HPMPC (**2**) and (*S*)-cHPMPC (**4**) (Table 4). All four compounds were most active against HCMV. Interestingly, a different pattern of activity was observed against both herpesviruses. Compounds **1**−**4** were approximately equally active against cowpox and vaccinia, but were considerably more active against HCMV than HSV-1.

The prodrugs **6**−**13** had antiviral potency (IC_{50} values) similar to the activity of the parent compounds **1**−**4** against all the viruses tested (Table 4). The activities of the prodrugs against cowpox virus,

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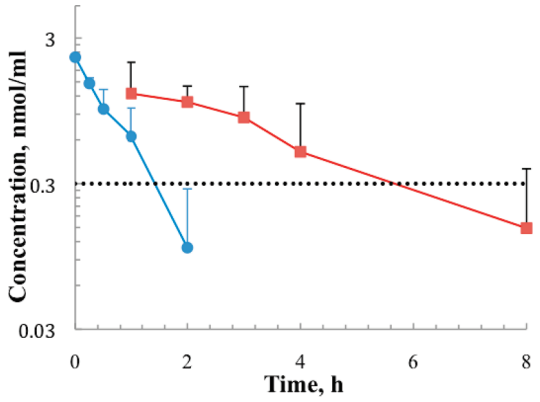

Figure 3. Concentration−time profiles of (L)-Tyr-NH-*i*-Bu cHPMPA (**11**) in mouse plasma. Concentration of total **1**-containing species (**3** and **11**) following intravenous (at a level of 1 mg/kg) (blue circle) or oral (at a level of 10 mg/kg) (red box) administration of **11**. Error bars represent standard deviation with a number of 5 animals per time point. The AUC values from the curves of total **1**-containing compounds were used to calculate the bioavailability. A horizontal line indicating a concentration in plasma of 0.3 μM, the IC_{50} of **11** in the HCMV assay (Table 4), is shown for reference.

vaccinia virus, and HSV-1 varied over a range of 1−50 μM, with the **1** (adenine)-based compounds **10**, **11**, **11′**, **13**, and **28** showing better activity against both poxviruses (1−8 μM) than **2** (cytosine)-based derivatives **6**−**9**, **12**, **8′** (4−50 μM). Tyrosine-based dipeptide derivatives **12** and **13** demonstrated antiviral activity against both pox viruses similar to parents **2** and **1**, in contrast to the reduced activity of (L)-Val-(L)-Ser-OMe cHPMPC and (L)-Leu-(L)-Ser-OMe cHPMPC reported previously.[18] Similar to our results with the adenine-based parent compound and prodrugs, alkoxyalkyl prodrugs of (*S*)-HPMPA and (*S*)-HPMPA itself were found to be more active against poxviruses than was (*S*)-HPMPC and prodrugs such as HDP-CDV.[12] In contrast to our results with tyrosine-based prodrugs, the alkoxyalkyl prodrugs of these acyclic nucleosides were considerably more active against poxviruses and HCMV.[12]

The similarity of the activity of the parent compunds and their prodrugs could be due to hydrolysis of the prodrugs to drug by enzymes present in cell culture media. In contrast, it could be due to similar uptake of the compounds and subsequent intracellular conversion of prodrug to drug. Additional studies are required to resolve these possibilities. Activity against HCMV of both adenine and cytosine compounds and their prodrugs was similar to or better than that of the positive control GCV, with IC_{50} values ranging from <0.1 to 0.55 μM.

The antiviral activities of the (L)-Tyr-NH-*i*-Bu cHPMPA (**11**) TFA and HCl salts were not significantly different, demonstrating that the TFA as counteranion did not influence the in vitro assay results. Somewhat similar activities were also observed for **8** and **11**, enriched with the S_p isomer, relative to the **8′** and **11′** diastereomeric mixtures enriched with the R_p isomer. It is noteworthy that (L)-Tyr-NH-*i*-Bu HPMPA (**28**), which is produced as a stable metabolite in the activation pathway of **11**, had antiviral activity against all the viruses, with IC_{50} values in the same range as prodrug **11** itself. These data suggest that metabolite **28** is able to enter the cell and to be metabolized to **1**. Additionally, none of the compounds showed any significant cytotoxicity toward stationary (HFF) or growing (KB) cells up to a concentration of at least 100 μM (Table 4).

Transport Studies. Compound **11** (as the diastereomeric mixture enriched with the S_p isomer, de ≈ 62%) was evaluated for oral bioavailability. The drug was administered by oral gavage to mice at a dose of 10 mg/kg, and the levels of drug species were compared with those observed after intravenous injection (at a dose of 1 mg/kg) (Figure 3).

Plasma samples obtained from the mice were analyzed by LC−MS for prodrug **11** and its metabolites (L)-Tyr-NH-*i*-Bu HPMPA (**28**) and (*S*)-cHPMPA (**3**) as described in the Experimental Section. The total concentration of **1**-containing compounds was calculated based on the amounts of prodrug **11** and **3**, since no **28** was detected in analyzed plasma samples after either oral or intravenous routes of administration. In the oral uptake experiment approximately 80% of the **1**-containing species detected in plasma samples was identified as **3** and 20% as **11**.

Oral bioavailability was calculated from the ratio of oral AUC divided by the intravenous AUC data adjusted for dose (10 mg/kg oral vs 1 mg/kg iv). The oral uptake of the prodrug was significantly enhanced over the parent compound. The total bioavailability derived from the oral administration of **11** in this experiment was estimated to be 39% compared to <5% for the parent (*S*)-HPMPA.[33]

CONCLUSION

Eight novel (*S*)-HPMPC and (*S*)-HPMPA prodrugs that incorporate an amino acid or dipeptide promoiety conjugated to the cyclic form of the drugs via esterification by tyrosine side chain hydroxyl group have been synthesized and evaluated for stability, transport, and in vitro antiviral activity and cytotoxicity. The prodrugs were obtained as mixtures of two diastereomers, which had significant differences in stability. A convenient method for the partial conversion into the more stable R_p diastereomer of the prodrugs by a transesterification reaction from the corresponding S_p diastereomer was demonstrated. The tyrosine alkylamide esters of **3** and **4** have better stability than corresponding carboxylate ester derivatives. The tyrosine amide derivatives had a simple activation pathway, generating the cyclic form of the parent drug plus an acyclic form of the respective prodrug, which had in vitro antiviral activity as well. In vitro antiviral activities of the potential prodrugs were similar to the corresponding parent drugs **1** and **2** and did not depend on the prodrug phostonate ester stereochemistry (S_p vs R_p) or salt form (TFA vs HCl). The prodrugs were not cytotoxic in KB and HFF cells at concentrations up to 100 μM. The bioavailability of **11** in mice was ∼8× higher than that of the parent drug (39% versus <5%). Thus, an amino acid moiety, strategically linked to (*S*)-cHPMPA or (*S*)-cHPMPC to mask its anionic properties, significantly enhances intestinal transport, creating the possibility of an orally bioavailable form with low toxicity.

EXPERIMENTAL SECTION

^1H, ^{13}C, and ^{31}P NMR spectra were obtained on Varian Mercury 400, Varian 400-MR, Bruker AMX-500 two-channel NMR spectrometers, or on a Varian VNMRS-600 three-channel NMR spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) relative to internal CD_3OD (δ 3.34 ^1H NMR, δ 49.86 ^{13}C NMR) and $CDCl_3$ (δ 7.26 ^1H NMR, δ 77.36 ^{13}C NMR) or external 85% H_3PO_4 (δ 0.00 ^{31}P NMR). ^{31}P NMR spectra were proton-decoupled, and ^1H and ^{13}C coupling constants (J) are quoted in Hz. The following NMR abbreviations are used: s (singlet), d (doublet), m (unresolved multiplet), dd (doublet of doublet), ddd (doublet of doublet of doublet), br

(broad signal). The concentration of the NMR samples was in the range of 2−20 mg/mL. HR-MS spectra were recorded at the UCR High Resolution Mass Spectrometry Facility, Riverside, CA. The UV spectra were recorded using Beckman Coulter DU 800 spectrophotometer. Preparative HPLC was performed using Varian ProStar with a Shimadzu SPD-10A UV detector (0.5 mm path length). LC−MS was performed on Finnigan LCQ Deca XP Max mass spectrometer in positive mode with a Finnigan Surveyor PDA 158 Plus detector (1 cm path length) and MS Pump Plus, all controlled using Xcalibur software, version 2.0.7. (*S*)-HPMPC and (*S*)-HPMPA were synthesized in our laboratory as previously described[7,18] or purchased from Rasayan Inc., CA, U.S. The names of the compounds were assigned using ACD/Labs, version 12.0. The *tert*-butyloxycarbonyl (Boc) protected amino acid **14** (Boc-(L)-Tyr-OMe) was obtained from Aldrich. Compounds **15**−**19** (Boc-(D)-Tyr-OMe (**15**), Boc-(L)-Tyr-O-*i*-Pr (**16**), Boc-(D)-Tyr-NH-*t*-Bu (**17**), Boc-(L)-Tyr-NH-*i*-Bu (**18**), Boc-(L)-Val-(L)-Tyr-OMe (**19**)), were synthesized as described below. All other reagents were purchased from commercial sources and used as obtained, unless specified otherwise. Compounds **20**−**27** and **6**−**13** were obtained as diastereomeric mixtures. The ratio of diastereomers was determined based on ^{31}P NMR. Where possible, the characteristic parameters of the signals of S_P and R_P isomers are described separately. The assignment of the signals in ^{1}H NMR and ^{13}C NMR was done based on the analysis of coupling constants and additional two-dimensional experiments (HSQC, DEPT). The >95% purity of the final compounds **6**−**13**, **8′**, **11′**, **28** was confirmed using HPLC analysis. The log *D* values presented in Table 1 were calculated using Marvin Sketch, version 5.2.0.

Synthesis of Boc-(D)-Tyr-OMe (15) and Boc-(L)-Tyr-O-*i*-Pr (16). General Procedure. Esterification of (D)- and (L)-Tyrosine[24]. Thionyl chloride (29.0 mmol, 3.40 g) was added dropwise to 16.5 mL of MeOH cooled to −20 °C (for (D)-Tyr-OMe) or *i*-PrOH cooled to −20 °C (for (L)-Tyr-O-*i*-Pr), followed by addition of the appropriate amino acid ((D)- or (L)-tyrosine) (3.9 mmol, 0.70 g). After the mixture was stirred for 15 min at −20 °C, the reaction mixture was allowed to reach room temperature and stirred overnight (for (D)-Tyr-OMe) or heated at 100 °C (for (L)-Tyr-O-*i*-Pr). Volatiles were removed under vacuum to provide (D)-Tyr-OMe or (L)-Tyr-O-*i*-Pr as hydrochloride salts, which were used for the next step without further purification.

Boc Protection of (D)-Tyr-OMe or (L)-Tyr-O-*i*-Pr[34]. The hydrochloride of (D)-Tyr-OMe (3.4 mmol, 0.79 g) or (L)-Tyr-O-*i*-Pr (3.4 mmol, 0.89 g) was dissolved in EtOH (16 mL) under vigorous stirring at room temperature. $NaHCO_3$ (34.3 mmol, 2.88 g) and di-*tert*-butyl dicarbonate (3.4 mmol, 0.75 g) were added sequentially. The resulting suspension was stirred overnight at room temperature. Solids were removed by filtration, and the residue was washed with water. The organic phase was dried over Na_2SO_4 and concentrated under vacuum to afford compounds **15** and **16**.

Methyl *N*-(*tert*-Butoxycarbonyl)-(D)-tyrosinate (15). Yield 82%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.00 (d, J = 8.0 Hz, 2H, aromatic), 6.76 (d, J = 8.4 Hz, 2H, aromatic), 5.80 (br, 1H, OH), 5.02 (d, J = 8.0 Hz, 1H, NH), 4.57 (m, 1H, C**H**NH), 3.75 (s, 3H, OCH_3), 3.06 (dd, J = 14.4, 6.0 Hz, 1H, C**H**$_a$H$_b$), 3.11 (dd, J = 14.4, 5.6 Hz, 1H, CH$_a$**H**$_b$), 1.45 (s, 9H, $C(CH_3)_3$).

Isopropyl *N*-(*tert*-Butoxycarbonyl)-(L)-tyrosinate (16). Yield 83%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.03 (d, J = 8.0 Hz, 2H, aromatic), 6.76 (d, J = 8.0 Hz, 2H, aromatic), 5.06−5.01 (m, 2H, C**H**$(CH_3)_2$ and OH), 4.50 (m, 1H, C**H**NH), 3.05 (dd, J = 14.4, 6.0 Hz, 1H, C**H**$_a$H$_b$), 3.11 (dd, J = 14.4, 6.0 Hz, 1H, CH$_a$**H**$_b$), 1.46 (s, 9H, $C(CH_3)_3$), 1.27−1.24 (m, 6H, $CH(CH_3)_2$).

Synthesis of Boc-(D)-Tyr-NH-*t*-Bu (17) and Boc-(L)-Tyr-NH-*i*-Bu (18). Boc Protection of (D)-Tyrosine[35]. (D)-Tyrosine (11.0 mmol, 2.0 g) was dissolved in dioxane/H_2O (2:1, 75 mL), and then 1 N NaOH (24.3 mmol, 25 mL) and di-*tert*-butyl dicarbonate (12.1 mmol, 2.64 g) were added sequentially. After being stirred at room temperature for 2 h, the reaction mixture was acidified (pH 2.3) with 0.5 N HCl and extracted with EtOAc (50 mL × 3). The organic phase was washed with brine, dried over Na_2SO_4, and concentrated under vacuum, affording 3.0 g (99%) Boc-(D)-tyrosine, used in the next step without further purification,

Amidation of Boc-Protected (D)- and (L)-Tyrosine.[25] General Procedure. Boc-(D)-tyrosine or Boc-(L)-tyrosine (commercially available) (4.6 mmol, 1.30 g) was suspended in dry CH_2Cl_2 (20 mL), and the suspension was cooled to 0 °C before addition of *N*-hydroxybenzotriazole (HOBt) hydrate (6.0 mmol, 0.81 g) and Et_3N (5.1 mmol, 0.71 mL). The reaction mixture was kept at 0 °C for 15 min before 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) hydrochloride (6.0 mmol, 1.15 g) and *tert*-butylamine (5.1 mmol, 0.53 mL; Boc-(D)-Tyr-NH-*t*-Bu) or isobutylamine (5.1 mmol, 0.51 mL; Boc-(L)-Tyr-NH-*i*-Bu) were added sequentially. The reaction mixture was stirred at room temperature overnight. An additional 30 mL of CH_2Cl_2 was added, and the organic layer was washed successively with 0.5 M HCl (25 mL, Boc-(D)-Tyr-NH-*t*-Bu) or with 1.6 M citric acid (25 mL, Boc-(L)-Tyr-NH-*i*-Bu), saturated $NaHCO_3$ (25 mL), and saturated NaCl (20 mL). The organic phase was dried over Na_2SO_4 and concentrated under vacuum. Boc-(L)-Tyr-NH-*i*-Bu (**18**) was used in the next step without further purification, whereas crude Boc-(D)-Tyr-NH-*t*-Bu was further purified by silica gel column chromatography [hexane/EtOAc (7:3)] to afford pure **17**.

***N*$^\alpha$-(*tert*-Butoxycarbonyl)-*N*-*tert*-butyl-(D)-tyrosinamide (17).** Yield 32%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.10 (d, J = 8.4 Hz, 2H, aromatic), 6.80 (d, J = 8.4 Hz, 2H, aromatic), 5.62 (br, 1H, NH), 6.36 (br, 1H, NH), 5.21 (br, 1H, NH), 4.08 (m, 1H, C**H**NH), 3.07 (dd, J = 13.2, 5.2 Hz, 1H, C**H**$_a$H$_b$), 2.87 (dd, J = 13.2, 8.0 Hz, 1H, CH$_a$**H**$_b$), 1.46 (s, 9H, $C(CH_3)_3$), 1.25 (s, 9H, $NHC(CH_3)_3$).

***N*$^\alpha$-(*tert*-Butoxycarbonyl)-*N*-(isobutyl)-(L)-tyrosinamide (18).** Yield 89%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.97 (d, J = 8.3 Hz, 2H, aromatic), 6.69 (m, 2H, aromatic), 5.82 (br, 1H, NH), 5.03 (br, 1H, NH), 4.15 (dd, 1H, J = 13.4, 7.2 Hz, C**H**NH (Tyr)), 3.02−2.79 (m, 4H, C**H**$_a$H$_b$, CH$_a$**H**$_b$ (Tyr), C**H**$_2$ (*i*-Bu)), 1.58 (m, 1H, C**H**$(CH_3)_2$), 1.35 (s, 9H, $C(CH_3)_3$), 0.73 (dd, J = 5.8 Hz, 6H, $CH(CH_3)_2$).

Methyl *N*-(*tert*-Butoxycarbonyl)-(L)-valyl-(L)-tyrosinate (19)[26]. (L)-Tyrosine methyl ester hydrochloride (3.2 mmol, 0.74 g) and Boc-(L)-valine (3.2 mmol, 0.70 g) were dissolved in dry CH_2Cl_2 (30 mL). The reaction mixture was cooled to 0 °C before addition of HOBt hydrate (4.8 mmol, 0.65 g) and Et_3N (16.0 mmol, 2.3 mL). The reaction mixture was kept at 0 °C for 15 min before EDC·HCl (4.0 mmol, 0.77 g) was added. The reaction mixture was stirred at room temperature overnight. An additional 30 mL of CH_2Cl_2 was added, and the organic layer was washed successively with 1.6 M citric acid (25 mL), saturated $NaHCO_3$ (25 mL), and saturated NaCl (20 mL). The organic phase was dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by silica gel column chromatography [CH_2Cl_2/MeOH (98:2)] to afford 0.91 g (73% yield) of compound **19** spectroscopically identical to the known compound.[36] ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.98 (d, J = 8.5 Hz, 2H, aromatic), 6.93 (d, J = 8.5 Hz, 2H, aromatic), 6.25 (br, 1H, NH), 4.99 (br, 1H, NH), 4.79 (m, 1H, CH (Tyr)), 3.84 (m, 1H, CH (Val)), 3.68 (s, 3H, OCH_3), 3.01 (m, 2H, CH_2), 2.05 (m, 1H, C**H**$(CH_3)_2$), 1.42 (s, 9H, $C(CH_3)_3$), 0.89 (d, J = 7.0 Hz, 3H, CH_3), 0.85 (d, J = 7.0 Hz, 3H, CH_3).

Synthesis of 20−27. General Procedure. To a suspension of **1** or **2** (1.0 mmol) in dry DMF (5 mL) was added dry DIEA (10.0 mmol, 1.8 mL). The reaction flask was warmed by a heat gun to facilitate the dissolution of the HPMPC/HPMPA-DIEA salt. The volatiles were then removed under vacuum. To the residue, anhydrous DMF (5 mL), dry DIEA (10.0 mmol, 1.8 mL), the relevant amino acid **14**−**18** or dipeptide **19** (1.5 mmol), and PyBOP (2.0 mmol, 1.04 g) were added. The reaction mixture was stirred under N_2 at 40 °C for 2 h. The reaction was monitored by ^{31}P NMR, and additional portions of PyBOP were added as necessary. After reaction was complete, DMF and DIEA were removed under vacuum. The residue was extracted with diethyl ether

and purified by silica gel column chromatography [CH_2Cl_2, CH_2Cl_2/acetone (2:1) and CH_2Cl_2/acetone/CH_3OH (6:3:1)]. Solvents were removed under vacuum to furnish Boc-protected compounds **20**−**27** containing a small amount of HOBt (by ^{1}H NMR). The yields of the Boc-protected compounds **20**−**27** were estimated by ^{1}H NMR analysis.

Methyl *O*-[(5*S*)-5-[(4-Amino-2-oxopyrimidin-1(2*H*)-yl)methyl]-2-oxido-1,4,2-dioxaphosphinan-2-yl]-*N*-(*tert*-butoxycarbonyl)-L-tyrosinate (20). Yield 57%. Obtained from 1.00 g of **2** as a mixture of the diastereomers (S_P/R_P, 4:1). ^{1}H NMR (500 MHz, CD_3OD): δ 7.53 (d, J = 7.0 Hz, 0.8H, CH═C**H**−N (S_P)), 7.52 (d, J = 6.0 Hz, 0.2H, CH═C**H**−N (R_P)), 7.21−7.09 (m, 4H, aromatic), 5.85 (d, J = 7.5 Hz, 1H, C**H**═CH−N), 4.59 (ddd, J = 13.5, 13.5, 2.5 Hz, 0.8H (S_P)), 4.50−3.92 (m, 7H), 3.75 (dd, J = 14.5, 7.0 Hz, 0.2H, $CH_a\mathbf{H_b}N$ (R_P)), 3.65 (s, 3H, OCH_3), 3.06 (dd, J = 14.0, 4.5 Hz, 1H, $C\mathbf{H_a}H_b$(Tyr)), 2.86 (dd, J = 13.0, 9.0 Hz, 1H, $CH_a\mathbf{H_b}$(Tyr)), 1.33 (s, 9H, $C(CH_3)_3$). ^{31}P NMR (202 MHz, CD_3OD): δ 11.37 (0.80P (S_P)), 10.17 (0.20P (R_P)).

Methyl *O*-[(5S)-5-[(4-Amino-2-oxopyrimidin-1(2*H*)-yl)methyl]-2-oxido-1,4,2-dioxaphosphinan-2-yl]-*N*-(*tert*-butoxycarbonyl)-D-tyrosinate (21). Yield 47%. Obtained from 0.11 g of **2** as a mixture of the diastereomers (S_P/R_P, 3.8:1). ^{1}H NMR (400 MHz, CD_3OD): δ 7.62 (m, 1H, CH═C**H**−N), 7.28−7.15 (m, 4H, aromatic), 5.90 (m, 1H, C**H**═CH−N), 4.66 (ddd, J = 12.4, 12.4, 2.8 Hz, 0.8H, (S_P)), 4.56−3.99 (m, 7H), 3.80 (dd, J = 14.4, 7.6 Hz, 0.2H, $CH_a\mathbf{H_b}N$ (R_P)), 3.72 (s, 3H, OCH_3), 3.13 (dd, J = 14.0, 5.6 Hz, 1H, $C\mathbf{H_a}H_b$(Tyr)), 2.92 (dd, J = 13.6, 9.2 Hz, 1H, $CH_a\mathbf{H_b}$(Tyr)), 1.40 (s, 9H, $C(CH_3)_3$). ^{31}P NMR (162 MHz, CD_3OD): δ 11.40 (0.80P (S_P)), 10.21 (0.20P (R_P)).

Isopropyl *O*-[(5*S*)-5-[(4-Amino-2-oxopyrimidin-1(2*H*)-yl)ethyl]-2-oxido-1,4,2-dioxaphosphinan-2-yl]-*N*-(*tert*-butoxycarbonyl)-L-tyrosinate (22). Yield 35%. Obtained from 0.12 g of **2** as a mixture of the diastereomers (S_P/R_P, 3.4:1). ^{1}H NMR (400 MHz, CD_3OD): δ 7.61 (d, J = 7.6 Hz, 0.8H, CH═C**H**−N (S_P)), 7.59 (d, J = 7.6. Hz, 0.2H, CH═C**H**−N (R_P)), 7.31−7.15 (m, 4H, aromatics), 5.91 (d, J = 7.6 Hz, 1H, C**H**═CH−N), 4.98 (m, 1H, C**H**$(CH_3)_2$), 4.65 (ddd, J = 12.0, 12.0, 2.4 Hz, 0.2H (S_P)), 4.59−3.96 (m, 7H), 3.81 (dd, J = 14.0, 7.2 Hz, 0.2H, $CH_a\mathbf{H_b}N$ (R_P)), 3.10 (dd, J = 14.0, 6.0 Hz, 1H, $C\mathbf{H_a}H_b$ (Tyr)), 2.92 (dd, J = 14.0, 9.2 Hz, 1H, $CH_a\mathbf{H_b}$ (Tyr)), 1.41 (s, 9H, $C(CH_3)_3$), 1.26 (d, J = 6.0 Hz, 3H, $CHC\mathbf{H_3}$), 1.19 (d, J = 6.4 Hz, 3H, $CHC\mathbf{H_3}$). ^{31}P NMR (162 MHz, CD_3OD): δ 11.36 (0.77P (S_P)), 10.20 (0.23P (R_P)).

***O*-[(5*S*)-5-[(4-Amino-2-oxopyrimidin-1(2*H*)-yl)methyl]-2-oxido-1,4,2-dioxaphosphinan-2-yl]-N^{α}-(*tert*-butoxycarbonyl)-*N*-*tert*-butyl-D-tyrosinamide (23).** Yield 27%. Obtained from 0.12 g of **2** as a mixture of the diastereomers (S_P/R_P, 1.8:1). ^{1}H NMR (400 MHz, CD_3OD): δ 7.58 (d, J = 6.8 Hz, 0.6H, CH═C**H**−N, (S_P)), 7.55 (d, J = 7.6. Hz, 0.4H, CH═C**H**−N (R_P)), 7.31−7.14 (m, 4H, aromatic), 5.89 (d, J = 7.2 Hz, 0.4H, C**H**═CH−N (R_P)), 5.88 (d, J = 7.6 Hz, 0.6H, C**H**═CH−N (S_P)), 4.65 (ddd, J = 12.0, 12.0, 2.4 Hz, 0.6H (S_P)), 4.58−4.41 (m, 2H), 4.33 (dd, J = 14.8, 10.8 Hz, 0.4H (R_P)), 4.25−4.03 (m, 4H), 3.96 (dd, J = 14.4, 8.0 Hz, 0.6H, $CH_a\mathbf{H_b}N$ (S_P)), 3.82 (dd, J = 14.4, 7.2 Hz, 0.4H, $CH_a\mathbf{H_b}N$ (R_P)), 2.99 (m, 1H, $C\mathbf{H_a}H_b$ (Tyr)), 2.84 (m, 1H, $CH_a\mathbf{H_b}$ (Tyr)), 1.41 (s, 3.6H, $C(CH_3)_3$ (R_P)), 1.40 (s, 5.4H, $C(CH_3)_3$ (S_P)), 1.29 (s, 9H, $NH(C\mathbf{H_3})_3$). ^{31}P NMR (162 MHz, CD_3OD): δ 11.31 (0.64P (S_P)), 10.06 (0.36P (R_P)).

Methyl *O*-[(5*S*)-5-[(6-Amino-9*H*-purin-9-yl)methyl]-2-oxido-1,4,2-dioxaphosphinan-2-yl]-*N*-(*tert*-butoxycarbonyl)-L-tyrosinate (24). Yield 73%. Obtained from 0.75 g of **1** as mixture of the diastereomers (S_P/R_P, 4:1). ^{1}H NMR (400 MHz, CD_3OD): δ 8.25 (s, 1H, N═C**H**−N), 8.17 (s, 0.8H, N═C**H**−N (S_P)), 8.15 (s, 0.2H, N═C**H**−N (R_P)), 7.28−7.04 (m, 4H, aromatic), 4.76 (ddd, J = 11.8, 11.8, 2.6 Hz, 0.8H (S_P)), 4.62−4.07 (m, 7.2H), 3.73 (s, 0.6H, OCH_3 (R_P)), 3.72 (s, 2.4H, OCH_3 (S_P)), 3.15 (dd, J = 14.0, 5.3 Hz, 1H, $C\mathbf{H_a}H_b$(Tyr)), 2.91 (dd, J = 14.0, 9.2 Hz, 1H, $CH_a\mathbf{H_b}$(Tyr)), 1.41 (s, 1.8H, $C(CH_3)_3$ (R_P)), 1.39 (s, 7.2H, $C(CH_3)_3$ (S_P)). ^{31}P NMR (162 MHz, CD_3OD): δ 11.22 (0.80P (S_P)), 10.00 (0.20P (R_P)).

***O*-[(5*S*)-5-[(6-Amino-9*H*-purin-9-yl)methyl]-2-oxido-1,4,2-dioxaphosphinan-2-yl]-N^{α}-(*tert*-butoxycarbonyl)-*N*-(2-methylpropyl)-L-tyrosinamide (25).** Yield 76%. Obtained from 1.00 g of **1** as mixture of the diastereomers (S_P/R_P, 4.4:1). ^{1}H NMR (400 MHz, CD_3OD): δ 8.26 (s, 0.2H, N═C**H**−N (R_P)), 8.25 (s, 0.8H, N═C**H**−N (S_P)), 8.17 (s, 0.8H, N═C**H**−N (S_P)), 8.14 (s, 0.2H, N═C**H**−N (R_P)), 7.30 (d, J = 8.6 Hz, 2H, aromatic), 7.15 (d, J = 8.0 Hz, 1.6H, aromatic (S_P)), 7.11 (d, J = 8.6 Hz, 0.4H, aromatic (R_P)), 4.75 (ddd, J = 11.9, 11.9, 2.7 Hz, 0.8H (S_P)), 4.62−4.06 (m, 7.2H), 3.09−2.81 (m, 4H), 1.72 (m, C**H**$(CH_3)_2$), 1.41 (s, 1.8H, $C(CH_3)_3$ (R_P)), 1.39 (s, 7.2H, $C(CH_3)_3$ (S_P)), 0.86 (m, 6H, $CH(C\mathbf{H_3})_2$). ^{31}P NMR (202 MHz, CD_3OD): δ 11.17 (0.82P (S_P)), 9.90 (0.18P (R_P)).

Methyl *N*-(*tert*-Butoxycarbonyl)-L-valyl-*O*-[(5*S*)-5-[(4-amino-2-oxopyrimidin-1(2*H*)-yl)methyl]-2-oxido-1,4,2-dioxaphosphinan-2-yl]-L-tyrosinate (26). Yield 65%. Obtained from 0.20 g of **1** as mixture of the diastereomers (S_P/R_P, 4:1). ^{1}H NMR (400 MHz, CD_3OD): δ 7.58 (d, J = 7.0 Hz, 0.80H, CH═C**H**−N (S_P)), 7.56 (d, J = 7.0 Hz, 0.20H, CH═C**H**−N (R_P)), 7.29−7.14 (m, 4H, aromatic), 5.88 (d, J = 6.9 Hz, 0.20H, C**H**═CH−N (R_P)), 5.87 (d, J = 7.5 Hz, 0.80H, C**H**═CH−N (S_P)), 4.73−3.83 (m, 8H), 3.71 (s, 3H, OCH_3), 3.18 (dd, J = 13.9, 5.4 Hz, 1H, $C\mathbf{H_a}H_b$(Tyr)), 3.02 (dd, J = 13.9, 8.5 Hz, 1H, $CH_a\mathbf{H_b}$(Tyr)), 1.96 (m, 1H, C**H**$(CH_3)_2$), 1.47 (s, 9H, $C(CH_3)_3$), 0.90 (m, 6H, $CH(C\mathbf{H_3})_2$). ^{31}P NMR (162 MHz, CD_3OD): δ 11.28 (0.80P (S_P)), 10.09 (0.20P (R_P)).

Methyl *N*-(*tert*-Butoxycarbonyl)-L-valyl-*O*-[(5*S*)-5-[(6-amino-9*H*-purin-9-yl)methyl]-2-oxido-1,4,2-dioxaphosphinan-2-yl]-L-tyrosinate (27). Yield 63%. Obtained from 0.10 g of **1** as mixture of the diastereomers (S_P/R_P, 16:1). ^{1}H NMR (400 MHz, CD_3OD): δ 8.25 (s, 1H, N═C**H**−N), 8.17 (s, 0.9H, N═C**H**−N (S_P)), 8.14 (s, 0.1H, N═C**H**−N (R_P)), 7.27−7.09 (m, 4H, aromatic), 4.78−3.83 (m, 9H), 3.71 (s, 0.2H, OCH_3 (R_P)), 3.70 (s, 2.7H, OCH_3 (S_P)), 3.17 (dd, J = 13.9, 5.4 Hz, 1H, $C\mathbf{H_a}H_b$(Tyr)), 3.02 (dd, J = 14.0, 8.6 Hz, 1H, $CH_a\mathbf{H_b}$(Tyr)), 1.95 (m, 1H, C**H**$(CH_3)_2$), 1.47 (s, 9H, $C(CH_3)_3$), 0.90 (m, 6H, $CH(C\mathbf{H_3})_2$). ^{31}P NMR (202 MHz, CD_3OD): δ 11.16 (0.94P (S_P)), 9.80 (0.06P (R_P)).

Boc Deprotection. General Procedure. TFA (4 mL) was added to a solution of the Boc-protected derivatives (**20**−**27**) (0.7 mmol) dissolved in dry CH_2Cl_2 (4 mL). After the mixture was stirred overnight at room temperature, volatiles were removed under vacuum. The residue was purified by silica gel column chromatography [CH_2Cl_2/MeOH (10:1.5), with addition of 0.5% v/v TFA]. After evaporation of the solvents, compounds **6**−**13** were precipitated with diethyl ether, filtered, and dried in vacuum to give TFA salts of the final products as white powders.

Transformation of Compounds 6−13 from TFA Salts into Hydrochloride Salts. General Procedure. The TFA salt of **6**, **10**, or **11** (0.4 mmol) was dissolved in MeOH (20 mL) at −20 °C with stirring, followed by addition of 0.2 M HCl/MeOH (20 mL) with cooling to −20 °C. The volatiles were evaporated under vacuum. The procedure was repeated twice. After the third addition and evaporation of the HCl/MeOH solution, the residue was dried under vacuum and reprecipitated with diethyl ether as described above for the TFA salts. The yields of the products **6**−**13** were calculated on the basis of the content of active compound measured by its UV spectrum. UV determinations of the active compound content in salts **6**−**13** used the following extinction coefficients: **1** (ε = 14 191 at 260 nm, pH 7.0), **2** (ε = 9000 at 274 nm, pH 7.0), and tyrosine (ε = 612 at 260 nm and ε = 667 at 274 nm, pH 7.0).

HPLC Analysis of Compounds 6−13. HPLC of **6**−**13** was performed on a Varian Microsorb-MV C_{18} HPLC column (5 μm, 250 mm × 4.6 mm) with a 0−30% CH_3CN gradient in ammonium acetate buffer, pH 5.5, at a flow rate of 1.0 mL/min. The UV detector was operated at 274 or 260 nm for **2** or **1** derivative, respectively.

Methyl *O*-[(5*S*)-5-[(4-Amino-2-oxopyrimidin-1(2*H*)-yl)methyl]-2-oxido-1,4,2-dioxaphosphinan-2-yl]-L-tyrosinate (6). Yield 58%. Obtained as a hydrochloride salt; mixture of two diastereomers (S_P/R_P, 3.8:1).

^{1}H NMR (400 MHz, CD_3OD): δ 7.94 (m, 1H, CH═C**H**−N), 7.38−7.24 (m, 4H, aromatic), 6.10 (d, J = 8.0 Hz, 1H, C**H**═CH−N), 4.68 (ddd, J = 13.8, 13.8, 2.8 Hz, 0.8H, C**H$_a$**H$_b$O (S_P)), 4.61−4.52 (m, 1.2H, C**H$_a$**H$_b$O (R_P) and CH$_a$**H$_b$**O), 4.47 (dd, J = 14.8, 7.6 Hz, 0.8H, C**H$_a$**H$_b$P (S_P)), 4.44−4.35 (m, 1.2H, C**H$_a$**H$_b$P (R_P) and C**H**NH$_2$), 4.32−4.23 (m, 2H, CHO, CH$_a$**H$_b$**P (S_P) and C**H$_a$**H$_b$N (R_P)), 4.19−4.13 (m, 1H, CH$_a$**H$_b$**P (R_P) and C**H$_a$**H$_b$N (S_P)), 4.10 (dd, J = 14.5, 8.0 Hz, 0.8H, CH$_a$**H$_b$**N (S_P)), 3.86 (s, 0.6H, OCH_3 (R_P)), 3.85 (s, 2.4H, OCH_3 (S_P)), 3.84 (m, 0.2H, CH$_a$**H$_b$**N (R_P)), 3.30 (m, 1H, C**H$_a$**H$_b$ (Tyr)), 3.21 (m, 1H, CH$_a$**H$_b$** (Tyr)). ^{31}P NMR (162 MHz, CD_3OD): δ 12.01 (0.79P (S_P)), 10.39 (0.21P (R_P)). HRMS: m/z calcd 439.1387 $(M + H)^+$, found 439.1378 $(M + H)^+$. HPLC: t_R, 24.05 min.

Methyl *O*-[(5*S*)-5-[(4-Amino-2-oxopyrimidin-1(2*H*)-yl)methyl]-2-oxido-1,4,2-dioxaphosphinan-2-yl]-(D)-tyrosinate (7). Yield 50%. Obtained as a hydrochloride salt; mixture of two diastereomers (S_P/R_P, 2.7:1). ^{1}H NMR (400 MHz, CD_3OD): δ 7.89 (d, J = 7.6 Hz, 0.7H, CH═C**H**−N, (S_P)), 7.86 (d, J = 8.4 Hz, 0.3H, CH═C**H**−N (R_P)), 7.37−7.23 (m, 4H, aromatic), 6.08 (d, J = 7.6 Hz, 1H, C**H**═CH−N), 4.68 (ddd, J = 12.4, 12.4, 2.4 Hz, 0.7H, C**H$_a$**H$_b$O (S_P)), 4.63−4.50 (m, 1.3H, C**H$_a$**H$_b$O (R_P) and CH$_a$**H$_b$**O), 4.47 (dd, J = 15.2, 8.0 Hz, 0.7H, C**H$_a$**H$_b$P (S_P)), 4.36 (m, 1.3H, C**H$_a$**H$_b$P (R_P) and C**H**NH$_2$), 4.30−4.24 (m, 2H, CHO, CH$_a$**H$_b$**P (S_P) and C**H$_a$**H$_b$N (R_P)), 4.24−4.13 (m, 1H, CH$_a$**H$_b$**P (R_P) and C**H$_a$**H$_b$N (S_P)), 4.08 (dd, J = 14.4, 7.6 Hz, 0.7H, CH$_a$**H$_b$**N (S_P)), 3.85 (s, 0.9H, OCH_3 (R_P)), 3.84 (s, 2.1H, OCH_3 (S_P)), 3.83 (dd, J = 13.9, 8.1 Hz, 0.3H, CH$_a$**H$_b$**N (R_P)), 3.28 (m, 1H, C**H$_a$**H$_b$ (Tyr)), 3.20 (m, 1H, CH$_a$**H$_b$** (Tyr)). ^{31}P NMR (162 MHz, CD_3OD): δ 11.97 (0.73P (S_P)), 10.39 (0.27P (R_P)). HRMS: m/z calcd 439.1377 $(M + H)^+$, found 439.1387 $(M + H)^+$. HPLC: t_R, 24.13 min (R_P), 24.54 min (S_P).

Isopropyl *O*-[(5*S*)-5-[(4-Amino-2-oxopyrimidin-1(2*H*)-yl)methyl]-2-oxido-1,4,2-dioxaphosphinan-2-yl]-(L)-tyrosinate (8). Yield 22%. Obtained as TFA salt; a mixture of two diastereomers (S_P/R_P, 2.6:1). ^{1}H NMR (400 MHz, CD_3OD): δ 7.79 (d, J = 7.2 Hz, 0.7H, CH═C**H**−N (S_P)), 7.76 (d, J = 7.6 Hz, 0.3H, CH═C**H**−N (R_P)), 7.33−7.18 (m, 4H, aromatic), 6.01 (m, 1H, C**H**═CH−N), 5.03 (m, 1H, C**H**$(CH_3)_2$), 4.60 (ddd, J = 12.4, 12.4, 2.8 Hz, 0.7H, C**H$_a$**H$_b$O (S_P)), 4.56−4.39 (m, 1.3H, C**H$_a$**H$_b$O (R_P) and CH$_a$**H$_b$**O), 4.42 (dd, J = 14.4, 7.2 Hz, 0.7H, C**H$_a$**H$_b$P (S_P)), 4.37 (dd, J = 15.6, 11.2 Hz, 0.3H, C**H$_a$**H$_b$P (R_P)), 4.27−4.19 (m, 3H, C**H**NH$_2$, CHO, CH$_a$**H$_b$**P (S_P) and C**H$_a$**H$_b$N (R_P)), 4.13−3.99 (m, 1.7H, CH$_a$**H$_b$**P (R_P), CH_2N (S_P)), 3.80 (dd, J = 12.4, 7.6 Hz, 0.3H, CH$_a$**H$_b$**N (R_P)), 3.24−3.13 (m, 2H, CH_2(Tyr)), 1.25−1.23 (m, 3H, CH_3), 1.18−1.16 (m, 3H, CH_3). ^{31}P NMR (162 MHz, CD_3OD): δ 11.78 (0.72P (S_P)), 10.19 (0.28P (R_P)). HRMS: m/z calcd 467.1700 $(M + H)^+$, found 467.1698 $(M + H)^+$. HPLC: t_R, 23.61 min (R_P), 24.05 min (S_P).

***O*-[(5*S*)-5-[(4-Amino-2-oxopyrimidin-1(2*H*)-yl)methyl]-2-oxido-1,4,2-dioxaphosphinan-2-yl]-*N*-*tert*-butyl-(D)-tyrosinamide (9).** Yield 53%. Obtained as a TFA salt; mixture of two diastereomers (S_P/R_P, 2.3:1). ^{1}H NMR (400 MHz, CD_3OD): δ 7.86 (d, J = 7.6 Hz, 0.7H, CH═C**H**−N (S_P)), 7.84 (d, J = 8 Hz, 0.3H, CH═C**H**−N (R_P)), 7.32−7.17 (m, 4H, aromatic), 6.04 (d, J = 7.6 Hz, 1H, C**H**═CH−N), 4.62 (ddd, J = 12.4, 12.4, 2.8 Hz, 0.7H, C**H$_a$**H$_b$O (S_P)), 4.56 (m, 0.3H, C**H$_a$**H$_b$O (R_P)), 4.48 (m, 1H, CH$_a$**H$_b$**O), 4.41 (dd, J = 14.4, 7.6 Hz, 0.7H, C**H$_a$**H$_b$P (S_P)), 4.35 (dd, J = 15.2, 11.2 Hz, 0.3H, C**H$_a$**H$_b$P (R_P)) 4.24−4.16 (m, 2H, CHO, CH$_a$**H$_b$**P (S_P) and C**H$_a$**H$_b$N (R_P)) 4.13−4.01 (m, 1.7H, CH$_a$**H$_b$**P (R_P), CH_2N (S_P)), 3.92 (m, 1H, C**H**NH$_2$), 3.80 (dd, J = 14.8, 8.4 Hz, 0.3H, CH$_a$**H$_b$**N (R_P)), 3.11−3.00 (m, 2H, CH_2(Tyr)), 1.24 (s, 2.7H, NHC(C**H**$_3$)$_3$ (R_P)), 1.23 (s, 6.3H, NHC(C**H**$_3$)$_3$ (S_P)). ^{31}P NMR (162 MHz, CD_3OD): δ 11.79 (0.70P (S_P)), 10.25 (0.30P (R_P)). HRMS: m/z calcd 480.2006 $(M + H)^+$, found 480.2004 $(M + H)^+$. HPLC: t_R, 20.50 min (R_P), 20.98 min (S_P).

Methyl *O*-[(5*S*)-5-[(6-Amino-9*H*-purin-9-yl)methyl]-2-oxido-1,4,2-dioxaphosphinan-2-yl]-(L)-tyrosinate (10). Yield 74%. Obtained as a hydrochloride salt; mixture of two diastereomers (S_P/R_P, 5.4:1). ^{1}H NMR (400 MHz, CD_3OD): δ 8.41 (s, 0.8H, N═CH−N (S_P)), 8.40 (s, 0.2H, N═CH−N (R_P)), 8.34 (s, 0.8H, N═CH−N (S_P)), 8.30 (s, 0.2H, N═CH−N (R_P)), 7.34−7.24 (m, 4H, aromatic), 4.78 (ddd, J = 12.1, 12.1, 3.0 Hz, 0.8H, C**H$_a$**H$_b$O (S_P)), 4.69 (dd, J = 15.0, 8.3 Hz, 0.8H, C**H$_a$**H$_b$N (S_P)), 4.61−4.34 (m, 5.4H, C**H$_a$**H$_b$O (R_P), C**H$_a$**H$_b$N (R_P), CH$_a$**H$_b$**N, CH$_a$**H$_b$**O, C**H$_a$**H$_b$P, CHO and C**H**NH$_2$), 4.24 (dd, J = 14.8, 4.3 Hz, 0.8H, CH$_a$**H$_b$**P (S_P)), 4.12 (dd, J = 15.3, 1.3 Hz, 0.2H, CH$_a$**H$_b$**P (R_P)), 3.86 (s, 0.6H, OCH_3 (R_P)), 3.85 (s, 2.4H, OCH_3 (S_P)), 3.29 (dd, J = 14.5, 6.2 Hz, 1H, C**H$_a$**H$_b$ (Tyr)), 3.18 (dd, J = 14.5, 7.4 Hz, 1H, CH$_a$**H$_b$** (Tyr)). ^{31}P NMR (202 MHz, CD_3OD): δ 11.77 (0.84P (S_P)), 10.19 (0.16P (R_P)). HRMS: m/z calcd 463.1489 $(M + H)^+$, found 463.1499 $(M + H)^+$. HPLC: t_R, 17.58 min (R_P), 18.47 min (S_P).

***O*-[(5*S*)-5-[(6-Amino-9*H*-purin-9-yl)methyl]-2-oxido-1,4,2-dioxaphosphinan-2-yl]-*N*-(2-methylpropyl)-(L)-tyrosinamide (11).** Yield 76%. Obtained as a hydrochloride salt; mixture of two diastereomers (S_P/R_P, 3.8:1). ^{1}H NMR (400 MHz, CD_3OD): diastereomer S_P, δ 8.42 (s, 1H, N═CH−N), 8.37 (s, 1H, N═CH−N), 7.32 (J = 8.6 Hz, 2H, aromatic), 7.19 (d, J = 8.6 Hz, 2H, aromatic), 4.76 (ddd, J = 12.1, 12.1, 2.7 Hz, 1H, C**H$_a$**H$_b$O), 4.70 (dd, J = 14.9, 8.3 Hz, 1H, C**H$_a$**H$_b$N), 4.60 (dd, J = 14.9, 3.5 Hz, 1H, CH$_a$**H$_b$**N), 4.54 (dd, J = 11.7, 7.4 Hz, 1H, CH$_a$**H$_b$**O), 4.49 (dd, J = 15.2, 7.0 Hz, 1H, C**H$_a$**H$_b$P), 4.42 (m, 1H, CHO), 4.23 (dd, J = 14.9, 4.3 Hz, 1H, CH$_a$**H$_b$**P), 4.04 (t, J = 7.4 Hz, 1H, C**H**NH$_2$), 3.17 (dd, J = 14.1, 7.6 Hz, 1H, C**H$_a$**H$_b$ (Tyr)), 3.12−3.05 (m, 2H, CH$_a$**H$_b$** (Tyr) and C**H$_a$**H$_b$ (*i*-Bu)), 2.92 (dd, J = 13.3, 7.0 Hz, 1H, CH$_a$**H$_b$** (*i*-Bu)), 1.69 (m, 1H, C**H**$(CH_3)_2$), 0.85 (d, J = 6.6 Hz, 3H, CH_3), 0.82 (d, J = 6.7 Hz, 3H, CH_3); diastereomer R_P data are summarized in the isomerization section. ^{31}P NMR (162 MHz, CD_3OD): δ 11.69 (0.79P (S_P)), 10.10 (0.21P (R_P)). HRMS: m/z calcd 504. 2119 $(M + H)^+$, found 504.2128 $(M + H)^+$. HPLC: t_R, 17.66 min (R_P), 18.32 min (S_P).

Methyl (L)-Valyl-*O*-[(5*S*)-5-[(4-amino-2-oxopyrimidin-1(2*H*)-yl)methyl]-2-oxido-1,4,2-dioxaphosphinan-2-yl]-(L)-tyrosinate (12). Yield 50%. Obtained as a TFA salt; mixture of diastereomers (S_P/R_P, 5.7:1). ^{1}H NMR (400 MHz, CD_3OD): diastereomer S_P, δ 7.84 (d, J = 7.5 Hz, 1H, CH═C**H**−N), 7.26 (d, J = 8.6 Hz, 2H, aromatic), 7.13 (d, J = 8.6 Hz, 2H, aromatic), 6.02 (d, J = 7.9 Hz, 1H, C**H**═CH−N), 4.73 (dd, J = 8.7 Hz, 5.9 Hz, 1H, CH (Tyr)), 4.62 (ddd, J = 13.7, 12.1, 2.8 Hz, 1H, C**H$_a$**H$_b$O), 4.48 (dd, J = 12.1, 7.5 Hz, 1H, CH$_a$**H$_b$**O), 4.41 (dd, J = 14.5, 8.0 Hz, 1H, C**H$_a$**H$_b$P), 4.22 (dd, J = 14.8, 3.1 Hz, 1H, CH$_a$**H$_b$**P), 4.20 (m, 1H, CHO), 4.11 (dd, J = 14.5, 3.5 Hz, 1H, C**H$_a$**H$_b$N), 4.03 (dd, J = 14.5, 7.8 Hz, 1H, CH$_a$**H$_b$**N), 3.68 (s, 3H, OCH_3), 3.64 (d, J = 5.5 Hz, 1H, C**H**NH$_2$), 3.19 (dd, J = 14.1, 5.9 Hz, 1H, C**H$_a$**H$_b$ (Tyr)), 3.01 (dd, J = 14.1, 5.9 Hz, 1H, CH$_a$**H$_b$** (Tyr)), 2.20 (m, 1H, C**H**$(CH_3)_2$), 1.06 (d, J = 6.6 Hz, 3H, CH_3), 1.01 (d, J = 6.7 Hz, 3H, CH_3); diastereomer R_P, δ 7.80 (d, J = 7.9 Hz, 1H, CH═C**H**−N), 7.28 (d, J = 8.6 Hz, 2H, aromatic), 7.17 (d, J = 8.6 Hz, 2H, aromatic), 6.02 (d, J = 7.9 Hz, 1H, C**H**═CH−N), 4.79−3.80 (m, 8H), 3.70 (s, 3H, OCH_3), 3.65 (d, J = 5.5 Hz, 1H, C**H**NH$_2$), 3.19 (m, 1H, C**H$_a$**H$_b$ (Tyr)), 3.01 (m, 1H, CH$_a$**H$_b$** (Tyr)), 2.20 (m, 1H, C**H**$(CH_3)_2$), 1.06 (d, J = 6.6 Hz, 3H, CH_3), 1.01 (d, J = 6.7 Hz, 3H, CH_3). ^{31}P NMR (202 MHz, CD_3OD): δ 11.70 (0.85P (S_P)), 10.03 (0.15P (R_P)). HRMS: m/z calcd 538.2061 $(M + H)^+$, found 538.2065 $(M + H)^+$. HPLC: t_R, 16.06 min (R_P), 16.43 min (S_P).

Methyl (L)-Valyl-*O*-[(5*S*)-5-[(6-amino-9*H*-purin-9-yl)methyl]-2-oxido-1,4,2-dioxaphosphinan-2-yl]-(L)-tyrosinate (13). Yield 54%. Obtained as a TFA salt; mixture of two diastereomers (S_P/R_P, 1.05:1). ^{1}H NMR (400 MHz, CD_3OD): δ 8.40 (s, 0.5H, N═CH−N (S_P)), 8.39 (s, 0.5H, N═C**H**−N, (R_P)), 8.34 (s, 0.5H, N═CH−N (S_P)), 8.30 (s, 0.5H, N═C**H**−N, (R_P)), 7.32−7.30 (m, 2H, aromatic), 7.18−7.15 (m, 2H, aromatic), 4.81−4.34 (m, 7H), 4.22 (dd, J = 15.1, 4.3 Hz, 0.5H, CH$_a$**H$_b$**P (S_P)), 4.11 (dd, J = 15.5, 1.6 Hz, 0.5H, CH$_a$**H$_b$**P (R_P)), 3.74, 3.72 (2s, 3H, OCH_3 (S_P and R_P)), 3.69, 3.67 (2d, J = 5.4 Hz, 1H, C**H**NH$_2$ (S_P and R_P)), 3.23 (m, 1H, C**H$_a$**H$_b$ (Tyr)), 3.05 (m, 1H, CH$_a$**H$_b$** (Tyr)), 2.24 (m, 1H, C**H**$(CH_3)_2$), 1.10, 1.09 (2d, J = 6.9 Hz, 3H, CH_3), 1.05, 1.04 (2d, J = 7.0 Hz, 3H, CH_3). ^{31}P NMR (202 MHz, CD_3OD): δ 11.54 (0.50P (S_P)), 9.98 (0.50P (R_P)). HRMS: m/z calcd 562.2174 $(M + H)^+$, found 562.2180 $(M + H)^+$. HPLC: t_R, 18.99 min (R_P), 20.35 min (S_P).

General Method for Isomerization. To a solution of the Boc-protected compounds (**22**, **25**) (0.2 mmol) and the appropriate Boc-protected amino acid (**16**, **18**) (0.02 mmol) in absolute DMF (5 mL) were added molecular sieves (0.4 nm). After 30 min, cesium carbonate (0.4 mmol, 130 mg) was added to the mixture under a nitrogen atmosphere and the reaction mixture was stirred for 1−3 days at room temperature until the of ratio of diastereomers was $S_P/R_P \approx 1{:}9$ (^{31}P NMR). Molecular sieves and cesium carbonate were removed by filtration, and DMF was evaporated under vacuum. The residue was deprotected without isolation using TFA/CH_2Cl_2 and purified as described in the general method for Boc deprotection.

Isopropyl *O*-[(5*S*)-5-[(4-Amino-2-oxopyrimidin-1(2*H*)-yl)methyl]-2-oxido-1,4,2-dioxaphosphinan-2-yl]-(L)-tyrosinate (8′, Enriched with Isomer R_P). Yield 27%. Obtained as a TFA salt, mixture of two diastereomers (S_P/R_P, 1:11.5). Diastereomer R_P, ^{1}H NMR (400 MHz, CD_3OD): δ 7.84 (d, J = 7.2 Hz, 1H, CH═C**H**−N), 7.32 (d, J = 8.2 Hz, 2H, aromatic), 7.25 (d, J = 7.8 Hz, 2H, aromatic), 6.05 (d, J = 8.0 Hz, 1H, C**H**═CH−N), 5.00−5.06 (m, 1H, C**H**-$(CH_3)_2$), 4.57−4.44 (m, 2H, CH_2O), 4.35 (dd, J = 15.2, 10.8 Hz, 1H, C**H**$_a$H$_b$P), 4.25−4.19 (m, 2H, C**H**NH_2 and C**H**$_a$H$_b$N), 4.16−4.05 (m, 2H, CHO and CH_a**H**$_b$P), 3.80 (dd, J = 14.4, 8.0 Hz, 1H, CH_a**H**$_b$N), 3.24−3.14 (m, 2H, C**H**$_2$(Tyr)), 1.23 (d, J = 6.4 Hz, 3H, C**H**$_3$), 1.17 (d, J = 6.4 Hz, 3H, C**H**$_3$). ^{13}C NMR (100.6 MHz, CD_3OD): δ 168.0 (CO), 160.5 (CO), 150.3 (CH═**C**H−N), 149.1 (d, J = 7.7 Hz, COP, aromatic), 148.0 (CNH_2), 131.8 (C, aromatic), 130.9 (2CH, aromatic), 120.6 (d, J = 4.6 Hz, 2CH, aromatic), 92.9 (**C**H═CH−N), 73.6 (d, J = 5.4 Hz, CHO), 72.9 (d, J = 8.5 Hz, CH_2OP), 70.7 (**C**H$(CH_3)_2$), 61.7 (d, J = 143.3 Hz, CH_2P), 53.7 ($CHNH_2$), 48.1 (CH_2N), 35.3 (CH_2 (Tyr)), 20.4 (CH_3), 20.3 (CH_3). ^{31}P NMR (162 MHz, CD_3OD): δ 11.79 (0.08P (S_P)), 10.27 (0.92P (R_P)). HRMS: m/z calcd 467.1700 $(M + H)^+$, found 467.1689 $(M + H)^+$. HPLC: t_R, 23.58 min (R_P), 24.05 (S_P).

***O*-[(5*S*)-5-[(6-Amino-9*H*-purin-9-yl)methyl]-2-oxido-1,4,2-dioxaphosphinan-2-yl]-*N*-(2-methylpropyl)-(L)-tyrosinamide (11′, Enriched by Isomer R_P).** Yield 73%. Obtained as a hydrochloride salt; mixture of two diastereomers (S_P/R_P, 1:9). Diastereomer R_P, ^{1}H NMR (400 MHz, CD_3OD): δ 8.34 (s, 1H, N═CH−N), 8.23 (s, 1H, N═CH−N), 7.32 (d, J = 8.8 Hz, 2H, aromatic), 7.21 (d, J = 8.8 Hz, 2H, aromatic), 4.63 (ddd, J = 17.6, 11.7, 2.0 Hz, 1H, C**H**$_a$H$_b$O), 4.52 (dd, J = 14.3, 3.2 Hz, 1H, C**H**$_a$H$_b$N), 4.48 (ddd, J = 11.3, 11.3, 0.9 Hz, 1H, CH_a**H**$_b$O), 4.41 (dd, J = 14.7, 7.4 Hz, 1H, CH_a**H**$_b$N), 4.38 (m, 1H, CHO), 4.35 (dd, J = 15.3, 11.2 Hz, 1H, C**H**$_a$H$_b$P), 4.10 (dd, J = 15.3, 1.2 Hz, 1H, CH_a**H**$_b$P), 4.03 (t, J = 7.7 Hz, 1H, C**H**NH_2), 3.17 (dd, J = 14.1, 7.6 Hz, 1H, C**H**$_a$H$_b$ (Tyr)), 3.12−3.05 (m, 2H, CH_a**H**$_b$ (Tyr) and C**H**$_a$H$_b$ (*i*-Bu)), 2.92 (dd, J = 13.2, 7.0 Hz, 1H, CH_a**H**$_b$ (*i*-Bu)), 1.69 (m, 1H, C**H**$(CH_3)_2$), 0.85 (d, J = 6.8 Hz, 3H, CH_3), 0.82 (d, J = 6.7 Hz, 3H, CH_3). ^{13}C NMR (100.6 MHz, CD_3OD): δ 168.2 (CO), 152.4 (C^6, adenine), 149.4 (C^4, adenine), 149.3 (d, J = 6.4 Hz, COP, aromatic), 146.8 (N═CH−N), 144.2 (N═CH−N), 132.2 (C, aromatic), 131.2 (2CH, aromatic), 120.8 (d, J = 4.6 Hz, 2CH, aromatic), 118.5 (C^5, adenine), 74.2 (d, J = 5.5 Hz, CHO), 73.2 (d, J = 8.6 Hz, CH_2O), 62.4 (d, J = 144.0 Hz, CH_2P), 54.5 ($CHNH_2$), 47.1 (**C**H_2CH$(CH_3)_2$), 43.2 (CH_2N), 36.8 (**C**$H_2C_6H_4$), 28.3 (**C**H$(CH_3)_2$), 19.3 ($2CH_3$). ^{31}P NMR (162 MHz, CD_3OD): δ 11.70 (0.10P (S_P)), 10.14 (0.90P (R_P)). HRMS: m/z calcd 504. 2119 $(M + H)^+$, found 504.2119 $(M + H)^+$. HPLC: t_R, 17.55 min (R_P), 18.26 (S_P).

Conversion of (L)-Tyr-NH-*i*-Bu cHPMPA (11) into (L)-Tyr-NH-*i*-Bu HPMPA (28) and cHPMPA (3). The TFA salt of **11** (0.2 mmol, 0.15 mg) was dissolved in 8 mL of 14.8 M NH_4OH. The reaction mixture was heated at 38 °C for 1 h (reaction process was monitored by LC−MS). Products were purified on a Varian Dynamax Microsorb 100-8 C_{18} HPLC column (5 μm, 41.4 mm × 250 mm) using a gradient of 0−30% ACN in ammonium acetate buffer, pH 5.5, with a flow rate of 80 mL/min. The products were detected at 260 nm and collected. Solvents were removed under reduced pressure, and the samples were lyophilized to give **28** and **3** as white powders.

***O*-[[[[(2*S*)-1-(6-Amino-9*H*-purin-9-yl)-3-hydroxypropan-2-yl]oxy]methyl](hydroxy)phosphoryl]-*N*-(2-methylpropyl)-(L)-tyrosinamide (28).** Yield 56%. ^{1}H NMR (400 MHz, CD_3OD): δ 8.17 (s, 1H, N═CH−N), 8.14 (s, 1H, N═CH−N), 7.09 (d, J = 8.4 Hz, 2H, aromatic), 7.01 (d, J = 8.4 Hz, 2H, aromatic), 4.44 (dd, J = 14.7, 3.9 Hz, 1H, C**H**$_a$H$_b$N), 4.37 (dd, J = 14.5, 6.8 Hz, 1H, CH_a**H**$_b$N), 3.86−3.62 (m, 5H, C**H**NH_2, C**H**$_a$**H**$_b$O, C**H**O, CH_a**H**$_b$P), 3.51 (dd, J = 12.5, 4.2 Hz, 1H, CH_a**H**$_b$P), 3.12−2.83 (m, 4H, C**H**$_2$(Tyr), C**H**$_2$ (*i*-Bu)), 1.73 (m, 1H, C**H**$(CH_3)_2$), 0.87 (d, J = 6.7 Hz, 3H, CH_3), 0.85 (d, J = 6.0 Hz, 3H, CH_3). ^{31}P NMR (202 MHz, CD_3OD): δ 13.25. HRMS: m/z calcd 522.2224 $(M + H)^+$, found 522.2224 $(M + H)^+$.

(5*S*)-5-[(6-Amino-9*H*-purin-9-yl)methyl]-1,4,2-dioxaphosphinan-2-ol 2-Oxide (3). Yield 35%. ^{1}H NMR (400 MHz, D_2O): δ 8.17 (s, 1H, N═CH−N), 8.14 (s, 1H, N═CH−N), 4.28−4.01 (m, 5H), 3.79 (dd, J = 14.1, 8.4 Hz, 1H), 3.54 (dd, J = 14.1, 2.3 Hz, 1H). ^{31}P NMR (202 MHz, D_2O): δ 8.98 (s).[37]

Stability Assays. Description of Analytical LC−MS Conditions. LC−MS analysis of compounds **6**−**13** was performed on a Varian Microsorb-MV C_{18} HPLC column (5 μm, 250 mm × 4.6 mm) with a 0−30% CH_3CN gradient in ammonium acetate buffer, pH 5.5, at a flow rate of 1.0 mL/min. The mass spectrometer equipped with an ESI source was operated in the positive-ion mode. MS parameters were optimized to the following: sheath gas (N_2) flow rate 20 arb, I spray voltage 5 kV, capillary temperature 275 °C, capillary voltage 35 V, tube lens offset 55 V. Full scan mass spectra were recorded over a range of m/z 200−600. The UV detector was operated at 274 or 260 nm for **2** or **1** derivative, respectively.

Chemical and Enzymatic Stability of Compounds 6−13. pH-Dependent Phosphate Buffer Stability. Compounds **6**−**13** were incubated in 200 mM phosphate buffer at pH 6.5 and 7.4 at 37 °C in a water bath. The incubation mixture was prepared by dissolving 1 mg/mL stock solution of compounds **6**−**13** in H_2O in preheated buffer solutions (1 mL). Aliquots of 25 μL were withdrawn at appropriate intervals and analyzed by LC−MS using conditions described in the previous section.

Stability of Prodrugs in Rat Tissue Intestinal Homogenates at pH 6.5. The enzymatic stability of compounds **6**−**13** was investigated by exposing the compound to tissue homogenates, obtained from intestine of Sprague−Dawley rats by the method described below.

The tissue sample was placed into a 50 mL conical tube into which 15 mL of 0.1 M phosphate buffer at pH 6.5 was added. The tissue was homogenized with an Omni International GLH homogenizer at a setting of 6 for 10 min. Aliquots of homogenate (1 mL) were transferred to microcentrifuge tubes, centrifuged at 14000*g* for 15 min, and stored at −80 °C until use. Total protein content was determined using a protein assay kit (BioRad).

The incubation mixture for enzymatic stability study of compounds **6**−**13** was prepared by dissolving 400 μL of 1 mg/mL stock solution in 260 μL of phosphate buffer at pH 6.5 and 140 μL of intestinal homogenate. The mixture was incubated at 37 °C in a water bath. Samples (25 μL) were drawn at appropriate intervals mixed with methanol (25 μL) and centrifuged (2 min at 6000 rpm). Aliquots of 25 μL of the supernatant liquid were analyzed by LC−MS using conditions described in the previous section

Transport Studies. (L)-Tyr-NH-*i*-Bu cHPMPA (**11**) was administered to mice intravenously or orally. Because of the sensitivity of **1** assay and the limited plasma volume of the mouse, one mouse represented one time point in these experiments. At predetermined time points after administration groups of 5 mice within the cohort were sacrificed. Blood samples were withdrawn by cardiac puncture, processed to recover the plasma, and stored at −20 °C. For the intravenous injection experiment mice (CFW Swiss−Webster) 4 weeks old and weighing 25 g were injected intravenously with drug sample in saline at a dose level of 1 mg equiv (of **1**)/kg. Plasma samples were collected at 0.25, 0.5, 1, 2, 4 h after injection. For the oral gavage experiment mice (CFW Swiss−Webster)

4 weeks old and weighing 25 g were fasted for 18 h with free access to water. Solution of drug sample in water was administered by gavage needle at a dose level of 10 mg equiv (of **1**)/kg. Plasma samples were collected at 1, 2, 3, 4, 8 h after administration of the oral dose. Systemic plasma concentrations of the administered prodrug **11** and metabolites were determined using LC−MS analysis as described below.

Samples for analysis were prepared on a cation exchange solid-phase cartridge (Varian Bond Elut SCX, 100 mg, 1 mL). A 200 μL aliquot of sample was combined with 100 μL of internal standard (IS) solution (62 nmol/mL (*S*)-HPMPC ethyl ester), acidified with 700 μL of 1% TFA, and loaded onto the activated SPE cartridge. The cartridge was washed with 1 mL of 1% TFA in water, 1 mL of methanol and then eluted with 2.5 mL of 5% NH_4OH in methanol. The solvent was evaporated at 40 °C under a gentle stream of N_2, and the residue was reconstituted in 100 μL of water and then analyzed by LC−MS.

LC−MS analysis was performed on a Varian Microsorb MV 100-3 HPLC column (5 μm, 50 mm × 4.6 mm) with a 0−30% CH_3CN gradient in ammonium acetate buffer, pH 5.5, at a flow rate of 0.4 mL/min. The injection volume was 100 μL. The mass spectrometer equipped with an ESI source was operated in the positive-ion mode. MS parameters were optimized to the following: sheath gas (N_2) flow rate 30 arb, I spray voltage 5 kV, capillary temperature 390 °C, capillary voltage 7 V, tube lens offset 15 V. Full scan mass spectra were recorded over a range of m/z 200−600. UV detector was operated at 260 nm.

Stock solutions of prodrug **11**, its metabolites **3** and **28**, and internal standard were prepared by dissolving the accurately weighed compounds in water. The standard solutions were then serially diluted to prepare calibration and QC samples. Calibration curves were constructed by plotting the ratio of the compound's response to the response of IS versus concentration of the calibration standards. The detection of prodrug **11** was performed by an indirect method based on the molecular peak of (*S*)-HPMPA dimethyl ester produced in ~85% yield as a result of the treatment of compound **11** in plasma sample with methanolic ammonia during SPE procedure.

The oral bioavailability (F) of the total **1**-containing species was calculated from the AUC of the oral dose divided by the AUC of the intravenous dose as follows: $F = [(AUC_o)/(dose_o)]/[(AUC_{iv})/(dose_{iv})]$.

Antiviral and Cytotoxicity Assays. Propagation of Cells and Virus. The routine growth and passage of KB cells were performed in monolayer cultures using minimal essential medium (MEM) with either Hanks salts [MEM(H)] or Earle salts [MEM(E)] supplemented with 5% fetal bovine serum. Cells were routinely enumerated with a Coulter counter model ZF equipped with 100 mm orifice. KB cells were plated at 1 × 10^5 cells/well using 24-well cluster dishes. The routine growth and passage of primary FFF (HFF) cells and methods for propagation and titration of virus have been previously described by Turk et al.[38] The plaque-purified P_0 isolate of the Towne strain of HCMV was kindly provided by Dr. Mark Stinski, University of Iowa. Vaccinia virus strain Copenhagen and cowpox virus strain Brighton stock pools were obtained from Dr. Earl R. Kern, University of Alabama at Birmingham. HSV-1 KOS strain was kindly provided by Dr. Sandra K. Weller, University of Connecticut. Viral pools were prepared in HFF cells and were diluted to provide working stocks. All viruses were titered using monolayer cultures of HFF cells.[39] Following incubation for 3 days (HSV-1 and poxviruses) or 10−12 days (HCMV), cells were fixed and stained with 0.1% crystal violet in 20% methanol and macroscopic plaques (herpes simplex and poxviruses) or microscopic plaques (HCMV) enumerated.

Assays for Antiviral Activity. The effects of compounds on the replication of all the viruses were measured using plaque reduction assays.[10,38] Briefly, for poxviruses, virus used was diluted in MEM containing 10% FBS to a desired concentration that would give ~50 plaques per well in six-well cluster plates. After a 1 h incubation period, an equal amount of 1% agarose was added to an equal volume of each drug dilution (100 μM and ending with 0.03 μM in a methocel overlay). The drug−methocel mixture was added, and the plates were incubated for 3 days, after which cells were stained with 0.1% crystal violet in 20% methanol. Similar techniques were used for HSV-1 and HCMV differing in that 100 plaques were used per well in 24-well cluster plates and incubation was approximately 3 days for HSV-1 and 10 days for HCMV. Drug effects were calculated as a percentage of the reduction in plaque number in the presence of each drug concentration compared to the numbers obtained in the absence of drug. Cidofovir ((*S*)-HPMPC, CDV, **2**) and ganciclovir (GCV) were used as positive controls in experiments with poxviruses and HCMV, respectively. Acyclovir (ACV) was used as a control for experiments with HSV-1. The 50% inhibitory concentrations (IC_{50}) were calculated from the regression lines using the methods described by Goldstein.[40] Samples containing positive controls were used in all assays.

Cytotoxicity Assays. Effects of all compounds on HFF cells used in plaque reduction assays were scored visually for cytotoxicity. Cytotoxicity to KB cell growth was tested using a colorimetric assay. In HFF cells, cytopathology was estimated at 20- to 60-fold magnification in areas of the assay plate not affected with virus infection and scored on a 0 to 4+ basis. Cells were scored on the day of staining. In KB cells, the effect of compounds during two population doublings of KB cells was determined by crystal violet staining and spectrophotometric quantization of dye eluted from stained cells as described earlier.[39] Briefly, 96-well cluster dishes were plated with KB cells at 5000 cells per well. After overnight incubation at 37 °C, test compound was added in triplicate at eight concentrations. Plates were incubated at 37 °C for 48 h in a CO_2 incubator, rinsed, fixed with 95% ethanol, and stained with 0.1% crystal violet. Acidified ethanol was added, and plates were read at 570 nm in a spectrophotometer designed to read 96-well ELISA assay plates. Dose−response relationships were constructed by linearly regressing the percent inhibition of parameters derived in the preceding sections against log drug concentrations. The 50% inhibitory concentrations were calculated from the regression lines using the methods described by Goldstein.[40]

■ ASSOCIATED CONTENT

Supporting Information. NMR spectra of the intermediates and the prodrugs; purity data, HPLC traces, HRMS spectra of the prodrugs; and stability profiles of **11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

(*S*)-HPMPC, 1-(*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl]-cytosine; (*S*)-HPMPA, 1-(*S*)-[3-hydroxy-2-(phosphonomethoxy) propyl]adenine; HPMP, 3-hydroxy-2-(phosphonomethoxy)propyl; ANP, acyclic nucleoside phosphonate; POM, pivaloyloxymethyl; SATE, *S*-acylthioethyl; HDP, hexadecyloxypropyl; HCMV, human cytomegalovirus; HSV-1, herpes simplex virus type 1; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HOBt, *N*-hydroxybenzotriazole; DIEA, diisopropylethylamine; PyBOP, benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate; RP, reverse phase;

DKP, diketopiperazine; ACV, acyclovir; GCV, ganciclovir; SPE, solid phase extraction; MEM, minimal essential medium

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