Journal of Medicinal Chemistry

ARTICLE

pubs.acs.org/jmc

Dipeptidyl Peptidase IV Dependent Water-Soluble Prodrugs of Highly Lipophilic Bicyclic Nucleoside Analogues

Alberto Diez-Torrubia,[†] Jan Balzarini,[‡] Graciela Andrei,[‡] Robert Snoeck,[‡] Ingrid De Meester,[§] María-José Camarasa,[†] and Sonsoles Velázquez*[,†]

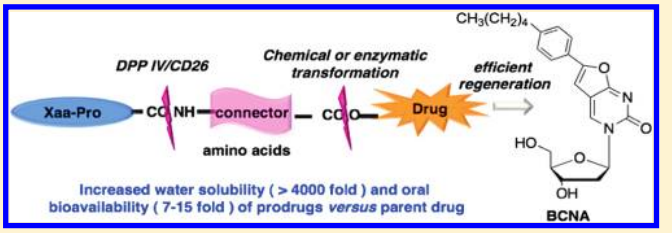
[†]Instituto de Química Médica (C.S.I.C.), Juan de la Cierva 3, E-28006 Madrid, Spain

[‡]Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

[§]Laboratory of Medical Biochemistry, University of Antwerp, Universiteitsplein 1, B-2610 Antwerp, Belgium

Supporting Information

ABSTRACT: We present the first report of the application of the dipeptidyl peptidase IV (DPPIV/CD26) based prodrug approach to hydroxy-containing drug derivatives. In particular, we applied this strategy to the highly lipophilic antiviral drug family of bicyclic furanopyrimidine nucleoside analogues (BCNA) in order to improve their physicochemical and pharmacokinetic properties. Our stability data demonstrated that the prodrugs efficiently release the parent BCNA drug upon selective conversion by purified DPPIV/CD26 and by soluble DPPIV/CD26 present in bovine, murine, and human serum. Vildagliptin, a specific inhibitor of DPPIV/CD26, was able to completely block the hydrolysis of the prodrugs in the presence of purified DPPIV/CD26 human, murine, and bovine serum. Several novel prodrugs showed remarkable increases in water solubility (up to more than 3 orders of magnitude) compared to the poorly soluble parent drug. We also demonstrated a markedly enhanced oral bioavailability of the prodrugs versus the parent drug in mice.



INTRODUCTION

Dipeptidyl-peptidase IV (DPPIV/CD26) belongs to a unique class of membrane-associated peptidases and is identical to the lymphocyte surface glycoprotein CD26.[1] It is endowed with an interesting (dipeptidyl) peptidase catalytic activity, and it has high hydrolytic selectivity for natural peptides carrying a proline, or to a lesser extent an alanine, at the penultimate position of the N-terminus end. Instead, it tolerates a much wider range of amino acid residues at the N-terminal end. A free, nonsubstituted amino group on the ultimate (amino terminal) amino acid position is one of the prerequisites for substrate recognition by the enzyme.[2−4] The enzyme is not only expressed on a variety of leukocyte cell subsets but also on several types of epithelial, endothelial, and fibroblast cells. A soluble form of the enzyme is detected in plasma and cerebrospinal fluid at low amounts.[5,6]

In 2005, it was shown, for the first time, that a synthetic small molecule [GPG-NH_2 (glycyl-prolyl-glycynamide)] can be converted to an antiviral drug through the specific action of DPPIV/CD26.[7] This was the first demonstration that a differentiation/activation leukocytic enzyme marker acts as a highly specific and obligatory activator of a synthetic anti(retro)viral prodrug that is otherwise inactive as such. On the basis of this study, we previously reported[8,9] a novel type of prodrug approach that could be applied to improve the solubility and formulation properties of therapeutic agents. In our approach a di- (or oligo)peptide moiety was linked to a free amino group of a non-peptide drug through an amide bond, which is specifically cleaved by the endogenous DPPIV/CD26 activity (Figure 1).

For proof-of-concept of this novel prodrug technology, we focused on the lipophilic TSAO derivatives[10−12] that potently inhibit HIV-1 replication. This lentivirus infects lymphocytes that abundantly express DPPIV/CD26 in their membrane. In particular, the N-3 aminopropyl TSAO-T derivative was chosen as model compound because its primary amine functionality would allow the formation of an amide bond between the prodrug peptide moiety and the TSAO molecule. A variety of dipeptidyl and tetrapeptidyl amide prodrugs of NAP-TSAO-T derivatives deprotected at the peptide N-terminus were synthesized and studied. Our data revealed that purified DPPIV/CD26 could specifically recognize these prodrugs as efficient substrates to be converted to the parent compound. Interestingly, it was possible to modify the enzymatic and serum hydrolysis rate (half-life) of the prodrug conjugates by changing the nature and the length of the peptide promoiety.[8,9]

We recently extended the applicability of this prodrug approach to a variety of amine-containing drugs.[13] Our studies demonstrated that XaaPro dipeptides linked to a free amino group present on an aromatic ring [6-aminoquinoline (6-AQ)] or on a sugar entity (doxorubicin) behave as prodrugs that efficiently release the parent drug upon conversion by purified DPPIV/CD26 and soluble DPPIV/CD26 present in bovine

Received: December 22, 2010
Published: February 18, 2011

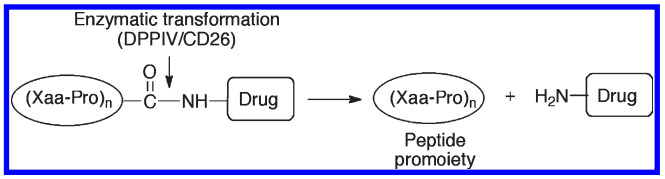


Figure 1. [(Xaa-Pro)$_n$]-[drug] conjugates cleavable by DPPIV/CD26.

and human serum. When the amino group is present on a heterocyclic pyrimidine or purine ring (i.e., cytarabine or vidarabine), the dipeptide derivatives are chemically unstable, whereas the tetrapeptide derivatives were much more stable in solution and efficiently converted to the parent drug by the action of DPPIV/CD26. Thus, our studies revealed that a wide variety of drug types containing a free amino moiety on an aromatic, carbohydrate, or heterocyclic pyrimidine or purine ring could be derivatized with di- or tetrapeptides to become efficient substrates for the dipeptidyl peptidase activity of DPPIV/CD26.[13] Vildagliptin, a specific inhibitor of DPPIV/CD26, was able to completely block the hydrolysis of the prodrugs in the presence of purified CD26 but also in human and bovine serum. Furthermore, we could demonstrate that the 6-AQ and vidarabine peptide prodrugs exhibited a considerably higher water solubility than the parent compounds.[13]

On the basis of these promising results with amino-containing drugs, we now successfully expand for the first time our prodrug strategy to hydroxy-containing drug derivatives. In amine-containing drugs, the peptide promoiety is attached directly to the amino group of the drug through an amide bond that is specifically cleaved by DPPIV/CD26 (Figure 1). In hydroxy-containing drugs, however, this is not possible, and for the design of these novel prodrugs a heterobifunctional connector is required between the peptide promoiety and the hydroxyl group of the drug. As connector in the proposed conjugates (general formula **I**, Figure 2), an amino acid was chosen to conjugate via its amine function to the acid end of the dipeptide and via its acid function to the OH group of the drug through a metabolically labile ester linkage. Thus, the release of the parent drug from the designed novel prodrugs would involve a two-step mechanism: (1) DPPIV/CD26-mediated hydrolysis to release an amino acid ester of the drug and (2) subsequent chemical or enzymatic cleavage of the ester bond of this intermediate resulting in the formation of the free parent drug and the connector.

As hydroxy-containing drugs, in particular, we wanted to apply this strategy to bicyclic furanopyrimidine nucleoside analogues (BCNAs) which represent a family of highly lipophilic antivirals, which display high inhibitory potency and unusual selectivity against varicella zoster virus (VZV).[14−16] The most potent anti-VZV prototype compound is the *p*-pentylphenyl BCNA analogue Cf1743[15] (**1**) (Figure 2), which exhibits activity against a broad range of VZV isolates at low nanomolar concentrations and little or no detectable toxicity at micromolar concentrations. However, the clinical potential of this highly lipophilic compound is mainly limited by its low oral bioavailability. Moreover, this compound has a poor aqueous solubility. Formulation-based approaches were successful in improving the solubility of **1** (Cf1743) but did not have a significant impact on oral pharmacokinetics.[17] To overcome this problem, a prodrug approach was successfully designed. The most promising prodrug that emerged from these studies proved to be the HCl salt of the 5′-valyl ester, designated as FV-100, which was endowed with a promising stability profile and a significant enhancement of

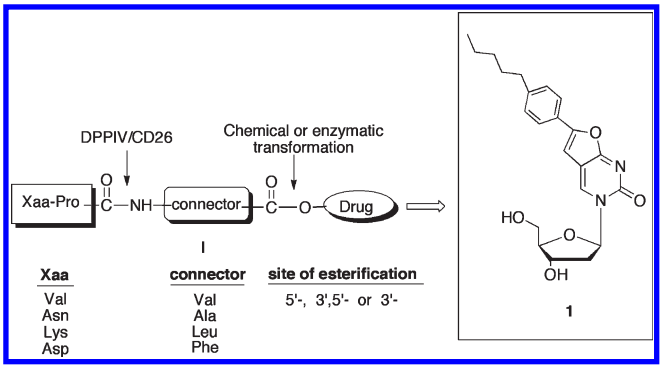


Figure 2. Design of DPPIV/CD26-cleavable prodrugs of the BCNA compound **1** of general formula **I**.

both water solubility and oral bioavailability.[17,18] This compound is currently subject of phase II clinical trials.

In the present study, we report the application of the DPPIV/CD26-based prodrug approach to the prototype BCNA drug **1** in order to improve its physicochemical and pharmacokinetic properties. The design and synthesis of a broad variety of water-soluble prodrugs of **1** (Figure 2) and evaluation of the pharmacokinetic properties of the prodrugs including chemical and enzymatic stability (cleavage rates), water solubility, and oral pharmacokinetics have been investigated. The results of our studies on the effect of the site of esterification, the number of peptide promoieties, and the influence of the nature of the peptide promoiety on the rate of hydrolysis and pharmacokinetics of these novel prodrugs are now reported. Several of the synthesized prodrugs combine high aqueous solubility and stability with an efficient and selective conversion of the prodrug to the biologically active parent compound in the presence of DPPIV/CD26. Moreover, plasma levels of **1** after oral administration of selected prodrugs to mice proved markedly higher than their plasma levels obtained after oral administration of the parent drug. Therefore, the data obtained for the synthesized prodrugs of the highly insoluble and poor bioavailable BCNA analogue **1** may form the basis and rationale for the synthesis of similar prodrug types of molecules that suffer from poor solubility and/or unfavorable pharmokinetic (i.e., poor bioavailability) properties.

RESULTS AND DISCUSSION

Chemistry. Our initial efforts focused on the preparation of the model thymidine prodrug **6** (Scheme 1) to explore the viability of the prodrug approach in hydroxy-containing drugs. In this model system, the dipeptide ValPro sequence (efficiently recognized by DPPIV/CD26 in natural peptides[2,3] and in previous [XaaPro]-[drug] conjugates[8,13]) is attached to the hydroxyl group of the 5′-position of the nucleoside through L-valine as the connector. L-Valine was first chosen as connector, as it has been successfully used as a promoiety to enhance its intestinal absorption by the h-PEPT1 transporter (e.g., valacyclovir and valgancyclovir).[19−21] The synthesis of the proposed conjugate involved a four-step procedure: (i) regioselective acylation of the 5′-OH position of the nucleoside with conveniently protected R-Val-OH, (ii) deprotection of the amino group of the amino acid ester intermediate of the nucleoside, (iii) coupling of the 5′-Val-thymidine intermediate with the commercially available dipeptide R-Val-Pro-OH (formation of

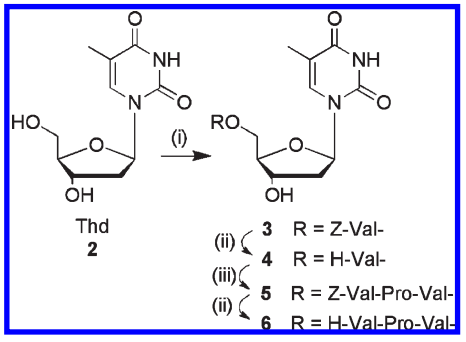
a N-protected tripeptide conjugate), and finally (iv) N-deprotection of the tripeptide ester of the nucleoside.

Initial attempts of coupling of thymidine (**2**) with Z-Val-OH using conventional coupling method (DCC/DMAP) gave a mixture of 3′-, 5′-, and 3′, 5′-di-*O*-valyl ester thymidine derivatives (data not shown). The regioselective coupling of amino acids on the 5′ position of a nucleoside via Mitsunobu condensation[22,23] was next explored. Thus, condensation of thymidine (**2**) with Z-Val-OH in the presence of Ph_3P and DIAD in dry THF at room temperature overnight under argon afforded exclusively the 5′-valyl nucleoside **3** in 49% yield. However, repeated chromatographic purifications were required to separate the desired material from the byproduct triphenylphosphine oxide and the reduced azodicarboxylate. Improved procedures for the Mitsunobu coupling reaction that facilitate purification of the desired products have been previously described.[24−26] Among these, the combined use of polymer-supported triphenylphosphine (PS-PPh_3) and the acid-labile di-*tert*-butyl azodicarboxylate (DBAD) allows the destruction/removal of reagent byproduct through simple filtration and evaporation after an acid workup.[26] By use of this combination of reagents, thymidine was coupled with Z-Val-OH in dry THF at room temperature overnight. The crude mixture was then treated with 4 M HCl in dioxane for 1 h, filtered, and washed with aqueous HCl. The 5′-valyl ester intermediate **3** was isolated in 86% yield after purification by short flash column chromatography. Catalytic hydrogenation of **3** in the presence of 10% Pd/C in methanol afforded the N-deprotected valyl ester nucleoside derivative **4** in 93% yield. Coupling of **4** with Z-Val-Pro-OH in the presence of BOP and TEA yielded the N-protected tripeptide ester conjugate **5** in moderate yield (44%). Removal of the benzyl groups by catalytic hydrogenation of **5** in the presence of 10% Pd/C in methanol afforded the desired model prodrug **6** in good yields (90%).

Next, the regioselective synthesis of the 5′-Val-Pro-Val prodrug **13** (Scheme 2) was carried out in a way similar to that described for the model thymidine conjugate **6**. Thus, reaction of the parent drug **1**, which was synthesized by the previously described method,[17] with Z-Val-OH under the above Mitsunobu mentioned conditions (PS-PPh_3 and DBAD) gave the 5′-valyl ester intermediate **7** in 66% yield. It is noted that acidic workup with aqueous HCl could not be used in this case because such acidic conditions led to partial base cleavage. Catalytic hydrogenation of **7** (H_2, Pd/C) followed by coupling Z-Val-Pro-OH under standard conditions (BOP and TEA) gave a mixture of the desired tripeptide ester conjugate **8** in low yield (27%) together with the unexpected acyclic derivative **9** (32% yield). Ring-opening of the furan ring and subsequent reduction of the double bond at the C-5 substituent under the catalytic hydrogenation conditions could explain formation of compound **9**. Scarce ring-opening reactions of furanopyrimidine nucleoside derivatives with nucleophiles have been previously described.[27−29] The absence of fluorescence in the unexpected product **9** clearly indicated that the product did not contain a bicyclic base moiety. Its structure was unequivocally assigned on the basis of its spectroscopic and analytical data. The ^1H NMR spectrum for **9** showed the disappearance of the characteristic signals of the furanopyrimidine moiety (two singlets at δ 7.18 and 8.71 ppm assigned to H-4 and H-5) and the presence of two new singlets (one broad singlet at 10.08 ppm and a narrow one at 7.20 ppm corresponding to the NH-3 and H-6) and new multiplets between 2.5 and 2.8 ppm corresponding to the new -CH_2CH_2- system at the C-5 substituent.

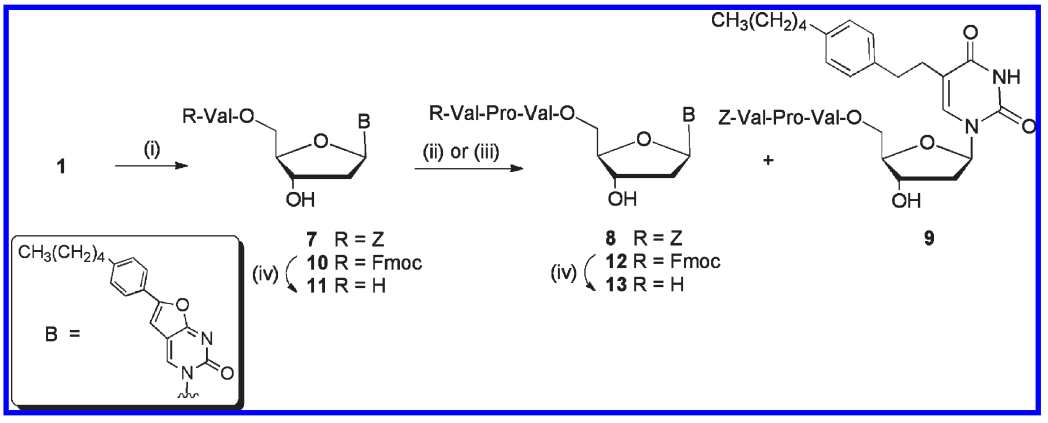
An alternative Fmoc strategy for the synthesis of the target prodrug **13** is depicted in Scheme 2. Thus, coupling of **1** with Fmoc-Val-OH under the above-mentioned optimized Mitsunobu conditions followed by reaction with piperidine in DMF

Scheme 1. Synthesis of 5′-Val-Pro-Val Thymidine Model Prodrug 6[a]



[a] Reagents: (i) (a) Z-Val-OH, DBAD, PS-PPh_3, THF; (b) 4 M HCl in dioxane; (ii) H_2, Pd/C, HCl (cat.), CH_3OH; (iii) Z-Val-Pro-OH, BOP, TEA, CH_2Cl_2.

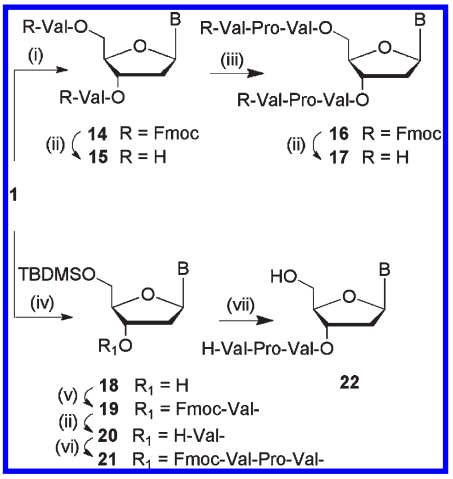
Scheme 2. Synthesis of 5′-Val-Pro-Val Prodrug 13[a]



[a] Reagents: (i) Z-Val-OH or Fmoc-Val-OH, PS-PPh_3, DBAD, THF; (ii) (a) H_2, Pd(C), HCl (cat.), CH_3OH; (b) Z-Val-Pro-OH, BOP, TEA, CH_2Cl_2; (iii) (a) piperidine 10%, DMF; (b) Fmoc-Val-Pro-OH, BOP, TEA, CH_2Cl_2; (iv) piperidine 10%, DMF.

dx.doi.org/10.1021/jm101624e |*J. Med. Chem.* 2011, 54, 1927–1942

Scheme 3. Synthesis of 3′,5′-Val-Pro-Val and 3′-Val-Pro-Val Prodrugs 17 and 22[a]

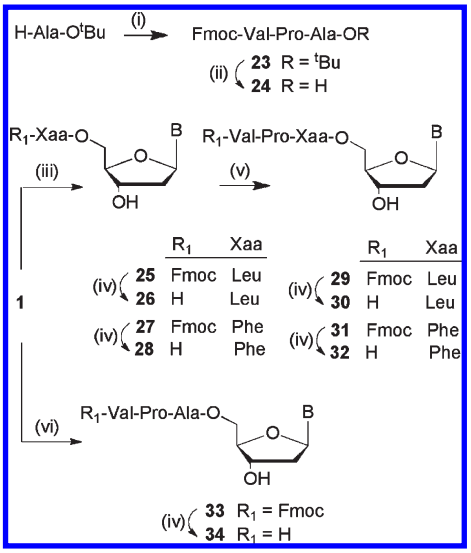


[a] Reagents: (i) Fmoc-Val-OH, DCC, DMAP, DMF; (ii) piperidine 10%, DMF; (iii) Fmoc-Val-Pro-OH, BOP, TEA, CH_2Cl_2; (iv) TBDMSCl, Py; (v) Fmoc-Val-OH, DCC, DMAP, DMF; (vi) Fmoc-Val-Pro-OH, BOP, TEA, CH_2Cl_2; (vii) (a) HCl 0.1 N, MeOH; (b) piperidine 10%, DMF.

for 5 min gave the 5′-valyl nucleoside derivative (**11**)[17] in good yields after chromatographic purification. Intermediate **11** was then reacted with Fmoc-Val-Pro-OH in the presence of BOP and TEA to give the N-protected tripeptide ester conjugate **12** in 74% yield. Final removal of the Fmoc group by brief treatment of **12** with piperidine afforded the desired final deprotected prodrug **13** in 89% yield. The biological results (see below) obtained with the prodrug **13** encouraged us to prepare novel series of Cf1743 prodrugs.

To evaluate the effect of the site of esterification on the rate of hydrolysis and pharmacokinetic properties of the prodrugs, the synthesis of the 3′,5′-diester and 3′-monoester prodrugs **17** and **22** was next investigated (Scheme 3). Reaction of **1** with an excess of Fmoc-Val-OH (3 equiv) under standard coupling conditions (DCC/DMAP) in dry DMF afforded the protected 3′,5′-di-*O*-valyl ester intermediate **14** in 70% yield after chromatographic purification. The Fmoc groups were deprotected by treatment with piperidine to give the N-deprotected valyl intermediate **15** in excellent yield which was finally converted into the target 3′,5′-di-*O*-tripeptide ester derivative **17** (Scheme 3) in good yield by coupling with Fmoc-Val-Pro-OH in the presence of BOP/TEA followed by N-deprotection with piperidine. The synthesis of the 3′-monoester prodrug **22** was carried out in a way similar to that described for the corresponding 5′-monoester prodrug **13** starting from the 5′-silyl protected derivative **18** (Scheme 3). Thus, reaction of **1** with TBDMSCl in anhydrous pyridine at room temperature for 48 h provided the 5′-silyl protected nucleoside **18** in 63% yield after flash column chromatography. Condensation of **18** with Fmoc-Val-OH in the presence of DCC/DMAP followed by treatment with piperidine yielded the N-deprotected 3′-valyl ester intermediate **20** in good yield. Coupling of **20** with Fmoc-Val-Pro-OH under standard conditions (BOP/TEA) provided the tripeptide ester prodrug **21** in 80% yield. Finally, treatment of intermediate **21** with 0.1 N HCl in MeOH (5′-silyl deprotection) followed by reaction with piperidine (Fmoc removal) gave the fully deprotected 3′-tripeptide target conjugate **22** in good yields (Scheme 3).

Scheme 4. Synthesis of 5′-Val-Pro-Xaa Prodrugs 30, 32, and 34 Modified at the Connector Xaa Residue[a]

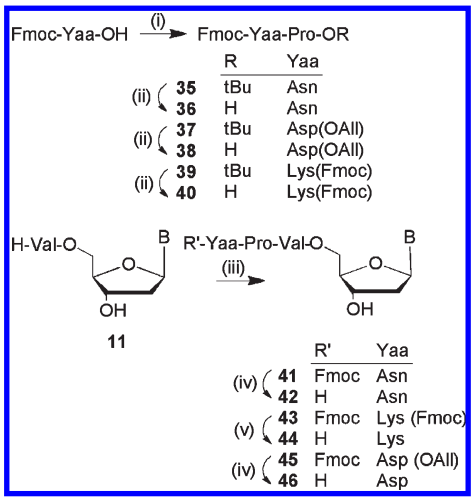


[a] Reagents: (i) Fmoc-Val-Pro-OH, BOP, TEA, CH_2Cl_2; (ii) TFA, CH_2Cl_2; (iii) Fmoc-Xaa-OH, PS-PPh_3, DBAD, THF; (iv) piperidine 10%, DMF; (v) Fmoc-Val-Pro-OH, BOP, TEA, CH_2Cl_2; (vi) Fmoc-Val-Pro-Ala-OH (**24**), PS-PPh_3, DBAD, THF.

A novel series of prodrugs (compounds **30**, **32**, and **34**, Scheme 4) was next prepared where the valine used as connector was replaced by different natural amino acids (Xaa) with hydrophobic (Ala, Leu) or aromatic residues (Phe). The synthesis of prodrugs **30** and **32** (Scheme 4) was carried out following a four-step procedure similar to that described for the synthesis of prodrug **13**. Thus, condensation of **1** with the appropriate Fmoc-Xaa-OH under the above-mentioned Mitsunobu conditions (PS-PPh_3 and DBAD) provided the protected amino acid ester intermediates **25** and **27** in 53% and 56% yield, respectively, that were subsequently reacted with piperidine to yield the unprotected amino acid ester intermediates **26** and **28** in excellent yields. Coupling of intermediates **26** and **28** with Fmoc-Val-Pro-OH in the presence of BOP and TEA followed by removal of the Fmoc group with piperidine afforded the desired final deprotected conjugates **30** and **32** in good yields. For the synthesis of the corresponding conjugate **34**, condensation of **1** with Fmoc-Ala-OH·H_2O was first attempted using similar Mitsunobu conditions, but only starting material was recovered. As an alternative, **1** was directly coupled with Fmoc-Val-Pro-Ala-OH **24** (previously prepared using standard coupling/deprotection protocols) under the optimized Mitsunobu conditions (PS-PPh_3 and DBAD). Although longer reaction times were needed in this case (6 days), the desired protected tripeptide prodrug **33** could be obtained in 58% yield using this strategy. Further treatment of **33** with piperidine afforded the fully deprotected tripeptide ester derivative **34** in 74% yield.

As will be discussed below, it appears that the chemical stability of the ester bond in the prodrugs is greatly influenced by the nature of the amino acid used as connector. The highest chemical stability, together with an adequate activation profile of the prodrug bearing a Val residue as the connector (compound **13**) compared to the prodrugs **30**, **32**, and **34** (bearing a Leu, Phe, and Ala residue, respectively), prompted us to maintain the Val residue as connector for the subsequent modifications of prodrugs of Cf1743.

Scheme 5. Synthesis of 5′-Yaa-Pro-Val Prodrugs 42, 44, and 46 Modified at the N-Terminal Residue of the Peptide Promoiety[a]



[a] Regents: (i) H-Pro-O^{t}Bu, BOP, TEA, CH_2Cl_2; (ii) TFA, CH_2Cl_2; (iii) Fmoc-Yaa-Pro-OH, BOP, TEA, CH_2Cl_2; (iv) piperidine 10%, DMF; (v) (a) $Pd(PPh_3)_4$, $PhSiH_3$, CH_2Cl_2; (b) piperidine 10%, DMF.

Finally, a novel series of prodrugs (compounds **42**, **44**, and **46**, Scheme 5) was prepared to diversify the half-life and to study the physicochemical and pharmacokinetic properties of these prodrugs. In these conjugates the Val linker was maintained but the N-terminal Val residue was changed by polar residues such as Asn (compound **42**), Lys (compound **44**), and Asp (compound **46**). It is noted that the selected dipeptide sequences YaaPro were also recognized by DPPIV/CD26 in natural peptides[2,3] and in previous [YaaPro]-[drug] conjugates[8] with different conversion rates to the parent compounds. The synthesis of compounds **42**, **44**, and **46** (Scheme 5) was carried out in a way similar to that described for prodrug **13** starting from the appropriate dipeptide derivatives Fmoc-Yaa-Pro-OH and the 5′-valyl nucleoside derivative **11**. Noncommercially available dipeptides Fmoc-Asn-Pro-OH (**36**), Fmoc-Asp-(OAll)-Pro-OH (**38**), and Fmoc-Lys-(Fmoc)-Pro-OH (**40**) were prepared using standard peptide coupling/deprotection procedures. Next, the appropriate dipeptide derivatives Fmoc-Yaa-Pro-OH (**36**, **38**, and **40**) were reacted with the 5′-valyl nucleoside derivative **11** in the presence of BOP and TEA to give the protected intermediate conjugates **41**, **43**, and **45** in good yields (72−82%). Removal of the Fmoc groups in the presence of piperidine in DMF afforded the desired final deprotected prodrugs **42** and **44** in good yields. For the synthesis of the corresponding conjugate **46**, simultaneous deprotection of the aspartic allyl ester group and the base labile Fmoc was first attempted by using a mild palladium(0)-catalyzed allyl transfer to various amines (such as morpholine or pyrrolidine) as the accepting nucleophile.[30,31] However, treatment of protected prodrug **45** in the presence of catalytic amounts of $Pd(PPh_3)_4$ and morpholine in dry THF under an argon atmosphere at room temperature for 1 h provided low yields of the desired fully deprotected conjugate **46** because of ring-opening of the furan ring of the bicyclic heterocyclic base of the nucleoside as determined by HPLC−MS analysis. Among the wide variety of allyl group scavengers that have been previously described, phenyltrihydrosilane ($PhSiH_3$) was next selected as a neutral allyl group scavenger.[32] Thus, palladium catalyzed deprotection of prodrug **45** in the presence of a catalytic amount of $Pd(PPh_3)_4$ and $PhSiH_3$ as hydride donor (acting as allyl group scavenger) in dry CH_2Cl_2 at room temperature for 1 h, followed by brief treatment with piperidine, afforded the desired deprotected conjugate **46** in 83% yield.

The target prodrugs were characterized by ^1H and ^{13}C NMR, mass spectrometry, and microanalysis, all data confirming the structure and purity of the novel compounds.

Aqueous Solubility and Chemical Stability. Aqueous drug solubility has long been recognized as a key molecular property in pharmaceutical science and is important for efficient drug delivery, transport, and biodistribution. The aqueous solubilities of the DPPIV-dependent prodrugs of compound **1** were measured at 25 °C (Table 1). Most of the compounds were highly stable in PBS buffer at pH 7.4 (>95% intact prodrug after 24 h at room temperature, Table 1), and thermodynamic solubilities have been measured after 24 h. However, the prodrugs **30**, **32**, and **34** in which the valine residue connector was replaced by other amino acids (Leu, Phe, or Ala, respectively) showed markedly lower chemical stability (52−74% intact prodrug after 24 h in PBS) (Table 1). For this reason, their aqueous solubility was measured after 4 h to minimize the error due to instability of these derivatives. As shown in Table 1, all novel prodrugs showed dramatic increases in water solubility compared to the sparingly soluble parent drug solubility. Values ranged from 1.1 to 77 mg/mL, which correspond to a 64- to 4.294-fold higher water solubility than that of the parent drug **1**. No close correlation was observed between the water solubility values and the structure of the prodrugs or the ClogP values. In general, prodrugs **42**, **44**, and **46** containing polar N-terminal residues (capable of hydrogen bonding with water molecules) such as Asn, Lys, and Asp, respectively, ranked among the most water-soluble compounds (2.186-fold, 3.061-fold, and 838-fold solubility increases, respectively). However, the 3′,5′-diester prodrug **17** bearing twice the less polar ValProVal peptide promoiety showed the highest increase in solubility compared with the parent drug (4.294-fold increase) (Table 1).

Biological Studies. *Anti-VZV Activity of Peptide Prodrugs in HEL Cell Cultures.* The variety of peptide prodrugs has been evaluated for their anti-VZV activity in HEL cell cultures. The parent compound **1** inhibited VZV-induced cytopathicity at an EC_{50} of 0.003 μM (both YS and OKA strains) (Table 2). The 5′-Val-Pro-Xaa prodrugs **30**, **32**, and **34** inhibited VZV within the same order of magnitude as the parent drug (EC_{50}: 0.003−0.013 μM). Also, the 5′-Yaa-Pro-Val prodrugs **42**, **44**, and **46** showed substantial antiviral activity as well as the 5′-, 3′-, and 3′,5′-Val-Pro-Val prodrugs (**13**, **17**, and **22**). These findings indicate that the peptide prodrugs of compound **1** are invariably efficiently converted to the parent drug in the virus-infected cell cultures (Table 2) over the course of the antiviral assays (∼5 days).

Enzymatic Stability Studies of Peptide Prodrugs. In the first series of experiments, the 5′-, 3′-, and 3′,5′-Val-Pro-Val prodrugs (compounds **13**, **22**, and **17**) were exposed to purified DPPIV/CD26, 10% human serum (HS), or 10% bovine serum (BS) for different time periods (up to 24 h). Exposure to purified DPPIV/CD26 revealed hydrolysis of the prodrugs to the corresponding valine derivatives, followed by further conversion to the parent compound. The latter observations point to a certain instability of the valine (5′-, 3′-, or 3′,5′-) derivative of compound **1** (spontaneous conversion to parent compound). Exposure of these compounds to HS or BS revealed the appearance of the same type of metabolites but often a more extensive conversion

Table 1. Aqueous Solubility and Chemical Stability of 5′-Yaa-Pro-Xaa Prodrugs and Parent Compound 1

compd	Yaa	Xaa (connector)	site of esterification	ClogP [a]	aqueous solubility (mg/mL) [b]	fold increase of solubility [c]	chemical stability (% remaining at 24 h) [d]
1 (parent compound)				2.48	0.018		
13	Val	Val	5′	2	1.2	68	>95
17	Val	Val	3′, 5′	1.77	77	4294	>95
22	Val	Val	3′	1.97	5.2	290	>95
30 [e]	Val	Leu	5′	2.12	14	766	69
32 [e]	Val	Phe	5′	2.53	1.1	64	52
34 [e]	Val	Ala	5′	1.63	6.1	337	74
42	Asn	Val	5′	1.06	39	2186	>95
44	Lys	Val	5′	0.41	55	3061	>95
46	Asp	Val	5′	1.3	15	838	>95

[a] ClogP values were obtained by using the ALOGPS 2.1 program (www.vcclab.org/lab/alogps/). [b] Thermodynamic water solubilities measure at 25 °C after 24 h. Values are typically the mean of two measurements, with variations of <20%. [c] Fold increase in water solubility relative to the parent drug **1**. [d] Chemical stability in PBS buffer at pH 7.4 as determined by HPLC after 24 h at room temperature. [e] Aqueous solubility was determined after 4 h instead of 24 h because of the lower chemical stability of these prodrugs.

Table 2. Anti-VZV Activity of Peptide Prodrugs in HEL Cell Cultures

	EC_{50} [a] (μM)			
compd	VZV (YS)	VZV (OKA)	CC_{50} [b] (μM) (HEL)	MCC [c] (μM) (HEL)
1: parent compound	0.004	0.003	≥100	>20
13: 5′-Val-Pro-Val prodrug	0.154	0.089	>100	>2
17: 3′,5′-di-Val-Pro-Val prodrug	0.260	0.127	50	>5
22: 3′-Val-Pro-Val prodrug	0.016	0.018	17	60
30: 5′-Val-Pro-Leu prodrug	0.006	0.009	31	>0.5
32: 5′-Val-Pro-Phe prodrug	0.010	0.013	40	>0.5
34: 5′-Val-Pro-Ala prodrug	0.003	0.006	44	>0.5
42: 5′-Asn-Pro-Val prodrug	0.085	0.042	56	>5
44: 5′-Lys-Pro-Val prodrug	0.022	0.018	29	>5
46: 5′-Asp-Pro-Val prodrug	0.073	0.032	52	>5

[a] 50% effective concentration or compound concentration required to inhibit VZV-induced cytopathicity by 50%. [b] 50% cytostatic concentration or compound concentration required to inhibit HEL cell proliferation by 50%. [c] Minimal cytotoxic concentration or compound concentration required to afford a microscopical alteration of HEL cell morphology.

to parent compound **1** (presumably due to the presence of hydrolytic enzymes in the sera) (Figure 3a). It is noted that in the HPLC chromatograms of especially 3′,5-di-Val-Pro-Val prodrug **17** (Figure 3a), one more metabolite eluting slightly later than the prodrug and parent compound **1** appears in function of time. It is rather minor in quantity and may represent one or more intermediates that may eventually appear upon conversion of the prodrug to the parent compound. In the second series of experiments, three 5′-Yaa-Pro-Val prodrug derivatives (**42**, **44**, and **46**) were investigated (Figure 3b). Although all of them were converted to the 5′-valyl intermediate and eventually to the parent compound **1**, the 5′-Asp-Pro-Val **46** was clearly less efficiently hydrolyzed by DPPIV/CD26, an observation that was most evident in the HS and BS samples. This higher stability is in agreement with literature data and our earlier observations that [Asp-Pro]-based prodrugs are less good substrates for CD26 than [Val-Pro]- or [Lys-Pro]-based prodrugs.[2,8] In a third series of experiments, three 5′-Val-Pro-Xaa prodrugs (**30**, **32**, and **34**) were investigated (Figure 3c). These prodrugs, although less stable in PBS than the other compounds mentioned above, were more efficiently converted to the parent drug than the 5′-Yaa-Pro-Val prodrug series.

*Hydrolysis of Peptide Prodrugs of Compound **1** in the Presence of a Specific Inhibitor of DPPIV/CD26.* Compounds **6** and **13** (5′-Val-Pro-Val thymidine and compound **1** prodrugs) have been subjected to conversion studies upon exposure to purified DPPIV/CD26 or human, bovine, and murine (only for **13**) serum (20% in PBS) in the presence of varying concentrations of vildagliptin, a selective and potent DPPIV/CD26 inhibitor (Figure 4, panels a and b). Human serum was used to enable comparison of the DPPIV/CD26-hydrolytic activity with the other sera included in our study and thus showed the relevance of the obtained data in animal sera to the human situation. Bovine serum was used to enable correlation with the activity of the compounds in cell culture, since the cell culture medium used for the antiviral experiments contained 10% bovine serum. Murine serum was used to enable correlation with the In Vivo (mice) data and to ascertain that the data obtained in murine serum (known to contain an abundant amount of proteases) were comparable to those obtained in the other (especially human) sera. The tripeptide prodrugs were efficiently and time-dependently converted to the 5′-Val intermediates, respectively. Interestingly, vildagliptin dose-dependently inhibited the hydrolysis of both prodrugs **6** and **13**. In fact, at 2.5 μM inhibitor, the DPPIV/CD26-catalyzed hydrolysis of **13** was virtually

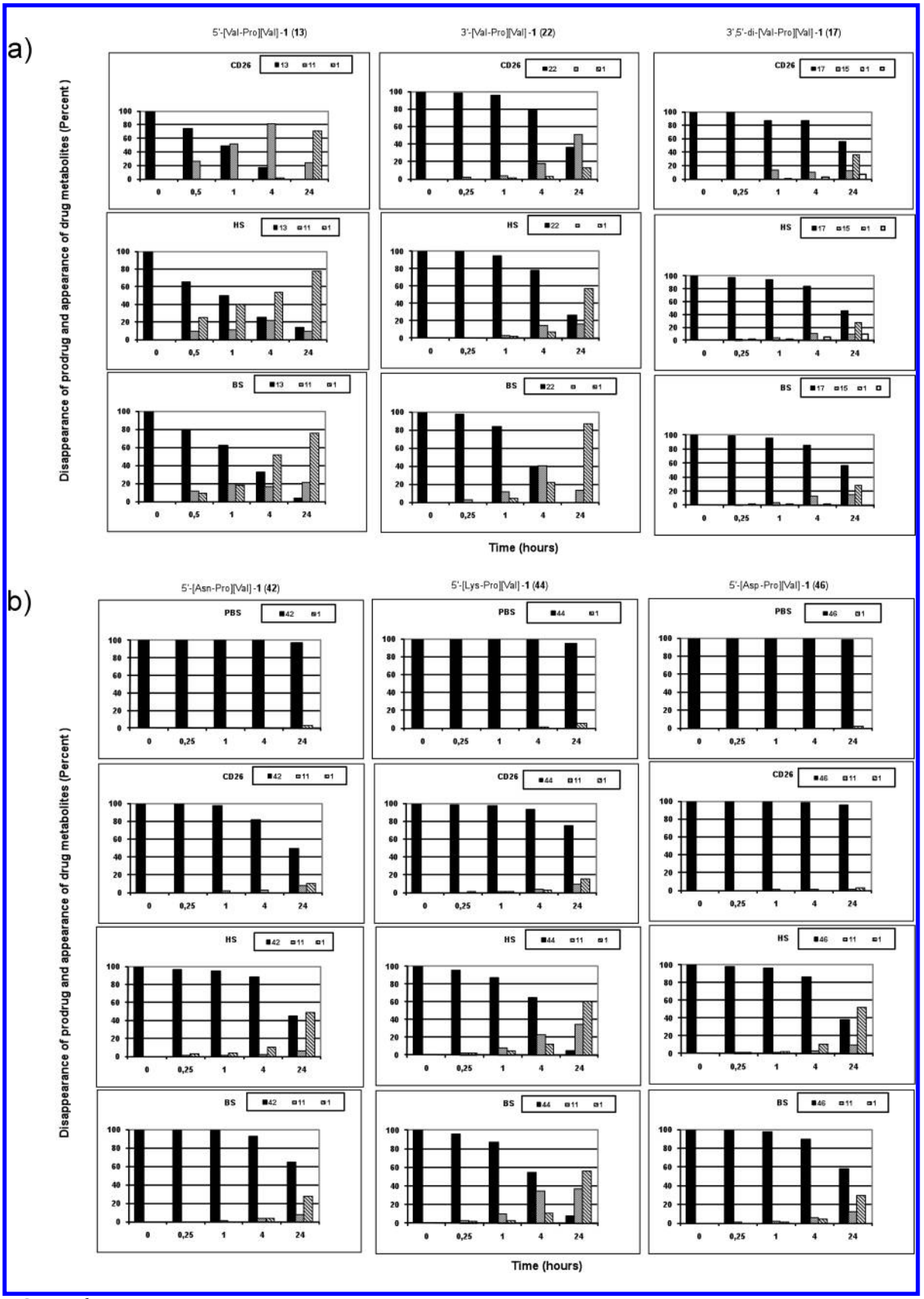


Figure 3. Continued

dx.doi.org/10.1021/jm101624e |*J. Med. Chem.* 2011, 54, 1927–1942

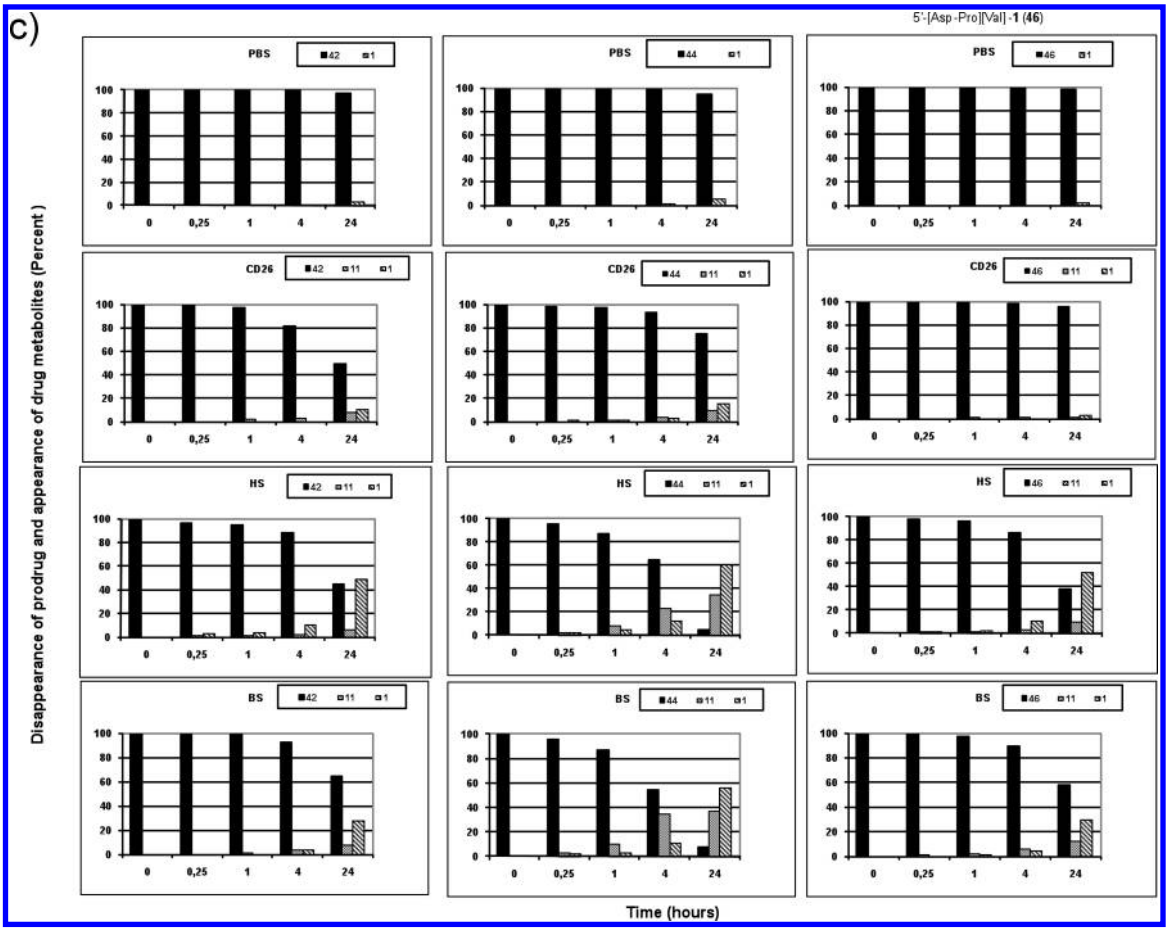


Figure 3. (a) Conversion of 5′-, 3′-, and 3′,5′-Val-Pro-Val prodrugs (**13**, **22**, and **17**) to the corresponding valyl intermediates and parent drug **1** in the presence of purified DPP IV/CD26, bovine, and human serum (20%). The open bars (□) in (a) represent unidentified metabolite(s). (b) Conversion of 5′-Yaa-Pro-Val prodrugs (**42**, **44**, and **46**) to 5′-Val intermediate and parent drug in the presence of purified DPP IV/CD26, bovine, and human serum (20%). (c) Conversion of 5′-Val-Pro-Xaa prodrugs (**30**, **32**, and **34**) to 5′-Xaa intermediates and parent drug in the presence of purified DPP IV/CD26, bovine and human serum (20%).

completely blocked, not only in the presence of purified enzyme but also in the presence of (20%) human, bovine, and murine serum. These findings point to a highly specific conversion of these types of prodrugs by DPPIV/CD26. It is noted that the murine serum expressed a somewhat higher DPPIV/CD26 activity (∼2-fold after 15 min compared to human serum). This higher activity may be related to the ∼2.5- to 5-fold higher concentration of vildagliptin required to afford a similar inhibitory potency of CD26-catalyzed prodrug conversion in murine versus human serum (Figure 4a).

Recently, several classes of compounds (the so-called gliptins, including vildagliptin) have been developed as novel oral glucose-lowering agents to be used to treat patients with type 2 diabetes mellitus. These drugs can be safety administered to such patients without significant side effects.[33−35] Interestingly, poor if any drug−drug interactions have been observed for the gliptins. Although caution should be given to combine DPPIV/CD26 inhibitors with DPPIV/CD26-dependent prodrugs (this combination may potentially delay prodrug cleavage to the parent drug and/or may negatively effect its impact on glucose levels), the DPPIV/CD26-dependent prodrug approach may be applicable to a much broader range of diseases to eventually deliver effective drugs with unfavorable PK properties when used as its nonderivatized parental drug as such.

The hydrolytic release of the Val-Pro dipeptide in CaCo-2 cell cultures from the 5′-Val-Pro-Val prodrug **13** and the effect of vildagliptin on this DPPIV/CD26-driven prodrug conversion has also been studied. CaCo-2 cells are human colon carcinoma cells that are generally used to study the potential oral bioavailability of candidate drug compounds. It is well-known that colon cells express high amounts of DPPIV/CD26 but also the pepT1 receptor that recognizes valine and valine-based compounds for their internalization at the apical side and subsequent release of valine and excretion of the parent (valine-lacking) drug at the basolateral side. We found prodrug **13** very efficiently hydrolyzed (80% of prodrug disappeared within 15 min of incubation), and the DPPIV/CD26-driven hydrolysis was efficiently inhibited by vildagliptin applied at 25 μM. Longer incubation times of the CaCo-2 cells with prodrug and vildagliptin decreased the inhibitory efficiency of the DPPIV/CD26 inhibitor and resulted in a time-dependent hydrolysis of the intact drug presumably due to the overwhelming amounts of DPPIV/CD26 present on the CaCo-2 cells (Figure 5).

*Release of Compound **1** at the Baselateral Side of Prodrug 13-Exposed CaCo-2 Cell Cultures.* CaCo-2 cells have also been seeded in 24-well microtiter plates and grown until confluency. Then prodrug **13** was added to the cell cultures (apical side) and

dx.doi.org/10.1021/jm101624e |*J. Med. Chem.* 2011, 54, 1927–1942

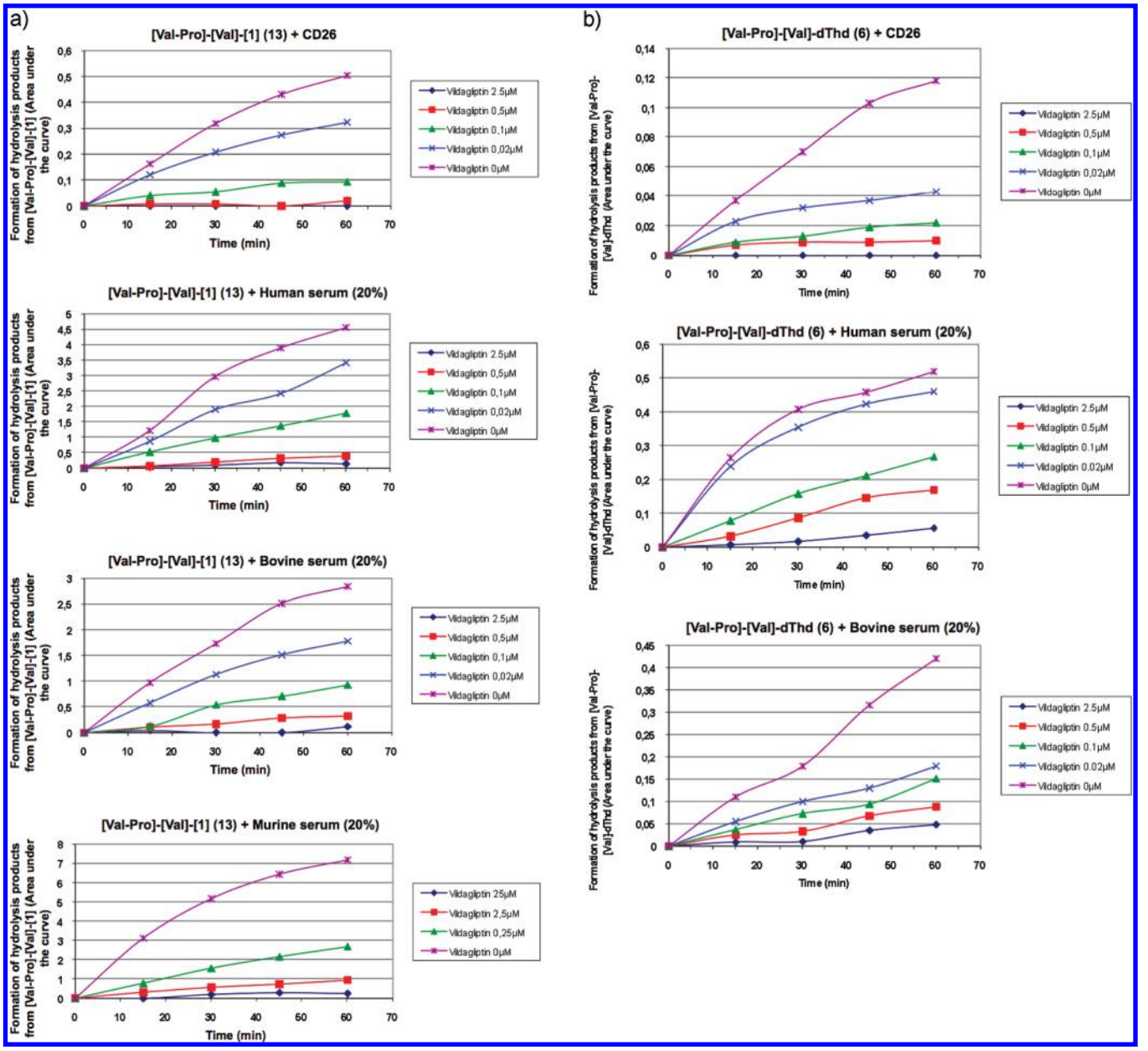


Figure 4. (a) Hydrolysis of prodrug **13** in the presence of a specific inhibitor of DPP IV/CD26. (b) Hydrolysis of thymidine prodrug **6** in the presence of a specific inhibitor of DPP IV/CD26.

the appearance of prodrug and parent compound **1** at the basolateral side of these cell cultures was quantified. Interestingly, whereas administration of 100 μM parent compound **1** to the CaCo-2 cell cultures resulted in a limited appearance of this drug at the baselateral side, the cells more efficiently released **1** from prodrug **13** in function of time. No significant amounts of prodrug were found to appear at the basolateral side, pointing (i) to the potential oral bioavailability of the prodrug and (ii) to an efficient hydrolysis and processing/transport of the (pro)drug by the CaCo-2 cell cultures.

In Vivo Experiments in Mice (with Selected Peptide Prodrugs). Several peptide prodrugs of compound **1** have been examined for their oral bioavailability compared with the parent compound **1** in mice. Plasma levels of **1** were determined at different time points after oral gavage of the peptide drugs. In none of the experiments, intact prodrug could be detected in the plasma of the exposed mice. As evident from Figure 6, parent compound **1** has a low oral bioavailability. However, the 5′-Val-Pro-Val prodrug **13** showed a markedly increased oral bioavailability. Its bioavailability (average of three independent experiments) was higher than if the 5′-Val intermediate **11** was administered to the mice (Figure 6). When the 5′-Val-Pro-Val, 3′,5′-di-Val-Pro-Val, and 3′-Val-Pro-Val derivatives **13**, **17**, and **22** were compared, oral bioavailability of all prodrugs was markedly increased to a comparable extent (Table 3). Also, the 5′-Val-Pro-Leu and the 5′-Lys-Pro-Val prodrugs **32** and **40** showed a comparable increased oral bioavailability (Table 3).

■ CONCLUSIONS

We successfully developed water-soluble prodrugs of the highly lipophilic BCNA compound **1**. In some cases, the water

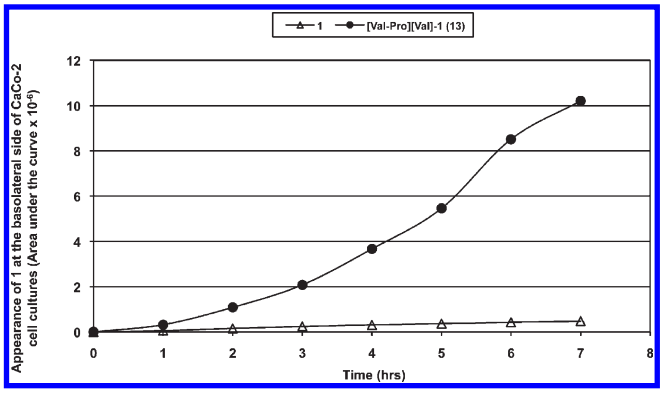


Figure 5. Appearance of parent compound **1** at the basolateral side of CaCo-2 cell cultures upon incubation of the cells by compound **1** or the 5′-Val-Pro-Val **13** prodrug at the apical side of the CaCo-2 cell cultures.

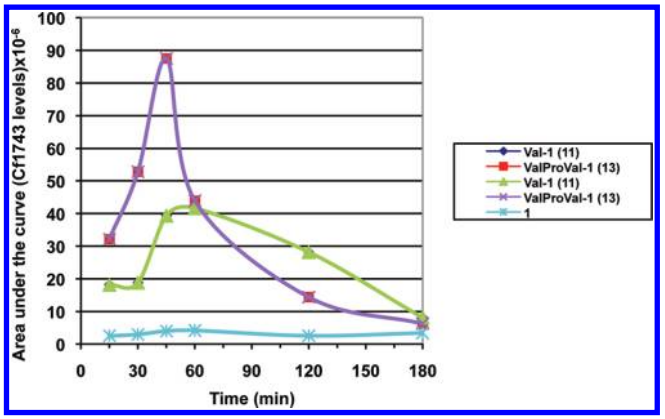


Figure 6. Administration of 5′-Val-Pro-Val (**13**), 5′-Val intermediate (**11**), and parent drug to mice by oral gavage.

solubility could be boosted by more than 3 orders of magnitude when compared to the parent drug. DPPIV/CD26 was solely responsible for the efficient hydrolysis of the dipeptide moiety from the prodrugs, since the DPPIV/CD26-specific inhibitor vidagliptin could completely block the enzymatic conversion in human, bovine, and murine serum. We also demonstrated a markedly enhanced oral bioavailability of the prodrugs versus the parent drug in mice that were receiving the prodrugs by oral gavage. The DPPIV/CD26-based prodrug approach may therefore prove useful to increase solubility and oral bioavailability of lipophilic drugs with poor systemic absorption characteristics exemplified by the BCNA compound **1**. Since compound **1** served as a prototype drug in our studies to demonstrate the power and usefulness of this novel DPPIV/CD26-based prodrug concept, the method may now be extended to other unrelated drugs on the condition that they contain a free hydroxyl moiety.

EXPERIMENTAL SECTION

Chemical Procedures. Microanalyses were obtained with a Heraeus CHN-O-RAPID instrument and the analytical results were within 0.4% of the theoretical values. Electrospray mass spectra were measured on a quadropole mass spectrometer equipped with an electrospray source (Hewlett-Packard, LC/MS HP 1100). Spectra were recorded with a Varian Inova-300 or Varian Inova-400 spectrometers operating at 300 or at 400 MHz for ^{1}H NMR and at 75 or at 100 MHz for ^{13}C NMR

Table 3. Fold-Increased Oral Bioavailability of the Tripeptide Prodrugs versus Parent Compound 1

compd	fold-increased plasma levels of parent compound **1** within 0 and 180 min after oral gavage of the prodrug[a]
13: 5′-Val-Pro-Val prodrug	13
22: 3′-Val-Pro-Val prodrug	7
17: 3′, 5′-di-Val-Pro-Val prodrug	12
44: 5′-Lys-Pro-Val prodrug	15
30: 5′-Val-Pro-Leu prodrug	11
1: parent compound	

[a] Seven time points (15, 30, 45, 60, 90, 120, 180 min) were taken into consideration for each compound.

with Me_4Si as internal standard. Analytical thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} (Merck). Separations on silica gel were performed by flash column chromatography with silica gel 60 (230−400 mesh) (Merck) or preparative centrifugal circular thin layer chromatography (CCTLC) on a Chromatotron (Kieselgel 60 PF_{254} (Merck), layer thickness of 1 mm, flow rate of 5 mL/min). Liquid chromatography was performed using a force flow (flash chromatography) Horizon HPFG system (Biotage) with Flash 25 or 40 silica gel cartridges. The dipeptide derivatives Cbz-Val-Pro-OH and Fmoc-Val-Pro-OH were purchased from Bachem Feinchemikalien. Compound **1** was synthesized as previously described.[17] The purity of novel compounds was determined to be >95% by elemental analysis.

5′-*O*-[*N*-(Benzyloxycarbonyl)valyl]thymidine (3). Under an argon atmosphere, to a solution of thymidine **2** (150 mg, 0.62 mmol) in anhydrous THF (6 mL), Cbz-Val-OH (311 mg, 1.24 mmol), polymer-bound triphenylphosphine (PS-PPh_3) (413 mg, 1.24 mmol), and di-*tert*-butyl azodicarboxylate (DBAD) (285 mg, 1.24 mmol) were added. The reaction mixture was stirred at room temperature for 15 h. After this time, 4 M HCl solution in dioxane (4 mL) was added, and the mixture was stirred for 1 h. The resin was filtered off, washed with ethyl acetate, and the filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in ethyl acetate (20 mL) and washed with 4 M HCl (3 × 20 mL). The organic layer was dried (Na_2SO_4), filtered, and evaporated to dryness. The final residue was purified by flash chromatography (CH_2Cl_2/MeOH, 10:1) to give 255 mg of **3** (86%) as a white foam. MS (ESI$^+$): *m*/*z* 476.3 (M + 1$^+$). Anal. for $C_{23}H_{29}N_3O_8$: C, H, N.

5′-*O*-(Valyl)thymidine (4). To a solution of compound **3** (75 mg, 0.158 mmol) in ethyl acetate (3 mL), Pd(OH_2) (23 mg, 30% w/w) was added. The reaction mixture was hydrogenated at 20 psi at room temperature for 2 h. The reaction mixture was filtered, and the filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in water and liophilized to give 50 mg of **4** (93%) as a yellow foam. MS (ESI$^+$): *m*/*z* 342.1 (M + 1$^+$), 364.1 (M + Na^+), 683.2 (2M + 1$^+$). Anal. for $C_{15}H_{23}N_3O_6$: C, H, N.

5′-*O*-[*N*-(Benzyloxycarbonyl)valylprolylvalyl]thymidine (5). To a solution of **4** (125 mg, 0.32 mmol) in CH_2Cl_2 (3 mL), Cbz-Val-Pro-OH (132 mg, 0.38 mmol), (benzotriazol-1-yl-oxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) (168 mg, 0.38 mmol), and TEA (0.048 mL, 0.38 mmol) were successively added. The reaction mixture was stirred at room temperature for 15 h. The solvent was removed under reduced pressure, and the residue was dissolved in ethyl acetate (20 mL), washed with 10% aqueous citric acid (3 × 20 mL), 10% aqueous $NaHCO_3$ (3 × 20 mL), water (3 × 20 mL), and brine (3 × 20 mL). The organic layer was dried (Na_2SO_4), filtered, and evaporated to dryness. The final residue was purified by CCTLC on the Chromatotron (CH_2Cl_2/MeOH, 10:1) to give 97 mg of protected

conjugate **5** (44%) as a yellow foam. MS (ESI^+): *m/z* 672.3 ($M + 1^+$). Anal. for $C_{33}H_{45}N_5O_{10}$: C, H, N.

5′-*O*-(Valylprolylvalyl)thymidine (6). According to the deprotection procedure described for compound **4**, protected conjugate **5** was hydrogenated to give 41 mg of **6** (90%) as a yellow foam. ^1H NMR (300 MHz, acetone-d_6): δ 0.83−0.97 (m, 12H, 2γ-CH_3, Val_1 and Val_2), 1.84 (s, 3H, CH_3-5), 1.87−2.12 (m, 5H, β-CH, Val_1, β-CH_2, Pro, γ-CH_2, Pro), 2.21−2.32 (m, 3H, H-2′, β-CH, Val_2), 3.47−3.77 (m, 2H, δ-CH_2, Pro), 3.94 (d, 1H, α-CH, Val_1, *J* = 8.3 Hz), 4.07−4.38 (m, 4H, H-5′, H-4′, α-CH, Val_2), 4.48 (m, 1H, H-3′), 4.57 (m, 1H, α-CH, Pro), 6.29 (t, 1H, H-1′, *J* = 6.7 Hz), 7.47 (s, 1H, H-6). ^{13}C NMR (100 MHz, acetone-d_6): δ 11.9 (C−C_5), 17.7, 18.6, 18.8, 19.3 (4C-γ, Val_1 and Val_2), 25.1 (C-γ, Pro), 27.5 (C-β, Pro), 31.1, 31.7 (2C-β, Val_1 and Val_2), 39.4 (C-2′), 47.2 (C-δ, Pro), 58.0 (C-α, Val_2), 60.1 (C-α, Pro), 64.5 (C-α, Val_1), 70.5 (C-3′), 71.4 (C-5′), 84.4, 85.2 (C-1′, C-4′), 110.5 (C-5), 136.0 (C-6), 150.6 (C-2), 163.5 (C-4), 171.4, 171.8, 171.9 (C═O, Val_1, C═O, Val_2, C═O, Pro). MS (ESI^+): *m/z* 538.3 ($M + 1^+$). Anal. for $C_{25}H_{39}N_5O_8$: C, H, N.

3-[5′-*O*-[*N*-(Benzyloxycarbonyl)valyl]-2′-deoxy-β-D-ribofuranosyl]-6-(*p*-pentylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (7). Following the procedure described for thymidine derivative **3**, compound **1** (100 mg, 0.25 mmol) was reacted with Z-Val-OH (125 mg, 0.5 mmol), PS-PPh_3 (183 mg, 0.55 mmol) and DBAD (116 mg, 0.5 mmol). The resin was filtered off, washed with ethyl acetate, and the filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in ethyl acetate (20 mL) and washed with brine (1 × 20 mL). The organic layer was dried (Na_2SO_4), filtered, and evaporated to dryness. The final residue was purified by flash chromatography (CH_2Cl_2/MeOH, 20:1) to give 105 mg of the valyl derivative **7** (66%) as a white foam. MS (ESI^+): *m/z* 632.3 ($M + 1^+$). Anal. for $C_{35}H_{41}N_3O_8$: C, H, N.

3-[5′-*O*-[*N*-(Benzyloxycarbonyl)valylprolylvalyl]-2′-deoxy-β-D-ribofuranosyl]-6-(*p*-pentylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one and 5′-*O*-[*N*-(Benzyloxycarbonyl)valylprolylvalyl]-2′-deoxy-5-[2-(*p*-pentylphenyl)ethyl]uridine (8 and 9). A solution of **7** (125 mg, 0.2 mmol) in ethyl acetate (3 mL) was hydrogenated at 20 psi at room temperature in the presence of $Pd(OH_2)$ (38 mg, 30% w/w) for 2 h. After this time, TLC showed the disappearance of starting material and the formation of a mixture of two compounds. The catalyst was filtered off, and the filtrate was evaporated to dryness under reduced pressure. A solution of the residue in CH_2Cl_2 (3 mL) was treated with Cbz-Val-Pro-OH (84 mg, 0.24 mmol), BOP (106 mg, 0.24 mmol), and TEA (0.033 mL, 0.24 mmol) for 15 h following the coupling procedure described for thymidine derivative **5**. The final residue was purified by CCTLC on the Chromatotron (CH_2Cl_2/MeOH, 20:1). The fastest moving fractions afforded 53 mg of the unexpected acyclic derivative **9** (32%) as a yellow foam. ^1H NMR (400 MHz, acetone-d_6): δ 0.85−1.00 (m, 15H, 2γ-CH_3, Val_1 and Val_2, CH_3), 1.27−1.39 (m, 4H, CH_2), 1.57 (m, 2H, CH_2), 1.89−2.14 (m, 7H, 2β-CH, Val_1 and Val_2, β-CH_2, Pro, γ-CH_2, Pro, H-2′a), 2,20 (m, 1H, H-2′b), 2.53−2.65 (m, 4H, CH_2, CH_2-Ph), 2.76 (m, 2H, CH_2-5), 3.61−3.83 (m, 2H, δ-CH_2, Pro), 4.02- 4.38 (m, 6H, 2H-5′, H-4′, H-3′, 2α-CH, Val_1 and Val_2, OH), 4.56 (m, 1H, α-CH, Pro), 5.03 and 5.07 (AB system, 2H, CH_2, Cbz, *J* = 12.5 Hz), 6.23 (t, 1H, H-1′, *J* = 6.2 Hz), 6.32 (d, 1H, NH, Val_1, *J* = 8.8 Hz), 7.09−7.35 (m, 9H, Ar, Ar, Cbz), 7.20 (s, 1H, H-6), 7.64 (d, 1H, NH, Val_2, *J* = 8.2 Hz), 10.08 (bs, 1H, NH-3). ^{13}C NMR (100 MHz, acetone-d_6): δ 13.7 (CH_3), 17.7, 17.8, 18.8, 19.1 (4C-γ, Val_1 and Val_2), 22.5 (CH_2), 25.1 (C-γ, Pro), 28.2, 28.6, 29.8, 31.0, 31.5, 31.6 (3CH_2, C-β, Pro, C-C_5, C-*ipso*-C), 34.3, 35.4 (2C-β, Val_1 and Val_2), 39.4 (C-2′), 47.7 (C-δ, Pro), 58.0, 58.1 (C-α, Val_2, C-α, Pro), 59.9 (C-α, Val_1), 64.6 (C-5′), 66.1 (CH_2, Z), 71.5 (C-3′), 84.4 (C-4′), 85.3 (C-1′), 113.8 (C-5), 127.9, 128.0, 128.5, 128.6, 128.7, 128.9 (4C-Ar, Cbz, C-Ha, C-Hb), 136.6 (C-6), 138.9, 140.4 (*ipso*-C, *para*-C), 150.5 (C-2), 156.5 (C═O, Z), 163.2 (C-4), 171.4, 171.6, 171.7 (C═O, Val_1, C═O, Val_2, C═O, Pro). MS (ESI^+): *m/z* 832.3 ($M + 1^+$), 854.3 ($M + Na^+$), 1685.6 ($2M + Na^+$). Anal. for $C_{45}H_{61}N_5O_{10}$: C, H, N.

From the slowest moving fractions, an amount of 45 mg (27%) of compound **8** as a yellow foam was isolated. ^1H NMR (400 MHz, acetone-d_6): δ 0.84−0.95 (m, 15H, 2γ-CH_3, Val_1 and Val_2, CH_3), 1.23−1.35 (m, 4H, CH_2), 1.62 (m, 2H, CH_2), 1.92−2.17 (m, 6H, β-CH, Val_1 and Val_2, β-CH_2, Pro, γ-CH_2, Pro), 2,34 (m, 1H, H-2′a), 2.58−2.66 (m, 3H, H-2′b, CH_2), 3.65−3.82 (m, 2H, δ-CH_2, Pro), 4.23−4.52 (m, 6H, 2H-5′, H-4′, H-3′, α-CH, Val_1, OH), 4.57 (m, 1H, α-CH, Val_2), 4.68 (m, 1H, α-CH, Pro), 5.04 and 5.08 (AB system, 2H, CH_2, Cbz, *J* = 12.6 Hz), 6.18 (d, 1H, NH, Val_1, *J* = 8.2 Hz), 6.27 (t, 1H, H-1′, *J* = 6.0 Hz), 7.18 (s, 1H, H-5), 7.30−7.37 (m, 7H, Ar, Cbz), 7.23 (d, 1H, NH, Val_2, *J* = 7.5 Hz), 7.79 (AA′BB′ system, 2H, Ar, *J* = 8.2 Hz), 8.71 (s, 1H, H-4). ^{13}C NMR (100 MHz, acetone-d_6): δ 13.6 (CH_3), 17.6, 18.1, 18.7, 19.1 (4C-γ, Val_1 and Val_2), 22.5 (CH_2), 25.1 (C-γ, Pro), 28.5, 30.8, 31.0, 31.2 (3CH_2, C-β, Pro), 31.5, 35.6 (2C-β, Val_1 and Val_2), 41.8 (C-2′), 47.7 (C-δ, Pro), 58.1, 58.6 (C-α, Val_2, C-α, Pro), 59.9 (C-α, Val_1), 64.1 (C-5′), 66.1 (CH_2, Cbz), 70.5 (C-3′), 85.3 (C-4′), 88.5 (C-1′), 99.0 (C-5), 107.5 (C-4a), 125.0, 126.8, 127.9, 128.0, 128.1 128.6 (4C−Ar, Cbz, C-Hb, *ipso*-C), 129.2 (C-Ha), 137.1 (C-4), 144.6 (*para*-C), 154.4, 154.8 (C-6, C═O, Cbz), 156.5 (C-2), 171.5, 171.7, 172.0, 172.2 (C═O, Val_1, C═O, Val_2, C═O, Pro, C-7a). MS (ESI^+): *m/z* 828.3 ($M + 1^+$), 850.3 ($M + Na^+$), 1677.6 ($2M + Na^+$). Anal. for $C_{45}H_{57}N_5O_{10}$: C, H, N.

3-[2′-Deoxy-5′-*O*-[*N*-(fluorenylmethoxycarbonyl)valyl]-β-D-ribofuranosyl]-6-(*p*-pentylphenyl)-2,3-dihydrofuro[2,3-*d*]-pyrimidin-2-one (10). According to the procedure described for compound **7**, a solution of compound **1** (100 mg, 0.25 mmol) in anhydrous THF (3 mL), under an argon atmosphere, was reacted with Fmoc-Val-OH (170 mg, 0.5 mmol) in the presence of PS-PPh_3 (184 mg, 0.55 mmol) and DBAD (118 mg, 0.5 mmol). The final residue was purified by CCTLC on the Chromatotron (CH_2Cl_2/MeOH, 40:1) to give 110 mg of compound **10** (61%) as a white foam. MS (ESI^+): *m/z* 720.2 ($M+1^+$), 742.2 ($M+Na^+$), 1439.0 ($2M + 1^+$). Anal. for $C_{42}H_{45}N_3O_8$: C, H, N.

3-(2′-Deoxy-5′-*O*-valyl-β-D-ribofuranosyl)-6-(*p*-pentylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (11). To a solution of **10** (143 mg, 0.2 mmol) in DMF (4 mL) piperidine (0.2 mL, 2.0 mmol) was added. The mixture was stirred at room temperature for 5 min and evaporated to dryness under reduced pressure. The residue was purified by CCTLC on the Chromatotron (CH_2Cl_2/MeOH, 20:1) to give 88 mg of **11** (89%) as a yellow foam. ^1H NMR (300 MHz, acetone-d_6): δ 0.88−0.95 (m, 9H, γ-CH_3, Val, CH_3), 1.31−1.36 (m, 4H, CH_2), 1.59−1.67 (m, 2H, CH_2), 1.84 (m, 1H, β-CH, Val), 2.25 (m, 1H, H-2′a), 2.55−2.72 (m, 3H, CH_2, H-2′b), 3.94 (d, 1H, α-CH, Val, *J* = 6.8 Hz), 4.26- 4.59 (m, 5H, 2H-5′, H-4′, H-3′, OH), 6.39 (t, 1H, H-1′, *J* = 6.4 Hz), 7.02 (s, 1H, H-5), 7.34 and 7.73 (AA′BB′ system, 2H, Ar, *J* = 8.5 Hz), 8.74 (s, 1H, H-4). ^{13}C NMR (100 MHz, acetone-d_6): δ 13.9 (CH_3), 17.3, 19.2 (2C-γ, Val), 22.8 (CH_2), 30.2, 31.4, 32.6 (3CH_2), 35.9 (C-β, Val), 41.9 (C-2′), 60.6 (C-α, Val), 64.0 (C-5′), 70.2 (C-3′), 85.9 (C-4′), 88.5 (C-1′), 98.6 (C-5), 107.9 (C-4a), 125.2 (C-Hb), 126.9 (*ipso*-C), 129.6 (C-Ha), 137.4 (C-4), 145.2 (*para*-C), 154.7 (C-6), 155.3 (C-2), 171.8 (C-7a), 175.7 (C═O, Val). MS (ESI^+): *m/z* 498.3 ($M + 1^+$), 995.5 ($2M + 1^+$). Anal. for C $C_{27}H_{35}N_3O_6$: C, H, N.

3-[2′-Deoxy-5′-*O*-[*N*-(fluorenylmethoxycarbonyl)valylprolylvalyl]-β-D-ribofuranosy]-6-(*p*-pentylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (12). A solution of compound **11** (95 mg, 0.19 mmol) in CH_2Cl_2 (5 mL) was reacted with Fmoc-Val-Pro-OH (100 mg, 0.23 mmol), BOP (101 mg, 0.23 mmol), and TEA (0.032 mL, 0.23 mmol) according to the coupling procedure described for compound **5**. The final residue was purified by CCTLC on the Chromatotron (CH_2Cl_2/MeOH, 25:1) to give 130 mg of **12** (74%) as a white foam. MS (ESI^+): *m/z* 916.3 ($M + 1^+$), 938.0 ($M + Na^+$). Anal. for $C_{52}H_{61}N_5O_{10}$: C, H, N.

3-[2′-Deoxy-5′-*O*-(valyl-prolyl-valyl)-β-D-ribofuranosyl]-6-(*p*-penthylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (13). Following the deprotection procedure described for **11**, a solution of compound **12** (120 mg, 0.13 mmol) in DMF (3 mL) was treated with piperidine (0.15 mL, 1.5 mmol). The final residue was purified by CCTLC on the Chromatotron (ethyl acetate/MeOH, 10:1) to give 81 mg of **13** (89%) as a white solid after trituration with Et_2O. Mp: 76−79 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ 0.78−0.97 (m, 15H, 2γ-CH_3, Val_1 and Val_2, CH_3), 1.17−1.41 (m, 4H, CH_2), 1.65 (m, 2H, CH_2), 1.83−2.35 (m, 7H, 2β-CH, Val_1 and Val_2, β-CH_2, Pro, γ-CH_2, Pro, H-2′a), 2.59−2.70 (m, 3H, H-2′b, CH_2), 3.51−3.78 (m, 2H, δ-CH_2, Pro), 3.93 (d, 1H, α-CH, Val_1, J = 8.1 Hz), 4.32−4.58 (m, 7H, 2H-5′, H-4′, H-3′, α-CH, Val_2, α-CH, Pro, OH), 6.31 (t, 1H, H-1′, J = 5.8 Hz), 7.25 (s, 1H, H-5), 7.33 (AA′BB′ system, 2H, Ar, J = 8.5 Hz), 7.75−7.86 (m, 3H, Ar, NH, Val_2), 8.71 (s, 1H, H-4). ^{13}C NMR (75 MHz, acetone-d_6): δ 13.9 (CH_3), 18.4, 19.0, 19.1, 19.6 (4C-γ, Val_1 and Val_2), 22.8 (CH_2), 25.4 (C-γ, Pro), 28.2, 31.0, 31.4, 31.8 (3CH_2, C-β, Pro), 32.5 (C-β, Val_2), 42.0 (C-2′), 47.6 (C-δ, Pro), 58.8 (C-α, Val_2), 60.1, 60.4 (C-α, Val_1, C-α, Pro), 64.4 (C-5′), 70.8 (C-3′), 85.6 (C-4′), 88.8 (C-1′), 99.4 (C-5), 108.2 (C-4a), 125.3 (C-Hb), 127.1 (*ipso*-C), 129.5 (C-Ha), 137.5 (C-4), 144.9 (*para*-C), 154.8 (C-6), 155.1 (C-2), 168.9, 172.0, 172.3, 172.7 (C═O, Val_1, C═O, Val_2, C═O, Pro, C-7a). MS (ESI$^+$): m/z 694.3 (M + 1$^+$), 716.5 (M + Na$^+$), 1387.7 (2M + 1$^+$), 1409.6 (2M + Na$^+$). Anal. for $C_{37}H_{51}N_5O_7$: C, H, N.

3-[2′-Deoxy-3′,5′-bis-*O*-[*N*-(fluorenilmethoxycarbonyl)-valyl]-β-D-ribofuranosyl]-6-(*p*-pentylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (14). To a solution of compound **1** (188 mg, 0.47 mmol) in DMF (4 mL) at 0 °C, DMAP (17 mg, 0.14 mmol), Fmoc-Val-OH (481 mg, 1.42 mmol), and DCC (292 mg, 1.42 mmol) were successively added. The reaction mixture was stirred at room temperature for 15 h. The white solid was filtered off, washed with DMF, and the solvent was evaporated to dryness. The filtrate was dissolved in ethyl acetate (20 mL) and washed with 10% aqueous citric acid (3 × 20 mL), 10% aqueous $NaHCO_3$ (3 × 20 mL), water (3 × 20 mL), and brine (3 × 20 mL). The organic layer was dried (Na_2SO_4), filtered, and evaporated to dryness. The final residue was purified by flash column chromatography on silica gel (hexane/ethyl acetate, 3:1) to give 343 mg of **14** (70%) as a white foam. MS (ESI$^+$): m/z 1041.3 (M + 1$^+$). Anal. for $C_{62}H_{64}N_4O_{11}$: C, H, N.

3-(2′-Deoxy-3′,5′-bis-*O*-valyl-β-D-ribofuranosyl)-6-(*p*-pentylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (15). Following the deprotection procedure described for compound **11**, a solution of **14** (330 mg, 0.32 mmol) in DMF (12 mL) was treated with piperidine (0.6 mL, 6.0 mmol). The final residue was purified by CCTLC on the Chromatotron (CH_2Cl_2/MeOH, 20:1) to give 181 mg of **15** (95%) as a yellow foam. MS (ESI$^+$): m/z 597.3 (M + 1$^+$), 1193.7 (2M + 1$^+$). Anal. for $C_{32}H_{44}N_4O_7$: C, H, N.

3-[2′-Deoxy-3′,5′-bis-*O*-[*N*-(fluorenilmethoxycarbonyl)-valylprolylvalyl]-β-D-ribofuranosyl]-6-(*p*-pentylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (16). Following the coupling procedure described for compound **5**, a solution of **15** (113 mg, 0.19 mmol) in CH_2Cl_2 (5 mL) was reacted with Fmoc-Val-Pro-OH (198 mg, 0.45 mmol), BOP (201 mg, 0.45 mmol), and TEA (0.063 mL, 0.45 mmol) at room temperature for 15 h. The final residue was purified by CCTLC on the Chromatotron (CH_2Cl_2/MeOH, 40:1) to give 184 mg of **16** (68%) as a yellow oil. MS (ESI$^+$): m/z 1434.4 (M + 1$^+$). Anal. for $C_{82}H_{96}N_8O_{15}$: C, H, N.

3-(2′-Deoxy-3′,5′-bis-*O*-valylprolylvalyl-β-D-ribofuranosyl)-6-(*p*-pentylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (17). Following the deprotection procedure described for compound **11**, a solution of **16** (164 mg, 0.11 mmol) in DMF (12 mL) was reacted with piperidine (0.25 mL, 2.5 mmol). The final residue was purified by CCTLC on the Chromatotron (CH_2Cl_2/MeOH, 10:1) to give 98 mg of **17** (87%) as a white solid after trituration with Et_2O. Mp: 85−89 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ 0.88−1.02 [m, 27H, 4γ-CH_3, (Val_1, Val_2)-3′, (Val_1, Val_2)-5′, CH_3], 1.29−1.40 (m, 4H, 2CH_2), 1.65 (m, 2H, CH_2), 1.72−2.57 [m, 14H, 2H-2′, 4β-CH, (Val_1, Val_2)-3′, (Val_1, Val_2)-5′, 2β-CH_2, Pro-3′, Pro-5′, 2γ-CH_2, Pro-3′, Pro-5′], 2.67 (t, 2H, CH_2, J = 7.7 Hz), 3.60−4.58 (m, 13H, 2H-5′, H-4′, 4α-CH, (Val_1, Val_2)-3′, (Val_1, Val_2)-5′, 2α-CH Pro-3′, Pro-5′, 2δ-CH_2, Pro-3′, Pro-5′), 5.45 (m, 1H, H-3′), 6.35 (m, 1H, H-1′), 7.20 (s, 1H, H-5), 7.34 and 7.70 (AA′BB′ system, 2H, Ar, J = 8.1 Hz), 7.75−7.89 (m, 2H, 2NH Val_2-3′, Val_2-5′), 8.68 (s, 1H, H-4). MS (ESI$^+$): m/z 989.5 (M + 1$^+$). Anal. for $C_{52}H_{76}N_8O_{11}$: C, H, N.

3-[5′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxy-β-D-ribofuranosyl]-6-(*p*-pentylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (18). To a solution of compound **1** (200 mg, 0.5 mmol) in anhydrous pyridine (4 mL), TBDMSCl (302 mg, 2.0 mmol) was added. The reaction mixture was stirred at room temperature under an argon atmosphere for 48 h. The solvent was removed under reduced pressure. The residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH, 40:1) to give 160 mg of **18** (63%) as a white foam. MS (ESI$^+$): m/z 513.3 (M + 1$^+$), 535.3.3 (M + Na$^+$), 1025.5 (2M + 1$^+$). Anal. for $C_{28}H_{40}N_2O_5Si$: C, H, N.

3-[5′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxy-3′-*O*-[*N*-(fluorenilmethoxycarbonyl)valyl]-β-D-ribofuranosyl]-6-(*p*-pentylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (19). To a solution of **18** (170 mg, 0.33 mmol) in DMF (3 mL), DMAP (12 mg, 0.1 mmol), Fmoc-Val-OH (225 mg, 0.66 mmol) and DCC (137 mg, 0.66 mmol) were successively added at 0 °C. The reaction mixture was stirred at room temperature for 2 h. The white solid was filtered off, washed with DMF, and the solvent was evaporated to dryness. The residue was dissolved in ethyl acetate (20 mL) and washed with 10% aqueous citric acid (3 × 20 mL), 10% aqueous $NaHCO_3$ (3 × 20 mL), water (3 × 20 mL), and brine (3 × 20 mL). The organic layer was dried (Na_2SO_4), filtered, and evaporated to dryness. The final residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH, 60:1) to give 230 mg of **19** (83%) as a white foam. MS (ESI$^+$): m/z 834.3 (M + 1$^+$). Anal. for $C_{48}H_{59}N_3O_8Si$: C, H, N.

3-[5′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxy-3′-*O*-valyl-β-D-ribofuranosyl]-6-(*p*-pentylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (20). Following the deprotection procedure described for **11**, compound **19** (179 mg, 0.22 mmol) was reacted with piperidine (0.25 mL, 2.5 mmol). The final residue was purified by CCTLC on the Chromatotron (CH_2Cl_2/MeOH, 20:1) to give 122 mg of **20** (93%) as a yellow foam. MS (ESI$^+$): m/z 612.4 (M + 1$^+$), 634.3 (M + Na$^+$), 1224.7 (2M + 1$^+$). Anal. for $C_{33}H_{49}N_3O_6Si$: C, H, N.

3-[5′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxy-3′-*O*-[*N*-(fluorenilmethoxycarbonyl)valylprolylvalyl]-β-D-ribofuranosyl]-6-(*p*-pentylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (21). Following the coupling procedure described for compound **5**, a solution of **20** (81 mg, 0.13 mmol) in CH_2Cl_2 (2 mL) was treated with Fmoc-Val-Pro-OH (69 mg, 0.16 mmol), BOP (70 mg, 0.16 mmol), and TEA (0.022 mL, 0.16 mmol). The final residue was purified by CCTLC on the Chromatotron (CH_2Cl_2/MeOH, 50:1) to give 109 mg of **21** (80%) as a white foam. MS (ESI$^+$): m/z 1030.3 (M + 1$^+$), 1052.6 (M + Na$^+$). Anal. for $C_{58}H_{75}N_5O_{10}Si$: C, H, N.

3-[2′-Deoxy-3′-*O*-(valylprolylvalyl)-β-D-ribofuranosyl]-6-(*p*-pentylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (22). Compound **21** (109 mg, 0.11 mmol) was dissolved in MeOH (2 mL), and a solution of 0.1 N HCl in MeOH (1.1 mL, 0.11 mmol) was added. The reaction mixture was stirred at room temperature for 2 h, and the solvent was evaporated to dryness. The residue was dissolved in CH_2Cl_2 (40 mL) and washed with a 10% aqueous $NaHCO_3$ (3 × 20 mL). The organic layer was dried, filtered, and evaporated to dryness. The residue was dissolved in DMF (2 mL). Piperidine (0.10 mL, 1.0 mmol) was added, and the mixture was stirred at room temperature for 5 min. The solvent was removed under reduced pressure and the

residue was purified by CCTLC on the Chromatotron (CH_2Cl_2/MeOH, 15:1) to give 50 mg of **22** (62%) as a white solid after trituration with Et_2O. Mp: 71−72 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ 0.83−1.02 (m, 15H, 2γ-CH_3, Val_1, Val_2, CH_3), 1.29−1.39 (m, 4H, $2CH_2$), 1.65 (m, 2H, CH_2), 1.88−2.48 (m, 8H, 2β-CH, Val_1,Val_2, β-CH_2, Pro, γ-CH_2, Pro, 2H-2′), 2.66 (t, 2H, CH_2, J = 7.5 Hz), 3.53−3.76 (m, 3H, α-CH, Val_1, δ-CH_2, Pro), 3.93−4.55 (m, 6H, 2H-5′, H-4′, α-CH Val_2, α-CH, Pro, OH), 5.43 (m, 1H, H-3′), 6.37 (m, 1H, H-1′), 7.05 (s, 1H, H-5), 7.34 and 7.74 (AA′BB′ system, 2H, Ar, J = 8.5 Hz), 7.69 (d, 1H, NH, Val_2, J = 7.9 Hz), 8.86 (s, 1H, H-4). ^{13}C NMR (100 MHz, acetone-d_6): δ 14.0 (CH_3), 17.3, 18.3, 18.9, 19.7 (4C-γ, Val_1 y Val_2), 22.0 (CH_2), 24.5 (C-γ, Pro), 28.9, 29.8, 30.4, 30.8, 31.6 ($3CH_2$, C-β, Pro, C-β, Val_2), 34.9 (C-β, Val_1), 38.6 (C-2′), 46.7 (C-δ, Pro), 57.3 (C-α, Val_2), 58.7 (C-α, Val_1), 61.0 (C-α, Pro), 62.4 (C-5′), 74.4 (C-3′), 83.7 (C-4′), 87.7 (C-1′), 98.7 (C-5), 107.3 (C-4a), 124.6 (C-Hb), 125.8 (*ipso*-C), 129.1 (C-Ha), 137.7 (C-4), 144.2 (*para*-C), 153.8, 154.1 (C-6, C-2), 171.2, 172.0, 172.5, 173.7 (C-7a, C═O, Val_1 and Val_2, C═O, Pro). MS (ESI$^+$): m/z 916.3 (M + 1$^+$), 938.3 (M + Na^+). Anal. for $C_{37}H_{51}N_5O_8$: C, H, N.

3-[2′-Deoxy-5′-*O*-[*N*-(fluorenylmethoxycarbonyl)leucyl]-β-D-ribofuranosyl]-6-(*p*-pentylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (25). Following the coupling procedure described for compound **7**, compound **1** (186 mg, 0.46 mmol) was reacted with Fmoc-Leu-OH (330 mg, 0.93 mmol), DBAD (215 mg, 0.93 mmol), and PS-PPh_3 (341 mg, 0.93 mmol) in anhydrous THF (3 mL). The final residue was purified by CCTLC on the Chromatotron (CH_2Cl_2/MeOH, 40:1) to give 180 mg of **25** (53%) as a white foam. MS (ESI$^+$): m/z 734.3 (M + 1$^+$), 1467.4 (2M + 1$^+$). Anal. for $C_{43}H_{47}N_3O_8$: C, H, N.

3-(2′-Deoxy-5′-*O*-leucyl-β-D-ribofuranosyl)-6-(*p*-pentylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (26). Following the deprotection procedure described for compound **11**, a solution of **25** (175 mg, 0.24 mmol) in DMF (5 mL) was treated with piperidine (0.25 mL, 2.5 mmol). The final residue was purified by CCTLC on the Chromatotron (CH_2Cl_2/MeOH, 15:1) to give 110 mg of **26** (90%) as a yellow foam. MS (ESI$^+$): m/z 512.3 (M + 1$^+$), 1023.5 (2M + 1$^+$). Anal. for $C_{28}H_{37}N_3O_6$: C, H, N.

3-[2′-Deoxy-5′-*O*-[*N*-(fluorenylmethoxycarbonyl)valylprolylleucyl]-β-D-ribofuranosy]-6-(*p*-penthylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (29). Following the coupling procedure described for compound **5**, a solution of **29** (66 mg, 0.13 mmol) in CH_2Cl_2 (2 mL) was reacted with Fmoc-Val-Pro-OH (67 mg, 0.15 mmol), BOP (68 mg, 0.15 mmol), and TEA (0.021 mL, 0.15 mmol). The final residue was purified by CCTLC on the Chromatotron (CH_2Cl_2/MeOH, 30:1) to give 104 mg of **41** (87%) as a white foam. MS (ESI$^+$): m/z 930.3 (M + 1$^+$), 952.3 (M + Na^+). Anal. for $C_{53}H_{63}N_5O_{10}$: C, H, N.

3-[2′-Deoxy-5′-*O*-(valylprolylleucyl)-β-D-ribofuranosyl]-6-(*p*-penthylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (30). Following the deprotection procedure described for compound **11**, to a solution of compound **29** (100 mg, 0.11 mmol) in DMF (2 mL) piperidine (0.1 mL, 1.0 mmol) was added. The final residue was purified by CCTLC on the Chromatotron (CH_2Cl_2/MeOH, 15:1) to give 62 mg of **30** (82%) as a white solid after trituration with Et_2O. Mp: 78−82 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ 0.77−0.91 (m, 15H, CH_3, γ-CH_3, Val, δ-CH_3, Leu), 1.28−1.36 (m, 4H, $2CH_2$), 1.57−1.79 (m, 5H, β-CH_2, Leu, γ-CH, Leu, CH_2), 1.83−2.33 (m, 6H, β-CH, Val, β-CH_2, Pro, γ-CH_2, Pro, H-2′a), 2.66 (m, 3H, CH_2, H-2′b), 3.52−3.71 (m, 2H, δ-CH_2, Pro), 3.91 (d, 1H, α-CH, Val, J = 8.4 Hz), 4.33−4.56 (m, 6H, H-4′, H-3′, H-5′, α-CH, Leu, α-CH, Pro), 6.29 (t, 1H, H-1′, J = 6.0 Hz), 7.18 (s, 1H, H-5), 7.33 and 7.84 (AA′BB′ system, 2H, Ar, J = 8.4 Hz), 7.78 (d, 1 H, NH, Leu, J = 7.8 Hz), 8.74 (s, 1H, H-4). ^{13}C NMR (75 MHz, acetone-d_6): δ 13.6 (CH_3), 18.6, 19.4 (2C-γ, Val), 21.3, 21.4, 22.5 (2C-δ, Leu, CH_2), 24.7, 25.2 (C-γ, Pro, C-γ, Leu), 28.1, 31.2, 31.5, 31.6 ($4CH_2$, C-β, Pro), 35.6 (C-β, Val), 40.5 (C-β, Leu), 41.8 (C-2′), 47.3 (C-δ, Pro), 51.5 (C-α, Leu), 59.7, 60.6 (C-α Pro, C-α, Val), 64.2 (C-5′), 70.5 (C-3′), 85.4, 88.6 (C-1′, C-4′), 99.1 (C-5), 107.8 (C-4a), 125.1, 126.7 (C-Hb, *ipso*-C), 129.2 (C-Ha), 137.1 (C-4), 144.5 (*para*-C), 154.4, 154.8 (C-6, C-2), 171.4, 172.0, 172.4, 172.7 (C═O, Val, C═O, Leu, C═O, Pro, C-7a). MS (ESI$^+$): m/z 708.3 (M + 1)$^+$, 730.3 (M + Na)$^+$, 1416.8 (2M + 1)$^+$. Anal. for $C_{38}H_{53}N_5O_8$: C, H, N.

3-[2′-Deoxy-5′-*O*-[*N*-(fluorenylmethoxycarbonyl)phenylalanyl]-β-D-ribofuranosyl]-6-(*p*-pentylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (27). Following the coupling procedure described for compound **7**, a solution of compound **1** (250 mg, 0.628 mmol) in anhydrous THF (6 mL) was reacted with Fmoc-Phe-OH (487 mg, 1.256 mmol), DBAD (295 mg, 1.256 mmol), and PS-PPh_3 (461 mg, 1.382 mmol). The final residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH, 22:1) to give 265 mg of **27** (56%) as an amorphous solid. MS (ESI$^+$): m/z 768.3 (M + 1$^+$), 770.2 (M + Na^+). Anal. for $C_{46}H_{45}N_3O_8$: C, H, N.

3-(2′-Deoxy-5′-*O*-phenylalanyl-β-D-ribofuranosyl)-6-(*p*-pentylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (28). Following the deprotection procedure described for compound **11**, a solution of **27** (237 mg, 0.309 mmol) in DMF (8 mL) was treated with piperidine (0.4 mL, 4.0 mmol). The final residue was purified by CCTLC on the Chromatotron (CH_2Cl_2/MeOH, 15:1) to give 143 mg of **28** (85%) as a white foam. MS (ESI$^+$): m/z 546.3 (M + 1$^+$), 568.3 (M + Na^+), 1091.5 (2M + 1$^+$). Anal. for $C_{31}H_{35}N_3O_6$: C, H, N.

3-[2′-Deoxy-5′-*O*-[*N*-(fluorenylmethoxycarbonyl)valylprolylphenylalanyl]-β-D-ribofuranosy]-6-(*p*-penthylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (31). Following the coupling procedure described for compound **5**, a solution of **28** (90 mg, 0.165 mmol) in CH_2Cl_2 (2 mL) was reacted with Fmoc-Val-Pro-OH (86 mg, 0.198 mmol), BOP (88 mg, 0.198 mmol), and TEA (0.028 mL, 0.198 mmol). The final residue was purified by CCTLC on the Chromatotron (CH_2Cl_2/MeOH, 35:1) to give 126 mg of **31** (79%) as a white foam. MS (ESI$^+$): m/z 964.3 (M + 1$^+$). Anal. for $C_{56}H_{61}N_5O_{10}$: C, H, N.

3-[2′-Deoxy-5′-*O*-(valylprolylphenylalanyl)-β-D-ribofuranosyl]-6-(*p*-penthylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (32). Following the deprotection procedure described for compound **11**, a solution of **31** (115 mg, 0.119 mmol) in DMF (3 mL) was reacted with piperidine (0.15 mL, 1.5 mmol). The final residue was purified by CCTLC on the Chromatotron (CH_2Cl_2/MeOH, 20:1) to give 79 mg of **32** (90%) as a white solid after trituration with Et_2O. Mp: 79−81 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ 0.76−0.92 (m, 9H, γ-CH_3 Val, CH_3), 1.27−1.36 (m, 4H, $2CH_2$), 1.58−1.68 (m, 2H, CH_2), 1.74−2.10 (m, 5H, β-CH_2, Pro, γ-CH_2, Pro, H-2′a), 2.17−2.29 (m, 1H, β-CH, Val), 2.47−2.5 (m, 1H, H-2′b), 2.64 (t, 1H, CH_2, J = 7.8 Hz), 3.00−3.22 (m, 2H, β-CH_2, Phe), 3.94 (d, 1H, α-CH, Val, J = 8.4 Hz), 4.01 (m, 1H, H-3′), 4.25−4.66 (m, 5H, H-5′, H-4′, α-CH, Pro, α-CH, Phe), 6.28 (t, 1H, H-1′, J = 6.0 Hz), 7.11−7.20 (m, 5H, Ar, Phe), 7.22 (s, 1H, H-5), 7.31 (AA′BB′ system, 2H, Ar, J = 8.1 Hz), 7.82−7−88 (m, 3H, Ar, NH, Phe), 8.69 (s, 1H, H-4). ^{13}C NMR (75 MHz, acetone-d_6): δ 15.3 (CH_3), 20.2, 21.0 (2C-γ, Val), 21.3 (CH_2), 26.7 (C-γ, Pro), 29.1, 32.7, 33.1, 33.2 ($3CH_2$, C-β, Pro), 37.2 (C-β, Val), 39.1, 39.2 (C-β, Phe, C-β, Pro), 43.4 (C-2′), 48.9 (C-δ, Pro), 55.7 (C-α, Phe), 56.7 (C-α Pro), 61.8 (C-α, Val), 65.9 (C-5′), 72.2 (C-3′), 86.0 (C-4′), 90.2 (C-1′), 100.8 (C-5), 109.7 (C-4a), 126.7, 128.3, 128.6, 130.2, 130.8, 130.9, 138.4, 138.8 (5C−Ar, Phe, C-Hb, *ipso*-C, C-Ha), 146.1 (*para*-C), 156.1, 156.5 (C-6, C-2), 173.1, 173.4, 173.5, 174.0 (C═O, Val, C═O, Leu, C═O, Pro, C-7a). MS (ESI$^+$): m/z 742.3 (M + 1$^+$), 764.3 (M + Na^+), 1484.7 (2M + 1$^+$). Anal. for $C_{41}H_{51}N_5O_8$: C, H, N.

3-[2′-Deoxy-5′-*O*-[*N*-(fluorenylmethoxycarbonyl)valylprolylalanyl]-β-D-ribofuranosy]-6-(*p*-penthylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (33). Following the coupling procedure described for compound **7**, a solution of compound **1** (200 mg, 0.502 mmol) in anhydrous THF (5 mL) was reacted with Fmoc-Val-Pro-Ala-OH (510 mg, 1.0 mmol), DBAD (231 mg, 1.0 mmol), and PS-PPh_3 (368 mg, 1.105 mmol) under Ar at room temperature for 6 days.

The final residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH, 20:1) to give 259 mg of **33** (58%) as a yellow foam. MS (ESI^+): m/z 888.3 ($M + 1^+$), 910.3 ($M + Na^+$). Anal. for $C_{50}H_{57}N_5O_{10}$: C, H, N.

3-[2′-Deoxy-5′-*O*-(valylprolylalanyl)-β-D-ribofuranosyl]-6-(*p*-penthylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (34). Following the deprotection procedure described for compound **11**, a solution of **33** (255 mg, 0.287 mmol) in DMF (6 mL) was treated with piperidine (0.3 mL, 3.0 mmol). The final residue was purified by CCTLC on the Chromatotron (CH_2Cl_2/MeOH, 10:1) to give 140 mg of **34** (74%) as a white solid after trituration with Et_2O. Mp: 75−78 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ 0.78−0.93 (m, 9H, γ-CH_3 Val, CH_3), 1.36 (m, 4H, $2CH_2$), 1.38 (d, 3H, β-CH_3 Ala, J = 7.1 Hz), 1.65 (m, 2H, CH_2), 1.81−1.99 (m, 4H, β-CH_2, Pro, γ-CH_2, Pro), 2,19−2.34 (m, 2H, β-CH, Val, H-2′a), 2.60−2.71 (m, 3H, H-2′b, CH_2), 3.47−3.62 (m, 2H, δ-CH_2, Pro), 3.91 (d, 1H, α-CH, Val, J = 8.3 Hz), 4.31−4.56 (m, 6H, H-5′, H-4′, H-3′, α-CH, Ala, α-CH, Pro), 6.28 (t, 1H, H-1′, J = 6.1 Hz), 7.19 (s,1H, H-5), 7.34 and 7.86 (AA′BB′ system, 2H, Ar, J = 8.3 Hz), 7.79 (d, 1H, NH, Ala, J = 7.0 Hz), 8.71 (s, 1H, H-4). ^{13}C NMR (100 MHz, acetone-d_6): δ 13.7 (CH_3), 16.8(C-β, Ala), 18.6, 19.3 (2C-γ, Val), 22.5 (CH_2), 25.1 (C-γ, Pro), 28.4, 31.1, 31.5, 31.6 ($3CH_2$, C-β, Pro), 35.6 (C-β, Val), 41.7 (C-2′), 47.3, 48.7 (C-δ, Pro, CH Fmoc), 58.0 (C-α, Ala), 59.6 (C-α, Val), 60.0 (C-α, Pro), 64.2 (C-5′), 70.1 (CH_2, Fmoc), 70.4 (C-3′), 85.4 (C-4′), 88.6 (C-1′), 99.0 (C-5), 107.9 (C-4a), 125.0 (C-Hb), 126.7 (*ipso*-C), 129.2 (C-Ha), 137.2 (C-4), 144.5 (*para*-C), 154.5, 154.8 (C-6, C-2), 171.4, 171.9, 172.4, 172.8 (C═O, Val_1, C═O, Val_2, C═O, Pro, C-7a). MS (ESI^+): m/z 666.3 ($M + 1^+$), 688.3 ($M + Na^+$), 1331.6 ($2M + 1^+$). Anal. for $C_{35}H_{47}N_5O_8$: C, H, N.

3-[2′-Deoxy-5′-*O*-[*N*-(fluorenylmethoxycarbonyl)asparagylprolylvalyl]-β-D-ribofuranosy]-6-(*p*-penthylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (41). Following the coupling procedure described for compound **5**, a solution of **11** (150 mg, 0.30 mmol) in CH_2Cl_2 (3 mL) was reacted with Fmoc-Asn-Pro-OH (163 mg, 0.36 mmol), BOP (160 mg, 0.36 mmol), and TEA (0.050 mL, 0.36 mmol). The final residue was purified by precipitation in Et_2O to give 231 mg of **41** (82%) as a white solid. Mp: 93−95 °C. MS (ESI^+): m/z 931.5 ($M + 1^+$), 953.5 ($M + Na^+$). Anal. for $C_{51}H_{58}N_6O_{11}$: C, H, N.

3-[5′-*O*-(Asparagylprolylvalyl)-2′-deoxy-β-D-ribofuranosy]-6-(*p*-penthylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (42). Following the deprotection procedure described for **11**, to a solution of compound **41** (197 mg, 0.211 mmol) in DMF (5 mL) piperidine (0.25 mL, 2.5 mmol) was added. The final residue was purified by precipitation in Et_2O to give 146 mg of **42** (90%) as a white solid. Mp: 97−99 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.83−0.91 (m, 9H, γ-CH_3, Val, CH_3), 1.24−1.32 (m, 4H, $2CH_2$), 1.58 (m, 2H, CH_2), 1.69−1.86 (m, 3H, β-CHa, Pro, γ-CH_2, Pro), 1.99−2.10 (m, 2H, β-CH, Val, β-CHb, Pro), 2.17−2.46 (m, 4H, β-CH_2, Asn, H-2′), 2.61 (t, 2H, CH_2, J = 7.5 Hz), 3.49−3.65 (m, 2H, δ-CH_2, Pro), 4.07 (t, 1H, α-CH, Val, J = 7.2 Hz), 4.13−4.37 (m, 5H, H-5′, H-4′, H-3′, α-CH, Asn), 4.48 (m, 1H, α-CH, Pro), 5.59 (m, 1H, OH-3′), 6.20 (t, 1H, H-1′, J = 6.0 Hz), 6.84 (bs, 1H, NHa, Asn), 7.16 (s, 1H, H-5), 7.31 and 7.75 (AA′BB′ system, 2H, Ar, J = 8.1 Hz), 7.43 (bs, 1H, NHb, Asn), 8.25 (d, 1H, NH, Val, J = 7.2 Hz), 8.60 (s, 1H, H-4). ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.4 (CH_3), 19.1, 19.4 (C-γ, Val), 22.5 (CH_2), 24.8 (C-γ, Pro), 28.1, 29.4, 301, 30.9 ($3CH_2$, C-β, Pro), 35.4 (C-β, Val), 41.2 (C-2′), 46.7 (C-δ, Pro), 50.2 (C-β, Asn), 58.7 (C-α, Val), 59.4 (C-α, Pro), 64.5 (C-5′), 70.3 (C-3′), 85.1 (C-4′), 88.3 (C-1′), 99.3 (C-5), 107.8 (C-4a), 125.1, 126.4 (C-Hb, *ipso*-C), 129.6 (C-Ha), 138.1 (C-4), 144.6 (*para*-C), 154.2, 154.4, 156.4 (C-6, C-2), 171.6, 171.9, 172.7, 173.1, 173.8 (C═O, Val, 2C═O, Asn, C═O, Pro, C-7a). MS (ESI^+): m/z 709.7 ($M + 1^+$), 731.5 ($M + Na^+$). Anal. for $C_{36}H_{48}N_6O_9$: C, H, N.

3-[2′-Deoxy-5′-*O*-[[*N*,N^6-di(fluorenylethoxycarbonyl)lysyl]-prolylvalyl]-β-D-ribofuranosy]-6-(*p*-penthylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (43). Following the coupling procedure described for compound **5**, a solution of **11** (117 mg, 0.24 mmol) in CH_2Cl_2 (4 mL) was reacted with Fmoc-Lys(Fmoc)-Pro-OH (194 mg, 0.28 mmol), BOP (125 mg, 0.28 mmol), and TEA (0.039 mL, 0.28 mmol). The final residue was purified by CCTLC on the Chromatotron (CH_2Cl_2/MeOH, 25:1) to give 197 mg of **43** (72%) as a white foam. MS (ESI^+): m/z 1167.8 $(M + 1)^+$, 1189.9 $(M + Na)^+$. Anal. for $C_{68}H_{74}N_6O_{12}$: C, H, N

3-[2′-Deoxy-5′-*O*-(lysylprolylvalyl)-β-D-ribofuranosyl]-6-(*p*-penthylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (44). Following the deprotection procedure described for compound **11**, to a solution of **43** (140 mg, 0.121 mmol) in DMF (6 mL) piperidine (0.3 mL, 3.0 mmol) was added. The final residue was purified by precipitation in Et_2O to give 82 mg of **44** (95%) as a yellow solid. Mp: 68−71 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.840.92 (m, 9H, γ-CH_3, Val, CH_3), 1.14−1.36 (m, 10H, $2CH_2$, β-CH_2, Lys, γ-CH_2, Lys, δ-CH_2, Lys), 1.59 (m, 2H, CH_2), 1.72−2.0. (m, 3H, β-CH, Val, β-CH_2, Pro), 2,22 (m, 1H, H-2′a), 2.32−2.45 (m, 3H, γ-CH_2, Pro, H-2′b), 2.61 (t, 2H, CH_2, J = 6.0 Hz), 3.15 (m, 2H, ε-CH_2, Lys), 3.41−3.61 (m, 2H, δ-CH_2, Pro), 4.02- 4.38 (m, 6H, H-5′, H-4′, H-3′, α-CH, Val, α-CH, Lys), 4.51 (m, 1H, α-CH, Pro), 6.19 (t, 1H, H-1′, J = 6.0 Hz), 7.08 (s, 1H, H-5), 7.32 and 7.80 (AA′BB′ system, 2H, Ar, J = 8.0 Hz), 8.30 (m, 1H, NH, Val), 8.67 (s, 1H, H-4). ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.9 (CH_3), 18.8, 19.0 (2C-γ, Val), 21.7 (CH_2), 24.5 (C-γ, Pro), 28.6, 28.7, 29.6, 30.4, 30.7, 30.8, 31.3 ($3CH_2$, C-β, Pro, C-β, Lys, C-γ, Lys, C-δ, Lys), 34.9 (C-β, Val), 40.8 (C-ε, Lys), 46.5 (C-2′), 50.0 (C-δ, Pro), 52.0 (C-α, Lys), 56.8 (C-α, Val), 58.7 (C-α, Pro), 63.9 (C-5′), 69.6 (C-3′), 84.5 (C-4′), 87.9 (C-1′), 98.9 (C-5), 107.2 (C-4a), 124.7 (C-Hb), 125.9 (*ipso*-C), 129.0 (C-Ha), 137.5 (C-4), 144.1 (*para*-C), 153.7, 153.9 (C-6, C-2), 171.1, 171.6, 172.5, 174.2 (C═O, Val, C═O, Lys, C═O, Pro, C-7a). MS (ESI^+): m/z 723.7 ($M + 1^+$), 745.5 ($M + Na^+$). Anal. for $C_{38}H_{54}N_6O_8$: C, H, N.

3-[5′-*O*-[[O^4−Allyl-*N*-(fluorenylmethoxycarbonyl)aspartyl]-prolylvalyl]-2′-deoxy-β-D-ribofuranosy]-6-(*p*-penthylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (45). Following the coupling procedure described for compound **5**, a solution of **11** (170 mg, 0.34 mmol) in CH_2Cl_2 (4 mL) was reacted with Fmoc-Asp(OAll)-Pro-OH (181 mg, 0.34 mmol), BOP (182 mg, 0.41 mmol), and TEA (0.057 mL, 0.41 mmol). The final residue was purified by CCTLC on the Chromatotron (CH_2Cl_2/MeOH, 20:1) to give 259 mg of **45** (78%) as a white foam. MS (ESI^+): m/z 972.3 ($M + 1^+$), 994.2 ($M + Na^+$), 1943.6 ($2M + 1^+$). Anal. for $C_{54}H_{64}N_5O_{12}$: C, H, N.

3-[5′-*O*-(Aspartylprolylvalyl)-2′-deoxy-β-D-ribofuranosy]-6-(*p*-penthylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (46). To a solution of compound **45** (135 mg, 0.140 mmol) in anhydrous CH_2Cl_2, $Pd(PPh_3)_4$ (16 mg, 0.014 mmol) and $PhSiH_3$ (34 mL, 0.28 mmol) were added. The reaction mixture was stirred at room temperature for 1 h, and the solvent was evaporated to dryness under reduced pressure. The residue was dissolved in DMF (3 mL), and piperidine (0.15 mL, 1.5 mmol) was added. After the mixture was stirred at room temperature for 5 min, the solvent was evaporated to dryness under reduced pressure. The final residue was purified by precipitation in ethyl acetate to give 82 mg of **46** (83%) as a yellow solid. Mp: 118−122 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.84−0.90 (m, 9H, γ-CH_3, Val, CH_3), 1.28 (m, 4H, $2CH_2$), 1.58 (m, 2H, CH_2), 1.74−1.88 (m, 3H, γ-CH_2, Pro, β-CHa, Pro), 2.04−2.10 (m, 2H, β-CH, Val, β-CHb, Pro), 2,16−2.46 (m, 2H, H-2′), 2.61 (t, 2H, CH_2, J = 7.6 Hz), 3.21−3.41 (m, 2H, β-CH_2, Asp), 3.52−3.69 (m, 2H, δ-CH_2, Pro), 3.97−4.05 (m, 2H, H-4′, α-CH, Asp), 4.12 (m, 1H, α-CH, Val), 4.23−4.26 (m, 3H, H-5′, H-3′), 4.48 (m, 1H, α-CH, Pro), 6.20 (t, 1H, H-1′, J = 6.0 Hz), 7.17 (s, 1H, H-5), 7.34 and 7.74 (AA′BB′ system, 2H, Ar, J = 8.0 Hz), 7.00 (d, 1H, NH, Val, J = 7.6 Hz), 8.59 (s, 1H, H-4). ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.9 (CH_3), 18.5, 19.0 (2C-γ, Val), 21.9 (CH_2), 24.4 (C-γ, Pro), 29.0, 29.7, 30.4, 30.9 ($3CH_2$, C-β, Pro), 34.9 (C-β, Val), 37.2 (C-β, Asp), 42.8 (C-2′), 46.6 (C-δ, Pro), 49.3 (C-α, Asp), 58.0 (C-α, Val), 59.1 (C-α, Pro),

64.0 (C-5′), 69.7 (C-3′), 84.5 (C-4′), 87.7 (C-1′), 98.7 (C-5), 107.2 (C-4a), 124.5, 125.8 (C-Hb, *ipso*-C), 129.1 (C-Ha), 137.6 (C-4), 144.2 (*para*-C), 153.8, 154.0 (C-6, C-2), 171.1, 171.3, 171.8, 172.0 (C═O, Val_1, C═O, Val_2, C═O, Pro, C-7a). MS (ESI^+): *m*/*z* 710.5 ($M + 1^+$), 732.5 ($M + Na^+$), 1420.1 ($2M + 1^+$). Anal. for $C_{36}H_{47}N_5O_{10}$: C, H, N.

Biological Methods. *Compounds and Enzymes.* Human soluble CD26 was purified as described and kindly provided by I. De Meester and A.-M. Lambeir (Antwerp, Belgium) or obtained from Sigma (St. Louis, MO). Fetal bovine serum (FBS) was obtained from Integro (Dieren, The Netherlands) and human serum provided by the Blood Bank, Leuven, Belgium.

Antiviral Activity Assays. The compounds were evaluated against varicella zoster virus (VZV) strains Oka and YS. The antiviral assay was based on inhibition of virus-induced cytopathicity (plaque formation in human embryonic lung (HEL) fibroblasts). Confluent cell cultures in microtiter 96-well plates were inoculated with 20 plaque forming units (PFU) of the virus. After a 1−2 h adsorption period, residual virus was removed, and the cell cultures were incubated in the presence of varying concentrations of the test compounds. Viral plaque formation was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. Antiviral activity was expressed as the EC_{50} or concentration required to reduce virus-induced cytopathogenicity or viral plaque formation by 50%.

Conversion of Tripeptidyl Prodrugs of Compound ***1*** *to the Corresponding Parent Compound.* The test compounds have been evaluated for their substrate activity against purified CD26, human serum (HS), and bovine serum (BS) in Eppendorf tubes. The 400 μL reaction mixtures contained 50 μM test compound (tripeptidyl prodrugs of compound **1**) in PBS (containing 0.1% DMSO). The reaction was started by the addition of purified CD26 (1.5 mU) or 20% of HS (in PBS) or BS (in PBS) at 37 °C. At different time points (as indicated in the figures) 100 μL was withdrawn from the reaction mixture, added to 200 μL of cold methanol, and put on ice for ∼10 min. Then the mixtures were centrifuged at 13 000 rpm for 5 min at 4 °C and 250 μL supernatant was analyzed by HPLC on a reverse phase RP-8 column, using following buffers and gradients.

Buffer A: 50 mM NaH_2PO_4 + 5 mM heptanesulfonic acid, pH 3.2. Buffer B: acetonitrile. Gradient A: 2 min 98% A + 2% B; 6 min linear gradient to 80% A + 20% B; 2 min linear gradient to 75% A + 25% B; 2 min linear gradient to 65% A + 35% B; 18 min linear gradient to 50% A + 50% B; 5 min 50% A + 50% B; 5 min linear gradient to 98% A + 2% B; 5 min equilibration at 98% A + 2% B.

Gradient B: 2 min 98% A + 2% B; 6 min linear gradient to 80% A + 20% B; 2 min linear gradient to 75% A + 25% B; 2 min linear gradient to 65% A + 35% B; 8 min linear gradient to 50% A + 50% B; 10 min 50% A + 50% B; 10 min linear gradient to 20% A + 80% B; 5 min 20% A + 80% B; 15 min linear gradient to 98% A + 2% B; 5 min 98% A + 2% B.

The gradients allowed separation of the tripeptidyl compound **1** prodrugs from the corresponding metabolites and parent compound **1**.

Exposure of CaCo-2 Cell Cultures to Compound ***1*** *and Its [Val-Pro]-[Val] Prodrug.* Human colon carcinoma CaCo-2 cells were seeded in the upper cups of dual-chamber 24-well trays at 1.5×10^5 cells/well in 500 μL of DMEM containing 10% fetal bovine serum. The bottom cups contained the same medium (without cells). After the cultures in the upper cups became confluent (∼3 days after seeding), the medium was carefully aspirated and replaced by 800 μL of DMEM containing either compound **1** or the 5′-Val-Pro-Val prodrug **13** at 100 μM. In the bottom wells, the medium was replaced by PBS. At different time points (i.e., 0, 1, 2, 3, 4, 5, 6, 7, and 24 h), 50 μL supernatant was removed from both the upper and bottom cup and added to 100 μL of cold methanol. After centrifugation, the drugs and drug metabolites in the methanolic supernatants were quantified by HPLC analysis as described above.

Administration of Compounds to Mice by Oral Gavage. Separate groups of female mice received equimolar doses of compounds **1** (25 mg), **13**, **17**, **22**, **30**, and **44** as a single oral gavage dose formulated in 0.5% carboxymethylcellulose. The mice were serially sacrificed at time-points ranging from 0.25 to 3 h after dosing (3 mice/time-point), and plasma samples were taken and analyzed for compound **1** concentration by HPLC with fluorescence detection. Results are reported as relative peak areas for compound **1**, which assumes that peak area is directly proportional to concentration over the ranges of concentrations.

■ ASSOCIATED CONTENT

Supporting Information. Results from elemental analysis data for novel compounds **3**−**46**; chemical procedures for the synthesis and identification of novel tri- and dipeptide derivatives **23**, **24**, and **35**−**40**; ^1H NMR and ^{13}C NMR chemical shifts assigments of compounds **3**−**5**, **7**, **10**, **12**, **14**−**16**, **18**−**21**, **25**−**29**, **31**, **33**, **41**, **43**, and **45**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
*Phone: (+34)-912587458. Fax: (+34)-91 5644853. E-mail: iqmsv29@iqm.csic.es.

■ DEDICATION

This paper is dedicated to the memory of Prof. Rafael Suau who died on November 11, 2010.

■ ACKNOWLEDGMENT

We thank Ria Van Berwaer, Lizette van Berckelaer, Lies Van den Heurck, Steven Carmans, and Anita Camps for excellent technical assistance. We also thank the Spanish MEC/MICINN (Project SAF2009-13914-C02), the Comunidad de Madrid (Project BIPEDD-CM ref S-BIO-0214-2006), and the K. U. Leuven (GOA No. 10/014) for financial support. We very much appreciated the stimulating discussions on the prodrug approach with Prof. C. McGuigan (Cardiff, U.K.).

■ ABBREVIATIONS USED

6-AQ, 6-aminoquinoline; BCNA, bicyclic nucleoside analogue; BOP, 1-benzotriazolyloxy-tris-dimethylaminophosphonium hexafluorophosphate; BS, bovine serum; CCTLC, circular thin layer chromatography; CD26, cluster of differentiation 26; DBAD, di-*tert*-butyl azodicarboxylate; DCC, *N*,*N*′-dicyclohexylcarbodiimide; DIAD, diisopropyl azodicarboxylate; DMAP, 4-dimethylaminopyridine; DPPIV, dipeptidyl peptidase IV; FBS, fetal bovine serum; GPG-NH_2, glycyl-prolyl-glycynamide; HEL, human embryonic lung; hPEPT1, human intestinal oligopeptide transporter 1; HS, human serum; NAP, N-3 aminopropyl; PBS, phosphate buffered saline; PS-PPh_3, polymer-bound triphenylphosphine; TBDMSCl, *tert*-butyldimethylsilyl chloride; Thd, thymidine; TSAO, (*tert*-butyldimethylsilyl-β-D-ribofuranosyl)-3′-spiro-4″-amino-1″,2″-oxathiole 2″,2″-dioxide; VZV, varicella zoster virus

■ REFERENCES

(1) Fox, D. A.; Hussey, R. E.; Fitzgerald, K. A.; Acuto, O.; Poole, C.; Palley, L.; Daley, J. F.; Schossman, S. F.; Reinhert, E. L. Ta1, a novel 105 kDa human T cell activation antigen defined by a monoclonal antibody. *J. Immunol.* **1984**, *133*, 1250–1256.

(2) De Meester, I.; Vanhoof, G.; Lambeir, A.-M.; Scharpe, S. CD26, let it cut or cut it down. *Immunol. Today* **1999**, *20*, 367–375.

(3) Lambeir, A.-M.; Durinx, C.; Scharpe, S.; De Meester, I. Dipeptidyl-peptidase IV from bench to bedside: an update on structural properties, functions and clinical aspects of the enzyme DPPIV. *Crit. Rev. Clin. Lab. Sci.* **2003**, *40*, 209–294.

(4) Yaron, A.; Naider, F. Proline-dependent structural and biological properties of peptides and proteins. *Crit. Rev. Biochem. Mol.* **1993**, *28*, 31–81.

(5) Mentlein, R. Dipeptidyl-peptidase IV (CD26)-role in the inactivation of regulatory peptides. *Regul. Pept.* **1999**, *85*, 9–24.

(6) Mentlein, R. Cell-surface peptidases. Cell-surface peptidases. *Int. Rev. Cytol.* **2004**, *235*, 165–202.

(7) Balzarini, J.; Andersson, E.; Schols, D.; Proost, P.; Van Damme, J.; Svennerholm, B.; Horal, P.; Vahlne, A. Obligatory involvement of CD26/dipeptidyl peptidase IV in the activation of the antiretroviral tripeptide glycylprolylglycinamide (GPG-NH_2). *Int. J. Biochem. Cell Biol.* **2004**, *36*, 1848–1859.

(8) García-Aparicio, C.; Bonache, M.-C.; De Meester, I.; San-Félix, A.; Balzarini, J.; Camarasa, M.-J.; Velázquez, S. Design and discovery of a novel dipeptidyl-peptidase IV (CD26)-based prodrug approach. *J. Med. Chem.* **2006**, *49*, 5339–5351.

(9) García-Aparicio, C.; Diez-Torrubia, A.; Balzarini, J.; Lambeir, A.-M.; Velázquez, S.; Camarasa, M.-J. Efficient conversion of tetrapeptide-based TSAO prodrugs to the parent drug by dipeptidyl-peptidase IV (DPPIV/CD26). *Antiviral Res.* **2007**, *76*, 130–139.

(10) Balzarini, J.; Pérez-Pérez, M. J.; San-Félix, A.; Schols, D.; Perno, C. F.; Vandamme, A. M.; Camarasa, M. J.; De Clercq, E. 2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)pyrimidine (TSAO) nucleoside analogues: highly selective inhibitors of human immunodeficiency virus type 1 that are targeted at the viral reverse transcriptase. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 4392–4396.

(11) Camarasa, M. J.; Pérez-Pérez, M. J.; San-Félix, A.; Balzarini, J.; De Clercq, E. 3′-Spironucleosides (TSAO derivatives), a new class of specific human immunodeficiency virus type 1 inhibitors: synthesis and antiviral activity of 3′-spiro-5″-[4″-amino-1″,2″-oxathiole-2″,2″-dioxide]pyrimidine nucleosides. *J. Med. Chem.* **1992**, *35*, 2721–2727.

(12) Camarasa, M. J.; San-Félix, A.; Velázquez, S.; Pérez-Pérez, M. J.; Gago, F.; Balzarini, J. TSAO compounds: the comprehensive story of a unique family of HIV-1 specific inhibitors of reverse transcriptase. *Curr. Top. Med. Chem.* **2004**, *4*, 945–963.

(13) Diez-Torrubia, A.; García-Aparicio, C.; Cabrera, S.; De Meester, I.; Balzarini, J.; Camarasa, M. J.; Velázquez, S. Application of the dipeptidyl-peptidase IV (DPPIV/CD26)-based prodrug approach to different amine-containing drugs. *J. Med. Chem.* **2010**, *53*, 559–572.

(14) McGuigan, C.; Yarnold, C. J.; Jones, G.; Velázquez, S.; Barucki, H.; Brancale, A.; Andrei, G.; Snoeck, R.; De Clercq, E.; Balzarini, J. Potent and selective inhibition of varicella-zoster virus (VZV) by nucleoside analogues with an unusual bicyclic base. *J. Med. Chem.* **1999**, *42*, 4479–4484.

(15) McGuigan, C.; Barucki, H.; Blewet, S.; Carangio, A.; Erichsen, J. T.; Andrei, G.; Snoeck, R.; De Clercq, E.; Balzarini, J. Highly potent and selective inhibition of varicella-zoster virus by bicyclic furopyrimidine nucleosides bearing an aryl side chain. *J. Med. Chem.* **2000**, *43*, 4993–4997.

(16) Mc Guigan, C.; Balzarini, J. Aryl furano pyrimidines, the most potent and selective anti-VZV agents reported to date. *Antiviral Res.* **2006**, *71*, 149–153.

(17) McGuigan, C.; Pathirana, R. N.; Migliore, M.; Adak, V.; Luoni, G.; Jones, A. T.; Diez-Torrubia, A.; Camarasa, M. J.; Velázquez, S.; Henson, G.; Snoeck, R.; Andrei, G.; Balzarini, J. Preclinical development of bicyclic nucleoside analogues as potent and selective inhibitors of varicella zoster virus. *J. Antimicrob. Chemother.* **2007**, *60*, 1316–1330.

(18) McGuigan, C.; Balzarini, J. FV100 as a new approach for the possible treatment of varicella-zoster virus infection. *J. Antimicrob. Chemother.* **2009**, *64*, 671–673.

(19) (a) Colla, L.; De Clercq, E.; Busson, R.; Vanderhaeghe, H. Synthesis and antiviral activity of water-soluble esters of acyclovir [9-[(2-hydroxyethoxy)methyl]guanine]. *J. Med. Chem.* **1983**, *26*, 602–604. (b) Beauchamp, L. M.; Orr, G. F.; De Miranda, P.; Burnette, T.; Krenitsky, T. A. Amino acid ester prodrugs of acyclovir. *Antivir. Chem. Chemother.* **1992**, *3*, 157–164.

(20) (a) Beutner, K. R. Valacyclovir: a review of its antiviral activity, pharmacokinetic properties, and clinical efficacy. *Antiviral Res.* **1995**, *28*, 281–290. (b) MacDougall, C.; Guglielmo, B. J. Pharmacokinetics of valacyclovir. *J. Antimicrob. Chemother.* **2004**, *53*, 899–901.

(21) Cocohoba, J. M.; McNicholl, I. R. Valganciclovir: an advance in cytomegalovirus therapeutics. *Ann. Pharmacother.* **2002**, *6*, 1075–1079.

(22) Montero, J.-L.; Criton, M.; Dewynter, G.-F.; Imbach, J.-L. Aminoacylation of nucleosides. Mitsunobu conditions versus chemoenzymatic route. *Tetrahedron Lett.* **1991**, *32* (39), 5357–5358.

(23) Wei, Y.; Pei, D. Activation of antibacterial prodrugs by peptide deformylase. *Bioorg. Med. Chem.* **2000**, *10*, 1074–1076.

(24) Reynolds, A. J.; Kassiou, M. Recent advances in the Mitsunobu reaction: modifications and applications to biologically active molecules. *Curr. Org. Chem.* **2009**, *13* (16), 1610–1632.

(25) Dandapani, S.; Curran, D. P. Separation-friendly Mitsunobu reactions: a microcosm of recent developments in separation strategies. *Chem.—Eur. J.* **2004**, *10*, 3130–3138.

(26) Pelletier, J. C.; Kincaid, S. Mitsunobu reaction modifications allowing product isolation without chromatography: application to small parallel library. *Tetrahedron Lett.* **2000**, 797–800.

(27) Woo, R. B.; Meyer, Jr.; Gamper, H. B. G/C-modified oligodeoxynucleotides with selective complementarity: synthesis and hybridization properties. *Nucleic Acids Res.* **1996**, *24*, 2470–2475.

(28) McGuigan, C.; Pathirana, N.; Jonse, G.; Andrei, G.; Snoeck, R.; De Clercq, E.; Balzarini, J. *Antiviral Chem. Chemother.* **2000**, *11*, 343–348.

(29) Loakes, D.; Brown, D. M.; Salisbury, S. A.; McDougall, M. G.; Neagu, C.; Nampalli, S.; Kumar, S. Synthesis and some biochemical properties of a novel 5,6,7,8-tetrahydropyrimido[4,5-*c*]pyridazine nucleoside. *Helv. Chim. Acta* **2003**, *86*, 1193–1204.

(30) Friedrich-Bochnitschek, S.; Waldmann, H.; Kunz, H. Allyl esters as carboxy protecting groups in the synthesis of *O*-glycopeptides. *J. Org. Chem.* **1989**, *54*, 751–756.

(31) Deziel, R. Mild palladium(0)-catalyzed deprotection of allyl esters. A useful application in the synthesis of carbapenems and other β-lactam derivatives. *Tetrahedron Lett.* **1987**, *28* (38), 4371–4372.

(32) Dessolin, M.; Guillerez, M. C.; Thieriet, N.; Guibé, F.; Loffet, A. New allyl group acceptors for palladium catalyzed removal of allylic protections and transacylation of allyl carbamates. *Tetrahedron Lett.* **1995**, *36*, 5741–5744.

(33) Scheen, A. J. Dipeptidylpeptidase-4 inhibitors (gliptins): focus on drug−drug interactions. *Clin. Pharmacokinet.* **2010**, *49*, 573–588.

(34) Gerich, J. DPP-4 inhibitors: what may be the clinical differentiators? *Diabetes Res. Clin. Pract.* **2010**, *90*, 131–140.

(35) Keating, G. M. Vildagliptin: a review of its use in type 2 diabetes mellitus. *Drugs* **2010**, *70*, 2089–2112.