DOI: 10.1002/cmdc.201200295

Dipeptidyl Peptidase IV-Activated Prodrugs of Anti-Varicella Zoster Virus Bicyclic Nucleoside Analogues Containing Different Self-Cleavage Spacer Systems

Alberto Diez-Torrubia,[a] Silvia Cabrera,[a] Ingrid De Meester,[b] María-José Camarasa,[a] Jan Balzarini,[c] and Sonsoles Velázquez*[a]

Dedicated to Prof. José Luis García Ruano on the occasion of his 65th birthday

A new type of double prodrug of the antiviral family of bicyclic nucleoside analogues (BCNA) bearing cyclization self-cleavage spacers between the Val-Pro dipeptide sequence as well as the parent compound were synthesized and evaluated with regard to activation by the DPPIV/CD26 enzyme and for their stability in human and bovine serum. In buffer solution, carbamate and ester prodrugs were found to be chemically stable. Most prodrugs containing a dipeptidyl linker efficiently converted into the BCNA parent drug. In contrast, the Val-Pro alkyldiamino prodrugs converted predominantly into their alkyldiamino prodrug intermediates in the presence of CD26 and human serum. A marked increase in water solubility was observed for all prodrugs. In contrast to the parent compound, a tetrapeptide prodrug containing the Val-Val dipeptide as a self-cleavage spacer released substantial amounts of the BCNA parent drug at the basolateral side of Caco-2 cell cultures and exhibited 15- to 20-fold increased bioavailability in mice relative to the poorly bioavailable parent compound.

Introduction

Dipeptidyl peptidase IV (DPPIV, also termed CD26) is a serine peptidase that is part of the prolyl oligopeptidase superfamily, which removes X-Pro (and to a lesser extent X-Ala) dipeptides from the N-terminus of natural peptides, including many chemokines, neuropeptides, and peptide hormones.[1–4] This enzyme occurs in two forms, an extracellular membrane-bound peptidase (highly expressed on endothelial cells, epithelial cells, and lymphocytes) and a soluble form that is found in plasma.[5,6]

In 2005, it was first demonstrated that a synthetic small molecule [GPG-NH_2, (glycylprolylglycinamide)] can be converted into an antiviral drug (glycinamide) through the specific action of DPPIV/CD26.[7] Based on these results, we previously reported[8] a novel type of DPPIV/CD26-directed prodrug technology that can be applied to improve the solubility and/or bioavailability of therapeutic agents. With our technology, the prodrugs are conjugates of a therapeutic drug and a di- or oligopeptide moiety that is cleavable by the DPPIV/CD26 activity. This approach was first successfully applied to a broad variety of amine-containing drugs having a free amino group on their alkyl chains (N-3 aminopropyl derivative of the anti-HIV TSAO compounds)[9,10] as well as on heteroaromatic (6-aminoquinoline), carbohydrate (doxorubicin), heterocyclic pyrimidine (ara-C and TSAO-m5C), and purine rings (ara-A).[11] In these prodrugs, the compounds/drugs and the peptide moiety are directly coupled via an amide bond which is specifically cleaved by DPPIV/CD26 (Figure 1). Our data revealed that purified DPPIV/CD26 could recognize these prodrugs as substrates and efficiently release the parent compound. Interestingly, it was possible to modulate the enzymatic and serum hydrolysis rates (half-lives) of the prodrug conjugates by changing the nature and the length of the peptide promoiety.[8,12]

We also recently reported[13] the application of this prodrug approach to hydroxy-containing drugs, in particular, the furanopyrimidine bicyclic nucleoside analogues (a prototype of which is designated Cf1743 (**1**), Figure 2), which represent a promising new family of antivirals with extreme potency and specificity for varicella zoster virus (VZV) infections but have

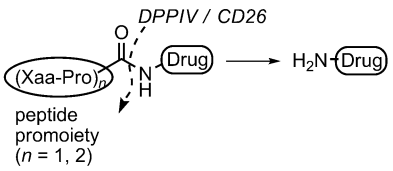


Figure 1. Application of the DPPIV/CD26-based prodrug approach to amine-containing drugs.

[a] *Dr. A. Diez-Torrubia, Dr. S. Cabrera, Prof. M.-J. Camarasa, Dr. S. Velázquez*
Instituto de Química Médica (CSIC)
Juan de la Cierva 3, 28006 Madrid (Spain)
E-mail: sonsoles@iqm.csic.es

[b] *Dr. I. De Meester*
Laboratory of Medical Biochemistry
University of Antwerp, Universiteitsplein 1, 2610 Antwerp (Belgium)

[c] *Prof. J. Balzarini*
Rega Institute for Medical Research
Katholieke Universiteit (KU) Leuven
Minderbroedersstraat 10, 3000 Leuven (Belgium)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.201200295.

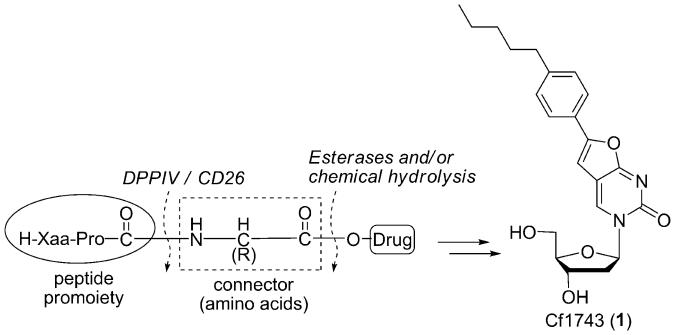


Figure 2. Two-step activation of DPPIV/CD26-based prodrugs of hydroxy-containing drugs.

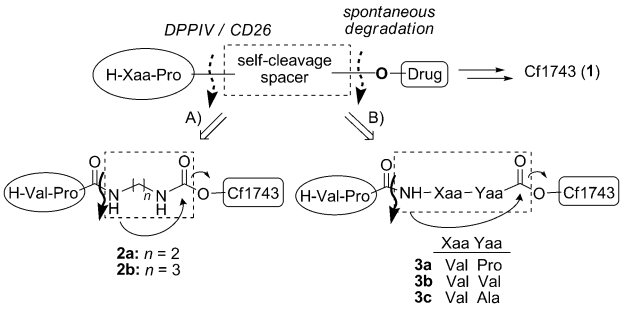


Figure 3. General approaches for the design of DPPIV/CD26-activated prodrugs of antiviral Cf1743 bearing cyclization connectors as self-cleavage spacers. Structure of target prodrugs **2a** and **b** and **3a**–**c**.

unfavorable pharmacokinetic properties.[14,15] Thus, our purpose was twofold: to study for the first time the applicability of this strategy to hydroxy-containing drugs, and to improve the poor aqueous solubility and low oral bioavailability of these highly lipophilic antiviral drugs. In hydroxy-containing drugs, the peptide sequence cannot be linked directly to the hydroxy group through an ester bond because the DPPIV/CD26 enzyme specifically recognizes amide bonds. Thus, heterobifunctional spacers such as amino acids were designed for attachment to the dipeptide through an amide bond (cleavable by DPPIV/CD26) and for attachment to the OH group of the drug through a metabolically labile ester linkage (Figure 2). Liberation of the parent drug from these tripartite prodrugs should therefore take place through a two-step hydrolysis sequence, first involving DPPIV/CD26-mediated enzymatic cleavage of the terminal dipeptide, followed by chemical or enzymatic (esterase-catalyzed) hydrolysis of the ester bond (Figure 2). Our data proved that the prodrugs efficiently release the parent drug upon selective conversion by purified DPPIV/CD26, as well as by soluble DPPIV/CD26 present in bovine and human serum. DPPIV/CD26 was solely responsible for the efficient hydrolysis of the dipeptide moiety from the prodrugs as the DPPIV/CD26-specific inhibitor vildagliptin was able to completely block the enzymatic conversion in human, bovine and murine serum. Several prodrugs showed remarkable increases in water solubility and markedly enhanced oral bioavailability in mice relative to the parent free compound.[13a]

Based on these encouraging results, we decided to explore self-cleavage spacers in the design of novel two-step activation prodrugs of the antiviral Cf1743 (Figure 3), attached to the 5′-hydroxy group of the nucleoside through a carbamate or ester linkage. They were designed to undergo enzymatic activation via DPPIV/CD26, followed by spontaneous release of the parent drug. In this way, ideally, and contrary to what occurred when amino acids were used as spacers in our previous studies,[13] final generation of the free drug may not be dependent on enzymatic hydrolysis (esterase-catalyzed) of the ester bond.

Cyclization self-cleavage spacers were selected.[16] We first turned our attention to the prodrugs of Cf1743 (**2a** and **b**), incorporating ethylene- and propylenediamine spacers[17] linked to the dipeptide moiety via an amide bond and to the 5′-hydroxy group of the nucleoside via a carbamate bond (Figure 3, approach A). After DPPIV/CD26 hydrolysis the spacer should spontaneously cyclize to give a cyclic urea derivative, thereby releasing free Cf1743.

Second, peptide ester prodrugs **3a**–**c** containing a dipeptide cyclization spacer (XaaYaa) between the dipeptide and CF1743 were prepared (Figure 3, approach B). These tetrapeptide prodrugs were designed to release the parent compound through an initial enzymatic conversion step (DPPIV/CD26), followed by spontaneous cyclization of the dipeptide conjugate intermediate via diketopiperazine (DKP).[16a,18–20] It is well documented in the literature that dipeptide-based ester prodrugs containing a C-terminal proline residue readily cyclize to the DKP due to the geminal-dimethyl effect.[21] The rate of drug release also depends on the size of the side chains of the dipeptide carrier, that is, the bulkier the amino acids that are incorporated in the dipeptide backbone, the slower the drug release.[18,22] Based on these reports, we incorporated prodrugs **3a**–**c**, bearing dipeptides (XaaYaa) with a C-terminal proline residue and/or (β-branched) amino acids of different molecular size, as cyclization spacers in order to diversify the half-lives of the prodrugs.

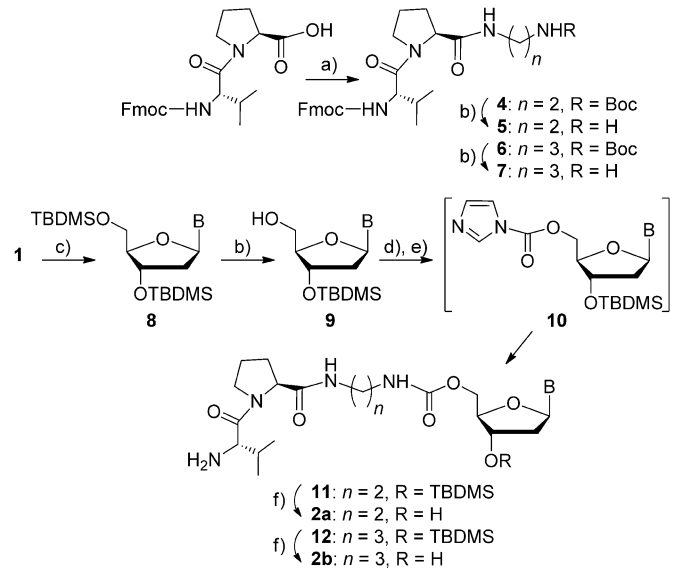
We herein describe the synthesis, water solubility, and chemical and enzymatic stability studies of these novel prodrugs of antiviral compound Cf1743 (**1**). In addition, permeability/transport across colon carcinoma Caco-2 cell layers with selected prodrugs and oral bioavailability in vivo in one selected tetrapeptide prodrug were also studied.

Results and Discussion

Synthesis

The target prodrugs were prepared by coupling of the 5′-hydroxy group of the antiviral nucleoside and the spacer end of the appropriate dipeptide-spacer moiety. Val-Pro was selected as the dipeptide for the prodrugs because it is efficiently recognized by DPPIV/CD26, as shown in previous studies.[8,12,13]

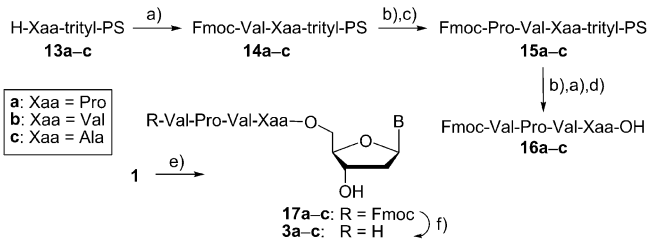
We first focused on the synthesis of carbamate prodrugs **2a** and **b**, bearing diamine self-cleavage spacers. The required dipeptide-spacer amines **5** and **7** (Scheme 1) were prepared in 80% and 95% yield by standard coupling of commercially available Fmoc-Val-Pro-OH with *N*-Boc-ethylenediamine or *N*-Boc-propylenediamine in the presence of dicyclohexylcarbodii-



Scheme 1. Synthesis of carbamate prodrugs **2a** and **b** from 3′-protected nucleoside **8**. *Reagents and conditions:* a) NH_2-$(CH_2)_n$-NHBoc, BOP, Et_3N, CH_2Cl_2, RT; b) HCl (1.25 M), MeOH, RT; c) TBDMSCl, imidazole, DMAP, DMF, RT; d) 1. CDI, Et_3N, DMF, RT; 2. H_2O; e) compound **5** or **7**, 40 °C; f) TBAF (1.1 M), THF, RT.

mide (DCC) and triethylamine (TEA), followed by acid hydrolysis of protected dipeptide intermediates **4** and **6** in a 1.25 M HCl solution. To introduce the carbamate linkage between the nucleoside and the dipeptide-spacer moiety, we needed to selectively activate the 5′-hydroxy group of the antiviral nucleoside. Initial attempts to form 5′-carbamate prodrug **2a** were carried out by in situ activation of unprotected nucleoside **1**, followed by reaction of the activated intermediate with dipeptide-spacer amine **5**. However, activation of nucleoside **1**, either with 4-nitrophenyl chloroformate and TEA or with carbonyldiimidazole (CDI) and pyridine in dry DMF, followed by reaction of the carbonate intermediates with amine **5** at room temperature afforded mixtures of the 5′- and 3′-carbamates in low yields along with starting material as the major compound. We next investigated an alternative approach for the synthesis of 5′-carbamate **2a** using the 3′-*tert*-butyldimethylsilyl (TBDMS)-protected nucleoside **9** as starting material (Scheme 1). The synthesis of protected nucleoside **9** was carried out in high yield by treatment of the fully TBDMS protected nucleoside **8** with 1.25 M HCl solution in methanol. Activation of the nucleoside was performed by treatment of **9** with an excess (5 equiv) of CDI and TEA at room temperature. After quenching the excess reagent through addition of water, the activated intermediate **10** was reacted directly with peptide-spacer amine **5** at 40 °C for 24 h to afford the 3′-TBDMS-protected carbamate **11** in good yield (51% over the two steps). Under these conditions, simultaneous deprotection of the Fmoc group was observed, likely due to the imidazole generated in the reaction media. The silyl protecting group was readily removed by reaction of **11** with 1.1 M TBAF solution in THF at room temperature to give the desired fully deprotected prodrug **2a** in 63% yield. Similarly, treatment of activated nucleoside **10** with the corresponding peptide–propylenediamine spacer (compound **7**), followed by TBDMS deprotection, afforded final target prodrug **2b** in good yield.

Tetrapeptide prodrugs **3a–c**, bearing a variety of dipeptide cyclization spacers, were next prepared by coupling of appropriate tetrapeptide sequences to parent nucleoside **1** (Scheme 2). The tetrapeptide pro-moieties **16a–c** were pre-



Scheme 2. Synthesis of tetrapeptide ester prodrugs **3a–c**. *Reagents and conditions:* a) Fmoc-Val-OH, HCTU, DIEA, DMF, RT; b) piperidine (20%), DMF; c) Fmoc-Pro-OH, HCTU, DIEA, DMF, RT; d) CH_2Cl_2/TFA (95:5), RT; e) Fmoc-Val-Pro-Val-Xaa-OH (**16a–c**), PPh_3, DBAD, DMF, 80 °C, MW; f) piperidine (5%), DMF.

pared in good yields by solid-phase synthesis using an Fmoc strategy on a 2-chlorotrityl polystyrene resin as previously described (see Supporting Information).[12] Regioselective coupling of these peptides on the 5′ position of the parent nucleoside via Mitsunobu condensation was first investigated in a similar way to that described previously for the 5′-O-acylation of **1** with amino acids.[13] Disappointingly, reaction of **1** with Fmoc-Val-Pro-Val-Pro-OH (**16a**) in the presence of PPh_3 and di-*tert*-butylazodicarboxylate (DBAD) in dry DMF (under argon atmosphere) at room temperature for several days failed to give 5′-tetrapeptide prodrug **17a**. However, treatment of **1** with pro-moiety **16a** under the aforementioned Mitsunobu conditions (PPh_3 and DBAD) under microwave irradiation (MW, 80 °C) for 8 h afforded desired tetrapeptide prodrug **17a** in 45% yield after chromatographic purification.

The syntheses of compounds **17b** and **c** were carried out in a similar manner, starting from the appropriate tetrapeptide derivatives (**16b** and **c**) (Scheme 2). Removal of the Fmoc group by brief treatment of **17a–c** with piperidine afforded the target-deprotected prodrugs **3a–c** in good to excellent yields (76–97%). The target prodrugs were characterized by ^1H and ^{13}C NMR, mass spectrometry, and microanalysis; all data confirmed the structure and purity of the novel compounds.

Water solubility studies

In addition, the water solubility of the [Val-Pro]-[self-cleavage spacer]-[Cf1743] prodrugs **2a**, **2b**, and **3a–c** were determined and compared with that of the poorly soluble parent compound (Table 1). All prodrugs dramatically improved the water solubility. Values ranged from 7.810 to 32.469 mg mL^{-1}, corresponding to 434- to 1804-fold higher water solubility in comparison with the poorly soluble parent drug Cf1743 (0.018 mg mL^{-1}). These results further support the applicability of the DPPIV/CD26 prodrug approach for increasing the water

Table 1. Experimental aqueous solubility and other physicochemical parameters of [Val-Pro]-[self-cleavage spacer]-[Cf1743] prodrugs **2a**, **2b**, **3a–c**, and parent compound **1**.

Compd	Aqueous solubility [mg mL^{-1}][a]	Increase[b]	log *S*	Clog *P*[c]	t_R[d] [min]	mp [°C]
Cf1743 (**1**)	0.018 ± 0.004	–	−4.35	2.48	35.7	204–207
2a	32.469 ± 0.303	1804	−1.32	2.6	31.6	84–86
2b	29.838 ± 0.167	1658	−1.37	–	–	–
3a	10.855 ± 0.229	603	−1.86	3.27	35.8	108–110
3b	7.810 ± 0.329	434	−2.01	3.60	37.6	113–115
3c	9.089 ± 0.394	505	−1.93	3.09	34.3	119–121

[a] Thermodynamic water solubility measured at 25 °C after 24 h; values are typically means of three measurements. [b] Fold increase in water solubility relative to parent drug **1**. [c] Calculated log *P* values obtained with the ALOGPS 2.1 program (http://www.vcclab.org/lab/alogps/). [d] HPLC retention times determined with a reversed-phase column [Lichro Card (LiChrospher 60 RP-select B; 5 μm; Merck)] (4.6 mm × 250 mm).

solubility of hydrophobic drugs. In general, the aqueous solubility of small molecules is influenced by their hydrophobicity (log *P*).[23] Thus, a classical strategy for improving aqueous solubility has been the introduction of hydrophilic groups into molecules. However, this approach is far from being universally effective. It has also been proposed that disruption of crystal packing could be an alternative method for improving aqueous solubility.[24] In an attempt to explain the improved solubility of prodrugs **2a**, **2b**, and **3a–c**, the effect of changes in hydrophobicity as well as modification of crystal packing of prodrugs versus parent drug was examined next. To clarify the contributions of the different mechanisms, physicochemical parameters including Clog *P*, retention time on reversed-phase HPLC (related to hydrophobicity), and melting point values (related to crystal packing) were evaluated (Table 1).

These physicochemical parameters and log(solubility) (log *S*) were plotted as shown in Figure 4. In all cases, log *S* was more strongly correlated with melting point values (Figure 4a) than with hydrophobicity (Clog *P*) and retention time parameters (Figure 4b,c). Thus, the increase in aqueous solubility does not seem to be caused by a decrease in hydrophobicity. Several reports have examined the relationship between solubility and crystal packing or melting point values.[24] Despite the limited number of prodrug analogues examined in our study, it became clear that our results represent another example in which disruption of crystal packing could play an important role in improved aqueous solubility.

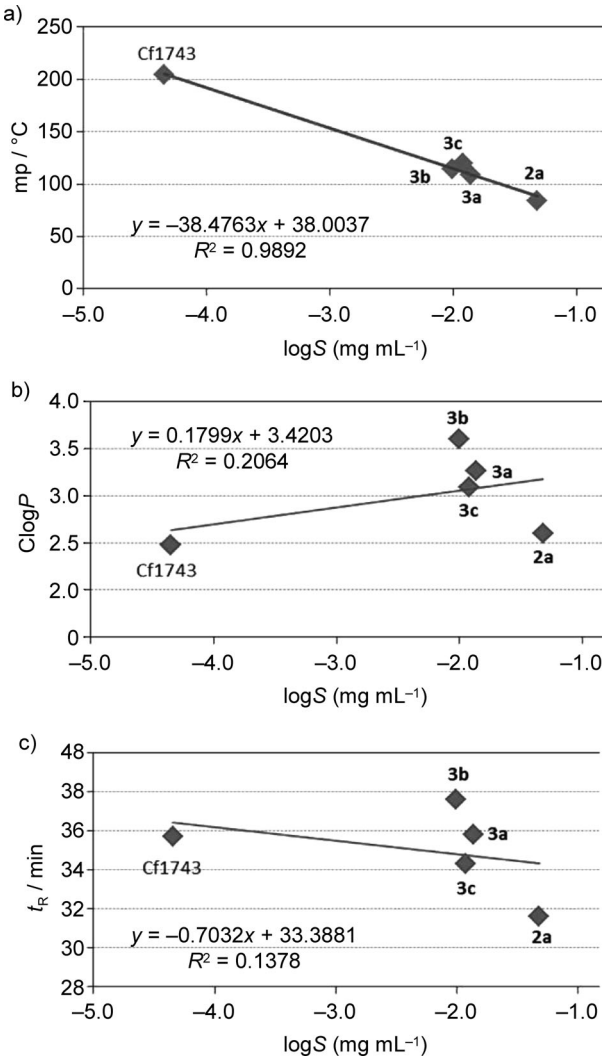


Figure 4. Relationships between experimentally observed solubility values and physicochemical data of prodrugs **2a**, **3a–c**, and parent compound **1**: a) melting point; b) Clog *P*; c) HPLC retention time (t_R).

Biological evaluation

The Cf1743 prodrugs were examined for their stability in PBS (pH 7.4), their susceptibility to degradation by CD26 enzymatic activity, and their conversion into parent compound in human and bovine serum (20% in PBS). Two prodrugs of Cf1743 were investigated in which the terminal Val-Pro dipeptide was linked to the parent molecule through an ethylenediamino (**2a**) or propylenediamino (**2b**) spacer (figures S1 and S2, Supporting Information). Both prodrugs were fully stable in PBS. Exposure to CD26 or human serum revealed time-dependent conversion into an intermediate that lacked the terminal Val-Pro moiety. After 4 h, virtually complete conversion into this intermediate was observed (figures S1 and S2, panels B and C). Interestingly, in bovine serum, the prodrugs not only converted into the alkyldiamino-Cf1743 intermediate, but a time-dependent release of the parent Cf1743 drug was also observed (figures S1 and S2, panel D). In fact, after 24 h, almost 40% Cf1743 was formed from the **2a** prodrug, and > 95% Cf1743 was derived from the **2b** prodrug. This means that the spacer was released (either spontaneously or enzymatically) from the **2a** and **2b** prodrugs in bovine serum, but not in human serum. When the **2b** prodrug was exposed to bovine serum in the presence of vildagliptin, a potent inhibitor of CD26 activity, no intermediate was released from the prodrug, nor could the parent drug (Cf1743) be detected (data not shown). These observations revealed that the eventual release of Cf1743 from the prodrug can only be performed after the dipeptide moiety has been released from the prodrug. Thus, the enzyme responsible for the even-

tual release of Cf1743 must specifically act on the alkyldiamino Cf1743 conjugate. Similar observations were observed with bovine serum that was extensively dialyzed in PBS to remove any small molecules from the enzyme preparation. This points to the lack of necessity of participation of additional small organic molecule substrates such as amino acids or sugars in the enzymatic conversion reaction.

The Val-Pro-Val-Pro-Cf1743 tetrapeptide prodrug (**3a**) proved stable in PBS (Figure 5a) but was highly susceptible to CD26-catalyzed hydrolysis of the parent compound (Figure 5b). Conversion of the tetrapeptide to the parent compound proceeded very efficiently, and no intermediary Val-Pro dipeptide prodrug was observed during the conversion process to parent drug Cf1743. Also, human and bovine serum efficiently released the parent compound without detectable release of the dipeptide intermediate (Figure 5c,d).

Next, Val-Pro-Val-Val-Cf1743 (**3b**) was studied for stability in PBS and CD26 susceptibility against the purified enzyme and in serum. While very stable in PBS (only ~6% conversion into the parent drug after 24 h) (Figure 6a), the tetrapeptide prodrug was rapidly converted into Val-Val-Cf1743 and Cf1743 (60% and 13%, respectively, after 1 h) in the presence of CD26. After 24 h, all of the prodrug was converted into a 40:60 mixture of Val-Val-Cf1743 and Cf1743 (Figure 6b). Human and bovine serum (Figure 6c,d) showed a similar conversion profile. After 24 h, appearance of Cf1743 in the serum was still inferior to formation of the Val-Val-Cf1743 intermediate. When similar incubations with Val-Val-Cf1743 were performed, the dipeptide prodrug was quite stable in PBS as well as in the CD26 assay and in serum incubations (Figure S3). Only after 24 h incubation was the Val-Val-Cf1743 dipeptide prodrug further converted into Cf1743, to a limited extent.

Upon investigation of the tetrapeptide Val-Pro-Val-Ala-Cf1743 (**3c**), the formation of small amounts of Cf1743 in PBS was observed as a function of time (figure S4, panel A, Supporting Information). However, CD26 efficiently converted the tetrapeptide prodrug to Cf1743. Only trace amounts of the intermediary dipeptide Val-Ala-Cf1743 could be observed (figure S4, panel B). Most likely, CD26 also cleaved the Val-Ala dipeptide from the dipeptide prodrug intermediate to release Cf1743. The Val-Ala dipeptide has indeed been shown to act as a substrate recognition motif for CD26.[22] Both human and bovine serum also efficiently and time-dependently converted the tetrapeptide prodrug to Cf1743 (figure S4, panels C and D).

To determine the potentially increased oral bioavailability of the prodrugs compared with the poorly oral bioavailable parent drug Cf1743, uptake and release of the tetrapeptide prodrug Val-Pro-Val-Val-Cf1743 (**3b**) and its parent Cf1743 were investigated in confluent Caco-2 monolayer cell cultures. In this assay model, the drugs were exposed at the apical side of the cell culture monolayer, and release of the parent drug (or prodrug/intermediates) at the basolateral side of the cell cultures was determined as a function of exposure time (Figure 7). Whereas Cf1743 was poorly transported through the Caco-2 monolayers, exposure of equimolar amounts of the tetrapeptide prodrug **3b** resulted in a pronounced appearance of Cf1743 in the basolateral compartment. Also, measurable

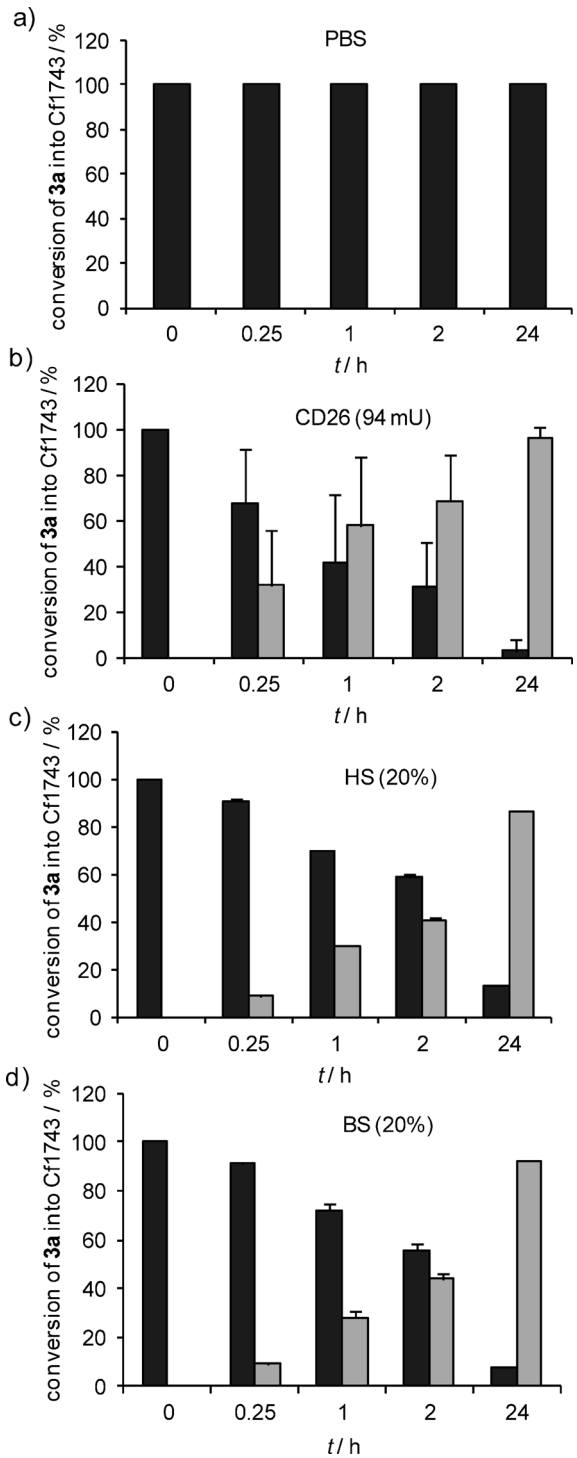


Figure 5. Stability of [Val-Pro][Val-Pro]-Cf1743 (**3a**; ■) in a) PBS and its conversion into parent drug Cf1743 (■) in the presence of b) purified CD26, c) human serum (HS), and d) bovine serum (BS).

amounts of the dipeptide intermediate (Val-Val-Cf1743) were also observed to be released at the basolateral side of the Caco-2 cell cultures, but these prodrug levels were invariably well below the Cf1743 amounts.

These data indicate a markedly increased potential for oral bioavailability of the tetrapeptide prodrug versus parent drug

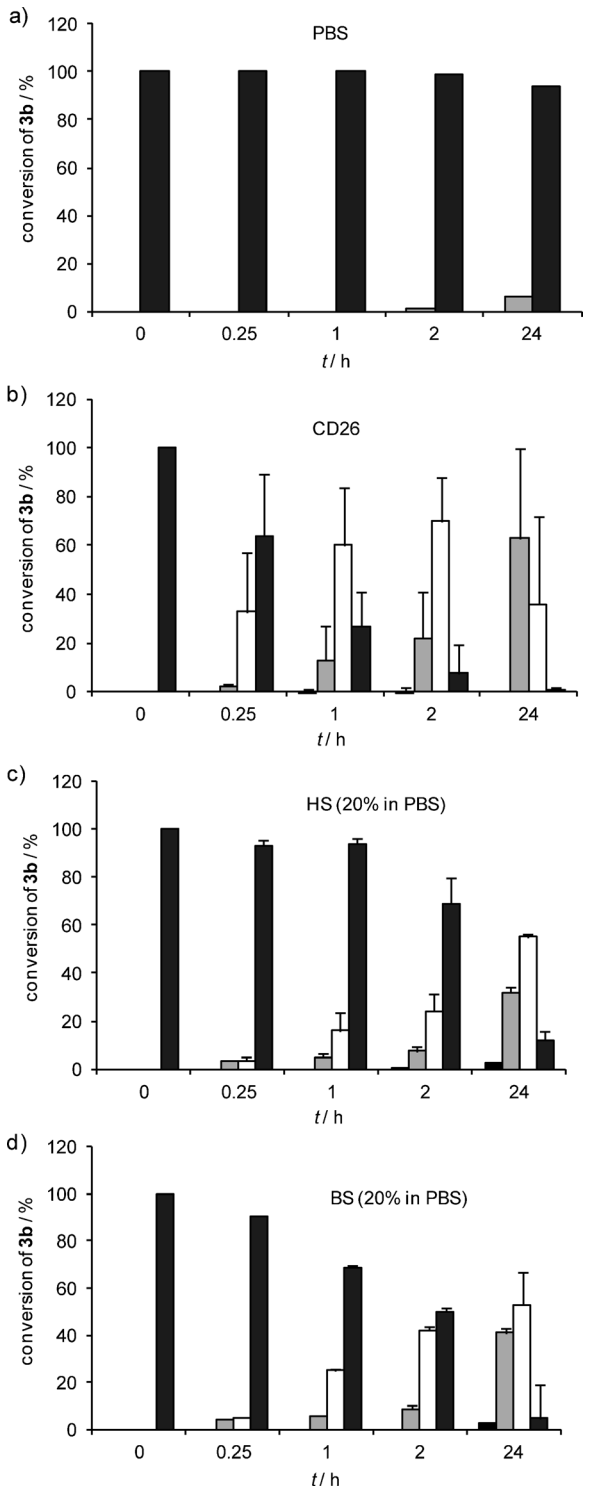


Figure 6. Stability of [Val-Pro][Val-Val]-Cf1743 (**3b**; ■) in a) PBS and its conversion into parent drug Cf1743 (■) and intermediates [Val]-Cf1743 (■) and [Val-Val]-Cf1743 (□) in the presence of b) purified CD26, c) human serum (HS), and d) bovine serum (BS).

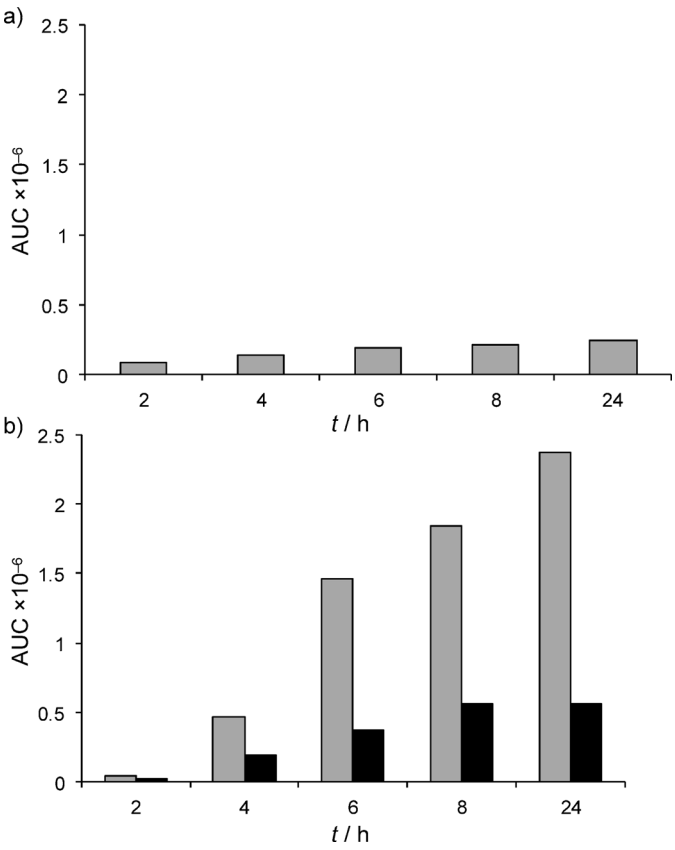


Figure 7. Appearance of Cf1743 (■) and [Val-Val]-Cf1743 (■) at the basolateral side of monolayer Caco-2 cell cultures exposed to a) Cf1743 or b) [Val-Pro][Val-Val]-Cf1743.

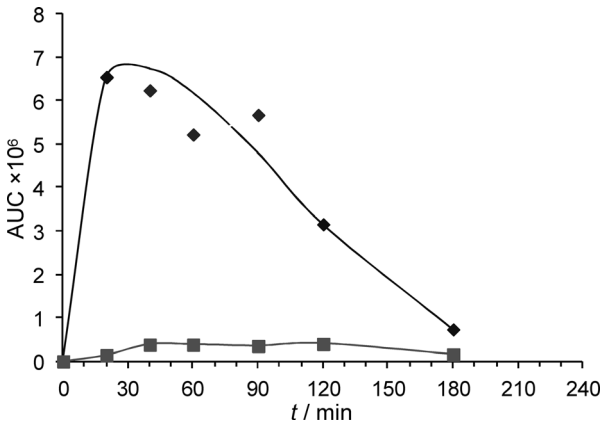


Figure 8. Oral bioavailability of equimolar drug concentrations (25 mg mL^{-1} Cf1743 (■) or 50 mg mL^{-1} [Val-Pro][Val-Val]-Cf1743 (**3b**; ◆)) when administered to mice by oral gavage.

Cf1743. Indeed, when Balb/C mice were given Val-Pro-Val-Val-Cf1743 (**3b**) or Cf1743 by oral gavage at 25 mg kg^{-1}, pronounced levels of Cf1743 appeared in the plasma of the tetrapeptide prodrug-exposed mice (Figure 8). It was estimated that the tetrapeptide prodrug of Cf1743 (**3b**) was at least 15- to 20-fold more orally bioavailable than the parent drug. In this respect, the oral bioavailability of the Val-Pro-Val-Val-Cf1743 prodrug was superior ($\leq$2-fold) to the bioavailability previously observed for Val-Pro-Val-Cf1743.[13a] It should also be noted that only Cf1743 was detected in the murine plasma and not any tetrapeptide prodrug or dipeptide (Val-Val) intermediate (Figure 8).

Conclusions

We have demonstrated that prodrugs of the bicyclic nucleoside Cf1743 containing diamines or dipeptide cyclization spacers are efficient substrates for the dipeptidyl-peptidase activity of DPPIV/CD26. Prodrugs **3a**–**c**, based on dipeptide cyclization spacers, efficiently released the parent nucleoside upon hydrolysis by DPPIV/CD26. All novel prodrugs had substantially increased water solubility, which proved closely and inversely correlated to decreased melting point values of the (pro)drugs. A marked increase in transport through Caco-2 monolayers and oral bioavailability was observed for the Val-Pro-Val-Val tetrapeptide prodrug of Cf1743 (**3b**). Consequently, tetrapeptide ester prodrugs of Cf1743 represent a new type of efficient DPPIV/CD26-based prodrugs that could be extended to other (lipophilic) hydroxy-containing drugs with poor solubility and/or unfavorable pharmacokinetic (i.e., poor bioavailability) properties.

Experimental Section

Chemical procedures

General: Microanalyses were obtained with a Heraeus CHN-O-RAPID instrument, and analytical results were within 0.4% of theoretical values. Electrospray mass spectra were measured on a quadrupole mass spectrometer equipped with an electrospray source (Hewlett Packard, LC/MS HP 1100). Spectra were recorded with Varian Inova-300 or Varian Inova-400 spectrometers operating at 300 or 400 MHz for ^{1}H NMR and at 75 or 100 MHz for ^{13}C NMR with Me_4Si as an 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 (Kiesegel 60 PF_{254} gipshaltig (Merck), layer thickness 1 mm, flow rate 5 mL min^{-1}. Liquid chromatography was performed using a force flow (flash chromatography) Horizon HPFG System (Biotage) with Flash 25 or 40 silica gel cartridges.

Microwave reactions were performed using the Biotage Initiator 2.0 single-mode cavity instrument from Biotage (Uppsala). Experiments were carried out in sealed microwave process vials using the standard absorbance level (400 W maximum power). The temperature was measured with an IR sensor on the outside of the reaction vessel.

The dipeptide derivative Fmoc-Val-Pro-OH was purchased from Bachem Feinchemikalien. H-Pro-2-chlorotrityl resin was purchased from GL Biochem (Shanghai), H-Val-2-chlorotrityl resin and H-Ala-2-chlorotrityl resin were purchased from Irish Biotech, and HCTU was purchased from Novabiochem. Cf1743 (**1**) was synthesized as previously described.[25] The purity of novel prodrugs was determined to be >95% by elemental analysis.

Fmoc-Val-Pro-NH-$(CH_2)_2$-NH-Boc (4): A solution of Fmoc-Val-Pro-OH (500 mg, 1.14 mmol) in CH_2Cl_2 (10 mL) was reacted with Boc-NH-$(CH_2)_2$-NH_2 (0.181 mL, 1.14 mmol), 1-benzotriazolyloxy-tris-dimethylamino-phosphonium hexafluorophosphate (BOP) (608 mg, 1.37 mmol) and Et_3N (0.192 mL, 1.37 mmol). The reaction mixture was stirred at room temperature for 15 h, and the solvent was removed under reduced pressure. The residue was dissolved in EtOAc (30 mL) and washed with 10% aqueous citric acid (3× 20 mL). The aqueous layer was basified with NH_3 and was extracted with CH_2Cl_2 (5×20 mL). The organic layer was dried (Na_2SO_4), filtered, and evaporated to dryness. The final residue was purified by flash chromatography (HPFC) in a Biotage SP1 to give 522 mg of **4** (80%) as a white foam: ^{1}H NMR (300 MHz, [D$_6$]acetone): $\delta=0.98$ (d, 3H, γ-CH_3, Val, $J=6.6$ Hz), 1.02 (d, 3H, γ-CH_3, Val, $J=6.6$ Hz), 1.44 (s, 9H, *t*Bu, Boc), 1.82–2.10 (m, 4H, β-CH_2, Pro, γ-CH_2, Pro), 2.18 (m, 1H, β-CH, Val), 3.16 (m, 2H, CH_2-N), 3.27 (m, 2H, N-CH_2), 3.62–3.87 (m, 2H, δ-CH_2, Pro), 4.19–4.41 (m, 5H, α-CH, Val, α-CH, Pro, CH_2-Fmoc, CH-Fmoc), 6.09 (m, 1H, NH-Boc), 6.64 (d, 1H, NH, Val, $J=8.8$ Hz), 7.32 (t, 2H, $H_{2,7}$-Fmoc, $J=7.5$ Hz), 7.41 (m, 3H, $H_{3,6}$-Fmoc, NH-CH_2), 7.72 (dd, 2H, $H_{1,8}$-Fmoc, $J=4.6$, 7.1 Hz), 7.86 ppm (d, 2H, $H_{4,5}$-Fmoc, $J=7.3$ Hz); MS (ESI$^+$): *m/z* 579.5 $[M+H]^+$, 601.5 $[M+Na]^+$.

Fmoc-Val-Pro-NH-$(CH_2)_2$-$NH_3^+Cl^-$ (5): A solution of HCl (1.25 M) in MeOH (3.16 mL, 3.95 mmol) was added to a solution of compound **4** (457 mg, 0.79 mmol) in THF (5 mL). The reaction mixture was stirred at room temperature for 6 h, and the solvent was evaporated to dryness to afford 388 mg of **5** (95%, white foam) as a hydrochloride salt: ^{1}H NMR (300 MHz, [D$_6$]acetone): $\delta=0.89$–94 (m, 6H, γ-CH_3, Val), 1.73–1.84 (m, 2H, β-CHa, Pro, γ-CHa, Pro), 1.89–2.10 (m, 3H, β-CHb, Pro, γ-CHb, Pro, β-CH, Val), 2.83 (m, 2H, CH_2-N), 3.31 (m, 2H, N-CH_2), 3.54–3.75 (m, 2H, δ-CH_2, Pro), 4.04 (t, 1H, α-CH, Val, $J=8.4$ Hz), 4.17–4.28 (m, 4H, α-CH, Pro, CH_2-Fmoc, CH-Fmoc), 7.31 (t, 2H, $H_{2,7}$-Fmoc, $J=7.2$ Hz), 7.41 (t, 2H, $H_{3,6}$-Fmoc, $J=7.5$ Hz), 7.60 (d, 1H, NH, Val, $J=8.7$ Hz), 7.73 (m, 2H, $H_{1,8}$-Fmoc), 7.88 (d, 2H, $H_{4,5}$-Fmoc, $J=7.5$ Hz), 8.15 (bs, 3H, NH_3^+), 8.31 ppm (t, 1H, CONH, $J=8.1$ Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=18.9$, 19.3 (2C-γ, Val), 25.0 (C-γ, Pro), 30.1 (C-β, Pro), 36.5 (C-β, Val), 38.9 (CH_2-N), 39.6 (N-CH_2), 47.0, 47.6 (C-δ, Pro, CH-Fmoc), 58.3 (C-α, Val), 60.1 (C-α, Pro), 66.0 (CH_2-Fmoc), 120.4 ($C_{4,5}$-Fmoc), 126.0 ($C_{1,8}$-Fmoc), 127.4 ($C_{2,7}$-Fmoc), 128.0 ($C_{3,6}$-Fmoc), 141.0, 144.2 (2C-Fmoc), 156.4 (C=O, Fmoc), 170.7, 172.5 ppm (C=O, Val, C=O, Pro); MS (ESI$^+$): *m/z* 515.5 $[M+H]^+$.

Fmoc-Val-Pro-NH-$(CH_2)_3$-NH-Boc (6): Fmoc-Val-Pro-OH (500 mg, 1.14 mmol) was reacted with Boc-NH-$(CH_2)_3$-NH_2 (0.200 mL, 1.14 mmol), BOP (608 mg, 1.37 mmol), and Et_3N (0.192 mL, 1.37 mmol) in CH_2Cl_2 (10 mL), according to the coupling procedure described for compound **4**. The final residue was purified by flash chromatography (HPFC) in a Biotage SP1 to give 595 mg of **6** (88%) as a white foam: ^{1}H NMR (300 MHz, [D$_6$]acetone): $\delta=0.98$ (d, 3H, γ-CH_3, Val, $J=6.6$ Hz), 1.02 (d, 3H, γ-CH_3, Val, $J=6.6$ Hz), 1.41 (s, 9H, *t*Bu, Boc), 1.58 (m, 2H, C-CH_2-C), 1.84–2.23 (m, 5H, β-CH_2, Pro, γ-CH_2, Pro, β-CH, Val), 3.04–3.37 (m, 4H, CH_2-N, N-CH_2), 3.62–3.89 (m, 2H, δ-CH_2, Pro), 4.18–4.40 (m, 4H, α-CH, Val, CH_2-Fmoc, CH-Fmoc), 4.43 (m, 1H, α-CH, Pro), 6.07 (m, 1H, NH-Boc), 6.64 (d, 1H, NH, Val, $J=8.8$ Hz), 7.32 (m, 3H, $H_{2,7}$-Fmoc, NH-CH_2), 7.41 (d, 2H, $H_{3,6}$-Fmoc, $J=7.5$ Hz), 7.73 (dd, 2H, $H_{1,8}$-Fmoc, $J=4.6$, 7.1 Hz), 7.85 ppm (d, 2H, $H_{4,5}$-Fmoc, $J=7.5$ Hz); MS (ESI$^+$): *m/z* 593.3 $[M+H]^+$, 615.1 $[M+Na]^+$.

Fmoc-Val-Pro-NH-$(CH_2)_3$-$NH_3^+Cl^-$ (7): A solution of HCl (1.25 M) in MeOH (3.70 mL, 4.63 mmol) was added to a solution of Fmoc-Val-Pro-NH-$(CH_2)_3$-NH-Boc **6** (549 mg, 0.93 mmol) in THF (5 mL). The reaction mixture was stirred at room temperature for 5 h, and the solvent was evaporated to dryness to afford 480 mg of **7** (98%, white foam) as a hydrochloride salt: ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=0.88$–94 (m, 6H, γ-CH_3, Val), 1.62–1.81 (m, 4H, β-CHa, Pro, γ-CHa, Pro, C-CH_2-C), 1.91–2.07 (m, 3H, β-CHb, Pro, γ-CHb, Pro, β-CH, Val), 2.78 (m, 2H, CH_2-N), 3.15 (m, 2H, N-CH_2), 3.57–3.73 (m, 2H, δ-CH_2, Pro), 4.05 (t, 1H, α-CH, Val, $J=8.1$ Hz), 4.19–4.28 (m, 4H, α-CH, Pro, CH_2-Fmoc, CH-Fmoc), 7.30 (m, 2H, $H_{2,7}$-Fmoc), 7.40 (t, 2H, $H_{3,6}$-Fmoc, $J=7.2$ Hz), 7.57 (d, 1H, NH, Val, $J=8.4$ Hz), 7.74 (m, 2H, $H_{1,8}$-

Fmoc), 7.86 (d, 2H, $H_{4,5}$-Fmoc, $J=7.2$ Hz), 8.17–8.19 ppm (m, 4H, NH_3^+, CONH); ^{13}C NMR (75 MHz, [D_6]DMSO): $\delta=18.5$, 19.0 (2C-γ, Val), 24.5 (C-γ, Pro), 27.1 (C-CH_2-C), 29.4 (C-β, Pro), 29.7 (C-β, Val), 35.4 (CH_2-N), 36.3 (N-CH_2), 46.6, 47.0 (C-δ, Pro, CH-Fmoc), 57.9 (C-α, Val), 59.6 (C-α, Pro), 65.6 (CH_2-Fmoc), 120.0 ($C_{4,5}$-Fmoc), 125.3 ($C_{1,8}$-Fmoc), 126.9 ($C_{2,7}$-Fmoc), 127.5 ($C_{3,6}$-Fmoc), 140.6, 143.7 (2C-Fmoc), 156.1 (C=O, Fmoc), 170.1, 171.9 ppm (C=O, Val, C=O, Pro); MS (ESI$^+$): m/z 530.1 $[M+H]^+$.

3-[3′,5′-di-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxy-β-D-ribofuranosyl]-6-(*p*-pentylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (8): Imidazole (773 mg, 11.35 mmol), DMAP (116 mg, 0.95 mmol), and TBDMSCl (1.426 g, 9.46 mmol) were added to a solution of compound **1** (1.506 g, 3.78 mmol) in dry DMF (15 mL). The reaction mixture was stirred at room temperature for 1 h, and the solvent was evaporated to dryness. The crude reaction mixture was dissolved in CH_2Cl_2 (30 mL) and washed with NH_4Cl (3×20 mL). The organic layer was dried (Na_2SO_4) and removed by filtration, and the solvent was eliminated under reduced pressure. The final residue was purified by flash chromatography (CH_2Cl_2/MeOH, 40:1) to yield 2.24 g of **8** (95%) as a yellow solid: mp: 89–90 °C (Et_2O); MS (ESI$^+$): m/z 627.5 $[M+H]^+$; Anal. calcd for $C_{34}H_{54}N_2O_5Si_2$: C 65.13, H 8.68, N 4.47; found: C 65.61, H 8.69, N 4.56.

3-[3′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxy-β-D-ribofuranosyl]-6-(*p*-pentylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (9): A solution of HCl (1.25 M) in MeOH (2.82 mL, 3.53 mmol) was added to a solution of **8** (1.84 g, 2.94 mmol) in MeOH (30 mL). The reaction mixture was stirred at room temperature for 2 h. The solid was filtered and was washed with MeOH to afford 1.20 g of **9** (80%) as a white solid: mp: 190–191 °C (MeOH); MS (ESI$^+$): m/z 513.5 $[M+H]^+$, 535.5 $[M+Na]^+$; Anal. calcd for $C_{28}H_{40}N_2O_5Si$: C 65.59, H 7.86, N 5.46; found: C 65.67, H 7.83, N 5.49.

3-[3′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxy-5′-*O*-[2-(valylprolylamino)ethylcarbamoyl]-β-D-ribofuranosyl]-6-(*p*-pentylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (11): Et_3N (0.204 mL, 1.46 mmol) and CDI (245 mg, 1.46 mmol) were added to a solution of compound **9** (150 mg, 0.29 mmol) in dry DMF (15 mL) under argon atmosphere. The reaction mixture was stirred at room temperature for 5 h and quenched by the addition of several drops of water. Then, a solution of Fmoc-Val-Pro-NH-$(CH_2)_2$-$NH_3^+Cl^-$ (**5**; 166 mg, 0.32 mmol) and Et_3N (0.05 mL, 0.35 mmol) in dry DMF (5 mL) were added. The reaction was stirred at 40 °C for 24 h. The solvent was evaporated to dryness, and the residue was dissolved in EtOAc (30 mL) and washed with 10% aqueous $NaHCO_3$ (3× 20 mL) and brine (3×20 mL). The organic layer was dried (Na_2SO_4) and removed by filtration, and the solvent was evaporated to dryness. The final residue was purified by CCTLC on the Chromatotron (CH_2Cl_2/MeOH, 20:1) to afford 118 mg of **11** (51%) as a yellow foam: MS (ESI$^+$): m/z 795.9 $[M+H]^+$; Anal. calcd for $C_{41}H_{62}N_6O_8Si$: C 61.94, H 7.86, N 10.57; found: C 62.25, H 7.80, N 10.61.

3-[2′-Deoxy-5′-*O*-[2-(valylprolylamino)ethylcarbamoyl]-β-D-ribofuranosyl]-6-(*p*-pentylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (2a): A solution of TBAF (1.1 M) in THF (0.180 mL, 0.198 mmol) was added to a solution of **11** (105 mg, 0.13 mmol) in THF (2 mL). The reaction mixture was stirred at room temperature for 1 h, and the solvent was evaporated to dryness. The final residue was purified by CCTLC on the Chromatotron (CH_2Cl_2/MeOH, 10:1) to yield 54 mg of **2a** (63%) as a yellow solid after trituration with Et_2O: mp: 84–86 °C (Et_2O); ^{1}H NMR (400 MHz, [D_6]DMSO): $\delta=0.79$ (t, 3H, CH_3, $J=7.0$ Hz), 0.81–0.88 (m, 6H, γ-CH_3, Val), 1.22–1.33 (m, 4H, $2CH_2$), 1.57 (m, 2H, CH_2), 1.69–1.78 (m, 3H, γ-CH_2, Pro, β-CHa, Pro), 1.86–2.00 (m, 2H, β-CHb, Pro, β-CH, Val), 2.18 (m, 1H, H-2′a), 2.43 (m, 1H, H-2′b), 2.61 (t, 2H, CH_2, $J=7.6$ Hz), 3.00–3.36 (m, 5H, N-CH_2-CH_2-N, α-CH, Val), 3.43–3.61 (m, 2H, δ-CH_2, Pro), 4.09 (m, 1H, H-4′), 4.16–4.28 (m, 4H, 2H-5′, H-3′, α-CH, Pro), 6.21 (t, 1H, H-1′, $J=$ 6.4 Hz), 7.19 (s, 1H, H-5), 7.25 (m, 1H, NHCOO), 7.31 and 7.73 (AA′BB′ system, 4H, Ar, $J=8.0$ Hz), 7.84 (m, 1H, CONH-CH_2), 8.51 ppm (s, 1H, H-4); ^{13}C NMR (100 MHz, [D_6]DMSO): $\delta=13.9$ (CH_3), 17.0, 19.6 (2C-γ, Val), 21.8 (CH_2), 24.5 (C-γ, Pro), 29.0, 30.3, 30.8, 31.1 ($2CH_2$, C-β, Pro, C-β, Val), 37.2 (CH_2), 34.8 (CH_2-N), 38.4 (N-CH_2), 40.7 (C-2′), 46.7 (C-δ, Pro), 57.1 (C-α, Val), 59.5 (C-α, Pro), 63.8 (C-5′), 70.3 (C-3′), 85.3 (C-4′), 87.8 (C-1′), 98.7 (C-5), 107.1 (C-4a), 124.5 (C-Hb), 125.8 (*ipso*-C), 129.0 (C-Ha), 137.5 (C-4), 144.0 (*para*-C), 153.7, 153.9 (C-6, NHCOO), 155.9 (C-2), 171.0, 171.1, 171.9 ppm (C=O, Val, C=O, Pro, C-7a); MS (ESI$^+$): m/z 681.3 $[M+H]^+$, 703.3 $[M+Na]^+$; Anal. calcd for $C_{35}H_{48}N_6O_8$: C 61.75, H 7.11, N 12.34; found: C 61.85, H 7.07, N 12.41.

3-[3′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxy-5′-*O*-[2-(valylprolylamino)propylcarbamoyl]-β-D-ribofuranosyl]-6-(*p*-pentylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (12): According to the procedure described for compound **11**, a solution of **9** (200 mg, 0.39 mmol) in dry DMF (15 mL) was treated with Et_3N (0.272 mL, 1.95 mmol) and CDI (3175 mg, 1.95 mmol). After 5 h, the reaction was quenched by the addition of several drops of water, then Fmoc-Val-Pro-NH-$(CH_2)_3$-$NH_3^+Cl^-$ (**7**; 166 mg, 0.32 mmol) was added. The reaction mixture was stirred at 40 °C for 48 h. The final residue was purified by CCTLC on the Chromatotron (CH_2Cl_2/MeOH, 15:1) to afford 192 mg of **12** (61%) as a yellow foam: MS (ESI$^+$): m/z 809.7 $[M+H]^+$, 831.7 $[M+Na]^+$; Anal. calcd for $C_{42}H_{64}N_6O_8Si$: C 62.35, H 7.97, N 10.39; found: C 62.59, H 7.93, N 10.45.

3-[2′-Deoxy-5′-*O*-[2-(valylprolylamino)propylcarbamoyl]-β-D-ribofuranosyl]-6-(*p*-pentylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (2b): Following the procedure described for compound **2a**, nucleoside **12** (157 mg, 0.19 mmol) was reacted with a solution of TBAF (1.1 M) in THF (0.266 mL, 0.292 mmol) for 1 h. The final residue was purified by CCTLC on the Chromatotron (CH_2Cl_2/MeOH, 10:1) to afford 92 mg of **2b** (68%) as a yellow foam: ^{1}H NMR (400 MHz, [D_6]DMSO): $\delta=0.86$ (t, 3H, CH_3, $J=7.2$ Hz), 0.89–1.00 (m, 6H, γ-CH_3, Val), 1.26–1.35 (m, 4H, $2CH_2$), 1.51–1.61 (m, 5H, γ-CHa, Pro, CH_2, C-CH_2-C), 1.72 (m, 1H, γ-CHb, Pro), 1.91 (m, 1H, β-CHa, Pro), 2.01–2.21 (m, 3H, β-CHb, Pro, β-CH, Val, H-2′a), 2.42 (m, 1H, H-2′b), 2.62 (t, 2H, CH_2, $J=7.6$ Hz), 2.98–3.06 (m, 4H, N-CH_2, CH_2-N), 3.03–3.19 (m, 2H, δ-CH_2, Pro), 3.41 (m, 1H, α-CH, Val), 3.71 (m, 1H, H-4′), 3.97 (m, 1H, α-CH, Pro), 4.08 (m, 1H, H-3′), 4.16–4.27 (m, 2H, 2H-5′), 6.21 (t, 1H, H-1′, $J=6.4$ Hz), 7.17 (s, 1H, H-5), 7.20 (t, 1H, NHCOO, $J=7.6$ Hz), 7.32 and 7.73 (AA′BB′ system, 4H, Ar, $J=$ 8.0 Hz), 7.97 (t, 1H, CONH-CH_2, $J=7.0$ Hz), 8.02 (bs, 2H, NH_2, Val), 8.52 ppm (s, 1H, H-4); MS (ESI$^+$): m/z 695.7 $[M+H]^+$, 718.8 $[M+Na]^+$; Anal. calcd for $C_{36}H_{50}N_6O_8$: C 62.23, H 7.25, N 12.10; found: C 62.07, H 7.27, N 12.18.

3-[2′-Deoxy-5′-*O*-[*N*-(fluorenylmethoxycarbonyl)valylprolylvalylprolyl]-β-D-ribofuranosyl]-6-(*p*-pentylphenyl)-2,3-dihydrofuro-[2,3-*d*]pyrimidin-2-one (17a): A solution of compound **1** (100 mg, 0.25 mmol), Fmoc-Val-Pro-Val-Pro-OH (**16a**; 199 mg, 0.314 mmol), PPh_3 (99 mg, 0.376 mmol), and DBAD (88 mg, 0.376 mmol) in dry DMF (3 mL) were placed in a microwave vessel under argon atmosphere. The reaction was irradiated at 80 °C for 8 h. After cooling, volatiles were evaporated to dryness. The residue was dissolved in EtOAc (30 mL) and washed with 10% aqueous $NaHCO_3$ (3×20 mL) and brine (3×20 mL). The organic layer was dried (Na_2SO_4) and filtered, and the solvent was evaporated to dryness. The final residue was purified by CCTLC on the Chromatotron (CH_2Cl_2/MeOH, 25:1) to afford 113 mg of **17a** (45%) as a white foam: MS (ESI$^+$): m/z

1013.9 $[M+H]^+$, 1035.9 $[M+Na]^+$; Anal. calcd for $C_{57}H_{68}N_6O_{11}$: C 67.57, H 6.76, N 8.29; found: C 68.02, H 6.70, N 8.31.

3-[2′-Deoxy-5′-*O*-(valylprolylvalylprolyl)-β-D-ribofuranosyl]-6-(*p*-pentylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (3a): Piperidine (0.1 mL) was added to a solution of **17a** (255 mg, 0.287 mmol) in DMF (2 mL). 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, 10:1) to yield 47 mg of **3a** (76%) as a white solid after trituration with Et_2O; mp: 108–110 °C (Et_2O); ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 0.84$–0.94 (m, 15H, 4γ-CH_3, Val_1 and Val_2, CH_3), 1.23–1.32 (m, 4H, 2CH_2), 1.54–1.62 (m, 2H, CH_2), 1.65–2.22 (m, 11H, 2β-CH, Val_1 and Val_2, 2β-CH_2, Pro_1 and Pro_2, 2γ-CH_2, Pro_1 and Pro_2, H-2′a), 2.43 (m, 1H, H-2′b), 2.61 (t, 2H, CH_2, $J = 7.6$ Hz), 3.58–3.72 (m, 4H, 2δ-CH_2, Pro_1 and Pro_2), 4.11 (m, 1H, H-4′), 4.23–4.29 (m, 2H, H-3′, α-CH, Val_1), 4.33- 4.46 (m, 5H, 2H-5′, α-CH, Val_2, 2α-CH, Pro_1 and Pro_2), 5.51 (bs, 2H, NH_2), 6.20 (t, 1H, H-1′, $J = 6.4$ Hz), 7.07 (s, 1H, H-5), 7.31 and 7.73 (AA′BB′ system, 4H, Ar, $J = 8.0$ Hz), 8.60 ppm (s, 1H, H-4); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta = 13.9$ (CH_3), 17.2, 18.0, 19.0, 19.1 (4C-γ, Val_1 and Val_2), 22.0 (CH_2), 24.7, 24.8 (2C-γ, Pro_1 and Pro_2), 28.7, 28.9, 30.2, 30.5, 30.8, 30.9 (2CH_2, 2C-β, Pro_1 and Pro_2, 2C-β, Val_1 and Val_2), 35.0 (CH_2), 40.6 (C-2′), 46.9, 47.1 (2C-δ, Pro_1 and Pro_2), 55.3 (C-α, Val_1), 56.4 (C-α, Val_2), 58.6, 59.1 (2C-α, Pro_1 and Pro_2), 64.1 (C-5′), 69.7 (C-3′), 84.5 (C-4′), 87.8 (C-1′), 98.7 (C-5), 107.2 (C-4a), 124.7 (C-Hb), 125.8 (*ipso*-C), 129.0 (C-Ha), 137.7 (C-4), 144.1 (*para*-C), 153.7, 153.9 (C-6, C-2), 170.0, 171.1, 171.3, 171.8, 172.0 ppm (C=O, Val_1, C=O, Val_2, C=O, Pro_1, C=O, Pro_2, C-7a); MS (ESI$^+$): *m/z* 791.7 $[M+H]^+$, 813.7 $[M+Na]^+$; Anal. calcd for $C_{42}H_{58}N_6O_9$: C 63.78, H 7.39, N 10.63; found: C 63.65, H 7.41, N, 10.66.

3-[2′-Deoxy-5′-*O*-[*N*-(fluorenylmethoxycarbonyl)valylprolylvalyl-valyl]-β-D-ribofuranosyl]-6-(*p*-pentylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (17b): Following the procedure described for compound **17a**, a solution of **1** (100 mg, 0.25 mmol), Fmoc-Val-Pro-Val-Val-OH (**16b**; 199 mg, 0.314 mmol), PPh_3 (99 mg, 0.376 mmol), and DBAD (88 mg, 0.376 mmol) in dry DMF (3 mL) under argon atmosphere was irradiated at 90 °C for 10 h. The final residue was purified by CCTLC on the Chromatotron (CH_2Cl_2/MeOH, 25:1) to obtain 163 mg of **17b** (64%) as a white foam: MS (ESI$^+$): *m/z* 1016.23 $[M+H]^+$; Anal. calcd for $C_{57}H_{70}N_6O_{11}$: C 67.44, H 6.95, N 8.28; found: C 67.25, H 7.11, N 8.36.

3-[2′-Deoxy-5′-*O*-(valylprolylvalylvalyl)-β-D-ribofuranosyl]-6-(*p*-pentylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (3b): A solution of compound **17b** (143 mg, 0.181 mmol) in DMF (4 mL) was reacted with piperidine (0.2 mL), according to the deprotection method described for compound **17a**. The final residue was purified by CCTLC on the Chromatotron (CH_2Cl_2/MeOH, 10:1) to afford 94 mg of **3b** (84%) as a white foam: ^{1}H NMR (400 MHz, [D$_6$]acetone): $\delta = 0.87$–0.99 (m, 18H, 3γ-CH_3, Val_1, Val_2 and Val_3, CH_3), 1.28–1.35 (m, 4H, 2CH_2), 1.62–1.66 (m, 2H, CH_2), 1.86–1.97 (m, 3H, γ-CH_2, Pro, β-CHa, Pro), 2.11–2.35 (m, 5H, 3β-CH, Val_1, Val_2 and Val_3, β-CHb, Pro, H-2′a), 2.62–2.67 (m, 3H, H-2′b, CH_2), 3.50–3.82 (m, 4H, δ-CH_2, Pro), 3.96 (d, 1H, α-CH, Val_1, $J = 8.6$ Hz), 4.33–4.50 (m, 5H, α-CH, Val_1, 2H-5′, H-4′, H-3′) 4.52–4.59 (m, 2H, α-CH, Pro, α-CH, Val), 6.33 (t, 1H, H-1′, $J = 6.2$ Hz), 7.23 (s, 1H, H-5), 7.34 and 7.77 (AA′BB′ system, 4H, Ar, $J = 8.2$ Hz), 8.69 ppm (s, 1H, H-4); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 15.0$ (CH_3), 18.9, 19.6, 19.9, 20.0, 20.4, 20.7 (6C-γ, Val_1, Val_2 and Val_3), 23.8 (CH_2), 26.5 (C-γ, Pro), 29.2 (2C-β, Pro), 30.7, 30.9, 31.7 (3C-β, Val_1, Val_2 and Val_3), 32.5, 32.8 (2CH_2), 37.0 (CH_2), 42.9 (C-2′), 48.5 (2C-δ, Pro), 59.3, 59.7 (2C-α, Val_2 and Val_3), 61.8 (C-α, Pro), 65.6 (C-5′), 72.0 (C-3′), 72.2 (C-α, Val_1), 86.6 (C-4′), 89.8 (C-1′), 100.3 (C-5), 109.1 (C-4a), 126.3 (C-Hb), 128.0 (*ipso*-C), 130.6 (C-Ha), 138.5 (C-4), 145.9 (*para*-C), 155.7, 156.1 (C-6, C-2), 172.8, 172.9, 173.0, 173.1, 173.3 ppm (C=O, Val_1, C=O, Val_2, C=O, Val_3, C=O, Pro, C-7a); MS (ESI$^+$): *m/z* 793.79 $[M+H]^+$; Anal. calcd for $C_{42}H_{60}N_6O_9$: C 63.62, H 7.63, N 10.60; found: C 63.55, H 7.51, N 10.56.

3-[2′-Deoxy-5′-*O*-[*N*-(fluorenylmethoxycarbonyl)valylprolylvalyl-alanyl]-β-D-ribofuranosyl]-6-(*p*-pentylphenyl)-2,3-dihydrofuro-[2,3-*d*]pyrimidin-2-one (17c): Following the procedure described for compound **17a**, a solution of **1** (80 mg, 0.20 mmol), Fmoc-Val-Pro-Val-Ala-OH **16c** (152 mg, 0.25 mmol), PPh_3 (79 mg, 0.30 mmol), and DBAD (70 mg, 0.30 mmol) in dry DMF (3 mL) under argon atmosphere was irradiated at 90 °C for 6 h. Additional amounts of tetrapeptide (0.12 mmol), PPh_3 (0.15 mmol), and DBAD (0.15 mmol) in DMF (0.5 mL) were then added, and the mixture was irradiated again at 90 °C for 6 h. The final residue was purified by CCTLC on the Chromatotron (CH_2Cl_2/MeOH, 20:1) to obtain 117 mg of **17c** (59%) as a white foam: MS (ESI$^+$): *m/z* 988.5 $[M+H]^+$; Anal. calcd for $C_{55}H_{66}N_6O_{11}$: C 66.92, H 6.74, N 8.51; found: C 67.10, H 6.91, N 8.66.

3-[2′-Deoxy-5′-*O*-(valylprolylvalylalanyl)-β-D-ribofuranosyl]-6-(*p*-pentylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one (3c): A solution of compound **17c** (82 mg, 0.083 mmol) in DMF (3 mL) was reacted with piperidine (0.15 mL), according to the deprotection method described for compound **17a**. The final residue was purified by CCTLC on the Chromatotron (CH_2Cl_2/MeOH, 10:1) to afford 61 mg of **3c** (97%) as a white foam: ^{1}H NMR (400 MHz, [D$_6$]acetone): ^{1}H NMR (400 MHz, [D$_6$]acetone): $\delta = 0.79$–1.00 (m, 15H, 4γ-CH_3, Val_1 and Val_2, CH_3), 1.28–1.44 (m, 7H, 2CH_2, β-CH_3, Ala,), 1.60–1.68 (m, 2H, CH_2), 1.86–2.00 (m, 5H, β-CH, Val, β-CH_2, Pro, γ-CH_2, Pro), 2.19–2.31 (m, 2H, β-CH, Val, H-2′a), 2.62–2.67 (m, 3H, H-2′b, CH_2), 3.52–3.84 (m, 2H, γ-CH, Pro), 3.99 (d, 1H, H-3′, $J = 8.5$ Hz), 4.29–4.56 (m, 7H, 2α-CH, Val_1 and Val_2, α-CH, Ala, α-CH, Pro, 2H-5′, H-4′), 6.29–6.33 (m, 1H, H-1′), 7.18 (s, 1H, H-5), 7.33 and 7.76 (AA′BB′ system, 4H, Ar, $J = 8.2$ Hz), 8.66 ppm (s, 1H, H-4); ^{13}C NMR (100 MHz, [D$_6$]acetone): $\delta = 14.7$ (CH_3), 17.7, 17.9, 18.5, 20.1, (C-β, Ala, 4C-γ, Val_1 and Val_2), 23.5 (CH_2), 26.2 (C-γ, Pro), 29.1 (C-β, Pro), 32.0, 32.2, 32.5, 32.8 (2CH_2, 2C-β, Val_1 and Val_2), 36.7 (CH_2), 42.7 (C-2′), 48.3 (C-δ, Pro), 49.6, 58.6, 58.9, 61.5 (4C-α, Ala, Val_1, Val_2, Pro), 65.3 (C-5′), 71.7 (C-3′), 86.4 (C-4′), 89.5 (C-1′), 100.0 (C-5), 108.9 (C-4a), 126.0 (C-Hb), 127.7 (*ipso*-C), 130.3 (C-Ha), 138.2 (C-4), 145.7, (*para*-C), 155.4, 155.8 (C-6, C-2), 172.4, 172.6, 173.1, 173.6 ppm (C=O, Val_1, C=O, Val_2, C=O, Ala, C=O, Pro, C-7a); EMAR (ESI$^+$) for $C_{40}H_{56}N_6O_9$ $[M+H]^+$; Calcd: 764.4182, found: 765.4189; Anal. calcd for $C_{40}H_{56}N_6O_9$: C 62.81, H 7.38, N 10.99; found: C 62.95, H 7.31, N 10.86.

Water solubility studies: Water solubility of the prodrugs and the parent compounds was determined by HPLC analysis. HPLC was carried out on a Waters 484 System using Novapack C18 reversed-phase column (flow rate: 1 mL min^{-1}; detection: UV 254 nm; gradient solvent system A/B (CH_3CN/H_2O): initial 15% A + 85% B, 5 min linear gradient to 25% A + 75% B, 5 min linear gradient to 35% A + 65% B, 10 min linear gradient to 45% A + 55% B, 5 min linear gradient to 60% A + 40% B, and 5 min linear gradient to 100% A). Excess amounts of the prodrug or of the parent drug were suspended in deionized water (pH 5.5), sonicated for 10 min at room temperature, then equilibrated overnight at room temperature. The samples were centrifuged at 14000 rpm in an Eppendorf microcentrifuge for 1.5 min at room temperature. An aliquot of the clear supernatant was removed and diluted to a concentration within the range of a five-point standard curve. Water solubility was calculated from each peak area of the solution by HPLC com-

pared with the sample dissolved in DMSO as the standard, the exact concentration of which is known.

Biological methods

Compounds and enzymes: Soluble human CD26 was purified as described[26] and kindly provided by I. De Meester and A.-M. Lambeir (Antwerp, Belgium) or obtained from Sigma–Aldrich (St. Louis, MO, USA). Fetal bovine serum (FBS) was obtained from Integro (Dieren, The Netherlands), and human serum was provided by the Blood Bank, Leuven, Belgium.

Conversion of Cf1743 prodrugs to the corresponding parent compound: Test compounds were 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 (prodrugs of Cf1743) in PBS containing 0.1% DMSO. The reaction was initiated by the addition of purified CD26 (1.5 mU), 20% HS (in PBS), or 20% 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 MeOH, and incubated on ice for 10 min. The mixtures were then centrifuged at 13000 rpm for 5 min at 4 °C, and 250 μL supernatant was analyzed by HPLC on a reversed-phase RP-8 column with fluorescence detection (excitation λ: 340 nm; emission λ: 415 nm), using the following buffers and gradients: buffer A: 50 mM NaH_2PO_4 + 5 mM heptanesulfonic acid, pH 3.2; buffer B: CH_3CN; gradient: 90% A + 10% B, 12 min linear gradient to 75% A + 25% B, 13 min linear gradient to 65% A + 35% B, 8 min linear gradient to 60% A + 40% B, 7 min linear gradient to 50% A + 50% B, 5 min 90% A + 10% B, 5 min equilibration at 90% A + 10% B.

*Exposure of Caco-2 cell cultures to Cf1743 and Val-Pro-Val-Val prodrug **3b***: Human colon carcinoma Caco-2 cells were seeded in the upper cups of dual-chamber 24-well trays at 1.5×10^5 cells per 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 Cf1743 or the 5′-Val-Pro-Val-Val prodrug at 100 μM. In the bottom wells, the medium was replaced by PBS. At different time points (i.e., 0, 2, 4, 6, 8, and 24 h), 50 μL supernatant was removed from both the upper and bottom cups and added to 100 μL of cold MeOH. 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 Balb/C mice received equimolar doses of Cf1743 (25 mg kg^{-1})and Val-Pro-Val-Val-Cf1743 (50 mg kg^{-1}) 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, and plasma samples were taken and analyzed for Cf1743 quantification by HPLC with fluorescence detection. Results are reported as relative peak areas for Cf1743, which assumes that peak area is directly proportional to drug concentration over the ranges of the tested different concentrations.

Acknowledgements

We thank Ria Van Berwaer for excellent technical assistance. We also thank the Spanish MEC/MICINN (Project SAF2009-13914-C02), the Comunidad de Madrid (Projects BIPEDD-CM ref. S-BIO-0214-2006 and BIPEDD-2-CM ref. S-2010/BMD-2457), and the KU Leuven (GOA No. 10/014) for financial support.

Keywords: CD26 · dipeptidyl peptidase IV · peptides · prodrugs · self-cleavage spacers

[1] D. A. Fox, R. E. Hussey, K. A. Fitzgerald, O. Acuto, C. Poole, L. Palley, J. F. Daley, S. F. Schlossman, E. L. Reinherz, *J. Immunol.* **1984**, *133*, 1250–1256.
[2] I. De Meester, G. Vanhoof, A.-M. Lambeir, S. Scharpe, *Immunol. Today* **1999**, *20*, 367–375.
[3] A.-M. Lambeir, C. Durinx, S. Scharpe, I. De Meester, *Crit. Rev. Clin. Lab. Sci.* **2003**, *40*, 209–294.
[4] A. Yaron, F. Naider, *Crit. Rev. Biochem. Mol.* **1993**, *28*, 31–81.
[5] R. Mentlein, *Regul. Pept.* **1999**, *85*, 9–24.
[6] R. Mentlein, *Int. Rev. Cytol.* **2004**, *235*, 165–202.
[7] J. Balzarini, E. Andersson, D. Schols, P. Proost, J. Van Damme, B. Svennerholm, P. Horal, A. Vahlne, *Int. J. Biochem. Cell Biol.* **2004**, *36*, 1848–1859.
[8] C. García-Aparicio, M.-C. Bonache, I. De Meester, A. San-Félix, J. Balzarini, M. J. Camarasa, S. Velázquez, *J. Med. Chem.* **2006**, *49*, 5339–5351.
[9] a) J. Balzarini, M. J. Pérez-Pérez, A. San-Félix, D. Schols, C. F. Perno, A. M. Vandamme, M. J. Camarasa, E. De Clercq, *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 4392–4396; b) M. J. Camarasa, M. J. Pérez-Pérez, A. San-Félix, J. Balzarini, E. De Clercq, *J. Med. Chem.* **1992**, *35*, 2721–2727.
[10] M. J. Camarasa, A. San-Félix, S. Velázquez, M. J. Pérez-Pérez, F. Gago, J. Balzarini, *Curr. Top. Med. Chem.* **2004**, *4*, 945–963.
[11] C. García-Aparicio, A. Diez-Torrubia, J. Balzarini, A.-M. Lambeir, S. Velázquez, M. J. Camarasa, *Antiviral Res.* **2007**, *76*, 130–139.
[12] A. Diez-Torrubia, C. García-Aparicio, S. Cabrera, I. De Meester, J. Balzarini, M. J. Camarasa, S. Velázquez, *J. Med. Chem.* **2010**, *53*, 559–572.
[13] a) A. Diez-Torrubia, J. Balzarini, G. Andrei, R. Snoeck, I. De Meester, M. J. Camarasa, S. Velázquez, *J. Med. Chem.* **2011**, *54*, 1927–1942; b) A. Diez-Torrubia, S. Cabrera, A.-M. Lambeir, J. Balzarini, M. J. Camarasa, S. Velázquez, *ChemMedChem* **2012**, *7*, 618–628.
[14] a) C. McGuigan, C. J. Yarnold, G. Jones, S. Velázquez, H. Barucki, A. Brancale, G. Andrei, R. Snoeck, E. De Clercq, J. Balzarini, *J. Med. Chem.* **1999**, *42*, 4479–4484; b) C. McGuigan, H. Barucki, S. Blewet, A. Carangio, J. T. Erichsen, G. Andrei, R. Snoeck, E. De Clercq, J. Balzarini, *J. Med. Chem.* **2000**, *43*, 4993–4997.
[15] a) J. Balzarini, C. McGuigan, *Biochim. Biophys. Acta Mol. Basis Dis.* **2002**, *1587*, 287–295; b) C. McGuigan, J. Balzarini, *Antiviral Res.* **2006**, *71*, 149–153.
[16] For reviews of cyclization-activated prodrugs, see: a) P. Gomes, N. Vale, R. Moreira, *Molecules* **2007**, *12*, 2484–2506; b) *Prodrugs: Challenges and Rewards, Part 1–2*, (Eds.: V. J. Stella, R. T. Borchardt, M. J. Hageman, R. Oliyai, H. Maag, J. W. Tilley), Springer, New York, **2007** and references therein.
[17] For examples of prodrugs bearing these types of diamine cyclization spacers, see: a) F. M. H. De Groot, L. W. A. Van Berkom, H. W. Scheeren, *J. Med. Chem.* **2000**, *43*, 3093–3102; b) W. S. Saari, J. E. Schwering, P. A. Lyle, S. J. Smith, E. L. Engelhardt, *J. Med. Chem.* **1990**, *33*, 97–101; c) P. Vlieghe, F. Bihel, T. Clerc, C. Pannecouque, M. Witvrouw, E. De Clercq, J. Salles, J. C. Chermann, J.-L. Kraus, *J. Med. Chem.* **2001**, *44*, 777–786.
[18] Dipeptides have been widely used as drug carriers for cyclization-activated prodrugs; for example, see: a) C. Santos, M. L. Mateus, A. P. Santos, R. Moreira, E. Oliveira, P. Gomes, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1595–1598; b) C. Santos, J. Morais, L. Gouveia, E. De Clercq, C. Pannecouque, C. U. Nielsen, B. Steffansen, R. Moreira, P. Gomes, *ChemMedChem* **2008**, *3*, 970–978.
[19] For other examples of dipeptide-based prodrugs, see: a) Y. Tsume, J. H. Hilfinger, G. L. Amidon, *Mol. Pharmaceutics* **2008**, *5*, 717–727; b) R. Jain, S. Duvvuri, V. Kansara, N. S. Mandava, A. K. Mitra, *Int. J. Pharm.* **2007**, *336*, 233–240 and references therein; c) R. S. Talluri, S. K. Samanta, R. Gaudana, A. K. Mitra, *Int. J. Pharm.* **2008**, *361*, 118–124.
[20] Two-step prodrugs have also been proposed, involving an initial enzymatic step leading to a dipeptide-based prodrug intermediate; for example, see: a) A. Mhaka, S. R. Denmeade, W. Yao, J. T. Isaacs, S. R. Khan, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2459–2461; b) S. F. Brady, J. M. Pawluczyk, P. K. Lumma, D. M. Feng, J. M. Wai, R. Jones, D. DeFeo-Jones,

B. K. Wong, C. Miller-Stein, J. H. Lin, A. Oliff, R. M. Freidinger, V. M. Garsky, *J. Med. Chem.* **2002**, *45*, 4706 – 4715; c) Y. Kohchi, K. Hattori, N. Oikawa, E. Mizuguchi, Y. Isshiki, K. Aso, K. Yoshinari, H. Shirai, M. Miwa, Y. Inagaki, M. Ura, K. Ogawa, H. Okabe, H. Ishitsuka, N. Shimma, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2241 – 2245.

[21] a) S. Capasso, A. Vergara, L. Mazzarella, *J. Am. Chem. Soc.* **1998**, *120*, 1990 – 1995; b) R. Keese, M. Meyer, *Tetrahedron* **1993**, *49*, 2055 – 2064; c) N. Ramachandran, A. K. Mitra, *J. Mol. Biol.* **1976**, *107*, 85 – 92.

[22] C. R. Santos, R. Capela, C. S. G. P. Pereira, E. Valente, L. Gouveia, C. Pannecouque, E. De Clercq, R. Moreira, P. Gomes, *Eur. J. Med. Chem.* **2009**, *44*, 2339 – 2346.

[23] a) C. Hansch, J. E. Quinlan, G. L. Lawrence, *J. Org. Chem.* **1968**, *33*, 347 – 350; b) S. C. Valvani, S. H. Yalkowsky, T. J. Roseman, *J. Pharm. Sci.* **1981**, *70*, 502 – 507.

[24] For a recent review, see: M. Ishikawa, Y. Hashimoto, *J. Med. Chem.* **2011**, *54*, 1539 – 1554 and references therein.

[25] C. McGuigan, R. N. Pathirana, M. Migliore, V. Adak, G. Luoni, A. T. Jones, A. Diez-Torrubia, M. J. Camarasa, S. Velázquez, G. Henson, R. Snoeck, G. Andrei, J. Balzarini, *J. Antimicrob. Chemother.* **2007**, *60*, 1316 – 1330.

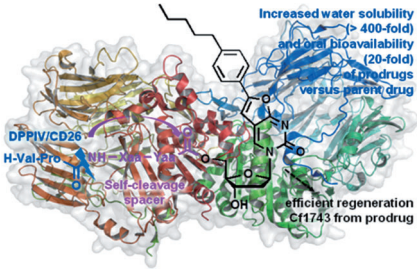
[26] I. De Meester, G. Vanhoof, A.-M. Lambeir, S. Scharpé, *J. Immunol. Methods* **1996**, *189*, 99 – 105.

Received: June 13, 2012
Published online on ■■ ■■, 0000

*A. Diez-Torrubia, S. Cabrera, I. De Meester, M.-J. Camarasa, J. Balzarini, S. Velázquez**

■■ – ■■

Dipeptidyl Peptidase IV-Activated Prodrugs of Anti-Varicella Zoster Virus Bicyclic Nucleoside Analogues Containing Different Self-Cleavage Spacer Systems



Better solubility and oral bioavailability! We describe a new type of water-soluble prodrug of anti-varicella zoster virus bicyclic nucleosides based on the cyclization self-cleavage spacers that efficiently release the parent nucleoside upon hydrolysis by DPPIV/CD26. A marked increase in transport through Caco-2 monolayers and in vivo oral bioavailability is reported.

