From 1-Acyl-β-lactam Human Cytomegalovirus Protease Inhibitors to 1-Benzyloxycarbonylazetidines with Improved Antiviral Activity. A Straightforward Approach To Convert Covalent to Noncovalent Inhibitors

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Starting from the structure of known β-lactam covalent human cytomegalovirus (HCMV) protease inhibitors and from the knowledge of the residues implicated in the active site of this enzyme, we designed a series of phenylalanine-derived 2-azetidinones bearing a 4-carboxylate moiety that could be apt for additional interactions with the guanidine group of the Arg165/Arg166 residues of the viral protease. Some compounds within this series showed anti-HCMV activity at 10−50 μM, but rather high toxicity. The presence of aromatic 1-acyl groups and a certain hydrophobic character in the region of the 4-carboxylate were stringent requirements for anti-HCMV activity. To go a step ahead into the search for effective HCMV medicines, we then envisaged a series of noncovalent inhibitors by simple deletion of the carbonyl group in the β-lactam derivatives to provide the corresponding azetidines. This led to low micromolar inhibitors of HCMV replication, with **17** and **27** being particularly promising lead compounds for further investigation, although their toxicity still needs to be lowered.

Introduction

Human cytomegalovirus (HCMV) is a ubiquitous member of the β-herpes virus family. Although HCMV infection of healthy children and adults is usually asymptomatic, it is a leading cause of birth defects[1] and may result in severe, often life-threatening diseases in immunocompromised (HIV)[2] or immunosuppressed (organ/bone marrow transplant) individuals.[3] Thus, an elevated percentage of kidney, liver, bone marrow, and heart/lung transplant recipients are affected by HCMV hepatitis and pneumonia, resulting in decreased graft and patient survival.[3] Although the use of antiretroviral therapies has significantly diminished the impact of HCMV diseases in AIDS patients, cessation of treatments in these patients led to recurrence or retinitis, colitis, and esophagitis, among other HCMV manifestations.[4]

Currently, five compounds have been licensed for the prophylaxis and treatment of HCMV infection: ganciclovir (GCV), foscarnet, cidofovir (CDV), valganciclovir, and fomivirsen.[5−7] Despite their antiviral potential, all these medications suffer from a number of drawbacks, such as dose-limiting bone marrow and kidney toxicity, as well as the emergence of single and multiple drug resistance.[8] This has prompted the search for other anti-HCMV agents that could be potent, safe, and orally bioavailable, with novel mechanisms of action. Apart from a few new inhibitors of the viral specific DNA polymerase,[9,10] different nucleoside derivatives as well as non-nucleoside inhibitors have been discovered, in recent years, to operate at different states of the replication cycle.[11−16] Among them, the indolizine-1-carboxamide MCV423, a highly effective anti-HCMV agent in vitro, acts on a step of the replicative cycle that precedes the DNA polymerase step, most likely at the intermediate early (IE) antigen synthesis.[14] The L-ribose benzimidazole derivative 1263W94 (maribavir), that appears to be targeted to the UL97 protein kinase, is at present under clinical trials.[15] More recently, a series of thiourea small molecule derivatives were identified as the most potent anti-HCMV agents described to date (nanomolar and subnanomolar inhibitory concentrations).[17−19] These compounds act very early in the replication cycle, inhibiting virion envelope fusion with the cell plasma membrane.[19]

A great part of the above-mentioned HCMV inhibitors were identified through the screening of various classes of compounds, but some approaches have also been directed toward the rational design of anti-HCMV therapies, one of the most relevant being the search for HCMV protease inhibitors. The HCMV pathogen encodes a 780-residue serine protease essential for capsid assembly and viral maturation.[20] The X-ray crystallography revealed that this protein has a backbone fold unique among serine proteases and an unusual catalytic triad formed by Ser132, Thr63, and Thr157 residues[21−23] that make this protein an attractive target for antiviral chemotherapy.[24−26] Overlays of the catalytic triad of HCMV protease with that of trypsin suggested that Arg165 and Arg166 are involved in stabilization of the oxyanion intermediate formed during the hydrolysis reaction. The importance of the side chain of these Arg residues in catalysis has been confirmed by site-directed mutagenesis studies.[27]

Since the active site of the HCMV protease has been identified, the search for inhibitors of this enzyme has become mainstream. Most of these inhibitors contain

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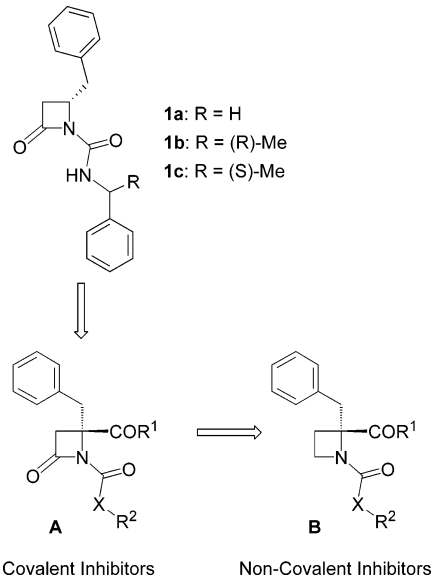
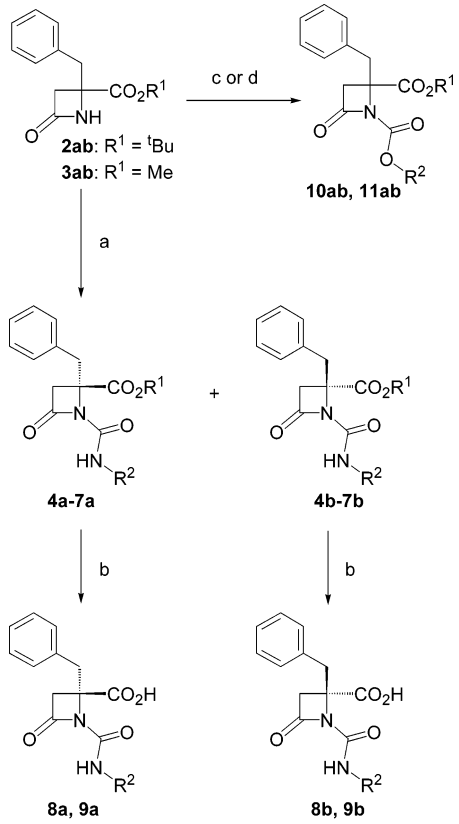
Chart 1



classical serine protease inhibitor motifs based on an activated carbonyl group, such as peptidyl keto-amides,[28,29] as well as mechanism-based inhibitors, such as oxazazinones[30,31] and pyrrolidine-5,5-*trans*-lactams.[32−35] Among the latter, a series of monocyclic β-lactams **1** (compound **1a** being the prototype, Chart 1) has resulted in highly potent derivatives in the isolated enzyme assay, but their efficacy in cell culture was quite limited, as for all described inhibitors of this enzyme.[36−40] Despite the vast efforts made in this field, none of these protease inhibitors has reached the clinic, most likely due to the fact that they covalently bind to the enzyme, as probably they also do to other macromolecules on their way to the target.

On the basis of the structure of the reported β-lactam inhibitors **1**[37] and of the residues implicated in the active site of the HCMV protease,[11] our first approach to potent anti-HCMV agents was directed to increase the contact points between the protein and the inhibitor. For such a purpose, a series of phenylalanine-derived 2-azetidinones of general formula **A** were designed (Chart 1). In comparison with model compounds **1**, these new β-lactams have an additional 4-carboxylate group that could be suitable for extra interactions with the guanidinium groups of the Arg165 or Arg166 residue of the viral protease. Although some compounds in this first series showed slightly improved anti-HCMV activity when compared to the leading β-lactam **1**a, these compounds are still less active than the reference compounds ganciclovir and cidofovir. For the development of therapeutically useful serine protease inhibitors with good pharmacological characteristics, it has generally been accepted that noncovalent inhibitors are preferred over covalent ones (even over covalent reversible inhibitors).[41,42] With this in mind, we envisaged a series of noncovalent inhibitors, the azetidine derivatives **B**, generated by deletion of the carbonyl lactam group in compounds **A**, while enough hydrogen bonds and ionic and/or van der Waals interactions were maintained with the biological target. Related serine trap deletion strategies have successfully guided the design of noncovalent inhibitors of some mammalian serine proteases.[41,42]

Scheme 1[a]



Compd.	R^1	R^2
4	^{t}Bu	Ph
5	^{t}Bu	CH(*R*-CH_3)Ph
6	^{t}Bu	CH(*S*-CH_3)Ph
7	Me	CH(*S*-CH_3)Ph
8	H	CH(*R*-CH_3)Ph
9	H	CH(*S*-CH_3)Ph
10	^{t}Bu	CH_2Ph
11	^{t}Bu	^{t}Bu

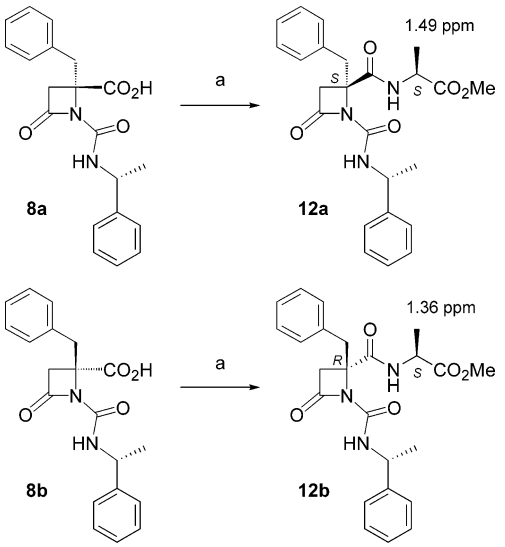
[a] Reagents and conditions: (a) R^2NCO/Cs_2CO_3/THF. (b) TFA/DCM. (c) $PhCH_2OCOCl$/DBU/DCM. (d) $(Boc)_2O$/TEA/DMPA/DCM.

This paper deals with the synthesis and evaluation of the above-mentioned series of compounds, azetidinones **A** and azetidines **B**,[43] and proposes a general approach to convert covalent viral protease inhibitors into noncovalent antiviral agents.

Results and Discussion

1-Acyl-β-lactam Derivatives. The starting *N*-unsubstituted 2-azetidinones **2ab** and **3ab** were prepared from H-Phe-O^{t}Bu and H-Phe-OMe, respectively, as previously described.[44] A moderate selectivity during the cyclization reaction, attributed to the occurrence of a "memory of chirality" phenomenon,[45,46] led to compounds **2** and **3** as mixtures of 4*S*:4*R* enantiomers in about a 2:1 **a**:**b** ratio. Taking into account that the acylation of related β-lactams with isocyanates generally proceeded in low yield,[37] we first investigated the reaction of azetidinone **2ab** with phenyl isocyanate under different conditions (Scheme 1). Among TEA/DMAP, DBU, and Cs_2CO_3, the latter base was found to be the most effective for the synthesis of compound **4ab**

Scheme 2[a]



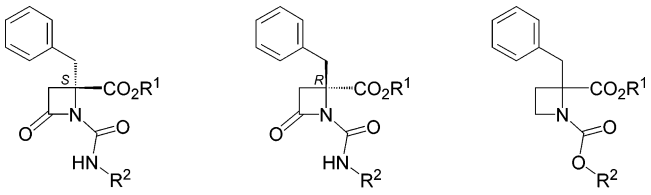
[a] (a) H-Ala-OMe/BOP/TEA/THF.

(82%). Using these optimized conditions, the reaction of compound **2** with (*R*)-1- and (*S*)-1-phenylethyl isocyanate afforded compounds **5ab** and **6ab**, respectively. These diastereoisomeric mixtures, which were easily separated by column chromatography, were obtained in approximately 1.5:1 **a**:**b** ratio, indicating some kinetic resolution during the acylation reaction. The methyl ester-containing derivatives **7a** (62%) and **7b** (29%) were prepared by treatment of **3** with (*S*)-1-phenylethyl isocyanate, followed by chromatographic resolution. Compounds **5a**, **5b**, **6a**, and **6b** were hydrolyzed to the corresponding free carboxylic acids **8a**, **8b**, **9a**, and **9b** by treatment with (1:4) TFA/CH_2Cl_2. The absolute configuration at C_4 in the main diastereoisomers **a** was assigned as *S*, since major enantiomers in **2** and **3** have *S* configuration, as previously established.[47] Nevertheless, to corroborate that the aliphatic−aromatic dipeptide rule[48,49] is also valid for these 1-acyl-β-lactams, compounds **8a** and **8b** were transformed into the corresponding Ala-dipeptide derivatives **12a** and **12b**. As expected, the signal corresponding to the β-H protons of Ala residue is more shielded in the *R*,*S*-dipeptide **12b** than in the *S*,*S*-analogue **12a** (Scheme 2),[48] while in HPLC the heterochiral derivative **12b** is more retained than the homochiral one.[49]

Finally, the 1-urethane-substituted β-lactams **10** and **11** were prepared as 2:1 *S*:*R* enantiomeric mixtures by treatment of azetidinone **2** with benzyl chloroformate and *tert*-butyl dicarbonate, respectively, as previously described.[50]

The target 1-acyl-β-lactams **4**−**11** were evaluated for their ability to inhibit the replication of HCMV in vitro,[14] and the results were compared to those obtained for the reference compounds ganciclovir and cidofovir (Table 1). For comparative purposes, the reported anti-HCMV activity of the model β-lactam **1a** was also included in the table. Compounds **4**−**11** did not interfere

Table 1. Activity of 1-Acyl-β-lactam Derivatives against Human Cytomegalovirus (HCMV) and Varicella-zoster (VZV) Virus in HEL Cell Cultures



			EC$_{50}$ (μM)					
			anti-HCMV activity[a]		anti-VZV activity[b]		cytotoxicity (μM)	
compd	R^1	R^2	AD-169	Davis	OKA (TK+)	07/1 (TK$^-$)	MCC[c]	CC$_{50}$[d]
4ab	^{t}Bu	Ph	50	50	33	13	200	118
5a	^{t}Bu	CH(*R*-CH_3)Ph	32	33	>50	23	200	70
5b	^{t}Bu	CH(*R*-CH_3)Ph	100	107	>50	>50	>200	>200
6a	^{t}Bu	CH(*S*-CH_3)Ph	35	35	37	20	200	184
6b	^{t}Bu	CH(*S*-CH_3)Ph	32	33	>50	14	200	164
7a	Me	CH(*S*-CH_3)Ph	>50	–	>50	>50	>50	>50
7b	Me	CH(*S*-CH_3)Ph	>50	–	>50	>50	50	>50
8a	H	CH(*R*-CH_3)Ph	>200	>200	>200	151	>200	>200
8b	H	CH(*R*-CH_3)Ph	>50	>50	>200	>200	200	>200
9a	H	CH(*S*-CH_3)Ph	>50	>50	>200	110	200	187
9b	H	CH(*S*-CH_3)Ph	>200	>200	>200	111	>200	>200
11ab	^{t}Bu	^{t}Bu	>200	>200	>200	>200	>200	>200
10ab	^{t}Bu	CH_2Ph	11	13	12	8	50	36
1a[e]			53	–	–	–	–	>250
ganciclovir			1.5	1.5	–	–	>1500	>150
cidofovir			0.6	1.0	–	–	>1400	>150
acyclovir			17	20	7.1	111	>1200	511
brivudin			>100	>100	0.03	103	1201	249

[a] Effective concentration required to inhibit by 50% the HCMV-induced cytopathicity in human embryonic lung (HEL) fibroblast cell cultures at 7 days postinfection, as described in ref 8c. Virus input was 100 plaque-forming units (PFU). [b] Effective concentration required to reduce VZV plaque formation after 5 days in HEL cell cultures by 50%, as compared to untreated controls. [c] Compound concentration required to cause a microscopically visible alteration of normal cell morphology. [d] Cytotoxic concentration required to reduce cell growth by 50%. [e] From ref 17b.

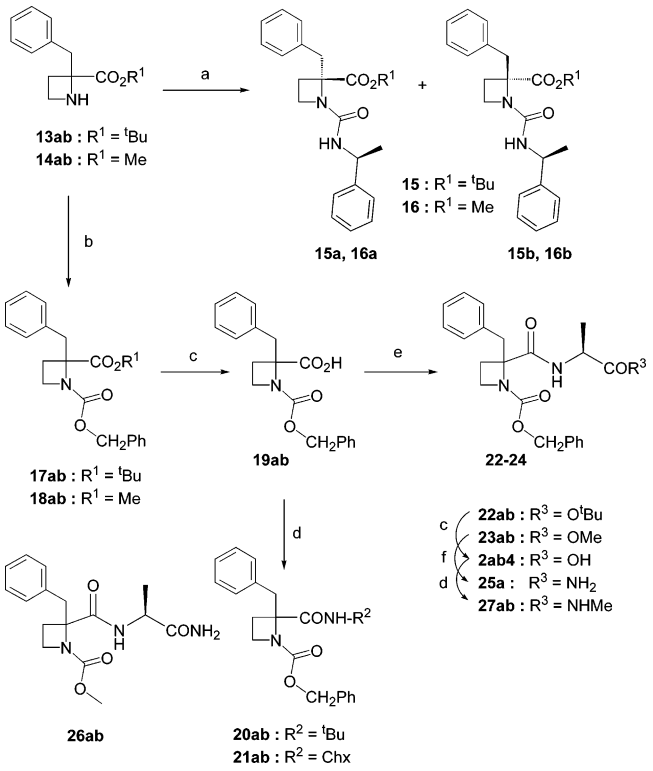
with the replication of a broad range of DNA or RNA viruses (data not shown), but some compounds showed moderate activity against VZV reference strains (Table 1).

As shown in Table 1, the phenylurea-substituted β-lactams **4** weakly blocked HCMV plaque formation with an EC_{50} value of 50 μM, fully comparable to that reported for the prototype 2-azetidinone **1a**. A slight increase in the antiviral activity was observed for compounds **5** and **6**, incorporating chiral α-methylbenzyl urea substituents at position 1. Evaluation of the anti-HCMV activity of 1′*R* and 1′*S* diastereoisomers **5a** and **6a** indicated no appreciable influence of the absolute configuration at the α-methylbenzyl moiety on the inhibition of viral replication. In contrast, a 4*S* configuration seems to be preferred, at least in the case of compounds bearing an *R*-α-methylbenzyl moiety (compare 4*S*-**5a** with 4*R*-**5b**). Although intriguing, the activity−stereochemistry relationships in our β-lactam series is quite similar to those found for model compounds **1**.[37] Thus, in compound **1b** the change of the C-4 configuration (*S* by *R*) provided an almost inactive compound, while inversion at the α-methylbenzyl chiral carbon led to a minor potency loss. The lack of activity of the methyl ester derivatives **7a** and **7b**, as well as of the free carboxylic acid analogues **8a**, **8b**, **9a** and **9b**, might suggest a role for the *tert*-butyl ester moiety, either as a bulky substituent for hydrophobic interaction with the viral target or by increasing cellular and/or nuclear membrane penetration. Nevertheless, since *tert*-butyl ester-hydrolyzing enzymes are uncommon, a higher enzymatic hydrolysis of methyl versus *tert*-butyl esters, to provide the inactive carboxylic derivatives, could also explain the inactivity of compounds **7**. Replacing the urea moieties in **4**−**6** by a benzyloxycarbonyl group to give **10** had a significant effect on the antiviral potency. Compound **10** showed the highest anti-HCMV activity within the β-lactam series, but its cell toxicity value was close to the EC_{50} data for viral inhibition. Since compound **10** is highly reactive toward *O*- and *N*-nucleophiles,[50] it could bind to many nucleophiles en route to the intended target, most likely resulting in toxic side effects.[41,42] Finally, the presence of an aromatic group at the 1-acyl substituent seems important for the antiviral activity, since the *tert*-butoxycarbonyl-substituted derivative **11** was completely inactive.

Almost in parallel to the inhibition of the HCMV replication, we also noted a modest anti-VZV activity for compounds **4ab**, **5a**, **6a**, **6b**, and **10ab**. Dual anti-VZV and anti-HCMV activity has also been described for a series of bicyclic furanopyrimidine deoxynucleosides.[51] Interestingly, compound **10ab**, which is the most active against HCMV, also proved to be the most active against VZV.

The precise mechanism of action of these 1-acyl-β-lactams against HCMV remains unclear. However, considering their structural analogy with model compounds **1**, we presumed that compounds **4**−**6** and **10** could inhibit HCMV replication in an irreversible manner through acylation of the virus serine protease. As other irreversible enzyme inhibitors, compound **10** is highly reactive toward nucleophiles,[50] and this reactivity could be masking, at least to some extent, its real antiviral effects. In fact, in the series of the potent 5,5-

Scheme 3[a]



[a] (a) R^2NCO/Cs_2CO_3/THF. (b) $PhCH_2OCOCl$/DCM. (c) TFA/DCM. (d) R^2NH_2/BOP/TEA/THF. (e) H-Ala-R^3/BOP/TEA/THF. (f) NH_3/MeOH.

trans-lactam serine protease inhibitors, a combination of low nanomolar potency against HCMV protease and sufficient plasma stability were strictly required to get compounds with low micromolar activity against the virus in the intact cells.[35]

1-Acylazetidine Derivatives. Following the idea that conversion of the 1-acyl-β-lactams into the corresponding azetidines could lead to more active and safe HCMV inhibitors, we first focused on removing the carbonyl lactam group from the hypothetically covalent inhibitors **6** and **10** to obtain the corresponding azetidine derivatives **15** and **17** (Scheme 3). After a positive proof of concept, some other azetidine derivatives were prepared to set up structure−activity relationships. Thus, the methyl ester derivatives **16** were designed as negative controls, since their 1-acylazetidinone counterparts **7a** and **7b** were inactive. On the other hand, the substitution of the O^{t}Bu group in **10** by NH^{t}Bu and NHChx to provide compounds **20** and **21** could serve to restrict the conformational flexibility, through the possibility of intramolecular hydrogen bonds, while a bulky hydrophobic group was kept at this point. The knowledge that the substrate cleavage site across all the herpesvirus proteases are highly conserved (Ala-Ser), led us to design azetidine derivatives **22**−**27**, which incorporate differently substituted Ala residues to allow access to the S1 subsite of the enzyme.[52]

The common starting azetidines **13ab** and **14ab** were prepared, as enantiomeric mixtures of approximately 2.5:1 **a**:**b** ratio, from suitable β-lactam precursors, following a chemoselective reduction method previously described.[53] As shown in Scheme 3, the treatment of azetidine derivatives **13** and **14** with (*S*)-1-phenylethyl isocyanate afforded the diastereoisomeric mixtures **15ab**

Table 2. Chemical Shifts and Temperature Coefficients of Amide Protons in Compounds **20**, **21**, and **27**

	δ 2-CONH				δ NHMe			
compd	$CDCl_3$ [a]	DMSO[a]	Δδ	Δδ/Δ*T*[b]	$CDCl_3$ [a]	DMSO[a]	Δδ	Δδ/Δ*T*[b]
20	7.81	7.72	0.09	−2.7				
21	7.86	7.85	0.01	−3.0				
27a[c]	8.18	8.23	−0.05	−2.8	6.34	7.81	1.47	−5.4
27b[c]	8.13	8.11	0.02	−2.9	6.34	7.90	1.56	−5.0

[a] Measured for the major trans rotamer (ppm). [b] Determined by least-squares linear regression analysis from measurements over the range 30−60 °C (seven points), in DMSO-d_6 (ppb/K). [c] Measured in approximately 4:1 enriched mixtures of each diastereoisomer.

and **16ab** in moderate to good yield. In this case, only compound **16ab** could be efficiently resolved into its corresponding diastereoisomers. In a similar way, acylation of **13** and **14** with benzyl chloroformate resulted in the corresponding Z-substituted derivatives **17ab** and **18ab**, as enantiomeric mixtures. Carboxamide derivatives **20ab** and **21ab** were prepared by reaction of the carboxylic derivative **19ab** with *tert*-butyl- and cyclohexylamine, respectively, using BOP as the coupling agent. A similar coupling reaction was conducted to prepare Ala dipeptide derivatives **22ab** and **23ab**, two diastereoisomeric mixtures that could not be chromatographically resolved. Compound **23** was then transformed into the corresponding free carboxylic and amide derivatives **24** and **25** by saponification and ammonolysis with NH_3/MeOH, respectively. It is interesting to note that, in the last reaction, the expected compound **25** was obtained as a single diastereoisomer, **25a**, along with a 1:1.1 diastereoisomeric mixture of compound **26ab**, resulting from the methanolysis of the Z group. Compound **24** was finally converted into the *N*-methylamide derivatives **27ab** by treatment with methylamine in the presence of BOP.

The examination of the ^{1}H NMR spectra of all these 1-acylazetidines evidenced a different conformational behavior among compounds with 2-carboxylic esters and 2-carboxamide derivatives. Thus, compounds **15**−**18** showed in $CDCl_3$ nearly equimolecular extent of cis and trans rotamers around the N^1−CO bond (38−50% cis). In contrast, the percentage of cis rotamer was lower than 15% for all the carboxamide derivatives (**20**−**27**) in the same solvent. We found that, even in a highly coordinating solvent, such as DMSO, the ratio of cis rotamer for the latter compounds did not exceed 25%, indicating a lower conformational flexibility of these derivatives compared with the carboxylic ester analogues. To ascertain if the restricted rotation around the N^1−CO bond could be due to the stabilization of folded conformations, we selected **20**, **21**, and **27** as model compounds to evaluate the differences in the amide NH protons chemical shifts between $CDCl_3$ and DMSO, as well as the temperature coefficients for these protons in DMSO, two parameters that could be related to the existence of intramolecular hydrogen bonds.[54] As shown in Table 2, the chemical values for the 2-CONH protons in $CDCl_3$ were in all cases >7 ppm; these values suffer an imperceptible variation in DMSO, and the temperature dependence of the chemical shifts are smaller than, or equal to, 3 ppb/K, as an absolute value. These observations are consistent with the participation of the 2-CONH proton of compounds **20**, **21**, and **27** in a stable intramolecular hydrogen bond, fixing a γ-turn conformation. In contrast, the NHMe amide protons in **27a** and **27b** showed chemical shift values and temperature coefficients that suggested non-hydrogen-bonded states.

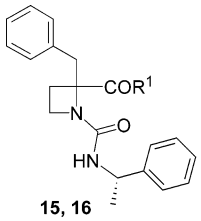
Compounds **15**−**17** and **20**−**27** were evaluated as inhibitors of the AD-169 and Davis HCMV strains, as previously described (Table 3).[14] As noted in Table 3, the 2-*tert*-butoxycarbonylazetidine derivative **15ab** retains the potency of its β-lactam equivalents **6a** and **6b**, but with a slightly increased cytostatic activity, while the methyl ester analogues **16a** and **16b** were inactive, as in the β-lactam series. Strikingly, compound **17** showed a considerable improvement in the antiviral activity with respect to **10** and all other β-lactamic inhibitors. Although certain toxicity persisted, the therapeutic index (ratio $CC_{50}/EC_{50} \geq 50$) was clearly improved with respect to the β-lactam analogue **10** (therapeutic index ≈ 3). Therefore, its antiviral selectivity was at least 10-fold increased. The EC_{50} value obtained for this azetidine derivative was similar to that of the standard reference compound cidofovir. Further modification of the structure of **17** by transformation to 2-carboxamide analogues **20** and **21** resulted in a 5−8-fold decrease in antiviral potency, probably due to the lower conformational flexibility of these latter compounds. The importance of a hydrophobic environment by the carboxylate in position 2 was suggested by the anti-HCMV activity of compound **22** and the inactivity of its analogues **23** and **24**, with a small methyl group and a free carboxylic acid, respectively. However, a high susceptibility toward enzymatic hydrolysis of the methyl ester derivative **23** could also take account for its inactivity. Contrastingly, the antiviral activity was restored with the primary 2-carboxamide derivative **25**, indicating that the NH_2 protons could be implicated in direct interactions with the biological target. Again, an increase in the hydrophobic character at this part of the structure, as in the NHMe derivative **27**, is followed by an enhancement of the activity and no signs of cytotoxicity at 50 μM. As previously noted for the parent β-lactams, the antiviral activity in the homologous azetidine series is dependent on the presence of an aromatic moiety within the substituent at position 1, as deduced from the lack of activity of compound **26**.

All compounds within this series, except for **15** and **21**, did not show notable inhibition of the cytopathicity of a broad panel of viruses, pointing to their selectivity as anti-HCMV agents.

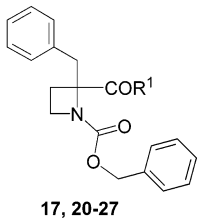
Conclusions

Starting from a series of described covalent inhibitors of the HCMV serine protease, we describe a two-step approach to develop more effective inhibitors of HCMV replication. First, we tried to introduce additional protein−inhibitor close contacts by incorporating carboxylate-related groups at position 4 of the model β-lactams and by fine-tuning the structure through other minor modifications. The second more challenging step was directed to remove the β-lactam ring carbonyl

Table 3. Activity of 1-Acylazetidine Derivatives against Human Cytomegalovirus (HCMV) and Varicella-zoster Virus (VZV) in HEL Cell Cultures



15, 16



17, 20-27

		EC_{50} (μM)					
		anti-HCMV activity[a]		anti-VZV activity[b]		cytotoxicity (μM)	
compd	R^1	AD-169	Davis	OKA (TK+)	07/1 (TK−)	MCC[c]	CC_{50} [d]
15ab	O^{t}Bu	40	33	36	25	400	57
16a	OMe	>20	–	50	50	>50	>50
16b	OMe	>20	–	>50	>50	>50	>50
17ab	O^{t}Bu	0.7	0.6	>2	>2	5	34
20ab	NH^{t}Bu	5.5	4.4	>3.2	>16	≥16	36
21ab	NHChx	5.9	3.2	7.8	5.1	≥16	37
22ab	Ala-O^{t}Bu	16	4.4	>16	>16	80	33
23ab	Ala-OMe	>80	>80	>80	>80	400	50
24ab	Ala-OH	>400	>80	>80	>80	≥400	124
25a	Ala-NH_2	39	32	>80	>80	≥400	50
26ab	Ala-NH_2	>400	>400	>400	346	>400	>200
27ab	Ala-NH-Me	7.4	5.0	>50	>50	>50	>50
ganciclovir		1.5	1.5	–	–	>1500	>150
cidofovir		0.6	1.0	–	–	>1400	>150
acyclovir		17	20	7.1	111	>1200	511
brivudin		>100	>100	0.03	103	1201	249

[a] Effective concentration required to inhibit by 50% the HCMV-induced cytopathicity in human embryonic lung (HEL) fibroblast cell cultures at 7 days postinfection, as described in ref 8c. Virus input was 100 plaque-forming units (PFU). [b] Effective concentration required to reduce VZV plaque formation after 5 days in HEL cell cultures by 50%, as compared to untreated controls. [c] Compound concentration required to cause a microscopically visible alteration of normal cell morphology. [d] Cytotoxic concentration required to reduce cell growth by 50%.

group, responsible for the covalent acylation of the enzyme, in an attempt to convert covalent inhibitors to noncovalent ones. Following this strategy, we obtained compounds **17** and **27**, which showed activity at 0.6−7.4 μM against both strains of HCMV. These promising leads could represent the first class of noncovalent inhibitors for HCMV protease reported to date, although their exact mechanism of action remains to be determined. The high throughput screening of various libraries, followed by optimization, has allowed the discovery of a series of competitive, noncovalent HCMV protease inhibitors, but their ability to reduce HCMV replication in cell assays was not disclosed.[55] The rationale here used could be applied to other families of HCMV protease covalent inhibitors to explore its utility as a general approach for transforming covalent to noncovalent inhibitors.

Experimental Section

Chemistry. All reagents were of commercial quality. Solvents were dried and purified by standard methods. Amino acid derivatives were obtained from Bachem AG. Analytical TLC was performed on aluminum sheets coated with a 0.2-mm layer of silica gel 60 F_{254} (Merck), and preparative TLC was done on 20 × 20 glass plates coated with a 2 mm layer of silica gel PF_{254} (Merck). Silica gel 60 (230−400 mesh) (Merck) was used for flash chromatography. Melting points were taken on a micro hot stage apparatus and are uncorrected. ^{1}H NMR spectra were recorded with a Varian Gemini 200 or a Varian XL-300, operating at 200 and 300 MHz, respectively, using TMS as reference. Temperature coefficients were obtained from least-squares fits to data of 30, 35, 40, 45, 50, 55, and 60 °C in DMSO-d_6. ^{13}C NMR spectra were recorded with a Varian Gemini 200 or a Varian XL-300 operating at 50 and 75 MHz, respectively. Elemental analyses were obtained on a CH−O−RAPID apparatus. Optical rotations were determined with a Perkin-Elmer 141 polarimeter. Analytical HPLC was performed on a Waters Nova-pak C_{18} column (3.9 × 150 mm, 4 μm) with a flow rate of 1 mL/min and using a tunable UV detector set at 214 nm. Mixtures of CH_3CN (solvent A) and 0.05% TFA in H_2O (solvent B) were used as mobile phase. Compounds **2**, **3**, **10**, **11**, **13**, and **14** were prepared as previously described.[44,50,53]

General Procedure for the Reaction of *N*-Unsubstituted *β*-Lactams with Isocyanates. To a solution of the corresponding azetidinone (2.54 mmol) in dry THF (4 mL) was added Cs_2CO_3 (0.83 g, 2.54 mmol), followed by the corresponding isocyanate (3.05 mmol). After stirring at room temperature for 10−20 min, the solvent was eliminated, and the resulting residue was dissolved in EtOAc and washed with brine. The organic layer was separated, dried over Na_2SO_4, and evaporated to dryness. The obtained residue was purified on a silica gel column or on preparative plates, using the solvent system indicated in each case.

(4*R*,*S*)-4-Benzyl-4-*tert*-butoxycarbonyl-1-phenylaminocarbonyl-2-azetidinone (4ab). Flash chromatography: EtOAc/hexane (1:8). Yield: 82% (from **2ab** and phenyl isocyanate), solid. Mp: 88−90 °C (EtOAc/hexane). HPLC: t_R = 16.6 min (A:B = 50:50). ESI-MS: 403.2 $(M + Na)^+$. Anal. ($C_{22}H_{24}N_2O_4$) C, H, N.

(4*S*,1′*R*)-1-[(1′-Phenyl)ethyl]aminocarbonyl-4-benzyl-4-*tert*-butoxycarbonyl -2-azetidinone (5a). Preparative TLC: EtOAc/hexane (1:8). Yield: 50% (from **2ab** and *R*-(+)-(1-phenyl)ethyl isocyanate), as a solid. Mp: 44 °C (EtOAc/hexane). $[\alpha]_D$ = +123.37 (c = 1.01, $CHCl_3$). HPLC: t_R = 24.10 min (A:B = 45:55). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.36−7.2 (m, 10H, C_6H_5), 6.78 (d, 1H, NH, J = 8.05), 5.09 (m, 1H, 1′-H), 3.67 (d, 1H, 3-H, J = 14.6), 3.28 (d, 1H, 3-H, J = 14.6), 2.90 (s, 2H, 4-CH_2), 1.57 (d, 3H, 2′-H, J = 6.9), 1.36 (s, 9H, CH_3,

^{t}Bu). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.47 (COO), 165.33 (2-C), 149.20 (NCON), 142.93, 134.47, 130.49, 128.52, 128.41, 127.23, 127.18, 125.94 (Ar), 82.88 (C, ^{t}Bu), 60.77 (4-C), 49.36 (1′-C), 43.80 (3-C), 35.95 (4-CH_2), 27.52 (CH_3, ^{t}Bu), 22.29 (2′-C). ESI-MS: 409.2 $(M + 1)^+$. Anal. ($C_{24}H_{28}N_2O_4$) C, H, N.

(4*R*,1′*R*)-1-[(1′-Phenyl)ethyl]aminocarbonyl-4-benzyl-4-*tert*-butoxycarbonyl-2-azetidinone (5b). Preparative TLC: EtOAc/hexane (1:8). Yield: 33% (from **2ab** and *R*-(+)-(1-phenyl)ethyl isocyanate), as a foam. $[\alpha]_D = -145.93$ (c = 1.01, $CHCl_3$). HPLC: t_R = 23.10 min (A:B = 45:55). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.43−7.01 (m, 10H, C_6H_5), 6.78 (d, 1H, NH, J = 7.8), 5.08 (m, 1H, 1′-H), 3.64 (d, 1H, 3-H, J = 14.5), 3.21 (d, 1H, 3-H, J = 14.5), 2.91 (m, 2H, 4-CH_2), 1.54 (s, 9H, CH_3, ^{t}Bu), 1.53 (d, 3H, 2′-H, J = 6.9). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.66 (COO), 165.13 (2-C), 149.22 (NCON), 143.23, 134.14, 130.55, 128.61, 128.38, 127.34, 127.18, 125.90 (Ar), 83.07 (C, ^{t}Bu), 60.97 (4-C), 49.44 (1′-C), 43.87 (3-C), 35.84 (4-CH_2), 27.82 (CH_3, ^{t}Bu), 22.53 (2′-C). ESI-MS: 409.2 $(M + 1)^+$. Anal. ($C_{24}H_{28}N_2O_4$) C, H, N.

(4*S*,1′*S*)-1-[(1′-Phenyl)ethyl]aminocarbonyl-4-benzyl-4-*tert*-butoxycarbonyl-2-azetidinone (6a). Preparative TLC: EtOAc/hexane (1:5). Yield: 44% (from **2ab** and *S*-(−)-(1-phenyl)ethyl isocyanate), as a foam. $[\alpha]_D = +150.43$ (c = 1.14, $CHCl_3$). HPLC: t_R = 23.10 min (A:B = 45:55). ESI-MS: 409.2 $(M + 1)^+$. Anal. ($C_{24}H_{28}N_2O_4$) C, H, N. ^{1}H and ^{13}C NMR data identical to that of its enantiomer **5b**.

(4*R*,1′*S*)-1-[(1′-Phenyl)ethyl]aminocarbonyl-4-benzyl-4-*tert*-butoxycarbonyl-2-azetidinone (6b). Preparative TLC: EtOAc/hexane (1:5). Yield: 30% (from **2ab** and *S*-(−)-(1-phenyl)ethyl isocyanate), as a solid. Mp: 43 °C (EtOAc/hexane). $[\alpha]_D = -126.03$ (c = 0.92, $CHCl_3$). HPLC: t_R = 24.10 min (A:B = 45:55). ESI-MS: 409.2 $(M + 1)^+$. Anal. ($C_{24}H_{28}N_2O_4$) C, H, N. ^{1}H and ^{13}C NMR data identical to that of its enantiomer **5a**.

(4*S*,1′*S*)-1-[(1′-Phenyl)ethyl]aminocarbonyl-4-benzyl-4-methoxycarbonyl-2-azetidinone (7a). Flash chromatography: EtOAc/hexane (1:5). Yield: 62% (from **3ab** and *R*-(−)-(1-phenyl)ethyl isocyanate), as a syrup. $[\alpha]_D = +161.66$ (c = 0.54, $CHCl_3$). HPLC: t_R = 12.84 min (A:B = 40:60). ESI-MS: 367.1 $(M + 1)^+$. Anal. ($C_{21}H_{22}N_2O_4$) C, H, N.

(4*R*,1′*S*)-1-[(1′-Phenyl)ethyl]aminocarbonyl-4-benzyl-4-methoxycarbonyl-2-azetidinone (7b). Flash chromatography: EtOAc/hexane (1:5). Yield: 29% (from **3ab**, and *R*-(−)-(1-phenyl)ethyl isocyanate), as a syrup. $[\alpha]_D = -96.41$ (c = 0.94, $CHCl_3$). HPLC: t_R = 14.56 min (A:B = 40:60). ESI-MS: 367.1 $(M + 1)^+$. Anal. ($C_{21}H_{22}N_2O_4$) C, H, N.

General Procedure for the Hydrolysis of O^{t}Bu Esters. To a solution of the corresponding *tert*-butoxycarbonyl-2-azetidinone (0.26 mmol) in dry CH_2Cl_2 (1 mL) was added TFA (0.2 mL, 2.6 mmol). After a 2-h reaction, the solution was evaporated to dryness, coevaporating several times with CH_2Cl_2. The resulting residue was purified on a silica gel column using CH_2Cl_2:MeOH (30:1) as eluent.

(4*S*,1′*R*)-1-[(1′-Phenyl)ethyl]aminocarbonyl-4-benzyl-4-carboxy-2-azetidinone (8a). Yield: 90% (from **5a**), as a solid. Mp: 165−167 °C (EtOAc/hexane). $[\alpha]_D = +95.31$ (c = 0.38, $CHCl_3$). HPLC: t_R = 6.80 min (A:B = 40:60). ESI-MS: 353.1 $(M + 1)^+$. Anal. ($C_{24}H_{28}N_2O_4$) C, H, N.

(4*R*,1′*R*)-1-[(1′-Phenyl)ethyl]aminocarbonyl-4-benzyl-4-carboxy-2-azetidinone (8b). Yield: 80% (from **5b**), as a solid. Mp: 170−172 °C (EtOAc/hexane). $[\alpha]_D = -120.33$ (c = 0.38, $CHCl_3$). HPLC: t_R = 6.42 min (A:B = 40:60). ESI-MS: 353.1 $(M + 1)^+$. Anal. ($C_{24}H_{28}N_2O_4$) C, H, N.

(4*S*,1′*S*)-1-[(1′-Phenyl)ethyl]aminocarbonyl-4-benzyl-4-carboxy-2-azetidinone (9a). Yield: 98% (from **6a**), as a solid. Mp: 171−173 °C (EtOAc/hexane). $[\alpha]_D = +119.54$ (c = 0.81, $CHCl_3$). HPLC: t_R = 6.40 min (A:B = 40:60). ESI-MS: 353.1 $(M + 1)^+$. Anal. ($C_{24}H_{28}N_2O_4$) C, H, N.

(4*R*,1′*S*)-1-[(1′-Phenyl)ethyl]aminocarbonyl-4-benzyl-4-carboxy-2-azetidinone (9b). Yield: 72% (from **6b**), as a solid. Mp: 161−163 °C (EtOAc/hexane). $[\alpha]_D = -99.19$ (c = 0.81, $CHCl_3$). HPLC: t_R = 6.80 min (A:B = 40:60). ESI-MS: 353.1 $(M + 1)^+$. Anal. ($C_{24}H_{28}N_2O_4$) C, H, N.

General Procedure for the Preparation of Azetidinone-Containing Dipeptides. A solution of the corresponding 4-carboxy-2-azetidinone **8** (0.021 g, 0.06 mmol) in THF (1 mL) was successively treated with H-Ala-OMe·HCl (0.012 g, 0.085 mmol), BOP (0.037 g, 0.085 mmol), and TEA (0.024 mL, 0.17 mmol) and stirred overnight at room temperature. After evaporation, the residue was dissolved in EtOAc and washed with citric acid (10%), $NaHCO_3$ (10%), H_2O, and brine. The organic layer was dried over Na_2SO_4 and evaporated to leave a residue that was purified on a silica gel column, using EtOAc: hexane (1:5) as eluent.

(4*S*,1′*R*,1″*S*)-1-[(1′-Phenyl)ethyl]aminocarbonyl-4-[(1″-methoxycarbonyl)ethyl]aminocarbonyl-4-benzyl-2-azetidinone (12a). Yield: 81% (from **8a**), as a white solid. Mp: 72−74 °C (EtOAc/hexane). $[\alpha]_D = -83.6$ (c = 0.64, $CHCl_3$). HPLC: t_R = 7.32 min (A:B = 45:55). ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.72 (d, 1H, 4-CONH, J = 7.2), 7.46−6.94 (m, 11H, C_6H_5 and CONH), 5.11 (m, 1H, 1′-H), 4.58 (m, 1H, 1″-H), 3.78 (s, 3H, OMe), 3.59 (d, 1H, 3-H, J = 14.2), 3.43 (d, 1H, 4-CH_2, J = 16.5), 3.11 (d, 1H, 3-H, J = 14.2), 2.92 (d, 1H, 4-CH_2, J = 16.5), 1.55 (d, 3H, 2′-H, J = 7.0), 1.49 (d, 3H, 2″-H, J = 7.3). ESI-MS: 438.2 $(M + 1)^+$. Anal. ($C_{24}H_{27}N_3O_5$) C, H, N.

(4*R*,1′*R*,1″*S*)-1-[(1′-Phenyl)ethyl]aminocarbonyl-4-[(1″-methoxycarbonyl)ethyl]aminocarbonyl-4-benzyl-2-azetidinone (12b). Yield: 80% (from **8b**), as a white solid. Mp: 123−124 °C (EtOAc/hexane). $[\alpha]_D = +37.5$ (c = 0.45, $CHCl_3$). HPLC: t_R = 8.40 min (A:B = 45:55). ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.72 (d, 1H, 4-CONH, J = 6.9), 7.40−7.05 (m, 11H, C_6H_5 and 1-CONH), 5.13 (m, 1H, 1′-H), 4.51 (m, 1H, 1″-H), 3.76 (s, 3H, OMe), 3.72 (d, 1H, 3-H, J = 14.3), 3.42 (d, 1H, 4-CH_2, J = 16.5), 3.24 (d, 1H, 3-H, J = 14.3), 2.96 (d, 1H, 4-CH_2, J = 16.5), 1.59 (d, 3H, 2′-H, J = 6.9), 1.36 (d, 3H, 2″-H, J = 7.3). ESI-MS: 438.2 $(M + 1)^+$. Anal. ($C_{24}H_{27}N_3O_5$) C, H, N.

General Procedure for the Reaction of *N*-Unsubstituted Azetidines with Isocyanates. A solution of the corresponding azetidine **13ab** or **14ab** (0.62 mmol) in dry CH_2Cl_2 (2 mL) was treated with TEA (0.086 mL, 0.62 mmol) and *S*-(−)-(1-phenyl)ethyl isocyanate (0.74 mmol) and stirred at room temperature for 1 h. After evaporation of the solvent, the resulting residue was purified on a silica gel column using the eluent system indicated in each case.

(2*S*,R,1′*S*)-1-[(1′-Phenyl)ethyl]aminocarbonyl-2-benzyl-2-*tert*-butoxycarbonylazetidine (15ab). Eluent: CH_2Cl_2: EtOAc (15:1). Yield: 73% (from **13ab**), as a white foam. ESI-MS: 395.2 $(M + 1)^+$. Anal. ($C_{24}H_{30}N_2O_3$) C, H, N.

(2*R*,1′*S*)-1-[(1′-Phenyl)ethyl]aminocarbonyl-2-benzyl-2-methoxycarbonylazetidine (16a). Eluent: EtOAc:hexane (1:3). Yield: 53% (from **14ab**). Gummy solid. HPLC: t_R = 7.63 min (A:B = 40:60). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.33−7.06 (m, 11H, C_6H_5 and NH), 4.96 (m, 1H, 1′-H), 3.65 (s, 3H, OMe), 3.54 (m, 1H, 4-H), 3.29 (d, 1H, 4-CH_2, J = 13.7), 3.26 (m, 1H, 2-H), 3.04 (d, 1H, 4-CH_2, J = 13.7), 2.30 (m, 1H, 3-H), 2.08 (m, 1H, 3-H), 1.43 (d, 3H, 2′-H, J = 6.9). ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.27 (COO), 157.11 (NCON), 144.38, 134.94, 130.26, 128.34, 128.19, 126.88, 126.79, 125.99 (Ar), 71.39 (2-C), 52.38 (OMe), 49.18 (1′-C), 43.75 (4-C), 39.33 (2-CH_2), 23.29 (3-C), 22.58 (2′-C). ESI-MS: 353.2 $(M + 1)^+$. Anal. ($C_{21}H_{24}N_2O_3$) C, H, N.

(2*S*,1′*S*)-1-[(1′-Phenyl)ethyl]aminocarbonyl-2-benzyl-2-methoxycarbonylazetidine (16b). Eluent: EtOAc:hexane (1:3). Yield: 23% (from **14ab**), as a foam. $[\alpha]_D = -41.1$ (c = 0.43, $CHCl_3$). HPLC: t_R = 7.07 min (A:B = 40:60). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.31−7.11 (m, 11H, C_6H_5 and NH), 4.95 (m, 1H, 1′-H), 3.64 (s, 3H, OMe), 3.54 (m, 1H, 2-H), 3.39 (d, 1H, 4-CH_2, J = 13.8), 3.23 (m, 1H, 2-H), 3.07 (d, 1H, 4-CH_2, J = 13.8), 2.31 (m, 1H, 3-H), 2.09 (m, 1H, 3-H), 1.39 (d, 3H, 2′-H, J = 6.9). ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.04 (COO), 156.85 (NCON), 144.51, 135.16, 130.47, 128.43, 128.31, 126.98, 126.90, 125.92 (Ar), 71.34 (2-C), 52.49 (OMe), 49.22 (1′-C), 43.87 (4-C), 39.38 (2-CH_2), 23.69 (3-C), 22.90 (2′-C). ESI-MS: 353.2 $(M + 1)^+$. Anal. ($C_{21}H_{24}N_2O_3$) C, H, N.

General Procedure for the Synthesis of *N*-Benzyloxycarbonylazetidines. A solution of the corresponding azeti-

dine (0.56 mmol) in dry CH_2Cl_2 (3 mL), cooled at 0 °C, was treated with propylene oxide (0.595 mL, 8.4 mmol) and benzyl chloroformate (0.158 mL, 1.12 mmol). After reaction overnight at room temperature, the solvent was evaporated and the resulting residue was purified on a silica gel column as specified in each case.

(2*R*,*S*)-2-Benzyl-1-benzyloxycarbonyl-2-*tert*-butoxycarbonylazetidine (17ab). Eluent: EtOAc:hexane (1:4). Yield: 82% (from **13ab**), as a syrup. HPLC: t_R = 14.24 min (A:B = 50:50). ESI-MS: 382.2 $(M + 1)^+$. Anal. ($C_{23}H_{27}NO_4$) C, H, N.

(2*R*,*S*)-2-Benzyl-1-benzyloxycarbonyl-2-metoxycarbonylazetidine (18ab). Eluent: EtOAc:hexane (1:6). Yield: 77% (from **14ab**), as a syrup. HPLC: t_R = 5.38 min (A:B = 50:50). ESI-MS: 340.1 $(M + 1)^+$. Anal. ($C_{20}H_{21}NO_4$) C, H, N.

(2*R*,*S*)-2-Benzyl-1-benzyloxycarbonyl-2-carboxylazetidine (19ab). A solution of azetidine **18ab** (0.4 g, 1.18 mmol) in MeOH (8 mL) was treated with 2 N NaOH (1.32 mL, 2.36 mmol) and stirred at room temperature overnight. After evaporation, the residue was dissolved in H_2O, acidified with 1 M HCl to pH = 3, and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and evaporated to leave a residue that was used without further purification (0.35 g, 92%, foam). HPLC: t_R = 4.21 min (A:B = 40:60).

General Procedure for Coupling 2-Carboxyazetidine 19 with Amines and Amino Acids. A solution of compound **19ab** (0.124 g, 0.38 mmol) in dry CH_2Cl_2 (2 mL) was successively treated with the corresponding amine or α-amino ester (0.57 mmol), BOP (0.219 g, 0.49 mmol), and TEA (0.069 mL, 0.49 mmol, or 0.12 mL, 0.87 mmol, if the amine was in the hydrochloride form). After stirring overnight at room temperature, the solvent was eliminated. The residue was dissolved in EtOAc and washed with citric acid (10%), $NaHCO_3$ (10%), H_2O, and brine. The organic layer was separated, dried over Na_2SO_4, and evaporated to dryness. The obtained residue was purified on a silica gel column as indicated in each case.

(2*S*,*R*)-2-Benzyl-1-benzyloxycarbonyl-2-(*tert*-butyl)carbamoylazetidine (20ab). Eluent: gradient from 12 to 60% EtOAc in hexane. Yield: 67% (from **19ab** and *tert*-butylamine), as a white solid. HPLC: t_R = 25.23 min (A:B = 40:60). ^{1}H RMN (200 MHz, $CDCl_3$): δ 7.80 (br s, 1H, NH), 7.30 (m. 5H, C_6H_5), 7.16 (m, 5H, C6H5), 5.22 (d, 1H, OCH_2, J = 12.2), 4.99 (d, 1H, OCH_2, J = 12.2), 3.42 (d, 1H, 4-CH_2, J = 13.8), 3.38 (m, 1H, 4-H), 2.83 (d, 1H, 4-CH_2, J = 13.8), 2.72 (m, 1H, 4-H), 2.51 (m, 1H, 3-H), 2.01 (m, 1H, 3-H), 1.32 (s, 9H, ^{t}Bu). ESI-MS: 381.2 $(M + 1)^+$, 403.3 $(M + Na)^+$. Anal. ($C_{23}H_{28}N_2O_3$) C, H, N.

(2*S*,*R*)-2-Benzyl-1-benzyloxycarbonyl-2-(cyclohexyl)-carbamoylazetidine (21ab). Eluent: gradient from 10 to 60% EtOAc in hexane. Yield: 50% (from **19ab** and cyclohexylamine), as a syrup. HPLC: t_R = 8.51 min (A:B = 50:50). ^{1}H RMN (200 MHz, $CDCl_3$): δ 8.86 (d, 1H, NH, J = 7.8), 7.32 (m. 5H, C_6H_5), 7.25 (m, 5H, C6H5), 5.21 (d, 1H, OCH_2, J = 12.2), 5.01 (d, 1H, OCH_2, J = 12.2), 3.72 (m, 1H, 1-Chx), 3.41 (d, 1H, 4-CH_2, J = 14.0), 3.39 (m, 1H, 4-H), 2.85 (d, 1H, 4-CH_2, J = 14.0), 2.72 (m, 1H, 4-H), 2.51 (m, 1H, 3-H), 2.05 (m, 1H, 3-H), 1.82−1.14 (m, 10H, Chx). ESI-MS: 407.3 $(M + 1)^+$, 429.3 $(M + Na)^+$. Anal. ($C_{25}H_{30}N_2O_3$) C, H, N.

(2*S*,*R*,1′*S*)-2-Benzyl-1-benzyloxycarbonyl-2-[1′-(*tert*-butoxycarbonyl)ethyl]carbamoylazetidine (22ab). Eluent: EtOAc:hexane (1:4). Yield: 53% (from **19ab** and HCl·H-Ala-O^{t}Bu), as a syrup. HPLC: t_R = 34.69 and 30.61 min (A:B = 40:60). **a**:**b** ratio = 2.5:1. ESI-MS: 453.3 $(M + 1)^+$. Anal. ($C_{26}H_{32}N_2O_5$) C, H, N.

(2*S*,*R*,1′*S*)-2-Benzyl-1-benzyloxycarbonyl-2-[1′-(methoxycarbonyl)ethyl]carbamoylazetidine (23ab). Eluent: gradient from 2 to 20% EtOAc in CH_2Cl_2. Yield: 53% (from **19ab** and HCl·H-Ala-OMe), as a syrup. HPLC: t_R = 9.23 and 9.95 min (A:B = 40:60). **a**:**b** ratio = 2:1. ESI-MS: 411.2 $(M + 1)^+$. Anal. ($C_{23}H_{26}N_2O_5$) C, H, N.

(2*S*,*R*,1′*S*)-2-Benzyl-1-benzyloxycarbonyl-2-[1′-(carboxy)-ethyl]carbamoylazetidine (24ab). To a solution of compound **22ab** (50 mg, 0.11 mmol) in dry CH_2Cl_2 (1 mL) was added TFA (0.2 mL, 2.6 mmol). After a 2-h reaction, the solution was evaporated to dryness, coevaporating several times with CH_2Cl_2, to afford 51 mg (99%) of the title compound as a syrup. **a**:**b** ratio = 2.4:1. ESI-MS: 397.2 $(M + 1)^+$. Anal. ($C_{22}H_{24}N_2O_5 \cdot H_2O$) C, H, N.

(2*R*,1′*S*)-2-Benzyl-1-benzyloxycarbonyl-2-[1′-(carbamoyl)ethyl]carbamoylazetidine (25a). Compound **23ab** (30 mg, 0.07 mmol) was treated with a saturated solution of NH_3 in MeOH (5 mL), stirred at room temperature for 48 h, and evaporated. The reaction was repeated twice. The solution was evaporated to dryness, and the resulting residue was purified on a silica gel column using a gradient from 1 to 5% MeOH in CH_2Cl_2, to afford 7 mg (24%) of the title compound as a white solid. A second, more retained, component was identified as the diastereoisomeric mixture of compounds **26ab** (4 mg, 17%), as a white foam.

Compound **25a**: HPLC: t_R = 3.74 (A:B = 40:60). ESI-MS: 396.3 $(M + 1)^+$. Anal. ($C_{22}H_{25}N_3O_4$) C, H, N.

(2*S*,*R*,1′*S*)-2-Benzyl-1-methoxycarbonyl-2-[1′-(carbamoyl)ethyl]carbamoylazetidine (26ab). HPLC: t_R = 1.76 min (A:B = 40:60). **a**:**b** ratio = 1:1.1. ESI-MS: 320.2 $(M + 1)^+$. Anal. ($C_{16}H_{21}N_3O_4$) C, H, N.

(2*S*,*R*,1′*S*)-2-Benzyl-1-benzyloxycarbonyl-2-[1′-(methylcarbamoyl)ethyl]carbamoylazetidine (27ab). To a solution of compound **24ab** (31 mg, 0.095 mmol) in dry CH_2Cl_2 (1 mL) was successively added methylamine hydrochloride (13 mg, 0.19 mmol), BOP (0.127 g, 0.285 mmol), and TEA (0.04 mL, 0.285 mmol). The mixture was stirred at room temperature overnight and evaporated. The resulting residue was dissolved in EtOAc and washed with citric acid (10%), $NaHCO_3$ (10%), H_2O, and brine. The organic layer was separated, dried over Na_2SO_4, and evaporated. The residue was purified on a silica gel column using EtOAc:hexane (1:4) to yield 19.2 mg (60%) of the title compounds as a white solid. HPLC: t_R = 17.56 and 19.93 min (A:B = 30:70). **a**:**b** ratio = 4:1. Major isomer (2*R*,1′*S*): ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.18 (d, 1H, 1′-NH, J = 7.2), 7.32 (m, 5H, C_6H_5), 7.05 (m, 5H, C_6H_5), 6.34 (br s, 1H, CONH), 5.26 (d, 1H, OCH_2, J = 12.3), 4.96 (d, 1H, OCH_2, J = 12.3), 4.37 (m, 1H, 1′-H), 3.44 (d, 1H, 4-CH_2, J = 14.2), 3.42 (m, 1H, 4-H), 2.86 (d, 1H, 4-CH_2, J = 14.2), 2.77 (m, 1H, 4-H), 2.74 (d, 3H, N−CH_3, J = 4.9), 2.50 (m, 1H, 3-H), 2.11 (m, 1H, 3-H), 1.33 (d, 3H, 1′-CH3, J = 7.1). ^{13}C NMR (50 MHz, $CDCl_3$): δ 173.84 and 172.41 (NCO), 156.30 (NCOO), 136.05, 134.64, 130.34, 128.54, 128.35, 127.10 (Ar), 73.33 (2-C), 67.08 (OCH_2), 49.06 (1′-C), 44.84 (4-C), 39.01 (2-CH_2), 26.28 (N−CH_3), 23.56 (3-C), 17.34 (2′-C). Minor isomer (2*S*,1′*S*): ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.13 (d, 1H, 1′-NH, J = 7.2), 7.32 (m. 5H, C_6H_5), 7.05 (m, 5H, C_6H_5), 6.34 (br s, 1H, CONH), 5.22 (d, 1H, OCH_2, J = 12.2), 5.02 (d, 1H, OCH_2, J = 12.2), 4.37 (m, 1H, 1′-H), 3.42 (d, 1H, 4-CH_2, J = 14.0), 3.42 (m, 1H, 4-H), 2.94 (d, 1H, 4-CH_2, J = 14.0), 2.77 (m, 1H, 4-H), 2.72 (d, 1H, N−CH_3, J = 5.2), 2.50 (m, 1H, 3-H), 2.11 (m, 1H, 3-H), 1.38 (d, 3H, 1′-CH3, J = 7.1). ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.28 and 172.43 (NCO), 156.40 (NCOO), 136.07, 134.66, 130.40, 130.35, 128.57, 128.39, 127.12 (Ar), 73.40 (2-C), 67.04 (OCH_2), 49.12 (1′-C), 44.80 (4-C), 39.00 (2-CH_2), 26.30 (N−CH_3), 23.48 (3-C), 17.31 (2′-C). ESI-MS: 410.2 $(M + 1)^+$, 432.1 $(M + Na)^+$. Anal. ($C_{23}H_{27}N_3O_4$) C, H, N.

Antiviral Assays. HCMV. Confluent human embryonic lung (HEL) fibroblasts were grown in 96-well microtiter plates and infected with the human cytomegalovirus (HCMV) strains Davis and AD-169 at 100 PFU per well. After a 2-h incubation period, residual virus was removed and the infected cells were further incubated with medium containing different concentrations of the test compounds (in duplicate). After incubation for 7 days at 37 °C, virus-induced cytopathogenicity was monitored microscopically after ethanol fixation and staining with Giemsa. Antiviral activity was expressed as the EC_{50} or concentration required to reduce virus-induced cytopathogenicity by 50%. EC_{50} values were calculated from graphic plots of the percentage of cytopathogenicity as a function of concentration of the compounds.

VZV. The laboratory wild-type VZV strain Oka and the thymidine kinase-deficient VZV strain 07/1 were used. Confluent HEL cells grown in 96-well microtiter plates were inoculated with VZV at an input of 20 PFU per well. After a

2-h incubation period, residual virus was removed and varying concentrations of the test compounds were added (in duplicate). Antiviral activity was expressed as EC_{50}, the compound concentration required to reduce viral plaque formation after 5 days by 50% as compared with untreated controls.

Cytotoxicity Assays. Cytotoxicity measurements were based on the inhibition of HEL cell growth. HEL cells were seeded at a rate of 5×10^3 cells/well into 96-well microtiter plates and allowed to proliferate for 24 h. Then, medium containing different concentrations of the test compounds was added. After 3 days of incubation at 37 °C, the cell number was determined with a Coulter counter. The cytostatic concentration was calculated as the CC_{50}, the compound concentration required to reduce growth by 50% relative to the number of cells in the untreated controls. CC_{50} values were estimated from graphic plots of the number of cells (percentage of control) as a function of the concentration of the test compounds. Cytotoxicity was expressed as minimum cytotoxic concentration (MCC) or the compound concentration that causes a microscopically detectable alteration of cell morphology.

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Supporting Information Available: ^1H and ^{13}C NMR spectra of compounds **4ab**, **5a**, **7b**, **8a**, **8b**, **9a**, **9b**, **17a**, **18ab**, **19ab**, **22ab**, **23ab**, **24ab**, **25a**, and **26ab** and elemental analysis for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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