β-Lactam Derivatives as Inhibitors of Human Cytomegalovirus Protease

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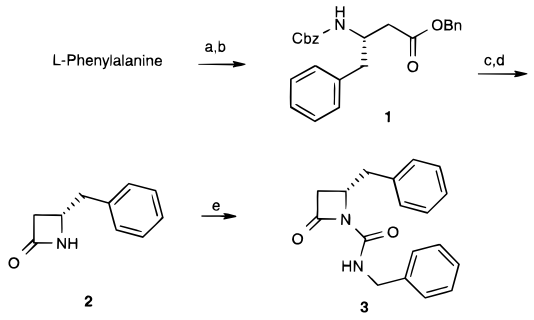
The development of novel monobactam inhibitors of HCMV protease incorporating a carbon side chain at C-4 and a urea function at N-1 is described. Substitution with small groups at the C-3 position of the *β*-lactam ring gave an increase in enzymatic activity and in stability; however, a lack of selectivity against other serine proteases was noted. The use of both tri- and tetrasubstituted urea functionalities gave effective inhibitors of HCMV protease. Benzyl substitution of the urea moiety was beneficial, especially when strong electron-withdrawing groups where attached at the para position. Modest antiviral activity was found in a plaque reduction assay.

Human cytomegalovirus (HCMV), a *β*-herpesvirus, is an opportunistic pathogen in immunocompromised individuals such as AIDS patients and organ transplant recipients. The current antiviral treatments for HCMV infection, ganciclovir, cidofovir, and foscarnet, inhibit the viral DNA polymerase; however, they demonstrate suboptimal efficacy and safety profiles.[1,2] Thus the need for effective, safe therapeutic agents for HCMV disease continues to exist. HCMV protease has become a viable target for antiviral chemotherapy because of its critical role in capsid assembly and viral maturation.[3] The HCMV UL80 gene encodes an 80-kDa precursor polyprotein whose N-terminal domain possesses proteolytic activity. Autocatalytic cleavage at the R-site (A.A.256) releases the N-terminal protease domain (N_o) from the full-length gene product. Cleavage also occurs near the C-terminus (A.A.643, M-site) of the polyprotein domain, a process which is essential for the production of mature infectious virions. Both N_o and the full-length protease precursor are catalytically active.

HCMV protease is a serine protease[4] which has little homology with other serine proteases.[5] Biochemical and mutagenesis studies have identified Ser 132 and His 63 as two residues involved in the catalytic machinery.[4] Recent reports of the crystal structure of HCMV protease[6] have identified the third member of the triad as His 157 and shown that the protease possesses a novel protein fold.

We have recently reported the development of peptidomimetic inhibitors of HCMV protease showing submicromolar potency in an enzymatic assay.[7] These compounds are substrate-based inhibitors incorporating an activated carbonyl group at the P1 position and are characterized by having marginal antiviral activity despite their potency as inhibitors of the enzyme (IC_{50} < 100 nM). It is reasonable to suggest that this poor activity could be due to the peptidic nature and large molecular weight of these inhibitors. This has prompted us to design small, nonpeptidic inhibitors of HCMV protease.[8] It has previously been reported that *β*-lactams are effective inhibitors of classical serine proteases such as human leukocyte elastase (HLE),[9] and we have recently shown that 4-thioalkyl *β*-lactams are potent HCMV protease inhibitors.[10] In this paper, we describe the design and synthesis of novel monobactams which are selective inhibitors of HCMV protease.

Scheme 1[a]



[a] Reagents and conditions: (a) isobutyl chloroformate, Et_3N, CH_2Cl_2, CH_2N_2/Et_2O; (b) AgOBz, Et_3N, BnOH, THF; (c) H_2, $Pd(OH)_2/C$ (20%), MeOH; (d) MsCl, $NaHCO_3$, MeCN, 60 °C; (e) LiHMDS, THF, BnNCO.

Chemistry

The methods used to synthesize the inhibitors are summarized in Schemes 1−5. Scheme 1 illustrates the overall procedure for generating the various 4-substituted *β*-lactams described herein. *N*-(Benzyloxycarbonyl)-L-phenylalanine was homologized via formation of the mixed anhydride, followed by reaction with diazomethane to give the corresponding diazomethyl ketone in 83% yield. A Wolff rearrangement was carried out using silver benzoate in the presence of benzyl alcohol to give the ester **1** in 63% yield. Palladium-catalyzed hydrogenolysis followed by methanesulfonyl chloride-mediated cyclization[11] in the presence of sodium bicarbonate gave the key intermediate **2**. Ureido formation was effected via deprotonation with LiHMDS in THF followed by addition of the appropriate isocyanate to give substituted ureas such as **3**. Other electrophiles such as phenoxycarbamates[12] or carbamoyl chloride derivatives were also used as needed.

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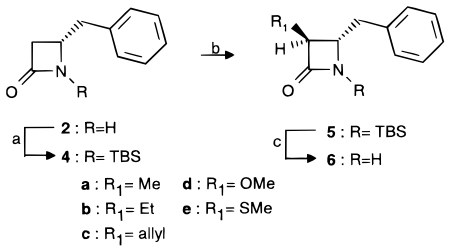
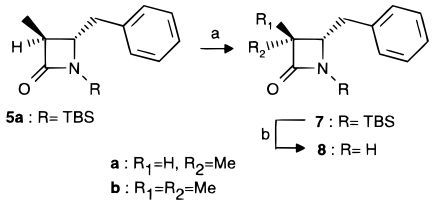
Scheme 2[a]



[a] Reagents and conditions: (a) TBSCl, DIEA, CH_2Cl_2; (b) LDA, THF, MeI or EtI or allyl bromide or O_2, $(MeO)_3P$, then CH_2N_2, SiO_2 or $(MeS)_2$; (c) CsF, MeOH.

Scheme 3[a]



[a] Reagents and conditions: (a) LDA, THF, then 2,6-di-*tert*-butylphenol or MeI; (b) CsF, MeOH.

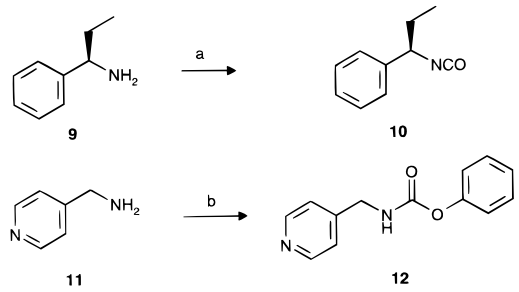
Inhibitors bearing a C-3 substituent can be easily prepared from intermediate **2** after protection as the *N*-*tert*-butyldimethylsilyl derivative (Scheme 2). Deprotonation of **4** with 2 equiv of lithium diisopropylamide (LDA) followed by alkylation with methyl iodide at −78 °C afforded selectively the 3β-methyl product **5a**. This methodology was also used to generate the corresponding 3β-ethyl and 3β-allyl derivatives (**5b**,**c**, respectively). Methyl ether **5d** was obtained by trapping the enolate with oxygen in the presence of trimethyl phosphite to provide the corresponding alcohol, which was treated with diazomethane in the presence of silica gel[13] (60% overall yield). Reaction of the enolate with methyl disulfide afforded the methylthio ether **5e** in 78% yield. Deprotection was achieved by exposure to cesium fluoride in MeOH to give intermediates **6a**−**e** which were further elaborated to the ureido derivatives as described above.

Inversion of the configuration at C-3 of **5a** was achieved via deprotonation (Scheme 3) with lithium diisopropylamide and quenching the resulting enolate with 2,6-di-*tert*-butylphenol[14] at −78 °C to give **7a** in a 28% yield. Quenching the above enolate with excess methyl iodide afforded the 3,3-dimethyl compound **7b** in 40% yield. Both compounds were deprotected using cesium fluoride in MeOH to afford **8a**,**b**, respectively.

Isocyanates which were not commercially available were prepared as described in Scheme 4. 1(*R*)-Phenylpropyl isocyanate (**10**) was prepared from the commercially available amine **9** by reaction with triphosgene. Alternatively, preactivation of amine **11** could be achieved using phenyl chloroformate[12] in the presence of triethylamine to give the corresponding phenoxycarbamate **12** in 42% yield after recrystallization.

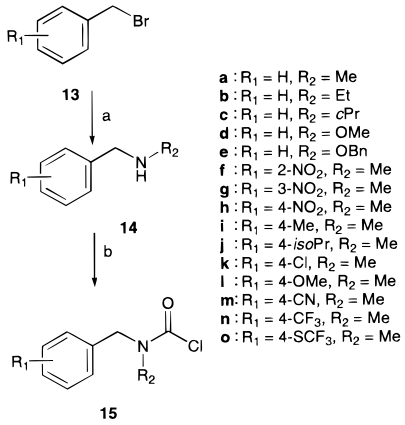
Noncommercial secondary benzylic amines could be readily prepared from the corresponding substituted benzyl bromides as exemplified in Scheme 5. The appropriate benzyl bromide **13** reacted with methylamine in ethanol to afford the corresponding secondary amine **14**, which was isolated as the hydrochloride salt.

Scheme 4[a]



[a] Reagents and conditions: (a) HCl/Et_2O, triphosgene, toluene; (b) PhOCOCl, Et_3N, CH_2Cl_2.

Scheme 5[a]



[a] Reagents and conditions: (a) $MeNH_2$, EtOH, HCl/Et_2O; (b) phosgene, DIEA, CH_2Cl_2.

Further reaction with phosgene and diisopropylethylamine (DIEA) in dichloromethane gave the desired amidoyl chloride.

Results and Discussion

We recently described the development of 4-thioalkyl β-lactams as HCMV protease inhibitors.[10] Preliminary investigations[15] of their mode of action suggested that these compounds did not inhibit HCMV protease via a "double hit" mechanism.[16] Mechanistic investigations of related HLE inhibitors had demonstrated that those compounds also did not inhibit by a "double hit".[17] These observations suggested to us that it might be possible that monobactams possessing a carbon appendage at C-4 would be effective HCMV protease inhibitors. This approach could be valuable since compounds incorporating such a structure would be less likely to cause irreversible inhibition of endogenous proteases. Compounds **17** and **18** were therefore prepared and tested for activity against HCMV protease. As shown in Table 1, replacement of the C-4 thiophenyl group[10] with a benzyl side chain resulted in only a 2-fold loss in potency (entries 1 and 2).[18] Compound **18**, in which the configuration at C-4 is *R*, was not an inhibitor of HCMV protease. These results prompted us to try to improve activity via modifications of the C-3 position of the β-lactam and to the urea moiety.

The structure−activity relationship (SAR) based on compound **17** began with an investigation of the effect of the nature of the urea moiety (Table 2). Removal of the alkyl group from the benzylic position of the urea gave a 2-fold drop in inhibitor activity (entries 1 and

Table 1. Monobactams with Various Substituents at C-4

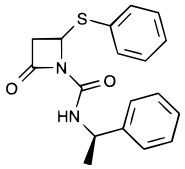
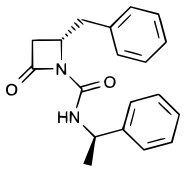
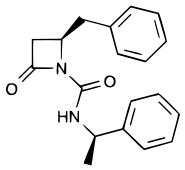
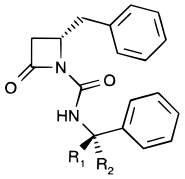
Entry	Compound	Structure	IC_{50} (μM)
1	**16**[10]		1.9
2	**17**		4.0
3	**18**		>75

Table 2. Modifications to the Benzylic Position of the Urea

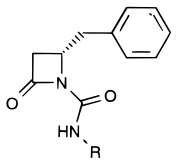


Entry	Compound	R_1	R_2	IC_{50} (μM)
1	**17**	Me	H	4.0
2	**3**	H	H	10
3	**19**	H	Me	42
4	**20**	Et	H	2.6
5	**21**	Pr	H	3.4

2). A 10-fold loss in activity was observed with inversion of the configuration at the benzylic position of the urea substituent (entries 1 and 3). Extension of the chiral alkyl side chain of urea **17** resulted in an increase in inhibitory activity relative to compound **17** (entry 4). Further expansion of this group did not significantly improve potency (entry 5).

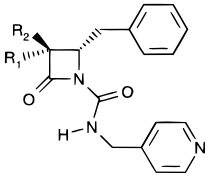
Other modifications to the urea function were also examined in an effort to establish the scope of the substituents which were tolerated at this position (Table 3). The use of a 2-phenylethyl group gave a 3-fold potency loss, whereas a simple phenyl led to almost a 4-fold increase in activity (entries 2 and 3). This increase in potency was accompanied, unfortunately, by a significant drop in the stability in cell culture media of compound **23** relative to compound **3**.[19] The use of ureas based on para-substituted anilines did not give an improvement in either activity or stability relative to compound **23** (entries 4−6). The incorporation of a cyclohexyl group gave an inhibitor with potency comparable to that of **3** (entry 7) accompanied by a concomitant drop in solubility (data not shown). The use of pyridyl-substituted ureas did not give an increase in activity, but there was an improvement in inhibitor

Table 3. Various Urea Substitutions



Entry	Compound	R	IC_{50} (μM)	$t_{1/2}$ (days)
1	**3**	Bn	10	1.5
2	**22**	CH_2-CH_2-Ph	24	1.5
3	**23**	Ph	2.2	<0.5
4	**24**	4(Me)-Ph	5.2	<0.5
5	**25**	4(OMe)-Ph	3.9	0.5
6	**26**	4(F)-Ph	3.7	<0.5
7	**27**	c-hex	8.4	1
8	**28**	CH_2-2-Py	33	1.5
9	**29**	CH_2-3-Py	10	1
10	**30**	CH_2-4-Py	12	1

Table 4. Effect of Substitutions at the C-3 Position



Entry	Compound	R_1	R_2	IC_{50} (μM)	$t_{1/2}$ (days)
1	**30**	H	H	12	1
2	**31**	Me	H	73	1.5
3	**32**	H	Me	13	1.5
4	**33**	Me	Me	>75	5
5	**34**	H	Et	60	2
6	**35**	H	Allyl	>75	1.5
7	**36**	H	OMe	2.1	<0.5
8	**37**	H	SMe	6.9	<0.5
9	**38**	Me	Me[a]	>300	5

[a] Compound **38** has an *R* configuration at C-4 of the *β*-lactam.

solubility relative to compound **3** (data not shown). Although aniline derivatives (**23**−**26**) were somewhat more active against HCMV protease than **3**, the fact that these compounds were less stable than **3** led us to continue further SAR studies on the benzylic series.

Previously it had been reported that the addition of substituents to the C-3 position of other monobactam inhibitors gave improvements in inhibitory activity, stability, and selectivity.[10,20] Substitutions at this position were therefore explored with the aim of improving both the potency and the stability profile of our compounds (Table 4). The addition of a methyl group cis to the C-4 benzyl group (**31**) resulted in a 6-fold drop in

Table 5. Selectivity Data for Various Monobactam Inhibitors

Entry	Compound	IC_{50} (μM) PPE	HLE	Chymotrypsin
1	**30**	>75	>75	>75
2	**31**	19	36	16
3	**32**	53	25	>75
4	**33**	45	3	>75
5	**34**	1.7	1.2	19
6	**35**	4.7	0.3	6
7	**36**	0.3	0.9	38
8	**37**	<0.2	<0.2	2.2

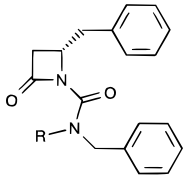
inhibitor activity, while the corresponding trans isomer **32** was equipotent with compound **30** (entries 1−3). Compounds **31** and **32** each demonstrated marginal improvements in stability. Disubstitution at the C-3 position gave a dramatic stability improvement (entry 4) which was incurred at the expense of inhibitory activity. Alkyl substituents other than methyl at C-3 led to a decrease in activity while maintaining stability in cell culture media (entries 5 and 6). The introduction of methoxy or methylthio moieties gave significant improvements in inhibitory activity, but these were accompanied by large losses in stability (entries 7 and 8). Previous results from the Merck group[21] had shown that disubstitution at the C-3 position of monobactams may lead to a shift in binding mode in which the opposite configuration at C-4 is favored. We therefore tested compound **38** to be sure that the inactivity of **33** was not due to a similar change. As shown in entry 9 however, activity could not be recovered in this manner.

Some of these compounds also displayed unsatisfactory selectivity profiles against other common serine proteases. The activity of the monobactam compounds was measured against porcine pancreatic elastase (PPE), human leukocyte elastase (HLE), bovine pancreatic α-chymotrypsin (BPC), and the cysteine protease human liver cathepsin B.[22] As shown in Table 5,[23] monobactam **30**, which is unsubstituted at C-3, was not an inhibitor of the other serine proteases tested. The introduction of methyl groups onto C-3 in either the α or β configuration or disubstitution at C-3 gave compounds which interacted with PPE and HLE (entries 2−4) and, in the case of **31**, with α-chymotrypsin. Substituents larger than methyl gave further decreases in selectivity (entries 5−8) particularly in the case of compound **37** which was a more potent inhibitor of PPE and HLE than of HCMV protease. For this reason the remaining SAR was performed without substituents at C-3.

As shown in Table 6, the addition of an *N*-methyl group to **3** gave a compound (**39**) with comparable activity toward HCMV protease. The use of alkyl substituents such as ethyl or cyclopropyl (entries 2 and 3) gave losses in activity. Although the use of alkoxy-substituted ureas gave small increases in inhibitor activity, this was accompanied by a loss of compound stability (entries 5 and 6).

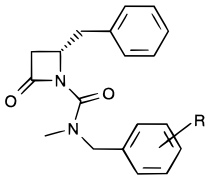
Extensive substitutions to **39** were carried out in order to try to increase the activity of this compound (Table 7). Nitro substituents were introduced onto the various positions of the urea benzyl function (entries 2−4). Addition of substituents onto the ortho and meta positions did not improve the potency; however, a 7-fold increase in potency was noted for the para-substituted nitro function (entry 4). Alkyl substituents induced a

Table 6. Incorporation of Tetrasubstituted Ureas



Entry	Compound	R	IC_{50} (μM)	$t_{1/2}$ (days)
1	**3**	H	10	1.5
2	**39**	Me	11	1.5
3	**40**	Et	42	2
4	**41**	c-Pr	>75	2
5	**42**	OMe	6.2	<0.5
6	**43**	OBn	13	<0.5

Table 7. Various Tetrasubstituted Urea Derivatives



Entry	Compound	R	IC_{50} (μM)
1	**39**	H	11
2	**44**	2-NO_2	11
3	**45**	3-NO_2	6.9
4	**46**	4-NO_2	1.5
5	**47**	4-Me	8.9
6	**48**	4-*iso*Pr	6.4
7	**49**	4-Cl	6.9
8	**50**	4-OMe	7.4
9	**51**	4-CN	6.0
10	**52**	4-NHAc	17
11	**53**	4-NH_2	20
12	**54**	4-CF_3	3.6
13	**55**	4-SCF_3	6.9
14	**56**	4-$SOCF_3$	8.7
15	**57**	4-SO_2CF_3	4.3

small improvement in inhibitory activity as did a chloro substituent (entries 5−7). Methoxy and cyano substitutions did not induce significant improvements in potency (entries 8 and 9), and other groups such as an acetamide or amine led to losses in activity (entries 10−11). Strong electron-withdrawing groups such as nitro or CF_3 (entry 12) produced the best results.

The apparent correlation between IC_{50} and the electron-withdrawing power of the para substituents suggested to us the possibility of observing a quantitative

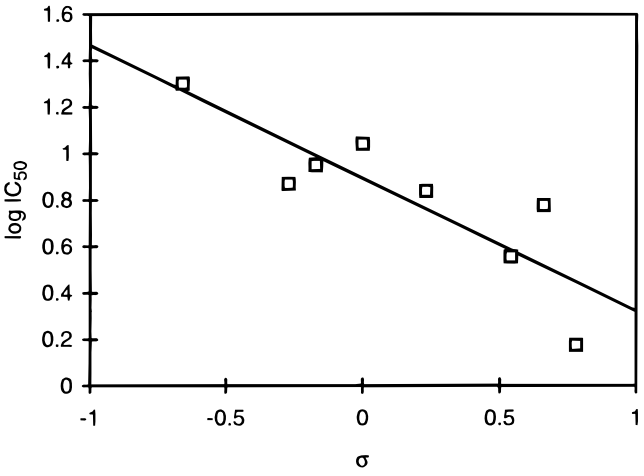


Figure 1. Plot of log IC_{50} (μM) vs σ_p for compounds **39**, **46**, **47**, **49**−**51**, **53**, and **54** ($r^2 = 0.74$).

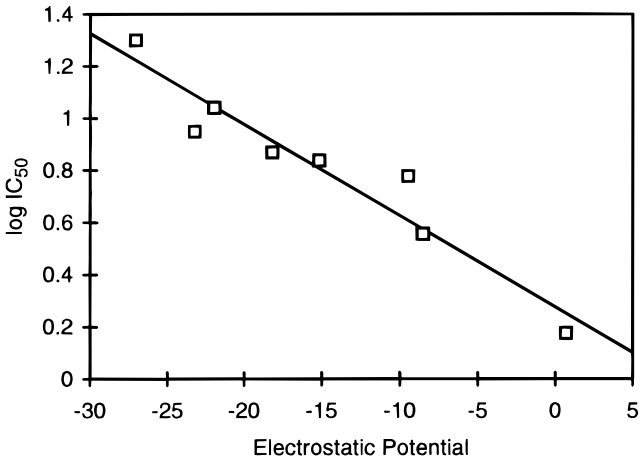


Figure 2. Plot of log IC_{50} (μM) vs ESP (kcal/mol) for compounds **39**, **46**, **47**, **49**−**51**, **53**, and **54** ($r^2 = 0.92$).

structure−activity relationship for substitutions at this position.[24] Shown in Figure 1 is a Hammett plot using readily available σ_p values.[25] Figure 2 shows a similar graph using electrostatic potential (ESP) values calculated at the electronic surface of the centroid of the ring[26] (see the Experimental Section). Both plots show modest correlations ($r^2 = 0.74$ and 0.92, respectively) suggestive of an electronic interaction between the enzyme and the aromatic ring. This correlation prompted us to try other strong electron-withdrawing groups which were chosen based upon their σ_p[25] and ESP values (entries 13−15). Unfortunately a large increase in potency was not realized, although the activity of the additional compounds examined did correlate approximately with the electron-withdrawing strength of the appendage.

The activity in cell culture of those compounds with IC_{50} values less than 10 μM was determined in a plaque reduction assay.[7] With the exception of compounds **27**, **42**, and **48**, all of the compounds tested were not cytotoxic, giving TC_{50} values greater than 250 μM. In general the EC_{50} values were greater than 100 μM, despite the fact that many compounds were very effective in the enzymatic assay. Five compounds showed more interesting activity in the plaque reduction assay. Compound **3** gave an EC_{50} of 53 μM with a toxicity window of at least 5-fold. Compound **42** showed similar activity ($EC_{50} = 60$ μM); however, the window between the activity and the cytotoxicity was smaller ($TC_{50} = 178$ μM). This compound was also somewhat unstable in the cell culture media (Table 6, entry 5), and so the EC_{50} value should be treated with caution. Compounds **46**, **51**, and **56** each gave results near 100 μM in the plaque reduction assay[27] and low-micromolar IC_{50} values. This suggests that improved cell culture activity could be realized by further improving the potency of these compounds, studies of which will be reported in due course.

Summary

The development of a new series of monobactam inhibitors of HCMV protease incorporating a benzyl side chain at C-4 has been described. Substitution at the C-3 position was tolerated and gave small increases in stability and enzymatic activity. These compounds were much less selective, however, than the corresponding inhibitors which were unsubstituted at C-3 and so the remaining SAR was performed with azetidinones which did not incorporate substituents at C-3. Substitution of the urea moiety suggested that benzyl groups were the best choice at this position. Both tri- and tetra-substituted ureas were effective with tetra substitution giving a slight stability advantage. Modification of the benzyl function indicated that strong electron withdrawing groups at the *para* position had the best activity. Preliminary mechanistic investigations indicate that these compounds are reversible and competitive inhibitors of HCMV protease and that inhibition involves the formation of an acyl-enzyme species.[15] Details of these studies will be published separately. Modest antiviral activity in a plaque reduction assay suggested that these compounds could potentially be useful antiviral agents. Studies are ongoing to improve the activity of the present series of inhibitors.

Experimental Section

Chemistry. Materials and Methods. Unless otherwise noted, materials obtained from commercial sources were used without further purification. In the last step, the reactions were done on an approximately 100-mg scale to give the inhibitor with the reported yield. ^{1}H NMR spectra were obtained on a Bruker AMX 400 spectrometer with tetramethylsilane as internal standard (δ scale) and in the given solvent. FAB mass spectra were recorded on an Autospec VG spectrometer. Column chromatography was performed either on silica gel (10−40 μm or 230−400 mesh ASTM, E. Merck) or by preparative HPLC using a Partisil 10 ODS-3, C18 preparative column (50 cm × 22 mm). Analytical HPLC was carried out in the following systems: system A, Vydac C18, 10-mm analytical column (24 cm × 4.6 mm), mobile phase acetonitrile/0.06% trifluoroacetic acid (TFA) in water/0.06% TFA; system B, Vydac C18, 10-mm analytical column (24 cm × 4.6 mm), mobile phase acetonitrile in 20 mM Na_2HPO_4 at pH 8.0. Analytical data for final compounds are given in Table 8.

3(*S*)-{{(Benzyloxy)carbonyl}amino}-4-phenylbutyric Acid Benzyl Ester (1). To a cooled solution (−10 °C) of *N*-(benzyloxycarbonyl)-L-phenylalanine (19 g, 62 mmol) in THF (300 mL) was added Et_3N (9.5 mL, 68 mmol). Isobutyl chloroformate (11 mL, 81 mmol) was added dropwise over 10 min. After 30 min at −10 °C and 30 min at room temperature, a solution of diazomethane in Et_2O (0.3−0.5 M, 500 mL) was added. The reaction mixture was stirred for 10 min and then purged with nitrogen for 2 h. The resulting white precipitate was removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was purified by flash

Table 8. Physical Data

Compound	HPLC A	HPLC B	Formula	Anal.	Compound	HPLC A	HPLC B	Formula	Anal.
3	99	96	$C_{18}H_{18}N_2O_2$	HRMS	**37**	99	98	$C_{18}H_{19}N_3O_2S$	HRMS
17	100	100	$C_{19}H_{20}N_2O_2$	C,H,N	**38**	100	100	$C_{19}H_{20}N_2O_2$	C,H,N
18	100	98	$C_{19}H_{20}N_2O_2$	C,H,N	**39**	100	95	$C_{19}H_{20}N_2O_2$	C,H,N
19	100	100	$C_{19}H_{20}N_2O_2$	C,H,N	**40**	95	96	$C_{20}H_{22}N_2O_2$	C,H,N
20	99	98	$C_{20}H_{22}N_2O_2$	C,H,N	**41**	99	98	$C_{21}H_{22}N_2O_2$	C,H,N
21	100	99	$C_{21}H_{24}N_2O_2$	C,H,N	**42**	100	99	$C_{19}H_{20}N_2O_3$	C,H,N
22	99	99	$C_{19}H_{20}N_2O_2$	C,H,N	**43**	97	91	$C_{25}H_{24}N_2O_3$	HRMS
23	100	100	$C_{17}H_{16}N_2O_2$	C,H,N	**44**	96	96	$C_{19}H_{19}N_3O_4$	HRMS
24	100	98	$C_{18}H_{18}N_2O_2$	C,H,N	**45**	96	97	$C_{19}H_{19}N_3O_4$	C,H,N
25	100	100	$C_{18}H_{18}N_2O_3$	C,H,N	**46**	99	99	$C_{19}H_{19}N_3O_4$	HRMS
26	100	100	$C_{17}H_{15}FN_2O_2$	C,H,N	**47**	96	95	$C_{20}H_{22}N_2O_2$	C,H,N
27	98	99	$C_{17}H_{22}N_2O_2$	C,H,N	**48**	99	98	$C_{22}H_{26}N_2O_2$	C,H,N
28	97	99	$C_{17}H_{17}N_3O_2$	C,H,N	**49**	100	99	$C_{19}H_{19}ClN_2O_2$	C,H,N
29	100	99	$C_{17}H_{17}N_3O_2$	HRMS	**50**	98	98	$C_{20}H_{22}N_2O_3$	HRMS
30	100	99	$C_{17}H_{17}N_3O_2$	HRMS	**51**	99	100	$C_{20}H_{19}N_3O_2$	HRMS
31	100	100	$C_{18}H_{19}N_3O_2$	C,H,N	**52**	94	99	$C_{21}H_{23}N_3O_3$	HRMS
32	96	95	$C_{18}H_{19}N_3O_2$	C,H,N	**53**	98	95	$C_{19}H_{21}N_3O_2$	HRMS
33	99	99	$C_{19}H_{21}N_3O_2$	C,H,N	**54**	99	97	$C_{20}H_{19}F_3N_2O_2$	C,H,N
34	100	96	$C_{19}H_{21}N_3O_2$	C,H,N	**55**	99	98	$C_{20}H_{19}F_3N_2O_2S$	C,H,N
35	98	99	$C_{20}H_{21}N_3O_2$	C,H,N	**56**	99	100	$C_{20}H_{19}F_3N_2O_3S$	HRMS
36	98	98	$C_{18}H_{19}N_3O_2$	HRMS	**57**	97	100	$C_{20}H_{19}F_3N_2O_4S$	HRMS

chromatography (20% EtOAc in hexane) to give 15.9 g (83% yield) of the desired diazo ketone as a yellow solid.

The diazo ketone (13 g, 43 mmol) and benzyl alcohol (4.7 mL, 45 mmol) were dissolved in THF (150 mL). Silver benzoate (977 mg, 4.29 mmol) in Et_3N (8.9 mL, 64 mmol) was added portionwise (vigorous gas evolution). After 30 min at room temperature, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc, and the solution was washed with H_2O and brine, dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography (15% EtOAc in hexane) to give **1** (11 g, 63% yield) as a white solid: ^{1}H NMR ($CDCl_3$) δ 7.40−7.10 (m, 15H), 5.27 (brd, J = 8.0 Hz, 1H), 5.14 (d, J = 12.2 Hz, 1H), 5.10 (d, J = 12.2 Hz, 1H), 5.06 (s, 2H), 4.30−4.20 (m, 1H), 2.93 (dd, J = 13.3, 6.5 Hz, 1H), 2.82 (dd, J = 13.3, 7.6 Hz, 1H), 2.57 (dd, J = 16, 5.5 Hz, 1H), 2.50 (dd, J = 16, 5.0 Hz, 1H); IR (KBr) υ 3323, 1711, 1691 cm^{-1}; FAB MS m/z 404.2 (MH^+), 426.2 ($M + Na^+$).

4(*S*)-Benzylazetidin-2-one (2). The 3(*S*)-{{(benzyloxy)-carbonyl}amino}-4-phenylbutyric acid benzyl ester (**1**) (10.97 g, 27.2 mmol) in MeOH (1 L) was stirred at room temperature for 7 h under hydrogen (1 atm) in the presence of 20% $Pd(OH)_2$/C (50 mg). The catalyst was removed by filtration through diatomaceous earth. The filtrate was concentrated under reduced pressure to give 3(*S*)-amino-4-phenylbutyric acid (4.53 g, 93% yield) as a white solid.

A suspension of $NaHCO_3$ (12.7 g, 152 mmol) in MeCN (1.55 L) was stirred and heated to gentle reflux. Freshly distilled methanesulfonyl chloride (2.15 mL, 27.8 mmol) was added, followed by the portionwise addition of the above acid (4.53 g, 25.3 mmol) over 5 h. After 16 h under reflux, the solid was removed by filtration at 60 °C, and the filtrate was concentrated under reduced pressure. The residual solid was triturated with EtOAc and filtered. The filtrate was concentrated and the residue purified by flash chromatography (40% EtOAc in hexane) to give **2** (2.20 g, 54% yield) as a white solid: ^{1}H NMR ($CDCl_3$) δ 7.35−7.17 (m, 5H), 5.83 (br s, 1H), 3.88−3.82 (m, 1H), 3.08 (ddd, J = 14.8, 5.0, 2.2 Hz, 1H), 2.98 (dd, J = 13.7, 5.7 Hz, 1H), 2.84 (dd, J = 13.7, 7.9 Hz, 1H), 2.70 (ddd, J = 14.9, 2.0, 1.3 Hz, 1H); IR (KBr) υ 3215, 3171, 1732 cm^{-1}; FAB MS m/z 323.1 (MH^+). Anal. ($C_{10}H_{11}NO$) C, H, N.

General Procedure for Ureido Formation. 4(*S*)-Benzyl-2-oxoazetidine-1-carboxylic Acid Benzylamide (3). To a solution of 4(*S*)-benzylazetidin-2-one (**2**) (200 mg, 1.24 mmol) in THF (4 mL) at −78 °C was added lithium bis(trimethylsilyl)-amide (1.36 mL, 1.36 mmol, 1 M in THF). After 10 min, benzyl isocyanate (180 mL, 1.49 mmol) was added. Stirring was continued at −78 °C for 45 min. The reaction mixture was diluted with EtOAc (50 mL), washed with aqueous $NaHSO_4$ (1 M) and brine, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash chromatography (30% EtOAc in hexane) to give **3** (56 mg, 15% yield) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 7.46 (t, J = 6.4 Hz, 1H), 7.36−7.21 (m, 10H), 4.36 (d, J = 6.4 Hz, 2H), 4.21 (m, 1H), 3.27 (dd, J = 11.8, 3.8 Hz, 1H), 3.06 (dd, J = 15.8, 5.6 Hz, 1H), 2.93 (dd, J = 11.8, 8.9 Hz, 1H), 2.73 (dd, J = 15.8, 3.0 Hz, 1H); IR ($CDCl_3$) υ 1769, 1700 cm^{-1}; FAB MS m/z 295.2 (MH^+); HRMS calcd for $C_{18}H_{19}N_2O_2$ 295.1447 (MH^+), found 295.1452.

4(*S*)-Benzyl-1-(*tert*-butyldimethylsilyl)azetidin-2-one (4). To a solution of 4(*S*)-benzylazetidin-2-one (400 mg, 2.48 mmol) in CH_2Cl_2 (8 mL) was added DIEA (648 μL, 3.72 mmol), followed by *tert*-butyldimethylsilyl chloride (411 mg, 2.73 mmol). The reaction mixture was stirred for 16 h at room temperature. The solvent was evaporated, and the residue was purified by flash chromatography (12% EtOAc in hexane) to give **4** (647 mg, 95% yield) as a white solid: ^{1}H NMR ($CDCl_3$) δ 7.34−7.15 (m, 5H), 3.77−3.70 (m, 1H), 3.25 (dd, J = 13.5, 3.5 Hz, 1H), 2.99 (dd, J = 15.5, 5 Hz, 1H), 2.70 (dd, J = 15.5, 2.5 Hz, 1H), 2.59 (dd, J = 13.5, 11 Hz, 1H), 1.01 (s, 9H), 0.31 (s, 3H), 0.29 (s, 3H); IR (KBr) υ 1725 cm^{-1}; FAB MS m/z 276.2 (MH^+) 298.2 ($M + Na^+$). Anal. ($C_{16}H_{25}NOSi$) C, H, N.

General Procedure for β-Lactam Alkylation: 4(*S*)-Benzyl-1-(*tert*-butyldimethylsilyl)-3(*S*)-methylazetidin-2-one (5a). To a solution of diisopropylamine (705 μL, 5.03 mmol) in anhydrous THF (12 mL) at −20 °C was added *n*-butyllithium (2.9 mL, 4.6 mmol, 1.6 M in hexane). After the reaction mixture was cooled to −78 °C, a solution of 4(*S*)-benzyl-1-(*tert*-butyldimethylsilyl)azetidin-2-one (**4**) (640 mg, 2.32 mmol) in THF (4 mL) was introduced, and the mixture was stirred at −78 °C for 15 min followed by addition of methyl iodide (488 mg, 214 μL, 3.44 mmol). After 10 min, the reaction mixture was poured into EtOAc (125 mL). The organic phase was washed with aqueous $NaHSO_4$ (1 M) and brine, dried ($MgSO_4$), filtered, and concentrated. The residual oil was purified by flash chromatography (6% EtOAc in hexane) to give **5a** (557 mg, 83% yield) as a pale-yellow solid: ^{1}H NMR ($CDCl_3$) δ 7.33−7.15 (m, 5H), 3.35 (ddd, J = 10.8, 3.8, 2.5 Hz, 1H), 3.21 (dd, J = 13.4, 3.8 Hz, 1H), 2.88 (qd, J = 7.5, 2.5 Hz, 1H), 2.60 (dd, J = 13.4, 10.8 Hz, 1H), 1.02 (d, J = 7.5 Hz, 3H), 1.00 (s, 9H), 0.31 (s, 3H), 0.27 (s, 3H); IR ($CHCl_3$) υ 1723, 1512, 1429 cm^{-1}; FAB MS m/z 290 (MH^+); HRMS calcd for $C_{17}H_{28}$-NOSi 290.1940 (MH^+), found 290.1932.

4(*S*)-Benzyl-1-(*tert*-butyldimethylsilyl)-3(*S*)-ethylazetidin-2-one (5b). Following the same alkylation procedure but replacing methyl iodide with ethyl iodide, **5b** was obtained in 82% yield: ^{1}H NMR ($CDCl_3$) δ 7.32−7.15 (m, 5H), 3.38 (ddd, J = 10.8, 4.0, 2.2 Hz, 1H), 3.22 (dd, J = 13.0, 3.8 Hz, 1H), 2.79 (ddd, J = 8.6, 5.7, 2.5 Hz, 1H), 2.57 (dd, J = 13.0, 10.8 Hz, 1H), 1.60−1.50 (m, 1H), 1.41−1.30 (m, 1H), 1.00 (s, 9H), 0.52 (t, J = 7.3 Hz, 3H), 0.32 (s, 3H), 0.27 (s, 3H); IR ($CHCl_3$)

υ 1723, 1472, 1255 cm^{-1}; FAB MS *m/z* 304 (MH^+); HRMS calcd for $C_{18}H_{30}NOSi$ 304.2097 (MH^+), found 304.2083.

4(*S*)-Benzyl-1-(*tert*-butyldimethylsilyl)-3(*S*)-allylazetidin-2-one (5c). Following the same alkylation procedure but replacing methyl iodide with allyl bromide, **5c** was obtained in 89% yield: ^1H NMR ($CDCl_3$) δ 7.25−7.13 (m, 5H), 5.34−5.25 (m, 1H), 4.88−4.75 (m, 2H), 3.44 (ddd, J = 10.6, 3.8, 2.5 Hz, 1H), 3.21 (dd, J = 13.2, 3.8 Hz, 1H), 2.91 (ddd, J = 7.8, 5.7, 2.2 Hz, 1H), 2.58 (dd, J = 13.2, 10.5 Hz, 1H), 2.21−2.09 (m, 2H), 1.01 (s, 9H), 0.31 (s, 3H), 0.27 (s, 3H); IR ($CHCl_3$) υ 1725, 1471, 1255 cm^{-1}; FAB MS *m/z* 316 (MH^+); HRMS calcd for $C_{19}H_{30}NOSi$ 316.2097 (MH^+), found 316.2091.

4(*S*)-Benzyl-1-(*tert*-butyldimethylsilyl)-3(*S*)-methoxyazetidin-2-one (5d). To a solution of diisopropylamine (800 μL, 5.7 mmol) in anhydrous THF (40 mL) at −20 °C was added *n*-butyllithium (3.6 mL, 5.7 mmol, 1.6 M in hexane). After 15 min, the reaction was cooled to −78 °C, and freshly distilled trimethyl phosphite (1.1 mL, 7.6 mmol) was added followed by a solution of **4** (1.05 g, 3.8 mmol) in THF (10 mL). A constant stream of oxygen was introduced, and the mixture was stirred at −78 °C for 3 h. The reaction was quenched with saturated aqueous NH_4Cl and extracted with EtOAc (120 mL). The organic layer was washed with brine, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash chromatography (10% EtOAc in hexane to 30% EtOAc in hexane) to give 4(*S*)-benzyl-1-(*tert*-butyldimethylsilyl)-3(*S*)-hydroxyazetidin-2-one (671 mg, 60% yield) as a white solid: ^1H NMR ($CDCl_3$) δ 7.25−7.21 (m, 5H), 4.51 (d, J = 2 Hz, 1H), 3.75−3.71 (m, 1H), 3.26 (br s, 1H), 3.20 (dd, J = 14, 3.8 Hz, 1H), 2.66 (dd, J = 14, 11.1 Hz, 1H), 1.00 (s, 9H), 0.31 (s, 3H), 0.29 (s, 3H).

To a solution of 4(*S*)-benzyl-1-(*tert*-butyldimethylsilyl)-3(*S*)-hydroxyazetidin-2-one (150 mg, 0.51 mmol) in Et_2O (70 mL) at 0 °C was added silica gel (40−60 μm, 9 g). The vigorously stirred mixture was treated with diazomethane in Et_2O (50 mL, 0.3−0.5 M solution). Once the yellow color had almost disappeared after about 15 min, additional diazomethane solution (20 mL) was added. This procedure was repeated several times until no more starting material could be detected by TLC (about 1.5 h). The reaction mixture was stirred for an additional hour at room temperature and then filtered and concentrated to give **5d** (157 mg, 99% yield) as a white solid which was used without purification: ^1H NMR ($CDCl_3$) δ 7.27−7.20 (m, 5H), 4.16 (d, J = 1.9 Hz, 1H), 3.67 (ddd, J = 11.1, 3.8, 1.9 Hz, 1H), 3.23 (dd, J = 13.5, 3.8 Hz, 1H), 2.94 (s, 3H), 2.57 (dd, J = 13.4, 11.1 Hz, 1H), 1.01 (s, 9H), 0.33 (s, 3H), 0.31 (s, 6H).

4(*S*)-Benzyl-1-(*tert*-butyldimethylsilyl)-3(*S*)-(methylthio)azetidin-2-one (5e). Following the same alkylation procedure but replacing methyl iodide with dimethyl disulfide, compound **5e** was obtained in 78% yield: ^1H NMR ($CDCl_3$) δ 7.36−7.20 (m, 5H), 3.75 (d, J = 2.6 Hz, 1H), 3.66 (ddd, J = 10.5, 3.8, 2.2 Hz, 1H), 3.29 (dd, J = 13.7, 3.8, 1H), 2.67 (dd, J = 13.7, 10.5 Hz, 1H), 1.82 (s, 3H), 1.03 (s, 9H), 0.34 (s, 3H), 0.31 (s, 3H); IR (KBr) ν 1733 cm^{-1}; FAB MS *m/z* 322.2 (MH^+), 344.2 ($M + Na^+$). Anal. ($C_{17}H_{27}NOSSi$) C, H, N.

General Procedure for *N*-Silyl Cleavage: 4(*S*)-Benzyl-3(*S*)-methylazetidin-2-one (6a). To a solution of 4(*S*)-benzyl-1-(*tert*-butyldimethylsilyl)-3(*S*)-methylazetidin-2-one (557 mg, 1.92 mmol) in MeOH (25 mL) at 0 °C was added cesium fluoride (439 mg, 2.89 mmol). After 1 h the solvent was evaporated under reduced pressure, and the residue was dissolved in EtOAc. The organic phase was washed with H_2O and brine, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash chromatography (50% EtOAc in hexane) to give **6a** (239 mg, 71% yield) as a white solid: ^1H NMR ($CDCl_3$) δ 7.35−7.17 (m, 5H), 5.78 (br s, 1H), 3.46 (ddd, J = 8.0, 5.9, 2.0 Hz, 1H), 2.98 (dd, J = 13.5, 5.9 Hz, 1H), 2.91 (qd, J = 7.3, 2.0 Hz, 1H), 2.84 (dd, J = 13.5, 8.0 Hz, 1H), 1.26 (d, J = 7.3 Hz, 3H); IR ($CHCl_3$) ν 1751, 1601, 1514 cm^{-1}; FAB MS *m/z* 176 (MH^+); HRMS calcd for $C_{11}H_{14}NO$ 176.1075 (MH^+), found 176.1079.

4(*S*)-Benzyl-3(*S*)-ethylazetidin-2-one (6b). Following the same deprotection procedure, compound **6b** was obtained in 88% yield: ^1H NMR ($CDCl_3$) δ 7.34−7.17 (m, 5H), 5.75 (br s, 1H), 3.53−3.50 (m, 1H), 2.94 (dd, J = 13.7, 6.0 Hz, 1H), 2.87 (dd, J = 13.5, 7.6 Hz, 1H), 2.86−2.81 (m, 1H), 1.82−1.71 (m, 1H), 1.66−1.56 (m, 1H), 0.87 (t, J = 7.6 Hz, 3H); IR ($CHCl_3$) ν 1753, 1216 cm^{-1}; FAB MS *m/z* 190.1 (MH^+); HRMS calcd for $C_{12}H_{16}NO$ 190.1232 (MH^+), found 190.1238.

4(*S*)-Benzyl-3(*S*)-allylazetidin-2-one (6c). Following the same deprotection procedure, compound **6c** was obtained in 100% yield: ^1H NMR ($CDCl_3$) δ 7.30−7.15 (m, 5H), 5.79 (s, 1H), 5.74−5.64 (m, 1H), 5.10−5.00 (m, 2H), 3.55 (ddd, J = 8.3, 5.7, 1.9 Hz, 1H), 2.99−2.93 (m, 2H), 2.83 (dd, J = 13.7, 8.3 Hz, 1H), 2.51−2.45 (m, 1H), 2.38−2.30 (m, 1H); IR ($CHCl_3$) ν 1755, 1496, 1371 cm^{-1}; FAB MS *m/z* 202 (MH^+); HRMS calcd for $C_{13}H_{16}NO$ 202.1232 (MH^+), found 202.1225.

4(*S*)-Benzyl-3(*S*)-methoxyazetidin-2-one (6d). Following the same deprotection procedure, compound **6d** was obtained in 100% yield: ^1H NMR ($CDCl_3$) δ 7.36−7.18 (m, 5H), 6.00 (br s, 1H), 4.26 (t, J = 1.9 Hz, 1H), 3.80 (ddd, J = 7.8, 6.2, 1.6 Hz, 1H), 3.34 (s, 3H), 2.98 (dd, J = 14, 6.2 Hz, 1H), 2.88 (dd, J = 14, 7.8 Hz, 1H).

4(*S*)-Benzyl-3(*S*)-(methylthio)azetidin-2-one (6e). Following the same deprotection procedure, compound **6e** was obtained in 97% yield: ^1H NMR ($CDCl_3$) δ 7.73−7.20 (m, 5H), 5.82 (br s, 1H), 3.78−3.74 (m, 2H), 3.09 (dd, J = 14.0, 5.4 Hz, 1H), 2.89 (dd, J = 14.0, 8.0 Hz, 1H), 2.09 (s, 3H); IR ($CDCl_3$) ν 1733 cm^{-1}; FAB MS *m/z* 208 (MH^+).

4(*S*)-Benzyl-1-(*tert*-butyldimethylsilyl)-3(*R*)-methylazetidin-2-one (7a). Using the same procedure for enolate formation as in **5a** followed by addition of 2,6-di-*tert*-butylphenol gave **7a** in 28% yield: ^1H NMR ($CDCl_3$) δ 7.32−7.17 (m, 5H), 4.07−4.03 (m, 1H), 3.40−3.33 (m,1H), 3.12 (dd, J = 14.9, 3.5 Hz, 1H), 2.86 (dd, J = 14.9, 11.1 Hz, 1H), 1.15 (d, J = 7.6 Hz, 3H), 1.00 (s, 9H), 0.27 (s, 3H), 0.25 (s, 3H); IR ($CHCl_3$) ν 2944, 1725 cm^{-1}; HRMS calcd for $C_{17}H_{28}NOSi$ 290.1940 (MH^+), found 290.1944.

4(*S*)-Benzyl-1-(*tert*-butyldimethylsilyl)-3,3-dimethylazetidin-2-one (7b). Using the same procedure for enolate formation as in **5a** followed by addition of excess MeI gave **7b** in 40% yield: ^1H NMR ($CDCl_3$) δ 7.33−7.15 (m, 5H), 3.74 (dd, J = 11.1, 3.8 Hz, 1H), 3.15 (dd, J = 14.8, 3.8 Hz, 1H), 2.82 (dd, J = 14.8, 11.1 Hz, 1H), 1.21 (s, 3H), 1.10 (s, 3H), 1.01 (s, 9H), 0.27 (s, 3H), 0.24 (s, 3H); IR ($CHCl_3$) ν 1723, 1259, 1178 cm^{-1}; FAB MS *m/z* 304 (MH^+); HRMS calcd for $C_{18}H_{30}NOSi$ 304.2097 (MH^+), found 304.2090.

4(*S*)-Benzyl-3(*R*)-methylazetidin-2-one (8a). Following the same deprotection procedure, compound **8a** was obtained in 98% yield: ^1H NMR ($CDCl_3$) δ 7.35−7.17 (m, 5H), 5.76 (br s, 1H), 3.94−3.90 (m, 1H), 3.41−3.34 (m, 1H), 2.93 (dd, J = 13.7, 4.1 Hz, 1H), 2.73 (dd, J = 13.8, 10.2 Hz, 1H), 1.30 (d, J = 7.6 Hz, 3H); IR ($CHCl_3$) ν 2900, 1753 cm^{-1}; HRMS calcd for $C_{11}H_{14}NO$ 176.1075 (MH^+), found 176.1066.

4(*S*)-Benzyl-3,3-dimethylazetidin-2-one (8b). Following the same deprotection procedure, compound **8b** was obtained in 31% yield: ^1H NMR ($CDCl_3$) δ 7.34−7.17 (m, 5H), 5.60 (br s, 1H), 3.57 (dd, J = 10.0, 4.1 Hz, 1H), 2.94 (dd, J = 13.8, 4.4 Hz, 1H), 2.72 (dd, J = 13.7, 9.8 Hz, 1H), 1.35 (s, 3H), 1.27 (s, 3H); IR ($CHCl_3$) ν 1757, 1428 cm^{-1}; FAB MS *m/z* 190 (MH^+); HRMS calcd for $C_{12}H_{16}NO$ 190.1232 (MH^+), found 190.1226.

General Procedure for Isocyanate Formation: 1(*R*)-Phenylpropyl Isocyanate (10).[9] To a solution of 1(*R*)-phenylpropylamine (**9**) (14.3 g, 106 mmol) in Et_2O (102 mL) was added a 1.0 M solution of HCl/Et_2O (212 mL, 212 mmol). The resulting solution was stirred for 30 min and then evaporated to dryness on a rotary evaporator. The resulting white hydrochloride salt was suspended in toluene (200 mL). Triphosgene was added (11.7 g, 39.3 mmol), and the resulting suspension was stirred at reflux for 3 h and at room temperature for 18 h. The reaction mixture was concentrated and the final volume adjusted to 200 mL with toluene giving a final concentration of 0.53 M. The resulting isocyanate solution was used as such. An aliquot (170 mL) was concentrated to give a colorless oil: ^1H NMR ($CDCl_3$) δ 7.36−7.22 (m, 5H), 4.50 (t, J = 6.7 Hz, 1H), 1.82 (q, J = 7.3 Hz, 2H), 0.94 (t, J = 7.3 Hz, 3H).

General Procedure for the Preparation of Phenoxycarbamate Derivatives: 4-{{(Phenoxycarbonyl)amino}methyl}pyridine (12). To a solution of 4-(aminomethyl)pyridine (**11**) (10.7 g, 98.5 mmol) in CH_2Cl_2 (245 mL) at 0 °C was added Et_3N (14.2 mL, 19.9 g, 197 mmol), followed by a dropwise addition of phenyl chloroformate (14.8 mL, 18.5 g, 118 mmol). After stirring for 1 h, the resulting mixture was diluted with EtOAc (1.5 L). The organic phase was washed twice with water and then brine, dried ($MgSO_4$), and concentrated under reduced pressure. Purification of the residue by chromatography (gradient EtOAc to 10% MeOH/$CHCl_3$) gave a yellow solid which was recrystallized from EtOAc/hexane (2:1) to yield the desired compound **12** (9.55 g, 42% yield) which was used immediately without further purification: ^{1}H NMR ($CDCl_3$) δ 8.61 (d, J = 5.7 Hz, 2H), 7.40−7.15 (m, 7H), 5.61 (br s, 1H), 4.50 (d, J = 6.4 Hz, 2H).

General Procedure for the Preparation of Carbamoyl Chlorides: *N*-Methyl-*N*-{{4-(trifluoromethyl)phenyl}methyl}carbamoyl Chloride (15n). To a solution of {4-(trifluoromethyl)phenyl}methyl bromide (**13n**) (20.0 g, 83.7 mmol) in EtOH was added methylamine (100 mL of 40% aqueous solution, 1.3 mol). After 2 h, the reaction was concentrated under reduced pressure. The aqueous phase was extracted with EtOAc (2 × 100 mL). The combined organic phases were washed with 5% aqueous $NaHCO_3$ solution and then brine, dried ($MgSO_4$), filtered, and evaporated to dryness. The resulting residue was dissolved in HCl/dioxane (4 N, 100 mL). The solvent was removed under reduced pressure. The resulting solid was triturated with Et_2O and collected by suction filtration to provide hydrochloride salt **14n** (17.0 g, 90% yield) as a white solid. The salt was suspended in CH_2Cl_2 (150 mL), and the suspension was cooled to 0 °C. DIEA (30.2 mL, 173 mmol) was added, followed by the addition of a phosgene solution in toluene (1.93 M, 55 mL, 105.7 mmol). After 2 h at 0 °C, the reaction mixture was concentrated, and the resulting thick gum was extracted with Et_2O. Evaporation of the Et_2O extract gave a light-yellow oil which was purified by flash chromatography (10% EtOAc in hexane) to give **15n** as a pale-yellow oil (16.0 g, 84% yield) which was used immediately without further purification: ^{1}H NMR ($CDCl_3$) δ 7.59 (m, 2H), 7.33 (m, 2H), 4.72 and 4.58 (2s, 2H), 3.04 and 2.97 (2s, 3H).

4(*S*)-Benzyl-2-oxoazetidine-1-carboxylic Acid (4-Pyridinylmethyl)amide (30). Following the same procedure as for compound **3** but using **12** as the reactant, compound **30** was obtained in 81% yield as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (m, 2H), 7.38−7.12 (m, 7H), 7.01 (m, 1H), 4.47 (m, 2H), 4.29 (m, 1H), 3.41 (dd, J = 14.0, 3.0 Hz, 1H), 2.99 (dd, J = 16.2, 5.8 Hz, 1H), 2.93 (dd, J = 14.0, 8.4 Hz, 1H), 2.73 (dd, J = 16.2, 2.9 Hz, 1H); IR ($CDCl_3$) υ 3357, 1764, 1694 cm^{-1}; FAB MS m/z 296.1 (MH^+); HRMS calcd for $C_{17}H_{18}N_3O_2$ 296.1399, found 296.1408.

4(*S*)-Benzyl-2-oxoazetidine-1-carboxylic Acid *N*-Methyl-*N*-{{4-(trifluoromethyl)phenyl}methyl}amide (54). To a solution of 4(*S*)-benzylazetidin-2-one (**2**) (110 mg, 0.68 mmol) in THF (6 mL) at −5 °C was added potassium bis(trimethylsilyl)amide (1.43 mL, 0.717 mmol, 0.5 M in toluene). After 20 min the reaction mixture was added via cannula to a solution of **15n** (860 mg, 3.4 mmol) in THF (6 mL) at −78 °C. The reaction mixture was stirred for 2 h during which time the temperature rose to −20 °C. The reaction was then quenched with brine, and the mixture was diluted with EtOAc. The aqueous phase was extracted twice with EtOAc, and the combined organic layers were washed with brine, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash chromatography (20% EtOAc in hexane) to give compound **54** (102 mg, 40% yield) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, J = 7.9 Hz, 2H), 7.31 (d, J = 7.9 Hz, 2H), 7.26−7.10 (m, 5H), 4.80−4.53 (m, 2H), 4.41 (m, 1H), 3.15 (dd, J = 14.8, 3.8 Hz, 1H), 2.85 (s, 3H), 2.84 (m, 2H), 2.65 (dd, J = 14.8, 3.5 Hz, 1H); IR (neat) υ 1778, 1665 cm^{-1}; FAB MS m/z 377 (MH^+); HRMS calcd for $C_{20}H_{20}F_3N_2O_2$ 377.1477, found 377.1488.

4(*S*)-Benzyl-2-oxoazetidine-1-carboxylic Acid *N*-Methyl-*N*-{(4-aminophenyl)methyl}amide (53). Azetidinone **46** (59 mg, 0.17 mmol) in EtOH (5 mL) was stirred under 1 atm of hydrogen for 2 h in the presence of 10% Pd/C (30 mg). The catalyst was removed by filtration through microfiber. The filtrate was concentrated under reduced pressure to give a crude material which was purified by preparative HPLC. The resulting TFA salt was suspended in EtOAc and washed with saturated $NaHCO_3$ to give, after drying over $MgSO_4$ and removal of solvent, **53** as a yellow foam (45.4 mg, 84%): ^{1}H NMR ($CDCl_3$) δ 7.30−7.23 (m, 3H), 7,18−7.14 (m, 2H), 7.07 (d, J = 7.9 Hz, 2H), 6.67 (d, J = 7.9 Hz, 2H) 4.50−4.40 (m, 3H), 3.22 (dd, J = 15.9, 3.5 Hz, 1H), 2.93−2.84 (m, 5H), 2.67 (dd, J = 15.9, 3.8 Hz, 1H); IR (neat) υ 3456, 3360, 1773, 1662 cm^{-1}; FAB MS m/z 324 (MH^+), 346 (M + 23); HRMS calcd for $C_{18}H_{22}N_3O_2$ 324.1712 (MH^+), found 324.1700.

4(*S*)-Benzyl-2-oxoazetidine-1-carboxylic Acid *N*-Methyl-*N*-{(4-acetamidophenyl)methyl}amide (52). A solution of azetidinone **46** (26 mg, 0.07 mmol) and excess acetic anhydride in THF (5 mL) was stirred under 1 atm of hydrogen for 24 h in the presence of 10% Pd/C (10 mg). The catalyst was removed by filtration through microfiber. The filtrate was concentrated under reduced pressure to give a colorless syrup which was purified by preparative TLC (EtOAc) to afford **52** as a colorless gel (15.9 mg, 60%): ^{1}H NMR ($CDCl_3$) δ 7.49 (d, J = 8.3 Hz, 2H), 7.32−7.20 (m, 4H), 7.19−7.14 (m, 3H), 4.57−4.42 (m, 3H), 3.21 (dd, J = 13.7, 3.2 Hz, 1H), 2.95−2.85 (m, 5H), 2.69 (dd, J = 15.9, 3.5 Hz, 1H), 2.19 (s, 3H); IR (neat) υ 3316, 1776, 1666 cm^{-1}; FAB MS m/z 366 (MH^+), 388 (M + 23); HRMS calcd for $C_{21}H_{24}N_3O_3$ 366.1819 (MH^+), found 366.1826.

4(*S*)-Benzyl-2-oxoazetidine-1-carboxylic Acid *N*-Methyl-*N*-{{4-(trifluoromethylsulfinyl)phenyl}methyl}amide (56) and 4(*S*)-Benzyl-2-oxoazetidine-1-carboxylic Acid *N*-Methyl-*N*-{{4-(trifluoromethylsulfonyl)phenyl}methyl}amide (57). To a solution of azetidinone **55** (130 mg, 0.32 mmol) in MeOH/water/acetone (1:3:2) (6 mL) was added Oxone (1.27 g, 2.08 mmol). After stirring at room temperature overnight, the methanol and acetone were removed by rotary evaporation. The residue was diluted with EtOAc and filtered. The filtrate was washed with saturated $NaHCO_3$ and brine and dried over $MgSO_4$. Flash chromatography (30% EtOAc in hexanes) afforded 122 mg of a mixture of the desired compounds which were separated by preparative HPLC (**56**, 39.4 mg, 29%; **57**, 43.9 mg, 31%). **56**: ^{1}H NMR ($CDCl_3$) δ 7.73 (d, J = 8.3 Hz, 2H), 7.44 (d, J = 8.3 Hz, 2H), 7.25−7.18 (m, 3H), 7.12 (m, 2H), 4.73−4.49 (m, 2H), 4.44−4.38 (m, 1H), 3.14 (dd, J = 13.7, 3.8 Hz, 1H), 2.94 (s, 3H), 2.89 (br m, 1H), 2.88 (dd, J = 16.2, 6.0 Hz, 1H), 2.64 (dd, J = 16.2, 3.8 Hz, 1H); IR (neat) υ 1776, 1668 cm^{-1}; FAB MS m/z 425 (MH^+); HRMS calcd for $C_{20}H_{19}F_3N_2O_3S$ 425.1147 (MH^+), found 425.1133. **57**: ^{1}H NMR ($CDCl_3$) δ 7.96 (d, J = 8.3 Hz, 2H), 7.48 (d, J = 8.3 Hz, 2H), 7.26−7.19 (m, 3H), 7.12−7.10 (m, 2H), 4.76−4.57 (br m, 2H), 4.43−4.39 (m, 1H), 3.14 (dd, J = 13.7, 4.2 Hz, 1H), 2.96 (s, 3H), 2.90 (dd, J = 15.9, 6.0 Hz, 1H), 2.95−2.82 (brm, 1H), 2.67 (dd, J = 15.9, 3.5 Hz, 1H); IR (neat) υ 1778, 1670 cm^{-1}; FAB MS m/z 441 (MH^+); HRMS calcd for $C_{20}H_{19}F_3N_2O_4S$ 441.1096 (MH^+), found 441.1080.

Electrostatic Potentials. The urea benzyl portion of **39** was modeled as toluene, and compounds **46**−**57** were modeled as the corresponding para-substituted toluenes. Equilibrium geometries were calculated using the AM1 semiempirical Hamiltonian[28] as implemented in Spartan 4.1.1[29] on an SGI Challenge L. The electron density and electrostatic potential were calculated on grids of 0.5-Å resolution. The total electron density was plotted as an isosurface of value 0.002 e/au^3 representing the molecular surface, and the electrostatic potential was mapped onto that surface as a property. Electrostatic potentials (in kcal/mol) were then measured as the minimum potential on the surface at the center of the phenyl ring.[26]

Biochemistry. Material and Methods: Fluorescence measurements were recorded on a Perkin-Elmer LS-50B spectrofluorimeter equipped with a plate reader accessory. UV measurements were recorded on a Thermomax microplate reader from Molecular Devices Corp., Menlo Park, CA.

HCMV N_o Protease Assay: HCMV N_o protease[30] was assayed with an internally quenched fluorogenic substrate based on the maturation cleavage site (Abz-VVNASSRLY(3-NO_2)R-OH, $k_{cat}/K_M = 260\ M^{-1}\ s^{-1}$). The fluorescence increase upon cleavage of the Ala−Ser amide bond was monitored using excitation $\lambda = 312$ nm (slit 2.5 nm) and emission $\lambda = 415$ nm (slit 5 nm). A protocol adaptable to a 96-well plate format was designed for the determination of IC_{50} values of inhibitors. Briefly, HCMV N_o was incubated for 2.5 h at 30 °C in the presence of the substrate with a range of sequentially diluted inhibitor concentrations (300−0.06 μM depending on the potency of each compound). After this period, enzymatic hydrolysis of the fluorogenic substrate in the absence of inhibitor led to about a 30% conversion. Quenching was not required before fluorescence measurement since the total scanning time by the plate reader accessory was brief relative to the duration of the reaction. The aqueous incubation buffer contained 50 mM tris(hydroxymethyl)aminomethane·HCl, pH 8, 0.5 M Na_2SO_4, 50 mM NaCl, 0.1 mM EDTA, 1 mM tris(2-carboxyethyl)phosphine·HCl, 3% v/v DMSO, and 0.05% w/v casein. The final concentrations of HCMV N_o protease (expressed in terms of total monomer concentration) and substrate were 100 nM and 5 μM, respectively. IC_{50} values were obtained through fitting of the inhibition curve to a competitive inhibition model using SAS NLIN procedure. The mode of inhibition was determined by measurements of the initial rates (in cuvettes) at various substrate concentrations in the buffer as described above. The IC_{50} values listed were obtained according to this assay.

Plaque Reduction Assay: Hs-68 cells (ATCC # CRL 1635) were seeded in 12-well plates at 83 000 cells/well in 1 mL of DMEM medium (Gibco Canada Inc.) supplemented with 10% fetal bovine serum (FBS; Gibco Canada Inc.). The plates were incubated for 3 days at 37 °C to allow the cells to reach 80−90% confluency prior to the assay. The medium was removed from the cells by aspiration. The cells were then infected with approximately 50 PFU of HCMV (strain AD169, ATCC VR-538) in DMEM medium (commercially available) supplemented with 5% inactivated FBS (assay medium). The virus was allowed to adsorb to cells for 2 h at 37 °C. Following viral adsorption, the medium was removed from the wells by aspiration. The cells were then incubated with or without 1 mL of appropriate concentrations of test reagent in assay medium. Occasionally, test compounds were added 24 h postinfection. After 4 days of incubation at 37 °C, the medium was exchanged with fresh medium containing test compound, and 4 days later the cells were fixed with 1% aqueous formaldehyde and stained with a 2% violet solution in 20% ethanol in water. Microscopic plaques were counted using a stereomicroscope. Drug effects were calculated as a percent reduction in the number of plaques in the presence of each drug concentration compared to the number observed in the absence of drug. Ganciclovir was used as a positive control in all experiments.[31]

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Supporting Information Available: Full tabulation of ^{1}H NMR, FAB MS, IR, and HRMS for all inhibitors not described in the Experimental Section (11 pages). Ordering information is given on any current masthead page.

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