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Synthesis and Antiviral Activity of Monobactams Inhibiting the Human Cytomegalovirus Protease

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Abstract—A series of monobactam inhibitors of HCMV (N_o) protease bearing a heterocycle linked by a methylene group at C-4 is described. Inhibitors containing a heterocycle such as a 2-furyl, 2-thiophenyl, 4-methyl-2-tetrazole and 2-benzothiazole were found to be active in a plaque reduction assay. Furthermore, 2-benzothiazole derivatives were shown to inhibit the HCMV protease activity inside cells by using a cell transfection assay, indicating that their antiviral activity in the plaque reduction assay could be attributed to protease inhibition.

Introduction

The human cytomegalovirus (HCMV), a member of the herpesvirus family, is an opportunistic pathogen in immunocompromised individuals including AIDS patients and organ transplant recipients.[1,2] An active infection with HCMV can result in disseminated disease, pneumonitis, retinitis, oesophagitis and colitis. Treatments are available, however, the performance of these drugs is far from ideal. Therefore, the need for alternative therapies for HCMV infections continues to exist.

The cytomegalovirus, expresses late in the virus life cycle, a protease that is essential for viral replication.[3,4] The protease is expressed as a precursor protein (UL80 gene product) together with the assembly protein. The assembly protein substrate is also independently expressed in approximately 10-fold excess (UL80.5 gene product). This serine protease[5–7] is necessary for the maturation of the viral capsid. Cleavage occurs to remove a fragment at the C-terminal end of the UL80 and UL80.5 gene products (M-site, Fig. 1), and also at a position located near the center of the precursor (R-site) to excise the catalytic domain (N_o). In addition to the M- and R-sites, two more cleavage sites (I and m) are known for the protease, both of which are located within the catalytic domain. Both N_o and the full-length protease precursor (UL80 gene product) are catalytically active.

It has been found recently that HCMV protease is uniquely folded and possesses a novel catalytic triad.[8–11] This enzyme has become an attractive target for inhibition in antiviral chemotherapy.[3,4] Strategies that have been employed include the use of peptidyl-activated carbonyls,[12] aryl trifluromethyl ketone derivatives[13] and other small molecule inhibitors.[14–17] More recently, we and others have described the use of monobactam inhibitors.[18–21] Mechanistic studies of this class of compounds indicate that inhibition involves processing of the β-lactam via an acyl enzyme complex.[18] While several of these monobactams have exhibited submicromolar potency in enzymatic assays,[19,21] antiviral activity in cell culture has so far been modest. In this paper we describe the preparation of a series of monobactam inhibitors with low micromolar activity in a plaque reduction assay. Furthermore, by using a cell based assay, these compounds have been shown to inhibit protease activity in transfected cells, supporting the fact that the observed antiviral properties were effected through a decrease in protease activity.

Chemistry

The synthesis of inhibitors **10**–**16** and **19**–**22** proceeded through common intermediate **4** (Scheme 1) which was readily prepared on a large scale from the commercially available serine derivative **1**. One carbon extension was

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achieved by an Arndt–Eistert synthesis using silver benzoate to catalyze the Wolff rearrangement. Although the latter reaction could be done in the presence of benzyl alcohol to obtain **2** directly, in practice it was found to be more convenient to prepare the corresponding acid, followed by the esterification. Removal of the Boc group under acidic conditions and cyclization using *t*BuMgCl[22,23] afforded monobactam **3**. Protection using a TIPS group[24] followed by removal of the benzyl ether gave key intermediate **4**.

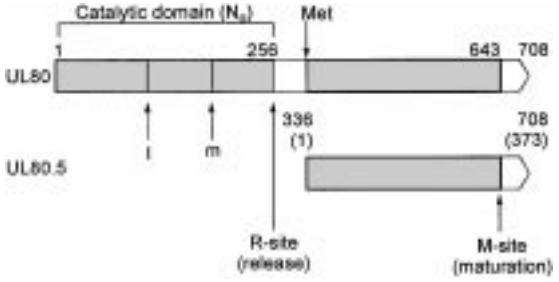
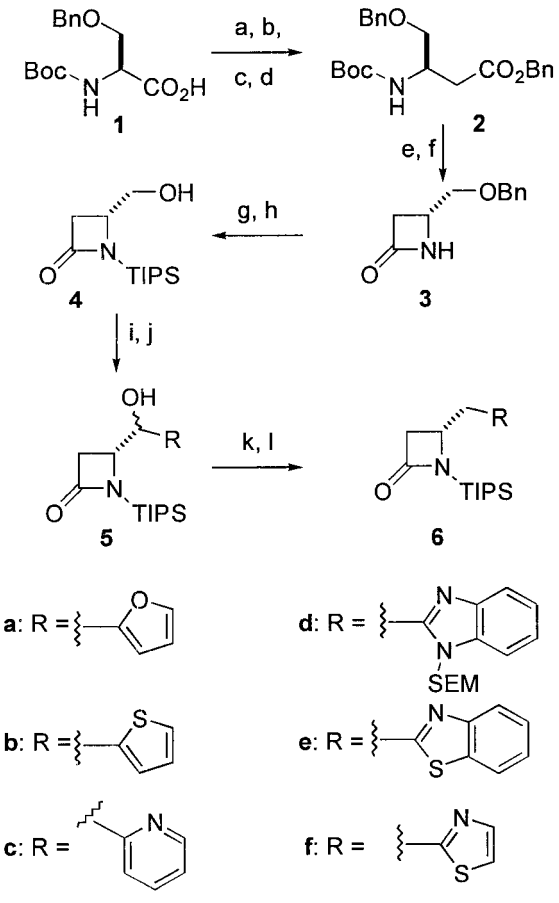


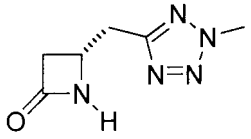
Figure 1. Genetic organisation of the HCMV protease and assembly proteins.



Scheme 1. *Reagents and conditions:* (a) IBCF, Et_3N, THF, 0°C; (b) CH_2N_2, 0°C; (c) AgOBz, Et_3N, THF, H_2O; (d) BnBr, DBU, CH_3CN, 25°C, 65% from **1**; (e) 4 N HCl dioxane, 25°C; (f) TMS-Cl, Et_3N, Et_2O then *t*BuMgCl, 0°C to rt, 76% from **2**; (g) TIPSOTf, 2,6-lutidine, CH_2Cl_2, 0°C; (h) H_2, $Pd(OH)_2$, THF, 84% from **3**; (i) Dess–Martin periodinane, CH_2Cl_2, H_2O, 100%; (j) heterocycle, BuLi, −78°C, 52–76%; (k) Im_2CS, DMAP, CH_2Cl_2; (l) Ph_3SnH, AIBN, benzene, △.

Dess–Martin[25] oxidation of **4** to the corresponding aldehyde was carried out using the modification recently described by Schreiber.[26] Failure to use this modification led to non-reproducible yields, especially on larger scales. Exposing the intermediate aldehyde to the appropriate lithio heterocycle gave the desired alcohols **5a–f** as mixtures of epimers. The use of the TIPS function on the β-lactam nitrogen was found to be necessary for successful introduction of the heterocycle and subsequent deoxygenation. The corresponding aldehyde protected at nitrogen by a TBS group gave good yields only when non-nitrogen containing heterocycles such as furan were used. The more basic lithio derivative of benzothiophene afforded very low yields of the desired product due to the formation of side products arising from migration of the silyl group onto oxygen.

The deoxygenation of alcohols **5a–f** was accomplished by treatment with 1,1′-(thiocarbonyl)diimidazole followed by reduction of the thiocarbamate with Ph_3SnH. The use of Ph_3SnH rather than Bu_3SnH greatly simplified the purification of the desired products **6a–f**. Removal of the TIPS group from **6a–f** using CsF in methanol was followed by treatment with KHMDS and the appropriate amidyl chloride[20,21] to afford the desired protease inhibitors with the general structure **8** (Scheme 2). The tetrazole containing derivatives were derived from **9**[27,28] in a similar manner. Compounds **15** and **16** were obtained after a final deprotection using TFA in CH_2Cl_2.



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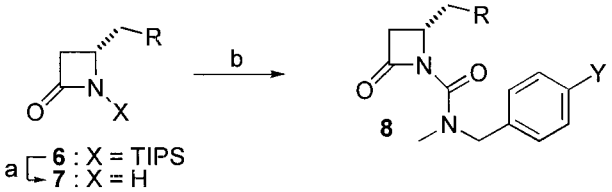
Cell based assay

The recombinant vaccinia virus T7 RNA polymerase transient expression system was used to express the enzyme and the substrate in COS-7 cells.[29] The expression of the UL80 gene encoding the protease precursor (Pra) was used to study the effect of protease inhibitors on the processing of the precursor at the M-site giving rise to the product (Prb) (Fig. 2). The processing of the assembly protein precursor by the protease was accomplished by expressing the catalytic domain of the protease (N_o) and the assembly protein precursor (pAP) in the same cell. The cells were treated with increasing concentrations of protease inhibitors for a period of 2 h after which they were metabolically labeled with [^{35}S]-Met and -Cys for an additional 2 h. The inhibitors were



Scheme 2. *Reagents and conditions:* (a) CsF, CH_3OH; (b) KHMDS, amidyl chloride, THF.

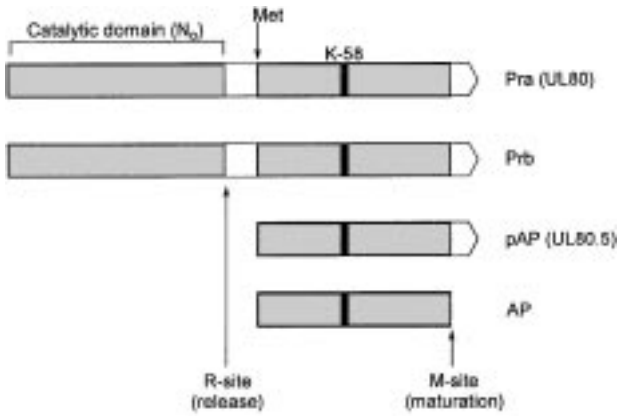


Figure 2. HCMV UL80 and UL80.5 gene products used to transfect COS-7 cells and the gene products analyzed after treatment with inhibitors.

present during the labeling period. The labeled proteins were immunoprecipitated using a polyclonal antiserum (K-58) which recognized an amino acid sequence common to Pra, Prb, pAP and AP as illustrated in Figure 2. The percent processing of the precursor in each sample was used to calculate the percent inhibition obtained for each concentration of inhibitors.

Results and Discussion

The identification of 4-thioalkyl-β-lactams as inhibitors of HCMV protease has been recently described.[19] Mechanistic investigations into the mode of action of these compounds led to the development of monobactams incorporating a hydrocarbon substituent at the C-4 position.[20] These compounds were effective HCMV protease inhibitors and were more stable in cell culture media than the 4-thioaryl series. By introducing various heterocycles linked by a methylthio substituent at the C-4 position, both enzymatic and cell culture activity could be improved.[21] Although these modifications led to inhibitors with sub-micromolar potency in the enzymatic assay and with activities between 50 and 80 μM in the plaque reduction assay, further improvements in the potency of this series were difficult to achieve. A possible explanation for this is that the C-4 heterocycle was located too distal from and in a non-optimal orientation relative to the β-lactam nucleus. Employing a one carbon tether would modify this while reducing the degrees of freedom available to the heterocyclic pharmacophore. Compounds **10**–**22** (Table 1) were, therefore, prepared and evaluated. Monobactams containing each heterocyclic group (R) were prepared with an *N*-methyl-*N*-benzyl urea function substituted in the *para* position of the phenyl ring by a CF_3 or NO_2 group (X).[20] These two ureas had been shown previously to impart the most activity to the monobactam series, and in the case of 4-methylthioheterocycles, to give the best potency in the cell culture assay.[21] Those inhibitors containing furan, thiophene, 2-methyltetrazole, thiazole and benzothiazole at the C-4 position of the β-lactam all had potencies between 0.7 and 7.1 μM. Compounds **14**–**16**, which incorporated pyridine or benzimidazole residues, did not give good activity in the enzymatic assay. Of those series which were active, compounds possessing a nitro group on the benzyl urea moiety were two to four times more potent than the corresponding CF_3 analogues, a result that is in agreement with previous observations.[20]

The present compounds all showed good selectivity towards other serine proteases such as human leukocyte elastase (HLE), porcine pancreatic elastase (PPE) and the cysteine protease human liver cathepsin B (cat-B). In all these cases the IC_{50} values were found to be above the solubility limit of the assay. Some of these compounds, however, were less selective towards bovine pancreatic α-chymotrypsin (BPC). It is interesting to note the effect of different substituents on the observed selectivity. For example, furan-containing inhibitors (**10** and **11**) did not significantly inhibit BPC (IC_{50} >75 μM), while compounds **12** and **13**, bearing a thiophene substituent showed modest selectivity: three and tenfold, respectively. The best selectivity was observed with the benzothiazole derivatives **19** and **20**, which were 16- and 80-fold, respectively, more active against HCMV protease.

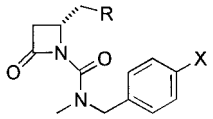
The antiviral activity of these compounds was determined in a plaque reduction assay. In a few cases, low solubility prevented an accurate determination of the antiviral activity and/or the cytotoxicity. However, for the reported cytotoxicity values, examination of the curves indicated that the TC_{50}'s were all well above the solubility limit. Five compounds (**10**, **11**, **13**, **17**, and **18**) had EC_{50} values below 150 μM, and derivatives **19** and **20** were the most active of the series ($EC_{50} = 11$ and 30 μM, respectively).[30]

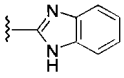
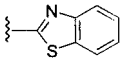
Protease inhibitors **19** and **20** were evaluated for their ability to inhibit the processing of the protease precursor as well as the assembly protein precursor in cells. In order to measure the effect on these events independently, the recombinant vaccinia virus T7 RNA polymerase transient expression system was used to express the enzyme (N_o) and the substrates (Pra and pAP) in COS-7 cells.[31,32] Figure 3 illustrates the inhibitory effect of **19** and **20** on the processing of the protease precursor (Pra). Both compounds exhibited similar effects on processing at the M-site with 70% inhibition at the maximum concentration tested. Both compounds also inhibited the processing of the assembly protein precursor (pAP) by the catalytic domain of the enzyme (Fig. 4). The effect on the processing of the assembly protein precursor (pAP) occurred at concentrations similar to that observed for the inhibition of the protease precursor processing. These data suggest that the β-lactam CMV protease inhibitors were active against the precursor as well as the mature form of the protease in cells. Furthermore, the concentrations at which protease inhibition was achieved were in the same range as those observed for the antiviral activities (EC_{50}).

Conclusions

A series of monobactam inhibitors of HCMV protease bearing a heterocycle linked by a methylene group at

Table 1. Biological activities of monobactam HCMV protease inhibitors



Compound	R	X	IC$_{50}$ (μM) HCMV[a]	IC$_{50}$ (μM) BPC[b]	EC$_{50}$[c] (μM)	TC$_{50}$[d] (μM)
10		CF_3	7.1	> 75	59	> 100
11		NO_2	3.1	> 75	143	> 261
12		CF_3	4.5	14	> 16	> 16
13		NO_2	1.3	15	70	> 79
14		CF_3	63	> 300		
15		CF_3	> 50	> 50		
16		NO_2	> 75	> 75		
17		CF_3	5.7	> 300	94	> 195
18		NO_2	2.7	27	100	> 133
19		CF_3	2.7	43	11	> 25
20		NO_2	0.7	57	30	> 51
21		CF_3	4.9	12	> 77	> 116
22		NO_2	2.5	8	> 29	> 29

[a] HCMV protease inhibition.
[b] Bovine pancreatic α-chymotrypsin inhibition.
[c] Plaque reduction assay. Ganciclovir was used as a positive control (EC$_{50}$ = 1.23 μM). See Experimental.
[d] Cytotoxicity as determined by the MTT method.[36]

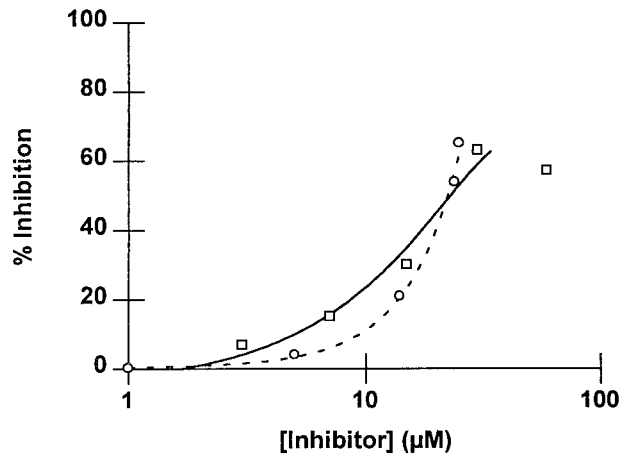


Figure 3. Plot showing the inhibition of autoprocessing of the protease precursor (Pra) in transfected cells. The effect of compounds **19** (○) and **20** (□) at various concentrations is illustrated.

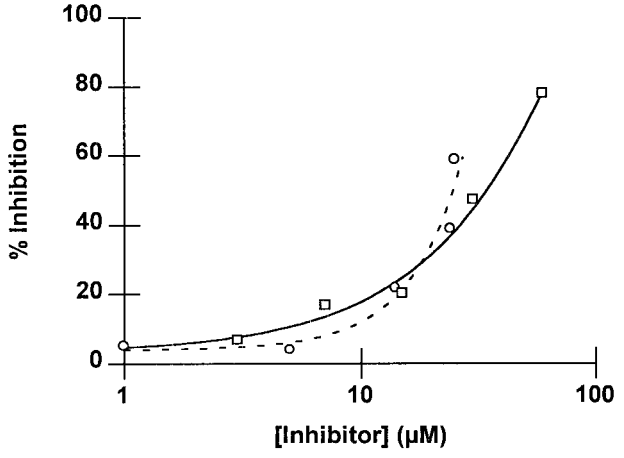


Figure 4. Plot showing the inhibition of processing of the assembly protein precursor (pAP) by the catalytic domain of HMCV protease (N_o) in transfected cells. The effect of compounds **19** (○) and **20** (□) at various concentrations is illustrated.

C-4 has been described. Inhibitors containing heterocycles such as a 2-furyl, 2-thiophenyl, 4-methyl-2-tetrazole, or 2-benzothiazole were active in a plaque reduction assay. Furthermore, 2-benzothiazole derivatives (**19** and **20**) were shown to inhibit the activity of HCMV protease inside cells by using a novel cell transfection assay, indicating that their antiviral activity in the plaque reduction assay could be attributed to protease inhibition.

Experimental

General methods

Unless otherwise noted, materials obtained from commercial sources were used without further purification. All reactions were carried out under inert atmosphere. In the last step, the reactions were performed on approximately 100 mg scale to give the reported yields. ^{1}H NMR spectra were obtained on a Bruker AMX 400 spectrometer with tetramethylsilane as internal standard (δ scale) and in the solvent given. FAB mass spectra were recorded on an Autospec VG spectrometer. ES mass spectra were recorded on a Quattro II 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, C_{18} preparative column (50 cm×22 mm). Analytical HPLC was carried out in the following systems; System A: Vydac C_{18}, 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 C_{18}, 10 mm analytical column (24 cm×4.6 mm); mobile phase, acetonitrile in 20 mM aqueous Na_2HPO_4 at pH 8.2.

3(*R*)-4-Benzyloxy-3-*tert*-butoxycarbonylamino-butyric acid benzyl ester (2). To a solution of **1** (18.59 g, 63.16 mmol) and Et_3N (9.2 mL, 66.3 mmol) in THF (126 mL) at 0°C was added dropwise isobutyl chloroformate (9.0 mL, 69.5 mmol). After stirring for 40 min, an excess of ethereal CH_2N_2 was added and stirring was continued for an additional 1.5 h. Sufficient glacial acetic acid was then introduced to destroy excess CH_2N_2, the solution was filtered and the filtrate washed with saturated $NaHCO_3$ and brine and dried over $MgSO_4$. Removal of solvent under reduced pressure gave the desired diazoketone (20.17 g, 85%). ^{1}H NMR ($CDCl_3$) δ 7.38–7.29 (m, 5H), 5.56 (br, 1H), 5.41 (br, 1H), 4.53 (s, 2H), 4.33 (br, 1H), 3.87 (dd, J=9.2, 3.2 Hz, 1H), 3.62 (dd, J=9.5, 5.1 Hz, 1H), 1.46 (s, 9H). This material (5.12 g, 16.0 mmol) was dissolved in THF (32 mL) and water (5.8 mL). Silver benzoate (367 mg, 1.6 mmol) in Et_3N (3.4 mL) was then added slowly (CAUTION: nitrogen gas evolution). After stirring at room temperature for 2 h the solution was diluted with ether and washed twice with 10% HCl, once with brine and dried over $MgSO_4$. Evaporation of the solvent gave the crude acid which was dissolved in CH_3CN (32 mL) containing benzyl bromide (2.1 mL, 17.6 mmol) and DBU (2.88 mL, 19.2 mmol). After stirring for 2 h at room temperature, the mixture was diluted with ether washed twice with 10% citric acid, twice with $NaHCO_3$, once with brine and dried over $MgSO_4$. Removal of solvent under reduced pressure gave the crude ester which was purified by flash chromatography (10% EtOAc in hexanes) to deliver the desired ester **2** (12.4 g, 77%). $[\alpha]_D^{25}$ + 5.0 (*c* 1.11, $CHCl_3$); IR (neat) 3364, 1713 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.39–7.28 (m, 10H), 5.16 (br, 1H), 5.10 (s, 2H), 4.51 (d, J=12.1 Hz, 1H), 4.47 (d, J=12.4 Hz, 1H), 4.20 (br, 1H), 3.58 (dd, J=9.2, 3.8 Hz, 1H), 3.52 (dd, J=9.5, 4.1 Hz, 1H), 2.75–2.65 (m, 2H), 1.45 (s, 9H); MS (ES) 400 (M + H); 422 (M + 23); HRMS calcd for $C_{23}H_{30}NO_5$ (M + H) 400.2124, found 400.2113.

4-(*R*)-4-Benzyloxymethyl-azetidin-2-one (3). The Boc protected amino acid ester **2** (8 g, 20 mmol) was stirred in 4 N HCl/dioxane (75 mL) for 1 h at which time TLC indicated that the reaction was complete. The solution was concentrated and the residue dissolved in a small volume of water. A 1 M solution of K_2CO_3 (30 mL) was then introduced and the mixture was extracted twice with EtOAc. Drying over $MgSO_4$ followed by removal of solvent gave the desired amine as yellow oil (5.95 g, 99%). A solution of the amine (5.9 g, 19.7 mmol) in ether (26 mL) was treated at 0°C with TMS-Cl (2.80 mL, 21.7 mmol) and 10 min later with Et_3N (3.3 mL, 23.6 mmol). The resulting suspension was stirred at 0°C for 1 h. Triethylamine hydrochloride was removed by filtration and the filtrate was diluted to 0.25 M with ether and placed in an ice bath. Slow addition of *tert*-butylmagnesium chloride (12.8 mL of a 2 M solution in ether, 25.6 mmol) was followed by stirring at 0°C for 1 h and overnight at room temperature. The reaction was quenched by the addition of saturated NH_4Cl (5 mL) and 10% HCl (10 mL) and extracted twice with CH_2Cl_2. The combined organic extracts were washed with saturated $NaHCO_3$ and brine and dried over $MgSO_4$. Flash chromatography (25–60% EtOAc in hexane) afforded **3** as yellow oil (2.87 g, 76%). $[\alpha]_D^{25}$ −42.9 (*c* 1.02, $CHCl_3$); IR (neat) 3262, 1759 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.39–7.30 (m, 5H), 6.03 (br, 1H), 4.56 (s, 2H), 3.86–3.81 (m, 1H), 3.67 (dd, J=10.4, 4.6 Hz, 1H), 3.48 (dd, J=9.5, 7.6 Hz, 1H), 3.04 (ddd, J=14.9, 5.4, 1.9 Hz, 1H), 2.65 (ddd, J=14.6, 2.2, 1.6 Hz, 1H); MS (ES) 192 (M + H); 214 (M + 23); HRMS calcd for $C_{11}H_{14}NO_2$ (M + H) 192.1025, found 192.1028.

4-(*R*)-4-Hydroxymethyl-1-triisopropylsilanyl-azetidin-2-one (4). Triisopropylsilyl trifluromethanesulfonate (1.07 mL, 3.98 mmol) was added via syringe to a solution of **3** (585 mg, 3.06 mmol) and 2,6-lutidine (0.89 mL, 7.65 mmol) in CH_2Cl_2 (6 mL) at 0°C. After stirring for 3 h, the solution was diluted with EtOAc and washed with water, twice with 10% citric acid, twice with $NaHCO_3$ once with brine and dried over $MgSO_4$. Evaporation of the solvent and flash chromatography (10% EtOAc in hexanes) gave the desired silyl amide as a colorless oil (927 mg, 87%). $[\alpha]_D^{25}$ + 33.1 (*c* 1.08, $CHCl_3$); IR (neat) 1744 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.33–7.22 (m, 5H), 4.48 (s, 2H), 3.74 (ddd, J=10.8, 5.7, 2.9 Hz, 1H), 3.59 (dd, J=9.9, 5.1 Hz, 1H), 3.47 (dd, J=9.9, 5.7 Hz, 1H), 3.12 (dd, J=15.3, 5.7 Hz, 1H), 2.76 (dd, J=15.3, 2.5 Hz, 1H), 1.31 (sept, J=2.6 Hz, 3H), 1.06 (d, J= 7.3 Hz, 9H), 1.02 (d, J=7.3 Hz, 9H); ^{13}C NMR ($CDCl_3$) δ 173.4, 137.9, 128.6, 128.0, 127.9, 73.6, 73.3, 48.8, 42.2, 18.4, 18.2, 12.0; MS (ES) 348 (M + H); 370 (M + 23); HRMS calcd for $C_{20}H_{34}NO_2Si$ (M + H) 348.2359, found 348.2371. A mixture of the benzyl ether so obtained (3.80 g, 10.9 mmol) and 10% $Pd(OH)_2/C$ (300 mg) in THF (100 mL) was stirred under an atmosphere of hydrogen for 5 h. Filtration through Celite® and removal of the solvent gave pure **4** (3.38 g, 96%) after flash chromatography (25% EtOAc in hexanes). $[\alpha]_D^{25}$ + 41.5 (*c* 1.06, $CHCl_3$); IR (KBr) 3380, 1707 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.88–3.82 (m, 1H), 3.77–3.69 (m, 2H), 3.15 (dd, J=15.3, 5.4 Hz, 1H), 2.88 (dd, J=15.3, 2.2 Hz, 1H), 1.83 (dd, J=7.0, 4.5 Hz, 1H), 1.37 (sept,

$J = 7.6$ Hz, 3H), 1.14 (d, $J = 7.3$ Hz, 9H), 1.11 (d, $J = 7.3$ Hz, 9H); ^{13}C NMR ($CDCl_3$) δ 173.6, 65.2, 50.3, 41.5, 18.4, 18.3, 12.0; MS (ES) 258 (M + H); 280 (M + 23).

4-(*R*)-4-(Hydroxy-thiazol-2-yl-methyl)-1-triisopropylsilanyl-azetidin-2-one (5f). To a solution of β-lactam alcohol **4** (1.08 g, 4.2 mmol) and Dess–Martin periodinane (2.68 g, 6.3 mmol) in dry CH_2Cl_2 (50 mL) was added dropwise reagent grade CH_2Cl_2 (30 mL). After stirring for 1 h, 50 mL of 10% $Na_2S_2O_3$ and 50 mL of saturated $NaHCO_3$ was added and the resulting mixture stirred vigorously until both layers were clear. The mixture was diluted with ether and the combined organic phases washed with satd $NaHCO_3$, brine and dried over $MgSO_4$. Removal of solvent under reduced pressure gave the desired aldehyde as a white solid (1.08 g, 100%). Thiazole (0.45 mL, 6.3 mmol) was dissolved in THF (40 mL) and the resulting solution cooled to −50°C. *n*BuLi (4.9 mL of a 1.2 M solution in hexanes, 5.88 mmol) was added and stirring was continued for 30 min. The solution was then cooled further to −78°C and the previously prepared aldehyde was slowly introduced as a solution in THF (8 mL). TLC analysis after 20 min indicated that the reaction was complete. The reaction was quenched by the addition of acetic acid (0.4 mL) in THF (3 mL), saturated NH_4Cl was added and the mixture extracted with ether. The combined ethereal extracts were washed with brine and dried over $MgSO_4$. Purification by flash chromatography (10% EtOAc in benzene) gave the desired alcohol **5f** (1.08 g, 76%) as a mixture of isomers. HPLC (A) 96%, (B) 97%; mp 79–81°C; IR (KBr) 1738, 1719, 1682 cm^{-1}; ^1H NMR ($CDCl_3$) δ **isomer A**: 7.77 (d, $J = 3.2$ Hz, 1H), 7.34 (d, $J = 3.2$ Hz, 1H), 5.32 (dd, $J = 3.5$, 2.2 Hz, 1H), 4.23–4.20 (m, 1H), 3.07 (dd, $J_{AB} = 15.3$, $J_{AX} = 2.7$ Hz, 1H), 2.94 (d, $J = 3.2$ Hz, 1H), 2.86 (dd, $J_{AB} = 15.3$, $J_{BX} = 5.5$ Hz, 2H), 1.46–1.37 (m, 3H), 1.20–1.15 (m, 18H); **isomer B**: 7.76 (d, $J = 3.2$ Hz, 1H), 7.36 (d, $J = 3.2$ Hz, 1H), 4.97 (dd, $J = 7.6$, 5.1 Hz, 1H), 3.97–3.93 (m, 1H), 3.57 (d, $J = 5.1$ Hz, 1H), 3.09 (dd, $J_{AB} = 15.6$, $J_{AX} = 5.7$ Hz, 1H), 2.81 (dd, $J_{AB} = 15.6$, $J_{BX} = 7.8$ Hz, 2H), 1.57–1.46 (m, 3H), 1.17–1.12 (m, 18H); MS (ES^+) 341 (M + H), 363 (M + 23); HRMS calcd for $C_{16}H_{29}N_2O_2SSi$, 341.1719, found 341.1722.

4-(*R*)-4-(Furan-2-yl-hydroxy-methyl)-1-triisopropylsilanyl-azetidin-2-one (5a). This compound was prepared from **4** (1.15 g, 4.47 mmol) and furan (0.65 mL, 8.94 mmol) in 53% yield (760 mg) using a procedure similar to that described for compound **5f** except that the condensation with furan was done in ether at −20°C. HPLC (A) 100%, (B) 98%; IR (neat) 1736 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.39 and 7.25 (2×m, 1H), 6.37–6.28 (m, 2H), 5.01 and 4.67 (2×m, 1H), 3.96 (m, 1H), 3.25 and 2.65 (2×m, 1H), 3.11–2.97 (m, 1H), 2.11 (m, 1H), 1.55–1.32 (m, 3H), 1.18-1.10 (m, 18H); MS (ES^+) 324 (M + H); HRMS calcd for $C_{17}H_{30}NO_3Si$ (M + H), 324.1995, found 324.2004; Anal. calcd for $C_{17}H_{29}NO_3Si$: C, 63.12; H, 9.04; N, 4.33. Found: C, 63.34; H, 9.42; N, 4.50.

4-(*R*)-4-(Hydroxy-thiophen-2-yl-methyl)-1-triisopropylsilanyl-azetidin-2-one (5b). This compound was prepared from **4** (160 mg, 0.62 mmol) and thiophene (0.07 mL, 0.94 mmol) in 70% yield (147 mg) using a procedure similar to that described for compound **5f**. HPLC (A) 100%, (B) 97%; mp 112–114°C; IR (neat) 1711 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.30–7.28 (m, 1H), 7.05–6.98 (m, 2H), 4.86–4.84 (m, 1H), 3.89–3.85 (m, 1H), 3.06–3.00 (m, 1H), 2.55 (dd, $J = 15.6$, 2.7 Hz, 1H), 2.15 (s, 1H), 1.62–1.37 (m, 3H), 1.25–1.13 (m, 18H); MS (ES^-) 338 (M − H); HRMS calcd for $C_{17}H_{30}NO_2SSi$ (M + H), 340.1767, found 340.1773.

4-(*R*)-4-(Hydroxy-pyridin-2-yl-methyl)-1-triisopropylsilanyl-azetidin-2-one (5c). This compound was prepared from **4** (200 mg, 0.78 mmol) and 2-bromopyridine (185 mg, 1.17 mmol) using a procedure similar to that described for compound **5f**. The reaction was carried out in CH_2Cl_2 at −78°C.[33] HPLC (B) 96%; IR (neat) 1719 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.59–8.54 (m, 1H), 7.68 (m, 1H), 7.32–7.23 (m, 4H), 5.06–5.04 and 4.67 (2×m, 1H), 4.11 and 4.07 (2×d, $J = 7.0$ and 4.5 Hz, 1H), 4.01–3.98 and 3.85–3.81 (2 m, 1H), 2.98–2.88 (m, 1H), 2.66–2.59 (m, 1H), 1.57–1.42 (m, 3H), 1.23–1.13 (m, 18H); MS (ES^+) 335 (M + H); HRMS calcd for $C_{18}H_{31}N_2O_2Si$ (M + H), 335.2155, found 335.2163.

4-(*R*)-4-{Hydroxy-[1-(2-trimethylsilanyl-ethoxymethyl)-1*H*-benzoimidazol-2-yl]-methyl}-1-triisopropylsilanyl-azetidin-2-one (5d). This compound was prepared from **4** (1.03 g, 4.03 mmol) and 1-[[2-(trimethylsilyl)ethoxy]-methyl]-1*H*-benzimidazole[34] (1.56 g, 6.05 mmol) in 52% yield (1.05 g) using a procedure similar to that described for compound **5f**. IR (neat) 3226, 1734 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.77 (dd, $J = 6.4$, 1.6 Hz, 0.3H), 7.74 (dd, $J = 7.6$, 1.9 Hz, 0.7H), 7.46 (dd, $J = 6.7$, 1.6 Hz, 0.3H), 7.41 (dd, $J = 6.4$, 0.9 Hz, 0.7H), 7.36–7.29 (m, 2H), 5.70 (d, $J = 11.4$ Hz, 0.7H), 5.68 (d, $J = 11.1$ Hz, 0.3H), 5.56 (d, $J = 11.1$ Hz, 0.7H), 5.54 (d, $J = 11.1$ Hz, 0.3H), 5.32 (t, $J = 8.6$ Hz, 0.3H), 4.92 (dd, $J = 8.6$, 5.4 Hz, 0.7H), 4.42 (ddd, $J = 8.9$, 6.0, 2.9 Hz, 0.7H), 4.32 (dt, $J = 5.1$, 2.5 Hz, 0.3H), 4.00–3.97 (m, 0.3H), 3.93 (s, 0.7H), 3.64–3.57 (m, 2H), 3.31 (dd, $J = 15.6$, 2.5 Hz, 0.3H), 3.17 (dd, $J = 15.6$, 6.0 Hz, 0.7H), 3.11 (dd, $J = 15.6$, 5.4 Hz, 0.3H), 2.59 (dd, $J = 15.6$, 2.9 Hz, 0.7H), 1.62 (septet, $J = 7.3$ Hz, 2H), 1.44 (septet, $J = 7.6$ Hz, 1H), 1.17–1.10 (m, 18H), 0.96–0.90 (m, 2H), −0.02 (s, 0.7H), −0.03 (s, 0.3H); MS (ES^+) 504 (M + H); HRMS calcd for $C_{26}H_{46}N_3O_3Si_2$, 504.3078, found 504.3004; Anal. calcd for $C_{26}H_{45}N_3O_3Si_2$: C, 61.98; H, 9.00; N, 8.34. Found: C, 61.56; H, 9.32; N, 8.24.

4-(*R*)-4-Thiazol-2-yl-methyl-1-triisopropylsilanyl-azetidin-2-one (6f). Alcohol **5f** (1 g, 2.94 mmol) and 1,1′-(thiocarbonyl)diimidazole (1.56 g, 8.82 mmol) were stirred together with DMAP (357 mg, 2.94 mmol) in CH_2Cl_2 (30 mL) for 18 h. Removal of the solvent and flash chromatography (50% EtOAc in hexanes) gave the desired thiocarbamate (1.26 g, 95%) which was immediately dissolved in benzene (56 mL, 0.05 M) containing Ph_3SnH (1.47 g, 4.2 mmol) and AIBN (92 mg, 0.56 mmol). The solution was degassed (two freeze–thaw cycles) and refluxed for 45 min. Removal of solvent and flash chromatography (10% EtOAc in hexanes the 30% EtOAc in hexanes) gave **6f** as a colorless oil (793 mg,

83%). HPLC (A) 97%, (B) 97%; $[\alpha]_D + 85°$ (*c* 0.55, $CHCl_3$); IR ($CHCl_3$) 1730 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.73 (d, $J = 3.5$ Hz, 1H), 7.25 (d, $J = 3.5$ Hz, 1H), 4.08–4.02 (m, 1H), 3.64 (dd, $J_{AB} = 14.5$, $J_{AX} = 3.6$ Hz, 1H), 3.23 (dd, $J_{AB} = 15.6$, $J_{ax} = 5.4$ Hz, 1H), 3.11 (dd, $J_{AB} = 14.5$, $J_{AX} = 10.7$ Hz, 1H), 2.85 (dd, $J_{AB} = 15.6$, $J_{AX} = 2.5$ Hz, 1H), 1.41–1.34 (m, 3H), 1.18–1.13 (m, 18H); MS (ES^+) 325 (M + H); HRMS calcd for $C_{16}H_{29}N_2OSSi$, 325.1770, found 325.1764; Anal. calcd for $C_{16}H_{28}N_2OSSi$: C, 59.21; H, 8.70; N, 8.63. Found: C, 59.20; H, 8.98; N, 8.66.

4-(*S*)-4-Furan-2-yl-methyl-1-triisopropylsilanyl-azetidin-2-one (6a). This compound was prepared from **5a** in 71% yield using a similar procedure to that described above for **6f**. $[\alpha]_D^{25} + 70°$ (*c* 0.95, CH_2Cl_2); IR (neat) 1745 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.32 (m, 1H), 6.29 (m, 1H), 6.06 (m, 1H),3.86 (m, 1H), 3.20 (m, 2H), 2.73 (m, 2H), 1.41–1.33 (m, 3H), 1.16–1.11 (m, 18H); MS (ES^+) 308 (M + H); HRMS calcd for $C_{17}H_{30}NO_2Si$ (M + H), 308.2046, found 308.2056.

4-(*R*)-4-Thiophen-2-yl-methyl-1-triisopropylsilanyl-azetidin-2-one (6b). This compound was prepared from **5b** in 67% yield using a similar procedure to that described above for **6f**. HPLC (A) 100%; $[\alpha]_D^{25} + 50°$ (*c* 0.80, CH_2Cl_2); IR (neat) 1744 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.17 (d, $J = 5.1$ Hz, 1H), 6.95 (dd, $J = 5.1$, 3.2 Hz, 1H), 6.83 (d, $J = 3.2$ Hz, 1H), 3.83–3.79 (m, 1H), 3.45 (dd, $J = 14.3$, 3.5 Hz, 1H), 3.16 (dd, $J = 15.5$, 5.4 Hz, 1H), 2.87 (dd, $J = 14.3$, 11.1 Hz, 1H), 2.76 (dd, $J = 5.6$, 3.0 Hz, 1H), 1.38 (m, 3H), 1.15 (m, 18H); MS (ES^+) 324 (M + H); HRMS calcd for $C_{17}H_{30}NOSSi$ (M + H), 324.1817, found 324.1808.

4-(*S*)-4-Pyridin-2-yl-methyl-1-triisopropylsilanyl-azetidin-2-one (6c). This compound was prepared from **5c** in 91% yield using a similar procedure to that described above for **6f**. HPLC (B) 99%; $[\alpha]_D^{25} + 249°$ (*c* 0.57, CH_2Cl_2); IR (neat) 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.54 (d, $J = 4.8$ Hz, 1H), 7.62 (dt, $J = 7.7$, 2.0 Hz, 1H), 7.17–7.11 (m, 2H), 4.09–4.03 (m, 1H), 3.49 (dd, $J = 14.6$, 5.2 Hz, 2H), 3.08 (dd, $J = 15.5$, 5.3 Hz, 1H), 2.84–2.74 (m, 1H), 1.46–1.35 (m, 3H), 1.22–1.14 (m, 18H); MS (ES^+) 319 (M + H); HRMS calcd for $C_{18}H_{31}N_2OSi$ (M + H), 319.2206, found 319.2223.

4-(*R*)-1-Triisopropylsilanyl-4-[1-(2-trimethylsilanyl-ethoxymethyl)-1*H*-benzoimidazol-2-yl-methyl]-azetidin-2-one (6d). This compound was prepared from **5d** in 82% yield using a similar procedure to that described above for **6f**. $[\alpha]_D^{25} + 78°$ (*c* 0.61, $CHCl_3$); IR (neat) 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.76–7.74 (m, 1H), 7.69–7.67 (m, 1H), 7.49–7.40 (m, 2H), 5.48 (s, 2H), 4.41–4.36 (m, 1H), 3.58–3.53 (m, 3H), 3.40 (dd, $J = 15.9$, 5.4 Hz, 1H), 3.04 (dd, $J = 15.6$, 11.1 Hz, 1H), 2.85 (dd, $J = 15.6$, 2.5 Hz, 1H), 1.42 (septet, $J = 7.3$ Hz, 3H), 1.20 and 1.17 (2×d, $J = 7.3$ Hz, 18H), 0.93 (t, $J = 7.9$, 2H), −0.02 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 173.3, 151.5, 136.3, 135.3, 130.6, 129.3, 123.4, 122.9, 119.8, 109.4, 72.5, 67.0, 47.6, 45.6, 34.9, 18.5, 18.4, 18.0, 12.0, −1.3; MS (ES^+) 488 (M + H), 510 (M + 23); HRMS calcd for $C_{26}H_{46}N_3O_2Si_2$ (M + H), 488.3129, found 488.3145.

4-(*R*)-4-Benzothiazol-2-yl-methyl-1-triisopropylsilanyl-azetidin-2-one (6e). This compound was prepared from **5e** in 71% yield using a similar procedure to that described above for **6f**. HPLC (A) 100%, (B) 100%; $[\alpha]_D + 81°$ (*c* 0.61, $CHCl_3$); IR ($CHCl_3$) 1745 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.00 (d, $J = 7.6$ Hz, 1H), 7.87 (d, $J = 7.9$ Hz, 1H), 7.51–7.46 (m, 1H), 7.41–7.37 (m, 1H), 4.19–4.13 (m, 1H), 3.73 (dd, $J_{AB} = 14.6$, $J_{AX} = 3.8$ Hz, 1H), 3.19 (dd, $J_{AB} = 14.6$, $J_{AX} = 10.5$ Hz, 1H), 3.28 (dd, $J_{AB} = 15.6$, $J_{AX} = 5.4$ Hz, 1H), 2.91 (dd, $J_{AB} = 15.6$, $J_{BX} = 2.5$ Hz, 1H); MS (ES^+) 375 (M + H), 397 (M + 23); HRMS calcd for $C_{20}H_{31}N_2OSSi$, 375.1926, found 375.1939; Anal. calcd for $C_{20}H_{30}N_2OSSi$: C, 64.12; H, 8.07; N, 7.46. Found: C, 64.19; H, 8.13; N, 7.40.

4-(*R*)-4-Thiazol-2-yl-methyl-azetidin-2-one (7f). Lactam **6f** (743 mg, 2.29 mmol) was stirred in methanol (20 mL) containing CsF (418 mg, 2.75 mmol) at room temperature for 1 h. The methanol was then removed, the residue suspended in EtOAc and washed twice with brine. Drying over $MgSO_4$ and flash chromatography (EtOAc) gave **7f** (269 mg, 70%) as a colorless oil which solidified upon standing. Mp 56°C; $[\alpha]_D$ −52° (*c* 0.51, $CHCl_3$); IR (KBr) 1739 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.73 (d, $J = 3.5$ Hz, 1H), 7.26 (d, $J = 3.5$ Hz, 1H), 6.13 (br, 1H), 4.12–4.07 (m, 1H), 3.39 (dd, $J_{AB} = 15.3$, $J_{AB} = 5.0$ Hz, 1H), 3.27 (dd, $J_{AB} = 15.3$, $J_{AX} = 8.3$ Hz, 1H), 3.21–3.16 (m, 1H), 2.80–2.76 (m, 1H); MS (ES^+) 169 (M + H), 191 (M + 23); HRMS calcd for $C_7H_{10}N_2OS$, 169.0436, found 169.0440; Anal. calcd For $C_7H_9N_2OS$: C, 49.98; H, 4.79; N, 16.65. Found: C, 49.75; H, 4.74; N, 16.43.

4-(*S*)-4-Furan-2-yl-methyl-azetidin-2-one (7a). Lactam **6a** (270 mg, 0.88 mmol) was treated with CsF as described above to give **7a** (120 mg, 90%) as a white solid. HPLC (A) 99%, (B) 99%; $[\alpha]_D^{25}$ −118° (*c* 0.28, CH_2Cl_2); IR (neat) 1742 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.34 (dd, $J = 1.9$, 0.6 Hz, 1H), 6.31 (dd, $J = 3.2$, 1.0 Hz, 1H), 6.08 (dd, $J = 3.2$, 1.0 Hz, 1H), 5.89 (br, 1H), 3.90 (m, 1H), 3.13 (ddd, $J = 15.0$, 4.8, 1.9 Hz, 1H), 3.00 (dd, $J = 14.9$, 5.4 Hz, 1H), 2.91 (dd, $J = 14.9$, 5.4 Hz, 1H), 2.72 (ddd, $J = 15.0$, 2.5, 1.6 Hz, 1H); MS (ES^+) 152 (M + H); HRMS calcd for $C_8H_9NO_2$, 155.0633, found 155.0634.

4-(*R*)-4-Thiophen-2-yl-methyl-azetidin-2-one (7b). Lactam **6b** (260 mg, 0.81 mmol) was treated with CsF as described above to give **7b** (120 mg, 92%) as a white solid. HPLC (A) 99%, (B) 95%; $[\alpha]_D^{25}$ −14° (*c* 0.85, CH_2Cl_2); IR (neat) 1764 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.18 (dd, $J = 5.4$, 1.3 Hz, 1H), 6.96 (dd, $J = 5.1$, 3.5 Hz, 1H), 6.84 (m, 1H), 5.98 (br, 1H), 3.89–3.84 (m, 1H), 3.18 (dd, $J = 14.9$, 5.7 Hz, 1H), 3.14–3.06 (m, 2H), 2.73–2.68 (m, 1H); MS (ES^+) 168 (M + H); HRMS calcd for $C_8H_{10}NOS$, 168.0483, found 168.0489.

4-(*S*)-4-Pyridin-2-yl-methyl-azetidin-2-one (7c). Lactam **6c** (550 mg, 1.73 mmol) was treated with CsF as described above to give **7c** (230 mg, 82%) as a white solid. HPLC (B) 99%; mp 89-90°C; $[\alpha]_D^{25}$ −204° (*c* 0.60, CH_2Cl_2); IR (neat) 3167, 1739 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.54 (d, $J = 4.7$ Hz, 1H), 7.63 (dt, $J = 7.6$, 1.6 Hz, 1H), 7.17 (d, $J = 6.4$ Hz, 1H), 7.16 (t, $J = 7.6$ Hz, 1H), 6.08 (br, 1H), 4.13-4.05 (m, 1H), 3.17–3.11 (m, 2H), 3.04 (dd,

J = 14.6, 8.4 Hz, 1H), 2.77–2.73 (m, 1H); MS (ES^+) 163 (M + H); HRMS calcd for $C_9H_{11}N_2O$, 163.0871, found 163.0875; Anal. calcd for $C_9H_{10}N_2O$: C, 66.65; H, 6.21; N, 17.27. Found: C, 66.68; H, 6.10; N, 16.93.

4-(*R*)-4-[1-(2-Trimethylsilanyl-ethoxymethyl)-1*H*-benzoimidazol-2-yl-methyl]-azetidin-2-one (7d). Lactam **6d** (445 mg, 0.91 mmol) was treated with CsF as described above to give **7d** (240 mg, 79%) as a white solid. Mp 68°C; $[\alpha]_D^{25}-74°$ (*c* 0.67, $CHCl_3$); IR (KBr) 3177, 1745 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.73–7.69 (m, 1H), 7.42–7.39 (m, 1H), 7.37–7.26 (m, 2H), 6.51 (s, 1H), 5.48 (s, 2H), 4.34–4.28 (m, 1H), 3.56 (dd, J = 8.3, 8.3 Hz, 2H), 3.35 (dd, J = 16.2, 4.1 Hz, 1H), 3.28 (ddd, J = 14.9, 5.1, 1.9 Hz, 1H), 3.12 (dd, J = 15.9, 9.2 Hz, 1H), 2.82 (ddd, J = 14.9, 2.2, 1.3 Hz, 1H), 0.91 (dd, J = 8.0, 8.0 Hz, 2H), −0.04 (s, 9H); MS (ES+) 332 (M + H), 354 (M + 23); HRMS calcd for $C_{17}H_{26}N_3O_2Si$, 332.1794, found 332.1806; Anal. calcd for $C_{17}H_{25}N_3O_2Si$: C, 61.60; H, 7.60; N, 12.68. Found: C, 61.48; H, 7.79; N, 12.46.

4-(*R*)-4-Benzothiazol-2-yl-methyl-azetidin-2-one (7e). Lactam **6e** (819 mg, 2.19 mmol) was treated with CsF as described above to give **7e** (330 mg, 69%) as a white solid. mp 115°C; HPLC (A) 100%, (B) 100%; $[\alpha]_D-66°$ (*c* 0.41, $CHCl_3$); IR ($CHCl_3$) 1768 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.98 (d, J = 7.9 Hz, 1H), 7.87 (d, J = 7.9 Hz, 1H), 7.51–7.47 (m, 1H), 7.42–7.38 (m, 1H), 6.20 (br, 1H), 4.23–4.18 (m, 1H), 3.48 (dd, J_{AB} = 15.5, J_{AX} = 4.6 Hz, 1H), 3.36 (dd, J_{AB} = 15.5, J_{AX} = 8.4 Hz, 1H), 3.24 (ddd, J = 14.9, 5.1, 2.2 Hz, 1H), 2.85–2.81 (m, 1H); MS (ES^+) 219 (M + H); HRMS calcd for $C_{11}H_{11}N_2OS$, 219.0592, found 219.0599; Anal. calcd for $C_{11}H_{10}N_2OS$: C, 60.53; H, 4.62; N, 12.83. Found: C, 60.82; H, 4.33; N, 12.86.

4-(*S*)-4-Furan-2-yl-methyl-2-oxo-azetidine-1-carboxylic acid methyl-(4-trifluoromethyl-benzyl)-amide (10). Lactam **7a** (50 mg, 0.33 mmol) was dissolved in THF (5 mL) and cooled to −50°C. A 0.5 M solution of KHMDS in toluene (0.73 mL, 0.36 mmol) was added and the resulting solution was stirred at −50°C for 20 min. This solution was then added rapidly via cannula to a THF solution of amidyl chloride (250 mg, 1 mmol) at −50°C. The resulting pale-yellow mixture was stirred for 10 min and then quenched by the addition of saturated NH_4Cl. The reaction mixture was diluted with ether, washed twice with water, once with brine and dried over $MgSO_4$. The crude residue was purified by flash chromatography (25% EtOAc in hexanes to 50% EtOAc in hexanes) to provide compound **10** (86 mg, 71%) as a pale-yellow gum. HPLC (A) 100%, (B) 100%; $[\alpha]_D^{25}-39°$ (*c* 0.31, CH_2Cl_2); IR (neat) 1778, 1669 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.61 (d, J = 8.3 Hz, 2H), 7.38 (d, J = 8.3 Hz, 2H), 7.29 (d, J = 1.0 Hz, 1H), 6.30 (dd, J = 3.2, 1.9 Hz, 1H), 6.10 (dd, J = 3.2, 1.0 Hz, 1H), 4.62–4.58 (m, 2H), 4.47–4.42 (m, 1H), 3.21–2.82 (m, 4H), 2.96 (s, 3H); MS (ES^+) 367 (M + H); HRMS calcd for $C_{18}H_{18}F_3N_2O_3$ (M + H), 367.1269, found 367.1263; Anal. calcd For $C_{18}H_{17}F_3N_2O_3$: C, 59.02; H, 4.68; N, 7.65. Found: C, 59.16; H, 4.74; N, 7.45.

4-(*S*)-4-Furan-2-yl-methyl-2-oxo-azetidine-1-carboxylic acid methyl-(4-nitro-benzyl)-amide (11). This compound was prepared from **7a** (46 mg, 0.31 mmol) using a procedure similar to that described above for compound **10**. Compound **11** was obtained as an oil (60 mg, 57%) after flash chromatography (50% EtOAc in hexanes). HPLC (A) 99%, (B) 99%; $[\alpha]_D^{25}-126°$ (*c* 0.27, CH_2Cl_2); IR (neat) 1777, 1668 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.21 (d, J = 8.9 Hz, 2H), 7.43 (d, J = 8.9 Hz, 2H), 7.30 (d, J = 0.7 Hz, 1H), 6.30 (dd, J = 2.9, 2.0 Hz, 1H), 6.10 (dd, J = 2.9, 0.7 Hz, 1H), 4.71–4.61 (m, 2H), 4.45 (m, 1H), 3.21–2.82 (m, 4H), 2.97 (s, 3H); MS (ES^+) 344 (M + H); HRMS calcd for $C_{17}H_{18}N_3O_5$, 344.1246, found 344.1251; Anal. calcd for $C_{17}H_{17}N_3O_5$: C, 59.47; H, 4.99; N, 12.24. Found: C, 59.80; H, 5.06; N, 11.97.

4-(*R*)-4-Thiophen-2-yl-methyl-2-oxo-azetidine-1-carboxylic acid methyl-(4-trifluoromethyl-benzyl)-amide (12). This compound was prepared from **7b** (30 mg, 0.18 mmol) using a procedure similar to that described above for compound **10**. Compound **12** was obtained as an oil (40 mg, 50%) after flash chromatography (50% EtOAc in hexanes). HPLC (A) 97%, (B) 95%; $[\alpha]_D^{25}+249°$ (*c* 0.25, CH_2Cl_2); IR (neat) 1779, 1668 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.61 (d, J = 7.9 Hz, 2H), 7.38 (d, J = 7.9 Hz, 2H), 7.18 (dd, J = 5.4, 1.3 Hz, 1H), 6.95 (dd, J = 5.4, 3.2 Hz, 1H), 6.84 (dd, J = 3.2, 1.3 Hz, 1H), 4.78–4.60 (m, 2H), 4.49–4.44 (m, 1H), 3.42 (dd, J = 14.9, 3.8 Hz, 1H), 3.19 (m, 1H) 2.99 (s, 3H), 2.98 (dd, J = 15.9, 6.1 Hz, 1H), 2.76 (dd, J = 15.9, 3.8 Hz, 1H); MS (ES^+) 383 (M + H); HRMS calcd for $C_{18}H_{18}F_3N_2O_2S$ (M + H), 383.1041, found 383.1052.

4-(*R*)-2-Oxo-4-thiophen-2-yl-methyl-2-oxo-azetidine-1-carboxylic acid methyl-(4-nitro-benzyl)-amide (13). This compound was prepared from **7b** (30 mg, 0.18 mmol) using a procedure similar to that described above for compound **10**. Compound **13** was obtained as a pale-yellow syrup (32 mg, 50%) after flash chromatography (50% EtOAc in hexanes). HPLC (A) 99%, (B) 99%; $[\alpha]_D^{25}-141°$ (*c* 0.26, CH_2Cl_2); IR (neat) 1661, 1607 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.21 (d, J = 8.5 Hz, 2H), 7.43 (d, J = 8.5 Hz, 2H), 7.18 (dd, J = 5.1, 1.0 Hz, 1H), 6.96 (dd, J = 5.1, 3.5 Hz, 1H), 6.85 (dd, J = 3.5, 1.0 Hz, 1H), 4.74–4.64 (m, 2H), 4.47 (m, 1H), 3.41 (dd, J = 14.9, 3.8 Hz, 1H), 3.23 (dd, J = 14.9, 7.0 Hz, 1H), 3.02 (s, 3H), 3.00 (dd, J = 17.1, 6.1 Hz, 1H), 2.79 (dd, J = 15.9, 3.8 Hz, 1H); MS (ES^+) 360 (M + H); HRMS calcd for $C_{17}H_{18}N_3O_4S$ (M + H), 360.1018, found 360.1010.

4-(*S*)-4-Pyridin-2-yl-methyl-2-oxo-azetidine-1-carboxylic acid methyl-(4-trifluoromethyl-benzyl)-amide (14). This compound was prepared from **7c** (50 mg, 0.31 mmol) using a procedure similar to that described above for compound **10**. Compound **14** was obtained as a pale-yellow syrup (70 mg, 61%) after flash chromatography (90% EtOAc in hexanes). HPLC (A) 100%, (B) 100%; $[\alpha]_D^{25}+167°$ (*c* 0.24, CH_2Cl_2); IR (neat) 1777, 1668 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.51 (dd, J = 4.5, 1.3 Hz, 1H), 7.62–7.51 (m, 3H), 7.39 (d, J = 8.0 Hz, 2H), 7.17–7.14 (m, 2H), 4.61 (br, 1H), 4.61–4.56 (m, 2H), 3.35 (dd, J = 13.7, 4.2 Hz, 1H), 3.15 (br, 1H), 3.01–2.99 (m, 2H), 2.94 (s, 3H); MS (ES^+) 378 (M + H); HRMS calcd for $C_{19}H_{19}F_3N_3O_2$(M + H), 378.1429, found 378.1420.

4-(*R*)-4-(1*H*-Benzoimidazol-2-yl-methyl)-2-oxo-azetidine-1-carboxylic acid methyl-(4-trifluoromethyl-benzyl)-amide (15). This compound was prepared from **7d** using a procedure similar to that described above for compound **10**. Final deprotection was accomplished by stirring in 2/1 TFA/CH_2Cl_2 for 6 h. Removal of solvent followed by dissolution in EtOAc, washing with $NaHCO_3$ and brine and drying over $MgSO_4$ gave **15** (12.7 mg, 40%) as a white solid after flash chromatography (10% acetone in EtOAc). Mp 232°C (dec); HPLC (A) 100%, (B) 92%; $[\alpha]_D$ +22° (*c* 0.46, CH_3CN); IR ($CHCl_3$) 1729, 1655 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.23–8.21 (m, 1H), 7.72–7.69 (m, 1H), 7.54 (d, J = 8.0 Hz, 2H), 7.41–7.38 (m, 2H), 7.29 (d, J = 8.3 Hz, 2H), 4.71–4.64 (m, 1H), 4.51 (s, 2H), 4.46 (d, J = 6.4 Hz, 1H), 3.47 (dd, J_{AB} = 16.8, J_{AX} = 4.4 Hz, 1H), 3.33 (dd, J_{AB} = 16.8, J_{BX} = 6.4, 1H), 3.15 (dd, J_{AB} = 17.3, J_{AX} = 3.7 Hz, 1H), 3.11 (dd, J_{AB} = 17.3, J_{BX} = 6.4, 1H), 2.81 (s, 3H); MS (ES^+) 417 (M + H), 439 (M + 23); HRMS calcd for $C_{21}H_{20}F_3N_4O_2$ (M + H), 417.1538, found 417.1521.

4-(*R*)-4-(1*H*-Benzoimidazol-2-yl-methyl)-2-oxo-azetidine-1-carboxylic acid methyl-(4-nitro-benzyl)-amide (16). This compound was prepared from **7d** (79 mg, 0.24 mmol) using a procedure similar to that described above for compound **10**. Final deprotection was accomplished by stirring in 2/1 TFA/CH_2Cl_2 for 6 h. Removal of solvent followed by dissolution in EtOAc, washing with $NaHCO_3$ and brine and drying over $MgSO_4$ gave **16** (18 mg, 19%) as a white solid after flash chromatography (10% acetone in EtOAc). Mp 194°C; HPLC (A) 100%, (B) 95%; $[\alpha]_D$ +32° (*c* 0.45, CH_3CN); IR ($CHCl_3$) 1729, 1657 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.21–8.19 (m, 1H), 8.13–8.11 (m, 1H), 7.71–7.69 (m, 1H), 7.40–7.37 (m, 2H), 7.33 (d, J = 8.9 Hz, 2H), 4.71–4.64 (m, 1H), 4.55 (s, 2H), 4.45 (d, J = 6.0 Hz, 1H), 3.46 (dd, J_{AB} = 16.9, J_{AX} = 4.2 Hz, 1H), 3.35 (dd, J_{AB} = 16.9, J_{BX} = 6.0, 1H), 3.14 (d, J = 5.1 Hz, 2H), 2.84 (s, 3H); MS (ES^+) 394 (M + H), 416 (M + 23); HRMS calcd for $C_{20}H_{20}N_5O_4$, 394.1515, found 394.1528.

4-(*R*)-4-(2-Methyl-2*H*-tetrazol-5-yl-methyl)-2-oxo-azetidine-1-carboxylic acid methyl-(4-trifluoromethyl-benzyl)-amide (17). This compound was prepared from **8** (40 mg, 0.24 mmol) using a procedure similar to that described above for compound **10**. Compound **17** was obtained as a pale-yellow oil (49 mg, 55%) after flash chromatography (50% EtOAc in hexanes). HPLC (A) 100%, (B) 99%; IR (neat) 1779, 1669 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.56 (d, J = 8.0 Hz, 2H), 7.35 (d, J = 8.0 Hz, 2H), 4.70–4.49 (m, 3H), 4.21 (s, 3H), 3.40 (dd, J = 15.0, 4.1 Hz, 1H), 3.28 (dd, J = 15.0, 6.5 Hz, 1H), 3.03 (dd, J = 16.0, 6.0 Hz, 1H), 2.90 (s, 3H), 2.83 (dd, J = 16.0, 3.7 Hz, 1H); MS (FAB) 383 (M + H); HRMS calcd for $C_{16}H_{18}F_3N_6O_6$, 383.1444, found 383.1452.

4-(*R*)-4-(2-Methyl-2*H*-tetrazol-5-yl-methyl)-2-oxo-azetidine-1-carboxylic acid methyl-(4-nitro-benzyl)-amide (18). This compound was prepared from **8** (50 mg, 0.29 mmol) using a procedure similar to that described above for compound **10**. Compound **18** was obtained as a pale-yellow syrup (54 mg, 53%) after flash chromatography (50% EtOAc in hexanes). HPLC (A) 96%, (B) 96%; IR (neat) 1779, 1669 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.22 (d, J = 8.6 Hz, 2H), 7.47 (d, J = 8.9 Hz, 2H), 4.73–4.56 (m, 3H), 4.29 (s, 3H), 3.43 (dd, J = 15.0, 4.5 Hz, 1H), 3.36 (dd, J = 15.0, 6.4 Hz, 1H), 3.09 (dd, J = 15.9, 6.0 Hz, 1H), 2.99 (s, 3H), 2.88 (dd, J = 16.2, 3.5 Hz, 1H); MS (FAB) 360 (M + H); HRMS calcd for $C_{15}H_{18}N_7O_4$, 360.1420, found 360.1414.

4-(*R*)-4-Benzothiazol-2-yl-methyl-2-oxo-azetidine-1-carboxylic acid methyl-(4-trifluoromethyl-benzyl)-amide (19). This compound was prepared from **7e** (141 mg, 0.65 mmol) using a procedure similar to that described above for compound **10**. Compound **19** was obtained as a pale-yellow syrup (153 mg, 55%) after flash chromatography (40% EtOAc in hexanes). HPLC (A) 100%, (B) 100%; $[\alpha]_D$ +91° (*c* 0.49, $CHCl_3$); IR ($CHCl_3$) 1782, 1669 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.95 (d, J = 8.3 Hz, 1H), 7.84 (d, J = 7.9 Hz, 1H), 7.59 (d, J = 8.3 Hz, 2H), 7.49–7.45 (m, 1H), 7.41–7.37 (m, 3H), 4.72–4.61 (m, 3H), 3.69 (dd, J_{AB} = 15.1, J_{AX} = 4.1 Hz, 1H), 3.52 (dd, J_{AB} = 15.1, J_{AX} = 7.3 Hz, 1H), 3.12 (dd, J_{AB} = 16.2, J_{AX} = 6.3 Hz, 1H), 3.06 (dd, J_{AB} = 16.2, J_{AX} = 3.6 Hz, 1H), 2.98 (s, 3H); MS (ES^+) 434 (M + H); HRMS calcd for $C_{21}H_{19}F_3N_3O_2S$, 434.1150, found 434.1164.

4-(*R*)-4-Benzothiazol-2-yl-methyl-2-oxo-azetidine-1-carboxylic acid methyl-(4-nitro-benzyl)-amide (20). This compound was prepared from **7e** (70 mg, 0.32 mmol) using a procedure similar to that described above for compound **10**. Compound **20** was obtained as a pale-yellow syrup (44 mg, 33%) after flash chromatography using TLC grade silica gel (60% EtOAc in hexanes). HPLC (A) 100%, (B) 99%; $[\alpha]_D$ +96° (*c* 0.60, $CHCl_3$); IR ($CHCl_3$) 1782, 1670 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.18 (d, J = 8.9 Hz, 2H), 7.94 (d, J = 8.3 Hz, 1H), 7.85 (d, J = 7.6 Hz, 1H), 7.50–7.38 (m, 4H), 4.75–4.64 (m, 3H), 3.67 (dd, J_{AB} = 15.0, J_{AX} = 4.0 Hz, 1H), 3.56 (dd, J_{AB} = 15.0, J_{AX} = 6.8 Hz, 1H), 3.17–3.05 (m, 2H), 3.01 (s, 3H); MS (ES^+) 411 (M + H); HRMS calcd for $C_{20}H_{19}N_4O_4S$, 411.1127, found 411.1117; Anal. calcd for $C_{20}H_{18}N_4O_4S$: C, 58.53; H, 4.42; N, 13.65. Found: C, 58.29; H, 4.40; N, 13.45.

4-(*R*)-4-Thiazol-2-yl-methyl-2-oxo-azetidine-1-carboxylic acid methyl-(4-trifluoromethyl-benzyl)-amide (21). This compound was prepared from **7f** (53 mg, 0.32 mmol) using a procedure similar to that described above for compound **10**. Compound **21** was obtained as a pale-yellow oil (64 mg, 53%) after flash chromatography using TLC grade silica gel (50% EtOAc in hexanes). HPLC (A) 100%, (B) 100%; $[\alpha]_D$ +72° (*c* 0.55, $CHCl_3$); IR ($CHCl_3$) 1781, 1668 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.72 (d, J = 3.2 Hz, 1H), 7.62 (d, J = 8.3 Hz, 2H), 7.40 (d, J = 7.9 Hz, 2H), 7.25 (d, J = 3.5 Hz, 1H), 4.75–4.55 (m, 3H), 3.59 (dd, J_{AB} = 14.9, J_{AX} = 4.2 Hz, 1H), 3.45 (dd, J_{AB} = 14.9, J_{AX} = 6.9 Hz, 1H), 3.10–3.01 (m, 2H), 2.97 (s, 3H); MS (ES^+) 389 (M + H), 406 (M + 23); HRMS calcd for $C_{17}H_{17}F_3N_3O_2S$, 384.0994, found 384.0987; Anal. calcd For $C_{17}H_{16}F_3N_3O_2S$: C, 53.26; H, 4.21; N, 10.96. Found: C, 53.08; H, 4.29; N, 10.88.

4-(*R*)-4-Thiazol-2-yl-methyl-2-oxo-azetidine-1-carboxylic acid methyl-(4-nitro-benzyl)-amide (22). This compound

was prepared from **7f** (50 mg, 0.30 mmol) using a procedure similar to that described above for compound **10**. Compound **21** was obtained as a pale-yellow oil (40 mg, 37%) after flash chromatography using TLC grade silica gel (60% EtOAc in hexanes). HPLC (A) 100%, (B) 99%; $[\alpha]_D$ +66° (*c* 0.62, $CHCl_3$); IR ($CHCl_3$) 1781, 1670 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.23–8.20 (m, 2H), 7.72 (d, J = 3.2 Hz, 1H), 7.46 (d, J = 8.6 Hz, 2H), 7.26 (d, J = 3.2 Hz, 1H), 4.68–4.57 (m, 3H), 3.58 (dd, J_{AB} = 14.9, J_{AX} = 4.1 Hz, 1H), 3.47 (dd, J_{AB} = 14.9, J_{AX} = 6.8 Hz, 1H), 3.11–3.03 (m, 2H), 2.99 (s, 3H); MS (ES^+) 361 (M + H), 383 (M + 23); HRMS calcd for $C_{16}H_{17}N_4O_4S$, 361.0971, found 361.0958; Anal. calcd for $C_{16}H_{16}N_4O_4S$: C, 53.32; H, 4.47; N, 15.55. Found: C, 53.06; H, 4.43; N, 15.35.

Enzymatic and plaque reduction assays

IC_{50} and EC_{50} values were determined by previously published methods.[12,20,35] Each reported IC_{50} value represents the mean of at least 3 determinations. The reproducibility of the enzymatic assay was gauged using Ac-Gly-Tbg-Val-Asn-Ala(CF_3) as a standard. This compound gave an IC_{50} of 8.28 ± 0.27 (95% confidence limit based on $2s_m$) on 302 determinations. Ganciclovir was used as a positive control in the plaque reduction assay and gave an EC_{50} of 1.23 ± 0.07 mM (95% confidence limit based on $2s_m$) on 82 determinations. The cytotoxicity of drugs (TC_{50}) was determined with the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT)[36] under the same cell culture assay conditions used in the plaque reduction assay.

Cell based assay

The coding sequences for the HCMV UL80 (protease precursor) and UL80.5 (assembly protein precursor) genes were amplified from HCMV genomic DNA by PCR and cloned into pCR3 plasmid vector (Invitrogen) which can direct expression of protein under the control of the T7 promoter in mammalian cells.[29] For expression of the catalytic domain of the protease, the DNA sequence encoding amino acid 1-256 of the UL80 gene was amplified by PCR using HCMV genomic DNA as a template and cloned in the pCR3 vector (Invitrogen).

COS-7, African green monkey kidney cells maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum were used for these experiments. COS-7 cells were infected with recombinant vaccinia virus v-TF7.3, which expresses bacteriophage T7 polymerase, at a m.o.i of 5 PFU/cell and incubated at 37°C for 1 h. The cells were then transfected with either the plasmid expressing the protease precursor or cotransfected with the plasmid for the assembly protein precursor and that for the catalytic domain of the protease using calcium phosphate mediated transfection.[37] Sixteen hours post transfection, the cells were exposed to protease inhibitors for a period of 2 h prior to metabolic labeling. The protease inhibitors were dissolved in DMSO and diluted to the desired concentrations in tissue culture medium. The cells were incubated in cysteine and methionine deficient DMEM for 20 min and labeled for 2 h with Easy Tag Express (100 μCi/mL), NEN. Cells were lysed in ice-cold RIPA buffer containing protease inhibitors. The samples were centrifuged and the supernatant subjected to immunoprecipitation. Total [^{35}S] incorporation into protein was determined using TCA precipitation on Whatman chromatography paper (Et-31). Aliquots representing the same number of counts were incubated with 10 μL of rabbit K-58 polyclonal antibody coupled to anti rabbit IgG-conjugated magnetic beads (Dynal). K-58 serum was produced using a peptide representing amino acids 196-213 of HCMV assembly protein. The immunoprecipitated proteins were solubilized in SDS sample buffer and denatured by heating at 95°C for 5 min. Samples were electrophoresed on SDS-polyacrylamide gels. The gels were dried and exposed to phosphor-autoradiography. The quantitation of the radioactivity contained in the precursor and the product was done using a phosphoimager (Molecular Dynamics).

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